continue to evade traditional techniques, such as x-ray crystallography and NMR. A spin-label "motion-on-a-cone" model was used during *de novo* folding of T4-lysozyme and αA-crystallin, which resulted in full-atom models at 1.0Å and 2.6Å to the crystal structures, respectively (Alexander *et al* 2008). This spin-label model and already-existing EPR distance data have been used to generate EPR distance and accessibility knowledge-based potentials, which can be implemented as folding constraints into Rosetta. In addition, we have introduced a rotamer library of the methanethiosulfonate spin-label (MTSSL). Spin-label rotamers have been derived from conformations observed in crystal structures of spin-labeled T4-lysozyme. The method was benchmarked using a set of proteins where the spin-label was positioned at various levels of exposure. The results indicate that the method is able to recover important aspects of spin-label orientation with up to 0.4Å RMSD. In particular, experimental distances and distance distributions observed for T4-lysozyme were reproduced with relatively high accuracy.

2388-Pos

Accurate Loop Generation of Protein Structures using Distance-Guided Sequential Monte Carlo Sampling Method

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Generating accurate structures of loops is a critical step in constructing protein structural models. Although much progress has been made in loop modeling, currently only loops with length less than 15 residues can be modeled effectively, regardless whether a database-search method or an ab initio loop generation method is used. Here we describe a new approach, called Distance-guided Sequential Monte Carlo (DSMC) method for generating long loops of accurate conformations. With further refinement using the CCD (Cyclic Coordinate Descent) method of Canutescu and Dunbrack, our approach works well for generating loops up to length 20, with local RMSD to the native loop conformation <3Å in some cases for length 20.

(AA.Canutescu and RL Jr.Dunbrack. Cyclic coordinate descent: A robotics algorithm for protein loop closure. 2003. Protein Sci.12(5):963-72)

2389-Pos

Antagonist-Binding Conformation of the Dopamine D2S Receptor

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The dopamine D2S receptor was modeled by reference to the β2 Adrenergic receptor crystal structure. Both are monoamine binding neurotransmitter receptors; the two receptors have high sequence similarity (> 85%) except for extracellular loop 2, which has 12 extra amino acids in the β2 Adrenergic receptor. Distance constraints were employed to reconstruct the conserved disulfide bridge Cys107 to Cys182 as well as extracellular loops 1 and 2 such that genetically conserved residue pair interactions are maintained in the D2S receptor when analogous packing occurs in the β2 Adrenergic receptor. Loops were rebuilt by first noting the positions of three template atoms at the N-terminal and C-terminal boundaries of the loop. Intermediate peptide conformers were searched and low energy states were filtered to replicate known distances between atoms in the N-terminal and C-terminal boundary templates. The adjusted homology model was refined by energy minimization, subject to weights that preserve an important salt bridge and a genetically conserved aromatic cluster. Receptor movement as large as 4 Ångstroms is necessary before the 0.3 nM D_{2S} antagonist spiperone can dock. The likely spiperone-binding receptor state was identified by an inverse docking strategy that packs flexible receptor fragments around the ligand. This binding site template then implies a set of distance constraints that can be used to reshape the full conformation of the apo receptor. Yet even a receptor that is explicitly reshaped to fit spiperone will not accommodate this ligand unless thorough search is done for variants of extracellular loop 2 that border the binding site

2390-Pos

Alloxan Derivatives as Inhibitors of Matrix Metalloproteinase-2: Theoretical Calculations and Experimental Results

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Matrix metalloproteinases (MMPs) are a family of structurally related zinc-containing endopeptidases involved in tissue remodelling and degradation of the extracellular matrix. The failure of common synthetic inhibitors makes the design of new selective and potent MMP inhibitors an extreme challenge in health care for the treatment of various pathological states such as inflammation, arthritis, and cancer. In this view, an over-expression of MMP-2 is supposed to be responsible for the occurrence of many different human tumours and inflammatory processes involving the hydrolysis of the type IV collagen, the main component of the basement membrane. A series of studies therefore focused on the design of new potential inhibitors biased towards MMP-2: campaigns of molecular virtual screening of several large chemical libraries resulted in a number of attractive hits. Interestingly, a shortlist of alloxan-like structures was selected with inhibition constants in the nM range. In this respect, we investigated a series of complexes of MMP-2 with alloxan inhibitors by thermodynamic integration in all atoms molecular dynamics simulations. We thus obtained quantitative differences in binding free energies for a list of alloxan compounds. On this basis, we were able to elucidate the molecular rationale for the remarkable inhibition exerted by these compounds with the ultimate aim of driving the synthesis of new more potent and selective derivatives that are at present awaiting for further experimental investigations through enzymatic assays.

2391-Pos

Theoretical Identification of Structural Elements for Stabilizing a Cavity Present in the Entrails of the Human Aryl Hydrocarbon Receptor Dioxin Binding Domain

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The aryl hydrocarbon receptor (AhR) is a transcription factor activated by structurally-diverse ligands including dioxins, which are known to be nongenotoxic carcinogens and are referred to as an environmental hazard due to their toxicities. Despite of the serious effects, experimental structures of AhR have not been determined so far; accordingly, the binding mode of the dioxin in AhR has still remained to be elucidated. In this study, we constructed a structural model of the ligand binding domain of the human AhR (hAhR) for the first time, employing homology modeling techniques coupled to molecular dynamics (MD) simulations.

As a result of the homology modeling phase, we have identified a cavity present in the core region of hAhR. The cavity size is significantly larger than those in the closely-related proteins, HIF-2α and ARNT, even though their folds are very similar. This may lead to a remarkable instability of the protein; we examined mechanisms to hinder such instability. In the early stages of the MD simulation, the cavity size is dynamically changed, whereas it is subsequently converged (stabilized) and seems to be enough to accommodate a dioxin molecule. This stabilization seems to be brought about through the insertion of Gly319 located on a flexible loop (i.e., in the closely-related proteins, this Gly residue is replaced). Actually, in the MD simulation, the Gly-insertion induces a rearrangement of the core packing, thereby leading to a new stacking with respect to Tyr322 (on the above-mentioned flexible loop) and the Phe295 and His337 residues. This rigid structural element still contributes to the core, and thus, may critically stabilize even the larger cavity in the interior of the protein, thereby yielding the capability of the ligand-transport.

2392-Pos

Applying Thermodynamics to Fragment-Based Drug Development

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Antibiotic resistance is a growing problem within the United States, necessitating the need to develop new antimicrobial agents. It has been estimated that within 10 years of an antibiotic entering the market, significant resistance will appear in target bacteria. Exacerbating this problem, many major pharmaceutical companies are not developing new antimicrobial agents, relying on biotech and universities to discover new classes of antibiotics. As a result, only 1% of drugs in clinical trials in 2004 were antibiotics. Because of this, there needs to be a greater push for the design of new antibiotics and antimicrobials to replace obsolete ones as well as development of new, more effective approaches for drug discovery. We have been using a fragment-based approach to identify potential inhibitor building blocks for two bacterial enzymes. Potential building blocks are tested for their ability to inhibit enzyme function and the thermodynamics of binding are investigated by calorimetry. Combinations of these fragments will be combined to develop potential new classes of antibiotics.

2393-Pos

A Unified Protein Docking Procedure with a Shape Complementarity Screening using 3D Zernike Descriptors

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Protein-protein interactions are a pivotal component of many biological processes. Knowing the tertiary structure of a protein complex is therefore essential for understanding the interaction mechanism. Experimental techniques to solve the structure of the complex are, however, often difficult. To this end,

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