

From proteins to people: an open toolkit to accelerate Huntington's disease research

Rachel J. Harding¹, Peter Loppnau¹, Suzanne Ackloo¹, Alexander Lemak², Ashley Hutchinson¹, Brittany Hunt¹, Alex S. Holehouse³, Jolene C. Ho¹, Lixin Fan⁴, Leticia Toledo-Sherman⁵, Alma Seitova¹, Cheryl H. Arrowsmith^{1,2}



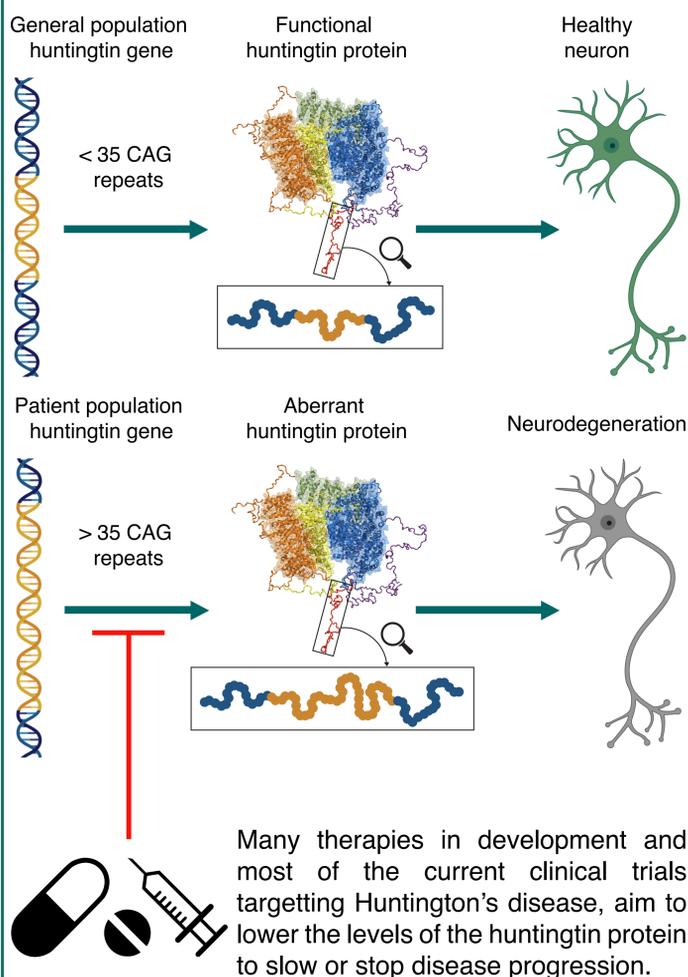
¹Structural Genomics Consortium, University of Toronto, MaRS South Tower, 101 College Street, Toronto, Ontario M5G 1L7, Canada, ²Princess Margaret Cancer Centre and Department of Medical Biophysics, University of Toronto, Toronto, Ontario M5G 1L7, Canada, ³Department of Biomedical Engineering and Center for Biological Systems Engineering, Washington University in Saint Louis, Saint Louis, Missouri 63130, USA, ⁴Basic Science Program, SAXS Core facility of National Cancer Institute, Frederick National Laboratory for Cancer Research, Frederick, MD 21701, USA, ⁵CHDI Foundation, 6080 Center Drive, Suite 700, Los Angeles, CA 90045, USA

Summary

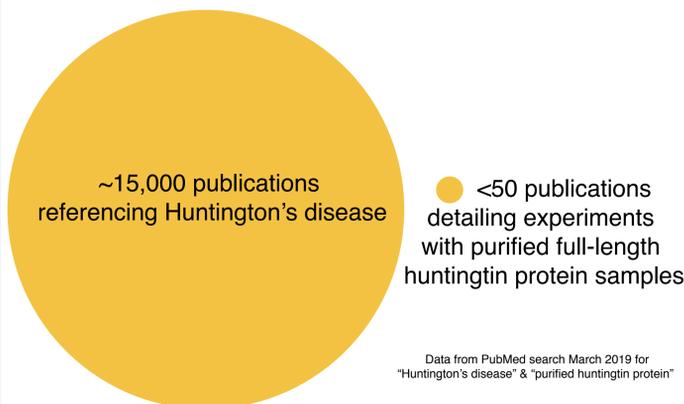
Huntington's disease research has been hampered by a paucity of openly available biochemical tools to facilitate research into this devastating disease and help develop new therapeutics. This project aimed to generate resources for the Huntington's disease research community, available without restriction, with the hope of accelerating research of the huntingtin protein which functions aberrantly in patients with this disease.

The mechanism of Huntington's disease remains incompletely understood

Huntington's disease patients have an expansion mutation in a CAG-repeat region of the huntingtin gene. This mutation leads to neuronal cell dysfunction and progressive neurodegenerative decline with complex psychological, cognitive and physical symptoms. There are no disease modifying therapies available for Huntington's disease.



