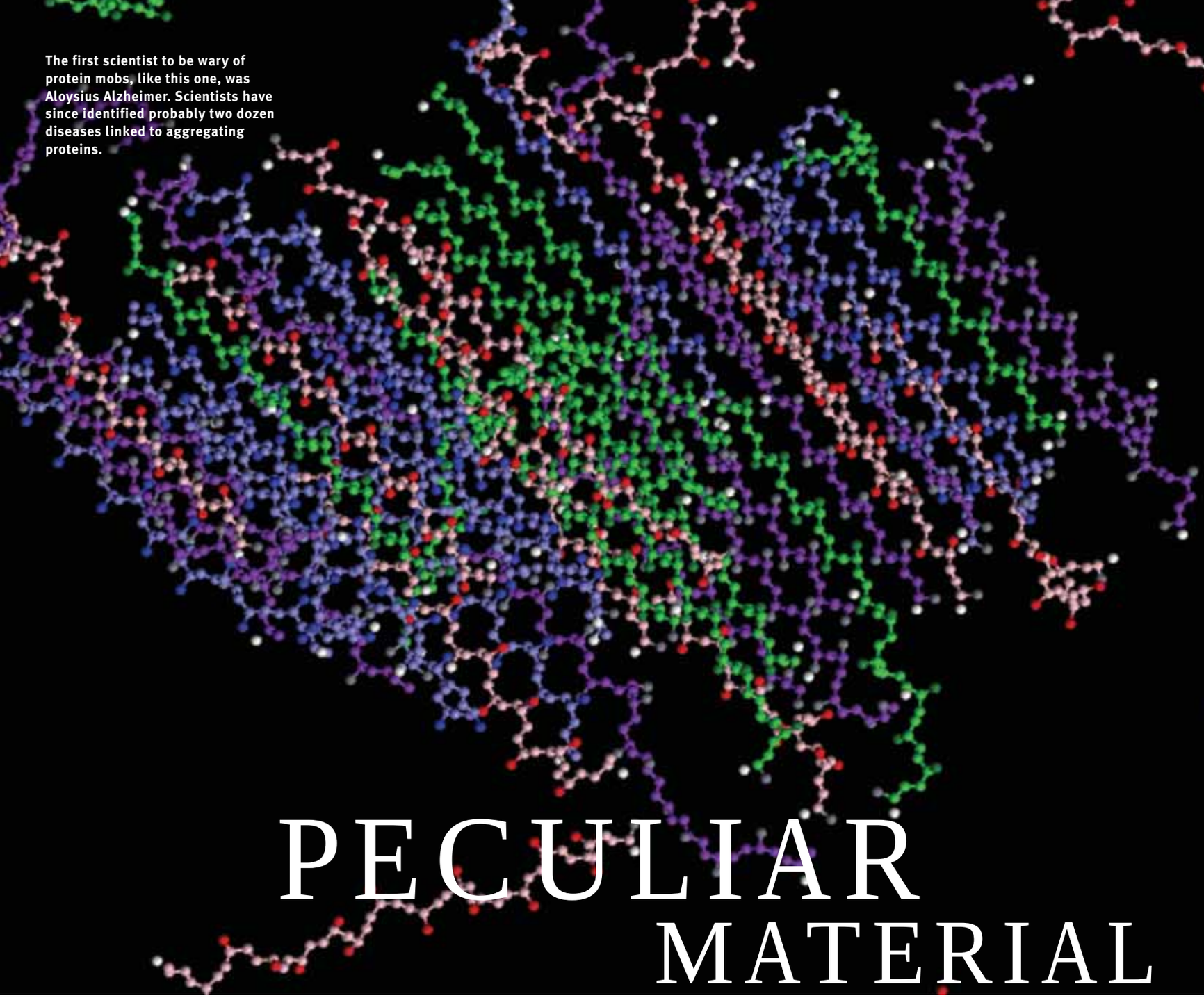


The first scientist to be wary of protein mobs, like this one, was Aloysius Alzheimer. Scientists have since identified probably two dozen diseases linked to aggregating proteins.

