

**Project:**

Biophysical investigation of purified HTT protein samples

**Experiment:**

Purification of HTT<sup>76-3060</sup>-HAP40<sup>1-371</sup> from Sf9

**Date completed:**

2019/10/21

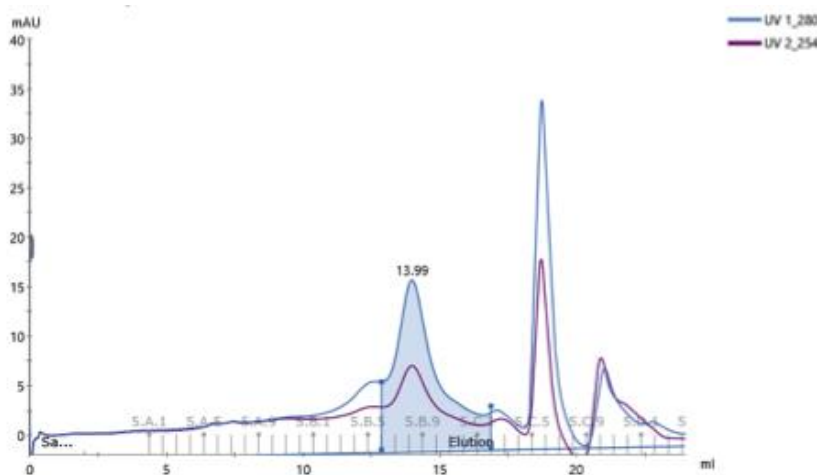
**Rationale:**

Purification of HTT<sup>76-3060</sup>-HAP40<sup>1-371</sup> for structural analysis

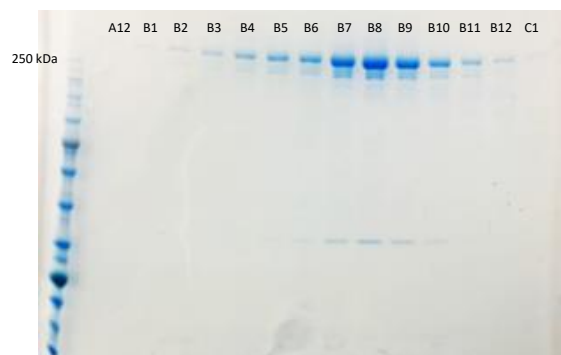
**Experimental approach for Sf9 cell purification of HTT:**

Growth: Sf9 production of TOC009E01-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<sub>2</sub> 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.



Retention (ml)	Area (ml*mAU)	Ext coeff. (mg ml <sup>-1</sup> cm <sup>-1</sup> )	Fraction(s)	Volume (ml)	Amount (mg)	Concentration (mg/ml)
13.986	31.81	0.780	5.B.6 - 5.C.1	4.001	0.204	0.051



B7-C1 -> 4 mg/mL A<sub>260/280</sub> ~0.59 (10 µL x 5) 0.2 mg total