play a significant role in both non-polar and polar binding, which is comparable to water reorganization energy. For both solutes, the entropy of water reorganization is predicted to favor binding in agreement with the classical view of the ''hydrophobic effect'', which is countered by ligand entropy. Depending on the specifics of the binding pocket, both energy-entropy compensation and reinforcement mechanisms are observed. Notable is the ability to visualize the spatial distribution of the thermodynamic contributions to binding at atomic resolution, opening up exciting avenues for mechanistic investigations of protein-ligand binding.

1081-Pos Board B32

Markovian Milestoning for Computing Entry, Exit, and Internal Diffusion Rates of Ligands in Proteins

Tang-Qing Yu[1], Anthony Bucci[2], Eric Vanden-Eijnden[1], **Cameron Abrams**[2].
[1]Courant Institute of Mathematical Sciences, New York University, New York, NY, USA, [2]Chemical and Biological Engineering, Drexel University, Philadelphia, PA, USA.

Measuring diffusion rates of ligands plays a key role in understanding the kinetic processes inside proteins. For example, although many molecular simulation studies have reported free energy barriers to infer rates for CO diffusion in myoglobin (Mb), they typically do not include direct calculation of diffusion rates because of the long simulation times needed to infer these rates with statistical accuracy. We show in this talk how to apply Markovian milestoning along minimum free-energy pathways to calculate diffusion rates of both CO inside Mb and O2 inside monomeric sarcosine oxidase (MSOX). In Markovian milestoning, one partitions a suitable reaction coordinate space into regions and performs restrained molecular dynamics in each region to accumulate kinetic statistics that, when assembled across regions, provides an estimate of the mean first-passage time between states. We show here that the milestones can be chosen from a Voronoi tessellation defined by discrete centers taken from minimum free-energy pathways computed using the single-sweep reconstruction method. In the case of CO/Mb, we find that, although simulations predict the existence of many potential portals that connect the solvent phase with the distal pocket (DP, the site of CO attachment to the heme iron), the so-called ''histidine gate'' pathway is kinetically dominant for both CO entry and exit by approximately a factor of ten, in qualitative agreement with existing experimental data. Our calculations also semi-quantitatively agree with experiment, predicting a 60-ns mean exit time for CO from the DP and a mean entry time of 50 microsec under CO-saturating conditions. The Markovian milestoning approach seems from these results to be a promising way to estimate biomolecule-related transition rates, and ongoing work involves using it to time conformational changes.

1082-Pos Board B33

Improving Small Molecule Docking for Bcl-xL via Accelerated Molecular Dynamics with Cosolvent

Andrew J. Kalenkiewicz[1], Chao-Yie Yang[2], Barry J. Grant[1].
[1]Department of Computational Medicine & Bioinformatics, University of Michigan, Ann Arbor, MI, USA, [2]Department of Internal Medicine, Hematology and Oncology Division, University of Michigan, Ann Arbor, MI, USA.

The anti-apoptotic factor Bcl-xL operates by binding and sequestering a range of pro-apoptotic factors that otherwise promote programmed cell death. Overexpression of Bcl-xL and its anti-apoptotic relatives is common in many human cancers. Consequently, extensive efforts are being directed to developing inhibitors of Bcl-xL protein-protein interactions. Here we describe the development of an improved workflow for utilizing experimental and simulated conformations in structure-based inhibitor design strategies. By employing computational docking of known small molecule inhibitors we demonstrate that certain methods of enhanced sampling molecular dynamics (MD) are more amenable to productive docking studies. In particular, structural ensembles derived from both accelerated MD (aMD) and MD in the presence of an organic cosolvent generally scored better than those from equivalent conventional MD. Furthermore, combined cosolvent aMD simulations yielded better average and minimum docking scores for known binders than an ensemble of 80 high-resolution experimental structures. A detailed analysis of the docked conformations indicates that the improved scores resulted from an increased flexibility of the helices flanking the main Bcl-xL binding groove. This enhanced conformational sampling allowed cosolvent to penetrate more deeply into the binding pocket and stabilize conformations not evident in conventional simulations. We believe this approach could potentially be used to identify protein-protein interaction inhibitors for other systems, and would be particularly beneficial against those targets for which there is less accumulated structural data.

1083-Pos Board B34

Free Energy Landscape of the Michaelis Complex of Lactate Dehydrogenase: A Network Analysis of Atomistic Simulations

Xiaoliang Pan, Steven D. Schwartz.
Chemistry and Biochemistry, University of Arizona, Tucson, AZ, USA.

It has long been recognized that the structure of a protein is a hierarchy of conformations interconverting on multiple time scales. However, the conformational heterogeneity is rarely considered in the context of enzymatic catalysis in which the reactant is usually represented by a single conformation of the enzyme/substrate complex. Lactate dehydrogenase (LDH) catalyzes the interconversion of pyruvate and lactate with concomitant interconversion of two forms of the cofactor nicotinamide adenine dinucleotide (NADH and NAD+). Recent experimental results suggest that multiple substates exist within the Michaelis complex of LDH, and they are catalytic competent at different reaction rates. In this study, millisecond-scale all-atom molecular dynamics simulations were performed on LDH to explore the free energy landscape of the Michaelis complex, and network analysis was used to characterize the distribution of the conformations. Our results provide a detailed view of the kinetic network the Michaelis complex and the structures of the substates at atomistic scale. It also shed some light on understanding the complete picture of the catalytic mechanism of LDH.

1084-Pos Board B35

Dissecting Ligand Binding Sites : A Layer at a Time

Anasuya Dighe[1], Nagasuma Chandra[2], Saraswathi Vishveshwara[3], G.K. Ananthasuresh[4].
[1]IISc Mathematics Initiative, Indian Institute of Science, Bangalore, India, [2]Department of Biochemistry, Indian Institute of Science, Bangalore, India, [3]Molecular Biophysics Unit, Indian Institute of Science, Bangalore, India, [4]Department of Mechanical Engineering, Indian Institute of Science, Bangalore, India.

Biological phenomena at a molecular level are a consequence of complex interplay of interactions between ligands and their associated protein targets. Such interactions are localized to defined protein regions termed as binding sites (residues within ~4.5Å from ligand). Numerous cases exist wherein proteins of diverse folds bind to similar ligands. We undertake a study with an objective to quantify similarities in binding sites, that utilize the structural information of a binding site at atomistic detail, in order to examine extension of such similarities to additional regions surrounding the binding site. We find that 68% and 22% of high scoring comparisons among single-domain and multi-domain proteins respectively, exhibit similarity at the binding site that extrapolates to regions beyond it (an additional 4.5Å from binding site). Such similarities in binding sites as well as their extension to adjoining regions are analysed along with residue-wise correspondences and pairwise structural alignment of binding sites. Sequence identity and ligand topology are found to be independent of the extent of binding site similarity. Statistical validation of these findings re-iterates their relevance by means of p-value calculation as well as decoy site generation. Occurrences of similarity beyond the binding site are also further investigated using Protein Side-chain Networks (PScN). Such networks are created wherein each node represents amino acid residues, and an edge is constructed based on interaction strength between side chain of non-covalently bonded residues. PScNs lead to the identification of densely connected modules i.e cliques and communities in regions of binding site and surrounding areas. Thus, a combinatorial approach capturing atomistic details of binding site architecture and global perspectives of ligand-binding by PScNs could aid in providing valuable insights to current understanding of molecular recognition events.

1085-Pos Board B36

Encounter and Binding of Camp at the Binding Domain of Mlok1

Béla Voß[1], Ulrich Benjamin Kaupp[2], Helmut Grubmüller[1].
[1]Departement of Theoretical and Computational Biophysics, Max Planck Institute for Biophysical Chemistry, Göttingen, Germany, [2]Molecular Sensory Systems, Center of Advanced European Studies and Research, Bonn, Germany.

Ligand-protein binding processes play an essential role in biological systems, be it signalling immune response or enzymatic activity. An experimentally well-studied system is the binding of cyclic adenosine monophosphate (cAMP) at the cyclic nucleotide binding domain (CNBD) of the bacterial potassium channel MloK1. The channel's conductivity is modulated by cAMP binding and is prototypical for cyclic nucleotide gated ion channels.
Two models were postulated to describe the binding process: The first is a two-step model consisting of bulk-surface diffusion and surfing-binding site rolling. The second consists of a diffusion process into a binding funnel combined with stochastic barrier crossing.
Here we present a comprehensive molecular dynamics study of the primary cAMP binding events, i.e. the ligands path to the binding site in the CNBD,

to identify substates, establish a model of the ligand pathways and to predict an effective on-rate for the binding process. Available experimental high precision measurements of the binding kinetics render the system suitable for comparison.
We identified multiple surface substates that play a crucial role for the dynamics for high ligand concentrations. Using rate estimates from the MD simulations and extrapolation to low, experimentally accessible ligand concentrations we ruled out the two-step models. We quantified the conformational confinement and the probability of binding barrier crossing to obtain values for the funnel model. The overall estimate for the on-rate rate constant was estimated to be 30/(μs*mol/l), which is in excellent agreement with the experimental measurements.

1086-Pos Board B37
Hofmeister Ion and Cosolvent Effects on the Structure, Aggregation, and Backbone Solvation of RecA
Taylor P. Light, Karen M. Corbett, Michael A. Metrick, Gina MacDonald.
Department of Chemistry and Biochemistry, James Madison University, Harrisonburg, VA, USA.
RecA is an *Escherichia coli* protein that catalyzes the strand exchange reaction utilized in DNA repair. Previous studies have shown that the presence of salts influence RecA activity, aggregation, and stability. Here we utilized attenuated total reflectance Fourier-transform infrared (ATR-FTIR) spectroscopy and circular dichroism (CD) to further investigate how various Hofmeister salts and cosolvents alter RecA structure, aggregation, and solvation. Spectroscopic studies performed in water and deuterium oxide suggest that salts alter amide I (or I') and amide II (or II') vibrations arising from the protein backbone. Specific infrared vibrations that may arise from protein-solvent interactions were identified. Infrared vibrations that correlate with protein desolvation were observed in the presence of strongly hydrated SO_4^{2-} anions. The vibrations that correlate with protein solvation were observed in the presence of weakly hydrated Cl^- and ClO_4^- anions. Additional experiments were performed under solution conditions known to influence protein-solvent and protein-water interactions. An increase in the infrared frequency of amide I (or I') correlated with increasing concentrations of trifluoroethanol (TFE) and sucrose. This result suggests an increase in desolvation of the amide backbone with an increase in the concentration of cosolvents. Additionally, increasing concentrations of TFE resulted in an increase in RecA aggregation. These results show that salts and cosolvents alter the solvation water surrounding proteins and influences overall structure and aggregation.

1087-Pos Board B38
Foldamer-Based Enantioselective Targeting of Islet Amyloid Polypeptide
Sunil Kumar, Diana Schlamadinger, Andrew Miranker.
MB and B, Yale University, New Haven, CT, USA.
A novel approach is developed where oligoquinoline based foldamers are designed and synthesized to stabilize the dynamic behavior of the pre-amyloid toxic state of the diabetes related protein, IAPP. Among small molecule foldamers, oligoquinolines have an unusually stable, non-covalent structure. The surface of oligoquinolines can be derivitized without perturbing its fold. This enables design of a surface that can target polypeptides. Here, we show the utility of this scaffold on protein misfolding from intrinsically disordered precursors. Amyloid self-assembly derived from misfolding is a hallmark for a group of diseases include Alzheimer's, Parkinson's, and type 2 diabetes (T2D). In the latter, islet amyloid polypeptide (IAPP) accumulates as fibers in the pancreatic β-cells. However, it has been shown that fibers are not the dominant toxin, rather it is the membrane bound α-helical intermediates of IAPP that give rise to pre-amyloid toxicity. Targeting the heterogeneous ensemble of dynamic protein structures, such as IAPP, is often regarded as challenge for the creation of specific ligands. However, the use of a strongly structured foldamer permits the induction of structures in flexible species. Here we show oligoquinioline derivatives that bind pre-amyloid IAPP and show activity in both solution biophysics and cellular assays.

