Project:
Biophysical investigation of purified HTT protein samples

Experiment:
Purification of Q23 and Q54 HTT and HTT-HAP40 from Sf9

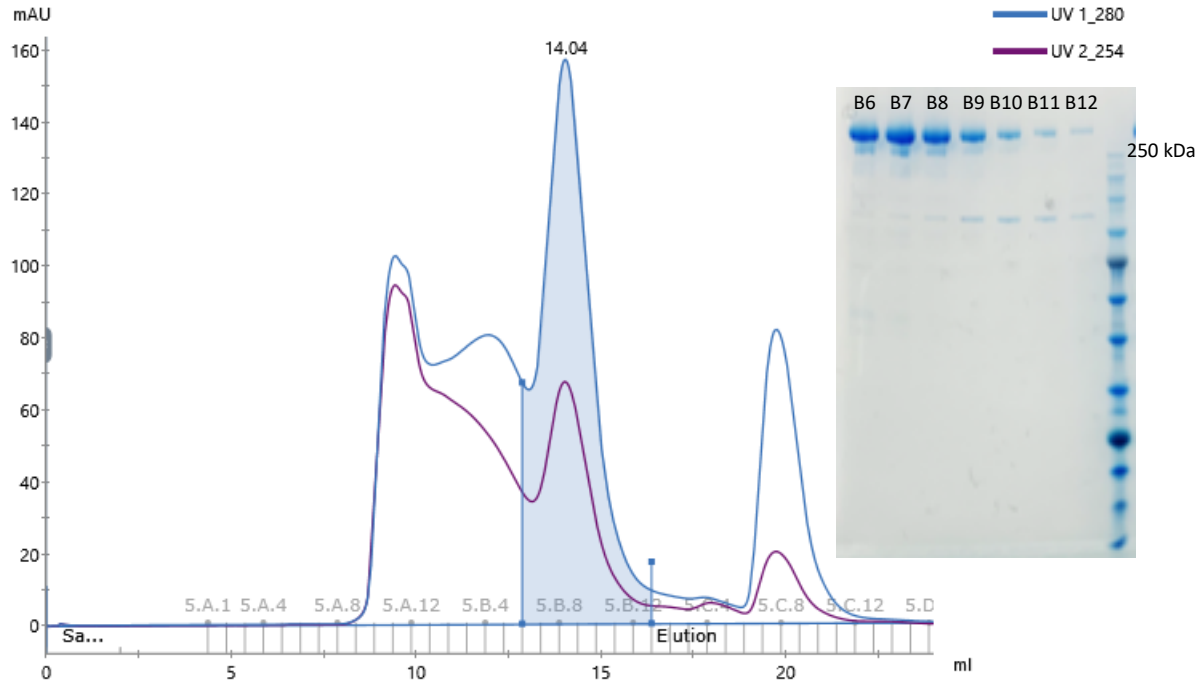
Date completed:
2019/10/07

Rationale:
Purification of HTT and HTT-HAP40 Q23 and Q54 for different biophysical and functional analyses

Experimental approach for Sf9 cell purification of HTT:
Growth: Sf9 production of TOC009D01 and TOC009D02 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: ~160 mL cell resuspension diluted to ~500 mL, rocked with benzonase + 2 mM $MgCl_2$ for 10 mins. Clarified lysate bound to 2 mL FLAG resin, washed in buffer and eluted in buffer + 250 µg/µL 3x FLAG peptide. Concentrated elution run on equilibrated Superose6 10/300 GL. Samples concentrated with MWCO 100,000 and flash frozen.

