Induced Fit and the Catalytic Mechanism of Isocitrate Dehydrogenase

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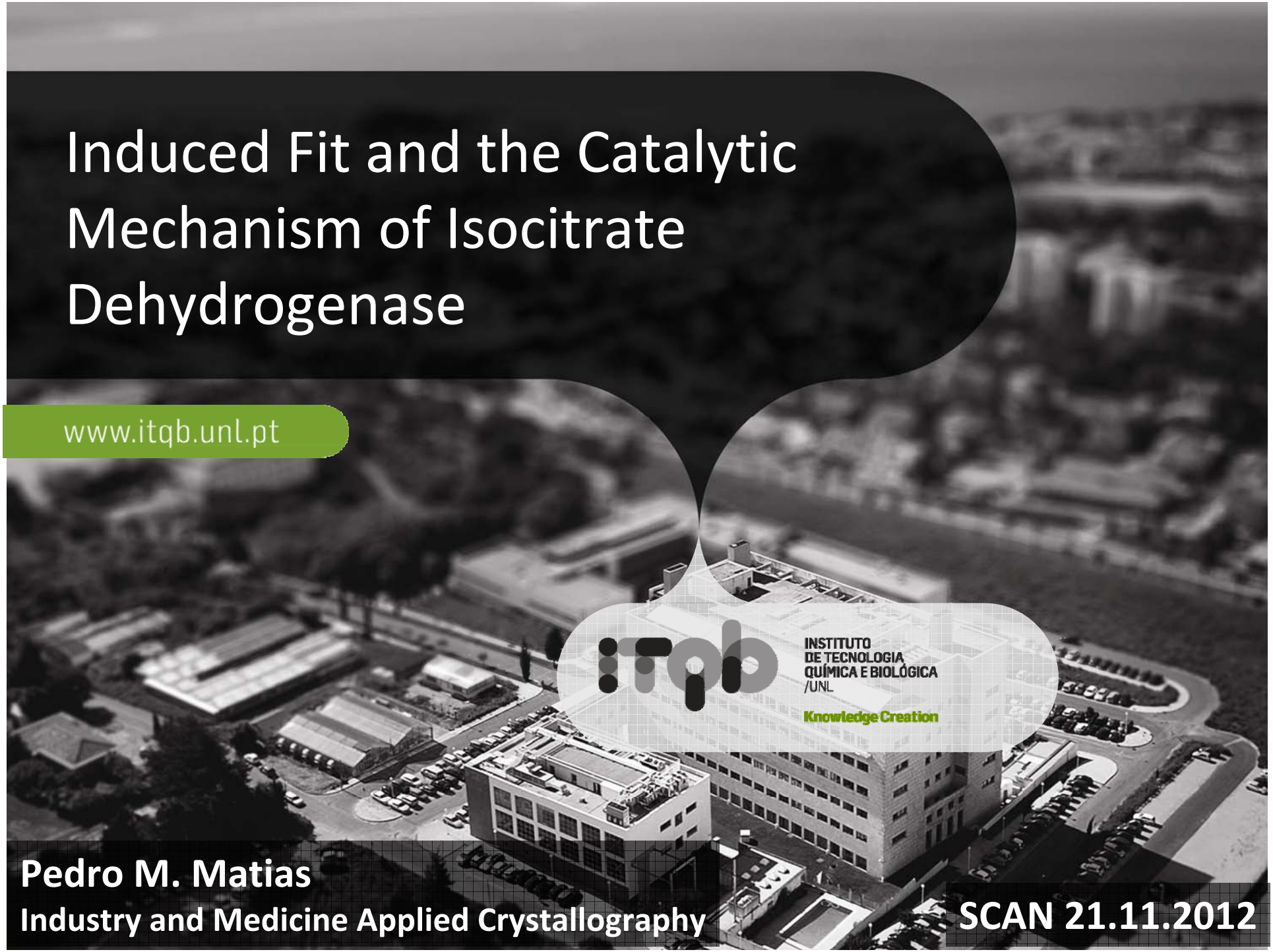


Pedro M. Matias
Industry and Medicine Applied Crystallography

SCAN 21.11.2012

Foreword

This presentation will cover in detail part of the PhD work of Susana Gonçalves, under my supervision.

This work began in the spring of 2008 in collaboration with Dr. Antony M. Dean and Dr. Stephen P. Miller (University of Minnesota) during Tony Dean's stay at IGC on sabbatical leave. This collaboration was brought about by Prof. Maria Arménia Carrondo.

At the time, Susana was struggling with the purification and crystallization of the proteins that were part of her thesis plan and the general idea was:

"This protein is very easy to crystallize, therefore the structural work should be straightforward."

It didn't take very long to find out how wrong we were.

Introduction to IDH

Isocitrate dehydrogenase (IDH):

- belongs to a large superfamily of decarboxylating dehydrogenases
- is involved in the citric acid (Krebs) cycle
- converts 2*R*,3*S*-isocitrate into α-ketoglutarate

5 known isoforms of IDH:

- IDH1, soluble, $NADP^+$ dependent [EC 1.1.1.42]
- IDH2, mitochondrial, $NADP^+$ dependent [EC 1.1.1.42]
- IDH3A, IDH3B and IDH3G are NAD^+ dependent [EC 1.1.1.41]

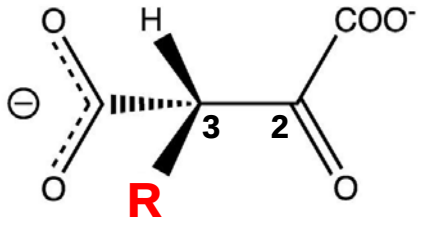
Introduction to IDH

To date, only 3D structures of **IDH1 isoforms** are known (human, bacterial, yeast).

IDH1 is regulated by phosphorylation (at Ser 113 in *E. Coli*) - prevents isocitrate binding.

Mutations of **IDH1** and **IDH2** have been found in some human cancer types.

Introduction to IDH



A substituted **2R-malate** core is the subtrate of decarboxylating dehydrogenases

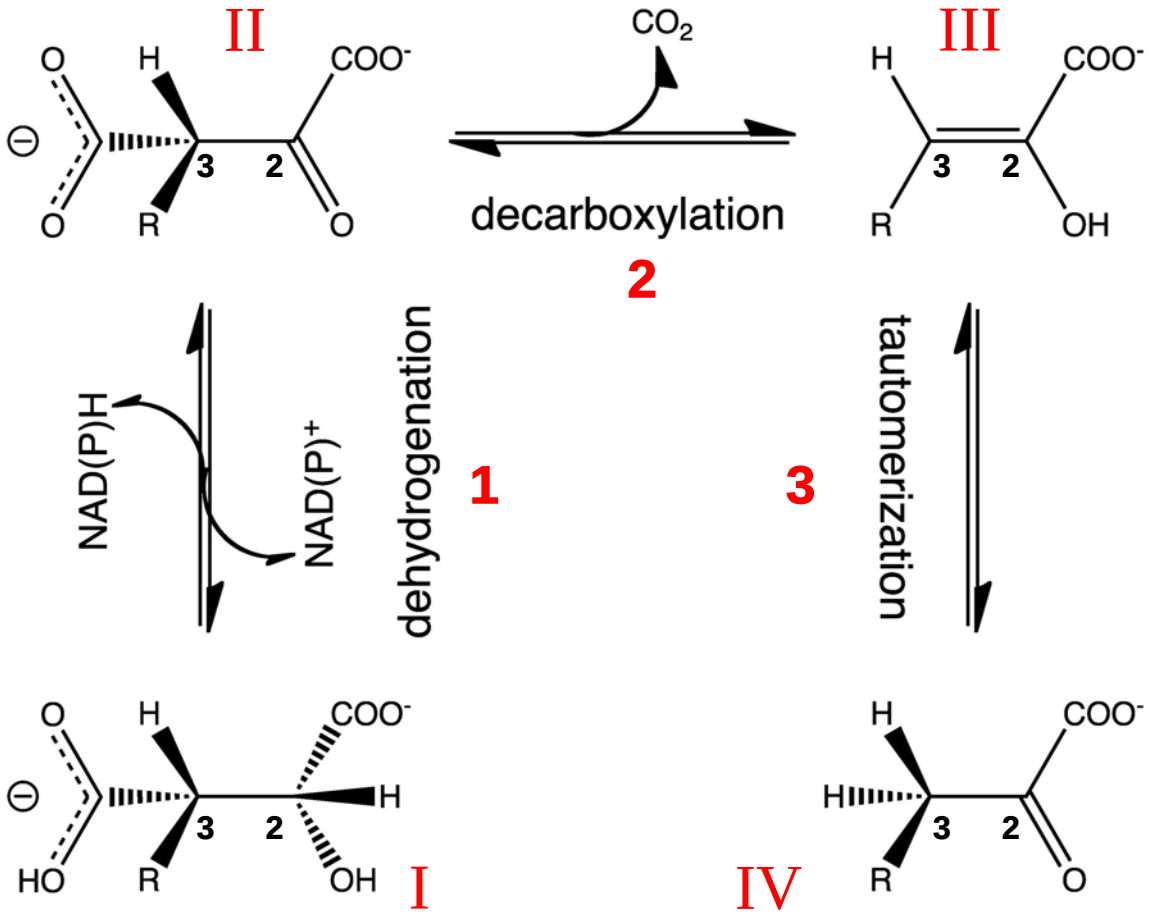
Decarboxylating dehydrogenase superfamily members:

- isocitrate (**R** = CH_2COO^-) [2*R*,3*S*]
- isopropylmalate (**R** = $CH(CH_3)_2$)
- homoisocitrate (**R** = $CH_2CH_2COO^-$)
- tartrate (**R** = OH)

Introduction to IDH

Decarboxylating dehydrogenases use a common 3-step mechanism:

1. Dehydrogenation at **C2**
2. Decarboxilation at **C3**
3. Tautomerization of the enol intermediate to the keto product



Introduction to IDH

All known IDHs require a divalent ion (Mg^{2+}) for catalysis

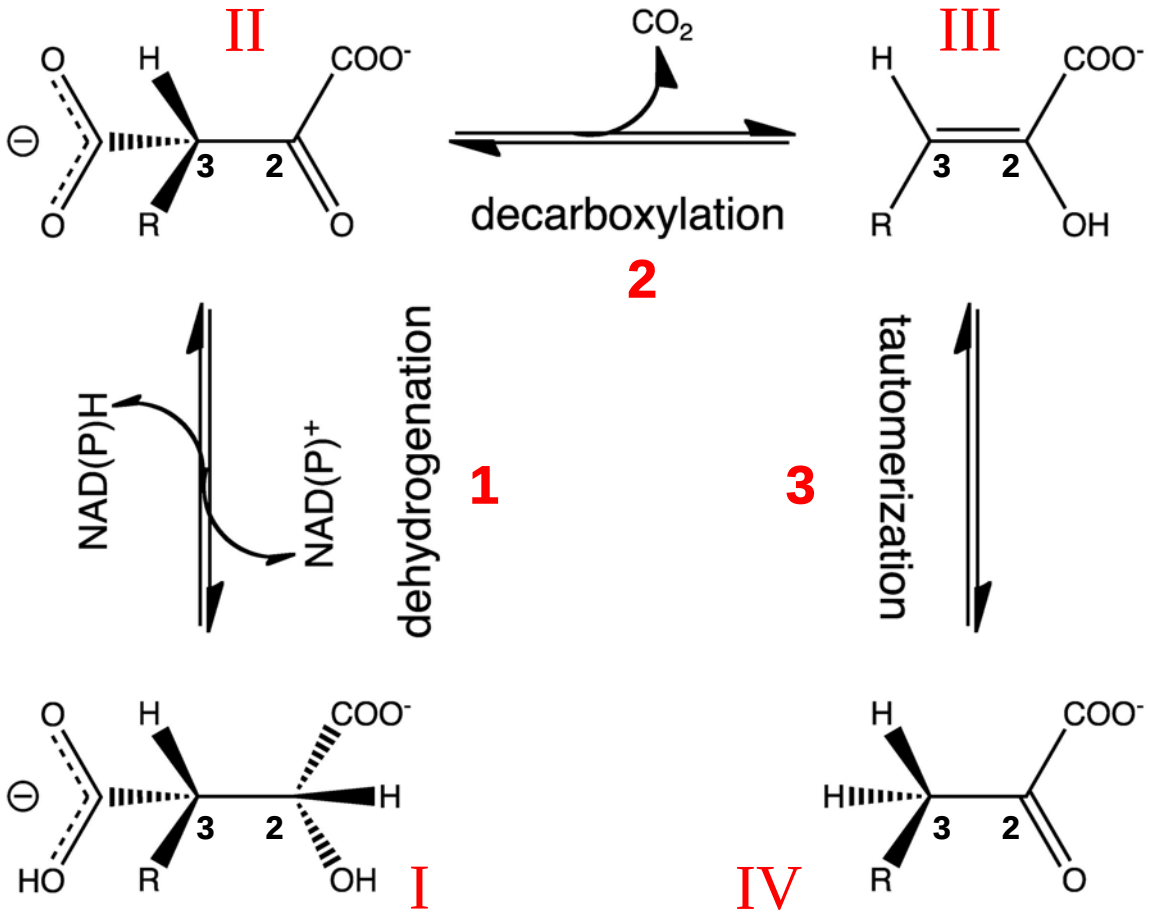
Mechanism requires:

Catalytic base
(proton abstraction from C2)

Catalytic acid
(protonation of C3 after decarboxylation)

Conflicting hypotheses have been made regarding their identities

3-step mechanism of IDH[1]:
(**R** = CH_2COO^-)



Introduction to IDH

E. Coli IDH (416 aa) is very easy to purify and crystallize.

In *wt E. coli* IDH, using **Ca^{2+}** as co-catalytic metal ion lowers K_{cat} by more than 2500-fold.

This was used in attempts to obtain 3D structures of a **pseudo-Michaelis complex**: **wtIDH:$NADP^+$:ICT:Ca^{2+}** by soaking and co-crystallization.

The **K100M** mutation in *E. coli* IDH reduces K_{cat} by a factor of 20,000.

This mutant was also used in attempts to obtain a 3D structure of a **pseudo-Michaelis complex**: **K100M IDH:$NADP^+$:ICT:Mg^{2+}** by soaking and co-crystallization.

Introduction to IDH

There are **27 crystal structures** of *E. Coli* IDH in the PDB:

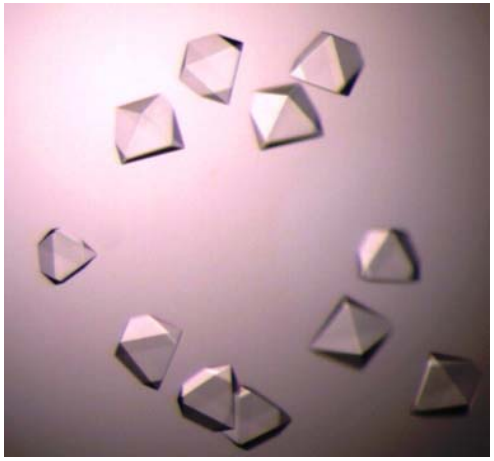
- None are representative of a true **pseudo-Michaelis complex**
- None are representative of a true **product complex**

Preparation of *E. coli* IDH crystal soaks

E. Coli IDH (wt and K100M) was produced in the U.S.A. by S. P. Miller

Crystallization buffer for *wt* IDH:
1.85 M $(NH4)_2SO_4$, 50 mM citric acid/Na_2HPO_4 pH 5.8, 0.1 M NaCl, 0.2 M DTT

Crystallization buffer for K100M IDH:
1.85 M $(NH_4)_2SO_4$, 50 mM citric acid/Na_2HPO_4 pH 5.2, 0.1 M NaCl, 0.2 M DTT



Crystals of ***wt* IDH** were soaked in solutions containing 52 mM Ca^{2+}, 300 mM isocitrate and 400 mM $NADP^+$ or thio-$NADP^+$ (1/2 ~ 3 hrs)

Crystals of **K100M IDH** were soaked in solutions containing 52 mM Mg^{2+} or Ca^{2+}, 300 mM isocitrate or 77 mM α-ketoglutarate, and 10 mM NADPH or 400-500 mM $NADP^+$ or thio-$NADP^+$ (1/2 ~ 3 hrs)

Data collection and structure refinement

A total of **27 datasets** were collected from IDH crystal soaks:
- 18 *in-house* (11 at room temperature)
- 6 at the ESRF (Grenoble, France)
- 2 at the SLS (Villigen, Switzerland)
- 1 at Diamond (Didcot, U.K.)

Data resolution: between **2.7** and **1.65** Å.
Data processing: XDS/CCP4 (synchrotron) and Proteum Suite (in-house)

IDH crystalizes in the tetragonal space group $P4_32_12$,
with cell parameters a ≈ 105 Å and c ≈ 150 Å

Structure determination - Molecular Replacement with PHASER
using PDB entry 1ai2 (Mesecar *et al.*, 1997) as the search model

Preliminary refinement – REFMAC5 in the CCP4 suite

Data collection and structure refinement

Several **problems** were faced and solved:

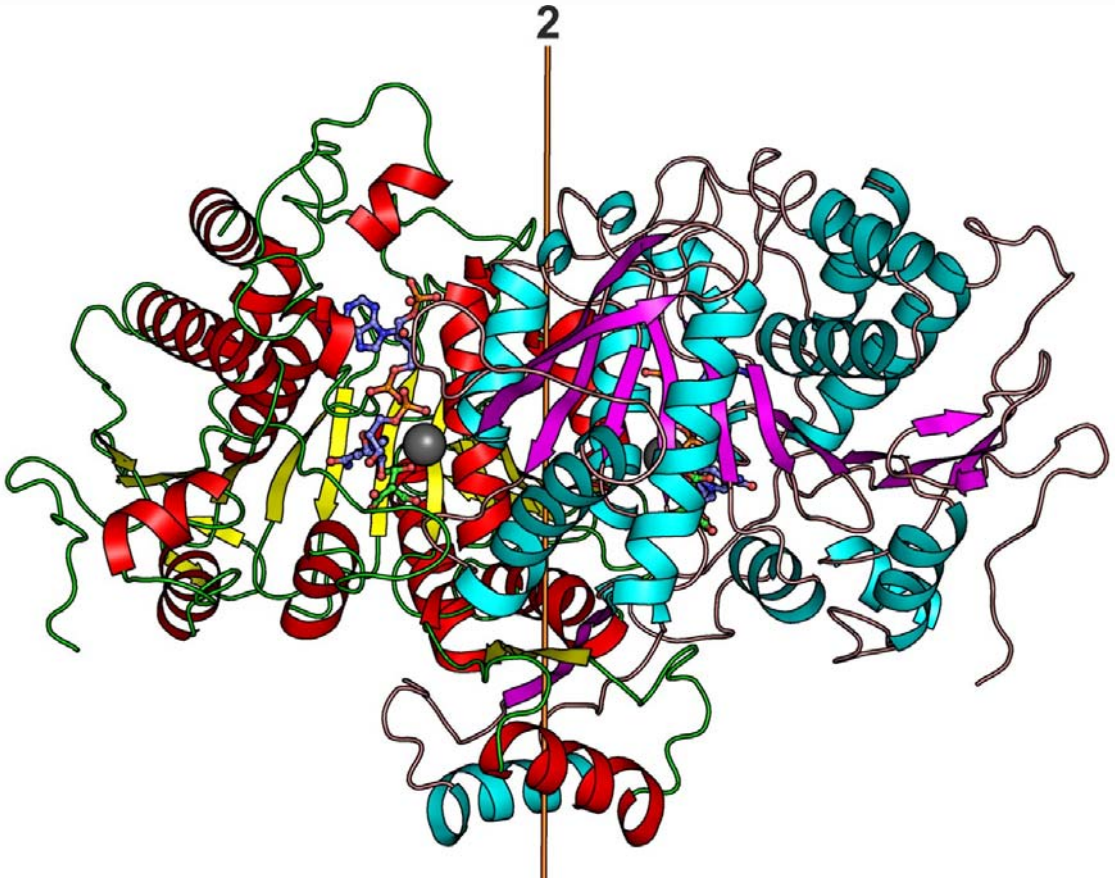
1. difficulty in finding proper **cryoprotecting conditions**
2. extreme **radiation damage** at RT (typical crystal lifetime ~ 2hrs)
3. no $NADP^+$ **binding**
4. **hydrolyzed** $NADP^+$ at the active site

6 datasets were selected for full structure refinement with PHENIX:

• **For the first time**, "**fully-closed**" enzyme conformations were obtained for one ***wt* IDH crystal soak** (**pseudo-Michaelis complex**) and one **K100M IDH crystal soak** (**product complex**)

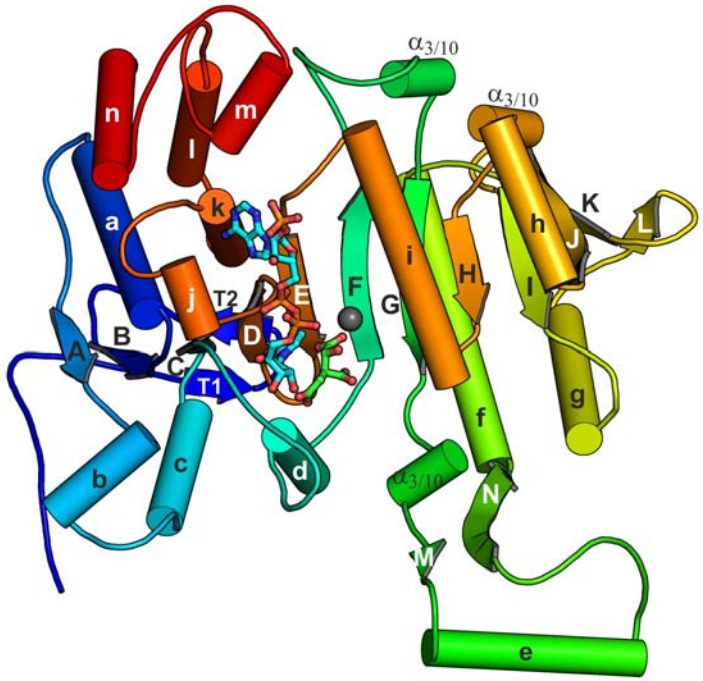
• The results **confirm** the details of the IDH catalytic mechanism proposed by Aktas and Cook (2009)

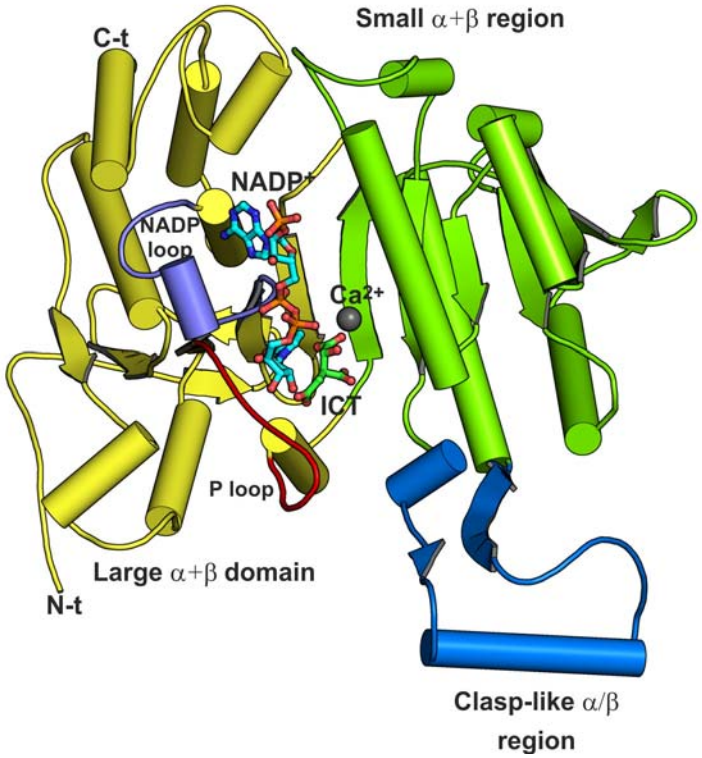
The 3D structure of *E. coli* IDH



- *E. Coli* IDH is a **homodimer**
- In nearly all published crystal structures the dimer is crystallographic.
- Each active site is formed by residues from both monomers

The 3D structure of *E. coli* IDH





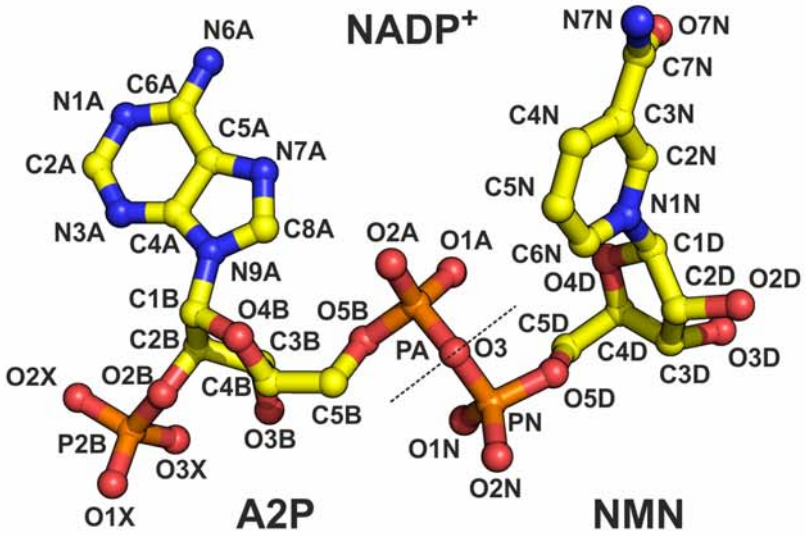
Domain structure of *E. Coli* IDH (1-416):

- Domain I, large α + β domain (1-124 and 318-416)
- Domain II, small α/β domain (125-317), includes a clasp-like α/β region

The 3D structure of *E. coli* IDH

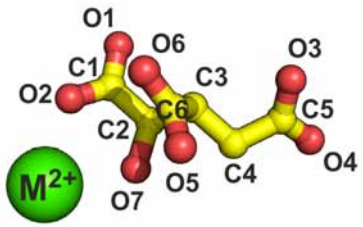
Cofactor

$NADP^+$ – β-Nicotinamide Adenine Dinucleotide Phosphate



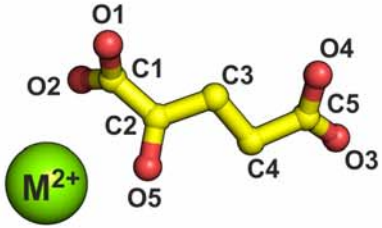
A2P – Adenosine 2',5'-diphosphate
NMN – Nicotinamide mononucleotide

Substrate



ICT

Product



AKG

ICT – 2*R*,3*S*-isocitrate
AKG – α-ketoglutarate
M^{2+} – co-catalytic metal ion

Structural dynamics and induced fit in IDH

In a crystal structure, IDH can be in **3** different conformations:

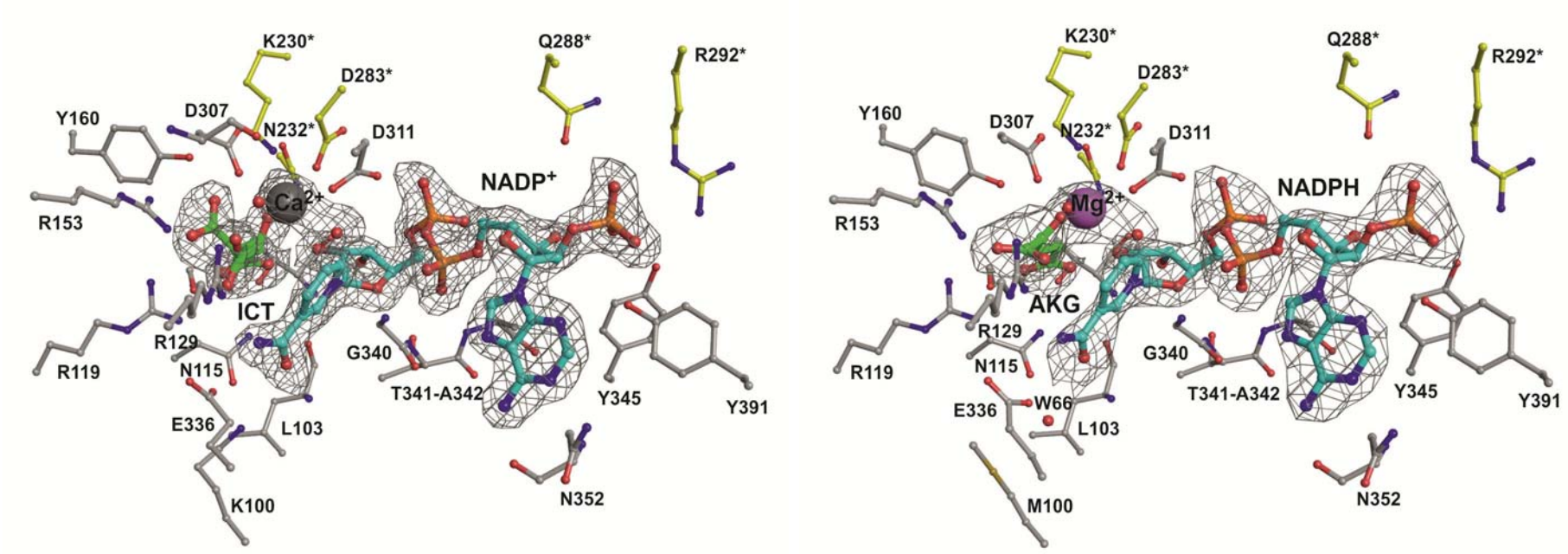
- **Open** (*E. coli* apo-isoform, PDB entry 1sjs, Finer-Moore et al, 1997)
- **Quasi-closed** (ALL other published *E. coli* IDH structures to date)
- **Fully closed** (4 IDH structures, 2 *E. coli* IDH structures from this work)

Structural dynamics and induced fit in IDH

The enzyme conformation can be evaluated by the **relative orientation** between Domain I (large) and Domain II (small) (**DYNDOM**/**LSQKAB** in the CCP4 suite):

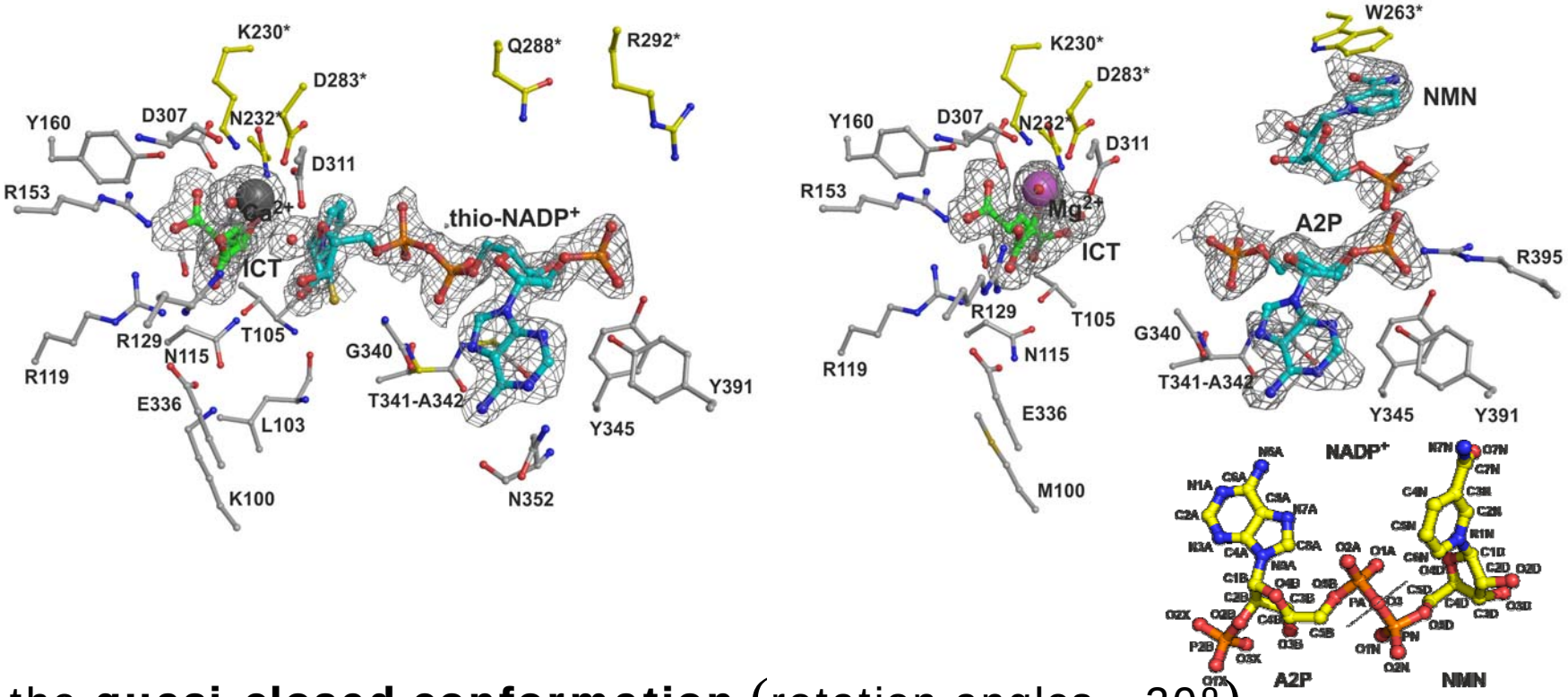
1. Superpose domain II of the **open isoform** with that of the test structure
2. Next, superpose domains I of both structures – obtain **rotation angle** of Domain I in the open structure needed to obtain the test structure
3. DYNDOM also identifies **rotation axes** and **hinge residues**
4. DYNDOM fails for small angles and non-identical structures – LSQKAB can be used instead after structural alignment in COOT

