

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 α A-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 β 2 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 β 2 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 β 2 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 Ångströms 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 respon-

sible 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-2 α 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,