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PECULIAR MATERIAL

DISBANDING UNRULY MOBS OF PROTEINS

BY JIM SWYERS

In 1901, a middle-aged man named Karl Deter reluctantly took his wife, Auguste, to the nearby Asylum for the Insane and Epileptic in Frankfurt, Germany. He did so on the advice of his family doctor, after months of witnessing her dramatic and tumultuous descent into a personal hell. The doctor's prescription read:

Auguste D. has been suffering for a long time from weakening of memory, persecution mania, sleeplessness, restlessness. She is unable to perform any physical or mental work. Her condition needs treatment from the local mental institution.

At the asylum, the physician assigned to care for her, Aloysius Alzheimer, found Auguste D., as he would always refer to her, to be in good physical condition. However, as he noted in his report, there was something terribly wrong with her mind:

Her ability to observe is severely disturbed. If one shows objects to her, she names these usually correctly, but immediately thereafter she has forgotten everything. When reading, she drifts from one line to another, reads by spelling or with senseless intonation; when writing, she repeats individual syllables repeatedly, drops others, and bogs down rather quickly. When speaking, she often uses phrases of embarrassment, some paraphasic expressions (creamer instead of cup), sometimes she gets stuck (in speaking). Some questions she obviously does not understand. She does not comprehend anymore the usage of certain objects.

Although Auguste D.'s symptoms fit the diagnosis of senile dementia—a general term used back then to describe the condition of elderly people who were too mentally impaired to care for themselves—because she was only 51 and the onset of her dementia was so rapid, Alzheimer, a psychiatrist and neuropathologist, became convinced that her symptoms were not age related. Rather, he suspected something far more aggressive and destructive was assaulting her brain.

As he watched her progressive decline in the following weeks and months, he became equally convinced that whatever it was, it would do irreversible damage to her brain and eventually claim her life. This conviction was based on his experience in examining the brains of dozens of other patients who had died while living at the asylum.

Alzheimer had honed his forensic skills while working with Franz Nissl, a neurologist who'd joined the asylum faculty shortly after Alzheimer's arrival in 1889. Even before landing in Frankfurt, Nissl was well known for developing a method of staining nerve cells. (The Nissl protocol is still in use today in laboratories throughout the world.)

Alzheimer and Nissl used this stain to look for clues about the potential causes of a number of illnesses, including epilepsy, dementia, and cerebral palsy. They made detailed case studies of a total of 170 asylum patients and postmortem examinations of their brains before Nissl left in 1895 for a position in the clinic of the famed Emil Kraepelin at the University of Heidelberg.

During their time together in Frankfurt, Alzheimer and Nissl became confidants as well as collaborators. Alzheimer frequently wrote to Nissl about Auguste D.

By the time Alzheimer joined Nissl in Kraepelin's clinic in Heidelberg, he had become obsessed with Auguste D.'s case. From Heidelberg, he sent regular messages inquiring about her welfare. As one dispatch to him reported, Auguste D. was in the final throes of the disease:

She is completely stupefied, always lying in bed with legs drawn up. Regularly soiled with urine and faeces: never says anything, mutters to herself, has to be fed.

A year later, when Kraepelin was appointed professor of psychiatry at the Royal Psychiatric Clinic in Munich, Alzheimer went with him while Nissl stayed in Heidelberg, assuming Kraepelin's old position.

When news reached Alzheimer in 1906 that Auguste D. had died at age 55, he wasted little time submitting a request to the asylum in Frankfurt for her brain and medical records. It was granted. Using Nissl's stain, he finally got to see Auguste D.'s killer under the microscope.

He described it in his notes as a dark "peculiar material" deposited inside and around her nerve cells. Although he wasn't quite sure what the material was, he had little doubt it had caused her aggressive dementia. He became even more convinced when he found similar deposits among the brain cells of two other people who'd suffered from early onset dementia.

Medical researchers would eventually show that these peculiar deposits, or "aggregates," that Alzheimer saw in Auguste D.'s brain cells consist of naturally occurring proteins. They would also show that the protein aggregates are toxic to cells, particularly brain and nerve cells. It turns out that protein aggregation is responsible for some of the most destructive neurological diseases known to humankind, including amyotrophic lateral sclerosis, Huntington's disease, and prion diseases like Creutzfeldt-Jakob.

Protein-aggregate diseases occur in other tissues besides nerve cells. In sickle cell disease, protein aggregates damage red blood cells. In type 2 diabetes, they taint the pancreatic cells. (There is a high prevalence of Alzheimer's disease among people with type 2 diabetes, prompting some to believe that the two conditions are closely related.)

