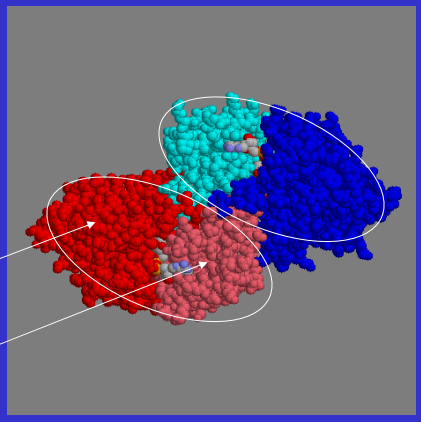
Simulations and experiment support role of loop in liver alcohol dehydrogenase as a NAD+-activated switch for domain closure

Steven Hayward

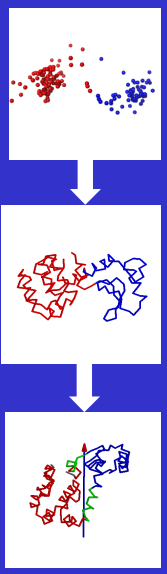
School of Computing Sciences, University of East Anglia, Norwich, U.K.

LADH

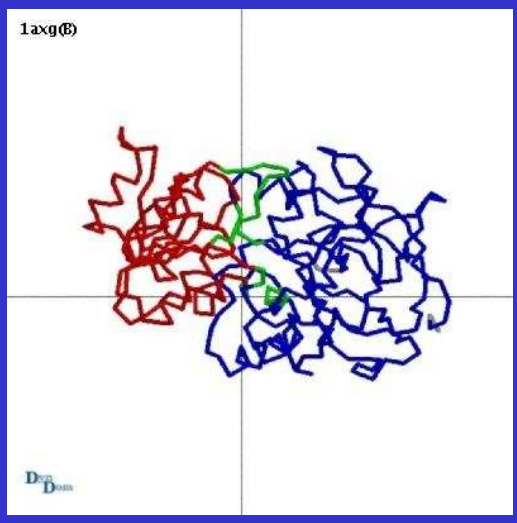
- Enzyme, EC 1.1.1.1
- Catalyses reaction of alcohol to aldehyde using co-enzyme NAD+
- Homodimer
- Each subunit 374 residues
- Each subunit comprises two domains
- NAD+ induces domain closure



NAD+-induced Domain Closure



DynDom result



Hayward & Berendsen, 1998. "Systematic Analysis of Domain Motions in Proteins:New Results on Citrate Synthase and T4 Lysozyme" *Proteins*, Vol 30, 144-154

DynDom Database (http://www.cmp.uea.ac.uk/dyndom)

There are 72 LADH protomer structures in single family.

They separate into two tight conformational clusters corresponding to the open and closed domain structures.

All closed structures are liganded with NAD+ or analogue.

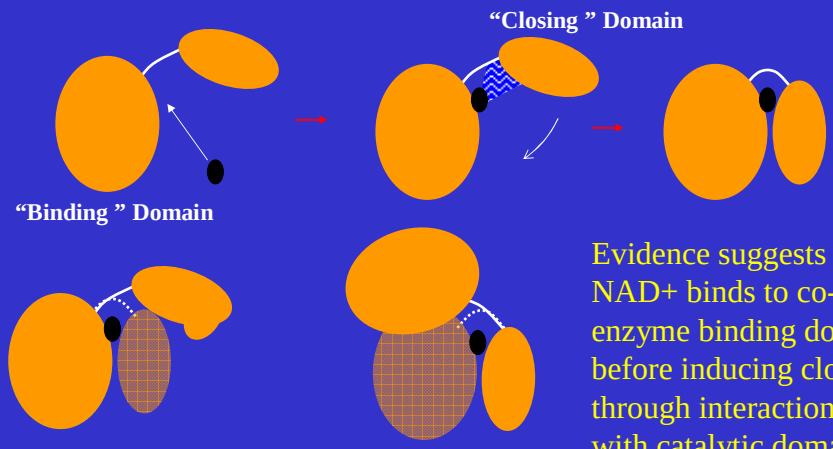
All open structures are either unliganded or liganded with molecule considerably different to NAD, or mutants.

