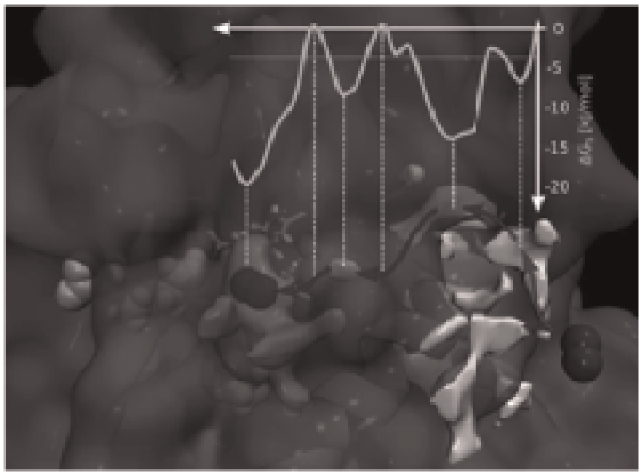

Protein Dynamics - II

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

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Board B46

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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Board B47

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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Board B48

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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Board B49

The possible relationship between the thermal stability and the catalytic power of enzymes is of great current interest. Particularly, it has been suggested that thermophilic or hyperthermophilic (Tm) enzymes have lower catalytic power at a given temperature than the corresponding mesophilic (Ms) enzymes, because the thermophilic enzymes are less flexible (assuming that flexibility and catalysis are directly correlated). These suggestions presume that the reduced dynamics of the thermophilic enzymes is the reason for their reduced catalytic power. The present paper takes the specific case of dihydrofolate reductase (DHFR) and explores the validity of the above argument by simulation approaches. It is found that the Tm enzymes have restricted motions in the direction of the folding coordinate but this is not relevant to the chemical process since the motions along the reaction coordinate are perpendicular to the folding motions. Moreover, it is shown that the rate of the chemical reaction is determined by the activation barrier and the corresponding reorganization energy, rather than by dynamics or flexibility in the ground state. In fact, as far as flexibility is concerned, we conclude that the displacement along the reaction coordinate is larger in the Tm than in the Ms and that the general trend in enzyme catalysis is that the best catalyst has less motion during the reaction. The relationship between thermal stability and catalysis appears to reflect the fact that in order to obtain small reorganization energy it is necessary to invest folding energy into the overall preorganization process. Thus, the improved catalysts are less stable. This trend is clearly observed in the present case.

218-Pos Modular Organization and Dynamics of the Budding Yeast Polarity Protein Network

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Board B50

More than 110 proteins which physically localize to the bud tip of budding yeast have been identified to play a role in polarity establishment. It is interesting to divide these proteins into groups and study the dynamics of these proteins.

Based on the physical interaction data from Saccharomyces Genome Database (SGD), we used a simulated annealing algorithm for modulization to define 5 functional modules (Signaling module, Transport module, endocytic module, Mitotic exit module and Exocytosis module) in the interaction network formed by these 110 proteins. Every protein is assigned a universal role according to its pattern of connections. Most proteins in the network are either ultra-peripheral nodes or peripheral nodes. Cdc42p in the Signaling module is a Connector Hub for the network, which means that it is not only a hub inside its own module, it also connects some other modules as a connector. This is consistent with Cdc42p's key role in polarity establishment.

How does the dynamics of polarity proteins contribute to polarity maintenance? We characterized the dynamics of 26 GFP-tagged proteins in Signaling module and Transport module in a systematic way, using iFRAP (inverse Fluorescence Recovery After Photobleaching) and calibrated confocal microscopy. With Metamorph and house-made programs, we quantified the iFRAP images systematically and obtained several parameters which quantify the dynamics of these proteins. Based on these parameters, we set up one model to describe and predict the dynamics of these polarity proteins.

On average, the proteins in the Signaling module are more dynamic than the proteins in Transport module. T-tests under both equal and unequal variance assumption show that T-Half values for these two modules are significantly different.

219-Pos Time-resolved Fluorescence Anisotropy Decay Reveals Loop Dynamics Of The Extra-cellular Domain Of Human Tissue Factor

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Board B51

Vascular injury leads to exposure of Tissue Factor (TF), an intrinsic membrane glycoprotein, to blood. Association between its extracellular domain and factor VIIa, a circulating protease, results in VIIa activation, initiating the coagulation cascade. We have been investigating VIIa activation at the molecular level by time-resolved fluorescence anisotropy. Our previous studies have shown that association between the extracellular, soluble domain of TF (sTF; residues 1-219) and VIIa eliminates segmental flexibility in VIIa. The current studies report on dynamics of sTF residues 80–91, which form a loop not resolved in the crystal structures of sTF or the sTF:VIIa complex. To determine the possible role of this loop in VIIa activation, we substituted two residues in sTF (E84 and T121) with cysteine, introducing sites for thiol-specific probes. In the sTF: VIIa complex, T121 is distant from the catalytic domain of VIIa, where no loops are present in sTF. Time-resolved fluorescence anisotropy decay experiments of mutant T121C alone, or in complex with VIIa, showed two correlation times, one reflecting rapid, sub-nanosecond probe motions and one reflecting global motions of T121C alone or in complex with VIIa. The other residue, E84, is located within the loop described above. Mutant E84C alone and in complex with VIIa showed, in addition to the fast correlation time due to probe motions, two longer correlation times: one is similar to the times for global motions of mutant T121C when alone or in complex with VIIa; the other is intermediate between the times for global motions and the times for rapid probe motions, and thus is associated with loop motions. When sE84C is bound to VIIa, this intermediate correlation time is slower. This indicates a decrease in the loop mobility when sTF interacts with VIIa.

