Programme and Abstracts

Membrane Proteins: Structure and Function
MGMS Spring Meeting

6 - 8 April 2011
Lady Margaret Hall, University of Oxford, UK

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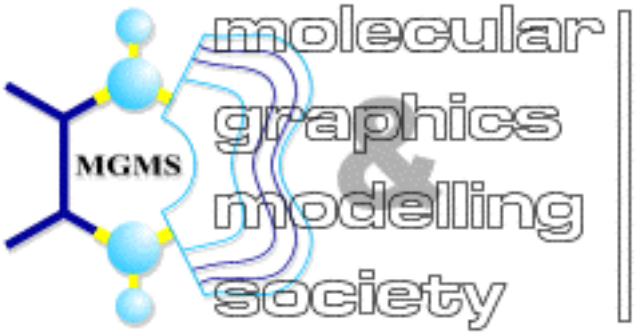

Wednesday 6th April

9am onwards	**Registration**
10.30-11.00	**Coffee/Tea**
11.00-11.10	**Welcome: Dr Phil Biggin. University of Oxford.**

SESSION I **Chair: Dr Phil Biggin, University of Oxford.**

11.10-11.50	**Pierre-Jean Corringer.** Institut Pasteur. 'X-ray structures of general anaesthetics bound to a bacterial channel-receptor homologous to nAChRs'.
11.50-12.10	**Select oral presentation: Charlotte Colenso.** University of Bristol. 'Interpreting Experimental Analysis of the hERG Potassium Channel using Molecular Dynamics Simulations and in silico Docking'.
12.10-12.50	**Isabel Bermudez.** Oxford Brookes University. 'An additional ACh binding site at the α4/α4 interface of the $(\alpha4\beta2)_2\alpha4$ nicotinic acetylcholine receptor confers stoichiometry-specific properties'.
12.50-14.00	**LUNCH**

SESSION II **Chair: Oliver Beckstein,** University of Oxford.

14.00-14.40	**Lucia Sivilotti.** University College London. 'Stumbling to the open state: probing the activation trajectory of glycine receptors'.

WEDNESDAY

WEDNESDAY

14.40-15.20	**Raimund Dutzler.** University of Zurich. 'The structural basis of open channel block in a prokaryotic pentameric ligand-gated ion channel'.
15.20-16.00	**Tea/Coffee**
16.00-16.40	**Jette Kastrup.** University of Copenhagen. 'Ionotropic glutamate receptors and their interactions with ligands – lessons from crystal structures'.
16.40-17.20	**Simon Ward.** University of Sussex. 'AMPA receptor positive modulators: from crystal structure to clinical trial'.

17.20-1800	**MGMS AGM**
18.00-19.00	**Posters/Exhibition and Wine Reception**
19.00	**Dinner**
20.00	**Bar is open**

Thursday 7th April

08.30	**Scalalife Demonstration with Mihai Duta.** Scalalife is pan-european unifying project to aid development of scalable software on HPC resources for the life-sciences.

SESSION III	**Chair : Charlie Laughton,** University of Nottingham.
09.05-09.40	**Phil Biggin.** University of Oxford. 'Using simulation to probe how glutamate receptors open'
09.40-10.20	**David Jane.** University of Bristol. 'Discovery of subunit selective kainate receptor antagonists'.
10.20-10.40	**Anny-Odile Colson.** Novartis. 'Structure and Ligand Based Design in the Identification of Chemokine Receptor Antagonists'.
10.40-11.20	**Coffee/Tea and Posters**
11.20-11.40	**Peter Hildebrand.** Charité Universitätsmedizin Berlin. 'The rhodopsin model: Crystal structures and theoretical considerations'.
11.40-12.00	**Dahlia Goldfield.** Columbia University. 'Successful prediction of the intra and extracellular loops of 4 G-protein-coupled receptors'.
12.00-12.40	**Ron Dror.** D.E. Shaw Research. 'Elucidating Membrane Protein Function through Long-Timescale Molecular Dynamics Simulations'.
12.40-14.00	**LUNCH + Posters**

THURSDAY

SESSION IV **Chair: Chris Reynolds,** University of Essex.

14.00-14.40	**Richard Law.** Evotec. 'Histamine GPCR family antagonists by fragment screening and molecular modelling'.
14.40- 15.20	**Chris Tate.** LMB, Cambridge. 'Conformational thermostabilisation of GPCRs to facilitate structure determination'.
15.20-16.00	**Tea/Coffee**
16.00-16.20	**Slawomir Flipek.** University of Warsaw. 'The action of molecular switches in complexes of GPCRs with agonists and antagonists'.
16.20-16.40	**Stefano Vanni.** EPFL Lausanne. 'The role and mechanism of proton transfer reactions along the activation pathway of GPCRs'.
16.40-17.00	**Krzysztof Jozwiak.** Medical University of Lublin. 'Interactions between stereoisomers of fenoterol and β2 adrenergic receptor. A molecular dynamics study'.
17.00-17.20	**Jana Selent.** Universitat Pompeu Fabra. 'Progresses and challenges in the prediction of gpcrs: d3 receptor in complex with eticlopride'.

17.20-19.00	**Posters/free time.**
19.00	**Canapes/pre-dinner drinks in the Old Library.**
19.30	**Gala Dinner.**

Friday 8th April

SESSION V	**Chair Mark Sansom,** University of Oxford.
09.00-9.40	**So Iwata.** Imperial/Diamond. 'Transporter Protein Structure.'
09.40-10.20	**Lucy Forrest.** MPI Frankfurt. 'The role of structural symmetry in secondary transport'.
10.20-10.40	**James Zook.** Arizona State University. 'Structural Investigations of Membrane Protein OEP16'.
10.40-11.10	**Coffee/Tea**
11.10-11.50	**Geoffrey Chang.** Scripps Research Institute. 'Structures of Multidrug Resistance Transporters'.
11.50-12.10	**Andreas Kukol.** University of Hertfordshire. 'United-atom lipid models for molecular dynamics simulation of membrane protein'.
12.10-12.40	**Jason Schnell.** University of Oxford. 'Structural studies of a dual topology regulator of melanocortin receptors'.
12.40-14.00	**LUNCH**

SESSION VI	**Chair: Phil Fowler,** University of Oxford.
14.00-14.40	**Antoinette Killian**. Utrecht University. 'Designed transmembrane peptides as tools to understand lipid/protein interactions'.
14.40- 15.20	**Andreas Klamt.** Cosmologic. COSMOmic: 'A novel approach for modelling molecules in biomembranes'.
15.20-15.30	**CLOSE OF MEETING**

S01. An additional ACh binding site at the α4/α4 interface of the $(\alpha4\beta2)_2\alpha4$ nicotinic acetylcholine receptor confers stoichiometry-specific properties

Isabel Bermudez
School of life Sciences, Oxford Brookes University, Oxford, UK.

The α4β2 nicotinic acetylcholine (nACh) receptor assembles in two alternate forms, $(\alpha4\beta2)_2\alpha4$ and $(\alpha4\beta2)_2\beta2$, which display stoichiometry-specific agonist sensitivity. Being heteromeric Cys loop ligand gated ion channels (LGIC), α4β2 receptors are activated by binding of agonist to sites located at the β2(+)/β2(-) and α4(+)/α4(-) interfaces. Because these interfaces are present in both stoichiometries it is unlikely that structural differences conferring specific properties to $(\alpha4\beta2)_2\alpha4$ and $(\alpha4\beta2)_2\beta2$ and receptors reside here. In contrast, the accessory subunit can be either α4 or β2, leading to stoichiometry-specific β2(+)/β2(-) and α4(+)/α4(-) interfaces. Using fully concatenated $(\alpha4\beta2)_2\alpha4$ nACh receptors in conjunction with structural modelling, chimeric receptors and functional mutagenesis, we have identified an additional agonist site at the α4(+)/α4(-) interface and account for the stoichiometry-specific agonist sensitivity of the $(\alpha4\beta2)_2\alpha4$ receptor. The additional agonist site occupies a region that also contains a potentiating Zn^{2+} binding site. However, unlike Zn^{2+}, the agonist site influences agonist responses by directly contributing to channel gating. By engineering a receptor with a C226S mutation to provide a free cysteine in loop C in the + side of the α4(+)/α4(-) interface, we demonstrated that the ACh responses of the additional agonist site were attenuated or enhanced following treatment with the sulfhydryl reagent MTSET or MTSEA, respectively. These findings suggested that agonist occupation of the site at the α4(+)/α4(-) interface leads to channel gating through a coupling mechanism involving a conformational switch in loop C. The MTS reagents had similar effects on α4(+)/β2(-) interface agonist sites with a free Cys in loop C. Homomeric Cys loop receptors are activated similarly by three agonist molecules, however, because of agonist site heterogeneity in $(\alpha4\beta2)_2\alpha4$ receptors, the overall effect of a third agonist site on receptor properties differs in $(\alpha4\beta2)_2\alpha4$ receptors.

Notes:

S02. Structures of Multidrug Resistance Transporters

Geoff Chang
Scripps Research Institute

P-glycoprotein (Pgp) detoxifies cells by exporting hundreds of chemically unrelated toxins but has been implicated in multidrug resistance in the treatment of cancers. Substrate promiscuity is a hallmark of Pgp activity, thus a structural description of polyspecific drug-binding is important for the rational design of anticancer drugs and MDR inhibitors. The x-ray structure of apo-Pgp at 3.8 Å reveals an internal cavity of ~6,000 $Å^3$ with a 30 Å separation of the two nucleotide binding domains (NBD). Two additional Pgp structures with cyclic peptide inhibitors demonstrate distinct drug binding sites in the internal cavity capable of stereo-selectivity that is based on hydrophobic and aromatic interactions. Apo- and drug-bound Pgp structures have portals open to the cytoplasm and the inner leaflet of the lipid bilayer for drug entry. The inward-facing conformation represents an initial stage of the transport cycle that is competent for drug binding. We will present our latest findings on P-glycoprotein and present strategy on obtaining other conformations, extending the diffraction resolution, and new co-crystal structures with inhibitors/drugs.

Transporter proteins from the multidrug and toxic compound extrusion (MATE) family play vital roles in metabolite transport in plants, directly affecting crop yields worldwide. MATEs also mediate multidrug resistance (MDR) in bacteria and mammals, modulating the efficacy of many pharmaceutical drugs used in the treatment of a variety of diseases. MATEs couple substrate transport to electrochemical gradients and are the only remaining class of MDR transporters whose structure has not been determined. The X-ray structure of the MATE transporter NorM from Vibrio cholera reveal an outward-facing conformation with two portals open to the outer leaflet of the membrane and a unique topology distinct from any other known MDR transporter. A cation-binding site in close proximity to residues previously deemed critical for transport has also been observed. This conformation likely represents a stage of the transport cycle with high-affinity to monovalent cations and low-affinity to substrates.

Notes:

S03. X-ray structures of general anaesthetics bound to a bacterial channel-receptor homologous to nAChRs

Pierre-Jean Corringer[1], Hugues Nury[1,2], Catherine Van Renterghem[1], Yun Weng[3], Alphonso Tran[3], Marc Baaden[4], Virginie Dufresne[1], Jean-Pierre Changeux[5], James M. Sonner[3] & Marc Delarue[2]

[1]Institut Pasteur, Groupe Récepteurs-Canaux, CNRS URA 2182, Paris, France. [2]Institut Pasteur, Unité de Dynamique Structurale des Macromolécules, CNRS URA 2185, Paris, France. [3]Department of Anesthesia and Perioperative Care, University of California, San Francisco, USA. [4]Institut de Biologie Physico-Chimique, CNRS UPR 9080, Paris, France. [5]Institut Pasteur & Collège de France, Paris, France.

General anaesthetics have enjoyed long and widespread use but their molecular mechanism of action remains poorly understood. There is good evidence that their principal targets are pentameric ligand-gated ion channels (pLGICs) such as inhibitory $GABA_A$ (γ-aminobutyric acid receptor A) and excitatory nicotinic acetylcholine receptors (nAChRs), which are respectively potentiated and inhibited by these allosteric effectors. The bacterial homologue from *Gloeobacter violaceus* (GLIC), whose X-ray structure was recently solved, is also sensitive to clinical concentrations of general anaesthetics. We recently solved the crystal structures of the complexes propofol/GLIC and desflurane/GLIC. These reveal a common general-anaesthetic binding site which pre-exists in the apo-structure in the upper part of the transmembrane domain of each protomer. Both molecules establish van der Waals interactions with the protein; propofol binds at the entrance of the cavity whereas the smaller, more flexible, desflurane binds deeper inside. Mutations of some amino acids lining the binding site profoundly alter the ionic response of GLIC to protons, and affect general-anaesthetic pharmacology. Simulations of molecular dynamics, performed on the wild type and two GLIC mutants, highlight differences in mobility of propofol in its binding site and help to explain these effects. These data provide a novel structural framework for the design of general anaesthetics and of allosteric modulators of brain pLGICs, including nAChRs. In addition, they give insights into the gating mechanism occurring in this family of channel, that involve transmission of conformational reorganization from the extracellular domain to the transmembrane domain.

Notes:

S04. Elucidating Membrane Protein Function through Long-Timescale Molecular Dynamics Simulations

Ron O. Dror
D. E. Shaw Research

Recent advances in hardware, software, and algorithms for molecular dynamics (MD) simulations have brought previously inaccessible simulation timescales within reach, allowing the use of such simulations to address a substantially broader set of questions regarding protein function. MD has proved particularly useful in elucidating the functional mechanisms of membrane proteins, whose dynamics are especially difficult to characterize experimentally. Here, we illustrate the utility of state-of-the-art high-performance MD simulations in the study of membrane proteins, using as examples a G-protein–coupled receptor, a transporter, and an ion channel. In each case, we employed MD either to deduce an atomic-level mechanism for protein function or to reconcile apparent discrepancies among recent experimental observations.

Notes:

S05. The structural basis of open channel block in a prokaryotic pentameric ligand-gated ion channel

Raimund Dutzler
Department of Biochemistry, University of Zurich

The flow of ions through cation selective members of the pentameric ligand-gated ion channel family is inhibited by a structurally diverse class of molecules that bind to the transmembrane pore in the open state of the protein. To obtain insight into the mechanism of channel block we have investigated the binding of positively charged inhibitors to the open channel of the bacterial homologue GLIC by X-ray crystallography and electrophysiology. Our studies reveal the location of two regions for interactions, with larger blockers binding in the center of the membrane and divalent transition metal ions binding to the narrow intracellular pore entry. The results provide a structural foundation of the interaction with inhibitors that is relevant for the entire family.

Notes:

S06. A role for structural symmetry in secondary transport

Lucy Forrest
Max Planck Institute of Biophysics

Alternating-access mechanisms of transport require at least two distinct conformations of the protein. X-ray crystallographic data for several secondary transporters from diverse families support this hypothesis and provide key insights into specific transport mechanisms. Nevertheless, to date, many of these structures have been solved for only one state in the transport cycle. An intriguing feature of these structures is the presence of internal repeats with inverted topologies with respect to the membrane. We show using computational modelling of three different secondary transporter families that these structural repeats encode two degenerate conformations of the same protein. Experimental suport for the models is available in the form of biochemical accessibility measurements, spectroscopic data, and cross-linking studies, as well as of recent X-ray structural data. The implications of these results will be discussed in terms of a general role of inverted-topology repeats – and of symmetry – in the pseudo-symmetric alternating-access mechanism of secondary transport.

Notes:

S07. Molecular mechanism of transport proteins.

So Iwata
Imperial College London

Membrane transporters form the second largest family among these membrane proteins; it is known that 5-12% of genes in the genomes sequenced to date encode membrane transporters. However, the structure determination of membrane transporters remains extremely challenging. Particularly molecular transport mechanism has been little studied because of a lack of transporters in different conformations. Tthe sodium-benzylhydantoin transport protein Mhp1 from Microbacterium liquefaciens provided a perfect opportunity to understand the conformational dynamics of the molecular transport.

We solved structures of the inward-facing, outward-facing and occluded states of the transporter. From analyses of the three structures and molecular dynamics simulations, we could propose a feasible mechanism for the transport cycle in Mhp1. In the conference, I would also address our new structure on PepTSo. This is a bacterial homologue of PepT1 and PepT2 that utilize a proton gradient to drive the uptake of di- and tri-peptides in the small intestine and kidney, respectively. They are the major routes by which we absorb dietary nitrogen and many orally administered drugs. We have solved the crystal structure of PepTSo in a ligand-bound occluded state. Comparing with other MFS transporters supported by various simulations, we have proposed a possible peptide transport mechanism of this transporter.

Notes:

S08. Discovery of subunit selective kainate receptor antagonists

David E. Jane
School of Physiology and Pharmacology, MRC Centre for Synaptic Plasticity, Medical Sciences Building, University of Bristol,

Kainate receptors (KARs) are a subtype of a family of ligand-gated ion channels activated by the fast excitatory neurotransmitter L-glutamate. KARs are tetrameric assemblies of GluK1-5 subunits. The major focus of our research is on the development of pharmacological tools for KARs that can be used to study the function of these receptors. Structural modification of the natural product willardiine led to the development of (S)-5-iodowillardiine, a selective agonist of GluK1-containing KARs. Additional structure-activity relationship studies around the willardiine structure facilitated the development of N3-substituted willardiine derivatives as KAR antagonists (Jane et al., 2009). Fine tuning of the structure of these first generation antagonists enabled the development of GluK1-containing KAR antagonists such as UBP296, UBP304 and UBP310. Characterisation in a functional assay on recombinant KAR and AMPAR subunits showed that UBP310 blocked human GluK1 (Ki value 10 nM), but not GluK2 or GluA2. An X-ray crystal structure of UBP310 in complex with the ligand binding domain (LBD) of GluK1 (Mayer et al., 2006) provided structural information that could be used to develop a nanomolar potent antagonist, ACET (Ki value for antagonism of recombinant human GluK1 1.4 nM). In addition, the structural information could be used to understand why UBP310 and ACET are selective for GluK1 versus GluK2 and AMPARs. We have recently reported the structures of the GluK1 LBD in complex with three high affinity antagonists UBP315, UBP318 and the structurally unrelated decahydroisoquinoline derivative LY466195. These structures revealed a much wider variation in ligand receptor interactions and LBD closure than found in our previous studies with the GluK1 antagonists (Alushan et al., 2011).
In an electrophysiological assay using fast application of glutamate, UBP310 and ACET potently blocked GluK3 but not GluK2 or GluK2/GluK3. The radiolabelled ligand [3H]UBP310 binds to GluK1 with low nanomolar affinity (KD = 21 ± 7 nM) but shows no specific binding to GluK2. However, [3H]UBP310 also binds to GluK3 (KD = 650 ± 19 nM) but with_30-fold lower affinity than that observed for GluK1. Non-conserved residues in GluK1–3 adjudged in modelling studies to be important in determining the GluK1 selectivity of UBP310 were point-mutated to switch residues between subunits (Atlason et al., 2010). None of the mutations altered the expression or trafficking of KAR subunits. Whereas GluK1-T503A mutation diminished [3H]UBP310 binding, GluK2-A487T mutation rescued it. Likewise, whereas GluK1-N705S/S706N mutation decreased, GluK3-N691S mutation increased [3H]UBP310 binding affinity. These data show that, as predicted in modelling studies, Ala487 in GluK2 and Asn691 in GluK3 are important determinants in reducing the affinity of UBP310 for these subunits.
We have shown that the piperazine-2,3-dicarboxylic acid derivative UBP161 is a selective antagonist of GluK1 versus GluK2, GluK3 and AMPA receptors, though it also blocks NMDA receptors. The GluK1 versus GluK3 selectivity of UBP161 is superior to that observed with willardiine based derivatives. Modelling studies revealed structural features required for the binding of UBP161 to the LBD of GluK1 subunits and suggested that S674 in GluK1 was vital for antagonist activity. Consistent with this hypothesis, replacing the equivalent residue in GluK3 (alanine) with a serine imparts UBP161 antagonist activity. The subunit selective KAR antagonists we have discovered so far will be useful pharmacological tools to probe the functions of KARs in the central nervous system and the roles of these receptors in neurological disorders.

