and RNA degradation. DEAD box helicases form the largest class of RNA helicases. Structurally, they consist of a conserved helicase core comprising two RecA-like domains connected by a flexible linker. In some cases, N- or C-terminal regions flanking the core confer substrate specificity or mediate interactions with other proteins. Most of the conserved helicase signature motifs line the cleft between the two core domains, and an opening and closing of this cleft during the helicase cycle has been postulated but not shown experimentally.

Single molecule FRET experiments with the *B. subtilis* DEAD box helicase YxiN carrying donor and acceptor fluorophores on different sides of the inter-domain cleft reveal an open helicase conformation in the absence of nucleotides, or in the presence of ATP, or ADP, or RNA. In the presence of ADP and RNA, the open conformation is retained. By contrast, cooperative binding of ATP and RNA leads to a compact helicase structure, proving for the first time that the ATP and ADP bound states of RNA helicases display substantially different structures only when the RNA substrate is bound. These results establish a closure of the inter-domain cleft in the helicase core at the beginning of the unwinding reaction. Furthermore, they open up avenues to follow ATP-induced conformational changes in the catalytic cycle of RNA helicases in real time and to understand the role of these movements in RNA unwinding. Altogether, they suggest a conserved mechanism of energy conversion among DEAD box helicases across kingdoms.

188-Pos The Subtle Nature of Allosteric Activation is Highlighted by Fluorescence Spectra and Anisotropy Data From Variants of Glycogen Phosphorylase b

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Board B20

Glycogen phosphorylase contains nine native tryptophan residues per subunit as well as a cofactor, pyridoxal-5-phosphate, at the active site. The fluorescence of these intrinsic fluorophores can be used as a reporter on structural alterations due to allosteric activation by AMP as well as mutations to the primary structure. Intrinsic tryptophan spectra from the truncate Δ2-17 and the site directed mutant K544E (both previously shown to have altered kinetic properties) match the spectra for wild-type glycogen phosphorylase b indicating that the over all structures are similar. The pyridoxal-5-phosphate cofactor remains stabily bound to a lysine side chain via a Shiff's base in the active site. The Shiff's base exists as an equilibrium between two forms. The spectral properties of the cofactor depend on the form of Shiff's base, and the equilibrium between forms is sensitive to the local environment at the active site. In wild-type phosphorylase b, the cofactor spectrum is altered due to the binding of AMP at the allosteric site. The spectra for both Δ2-17 and K544E are distinct from wild-type and do not show sensitivity to AMP despite being activated to similar extents. Steady-state anisotropic data confirms that the changes seen with each mutant are unique from each other and wild-type further confirming that the alterations at the active site are different for each mutant and are independent of activation. These data highlight the subtle nature of allosteric activation and indicate that perturbations due to binding of an allosteric ligand can be specific to only a portion of the active site rather than requiring some global alteration of tertiary structure.

This work was supported by the NIH funded CBI Training Grant, NIH grant GM33261, and the Welch Foundation.

189-Pos The Role of the Quaternary Shift in the Allosteric Regulation of Phosphofuctokinase from B. Stearothermophilus

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Board B21

Phosphofructokinase (PFK) is an important regulatory enzyme in glycolysis that catalyzes the conversion of fructose-6-phosphate (F6P) and MgATP to fructose-1,6-bisphosphate and MgADP. *Bacillus stearothermophilus* PFK (BsPFK) is a homotetramer that is allosterically inhibited by phosphoenolpyruvate (PEP) which binds along one dimer-dimer interface while F6P binds along the other dimer-dimer interface. The only overall quaternary structure deviation between the substrate bound and the inhibitor bound structures of wild-type BsPFK is a quaternary shift, which is defined as a 7° rotation about the substrate binding site interface. Located along the substrate binding interface and involved in numerous inter-subunit hydrogen bonds is the residue D12, which is a completely conserved residue among 149 prokaryote PFKs and is not directly involved in catalysis or the binding of any ligands. When compared to wild-type, D12A BsPFK shows a 100 fold increase in the binding affinity for PEP, a 50 fold decrease in the binding affinity for F6P, and surprisingly the coupling is not greatly affected. Two crystal structures of D12A BsPFK have been solved, with one enzyme structure bound to its inhibitor PEP and the other enzyme structure free of any ligands. These two crystal structures allow for a direct comparison of PEP binding in D12A BsPFK. Both of the structures reveal the enzyme in the inhibitor bound conformation. These structural data along with the kinetics data for D12A BsPFK suggest that the role of the quaternary shift may be involved more in ligand binding than in the allosteric response of the enzyme, contrary to previous proposals. Funding came from NIH grant GM33261 and the Welch Foundation.