To date, scientists have found at least two dozen protein-aggregate disorders, each with its own distinct course and symptoms. Although it has been more than a century since Alzheimer first discovered one, which now bears his name, only recently has medical science understood enough about what makes proteins aggregate to begin developing strategies for fighting back. Progress at the University of Pittsburgh in understanding the cell's normal protein quality-control apparatus is giving scientists new insights into why this process fails in protein aggregate diseases and how it might be bolstered to prevent bandit proteins from causing problems in the first place.

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Fran Welek had a baby boy in the mid-1980s in Columbia, Mo. She and her husband, John, named him Sean. At six weeks of age, Sean's skin turned yellow, and his liver swelled. Doctors at the hospital, where Fran Welek also worked as a registered nurse, diagnosed Sean with hepatitis but were unable to determine its cause. They couldn't find any bacteria or viruses known to cause hepatitis in the newborn's bloodstream.

So the doctors took a biopsy of the infant's liver and sent it off for testing. A few weeks later, the diagnosis came back. Sean had a genetic condition called alpha-1-antitrypsin (A1AT) deficiency, which is sometimes fatal. A1AT is an enzyme inhibitor made in the liver and released

JIM JUDKIS



David Perlmutter (left) and Jeffrey Brodsky are working on ways to stop proteins from aggregating, a process that causes many disabling diseases.

into the blood, where it finds its way to the lungs and protects them from inflammation caused by infection and inhaled irritants such as tobacco smoke. A1AT deficiency was first discovered in adults with an aggressive form of emphysema. It was later found that some people with the deficiency end up with abnormal protein aggregates, typically called globules, in the liver—resulting in inflammation and swelling, as Sean experienced. It is the most common genetic cause of liver disease in children. About a quarter of patients with liver problems require a transplant to survive.

Welek and her husband were in "total shock" about the diagnosis. Not only had they never heard of the disease, neither had Sean's doctors in Columbia. Although his hepatitis would eventually subside, Sean would still be a very sick little boy, often requiring hospitalizations to control his symptoms, which included bruising, frequent nosebleeds, and swelling in his feet and legs. Because their doctors knew so little about the disease or how to manage it, the Weleks felt completely alone and were fearful for their son's life.

When Sean turned 3, the Weleks got a break. Sean's aunt was a resident at St. Louis Children's Hospital; there, she came across a patient who had the same diagnosis as her nephew. When she asked who the patient's doctor was, she was introduced to David Perlmutter, who turned out to be one of the leading experts on the disease.

Perlmutter—now chair of the Department of Pediatrics in the University of Pittsburgh School of Medicine, physician-in-chief at Children's Hospital of Pittsburgh, and scientific director of the Rangos Research Center—had begun caring for children with A1AT deficiency a few years earlier, while completing his own training at Children's Hospital Boston. When it came time to choose a project for his research fellowship, Perlmutter decided to explore A1AT deficiency. He was involved in another project at the time that involved the ability of cells to secrete proteins. He believed, somewhat erroneously, that the globules seen in A1AT-deficient liver cell biopsies were the result of a defective protein secretion mechanism.

Perlmutter began traveling all over New England collecting samples from children diagnosed with the deficiency. When he followed his mentor to Washington University School of Medicine in St. Louis, he continued that research. Before long, doctors from all over the Midwest were referring their A1AT-deficient patients to him.

Meeting Perlmutter not only gave the Weleks peace of mind, it also gave Sean a relatively normal life. "His doctors in Columbia said we needed to be very careful with him," says Welek, "and set limits for what he could do and couldn't do. But Dr. Perlmutter told us just to let him be a kid and have fun, and he would help us manage whatever happened. It was extremely comforting to find someone who could explain what was happening to Sean and give us the most up-to-date information on how to manage his condition."

The Weleks began taking Sean to see Perlmutter every six months and sometimes more often when he had flare-ups or complications.

Once, when Sean developed a bad infection, the Weleks rushed him to St. Louis. It looked as though Sean might not pull through. The whole family, including aunts and uncles, gathered to stand vigil. After a long night, Perlmutter emerged from Sean's room and assured everyone that the boy was going to be okay.

