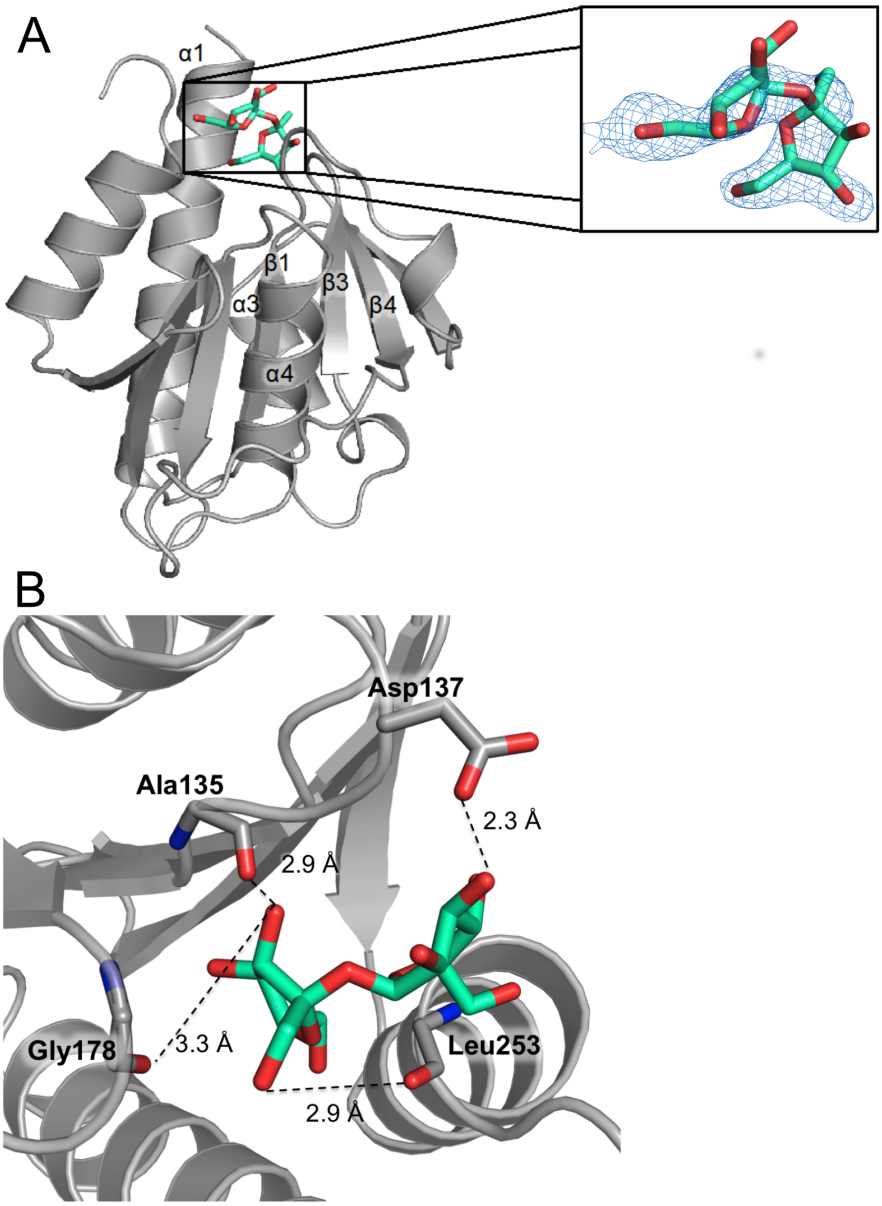
Supporting Information

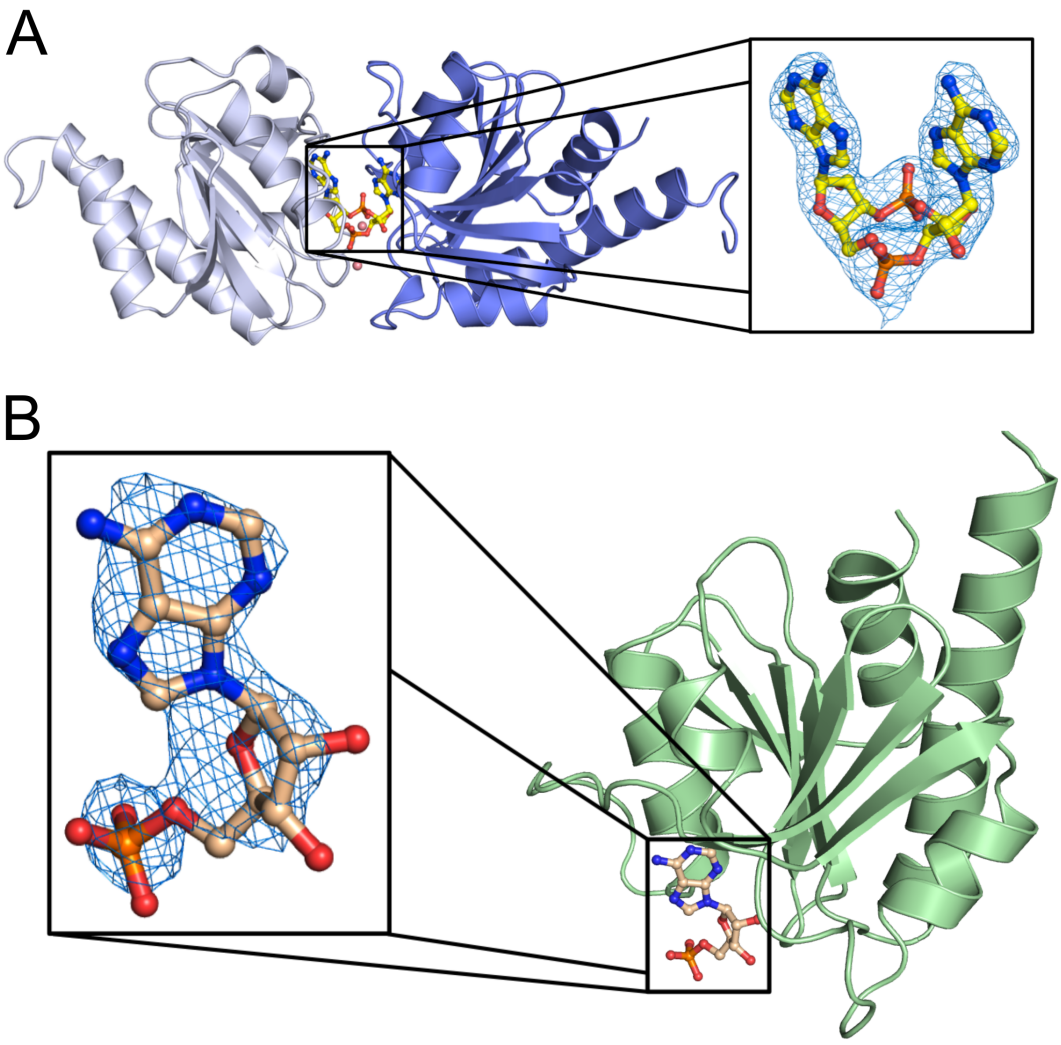
Crystal structures of the c-di-AMP synthesizing enzyme CdaA

Jana L. Heidemann[1], Piotr Neumann[1], Achim Dickmanns[1], and Ralf Ficner[1*]

