

SUPPLEMENTARY MATERIAL

Using cryo-EM to map small ligands on dynamic metabolic enzymes: Studies with glutamate dehydrogenase

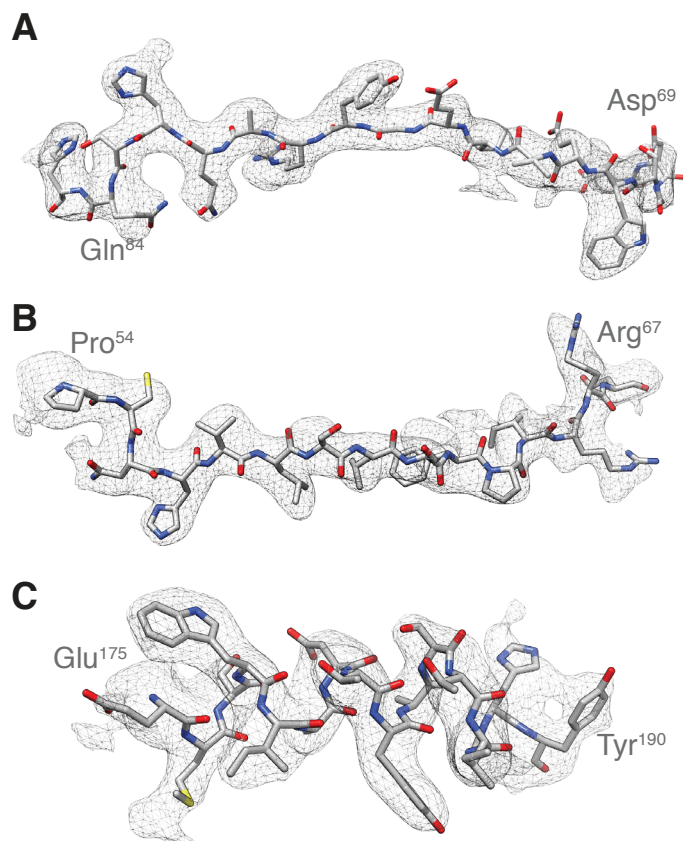
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Supplementary Table 1

Dataset	Micrographs	Total Particles	Particles Open*	Particles Closed*
GDH apo	500	22462	22462	---
GDH + NADH	1587	156801	34716	34926
GDH + GTP	380	66936	14793	20429
GDH + NADH + GTP	1264	39439	---	39439

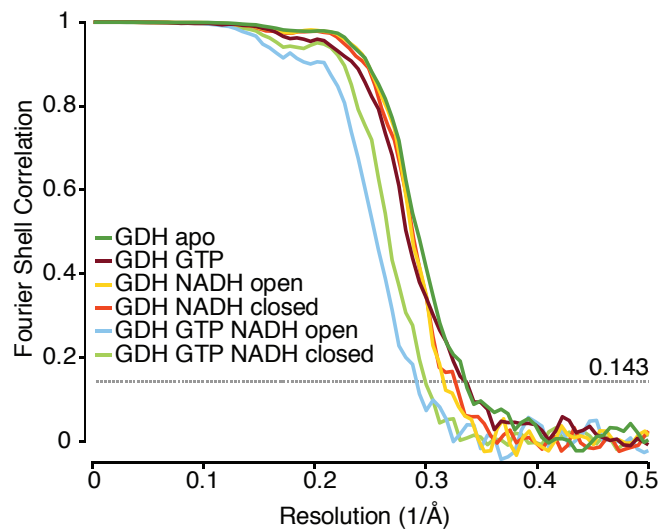
Supplementary Table 1: Statistics reported in this table represent the total number of micrographs and particles selected for the final 3D reconstructions shown in this manuscript. *Particle numbers shown here (as reported by RELION) for each reconstruction are also included in Table 2.

Supplementary Figure 1



Supplementary Figure 1: Selected regions of unliganded GDH in the open conformation, showing density maps with fitted model. (A, B) Density and model for two β -sheet regions, between Pro54 and Arg67 (B) and Asp69 and Gln84 (A). (C) Density and model for an α -helix at the interface between two protomers, below the catalytic site, showing a region that includes residues Gly175-Tyr190.

