

Our experience with a back-thinned FEI Falcon detector

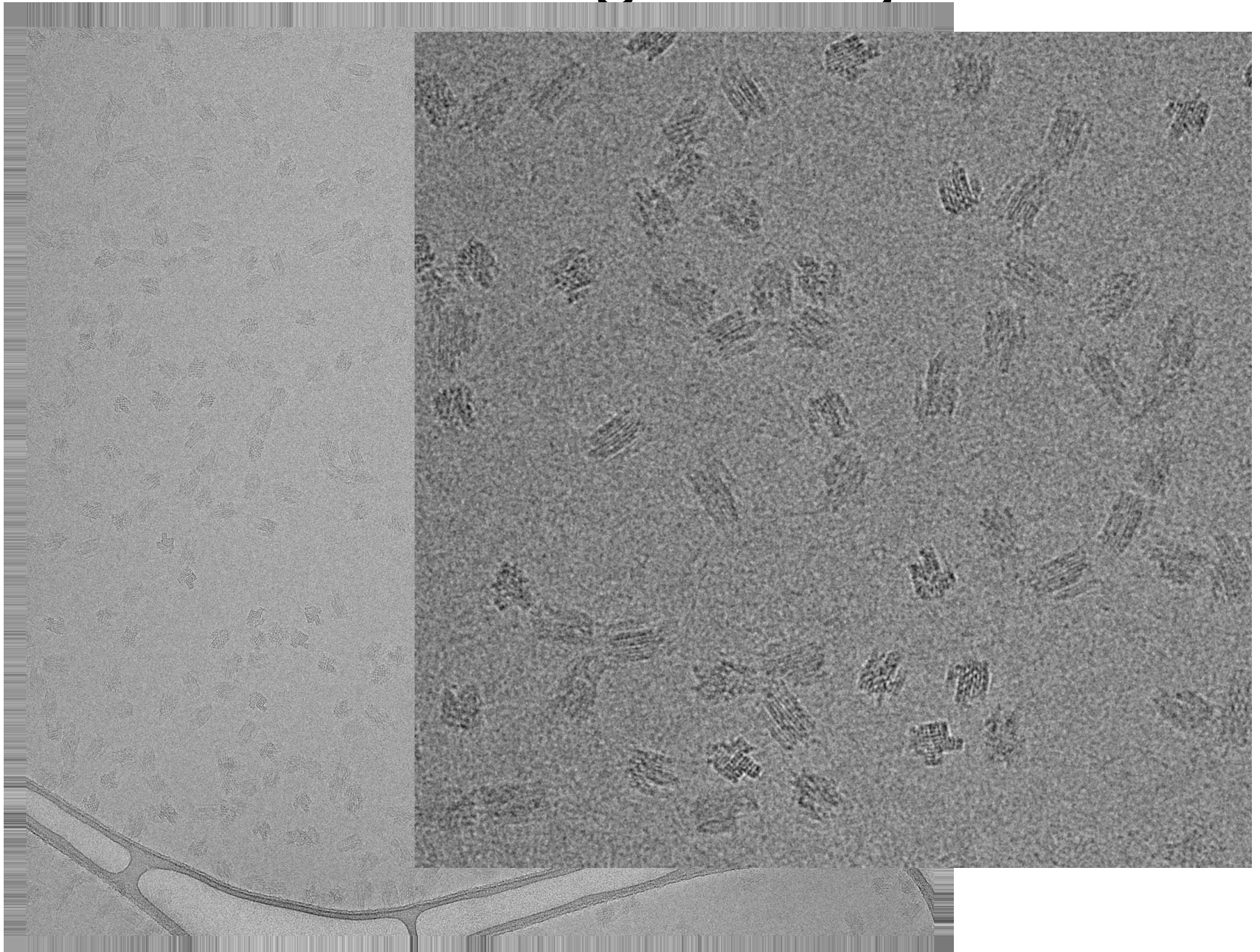
Xiao-chen Bai, Greg McMullan,
Sjors H.W. Scheres

MRC Laboratory of Molecular Biology

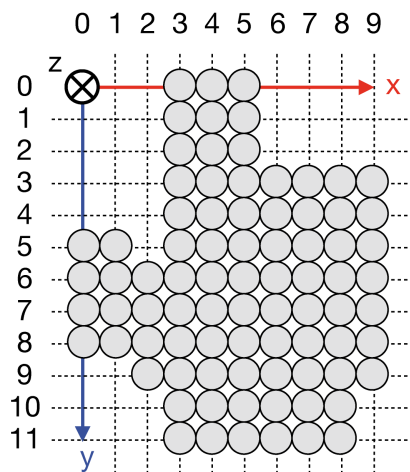
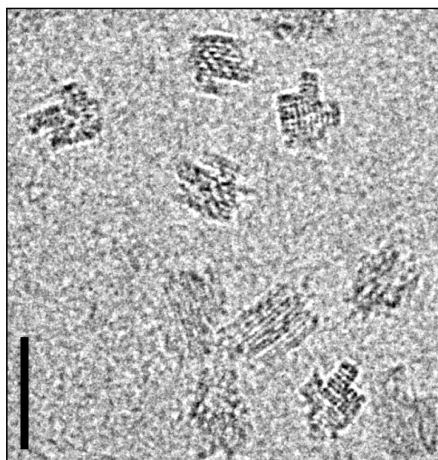
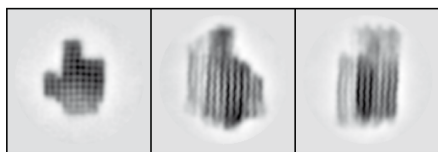
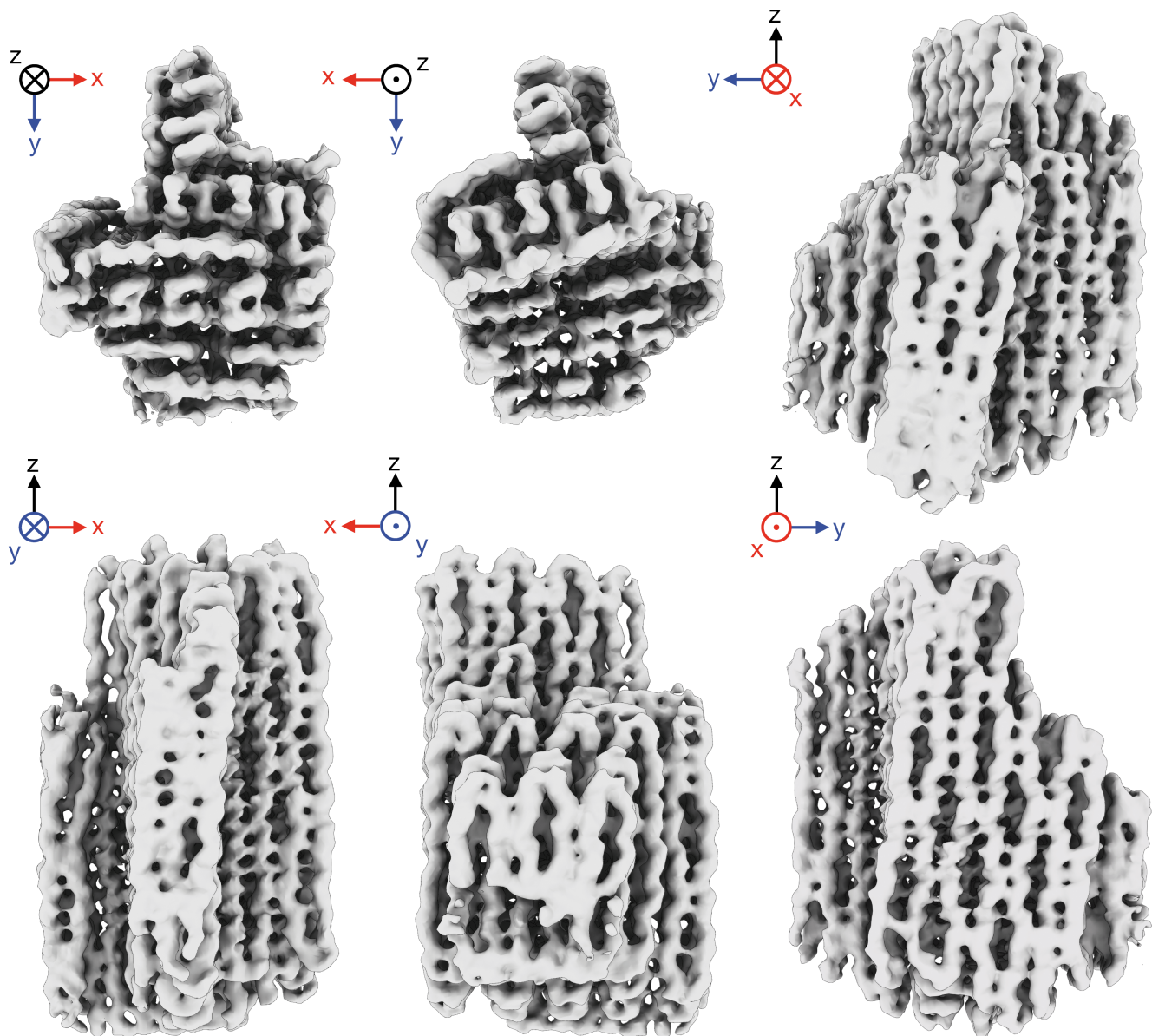
Three structures

- 4.8 MDa DNA-origami object (with Dietz group, TUM Munich)
 - 42,000x magnification (3.55 Å/pixel)
 - 456 micrographs (2 manual sessions)
- 70S ribosome (sample by Israel Sanchez, Ramakrishnan group, LMB)
 - 79,000x magnification (1.77 Å/pixel)
 - 180 tilted pairs (0-10°) of micrographs (2 manual sessions)
- 80S ribosome (sample by Israel Sanchez, Ramakrishnan group, LMB)
 - 79,000x magnification (1.77 Å/pixel)
 - 260 micrographs (1 manual session)

