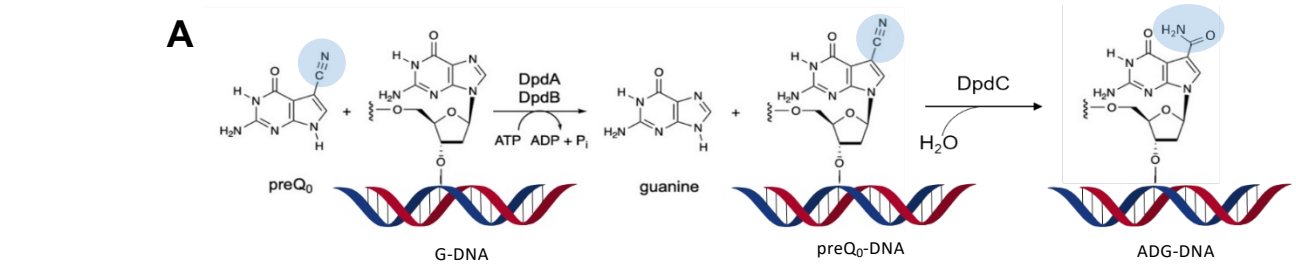

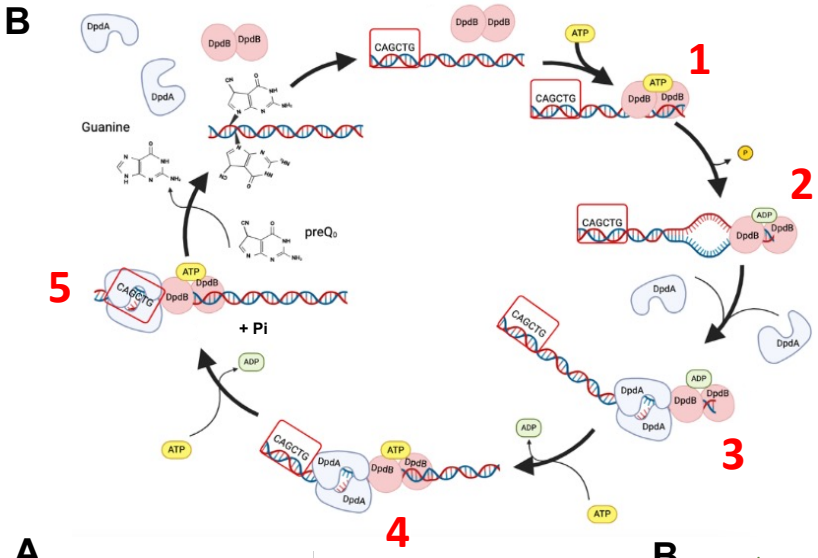

Figure 1. The biosynthetic pathway to 7-deazaguanines in DNA.
A) The pathway to the modification of DNA with 7-deazaguanines by the Dpd enzyme system. **B)** Proposed cycle highlighting the coordinated actions of DpdA and DpdB in modifying DNA through the insertion of the preQ$_0$ base, driven by ATP hydrolysis. The macromolecular complexes proposed for cryoEM studies in this proposal are numbered in red.