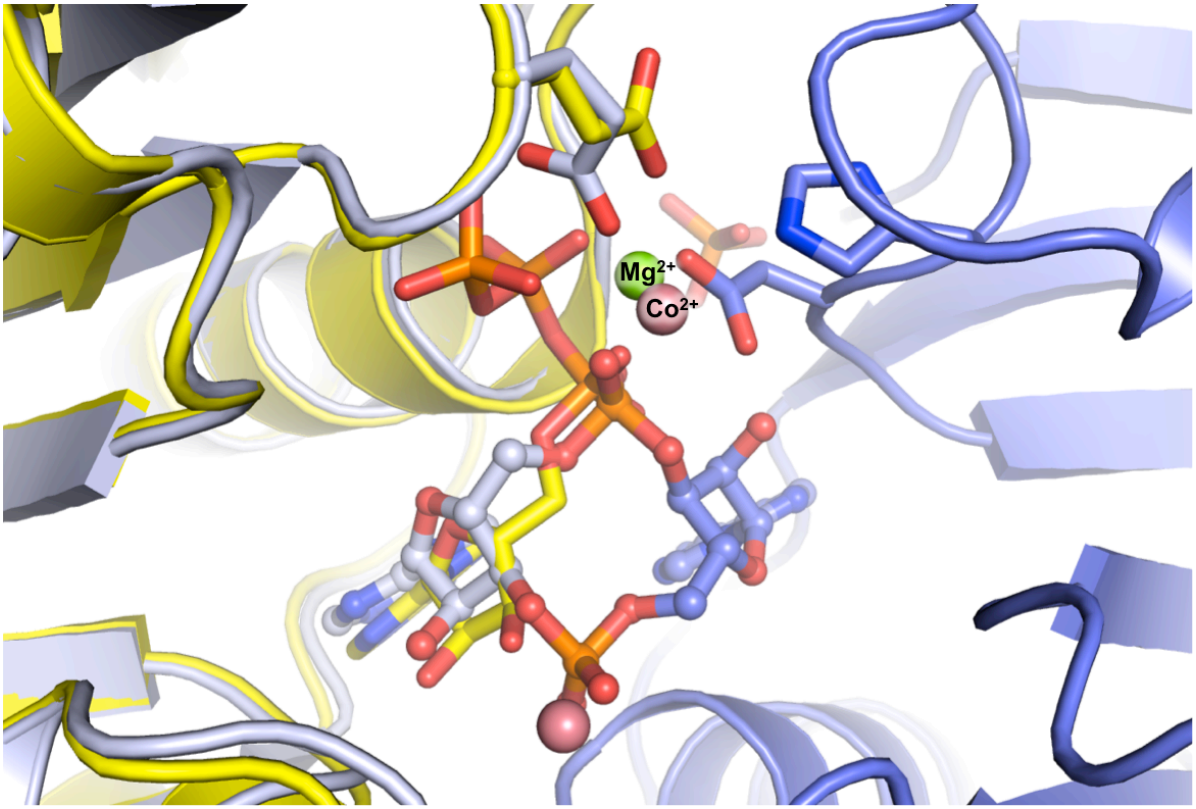
[1]Department of Molecular Structural Biology, Institute for Microbiology and Genetics, Georg-August-University Goettingen, 37077 Goettingen, Germany



