The Biodesign Institute at Arizona State University
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Executive Summary

The Biodesign Institute is the cornerstone of Arizona State University’s strategy to propel ASU to top-tier research university status by 2012. The State of Arizona has made this major investment in the Institute with the expectation that Biodesign will become a world-class, entrepreneurial research enterprise that:

1. Strengthens regional research and training capabilities
2. Drives technology transfer and regional economic development
3. Improves human health and quality of life

The Biodesign Institute pursues “use-inspired” research, aligning potential societal benefits with scientific knowledge and advances. This requires an approach not commonly found in academic settings. We start by identifying pressing societal needs where Biodesign has an assessed potential for significant impact. Then, we assemble teams whose collective expertise provides a mechanism to solve the identified challenge. Specifically, the Biodesign Institute is focusing on:

4. Improving health care through more personalized medicine
5. Outpacing the global threat of infectious diseases
6. Improving our environment through renewable energy and bioremediation

Addressing these complex problems requires large teams with a convergence of expertise in the biosciences, nanoscale engineering and advanced computing. Biodesign and ASU have significant depth and breadth of expertise in these areas. It amplifies these capabilities through strategic partnerships with other academic institutions and industry. Additionally, such large-scale efforts require sophisticated and sustained project management, and Biodesign provides this to a degree that is distinct among its peer institutions.

Thanks to this bold approach, rapid growth, and superb facilities, the Biodesign Institute will continue to be a magnet for top scientific and academic talent. ASU’s efforts dovetail with a statewide initiative to build a robust bioscience economy in Arizona. Our contribution is evident in three key areas:

Generating research funding: We secure significant external funding (more than $300 million in external funding since inception); reducing reliance on State funds;

Driving innovation leading to economic growth: Via ASU’s technology transfer office, Arizona Technology Enterprises, we have disclosed more than 279 inventions to date resulting in 178 provisional patents, 57 patent filings and 18 issued patents. This innovation is a critical component of efforts to attract and spin out new local companies. The formation of a new technology incubator, the Biodesign Impact Accelerator, will nurture Biodesign startup companies and will become a critical component in the successful translation of our discoveries into the marketplace. Additional information on the Impact Accelerator is included later in this document.

Workforce development: We help develop a home-grown workforce through hands-on laboratory training. To date, we’ve employed/trained 188 postdoctoral researchers and 301 graduate students; more than 170 have entered the workforce to date. We’ve also provided research experiences for 403 undergraduates, 95 high school students and 36 high school teachers.
SECTION ONE
Biodesign Institute Overview

Impetus
Our Approach
Our Team
Translation
Accomplishments
Rationale for ASU’s Investment in the Biodesign Institute

To succeed in the global economy, Arizona must educate its workforce, attract investments, build industries and further new knowledge in critical areas to differentiate Arizona from our national and international competitors. Though Arizona ranks 20th in the Kauffman Foundation 2008 State New Economy Index (Page 6), our state ranks 34th in Scientists and Engineers. Arizona has increased its position from 41st to 30th in Workforce Education since 2002, but we rank 11th in terms of job turnover. Improving our ability to provide a skilled workforce in emerging technologies and to innovate is essential in competing with other states in the growth of a new economy.

In 2002, ASU made a strategic commitment to form the Biodesign Institute as the flagship initiative to advance its scientific research and development capabilities in a way that would not simply replicate what other top research universities had done, but would establish a new gold standard for the American research university. This ambitious goal required a significant departure from traditional research approaches, and placed an atypical, goal-oriented responsibility on Biodesign to predict the trajectory of bioscience advances and establish programs in spaces not occupied by peers. This leapfrog approach is essential to a globally competitive biosciences industry cluster in Arizona.

The Bioscience industry sector remains one of the fastest growing and lucrative in the United States economy, so is highly competitive. Community leaders in Arizona have targeted this area for regional economic development by endorsing the “Arizona Bioscience Roadmap” which proposes an investment plan to expand this industry. Biotechnology, which is expected to be a major driving force behind the new economy of the 21st Century, is one of the most research-intensive industries in the world. ASU is expected to facilitate Arizona’s successful competition in this arena. Thus, there are high expectations and far-reaching goals established for the Institute.

“ I envision a community of scholars...guided by a focus on purpose.”

Michael Crow
A New American University

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The 2008 State New Economy Index ranks Arizona on a number of indicators, shown below:

<table>
<thead>
<tr>
<th>Category</th>
<th>2008 Ranking</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>20</td>
</tr>
<tr>
<td>Managerial, Professional and Technical Jobs</td>
<td>19</td>
</tr>
<tr>
<td>Workforce Education</td>
<td>30</td>
</tr>
<tr>
<td>Immigration of Knowledge Workers</td>
<td>50</td>
</tr>
<tr>
<td>Migration of Knowledge Workers</td>
<td>28</td>
</tr>
<tr>
<td>Manufacturing Value Added</td>
<td>7</td>
</tr>
<tr>
<td>Export Focus of Manufacturing and Services</td>
<td>7</td>
</tr>
<tr>
<td>Foreign Direct Investment</td>
<td>38</td>
</tr>
<tr>
<td>“Gazelle” Jobs</td>
<td>33</td>
</tr>
<tr>
<td>Job Churning</td>
<td>11</td>
</tr>
<tr>
<td>Fastest Growing Firms</td>
<td>12</td>
</tr>
<tr>
<td>IPOs</td>
<td>28</td>
</tr>
<tr>
<td>Entrepreneurial Activity</td>
<td>23</td>
</tr>
<tr>
<td>Online Population</td>
<td>29</td>
</tr>
<tr>
<td>Technology in Schools</td>
<td>40</td>
</tr>
<tr>
<td>Broadband/Telecommunications</td>
<td>22</td>
</tr>
<tr>
<td>Health IT</td>
<td>8</td>
</tr>
<tr>
<td>High-Tech Jobs</td>
<td>19</td>
</tr>
<tr>
<td>Scientists and Engineers</td>
<td>34</td>
</tr>
<tr>
<td>Industry R &amp; D Investment</td>
<td>18</td>
</tr>
<tr>
<td>Non-Industry R &amp; D Investment</td>
<td>27</td>
</tr>
<tr>
<td>Alternative Energy Use</td>
<td>16</td>
</tr>
<tr>
<td>Venture Capital</td>
<td>19</td>
</tr>
</tbody>
</table>

Leading a competitive academic research organization requires stringent analysis of the factors deemed most likely to determine future success. Contemporary academic research must be prepared to:

- Adapt organizationally to the blurring of boundaries between traditional intellectual disciplines (technology convergence);
- Recognize and master technology convergence as the driver of advanced research and prepare students given such convergence is driving new knowledge-based industries,
- Acknowledge and adapt to the globalization of research and education with intensifying competition from developing regions;
- Diversify research funding sources to supplement government funding, which is increasingly competitive and poorly adapted to the escalating scale and complexity of interdisciplinary research.

Biodesign is leveraging the investment in its infrastructure to address these challenges and attract top-notch research and administrative talent. It has crystallized an organizational structure that positions it to be nimble and competitive. The quality of research, refinement of the thematic focus areas and implementation of proposal development and project management processes have contributed to these capabilities. It has developed a five-year business plan that guides the strategic initiatives, investments and partnerships of the Biodesign Institute.
The Market

The broad bioscience industry is one of the fastest growing areas globally, and can be classified into four subsectors: 1) Agricultural Feedstock & Chemicals; 2) Drugs & Pharmaceuticals; 3) Medical Devices & Equipment; and 4) Research, Testing, & Medical Laboratories. Specific segments of this industry are well-positioned for expansion into the next decade. The field described as “biotechnology” leverages the latest innovations in molecular biology, genomics, genetic engineering, and other emerging fields such as “synthetic” biology. Biotechnology is having broad impact, cutting across all four traditional subsectors of the bioscience industry.

Biotechnology is considered “one of the most research-intensive industries in the world”. The table below shows the growth in recent biotech R&D expenditures and the number of biotech companies over eight years. According to the recent report, Biotech 2009, the R&D expenditures of public companies in 2008 was $99.5 billion.

### Burrill Public BioTech Companies Performance 2001-2008

<table>
<thead>
<tr>
<th>Year</th>
<th>2001</th>
<th>2002</th>
<th>2003</th>
<th>2004</th>
<th>2005</th>
<th>2006</th>
<th>2007</th>
<th>2008</th>
<th>Annual % Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>B$ Revenues*</td>
<td>39.0</td>
<td>42.7</td>
<td>47.4</td>
<td>59.5</td>
<td>71.5</td>
<td>82.6</td>
<td>89.6</td>
<td>99.5</td>
<td>11.05%</td>
</tr>
<tr>
<td>B$ R&amp;D Expense*</td>
<td>12.3</td>
<td>13.5</td>
<td>14.3</td>
<td>16.8</td>
<td>18.5</td>
<td>21.7</td>
<td>23.0</td>
<td>23.7</td>
<td>3.04%</td>
</tr>
<tr>
<td>B$ Net Income (Loss)*</td>
<td>-4.7</td>
<td>-11.6</td>
<td>-4.1</td>
<td>-4.4</td>
<td>-4.1</td>
<td>-3.2</td>
<td>-0.6</td>
<td>3.7</td>
<td>516.67%</td>
</tr>
<tr>
<td>B$ Market Capitalization*</td>
<td>383.0</td>
<td>224.0</td>
<td>344.0</td>
<td>400.0</td>
<td>490.0</td>
<td>492.0</td>
<td>454.0</td>
<td>404.0</td>
<td>-11.01%</td>
</tr>
<tr>
<td>No. of Companies</td>
<td>356</td>
<td>329</td>
<td>315</td>
<td>356</td>
<td>363</td>
<td>360</td>
<td>365</td>
<td>357</td>
<td>-2.19%</td>
</tr>
</tbody>
</table>

*$ Amounts are U.S. dollars in billions.

Market capitalization of biotechnology, the total value of publicly traded biotech companies at market prices, was $404 billion in 2008 dropping from a high of $492 billion in 2006, reflecting the downturn in global economic conditions. According to BioWorld, biotechnology raised more than $100 billion in new financing in the five-year span of 2003-2007. These biotechnology companies are expected to continue to be a major driving force behind the new economy of the 21st Century.

Technology Trends

The most compelling trend of the past decade — rapid and exponential technology convergence — is a key trajectory upon which Biodesign hopes to capitalize. The momentum of modern

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science and technology is being driven by the blurring of boundaries between the traditional disciplines, such as, biology, chemistry, physics, medicine, agriculture, ecology, engineering and computing. From the discovery of the DNA double helix in 1953 to the sequencing of the human genome in 1996, there has been a continuous revolution in genetics and molecular biology that is revealing the control mechanisms for key biological processes, while technological advances in other fields, particularly engineering and computing, have enabled the sophisticated instruments and analytical tools needed to study the design and dynamic processes of living organisms. Translating the resulting insights into useful products and services is at the core of biotechnology.

ASU and Biodesign have a distinct advantage in that our program was created with the knowledge of this trend. While peer institutions have had to restructure and “re-culture” for this challenge and opportunity, Biodesign was established around the convergent areas of ‘bio, nano and info.’

The Biodesign Institute is strategically-positioned to leverage converging technologies.

The leadership of the Biodesign Institute has recognized technology convergence as a linchpin in successful innovation and competitiveness for the future. It must be leveraged in an environment where there is a purposeful application of the technology to urgent societal needs and where the issues of feasibility and scalability are addressed early in the research and development process. This convergence leans heavily on the ability to collaborate, and this is an ASU strength that is rapidly becoming a hallmark for Arizona, as discussed in the following section.

**Competitive Landscape**

**U.S. and Global Trends**

Clearly, the challenges being addressed by the Biodesign Institute are global in nature and competitors are also increasingly global. Therefore, Biodesign must increase its awareness of the research and technology developments taking place around the world, particularly in developing countries that are significantly increasing their research investment — countries such as India and China.

Currently, the U.S. government and industry are the major investors in bioscience research and development and represent Biodesign’s most significant competition. In the U.S., the nationally recognized centers of excellence in bioscience research commercialization tend to be located along the east and west coasts, and have emerged around academic strongholds. Examples of
these respected and heavily endowed universities and research institutes include Maryland/DC’s bioscience cluster, which has formed around Johns Hopkins and the University of Maryland, and the Bay Area’s vibrant biosciences industry, which has formed around Stanford University (Bio-X). These institutions are heavily bolstered by investments made by their respective states. Business Facilities magazine has recently done a ranking of the different states and regions for their overall strength in biotechnology. They used government statistics and the latest State Bioscience Initiatives Report, prepared by the BIO and Battelle as their starting point, adding factors such as the amount of state R&D funding and venture capital investments; the level of concentrated occupational employment in biotech; tax exemptions specifically targeted to biotech; the number of biotech facilities; biotech patents generated; university grant funding; and bioscience higher education degrees.

Overall Biotechnology Strength in U.S. (based on 2007 data)
*Texas tied with Ohio in ranking

1. Pennsylvania
2. California
3. Massachusetts
4. Ohio
4.* Texas
6. Illinois
7. New Jersey
8. New York
9. Florida
10. Kansas

Noteworthy in this report was the recognition that Arizona was found to have the third highest 2007 annual investment of state and local funds into bioscience related research.

Funding for Bioscience Research by State (based on 2007 data)

<table>
<thead>
<tr>
<th>Rank</th>
<th>State</th>
<th>Research Expenditures</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Pennsylvania</td>
<td>$150 million</td>
</tr>
<tr>
<td>2.</td>
<td>New York</td>
<td>$120 million</td>
</tr>
<tr>
<td>3.</td>
<td>Arizona</td>
<td>$110 million</td>
</tr>
<tr>
<td>4.</td>
<td>Georgia</td>
<td>$80 million</td>
</tr>
<tr>
<td>5.</td>
<td>Kansas</td>
<td>$63 million</td>
</tr>
<tr>
<td>6.</td>
<td>Maine</td>
<td>$59 million</td>
</tr>
<tr>
<td>7.</td>
<td>Indiana</td>
<td>$50 million</td>
</tr>
<tr>
<td>8.</td>
<td>Ohio</td>
<td>$50 million</td>
</tr>
<tr>
<td>9.</td>
<td>Texas</td>
<td>$50 million</td>
</tr>
<tr>
<td></td>
<td>Washington</td>
<td>$40 million</td>
</tr>
</tbody>
</table>

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Pennsylvania set aside $150 million in 2007-2008 from its tobacco settlement allocation to support bioscience research. California, New Jersey, Ohio, Connecticut and New York have made major financial commitments to support the advancement of stem cell research. In California, Proposition 71 committed $3 billion in funding to the California Institute for Regenerative Medicine (CiRHM). Iowa allocated $100 million in 2008 for the Iowa Power Fund supporting research on clean bioenergy technology. Connecticut, Georgia, and Kansas each have established special financing programs to support commercial bioscience facilities. In the major biotechnology subsectors, Texas took the lead in agricultural feedstock and chemicals, followed by Illinois and Tennessee. In 2007, California edged out New Jersey in drugs and pharmaceuticals, as well as in research, testing and medical labs.

All of these trends have been significantly impacted by the recent economic recession. For example, the recent Arizona State budget has all but eliminated state support for Science Foundation Arizona, a tax-exempt organization launched with private and public support to deliver and manage an investment strategy in purpose-driven research and innovation for Arizona’s knowledge-based economy to grow and prosper. State support for SFAz will be important to regain as the economy improves, but, fortunately, the large stimulus-related infusion of funds to the federal granting agencies (i.e. NIH, NSF, and DOE) could offset the local losses given our track record of success in competing for this source of funding.

In the U.S., there are several top academic research institutions viewed as competitors and benchmarks for the Biodesign Institute. These include: BIO-X (Stanford University), Institute for Biotechnology & Life Sciences (Cornell), Institute for Quantitative Biomedical Research (U. of California System), Broad Institute (MIT & Harvard), Life Sciences Institute (U. of Michigan), Computational and Systems Biology (MIT), Institute for Genome Sciences and Policy (Duke), and the Lewis Siegler Institute (Princeton). These competitors are diverse in terms of size, maturity, levels of funding, and research management philosophies. However, they are recognized as successful based upon their national reputation and ability to attract competitive funding. We monitor the organizational design, research strategy, recruiting activity, public and media relations programs, and development efforts of these world-class institutions to set performance standards and identify approaches that can be emulated or surpassed by Biodesign.

Arizona Trends

Clearly, competition with such well-funded states and well-established institutional giants requires a highly innovative approach that does not follow the well-blazed trail. Arizona’s historic bioscience strengths are in the agricultural chemicals and medical devices segments, but it is gaining significant momentum in personalized medicine, cancer research, neurosciences, and bioengineering. The biosciences industry sector in the greater Phoenix area, the most populous area of the state, has emerging fields of proteomics, nanotechnology, and bioinformatics, in addition to aerospace and business services that continue to be important. Arizona and the Biodesign Institute must look for ways to leverage synergies between these industries.

ASU and Biodesign’s efforts dovetail with a statewide initiative to build a robust bioscience economy in Arizona. The focused statewide efforts began in 2002 with the establishment of a

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ten-year Bioscience Roadmap commissioned by the Flinn Foundation. Arizona’s investment in this initiative — $140 million per year — was based on the following anticipated returns:

- Greater availability of **new medical treatments** for Arizona residents.
- Opportunity to build a **higher-wage, skilled, and technology-driven** employment base overall, with added benefit of an industry that provides jobs at various skill levels.
- Ability to **build upon Arizona’s strengths**. While the State has fledgling bioscience competencies, it has some distinct advantages in high-tech, computing, and engineering fields, which are emerging as a critical component of modern bioscience discovery.
- Greater **stability for Arizona’s economy** with a sector that balances more cyclical industries.

The recent Battelle economic impact analysis confirmed the biosciences sector is becoming key contributor to Arizona’s economy.⁶ They found for 2007:

- The bioscience sector contributed $12.5 billion in economic activity to the Arizona economy.
- 87,415 bioscience workers — accounting for 2.5% of total state employment — earned $5.3 billion in compensation. This includes 16,000 new biosciences jobs since 2002, an increase of 23 percent. This is three times the national average.
- The total impact of the bioscience sector, taking into account the dollars spent on goods and services by bioscience companies and their workers, was $21.1 billion accounting for more than 155,600 jobs or 4% of all employment in the state.
- The result of this total impact of the bioscience sector was the generation of $765.7 million in state and local taxes.

In 2020, if the goals of the Arizona Bioscience Roadmap are attained, it is projected that:

- The bioscience sector will generate a total impact on economic activity of $34.3 billion, 62% higher than in 2007.
- 252,676 jobs will result from the total impact of the biosciences sector — an increase of 97,045 jobs or 62% higher from 2007 levels.
- The bioscience sector will generate from its total impact more than $1.2 billion in state and local taxes.⁶

Arizona is one of seven states where a majority of the existing biomedical/biotechnology firms have experienced employment or sales growth above 25 percent for 2007. In order to ensure a sufficient workforce to attract biotech companies, Arizona has established an aggressive workforce development plan. In 2004, a $950 million bond was issued for Maricopa Community Colleges of which $100 million for bioscience and healthcare training was identified to enhance the bioscience work force pipeline in the state.⁷

The Arizona plan has focused on advancing collaborations, emphasizing translational research, and building on areas of technology convergence within the biosciences — in fields like bioimaging, bioinformatics, genomics, and proteomics. ASU is leveraging the fact that this was previously an “unformed” part of Arizona’s economy and transform it into strength due to greater ability and willingness to form partnerships — even between rival universities and competing healthcare systems.

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Arizona’s progress against the statewide roadmap for the first several years is encouraging in some areas, but with clear areas for improvement in others. For example, one target specific to research funding was an increase in National Institutes of Health (NIH) funding equal to or greater than the average of the top ten states. The average funding growth for the top ten states in NIH funding was 23%. While ASU exceeded this metric significantly with a 30% increase, Arizona’s overall share of NIH funding increased at a rate of only 19%. ASU’s increases were almost entirely due to the advent of the Biodesign Institute. Because much of this is related to growth in the number of faculty (more than fifty new faculty members have been recruited since Biodesign’s founding), Biodesign’s business plan must encompass a strategy for continued growth in funding despite a natural leveling in faculty growth as the facility reaches capacity.
OUR APPROACH

Mission & Vision
Research Portfolio Themes
Funding Sources
Program Priorities
Start-up Opportunities
Core Competencies
Infrastructure
Mission & Vision

Our mission is to “Improve human health and the health of our planet.”

Our vision is to be the benchmark for excellence in use-inspired, collaborative research through understanding nature’s design of biological systems. Biodesign will be a catalyst for discovery and innovation through an environment that fosters integration and translation of research to useful applications. The research agenda is aligned to three major global challenges: advancing personalized medicine, outpacing infectious diseases and sustaining our environment.

A Bold New Approach

The Biodesign Institute has been designed as a “tightly-coupled” research enterprise as it will be bound by a common mission and vision and will be aligned through interdependency, unified program planning and unified metrics. This is distinct from most interdisciplinary research efforts, which use a more loosely structured “federation” model in which scientists contribute to projects based on their capabilities in a more time-limited, as needed fashion. Both models differ significantly from the traditional, investigator-driven academic research of the past, which is contrasted below:

<table>
<thead>
<tr>
<th>Traditional Approach</th>
<th>Biodesign Approach</th>
</tr>
</thead>
<tbody>
<tr>
<td>Societal needs drive the research focus — then the needed experts are engaged</td>
<td>Scientist's expertise drives the research focus</td>
</tr>
<tr>
<td>A team addresses multiple parts of the problem</td>
<td>Researcher addresses highly specific component of a problem</td>
</tr>
<tr>
<td>Applications — if pursued — emerge from the findings</td>
<td>Applications — if pursued — emerge from the findings</td>
</tr>
<tr>
<td>The area of application is clear throughout project; may be adapted by the findings.</td>
<td></td>
</tr>
</tbody>
</table>

A key to aligning the diverse resources of the Biodesign Institute to advance translational research is the targeting of specific, major global challenges. The Biodesign Institute has selected three areas of emphasis:

Research Portfolio Themes

1. Improving health care through more personalized medicine, with a focus on diagnostic technologies and cancer eradication;
2. Outpacing the global threat of infectious diseases; and
3. Improving our environment through renewable energy and bioremediation

The following two pages outline each of these areas.
**Personalized Medicine**

Biodesign has chosen to focus much of its efforts on target applications that may have profound benefits in improving medical outcomes. We are developing new molecular tools to assess disease risk and diagnose disease earlier and more accurately. Improved, accurate detection of diseases in their earliest stages will increase the prospect of better outcomes and reduce treatment costs. These approaches are part of a sea change to ‘Personalized Medicine,’ with diagnostic advances being key to defining the precise cause of a patient’s illness and allowing selection of the best available treatment for that individual. Personalized medicine will also embrace the importance of genetic differences in how individuals “metabolize” medicines, allowing dangerous side effects from medicines to be eliminated or reduced. This area of innovation also encompasses efforts to eradicate cancer. In addition to earlier, more accurate diagnoses, our efforts encompass such innovative concepts as a vaccine to prevent cancer.

**Outpacing Infectious Disease**

The leading causes of death reported by the WHO in 2002 were infectious diseases; specifically, more than 1,500 people worldwide die hourly from complications related to infections. Most result from six conditions: HIV/AIDS, malaria, measles, pneumonia, TB, and various diarrheal diseases. Children are particularly vulnerable. More than thirty new infectious diseases have been discovered in the last fifty years and new emerging infections such as SARS, avian flu and swine flu highlight the threat of pandemics.

At the same time, old foes are becoming resistant to antibiotics. More than 170,000 Americans will die this year from infections; more than twice the number occurring in 1980. Yet new antimicrobial drugs in the discovery pipeline are alarmingly scarce. For these reasons, outpacing infectious diseases through new vaccines, faster vaccine development technology and other methods is a major emphasis of Biodesign research.

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Renewable Energy and Bioremediation

According to a United Nations report, a staggering seventy percent of the earth’s land surface will be “destroyed, fragmented or disturbed” by 2023. Science can play a critical role in developing new solutions to limit and repair the destructive impact of human activities on the environment. Not only do we need clean air and water to survive, but environmental deterioration due to rapid urbanization of global society provides a “flashpoint” where infectious diseases and other public health threats could converge with devastating impact.

For these reasons, the Biodesign Institute includes research in alternative bioenergy sources along with bioremediation methods aimed at developing more sustainable systems. Alternative energy efforts focus on renewable bioenergy sources which do not increase the levels of greenhouse gases and do not deplete highly valuable, arable land. Bioremediation efforts encompass efforts to use microbes and other nature-inspired solutions to clean harmful contaminants from the environment.

Research Funding Sources

The various sources of funding for Biodesign programs and projects are tracked via the following categories:

1. Research Grants and Contracts
   a. Federal Research Grants and Contracts
   b. Arizona State or Local Government Research Grants and Contracts
   c. Private, For-Profit or Industrial Research Grants and Contracts
   d. Private, Non-Profit Grants, Contracts, and Gifts
2. ASU/Biodesign Internal Funding Sources and Revenues
3. Technology Research Initiative Fund (TRIF)
4. Biodesign Impact Accelerator & Technology License Fees
5. ASU Foundation (Philanthropic Gifts)

A large fraction of the Institute’s contractual funding comes via investigator- or center-initiated research in response to requests for proposals from federal agencies. These projects are the life-blood of the Institute, providing support to original research with target applications that connect national interests with the capabilities of our laboratories. Such projects allow us to employ and educate students as well as to advance the capabilities and capacities of the Institute. Such research often generates the novel ideas and serendipitous findings that open new research opportunities while contributing to the advancement of scientific understanding. We have assembled a support structure for assistance in proposal development and editing, which is particularly important for large, multi-investigator proposals. We use a system of review

processes to ensure quality proposals, which is important to positioning us with peers on review panels for these proposals and keeping our proposal funding rates high.

Depending on the size, complexity and level of cross-disciplinarity of the project, the Institute determines the best method of program and project management. Given that there are more than 50 projects underway at any given time, the majority of projects are managed by each principal investigator, with a high level of accountability and organizational alignment. For large or complex projects, the Institute brings to bear professional project managers. Specific processes may be defined in the research contract with the funding agency, and requires sophisticated systems for tracking multiple projects against time-sensitive milestones. The ability to successfully manage the logistical complexity of integrating diverse resources to meet aggressive performance milestones will continue to establish Biodesign as a world-class research enterprise capable of taking on very large research programs.

Research Program Priorities

<table>
<thead>
<tr>
<th>Signature Research Programs</th>
</tr>
</thead>
<tbody>
<tr>
<td>We recognize that prioritization of effort is critical to aligning internal resources, ensuring impact and promoting external recognition. Biodesign has chosen to focus on a limited number of signature programs against which the majority of discretionary resources will be targeted, encouraging projects within these areas. They reflect our three thematic areas of emphasis, but with a greater level of specificity:</td>
</tr>
<tr>
<td>1. Designing technologies for <strong>personalized diagnostics</strong> — predictive, preventative, and affordable monitoring of health and wellness</td>
</tr>
<tr>
<td>2. Designing solutions for <strong>cancer eradication</strong></td>
</tr>
<tr>
<td>3. Designing strategies and technologies for outpacing infectious diseases with an emphasis on <strong>novel vaccine development</strong> and <strong>new platforms for vaccine production</strong></td>
</tr>
<tr>
<td>4. Designing microbiology-based technologies for <strong>bioremediation</strong> and <strong>renewable energy</strong></td>
</tr>
</tbody>
</table>

The projects supporting these signature areas vary significantly in scope, maturity and external support. But, they can be categorized into two classifications relating to readiness for targeted application: 1) “Use-Inspired” and 2) “Exploratory.” These are discussed below and are outlined in the table on the following page.

**“Use-Inspired” Projects**— Because of the “use-inspired,” translational focus of Biodesign, most of the research projects have defined aims and target applications that we expect to lead to innovations with commercial application. These might also include more narrowly defined projects essential to contributing to the rapid translation of identified technical solutions. Use-inspired projects will range in maturity level—what identifies them as use-inspired is a reasonably clear area of application that is being pursued.

**“Exploratory” Projects** — This classification is for those projects that are very early-stage investigations or are emerging systems/technologies for which the best application pathway has yet be determined via scientific rigor. These may be fundamental investigations opening up a new technology or approach, or the translation of established technologies into new applications. Efforts in this area may be highly speculative and thus these projects may be viewed to be far from commercial application. However, success can have the potential to
attract additional external funding once the plausibility of the idea is confirmed or additional supporting evidence is generated.

<table>
<thead>
<tr>
<th>Signature Programs</th>
<th>Use-Inspired Projects</th>
<th>Exploratory Projects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Designing technologies for <a href="#">personalized diagnostics</a> — predictive, preventative, and affordable monitoring of health and wellness</td>
<td>▪ Protein Arrays for diagnostics</td>
<td>▪ Next Generation DNA Sequencing</td>
</tr>
<tr>
<td></td>
<td>▪ Mass Spec Immunoassay Diagnostics</td>
<td>▪ Human Performance Optimization Dark Genome</td>
</tr>
<tr>
<td></td>
<td>▪ Comparative Genomics</td>
<td>▪ Dark Proteome</td>
</tr>
<tr>
<td>Designing solutions for <a href="#">cancer eradication</a></td>
<td>▪ Prophylactic Breast Cancer Vaccine</td>
<td>▪ Presymptomatic Cancer Biomarkers</td>
</tr>
<tr>
<td></td>
<td>▪ Lung Cancer - Early Detection</td>
<td></td>
</tr>
<tr>
<td>Designing strategies and technologies for outpacing infectious diseases with an emphasis on <a href="#">novel vaccine development</a> and <a href="#">new platforms for vaccine production</a></td>
<td>▪ Pediatric Pneumonia Vaccine Norovirus Vaccine</td>
<td>▪ Rapid Vaccine Discovery System</td>
</tr>
<tr>
<td></td>
<td>▪ AIDS/HIV Research</td>
<td>▪ Synthetic Genes for Immunization</td>
</tr>
<tr>
<td></td>
<td>▪ Tackling Tuberculosis</td>
<td>▪ Microbes, Spaceflight and Human Health</td>
</tr>
<tr>
<td></td>
<td>▪ Coronavirus Vaccines</td>
<td></td>
</tr>
<tr>
<td></td>
<td>▪ Small Pox Vaccine</td>
<td></td>
</tr>
<tr>
<td>Designing microbiology-based technologies for <a href="#">bioremediation</a> and <a href="#">renewable energy</a></td>
<td>▪ Cyanobacteria for biolipid production</td>
<td>▪ Biomimetic Fuel Cells</td>
</tr>
<tr>
<td></td>
<td>▪ Water Contaminate Remediation</td>
<td>▪ Biohydrogen</td>
</tr>
</tbody>
</table>

**Emerging Programs**

We recognize the significant value of exploring areas beyond the borders of our signature programs that allows the Institute to evolve and expand into new areas of excellence. Like Signature Programs, projects associated with emerging programs can be classified as Use-Inspired or Exploratory, and they may also present “Start-Up” opportunities, which is explained in the next section.

<table>
<thead>
<tr>
<th>Signature Programs</th>
<th>Use-Inspired Projects</th>
<th>Exploratory Projects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Emerging programs with potential for signature status</td>
<td>▪ Microscale Life Sciences</td>
<td>▪ Nanoscale Scaffolds for Devices</td>
</tr>
<tr>
<td></td>
<td>▪ Higher Efficiency Solar Technology</td>
<td>▪ Safeguarding Against Nerve Toxins</td>
</tr>
<tr>
<td></td>
<td>▪ Environmental Sentinel Sensors</td>
<td>▪ Tear Sensor (for determining blood glucose levels)</td>
</tr>
<tr>
<td></td>
<td>▪ Therapies for Mitochondrial Diseases</td>
<td>▪ Novel <em>in vitro</em> RNA</td>
</tr>
</tbody>
</table>

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Designing Technologies for Personalized Diagnostics

The implementation of a frequently-used health monitoring system would give doctors a powerful new approach for diagnosing and treating disease. Novel technologies will rely on detecting predictive biosignatures of disease that can inform individuals when they are becoming ill. Physicians have shown that early detection is a key to not only prevention, but also to the successful treatment and management of many serious health conditions. For example, pre-tumor cells associated with cancer are much easier to eliminate than a solid tumor mass. Early treatment can also reduce the magnitude of damage to the body by the disease, improve drug response, and decrease a person’s recovery time.

Not only will early detection improve disease treatment, it also holds promise in dramatically reducing the current financial burden on the healthcare system. In the U.S., one out of every seven dollars is spent on health care. The management of chronic conditions such as heart and pulmonary disease, cancer, diabetes and depression accounts for 70% of these health care expenditures.

Biosignatures are an integrated set of biological indicators (also known as biomarkers) that are obtainable through assays of blood, saliva, tissue, or other biological samples. Biomarkers, in this context, are defined by the FDA as “any measurable diagnostic indicator that is used to assess the risk or presence of disease.” Monitoring the biosignatures of a healthy person provides baseline levels of their own biomarkers, which can be normalized and compared to the levels expected when specific diseases occur. Current approaches to personalized diagnostics only rely upon an average of biomarker levels within a given population sample. Once defined, for each altered state, individual samples of baseline versus the altered state of interest must be obtained via a controlled process for empirical identification of biomarkers of interest.

To assay the biomarker levels regularly, sensitive and accurate detection platforms need to be established. Possible devices could rely on protein-binding agents — artificial antibodies called synbodies, aptamers, or even synthetic protein-capture agents — that would capture and record the levels of the biomarkers present in the sample. Biomarkers of interest would need to be mapped to all potential disease-causing pathways and networks. These include extensive informatics solutions such as network reconstruction algorithms and the identification of all known disease heterogeneity. Device software would need to interpret the data and compare it to known fluctuations in certain biomarkers in order to assess the health status of the user.
Cancer disrupts lives, killing more than 7 million people worldwide each year. It is the second leading cause of death in the U.S. with half a million deaths each year. Treatment costs have also soared, now accounting for about 10% of the total U.S. healthcare costs.

Lung cancer is the number one cause of cancer death throughout the world. The WHO estimates that over 1.2 million new cases are diagnosed each year globally, and the prognosis remains dismal. In the United States, only about 15% of patients will survive 5 years after a primary diagnosis. The reason for the poor survival rate is largely due to late diagnosis — most lung cancers are detected when patients already experience symptoms, such as chronic cough, pain, bleeding, or shortness of breath. At this stage, tumors may have already metastasized to other parts of the body; causing death. It is increasingly accepted that early detection dramatically improves the prognosis for survival.

The world-wide search for lung cancer biomarkers has focused on an ever-increasing number of molecular elements of carcinogenesis at the genetic, epigenetic, and protein levels. Although some of these studies report high sensitivities, most of them lack reproducibility, and none of the proposed biomarkers has been validated in large scale clinical trials. When lung cells transform to cancer, they show very specific features that have been well characterized for many decades and can be microscopically diagnosed. Sputum sample cell cytology is clearly the cancer-detection method with the highest specificity in contrast with CT imaging. The great advantage of sputum cytology is that not only cancer cells but also cells that are on the verge of becoming cancerous (dysplasia) can be detected. If such pre-cancerous conditions are found, and the corresponding lesions are localized by bronchoscopy, they can be removed before the cancerous stage is reached. Further, the morphological signature of each precancerous cell corresponds to the point in time before true cancer is expected to develop. This information can be used to risk profile the patient, thus allowing for tailoring more costly and invasive methods to the diagnostic needs of each patient.

Despite its advantages, sputum cytology is currently not widely practiced for lung cancer screening. The main reason is low sensitivity — abnormal cells are often missed due to obscuration by mucus and other non-diagnostic cells. However, studies have shown that sensitivities of 80% to almost 100% can be achieved with a high level of scrutiny. Unfortunately, this potential is currently not fully exploited in sputum cytology as performed in clinical laboratories, since the level of scrutiny of most research studies cannot be applied in routine settings. Thus, our challenge is to achieve consistently high sensitivity for sputum diagnostics to catch cancer early.

We’re also attempting to eradicate cancer through protective vaccination. The goal is to develop a vaccine that would be administered to healthy people to prime their immune systems for the recognition and destruction of a tumor and other malfunctioning cells. It could also serve as a therapeutic treatment for early stage cancers.

It has been well established that cancers create foreign proteins that the immune system can recognize. The first cancer target we are exploring is breast cancer. The idea is to demonstrate that, if we could pre-immunize an individual with a collection of proteins that effectively
represent any foreign protein that a breast tumor would produce, the immune system would arm itself against breast cancer. If the platform technology proves successful, it could be applied to other cancers.

The Biodesign Institute and the Mayo Clinic Arizona are collaborating on the exploration of this research frontier, funded by an Innovator’s Award from the Department of Defense’s Breast Cancer Research Program and by the W.M. Keck Foundation. This epic endeavor brings the informatics, genomics, proteomics and immunology fields together to eradicate cancer.

The vaccine will take cancer cell surface antigens — the proteins and sugars from the exteriors of cells — and use them to provoke an immune response in a person. The cancer-related cell surface antigens are unique or are more abundant on cancer cells than on normal cells. These can act as markers for the body to recognize the dangerous cells. The immune system will initiate a cascade of events that will ultimately form antibodies to the antigens. This will allow the body to target cells with the antigens and destroy them before they can form tumors. This way, the dangerous cells can be eliminated without collateral damage to healthy cells. With success in cancer vaccine therapy, the technology might be used to produce a near-universal, prophylactic cancer vaccine that will help make cancer a disease of the past.

### Designing Strategies for Outpacing Infectious Diseases

In today’s global economy, international travel, rapid urbanization and other factors are facilitating the rapid spread of infectious diseases. The emergence and subsequent threat of SARS, H5N1 avian flu, and now the H1N1 swine flu, has taught us that our current methods of vaccine development are inadequate for outpacing the spread of infectious diseases. With the ever-present threat of new or mutating viruses looming in the not-so-distant future, we must minimize the time from pathogen discovery to vaccine delivery. A new platform for rapid vaccine development must emerge.

Biodesign is developing a suite of technologies to make vaccine development more systematic and predictable. High-throughput technologies for assembling pathogen genes and assessing their vaccine potential are a primary focus toward speedier vaccine development. Our development process is being streamlined on a pilot scale by addressing the agricultural need for a vaccine to the African Swine Fever Virus (ASFV). This highly contagious disease (unrelated to the current H1N1 swine flu) is responsible for high mortality in pigs and is now a threat to livestock worldwide. ASFV, previously confined to sub-Saharan Africa, made a jump to the Republic of Georgia in 2007 and then found its way into Armenia, which gave neighboring countries concern about their swine populations. Without a vaccine or a cure, the only way to prevent the spread of the disease is to destroy infected animals.

In addition, we will examine development of new vaccines against global killers such as TB, HIV, influenza and pneumonia. A vaccine to prevent pneumonia in newborns—a project funded primarily by the Bill and Melinda Gates Foundation—will be entering human clinical trials this year.
Among the exciting projects in this area is a multi-unit effort to optimize photosynthetic bacteria to produce sustainable, high-yield lipids with multiple uses, including fuel for conventional engines. The bacteria are grown in transparent tubes that can be constructed anywhere there is sufficient sunlight, so our demonstration project is called “Tubes in the Desert.” Bacteria-based lipids offer the following distinct benefits:

- Higher yield per reactor volume; an estimated hundred-fold increase over current sources of biofuel. Bacteria double in volume every 24–48 hours — faster than any plant can grow
- Does not require arable land; tube “crops” can be located anywhere there is sunlight
- Does not compete with food or commodity crops
- Requires less water than plant-based production
- Does not require fertilizer, so eliminates soil depletion and contamination concerns
- Has a simpler genetic structure than plants, resulting in higher quality control and less waste
- Allows less costly processing
- Is carbon-neutral; like a plant, the bacteria use carbon dioxide for growth
- Can be located in urban as well as rural areas, reducing transportation costs and associated environmental impact

The first phase of the project was funded in part by Science Foundation Arizona and included industry funding. This two-year effort resulted in significant advances in identifying new strains of photosynthetic bacteria with high yield potential, and included the design and construction of a sophisticated photobioreactor system to optimize growth. The photobioreactor, housed on a rooftop at ASU’s Tempe campus, has mathematical modeling tools applied for systems analysis and is the first step in exploring the industrial-scale feasibility of this approach. The next phase will be construction of a field-scale (2.5-acre) system located near a local power plant.

Other efforts within Biodesign include microbial-based systems for generating energy from waste and for cleaning dangerous contaminants from drinking water.

“Start-Up” Opportunities

As new intellectual property is identified and defined, including confirming the plausibility of a novel technology and identifying a business opportunity, there will be two primary pathways for the commercialization of the technology. One pathway is the traditional out-licensing of technology to industry. An alternative is the incubation of the technology in the Biodesign Impact Accelerator, which provides an additional source of funding to establish the commercial viability of a technology and the formation of a new start-up company. Those technologies that are judged to have the potential to be incubated in the Impact Accelerator are classified as our “Start Up” opportunities. Those technologies or companies actually selected into the Accelerator will be managed through the Accelerator management team and supervised by its Guidance Council.
Examples of promising “Start-Up” opportunities are two Biodesign efforts that received funding from Science Foundation Arizona in FY 2009 through their Small Business Catalytic Grants. These grants were specifically intended to facilitate the translation of promising technologies and the formation of new companies. One company was “VAXX” — created to advance a vaccine for Norovirus infections, a major cause of traveler’s illness afflicting the cruise industry and the military. A second was “Synbuild” — a contract service company established to produce synthetic genes for research purposes.

Core Competencies

To become the benchmark for integrative and translational research as well as remaining a ‘tightly coupled’ research institute, Biodesign will proactively manage and align its technical core competencies. A strategic “core competency” represents a specific research expertise or competency that is elevated to such a level of excellence that it is a source of sustained competitive advantage over other research programs or institutions which others would have difficulty in matching, let alone exceeding.

A “core competency” typically refers to an “over-arching” area of expertise which bundles or integrates multiple skills and capabilities (e.g. vaccine immunology, nanoscale sensors). We look to build these core competencies (or competitive advantages) within our defined innovation focus areas. The core competencies of the Institute are manifested through highly skilled and experienced faculty and staff and are managed through the ten centers that comprise Biodesign. The centers represent concentrations of expertise or functional knowledge. By linking the efforts of several centers, Biodesign aligns expertise and competencies toward its signature applications. The matrix below summarizes how various core competencies within the Biodesign centers and several collaborating programs within ASU are leveraged and directed at target signature applications with the expectation of building competitive advantage.

Selected Core Competencies Supporting Signature Programs

<table>
<thead>
<tr>
<th>Selected Core Competencies</th>
<th>Signature Programs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Personalized Diagnostics</td>
</tr>
<tr>
<td>Biosignatures/Diagnostics</td>
<td>●</td>
</tr>
<tr>
<td>Bioinformatics</td>
<td>●</td>
</tr>
<tr>
<td>Vaccinology/Immunology</td>
<td>●</td>
</tr>
<tr>
<td>Microbiology</td>
<td>●</td>
</tr>
<tr>
<td>Systems Biology</td>
<td>●</td>
</tr>
<tr>
<td>Molecular Assembly</td>
<td>●</td>
</tr>
<tr>
<td>Complex Signal Processing</td>
<td>●</td>
</tr>
<tr>
<td>Nanoscale Sensing</td>
<td>●</td>
</tr>
<tr>
<td>Photonics</td>
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</tr>
</tbody>
</table>
Infrastructure

The Institute is privileged to occupy superior facilities whose superb esthetic design qualities are matched by functional efficiency, encouraging the flexible workflow patterns that are an essential element of cross-disciplinary research.

Biodesign occupies a 13-acre site that serves as the eastern gateway to the ASU campus with the additional benefit of a location adjacent to the light-rail system for connection to the ASU downtown campus and future downtown biomedical facilities. The master plan for the Biodesign Institute research complex calls for four interconnected research buildings, totaling approximately 800,000 square feet of space. The first phase, Building A (~172,000 sq. ft.) was completed in December 2004. The second phase of similar size, Building B, was completed in January 2006. The next phase of construction, Buildings C and D, are projected to cover 420,000 gross square feet of new facilities.

The Biodesign Institute Master Plan

The first two buildings contain a number of specialized areas and laboratories including:

- low vibration areas
- low electromagnetic field areas
- Centers for Disease Control Biological Safety Level-3 Certification for Biological Select Agents (BSL-3, ABSL-3, ABSL-3Ag)
- femtosecond laser laboratory
- high-security vivarium capabilities and advanced surgical suites
The Biodesign Institute buildings have been designed to be flexible and to promote a unique culture of entrepreneurial research. To achieve this goal the buildings utilize an “open lab” concept. Each module of lab space is designed with the ability to be modified and reconfigured for future changes in research direction. The open, module delineated lab allows the progress of and change in research to proceed without physical constraint. Scientists, technicians, and students who use the labs create new design requirements in the space while evolving in response to required changes in the existing facilities simply by extending the amount of bench space needed and/or by rearranging instruments and equipment.

Interdisciplinary Science and Technology Building (ISTB) 5 Facility

The University implemented a $4.3 million refurbishment of the ISTB 5 facility to modernize laboratories and redress significant building code violations. This facility is occupied by the Center for Bioenergetics of the Biodesign Institute as well as researchers from the Department of Chemistry and Biochemistry. This has provided 11,000 square feet of additional space to Biodesign.

Commitment to Environmental Sustainability

The Institute was envisioned not only as a vanguard research facility but also as a world-class demonstration of ecological laboratory design. The LEED rating system of USGBC was utilized as the benchmarking framework for building performance. Both Buildings A and B have received high LEED ratings:

- Building A (Gold 2007)
- Building B (Platinum 2007) the first building in the state of Arizona to receive this rating

These outstanding features have resulted in thirteen other awards from local and national architectural, construction and civic organizations. Most notable in this context are:

- 2006 Laboratory of the Year Award from R&D Magazine
- 2007 Building of the Year and the Best New Construction Awards from the Associated General Contractors of America

Information Technology Investments

Modern research demands excellent computational capabilities as well as the ability to communicate with partners across the globe using high-tech methods of virtual exchange. It is increasingly recognized by funding agencies that large-scale projects rarely can be solved by single institutions. Large multi-million dollar awards now typically require that institutions coordinate multiple investigators and multiple institutions. In considering proposals, funding sponsors look at IT capabilities and the availability of resources such as an “Access Grid Room”

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that enables multiple location teleconferencing. In addition, networks must be highly secure and have high-throughput processing capabilities, along with large data storage capabilities.

The Institute has invested substantially to achieve the level of scale and response architecture needed to support vanguard research. Current capabilities are substantial, including:

- 53 servers with 238TB total raw storage
- fully redundant network, cooling and power systems
- secure off-site backup data storage
- comprehensive disaster recovery plan
- maintenance and provision of supercomputer services for TGen

Audio-Visual Communication Services

Sophisticated multi-media capabilities are integral to meeting the demands imposed on the modern research environment via dramatic knowledge expansion, burgeoning networked connectivity, and diverse digital media formats. Biodesign has implemented sophisticated AV capabilities to support educational and research linkages, both internally at ASU and with diverse external groups. Functional capabilities include:

- functional audio/video studio with full HD video broadcast audio
- high quality podcasting and streaming internet video
- sophisticated AV services to eleven conference rooms and an auditorium
- support of seven Tandberg video conferencing locations

Facility Security

Security technology is also an inevitable cost of operation for a large research institute. Biodesign must comply with the security regulations of a number of federal oversight agencies, including the Centers for Disease Control and the Office of Homeland Security. The U.S. government’s heightened awareness of bioterrorism has increased the security regulations on all bioscience research entities, especially those studying infectious disease. These mandates require a specified level of security personnel and protections. The Biodesign Institute is in compliance with these regulations. It is equipped with 78 security cameras with 60 terabytes of storage, which enables the facility to maintain thirty days worth of video, meeting all regulatory requirements. Contracted Security Officers provide 24 x 7 security in several shift rotations, including building access monitoring, CCTV monitoring, patrols and incident response and inquiry.
OUR TEAM

Administrative Leadership
Research Centers
Management and Organizational Structure

To fulfill its strategic aspirations, Biodesign is organized as a “tightly-coupled” Institute that purposefully aligns organizational resources to meet ambitious research goals that require the orchestrated linkage of different groups of researchers. In turn, the progress of teams toward milestones and performance goals requires a high level of executive leadership in planning and oversight. The organization of the Institute reflects these needs. Key organizational units include:

- Administrative Leadership Team
- Research Centers and Directors
- The Biodesign Guidance Council
- Institute Scientific Advisory Board
- Communication and Institutional Advancement
- Employee Health & safety
- Finance and Sponsored Projects Planning & Control
- Facility Services and Security
- Human Resources
- Impact Accelerator Management
- Information Technology Services
- Research Management Office

The Institute is built around research centers and each center consists of a director and faculty. These in turn are supported by research scientists and post-doctoral professionals. The teams also consist of graduate students (GRA) and under-graduate students.

Management and operations consist of functions not performed by ASU central services. These functions include administrators, finance, human resources, information technology, communications, research management office, facilities, and health and safety.

Table 8: Personnel by Function – January 2009
Total Personnel: 558

<table>
<thead>
<tr>
<th>Function</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Faculty</td>
<td>51</td>
</tr>
<tr>
<td>Scientist</td>
<td>110</td>
</tr>
<tr>
<td>Tech/Lab</td>
<td>44</td>
</tr>
<tr>
<td>Postdoc Researchers</td>
<td>54</td>
</tr>
<tr>
<td>Graduate Students</td>
<td>85</td>
</tr>
<tr>
<td>Students</td>
<td>97</td>
</tr>
<tr>
<td>Administration</td>
<td>117</td>
</tr>
</tbody>
</table>
Administrative Leadership

The Institute’s Administrative Leadership serves as the senior executive decision-making group for the Institute. Membership includes leaders from the administrative and research arms of the Institute. The Administrative Leadership’s principal duties are:

- Chart the Institute’s overall strategic agenda
- Ensure robustness of funding through efforts identified via the strategic business plan
- Set technical milestones and monitor performance metrics for programs
- Evaluate research center performance
- Review and approve all new faculty appointments in the Institute
- Approve appointments and promotions in the Institute’s Research Professoriate
- Prioritize and allocate funding across the research portfolio
- Monitor scientific trends and assess their implications for the competitiveness of the Institute’s portfolio
- Critique and rank proposals for seed funding
- Review and prioritize space allocations and major shared equipment requirements
- Ensure timely compliance with the Institute’s policies and procedures
- Ensure that administrative support is provided to research centers that allow them to operate efficiently and effectively

The Institute’s Executive Director heads the Institute and is chairman of the meetings for the Institute’s Administrative Leadership.

Executive Director — Alan Nelson, PhD

The Executive Director is appointed by and reports directly to an executive committee consisting of the President, the Provost, and the Vice President for Research and Economic Affairs. The Executive Director is responsible for the efficient execution of university policies and for overall leadership of the Institute. The roles and responsibilities of the Executive Director are to provide leadership, vision, and direction, to forge collaborative relationships, and to manage the funding of the Institute. The Executive Director will work in consultation with Administrative Leadership, the Biodesign Guidance Council and the Center Directors to establish the vision and strategic objectives of the Institute. The Executive Director will oversee the Institute staff and have overall management responsibility for the Institute’s Core Facilities. The Executive Director will share with members of the faculty and staff attached to the Institute, through discussion and other democratic procedures, the responsibility for Institute activities.

Dr. Nelson assumed the position of Executive Director of the Institute in March 2009, succeeding Dr. George Poste. Dr. Nelson, recruited from the University of Washington, is the developer of a number of medical innovations, including a landmark technology that dramatically improved detection of cervical cancer. In addition to leading the Biodesign Institute, he is a professor of bioengineering in the Ira A. Fulton School of Engineering.
Dr. Nelson’s career includes both academic and entrepreneurial successes. His company VisionGate has developed proprietary technology providing detailed 3-D images of cell structures, enabling lung cancer to be detected much earlier than current technology. He also founded NeoPath, Inc. (NPTH), conducting automated cervical cancer screening. His past academic roles include professorships at Harvard and MIT. He holds forty-five patents and has published more than one hundred scientific papers.

**Deputy Director — Neal Woodbury, PhD**

As the Deputy Director of the Biodesign Institute, Dr. Woodbury’s primary duties and responsibilities include the recruitment and retention of researchers, and interfacing with the Office of the Provost and/or the relevant deans and chairs on academic affairs related to the hiring and retention of tenured and tenure-track faculty. As a member of the strategic planning body for Biodesign, Dr. Woodbury is responsible for identifying and developing high-impact collaborative research programs that further the research mission of the Institute. He works with the Center Directors to expand research and ensure the effective utilization of space for research and compliance with all University and Biodesign Institute policies and procedures. In this role, Dr. Woodbury is the primary interface with the ASU’s Office of Research and Sponsored Projects Administration on pre-award activities. He also directs the Research Management Office (RMO) which is responsible for preparing and processing contract and grant proposals. Dr. Woodbury is also responsible for developing and maintaining relationships with the Institute’s research partners.

**Director of Finance and Planning — Jeffrey Darbut, MBA**

As the Director of Finance and Planning for the Biodesign Institute, Jeffrey Darbut oversees multiple administrative functions including finance, sponsored projects planning and control (post-award activities), facility maintenance, building security, employee health and safety, and information technology services. As a member of the strategic planning body for the Institute, Mr. Darbut is responsible for identifying and developing financial and business plans that further the research mission and business goals of the Institute. An important new area of involvement will be the creation of the “Biodesign Impact Accelerator” program to promote new start-up companies generated by Biodesign. Mr. Darbut will continue to work to develop and implement internal and external funding programs critical to the growth of the Institute.

**Director of Communication and Institutional Advancement — Kimberly Ovitt, APR**

As the Director of Communication and Institutional Advancement for the Biodesign Institute, Kimberly Ovitt oversees multiple programs including marketing, public relations, community outreach, employee relations, and fundraising. As a member of the strategic planning body for the Institute, Ms. Ovitt is responsible for identifying and developing communication efforts that further the research mission and business goals of the Institute. This includes enhancing relationships with the public at large, prospective donors, industrial, clinical and academic partners, the local business community, students, potential and current faculty and staff as well as the K-12 educational system.
Director of Human Resources — Melvin Holcom Ph.D., SPHR

As the Director of Human Resources for the Biodesign Institute, Melvin Holcom drives Biodesign’s commitment to the recruitment, retention and development of diverse faculty, classified and administrative staff, and academic and service professionals. He oversees employee orientation and training programs and other programs related to establishing the workplace culture identified in the business plan. Dr. Holcom provides customer service and creates business and customer partnerships that help further the research mission and business goals of the Institute while advancing the vision of the New American University.

The Biodesign Research Centers

The Research Centers represent units of highly specialized expertise, each led by a scientist of national/international stature. The research centers drive the core competencies of the Institute while managing specific disciplinary components within the network of programs and projects conducted at Biodesign. These centers are the nurseries for innovation where exploratory investigations of promising new ideas are pursued. The centers are organized to be flexible, adaptable, and responsive to keep pace with the accelerating advances in science and the increasing demands for new technology and broadened expertise.

Biodesign Institute Research Centers

<table>
<thead>
<tr>
<th>Center</th>
<th>Year Started</th>
<th>Director(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bioelectronics and Biosensors</td>
<td>2005</td>
<td>Dr. N. Tao (2008)</td>
</tr>
<tr>
<td>Bioenergetics</td>
<td>2006</td>
<td>Dr. S. Hecht</td>
</tr>
<tr>
<td>Bio-Optical Nanotechnology</td>
<td>2002</td>
<td>Dr. N. Woodbury</td>
</tr>
<tr>
<td>Ecogenomics</td>
<td>2007</td>
<td>Dr. D. Meldrum</td>
</tr>
<tr>
<td>Environmental Biotechnology</td>
<td>2005</td>
<td>Dr. B. Rittmann</td>
</tr>
<tr>
<td>Evolutionary Functional Genomics</td>
<td>2002</td>
<td>Dr. S. Kumar</td>
</tr>
<tr>
<td>Infectious Diseases and Vaccinology</td>
<td>2002</td>
<td>Dr. R. Curtiss</td>
</tr>
<tr>
<td>Innovations in Medicine</td>
<td>2005</td>
<td>Dr. S. Johnston</td>
</tr>
<tr>
<td>Personalized Diagnostics</td>
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<td>Sustainable Health</td>
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Following is a brief overview of each center and their team.
Center for Bioelectronics and Biosensors — Dr. Nongjian Tao

A variety of sensors ranging from embedded systems to handheld devices may one day analyze your breath for the first telltale signs of disease, identify pollutants in drinking water, or keep society safe by detecting trace vapors and signatures of explosives.

In the Center for Bioelectronics and Biosensors, research can be divided up into several key themes. Some of the technologies are focused on the detection of harmful chemicals that are a threat to the environment and human health. Others look inside the body for markers or presence of disease. Still others focus on the detection of human-made threats.

In order to detect the presence of specific chemicals in the environment, or biomarkers in the body, extremely sensitive and selective sensors are required. An accurate and fail-proof early warning system for chemical and biological warfare agents and explosives also require sensors. In spite of many years of research, few systems can meet these challenges.

To achieve these goals, this center utilizes a multi-scale, multi-technology and system-level approach. The team explores and integrates device and material functions from the nano- to micro- to macroscale. They hybridize different sensing platforms, including electrical, electrochemical, mechanical and optical signal transductions, to achieve results that a single sensor alone cannot deliver. They use a system-level approach that optimizes devices from sample collection and sensing elements to signal processing and communication to deliver a complete solution to real-world problems.

The Bioelectronics and Biosensors research team can accomplish this because it involves a diverse group of researchers and students from bioengineering, electrical engineering, device physics, chemistry and biochemistry, and materials science. Among its collaborators are organic chemists and theoreticians from around the world, and industry that include researchers from Motorola, Intel, Dial, Biosensing Instruments among others.

About Dr. Nongjian Tao

Director, The Biodesign Institute, Bioelectronics and Biosensors and Professor, Ira A. Fulton School of Engineering, Electrical Engineering

Dr. Tao joined the ASU faculty as a professor of electrical engineering and an affiliated professor of chemistry and biochemistry in August 2001. Before that, he worked as an assistant and associate professor at Florida International University. He holds five U.S. patents, has published 160 refereed journal articles and book chapters and has given over 150 invited talks and seminars worldwide.

Dr. Tao holds expertise in molecular electronics, nanostructured materials and devices, chemical and biological sensors, interfaces between biological molecules and solid materials, and electrochemical nanofabrications. He received a B.S. in Physics at Anhui University in 1984 and his Ph.D. in Electrical Engineering at Arizona State University in 1988.
Center for Bioelectronics and Biosensors, Continued

Other Team Members

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<td>Vinay Janthakahalli, N.</td>
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Center for BioEnergetics — Dr. Sidney Hecht

The health of every living organism is dependent on metabolism, a basic process of life that captures and releases the energy contained in foods we eat to help fuel the body. Within nearly every cell type in the body are tiny, pill-shaped structures called mitochondria. These are the powerhouses for the cells, allowing proper growth, enabling the organs and muscles of the body to function effectively, and providing us with the energy needed for good health. Defects in mitochondrial function can result in serious, often fatal, diseases.

The Center for BioEnergetics focuses on improved diagnoses and treatments for diseases caused by impaired energy metabolism. The majority of these diseases are degenerative and affect children and young adults. They include heart, liver or kidney disease; diabetes; poor growth; loss of muscle function; vision and hearing problems; developmental delays or mental retardation; respiratory and gastrointestinal disorders; and dementia. So in addition to impacting children, impairment to the mitochondria has been implicated as a factor in aging. It is associated with Parkinson's disease, atherosclerotic heart disease, stroke, Alzheimer's disease and cancer. On the other end of the spectrum, optimal mitochondrial function has been linked with peak physical performance, such as that exhibited by top athletes.

Dr. Hecht has more than three decades of experience in academic and industrial research positions as a biological chemist and drug designer, and also serves on the boards of several biotech companies. In 2005, he co-founded Edison Pharmaceuticals with Dr. Guy Miller. The company focuses on drugs for inherited mitochondrial diseases. Due to widely ranging symptoms and early lack of understanding of the root cause of these symptoms, mitochondrial diseases have historically been classified into discreet groupings of diseases, such as Friedreich's Ataxia, that are relatively rare. This meant little effort has been put into drug discovery and treatment. Yet, together, the more than 40 mitochondrial diseases comprise a significant human and health care burden.
About Dr. Sidney Hecht

Director, The Biodesign Institute, BioEnergetics and Professor, College of Liberal Arts and Sciences, Chemistry and Biochemistry

Dr. Hecht researches diseases caused by defects in the body’s energy production processes. Energy production is similar mechanistically to other molecular processes that he has studied extensively. Dr. Hecht played a key role in the development of Hycamtin, a drug used to treat ovarian and lung cancer, as well as the study of the mechanism of the anti-tumor agent bleomycin.

In a career spanning more than three decades, Dr. Hecht has held both academic and industrial research positions. He joins ASU from the University of Virginia, where he was a professor of both chemistry and biology. From 1981 to 1987, he concurrently held leadership positions in research and development for Smith Kline and French Laboratories.

Prior to his twenty-eight years at the University of Virginia, Dr. Hecht was a faculty member at MIT. He is the co-founder of Edison Pharmaceuticals, a pharmaceutical company focusing on inherited mitochondrial disorders. He earned a B.A. in Chemistry from the University of Rochester and a Ph.D. in Chemistry with emphasis in Biochemistry from the University of Illinois.

Other Team Members

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Center for Ecogenomics — Dr. Deirdre Meldrum

The Center for Ecogenomics leverages discovery and understanding of microbial populations in harsh environments such as oceans to give insights into biogeochemical cycles, metabolic capabilities, and complex interactions that affect the health of our planet. Diagnosing, understanding, and predicting cell function or dysfunction are key elements in gaining a better understanding of disease and other threats to human health. This cellular biology knowledge is essential for developing the links between genomics, cell function, and disease. Understanding these interrelationships will aid in the development of diagnostic tools to measure the health status across all dimensions of human health, from defects in single cells to alterations in the normal function of tissues and organs. Such knowledge could lead to the early diagnosis of major illnesses such as cardiovascular disease, cancer and stroke.

The Center serves as a headquarters to the Microscale Life Sciences Center (MLSC), a National Institutes of Health Center of Excellence in Genomic Science. Its researchers study different types of cell models to link cell genomics to metabolic and biochemical characteristics. Researchers in the center address cell-to-cell variations in physiological parameters by conducting studies to quantify cellular activities such as respiration and protein expression at the single-cell level. By refining the MLSC’s microsystem-based platforms for measuring gene expression and physiological parameters, research can progress to include cell to cell interaction studies, in vivo tissue measurements and in vivo imaging for detection and monitoring.
The Center also houses a portion of the work conducted by NEPTUNE, a project to construct a cabled underwater observatory in the northeast Pacific Ocean with high bandwidth and power for real-time oceanographic observations and experiments. The research team is developing sensing devices and other instruments to gain knowledge of the biological, chemical and physical environments at microbial levels on the sea floor and in the overlying water column.

About Dr. Deirdre Meldrum

Director, The Biodesign Institute, Ecogenomics and Professor and Dean, Ira A. Fulton School of Engineering

In addition to her roles at ASU, Dr. Meldrum directs a National Institutes of Health Center of Excellence in Genomic Science. Her research interests include genome automation, microscale systems for biological applications, ecogenomics, robotics and control systems. Before joining ASU in 2007, Meldrum had been on the faculty of the University of Washington in Seattle since 1992, where she founded and directed UW's Genomation Laboratory. She is a member of the National Advisory Council for Human Genome Research which advises the Department of Health and Human Services, the National Institutes of Health and the National Human Genome Research Institute.

Dr. Meldrum’s expertise lies with genome automation, microscale life sciences, single cell analyses, and robotics. She received her B.S. in Civil Engineering at the University of Washington in 1983, an M.S. in Electrical Engineering in 1985 from Rensselaer Polytechnic Institute and a Ph.D. in Electrical Engineering from Stanford University in 1993.

Other Team Members

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Center for Environmental Biotechnology — Dr. Bruce Rittmann

The Center for Environmental Biotechnology focuses on developing microbiological systems that capture or develop renewable resources and also prevent or clean up environmental pollution. Center researchers combine engineering with microbiology, molecular biology, and chemistry in order to gain an integrated understanding of how microbial ecosystems work and can be controlled to reclaim polluted water, generate energy from waste substances, and to improve public health and sustainability.

Microorganisms, as part of their normal life, do things that provide services to society and to improve environmental quality. They degrade contaminants that pollute water, air or soil. They
transform waste materials into valuable renewable resources. All of this they do in natural
communities of different types of microorganisms living and working together. The
microorganisms, the communities, and the services are the subjects for the Center for
Environmental Biotechnology.

The Center performs research that ranges from fundamentals of biochemistry, genomics, and
microbial ecology to field testing of technologies it develops. The Center's strategy is to
integrate basic science with engineering and fundamentals with applications in all aspects of its
research.

The Center performs scientific research to understand what various microorganisms do and
what constitutes a good environment in which they can do it. The Center uses sophisticated
tools and techniques, including DNA and RNA detection and analysis methods, chemical tools
and mathematical models to identify microbes and their native functions.

The Center then designs technologies that enable the microorganisms to deliver services for a
specific purpose, such as bioremediation or renewable energy. Recently, the Center has
embarked on applying this science to health care applications, e.g., they are studying the impact
of microbial communities in the human gut on obesity.

About Dr. Bruce Rittmann, Regents Professor

Director, The Biodesign Institute, Environmental Biotechnology and Professor, Ira A. Fulton
School of Engineering, Civil, Environmental, and Sustainable Engineering and Professor, Ira A.
Fulton School of Engineering, Chemical Engineering

Dr. Rittmann is an international leader in the use of microbial communities to provide services to
society. Those services include pollution clean-up, treatment of water and wastewater, capture
of renewable energy, and directly improving human health. Dr. Rittmann was elected to the
National Academy of Engineering in 2004. He is a Fellow of the AAAS, a recipient of the Clarke
Prize for Outstanding Achievement in Water Science and Technology, a winner of the Huber
Research Prize from ASCE, and one of the world's most highly-cited researchers (Source: ISI).

Dr. Rittmann’s expertise is in Environmental Biotechnology Biofilms Microbial Ecology. He
received a B.S. in Civil Engineering in 1974 from Washington University in St. Louis, an M.S. in
Environmental Engineering, also at Washington University in 1974, and his Ph.D. in 1979 in
Environmental Engineering from Stanford University.

Other Team Members

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<td>Hagner, Diane M</td>
<td>Research Laboratory Mgr</td>
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Center for Evolutionary Functional Genomics — Dr. Sudhir Kumar

Scientists in the Center for Evolutionary Functional Genomics are utilizing innovations in information technology to develop tools for exploring long-standing biological questions. The recent ability to obtain the complete DNA sequence information of any organism — its genome — has allowed researchers to map and explore life’s genetic blueprints and the evolution thereof. The Center strives for excellence in several core research areas: comparative genomics, computational developmental biology, and software and database development.

To facilitate exploration, scientists have developed easy-to-use computer software for the analysis of genomic databases. The Center is also using comparative sequence analysis to understand the mechanisms and functional impact of evolutionary change within and across species, particularly how the genes and genomes of humans and other organisms change over time. Through analysis and characterization of interspecific genomic variation, researchers are gaining insights into the emergence and prevalence of human disease factors. Using large-scale comparative sequence analysis techniques, they are building a timescale of life in an effort to reconstruct the evolutionary history of species.

The fusion of computer science and biology holds promise for other applications to benefit human health. The dramatic growth in the scale and complexity of biological information will drive market demands for new informatics tools for data annotation, mining and visualization to optimize information retrieval and productive utilization in diverse information sciences and industrial applications. Finding solutions to these questions will influence progress in both health-related research and information technology. The rapidly-growing bioinformatics market is estimated to be as large as $37 billion.

About Dr. Sudhir Kumar

Director, The Biodesign Institute, Evolutionary Functional Genomics and Professor, College of Liberal Arts and Sciences, School of Life Sciences

Dr. Kumar leads a team of interdisciplinary scientists who are developing new computer-based methods of studying and analyzing the tens of thousands of genes in humans and related species, enabling researchers to learn their functions and origins. Dr. Kumar is a renowned expert in the field of evolutionary bioinformatics, who received an Innovation Award in Functional Genomics from the Burroughs Wellcome Fund in 2000. In 2004 he joined the elite ranks of most-cited researchers, being among the top ten in number of citations in the field of computer science over the last decade. Among his more than seventy papers and books are three “Hot Papers,” which were cited among the most of any in their fields.

Dr. Kumar is an interdisciplinary scientist who brings the problem-solving skills from his undergraduate engineering background together with his knowledge of evolutionary genetics from his doctoral work to tackle long-standing problems in functional genomics and evolutionary biology. He has made pioneering efforts in developing bioinformatics tools and databases for the analysis of gene expression patterns from early stages of the fruit fly development. He has also conducted breakthrough work using protein molecular clocks to illuminate the Evolutionary Timescale of Life. Over the last decade, Dr. Kumar has led the team that developed the Molecular Evolutionary Genetics Analysis (MEGA) software in order to make useful methods of comparative sequence analysis easily accessible to the scientific community for research and education. His research is funded by National Institutes of Health and National Science Foundation, among other agencies.
Dr. Kumar is a professor of biology at ASU, where he teaches undergraduate-level evolutionary biology and graduate-level evolutionary genomics classes. He is a standing member of the NIH review panel and a member of many journal editorial boards, including *Molecular Biology and Evolution, Genome Research, Evolutionary Bioinformatics Online*, and *Gene: Functional Genomics*. Dr. Kumar is also currently the webmaster for *Society for Molecular Biology and Evolution* and *American Genetic Association*. He received his B.E. in Electrical/Electronics Engineering and M.Sc. in Biological Sciences from Birla Institute of Technology and Sciences in India. He earned his Ph.D. in Genetics from Pennsylvania State University.

Other Team Members

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**Center for Infectious Diseases and Vaccinology — Dr. Roy Curtiss, III**

Infectious disease causes 35% of deaths worldwide and is the world’s biggest killer of children and young adults. In the Center for Infectious Diseases and Vaccinology, researchers are focused on basic bacterial and viral infectious disease processes as well as the design and use of vaccines and protein therapeutics to combat infectious diseases. Methods include the use of recombinant attenuated bacteria and viruses as well as genetically modified plants, and transferring this technology to the developing world to help fight diseases.

The center’s research efforts seek to understand the mechanisms of host-pathogen interactions leading to disease as well as latency and induction versus evasion/suppression of mucosal, systemic, and cellular immunities. By identifying and characterizing protective antigens from established and emerging pathogens, and by modifying them to enhance induction of optimal immune responses, researchers are able to evolve mechanisms for producing and delivering vaccines using plants and attenuated live bacteria and viruses.

Center researchers also seek to establish the cellular and systemic mechanisms by which animal and human hosts respond immunologically to pathogens and to vaccines. The researchers’ goal is to combine these efforts in several different methods of vaccine delivery to develop heterologous prime boost vaccines to enhance productivity of agriculturally important farm animals and ultimately to improve human health throughout the world.

**About Dr. Roy Curtiss III**

Director, The Biodesign Institute, Infectious Diseases and Vaccinology and Professor, College of Liberal Arts and Sciences, School of Life Sciences

Dr. Curtiss is a leader in exploring the genetic basis by which bacteria colonize invade and induce disease. A member of the prestigious National Academy of Sciences, Dr. Curtiss seeks to create vaccines that are safe and effective. Most recently, his focus has been on the design, construction and evaluation of recombinant genetically modified Salmonella vaccine strains as immunizing vectors to deliver protection against disease.
Specifically, current endeavors are directed at developing vaccines to prevent infections by Streptococcus pneumoniae, Mycobacterium tuberculosis, Clostridium perfringens, eresinia pestis, enteric pathogens such as Salmonella, Escherichia, Shigella and Yersinia, Listeria monocytogenes, hepatitis B virus, influenza viruses (human and avian) and Eimeria species. In addition to his extensive knowledge of bacterial genetics, Dr. Curtiss has considerable expertise in avian plant and phage genetics. His body of published work includes more than 250 reviewed articles and he has been issued numerous patents for his discoveries, including one for the use of recombinant avirulent Salmonella, Escherichia and Salmonella-Escherichia hybrids as antigen delivery vectors to induce mucosal, systemic and cellular immunity.

Before coming to ASU, Dr. Curtiss was the George William and Irene Keochig Freiberg Professor of Biology at Washington University where he chaired the Department of Biology from 1983–1993. In addition to his academic pursuits, Dr. Curtiss founded MEGAN Health Inc., where he served as a member of the board of directors until 2000.

Curtiss is a member of the American Society for Microbiology Finance Committee, the National Academy of Sciences, and the NIH Vaccine Research Study Section. He chairs the Board of Executive Editors, *Escherichia coli & Salmonella: Cellular and Molecular Biology*, ASM Press. He earned a B.S. from Cornell University and a Ph.D. from the University of Chicago.

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Center for Innovations in Medicine — Dr. Stephen A. Johnston

Today’s medical science creates effective treatments for diseases and injuries by building on existing discoveries and knowledge. This incremental, improvement-focused approach is a useful means of meeting the urgent needs of patients diagnosed with life-threatening diseases.

Equally important, however, is research that attempts to transform our understanding of disease. In many cases, innovation requires that we put aside what we think we know and start fresh.

This is the tactic used by the Center for Innovations in Medicine. With such an unconventional approach, the possibilities are limitless, from creating a single vaccine that prevents virtually all types of cancer to treating oncoming illness before experiencing any of the symptoms. This methodology allows us to approach problems in ways that have never been attempted before.

The expertise of the Center is built on a history of innovation. It’s efforts focus on the improvement of medical diagnostics and treatment, and the prevention of disease. Projects include a presymptomatic diagnostic device and vaccine to prevent multiple forms of cancer. Significant innovations, inventions and discoveries by the Center's investigators and collaborators include:
- Mitochondrial transformation: First stable introduction of DNA into this organelle.
- Pathogen-derived resistance: Simple method to make cells resistant to viral infection.
- TEV protease: A widely used protease for proteomic research.
- Gene gun: a simple device to “shoot” gene-coated gold microprojectiles into cells of intact animals.
- Genetic immunization: Method to immunize by shooting in plasmids encoding antigens.
- Expression Library Immunization: Technique to reduce a pathogen genome to vaccines.
- Linear Expression Elements: System to transform cells without cloning genes.
- High-throughput antibody production: System to make antibodies in high number.

Like all research, the ultimate importance lies in the potential to save lives and improve the quality of life.

About Dr. Stephen A. Johnston

Director, The Biodesign Institute, Innovations in Medicine and Professor, College of Liberal Arts and Sciences, School of Life Sciences

Dr. Johnston’s expertise resides in drug targeting, vaccine technology, cancer treatment, and the presymptomatic diagnosis of cancers through identifying the biosignatures of disease. Dr. Johnston joined ASU from the University of Texas Southwestern Medical Center (UTSW) where he was Professor of Internal Medicine and the Eugene Tragus Chair in Cardiology. He and three colleagues founded the Center for Biomedical Inventions at UTSW in 1998 where Johnston served as director of the center until his decision to join ASU. The Center was among the first to bring a broad group of disciplines together to create solutions to basic problems in medicine. Since its establishment in 1998, the Center for Medical Innovations in Dallas has generated over $1,000,000 in grants, published over 200 publications in leading research journals, was granted 14 patents and formed two spinout companies. Dr. Johnston earned his Ph.D in 1981 in Genetics and Plant Genetics/Plant Breeding at the University of Wisconsin/Madison.
Other Team Members

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Center for Personalized Diagnostics —Dr. Joshua LaBaer

The Virginia G. Piper Center for Personalized Diagnostics is an integral component of the Arizona-based Partnership for Personalized Medicine connecting Biodesign, the Translational Genomics Research Institute, and the Fred Hutchinson Cancer Research Center. This partnership is sponsored through a $45 million grant from the Virginia G. Piper Charitable Trust and the Flinn Foundation. The Center is supported through $10 million from this grant.

The goals and objectives for this newly created center are to drive the diagnostic activities of the Piper Partnership for Personalized Medicine. The Center will connect the efforts and capabilities of the Biodesign Institute/ASU and TGen, without replicating existing resources but rather leveraging them to generate the greatest impact. The Center will also link in other Arizona institutions and initiatives, including the Arizona Proteomics Alliance (AZPA) and the Health Research Alliance of Arizona.

The Center will serve as a hub for intellectual activities and collaborative endeavors related to personalized diagnostics and as a convening point for all stakeholders in this effort. It will bring together scientific, research, and policy expertise in the fields of proteomics, imaging, nanotechnology, computing, informatics, and health economics to:

- Pursue the discovery of biomarkers (primarily but not exclusively in the form of proteins identified in blood samples)
- Translate these biomarkers into molecular diagnostic tools
- Conduct multiple demonstration projects to illustrate and document the value of adoption of these tools into common clinical practice, and
- Drive the development and commercialization of these tools and other related intellectual property
About Dr. Joshua LaBaer

Joshua LaBaer, M.D., Ph.D. is a founder and Director of the Institute of Proteomics at Harvard Medical School. He attended the University of California at Berkeley as an undergraduate and completed medical school and graduate school at the University of California, San Francisco where he studied steroid regulation of DNA transcription and protein-DNA interactions. He completed his internship and residency at the Brigham and Women’s Hospital and a clinical fellowship in Oncology at the Dana-Farber Cancer Institute in Boston. At the Institute of Proteomics he has led the effort to build recombination based clone sets for human genes and other model organisms, developed high throughput method for protein expression and purification, executed high throughput screens of gene expression in mammalian cells and invented novel protein microarray technologies. Dr. LaBaer is an associate editor of the *Journal of Proteome Research*, a member of the Scientific Advisory Committee for the Proteome Society and a founding member of the Human Proteome Organization.

Other Team Members

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Center for Single Molecule Biophysics — Dr. Stuart Lindsay

Single molecule biophysics lies at the confluence of molecular medicine and nanotechnology. The Center for Single Molecule Biophysics uses nanotechnology to study the physical processes on which life is based using the simplest model systems — those that exist on the level of a single molecule or several molecules. By doing this, scientists can gain a better understanding of gene regulation, molecular signaling, and molecular transport in cells that will lead to improved biosensors and other new technologies.

The Center seeks to better understand the physical basis of life by studying the individual molecules that comprise living cells. Researchers at the Center are using scanning probe microscope methods to measure and manipulate individual molecules. The premise is that single molecules are simple enough to be modeled accurately with computer simulations, yet complex enough to reflect the variations that are important to their function.

The Center is also dedicated to advancing the latest techniques for research on the single molecule scale and to translating discoveries into new tools and applications that are relevant to promoting health and outpacing disease. With a clearer understanding of the individual components that comprise integrated living systems, researchers are better able to grasp the many complex interactions between gene products (molecules) that cause disease. By learning from nature’s repertoire of experiments on its own “nanomachines,” researchers at the Center are figuring out how to craft components for machinery of never before realized complexity and sophistication.
About Dr. Stuart Lindsay, Regents Professor

Dr. Lindsay specializes in Biophysics at the molecular level and scanning probe microscopy. Much of his work is aimed at speedier diagnosis as well as to medical breakthroughs to understand and cure many diseases. He holds twenty-seven patents and is a technology advisor for the Atomic Force Microscope Division of Agilent Technologies. Agilent has acquired Molecular Imaging Corporation, which he co-founded in 1993.

Dr. Lindsay’s lab conducts innovative research in biological physics, molecular electronics, solar energy and condensed matter physics. The Lindsay Lab researchers are interested in how genes work, and study the way in which proteins change DNA structure to switch genes on and off. They are also interested in the chemistry and physics of the liquid-solid interface and are trying to understand electrochemical and charge transfer processes at the single-molecule level. One project that Dr. Lindsay is pursuing is a new method of DNA sequencing to allow much faster and cheaper sequencing of individual human genomes. His radical approach involves using Atomic Force Microscopy (AFM), which is customarily used to analyze the surface structure of materials at molecular resolution with the ultra-small tip of a sensitive probe, in combination with naturally occurring ring-shaped sugar molecules called cyclodextrins. Dr. Lindsay believes that the ring molecules, when paired with the AFM probe tip, can effectively be used as sensors to "read" the sequence of nucleic acid code (DNA "bases") in the human genome that comprises many millions of bases.

Dr. Lindsay also constructs specialized scanning probe microscopes in collaboration with Molecular Imaging Corporation. His twenty-seven patents include one in 2004 for “Devices based on molecular electronics.”

After receiving his Ph.D. in Physics from the University of Manchester, Dr. Lindsay spent two years as a consultant at Philips Industries in London before joining the faculty at ASU. He has been at ASU for twenty-five years. His body of published work comprises over 138 articles in peer-reviewed journals and many book chapters and refereed conference papers.

Dr. Lindsay sits on the editorial boards of Biophysical Journal and AIP Press International Series in Basic and Applied Biological Physics. He also holds the position of Associate Editor for Probe Microscopy at Ultramicroscopy, and Associate Editor for the Americas at Nanobiology. He is a fellow of the American Association for the Advancement of Science and the American Physical Society.

Other Team Members

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Center for Sustainable Health — Dr. Leland Hartwell, Nobel Laureate, & Dr. Michael Birt

In September 2009, Nobel Prize winner Dr. Leland “Lee” H. Hartwell was recruited to ASU to co-direct the Center for Sustainable Health, an expansive effort to improve the effectiveness of health care while reducing its costs. Hartwell becomes the first Nobel Prize recipient in medicine to serve a faculty appointment at an Arizona university.

Dr. Hartwell and co-director Dr. Michael Birt are currently establishing the Center, funded with a $2.5 million gift from the Arizona-based Virginia G. Piper Charitable Trust. The Center for Sustainable Health will identify biomarkers—early indicators of disease—to enable personalized, pre-symptomatic diagnoses, working in close collaboration with other Biodesign centers.

A key aspect of the center’s efforts will be redefining health outcomes metrics, encompassing expanded considerations such as the environmental, educational and socio-political impacts on health. Co-director Dr. Michael Birt is a health policy expert.

Dr. Hartwell is no stranger to Arizona, having served as executive chairman of the Partnership for Personalized Medicine since its creation. The partnership includes the Biodesign Institute, Translational Genomics Research Institute (TGen), and Seattle’s Fred Hutchinson Cancer Research Center. Hartwell currently is president and director of the Hutchinson Center.

An impetus for the center is the current lack of definition and defined metrics for optimal human health. The team will seek to define human health, both physical and mental, and to identify metrics that accurately measure health for individuals and populations. This goal will build on current research efforts in medicine, psychology, economics and sociology seek to define molecular, cellular and behavioral metrics of health and disease. Science must define the metrics and policy makers must implement them.

The center will work at the interface of three areas to ensure that science and policy are optimized:

1. Medicine: Science is currently defining human biology from molecules to behavior. This vast body of growing information has yet to be distilled into a set of measurable quantities that can guide medical care most effectively. We need measures of health as well as disease and metrics that guide cost-effective and equitable interventions to keep people healthy, detect early onset of disease, and monitor treatment. The current system of science innovation, commercial development, government regulation, and clinical implementation that has been effective for therapeutics is completely ineffective for diagnostics. Our goal is to create an effective pathway that leads from the discovery of key health metrics to their implementation in medicine.

2. Society: Sustainability science is currently defining the environmental and social parameters that interact in the complex system comprising human activity and the global environment. The future of human health on the planet will depend upon defining the metrics by which society functions and optimizing these for human health. The current world metric – economic growth – is leading to a devastating deterioration of the human habitat and gross inequities that preclude sustainability. We seek to define the metrics of a sustainable and nurturing environment and understand how science and policy can be integrated to optimize human well being.
3. Education: Science becomes policy through a process of education whereby the knowledge of science is transmitted to policy makers with the implied or active consent of the public. Bad policy is often the result of poor education yet few policy makers and even fewer members of the public understand the scientific basis of a sustainable life style. Advances in information technology now permit effective education through direct communication of scientists to policy makers and the public, aided and enhanced by broad public participation in debate and evaluation. We seek to improve education at all levels by engaging the best science on sustainable human health with the optimum means of disseminating education.

About Dr. Hartwell

Dr. Hartwell was awarded the Nobel Prize in physiology or medicine in 2001 for his discoveries of a specific class of genes that control the cell cycle. The cell cycle controls all aspects of cell growth and division. By identifying "checkpoint" genes that determine whether a cell is dividing normally, Hartwell provided important clues to cancer, which arises from abnormal, uncontrolled cell growth.

His interests for the past several years have turned to how researchers can use the enormous knowledge that has accumulated during the last 50 years in genetics and biochemistry to improve molecular diagnostics to benefit human health. By using powerful protein biomarkers, scientists hope to identify individuals at high risk for disease, detect cancer and other diseases at an early stage when they can be cured, and to provide improved prognostic information and better therapeutic response.

Other honors in Hartwell’s career include the Albert Lasker Basic Medical Research Award, the Gairdner Foundation International Award, and the Alfred P. Sloan Award in cancer research. He is a member of the National Academy of Sciences.

About Dr. Birt

Dr. Birt is an internationally renowned health care policy leader. Birt is senior vice president, Health and Society at The National Bureau of Asian Research; executive director of the Pacific Health Summit; and executive director of the Forum for Personal Health. He also holds the position of affiliate investigator at Fred Hutchinson Cancer Research Center. He has consulted for many of the world’s leading health care, medical technology and consumer product companies. At ASU, he also will serve as professor of practice in the School of Health Care Management and Policy in the W.P. Carey School of Business.

Dr. Birt has extensive experience in the private sector and the academic world as well as nonprofits. He has served academic appointments at the University of Washington and Wellesley College. His private sector experience includes the successful launch, development and stock sale of a leading U.S.-Asia biomedical business development company (a U.S.-registered corporation with offices in Japan, Korea, and Taiwan). He co-founded and led a
TRANSLATION

Collaboration & Partnerships
Technology Transfer
Collaboration and Partnerships

Critical to the growth and success of the Biodesign Institute will be effective partnering with groups both internal and external to ASU.

Internal Collaboration

Many programs at ASU interface with the Biodesign Institute and provide opportunities for complementary efforts toward our mutual goals. The establishment of the guidance council will further these relationships. Current close affiliations exist with:

- School of Life Sciences
- Ira. A. Fulton School of Engineering
- Center for the Study of Law, Technology, and Science
- Consortium for Science, Policy and Outcomes
- Center for Nanotechnology and Society

Arizona Partners/Collaborators

Although many of the Biodesign Institute’s activities are oriented to national and international perspectives, priority has also been given to building productive research collaborations with Arizona-based organizations. These embrace the following activities:

- **The Translational Genomics Research Institute (TGen).** Several TGen researchers hold joint appointments at ASU and Biodesign. Biodesign and TGen are co-participants in the Center of Medical Countermeasures against Radiation (CMCR) grant led by Columbia University (approximately $9M over 5 years to Biodesign and TGen).
- **Virginia G. Piper Partnership for Personalized Medicine.** Biodesign and TGen have a major collaborative agreement with the Fred Hutchinson Cancer Research Center in Seattle to form the new Center for Personalized Diagnostics, catalyzed by $45 million in grants from the Virginia G. Piper Charitable Trust and the Flinn Foundation.
- **Mayo Clinic.** Biodesign is an active contributor to the Mayo Clinic — ASU Center for Cancer-related Convergence, Cooperation and Collaboration agreement (MAC-5). We have a major research collaboration to develop a prophylactic cancer vaccine.
- **BIO5 at the University of Arizona.** The Institute has partnered with BIO5 on projects including diabetes biomarkers, asthma, Valley Fever, and the rational design of molecular therapeutics. While TRIF allocation shortfalls have affected these projects, it established helpful working collaborations.
- **Science Foundation Arizona (SFAz)** The creation of SFAz represents a crucial development in the ability of Arizona to achieve competitive status in the global research stakes. The Institute was successful in capturing funding in all of the new funding categories launched by SFAz in its first year of operations. Funding of Biodesign Institute activities represented 43% of the total monies awarded to ASU from SFAz, including the largest single investment to date by SFAz to accelerate the Tubes in the Desert project.
- **Arizona Proteomics Alliance (AZPA).** The Alliance is a partnership of Arizona institutions committed to advance regional proteomics capabilities and research. ASU and Biodesign are charter members of AZPA, which seeks to place Arizona in the forefront of proteomics research capabilities with broad impact on biomedical research, as well as the emerging fields of systems biology and personalized medicine. Member institutions include ASU,
Banner Health, Barrow Neurological Institute, Carl T. Hayden VA Medical Center, Intrinsic Bioprobes, Mayo Clinic, Sun Health Research Institute, TGen, and the University of Arizona. Biodesign hosted the first AZPA Integrated Biosystems Research Symposium in 2007.

- **Health Research Alliance Arizona (CTSA).** ASU and Biodesign are partnering with the UA and all the major clinical research organizations in Arizona in efforts to secure federal support through a Clinical and Translational Science Award (CTSA) leveraging the statewide Health Research Alliance Arizona. Biodesign researchers are helping to coordinate the Core Technologies and Critical Translational Technologies modules in this program.

- **Scottsdale HealthCare and the City of Scottsdale.** Both of these organizations collaborated with researchers from the Institute’s Center for Applied Nanobioscience (ANBC) and ASU’s Departments of Industrial Design and Visual Communication Design to develop concepts for “Bioterrorism and Radiation Tools” in support of the Coyote Crisis Campaign disaster response simulation exercises during the Spring of 2006.

- **Biodesign Adjunct Faculty Members.** Biodesign fosters relationships with more than seventy individual researchers across the state and country through our adjunct faculty program. This program is intended to advance the research program of the individual, as well as nurture collaborations.

**External Economic Development Partnerships**

A broad coalition of elected, civic and community leaders have recognized the importance of attracting leading-edge research to Arizona as an engine for new economic development, as well as the development and retention of a highly-skilled work force. Biodesign actively supports these efforts through its involvement with regional economic development organizations:

- **Arizona BioIndustry Association.** Biodesign is a participating member of this trade association which promotes the development of a robust bioscience industry in Arizona. A Biodesign administrator serves as Chairman of the Board for this association. Biodesign has been a major supporter of Association events.

- **Greater Phoenix Economic Council, GPEC.** Biodesign provides active support to this regional economic development organization on many fronts. Biodesign mentored managers from GPEC on the regional research capabilities and bioscience/biotechnology industrial issues. Biodesign has also actively participated in GPEC’s efforts to recruit new companies to Arizona by conducting tours and research review meetings at the Institute. Bioscience companies recruited successfully include: Covance, InNexus, Abraxis, and Insys.

- **Arizona Bioscience Roadmap.** Biodesign is an active participant in this strategic planning and coordination effort sponsored by the Flinn Foundation in collaboration with the Battelle Institute. Biodesign administrators and researchers serve on the steering committee and several of the research sub-committees to advance the bioscience areas of strategic interest to Arizona.

**Industrial Partnerships**

The grants awarded to Biodesign include many partnership research and development agreements with industry. We anticipate growing these partnerships, with such collaborations becoming a significant portion of our research portfolio. A sampling of current industrial partners includes:

- Agilent
- Boeing
- BP
- Corning
- Edison Pharmaceuticals
- Forensic Science Service
- General Dynamics
- Honeywell
Arizona Technology Enterprises, AzTE

Arizona Technology Enterprises, AzTE, a wholly-owned subsidiary of the ASU Foundation, was established for the purpose of managing ASU’s intellectual property. AzTE drives the transfer of discoveries and innovation from ASU’s labs to the marketplace through technology partnering and the creation of new technology-based ventures. AzTE identifies Biodesign technology that fills market needs to enable the right partners to take these innovations to the marketplace. AzTE focuses on creating long-term partnerships with industry around our core technology expertise. AzTE is organized into three business teams: Venture Development, Life Sciences, and Physical Sciences. AzTE views the Biodesign Institute as a major strategic imperative as it pursues its mission of generating a return on ASU innovations. Therefore, close communication ties with AzTE are maintained.

The AzTE executive team is comprised of industry and university veterans with years of professional experience in technology evaluation, product development, technology marketing, capital formation, operations/management, IP protection, industry relationships, and licensing and commercialization. AzTE performs at least three vital functions, each of which can be pursued in parallel:

- AzTE works to transform discoveries into valuable intellectual property. Possible inventions disclosed to AzTE are evaluated for their commercial potential. High-potential technologies are chosen for further investment in the form of patent protection. The scope of this protection is determined by a technology’s market potential in specific fields-of-use and geographic territories.
- AzTE determines and pursues the most appropriate commercialization strategy. Incremental technologies that build on the technologies of existing market players represent licensing opportunities. In contrast, technologies that have the potential to disrupt or revolutionize existing markets may represent new company start-up opportunities.
- An essential function AzTE performs is to provide or otherwise locate “gap funding” for promising new technologies that are not quite ready to attract the interest of commercial partners.

In August of 2009, the Biodesign Institute with AzTE introduced the Biodesign Impact Accelerator, an initiative to accelerate the translation of scientific breakthroughs into beneficial societal impacts and to dramatically increase economic returns to the Arizona community. This program is discussed in detail in a later section.
ACCOMPLISHMENTS

Major Funding Awards
Performance Metrics and Financial
Major Awards

Since inception, major awards and philanthropic investments include:

- $45 million to develop personalized diagnostics initiative (in partnership with TGen)
- $18 million for National Center for Excellence in Genome Sciences
- $14.8 million Gates Foundation Grand Challenge Award for a pneumonia vaccine
- $14 million to create solar-generated biofuels*
- $9 million for Biodesign and TGen to rapidly detect radiation exposure
- $7.4 million for plant-based HIV research
- $6.9 million to develop a breast cancer vaccine, plus $1.2 million to apply it to other cancers
- $6.1 million to create an improved integrated micro-fluidic device for criminal forensics
- $5.5 million to develop a safer smallpox vaccine
- $5.2 million to modify photosynthetic bacteria to secrete fatty acids for biofuel
- $5 million for graduate student and post-doctoral education
- $4.7 million to generate a biodiesel fuel from photosynthetic bacteria
- $3.2 million to create a rapid test for urinary tract infections
- $3.2 million for vaccine against Tularemia, a possible biothreat
- $2.7 million for production of nerve agent antidotes
- $2.5 million to establish the Center for Sustainable Health, an effort to improve health care while reducing its cost
- $2.5 million to generate hydrogen fuel using photosynthetic bacteria
- $2 million for projects with UA targeting asthma and Parkinson’s Disease
- $1.9 million to create new bioinformatics tools
- $1.5 million to discover a plant-based treatment for West Nile virus
- $1.2 million to study impact of space flight on human health
- $1.2 million to discover hidden proteins important in human health
- $1.2 million to develop nanomachines that may detect neurological disorders
- $1.1 million to use nanotechnology in improving solar energy
- $1 million to improve the detection of explosives
- $3.4 million to apply laws of physics to cancer studies*

*Project led by another ASU Dept. with Biodesign participation

---

Since inception (FY03-09), the Biodesign Institute has:

- Generated $300 million in research funding
- Created 500 new jobs and recruited 60 new faculty
- Disclosed more than 279 inventions, resulting in 178 provisional patents, 57 patent filings, and 18 issued patents
- Spun out 4 companies
- Provided hands-on research experiences for:
  - 188 postdoctoral researchers and 301 graduate students. More than 170 of these entering the workforce
  - 403 undergraduates, 95 high school students, and 36 high school teachers
Performance Metrics & Financials

The financial projections are based on, and assume, the successful implementation of the strategies. Both the business plan and the financial projections reflect numerous assumptions, including various ones regarding the anticipated future performance of the Biodesign Institute, federal funding, general business and economic conditions, as well as other matters, most of which are beyond the control of the Institute or Arizona State University.

While the projections were prepared in good faith and the assumptions, when considered on an overall basis, are believed to be reasonable in light of the current circumstances, it is important to note that there can be no assurance that such assumptions will be realized, and users must make their own determinations as to the reasonableness of such assumptions as well as the reliability of the projections. Therefore, although the financial projections are presented with numerical specificity, the actual results achieved during the financial projection period will vary and some of the variations could be material. Accordingly, no representation can be or is being made with respect to the accuracy of the financial projections or the ability of the Biodesign Institute to achieve the projected results of operations.

Return on Investment

Return on Investment includes federal and non-federal awards, royalty income and foundation funding for Biodesign and capacity building project investments. The sum of these three components (the Return total) is then divided by the related TRIF expenditures for the fiscal year to arrive at the Return on Investment ratio included in the financial table below.

<table>
<thead>
<tr>
<th></th>
<th>FY2010 Projection</th>
<th>FY2011 Projection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Federal &amp; non-federal grants</td>
<td>$58,800,000</td>
<td>$67,600,000</td>
</tr>
<tr>
<td>Royalty income</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Foundation funding</td>
<td>500,000</td>
<td>500,000</td>
</tr>
<tr>
<td>Return total</td>
<td>$59,300,000</td>
<td>$68,100,000</td>
</tr>
<tr>
<td>Return on investment</td>
<td>3.3:1</td>
<td>4.2:1</td>
</tr>
</tbody>
</table>

Technology Transfer

These measures provide results for ASU’s technology transfer arm, Arizona Technology Enterprises (AzTE). Measures for inventions, patents and licensing activity are included. AzTE facilitates development of ASU’s intellectual property, promotes industrial linkages, drives technology marketing, and accelerates transition of ASU discoveries into the marketplace.
**Technology Transfer, Continued**

<table>
<thead>
<tr>
<th></th>
<th>FY2010 Projection</th>
<th>FY2011 Projection</th>
</tr>
</thead>
<tbody>
<tr>
<td>New invention disclosures</td>
<td>150</td>
<td>151</td>
</tr>
<tr>
<td>New patent applications filed</td>
<td>160</td>
<td>162</td>
</tr>
<tr>
<td>New patents issued</td>
<td>21</td>
<td>21</td>
</tr>
<tr>
<td>Total start-up companies licensing ASU technology</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Licenses or options signed (as indication of technology adoption by industry)</td>
<td>33</td>
<td>34</td>
</tr>
</tbody>
</table>

**Workforce Contributions**

Workforce Contributions measures show the impact of TRIF funding and research participation by undergraduate students, graduate students, and post-doctoral appointments within the TRIF projects. The measures also include the number of graduate students and post-doctoral researchers leaving ASU to enter the workforce.

<table>
<thead>
<tr>
<th></th>
<th>FY2010 Projection</th>
<th>FY2011 Projection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Post-doctoral appointments</td>
<td>81</td>
<td>93</td>
</tr>
<tr>
<td>Post-doctoral researchers leaving to enter the workforce</td>
<td>12</td>
<td>13</td>
</tr>
<tr>
<td>Graduate students employed</td>
<td>253</td>
<td>278</td>
</tr>
<tr>
<td>Graduate students earning degrees and entering the workforce</td>
<td>45</td>
<td>47</td>
</tr>
<tr>
<td>Undergraduate students involved</td>
<td>214</td>
<td>225</td>
</tr>
</tbody>
</table>

**Partnerships & Collaborations**

Partnerships/Collaborations are an important component for growth of the Biodesign Institute and capacity building project initiatives. Increasing involvement with non-government and non-ASU researchers such as the Bill & Melinda Gates Foundation, Mayo Clinic, Genomics Research Institute (TGen) and Barrows Neurological Institute provides additional opportunities to expand and enhance ASU research.

<table>
<thead>
<tr>
<th></th>
<th>FY2010 Projection</th>
<th>FY2011 Projection</th>
</tr>
</thead>
<tbody>
<tr>
<td># of Biodesign research grants/contracts involving funding from non-government entities</td>
<td>35</td>
<td>39</td>
</tr>
<tr>
<td># of Biodesign research grants/contracts involving subcontracts to non-ASU researchers</td>
<td>6</td>
<td>8</td>
</tr>
</tbody>
</table>
## Financial Summary

### Revenue & Expenditures

<table>
<thead>
<tr>
<th></th>
<th>FY2010 Projection</th>
<th>FY2011 Projection</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Revenue</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carry forward</td>
<td>$1,862,600</td>
<td>-</td>
</tr>
<tr>
<td>Current Period TRIF revenue</td>
<td>16,053,600</td>
<td>$16,053,600</td>
</tr>
<tr>
<td><strong>Total Revenue</strong></td>
<td>17,916,200</td>
<td>16,053,600</td>
</tr>
<tr>
<td><strong>Operating Budget</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Personal services</td>
<td>7,499,800</td>
<td>6,549,900</td>
</tr>
<tr>
<td>Employee related expenses</td>
<td>1,764,700</td>
<td>1,541,100</td>
</tr>
<tr>
<td>Operating expenses</td>
<td>5,441,000</td>
<td>4,751,900</td>
</tr>
<tr>
<td><strong>Total operating budget</strong></td>
<td>14,705,500</td>
<td>12,842,900</td>
</tr>
<tr>
<td><strong>Capital Budget</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Building renovations</td>
<td>2,210,700</td>
<td>2,210,700</td>
</tr>
<tr>
<td>Debt service</td>
<td>1,000,000</td>
<td>1,000,000</td>
</tr>
<tr>
<td><strong>Total capital budget</strong></td>
<td>3,210,700</td>
<td>3,210,700</td>
</tr>
<tr>
<td><strong>Total Expenditures</strong></td>
<td>$17,916,200</td>
<td>$16,053,600</td>
</tr>
</tbody>
</table>
Section Two

Making it Real

Biodesign Impact Accelerator
Executive Summary

The goal of the Biodesign Institute’s Impact Accelerator is to increase the success rate and the speed and efficiency of commercial translation of promising ASU technologies toward societal impact. Biodesign’s initial technology focus areas are:

- Improving health care through more personalized medicine
- Combating the global threat of infectious diseases
- Improving our environment through renewable energy and bioremediation.

The Biodesign Impact Accelerator (BIA) will address the primary impediments to the successful commercialization of university technologies: 1) the lack of early stage investment to develop promising technologies, 2) the low efficiency of development efforts to commercialize novel technologies, and 3) the intellectual property license agreement that is often unfriendly to the start-up company. The strategy is executed primarily through the formation of new companies that hold licensed ASU intellectual property and are incubated within the state-of-the-art facilities of the Biodesign Institute. A disciplined, milestone driven approach to development that focuses on technology risk reduction, the use of expert development and project management personnel combined with shared facilities and administrative services are the primary drivers of improved efficiency. The goal of the BIA program is to generate a pipeline of new companies, Newcos, graduate from the BIA when the technology is mature enough to launch a stand-alone company or to achieve an exit valuation creating an attractive ROI through direct acquisition.

The Biodesign Impact Accelerator program is being implemented through a partnership with Arizona Technology Enterprises, AzTE, the technology transfer and marketing arm of ASU and a wholly-owned subsidiary of the ASU Foundation. The BIA applies a groundbreaking strategy by allowing philanthropic donors to finance value creating technologies initially through the ASU Foundation. This seed funding will be provided to advance the most promising technologies to obtain early proof-of-concept and confirmation of customers’ needs and market opportunities. Then, continuing funding through the BIA Private Equity Fund would be applied to develop those technologies that meet rigorous business criteria, as the the newly formed Newco is provided with sustaining resources (space, equipment, personnel and oversight). The Newcos would be housed within the well-outfitted facilities of the Biodesign Institute.

The screening process involves the preparation of a preliminary business plan that is evaluated by an “Entrance Exam” Committee composed of technical and entrepreneurial experts. Newcos admitted to the BIA will be set-up as C-corporations with 100% of voting shares held by the ASU Foundation. Each Newco will be assigned all necessary IP through a unique license agreement with AzTE that is exclusive, world-wide and royalty-free in exchange for equity in the form of a Cash Distribution Agreement that is triggered upon a liquidity event. All other stakeholders will participate in the Cash Distribution Agreement according to their relative contributions.

Newcos will be managed against a value creating, risk reducing graduation plan overseen by an expert "Graduation" Committee. The graduation or launch event for a Newco is expected to provide a return to the BIA and investors several times the initial investment. The preferred graduation event is an acquisition. The return on investment obtained by the BIA would then be re-invested in the Newco pipeline to ultimately achieve self-sufficiency.

The formation of the BIA was approved by the Arizona Board of Regents, announced and staffed in July 2009. The Biodesign Institute’s Executive Director, Dr. Alan Nelson, serves as the
Executive Director of the Impact Accelerator program that will be housed initially in 10,000 sq. ft. of prime laboratory space within the Biodesign Institute. An experienced management team for the BIA has been put in place by Dr. Nelson. Initial funding of $5.0 million was provided by a philanthropic donor through the ASU Foundation, and this seed funding will allow the start-up of several new companies. An NIH C06 facilities grant proposal was submitted, seeking $10M of funding to create an additional 21,000 sq. ft. of new laboratory space at SkySong in Scottsdale, Arizona. The BIA program will expand by seeking further sources of investment through a dedicated BIA Private Equity Fund that invests solely in the pipeline of Newcos.
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1.0 Impact Accelerator Program Description

1.1 Overview

The Biodesign Institute’s Impact Accelerator will expedite the multiple development stages that a research discovery must navigate in moving from conception toward a proven and marketable technology. The BIA applies a unique and groundbreaking strategy by allowing the ASU Foundation to directly invest into value creation with ASU Intellectual Property. Direct investment and allocation of Biodesign development resources circumvents the prevalent delays in development that plague typical university tech transfer and differentiates the BIA from traditional technology incubators. This strategy is executed primarily through the formation of startup companies, Newcos, incubated within the facilities of the Biodesign Institute. A disciplined, milestone driven approach to development that focuses on risk reduction and the use of expert resources is the primary driver of improved efficiency. The emerging Newco will be technologically mature enough for direct acquisition or be launched as stand-alone company.

The multiple stages of the technology translation process will be expertly managed. Through the collaboration between the Biodesign Institute and Arizona Technology Enterprises (AzTE) – the technology transfer arm of ASU – resources will be available to examine the potential utility and scope of candidate discoveries ensuring that appropriate intellectual property is secured for promising inventions. This will demand an understanding of customer and patient needs, the competitive technology landscape and business opportunities.

Seed and follow-on funding will be provided through the Biodesign Institute on a competitive basis for promising technologies to obtain early proof-of-concept (technical plausibility) and confirmation of customers’ needs and market opportunities while being developed through a disciplined, risk reduction strategy. This assures gap funding and support for companies in their most critical, nascent stage. Thus, the BIA represents a significant deviation from the normal pattern of academic technology transfer that is typically limited to patenting a disclosed technology and licensing the technology directly to industry when it is in a very immature form. The graduation for a startup company is expected to provide a return to the BIA several times the initial investment. These revenues obtained by the BIA would then allow further investment in other early stage technologies emerging at ASU.
1.2 Mission and Vision

Our Mission
Our Mission is to accelerate the translation of promising ASU bioscience innovations by forming and funding start-up companies and providing focused resources to manage risk-reduction in their earliest, most critical stages of development.

Our Vision
Our Vision is to establish a bold new approach that optimizes the translation of bioscience research into valuable commercial innovations. The Biodesign Impact Accelerator will nurture ASU start-up companies through a milestone driven, risk reducing program to facilitate successful commercialization. Our support includes access to funding, technical expertise, first-class facilities, disciplined management, expert mentoring, networking and other essential resources. The return-on-investment from successful graduates will fuel expanded support for additional start-ups.

1.3 Our Products: Newcos

The Biodesign Impact Accelerator program is designed to improve the speed and efficiency of ASU’s technology translation and its eventual impact on our community. Analysis has identified several bottlenecks that impede the progress of promising university discoveries toward translation and ultimate commercialization (See Section 2: Market and Opportunity Analysis). These major impediments are being addressed by the Biodesign Impact Accelerator (BIA) program and our solution; the formation and funding of new company startups, Newcos, is detailed in this Business Plan.

The BIA program is linked to the many research initiatives currently supported within the ASU and the Biodesign Institute, and it defines our mechanism for moving the most promising of our discoveries into development and eventual commercialization. The BIA has selected an initial focus on a limited numbers of areas within the broader scope of the Biodesign Institute:

- Vaccines
- Bioinformatics
- Personalized medicine diagnostics
- Medical devices
- Environmental technology

The Impact Accelerator program is being implemented through a partnership with Arizona Technology Enterprises, AzTE; the technology marketing arm of ASU. When opportunities are identified, the BIA will have the ability to form a startup company, Newco, that will hold the IP for a promising technology through a license agreement with AzTE that is exclusive, world-wide and royalty free in exchange for the equity held by the ASU Foundation in the Newco. For most foreseeable circumstances, the ASU Foundation would hold 100% of the equity shares in a BIA start-up Newco. This is a novel structural agreement for university IP that eliminates several of the impediments to the early development of a technology, as it avoids the persistent delays in identifying and negotiating with funding sources for university start-ups and provides expert management oversight to the incubated companies. Thus, rather than waiting for outside investment to start a Newco or license a technology, the Biodesign Impact Accelerator performs the technology risk-reduction process that is critical to making the technology/innovation more
attractive to eventual investors or acquirers – ensuring forward progress of the innovation and rapid financial and job creation returns benefitting ASU and the State of Arizona.

BIA expenses will be funded through external sources that include: federal granting agencies, industrial partners, foundations and individual philanthropic investments, venture investors and the liquidity returns on companies that graduate.

There are 6 key organization elements to the operation of the BIA:

1) The Biodesign technology portfolio management process
2) The multiple BIA start-up companies (Newcos)
3) Biodesign facilities and administrative support
4) BIA core technology resources
5) The BIA management team
6) The BIA advisory groups and boards.

The roles and operation of these elements are detailed in Section 3: Operational Strategies, and Section 4: Management and Organization.

The BIA management team will be overseen by the BIA advisory board composed of members with significant technical, legal and entrepreneurial experience. The selection of companies to enter the Accelerator will be made by an “Entrance Exam” Committee appointed by the Biodesign Executive Director. This Committee will have the specialized expertise in the technical and business areas of interest to ensure proper screening and selection. When new companies are selected and formed, a management team will be appointed and the necessary resources allocated. The Biodesign Institute will provide facilities and specified administrative support to each Newco on a no-cost basis. The BIA core technology resources can be deployed to each Newco on an as-needed, contracted, and cross-charged basis. Scientists, engineers and technicians in this core technology resource team with the specific expertise needed to advance a novel therapy, diagnostic or device will be allocated to each Newco for the fraction of time needed to achieve Newco risk reduction milestones. The company, working with this core team and the resources in the Biodesign Institute and ASU, will implement a milestone driven program, focused on addressing the primary technical and performance risks. This process will ensure that measureable risk reduction milestones are achieved, and access of enterprise efficiencies is integral to the development effort.

Specialized facilities will allow the production of early prototypes or materials testing for rigorous early feasibility assessment. GMP and GLP expertise, along with a carefully managed IRB process and a selected group of clinical collaborators will facilitate pre-clinical evaluations. Regulatory expertise will be consulted to ensure that documentation and regulatory filing requirements are being met throughout the development program.

All startup companies, Newcos, will be managed against a graduation plan and will have a set of identified preferred acquiring partners. The preferred exit strategy is an acquisition that will result in an immediate and significant return to the BIA and recruited investors. A “Graduation” Committee will oversee the exit of the Newco from the BIA. The value proposition return on investment cash generated at exit will be distributed according to the “Cash Distribution Agreement” in place between the defined Newco stakeholders. Biodesign’s allocation will fuel the continuing investments in the pipeline of additional technologies.
2.0 The Market and Opportunity Analysis

Background
Over the nearly thirty years since the Baye-Dohl Act, universities have attempted the technology transfer and commercialization process by employing various methodologies that have proven to be only marginally successful. A national analysis has identified several impediments to the progress of promising university discoveries toward translation and ultimate commercialization. These impediments can be traced to three major issues:

1. The lack of funding and resources to support early stage technology development
2. Low efficiency and poor track record for success in development and commercialization of novel technologies
3. Onerous IP license terms that unnecessarily burn precious cash and encumber future revenues with royalty payments.

The lack of available funding has caused promising technologies to languish, as there are often insufficient resources to address the critical needs and barriers relevant to the translation and commercialization of a technology. Consider the following data from the Biotechnology Industry Organization (BIO)\(^1\) that is impacting early stage investment:

- In 2008 the amount of capital raised by biotechnology companies across the U.S. fell 55 percent relative to 2007;
- There were no funds raised by US biotechs through Initial Public Offerings (IPOs) from January 2008 through August 2009, as compared with 41 companies raising $1.9 billion in 2007 and 32 companies raising $1.7 billion in 2006;
- Presently, 38% of public biotech companies have less than one year’s cash on-hand, and 26% have under 6 months’ cash.

The Biodesign Institute has developed a vehicle to cross this “valley of death” by adding a bridge over the funding gap between discovery and delivery. The high cost of developing innovations like vaccines, in-vitro diagnostic devices, bio-fuels, and other important advances causes investors in early-stage companies to demand significant proof-of-concept and technology risk reduction that typically are not addressed by the university. Universities generally are not set-up to nurture research through this critical development phase of the commercialization pipeline, so even the most promising university research may not achieve sufficient maturity to attract investment. In addition, the high costs and time-consuming efforts for licensing university IP are additional barriers to timely investment and development.

The low efficiency of start-up development efforts and poor rate of success for technologies is often a result of several factors:

- Lack of sufficient, early proof-of-concept, plausibility testing of novel ideas
- Inexperience and lack of breadth in the management of development efforts
- Lack of understanding of the development hurdles, the design requirements and the documentation requirements for application and commercialization of new technologies, therapies and biomedical devices.

Lack of capabilities and facilities to produce devices, and test materials under the appropriate guidelines, e.g., ISO 9000, Good Laboratory Practices (GLP), Good Clinical Practice (GCP) or Good Manufacturing Practices (GMP)

Lack of understanding of the market dynamics and commercialization issues in the targeted market segment

Lack of focus on the critical risk factors that are deterministic of potential success

The inability to recognize the need to terminate a development effort that is advancing down a blind alley.

The impediments to commercial success noted above are being addressed by the Biodesign Impact Accelerator program.

**What Startup Companies Need**
In order to alleviate the obstacles identified, there are several things needed by a new company start-up to improve the probabilities of success. These include, though are not limited to:

- A strong core technology with IP protection
- Resources sufficient to achieve critical mass to expedite development
- Access to needed research – equipment, expertise, networks
- Incubation lab and office facilities
- Mentoring and consulting services
- Strong management skills
- Well developed business plan and IP strategy
- Legal support for set-up and financial direction
- Access to financing (e.g., Venture Capital, partner investments, acquisition targets).

Thus, our Operational Strategies (Section 3) defines our plan to address these needs.

### 3.0 Operational Strategies

The goal of the Biodesign Impact Accelerator is to increase the speed of commercial translation of promising ASU technologies and their eventual impact on our community. Our operational strategy will proactively address major issues that impede successful commercialization of university technologies:

1) the lack of early stage investment to develop promising technologies, and

2) the low efficiency and poor rates of success for the efforts to develop focused, high impact products with paying customers.

The principle strategy of the BIA is to take immature, early-stage technologies and guide them through a development process focused on end-use products providing societal benefit and the creation of jobs in the State of Arizona. We will achieve this through the judicious funding and synergistic use of Biodesign Institute infrastructure. This will accelerate the launch of new start-up companies around our most promising technologies. The purpose of these efforts is to advance the company and its product to the stage where there is sufficient reduction in commercial risk and increase in commercial value that the technologies and companies are acquired or can exit from the BIA as viable, stand alone spin-outs.
Figure 1 provides an overview of the prototypical BIA technology process flow from inventive conception to a profitable company enterprise. Through collaboration between the BIA program and Arizona Technology Enterprises, AzTE, resources will be made available to examine the potential utility and scope of early stage discoveries ensuring that appropriate intellectual property protection is secured for promising inventions. Seed funding will be provided through the Biodesign Institute on a competitive basis for promising technologies to obtain early proof-of-concept (technical plausibility) and confirmation of customers’ needs and market opportunities, meeting the rigorous screening criteria described in Section 3.3. BIA will form a startup Newco and provide supplemental resources (funding, space, personnel and oversight) to advance the development of the technology. The startup Newco will hold the IP through an agreement with AzTE for an exclusive, world-wide, royalty free license in exchange for equity held by the ASU Foundation that generally, would hold all the equity shares in a BIA Newco. The development effort will employ a disciplined, milestone driven, risk reduction strategy.

- "Use-inspired" research supported by grant and contracts
- Novel ideas and discoveries are submitted for patent consideration.
- Preliminary assessment of technical plausibility and commercial opportunity. Potential "Seed" funding or targeted grant proposals.
- Proposal providing assessment of potential commercial value and development of preliminary business plan.
- Selection of a Newco to enter BIA program.
- Support development, implement IP strategy, manage to milestones, validate business models, reduce risk.
- Newco or technology leaves BIA program - is acquired by another company or forms a spin out.
Figure 1: Overview -- Biodesign Impact Accelerator (BIA) Activities Flow Diagram

All startup companies will be managed against a graduation plan with two potential exit strategies: 1) an acquiring partner or partners, or 2) a precisely defined spin-out plan. The preferred exit is an acquisition that results in an immediate and significant return to the BIA and recruited investors. The "Graduation" Committee will oversee the exit of the Newco from the BIA. The value proposition return on investment cash generated at exit will be distributed according to the “Cash Distribution Agreement” in place between the defined stakeholders. The Biodesign Institute’s distribution allocation will provide sustaining funds for continued investment in the technology development pipeline, generating more companies with high impact products.

There are 6 key organizational elements to the operation of the BIA:

1) The Biodesign Technology Portfolio Management Process (Section 3.2)
2) The multiple BIA startup companies (Newcos) (Section 3.4)
3) Biodesign Facilities and Administrative Support (Section 3.6)
4) BIA Core Technology Resources (Section 3.7)
5) The BIA Management Team (See Section 4)
6) BIA Advisory Groups (See Section 4)
   a. The BIA Advisory Board
   b. “Entrance Exam” Committee
   c. Graduation Committee.

3.1 Kick-off of BIA Development

The Biodesign Impact Accelerator Program was formally launched on July 16, 2009 with an announcement by Executive Director, Dr. Alan Nelson, to Biodesign employees and research partners that BIA would begin soliciting and screening promising technologies. The screening process is directed towards identifying technology and market maturity level. The process will identify sufficiently mature technologies for Newco company launch. The announcement came after formal consent by the Arizona Board of Regents (ABOR) that allows ASU owned IP to be licensed to BIA start-ups on a cash free, royalty-free basis (See Section 3.5). The launch of the Biodesign Impact Accelerator was announced to the public in a press release on August 6, 2009. The Management Team had been put in place and the process for selection of start-up companies to enter the BIA had been defined (Section 3.3). The first screening round schedule was also announced as:

- August 7, 2009: Letters-of-Intent due from researchers sponsoring technologies for admission to the BIA.
- August 17: Investigators with the most promising technologies were invited to prepare an “Entrance Exam” proposal and to attend a “Spartan Entrepreneurial Workshop.”
- August 17 – September 16: Writing preliminary draft of “Entrance Exam” proposal.
- September 17 – 19: Spartan Entrepreneurial Workshop was conducted by the ASU Technopolis Program to educate sponsors about the demands of a bioscience start-up and the operation of the BIA and to help them prepare their proposals.
- September 25: Deadline for submission of full “Entrance Exam” proposal.
Letters-of-Intent were received for 17 technologies in various stages of development. Of these, ten technology teams were invited to attend the Spartan Entrepreneurial Workshop for this first selection of potential companies. Nine “Entrance Exam” proposals were received for evaluation by the “Entrance Exam” Committee. Committee review and ranking was completed in October. Committee recommendations place each technology at its appropriate level of maturity within the Biodesign Technology Development Pipeline. Those technologies with sufficient maturity for pre-launch development were identified by the Entrance Exam Committee. The diversity of technologies proposed included:

- Portable breath sensor to diagnose and monitor asthma.
- Vaccine for Norovirus (common stomach flu) produced by engineered tobacco plants.
- Instrument systems for analysing, monitoring, and performing remediation on groundwater.
- Synthetic antibodies (affinity ligands) for low-cost, high performance protein purification and detection.
- Molecular diagnostics platform to simultaneously analyse proteins and DNA with high sensitivity.
- Instrument producing high resolution 3-D scaffolds for cell growth and tissue engineering
- Instrumentation to analyse the electrical activity of the brain to monitor, predict and control epileptic seizures.
- Non-invasive monitoring system for diabetics that measures the glucose level in tears.
- An analytical and diagnostic platform to measure antibody signatures utilizing high-density protein arrays.

Those technologies selected for acceleration will work with BIA management to develop a comprehensive business model and project plan. Combined, the business model and project plan will expose resource requirements and project risk prior to new company launch. The first companies are expected to enter the BIA program in January 2010.

Funding for the seed stage of the BIA has come through a $5M philanthropic grant through the ASU Foundation.

Facilities have been allocated within the Biodesign Institute to address company development needs. Accessing these allocated facilities is a transitional activity that will be managed on company-to-company basis (See Section 3.6). In July 2009, Biodesign submitted a grant proposal to the NIH Extramural Research Facilities Improvement Program (C06) requesting $10 million to renovate 21,000 sq. ft. in the SkySong building to accommodate anticipated growth in the BIA program. Awards will be announced in December 2009.
3.2 Biodesign Technology Portfolio Management Process

The Biodesign Technology Portfolio Management Process is intended to guide the flow of technology from early conception to commercial products with focused and purposeful oversight. The Biodesign Technology Portfolio is actively managed by the Institute Executive Director and Deputy Director. In general, all promising technologies begin with innovative concepts that require sustained investment and focused effort to realize any end-use value beyond the laboratory setting. Such novel ideas generally percolate up from the broad discovery efforts funded through sponsored research contracts to the Biodesign Institute. As indicated in Figure 1, much of this may be engaged through standard grant mechanisms. However, a gap often exists between what is funded as “new research” and a legitimate “commercial venture.” Ideally, novel technical ideas are strategically moved through a development pipeline (typical of industrial settings) with continuous focus on value-added effort with concomitant risk-reduction always mindful of return-on-investment. The Biodesign Technology Portfolio Management Process and the BIA exist to comprehensively manage this effort across the programs of the Biodesign Institute. The earliest stage is managed by maintaining an inventory of technologies and invention disclosures that are actively updated, reviewed and mined to identify nascent technology with potential for commercial application. Such potential will often be determined by a preliminary market or commercial analysis. The progression of promising ideas is often stymied by the lack of a demonstration of technical plausibility (preliminary proof-of-concept). Such efforts are infrequently funded by traditional granting agencies. In such cases, Biodesign will attempt to secure necessary “seed” funding for promising ideas from a variety of available sources. Such sources could be competitive “seed” funding grants through ASU, seed funding from Angel funds, grants from philanthropic organizations, and federal SBIR and STTR grants (which would require the formation of a receiving company). Biodesign will also explore sponsored research agreements with industrial partners. With a demonstration of technical plausibility and some evidence of commercial potential suggesting the technology could meet the BIA criteria and become a candidate for formal entrance to the BIA through our “Entrance Exam” process.

3.3 Selection of Company Candidates

Program Entrance Criteria

The decision to advance a technology within the Impact Accelerator program is determined through a screening and selection process with recommendations made by the “Entrance Exam” Committee whose recommendations are forwarded to the BIA Board with final Technology Development Pipeline classification, and BIA action directive made by the Biodesign Executive Director. Entrance to the BIA is based upon the following criteria:

- Strategic Alignment with Biodesign and ASU.
- IP Assignable to ABOR (tangible property value, may include proprietary “know-how”).
- Proof-of-Concept for technical innovation is complete (moving from discovery phase).
- Development path and milestones for risk reduction are defensible.
- Preliminary market/needs analysis is strong.
- Commercial opportunities and graduation options are rational and defensible.
- Inventors agree to IP assignment to Newco (signed Letter-of-Intent).
- Return-on-Investment for anticipated development and patent costs exceed 4:1.
- Newco at graduation meets minimum value requirements (Valuation >$10 million, projected NPV >$20 million).
The key characteristics of the new companies, Newcos, selected and supported in the Impact Accelerator program will be:

- C-Corporation, Common shares only, Shared minimum Board of Directors
- Cash free intellectual property (IP) license from AzTE
- Equity held by the ASU Foundation
- Acquisition Driven Business Plan
- Risk Reduction Management to Milestones
- Dedicated Project Management
- Shared Development Team
- Targeted Acquirer
- Cash Distribution Agreement for Exit

More details on the Program Entrance Criteria are contained in Appendix A.

**Entrance Exam Preparation:**

The “Entrance Exam” would involve the completion of a proposal by a sponsoring team that addresses the benefits of the technology, the market potential, the development plan and any remaining known uncertainties. The Entrance Exam will address all the key criteria described above for the selection of a technology. Details on the Entrance Exam Preparation are contained in Appendix B. The overriding selection criteria will be whether the Impact Accelerator can multiply the value of a nascent technology by several times through efficient investment and focused development over a limited time period (three to five years).

The BIA anticipates selecting between 3-5 technologies for acceleration from the initial set of candidates. Selection is competitive. Acceleration rate and partnering strategies are dependent on resources available. Subsequent calls for applications will be made at ~6 month intervals.

**3.4 BIA Startup Companies**

Each Newco formed within the BIA will be a C-Corporation, with Common Shares only. In nearly all cases, the exclusive shareholder will be the ASU Foundation via AzTE. Each Newco will be governed by the procedures defined in the Articles of Incorporation when it is formed. These articles will follow a standard and flexible template appropriate to the State in which the company is incorporated. Oversight for each will be provided by a Board of Advisors selected by the BIA and responsible to the shareholders. Directors may serve on multiple Newco Boards. Each Newco will have an identified Management Team.

**3.4.1 Newco Management**

Each Newco will have an appointed Management Team consisting of the following functions:

- Leader [President and Chief Executive Officer (CEO)]
- Finance [Chief Financial Officer (CFO)]
- Technical lead [Chief Scientific Officer (CSO)]

Additional members of the management team can be added as deemed necessary. The Leader is appointed by the Board of Advisors and is responsible for the selection and appointment of all other members of the management team and all additional staffing for the Newco. The Leader
will have the authority to make contractual obligations and agreements on behalf of the Newco. Typically, Executive Director of the BIA will serve in that role.

For efficiency, Management Team Members may be deployed to provide services to multiple companies in the BIA. Each Newco will also have a Board of Scientific Advisors with expertise to provide valuable advice to the development process for the company.

### 3.4.2 Cash Distribution Agreement

The normal and preferred structure for ownership of a start-up company, Newco, entering the BIA will be through all shares being held by the ASU Foundation via AzTE. Stakeholder compensation derivable from the sale or graduation of a startup will be allocated according to a “Cash Distribution Agreement” with provisions specific to that Newco. This agreement will be utilized to define the percentage of cash income derived that will go to inventors, AzTE, Bodesign, investors, researchers, key managers, scientific consultants and all other identified participants. This agreement will be established on formation of the Newco and may be amended by action of the Board of the company until a distribution event has occurred.

![Diagram of Cash Distribution on Liquidation of Equity in Newco.]

#### Figure 2: Diagram of Cash Distribution on Liquidation of Equity in Newco.

### 3.5 Intellectual Property

ASU producers of IP who participate in the BIA Program will waive and release their rights under the normal ABOR IP Policy as they relate to sharing and distribution of revenue for creators and their technologies. In place of those rights, participating university employees and students will receive about five percent (5%) of net cash income attributable to intellectual property created by them and licensed or otherwise commercialized under the Program. For the purpose of this calculation “Net Cash Income” means gross revenues actually received from
licensing or other commercialization of intellectual property less the following fees and costs: 1) an administrative fee of 15% and 2) all unreimbursed costs incurred by ASU or Arizona Technology Enterprises (AzTE) in protecting, licensing, maintaining, and litigating rights in the intellectual property at issue.

**Licensing of Intellectual Property to BIA Newco Start-up**

The Biodesign Impact Accelerator program is built on the premise that valuable Intellectual Property (IP) has been generated at ASU and has been assigned to the Arizona Board of Regents (ABOR) to be managed by AzTE. The start-up Newco will hold the IP through an agreement with AzTE for an exclusive, world-wide, royalty free license in exchange for the equity held by the ASU Foundation/AzTE. This license agreement will be made between the startup and AzTE and will become effective only after the Newco is incorporated and the equity shares are assigned the ASU Foundation. This license agreement will ensure access to all background and enabling patents and technical “know how” owned by ABOR for the defined field-of-use. With any acquisition or spin-out of the Newco, the license for the IP would transfer to the acquiring company. If the Newco should cease to operate, all IP licensed would revert back to ABOR.

**Management of IP by BIA Newco Start-up**

After the formation of a Newco within the BIA program, all new IP generated by research and development efforts supported by or in support of the Newco will be assignable to the Arizona Board of Regents. “Invention disclosures” will be filled through AzTE consistent with the current procedures in place for all ASU inventors. AzTE will manage the disposition of all disclosures and the patent filling process. This new IP will be licensed to the Newco on a non-cash, exclusive, world-wide, royalty-free basis in the field-of-use for the defined business of the Newco startup. All other anticipated fields-of-use rights will remain the property of ABOR unless specifically negotiated for by the Newco.

**3.6 Facilities / Administrative Support provided by Biodesign**

**3.6.1 Use of Biodesign Institute Facilities**

In the first phase of BIA launch, the development efforts supporting each Newco will be incubated within the facilities of the Biodesign Institute. Company development laboratory space, office space, conference rooms, and laboratory equipment will be provided in accordance with the project plan. These resources will be provided to the Newco on a no-cost basis while it remains within the BIA program.

The Biodesign Institute is privileged to occupy superior facilities whose superb esthetic design qualities are matched by functional efficiency, encouraging the flexible workflow patterns that are an essential element to multidisciplinary, translational research.

Biodesign occupies a 13-acre site that serves as the eastern gateway to the ASU campus with the additional benefit of a location adjacent to the light-rail system for connection to the ASU downtown campus and future downtown biomedical facilities. **Building A (~172,000 sq. ft.)** was
These two buildings contain a number of specialized areas and laboratories including:

- Low vibration areas.
- Low electromagnetic field areas.
- Centers for Disease Control Biological Safety Level-3 Certification for Biological Select Agents (BSL-3, ABSL-3, ABSL-3Ag).
- Femtosecond laser laboratory.
- High-security vivarium capabilities and advanced surgical suites.

The Biodesign Institute buildings have been designed to be flexible and to promote a unique culture of entrepreneurial research. To achieve this goal the buildings utilize an “open lab” concept. Each module of lab space is designed with the ability to be modified and reconfigured for future changes in research direction.² The open, module delineated lab allows the progress of and change in research to proceed without physical constraint. Scientists, technicians, and students who use the labs create new design requirements in the space while evolving in response to required changes in the existing facilities simply by extending the amount of bench space needed and/or by rearranging instruments and equipment.

Facility Security. Security technology is also an inevitable cost of operation for any large research enterprise. Biodesign must comply with the security regulations of a number of federal oversight agencies, including the Centers for Disease Control and the Office of Homeland Security. The U.S. government’s heightened awareness of bioterrorism has increased the security regulations on all bioscience research entities, especially those studying infectious disease. These mandates require a specified level of security personnel and protections. The Biodesign Institute is in compliance with these regulations. It is equipped with 78 security cameras with 60 terabytes of storage, which enables the facility to maintain thirty days worth of video, meeting all regulatory requirements. Contracted Security Officers provide 24 x 7 security in several shift rotations, including building access monitoring, CCTV monitoring, patrols and incident response and inquiry.

² Lord, L., Perspectives in Laboratory Design, Chapter 5, 1999.
3.6.2 Administrative Support Services
In addition to lab space and facilities, each Newco will have access to the following administrative support services on a no-cost basis:

- Administrative assistance (reception, mail, and copy services).
- Accounting services.
- Telecommunications, Information Technology and Computer Support (telephones, e-mail, teleconferencing, video conferencing).
- Web site development.
- Logos and business templates.
- Project management software.
- Business plan development support.
- Grant writing assistance.
- Library and research services.
- Lab Safety and OSHA training.
- Invention disclosures, patent writing & filing support.
- Investor presentation help.
- Education, mentors, seminars, market research.

3.7 Contracted Resources
In addition to the staffing and resources provided within the Biodesign institute, each Newco is likely to require additional services not currently available with Biodesign or the university. Such services might include specialized consulting services, limited development contracts, consumer or market research contracts, animal research contracts, and clinical or safety research contracts. In preparation for graduation, additional resources may be anticipated to:

- Conduct market research and develop Newco valuation.
- Determine “freedom-to-operate” and consolidate additional, required IP.
- Access acquisition targets or identify investment sources.
- Recruit management and an advisory board for a spin-out.

Funding for such services will be provided to the Newco by the BIA program when need is established and will be treated as a contracted cross-charge to the company.

3.8 Newco Graduation
The new companies formed by the Impact Accelerator will be managed against a plan to graduate from the program. The target is, on average, one Newco or technology graduating each year. Company graduation could be in the form of a sustainable local spin-out company moving to new quarters or the acquisition/merger of the Newco by another company. The Graduation Committee will oversee this process. Resources will be deployed to determine whether acquisition or spin-out is the best graduation strategy. A transition management team would also be put in place to develop and execute the final graduation plan.
4.0 Management and Organization

The management of the Biodesign Impact Accelerator will be provided by an experienced management team consisting of Biodesign Institute personnel:

a. Executive Director (President & CEO): Dr. Alan Nelson  
b. General Manager (COO): TBD (search active), full-time  
c. Director of Finance (CFO): Jeffrey Darbut  
d. Director of Business Development: Michael Mobley, full-time  
e. Director of Communications: Kimberly Ovitt  
f. Director of Human Resources: Melvin Holcom  
g. Director of Technology Development: Mark Holl, full-time

The Executive Director (President and CEO) will oversee all business and operational activities of the BIA. The Executive Director will appoint all other members of the management team in coordination with the General Manager and will have final approval of all members appointed to the BIA Advisory Groups.

The General Manager (GM) will oversee all business and operational activities of the BIA on a day-to-day basis. The GM will work closely with the management team to insure that the BIA mission is achieved within the BIA charter. The GM will work closely with the executive director in representing the BIA to external political and private entities for the purpose of raising funds, educating the larger societal community about the BIA mission.

The Director of Finances (CFO) will be responsible for the finances and accounting requirements of the BIA and will oversee these activities for each of the Newcos in the BIA.

The Director for Business Development will manage the marketing and commercialization efforts for the BIA, specifically developing the commercialization plans, contacts, and partners for each Newco to facilitate the graduation or acquisition of the Newco.

The Director of Communications will oversee the communications, marketing and public relations strategy for the BIA and each Newco to successfully brand our initiative and the individual Newcos and aid in the fund raising efforts of the BIA.

The Director of Human Resources will oversee all hiring and employment issues for the BIA and the Newcos and ensure the BIA is in compliance with the HR policies of ASU.

The Director of Technology Development will provide cross-disciplinary integration functions associated with developing and maintaining the BIA technology pipeline; and new company definition, development, and transition to graduation. In performing these responsibilities the Director of Technology Development and will oversee project management functions, manage the Core Technology Resources for the BIA, and deployment these resources to support the risk reducing, development strategies of the Newcos within the BIA.
4.1 Advisory Groups

Mentoring and advice for critical decisions will be provided by three BIA Advisory Groups.

4.1.1 BIA Advisory Board

This Board consists of experienced entrepreneurs and technical advisors appointed by the Director of the Biodesign Institute to provide expert guidance to the BIA program. The current Advisory Board members are:

- Alan Nelson, Ph.D. (Chair) – Executive Director, The Biodesign Institute
- Ken Noonan, Ph.D. (Co-Chair) – Partner, Advanced Technology Ventures
- Augustine Cheng – Managing Director, Arizona Technology Enterprises
- Rick Shangraw, Ph.D. – Vice President, ASU Research and Economic Affairs
- William Harris, Ph.D. – President, Science Foundation Arizona.
- Keith Stoneback – Chief Executive Officer, Agile Sciences.

4.1.2 Entrance Exam Committee

The “Entrance Exam” Committee is appointed by the President and CEO of the BIA with primary responsibility to oversee the process for screening technologies and selecting candidates to recommend to the BIA Board and the President and CEO for advancement into the BIA program. Entrance to the BIA program indicates that the company formation process will be chartered. If during the process of detailed project planning, technology, IP, and market planning fatal flaws are discovered, then the formation of the Newco will be ramped down and terminated. This Committee consists of experienced experts in technology development, technology commercialization, and venture financing. The President and CEO is an ex officio member of this Committee.

Current Committee Members include:

- Michael Mobley, Ph.D. (Chair) – Director for Business Development, BIA.
- Dan O’Neill – Director of Entrepreneurial and Research Initiatives for Innovation and Entrepreneurship at ASU Technopolis.
- George Andrews – Private fund manager.
- George Leone – Patent counsel for In-vitro Diagnostics (IVD) and remote sensing/vision.
- Dick Rhode – Corporate and contract counsel.
- Steve Gately, Ph.D. – Chief Scientist at TD2; expert in clinical trial management and FDA compliance.
- Mark Holl, Ph.D. – Associate Director of Development, BIA.
- Herb Goronkin – Partner, Lux Capital Management.
- Ron King, Ph.D. – President, Catapult Technology Accelerator.
- Mark Schwartz, Ph.D. – CEO Bayhill Therapeutics.
4.1.3 Graduation Committee

The Graduation Committee is appointed by the President and CEO of the BIA with primary responsibility to oversee the progress and exit planning for the individual Newcos in the BIA. This committee will assess the milestone progress of each Newco and advise the CEO on perceived points of risk and potential next step strategies. The Committee will advise on the multiple components of an optimal exit strategy – timing, approach, readiness, and valuation. If an acquisition is anticipated, the Committee will advise on acquisition targets and methods of engagement and will oversee and advise on all negotiations and transfer agreements associated with the graduation of a Newco. The Graduation Committee will be appointed when the first class of startup companies have been selected and enter the BIA program.

5.0 Marketing and Promotion Plan

The Marketing and Promotion Plan for the BIA will be managed by the Director of Communications and the Communications Team of the Biodesign Institute to leverage the synergy with the Institute’s other programs. The Biodesign Institute pursues a comprehensive promotion strategy, employing strategic methods to maximize service efficiency in the areas of community/public relations, media relations, interactive communication and marketing. It produces an annual Communication Plan against which efforts are measured to ensure accountability. The efforts in support of the BIA will become an integral part of that plan. In addition, the Biodesign Institute maintains an active media relations effort that encompasses local, national, and international media aimed at the general public, the business community, and the scientific community. This effort will also be leveraged to promote the BIA and the activities of the companies in the BIA. As each Newco in the BIA begins to develop its own marketing and promotion strategy, the resources of the Biodesign Institute will be used to guide this process and assist in obtaining the additional resources necessary to execute the strategy.

6.0 Financial Requirements

The Biodesign Impact Accelerator will manage a portfolio of startup companies and a sustaining innovation technology development pipeline. The process of launching the BIA as an initiative will be a ramped effort over an anticipated 5 year period. During this time period we will manage ~10,000 sq. ft. of dedicated BIA space within the Biodesign Institute on the Arizona State University campus, and up to ~21,000 sq. ft. of dedicated BIA space at Skysong in Scottsdale. These launch activities will require phased application of both contract assets for facilities modifications and improvements, as well as investments in capital assets to prepare the space for use. The scope of efforts and rate of deployment will be proportionate the facilities, personnel, financing available for disposition. In addition, each Newco formally entering the BIA will require direct and subcontracted R&D support. At this point, prior to performing the project management planning phase, we estimate these expenses in generic terms, for a focused diagnostic instrument development effort of moderate complexity. Below in Figure 3 is a chart estimating typical Newco R&D expenses by year of development. This chart assumes an exit or sale of the company in year 5. Subcontracted R&D would be those resources that must be accessed that are outside of the university. This might include consultants, market research, legal costs, contracted production or clinical testing. This chart provides an estimate of total cash investments of ~$4 million. It is clear some Newcos will require less time or more time in the accelerator and that there can be a large variation in the annual R&D expenses. Similarly, there could be much more or much less expense required for subcontracted support. These
estimated expenses would not include the contributions Biodesign is making through oversight, the use of University facilities and administrative support.

Figure 3: Chart estimating typical R&D Expenses (BIA Internal and Subcontracted) by year for a Newco.

Thus, it has been very roughly estimated that each company accelerated will require in the range of $3 - 5 million in initial investment to take it to a graduation event. Therefore, an initial portfolio of 3 to 5 company start-ups will require initial cash financial commitments of $15 to 25 million.

Figure 4 contains a chart that summarizes the cash expenditures associated with the ongoing operation of the BIA. These projections are over the first 7 years, reaching an annual expense level of about $5 million after 3 years. The key assumptions are that, on average, the Newcos will incur about $4 million in development costs and they graduate in their fifth year. Additional Newcos are added as others graduate or are terminated such that expenditures are maintained at a target level. It is estimated that $5 million in annual expenditures will maintain 6 Newcos in the accelerator. Each additional Newco would require approximately $800,000 per year in additional funding.
Revenues are generated from the graduation of the Newcos through their acquisition and a cash distribution or the formation of a spin-out with revenues back to the BIA and AzTE. For financial modeling purposes, we’ve estimated that the Newcos will graduate in their fifth year of development. To develop a Cash Flow analysis based upon the expenses projected in Figure 4 we must also estimate the returns on the sale of these Newcos and probability weight their success. The analysis shown in the chart on Figure 5 makes an assumption that the return on our investment will average 4:1, but that this return is discounted by a probability of success for each Newco entering the accelerator which is taken to be 50%. These factors together project an average ROI for our portfolio of about 2:1. This same average ROI can be derived from higher risk investments (25% probability of success) and higher anticipated return (8:1). This represents just one possible scenario among many, but it does illustrate how the BIA can move into a positive cash flow situation in 7 years. In this scenario, the BIA would be about $16 million in the red in year 4 before there is a revenue stream. This model analysis is very sensitive to both the probability of success estimate for the Newcos and the ROI. This points to the criticality of maximizing both parameters by only investing in those efforts that efficiently reduce risk and therefore maximize our ROI.
Figure 5: Cash flow modeling for the BIA assuming expenses from Figure 4, an average 4:1 ROI and 50% probability of success for our Newco investments.

7.0 Sources of Financing

The BIA is a use-inspired technology development enterprise having a broad portfolio. Within the charter of the Biodesign Institute, BIA acts as a focusing lens for technologies that emerge from mission driven research endeavors. Given the time horizon requirements associated with accelerating this diverse portfolio of companies of varying complexity and resource demands, a financing portfolio of equally diverse source of origin is needed.

The BIA will develop a finance portfolio comprising a mixture of sources, inclusive of: federal and state granting agencies (including SBIR/STTR sources), industrial partners, foundations, individual philanthropic investments, and venture investors. The channeling organization for external investors will be Arizona Technology Enterprises, AzTE. Figure 6 diagrams the flow of funding and investments through AzTE to support the development efforts of the Newcos.
Figure 6: External Funding Mechanisms for Newcos in the BIA.
Appendix A: Program Entrance Criteria

The Biodesign Impact Accelerator will invest in innovative research plans and brilliant people. Although individual circumstances will be different, we anticipate initial funding commitments of up to 24 months of operation based on risk-reduction milestones. We recognize that technology development is rarely linear or predictable. Thus we will work to facilitate the adjustment of business plans as progress is made to ensure that most relevant milestones are correctly set and achieved.

This document presents the criteria for screening, selecting, and managing technologies that will enter the Biodesign Impact Accelerator (“BIA”). It also defines the structure a Newco will have that enters into the BIA. A candidate technology will include Intellectual Property (IP) assets that will generally consist of patents granted, patents pending, proprietary methods (technical know-how) and invention disclosures. These are expected to have significant, derivable value. It is recognized that candidate technologies may cover a broad spectrum of technical maturity and valuations. Regardless, the BIA has a simple template for technology entrance. Prior to BIA entrance, all inventors and applicants will have a full knowledge their future relationship to the Newco and full disclosure of their rights as they pertain to a future company graduation event. All discussions and agreements will be up-front.

Use-inspired Selection Criteria

The BIA entrance decision will be based upon the following use-inspired criteria that must be addressed in an entrance exam proposal. The BIA will provide a template for these proposals. A technology portfolio that is a candidate for BIA launch will have:

1. Strategic alignment with Biodesign and ASU;
2. IP assignable to ABOR (tangible property value);
3. Proof-of-concept for technical innovation (discovery phase is complete);
4. Preliminary development plan and well defined risk-reduction milestones;
5. Preliminary market analysis identifying the commercial market size, market need (market penetration potential), and projected company valuation at risk reduction milestones;
6. Inventor(s) agreement to assign all IP to the newly formed BIA company (signed letter-of-agreement for assignment from AzTE);
7. Founder(s) and shareholder agreement to complete divestment of all company shares in return for negotiated return on present valuation at time of BIA company sale through the BIA cash distribution agreement mechanism (Applies only in the case of existing companies that are BIA entrance candidates);
8. Projected Return-on-Investment (ROI) for anticipated development, administrative, and legal (including patent) costs exceeding 4:1;
9. Graduation (liquidity event) minimum value requirements of valuation >$10 million, and projected Net Present Value (NPV) >$20 million.
These are minimum requirements and, as entrance is competitive, fulfilling these requirements does not guarantee entrance. The review and selection of candidates will be done by an “Entrance Exam” committee reporting to the Biodesign Executive Director, Dr. Alan Nelson.

**Biodesign Impact Accelerator Start-up Criteria**

All newly formed BIA companies will have the following attributes intended to manage risk reduction and maximize incremental value:

1. All equity is held by Arizona Technology Enterprises (AzTE). Opportunities with a minority ownership will not be considered by the BIA;
   a. C-Corporation formed, common shares only
   b. BIA assigned Board of Directors
2. Cash free intellectual property (IP) license from AzTE (no fees or royalties);
   a. Exclusive, and
   b. Worldwide rights in field-of-use
3. Risk-reduction management to key value-driving milestones;
4. Management, Development Team, and Advisors appointed by BIA;
   a. Defined project management staffing
   b. Shared core development team
   c. Established boundaries between Biodesign research and BIA development activities
   d. Work processes conforming to the strict requirements of the products category being developed (e.g., FDA regulations, GMP, GLP, HIPPA, etc.)
   e. Defined on-going role for inventors and/or founders
5. Acquisition-driven business plan;
6. Targeted acquirer or well defined exit strategy; and
7. Cash from anticipated liquidity event would be distributed to defined stakeholders in accordance with a pre-defined cash distribution agreement.
   a. Inventors receive five (5%) of net income attributable to intellectual property created by them and licensed or otherwise commercialized under the Program

Participation in the cash distribution at the liquidity event is based upon a contractual agreement initiated before a company enters the BIA. For entry to the BIA, all identified inventors to a transferable technology must sign a “Revenue-Sharing Requirements for the ASU Biodesign Impact Accelerator” agreement communicating they wish to participate in the BIA program and will agree to abide by its terms and conditions.

**Required Access to Enabling IP**

Where tangible background IP is required by the company, access to that IP can come through AzTE. In cases where the IP resides outside AzTE, the IP will be obtained through a negotiated percent allocation in the cash distribution agreement with the third party and/or through a license agreement deferring any cash payments until after revenue generation.
Consideration of BIA Entrance by a Pre-Existing Company

Where a spin-out company has already been formed that owns Intellectual Property critical to the advancement of the technology through BIA, there is one option: the pre-existing company will negotiate conversion of all equity in the form of IP and shares at present value in return for a negotiated percent allocation in the cash distribution agreement.

All agreements on IP assignments, present valuation of pre-existing company shares, and associated cash distribution agreement must be in place before a company can be supported within the BIA.

These criteria can be modified from time to time by the Executive Director of the Biodesign Institute.

Revision: November 7, 2009
Appendix B: “Entrance Exam” Preparation

This summarizes the information required for consideration of a proposed technology or company’s entrance into the Biodesign Impact Accelerator.

The entrance decision will be based upon the following criteria which should be addressed in the entrance exam proposal. The review and selection of candidates will be done by an “Entrance Exam” committee reporting to the Biodesign Executive Director, Alan Nelson.

Criteria:

1. Strategic Alignment with Biodesign and ASU
2. IP Assignable to ABOR (tangible property value)
3. Proof-of-Concept for technical innovation is completed (not in discovery phase)
4. Development path and milestones for risk reduction are defined
5. Preliminary market/needs analysis is completed
6. Commercial opportunities and graduation options are defined
7. Inventors agree to IP assignment to company (signed Letter-of-Intent for assignment)
8. Return-on-Investment for anticipated development and patent costs exceed 4:1
9. Company at graduation meets minimum value requirements (Valuation >$10 million, projected Net Present Value (NPV) >$20 million)

(These are minimum requirements and, as entrance is competitive, fulfilling these requirements does not guarantee entrance.)

Impact Accelerator Entrance Exam Procedure:
Applications for entrance into the Impact Accelerator should follow the general outline below. If a company business plan has already been prepared, it can be submitted, but it should include all the elements described in this outline to allow examination against stated criteria. If there is intent to prepare an entrance exam proposal, a letter of intent should be forwarded to obtain assistance and to allow an early inventory of potential companies. This LOI should be less than three pages and contain the title, list of key team members, a brief description of the product or innovation, the status of the IP, and a brief description of the envisioned business opportunity. Please submit the letter of intent and entrance exam proposal in electronic form (Word Document or pdf) to Mike Mobley, mike.mobley@asu.edu who will confirm receipt.

Outline

Cover Page to include:

Biodesign Impact Accelerator Entrance Exam

Name: Name of Company or Initiative

Title: (Should provide description of primary technical innovation)

Company Mission Statement: (One sentence that defines what the company intends to accomplish)
Technical Contacts: (List those proposing this Accelerator entrance -- inventors and researchers familiar with the innovation and relevant qualifications and contact information; clarify inventors on relevant IP)

Business Advisor: (Contact providing market analysis support, business modeling support and/or commercialization direction, contact information).

Revision Date:

Beginning on Page 2: (complete document with these outline elements)
(Footer to contain page #)

1. Company Overview/Business Model (1 or 2 paragraphs)

(Brief description of anticipated company and business. What are the products? - services, therapeutics, devices, reagents? What is the market segment? What need is being met? What is the competitive proposition?)

2. Product/Innovation Description (< 2 pages)

(Detailed description of products and innovation -- known potential advantages and disadvantages. How does this innovation align with the strategic interests of Biodesign and ASU? What are the uncertainties in performance or in market demands? What are the competitive or alternative approaches? What is the Intellectual Property position? Is there freedom-to-operate or is additional background IP required? (A confidential assessment of IP may be required from AzTE or outside legal counsel). What is the greatest technical risk?)

3. Market/Commercial Opportunity (< 2 pages)

(Describe the market segment – its size, structure, growth and major competitors. Describe the anticipated market penetration model or anticipated commercialization path and growth for the product innovation. Clarify who is the customer (user) and who is the payer. (Will reimbursement be required? If so, how will it be approved?) If there are multiple potential paths, describe how options will be selected. If the innovation will occupy a special niche, describe the size and characteristics of that niche. What are the projected costs, revenues and profitability associated with the innovation? What are the major market or commercial uncertainties?)

4. Development Plan (< 3 pages)

(What is the current status of development? Briefly describe the sequential work plan that addresses the technical and performance uncertainties of the innovation. How are the technical risks to be addressed? (Provide sequential risk reduction milestones.) Describe how the product specifications will be defined. Describe the milestones, the timetable and resources required for each phase of the plan. Describe how the commercialization model will be validated (customer feedback, testing plans). What will be the monetary investments required and on what schedule? Complete a pro forma spreadsheet on costs and investments (attached).

Resources: Describe what potential resources are available at ASU (individuals, labs and instruments) and those that must be accessed outside of ASU. Are there special requirements (e.g. radioactive or hazardous materials, cleanrooms, GMP facilities)?
5. Regulatory Pathway (< 1 page)

(Describe the plan for regulatory clearance. Clarify what regulatory pathways will be followed (e.g. NDA, PMA, 510(k)). Are there special record keeping requirements? Are there governing Quality Control or manufacturing standards (e.g. ISO 9000)? Will IRB approvals be required? Will there be animal research and how will it be monitored?)

6. Return-on-Investment and Exit Strategy (< 2 pages)

(How will the company graduate from the Accelerator? What will the value of the company be at graduation? What further investments may be required to commercialize innovation after planned graduation? Based upon direct costs and opportunity costs (e.g. space and facilities) for the Accelerator, what is the anticipated ROI at graduation? Is there a long-term revenue stream? What is the Net Present Value (7-year for device, 10-year for therapeutic) of the initiative anticipated at graduation? Are there other ROI impacts from this innovation that are not monetary that should be considered in the valuation (e.g. societal impacts, orphan disease treatments, follow-on products)?

Appendices

(Supporting charts or tables that clarify the market, the cost and revenue projections, the investment plan, or pro forma projections can be added as appendices.)
SECTION THREE
Research Project Examples

Vaccines
Presymptomatic Diagnosis & Personalized Medicine
Bioenergy & Bioremediation
Vaccines
Salmonella Anti-Influenza DNA and Antigen Delivery Vaccine Platform

Coordinator:

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The Challenge

Approximately 14.8 million deaths globally are due to infectious diseases with millions more due to secondary effects of infections (18). If there were universal vaccinations for these diseases (as strived for in the Children’s Vaccine Initiative), 5.6 million of these deaths could be prevented (57). However, there are many other infectious diseases for which no effective vaccines are available and others that are available see only limited use due to high costs and/or instability. Among these, influenza remains one of the most significant infectious diseases worldwide averaging about 40,000 deaths per year in the US alone (50, 51). Over the past few years H5, from avian influenza (H5N1) has been the major cause for concern. Highly pathogenic avian H5N1 influenza A virus has caused influenza outbreaks in poultry and migratory birds in Asia, Europe and Africa. Subsequently, human H5N1 disease with high fatality rates has also been reported in Southeast Asia.

The very recent H1N1 influenza virus, a reassortment with genome segments from avian, human and both North American and Eurasian swine influenza viruses, has infected humans in over 100 countries (39) leading WHO to declare a pandemic alert level to phase 6 (63). Although it is less severe than H5N1, human to human transmissibility of H1N1 is substantially higher than that of seasonal flu and could potentially result in a global pandemic (20).

In assessing the impact of either “seasonal” or pandemic influenza, one must appreciate that the world human population is more than five times greater today than in 1918 when the last very severe influenza pandemic occurred. In addition, global movement of people and goods by air further accelerates and increases the probability of transmission. In the current pandemic, CDC has estimated that at least one million people have already been infected in the US (1).

A critical aspect of pathogenesis of influenza virus infection is the susceptibility of the individual to secondary bacterial infections that seem to complicate the illness and lead to overall higher mortality rate due to influenza pandemics. WHO has also estimated that 3.7 million annual global deaths are caused by respiratory infections (57) and these are in addition to 2 million deaths caused by tuberculosis (57). Two million of these deaths due to respiratory disease are caused by Streptococcus pneumoniae infections in children under five years of age (57). Influenza is certainly a contributor to respiratory disease globally and although the estimated annual mortality of 600,000 to one million (45) is significant, the morbidity and lost-time associated with infection has a larger economic impact (41). It has also been shown that infection with influenza predisposes individuals to infection with bacteria causing pneumonia (62) with S. pneumoniae being most frequent (16) to result in 20 percent of the deaths associated with influenza infections (16).

The most frequent serious complications of influenza are pulmonary and fall into 4 categories: primary influenza pneumonia, secondary bacterial pneumonia, pneumonia due to unusual pathogens or to being immunocompromised, and exacerbations of chronic pulmonary diseases (47). Although rhinoviruses and coronaviruses account for the majority of pathogens that exacerbate chronic lung disease, influenza also may account for as much as 25% (62).

Finally, virus destruction of respiratory epithelium may also increase bacterial adhesion. Influenza and parainfluenza viruses possess neuraminidase (NA) activity, which appears to increase bacterial adherence after viral pre-incubation (47). Experimental studies demonstrate that viral NA exposes pneumococcal receptors on host cells by removing terminal sialic acids and inhibition of viral NA activity reduces adherence and invasion of S. pneumoniae, independently of effects on viral replication. Clinical studies reveal that influenza vaccination reduces the incidence of secondary bacterial respiratory tract infections (46).
Our Platform

Because of these considerations and the synergisms in exasperating consequences of infections due to multiple respiratory pathogens, our lab is focused on the design, construction and evaluation of recombinant attenuated Salmonella vaccines (RASVs) against Mycobacterium tuberculosis, S. pneumoniae and influenza virus. In this regard, we have been granted an IND license by FDA to conduct phase I human clinical trials to evaluate the safety of three attenuated S. Typhi vaccine strains delivering pneumococcal protective protein antigens.

We also plan to vary the S. enterica serotype used as the vector strain for antigen and DNA vaccine delivery. S. Typhi still causes 20-30 million infections per year with a significant mortality in underprivileged countries (11, 58, 60). However, with the available Ty21A (35) and Vi (33) vaccines to prevent S. Typhi infections increasingly used leading to the discontinuance in use of the killed TAB vaccine (5, 14, 56, 59) that gave modest control to S. Paratyphi A and B caused diseases (2), there has been an appreciable increase in enteric fever caused by S. Paratyphi A strains. For this reason, we are developing improved attenuated S. Paratyphi A vaccine vectors in parallel with our efforts with the S. Typhi vectors we will soon be evaluating in clinical trials. We hope to have a S. Paratyphi A vectored vaccine go into clinical trials in 2010 and based on results will decide whether to use it or a S. Typhi vaccine vector for our two proposed RASV-Influenza vaccines. The use of both vector systems will hopefully provide additional means to lessen global infection and disease caused by both S. Typhi and S. paratyphi A.

In 1981, Formal et al. (19) reported the first construction of a live recombinant attenuated Salmonella vaccine (RASV). They used the live attenuated S. Typhi Ty21a vaccine as host for a conjugationally transferred plasmid from Shigella sonnei specifying an LPS O-antigen. The vaccine was not very efficacious (6) but neither have recent live recombinant attenuated S. Typhi vaccines (RAStyV) using a diversity of sophisticated designs for presentation of protective antigens from numerous bacterial, viral and parasitic pathogens (22, 28, 43, 48, 54). In contrast, during this same time period, much progress was achieved in developing safe efficacious live attenuated Salmonella vaccines for poultry (7), swine (3), cattle (52,53) and humans (9, 15, 25, 26, 37, 49, 55) and the vaccines for poultry and swine are licensed and used internationally to prevent infection with broad host range and host-species adapted Salmonella serotypes.

In 1997, we (44) began to understand one reason for the poor results with live RAStyVs expressing protective antigens from a diversity of pathogens. In 1992, it was reported (44) that rpoS mutations attenuated S. Typhimurium and this led shortly thereafter to the demonstrated role of RpoS in regulating genes on the S. Typhimurium virulence plasmid (8, 24, 34). S. Typhimurium strains with rpoS mutations have markedly diminished abilities to colonize the Peyer’s patches in mice (17) and since virulence plasmid-cured S. Typhimurium colonizes the Peyer’s patches as well as wild-type strains (23), chromosomal genes controlled by RpoS must be of critical importance for Peyer’s patch colonization. We thus theorized that rpoS mutations not only attenuate Salmonella but also likely lessen their immunogenicity. This fits with past experiences when it was reported that S. Typhi Ty2, which had been used by all groups constructing RAStyVs to evaluate in humans, was an rpoS mutant (10). In this regard, rpoS mutants are much less immunogenic when administered orally or intranasally than wild-type strains. We have started our human phase I trial that will enable us to compare isogenic strains of S. Typhi Ty2 RpoS-, its RpoS- derivative and ISP1820 RpoS+ all delivering the pneumococcal PspA antigen specified by a high-copy number plasmid. The results of these studies will determine the best S. Typhi vector strain to use for future human vaccines.

During the past seven years, our group has endeavored to develop a vastly improved array of means to enhance the safety, efficacy and utility of Salmonella antigen and DNA vaccine delivery technologies. Many well known means for attenuation of Salmonella diminish their ability to withstand stresses encountered in the GI tract after oral immunization or have
mutations that impair their ability to attach to, invade into and survive in the GALT (12).
Collectively, these attributes lead to diminished immunogenicity.

Thus we have developed seven usable means to achieve regulated delayed attenuation in vivo such that the vaccine at the time of immunization exhibits almost the same abilities as a fully virulent wild-type strain to contend with stresses and successfully reach effector lymphoid tissues before display of attenuation to preclude onset of any disease symptoms (13). Since high-level expression of protective antigens by RASV strains also imposes an energy demand that decreases growth, fitness and ability to colonize lymphoid tissues resulting in further attenuation and reduced immunogenicity, we developed means to achieve regulated delayed protective antigen synthesis in vivo (36, 61) that is compatible with use of the regulated delayed attenuation strategies. Also to enable multiple antigens to be delivered from the same vaccine strain, we have developed two new balanced-lethal vector-host systems to use in conjunction with the system originally developed (21).

The two new balanced-lethal vector strategies are based on required needs for D-alanine and muramic acid, two additional essential unique constituents of the rigid peptidoglycan layer of the bacterial cell wall. We recently developed a series of balanced-attenuation vector-host combinations to further expand the repertoire of vector-host systems available for use in vaccine strains. We have thus made chromosomal deletion mutations for the entire aroA, aroC, aroD and ilvE genes, any one of which will attenuate Salmonella and render it immunogenic. We then have the wild-type alleles AroA+, AroC+, AroD+ and IlvE+ on multi-copy plasmids to complement these deletion mutations. These plasmids enhance safety since loss in vivo would increase the degree of vaccine attenuation (27). These new vector systems are described below. We have also introduced mutations to decrease induction of inflammatory intestinal responses, that eliminate biofilm formation to preclude persistent colonization (especially of the gall bladder) and that decrease inter- and intra-plasmidic recombination.

We have also made mutations that decrease antibody responses to Salmonella serotype-specific and surface immunodominant antigens to allow reuse of the vector system for multiple vaccines (13) but will not use these strategies for the vaccine to be used in these studies, but defer use of these technologies until developing the S. Typhi or S. Paratyphi A strains to use in human vaccine trials. We have also made good progress and are able to use S. Typhimurium strains with the properties described above to infect day-of-birth mice at doses of 3-4 x 10⁸ CFU with 100% survival and have demonstrated induction of protective immune responses in these mice. Our last accomplishment has been to design and construct a system for regulated delayed lysis in vivo that can deliver a bolus of antigen to augment induction of immunity (32). This strategy, which is also a means for regulated delayed attenuation, confers complete biological containment with no persistence in vivo or survivors if shed.

Our goal for an influenza vaccine is induction of specific immunity via neutralizing antibodies (HA encoded by DNA vaccine vectors) as well as broad-based immunity (antibodies to M2e and/or CTL responses to conserved NP) capable of at least partially controlling heterologous influenza strains. We propose to deliver these antigens individually to assess their role in protective immunity as well as in a mixture by a single RASV strain. The CTL responses wane within months of infection and correlate directly with loss of immunity to heterologous influenza virus infection. In this regard, we believe that delivery of conserved influenza antigens by means that are proficient at inducing cell-mediated immunity, such as by use of the Salmonella regulated lysis system, will likely induce a more lasting and effective cellular immunity (30, 31, 40).

