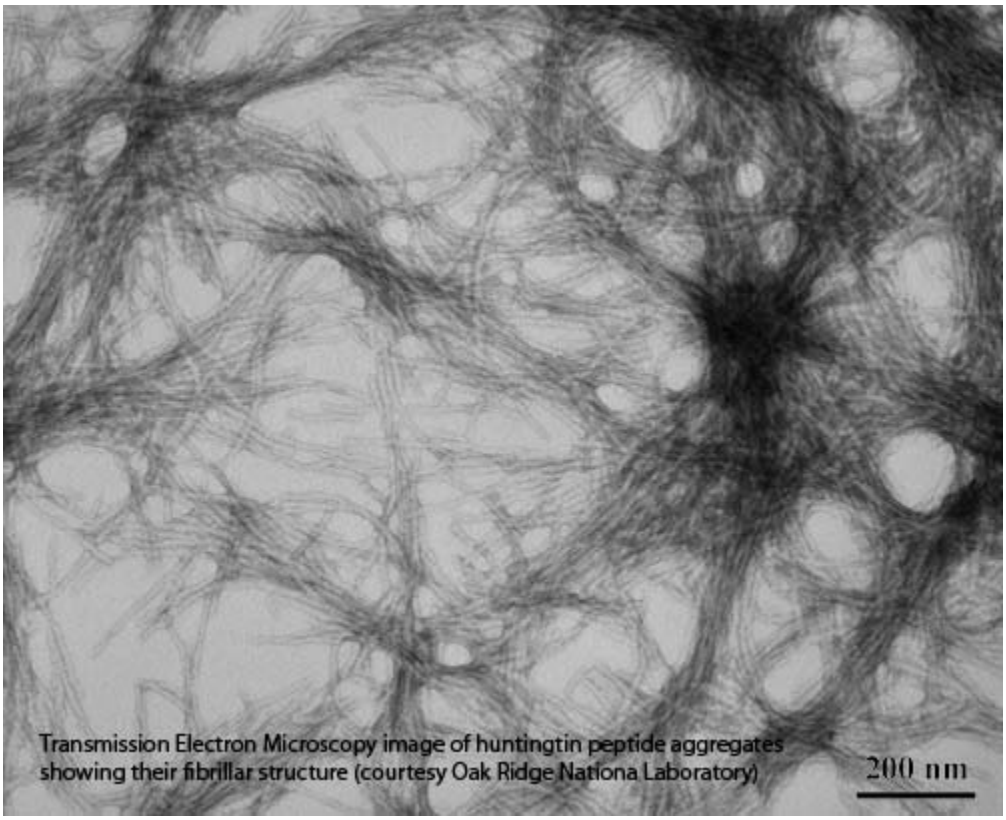
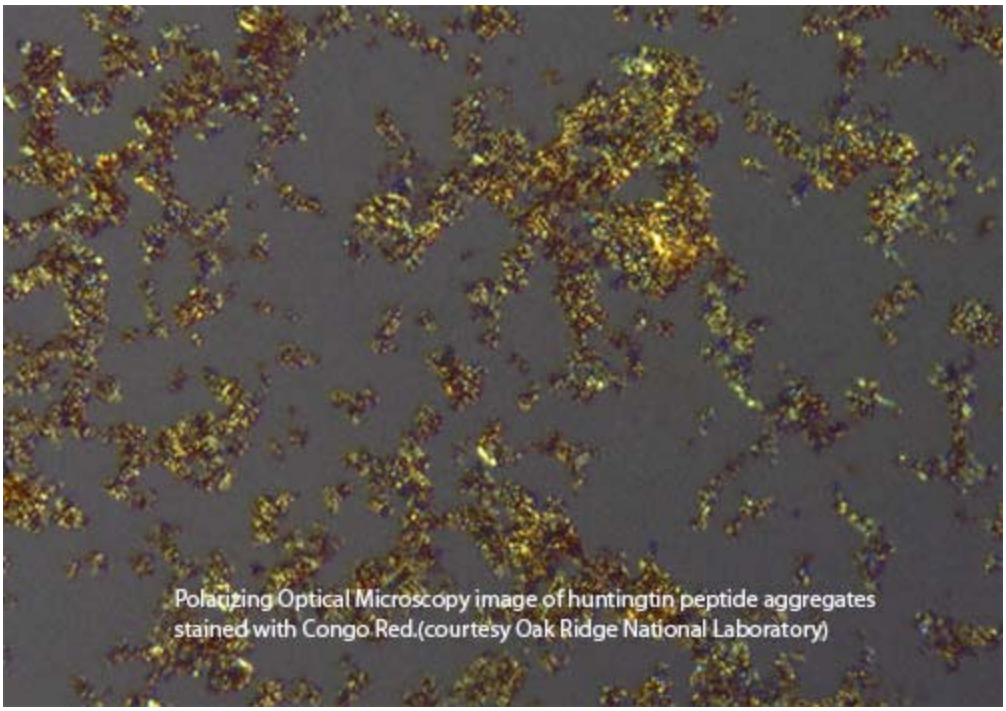
Huntingtin's Protein Aggregates Visualized at Oak Ridge National Laboratory



A new study sheds light on the protein aggregation that is a hallmark of Huntington's disease and other neurodegenerative disease. The Huntington's disease protein aggregates were first discovered in 1997 in the R6/2 mice and confirmed in postmortem brain tissue donated by HD patients. At first, some researchers thought that the aggregates might be the central pathology of the disease and hoped that a drug could be discovered or developed that would dissolve the protein clumps and treat the disease.