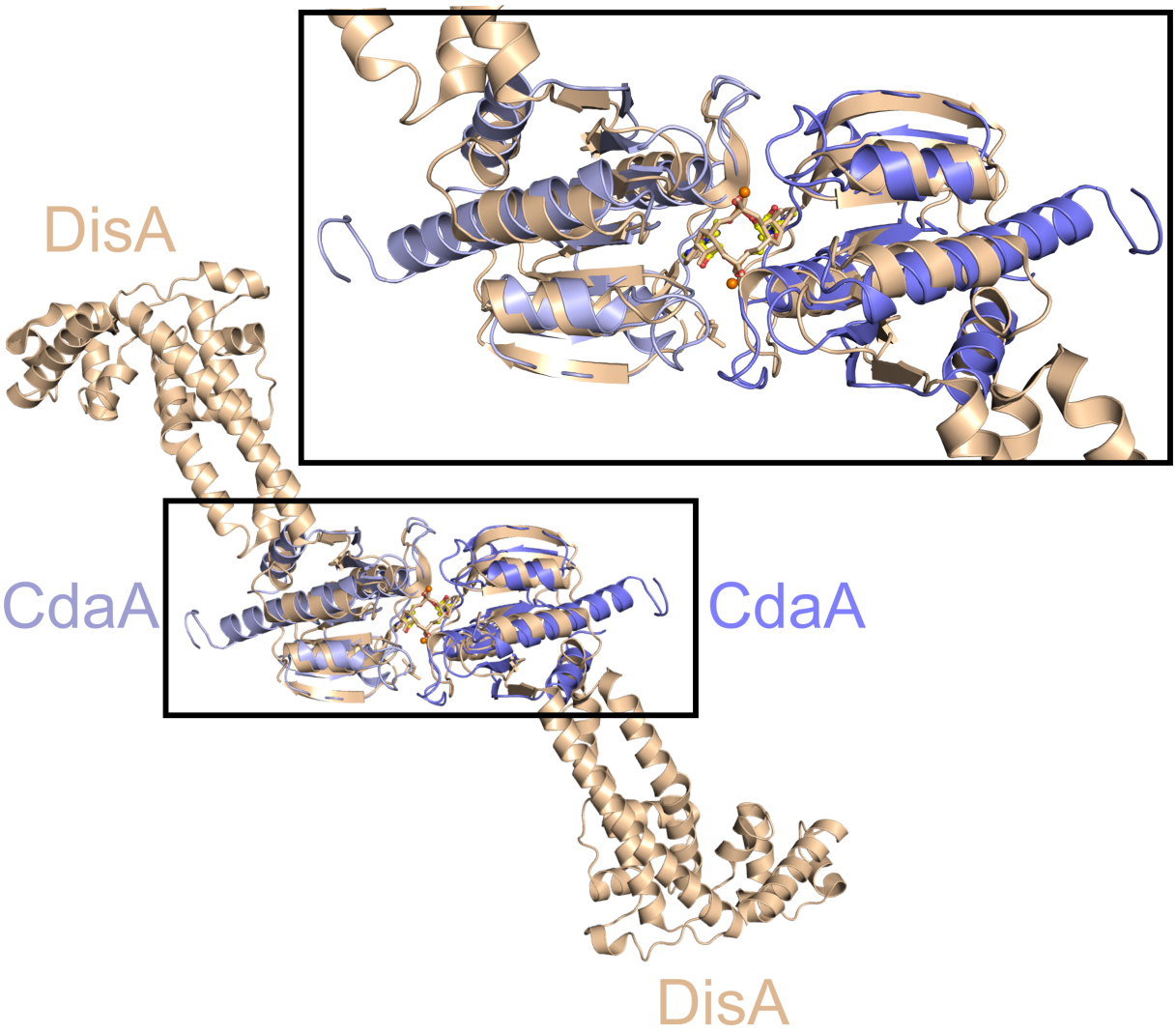
Supporting Figure S1: Crystal structure of apo Δ100CdaA with a bound sucrose molecule. (**A**) Δ100CdaA monomer is depicted in cartoon mode (light grey). The sucrose molecule (represented as sticks with carbons in green cyan and oxygen in red) is bound in a cavity formed by helix α1, loop connecting β1 and α3, loop between β3 and β4, and helix α3. An omit mFo-DFc electron density map (blue mesh) is contoured at a sigma level 3.0. (**B**) Detailed view of the sucrose binding. Amino acids (sticks, carbon in gray, oxygen in red and nitrogen in dark blue) that are involved in sucrose binding are shown in stick mode, and hydrogen bonds are indicated by dashed lines.



