SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure 1. Aligned sequences of yeast IDH1 (top) and IDH2 (bottom) with isocitrate dehydrogenase from *Escherichia coli* [ICD, pdb 1PB1, Mesecar, A. D., and Koshland, D. E., Jr. (2000) *Nature* **403**, 614-615], homoisocitrate dehydrogenase from *Thermus thermophilus* (HICD, pdb 1X0L) (37) and isopropyl malate dehydrogenase from *Sulfolobus tokodaii* (IMDH, pdb 1WPW) (Hirose *et al*. unpublished). The alignment of these sequences obtained from a BLAST search was modified based on the structural data and prepared using ESPript [Gouet, P., Robert, X., and Courcelle, E. (2003) Nucleic Acids Res. 31, 3320-3323]. Red and green arrows/helices represent secondary structural elements for IDH1 and IDH2, respectively. Identical residues are boxed and highlighted in blue. Similar residues are boxed and highlighted in yellow. Indicated are the residues that form the catalytic sites (C), regulatory sites (R in red), and polar interactions in the clasp β-barrel (I) of yeast IDH. Residues that participate in AMP binding in the regulatory site are shown as light blue (R) symbols.

Supplementary Figure 2: Crystal packing of the IDH heterooctamer in the citrate-bound C2 and unliganded P1 crystal forms. a) "Columns" of IDH heterooctamers are shown running vertically in the plane of the page. Consecutive heterooctamers in each column are shown in different colors. In the left image, three adjacent columns of citrate-bound IDH in space group C2 are shown with heterooctamers colored pink and green. In the right image, three adjacent columns of unliganded IDH in space group P1 are shown with heterooctamers colored blue and yellow. The columns are in approximately the same orientation such that pink and blue heterooctamers and green and yellow heterooctamers are in equivalent positions in each column. Note that the columns are slightly translated with respect to each other in the two crystal forms causing them to engage in different protein-protein interactions horizontally in the plane of the page. b) "Columns" of IDH heterooctamers looking end-on. The view is

rotated 90^{o} relative to panel a). The ends of five columns are shown for the citrate-bound IDH structure in space group C2 on the left and the ends of six columns are shown for the unliganded IDH structure in space group P1 on the right. The translation of columns relative to each other in the two crystal forms is evident. c) Superposition of three consecutive heterooctamers in a column coming from each crystal form. The color coding is as in panels a) and b).

Supplementary Figure 3: Secondary structural elements in yeast mitochondrial NAD^{+}-specific IDH. a) The yeast IDH1/IDH2 heterodimer. The regulatory IDH1 subunit is shown at the left, with α-helices colored light pink and β-strands colored yellow. The catalytic IDH2 subunit is shown at the right and is colored slate blue. The homologous IDH1 and IDH2 subunits of the heterodimer each contain 12 β-strands and 11 α-helices related by an approximate 180^{o} axis of rotation. b) The β-strands in the IDH1/IDH2 heterodimer. Strands A-H from each subunit form a continuous 10-standed β-sheet. In each subunit, the outermost β-strand is designated "A" and the innermost β-strand is designated "J". β-strands K and L are offset from the 10-stranded β-sheet in each subunit and associate to form a 4-stranded β-sheet centered on the pseudo-two-fold axis of rotation. c) The α-helices in the IDH1/IDH2 heterodimer. Helices *a*-*k* are labeled sequentially in the order they appear in the primary sequence of each subunit.

Supplementary Figure 4: Structural alignment of the unliganded yeast NAD^{+}-specific IDH heterodimer with the homologous *E. coli* $NADP^{+}$-specific IDH homodimer (pdb code 1PB1) (49) in three orthogonal views. a) Superposition of yeast IDH (purple) with *E. coli* IDH (light green). The view is along the yeast IDH pseudo-molecular two-fold axis of rotation, which corresponds to the true two-fold axis of rotation in the *E. coli* homodimer. Structural elements differing in the *E. coli* and yeast IDH structures are highlighted by showing the *E. coli* elements in yellow. The "clasp" β-strands that form one-half of the yeast heterotetrameric interface are in the very center of the image and are shown in red. Residues

78-102 in IDH1 that undergo a conformational change upon isocitrate binding site are also shown in red. The corresponding residues in the *E. coli* structure form a loop that is similar to the conformation of these residues in the liganded yeast IDH structure. b) This view is rotated 90^{o} relative to panel a) around an axis that runs horizontally in plane of the page. Note that the *E. coli* IDH structure contains two α-helices (yellow) that have been lost in yeast IDH to be replaced by β-strands K and L (red) that comprise the yeast heterotetrameric interface. c) This view is rotated 90^{o} relative to panel b) and 180^{o} relative to panel a) around an axis that runs horizontally in the plane of the page. The four-helix bundle at the heart of the heterodimeric yeast IDH and homodimeric *E. coli* interfaces is located at the very center of the image.

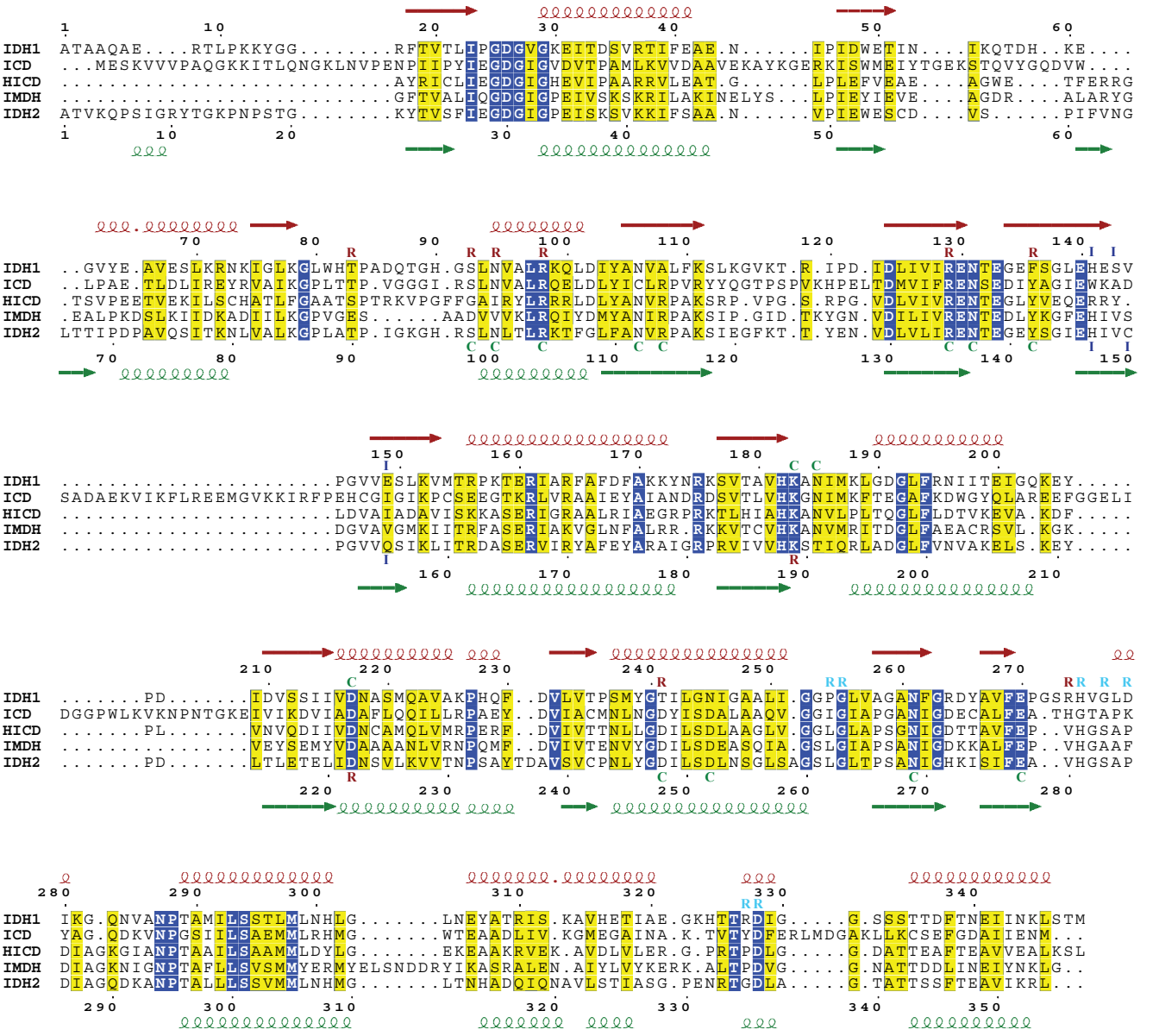
Supplementary Figure 5: Participation of residues of the 4-helix bundle in allosteric communication in the IDH heterodimer. a) The ligand-bound yeast IDH structure. IDH1 is shown in yellow and IDH2 is shown in pink. Upon binding of (iso)citrate and AMP, Thr241 and Asn245 of IDH1 helix *h* make hydrogen bonds to these ligands. Asn223 from helix *g* of IDH2 makes a hydrogen bond to the phosphate moiety of AMP. The binding of citrate and AMP in the regulatory site therefore is likely communicated through the four-helix bundle at the heterodimer interface to the active site. Previous kinetic and mutagenesis studies in which apolar residues in the core of the 4-helix bundle were altered slightly resulted in yeast IDH proteins with altered allosteric properties (35)**.** b) The unliganded yeast IDH structure in the same orientation as panel a). IDH1 is shown in pale green and IDH2 is shown in slate blue. c) Superposition of the liganded and unliganded IDH structures shown in the same orientation as in panels a) and b).

