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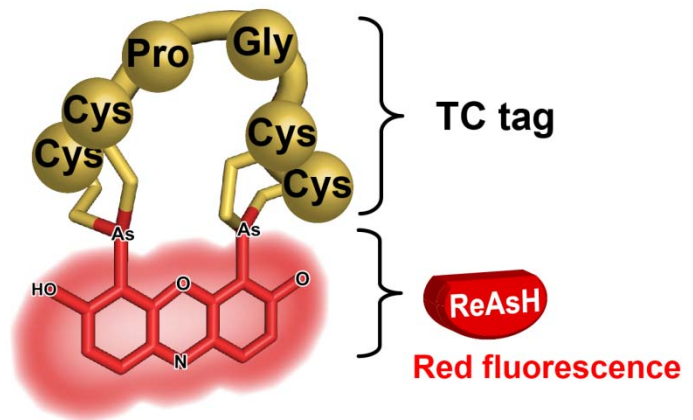
Protein misfolding, aggregation and neurodegenerative disease

Cells have an elaborate network of mechanisms to keep proteins folded and suppress their inappropriate aggregation. This phenomenon, known as proteostasis, is essential for the health and survival of cells. However, in more than 40 human diseases, such as Huntington's and Alzheimer's, protein misfolding and aggregation events escape proteostasis which leads to cellular dysfunction and degeneration. Our laboratory examines what happens to proteins in cells that are prone to misfolding and aggregation. We examine the conformation of misfolded proteins in the cell, their aggregation properties in the cell and mechanisms by which cells use to combat inappropriate aggregation.

Techniques we use

Our laboratory is truly multidisciplinary and we offer training in a wide range of biophysical, cell biology and genetics approaches. Typical methodologies include:

- Targeted mutagenesis of proteins to probe structure and function relationships
- Application of new fluorescent sensors of protein conformations in the cell
- New flow cytometry approaches to track how proteins aggregate in individual cells
- Mammalian cell culture and confocal microscopic imaging to observe protein localization and changes
- *Drosophila* cell biology to study aggregation *in vivo* (with Dr Leonie Quinn)
- Analytical ultracentrifugation using fluorescence detection to study aggregation in the cell
- Protein expression and purification to study intrinsic structural features of disease causing proteins.



TC sensor technology. Used to discriminate different conformations of huntingtin protein directly in live cells (1).

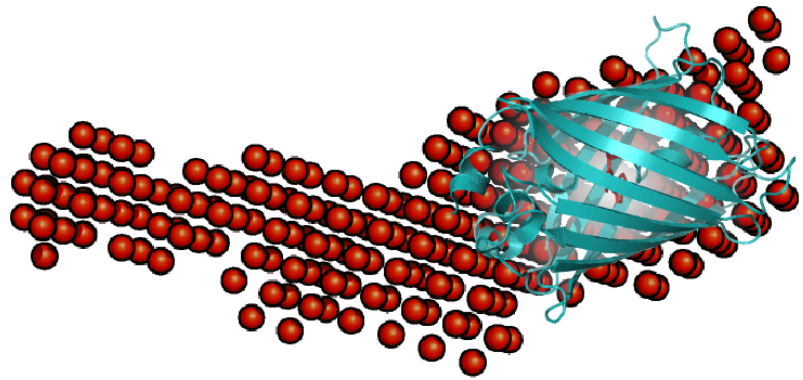
Projects:

Identification of intracellular “protective” mechanisms against mutant Huntingtin aggregation

This project aims to identify the cellular machinery that assists cells to package and process mutant huntingtin, which causes Huntington's disease, as they travel to large clusters of aggregates called inclusions. Proteins involved in the cytoskeleton will be investigated among several other candidate proteins that have been previously identified as modifiers of HD pathology in animal models of the human disease.

Structural analysis of oligomers formed in mammalian cells

This project involves determining the structural and biochemical properties of oligomers of mutant huntingtin protein and how they assemble into inclusions. The work will use live cell imaging and advanced fluorescence imaging and flow cytometry techniques.



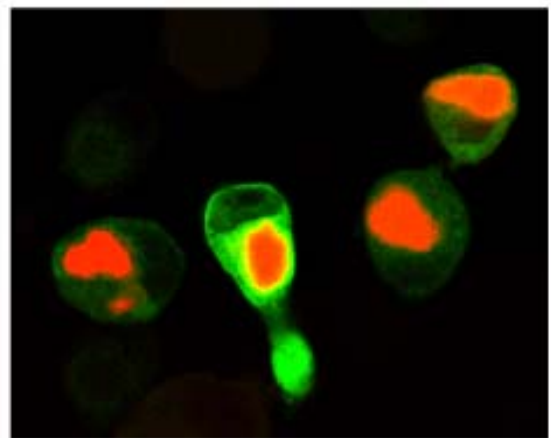
A bead model of the Huntingtin monomer fused to CFP. Derived from our small angle x-ray scattering experiments with Dr Mulhern.