Although avian influenza viruses (AIV) do not typically replicate efficiently in humans (4) H5N1, H7N7 and H9N2 subtypes have caused human infections. Culling of more than 1 million poultry in Asia could not prevent new cases from occurring and in spite of preventive measures repeated H5N1 infections were reported in Hong Kong in 2001, 2002 and 2003. Due to zoonotic potential of the disease it is of utmost importance to control the AIV infections in poultry. Such
Salmonella Anit-Influenza DNA and Antigen Delivery Vaccine Platform

efforts require prophylactic measures like vaccination. So far, vaccines have been used to successfully control highly pathogenic AI infections in both Pakistan and Mexico (42). We have developed a novel 8-unit plasmid that carries the entire influenza viral genome segments to generate the virus in cultured chicken cells. It is designed to carry the HA and NA gene from AIV on a background of a mouse adapted strain for vaccination of poultry. Its use would not only eliminate the use of embryonated eggs or cell culture but the ease of manipulation would allow the generation of new seed virus strains in just two weeks time by replacing the HA and NA genes of a new strain, as compared to months required for production of vaccine in eggs. This technology has the potential to supersede the traditional methods in the face of an outbreak such as the current swine flu (H1N1).

Impact

The annual economic burden of influenza epidemics is also enormous. During a typical year in the United States, 30,000 to 50,000 persons die as a result of influenza virus infection, and the global death toll is about 20 to 30 times higher than the toll in this country (45). Based on the 2003 US population, annual influenza epidemics result in an average of 610,660 lifetime-years lost, 3.1 million hospitalized days, and 31.4 million outpatient visits with the total direct medical costs averaging up to $10.4 billion annually. Projected lost earnings due to illness and loss of life amounted to $16.3 billion annually. The total economic burden of annual influenza epidemics using projected statistical life values amounted to $87.1 billion (41). The aforementioned socio-economic factors make influenza one of the critical infectious agents and hence a vaccine to prevent the resulting pandemics is highly warranted.

A pandemic outbreak will spread much faster than it will take for pandemic vaccines to be produced and distributed. The currently used influenza vaccines have serious drawbacks and limitations. Because these vaccines must be made in embryonated chicken eggs, it takes 6-9 months to make sufficient quantities of the vaccines, which is unacceptable in the event of a pandemic. Also of concern is the inability to use this technology to make vaccines containing avian influenza virus attributes, a major likely future necessity. In addition, the current technology results in one dose of vaccine per fertile egg and is limited to 565 million doses/year (29). It would require 60 million hens and 4 million roosters to immunize the world’s population, which is about 20-30 times the current global vaccine production capacity.

Another drawback to these vaccines is the cost. The cost of manufacturing the live attenuated virus vaccine is high and each dose sells for $25, although the consumer can pay up to $75 to be vaccinated. Thus, the need is present for a more cost-effective influenza vaccine that can be manufactured quickly and can protect from currently circulating influenza strains as well as from yet to emerge pandemic strains.

Salmonella-based vaccine administration is needle-free, easy and inexpensive to manufacture (10 doses/penny for poultry vaccines and 10 cents/dose for human vaccines). If successful our efforts would ensure development of prepandemic vaccines that can induce broad cross-protective responses and that can be administered as soon as a pandemic is declared or even before, in order to successfully prime the immune system and allow for a rapid and protective antibody response with one dose of the pandemic vaccine. We also believe that our developed Global Access Strategy dealing with discovery, development, intellectual property, manufacture, distribution and use will contribute to our success in making these vaccine technologies and RASVs available throughout the world to combat global infectious diseases (38).
Bibliography and References Cited


A New Platform for Rapid Vaccine Discovery

Participants:

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Background
Policy-makers and scientists have long recognized that the most financially and medically efficient defense against infectious disease is vaccination. However the acquisition of efficacious products that meet increasing expectations for safety and affordability has proven difficult. Concomitantly, the emergence of new pathogens and re-emergence of old ones highlight the global need for many new products.

African Swine Fever Virus (ASFV) is the only Asfarviridae family member and is included in the nucleo-cytoplasmic, large, DNA (NCLDV) virus superfamily. The NCLDV also includes the poxvirus family, of which smallpox is the most notorious member. Members of this superfamily carry the largest known viral genomes and display complex life cycles. Large DNA viruses have a number of mechanisms for host immune evasion. For instance ASFV encodes proteins that inhibit signaling through immune activation pathways in infected macrophages. Other factors inhibit apoptosis of infected cells so as to facilitate production of progeny virions. In addition, many different isolates occur; animals surviving an infection can be protected from members of the same clade, but are not protected from different ones.

ASFV is highly contagious; it causes an acute hemorrhagic fever in domestic pigs, but persistently infects its natural hosts: warthogs, bushpigs and soft ticks. The virus is endemic in Africa but non-African outbreaks also occur such as those on the Iberian Peninsula from 1960 to mid-90s. ASFV can become established in local arthropod species and asymptomatic wildlife reservoirs, making both domestic and sylvatic transmission cycles difficult to control. Mortality rates of up to 100% are observed; a domestic herd can be eliminated in 5 to 10 days. There is currently no treatment, vaccine, or disease model. ASFV represents a significant trans-boundary disease due to the severe economic and social impact resulting from loss of domestic pig populations and bans on export of animals and pork products. The short incubation period, high mortality rate, and environmental stability of the virus cause concern of ASFV as an agricultural bioterror agent. The potential impact of disease prevention, the complexity of the pathogen, and the ability to evaluate candidates in the relevant host contribute to selection of ASFV for demonstration of our new vaccine discovery platform.

Approach
We present an approach to vaccine discovery based on forcing a pathogen to unnaturally expose its immunome and then using this far broader repertoire of host activity to profile, and ultimately control, immune protection. By vaccinating with expression libraries encoding all proteins of a pathogen, antigens that do not typically elicit responses following infection or live vaccine administration can be immunogenic in this unique presentation and stochiometry. This group of immunogens is potentially useful in a vaccine despite not being infection-stimulated because the level and context of exposure necessary to activate a naive immune cell is far more stringent than that needed for later recognition by pre-formed effectors. This collective natural and unnatural set of reactivities is identified in a first round of immune assays. Immunogens are next characterized and sorted by either similarity or balance (diversity). Each of these categories is finally functionally sampled in challenge studies to identify those corresponding with protection.

Our objective is to obtain a safe and effective component vaccine against ASFV that is
cross-protective among clades. We will demonstrate the feasibility of the program using the Georgian isolate since this has been the agent of the most recent outbreaks. Once candidates are identified, we will survey broader isolate protection. Multiple antigens will be included if advantageous; since we intend to employ genetic immunization and live-vector constructs, multivalent formulation issues associated with proteins are circumvented.

The antigen discovery phase will be conducted with a gene-prime and vaccinia-boost regimen; however the final vaccine product is envisioned to be swinepox-vectored. In parallel with antigen discovery, another set of experiments will be conducted to develop a marked, attenuated swinepox-based vaccine vector for optimal, inexpensive delivery of the newly identified immunogens. Such a vaccine could be used to both protect domesticated animals and limit infection of feral pigs, by virtue of exposure, which serve as otherwise uncontrolled reservoirs of disease. This has been achieved with rabies virus in Europe. It is important to note that these two subprojects can be conducted largely independently and then brought together. The advantage of the swine-pox vector over vaccinia is its greater efficiency of pig infection and its inability to infect humans, or any other species. Its advantage over an attenuated ASFV is its broader potential for antigen presentation, its better safety profile, and the lack of interference with diagnostic assays.

1. Exposure and Sorting of the ASFV Immunome. Development of an effective vaccine for ASFV appears to be immunologically feasible because animals vaccinated with weakened strains are protected against subsequent lethal challenge (Lewis 2000, Oura 2005); however, the mechanism of immunity is unclear. For example, there is evidence that antibodies are sufficient for protection (Barderas 2001), and also studies showing that T cells are necessary (Oura 2005). There are two dominant antigens that induce neutralizing antibodies, p54 and p30, but conflicting data as to their importance relative to protection (Neilan2004, Barderas 2001). To circumvent this lack of understanding, we propose a systematic approach to the discovery of protective viral components based on exposing and then sorting the complete ASFV immunome recognized by the porcine host. This approach is built from technologies that have been developed by our labs at the Biodesign Institute at Arizona State University (ASU) to screen genomes for protective antigens and manipulate live vectors and also the strong expertise and productive history at Pirbright labs in working with this viral disease.

We obtained the Georgian ASFV responsible for the recent outbreaks in the Caucuses and Eurasia and have completed its sequencing and annotation; no other members of this clade had been previously analyzed. We used these results to build its ~180 ASFV genes. The modestly-sized ASFV genome provides us the opportunity to demonstrate our process; however, our high-throughput (HTP) protocol is scalable to thousands of genes. The ASFV genes will be built and placed in three expression systems. First, we will use our linear expression element (LEE) technology to assemble each gene for mammalian-promoter driven expression. LEEs enable recombinant-DNA engineering to be conducted in vitro, eliminating bacterial cloning steps and making it highly adaptable to robotic protocols. Second, the ORFs will also be assembled into a vaccinia-based expression vector. Our team has engineered a strain of vaccinia that contains a negative selectable marker linked to a reporter gene. This increases recombinant frequencies sufficiently high so as to identify viruses without plaque cloning. We will use these two libraries of ASFV genes in a prime/boost regimen since this strategy has frequently been shown to stimulate enhanced and more robust immune responses than any single modality.
regimen. The mammalian and vaccinia expression libraries will be partitioned into 5 sub-libraries of ~40 components each and used as inocula for groups of outbred pigs. The libraries will be randomly partitioned a second time, so as to increase the opportunity for separation of any immune suppressing factors from proteins upon which they act. The 10 groups of sub-library immunized animals will serve two purposes. First, they will provide the reagents (sera and PBMCs) for comprehensive immune analyses, and second, the sub-libraries will be challenged with virus to sample their protective potential. Additional groups will include pigs immunized with i) the complete library, ii) a protective avirulent isolate, and iii) naive animals.

Antigen for the proteome-scale immune assays will be generated from the third ASFV expression library. The technology for robust, high throughput (HTP) protein production and purification of tens of micrograms of purified antigen material has been developed in our labs at ASU. The genes will be assembled into T7 promoter-driven LEEs and used as template for in vitro transcription/translation reactions. These polypeptides will be used individually to assay antigen-specific cytokine release activities of T cells harvested from PBMCs of the immunized pigs. For broad-based B cell analyses, sera reactivities will be determined on a new proto-array recently developed in our labs. These microchips will be printed with each of the in vitro translated viral polypeptides in such a way as to maintain their natural folded structures. This survey of B cell activities relative to each ASFV genome-encoded component will create a first level of response profiling. All B cell immunogens will be identified. Likewise T cell reactivities will be generally identified in ELISpot interferon assays. By comparing the library groups’ immunogens to that of the live virus, normally hidden immunogens will be identified. All polypeptides identified as immunogenic will be tested in a second level of immune profiling. These will be further characterized in functional assays such as for cytotoxicity and viral neutralization. This collection of data will be used to categorize viral components into “bins” by their response profiles and then rank-sort them within these bins. The number of bins will be optimally decided based on the level of data stratification.

2. Identification of protective antigen sets. If any partial protection is conferred by any of the sets of library inocula, we will consider the pool to hold potential value for antigen discovery. Even if no protection is measured with the context of these complex pools (~40 components), protection may be subsequently measured as individual immunogens are identified and tested. In addition to subunit vaccine discovery, availability of the ASFV proteome, along with characterization of the ASFV immunome, will allow others to i) develop improved immunological tests for diagnosis, and ii) identify and characterize ASFV isolates in the field.

To identify vaccine candidates, the sorted immunome will be sampled. Top ranked immunogens from each bin will be evaluated in challenge-protection assays. To evaluate the importance of immune balance, the performance of a pool of antigens comprised of the top-most ranked immunogen from each bin will be tested. Animals will be immunized, immune-profiled as before and then challenged with virulent virus. This will enable the correlation between response profiles and disease protection to be established. Any correlations with protection among these antigen sets will be confirmed by testing others from the same category.

3. Vector development. We believe that a modified swinepox-based vaccine has the potential to be a potent, safe, marked vector for vaccination against ASFV (Tripathy 1999). Swinepox naturally infects porcine species and has been shown to induce potent immune responses in domesticated pigs. However, swinepox does not infect humans, thus obviating the
A New Platform for Rapid Vaccine Discovery

Concerns of using a modified human pathogen, such as vaccinia virus, as a livestock vaccine. We are proposing to apply technology that we have developed and characterized for vaccinia virus to attenuate swinepox, without decreasing immunogenicity of the vector (Brandt 2005, Jentarra 2008, Vijaysri 2008). Attenuation will also limit the potential spread of vaccine to non-target wildlife species. Since this technology is already in place in our labs for vaccinia, we will use this virus as live vector for the discovery prong of the project. However, we believe swinepox is a preferable viral vector for the final product, as argued above.

Swinepox will be attenuated by deleting the poxvirus virulence gene E3L. Mutations of E3L in vaccinia virus are the most attenuating of any known single gene (Vijaysri 2008). Vaccinia virus deleted of E3L is apathogenic in all but the most sensitive animal models, and is attenuated by at least 6 logs compared to the wild-type parental virus. Despite being highly attenuated, this virus induces potent pro-inflammatory signal transduction, potent pro-inflammatory gene expression, and potent cell-mediated and antibody responses (Jentarra 2008, Langland 2006). The swinepox E3L homologue contains 35% sequence identity with vaccinia virus E3L, and both functional domains of E3L are highly conserved.

The swinepox E3L homologue will be deleted from swinepox and replaced with a novel antibiotic-sensitivity cassette that we have developed. A cassette containing ASFV protective genes will be inserted in place of the antibiotic-sensitivity cassette in the E3L locus. Attenuation will be determined by infection of pigs with swinepox deleted of E3L. Induction of pro-inflammatory signal transduction and pro-inflammatory gene expression will be determined after infection of HeLa cells, or after infection of pig kidney cells. Since the vaccine will not express all ASFV antigens, but only protective antigens, it will be feasible to differentiate immunized animals from infected animals. Likewise, since the swinepox vector will contain a deletion of the E3L gene, which in vaccinia virus is a potent B cell antigen, vaccinated animals can be differentiated from naturally swinepox infected animals. Antigen expression will be determined in pig kidney cells. Immunogenicity and efficacy will be tested by vaccination of pigs and challenge with a lethal dose of ASFV. If we find that a DNA-prime and viral boost regimen (as used in the screens) is optimal ten this can be implemented, too. Challenged animals will be monitored for morbidity and mortality, and for viral load.

Management Plan
Bertram Jacobs is the principal investigator (PI) on this project. He will be responsible for production of the vaccine-vectored expression library and development of swinepox as an ASFV vaccine delivery vehicle. Co-PI Kathryn Sykes will be responsible for the design and construction of the gene library and its use in immunizing animals. She will generate a protein-antigen library for use in immune assays. She will also responsible for conducting sera assays by ELISA and protein-arrays and the analyses of immune patterns. Co-PI Linda Dixon is head of the ASFV group at the UK Institute for Animal Health at Pirbright. Dr. Dixon will be overseeing all of the pig immunization and challenge experiments. She will also be responsible for the cellular immune analyses. A dedicated project manager is responsible for progress towards research milestones. All participants meet at least biweekly via videoconference to review objectives and experimental progress. The Biodesign Institute has an information management plan that covers Material Transfer Agreements, Non-Disclosure Agreements, and Arizona State University has a dedicated office for intellectual property protection (Arizona Technology Enterprises).
References


Cancer Vaccine

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Summary

We believe it is possible to take this one step further – to vaccinate to prevent cancer. Our premise is that cancer can be treated like an infectious disease by pre-mobilizing the immune system to kill the tumor when it arises. The success of the concept rests on whether tumors have recurrent, common peptides that are tumor specific. The revolution in genomics and proteomics technology now makes it possible to determine if this is true.

We have launched a multi-prong effort involving genomics, proteomics and immunology to screen tumors for the variants that would constitute the vaccine. Our goal is to find all variants that reoccur in tumors (at least 1% over all major tumor types) that are not in normal cells, or at least not recognized immunologically by the normal immune system. Importantly, any variants that reoccur in the screen in Project 1 are candidates for a prophylactic vaccine. As far as we know we are the only group working on this type of vaccine.

Our immunological analysis of sera from patients already indicate that patients often have tumor-specific immune responses in common. This indicates that there are common, immuno-reactive antigens produced by different tumors. So in principle this approach should work.

Status: A group of researchers in proteomics, genomics, informatics, clinical diagnostics, immunology and molecular biology have been assembled to work on this project. Significant funding from DoD and the Keck Foundation have been secured. The process for conducting almost all facets of the screen have been created. To date tumor variants that would provide ~10% prevention of cancer in the general population have been found. Our goal is to assemble a vaccine with at least 30% coverage of the 4 major cancers before initiating a Phase I safety trial.
Presymptomatic Diagnostics and Personalized Medicine
Immunosignaturing for Diagnostics and Discovery

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Overview:
It is becoming increasingly clear that the immune system is exquisitely sensitive to changes in the body. The traditional view of it only responding adaptively to infection is wholly inadequate. It at least is an early indicator of almost all chronic diseases. The challenge is to invent the technology that can translate this potential into practical clinical value.

Our Plan:
Antibodies respond to all types of antigens – proteins (normal and abnormal), sugar, peptides, lipids and post-translation modifications. These reactions signal that the immune system is responding to something the body perceives as a threat. We have evidence that the antibodies in an individual change in profile within a day of infection. The most advanced technology to date to “read” the complexity of the antibody response is to array native proteins on slides and then react them with the serum of patients. These arrays have yet to become comprehensive (approximately 8,000 of 25,000 human proteins to date) and are very expensive to produce. Though the results with these arrays have been impressive in sensing auto antibodies, they are clearly inadequate. Our overall goal is to develop an inexpensive system that can comprehensively read out the antibody composition in a person’s serum from a drop of blood.

We are developing a totally different approach. We create arrays of peptides that can react with the antibodies. We have two phases of this effort. One is to make arrays of random sequence peptides. We currently produce spotted arrays with 10,000 peptides of 20 amino acids of known sequence. The cost is 1/30th to 1/60th the cost of the current state of the art. This is important as it makes large clinical studies feasible. These arrays are in regular production and being used in clinical analysis.

Phase II of the array production is to make much higher density silica arrays. These arrays will consist of 1-5 million peptides that the human genome could produce. They will comprehensively read out almost any immune response to a peptide, but will also be amenable to mass production by standard microfabrication. The goal is to have a simple, inexpensive chip-based system that could read out the immune diagnosis. These chips could include the proteomes of all major human pathogens. Dr. John Rajasekaran who developed this process at Intel has now joined Biodesign to re-establish this fabrication system. Intel has donated the equipment for the effort.

Of course, the antibodies that are evident on the array have value in themselves. Most directly, they could be developed as a therapeutic against an infection or tumor. In collaboration with Pacific Northwest National Laboratory, we plan to develop a protocol to read the antibodies from the array.

Status:
Over 10,000 of the phase I arrays have been produced and tested with the blood from hundreds of patients and normal individuals. Serum of animals and people (apx. 800) that includes control groups and subjects with, lupus; breast, brain, and pancreatic cancer; influenza; Valley fever; and asthma have been screened. In each case, the analysis groups the disease profiles
together and further subdivides the patients within each group. It has proven a remarkably robust and informative system to date.

The peptides from the array can be a source for vaccines. We have demonstrated with several biothreat agents that the immune response to the pathogen can be detected before symptoms occur. Further, the peptides that are reactive on infection can themselves serve as vaccines to create an immune response to the pathogen. This system suggests a very fast protocol to develop vaccines. It can also be used to characterize the immune response to vaccines.

The silicon based system for high-density arrays is being established. The scientific team has been organized and a clean room obtained.

**Need:**
This system should be tested against a variety of infectious and chronic diseases for diagnostic potential. These trials can be initially on historic blood samples, but then will evolve to prospective clinical studies. The new versions of phase I chips and the phase II chips will be developed.

**Summary:**
Immunosignature diagnosis has many advantages over the existing, common approaches to biomarker diagnosis. This approach is limited only to the extent the immune system “reads” health status and that chips can be produced that display this information.
Novel Technologies for Rapid Diagnosis

Coordinator:

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A New Technology for Rapid Diagnosis of Viral Infection

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The Challenge: Viral infection is one of the major causes of human diseases, and rapid diagnosis of viral infection is critical for prompt treatment and containment of the diseases. However, existing diagnostic methods for viral infection are slow and expensive because they require multiple and complicated steps, including sample collection, culture, extraction, purification, staining and imaging, which limit their applications as effective clinical tools in many cases. Current clinical practice is still largely based on the symptoms of the patient, which have many limitations, such as accuracy. The lack of accuracy may result in delayed and improper treatment of the patient, and has other consequences. For example, due to the difficulty of distinguishing the swine flu from the common flu based solely on the symptoms, H1N1 flu vaccine is recommended for those may already had the swine flu, which contributes to the vaccine shortage and the difficulty for stopping the flu pandemic. A rapid and affordable method for diagnosis the type and strain of viral infection is in needed.

Our Solution: Develop a simple and affordable diagnostic method for rapid detection and identification of viruses using a novel combined optical mass and affinity measurement of individual viral particles in their native state.

Approach: The invention of optical microscopy revolutionized the diagnosis of bacteria infection. Optical microscopy is simple to use, affordable and can resolve and identify a single bacterium particle in its native states with minimal sample preparation. However, the size of virus (20-300nm) is much smaller than the resolution limit of the conventional optical microscopes. The present project is aimed at developing a new tool to detect and identify viruses by measuring the optical mass and affinities at single virus level using surface plasmon resonance (SPR). SPR is well known as a label-free technology for real time measurement of biomolecular interactions, but the conventional SPR technology lacks the resolution to resolve single viral particles. To overcome the limit, an objective-based SPR microscopy, together with electrochemical signal enhancement, will be developed (see Figure below). Our technology has the following unique advantages:

- **Highly sensitive**: Due to the large optical enhancement and electrochemical amplification, single viral particles from a complex sample can be directly detected without the need of time-consuming culture steps.
- **Highly specificity**: Simultaneous measurement of optical mass and affinity/binding to specific antibody, plus spatially resolving the viral particles from other macro molecules in the sample (from blood or other body liquid), the type and strain of viral particles can be identified within minimal sample preparation requirement.
- **Rapid detection**: It can be operated like a conventional optical microscope that detects and identifies virus by simple “looking” at both the optical mass and binding affinity to antibodies in real-time. The anticipated time is in minutes, instead of hours and days required by other technologies.

![SPR image of 20nm Gold nanoparticles](image)
Likelihood of Success: Preliminary study shows that nanoparticles with size comparable or smaller than virus can be imaged with the objective-based SPR microscopy. A SPR image of 20nm gold nanoparticles is shown above. Theoretical calculations and existing experimental evidence have shown that a single protein difference between viruses can be detected by the proposed technology.

Anticipated Outcome: If success, the proposed technique can be developed into an affordable optical microscope-like device that could be placed in doctor's office or clinical labs, for quick detection and identification of viruses by simply “looking” at the size of individual virus and binding of individual virus to corresponding antibodies with minimal sample preparation. The method could also be used for diagnostic of bacteria infection, and used as a research tools for quantitative characterization viruses structures and functions based on the protein abundance, and real-time and label-free study of virus-host interactions and the interaction kinetics at single virus level.
SPECIFIC AIMS – This project’s focus is on obtaining clinically relevant data to distinguish a potential infectious disease from a patient’s blood sample (~1 ml) in a culture-free process thereby in a rapid (<1 hr) manner.

IMPACT - Sickness and death caused by nosocomial or health-care associated infections (HAI’s) and communicable diseases are prevalent in all areas of the world. These infections lead to approximately nine million deaths worldwide annually (World Health report 2008, WHO). In the US, HAI’s associated with Methicillin resistant *Staphylococcus aureus* (MRSA) alone resulted in 18,650 deaths in 2005¹ and an average cost of $12,197 per infection in a study from 2001 to 2006². Most HAI’s are the result of approximately eight bacteria in general.

What is needed is a system to gather and handle the sample in a single device; a simple to operate, self-contained system; culture free technique for rapid results; a means to gather and present data in a manner consistent with the training of the physicians who would use the device; an integrated detection system allowing for reproducible sample examination; and have a selection of typical bacterial infectious agents. The goal is to not trade off between rapid results and sensitivity by combining modern technologies with the gold standard. Here we present a novel, integrated, technological approach to provide physicians with a means to identify a possible bacterial infection. We propose an integrated system consisting of disposable cartridges that can perform typical clinical tests when inserted into a mini-microscope-user interface (Fig. 1).

APPROACH – The device works on a simple disposable cartridge-like device where 1 ml of patient sample is filtered/separated, then the bacteria are captured by molecular recognition elements (highly specific antibodies) thereby concentrating the bacteria onto a fixed focal plane, a Gram stain and wash is then applied and imaging (fixed magnification) is performed while the attending physician receives clinical test results such as Gram positive/negative, Oxidase positive/negative, Catalase positive/negative, size, shape, motility, etc. The next critical steps are: integrating the Gram staining steps into the cartridge demonstrating separation from blood sample, and integrating the optics into the system.

LIKELIHOOD OF SUCCESS – Our preliminary steps have rendered a Gram stain on an immobilized (captured) E. coli cell sample. We have developed a mass manufacture-able approach to fabricate the cartridges. We are integrating a USB plug and play CCD (fixed 500x) microscope onto a system to image our capture area of our device. Challenges that remain might include increasing capture efficiency, this can be solved by slowing down the flow rate or changing the channel geometries to optimize capture; current Gram stain has too many steps and reagents which we are replacing with a 1-step fluorescent Gram stain that can even distinguish live/dead cells; and typical systems integration which could require us to fabricate a plug and play device connected to a laptop for first system prototype.

1. Abstract

Diabetes is a chronic disease in which the body does not produce or properly use insulin, a hormone needed to convert sugar, starches and other food into energy for normal body functions. Diabetes currently affects 18.2 millions Americans and the number of diabetes patients is increasing at an annual rate of 5% since 1990. The cause of diabetes is not yet clear, although both genetics and external factors such as obesity and lack of exercise appear to play roles. Traditional diabetes diagnosis is based on the detection of blood glucose and blood glycosylated hemoglobin levels. However, these parameters do not reflect the use of active metabolic routes of diabetics, and even worse they do not reflect the exposure of diabetics to poisoning metabolic products that can cause diabetic coma (ketoadidosis). A recent breakthrough in diabetes research is the discovery of a diabetic metabolism indicator (biomarker) in exhaled breath and skin – acetone. This leads to the possibility of a non-invasive device that could dramatically improve diabetes diagnosis and patients’ quality of life (Fig. 1A):

- **Clinical studies, diagnosis, treatment and management of diabetes:** Combining the detection of exhaled acetone with existing glucose-related analytical tools and emerging therapeutic methods can deeply impact diabetes clinical studies, diagnosis, treatment, management and eventually, cure.

- **Screening of diabetes in newborn babies:** Since acetone is a volatile molecule that permeates through skin, the non-invasive diagnostic device can be used to screen diabetes in newborn babies, allowing earliest possible treatment and management of the babies.

- **Monitoring efficiency of fat burning in obesity:** Acetone is also a good indicator that measures the effectiveness of fat burning in carbohydrate-lacking diets for non-diabetic individuals.

The operation of the proposed device is similar to regular glucometers but much easier, less painful and with higher functionalities. A gaseous sample is provided to the device instead of blood. The device has four distinct functions (Fig.1B):

- **1-** It includes a sensor that monitors exhaled acetone (eAc) in real-time. The device can be use to measure acetone either in breath or skin (forearms of babies).

- **2-** It includes a personal database of measured eAc values to track the history and temporal eAc levels for each patient. The database will be used for efficient treatment and management of the patient.

- **3-** It comprises a wireless chip that transmits the eAc data to medical professionals instantaneously. This will allow the patient to receive immediate and appropriate treatment.

- **4-** It has a software algorithm that assists health care professionals to recommend proper dosage of medicine (e.g. insulin) or calories to be consumed based on the acetone levels.

All these features are achieved by integrating novel sensing principles, signal transduction/processing electronics, wireless communication, and functional interface software into a single unit. **The basic principle and functions of this novel device have been established at ASU.** The team will optimize the device and carry out clinical trials via collaboration with Mayo Clinic.
**The Anticipated Outcome:** This device is expected to become an integral part of future clinical studies, diagnosis, treatment and management of diabetes patients. It will also have a significant impact on obesity.

**2. Team and bio-sketches**

A team of chemists, physicists, engineers and clinicians at the Biodesign Institute, Arizona State University (ASU) and the Mayo Clinic, Scottsdale, AZ will carry out this project. The ASU team, Drs. Forzani, Tao and Tsow will lead the effort for the device development and construction. Dr. Forzani has 10 years of experience in chemical and biosensors. She has developed and validated devices for environmental and health care-related analytes. She is inventor of 3 IPs
related to this project. Dr. Tao, Professor of Electrical Engineering, Director of Center for Bioelectronics and Biosensors, Biodesign Institute, has worked for more than 15 years on chemical sensors and related projects with over 160 refereed publications and 10 patents in the area. Dr Forzani and Tao are inventors of an exhaled breath indicator sensor that uses similar principles to the proposed acetone sensor. Dr Tsow, is an expert in the design of miniaturized electronics for sensors. He has 5 years of experience integrating sensing devices, and working with Drs. Forzani and Tao. All of them will work together with Mayo Clinic – Arizona’s medical doctors working with Laurence J. Miller, M.D., Director of Research, in the development of the new eAc device. Mayo Clinic team will perform functional validation of the eAc device involving healthy, on-diet and diabetic individuals. The group will work together to improve device design and maximize functionality of the system.
Wearable wireless breath monitor for asthma diagnosis and management
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N.J. Tao, PhD, Arizona State University
Francis Tsow, PhD, Arizona State University
Richard Robbins, MD, Carl T Hayden VA Medical Center, Phoenix

1. Abstract
Asthma affects 20 millions Americans and is increasing at a rate of about 20% every year. It is responsible for 14.6% of adult missed workdays and contributes to approximately 5,000 deaths annually. The exact cause of asthma is still unclear, making it difficult to prevent. In addition, the disease is difficult to diagnose correctly and is still considered as incurable. Current detection technologies are bulky, expensive and difficult to operate/maintain. The doctors need a more effective diagnostic and treatment technology while the patients need a user-friendly management tool. A recent breakthrough in asthma research is the discovery and validation of an asthmatic condition indicator (biomarker) in exhaled breath - nitric oxide (eNO). This opens a door to new generation diagnostic device. Combining the detection of asthma biomarker with effective therapeutic methods will have a profound impact on asthma treatment and patient management. In addition, the diagnostic tool can further help medical professionals to study the cause of asthma and new drugs for asthma treatment and cure.

The operation of the proposed device is similar to the common inhaler asthma patients are familiar with. The device features four distinct integrated functions (Fig.1):

- First, it includes a miniaturized sensor that monitors exhaled nitric oxide (eNO). eNO has been approved by the FDA as an asthma biomarker. The ability to monitor it in real-time can assist doctors to diagnose asthma and to assist patients to manage their asthma treatment.
- Second, it includes a database to track the history and temporal eNO levels for each patient. This will allow medical professionals to provide personalized medication and management to different patients.
- Third, it can wirelessly transmit the eNO data to medical professionals instantaneously, assisting them to provide proper attention and advice to the needed patients.
- Finally, it includes interface software for health care professionals to input recommended medication dosage based on the eNO levels.

All these features are achieved by integrating novel sensing principles, signal transduction/processing electronics, wireless communication, and functional interface software into a single unit.

The science behind this novel device has been developed in our laboratory and preliminary studies have demonstrated outstanding capability to evaluate asthma as well inflammatory processes in respiratory tract. Further optimization can make it ready for rigorous clinical use.

The Anticipated Outcome: This device is expected to become an integral part of typical asthma treatment/management and on-the-spot diagnosis; being able to go from bench to bedside in a relatively short time. It will improve the efficiency and accuracy of asthma diagnosis, reduce the risk of asthma attack, and minimize medication overdoses by proper treatment. This new tool will also aid to accelerate discovery of new drugs for more efficient asthma management and, eventual asthma cure.
Fig. 1. Proposed eNO device and schematic illustration of the use. Top: The device is paired to the patient’s cell phone via wireless communication, which receives, processes, and communicates data to the patient and to the health care professional, who can follow up patient’s asthma status and provide feedback for disease control and treatment. Bottom: The device can also be used in doctors’ office or hospital to assist accurate diagnosis.

2. Team and bio-sketches
A team of chemists, physicists, engineers and clinicians at the Biodesign Institute, Arizona State University (ASU) and the Carl T Hayden VA Medical Center, Phoenix will carry out this project. The ASU team, Drs. Forzani, Tao and Tsow will lead the effort for the device development and construction. Dr. Forzani has 10 years of experience in chemical and biosensors. She has developed and validated devices for environmental and health care-related analytes. She is inventor of 3 IPs related to this project. Dr. Tao, Professor of Electrical Engineering, Director of Center for Bioelectronics and Biosensors, Biodesign Institute, has worked for more than 15 years on chemical sensors and related projects with over 160 refereed publications and 10 patents in the area. Dr Forzani and Tao are inventors of the eNO sensor. Dr. Robbins, MD, will help the ASU team to design the eNO device and the sample collector, and to perform clinical tests. Dr. Robbins has more than 15 years’ experience working with diagnostic tools and methods for asthma and pulmonary diseases as well as population studies and clinical trials. He has over 175 publications, including the first description of increased eNO in asthmatic patients,1 which currently has over 880 citations. He has also participated in the development of a desktop-type system to measure eNO by chemiluminescence, which has become a gold standard method. Drs. Forzani, Tao, Tsow and Robbins will work together in the development of the new eNO device. Dr. Robbins and his team will perform functional validation of the eNO device involving healthy and asthmatics individuals. The group will work together to improve device design and maximize functionality of the system.

The Carbon Nanotube Nanopore: A Needle in a Haystack Sensor

Coordinator:

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Our Platform

The ability to detect a single molecule is elusive, yet necessary. Single molecule detection has several critical applications that range from the fields of healthcare to basic research. For example, the detection of a single molecule that is correlated with septicemia would be critical to the early diagnosis and effective treatment of the condition. The technology can also be used to interrogate the genome of model organisms to better understand how single base changes in DNA and RNA relate to form and function. Dr. Lindsay’s research group has created a nanofluidic device in which two microfluidic reservoirs are connected by just one single walled carbon nanotube (SWCNT). This device serves as a sample preconcentrator and is designed to act as a single molecule channel and electrochemical detection device. Furthermore, it needs no special nanofabrication for manufacture of the device.

Our Approach

The SWCNT acts as a “pipe” that conducts single molecules into a detector that then measures the charge and conductance signature of that molecule. This creates a molecular fingerprint for every molecule tested. This information can eventually become a library used for single molecule detection. Nanofluidic devices based on single SWCNTs open up a whole new avenue for combining nanofluidic nanopore analysis with nanoelectrochemistry and nanoelectronics. SWCNTs are intrinsically sensitive to single molecule interactions, yet they can be grown and processed by conventional CVD and fabrication processes. They are one of the few nanoscale devices that require no special nanofabrication. Transport in SWCNTs has already been shown to demonstrate completely unexpected phenomena. Simulations suggest that the (several nA) signals are generated primarily by electroosmosis. At 1 M salt, there are about 100 ions in the tube (2nm diameter, 2 microns long) at any one time, so this level of current would require that the contents of the tube empty 6 x 107 times per second. This corresponds to a flow rate of 2 x 10-13 L/s. Thus, the detection of a single molecule would correspond to pM concentrations detected in 1s (this estimate is consistent with the observed sensitivity for DNA). The sensitivity would increase in direct proportion to the accumulation time. The ability to combine several detection modes in the same device (ion current detection, retention time on a recognition reagent, electrochemical signal, electronic signal) opens up new possibilities for multi-parameter measurement, and with it, increased selectivity.

It is not at all obvious that SWCNTs will transport water, let alone solutions containing ions and macromolecules. Yet a several-thousand-fold enhancement of the pressure-driven flow of water has been reported in membranes consisting of > 106 tubes (1) and DNA transport has been predicted with MD simulations (2). Stimulated by these observations, we have constructed devices in which just one SWCNT bridges a gap between two microfluidic reservoirs (see Figure 1). This allows electrical measurement of ionic flow through the interior of a SWCNT for the first time. Furthermore, because the SWCNTs are themselves conductive (or readily turned on to be so) this device permits direct measurement of electronic and electrochemical currents using the SWCNT as a nanoelectrode (Figure 1D) contacted via an electrode buried under the fluid barrier. We have created a nanopore that is, itself, a nanoelectrode. In addition, the micro-nano fluidic junction acts as a remarkable preconcentrator for molecules that accumulate at the entrance to the nanochannel (3).

With a series of careful control experiments that eliminate the possibility of leakage paths, we have demonstrated that ionic currents can be measured through the interior of the SWCNT itself. The ionic current is much larger than would be predicted on the basis of bulk ion mobility and the known dimensions of the SWCNT. Simulations by our collaborators at Oak Ridge National Laboratories suggest that charge accumulates in the SWCNT, leading to electroosmotic flow, greatly enhancing ionic current. Values of charge deduced by fitting the
tube conductance (Figure 2a) accurately predict the dependence of conductance on tube diameter (Figure 2b).

Placing small DNA oligomers in the input reservoir results in a remarkable pulse-like signal, only observed when the output reservoir is biased positive (Figure 3). Quantitative polymerase chain reaction (q-PCR) confirms translocation of DNA in tubes of diameter > 2nm and the number of molecules translocated correlates with the number of current spikes observed in the ionic current.

Finally, we have measured the electronic properties of one of the tubes directly, using a pair of Pd electrodes in contact with the SWCNT under the fluid barrier. The tube was a semiconductor, but it was turned on at all biases once the interior was wetted, returning to its original semiconducting state only after drying in a high vacuum. Thus the electronic properties of the tube itself are sensitive to the fluid contained within it.

We are investigating combined nanopore/nanoelectrodes by:

1. Improving the yield of devices to speed up the rate at which measurements can be made.

2. Further characterizing ionic current and the mechanism of enhanced ion flow. In particular, we will measure charging of the tube directly with a buried electrode connected to a sensitive electrometer.

3. Exploring transport in various solvents and in the presence of carrier reagents used to electrophorese uncharged targets like nitro-aromatic explosives.

4. Exploring “nanopore” types of current-blockade (or enhancement) signal in the ionic current using explosive molecules complexed with carriers, models for soluble chemical warfare agents and small peptides.

5. Exploring current blockade signals with recognition reagents tethered to the ends of the SWCNT (Figure 1D). For example, a single stranded DNA hybridizing to a characteristic part of the genome of a pathogen could be tethered to the end of the tube. Capture of a target molecule might be signaled by a very large current blockade.

6. Characterizing the SWCNT as a nanoelectrode using well-understood redox couples. These molecules will also allow us to characterize and quantify preconcentration at the micro-nano interface. We will subsequently explore nitroamines and nitro esters and peroxide explosives converted to hydrogen peroxide.

7. Characterizing changes in the electronic properties of the tube itself using a pair of buried electrodes to monitor electronic conductivity of the tube as target molecules are
transported through it.

The fabrication steps we currently use are shown in Figure 1A-C. The reservoirs on each side of the barrier are electrically contacted with Ag/AgCl reference electrodes. Proposed process improvements include the use of SiN device substrates and barriers for compatibility with a larger range of solvents and pH's. We will also explore other barrier materials (such as ZEP). We expect that yield will be improved using a protective SiO$_2$ layer over the SWCNTs that will be removed in a final step with a buffered oxide etch. Future devices will be fabricated with up to four electrodes in contact with the SWCNT under the barrier (for four-point probe measurements).

The potential for preconcentration of target molecules is illustrated by the fact that the spike rate for DNA was found to be independent of DNA concentration down to 1 pM (the lowest concentration tried so far). The preconcentration is evidenced by the fact that the wait time for the onset of the spike signal was inversely proportional to the concentration of DNA. At 1 pM DNA, only 8 molecules would occupy the input reservoir prior to electrophoretic accumulation and concentration.

Personalized Assessment of Risk of Tobacco-Related Diseases and Opportunities for Early Diagnosis and Treatment

Coordinators:

Drs. Claudia Henschke and David Yankelevitz

The Biodesign Institute, Arizona State University
PERSONALIZED ASSESSMENT OF RISK OF TOBACCO-RELATED DISEASES AND OPPORTUNITIES FOR EARLY DIAGNOSIS AND TREATMENT

Medical science is now in the earliest phase of developing new approaches to therapeutics based on personalized treatments as determined by molecular and genetic profiling of the individual patient. This dream has been made possible by the rapid advances in molecular biology and genomics. Parallel to those developments has been the tremendous advances in imaging technology, and these two approaches can now be used jointly to characterize an individual. The ability to link information provided by molecular profiles along with imaging and clinical profiles represents a major step forward. Key to development of these approaches is access to a large well characterized database that contains clinical information, high quality image data, and access to molecular markers.

Key Investigators: C. Henschke, Ph.D., M.D., Professor of Biodesign Institute, Arizona State University; David Yankelevitz, M.D., Professor of Biodesign Institute, Arizona State University; P. Boffetta, M.D. Professor and Deputy Director, Tisch Cancer Institute, Mount Sinai School of Medicine and Vice President of International Prevention Research Institute.

Mission: To develop an international consortium of institutions in countries at different stages of the epidemic of tobacco-related diseases to provide imaging and biospecimens for identification and validation of markers for risk assessment of the diseases, to personalize the diagnostic workup needed for these diseases and the subsequent treatment. The focus is on the three major killers – cardiovascular disease, emphysema and chronic airway diseases, and lung cancer – which account for vast majority of tobacco-related premature deaths in the United States.

Background: Our research to date has focused on the early detection and treatment of lung cancer (1-4). We have developed an international collaboration of 58 institutions in 9 countries and to date, have enrolled 50,000 participants at major cancer centers in Italy, Spain, Switzerland, Canada, Taiwan, Korea, China, Japan and the United States following a well-defined protocol (5, 6). This enrollment is currently being expanded to include the state of Arizona and the entire country of Luxembourg. Conferences and Steering Committee meetings have been held every 6 months since 1999 (7) where the protocol is reviewed and updated to incorporate technologic and knowledge advances (8-10). In addition, we have established and coordinated a large scale consortium of molecular and genetic epidemiology of lung cancer (ilcco.iarc.fr) (11).

For all participants in the early detection studies there is detailed background and clinical information, high quality low-dose CT scans of the chest and other imaging studies, psychologic/behavioral measures using Short Form 12 (SF-12), a standardized international instrument, and pathology specimens. A pathology protocol (12,13) specifies the pathology specimen preparation and ultimate review by a panel of expert pulmonary pathologists (14-16). The sites are all linked together by a web-based infrastructure and all of the data including the CT images is held in a central repository. This database represents the largest imaging database ever performed. The cost to date of developing the infrastructure and accumulating the database is estimated to be more than $130 million.
Using the database we have been able to perform personalized risk analysis. For example, based on age and smoking history we can predict a person’s risk of being diagnosed with lung cancer (17). We have also been able to demonstrate an important inter-relationship between lung cancer, emphysema and coronary artery disease as these can all be diagnosed on CT scans (18-20) and this idea supports the theory that there is a common pathway for these diverse illnesses, a leading candidate is inflammation. We are now exploring the use of the images to help modify the risk assessment (21). For example, if a person has had a CT scan and we know the person’s risk factors for lung cancer we can perform computer aided analysis of the images to assess the degree and distribution of emphysema to then update the person’s risk for developing cancer beyond what can be predicted by simply using clinical information such as age and smoking history. It is clear that people respond differently to tobacco smoke and the way in which they respond will influence their susceptibility for development of other diseases, and this information can be ascertained by analysis of the CT images. For this effort imaging software tools were developed which could be used to evaluate the entire database of images and thus update the risk profile for each participant. We have developed a variety of tools for these purposes, including quantitative assessment of nodule growth, the extent of emphysema, the degree of airway inflammation, and the extent of coronary artery calcification (22-33). The database itself continues to grow both in terms of the number of participants and their long-term outcome. As it is continually being updated, it reflects state-of-the-art imaging and diagnostic workup and provides individualized assessment.

The initial focus has been on lung cancer in high-risk individuals (smokers and former smokers), but the database also includes individuals who have occupational and other exposures, including second hand smoke exposure which leads to 25% of the lung cancers being identified in individuals who have never smoked (34). In addition, detailed clinical, genetic, and epidemiological characteristics are available for the patients (N>15,000) included in the ILCCO consortium, and high-quality tumour samples are available for a large proportion of them for further molecular characterization (11).

**Significance:** While our network has focused primarily on clinical and image based analyses, we will now develop the infrastructure to manage the collection of bio-specimens. In this way, each of our sites will collect biologic samples under a common protocol. This will provide a unique opportunity as currently there is no large database of this type that will allow for collection of the full range of information, molecular and image-based. Our collaborators at Biodesign have already developed a network for collection of bio-specimens throughout the state of Arizona, as well as the entire country of Luxembourg. It is now our plan to add our imaging infrastructure to these new sites and to leverage the existing bio-specimen collection system to 20 sites so as to have international participation in this effort.

The flexibility of the research paradigm allows for incorporation of projects to answer critical diagnostic and treatment questions in a multi-institutional, multi-disciplinary, and timely fashion. Thus, new diagnostic modalities (e.g., biomarkers obtained from biospecimens, new imaging tests) can be evaluated and treatment trials can be performed (e.g., non-randomized and
randomized trials of different smoking cessation approaches (35-37), treatment approaches (e.g., lobectomy vs. limited resection vs. non-surgical treatment) (38, 39).

**Goals:** We now have a worldwide data collection system with one of the world’s largest legacy databases (collection of data has been accruing for 15 years), we have the tools to interrogate the database, and we are focusing on three of the illnesses that account for the majority of deaths in the United States and abroad.

Currently, we do not have participating sites in India, Russia, and Africa, but we are actively selecting institutions in these countries which will be invited to join the project. This is important as it would allow for assessment of varying patterns of the global epidemic of lung cancer, emphysema, and cardiovascular diseases and allow for assessment of innovative approaches which might differ widely in the various settings. For example, some of the participating institutions are in countries actively seeking to decrease smoking exposure but the absolute number of cancer cases will continue to increase nevertheless as the population increases, while other countries are rapidly changing from traditional habits to Western ones and thus have an increasing epidemic until preventive measures are put in place. Combining the capability to compare imaging and non-imaging biomarkers will allow for deeper insight and understanding of the relevant factors in the development of these diseases and thus provide new insights into prevention and early detection of these diseases. The researchers have in depth experience in performing clinical studies in countries throughout the world. The study design that has been developed for this research presents a new paradigm for rapid assessment of the usefulness of various diagnostic methods, the resulting mortality reduction (40), and the legacy database provides an excellent source for case-control and other types of retrospective studies.

It has been a particular strength of the Biodesign Institute to look for opportunities as the cross section of different fields of research. In this endeavor, we are now leveraging the two most powerful areas of technologic development in medical research, molecular markers and imaging markers under the broad rubric of “biosignatures”.
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Applying a Novel Protein Microarray Technology to Develop New Diagnostics for Rheumatoid Arthritis and Multiple Sclerosis

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Autoantibodies as biomarkers in diseases

Many human diseases manifest circulating antibodies that bind to self proteins, called autoantibodies. These autoantibodies have long been recognized as potentially useful biomarkers for diagnosing diseases. This is most notable among a series of often debilitating and chronic autoimmune diseases that include rheumatoid arthritis, multiple sclerosis, lupus, inflammatory bowel disease, type I diabetes and many others, for which autoantibody measurements are used routinely as diagnostic tests. Autoantibodies are stable, highly specific, and easily accessible, especially during the early stage of disease when the inducing antigen is localized and minimal but the patient’s immune response affords a dramatic amplification of signal in the form of antibodies being released in the circulation that can be readily detected with well-validated secondary reagents.

Autoantibodies may also prove useful in the recognition of cancer, even at early stages. The autoantibody responses in cancer are not as pronounced as they are in autoimmune diseases, and their typical sensitivities are in the 10-20% range, but they tend to be quite specific. In recognition of this application to cancer, there has already been and continues to be a strong and well-funded effort in the cancer research community over the past decade to identify autoantibody immune signatures in cancer against tumor antigens as a potential means to make inroads in early cancer diagnosis. Two major National Cancer Institute efforts, the Early Detection Response Network (EDRN) and the Clinical Proteomic Technologies of Cancer (CPTAC) have major programs in this area and our laboratory is an ongoing participant in both of these efforts.

However, despite direct relevance, the application of state-of-the-art technologies to do autoantibody research in autoimmune disease has significantly lagged behind. This is particularly unfortunate because more than cancer, the autoantibodies in autoimmune diseases are not only potential diagnostics, but they also play pivotal roles in the actual cause of the disease. Therefore, identifying new autoantibodies for rheumatoid arthritis or multiple sclerosis, would not only aid us in making earlier and more accurate diagnosis, but also shed light on how the autoimmune process for these diseases causes so much tissue damage. Thus, there is an unmet need in the discovery of autoantibodies in the autoimmune diseases:

1. Autoimmune disease is common. There are approximately 2.5 million patients with rheumatoid arthritis in the USA today and 400,000 patients with multiple sclerosis;
2. Compared to cancer, there is relatively little funding applied to autoimmune antibody discovery;
3. Autoantibodies have stronger signals and greater prevalence than in other diseases, thus simplifying discovery;
4. In autoimmune disease, antibodies themselves play a role in the morbidity from the illness, thus discovery here might not only result in useful diagnostic tests, but also new therapeutic strategies;
5. The chronic nature of autoimmune disease means that it would particularly benefit from diagnostic tests that can be used over time to monitor the illness and response to therapy.

Autoantibody signatures in patients with autoimmune disease

Autoimmune disease comprises a wide variety of systemic or organ-specific inflammatory diseases, characterized by aberrant activation of immune cells to target self tissues. Autoimmunity arises from complex interactions of genetic factors and environmental
factors, and there is substantial heterogeneity in the clinical manifestations, disease courses and outcomes among patients(1). However, virtually all autoimmune diseases are associated with circulating autoantibodies, and autoimmunity is also the underlying cause of the disease in most of these diseases. Therefore, we expect identification of the targets of antibodies to benefit clinical care of patients with autoimmune diseases from several perspectives.

First of all, the identification of autoantibody signatures during early disease development will lead to early diagnosis and effective therapeutic intervention. For example, early and aggressive treatment of rheumatoid arthritis can prevent cartilage damage (2). Delay in diagnosis, and thus delayed treatment, results in increased tissue damage. Disease specific autoantibodies are found in serum samples many years before disease onset (3, 4). Identification of such autoantibody signatures might allow diagnosis of pre-symptomatic patients and administration of timely immunological intervention that will either avert disease from progressing to life-threatening condition or allow complete disease prevention (5, 6). Furthermore, autoantibody signatures may also help precise diagnosis by stratifying patients into groups of different diseases types/subtypes and/or different responsiveness to certain treatment so that each group of patients will receive the most appropriate treatment, that is to enable the application of personalized medicine. Last, autoantibodies may be directly responsible for many of the clinical manifestations of autoimmune disease, and even in those situations where they are not, disease specific autoantibody signatures will definitely help understand the etiology and pathogenesis of autoimmune disease and promote effective therapeutic strategies.

Experimental Design

Rationale

The currently known autoimmune antigen targets, such as Ro/SSA, La/SSB, Sm, RNP, Scl-70 and Jo-1, have limited specificity for a particular disease and do not allow for a precise diagnosis (7-9). The traditional hypothesis-driven one-protein-at-a-time approach has not been effective in identifying specific autoantigen targets, and the targets for most autoimmune diseases remain poorly understood despite decades of research (10). With the development of proteomics, the high-throughput study of all proteins simultaneously, recent efforts on the application of a new technology referred to as protein microarrays to autoimmune response profiling (11-14) have been successful in identifying signatures for different diseases and disease subtypes, although only a small antigen repertoire of “usual suspects” was interrogated in these studies. This initial success bodes well for the tremendous potential of unbiased large scale profiling studies of autoantigen targets in autoimmune diseases, where antibody reactivity against tens of thousands of proteins can be tested in parallel. Our innovative NAPPA platform is uniquely suited for high-throughput screening of autoantibodies in serum samples at the proteome level. NAPPA has been used successfully to profile disease humoral immune response in cancer and infectious diseases as detailed in Technology Background section. A similar strategy will be adapted to the autoimmune disease study.

Goal

Our overall goal is to identify autoantibody signatures in patients with various autoimmune diseases for early diagnosis and patient stratification.

Sample sources

Serum samples for this study will be provided by Dr. Gerald Nepom, director of Benaroya Research Institute at Virginia Mason (BRI) (Seattle, Washington). BRI is an international leader in immune system and autoimmune disease research. BRI is one of the few
research institutes in the world dedicated to finding causes and cures to eliminate autoimmune
diseases including Type 1 diabetes, arthritis, lupus, multiple sclerosis, scleroderma and many
others.

Our close collaboration with Dr. Nepom will ensure not only the availability of high quality
clinical samples, which is essential for the rigor of any biomarker discovery, but also the
identification of research projects that have direct clinical relevance and the translation of
research discoveries to patient care.

We will initially focus on rheumatoid arthritis and multiple sclerosis as our first test
studies. There is evidence for a role of autoantibodies in both diseases. Both are common
diseases in the USA and both have strong needs for better diagnostic tests. Multiple sclerosis,
in particular, is a very difficult disease to diagnose, often requiring repeated brain scans. The
availability of a blood test for this disorder would be of exceptional value.

Study plan

1. Identification of autoantibody signatures in symptomatic patients
   Serum samples from patients with different autoimmune diseases and healthy controls
   will be screened on our NAPPA platform with our collection of ~10,000 human genes to
   identify sets of autoantibodies that are specific to certain disease or disease subtype.

2. Identification of autoantibody signatures in pre-symptomatic patients
   Serum samples collected from patients diagnosed with autoimmune disease at various
time points prior to diagnosis will be screened on our NAPPA platform. This is to identify a
set of autoantibodies as signatures that can accurately predict the future occurrence of
disease.

