It takes eight minutes for a photon of light to travel the 93 million miles from the sun to the Earth’s surface. A green plant needs only a few seconds to capture the energy in that light, process it, and store it in the form of a chemical bond.

The amazing process for converting light energy to stored energy is called photosynthesis.

Photosynthesis as a process includes some of the fastest known chemical reactions. The most important events in a photosynthetic reaction occur in trillionths of a second. Measuring such short-lived events, and understanding the chains that link them together, demand some of the most precise experiments and exact measurements technology currently allows.

The stakes are high. Ultimately, almost all life on our planet is fueled by the power of green—green chlorophyll, that is. That is one reason why in 1988, the National Science Foundation and the U.S. departments of Agriculture and Energy funded the creation of Arizona State University’s Center for the Study of Early Events in Photosynthesis. The center includes more than 20 scientists from the departments of chemistry and biochemistry, botany, and teams of graduate and undergraduate students. All contribute brain power toward unraveling the exact chain of events that occurs in those first trillionths of a second. ASU researchers use magnets and microwaves, lasers and spectrosopes, petri dishes, ultracentrifuges, and a bevy of sophisticated instruments in their work. To get information, they peer into the infinitesimal nooks and crannies of biological molecules. They watch. They record. They measure. They experiment. They learn.
The molecular structure green plants use to capture energy from sunlight is complex. Below is a schematic view of the structure of the photosynthetic reaction center of the purple bacterium Rhodobacter sphaeroides. The spirals show the enclosing protein helixes, and the bright dot marks the position of the iron atom which is the site of the important catalytic reactions.
lying functional and chemical similarities.

“What we find is that there really are just two classes,” Blankenship says. “One of them we call the pheophytin quinone. The other is the iron-sulfur type of reaction center.”

Primitive purple photosynthetic bacteria have the pheophytin quinone reaction center, which chemically resembles the photosystem II of higher plants. Green sulfur and heliobacteria have only the iron-sulfur photosystem, which resembles photosystem I. However, none of these more primitive photosynthetic forms can make oxygen.

“The really big advance in the history of the Earth is the evolution of plants’ ability to produce oxygen,” Blankenship says. “That’s what made it possible for organisms like us to do respiration. However, the evolutionary source of this activity is still a mystery.”

Kissing Cousins

The cellular machinery that constructs proteins from the instructions given in the cell’s DNA has a great deal of biochemical similarity—not just across species, but across kingdoms. For example, genetic engineers know that it is possible to insert the human gene for insulin into Escherichia coli bacteria. The bacteria then become living factories that can produce large quantities of insulin. Such work is the essence of biotechnology.

But such abilities also give the evolutionary detective new tools with which to dig out bits of information. For example, scientists can now analyze sequences of life’s master molecules, DNA and RNA, as well as the proteins that they issue orders and blueprints to build. More importantly, it is possible to assess how long ago it was that these proteins arose from the same “parent” DNA or RNA.

Blankenship compares the reaction centers in various bacteria and plants alive today. The goal is to determine statistically how closely these reaction centers are related. The ASU botanist Wim Vermaas did experiments to analyze the sequences of the reaction centers in recently discovered primitive photosynthetic organisms called heliobacteria. They were the first to demonstrate that a very primitive complex still exists in Nature.

The Cake Of All Possible Ingredients

Today, scientists understand fairly well which molecules make up cells, and to a greater extent, how those molecules work. The basic molecular building blocks of cells come in four types: proteins, nucleic acids, lipids, and polysaccharides. Proteins are built from chains of 20 different amino acids, which assembled in different sequences. Proteins fold into complex three dimensional shapes that provide cellular structure and framework. They also form the machinery that does most of the work.

Nucleic acids come in two forms, DNA and RNA. They store, transfer, and read information. The DNA molecule is made up of two sugar-phosphate strips holding chains of four chemical bases: adenine (A), thymine (T), cytosine (C), and guanine (G). The bases bond (A only with T; C only with G) to join the strips in the famous double-helix structure. Humans have about 3 billion of these bonded base pairs. Every single human cell contains a yard of DNA, tightly coiled.

