Large scale expression and purification of full-length huntingtin Q23 with HAP40 from baculoviral expression system production in sf9 insect cells – 2018/07/11

Rationale:

Purified huntingtin samples with and without stabilising binding partners (HAP40) are required for use in structural and functional studies, in particular SAXS experiments.

Growth:

3:1 HTT Q23 (FLAG-tagged, TOC009:D01) to HAP40 (His-tagged, TOC011:C01) virus ratios used for 8 L BVES sf9 production each. Cells were harvested by centrifugation at 4000 rpm, 10 mins, 4 °C (Beckman JLA 8.1000). HTT-HAP40 cell pellets were resuspended in ~400 mL of 50 mM Tris pH 8, 300 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

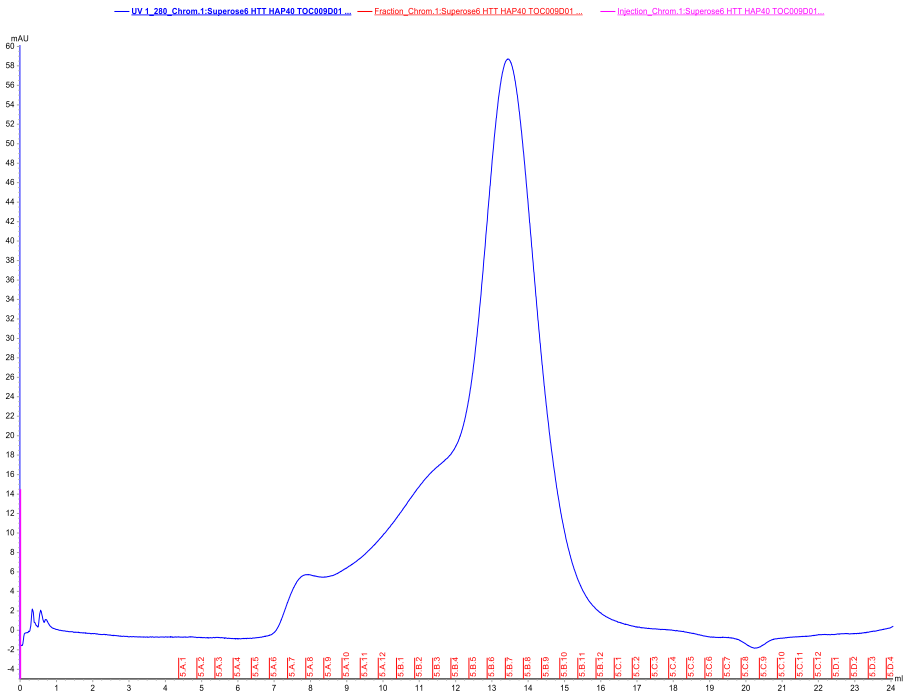
Purification:

Cell pastes were thawed and resuspended in ~500 mL total 50 mM Tris pH 8, 300 mM NaCl, 0.5 % (v/v) Tween-20 supplemented with 1 x protease inhibitors supplemented with benzonase. The lysate was clarified by centrifugation at 15,000 rpm for 1 hour (JLA16.2500) and then bound to 5 mL anti-FLAG resin (Sigma M2) at 4 ˚C with rocking for 2 hours (flow through – FT). Resin was washed with 2 x 250 mL 50 mM Tris pH 8, 300 mM NaCl (wash – W1 and W2). HTT-HAP40 protein was eluted with ~2x10 mL resuspension buffer supplemented with 200 µg/mL 3xFLAG peptide (elution – E1 and E2). The sample was then rocked with 1 mL Ni-NTA at 4 ˚C with rocking for 30 mins (flow through – FT2). Ni-NTA beads were washed with 2x50 mL 50 mM Tris pH 8, 300 mM NaCl, 15 mM imidazole (wash – W3 and W4) and then eluted with 50 mM Tris pH 8, 300 mM NaCl, 300 mM. The elution was concentrated to 1 mL (elution concentrated – E3) and run on Superose6 10/300 GL column in 20 mM Hepes pH 7.4, 300 mM NaCl, 1 mM TCEP, 5 % (v/v) glycerol. Samples were run on SDS-PAGE 4-20 % tris-glycine gel of the purification process. Fractions of the peak (B5-B9) were concentrated then aliquoted and flash frozen in $N_{2\ (l)}$.

Yield:

3.8 mg/mL, 12 x 20 µL aliquot

Gel Filtration:



SDS-PAGE: