MGMS and RSC MMG
Young Modellers' Forum 2011
ORAL PRESENTATIONS

Programme of Presentations

9.00 – 9.30	**Coffee and Registration**
9.30 – 9.40	**Welcome and Introduction**
9.40 – 10.00	**Flexibility Controls Specificity of Snake Venom Metalloproteases** Hannes Wallnoefer, *University of Innsbruck, Austria*
10.00 – 10.20	**Flipping Inositol Phosphates: A Molecular Dynamics Approach to Understanding the Selectivity of Protein Kinase B** Sarah Rosen, *Imperial College London*
10.20 –10.40	**Protein-ligand binding free energies from large-scale DFT calculations with the ONETEP program** Stephen Fox, *University of Southampton*
10.40 – 11.00	**Combination of structure- and ligand-based virtual screening for the discovery of novel non-LBP antiandrogens as chemical tools to treat prostate cancer** Laura Caboni, *Trinity College Dublin, Ireland*
11.00 – 11.20	**Atomic Detail Studies of P-Glycoprotein and Drug Permeation in Model Membranes** Jerome Ma, *University of Oxford*
11.20 – 11.40	**From Development to Application: Molecular Mechanical Study of Halogen Bonding in Drug Discovery** Mahmoud Ibrahim, *University of Manchester*
11.40 – 13.30	**Lunch and Poster Session**
13.30 – 13.50	**Druggability and structural analysis of bromodomain acetylated-lysine binding sites** Lewis R. Vidler, *Institute of Cancer Research*
13.50 – 14.10	**Advances in Free Energy Calculation: The Enveloping Distribution Sampling Method** Sereina Riniker, *ETH Zurich, Switzerland*
14.10 – 14.30	**Metabolite Space and Metabolite-Likeness** Julio Peironcely, *Leiden University/TNO, The Netherlands*
14.30 – 15.00	**Tea**
15.00 – 15.20	**A Ligand-Assisted Proton Shuttle (LAPS) Mechanism: Using Coordinated Acetate Ligands to Affect Chemical Change** David G. Johnson, *University of York*

15.20 – 15.40	**Interpreting Experimental Analysis of the Human Ether-a-go-go Related Gene Product (hERG) with Molecular Dynamics and *in-silico* Docking** Charlotte Colenso, *University of Bristol*
15.40 – 16.00	**A Novel Computational Approach to Fragment-Based Drug Discovery** Michael Bodnarchuk, *University of Southampton*
16.00	**Fun event – "Chemical Catchphrase"**
16.30	**Judges Deliberations**
16.45	**Prize Presentations**
17.00	**End**

Talk 1

Flexibility Controls Specificity of Snake Venom Metalloproteases

Hannes G. Wallnoefer[†], Torsten Lingott[‡], José M. Gutiérrez[§], Irmgard Merfort[‡] and *Klaus R. Liedl*[†]

[†]*Institute of General, Inorganic and Theoretical Chemistry, University of Innsbruck, Innrain 52a, A-6020 Innsbruck, Austria*
[‡]*Department of Pharmaceutical Biology and Biotechnology, University of Freiburg, Stefan-Meier-Str. 19 (VF), D-79104 Freiburg,Germany*
[§]*Instituto Clodomiro Picado, Facultad de Microbiología, Universidad de Costa Rica, San José, Costa Rica*

Protein-Protein interfaces have crucial functions in many biological processes. The large interaction areas of such interfaces show complex interaction motifs. Even more challenging is the understanding of (multi-)specificity in protein-protein binding. Many proteins can bind several partners to mediate their function[1]. A perfect paradigm to study such multi-specific protein-protein interfaces are snake venom metalloproteases (SVMPs). Inherently, they bind to a variety of basement membrane proteins of capillaries, hydrolyze them, and induce profuse bleeding. However, despite having a high sequence homology, some SVMPs show a strong hemorrhagic activity, while others are (almost) inactive[2]. Our results indicate that the activity to induce hemorrhage, and thus the capability to bind the potential reaction partners, is related to the backbone flexibility in a certain surface region[2]. A subtle interplay between flexibility and rigidity of two loops seems to be the prerequisite for the proteins to carry out their damaging function. Presumably, a significant alteration in the backbone dynamics makes the difference between SVMPs that induce hemorrhage and the inactive ones.

References:
[1] Han, J.D., Hao, T., Goldberg, D. S., Berriz, G. F., Zhang, L. V., Dupuy, D., Walhout, A. J. M., Cusick, M. E., Roth, F. P., Vidal, M., Evidence for dynamically organized modularity in the yeast protein-protein interaction network. *Nature*, 2004, **430**, 88-93.
[2] Wallnoefer, H. G., Lingott, T., Gutiérrez, J. M., Merfort, I., Liedl, K. R., Backbone Flexibility Controls the Activity and Specificity of a Protein−Protein Interface: Specificity in Snake Venom Metalloproteases. *J Am Chem Soc*, 2010, **132**, 10330-10337.

Talk 2

Flipping Inositol Phosphates: A Molecular Dynamics Approach to Understanding the Selectivity of Protein Kinase B

Sarah Rosen, *Piers R. J. Gaffney*, and *Ian R. Gould*

Institute of Chemical Biology and Department of Chemistry, Imperial College London, Exhibition Road, London, SW7 2AZ.

Protein kinase B (PKB) is a serine/threonine kinase at the focus of many cell signalling pathways. Upon activation, PKB acts as a regulator for a variety of downstream proteins which play critical roles in apoptosis, cell cycle progression and metabolism. Dysregulation of PKB is consequently associated with a number of human diseases including cancer, type-2 diabetes and schizophrenia [1].

It is of great interest to understand the activation process of PKB. A key stage in this activation is PKB's translocation to the plasma membrane, which is driven by binding of its pleckstrin homology (PH) domain to the inositol phosphate head-group of PtdIns(3,4,5)P_3 or PtdIns(3,4)$_2$. The selectivity of PKB for these 3-phosphorylated phosphoinositides, over the relatively abundant PtdIns(4,5)P_2, is critical for regulation of its activity.

We have used a total of 3μs of molecular dynamics (MD) simulations to better understand the relative affinity of the PKB PH domain for the inositol phosphate head-groups of PtdIns(3,4,5)P_3, PtdIns(3,4)P_2 and PtdIns(4,5)P_2. The length of our simulations, allow us to analyse and visualise the behaviour of these interactions over a biologically relevant time-scale.

Our computational models successfully mirror PKB's widely accepted preference for 3-phosphorylated phosphoinositides, as highlighted in numerous experimental studies. Moreover, our models also predict binding modes for these ligands in good agreement with crystallographic and mutagenesis data. In addition to providing a supporting role to experimental work, we highlight the use of our MD simulations to rationalise experimental data. It has previously been reported that Ins(1,4,5)P_3, the head-group of PtdIns(4,5)P_2, binds to the PKB PH domain with a relatively high affinity, comparable to that of the head-group of PtdIns(3,4,5)P_3 [2]. This finding has puzzled many, due to the markedly different binding affinities of the parent lipids. Our simulations have led us to hypothesise that when not bonded to a phosphatidate tail, Ins(1,4,5)P_3 binds in an orientation in which its inositol ring is flipped 180° with respect to the 3-phosphorylated inositol phosphate ligands, and its parent lipid.

We are now currently extending this work to consider a PKB mutant reported in multiple cancers. This PKB mutant constitutively targets the plasma membrane due to its broadened lipid selectivity. We hope that the atomic-level view available from MD simulations will help elucidate the mechanism behind this.

References:

[1] Sale, E. M. And Sale G.J., Protein Kinase B: Signalling Roles and Therapeutic Targeting, *Cellular and Molecular Life Sciences*., (2008), **65**, 113-127.

[2] Frech, M. *et al*., High Affinity Binding of Inositol Phosphates and Phosphoinositides to the Pleckstrin Homology Domain of RAC/Protein Kinase B and Their Influence on Kinase Activity, *The Journal of Biological Chemistry*, (1997), **272**, 8474-8481.

Talk 3

Protein-ligand binding free energies from large-scale DFT calculations with the ONETEP program

Stephen Fox[a], Thomas Fox[b], Christofer S. Tautermann[b,] and Chris-Kriton Skylaris[a]

[a] *School of Chemistry, University of Southampton, Highfield, Southampton SO17 1BJ, UK*
[b]*Boehringer Ingelheim Pharma GmbH & Co. KG, Lead Identification and Optimization Support, 88397 Biberach, Germany*

The calculation of binding free energies for small molecules to proteins is a very important problem in drug optimisation and many approaches have been developed [1]. The Molecular Mechanics Poisson-Boltzmann Surface Area (MM-PBSA) is one such method, in which the free energy of binding is obtained as a sum of the differences in energies of the complex, receptor and ligand (ΔH), and the differences in solvation energies (ΔG_{solv}), averaged over a structural ensemble taken from a Molecular Dynamics simulation. Conventional methods use classical force fields to calculate the energies in vacuum and the PBSA implicit solvent model for the solvation energies. This can be a significant limitation to the accuracy of this method due to the empirical nature of the force field used to describe the system, and the neglect of electrons in force fields, which leads to the inability to properly describe polarisation or to account for electron transfer. In an attempt to overcome these limitations, we calculate binding free energies using large-scale Density Functional Theory (DFT) calculations with the ONETEP program [2] on the entire system. Solvation energies are obtained from a minimum parameter implicit solvent approach[3] within the self-consistent calculation procedure. We present a preliminary application of this QM-PBSA[4,5] method on a number of small aromatic ligands bound in the polar cavity of the T4-lysozyme mutant L99A/M102Q [6]. Our DFT calculations are performed a with near-complete basis set and include more than 2600 atoms.

References:

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[2] C.-K. Skylaris, P. D. Haynes, A. A. Mostofi and M. C. Payne, *J. Chem. Phys.* **122**, 084119 (2005).
[3] J. Dziedzic, H. H. Helal, C.-K. Skylaris, A. A. Mostofi and M. C. Payne, *Europhysics Letters* **95**, 43001 (2011)
[4] D. J. Cole, C.-K. Skylaris, E. Rajendra, A. R. Venkitaraman and M. C. Payne. *Europhysics Letters*, **91,** 37004 (2010)
[5] S. Fox, H. Wallnoefer, T. Fox, C. Tautermann and C.-K. Skylaris. *J. Chem. Theor. Comput.*, **7**, 1102 (2011).
[6] B. Q. Wei, W. A. Baase, L. H. Weaver, B. W. Matthews and B. K. Shoichet, J. Mol. Biol. **322**, 339, (2002).

Talk 4

Combination of structure- and ligand-based virtual screening for the discovery of novel non-LBP antiandrogens as chemical tools to treat prostate cancer

Laura Caboni[a], Fernando Blanco[a], Darren Fayne[a], D. Clive Williams[b] and David G. Lloyd[a]

[a]*Molecular Design Group, School of Biochemistry and Immunology, Trinity Biomedical Sciences Institute, Trinity College Dublin, Dublin 2, Ireland*

[b]*School of Biochemistry and Immunology, Trinity Biomedical Sciences Institute, Trinity College Dublin, Dublin 2, Ireland*

Current treatment of prostate cancer (PCa) typically involves administration of 'classical' antiandrogens, competitive inhibitors of natural androgen receptor (AR) ligands, dihydrotestosterone (DHT) and testosterone (tes), for the ligand binding pocket (LBP) in the C-terminal ligand binding domain (LBD) of the AR. However, prolonged LBP-targeting can often lead to androgen resistance and alternative therapies are urgently required. We provide supporting evidence that alternative non LBP-mediated modulation of the AR with selective small molecule scaffolds can directly disrupt the AR:Coactivator interaction, affording the possibility to overcome the problem of androgen resistance [1].

We report the identification and characterization of a novel series of diarylhydrazides as selective disruptors of the AR interaction with coactivators through application of structure- and ligand-based virtual screening and time-resolved fluorescence energy transfer (TR-FRET) techniques.

Structural information was provided by the available X-ray crystal structures of AR with different coactivator peptides [2]. A 3D consensus pharmacophore was built containing coactivators features and vendor databases of small molecules (~$3x10^5$ molecules) were screened to obtain small molecules satisfying the structural requirements for AR-coactivator interaction.
Compounds demonstrate full ('true') antagonism in AR with low micromolar potency, high selectivity over both the Estrogen Receptors alpha and beta (ER□ and ER□) and the Glucocorticoid Receptor (GR) and only micromolar partial antagonism of the Progesterone Receptor (PR).

These data provide supporting evidence for non-LBP intervention as an alternative approach or in combination to classical PCa therapy.

References:
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[2] Hur, E., et al., Recognition and accommodation at the androgen receptor coactivator binding interface. *PLoS Biol*, **2004**. 2(9):E274.

Talk 5

Atomic Detail Studies of P-Glycoprotein and Drug Permeation in Model Membranes

Jerome Ma, Jason Schnell and Phil Biggin

Biochemistry Department, University of Oxford, South Parks Road, Oxford, OX1 3QU

The ability of drugs acting within the central nervous system (CNS) to cross the bloodbrain-barrier (BBB) is crucial to their effectiveness. The BBB lies at the interface between the blood and brain and very effectively restricts the movement of many therapeutically useful drugs from the blood into the brain. A major factor contributing to CNS penetration of a drug is its permeability through the BBB membranes. We have examined the dynamics and localisation of several CNS-acting drugs in model lipid bilayers using both atomistic molecular dynamics (MD) simulations and NMR techniques. The localisation and orientation of these drugs were shown to be dependent on both protonation state and lipid composition of the bilayer.

Permeation across the BBB is also complicated by the presence of efflux transporter proteins such as P-glycoprotein (Pgp), which are expressed in the BBB membranes and able to actively export drugs. There is growing evidence that Pgp binds drugs from within the membrane, rather than from the cytoplasm. We have been using atomistic MD to compare the dynamics of the recently published X-ray structure of inhibitor bound Pgp (PDB: 3G60) with apo Pgp. Initial results suggest that the presence of the inhibitor induces conformational changes in the nucleotide binding domains of Pgp that prevents ATP hydrolysis. We discuss how the results our drug localisation studies can be combined with the structure and dynamics of Pgp to provide insight into the mechanism of drug export.

Talk 6

From Development to Application: Molecular Mechanical Study of Halogen Bonding in Drug Discovery

Mahmoud A. A. Ibrahim,[a,b]

[a]*School of Chemistry, University of Manchester, Oxford Road, Manchester M139PL, United Kingdom*
[b]*Chemistry Department, Faculty of Science, Minia University, Minia 61519, Egypt*

Quantum mechanical and experimental studies on halogen atoms in crystal structures revealed a dual behavior of halogens, as Lewis acid (donor) in formation of a halogen bond and as Lewis base (acceptor) in formation of a hydrogen bond. The dual behavior of halogens is attributed to the anisotropic distribution of the charge density on the halogen atom, resulting in the formation of a positive cap (called σ-hole) centered on the A–X axis. The current research is the first reported molecular mechanical (MM) study of halogen bonding, the positive region centered on the halogen atom was represented by an extra-point (EP) of charge [1]. The developed MM approach was examined in geometrical optimization and binding energy calculation of 27 halogen-containing molecules complexed to various Lewis bases. The MM results were in good

agreement with the corresponding quantum mechanical data. A comparison between the performance of the developed MM approach and the corresponding performance of six semiempirical molecular orbital methods in describing halogen bonding were carried out [2]. The results revealed that MM gives better halogen bond properties in terms of bond length and binding energy than do the studied semiempirical methods.