220-Pos Model Cysteine-containing Peptides Indicate Sensitivity of cysteine vibrations to conformational distribution, and specific side-chain interactions

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Board B52

Model cysteine-containing peptides with established secondary structures were analyzed by infrared spectroscopy to investigate the utility of the SH stretching mode of cysteine and the CN stretching mode of cyanylated cysteine to report local structure and dynamics. In a helical peptide displaying a cooperative helix-coil transition, a strategically placed cysteine reports the site-specific conformational distribution. In a model beta-sheet peptide, the cysteine bands respond to specific interactions with hydrogen bonding partners on an opposing strand and report on the assembly of the local folded structure. In each case, complex line shapes are observed due to solvation and multiple contributing structures. These model systems provide a baseline for the use of such cysteine-based local vibrational probes in larger protein systems.

221-Pos Isotopic labeling of the GTPases Ran and Rap for mechanistic studies by trFTIR

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Board B53

GTP-binding proteins of the Ras-superfamily function as switches for e.g. signal cascades in cells. They cycle between the GDP bound “OFF” state and the GTP bound “ON” state. The latter interacts with effectors and transduces the signal. The intrinsic hydrolysis reaction is very slow but it can be accelerated 10^5-fold by GTPase activating proteins (GAPs) [1].

To analyze the GAP-catalysed GTPase reaction mechanism of Ran (nucleocytoplasmatc transport) and Rap (cell adhesion), time resolved Fourier Transform Infrared spectroscopy (trFTIR) [2] was applied. In order to gain information on the reaction mechanism it is important to assign the bands in the spectra to individual groups within the protein. This was achieved by incorporation of isotopic labeled amino acids in the proteins using M9-media for overexpression in *E. coli*. The correct labeling was checked by mass spectrometric analysis. In cases of spreading of the label into other amino acids or ineffective labeling, auxotroph *E. coli* strains were used [3].

Ras is activated by a GAP with an arginine-finger [4]. Opposed to this RanGAP and RapGAP are using an asparagine-thumb [5, 6] to activate GTP hydrolysis by Ran and Rap. Here we present comparisons of the GTPases Ras, Ran and Rap by means of isotopically labeled proteins.

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222-Pos Dynamics Of Eco109i Studied by Small-angle X-ray Scattering Experiments and Molecular Dynamics Simulations

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Yokohama City University, Yokohama, Japan

Board B54

A knowledge of protein dynamics in solution and its hydration structure is a prerequisite of understanding how proteins function. The combination of small-angle X-ray scattering (SAXS) experiments and molecular dynamics (MD) simulations can provide such knowledge at an atomic resolution.

In this study, we carried out a 150-ns MD simulation of the DNA-free form of EcoO109I, a type II restriction endonuclease, with explicit water at 298 K. During the simulation, the two catalytic domains fluctuated largely, and the cooperative motion between the two-dimerization domains was observed. The average conformation is more open than the crystal structure, and the distance between the two catalytic domains was large enough to bind DNA. The SAXS profile calculated from trajectories of the MD simulation including the scattering from explicit water molecules shows an excellent agreement with the experimental SAXS profile. This suggests that the open structures observed in the MD simulation are consistent with the experimental solution structure.

223-Pos All-atom Molecular Dynamics Simulations Of Hemoglobin Reveal The Dynamic Nature Of The Low Oxygen Affinity State

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Board B55

MD simulations performed on oxy- and deoxy HbA models in the presence and absence and biphosphoglycerate (BPG) show high-frequency (500MHz) fluctuations occur in helices E and F of oxy-Hb, the amplitude of which is larger in the deoxy and BPG-bound models. We propose that these amplitude variations can lower the oxygen affinity via positive homotropic and negative heterotropic allosteric effects, respectively, allowing Hb to reversibly modulate the positions of the distal and proximal His relative to the heme Fe. These changes in tertiary structure may result in reversible modulations of oxygen-affinity in both deoxy- and oxy-Hb with/without BPG. We speculate that the free energy of BPG binding may be stored as conformational entropy in the helical fluctuations of both deoxy- and oxy-Hbs. In this view, the difference in conformational entropy between deoxy- and oxy-Hbs corresponds to the free energy of cooperativity during the oxygenation/deoxygenation process rather than the free energy difference between the T- and R-quaternary structures.