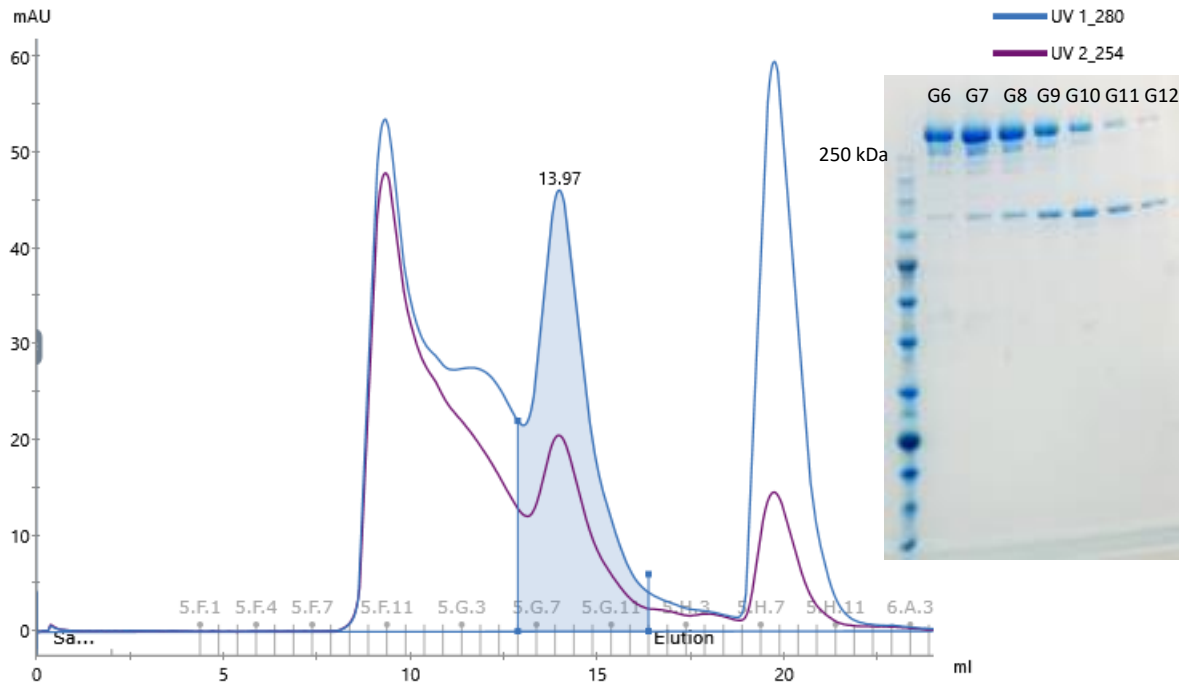
Q23:



Retention (ml)	Area (ml*mAU)	Area %	Ext coeff. (mg ml $^{-1}$ cm $^{-1}$)	Fractions	Volume (ml)	Amount (mg)	Concentration (mg/ml)
14.036	251.1	100	0.780	5.B.6 - 5.B.12	3.501	1.610	0.460

Peak B6-C1 -> 4 mg/mL $A_{260/280}$ ~0.58 (10 µL x 20, 20 µL x 6) -> 1.3 mg total

Q54:



Retention (ml)	Area (ml*mAU)	Area %	Ext coeff. (mg ml $^{-1}$ cm $^{-1}$)	Fractions	Volume (ml)	Amount (mg)	Concentration (mg/ml)
13.975	79.83	100	0.780	5.G.6 - 5.G.12	3.501	0.512	0.146

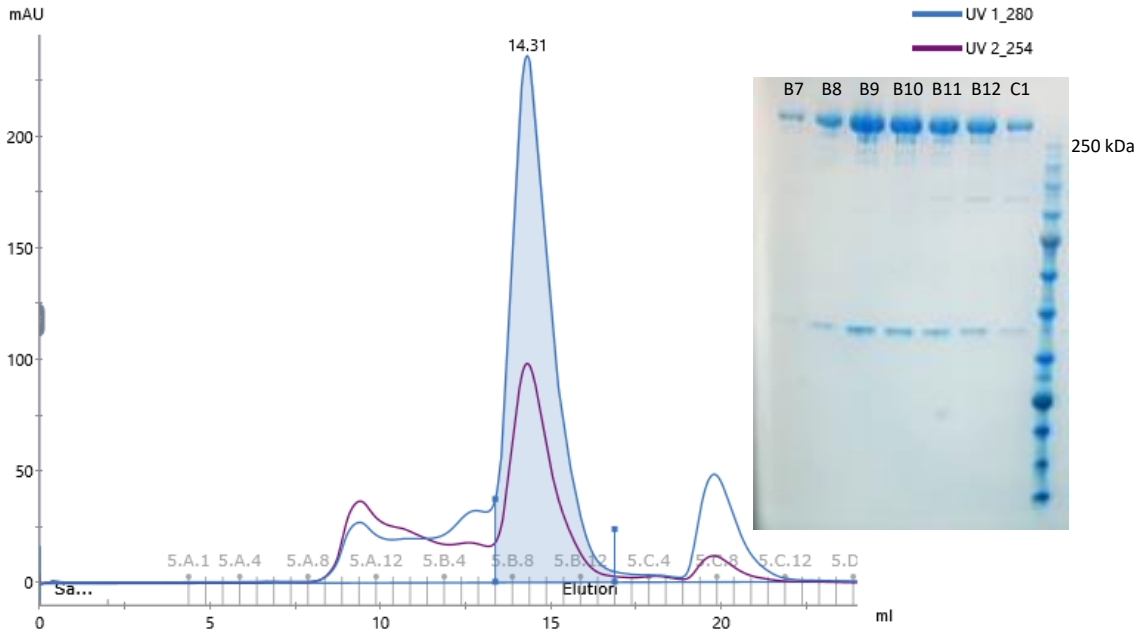
Peak G6-H1 -> 2.7 mg/mL $A_{260/280}$ ~0.60 (10 µL x 15) -> 0.4 mg total