It was also found that the EP inclusion on the halogen atom resulted in an improvement in the accuracy of the electrostatic-potential derived charges. The solvation free energies of halobenzene molecules relative to benzene were calculated with and without EP inclusion to assess the accuracy of the developed approach and compared to the corresponding experimental and semiempirical data. Molecular mechanical study of halo derivatives of benzotriazole complexed to cyclin-dependent protein kinase 2 (CDK2) was performed, and MM-PB(GB)SA binding energies were calculated as a case study in finding potent halogenated inhibitors that can serve as antitumor drugs.

References:
[1] Ibrahim, M. A. A., Molecular mechanical study of halogen bonding in drug discovery, *J. Comput. Chem.*, (2011), **32**, 2564-2574.
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Talk 7

Druggability and structural analysis of bromodomain acetylated-lysine binding sites

Lewis R. Vidler, *Nathan Brown* and *Swen Hoelder*

Cancer Research UK Cancer Therapeutics Unit, The Institute of Cancer Research, 15 Cotswold Road, Sutton, Surrey SM2 5NG,United Kingdom.

The bromodomain family of proteins represent one of the readers of the epigenetic code, binding histone acetylated-lysine to a conserved site. Through discovery of potent inhibitors of the BET-family of bromodomains, members of this class of proteins have been demonstrated to be druggable[1].

Here, we hypothesize that there are inherent differences in druggability between the different bromodomain members and not all members are as druggable as the BET-family.

To test this hypothesis, initially the druggability of all available bromodomain crystal structures from the Protein Data Bank were assessed using SiteMap. This method represents a common approach for this sort of analysis with a detailed validation published[2].

SiteMap yielded a wide range of druggabilities across the family of bromodomains with the BET-family scoring highly. Significantly, the scores attained were able to be related back to the features of the binding pocket and subsequently clustered based on these features. This clustering was then compared to the phylogenetic tree generated from whole sequence similarity.

This represents the first analysis of this type for which the protein family in question spans a wide range of druggabilities and demonstrates the necessity for the analysis of structure and not just a score.

References:
[1] Oliver, S. S. and Denu, J. M., Disrupting the Reader of Histone Language, *Angew. Chem. Int. Ed.*, (2011), **50**, 5801-5803.
[2] Halgren, T.,A., Identifying and Characterizing Binding Sites and Assessing Druggability, *J. Chem. Inf. Model*., (2009) 49, 377-389

Talk 8

Advances in Free Energy Calculation: The Enveloping Distribution Sampling Method

Sereina Riniker and *Wilfred F. van Gunsteren*

Laboratory of Physical Chemistry, ETH Zurich, Wolfgang Pauli Str. 10, 8093 Zurich, Switzerland.

Enveloping distribution sampling (EDS) allows the estimation of free energy differences from a single simulation by construction of a reference state, which envelopes the regions of phase space important to the various end states of interest [1]. The Hamiltonian of the reference state can be modified by two kind of parameters, ensuring optimal sampling. The parameters are the smoothness parameter s, which decreases the barrier between the end states, and the energy offset parameters, which allow equal sampling of all end states. Recently, a new iterative procedure was introduced to derive these parameters in an automatic way [2].

The method is applied to estimate the relative binding free energies of 17 tetrahydroisoquinoline derivatives which act as inhibitors for phenylethanolamine N-methyltransferase (PNMT) and the results are compared to those calculated by the thermodynamic integration (TI) method. A very good agreement between TI and EDS free energy differences was observed at a comparable computational cost. As EDS avoids the specification of a λ-dependence, it is an valuable alternative to TI simulations.

References:
[1] Christ, C. D. and van Gunsteren, W. F., Comparison of three enveloping distribution sampling Hamiltonians for the estimation of multiple free energy differences from a single simulation, *J. Comput. Chem.*, (2009), **30**, 1664-1679.
[2] Riniker, S., Christ, C. D., Hansen N., Nair, P. C., Mark, A. E. and van Gunsteren, W. F., Comparison of enveloping distribution sampling and thermodynamic integration to calculate binding free energies of phenylethanolamine N-methyltransferase inhibitors., *J. Chem. Phys.*, (2011) **135**, 024105.