Supplementary Figure 6: The local environment near IDH2 Cys150 in the unliganded yeast IDH structure. a) IDH2 Cys150 residues form a disulfide bond at the heterotetrameric interface. IDH2

residues forming the clasp β-barrel are shown in cyan. IDH1 residues are shown in green. The N-termini of IDH1 helix *e* are shown as ribbons with side chains represented as sticks. The Sγ atoms of IDH2 Cys150 are "sunken" into the clasp β-barrel and are surrounded by apolar side chains. These include IDH2 Val149, Pro151, and Val153 and IDH1 Val153 and Pro157. Each IDH2 Cys150 Sγ atom is positioned on the long axis of helix *e* coming from its cognate IDH1 subunit in the heterodimer. The combination of the positive charge coming from basic residues at the N-terminus of the uncapped helix *e* coupled with the generally apolar environment is predicted to substantially lower the pKa values of these Cys150 residues. b) Surface representation of the image shown in panel a). The colors reflect the type of atom. Nitrogen atoms are blue, oxygen atoms are red, sulfur atoms are yellow and carbon atoms are gray. Note that each Sγ atom of Cys150 is in contact with carbon atoms only.

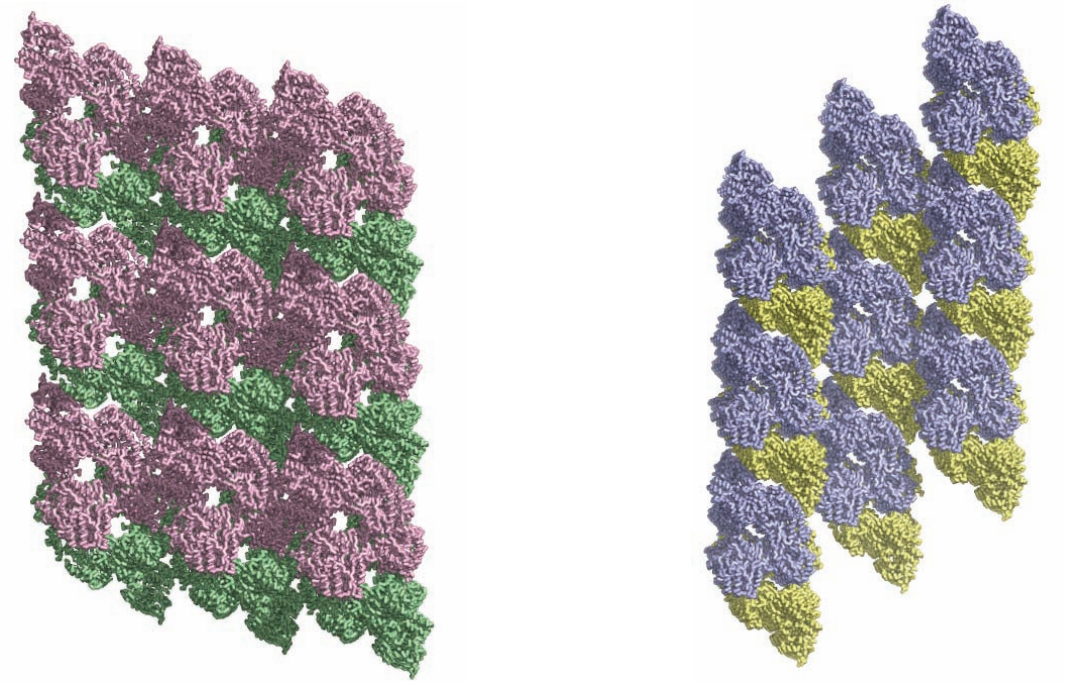
Supplementary Figure 7: The local environment near IDH2 Cys150 in the citrate-bound yeast IDH structure. The view and the color scheme is the same as in **Supplementary Figure 6** except that the N-terminus of IDH1 chain C from the cognate heterotetramer of the heterooctamer is shown as orange sticks. a) The disulfide bond observed between IDH2 Cys150 residues in the unliganded structure is reduced and the positively charged primary amino group of IDH1 chain C resides 3.9 Å from one of the Cys150 Sγ atoms. The proximity of helix *e* from the cognate IDH1 subunit and the IDH1 chain C amino terminus are predicted to stabilize the thiolate form of the Cys150 side chain. b) Surface representation of the image shown in panel a). The colors reflect the type of atom. Nitrogen atoms are blue, oxygen atoms are red, sulfur atoms are yellow and carbon atoms are gray.

