studies of the mechanism, we were able to quantitatively reconstruct the elementary steps as well as the energetic pathways along the AK's enzymatic cycle. The mechanistic roles of AK's stochastic lid dynamics were found to engage in conformation gating, shuffling of reaction pathways, and dynamical induced fit.

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Two-colors Photo-Switching of E222Q-GFPMut2 Mutant by Fluorescence Correlation Spectroscopy
Valentina Quercioli, Chiara Bosisio, Maddalena Collini, Laura D'Alfonso, **Giuseppe Chirico**.
Dep. Physics, Milan, Italy.
GFP mutants display complex photodynamics whose properties can be tuned even by single mutations of the chromophore or the protein backbone. Some GFP mutants can be photo-activated (paGFP) or photo-switched (E222Q mutants). Although photo-activable mutants are valuable tools in nanoscopy studies and have been already applied in this field, the photo-switching behavior of some GFP mutants has not been yet exploited in biological imaging.
We report here the characterization of the two-color enhancement of the E222Q mutant of the GFPMut2 protein aimed to its application in cellular imaging. The anionic fluorescence output is enhanced when the GFP is irradiated simultaneously at 390-440 nm. By fine tuning the 488 nm direct anionic *excitation* and the UV-blue *irradiation*, the GFP mutant emission can be enhanced up to 2.5 times. The maximum switching efficiency occurs at 420 nm and display a marked pH dependence.
Moreover, we have characterized the activation time of this process by modulating the irradiation or excitation beams. By means of Fluorescence Correlation Spectroscopy methods under modulated irradiation in the UV-blue range of the spectrum, we are able to measure the activation times of the switching process that lie in the 10-100 ms range. We present a simple two states model and analyze it by Laplace Transform methods to obtain a validation of the proposed model and a direct estimate of the activation times.
Finally we discuss possible applications of this behavior in fluorescence imaging and direct studies of intracellular dynamic processes.

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Conformational Transitions Of Disordered Proteins Associated With Different Redox States Of Di-thiol Pairs
Samuel W. Fan, Richard A. George, Naomi L. Haworth, Lina L. Feng, Jason Y. Liu, **Merridee A. Wouters**.
Victor Chang Cardiac Research Institute, Sydney, NSW, Australia.
Because of their enhanced reactivity, physiologically important redox-active disulfides are also more susceptible to cleavage/oxidation under non-physiologic conditions. For instance, redox-active disulfides are prone to cleavage by synchrotron radiation during the process of X-ray structure determination. Here we mined the Protein Data Bank for highly similar proteins that have been solved in multiple redox states - i.e. disulfide-bonded in one structure and reduced in another. Some of these protein pairs exhibited order/disorder transitions. Disorder-to-order transitions have previously been observed upon binding of ligands. Acquisition of order upon binding of ligands concomitant with disulfide formation was apparent for the oxidoreductase gdhB, where the disulfide straddled part of the PQQ binding site, and the RNA sulfuration enzyme *Ec*TrmU, where the disulfide straddles the tRNA-binding site. However, the reverse was true for the *Thermotoga maritima* tRNA-processing enzyme, Psi55s, where significant disorder of the protein chain concomitant with disulfide reduction occurred upon binding of the tRNA substrate fragment. The introduction of disorder may facilitate further co-operative binding of the RNA and protein after the initial docking step. A subset of Redox Pair proteins exhibiting order/disorder transitions correlated with disulfide redox status may contain regions of disorder in excess of 20% of the protein chain. All the proteins in this group exist as dimers with the other monomer being more ordered. The proteins may adopt a physiologically-relevant Molten Globule state as part of their function. The oxygen-rich sequences of the disordered regions of proteins of the Redox Pair dataset seem to represent a novel type of disordered sequence not previously recognized. Finally, disordered regions are posttranslationally modified by acetylation, glycosylation, methylation and phosphorylation which may regulate the order/disorder transition. Redox-activity of disulfides should be added to this list of posttranslational modifications.