3. Identification of autoantibody signatures in relation of treatment-responsiveness
   Serum samples collected from patients with autoimmune disease prior to
   immunotherapy will be screened on our NAPPA platform. By comparing the reactivity
   pattern between the patient group responsive to treatment and the non-responsive group,
   we will identify autoantibody signatures that can accurately predict patients’ responsiveness
to therapeutic intervention.

Technology background

Using protein microarrays to present candidate antigens

Protein microarrays offer the ideal platform to present antigens to identify new immune
responses (15, 16). Protein microarrays are microscopic tools in which thousands of different
proteins are displayed with high spatial density on a microscopic surface. In this context, small
sample volumes can be added to the slide in order to detect responses against any of the
proteins displayed there. In comparison to traditional enzyme linked immunosorbent assays
(ELISAs), protein microarrays are capable of presenting and assessing hundreds to thousands
of antigens simultaneously. The responses are rapidly identified because the address of each
protein is known in advance and there are no representation issues; all proteins, even rare
ones, are represented equally (usually in duplicate). The proteins are arrayed on a single
microscope slide requiring only a few microliters of serum per assay. Known antigens as well
as predicted antigens can be included to generate a comprehensive protein antigen array. An
additional advantage of this approach is that the data can be evaluated to look for both
informative individual antigens as well as for patterns of antigen responses with good predictive
value. Moreover, the coupling of clustering algorithms with the simultaneous comparison of
many antigen responses lends itself well to the determination of antigens with concordant and independent responses.

Despite their potential of immense power and the early demonstrations of feasibility, protein microarrays are not yet widely used. This is largely due to the labor and technical issues associated with producing, purifying, and assessing the quality control of proteins for spotting on the array. Traditional production of antigen microarrays, for example, requires that hundreds of different human proteins are purified for spotting on the array. The expression and purification of mammalian proteins is tedious, costly and often unsuccessful. In addition, quality issues, such as more than 1000 fold differences of protein-spotting concentration across the different proteins on the same array and batch-to-batch variations, are major concerns.

The difficulty in producing and purifying proteins for arrays has led to the alternative use of arrays of short peptides (5-20 amino acids) instead of full length proteins. These are less costly to produce and do not suffer as much from differences in amount from one peptide to another. However, the shapes of short peptides do not always reflect those of natural proteins, and studies to date have suggested that non-specific binding is a problem for peptides, making it difficult to detect reproducible signal from the background noise. Moreover, especially in the context of autoimmune disease where antibodies bind naturally to full length proteins, it would be better to look for signal of binding to full length proteins.

To address these concerns, we have developed a novel protein microarray technology, termed Nucleic Acid Protein Programmable Array (NAPPA, US Patent No. 6,800,453), which enables the study of full length proteins while circumventing many of the challenges in producing traditional protein microarrays. Moreover, we have already demonstrated the utility of this platform for the identification immune responses in infected patients and in observing autoimmune responses in cancer.

**Nucleic Acid Programmable Protein Array**

As reported in our initial paper in Science, we do not purify and print full length proteins, but instead the NAPPA approach entails printing the DNA that encodes the full length gene for the proteins on the array (17). This creates a stable array that can be stored dry for many months on the bench top. At the time of the experiment, when proteins are desired, a cell free extract is added to the microarray that then manufactures the proteins on the surface of the array. The resulting proteins have been engineered to contain a signal that will allow their immediate capture to the same spot where their gene was printed. This approach eliminates the need to express and purify proteins separately and produces fresh proteins “just-in-time” for the assay, abrogating concerns about protein stability during storage. Proteins produced in this manner are not only full length, but in all cases where we have tested, they fold naturally and retain their enzymatic activities.

Our laboratory already has ample gene content available for NAPPA to present 10,000 different human proteins, about half of the genes in the human. The NAPPA method has been demonstrated on more than 15,000 proteins including all of the proteins of cholera and tularemia. Our laboratory has the capacity to produce more than 10,000 arrays per year.

**Demonstrating robustness for clinical research**

In order to be useful in clinical research, it was essential to demonstrate that our tool is both robust and reproducible enough to allow the reliable comparisons between different samples from different patients and controls. To that end, our recent publication in Nature Methods (18) demonstrated that:
1. Sufficient protein levels are displayed,
2. The proteins are full length,
3. There is relatively minimal variation in the levels of displayed proteins,
4. There is minimal variation from array to array,
5. There is no significant bias against protein classes (e.g., all protein types are displayed),
6. There is minimal zone variation from one part of the array to the other,
7. There is minimal cross contamination from feature to feature, and
8. The proteins are functional.

Finally, we have established a protein array manufacturing pipeline that can produce enough arrays to do large scale studies on serum. We have demonstrated that our NAPPA arrays possess the properties needed to serve as the ideal platform for screening large patient populations for antibody biomarkers. To our knowledge, NAPPA is the first non-protein printing method to produce over a thousand unique proteins on a microarray surface.

**Strong protein signals and quantitative reproducibility**

Given that we will want to confirm statistically even potentially weak signals, large numbers of patient and control sera will be tested. Thus, we need reproducibility both within the array and from array to array. To assess protein yield and reproducibility, we printed a test array of 96 genes (Figure 1a), as well as non-expressing plasmid DNA as a negative control and a concentration series of purified recombinant protein. Using an antibody that binds to a tag on all of our proteins, we confirmed protein signal for 99% of the 96 printed genes (3 s.d. > non-expressing plasmid; Figure 1a, b). By comparing our signals to control spots with known amounts of protein, we demonstrated that the average protein yield was 9 fmol per feature, which is more than most protein printing methods. Most importantly for clinical research, protein display was uniform and reproducible between replicates within an array ($R^2 = 0.95$) and between duplicate arrays ($R^2 = 0.96$; Figure 1c). This shows that comparisons between arrays are likely to be legitimate.

![Figure 1. Test array. (a) A test array representing 96 unique genes was printed and stained with PicoGreen dye to assay DNA binding (left) or anti-GST for expressed proteins (right). (b) The amount of protein produced in each feature. (c) Average intra-array (left) and inter-array (middle and right) correlation of signal was >0.95.](image-url)
Consistent full-length protein display without class biases

In order to successfully screen for novel autoimmune antibodies, the ideal protein microarray will display full length protein for all protein types at consistent levels. As an artifact of the unpredictable purification process, most protein arrays manufactured by traditional methods of printing purified proteins often display proteins that span a 1000 fold range in amounts. This biases the assay towards the abundant proteins. To demonstrate a more even distribution of proteins using our method, we tested 1,000 full length human genes (19, 20). We confirmed high quality printing by probing the arrays with a picogreen (a DNA binding dye) and detected DNA signal for 99% of the samples (coefficient of variation, CV = 18%; Figure 2a,b). We measured protein using an antibody against a tag that is present on all proteins. This tag is placed intentionally at the end of the protein. This way, confirming presence of the tag proves that the protein is full length. Compared with traditional methods where the variation is often over a 1000 fold range, the protein signals for NAPPA were all within a ten-fold range, with 92% of the proteins landing within 2 fold of the average. This demonstrated that the arrays show consistent levels of proteins, regardless of protein type, even including the membrane proteins that are likely to be on the surfaces of most cells.

In our Nature Methods paper (18), we also demonstrated that there was minimal zone variation across the array and showed that there was minimal cross talk between features on the array.

Proteins are functional on the arrays

There are no simple tests to confirm protein folding, and function must be tested on a protein-by-protein basis. To test for true protein interactions, we printed an array expressing 647 unique genes in duplicate, including 449 genes that we had not previously tested (Figure 3). We demonstrated three different proteins appropriately bound to their known targets on the array, demonstrating that the proteins retained their functional folding. All of the interaction pairs we tested behaved as expected.

Figure 2. High-density array. (a) A high density array with DNA representing 1,000 unique human genes. (b) Plot of protein yield (Y-axis) v. DNA yield (X-axis). Most of the DNA (98%) and protein signals (92%) were within two fold of their respective means. (c) Success of protein signal was examined by protein class and size and shows little bias.
Autoantibody detection in cancer patients

In work that we published in a leading proteomics journal, the Journal of Proteome Research, we confirmed that known and novel autoimmune antibodies are specifically detected by microarray, by selecting over 1700 antigen clones from our DNASU repository to build a custom high density antigen array, which was printed onto a derivatized glass surface (21). The gene collection represented 1116 cancer related genes of which 539 were linked to breast cancer, as determined by our literature mining tool, MedGene (22). The success of protein expression and capture on the array was measured by probing the array with an antibody that recognizes the capture tag on every antigen, Figure 4A. We determined that 90% of the proteins had readily detected protein signal over background (10% above the average of non antigen containing spots).

To detect an immune response, we probed the high density cancer antigen arrays with serum from a healthy control and sera from patients with melanoma, ovarian cancer, and breast cancer. As a positive control, we again used the Epstein–Barr nuclear antigen (EBNA), a protein from the virus that causes mononucleosis to which >90% of the population has a response. In Figure 4B, we detected responses to the EBNA antigen from the control, breast, and ovarian cancer sera. The serum from the melanoma patient did not have detectable antibodies to EBNA, although a strong response to a well-documented melanoma antigen called ML-IAP (melanoma inhibitor of

Figure 3. Protein interactions on high density arrays. (a) An array containing DNA for 647 genes. (b) Detection of specific interactions between Jun / Fos and p53 / MDM2 on NAPPA arrays.

Figure 4. Development of custom tumor antigen high-density programmable protein microarrays for the detection of autoantibodies in patient sera. (A) An array with over 1700 candidate tumor antigens. (B) The arrays were probed with sera from a healthy individual, and patients with melanoma, breast, and ovarian cancer.
apoptosis protein) was detected, Figure 4B (ii) (23). For breast and ovarian cancer sera, we detected responses to the tumor antigen p53 as well as other potential cancer antigens, Figure 4B (iii,iv). Responses to EBNA1, p53, survivin, PCNA, and ML-IAP were confirmed by purification of his-tagged or GST-tagged recombinant proteins for standard ELISA as well as immunoblotting for p53.

**Employing our NAPPA Arrays in new clinical research**

**Detecting common immune responses in infection for use in diagnostics and vaccine development**

In the course of normal infections, patients produce a humoral antibody response to the micro-organism. This response is often exploited in the development of vaccines to prevent future infection. However some organisms have remained recalcitrant to the development of vaccines, in part because it has proved difficult to identify which bacterial or viral proteins induce a strong immune response. *Pseudomonas aeruginosa* is one such organism that is responsible for potentially life threatening infections in individuals with compromised defense mechanisms, nosocomial infections and those with cystic fibrosis. Although a number of immunogenic proteins are known, no effective vaccine has been approved yet. In a recent publication in Infections and Immunity (24), we exploited our comprehensive microarray tool to study the immune responses of patients infected with pseudomonas. We used our arrays to display ALL

**Serum Responses to Individuals Infected with Pseudomonas**

![Figure 5](image)

*Figure 5.* Serum responses to protein arrays displaying all outer membrane proteins of pseudomonas. Top. Serum responses are shown from selected patients with documented pseudomonal infections who either had cystic fibrosis or not (as indicated) as well as healthy controls. All proteins are present on the arrays in duplicate and all duplicates agreed. Bottom. Duplicate arrays from some individuals were tested on different days to demonstrate that responses were reproducible and consistent.
of the proteins that pseudomonas has on its cell surface and probed the arrays with serum from patients and controls to identify which proteins are specifically detected in infection. Because these were all membrane proteins, ours was simply the only tool capable of displaying these proteins for such an experiment. As shown in Figure 5, the same proteins were recognized in the sera from patient to patient and were not observed in the controls. From this study we reported 12 proteins that trigger an adaptive immune response in numerous cystic fibrosis and acutely-infected patients, providing valuable information about which bacterial proteins are actually recognized by the immune system during the natural course of infection. One of the advantages of this approach is that as soon as the responses are observed on the arrays, we can unequivocally identify which protein is the responsible antigen.

**Application of this Strategy for cancer biomarker detection**

In more recent work we have had a primary goal for the development of a biomarker panel for early breast cancer detection. This work represents one of the largest biomarker discovery efforts in our lab and demonstrates the stepwise approach we take to identifying and validating diagnostic markers. We obtained sex- and age-matched (± 2 yrs) serum sets of early-stage breast cancer patients that included 325 cases and 325 controls. All samples were obtained at the time of routine mammography, prior to the diagnosis of cancer. These samples were all collected under a standard sample collection protocol to control for variations in collection techniques. This is the most stringently collected case/control sera from screening mammography that we know of. They were randomly divided into a test set for the **preliminary screen** (50/50), the **training study** (100/100) and a **blinded validation study** (100/100) (Figure 7). Cases from Fox Chase were 49% stage I, 48% stage II, and 3% stage III. Cases were not selected for tumor subtype because Her2 status was not available on all samples, so that tumor subset analyses could not performed on these data. Of the samples in which Her2 was known, they were 73% ER or PR+, 33% Her2+, and 15% ER-/PR-/Her2-, reflecting the incidence of breast cancer in this screening population. To control for benign breast disease, we obtained an independent set of age-matched serum sets of early-stage invasive breast cancer patients and benign breast disease controls from Dr. Jeff Marks at Duke University (100 cases/100 controls), which were randomly divided into the **training study** (50/50) and the **validation study** (50/50).

i. **Antigen discovery data analysis**

We performed our screen in three discrete steps outlined in Figure 6. First, we did a preliminary screen of a test set of 50 cases/50 control sera on 7500 human antigens, with the goal to eliminate antigens that showed no differences between cases and controls. This analysis was performed by our biostatistician, Dr. Garrick Wallstrom, who is an associate professor of statistics. Based on this analysis, we eliminated 90% of the antigens as either having no signal in either cases or controls (most of them), or having similar signals in both.

For a training study, we then tested the top 750 candidate antigens with a fully independent set of age-matched sera consisting of 100 controls and 100 patient sera from Fox Chase and 50 controls with benign breast disease and 50 patient sera from Duke University. A careful normalization process was performed on the raw data to account for non specific patient differences. For each
antigen we used quantile regression to compare the normalized intensities of cases and controls, accounting for center and array batch differences. We tested the hypothesis that the 90th percentile of cases exceeds that of controls and computed a p-value.

Statistical Conclusion From these statistical training studies, we constructed a 32 antigen biomarker panel by selecting antigens with: a) p-values less than 0.075 and b) estimated 90th percentile of normalized intensities for cases greater than one. For each antigen in the panel we computed its sensitivity at 95% specificity.

ii. Blinded confirmation of 32-antigen biomarker panel

In doing such large scale studies, there is a significant danger of overfitting the data. That is, if enough variables are tested, there is a finite probability that one such test will appear to be significant even if it were reached on chance alone. By far, the best way to control this possibility is to repeat the study on samples that are blinded to the researcher and the statistician to see if the tests still predict disease. The blinded study must be done on different samples from the ones used in all previous studies. We then tested the antigen panel on blinded independent validation assays. The sera were fully independent and of identical composition as the training set sera (100 cases/controls from Fox Chase and 50 cases/controls from Duke University) and the arrays were identical. For each antigen, we used the threshold that yielded 95% specificity on the training set.

Blinded Validation Conclusion All antigens maintained high levels of specificities (>89%) in our blinded study. The overall sensitivities of each biomarker varied and were typically between 2-20%. This low sensitivity is characteristic for autoantibodies for cancer and is documented in numerous previous studies. This is one reason for electing to study autoimmune disease where responses are expected to be much higher.

iii. Breast Cancer Conclusion

In these experiments, which we are preparing for a manuscript, we have used our protein microarray technology to study over 600 sera to identify a panel of 32 antigens with high specificity and moderate sensitivity for breast cancer. These antigens have been tested in a blinded study of entirely independent samples and still retain their high specificity for cancer. Moreover, an examination of these genes suggests a strong correlation with both overexpression in cancer and involvement in pathways related to cancer.

References
Population-Based Proteomic Investigation of Type 2 Diabetes Mellitus: Biomarkers for Diagnosis and Monitoring

Coordinator:

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Background and Significance

Type 2 Diabetes Mellitus

It is estimated that diabetes afflicts nearly 24 million Americans, with nearly one third of these individuals unaware that they are affected by the disease. Of the 24 million individuals with diabetes, approximately 95% of these cases are Type 2 Diabetes Mellitus (T2DM), almost 23 million people. Complications of diabetes are conservatively estimated to be the sixth leading cause of death in the U.S., and are found to occur disproportionately in minority populations. The prevalence of diabetes increased by ~ 50 % over the decade from 1990 to 2000, is estimated to double in the next forty years, and by many accounts is considered a pandemic threat within the nation with regards to increased mortality, decreased quality of life and escalating costs in healthcare. In 2007, the estimated total national cost of diabetes was $174 billion, with a majority of that amount spent solely on medical expenditures [5]. In addition to being a significant contributing factor to atherosclerosis and other adverse cardiovascular disease (CVD) outcomes [6, 7], diabetes results in 12,000 – 24,000 new cases of blindness each year, is responsible for ~150,000 patients with end-stage kidney disease and accounts for 60% of non-traumatic lower limb amputations – 82,000 in 2002 [8].

Current Markers for T2DM

Currently, two “gold-standard” biomarkers are commonly used in the detection and monitoring of T2DM; blood glucose and glucose-modified hemoglobin (HbA1c). These two markers are essentially a direct (glucose) and indirect (HbA1c) monitor of elevated glucose in the blood stream. Glucose is an immediate measurement of elevated blood glucose, and is used in both assisting diagnosis and monitoring of treatments for T2DM. HbA1c is a measurement of longer-term exposure to elevated blood glucose – the time-scale is generally equated with the in vivo half-life of hemoglobin (60-90 days) – and is typically used in monitoring glycemic control related to the management of T2DM. Both analyses rely on the accurate measurement of relatively small quantitative changes in the target biomarker. During fasting blood sugar tests, a blood glucose level of < 100 mg/dL is considered normal, whereas levels greater than 126 mg/dL are consistent with T2DM [9]; an approximately 25% change in concentration. Similar increases are associated with oral glucose tolerance tests (OGTT), where < 140 mg/dL is considered normal and > 200 mg/dL is indicative of diabetes (an ~ 40% change) [9]. Instead of measuring an absolute concentration, glycated hemoglobin is measured relative to total hemoglobin. HbA1c values of < 6% are the target values for normal individuals or diabetics undergoing treatment, whereas values greater than 7% are indicative of poor management and may warrant change in treatment [9]. There are also intermediate ranges in these values (i.e., fasting glucose of 100 – 125 mg/dL; OGTT = 140 – 200 mg/dL; and HbA1c = 6 – 7 %) that are often used in classifying the early stages of T2DM – a high-risk, or “pre-diabetic” state.

Evidence for the Need of HbA1c Alternatives

Hemoglobin A1c from blood has been widely used as a test for assessing glycemic control and the risk of diabetic complications. It is a relative measurement of the fraction of hemoglobin that is glycated and acts as a physiologic integrator to assess longer-term glycemic control over the lifespan of the red blood cells (t1/2 ~90 days). However, this test is not sufficiently sensitive to detect the range of glucose values typically seen in pre-diabetes or new-onset type 2 diabetes. In the UKPDS study [10], HbA1c only showed a weak and statistically insignificant relation to macrovascular disease. The usefulness of HbA1c in assessing and managing T2D was questioned over 20 years ago [11] and remains a matter of doubt and debate. While observational studies suggest that for every 1% increase in HbA1c, there is an 18% increase in CVD risk [12]; recent large randomized trials (ACCORD, ADVANCE, and VADT) have failed to translate tight control of HbA1c into cardiovascular benefit [13-15]. One limitation of HbA1c is that its assessment requires accurate measurement over a relatively small quantitative range. For instance, HbA1c values of < 6% would be expected in normal individuals, whereas values greater than 7% would often indicate sub-optimal glycemic control such that changes in treatment would be considered. Thus, a difference in HbA1c of 1 % is considered clinically significant [9]. Moreover, evidence is accumulating that postprandial hyperglycemia contributes to endothelial damage via...
oxidative stress [16], and that large and repeated excursions in postprandial glycaemia may be important in the development and progression of atherosclerosis [17]. These transient glucose excursions are likely not well represented in an HbA1c measurement yet may represent an important independent risk factor and causative agent in T2DM related to CVD [18]. Collectively, these issues illustrate the limitations in using HbA1C (and probably any other single marker) in directing patient therapy, assessing patient risk or guiding clinical trials. Consequently, we maintain that diabetes-biomarker development would benefit from the accurate characterization and analysis of several changes in proteome composition considered together, rather than depending on finding a dramatic difference in a single marker.

**Diabetes Definition Revisited: Need for Biomarkers in the Setting of Prediabetes and Early Diabetes**

More than 5 million adults in the United States have undiagnosed T2DM—a major risk factor for cardiovascular diseases and stroke, and another 38 million with pre-diabetes are at increased risk for developing diabetes and cardiometabolic complications [19]. The lack of a simple and reliable test to predict diabetes and diabetic complications has hindered identification of these high-risk individuals as well as the prudent and effective targeting of therapies. Indeed, predicting the likelihood of diabetic cardiovascular complications poses a significant challenge. Current diabetes biomarkers include fasting glucose, 2-hr glucose tolerance test and glycated hemoglobin (HbA1c). Both WHO and the American Diabetes Association use a fasting plasma glucose (FPG) of 126 mg/dl or higher to define diabetes. While this definition conforms to the traditional “glucocentric” view of diabetes and diabetic complications, it is becoming increasingly evident that individual and integrated glucose measurements are only modest predictors of diabetes and diabetic complications [20, 21]. Numerous factors other than hyperglycemia, including oxidative stress, dyslipidemia, inflammation and altered coagulation likely play important roles in the development of diabetic complications [22]. The fact that these and other factors, not necessarily related to hyperglycemia, are not adequately captured with current biomarkers may explain their less than optimal predictive power. Alternatively, if unique protein modifications resulting from these additional factors and pathways are identified, efforts to assess clinical risk and predict poor outcomes will be greatly enhanced.

**Need for Novel Orthogonal Biomarkers for Prediction of Cardiovascular Disease**

Data from the Diabetes Prevention Program and other studies have found a higher prevalence of retinopathy in subjects with impaired glucose tolerance even when associated with low and normal FPG. However, the oral glucose tolerance test (OGTT) for diagnosis of diabetes and pre-diabetes is inconvenient, requires fasting, and is not sufficiently reproducible. Moreover, only one third of subjects with impaired glucose tolerance go on to develop T2DM [19]. Interestingly, while even modest IGT is predictive of cardiovascular risk [23], several recent large randomized clinical trials (ACCORD, ADVANCE, and VADT) aimed at tight glycemic control did not ameliorate cardiovascular and microvascular risk or mortality [13-15]. So while diabetes, which is defined by hyperglycemia, is a major risk factor for cardiovascular morbidity and mortality, lowering glucose alone does not necessarily improve clinical outcomes. Certainly, the belief that diabetes represents a well defined glycemic threshold that is predictive of clinical risk is losing favor. In the Whitehall study, coronary mortality was increased with post-load glucose levels well within the prediabetes range [23]. The diabetes threshold for fasting glucose of 126 mg/dl is also based on the underlying assumption that there is a clear glycemic threshold that separates people at high and low risk of diabetic microvascular complications. Recent data, however, fails to identify a clear glycemic threshold even for retinopathy that is consistent across populations [24].

Thus, there is a need for orthogonal (uncorrelated) biomarkers to accurately monitor across the continuum of healthy – to – T2DM – to – CVD. This need is highlighted in the NHLBI Clinical Proteomics Programs statement [25] and illustrated by recent findings from the Framingham Heart Study [1]. Wang et al evaluated 10 contemporary biomarkers of cardiovascular disease in over 3,000 study participants who were followed for development of cardiovascular disease for almost 10 years. Among the 10 biomarkers measured, many were significant predictors of cardiovascular events (B-type natriuretic peptide, urinary albumin excretion) and mortality (C-reactive protein, B-type natriuretic peptide, urinary
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Fig. 1. The lack of predictive power of current biomarkers in the T2DM/CVD continuum.

Building Multidimensional Views of T2DM and CVD

Both T2DM and CVD are systemic heterogeneous diseases, each comprised of numerous subtypes with their own idiosyncrasies in diagnosis, monitoring and treatment. Thus, multiple markers aligned with both the common and specific pathobiologies of metabolic syndrome [26], diabetes [3, 27] and CVD [22] are needed to evaluate an individual as he/she transitions through this continuum. From a biomarker development point-of-view, these observations point toward unifying a number of markers appropriately aligned with upstream causes, and downstream effects of T2DM and CVD (i.e., taking a multidimensional biomarker development approach that is aligned with the pathobiologies of the diseases), and using multi-marker data to implement and monitor treatment to negate major outcomes (death or disability). Notably, the effects of T2DM and CVD can be prevented (or at least delayed) through early detection, repeated and progressive monitoring and the administration of the most effective treatments. Non-drug treatment regimes for T2DM focus on lifestyle intervention in the form of diet modification, weight loss and exercise regiments [28-33]. Classical drug treatment of aggressive T2DM is through sulfonylureas or metformin [34, 35], as well as formulations of short- and long-acting forms of insulin [36, 37]. More recently, new drugs, as typified by dipeptidyl peptidase IV inhibitors (e.g., Januvia and Galvus) have shown great promise in controlling blood glucose levels [38]. Moreover, there are currently in excess of 350 drug candidates in development making diabetes second only to cancer in health-related R&D focus [39]. Included in the cardiovascular medication arsenal are; statins, ACE–inhibitors, anti-oxidants, anticoagulants and vasodilators. As with the T2DM medications, each is generally administered to a different sub-type of CVD and oftentimes used in combination with other medications.

Across the T2DM/CVD continuum, such multi-marker (-dimensional) panels reach their ultimate value in defining subtypes for treatment options, determining an individual’s responsiveness to such treatments and viewing the overall health status of the individual. For instance, at the early stages of T2DM (pre-T2DM stemming from e.g., impaired glucose tolerance (IGT) vs. fasting glucose tolerance (FGT)), a legitimate question is whether an individual will respond better to lifestyle changes (changes in diet and exercise) or to the administration of metformin. Today, there are no discrete markers used for such prediction. Once on medication (stark-T2DM), is there a need to adjust from metformin to insulin analogs or DPP-IV inhibitors? Ideally, matching such T2DM sub-types (e.g., overactive DPP-IV or hyperinsulinemia) with the different treatments is guided by “sub-type” markers; however, other than HbA1c (which is not used to make these distinctions), there are no markers that are routinely used in evaluating this transition. Finally, as T2DM breaches into CVD, the “glucose paradox” is encountered. As described by Libby and Plutzky [40] “A number of well-conducted clinical trials, such as the University Group Diabetes Program (UGDP) and the United Kingdom Prospective Diabetes Study (UKPDS), among others, have found only limited, if any, relationship between glycemic control and diabetic macrovascular manifestations”. As noted above, in the worst example of the “glucose paradox”, coronary mortality was increased with post-load glucose levels well within the prediabetes range (as monitored using HbA1c) [23]. We believe that the investigations proposed here stand to significantly impact these collective dilemma by providing the means to monitor the health status of individuals across T2DM/CVD such that their subtypes can be matched with the most appropriate treatments.
Ongoing Studies

The studies described here stem from ongoing research where we have followed these insights and used proteomics approaches to identify specific proteins and variants indicative of T2DM that cross over into CVD complications. In particular, we have used targeted approaches to monitor for qualitative/semi-quantitative differences in plasma proteins via population-based studies (see next sections). To date, we have identified several candidate markers, which when used together are able to differentiate healthy from T2DM cohorts with high accuracy, and additionally dissected the T2DM/CVD crossover into eight rudimentary subtypes (high-low values for glycation, oxidative stress and insulin signaling – see Preliminary Data). Here we propose to expand these studies along other axes through a multi-step systematic process designed to translate initial discoveries beyond basic research and closer to clinical use.

Targeted Proteomics Technologies

Over a decade ago, we introduced Mass Spectrometric Immunoassay (MSIA) as a technique for detecting and identifying peptides, proteins and their variants present in biological fluids [41]. The approach is based on the isolation of analytes from biological milieu using immobilized antibodies, followed by release of the analytes and analysis using matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS), and more recently using electrospray ionization mass spectrometry (ESI-MS). Our original papers describe methods for qualitative determination and identification of analytes, quantitative methodologies and multiplexed MSIA where multiple antibodies are used to target multiple analytes simultaneously [41]. To simplify the approach we introduced the use of solid supports built into pipette tips for protein isolation prior to mass spectrometry [42], and in 1996 founded a company to develop and manufacture the devices needed for high-throughput MSIA used in population screening and biomarker development [43]. When needed, these devices are able to address large volumes of biofluid and are readily used with 96-well (parallel) robotic workstations. Using a high throughput MSIA system (see Fig. 2) we can reach throughputs of ~500 analyses/day [44].

Population-Based Investigation of Disease – Biomarker Development Workflow

In past work, we have described a progressive biomarker development workflow capable of producing multiplexed assays for disease detection [45, 46, 49]. Fig. 3 shows a general scheme for this progression, where “discovered” biomarkers are progressively challenged (verified) before assembly into assay/data evaluation

![Fig. 2. HT-MSIA. A) Robotics platform showing extraction, rinsing and micro-elution stations. B) Proteins extracted from 96 samples eluted (in parallel) onto MALDI target. C) Arrayed samples drying in preparation for MALDI-TOFMS. Alternatively, captured proteins are eluted into titer plates for LC-ESI-MS analyses.](image-url)
combinations as a prelude to clinical challenge. In operation, we illustrated this process by developing a three-marker multiplexed assay (and accompanying methods of data analysis) capable of differentiating individuals suffering from myocardial infarction (MI) from healthy individuals. By following this workflow, we were able to produce biomarkers, targeted multiplexed assays and methods of data analysis possessing (putative) clinical specifications exceeding conventional MI-assays. In doing so, we believe that this study was among the first such proteomics-based investigations that iteratively proceeded from discovery – to – assay design – to – cohort challenge. Key aspects derived from the study were: 1) The verification of (presumably significant) discoveries, 2) The amalgamation of determinants and methods of analysis into unified assays, and, 3) The progressive challenge of findings on increasingly larger populations. In practice, when performing this study we demonstrated a step-wise workflow that translated from initial discovery and identification, to verification of findings on larger populations, to assay design/data analysis and challenge. The Preliminary Studies and Research Design and Methods given here follow this workflow with the intent of producing verified biomarkers, assays and methods of analysis that are suitable for use in monitoring T2DM and CVD comorbidities.

**Preliminary Studies**

**Population Proteomics and Type 2 Diabetes**

Preliminary investigations were performed using cohorts consisting of 50 healthy individuals (not known to have ailments), and 52 T2DM patients (comprised of 37 individuals diagnosed as T2DM and treated through diet, exercise and non-insulin drugs, and 15 insulin-dependent individuals who were diagnosed as T2DM and treated through administration of insulin). To date, we have produced 15 blood-borne markers (proteins & protein variants), each able to differentiate healthy from T2DM with moderate – to – high accuracy. Table 1 gives a summary of the markers found to date (reported in detail below). The markers are categorically indicative of: 1) Glycation, 2) Differential oxidative stress, and 3) Microheterogeneity in the insulin-signaling pathway. It is important to note that all of the markers are due to the relative modulation of PTM’s associated with physiological pathways known to be influential in the diagnosis or treatment of T2DM and CVD comorbidities (e.g., oxidative stress related to macrovascular complications).

<table>
<thead>
<tr>
<th>Protein</th>
<th>Category</th>
<th>Observation (Ave: healthy vs T2D)</th>
<th>ROC (Area under curve)</th>
<th>Comments (See below)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beta-2-microglobulin</td>
<td>Glycation</td>
<td>0.7 vs. 2.5%</td>
<td>0.84</td>
<td>1</td>
</tr>
<tr>
<td>Cystatin C</td>
<td>Glycation</td>
<td>1.0 vs. 3.8%</td>
<td>0.93</td>
<td>1</td>
</tr>
<tr>
<td>GcG</td>
<td>Glycation</td>
<td>0.9 vs. 4.8%</td>
<td>0.98</td>
<td>1, 2</td>
</tr>
<tr>
<td>Albumin</td>
<td>Glycation</td>
<td>13 vs 27%</td>
<td>0.93</td>
<td>1, 3</td>
</tr>
<tr>
<td>Hem A&amp;B</td>
<td>Glycation</td>
<td>3.1 vs. 6.3% (b-chain) 1.7 vs. 3.4% (a-chain) 8.2 vs. 13.6% (b-chain; + 120 Da)</td>
<td>0.88 – value using all Hemoglobin variants</td>
<td>1, 4</td>
</tr>
<tr>
<td>Albumin</td>
<td>Oxidation</td>
<td>40 vs. 25%</td>
<td>0.96</td>
<td>5</td>
</tr>
<tr>
<td>TTR</td>
<td>Oxidation</td>
<td>1.5 vs. 30%</td>
<td>0.99</td>
<td>6</td>
</tr>
<tr>
<td>Apo AI</td>
<td>Oxidation</td>
<td>30 vs. 55%</td>
<td>0.80</td>
<td>7</td>
</tr>
<tr>
<td>Apo CI</td>
<td>Oxidation</td>
<td>8.8 vs.28.9%</td>
<td>0.98</td>
<td>8</td>
</tr>
<tr>
<td>C-peptide</td>
<td>Enzymatic</td>
<td>4.8 vs. 9.0%</td>
<td>0.85</td>
<td>9</td>
</tr>
<tr>
<td>Insulin</td>
<td>Enzymatic</td>
<td>4.1 vs. 10.8</td>
<td>0.81</td>
<td>10</td>
</tr>
</tbody>
</table>

1) Used in combination with other glycation markers to produce a static glycation index, or when viewed versus the in vivo lifetime of the proteins, a dynamic glycation index indicative of changes in glycation over ~ 3-months.

2) The GcG assay also readily yields genotype data that is correlated with T2DM.

3) In addition to mono-glycated, di-glycated albumin is observed differentially in T2DM cohorts.

4) The hemoglobin MSIA detects HbA1c, as well as a second PTM of hemoglobin B-chain (at + 120 Da) and glycation of the A-chain (+ 162 Da).

5) Differential oxidation is monitored as depletion of the native form relative to all modified forms (e.g., cysteinylation at +119 Da). Differential glycation is also monitored (simultaneously) using this assay.

6) Differential oxidation is increased sulfonation (+80 Da) occurring at cys10.
Glycation Markers – GcG, b2m, cysC, Alb, Hem A&B

Gc-Globulin or GcG (also known as Vitamin D binding protein) is a plasma protein with a nominal molecular weight of ~ 51 kDa and an estimated concentration in plasma of 200-600 mg/L [81]. It is known to be present in human populations as three high-frequency allelic variants, Gc-1F, Gc-1S and Gc-2, as well as other low-frequency variants [81]. Major biological roles for GcG include vitamin D metabolite transport [82, 83], fatty acid transport [84, 85], actin sequestration [86] and macrophage activation [86, 87]. Modification of this protein may thus constitute a biological event of wide-sweeping consequence.

Fig. 4 is given to illustrate the extent of information resulting from the GcG assay. Shown are overlays of deconvoluted ESI mass spectra resulting from the analysis of GcG from three individuals. Signals are observed for three genomic variants that were commonly observed during the course of study. Indicated are Gc-1F (MW_{calc} = 51188.2), Gc-1S (MW_{calc} = 51202.2) and Gc-2 (MW_{calc} = 51215.3 Da). The determined masses (for all samples analyzed in this manner) were within 2 Da of the calculated values. The three other genotypes that were observed at high frequency during study were heterozygous combinations of these three genotypes, i.e., Gc-1F/1S, Gc-1F/2 and Gc-1S/2. On occasion, other genotypic variants were observed throughout the study (indicated by variant), however, at low frequency within the populations under investigation. Also indicated are posttranslational modifications, namely O-linked glycosylation [(NeuAc){(Gal)}_{1} (GalNAc)]_{1} trisaccharide]. Notably, the glycosylation signals were observed at consistent mass shifts relative (dm = +656 Da) to the Gc-1F and Gc-1S genotypes, but not the Gc-2 genotypes. This observation is consistent with the protein originating from the GcG-2 genotype lacking the preferred site of O-linked glycosylation (Thr{420} changed to Lys{420}). A full report of the structural characterization of GcG in populations is given in [88].
Additionally, Fig. 5 shows spectral overlays of GcG from two individuals (both of genotype Gc1F1/F1), healthy (green) and T2DM (red). Observed in the spectra originating from the individuals having T2DM are increased levels of signals at 162 Da greater mass than the native GcG. This shift in molecular weight corresponds to that expected to result from (non-enzymatic) addition of a 1-deoxyfructosyl adduct, which is consistent with elevated blood glucose levels associated with T2DM. Viewed as groups, the mean level of glycated GcG (integrated ion signal) in the T2DM cohorts is ~ 4-5-fold greater than that found in the healthy individuals (see Fig. 5 inset). In further population-based screening using MALDI-TOFMS MSIA, glycated variants of three other plasma proteins – beta2-microglobulin (b2m) (the light chain of the Class I major histocompatibility complex, normally present in plasma at ~ 1 mg/L), cystatin C (cysC) (a cysteine protease inhibitor, normally present in plasma at ~ 0.1 mg/L) and hemoglobin (both A and B chains) – were found at elevated levels in T2DM cohorts. Fig. 5 shows spectral overlays resulting from the additional three assays, with spectra obtained from healthy individuals given in green and T2DM given in red. Common to all spectra are signals due to wild-type proteins [b2m: m/z = 11,730 Da; four forms of cysC: N-terminal desSSP: m/z = 13,073 Da, N-term. desS; m/z = 13257 Da or 13273 Da, w/o or w/ hydroxyproline, respectively; native cysC: m/z = 13,344 Da; hydroxyproline cysC: m/z = 13360 Da; hemoglobin a-chain: m/z = 15,128 Da and hemoglobin b-chain: m/z = 15,869 Da] plus matrix adducts (sinapinic acid; at δm/z = 207 & 224 Da). Similar to the GcG analyses, increased levels of glycation – indicated by signals at 162 Da greater in molecular weight than the parent proteins – are observed in the spectra originating from the individuals having T2DM. Viewed as groups, the level (relative ion signals) of glycated variant in the T2DM cohorts was 2-5-fold greater than that found in the healthy individuals (Fig. 5 insets).

Oxidative Stress Markers – Alb, ApoAI, ApoC, TTR

In a manner similar to protein glycation, differential oxidation was observed in a number of proteins. Fig. 6 shows overlays of albumin (Alb), Apolipoprotein Al (ApoAI), Apolipoprotein CI (ApoC) and transthyretin (TTR) taken from healthy (green) and T2DM (red) individuals. Albumin and TTR exhibit differential oxidation at their free cysteines in the form of, respectively, cysteinylation (dm = 119 Da) and sulfonation (dm = 80 Da) – (also observed in the albumin spectra are signals due to differential glycation). Oxidation of the apolipoproteins occurred predominantly in the form of sulfoxide formation at the free methionines (three in ApoAI and one in ApoCI). Also observed in the ApoCI spectra is a signal due to the truncation of two n-terminal amino acids from the intact species. In addition to apolipoprotein modulation relevant to CVD [89], and the additional wealth of information stemming from the NIH Cardiovascular Proteomics Centers [90], a direct point of consistency is has been reported recently by Brock et al, who have shown that increased oxidation of specific Met residues in plasma ApoAI in Type I Diabetic patients (relative to that of healthy patients) is statistically significant [91].

On a technical note, it is easy to overlook differential oxidation during proteomics studies. However, as has been commented on by Paulovich et al [92], differential modulation of a number of general disease-related proteins (e.g., lipoproteins, inflammatory and acute-phase proteins) are largely unexplored and have yet to be studied systematically (using large-scale proteomics approaches) for overall usefulness in disease discrimination. As shown here, the investigation of microheterogeneity adds an

Fig. 6. Mass spectral overlays of (as indicated) Alb, ApoAI, ApoC and TTR taken from healthy (green) and T2DM patients (red). Elevated oxidation is indicated in T2DM relative to healthy individuals. Insets: Box plot distributions showing % oxidation (measured per protein as the integral of glycated ion signal normalized to the integral of all species) healthy (green; n = 50) and T2DM (red; n = 52).
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extra discriminating capability to proteins already implicated in T2DM and CVD susceptibility. We note, however, that care must be taken to align these findings with biological underpinnings. Thus, for all the data described above, the T2DM and corresponding age and gender matched healthy control samples were collected, on average, 2 years prior to analysis and stored frozen at -80 °C until analyzed as described. Cohorts were treated equivalently during the analysis process. Thus, it is reasonable to expect that both the native (i.e., reduced) and oxidized forms of the proteins under study remain in the respective form in which they were collected (either reduced or oxidized) for extended periods of time when stored frozen, and that, correspondingly, differences in the relative abundances of the oxidized protein forms are due to the disease state under study and not sample handling artifacts. We recently this expectation experimentally by showing that samples stored at -80 °C for four years do not display “artificial” oxidation of TTR, albumin, Apo A-I, or Apo C-I, and that prolonged exposure of plasma to air at room temperature (at least 48 hours) does not result in any significant changes in oxidation of the target proteins.

**Signaling – C-peptide and Insulin**

Both glycation and oxidative stress present themselves as positive mass shifts relative to the target proteins. We have also observed negative mass shifts – i.e., truncations – in certain proteins that correlate with T2DM. Briefly, (reflectron) MALDI-TOFMS MSIA assays for C-peptide (C-pep) and insulin (Ins) were developed for use in the studies described here. Upon initial screening in populations, truncated variants of C-pep, insulin and insulin analogs were identified and observed to correlate with the T2DM cohort. Fig. 7 shows negative-ion MSIA spectrum qualitatively representative of those obtained for the individuals investigated in this study. Observed in the spectra are intact C-peptide at monoisotopic m/z = 3017.50 Da, and signals two other signals registering at m/z = 2888.49 Da and 2817.45 Da. With 10 ppm mass accuracy, accompanied by partial sequencing using MALDI-TOF/TOFMS, these signals were identified as C-peptide, C-pep(2-31), and C-pep(3-31), respectively. These three signals were observed universally throughout both the healthy and T2D cohorts. A heterozygous point mutation C-pep Ala18Glu was observed once in the cohorts (healthy female). Spectral data from each individual were subjected to relative quantitative analysis by normalizing the ion signal of each qualitatively different species to the total signal from all species. The relative ion signal for each species was then evaluated with respect to the presence of T2DM by grouping data from individuals into their respective cohorts. C-pep(2-31) showed little difference between the healthy and T2DM cohorts. However, the relative contribution of C-pep(3-31) was found to be comparatively different between the two cohorts. Fig. 7 inset shows a histogram comparing the frequency of occurrence between the two cohorts for the relative ion signal of C-pep(3-31). A broad distribution averaging ~ 9.0% (average of all individuals in the cohort) was observed for the T2DM cohort, as compared to a narrow distribution averaging ~ 4.8% observed for the healthy cohort.

In a similar manner, insulin MSIA was performed on the cohorts. Shown in Fig. 8 are two exemplar spectra taken from a healthy (green) and insulin-dependent T2DM patient (red). Intact endogenous insulin is observed to register in both individuals at m/z_{true} = 5,808.4 Da. In addition, insulin homologs of Lantus (insulin glargine; mw = 6,063.7) and Novolog (insulin aspart; mw = 5831.6) are observed as discrete signals in the T2DM individual (in accordance with his medical records). In accordance with
known physiological processing Lantus is observed to degrade initially by the removal of two C-terminal arginine residues, and then a subsequent Thr residue (from the c-terminus of the b-chain) [93]. No noticeable degradation products were observed to align with Novolog sequence, however, an endogenous insulin variant was identified (throughout the cohorts) as a truncation of the b-chain C-terminal residue (Des(B30) HI). Similar to the C-pep(3-31), this truncated variant was present at higher relative contribution and frequency in the T2DM cohort (Fig. 8 inset).

**Data Evaluation**

**Single Assay: GcG Genotype and Glycation**

An advantage of performing the MS-based GcG assay is that both genotype and protein phenotype (glycation) data are obtained in a single analysis – each metric independently having value toward T2DM detection and monitoring. At the time of this writing, we are not aware of a *single-analysis assay* that is capable of producing equivalent data. This said, a pertinent question arises as to the value of using both the GcG genotyping and glycation in combination. Upon evaluating only the genotyping data from all cohorts (n = 102 individuals), the Gc-1S allele (genotypes Gc-1S/1S, Gc-1F/1S and Gc-1S/2) was found predominantly in the T2DM cohorts, and the allelic frequency observed to increase by ~500% in the T2DM cohorts relative to the healthy cohorts [Chi-squared test: (2 sample donor types x 3 major GcG alleles; α = 0.01; 2 degrees of freedom; χ² = 49.6, p < 0.0001; Cramer’s V = 0.474)]. Combining this knowledge of genotype stratification with the addition glycation data from the assay yields Fig. 9, where each point stems from a single analysis performed on a given individual [healthy (green) and T2DM (red)]. Defined on the X-axis are the six major genotypes of GcG. Given on the Y-axis is the relative abundance of the glycated GcG found in the individuals. Dashed lines highlighted by gray areas are given to mark reference levels that best separate healthy from T2DM as a function of glycated GcG, and ranges that may indicate individuals adequately managing T2DM (or pre-T2DM). *With the exception of a few outliers, there appears to be a genotype-dependent threshold above which glycated GcG levels are indicative of T2DM.*

The prospects of this sort of (single-analysis) genotype-protein phenotype assay are exciting. Prophetically, such an assay finds value by: 1) Indicating the likelihood of developing T2DM, 2) Detecting T2DM, and 3) Monitoring the progression (and/or effect of treatment) of T2DM on a personalized level. Essentially, the X-axis may be interpreted on its own as the predisposition for T2DM based on genotyping – i.e., the measurement of a genetic risk factor that an individual may develop T2DM within his/her lifetime, with Gc-1S genotypes being more disposed to T2DM. A genotype-dependent threshold for glycation (as an indicator of T2DM) yields a more personalized assay that is able to stratify an individual within the general population based on the initial risk factor as well as the presence of the pathophysiological marker of T2DM – i.e., *using the two values in combination to more accurately indicate when an individual has developed*...
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T2DM and how he/she is responding to treatment. Such stratification is often viewed as an essential component of personalized medicine. However, more studies are needed to substantiate the validity of using such a GcG genotype/phenotype assay for stratification of T2DM populations.

**Univariate Verification and Multidimensional Analysis**

Each glycation and oxidative stress marker was evaluated using receiver operating characteristic (ROC) curves, which reflect the ability of the marker to differentiate healthy from T2D across all possible assay cutoff values. Fig. 10 shows ROC curves for eight of the markers given in Table 1. As a point of consistency, the area under the curve (AUC) for glycated hemoglobin is 0.88, which is in good agreement with literature values of 0.90 reported for HbA1c [94]. AUC values for other markers range between 0.80 – 0.99, indicating each can be used as an independent marker to differentiate between healthy and T2DM. However, a generally accepted convention in “omics” analyses is to use multivariate data in building classification rules able to distinguish healthy from disease individuals. To this end, the data were subjected to principle components analysis (PCA) for the purpose of creating a soft independent modeling of class analogies (SIMCA) classification (using commercially available software: The Unscrambler; Camo Software, Inc., Woodbridge, NJ). Fig. 11 shows the results of plotting PC1 from glycation data versus PC1 from oxidation data. Viewed simply, the healthy individuals cluster in the low-glycation, low-oxidation quadrant – i.e., a quadrant of “healthy” glycation and oxidation, which serves as the point of reference for T2DM diagnosis, as well as is the target for treatment of T2DM once diagnosed. Most of the individuals in the T2DM cohorts fall into the high-glycation, high-oxidation quadrant. Interestingly, though, ~20% of the T2DM cohort falls in the low-glycation, high oxidation quadrant (see possible explanation below). Also, the high glycation/low oxidation quadrant does not escape our attention as a possible quadrant (or, with the addition or more axes, octant, etc.) of high-risk, “pre-T2DM” detection.

This form of orthogonal dimensionality – viewing two components contributing to T2DM and CVD comorbidities (i.e., glycation and oxidative stress) – opens up the possibility of creating additional axis in the T2DM/CVD continuum. Based on the data given above, a third axes regarding signaling involved in glucose homeostasis presents itself – potentially aligned with IR, IGT and hyperinsulinemia. When treated in the PCA manner described above, data from the signaling data shown in Figs. 7 & 8 yields an ROC AUC of > 0.80, indicating good dispersion between the healthy and T2DM cohorts. Incorporating these data with those shown in Fig. 11, produces Fig. 12, where we have created a three dimensional view describing eight octants related to the T2DM/CVD cross over. Notably, healthy individuals cluster in the low glycation/oxidative stress/signaling octant, and T2DM scatter away from the origin along the three dimensions. All T2DM individuals exhibit high oxidative stress, ~ 70% of the T2DM cohort exhibits high
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Nelson, Randall W.

glycation and ~ 50% of the individuals exhibit perturbations in the insulin signaling pathway. Viewing the individuals marked in Figs. 11 & 12, Individual's 1, 3 & 5 are observed at relatively low values along the signaling axis. Individual's 2 & 4 are observed to enter significantly into higher variation in the signaling peptides (with Individual 2 representing the worst of all three axes). Note that such difference between T2DM individuals are to be expected due to the heterogeneity of the disease, and/or the physiological position of an individual in the progression of T2DM/CVD. In repeated use, such multi-dimensional analysis will allow an individual to be tracked longitudinally for use in monitoring T2DM and CVD and aiding the administration of tailored interventions (e.g., diet/exercise, metformin, insulin cocktails, DPP-IV inhibitors, statins, anti-

oxidants, ACE inhibitors and other hypertension drugs).

**Longitudinal Glycation Monitoring**

It is important to note that the axes in Fig. 12 represent the compression of multiple data points into a single data value indicating the total contribution of either glycation, oxidative stress or signaling. As such, additional information can be extracted from each point through different treatment of the data. As an example, temporal fluctuations in glycation can be viewed by correlation with the *in vivo* lifetime of the proteins. Fig. 13 illustrates the possibility of building such a “half-life clock”. Shown are plots of relative glycation versus time prior to sampling. The *in vivo* lifetimes of the markers are ~ 0.5, 2, 85, 550 and 2000-hours for b2m, cysC, GcG, Alb and Hem (A&B), respectively. The colored dashed lines link the average values found for the glycated proteins during the analysis of the healthy (green) and T2DM (red) cohorts. Also given are data from five individuals indicated in Figs. 11 & 12. For Individual 5, all markers are lower than the average values of the regimented non-insulin based treatment. Individual 4 exhibits roughly the same profile, except with elevated glycation in the most recent past (and with reference to Fig. 11, also exhibits a relatively higher oxidative stress value). At the other extreme, Individual 1 is either not properly administering his treatment, or the treatment itself is not correct. Similarly, 1-2 months into the past, Individual 2 exhibits (extreme) elevated glycation, but within the past week has begun to reduce glycation to a comparatively lower level (and with reference to Fig. 12, lower oxidative stress is also observed). Individual 3, not previously diagnosed with T2DM, is observed to fluctuate in and out of the T2DM levels, illustrative of a borderline, or “pre-T2DM” state. Finally, it should be noted that Individual’s 3, 4 & 5 all exhibit roughly the same glycation index as measured using glycated hemoglobin, but follow noticeably different trajectories in the time leading up to blood draw.

These time-dependent markers allow an unusually detailed view of an individual’s glycation status based on...
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Alignment with the Pathobiology of Diabetes Complications

Foremost, excessive blood glucose levels – traditionally measured via glucose or HbA1c – are used almost exclusively in monitoring diabetics. Thus, in the simple context of elevated blood glucose, our additional glycated-protein markers make sense; albeit they provide a much more granular view (temporally) of an individual than the two traditional markers. However, we can additionally begin to align our findings with greater unified mechanism of the pathobiology of diabetes complications [3, 27]. A component of this unified mechanism indicates a propensity for formation of intracellular and plasma-borne advance glycation endproducts (AGE), either in precursor of mature forms. Relative to our studies, the precursor forms are viewed in the data given above as either the x-axis of Fig. 12, or Fig. 13. Downstream effects of the AGE products range from accumulation in arterial and microvascular plaques (e.g., b2m and cysC) to inter-cellular signaling linked to the expression of cytokines, growth factors and general inflammatory proteins. Study of the glycated-proteins – to – AGE transition opens up additional avenues for investigating diabetes/CVD complications, as well as illustrates putative downstream markers for further investigation (e.g., structural/quantitative modulation of cytokines, growth factors and general inflammatory proteins). Regarding oxidative stress, hyperglycemia-induced production of superoxide begins to explain our results [3, 27]. Oxidized lipids (from superoxide) are known to oxidize apolipoproteins, generally by direct oxidation at methionine groups (i.e., addition of O) [95]. Presently, the best explanation for differential cysteinylation and sulfonation is global change in the redox potential of blood due to superoxide production, although other mechanisms are being investigated.

Interestingly, the glycation-oxidative stress axis lies at the heart of other studies investigating the relationship between glucose variability and oxidative stress [96]. These studies indicate a correlation between acute glucose excursions and oxidative stress products, in particular during the postprandial period (as indicated in Fig. 13). Thus, these studies suggest that two individuals can experience significantly different levels of oxidative stress (due to e.g., glucose spikes versus constant levels), while exhibiting the same level of glycation – as measured using a long-term integrator such as HbA1c. Thus, a possible explanation for the sub-group shown in Fig. 12 – i.e., the 20% of T2D in the low glucose, high oxidative stress quadrant – is sustained level of oxidative stress induced by relatively low average-level, but spurious hyperglycemia. Because of the clear connection between oxidative stress and cardiovascular complications [6], the glycation/oxidation tandem assay, as well as the temporal monitoring of glycation shown in Fig. 13, are lead candidates for studying T2D/CVD comorbidities and assessing CVD risk reduction through appropriate treatment.