Protein Dynamics - I

190-Pos Structural Characterization Of Engineered pH-sensitive Allostery

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Board B22

Conformational changes in proteins are crucial to many biochemical reactions and enable the precise control of protein function. The engineering of effector-induced conformational changes into proteins and biomaterials is also envisioned to allow for the remote- or autonomous control of engineered catalytic processes or assemblies.

We here present a structural investigation of engineered protein molecules that switch conformations in response to pH changes. The chargeable residues aspartate, histidine and cysteine were engineered into glutathione-S-transferase of Shistosoma japonicum respectively. The residues were introduced into the hydrophobic core of the protein to selectively destabilize its structure - and thus function - in a pH-dependent manner. Subsequent rounds of combinatorial design are used to customize function to a defined pH condition. In contrast to the wild-type protein, the spectroscopic characterizations of substrate binding confirm the specific pH-dependent binding to glutathione. The crystal structures of the mutant enzymes demonstrate the pH-dependent structural flexibility of the mutation site. The methodology described here may provide a general approach to design "external control" into a biochemical process.

191-Pos Local Structural Fluctuations Determine Global Pathways Of Oxygen Migration In Myoglobin

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Board B23

Myoglobin is a globular protein involved in oxygen storage and transport. No consensus yet exists on the atomic level mechanism by which small non-polar ligands move between surrounding solvent and its binding site on the heme group buried inside the protein. This study uses multi-microsecond, room temperature molecular dynamics simulations to complement experiment with a complete atomic-level picture of ligand migration. Specifically, we characterize:

(i) Explicit full trajectories in which the CO ligand shuttles between the internal binding site and the solvent; and
(ii) Pattern and structural origins of transient voids available for ligand migration.

The computations are performed both in wild-type myoglobin and in V68F myoglobin mutant, which is experimentally known to slow ligand binding kinetics.

The map of ligand visitation sites explicitly observed in the simulated trajectories closely follows the independent mapping of integrated free volume fluctuations. This remarkable consistency points to localized transient thermal fluctuations that occur in the protein structure (on picosecond time-scale) as the primary physical origin of the migration pathways for small non-polar ligands inside the protein. There are two discrete dynamical pathways for ligand migration in myoglobin. The natural propensity of larger fluctuations to occur in the "softer" domains of the protein matrix – in-between the helices and in the loop regions – determine the preferential location of the pathways relative to protein scaffold.

192-Pos Conformational characterization of peptides by UV resonance Raman spectroscopy

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Board B24

As protein function and structure are intimately linked, rapid spectroscopic methods for assessing conformational changes in proteins are actively being developed. UV resonance Raman (UVRR) is one of the most broadly useful of these methods. Excitation at 204 nm probes backbone vibrations, including the amide III region around 1200 cm-1. Previous work by the Asher group has established a close relationship between the intensity and peak position of UVRR bands in the amide III region and the backbone dihedral angle psi, enabling us to detect the formation of a single helical turn. Our goal is to detect conformational changes at the single residue level by lineshape analysis of the amide III band.

The current work extends this project to new peptides, such as the model system A9RA3EA5RA2. In this peptide, the alpha helical conformation is stabilized, under neutral pH conditions, by a salt bridge between residues 10 and 14 and between residue 20 and the C-terminal carboxyl group. The relationship between conformation and the amide III spectrum is probed by CD and UVRR spectroscopy, while the conformational dynamics are investigated by nanosecond temperature jumps followed by time-resolved UVRR.

193-Pos Dynamic Transition in Proteins: The 'Mystery' Solved

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Board B25

Hydrated proteins, DNA and RNA exhibit a sudden sharp rise in their mean-squared atomic displacements $<x^2>$ at temperatures above 200–230K known as the "dynamic transition". Despite significant efforts in experimental and computational biophysics, origin of the dynamic transition and its relationship to the protein activity remains elusive. Combining dielectric spectroscopy and neutron scattering techniques, we are able to follow protein dynamics over an extremely broad frequency and temperature range. We identified structural relaxation of the protein and demonstrated that it exhibits smooth slightly non-Arrhenius temperature variation. We

did not observe any indication of the cusp-like behavior around T~220K neither in protein nor its hydration water. We demonstrated the sharp rise in $<x^2>$ is just a result of the protein structural relaxation process reaching the limit of the experimental frequency window of the instrument at T~200–230K.

194-Pos Protein thermodynamics structure theory

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Board B26

The study of protein dynamics leads to the establishment of protein thermodynamics structure theory. A protein is not a global thermodynamics system, but composed of many sub-systems of thermodynamics, named potherse. The change of potherse, which shows cooperation in view of biochemistry, is phasic in view of physics. The allostery phenomenon occurs in the coupling or interaction between different potherses and conformational waves could be produced in the coupling between different potherses or proteins. The protein function-structure relation, as well as protein inner motion-function (dynamic-function) relation, can be fully and logically interpreted by applying principles of protein thermodynamics structure theory and system logic. The protein thermodynamics structure is the fundamental theory of protein and bioinformatics. In addition, the protein thermodynamics structure theory is axiomatic. The combination of protein three-dimensional structure theory and thermodynamics structure theory can account for all our knowledge about protein science.

195-Pos Poly-L Stereochemistry and Conformational Landscapes of Protein Folding. Explicit Solvent MD of Poly-L and Alternating-L, D Octa-alanines

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Board B27

Protein molecular folds are poly-L specific in stereochemical structure. Examining possible relevance for protein folding principles, poly-L and alternating-L, D Ac-Ala_8-NHMe are submitted to molecular dynamics with gromos96 force field at 298 K over ~200 ns in water. The microstates enumerated to 1.5 Å cut off are in good approximation of equilibrium, but only 1% of the expectation for a random coil. Specific in ϕ, ψs, the microstates are folded in overall hairpin-like morphologies, shorter in contact order, encompassing sterically and electrostatically less favorable ϕ, ψs when in poly-L rather than alternating-L, D structure. The folding transitions encompass all four ϕ, ψ quadrants in poly-L microstates, but are confined to mainly the sterically and electrostatically favored ϕ, ψs in alternating-L, D microstates. The conformational diffusion and kinetics of conformational folding in an order of magnitude slower in the poly-L microstates presumably due to topological blocks in ϕ, ψ space. Overall, stereochemistry defines both specificity and speed of conformational folding in polypeptide structure by defining the relationship between through-space electrostatics and short-ranged hydrogen bonding between peptide dipoles. Mutually conflicted or harmonious, stereochemical structure in peptide dipolar interactions could be responsible for many of features of protein folding thermodynamics and kinetics. The implications for a possible backbone-based protein-folding theory are discussed.

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196-Pos

Board B28

WITHDRAWN

197-Pos Molecular Dynamics Simulations Of The Allosteric Regulation Of Pyruvate Kinase

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Board B29

Binding of an effector molecule at one site of an allosteric protein triggers functional changes at a second site. Using molecular dynamics simulations, we studied the behaviour of allosteric l. mexicana pyruvate kinase in response to binding of different combinations of activator and effector molecules (fructose-2,6-biphosphate and phosphoenol pyruvate, respectively). We discuss the role of entropy as driving force of the observed structural rearrangements and present the effects of mutations of a number of putative key residues suggested beforehand on the basis of experimental activity measurements.

198-Pos Viscoelastic Models to Elucidate Time Scales in Proteins: Allostery, Memory Effects, and Synchronizability

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Board B30

We study the dynamics of folded proteins using all-atom and coarse-grained approaches. Our main goal is to understand how functionality is achieved once the protein folds into its three-dimensional structure. In all-atom models, we carry out classical molecular dynamics (MD) simulations in water and glycerol at different temperatures, where the protein maintains its structure, but not necessarily its function (1). The solvated system is modeled in two distinct states whereby the protein is decoupled from the bulk solvent at low temperatures, and communicates with it through a vicinal layer at physiological temperatures (2). The protein adapts different conformational routes for organizing the required coupling to a specific solvent, which is achieved by adjusting the amount of conformational jumps in the surface-group dihedrals. We also develop a viscoelastic network model to identify the time scales of the internal motions of proteins. We find that while most of the residues retain their relaxation times, few that reside far from the binding site display remarkable changes in rate, implying their role in allosteric behavior (3). We further deduce the autocorrelation of distance fluctuations that are also observed by single-molecule experiments (4). The model with a time-dependent friction kernel and a high-friction coefficient paves the way to study edge-to-edge distance correlations whose full relaxation is not achievable by MD simulations. We observe that the synchronizability (5) of weighted residue networks (6) are found to be associated with enzymatic activity.

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199-Pos Simulation of the AOT Reverse Micelle Self-Assembly with and without alpha helical peptide

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Board B31

We use a peptide/reverse micelle system to study the dynamics of proteins in a crowded environment. Full-atom molecular dynamics simulations are conducted for bis-(2-ethylhexyl) sulfosuccinate (AOT) reverse micelle self-assembly in the AOT-water-Isooctane-Ion (Na^+ or K^+) system with and without peptide for over one hundred nanoseconds. Water and AOT molecules are randomly distributed in the Isooctane solvent initially with water to AOT ratio 6. AOTs spontaneously form reverse micelles with a water pool inside. The self-assembly process can be divided into three phases:

1. formation of clusters of AOT, water and sodium within hundreds of picoseconds,
2. formation of small reverse micelle with water inside through the cluster aggregation within few nanoseconds,
3. very slow formation of large reverse micelles by collision of small reverse micelles.

The driving force for the first two phases is electrostatic force (hydrophilic aggregation), the driving force for the third phase is van der Waals attraction force between the hydrocarbon tails. The reverse micelles formed in the simulation have different sizes. Small reverse micelles have spherical shape while large reverse micelles have elongated shape. In another simulation the YGAKAAAAKAAAAKAAAAKAAAAG alpha-helical peptide is included in the system in addition to AOT and water molecules. Peptide-surfactant complex formed within hundreds of picoseconds. The peptide sets on the surface of the biggest reverse micelle composed as part of the interface for water and isooctane. Charged side chains of the peptide point into the water core of the reverse micelle. The peptide also serves as a bridge to connect two reverse micelles before they combine together. The conformation of peptide remains alpha-helical through the whole self-assembly process.

200-Pos Binding And Folding Dynamics Of The DLin-7 / Stardust Complex Measured By Single-Molecule Fluorescence Spectroscopy

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Board B32

Cellular differentiation is frequently regulated by multiprotein complexes where the spatial proximity of the components facilitates biological function. There is immense interest in isolating the individual components involved as well as determining how their interaction regulates differentiation. Multiparameter fluorescence detection nowadays allows direct observation of molecular processes at the single molecule level.

In Drosophila melanogaster the obtained complex is located in the subapical region (SAR) of embryonic epithelia and composed of the four proteins DPATJ and DLin-7, Stardust (Sdt), and Crumbs (Crb). The scaffold protein Sdt contains two subsequent L27 modules, which mediate the interaction with DPATJ and DLin-7 through their L27 domains. The trans-membrane protein Crb binds to the PDZ domain of Sdt with its cytoplasmic tail.

In this work we study the Sdt / DLin-7 interaction. In single molecule experiments the DLin-7 protein shows two conformational states. According to a structural similarity model, we assign one state as folded and the other as unfolded state. Probability distribution analysis (PDA) shows that the distance distribution of both conformational states is broader than the experimental shot noise limit. The interconversion between the two states is slow and occurs on a time scale longer than milliseconds. We investigate the question that the biomolecular flexible binding process is possible.

The addition of binding partner Sdt results in a broadening of the observed fluorescence resonance energy transfer (FRET) efficiency distribution. Analysis by PDA using a dynamic two state model shows an increase in the folding rate of DLin-7. This kinetic effect

suggests that the binding reaction proceeds via an induced fit mechanism and not via a lock-and-key-system, i.e. binding and conformational folding are coupled. Dynamic PDA allows for the direct measurement of the protein folding rate in complexes.

201-Pos Large Scale Domain Motion of Activated STAT3 using Molecular dynamics Simulations

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Board B33

Signal transducers and activators of transcription factors 3 (STAT3) is one of STAT family proteins that mediate the response to cytokines, growth factors and hormonal factors. Aberrant activity of STAT3 leads to dysregulated growth, survival and angiogenesis, whereas STAT1 which is another subtype STAT is a tumor suppressor. Therefore specific targeting of STAT3 has a high potential for cancer therapy. STAT3, a multidomain protein, gets activated via phosphorylation of Tyrosines in the SH2 domain that leads to formation of a homodimer. Crystal structure of the STAT3 homodimer is available at 2.25 Å resolution. To identify druggable sites in STAT3, we have studied the large scale domain motions in STAT1 and STAT3 if any, using molecular dynamics simulations in explicit solvent. Principal component analysis combined with clustering analysis shows four distinct substates for STAT3 from the 50ns MD simulation trajectory of STAT3. The dominant domain motion shows a scissor like mode upon binding DNA, that enhances the interaction of the SH2 domains in the homodimer. Free energy calculations showed that this large scale domain motion of STAT3 protein stabilized the STAT3/DNA complex. Similar results will be presented for STAT1 and the dynamics of STAT1 will be compared to STAT3.

202-Pos Helix Kinking, Conformational Heterogeneity, and Blockage of the M2 Proton Channel: Roles of Water Molecules and Amantadine Binding

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Board B34

The M2 proton channel is essential for the life cycle of the influenza A virus. The antiviral drug amantadine inhibits the M2 channel by binding to the transmembrane helix (TMH). To gain insight into the structure, dynamics, and function of the channel, we performed molecular dynamics (MD) simulations of tetrameric M2-TMH in a DMPC bilayer, both in apo form and in amantadine-bound form. The simulation of the apo form revealed three natural tendencies of M2-TMH. First, the THM can kink near G34, perhaps due to backbone flexibility of glycine. The short sidechain of G34 then allows for the close approach of water molecules, which make up the backbone hydrogen bonds disrupted by the kinking. Second, M2-THM is flexible and there is a large degree of heterogeneity in conformational sampling among the four subunits. Third, V27 from the four subunits can serve as a gate near the channel entrance, which breaks the water wire down the channel pore. The gate arises from physical occlusion and the elimination of hydrogen bonding partners for water molecules. In the presence of amandatine, M2-TMH becomes more rigid and conformational sampling among the four subunits becomes more homogeneous, in agreement with NMR results [Hu et al. (2007) *Biophys. J.* ***93***:276]. The kinking of the THM persists in the amandatine-bound form, supporting the notion that kinking is an intrinsic property of M2-THM. Importantly, the gate formed by V27 is reinforced by the nonpolar portion of amantadine and the water wire is now much more likely to break. This suggests a novel role of V27 in the inhibition of the M2 proton channel by amamtadine and a possible explanation for amandatine-resistant mutations on V27.

203-Pos Characterization of Conformational Motions in Proteins Using the Gaussian Network Model

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Board B35

Near-native conformational dynamics of proteins is of crucial importance for elucidating protein function. Coarse-grained models such as the Gaussian Network Model (GNM) and the Anisotropic Network Model (ANM) are computationally efficient tools used to predict the experimental Debye-Waller factors and various cross-correlations of residue fluctuations in native proteins. The GNM does not provide directions of residue motions because of its isotropic Hamiltonian whereas the ANM expresses residue fluctuations relative to an external coordinate system. In this project, we study the underlying assumptions of the GNM and ANM models and discuss their limits of validity and suggest a statistical mechanical model to generalize the GNM to yield information on residue motions in a laboratory fixed coordinate frame. Specifically, we compare the eigenmodes of the two models. We validate our results by using trajectories obtained from extensive molecular dynamics simulations. Our systematic approach is based on the following steps:

(i) Using modal decomposition, we obtain the n components of the inverse Kirchoff matrix of GNM.
(ii) Assuming that residue fluctuations preserve their directions for each mode, validated by MD simulations, we calculate directions of residue motions relative to a known internal coordinate system of the protein. This requires the solution of a set of nonlinear system of equations.
(iii) we cluster the 3n eigenvectors of the ANM into n groups and match these with the n eigenvectors of the GNM. This matching provides insights into the assumptions of the two

models. We then show possible generalizations for different elastic network models (ENM) of varying levels of complexity.

204-Pos Three States Conformational Change In Membrane Embedded Beta Barrels

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Board B36

Despite the popular view of the structural rigidity of membrane embedded beta barrels. We discovered that for at least two different categories of beta barrels (two autotransporters and a monomer porin), there are three distinct structural ensembles, termed as the ground state ensemble, the 1st excited state ensemble and the 2nd excited state ensemble. Perfect correlations are observed for the beta strand backbones and extracellular loops. The structural diversity of loops are unleashed only when the beta strands are in one of the excited states. Our non-equilibrium molecular dynamics simulation studies suggests that such conformational change are critical in facilitating substrate translocation through beta barrels. We speculate such conformational change are a conserved feature for all beta barrel channels and are important for their tranport functions in outer membranes of gram-negative bacteria.

205-Pos Minimum Structure Necessary For Protein Dynamical Transition

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Board B37

The biomolecular dynamical transition is a rapid increase in the temperature dependent average atomic mean square displacement, $<x^2>$, at ~200 K. The increase in structural flexibility coincides with the onset of function in some systems. Among the possible origins of the effect are: thermally activated small scale diffusive motions , or an intrinsic transition in the biological water adjacent to the biomolecule . While the effect is often measured using neutron quasi-elastic scattering, terahertz dielectric response is also sensitive to the rapid change in flexibility of the system (1). This sensitivity arises from either the relaxational loss from picosecond diffusive motions, or low frequency structural vibrational mode absorption. Recently we found the dynamical transition is still present for hen egg white lysozyme denatured in 6 M guanidine hydrochloride, indicating tertiary structure is not necessary for the effect. Here we explore the minimum amount of structure required for the transition and how the transition depends on side chain composition. Terahertz (0.2–2.0 THz) complex dielectric response is measured as a function of temperature (80 – 294 K) for poly-alanine and poly-glycine solutions with chain lengths varying from single monomers to those sufficient for α-helix formation. A 220 K transition is found for ~ α- helix polyalanine, whereas the transition is no longer apparent for 3-mers. The results suggest diffusive side chain motion is not sufficient to account for the effect.

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206-Pos A Non-equilibrium Dynamic Mechanism For The Allosteric Effect

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Board B38

Allosteric regulation is often viewed as thermodynamic in nature. However protein internal motions during an enzymatic reaction cycle can be slow hopping processes over numerous potential barriers. We propose that regulating molecules may function by modifying the nonequilibrium protein dynamics. The theory predicts that an enzyme under the new mechanism has different temperature dependence, waiting time distribution of the turnover cycle, and dynamic fluctuation patterns with and without effector. Experimental tests of the theory are proposed.

207-Pos Inherent Flexibility and Conformational Transition in Calmodulin N-Terminal Domain from Variational Approach

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Board B39

The key to understand a protein's function often lies in its conformational dynamics. We develop a coarse-grained variational model to investigate the interplay between structural transition, conformational flexibility and function of N-terminal calmodulin (nCaM) domain. In this model, the two energy basins corresponding to the "closed" apo conformation and "open" holo conformation of nCaM domain are connected by an interpolation parameter. Results from our model are in very good agreement with recent experimental and molecular dynamics simulation studies of CaM protein. We found that, the calcium binding loops, helix-linker and termini are very flexible in this protein domain. In addition to the two terminal helices A and D, helix C is also found to be very flexible in this domain. The N-terminal part in calcium binding loops I and II shows higher flexibility than the C-terminal part with small beta-sheet structure. Binding loop II in the structure with higher flexibility and backbone mobility than the binding loop I probably dominates the

conformational transition. Residues numbered 31–49 shows a large structural change when the calmodulin domain opens. The equilibrium populations of the two states are found to be about 94% "closed" and 6% "open" conformation. Furthermore, assuming a prefactor of 1 per microsecond gives a conformational transition rate from close to open of 40,000 per second.

208-Pos Mobility of a Loop of a B. subtilis Carboxylesterase and its Effect on Substrate Conversion

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Board B40

Carboxylesterase (CEs) are ubiquitous enzymes responsible for the detoxification of xenobiotics. CEs can metabolize and hydrolyze a variety of esterified drugs, including the anticancer agent CPT-11. The specificity of CEs for a particular substrate or inhibitor depends on the enzyme's molecular structure and the dynamics of conformational substructures when a substrate is bound. We have used a series of biophysical techniques to understand differences in substrate selectivity of CEs. First, we used molecular dynamics simulations (MD) to identify the loop region of high fluctuation in a CE from B. subtilis. Second, we generated a T->C mutation at specific amino acid residue (T422C) that is located both on this flexible loop region and near the active site. This Cys residue was then used to label the enzyme with the fluorophore IAEDANS. Next, we examined the dynamics of the IAEDANS attached to the loop by using fluorescence anisotropy measurements (FAD). Finally we compared the FAD data with MD studies to confirm the enzyme structure fluctuations. Our hypothesis is that the molecular dynamics of this loop region is correlated with substrate conversion efficiency for selected CEs. These experiments provide the first data toward testing this hypothesis.

209-Pos Single-molecule Protein Conformational Dynamics of a Pyrophosphokinase In Action

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Board B41

Enzymatic reactions are traditionally studied at the ensemble level, despite significant static and dynamic inhomogeneities. Subtle conformational changes play a crucial role in protein functions, and these protein conformations are highly dynamic rather than being static. Using only a static structural characterization, from an ensemble-averaged measurement at equilibrium, is often inadequate in predicting dynamic conformations and understanding correlated protein functions in real time. We applied single-molecule spectroscopy under physiological conditions to study the mechanisms and dynamics of enzymatic phosphorylation reactions involved with a pyrophosphokinase, HPPK. Enzymatic reaction turnovers and the associated structure changes of individual protein molecules were observed simultaneously in real-time by single-molecule FRET detection. We obtained the rates for single-molecule conformational active-site open-close fluctuations and correlated enzymatic reactions. The overall reaction rates were found to vary widely from molecule-to-molecule. We have demonstrated a specific statistical analysis to reveal single-molecule FRET anti-correlated fluctuations from a high background of fluorescence correlated thermal fluctuations. Our new approach is applicable to a wide range of single-molecule FRET measurements for protein conformational changes in enzymatic reactions and cell signaling.

210-Pos Fluorescence Techniques For Studying Live Cell Protein Dynamics Reveal Strong Chromatin Tethering Of HIV-1 Integrase By Human LEDGF/p75

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The retroviral replication cycle includes a step where the viral genome is incorporated into that of the host cell, by the action of the enzyme integrase (IN). Nowadays it is accepted that the integrase of HIV-1, the virus that causes AIDS, has a human co-factor, LEDGF/p75. In contrast to the cellular function of LEDGF/p75, a transcriptional co-activator, the exact viral function is poorly understood.

We studied eGFP-tagged LEDGF/p75 with cellular fluorescence correlation spectroscopy (FCS), to gain insight in the cellular dynamics of the protein. In the nucleus the diffusion of the protein was a lot slower than in the cytoplasm, and two-thirds of the protein population was present in a second slow component. We concluded that LEDGF/p75 is slowed down by dynamic binding to the chromatin. This result is in line with known information on transcription factors. We then investigated the dynamics of the non-chromatin-bound protein using domain-deletions and site-directed mutagenesis. We found that free LEDGF/p75 shows anomalous diffusion both in the cytoplasm and nucleus, most likely due to its large size and multi-domain structure.