Lipids are fatty acids, short strings of atoms that combine to form watertight layers. Lipids protect the cell’s insides from the trials and tribulations of the outside environment. Polysaccharides form the cell’s energy storehouses.

Every cell is 70 percent water. Most biological molecules are either hydrophilic (water loving) or hydrophobic (water fearing). Lipids have a water loving end. They orient themselves towards the inside or outside of the cell, and pile back-to-back, to form a watertight layer.

One gene codes for one protein, while every three bases of the gene code for one of the amino acids in the protein. To modify a protein, one has to displace or add an amino acid to the string that makes up that protein. Sometimes, Nature does this accidentally by making copying errors in DNA. A string of DNA might look like this: G G C T A A T G C A. But one base might be changed, added, or deleted. The cell’s copying mechanisms usually are quite accurate. Mistakes occur only at the rate of 1 in 100,000 to 1 in one million bases.

Genetic engineers work to modify DNA sequences. Their intent is to co-opt the cell into building modified proteins. The challenge is to find the means of inserting modifications that are both accurate and that take hold inside the cell.

Wim Vermaas likes to juggle amino acid residues, the 20 medium-sized molecules which, when connected in millions of configurations, make proteins. He uses a complicated apparatus called an oligonucleotide synthesizer to assist his juggling.

“This machine makes pieces of DNA,” says Vermaas, a professor of botany. “We can design oligonucleotides that are degenerate in regions. That means we can make a zillion different molecules. The catch is that a quarter of them have an A in one position, while another quarter have a C in the same position, another have a G, and the final quarter have a T. We can do that at multiple positions.”

The manipulation allows the scientists to have a large number of different DNA sequences represented. Each bit of DNA has...
Robert Blankenship uses techniques similar to those used by scholars who study ancient manuscripts such as the Bible or The Iliad. The objects of his analysis are not writings, however; they are tiny twisted bits of protein. The ASU chemist searches for evolutionary relationships among the proteins.

Imagine, if you will, a manuscript written 3,000 years ago. The original manuscript does not exist, only copies. In ancient times, the only way to make a copy was for someone trained in reading and writing, perhaps a monk, to painstakingly transcribe the information, letter by letter.

Often, these human copiers were trained to copy but not to understand, much like a Xerox machine or the cellular machinery which duplicates DNA. If one letter were substituted for another, it might not be noticed, but that mistake would likely be preserved on subsequent duplications.

Clues as to what the original text looked like can be gleaned from the study of copies and the differences between them. Note the following three sentences:

SENTENCE 1: The great white lion ate the angry man.
SENTENCE 2: The great golden lion ate the hapless man.
SENTENCE 3: The great white lion smote the hapless man.

By choosing the most common words in the sentences, one can construct a hypothetical ancestor sentence, like this:

NEW SENTENCE: The great white lion ate the hapless man.

The same follows for copies of genetic material. Imagine three sequences of RNA coding for amino acids that are similar to each other.

Parts of them share sequences, as follows:
SEQUENCE 1: AUG UUU CUC ACA GCA
SEQUENCE 2: AUG UCU CUC AAA GCA
SEQUENCE 3: AUG UUU CCC AAA GCA

One can build a sequence that combines features that are most common:
NEW SEQUENCE: AUG UUU CUC AAA GCA

It is likely that the ancient original sequence looked more like the newly generated sequence than any of the existing modern sequences.

Blankenship and his students use powerful computers to analyze significant chunks of the genetic material in the various photosystems now flourishing in plants. One section they study is called the heterodimeric complex.

“The heterodimeric complex undoubtedly occurred by having a single gene that duplicated and then diverged to form a pair,” Blankenship explains. “This is what happens in photosystem I, photosystem II, in purple bacteria, and so on. And it’s clear that this happened at least three different times, because the two halves of the complex are more similar to each other than they are to another complex.”

For example, in photosystem I, the two parts of the heterodimeric complex are very similar to each other. They are about 50 percent identical. But they are only about 10 or 20 percent identical to the complex in photosystem II.

“The two halves of the photosystem II complex are about 30 percent identical to each other,” he continues. “What happened? We think that this gene duplication and divergence happened at least three or four different times to form these separate classes of reaction centers.” —John Svetlik

Cells are the building blocks of every living thing. Proteins in turn serve as the machinery of cells and determine cellular type and activity. DNA within cells specifies the proteins made by the cell.

