



MGMS and RSC MMG Young Modellers' Forum 2009

POSTER PRESENTATIONS

Poster 1

Modelling the Hydroxylation of Diclofenac in Human Cytochrome P450 Enzymes

Kerensa Houghton, Christine Bathelt, Richard Lonsdale, Jeremy Harvey and *Adrian J. Mulholland*

*Centre for Computational Chemistry, School of Chemistry, University of Bristol,
Cantock's Close, Bristol, BS8 1TS*

Cytochromes P450 (CYP) are ubiquitous, haem-containing enzymes which catalyse oxygen insertion reactions by activation of molecular oxygen. Since P450 isozymes are involved in over 90% of known drug oxidations, they are of considerable interest when studying drug metabolism. P450 enzymes are often capable of binding to many different substrates due to a large degree of flexibility in their active sites. If two or more co-administered drugs are substrates of the same isozyme, the rate of metabolic clearance of at least one of the drugs is likely to be affected. This can lead to adverse drug reactions.

The hydroxylation of diclofenac by P450 enzymes provides a well characterised test case to investigate the ability of combined quantum mechanical/ molecular mechanical (QM/MM) techniques [1] to predict the metabolism and adverse drug reactions of new drugs. Diclofenac is a nonsteroidal antiinflammatory drug (NSAID) widely used as an analgesic and antipyretic as well as to treat inflammatory conditions 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 [2]. 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 [3]. Molecular dynamics 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 reaction process as well as the mobility and conformational behaviour of diclofenac in this enzyme active site.

References:

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Poster 2

***In Silico* Design of Potential HIF-1 α : p300 Interaction Inhibitors**

Tigran Abramyan, Jarmila Husby, Shozeb Haider, Giovanna Zinzalla and David E. Thurston

School of Pharmacy, University of London

The hypoxia inducible factor 1 (HIF-1) is a heterodimeric transcription factor that plays a key role in development and growing tumor's adaptation to hypoxia. This process is mediated by activation of hypoxia responsive genes by the dimeric HIF-1 in complex with the CBP and p300 transcriptional coactivators. Inhibition of HIF-1 activity significantly decreases tumor growth, vascularization, and energy metabolism. HIF-1 is then considered as suitable molecular target for anticancer drug discovery, as its function can be potentially specifically inhibited by blocking the protein-protein interaction between Carboxy-Terminal Activation Domain (CTAD) of HIF-1 α and p300/CBP Cysteine/Histidine-rich domain (CH1).

Both structure-based and fragment-based approaches and *in silico* screening methods were utilized to design small molecules that might show a promise as potential inhibitors of the HIF-1 α :p300 interaction. Upon identification, these molecules were docked into the protein target, CH1 p300, using DOCK6. Key interactions between the docked molecules and the macromolecular target were determined and further analyzed by 3D-pharmacophores.

CITED2 is an ubiquitously expressed nuclear protein that competes with HIF-1 α for binding to the CH1 p300/CBP. A comparison study was simultaneously carried out with CITED2:p300 CH1 protein complex to provide further insight into the search for a potent potential inhibitor of HIF-1 α :p300 complex formation.

Poster 3

QSARs for a Series of Aminoquinoline Compounds against Chloroquine Sensitive and Resistant Strains of the Malaria Parasite, Along with Hit Expansion Methods to Find Potential Lead-Like Compounds

Alexandre S. Lawrenson and *Neil G. Berry*

Robert Robinson Laboratories, Department of Chemistry, University of Liverpool, Liverpool, L69 7ZD, UK

Malaria is responsible for roughly one million deaths annually, and with increased resistance of the parasite to established drugs such as chloroquine, continued efforts in this area are essential.

Quantitative structure-activity relationships (QSAR) can be used to correlate structural or physicochemical properties of molecules (through the use of molecular descriptors) with a measured property, such as biological activity.¹

In our study we used QSAR techniques to develop statistically significant models for a series of aminoquinoline compounds. The compounds had been tested against both a chloroquine sensitive and resistant strain of the *P.falciparum* malaria parasite. Models were developed using a host of machine learning techniques² (MLR, PLS, *k*NN) to produce linear relationships for both strains of the parasite.

Several molecular descriptors were found to be of particular importance within the models for each strain. Comparisons were made in an attempt to interpret the molecular descriptors with reference to a previously proposed mechanistic model. The hydrophilic factor was found to be crucial in explaining the difference in activity of the two strains, supporting previous findings in the literature which discuss possible chloroquine resistance mechanisms.

Our current work involves using a variety of hit expansion methods such as TurboSimilarity searching, Bayesian classification and PCA models to screen the Zinc lead-like library. From this we hope to identify potential lead-like structures which are active against a novel antimalarial target, feeding the results into synthetic efforts to ultimately refine the molecular design loop.

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Poster 4

Catalytic Activity of Fatty Acid Amide Hydrolase: Non-Empirical Analysis of Differential Transition State Stabilization

Ewa Chudyk^{1,2}, Edyta Dyguda-Kazimierowicz¹, Karol M. Langner¹, W. Andrzej Sokalski¹, Jitnapa Sirirak² and Adrian J. Mulholland²

¹*Institute of Physical & Theoretical Chemistry, Wrocław University of Technology, Wyb. Wyspińskiego 27, 50-370 Wrocław, Poland,*

²*Centre of Computational Chemistry, School of Chemistry, University of Bristol, Bristol BS8 1TS, UK*

Fatty acid amide hydrolase (FAAH), an enzyme discovered during research on the influence of marijuana on the human brain, deactivates neurotransmitters responsible for numerous symptoms of nervous system diseases. One of its substrates is oleamide, which induces sleep, antianxiety effects, inflammatory and pain states, among others. Such a crucial role in living organisms requires more detailed knowledge of the molecular basis of FAAH-catalyzed oleamide hydrolysis in order to rationally control the latter.

In this contribution we analyzed the factors determining FAAH catalytic activity within the framework of Differential Transition State Stabilization (DTSS) theory. Following the DTSS approach, stronger binding of an enzyme with the transition state relative to the reactants results in a lower activation energy barrier and in overall reaction rate increase. We further partitioned the differential transition state stabilization energy according to a variation-perturbation scheme into the electrostatic, exchange, delocalization and correlation contributions. Wherever electrostatic effects are dominant, these results can be generalized in the form of catalytic fields [1].

DTSS analysis of the first step of the FAAH-catalyzed oleamide hydrolysis was performed based on the mechanism proposed in Ref. 2. In addition to the reactive conformation of an enzyme-substrate complex, we also considered three conformations described there as unreactive. Remarkably, the height of the reactive reaction barrier coincides with the amount of DTSS energy calculated herein. These results along with the catalytic contribution of particular FAAH residues are discussed.

Thus, both the catalytically important active site residues and physical nature of preferential transition state binding are determined, providing detailed insight into this enzyme's catalytic mechanism. This is especially useful when planning mutagenesis experiments and/or for rational inhibitor design. Finally, approximate, yet well-founded models can be constructed to predict the influence of mutations on enzymatic activity.

References:

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Poster 5

RNA- Small Molecules Complexes Database

Irena Sani¹ and *Andrew C.R. Martin*²

¹*Department of Crystallography, Birkbeck College, Malet Street, London WC1E 7HX*

²*Biomolecular Structure & Modelling Unit, University College London, Gower Street, London, WC1E 6BT*

RNA molecules, like proteins, bind to a large number of ligands with high selectivity and affinity, rendering RNA as an attractive target for drug design. The aim is to develop a functional database for RNA - small molecules interactions and to study relationship between RNA and ligands.

The motivation to create an automated RNA-ligands database is due to the dramatic growing of RNA – ligands structures, especially in the last few years. In this project, we plan to create a web source, database driven to describe the most important features of RNA- ligands complexes. We employ bioinformatics approaches to characterize properties of these interactions. RNAView program annotates secondary and tertiary RNA structure elements while LIGPLOT calculates hydrogen and hydrophobic interactions. As of May 2009, in PDB database we collected 802 RNA – ligands complexes where 82 of them represented NMR structures and the rest of 720 X- RAY structures.

The RNA-ligands complexes, web - based database will contribute for further understanding of molecular recognition mechanism of RNA - small molecules interactions.

Poster 6

Large Scale Molecular Simulations using Brownian Dynamics with Boundary Element Method Electrostatics.

David Fallaize^{1,4}, Prof. John E. Ladbury² and Dr. Mark A. Williams^{3,4}

¹ *Centre for Maths and Physics in the Life Sciences and Experimental Biology (CoMPLEX), University College London*

² *Department of Biochemistry & Molecular Biology, University of Texas M.D. Anderson Cancer Center, Houston*

³ *Institute of Structural and Molecular Biology, School of Crystallography, Birkbeck, University of London*

⁴ *Research Department of Structural and Molecular Biology, University College London*

When people think of molecular simulations they commonly think of Molecular Dynamics (MD). Unfortunately MD is limited in both time and length scales to a level several orders of magnitude lower than that required to simulate realistically complex biological systems, for example the early events in the T-Cell Receptor signalling pathway. In order to simulate such a system it is necessary to use a less detailed model of the individual proteins, and in particular a simplified representation of the solvent molecules, compared to the all-atom approach of MD.

Brownian Dynamics operates on a scale beyond MD where proteins are replaced by diffusing rigid bodies and the solvent is represented by a uniform dielectric continuum. A particularly challenging aspect of implementing a Brownian Dynamics simulator is the accurate modelling of electrostatic interactions between proteins, which requires solving the Poisson-Boltzmann Equation.

This poster describes the practical implementation of a Brownian Dynamics simulator using Boundary Element Method (BEM) electrostatics, which we hope to use for large scale simulations.

Poster 7

Molecular Dynamics Using Cooperative Replicas: Folding of Trp-cage Mini-protein

Neil J. Bruce and *Richard A. Bryce*

School of Pharmacy and Pharmaceutical Science, University of Manchester, Oxford Road, Manchester, M13 9PT, UK.

All-atom molecular dynamics simulations of biological systems offer the potential to provide insights into many interesting biological processes, such as protein folding and large scale conformational change. Unfortunately, this potential is hampered by the limitations of current computing power and the simulation timescales required to study many dynamic biomolecular processes.

The limiting factors preventing the simulation of many systems of interest are the number of force evaluations required per simulation time step, and the number of steps required for the simulation to traverse the conformational space of the system. The first factor can be addressed by using a simplified model of the system. However the loss of all-atom resolution, may lead to erroneous behaviour in some systems. The latter factor can be countered by smoothing the potential energy surface of the system, preventing the simulation from becoming trapped in high energy minima.

A number of techniques exist that seek to smooth the energy surface of the system: a protocol, developed by Huber and van Gunsteren and named SWARM-MD,¹ smoothes the potential energy surface of the system through the use of a swarm of multiple interacting simulation replicas, that are driven towards the average of the swarm members. For the first time, we apply this approach to the simulation of biomolecular systems, evaluating its performance in modelling the folding of model peptides, including Trp-cage miniprotein in aqueous solvent. Implications and future directions will also be discussed.