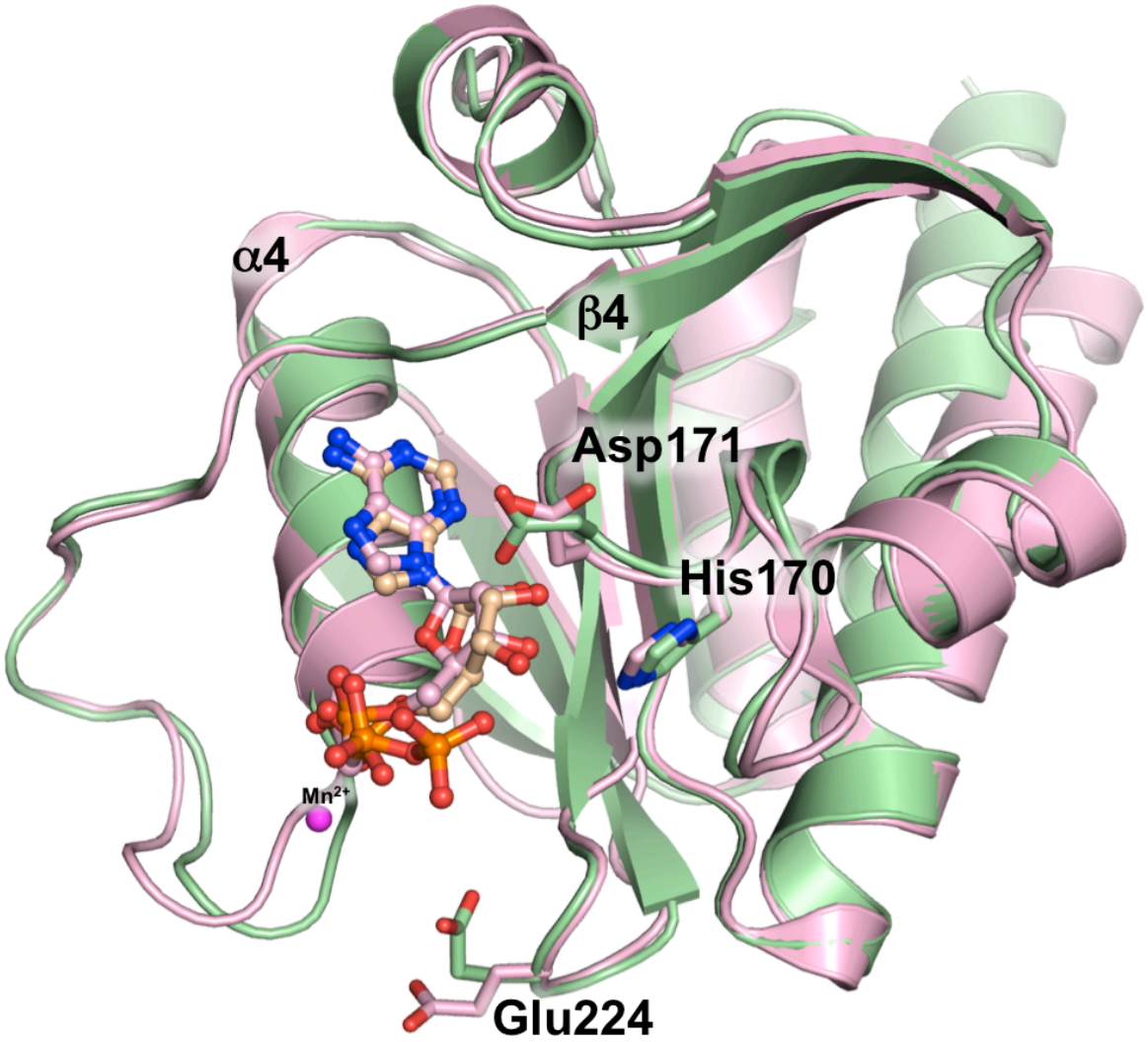
Supporting Figure S2: Crystal structure of Δ100CdaA with a bound c-di-AMP. (**A**) The crystallographic homo-dimer forming the catalytically active form of CdaA with bound c-di-AMP is depicted as ribbon cartoon (light blue and dark blue). The two monomers are related by a crystallographic two-fold symmetry axis. The difference electron density mFo-DFc omit map contoured at 3 σ revealed the presence of a c-di-AMP molecule (sticks; carbon in yellow, phosphate in orange, oxygen in red and nitrogen in blue) and a Co^{2+} ion (red sphere) in the active site. (**B**) The active site of the second CdaA molecule (light green/cartoon mode) present the asymmetric unit, which does not form an active dimer, is loaded with an AMP molecule (Carbon in wheat, rest coloured as in A). The omit mFo-DFc electron density map (blue mesh) is contoured at a 3 σ.



