

MGMS and RSC MMG Young Modellers' Forum 2008

ORAL PRESENTATIONS

Talk 1

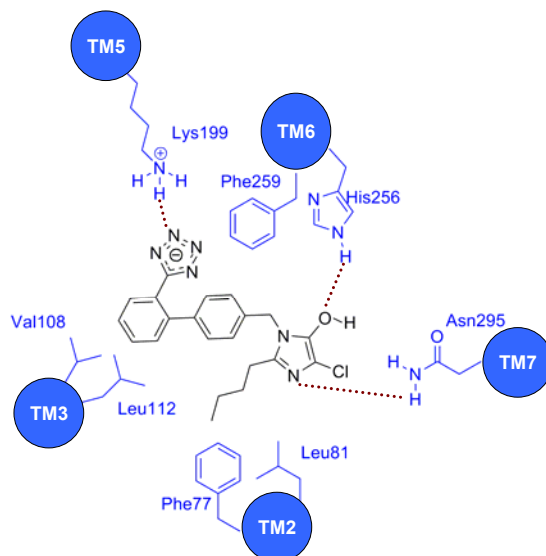
Where Does the Tetrazole Belong? Insight to the Binding Pose of AT1 Antagonists Using Homology Modelling, Molecular Dynamics, and Docking

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The angiotensin II type I (AT1) receptor is a class A GPCR that mediates the renin-angiotensin system (RAS), a well-characterized pathway that regulates blood pressure and maintains water-salt homeostasis [1]. Through the release and progressive cleavage of several precursor proteins, the octapeptide angiotensin II binds to AT1, causing vasoconstriction. A class of non-peptide AT1 antagonists, called “sartans”, serve to block angiotensin II from binding to AT1 and have been used to successfully treat hypertension. Most sartans contain a biphenyltetrazole moiety which is crucial for binding activity.

The ability to design more effective AT1 antagonists is of great pharmaceutical interest and relies on understanding the interactions between binding site residues and existing sartan compounds. There is, however, a great divide in the scientific community as to the role of Lys199 in binding. Many experimental and computational studies have determined that the acidic tetrazole ring of sartans interacts with the positively charged Lys199 residue in AT1. Other research indicates that this is not the case. So the question endures: How does the tetrazole ring fit in the binding site?



In order to address this question, a comparative model of the AT1 receptor was constructed using the newly crystallized β 2-adrenergic receptor as a template. This structure was relaxed using molecular dynamics in an explicit solvent and lipid environment. We used diverse AT1 antagonists which have never been computationally examined before and integrated both SAR data and binding affinity trends to guide docking studies. The results agree well with experimental information and suggest a novel binding orientation, involving a pi-stacking interaction with His256 and a clear interaction between tetrazole and Lys199.

Reference:

[1] Kang, PM *et al.* Angiotensin-II Receptor Antagonists – A New Approach to Blockade of the Renin-Angiotensin. *American Heart Journal*. **1994**, 127(5), 1388-1401.

Talk 2

The Determinants of Protein Kinase Inhibitor Selectivity

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Protein kinases are of considerable interest to the pharmaceutical industry. The major challenge for kinase inhibitor design is the difficulty of obtaining selectivity, which makes many kinase inhibitors unsuitable as drugs. In order to understand selectivity we have studied the relationship between the structures of protein kinases and their ability to bind promiscuous ligands. We have examined the structures around the pockets of a set of high quality structures in order to investigate what inhibitor a kinase can bind and what

is required for kinase binding to an inhibitor. We report a novel method using the Mantel test¹ which can group kinases based on the similarity in spatial arrangement of the side chains. Our shape-based phylogenetic tree shows that similarity in shape alone can sometimes determine the ability to bind a set of ligands, regardless of the overall sequence similarity. Our study of the similarities and differences in the shapes of the pockets indicates what is required for most kinases to bind the promiscuous inhibitor, staurosporine. We examine the similarity in the shape of the pocket by collecting atoms that are conserved in their relative positions to staurosporine and show that some atoms are recruited to staurosporine via an induced-fit mechanism, which contributes to staurosporine promiscuity. Finally, we focus on the differences in distance matrices among the pockets and show how the side chains play roles in binding affinities. Based on our QSAR approach, our selected set of distances shows high correlation to binding affinities and we demonstrate that the tighter binding is caused by the closure of the N-lobe and C-lobe and the larger size of the gatekeeper and gatekeeper+2 residues. The numbers of ionic interactions and hydrogen bonds around the methylamine of staurosporine also determine the magnitudes of the binding affinities.

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Talk 3

On the Diversity of Physicochemical Environments Experienced by Identical Ligands in Binding Pockets of Unrelated Proteins

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Introduction: Most function prediction methods that identify cognate ligands via binding site analysis, work on the principle of molecular complementarity that is they assume that the ligand has complementary geometrical and physicochemical properties to the binding site and that similar binding sites will bind similar ligands¹. This assumption however does not hold for all protein ligand interactions. Here we present an analytic continuation of our previous work² on the geometrical variation in protein binding pockets and ligands by analysing binding-pocket / ligand-complexes with respect to their electrostatic potential and hydrophobicity environmental score.

Methods: The computer program CleftXplorer was developed to automate the analysis of protein binding pockets, firstly by the description of their geometrical shape² and secondly by the calculation of the protein's physicochemical properties on binding pocket or ligand surfaces. Through calling a number of established software packages and novel implementations, the program is able to calculate electrostatic potentials, hydrophobicity scores, hydrogen bond acceptor and donor and van der Waals potential energies.

Results: The analysis of 100 protein binding sites binding one of nine cognate ligands revealed that protein molecules often require the assistance of neighbouring chemical compounds to overcome physicochemical properties that are unfavourable for the binding of the ligand. Furthermore within ligand sets the physicochemical properties ligands experience when bound to different binding pockets vary enormously in their absolute values. This high variation reflects large energy fluctuations including changes in the sign of the potentials for corresponding atoms in a ligand set. Repulsive electrostatic potentials in particular on aromatic rings were often overridden by attractive aromatic interactions - in particular with π -CH and π - π interactions.

Discussion: The results in this manuscript highlight the complexity and diversity of even simple protein-ligand interactions, created by the interplay of various factors and forces. Nature has evolved in parallel multiple binding solutions for the same ligand, such that the same ligand can bind in one binding site by exploiting electrostatic interactions and in an other via entropic contributions etc. For a protein and its function it does not matter how the ligand binds to its receptor, but that it binds. This complexity will provide a difficult challenge for computational biology, be it for the accurate calculation of binding free energies or the derivation of more empirical approaches.

References:

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Talk 4

Protein-Protein Interactions as Drug Targets: a View from the Binding Site

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Protein-protein interactions (PPIs) are critical for all aspects of cellular function, a role that is further underlined by their involvement in a large number of disease pathways, and thus they represent attractive targets for therapeutic intervention. A number of small molecules have been successful in targeting and disrupting protein-protein interactions. It is observed that these small molecules bind deeper within the contact surface of the target protein, with higher ligand efficiencies[1]. We address the differences between proteins that bind marketed drugs and the proteins that are currently targeted by small molecule protein-protein interaction inhibitors.

We use an energy based cleft detection algorithm (Q-SiteFinder[2]) as a tool to define and characterise the extent of binding site pockets on the protein surface. The aim is to compare and contrast different classes of protein interactions specifically focussing on the properties of occupied pockets i.e. known protein binding sites. We find that in general protein-protein interactions occur in a number of small pockets[3] (54 \AA^3) compared to current marketed drugs, which occur predominantly in a single large pocket[2] (260 \AA^3). PPI inhibitors are shown to occur in relatively small pockets[3] (77 \AA^3) when compared to current marketed drugs, thus they appear to have properties resembling those of protein-protein interactions, whilst lacking some of those relating to typical marketed drugs.

Our results suggest that drug discovery methods that target several small pockets are likely to increase the chances of success in this new field of therapeutics[3].

References:

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- 4 Fuller, J.C., Burgoyne, N.J. and Jackson, R.M. Manuscript submitted.

Talk 5

Entropic Cost of Molecular Association

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The non-covalent association of molecules is of central importance in biology and pharmacology. Methods of predicting the affinity of such associations would be of great practical value in many drug discovery programmes. In this work a new method is presented to calculate the translational and rotational entropy loss on binding. Molecular dynamics simulations of the bound and unbound ligand are performed. The average magnitudes of the forces and torques acting on the ligand along its principal axes are evaluated from which the entropy is then calculated. The difference in entropy of the bound and free states gives the entropy change on binding. Entropic contributions from the protein and solvent are the subject of future work. The systems chosen for study are benzene bound to T4 lysozyme, benzamidine to trypsin, 2-butanone to FKBP, water to barnase and bovine pancreatic trypsin inhibitor and 2-methoxy-3-isopropylpyrazine to MUP. The entropy change for binding in these systems are found to range from 0 to $-12 \text{ J K}^{-1} \text{ mol}^{-1}$ for water at 55.6 M standard concentration and -58 to $-83 \text{ J K}^{-1} \text{ mol}^{-1}$ for the other ligands at 1M standard state. Corresponding components are difficult to obtain by experiment and are only available for the MUP system in which we obtain good agreement.

Talk 6

Haptic Technology in Drug Design: Breaching the Accessibility Barrier

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Structure-based drug design is a creative process that displays several features that make it closer to human reasoning than to machine automation. However, very often the user intervention is limited to the preparation of the input and analysis of the output of a computer simulation. In some cases, allowing human interactive intervention directly in the process could improve the quality of the results by applying the researcher's intuition directly into the simulation. Haptic technology has been previously explored as an useful

method to interact with a chemical system. However, the need for expensive hardware and the lack of accessible software have limited the use of this technology to date.

The release of low-end haptic devices for the computer games market has recently made this technology very accessible. We implemented a haptic-driven molecular mechanics environment based on MMFF94 and Chemscore in an open source molecular modelling software (Zodiac) freely available to the public. In this simulations the user can test his/her hypotheses in real time, while gathering informations about the energy profile of a binding site in an intuitive way, by feeling the total force resulting from the protein-ligand interaction on his/her hand.

The software also supports popular devices such as the Nintendo Wii controller, using its rumble pack and a visual/haptic sensory illusion to provide pseudo-directional haptic feedback.

The affordability of the software/hardware combination makes this technology also extremely suitable for teaching purposes, where a large number of workstations is required and cost is usually a limiting factor.

References:

1. Persson, p. B., Cooper, m. D., Tibell, L. A. E., Ainsworth, S., Ynnerman, A., Jonsson, B. H. Designing and evaluating a haptic system for biomolecular Education. IEEE Virtual Reality Conference 2007, 171 – 178.
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Talk 7

Coarse-Grain Protein Modelling with Hamiltonian Replica Exchange

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Conformational change in a protein can be a key step in its biochemical function. The motion can vary from the subtle rearrangements of flexible loops through to large domain rearrangements as observed in some allosteric effects. Computer simulation by molecular dynamics has the potential for studying in detail the mechanisms of protein conformational change. Unfortunately, the time-scale of many conformational change events is of the order of milli-seconds whereas standard atomic level molecular simulations are performed at the nanosecond time-scale.

The approach of coarse-grained modelling provides a possible way to solve the aforementioned difficulties. By grouping atoms into coarser sites, the number of degrees of freedom required to represent a given system can be significantly reduced, thus decreasing the number of interactions to be evaluated. The long-range electrostatic interactions, which are the most time-consuming part of an atomistic simulation, can also often be collapsed into a short-range interaction at a coarse grain level. However, by their very nature coarse grain methods remove the individual location of atoms and an approach must be devised to map the atoms back rigorously. To accomplish this, Hamiltonian replica exchange may be used, whereby coordinate information is exchanged between levels, using a metropolis Monte Carlo test.

This talk will summarise a range of simple coarse-grain models which have been implemented and discuss the use of a Hamiltonian replica exchange protocol which can combine atomistic and coarse-grain simulations, providing a useful way in which phase space can be sampled efficiently.

