**Project:**

High resolution structural analysis of purified HTT samples

**Experiment:**

Analysis of dephosphorylated HTT samples by cryoEM analysis

**Date completed:­**

2019/05/01

**Rationale:**

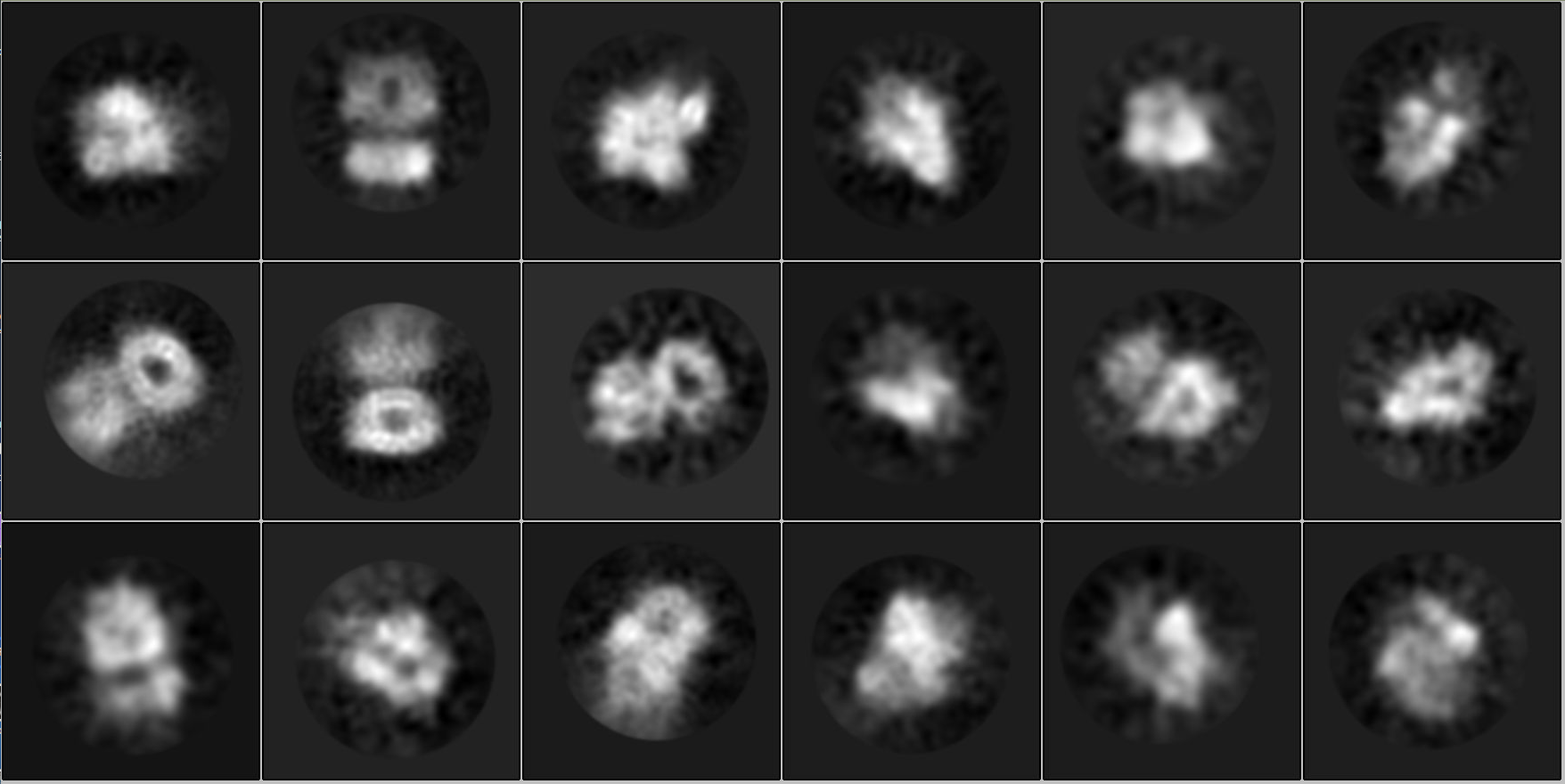
We now have a good understanding of the global structure of HTT when in complex with HAP40 and our MALS and SAXS data suggest that apo HTT is likely self-associating and heterogenous in nature, despite high levels of purity. Dephosphorylating HTT from Sf9 cell production could alter its global structure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