Closed (62)

Open(10)

Qi G., R. A. Lee, S. Hayward 2005. A comprehensive and non-redundant database of protein domain movements. *Bioinformatics.* 21(12):2832-2838

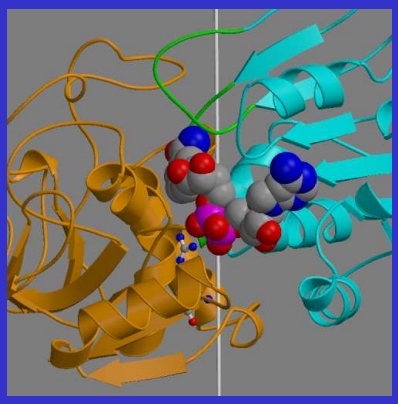
Sequential Model of Binding and Actively Induced Closing

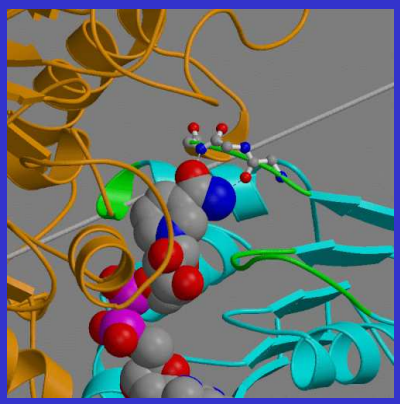


Evidence suggests that NAD+ binds to co-enzyme binding domain before inducing closure through interactions with catalytic domain

Hayward S. 2004. Identification of specific interactions that drive ligand-induced closure in five enzymes with classic domain movements. *J. Mol. Biol.* 339:1001-1021

Closure-inducing Residues





Arg369-NAD+ interaction **Ala317-, Phe319-NAD+ interaction**

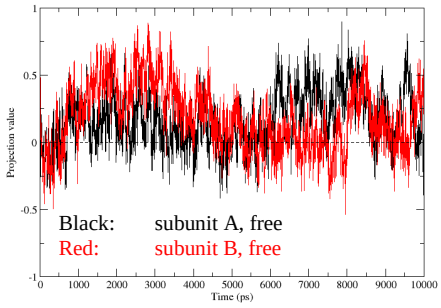
Hayward S. 2004. Identification of specific interactions that drive ligand-induced closure in five enzymes with classic domain movements. *J. Mol. Biol.* 339:1001-1021

MD Simulations

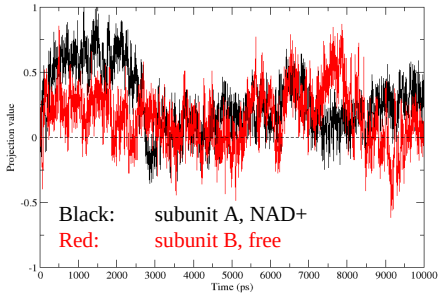
- Performed using AMBER 7.0
- Full dimeric LADH molecule + water =approx 70,000 atoms
- In total five 10 ns simulations were performed
- NAD+ modelled onto co-enzyme-binding domain of open

S.Hayward, A. Kitao, “Molecular dynamics simulations of NAD+-induced domain closure in horse liver alcohol dehydrogenase”, Biophysical Journal, 91: 1823-1831, 2006

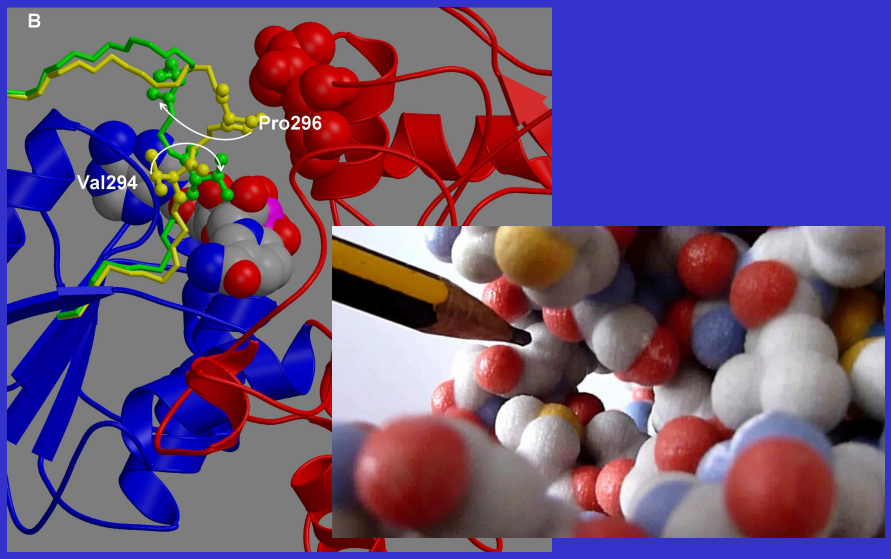
No NAD+ present in either subunit



NAD+ present in subunit A only



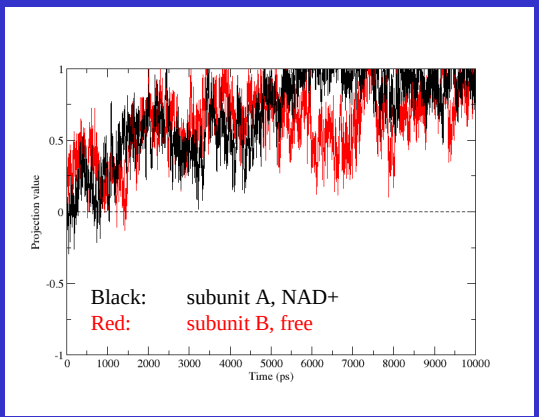
Loop (290-300) would appear to block domain closure



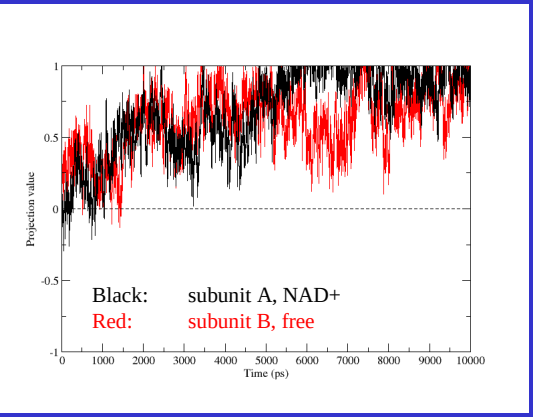
Loop modelled as in closed X-ray structure

"Closing" Trajectory

NAD+ present in subunit A only



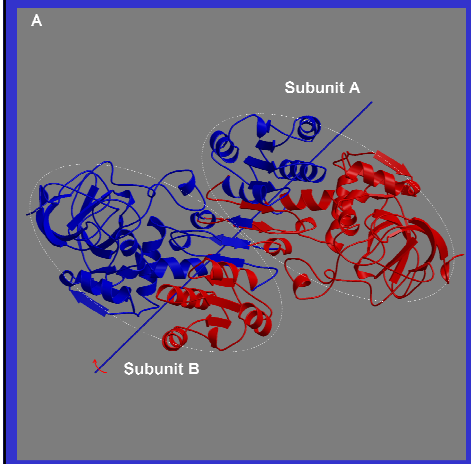
Cooperative domain closure

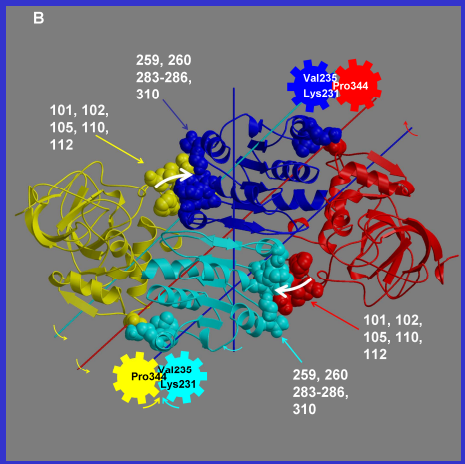


Zero time lag correlation in projection value is 0.38

Over first 5 ns it is 0.46

Cooperative domain closure – DynDom Analysis



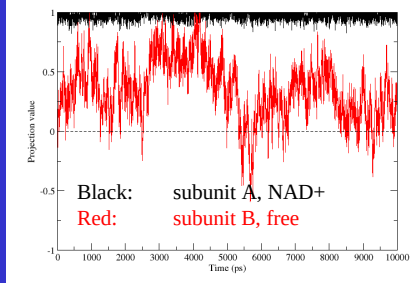


Purpose of Loop

Start:

cooperative forces

Final:

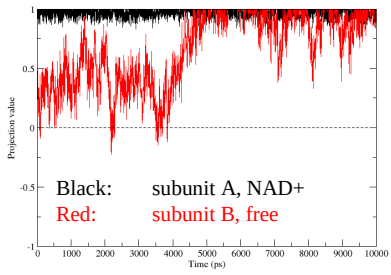


Loop as in open

Start:

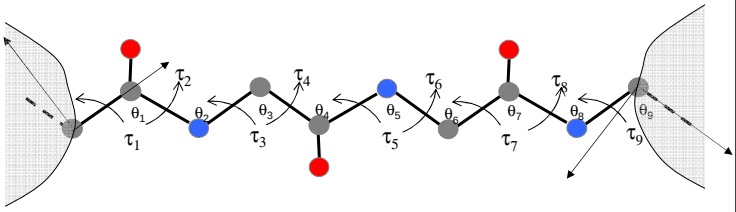
cooperative forces

Final:



Loop modelled as in closed

Local Deformation-
Linear Inverse-Kinematics Method



$$\delta\boldsymbol{\varphi} = \sum_{i=1}^{N_b} \delta\tau_i \mathbf{n}_i$$

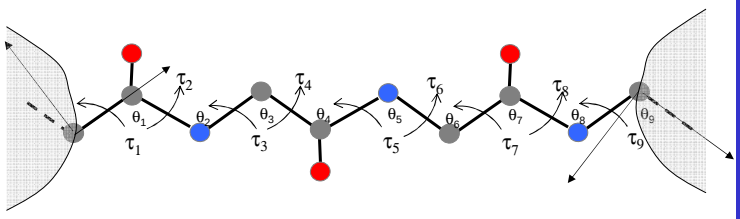
Rotation vector for rotation of C-terminal flank relative to N-terminal flank.

$$\delta\mathbf{d} = \sum_{i=1}^{N_b-1} \delta\tau_i \mathbf{n}_i \times \left(\sum_{j=i+1}^{N_b} \mathrm{r}_j \mathbf{n}_j \right) = \sum_{i=1}^{N_b-1} \delta\tau_i \mathbf{D}_i$$

Displacement vector

S.Hayward, A.Kitao, “Effect of end constraints on protein loop kinematics”, Biophysical Journal, 98(9), 1976-1985, 2010

From vector equations to matrix equations



$$\mathbf{n}_i = \prod_{j=1}^{i-1} \mathbf{A}_j \begin{pmatrix} 1 \\ 0 \\ 0 \end{pmatrix}$$

$$\mathbf{A}_j = \begin{pmatrix} -\cos\theta_j & -\sin\theta_j & 0 \\ \sin\theta_j \cos\tau_j & -\cos\theta_j \cos\tau_j & -\sin\tau_j \\ \sin\theta_j \sin\tau_j & -\cos\theta_j \sin\tau_j & \cos\tau_j \end{pmatrix}$$

$$\begin{pmatrix} \boldsymbol{\delta\varphi} \\ \boldsymbol{\delta d} \end{pmatrix} = \sum_{i=1}^{N_b} \begin{pmatrix} \mathbf{n}_i \\ \mathbf{D}_i \end{pmatrix} \delta\tau_i = \begin{pmatrix} \mathbf{n}_1 & .. & \mathbf{n}_{N_b-1} & \mathbf{n}_{N_b} \\ \mathbf{D}_1 & .. & \mathbf{D}_{N_b-1} & \mathbf{0} \end{pmatrix} \boldsymbol{\delta\tau} = \mathbf{Y}(\boldsymbol{\tau})\boldsymbol{\delta\tau}$$

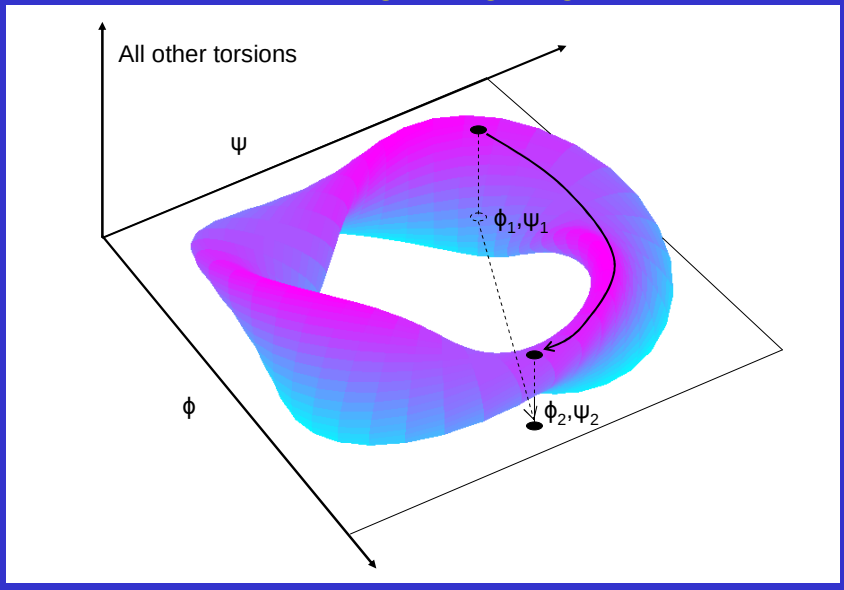
Null space condition for no movement of C-terminal end group relative to N-terminal end group

$$\mathbf{Y}(\boldsymbol{\tau})\boldsymbol{\delta\tau}^0 = \mathbf{0}$$

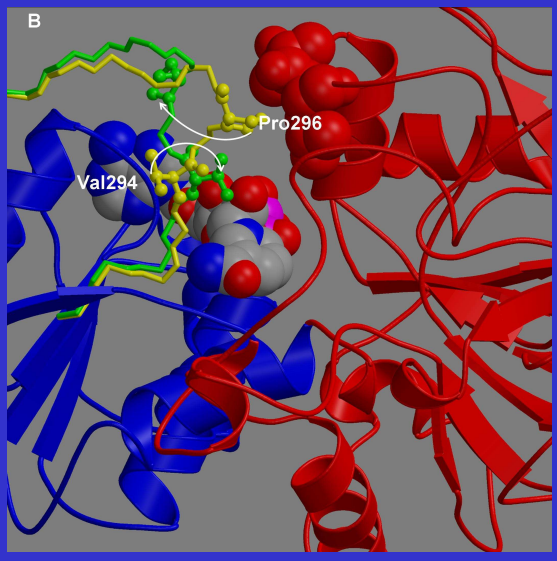
where $\boldsymbol{\delta\tau}^0 = \left(\boldsymbol{\delta\tau}_1^0 \quad \boldsymbol{\delta\tau}_2^0 \quad .. \quad \boldsymbol{\delta\tau}_j^0 \quad .. \quad \boldsymbol{\delta\tau}_{N_{\phi\psi}-6}^0\right)$ $N_{\phi\psi} - 6$ nullvectors

To constrain particular torsions simply remove corresponding columns from matrix **Y**.

Torsion Angle Targeting



The loop 290-301 contains a "rigid arm"



Gly293-Val294-Pro295-Pro296

We proposed that the ProPro motif creates a rigid arm constraining:

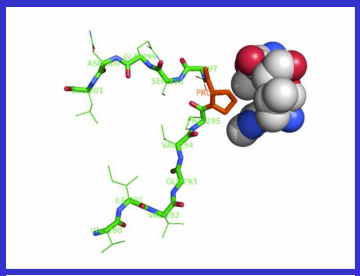
ψ 294, ϕ295, ψ295, ϕ296

This communicates rotation of Val294 to contact NAD+ to the block to closure at Pro296.

