

Project:

High resolution structural analysis of purified HTT samples

Experiment:

Analysis of dephosphorylated HTT samples for cryoEM analysis and further sample preparation

Date completed:

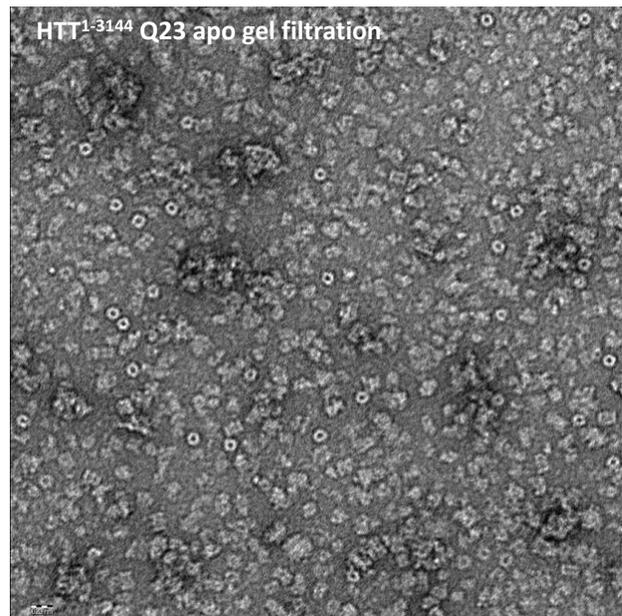
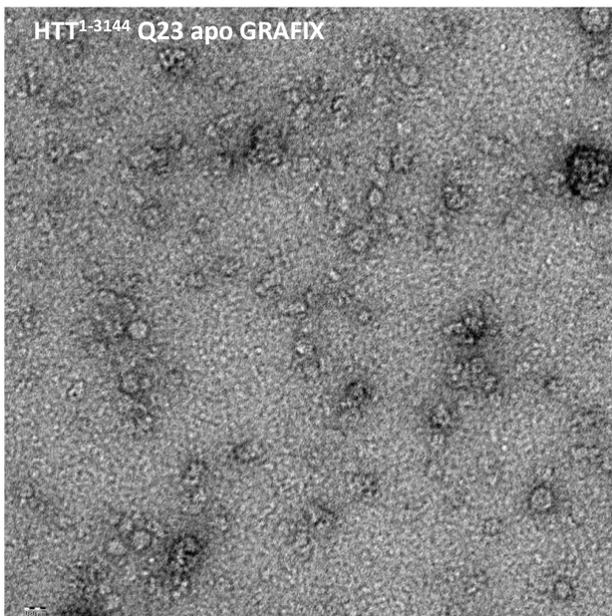
2019/04/15

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.

Analysis of previous samples:

Previously, dephosphorylated samples were generated <https://zenodo.org/record/2601987> and sent to the laboratory of Prof. Susan Lea. Postdoctoral fellow Dr. Justin Deme generated grids of the samples. Unfortunately the samples were insufficient concentration to see any particles in the ice of the cryo grids. However, the samples were concentrated to see particles via negative staining:



The gel filtration purified sample shows disperse and structured particles which is very promising. I now aim to scale up the purification of these samples so there is sufficient material for Justin to generated cryo grids for EM analysis.

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

Experimental approach:

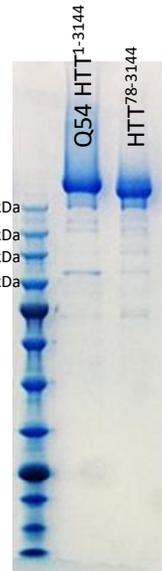
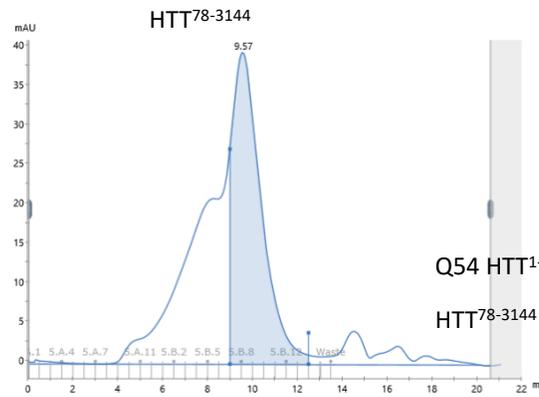
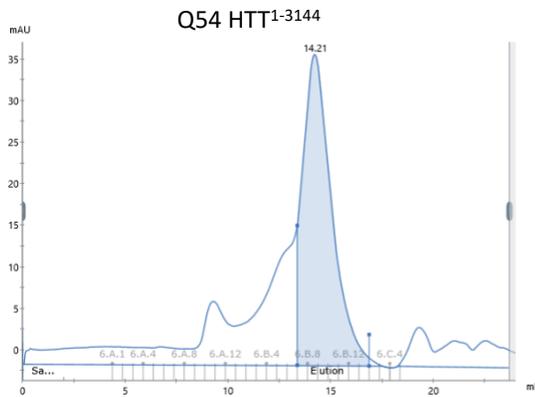
Dephosphorylation:

- ~1000 µg each of HTT Δexon1 and HTT Q54 (100x the NEB standard reaction) in total reaction volume ~500 µL 10,000 U of λ phosphatase with 1 x PMP buffer (NEB) and 1 x MnCl₂. Incubate at 25°C for 1 hour or overnight.

Gel filtration:

- Samples applied to Superose6 10/300 GL and run at 0.4 mL/min in 20 mM Hepes pH7.4, 300 mM NaCl, 5% glycerol (v/v), 1 mM TCEP. Monomer peak (highlighted in blue) concentrated (MWCO 100,000) and flash frozen.

1 Hour Treatment:

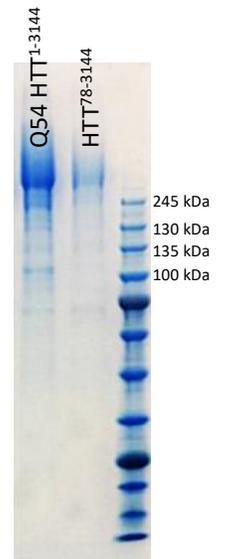
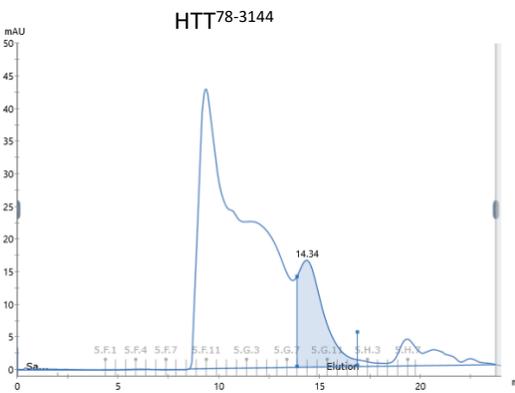
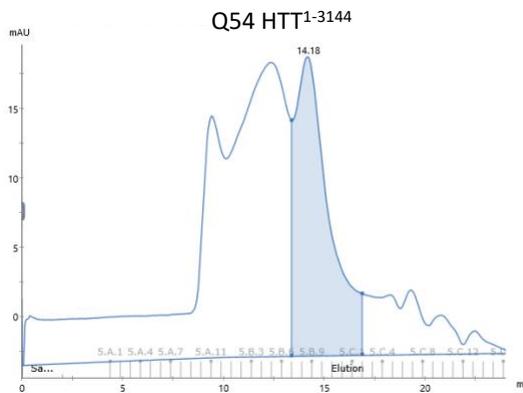


Final samples shipped to Oxford:

Q54 HTT¹⁻³¹⁴⁴ - 5 mg/mL, 4 x 10 µL

HTT⁷⁸⁻³¹⁴⁴ - 3.7 mg/mL, 4 x 10 µL

Overnight Treatment:



Final samples shipped to Oxford:

Q54 HTT¹⁻³¹⁴⁴ - 4.2 mg/mL, 4 x 6 µL

HTT⁷⁸⁻³¹⁴⁴ - 2.4 mg/mL, 4 x 7 µL