Project:
High resolution structural analysis of purified HTT samples

Experiment:
Preparation of dephosphorylated HTT samples for cryoEM analysis

Date completed:
2019/03/20

Rationale:
We now have a good understanding of the global structure of HTT when in complex with HAP40 and our MALS and SAXS data suggest that apo HTT is likely self-associating and heterogenous in nature, despite high levels of purity. Dephosphorylating HTT from Sf9 cell production could alter its global structure sufficiently to allow high resolution structure determination by cryoEM.

NB: This work is directed via discussions and collaboration with the Lashuel group at EPFL.

Experimental approach:

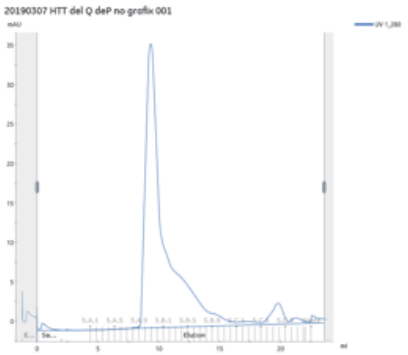
1. 24 hour reaction set up:

Dephosphorylation:

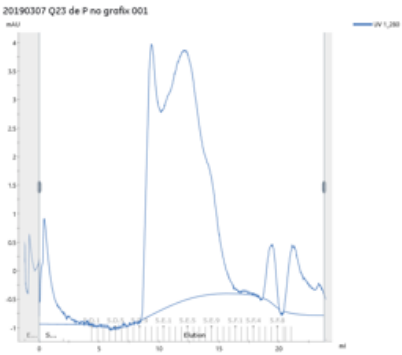
- ~800 µg each of HTT Δexon1, HTT Q23 and HTT Q54 (80x the NEB standard reaction) in total reaction volume ~500 µL 8000 U of λ phosphatase with 1 x PMP buffer (NEB) and 1 x $MnCl_2$. Incubate overnight at 25˚C

Gel filtration:

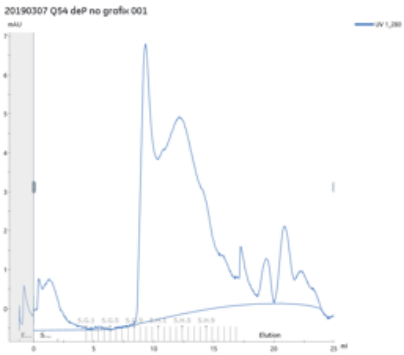
- ~400 µg each applied to Superose6 10/300 GL – monomer peak concentrated and flash frozen. Sample shows redistribution into different species seen on GF previously.