Live cell imaging of huntingtin aggregation in *Drosophila* (with Dr Leonie Quinn, Dept of Anatomy and Cell Biology)

We have developed *Drosophila* models for HD to determine how defects in protein conformation and aggregation affect neural functioning. The aim of this project is to determine the physiological changes that lead to HD *in vivo*.

Determining the interplay between two “garbage disposal” mechanisms for problematic proteins

This project will investigate the processes that cells use to process unsalvageable proteins, such as mutants that cannot fold properly. In this project, the student will study how these mechanisms operate using specially designed “problem” proteins and a collection of disease associated proteins from a range of neurodegenerative diseases.



Heat shock chaperones (green) and huntingtin inclusions (red) in mouse neuroblastoma cells.

Recent publications:

1. Polling, S., Hill, A. F., and Hatters, D. M. (2012) Polyglutamine aggregation in Huntington and related diseases, in *Tandem Repeat Polymorphisms: Genetic Plasticity, Neural Diversity and Disease* (Hannan, A. J., Ed.), Landes Bioscience, Austin.
2. Hatters, D. M., and Griffin, M. D. (2012) Diagnostics for amyloid fibril formation: where to begin., in *Protein Folding, Misfolding, and Disease* (Hill, A. F., Cappai, R., Barnham, K., and Bottomley, S. P., Eds.), Humana Press.
3. Mok, Y. F., Ryan, T. M., Yang, S., Hatters, D. M., Howlett, G. J., and Griffin, M. D. (2011) Sedimentation velocity analysis of amyloid oligomers and fibrils using fluorescence detection. *Methods* 54, 67-75.
4. Irtegun, S., Ramdzan, Y. M., Mulhern, T. D., and Hatters, D. M. (2011) ReAsH/FlAsH labeling and image analysis of tetracysteine sensor proteins in cells. *J. Vis. Exp.*, Accepted 3 Jan, 2011.
5. Bernoux, M., Ve, T., Williams, S., Warren, C., Hatters, D., Valkov, E., Zhang, X., Ellis, J. G., Kobe, B., and Dodds, P. N. (2011) Structural and functional analysis of a plant resistance protein TIR domain reveals interfaces for self-association, signaling, and autoregulation. *Cell Host Microbe* 9, 200-11.
6. Tetali, S. D., Budamagunta, M. S., Simion, C., den Hartigh, L. J., Kalai, T., Hideg, K., Hatters, D. M., Weisgraber, K. H., Voss, J. C., and Rutledge, J. C. (2010) VLDL lipolysis products increase VLDL fluidity and convert apolipoprotein E4 into a more expanded conformation. *J Lipid Res* 51, 1273-83.
7. Ramdzan, Y. M., Nisbet, R. M., Miller, J., Finkbeiner, S., Hill, A. F., and Hatters, D. M. (2010) Conformation sensors that distinguish monomeric proteins from oligomers in live cells. *Chem Biol* 17, 371-9.
8. Olshina, M. A., Angley, L. M., Ramdzan, Y. M., Tang, J., Bailey, M. F., Hill, A. F., and Hatters, D. M. (2010) Tracking Mutant Huntingtin Aggregation Kinetics in Cells Reveals Three Major Populations That Include an Invariant Oligomer Pool. *J. Biol. Chem.* 285, 21807-21816.
9. Hatters, D. M., Voss, J. C., Budamagunta, M. S., Newhouse, Y. N., and Weisgraber, K. H. (2009) Insight on the Molecular Envelope of Lipid-bound Apolipoprotein E From Electron Paramagnetic Resonance Spectroscopy. *J. Mol. Biol.* 386, 261-71.
10. Coleman, B. M., Nisbet, R. M., Han, S., Cappai, R., Hatters, D. M., and Hill, A. F. (2009) Conformational Detection of Prion Protein with Biarsenical Labeling and FlAsH Fluorescence. *Biochem Biophys Res Commun* 380, 564-8.
11. Hatters, D. M. (2008) Protein Misfolding Inside Cells: The Case of Huntingtin and Huntington's Disease. *IUBMB Life* 60, 724-8.