Despite a long and intense search, the nature and identity of the low oxygen-affinity state has eluded experimental detection and sidetracked efforts to understand the molecular mechanism of allostery of Hb. Our results reveal the dynamic nature of the low oxygen-affinity state of Hb and we propose that oxygen- and effector-binding lead to dynamic tertiary structural changes which determine the oxygen-affinity, independent of Perutz's T-/R-quaternary switch. The identification of the dynamic nature of the low-affinity state of Hb allows us to redefine the allosteric mechanism of Hb as a Dynamic Allostery model that is consistent with the Monod-Wyman-Changeux (MWC) model of an allosteric enzyme.

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224-Pos Understanding The Different Mechanical Strength Of Homologous Proteins By 3D Modeling And Steered Molecular Dynamics

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Board B56

The mechanical properties of proteins are of crucial importance for the living cell. Previous studies have shown several topology, especially those including beta sheet patches along the pulling direction, have strong mechanical resistance. However, for proteins belong to the same topology, their mechanical resistance varies a lot sometimes. We performed homology based tertiary structure prediction and steered molecular dynamics simulation to investigate the different mechanical resistance of these proteins.

Titin is a 3 mega-dalton protein functioning as a molecular spring. It connecst the M-line and the Z-disk of the sarcomere. Titin consists of a head to tail linear arrangement of immunoglobulin (Ig) and fibronectin type III (fnIII) domains interconnected with linkage sequences. We stretched this single Ig domain in solution with constant velocity. The unfolding kinetics of I32 were examined to find out why I32 has stronger forces than I27.

225-Pos Origins of the Nuclear Localization of the Protein Ran in Interphase Live Cells

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Board B57

The small GTPase Ran is a key regulator of nuclear export and import of macromolecules through the nuclear pore complex (NPC) channel. To examine how Ran distribution and mobility contributes to its nuclear localization and its role in nuclear transport, we analyzed the behavior of EYFP-Ran fusion protein in HeLa cells using several quantitative fluorescence techniques. Similar to the endogenous Ran, on average 70% of EYFP-Ran localized to the nucleus. The majority of EYFP-Ran were freely diffusing and a smaller population of the protein displayed significantly slower mobility, resulting in an overall average diffusion coefficient of 5 $\mu m^2/s$. In contrast to previous reports, we observed no immobile fractions of EYFP-Ran in cells. The fraction of slower moving EYFP-Ran was larger in nuclei, apparently due to multiple ligands that are expected to bind the GTP-bound Tan prevailing in nuclei. The subdiffusive mobility of Ran GTP may therefore contribute to the nuclear retention of Ran. From the relative concentration o f EYFP-Ran accumulating at the nuclear envelope we estimated that in average ~300 endogenous Ran molecules are present around each nuclear pore complex. The relatively long residence time of Ran at the nuclear envelope (~20 ms) suggests that a significant fraction of Ran maybe recruited to the nuclear membrane by interactions that do not result in the transport of Ran through the NPC channel.

226-Pos How Well Can We Understand Large-Scale Protein Motions Using Elastic Network Modes?

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Board B58

We use a coarse-grained elastic network model (ENM) to study conformational transitions to address the following questions: How well can a conformational change be predicted by the mode motions? Is there a way to improve the model to get better results? To answer these questions, we use a dataset of 170 pairs having "open" and "closed" structures from Database of Macromolecular Movements (http://molmovdb.org/). Our results show that the conformational transitions fall into three categories:

1. the transitions that can be explained well by ENM;
2. the transitions that are not explained well by ENM, but the results are significantly improved after considering the rigidity of some residue clusters and modeling them accordingly; and
3. the intrinsic nature of these transitions, specifically the low degree of collectivity, prevents their conformational changes from being represented well with any ENM.

The results thus indicate that the applicability of ENM for explaining conformational changes is not limited by the size of the protein or even the scale of the conformational change. Instead, it depends strongly on how collective the transition is.

227-Pos Rigid Body Analysis of A. Aeolicus Argonaute

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Board B59

Members of the Argonaute family of proteins have been implicated as the primary effectors in the RNA interference pathway, in which genes are silenced post-transcriptionally by inactivating mRNA on a sequence-specific basis. An Argonaute protein complexed to a short template RNA strand serves as the minimal RNA Induced Silencing Complex (RISC). Argonaute proteins tend to have a large, globular structure with many physical degrees of freedom because each atom can move in three dimensions. Consequently, a full description of the dynamics of the structure is of high complexity. We construct a simplified description of the dynamics of two Argonaute proteins (from Aquifex aeolicus and Pyrococcus furiosus respectively) by analyzing a trajectory of the molecule's fluctuations generated by the method of molecular dynamics. The protein is partitioned, in a hierarchical manner, into several distinct domains which are defined by their collective essential modes of motion as well as the pairwise proximity of their component atoms. This reduced representation can then provide a framework for comparing the dynamics of the proteins. Notably, the scope of this work is not limited to Argonaute. Our methodology provides a framework for creating a simplified rationalization of complex protein motions.

228-Pos A Normal Mode and Molecular Dynamics study of the Chaperonin Folding Machine

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Board B60

Most proteins are highly dynamic, and often large-amplitude deformations are crucial for their function. Information regarding protein dynamics is thus required to establish the important link between structure and function. A wide range of studies in recent years has shown that these large-amplitude motions can be well predicted by simplified normal mode analysis (NMA), especially for supra molecular complexes. Another method is the principal component analysis (PCA) of molecular dynamics (MD) trajectories. To predict such global motions in multimeric protein complexes it has been standard practice to perform NMA on the monomeric forms. This procedure is expected to yield the higher frequency modes of the multimeric complex. These modes are informative, but in many cases constraints, which might be important for the monomeric dynamics, are left out. We have developed a strategy to investigate the monomeric dynamics in a multimeric scale. In other words; can we find the normal modes from the monomeric calculation in the multimeric set of modes, and in which frequency range? And, which type of monomeric modes are the most energetically favorable in the multimeric scale?

In order to answer these questions we have chosen the well studied chaperonin GroEL-GroES complex. This is a multimeric molecular machine that assists in the folding of a wide range of substrate proteins. To carry out its work, the protein subunits undergo large conformational changes. We present here a study of the dynamics of this protein complex which consists of ~110.000 atoms. NMA on different units of the macromolecule and a principal component analysis of a 50 ns-MD simulation have been performed. Different approaches have been used to analyze the global motions within the complex and the results will be comparatively discussed.

229-Pos Solvent Viscosity and Peptide Helix Enantiomerization Dynamics

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Board B61

We have used ^{13}C dynamic NMR spectroscopy to determine rate constants for the conformational dynamics of a 3_{10}-helical hexameric peptide, Z-$(Aib)_6$-OtBu (Aib = residue of α-aminoisobutyric acid). Because the strongly helix-promoting Aib residue is achiral, oligomers of Aib will form left- and right-handed helices with equal probability. Furthermore, these helices interconvert, through a large number of single bond rotations, between left- and right-handed helical forms on a timescale that is measurable via ^{13}C dynamic NMR. We have measured rate constants for this interconversion in a series of solvents of varying viscosities, including small, 1-, 2-, and 4-carbon alcohols, measured at temperatures between ~ 3°C and ~ 43 °C. We have observed that the solvent viscosity limits the rate of the conformational dynamics of this peptide in a 1/ђ fashion, consistent with Kramers' diffusional model of reaction dynamics in a viscous medium. The interpretation of the measured rate constants will be considered within Kramers' theory and alternative theories of condensed-phase conformational dynamics.

230-Pos Allosteric Communication in a Homotetramer: The Inside Story

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Board B62

EcPFK is a homotetrameric enzyme with a single active site and a single allosteric site per subunits. It has 28 potential site-site interactions, 16 heterotropic interactions and 12 homotropic interactions. These interactions in turn derive from multiple copies of 6 potentially unique homotropic interactions and 4 potentially unique heterotropic interactions. The four heterotropic interactions are named by the distance between one active site and one allosteric site, 23Å, 30Å, 33Å, and 45Å. Making hybrid tetramers of EcPFK is able to dissect a single heterotropic interaction. Four heterotropic interactions were isolated by this manner and shown that in sum they can account for all the heterotropic interactions in the wild type enzyme. Moreover, each interaction has a unique magnitude, and its relative contribution to the total is different for both MgADP activation and PEP inhibition. The 23Å interaction has the highest contribution for inhibition. The 33Å interaction is the strongest one for activation. The hybrid method has been modified to remove the single trptophan in the mutated subunits so that the trptophan remaining in the native subunit involved in a single heterotropic interaction. By doing mutagenesis on conservative phenylalanine or tyrosine, the trptophan has been placed in various positions in

EcPFK. Each tryptophan is a fluorescence probe that can monitor the response to ligand binding by doing steady-state anisotropy experiment. The results suggest that the 23Å allosteric interaction involves the perturbation of side-chain dynamics both near and quite far away from the respective ligand binding sites.

Supported by grants from the NIH (GM033216) and the Welch Foundation (A1543).

231-Pos Characterization of Phenylalanine Based Short Peptides at Varying pHs, Temperatures, and Concentrations via Electronic Circular Dichroism and Absorption Spectroscopy

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Board B63

Previously it was shown that phenylalanine based short peptides form nanotubes, spheres and gels in water. In order to elucidate the underlying aggregation process, we investigated diphenylalanine (FF), glycine-phenylalanine-glycine (GFG), and alanine-phenylalanine-alanine (AFA) by electronic circular dichroism and absorption spectroscopy. Our data indicate the presence of a weak absorption band at 230 nm, which exhibits a rather strong positive Cotton effect. Based on preliminary time-dependent DFT calculations we currently assign it to a backbone → F charge-transfer transition. Its rotational strength is temperature and pH dependent. At neutral pH, with varying temperatures (5°C to 95°C), the phenylalanine group rotates and produces a positive cotton band at low temperatures, a couplet at intermediate temperatures, and a negative cotton effect at high temperatures. This effect is reduced at acidic pH, the positive cotton band only decreasing in magnitude with increasing temperature. Lastly, our measurements capture the change in the circular dichroism signal of the peptides studied with increasing concentrations. These changes are caused by aggregation present at higher concentrations.

232-Pos Determination of the Role of Reduced Steric Hindrance in Mediating the Activation Barriers to H/D Exchange in an Eight-Residue Hydrophobic, Helical Peptide

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Board B64

The physical characteristics of amino acids play critical roles in determining protein structure and flexibility. The α, α-dialkylated amino acid α-methylalanine (Aib) is notable for stabilizing helical structures as a result of its significant sidechain steric hindrance. One measure of flexibility in structures such as helices is the strength of hydrogen bonds between amino acids. We are investigating the effects of steric hindrance on flexibility by preparing a series of octameric peptides composed mainly of Aib but with strategically placed alanine residues, and analyzing the strengths of the hydrogen bonds by ^{1}H NMR. The pseudo-first order kinetics for the exchange of amide protons with methanol deuterons are measured for each amide at various temperatures. The activation energy for the exchange reaction corresponding to each proton is used to determine which protons are more exposed to the solvent. More exposed protons are an indication of weaker hydrogen bonding, and thus greater helix flexibility. Previous results on a peptide with two contiguously placed Ala residues (AA45) indicate that the reduction in steric hindrance at the Ala-Ala site results in a significant local perturbation of the helical structure and flexibility. In this study, we have prepared a similar peptide, but with two central Ala residues separated by one full helical turn (AA36). The results on this peptide should shed light on the long or short range effects of steric hindrance on helix structure and flexibility.

233-Pos Imaging Htt protein aggregation in cells by fluctuation analysis

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Board B65

The aggregation of the huntingtin protein is thought to influence Huntington's disease pathogenesis. We performed experiments in transfected COS 7 cells using human Htt exon 1 with varying lengths of polyglutamine fused to GFP (Httex1 97QP-GFP, Httex1 46Qp-GFP, Httex1 25QP-GFP). Expression of the Htt protein with different glutamine lengths results in the formation of visible fibrils in cytosol and in the nucleus. The macroscopic formation of large aggregates depends on the glutamine sequence lengths. Using N&B fluctuation spectroscopy analysis performed simultaneously on the entire cell, we followed the kinetics of aggregate formation. In this analysis the size of the aggregates is deduced from the brightness (B) map. This map shows different aggregate sizes in the cytosol and in the nucleus. In a first phase, large aggregates and fibrils appear in the cytosol followed by aggregation in the nucleus. After several hours, the apparent size of aggregates in the nucleus surpasses the size of the aggregates in the cytosol. At this point in the cell cycle, a very large immobile aggregate forms in the cytosol. In the presence of this large "solid like" aggregate, the overall fluorescence intensity as well as the size of aggregates decreases in the rest of the cytosol. After some time, the intensity and the size of aggregates also decrease in the nucleus. It seems that the very large aggregate accumulates most of the cellular protein. The apparent lag time of the changes in the nucleus with respect to the cytosol indicates a barrier for the transport of the protein in and out of the nucleus. Our findings suggest a model for a dynamic sequence of phases of Htt fibril formation in living cells.

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234-Pos The Role Of Amino Acids In The Reaction Mechanism Of Ras By trFTIR And Isotopic Labeling

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Board B66

The small GTPase Ras plays an important role in the signal transduction of cellular processes during proliferation and differentiation. These are controlled by the switch from the active GTP bound form to the inactive GDP bound form of Ras, which can be catalyzed by GTPase activating proteins (GAPs). Ras is the prototype for the superfamily of GTPases. As an oncogenic protein mutations can induce cancer. [1, 2]

To analyze the reaction mechanism time-resolved Fourier-transform infrared (trFTIR) difference spectroscopy was applied [3]. To start a controlled GTPase reaction caged-pHP-GTP was photolysed by laser irradiation. In order to obtain information the reaction mechanism, the absorptions have to the assigned to individual groups within the protein. For this, isotopically labeled amino acids were integrated during the expression in E. coli into Ras [4]. Most of the important amino acids in Ras were successfully labeled. First assignments were done for threonine and tyrosine. The C=O backbone vibration at 1689 cm-1 of Thr35 can be used as a marker band of switch I [5]. Further, the GAP of Ras (NF1) was successfully labeled. Here we could assign the aborption of the important Arg-finger and characterized details for the catalyzed reaction mechanism. The "off"-conformation of Ras is only obtained after the Pi-release.

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235-Pos Normal Mode Directed Exploration of Conformation Space as a Tool for Studying Macromolecular Interactions

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Protein structures are frequently represented near an equilibrium by a single set of coordinates (e.g. from x-ray crystallography). This is an effective way to visualize the conformational shape and identify the internal structural linkages. In order to study the interactions between a protein and another (macro)molecule it is necessary to consider an ensemble of accessible states. Sampling techniques, pathway simulations and experimental methods have all been employed, but often are limited by computational requirements or experimental resolution. A new exploration method, based on cluster normal mode analysis (cNMA), is presented. This method, called cNMA Search, is used to populate conformational space around HIV protease.

