selectivity of inhibitory ligands, their susceptibility to resistance mutations, and the discovery of putative allosteric binding sites. Markov state models have recently emerged as a practical computational approach to the enumeration of protein conformational states, and can be constructed by aggregating the data from multiple, independent, short molecular dynamics trajectories in a statistical fashion. We aim to apply this technique to the entire human kinome, simulating each protein kinase catalytic domain using a range of high performance compute resources, including the distributed simulation framework, Folding@Home. In combination with recent developments in GPU-accelerated simulation algorithms, this approach allows us to obtain aggregate trajectory lengths on the order of milliseconds. An automated software pipeline provides the ability to quickly generate multiple starting configurations for each kinase, while a central database of publicly available kinase data has been set up and used for tasks such as the selection of catalytic domain sequences and the assignment of relative priorities to each kinase. In parallel with our computational approach, we are working towards expressing a diverse range of kinases in bacterial systems, and scaling up a fluorescence-based assay to plate format for direct measurement of kinase inhibitor binding affinities. Our poster will present preliminary results from these efforts.

3316-Pos Board B44

Docking Benchmark Set of Protein Models

Ivan Anishchanka[1,2], Petras J. Kundrotas[1], Alexander V. Tuzikov[2], Ilya A. Vakser[1,3].
[1]Center for Bioinformatics, The University of Kansas, Lawrence, KS, USA, [2]National Academy of Sciences of Belarus, Minsk, Belarus, [3]Department of Molecular Biosciences, The University of Kansas, Lawrence, KS, USA.

Protein docking is a computational procedure for predicting the 3D structure of protein complexes. Docking has been extensively benchmarked on experimentally determined protein structures. However, studies of protein-protein interactions increasingly involve modeled structures of the individual interactors. These structures are inherently less accurate than the X-ray structures. Thus, the utility of docking procedures, when applied to protein models, should be thoroughly tested in benchmarking studies. Such benchmark set of protein models was developed as part of the DOCKGROUND resource (http://dockground.bioinformatics.ku.edu). The set contains 63 complexes with each monomer represented by six models with a pre-defined Ca RMSD from the native structure (1, 2, … 6 Å). The models were generated by a combination of homology modeling and Nudged Elastic Band method. A new, extended set of protein models was recently built for 165 nonredundant hetero complexes from DOCKGROUND. For more realistic representation of the models, they were generated exclusively by I-TASSER protein modeling package. The benchmark sets were used in the assessment of protein docking methodologies.

3317-Pos Board B45

Structural Similarity in Modeling of Homodimers

Petras Kundrotas[1], Ilya Vakser[1], Joël Janin[2].
[1]University of Kansas, LAWRENCE, KS, USA, [2], Université Paris-Sud 11, Orsay, France.

Template-based methods, which utilize known protein structures, are commonly employed to model individual proteins from their sequences. Here we benchmark a template-based method, previously proposed for modeling hetero-dimeric complexes, on sets of homodimeric assemblies. The method is based on structural alignment of assembly subunits and identifies templates for the vast majority of the test targets. In many cases, the target-template pairs have sequence identity too low for reliable detection by sequence-based methods. An overall dimer geometry as well as interface residue contacts are correctly reproduced for almost half of the targets. We present analysis of the obtained models and their templates, which revealed incorrectly determined quaternary structure for a number of entries in the Protein Data Bank.

3318-Pos Board B46

Three-Dimensional Structure of the 54-Kda Subunit of the Chloroplast Signal Recognition Particle using Molecular Modeling

Rory Henderson, Mercede Furr, Srinivas Jayanthi, Alicia Brown, Robyn Goforth, Ralph Henry, T.K.S. Kumar.
University of Arkansas, Fayttevile, AR, USA.

The chloroplast signal recognition particle is a heterodimeric complex of the 54kDa cytosolic signal recognition particle homologue (cpSRP54), and a novel 43kDa subunit (cpSRP43). While a nearly complete three-dimensional structure of cpSRP43 has been obtained, no structure is yet available for cpSRP54. The three-dimensional structure for cpSRP54 could provide valuable information for the rationalization of the extensive information already available regarding its function, and in the understanding of the as yet undetermined mechanism of light harvesting chlorophyll binding protein's (LHCP) insertion into the thylakoid membrane. In this study, we developed an in silico, three-dimensional structural model of cpsRP54 using a combination of homology modeling, de novo structure prediction and molecular dynamics simulation. The resulting structure is consistent with the known properties of the protein and sheds new light on some of the mechanistic details of its functioning.

3319-Pos Board B47

A Molecular Dynamics Simulation Study of Outer Membrane Phospholipase a (OMPLA) Structure and Dynamics in an Asymmetric Lipopolysaccharide Membrane

Emilia L. Wu[1], Patrick J. Fleming[2], Jeffery B. Klauda[3], Karen G. Fleming[2], Wonpil Im[1].
[1]University of Kansas, Lawrence, KS, USA, [2]John Hopkins University, Baltimore, MD, USA, [3]The University of Maryland, College Park, MD, USA.

The outer membrane of Gram-negative bacteria is a unique and highly asymmetric lipid bilayer composed of phospholipids in the inner leaflet and mostly lipopolysaccharide (LPS) in the outer leaflet. Outer membrane phospholipase A (OmpLA) is an integral membrane enzyme in Escherichia coli. The structure of monomeric OmpLA consists of a 12-stranded antiparallel β-strands with a convex and a flat side, six loops at the extracellular side and five turns at the periplasmic side of the membrane. Utilizing the latest C36 CHARMM lipid and carbohydrate force field, we have constructed a model of OmpLA embedded in an asymmetric lipid bilayer with rough LPS molecules (without O-antigen) in one leaflet and phosphatidylethanolamine, phosphatidylglycerol, and cardiolipin in the other leaflet to model the realistic outer membrane environment. The simulation results will be discussed in terms of the key structural properties of the bacterial outer membrane including hydrophobic thickness, area per lipid, and acyl chain order parameter. We will also show the difference of OmpLA structure and dynamics compared to that in a DLPC bilayer. At the same time a comparison between simulations with different numbers of LPS molecules on the outer leaflet will elucidate the potential technical difficulties in building asymmetric bilayer.

3320-Pos Board B48

Modular Platform for Biomolecular Modeling and Simulations

Dominik Gront.
Faculty of Chemistry, University of Warsaw, Warsaw, Poland.

Computational software has been a cornerstone of many biological sciences such as biophysics, bioinformatics or biomolecular modelling in general. The last few decades witnessed numerous software packages that implemented newly emerging methods and algorithms. In parallel with the development of methods to solve particular scientific problems, the general picture how a suite of computational software should be constructed also evolved.
Here we present the design, implementation and functionality of BioShell[1,2] software - a versatile package for biomolecular modelling. Its functionality ranges from processing structural and sequence databases to sampling conformations both in Cartesian and alignment space. Highly modular structure facilitates easy extension of the package. Its modules may be conveniently bound by a high-level Python script into a single pipeline.
One of the newest BioShell applications is three-dimensional threading. A Monte Carlo search scheme samples the conformational space of alignments between a query sequence and a template structure. In another example, BioShell modules were used to build a simple computational model of RNA molecules.

1. D. Gront, and A. Kolinski, Bioinformatics, 2005, 22, 621-622.
2. D. Gront, and A. Kolinski, Bioinformatics, 2008, 24, 584-585.

3321-Pos Board B49

A Global Machine Learning Based Scoring Function for Protein Structure Prediction

Eshel Faraggi[1,2], **Andrzej Kloczkowski**[2,3].
[1]Department of Biochemistry and Molecular Biology, Indiana University School of Medicine, Indianapolis, IN, USA, [2]Battelle Center for Mathematical Medicine, Nationwide Children's Hospital, Columbus, OH, USA, [3]Department of Pediatrics, The Ohio State University, Columbus, OH, USA.

We present a knowledge-based function to score protein decoys based on their similarity to native structure. A set of features is constructed to describe the structure and sequence of the entire protein chain. Furthermore, a qualitative relationship is established between the calculated features and the underlying electromagnetic interaction that dominates this scale. The features we use are associated with residue-residue distances, residue-solvent distances, pairwise knowledge based potentials and a four-body potential. In addition we introduce a new target to be predicted, the fitness score, which measures the similarity of a model to the native structure. This new approach enables us to obtain information both from decoys and from native structures. It is also devoid of

previous problems associated with knowledge-based potentials. These features were obtained for a large set of native and decoy structures and a back-propagating neural network was trained to predict the fitness score. Overall this new scoring potential proved to be superior to the knowledge-based scoring functions used as its inputs. In particular, in the latest CASP (CASP10) experiment our method was ranked third for all targets, and second for freely-modeled hard targets among about 200 groups for the top model predictions. Ours was the only method ranked in the top three for all targets and for hard targets. This shows that initial results from the novel approach are able to capture details that were missed by a broad spectrum of protein structure prediction approaches.

3322-Pos Board B50

New Insights on the Mechanism of Action of Ice-Binding Proteins

Ran Drori[1], Yeliz Celik[2], Peter L. Davies[3], Ido Braslavsky[1,2].
[1]Institute of Biochemistry, Food Science and Nutrition, The Robert H. Smith Faculty of Agriculture, Food and Environment , The Hebrew University of Jerusalem, Rehovot, Rehovot, Israel, [2]Department of Physics, Ohio University, Athens, OH, USA, [3]Department of Biomedical and Molecular Sciences, Queen's University, Kingston, ON, Canada.

Ice-binding proteins (IBPs) aid the survival of cold-adapted organisms by inhibiting the growth of endogenous ice crystals. The binding of IBPs to ice causes a separation between the melting point and the freezing point of the ice crystal (Thermal hysteresis, TH). Although the discovery of IBPs was more than 45 years ago, the mechanism of action is still unclear. For moderately active IBPs it is known that thermal hysteresis values increase with annealing time between ice and IBP before cooling is started. We have extended this observation to hyperactive IBPs. Using a custom-made nanoliter osmometer and a novel microfluidics system [1], we show that the exposure time of crystals to IBPs is a crucial factor and that their activity could be increased up to 40-fold over long periods of time (13 h). We found that each IBP has a different time-dependent behavior thus the structure of the IBP molecule brings about a distinct kinetic behavior. Using our cooled microfluidic system, we show that hyperactive IBPs accumulate progressively on the basal plane, and rapidly on the prism plane. Fluorescence intensity analysis showed that the distance between IBPs on the ice surface is 7-20 nm (the ice-binding site of the protein is 3 nm long), and a correlation between the surface density to the measured TH activity was obtained. Microfluidic solution exchange experiments show that both moderate and hyperactive IBPs prevent the growth of ice crystals after the removal of the protein solution. These results have a significant contribution to understanding the IBP mechanism and can be helpful in applying these proteins in different fields.

Funded by ISF, CIHR and ERC.

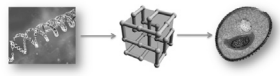
1) Celik, Y., Drori, R., et al., Proc Natl Acad Sci U S A, 2013. 110(4): p. 1309-14.

3323-Pos Board B51

Can a Protein's Evolutionary Fate be Predicted from its Structure?

Amy I. Gilson, Eugene I. Shakhnovich.
Harvard University, Cambridge, MA, USA.

A fundamental question in evolution is the role of a protein's 3D structure in determining its evolvability. We observe that different protein structures vary widely in their ability to respond adaptively to simulated selective pressures, even with the aid of folding chaperones. This variation that cannot be explained by factors such as stability, that are already understood to promote evolvability. In these simulations, lattice proteins evolve in a crowded, cell-like environment with complex dynamics between protein structure, protein-protein interactions, and cellular fitness, while remaining exactly solvable. We develop new structural metrics predictive of a protein's evolvability and corroborate the conclusions with analysis of protein folds in nature.



3324-Pos Board B52

Probing an Ancient Protein's Dynamics with NMR

Marc S. Hoemberger[1], Christopher G. Wilson[1], Dorothee Kern[2].
[1]Biochemistry and Biophysics, Brandeis University, Waltham, MA, USA, [2]Biochemistry, Brandeis University, Waltham, MA, USA.

The tyrosine kinases Abl and Src are very similar in function and fold. However, the cancer drug imatinib exhibits highly selective binding for Abl compared to Src. For a long time it was assumed that these differences arise through a conformational selection mechanism of a DFG-loop, which allows binding of imatinib only if it is in the correct conformation. Recently, an additional induced fit step in the process of imatinib binding has been shown to account for the differences in binding of the drug to Abl in comparison with Src. To investigate the binding mechanism of imatinib to Abl we used resurrected enzymes along the nodes in a phylogenetic tree between the common ancestor of Abl and Src and the contemporary Abl kinase. We show that the binding affinities of each of the four resurrected ancestor enzymes are gradually increasing from the common ancestor towards the contemporary Abl and that the mechanism of binding is comprised of a conformational selection step followed by an induced fit step. To probe the conformational changes the enzyme undergoes upon imatinib binding, we use CPMG relaxation dispersion experiments. We show that most of the exchanging residues are in an intermediate or fast time regime. Combining the CPMG data with molecular dynamics will allows us to get a more detailed look at which residues play an import role for the selectivity of imatinib towards Abl kinase.

3325-Pos Board B53

Exploring the Energy Landscape through Ancestral Proteins

Shion An, Kathryn M. Hart, Susan Marqusee.
UC Berkeley, Berkeley, CA, USA.

How does a protein's energy landscape change over an evolutionary time scale, and could a study of ancestral sequences reveal novel features of the landscape? Although there has been significant advances protein structure prediction from its primary sequence, extracting features of the entire energy landscape remains a challenge. There is limited understanding on how variations in the sequence that take place over the course of an evolutionary time scale change the landscape to yield novel properties and function. Past research on the landscape has focused on extant proteins, which represent only a small portion of proteins that have existed since the origin of life. Examining the ancestors of current proteins should provide additional insight into the properties of proteins and the process of protein evolution, and ultimately, further our understanding of the depth of information encoded within an amino acid sequence.

We conducted an ancestral sequence reconstruction of ribonuclease H1, a biophysically well-characterized protein. A total of seven ancestral proteins along the mesophilic and thermophilic lineages were constructed and studied. This study reports on the characterization of the folding pathway and stability of the last common ancestral protein between E. coli and T. thermophilus ribonucleases H.

3326-Pos Board B54

The Effective Temperature of Mutations: A General Mechanism for the Congruent Evolution of Robustness

Gergely J. Szöllősi, Imre Derényi.
"Lendület" Biophysics Research Group, ELTE-MTA, Budapest, Hungary.

Genetic robustness is critical to the understanding of evolution, as phenotypically expressed variation is the fuel of natural selection. The origin of robustness, whether it evolves directly by natural selection or it is a correlated byproduct of other phenotypic traits, is unresolved. Examining microRNA (miRNA) genes of several eukaryotic species, Borenstein and Ruppin [1] showed that the structure of miRNA precursor stem-loops exhibits significantly increased mutational robustness in comparison with random RNA sequences with the same stem-loop structure. Introducing a novel measure of robustness based on the equilibrium thermodynamic ensemble of secondary structures of miRNA precursor sequences, we demonstrated [2] that, (i) miRNA are significantly more tolerant with respect to thermal and mutational perturbations (exhibit enhanced thermodynamic and mutational robustness) than samples of random RNA sequences with the same structure, (ii) the biophysics of RNA folding induces a high level of correlation between the responses to mutational and thermodynamic perturbations.

Motivated by the striking similarity between the effects of mutational and thermodynamic perturbations that the observed high level of correlation implies, here we attempt to quantify this similarity. Extending our study to lattice proteins we demonstrate that the effects of mutations can very generally and with unanticipated precision be described as an effective temperature increase of the thermodynamic ensemble of structures [3]. This result presents a general mechanism for the congruent evolution of thermodynamic and mutational robustness in the context of molecular phenotypes, and provides an explanation of how increased stability can facilitate opportunities for molecular innovation by facilitating increased neutral variation.

[1] Borenstein & Ruppin (2006) Direct evolution of genetic robustness in microRNA. PNAS.
[2] Szollosi & Derenyi (2009) Congruent evolution of genetic and environmental robustness in microRNA. Mol. Biol. Evol.
[3] Szollosi & Derenyi (in preperation) The effective temperature of mutations and the congruent evolution of robustness.