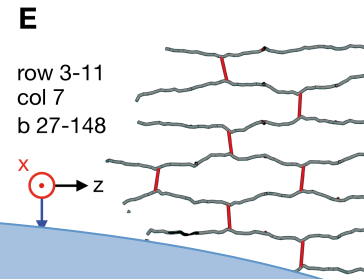
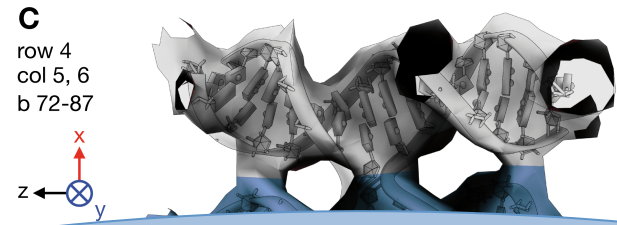
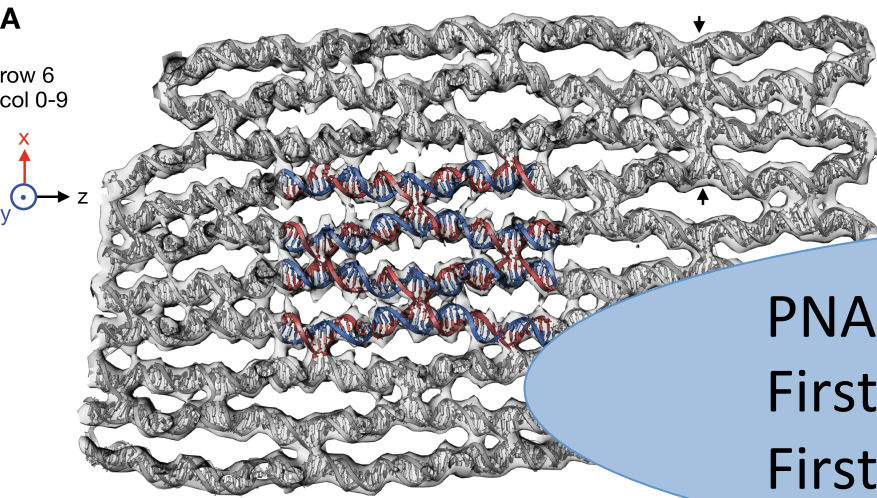
3D DNA origami object



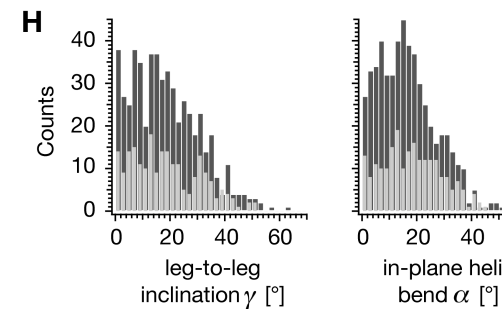
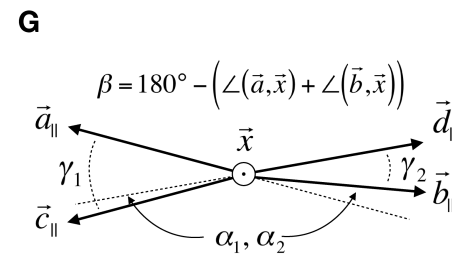
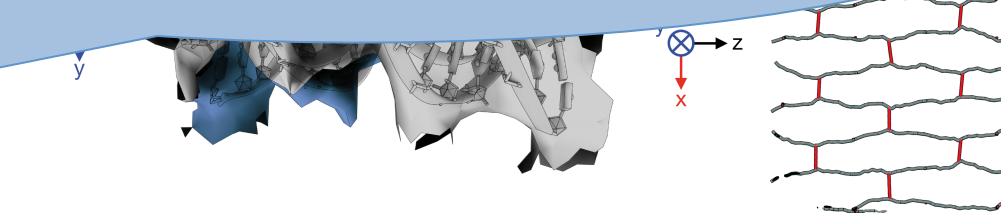
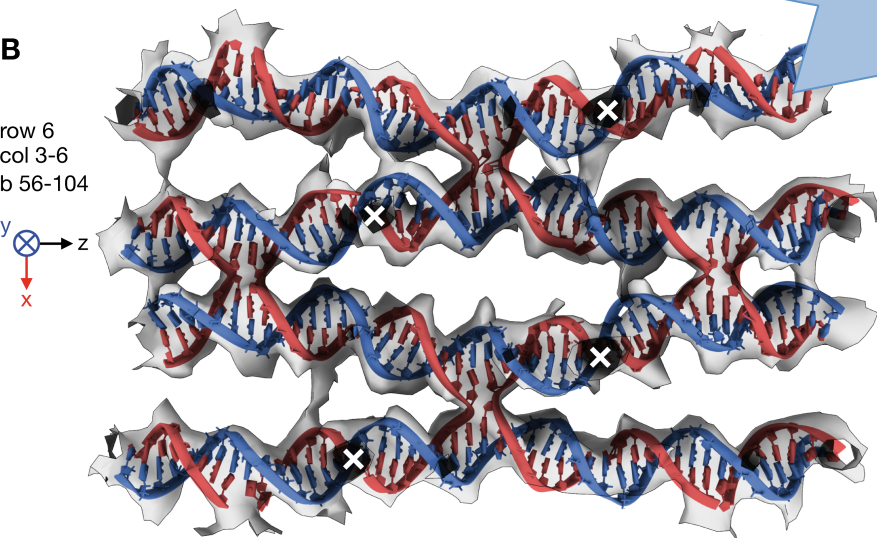
28k particles: 11.5 Å map