1088-Pos Board B39
Structural Dynamics of Proteins using Novel Visible Fluorescence Probes
Haifeng Pan.
East China Normal University, Shanghai, China.
Fluorescence spectroscopy has been widely used in life science. In this paper, some fluorescent molecules have been designed and synthesized to interact with biological thiol groups, in order to further study protein structural changes at different environment. 4-(2-(1,10c-dihydropyren-1-yl)vinyl)-1-methylpyridinium iodide (4-PBPMI) and 2-(2-(1,10c-dihydropyren-1-yl)vinyl)-1-methylpyridinium iodide (2-PBPMI), whose absorption and fluorescence spectra are in ultraviolet and visible region respectively, have been shown to very sensitively probe L-Cysteine (L-Cys) due to the strong interaction between thiols and vinyl. We reported here the systematic measurements of steady state fluorescence of PBPMIs in the mixtures with various proportions of glycerol and water, and time resolved fluorescence decay using a time correlated single photon counting apparatus on the 100ps to 30ns time scale. The probes are excellent to monitor the L-Cys concentrations and environment simultaneously. For example, with the increasement of the concentration of L-Cys, the emission intensities of PBPMIs in ultraviolet got a great enhancement as well as absorptions. Meanwhile, in visible region, the intensities were decreased sharply. Further experiments and discussion will be made to study structural dynamics of proteins with disulfide bonds
and free thiols. The results may motivate additional experimental and theoretical research on the molecular-level design of luminescent probes in biophysics.

1089-Pos Board B40
Intrinsic Thermodynamics - Structure Correlation of Carbonic Anhydrase Inhibitors
Daumantas Matulis, Asta Zubrienė, Lina Baranauskienė, Alexey Smirnov, Vaida Morkūnaitė, Joana Smirnovienė, Miglė Kišonaitė, Povilas Norvaišas, David D. Timm.
Institute of Biotechnology, Vilnius, Lithuania.
For the design of selective inhibitors towards 12 active human carbonic anhydrase (CA) isoforms, a map correlating thermodynamics of inhibitor binding with cocrystal structures was created. A series of over 600 inhibitors were chemically synthesized. Their binding to CA isoforms was measured by three biophysical and biochemical techniques, namely, isothermal titration calorimetry (ITC), fluorescent thermal shift assay (FTSA), and enzymatic inhibition methods. A database of over 3000 binding reactions, including the intrinsic Gibbs free energies and sometimes the enthalpies, entropies and heat capacities, was assembled. Intrinsic energetics of binding was determined by accounting for the linked protonation-deprotonation reactions of both protein and ligand. Together with over 80 cocrystal structures, this database provided insight into some general issues of protein-ligand interactions, especially the enthalpy - entropy compensation and isoform-selective ligand design. Parts of some ligands contained flexible tailgroups that correlated with increased intrinsic entropy of binding. All designed ligands exhibited enthalpy-driven binding with minor but important entropic contributions. The method is useful for the design of ligands with appropriate affinities and selectivities.

1090-Pos Board B41
Zebrafish Larvae as Model System to Study Possible Toxicity of Silver Nanoparticles at Cytoskeletal Level by Means of Advanced Microscopy
Marta d'Amora[1,2], Abdelrasoul N. Gaser[1], Zeno Lavagnino[1], Giuseppe Sancataldo[1,3], Francesca Cella Zanacchi[1], Alberto Diaspro[1,2].
[1]Nanophysics, Istituto Italiano di Tecnologia, Genova, Italy, [2]Nikon Imaging Center, Istituto Italiano di Tecnologia, Genova, Italy, [3]Department of Physics, University of Genova, Genova, Italy.
Previous studies on the toxicity induced by Ag NPs of different size on zebrafish during development have almost exclusively characterized macroscopic effects, in particular anomalies in the spontaneous movements, hatching rate/time disturbance and the presence of different malformations [1][2]. However little is known about a possible interaction of Ag NPs with subcellular structures.
In this study we focus our attention on the biological effects of small-sized silver nanoparticles (8-10nm) through a multi-level investigation on zebrafish larvae. The use of these model organisms and the possibility to image them using advanced microscopy techniques represent an optimal opportunity to screen toxicity effects of nanoparticles at microscopic level.
In particular, the use of advanced microscopy techniques (such as confocal and selective plane illumination microscopy (SPIM)) [3] [4] allows for 3D high resolution imaging of possible effects of nanoparticles on the cytoskeletal architecture. With this approach high cellular and sub-cellular resolution can be achieved. So far, we have investigated the possible damage induced by Ag NPs in the structure of both tubulin and filamentous actin (F-actin) in zebrafish larvae.
(1) Asharani P.V. et al., Nanotechnology; 19(25):255102 (2008)
(2) Powers M. et al., Neurotoxicology and Teratology; 32(3): 391-397 (2011)
(3) Huisken J. et al., Science; 305: 1007-1009 (2004)
(4) Lavagnino Z. et al., Optics Express; 21(5): 5998-6008 (2013)