Pursuit of a high resolution structure of full-length huntingtin by cryo-electron microscopy

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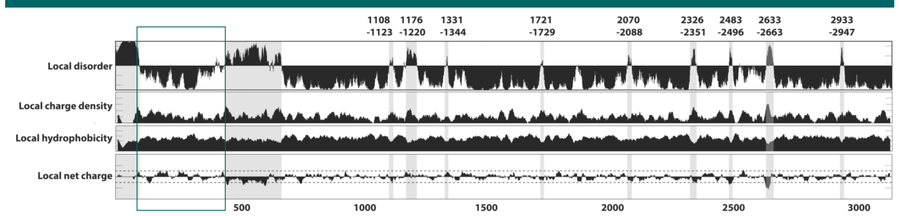
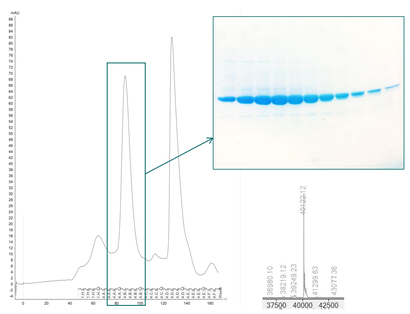
Abstract:

Huntington's disease is hallmarked by the CAG expansion of the huntingtin gene. How the corresponding polyQ expansion affects the structure of the encoded huntingtin protein remains poorly understood in the absence of a high resolution full-length protein structure. Huntingtin is a large, monomeric protein of 350 kDa, an ideal size for electron microscopy based structural biology methods. Using protein derived from a baculovirus expression system, we have successfully calculated a new protein envelope of huntingtin at ~15 Å resolution by negative stain electron microscopy. This reveals a claw-shaped molecule with a large central cavity. Grids of the protein sample have been optimized to produce a disperse array of homogenous protein particles in a fine vitreous ice layer for analysis by cryo-electron microscopy. A high resolution dataset has been collected on a Krios instrument and work is continuing in pursuit of this protein structure. This project is part of the open notebook labscribbles, through which methods and data are freely shared through the repository Zenodo under a CC-by license in real time in an effort to catalyse research in this area.

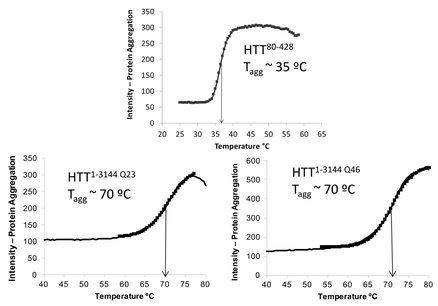
Huntingtin$^{1-3144}$ is stable *in vitro*



Region 80-428 is predicted to be folded – Classification of Intrinsically Disordered Ensemble Regions (CIDER)



Huntingtin$^{80-428}$ cloned into pFBOH-MHL can be successfully purified by cobalt affinity chromatography and gel filtration



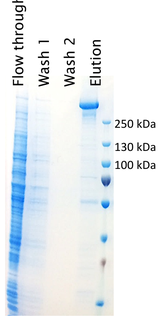
Despite extensive buffer screening, huntingtin$^{80-428}$ is unstable with a low calculated T_{agg}. Full-length huntingtin is more stable.

Expression and purification of full-length huntingtin

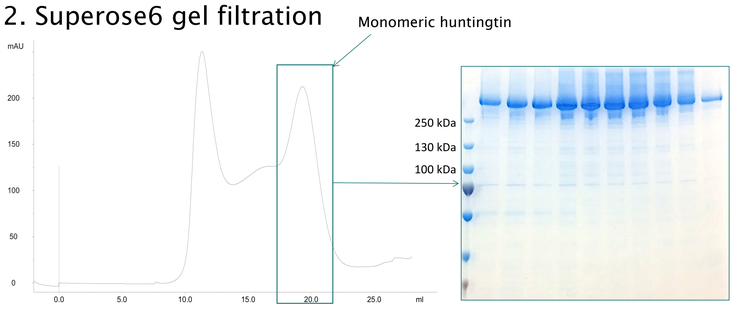
Expression systems were provided by collaborators and generated in house. Purification protocols were optimised from the published literature: Vijayvargia et al (2016) eLIFE, Huang et al (2015) PLoS One. Yields decrease with increased polyQ length.

Expression System	PolyQ	Tags	Vector	Pubmed ID	Acknowledgements	Comments
Baculovirus sf9	23, 46, 78	N-terminal His and FLAG tags – cleavable by TEV	pFASTBAC	27003594	Ihn Sik Seong (MGH, Harvard)	Final yield ~ 250-1000 µg per 1 L culture
HEK293 Tet-ON (adherent)	17, 46	C-terminal His and FLAG	pTRE-Tight-BI-AcGFP1	25799558	Stefan Kochanek (Ulm University)	Final yield ~ 2-4 µg per T-160 flask
Baculovirus sf9 or HEK293 (suspension adapted)	23, 79, 145	N-terminal His and FLAG tags	BacMam	N/A	Peter Loppnau, Alma Seitova (SGC Toronto)	Cloned, expression to be validated.
Baculovirus sf9 or HEK293 (suspension adapted)	23, 79, 145	C-terminal His and FLAG tags	BacMam	N/A	Peter Loppnau, Alma Seitova (SGC Toronto)	Cloning underway