Torsion Angle Targeting

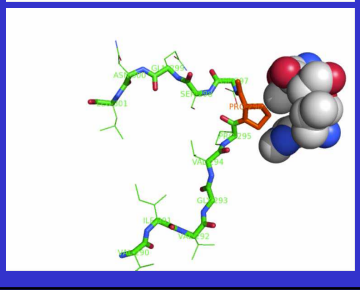
Starting from open structure target torsions ϕ291, ψ291, ϕ292, ψ292, ϕ293, ψ293, ϕ294 to their values in the closed structure.

ψ294, ϕ295, ψ295, ϕ296 constrained (WT)



ψ294, ϕ295, ψ295, ϕ296 unconstrained

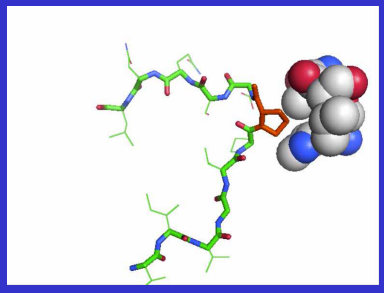
(Pro295nonPro Pro296nonPro double mutant)



Starting from open structure target torsions ϕ291, ψ291, ϕ292, ψ292, ϕ293, ψ293, ϕ294 to their values in the closed structure.

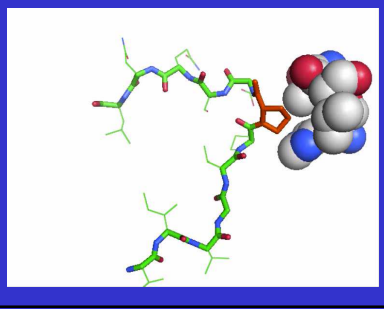
ψ295, ϕ296 unconstrained

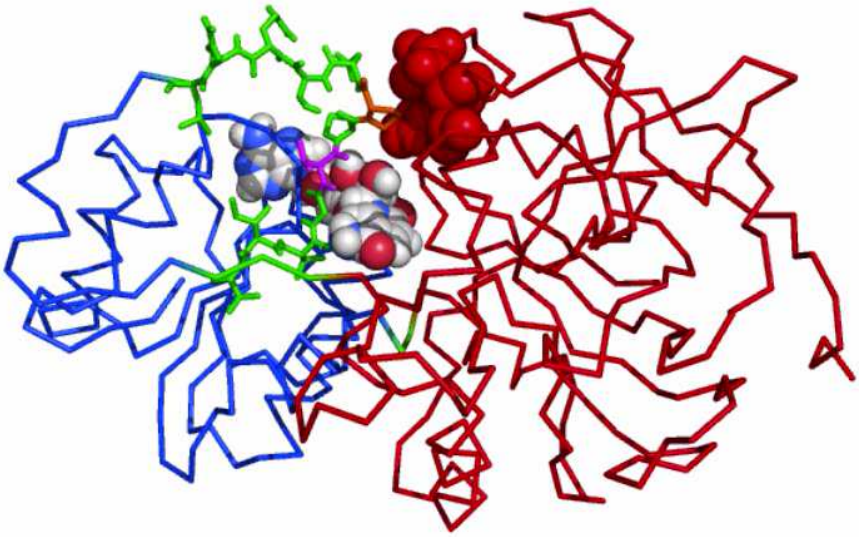
(Pro296nonPro mutant)



ψ294, ϕ295 unconstrained

(Pro295nonPro mutant)





Conclusions

- Domain closure in LADH is driven by specific interactions between NAD+ and residues on the catalytic domain.
- The loop appears to block domain closure in the absence of NAD+.
- A cooperative mechanism acts between the subunits.
- Using a linear inverse-kinematics technique we have confirmed that the Pro-Pro motif on the loop creates a rigid arm for communicating the presence of NAD+ to the blocking region.
- This shows that in this enzyme a there is a NAD+ activated switch for domain closure.

Acknowledgements

Akio Kitao
Guoying Qi
Guru Prasad Poornam
Richard Lee

Applying forces to an elastic network model using haptic feedback

Download (mouse version also) from http://www.haptimol.com

Stocks, M. B., Laycock, S. D., Hayward, S., "Applying forces to elastic network models of large biomolecules using a haptic feedback device", Journal of Computer-Aided Molecular Design, 25, 203-211, 2011.