**Analysis of samples:**

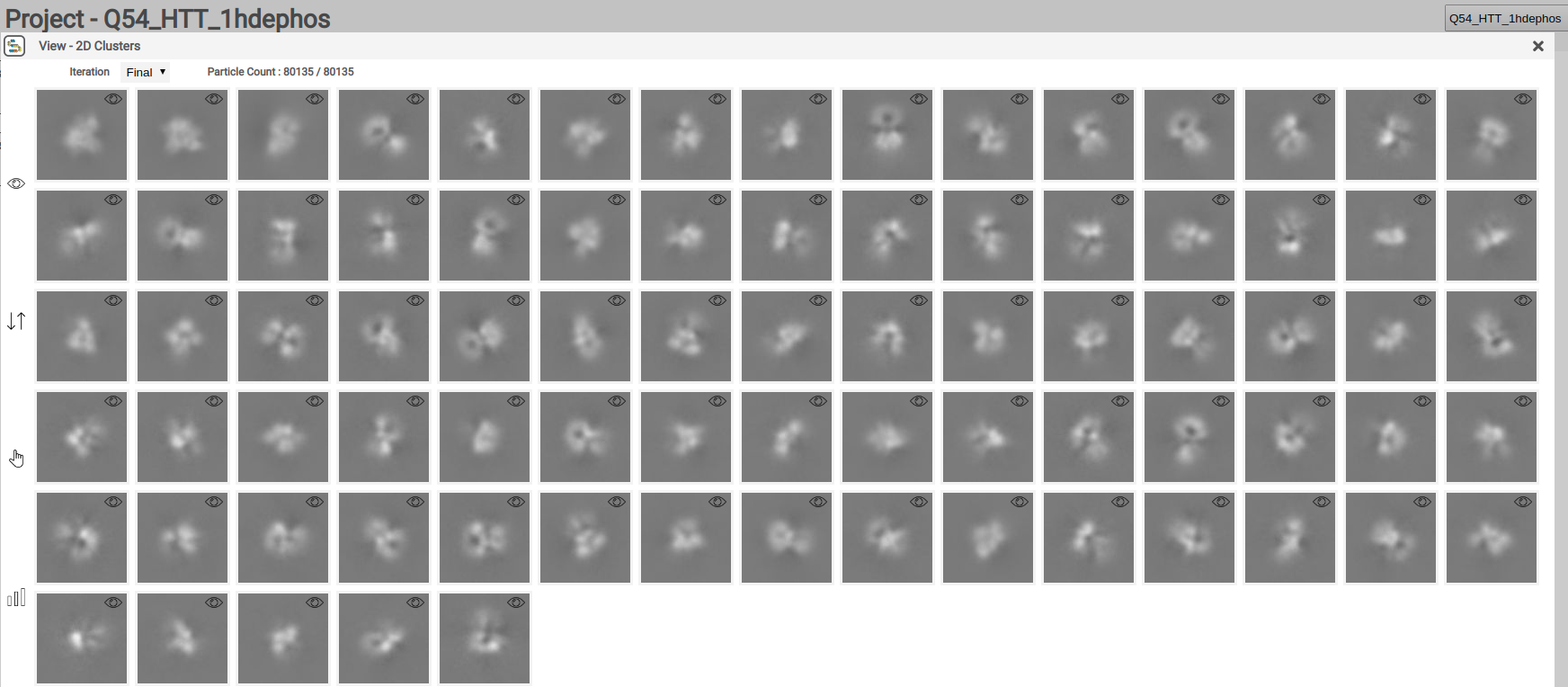
Previously, dephosphorylated samples were generated and sent to the laboratory of Prof. Susan Lea - https://zenodo.org/record/3234163. Postdoctoral fellow Dr. Justin Deme performed the following experiments and analysis:

* 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

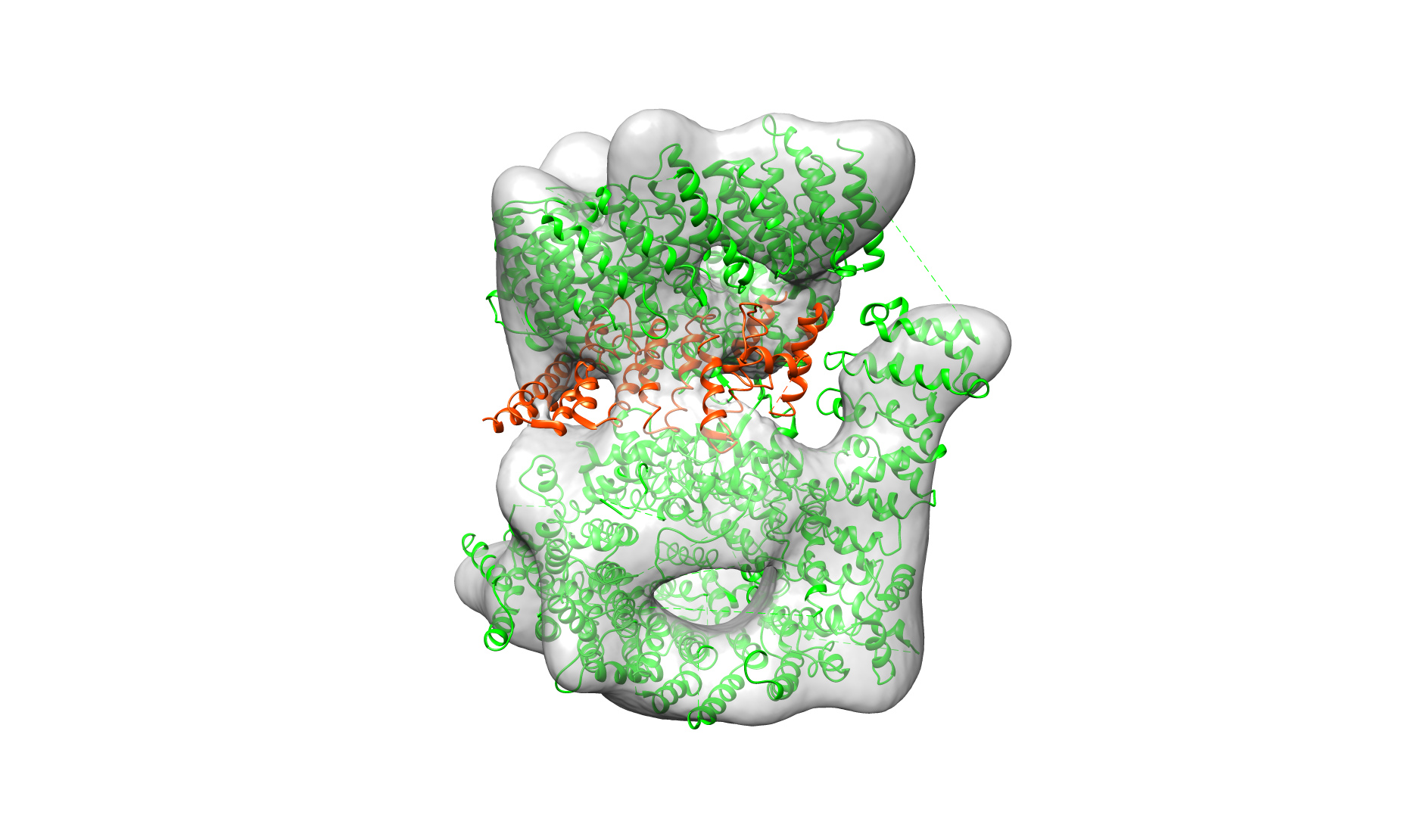
2D Classification from RELION:



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.