Supplementary Information

Structural and biochemical insights into the inhibition of *Mycobacterium tuberculosis* Cyclic Dinucleotide Phosphodiesterase by a sulfur-modified cyclic dinucleotide analog

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Supplementary Figures

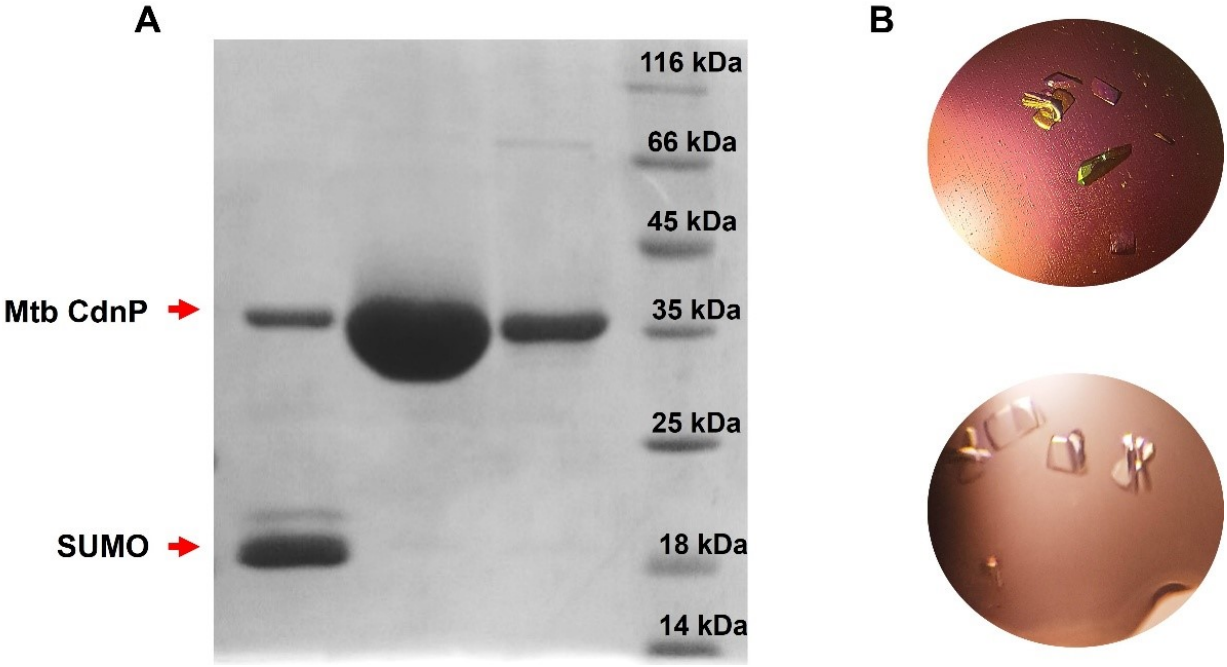

Figure S1. Purification and crystallization of M. tuberculosis CdnP. (A) SDS-PAGE analysis of gel filtration–purified recombinant CdnP. (B) Crystal of the CdnP–ES-2'3'–cAAMP complex grown at 20 °C.

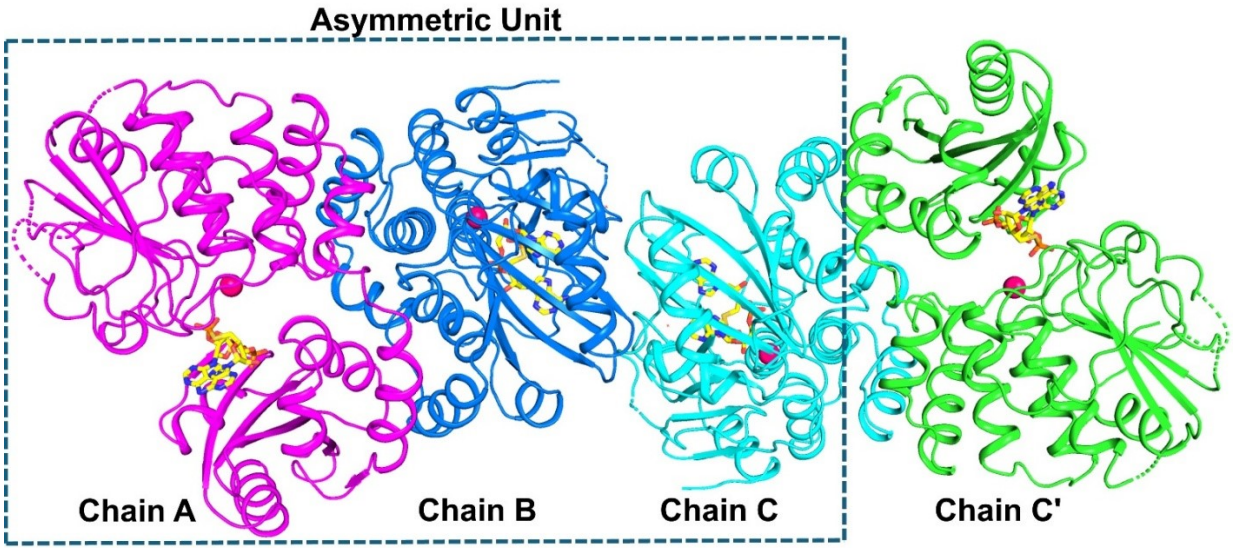

Figure S2. Biological assembly of CdnP. The functional dimer of CdnP is formed in two distinct ways: one dimer is assembled from two protomers (chains A and B) within the

asymmetric unit, while a second dimer is formed by chain C and its crystallographic symmetry mate (chain C′).

