computational protein-protein docking approaches can provide a useful alternative to address this issue. We present a novel protein docking algorithm, VDOCK, based on the use of 3D Zernike descriptors (3DZD) as regional features of molecular shape. The key motivation of using these descriptors is their invariance to transformation, in addition to a compact representation of local surface shape characteristics. In our previous works we have shown that 3DZD are suitable for comparing global/local protein surface shape and surface physicochemical properties to quantify their similarity. Here we apply 3DZD for quantifying surface complementarity. Docking decoys are generated using geometric hashing, which are then initially screened by a shape-based scoring function that incorporates buried surface area and 3DZD. The benchmark studies show that 3DZD are not only efficient in identifying shape complementarity for bound docking cases but superior to other existing methods in accommodating a certain level of flexibility of the protein surface in unbound docking cases, taking advantage of 3DZD's controlable resolution of the surface description. In the next stage, generated docking decoys are evaluated using a physics-based scoring function. The weighting factors to combine these terms are trained using several different target metrics on a large dataset of docking decoys. Additional information and steps for selecting models are also employed, which include protein-protein interaction site predictions and optimization of global and side-chain conformations.

2394-Pos

A Physics-Based Iterative Method to Extract Distance-Dependent All-Atom Potentials for Protein Structure Prediction

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One of the challenges in protein structure prediction is the development of an accurate scoring function that yields a global minimum free energy for the native state. Although the knowledge-based scoring function has proven to be a successful scoring approach to protein structure prediction, there exists a hurdle in deriving knowledge-based potentials. Namely, the ideal reference state is inaccessible. In this work, we have developed a general physics-based iterative method to extract distance-dependent all-atom potentials for protein structure prediction. Our method circumvented the long-standing reference state problem. The derived scoring function was extensively evaluated with three diverse test sets, and showed significant improvement over other well-known scoring functions. The results suggest the efficacy of our scoring function for protein structure prediction.

2395-Pos

Using Molecular Simulations to Screen for Antibiotics with Enhanced Permeation Properties through Bacterial Pores

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Gram-negative bacteria are protected by an outer membrane and to function, antibiotics have to diffuse through outer membrane channels, or porins, such as OmpF. Previously, we revealed the complete permeation pathways of β-lactams antibiotics, such as ampicillin, using all atom accelerated molecular dynamic simulations and found remarkable agreement with experimental results(1). Here we follow the paradigm for selecting antibiotics with better permeation properties via computer simulations. In-depth analysis of the simulations revealed the key determinants for the diffusion of ampicillin through OmpF: a subtle balance of interactions at the constriction region of the channel compensates the loss of entropy of the antibiotic and facilitates its diffusion. The simulations were then repeated using porins mutated in their key interacting residues, such as Asp113, and the drastic changes found in ampicillin permeation confirmed our hypothesis. Guided by these results, we then predict that an antibiotic that would interact differently with OmpF, such as penicillin-G that lacks the ampicillin positive group, would translocate faster. This is confirmed by the calculation of the effective energy barriers for translocation, and importantly, we are able to validate the predictions by a wide range of experiments using electrophysiology, spectroscopy and swelling assays techniques. We conclude by drawing, the complete inventory of the rate-limiting interactions and map them on both the porin and antibiotics structure. Finally we show how our multi-scale approach can help rational antibiotics design and screening as we extend it to (i) novel antibiotics of pharmaceutical and therapeutical interest and (ii) homology modeled porins from novel pathogenic strains which shows interesting antibiotic resistance profiles.

1. Hajjar, E., et. al. Bridging time and length scales: from macroscopic flux to molecular mechanism of antibiotics diffusion through porins. Biophys. J. (minor revisions).

2396-Pos

Simulation Study of Stapled Alpha-Helical P53 Peptide Analogs:probing the Relationship between Structural Stability and Biological Potency

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Reactivation of the p53 cell apoptosis pathway through inhibition of the p53-hDM2 interaction is known to be a viable approach to suppressing tumor growth in many human cancers and stabilization of the helical structure of p53 analogs via a hydrocarbon cross-link (staple) has been found to lead to increased potency and inhibition of protein-protein binding. However, details of the structure and dynamic stability of the stapled peptides and their relationship to the nature and location of hydrocarbon linker are not well understood. Here, we use extensive molecular dynamics simulations to study a series of stapled α−helical peptides over a range of temperatures in solution. The peptides are found to exhibit substantial variations in predicted helicities that are in good agreement with the experimental values. In addition, we find significant variation in local structural flexibility of the peptides with the position of the linker, which appears to be more closely related to the observed differences in activity than the absolute helical stability. These simulations provide new insights into the design of α−helical stapled peptides and could aid in the development of potent inhibitors of protein interfaces.

