

**Fig. 1.** A representative cryo-EM image of the *E. coli* RNAP initiation complex primed with a cap. The image was obtained on Krios2 at NYSBC.

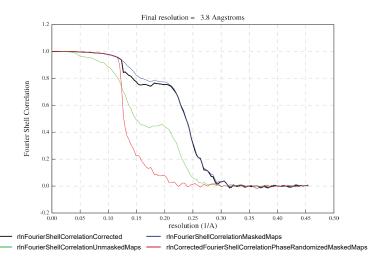


Fig. 3. Graph showing FSC of half-maps after 3D refinement.

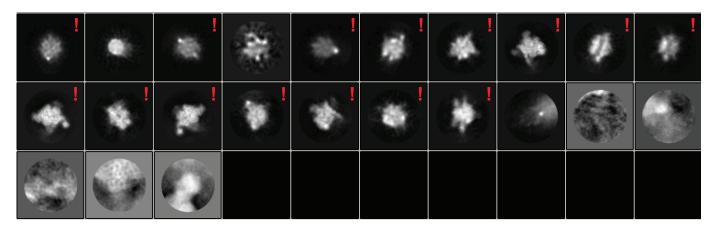


Fig. 2. 2D classification of 109,000 particles after initial cleanup. Particles from marked 2D classes were used for 3D reconstruction.

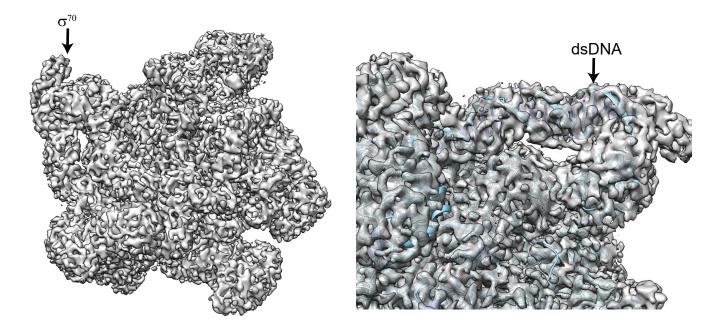


Fig. 4. Final cryo-EM map of the *E. coli* transcription initiation complex. The map shows RNAP bound to the  $\sigma^{70}$  factor.

**Fig. 5.** Fragement of the cryo-EM map showing a good fit of the RNA polymerase and template DNA models.