Figure 1. Preliminary reconstruction of different assemblies of CasTn7, corresponding to three different samples, ranging between 4-6 Å resolution. Asterisk(*) indicates sample demonstrating severe preferred orientation problem. Arrow indicates region of high variability that would benefit from additional data and focused refinement.
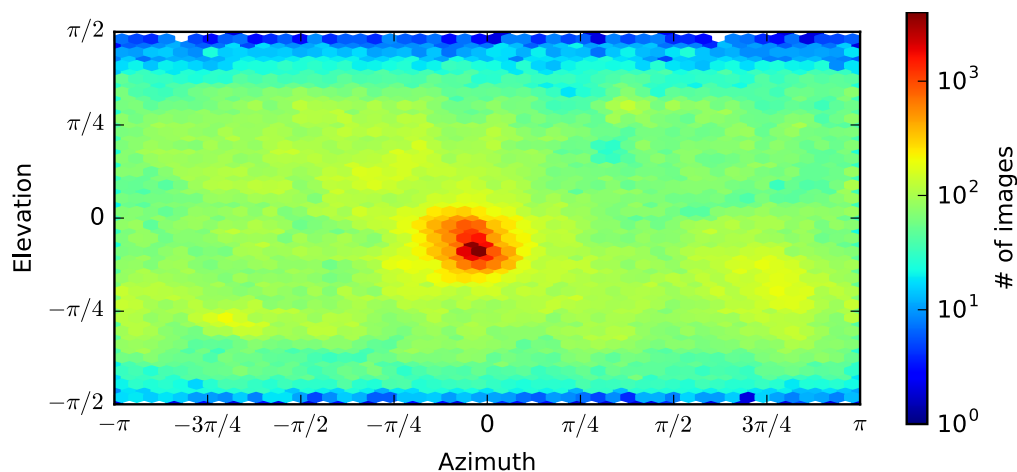

Figure 2. Orientation distribution (untilted, from cryosparc) for the problematic sample shown in Figure 1 (indicated by asterisk)
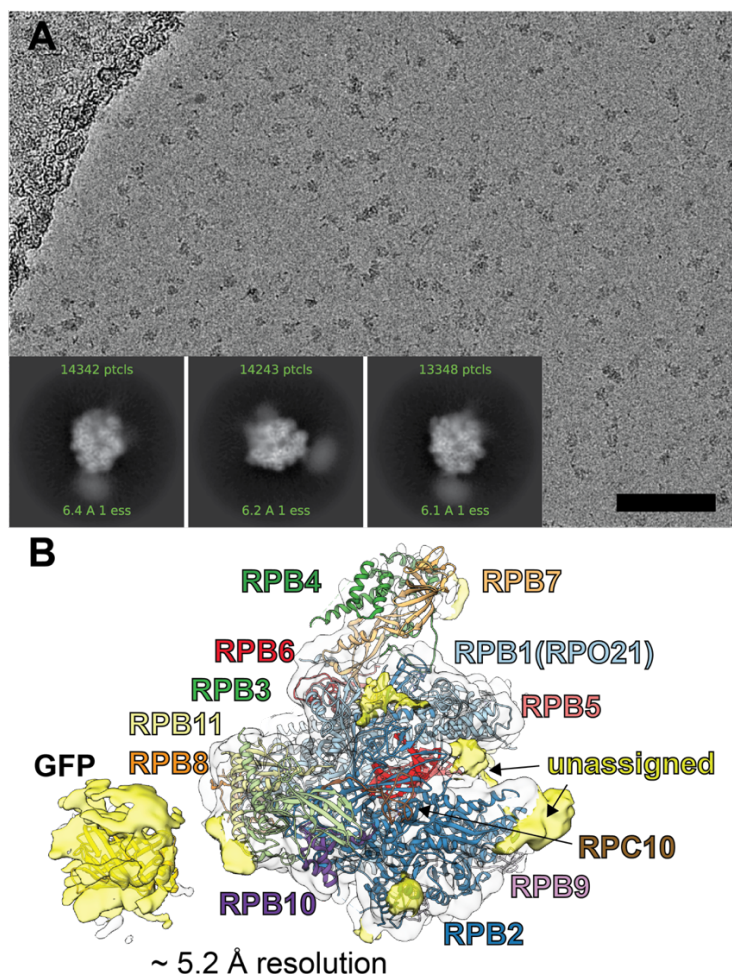
Figure 3. Preliminary data for endogenous RNA pol II purified from yeast (*S. Pombe*). **A.** Representative cryo-EM image of RNA pol II indicates that particle density on graphene oxide is sufficient for high-resolution cryo-EM data collection (average around 400 particles/image). **B.** preliminary reconstruction from the dataset reaches 5 Å resolution from a 170,000 particle dataset (initial particle numbers).

Sample name	Assembly state	Number of particles	Estimated resolution (Å)
Initial	Incomplete	81,327	-
	Complete	65,365	~ 6
Intermediate	Incomplete	81,368	-
	Complete	85,434	3.8
	Inhibited	38,049	-
Active	Incomplete	73,662	-
	Complete	83,090	4.5
	Inhibited	47,279	~ 6

Table 1. Dataset statistics and 3D sorting reveal extensive heterogeneity and are resolution-limiting. Significant structural heterogeneity within each sample results in resolution-limiting numbers of particles when short data-collection sessions are used (1 day on an Arctica/K3 for each sample).