Experimental approach for Sf9 cell purification of HTT-HAP40:

Growth: Sf9 production of TOC009D01-TOC011C01 (1:1) and TOC009D02-TOC011C01 (1:1) 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: ~160 mL cell resuspension diluted to ~500 mL, rocked with benzonase + 2 mM $MgCl_2$ for 10 mins. Clarified lysate bound to 2 mL FLAG resin, washed in buffer and eluted in buffer + 250 µg/µL 3x FLAG peptide. Concentrated elution run on equilibrated Superose6 10/300 GL. Samples concentrated with MWCO 100,000 and flash frozen.

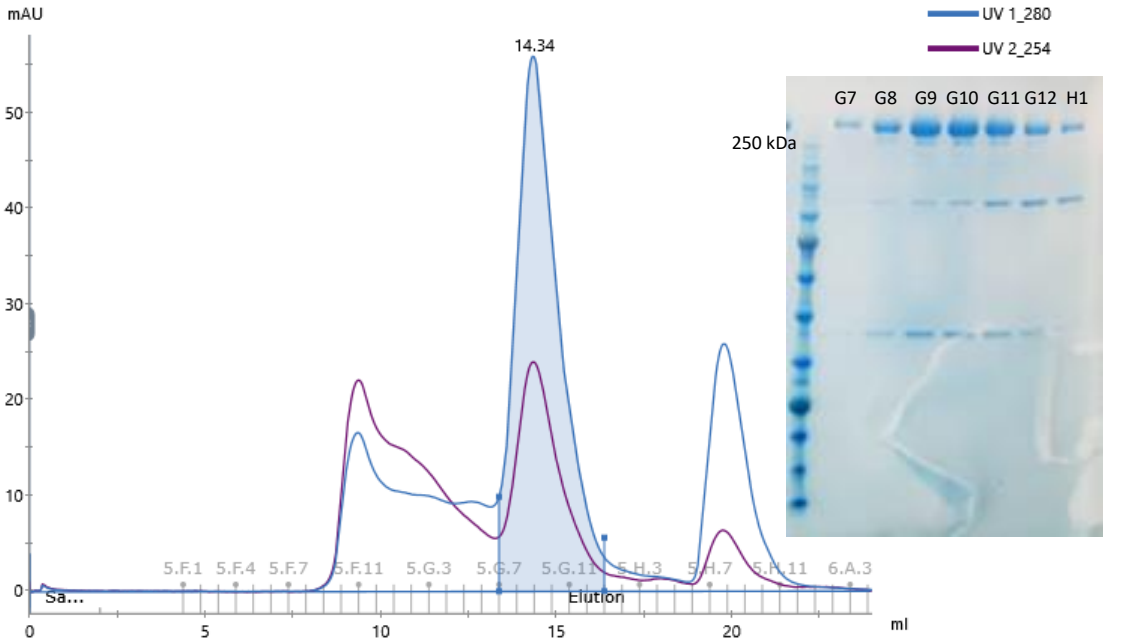
Q23:



Retention (ml)	Area (ml*mAU)	Area %	Ext coeff. (mg ml $^{-1}$ cm $^{-1}$)	Fractions	Volume (ml)	Amount (mg)	Concentration (mg/ml)
14.308	328.3	100	0.750	5.B.7 - 5.C.1	3.501	2.189	0.625

B7-C1 -> 4 mg/mL $A_{260/280}$ ~0.59 (10 µL x 25, 20 µL x 14) -> 2.1 mg total

Q54:



Retention (ml)	Area (ml*mAU)	Area %	Ext coeff. (mg ml $^{-1}$ cm $^{-1}$)	Fractions	Volume (ml)	Amount (mg)	Concentration (mg/ml)
14.341	80.03	100	0.750	5.G.7 - 5.G.12	3.001	0.534	0.178

G7-H1 -> 4 mg/mL $A_{260/280}$ ~0.63 (10 µL x 12) -> 0.5 mg total