380-Pos Board B259
"Arrhenius Approach to Study Kinetics of Fresh Egg Protein"
Dipti Sharma.
UML, Lowell, MA, USA.
This study explores an interesting denaturing kinetics of fresh egg proteins following Arrhenius behavior. Fresh egg white-protein (2mg) was used in a sealed cell for scanning using calorimetric technique. Heating scans were performed from 10° C to 100° C at different heating ramp rates varying from 1 to 20° C/min. All environments were kept identical for all runs to compare parameters (temperature, enthalpy, heat energy). An endothermic peak was found on heating scan showing denaturing of protein. As heating ramp rate increases, the denature peak shifts towards higher temperature. This peak shift follows Arrhenius behavior and shows an activated denaturing kinetics of the white egg protein. This peak was also compared with the water to avoid water effects. Cooling scan and second heating scan were also performed for the samples and no residue of peak was found which clarifies that the protein was completely denatured after first heating. The denaturing peak shifts linearly with the ramp rate and temperature and gives activation energy of this transition. Behavior of denaturing peak can be explained in terms of Arrhenius theory.

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NMR Dynamics Of PSE-4 β-lactamase: An Interplay Of ps-ns Order And μs-ms Motions In The Active Site
Sébastien Morin, Stéphane M. Gagné.
Université Laval, Québec, QC, Canada.
Class A beta-lactamases are involved in antibiotics resistance, a persistent phenomenon in medicine and agriculture. Many kinetics and structural studies have been reported. However, comprehension of their serine-based mechanism is incomplete. Studying the dynamics of these enzymes and relating it to the considerable structural and functional data available could provide more insights. Indeed, dynamics on different timescales has been shown to be central to proteins function.
Beta-lactamases TEM-1 and PSE-4 are studied by NMR and molecular dynamics (MD), both atomistic methods to protein dynamics. TEM-1 is a traditional class A beta-lactamase for which a dynamic study by NMR has been reported recently. On the other hand, PSE-4 is a member of the subclass of carbenicillin hydrolyzing beta-lactamases. Both enzymes share high identity (42%) and structural homology (1.3 Å backbone RMSD).
We present an overview of the work done on PSE-4 by NMR. This includes amide exchange as well as 15N spin relaxation data. Analysis is performed using the Lipari & Szabo model-free formalism. Moreover, the assessment of datasets consistency, a prerequisite for united data analysis, is discussed. Finally, comparisons are made with the homologous TEM-1. It turns out that both beta-lactamases share high backbone order on the picosecond-nanosecond timescale, especially around the active site. Moreover, evidence of slow microsecond-millisecond motions around the active site points toward important dynamics arising on the catalysis timescale.
In the near future, relaxation dispersion experiments will aim at quantifying the slow microsecond-millisecond motions detected with backbone 15N spin relaxation. Moreover, experiments will be performed to assess the influence of substrate (or inhibitor) binding on the dynamics. Finally, clinically-relevant mutants will be studied to link their increased activity to possible changes in dynamics.

Protein Assemblies

382-Pos Board B261
SEDPHAT - An Analysis Platform for the Biophysical Analysis of Reversibly Assembled Multi-protein Complexes in Solution
Patrick H. Brown.
NIBIB, National Institutes of Health, Bethesda, MD, USA.
Multi-protein complexes are ubiquitous in cellular activities, and found in metabolism, transcription control, and intracellular signaling and motility. In many areas, intense research is devoted to understanding the functional mechanism of these complexes, for example, dissecting the energetics of the total assembly process from the individual components to form the functional active multi-protein complex. This is particularly difficult for complexes that are only transiently assembled by relatively low-affinity interactions. Our research is devoted to developing new biophysical methods for the characterization of the number, size and hydrodynamic shape of protein complexes as well as quantifying the dynamics of their assembly and disassembly from purified components in solution. One approach utilized is to make measurements from orthogonal perspectives of the reaction coordinate and to integrate these data in a global analysis. To this end, we have developed the analysis software platform SEDPHAT that allows us to analyze globally multiple data sets collected from one of several different biophysical techniques. We present here an overview of the analytical tools available in this software.