Talk 9

Metabolite Space and Metabolite-Likeness

Julio E. Peironcely[1,2,3], Theo Reijmers[2,3], Leon Coulier[1,3], Andreas Bender[4] and Thomas Hankemeier[2,3]

[1]*TNO Research Group Quality and Safety, Zeist, The Netherlands*
[2]*Division of Analytical Biosciences, Leiden/Amsterdam Centre for Drug Research, Leiden University, Leiden, The Netherlands*
[3]*Netherlands Metabolomics Centre, Leiden, The Netherlands*
[4]*Unilever Centre for Molecular Science Informatics, Department of Chemistry, University of Cambridge, Cambridge, United Kingdom*

While the entirety of 'Chemical Space' is huge (and assumed to contain between 10^{63} and 10^{200} 'small molecules'), distinct subsets of this space can nonetheless be defined according to certain structural parameters. An example of such a subspace is the chemical space spanned by endogenous metabolites, defined as 'naturally occurring' products of an organisms' metabolism. In order to understand this part of chemical space in more detail, we analyzed the chemical space populated by human metabolites in two ways. Firstly, in order to understand metabolite space better, we performed Principal Component Analysis (PCA), hierarchical clustering and scaffold analysis of metabolites and non-metabolites in order to analyze which chemical features are characteristic for both classes of compounds. Here we found that heteroatom (both oxygen and nitrogen) content, as well as the presence of particular ring systems were able to distinguish both groups of compounds. Secondly, we established which molecular descriptors and classifiers are capable of distinguishing metabolites from non-metabolites, by assigning a 'metabolite-likeness' score. It was found that the combination of MDL Public Keys and Random Forest exhibited best overall classification performance with an AUC value of 99.13%, a specificity of 99.84% and a selectivity of 88.79%. This performance is slightly better than previous classifiers; and interestingly we found that drugs occupy two distinct areas of metabolite-likeness, the one being more 'synthetic' and the other being more 'metabolite-like'. Also, on a truly prospective dataset of 457 compounds, 95.84% correct classification was achieved. Overall, we are confident that we contributed to the tasks of classifying metabolites, as well as to understanding metabolite chemical space better. This knowledge can now be used in the development of new drugs that need to resemble metabolites, and in our work particularly for assessing the metabolite-likeness of candidate molecules during metabolite identification in the metabolomics field.

Talk 10

A Ligand-Assisted Proton Shuttle (LAPS) Mechanism: Using Coordinated Acetate Ligands to Affect Chemical Change

David G. Johnson, Christine E. Welby, Jason M. Lynam and John M. Slattery

The University of York, Heslington, York, YO10 5DD.

The flexibility of transition metals to assume different oxidation states and coordinate organic ligands in a variety of ways is the basis for the activity of many synthetic homogeneous

catalysts. This contribution will discuss how computational methods have allowed us to gain insight into the mechanisms of reactions involving organometallic complexes where the ligand periphery around the metal centre plays as important a role as the metal itself, specifically the functionality of acetate ligands coordinated to a ruthenium bis-triphenylphosphine centre.

A DFT investigation was undertaken to identify the mechanism of tautomerisation of a terminal alkyne into a vinylidene complex on reaction with *cis*-[Ru(κ^2-OAc)$_2$(PPh$_3$)$_2$]. The potential energy surface (PES) for the tautomerisation showed the lowest energy pathway to be one where the bound acetate ligand acted as both Brønsted base and acid to transfer a hydrogen from one part of the alkyne to another during the tautomerisation. This process was subsequently termed a ligand-assisted proton shuttle LAPS mechanism [1].

A further mechanism was studied using a similar ruthenium complex, *trans*-[Ru (κ^1-OAc)(κ^2-OAc)(CO)(PPh$_3$)$_2$] which contains a carbonyl ligand. An experimental study showed that reaction of this complex with PhC≡CH leads to formation of an acetylide complex and free acetic acid. If an excess of alkyne is used a β-styryl ester is formed (from the coupling of acetic acid and the alkyne). It was shown *via* a DFT study that while a LAPS-type mechanism was occurring, the corresponding acetylide complex and free acetic acid were a significant thermodynamic sink for the system. Alkyne substituent and solvent effects were also investigated with a view to understanding the regiochemical outcome of the reaction.