DNA is a huge molecule, but still too small to be visible through any optical microscope. Strings of DNA are made of interlocked pairs of bases—guanine and cytosine, adenine and thymine. The bases stick to each other because they have a slight electric charge. Only GC and AT pairs fit together. This preserves the sequence of bases when DNA replicates.

RNA reads the sequence of DNA bases by matching the shape of the bases as a key fits a lock. Short segments of RNA carry and link specific amino acids to form proteins.

Different proteins result from different sequences of amino acids. In this way the DNA base sequence forms a code that directs the assembly of proteins.
the same sequences at places where Vermaas wants them to be the same, but all have different sequences at other places.

Vermaas needs the power of such a technique. He investigates how photosystem ii moves electrons across the cell membrane. His approach is a little like Thomas Edison building the light bulb--he tries everything to see what works. His organism of choice is a cyanobacterium.

By creating large numbers of bacterial mutants, Vermaas learns exactly which amino acid residues are important for electron transport, and which residues can be replaced without any significant change in function. Part of this research is guided by intuition, part of it is blind. Vermaas and his group have made predictions about how changes in the structure of the protein are likely to affect the performance of electron transfer.

Typically, Vermaas modifies dna sequences of no more than 15 bases at a time. The dna chains he works with may stretch past 3,000 base pairs. He is able to attach short modified sequences to longer chains of dna. He then plugs the modified chains directly into the genome of cyanobacteria. “pcr lets me amplify dna very specifically from specific genes,” he says. These new strings are amplified in E. coli bacteria, and introduced into cyanobacteria. The cyanobacteria have been engineered to lack the functional part of the protein Vermaas is trying to replace.

Cyanobacteria reproduce at stunning rates. As a result, within days, Vermaas has many new varieties of photosystems at his disposal. He tests them using other sophisticated techniques such as electron paramagnetic resonance imaging and laser spectroscopy.

Using laser spectroscopy, scientists fire extremely short pulses of light at the organisms. They then measure how much light is absorbed, how much is reflected, how much fluoresces back out, and how much the spectrum is modified. Vermaas uses the technique to determine the function and efficiency of his newly created mutants.

How a Skein Of Yarn Makes a Sweater

Machines are used to determine the sequence of amino acids that make up a protein. The process is routine. However, determining the geometry of how an amino acid chain folds is one of the most difficult tasks facing scientists today.

The technique of choice is x-ray diffraction. Scientists try to rebuild a protein’s structure based on the pattern of interference generated by sending x-rays through a sample.

James Allen uses x-ray diffraction to study the photosynthetic reaction center of Rhodobacter sphaeroides, a purple bacterium. X-ray diffraction analysis is very difficult.

“We have to do what’s called a Fourier transform,” the asu chemistry professor explains. “The result is an electron density map. This map tells us the location of all the electrons in the structure. Then we must interpret that information in terms of atoms.”

Density map information does not tell the scientist what type of atom is present, only that an atom is there. For Allen to know what is an oxygen and what is a carbon requires knowing the amino acid sequences. In this case, the sequencing was provided by his wife, a su chemist JoAnn Williams.

“It’s easy to grow and isolate the protein from this particular bacterium,” Williams says. “We did this work in the early 1980s (at University of California, San Diego).”

Teamwork was key to their lab’s success. “All of these techniques were just being developed at the same time. That’s what made it so powerful,” Allen says. “We got the sequence, and genetic work was being done at the same time as the three-dimensional work.”

Their achievement is close to unique. The reaction center protein structure is enormously complicated. “There are a thousand amino acids. That’s more than 10,000 atoms. There’s only structural information for two reaction centers. This is one of them,” Allen adds.

The structural knowledge is broadly applicable. Allen says that the reaction center from purple bacteria is ancestral to photosystem ii.

Deciphering the structure of membrane proteins is a dirty problem. Neal Woodbury takes it as a personal challenge.

“In order to pull proteins out of the membrane, we must replace that membrane with something else that acts like a membrane,” the chemistry professor explains.

