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Induced Fit and the Catalytic Mechanism of Isocitrate Dehydrogenase[†]

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Abstract

NADP⁺ dependent isocitrate dehydrogenase (IDH; EC 1.1.1.42) belongs to a large family of α -hydroxyacid oxidative β -decarboxylases that catalyze similar three-step reactions, with dehydrogenation to an oxaloacid intermediate preceding β -decarboxylation to an enol intermediate followed by tautomerization to the final α -ketone product. A comprehensive view of the induced fit needed for catalysis is revealed on comparing the first “fully closed” crystal structures of a *pseudo*-Michaelis complex of wild-type *Escherichia coli* IDH (*Eco*IDH) and the “fully closed” reaction product complex of the K100M mutant with previously obtained “quasi-closed” and “open” conformations. Conserved catalytic residues, binding the nicotinamide ring of NADP⁺ and the metal-bound substrate, move as rigid bodies during domain closure by a hinge motion that spans the central β -sheet in each monomer. Interactions established between Thr105 and Ser113, which flank the “phosphorylation loop”, and the nicotinamide mononucleotide moiety of NADP⁺ establish productive coenzyme binding. Electrostatic interactions of a Lys100-Leu103-Asn115-Glu336 tetrad play a pivotal role in assembling a catalytically competent active site. As predicted, Lys230* is positioned to deprotonate/reprotonate the α -hydroxyl in both reaction steps and Tyr160 moves into position to protonate C3 following β -decarboxylation. A proton relay from the catalytic triad Tyr160-Asp307-Lys230* connects the α -hydroxyl of isocitrate to the bulk solvent to complete the picture of the catalytic mechanism.

[†]This work is based in part on experiments performed at the ID14-4 and ID23-1 beamlines of the European Synchrotron Radiation Facility (ESRF) in Grenoble, France and at the PXIII beamline of the Swiss Light Source (SLS), Paul Scherrer Institute in Villigen, Switzerland. The research leading to these results received funding from the European Community’s Seventh Framework Programme (FP7/2007-2013) under grant agreement n.º 226716, and Fundação para a Ciéncia e Tecnologia (Portugal) grants PEst-OE/EQB/LA0004/2011 and SFRH/BD/23222/2005 to SG. Funding was also provided by grant GM060611 from the NIH, and the 2009 Oeiras-Professor Doutor António Xavier Scientific Award and an FCT Visiting Professor Scholarship to AMD during his sabbatical in Portugal.

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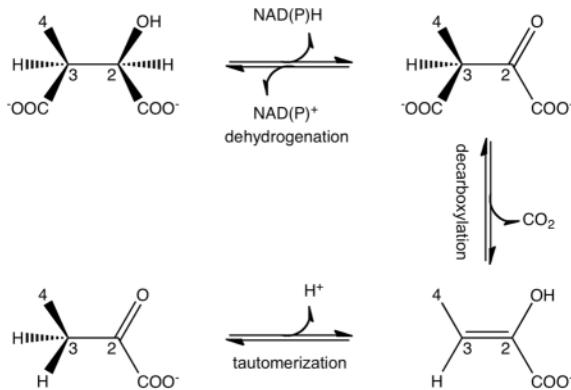
ACCESSION CODES

Coordinates and structure factors were deposited in the Protein Data Bank in Europe (PDBe, <http://www.pdbe.org>). The accession codes for the coordinate entries and the respective structure factors are: 4aj3 and r4aj3sf for *wt* [Ca²⁺:ICT:NADP], 4ajs and r4ajss for K100M [Mg²⁺:ICT:NADP]⁺ × 1, 4ajr and r4ajrsf for K100M [Mg²⁺:ICT:NADP]⁺ × 2, 4ajc and r4ajcsf for K100M [Ca²⁺: α -KG:NADPH], 4ajb and r4ajbsf for K100M [Mg²⁺:ICT:thio-NADP⁺], 4aja and r4ajasf for *wt* [Ca²⁺:ICT:thio-NADP⁺].

SUPPORTING INFORMATION

Composition of the soaking solutions, the duration of each soak and the final ligand concentrations for each dataset (Table S1) and a ribbon diagram of the biological homodimer in the [Ca²⁺:ICT:NADP] crystal structure (Figure S1). This material is available free of charge via the Internet at <http://pubs.acs.org>.

Escherichia coli isocitrate dehydrogenase [IDH; 2R,3S-isocitrate:NADP⁺ oxidoreductase (decarboxylating); EC 1.1.1.42] belongs to a large superfamily of decarboxylating dehydrogenases evolutionarily unrelated to all other enzymes¹. Each family member uses a three-step mechanism to sequentially catalyze a dehydrogenation at the C2 and a decarboxylation at the C3 of the 2R-malate core common to all substrates, with tautomerization of the enol intermediate to produce the ketone product, viz:



The substrates differ at the 4 position: isocitrate (4 = $-\text{CH}_2\text{COO}^-$) is an intermediate in Krebs' cycle, isopropylmalate (4 = $-\text{CH}(\text{CH}_3)_2$) is an intermediate in leucine biosynthesis, homoisocitrate (4 = $-\text{CH}_2\text{CH}_2\text{COO}^-$) is an intermediate in the α -amino adipate pathway for lysine biosynthesis in fungi² and tartrate (4 = $-\text{OH}$) is catabolized by *Pseudomonas putida* and *Agrobacterium vitis*^{3,4}. Superpositioned active sites reveal that functional residues interacting with the 2R-malate core are invariant, even among highly divergent family members⁵.

Classic work on IDH catalysis^{6–12} established that hydride transfer from C2 to the *re* face of the nicotinamide C4 precedes facile decarboxylation at C3 of the oxaloacid intermediate. Recent work determined that decarboxylation produces an enol intermediate that tautomerizes to the final ketone product⁵. All known IDHs require a divalent metal cation, such as Mg²⁺, for catalysis. Structural studies of IDH confirm the expected bidentate chelation of Mg²⁺ by the C1 carboxylate and C2 hydroxyl of the substrate needed to stabilize the negative charges formed on the hydroxyl oxygen during the transition states of both steps^{6,13}. IDH ternary complex structures also confirm that hydride transfer is to the *re* face of the nicotinamide C4^{14–16}.

Less certain are the identities of the catalytic base, needed to initiate dehydrogenation by abstracting the proton from the isocitrate C2 hydroxyl, and of the catalytic acid, needed to protonate C3 following decarboxylation. The original suggestion^{13,17} that Asp283* acts as the base, always dubious given that chelation to Mg²⁺ should reduce its pK_a far below that required for function under physiological conditions, failed to receive support upon detailed investigation¹⁸. A structurally conserved water has also been proposed as the catalytic base^{19,20}, with a proton relay system exiting the active site to allow exchange with bulk solvent. However, no mechanism to reduce the water's pK_a to within a plausibly physiological range was proposed. Replacing the active site Tyr160 by phenylalanine compromised overall activity with isocitrate; decarboxylation of oxalosuccinate was unaffected while its reduction to isocitrate was severely lowered²¹. These results suggested a role for Tyr160 in dehydrogenation, although in all binary complexes of IDHs^{19,22–25} and IMDH²⁶, and in the ternary complexes of IDH^{20,23,27–32} and TDH³³, the tyrosine side chain is in no position to abstract the proton from the isocitrate C2 hydroxyl. Based on its geometry and spatial proximity to isocitrate in a binary complex, Lys230* was proposed to

be the proton donor following decarboxylation¹³. Support for this conjecture was obtained upon replacing Lys230* by methionine which greatly compromised overall activity; decarboxylation of oxalosuccinate was obliterated while its reduction to isocitrate was barely affected²¹. By contrast, detailed kinetic studies of mutations at the equivalent sites in pig heart IDH (Tyr140 and Lys212*) suggest Tyr160 is the acid that protonates the substrate after decarboxylation, with Lys230* playing a supporting role³⁴. Recent work on homoisocitrate dehydrogenase⁵ has proposed that Lys199* and Tyr126 (equivalent to Lys230* and Tyr160 in *EcoIDH*) work as a pair, with Lys199* abstracting the proton directly from the C2 hydroxyl during dehydrogenation to the oxaloacid, then reprotonating the hydroxyl during C3 decarboxylation to the enol, and then once again abstracting the proton as Tyr126 protonates C3 to the ketone product.

The confusion surrounding the identities and exact roles of the active site residues is partly attributable to the absence of crystal structures detailing the active site during catalysis. A particular difficulty with *EcoIDH* (and by inference, other family members too) is that it undergoes substantial conformational changes upon substrate binding, not to the productive Michaelis complex, but rather to an intermediate nonproductive position. Structural data, though informative, may be misleading. Kinetic data from site directed mutants may also mislead when other active site residues partially compensate for loss of a functional group. Here, we determine the structures of several new ternary complexes of wildtype *EcoIDH* and a catalytically compromised mutant K100M³⁵ to better resolve the conformational changes needed to produce a productive Michaelis complex and reconcile conflicting hypotheses regarding the catalytic mechanism.

MATERIALS AND METHODS

Protein production and purification

E. coli strain STΔ*icd*³⁵ was transformed with plasmid (pIDH[wildtype] or pIDH[K100M] containing *E. coli* *icd* wildtype and Lys100Met mutant genes respectively) and grown at 37° to full density in 5 liters of broth (12 g tryptone, 24 g yeast extract, 4 g glycerol, 17 mM KH₂PO₄, 72 mM K₂HPO₄ and 5 mg/L tetracycline) in a 6 L NBS BioFlow 3000 fermentor (University of Minnesota Biotechnology Resource Center). Cells were harvested by centrifugation and the paste flash frozen between blocks of dry ice and stored at -80° until needed.

Fifty g of cell paste were resuspended in 150 mL of extract buffer (10 mM KH₂PO₄, 0.5 M NaCl, 2 mM MgCl₂, and 2-mercaptoethanol, pH 7.7) and stirred for 1 hr at room temperature to allow IDH to become fully dephosphorylated. The suspension was then chilled to 4°C, sonicated and the cell debris removed by centrifugation. Enzymes were purified by a standard protocol³⁶ involving ammonium sulfate precipitation, DEAE (Pharmacia) anion chromatography and affinity chromatography using Affi-Gel Blue (BioRad). Protein concentrations were determined by the method of Bradford³⁷ using BSA as a standard. All preparations were 98% free of contaminating enzymes as judged by Coomassie Blue staining following SDS PAGE.

Crystallization of WTIDH and IDH (K100M)

Concentrated protein solutions of wIDH (35 mg/mL) and IDH (K100M) (45 mg/mL) were diluted to 20 mg/mL in a storage buffer solution containing 0.9 mM citric acid, 3.5 mM Na₂HPO₄ pH 6.0, 100 mM NaCl, 0.02% NaN₃ and 2 mM DTT. Crystal growth optimization was carried out using the hanging drop vapor diffusion method by screening 24 different crystallization solutions with varying pH and (NH₄)₂SO₄ concentrations close to the conditions established by Hurley and co-workers³⁸. The best crystallizations were obtained

in a solution of 1.85M $(\text{NH}_4)_2\text{SO}_4$, 50 mM citric acid/ Na_2HPO_4 , 0.1 M NaCl and 0.2 M DTT at pH 5.8 for *wt*IDH and at pH 5.2 for IDH (K100M). Protein crystals for ligand soaking and diffraction data collection were also obtained by the hanging drop vapor diffusion method. 2 μL drops were set up at 293 K in a XRL 24-well crystallization plate (Molecular Dimensions), by mixing native and mutant protein solutions with their respective crystallization solutions in a 1:1 ratio of protein to reservoir solution, and equilibrated against 500 μL of crystallization solution in the reservoir. Single crystals with tetragonal bipyramidal shape developed within 5 days, reaching dimensions of 500 $\mu\text{m} \times 200 \mu\text{m} \times 100 \mu\text{m}$ for *wt*IDH and of 300 $\mu\text{m} \times 150 \mu\text{m} \times 75 \mu\text{m}$ for IDH (K100M).

Crystal soaking procedure

Crystals of *wt*IDH and IDH (K100M) were transferred from their mother liquor to the final soaking solution in either one or two steps. In the two-step procedure the crystals were first soaked in Solution 1 (1.58 M $(\text{NH}_4)_2\text{SO}_4$, 156 mM NaHEPES pH 6.0, 52 mM Ca^{2+} or Mg^{2+} , 300 mM 2R-isocitrate) and then transferred to Solution 2 (1.58M $(\text{NH}_4)_2\text{SO}_4$, 156 mM NaHEPES/MES pH 7.5~8.0, 52 mM Ca^{2+} or Mg^{2+} , 300 mM 2R-isocitrate or α -ketoglutarate, 500–400 mM NADP $^+$ or NADPH or thio-NADP $^+$), whereas the one-step procedure involved only Solution 2. All the soaks were done at 293 K. Crystals were then cryo-protected by transferring them directly into an artificial mother liquor with the same composition as that of soaking solution 2 supplemented with 25% glycerol, and flash-cooled in liquid nitrogen prior to data collection. For each dataset, the composition of the soaking solutions and the final ligand concentrations, as well as the duration of each soak, are described in Table S1.