In summary, this presentation will show how DFT methods provided new insights into catalytic reactions, including non-covalent base-assisted proton transfer and organic ligand rearrangement.

References:
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Talk 11

Interpreting Experimental Analysis of the Human Ether-a-go-go Related Gene Product (hERG) with Molecular Dynamics and *in-silico* Docking

Charlotte Colenso[a], Yi Hong Zhang[b], Richard B. Sessions[a], Jules C. Hancox[b] and Chris E. Dempsey[a]

[a] School of Biochemistry, [b] School of Physiology and Pharmacology, University of Bristol, University Walk, Clifton, Bristol BS8 1TD

hERG encodes ion channels that conduct the rapid component of the delayed rectifier current, I_{Kr} in human heart. These channels are key components of cardiac action potential repolarization; genetic mutation and drug blockade disrupt normal channel function which may lead to fatal cardiac arrhythmias. In the absence of a crystal structure, a molecular model is required to interpret hERG function in structural terms. We describe a hERG model based on a $K_v1.2/K_v2.1$ 'paddle chimera' structure in the open state [1]. We employ molecular dynamics simulations within a lipid bilayer to investigate the effects of mutations L532P and G648A on channel properties and gating mechanisms. Principal component analysis (after 150 ns) indicates the Leu

to Pro mutation perturbs the S4 transmembrane helix, providing insight into the electrophysiological effects of this mutation [2]. A preliminary normal mode analysis indicates that both mutations result in different deformation energy and atomic fluctuation profiles compared with wild-type. We have also performed *in-silico* alanine-scanning mutagenesis to assess docking of amiodarone, a class III antiarrhythmic agent, with a MthK based homology model of the hERG pore region. The analysis broadly agrees with an electrophysiological study of amiodarone block of hERG mutant channels, supporting the reliability of open channel hERG homology models for interpreting experimental data.

References:
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Talk 12

A Novel Computational Approach to Fragment-Based Drug Discovery

Michael S. Bodnarchuk[a], Russell Viner[b] and Jonathan W. Essex[a]

[a] *School of Chemistry, University of Southampton, Southampton SO17 1BJ*
[b] *Jealott's Hill International Research Centre, Bracknell, Berkshire RG42 6EY*

High-Throughput Screening (HTS) is a widely used approach in drug discovery, where large numbers of compounds are screened against a drug target. One problem with this approach is that the hit rate is often poor, whilst another lies in the typically large molecular weight of the resultant hits. Since optimisation often requires the addition of more molecular weight and lipophilicity, the chances of the end drug obeying Lipinski's rules are typically low [1].

Fragment-Based Drug Discovery (FBDD) has emerged in the last 20 years as a valuable tool for the medicinal chemist. The method screens low weight compounds (typically less than 250 Da), allowing the chemical space to be screened more effectively. Since the fragments are less complex than the compounds screened in HTS, the probability of finding a good match between a fragment and the target, and therefore the hit rate, is higher than for larger molecules. The resultant hits typically display greater ligand efficiency (binding affinity per heavy atom) than HTS hits, allowing the medicinal chemist to carefully optimise the fragments to minimise the molecular weight of the end drug molecule.

Various computational approaches to FBDD have been described in the literature, although most of them suffer from several limitations. The two major drawbacks to most approaches lie in the lack of consideration of water competition during the simulation, and the adoption of a rigid binding site approximation. In this work, the Just Add Water Molecules (JAWS) methodology [2] has been modified to take into account most of the known limitations of computational FBDD approaches, allowing the prediction of fragments whilst competing against water. The methodology has been tested upon factor Xa, highlighting the key structural elements of known

factor Xa inhibitors. The observed results are based upon the binding free energy of each fragment, allowing competiting fragments to be ranked in terms of affinity.

References:
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