Woodbury shares laboratory resources with Allen and Williams. The scientists use a technique called site-directed mutagenesis to...
Acrobats On Apeze

Part of what gives proteins their capacities for work are the metal atoms suspended within them. For example, human hemoglobin holds four iron ions, in a coordinating complex. Oxygen binds to this complex tightly enough for a ride through our bloodstream, but loosely enough to be taken away by needy cells.

There are two types of photosynthesis reaction centers in higher plants. Both photosystem I and II work in concert. Metal ions are suspended in both classes of reaction centers. The metals are manganese and iron.

The atoms from which they are suspended by chemical bonds are called ligands. The metal ions are said to reside in coordination spheres made of the metal and some of the lighter elements, such as oxygen, nitrogen, carbon, and hydrogen. In photosynthetic organisms, the coordinated metal ions are crucial to the transfer of electrons.

Russ LoBrutto runs the Electron Paramagnetic Resonance (EPR) Imaging Facility deep in the sub-basement of the ASU Life Sciences C-wing. His doctorate is in biophysics. He has worked in a number of different environments, including a medical school.

LoBrutto’s work combines a deep knowledge of molecular biology, chemistry, and quantum physics, all in the name of characterizing the structures of metal-ligand complexes and of free-radical centers. Unpaired electrons generated when a chemical bond is broken. He works too discover how proteins do their jobs. His services are used by most of the scientists in ASU’s Center for the Study of Early Events in Photosynthesis, both botanists and chemists.

Electron Paramagnetic Resonance (EPR) is one way to examine the structures of metal-ligand coordination complexes, and how these structures change during the protein’s functional cycle. If the metal atoms have unpaired electrons in their outermost shells, it is possible to do some trickery to help them give up their secrets.

By aligning the unpaired electrons of the metals with a strong magnetic field, and adding electromagnetic energy with microwaves, LoBrutto causes the proteins to absorb a weak but detectable spectrum of radiation. The spectrum tells him what type of metal ion is present, and what is its coordination environment in the protein.

One of the basic attributes of electron is “spin,” but the name doesn’t quite refer to our usual conception. Around an atom, electrons are said to inhabit “orbitals,” spaces which denote both a fairly distinct place and a certain energy level for the electron. Given enough energy, an electron can hop up to a higher orbital.

Spin states are limited to two: “up,” or against the applied magnetic field, and “down,” or with the field. Adding energy to an electron can cause it to flip its spin to the opposite state.

Whereas many EPR facilities can detect only the signals of the electrons flipping their spins, ASU’s facility has an additional capability. By adding radiofrequency energy to the sample in the microwave cavity, LoBrutto can cause nuclear spins in the protein to flip states as well. These nuclear flips are detected as momentary perturbations of the EPR spectrum. This technique is called Electron-Nuclear Double Resonance, or ENDOR.

The EPR signals from a protein molecule are often hard to interpret because the proteins are randomly oriented in the tube, and each orientation gives a slightly different spectrum. The result of combining all possible orientations can be a featureless blob. But by using ENDOR, the information on ligand identities and distances, which is lost in ordinary EPR due to orientation effects, can frequently be recovered.

LoBrutto also has constructed a special type of EPR spectrometer in which very intense microwave pulses, lasting 10-20 billions of a second, replace the weak, steady microwaves used in ordinary EPR or ENDOR. This method, while tricky, is sometimes the best way to regain lost information on metal ligands in randomly-oriented protein samples.

LoBrutto and five other ASU investigators recently were awarded a large NSF grant that will bring ASU’s facility to the state of the art in pulsed EPR technology. The facility’s growth will help Photosynthesis Center scientists to know more of the internal details of the molecules they are studying, and to better understand exactly how those molecules do their jobs.—John Svetlik

Micro machinery

The photosynthetic reaction centers use light energy to drive electron transfer reactions. The goal of this process is to convert the energy from the light into a form that can be used by the living organism. But initially, much of the light energy is used to make the inner most vesicles of the chloroplast much more acidic than their surroundings. Although this does serve as a way to store the energy, it has some severe limitations. First, the protons that cause the acidity rapidly leak out of the vesicles.
Hunting for Mutants

"People have been trying to refine techniques for transforming organelles. The gene gun is a method that works."

