

MGMS and RSC MMG Young Modellers' Forum 2010

ORAL PRESENTATIONS

Programme of Presentations

9.00 – 9.30	Coffee and Registration
9.30 - 9.40	Welcome and Introduction
9.40 – 10.00	Talk 1 (Paul Ashford)
10.00 – 10.20	Talk 2 (Sabrina Beniken)
10.20 – 10.40	Talk 3 (Andrew Guy)
10.40 – 11.00	Talk 4 (Ilenia Giangreco)
11.00-11.20	Talk 5 (Julie Roy)
11.20-11.40	Talk 6 (Julia Fischer)
11.40-13.30	Lunch and Poster Session
13.30 – 13.50	Talk 7 (Ignasi Buch)
13.50 – 14.10	Talk 8 (Alex Lawrenson)
14.10 – 14.30	Talk 9 (Michèle Schulz)
14.30 – 15.00	Tea
15.00 - 15.20	Talk 10 (Kerensa Houghton)
15.20 – 15.40	Talk 11 (Hui-Chung Tai)
15.40 – 16.00	Talk 12 (Waldemar Striker)
16.00	Fun event
16.30	Judges Deliberations
16.45	Prize Presentations
17.00	End

Talk 1

A method for visualising transient binding sites on protein surfaces

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In order to bind a drug with suitable specificity and affinity, a protein requires a suitably sized and shaped binding pocket. Given a 3D protein structure from the Protein Data Bank (PDB) various tools exist that predict putative binding pockets using geometric methods that systematically scan the surface for cavities, such as PASS¹ or LIGSITE².

However, proteins in solution are dynamic entities that explore conformational space over time due to rearrangement of structural features, such as side chain motion, local backbone flexibility and larger domain shifts. It follows that pocket prediction based on static structures may not give the complete picture – some pockets may change shape or open and close over time – i.e. they are transient.³ A protein's conformational space can be examined using different approaches: For example, existing software tools allow Essential Dynamics (ED) simulations of a protein generating a set of feasible conformations based on geometric constraints; conformational ensembles can also be provided by solution NMR experiments. Alternative evidence is provided by analysing multiple structures of the same protein from the PDB, which may have different conformations due to variations in ligands or solvent conditions. Finally, members of a homologous superfamily can provide a set of functionally conserved structural variants.

We present a method that visualises surface patches of proteins that show evidence of transient binding pockets. It is based on meta-analysis of existing geometric pocket prediction algorithms applied to any of the protein data sets highlighted above. The output is mapped to a single structure as a residue-level probability score that represents the likelihood of being part of a predicted binding pocket. This is then visualised with suitable software and filtered to indicate transient regions.

Results of particular interest are where the bound and unbound forms of the protein are markedly different due to conformational changes in the binding site; this is particularly relevant to known inhibitors of protein-protein interactions such as interleukin-2 (IL2) and its receptor; we show how the method highlights transient residues from unbound structures based on analyses of a set of homologues or related ED simulations. Other systems studied include simulated variations of α -1-antitrypsin, solution NMR ensembles of ubiquitin and analysis of heterogeneity across 72 structures of HIV Reverse Transcriptase compared with ED simulated conformational variation.

References:

- [1] Brady, G. P., Jr. & Stouten, P. F. (2000). Fast prediction and visualization of protein binding pockets with PASS. *J Comput Aided Mol Des* 14(4): 383-401.
- [2] Huang, B. & Schroeder, M. (2006). LIGSITEcsc: predicting ligand binding sites using the Connolly surface and degree of conservation. *BMC Struct Biol* 6: 19.
- [3] Eyrisch, S. & Helms, V. (2007). Transient pockets on protein surfaces involved in protein-protein interaction. *J Med Chem* 50(15): 3457-3464.