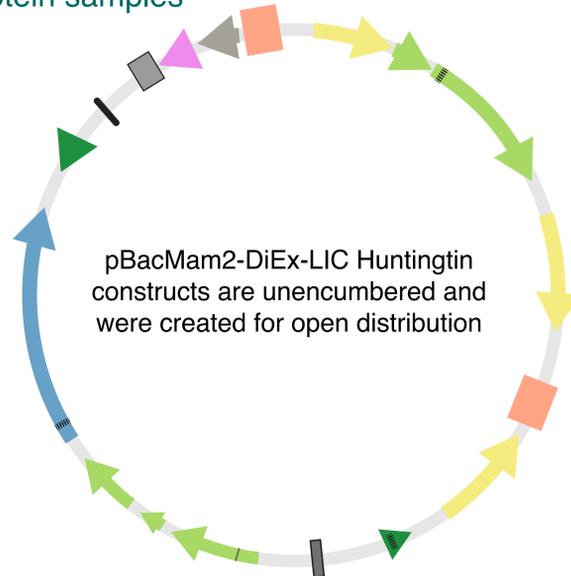
However, we still don't know very much about the physiological function of this critical protein molecule.



Huntingtin is in the top 1% of human genes, encoding a 3000+ amino acid protein. This creates many technical barriers to make huntingtin protein samples.

Aim: Design and characterise an open toolkit of Huntington's disease research resources for biochemical investigation of huntingtin protein

An open-source toolkit to make huntingtin protein samples



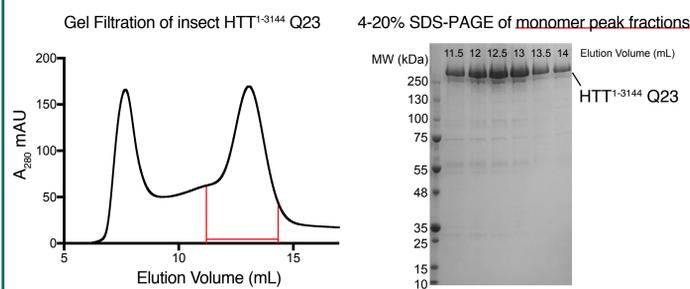
Constructs with different expansions were generated to allow expression and purification of huntingtin proteins reflecting different populations:

General population	15, 19, 23, 24, 25, 30
HD patients	36, 42, 48
Juvenile HD patients	51, 52, 54, 60, 66, 73, 78, 79, 85
Extreme expansions	109, 139, 142, 145

pBacMam constructs permit flexible expression in either insect or mammalian cells to suit the needs of different labs.

Suspension eukaryotic culture systems allow scalable production of protein, facilitating purification of large quantities of material, needed for subsequent biochemical analysis.

Huntingtin samples can be simply purified with a 2-step protocol using FLAG-affinity chromatography and gel filtration. This yields highly pure huntingtin samples.



Protein samples were thoroughly characterised and validated:

- ✓ Protein fold assessed by differential static light scattering
- ✓ Monodispersity assessed by analytical gel filtration and MALS
- ✓ Function assessed by Huntingtin-associated protein of 40 kDa (HAP40) complex formation
- ✓ Post-translational modification motifs mapped by mass spectrometry

References

The cryo-electron microscopy structure of huntingtin. Guo *et al* (2018) Nature
 Open lab notebooks: good for science, good for society, good for scientists. Schapira *et al* (2019) F1000
 Toolkit of HTT protein resources. Harding *et al* (2019) JBC
 Huntingtin Lowering Strategies for Disease Modification in Huntington's Disease. Tabrizi *et al* (2019) Neuron

Sharing the toolkit with the community

All data from this project are available through the LabScribbles open lab notebook

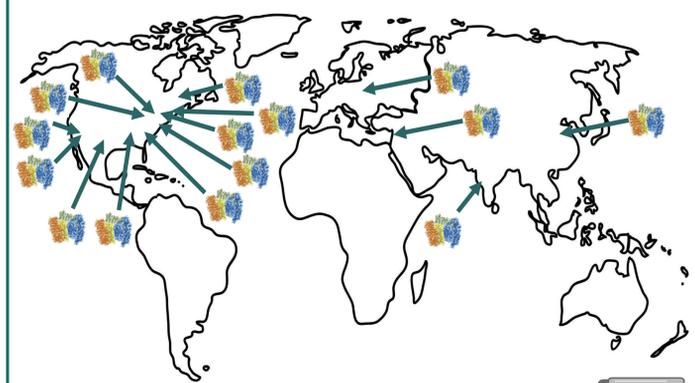
1. Experiments completed in lab
2. Materials, methods, data and analysis uploaded to Zenodo LabScribbles community
3. Experiment lay summary linking to Zenodo data
4. Share open notebook post via Twitter @LabScribbles

pBacMam Huntingtin constructs are shared via:



- Since December 2018:
- 40+ plasmid samples requested
 - 15 research groups requesting samples
 - 16 different constructs (repeat-lengths) requested

Plasmid samples have been shared with the international Huntington's disease research community



Purified huntingtin protein samples have been shared with 10+ academic labs through collaboration.

Protein samples have been shared with multiple biotech and pharma companies via CRO.

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