Project:
Biophysical investigation of purified HTT protein samples

Experiment:
Optimisation of HTT-HAP40 purification using heparin affinity chromatography

Date completed:
2019/04/02

Rationale:
Previous attempts to generate a much purer and homogenous HTT-HAP40 sample showed that the complex can bind heparin resin. In addition, I will incorporate helpful suggestions from scientists at the CHDI Palm Springs meeting full-length HTT research breakout group i.e. ATP wash to remove HSP proteins. The experiment will include 3 affinity chromatography steps with FLAG, heparin and NiNTA resin and finally a gel filtration step.

Experimental approach:

Lysis:
Cell pastes (1 L of culture) were thawed and diluted to 200 mL with 20 mM Hepes pH 7.4, 500 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)

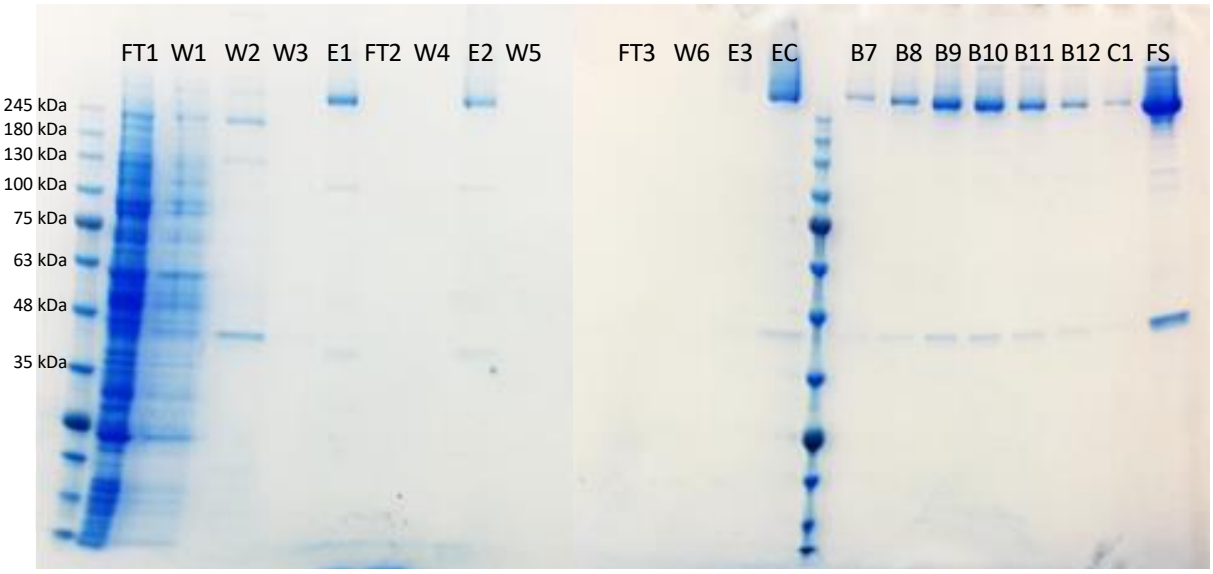
FLAG:
Clarified lysates then bound to 5 mL anti-FLAG slurry (Sigma M2) at 4 ˚C with rocking for 2 hours (flow through – FT1). Lysate-resin mix was transferred to open column (BioRad) and the resin then washed with 250 mL 20 mM Hepes pH 7.4, 300 mM NaCl, 5 % glycerol (wash – W1), 250 mL 20 mM Hepes pH 7.4, 400 mM KCl, 5 % glycerol, 5 mM $McCl_2$, 5 mM ATP (wash - W2) and then 50 mL 20 mM Hepes pH 7.4, 50 mM KCl, 5 % glycerol (wash – W3). HTT protein was eluted with ~16 mL 20 mM Hepes pH 7.4, 50 mM KCl, 5 % glycerol supplemented with 250 µg/mL 3xFLAG peptide (elution – E1).

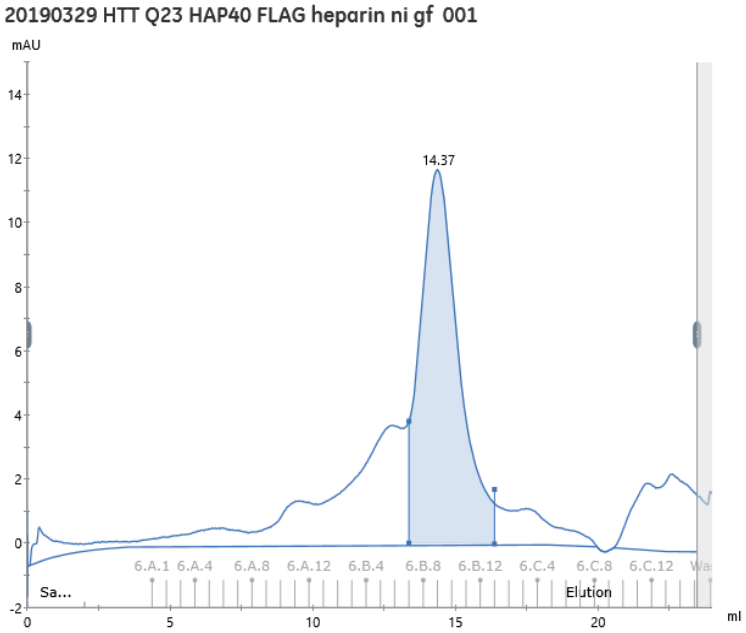
Heparin:
FLAG elution then bound to 2 mL heparin slurry at 4 ˚C with rocking for 30 mins (flow through – FT2). Lysate-resin mix was transferred to open column (BioRad) and the resin then washed with 50 mL 20 mM Hepes pH 7.4, 50 mM KCl, 5 % glycerol (wash – W4) and protein eluted with 25 mL 20 mM Hepes pH 7.4, 200 mM KCl, 5 % glycerol (elution – E2) and then cleaned with 25 mL 20 mM Hepes pH 7.4, 1 M KCl, 5 % glycerol (wash – W5).

NB: unfortunately after this step I had to leave early as I was feeling unwell so the experiment was resumed the following day.

Ni:
Heparin elution bound to 1 mL NiNTA slurry at 4 ˚C with rocking for 30 mins (flow through – FT3). Lysate-resin mix was transferred to open column (BioRad) and the resin then washed with 25 mL 20 mM Hepes pH 7.4, 300 mM NaCl, 5 % glycerol, 5 mM imidazole (wash – W6) and protein eluted with 10 mL 20 mM Hepes pH 7.4, 300 mM NaCl, 5 % glycerol, 250 mM imidazole (elution – E3).

GF:
The elution was concentrated to 1 mL (elution concentrated – EC) 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.





Retention (ml)	Area (ml* mAU)	Ext coeff. (mg ml $^{-1}$ cm $^{-1}$)	Fraction (s)	Volume (ml)	Amount (mg)	Concentration (mg/ml)	Conductivity (mS/cm)
14.373	18.09	0.780	6.B.7 - 6.B.12	3.000	0.116	0.039	26.21

Although the yield is lower than I have successfully purified previously, the protocol yields clean and largely monodisperse sample. I hope that if all steps were completed in one day, the gel filtration profile would be even further improved.