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mechanical clocks, explains directional coupling. We propose that similar metal-dependent conformational coupling occurs in other transducing NTPases that also couple catalysis of NTP utilization to functionally relevant conformational changes.

### 248-Pos Board B34

### Dynamics of NADH Cofactor Binding to B. Stearothermophilus Lactate Dehydrogenase

**Nickolay Zhadin**[1], Kara Lodewyks[2], Beining Nie[1], Ruel Desamero[3], Robert Callender[1].
[1]AECOM, Bronx, NY, USA, [2]U. Manitoba, Winnipeg, MB, Canada,
[3]York College, CUNY, Jamaica, NY, USA.

Laser induced temperature jump relaxation spectroscopy was used to study NADH nucleotide cofactor binding to lactate dehydrogenase from Bacillus Stearothermophilus thermophylic bacteria (bsLDH). The tryptophan fluorescence T-jump kinetics, that are determined almost exclusively by NADH binding/unbinding, show only one exponential relaxation component, while the NADH fluorescence T-jump kinetics, that report on NADH (un)binding and conformation changes around the active site, display three distinct relaxation components. The mid-range NADH relaxation rates are very close to the relaxation rates of the tryptophan kinetics, and their dependence on the total concentration of free ligand and enzyme shows a sharp initial increase and much slower rise at higher concentrations. The slow NADH relaxation rates are nearly independent of the total free ligand and enzyme concentration. Our kinetic data can be best described by a model in which NADH binding to the binding pocket of bsLDH is followed by a conformation change, presumably closing of the active site loop, and additional weak NADH binding to the binary complex bsLDH·NADH in the open-loop conformation only. The presumed open and closed loop conformations, that can be considered as competent and incompetent for further substrate binding, interconvert with relaxation time of about 1 ms.

### 249-Pos Board B35

### The Biomolecular Channelling of a Reactive Intermediate

**Natalie E. Smith**, Paul V. Attwood, Ben Corry, Alice Vrielink.
University of Western Australia, Perth, Australia.

The channelling of intermediates through buried molecular tunnels in multi-enzyme subunits is a topic of considerable interest as it is thought to occur in many different enzymes. This process shields reactive or poisonous intermediates from the rest of the organism and ensures direct, rapid transport from one active site to the other. Many of these molecular channels have been identified using techniques such as X-ray crystallography, but little is known about the internal mechanisms they use to transport their intermediates. In this case, computational and kinetic methods have been applied to study the bifunctional enzyme 4-hydroxy-2-ketovalerate aldolase-aldehyde dehydrogenase (acylating) (DmpFG) and its proposed channelling activity. Previous crystallographic studies have found that DmpFG has a 29 Å channel linking its two active sites. This raises questions about whether it functions as a conduit for the poisonous intermediate acetaldehyde. in the case of this project computational techniques, including both standard molecular dynamics and metadynamics, have been used to investigate whether it is energetically feasible for the intermediate to be transported in this way between the two active sites and to verify the role of proposed checkpoints at the entry and exit of the channel. In this study, we found that it is indeed energetically favourable for acetaldehyde to be transported in this way through the protein and that the directionality of the channelling event alters dependent on what is bound in the active site of one of the subunits. The kinetic assays on wild type DmpFG are designed to determine the kinetic parameters of both the active site 1 and 2 reactions and the channelling event. Both computational and kinetic data will be presented.

### 250-Pos Board B36

### Probing the Pro- and Anti-Coagulant Interactions of Thrombin

**Nicholas A. Treuheit**, Elizabeth A. Komives.
UC San Diego, San Diego, CA, USA.

Thrombin is a serine protease that is central to the coagulation cascade. It functions both as a pro-coagulant, through the cleavage of fibrinogen and protease-activated receptors, and as an anti-coagulant primarily through the thrombomodulin (TM)-mediated cleavage of Protein C which functions as negative feedback for thrombin production. Fully understanding the physical behavior of thrombin is critical for the treatment of a number of blood related illnesses such as strokes and sepsis. in our research we seek to better understand the allosteric conversion of thrombin from pro- to anti-coagulant using various biophysical techniques. Using Isothermal Titration Calorimetry, we have shown a thermodynamic connection between the anion-binding exosite I (ABEI) and the active site in both active and inhibited forms of thrombin. A fluorescent active site analog, DAMPA, binds with enthalpies differing by up to 1.7 kcal/mol between thrombin and TM-bound thrombin. However, this change in enthalpy is offset by an entropic compensation resulting in an unchanged binding free energy. Furthermore, a similar change is seen when a DNA aptamer for ABEI binds to free and covalently inhibited thrombin. Additionally, we have developed new constructs of thrombomodulin that demonstrate similar binding affinity and effectiveness to natural thrombomodulin, which together with fluorescent and calorimetric characterization techniques presents a strong new tool for further illuminating the interactions of thrombin and thrombomodulin.