In 1995, when Sean was 10, his liver failed and he required a transplant. Perlmutter was there to help the Weleks through the ordeal. The transplant was successful, and Sean, who is now a scout for an NFL team, has had very few complications and no hospitalizations since.

Of course, a liver transplant is a life-altering procedure that often requires long-term use of antirejection drugs, which can lead to other serious health problems. For some 20 years now, Perlmutter has been looking for a way to stop A1AT deficiency before it can destroy patients' livers.

Early on, he says, he began to wonder why only about 10 percent of children with the deficiency get liver disease.

"The more children I saw with the deficiency, the more I wondered why the other 90 percent of children with this deficiency don't develop liver disease," he says.

Despite Alzheimer's remarkable finding, for more than 50 years after his untimely death in 1915 his discoveries were largely overlooked by psychiatry. All eyes were turning instead toward the theories and writings of Kraepelin's contemporary and rival, Sigmund Freud.

Unlike Kraepelin, Freud believed that most psychiatric diseases were caused by emotional trauma or repressed sexual desires and not by organic disease. Whereas Kraepelin sought evidence to support or refute his theories, Freud held up his theories as infallible and had little tolerance for those who questioned them. He even had dissidents expelled from their posts.

As Freud's doctrines began to dominate psychiatry, Alzheimer's reports and others that proposed a biological cause of psychiatric illness would become cold cases for decades. It wasn't until the 1970s, some 30 years after Freud's death, that the tide began to shift toward Kraepelin's model of psychiatric disease and Alzheimer's writings were rediscovered.

We now know the plaques that Alzheimer first observed in the spaces between Auguste D.'s brain cells consist mostly of beta amyloid, a fragment of a protein called amyloid precursor protein that occurs naturally in nerve cells. (Many now refer to aggregate diseases as amyloid diseases.) The tangles found inside the brain cells of Alzheimer's disease (AD) patients turned out to consist mostly of a protein called tau. In normal cells, tau is part of a structure called a microtubule, which helps support the cell's architecture and also transports molecules back and forth. In AD cells, however, tau is twisted out of shape, which badly contorts the microtubule, causing the cell to become sick or die. Tau is the most commonly found protein in neurodegenerative diseases.

We also know now that most protein aggregate diseases involve misshapen proteins, which appear, at least in some cases, to be caused by genetic mutations in the protein. Scientists believe that the more "destabilizing" the mutation, the more likely protein will gather together into an unruly mob.

Such destabilizing mutations do not always alter the structure or function of the protein immediately. Rather, they may predispose it to adopt an inappropriate structure and muck things up later on. This is why the symptoms of many of these conditions do not show up until adulthood or, as in Auguste D.'s case, middle age.

In some protein aggregate diseases, it is not a genetic mutation but the excess production of a protein that leads to its aggregation. In others, rogue proteins called prions set off the clumping phenomenon.

Regardless of the biological instigator, treatment strategies for protein aggregate diseases have met with little success.

In 1996, Perlmutter reported the discovery of an apparatus in cells that might be harnessed for degrading the globules that caused so much trouble for Sean and his other young patients. In a genetically engineered human fibroblast cell, he found that a well-known degradative enzyme called the proteasome played a role in the disposal of the mutant A1AT protein.

This was a surprise. The mutant A1AT protein accumulates in a membrane-bound compartment of the cell called the endoplasmic reticulum (ER). There, everyone supposed, it would stay put or the ER's own system would dispose of it as required. The proteasome resides in the cytoplasm, the fluid part of the cell that surrounds the ER, the nucleus, and other membrane-bound subcellular compartments. Perlmutter's results implied that the mutant A1AT was somehow transported out of the ER and into the cytoplasm.

At almost the same time, Jeffrey Brodsky, now the Avinoff Professor of Biological Sciences at Pitt, reported that the proteasome played a key role in disposal of the mutant A1AT protein in yeast cells. By manipulating genes in yeast, Brodsky made a powerful case for the proteasomal pathway.

Evidence was mounting: Others were also finding misshapen ER proteins could be degraded in the cytoplasm. Yet when Perlmutter first tried to report his findings, he encountered a great deal of skepticism. His observations ran counter to the then-prevailing notion that an ER protein would be taken care of by the ER exclusively.

"No one remembers now how heretical that finding was back then. But, it was a bear getting that paper published because no one wanted to touch it," he says.