Andrew Webber shows a vial of genetic ammo ready to be loaded into the pneumatic gene gun.

Scott Bingham is a hunter. His quarry is mutant DNA. His hunting ground is a petri dish. Bingham works closely with ASU botanist Andrew Webber. Their quarry of choice is an organism called Chlamydomonas reinhardtii. Lately, his efforts have focused on creating new types of selectable markers, means by which one can ensure that a particular new mutation has taken hold within the target organism.

There are many steps to creating a useful selectable marker. One starts with natural selection itself.

"When we try to select the mutant that's resistant to a compound like norfluorozone (a common herbicide), we can generally put about 10 million cells on one plate, in one little petri dish. And from those 10 million cells we can sometimes pick up five or 10 mutants on one plate that might be resistant to a particular compound. That's basically what we've done with Chlamydomonas," Bingham says.

Colonies that have resistance are easy to spot. "If you see something that's very round all by itself on a plate, eventually after a week or two, that arose from one single cell that became resistant," Bingham says. "We just pick them off the plate with a toothpick. We restreak them on another plate that contains the same selective agent. This is to make sure that it's really resistant to the agent that we've been using."

Because DNA does occasionally make copying errors, given 10 million cells, some errors have already occurred. The resistance to the herbicide fluorozone turns out to be a single modified base pair in the strand of millions.

This particular mutation is not regularly seen in Nature, because it causes somewhat nasty side effects. But in the harsh environment of a petri dish flooded with norfluorozone, it's just what Chlamydomonas ordered.

"This gene, once isolated through a rather arduous series of steps, can be attached to the plasmid vector carrying the new protein sequence being fired into Chlamydomonas. It becomes possible to selectively kill off those algae that have not received the modification. Through successive generations, Bingham can breed only those cells with a strong resistance. As a result, a sizable percentage of chlorophylls will have the desired trait."

Bingham has also turned his attention and expertise to other organisms. "We're also using Chlorella, which is an organism of much more industrial significance. It's actually used to make many commercial products," he adds. For example, Chlorella is used to make stable isotopically labeled chemicals.

"We use the organisms to make products. For instance, glucose is a sugar. It's not ordinary glucose because the algae can take up carbon 14, instead of carbon 12," Bingham explains. "Carbon 14 is weakly radioactive. It can be traced through organisms that feed on this modified glucose.

"The creation of herbicide-resistant markers is particularly important in work on plants. Antibiotics are often used as markers in bacteria, but have their drawbacks. Herbicide resistance can be reproduced by Escherichia coli without affecting it. It is quite a useful trait to have."

—John Svetlik

Energy is lost quickly, much like a car battery that won't hold a charge.

The second problem is that few biological processes in a living organism can tap into this energy source. For example, try to run all your electrical devices designed for 110 volts off of a 12-volt car battery. Without adapters, it cannot be done.

To get around these problems, photosynthetic systems contain a large protein complex called $F_0F_1\gamma$-ATP synthase. The protein allows protons to flow through the $F_0$ portion and use the energy to convert adenosine diphosphate (adenosine triphosphate). Think of at p has high test gasoline for cells. at p is very stable, making it ideal for energy storage.

An ASU chemist Wayne Frasch studies the ATP synthase enzyme. He is excited. Scientists recently completed the x-ray crystal structure of the enzyme's $F_1$ portion. This portion is responsible for at p synthesis. Frasch says the portion looks like an orange with six protein subunit slices that surround another protein subunit. Each of the slices contains a binding site for at p, but only three of them are responsible for the conversion of a dp and phosphate into at p. The function of the other three slices remains unknown.

"Each of the slices has what appears to be a lever at the bottom. This structure is provocative in that it appears that the central subunit might be around the inside, perhaps in response to the movement of protons," Frasch explains.

As it spins, this central unit might raise and lower the levers in a manner that might cause at p to be formed. "If correct, the enzyme would be a mechanically-driven, molecular pump!" he adds.

X-ray crystal structures provide only a snapshot view of something that must move in many complex ways to function. Frasch is able to follow many of these important structural changes through a combination of epr spectroscopy and genetic engineering using the gene gun on Chlamydomonas.

