**2382-Pos**
***de Novo* Protein Structure Prediction using Fragment Based Potential and Conformational Space Annealing**
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De novo prediction of the protein structure, modeling the native structure of a protein from its sequence information when homologous protein structures are not available from the database, is one of the most challenging problems in biophysics. Here, we have employed a coarse-grained multibody energy function approach. A sequence-specific continuous energy function is constructed from the structural and environmental information extracted from fragments. The original algorithm[1] was coupled with Langevin molecular dynamics for searching low energy structures, and when applied to "free modeling" and hard "template-based modeling" targets from CASP7, the prediction results were quite comparable to existing methods. In this study, first, the conformational space annealing (CSA) method is implemented with the above-mentioned energy function. CSA has been quite efficient in searching diverse low-energy solutions in various multiple-minima problems. As expected, lower energy model structures are obtained by CSA over Langevin molecular dynamics approach. However, lower energy structures were not necessarily better in their structural quality. We have carried out energy function optimization by adjusting weight parameters of the function. Details of the parameter optimization as well as application of the newly obtained energy function to target proteins not included in the optimization will be provided.
1. Sasaki TN, Cetin H, Sasai M: A coarse-grained Langevin molecular dynamics approach to de novo protein structure prediction. *Biochem. Biophys. Res. Comm.* 2008, 369:500-506.

**2383-Pos**
**Iterative Assembly of Protein Fragments**
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MASH (Magical Assembly of Sheets and Helices) is a computational method for iterative assembly of protein secondary structure fragments into native-like tertiary structures. For a given pair of fragments, we select a pair of hydrophobic residues (in the case of helix assembly) or an H-bond donor/acceptor pair (in the case of beta strands). We then position the two fragments so that the selected residues are in close proximity to one another. Finally, we attempt to connect the two fragments with a robotics-based loop-closure algorithm. Fragment pairs that have steric clashes or unclosable loops are discarded. We repeat the above procedure to generate an ensemble of fragment pairings, which we then score on the basis of radius of gyration and solvent accessible surface area.

**2384-Pos**
**Template-Based Protein Modeling using Global and Local Templates**
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For successful template-based protein modeling, it is important to identify relevant template proteins to the target sequence and then to generate proper multiple sequence alignment (MSA) between the target and the templates. However, in many cases, the templates obtained by global sequence search do not provide relevant structural information for local regions represented by gaps in the MSA. We have developed a method to improve the modeling accuracy of such regions by detecting unreliable local regions and utilizing local templates that can provide more reliable structural information for those regions. Our approach takes the following steps. First, a new scoring scheme that utilizes a modified information score is employed to detect unreliable local regions. Second, local templates that are aligned to the local regions more reliably are identified. Finally, the local templates are combined with the global templates to produce better 3D models. With newly obtained MSA containing global as well as local templates, protein 3D models are generate by a recently proposed model-building technique, MODELLER-CSA.

**2385-Pos**
**Determination of the Pseudo-Atomic Structure of Nuclear Pore Complex (NPC) Components by Small Angle X-Ray Scattering (SAXS) and Computational Modeling**
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The Nuclear Pore Complex (NPC, ~50 MDa) is the sole passageway for the transport of macromolecules across the nuclear envelope. The pore plays a key role in numerous critical cellular processes such as transcription, and many of its components are implicated in human diseases such as cancer. Previous works provided the first description of the macromolecular architecture of the yeast NPC. This structure defined the relative positions and proximities of its 456 constituent nucleoporin (nup) proteins, based on spatial restraints derived from experimental data. Further elucidation of the evolutionary origin and transport mechanism of the NPC will require higher resolution information. To help improve upon the resolution and accuracy of the NPC structure, we obtained small angle x-ray scattering (SAXS) data.
We prepared sets of single protein, protein domain, and small NPC sub-complex samples for SAXS analysis, because producing crystal structures for many of the proteins has proven difficult. We generated SAXS profiles for individual proteins or sub-complexes in solution, which provide shape information. This shape information generated by SAXS can in turn be used to improve atomic homology models for individual proteins or complexes.
We apply our Integrated Modeling Platform (IMP) software to incorporate a diverse set of experimental data, including SAXS spectra, as spatial restraints, to determine the three dimensional structures of these sub-complexes and proteins by simultaneously minimizing violations of all of the restraints.
We specifically focus on components of two sub-complexes, the 7-protein Nup84 sub-complex, and the 4-protein Nup170 sub-complex, for which complementary experimental data are available. For each SAXS profile, we utilize a score that evaluates a model structure based on the deviation between the experimental profile and a calculated profile for the model.

**2386-Pos**
**Protein Structure Determination by Molecular Replacement using High-Accuracy Protein Structure Modeling**
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Molecular replacement (MR) technique is to solve the phase problem in x-ray crystallography. Currently, many computational methods are available and they can provide MR solutions when a suitable 3D model of the target molecule is available. In practice, the success of the MR method is limited by various factors including unavailability of suitable 3D models and the ambiguity of crystallographic parameters. Accurate 3D modeling of the target molecule can provide a breakthrough in these cases. Recently, we have developed a protein 3D modeling method which can provide accurate protein 3D models both in backbones and side-chains. In addition, the method provides a variety of protein 3D models with structural variation. In recent blind tests, CASP7 and CASP8 experiments, the method produced the very top quality protein 3D models for high-accuracy template-based modeling targets. We combined this modeling method with a MR technique and successfully determined two protein structures, which could not be determined using conventional methods with available protein templates. It appears that high-accuracy protein 3D modeling for backbones as well as side-chains can boost up the success rate of molecular replacement technique allowing us to solve the phase problem in x-ray crystallography without requiring additional experiments with high cost efforts.

**2387-Pos**
**Rosettaepr: Developing Protein Structure Prediction Methods using Sparse SDSL-EPR Data**
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Site-Directed Spin-Labeling Electron Paramagnetic Resonance (SDSL-EPR), in combination with the Rosetta protein folding algorithm (Rohl *et al* 2004), could serve as an alternative method in structure elucidation of proteins that

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,