A**B****C****D**

28k particles: 11.5 Å map

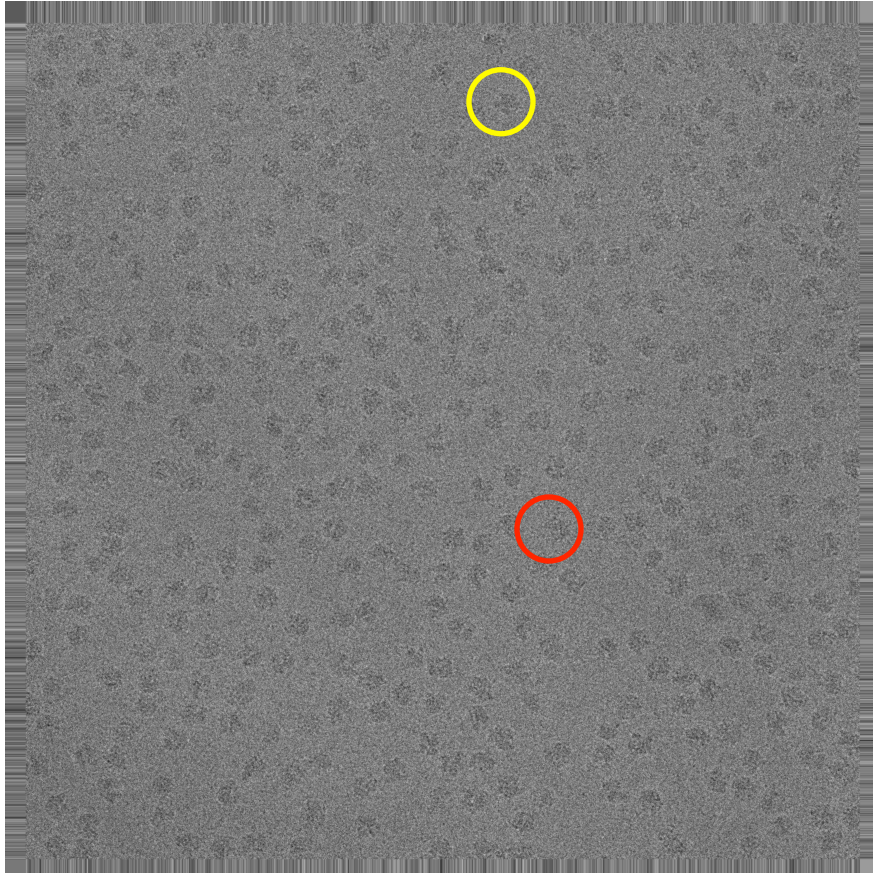


PNAS, *in press*
First structure on (back-thinned) Falcon
First genome in the PDB ;-)

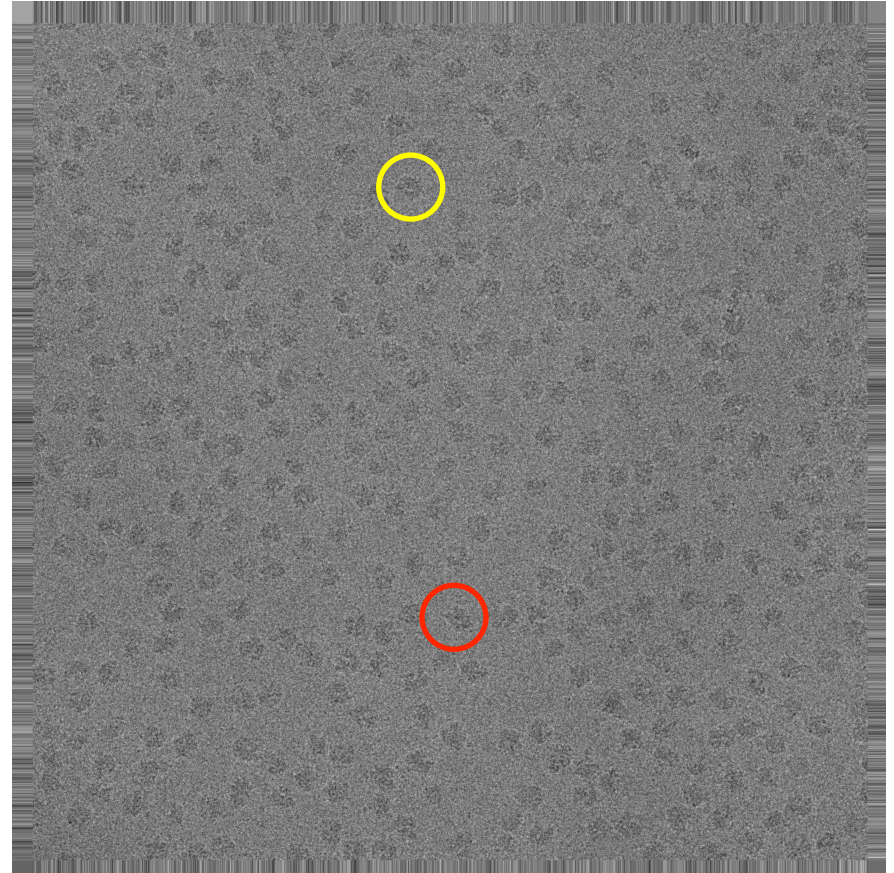


70S *E.coli* ribosome

0°

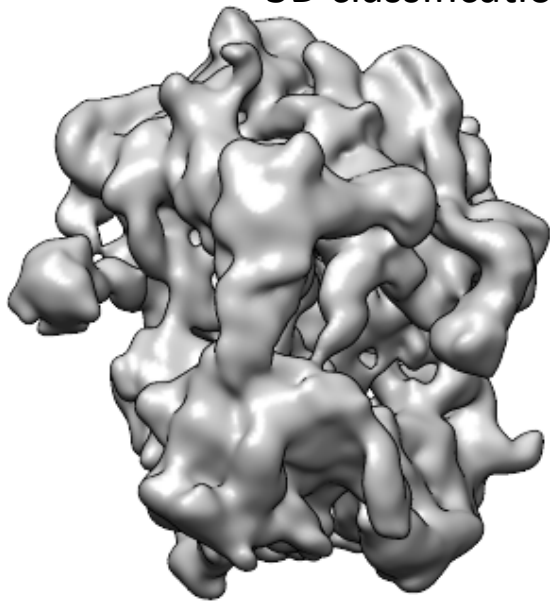


10°

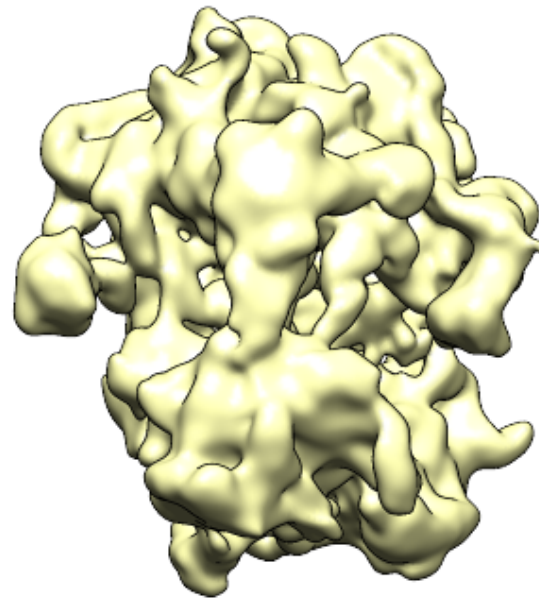


24K pairs of particles from 180 micrograph pairs

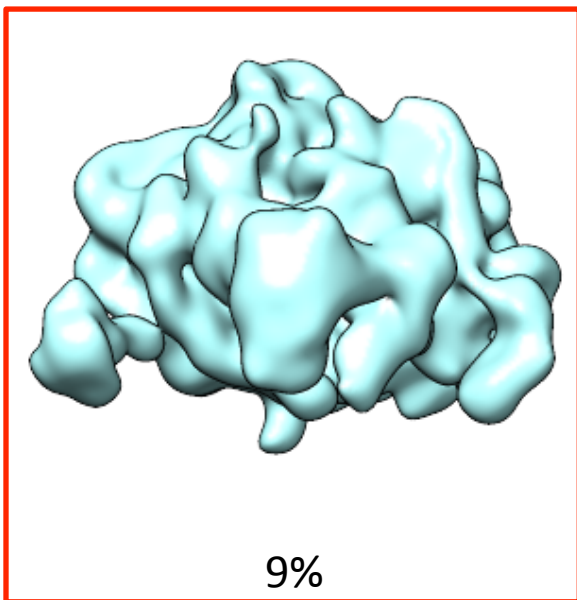
3D classification of 70s by Relion (Round 1)



