BCL::PharmMap (unpublished), and contain information about hydrogen bonding, steric bulk, and polarizability. Discrete cartesian grids describing the hydrogen bonding ability, steric bulk and polarizability of the ligand binding site are overlaid on the protein structure, and these grids are used to score the initial placement of the ligand prior to h fine grained docking. As the scoring grids are precomputed, ligand scoring is extremely fast, and thorough Monte Carlo sampling of the ligand binding site can be rapidly performed before to fine grained ligand docking.
This rapid initial sampling makes it possible to predict accurate binding poses with a significantly smaller amount of fine grained sampling, decreasing the amount of CPU time necessary to predict a single binding interaction, and increasing the practicality of structure based virtual High Throughput Screening (vHTS). The integration of structure based and ligand based vHTS techniques allows the full range of pharmacological information surrounding a target and drug scaffold to be considered in a single approach. This technique can be used to rapidly develop small focused libraries for High Throughput Screening, increasing the hit rate and decreasing the number of compounds that need to be purchased for testing.

2133-Pos Board B119
Rational Design of Early Premortem Alzheimer's Disease Diagnostic Agents
Katryna K. Cisek, Jeff A. Kuret.
Alzheimer's disease (AD) is a global burden; it affects over five million people in the US, and nearly 30 million worldwide. It is a progressive neurodegenerative disease that develops over many years before cognitive and behavioral symptoms appear. During that time, characteristic lesions called neurofibrillary tangles (NFTs) and senile plaques accumulate in the brain. Although the presence of both plaques and tangles defines AD, NFTs have special utility for diagnosis because their appearance correlates strongly with neurodegeneration and decline in memory. In fact, the spatial distribution of NFTs is the gold standard of postmortem assessment and AD staging. A challenge for porting NFT detection to premortem diagnosis is the identification of a potent and selective NFT probe for a whole brain imaging technique, such as Positron Emission Tomography (PET). Thus, the factors essential for generating differential binding affinity for various proteinaceous deposits must be elucidated.
To identify the major sources of binding affinity for protein filaments, a family of 50 benzothiazole derivatives disclosed in patent literature was investigated using a computational approach. The published values for compound potency on synthetic aggregates composed of Abeta peptide and insulin using Thioflavin T displacement fluorescence assays were rationalized via calculated molecular properties using both partial and multiple least squares (PLS, MLS) regression methods. Correlation of over 280 molecular properties through PLS analysis identified molecular polarizability, hydrophobicity, valence connectivity, and the rotatable bond fraction as top ranked determinants of binding affinity. MLS revealed that the correlation coefficient for calculated vs. measured potency exceeded 0.7 when just these four descriptors were used to build the structure-activity relationship. Most importantly, the analysis revealed that polarizability and hydrophobicity were most important for generating differential binding affinity for Abeta relative to insulin filaments ($p < 0.01$, and 0.001, respectively).

2134-Pos Board B120
MloK1 Ligand Binding Simulations: Induced Fit Versus Conformational Selection
Béla Voß, Helmut Grubmüller.
Many ion channels such as the MloK1 channel are steered by ligand binding via conformational changes. Mainly two binding mechanisms have been proposed, induced fit and conformational selection. Using molecular dynamics simulations, we studied the ligand binding mechanism of cyclic adeonise monophosphate at the cyclic nucleotide binding domain of the MloK1 ion channel of Mesorizobium loti. For this binding domain, crystal structures are available for both the ligand free as well as for the ligand bound conformation, rendering the system an ideal prototype for studying the binding process by atomistic simulations.
In the simulations, spontaneous binding was observed. Furthermore, reaction coordinates for the ligand binding as well as for the associated conformational change of the protein were determined. Together with potentials of mean force along these coordinates calculated by umbrella sampling, the results show a relatively fast ligand binding kinetics and a larger free energy barrier for the subsequent conformational change. Calculated free energy differences between both protein conformations during the absence of a ligand allow to distinguish between conformational selection and induced fit.

2135-Pos Board B121
Phospholipid Binding and Membrane Attachment of the Osh4 Protein
Brent Rogaski, Jeffery B. Klauda.
Osh4 is an oxysterol binding protein (OSBP) homologue found in yeast that is essential for the intracellular transport of sterols and for cell life. It has been proposed that Osh4 acts as a lipid transport protein, capable of binding a single sterol residue within a hydrophobic binding pocket and transporting it, against a concentration gradient, from the endoplasmic reticulum to the plasma membrane. Phosphoinositides (PIPs) are thought to stimulate sterol transfer by binding to the Osh4 protein surface.
In order to study how the Osh4 protein attaches to the plasma membrane, possible lipid binding sites were investigated through the use of blind docking techniques. Model ligand compounds for phosphatidylcholine, phosphatidylserine, and two PIP [$PI(4,5)P_2$ and $PI(3,4,5)P_3$] head groups were docked against several conformational snapshots of the Osh4 surface to determine possible regions favorable to interact with plasma membrane lipids. These conformational snapshots were taken from two 25-ns molecular dynamics simulations of the Osh4 protein complexed with ergosterol and two complexed with 25-hydroxycholesterol. The PIP models frequently docked to a lysine-rich region on an exposed portion of the protein's β-barrel. This region is bounded by a flexible surface loop that is believed to be important for Osh4-membrane binding.
Osh4-membrane interaction was also investigated through molecular dynamics simulations of a combined membrane and protein system. Model sterol-acceptor and sterol-donor membranes were constructed using CHARMM-GUI and equilibrated for 25 ns. Key residues of the Osh4 protein that were identified during the blind docking tests, as well as residues known to be in close contact with these membranes upon binding, were placed parallel to the membrane surface. Ultimately, understanding how Osh4 attaches to cellular membranes will lead to a clear understanding how this protein transports sterols *in vivo*.

2136-Pos Board B122
Influence of Ligand Chirality on the Catalytic Efficiency of Human 3-Phosphoglycerate Kinase
Zoltan Palmai, David Perahia, Corinne Lionne, Judit Fidy, Erika Balog, Laurent Chaloin.
L-nucleoside analogues form an important class of antiviral and anticancer drug candidates. To be pharmacologically active, they need to be phosphorylated in multiple steps by cellular kinases. Human phosphoglycerate kinase (hPGK) was shown to exhibit low specificity for nucleotide diphosphate analogues and its catalytic efficiency in phosphorylation was also affected. Revealing the mechanism of action and functional motions of hPGK gains importance in in silico drug-design to provide efficient phosphorylation process.
To elucidate the effect of ligand chirality on dynamics and catalytic efficiency, molecular dynamics simulations were performed on four different nucleotides (D-/L-ADP and D-/L-CDP) in complex with hPGK and 1,3-bisphospho-D-glycerate (bPG). The simulation results confirm high affinity for the natural substrate (D-ADP), while L-ADP shows only moderate affinity for hPGK. The observed short residence time of both CDP enantiomers at the active site suggests very weak binding affinity which may result in poor catalytic efficiency shown for hPGK with D-/L-CDP. Analysis of the simulations unravels important dynamic conditions for efficient phosphorylation replacing the single requirement of a tight binding. These are: 1) over the strength of the binding, the flexibility of the substrate within the binding site gains importance, especially for the phosphate groups; 2) the hinge bending motion of the domains upon substrates binding should be more correlated and directional, and consequently should imply a lower number of hinge residues; 3) the nucleotide binding site should have an increased flexibility allowing significant dynamic freedoms for the substrates.

2137-Pos Board B123
Point Mutation I261M Affects the Dynamics of BVDV and its Interaction with Benzimidazole Antiviral 227G
Shailendra Asthana, Saumya Shukla, Paolo Ruggerone, Matteo Ceccarelli, Gabriele Giliberti, Paolo La Colla, **Attilio Vittorio Vargiu**.
Bovine viral diarrhea virus (BVDV) is a Pestivirus of the Flaviviridae family and represents a major viral pathogen in cattle and other ruminants. Infection with BVDV can result in a wide assortment of disease manifestations including resorption, mummification, or abortion of the dead fetus. Recently the point mutation I261M on the thumb domain was shown to confer resistance to BDVD against 227G and other benzimidazole compounds. Here we investigated the role of this mutation by using a multidisciplinary protocol, not involving free energy calculations on structures of the mutated complex which are taken a priori similar to those of the wild one. Namely, we firstly performed MD simulations on the wild and mutated BVDV RdRp proteins in

aqueous solution. Then, we selected representative equilibrium conformations by performing a cluster analysis, and ran docking calculations of 277G on representative of the 5 most populated clusters of each protein. Finally, we performed MD simulation on selected complexes as to assess structural and dynamical differences between wild and mutated 227G-protein adducts.

Interestingly, the mutation affects the structure and the dynamics of the protein, particularly in the region of binding of the ligand, and this results in a different binding site of 227G with respect to the wild protein. Moreover, while 227G closes the entrance to the RNA strand in the case of the wild protein, a gate and a channel leading to the catalytic site are still present in the mutated complex. These results could offer a possible molecular explanation of the resistance mechanism by mutation I261M.

2138-Pos Board B124

Study of the Cooperativity of Calcium Binding in Calbindin D9k using 2D Replica-Exchange Umbrella Sampling

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Calbindin D_{9k}, a small protein possessing a pair of EF-hands that binds two calcium ions in a cooperative fashion, is chosen as a model system for studying the molecular mechanism of cooperativity.[1] Binding is characterized in terms of a potential of mean force (PMF) as a function two variables: the distance r between the ion, and the binding pocket, and the root-mean-square deviation (RMSD) of the conformation of the EF-hand relative to its ion-bound structure. The PMF is calculated using a novel two-dimensional replica-exchange molecular dynamics (MD) umbrella sampling scheme, which is developed and implemented in the program CHARMM to increase the configuration space sampling.

Using 2048 replicas on Blue Gene/P, the 2D-PMF/REMD calculation for the binding the second calcium ion converges within 800 ps, with an exchange probability of 30%. In contrast, standard umbrella sampling PMF/MD simulations without replica-exchange did not converge, even after 1.2 ns. The absolute binding free energy of a second ion to a singly occupied calbindin calculated from the 2D-PMF following the statistical mechanical formulation of noncovalent association[2] is −9.4 kcal/mol, in excellent agreement with the experimental value[3]. The 2D-PMF/REMD simulations will be extended to provide important information about the molecular basis of calcium binding cooperativity.

Footnotes

[1] Marchand S. and Roux B. Protein Struct. Funct. Genet. 1998, 33, 265-284.
[2] Woo H. and Roux B., PNAS, 2005, 19, 6825.
[3] Linse S. *et al*. Biochemistry 1991, 30, 154.

2139-Pos Board B125

Differential Binding Affinities of Anti-Apoptotic MCL-1 and A1 Proteins for the Pro-Apoptotic BH3 Peptides: Understanding the Molecular Basis using MD Simulations

Vivek Modi, Dilraj Lama, Ramasubbu Sankararamakrishnan.

The Bcl-2 family of proteins plays a crucial role in the regulation of Apoptosis through heterodimerization between pro- (e.g. Bim, Puma, Bad) and anti-apoptotic (e.g. Bcl-X_L, MCL-1, A1) members. The anti-apoptotic proteins however display different binding specificities and affinities towards the BH3 peptides of pro-apoptotic partners. The current study investigates the differences in binding affinities of peptides derived from pro-apoptotic proteins towards MCL-1 and A1 using MD simulations. Simulations each for a period of 100 ns has been carried out for MCL-1/A1 proteins in complex with Puma and Bad BH3 peptides. Stability of non-covalent interactions between the peptides and the protein was analyzed. The MCL-1-Puma complex simulation reveals the presence of very strong interactions between the acidic residues at the N-terminus of Puma with the basic residues present in helix H3 of protein. Moreover, the helix H4 & H6 exhibit very stable hydrophobic contacts with the C-terminal residues of Puma. The equivalent interactions are absent in A1: Puma complex simulation but it reveals the presence of a strong hydrophilic contact involving the ARG from ''LR'' motif of the peptide. These observations collectively may account for the slightly higher binding affinity of Puma towards MCL-1. In the simulation of the modeled MCL-1-Bad complex, interestingly we did not observe any stable interactions including those involving the ARG of the 'LR motif' with the protein. Involvement of ''LR'' motif in peptide-protein interactions was previously hypothesized to be important in high binding affinity of Bad towards Bcl-X_L [1]. The negligible affinity of Bad towards MCL-1 can be attributed to these factors.

[1] Lama and Sankararamakrishnan, *Proteins***73**, 492-514 (2008)

2140-Pos Board B126

The Two-Pathway Model of the Biological Catch-Bond as a Limit of the Allosteric Model

Yuriy Pereverzev, Oleg Prezhdo, Evgeni Sokurenko.