In an attempt to study the intracellular interaction of LEDGF/p75 and HIV-1 integrase (IN), we used fluorescence cross-correlation spectroscopy (FCCS). When co-overexpressing mRFP1-tagged IN, the diffusion time of the intranuclear complex of the two proteins is situated on the hundreds-of-milliseconds timescale, resulting in strong photophysical bleaching of the fluorescence. By specifically decreasing the affinity of the complex for the chromatin using site-directed mutagenesis of the main chromatin-binding domain of LEDGF/p75, the apparent diffusion time of the protein complex decreased, allowing for the quantification of the interaction with

FCCS. These results show the possibility to dissect diffusion and exchange kinetics when performing FCS or FCCS.

211-Pos A Possible Motional Mode in Chemoreceptor Cytoplasmic Domain, And Its Potential Role in Signal Transduction

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A possible degree of motional freedom was found in a long, antiparallel 4-helix bundle structure, the cytoplasmic domains of bacterial chemoreceptors. Simulation shows that each helix can move along the helical axis direction by 1-1.5Å, without interrupting the side chain interdigitation. This motion must be accompanied by a small number of side chain rotations at involved residue interfaces (a-a, g-g, d-d, g-d, and a-d). However, it does not require shearing motion of most side chains, as what the well-known shear mechanism would imply. Structural 4-helix bundles such as GCN4 usually do not exhibit such a freedom.

Within this "free play", the governing interaction is likely the hydrogen-bonding interaction between water and helix backbone. This dictates that the free energy is minimized at both ends, which defines two states. The existence of a two-state system in 4-helix bundles was experimentally confirmed by a small 4-helix domain, the $P1_{short}$ domain of CheA in *Thermotoga Maritima* (Quezada et al., J. Mol. Biol. *341*, 1283–1294). The atomic resolution crystal structure shows the coexistence of both states as structural heterogeneity, with their positions defined by the "free play" boundary. The results suggest that the fundamental requirements of signal transduction - two discrete states with small energy bias - is achievable via a deliberate design of protein sequence. Thus it seems that this motional mode is a plausible candidate for the long-sought mechanism of long distance signal transduction in bacterial chemoreceptors. The model is qualitatively consistent with literature chemoreceptor mutagenesis results.

212-Pos The Allosteric Role of the Ca++ Switch in Adhesion and Elasticity of C-cadherin

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Board B44

Cadherin proteins involved in adhesion, signaling, and mechanical processes are characterized by extracellular domains containing a variable number of heterogeneous "repeats" arranged in series. These cadherin repeats feature unique linker regions with calcium binding motifs. While it is well known that the extracellular repeats of cadherin proteins mediate cell-cell adhesion in a calcium-dependent manner, the molecular mechanisms behind the influence of calcium in adhesion dynamics and cadherin's mechanical response are not well understood. Here we present results from molecular dynamics simulations showing how calcium ions control the structural integrity of cadherin's linker regions, thereby affecting cadherin's equilibrium dynamics, the availability of key residues involved in cell-cell adhesion, and cadherin's mechanical response. The all-atom, multi-nanosecond molecular dynamics simulations involved the entire C-cadherin extracellular domain solvated in water (a 345,000 atom system). Equilibrium simulations show that the extracellular domain keeps its crystal conformation (elongated and slightly curved) when calcium ions are present. In the absence of calcium ions, however, it assumes a disordered conformation. The conserved residue Trp2, which is thought to insert itself into a hydrophobic pocket of another cadherin molecule (thereby providing the basis for cell-cell adhesion) switches conformation from exposed to intermittently buried upon removal of calcium ions. Furthermore, the overall mechanical response of C-cadherin's extracellular domain is characterized at low force by changes in shape (tertiary structure elasticity), and at high force by unraveling of secondary structure elements (secondary structure elasticity). This mechanical response is modulated by calcium ions at both low and high force, switching from a stiff, rod-like to a weak, entropic-like behavior upon removal of ions. The simulations provide an unprecedented molecular view of calcium mediated allostery in cadherins, also illustrating the general principles of linker mediated elasticity of modular proteins.

213-Pos Dioxygen Enters Active Site of 12/15-Lipoxygenase via Dynamic Oxygen Access Channels

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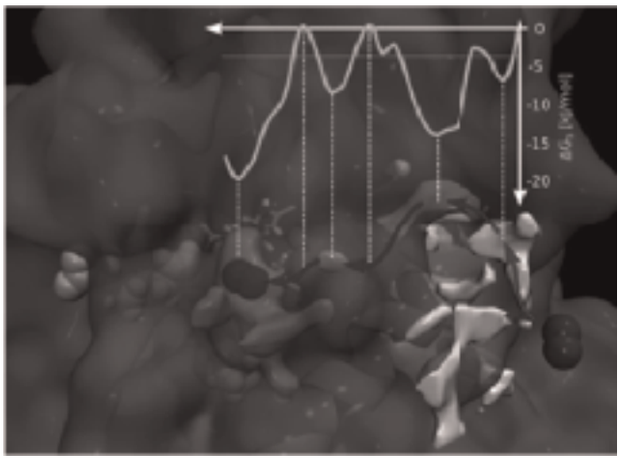
Board B45

Cells contain numerous enzymes using molecular oxygen for their reactions. Often, their active sites are buried deeply inside the protein, which raises the question whether there are specific access channels guiding oxygen to the site of catalysis. Choosing 12/15-lipoxygenase as a typical example for such oxygen-dependent enzymes, we determined the oxygen distribution within the protein and defined potential routes for oxygen access. For this purpose, we have applied an integrated strategy of structural modeling, molecular dynamics simulations, site-directed mutagenesis, and kinetic measurements. First, we computed the 3D free-energy distribution for oxygen, which led to identification of different oxygen channels in the protein. All channels connect the protein surface with a region of high oxygen affinity at the active site, localized opposite to the nonheme iron, providing structural explanation for the reaction specificity. The catalytically most relevant path can be obstructed by L367F exchange. The blocking mechanism is explained by reordering the hydrogen-bonding network of water molecules. Our results provide strong evidence that the main route for oxygen access to the active site follows a channel formed by transiently interconnected cavities whereby opening and closure are governed by side chain dynamics.



Protein Dynamics - II

214-Pos How Dynamics Govern The Role of Selectivity in PDZ Domain Protein Interactions?

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We studied specificity and promiscuity of the most common protein-protein interaction domain protein, PDZ domain using dynamic approach. First we identify the dynamics driving class I and class II peptide binding in PDZ domain proteins using a modified elastic network model. In this model, we introduce specificity for each single parameter constant using statistical potential and also modified network that makes possible to include solvation effect implicitly. The new elastic network model is shortly referred to as specific Gaussian Network Model (s-GNM). s-GNM is successful to identify changes in binding affinity upon mutation. Then we implement the binding induced collective motion obtained from s-GNM into all-atom molecular conformational sampling methods. This multi-scale approach enables us to compute the binding energy, free energy profiles and pathways for conformational change of PDZ domain proteins.

215-Pos Cyanylation Of Active-site Cysteines In Creatine Kinase Provides Site-specific Infrared Probes Of Local Structure And Dynamics

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Creatine kinase is an asymmetrical homodimer in solution, with a reactive thiol group in each subunit. Reaction with 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) followed by complete cyanolysis yields an enzyme derivative with both active cysteine residues cyanylated, while 2-nitro-5-thiocyanobenzoic acid (NTCB) instead selectively cyanylates only one subunit. These reactions have been used to generate two cyanylated creatine kinase derivatives. The cyanide stretching modes absorb in the infrared at ca. 2160 cm^{-1}; the observed lineshapes were fit to pseudo-Voigt and multiple Gaussian profiles to quantify the shapes of the absorption peaks. The differing CN lineshapes in the two cyano-protein derivatives indicate a difference in the local environments surrounding the two reactive cysteines. These data also contain some of the first evidence that introduction of thiocyanate into proteins generates a site-specific infrared probe of both the local structural environment and of dynamics due to local solvation.

216-Pos Protein dynamics of Rubisco mutants from Chlamydomonas Reinhardtii

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Ribulose 1,5 bisphosphate carboxylase/oxygenase (Rubisco) is the most abundant enzyme on earth and is responsible for incorporation of atmospheric CO2 into the biosphere. It does this by converting its substrate ribulose 1,5-bisphosphate to 3-phosphoglycerate. Rubisco is a relatively inefficient catalyst because of its low turnover number and its inherent oxygenase activity, which leads to a significant loss of carbon to the atmosphere. Engineering this enzyme to improve crop growth has been a long held research goal.

Studies on the dynamics of Rubisco enzyme and several of its mutants reveal the origin of their different rates of catalysis. Despite their structural similarity - the mutant structures are virtually the same as wild type protein - they show clearly different dynamics, influencing their enzymatic activity.

This study uses Molecular Dynamics calculations, combined with Essential Dynamics analysis, to successfully show the origins of the difference in catalitic efficiency and also provides a way to look at the effects of single mutations without the need for wet lab cloning and expression.

217-Pos On the Relationship between Thermal Stability and Catalytic Power of Enzymes

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The possible relationship between the thermal stability and the catalytic power of enzymes is of great current interest. Particularly,