

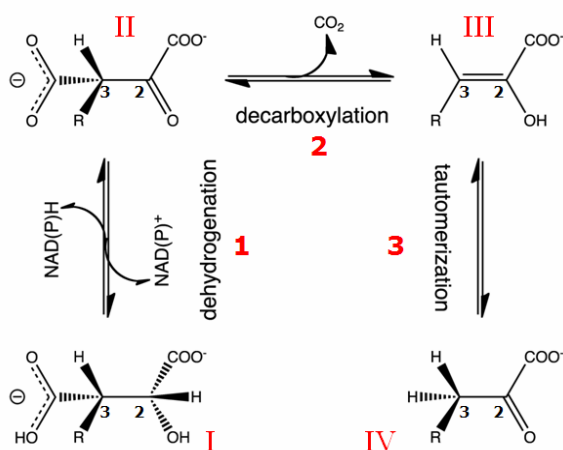
## Induced Fit and the Catalytic Mechanism of Isocitrate Dehydrogenase

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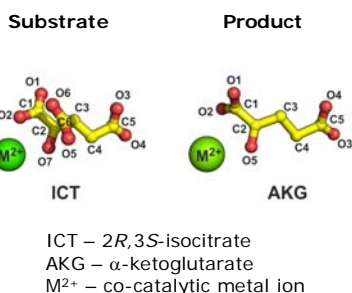
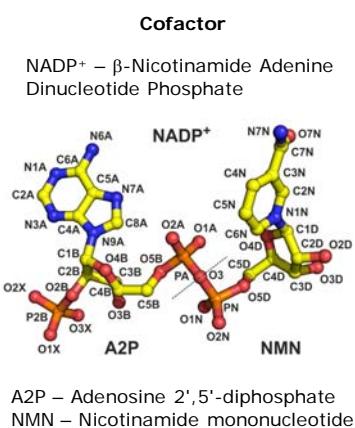
This work is based in part on experiments performed at the ID14-4 and ID23-1 beamlines of the European Synchrotron Radiation Facility (ESRF) in Grenoble, France and at the PXIII beamline of the Swiss Light Source (SLS), Paul Scherrer Institute in Villigen, Switzerland. The research leading to these results received funding from the European Community's Seventh Framework Programme (FP7/2007-2013) under grant agreement n.°226716, and Fundação para a Ciência 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.

NADP<sup>+</sup> dependent isocitrate dehydrogenase isoform 1 (IDH1; EC 1.1.1.42) belongs to a large family of  $\alpha$ -hydroxyacid oxidative  $\beta$ -decarboxylases that catalyze similar three-step reactions, with dehydrogenation to an oxaloacid intermediate preceding  $\beta$ -decarboxylation to an enol intermediate followed by tautomerization to the final  $\alpha$ -ketone product.



IDH1 is involved in the citric acid (Krebs) cycle and converts 2R,3S-isocitrate into  $\alpha$ -ketoglutarate. A comprehensive view of the induced fit needed for catalysis is revealed on comparing the first fully closed conformations of *Escherichia coli* IDH1 in crystal structures of a pseudo-Michaelis complex of wild-type *E. coli* IDH1 and of a reaction product complex of the K100M mutant with previously obtained quasi-closed and open conformations. As previously predicted by Aktas and Cook (2009), Lys230\* is positioned to deprotonate/reprotonate the  $\alpha$ -hydroxyl in two of the reaction steps and Tyr160 moves into position to protonate C3 following  $\beta$ -decarboxylation. A proton relay from the catalytic triad Tyr160-Asp307-Lys230\* connects the  $\alpha$ -hydroxyl of isocitrate to the bulk solvent to complete the picture of the catalytic mechanism.

### The 3-step mechanism of IDH1: {R = CH<sub>2</sub>COO<sup>-</sup>}



### The *E. Coli* IDH1 homodimer

