BY LINLEY ERIN HALL  DURING A BIOPSY, SURGEONS REMOVE SMALL PIECES OF TISSUE FROM A DISEASED ORGAN. THEY STUDY THE PIECE TO LEARN MORE ABOUT THE ILLNESS. BRAIN DISEASE IS DIFFERENT. DOCTORS CAN'T JUST REMOVE A PIECE OF BRAIN TO STUDY. IMAGE: ALPHA-SYNUCLEIN IS A PROTEIN FOUND IN THE BRAIN. PATIENTS WITH DEGENERATIVE BRAIN DISEASE OFTEN DEVELOP FIBRILS, STRING TANGLES OF PROTEIN THAT CAN DISRUPT NEURAL CELL FUNCTION.
The memory loss begins slowly: a lunch date here, an acquaintance’s name there. But this is not the simple forgetfulness that often accompanies old age.

As the years go by, more and more things slip away,

from one’s address to the identity of one’s children.
Finding the Right Antibody

Scientists use atomic force microscopy (AFM) as a powerful tool to get a better look at molecules and atoms. The AFM has a resolution of one picometer (one trillionth of a meter). The instrument is crucial to Michael Sierks' research. The ASU scientist is trying to find antibodies that bind to clumps of proteins important in neurodegenerative diseases. Scientists refer to these clumps as aggregates. The microscopes most people remember from biology class use lenses to magnify an image. The atomic force microscope works very differently. A very small lever is dragged over a surface. The deflection of the lever, both up and down, is used to create an image of the surface. In Sierks' research, the surface is made of mica, a shiny mineral composed of aluminum and silicon.

The scientist immobilizes protein aggregates of varying sizes on the mica. The aggregates have been incubated with a solution containing phages. Different antibodies are attached to the phages. Phages are viruses that infect bacteria. The phages in Sierks' work are genetically engineered so that an antibody of interest is fused to a protein that is part of the phage's outer coating. The phage also contains the DNA that codes for the antibody. When the phages come in contact with the protein aggregates, some of the antibodies will bind to the aggregates. The researchers wash away the unbound phages, then use the AFM to visualize which phages are stuck to which aggregates. The phages that specifically bind to one kind of aggregate are then removed.

Phages and other viruses cannot make their own proteins. Instead, they inject their DNA into other cells. They take over the cells and turn them into machines for making the components of new phages. In this case, the ASU researchers use the phages that bound to the aggregates to infect bacteria, which then produce copies of the antibody protein from the DNA in the phage. “The protein we obtain is then purified and used for further testing against the aggregates to determine its functionality.” The purified protein may now be used as a potential therapeutic, explains Sharareh Emadi, a post-doctoral researcher in Sierks' lab. “We’ve isolated many antibodies that can inhibit aggregation well,” Sierks adds. “We’re just now starting with animal models; that’s where the surprises will probably arise.”

“Linley Erin Hall

We need a therapeutic that we can use for a long time that won’t be toxic. Antibodies are good because they don’t generate an immune response.”
Alzheimer’s disease is devastating for many families. Michael Siers is an associate professor of chemical and materials engineering at Arizona State University. He lost his father to Alzheimer’s disease some years ago. The experience prompted Siers to change his research to focus on treatments for neurodegenerative diseases.

Neurodegenerative diseases progressively worsen the functioning of the nervous system in some way. For example, patients with Alzheimer’s disease lose their memory. Sufferers of Parkinson’s disease, on the other hand, lose motor control. They suffer with tremors and/or partial paralysis.

Siers’ approach to the problem utilizes man-made variants of molecules that the body naturally produces to fight disease. “As the population ages and life expectancy increases, more and more people will get these debilitating diseases that require enormous amounts of money to treat,” Siers says. “If we can simply delay the incidence by three to five years, it would have a huge impact on both cost and quality of life.”

