Project:
Biophysical investigation of purified HTT protein samples

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

Date completed:
2019/09/16

Rationale:
To purify HTT-HAP40 Q23 and Q54 in PBS to see if this reduces nucleic acid contamination.

Experimental approach for Sf9 cell purification:
Growth: Sf9 production of 1:1 TOC009D01:TOC011C01 and 1:1 TOC009D02: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 PBS + 300 mM KCl + 5% glycerol freeze-thawed and rocked with benzonase + 2 mM $MgCl_2$ for 10 mins. Clarified lysate bound to 2 mL FLAG resin, washed in PBS + 300 mM KCl + 5% glycerol and eluted in PBS + 300 mM KCl + 5% glycerol + 250 µg/µL 3x FLAG peptide. Elution bound to 1 mL NiNTA, washed with PBS + 300 mM KCl + 5% glycerol + 10 mM imidazole and then eluted with PBS + 300 mM KCl + 5% glycerol + 300 mM imidazole. Concentrated elution run on equilibrated Superose6 10/300 GL in 20 mM HEPES pH7.4, 300 mM NaCl, 5 % glycerol (v/v), 1 mM TCEP.

Conclusions:
Q23 -> 3.3 mg/mL $A_{260/280}$ ~0.59 (10 µL x 8) Q54 -> 2.7 mg/mL $A_{260/280}$ ~0.6 (10 µL x 8)
Yields low and samples only ~75 % pure – PBS does not improve the purification.

Superose6 10/300 GL gel filtration runs: