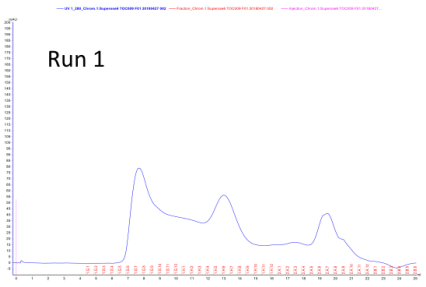
Full-length huntingtin Q19, Q23, Q42 and Q54 expression and purification – 2018/04/23

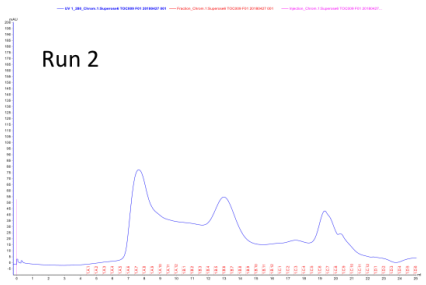
Rationale:
Purified huntingtin samples of different polyQ lengths are required for use in structural and functional studies.

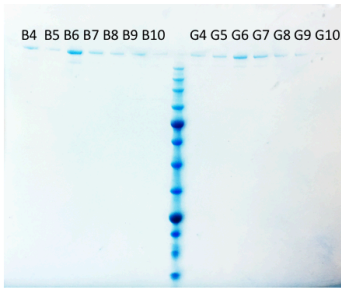
Growth:
8 L BVES production each for TOC009-F01 (Q19), D01 (Q23), F06 (Q54) and D02 (Q54) – all HTT^{1-3144} pBACMAM C-terminal FLAG. sF9 cells were harvested by centrifugation, resuspended in ~200 mL 50 mM Tris pH 8, 500 mM NaCl supplemented with 1 x protease inhibitors and then the pastes were stored at -80 ˚C prior to purification. Full methods are here: https://zenodo.org/record/154611

Purification:
Cell pastes were thawed and diluted to 500 mL with 50 mM Tris pH 8, 500 mM NaCl and supplemented with benzonase. NB: freeze-thaw cycle for cells is sufficient for lysis. Lysates were clarified by centrifugation and then bound to 5 mL anti-FLAG resin (Sigma M2) at 4 ˚C with rocking for 2 hours. Resins were washed with 1000 mL 50 mM Tris pH 8, 500 mM NaCl. HTT protein was eluted with ~20 mL resuspension buffer supplemented with 200 µg/mL 3xFLAG peptide. Samples were concentrated and run as 1 mL injections on Superose 6 10/300 GL column in 20 mM Hepes pH 7.4, 300 mM NaCl, 1 mM TCEP, 5 % (v/v) glycerol. Monomer peaks were concentrated then aliquoted and flash frozen in $N_{2\,(l)}$.

TOC009-F01 (Q19)

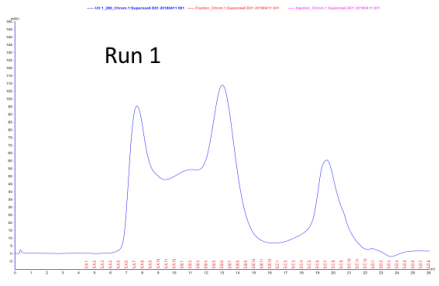


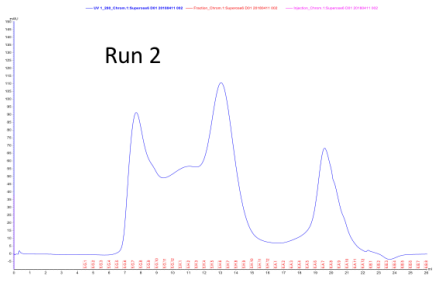


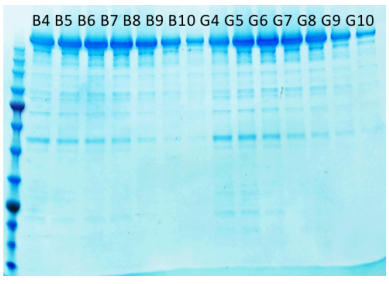


Peak shape from elution is the typical huntingtin distribution. Yields good and sample clean -> 3.0 mg/mL – 11 x 40 µL aliquots.

TOC009-D01 (Q23)

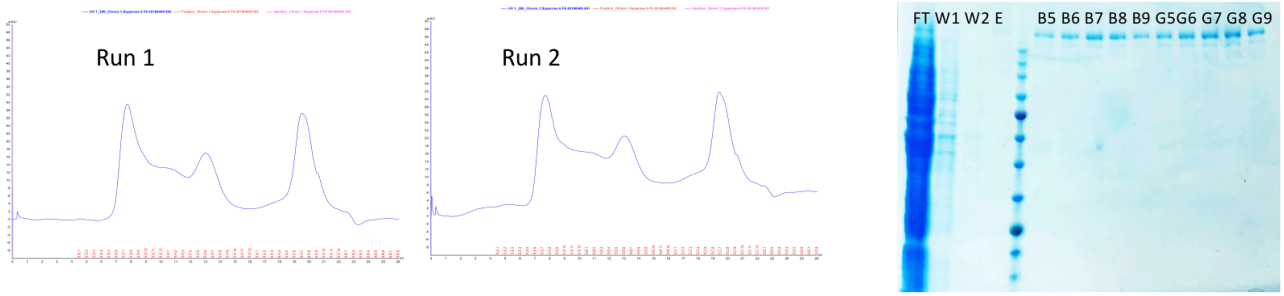






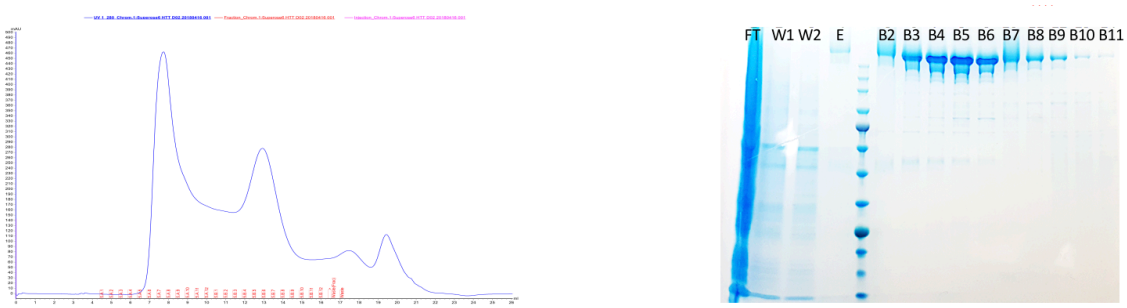
Peak shape from elution is the typical huntingtin distribution. Yields good and sample clean -> 3.7 mg/mL – 16 x 40 µL aliquots.

TOC009-F06 (Q42)



Peak shape from elution is the typical huntingtin distribution. Yields good and sample clean -> 2.5 mg/mL – 6 x 20 µL aliquots.

TOC009-D02 (Q54)



Peak shape from elution is the typical huntingtin distribution. Yields good and sample clean -> 5.2 mg/mL – 16 x 40 µL aliquots.