Supplementary Material: Structure of a Highly $NADP^+$-Specific Isocitrate Dehydrogenase

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Structure Analysis

GenBank/NCBI database accession codes and organism names for "representative sequences" of (putative) monomeric IDHs that were used in sequence alignments were as follows (if more than one relevant sequence for a particular organism was available, the first in the default order at the NCBI website was typically used): *Acinetobacter calcoaceticus*, ZP_06056020; *Azotobacter vinelandii*, BAA11169; *Campylobacter jejuni*, YP_002343962; *Chlorobium limicola*, BAC00856; *Colwellia maris*, BAA03134; *Colwellia psychrerythraea*, BAE92863; *Corynebacterium diphtheriae*, CAE49148; *Corynebacterium glutamicum*, YP_224955; *Francisella tularensis*, YP_667588; *Mycobacterium bovis*, NP_853736; *Mycobacterium leprae*, CAA17935; *Mycobacterium tuberculosis* H37Rv, NP_214580; *Neisseria meningitidis* alpha14, YP_003083023; *Pseudomonas aeruginosa*, NP_251314; *Pseudomonas fluorescens*, YP_002873363; *Pseudomonas psychrophila*, BAH80317; *Ralstonia eutropha*, YP_841443; *Rhodomicrobium vannielii*, YP_004012323; *Streptomyces lividans* TK24, ZP_05522130; *Vibrio cholerae*, NP_230786; and *Vibrio parahaemolyticus*, NP_797390. Accession codes for three other sequences used for the putative monomeric IDH from *Ralstonia eutropha* were CAJ96713, YP_298398 and AAZ63554.

Comparison of CgIDH and AvIDH Structures

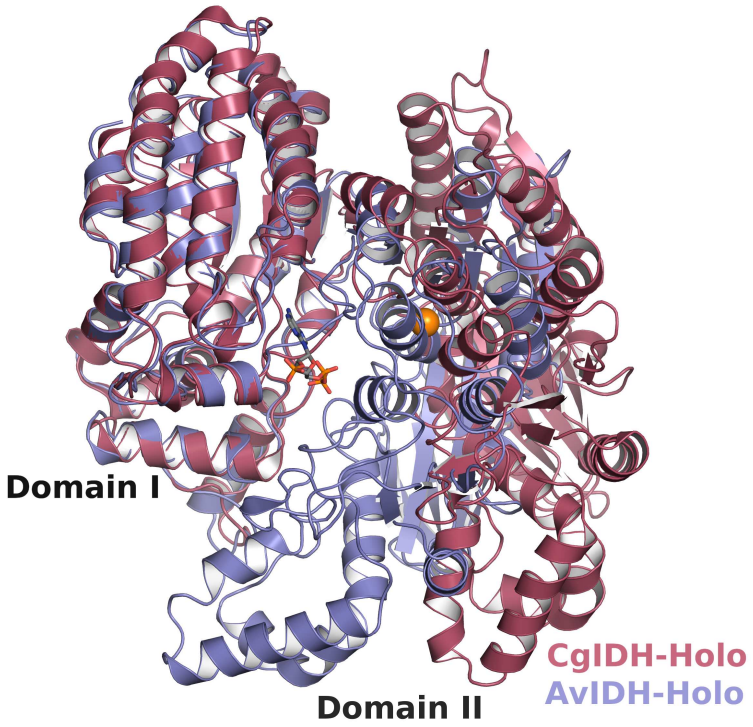

Figure 1: Structural comparison of the CgIDH and AvIDH holoenzyme. Domains I of CgIDH-Holo (maroon) and AvIDH-Holo (blue) are superimposed, with Domains II showing a 36° hinge-bending movement relative to each other. Also shown are Mg^{2+} (orange ball) and $NADP^{+}$ (stick model) bound to CgIDH-Holo. AvIDH-Holo coordinates are from PDB ID 1J1W (Yasutake *et al.*, 2003).