The signaling axis potentially links into IR, IGT, decreased beta-cell activity or hyperinsulinemia, and on the therapeutic side, guides the administration of insulin or non-insulin based drugs1. Regarding C-peptide, only one other variant – in the form of C-peptide(1-26) – had been reported in the literature [97, 98]. To our knowledge, N-terminally truncated forms of C-peptide have yet to be reported or studied. Several insulin variants have been previously characterized with the aid of mass spectrometry as far back as the late 1980’s [99]. Most pertinent to our findings, other investigators have also begun characterizing insulin microheterogeneity in human body fluids and observed elevated levels of Des30 HI

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1 Note that insulin and c-peptide (without microheterogeneity) are used as an initial indication of insulin resistance and hyperinsulinemia – see e.g., http://www.jewishhospitalcincinnati.com/cholesterol/Research/insulin_resistance.html.
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in urine obtained from diabetic and insulin-treated individuals [100]. The biological underpinnings of both C-peptide and insulin degradation remain unclear; however, proteolytic processing via exopeptidases may be partially responsible. For example, structural and biophysical characterization of dipeptidyl peptidase IV (DPP-IV; EC 3.4.14.5) [101], a multifunctional type II transmembrane serine peptidase, reveals a preferred enzyme specificity of Xaa-Pro or Xaa-Ala from the amino termini of peptide hormones. Observation of C-peptide(3-31) is thus consistent with DPP-IV cleavage at the N-terminal GluAla- of C-peptide, and a possible explanation for the elevated levels observed in the diabetic population may be abnormal DPP-IV activity in the diabetic population. Moreover, DPP-IV is a critical enzyme in regulating physiological glucose homeostasis via N-terminal cleavage of peptide hormones such as glucagon-like peptide-1 (GLP-1) and glucose-dependent insulino tropic polypeptide (GIP) [102], and as such, control of DPP-IV activity via drug formulations is a major focus of new T2DM drug development [103-105]. Consequently, as we have recently demonstrated during the study of BNP [77], the rigorous investigation of signaling peptides susceptible to enzymatic degradation opens up additional avenues for accurately defining monitoring signatures to investigate molecular responses relative to drug treatment.

Summary

Presently, we have studied the 10 proteins listed in Table 1, which, due to the microheterogeneity of the proteins results in multiple different forms of the proteins, several of which can be used in the study of T2DM and CVD comorbidities. In evaluating the data and aligning with the pathobiologies of metabolic syndrome, diabetes and CVD, we are beginning to understand biomarker development in a multidimensional framework. With reference to Fig. 14, the three initial “dimensions” of investigation – glycation, oxidative stress and signaling (in the insulin pathway) – have matured to panels of multiple markers that can be used in different modes of evaluation. In particular, when used orthogonally the data produce eight octants of investigation, i.e., low & high values for the three metrics. These octants can be used immediately to monitor multi-modal contributions to diabetes and comorbidities – e.g., glycation & oxidative stress used in recognizing the transition from stark T2DM – to – CVD (used in accessing CVD risk), or, all three used in optimizing T2DM & CVD therapeutic interventions. In future research, additional panels will be developed that tune into other factors involved in the T2DM/CVD crossover. In a progressive manner, assays and determinants from these discovery efforts will be applied in our collaborative studies of T2DM and CVD cohorts, with the overall intent of describing (through multiple markers) the transition from healthy – to – T2DM – to – CVD outcome.

Fig. 14. Multidimensional framework for biomarker development in diabetes and comorbidities. Panels of assays are developed for axes addressing different aspects of the pathobiology of diabetes and comorbidities, see ref. [3]. Panels for glycation and oxidative stress have matured, a nascent signaling axis is under construction and others will be added during the course of these investigations.
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Population-Based Proteomic Investigation of Type 2 Diabetes Mellitus

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Single Cell Analyses: From Physiology to Cancer Diagnostics

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**Live Single Cell Analysis**

We design and develop cutting edge technologies for multi-parameter analysis of single cells, and apply these technologies to the understanding of cellular heterogeneity and its role in disease pathways. Our goal is to elucidate pathways to disease states directly at the individual cell level, such that genotype, and phenotype, encompassing both the transcriptome and proteome, can be related to cell fate. Specifically, we are targeting two model systems: We are using the premalignant condition Barrett’s esophagus (BE) as a model system for investigating heterogeneity at the level of the single cell during neoplastic progression. We are using macrophages as a primary cell target to investigate the ability of proinflammatory stimulation to trigger cell death via either the non-inflammatory apoptosis or the proinflammatory pyroptosis pathways.

While the field of single cell analysis has evolved from an emergent concept to a maturing practice with many entrants, we have been developing systems and methods that address key measures of metabolic activity that cannot be addressed in current high-throughput microplate formats. Multiparameter analysis of cellular response using spectrally resolved fluorescence techniques allows for broad diffusional equilibration of the macro environment surrounding a field of cells. This is generally applicable to a wide array of questions in many biological fields. However, these approaches are critically limited in ways specifically linked to the lack of diffusional control in the microscale environment surrounding target cells of interest.

The MLSC has developed a microsystem-based platform with core capabilities for measuring gene expression and physiological parameters including oxygen metabolism and cytokine production rates in individual cells. Multiple individual cells can be observed in parallel, with each cell being spatially and diffusional isolated. Nanoscale electronic and photonic detection methods have been developed and are being integrated into our multi-well analysis cassettes for high throughput experiments. The systems have been used for repeatable and reproducible observations of oxygen draw down rates for single cells. We have performed single cell protein signature measurements, and RT-LATE-PCR measurements of mRNA levels in single cells and small groups of normal and neoplastic cells. Automation and integration of the modules is in progress.

**3D Cancer Cell Imaging to Correlate Nuclear Shape and Chromatin Texture with Neoplastic Progression Stage**

In this research we are investigating how cancer progression stage affects the shape of the nuclear envelope and the distribution and texture of chromatin within cancer cell nuclei. Early evidence suggests that the causative relationship may be the other way around: That the arrangement of DNA in the nucleus may be implicated in aberrant gene expression patterns, for example, by silencing of tumor suppressor genes.

We have acquired and are further developing a new technology for 3D cancer cell imaging; either cultured cells or cells from patient biopsies. The instrument, called the cell CT, is shown below in the left
image. It is especially useful for elucidating subtle changes in nuclear architecture, including the shape of the nuclear membrane and the arrangement of heterochromatin within the nucleus. It is thought that abnormal nuclear membrane shape features, including deviation from spherical shape, infoldings, invaginations and protrusions, and abnormal distribution of chromatin within the nucleus, including coarse, clumpy chromatin and multiple nucleoli, are among the hallmarks of cancer. Cell CT is an ideal modality for further investigation of this important hypothesis.

We will image cells across the neoplastic progression spectrum—from normal (shown in panel a below), to metaplasia to mild, moderate and severe dysplasia (shown in panels b, c and d below), to invasive adenocarcinoma—to produce 3D images optimized to quantify nuclear morphometry, including shape and texture. We will segment features which capture shape and texture parameters from the 3D data sets, and derive a feature vector sensitive to cancer progression stage. We will determine which features are most powerful in predicting cancer progression stage.

It is now clear that cancer is much more than a disease merely of the genome, and that “following the genes” alone will not provide adequate understanding to defeat cancer. This research summarized above may have important implications for cancer prevention, diagnosis and therapy, since it will add to the information we have about the involvement of epigenetic factors in cancer initiation and progression.
Evolutionary Medicine of Personal Mutations to Predictive Disease Propensity and Drug Response

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Summary
As the cost of DNA sequencing continues to drop, we are now moving beyond one genome per species to one genome per individual. Already, complete genomes of many individual humans have been sequenced, and projects are underway to expand that number to over a thousand, or even 100,000. In response to this rapid technological growth, the National Institutes of Health, particularly the National Cancer Institute, is predicting that personal genomes will soon become a regular component of our health records. The profiling of many individuals’ personal genomes has now established that such individual health records will contain over a million mutations per person! Thousands of these mutations are found in proteins, hundreds of which appear in genes that are already known to be strongly associated with heritable diseases. These mutations are now providing an opportunity to create a bar-code description of the health status of each individual in the population. Predicting the potential functional consequences of each mutation and their use in predicting response to a given drug regimen presents a grand challenge in biomedicine.

The Evolutionary Biomedicine Program in the Center for Evolutionary Functional Genomics @ the Biodesign Institute is taking a transdisciplinary approach that integrates experimental and bioinformatics platforms with a goal to discern disease propensities of novel mutations. We apply the principles of Darwinian evolution in our experimental research in the laboratory (molecular evolution in the test tube) and integrate them with novel in silico prediction methods that capitalize on the outcomes of Nature’s laboratory in order to: [a] identify parts of the human genome that encode proteins using classical and non-classical translation mechanisms, and [b] determine the likelihood that mutations will affect protein function. Our evolutionary medicine research efforts have already yielded many exciting results, including the discovery of signature sequence motifs in the human genome that have an ability to initiate non-classical translation (Translation Enhancer Elements, TEEs). Genomic locations of TEEs are revealing novel proteins that are part of a shadow proteome encoded by the human genome. With this discovery, we have begun to explore the functional consequences of mutations found in previously unknown proteins and their functional role in the genome.

On a global scale, we are conducting evolutionary bioinformatics analysis on mutations protein-wide in the context of the Long-Term (among-species) Evolutionary (LTE) profiles of positions harboring these mutations. The LTE profiles hold the promise of forecasting which in silico predictions are most likely to be correct, and capable of disrupting normal cellular function. Such in silico predictions have become the mainstay of genomics and biomedical research, because it is not yet possible to experimentally evaluate even a small fraction of the known mutations, but also those that continue to be discovered by individual genome sequencing projects. LTE profiles are revealed from multi-species sequence comparisons, and we have already demonstrated that LTE profiles will enable scientists to optimize the accuracy of mutation predictions. Our current efforts are focused on the large-scale analysis of Mendelian, complex, and somatic cell mutations and their role in human cancer.

In summary, our Evolutionary Medicine efforts, coupled with ASU’s broader efforts in Personalized Medicine, tackle the next-generation challenges of Biomedicine. To this end, we are leveraging our existing in vitro and in silico capabilities as we strive to discover functional parts of the human genome, along with the role that individual mutations play in human health and disease. This research emphasis combined with the technology development of discovery tools for the analysis of human genome and pathogens places ASU in the ranks of leaders in innovative genome research.
Rapid Single Molecule Reads of Genomes

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The Vision

DNA sequencing needs to become cheap, fast and easy in order to enable sequencing of genomes from just a few cells in individual tissues, and do so on a large population of subjects. Long reads (hundreds of kilobases) are needed to unravel long-range genomic complexity (insertions, deletions, inversions and repeats). Modified bases need to be identified so as to read epigenetic markings (5-methyl C) and oxidative damage (oxy-G). In this way, we will understand not only the genomic rearrangements brought about by meiotic recombination, but also the changes (epigenetic and oxidative damage) that accompany cell differentiation, specialization and the development of pathologies over time.

Achieving these goals requires (1) Single molecule sequencing so that modified bases are not lost to PCR amplification; (2) A method free of costly labels and sample preparation; (3) A method capable of long contiguous reads so that long-range structure is not lost in assembly algorithms. Nanopore sequencing might be capable of delivering the required performance. A nanopore is an orifice so small that when a DNA molecule is driven through it, it is forced to translocate one base at a time. If a suitable sequence read out can be found, an entire genome could be read from one molecule. This project is developing both a suitable readout and a nanopore that can incorporate it.

Our Platform

We have developed two technologies to achieve fast reads of sequence from a nanopore: (a) Single molecule reads that identify DNA bases by means of an electron tunneling current. (b) Carbon nanotube fluidic devices that serve both as electrodes (for tunneling readouts) and as nanopores (that automatically aligned with an electrode).

(a) Tunneling Readout. The operation of our electron tunneling readout is illustrated in Figure 1. Two electrodes, functionalized with a reagent containing both hydrogen bond donors and hydrogen bond acceptors, are held at a controlled separation. The electrodes are close enough to sense electron tunneling currents, but separated enough so that the reagents on the two electrode surfaces do not interact. When DNA bases are introduced into the system, they bridge the gap by means of specific hydrogen bonding arrangements. The binding of a base by the functional groups on the electrode surfaces is accompanied by a pulse of tunnel current. The distributions of tunnel current pulse heights obtained from the binding of deoxy-Cytidine (C) and Thymidine (T) in the functionalized tunnel gap are shown by the red bars in Figure 2. Clearly, C and T can be distinguished in a single molecule read by this method. In contrast, if the electrodes are not functionalized with the hydrogen bonding reagent, then a very broad distribution of tunnel current spikes occurs (green bars in Figure 2) as every possible orientation of the molecule contributes to the
readout. Thus we have the basis of a rapid single base readout. Preliminary data indicates that the reader can obtain a distinct signal from all four bases and, in addition, 5-methyl-Cytidine.

(b) A manufacturable device

While it appears that a tunneling readout will work, the final device must be capable mass-production, like a computer chip, if costs are to be reduced significantly. To this end, we have recently demonstrated that DNA can be translocated through single-walled carbon nanotubes (SWCNTs)\(^4\) nanostructures that are readily grown on a silicon wafer by chemical vapor deposition (CVD). We have fabricated devices in which one SWCNT spans a barrier between two fluid reservoirs, growing the SWCNTs on silicon wafers by CVD and forming the fluid reservoirs with lithography. The SWCNTs are opened in the exposed reservoirs using an oxygen plasma etch. DNA is passed through the SWCNT by flowing an ionic current from one reservoir to the other. Translocation of DNA from the negatively biased reservoir to the positively-biased reservoir is marked by large pulses of ionic current. These are large enough to serve as signals that could be used to control the speed of DNA translocation.

The Future

We will improve the readout chemistry to achieve reads at a high level of confidence from all four bases and from modified bases (particularly 5-methyl C) and develop specifications for the required tunnel gap. We will use these specifications to build devices in which tunnel gaps are fabricated into individual SWCNTs, eventually opening the way for mass-production of computer-chip like devices capable of rapid single molecule reads of long fragments of genomes. When combined with the ability to read epigenetic markings, we expect that such devices will alter the way we measure, and subsequently treat, human diseases.

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**Figure 2:** Distribution of tunnel current spike amplitudes in a functionalized tunnel junction (red) and with bare electrodes (green) for C and T bases.


Using the Intestinal Microbial Community to Diagnose and Manage Human Disease

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Using the Intestinal Microbial Community to Diagnose and Manage Human Diseases

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Background

One of the most diverse ecosystems is the microbial community that inhabits the human intestine. Because this microbial community has an intimate relationship with its human host, recent findings that the makeup of the intestinal community can affect human health come as no surprise to those who have thought about the issue. For example, our team's recent findings, published in the Proceedings of the National Academy of Sciences (Zhang et al., 2009), showed that obese individuals uniquely harbor fermenting bacteria and methanogens in their intestines, and these microorganisms form a syntrophy that generates acetic acid, which is rapidly taken up through the intestinal wall and turned into fat. Obesity is epidemic in the United States: More than half of the U.S. population is overweight, and nearly 1 in 3 adults are clinically obese. Obesity and its associated co-morbidities threaten to overwhelm an already overburdened healthcare system. The relationship to diabetes is well known, but a recent study by the American Institute for Cancer Research links obesity to seven forms of cancer.

The intestinal community of microorganisms seems to be related to several other widespread diseases. Crohn’s Disease, Irritable Bowel Disease, and Autism Spectrum Disorders (ASD) also appear to be connected. For example, gastrointestinal problems are common with ASD and significantly contribute to ASD behavioral symptoms. Attention has been given to presence and abundance of Clostridium groups in the intestines of autistic children, and levels of Clostridium histolyticum have been detected to be higher in ASD children compared to typical children.

Objectives and Outcomes

The over-arching objective of the proposed research is to define the unique characteristics of microbial communities that occur in human subjects who display certain widespread diseases. This research begins by utilizing high-throughput and quantitative tools to identify microorganisms that are strongly associated with the diseases. The results are then interpreted using the concepts and tools microbial ecology so that we understand the metabolic functions of the important microbial strains, how they interact with each other, and how they interact with the human host, particularly in regard to the human diseases.

The characteristics and ecological framework will then lead to two profound outcomes that can improve the efficacy of the health-care system and, ultimately, human health. The first outcome is a diagnostic tool to help identify people with certain diseases based on the composition of their intestinal microbial community. Much like sampling the individuals DNA or proteins, sampling the microbial
community offers a means of personalized diagnosis. The second outcome is to develop non-invasive, microorganism-oriented therapies to eliminate undesired microorganisms associated with diseases. Because of our ecological approach, we ought to be able to define ways to manage the microbial community of the intestine by selecting for desired strains and against undesired ones. The ecological-management strategies could involve diet, pre-biotics (e.g., fiber), pro-biotics (i.e., microorganisms), specific antibiotics, and other approaches that may appear as we understand the human-community interactions better.

**Technical Approach**

The research is based on collaboration between our team in the Center for Environmental Biotechnology and leading medical researchers. The Center’s team brings to bear world-leading expertise in all of the key factors needed to understand and manage microbial communities (e.g., Rittmann et al., 2008): genomics, proteomics, and mathematical modeling. We already have a fruitful collaboration with gastroenterology researchers at the Mayo Clinic in Scottsdale (Dr. John DiBaise’s team) and are prepared to collaborate with others.

We believe that the most efficient approach is to study several diseases that have apparent microbial connections in parallel and using similar tools. The research strategy for any of them involves these steps:

1. Analyze the luminal and mucosal gut microbiota in humans showing the disease of interest, as well as in non-diseased controls.
2. Determine metabolic functions of the microorganisms associated uniquely with the specific disease.
3. Formulate plausible cause-and-effect relationships among the metabolism of the key microorganisms, their syntrophies, and the disease symptoms.
4. Develop a mathematical model to describe growth and metabolism of the key microbial types in the gut, with special emphasis on syntrophic relationships and factors that can cause disease symptoms.
5. Determine gene transcripts and proteins/peptides associated with the intestinal microbiome in humans showing the disease of interest.
6. Devise microorganism-oriented methods to diagnose disease onset or proclivity.
7. Use ecology and modeling information to create a rational design of a diet, pre-biotic, pro-biotic, or other means to manage the microbial community.

**References**


Multifunctional Radical Quenchers for the Treatment of Friedreich’s Ataxia and Other Mitochondrial Respiratory Chain Disorders

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Executive Summary

This project is focused on the development of drugs to treat Friedreich’s ataxia. The strategy is based on the belief that the absence of frataxin in Friedreich’s Ataxia patients leads to progressive mitochondrial dysfunction and associated oxidative stress, resulting in increasing degradation of the mitochondrion, especially the mitochondrial membranes, and in diminished ATP production. The objectives of this project involve the synthesis and evaluation of potential drugs by implementation of a strategy for which proof-of-principle has been obtained. The drugs are designed to blunt mitochondrial degradation resulting from oxidative stress, and to augment ATP production in partially dysfunctional mitochondria.

The specific aims of this proposal include

- the synthesis of coenzyme Q analogues (MRQs; Multifunctional Radical Quenchers) that are capable of carrying out at least three functions catalytically in dysfunctional mitochondria, namely
  - oxidizing superoxide to oxygen
  - quenching carbon-centered lipid radicals
  - augmenting/restoring ATP production in (partially) dysfunctional mitochondrial electron transport chains
- demonstration that the compounds work as envisioned in carrying out each of the above functions
- optimizing the structures for each of the above functions
- working with collaborators to evaluate the MRQs
- final structure optimization to enable clinical development including
  - side chain optimization to enable exogenous delivery as a therapeutic agent
  - side chain optimization to maximize function within the electron transport chain
  - optimization of structure to achieve low toxicity and suitable pharmacokinetic parameters
  - optimization of structure to assure drugability and acceptable cost of therapy

Realization of these specific aims will enable the design and clinical development of coenzyme Q analogues capable of (1) protecting the mitochondria and other organelles in Friedreich’s patients from self-inflicted damage resulting from mitochondrial dysfunction, and (2) augmenting ATP production.
Multifunctional Radical Quenchers for the Treatment of Friedreich’s Ataxia and Other Mitochondrial Respiratory Chain Disorders

Project Objectives

The objectives for this project are based on the belief that the absence of frataxin in Friedreich’s Ataxia patients leads to progressive mitochondrial dysfunction and concomitant oxidative stress, resulting in increasing degradation of the mitochondrion, especially the mitochondrial (and cell) membranes, and probably in diminished ATP production.

The objectives of this project involve the implementation of a strategy, for which proof-of-principle has already been obtained, to develop therapeutic agents that can be used to blunt mitochondrial degradation resulting from oxidative stress, and to augment ATP production in partially dysfunctional mitochondria. These agents should be broadly applicable to the treatment of diseases involving mitochondrial dysfunction within the electron transport chain. The specific compounds to be prepared are structures analogues of coenzyme Q\textsubscript{10}, which are specifically enabled to mediate one-electron trafficking through the mitochondrial electron transport chain.

Background/Preliminary Data

Friedreich's ataxia is a severe neurodegenerative and cardiodegenerative condition. It is characterized by progressive ataxia of the limbs, muscle weakness, dysarthria, skeletal deformities and cardiomyopathy. In common with other mitochondrial diseases, there are no effective treatments today for Friedreich’s ataxia.\textsuperscript{1} While the biochemical basis of the disease is still under investigation, it is strongly associated with insufficient frataxin.\textsuperscript{2} In the majority of patients the insufficiency of frataxin is a consequence of an intronic GAA triplet repeat expansion in the gene for frataxin, which results in a significant decrease in its mRNA levels, and ultimately in protein levels as well.\textsuperscript{3,4} Frataxin acts as an iron chaperone during heme biosynthesis\textsuperscript{5} and has been shown to be capable of stimulating the \textit{in vitro} assembly of heme and Fe-S clusters.\textsuperscript{6-8} Frataxin can interact physically with mitochondrial electron transport chain proteins, as well as with mitochondrial aconitase (which contains an Fe-S cluster).\textsuperscript{9,10} Therefore, frataxin deficiency results in disruption of cellular iron homeostasis, with a progressive iron accumulation in the mitochondrion, and a deficiency in heme and Fe-S clusters.

It is believed that a deficiency in frataxin leads to compromised mitochondrial respiratory chain function through a failure to assemble one or more Fe-utilizing proteins; one or more Fe-S clusters in the mitochondrial respiratory complexes are likely to represent a critical locus. In fact, diminished function of these complexes has been noted in Friedreich’s ataxia patients.\textsuperscript{11} The loss of mitochondrial respiratory chain function can lead to diminished ATP production, while the accumulation of Fe in the mitochondria makes the organelle highly susceptible to oxidative damage by reactive oxygen species, whose concentration increases concomitant with the decrease in respiratory chain function. There is compelling evidence from multiple types of studies\textsuperscript{12} that while oxidative damage is not the primary lesion in Friedreich’s ataxia, oxidative stress helps to drive disease progression. Therefore, strategies to overcome oxidative stress should blunt disease progression and provide effective therapy. This strategy parallels the way in which most marketed therapeutic agents have been designed and developed for the treatment of chronic diseases of complex etiology.

Mitochondria are organelles found within eukaryotic cells that are responsible for a number of metabolic transformations and regulatory functions. They produce much of the ATP employed by eukaryotic cells. There are numerous serious metabolic disorders that involve mitochondrial dysfunction, and a number of these involve the mitochondrial respiratory chain. The goal of this
project is to understand function and dysfunction within the mitochondrial respiratory apparatus from a chemical perspective, and to develop and implement strategies to blunt the deleterious effects of mitochondrial dysfunction, and compensate for diminished ATP production. In particular, we propose to develop chemical tools to enable single electron trafficking within the mitochondrial electron transport chain, which we believe can be used to restore electron transport and ATP production in (partially) dysfunctional mitochondria while preventing the progression of organelle degradation.

Although the focus of this proposal is Friedreich’s ataxia, it may be noted that there are a number of mitochondrial diseases resulting from both nuclear and mitochondrial genetic defects, and that the underlying biochemistries of these diseases tend to be rather similar. They include increased lactate production, diminished respiration and ATP production, and reflect the consequences of oxidative stress. Therefore, it is believed that the studies proposed here may well find utility for the treatment of mitochondrial disorders in addition to Friedreich’s ataxia.

The compounds proposed for development here may be regarded as special quinone analogues of coenzyme Q and tocopherol. In fact, it is known that a neurodegenerative disease involving mutations in \( \alpha \)-tocopherol transfer protein associated with vitamin E deficiency produces a clinical condition similar to Friedreich’s ataxia.\(^{13}\) This argues strongly for the identification of quinone analogues that function more efficiently within the mitochondrial respiratory chain to treat Friedreich’s ataxia patients.

**The Mitochondrial Electron Transport Chain**

The mitochondria are organelles found within eukaryotic cells that are responsible for a number of metabolic transformations and regulatory functions. They produce much of the ATP employed by eukaryotic cells. Energy released from the citric acid (Krebs) cycle in the mitochondrial matrix enters the mitochondrial electron transport chain as NADH (complex I) and FADH\(_2\) (complex II). These are the first two of five protein complexes involved in ATP production, all of which are located in the inner mitochondrial membrane. Electrons derived from NADH (by oxidation with a NADH-specific dehydrogenase) and FADH\(_2\) (by oxidation with succinate dehydrogenase) travel down the respiratory chain, releasing their energy in discrete steps by driving the active transport of protons from the mitochondrial matrix to the intermembrane space (i.e., through the inner mitochondrial membrane). The electron carriers in the respiratory chain include flavins, protein-bound iron-sulfur centers, quinones, cytochromes and copper. There are two molecules that transfer electrons between complexes: coenzyme Q (complex I → III, and complex II → III) and cytochrome c (complex III → IV). The final electron acceptor in the respiratory chain is O\(_2\), which is converted to H\(_2\)O in complex IV.

In a functional mitochondrion, transport of two electrons through complex I results in the transport of 4 H\(^+\) into the intermembrane space. Two more H\(^+\) transfers to the intermembrane space result from electron transport through complex III, and four more H\(^+\) transfers from electron transport through complex IV. The 10 electrons transported to the intermembrane space create a proton electrochemical gradient; they can return to the mitochondrial matrix via complex V (ATP synthase), with the concomitant conversion of ADP to ATP. It is interesting that no H\(^+\) is transferred to the intermembrane space as a consequence of electron transport through complex II. Therefore, 2e\(^-\) transfer from FADH\(_2\) (complex II → complex III → complex IV) results in the transport of only 6 protons, compared with 10 protons resulting from 2e\(^-\) transfer from NADH (complex I → complex III → complex IV), with correspondingly less ATP produced. Each glucose molecule metabolized by glycolysis produces 12 electrons; these are converted to 5 NADH molecules and 1 FADH\(_2\) via the Krebs cycle in the mitochondrial matrix. The 5 NADH molecules employed in mitochondrial electron transport produce about 25 ATPs, while the single FADH\(_2\) affords only about 3 ATP molecules. (There are another 4 molecules of ATP derived from glucose metabolism – 2 during glycolysis and 2
in the Krebs cycle). While this analysis underscores the importance of complex I involvement in normal ATP production, it also tends to obscure certain metabolic realities/uncertainties that may offer important opportunities for therapeutic intervention. One metabolic reality is that complex I, while important quantitatively for ATP production in normal mitochondria, is not essential for all mitochondrial ATP production. Electrons can enter the electron transport chain at the level of coenzyme Q (either from complex II or from fatty acid oxidation), producing about 60% as much ATP as would have resulted had they entered the electron transport chain at complex I. While the flux of electrons that normally enter the individual mitochondrial complexes, ultimately passing through coenzyme Q, is probably dictated largely by the availability of electrons derived from NADH, FADH$_2$ and fatty acid oxidation, the actual intrinsic capacity of the individual pathways does not appear to have been studied carefully.

While the normal electron carriers involved in the respiratory chain are coenzyme Q and cytochrome c, there is good evidence that other electron carriers are not only present, but also essential for mitochondrial function. One particularly intriguing example is the vitamin E metabolite $\alpha$-tocopherol quinone, the semiquinone radical of which has been suggested to be required as a cofactor for mitochondrial fatty acid desaturases in the carnitine-dependent mitochondrial desaturation-elongation pathway. Pathophysiological states produced by impaired $\alpha$-tocopherol-dependent desaturase function include ataxia, myopathy, retinopathy and sterility. Interestingly, while vitamin E is nature’s most efficient protector of the cell membrane against lipid peroxidation, the oxidized form ($\alpha$-tocopherol quinone) blocked glutathione-dependent protection of lipid membranes, and thus has the potential to act as a pro-oxidant. In fact, $\alpha$-tocopherol quinone is being developed by Penwest as a treatment for diseases of the mitochondrial respiratory chain. The metabolic cycle for vitamin E as an anti-oxidant and pro-oxidant is shown in Fig. 1.

Figure 1. Two-electron oxidation of $\alpha$-tocopherol produces $\alpha$-tocopherol quinone, which can be reduced to $\alpha$-tocopherol hydroquinone. It has been shown that $\alpha$-tocopherol quinone is converted to $\alpha$-tocopherol in situ, possibly via $\alpha$-tocopherol hydroquinone.

One implication of $\alpha$-tocopherol function within mitochondria as an enzyme cofactor is that other coenzyme Q-like species may be able to do so as well, i.e. that appropriate coenzyme Q or tocopherol analogues might be a useful vehicle for therapeutic intervention. It may be noted that $\alpha$-tocopherol itself may not be optimal for this task as $\alpha$-tocopherol quinone has been noted to be an inhibitor of ubiquinone function within complex III.

Mitochondrial Electron Transport Chain Function and Dysfunction: A Chemical View

In functional mitochondria, a few experimental parameters can be measured readily, reflecting mitochondrial respiration. These include NADH and O$_2$ consumption, and ATP production. Less readily measured are the electrons that flow through the electron transport chain, thereby consuming oxygen, and producing H$_2$O and ATP. The electrons within the mitochondria can really only be measured when they are associated with one of the mitochondrial electron carriers such as coenzyme Q. In humans, this mitochondrial coenzyme is present as coenzyme Q$_{10}$, which has a 50-
carbon C-substituent that renders the molecule virtually insoluble in water (calculated octanol-water partition coefficient >10^{20}). Nonetheless, as discussed below, the use of coenzyme Q analogues has permitted some insight into the nature of mitochondrial electron transport.

In dysfunctional mitochondria, one can still carry out the same types of measurements as noted above for functioning mitochondria. If the flow of electrons through complex I is interrupted several measured parameters should change. These include diminished consumption of NADH (measured as increased lactate through pyruvate reduction) and diminished ATP production. Since electrons will not flow as efficiently from complex I to coenzyme Q, the concentration of this reduced coenzyme will diminish. Interestingly, a new pathway for oxygen consumption is created. While oxygen is not converted as efficiently to water in complex IV (an overall four electron reduction of each oxygen molecule), much of the flow of electrons into a defective complex I is redirected to oxygen, with the production of superoxide (a one electron reduction of each oxygen). Thus the stoichiometry of oxygen utilization is altered. The production of superoxide by mitochondria actually occurs to some extent even in normal mitochondria, but is a much more frequent event in mitochondria containing defects in the respiratory chain. Superoxide is one form of reactive oxygen species (ROS). Superoxide itself is not believed to react readily with biological molecules such lipid membranes, proteins and DNA, and actually functions as a signaling molecule for the regulation of certain cellular processes. Biologically, the main fate of superoxide (O_{2}^{-}) is a disproportionation reaction with itself to produce peroxide (H_{2}O_{2}) and oxygen, i.e.

\[ 2 \text{O}_2^- + 2\text{H}^+ \rightarrow \text{H}_2\text{O}_2 + \text{O}_2 \]

This reaction occurs spontaneously, and can also be catalyzed by superoxide dismutase. Superoxide can also be reduced to peroxide in a monovalent process. Like superoxide, hydrogen peroxide is also not intrinsically deleterious to cellular macromolecules, and is actually essential to the function of a number of enzymes. However, in the presence of metal ions such as iron and copper, hydrogen peroxide is converted to hydroxyl radical (HO•) and hydroxide ion (OH\text{^-}) according to the Fenton reaction, i.e.

\[ \text{HOOH} + \text{Fe}^{2+} \rightarrow \text{Fe}^{3+} + \text{HO}• + \text{OH}^- \]

Hydroxyl radicals are very highly reactive, capable of reacting with virtually any biological molecule, including DNA, proteins and lipids. Hydroxyl radicals can also diffuse through cells readily, and their ability to damage cells is limited only by the distance that they travel before they react. Hydroxyl radicals can also react with superoxide, producing singlet oxygen (\(^{1}\text{O}_2) + \text{OH}^{-}\), another highly reactive form of ROS that damages cellular macromolecules and assemblies. One particularly deleterious and well studied reaction mediated by hydroxyl radicals is the abstraction of hydrogen atoms (H•) from membrane lipids, forming a carbon-centered radical (R•). This radical

\[ \text{HO}\cdot + \text{RH (lipid)} \rightarrow \text{R}\cdot + \text{H}_2\text{O} \]

\[ \text{R}\cdot + \text{O}_2 \rightarrow \text{ROO}\cdot \]

\[ \text{ROO}\cdot + \text{RH} \rightarrow \text{ROOH} + \text{R}\cdot \]

can readily react with oxygen, forming a hydroperoxy radical (ROO•). The hydroperoxy radical is also highly reactive, and can abstract another hydrogen atom from the membrane lipid, producing another carbon-centered radical (which can undergo precisely the same chemistry), ultimately producing a chain reaction affording many oxidative lesions in the membrane lipids from a single hydroxyl radical (lipid peroxidation). It is for this reason that lipid peroxidation likely represents a major process by which cellular and mitochondrial membranes are degraded in cells containing (partially) dysfunctional mitochondria. The observed accumulation of lipofuscin in Friedreich’s ataxia patients is fully consistent with the thesis that lipid peroxidation is a central process that drives disease progression.\(^{22}\)
It may be noted that while all lesions in the mitochondrial electron transport chain that affect mitochondrial dysfunction will result in elevated levels of superoxide, some types of lesions may be expected to produce more functional damage. The latter would certainly include Friedreich’s ataxia, in which suboptimal levels of the protein frataxin (which is responsible for cellular iron homeostasis\textsuperscript{23}) results in an accumulation of Fe\textsuperscript{2+/3+} within the mitochondria, and contributes instead to the Fenton chemistry noted above. Likewise, disorders such as amyotrophic lateral sclerosis are associated with a deficiency in the detoxifying enzyme superoxide dismutase, and will have greatly enhanced concentrations of the ROS discussed above.

One poorly studied parameter of mitochondrial electron transport is whether the process is best characterized as involving one or two electron transfers. The is important because NADH is an obligatory two-electron donor, and coenzyme Q and cytochrome c participate in two-electron redox cycles, as does FADH\textsubscript{2}. Virtually all publications represent the processes in which these species participate as involving a net two electron change. However, FADH\textsubscript{2} may (and generally does) transfer its reducing equivalents as single electrons. Further, the Q cycle in complex III clearly involves single-electron transfers and, as noted above, the semiquinone radical of \(\alpha\)-tocopherol quinone (i.e., the form that is the one-electron intermediate between \(\alpha\)-tocopherol quinone and \(\alpha\)-tocopherol hydroquinone, \textit{vide infra}) has been posited to function within the mitochondrion and likely interacts with the electron transport chain to attain that oxidation state. Reduced cytochrome c is known to transfer electrons one at a time to cytochrome c oxidase, the enzyme responsible for the final step in respiration. Finally, the accumulation of electrons within dysfunctional mitochondria (producing reductive stress) is relieved substantially by (one-electron) reduction of oxygen to superoxide (\textit{vide supra}). Thus, while the electron transport chain has the \textit{capacity} to transfer two electrons by virtue of the redox cycles of most of its participants, it is not clear that it necessarily must do so to function. From a medicinal chemistry perspective, the (one- or two-electron) nature of mitochondrial electron transport is critical, as it defines the nature of the coenzyme Q surrogates which may be of utility for therapeutic intervention. Two obvious chemical strategies that could ameliorate the cellular damage noted above include removal of “excess” electrons from dysfunctional mitochondria before they react with oxygen (or at least before the formed superoxide is converted to deleterious ROS), and the use of a radical scavenger to quench the chain reaction in lipid peroxidation noted above.

\textit{Electron Acceptors/Donors in Mitochondrial Systems}

A number of \(p\)-benzoquinones have been studied for their effects within the mitochondrial electron transport chain, and for their ability to confer protection against oxidative damage. As shown in Fig. 2, this includes the coenzyme Q analogue idebenone. Coenzyme Q\textsubscript{10} is the normal cofactor supporting electron transport in human mitochondria. However, due to its extremely poor water solubility it is taken up minimally by isolated mitochondria, and is unable to restore normal respiration or growth if administered exogenously to (yeast or human) cells containing mitochondria deficient in coenzyme Q. Nonetheless, coenzyme Q\textsubscript{10} has been employed in a number of clinical trials, with some apparent success. The utility of CoQ\textsubscript{10} has been explored clinically for the treatment of mitochondrial diseases as well as a number of other conditions, including heart failure, muscular dystrophy, and mitochondrial diseases. Other than for patients having a preexisting CoQ\textsubscript{10} deficiency, it appears that while CoQ\textsubscript{10} does elicit a biomarker response\textsuperscript{24} consistent with potential clinical utility, there has been an absence of clear-cut benefit.\textsuperscript{25} Given CoQ\textsubscript{10}’s biomarker response and favorable
safety profile, several investigators have sought to improve the clinical results by enhancing the pharmacokinetic properties of CoQ\textsubscript{10}. The focus of these efforts has been to deliver higher concentrations by altering the lipophilic side chain of CoQ\textsubscript{10}. These efforts are exemplified by idebenone (Fig. 2), a CoQ analogue having a calculated octanol/PBS partition coefficient of about 3,100 (vs >10\textsuperscript{20} for coenzyme Q\textsubscript{10}).\textsuperscript{21} While it is not targeted to mitochondria, it can accept electrons from complex I and restore respiration in ubiquinone-deficient and rotenone-blocked mitochondria (eg, Fig 4 below). Idebenone has been evaluated in clinical trials for patients with neurologic, dermatologic, and mitochondrial diseases\textsuperscript{26} and elicits a positive biomarker response in multiple organ systems. While the results of clinical trials with idebenone have been mixed, the drug is approved for the treatment of Friedreich’s ataxia and other mitochondrial disorders in Canada (tradename Catena).

MitoQ\textsubscript{10} (calculated octanol/PBS partition coefficient about 2,500)\textsuperscript{21} was designed to accumulate in mitochondria by virtue of its positively charged triphenylphosphonium side chain, and has been shown to accumulate about 500-fold within mitochondria.\textsuperscript{27-29} It does not accept electrons from complex I, but exhibits side chain length-dependent electron acceptance from complex II. It has also been reported not to transfer electrons to complex III,\textsuperscript{21} ostensibly for physicochemical reasons relating to the effect of the triphenylphosphonium side chain on the concentration of the mitoQ’s within the membrane core.\textsuperscript{21} In spite of its lack of interaction with complexes I or III, mitoQ\textsubscript{10} has been shown to protect Friedreich’s ataxia fibroblasts from oxidative stress more effectively than untargeted antioxidants.\textsuperscript{30} Given its inability to accept electrons from complex I,\textsuperscript{21} the only logical mechanism by which this could occur within the electron transport chain is via relief of reductive stress in complex II. This may relate to its ability to prevent peroxidation of lipid mitochondrial membranes.\textsuperscript{29}

Another authentic one-electron process mediated by a molecule related to the \(p\)-benzoquinones is shown below in Figure 3. It is the reaction by which \(\alpha\)-tocopherol quenches the chain reaction that leads to lipid peroxidation. The formed \(\alpha\)-tocopherol radical is resonance stabilized, strongly contributing to its facile formation concomitant with lipid radical quenching. Thus \(\alpha\)-tocopherol appears to function within the mitochondrion as the semiquinone radical of \(\alpha\)-tocopherol quinone (\textit{vide supra}), and is also a powerful quencher of lipid peroxidation.

Figure 3. Quenching of radical-mediated lipid peroxidation by \(\alpha\)-tocopherol. \(R^*\) is a lipid radical.

In order to ensure that tocopherol quinone-type molecules can function in the mitochondria, we prepared the idebenone analogues shown in Figure 4. These molecules were tested by Dr. Gino Cortopassi (UC Davis) and found to restore oxygen consumption in cells treated with amounts of rotenone sufficient to partially block complex I function. They worked about as well as idebenone, indicating no mitochondrial bias against tocopherol quinone-type mediators. In fact, tocopherol quinone-type compounds have been found to function as mediators of mitochondrial electron transport.\textsuperscript{31} In comparison, none of four tested mito Q analogues (differing in side chain length) had any significant effect in restoring oxygen consumption (Figure 4). While Figure 4 does not measure
ATP production per se, it is logical to think that increased oxygen consumption in complex IV will result in incremental ATP production by mitochondrial ATP synthase.

Figures 4 and 5 illustrate idebenone analogues prepared and tested for their ability to support mitochondrial oxygen consumption. Coenzyme Q participates in a two electron redox cycle. However, the semiquinone radical shown in Fig. 5 represents an intermediate resulting from 1-e⁻ transfer. Given that the reductive stress (build-up of electrons) encountered initially in mitochondrial dysfunction is a one electron process, as is lipid peroxidation, ubiquinone-like carriers of single electrons could well find utility in dealing with reductive stress. While the semiquinone radical shown in Figure 5 would not be anticipated to be especially stable, one can envision ubiquinone analogues better equipped to transport single electrons. These include molecules in which the one-electron reduced intermediate is stabilized by dipole interactions, substituent effects, resonance effects or captodative effects. We seek molecules designed to traffic single electrons, and which can (i) accept electrons from superoxide, (ii) donate electrons to complex III and (iii) quench carbon-centered lipid radicals. We denote these compounds Multifunctional Radical Quenchers (MRQs), and believe that they can effectively protect mitochondria, cells and organisms from oxidative stress. The pyridinols shown in Figure 6 have been reported to be superior quenchers of lipid peroxidation, and we believe that these can form the basis for developing effective MRQs. Each of these undoubtedly quenches lipid peroxidation by donating H⁺ from the phenolic OH group, in analogy with tocopherol (cf Figure 3). It may be noted that the resulting...
phenolic radicals (shown in protonated form in Figure 7) are extensively resonance stabilized, and potentially capable of one-electron oxidation/reduction.

Figure 7. Electron transfer chemistry of 2,4-dimethyl-6-(dimethylamino)-3-hydroxypyridine, illustrating two putative one electron redox processes. Resonance contributors capable of stabilizing the putative radical cation intermediate are illustrated.

Viehe and his coworkers pioneered the study of steric and electronic factors that contribute to the stability of radicals, such as quinone radicals. They found that there were steric effects of the substituents attached to the carbon atom bearing the unpaired electron, and that these were mainly kinetic in nature. The electronic effects of the substituents were mainly thermodynamic in nature, and included a remarkable captodative effect, in which exceptional stabilization was achieved when the carbon bearing the unpaired electron was substituted both by an electron withdrawing and electron-donating substituent. An example is shown below in the structure to the right:

It may be noted that the structure to its left is simply a vinylogous example of the captodative effect, and that it is the deprotonated form of one of the resonance contributors shown in Figure 7.

Data/Analysis

Several compounds have been prepared to test this strategy, including analogues A, B and C. These compounds employ redox cores whose reduced forms have been shown to strongly quench lipid radicals. Compounds A and B are relatively simple analogues of idebenone, predicted to have
somewhat altered redox properties. These compounds have been tested for their ability to quench lipid peroxidation. As illustrated in Figure 8 for compound C, this compound was much more effective than \( \alpha \)-tocopherol in quenching lipid peroxidation, as would have been predicted (ref. 15 and Figure 6). The phytol side chain in C was found to be very important for quenching lipid peroxidation in this assay system; the core redox structure was much less effective. In the same assay system, compound B was about as effective as C, but compound A afforded complete protection at 12.5 \( \mu \)M concentration.

Compounds A, B and C were also studied for their ability to quench superoxide. This was done by assaying the effects of A-C on CEM cells that had been treated with 5 mM diethyl maleate (DEM) to (curve 1) and compound C (curves 2-5, at 5, 12.5, 25 and 50 \( \mu \)M concs, respectively). The blue (top) curve was an untreated sample; the pink (lowest) curve was a sample treated with the radical initiator 2,2'-azobis(2-amidinopropane)dihydrochloride (AAPH). The results show that compound C quenches lipid peroxidation in a concentration-dependent fashion.

chemically deplete glutathione in the cells, thus exposing them to increased oxidative stress. As shown in Fig. 9, A-C all protected the cells when used at 5 \( \mu \)M concentration. In comparison, \( \alpha \)-tocopherol (a lipid peroxidation quencher) was ineffective, and both the oxidized and reduced forms of idebenone had only limited effect. Compound A also afforded complete protection when used at 1 \( \mu \)M concentration. The quenching of ROS had the expected cytoprotective effect on the cultured CEM cells; as shown in Fig. 10, this was shown to be superior to that achieved with ideibenone (EC\(_{50}\) values for A, B and idebenone were 202±23 nM, 300±22 nM and 765±36 nM, respectively). The effects of A, B and C in protecting cells from ROS and the cytotoxic effects of oxidative stress at high nM/low \( \mu \)M concentrations contrasts sharply with the mM concentrations of the endogenous reagent glutathione that nature uses for the same purpose. It suggests that MRQs such as A, B and C must function catalytically to achieve their effects.

![Fig. 9. Flow cytometric histograms of ROS production in CEM cells. The cells were treated with DEM (5 mM) and test compounds (5 \( \mu \)M) as noted. The cells were loaded with dichlorofluorescein diacetate (10 \( \mu \)M) and green fluorescence was measured. A shift to the right indicates oxidation.](image)

![Fig. 10. Effect of A, B and idebenone on the viability of cultured CEM cells treated with diethyl maleate (DEM).](image)
As regards the putative catalytic activity of compounds A, B and C, we posit a catalytic cycle (illustrated in Figure 12) in which these compounds quench lipid peroxidation by donating H•, generating a radical which can be protonated to form a resonance-stabilized radical cation, the latter of which is capable of reacting with superoxide to regenerate the starting compound.

Figure 11. Effect of compounds in maintaining the viability of cultured skin fibroblasts from a Friedreich’s ataxia patient that had been pretreated with the oxidant buthionine sulfoximine (BSO). Blue curve, compound A; red curve, idebenone; green curve, redox core of compound A.

The radical cation is the one-electron reduced form of the compound between the hydroquinone and quinone forms of compound A and should, if its redox potential is appropriate, be able to transfer an electron to complex III. However, for compound A the putative radical cation is actually quite oxidizing in comparison with idebenol. The consequence of this reduction potential is that the radical cation of compound A would be predicted to be very efficient at scavenging superoxide, consistent with the results actually obtained in Figures 9-11. The extraordinarily efficient behavior of compound A as a quencher of lipid peroxidation argues that the parent compound has a favorable energy for dissociation of the phenolic O-H bond. These results argue strongly that compounds A – C, and especially compound A, are capable of catalytically carrying out at least two of the functions desired for our MRQs (multifunctional radical quenchers), namely:

- accepting electrons from superoxide
- quenching carbon-centered lipid radicals

However, the potential for the redox core of compound A argues that compound A itself should not readily reduce mitochondrial complex III, which is the third technical goal for our MRQs.

The reduction potentials of mediators in the mitochondrial electron transport chain become more positive as one proceeds down the chain. Thus cytochrome c, the carrier of electrons between mitochondrial complexes III and IV, has a redox potential of +0.23V, and is capable of accepting...
electrons from (i.e. capable of oxidizing) superoxide (redox potential \(-0.16V\)). This is illustrated in Figure 13, in which superoxide is generated by the use of xanthine oxidase, and efficiently reduces cytochrome \(c\). While the reduction of cytochrome \(c\) by superoxide does not result in the optimal production of ATP (4 protons are pumped across the mitochondrial membrane by virtue of two electrons transiting through complexes I and IV, 2 protons are pumped for two electrons transiting through complex III), the reduction of cytochrome \(c\) by superoxide will serve to relieve potential reductive stress, and can produce up to 40% as much ATP as that produced by fully functional mitochondria. Compound \(A\) does not reduce cytochrome \(c\) as efficiently as superoxide (due to its reduction potential), but it does clearly reduce the cytochrome, and will thus produce some ATP. The challenge is to create coenzyme Q analogues that function at somewhat less positive potential than compound \(A\), and which thereby reduce cytochrome \(c\) more efficiently, and hopefully can reduce complex III as well. If realized experimentally, this would enable mitochondria with defects in complexes I/II to produce up to 60% as much ATP as that produced by fully functional mitochondria.

Figure 13. Reduction of cytochrome \(c\) by superoxide and compound \(A\).

It may be noted that compounds \(B\) and \(C\) were also found to be capable of reducing cytochrome \(c\). The activity of compound \(B\) is of special interest in this regard, as it has a redox potential of \(+0.23V\), i.e. the same as that of cytochrome \(c\). However, the reoxidation of this compound in aqueous media (in the cyclic voltammogram, at 1 mM concentration) was inefficient, suggesting that this compound may be able to undergo side reactions. Idebenol (the hydroquinone form of idebenone) was also able to reduce cytochrome \(c\), as expected, although idebenone functioned inefficiently even in the presence of xanthine oxidase.

**Future Studies**

Our plan reflects the objectives of the project, which all deal with the preparation and characterization of analogues of coenzyme Q that enable one-electron trafficking within the respiratory chain. These are the multifunctional radical quenchers (MRQs) described above.

**Synthesis and Evaluation of MRQs Capable of Mediating the Three Desired Functions in Radical Quenching**

Medicinal chemistry functions by an iterative process in which the results obtained with compounds prepared initially inform the choice of compounds for subsequent preparation. As noted above, we have already identified compounds that accept electrons from superoxide and quench lipid peroxidation in what we believe to be a catalytic fashion. These compounds reduce cytochrome \(c\), but not with high efficiency. We suspect that they reduce complex III poorly, if at all, and some of them may actually inhibit complex III function. Accordingly, our initial objective is the preparation of compounds that retain their ability to quench superoxide and carbon-centered lipid radicals, but which have an enhanced ability to reduce complex III and cytochrome \(c\). We believe that this can be accomplished in part by shifting the redox potential to a somewhat less positive value. However, the compounds prepared need to have an O-H bond dissociation energy that enables efficient quenching of carbon-centered lipid radicals; the ring N atom by which compounds \(A\) and \(B\) differ is known\(^{34}\) to
affect bond dissociation energies. Further, the compounds prepared need to be stable under conditions of redox cycling, unlike compound B (vide supra), and also under ambient conditions.

There are a number of ways to accomplish this, one of which is simply to prepare compounds analogous to compound A in function, but which are more electron rich. The prepared compounds will be evaluated for their abilities to quench ROS (principally superoxide) in a cellular context as shown in Figure 9, and for their abilities to quench carbon-centered lipid radicals as shown in Figure 8. The reference compounds in these assays will be compounds A and B. Each compound will also be tested for its ability to confer protection from oxidative stress to cultured cells such as CEM cells (Figure 10) and fibroblasts from Friedreich’s ataxia patients (Figure 11). We wish to identify compounds that can restore/augment ATP production, and so will also evaluate compounds for their effects in transferring electrons to complex III and reducing cytochrome c, as well as ATP production per se. In collaboration with Gino Cortopassi, we will continue to evaluate compounds for their ability to induce oxygen consumption in mitochondria partially blocked with rotenone, or in mitochondria from patients with diseases of the mitochondrial respiratory chain. ATP production will be monitored, as described below. We hope that our new analogues will retain the favorable properties of compounds A and B as regards oxidation of superoxide and quenching of carbon-centered lipid radicals, but will also transfer electrons to complex III and to cytochrome c. We hope that they will also increase ATP production in rotenone-blocked normal mitochondria, and mitochondria from patients with diseases of the mitochondrial respiratory chain.

All of the above analogues envision the use of the idebenone side chain (i.e. a ten carbon unbranched substituent with a primary alcohol at the terminus). The success of idebenone in many cell free, cellular and animal model systems, in addition to its reasonable success in clinical trials, argues for the use of this substituent, at least until some better substituent is identified. As a practical matter, the preparation and evaluation of idebenone analogues with altered side chains is straightforward experimentally. Figure 14 illustrates a tricyclic intermediate, accessible in a single step from commercially available CoQ0, that permits facile placement of side chains with numerous chain

![Figure 14. Preparation of idebenone analogues having altered side chains.](image)

length and functional groups within the side chains. We are making a significant number of these for evaluation. The initial evaluation of these analogues will involve their ability to confer protection to cultured cells subjected to oxidative stress (i.e., as in Figures 10 and 11). The most promising analogues will be evaluated using all of the assay systems described above, and the best will be used for further modification of the optimized analogues of compounds A, B and C.
Evaluation of the Biochemical and Biological Functions of the MRQs

Essentially all of the biochemical techniques required for analogue evaluation have already been implemented within the Center for BioEnergetics, or by our collaborators, as may be judged from the data actually obtained in the several figures above. In particular, Dr. Gino Cortopassi (UC Davis) has been helping to guide our efforts by measuring the ability of our MRQs to support oxygen consumption (Figure 4).

We find methods for measuring ATP production to be the least well developed, and plan to explore a number of approaches to achieve robust, reproducible results. Several approaches are available to measure mitochondrial ATP production. The luciferase-luciferin system has been previously used for measurement of electron transport-linked ATP synthesis in intact mitochondria and SMPs. However, because submitochondrial particles are prepared in the presence of 1 mM ATP, the use of this assay has an intrinsically high background. Unless large amounts of ATP are being produced, an HPLC assay is preferred. ATP is formed not only through mitochondrial oxidative phosphorylation, but also through other metabolic pathways such as glycolysis and adenylate kinase. Therefore, $P^1$, $P^5$-di(adenosine) pentaphosphate, an inhibitor of adenylate kinase, will be used.

We will also measure the ability of the analogues to restore respiration in ubiquinone-depleted SMPs. Ubiquinone-depleted submitochondrial particles will be prepared from bovine heart mitochondria as described by Ernster. The particles will be lyophilized and extracted repeatedly with pentane to remove ubiquinone. The ubiquinone-depleted particles should exhibit very low or no NADH oxidase activities, but these can be restored upon the re-incorporation of ubiquinone to the levels found in the lyophilized particles prior to pentane extraction. Our analogues can then be tested for their ability to restore NADH oxidase activity in ubiquinone-depleted SMPs.

