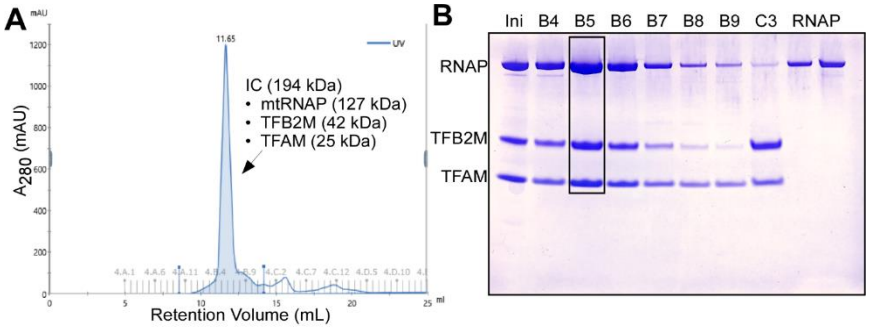

Figure 1. Size exclusion chromatography. The chromatogram of the gel filtration of assembled IC (A) and the corresponding SDS-PAGE (B) of the peak fractions reveal efficient assembly of the IC.

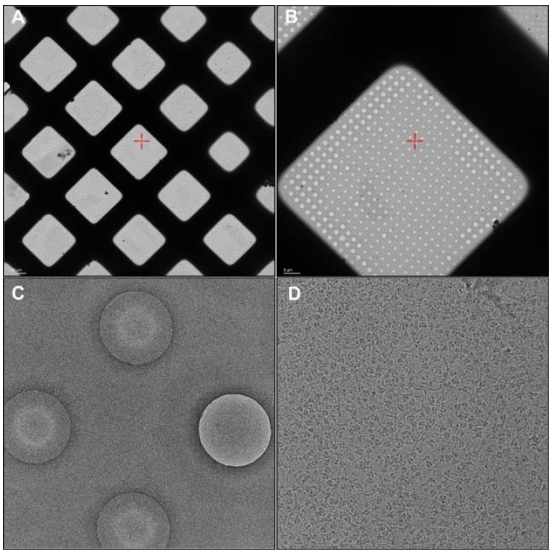

Figure 2. Cryo-EM grid preparation. Sample images of the assembled IC on Quantifoil R1.2/1.3 Copper grids using the Atlas (A), Grid Square (B), Foil hole (C) and Data Acquisition (D) presets captured in EPU cryo-EM imaging software.

