Huntingtin Q23 with FLAG antibody Grafix in 0-1 % Glutaraldehyde – 26th January 2018

Rationale:

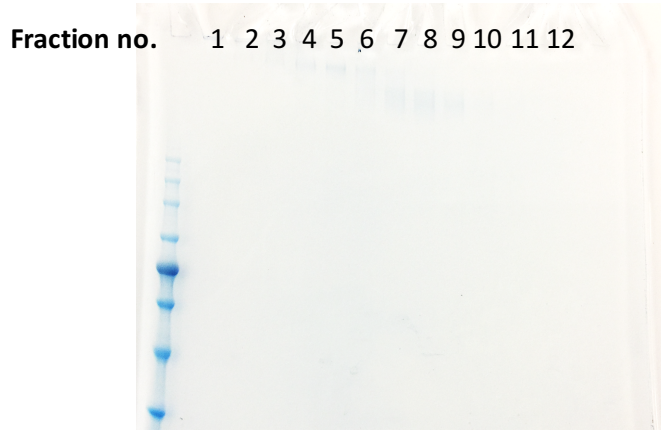
Previous efforts to generate a cryo electron microscopy sample of full-length huntingtin (HTT) indicated that sample conformational heterogeneity and flexibility limited envelope resolution. In an effort to constrain the HTT protein conformation, I modified the gradient fixation conditions in which the sample is generated to increase the cross-linking of the protein molecule. To identify the N and C termini, FLAG antibody (Ab) complex samples of N and C FLAG tagged HTT samples were generated. Additional sample is required for the EM scientists who are generating samples and running the experiments in UK.

Procedure:

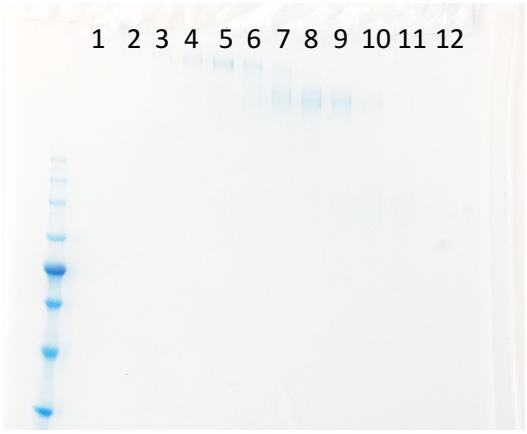
1. 2 x 30 mL gradients were poured in SW32.Ti compatible tubes:
   Heavy: 20 mM Hepes pH 7.4, 300 mM NaCl, 15 % (w/v) sucrose, 1 % (v/v) glutaraldehyde (previously 0.5 % (v/v) glutaraldehyde)
   Light: 20 mM Hepes pH 7.4, 300 mM NaCl, 5 % (w/v) sucrose
2. HTT:FLAG antibody mixed in ~equimolar ratio (400 µg of HTT Q23 (TOC009:D01 - HTT^{1-3144} pBACMAM C-terminal FLAG) + 170 µg FLAG Ab - Sigma https://www.sigmaaldrich.com/catalog/product/sigma/f3165?lang=en&region=CA) and incubated on ice 30 mins before application to 2 tubes (#1 and 2)
3. Tubes spun in ultracentrifuge at 25,000 rpm for 16 hours at 4 ˚C in SW32.Ti swing bucket rotor
4. Samples fractionated (heavy to light) into 15 fractions ~ 2 mL each. Samples of fractions 1-12 run on 4-20 % tris-glycine SDS-PAGE (run at 225 V for 2 hours at 4 ˚C).

C-terminal bound FLAG antibody:

Tube 1



Tube 2



5. Fractions corresponding to monomer HTT + Ab (fraction 7, 8 and 9 for both tubes) were quenched by addition of 100 mM Tris pH 8 and then dialysed against 3 x 4 L of 20 mM Hepes, 150 mM NaCl (dialysis buffer changed every 3 hours then left overnight) with snakeskin MWCO 10,000.
6. Samples concentrated with MWCO 100,000 spin concentrators. 5 ul x 6 aliquots @ 1.8 mg/mL