Project:

Biophysical investigation of purified HTT protein samples

Experiment:

Purification of Q23 and Q54 HTT from EXPI293F

Date completed:

2019/09/30

Rationale:

Purify HTT Q23 and Q54 for mass spectrometry analysis

Experimental approach for Sf9 cell purification:

Growth: ~250 mL EXPI293F production of TOC009D01 (Q23) and TOC009D01 (Q54) harvested by centrifugation and resuspended in 40 mL buffer each: 20 mM HEPES pH7.4, 300 mM NaCl, 5 % glycerol (v/v) supplemented with protease inhibitors.

Purification: ~cell resuspension thawed and sonicated for ~3 mins and rocked with benzonase + 2 mM MgCl₂ for 10 mins. Clarified lysate bound to 0.5 mL FLAG resin, washed in buffer and eluted in buffer + 250 μ g/ μ L 3x FLAG peptide. Concentrated elution flash frozen.

Q23 -> 2.4 mg/mL - 60 μL Q54 -> 1.7 mg/mL - 45 μL