Supplementary Figure 1



Supplementary Figure 2

a)



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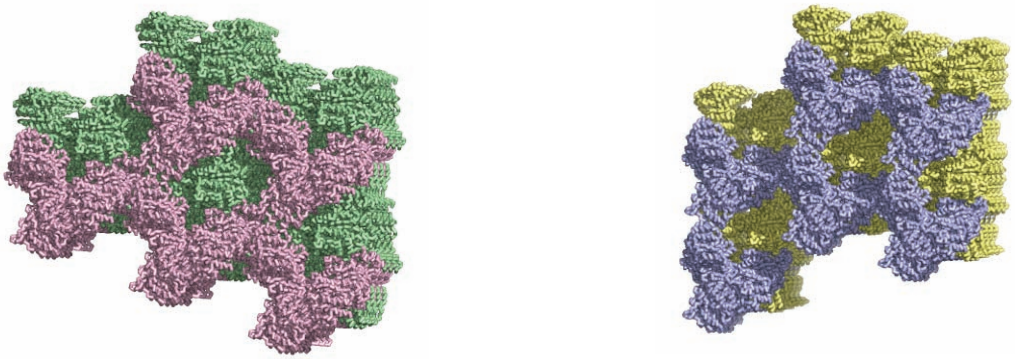

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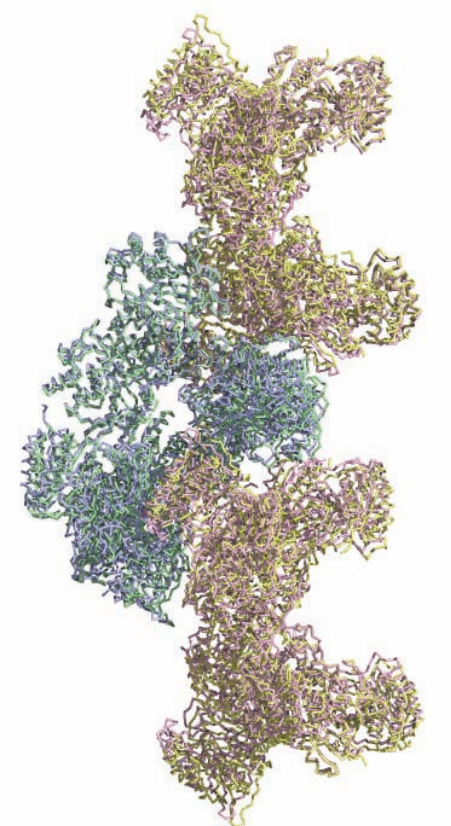