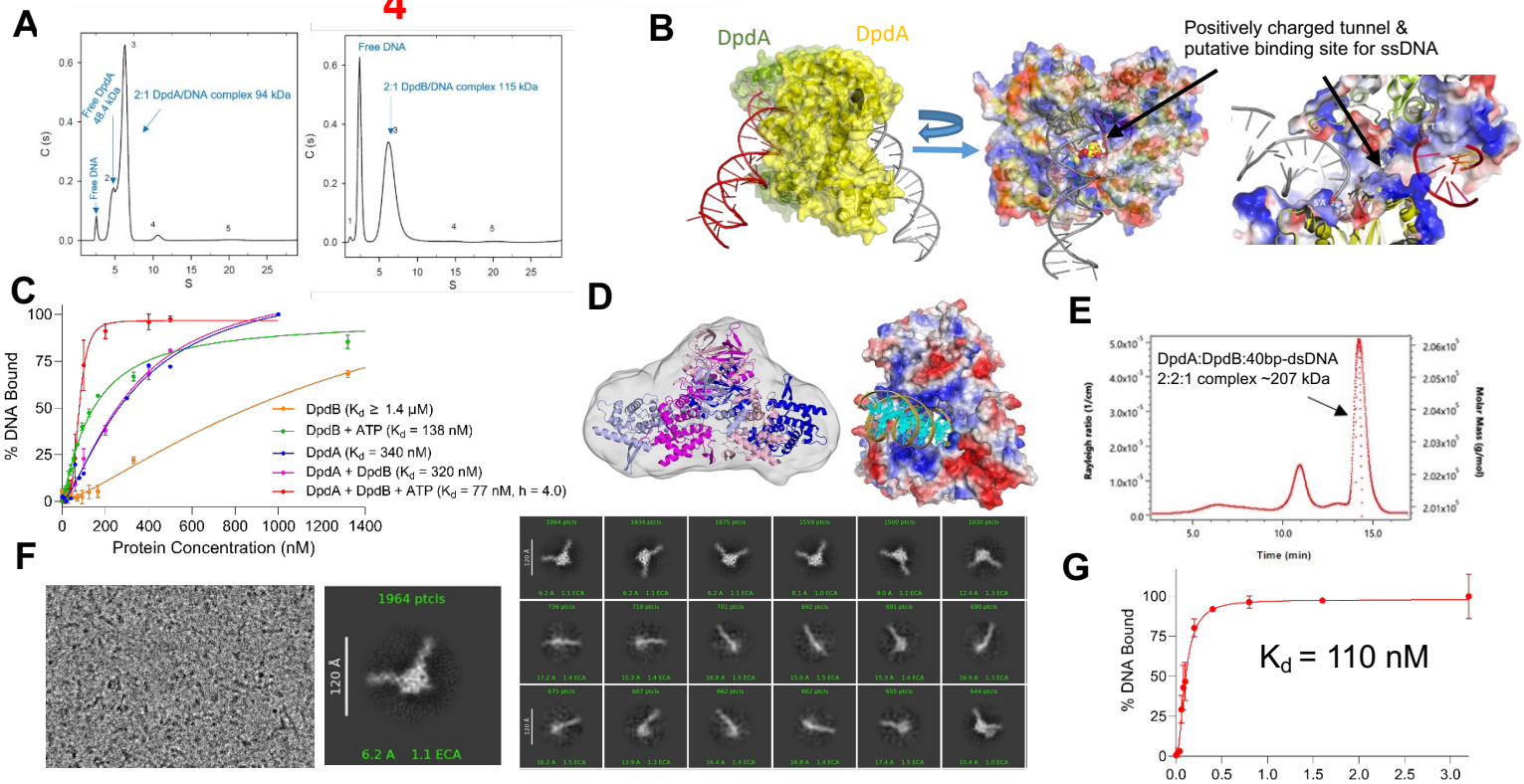

Figure 2. Macromolecular complexes in the Dpd pathway and supporting data. **A)** Analytical ultracentrifugation of DpdA and DpdB bound to 20-bp DNA indicates 2:1 complexes. **B)** Crystal structure of DpdA homodimer bound to 20-bp DNA reveals DNA bending and a positively charged inter-subunit tunnel, potentially a ssDNA binding site. **C)** Fluorescence polarization binding assays show ATP-dependent 10-fold DpdB binding enhancement to 40-bp DNA and 4-fold DpdA enhancement (orange/green and blue/red lines, respectively). **D)** SAXS envelop of DpdB displays ParB-fold dimer (left) and our model of how it clamps onto dsDNA (right). **E)** SEC-MALS of DpdA/DpbB/40bp-DNA confirms Mr of a 2:2:1 complex. **F)** cryogenic electron micrograph of a sample containing DpdA/DpdB/40bp-DNA at 2.5 uM with ATP (left), some 2D classes (right), and the 2D class representing DpdA dimer bound to DNA (middle). **G)** High-affinity DpdC binding to 20-bp DNA by fluorescence-polarization assay. **H)** SEC-MALS of DpdC/40-bp DNA confirms 2:1 complex Mr (left); SAXS envelop supports this stoichiometry (right).