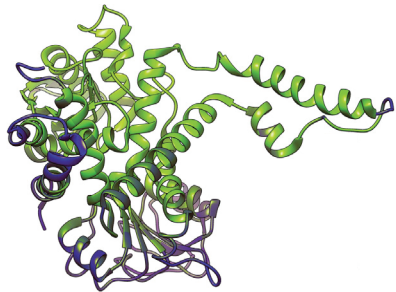
Supplementary Figure 2



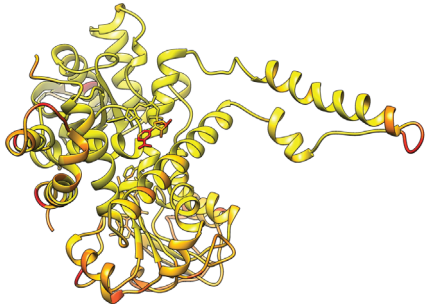
Supplementary Figure 2: Fourier shell correlation (FSC) plots for GDH maps in apo (dark green), GTP-bound (magenta), NADH-bound open (yellow), NADH-bound closed (red), GTP- and NADH-bound open (cyan) and GTP- and NADH-bound closed (light green) states. Resolutions at an FSC value of 0.143 are listed in Table 2.

Supplementary Figure 3

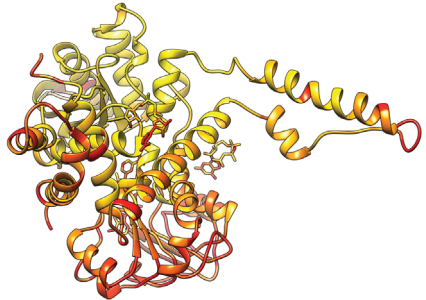
A Open X-ray Structure (PDB 1NR7)



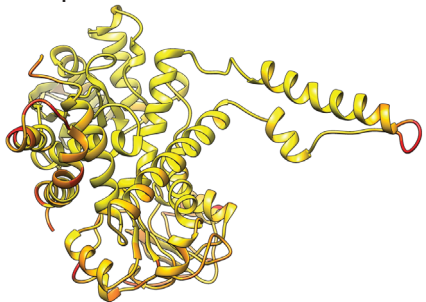
B Open NADH



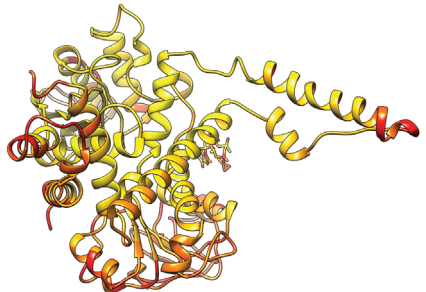
C Open GTP-NADH



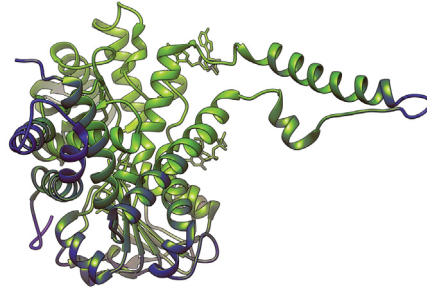
D Open Apo



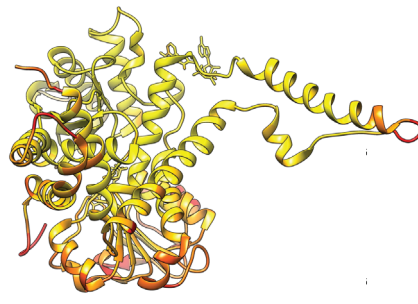
E Open GTP



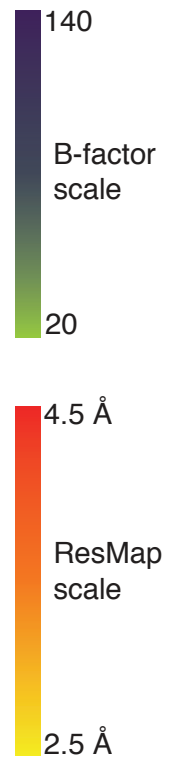
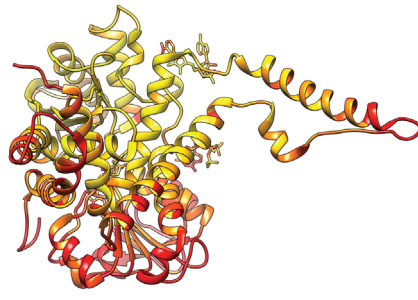
F Closed X-ray Structure (PDB 3MW9)



G Closed NADH

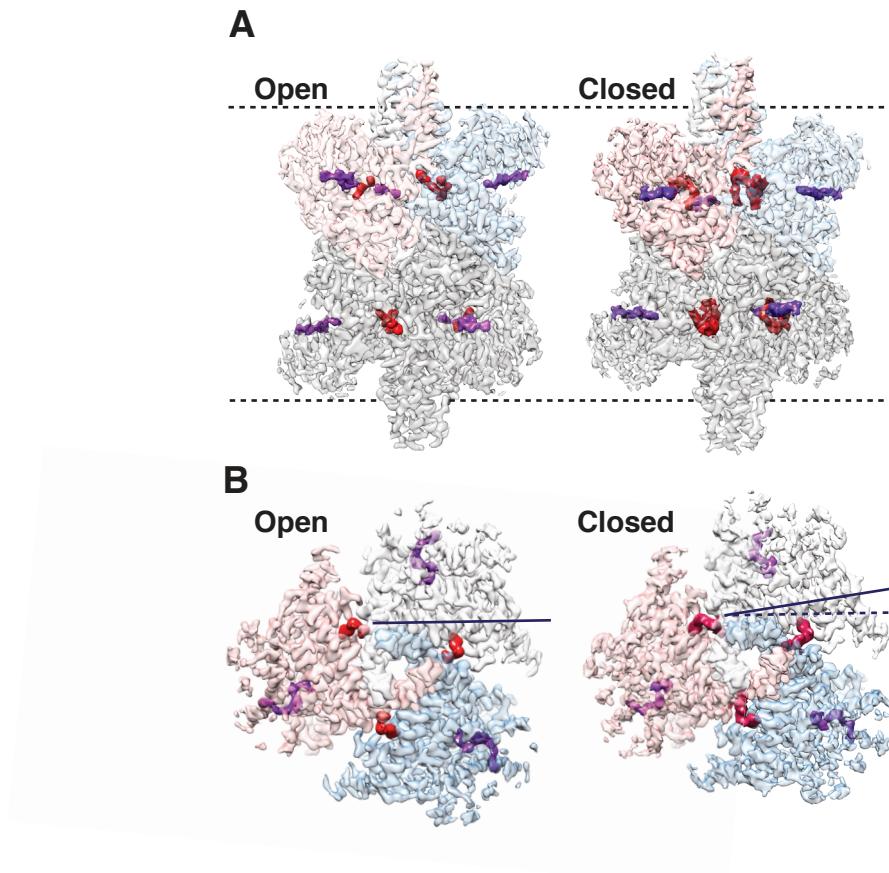


H Closed GTP-NADH



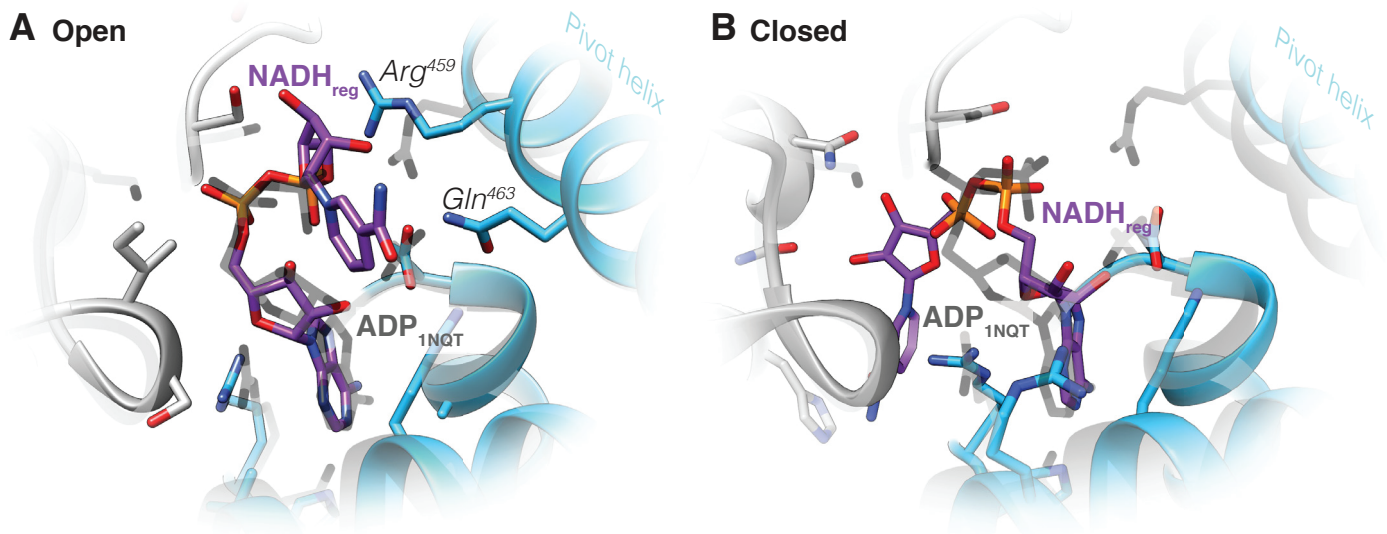
Supplementary Figure 3: Structural resolution variation for cryo-EM models generated in this study is similar to the B-factors reported for open and closed GDH X-ray structures. (A-E) Open GDH structures. (A) B-factor values for an unliganded X-ray structure (PDB 1NR7). (B-E) ResMap values applied to the model of a single protomer for open GDH cryo-EM structures with NADH (B), with GTP and NADH (C), unliganded (D), and with GTP alone (E). (f-h) Closed GDH structures. (F) B-factor values for an X-ray structure with NADH/GTP/Glu (PDB 3MW9). (G-H) ResMap values for closed GDH cryo-EM structures with NADH (G) and NADH and GTP (H). B-factor values are on a scale from 20 (green) to 140 (purple). ResMap values are from 2.5 Å (yellow) to 4.5 Å (red).

Supplementary Figure 4



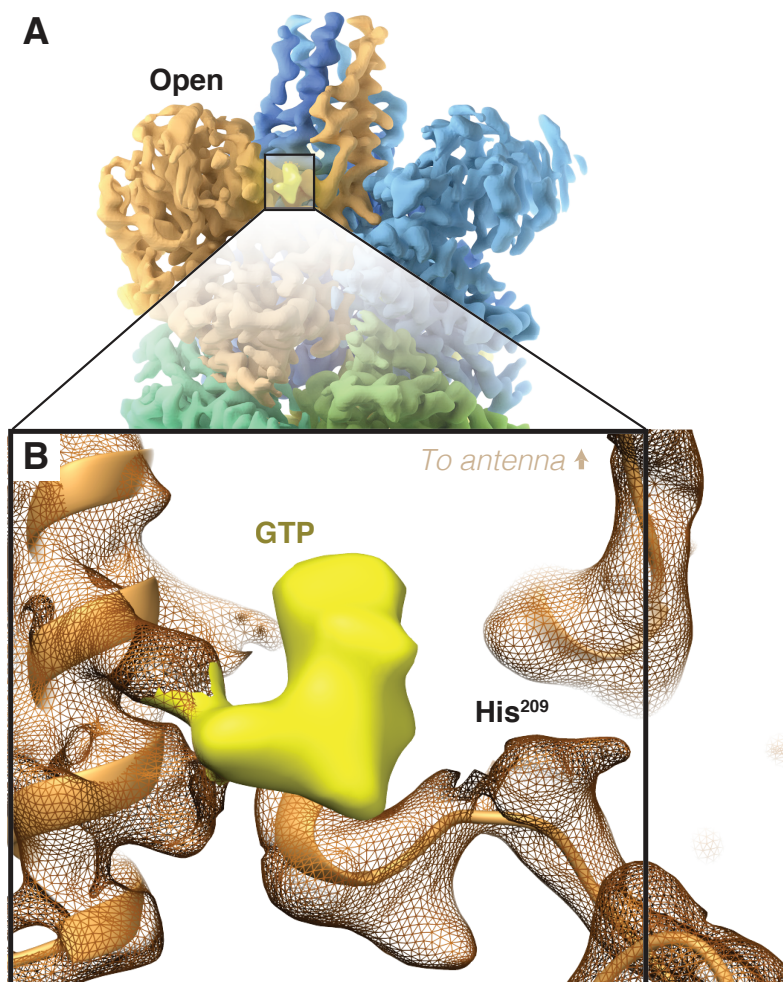
Supplementary Figure 4: Localization of NADH density in open and closed conformations. Densities for 12 NADH molecules are clearly identified in both open and closed conformational states. Side views (A) and top views (B) are shown.

Supplementary Figure 5



Supplementary Figure 5: Comparison of NADH and ADP conformations in open and closed states. (A) In the open state, NADH binds to the catalytic site in a similar orientation to ADP, as seen in the overlay of the regulatory site from the model of GDH bound to ADP (PDB 1NQT, gray) with a model derived from the open state of GDH bound to NADH (purple). The adenine moiety of NADH aligns with ADP in the open state. (B) Overlay of the regulatory site bound to ADP (PDB 1NQT, gray) with model derived from the cryo-EM structure of the closed state of GDH bound to NADH (purple) shows that the nicotinamide moiety of NADH is oriented differently in the closed state than ADP in the open state.

Supplementary Figure 6



Supplementary Figure 6: Cryo-EM structure of the GDH-GTP complex. (A) Density map of GTP-bound GDH, demonstrating evidence for bound GTP (yellow) and the unexpected finding that the protein is still maintained in the open conformation. (B) Model of the GTP-binding site. The orientation of His209 is similar to that observed in the open, but not closed NADH-bound conformations.