Project:
High resolution structural analysis of purified HTT samples

Experiment:
Analysis of dephosphorylated HTT samples by cryoEM analysis

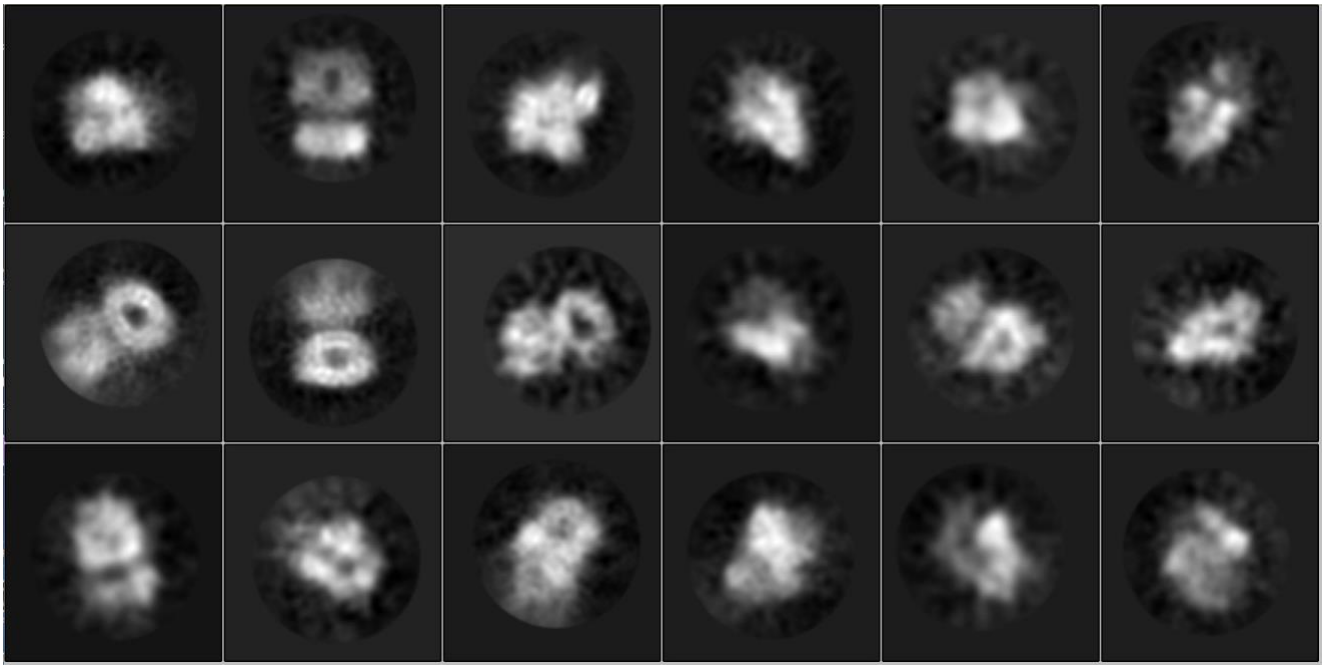
Date completed:
2019/05/01

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 samples:
Previously, dephosphorylated samples were generated and sent to the laboratory of Prof. Susan Lea - https://zenodo.org/record/3234163. Postdoctoral fellow Dr. Justin Deme performed the following experiments and analysis:

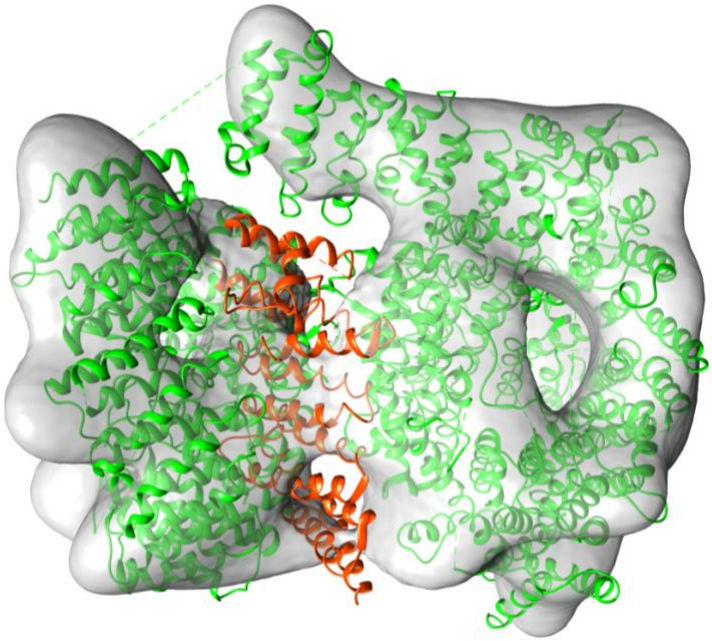
- The Q54 HTT 1h dephosphorylated sample at 0.4 mg/ml was applied to 300 mesh 1.2/1.3 quantifoils.
- Protein particles clearly visible this time but the nanodrop read 3.5 mg/ml for this sample, so possibly some loss (30%) from freeze-thawing.
- Particles appeared to be somewhat sharper than previous so a ~100 k particle dataset was collected
- 2D classification was performed using SIMPLE or RELION

2D Classification from RELION:



2D Classification from SIMPLE:

- Comparing this set with previous HTT sets, this sample probably gave better 2D averages. Structure in the N-HEAT domain is visible but the C-HEAT domain appears to be more floppy and blurred out.
- A low resolution (20 Å) map was generated using a lowpass-filtered version of the HTT-HAP40 structure as reference and fit in HTT-HAP40 model (see attached). On the whole, the overall shape is consistent with the HAP40-bound complex, though density for HAP40 is missing (it is not present in the sample).



Next Steps:

The samples are much better quality than anything previous analysed for apo HTT suggesting high quality and homogenous protein sample.

It could be that the 1 hour phosphorylation treatment is insufficient to collapse the structure fully – try the overnight sample next.