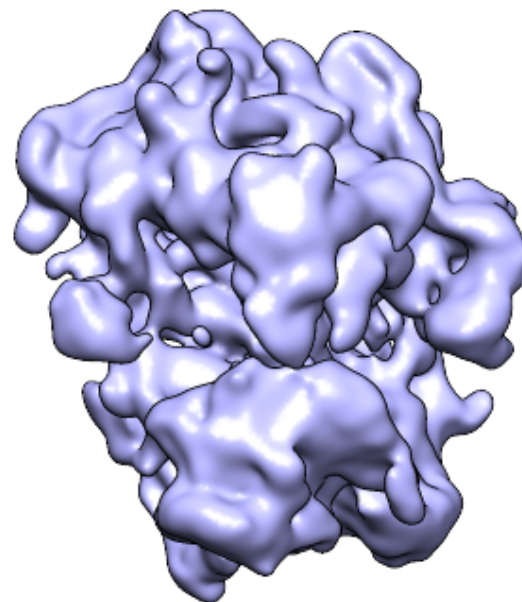
31%



29%

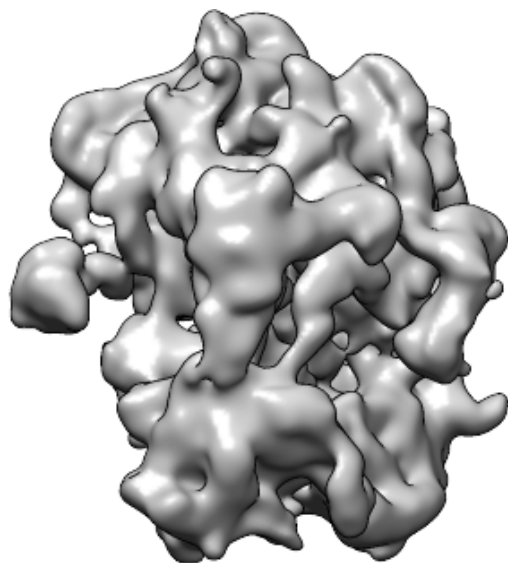


9%

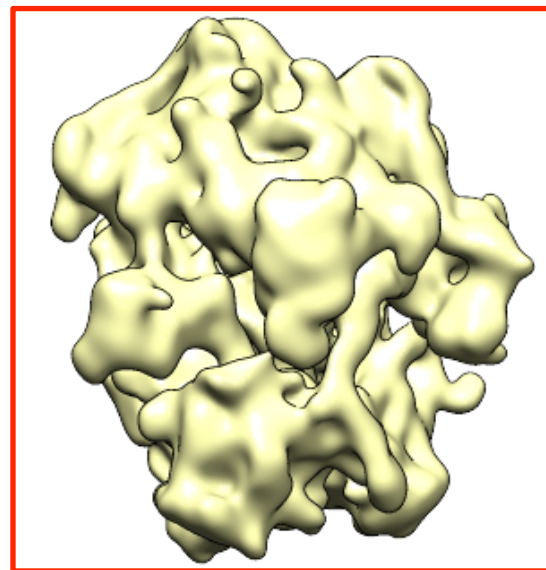


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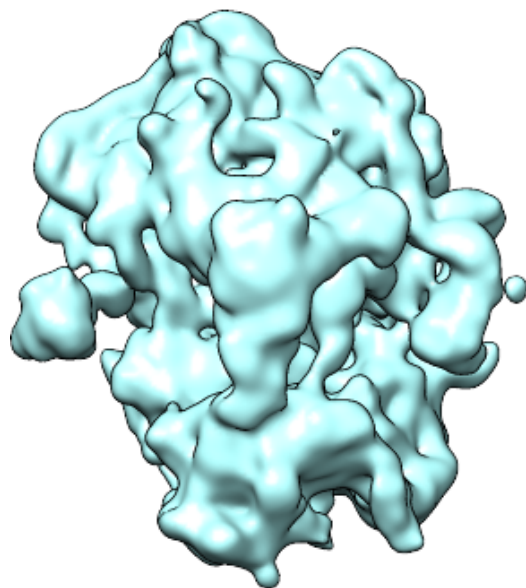
3D classification of 70s by Relion (Round 2)