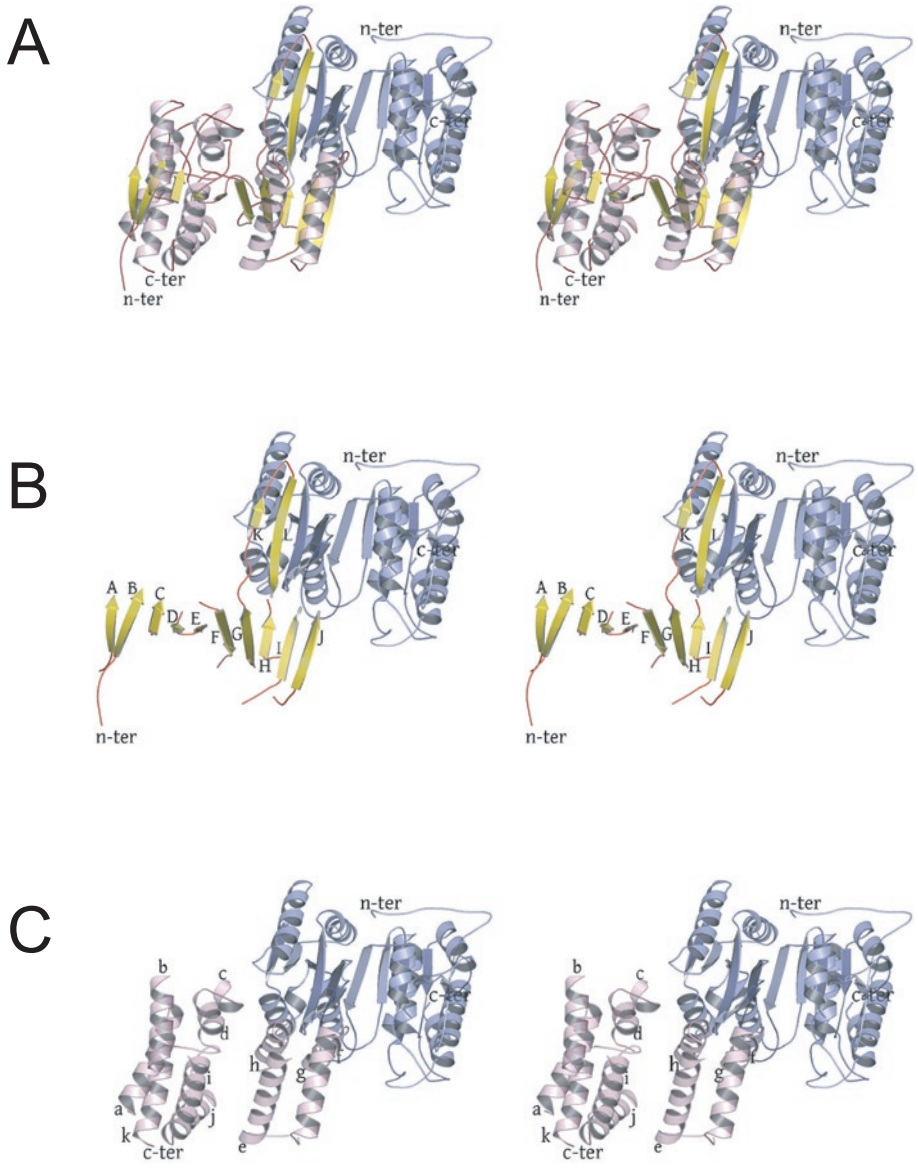
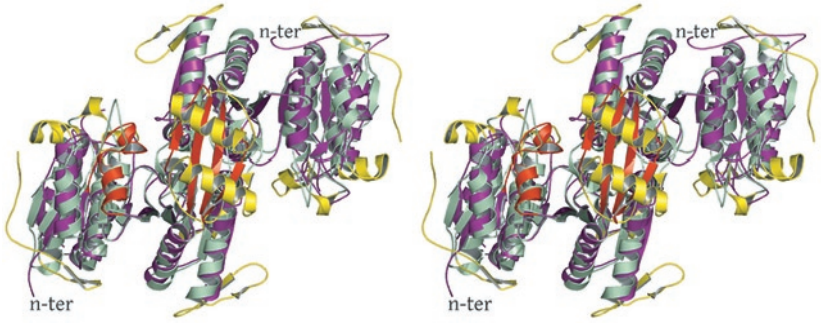


Supplementary Figure 3



Supplementary Figure 4

A



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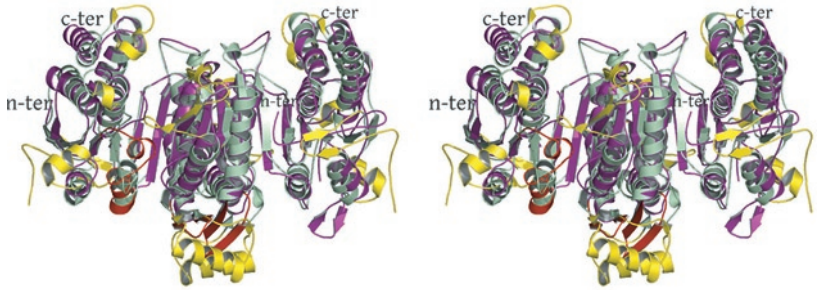