We are hopeful that the analogues will also support ATP production in intact cells. In order to determine this definitively, we will have to obtain (or construct) a cell line deficient in CoQ biosynthesis. Even in the absence of such cells, we may be able to demonstrate the special properties of the compounds by treating cultured cells with a complex I toxin, and then demonstrating restoration of ATP production with the tocopherol analogues. Obviously, we will also treat cultured cells derived from Friedreich’s ataxia patients with the analogues, and determine whether we can detect increased ATP production, decreased ROS and less damage to lipid membranes and DNA (vide infra). The ability of the treated cells to withstand oxidative stress will also be measured. We will determine the protective effects of our analogues in a well established cellular model of Friedreich’s ataxia. A fibroblast cell line derived from a donor with FRDA can be obtained commercially (Coriell Cell Repositories (Camden, NJ, USA; catalog no GM04078)). In these cells, It has been shown that exposure to the glutathione synthesis inhibitor L-buthionine sulfoximine (BSO) leads to a 70% decrease in the glutathione content of FRDA and control cells. Partial GSH depleted Friedreich’s ataxia fibroblasts die within 24-48 hr of the treatment due to endogenously generated oxidative stress. Previously, the BSO-induced death of FRDA fibroblasts was blocked by exogenous antioxidants such as mitoQ, Fe-Aox29 and small-molecule glutathione peroxidase mimetics, making this a good model to test the potency of our compounds. Cell viability can be determined using double-fluorogenic labeling as described. Another cellular model of Friedreich’s ataxia that provides the opportunity to generalize observations obtained with the fibroblasts above is a Friedreich’s ataxia lymphocyte cell line (GM15850) that we have also found to be useful for such measurements.

Another valuable method for characterizing our MRQs involves the use of engineered yeast mutants having lesions at characterized loci in the electron transport chain. Dr. Robert Wilson (Univ.
Pennsylvania) has agreed to assist us in evaluating the function of our MRQs in yeast, and a first set of MRQs is already in his hands for evaluation. Dr. Wilson’s laboratory also has mouse fibroblasts expressing (only) wild-type human frataxin or human frataxin with disease-associated missense mutations. These cell lines will facilitate evaluation of our MRQs for efficacy and potency of action.

Once optimized MRQs are in hand, we will prepare quantities of the most promising candidates sufficient for testing in animal models of Friedreich’s ataxia. Discussions at the most recent FA Therapeutics symposium, held in Philadelphia during summer 2009, and in follow up conversations with FARA representatives suggest that animal model(s) well suited to test compounds that function by ameliorating oxidative stress are under development and should be available in parallel with our optimized MRQs. We will make our compounds available to whichever laboratories are best equipped with animal models suitable for their evaluation.

Structure Optimization to Enable Clinical Development

Part of the work required to optimize the structures of the MRQs will take place as part of the iterative process described above. This certainly includes optimization of the side chain to enable exogenous delivery consistent with effective function in the mitochondrial electron transport chain. Those efforts will certainly take cognizance of chemical functional groups consistent with low toxicity, and will avoid those groups known to be associated with toxic responses, including inducers and inhibitors of cytochromes P450. Side chain optimization will be carried out in part to achieve good pharmacokinetic parameters, but verification of success will be clear only when the testing in animal models begins. It is beyond the scope of the current proposal to study the metabolic stability of the lead MRQs, but we will provide analytical methods that can be used to test bioavailability in animals. Fortunately, many of the (hydro)quinone analogues that we are studying have distinctive ultraviolet spectra which should facilitate this task, and a number of them are fluorescent. It is anticipated that the finalized MRQs will be orally available, but this will also have to be verified during the animal studies.

Parameters that need to be interrogated carefully before optimization can be considered complete include the physicochemical properties of the MRQs. The MRQs that we are studying include both oils and solids, and we need to assure that they can be purified easily (preferably without chromatography) so that large quantities can be prepared at an acceptable cost. If the final optimized MRQs happen not to be crystalline, this may be achievable by identifying a crystalline derivative that can be purified by crystallization and then converted to the parent MRQ efficiently under mild conditions. The MRQs presently being studied all seem to have reasonable stability under ambient conditions, but this property will have to be studied much more carefully for both the solid (or oil) form of the optimized MRQs, and also for aqueous solutions.

The synthetic routes used to prepare the parent MRQs will be optimized to be short, efficient and not involving any reagent that would be problematic for ultimate scale up to multikilogram quantities, or which would add unreasonably to the final cost of the MRQs. While it appears that the MRQs may function catalytically once they have reached the site of action, to achieve mitochondrial concentrations useful for therapy they will nonetheless probably have to be used at reasonable doses to be effective. Idebenone is marketed in Canada for the treatment of Friedreich’s ataxia as tablets containing 150 mg of the API (active pharmaceutical ingredient). It is in phase I clinical trials for the treatment of Duchenne Muscular Dystrophy and is being evaluated at single doses up to 900 mg. It is reasonable to anticipate that our optimized MRQ may be tested within the same approximate dose range.
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Synthetic Antibodies for Diagnostics and Personalized Therapeutics

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Overview: Antibodies have been and continue to be immensely valuable tools. They are essential for research in biology and now constitute a large portion of all new therapeutics. Even before the invention of monoclonals they were mainstays in diagnosis and therapy. The advent of the ability to create large amounts of pure antibodies revolutionized discovery biology and therapeutics. New applications are continually being developed. For example, the ability to make bifunctional antibodies has opened a whole new vista in therapeutics.

However, antibodies have clear limitations. Production in living systems make them physically labile and costly to produce. Engineering them for specific capabilities is difficult and time-consuming. Relative to large scale proteomics applications, they are not amenable to high throughput production. These and other limitations have spawned a number of strategies to make antibodies better or make better antibodies. Many of these processes still involve production in living systems and most center on screening large libraries of ligands.

Our Plan: We have developed two versions of a basic strategy to make synthetic ligands. Our goal was to create a system that would be simple, robust, high throughput and inexpensive. In addition the ligands would be created chemically and be amenable to manipulations.

The basic strategy is quite simple. Instead of screening large libraries we screen a pre-made stock of 10,000 peptides. Two peptides that bind different surfaces of a target are found in a highthroughput screen. These two peptides are then linked by a small universal peptide to create a binding agent (Synthetic Antibody = Synbody) with affinity and specificity rivaling antibodies or better. A simple mutation protocol applied to the peptide arms can increase the affinity and/or specificity dramatically. The protocol is remarkably robust and flexible. Selection can be conducted under any condition and ligands for multimeric targets readily developed.

Forward Process: In this process the target is chosen and then it is screened against all 10,000 peptides. This screen can be in solution via SPR or on slides. Two peptides binding two different areas of the target are chosen and then the proper linker length selected by a second screen. In parallel one or both peptide arms are mutagenized to create a 10-100 fold increase in binding and specificity.

Reverse Process: Peptides are chosen that recognize surfaces commonly on proteins. A large collection of synbodies are made with combinations of these peptides linked at different distances. These synbodies are then screened on arrays of spotted human proteins to find the target of the synbody. Our new breakthroughs in gene synthesis, in vitro translation and spotting protocols, allows facile and inexpensive creation of human or other species/pathogen protein arrays for screening. Once a synbody for a protein is identified it can be improved as needed through mutagenesis. An alternative and complementary protocol is to use each synbody in a pull-down assay from human cellular extracts. The proteins precipitated are identified by mass spectrometry. The synbody is again improved if necessary by mutagenesis. This is a very simple and inexpensive method to develop ligands. Though it is a random process in developing each ligand-target set, it is sufficiently high throughput to create a large number of ligands quickly.
The synbodies from these protocols can be used in research, diagnosis, imaging or as leads for therapeutics.

**Status:** All the protocols and resources are in place to effect these processes. We have invested considerably over the last 3 years to test and validate these concepts, procure the special equipment and hire/train the personnel. We are prepared to initiate and pursue vigorously the production of synbodies to the human proteome and our pathogens.

**Need:** A phase II chip of 1M peptides will be created to facilitate the screening. Otherwise, funding is needed only to initiate the production of synbodies on a large scale.

*We are ready to initiate a project to make ligands to the human proteome in less than 5 years.*
Bioenergy, Biofuels and Bioremediation
Cyanobacteria Designed for Solar-Powered, Highly Efficient Production of Petroleum Substitutes

Coordinator:

Willem Vermaas, School of Life Sciences and Center for Bioenergy and Photosynthesis

wim@asu.edu

(480)965-6250
Our Vision and Platform

Our atmosphere’s increasing concentration of CO$_2$ is linked to global climate change, negative impacts on the Earth’s ecosystems, and mounting stress on social and political structures. The use of fossil fuels is the single largest contributor to this problem. The urgency of the situation is driving the search for alternative fuels, including plant-based biofuels. Unfortunately, biofuel crops compete for precious food resources and consume enormous amounts of water for a relatively low yield of fuel. We need a truly renewable and domestic fuel source that protects our valuable soil and water resources. Arizona State University (ASU) has built an ongoing research program to develop a modern biotechnological process for microorganism-based photobioenergy which can overcome the barriers of plant-based biofuels.

Our platform focuses on the use of the cyanobacterium *Synechocystis* sp. PCC 6803 as a biocatalyst for solar energy conversion. Cyanobacteria are excellent organisms for the production of carbon-neutral and sustainable biofuel. Unlike the vast majority of algae, the genome of *Synechocystis* is relatively easy to manipulate, and the absence of organellar compartmentation simplifies metabolic engineering efforts. Cyanobacteria are efficient at converting solar energy into lipids, and unlike energy crops, they can be grown on non-arable land.

This work has set the stage for research and development to efficiently produce petrochemical substitutes using solar energy and CO$_2$ by this cyanobacterium. The natural photosynthetic process adapted in this project utilizes CO$_2$ from the atmosphere or from CO$_2$-emitting industries; thereby, this project will enable the sequestration of CO$_2$. In addition, the photosynthesis process uses the abundant energy from the sun as its main energy input and, over time, will eliminate the need for fossil fuels for the production of biofuel-compatible feedstock. Finally, the systems proposed here follow a development pathway starting with generation of high-value products leading the development and optimization of technologies that ultimately define a new high-efficiency platform for the production of biofuels via photosynthetic microorganisms.

Traditionally, products from microbial biomass are obtained by harvesting the organism and extracting the product from the biomass, adding cost and complexity, and creating waste. Our platform aims at a new paradigm, sunlight-driven production and secretion of biofuel-compatible feedstock without significant growth and biomass production. Our experimentally supported concept, eliminating major biomass production, enables the highly efficient conversion of solar energy into biofuel-compatible feedstock with efficiencies that are expected to be closer to the maximal theoretical limit of 28%. The photosynthetic organism, modified to make and secrete products of our choice, thus acts as a light-driven biocatalyst: it enables the process, but is not used up or altered in the process. This eliminates most waste and yields a much more economical and environmentally responsible path toward biofuel production than can be accomplished by traditional methods.

Our Approach

Our approach toward development and optimization of cyanobacteria as photobiocatalysts for solar-powered CO$_2$ reduction and fuel production represents a critical step forward in the efficient production of renewable biofuels by photosynthetic microorganisms. Our platform can be developed into at least three different technologies for production of biofuels and other petroleum substitutes.

**Fatty acids.** The transformative concept of this research program is to use cyanobacteria as biocatalysts using solar energy and CO$_2$ to produce fatty acids that the cyanobacteria
Cyanobacteria Designed for Solar-Powered Highly Efficient Production of Petroleum Substitutes

secrete, without major increases in cyanobacterial biomass. Fatty acids are then used for fuel production. In this project we utilize metabolic engineering to maximize fatty acid production and secretion in the cyanobacterium *Synechocystis* sp. PCC 6803 and minimize the energy diverted to the growth of the organism by using cultures that ideally are in stationary phase but that remain physiologically competent. Efficient fatty acid production is then partnered with technologies that efficiently transform these fatty acids into liquid transportation fuels.

We aim at secreting fatty acids from the cyanobacterial cells throughout their life cycle, including in stationary phase, which yields two major advantages: (1) more of the harvested energy can be used for fatty acid and lipid production as less energy needs to be applied toward growth (cells continue to produce fatty acids even in stationary phase), and (2) fatty acid secretion minimizes downstream processing complexity and cost of production. In this approach, as continuous and rapid growth of the culture is not required, the main inputs are CO$_2$ and light, and the main output is fatty acid. Thus, the transformative breakthrough in our approach is that the cyanobacteria are not a biomass product that must then be processed; they are a biocatalyst and a cellular factory for the products (fatty acids) that can be directly harvested (Figure 1).

This approach is in a more advanced stage. It has been awarded over $5 million in funding through the Department of Energy’s Advanced Research Projects Agency-Energy (ARPA-E) primarily for development and optimization of the cyanobacteria, but it will require additional funding to scale up the process to be applicable at a commercial scale. This part of the work is in collaboration with primarily Prof. Roy Curtiss III (Director, Center for Infectious Diseases and Vaccinology at the Biodesign Institute), Prof. Bruce Rittmann (Director, Center for Environmental Biotechnology at the Biodesign Institute), and Prof. William Roberts (Director, Applied Energy Research Laboratory at North Carolina State University).

Isoprene. This project focuses on efficient synthesis of isoprene, a petrochemical feedstock substitute, by photosynthetic microbes using solar energy and CO$_2$. Isoprene is a major chemical commodity that is currently produced primarily from imported oil. The cyanobacterium *Synechocystis* sp. PCC 6803 is being modified to carry the isoprene synthase gene to enable efficient isoprene production. In this system less energy is expended on growth and maintenance of the organism, and more energy is devoted to production of isoprene, which diffuses out of the cells and can be harvested independent from cells. This results in a highly efficient pathway to the renewable production of isoprene (Figure 2).

![Figure 1. Continuous solar-driven fatty acid production by cyanobacteria in a photobioreactor. CO$_2$ and sunlight are the main inputs, and secreted fatty acids are the main output.](image1)

![Figure 2. Schematic representation of solar-driven isoprene production from CO$_2$ by cyanobacteria. Our goal is to produce isoprene independent of growth of the organism, so that biomass production is limited and isoprene production is optimized (figure provided by our partner, Acidophili/ZuvaChem).](image2)
In this arrangement, *Synechocystis* is a photosynthetic biocatalyst for isoprene production, and we strive to minimize biomass generation. For application at a larger scale, photobioreactors (PBRs) will need to be designed that carry a low cost of materials and construction and that will reflect infrared radiation that would cause heating. Isoprene-specific constraints for PBR design include compatibility of the materials with isoprene and with the isoprene-harvesting process. We will also optimize processes to efficiently harvest isoprene from the PBR. Absorption of the isoprene in an organic phase inside the PBR provides promising options that we will evaluate and optimize.

Solar-driven cyanobacterial isoprene production is in development, but it could represent a quantum leap forward in efficient biological production of petrochemical feedstock replacements. This new method may result in isoprene production rates of 20 g/m$^2$/day or more, which corresponds to the sequestration of 64 g/m$^2$/day of CO$_2$. Therefore, the estimated 170,000 metric tons of isoprene produced in the United States each year could be produced on as little as 7,200 acres of non-arable land and would sequester 540,000 metric tons of CO$_2$. We estimate that worldwide isoprene production, currently at approximately 800,000 metric tons/year and valued at $1-2$ billion, would expand with a new low-cost source of high-purity renewable isoprene to well over 5,000,000 metric tons/year, further advancing the impact of this new highly efficient renewable process.

**Isoprenoids.** Isoprenoids are natural polymers of isoprene precursors, and include many high-value products such as carotene (a colorant and anti-oxidant), astaxanthin (a natural colorant), squalene (the precursor to steroids), menthol (a fragrant and flavorful essential oil), artemisinin (an anti-malaria drug) and taxol (an anti-cancer drug). The natural abundance of total isoprenoids in *Synechocystis* already is a few % of the dry weight, and this can be further enhanced by metabolic engineering. Moreover, genes may be introduced to enable the synthesis of specific valuable compounds.

To enhance isoprenoid production, we will over-express the existing isoprenoid biosynthesis pathway and introduce a new one. This approach already has proven to be successful with yeast and the bacterium *E. coli*, and the cyanobacterial system already is set up for much higher isoprenoid biosynthesis rates than either yeast or *E. coli*. We already have modified and enhanced the content of carotene and related compounds in cyanobacteria, and similarly could introduce genes to enable production of other high-value isoprenoids.

The transformative focus of our research greatly enhances the efficiency with which solar energy can be used for production of biofuels, other petroleum substitutes such as isoprene, and valuable compounds such as isoprenoids. Current approaches depend on growth of photosynthetic organisms that are subsequently harvested and extracted. For at least fatty acids and isoprene our production system should be largely independent from biomass generation, thus boosting production efficiencies. Our photosynthesis-based approach utilizes CO$_2$ from the atmosphere or from CO$_2$-emitting industries, and uses the abundant energy from the sun as its main energy input. As Arizona is blessed with much sunshine and large areas of non-arable land that are suitable for culturing photosynthetic microbes but not agricultural crops, this is a natural and excellent fit of location, research and application potential, environmental stewardship, and energy security enhancement.
Advanced Photobioreactors for Massive Bioenergy Production

Coordinator:

Dr. Bruce Rittmann, Center for Environmental Biotechnology

The Biodesign Institute

Bruce.Rittmann@asu.edu
**The Challenge:** The greatest challenges facing human society today is replacing fossil fuels with an alternative that provides similar energy services, but is renewable and carbon-neutral. Today, fossil energy is consumed at a rate of over 10 terrawatts (TW).\(^1\) Society’s goal must be to cut fossil fuel use to no more than about 3 TW if we are to suspend the rise in CO\(_2\).\(^2\) Therefore, we need at least 7 TW of renewable, C-neutral energy to replace all that fossil fuel.

Dr. Bruce Rittmann leads a team at that has developed bench and pilot systems for growing photosynthetic microorganisms optimized for high-yield production of biomass that can be converted to a range of renewable biofuels, including jet and diesel fuels. Ta picture of the pilot system is shown below. Located on the roof of the Engineering Research Center, it has 2,000 L of sunlight-exposed tubes. Besides high areal yield, the microbe-bases systems do not require arable land, do not compete with food or commodity crops, and require substantially less water than plant-based biofuels. Based on known photosynthesis rates of cyanobacteria, large-scale cultivation of these photosynthetic bacteria could produce enough high-energy biomass to replace the entire 7 TW of fossil-fuel use if only 200,000 – 400,000 square miles of sunny land area were devoted to this goal; this is roughly the size of Texas.

**The Approach:** The essential technological step for making microbial photobioreactors economically and environmentally realistic is designing a system that recycles water and nutrients to the bacterial “crops” while collecting the biomass when it is growing very rapidly. One key to the approach is continuous harvesting of the biomass solids by membrane filtration. Currently, biomass harvesting is a labor-intensive process that results in water waste that, magnified at the scale of the proposed energy need, is the problem Dr. Rittmann and team wish to solve.

Dr. Rittmann has developed a novel strategy for the design and operation of photobioreactors so that they can generate photosynthetic biomass at a very high rate with low water consumption. The photosynthetic bacteria are grown in a water-based photobioreactor from which they are harvested as a concentrated slurry. The lipid portion of the harvested biomass is converted into feedstock for transportation fuel, while the rest of the biomass is converted into methane or hydrogen gas. The water and nutrients are recovered and recycled.

Dr. Rittmann has already constructed and tested the bench-top PBR part of the system. What is needed now is to integrate the PBR with an MFS so that the strategy of low-SRT and high HRT can be experimentally tested and optimized. Thus, the research for the Photobioreactor + Membrane-Filtration System will be to design, build, and systematically
evaluate (by experiments and modeling) the PBR+MFS process by linking a MFSs to existing and newly constructed PBRs.

Experimentation will be carried out with the photosynthetic bacterium *Synechocystis* PCC6803, since Dr. Rittmann has extensive experience working with PCC6803 in the bench-top PBRs, and it has ideal physiological characteristics for achieving high photosynthetic productivity. However, the principles advanced by this research will apply to other photosynthetic microorganisms, including algae. Experimental research will involve series of experiments in which the flow rates, incident light intensity, and nutrient (C, N, and P) supply rates are controlled in ways that we expect will lead to high biomass concentration and productivity, but low water use. Measurements will include the biomass concentrations in the reactor and the harvested flow, concentrations of the nutrients (C, N, and P), pH, and temperature. The light intensity inside the PBR will be computed from the Beer-Lambert law and the known light-absorption coefficient for PCC6803. The microbial community will be assayed for PCC6803 and other types of microorganisms using a range of genomics techniques that are well developed in Dr. Rittmann’s center.¹

In addition to the research directly on the PBR+MFS, the team will integrate the energy- and nutrient-capture technologies needed to gain the full benefit of massive biomass production. This will involve innovative and environmentally benign means to extract lipids for making transportation fuels, to capture the energy in the non-lipid biomass as methane or hydrogen gas, and to capture and recycle the N and P nutrients.

**The Goal and Outcomes:** The goal is to advance the PBR+MFS system so that it is ready for testing at the proto-commercial scale. Dr. Rittmann already has linkages to partners who can supply large-scale, low-cost PBR systems and who have sites that can provide the needed CO₂ source. They will be brought into the team as the PBR+MFS systems move to field-scale testing. Once the system has been tested and refined at the proto-commercial scale, it can be phased in as a means to generate enough biomass energy that our society’s use of fossil fuel is scaled back enough to stop the advance of global warming, to replace dwindling supplies of petroleum, and to reduce geopolitical tensions that stem from over-reliance on fossil fuels.

**References**

Renewable BioHydrogen from Animal Wastes

Coordinator:

Dr. Bruce Rittmann, Center for Environmental Biotechnology
The Biodesign Institute

Bruce.Rittmann@asu.edu
Background and Goal: Animal wastes represent a large potential source of renewable energy that is currently being wasted. When the energy-rich content of the animal wastes is not captured, these wastes can create severe environmental problems. Therefore, finding a way to harvest the energy value of animal wastes will reap dividends in terms of developing renewable energy that can displace fossil fuels and in terms of improving environmental quality.

An exciting new development within the field of environmental biotechnology, the microbial electrolysis cell (MEC), offers the possibility of achieving both goals in the most efficient way possible. The MEC allows the conversion of organic wastes into renewable hydrogen (H₂). H₂ produced from organic wastes can be used as transportation fuel or coupled with a chemical fuel cell to generate electricity. It also becomes a renewable feedstock to the chemical and petrochemical industries. Developing this renewable and sustainable source of H₂ will enhance the economic foundation of agricultural areas while reducing our society's reliance on fossil fuels.

The goal of this project is to advance the MEC from an exciting research discovery to a technology that is ready for large-scale implementation at facilities that produce large amounts of animal waste, such as hog and poultry farms.

Description of the Technology: Like all fuel cells, the MEC works on the principle of combustion-less oxidation of a fuel at an anode, with the electrons transferred to the anode, passing through an electrical circuit, and ultimately being transferred to an electron acceptor at the cathode. Figure 1 illustrates how an MEC is used as a source of renewable H₂ fuel. The left compartment contains the anode, which is a conducting solid, usually graphite. The organic fuel (represented as C₂H₄O₂) is fed continuously to the anode compartment in liquid solution or slurry. Bacteria that live as a biofilm attached to the anode, the anode-respiring bacteria (ARB), oxidize C₂H₄O₂ (i.e., remove electrons from it) and transfer the electrons to the anode via conduction through the biofilm matrix. The electrons flow from the anode to the cathode, where a potentiostat is used to control the potential (or voltage) at the cathode to one favorable for the half reaction: 2H⁺ + 2e⁻ → H₂. To maintain charge neutrality, protons (H⁺) also transfer from the anode to the cathode, often via a special proton-exchange membrane (PEM), although other options are possible.
**Technical Approach:** The research and development plan outlined here is based on the world-leading foundation established by the MEC research team in the Center for Environmental Biotechnology at ASU. It is designed to move rapidly and efficiently from the current bench-scale systems to larger lab-scale systems. These systems will enable basic research and inform the design needed for pilot-scale implementation in the field at a large animal farm. The research and development plan is further oriented towards an integrated MEC system. Process integration continues to be a key technical challenge impeding the advancement of this technology to implementation. Focusing on integration from the beginning is, therefore, essential for understanding and optimizing all of the components.

Multiple generations of prototypes will be designed, constructed, and evaluated to allow us to benefit from knowledge gained in the previous stage, while continually advancing the size and sophistication of the MEC systems. In years 1 and 2, we will start with ~20 liter lab-scale prototypes and will progress to ~60-liter prototypes in years 3 and 4. Research components that will be included are (Figure 2): pre-treatment and fermentation of the animal wastes to make the organic material more bioavailable for the ARB on the anode; anode configuration, materials, and operations strategy; cathode configuration, materials, and operations strategy; and integration of the anode and cathode with or without a membrane. All components of the MEC system will be tested and improved upon based on the results of prototype evaluation, and the systems will become more completely integrated from generation to generation.

The project team brings together technical experts within in the Biodesign Institute, the Ira A. Fulton Schools of Engineering, and the College of Technology and Innovation. This team provides deep expertise in microorganism-based processes, microbial ecology, and electrochemical systems and materials. This diverse set of technical experts, brought together with engineering integration and program management, provides the necessary foundation for translating MEC research from the lab to the field.

**Outcomes:** The research plan will create integrated MEC systems ready for field testing at animal farms. The implementation of MECs at large-scale animal farms will create a large value stream of renewable H\textsubscript{2} fuel that simultaneously helps to reduce our nation’s dependence on fossil fuels and eliminates a serious environmental-contamination hazard.
PART 4
EXAMPLE CVs
SECTION FOUR

Selected Faculty & Leadership CVs
BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed for Form Page 2.
Follow the sample format for each person. DO NOT EXCEED FOUR PAGES.

NAME
Alan Nelson

POSITION TITLE
Professor of Bioengineering at ASU
Director, the Biodesign Institute at ASU

eRA COMMONS USER NAME (credential, e.g., agency login)
alannelson

EDUCATION/TRAINING  (Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)

<table>
<thead>
<tr>
<th>INSTITUTION AND LOCATION</th>
<th>DEGREE</th>
<th>YEAR</th>
<th>FIELD OF STUDY</th>
</tr>
</thead>
<tbody>
<tr>
<td>University of Southern California, Los Angeles, CA</td>
<td>B.A.</td>
<td>1972</td>
<td>Physics</td>
</tr>
<tr>
<td>University of California, Berkeley, CA</td>
<td>M.S.</td>
<td>1976</td>
<td>Geophysics</td>
</tr>
<tr>
<td>University of California, Berkeley, CA</td>
<td>Ph.D.</td>
<td>1980</td>
<td>Biophysics</td>
</tr>
</tbody>
</table>

A. Positions and Honors

Positions and Employment
1980-1985  Assistant Professor, Department of Nuclear Engineering, MIT
1981-1986  Director, Whitaker Laboratory of Biomedical Imaging, MIT
1983-1986  Endowed Chair for Biomedical Engineering, W.M. Keck Foundation, MIT
1984-1986  Associate Professor, Dept. of Nuclear Engineering (MIT) and Health Sciences and Technology (Harvard)
1985-1986  Visiting Fellow of The British Royal Society, Sabbatical
1986-1989  Associate Professor, Center for Bioengineering, UW; Adjunct in Pathology, Radiology and EE
1989-1991  Tenured Associate Professor, Center for Bioengineering, UW; Adjunct as Above
1989-1991  Director, Center for Information Systems Optimization, UW
1991-1999  President and CEO, NeoPath, Inc., Redmond, WA
1999-2000  Executive Chairman, TriPath Imaging, Inc., Burlington, NC
1991-2009  Affiliate Professor, Center for Bioengineering and Mechanical Engineering, UW
2001-2009  Founder, Chairman and CEO, VisionGate, Inc., Gig Harbor, WA
2009-  Professor, Bioengineering, ASU
2009-  Director, the Biodesign Institute, ASU

Other Experience, Professional Memberships and Board Directorships
1984-1986  Swedish Embassy, Office of Science and Technology, Science Advisor
1995-1997  Director, Washington Biotechnology and Biomedical Association
1996-1998  Director, Washington Technology Center, UW
1996-2009  Director and Executive Board Member, Technology Alliance of Washington
1998-  Fellow of the American Institute for Medical and Biological Engineering
1999-2006  Board of Advisors, Conus Fund, NY
2003-2004  Washington Alliance of Angles

Honors and Certifications
1983  W. M. Keck Foundation Endowed Professorship Chair, MIT
1984  MIT Graduate Student Council Teaching Award
1984  American Nuclear Society Outstanding Professor Award
1986  Visiting Fellow to The British Royal Society
1986  Honorary Professorships at Queens University of Belfast and the University College of Dublin
1990  IBM Supercomputing Award, National 1st Prize
B. Selected peer-reviewed publications (From among 51 peer-reviewed publications)


C. Research Support
None applicable.
BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. DO NOT EXCEED FOUR PAGES.

NAME
Woodbury, Neal W.

POSITION TITLE
Professor of Chemistry and Biochemistry

eRA COMMONS USER NAME (credential, e.g., agency login)
NWOODBURY

EDUCATION/TRAINING (Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)

<table>
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<th>INSTITUTION AND LOCATION</th>
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<tbody>
<tr>
<td>University of California at Davis; Davis, CA</td>
<td>B.S.</td>
<td>1979</td>
<td>Biochem. &amp; Biophys</td>
</tr>
<tr>
<td>University of Washington; Seattle, WA</td>
<td>Ph.D</td>
<td>1986</td>
<td>Biochemistry</td>
</tr>
<tr>
<td>Carnegie Inst. of Washington; Stanford, CA</td>
<td>Postdoc</td>
<td>1986-1987</td>
<td>Plant Biology</td>
</tr>
<tr>
<td>Stanford University; Stanford, CA</td>
<td>Postdoc</td>
<td>1987-1988</td>
<td>Molecular Biology</td>
</tr>
</tbody>
</table>

1. Positions and Honors

Research and professional positions:

1979 - 1986 University of Washington, Graduate research with W. Parson
1986 - 1987 Carnegie Institute of Washington, Dept. of Plant Biology, Postdoctoral research with W. Thompson
1987 - 1988 Stanford University, Postdoctoral research with S. Boxer
1987 - 1994 Arizona State University, Assistant Professor of Chemistry
1994 - 1998 Associate Professor of Chemistry and Biochemistry
1998 - present Professor of Chemistry and Biochemistry
1997 - 2000 Director, Photosynthesis Center
1996 – 2002 Director, NSF RTG Optical Biomolecular Development Program
2000 – 2008 Director, NSF IGERT Biomolecular Nanotechnology
2002 – present Director, Center for BioOptical Nanotechnology, The Biodesign Institute at ASU
2008 – present Deputy Director, Biodesign Institute at Arizona State University

Honors and other professional activities:

1985 NSF Postdoctoral Fellowship in Plant Molecular Biology
1991 NSF Presidential Young Investigator Award
1995 - 2001 Director, NSF RTG in Biomolecular Devices
1997 NSF Panel for Instrument Development for Biological Research
1997 - 2000 Director, ASU Photosynthesis Center
1998 - 2001 NSF Molecular Biophysics Panel
1998 Biodesign Institute Development Panel Member
1999 NSF Integrative Graduate Education and Research Training Panel
2001 - present Director, NSF IGERT in Biomolecular Nanotechnology
2002 – present Photochemistry and Photobiology, Associate Editor
2002 – present NSF Integrative Graduate Education and Research Training Panel
2002 – present Director, Center for BioOptical Nanotechnology, The Biodesign Institute at ASU
2004 NSF Biophysics Panel
2004 Outstanding Supervisor Award
2004 – present NSF MPS-MCB Joint Review Panel member
2004-2005 American Chemical Society Panel Member
2005 - present Chair of the Biodesign Personnel Committee
2005 National Academy of Science Workshop
    Chemical Imaging Committee Member
2006 Regents’ Professor Nominating Committee
2007 Professor of the Year Nominee
2008 Professor of the Year Nominee
2008 Gary Krahenbuhl Difference Maker Award, recipient
2. Selected peer-reviewed publications (10 selected).

3. Research Support
Completed Research Support
09/15/01 – 08/31/08  NSF-Directorate for Education & Human Resources
IGERT: OPTICAL BIOMOLECULAR DEVICES: FROM NATURAL PARADIGMS TO PRACTICAL APPLICATIONS.
03/01/02 – 02/28/07 NSF-Directorate for Biological Sciences
Controlling the pathway of electron transfer in bacterial reaction centers
07/01/03 – 06/30/06 NSF-Directorate for Mathematical and Physical Sciences
Characterization of DNA-Protein interactions at the single molecule level
02/15/06 – 01/31/09 NSF-Division of Chemistry (Co-I)
Purchase of an Instrument for Ultrafast, multidimensional fluorescence detection and Imaging
06/01/06 – 05/31/07 NSF-Division of Physics
SGER: Merging single molecule spectroscopy and molecular simulation
09/01/06 – 08/31/07 Molecular Imaging Corporation
CRADA-Mod26
03/30/07 – 09/30/08 Science Foundation Arizona
CAA: Factor Binding Dynamics on Promoters

Ongoing Research Support
07/01/2005 – 06/30/2009 Department Of Energy
Combinatorial development of water splitting catalysts based on the oxygen evolving complex of Photosystem II
4/01/2007–3/31/2012 National Science Foundation
Protein control of electron transfer pathways in photosynthesis
### BIOGRAPHICAL SKETCH

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<table>
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<tr>
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<th>POSITION TITLE</th>
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<tbody>
<tr>
<td>Charles J. Arntzen, Ph.D.</td>
<td>Regent’s Professor and Florence Ely Nelson Presidential Chair</td>
</tr>
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</table>

| eRA COMMONS USER NAME (credential, e.g., agency login) | carntzen |

#### EDUCATION/TRAINING

*Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.*

<table>
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<tr>
<td>University of Minnesota, Minneapolis, Minnesota</td>
<td>B.S.</td>
<td>1965</td>
<td>Plant Physiology</td>
</tr>
<tr>
<td>University of Minnesota, Minneapolis, Minnesota</td>
<td>M.S.</td>
<td>1967</td>
<td>Plant Physiology</td>
</tr>
<tr>
<td>Purdue University, Lafayette, Indiana</td>
<td>Ph.D.</td>
<td>1970</td>
<td>Cell Physiology</td>
</tr>
<tr>
<td>Charles F. Kettering, Lab, Yellow Spring, Ohio</td>
<td>Post-Doc.</td>
<td>1969-70</td>
<td>Photosynthesis</td>
</tr>
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</table>

#### Professional Experience

1969-80 | Associate Professor, Department of Botany, University of IL
1973-74 | Research Scientist, Laboratoire de Photosynthese du CNRS, Gif-sur-Yvette, France
1976-80 | Plant Physiologist, USDA/SEA, Urbana, IL
1976 | NATO Scientist, Laboratoire de Photosynthese du CNRS, Gif-sur-Yvette, France
1980-84 | Director, MSU-DOE Plant Research Laboratory, Michigan State University
1981 | ANU Fellow, Australian National University, Canberra, Australia
1983 | Visiting Scientist, Academia Sinica, Beijing, China
1984-88 | Director, Plant Science and Microbiology, DuPont Central Research & Development Department, and Director, Biotechnology Research, DuPont Agricultural Products Division, Wilmington, DE
1988-95 | Deputy Chancellor for Agriculture, Dean, College of Agriculture and Life Sciences, Director, Texas Agricultural Experiment Station, Texas A&M Univ., College Station, Texas; Professor, Dept. of Biochemistry and Biophysics; Director, Plant Biotechnology Program, Institute of Biosciences and Technology, Texas A&M Univ., Houston, TX
1995-2000 | President and CEO, Boyce Thompson Institute for Plant Research, Inc. and Adjunct Professor, Cornell University
2000-Present | Florence Ely Nelson Presidential Chair in Plant Biology and (since 2004) Regent’s Professor, Arizona State Univ., Tempe, AZ

#### Honors and Awards

1979 | Charles Albert Shull Award for Outstanding Research in Plant Physiology
1980 | Award for Superior Service, US Department of Agriculture
1980 | Award of Excellence, Weed Science Society of America
1983 | Elected Member of the National Academy of Sciences, USA
1984 | Elected Fellow of the National Academy of Sciences, India
1985 | Elected President, American Society of Plant Physiologists
1990-1997 | Member, Board of Governors of the University of Chicago for Argonne National Laboratory. Member Executive Comm.; Chair of Scientific/Technical Advisory Board
1991-1993 | Member and Chairman, NIH Biotechnology Policy Board
1991-1998 | *SCIENCE* Editorial Board Member
1994 | Dennis Robert Hoagland Award, American Association of Plant Physiologists
1994 | Elected Fellow, AAAS
1997 | Doctor of Science *honoris causa*, Purdue University, School of Science
2001-2009 | President George W. Bush’s Council of Advisors on Science and Technology, and Presidential appointee to the National Nanotechnology Advisory Board (in 2004)
2003 | Doctor of Science *honoris causa*, University of Minnesota
2004 | American Society of Plant Biology Leadership in Science Public Service Award
2006 | Botanical Society of America Centennial Award
2007 | Elected Fellow of American Society of Plant Biologists
2008 | Doctor of Science *honoris causa*, Hebrew University of Jerusalem
Representative Publications:


Current Funding (Active)

USDA 08060498 07/01/08-06/30/2011
USDA-U.S. Department of Agriculture
Development of a robust assay for infective Noroviruses, for use in food safety diagnostics
The goal of this project is to extend the reported discovery of a replication method for human norovirus with the ultimate goal being to develop a functional in vitro assay for noroviruses.
Role: Co-PI (C. Nickerson=PI)

JHSF 08080923 09/01/08-05/31/10
Japan Health Science Foundation
Production of hepatitis E virus-like particles by tobacco mosaic virus vector
This project uses transient expression with plant viral vectors to optimize production of HEV capsid protein VLP. Role: Co-I (H. Mason=PI)

SFAZ 08080969 07/01/08-12/31/09
Science Foundation Arizona
Commercialization of Norovirus Vaccine Technology in an Arizona spin-off Company
The proposed research supports product validation for a bivalent norovirus vaccine.
Role: PI

NIH 1 U54 NS058183-03 09/30/06 – 05/31/11
Rapid and Large Scale Plant-based Production of Catalytic Nerve Agent Bioscavengers Major Goal: Optimization for plant expression of BChE and PON1 gene sequences (Human enzymes or derivatives) for chemical warfare counter measures. Dr. Tsafirir Mor is the ASU PI on Project 5 of this multi-center project; David Lenz of US AMRICD is overall PI. C. Arntzen is co-PI on ASU subcontract.

NIH U19AI066332-03 (PI-Arntzen) 07/01/08-12/31/09
Plant-derived Vaccines against Hepatitis C Cooperative Research Center
Major Goal: To test the capacity of plant cells and tissues to express genes derived from the Hepatitis C virus; to isolate the HCV-proteins from plant tissues and test them for their ability to stimulate immune responses in pre-clinical animal trials.

NIH U19 Al 062150-04 (PI-Arntzen) Currently in no cost extension 09/01/04 – 08/30/10
Plant Made Microbicides and Mucosal Vaccines for STIs
Goals: One goal of this project is to design and produce mucosal vaccines in plant expression systems for sexually transmitted viral diseases and to test these vaccines in pre-clinical animal trials and in human trials. A second goal is to produce mABs in plants which neutralize sexually transmitted viruses or which block viral receptors, and test them in human trials using vaginal delivery.

NIH U01AI061253-01 (PI-Arntzen) Currently in no cost extension 3/15/05 – 2/28/09
Development of a Vaccine for Ebola Virus in Plant System
Major goal: To develop plant-expressed monoclonal antibody fusion proteins as a vaccine against Ebola virus.

Completed funding (past three years):

1 S10 RR023652-01 (PI-Arntzen) 03/01/07 – 2/29/08
HHS-NIH
BiaCore Instrument Purchase
Major Goal: Equipment purchase

DAMD 17-02-2-0015 (PI-Arntzen) 06/01/02 – 07/31/07
USAMRIID/DOD Army Research Office
Plant Production of Vaccines and Antibodies for Protection against Biowarfare Agents
Major Goal: To produce plant-based vaccines and antibodies for the protection of war fighters against biowarfare agents, specifically Ebola and *Yersinia pestis* (agent of bubonic and pneumonic plague).

0114712 (Co-PI Arntzen) 09/01/01 – 10/30/06
NSF
Phase III: Western Alliance to Expand Student Opportunities
Major Goal: To expand student opportunities at the undergraduate level and facilitate transition to graduate school levels
BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2. Follow this format for each person. DO NOT EXCEED FOUR PAGES.

NAME
Sudhir Kumar

POSITION TITLE
Professor, School of Life Sciences
Director, Center for Evolutionary Functional Genomics, Biodesign Institute

eRA COMMONS USER NAME (credential, e.g., agency login)
skumar

EDUCATION/TRAINING (Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)

<table>
<thead>
<tr>
<th>INSTITUTION AND LOCATION</th>
<th>DEGREE</th>
<th>YEAR(s)</th>
<th>FIELD OF STUDY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birla Institute of Technology and Sciences</td>
<td>B. Engg.</td>
<td>1990*</td>
<td>Electrical &amp; Electronics</td>
</tr>
<tr>
<td>Birla Institute of Technology Sciences</td>
<td>M. Sc.</td>
<td>1990*</td>
<td>Biological Sciences</td>
</tr>
<tr>
<td>Pennsylvania State University (USA)</td>
<td>Ph. D.</td>
<td>1996</td>
<td>Genetics</td>
</tr>
<tr>
<td>Pennsylvania State University (USA)</td>
<td>Postdoctoral</td>
<td>1996-1998</td>
<td>Molecular Evolution</td>
</tr>
</tbody>
</table>

*Completed concurrently under a dual degree program

A. Positions and Honors

Positions and Employment
1991 – 1996 Research Assistant, Department of Biology, Penn State University (PSU)
1996 – 1998 Postdoctoral Fellow, Department of Biology, PSU
1998 – 2002 Assistant Professor, Department of Biology, Arizona State University, Tempe (ASU)
2002 – 2006 Associate Professor, School of Life Sciences, ASU
2003 – Director, Center for Evolutionary Functional Genomics, Biodesign Institute, ASU
2006 – Professor, School of Life Sciences, ASU

Awards and Honors
2000 Innovation Award in Functional Genomics, Burroughs Wellcome Fund (BWF)
2000 Hot Paper in Biology (Nature 392:917-920), Essential Science Indicators, Web of Knowledge, Science Citation Index (SCI)
2004 – Top-10 most-cited scientist with publications categorized in Computer Sciences, SCI
2004 Hot Paper in Biology (Bioinformatics 17:1244-1245), SCI
2004 Hot Paper in Computer Science (Bioinformatics 17:1244-1245), SCI
2006 – 2007 Hot Paper in Computer Science (Briefings in Bioinformatics 5:150-163), SCI
2007 – 2008 Visiting Fellowship Award, Japanese Society for Promotion of Science (JSPS) Competition
2007 – 2009 Current Classic, Computer Sciences (Briefings in Bioinformatics 5:150-163), SCI
2008 Fellow, American Association for the Advancement of Science (AAAS)
2009 Honorary Professor, Computer Sciences, ASU
2009 Finalist, Governor’s Celebration of Innovation Award, Arizona (winner selection pending)

Current Editorial Activities
2004 – Editorial Board, Molecular and Developmental Evolution
2005 – Associate Editor, Molecular Biology & Evolution
2005 – Associate Editor, Evolutionary Bioinformatics Online
2006 – Editorial Board, Genome Research

Other Experiences and Professional Memberships
2006 – Member, BioData Management and Analysis Study Section, NIH, USA
2006 – 2009 Informatics Advisory Committee, National Center for Evolutionary Synthesis (NEScent), USA
2006 Organizer, Annual meeting of the Society for Molecular Biology and Evolution, Arizona State University, Tempe, Arizona (May 24 – 28).
2004 – 2006 Secretary, Society for Molecular Biology and Evolution (Elected)
2004 – 2008 Webmaster, Society for Molecular Biology and Evolution
2004 Organizer, Symposium on Evolutionary and Population Genomics in the Future of Statistics Conference at Indian School of Business, Hyderabad, India (December 29–January 1)
1999 – 2007 Webmaster, American Genetic Association

B. Selected publications (in reverse chronological order)


49. Nei M, Kumar S & Takahashi K (1998) The optimization principle in phylogenetic analysis tends to give incorrect topologies when the number of nucleotides or amino acids used is small. *PNAS (USA)* 95:12390-7.


C. Research Support (Ongoing)

R01 HG002096 Kumar (PI) 01/01/2000 – 04/30/2010 NIH/NHGRI

**Comparative molecular sequence analysis**

Development of techniques and tools for comparative analysis of DNA and protein sequences

R01 HG002516 Kumar (PI) 07/11/2003 – 06/30/2010 NIH/NHGRI

**Computational analysis of gene expression pattern images**

Developing tools and techniques for comparative analysis of *in situ* gene expression patterns (fruit fly)

DBI- 0548366 Fagan (PI) 07/01/2006 – 06/30/2010 NSF-DBI

**Developing a bioinformatics database for stoichioproteomics**

Developing a database containing the elemental composition of the amino acid side chains

IIS- 0612069 Ye (PI) 08/01/2006 – 07/31/2010 NSF-IIS

**SEI: Machine learning approaches for biological image informatics**

Developing new machine learning methods for analysis of gene expression patterns images

R01 GM081066 Kumar (PI) 08/01/2007 – 07/31/2010 NIH/NIGMS

**Re-engineering the MEGA software package**

Refactoring/ upgrading of the popular MEGA software package to adapt to new architectures/ paradigms (with ARRA supplement from 2009-2011)

R01 GM085530 Chaput (PI) 08/01/2008 – 07/31/2012 NIH/NIGMS

**Discovering a Hidden Proteome in the Human Genome**

Identifying genomic segments of the human DNA with capacity to initiate non-classical translation

DBI-NSF Hedges (PI) 08/01/2009 – 07/31/2012 NSF-DBI

**Bioinformatics of Molecular Timetrees**

The focus is on building a knowledgebase of species divergence times inferred from molecular data.
NAME
Deirdre R. Meldrum

POSITION TITLE
Dean, Ira A. Fulton School of Engineering
Director, Center for Ecogenomics, Biodesign Inst.
Professor of Electrical Engineering

eRA COMMONS USER NAME
deedee

EDUCATION/TRAINING
(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)

INSTITUTION AND LOCATION DEGREE YEAR(s) FIELD OF STUDY
University of Washington, Seattle, WA B.S. 1978-83 Civil Engineering
Rensselaer Polytechnic Institute, Troy, WA M.S. 1983-85 Electrical Engineering
Stanford University, Stanford, CA Ph.D. 1987-93 Electrical Engineering

A. Positions and Honors

Positions and Employment
1979 Engineering Co-op Student, Puget Sound Naval Shipyard, Bremerton, WA (designed & inspected foundations on Navy ships and submarines.)
1980-1981 Engineering Co-op Student, NASA Johnson Space Center, Houston, TX (Shuttle Mission Simulator instructor for astronauts.)
1982-1983 Design Engineer, Washington State Department of Transportation, Seattle, WA
1983-1984 Teaching Assistant, Department of Electrical Engineering, Rensselaer Polytechnic Institute, Troy, NY (courses in Probability, Discrete Time Systems, Linear Systems, & General Engr. Lab)
1984 Summer Intern, Galileo Flight Test Group, Guidance & Control Section, Jet Propulsion Laboratory, Pasadena, CA (tested the Galileo spacecraft flight software with flight hardware in the loop.)
1985-1987 Member of Technical Staff, Machine Intelligence Systems Group, Automated Systems Section, Jet Propulsion Laboratory, Pasadena, CA (developed algorithms for robot control, flexible space structure control, & identification. Tested identification algorithms on a 12-rib flexible antenna experiment.)
1987-1988 Graduate Research Assistant, Department of Electrical Engineering, Stanford University (investigated adaptive time-optimal control of flexible structures, advisor Gene F. Franklin)
1988-1989 Teaching Assistant, Department of Electrical Engineering, Stanford University (courses in Digital Signal Processing, Feedback Control Design, Electromagnetic Fundamentals, and Electromagnetic Waves)
1989-1992 NASA Fellow and Amelia Earhart Fellow, Stanford University (Obtained new results in adaptive control of multi-link serial manipulators using spatial operator algebra.)
1992-1998 Assistant Professor, Dept. of Electrical Engineering, Univ. of Washington
1997-1998 Adjunct Assistant Professor, Dept. of Bioengineering, Univ. of Washington
1998-2001 Associate Professor, Dept. of Electrical Engineering, Univ. of Washington
2001-2006 PI & Director (with M. Lidstrom), NIH Center of Excellence in Genomic Science (CEGS) Microscale Life Sciences Center (MLSC)
Professor, Dept. of Electrical Engineering, Univ. of Washington
Adjunct Professor, Dept. of Bioengineering, Univ. of Washington
Adjunct Professor, Dept. of Mechanical Engineering, Univ. of Washington
2007-present Dean, Ira A. Fulton School of Engineering, Arizona State University
Director, Center for Ecogenomics, Biodesign Institute, Arizona State University
Professor of Electrical Engineering, Arizona State University

Other Experience and Professional Memberships
Member, IEEE Robotics & Automation Technical Committee on Automation, 3/97-present; Region 6 Representative, IEEE Engineering, Medicine, & Biology Society (EMBS) Advisory Committee, 1999-2000;

Honors
1989-1991 Zonta International Amelia Earhart Fellowship
1989-1992 NASA Graduate Student Researchers Fellowship
1993-present Sigma Xi, The Scientific Research Society, full member
1993 SAE Ralph R. Teetor Educational Award
1993-1998 NIH NCHGR Special Emphasis Research Career Award
1995 NAE First Annual Symposium on Frontiers of Engineering (1 of 95 invited participants)
1996-2001 Presidential Early Career Award for Scientists and Engineers (nomination by NIH)
"for recognition of innovative research utilizing a broad set of interdisciplinary approaches to advance DNA sequencing technology." [President William Clinton]
1997 University of Idaho Honors Convocation Speaker
2000 Finalist (1 of 4) Best Automation Paper, International Conf. on Robotics and Automation
2000-2004 Member, Peer Review Oversight Group (PROG), Office of the Director, National Institutes of Health (NIH)
2000-2003 Member, Scientific Advisory Board, Joint Genome Institute (JGI), Department of Energy (DOE)
2001-2006 Director (with M. Lidstrom), NIH NHGRI Center of Excellence for Genomic Science (CEGS), Microscale Life Sciences Center (MLSC)
2001-present Faculty of 1000 evaluator of life sciences literature for Genomics Section
2003 Fellow of the American Association for the Advancement of Science (AAAS)
2004 Fellow of the Institute for Electrical and Electronic Engineers (IEEE)
2004 NSF Women Engineering Leadership Advanced Institute participant, Syracuse, New York
2005 Keck Foundation, National Academies' Futures Initiative, Conference Planning Committee
2005-2008 DHHS NIH NHGRI National Advisory Council for Human Genome Research
2006-2007 IEEE Robotics and Automation Society, Distinguished Lecturer
2007-present Microsoft Research, Advisory Board for External Research and Programs

B. Selected peer-reviewed publications (in chronological order)


Patents Granted

C. Research Support

Ongoing Research Support

5 P50 HG002360-05 Meldrum (PI) 8/1/01 – 7/31/11
NIH/NHGRI
CEGSTech: Integrated Biologically-Active Microsystems (01-06)
CEGS: Microscale Life Sciences Center (MLSC) (06-11)

The goal is to develop cutting edge technology for multi-parameter analysis of single cells, & apply this technology to the understanding of biological questions characterized by cellular heterogeneity. Our focus is on disease pathways, & our vision is to address pathways to disease states directly at the individual cell level, at increasing levels of complexity that progressively move to an in vivo understanding of disease. Two model systems, pro-inflammatory cell death (pyroptosis) and neoplastic progression of Barrett's esophagus, are addressed. Investigators are D. Meldrum, M. Lidstrom, K. Böhringer, L. Burgess, B. Cookson, N. Dovichi, M. Holl, A. Jen, B. Parviz, and B. Reid.

2 R01 HG01497-08 Meldrum (PI) 5/1/02 - 09/30/09
NIH/NHGRI
Microscale Instrument Development for Genomic Analysis

The major goal of this proposal is to design and build integrated and automated microsystems to study for genomic signatures at the single cell level.

1R01 GM088818-01 (Yan) PI 8/1/09-5/31/14
NIH/NIGMS
Water Soluble Nanoarrays for Single Cell Proteomics
LaBaer, Joshua

BIOGRAPHICAL SKETCH

NAME
Joshua LaBaer, M.D., Ph.D.

POSITION TITLE
Director, Virginia G. Piper Center for Personalized Diagnostics

eRA COMMONS USER NAME
JLABAER

EDUCATION/TRAINING (Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)

<table>
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<th>YEAR(s)</th>
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</tr>
</thead>
<tbody>
<tr>
<td>University of California, Berkeley</td>
<td>B.S.</td>
<td>1981</td>
<td>Nutritional Sciences</td>
</tr>
<tr>
<td>University of California, San Francisco</td>
<td>Ph.D.</td>
<td>1989</td>
<td>Biochemistry &amp; Biophysics</td>
</tr>
<tr>
<td>University of California, San Francisco</td>
<td>M.D.</td>
<td>1990</td>
<td>Medicine</td>
</tr>
</tbody>
</table>

A. Positions and Honors.

Positions:
1979-81: Undergraduate Honors Research, Dept. of Nutritional Sciences, UC Berkeley; laboratory of Dr. Leonard F. Bjeldanes, Professor of Nutritional Sciences. Induction of cytochrome P450-related enzymes
1981-90: Medical Scientist Training Program, University of California, San Francisco
1982: Laboratory of Dr. J. Michael Bishop, Professor of Microbiology and Immunology, Nobel Laureate in Medicine 1988. Gene amplification of oncogenes in tumor cell lines.
1984: Laboratory of Dr. Anthony L. DeFranco, Professor of Microbiology and Immunology. Mechanisms of B lymphocyte activation by anti-receptor antibodies.
1990-91: Intern in Internal Medicine, Brigham and Women's Hospital, Boston, MA
1991-92: Resident in Internal Medicine, Brigham and Women's Hospital, Boston, MA
1992-93: Clinical Fellow in Oncology, Dana-Farber Cancer Institute, Boston, MA
1994-99: Postdoctoral fellow of Dr. Ed Harlow, American Cancer Society Professor of Genetics, Molecular Biologist, Massachusetts General Hospital. Cell cycle regulation.
1994 - 1999: Instructor in Medicine, Harvard Medical School
1999- : Director, Harvard Institute of Proteomics, Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School
2009: Otto Hertz Memorial Lectureship
2009: Board of Advisors, National Institutes of Health – National Cancer Institute
2009- : Director, Virginia G. Piper Center for Personalized Diagnostics, The Biodesign Institute, Arizona State University

Honors and Awards:
1981: Phi Beta Kappa, UC Berkeley
1981: Departmental Citation for Most Outstanding Student Research, Department of Nutritional Sciences, UC Berkeley
1981: Graduation with Highest Honors, UC Berkeley
1981: University Medal for Most Distinguished Graduating Senior, UC Berkeley
1985: Dean’s Prize for Student Research, UCSF
1986-87: Chancellor’s Fellowship, UCSF
1989: Elected to Alpha Omega Alpha, UCSF
2001-04 Arthur and Rochelle Belfer Foundation Awardee, Breast Cancer Research Foundation
2005- : Breast Cancer Research Foundation
2007: Kavli Frontiers of Science Symposium Recipient

B. Selected peer-reviewed publications (in chronological order).


25. LaBaer, Joshua PHS 398/2590 (Rev. 02/06) Page 18 Biographical Sketch Format Page


C. Research Support.

**Ongoing Research Support**


NIH Development and Implementation of a Materials Repository for the PSI

In the course of protein structural studies, the Protein Structure Initiative (PSI) has produced thousands of useful protein expression clones that they plan to share with the community through a proposed PSI-Materials Repository. The Harvard Institute of Proteomics (HIP) has more than five years of experience in the production, sequence verification, distribution and use of tens of plasmids encoding whole genomes for biodefense related organisms to thousands of human genes. HIP will use its expertise to create the PSI-Materials Repository to give their scientific community access to the PSI protein expression clones.