The following stock solutions were used to prepare the soaking solutions: 1 M NaHEPES pH 7.5 (Sigma-Aldrich), 1 M MES-NaOH (Sigma-Aldrich) pH 6.3, 2 M MgCl_2 (Sigma-Aldrich); 2 M CaCl_2 (Sigma-Aldrich); 2 M ICT (Fluka) in 11% NH_4OH (Merck) pH 6.0; 0.5 M α -KG (Sigma-Aldrich) in 0.1 M MES-NaOH (Sigma-Aldrich) pH 6.3; and 0.24 M β -NADPH (Sigma-Aldrich) in 166 mM α -KG (Sigma-Aldrich), 166 mM MES-NaOH (Sigma-Aldrich) pH 6.3 and 1.54 M $(\text{NH}_4)_2\text{SO}_4$ (Merck). $\text{Na}_2\beta\text{-NADP}^+$ (Fluka) and $\text{Na}\beta\text{-thio-NADP}^+$ (Sigma-Aldrich) were added directly to soaking solution 1 (see Table S1).

Data collection and processing

Datasets of the different IDH crystal soaks were collected at 100 K using different X-ray sources and detector systems. The *wt* [Ca^{2+} :ICT:NADP $^+$] and K100M [Ca^{2+} : α -KG:NADPH] datasets were measured *in-house*, using a Bruker-AXS Proteum Pt135 CCD detector system coupled to a Bruker-AXS Microstar-I rotating anode X-ray generator with Montel mirrors. The data were integrated with SAINT and scaled with SADABS as part of the Bruker-AXS Proteum Software Suite. Diffraction data statistics were obtained with XPREP (Bruker-AXS) and are listed in Table 1. The K100M [Mg^{2+} :ICT:NADP $^+$] $\times 1$ and K100M [Mg^{2+} :ICT:NADP $^+$] $\times 2$ datasets were collected at the ESRF (Grenoble, France) ID14-4 and ID23-1 beamlines respectively, using an ADSC Quantum Q315r detector. The K100M [Mg^{2+} :ICT:thio-NADP $^+$] and the *wt* [Ca^{2+} :ICT:thio-NADP $^+$] datasets were recorded at the SLS (Villigen, Switzerland) PXIII beamline, using a MAR225 detector. For the datasets collected at synchrotron X-ray sources, the diffraction images were processed with XDS³⁹, and the data collection statistics are included in Table 1. All diffraction data were further processed with the CCP4 Program Suite⁴⁰. Two preliminary datasets, replicates of *wt* [Ca^{2+} :ICT:NADP $^+$] and K100M [Mg^{2+} :ICT:NADP $^+$] $\times 1$ datasets, were initially collected *in-house* at 293K and the respective crystal structures were solved by Molecular Replacement with PHASER⁴¹, using the coordinates of the PDB entry 1ai2²⁷ as the search model. The solutions were partially refined (our unpublished results) and subsequently used as search models to solve the crystal structures herein described by the

Molecular Replacement with PHASER. Although Matthews coefficient calculations⁴² suggested the presence of 2 molecules in the asymmetric unit of all crystal structures, with V_m values of about $2.2 \text{ \AA}^3 \text{Da}^{-1}$ and a predicted solvent content of about 45%, the results of the Molecular Replacement calculations showed that, as in all *EcoIDH* crystal structures reported to date (see “Results”), only one *wIDH* or IDH (K100M) monomer was present in the asymmetric unit of each crystal structure, corresponding to V_m values and estimated solvent contents of *ca.* $4.5 \text{ \AA}^3 \text{Da}^{-1}$ and 72.4 % respectively.

Crystallographic refinement

The structures were refined using the amplitude-based Maximum-Likelihood target function with automatic weight optimization procedure as implemented through the graphics user interface of the PHENIX v.1.6.4 software package⁴³. The initial refinement step consisted of five macrocycles, the first of which comprised a rigid body refinement followed by individual coordinate and isotropic atomic displacement parameter (ADP) refinement. In the second and fourth macrocycles, a simulated annealing refinement (cartesian and torsion angles) was carried out using the default parameters, followed by individual coordinate and ADP refinement of individual isotropic B-factors. Monomer library descriptions for new ligands were created with LIBCHECK⁴⁴ through the smiles translator interface in Coot⁴⁵. Ligand restraints were further reviewed using JLigand⁴⁶. During refinement, the models were periodically inspected and corrected with Coot against sigmaA-weighted $2|F_o|-|F_c|$ and $|F_o|-|F_c|$ electron density maps. Inclusion of ordered solvent molecules was done with ARP/wARP⁴⁷, followed by inspection in Coot. In the final refinement, automatic atom occupancy refinement was included in the protocol. The stereochemical quality of each model was assessed with MolProbity⁴⁸. A summary of the refinement statistics, model composition and stereochemical quality is presented in Table 2. Coordinates and structure factors were deposited in the Protein Data Bank in Europe⁴⁹. The coordinate accession codes are also included in Table 2.

Structure analysis

Structures were superimposed at their corresponding domains I and II (see “Structural analysis” in the “Results” section for definitions of domains I and II), and r.m.s. deviations between superimposed atoms were calculated using the CCP4 suite program LSQKAB⁵⁰. Domain rotations and hinge points for *EcoIDH* crystal structures were determined using the program DynDom⁵¹ as implemented through the CCP4i graphics user interface⁵² with default parameters. Using the “open” apo-form of EcoIDH (PDB 1sj)⁵³ as a reference, the small domains were first aligned and then the positions of the large domains were compared. DynDom calculations failed for small rigid body rotations and so LSQKAB calculations⁵⁰ in CCP4i were used to first superimpose the small domains and then use the resulting transformed coordinate file to superimpose the large domains. LSQKAB calculations were also used to compare crystal structures from different organisms. The analysis of the residues involved in the binding of NADP⁺/NADPH and ICT/α-KG (as a metal-bound ligand complex), was done by least-squares superposition of domains I and II respectively, using the CCP4 suite program LSQKAB. The calculation of the r.m.s. deviations between the superimposed ligands was done by matching them using the least-squares superposition function implemented in Coot⁴⁵. Comparison of the IDH structures reported herein with their closest homologues was carried out using the Secondary Structure Matching (SSM) method⁵⁴ as implemented in the CCP4 suite. Distances and angles were calculated using Coot. Crystal-packing contacts were determined with the CCP4 suite program NCONT. Most of the CCP4 suite programs were used through the CCP4 graphical user interface⁵².

Figures were generated using the PyMOL Molecular Graphics System, Version 1.4.1 (Schrödinger, LLC).

RESULTS

Structural overview

Both *wt*IDH and IDH (K100M) crystallized in the tetragonal space group P4₃2₁2 with one monomer in the asymmetric unit (Figure 1A). The biological unit is a homodimer with subunits related by a binary crystallographic axis (Figure S1). The monomers fold as previously described³⁸ with a large $\alpha+\beta$ domain (residues 1–124 and 318–416) and a small α/β domain (residues 125–317) that includes a distinctive clasp-like α/β sub-domain (residues 158–202) (Figure 1B). Secondary structure elements are referenced using Hurley's nomenclature³⁸ with residues in the second subunit denoted by an asterisk (*).

We designate different datasets, and the corresponding crystal structures, based on the different protein crystals and ligands in which they were soaked: A [B:C:D], with A either *wt* for *wt*IDH or K100M for IDH (K100M); B either Mg²⁺ or Ca²⁺; C either isocitrate (ICT) or α -ketoglutarate (α -KG); and D either NADP⁺, thio-NADP⁺ or NADPH. The suffixes $\times 1$ and $\times 2$ distinguish datasets collected from different crystals of a given soak. Note that α -ketoglutarate is bound in the K100M [Mg²⁺:ICT:NADP⁺] $\times 2$ ternary product complex, the enzyme having turned over the substrate *in crystallum*.

To date, there are 27 crystal structures of *E. coli* isocitrate dehydrogenase in the Protein Data Bank (Table 3). Secondary structure matching against the entire PDB, carried out with SSM⁵⁴ as implemented at the European Bioinformatics Institute (<http://www.ebi.ac.uk/PDBeFold>) and using coordinates from the *wt* [Ca²⁺:ICT:NADP] and K100M [Mg²⁺:ICT:NADP⁺] $\times 1$ crystal structures, also recovered several IDHs from other organisms with high structural similarity to *EcoIDH*. The four crystal structures displaying the lowest r.m.s. deviations were selected for further analysis and comparison with our *EcoIDH* crystal structures (Table 3).

We first describe the large intra- and inter-domain conformational changes upon ligand binding and then describe the details of the active site in the catalytically productive “fully closed” conformations of *EcoIDH*.

Intra-domain dynamics

east-squares superpositions of the large domain in all published crystal structures of *EcoIDH* against that of the apo-isoform (PDB 1sj) ⁵³ (Table 4) show larger variations (0.5 to 1.3 Å C^α r.m.s. deviations) than similar calculations for the small domain (0.3 to 0.4 Å C^α r.m.s. deviations, with the notable exception of 0.8 Å for 1ika). In the large domain, the main structural differences are located in those residues that define the adenosine 2',5'-diphosphate binding pocket (residues 336–352 of the NADP-binding loop, residues 100–113 of the phosphorylation loop, residues 114–122 at the N-terminus of helix “d” and residues 71–79 that comprise helix “l”). In the small domain, deviations are confined to the loop linking β -strand “K” to the disordered β -strand “L” (residues 259–261) at the end of the sheet. Local structural differences attributable to crystal packing are present only at the interface defined by helix “h” and the phosphorylation loop of a second homodimer related by crystallographic symmetry. We thus attribute intramolecular structural rearrangements to substrate binding during the soaking assays.

Inter-domain hinge dynamics

Changes between the conformational states of *EcoIDH* (classified as “open”, “quasi closed” and “fully closed”) are attributable to rigid body rotations about an axis that lies approximately parallel to, and between, β -strands “E” and “F” located between the large and small domains (Figure 2A and 2B). *EcoIDH* apo-isoform (PDB 1sj) ⁵³, *EcoIDH* bound to

E. coli isocitrate dehydrogenase kinase/phosphatase (PDB 3lcb)⁵⁵ and *Aeropyrum pernix* IDH (PDB 1×kd, chain A)²⁰ adopt the “open” conformation. The vast majority of IDH structures listed in Tables 4 and 5, including those purporting to represent or mimic *pseudo*-Michaelis complexes of *Eco*IDH (PDB 1ide²³, 1ai2 and 1ai3²⁷ and 1hj6²⁹), exhibit a nonproductive “quasi-closed” conformation, intermediate between the “open” and “fully closed” forms, with the large domain rotated by *ca.* 20° relative to the “open” apo-*Eco*IDH structure. In our “quasi-closed” structures and several others (Table 3) a sulphate ion from the crystallization solution interacts with the phosphorylation loop, possibly contributing to the incomplete closure of the large domain. Our “fully closed” *wt* [Ca²⁺:ICT:NADP⁺] *pseudo*-Michaelis and K100M [Mg²⁺:ICT:NADP⁺] ×2 ternary product complexes show *ca.* 24.5° large domain rotations relative to the “open” conformation (Figure 2E). Though they differ in detail, both are similar to the nonproductive ternary complexes of *Aeropyrum pernix* IDH with isocitrate and NADP⁺ (PDB 1×kd, chain B)²⁰ and *Acithiophilus thiooxidans* IDH with citrate and NAD⁺ (PDB 2d4v)⁵⁶ (Figure 2E). The closure of the active site, as the large domain rotates from the “open” to the “quasi-closed” and then to the “fully closed” positions to form a catalytically competent ternary *pseudo*-Michaelis complex, is illustrated in Figure 3.

NADP⁺ and thio-NADP⁺ Binding

Continuous electron densities were observed for the NADP(H) ligands in both “fully closed” *wt* [Ca²⁺:ICT:NADP⁺] and K100M [Mg²⁺:ICT:NADP⁺] ×2 complexes (Figures 4A and 4B). Isotropic atomic displacement parameters of NADP(H) atoms were comparable to those of the side-chains interacting directly with the ligands. Bound NADP(H) adopts the same catalytically productive conformation in both structures (r.m.s. deviation of 0.14 Å for all ligand atoms).