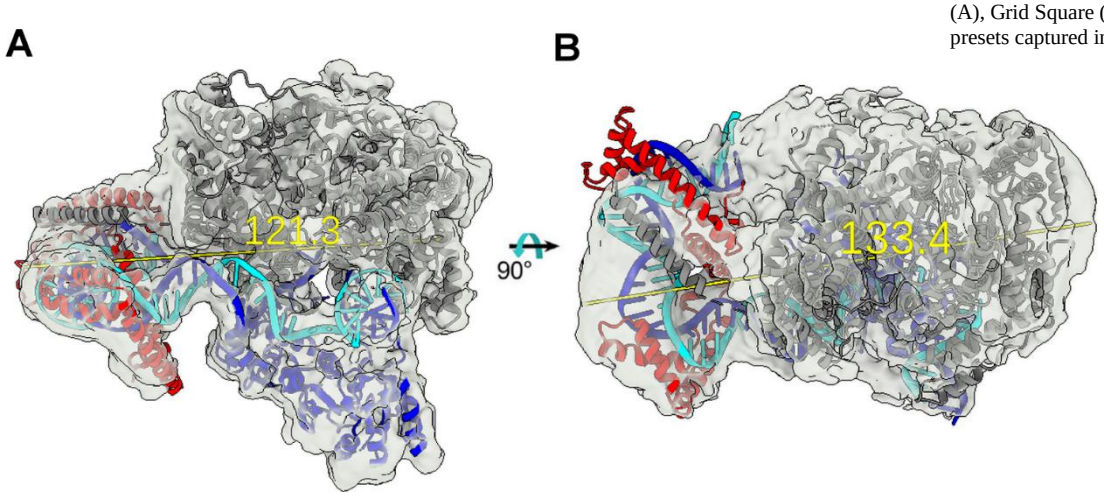

Figure 3. A high-resolution structure generated from particles containing the full initiation complex composed of mtRNAP (grey), TFB2M (blue), TFAM (red), and promoter DNA (blue, cyan) superimposed on the published crystal structure of the IC (PDB:6ERP). Non-uniform 3D-refinement in CryoSPARC reported a 3.81Å resolution map (A) which shows electron density for TFAM in a different conformation compared to the published crystal structure (B).

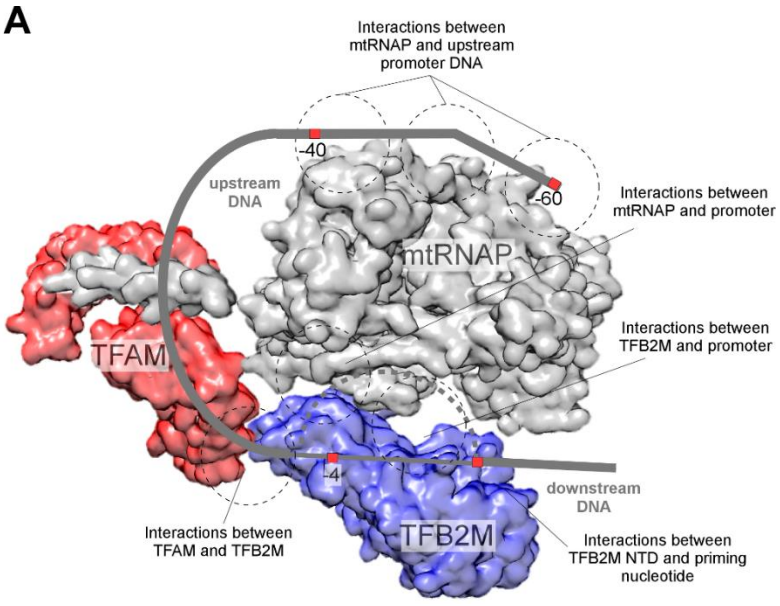

Figure 4. Schematic of the initiation complex annotated with potential interactions revealed by cryo-EM.

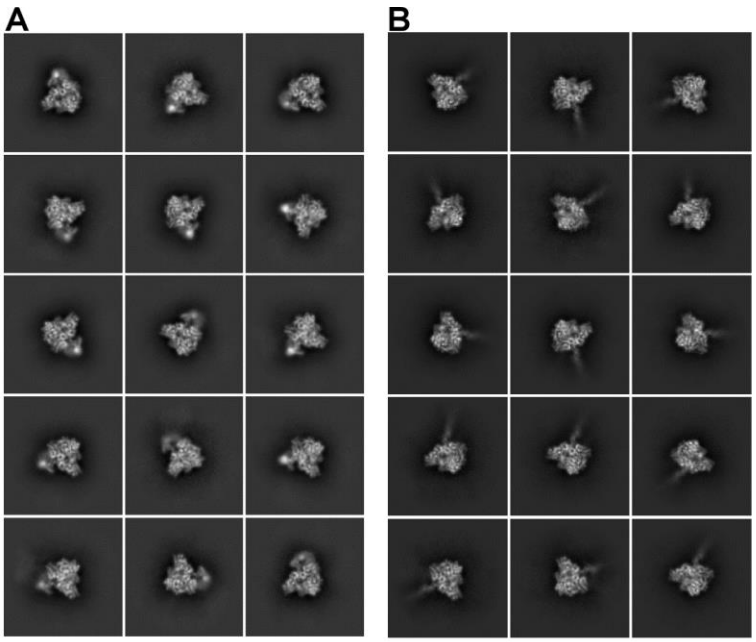

Figure 5. The fifteen most populated classes from 2D classification of selected particles containing the full initiation complex (A) and the partial complex lacking TFAM (B).