Brodsky's team had a similar experience. He reports that he and his coauthors were so nervous about their finding that they omitted it from the initial draft of the paper. They only put it back in response to one reviewer's questions.

"The overwhelming, prevailing belief at the time was that the mechanism for getting rid of [such] unwanted or defective proteins resides entirely in the endoplasmic reticulum. However, our data showed that misshaped proteins were somehow getting out of the endoplasmic reticulum and being degraded in the cytoplasm of the cell. It was both exciting and scary at the same time," Brodsky says.

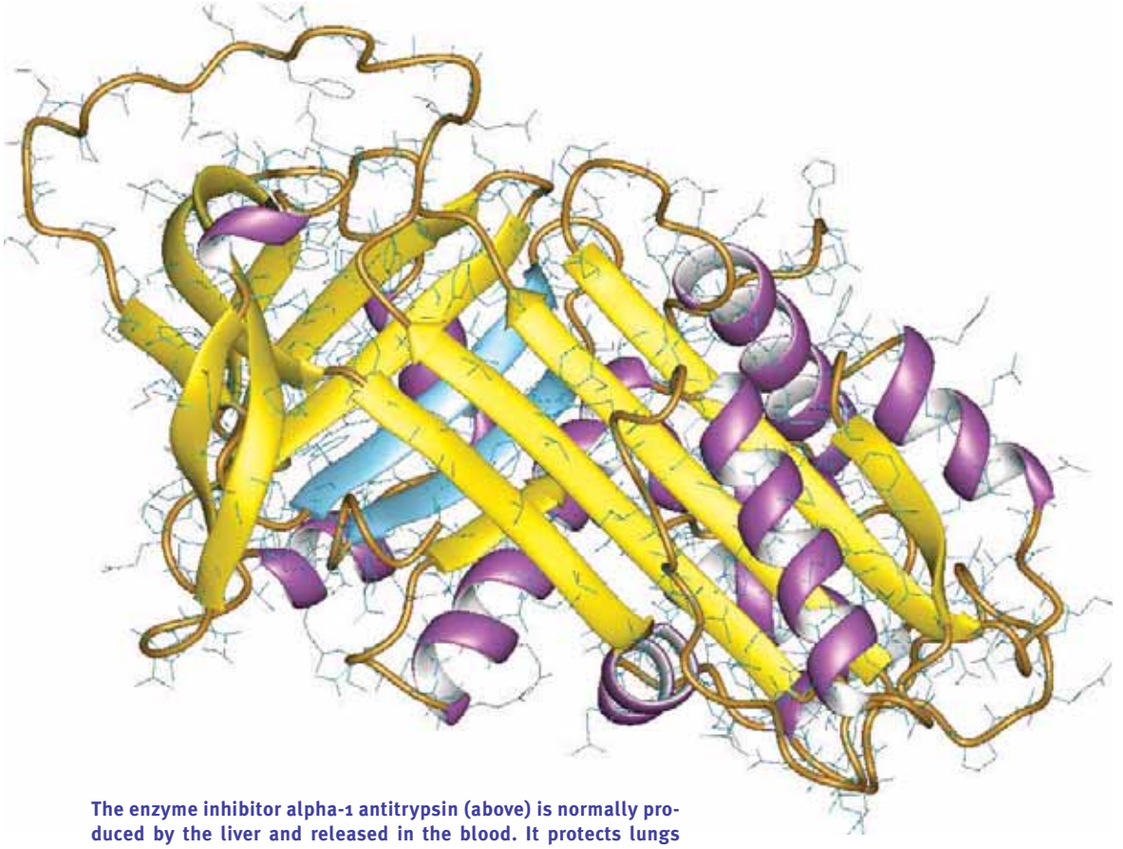
"It was heretical at the time. Now it's dogma," says Perlmutter.

Just as Alzheimer, Nissl, and Kraepelin helped launch a new field of research, Perlmutter and Brodsky were contributing to a new area of investigation into how mutant proteins were degraded in the cytoplasm of the cell by the proteasome.

In 1998, Perlmutter and Brodsky finally got the chance to meet and compare notes when Brodsky visited Perlmutter's lab in St. Louis as part of a grant review team. Although they initially viewed each other as potential rivals, they soon recognized they could complement each other. Perlmutter had the experience treating people that Brodsky knew would be critical in someday bringing his laboratory findings to the clinic. Brodsky had the in-depth understanding of cell and molecular biology that Perlmutter needed to fully comprehend the mechanisms for degrading proteins in the cell. Perlmutter works with mammalian cell systems and models. Brodsky works with yeast. The synergy was palpable to each.

