1062-Plat
Regulation of Enzymatic Activity Occurs by Selection of Discrete Activity States
Nikos S. Hatzakis, Li Wei, Sune K. Jorgensen, Andreas H. Kunding, Per Hedegaard, Dimitrios Stamou.
Nanoscience Center University of Copenhagen, Copenhagen, Denmark.
Tight regulation of enzymatic activity is crucial for controlling a multitude of fundamental cellular processes. Yet the molecular level details underlying regulation often remain poorly understood (1). Here we employed single molecule activity studies to dissect the mechanistic origin of regulation of enzymatic activity (2). As a model system we employed a lipase and measured its activity as a function of accessibility to surface tethered liposomes (3,4), which are known regulators of its activity. Our results surprisingly revealed that the lipase does not follow a continuous distribution of activity rates (5,6) but rather oscillates between 2 states of different activity as the majority of signaling protein and ion channels studies (1). We report here the first time both the interconversion rates between the 2 states and their inherent activity. Based on these we calculated the energetic landscape of the entire reaction pathway and identified that regulation of enzymatic activity does not occur via an increase in the enzyme inherent activity but rather predominantly originates from increased time spent on the highly active state. Because we found a number of structurally unrelated enzymes to exhibit an analogous behavior we anticipate that oscillation between a discrete number of activity states rather than a between a continuous distribution of them might be a generic mechanism potentially facilitating regulation of enzymatic activity.
1. Gardino A.K et al., Cell, 139, 1109 (2009).
2. N. S Hatzakis et al., submitted

1063-Plat
Decay of Compound ES in Dehaloperoxidase-Hemoglobin
Matthew K. Thompson1, Stefan Franzen1, Reza A. Ghiildi1, Brandon J. Reeder2, Dimitri A. Svitenson2.
1North Carolina State University, Raleigh, NC, USA, 2University of Essex, Colchester, United Kingdom.
Dehaloperoxidase (DHP) is a respiratory hemoglobin (Hb) that catalyzes the conversion of trihalophenols to dihaloquinones in the presence of hydrogen peroxide. Ferric heme states of the resting DHP and the free radical intermediates formed under H2O2 treatment were studied by low temperature EPR spectroscopy in the range of reaction time of 50 ms - 2 min at three different pH forms. This over-expression is thought to be one of the evolutive adaptations linked to Tyr34. A kinetic model of the experimental data suggests that formation of Compound RH or the Tyr38 radical are two alternative routes of Compound ES decay. The detached product shows a pH dependent formation of covalent heme-to-protein cross-links. The stable DHP Compound RH formed under H2O2 in the absence of substrates is proposed to be a state with the ferric heme covalently crosslinked to Tyr[34]. A reaction model of the former data suggests that formation of Compound RH or the Tyr38 radical are two alternative routes of Compound ES decay. Which route is taken depends on the formation of His55: in the less populated closed conformation, the Tyr38 radical is formed, but in the major open conformation, Compound ES decays yielding Compound RH, a product of safe termination of the two oxidizing equivalents of H2O2 when no substrate is available.

1064-Plat
Human Myoglobin: Two Isomers that Differ at Single Residue. Their Different Dynamics Suggest Distinct and Complementary Role
Mariano A. Scoccafano1,2, Enrico Spiga1, Mariano Casu1, Paolo Ruggerone1,2, Matteo Cecarelli1,2.
1Department of Chemical Sciences - University of Cagliari, Monserrato, Italy, 2Sardinian Laboratory for Computational Material Science, CNR/INFM, Monserrato, Italy, 3Swiss Federal Institute of Technology, Lausanne, Switzerland, 4Department of Physical Sciences - University of Cagliari, Monserrato, Italy.
In Human up to five different myoglobin isoforms are expressed. Iso-I (~75-80%) and iso-II (~15-20%) are the more expressed ones and differ only at the 54th position, K54 and E54 respectively. It has been reported that myoglobin concentration in muscles is higher for high-altitude natives than sea-level populations, and that only iso-II is over-expressed among the myoglobin isoforms. This over-expression is thought to be one of the evolutive adaptation to the high-altitude hypoxic environment. Since it is widely accepted that myoglobin is not only an oxygen storage/deliver system, iso-II over expression has been related to other functions such as NO scavenger and/or nitrite reductase. In this work Molecular Dynamics simulations were applied to study the dynamics of 54K and 54E human myoglobins. Statistical analysis of internal cavities and their interconnections helped to highlight and compare the intrinsic dynamic behavior of these two proteins. Furthermore, the role of the solvent in the mutation proximity has been investigated and it was found that water molecules can quasi-allostERICally modulate the dynamics of myoglobin distant region. Important differences have been found especially at the histidine gate, even if the two myoglobin isoforms differ at only one residue that, indeed, is not located at the gate itself. These differences suggest that the two more expressed human myoglobins might have a distinct and complementary role. This would well fit the literature scenario where NO scavenging is thought to be important at normoxic condition to retain mitochondrial respiration efficacy, while nitrite reduction is believed to be crucial under hypoxic stress. NO release by myoglobin would decrease mitochondrial oxygen consumption preserving the optimal O2 gradient across the cell but also ensuring sufficient ATP synthesis.

1065-Plat
Protein Flexibility and Energy Flow During Enzyme Catalysis
Arvind Ramanathan1, Jose M. Borrego2, Chakra S. Chennubhotla2, Pratul K. Agarwal1.
1Oak Ridge National Lab, Oak Ridge, TN, USA, 2University of Pittsburgh, Pittsburgh, PA, USA.
Enzymes are dynamic molecules. Although in the past enzymes were viewed as static entities, recent evidence from experimental, theoretical and computational work indicates that protein dynamics play a significant role in enzyme catalytic activity. Investigations of the free energy profile for several proteins such as cyclophilin A and dihydrofolate reductase have revealed a network of protein motions that promote catalytic activity. Results indicate that these reaction-promoting motions are conserved as part of the enzyme fold across several species, even though they have low sequence similarity. Extending our study to a superfamily of enzymes, namely the dinucleotide binding Rossman Fold proteins (DBRP), shows that in spite of having very low sequence homology and different structural features, the overall intrinsic dynamical flexibility of the superfamily is remarkably well preserved with respect to the catalytic step. The conformational coupling observed between exterior surface regions with the active site entails energetic coupling between them. To characterize this energetic coupling, we used an integrated information theoretic and biophysical approach to analyze residues that may constitute pathways through which energy may propagate from the flexible exterior surface regions to the active site of the protein. Our results reveal significant similarities in the energy flow pathways within the DBRP super-family. This study provides specific insights into how the DBRP super-family of proteins has evolved to catalyze hydride transfer reactions.

PLATFORM AD: Molecular Dynamics

1066-Plat
Steered Molecular Dynamics Simulation of Kinesin Detachment from the Microtubule Surface
Tran Trinh, Christian Lastoskie, Tamir Epstein, Martin Philipson. University of Michigan, Ann Arbor, MI, USA.
The effects of timescale and force transducer stiffness on kinesin detachment from the microtubule surface, as represented by the alpha-beta tubulin dimer, have been investigated using Steered Molecular Dynamics (SMD) simulations. By decreasing the pulling speed and using a softer spring in the SMD simulations, a kinesin detachment force in the range of 100 to 150 pN is obtained. This result is in better agreement than previous SMD calculations with experimental measurements of the detachment force obtained using optical traps and atomic force microscopy. Moreover, the differences between the simulated and experimentally measured kinesin detachment forces may be attributed to the experimental configuration a microbead attached to an 80 nanometer coiled-coil stalk. The stalk acts as a long moment arm on the neck linker of kinesin, thus causing underestimation of the actual detachment force at the kinesin-microtubule interface by a factor of 5 to 7. Taking into account the effect of the moment arm, the experimental results suggest an actual stall force for kinesin-microtubule binding in the range of 60 to 150 pN. This range of forces brackets the detachment force calculated in the SMD simulations. It was also noted that different mechanisms of kinesin detachment from the tubulin dimer are observed for different selected values of the SMD pulling parameters. Specifically, at low pulling speed and for a soft spring stiffness, the detachment process reveals conformational changes that involve the translocation and rotation of the kinesin head as well as the switch II region.
Switch II has been identified as the main binding region between kinesin and the microtubule surface.

1067-Plat
Molecular Dynamics Simulations of Monomeric and Dimeric NAC in Alpha-Synuclein at Various Temperatures
Hongyi Yang1, James C. Patterson2,3
1UAB, Birmingham, AL, USA, 2UAB, BIRMINGHAM, AL, USA.

The aggregation of z-Synuclein (zS), a 140-residue presynaptic protein, is thought to be the primary step in the pathology of Parkinson’s disease. Several studies have suggested that residues 61-95 of zS, known as the NAC region, may play a critical role in promoting the aggregation of the full-length zS. Furthermore, the first eighteen residues of NAC (i.e., residue 61-79) seem to be essential for the self-assembly of NAC. To better understand the dynamic structure of these peptides and mechanism by which they aggregate, molecular dynamics (MD) simulations have been performed on the NAC region of zS. In our MD simulations, the conformational changes of NAC are more sensitive to increased temperatures than the full-length zS. The initial monomeric NAC model, containing z-helix through the entire sequence, was obtained from the NMR minimized average structure of zS (PDB ID = 1XQ8). The dimeric peptide models were constructed through docking. Atomistic simulations for both monomeric and dimeric NAC in explicit water were conducted for 50 ns at 300 K and 372 K using the CHARMM22/CMAP force field. In simulations with monomeric NAC, the secondary structure of residues 74-84 and 87-92 were largely unchanged at both 300 K and 372 K, while residues 61-73 lost the initial helical structure much faster at 372 K than at 300 K. In simulations with dimeric NAC, a short region encompassing residues 64-71 appeared as a higher propensity to form β structures than other regions. Inter-chain β-sheet structure was observed in this region at the beginning of the simulation at 372 K; however, this β-sheet was interrupted when the backbone of one peptide chain folded up, largely due to the electrostatic attraction between residue 61 (i.e., Glu) and 80 (i.e., Lys).

1068-Plat
How Biomolecules Influence Water Structure and Dynamics
Ana Vila Verde1,2, R. Kramer Campen1
1FOM institute AMOLF, Amsterdam, Netherlands, 2University of Minho, Department of Physics, Braga, Portugal.

Interactions between solutes and water impact both water structure and structural dynamics as well as solute properties (e.g., conformational fluctuations of proteins). To understand these interactions we investigate water near disaccharides using classical atomic molecular dynamics simulations. Disaccharides show topological and chemical complexity characteristic of larger biomolecules but are sufficiently small to permit detailed study. We observe that increases in hydrophobicity precisely map slow down in water translation and rotation of local water populations. In line with recent studies of proteins, we find that chemically similar functional groups may interact differently with water depending on neighboring functional groups.

To explain these observations we examine the mechanism of hydrogen bond exchange for waters hydrogen bonded to other waters but within the sugar first solvation shell, as well as waters hydrogen bonded to the sugar. Recent work showed that water in bulk rotates through large angular jumps that pass through bifurcated hydrogen bond intermediates and that rotational rates are rationalized through transition state theory. Previous reports found that the rotational slow down of water near small solutes can be predicted from changes in the accessible transition state volume or the enthalpy of the hydrogen bonds. For our larger solutes we find that accounting for the transition state volume alone overestimates water rotational slow down. Differences in hydrogen bond enthalpy are also insufficient to predict rotational slowdown. Water slowdown can only be understood by additionally accounting for subtle changes in the free energy landscape associated with water rotation - reduction in the number of available reactant states and broadening of the transition state barriers. The presence of solutes of even moderate size thus affects water dynamics in ways difficult to predict using simple scaling considerations from bulk, making water response system dependent.

1069-Plat
Exploring Signal Transduction Mechanism of HAMP Domains via Molecular Dynamics and Metadynamics
Lizhe Zhu, Peter G. Bolhuis, Jocelynne Vreeede
University of Amsterdam, Amsterdam, Netherlands.

The HAMP domain is a linker region in prokaryotic sensor proteins which functions in two-component signal transduction pathways. HAMP exhibits a parallel coiled coil motif comprising four helices and transfers the signal from the sensor domain to the transmitter domain. We performed MD simulations of a number of HAMP structures in isolation. These structures include wild type and two mutants (A291V, A291F) of HAMP from A. fulgidus (PDB 2ASW) and three HAMPS in the triHAMP chain (PDB 3LRN) from the P. aeruginosa soluble receptor Acr2. Our simulations show that a) 2ASW retains its N-terminal structural integrity in isolation; b) mutants of 2ASW exhibit slight changes in β-strand flexibility but relax fast to NMR 2ASW if mutations are removed; c) while the N-terminal HAMP structure in the triHAMP chain is stable when isolated, the C-terminal domain and the inner HAMP show extensive rearrangements, indicating that the N-terminal constrains the flexibility of the triHAMP complex. Using MD in combination with adaptive biasing potentials, known as the metadynamics method, we explored different features of the signal transduction mechanism of HAMP. Our results indicate that HAMP can transfer signals by changing the tilt angle, the hydrophobic packing in the core of the complex and the rotation angle of the helices. The implications of these results for signal transduction proteins containing HAMP are discussed.

1070-Plat
Dominant Site Involved in Inhibition of Targets by Anesthetics Suggested Through Novel Free Energy Perturbation Calculations
David LeBard1, Grace Brannigan1, Jerome Henin2, Roderic Eckenhoff1, Michael Klein1
1Temple University, Philadelphia, PA, USA, 2CNRS - Marseille, Marseille, France.

It is well-established that general anesthetics bind directly to many of their targets, but the location of binding sites in a well-investigated target family, the cys-loop receptors, remains unresolved. Although the structure of eukaryotic cys-loop receptors has proven challenging to determine cryo-structurally, a recent prokaryotic anesthetic-sensitive member of the family (GLIC) has been crystallized in complex with volatile and injected anesthetics. Detergent molecules occupy the pore of both the apo and liganded GLIC receptor, preventing any direct observation of pore-block by anesthetics from the crystal structure. In recent microscopic atomistic molecular dynamics (MD) simulations of flooding by isoflurane of both the eukaryotic nicotinic acetylcholine receptor (nAChR) and GLIC, we observed two isoflurane molecules bind to the pore of each receptor. Circumventing the experimental obstacles, computational measurements of binding affinity to the pore site provide an alternate method for determining the relevance of pore-block to GLIC inhibition by anesthetics. In addition to a calculated binding affinity for isoflurane in the pore that corresponds well to the micro-molar concentrations at which inhibition is observed, we also present novel measurements using the alchemical free energy perturbation method (FEP) to quantify negative cooperativity between the two isoflurane molecules in the pore. The low Hill coefficient (Kt=0.5) calculated in this manner corresponds to that measured experimentally within 10%. The results suggest that the pore is probably occupied in GLIC exposed to experimental concentrations of isoflurane, and pore-block may provide the dominant source of inhibition.