383-Pos Board B262
Probing the Heterogeneity in the Distribution of Binding Properties of Immobilized Surface Sites through Bayesian Analysis
Huaying Zhao, Inna I. Gorshkova, Juraj Svitel, Faezeh Razjouyan, Peter Schuck.

NIBIB/National Institutes of Health, Bethesda, MD, USA.
When studying the binding of ligands to macromolecules immobilized at a surface, the question arises whether the surface binding sites are heterogeneous, as one might expect considering many factors such as intrinsic surface roughness, nonuniform density distribution of polymeric linkers and nonuniform chemical attachment producing different protein orientations and conformations. We previously developed a computational tool to determine the distribution of affinity and kinetic rate constants from the analysis of experimental surface binding data. In order to avoid an ill-posed computational analysis, the previous approach used a regularization strategy assuming a priori an equal probability for all binding constants, which results in the broadest possible distribution of all that are consistent with the data. In the present work, we implemented a Bayesian approach of regularization to incorporate the opposite assumption, i.e. that the surface sites a priori are expected to be uniform (as expected in free solution). The data analysis with this new approach results in the narrowest distribution given the experimental data. We applied this method to several protein systems immobilized on a carboxymethyl dextran surface and with protein interactions measured by surface plasmon resonance. The obtained distributions are highly reproducible. The results demonstrate microheterogeneity of the binding sites on the surface, in addition to broad populations of significantly altered affinity. The variation of immobilization conditions and the total surface density of immobilized sites indicates a considerable impact of these parameters on the distribution of the surface binding sites.

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Proteomic Analysis of KvLQT1 and HERG-associated Proteins

Hitesh K. Jindal, Gideon Koren.
Rhode Island Hospital, Providence, RI, USA.
Background: We have generated LQT1 and LQT2 rabbit models by overexpressing the Flag-tagged pore mutant KvLQT1-Y315S or HERG-G628S, in the heart of transgenic rabbits. We undertook a proteomics approach in order to identify KvLQT1 as well as HERG-interacting proteins and differences in protein expression in the hearts of these transgenic rabbits.
Methods: Triton X-100 solubilized heart membranes were subjected to immuno-precipitation using anti-FLAG antibody. The FLAG-immunoprecipitated KvLQT1 and HERG-bound complexes were resolved by SDS-PAGE and stained with Commassie blue. Upon resolution, 15 protein bands each corresponding to KvLQT1 and HERG-interacting proteins were obtained. Individual protein bands were destained, excised and digested with trypsin. The resulting peptide mixtures were analyzed by high pressure liquid chromatography coupled with electrospray ionization tandem mass spectrometry. The results from mass spectra were searched against the mouse, rat, and human genomic data base using the SEQUEST software. 2-D DIGE technique in conjunction with proteomic studies was employed to investigate the differences in protein expression in the hearts of transgenic rabbits.
Results: Our results revealed that both channels were precipitated. In addition, several unique potential KvLQTI and HERG-interacting proteins along with the previously known KvLQT1-interacting protein such as calmodulin, and HERG-interacting proteins, such as Hsc70 and Hsp90 were detected. Our results also showed that there were four protein bands with varying molecular weights which were common for KvLQT1 and HERG-bound complexes, suggesting potential common KvLQT1 and HERG-interacting proteins. Furthermore, the results of 2D-DIGE have revealed the upregulation of several proteins in KvLQT1 transgenic rabbit heart.
Conclusions: We have identified potential unique proteins that interact with either KvLQT1 or HERG, as well as the several potential proteins that interact with both of these channels. These interacting proteins might play an important role in regulating the trafficking and localization of these channels.

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Computer Simulation of Protein-Protein Association Processes