The cNMA method effectively captures the low frequency modes that are most relevant to large structure motions. cNMA Search allows conformational change along each mode's axis to produce a first level ensemble. Each of these conformations is then used in a similar fashion to produce a second level ensemble. The continued iteration of this procedure yields a set of conformations distributed around the original equilibrium structure.

Each structure in the final ensemble is defined by a sequence of mode shapes. The corresponding series of structures spanning across the ensemble levels is equivalent to a guided extrapolation of the original equilibrium structure. Since each step in this extrapolation follows a mode shape relevant to the current conformation, the pathway restricts itself to an exploration of the accessible subspace of the entire conformation space, whereas pure sampling methods may interrogate inaccessible regions.

Following the presentation of cNMA Search, the HIV protease conformation set is analyzed. The distribution of conformations is compared to that of NMR ensembles. The conformations are also classified by geometric features.

236-Pos Helical Peptide Conformational Excursions Measured by H/D Exchange

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Board B68

We have characterized the conformational propensities of short oligomers of α-aminoisobutyric acid (Aib) via hydrogen/deuterium (H/D) exchange kinetics in methanol solvent. The Aib residue has geminal methyl side chains which restrict the conformation of this residue to the helical region of the Ramachandran map. Small differences in ϕ, ψ angles result in distinct intramolecular hydrogen bonding patterns: i/i+3 (3_{10} helix) and i/i+4 (α-helix). These two hydrogen bonding patterns may be distinguished, in principle, via H/D exchange experiments. For a tetramer of Aib C-terminated by a tert-butyl ester, we have observed only two classes of exchange rates, suggestive of two limiting classes of amide hydrogen atoms found within the peptide: intramolecularly hydrogen-bound and solvent-exposed. In contrast, a tetramer C-terminated by β-alanyl-OtBu displays an amide with an intermediate exchange rate, suggesting that excursions of this tetramer to an α-helical form exposes an amide hydrogen to exchange. 2-D NMR assignment of the amide hydrogen atoms confirm that the hydrogen with an intermediate exchange rate is indeed the proton that is solvent exposed in an α-helix but not in a 3_{10} helix. Results for tetramers and octamers of Aib with varying C-terminal protecting groups implicated in the α-/3_{10} helix equilibrium will be discussed.

237-Pos Monitoring The C Terminal Loop In Inosine Monophosphate Dehydrogenase (IMPDH) Using Fluorescence Correlation Spectroscopy

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Inosine monophosphate dehydrogenase (IMPDH) catalyzes the oxidation of inosine monophosphate (IMP) to xanthosine monophosphate (XMP), a required step in de novo guanine nucleotide biosynthesis. X-ray crystal structures of the IMPDH homotetramer reveal that the C terminal loop of one monomer interacts with the active site of an adjacent monomer. These interactions, as well as the flexibility of the C terminal loop, are dependent on what substrates, cofactors and/or inhibitors are bound in the active site. Models of IMPDH activity, therefore, suggest that loop motions play a critical role in catalysis. In order to directly monitor the role of loop motions in the IMPDH catalytic cycle, we have added a 12 residue “Q tag” to the C terminus of IMPDH from the parasite Trichomonas foetus. The Q tag is recognized by the enzyme transglutaminase and can be specifically labeled with cadaverine derivatized fluorescent dyes. Changes in the dynamics of labeled C terminal loops are monitored in real time using fluorescence correlation spectroscopy (FCS), and FCS experiments reveal substrate dependent changes in the dynamics of the C terminal loop supporting existing models for IMPDH activity.

238-Pos Auto-inhibition In The Multi-domain Protein Pick1 Revealed By Dynamic Models Of Its Quaternary Structure

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PICK1 (protein interacting with C kinase 1) is a two-domain protein involved in membrane protein trafficking. Its N-terminal domain is a PDZ that binds to the C-termini of proteins in a sequence specific manner, and its C-terminal domain belongs to the BAR family. The cellular localization of PICK1 is determined by the interaction between its BAR domain dimer and phospholipid membranes, but only after the BAR domains are freed from the inhibitory effect of their cognate PICK1-PDZ domains. To reveal the structural context of the PICK1 auto-inhibition mechanism, we developed a model of a PICK1 dimer complex with protein-protein docking tools starting from the crystal structure of the PICK1-PDZ and a homology model of the BAR domain. In this multi-domain model of PICK1, two PDZ domains occlude the lipid binding surface of the BAR dimer. To explore the role of PDZ ligand binding on the stability of this multi-domain complex, we studied the behavior of the PICK1-PDZ domain in isolation, in its dimeric complex with the other PICK1 domains, and/or bound to a ligand with several molecular dynamics simulations. Residues near the ligand binding site were found to be dynamically coupled to the residues forming the complex interface. Moreover, ligand binding changed the dynamics of the interaction between the PDZ domain and the BAR dimer. Thus, there is an entropic cost for the PDZ/BAR complex formation, which is enhanced when the PDZ domain binds a C-terminal peptide from the dopamine transporter, but not one corresponding to PKC-alpha. These differences correspond to the physiological selectivity of the PICK1 protein, suggesting that allosteric changes in the PICK1-PDZ can contribute to the release of the auto-inhibition of the PICK1-BAR domain and determine the physiological function of the protein.