Researchers believe that neurodegenerative diseases are caused by aggregation of particular proteins in the brain. Proteins are made of long chains of amino acids that fold into a wide variety of shapes. Most proteins function on their own, as monomers. Others join with proteins of the same type as aggregates of various sizes. Keratin, for example, is a naturally aggregating protein. The bonds between keratin molecules help give hair and fingernails their strength.

In neurodegenerative diseases, however, proteins in the brain that should not clump together begin to do so. In Parkinson’s, the protein is called alpha-synuclein. It forms clumps called Lewy bodies inside brain cells that control movement. The problematic protein in Alzheimer’s disease is called beta-amyloid. It clumps together outside brain cells. Researchers are searching for ways to prevent these aggregates from forming and to make existing aggregates less toxic.

Studying neurodegenerative diseases is difficult. For example, if someone has a disease of a particular organ, doctors can do a biopsy and remove a small piece of tissue. They then study that piece to learn more about the illness. Brain diseases are different. A surgeon can’t just remove a piece of brain to study.

“We can’t study a Parkinson’s patient’s brain while the person is living. We have to use models. But we don’t have any good models that mimic Parkinson’s disease,” Siers explains. “We can get mice to overproduce alpha-synuclein, for example, but it’s not toxic to their neurons in the same way as in humans.”

However, based on results from test tube studies, researchers do know that not all aggregates are equal. They can take two forms: One is called an oligomer. An oligomer is made of just a few alpha-synuclein or beta-amyloid molecules. The other is the fibril form. A fibril can be made of hundreds of molecules.

“One everyone thought that the fibrils were the problem,” Siers says. “Now it seems that the intermediate oligomers are the toxic species, at least in test tubes.”

Siers’ and his ASU colleagues are searching for antibodies that will bind to aggregates. Antibodies are molecules produced by the body in response to something foreign, such as a bacteria or virus. They’re part of the immune system, which fights disease.

Researchers can also make artificial antibodies in the laboratory. Siers exposes a wide variety of man-made antibodies to samples of aggregates and sees which ones bind to the clumps of protein.

Siers thinks that the antibodies could prevent aggregates from harming patients in two different ways. They could bind to the monomer and prevent aggregation altogether. Or, they could bind to the oligomer in a way that makes it non-toxic. The latter might involve promoting fibril formation or signaling the cell to break down the oligomer. “We need a therapeutic that we can use for a long time that won’t be toxic,” Siers says. “Antibodies are good because they don’t generate an immune response.”

One challenge the researchers face is that natural antibodies circulate in the bloodstream and never enter cells. This is fine for beta-amyloid, which aggregates outside cells. But the researchers must find a way to deliver alpha-synuclein antibodies to the interior of cells.

To solve the problem, Siers is working with Dr. Anne Messer, director of the Molecular Genetics Program at the New York State Department of Health’s Wadsworth Center. The current strategy is to insert the DNA that codes for the artificial antibody into a virus that has been modified so that it cannot harm humans. The virus then injects the DNA into a nerve cell, where cellular machinery uses it to produce the desired antibody. Once inside the cell, the antibody can prevent formation of alpha-synuclein aggregates and thus Lewy bodies.

Siers says that having an antibody-based drug for Alzheimer’s or Parkinson’s disease is still many years in the future. The ASU researchers have identified many antibodies that bind to aggregates of beta-amyloid and alpha-synuclein. However, they still must test these antibodies in animals before they even think about human trials. Even so, the work suggests that a brighter future for patients with neurodegenerative diseases may be possible.

“Diseases like these hit close to home — you get a sense of urgency,” says Hedieh Barkhdarian, a graduate student in Siers’ lab. “My parents are becoming older, so it’s significant for me because of that.”

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For more information, contact Michael Siers, Ph.D., Department of Chemical and Materials Engineering, 480.965.2828. Send e-mail to siers@asu.edu

Or visit the Biodesign Institute web site at http://biodesign.asu.edu/