Project:
Biophysical investigation of purified HTT protein samples

Experiment:
Large-scale purification of Q23 and Q54 HTT-HAP40 from Sf9 expression system

Date completed:
2019/07/29

Rationale:
HTT samples are to be purified from different systems for subsequent biophysical and structural studies.

Experimental approach:
Harvest: Sf9 cultures produced as described previously (Harding *et al* 2019 JBC) were harvested at 4000 rpm 15 mins 4 °C (Beckman JLA8.1000). Harvested pellets were resuspended in ~45 mL buffer (20 mM Hepes pH 7.4, 300 mM NaCl, 5 % glycerol) per 1 L of cells harvested and supplemented with protease inhibitor mix. Pellets were flash frozen in liquid nitrogen and stored at -80 °C prior to protein purification.

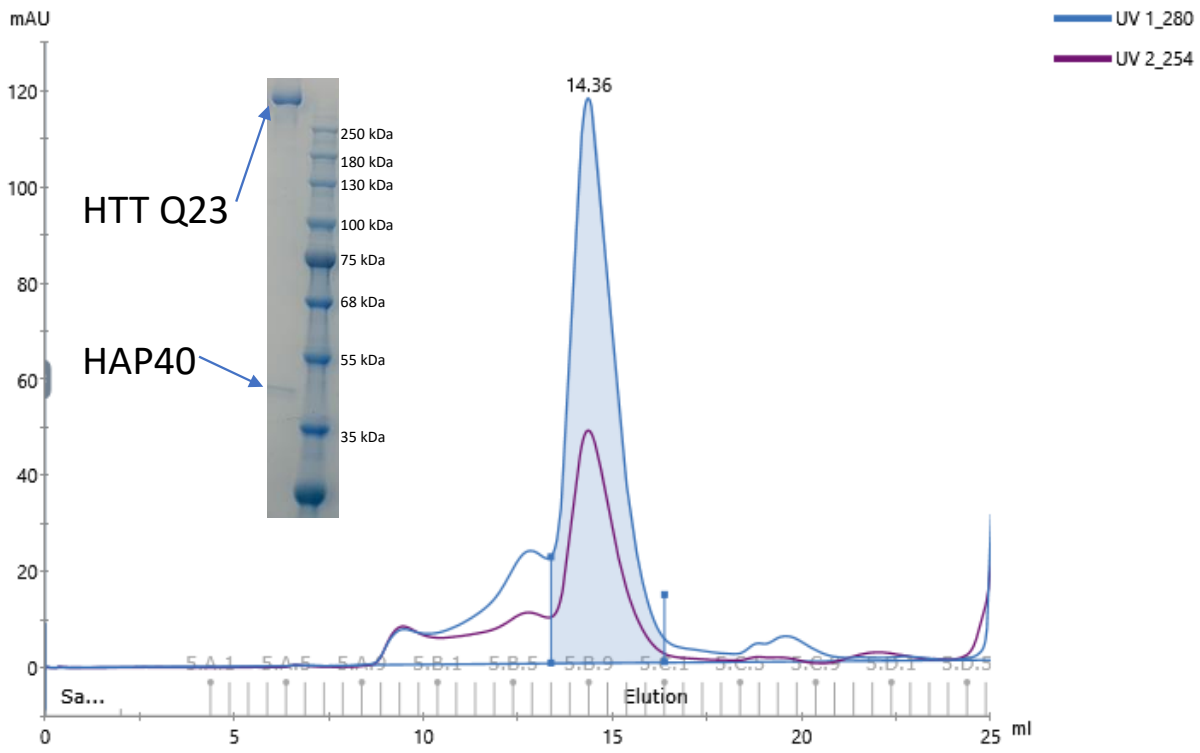
Lysis: Cell paste equivalent to 4 L of culture was thawed and diluted to 500 mL with 20 mM Hepes pH 7.4, 300 mM NaCl, 5 % glycerol and supplemented with benzonase. NB: freeze-thaw cycle for cells is sufficient for lysis. Lysates were clarified by centrifugation at 15,000 rpm, 1 h, 4 °C (Beckman JLA16.2500)

FLAG: Clarified lysates then bound to 5 mL anti-FLAG slurry (Sigma M2) at 4 ˚C with rocking for 2 hours (flow through – FT). Lysate-resin mix was transferred to open column (BioRad) and the resin then washed with 2 x 250 mL 20 mM Hepes pH 7.4, 300 mM NaCl, 5 % glycerol (wash – W). HTT protein was eluted with ~16 mL 20 mM Hepes pH 7.4, 300 mM NaCl, 5 % glycerol supplemented with 250 µg/mL 3xFLAG peptide (elution – E).

Ni: FLAG elution bound to 1 mL NiNTA slurry at 4 ˚C with rocking for 30 mins (flow through – FT2). Protein-resin mix was transferred to open column (BioRad) and the resin then washed with 50 mL 20 mM Hepes pH 7.4, 300 mM NaCl, 5 % glycerol, 5 mM imidazole (wash – W7) and protein eluted with 20 mL 20 mM Hepes pH 7.4, 300 mM NaCl, 5 % glycerol, 250 mM imidazole (elution – E2).

GF: The elution was concentrated to 1 mL (elution concentrated – EC) with spin concentrators MWCO 100,000 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 analysed by 4-20 % tris-glycine SDS-PAGE.

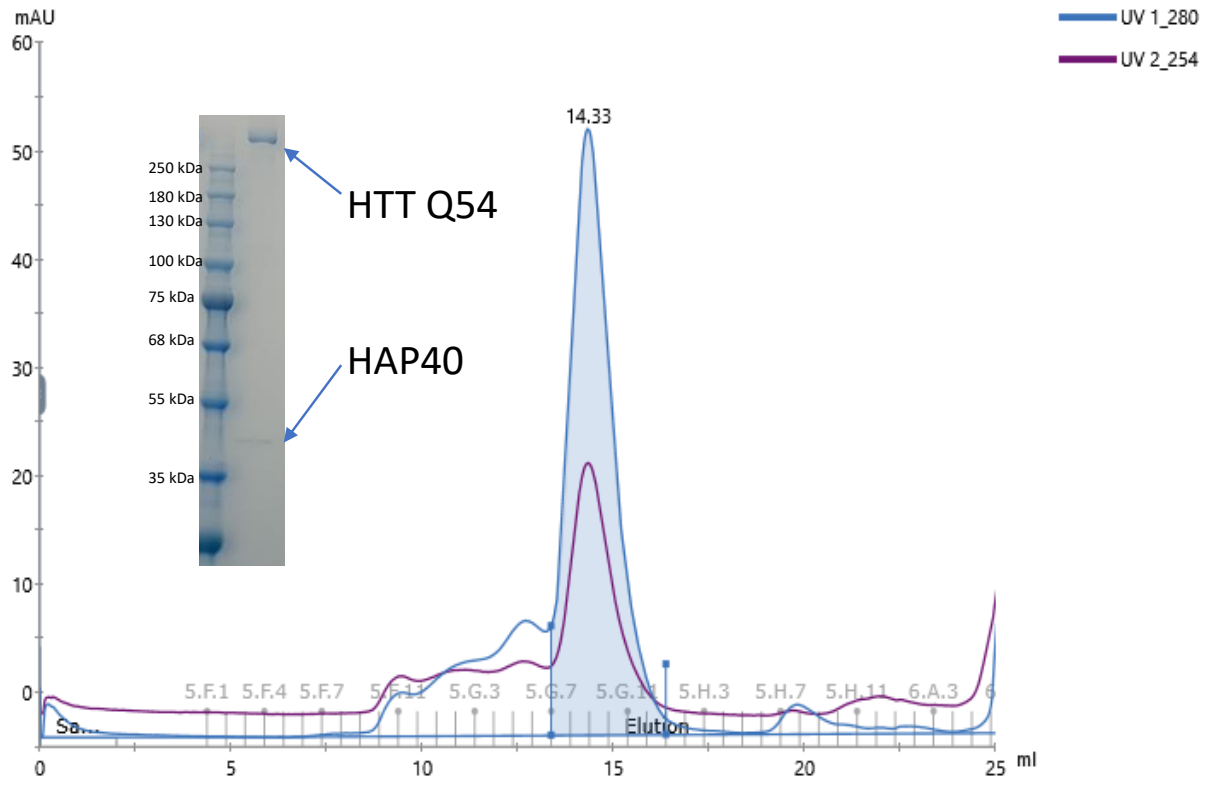
HTT-HAP40 Q23:



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	14.355	162.5	100.00	0.750	5.B.7 - 5.B.12	3.001	1.083	0.361	25.70

The monomer peak was concentrated (MWCO 100,000) and flash frozen:
Final yield: ~1 mg
Concentration: 4 mg/mL
Aliquots: 10 µL x 25
A260/280: 0.59

HTT-HAP40 Q54:



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	14.328	73.33	100.00	0.750	5.G.7 - 5.G.12	3.000	0.489	0.163	26.02

The monomer peak was concentrated (MWCO 100,000) and flash frozen:
Final yield: ~0.5 mg
Concentration: 5.5 mg/mL
Aliquots: 10 µL x 9
A260/280: 0.59