Alushin, GM, Jane, DE, Mayer, M.L. (2011) *Neuropharmacology* 60, 126-134.
Atlason, PT, Scholefield, CL, Eaves, RJ, Mayo-Martin, MB, Molnar, E, Jane, DE (2010) *Mol. Pharm.* 78, 1036–1045.
Jane DE, Lodge, DL, Collingridge, GL (2009) *Neuropharmacology* 56, 90-113.
Mayer ML, Ghosal A, Dolman NP, Jane DE (2006) *J. Neurosci.* 26, 2852-2861.

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S09. Ionotropic glutamate receptors and their interactions with ligands – lessons from crystal structures

Jette Sandholm Kastrup
Department of Medicinal Chemistry, Faculty of Pharmaceutical Sciences, University of Copenhagen,
Email: jsk@farma.ku.dk

Ionotropic glutamate receptors (iGluRs) comprise a family of ligand-gated ion channels, mediating most fast synaptic transmission in the central nervous system. iGluRs play an important role for the development and function of the nervous system, and are essential in learning and memory. However, these receptors are also implicated in or have causal roles for several brain disorders, e.g. epilepsy and Alzheimer's disease. The involvement of iGluRs in neurological diseases has stimulated widespread interest in their structure and function.

Since publication in 1998 of the first crystal structure of a soluble construct of the ligand-binding domain (LBD) of the AMPA-type iGluR named GluA2 [1], many structures have been determined of representatives for each of the four classes of iGluRs by us and others. Recently, a crystal structure of full-length GluA2 was determined in complex with an antagonist [2], and a comparison of the full-length GluA2 structure with the soluble GluA2 LBD structure shows that the soluble protein is a good model system of the full length receptor as the dimeric unit is very similar. The structures of wild type and mutant GluA2 LBD proteins, combined with functional data, have led to models for receptor activation and desensitization by agonists, inhibition by antagonists and block of desensitization by positive allosteric modulators, as well as provided some understanding of subunit selectivity [3]. The structural results together with functional studies are forming a powerful platform for design of new selective drugs.

[1] Armstrong, N., Sun, Y., Chen, G. Q., Gouaux, E., 1998. Structure of a glutamate-receptor ligand-binding core in complex with kainate. Nature 395, 913-917.
[2] Sobolevsky, A. I., Rosconi, M. P., Gouaux, E., 2009. X-ray structure, symmetry and mechanism of an AMPA-subtype glutamate receptor. Nature 462, 745-756.
[3] Pøhlsgaard, J., Frydenvang, K., Madsen, U., Kastrup, J.S., 2011. Lessons from more than 80 structures of the GluA2 ligand-binding domain in complex with agonists, antagonists and allosteric modulators. Neuropharmacology 60,135-150.

Notes:

S10. Designed transmembrane peptides as tools to understand lipid/protein interactions

Antoinette Killian
University of Utrecht

Interactions between proteins and lipids form the basis of virtually all membrane processes, but on a molecular level they are still poorly understood. A powerful tool to uncover basic principles of protein-lipid interactions is the use of designed peptides as models for the membrane spanning segments of membrane proteins. Such peptides can be incorporated in synthetic lipid bilayers and properties of peptides as well as lipids can be systematically varied, enabling detailed analysis of the systems by experimental and computational approaches. Questions that can be addressed are for example how the lipid environment influences the structure and dynamics of transmembrane helices, how tryptophans as residues that flank the transmembrane segments can anchor helices at the lipid-water interface, how the length and hydrophobicity of the helices influence the organization and dynamics of membrane lipids and how lipids influence helix-helix interactions. As a further step towards mimicking membrane proteins, one can covalently link two or more of such peptides and compare the helix-lipid interactions of monomers and oligomers. We will discuss recent results obtained on these relatively simple peptide/lipid model systems and we will discuss future prospects and challenges concerning the use of these systems as tool to understand membrane structure and function.

Notes:

S11. COSMOmic: A novel approach for modelling molecules in biomembranes

Andreas Klamt,
COSMOlogic GmbH&CoKG, Leverkusen and Inst. of Phys. And Theor. Chemistry, Universtiy of Regensburg

COSMO-RS is quantum-chemically based method for the prediction of thermodynamic properties of pure and mixed homogeneous fluids. COSMO-RS calculates the thermodynamic data from molecular surface polarities, which result from DFT calculations of the individual components of the mixture. The different interactions of molecules in a liquid, i.e. electrostatic interactions, hydrogen bonding, and hydrophobic interactions are represented as functionals of surface polarities of the partners. Using a rigorous and efficient statistical thermodynamics method for such pair-wise surface interactions, COSMO-RS finally converts the molecular polarity information into standard thermodynamic data of fluids, i.e. vapor pressures, activity coefficients, excess properties, etc. COSMO-RS meanwhile is widely validated and accepted for the predictive exploration of fluid phase thermodynamics.

COSMOmic is an extension of COSMO-RS, which approximates self assembled structures as bio-membranes or micelles as layered liquids with different polarity composition in each layer. In this way, it enables the calculation of the distribution and of free energy profiles of solutes in biomembranes and other self-assembled structures in a much more efficient, and most likely at least as rigorous way as the standard force-field based simulation techniques. Application examples of this novel COSMOmic software for the partition coefficients and free energy barriers of solutes in bio-membranes and micelles will be shown.

Notes:

S12. Histamine GPCR family antagonists by fragment screening and molecular modelling

Richard J Law, Thomas Hesterkamp, Andreas Kahrs, Mark Whittaker, Sandeep Pal, Alexander Heifetz, David Hallett.

Computational Chemistry Evotec Abingdon Oxfordshire United Kingdom, Evotec AG Hamburg Germany, Evotec UK Abingdon United Kingdom

Screening of low molecular weight weak binders, "fragments", and obtaining hits is a well understood process that can be achieved by many different assay techniques. Less well defined is how to proceed once a hit is obtained. Computational chemistry, and the application of multiple techniques, plays a vital role in understanding and ranking the many potential routes for fragment expansion design. We have applied a small fragment collection to the screening of three histamine receptors with the goal to identify subtype specific antagonists. The rationale for doing this is to cover as much chemical diversity as possible in a small screening effort and to meet the chemical space of the natural ligand, histamine. We have followed up on the resulting fragment hits by building H3 and H4 receptor models based on similarity to known GPCR crystal structures and optimized using a series of molecular dynamics procedures. These models were used for a docking procedure to reveal the bioactive conformation of the bound ligands, with a view to structure-guided fragment-to-lead expansion. A subsequent shape-based analogue search provided a short list of hits from which we have been able to obtain novel sub-micromolar and lead-like H3 and H4 antagonists. Also show other examples of successful GPCR modelling for Bradykinin-1 and MCH-1.

Notes:

S13. Using simulation to probe how glutamate receptors open

Philip Biggin and Ranjit Vijayan
Department of Biochemistry, University of Oxford

Ionotropic glutamate receptors are modular ligand-gated cation channels that modulate the vast majority of excitatory neurotransmission in the central nervous system. A series of X-ray crystal structures of the amino terminal domain (ATD), ligand binding domain (LBD) and finally the full-length receptor has provided atomic level insights into subunit organization and interfaces of the receptor. However, the open state of the channel is currently unknown. The free energy of ligand binding in the LBD is believed to drive the opening of the transmembrane pore. From the ligand-free (LBD open) and ligand-bound (LBD closed) structures of the LBD, we derived possible transition pathways using dynamic importance sampling. Simulations indicate that the LBD closing event is associated with the rearrangement of a salt bridge in the "hinge" region of the LBD. These transition pathways were then used to drive channel opening using restrained and equilibrium molecular dynamics simulations. In spite of near four-fold symmetry in the transmembrane domain (TMD), results suggest that the channel opens asymmetrically reflecting the dimer-of-dimer assembly of the extracellular domains and structural differences of the LBD-TMD linkers.

Notes:

S14. Structural studies of a dual topology regulator of melanocortin receptors

Jason R. Schnell & Barbara Sladek
Department of Biochemistry, University of Oxford

The melanocortin receptors (MCRs) are a sub-family of five class A G-protein coupled receptors involved in a wide range of biological activities that include skin pigmentation, the adrenaline response, and energy homeostasis. The melanocortin receptor accessory protein (MRAP) is an integral membrane protein that homodimerises with a dual topology, and directly associates with all MCRs, but differentially affects receptor trafficking to the cell surface and activation by ligand. MCR-2, the receptor for the pituitary hormone ACTH that leads to a stress response is unique in its requirement for MRAP to both traffic from the endoplasmic reticulum to the cell surface and to activate. Our work seeks to understand the connection between the structure of MRAP and its role in assembly and activation of MCR-2. The structure and homodimerisation of MRAP solubilised in detergent micelles have been studied using a range of biophysical techniques. Residues in the transmembrane and the K-rich juxtamembrane domain have been established as critical for MRAP dimer formation. In addition, the structural propensities of extra-membrane domains responsible for MCR-2 activation have been determined.

Notes:

S15. Stumbling to the open state: probing the activation trajectory of glycine receptors

Lucia Sivilotti
Department of Neuroscience, Physiology & Pharmacology, University College London

Among all channels in the Cys-loop superfamily, Glycine receptors are probably the most amenable to single channel measurements, because of their large conductance and the absence of agonist self-block. I shall briefly review how our method (global mechanism fitting to large datasets of single channel records) led us to detect and characterize an intermediate 'flip' state, that follows neurotransmitter binding and precedes opening in this channel. This reaction intermediate is important in accounting for agonist efficacy in the nicotinic superfamily. Our new data show that the passage of the channel along the activation reaction can be slowed by a human channelopathy mutation in the transduction pathway. This raises the hope that we will be able to probe and characterise the coarse-grained wave of activation detected by other methods (i.e. linear free energy relations).

Notes:

S16. Conformational thermostabilisation of GPCRs to facilitate structure determination

Christopher G. Tate
MRC Laboratory of Molecular Biology Hills Road, Cambridge CB2 0QH

Structural studies of mammalian membrane proteins, such as transporters and G protein-coupled receptors, are hampered by their lack of stability in detergents. This is manifested by a loss of activity during purification, often accompanied by aggregation, even when mild detergents such as dodecylmaltoside (DDM) are used. The situation is exacerbated during the final stages of purification, where the membrane protein is often transferred into short-chain detergents that are more suitable for crystallization, but are more destabilizing than DDM. We have developed a mutagenic strategy combined with a radioligand binding assay to isolate thermostable mutants of GPCRs[1-3]. This initially led to the structure determination of the thermostabilised turkey β-1 adrenergic receptor mutant (β 36-m23) in complex with the antagonist cyanopindolol at 2.7 Å resolution[4], with a further 8 structures recently refined with different antagonists and agonists bound[5]. Recent work has also demonstrated the feasibility of transferring the thermostabilising mutations to related receptors[6] and enhancing the thermostability of β 36-m23 further, which resulted in better ordered crystals. The thermostabilisation strategy and its applications will be discussed.

1 Serrano-Vega, M. J., Magnani, F., Shibata, Y., and Tate, C. G., Conformational thermostabilization of the b1-adrenergic receptor in a detergent-resistant form. Proc Natl Acad Sci U S A 105, 877 (2008).
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Notes:

S17. AMPA receptor positive modulators: from crystal structure to clinical trial

Simon Ward
Now at Sussex..

The AMPA receptor subtype of ionotropic glutamate receptors mediates the majority of fast, excitatory signalling within the central nervous system. AMPA receptor-mediated cell depolarization leads to calcium influx via NMDA receptors and the induction of synaptic plasticity, which is thought to be associated with cognitive processing. Furthermore, there is considerable evidence to support the hypothesis that a hypoglutamatergic state may underlie the symptoms of many disease states, including schizophrenia. Re-instating glutamatergic function in schizophrenia could be achieved by positive modulation of AMPA receptors. AMPA receptor positive modulators slow the rate at which the receptor (a) desensitizes in the continued presence of glutamate, or (b) deactivates after removal of glutamate. The resulting prolongation of glutamatergic currents promotes synaptic transmission and plasticity.

AMPA receptors consist of a family of hetero-tetrameric receptors arising from four genes, each of which encodes a distinct receptor. The many different subunit permutations make possible the functional and anatomical diversity of AMPA receptors throughout the CNS. AMPA receptor subunit stoichiometry influences the biophysical and functional properties of the receptor by modifying parameters such as receptor kinetics, channel open time, internalization and trafficking to, and from, the post-synaptic membrane.
A number of chemically diverse AMPA receptor positive modulators have been identified which potentiate AMPA receptor-mediated activity and some of these molecules have been advanced to Phase II clinical trials, although none yet have successfully progressed further.
This presentation outlines work to identify a chemically distinct series of AMPAR positive modulators addressing the challenges created by the heterogeneity of the AMPA receptor populations and the development of structure activity relationships (SAR) driven by homomeric, recombinant systems on high throughput platforms. In particular, the role of X-ray crystallography will be described in guiding the selection and prioritisation of targets through the lead optimisation process and ultimately delivering a novel drug candidate for clinical evaluation.

Notes:

P07. Successful prediction of the intra and extracellular loops of 4 G-protein-coupled receptors

Dahlia A. Goldfeld, Kai Zhu, Richard A. Friesner
Department of Chemistry/Columbia University

We present de novo results of the restoration of all crystallographically available intra and extracellular loops of four G-protein-coupled receptors (GPCRs): bovine rhodopsin, the turkey Beta-1 adrenergic receptor, and the human Beta-2 adrenergic and A2A adenosine receptors. We use our Protein Local Optimization Program (PLOP), which samples conformational space to build sets of loop candidates and then discriminates between them using our physics based, all-atom energy function with implicit solvent. We also discuss a new membrane method developed for GPCR loops that interact, either in the native structure or in low energy false positive structures, with the membrane, and thus exist in a multi-phase environment not previously incorporated in PLOP. Our results demonstrate a significant advance over previous work reported in the literature, and of particular note we are able to accurately restore the extremely long second extracellular loop which is key for GPCR ligand binding. These results represent an encouraging start for the more difficult problem of accurate loop refinement for GPCR homology modeling.

Notes:

P08. Simulation studies for the investigation of the interactions between the amyloid-β (Aβ) peptide and lipid bilayers

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The etiology of Alzheimer's disease is linked to interactions between amyloid-β (Aβ) and neural cell membranes, causing membrane disruption and increased ion conductance. It was shown that Aβ1-42 stably incorporates into the plasma membrane [1]. The effects of Aβ on lipid behavior have been characterized experimentally, but structural and causal details are lacking. Simulations of Aβ in a bilayer may provide the information necessary to explain the toxicity of Aβ.
We have employed atomistic MD simulations totaling over 7.5 microseconds in simulation time to investigate the behavior of Aβ1-42 in zwitterionic POPC and DPPC, and anionic POPG bilayers. For the initial Aβ1-42 structures we used a transmembrane β-sheet (monomer and tetramer) resulting from basin-hopping global optimization in an implicit membrane [2] and a helical structure obtained from an NMR study in an apolar solvent [3]. Mutational studies were performed for the β-sheet to assess the role of charged residues on stability of this transmembrane structure. Finally, we performed umbrella sampling MD simulations to investigate the insertion of Aβ1-42 and the more toxic E22G mutant into the POPC lipid bilayer. In all MD simulations Aβ1-42 remained embedded in the bilayer, with slow unfolding of the peptide monomer in the bilayer occuring in some cases. The N-terminal segment of the peptide outside the membrane strongly interacts with the lipid headgroups, leading to a disordering of the headgroup arrangement. We observe β-sheet and coil structures being able to draw water molecules into the membrane, while no water molecules enter the hydrophobic core when the helix is stable. We find increased stability for the β-sheet tetramer, due to interpeptide interactions, and for the E22G mutant, due to the reduced charge in the membrane core. These studies give us insight into the mechanism of the interaction between Aβ1-42 and lipid membranes. We discuss our findings in terms of neuronal toxicity of the various Aβ mutants.
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Notes:

P09. Membrane topology of the Transient Receptor Potential Ca^{2+} Channel TRPC5

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Department of Pathophysiology, Shenyang Medical College, China

TRP (Transient receptor potential) channels form ion conducting channels which are activated following receptor stimulation. TRPs contain 6TM subunits that assemble as tetramers to form cation-permeable pores. They were thought to adopt the topology similar to Kv channels. To update, two possible topologies of TRPC channel has been proposed, TRPC1 and TRPC3 models. The discrepancies are the first 3 segments. TRPC3 model thought that the first S1 segment is positioned in the cytosol and S2-S3 segments are integrated into membrane. In contrast, TRPC1 model shows that the first S1-S2 is integrated into membrane and S3 is positioned in the cytosol. To make clear the topology of TRPC channels, we assessed the biogenesis and topology of TRPC5 using glycosylation and truncation methods (N-terminal region deleted). We found that, in vitro, TRPC5 may adopt two mixed topologies, TRPC1 model and TRPC3 model. Similar results were found in TRPC1, TRPC3 and hTRPM2 channels. TRP channels have ankyrin domains in the N-terminal region, which may play important roles on the multimerization of the channel. Thus, we hypothesize that TRPC channel may take the topology of TRPC3 model; the multimerization formation of the N-terminal region of TRP channels may constrain the S1 segment located in the cytosol, which likely meets with the requirement of the space between ankirin region and the end of the first TM. Since S1 has a moderate topogenic function, it is also a key factor affecting membrane topology of the following segments.

Notes:

P10. United-atom lipid models for molecular dynamics simulation of membrane protein

Andreas Kukol
School of Life Sciences, University of Hertfordshire

Molecular dynamics (MD) simulations of biomolecules are performed at various levels of atomic detail from all-atom models to coarse grained models. United-atom models are at an intermediate level, in which non-polar hydrogen atoms are subsumed into their adjacent carbon-atom reducing the number of particles for a DPPC lipid molecule by 60%. United-atom models of the lipids DPPC, DMPC, POPC and POPG in the GROMOS96 53a6 force field were developed, that reproduce experimental properties of lipid bilayers without assumption of a constant surface area or inclusion of surface pressure. In the absence of experimental data a surface per lipid area for the negatively charged POPG of 0.700±0.007 nm2 was obtained that is higher than 0.53 to 0.56 nm2 reported in previous simulation studies in the literature. It is argued that MD simulations of anionic bilayers may have underestimated the steric requirements of sodium counter ions entering the head group region in order to compensate the negative charge. The use of the DMPC GROMOS96 53a6 model was evaluated in an MD simulation of the ErbB2 transmembrane peptide and showed closer agreement with the NMR-derived structure than the GROMOS87 force field that was most commonly used for membrane protein simulations previously. Lipid topologies and coordinates are available via LipidBook (http://lipidbook.bioch.ox.ac.uk/).

Notes:

P11. Exploration of the human beta adrenergic receptors

Abigail Emtage[1], Phillip Stansfeld[2], Barrie Kellam[1], Peter Fischer[1], Mark Sansom[2] and Charles Laughton[1]
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The beta adrenergic receptors (β-ARs) are an important class of therapeutic targets. Of particular interest are the β1-AR and β2-AR subtypes. All clinical β 1-AR antagonists (β-blockers) used in the treatment of cardiovascular disease also have effects at the β2-AR which can cause exacerbation of airway disorders and other unwanted side effects. Obtaining selective ligands is therefore a highly desirable goal.

Using homology modelling methods and the latest crystal structure data, we have predicted the structure of the human β1-AR. There is little deviation in overall structure of the β1-AR model from its turkey counterpart (which was used as the template, pdb codes: 2VT4, 2Y00, 2Y01, 2Y02, 2Y03, and 2Y04)[1, 2]. To obtain a more detailed view of the differences between the β1 and β2 adrenergic receptors three main strands of work are underway: 1) The homology models are being validated and refined using docking studies with known ligands of varying efficacy and selectivity, with the aim of reproducing known structure-activity relationships. 2) The dynamics of the models in a lipid bilayer environment are being investigated, using multiscale molecular dynamics simulation methods[3]. 3) The structure of the β-AR binding pocket is being explored using Active Site Pressurization[4], to further the search for stable regions of the pocket, explore plasticity and map any differences between the subtypes of interest.

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P12. Cell-free expression yield enhancement by template optimization

Stefan Haberstock, Christian Roos, Volker Dötsch, Frank Bernhard
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Cell-free expression has developed into a versatile and efficient technique for the production of membrane proteins by avoiding some of the central drawbacks of classical in vivo expression systems like translocation system overload and target toxicity. We have established several expression modes specific for membrane proteins based on the tolerance of cell-free reactions for various artificial hydrophobic environments such as lipids, detergents or surfactants. High quality samples of even difficult targets such as G-protein coupled receptors (GPCRs) can be produced in less than a day and in amounts suitable for structural approaches. Initial expression rates of new targets often vary in the E. coli cell-free expression system starting from only few µg of protein up to several mg per ml of reaction mixture. Significant improvement of target expression is therefore frequently necessary and it is supported by the open accessibility of the reaction as well as by the implementation of PCR products as expression templates. We have established an expression template optimization strategy based on the screening of small nucleotide sequences (expression tags) inserted downstream of the ribosome binding site. We demonstrate a screening approach on sets of GPCRs and exemplify the complete construct optimization process.

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P13. Directing Cell-free Expressed Membrane Proteins into Lipid Bilayers: Evaluating the L-CF Mode

Christian Roos, Stefan Haberstock, Karsten Mörs, Friederike Junge, Volker Dötsch & Frank Bernhard *Institute for Biophysical Chemistry, Goethe-University, Frankfurt am Main*

Conventional protocols for the production of membrane protein (MP) samples inserted into defined lipid bilayers are time consuming and include protein solubilisation, purification procedures and in vitro reconstitution steps. Cell-free expression in presence of supplemented artificial liposomes (L-CF mode) would provide a much more straight-forward approach to shorten workload and time requirements by the direct co-translational integration of the nascent polypeptide-chain. We have evaluated requirements for the efficient L-CF expression of MPs in E. coli cell-free expression systems. A library of different MP-GFP fusions comprising transporters, channels and GPCRs was used as targets. Parameters analyzed for their effects on the co-translational MP translocation in the L-CF mode included (I) type of supplemented lipids; (II) size and composition of artificial liposomes; (III) lipid/detergent mixtures; (IV) nanodiscs; (V) translation rate and (VI) concentrations of system compounds. The L-CF expressed MPs have been characterized by insertion rates and functional activity. The results will help to define general guidelines for the efficient L-CF expression with regard to MP characteristics (size, number of TMS, topology etc.) in order to allow a time optimized expression and simultaneous lipid bilayer insertion of MPs of different origins.

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P14. Structural Investigations of Membrane Protein OEP16

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The 16kDa transmembrane amino acid transporter, Outer Envelope Protein (OEP16) is recombinantly expressed and investigated through CD, Light Scattering, and NMR spectroscopy. Using improved expression and purification techniques, it is possible to obtain the necessary amount and concentration for NMR spectroscopy studies. This new technique also allows for purification in several different detergent micelles. The search for optimal protein conditions for NMR studies involves CD analysis and 1H-15N HSQC NMR spectra, including secondary structure estimation as well as melting profiles. The multimeric state of OEP16 is studied through light scattering techniques. Due to the problem of spectral overlap of peaks in solution NMR caused by the narrow chemical shift distribution of alpha helical membrane proteins and slow correlation time of the large protein-micelle complex, more specialized labeling techniques are required to improve resolution quality. One such technique is via partial selective deuterium exchange as a way to observe only buried transmembrane regions. Initial studies show that the long correlation times of the large protein-micelle complex limit certain NMR experiments, but promise is shown with initial HNCA and NOESY-HSQC results.

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P15. Characterization of a truncated form of Lecithin Retinol Acyltransferase

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Ophtalmology / Université Laval

Lecithin retinol acyltransferase (LRAT) is a 230 amino acids membrane-associated protein that catalyzes the esterification of all-trans-retinol into all-trans-retinyl ester, an essential reaction in the vertebrate visual cycle. The present study was performed to investigate the enzymatic activity, the secondary structure and the membrane binding properties of a truncated and soluble form of LRAT (tLRAT). The mutant S175R responsible for retinis pigmentosa has also been studied. tLRAT and S175R mutants have been overexpressed in E. coli and purified. The enzymatic activity of both mutants was studied and their secondary structure was compared by performing circular dichroism measurements. Moreover, the membrane binding properties of both tLRAT and S175R were studied using lipid monolayers, a model membrane system. The maximal enzymatic activity of tLRAT is ~2300 mol of retinyl ester/min . mol of protein. The mutation S175R vanishes the enzymatic function of tLRAT. However, circular dichroism measurements show that tLRAT and the S175R mutant have the same major alpha helical content. Monolayer studies allowed to determine that both tLRAT and S175R strongly bind to membranes even though their N- and C-terminal hydrophobic peptides have been removed. In conclusion, all of these results suggest that the inhibition of the activity resulting from the S175R mutation could be explained a steric blocking of the active site rather than from protein denaturation or a lack of membrane binding.

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P16. In vitro refolding, oligomerisation and characterization of a *Brucella melitensis'* outer membrane protein.

G. Roussel[a], X. De Bolle[b], J. Wouters[a], E.A. Perpète[a] and C. Michaux[a].

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Membrane proteins play a central role in many cellular and physiological processes, and are therefore essential therapeutic targets. Their isolation and characterization both remain challenging issues in biochemistry. In this contribution, we describe the refolding of the porin Omp2a from Brucella melitensis a ß-barrel outer membrane protein (OMP). That is an intracellular pathogen infecting numerous mammalian species. Refolding is a crucial step to obtain a pure and native protein and to allow the study of its structure and function(s). From inclusion bodies, two methods were applied to refold the protein: (i) the usual screening that shows the mild detergent n-dodecylmaltopyranoside (DDM) to be the only one to refold Omp2a. (ii) an original protocol where the association of a denaturing detergent, sodium dodecylsulfate (SDS) and an amphiphilic cosolvent, 2-methyl-2,4-pentanediol (MPD) that leads to the refolded Omp2a. This new strategy was already successfully applied to PagP, a ß-barrel enzyme. The refolding course was monitored by SDS/PAGE and tryptophan intrinsic fluorescence. Several factors influencing the process (i.e. temperature, time, protein concentration and ionic strength) were optimized. It hypothesized that the refolding is a two-stage process, where unfolded monomers are first refolded and then associated into trimers. Finally, the three-dimensional characterization will be performed on crystals already obtained by both methods (DDM and SDS/MPD).

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MGMS Spring Meeting 2011

P17. **Lisa M Simpson, Ian D Wall and Christopher A Reynolds**
University of Essex

The recent crystal structure of a mutated ß1adrenergic receptor, ßAR-m23, resulted from careful selection of mutants designed through thermostabilization experiments. Here, we have we have used computation with a view to understanding and predicting stability. Analysis of molecular dynamics trajectories revealed that the ßAR-wt rigid core was centred on TM2/3 whereas in ßAR-m23, the core moved onto regions involved in activation, indicating that equilibrium had shifted towards the inactive state. The loops showed less movement in ßAR-m23 than in ßAR-wt; these are involved in packing interfaces within the periodic lattice and their stabilization may have contributed to the ease of crystallization. Correlation analysis showed that in the ßAR-wt, TM5, ICL3, and TM6 (key to activation) move as a separate domain, whereas in ßAR-m23 large correlated motions are disrupted by the anti-correlated behaviour of the loops. Most stabilizing mutations are within low connectivity regions known to be involved in activation. Evidence is presented that they function by locking the ß1-AR into an inactive state by disfavouring motion that leads to the active state. The remaining stabilizing mutations are within high connectivity regions and probably function by enhancing core stability.

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MGMS Spring Meeting 2011

P18. The class A – class B GPCR alignment

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Homology modelling of class A G-protein coupled receptors (GPCRs) has been greatly facilitated by the recent X-ray crystal structures of rhodopsin and the beta-adrenergic receptor, enabling a wealth of functional studies to be interpretted in the light of structure. For the class B GPCRs, which include medically important targets such as receptors for secretin and the calcitonin gene-related peptide (CGRP), this has not been an option because class A and class B GPCRs are remote homologues. Here we show that GCR1, the only well-characterized plant GPCR, has homology to both class A and class B GPCRs and that this property can be used to address the class A – class B alignment problem. By considering the alignment with GCR1, the variability amongst subsets of highly similar sequences for both class A and class B GPCRs and other physical properties, we have been able to align the transmembrane regions of class A and class B GPCRs. The resulting homology models of the CGRP receptor have been used to interpret site-directed mutagenesis results, indicating new similarities between class A and class B GPCRs. The significance of these results reside in the use of plant bioinformatics to interpret mamalian structure and function, the general usefulness of the class A – class B alignment, the generatoin of a CGRP homology model and the indication that variability amongst subsets of highly similar sequences may become a useful bioinformatics tool for aligning remote homologues.

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MGMS Spring Meeting 2011

P19. Design and Synthesis of Novel Inhibitors of Ion Channels and Transporters as Potential Drug Targets

Katie J. Simmons Colin W.G. Fishwick A. Peter Johnson
University of Leeds

Membrane proteins are essential to regulating biological activity within cells, giving us our sense of sight, the ability to breathe, convert nerve signals into actions and use nutrients for energy. They are ideal targets for the development of new therapies. Despite their natural abundance and clinical significance, the structures of fewer than one hundred independent transporter or channel proteins have been determined. These available X-ray crystal structures, along with homology modeling and molecular dynamics, has allowed us to identify a number of suitable membrane protein targets for the discovery of suitable small molecule modulators, using virtual high-throughput screening (vHTS) and de novo design campaigns. We have identified inhibitors of several membrane proteins using this approach, including the bacterial Leucine transporter and the integral membrane protein Mhp1, from Microbacterium liquefaciens. Both of these proteins are crucial in developing an understanding of the molecular mechanism of many human transporter proteins, which are implicated in glucose/galactose malabsorption, congenital hypothyroidism, Bartter's syndrome, epilepsy, depression, autism and obsessive-compulsive disorder. We have also targeted a number of aquaporin and aquaglyceroporin proteins, which form pores in the membrane of cells. If aquaporins could be manipulated, they could potentially solve medical problems such as fluid retention in heart disease and brain oedema after stroke. The aquaglyceroporin from Plasmodium falciparum (pfAQP) plays an important role in malarial biology by providing access to host serum glycerol pools and ensuring survival during osmotic stress. Using a vHTS screening approach we have identified several inhibitor of pfAQP, one of which also exhibits an IC50 against the P falciparum parasite of 840 nM in an intraerythrocytic assay.

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MGMS Spring Meeting 2011

P20. Progresses and Challenges in the Prediction of GPCRs: D3 Receptor in Complex with Eticlopride

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Research Program on Biomedical Informatics (GRIB), IMIM / Universitat Pompeu Fabra

Predicting the three-dimensional structure of G protein-coupled receptors (GPCRs) and the binding mode of potential ligands is still a challenging task in rational drug design. To evaluate the reliability of the GPCR structural prediction, only a couple of community-wide assessments have been carried out. Our participation in the last edition, GPCR DOCK 2010 assessment organized by the Scripps Institute of California [1], involved the blind prediction of the dopaminergic D3 receptor in complex with the D2/D3 selective antagonist eticlopride for which the crystal structure has been recently released (PDB 3PBL [2]). Here we present the applied methodology which resulted in a correctly predicted ligand pose of ecticlopride in the D3 receptor (first place in the GPCR DOCK 2010) as well as discuss encountered limitations of the modeling methods [3]. Overall, the success of our case study highlights the current progress in modeling GPCR complexes and underlines that in-silico modeling is nowadays a valuable complement in GPCR drug discovery.

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P21. Expression, purification and biophysical characterization of a 3-TM GPCR fragment

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Structure-based drug discovery is currently considered to be one of the most reliable and efficient methods for the design of novel drugs. Therefore, much effort has been undertaken to determine the structure of new potential drug targets. In this context, the G-protein coupled receptor protein family (GPCRs) represents one of the most important class of drug-targets. Unfortunately, only a limited number of GPCRs structures has been solved up to now, exclusively using X-ray crystallography (1). Besides crystallography, NMR spectroscopy is a powerful alternative (2,3). The study of GPCR fragments is justified mainly for two reasons: first, because a fragment can be considered as in independent folding unit (4) from which we can obtain structural informations (e.g. ligand binding); second, the work with the fragments will enable the establishment of robust protocols in our lab for the complete characterization the full-length receptor. In this context, we have started the project presented herein with the aim to determine the structure of different fragments and the entire human neuropeptide Y4 receptor, whose peptide ligands regulate several important functions including food intake, circadian rhythms, mood, blood pressure, intestinal secretion, and gut motility. The fragment consisting of N-TM1-TM2-TM3 helices has been expressed in fusion with a soluble tag that localizes it into the E.coli inner membrane, enabling the purification in the natively folded form.

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MGMS Spring Meeting 2011

P22. Structural studies of a ß1-adrenoceptor: thermostabilisation by mutagenesis improves crystal quality

Jenny Miller Tony Warne Pat Edwards Andrew Leslie Chris Tate
MRC Laboratory of Molecular Biology

G protein-coupled receptors are important drug targets for many diseases and neurological disorders due to their pivotal role in intercellular communication. Structural studies of these integral membrane proteins are essential for drug design and understanding the mechanism of receptor activation. However, X-ray crystallography of membrane proteins is challenging due to their instability in the detergents required for purification and crystallography. Our lab has developed a method for increasing the thermostability of membrane proteins by mutagenesis coupled to a radioligand binding assay, which has led to the structure determination of the thermostabilised turkey ß1-adrenoceptor, ß1AR-m23. Further stabilisation of ß1AR-m23 was attempted by reducing the movement of helices through the introduction of disulphide bonds, zinc bridges and salt bridges. In addition, leucine-scanning mutagenesis of ß1AR-m23 identified further thermostabilising mutations. Addition of the three most thermostabilising mutations to ß1AR-m23, to generate ß1AR-JM3a, improved thermostability by a further 14 °C. Crystallisation of cyanopindolol-bound ß1AR-JM3a in lipidic cubic phase reproducibly produced crystals that were about ten times larger than those obtained for ß1AR-m23 grown under similar conditions, with higher resolution diffraction (2.1 Å) than previously obtained. This suggests that increased thermostabilisation can improve both the size and diffraction quality of membrane protein crystals.

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MGMS Spring Meeting 2011

P23. Brownian dynamics simulations of FYVE domain association to PtdIns(3)P containing membranes

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The FYVE domain is a protein domain that associates to membranes containing phosphatidyl 3-phosphate (PtdIns(3)P). Once membrane bound, FYVE domains dimerise and facilitate recruitment of proteins involved in cell signaling. The FYVE domain is defined by three conserved elements that define positively charged binding site: an N-terminal WxxD; central RR/KHHCR; and a C-terminal RVC motif. The two histidine residues from the central motif are well conserved amongst FYVE domains. Protonation of these histidine residues, which is dependent on the cytosolic pH, regulates binding of FYVE domains to PtdIns(3)P containing membranes. In addition to the positive charge carried by the PtdIns(3)P binding site, membrane anchoring is also facilitated by insertion of the hydrophobic turret loop near the PtdIns(3)P binding pocket. There is a crystal structure of the head group of PtdIns(3)P (Ins(1,3)P) in complex with a FYVE domain which allows us to perform calculations of association rates (using Brownian dynamics techniques). Furthermore, we perform rate calculations of FYVE domains binding to both an idealised membrane, and a membrane with explicit consideration of PtdIns(3)P. The use of several different FYVE domain mutants allows us to evaluate the success of a Brownian dynamics method for performing association rate calculations for FYVE domains binding to biological membranes and whether one representation of a membrane is superior to another for this purpose. Acknowledgements The authors acknowledge the BMBF (German Federal Ministry of Education and Research) Virtual Liver Network and Klaus Tschira Stiftung for funding. The authors also wish to thank Vlad Cojocaru for provision of phospholipid simulation parameters.

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MGMS Spring Meeting 2011

P24. Membrane protein structure analysis and visualisation with MPLOT and PROVI

Alexander Rose, Dominic Theune
Charite Institute of Medical Physics and Biophysics

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MGMS Spring Meeting 2011

P25. Expression and Purification of Human Equilibrative Nucleoside Transporter-1 (hENT1) in *Saccharomyces cerevisiae*.

Shahid Rehan[1,2] and Veli-Pekka Jaakola[1,2]

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The SLC29 family of protein consists of four members of equilibrative nucleoside transporters (ENTs). ENTs are recently characterized and poorly understood group of membrane proteins that are important in uptake of endogenous nucleoside essential for nucleic acids and nucleotides synthesis. As ligands to widely distributed cell-surface adenosine receptors, they have direct regulatory role in many physiological processes. These proteins are also responsible for the cellular uptake of nucleoside analogue use to treat cancer, HIV and many other viral diseases.
We have recently cloned, expressed and successfully purified hENT1 protein in yeast Saccharomyces cerevisiae. Full length gene of hENT1 was cloned into 2µ GFP-fusion vector by homologous recombination and protein expression was monitored by whole cell and in-gel fluorescence, flow cytometery and hemocytometery. Over expression potential of hENT1 was evaluated by testing different expression conditions, induction time and temperature. Detergent solubilization screen was carried out with previously most successfully used detergents. IMAC purified protein was subjected to SDS-PAGE and detection through western bloting. Localization and monodispersity was analyzed by confocal microscopy and fluorescence size exclusion chromatography (FSEC) as well as static-light scattering. This study revealed that expression of hENT1 is optimum at 22 °C in URA-negative selective media. Maximum protein yield was achieved by incubating the culture for 17-18 hours. We are currently engaged with protein stability and ligand binding studies.