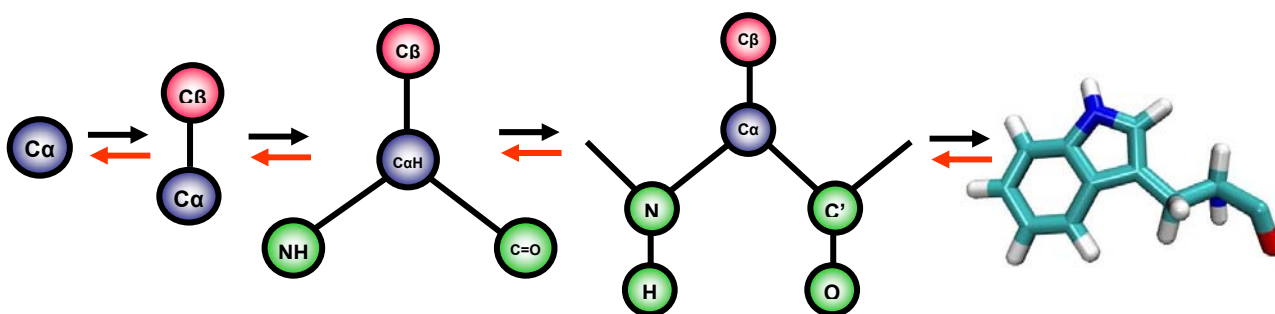


Figure 1: A ladder of coarse-grain models representing an amino acid. At each level the granularity of the model increases.

Talk 8

Classification Models for Aqueous Solubility in the Early Drug Discovery Phase

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Aqueous Solubility is a very important property in drug discovery. In the early phase insoluble compounds give wrong results in HTS, and medium throughput ADME-Tox. Insoluble compounds bought for validation of virtual screening campaigns are not very

useful. Although medium throughput solubility measurement methods are arising, it is impossible to measure hundreds of thousands of compounds in short time. Thus there is a clear need for fast and cheap in-silico solubility determination methods.

Although this has long been known, up to now it still seems impossible to predict aqueous solubility with sufficient accuracy. In a paper from 2007, Mueller et al showed that the best commercially available tools for solubility prediction have an $R^2 \sim 0.3$ on an internal test set of Bayer-Schering.^[1] This may on the one hand be attributed to the inconsistent training data set, i.e. the Huuskonen or PHYSPROP dataset,^[2] and on the other hand to the complexity of the physical nature of solubility.

Here we present the results of Classification models for aqueous solubility on a dataset of 844 druglike compounds measured in the same lab within Boehringer Ingelheim under equal conditions. We show that classification predictions are sufficient to tackle early phase solubility issues. We further show that in contrast to regression models the classification model is very reliable, on the training sets itself as well as on an independent validation set.

Finally a strategy for uniting predictions from several state-of-the art nonlinear classifiers (SVMs, BRANNs, random Forest) and different descriptor sets (Pharmacophore Fingerprints, MOE2D, ParaSurf, VolSurf) by a Meta-Classifer is presented. The Meta-Classifier exhibits significantly higher prediction power in terms of accuracy, ROC-area and probability accuracy.

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Talk 9

Towards High Level QM/MM Calculation of Protein-Ligand Binding Affinities

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In computer-aided drug design, accurately predicting the relative binding affinities of different ligands to a protein is one of the most fundamental and problematic tasks. Simulations employing techniques such as free energy perturbation and thermodynamic

integration offer the possibility of reliable predictions, but are currently limited to empirical molecular mechanics (MM) methods. These have significant limitations, because electrons are not treated explicitly, making it difficult to represent chemically important effects such as electronic polarisation and charge transfer. Combined quantum mechanics/molecular mechanics (QM/MM) methods have the potential to provide a better physical description of biomolecular interactions, but until recently, the large computational requirements of high-level QM/MM methods has prevented their routine use in biomolecular simulations.

We have studied the binding of biotin to the streptavidin protein. This system is interesting because it is one of the strongest non-covalent interactions seen in nature, and yet the origins are still not clearly understood. Ligand polarisation has been proposed to be significant¹, and QM/MM simulations are ideal to study this. Low level (AM1, PM3 and SCC-DFTB) QM/MM simulations show that whilst hydrogen bond residues do individually polarise biotin, the net effect, compared to polarisation in water, is negligible. Higher level calculations are desirable in this work, due to known limitations in these methods. This is now possible, using work by Woods *et al.*², which combines the Warshel free energy cycle^{3,4} and the Metropolis-Hastings algorithm⁵. 50,000 step *ab initio* QM/MM Monte Carlo simulations, on a QM water in bulk MM water, can be run within 2 days, using commodity processors. Work has been carried out to study the accuracy of these simulations, such that the method can be extended to protein systems. Initial findings suggest that TIP3P and TIP5P are not suitable models for use with QM/MM simulations, whilst the application of a simple scaling factor to the charges on TIP4P greatly improves the QM/MM prediction of the relative hydration free energies of water and methane. This work shows that the simulations could provide the desired accuracy-time trade-off. The findings have important implications for future QM/MM protein ligand free energy calculations, and the development of QM/MM methods, with particular emphasis on the better description of QM to MM interactions.

References:

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Talk 10

FapR Protein: Functional Study of a Potential Target for Antibiotics Against Gram Positive Bacteria

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In many infectious Gram+ bacteria, FapR (Fatty acids and phospholipids Regulator) represses almost all the genes involved in the synthesis of fatty acids necessary for the membrane formation [1]. Recently, the crystal structure of the C-Terminal domain has been solved. The complex with the positive effector, malonyl-coenzymeA, and the apo form reveal a conformational change upon binding [2]. On the one hand, a virtual screening was performed on the repressed form in an attempt to find an antagonist. Some of the molecules responded in a binding test. On the other hand, to better understand repression/derepression and discriminate agonists and antagonists, the transition between the holo and apo forms was studied. A specific method based on CPR algorithm was used and gave a plausible transition path. Analysis tools were developed to characterize the transition, the concerted motions and chronology of events. Network of interactions were identified with this method. Nevertheless, the transition appeared to follow a global, relatively simple and flexible mechanism.

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Talk 11

Wavelet Compression of GRID Fields for Similarity Searching and Virtual Screening

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One of the most revealing three-dimensional descriptors available for analysis of small molecules is the *molecular interaction potential* (MIP). Perhaps the most commonly used of these is the *GRID* field (Goodford, 1985), which is comprised of a discrete grid placed over the ligand for which potential interaction energies between the molecule and a *probe group* (e.g. water) are calculated at each vertex. A disadvantage of such a field is its large size and hence the demanding nature of the computations required for any studies which involve comparing two fields, one from each molecule. One way in which this is overcome is to extract features into a linear fingerprint (e.g. GRIND descriptors). However, this results in a loss of information and requires the selection of an appropriate set of parameters. Here we use wavelets to encode the entire field in a holistic manner.

We show that the nonstandard *Daubechies 4-tap wavelet transform* (D4 WT) can be exploited to represent finely sampled GRID maps requiring between 1.1% and 1.5% (to one decimal place) of the storage of the original fields. The efficacy of other wavelets and the fast Fourier transform (FFT) are also examined. The reduced representation can be used in ligand-based similarity searching without significant loss of accuracy compared with using the whole field. Nearly identical search results were observed when searching over sets of CDK2, ESR1 and HIV inhibitors also used by Chen et al (Chen et al., 2006). We also describe the impact of wavelet approximation methods upon the retrieval of active compounds from amongst a set of decoys, and a process for eliminating the method's dependence on generation of a pre-GRID alignment.

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Talk 12

Probing Enzyme Catalysis Using High Pressure Molecular Dynamics

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Enzymes are highly efficient catalysts, achieving rate enhancements of up to 10^{21} , however it is not yet fully understood how they work. The majority of enzyme reactions involve one or more hydrogen transfers, and these transfers can occur by a partial or full quantum mechanical tunneling mechanism. Morphinone reductase (MR) is an enzyme which employs a deep tunneling mechanism in its reductive half-reaction – the hydride transfer from the NADH-C4 atom to the FMN-N5 atom.¹ This mechanism is thought to be assisted by a promoting vibration that decreases the distance between the donor and acceptor atoms. Experimental work using the pressure dependence of kinetic isotope effects as a probe for hydrogen tunneling has suggested an increase in pressure, from 1 bar to 2 kbar, decreases the donor/acceptor distance.² Using pressure is a novel approach for probing hydrogen tunneling in enzymes. This complements the established use of temperature dependence. Pressure shifts the equilibrium of the system towards the tunneling ready configuration. To gain atomistic insight, molecular dynamics simulations were carried out to probe the rate of structural changes in MR as a function of pressure. The protein is shown to be stable across the range of pressures used. Trajectory analysis showed a decrease of 0.19 Å in the average distance between the donor and acceptor atoms, as the pressure is increased from 1 bar to 2 kbar. This is due to the pressure restricting the conformational space in which the nicotinamide can move. This work gives the first atomistic insight into the effect of pressure on the tunneling reaction, through a decrease in the tunneling distance.

References:

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