The enzyme uses a metal atom, usually magnesium, to bind the phosphate and the phosphate groups of adp in order to make the bond between them. By substituting the magnesium for vanadyl, which gives rise to an epr signal, the changes that occur to the metal as at p is made can be followed directly.

"Although we can't see all the changes that take place, it's like shining a flashlight on the very spot you want to see," Frasch says.
When amino acids crucial to this process are changed by genetic engineering, the changes are easily seen in the epr spectra from the vanadyl bound at the site. In this way, many of the subtle but important mechanistic features of the enzyme are coming to “light” (fitting for a photosynthetic process).

Getting Past Cellulose

Most of the scientists at a su’s Photosynthesis Center work on bacterial photosystems for very practical reasons. Bacteria are small, so small that millions can live inside an ordinary beaker. They reproduce so quickly that one organism can become a billion in less than week. Bacteria also contain a limited amount of d n a. New d n a can be inserted relatively easily by using sexual conjugation with ordinary E. coli bacteria.

But eukaryotic plants, the photosynthetic organisms that affect us directly, are more complex. Andrew Webber studies one of these eukaryotes.

The plant is a little simpler than some, but shares most of the important biochemical features of plants that cover hills and valleys. Chlamydomonas reinhardtii is a one-celled algae. Algae are similar to higher plants. Their cell walls contain a separate chloroplast. The chloroplast contains only 196,000 base pairs of d n a. Many of the proteins necessary for photosynthesis must be assembled outside the chloroplast and imported.

Webber studies photosystem i in C. reinhardtii. Doing the sort of genetic engineering so popular among his colleagues involves special challenges. Actually, it involves a gun. A ballistic gene gun, to be exact.

C. reinhardtii is a plant, not a bacterium. It has tough cellulose cell walls, just like the plants growing outside your window. Typical methods for inserting genetic material, such as bacterial conjugation, just won’t work.

For one thing, bacteria show no great efforts to mate with an organism from another kingdom. For another, the cell wall would probably prevent any such attempts.

“For years and years, people have been trying to refine techniques for transforming organelles,” Webber explains. “The gene gun is a method that works.”

Scientists mix purified engineered d n a with very small balls made of tungsten or gold. The balls are less than a micron in diameter. Consider that the smallest grain of sand on a beach is about 90 microns in diameter. The gene gun is fired using compressed helium. At sufficient helium pressure, a plastic membrane ruptures and fires the particle mixture down a barrel at the C. reinhardtii target. Success at creating mutants results through selection and reproduction.

“It is a numbers game,” Webber explains. “This is why we work on C. reinhardtii. We can grow a large number of cells, and it has the advantage of having only one chloroplast per cell.” The chloroplast itself is huge; taking up 70 percent of the cell’s volume. “When we shoot C. reinhardtii, there’s a good chance that the particle will go into the chloroplast as opposed to anywhere else.”

Inside the cell there are perhaps 80 copies of chloroplast d n a, and the new d n a reaches perhaps only one of those. But the new d n a is special. It carries a gene for antibiotic resistance. Webber simply kills off the algae that don’t contain the new gene.

Over time, numerous bouts of antibiotic treatment leave only algae that possess many copies of Webber’s gene for antibiotic resistance. They carry his genetic changes as well.

Webber wants to better understand the mechanisms of the photosystem i reaction center in higher plants, particularly the function of cofactors like the iron-sulfur complex and reaction center chlorophylls, which are essential for electron transfer.

Piece by piece, Webber replaces amino acids to find which ones are crucial to the process. Success is judged using methods like epr and laser spectroscopy.

Webber’s work, like most of the research conducted by his a su colleagues, is both basic and directed toward real-world applications. By better understanding the nuances of photosynthesis, scientists hope to learn enough to improve farming techniques, make the generation of electricity more efficient, engineer new drugs and, ultimately, better understand the nature of life itself.

Research at the Center for the Study of the Early Events in Photosynthesis is supported by the National Science Foundation and other federal agencies. For more information about work described here or about other projects, contact Director Wim Vermaas, Ph.D., 602.965.3698, or click on the Center’s home page at http://photoscience.la.asu.edu/photosyn