1167-Pos Board B77
Macromolecular Crowding Affects the Mechanical Unfolding Forces in Titin: The Size Effect
Marisa I. Roman, Gouliang Yang.
Macromolecules can occupy a large fraction of the volume of the cell and this affects many properties of the proteins inside the cell, such as thermal stability and rates of folding. We present a study comparing the effects of the size of molecular crowders on the unfolding forces of titin. We used an atomic force microscope based single molecule method to measure the effects of the crowding on the mechanical stability of this protein. We used dextran as the crowding agent with three different molecular weights, with concentrations varying from zero to 300 grams per liter in the buffer solution. The results show that the forces that are required to unfold molecules are enhanced when high concentration of dextran molecules is added to the buffer solution and also that there is a maximun force when the crowder size is comparable to the protein.

Protein Structure Prediction & Drug Design

1168-Pos Board B78
Computational Simulation of a Beta-helical Tubular Peptide in a Lipid Bilayer
Ayat Saeedi, Anahita Kyani, Armin Madadkar-Sobhani, **Bahram Goliaei**.
Peptides having a regularly repeating pattern of L and D amino acids adopt unique structures termed beta-helix. In beta helix structure, intramolecular hydrogen bonding looks like that in parallel β-sheets. The beta helix structure has been reported for both natural peptides, like gramicidin A, and syntetic peptides such as poly (D, L-γ -benzylglutamyl). Studies have shown a short beta sheet tetraicosa-peptide (VSLGLSIAFSVAVSIAWSFARSRG, where all As are D-alanine) accept beta-helical conformation in lipid bilayer similar to gramicidin A [4], called gramicidin-like channel (GLC).
In the present work, the GLC syntetic peptide has been modeled and simulated in different orientations with respect to a lipid bilayer. The aim of this study was to find the stable conformation of the peptide and understanding its key interactions with lipid bilayer. GROMOS force field implemented in GROMACS software, version 4.0 was employed for the molecular dynamics simulations. The results showed that this peptide adopted beta helical structure, in agreement with the previous studies. However, GLC was stable in the trans-membrane state during 20 ns molecular dynamics simulation. The findings of this study proved that the elctrostatic interactions between the polar residues of the peptide and the polar head group of the lipid bilayer are the most important interactions in the insertion of peptide into the membrane. Furthermore, simulation revealed that the hydrophobic interactions play a key role in the stability of GLC peptide in hydrophobic environment of lipid bilayer.

1169-Pos Board B79
Quality Assessment of Predicted Protein Structures by Using Molecular Dynamic Simulations
Jiong Zhang, Jingfen Zhang, Qingguo Wang, Yi Shang, Dong Xu, Ioan Kosztin.
In silico protein structure prediction using efficient fully automated servers continues to remain a challenging problem. While many of these servers can generate near-native structures, the lack of reliable structure quality assessment methods makes the identification of these structures problematic. The most common way of discriminating between predicted structures of a given protein is to employ either knowledge or physics based energy functions. Our recently developed MUFOLD-MD server uses an alternative ranking method of the predicted protein structures by testing their relative stability against gradual heating during all atom molecular dynamics (MD) simulations. We refer to this approach as the MD-Ranking (MDR) method. The MUFOLD-MD server consists of three sequential steps involving structure: generation, refinement and selection. First, by using sequence-profile alignment (e.g., PSI-BLAST) and profile-profile alignment (e.g., HHSearch) methods the query sequence is classified as either ''hard'' or ''easy'' target. For hard targets, models are generated using the Rosetta 3.1 software (ab initio method) and then ranked by using their Rosetta energy score. For easy targets, models are generated with the Multi-Dimensional Scaling (MDS) method and then ranked using the OPUS_Ca scoring function. Next, the structures (only hard targets) are refined by employing the ''relax'' mode in Rosetta 3.1. Finally, the MDR method is used to select the top 5 structures as the output of the server. Our MUFOLD-MD server was tested in both CASP8 and CASP9 competitions. Based on the official CAP8 results, MUFOLD-MD was ranked as number one server in the Free Modeling category.
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1170-Pos Board B80
Identification of ERK2-Substrate Protein Inhibitors via Virtual Screening, Biological Assays and X-Ray Crystallography
Taiji Oashi, Jun Zhang, Kimberly Burkhard, U. Deva Priyakumar, Edvin Pozharskiy, Paul Shapiro, Alexander D. MacKerell Jr.
Extracellular signal regulated kinases (ERK1/2) are involved in signaling events that regulate cell division and proliferation. Hyperactivation of ERK has been implicated in the pathogenesis of many human cancers. The F-site recruitment site (FRS) (L198, H230, Y231, L232, L235, and Y261) as well as common docking (CD) (D316 and D319) and ED (T157 and T158) domain in ERK2 is used to facilitate interactions with substrate proteins. Thus, small molecules targeting FRS and/or CD/ED domain have the potential to modulate ERK2 specific functions, potentially leading to the development of novel therapeutic agents. MD simulations of ERK2 from which structurally diverse conformations were selected were used to identify putative binding sites for low molecular weight compounds in the vicinity of the FRS and CD/ED sites. Identified sites were then targeted in individual database screens of over 1.5 million compounds. Following two levels of database screening, fingerprint based similarity clustering and analysis of physicochemical properties that maximize bioavailability, final compounds for biological assay were selected for each site. Inhibition of ERK2-specific phosphorylation was confirmed and dose-dependency was measured in several cancer cell lines using colony survival assays. Direct binding of active compounds to ERK2 was validated by fluorescence quenching experiments. ERK2 was crystallized in complex with several active compounds, showing binding in the critical site in FRS. These identified compounds provide novel tools to study the biological functions of ERK2 as well as act as lead compounds for the development of novel therapeutic candidates for cancer.

