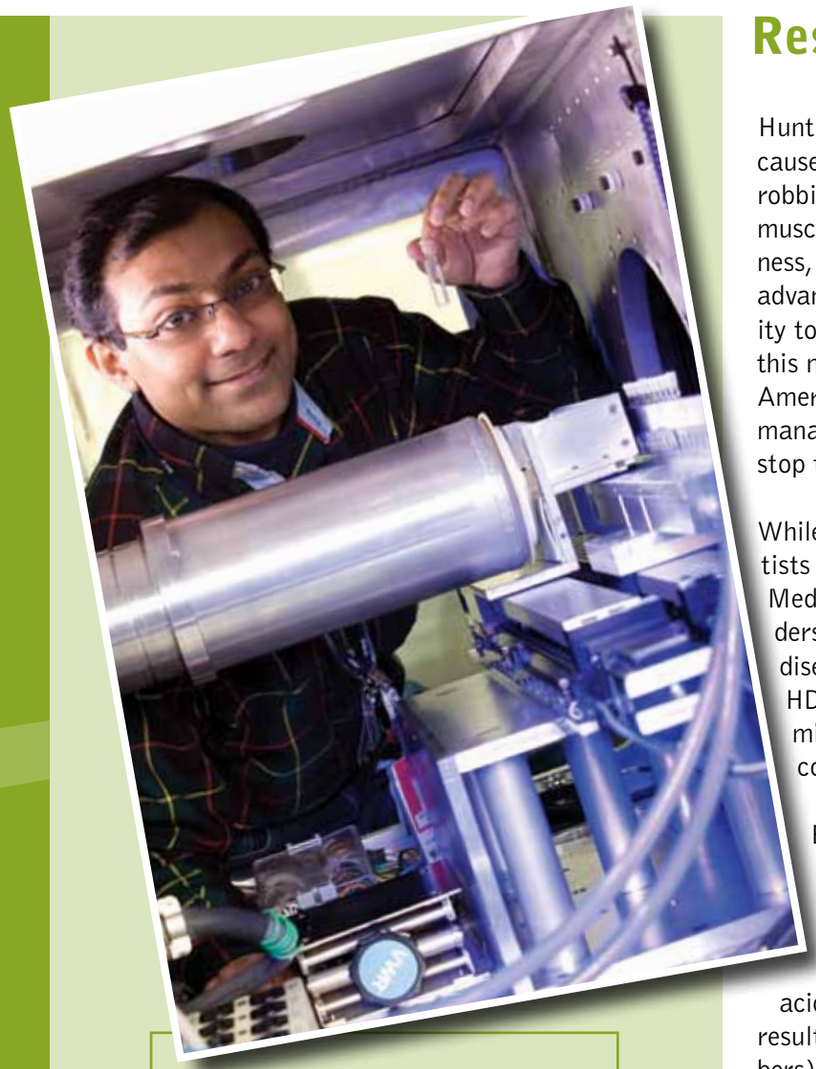
Researching a Cure for Huntington's Disease



Sai Venkatesh Pingali, Chemical Sciences Division, with an experiment sample at the Bio-SANS instrument.

Research on the molecular structure of proteins will help us better understand neurological and genetic diseases.

Huntington's disease (HD) is a genetic condition that causes certain brain cells to waste away, eventually robbing its victims of their ability to control their muscles. In its early stages, it can cause clumsiness, forgetfulness, and impaired speech. In its more advanced stages, it can take away a person's ability to walk, talk, and swallow. Available drugs for this neurological disease, first described in 1872 by American physician George Huntington, help patients manage the symptoms of HD, but they don't slow or stop the disease.

While there is no cure for HD, there is hope. Scientists at ORNL and the University of Tennessee (UT) Medical Center are using modern tools to better understand the mechanisms behind this neurological disease. Like Alzheimer's and Parkinson's diseases, HD is caused by a specific protein, huntingtin, that misbehaves. For all cases of HD, a defective gene codes for the troublesome protein.

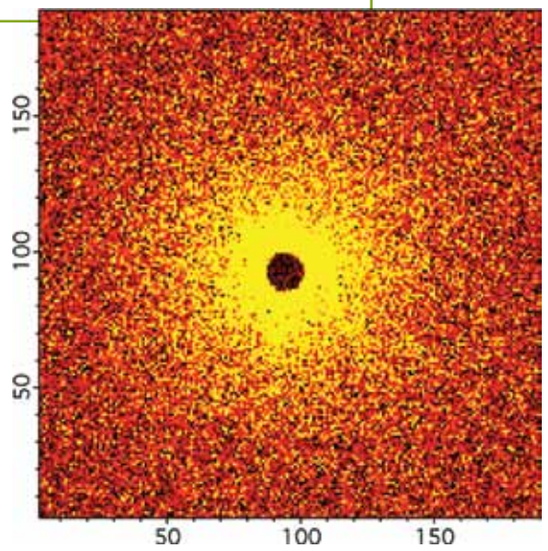
Each protein is made of a combination of amino acids in a sequence dictated by a gene. In a person suffering from HD, the HD protein in many brain cells contains an abnormally long sequence of 40 or more glutamine amino acids in succession. This abnormal repeat sequence results in the formation of fibrils (thin, threadlike fibers) that cause brain cells to deteriorate and die. In a healthy person's brain, the huntingtin protein has only 15 to 20 glutamines in repeat.