239-Pos Enthalpic And Entropic Stages In a-helical Peptide Unfolding, From Laser T-jump/uv Raman Spectroscopy

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In this work we report application of a novel kHz repetition rate solid-state tunable NIR (pump) and deep UV Raman (probe) laser system to study the dynamics of helix unfolding in Ac-GSPEA$_{3}$-KA$_4$KA$_4$-CO-D-Arg-CONH$_2$, a peptide designed for helix stabilization in aqueous solution. Its T-dependent UV resonance Raman (UVRR) spectra, excited at 197 nm for optimal enhancement of amide vibrations, were decomposed into variable contributions from helix and coil spectra. The helix fractions derived from the UVRR spectra and from far UV CD spectra were coincident at low T, but deviated increasingly at high T, the UVRR curve giving higher helix content. This difference is consistent with the greater sensitivity of UVRR spectra to local conformation than CD. After a laser-induced T-jump the UVRR-determined helix fractions defined mono-exponential decays, with time-constants of ~120 ns, *independent* of the final T ($T_f = 18$ to 61°C), provided the initial T (T_i) was held constant (6°C). However, there was also a prompt loss of helicity, whose amplitude increased with increasing T_f, thereby defining an initial enthalpic phase, distinct from the subsequent entropic phase. These phases are attributed to disruption of H-bonds followed by reorientation of peptide links, as the chain is extended. When T_i was raised in parallel with T_f (10°C T-jumps), the prompt phase merged into an accelerating slow phase, an effect attributable to the shifting distribution of initial helix lengths. Even greater acceleration with rising T_i has been reported in T-jump experiments monitored by IR and fluorescence spectroscopies. This difference is attributable to the longer range character of these probes, whose responses are therefore more strongly weighted toward the H-bond-breaking enthalpic process.

240-Pos Towards Quantitative Characterization of Interaction Energies in Cells

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Interest in protein-protein interactions is continually increasing due to the number of disorders and medical conditions caused by variations in these interactions. Characterization of these interactions can be obtained through a variety of techniques, through which the oligomeric state, stoichiometry ratio, equilibrium constants and kinetic rate constants can be determined. Forster resonance energy transfer (FRET) is one method used in studying protein-protein interactions. FRET is based upon the energy transfer from a donor to an acceptor, which is dependent upon the distance between the two. The length scale is between 1 to 10 nm which is approximately the same as that for protein-protein interactions; therefore, FRET is capable of yielding important information when fluorophores are attached to the proteins of interest. Here we made use of a FRET pair, an acceptor (mCherry) and donor (eYFP) fluorescent protein, to show that we could obtain the FRET efficiency (E) from the FRET observed in a confocal microscope. In addition, we showed how to determine the concentrations of the acceptor and donor. This is important because in a cellular system, protein levels cannot be tightly regulated; therefore, it is necessary to know the exact protein concentrations for quantitative measurements. Once the FRET efficiency and the concentrations are determined, the disassociation constant (Kd) for the protein interactions can be determined. The new insight into the protein-protein interaction could aid in drug design to correct problems caused by improper protein interactions.

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241-Pos The Conformational Dynamics of HIV-1 Reverse Transcriptase Revealed by Hydrogen/Deuterium Exchange and Mass Spectrometry

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Board B73

HIV-1 reverse transcriptase is an essential enzyme in the HIV lifecycle and a popular target for drug design. While there are numerous crystal structures of reverse transcriptase (RT) bound to various ligands and inhibitors, there is little experimental information on the conformational dynamics of RT in solution, even though dynamics are believed to play an important role in function. We studied the dynamics of RT using hydrogen/deuterium exchange and mass spectrometry. RT consists of a 66 kD subunit (p66) and a 51 kD subunit (p51), each of which is composed of multiple subdomains. While various crystal structures indicate that the fingers and thumb subdomains and RNase H domain of the p66 subunit can assume different orientations, H/D exchange indicates that the secondary structure within these sub-domains is stable. An important exception is the small beta-sheet K in p66 that lies at the base of the thumb domain. This beta sheet is highly flexible and marginally stable. Several residues in this region form contacts with non-nucleoside inhibitors bound to RT. Thumb domain motions are thought to play an important role in the RT replication cycle, and we suggest that the high degree of flexibility seen in beta sheet K facilitates thumb domain mobility. Despite the fact that the p51 subunit has a more compact tertiary structure than the p66 subunit, it shows similar rates of H/D exchange. Additionally, we find that several regions of the RNAse H domain are highly flexible despite containing significant secondary structure, which is consistent with an earlier NMR study of the isolated RNAse H domain.

242-Pos Exact Low-Force Kinetics from High-Force Single-Molecule Unfolding Events

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Mechanical forces play a key role in crucial cellular processes involving force-bearing biomolecules, as well as in novel single-molecule pulling experiments. We present an exact method that enables one to extrapolate, to low (or zero) forces, entire time-correlation functions and kinetic rate constants from the conformational dynamics either simulated numerically or measured experimentally at a single, relatively higher, external force. The method has two-fold relevance:

(i) to extrapolate the kinetics at physiological force conditions from molecular dynamics trajectories generated at higher forces that accelerate conformational transitions, and
(ii) to extrapolate unfolding rates from experimental force-extension single-molecule curves.