1171-Pos Board B81
Sifting a Massive Virtual Library of Peptide Ligands for an Optimal Binder to a Given Receptor
Gungor Ozer, Denise C. Enekwa, Shi Zhong, Stephen Quirk, Rigoberto Hernandez.
The identification of an optimal protein ligand that binds to a target is a difficult problem because the library contains more than a mole of ligands if only 18 residue sites are allowed to vary across all the naturally occurring amino acids. Such a library is far too large to specify explicitly. Instead, the so-called massive virtual library (MVL) is specified indirectly through a set of class rules. An efficient search method through the MVL, which incorporates the principles of sequence design, protein docking and statistical mechanics, has been recently introduced:* (i) a random sub-library is created according to the user defined pruning criteria, (ii) each member in the sub-library is docked to the target using AutoDock and ranked according to the binding free energies that are calculated with both AutoDock's scoring function and CHARMM non-bonded interaction energies, (iii) using the calculated free energies, Boltzmann weighted probabilities are assigned to each sequence, (iv) the weights are then used to select the next-generation MVL, and (v) iterate back to step (i) using the current MVL. The algorithm concludes when convergence occurs between the results from two subsequent rounds. For an 8 residue peptide design that binds to Deoxyribonuclease I, the convergence is achieved in as few as 16 iterations. The generated sequence was found experimentally to have high binding affinity selectively towards the desired target.
*S. Quirk, S. Zhong, and R. Hernandez, Proteins: Struct. Func. Bioinfo. 76, 693-705 (2009).

1172-Pos Board B82
Mechanisms of Interaction Between Lung Collectins and Influenza a Virus Hemagglutinin
Michael J. Rynkiewicz, Dong Luo, Nancy Leymarie, Erika C. Crouch, Kevan L. Hartshorn, James F. Head, Francis X. McCormack, Martin van Eijk, Joseph Zaia, **Barbara A. Seaton**.
The unpredictability and rapid appearance of new influenza A virus (IAV) strains pose major challenges for global health. Highly virulent strains can evolve quickly through genetic reassortment from animal IAV strains and spread rapidly through populations lacking immunity. Some pandemic IAV strains can kill patients rapidly, before the acquired immune system can respond, underscoring the importance of the innate response. The innate activity of pulmonary surfactant proteins A (SP-A) and D (SP-D) forms a front line defense against inhaled pathogens. SP-D targets IAV through lectin-based recognition of high-mannose glycans attached to specific glycosylation sites on viral hemagglutinin (HA) and neuraminidase (NA). This recognition drives HA binding, NA inhibition, and viral aggregation and neutralization. SP-A and glycosylated SP-D variants utilize different mechanisms involving sialic acid recognition by HA. Our studies aim to determine the molecular basis of these