Talk 2

Multiscale modelling of an artificial self-replicating system

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Self-replicating systems are defined as chemical systems which are able to template and catalyze their own synthesis. Self-replicators are of central interest for biologists as they can shed light on the study of pre-requisite for life on Earth and evolution. For chemists they represent the ultimate synthetic machine for nanoscale architecture. This project is about a new minimal self-replicator in which reactants A and B undergo a Diels-Alder reaction to form the autocatalytic product species C. The goal of this work is to support the experimental analysis and to provide a detailed insight into the system which may be used to design improved self-replicators. In the past self-replicating systems have been studied using static methods, no dynamical studies have been performed. This work is a new approach to study the dynamics of self-replication by modeling the system using a multiscale approach based on first principles. Car-Parrinello molecular dynamics (CP-MD) simulations of the constituent species A, B, and C as well as of the hydrogen-bonded complexes they form have been carried out. From the CP-MD trajectories dynamically averaged *ab initio* NMR chemical shifts have been calculated which have been used to assign experimental NMR spectra [1]. However, *ab initio* molecular dynamics are computationally demanding. Therefore, a classical force field that is able to reproduce the first principles dynamical data has been developed and applied to the study of the association and dissociation of A, B, and C in a chloroform solution

References:

1 A. Dieckmann, S. Beniken, C. Lorenz, N. Doltsinis and G. von Kiedrowski, *Journal of Systems Chemistry* 2010, **1**:10.

Talk 3

Translocation of biopolymers through protein nanopores: mechanistic insights from MD simulations.

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The translocation of biopolymers through nanopores is a ubiquitous process in biology. In recent years, inspired by biology, some of these processes have been manipulated for applications in bionanotechnology, e.g. as stochastic sensors and potential DNA sequencing devices¹. To date research into such protein nanopores has largely focused on α -hemolysin (α HL), a transmembrane exotoxin from *S. aureus*. In the present study, we have developed simplified models of the wildtype α HL pore and its mutants, in order to study the translocation dynamics of DNA and peptides under the influence of an applied electric field.

We show that interactions between rings of cationic amino acids and DNA backbone phosphates result in meta-stable tethering of nucleic acid molecules within the pore, leading us to propose a “binding and sliding” mechanism for translocation. We also observe folding of DNA into non-linear conformational intermediates during passage through the confined nanopore environment, helping to rationalize experimentally determined trends² in residual current and translocation efficiency for α HL and its mutants. Finally, we explore the translocation of peptides (both helical and extended) through our model pores as a model of protein transport.

References:

1. Branton et al. The potential and challenges of nanopore sequencing. *Nat. Biotechnol.* (2008) **26** (10), 1146-1153
2. Maglia, G., Restrepo, M.R., Mikhailova, E., Bayley, H. Enhanced translocation of single DNA molecules through α -hemolysin nanopores by manipulation of internal charge. *Proc. Nat. Acad. Sci.* (2008) **105** (50), 19720-19725

Talk 4

The use of Voronoi protein-ligand contact statistics in structure-based design

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Structure-based design methods rely heavily on a good understanding and representation of protein-ligand interactions. These interactions can be described in various ways, including molecular mechanics force fields, and empirical and knowledge-based scoring functions. Here, we will describe a knowledge-based method for scoring protein-ligand interactions based on contact statistics derived from protein-ligand complexes in the Protein Data Bank (PDB). The contacts are defined from a Voronoi analysis [1] of each protein-ligand complex in the PDB. The advantage of using the Voronoi approach is that in theory only the key contacts are found, and also that a contact area is defined for each protein-ligand contact. We have made a particular effort to ensure that the atom type assignments are as accurate as possible. Contact propensities were derived for each combination of ligand and protein atom types. Next, we will describe how we used the derived Voronoi contact statistics to filter protein-ligand docking results for fragments and drug-like ligands. Finally we will illustrate other potential applications of these data in structure-based design.

References:

[1] McConkey, B. J., Sobolev, V. and Edelman M. Quantification of protein surfaces, volumes and atom-atom contacts using a constrained Voronoi procedure, *Bioinformatics* (2002) **18**, 1365-1373.

Talk 5

Applications of Molecular Modelling (MD) studies and Quartz Crystal Microbalance (QCM) experiments to study the ligand recognition by the major urinary protein (MUP).

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The major urinary protein (MUP) is a member of the lipocalin family that binds small ligands in a deeply buried hydrophobic pocket. Detailed calorimetric studies have shown that ligand binding is driven by enthalpic effects, not the entropic effects that are commonly associated with hydrophobic association [1]. Previous studies have shown that this is due to ‘dewetting’ of the binding site cavity even in the absence of ligands, and have also characterised the complex dynamical changes that accompany ligand binding-features that may be correlated with NMR data[2]. A recent long-timescale MD study [3] has revealed that for wild type MUP water molecules are removed instantly from the binding site instantly except for a small volume with a normal water density around the hydroxyl group of tyrosine 120 (see figure 1). The paper has also shown where certain regions of MUP become more flexible whilst others remain rigid upon ligand binding.

In our current MD studies and QCM experiments we have used 2 mutants of MUP with altering hydrophobicity of the binding site (i.e. a more hydrophobic mutant where a tyrosine is replaced by a phenylalanine (Y120F) and a more hydrophilic mutant where an alanine is replaced by a serine (A103S)). For all systems the hydration of the binding cavity upon ligand binding has been analysed. Simulations predict that the Y120F mutant has no water molecules in the binding site before or after ligand binding. On the other hand the A103s mutant has 5 water molecules in the site which reduces to 3 upon ligand binding. These predictions are in excellent agreement with subsequent QCM experiments.

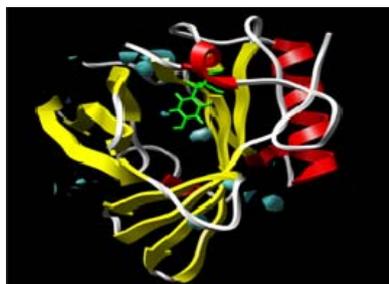


Figure 1: Binding cavity of wild type MUP with 1/3 of a water molecule tightly bound to tyrosine 120

References:

- [1] Bingham, R., Findlay, J. B. C., Hsieh, S., Kjellberg, A., Perazzolo, C., Phillips, S., Kothandaraman, S., Trinh, C.H., Turnbull, W.B., Bodenhausen, G., Homans, S.W. (2004) *J. AM. Chem. Soc.*, **126**: 1675-1681
- [2] Barratt, E., Bronowska, A., Vondrasek, J., Cerny, J., Bingham, R., R., Phillips, S., Homans, S. W. (2006) *J. Mol., Biol.*, **362**: 994-1003
- [3] Roy, J., Laughton, C.A., (2010) *Biophys. J.*, **99**: 218-226.

Talk 6

Organic enzyme cofactors: on their properties, intrinsic groupings and conformational variability

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Motivation: Many crucial biochemical reactions in the cell require not only enzymes for catalysis but also organic cofactors or metal ions. We investigate the properties, structures and conformational variability of organic enzyme cofactors and discuss their contribution to biocatalysis and implications for conformation-function relationships.

Methods: We use the CoFactor database as an underlying dataset and perform clusterings and principal component analyses to elucidate properties and intrinsic groupings among the cofactors and compare those to other metabolites in the cell. We further apply an RMSD analysis to compare the conformational variability of protein-bound cofactors to the theoretically possible variability using computer-generated conformations.

Results and Discussion: We show that, as expected, organic cofactors are on average significantly more polar and slightly larger than other metabolites in the cell, yet they cover the full spectrum of physicochemical properties found in the metabolome. Furthermore, we have identified intrinsic groupings among the cofactors, based on their molecular properties, structures and functions, which represents a new way of considering cofactors. Although some classes of cofactors, as defined by their physicochemical properties, exhibit clear structural communalities, cofactors with similar structures can have diverse functional and physicochemical profiles. Finally, we show that the molecular functions of the cofactors may duplicate reactions performed by inorganic metal cofactors and amino acids, the cell's other catalytic tools, but also provide novel chemistries for catalysis. We will also present our latest results on the conformational variability of cofactors.

References:

Fischer, J.D., Holliday, G.L. and Thornton, J.M. The CoFactor database: organic cofactors in enzyme catalysis, *Bioinformatics*. (2010). Epub ahead of print.

Fischer, J.D., Holliday, G.L., Rahman, S.A. and Thornton, J.M. The structures and physicochemical properties of organic cofactors in biocatalysis, *J. Mol. Biol.* (2010). In press.

Talk 7

Energetics, kinetics and binding pathway reconstruction for enzyme-inhibitor complex from high-throughput molecular dynamics simulations

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Recent advances of molecular simulation software on graphical processing units (GPUs) have made possible to perform high-throughput simulations of free molecular association between a protein and a ligand and fully describe the binding process.

We have performed hundreds of 100 ns long all-atom explicit-solvent MD simulations of free inhibitor binding to Trypsin obtaining around 30% of events of binding determined by a final RMSD less than 2 Å from the crystal structure. All simulations are performed using ACEMD [Harvey2009] (<http://multiscalelab.org/acemd>) on GPUGRID [Buch2010] (<http://gpugrid.net>) a worldwide distributed computing project. A total aggregate of around 13 μs of simulation data is used to build a Markov state model [Noé2008] representation of the binding of Benzamidine from which to obtain the standard free energy of binding and kinetic constants of association. Moreover, we have also characterised the binding pathway of Benzamidine which also allows us to identify those residues of Trypsin that participate in the overall process of recognition and binding.

In summary, we show the ability to describe the entire enzyme-inhibitor binding process as well as providing detailed quantitative information at the atomistic level. These results suggest that the approach may be ready to be applied in studying similar systems within the context of rational drug design.

References:

- Harvey M.J., G. Giupponi and G. De Fabritiis, ACEMD: Accelerated molecular dynamics simulations in the microseconds timescale, *J. Chem. Theory and Comput.* (2009) 5, 1632
- Buch I., M. J. Harvey, T. Giorgino, D. P. Anderson and G. De Fabritiis, High-throughput all-atom molecular dynamics simulations using distributed computing, *J. Chem. Inf. and Mod.* (2010) 50, 397
- Noé F. and S. Fischer, Transition networks for modeling the kinetics of conformational change in macromolecules. *Curr. Op. Struct. Biol.* (2008), 18, 154-162

Talk 8

Virtual Screening to Identify Novel Antimalarial Chemotypes

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Approximately 40% of the world's population are exposed to the risk of malaria, resulting in around one million deaths annually. Previous successes in attempting to eradicate the disease were only short lived due to increased resistance of the mosquito to insecticides, and of the parasite to established drugs such as chloroquine. Computer aided-drug design is an essential part of modern drug discovery, not least in the field of antimalarial chemotherapy.

Our research has been looking at the cytochrome bc₁ complex, which has been confirmed as an antimalarial target through the study of drugs such as floxacrine. We have used a range of virtual screening techniques to identify potential lead-like structures which are active against this target, based on a number of in-house compounds whose activities had previously been described. Techniques used included Fingerprint Similarity searching, TurboSimilarity searching, Substructure searching, Principle Component Analysis, Bayesian Classification and Decision Tree models. These methods were used to screen the Zinc lead-like library of compounds, with hits being scored based on factors such as physicochemical properties, and the number of methods by which they were identified.

Filters were applied to remove compounds which contained structural motifs that may produce unwanted biological interactions (toxicophores). The molecules spanned a wide range of chemical space so a diverse selection of compounds was chosen using clustering methods. Potentially interesting hits were purchased, and biological testing is currently ongoing against a number of assays. The results from this testing will be used to inform and refine the next phase of the molecular design loop.

Molecule docking has allowed us to improve our understanding of how these compounds elicit a response. Docking was performed on a PDB file containing the crystal structure of bovine bc₁, with Stigmatellin (a known bc₁ inhibitor) present in the active site. Using GOLD, the native ligand was removed and then docked back into the active to optimise our docking parameters. When the crystallographic water molecule was present in the active site, the docking solutions noticeably improved, and a number of interesting interactions were observed. The purchased compounds from virtual screening have since been docked into the active site, allowing us to identify important interactions, and to see whether there is a relationship between binding and activity.