Ongoing research has revealed that the issue is more complicated. The protein aggregates are not supposed to be there and are certainly a sign of the disease. However, the soluble HD protein appears to be more toxic than the aggregates so dissolving the large aggregates does not seem to be the answer.

Some researchers have hypothesized that the earliest form of aggregation might be toxic but they have been hard to study because they are small and transitory.



Polarizing Optical Microscopy image of huntingtin peptide aggregates stained with Congo Red.(courtesy Oak Ridge National Laboratory)

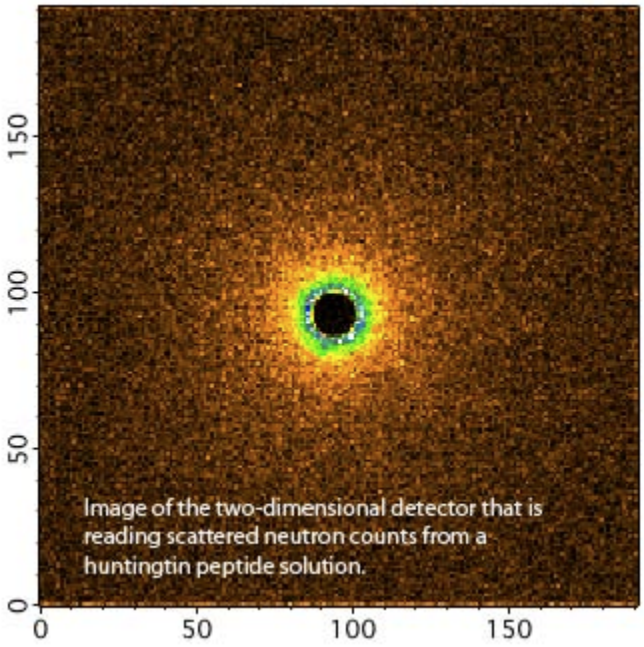
Christopher Stanley, a Shull Fellow in the Neutron Scattering Science Division at Oak Ridge National Laboratory, and Valerie Berthelier, a UT Graduate School of Medicine researcher who studies protein folding and misfolding in Huntington's, have used a small-angle neutron scattering instrument, called Bio-SANS, at ORNL's High Flux Isotope Reactor to explore the earliest aggregate species of the protein that are believed to be the most toxic.

At the HFIR Bio-SANS instrument, the neutron beam comes through a series of mirrors that focus it on the sample. The neutrons interact with the sample, providing data on its atomic structure, and then the neutrons scatter, to be picked up by a detector. From the data the detector sends of the scattering pattern, researchers can deduce at a scale of less than billionths of a meter the size and shape of the diseased, aggregating protein, at each time-step along its growth pathway.

Designing the study was a challenge. “My initial interest was advancing knowledge about protein structure but it was especially challenging to design a study where you have a transient structure to observe. You have to deal with the fact that it’s evolving and changing and that you have a heterogeneous population of aggregates,” said Dr. Stanley.

Dr. Stanley and colleagues didn’t observe the full length protein but rather took a portion of the front end of the protein, N terminal Exon 1, the first seventeen amino acids followed by the CAG repeats and the first proline stretch. They were able to detect aggregates as small as two and three molecules of this peptide, called dimers and trimers. These tiny aggregates appeared to be spherical. Over time, the monomers (the single molecules of the peptides) disappeared from the solution. Dimers and trimmers

appeared, followed by fibrils, string like aggregates of the peptide. Large aggregates appeared; they are bundles of fibers.



Dr. Stanley explained that this work could lead to therapeutic strategies. "The next step is to take drug molecules, molecules that you have some sort of idea are affecting toxicity from mouse or cell studies, and see how on a molecular level those drug molecules are affecting aggregation. Then you might be able to rationally design therapeutics to target the most toxic species of aggregates."

Dr. Stanley has received calls and emails from the HD community and is pleased to have done work that could help lead to treatments. "A lot of things that I have done have been interesting from a scientific perspective but this is especially meaningful because of its medical relevance. I hope my work contributes in a positive way to helping people with Huntington's disease. I have heard from family members of people with Huntington's disease and their stories are very touching. It's a nice feeling to know that people appreciate our work. I am used to working with other scientists and we have a common interest so that makes it fun, but hearing back from families gives added meaning to the work."

References:

Interview with Dr. Christopher B. Stanley

Christopher B. Stanley, Tatiana Perevozchikova, and Valerie Berthelier. *"Structural Formation of Huntingtin Exon 1 Aggregates Probed by Small-Angle Neutron Scattering."* Biophysical Journal Vol. 100 (May 2011):2504-12.

- Marsha L. Miller, Ph.D., June 3, 2011