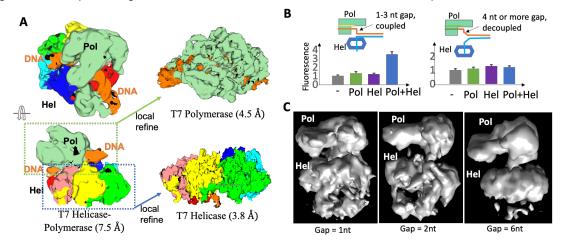


**Figure 1. Structural studies of T7 helicase. A.** Previous structures of T7 helicase-DNA complex at 3.2 Å. **B.** T7 helicase binding affinities to ssDNA with AP site at different location. The selected substrate for cryo-EM test is circled in red. **C.** 2D-classification of T7 helicase-DNA<sub>AP</sub> complex suggested disrupted rings. **D.E.** 3D classes of T7 helicase-DNA<sub>AP</sub> complex.



**Figure 2. Structural studies of T7 replisome. A.** Previous structures of T7 helicase-polymerase complex on a replication fork. The helicase and polymerase of the complex can be separately refined to near atomic-resolution. **B.** Fluorescence assays suggested T7 polymerase and helicase are coupled with 1-3 nt gap on the leading strand DNA, whereas become decoupled with 4nt or longer gap. **C.** Major 3D-classes of T7 replisome with 1, 2, and 6 nt gaps on the leading strand.

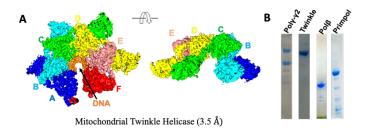


Figure 3. Structural studies of mitochondrial replisome. A. Previous structures of Twinkle helicase-DNA complex at 3.5 Å. **B.** Purified mitochondrial Twinkle helicase, Pol  $\gamma$  /  $\gamma$ 2, Pol  $\beta$ , and Primpol.