Recent years have witnessed great interest in the biophysics of receptor-ligand complexes, such as FimH/mannose, P,L,E-selectin/PSGL-1, etc., showing unusual dependence of complex lifetime on the strength of an applied external force. In contrast to the expected decrease in the lifetime with increasing force strength, the lifetimes of these complexes showed an initial growth, which was followed by the expected decay. This low force phenomenon was called catch-binding, and the transition between the unexpected low force behavior and the expected high force limit was called the catch-slip transition. We discuss various models for the catch-slip anomaly and show that the two-state allostery model [Thomas, W *et al.*, Biophys. J. **90**, 753 (2006); Pereverzev, YV *et al.*, Phys. Rev. E **79**, 051913 (2009)] can be transformed into the two-pathway model [Pereverzev, YV *et al.*, Biophys. J. **89**, 1446 (2005)]. We demonstrate that such transformation is possible when the relaxation time of the allostery site is much smaller than the characteristic decay time of the bound complex. This transition is considered for P-selectin/PSGL-1 and FimH/mannose complexes exposed to both constant and time-dependent forces. Good agreement between theory and experiment is obtained, and the relevant biophysical parameters of the catch-bonds are found.

Protein Folding & Stability II

2141-Pos Board B127

Revealing the Early Events of ACBP Folding by Ultrarapid Mixing

Li Zhu, Vincent A. Voelz, Olgica Bakajin, Vijay S. Pande, Lisa J. Lapidus.

ACBP (acyl-CoA binding protein) became an attractive model of protein folding due to its special 4-helix bundle structure and complex kinetics. Teilum and Roder et al (1) found there is an 80 us phase before its barrier-limited folding phase by using a mixer with 70 us dead time and attributed it to the formation of an intermediate. In this work, an ultrarapid mixer with a 5 us dead time combined with FRET measurement was applied to take a closer look at ACBP folding mechanism. The kinetics observed after dilution of high denaturant of various mutants with multiple pairs of FRET probes are similar to the previous measurement but are poorly fit by a single exponential. There is reasonable agreement with the prediction of Markov State Model (MSM) constructed from extensive MD simulations. MSM explains that this phase is due to a surprisingly slow acquisition of unfolded-state residual structure, not a single folding intermediate.

2142-Pos Board B128

Factors Defining Effects of Macromolecular Crowding on Protein Stability: an in vitro/in Silico Case Study using Cytochrome C

Alexander Christiansen, Margaret S. Cheung, Pernilla Wittung-Stafshede.

Previous experiments on two single-domain proteins showed that macromolecular crowding can stabilize dramatically towards heat perturbation and modulate native-state structure and shape. To assess the generality of this, we here tested the effects of the synthetic crowding agents on cytochrome c, a small single-domain protein. Using far-UV circular dichroism (CD), we discovered that there is no effect on cytochrome c's secondary structure upon additions of Ficoll or dextran (0-400 mg/ml, pH 7). Thermal experiments revealed stabilizing effects (5-10 °C) of Ficoll 70 and dextran 70: this effect was enhanced by the presence of low levels of guanidine hydrochloride (GuHCl) that destabilize the protein. When using a smaller dextran, dextran 40, the thermal effects were larger (10-20 °C). Together with previous data, we conclude that protein size, stability, conformational malleability and folding routes, as well as crowder size/shape, are key factors that modulate the net effect of macromolecular crowding on proteins.

2143-Pos Board B129

Osmolytes Induce Changes in the Conformational Landscape of a Model Peptide

Liel Sapir, Deborah E. Shalev, Daniel Harries.

Stabilizing osmolytes are naturally occurring small solutes known to shift proteins and peptides to their native folded conformation and are used by many organisms to counteract harsh environmental stress. The thermodynamic basis for osmolyte action is described in terms of their preferential interactions; however, the molecular mechanism has not yet been resolved. To gain insight into the effect of osmolytes on molecular interactions, the conformational landscape of a 16 amino acid model peptide, known to form a β-hairpin fold in solution, was explored by molecular dynamics (MD), replica exchange (REMD) simulations and NMR spectroscopy. The peptide was studied in pure water and in aqueous solutions of two prominent osmolytes: glucose and trehalose. The contribution of different free-energy components to the folding equilibrium