Structural dynamics and induced fit in IDH



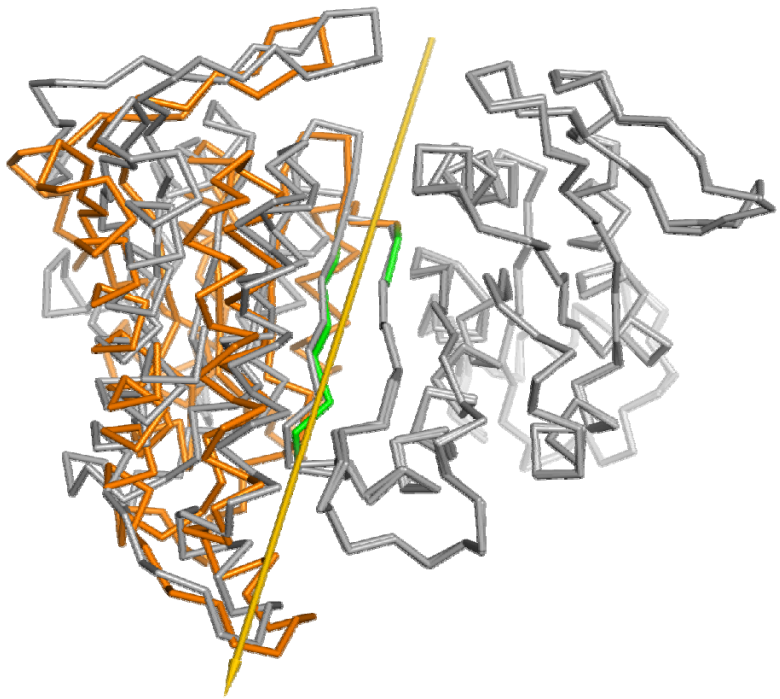
In the **fully closed conformation** (rotation angles larger than ~20º), the enzyme, co-factors and substrate can be in a **catalytically productive** conformation.

Structural dynamics and induced fit in IDH



In the **quasi-closed conformation** (rotation angles ~20°), the enzyme, co-factors and substrate are in a conformation that is **not** catalytically productive (**left**) and NADP+ may be partly **hydrolyzed** (**right**).

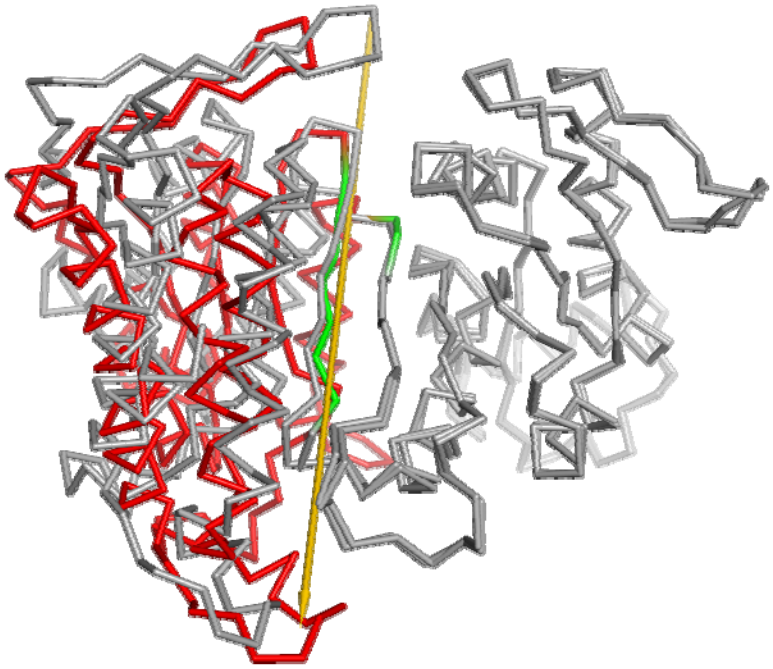
Structural dynamics and induced fit in IDH



Rotation axis is colored gold
Hinge residues are colored green
The rotation angle is **18.6º**

wt IDH structure (1sjs) in the open conformation (gray)
K100M IDH structure in the quasi-closed conformation (orange Domain I and gray Domain II)

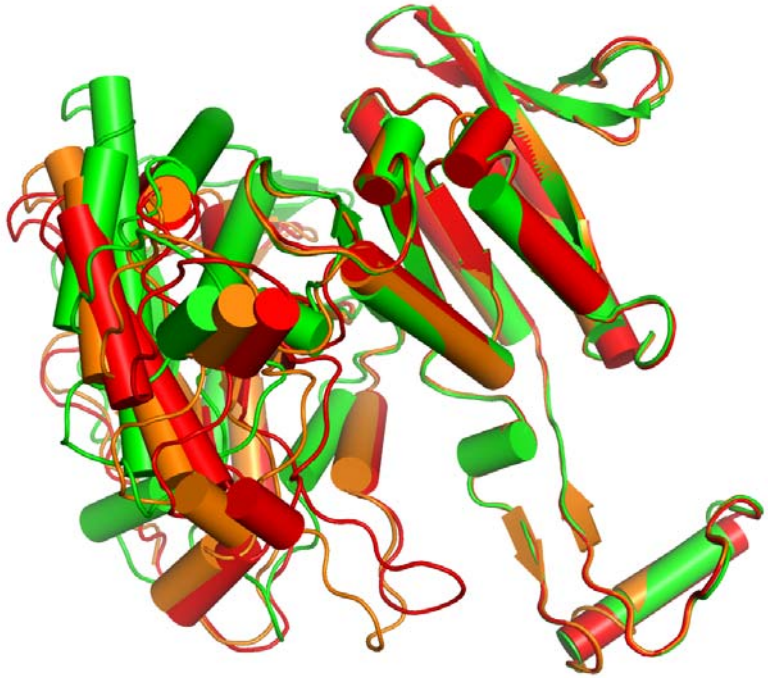
Structural dynamics and induced fit in IDH



Rotation axis is colored gold
Hinge residues are colored green
The rotation angle is **24.4º**

wt IDH structure (1sjs) in the open conformation (gray)
wt IDH structure in the fully closed conformation (red Domain I and gray Domain II)

Structural dynamics and induced fit in IDH

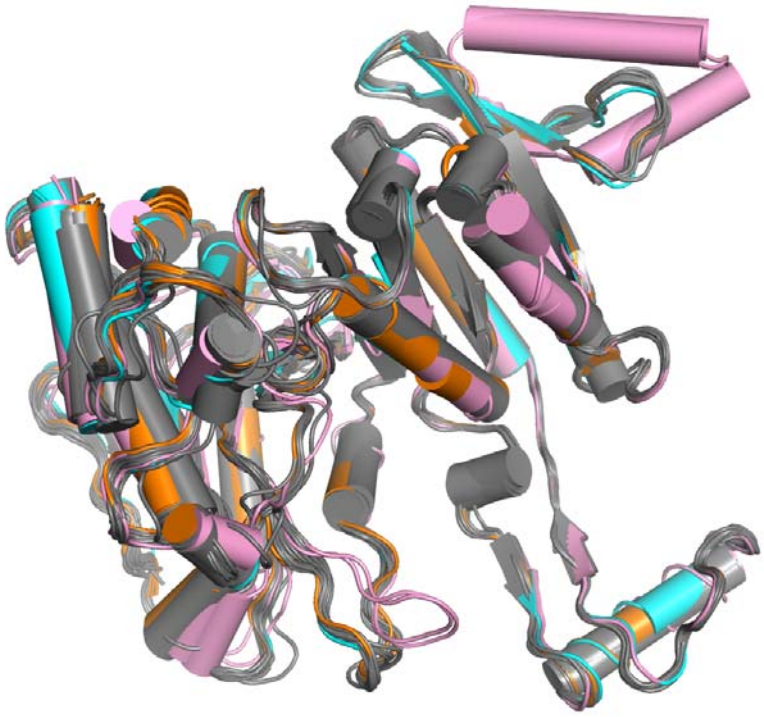


wt IDH structure (1sjs)
in the **open conformation**

K100M IDH structure
in the **quasi-closed conformation**

wt IDH structure
in the **fully closed conformation**

Structural dynamics and induced fit in IDH



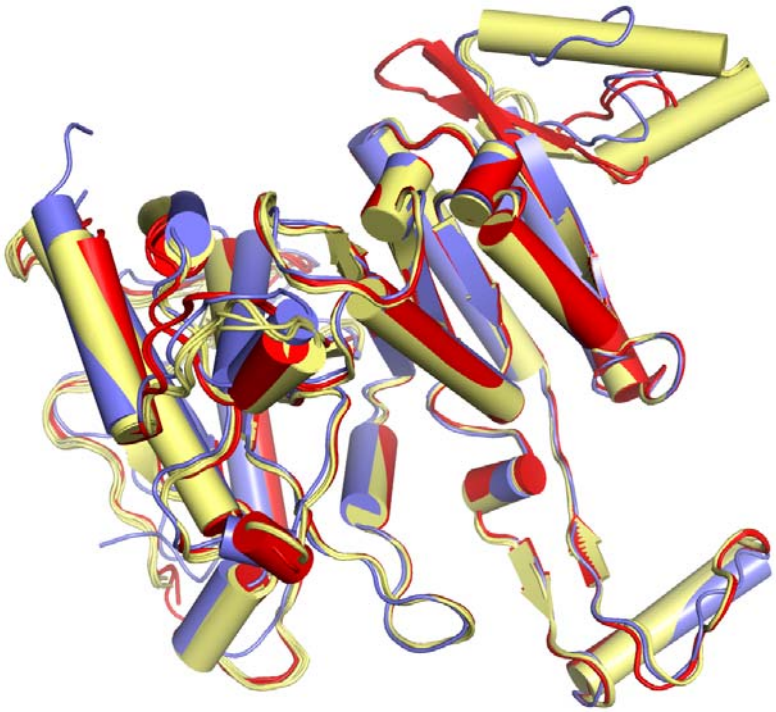
IDH structures
in the quasi-closed conformation:

E. coli *wt*/K100M (**this work**, **others**)

B. pseudomallei

B. subtilis (2 chains)

Structural dynamics and induced fit in IDH



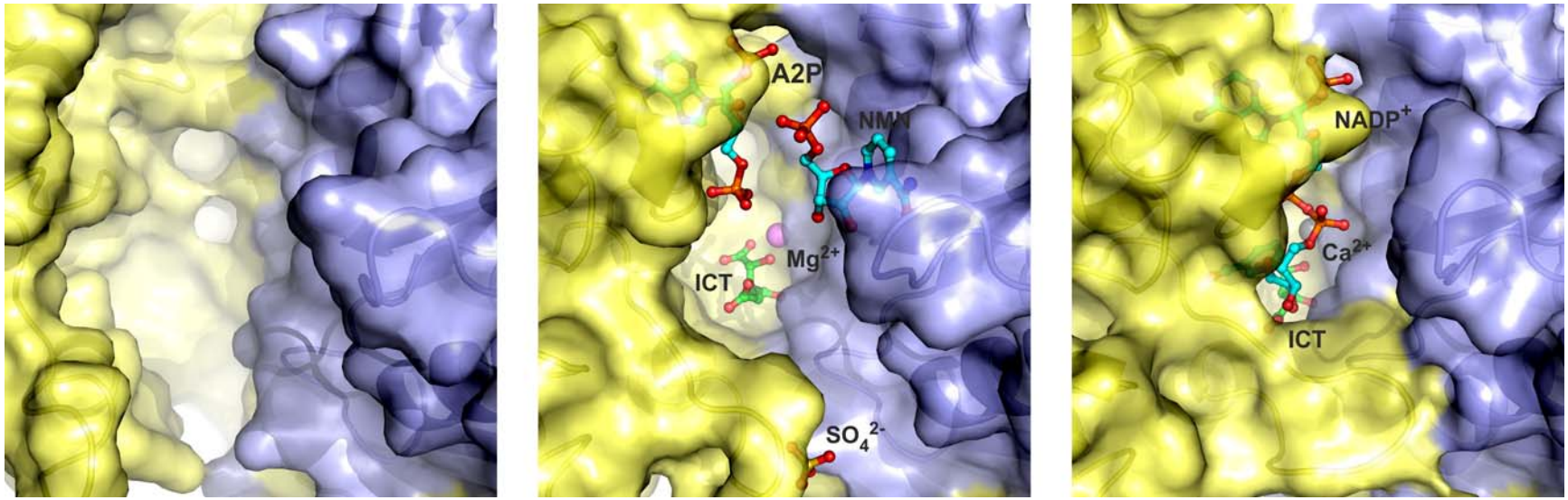
IDH structures
in the closed conformation:

***E. coli wt*/K100M** (this work)

A. pernix (chain B)

A. thiooxidans (4 chains)

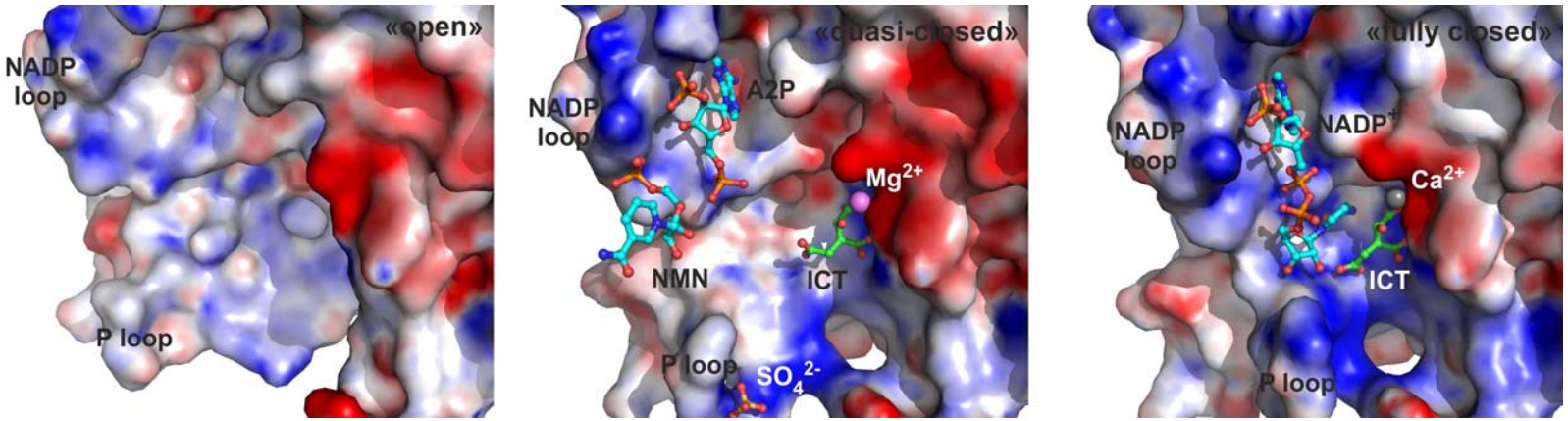
Structural dynamics and induced fit in IDH



Closure of the active site in *E. coli wt* IDH as the large domain rotates from the **open** (left) to the **quasi-closed** (centre) and **fully-closed** (right) conformations.

One IDH monomer is shown in yellow and the other in light blue.

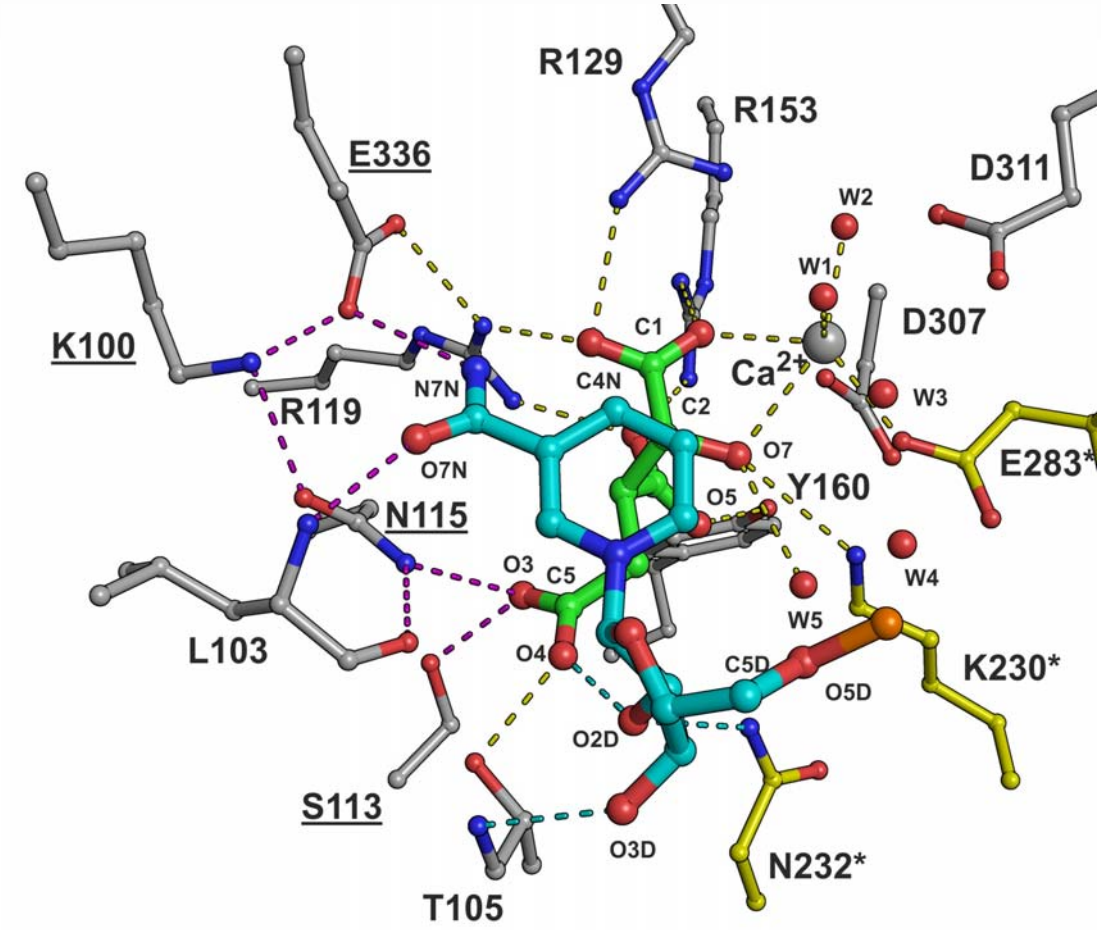
Structural dynamics and induced fit in IDH



Changes in the electrostatic potential landscape of the *E. coli wt* IDH active site from the **open** (left) to the **quasi-closed** (centre) and **fully-closed** (right) conformations.

The motion of the “NADP loop” and “P loop” is also evident.

The catalytic mechanism of IDH



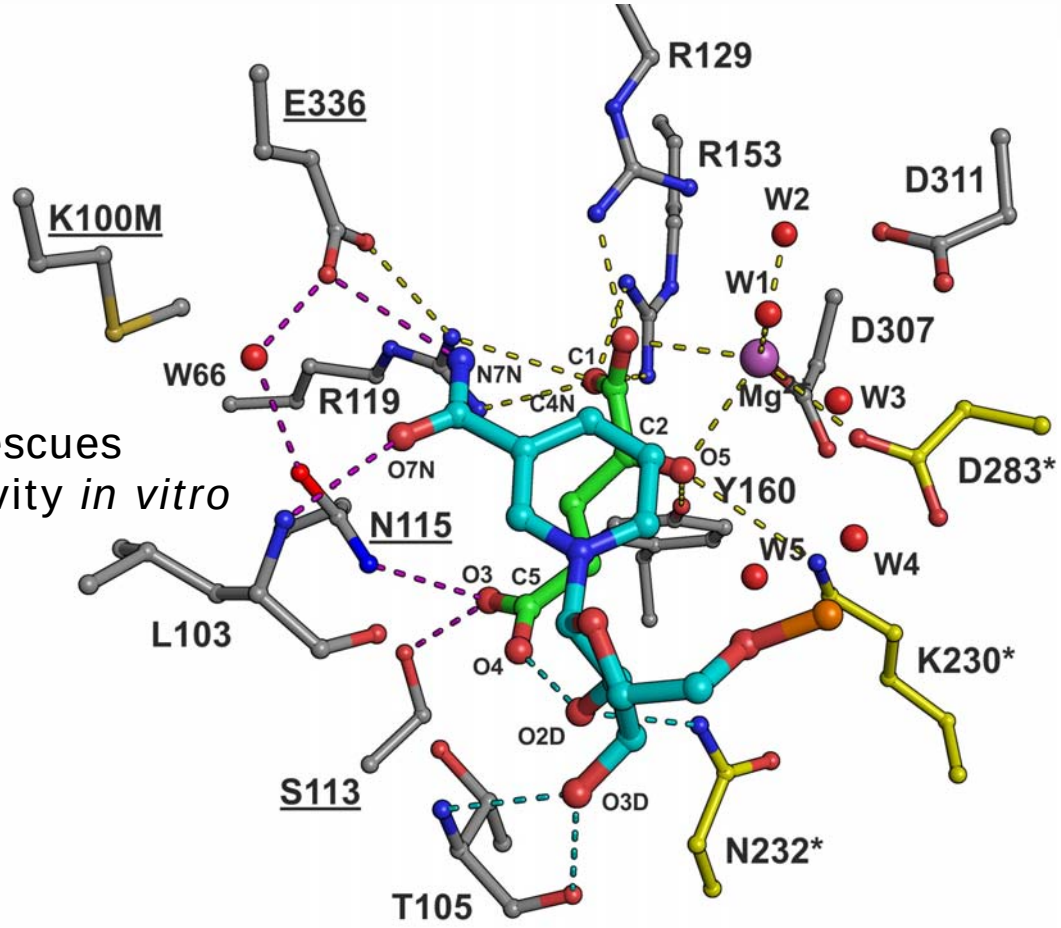
C2…C4N ~ 3 Å

The pseudo-Michaelis complex ***wt*** **IDH:NADP$^+$:ICT:Ca^{2+}**

The catalytic mechanism of IDH

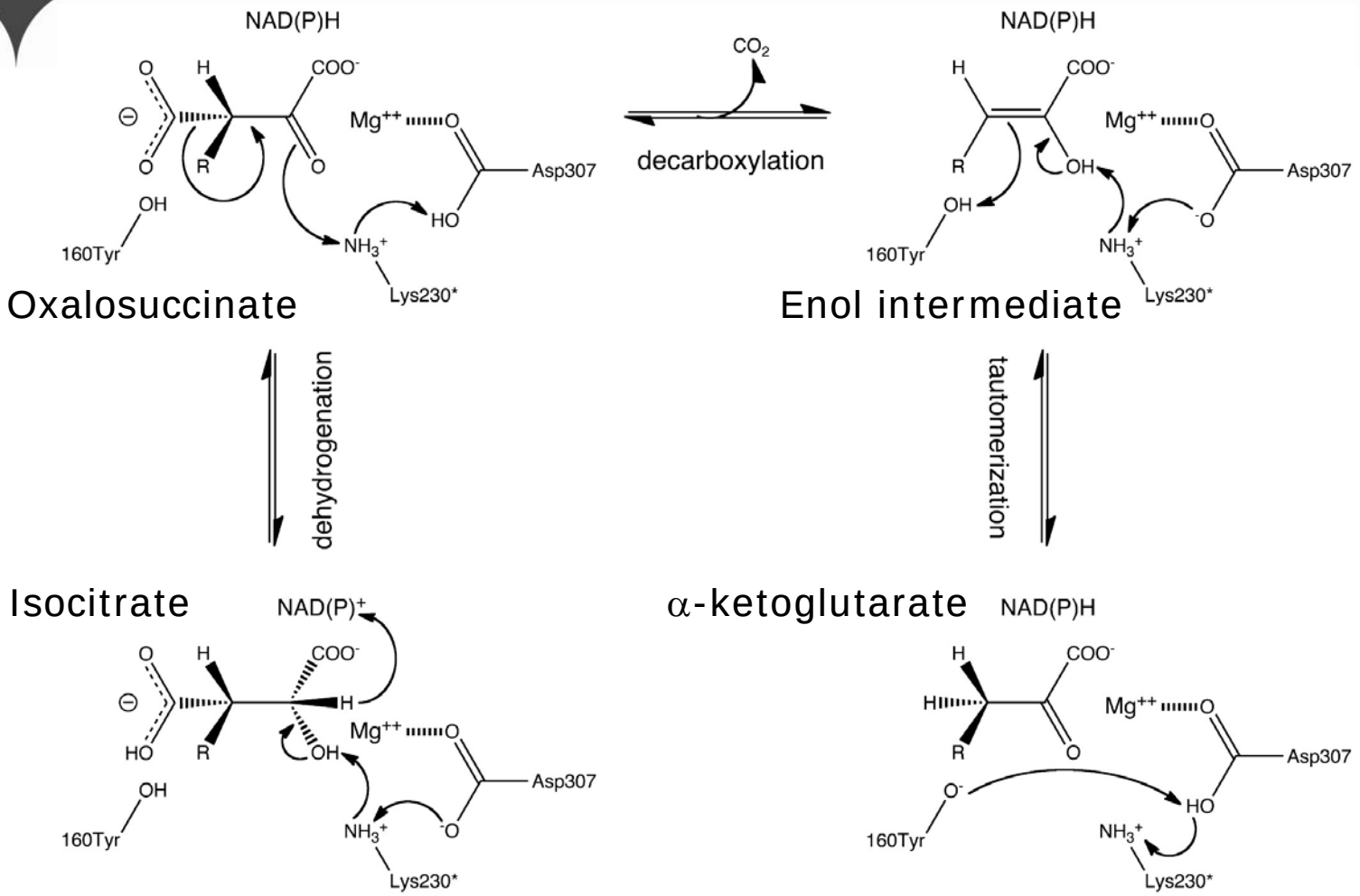
W66 ≈ NH_4^+ ?

NH_4^+ partially rescues K100M IDH activity *in vitro*



The product complex **K100M IDH:NADPH:AKG:Mg^{2+}**

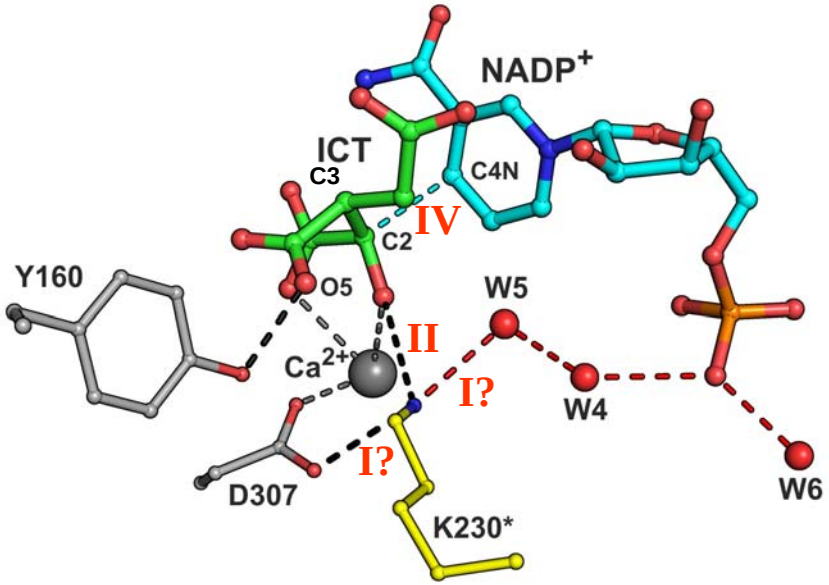
The catalytic mechanism of IDH

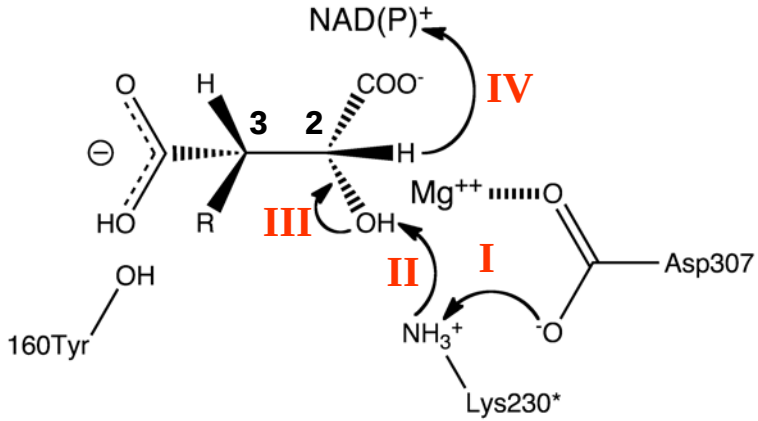


IDH mechanism proposed by Aktas and Cook (2009)

The catalytic mechanism of IDH

The pseudo-Michaelis complex ***wt* IDH:NADP$^+$:ICT:Ca^{2+}**





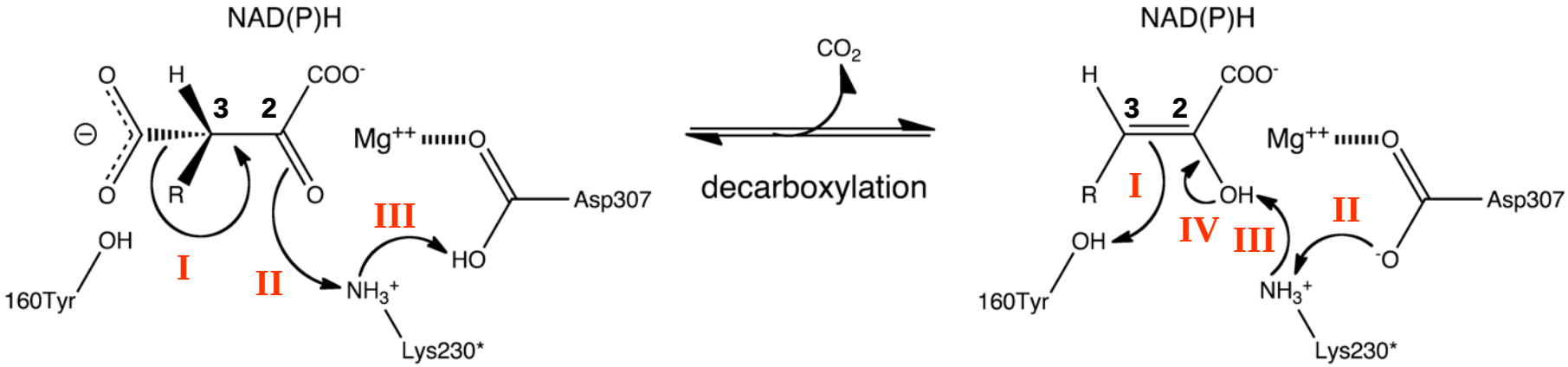
K230* is positioned (d = 3.2 Å) to initiate dehydrogenation by proton abstraction from OH at C2 (II)

D307 is hydrogen-bonded to K230* (d = 2.7 Å) (I)

C4N is poised to receive the hydride from C2 (d = 3.2 Å) (IV)

Isocitrate conversion to **oxalosuccinate**

The catalytic mechanism of IDH



Decarboxylation of oxalosuccinate (I)

C2 carbonyl reprotonated by K230* (II, III): **enol intermediate**

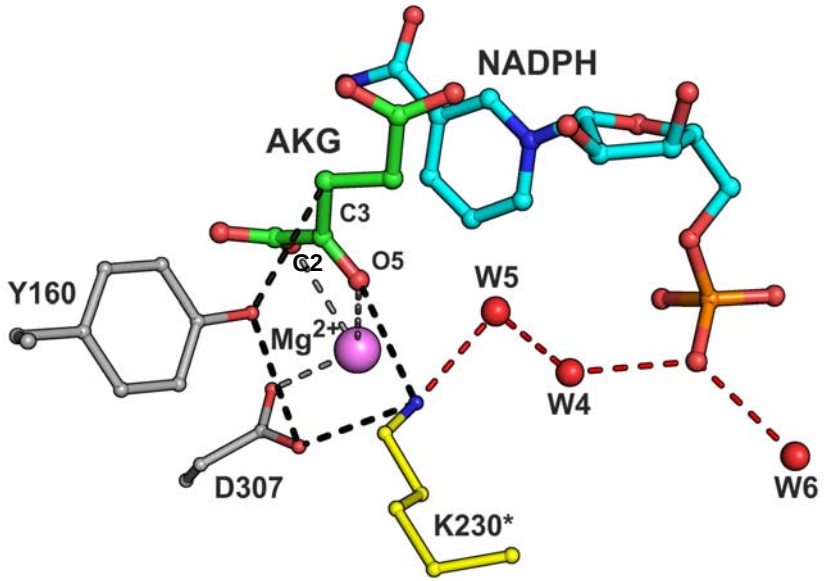
Tautomerization of the enol intermediate: **α-ketoglutarate**