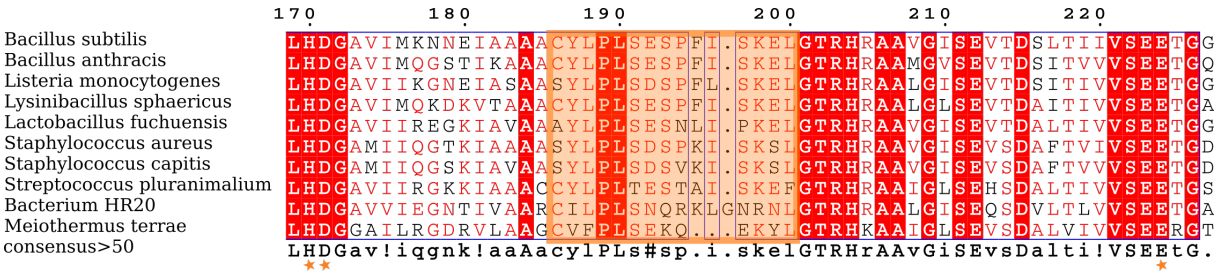
Supporting Figure S3: Superposition of a Δ100CdaA dimer with bound c-di-AMP and a Δ100CdaA monomer with bound ATP (PDB code: 4RV7). The overlay shows a slightly different positioning of the metal ions. Colouring of the Δ100CdaA dimer and the c-di-AMP is according to Figure 3. The Δ100CdaA monomer is depicted in ribbon cartoon mode (yellow). The bound ATP is shown in stick model (carbon in yellow, phosphate in orange, oxygen in red and nitrogen in dark blue) and the Mg^{2+} and Co^{2+} ions as a green and light pink sphere, respectively.



Supporting Figure S4: Superposition of DisA and Δ100CdaA homodimers. The catalytically active Δ100CdaA homo-dimer is shown as on Figure 3 (cartoon in blue and pale blue, c-di-AMP is depicted as colour coded balls and sticks, Co^{2+} ions are depicted as orange spheres). DisA homodimer is coloured wheat.



Supporting Figure S5: Superposition of *S. aureus* $DacA_{CD}$ (6GYX) and *L. monocytogenes* Δ100CdaA (6HVL) monomers. The Δ100CdaA monomer is shown as on Figure 4 (cartoon in pale green, AMP is depicted as colour coded balls and sticks. The $CacA_{CD}$ monomer is coloured pale pink, ApCpp is depicted in colour coded balls and stick mode, Mn^{2+} ion is shown as a magenta sphere. The amino acids His, Glu and Asp, which are involved in metal ion coordination and highly conserved in CdaA, are highlighted as sticks.



Supporting Figure S6: Sequence alignment of CdaAs from different organisms. The highly conserved residues that are involved in metal ion binding are labelled with orange stars at the bottom. The structurally divergent loop region connecting α-helix 4 and β-strand 4, which is in the close vicinity of the phosphate moiety of ATP, is marked with an orange box