2397-Pos

Synthesis of Kynapcins and Telephoric Acids as Prolyl Endopeptidase Inhibitors of Anti-Dementia Drugs

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Prolyl endopeptidase (PEP), a serine protease, is known to cleave a peptide substrate on the C-terminal side of a proline residue. Additionally, the PEP activity of Alzheimer's patients has been found to be significantly higher than that of the normal person. Therefore, the search for PEP inhibitors as anti-dementia drugs, which may play a crucial role in curing Alzheimer's disease, has attracted significant attention from the synthetic, biological, and medicinal comunities. Recently, Song et al. reported that two novel PEP inhibitors, benzofuran dimer kynapcin-24 (**1**) (IC_{50}, 1.14 μM) and pentacyclic polyozellin, as well as related compounds, were isolated from Polyozellus multiflex Murr. On the other hand, although propeptin (IC_{50}, 1.1 μM) has inhibition similar to **1**, it is a hydrophilic and large molecular weight peptide, which may make it difficult to penetrate the blood-brain barrier. With the difficulty of propeptin as a PEP inhibitor and the promise shown by drugs such as **1**, The synthesis of kynapcin-24, which can be isolated from the Korean mushroom *Polyozellus multiflex* Murr, is achieved in 12% overall yield from commercially available 3,4-dihydroxybenzaldehyde by a route in which the longest linear sequence is only 14 steps. The key transformations in the synthesis are the Cu-mediated and Pd-catalyzed coupling reactions of benzofuranyl iodide **12** with stannane **15**, and 5-endo-dig iodocyclization of a phenol propargyl ether. In addition, telephoric acids have also synthesized in high yields. Finally, the molecular model was examined the interactions of proteins and ligands as well.

Apoptosis

2398-Pos

FCS Studies of the Pore Formation by Protein BAX in Lipid Membranes

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BAX is a pro-apoptotic member of the BCL-2 protein family. At the onset of apoptosis, monomeric, cytoplasmic BAX is activated and translocates to the outer mitochondrial membrane, where it forms oligomeric pores. The biophysical mechanism of BAX pore formation and the structure of the BAX pore are not clear. To study the mechanism of BAX pore formation in lipid membranes we designed an *in vitro* system employing giant unilamellar vesicles (GUVs) and fluorescently labeled BAX combined with the single-molecule sensitivity technique, dual-focus scanning FCS. Use of scanning FCS in experiments, where two spectrally different populations of BAX molecules interact with

a GUV membrane, allowed correlation of the pore formation by BAX with the ability of BAX to oligomerize in lipid membranes. As a result, we show that BAX binds lipid membranes as a monomer and then undergoes oligomerization to form BAX pore protein-lipid complexes. FCS analysis of the populations of GUVs over a period of time showed that BAX pore complexes grow in size and increase in number with time. Analysis of the diffusion coefficients of these BAX complexes using Saffman-Delbruck theory estimates that the in-membrane hydrodynamic radius of a BAX pore complex ranges from 1 to 31 nm. Formation of BAX pore complexes in a lipid membrane is inhibited in the presence of BCL-XL (in-membrane BAX is 100% monomeric) and can be rescued by the addition of cut BID. We also show confocal 3D reconstructions of a giant BAX pore with fluorescent BAX accumulating in the edge of the pore. Lifetime of the giant BAX pore (5-10 min) together with the accumulation of BAX at the edge of the pore and the loss of surface tension in a GUV support the toroidal BAX pore model.

2399-Pos

Direct Activators BID & BIM Function Like Membrane Receptors for BAX & BCL-XL

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The BCL-2 protein family is a primary regulator of apoptosis and its interaction network converges at the pro-apoptotic BAX/BAK nexus. BAX has soluble, membrane-bound, and membrane-integrated forms that are central to the management of mitochondrial permeabilization. These states, which lead to BAX pore formation and cytochrome *c* egress, are modulated by anti-apoptotic multidomain and pro-apoptotic BH3-only proteins. Using purified recombinant BCL-2 proteins and defined liposomes, the soluble->membrane transitions and pore activity modulations have been characterized.

Direct activators cBID and BIM_S instigate BAX pore formation - a process inhibitable by BCL-X_L - and these oppositional functions are dosage-dependent. BIM_S is more efficient an activator than cBID; however, BIM_S-BAX activation is more susceptible to inhibition by BCL-X_L. Since the steps of BAX activation remain controversial, we investigated the kinetics of protein-membrane binding. BAX, cBID, BIM_S, and BCL-X_L are each capable of adsorbing to membranes, albeit with differing properties. These proteins' transitions to lipid bilayers include a rapid binding step that is reversible and distinct from a slower membrane integration step. BCL-X_L and BIM_S show a comparatively high rate of binding to membranes whereas BAX and cBID are substantially slower. The membrane-resident forms of each protein have comparably strong affinities for membranes indicating that the on-rate is most influential on their in-membrane concentrations.The difference in membrane on-rates between the direct activators potentially accounts for the disparity in their BAX activation efficacies. Intriguingly, the membrane-resident forms of cBID and BIM_S were capable of driving BAX and BCL-X_L to tight membrane affinity conformations. These activities were saturable, suggesting a protein-protein interaction rather than modulation of the bulk membrane environment. These data reveal receptor-like roles for cBID & BIM_S for soluble BCL-2 proteins during the initiation of apoptosis.