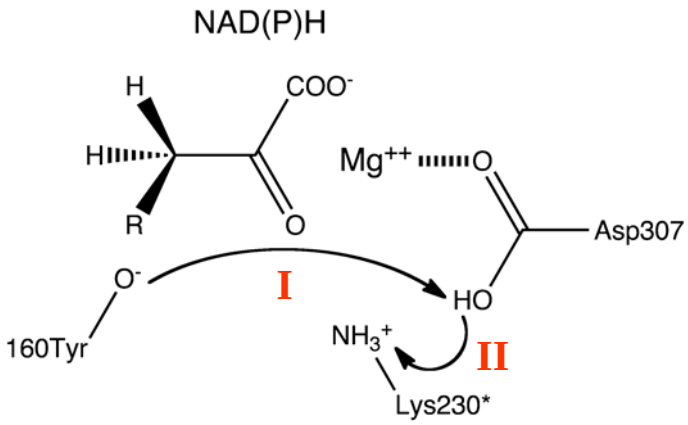
Y160 protonates C3 (I)

K230* deprotonates C2 hydroxyl (O5 in AKG) (II, III, IV)

The catalytic mechanism of IDH

The product complex **K100M IDH:NADPH:AKG:Ca^{2+}** was obtained by ICT turnover *in crystallum*





Y160 after C3 protonation (d = 3.4 Å)

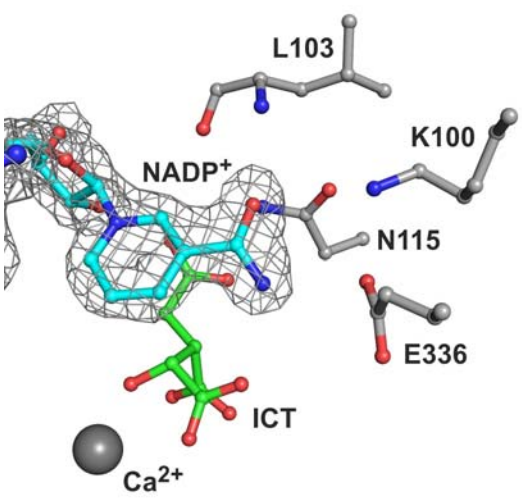
K230* after C2 hydroxyl deprotonation (O5 in AKG) (d = 3.3 Å)

Y160 re-protonation: D307 or a proton relay from bulk solvent (I, II)

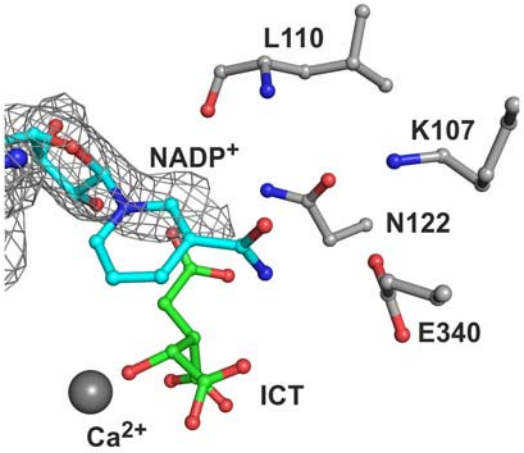
Conclusions

For the first time, “fully-closed” enzyme conformations were obtained:

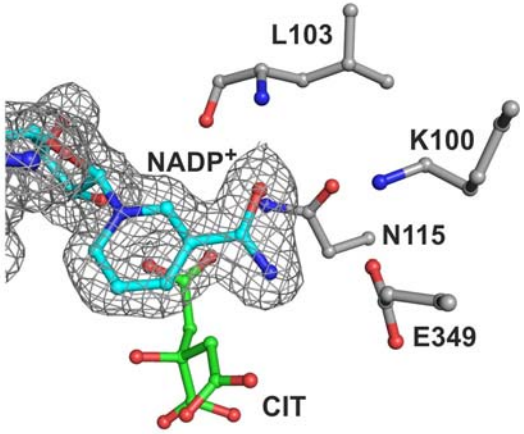
- one *wt* IDH crystal soak, representing a **pseudo-Michaelis complex**
- one K100M IDH crystal soak, representing a **product complex**



E. coli IDH
(this work)



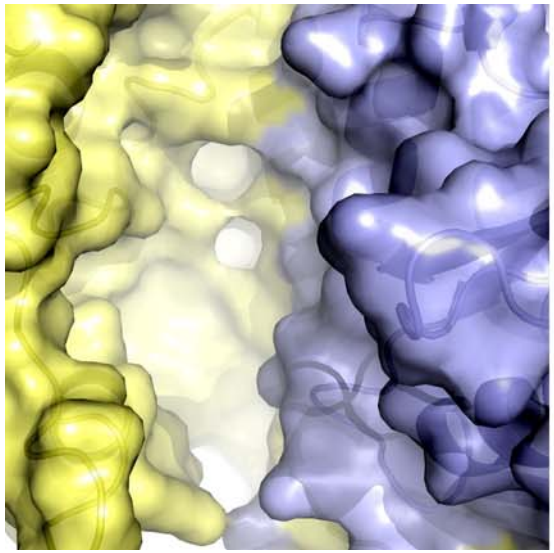
A. pernix IDH
(Karlstrom et al, 2005)



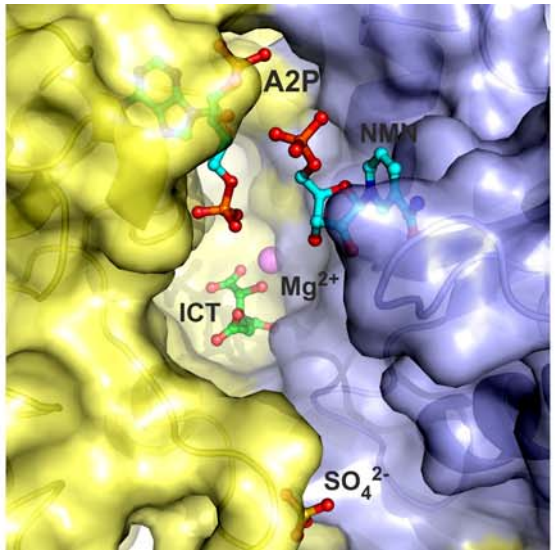
A. thiooxidans IDH
(Imada et al, 2008)

Conclusions

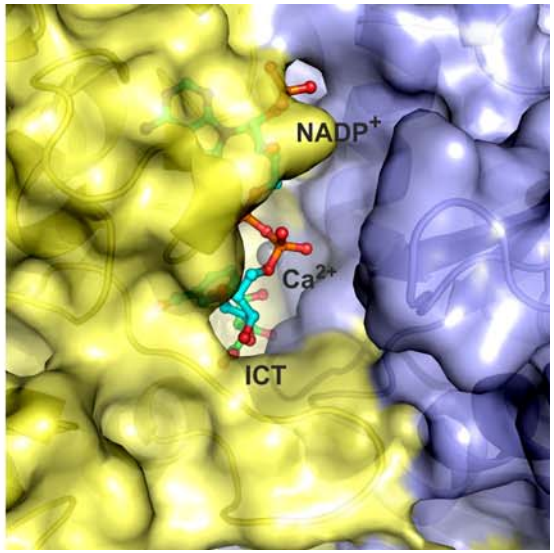
These “fully-closed” enzyme conformations provided a more complete picture of the **induced fit** needed for catalysis:



Open



Quasi-closed

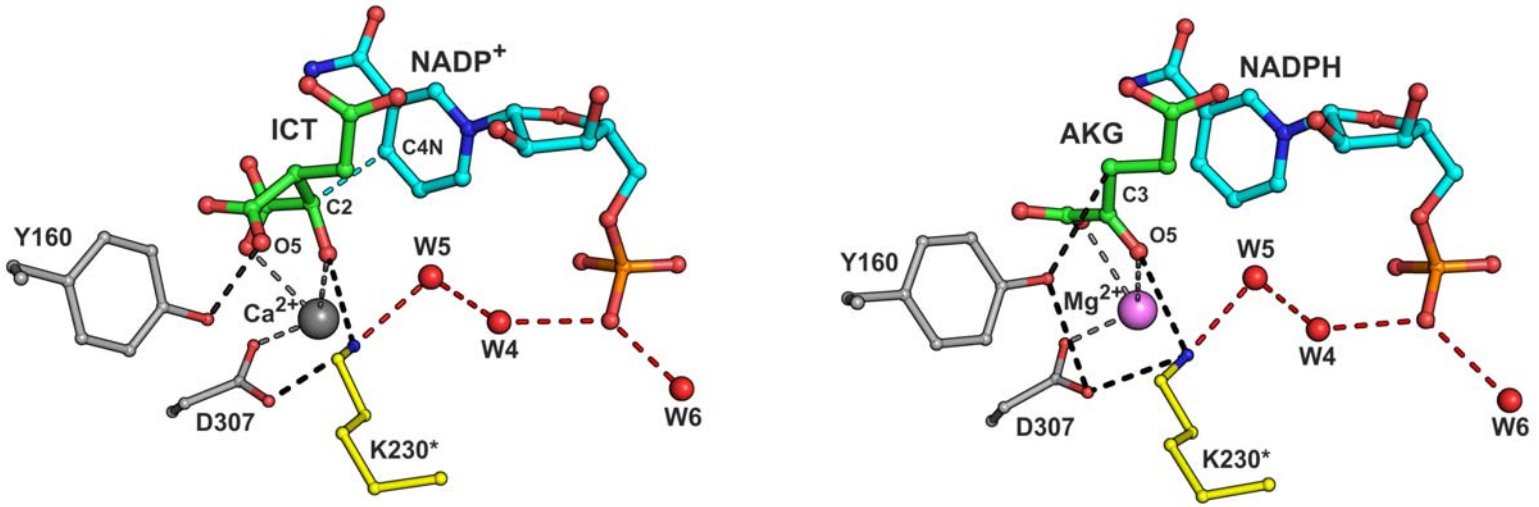


Fully closed

Conclusions

The “fully-closed” enzyme conformations **confirmed the details** of the IDH catalytic mechanism proposed by Aktas and Cook (2009):

- K230* is a **catalytic acid/base** active in all mechanism steps
- Y160 is a **catalytic acid** essential for the enol tautomerization
- D307 or a **proton relay** from bulk solvent balance the proton flow



S. Gonçalves, S. P. Miller, M. A. Carrondo, A. M. Dean and P. M. Matias, "Induced Fit and the Catalytic Mechanism of Isocitrate Dehydrogenase" (2012) Biochemistry, 51:7098–7115.