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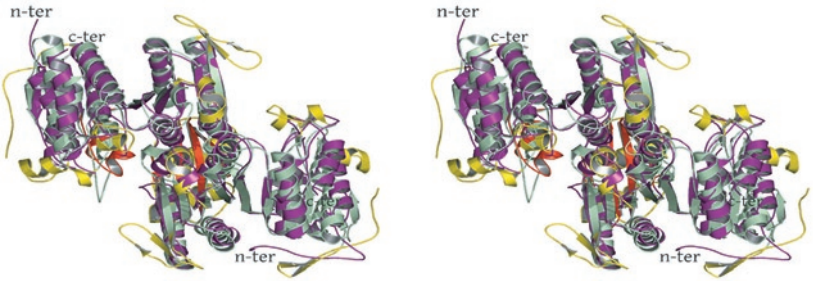
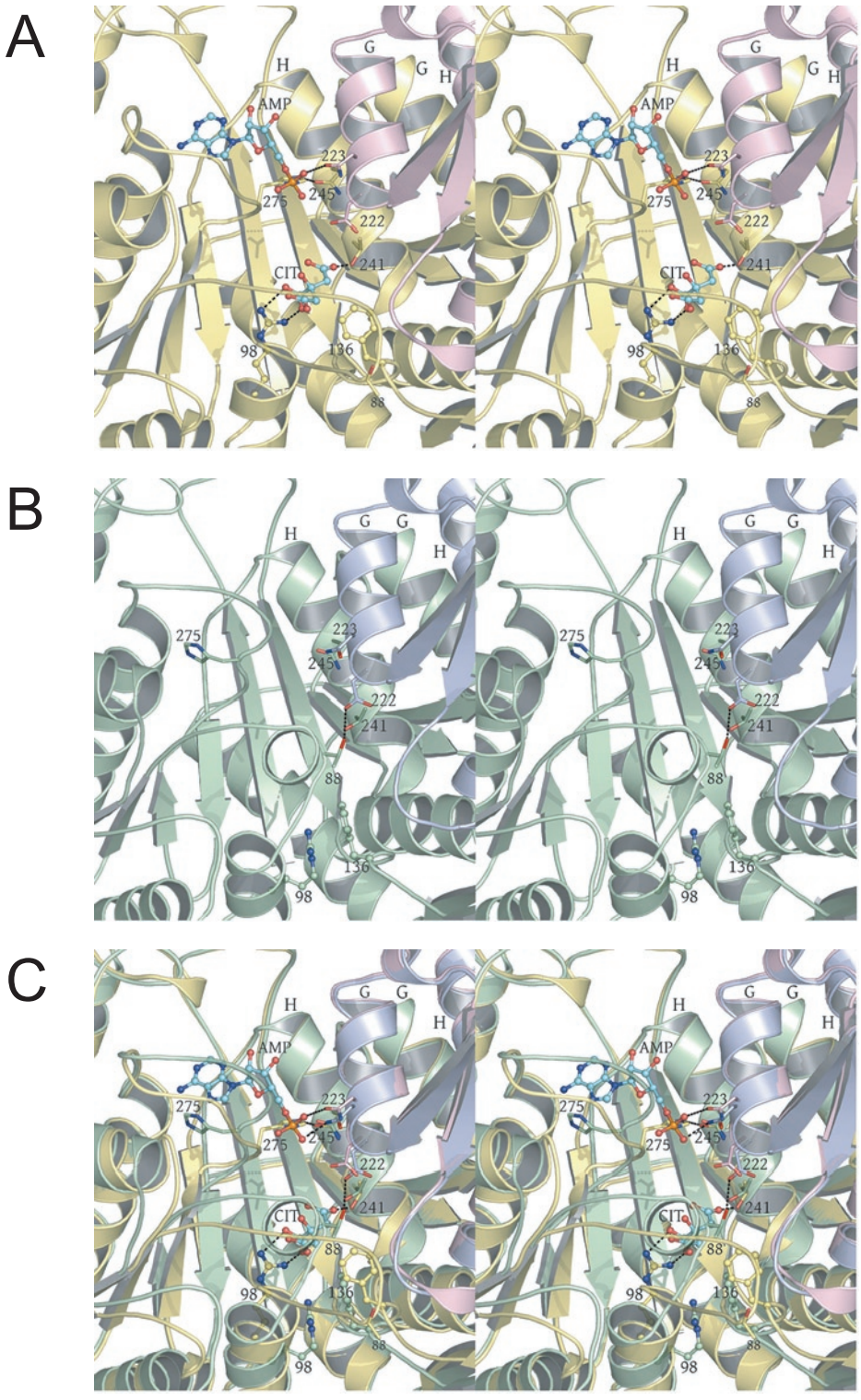
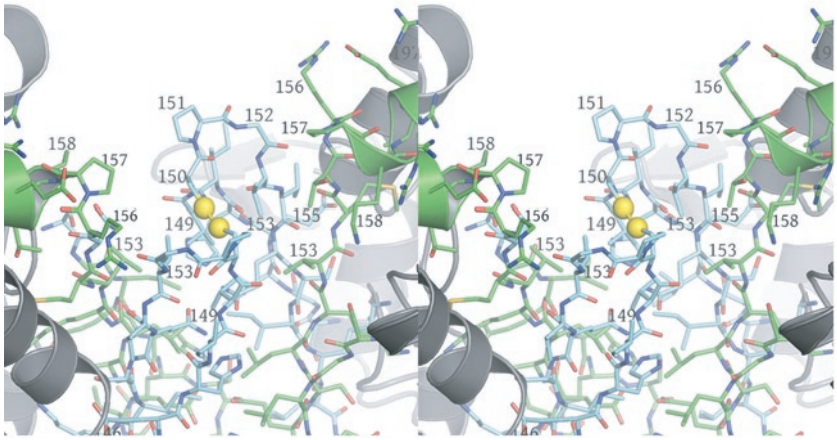