### 251-Pos Board B37

### Protein Dynamics Coupled to Electron Transfer in Cytochrome *C* Oxidase from *R. Sphaeroides* by Time-Resolved Surface-Enhanced 2D-IR-Absorption Spectroscopy

**Renate L.C. Naumann**[1], Christoph Nowak[1], Andreas Schwaighofer[1], Shelagh Ferguson-Miller[2], Robert B. Gennis[3], Wolfgang Knoll[1].
[1]AIT Austrian Institute of Technology GmbH, Vienna, Austria,
[2]Michigan State University, East Lansing, MI, USA, [3]University of Illinois, Urbana, IL, USA.

Time-Resolved Surface-Enhanced IR-Absorption Spectroscopy (tr-SEIRAS) has been performed on cytochrome *c* oxidase (C*c*O) from *R. sphaeroides*, thereby usingdirect electron transfer to inject electrons into the enzyme via the first electron acceptor, $Cu_A$. Direct ET offers the possibility to change the redox state repeatedly between oxidized and reduced by applying periodic potential pulses at different frequencies and to measure FTIR spectra as a function of time. A large variety of vibrational modes have thus been detected that could be tentatively assigned to conformational transitions of peptide groups associated with redox transitions of the cofactors. The time evolution of bands characteristic of redox transitions has been analyzed by fitting the experimental data to the sequential four-electron transfer model developed previously in the context of fast-scan voltammetry studies. From the agreement of kinetic data obtained from tr-SEIRAS and fast scan voltammetry, we conclude that protein dynamics coupled to electron transfer (ET) takes place in the ms time scale, particularly when the enzyme had previously been transformed into an activated state. 2D-IR auto-correlation maps have then been calculated thus spreading the data in the second dimension. A detailed analysis of synchronous in combination with asynchronous 2D IR spectra allowed us to discriminate conformational transitions of peptide groups that occur simultaneously with each other from those that are delayed in time. Hence from 2D-IR, the sequential order of conformational changes of single groups coupled to redox transitions could be established. Consequences for the mechanism of proton transport are discussed.

### 252-Pos Board B38

### Novel "Order-Disorder-Order" Mechanism in Adenylate Kinase Conformational Change

**Magnus Wolf-Watz**, Ulrika Olsson.
Umeå University, Umeå, Sweden.

Conformational changes are intimately coupled to the biological activity of many proteins. It is challenging to unravel the molecular mechanism of conformational change since intermediate states are short-lived and usually not observable by spectroscopic methods. Adenylate kinase (Adk) is an enzyme that undergoes a large conformational rearrangement in response to ATP binding, and serves as an excellent model to study the interplay between structure, dynamics and activity. We have studied the molecular mechanism of Adk conformational change (associated with ATP binding) with a combined protein engineering and spectroscopic approach. We identified a novel mechanism that includes local unfolding/refolding of a segment in the ATP binding subdomain in an otherwise folded enzyme. Thus, the mechanism for conformational change can be denoted as an ''order-disorder-order'' transition, where ''order-disorder'' transitions have been observed for other proteins previously. Our results show that the functional and folding energy landscapes of adk in fact are overlapping. This observation suggests that functional properties may be added to proteins by use of cooperative folding/unfolding transitions. in addition, we present data that conspire to define the structure of an initial and transient Adk-ATP complex. This accomplishment provides detailed knowledge of a structural state that is both difficult to capture and important for understanding of the catalytic power of Adk. Taken together we can portrait a detailed picture of the structural states that form the basis for Adk catalysis.
Reference:

Ulrika Olsson & Magnus Wolf-Watz. *Nature Communications*. **2010**.