Volkhard Helms, Mazen Ahmad, Alexander Spaar, Wei Gu.
Saarland University, Saarbrücken, Germany.
Protein-protein interactions are key components of most biological processes. About half of all cellular proteins appear to be parts of larger stable protein complexes whereas transient, pairwise protein-proteins interactions are crucial parts of bioenergetic and signal transduction pathways. Here, we will concentrate on fast assembling protein pairs where complementary electrostatic interactions accelerate the association processes by several orders of magnitude. Brownian and molecular dynamics simulations will be used to identify the energetic principles for these binding phenomena. First, we have studied the association free energy landscape for the barnase:barstar complex by Brownian Dynamics simulations [1]. We will use this system to introduce the concept of diffusional protein protein association on conformational energy landscapes. Interestingly, we found that single protein mutations can drastically alter the shape of the energy landscape and the location of the encounter complex. Secondly, unbiased molecular dynamics simulations were used to study the binding process of a proline-rich peptide to an SH3 domain [2]. In this case, stable complexes were formed within 20 - 130 ns of simulation. Depending on the orientation of the first contacts made, the peptide adopted one of three experimentally known binding modes on the adaptor domain. Association was found to be governed by the synergistic interplay of two types of driving forces for binding. The long-range electrostatic effects play the main role during diffusion and stabilize the transient complexes formed by the electrostatic parts in the interface. At short distances, this then enables partial dewetting at the interfaces to increase the probability for the collapse of the hydrophobic part of the interface and the convergence to the final specific complex.
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At Clinically Relevant Concentration Isoflurane and Desflurane Induce Abeta Oligomerization. Molecular Details from NMR Spectroscopy

Pravat K. Mandal[1], Vincenzo Fodale[2].
[1]National Brain Research Center, Gurgaon, India, [2]University of Messina, Messina, Italy.
Anesthetics could be a risk factor for Alzheimer disease (AD). Findings from other laboratories confirm that several commonly used inhaled anesthetics may cause brain damage that accelerates the onset of AD . Using nuclear magnetic resonance spectroscopy, we previously evidenced that at high concentration (higher than clinically relevant concentration), halothane and isoflurane interact with specific amino acid residues (G29, A30 and I31) and induce Aβ oligomerization. Our present study, performed at clinically relevant anesthetic concentration indicates that two popularly used inhaled anesthetics, isoflurane and desflurane, induce Aβ oligomerization by inducing chemical shift changes of the amino acid residues (G29, A30 and I31). Experimental data at clinically relevant concentration reinforce that perturbation of these three crucial residues indeed play important role for the induction of Aβ oligomerization. A working model for Aβ olifomerization due to isoflurane and desflurane is presented in Figure 1.
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Correspondence Dr. Pravat Mandal

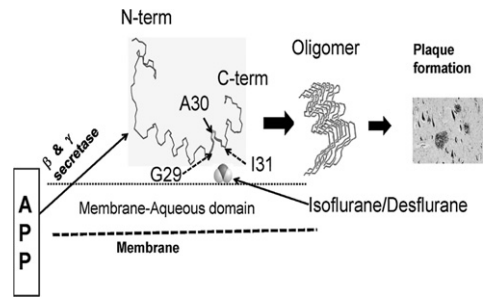

Figure 1 A plausible schematic diagram for the Aβ interactions with isoflurane and/or desflurane at a clinically relevant concentration that leads to oligomer formation. Aβ peptide is generated by the amyloid precursor protein (APP), by the action of β and γ secretase by the natural process, and the inhaled anesthetic interacts with three specific residues (G29, A30 and I31) and modulates Aβ oligomer formation, which are neurotoxic and produce plaque as seen in AD patients, on biopsy.

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Molecular Dynamics Simulations of a Single 11-Residue Beta-Sheet Adhesive and its Assembly

Ahlam N. Al-Rawi[1,2], Brigita Urbanc[3], Debabani Ganguly[1], Talat S. Rahman[2], Jianhan Chen[1], John Tomich[1].
[1]Kansas State University, Manhattan, KS, USA, [2]University of Central Florida, Orlando, FL, USA, [3]Drexel University, Philadelphia, PA, USA.
Thirty synthetic peptides with varying degrees of adhesion strength toward wood strips are prepared and tested as glues by checking adhesive strength at different pHs and curing temperatures. Lysine-Lysine-Lysine repeats on both the N- and C-termini of the hydrophobic core give the highest adhesion strength. In the absence of water, the peptide retains a β-sheet structure. Experimental results show that these peptides form a nano fiber-like structure in the absence of water. Simulations of three of the adhesive sequences, KKKFLIVIKKK, KKKIGSIIKKK,