Supplementary Figure 5



Supplementary Figure 6

A



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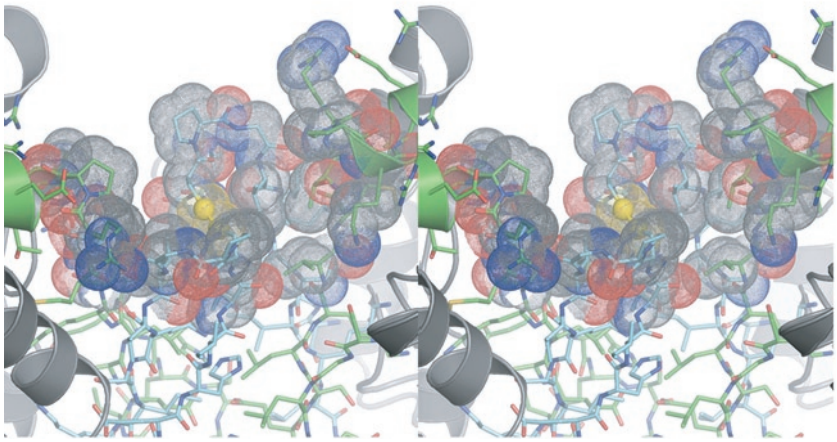
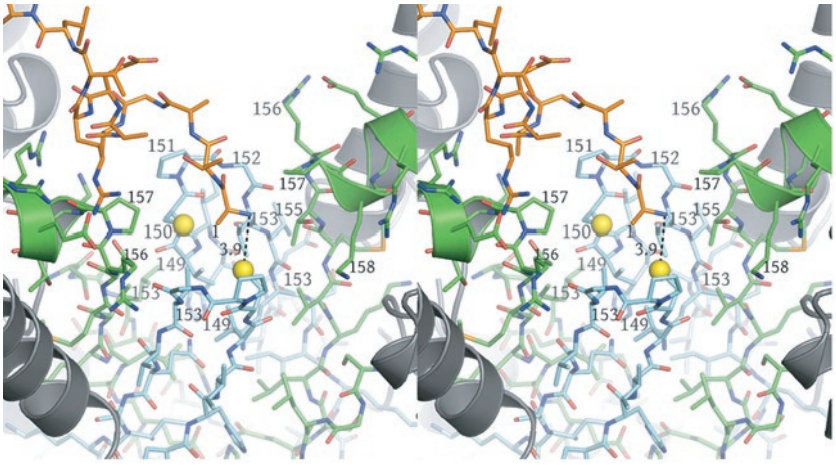


Supplementary Figure 7

A



B