Talk 9

Generation of a fragment library that maximally represents a compound collection

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In any screening process, the quality of the library dictates the quality of the hits that are found. For fragment screening ¹, there are additional constraints placed by the high concentrations required in screening and the need for appropriate synthetic routes to evolve the fragment into lead compounds. Most published fragment libraries are constructed using chemoinformatics pipelines that take available compounds and identify a representative subset that exclude reactive or toxic molecules and assess solubility and chemical diversity. The final step is usually a rather subjective review by medicinal chemists for synthetic tractability, to ensure any fragment hits can be evolved.

An alternative method for initial evolution of fragment hits is the so-called, SAR by catalogue approach, where available compounds are assayed that contain similar sub-structures to the fragment hits. This has been successful in rapidly establishing SAR for a hit series. This is of particular interest to academic groups as it provides access to potential hits without the need for synthesis.

The aim of this project is to devise and implement procedures to generate and characterise a fragment library designed for such SAR by catalogue. The library should contain fragments that maximally represent the features of compounds that can be purchased from available chemical databases. Five initial schemes were developed (implemented in Pipeline Pilot Student Edition ²) together with automated evaluation procedures to consider the quality of the libraries produced.

Here, the methods and our preliminary results from assembling and testing the library in screening against a range of protein targets will be described.

References:

- 1.) Schulz M. N., Hubbard R.E., Recent Progress in Fragment Based Ligand Discovery, *Curr Opin Pharmacol* (2009) **9(5)**:615-21
- 2.) Accelrys Software Inc., Pipeline Pilot Student Edition, *SciTegic, San Diego, USA* (2007)

Talk 10

QM/MM modelling of the hydroxylation of diclofenac by human cytochrome P450 enzymes

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Accurate prediction of drug metabolism is immensely important in pharmaceutical research. Knowledge of drug metabolites helps predict the ADME-Tox properties of new drugs. Additionally, insight into the mechanisms involved in enzyme catalysed chemical transformations is essential for the discovery of pro-drugs. Cytochromes P450 (CYP) are ubiquitous, haem-containing enzymes which catalyse oxygen insertion reactions by activation of molecular oxygen. Since P450 enzymes are involved in over 90% of known drug oxidations, they are of considerable interest when studying drug metabolism.

The oxidation of diclofenac by P450 enzymes provides a well-characterised test-case to investigate the ability of mixed quantum mechanical/ molecular mechanical (QM/MM) methods [1] to predict the metabolism of new drugs. Diclofenac is a non-steroidal anti-inflammatory drug (NSAID) widely prescribed for musculo-skeletal disorders such as rheumatoid arthritis. A number of P450 isozymes are involved in the metabolic clearance of diclofenac: CYP 2C9 hydroxylates diclofenac exclusively at the 4- position, CYP 3A4 catalyzes production of the minor metabolite 5-hydroxy-diclofenac, and CYPs 2C8, 2C18 and 2C19 produce varying ratios of both 4- and 5-hydroxy-diclofenac. Understanding the origins of the selectivity observed in the hydroxylation of diclofenac by different P450 isozymes is also important because diclofenac can be hepatotoxic and this may be related to the formation of 5-hydroxy-diclofenac as a metabolite.

Molecular dynamics (MD) simulations of diclofenac in the active site of CYP 2C9 have been performed. This has provided a basis for modelling the energy barrier of hydroxylation using density functional theory based QM/MM methods. The results provide detailed insight into the active binding modes of diclofenac in CYP2C9. The calculated reaction energy barriers compare well with experiment [2] and MD simulations have increased our understanding of the mobility and conformational behaviour of diclofenac in the CYP 2C9 active site.

References:

- [1] Bathelt C. M., Riddler L., Mulholland A. J., Harvey J. N., Mechanism and structure-reactivity relationships for aromatic hydroxylation by cytochrome P450. *Org. Biomol. Chem.* **2**, 2998-3005 (2004)
- [2] Ridderström M., Masimirembwa C., Trump-Kallmeyer S., Ahlefeldt M., Otterb C. and Andersson T. B., Arginines 97 and 108 in CYP2C9 Are Important Determinants of the Catalytic Function. *Biochem. Biophys. Res. Commun.* **270**, **3**, 983 (2000)

Talk 11

Photochemistry and DNA Interactions of Platinum Anticancer Agents: DFT/LFMM Studies

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Pt(IV) complexes are stable, inert pro-drugs which can be photoactivated to produce Pt(II) species which have promising anti-cancer activity. We show that TDDFT can be used as a tool to help tune the coordination environment to achieve desirable photochemical properties and further illuminate the mechanism of photoactivation. Exploration of *trans* influences, the nature of the axial and reducing ligands, and electron-withdrawing/donating substituents shows that the design of clinically-preferred platinum(IV) complexes that exhibit greater absorption at longer wavelengths can be achieved. Absorption maxima can be red-shifted by up to 32 nm, and moreover electron-withdrawing substituents on pyridine give rise to a "tail" in the absorbance in the 625–858 nm region.

Additionally, we have determined a set of new ligand field molecular mechanics (LFMM) parameters, which take into account d-electron effects, and carried out ligand field molecular mechanics dynamics (LFMD) simulations of the bifunctional binding of cisplatin and the major photoproduct of a novel platinum(IV) complex *trans,trans,trans*-[Pt(N₃)₂(OH)₂(py)₂] (1) to two nucleobases in DNA using the novel parameters. In the field of platinum-based drug design, DNA has most commonly served as the major target by inhibition of DNA replication and transcription. Formation of various cisplatin adducts greatly alters the structure of the target DNA in distinctive manners, which may activate different signal transduction pathways. Complementary to experimental work, molecular modelling is used to study the DNA distortions which are important to comprehend the mechanism of action of the drugs. We have shown that not only does LFMM yield bond distances in good agreement with the experimental and QM/MM structures, but LFMD can also accurately reproduce the experimentally observed structures of intrastrand and interstrand platinum–DNA complexes. Unlike cisplatin, the major photoproduct of complex 1 compresses DNA, which may affect nucleosome dynamics by binding and shortening linker DNA of chromatin as experiments have revealed that larger platinum compounds generally display a greater tendency to target the linker region of the nucleosomal DNA and have less access to nucleosomal core DNA.

References:

- (1) Farrer, N. J.; Woods, J. A.; Salassa, L.; Zhao, Y.; Robinson, K. S.; Clarkson, G.; Mackay, F. S.; Sadler, P. J. *Angew. Chem. Int. Ed.* **2010**, *49*, 1-5
- (2) Deeth, R. J.; Anastasi, A.; Diedrich, C.; Randell, K. *Coord. Chem. Rev.* **2009**, *253*, 795-816.

Talk 12

Structure-based virtual screening and validation of UGP inhibitors

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Introduction: The protozoan parasite *Trypanosoma brucei* is the causative agent of human African sleeping sickness and the cattle disease Nagana. The enzyme uridine diphosphate glucose pyrophosphorylase (UGP) is a genetically validated drug target for *T. brucei* (*Tb*) but no inhibitors are known so far¹. Therefore, we set out to discover inhibitors by performing a structure-based virtual screening followed by nuclear magnetic resonance (NMR), differential scanning fluorimetry (DSF), surface plasmon resonance (SPR), and coupled inhibition assay for hit validation.

Results: A virtual screening was performed using our in-house database of 5.2 million commercially available compounds. The molecules were docked into the binding pocket of *Tb*UGP using DOCK 3.5.54. Based on the estimated ligand efficiency and visual inspection of the docked hits, fifteen compounds were purchased and tested for binding with DSF, NMR and a coupled inhibition assay. From these screens one fragment-like compound was identified with an IC₅₀ of 36.7±5.9 µM and a hillslope of 0.8.

Discussion and future work: We have identified the first inhibitor for *Tb*UGP which can serve as a starting point to create more potent inhibitors. The next steps will be to test analogues of the inhibitor to derive structure-activity relationships and to establish suitable crystallisation conditions.

References:

- 1 Marino, K. *et al.* Identification, subcellular localization, biochemical properties and high-resolution crystal structure of *Trypanosoma brucei* UDP-glucose pyrophosphorylase. *Glycobiology*, doi:cwq115 [pii] 10.1093/glycob/cwq115 (2010).