At HFIR, Chris Stanley is using SANS to trace in real time the detailed formation of protein fibrils composed of glutamine repeats. Stanley, a Shull Fellow and biophysicist with a Ph.D. in polymer science, came to ORNL with the idea of conducting a study of the growth, or aggregation, of protein fibrils using the Bio-SANS instrument at HFIR. Stanley's mentor, Dean Myles, introduced him to a UT Medical Center researcher, Professor Valerie Berthelier, who is studying the protein specifically involved in Huntington's disease.

At the Graduate School of Medicine, UT Medical Center, Berthelier's group seeks to understand the mechanisms of protein folding and misfolding—correct and incorrect folding of a linear chain of amino acids into a three-dimensional protein. The researchers decipher how these processes are related to normal physiology and disease using chemical, biophysical, and cell biology approaches.

In their current work on HD, they hope to find what makes an aggregate toxic. By identifying small compounds that are capable of altering the shape of the aggregates, they think they will be able to understand the functional role of pre-fibrillar species in the HD

Two-dimensional SANS pattern from polyglutamine fibrils.



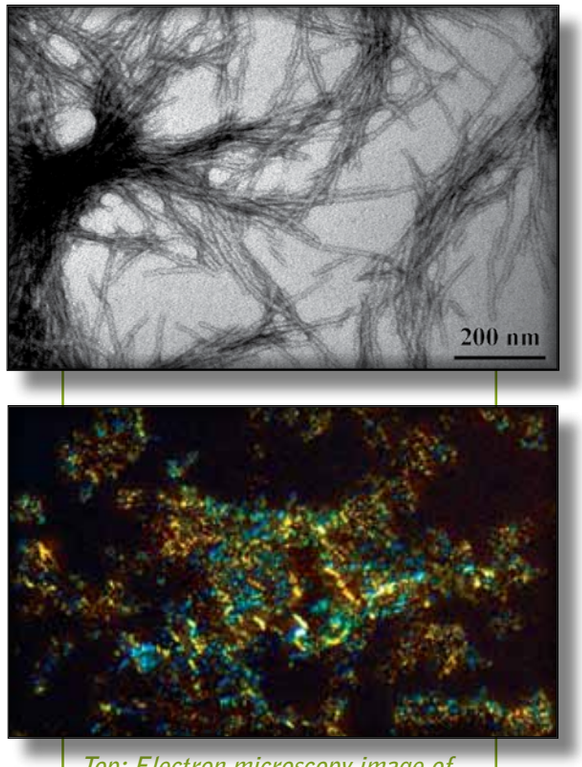
process. Berthelier and Stanley have had a fruitful collaboration so far.

"I am using small-angle neutron scattering to study the structural formation of fibrils to help her understand polyglutamine aggregation in Huntington's disease," Stanley says. "HD is caused by an abnormal polyglutamine expansion in the huntingtin protein, a huge biomolecule that has an interminable site that can be cleaved off as a protein fragment. The resulting small protein, or peptide, fragment misbehaves by aggregating into a threadlike fibril that can turn into neuronal inclusions in the brain, causing brain degradation."

In mouse cell studies conducted by other researchers, no strong correlation has been found between the amount of HD fibrils in the brain cells and real behavioral effects, such as loss of memory and muscular control.

"Something must be happening between the normal, well-behaved protein and the fully formed fibril that is toxic for the brain cells," Stanley says. "We are searching for early intermediates or unusual prefibrillar structures that can affect and potentially kill brain cells.

"With the Bio-SANS at HFIR, we can look at samples on a nanometer-length scale. We are doing SANS experiments to identify the structures that form over time with the hope of pinpointing unique structures along the pathway, and possibly even off the pathway, of fibril formation. Valerie and her team can target those unique structures and try to identify therapeutic compounds that inhibit the formation of unique toxic structures. We are trying to get a better handle on what is happening during the formation of the fibril and which part should be targeted for treatment."



Top: Electron microscopy image of polyglutamine fibrils. Bottom: Polarized optical image of stained polyglutamine fibrils.

Using time-resolved SANS, Stanley captured snapshots of the protein structure as it aggregated into fibrils and determined the rate of aggregation. In the experiment, synthetic huntingtin-like peptides having between 22 and 42 glutamines in the repeat were placed in a buffered salt solution in a quartz cuvette. The solution contained deuterated water that, for neutron scattering, provides a high contrast level to observe the scattering from the protein.

"We know when in time that protein structural elements change," Stanley says. "We can determine when changes in size and shape occur during aggregation to form the fibril. We can measure the radius of the cross section of the formed fibrils as well as their mass per length. In future kinetic studies, we will look for changes in the fibril's building blocks when therapeutic compounds are added."

"In future experiments, we're hoping to do a better job of mapping out the pathway of the fibril as it aggregates. We will be able to monitor a larger size range simultaneously in our time-resolved aggregation studies when we use the EQ-SANS instrument at SNS."

Another project that Stanley is engaged in is using SANS to study intrinsically disordered proteins that are responsible for transcription and translation—reading instructions from DNA and producing the requested protein. Because of its plasticity and flexibility, this protein type can bind to different binding partners.

Stanley surmises that the CREB (CAMP response element binding) binding protein, a large binding protein with a short glutamine repeat that performs transcription, might get trapped by an aggregating HD fibril, knocking out the large protein's central transcription function. If SANS can be used to find such a sequestered protein in an HD fibril, the result would suggest one way that a protein fibril could be responsible for the decline and death of a brain cell. Other researchers have speculated about this possible interaction where there are obvious implications for HD.

As more powerful scientific tools become available, HD patients and researchers remain hopeful that a breakthrough might turn an incurable disease into a curable one.