Electron densities corresponding to NADP(H) in the “quasi-closed” K100M [Mg²⁺:ICT:NADP⁺] ×1 and K100M [Ca²⁺:α-KG:NADPH] complexes were not continuous. In both complexes the adenosine 2',5'-diphosphate moieties (A2P) of NADP were similarly bound in the A2P binding pocket (Figure 4C; r.m.s. deviation of 0.10 Å) and refined with partial occupancies of 0.64 and 0.67, respectively. The isotropic atomic displacement parameters for the A2P atoms increased progressively from the adenine ring towards the 5'-phosphate, whereas those of the side-chains interacting directly with A2P remained approximately constant. Fragments corresponding to the nicotinamide mononucleotide (NMN) moiety of NADP, or the corresponding mononucleoside, were identified at the end of the β-sheet in the “quasi-closed” K100M [Mg²⁺:ICT:NADP⁺] ×1 and the “fully closed” K100M [Mg²⁺:ICT:NADP⁺] ×2 complexes. No similar fragment could be identified in the K100M [Ca²⁺:α-KG:NADPH] complex where only the A2P moiety was visible.

Continuous well-defined electron densities with low isotropic atomic displacement parameters were observed for the thio-NADP⁺ analogues in the *wt* [Ca²⁺:ICT:thio-NADP⁺] and K100M [Mg²⁺:ICT:thio-NADP⁺] complexes (Figure 4D), with refined occupancies of 0.79 and 0.75, respectively. Bound thio-NADP⁺ adopts the same catalytically unproductive conformation in both structures (r.m.s. deviation of 0.12 Å for all ligand atoms).

The adenosine-2',5'-biphosphate (A2P) binding site

The A2P binding site is defined by the interdomain 3/10 helix (residues 318–324) on one side of the adenine ring, the NADP-binding loop (residues 336–352) on the opposite side, and α-helix “I” (residues 390–397) which is oriented obliquely to the plane of the ring (Figure 1A). In all structures the adenine ring π-stacks with His339. The adenine ring and the endocyclic ribose oxygen adopt the typical *anti*-conformation with respect to each other

around the N-glycosidic bond, and the ribose ring binds in an approximate half-chair C2'-exo/C3'-endo conformation (Figure 5A)^{20, 27, 32, 57}. The nitrogen atoms of the adenine ring are involved in several hydrogen bonds: N6A to the carbonyl oxygen of Asn352, N1A to the amide nitrogen of Asn352, and N3A to water W10. This water forms part of a hydrogen-bonded chain of five solvent molecules (W10-W14), present only in the “fully closed” conformation of IDH (Figure 5A), linking α -helices “i” and “h*” to α -helix “I” of the large domain. The protein atoms involved in this network are Asp392 O^{δ2}, Gln287* N^{ε2}, Arg395 N^{η2}, and Asp392 O.

In all structures O1X and O3X of the 5'-phosphate group hydrogen bond to the side chain hydroxyls of Tyr345 and Tyr391, respectively. In the “fully closed” conformations additional hydrogen bonds form between O2X and Gln288* N^{ε2} and Arg292* N^ε, O3X and Arg292* N^{η1}, while W15 bridges O1X to Lys344 N and W10 bridges O2X to Gln287* O^{ε1} (Figure 5A). The PA centre of the pyrophosphate hydrogen-bonds via O1A and O2A to the backbone amide of residues Gly340 and Ala342 in the NADP-binding loop (Figure 5A). The NADP(H) pyrophosphate backbone, which adopts a PA-O3-PN-O5D dihedral angle of 92°, contributes to the assembly of a six atom ring formed by four water molecules, the C2 hydroxyl of isocitrate and the active site Mg²⁺ or Ca²⁺.

In the “quasi-closed” conformations the A2P binding site is displaced as a rigid-body, with interactions between the large domain and α -helix “h*” replaced by bulk solvent molecules and by Arg395 N^{η1}. The interaction with Gly340 is absent in structures with bound thio-NADP⁺, and the pyrophosphate backbone adopts a different conformation with PA-O3-PN-O5D dihedral angles of 133° in the K100M [Mg²⁺:ICT:thio-NADP⁺] complex and 114° in the *wt* [Ca²⁺:ICT:thio-NADP⁺] complex. This places the thio-nicotinamide mononucleotide at the entrance of the isocitrate binding pocket in a conformation flipped relative to NMN in the “fully closed” structures (Figure 4D).

The nicotinamide mononucleotide (NMN) binding site

The NMN binding site lies adjacent to the isocitrate binding pocket (Figure 5B). In both “fully closed” *wt* [Ca²⁺:ICT:NADP⁺] and K100M [Mg²⁺:ICT:NADP⁺] $\times 2$ complexes, NADP(H) binds in a bent conformation with the nicotinamide ring above the substrate binding pocket, its amide oriented towards the large domain. The ribosyl moiety is anchored to the phosphorylation loop, in a roughly half-chair C2'-exo/C3'-endo conformation, with a dihedral angle of 166° about the O5D-C5D phosphoester bond. The 3'-hydroxyl O3D hydrogen-bonds to Thr105 N and Thr105 O. The 2'-hydroxyl O2D interacts with a C5 carboxylate oxygen of isocitrate or α -ketoglutarate, as well as with Asn232* N^{δ2}. These interactions trigger a shift in the phosphorylation loop towards the active site pocket where it acts as a platform to bind NADP in a catalytically productive conformation (Figure 6).

In the *wt* [Ca²⁺:ICT:NADP⁺] *pseudo*-Michaelis complex, the amide N7N hydrogen bonds to the C1 carboxylate of isocitrate (3.1 Å) and Glu336 O^{ε1} (2.9 Å), while the O7N hydrogen bonds with Leu103 N (2.9 Å) and Asn115 N^{δ2} (3.0 Å) (Figure 5B). Similarly, in the K100M [Mg²⁺:ICT:NADP⁺] $\times 2$ ternary product complex the N7N is within van der Waals distance of the C1 carboxylate of α -ketoglutarate (3.8 Å) and hydrogen bonds to Glu336 O^{ε1} (3.2 Å), while the O7N hydrogen bonds with water W66 (2.9 Å, and near Lys100 N^ε in the *pseudo*-Michaelis complex) and Asn115 N^{δ1} (2.9 Å) (Figure 5C). The Lys100-Leu103-Asn115-Glu336 tetrad (Figure 5B and 5C) plays a central role both in anchoring the nicotinamide ring and in triggering a cascade of electrostatic interactions that propagate towards the hinge region of the central β -sheet, facilitating the full closure of the enzyme. In particular, Asn115 plays a pivotal role in anchoring both the NMN moiety and isocitrate in a conformation favorable to catalysis.

In both “fully closed” complexes, the nicotinamide ring lies with its C4N poised to receive the hydride from isocitrate C2 on its *re* face (Figure 5B and 5C). In structures with bound thio-NADP⁺, the entire thio-NMN moiety is rotated 166° about the O5D-C5D phosphoester bond. The C4N now points away from the active site while the *si* face of the nicotinamide ring lies against the C5 carboxylate of isocitrate. This flipped conformation is stabilized by a hydrogen bond from the N7N of the thioacetamyl to Thr105 O and interactions between the ribosyl-moiety of thio-NMN and waters that fill the NMN binding pocket.

Evidence that NADP⁺ might be hydrolysed in *EcoIDH* crystals was first obtained when a fragment corresponding to the missing NMN moiety was observed in the “quasi-closed” K100M [Mg²⁺:ICT:NADP⁺] × 1 (Figure 4C) and “fully closed” the K100M [Mg²⁺:ICT:NADP⁺] × 2 complexes. In both instances, the *si* face of the nicotinamide ring stacks against the aromatic ring of Trp263* with the carboxyamide stabilized by the loop formed by residues 258–261. Preliminary evidence for hydrolytic activity by *EcoIDH* was given by mass spectrometry analysis (unpublished results). These studies detected the presence of hydrolysed NADP⁺ products in re-dissolved tetragonal crystals of both *wt*IDH and K100M IDH soaked with NADP⁺, but not when NADP⁺ was added to the protein solutions. Despite their qualitative nature, these results suggest that the coenzyme hydrolysis occurs only in the “fully closed” or “quasi-closed” conformation of the enzyme. Further studies are required to establish the biological relevance, if any, of coenzyme hydrolysis by IDH.

The substrate binding pocket

As previously described¹³, isocitrate and α-ketoglutarate bind in a pocket located at the base of the cleft between hinge axis strands “E” and “F” and helices “i” and “h*” at the dimer interface (Figure 1A). In all structures a divalent metal ion, Mg²⁺ or Ca²⁺, chelates a C1 carboxylate oxygen and the C2 oxygen of bound isocitrate or α-ketoglutarate (a hydroxyl and a carbonyl, respectively) (Figure 5B and 5C). The conserved Arg119, Arg129 and Arg153 provide a binding scaffold for the C1 carboxylate – this occurs despite the 90° rotation around the C1-C2 σ-bond caused by the change in hybridization at C2 from sp³ in isocitrate to sp² in α-ketoglutarate (Figure 5B and 5C). A Lys230*-Tyr160 diad also participates in binding the C6 carboxylate (attached to the C3 of isocitrate) and C2 hydroxyl, respectively²¹. As with the NADP-binding pocket, the partial displacement of the large domain during hinge closure produces a positional shift in the substrate binding site, with preserved hydrogen bonds to the ligand C1 and C6 carboxylates.

Both isocitrate and α-ketoglutarate bind in “active” and “inactive” conformations, as reflected in the different C2-C3-C4-C5 dihedral angles: 139° (“active”) and 63° (“inactive”) for isocitrate, 172° (“active”) and 75° (“inactive”) for α-ketoglutarate. In the “active” conformation, observed in the “fully closed” *wt* [Ca²⁺:ICT:NADP⁺] *pseudo*-Michaelis complex (Figure 5B), the “fully closed” K100M [Mg²⁺:ICT:NADP⁺] × 2 ternary product complex (Figure 5C, this enzyme is active *in crystallum*) and the “quasi-closed” *wt* [Ca²⁺:ICT:thio-NADP⁺] complex, the C5 carboxylate hydrogen bonds with Ser113 O^γ, Thr105 O^γ and Asn115 N^{δ1}. In the inactive conformation, observed in the “quasi-closed” K100M [Mg²⁺:ICT:NADP⁺] × 1, the K100M [Mg²⁺:ICT:thio-NADP⁺] and the K100M [Ca²⁺:α-KG:NADPH] complexes, the C5 carboxylate hydrogen bonds to Ser113 O^γ and one or more waters.

The coordination number (CN) of Mg²⁺ changes from CN = 5 to CN = 6 as the enzyme shifts from the “quasi-closed” K100M [Mg²⁺:ICT:NADP⁺] × 1 and K100M [Mg²⁺:ICT:thio-NADP⁺] to the “fully closed” K100M [Mg²⁺:ICT:NADP⁺] × 2 position. With Ca²⁺, CN = 7 and CN = 6 in the “quasi-closed” *wt* [Ca²⁺:ICT:thio-NADP⁺] and K100M [Ca²⁺:α-KG:NADPH] complexes respectively, whereas CN = 6 in the “fully closed” *wt*

[Ca²⁺:ICT:NADP⁺] complex. The Ca²⁺ in the *wt* [Ca²⁺:ICT:NADP⁺] structure lies close to the Mg²⁺ in K100M [Mg²⁺:ICT:NADP⁺] × 2 structure, effectively mirroring the Michaelis-Menten complex (Figure 5B and 5C). In addition to binding the isocitrate or the α-ketoglutarate ligands, Mg²⁺ coordinates to Asp307 O^{δ1} and two water molecules, W1 and W2, the second a part of a conserved six-atom ring. This non-labile pentavalent coordination sphere is supplemented with a sixth ligand, Asp283* O^{δ2}, that completes the Mg²⁺ coordination sphere in the “fully closed” *wt* [Ca²⁺:ICT:NADP⁺] and K100M [Mg²⁺:ICT:NADP⁺] × 2 complexes. The Ca²⁺ coordination sphere also includes a transient ligand, Asp311 O^{δ2}, coordinated to the metal ion prior to full closure of the enzyme (Figure 5B).

DISCUSSION

Hinge dynamics and substrate-mediated structural rearrangements

Metal-dependent NAD(P)-linked hydroxyacid oxidative decarboxylases undergo structural rearrangements typical of many enzymes, including rigid body motions and substrate-induced conformational changes that modulate their catalytic activities⁵⁸. Studies of *EcoIDH* have identified basic mechanisms behind substrate-induced conformational changes^{21, 23, 29, 53}. However, crystallographic intermediate-trapping methods based on crystal soaks with substrates and substrate analogues have proved insufficient to identify all the residues involved, mostly because crystal packing is incompatible with the fast structural rearrangements taking place during catalysis.