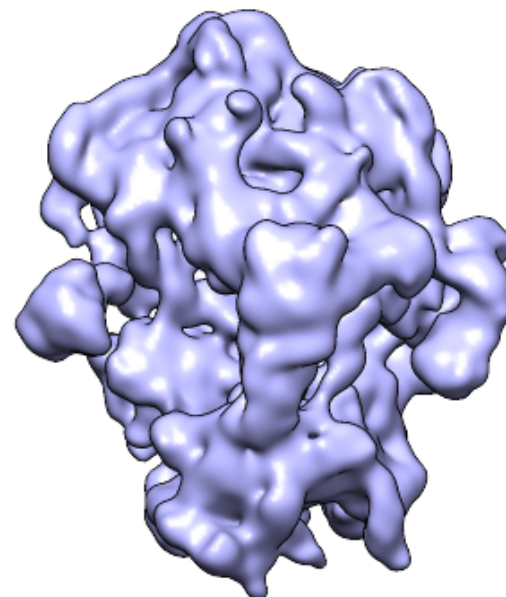
29%



19%

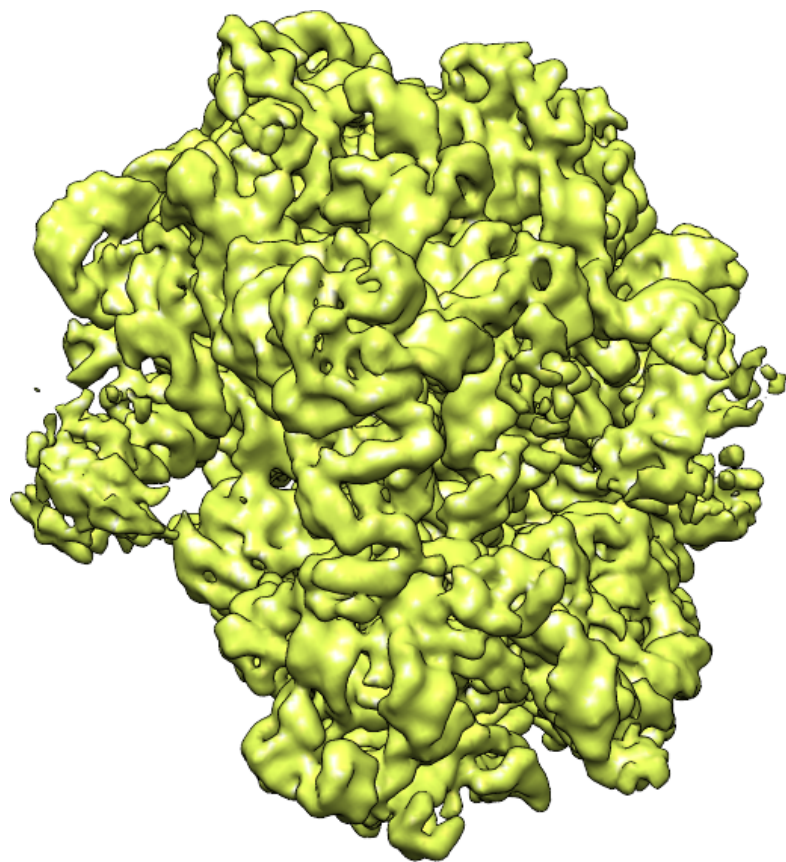


26%



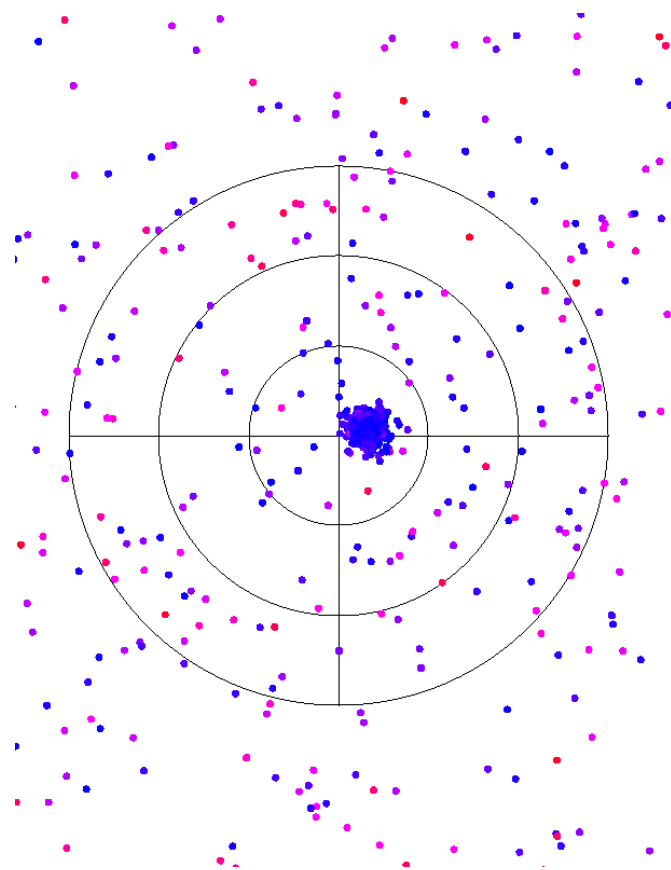
26%

Relion



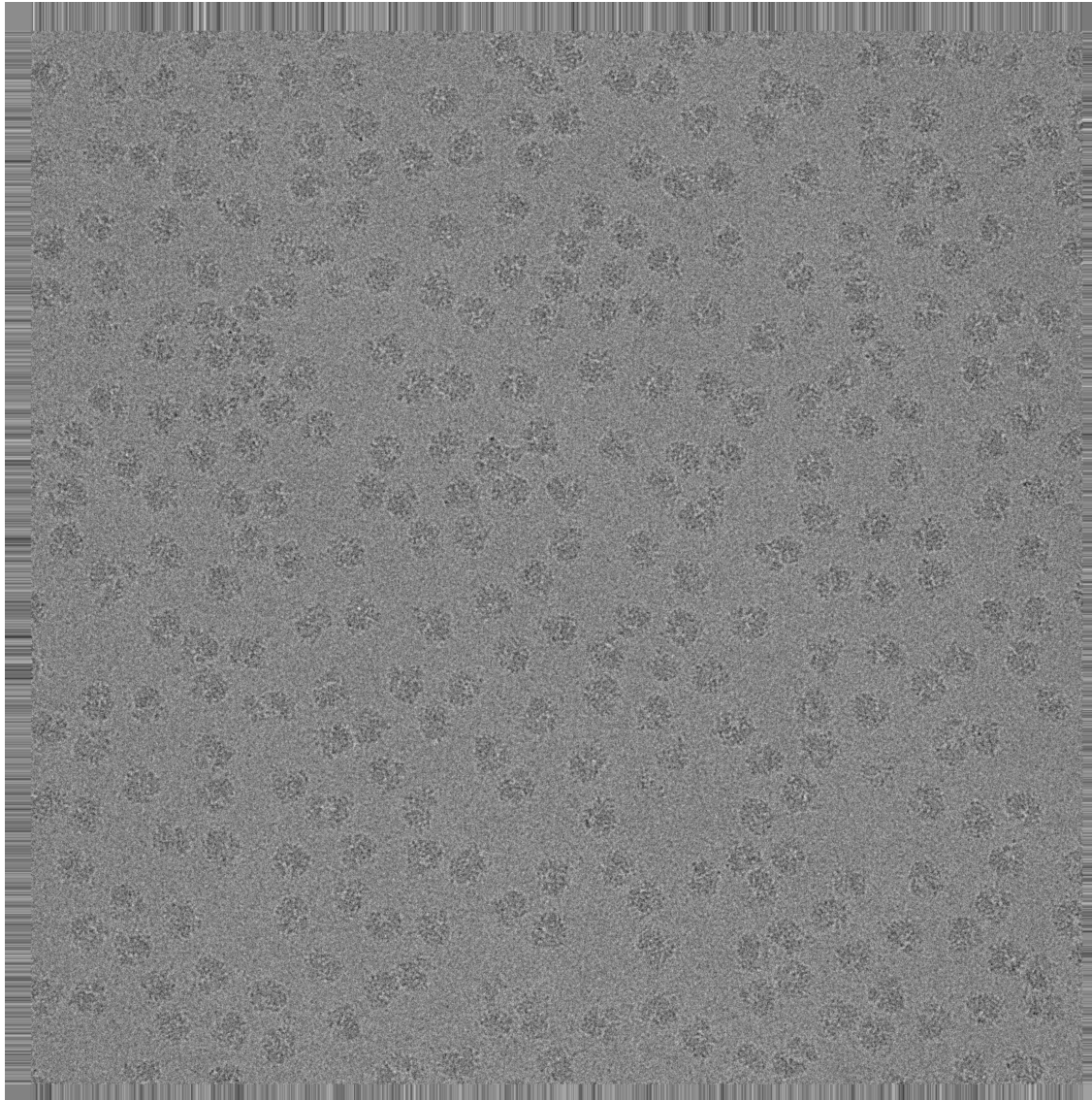
6.8Å resolution, 15K pairs of particles

Tiltpair test



Only 1K particles are not well aligned

80S *S. cerevisiae* ribosome