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MGMS Spring Meeting 2011

P26. **Jochen Ismer, Johanna Tiemann**
Charite, Institute of Medical Physics and Biophysics

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MGMS Spring Meeting 2011

P27. The rhodopsin model: crystal structures and theoretical considerations

Peter W Hildebrand, Patrick Scheerer, Klaus Peter Hofmann
Institute of Medical Physics and Biophysics / Charité

G protein-coupled receptors (GPCRs) are ubiquitous signal transducers in cell membranes and important drug targets. Interaction with extracellular agonists turns the seven transmembrane helix (7TM) scaffold of a GPCR into a catalyst for GDP and GTP exchange in heterotrimeric Gαβγ proteins. The model GPCR, rhodopsin, is activated by photoisomerisation of its retinal ligand. From the augmentation of biochemical and biophysical studies by recent high resolution 3D structures, its activation intermediates can now be interpreted as the stepwise engagement of protein domains. Rearrangement of TM5/TM6 opens a crevice at the cytoplasmic side of the receptor into which the C-terminus of the Gα-subunit can bind. The Gα C-terminal helix is used as a transmission rod to the nucleotide binding site. The mechanism relies on dynamic interactions between conserved residues and could therefore be common to other GPCRs.
(1-4)

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Notes:

MGMS Spring Meeting 2011

P28. **Heiko Bittner, Peter Hildebrand, Gerd Multhaup**
Institute of Medical Physics and Biophysics

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MGMS Spring Meeting 2011

P29. New Insights into the Human Histaminergic Type 4 Receptor (HRH4): Increasing HIT Rates of Antagonist

Chiara B.M. Platania[1§], Matteo Pappalardo[2], Danilo Milardi[3], Salvatore Guccione[1] and Anwar Rayan[4]

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Selective antagonists of the GPCR (G-Protein Coupled Receptor) human Histamine subtype 4 (hH4R) may have therapeutic significance in treating allergy, inflammation, autoimmune disorders, pain conditions and possibly cancer [1]. Different in silico techniques were used to increase the hit rate in virtual screening experiments, seeking for new hH4R antagonists.

First a "molecular activity index" was defined by the ILE technology [2] on the basis of optimized differences between H4R-antagonists vs. non-active molecules and used to screen the ZINC database. Highly indexed chemicals were picked out to be tested as H4R antagonists and docked to homology models using AutoDock 4, in order to both validate those models and find out which model better performs in the filtering of potentially H4R antagonists.

Two structural models of the hH4R were built. The first model is limited to the transmembrane-domain of the receptor whether the second one is the whole receptor bearing also the extracellular disulfide-bridge. Long term molecular dynamics (MD) simulations, in water and membrane environment, were used to optimize both the structures.

Multiple focusing combining chemoinformatics (ILE technology), MD and docking led to a very promising enrichment factor. The described approach will be further tested on other pharmacological targets.

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MGMS Spring Meeting 2011

P30. Interpreting Experimental Analysis of the hERG Potassium Channel using Molecular Dynamics Simulations and in silico Docking

Charlotte Colenso, Yi Hong Zhang, Richard B. Sessions, Jules C. Hancox and Chris E. Dempsey
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hERG potassium channels are responsible for conducting the rapid component of the delayed rectifier current, IKr in human heart. Genetic mutations and drug blockade disrupt normal channel function leading to cardiac arrhythmias which may result in sudden death. 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 Kv1.2/Kv2.1 'paddle chimera' structure in the open state. We employ molecular dynamics simulations within a lipid bilayer to investigate the effect of mutations on channel properties and gating mechanisms. We have also performed in-silico alanine-scanning mutagenesis to assess docking of amiodarone, a class III antiarrhythmic agent, with a KvAP 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.

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MGMS Spring Meeting 2011

P31. Improving Molecular Dynamics Sampling Using a Cooperative Swarm of Simulation Replicas

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While molecular dynamics simulations offer the potential to provide insights into many biological processes, current computational power can place limitations on the systems to which they can be applied. The rugged nature of the biomolecular free energy surface means that the time required to observe large scale conformational changes is often longer than is feasible to simulate. A number of techniques exist that seek to smooth the free energy surface while maintaining its representative features, allowing the simulation of longer timescale events. SWARM-MD, first described by Huber and van Gunsteren (1), attempts to smooth this surface of the system through the use of a swarm of multiple interacting simulation replicas that are driven towards the average conformation of the swarm members. We have successfully applied this approach to prediction of the native states of a series of model peptides (2), including Trp-cage miniprotein in aqueous solvent. In each case, the cooperation between replicas was found to improve the convergence of the simulations towards the native state. Extensions to this method and future directions will also be discussed. References: (1) Huber, T., van Gunsteren, W. F. SWARM-MD: Searching Conformational Space by Cooperative Molecular Dynamics, J. Phys. Chem. A, (1998), 102, 5937-5943. (2) Bruce, N. J., Bryce, R. A. Ab Initio Protein Folding Using a Cooperative Swarm of Molecular Dynamics Trajectories, J. Chem. Theory Comput., (2010), 6, 1925-1930.

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MGMS Spring Meeting 2011

P32. Possible mode of binding of allosteric potentiating ligands in nicotinic acetylcholine receptor

Katarzyna Targowska-Duda[1]; Hugo Arias [2]; Irving Wainer[3] ; Pawel Cimek [1]; Krzysztof Jozwiak[1]

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Nicotinic acetylcholine receptors (nAChRs) are archetypical members of the Cys-loop Ligand Gated Ion Channels superfamily. nAChRs are composed of five membrane proteins (subunits) oriented around a centrally located pore permeable to cations. In our studies we developed a panel of methods characterizing interactions of different classes of allosteric ligands with models of extracellular or membrane domain of nAChRs. In current project interactions of Allosteric Potentiating Ligands (APLs) with two models of extracellular part of nAChR. APLs enhance the activity of an agonist without producing effect by themselves. We used two different templates to generate nAChR models: (1) the acetylcholine binding protein (AChBP) obtained from Lymnaea stagnalis (PDB id: 1UV6) and (2) the pentameric ligand-gated ion channel originated from Gloeobacter violaceus (PDB id: 3EAM). Models (1) and (2) are considered to represent two different conformations, the close and the open, respectively. A series of APLs (galanthamine, physostygmine, codeine, serotonin, and ketamine and its metabolites) were docked to these two models. Comparison of simulated docking energies indicates that the interactions of APLs are stronger with the model in the open conformation. In open conformation models the ligands bind at the interface between two subunits whereas in close conformation models, interaction occurs at the outer surface of the receptor. This observation permits to postulate that APLs preferentially interact with the open state of nAChR. Thus, these compounds may stabilize the open conformation of the receptor increasing its sensitivity to agonists.

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MGMS Spring Meeting 2011

P33. Geometric Simulation of Ligand-gated Ion Channels

William Belfield, Daniel Cole, Mike Payne, Pak-Lee Chau, Kenji Mizuguchi, Mizuki Morita
William Belfield
University of Cambridge

A typical method of exploring phase space from an initial structure is molecular dynamics using an empirical force field. However, the necessity for a short timestep (~fs) to obtain an accurate trajectory renders some biological processes, which may occur on millisecond or greater timescales, inaccessible. An alternative technique is to employ a Lagrangian constraint based method. Here, instead of a trajectory advancing in time, a collection of conformers satisfying various constraints is generated. This approach is embedded in the software packages FIRST/FRODA as created by Thorpe, Wells et al. 1,2 Covalent and non covalent bonds are used as inputs to rigidity analysis to create a set of constraints that a valid conformer must meet. Here I show an example of the method being applied to a large scale conformational change of ELIC, a prokaryotic ligand gated ion channel. [1]D.J. Jacobs, A.J. Rader, M.F. Thorpe, and L.A. Kuhn, "Protein Flexibilty Predictions using Graph Theory", Proteins, 44, (2001) [2]S. Wells, S. Menor, B.M. Hespenheide and M.F. Thorpe, "Constrained geometric simulation of the diffusive motions in proteins", Phys. Bio., 2, S127 S136 (2005)

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MGMS Spring Meeting 2011

P34. Stereochemistry of Fenoterol Influences Ligand Specific Activation of B2 Adrenergic Receptor

Krzysztof Jozwiak, Anita Plazinska, Lawrence Toll, Lucita Jimenez, Anthony Yiu-Ho Woo, Rui-Ping Xiao, Irving W. Wainer

Department of Chemistry / Medical University of Lublin, Lublin, Poland

Fenoterol (FEN) is B2-adrenergic receptor (B2-AR) agonist that exists as four stereoisomers, (R,R)-FEN, (R,S)-FEN, (S,R)-FEN, (S,S)-FEN. We have characterized the agonist activity and binding affinities of the isomers and demonstrated that FEN activity is highly dependent upon stereoconfiguration with the relative order (R,R)-FEN>(R,S)-FEN>(S,R)-FEN>(S,S)-FEN. Subsequent Van't Hoff analysis revealed very different thermodynamic characteristic of binding as affinities of (S,S)- and (S,R)-isomers increased with temperature, affinity of (R,S)-FEN decreased while there was no significant effect on (R,R)-FEN binding. Thus, B2-AR interactions with (S,x)-isomers of FEN are almost entirely enthalpy driven whereas interactions with (R,x)-isomers are purely entropy driven. Stereochemistry also affects the G-protein coupling of the receptor. In cardiomyocyte contractility studies, addition of pertussis toxin had no effect on activity of (R,R)-FEN indicating that the receptor selectively couples Gs protein signaling upon binding of this stereoisomer. When (S,R)-FEN was studied, the addition of pertussis toxin significantly reduced the effect indicating that (S,R)- isomer activates both Gi and Gs pathways. Molecular modeling suggested that the Y308 residue of ß2-AR plays a role in this selectivity through the interaction with para-hydroxyl moiety of the (R,R)-FEN molecule. Recent data from binding, activity and G-protein selectivity studies with a Y308A B2-AR mutant support the computer-based postulate . The overall data demonstrate that stereochemistry of FEN molecule influences the magnitude of binding affinity, thermodynamics of local ligand - receptor interactions and the global mechanism of B2-AR activation.

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MGMS Spring Meeting 2011

P35. A Three-Ion Selectivity Filter Potential Energy Landscape of a Putative Open-Conductive KcsA

David Medovoy, Luis G Cuello, Eduardo Perozo, Benoit Roux
Institute for Biophysical Dynamics, University of Chicago

Potassium channels are tetrameric integral membrane proteins that passively allow rapid potassium conduction through the cell membrane while being strongly selective over monovalent cations. A narrow region known as the selectivity filter, conserved in both structure and sequence, presents a series of backbone carbonyls towards the permeating ion. Here, we have performed a full three-ion permeation free energy landscape calculation for the selectivity filter region, either for pure potassium conduction or for a single sodium chaperoned by potassium. Three-dimensional umbrella sampling Potential of Mean Force (PMF) calculations were performed both with and without adjusted carbonyl-ion interaction parameters, based on a new unpublished high-resolution crystal structure of KcsA in a conformation which includes both an open gate and a conductive filter.