Our cryo-trapped snapshots of the “fully closed” *wt* [Ca²⁺:ICT:NADP⁺] and K100M [Mg²⁺:ICT:NADP⁺] × 2 structures provide deeper insights into the structural conformations needed to produce productive ternary complexes. Both *wild* IDH and its K100M variant undergo a more complete hinge closure than the “quasi-closed” conformations previously reported (Figure 3). In the “quasi-closed” conformation, ligands bind at the pocket surface yielding non-productive complexes. In the “fully closed” conformation they move as rigid bodies to the inner pocket region as the large domain rotates further towards the small domain. The “fully closed” conformation is also distinguished from the “quasi-closed” conformation by two additional structural changes: a small displacement of the NADP-binding loop in the direction of the rotation (Lys 344 C^α moves by *ca.* 4 Å) and a shift in the phosphorylation loop towards the active site pocket (Gly 108 C^α moves by *ca.* 9 Å) which blocks access to bulk solvent while stabilizing the substrates in position for catalysis (Figure 6). Figure 6 also illustrates the changes in the electrostatic landscape of the catalytic pocket that result from the hinge motion upon forming a catalytically competent *pseudo*-Michaelis complex.

Our analysis shows that only *Acidithiophilus thiooxidans* IDH (PDB 2d4v, chains A, B, C, D)⁵⁶ and *Aeropyrum pernix* IDH (PDB 1×kd, chain B)²⁰ display the “fully closed” conformation of the enzyme (see Tables 4 and 5). However, the *Acidithiophilus thiooxidans* IDH structure lacks the catalytic metal ion and the ligands are an unnatural citrate and NAD⁺. Chain B of *Aeropyrum pernix* IDH is the most similar to our *wt* [Ca²⁺:ICT:NADP⁺] structure, the only notable difference being that the carboxamide group was modelled perpendicular to the nicotinamide ring (C2N-C3N-C7N-O7N dihedral angle of 95.6°) rather than nearly coplanar (27.5°). Unfortunately, and in contrast with our “fully closed” structure, the electron density of the nicotinamide ring is poorly defined (Figure 7). Our *wt* [Ca²⁺:ICT:NADP⁺] structure is thus the first complete example of a true *pseudo*-Michaelis complex in an IDH enzyme.

Cryo-trapped product ternary complexes with bound NADPH, α-ketoglutarate and Ca²⁺ have also been reported for *Saccharomyces cerevisiae* IDH isoform 1 (*ScerIDH1*, PDB

2qfx)³⁰ and the R132H mutant of human IDH isoform 1 (R132H *HsapIDH1*; PDB 3inm)³¹. Despite the different electrostatic environments of their active sites, both structures share features in common with our “fully closed” *wt* [Ca²⁺:ICT:NADP⁺] and K100M [Mg²⁺:ICT:NADP⁺] $\times 2$ complexes. In all cases, Thr105 and Ser113 (*EcoIDH* numbering) are conserved in the homologous enzymes and play pivotal roles in anchoring the C5 carboxylate group of isocitrate or α -ketoglutarate and the 3'-hydroxyl group O3D of the NMN.

Together with our “fully closed” *wt* [Ca²⁺:ICT:NADP⁺] and K100M [Mg²⁺:ICT:NADP⁺] $\times 2$ complexes of *EcoIDH*, these four “fully closed” structures confirm that the significance of the structural rearrangements in the phosphorylation loop of *EcoIDH* extends beyond its role in allowing access for regulatory phosphorylation, effectively an “on-off switch”^{53, 55, 59, 60}, to being directly involved in the assembly of productive ternary complexes and product release during the catalytic turnover.

The Lys100-Leu103-Asn115-Glu336 tetrad in *EcoIDH* anchors the nicotinamide ring in position for catalysis. As seen in the “fully closed” *wt* [Ca²⁺:ICT:NADP⁺] and K100M [Mg²⁺:ICT:NADP⁺] $\times 2$ complexes, Asn115 plays a central role in anchoring the C5 carboxylate of isocitrate and α -ketoglutarate, as well as helping orient the carboxamide of the nicotinamide ring (Figure 5B and F5C). In the “fully closed” K100M [Mg²⁺:ICT:NADP⁺] $\times 2$ structure, the now absent electrostatic contribution of the hydrogen bond between Lys100 and Glu336, which in turn anchors the nicotinamide ring in *wtIDH*, is rescued by a water molecule (W66) that is nearly coincident with the Lys100 N^c atom (Figure 5C). In fact W66 may be an NH₄⁺ cation ((NH₄)₂SO₄ was the precipitant). Not only is NH₄⁺ isoelectronic with H₂O, and therefore indistinguishable by X-ray crystallography at 2.7 Å resolution, but the local environment is negatively charged (provided by O7N of the NMN moiety and the carboxylate group of Glu336, and to a lesser extent by the carbonyl group of Gly101). Preliminary kinetic studies show that NH₄⁺ partially rescues activity in the K100M mutant (unpublished observation).

The active site

Replacing Mg²⁺ by Ca²⁺ reduces k_{cat} > 2500-fold²⁷. Theoretical studies based on small distance and angle perturbations of the C2 hydroxyl group of isocitrate and the C4N atom of the nicotinamide ring suggest that Ca²⁺ disturbs the optimal orientation (distance and angle) of the reacting molecular orbitals for hydride transfer²⁷. Yet, despite the higher ionic radius of Ca²⁺ (0.99 Å) compared to that of Mg²⁺ (0.65 Å), our results show that Ca²⁺ in the “fully closed” *wt* [Ca²⁺:ICT:NADP⁺] *pseudo*-Michaelis complex is geometrically equivalent to the Mg²⁺ metal ion observed in the “fully closed” K100M [Mg²⁺:ICT:NADP⁺] $\times 2$ ternary product complex (Figure 5B and 5C). Being heavier than Mg²⁺, Ca²⁺ might instead slow activity by affecting protein dynamics, perhaps by hindering mobility at the metal binding site during hinge motion. Kinetic data from *EcoIDH*³⁶, pig heart IDH¹² and the related homoisocitrate dehydrogenase in yeast¹⁰ support a steady-state kinetic mechanism with catalysis at least 10 times faster than the structural changes needed to set up the active site for catalysis.

The *wt* [Ca²⁺:ICT:NADP⁺] *pseudo*-Michaelis complex and the K100M [Mg²⁺:ICT:NADP⁺] $\times 2$ ternary complex with bound α -ketoglutarate and NADPH portray key structural changes in the active site during catalysis (Figure 5B and 5C). The most significant conformational changes take place at Tyr160 and Glu336. While the change at Tyr160 arises following β -decarboxylation, that at Glu336 likely results from substituting the amine group of Lys100 by a water molecule or a NH₄⁺ cation (W66).

Catalytic mechanism

Our structural data support, in detail, the catalytic mechanism proposed by Aktas and Cook (Figure 8A)⁵. Kinetic studies of the related homoisocitrate dehydrogenase (HIDH)⁶¹ suggest that an invariant Lys (Lys230* in *EcoIDH*) initiates dehydrogenation by abstracting the proton from the hydroxyl of isocitrate. Consistent with this proposal, and seen for the first time, Lys230* forms a hydrogen bond (3.2 Å) with the substrate hydroxyl in the fully “closed” *wt* [Ca²⁺:ICT:NADP⁺] *pseudo*-Michaelis complex (Figure 8B). Lys230* must be uncharged in order to act as a base. As predicted⁵ Asp307, a metal ligand, aids proton abstraction from the hydroxyl by hydrogen bonding to Lys230*. Conversion of isocitrate into oxaloacetate is fast³⁶ and, except for the observed ~90° rotation of the C1 carboxylate upon the change in hybridization at C2 from sp³ to sp², is accompanied only by minimal structural rearrangements in the active site pocket.

Next, decarboxylation of oxaloacetate produces an enol intermediate (Figure 8A), with the C2 carbonyl re-protonated by Lys230*. Tautomerization follows loss of CO₂. An invariant Tyr (Tyr160 in *EcoIDH*) approaches C3 for stereospecific protonation, while Lys230* once again abstracts the proton from the C2 hydroxyl to yield the α-ketoglutarate product. In the K100M [Mg²⁺:ICT:NADP⁺] × 2 ternary product complex, Lys230* hydrogen bonds to the C2 carbonyl while Tyr160 seems poised to (de)protonate C3, albeit 3.4 Å away (Figure 8C). Indeed, the near planar α-ketoglutarate suggests some double-bond character between C2 and C3 (reducing the C1-C2-C3-C4 dihedral angle from 20° to 0° does not greatly compromise the observed electron density). Although NMR studies detected the signature of α-hydroxyglutarate bound to pig heart IDH¹¹, we detected no activity towards this substrate by *E. coli* wildtype and the K100M mutant enzymes (unpublished observations). Thus, the observed electron density is probably an average dominated by the carbonyl form of α-ketoglutarate with lesser contributions from the enol form.

Aktas and Cook⁵ proposed that the IDH mechanism is similar to that of the malic enzyme, i.e., malate dehydrogenase (decarboxylating). In the malic enzyme a hydrogen bond exists between the catalytic Lys and Tyr, allowing dehydrogenation and decarboxylation to proceed without the need to exchange protons with other groups or with bulk solvent. Neither in the *wt* [Ca²⁺:ICT:NADP⁺] *pseudo*-Michaelis complex nor in the K100M [Mg²⁺:ICT:NADP⁺] × 2 ternary product complex is there evidence of a hydrogen bond between Lys230* and Tyr160, even though their functional groups lie in close proximity. However, both are close to Asp307 in the *wt* [Ca²⁺:ICT:NADP⁺] *pseudo*-Michaelis complex. If a proton is not exchanged directly between Lys230* and Tyr160 during enol conversion to α-ketoglutarate, then the exchange may be indirect via Asp307 (Figure 8A). Alternatively, extending from the Lys230*-Tyr160-Asp307 catalytic triad there is a proton relay comprising two water molecules (W5 and W4), the O2N oxygen from the NADP pyrophosphate backbone and a third water molecule (W6) that may balance proton flow between the active site and the bulk solvent during catalysis (Figure 8B and 8C). Although a proton relay of this kind has been proposed for the *pseudo*-Michaelis complex of *Aeropyrum pernix* IDH²⁰, our results provide new insights into the role played by the Lys230*-Asp307-Tyr160 catalytic triad during oxidative decarboxylation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ABBREVIATIONS

NADP⁺ β-Nicotinamide Adenine Dinucleotide Phosphate

NADPH	β-Nicotinamide Adenine Dinucleotide Phosphate (reduced)
thio-NADP⁺	β-thio-Nicotinamide Adenine Dinucleotide Phosphate
ICT	2R,3S-isocitrate
OXA	oxalosuccinate
α-KG or AKG	α-ketoglutarate
NMN	nicotinamide mononucleotide
thio-NMN	thionicotinamide mononucleotide
A2P	Adenosine 2',5'-diphosphate
IMP	isopropylmalate
IDH	isocitrate dehydrogenase
EcoIDH	<i>Escherichia coli</i> K21 IDH
wtIDH	<i>Escherichia coli</i> K21 IDH (wild type)
IDH (K100M)	<i>Escherichia coli</i> K21 IDH K100M mutant
AperIDH	<i>Aeropyrum pernix</i> IDH
BsubIDH	<i>Bacillus subtilis</i> IDH
R132H HsapIDH1	R132H mutant of human IDH isoform 1
SscrIDH	<i>Sus scrofa</i> (porcine) IDH
ScerIDH1	<i>Saccharomyces cerevisiae</i> IDH (mitochondrial NADP-dependent)
r.m.s	root mean square

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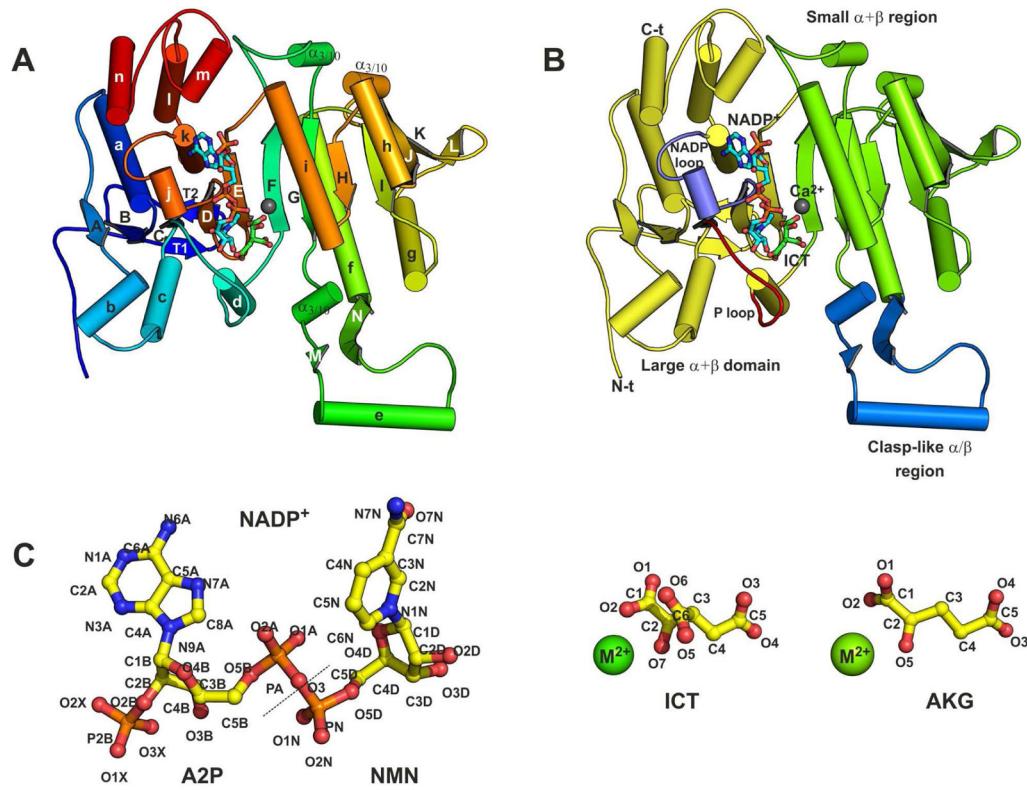


Figure 1. The *E. coli* IDH monomer and its ligands

A) Secondary structure: ribbon representation of the IDH monomer in the *wt* [Ca²⁺:ICT:NADP⁺] crystal structure, rainbow-color coded from N-terminal (blue) to C-terminal (red). The secondary structure elements are labeled according to Hurley's scheme: β-strands are denoted by capital letters and α-helices are labeled in lowercase letters 38. The T1 and T2 elements indicate the N-terminal β-hairpin. **B)** Domain structure: ribbon representation of the IDH monomer in the *wt* [Ca²⁺:ICT:NADP⁺] crystal structure, color coded according to the domain structure. Domain I (large α+β domain, residues 1–124 and 318–416) is colored light yellow. Domain II comprises the small α/β region (residues 125–157 and 203–317) represented in light green and a α/β clasp-like region (residues 158–202) displayed in marine blue. The phosphorylation and NADP-binding loops are highlighted in red and slate blue, respectively. The NADP⁺ and ICT ligands are drawn in ball-and-stick and Ca²⁺ is depicted as sphere. Atom colors are: gray for calcium, orange for phosphorus, blue for nitrogen and red for oxygen; carbon atoms from NADP⁺ and ICT are colored cyan and green, respectively. **C)** Ball-and-stick diagrams of the NADP⁺, ICT:M²⁺ and AKG:M²⁺ ligands in the IDH crystal structures showing the atom labeling scheme used in the text. M²⁺ represents either one of the Mg²⁺ or Ca²⁺ metal ions. In thio-NADP⁺ the sulfur atom S1 replaces O17. The dashed line at atom O5 in NADP⁺ marks the division between the A2P and the NMN moieties.

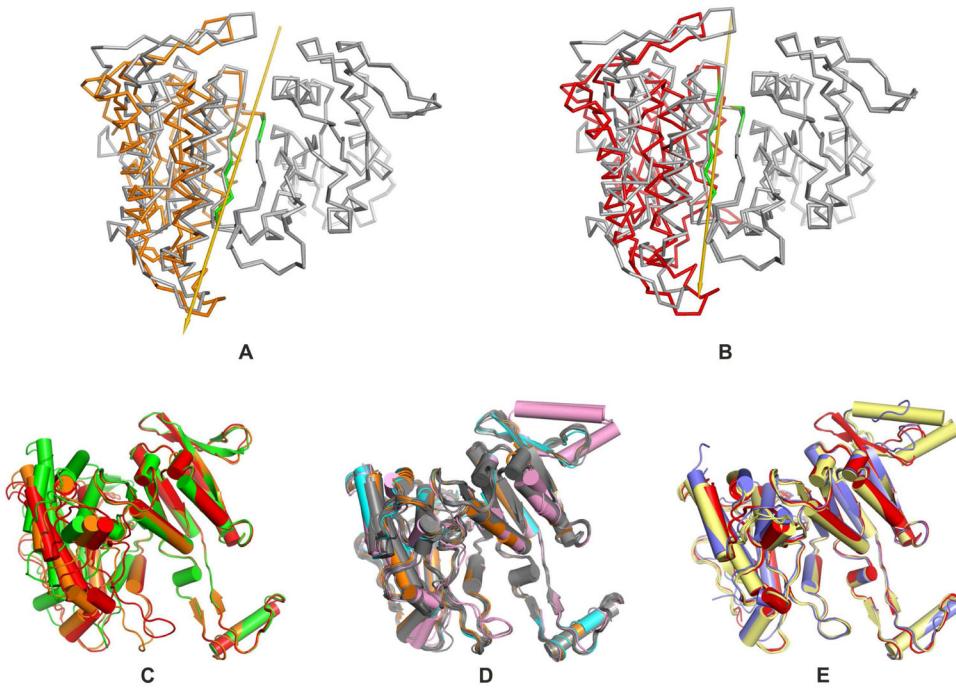


Figure 2. Interdomain hinge dynamics in *EcoIDH*

A) Ribbon diagram showing the large domain rotation between the open (PDB 1sjs, light gray) and quasi-closed (K100M [Mg²⁺:ICT:NADP⁺] ×1 complex, orange) conformations of *EcoIDH*. The rotation axis determined with DynDom is drawn in gold and the corresponding hinge residues are colored green. For clarity, the small domain is shown in light gray. **B)** Ribbon diagram showing the large domain rotation between the open (PDB 1sjs, light gray) and fully closed (*wt* [Ca²⁺:ICT:NADP] *pseudo*-Michaelis complex, red) conformations of *EcoIDH*. The rotation axis determined with DynDom is drawn in gold and the corresponding hinge residues are colored green. For clarity, the small domain is shown in light gray. **C)** Cartoon diagram of selected *EcoIDH* monomers from this work, illustrating the rigid-body hinge motion of the large domains after superposition of the small domains. The open conformation (PDB 1sjs) is represented in green, the quasi-closed conformation of the K100M [Mg²⁺:ICT:NADP⁺] ×1 complex is colored orange, and the fully closed conformation of the *wt* [Ca²⁺:ICT:NADP] *pseudo*-Michaelis complex is displayed in red. **D)** Cartoon diagram of IDH monomers in the quasi-closed conformation, after superposition of the small domains. The four complexes from this work are colored orange, the other *EcoIDH* monomers are shown in gray, *B. pseudomallei* IDH is displayed in cyan and *B. subtilis* IDH chains A and B are represented in pink. **E)** Cartoon diagram of IDH monomers in the fully closed conformation, after superposition of the small domains. The two complexes from this work are colored red, *A. pernix* IDH chain B is shown in slate blue and the 4 chains of *A. thiooxidans* IDH are represented in pale yellow. Panels **A** and **B** are drawn in the same orientation, different from that of panels **C–E**, to better show the location of the hinge residues and the orientation of the rotation axes.

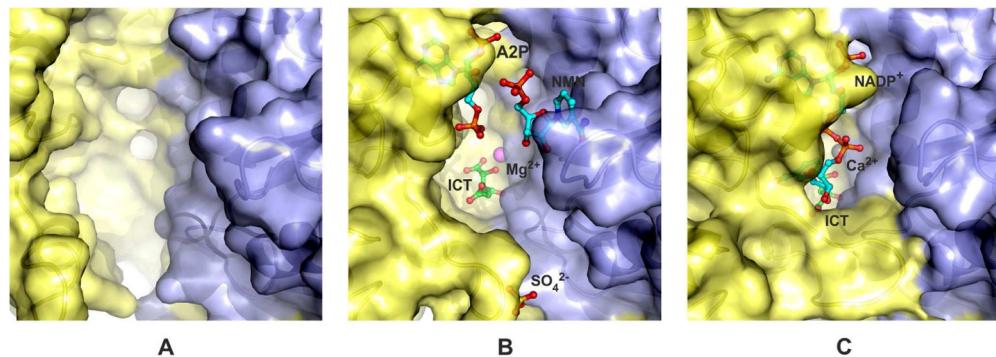


Figure 3. Closure of the active site as the large domain rotates from the “open” to the “quasi-closed” and “fully closed” positions

A) The “open” form (PDB 1sjs)⁵³. B) The “quasi-closed” form, represented by the K100M [Mg²⁺:ICT:NADP⁺] × 1 crystallographic dimer. C) The catalytically competent “fully closed” conformation, represented by the wt [Ca²⁺:ICT:NADP⁺] crystallographic dimer. The protein chains are shown as cartoons. The respective molecular surfaces are represented as semi-transparent and were calculated using a solvent probe radius of 1.4 Å. The surface of monomer A is colored light yellow and that of symmetry-related monomer B is displayed in light blue. Ligands are shown as ball-and-stick and Mg²⁺ and Ca²⁺ ions drawn as spheres. Atom colors are: violet for magnesium, gray for calcium, yellow for sulfur, orange for phosphorus, blue for nitrogen, red for oxygen, green for carbon atoms in ICT and cyan for carbon atoms in NADP⁺, A2P and NMN.

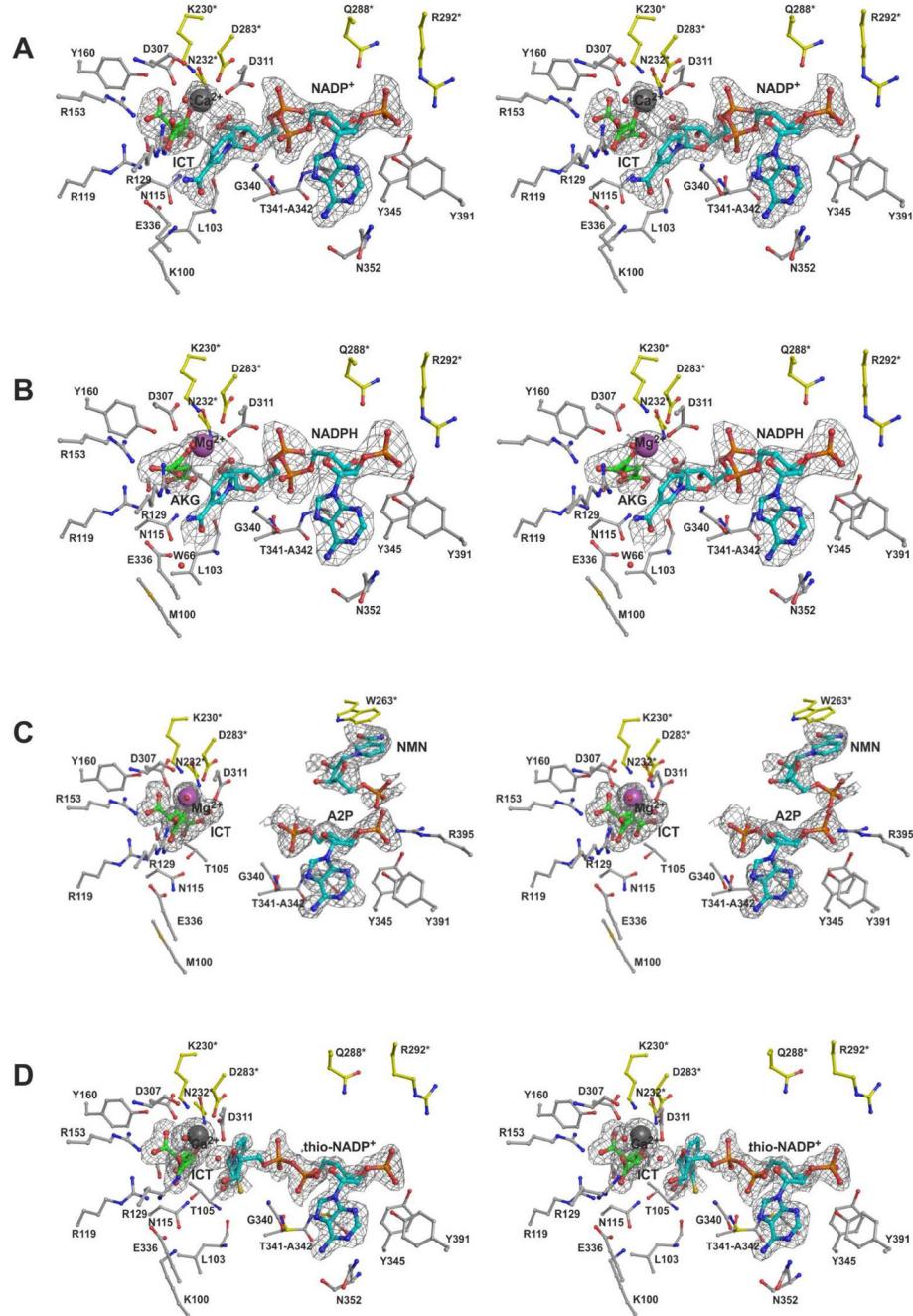


Figure 4. Stereo diagrams of ligands binding to *EcoIDH* crystal structures

A) *wt* [Ca^{2+} :ICT:NADP $^+$], **B)** K100M [Mg^{2+} :ICT:NADP $^+$] $\times 2$, **C)** K100M [Mg^{2+} :ICT:NADP $^+$] $\times 1$, **D)** *wt* [Ca^{2+} :ICT:thio-NADP $^+$]. Unbiased σ_A -weighted $|F_o| - |F_c|$ electron density maps covering the ligands in the catalytic pockets were obtained with PHENIX by simulated annealing in the absence of the ligands and water molecules located inside the catalytic pocket and coordinated either to Mg^{2+} or its analogue Ca^{2+} . The maps are represented as a light gray mesh and contoured at the 3σ level for all structures except for K100M [Mg^{2+} :ICT:NADP $^+$] $\times 1$ structure (C) where it is drawn at the 3σ level for the [Mg^{2+} :ICT] complex and at the 2σ level for the hydrolyzed NADP $^+$. Protein residues and polyatomic ligands are drawn as ball-and-stick, and the Mg^{2+} and Ca^{2+} metal ions and the