A year later, they met again at a scientific meeting. At a tavern, Perlmutter told Brodsky he was considering coming to Pitt. Brodsky encouraged him, and they began a

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The enzyme inhibitor alpha-1 antitrypsin (above) is normally produced by the liver and released in the blood. It protects lungs from inflammation caused by irritants like tobacco smoke. When it's missing in the body, the result can be emphysema. In some patients who lack the inhibitor, usually children, proteins aggregate in the liver and cause severe damage.

collaboration, and friendship, that continues to this day.

But first, another surprise was in store. Just before Perlmutter joined the Pitt faculty in 2000, his lab implicated a little-known process called autophagy in the cytoplasm's disposal system for mutant proteins.

Before this, most cell biologists believed autophagy was active only under stressful conditions, such as when cells are starving. The word means "self eating" and refers to the process cells use to consume and recycle structures in the cell when deprived of nutrients. Perlmutter was among the first to demonstrate that it might be active under normal conditions.

By 2006, both Perlmutter's and Brodsky's labs independently showed—through entirely different approaches—that autophagy degraded A1AT. At low levels, the defective protein was degraded by the proteasomal pathway. However, when they turned up the production of the defective protein to a very high level, it was degraded by autophagy.

They also demonstrated, says Perlmutter, "that cells lacking a normal autophagic response are susceptible to greater aggregation when they are exposed to too much of the mutated protein."

Autophagy seems to be the preferred pathway for cells to degrade misfolded and other defective proteins that tend to form into aggregates. Perlmutter now believes subtle defects in autophagic response could be among the processes that cause liver damage in 10 percent of patients with A1AT deficiency. He and Brodsky are searching for compounds that might restore this response in A1AT deficiency and other protein aggregate diseases.

To find such agents, they've enlisted the staff at the Molecular Libraries Screening Center at Pitt. This high-throughput center allows them to test thousands of compounds at once.

Perlmutter has also enlisted the help of a worm. Or more precisely, with Gary Silverman, Pitt professor of pediatrics, and Stephen Pak, a research assistant professor of pediatrics in the Silverman lab, he is developing a worm model of A1AT deficiency that develops protein aggregates. They are testing promising compounds to see if any degrade the aggregates without hurting the worms.

Although preliminary, Perlmutter says the results are promising. A man who chooses his words carefully, the 54-year-old Perlmutter is barely able to contain his enthusiasm when talking about the potential impact of his current work with Brodsky and Silverman—not only for A1AT deficiency: "We're cautiously optimistic about our chances of finding some interesting compounds that will have application to a number of protein aggregate diseases," he says.

Stimulating autophagy to chew up bad proteins in worms is one thing; doing it in people is something entirely different. For starters, worms don't have a blood-brain barrier, a membrane that controls the passage of substances from the blood into the central nervous system in humans. Countless researchers have been stymied in their efforts to develop treatments for diseases of the brain because the blood-brain barrier prevents entry to many large molecules.

Autophagy itself could present a problem. When autophagic activity is especially high, it induces cell death. So attempts to boost it could be risky.

These problems are surmountable, Brodsky and Perlmutter believe. They are screening for molecules that are small enough to pass through the blood-brain barrier. In addition, Brodsky notes, you can stimulate autophagy without going overboard:

"We have evidence that autophagy can be turned up slightly without causing cellular damage. Ultimately, however, the most effective approach to reversing or preventing protein aggregation in cells may involve treating these diseases much the way AIDS is treated today. A multipronged approach that combines therapies and fine-tunes them to the particular condition may be optimal."

Speaking with Brodsky and Perlmutter, it's easy to think that effective therapies are around the corner. It seems, at least, that if scientists keep collaborating this productively, we won't need to wait another century to find worthwhile approaches to treating protein aggregate diseases. The Brodsky/Perlmutter and Alzheimer/Nissl teams have, in a sense, mimicked the rogue proteins making up that "peculiar material"—they've shown that by banding together, they are a force to be reckoned with.

"I've always found that science works better when people combine their collective talents," says Perlmutter. ■