Large scale expression and purification of full-length huntingtin Q42 from baculoviral expression system production in sf9 insect cells – 2018/07/10

Rationale:

Purified huntingtin samples are required for use in structural and functional studies.

Materials and methods:

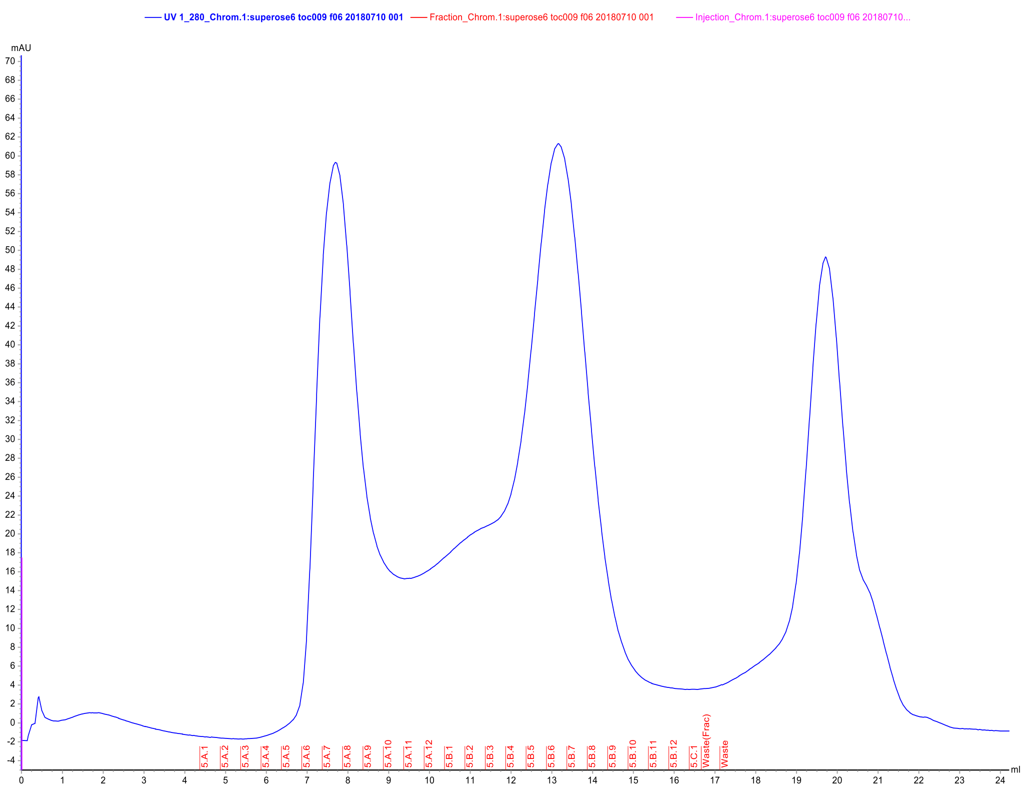
Growth: TOC009-F06 (Q42) P3 virus used for 16 L BVES sf9 production. Cells were harvested by centrifugation at 4000 rpm, 10 mins, 4 °C (Beckman JLA 8.1000). HTT cell pellets were resuspended in ~640 mL of 50 mM Tris pH 8, 500 mM NaCl. Cell resuspensions were spiked with protease inhibitor mix and then stored at -80 ˚C prior to purification. Full BVES production methods are here: https://zenodo.org/record/154611

Purification: Cell pastes were thawed and diluted to 1200 mL with 50 mM Tris pH 8, 500 mM NaCl and supplemented with benzonase and 1 x protease inhibitors. NB: freeze-thaw cycle for cells is sufficient for lysis. Lysates were clarified by centrifugation at 15,000 rpm, 1 h, 4 °C and then bound to 10 mL anti-FLAG resin (Sigma M2) at 4 ˚C with rocking for 2 hours (flow through – FT). Lysate-resin mix was transferred to 2 open columns (BioRad) and the resins then washed with 2 x 250 mL 50 mM Tris pH 8, 500 mM NaCl per column (wash – W1 and W2). HTT protein was eluted with ~15 mL resuspension buffer supplemented with 200 µg/mL 3xFLAG peptide per column (elution – E). Samples were concentrated and run as 1 mL injection on Superose 6 10/300 GL column in 20 mM Hepes pH 7.4, 300 mM NaCl, 1 mM TCEP, 5 % (v/v) glycerol. The monomer peak (~fractions B4-B10) was concentrated then aliquoted and flash frozen in $N_{2\,(l)}$. Samples were analysed by 4-20 % tris-glycine SDS-PAGE.

Yield:

1.9 mg/mL 15 x 20 µL aliquot

Gel filtration:



SDS-PAGE analysis of samples throughout the purification: