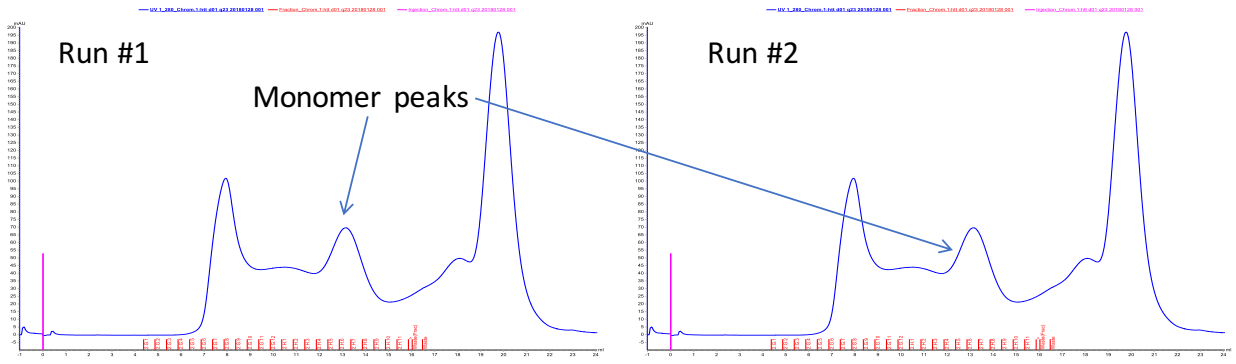
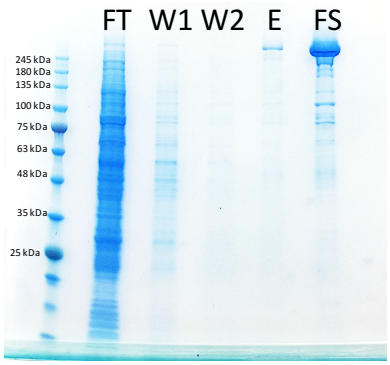
Full-length huntingtin Q23 expression and purification for SAXS experiments – 29th January 2018

Rationale:
Purified huntingtin sample is required for use in structural studies conducted by small-angle X-ray scattering (SAXS). In order to generate the best possible samples (monodisperse, not aggregated), the protein was freshly prepared before flash freezing and shipping on dry-ice to the APS.

Growth:
8 L BVES production for TOC009-D01 (HTT^{1-3144} pBACMAM C-terminal FLAG). 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 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. The lysate was clarified by centrifugation and then bound to 5 mL anti-FLAG resin (Sigma M2) at 4 ˚C with rocking for 2 hours. Resin was washed with 800 mL 50 mM Tris pH 8, 500 mM NaCl. HTT protein was eluted with ~25 mL resuspension buffer supplemented with 250 µg/mL 3xFLAG peptide. The sample was concentrated to 2 mL and run as 2 x 1 mL samples on Superose 6 10/300 GL column in 50 mM Tris pH 8, 500 mM NaCl.





Monomer peak concentrated to 4 mg/mL and then aliquoted and diluted with gel filtration buffer to make 4 x 200 µL samples at 4, 2, 1 and 0.5 mg/mL. The remaining sample was flash frozen as 4 x 5 µL aliquots at 4 mg/mL in $N_{2\,(l)}$.