$NADP^+$ Binding

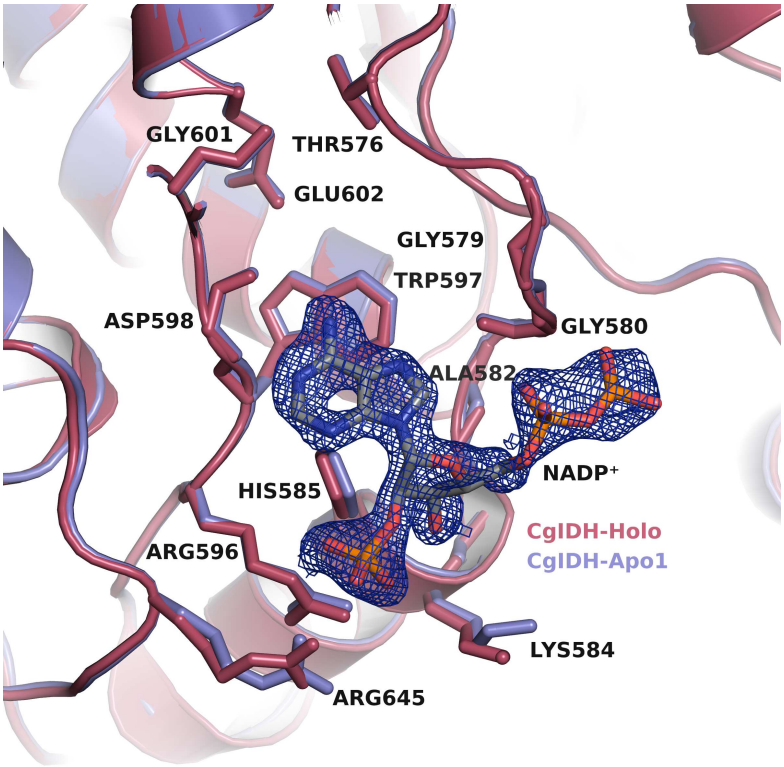

Figure 2: Overlay of CgIDH-Holo and Apo1 forms in the coenzyme binding region. CgIDH-Holo (maroon), -Apo1 (blue), with residues directly or indirectly interacting with $NADP^+$ (stick models). Also shown is the final *2mFo - DFc* map (blue) contoured at 1.0 σ level around $NADP^+$ atoms alongwith the final $NADP^+$ model (stick model). CgIDH-Apo1 coordinates are from PDB ID 2B0T (Imabayashi *et al.*, 2006).

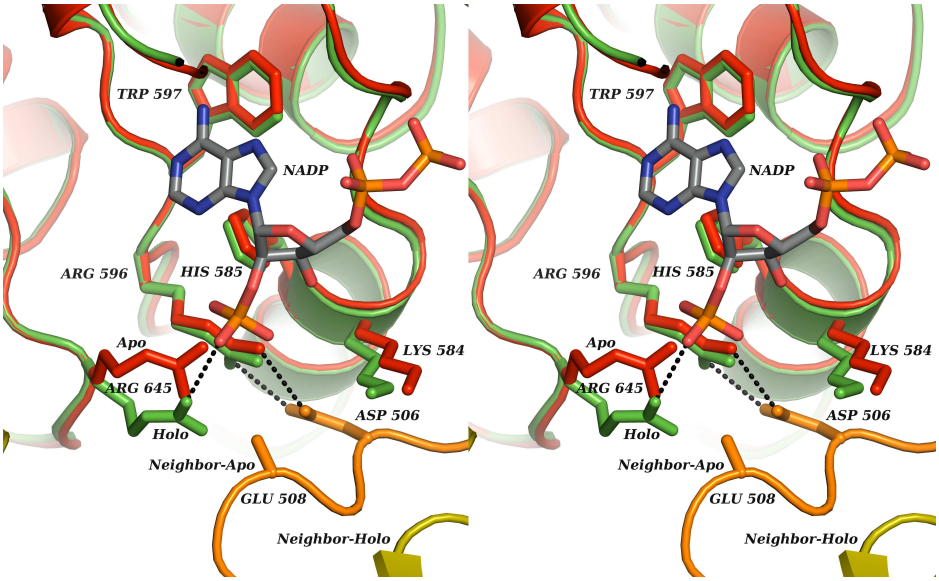

Figure 3: Stereo view of interference with $NADP^+$ binding in the apoenzyme. Domains I of CgIDH-Holo (green) & -Apo2 (red) molecules in the asymmetric unit in the current structure are superimposed; their symmetry-related neighbors (Neighbor-Holo, greenish yellow and Neighbor-Apo, orange, respectively) are also shown. Some of the hydrogen bonds formed (a double one between the side chains of Apo2 Arg596 and Neighbor-Apo Asp506, and one between the 2′-phosphate of $NADP^+$ and the Holo Arg645) are also shown (black dashes). The sidechain of Glu508 of Neighbor-Apo is disordered but well within interaction distance of the Arg645 side chain of the apo form. Neighbor-Holo lies farther from the coenzyme binding site than Neighbor-Apo.