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Poster 8

Molecular Docking and QSAR of Aplyronine A and Analogues: Potent Inhibitors of Actin

Abrar Hussain and Jonathan D. Hirst

School of Chemistry, University of Nottingham, University Park, Nottingham NG7 2RD

The actin cytoskeleton is essential in the development of all cells. However, it is also central to the growth and progression of cancers, thus making it a strategic target for the design and development of novel cancer therapeutic agents. The recent discovery of a series of highly specific, marine macrolides that are able to disrupt actin filament dynamics are considered as a potential basis for the design of such therapeutic agents.¹ Using AutoDock, we performed flexible docking simulations on analogues of the actin-binding macrolide, aplyronine A,² to gather insight into the binding modes and strength of binding of each analogue. In addition, we also carried out a 3D-QSAR study using our own implementation of the CoMSIA method. Our findings show the macrolide ‘tail’ to be fundamental for the depolymerisation effect of actin-binding macrolides and that it is the tail which forms the initial interaction with actin rather than the macrocycle, as previously believed. Docking energy scores for the compounds were highly correlated with actin depolymerisation activity. The 3D-QSAR models were predictive, with the best model giving a q^2 value of 0.85 and a r^2 of 0.94. Results from the docking simulations and the interpretation from QSAR “coeff*stdev” contour maps provide insight into the binding mechanism of each analogue and highlight key features that influence depolymerisation activity. Our results may aid the design of a putative set of analogues that can help produce efficacious and tolerable anti-tumour agents. Finally, using the best QSAR model, we have also made genuine predictions for an independent set of recently reported aplyronine analogues.³

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Poster 9

Simulation Studies of the Binding Properties of an Unusual Pleckstrin Homology Domain.

C.N. Lumb, P.J. Stansfeld and *M.S.P. Sansom*

Department of Biochemistry, University of Oxford, OX1 3QU

Although phosphoinositides make up only around 1% of the lipids in the cytoplasmic leaflet of the cell membrane they are of critical importance in many cell signalling pathways [1]. The precise pattern of phosphorylation on the inositol ring of the lipid headgroup, coupled with the variable negative charge, allows different phosphoinositides to recruit specific signalling proteins to the surface of the membrane. These signalling proteins often possess a well-defined phosphoinositide binding domain that allows them to dock effectively with a particular member of the phosphoinositide family, and one such recognition module is the pleckstrin homology (PH) domain [2]. Experimental studies have determined that the PH domain of Grp1, a guanine nucleotide exchange factor, exhibits pronounced specificity for its target phosphoinositide PI(3,4,5)P₃ and binds reversibly with high affinity, but the nature and extent of its interaction with the rest of the membrane remain unclear. We examine the membrane-associated states of Grp1-PH using atomistic molecular dynamics simulations to characterise the interaction of the protein with both the ligand and the membrane lipids. To probe the subsequent dissociation of the domain from the membrane we employ steered molecular dynamics simulations to 'pull' the protein away from the surface. We find that, in agreement with experimental results, protonation of a key histidine residue close to the binding site reinforces the interaction with the membrane. Grp1-PH also possesses an additional β -hairpin insertion that is not part of the canonical PH domain fold, and our simulations suggest that this unusual structural feature of the Grp1-PH domain may enhance the binding affinity of the domain *via* a secondary interaction with the lipid bilayer.

The authors are extremely grateful to Professor Tatiana Kutateladze and her colleagues at the University of Colorado, Denver for sharing their experimental data.

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Poster 10

The Sugar Shovel: The Role of Tryptophan in the Glycosyl Transfer Activity of *Trypanosoma cruzi* Trans-Sialidase

Felicity L. Mitchell, Steven M. Miles, Joao P. Neres, Elena V. Bichenkova and *Richard A. Bryce*

School of Pharmacy and Pharmaceutical Sciences, University of Manchester, Oxford Road, Manchester M13 9PT

Computational investigations into the active site plasticity of the *Trypanosoma cruzi* trans-sialidase enzyme presented here provide exciting molecular details on its mechanism of action. Varying degrees of movement of the Trp312 loop are observed as part of a carefully orchestrated multistep catalytic process. Although several key active site residues appear to be involved, flexibility of the Trp312-loop in particular seems to be a critical requirement of the sialic acid transfer mechanism. Key insights into the role of this loop flexibility and aromatic-carbohydrate interactions suggest a ‘molecular shovel’ action is employed to expel the donor aglycone leaving group from the catalytic site, and, by implication, subsequently position the acceptor aglycone into the catalytic site. The detailed information on sialyl transfer obtained herein is hoped to shed new light on the structural and mechanistic understanding of TcTS, and the inclusion of receptor flexibility is anticipated to support the structure-based design of inhibitors effective against this neglected but devastating tropical disease.