HTT Δexon1



HTT Q23



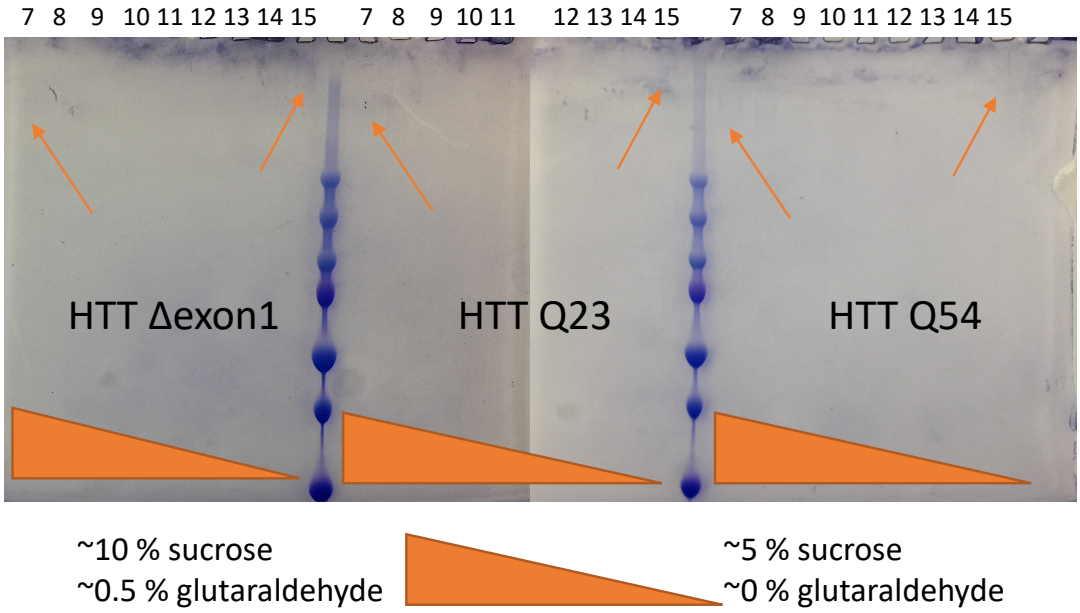
HTT Q54

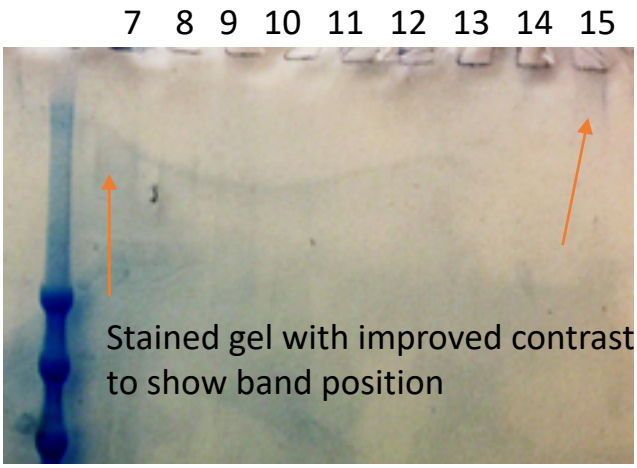
- Samples show aggregation and redistribution into different oligomeric species. Fractions corresponding to the monomer shoulder concentrated to ~ 20 µL and flash frozen to ship to Lea lab:
  HTT Δexon1 – 0.2 mg/mL HTT Q23 – 0.4 mg/mL HTT Q54 – 0.45 mg/mL

GRAFIX:

- ~400 µg each applied to 5-15 % (v/v) sucrose, 0-1 % glutaraldehyde 30 mL gradients in 20 mM HEPES pH 7.4, 300 mM NaCl. Samples run at 25,000 rpm Ti SW32.0 ultracentrifuge for 24 hours. Tubes fractionated ~15 x 2 mL and analysed by 4-20 % Tris-Glycine SDS-PAGE.
- Bands very faint due to low sample recovery (more visible in real life than in these gel images unfortunately despite my best staining efforts).
- Species seen at same position as monomer HTT (previously assessed by GF and EM analysis) in terms of fraction and gel position. Concentrated to ~20 µL and flash frozen.
- Species seen in lowest sucrose concentration (implying low MW) but shows poor gel migration (implying high MW). Not observed before and seen in all 3 samples. Concentrated to ~20 µL and flash frozen to ship to Lea lab:

Fractions 7-10 species:	HTT Δexon1 – 0.7 mg/mL	HTT Q23 – 1 mg/mL	HTT Q54 – 0.6 mg/mL
Fraction 15 species:		HTT Q23 – 0.3 mg/mL	HTT Q54 – 0.5 mg/mL





2. 1 hour reaction set up:

Dephosphorylation:

- ~200 µg each of HTT Q23 (20x the NEB standard reaction) in total reaction volume ~250 µL 2000 U of λ phosphatase with 1 x PMP buffer (NEB) and 1 x $MnCl_2$. Incubate 1 hour at 25˚C

GRAFIX:

- ~200 µg each applied to 5-15 % (v/v) sucrose, 0-1 % glutaraldehyde 30 mL gradients in 20 mM HEPES pH 7.4, 300 mM NaCl. Samples run at 25,000 rpm Ti SW32.0 ultracentrifuge for 24 hours. Tube fractionated ~15 x 2 mL and analysed by 4-20 % Tris-Glycine SDS-PAGE.
- Species seen in heaviest fractions but do not run at highest MW? Need to run on gel filtration….
- Could be due to aggregation of sample?