structural basis of this drastic functional diffrences are unknown The general objectives of our work are to address these issues and to progress towards a better understanding of the structural basis of resistin biology. Our primary focus is (i) to understand the similarities and differences between human and mouse resistin with respect to their structures and (ii) to infer, using computational approaches, putative functionally important residues. For that purpose we have applied known homology modelling approaches to build a comprehensive 3D model for human resistin using mouse crystallographic data as template. We further assessed the structural properties of this 3D model using molecular dynamics techniques. We importantly compared the properties of both mouse and human resistin structures. The structural status of conserved and non-conserved residues between mouse and human resistin were further investigated with particular emphasis on those residues involved in inter-chain contacts and those exposed on the surface. By identifying the few important residues from the above analysis, we further studied and compared the dynamic properties which provide important insights into structural and functional properties of resistin. Our work suggests that there are considerable differences in interchan interactions and contact surface area between human and mouse structures. Our work also suggests that considerable differences in N-terminal helical orientation in the human model.

3370-Pos Board B417

On Template Selection for Homology Modeling of G-Protein Coupled Receptors

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G-Protein Coupled Receptors (GPCRs) are a family of structurally similar integral membrane proteins that bind diverse ligands, from the size of a photon to small peptides. For several years the inactive conformation of Bos taurus rhodopsin has been the only GPCR crystal structure available at atomic resolution, thus serving as the most reliable template for homology modeling of other GPCRs. Over the past year, the atomic coordinates of several different new crystal structures of GPCRs (two of them encompassing some of the characteristic structural features that have often been attributed to GPCR activated states) have become available. Considering that acceptable models of the transmembrane (TM) regions of membrane proteins may be obtained for template sequence identities of 30% or higher, we investigated the extent to which current crystal structures of GPCRs are valuable templates for homology modeling of the TM regions of a dataset of non-redundant non-orphan non-olfactory Class A GPCRs from the human genome aligned using conserved functional residues in their TMs. While the recently solved crystal structures of beta-2 adrenergic receptor and mutant m23 beta-1 adrenergic receptor are calculated to be valuable templates for 16% and 18% of class A human GPCRs, respectively, our results indicate that the majority of GPCRs in the human genome needs better templates for their accurate homology modeling. Thus, our calculations point to specific GPCR targets whose crystal structures would be most beneficial to the majority of human GPCRs. Moreover, we suggest specific ways to improve GPCR modeling, including the use of hybrid templates.

3371-Pos Board B418

Protein Structure Prediction Without Optimizing Weighting Factors For Scoring Function

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Optimizing weighting factors for a linear combination of terms in a scoring function is a crucial step for success in developing a threading algorithm. Usually weighting factors are optimized to yield the highest success rate on a training dataset, and the determined constant values for the weighting factors are used for any target sequence. Here we explore completely different approaches to handle weighting factors for a scoring function of threading. Throughout this study we use a model system of gapless threading using a scoring function with two terms combined by a weighting factor, a main chain angle potential and a residue contact potential. We present three novel threading methods which circumvent training dataset-based weighting factor optimization. The basic idea of the three methods is to employ different weighting factor values and finally select a template structure for a target sequence by examining characteristics of the distribution of scores computed by using the different weighting factor values. Interestingly, the success rate of our approaches is comparable to the conventional threading method where the weighting factor is optimized based on a training dataset. Moreover, when the size of the training set available for the conventional threading method is small, our approach often performs better. In addition, we predict a target-specific weighting factor optimal for a target sequence by an artificial neural network from features of the target sequence. Finally, we show that our novel methods can be used to assess the confidence of prediction of a conventional threading with an optimized constant weighting factor by considering consensus prediction between them. Preliminary result of applying our approaches to docking is also presented.

3372-Pos Board B419

FRESS: an Efficient Monte Carlo Method for Biopolymer Structure Simulation

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An efficient exploration of the configuration space of a biopolymer is essential for its structure modeling and prediction. In this presentation, we report a new Monte Carlo method, Fragment Re-growth via Energy-guided Sequential Sampling (FRESS). We tested FRESS on hydrophobic-hydrophilic (HP) protein folding models in both two and three dimensions. For the benchmark sequences, FRESS not only found all the minimum energies obtained by previous studies with substantially less computation time, but also found new lower energies for all the three-dimensional HP models with sequence length longer than 80 residues. We also developed a new version of FRESS, mFRESS, whose performance will also be presented.

3373-Pos Board B420

Refinement Of Protein Model Structures In Explicit Solvent Using Biasing Potential Replica Exchange Simulations

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Comparative protein modeling of a target protein based on sequence similarity to a protein with known structure is widely used to provide structural models of proteins. Frequently, the quality of the target- template sequence alignment is non-uniform along the sequence: parts can be modeled with a high confidence, whereas other parts differ strongly from the template. In principle, molecular dynamics (MD) simulations can be used to refine protein model structures but it is limited by the currently accessible simulation time scales. We have used a recently developed biasing potential replica exchange (BP-Rex) MD method (Kannan, S. Zacharias, M. *Proteins* 2007, 66, 697-70) to refine homology modeled protein structure at atomic resolution including explicit solvent. In standard Rex-MD simulations several replicas of a system are run in parallel at different temperatures allowing exchanges at preset time intervals. In a BP-RexMD simulation replicas are controlled by various levels of a biasing potential to reduce the energy barriers associated with peptide backbone dihedral transitions. The method requires much fewer replicas for efficient sampling compared with standard temperature RexMD. It is also possible to focus the method to parts of a protein structure (segments of a model structure that may differ strongly from a template structure). Application to several protein structures indicates improved conformational sampling compared to conventional MD simulations. BP-RexMD simulations on several test cases starting from decoy structures deviating significantly from the native structure resulted in final structures in much closer agreement with experiment compared to conventional MD simulations.

3374-Pos Board B421

Protein Structure Refinement Using Physics-Based Models And Sampling

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Much progress has been made in predicting the three-dimensional structures of proteins from sequence alone. So far, the most successful prediction methods have been strongly bioinformatics-based, reliant on known templates or statistical features of solved protein structures. However, in cases of low homology or where templates require substantial editing, it has been challenging for bioinformatics methods to refine predictions better than the closest template [1]. Here, we discuss a strategy for refining structures generated by bioinformatics web servers using physics-based simulations, with an atomic physiochemical force field and canonical sampling at physiological temperature. Specifically, we use replica exchange molecular dynamics (REMD) simulations with an AMBER force field and implicit solvation model that we previously found to correctly stabilize short peptide [2] and small, single domain protein folds [3]. The present REMD simulations are seeded with different conformations, enabling simultaneous selection among and refinement of webserver structures. Periodic conformational clustering and re-seeding are also used to accelerate convergence. In addition, we narrow the sampling space by using restraints derived from the webserver structures to lock in common, high-confidence interactions, both in backbone secondary-structure preferences and in favorable hydrophobic interactions among side chains. These restraints are added in a manner congruent with hierarchical ''zipping'' folding behavior, where local structures form prior to global tertiary rearrangement. We demonstrate the success of the approach for a number of small proteins, and for several targets in

the CASP8 competition. This work suggests that physics-based simulations provide an important complement to bioinformatics structure prediction methods.

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3375-Pos Board B422

Accuracy of Ion Channels Homology Models is Significantly Improved by Symmetry-Restrained Molecular Dynamics Simulations

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Ion channels are an important target for drug development, however 3-D structures of biomedically relevant targets are usually unknown. Structural information required for structure-based drug design is often filled by homology models. Making models sufficiently accurate is challenging because few templates are available and these often have substantial structural differences. Most crystallized homo-oligomeric ion channels are highly symmetric, which dramatically decreases conformational space. In molecular dynamics (MD) simulations, channels deviate from the ideal symmetry and accumulate thermal defects. We have tested whether incorporating symmetry restraints in the MD simulations stage improves the accuracy of homology models. Our testing set consisted of three crystal structures of distantly related channels in closed conformation (KcsA, NaK and KirBac3.1), from which six homology models, two for each channel, were built using the remaining two structures as templates. These were embedded in POPC bilayer, solvated, and subjected to unrestrained MD simulations for 8 ns. Two approaches were then used to restore symmetry: 1) symmetry annealing gradually imposed symmetry through soft harmonic restraints during short 1ns simulations. 2) instantaneous symmetrization involved averaging the structures at a given timestep. Both techniques were followed by 8ns unrestrained simulation. This process was repeated three times. Our results show that the symmetry-annealing method improved the accuracy of homology models in 4 out of 6 cases, decreasing the RMSD against the x-ray structure by ~30%. The symmetricized models are also more stable during subsequent unrestrained simulations. The pore of the channel, which is the drug binding region, is improved most, making the modeled structures suitable for drug design. Instantaneous symmetrization produced effects similar to the gradual annealing, but was not as effective in mimicking the target protein's crystal structure and lowered structure stability.

3376-Pos Board B423

The combination of Small-Angle X-ray Scattering fitting and protein structure modeling in Integrative Modeling Platform

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We have shown that Small Angle X-ray Scattering (SAXS) data can be combined with protein structure modeling to determine the quaternary structures of multi-domain proteins and multi-subunit assemblies (Förster et al., J.Mol.-Biol., 2008, 382(4):p. 1089-1106). To maximize the utility of this approach, we further improved the scoring and sampling algorithms, and implemented them in our Integrative Modeling Platform (IMP) software (http://salilab.org/imp). This implementation will facilitate further integration of different kinds of data for determining the structures of proteins and their assemblies.

3377-Pos Board B424

Cryo-EM Guided de novo Protein Fold Elucidation

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Using cryo-electron microscopy (cryoEM) numerous sub-nanometer resolution density maps of large macromolecular assemblies have been reported recently. Although generally no atomic detail is resolved in these density maps, at 7 Å resolution α-helices are observed as density rods. Here we present the development of a computational protein structure prediction algorithm that incorporates the experimental cryoEM data as restraints. The placement of helices is restricted to regions where density rods are observed in the cryoEM density map. The Monte Carlo based protein folding algorithm is further driven by knowledge based energy functions.

The method has been benchmarked with ten highly α-helical proteins of known structure. The chosen proteins range in size from 250 to 350 residues. Starting with knowledge of the true secondary structure for these ten proteins, the method can identify the correct topology within the top scoring 10 models. With more realistic secondary structure prediction information, the correct topology is found within the top scoring 5 models for seven of the ten proteins. The algorithm has been applied to human adenovirus protein IIIa. This protein, for which there is no high resolution structure, is predicted to be highly α-helical. It is resolved in a 6.9Å resolution cryoEM adenovirus structure as a bundle of ~13 α-helical density rods.

3378-Pos Board B425

Membrane Protein Structure Determination by Coupling Sparse Experimental Data with Protein Structure Prediction Techniques

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Membrane protein structure determination by classic experimental methods such as X-ray crystallography and nuclear magnetic resonance (NMR) continues to be extremely challenging, as demonstrated by the extremely low proportion of such structures in the Protein Data Bank. However, more than 50% of pharmaceuticals target membrane proteins, and it is estimated that membrane proteins make up 30-40% of all proteins. Therefore, a novel method was developed for membrane protein structure determination, taking advantage of alternative experimental techniques which are not hindered by the size or environment of membrane proteins. These alternative experimental techniques, such as electron paramagnetic resonance (EPR) and cryo-electron microscopy, provide sparse or low resolution structural data but cannot alone uniquely define a protein's structure. In order to obtain atomic detail models, the method incorporates one or more types of sparse or low resolution experimental data into a protein structure prediction algorithm. The method was benchmarked on a set of membrane proteins with known structure using sparse or low resolution data. This demonstrated the feasibility of obtaining membrane protein models of biologically relevant quality. The method was then applied to the multidrug resistance membrane transporter protein EmrE, for which extensive EPR and electron density data exist, giving a model with a high confidence of being a valid structure for EmrE.

Voltage-gated K Channels-Gating III

3379-Pos Board B426

Voltage-Clamp Fluorimetry Of Kv1.2 Channels Show Two Unique Phases Of Quenching Associated With Channel Activation

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Voltage-clamp fluorimetry can be used to visualize real-time changes in protein structure relative to a locally introduced fluorophore, and thus enhance our understanding of ion channel gating. Since the crystal structure is available for Kv1.2, it seems important to compare the voltage-dependent fluorescence reports from this channel with those already recorded from Shaker potassium channels. In the absence of introduced cysteine residues, we were unable to record voltage-dependent fluorescence signals from wild-type channels incubated with tetramethylrhodamine maleimide (TMRM). However, signals were obtained from an introduced cysteine at A291 in the Kv1.2 S3-S4 linker. Depolarization resulted in two separate components of quenching, which may underlie two different conformational changes in the protein. A slow quenching phase was observed upon depolarization from a holding potential of −120 mV and was essentially complete by −50 mV. The rate of this quenching was not significantly voltage-dependent, with time constants between 35 ms at −70 mV and 29 ms at +80 mV. The voltage-dependence of the slow component suggests that it reports on conformational changes preceding opening. A more rapid quenching component was observed upon depolarizations positive to −40 mV, with time constants from 2-10 ms. It had a similar voltage-dependence to the conductance-voltage relationship for potassium currents through Kv1.2, suggesting that it may report on channel rearrangements associated with opening. Dissociation of channel gating charge movement from pore opening by the incorporation of the ILT triple mutation in the S4 domain abolished the fast phase of fluorescence quenching at potentials up to +80 mV. This suggests that the fast quenching reports on conformational changes associated with channel opening, and that slow fluorescence quenching reflects protein rearrangements occurring earlier in the gating process.

3380-Pos Board B427

Kinetics Of Open- And Closed-state Inactivation Of Kv1.5 At Low pH Or With Ni2+ Ions

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Previous studies have shown that Ni^{2+}_o and H^+_o inhibit Kv1.5 current by enhancing open state inactivation (OSI) and promoting closed state inactivation