water molecules are drawn as spheres. Atom colors are violet for magnesium, gray for calcium, orange for phosphorus, blue for nitrogen and red for oxygen; carbon atoms are colored cyan in NADP⁺, NADPH, thio-NADP⁺ and the hydrolysis products A2P and NMN, green in ICT and AKG, and light-gray and magenta in the protein residues from the first and second subunits of the biological homodimer respectively. For clarity, only the side-chains (starting at C^α) of the protein residues are represented, except where the main-chain atoms interact with the ligands. Due to the orientation chosen, S113 and occasionally T105 are obscured by the ligands and not clearly visible.

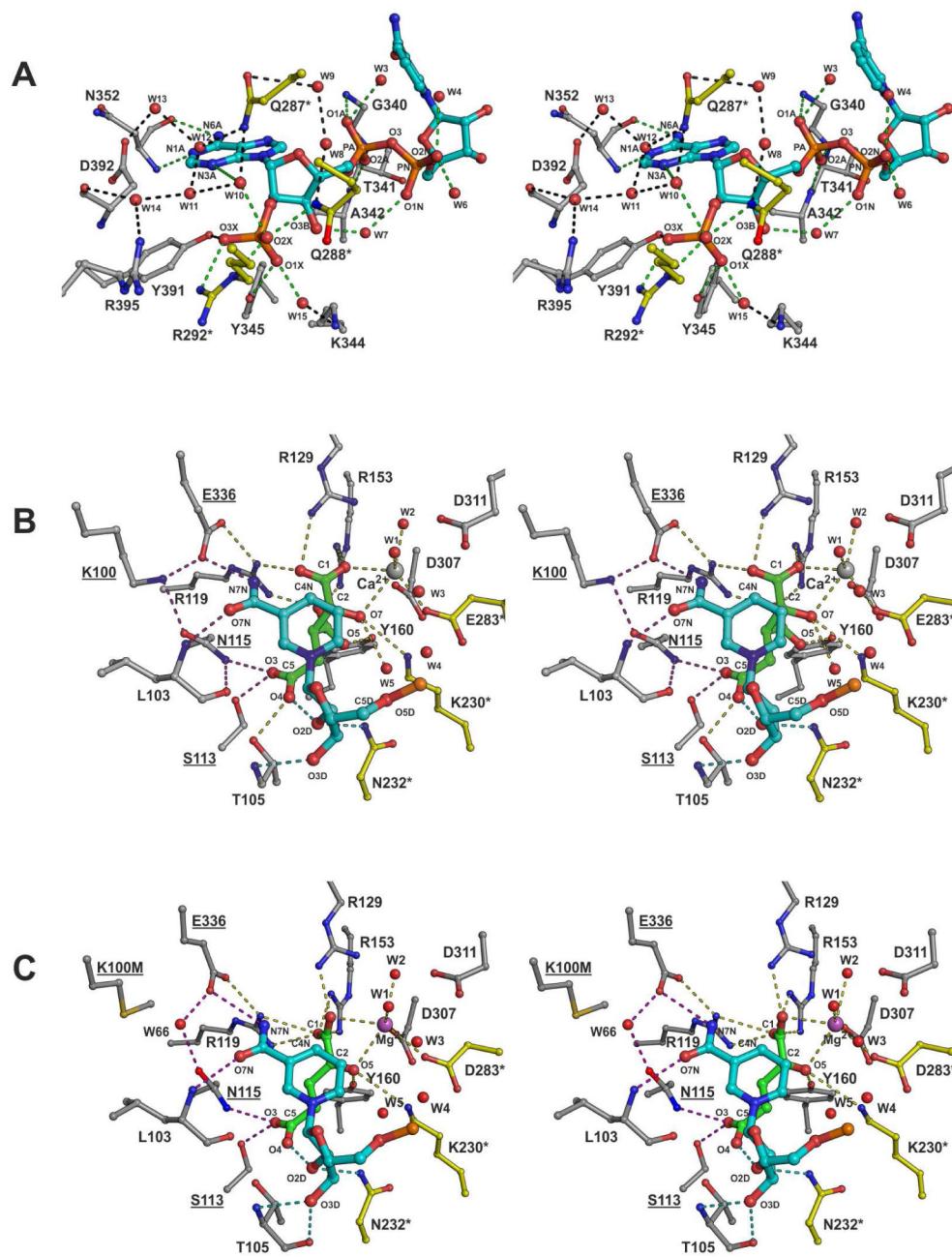


Figure 5. Stereo diagrams showing substrate and product binding in the “fully closed” *EcoIDH* crystal structures

A) The adenosine-2',5'-biphosphate (A2P) binding site in the *wt* [Ca^{2+} :ICT:NADP $^+$] structure. **B)** the nicotinamide mononucleotide (NMN) and Ca^{2+} -isocitrate binding sites in the *wt* [Ca^{2+} :ICT:NADP $^+$] structure. **C)** The nicotinamide mononucleotide (NMN) and Mg^{2+} : α -ketoglutarate binding sites in the K100M [Mg^{2+} :ICT:NADP $^+$] $\times 2$ structure. Side chains and substrates are shown as ball-and-stick with waters and Ca^{2+} shown as spheres. Dashed lines indicate hydrogen coordination bonds and are color-coded according to the interactions made: green with A2P, cyan with the ribosyl of NMN, yellow with Ca^{2+} -isocitrate, black for interactions between side-chains and waters, and purple for interactions anchoring the nicotinamide ring and the catalytic tetrad Ser113-Asn115-Lys100-Glu336

(with residue labels underlined). Carbon atoms are colored cyan for NADP, green for isocitrate, and light gray and yellow for the first and second subunits of *EcoIDH* respectively. Residues from the second subunit are labeled with an asterisk. Ligand atoms are labeled according to Figure 1C.

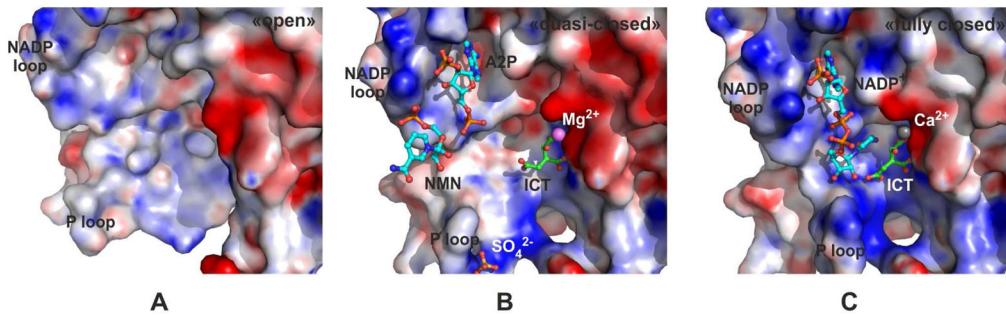


Figure 6. Changes in the electrostatic potential landscape of the *EcoIDH* active site between the “open”, “quasi-closed” and “fully closed” conformations

(A) The “open” form (PDB 1sjs)⁵³; (B) The “quasi-closed” form, represented by the K100M [Mg²⁺:ICT:NADP⁺] × 1 monomer; (C) The catalytically competent “fully closed” conformation, represented by the wt [Ca²⁺:ICT:NADP⁺] monomer. The electrostatic potential of each biological homodimer is represented at the molecular surface of the respective monomer (the second monomer is not shown for clarity). The atomic charges for each homodimer were calculated with PDB2PQR⁶² using the CHARMM22⁶³ all-atom force field for the protein atoms. The electrostatic distribution was determined with the APBS v0.4.0 PyMol plug-in⁶⁴ by applying the non-linear Poisson-Boltzmann method with T = 300 K, an ionic strength of 0.15 M, a solvent dielectric constant $\epsilon_s = 80$ and a protein dielectric constant $\epsilon_p = 6$. The molecular surface of each monomer was calculated using a solvent probe radius of 1.4 Å. The range of electrostatic potentials shown spans from -15 (red) to +15 kT/e (blue) units. Ligands are shown as ball-and-stick and the Mg²⁺ and Ca²⁺ ions are represented as spheres. Atom colors are: violet for magnesium, gray for calcium, yellow for sulfur, orange for phosphorus, blue for nitrogen, red for oxygen, green for carbon atoms in ICT and cyan for carbon atoms in NADP⁺, A2P and NMN.

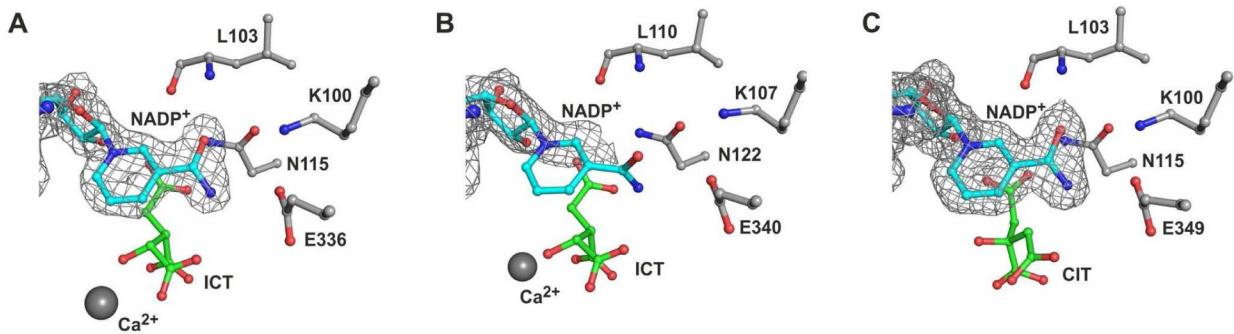


Figure 7. The nicotinamide ring of NADP⁺ in the fully closed IDH structures

A) wt [Ca²⁺:ICT:NADP⁺] (this work), B) *Aeropyrum pernix* IDH (PDB 1×kd, chain B)²⁰, C) *Acidithiophilus thiooxidans* IDH (PDB 2d4v, chain A)⁵⁶. σ_A -weighted $2|F_0|-|F_c|$ electron density maps covering NADP⁺ were calculated with COOT using the observed and final calculated structure factors. The structure factor data for PDB 1×kd and 2d4v were downloaded from the Protein Data Bank with COOT. The maps are represented as a light gray mesh and contoured at the 1.5 σ level. Protein residues and polyatomic ligands are drawn as ball-and-stick, and the Ca²⁺ metal ions are drawn as spheres. Atom colors are gray for calcium, orange for phosphorus, blue for nitrogen and red for oxygen; carbon atoms are colored cyan in NADP⁺, green in ICT and citrate (CIT), and light-gray in the displayed protein residues. For clarity, only the side-chains (starting at C^a) of the protein residues are represented, except where the main-chain atoms interact with the ligands.