260 micrographs

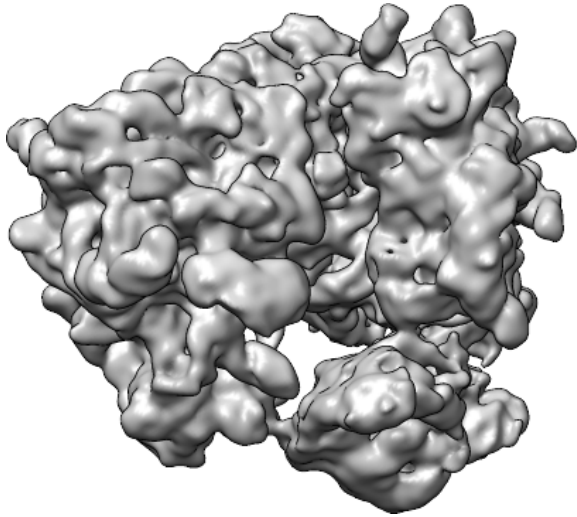
72,437 particles

First picture: noon

Last picture: 11pm

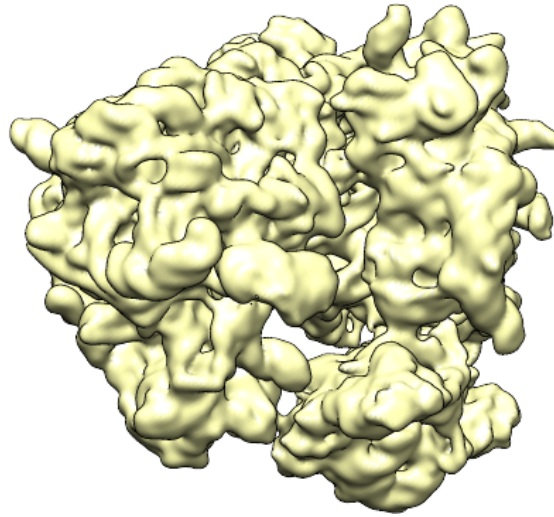
3D classification of 80s by Relion

Class 1



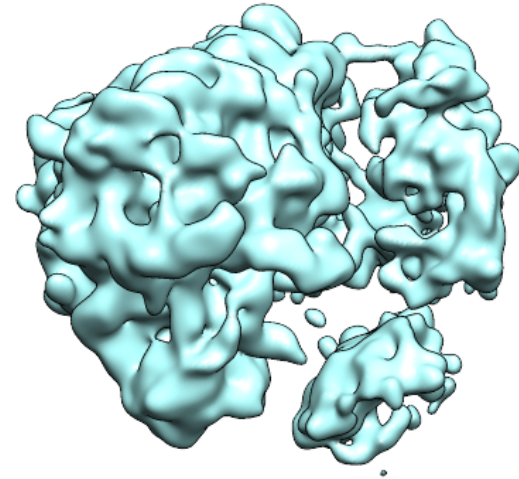
22,638 particles

Class 2



35,813 particles

Class 3



13,986 particles

80S ratchet movement

