Invited Talks

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RESCUING ONCOGENIC MUTANTS OF P53

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The tumour suppressor p53 controls the main defense of the cell against cancer. On detecting oncogenic stress, p53 orchestrates a chain of events leading to apoptosis. As a consequence, cancer cells must have p53 or its pathways inactivated or impaired by mutation. p53 is, therefore, an important target for drugs that can reactivate it. About 30% of the oncogenic mutants are temperature sensitive mutants that denature too rapidly and aggregate. I will describe how small molecules can be used to slow down the rate of spontaneous denaturation and inhibit aggregation. A particular oncogenic mutant of p53, Y220C, is a useful test-bed for potential drug development because the mutation of tyrosine to cysteine induces a druggable cavity far away from the functional binding sites of p53. The stability of Y220C can be altered by small molecules that do not bind to wild-type p53 and do not interfere with those functional regions. We are developing leads for novel anticancer drugs.

AUTODOCK HYDRATED DOCKING: A FORCEFIELD WITH DISCRETE DISPLACEABLE WATERS AND DESOLVATION ENTROPY FOR HYDRATED LIGAND DOCKING

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Water plays an essential role in ligand binding. When a small molecule binds to a protein, it must displace most of the waters occupying the binding cavity. However, rarely are all water molecules displaced. Some waters can be so strongly bound and conserved among similar proteins that from a ligand-docking perspective they are considered a part of the target structure, altering the binding site topography. For modeling these water molecules, a new computationally efficient forcefield and hydration docking method has been designed. It enables the automated prediction of waters mediating the binding of ligands with target proteins. The method presumes no prior knowledge of the apo or holo protein hydration state, and is potentially useful in the process of structure-based drug discovery. The hydration forcefield accounts for the entropic and enthalpic contributions of discrete waters to ligand binding, improving energy estimation accuracy and docking performance. The forcefield has been calibrated on 197 complexes and tested on 220 complexes, and further validated on cross-docking experiments. Docking results show a significant performance improvement when waters are involved in docking, confirming that the method is able to predict presence of weakly and strongly bound waters on a ligand basis. Up to 35 waters per ligand can be modeled with a very limited computational overhead.

STRUCTURE-BASED DE NOVO LIGAND DESIGN FOR PROTEIN TARGETS

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Parallel to structure based screening of molecular databases by molecular docking, algorithms for de novo drug design started to appear from late 1980s and early 1990s. However, de novo drug design approaches have resulted far less successful examples due to many practical limitations. Two of the major obstacles are: the synthesis accessibility of designed molecules and the requirement for highly accurate scoring function as the molecules designed need to be synthesized in the laboratory. In this talk, I will first give a summary for the de novo drug design approaches and then will introduce LigBuilder, a de novo drug design program developed in our laboratory. The first generation of LigBuilder (1.0-1.2) was developed in early 1990s and has been widely used. The new generation, LigBuilder 2.0 was recently developed to solve the problem of synthesis accessibility of designed compounds and the problem of multi-objective optimization to reduce false positives in ligand design [1]. LigBuilder 2.0 has been successfully applied in practical drug design projects [2]. Discussions for challenges in drug discovery based on systems biology will also be given.

References

1. Yuan, YX, Pei, JF, Lai, LH, LigBuilder 2: A Practical de Novo Drug Design Approach. J Chem Inf Model 2011, 51, 1083.
2. Ni, SS, Yuan, YX, Huang, J, Mao, XN, Lv, MS, Zhu, J, Shen, X, Pei, JF, Lai, LH, Jiang, HL, Li, J, Discovering Potent Small Molecule Inhibitors of Cyclophilin A Using de Novo Drug Design Approach. J Med Chem 2009, 52, 5295.

INTERACTING WITH PROTEIN INTERACTIONS

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Biology has become accessible to an understanding of processes that span from atom to organism. As such we now have the opportunity to build a spatio-temporal picture of living systems at the molecular level. Recent work in my laboratory addresses some of the issues that we confront in our attempts to create, interact with, and communicate physical representations of complex molecular environments.

In this talk I will discuss and demonstrate three levels of interaction with protein interactions: 1) human perceptual and cognitive interaction with complex structural information; 2) interaction and integration of disparate data sources to construct cellular environments at the molecular level; and 3) interaction of software tools that can bridge the disparate disciplines needed to explore, analyze and communicate the emergent holistic molecular view of living systems.

In order to increase the understanding and interaction with complex molecular structural information we have combined two evolving computational technologies: computer autofabrication, known as solid printing and augmented reality, the technology that combines real-world object with computer generated information. We create tangible models utilizing computer autofabrication. Each molecular model can be custom made, with an ease similar to that of printing an image on a piece of paper. Specific model assembly kits can be made with this technology to create “molecular Legos” that go well beyond the chemical models of the nineteenth and twentieth centuries. Augmented reality is used to combine computer-generated information with the physical models in the same perceptual space. By real-time video tracking of the models as they are manipulated we can superimpose text and graphics onto the models to enhance the information content and drive interactive computation.

We have recently developed AutoFill and AutoCell, automated technologies to construct the crowded molecular environment of living cells from structural information at multiple levels as well as bioinformatics information on levels of protein expression and other data. Utilizing a multi-algorithmic approach we can populate cytoplasm, membranes, organelles and multi-protein complexes within the same structural volume, resulting in three-dimensional representations of cellular environments that synthesize our current best knowledge of such systems, and which can become “community models” that evolve with new information and knowledge. Such environments can be used for multi-scale simulations of complex cellular molecular phenomena.

The communication of such complex structural information requires both extensive scientific knowledge as well as expertise in creating clear visualizations and interactive environments. To this end we have developed a method of combining our existing molecular modeling environment with several professional grade 3D modeling and animation programs such as Maya, Cinema4D and Blender. This gives both molecular scientists and professional scientific illustrators access to the best capabilities of both the science and art of molecular communication. In doing so it brings new, expanded capabilities to both communities.

References

Gillet, A., Sanner, M., Stoffler, D., Olson, A.J. (2005) Tangible interfaces for structural molecular biology. Structure 13:483-491.

Johnson, G.T., Autin, L., Goodsell, D.S., Sanner, M.F., Olson A.J. (2011) ePMV Embeds Molecular Modeling into Professional Animation Software Environments. Structure 19:293-303.

CHASING EHRLICH'S MAGIC BULLET - NUCLEIC ACID APTAMERS FOR THERAPY AND DIAGNOSTICS

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Highly specific molecular recognition between nucleic acids and proteins is a fundamental tenet of life. Artificial selection of a nucleic acid, whereby a particular target-binding nucleic acid is selected and evolved from a large unbiased random pool, was first achieved over 20 years ago. This process of in vitro molecular evolution towards the evolved nucleic acid aptamer was termed SELEX - systematic evolution of ligands by exponential enrichment – and has had major impact over the last two decades in both basic and applied sciences. One particular application of such selected nucleic acids is in medicine, and aptamers have been clinically proven over recent years. Our laboratory has been developing aptamers as a new therapeutic approach against skeletal disease and tuberculosis. We have also been developing aptamers as perhaps a better and cheaper way to diagnose malaria. Here, we discuss our progress with a particular focus on the biochemistry of the nucleic acid-protein molecular recognition events underpinning such potential medical applications.

MOVING IN NEW CIRCLES - EXPLOITING MACROCYCLES FOR DRUG DISCOVERY

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Macrocycles are found widely in nature where they fulfil numerous specific biological functions, but have been generally underexploited as drug molecules. In order to permit the further investigation of macrocyclic compounds, Ensemble Therapeutics has developed two complementary platforms for the rapid synthesis and screening of macrocycles (EnsemblinsTM) and are using macrocycles for the discovery of leads against challenging protein-protein interaction targets. The macrocycle collections have been used successfully for the discovery of compounds that interact with a number of important drug discovery targets. It has been shown that small molecule macrocycles can have desirable drug-like properties including solubility, membrane permeability and oral bioavailability, and offer a realistic alternative to biological therapeutics for protein-protein interaction targets.

STRUCTURE-BASED DISCOVERY OF INHIBITORS TOWARD THE SHIKIMATE PATHWAY IN HELICOBACTER PYLORI

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Persistent *Helicobacter pylori* infection in the human stomach causes gastritis and the development of severe gastrointestinal diseases including ulceration and/or carcinoma. Resistant H. pylori and other multiple antibiotics-resistant microbial infections have posed serious public health issues. The shikimate pathway is an attractive target for antibiotic discovery against multi-antibiotic–resistant pathogenic bacteria because it synthesizes chorismate, a precursor of aromatic amino acids and other aromatic compounds in bacteria, fungi, and plants, but not animals. We report a structure-based site-moiety screening method, orthSiMMap, to discover the inhibitors for a family of orthologous proteins in the shikimate pathway. Here, we utilized the orthSiMMap to pharmacologically interrogate orthologous shikimate kinases (SKs) from Mycobacterium tuberculosis and Helicobacter pylori. The derived apo/closed core site-moiety maps and the anchor scores were used to identify six potent inhibitors (IC50 <8.0 μM). Site-directed mutagenesis and analogues studies revealed that critical conserved interacting residues contribute to a given pocket-moiety interaction spot.

Crystal structures of HpSK•SO4, R57A, HpSK•shikimate-3-phosphate•ADP, and E114A•162535 show a characteristic three-layer architecture and a conformationally elastic region having R57, R116, and R132 occupied by shikimate/inhi4bitor, locking into an induced-fit form. These results illustrate a robust approach in identifying selective inhibitors and reveal insight to the active site chemistry of SKs and a new induced-fit mechanism by an inhibitor