Attempted heparin resin purification of HTT-HAP40 complex from Sf9 production – 14th January 2019

Aim:

The current protocol for HTT and HTT-HAP40 purification I am using requires a long incubation of clarified cell lysate with FLAG resin. To potentially improve yields and sample quality, it would perhaps be beneficial to have a quick heparin resin purification step prior to FLAG binding which may also remove contaminating nucleic acid material. To test this hypothesis, small-scale purification of Q23 HTT-HAP40 samples in different buffer systems will be carried out using heparin and FLAG affinity chromatography.

Method:

1. Lysis and clarification:

1 L Sf9 cell growth expressing TOC009-D01 (Q23 HTT^{1-3144} pBacMam2-Di-Ex-Lic) and TOC011-C01 ($HAP40^{371}$ pFBOH-MHL), harvested and resuspended in 45 mL of 20 mM HEPES pH 7.4, 300 mM NaCl, 2.5 % glycerol. Resuspension thawed and then diluted as follows: 15 mL cell paste diluted with 75 mL of lysis buffer containing 10 % glycerol, 1 mM TCEP, 1 mM EDTA, 1 mM PMSF, 1 mM benzamidine and benzonase with either 50 mM Bis-Tris pH 6.5, 50 mM HEPES pH 7.4 or 50 mM Tris pH 8.5. Diluted resuspensions freeze-thawed to lyse and then spun at 20,000 rpm, 20 mins, 4 ˚C (Beckman JA 25.50).

2. Heparin column:

Supernatant applied to equilibrated 5 mL Heparin FF column at ~1 mL/ min (flow through – FT) and then washed with 20 CVs of lysis buffer (wash – W). Stepped KCl elution of sample from the column with 50 mM Bis-Tris pH 6.5, 50 mM HEPES pH 7.4 or 50 mM Tris pH 8.5 with 5 % glycerol, 1 mM TCEP, 1 mM EDTA supplemented with 0.2, 0.4, 0.6, 0.8 then 1 M KCl (E.2, E.4, E.6, E.8, E1). Each buffer step was ~15 mL.

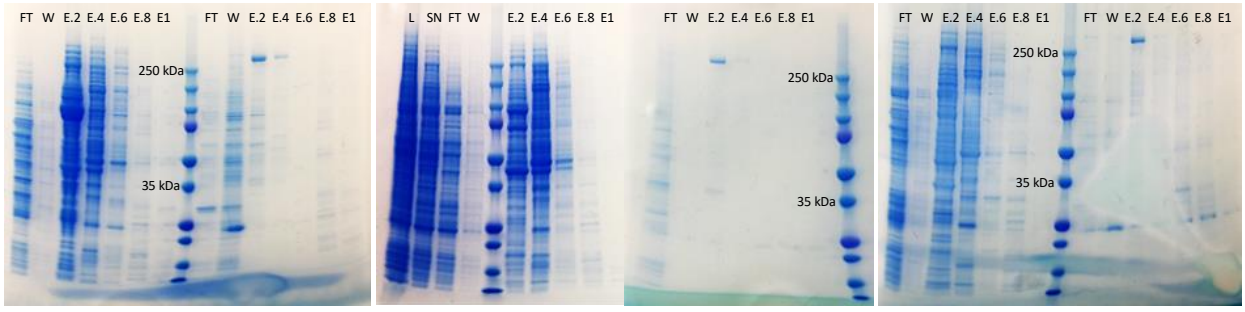
3. FLAG purification:

Add ~200 µL FLAG resin to the supernatant, wash and elution fractions and bind with rocking at 4 ˚C for 30 mins. Beads washed with 1 volume of 50 mM Tris pH 8, 300 mM NaCl and then protein eluted with 3 mL 100 mM glycine pH 3.

4. SDS-PAGE analysis:

Samples from the heparin purification and FLAG purification were analysed on 4-20 % Tris-Glycine SDS-PAGE and stained with Coomassie.

Results:



Heparin elution FLAG elution Heparin elution FLAG elution Heparin elution FLAG elution

50 mM Bis-Tris pH 6.5 50 mM HEPES pH 7.4 50 mM Tris pH 8.5

Conclusions:

HTT is not immediately obvious in any of the heparin affinity chromatography gel lanes suggesting the enrichment of HTT-HAP40 by this step is not that substantial however the HTT has been separated from the bulk of cell debris in the flow through and wash fractions. A band corresponding to HTT (~350 kDa) can be seen

in FLAG elution fraction E.2 corresponding to the 200 mM KCl for all 3 buffer conditions. The HEPES sample appears to be the cleanest and a faint band corresponding to HAP40 is visible on the SDS-PAGE (~40 kDa). No HTT protein is pulled-down in the heparin flow through or wash suggesting efficient binding. Elution of the HTT-HAP40 protein in only 200 mM KCl suggests the interaction with the resin is weak.

Next steps:
The results are promising that heparin affinity chromatography could provide a first clean up step to remove HTT-HAP40 from the bulk of cell debris in the clarified lysate. However, this method requires large scale dilution of the sample in order to ensure low ionic strength and effective heparin resin binding. This would mean applying litres of dilute clarified lysate to a 5 mL Heparin FF column which would take hours. I will next see if I can repeat this step using batch-binding of HTT-HAP40 to heparin resin.