Poster 11

How Point Mutations Confer Resistance in BCR-Abl – a Computational Study

Genevieve Clapton, Katrin Spiegel, Ian Craig and *Jon Essex*

University of Southampton, University Road, Southampton SO17 1BJ

Novartis Pharmaceuticals UK Ltd, Wimblehurst Road, Horsham, West Sussex RH12 5AB

Deviant protein kinases lead to the production of abnormal cells; one example of this is chronic myelogenous leukaemia (CML) caused by the irregularly active BCR-Abl kinase.

Gleevec was successfully developed as an ATP-competitive inhibitor of BCR-Abl and effectively used in the treatment of CML.¹ Unfortunately point mutations rapidly develop in BCR-Abl resulting in eventual resistance to Gleevec. These mutations can be located both locally within the ligand binding site and remotely from it in other regions of the kinase.² This suggests that there are two mechanisms by which resistance can be conferred.

The first is through disruption of protein-ligand binding. This serves to shift the binding equilibrium towards an unbound state. This may be observed in either the active or inactive kinase conformation depending on which state the inhibitor targets. Resistance of this nature is typically exemplified by mutations clustered in the ATP binding site. These mutations are not expected to induce any large scale conformational movements as the mutated residue and the inhibitor are in direct contact.

The second method by which resistance is mediated is by disruption of the active/inactive conformational equilibrium. Kinases are highly flexible, and undergo significant conformational change on activation. This mechanism involves a shift in the equilibrium towards the conformation less favoured by the inhibitor, thus hindering its binding.

This research project aims to explore the effect of point mutations within the BCR-Abl kinase system through the use of atomistic computer simulations. The overall objective is to develop a model capable of explaining why certain mutations confer resistance, and hence to enable the prediction of likely mutations. Rational proposals could then be made on how to modify ligands in order to overcome resistance.

Presented research is focused on mutations local to the binding site where methods such as molecular dynamics, free energy calculations and docking studies have been used.

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Poster 12

Activation of Integrins by Formation of a Complex with Talin and Membranes: a Coarse-Grained Molecular Dynamic Study

Antreas Kalli and *Mark Sansom**

**Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU, U.K.*

Integrins are major cell surface receptors that are crucial for a variety of cell migration and adhesion events. Integrins are ‘activated’ to a high affinity state by the intracellular protein talin, a process known as “inside-out activation”. Complex formation between the talin head domain and the β cytoplasmic tail as well as talin/membrane interactions are believed to have a crucial role in integrin activation but they have not been directly

observed. In this study, we use coarse-grained molecular dynamic simulations (CG-MD) to explore talin/membrane interactions and the importance of the membrane in the “inside-out activation” process. These interactions are also probed in the presence of the integrin α/β subunits to give a more complete picture. In this CG-MD method (1, 2) an approximate 4:1 mapping of heavy atoms (not H) to CG particles is used. The membrane binding properties of the talin head subdomains, F2 and F3, have been elucidated. Residues, which are important in the binding of talin to the membrane, have been proposed and we show, that mutations in residues in the binding surface reduce the affinity of the F2-F3 domain and change its orientation relative to the membrane. The binding surface was confirmed by CG-MD self-assembly simulations. Further, it is shown that the acidic lipids have an essential role in the binding of talin to the membrane. These results are evaluated by comparison with experimental studies and they are consistent with these data. Finally, this study demonstrates that CG-MD simulations are a valuable tool for exploring the orientation and the position of a membrane-bound protein relative to a lipid bilayer besides their radical simplification in the treatment of the electrostatic interactions.

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