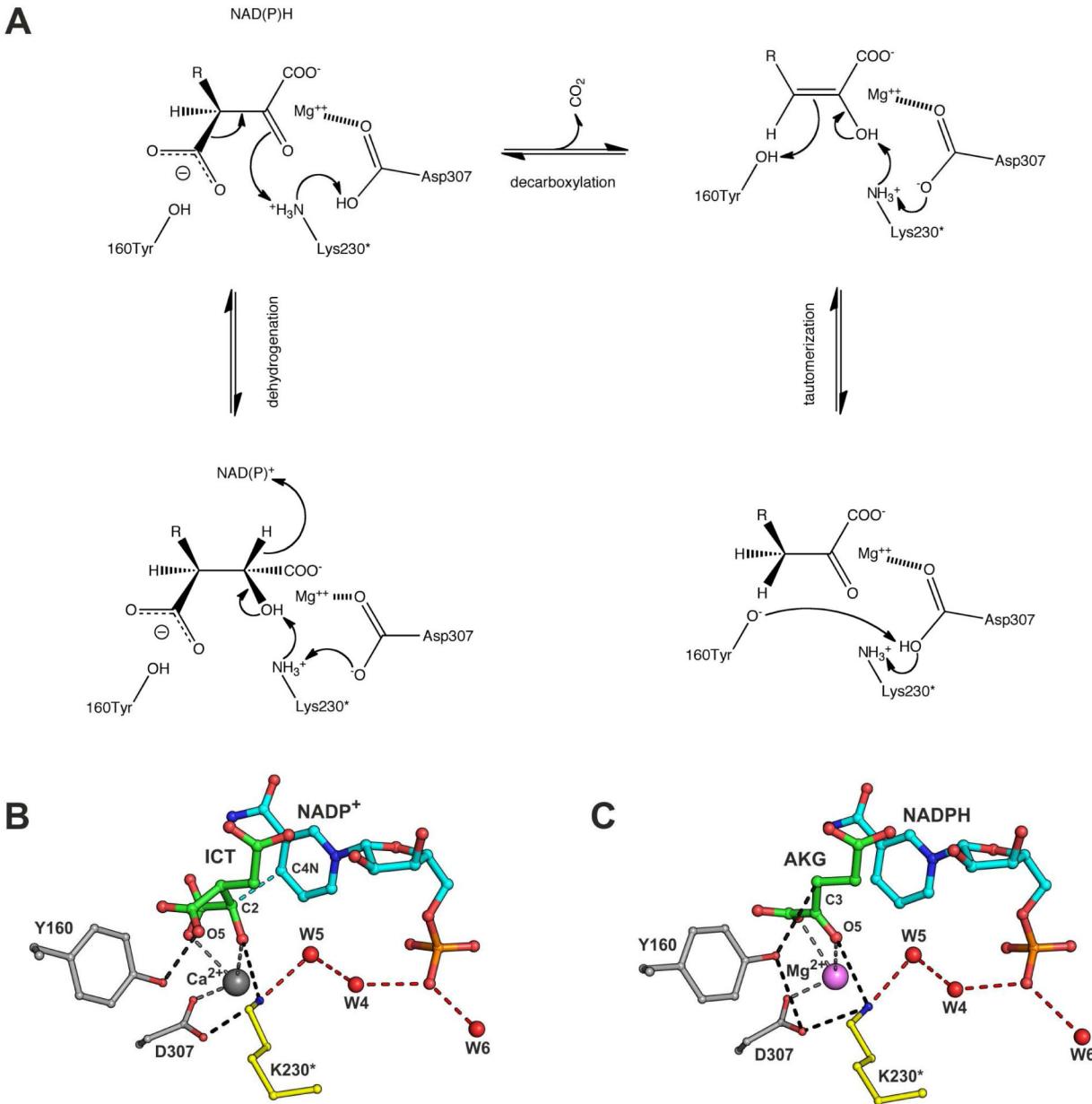


Figure 8. Structural evidence for the IDH catalytic mechanism after Aktas and Cook⁵
A) Scheme of the catalytic mechanism of isocitrate conversion to α -ketoglutarate in IDH. **B)** The *pseudo*-Michaelis complex of the *wt* [Ca²⁺:ICT:NADP⁺] structure mimics the dehydrogenation step, with Lys230* positioned to deprotonate the isocitrate C2 hydroxyl and the nicotinamide ring C4 poised to receive the C2 hydride. **C)** Products trapped in the K100M [Mg²⁺:ICT:NADP⁺] ternary complex mirror the reciprocal (de)protonations by Lys230* at the C2 carbonyl and Tyr160 at C3 during the tautomerization of α -ketoglutarate following decarboxylation of the oxaloacetate intermediate. Dashed lines indicate important interactions or hydrogen coordination bonds and are color-coded black for the protonation/deprotonation interactions during conversion of isocitrate into α -ketoglutarate, red for a proton relay possibly involved in acid/base catalysis, and gray/violet for interactions with the Ca²⁺/Mg²⁺ metal ion respectively. The catalytic triad residues and the ligands are shown as ball-and-stick with waters and metal ions shown as spheres. Atom

colors are gray for calcium, violet for magnesium, orange for phosphorus, blue for nitrogen and red for oxygen, with carbons colored cyan for NADP⁺/NADPH, green for isocitrate/α-ketoglutarate, and light gray and yellow for residues in the first and second subunits of *EcoIDH*, respectively.

Data collection and processing statistics^a**Table 1**

Data Sets	wt [Ca ²⁺ :ICl:NADP ⁺]	K100M [Mg ²⁺ :ICl:NADP ⁺]	K100M [Mg ²⁺ :ICl:NADP ⁺] × 1	K100M [Mg ²⁺ :ICl:NADP ⁺] × 2	K100M [Ca ²⁺ :α-KG:NADP ⁺]	K100M [Mg ²⁺ :ICl:thio-NADP ⁺]	wt [Ca ²⁺ :ICl:thio-NADP ⁺]
Beamline	In house	ESRF ID23-1	ESRF ID14-4	In house	SL S PXIII	SL S PXIII	SL S PXIII
Detector	Protein Pt.35	ADSC Q315r	ADSC Q315r	Protein Pt135	Mar225	Mar225	Mar225
Processing packages	ProteinSuite, CCP4	XDS, CCP4	XDS, CCP4	Protein Suite, XPREP, CCP4	XDS, CCP4	XDS, CCP4	XDS, CCP4
Wavelength (Å)	1.5418	0.9795	0.9535	1.5418	0.9792	0.9792	0.9792
Space group				P4 ₃ 2 ₁ 2			
Cell parameters (Å)	a = b = 105.30 c = 145.72	a = b = 103.58 c = 149.68	a = b = 105.79 c = 145.49	a = b = 103.68 c = 149.58	a = b = 103.05 c = 150.34	a = b = 104.01 c = 149.56	a = b = 104.01 c = 149.56
Resolution (Å)	47.10–1.90 (2.00–1.90)	50.00–1.80 (1.91–1.80)	60.00–2.69 (2.85–2.69)	46.37–2.30 (2.40–2.30)	48.74–1.90 (2.01–1.90)	50.0–1.80 (1.91–1.80)	50.0–1.80 (1.91–1.80)
Nr. observations	370445 (30740)	851107 (128548)	203269 (30161)	557631 (40200)	468880 (71214)	552349 (85244)	552349 (85244)
Nr. unique reflections	65181 (9149)	75372 (11833)	23556 (3636)	36981 (4332)	64372 (9910)	76263 (11806)	76263 (11806)
Completeness (%)	99.9 (100.0)	99.7 (98.5)	99.5 (97.5)	100.0 (100.0)	99.4 (96.1)	99.4 (96.7)	99.4 (96.7)
Multiplicity	5.7 (3.4)	11.3 (10.8)	8.6 (8.3)	15.1 (9.3)	7.3 (7.2)	7.2 (7.2)	7.2 (7.2)
<I/σ(I)>	11.2 (1.7)	12.3 (2.6)	15.2 (2.8)	24.8 (6.1)	18.2 (3.2)	17.7 (2.7)	17.7 (2.7)
R-merge (%) ^b	11.0 (54.0)	12.6 (70.8)	12.5 (83.5)	8.3 (31.4)	7.6 (54.5)	6.7 (62.6)	6.7 (62.6)
R-meas (%) ^b	—	13.1 (74.2)	13.3 (89.0)	—	8.2 (58.7)	7.2 (67.4)	7.2 (67.4)
R-pim (%) ^b	4.9 (34.8)	—	—	2.1 (10.8)	—	—	—
Wilson B (Å ²)	18.7	30.6	51.6	32.7	31.2	31.8	31.8

^aValues in parentheses refer to the highest resolution shell;^bR-merge = merging R-factor, $(\sum_{hkl} \sum_i |I_i(hkl) - \langle I(hkl) \rangle| / \sum_{hkl} \sum_i |I_i(hkl)|) \times 100\%$; R-meas = multiplicity independent R-factor, $\sum_{hkl} [N_{hkl}/(N_{hkl}-1)]^{1/2} \sum_i |I_i(hkl) - \langle I(hkl) \rangle| / \sum_{hkl} \sum_i |I_i(hkl)| \times 100\%$; 65; R-pim = precision independent R-factor, $\sum_{hkl} [1/(N_{hkl}-1)]^{1/2} \sum_i |I_i(hkl) - \langle I(hkl) \rangle| / \sum_{hkl} \sum_i |I_i(hkl)| \times 100\%$, where $I_i(hkl)$ is the observed intensity, $\langle I(hkl) \rangle$ is the average intensity of multiple observations from symmetry-related reflections, and N_{hkl} is their multiplicity.

Refinement Statistics

Table 2

	<i>wt</i> [Ca ²⁺ :ICT:NADP ⁺]	K100M [Mg ²⁺ :ICT:NADP ⁺] × 1	K100M [Mg ²⁺ :ICT:NADP ⁺] × 2	K100M [Ca ²⁺ :α-KG:NADPH]	K100M [Mg ²⁺ :ICT:NADP ⁺]	<i>wt</i> [Ca ²⁺ :ICT:NADP ⁺]
Resolution limits (Å)	44.81–1.90 (1.97–1.90)	46.34–1.80 (1.86–1.80)	52.90–2.70 (2.80–2.70)	44.30–2.30 (2.38–2.30)	48.90–1.90 (1.97–1.90)	46.5–1.80 (1.86–1.80)
R-factor ^a	0.190 (0.351)	0.170 (0.253)	0.160 (0.230)	0.175 (0.233)	0.165 (0.242)	0.183 (0.270)
nr. reflections ^b	61806 (6069)	71597 (6867)	22359 (2362)	34197 (3324)	61051 (5951)	72371 (6824)
Free R-factor ^c	0.224 (0.347)	0.19 (0.284)	0.210 (0.292)	0.210 (0.278)	0.181 (0.263)	0.204 (0.286)
nr. reflections ^b	3272 (324)	3768 (358)	1195 (119)	1822 (203)	3235 (308)	3802 (331)
Overall coordinate error estimate (Å) ^d	0.58	0.38	0.66	0.64	0.42	0.37
<i>Model completeness and composition (%)</i>						
Regions omitted	-	Met 1	Met 1	Met 1	Met 1	Met 1
Mg ²⁺ /Ca ²⁺	Ca ²⁺	Mg ²⁺	Mg ²⁺	Ca ²⁺	Mg ²⁺	Ca ²⁺
NADP ⁺ /NADPH/thio-NADP ⁺	NADP ⁺	-	NADPH	-	thio-NADP	thio-NADP
NMN moiety	-	1	1	-	-	-
A2P moiety	-	1	-	1	-	-
ICT/α-KG	ICT	ICT	α-KG	α-KG	ICT	ICT
SO ₄ ³⁻	-	1	-	1	1	1
Loop conformation	“fully closed”	“quasi-closed”	“fully closed”	“quasi-closed”	“quasi-closed”	“quasi-closed”
Non-hydrogen protein atoms	3674	3814	3412	3716	3809	3818
Disordered residues	5	6	-	7	9	7
Solvent molecules	374	504	127	429	490	507
<i>Mean B values (Å²)^e</i>						
Protein main-chain	27.2	29.5	25.3	27.08	27.8	29.07
Protein side-chain	30.0	33.4	29.5	30.40	31.7	32.57
Ions and ligands	27.7	40.7	40.2	47.8	38.3	33.0
Solvent	38.7	40.1	27.4	42.9	42.4	42.7
<i>Model r.m.s. deviations from ideality</i>						
Bond lengths (Å)	0.007	0.008	0.008	0.007	0.007	0.007
Bond angles (°)	1.088	1.041	1.139	1.101	1.079	1.086

	wf [Ca ²⁺ :ICT:NADP ⁺]	K100M [Mg ²⁺ :ICT:NADP ⁺] × 1	K100M [Mg ²⁺ :ICT:NADP ⁺] × 2	K100M [Ca ²⁺ :α-KG:NADPH]	K100M [Mg ²⁺ :ICT:NADP ⁺]	wf [Ca ²⁺ :ICT:NADP ⁺]
Chiral centers (Å ³)	0.070	0.072	0.069	0.073	0.073	0.075
Planar groups (Å)	0.004	0.004	0.004	0.005	0.005	0.004
<i>Model validation f</i>						
% Ramachandran outliers	0.0	0.0	0.5	0.0	0.0	0.0
% Ramachandran favored	96.9	97.6	96.1	96.9	96.9	97.1
% Rotamer outliers	0.0	0.9	2.4	0.9	0.3	0.3
C ^B outliers	0	0	0	0	0	0
Clash score	6.00	7.01	9.37	8.81	5.55	6.30
PDB accession ID	4aj3	4qis	4qir	4ajc	4ajb	4ajc