The theoretical formalism, based on stochastic path integral weights of Langevin trajectories, is presented for the constant-force, constant loading rate and constant-velocity modes of the pulling experiments. For

(i) applications are described for simulating the conformational isomerization of alanine dipeptide, and for
(ii) the single-molecule pulling of RNA is considered.

The ability to assign a weight to each trace in the single-molecule data also suggests a means to quantitatively compare unfolding pathways under different conditions.

243-Pos Perturbation-based Markovian Transmission Model for the Dynamics of GroEL-GroES

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Board B75

The study of the dynamics of a complex system is an important problem that includes large macromolecular complexes, molecular interaction networks, and cell functional modules. Large macromolecular complexes in cellular machinery can be modeled as a connected network, as in the elastic or Gaussian network models as demonstrated by Bahar and colleagues. Here we propose the Perturbation-based Markovian Transmission Model for studying the dynamics of signal transmission in macromolecular machinery. The initial perturbation is transmitted by Markovian processes, where the dynamics of the probability flow is analytically solved using the master equation. Due to the large size of macromolecular complexes, it is very difficult to obtain analytical time-dependent Markovian dynamics of all atoms from the first perturbation until stationary state. To overcome it, we decrease the level of complexity of the transition matrix using Krylov subspace method. This method is equivalent to integrating all eigen modes, and we show it can provide a globally accurate solution to the dynamics problem of signal transmission for very large macromolecular complexes with reasonable computational time. We studied the dynamics of the GroEL-GroES chaperone system by applying uniform perturbation to all residues of the X-ray structure. We predicted a set of pivot, messenger, and effector residues, each with distinct dynamic behavior. We also identified key residues involved in the multiple salt-bridge switches important for the allosteric transition in GroEL. Our predictions are in agreement with published experimental data and with the results of computer simulations that used different methodologies. We also studied a selective perturbation on the surface of ATP binding pocket and predicted the path of maximal probability flow of the signal. It demonstrated clear advantages in studying dynamic behavior of large systems, such as virus capsid, ribosome, or large allosteric proteins.

244-Pos Improvements In Mixing Time And Mixing Uniformity In Devices Designed For Studies Of Protein Folding Kinetics

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Using a microfluidic laminar flow mixer designed for studies of protein folding kinetics, we demonstrate a mixing time of 1 ± 1 μs with sample consumption on the order of femtomoles. We recognize two limitations of previously proposed designs:

1. size and shape of the mixing region, which limits mixing uniformity and
2. the formation of Dean vortices at high flow rates, which limits the mixing time.

We address these limitations by using as narrow a shape-optimized nozzle and by reducing the bend of the side-channel streamlines. The final design, which combines both of these features, achieves the best performance. We quantified the mixing performance of the different designs by numerical simulation of coupled Navier-Stokes and convection-diffusion equations, and experiments using fluorescence resonance energy-transfer (FRET)-labeled DNA. These mixers are currently being used for measurements of protein folding kinetics using FRET & tryptophan fluorescence.

244.01-Pos Characterization Of Protein Breathing Using Coordinated WAXS And NSE

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In aqueous solution some proteins undergo large-scale rigid body movement of secondary structures, subunits or domains - referred to as protein ‘breathing’ - that define a native-state ensemble of structures. These motions increase with decreasing protein concentration or increasing temperature. They are sensitive to the nature and concentration of solutes and other proteins and appear to be characteristic of individual proteins. We are using a combination of wide angle x-ray scattering (WAXS), neutron spin echo (NSE) spectroscopy and computational modeling to determine the spatial and temporal scales of these fluctuations including the size of the rigid bodies undergoing these motions; the spatial scale of these motions and the decay rates of the fluctuations.

Concentration-dependent intensity changes in WAXS data have been interpreted in terms of the increase in spatial scale of motion with decreasing concentration. Compared to reference structures at high concentration, hemoglobin in dilute solution undergoes fluctuations in which structural features of characteristic length 10 A exhibit relative motions of ~1.2 A. Myoglobin exhibits similar behavior.

NSE data collected to high Q values from solutions of hemoglobin also suggest relative motions of structural features with characteristic lengths of 10–20 A. Collection of data at lower concentrations will be carried out in order to correlate trends with those seen in the WAXS data.

These experiments are being used to construct a comprehensive description of the spatial and temporal properties of the slow, correlated motions that make up the native state ensemble of a protein in solution.

Physical Chemistry

245-Pos Do Zwitterions Contribute to the Ionic Strength of a Solution?

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Board B78

The question of whether zwitterions contribute to the ionic strength of a solution has been a topic of much confusion over the years. Some authors have assumed that zwitterions do not contribute to ionic strength, while others have assumed the opposite. We have used free solution capillary electrophoresis to answer this question.