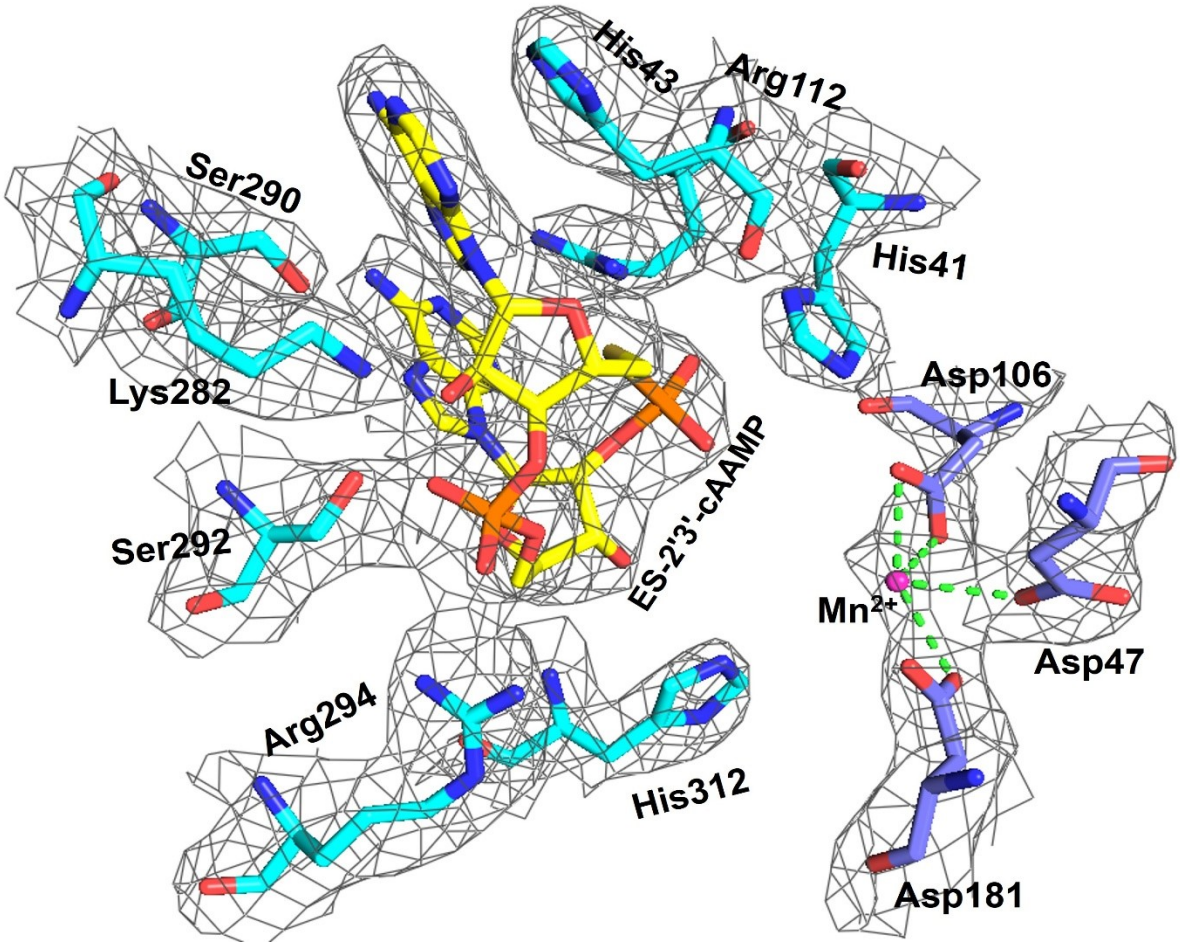

Figure S3. Electron density map of the Mn^{2+} ion and active-site residues. The 2Fo–Fc electron density map (grey mesh, contoured at 1.0 σ) is shown around the Mn^{2+} ion, catalytic Asp residue, and ES-2'3'-cAAMP-interacting residues corresponding to those shown in Fig. 5. The Mn^{2+}-coordinating residues are shown as light blue sticks, ES-2'3'-cAAMP-interacting residues as cyan sticks, and ES-2'3'-cAAMP as yellow sticks. Mn^{2+} coordination is indicated by green dashed lines. The stacking interaction is omitted for clarity.

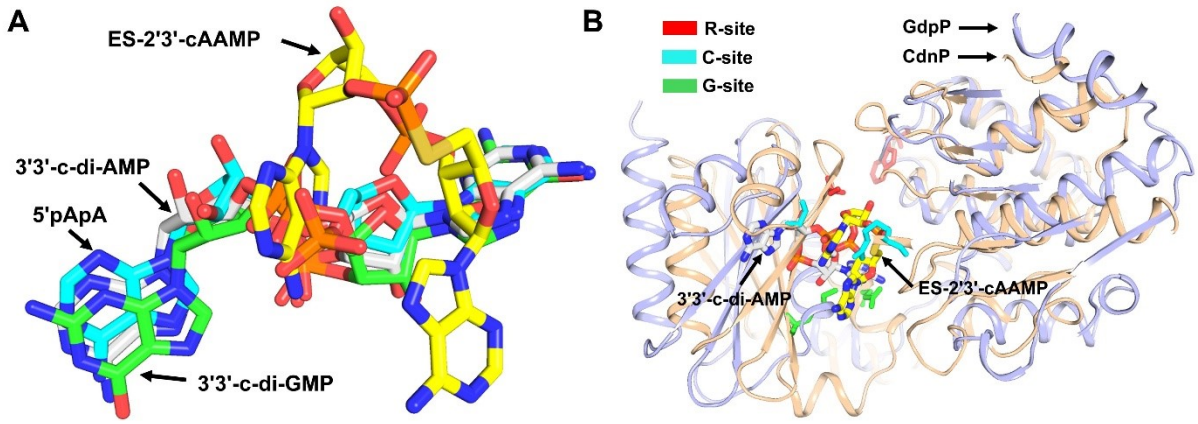

Figure S4. Distinct CDN Binding Conformations and Site Preferences in CdnP and GdpP. (A) The ES-2'3'–cAAMP bound to CdnP was superimposed with CDNs bound to GdpP. The 3'3'-c-di-AMP (PDB ID: 5XSN), 3'3'-c-di-GMP (PDB ID: 5XT3) and 5'pApA (PDB ID: 5XSP) exist in the extended conformations, while the ES-2'3'–cAAMP has a horseshoe-shape conformation. (B) 3′3′-c-di-AMP binds to GdpP at the GC site, whereas in CdnP it occupies the RC site. In contrast, ES-2'3'cAAMP binds to CdnP at the GC site. The R site in GdpP is not sufficiently spacious to accommodate a nucleoside moiety. R, C and G sites correspond to CdnP.