^aR-factor = $\Sigma hkl |||F_{\text{O}}|| - |F_{\text{C}}||| / \Sigma hkl |F_{\text{O}}|$, where |F_O| and |F_C| are the observed and calculated structure factor amplitudes, respectively;

^bNo σ(F_O) cutoff;

^cCross-validation R-factor computed from a randomly chosen subset of 5% of the total number of reflections were not used during the refinement;

^dMaximum-likelihood estimate;

^eCalculated from isotropic or equivalent isotropic B-values;

^fCalculated with MolProbity 4.8

Secondary Structure Matching comparisons for *E. coli* IDH**Table 3***Escherichia coli*K12

PDB id	Reference	Resolution (Å)	Year	Rmsd-1 (Å)	Rmsd-2 (Å)	Ligands
<i>Escherichia coli</i> K12						
4aj3	This work	1.90	2012	0.00	1.01	Ca ²⁺ , ICT, NADP ⁺
4ajr	This work	2.70	2012	0.26	1.03	Mg ²⁺ , ICT, NADP ⁺
4ajs	This work	1.80	2012	1.01	0.00	Mg ²⁺ , ICT, NADP ⁺
4aja	This work	1.80	2012	0.94	0.19	Ca ²⁺ , ICT, thio-NADP ⁺
4ajb	This work	1.90	2012	1.12	0.18	Mg ²⁺ , ICT, thio-NADP ⁺
4ajc	This work	2.30	2012	0.95	0.21	Ca ²⁺ , α-KG, NADPH
1sj8	53	2.42	1997	2.36	2.08	-
3icd	38	2.50	1989	0.98	0.33	-
4icd	66	2.50	1990	0.97	0.34	-
5icd	22	2.50	1990	1.04	0.32	Mg ²⁺ , ICT
6icd	22	2.80	1990	0.96	0.36	-
7icd	22	2.40	1990	0.95	0.36	-
8icd	22	2.50	1990	1.00	0.32	Mg ²⁺ , ICT
9icd	13	2.50	1991	0.90	0.35	NADP ⁺
1ika	67	2.70	1993	1.16	0.85	Ca ²⁺ , α-KG
1idc	23	2.50	1995	0.95	0.45	Mg ²⁺ , OXS
1idd	23	2.50	1995	1.04	0.46	-
1ide	23	2.50	1995	0.99	0.48	Mg ²⁺ , ICT, NADP ⁺
1idf	23	2.50	1995	0.99	0.51	-
1gro	24	2.50	1995	1.01	0.33	Mg ²⁺ , ICT
1grp	24	2.50	1996	1.01	0.31	Mg ²⁺ , ICT
1iso	68	1.90	1996	1.05	0.37	NAD ⁺
1ai2	27	1.90	1997	1.05	0.38	Ca ²⁺ , ICT, NADP ⁺
1a3	27	1.90	1997	1.03	0.37	Mg ²⁺ , ICT, NHDP ⁺
1b15	28	2.50	1998	1.04	0.39	Mg ²⁺ , α-KG, NADPH

PDB id	Reference	Resolution (Å)	Year	Rmsd-1 (Å)	Rmsd-2 (Å)	Ligands
1p8f	69	1.85	2000	1.03	0.16	Mg ²⁺ , ICT, SO ₄ ²⁻
1pb1	69	1.70	2000	0.97	0.21	ICT, SO ₄ ²⁻
1pb3	70	1.70	2000	0.98	0.19	-
1cw1	25	2.10	2000	1.01	0.24	Mn ²⁺ , ICT, SO ₄ ²⁻
1cw4	25	2.10	2000	1.03	0.24	Mn ²⁺ , α-KG, SO ₄ ²⁻
1cw7	25	2.60	2000	1.01	0.27	Mg ²⁺ , ICT, SO ₄ ²⁻
1hj6	29	2.00	2001	0.47	0.82	Mg ²⁺ , IPM, NADP ⁺
3lcb:C	55	2.90	2010	2.24	2.21	-
3lcb:D	55	2.90	2010	2.44	2.08	-
<i>Acidithiophilus thiooxidans</i> (UNIPROT Q8GAX0), 60.1% identity						
2d4v:A	56	1.90	2008	0.81	1.36	Citrate, NAD ⁺
2d4v:B				0.87	1.37	Citrate, NAD ⁺
2d4v:C				0.77	1.30	Citrate, NAD ⁺
2d4v:D				0.76	1.38	Citrate, NAD ⁺
<i>Burkholderia pseudomallei</i> Strain 1710b (UNIPROT Q31V82), 74.2% identity						
3dns	71	1.65	2011	0.92	0.62	-
<i>Aeropynum permix</i> ATCC 700893(UNIPROT Q9YE81), 45.2% identity						
1×kd:A	20	2.3	2005	2.24	1.94	Ca ²⁺ , ICT, NADP ⁺
1×kd:B				0.99	1.33	ICT, NADP ⁺
<i>Bacillus subtilis</i> Strain 168 (UNIPROT P39126), 65.0% identity						
1hq: A	72	1.55	2001	1.08	0.95	ICT
1hq: B				1.22	0.70	ICT

Rmsd-1 is calculated between C^α-atoms of matched residues at best 3D superposition of the query 4aj3 and target structures; Rmsd-2 is calculated between C^α-atoms of matched residues at best 3D superposition of the query 4aj5 and target structures; Ligand abbreviations: ICT, 2R,3S-isocitric acid/isocitrate; NADP⁺, β-Nicotinamide Adenine Dinucleotide Phosphate; NADPH, β-Nicotinamide Adenine Dinucleotide Phosphate (reduced); thio-NADP⁺, β-thio-Nicotinamide Adenine Dinucleotide Phosphate; NHDP, β-nicotinamide-(6-deamino-6-hydroxy-adenine)-dinucleotide phosphate; α-KG, α-ketoglutarate or 2-oxoglutarate; OXS, 2-oxosuccinate; IPM, isopropylmalate; NAD⁺, β-Nicotinamide Adenine Dinucleotide.

Summary of DynDom calculations for *E. coli* IDH crystal structures

Table 4

PDB id	Reference	Domain I r.m.s.d. (Å)	Domain II r.m.s.d. (Å)	Rotation (°)	Hinge residues	Conformation	NADP loop	P-loop
1sj5	53	-	-	0	-	Open	Out	Out
4aj3	This work	1.01	0.59	24.4	125–126, 323–329	Fully closed	In	In
4ajr	This work	0.99	0.56	24.5	125–127, 323–329	Fully closed	In	In
4ajs	This work	0.54	0.41	18.6	126–127, 322–327	Quasi-closed	Out	Out
4aja	This work	0.53	0.40	18.7	126–127, 324–328	Quasi-closed	Out	Out
4ajb	This work	0.57	0.42	18.3	126–127, 323–327	Quasi-closed	Out	Out
4ajc	This work	0.53	0.41	18.8	126–127, 323–325	Quasi-closed	Out	Out
3icd	38	0.56	0.36	19.7	126–128, 323–324	Quasi-closed	Out	Out
4icd	66	0.56	0.36	19.8	126–128, 323–324	Quasi-closed	Out	Out
5icd	22	0.56	0.43	19.4	125–127, 323–324	Quasi-closed	Out	Out
6icd	22	0.58	0.38	19.7	125–128, 323–324	Quasi-closed	Out	Out
7icd	22	0.56	0.38	19.8	126–128, 323–324	Quasi-closed	Out	Out
8icd	22	0.56	0.41	19.5	126–128, 324–327	Quasi-closed	Out	Out
9icd	13	0.55	0.36	19.8	126–127, 323–324	Quasi-closed	Out	Out
l1ka	67	0.97	0.84	20.0	126–127, 136, 323–328	Quasi-closed	Out	Out
l1dc	23	0.59	0.44	20.0	126–128, 134, 323–324	Quasi-closed	Out	Out
l1dd	23	0.67	0.45	19.7	126–127, 323–324	Quasi-closed	Out	Out
l1de	23	0.66	0.45	19.7	126–127, 323–328	Quasi-closed	Out ^a	Out
l1df	23	0.60	0.48	20.0	126–127, 323–328	Quasi-closed	Out	Out
1gro	24	0.56	0.38	19.5	126–128, 323–328	Quasi-closed	Out	Out
1grp	24	0.55	0.39	19.5	126–128, 323–328	Quasi-closed	Out	Out
1iso	68	0.65	0.40	19.2	126–128, 323–324	Quasi-closed	Out	Out
1ai2	27	0.65	0.42	19.2	126–128, 323–329	Quasi-closed	Out	Out
1ai3	27	0.65	0.41	19.2	126–129, 321–328	Quasi-closed	Out	Out
1bi5	28	0.60	0.47	19.2	126–127, 324–328	Quasi-closed	Out	Out
1p8f	69	0.57	0.43	18.6	126–127, 322–324	Quasi-closed	Out	Out
1pb1	69	0.57	0.41	18.8	126–128, 322–324	Quasi-closed	Out	Out
1pb3	70	0.54	0.38	18.8	126–128, 319, 322–324	Quasi-closed	Out	Out

PDB id	Reference	Domain I r.m.s.d. (Å)	Domain II r.m.s.d. (Å)	Rotation (°)	Hinge residues	Conformation	NADP loop	P-loop
1cw1	25	0.57	0.38	18.6	126–128, 319, 322–324	Quasi-closed	Out	Out
1cw4	25	0.57	0.38	18.6	126–128, 319, 322–324	Quasi-closed	Out	Out
1cw7	25	0.59	0.41	18.6	126–128, 319, 322–328	Quasi-closed	Out	Out
1hj6	29	0.78	0.57	19.7	126–128, 323–324	Quasi-closed	Out	Out
3lcb:C	55	1.29	0.38	2.4	^a	Open	Out	In
3lcb:D	55	1.28	0.38	1.4	^b	Open	Out	In

^abut with a different conformation;

^bDynDom failed due to very similar domain orientations. These values were obtained with LSQKAB.

Summary of Domain Motion calculations for selected IDHs

Table 5

PDB id	Reference	Domain II r.m.s.d. (Å)	Domain I r.m.s.d. (Å)	Rotation (°)	Conformation	NADP loop	P-loop
<i>Escherichia coli</i>							
1sj5	53	-	-	0°	Open	In	In
4aj3	This work	0.41	1.06	24.3	Fully closed	In	In
4ajr	This work	0.37	1.05	24.5	Fully closed	In	In
4ajs	This work	0.36	0.56	18.6	Quasi-closed	Out	Out
4aja	This work	0.33	0.56	18.7	Quasi-closed	Out	Out
4ajb	This work	0.37	0.60	18.3	Quasi-closed	Out	Out
4ajc	This work	0.35	0.54	18.9	Quasi-closed	Out	Out
1hj6	29	0.41	0.85	21.5	Quasi-closed	Out	In
9icd	13	0.29	0.57	19.7	Quasi-closed	Out	Out
<i>Acidithiophilus thiooxidans</i>							
2d4v:A	56	0.50	1.38	26.0	Fully closed	In	In
2d4v:B	0.48	1.37	26.1	Fully closed	In	In	
2d4v:C	0.51	1.36	25.0	Fully closed	In	In	
2d4v:D	0.50	1.36	25.7	Fully closed	In	In	
<i>Burkholderia pseudomallei</i>							
3dms	71	0.47	0.57	19.5	Quasi-closed	Out	Out ^a
<i>Aeropyrum pernix</i>							
1×kd:A	20	0.63	1.01	2.2	Open	In	Out ^a
1×kd:B	0.63	1.34	23.1	Fully closed	In	In	In
<i>Bacillus subtilis</i>							
1hq5:A	72	0.44	0.74	22.1	Quasi-closed	Out	In
1hq5:B	0.43	0.62	19.0	Quasi-closed	Out	In	In

^aincomplete loop in coordinate file.