Role: Co-PI

5 P30 CA06516-50 Howley (PI) 7/01/00 – 11/30/2010

NCI Cancer Center Support Grant

Supports a Core that provides DNA sequencing and Clone Resources for a multi-institutional cancer center.
Role: Site PI

2 P01 CA80111  LaBaer/Weinberg (PI)  3/16/04 – 1/31/09
NIH/NCI, Subcontract with Whitehead Institute
Mechanisms of Breast Development and Carcinogenesis (Core A)
Use a novel gene cloning strategy to produce master cDNA clones that will allow the members of the P01 to have easy access to large numbers of open reading frames important in the normal development of the breast tumor formation.
Role: PI

HHHS266200400053C  LaBaer (PI)  8/2/04 – 8/1/09
NIH/NCI
Identifying Targets for Therapeutic Interventions Using Proteomics Technology
This contract supports the capture of all the ORFs for two important biodefense pathogens, V. cholerae and B. anthracis, and to produce and study the protein for these organisms on protein microarrays.
Role: PI

Breast Cancer Research Foundation  LaBaer (PI)  10/1/01 – 9/30/08
Breast Cancer 1000 Project
Supports a postdoctoral position to develop and perform high-throughput screens in human breast epithelial cells to identify genes and pathways which contribute to hormone resistance in breast cancer.

1 U01 CA117374-01  LaBaer (PI)  8/11/05 – 7/31/10
NIH/NCI
Biomarker Detection Using NAPPA Tumor Antigen Arrays
The major goal of this project is to use the NAPPA protein microarray technology to discover autoantibodies that detect the presence of cancer in early breast cancer. Tumor antigen protein arrays programmed with genes for breast cancer will be probed with serum from patients with early stage breast cancer or normal controls to identify informative antigens.
Role: PI

U54 AI057159 (Kasper)  9/04/00-02/29/08
NIAID
New England Regional Center for Excellence for Biodefense and Emerging Infectious Diseases Research
The main goal of the project is to develop novel approaches and technologies for HTP production of proteins and protein complexes for structural analysis and any other application requiring mg amounts of proteins.

RO1 HG003828 (Larson)
HTS of Small molecule-protein interactions
Development of a nanomole array biosensor for use in screening and characterizing the interactions between small molecules and immobilized protein targets.

Juvenile Diabetes Research Foundation (LaBaer)  9/1/07-8/31/10
The use of protein microarrays to study autoimmunity and diabetes
The main objective of this project is to find new biochemical markers for T1D that can ultimately be used to provide for an earlier diagnosis of the disease. In addition these markers might find use in predicting the course of the disease or even subclassifying patients into groups that might respond better to specific therapies.

Completed Research Support

U01 DK07273  Magnuson (PI)  4/1/06 – 3/31/07
NIH/NIDDK, Subcontract with Vanderbilt University
Monoclonal Antibodies Directed toward Developing Beta Cell
The main objectives of this project are to develop a comprehensive panel of monoclonal antibodies directed against cell surface molecules expressed by endoderm and/or developing endocrine cells.
Role: PI

U01 DK56047  LaBaer (PI)  9/30/02 – 7/31/07
NIH/NIDDK, Subcontract with UPENN 539693
Functional Genomics of the Beta Cell
A collaboration with Klaus Kaestner (UPenn) and Chris Newgard (Duke) designed to build systems biology resources relevant to type 1 diabetes. Genes specific to the pancreas, especially the plasma membrane, have been cloned, sequenced and moved to expression vectors for high throughput functional experiments of tumor cells in culture.
Role: PI

R33 CA99191-03  LaBaer (PI)  5/1/04 – 4/30/07
NIH/NCI
Functional Proteomics of Breast Cancer
Supports the development of a novel protein array technology, Nucleic Acid-Programmable Protein Array (NAPPA); in which DNA clones are spotted, expressed using a mammalian cell-free system, and immobilized in situ.
Role: PI
NAME
Tao, Nongjian

POSITION TITLE
Professor & Center Director

eRA COMMONS USER NAME
TAONJT

EDUCATION/TRAINING (Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)

<table>
<thead>
<tr>
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<th>DEGREE (if applicable)</th>
<th>YEAR(s)</th>
<th>FIELD OF STUDY</th>
</tr>
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<tbody>
<tr>
<td>Arizona State University</td>
<td>Ph.D</td>
<td>1988</td>
<td>Biophysics</td>
</tr>
<tr>
<td>Anhui University</td>
<td>B.S.</td>
<td>1984</td>
<td>Physics</td>
</tr>
</tbody>
</table>

A. Positions and Honors.

Professional experience
2008 - present  Director, Center for Bioelectronics and Biosensors, Biodesign Institute at Arizona State University
2001 - present  Professor, Electrical Engineering & Chemistry, Arizona State University
1997- 2001      Associate Professor, Physics, Florida Int’l University.
                 Adjunct Professor, Chemistry, University of Miami.
1992 - 1997      Assistant Professor, Florida International University.
1988 – 1990      Postdoctoral Fellow, City College of City University of New York.

Professional service
• Co-Organizer, Symposium on Nanomaterials, Electrochemical Society Meeting, 2007.
• Chair, Electrochemical Society Annual Meeting, Washington DC., Oct. 8-12, 2007
• Chair, 9th International Mutation Detection Symposium, Xiamen, China, Sept 23-26, 2007.
• Co-Organizer, Symposium on Molecular-Scale Electronics, Material Research Society Meeting, 2005.
• Co-Organizer, Symposium on Nanoengineered Materials, Electrochemical Society Meeting, 2005.
• Chair, 12th International Colloquium on STM, Dec. 11, 2004.
• Science and Engineering Program, Executive Committee, Arizona State University, 2002-2004.
• Co-Organizer, Symposium of Scanning Probe Microscopy, Electrochemical Society Meeting, 2002.

Honors
• Arizona Technology Enterprise Innovator of the Year 2006.
• Alexander von Humboldt Research Award, 2004.
• Hellmuth Fischer Medal, Germany, 2003.
• Excellence in Research Award, Florida International University, 2000.
• Molecular Imaging's Young Microscopist Award, 1996.
• Over 150 invited and keynote talks.

B. Publications (selected from 160 peer-reviewed publications and 15 book chapters)


Patents and intellectual properties:

C. Research Support

**Ongoing Research Support:**
- 5U01ES016064, “A Wearable Wireless System for Real-Time Monitoring of Chemical Toxicants”,
  Goal: To develop and build a wearable wireless sensor system for quantitative and real-time measurement of multiple environmental toxicants at the point of contact based on microfabricated quartz tuning fork sensors. Such a system will be used in population studies for better understanding the role of gene-environment interactions in human diseases and for health disparities research.
  HHS-NIH-NIEHS-National Institute of Environmental Health, 8/15/07-8/14/11.
  NJ Tao (PI), Erica Forzani (Co-PI), Ray Tsui from Motorola (Co-PI),
  - AGR 8/7/07, “Integrated sensor for explosive detection based on multiple orthogonal detection schemes”
  Goal: To build an orthogonal system based of three sensing platforms for fast and real-time detection of explosives. The system includes preconcentrator, sensing platforms and a signal processing unit.
  MASIN Consortium, DIA, 7/20/2007 – 7/19/10
  Ray Tsui (PI), Joseph Wang, NJ Tao, Douglas Cochran and Kevin Linker
  - CHE-0243423, “Probing Single Redox Molecules and Ions with Nanoelectrodes”
  Goal: To study redox molecules and metal ions with nanoelectrodes.
  NSF, 3/1/06 - 2/28/09
  NJ Tao (PI)
• DE-FG03-01ER45943, “Electrical, thermal and optical properties of single molecule devices”

**Goal:** To study Electrical, thermal and optical properties of single molecules wired into electrical circuits.
DOE, 3/1/05 - 2/28/08
NJ Tao (PI)

**Completed Research Support (past 3 years)**

• 07-06126490, “Demonstration of Tuning Fork Technology for Dial Corporation: Development of chemical sensors for indoor air quality” - Phase II

**Goal:** To built an efficient system to detect mal-odors (sulfur derivative molecules) in indoor air. The system includes filters for interferents and a sensing element based on a tuning fork.
Dial Co., Scottsdale, AZ, 11/15/07 to 11/15/08.
NJ Tao (PI), Erica Forzani (Co-PI).

• “From Molecule Gated Nanowires toward Electron Transport with Single Molecules”

**Goal:** To develop a collaborative effort to study single molecule electronics.
Volkswagen Foundation, 5/1/05 - 4/31/08
Wandlowski (PI), Tao and Mayor

• ECS0304682, “Epitaxial Silicides for Nanoelectronics”

**Goal:** This is a Nano Interdisciplinary Research Team Award. The major goal is to understand electron transport properties of silicide nanowires and develop biosensor applications with silicides.
NSF, 7/1/03 - 6/30/07
Bennett (PI), Bird and Tao

• “Ballistic magnetoresistance in magnetic nanocontacts”

**Goal:** This is collaborative project between SUNY-Buffalo and Arizona State University to investigate magneto-transport properties of nanocontacts fabricated with electrochemical methods.
NSF, 6/1/03 - 5/30/07
Chopra (PI) and Tao (Co-PI)

• AF8650-06-C-7623, “A Rapid Screening Tool for Molecular Electronics”

**Goal:** The goal of this project is to develop a stable, fast and automated device for routine screening of molecules for electronic device applications.
DARPA, 04/01/06 – 9/31/07
NJ Tao (PI)

• “Chemical and Bio nanosensors”

**Goal:** Explore the capability of nanofabricated Silicon-based chips to produce nanosensors, including nanojunctions and single wall carbon nanotubes.
Motorola Co., Tempe, AZ, 8/1/04 – 7/31/06.
NJ Tao (PI), Erica Forzani (Co-PI).

• 07-06126490, “Demonstration of Tuning Fork Technology for Dial Corporation: Development of chemical sensors for indoor air quality” - Phase I,

**Goal:** To explore commercial polymers' properties to improve selectivity towards the detection of mal-odors (sulfur derivatives).
Dial Co., Scottsdale, AZ, 12/15/2006 - 04/01/07
NJ Tao (PI) and Erica Forzani (Co-PI).

• DE-FG03-01ER45943, “Probing Mechanical Properties of Single Molecules”

**Goal:** To develop a method to study mechanical properties of single molecules.
DOE, 3/1/05-3/31/08.
N.J. Tao (PI)
BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2. Follow this format for each person. DO NOT EXCEED FOUR PAGES.

NAME
Bruce E. Rittmann

POSITION TITLE
Director, Center for Environmental Biotechnology and Professor of Civil and Environmental Engr.

eRA COMMONS USER NAME
BRITTMAN

EDUCATION/TRAINING (Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)

<table>
<thead>
<tr>
<th>INSTITUTION AND LOCATION</th>
<th>DEGREE (if applicable)</th>
<th>YEAR(s)</th>
<th>FIELD OF STUDY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Washington University, St. Louis, MO</td>
<td>BS</td>
<td>1974</td>
<td>Civil Engineering</td>
</tr>
<tr>
<td>Washington University, St. Louis, MO</td>
<td>MS</td>
<td>1974</td>
<td>Environmental Engineering</td>
</tr>
<tr>
<td>Stanford University, Stanford, CA</td>
<td>PhD</td>
<td>1979</td>
<td>Environmental Engineering</td>
</tr>
</tbody>
</table>

A. Positions and Honors.

Positions
2005-now  Director, Center for Environmental Biotechnology, Biodesign Institute at Arizona State University
2005-now  Professor, Dept. of Civil and Environmental Engineering, Arizona State University, with an affiliate appointment in the Dept. of Chemical and Materials Engineering
1992-2004  John Evans Professor of Environmental Engineering, Dept. of Civil and Environmental Engineering, Northwestern University
1988-1992  Professor of Environmental Engineering, University of Illinois at Urbana-Champaign
1984-1988  Associate Professor of Environmental Engineering, University of Illinois at Urbana-Champaign
1980-1984  Assistant Professor of Environmental Engineering, University of Illinois at Urbana-Champaign
1979      Post-Doctoral Scholar and Lecturer, Stanford University
1979      Lecturer, San Jose State University, San Jose, CA
1975-1979  Graduate Research Assistant, Stanford University

Selected Honors
2009      Simon A. Freese Award and Lecture, American Society of Civil Engineers
2008      Faculty Achievement Award for Excellence in Defining Research, Nat. Science & Math, ASU
2004      Member, National Academy of Engineering
2004      Distinguished Lecturer, Association of Environmental Engineering and Science Professors
2002      ISI List of Most Highly Cited Researchers, Institute for Scientific Information
1999      Award of Excellence in Research and Development, Ontario Ministry Environment
1998      Founders Award, USA National Committee of Intl. Assoc. on Water Quality
1996      Fellow, American Association for the Advancement of Sciences
1994      A.R.I. Clarke Prize or Outstanding Achievement in Water Science an Technology, National Water Research Institute
1990-2004  Managing Editor, Biodegradation; Editor-in-Chief (1996-2004)
1990      Walter Huber Research Prize, American Society of Civil Engineers
1990      Academic Achievement Award, American Water Works Assn.
1987      University Scholar Award, Univ. of Illinois
1985      Xerox Faculty Research Award, Univ. of Illinois
1984      Presidential Young Investigator Award, National Science Foundation
B. Selected peer-reviewed publications. (For 2000-2008 and out of approximately 400 since 1978)


C. Research Support.

On-Going Research Support

OpenCEL LLC              06-01446        Rittmann (PI)      8/05-7/09
Capturing the Energy Value of Organic Wastes Made More Biodegradable by OpenCEL Electroporation Technology
Evaluates the microbial ecology and kinetics for converting organic waste streams to methane, biohydrogen, or electricity after pre-treatment with the OpenCEL electroporation technology.
Role: Principal Investigator

EPA          RD8332201         Westerhoff (PI)  1/07-04/10
Biological Fate and Electron Microscopy Detection of Nanoparticles in Wastewater Treatment
Evaluates the mechanisms affecting the fate of a range of nanomaterials that enter biological wastewater treatment facilities.
Role: Co-Principal Investigator

ESTCP (DoD)                 W912HQ07C0033        Rittmann (PI)           08/07-08/10
Assessment of the Natural Attenuation of NAPL Source Zone and Post-Treatment NAPL Source Zone Residual
Defines and demonstrates methods to evaluate natural attenuation of chlorinated solvents in source zones involving DNAPLs
Role: Principal Investigator

NSF                0651794                  Rittmann (PI)      4/07-3/10
Intimate Coupling of Photocatalysis and Biodegradation in a Photocatalytic Circulating-Bed Biofilm Reactor
Develops the scientific basis for a novel method to combine TiO$_2$-based photocatalysis with biodegradation in one reactor and for the purpose of biodegrading very recalcitrant organic contaminants.
Role: Principal Investigator

Methuselah Foundation          AGR07129310   Rittmann (PI)               8/07-8/09
Medical Bioremediation
Explores means to find and utilize microorganisms-derived enzymes to eliminate macromolecules that accumulate in human cells over a long time and are associated with diseases of aging, such as atherosclerosis and macular degeneration.
Role: Principal Investigator

CH2M-HILL             AGR01/12/08              Rittmann (PI)   08/07-9/09
AwwaRF TC Project 4131: Optimizing the Sustainability of Treatment Processes for Nitrate Removal in Inland Communities
Demonstrate at the pilot scale biological denitrification of groundwater.
Role: Principal Investigator

Awwa Research Foundation 4065                  Westerhoff (PI)  10/06-10/09
Organic Chloramine Formation in Water Distribution Systems and Influence on Nitrification and disinfection Efficacy
Explore the role of organic chloramines in drinking-water distribution systems in terms of promoting nitrification and having disinfection power.
Role: Co-Principal Investigator

Applied Process Technology, Inc.    AGR01/01/08          Rittmann (PI)    10/07-9/09
Advancing the Membrane Biofilm Reactor
Investigate fundamental mechanisms that control the effectiveness of the hydrogen-based membrane biofilm reactor for bio-reducing a range of oxidized contaminants found in water and wastewater.
Role: Principal Investigator

Applied Process Technology, Inc. AGR 01/01/08         Rittmann (PI) 5/08-11/08
MBfR Reduction of Selenate for Wastewater from the Scrubber of a Coal fired Power Plant
Determine the feasibility of bioreducing selenate in the wastewater from the scrubber of a coal-fired power plant. Set up two MBfRs and inoculate with high-salinity cultures; determine the kinetics for bioreduction of selenate; assess the microbial ecology in the biofilm
Role: Principal Investigator

Science Foundation Arizona and BP SRG0200-07 Rittmann (PI) 9/07-8/09
Cyanobacteria for Generating Solar-Powered, Carbon-Neutral, and Cost-Effective Bio-diesel
Carry out a proof-of-concept study for the production of renewable hydrocarbon-fuel feedstock based on capturing solar energy with photosynthetic bacteria
Role: Principal Investigator

Arizona Water Institute AWI-08-04 Brown (co-PI) 02/08-1/09
Evaluating Proposed Operational Practices for Control of Naegleria fowleri in Arizona’s Public Drinking Water Systems
Systematically look for the occurrence of the protozoan Naegleria fowleri in full-scale and pilot drinking-water distribution systems and correlate its occurrence to biofilm formation.
Role: co-Principal Investigator

Water Environment Research Foundation, as a sub-contract from Montgomery-Watson-Harza AGR08070839 Rittmann (PI) 10/07-4/09
Investigation of Membrane Bioreactor Effluent Water Quality and Technology
Develop a model for the water quality produced by membrane biofilm reactors
Role: Principal Investigator

Montgomery-Watson-Harza AGR08070839 Rittmann (PI) 01/06-2/09
Biological Destruction of Perchlorate and Nitrate in Ion Exchange Concentrate
Carry out bench-scale research to determine the kinetics and ecology for hydrogen-based reduction of nitrate and perchlorate in ion-exchange brine.
Role: Principal Investigator

Applied Process Technology, Inc. AGR05/02/08 Rittmann (PI) 5/08-5/09
Membrane Biofilm Reactor for Ex Situ Treatment of NDMA and Co-Contaminants
Determine the kinetics and ecology associated with bio-reduction of NDMA and co-contamiant using the hydrogen-based membrane biofilm reactor
Role: Principal Investigator

Intellectual Fusion Fund, ASU Foundation BT91109 Rittmann (PI) 3/08-02/13
Designer Organisms and Fuel Cells for Biohydrogen Production
Develop the science base for microbiological generation of renewable hydrogen gas
Role: Principal Investigator
BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors.
Follow this format for each person. DO NOT EXCEED FOUR PAGES.

NAME
Roy Curtiss III

POSITION TITLE
Professor, School of Life Sciences, School of Life Sciences and Director, Center for Infectious Diseases and Vaccinology

eRA COMMONS USER NAME
Roy_Curtiss

EDUCATION/TRAINING (Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)

<table>
<thead>
<tr>
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<th>FIELD OF STUDY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cornell University, Ithaca, NY</td>
<td>B.S.</td>
<td>1956</td>
<td>Agriculture</td>
</tr>
<tr>
<td>University of Chicago, Chicago, IL</td>
<td>Ph.D.</td>
<td>1962</td>
<td>Microbiology</td>
</tr>
</tbody>
</table>

RESEARCH AND PROFESSIONAL EXPERIENCE

2007-Present  Director, Center for Infectious Diseases and Vaccinology, The Biodesign Institute, Arizona State University, Tempe, AZ

2004-2007 Co-Director, Center for Infectious Diseases and Vaccinology, The Biodesign Institute, Arizona State University, Tempe, AZ

2004-Present  Professor of Life Sciences, School of Life Sciences, Arizona State University, Tempe, AZ

2005-Present  George William and Irene Koechig Freiberg Professor of Biology Emeritus, Department of Biology, Washington University, St. Louis, MO

1984-2005  George William and Irene Koechig Freiberg Professor of Biology, Department of Biology, Washington University, St. Louis, MO

1983-2005  Professor, Department of Biology, Washington University, St. Louis, MO

1993-2000  Founder, Member Board of Directors, MEGAN Health, Inc., St. Louis, MO

1998-1999  Vice President of Research, MEGAN Health, Inc., St. Louis, MO

1991-1994  Professor/Collaborator, Iowa State University, Ames, IA

1991-1994  Director, Center for Plant Science and Biotechnology, Washington University, St. Louis, MO

1983-1991  Prof of Cellular & Molecular Biol, Sch of Dental Med, Washington University, St. Louis, MO

1983-1993  Chairman, Department of Biology, Washington University, St. Louis, MO

1983-1987  Adjunct Professor, Department of Microbiology, UAB-Birmingham, AL

1982-1983  Acting Chairman, Department of Microbiology, UAB-Birmingham, AL

1981-1982  Vice Chairman, Department of Microbiology, UAB-Birmingham, AL

1981-1983  Director and Senior Scientist, Cystic Fibrosis Research Center, UAB-Birmingham, AL

1977-1983  Charles H. McCauley Professor of Microbiology, UAB-Birmingham, AL

1976-1981; 1983  Dir, Infectious Diseases Postdoctoral Trng Prog, UAB-Birmingham, AL

1975-1983  Dir, Molecular, Cellular & Med Genetics Postdoctoral Trng Prog, UAB-Birmingham, AL

1973-1982  Dir, Molecular Cell Biology Grad Program, UAB-Birmingham, AL

1972-1983  Prof of Microbiology, UAB-Birmingham, AL

1972-1983  Sr Scientist, Inst of Dental Research, UAB-Birmingham, AL

1972-1983  Sr Scientist, Comprehensive Cancer Ctr, UAB-Birmingham, AL

1971-1972  Interim & Trng Dir, UT-Oak Ridge Grad Sch of Biomed Sciences, Oak Ridge, TN

1970-1971  Assoc Dir, UT-Oak Ridge Grad Sch of Biomed Sciences, Oak Ridge, TN

1969-1972  Professor, UT-Oak Ridge Grad Sch of Biomed Sciences, Oak Ridge, TN

1967-1969  Lecturer, UT-Oak Ridge Grad Sch of Biomed Sciences, Oak Ridge, TN

1965-1972  Lecturer, Dept of Microbiol, Univ of Tenn-Knoxville, Knoxville, TN

1969-1972  Leader, Microbial Genetics & Radiation Micro Group, Biol Div, Oak Ridge Natl Lab, TN

1963-1972  Biologist, Biol Div, Oak Ridge Natl Lab, Oak Ridge, TN

1956-1958  Jr. Technical Specialist, Biology Div, Brookhaven Natl Lab, Upton, NY

1955-1956  Laboratory Instructor & Research Assistant, Dept Poultry Husbandry, Cornell Univ, Ithaca, NY

HONORS

NY State Regents Scholar (52-56); Esso 4-H Scholarship (52-56); Danforth Fellowship (55); University Fellow (58-60); NIH Pre-doctoral Fellow (60-62); Sigma Xi (62); Visiting Prof, Instituto Venezolano de Investigaciones Cientificas (69), Univ of Puerto Rico (72), Universidad Catolica de Chile (73), and Univ of Oklahoma (83); Honorary Member, Asociacion Chilena de Microbiologia (73); Editorial Bd Member (66-70) and Editor (70-76)
PUBLICATIONS (recent out of 288)


protection against *Streptococcus pneumoniae* infection following oral vaccination with attenuated *Salmonella* expressing PspA antigen. J. Immunol. **181**:6447-6455.


Dieye, Y., K. Ameiss, M. Mellata, and R. Curtiss III. 2009. The *Salmonella* pathogenicity island (SPI) 1 contributes more than SPI2 to the colonization of the chicken by *Salmonella enterica* serovar Typhimurium. BMC Microbiol. **9**:3.


Branger, C. G., J. Fetherston, A. Torres-Escobar, W. Sun, R. Perry, K. L. Roland, R. Curtiss III. 2009. Oral Vaccination with LcrV from *Yersinia pestis* KIM delivered by live attenuated *Salmonella enterica* serovar Typhimurium elicits a protective immune response against challenge with *Yersinia pseudotuberculosis* and *Yersinia enterocolitica*. Vaccine. Accepted

A live recombinant attenuated Salmonella anti-pneumococcal vaccine for newborns

To design, construct and evaluate novel innovative low-cost recombinant Salmonella vaccines expressing multiple protective Streptococcus pneumoniae antigens that are safe for fetuses, newborns, infants and immunocompromised or malnourished individuals and will induce after oral needle-free immunization protective immunity to diverse S. pneumoniae serotypes. Role: PI

R01 AI065779 NIH/NIAID 07/01/05-01/31/10 (no-cost extension)
Salmonella anti-influenza DNA & antigen delivery vaccine

Our objective is to design, construct and evaluate a novel inexpensive rapidly modifiable vaccine for oral needle-free vaccination to deliver DNA vaccines and protective antigens/epitopes to induce protective immunity, including mucosal immunity, in children and adults to prevent infection by influenza viruses with avian and human antigenic components. In addition to relying on inducing protective immunity to HA antigens, we will thoroughly investigate the potential to induce a longer lasting cross-protective cellular immunity by delivery of conserved antigens containing T-cell epitopes. The vaccine delivery system employs attenuated Salmonella strains with special newly developed features to maximize colonization of lymphoid tissues and that display a regulated delayed lysis in vivo phenotype to release DNA vaccines encoding influenza hemagglutinin antigens in the cytosol of host cells and deliver protective antigens/epitopes to other tissues. Role: PI

Science Foundation Arizona (Bruce Rittman, PI) 05/01/2007-07/31/09
Cyanobacteria for Generating Solar-Powered, Carbon Neutral and Cost-Effective Biodiesel
Role: Co-PI

2007-35201-18519 USDA NRICGP 08/15/07-08/14/10
Food safety vaccine of economic benefit to poultry producers to prevent Salmonella and APEC infections and foodborne transmission to humans.

Our objectives are to genetically engineer the S. Typhimurium vaccine strain to: (i) minimize induction of immune responses to serotype-specific antigens and maximize induction of cross-protective immunity to common related antigens of S. enterica strains of diverse serotypes; (ii) cure its virulence plasmid and insert the virulence plasmid spv operon into the chromosome; and (iii) express APEC antigens to enhance induction of cross-protective immunity to APEC serotypes. We will evaluate abilities of the different RASV strains to colonize lymphoid tissues in mice and chickens, exhibit biological containment attributes, induce mucosal, systemic and cellular immunities to different Salmonella and APEC serotypes and eliminate or reduce Salmonella colonization and APEC infection of poultry. Role: PI

COMPLETED RESEARCH SUPPORT
R01 AI057885 NIH/NIAID 01/01/04-12/31/09 (no-cost extension)
Attenuated Live and Recombinant Yersinia pestis Vaccines—Role: PI

U01 AI60557 NIH/NIAID 09/30/03-03/31/09
S. typhimurium Vaccine Against Bacterial Enteropathogens—Role: PI

ID-SS-0520-03 Ellison Medical Foundation 11/01/03-10/31/07
Providing an economic benefit to using a vaccine to enhance food safety and reduce antibiotic use—Role: PI

R01 AI024533 NIH/NIAID 01/01/87-05/31/07
Molecular Genetic Analysis of Salmonella Pathogenicity—Role: PI

2003-35204-16160 USDA NRICGP 08/01/03-07/31/06
Attenuated Salmonella Antigen and DNA Vaccine Delivery Vaccines Against Eimeria—Role: PI

R01 DE06669 NIH/NIDCR 04/01/83-04/30/06
Antigen Delivery System Design for Host Defense Analysis—Role: PI
BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

<table>
<thead>
<tr>
<th>NAME</th>
<th>POSITION TITLE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sidney M. Hecht</td>
<td>Director, Center for BioEnergetics</td>
</tr>
<tr>
<td></td>
<td>Professor of Chemistry</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>NAME</th>
<th>eRA COMMONS USER NAME (credential, e.g., agency login)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SIDNEYHECHT</td>
</tr>
</tbody>
</table>

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

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<th>DEGREE (if applicable)</th>
<th>YEAR(s)</th>
<th>FIELD OF STUDY</th>
</tr>
</thead>
<tbody>
<tr>
<td>University of Rochester, Rochester, NY</td>
<td>A.B.</td>
<td>1966</td>
<td>Chemistry</td>
</tr>
<tr>
<td>University of Illinois, Urbana, IL</td>
<td>Ph.D.</td>
<td>1970</td>
<td>Chemistry (Biochem.)</td>
</tr>
<tr>
<td>University of Wisconsin, Madison, WI</td>
<td>Postdoctoral</td>
<td>1970-1971</td>
<td>Molecular Biology</td>
</tr>
</tbody>
</table>

A. Positions and Honors

**Positions and Employment**
1970-1971 Research Associate, Laboratory of Molecular Biology, University of Wisconsin
1971-1975 Assistant Professor, Massachusetts Institute of Technology
1975-1979 Associate Professor, Massachusetts Institute of Technology
1978-2008 John W. Mallet Professor of Chemistry and Professor of Biology, University of Virginia
1981-1983 Vice President, Preclinical R&D, Smith Kline & French Laboratories
1983-1986 Vice President, Chemical R&D, Smith Kline & French Laboratories
1986-1987 Distinguished Fellow, Smith Kline & French Laboratories
2006-2008 Adjunct Professor and Director, Center for BioEnergetics, Arizona State University
2008- Professor and Director, Center for BioEnergetics, Arizona State University

**Other Experience and Professional Memberships**
1978 Visiting Professor, Max-Planck Institut für experimentelle Medizin, Göttingen
1979-1981 Center for Advanced Studies, University of Virginia
1984 T.Y. Shen visiting Professor, Massachusetts Institute of Technology
1985-1989 Member, Walter Reed Scientific Advisory Board
1986-1990 Member, California Institute of Technology Chemistry and Chemical Engineering Division Visiting Committee
1987-1991 Member, Advisory Board, Research Institute of Pharmaceutical Sciences, University of Mississippi
1989-1994 Member, Board of Directors, Harbor Branch Oceanographic Institution
1991 Professor Associé, Muséum National D’Histoire Naturelle, Paris
1991 Gastprofessor, Eidgenössische Technische Hochschule, Zurich
1992-1993 Chairman, Review panel for Laboratory of Medicinal Chemistry, National Cancer Institute
1992- Associate Editor, Journal of the American Chemical Society
1993-1995 Member, Board of Scientific Counselors, Division of Cancer Treatment, National Cancer Institute
1999 Chairman, Special Emphasis Review Panel, National Cancer Institute
2002 Search Committee, Laboratory of Medicinal Chemistry, National Cancer Institute

**Honors and Awards**
1967-1970 NIH Predoctoral Fellow
1970-1971 NIH Postdoctoral Fellow
1975-1980 NIH Career Development Awardee
1975-1979 Alfred P. Sloan Research Fellow
1977-1978 John Simon Guggenheim Fellow
1996     Arthur C. Cope Scholar Award, American Chemical Society
1996     Virginia’s Outstanding Scientist
1998     American Society of Pharmacognosy Research Achievement Award
2004     Fellow, American Association for the Advancement of Science
2007     Fellow, American Society of Pharmacognosy

27 Lectureships at Other Universities

B. Selected peer-review publications (selected from more than 385 publications)

R. D. Anderson, J. Zhou and S. M. Hecht, Fluorescence Resonance Energy Transfer Between Unnatural

B. M. Eisenhauer and S. M. Hecht, Site-Specific Incorporation of (Aminooxy)acetic Acid into Dihydrofolate
Reductase, Biochemistry, 41, 11472-11478 (2002).

L. M. Dedkova, N. E. Fahmi, S. Y. Golovine and S. M. Hecht, Efficient D-Amino Acid Incorporation into Protein

L. Tian, C. D. Claeboe, S. M. Hecht and S. Shuman, Guarding the Genome: Electrostatic Repulsion of Water

C. D. Claeboe, R. Gao and S. M. Hecht, 3'-Modified Oligonucleotides by Reverse DNA Synthesis, Nucleic

A. Cagir, S. H. Jones, R. Gao, B. M. Eisenhauer and S. M. Hecht, Luotonin A. A Naturally Occurring Human


L. Tian, C. D. Claeboe, S. M. Hecht and S. Shuman, Remote Phosphate Contacts Trigger Assembly of the


R. Gao, C. D. Claeboe, B. M. Eisenhauer and S. M. Hecht, Identification of Specific Phosphate Oxygen(s)
Important for DNA Cleavage by Human Topoisomerase I, Biochemistry, 43, 6167-6181 (2004).

Mullen, Identification of Small Molecule Synthetic Inhibitors of DNA Polymerase β by NMR Chemical Shift

G. J. Klarmann, B. M. Eisenhauer, Y. Zhang, K. Sitaraman, D. K., Chatterjee, S. M. Hecht and S. F. J. LeGrice,
Site- and Subunit-Specific Incorporation of Unnatural Amino Acids Into HIV-1 Reverse Transcriptase, Prot.


R. Gao, Y. Zhang, A. K. Choudhury, L. M. Dedkova and S. M. Hecht, Analogues of Vaccinia Virus DNA


C. Research Support

Ongoing Research

Friedreich's Ataxia Research Alliance – Hecht (PI) 5/15/08 – 8/31/09
Analysis and Restoration of Mitochondrial Function
This is a seed grant in support of a new research initiative involving the mitochondrial electron transport chain.

R01 CA116566 Hecht (PI) 2/1/07 – 1/31/10
NCI
Inhibitors of p90Rsk
The goals of this project include the identification and characterization of improved inhibitors of p90Rsk
Role: PI

Completed Research

R01 CA77284 Hecht (PI) 4/1/03 – 6/30/08
NCI
Synthesis of Bleomycin Group Antibiotics and Analogues
The goals of this project include the synthesis of bleomycin group antibiotics phleomycin and tallysomycin, as well as analogues having improved properties at the putative loci of therapeutic action. The latter is to be done in part through the synthesis of combinatorial libraries of BLMs. No renewal application is planned.
Role: PI

R01 CA77359 Hecht (PI) 4/1/03 – 3/31/08
NCI
Elaboration of Modified Proteins Using Misacylated tRNAs
The goal of this project is the elaboration and study of proteins containing structurally modified amino acids at single, predetermined positions. The proteins being studied include dihydrofolate reductase, firefly luciferase, HIV-1 protease and ribonuclease B. A renewal application has been submitted in revised form.
Role: PI
BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2. Follow this format for each person. DO NOT EXCEED FOUR PAGES.

NAME  Stephen Albert Johnston

POSITION TITLE  Professor, School of Life Sciences
Director, Center for Innovations in Medicine

eRA COMMONS USER NAME (credential, e.g., agency login)  sjohnston

EDUCATION/TRAINING  (Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)

<table>
<thead>
<tr>
<th>INSTITUTION AND LOCATION</th>
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<th>YEAR(s)</th>
<th>FIELD OF STUDY</th>
</tr>
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<tbody>
<tr>
<td>University of Wisconsin – Madison</td>
<td>B.S.</td>
<td>1975</td>
<td>Molecular Biology</td>
</tr>
<tr>
<td>University of Wisconsin – Madison</td>
<td>PhD</td>
<td>1976-1981</td>
<td>Genetic &amp; Plant Genetics/Plant Breeding</td>
</tr>
<tr>
<td>The Pennsylvania State University Medical Center</td>
<td>Postdoctoral</td>
<td>1981-1983</td>
<td>Biochemistry</td>
</tr>
</tbody>
</table>

A. Positions and Honors.

2007-Present  Director, Biological Design Graduate Program, ASU
2005-Present  Director, CIM, Biodesign Institute, Professor SOLS, ASU
1999-2005  Professor of Microbiology, UT-Southwestern
1998-2005  Director, Center for Biomedical Inventions, UT-Southwestern
1995-2005  Professor of Internal Medicine, UT-Southwestern
1995-2000  Professor of Biochemistry, UT-Southwestern
1993-2005  Eugene Tragus Chair in Molecular Cardiology
1990-1995  Associate Professor, Internal Medicine and Biochemistry, University of Texas-Southwestern Medical Center
1989-1990  Associate Professor of Biology and Biochemical Engineering, Duke University (Research Career Development Award)
1984-1989  Assistant Professor of Biology and Biochemical Engineering, Duke University
1982-1983  NIH Postdoctoral Fellow, Department of Biochemistry, The Pennsylvania State University Medical Center (with Dr. James E. Hopper), Research: The isolation and characterization of the GAL4 regulatory gene of yeast.
1981-1982  Rockefeller Postdoctoral Fellow (with Dr. James E. Hopper)
1975-1981  Graduate Student, Department of Genetics (with Dr. Oliver E. Nelson) and Program in Plant Breeding/Plant Genetics (with Dr. Robert E. Hanneman), University of Wisconsin-Madison. Thesis Title: The Role and Nature of Genic Balance in 1Endosperm Development.

Defense-Related Committees:

1997-Present  BioChem 20/20 Advisory Committee of Experts (ACE) for Defense Intelligent Agency (Founding member)
2003- Present  SAB of Western Regional Center of Excellence for Biodefense and Emerging Infectious Diseases
2004- Present  Member, Institute of Medicine Forum on Microbial Threats

B. Selected peer-reviewed publications (5 years).


C. Research Support.

ONGOING

HHSN266200500040C (Johnston) 3/2/06 – 9/29/10
NIH Tularemia Vaccine Development Team
Development of standardized animal models and immunoassays for the evaluation of newly identified candidate vaccines against tularemia.

Keck Foundation 2/1/07 – 1/31/10
Frameshifts as Cancer Vaccine Targets
To identify frameshift derived antigens that may be ideal cancer vaccine candidates in lung, colon, brain, pancreas and prostate tumors.

CDMRP – BCRP – Department of Defense 7/1/07 – 6/30/12
Towards Developing a Prophylactic Breast Cancer Vaccine
To identify and validate a sufficient number of neo-antigens to be included in a prophylactic breast cancer vaccine.
COMPLETED

HHSN266200500040C (Johnston) 3/2/06 – 3/1/09
NIH Tularemia Vaccine Development Team
Development of standardized animal models and immunoassays for the evaluation of newly identified candidate vaccines against tularemia.

NIH- WRCE - Pathogenesis Expression Core (Core H) – PI 9/1/03 - 2/29/08
5 U54 AI057156-04 To provide microarrays and antibodies related to biodefense projects in the RCE.

Wallace Research Foundation 1/1/08-12/31/08
H.B. Wallace Research Initiative in the Center for Innovations in Medicine Role: PI

University of Texas Medical Branch 3/1/08-2/28/09
Peptide arrays for antigen identification and diagnosis
To allow the systematic production of sugar antigen vaccine components and plug into the general systems approach to vaccinology. Role: PI

Arizona Alzheimer’s Consortium 7/1/08 – 6/30/09
AGR200737
To develop immunotherapy to Alzheimer’s disease. Role: Co-I
Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2. Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

<table>
<thead>
<tr>
<th>NAME</th>
<th>POSITION TITLE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stuart Lindsay</td>
<td>Professor of Physics and Chemistry</td>
</tr>
</tbody>
</table>

**eRA COMMONS USER NAME**
lindsay

**EDUCATION/TRAINING** *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

<table>
<thead>
<tr>
<th>INSTITUTION AND LOCATION</th>
<th>DEGREE (if applicable)</th>
<th>YEAR(s)</th>
<th>FIELD OF STUDY</th>
</tr>
</thead>
<tbody>
<tr>
<td>University of Manchester, Manchester, England</td>
<td>BS</td>
<td>1972</td>
<td>Physics</td>
</tr>
<tr>
<td>University of Manchester, Manchester, England</td>
<td>Ph.D</td>
<td>1976</td>
<td>Physics</td>
</tr>
</tbody>
</table>

**A. Positions and Honors.**

1975 - 1977    Research Fellow, University of Manchester. (Advisor: I.W. Shepherd)
1979 - 1984    Assistant Professor, Arizona State University.
1984 - 1989    Associate Professor, Arizona State University.
1985 - 1989    Associate Chairman, Department of Physics.
1994 - 1999    Vice President, Research and Development, Molecular Imaging Corp.
2000 - present Technology Advisor, Molecular Imaging Corp.
1989 - present Professor, Arizona State University.
1994 - 2000    Co-founder, Vice President R&D, Molecular Imaging Corp.
2002 - present Carson Presidential Chair, Arizona State University.
2002 - present Director, Center for Single Molecule Biophysics (ASU).
2002 - present Professor of Chemistry, Arizona State University.
2008 – present Regents' Professor

**Honors**


**US Patents:** 29 awarded to date.

**Textbook:** "Introduction to Nanoscience" S.M. Lindsay, Oxford University Press 2009.

**B. Selected peer-reviewed publications (in chronological order).**


C. Research Support

LINDSAY, S.
ACTIVE

0609362 Lindsay (PI) 09/01/06-08/31/10 NSF-ENG
NIRT: GOALI: Self Assembly at Photonic and Electronic Scales
The goal of this project is to determine if self-assembled DNA scaffold can be used to self-assemble complex photonic and electronic structures.
Role: PI

AGR 07/25/06 01/01/08-12/31/09
Bristol-Myers-Squibb Company Initial Imaging of BMS Materials
Role: PI

HG004378 Lindsay (PI) 08/01/07-07/31/2011 (NCE)
NIH
Sequencing by Recognition
The goal of this project is to explore a new approach to DNA “sequencing by recognition” in nanopores. It is based on a recent report of chemical recognition of the DNA bases via enhanced electron-tunneling when Watson-Crick hydrogen bonded base pairs form between a base-functionalized probe and a base on the DNA to be read.
Role: PI
HG004378  Lindsay (PI)  09/29/09-08/31/2011
NIH
Sequencing by Recognition
ARRA Supplement to current awarded project HG004378. The focus of this one is around transferring the
manufacturing of the carbon nanotube (CNT) and testing/characterization of translocation through the CNT and
the impact of the functionalizing the end of the CNT with a phosphate backbone clamp on translocation.
Role: PI

U54 CA143862  (Davies)  09/30/2009-08/31/2011
NIH-NCI
A Center for the Convergence of Physical Science and Cancer Biology
Role: Co I

CA125510 Lindsay (PI)  09/01/07-08/31/09
NIH
Mapping Epigenetic Modifications at the Nanoscale: Aptamers for microscopy
The goal of this project is to develop a technique for mapping post-translational chemical modifications of
histones in chromatin by direct imaging of single molecules.
Role: PI

RC2HG005625 Lindsay (PI)  09/30/09- 07/31/2011
NIH
Carbon Nanotubes: A New Synthetic Nanopore for Sequencing

EB005844 Lindsay (PI)  09/01/06-08/31/09
NIH
Water –Soluble Arrays for Personalized Medicine
The goal of this project is to integrate two new technologies to develop a radical new approach to making,
utilizing and reading DNA arrays.
Role: PI

N0014-09-1-1118 Yan (PI)  08/09/09-08/08/13
DOD-ONR
DNA Based Three- Dimensional Nanofabrication
Role: Co I

021420-001  Lindsay (PI)  08/18/08-08/18/10 (NCE)
HHS-NIH-NHGRI
Sequencing by Recognition – A test device
Building a test device for DNA translocation
Role: PI

Completed Projects

R01 CA085990 Lindsay (PI)  04/01/01-03/31/07
HHS-NIH-NCI  $1,423,970
New SPM Methods to study Chromatin Remodeling

0103175  Lindsay (PI)  08/15/01-07/31/06
NSF-ENG  $1,200,000
Nanoscale Molecular Opto-Electronics
<table>
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<tr>
<th>Project ID</th>
<th>PI</th>
<th>Start Date - End Date</th>
<th>Principal Funding Agency</th>
<th>Amount (USD)</th>
<th>Description</th>
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<tbody>
<tr>
<td>0321343</td>
<td>Lindsay (PI)</td>
<td>08/15/03-07/31/06</td>
<td>NSF-ENG</td>
<td>$465,000</td>
<td>MRI: Acquisition of a Dual Beam Focused Ion Beam System for Nanostructures that Interface to Molecules</td>
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<tr>
<td>1R21HG003061</td>
<td>Lindsay (PI)</td>
<td>05/14/04 – 04/30/08</td>
<td>HHS-NIH</td>
<td>$558,152.00</td>
<td>Molecular Reading Head for Single Molecule DNA Sequencing</td>
</tr>
</tbody>
</table>
BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors.
Follow this format for each person. DO NOT EXCEED FOUR PAGES.

NAME
HENSCHKE, Claudia

POSITION TITLE
Professor of Radiology

eRA COMMONS USER NAME
CHENSCHKE

EDUCATION/TRAINING (Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)

<table>
<thead>
<tr>
<th>INSTITUTION AND LOCATION</th>
<th>DEGREE</th>
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<th>FIELD OF STUDY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Southern Methodist University, Dallas, TX</td>
<td>BA</td>
<td>1962</td>
<td>Mathematical, Mathematics</td>
</tr>
<tr>
<td>Southern Methodist University, Dallas, TX</td>
<td>MS</td>
<td>1966</td>
<td>Mathematical Statistics</td>
</tr>
<tr>
<td>University of Georgia, Athens, GA</td>
<td>PhD</td>
<td>1969</td>
<td>Mathematical Statistics</td>
</tr>
<tr>
<td>Howard University Medical School, Wash, DC</td>
<td>MD</td>
<td>1977</td>
<td>Medicine</td>
</tr>
<tr>
<td>Harvard Med. School, Brigham and Women’s, MA</td>
<td>Resident</td>
<td>1981</td>
<td>Radiology</td>
</tr>
<tr>
<td>Harvard Med. School, Brigham and Women’s, MA</td>
<td>Fellow</td>
<td>1981</td>
<td>Radiology</td>
</tr>
</tbody>
</table>

A. Positions and Honors

Positions and Employment
1969-1971 Assistant Professor of Statistics and Computer Science, University of Georgia
1972-1974 Assistant Professor of Biostatistics, School of Medicine, Georgetown University
1977-1977 Clinical Assistant Professor of Biostatistics, School of Medicine, Georgetown University
1982-1983 Instructor in Radiology, Harvard Medical School and Associate Radiologist, Brigham & Women’s
1983-1983 Co-Director, Thoracic Division, Department of Radiology, Brigham and Women’s Hospital
1983-1983 Assistant Professor of Radiology, Harvard Medical School
1983-1987 Assistant Professor of Radiology, Cornell University Medical College
1987-1992 Associate Professor of Radiology, Cornell University Medical College
1987-1992 Associate Professor of Radiology with Tenure, Cornell University Medical College
1988-1992 Section Chief, Chest Imaging, The New York Hospital-Cornell Medical Center
1995-2002 Chief, Health Policy and Technology Assessment, NYH-CUMC
1992-2009 Chief, Chest Imaging and Attending Radiologist, The New York Hospital-Cornell Medical Center
1992-2009 Professor of Radiology, Cornell University Medical College
2009-present Research Professor, Biodesign Institute, Arizona State University
2009-present President, Early Diagnosis and Treatment Research Foundation

Other Experience and Professional Memberships
1972-1974: Statistical Consultant for National Clinical Trial of Narcotic Antagonists, National Academy of Sciences; Statistical Consultant for National Clinical Trial of Long Acting Methadone, Veterans’ Administration; Consultant for Health Manpower Planning, Bureau of Health Manpower Education (NIH); Consultant for Planning and Construction of Howard University Hospital, Howard Medical School
1986-1996: Consultant on Clinical Trials and Statistics, Rockefeller University
2005-2009: Member of NCI Biomedical Imaging Technology (BMIT) Study Section

Honors
B. Selected peer-reviewed publications (in chronological order). Selected from total of 250.

C. Research Support

Ongoing Research Support

2 U01-CA97431-05 (Kimmel) 09/02-08/10 NCI Model Lung Cancer: Risks, Progression, and Screening
This is a subcontract to provide limited data to the grant to construct a statistical model of lung cancer, involving genetic and behavioral determinants of susceptibility progression of the disease from precursor lesions through early localized tumors to disseminated disease.
There is no overlap with the present application.

1 R01 HL75476-04 (Barr) 04/04-03/09 NIH Fish Oil Intake: Biomarkers and Change in Lung Function
The goal of this project is to analyze initial CT scans and subsequent ones of 550 current and former smokers enrolled at Columbia to determine the extent of emphysema and change over time and the correlation with the spirometry.
There is no overlap with the present application.

6218 (Henschke) 01/05-12/09 American Legacy Foundation Smoking Cessation in the Context of CT Screening for Lung Cancer. The goal of this project is to randomize older smokers who present for CT screening for the early detection of lung cancer into one of two smoking cessation approaches.
There is no overlap with the present application.

FAMRI-IELCAP (Henschke) 01/07-12/11 Flight Attendants Medical Research Institute FAMRI-IELCAP Collaborative Network. The goal of this project is to determine the probability of specific respiratory diseases (emphysema, chronic bronchitis, bronchiectases, focal pneumonia, lung and mediastinal cancer), cardiovascular diseases, and other diseases among study participants who have never smoked, including the way in which this probability relates to the indicators of risk, CT findings and quantitative lung health indices (e.g., age, second-hand smoke exposure, nodules) in order to develop appropriate clinical (screening and treatment) programs.
There is no overlap with the present application.

Completed Research Support

5U01 CA91100-05 (Yankelevitz) 7/1/01-6/31/06 NCI Development and CAD Assessment of a PN Database
The goal of this grant was to form a collaborative group of institutions to develop a large, high-quality internet-accessible spiral computed tomography (CT) image database of pulmonary nodules to develop improved methods for early detection and screening for lung cancer.
There is no overlap with the present application.

R33 CA 101110-01 A1 (Reeves) 09/04-08/09 NCI Computer Detection of Nodules of the Lung
This project developed a system for the detection of pulmonary nodules from whole-lung CT scans considering nodule subtypes and the effect of CT scan parameters on the system performance. Model based verification methods were used to reduce the number of false positives.
There is no overlap with the present application.

5 R01 CA78905-06 (Yankelevitz) 9/99-8/09 NCI Repeat CT Imaging for Diagnosis of Lung Cancer: Second Order Considerations. The goal of this project was to determine growth rates of small nodules seen on CT scans, whether on screening tests or incidentally in routine clinical practice. This approach would decrease the potential invasive procedures required in routine care as well as screening.
There is no overlap with the present application.
BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors.
Follow this format for each person.  DO NOT EXCEED FOUR PAGES

NAME
YANKELEVITZ, David

POSITION TITLE
Professor of Radiology

eRA COMMONS USER NAME
DYANKELEVITZ

EDUCATION/TRAINING (Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)

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<tr>
<td>Brooklyn College of the City of New York, NY</td>
<td>BS</td>
<td>1977</td>
<td>Mathematical Statistics</td>
</tr>
<tr>
<td>Downstate Medical Center, Brooklyn, NY</td>
<td>MD</td>
<td>1981</td>
<td>Medicine</td>
</tr>
<tr>
<td>Staten Island Hospital, New York</td>
<td>Intern</td>
<td>1982</td>
<td>Internal Medicine</td>
</tr>
<tr>
<td>Long Island College Hospital, Brooklyn, NY</td>
<td>Resident</td>
<td>1984</td>
<td>Radiology</td>
</tr>
<tr>
<td>New York Hospital-Cornell Medical Center, NY</td>
<td>Resident</td>
<td>1986</td>
<td>Nuclear Medicine</td>
</tr>
<tr>
<td>New York Hospital-Cornell Medical Center, NY</td>
<td>Fellow</td>
<td>1987</td>
<td>Thoracic Radiology, CT and Ultrasound</td>
</tr>
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A. Positions and Honors

Positions and Employment
1982-1984  Emergency Room Physician, Parkway Hospital
1984  Associate Adjunct Professor in Radiology, Long Island University
1986-1987  Fellow in Radiology, Cornell University Medical College
1987-1991  Assistant Attending Radiologist, The New York Hospital-Cornell Medical Center
1987-1991  Assistant Professor of Radiology, Cornell University Medical College
1991-2002  Director of Radiology, Community Hospital of Brooklyn
1993-1998  Associate Attending Radiologist, The New York Hospital-Cornell Medical Center
1993-1998  Associate Professor of Radiology, Cornell University Medical College
1998- Present Professor of Radiology, Cornell University Medical College, Attending Radiologist, NYH-CUMC
2009-present  Research Professor, Biodesign Institute, Arizona State University

Other Experience and Professional Memberships
1987  Society of Thoracic Radiology
1988  Radiologic Society of North America
1992  American College of Radiology
1997  Expert Panel of the American College of Radiology, Chest Imaging
2002  European Thoracic Society, Int’l Ass’n for the Study of Lung Cancer

Awards
2002  Connie and Priscilla Mack Cancer Advocacy Award
2006  SUNY Brooklyn 25th Anniversary Radiology Award

B. Selected peer-reviewed publications (in chronological order)
52. The I-ELCAP Investigators (Henschke CI, Principal Investigator). CT Screening for Lung Cancer: The relationship of disease stage to tumor size. Arch Int Med 2006;166:321-325
59. I-ELCAP Investigators (Henschke CI, Principal Investigator). Women's susceptibility to tobacco carcinogens and survival after diagnosis of lung cancer. JAMA 2006; 296:180-184
60. The I-ELCAP Investigators (Henschke CI, Principal Investigator). Survival of Patients with Stage I lung cancer detected on CT screening. NEJM 2006; 355:1763-1771

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