2400-Pos

Biophysical Insights into Bax Oligomerization and Membrane Insertion

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The BCL2 family of proteins tightly regulates the delicate balance between life and death. Bax, a proapoptotic member of this family, acts as the penultimate factor in the apoptotic cascade by releasing apoptogenic factors such as Cytochrome C from the mitochondrial lumen. The mechanism of mitochrondrial permeabilization by BAXis not well defined. What is known is that BAX translocates to and aggregates at the outer mitochondrial membrane before cytochrome C is released, implying the insertion of the protein occurs after the aggregation event. In this work, we have evaluated the function of the oligomerization state of BAX on the insertion of the protein into artificial membranes.

2401-Pos

Bax Pore Formation: From Activation to Oligomerization

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Many of the known apoptotic pathways involve mitochondrial membrane permeabilization, just upstream of caspase activation and cell death. This key step is controlled by the Bcl-2 family of proteins, and revolves around the action of pore-forming family member Bax, which inserts in the outer mitochondrial membrane in response to apoptotic stimuli, oligomerizes and form pores. In its soluble form, Bax is known to be monomeric and to adopt a globular α-helical structure, however, little is known about its structure (or structures) when bound to the membrane, or about the stoichiometry of its membrane oligomers. We used an in vitro system consisting of 25 nm radius liposomes prepared with a lipid composition mimicking the mitochondrial membrane, in which recombinant purified full-length Bax was inserted via activation with purified tBid. We looked at the distribution of the protein on the liposomes using both fluorescence fluctuation techniques and small-angle neutron scattering. We found that although tBid activation is necessary to set off insertion of Bax into the membrane of the liposomes, Bax auto-activation plays an important role in the formation of the membrane oligomers. We observed that part of the protein inserts in the lipid bilayer, but that a significant amount of the protein mass protrudes above the membrane. This is in contrast to predictions that all of the membrane-associated Bax states are umbrella-like, with the protein's α-helices either inserted in or arranged parallel to the membrane. Upon protein insertion we also detect a thinning of the lipid bilayer, accompanied by an increase in liposome radius, an effect reminiscent of the action of antimicrobial peptides on membranes.

2402-Pos

Conformations and Interactions of BCL-2 Family Proteins: Implications For Apoptosis

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The BCL-2 family proteins are major regulators of mitochondrion-dependent programmed cell death. They includes both pro-death and pro-life proteins, which exert their activities through physical interactions with each other with other non-homologous proteins, and with intracellular membranes. The BH3-only cytotoxic protein BID is activated by caspase-8 cleavage upon engagement of cell surface death receptors. The resulting C-terminal fragment, tBID, translocates to mitochondria, triggering the release of cytotoxic molecules and cell death. The activity of tBID is regulated by its interactions with pro-survival BCL-XL and pro-death BAX, both in the cytosol and at the mitochondrial membrane. Using NMR spectroscopy we show that full length BCL-XL is soluble and monomeric in aqueous solution. Its hydrophobic C-terminal tail, which is predicted to form a transmembrane helix in lipid membranes, folds back to interact with the hydrophobic pocket known to bind the BH3 domains of pro-apoptotic proteins. The presence of the C terminus reduces the binding affinity of BCL-XL for BH3 domains 4-fold, compared to the affinity of truncated BCL-XL. The activated pro-apoptotic protein tBID adopts an α-helical but dynamically disordered conformation in solution. However, its three-dimensional conformation is stabilized when tBID engages its BH3 domain in the BH3-binding hydrophobic groove of BCL-XL to form a stable heterodimeric complex. Studies in lipid micelles show that the proteins' conformations and interactions are dramatically different in the presence of lipids.

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2403-Pos

Membrane-Targeted Soluble Form of BAK Unfolds Like an Umbrella Upon Pore-Formation

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Mitochondrial permeabilization by the pore-forming Bcl2 (B-cell lymphoma-2) proteins such as BAX or BAK constitutes a key regulatory step in the apoptotic processes. Based on the structural similarity of these to the pore-forming bacterial proteins such as colicin and the transmembrane domain of diphtheria toxin, it has been hypothesized that BAX or BAK undergoes conformational changes upon pore formation, in which the hydrophobic helical hairpin structure found in these proteins is unwrapped and inserts into the membrane. We have developed a liposomal system that recapitulates the membrane-permeabilization by BAK through pore-formation. Using a Ni(II)-nitriloacetic acid liposomal system which can conjugate hexa-histidine tagged proteins to the surface of the simulated outer mitochondrial membrane surface, we demonstrate that nanomolar concentrations of BAK when targeted to the membrane surface can efficiently permeabilize the membrane by forming large pores. Using pairs of spin labels introduced at various positions in BAK, we measured the distances between them in the BAK protein before and after pore-formation in the Ni-NTA-liposomal system. The distance between spin labeled residues 55R1 and 146R1, which are located at the αH1-αH2 loop and at the tip of the αH5-αH6 helical hairpin loop, respectively, changes from approximately