Project:
Biophysical investigation of purified HTT protein samples

Experiment:
Large-scale purification of Q23 HTT-HAP40 from Sf9 expression system with contaminating nucleic acid material

Date completed:
2019/09/16

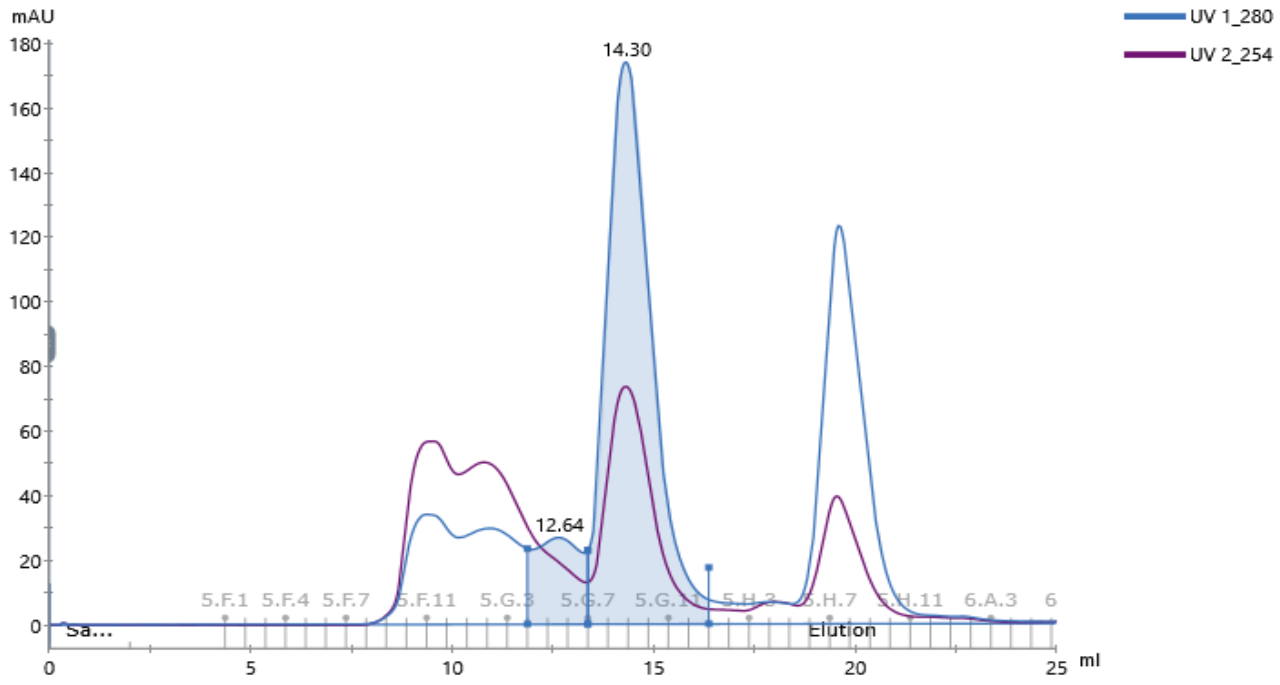
Rationale:
To purify HTT-HAP40 + Sf9 derived nucleic acid material for cryoEM analysis

Experimental approach for Sf9 cell purification:
Growth: Sf9 production of 1:1 TOC009D01:TOC011C01 harvested by centrifugation and resuspended in 40 mL buffer/L growth: 20 mM HEPES pH7.4, 300 mM NaCl, 2.5 % glycerol (v/v) supplemented with protease inhibitors.

Purification: ~80 mL cell resuspension diluted to ~250 mL in resuspension buffer, sonicated for ~3 mins and rocked with benzonase + 2 mM $MgCl_2$ for 10 mins. Clarified lysate bound to 2 mL FLAG resin (flow through - FT), washed in buffer (wash - W1 and W2) and eluted in buffer + 250 µg/µL 3x FLAG peptide (elution - E). Concentrated elution run on equilibrated Superose6 10/300 GL. Samples concentrated with MWCO 100,000 and flash frozen.

Peak A - G4-G6 -> 4.1 mg/mL $A_{260/280}$ ~1.2 (5 µL x 11 aliquots) NB: heavy nucleic acid contamination
Peak B - G7-H1 -> 6.5 mg/mL $A_{260/280}$ ~0.6 (5 µL x 5, 10 µL x 6, 20 µL x 6 aliquots)

Superose6 10/300 GL gel filtration run:



Peaks from Superose6 10/300 GL gel filtration run:

Peak	Retention (ml)	Area (ml*mAU)	Area %	Ext coeff. (mg ml $^{-1}$ cm $^{-1}$)	Fraction(s)	Volume (ml)	Amount (mg)	Concentration (mg/ml)	Conductivity (mS/cm)
Peak A	12.638	36.70	14.02	0.750	5.G.4 - 5.G.6	1.499	0.245	0.163	27.03
Peak B	14.302	225.1	85.98	0.750	5.G.7 - 5.G.12	3.000	1.500	0.500	27.01

4-20% Tris-Glycine SDS-PAGE analysis of samples throughout purification: