Project:
Biophysical investigation of purified HTT protein samples

Experiment:
Large-scale stringent purification of Q23 HTT and HTT-HAP40 using heparin affinity chromatography

Date completed:
2019/05/22

Rationale:
Previous attempts to generate a much purer and homogenous HTT-HAP40 sample showed that the complex can bind heparin resin. In small-scale experiment, I also incorporated 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 included 3 affinity chromatography steps with FLAG, heparin and NiNTA resin and finally a gel filtration step and yielded a highly pure HTT-HAP40 sample: https://zenodo.org/record/3234174. I now want to try this purification with apo HTT.

Experimental approach for each purification:
Lysis: Cell paste (8 L of culture) was thawed and diluted to 750 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)

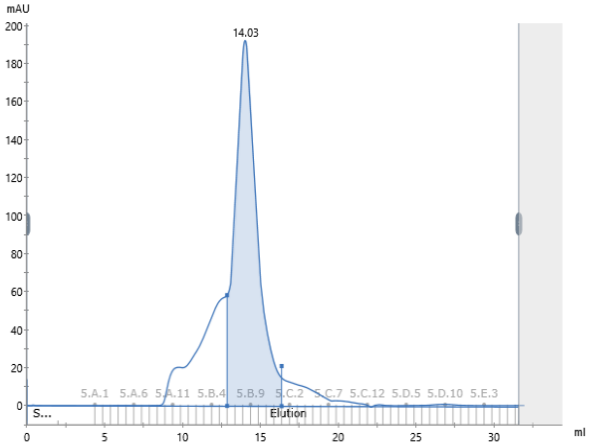
FLAG: clarified lysates then bound to 5 mL anti-FLAG slurry (Sigma M2) at 4 ˚C with rocking for 2 hours (flow through – FT1). Lysate-resin mix was transferred to open column (BioRad) and the resin then washed with 500 mL 20 mM Hepes pH 7.4, 300 mM NaCl, 5 % glycerol (wash – W1), 500 mL 20 mM Hepes pH 7.4, 400 mM KCl, 5 % glycerol, 5 mM McCl2, 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 ~30 mL 20 mM Hepes pH 7.4, 50 mM KCl, 5 % glycerol supplemented with 250 µg/mL 3xFLAG peptide (elution – E1).

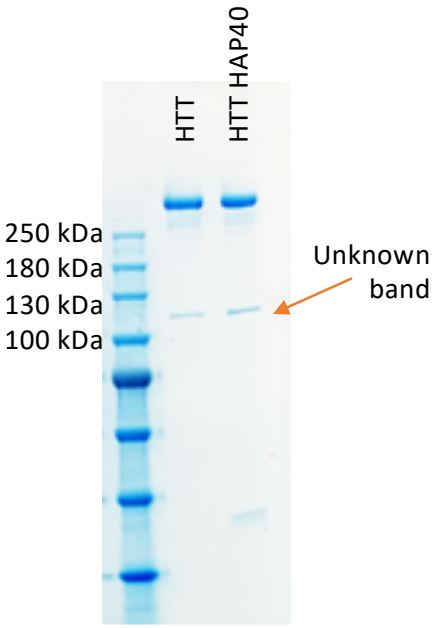
Heparin: FLAG elution then bound to 2 mL heparin slurry at 4 ˚C with rocking for 30 mins (flow through – FT2). Lysate-resin mix was transferred to open column (BioRad) and the resin then washed with 50 mL 20 mM Hepes pH 7.4, 50 mM KCl, 5 % glycerol (wash – W4) and protein eluted with 50 mL 20 mM Hepes pH 7.4, 200 mM KCl, 5 % glycerol (elution – E2) and then cleaned with 50 mL 20 mM Hepes pH 7.4, 400 mM KCl, 5 % glycerol (wash – W5) and 50 mL 20 mM Hepes pH 7.4, 1 M KCl, 5 % glycerol (wash – W6).

Ni (only HTT-HAP40 samples): 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 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 – E3).

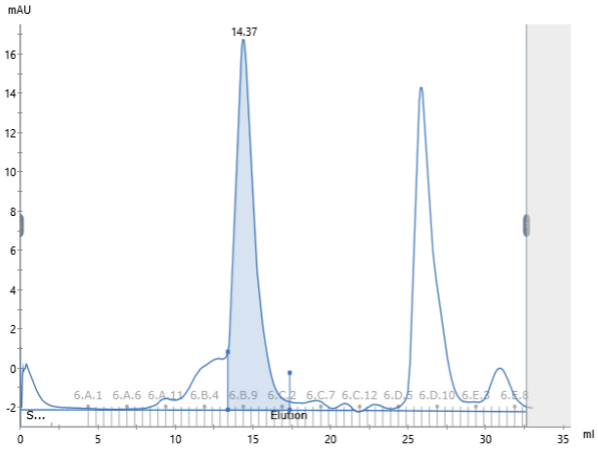
GF: The elution was concentrated to 1 mL (elution concentrated – EC) and run on Superose6 10/300 GL column in 20 mM Hepes pH 7.4, 300 mM NaCl, 1 mM TCEP, 5 % (v/v) glycerol. Samples were analysed by 4-20 % tris-glycine SDS-PAGE.

Q23 HTT – 2.2 mg protein

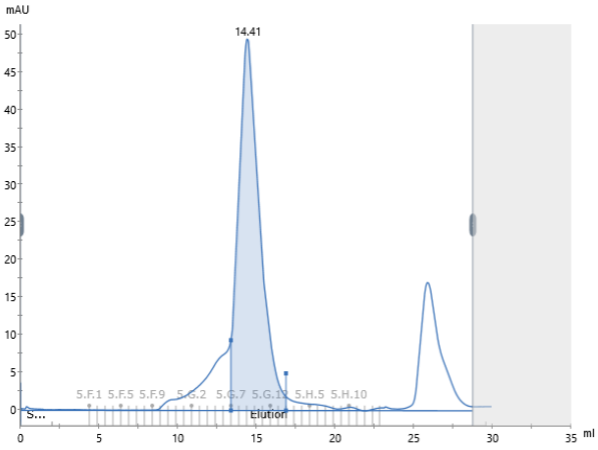




Q23 HTT-HAP40 run 1



Q23 HTT-HAP40 run 2 – 0.6 mg protein total



The monomer peak was concentrated (MWCO 100,000) and flash frozen at 4 mg/mL in 5, 10 and 20 µL aliquots.