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Susana Gonçalves

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ITQB, Universidade Nova de Lisboa, Oeiras, Portugal

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Biotechnology Institute, University of Minnesota, St. Paul, MN 55108, USA

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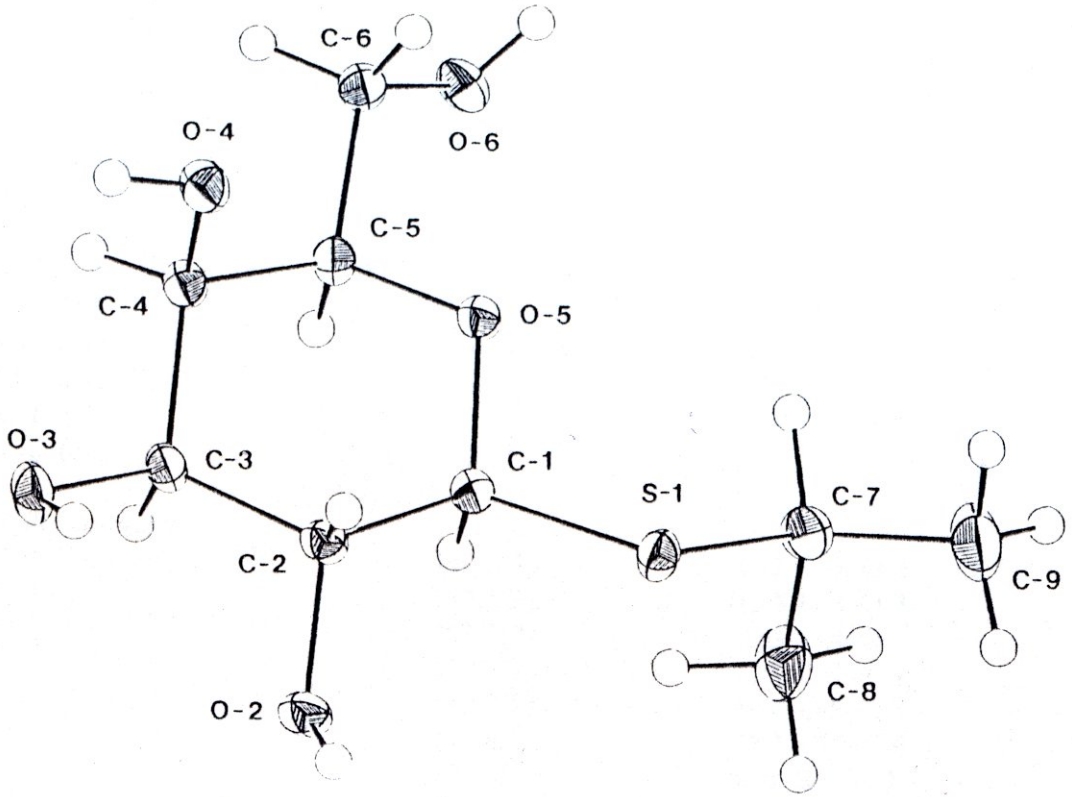
C-6
O-6
O-4
C-5
C-4
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O-3
C-3
C-1
S-1
C-7
C-9
C-2
C-8
O-2

Isopropyl β-D-1-thiogalactopyranoside

From Wikipedia, the free encyclopedia

Isopropyl β-D-1-thiogalactopyranoside, abbreviated **IPTG**, is a molecular biology reagent.

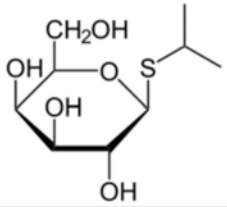
This compound is used as a molecular mimic of allolactose, a lactose metabolite that triggers transcription of the *lac* operon. Unlike allolactose, the sulfur (S) atom creates a chemical bond which is non-hydrolyzable by the cell, preventing the cell from "eating up" or degrading the inductant; therefore the IPTG concentration remains constant. For induction, a sterile 1 M solution of IPTG is typically added by 1:1000 dilution into a logarithmically growing bacterial culture. Different final concentration of IPTG may be used.

IPTG binds to the lac repressor and releases the tetrameric repressor from the lac operator in an allosteric manner, thereby allowing the transcription of genes in the lac operon, such as the gene coding for beta-galactosidase, a hydrolase enzyme that catalyzes the hydrolysis of β-galactosides into monosaccharides. One advantage of IPTG for *in vivo* studies is that since it cannot be metabolized by *E. coli* its concentration remains constant and the rate of expression of *lac p/o*-controlled genes, is not a variable in the experiment. IPTG intake is independent on the action of lactose permease, since other transport pathways are also involved.[1]

In cloning experiments, colonies that have been transformed with the recombinant plasmid rather than a non-recombinant need to be identified. X-gal is a substance that can be metabolised by beta-galactosidase to produce a blue product. Thus cells expressing beta-galactosidase grown in the presence of X-gal and IPTG (to induce the expression) will turn blue. Where a DNA fragment has been inserted into the LacZ (one of the genes for beta-galactosidase) there will be no action upon X-gal and the cells will not turn blue, thus identifying the cells that carry recombinant plasmid rather than non-recombinant plasmid.

Many regulatory elements of the *lac* operon are used in inducible recombinant protein systems; IPTG is an effective inducer in the concentration range of 100 μM to 1.5 mM. Concentration used depends on the strength of induction required, as well as the genotype of cells or plasmid used - if *lacI*^{q}, a mutant that over-produces the lac repressor, is present, then a higher concentration of IPTG may be necessary.

Isopropyl β-D-1-thiogalactopyranoside



Identifiers	
CAS number	367-93-1
PubChem	656894
MeSH	Isopropyl+Thiogalactoside
Properties	
Molecular formula	$C_9H_{18}O_5S$
Molar mass	238.3 g mol^{-1}

Except where noted otherwise, data are given for materials in their standard state (at 25 °C, 100 kPa)

Infobox references

See also

- X-gal

References

1. ^ Hansen LH, Knudsen S, Sørensen SJ (June 1998). "The effect of the lacY gene on the induction of IPTG inducible promoters, studied in *Escherichia coli* and *Pseudomonas fluorescens*" (http://link.springer-ny.com/link/service/journals/00284/bibs/36n6p341.html) . *Curr. Microbiol.* **36** (6): 341–7. doi:10.1007/s002849900320 (http://dx.doi.org/10.1007%2Fs002849900320) . PMID 9608745 (http://www.ncbi.nlm.nih.gov/pubmed/9608745) . http://link.springer-ny.com/link/service/journals/00284/bibs/36n6p341.html.

External links

- IPTG's description on the Acros website (http://www.acros.com/DesktopModules/Acros_Search_Results/Acros_Search_Results.aspx?search_type=CatalogSearch&SearchString=iptg)

Retrieved from "http://en.wikipedia.org/w/index.php?title=Isopropyl_%CE%B2-D-1-thiogalactopyranoside&oldid=464771748"
Categories: Carbohydrates | Molecular biology | Biochemistry stubs

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THE CRYSTAL STRUCTURE OF ISOPROPYL 1-THIO-β-D-GALACTO-PYRANOSIDE MONOHYDRATE AT 123 K

PEDRO M. MATIAS AND GEORGE A. JEFFREY
Department of Crystallography, University of Pittsburgh, Pittsburgh, Pennsylvania 15260 (U.S.A.)
(Received July 30th, 1985; accepted for publication in revised form, January 27th, 1986)

ABSTRACT

The crystal structure of isopropyl 1-thio-β-D-galactopyranoside monohydrate is orthorhombic, $P2_12_12_1$, $Z = 4$, with cell dimensions at 123 K [293 K] of $a = 7.983(1)$ [8.037(1)], $b = 24.574(5)$ [24.709(4)], $c = 6.329(1)$ [6.3736(8)] Å, $V = 1241.84$ [1265.71] Å^3. The calculated and measured density is $D_x = 1.371$ [1.345] g cm^{-3}, $D_m = [1.340]$ g cm^{-3}. Diffraction data were obtained with CuKα radiation and a Nonius CAD-4 diffractometer. The structure was solved by using MULTAN, and refined to $R(F^2) = 0.051$, $R_w(F^2) = 0.078$, $R(F) = 0.029$, $S = 1.16$ for 1502 reflections. The molecule has the $^4C_1$(D) conformation. The orientation of the primary alcohol group is *gauche/trans*, and that about the glycosidic C–S bond is (−)*synclinal* relative to the ring C–O bond. Although this compound does not form thermotropic liquid crystals, it has two crystal-to-crystal phase-transitions, at 70 and 104°, prior to melting at 126°. The crystal structure has a characteristic, amphiphilic, head-to-head bilayer molecular packing, with intercalated alkyl groups. The water molecule is included in the hydrogen-bond structure that links the galactoside moieties.

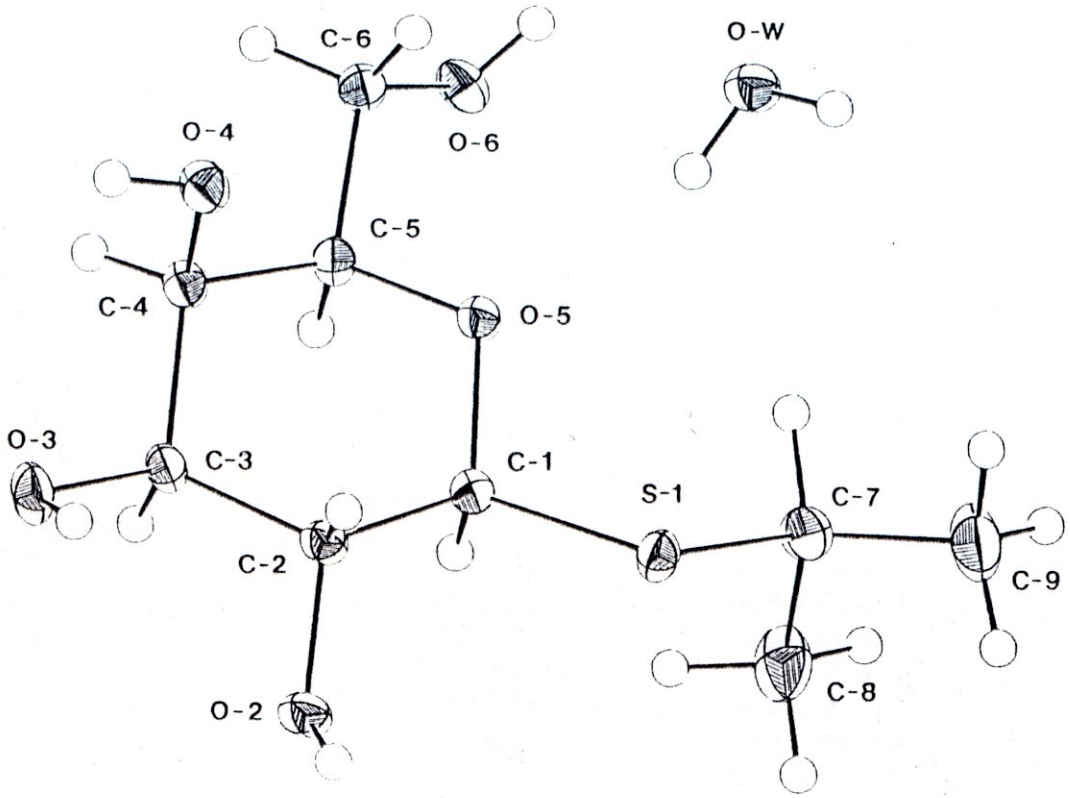

Fig. 1. Atomic notation and thermal ellipsoids (50% probability) for isopropyl 1-thio-β-D-galacto-pyranoside monohydrate at 123 K.