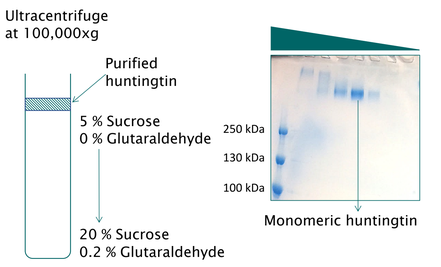
1. FLAG-bead purification



2. Superose6 gel filtration

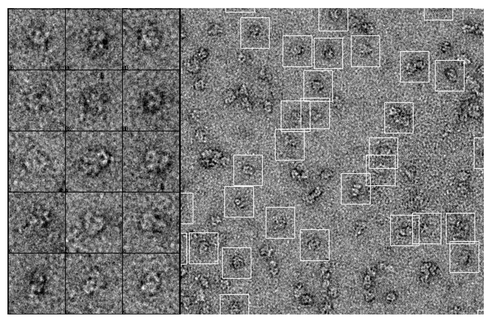


3. Gradient fixation and fractionation

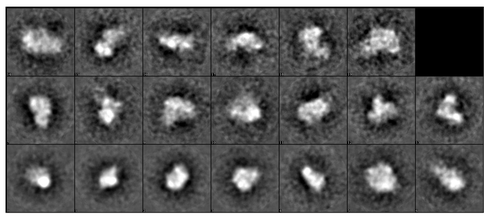


Negative stain electron microscopy of huntingtin

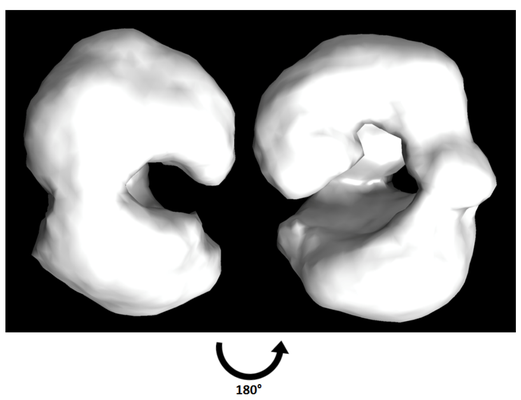
Negative stain electron microscopy grids generated of huntingtin protein samples of different polyQ lengths (Q23 or Q46), derived from different expression systems (mammalian or insect) and different construct tags (N-terminal, C-terminal or cleaved). No significant differences are observed at the point of generating 2D class averages. Analysis is ongoing.



Uranyl acetate negatively stained huntingtin protein particles



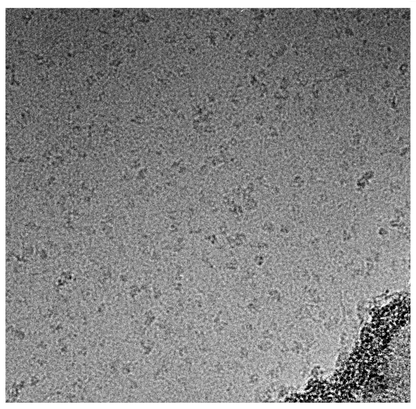
2D class averages calculated in SIMPLE from 10k particles collected on FEI Arctica.



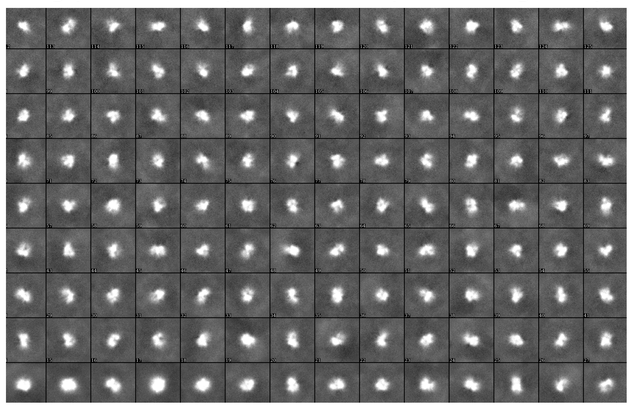
Refined ab initio 3D reconstruction based on particles alignments refined at the level of class averages and mapped back to the particles

Cryo electron microscopy analysis of huntingtin Q23

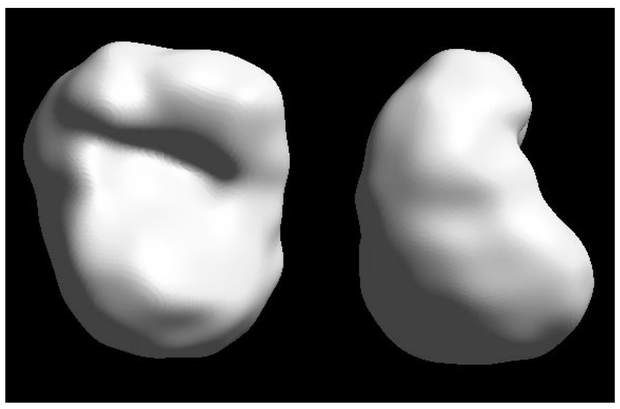
Initial data collection comprises just 900 micrographs, corresponding to ~ 36,000 particles. The data processing results suggest conformational heterogeneity of the sample, likely due to the flexible nature of huntingtin. Larger particle sets are required to investigate further. Formation of a complex with validated huntingtin binding partners may resolve sample heterogeneity through constraint of the protein conformation.



Huntingtin particles in cryo-micrograph



2D class averages calculated in SIMPLE from 36k particles collected on a Titan Krios with CS corrector.



Preliminary ab initio 3D reconstruction based on particles alignments refined at the level of class averages and mapped back to the particles

Open science initiatives

This project is run using a real-time open notebook. Full methods and results can be found on Zenodo via labscribbles.com

All data is available through a Creative Commons Attribution License 4.0.

SGC generated expression constructs are freely available upon request.

Huntingtin protein samples are enthusiastically shared on the basis of a collaborative agreement with any interested research groups and the SGC.

All enquiries to be sent to rachel@labscribbles.com

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