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MGMS Spring Meeting 2011

P36. Investigation of the Integrin Inside-out Activation Mechanism

Antreas Kalli, Iain D. Campbell, Mark S.P. Sansom
Department of Biochemistry, University of Oxford

Integrins are major cell surface receptors that are crucial for a variety of cell migration and adhesion events. The formation of a complex between the talin head domain and the integrin ß tail, in addition to talin/membrane interactions, activate integrins. In this study, multi-scale molecular dynamics (MD) simulations were used to probe the talin F2-F3/membrane and talin F3/ß interactions in a POPC/POPG bilayer. A reorientation of the talin F2-F3 domain followed by a large increase in the tilt angle of the ß tail relative to the bilayer normal was observed. Furthermore, our simulations demonstrate that mutation of four basic residues in the talin F2 domain reduces the affinity of talin F2-F3 for the membrane and changes its orientation relative to the bilayer surface. This perturbed orientation is expected, in turn, to perturb talin/integrin interactions. During the simulations, enrichment of the F2-F3 binding surface with anionic lipids reveals an important role for negatively charged moieties in the membrane. On the basis of these simulations, a model for disruption of integrin a/ß transmembrane interactions is proposed in which a large increase in the tilt angle to the ß tail upon talin binding weakens the a/ß transmembrane association and thus leads to integrin tail separation.

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MGMS Spring Meeting 2011

P37. From Coarse-Grained to Atomistic: A Serial Multi-Scale Approach to Membrane Protein Simulations

Phillip J. Stansfeld **Mark S.P. Sansom**
Biochemistry, University of Oxford

Coarse-Grained molecular dynamics (CGMD) simulations provide a means for assessing the assembly and interactions of molecular complexes at a reduced level of representation, allowing longer and larger simulations. Nonetheless, the traditional means for evaluating molecular structure is at the level of the atom. Here we describe a fragment-based protocol for converting the representation of a whole molecular system from CG to atomistic. As well as allowing visualization of the protein-lipid interactions on the atomistic level, this procedure also facilitates a protocol for generating an accurate and well equilibrated membrane-protein complex; suitable for commencing atomistic molecular dynamics simulations. We apply the methodology to ten systems, extracted from the CGDB (http://sbcb.bioch.ox.ac.uk/cgdb/), that contain proteins of distinct family, size and complexity. Simulations are assessed in terms of protein conformational drift, lipid/protein interactions, and lipid dynamics. The protocol enables high throughput multi-scale simulations of membrane protein structures within biological membranes; proving a tool to enhance our understanding of membrane proteins interactions with complex lipid environments.

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MGMS Spring Meeting 2011

P38. Computational studies of large conformational changes in proteins

Oliver Beckstein, Lukas Stelzl, Mark Sansom
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Many proteins function by cycling between different conformational states; examples are molecular motors, enzymes, ion channels, or transmembrane transporters. Studying such transitions experimentally is difficult because the duration of the actual transition event is very short compared to the dwell time in stable and meta-stable states low-energy states. Unbiased equilibrium molecular dynamics (MD) simulations, which can yield unprecedented detail for molecular systems, do not yet typically reach the time scales of milliseconds to seconds necessary to observe a transition. Using a range of examples including the transmembrane transporters Mhp1 and LacY and the enzyme adenylate kinase I will show how macromolecular transitions can be studied computationally if additional information is available. Methods include equilibrium MD, umbrella sampling free energy calculations, and the dynamic importance sampling (DIMS) method to generate ensembles of candidate transition pathways between known endpoints.

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MGMS Spring Meeting 2011

P39. A Novel Approach For Predicting GPCR Oligomerization Based on Classical Protein-Protein Docking and Surface Roughness

Agnieszka A. Kaczor[1,2], Pau Carrió[1], Ramon Guixá[1], Cristian Obiol-Pardo[1], Jana Selent[1] and Manuel Pastor[1]
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Dimerization or oligomerization of G-protein coupled receptors (GPCRs) is a well-established concept, which may lead to the re-evaluation of many ligands acting on these receptors. A large number of experimental data has confirmed the phenomenon of GPCRs oligomerization with physiological and pharmacological significance.
The aim of this work is the elaboration of an efficient protocol for improvement of classical protein-protein docking approaches [1] for modeling GPCRs dimers. Three dimers are studied: the semiempirical model rhodopsin-rhodopsin homodimer [2], the model mGluR2-5HT2A receptor heterodimer [3] and the recently published crystallographic model of the chemokine CXCR4-CXCR4 receptor homodimer [4].
The method involves the application of Rosetta software [5] for obtaining populations of dimers with all the possible interfaces. In order to sort out unrealistic solutions, we apply geometrical constraints. The obtained membrane-compatible solutions are scored by their interface surface area and some parameters derived from the surface roughness calculated as fractal dimension. The best models are minimized and the whole cycle is iteratively repeated until the results converge to a consistent dimer structure. Using this elaborated protocol, we succeeded in reproducing experimentally obtained structures of GPCR dimers, suggesting that this protocol can produce realistic solutions, and be used as a tool for using GPCR dimers as novel drug target.

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MGMS Spring Meeting 2011

P40. Fractal Properties of GPCRs - Part I
Analysis of Surface Roughness

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The fractal nature of proteins manifests itself in the way proteins vibrate and in the manner they fill space [1]. For natively folded proteins it can be described by broken dimensions: the fractal and spectral dimensions [2].
In this study we calculate the fractal dimensions of the residues forming transmembrane helices of β2 adrenergic receptors, which can be used as a measure of surface roughness [3]. In addition, we determine the exposure ratio of each helix residue. Our results show that both roughness and exposure ratio are periodic along the helices which results from helix structure and which is in accordance with earlier results by Renthal [4]. Moreover, the most exposed residues exhibit the highest roughness in the regions where they are surrounded by membrane lipids whereas are much smoother in the intracellular and extracellular termini of the helices where they are embedded in water. This result can be justified by thermodynamic reasons. Alkyl chains, when situated adjacent to a rough surface adopt a thermodynamically favored conformation that is rich in kinks (i.e., gauche-trans-gauche) which is less common when alkyl chains are packed against a smooth surface [4]. Thus, the entropy of the alkyl chains increases when they neighbor with a rough protein surface in comparison with a smooth one. Furthermore, by interaction along smooth surfaces, transmembrane helices exclude these regions from the interactions with lipids, decreasing the overall free energy of the membrane.

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Notes:

MGMS Spring Meeting 2011

Membrane Proteins: Structure and Function Poster and Oral Abstracts 52

P41. Fractal Properties of GPCRs - Part II
Identification of Classical and Novel Binding Sites

Agnieszka A. Kaczor[1,2], Pau Carrió[1], Ramon Guixá[1], Cristian Obiol-Pardo[1], Manuel Pastor[1] and Jana Selent[1]

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Protein surface roughness is a structural property, which was shown to be associated with binding surfaces [1]. It has been demonstrated that small molecule binding sites are usually much rougher than the average for the whole protein. Identification of specific patterns of roughness is a sensitive and reliable tool to detect the binding sites [1]. Surface roughness can be quantified by the fractal dimension df, which denotes the rate of change in the protein's surface area with respect to the yardstick or probe size used to measure it [2].

In this study, we explore the fractal properties of G-protein coupled receptors (GPCR) by calculating the surface roughness expressed as the fractal dimension df for helical regions. We mapped the obtained values of the fractal dimension on the receptor structures to identify small molecule binding sites. We found significantly higher roughness for the cavities forming the orthosteric binding sites, the allosteric binding sites (e.g. sodium binding sites), and patches for cholesterol binding. Additional patches of residues, not yet considered as binding sites for small molecules, were also found.

The obtained results confirm the earlier observations about the increased roughness of small molecule binding sites. We believe that this approach might be helpful in structure-based discovery of novel classes of GPCR modulators.

References:
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MGMS Spring Meeting 2011

Membrane Protein Structure and Function Poster and Oral Abstracts

P42. Fractal Properties of GPCRs - Part III
Is the Oligomerization Interface Smooth or Rough?

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The classical idea that G-protein coupled receptors (GPCRs) operate as monomeric entities is increasingly giving way to the paradigm of dimerization-dependent GPCR function. Experimental and computational efforts have led to the concept that GPCRs exist not only as homodimers and heterodimers, but also that their oligomeric assembly could have important functional roles.

Although the first X-ray structure of functional GPCR dimer has been recently deposited in the Protein Data Bank for the chemokine CXCR4 receptor, the questions about (i) the driving forces of GPCR dimerization as well as (ii) the number of possible oligomerization interfaces remain still open for the majority of GPCRs.

In this respect, fractal properties expressed as fractal dimension proportional to surface roughness can be used to identify binding sites at the protein surface. From a thermodynamic point of view it is favorable that transmembrane α-helical proteins bury their smooth regions in intramolecular interactions between helices and expose their rough residues to the contact with the alkyl chains of membrane lipids. Controversially for intermolecular contacts (protein oligomers), Pettit and Bowie [1] postulated that protein-protein interfaces are a little rougher compared to other regions of the protein chain but still less rough than small molecule binding sites.

To address these problems, we analyze the roughness patterns of the oligomerization interface for the chemokine CXCR4 receptor as found in the crystal structure of its dimer. The calculation of the fractal dimension of the interface forming residues of both monomers, shows that the TMV-TMVI interface of the chemokine CXCR4 is rougher than the average protein surface for both monomers which is consistent with earlier results [1]. Interestingly, a second region of higher roughness was identified in the protein surface at the intracellular half of TMII, III, and IV indicating an evolutionary conserved binding site for cholesterol which could mediate oligomerization [2].

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MGMS Spring Meeting 2011

P43. Structure and Ligand Based Design in the Identification of Chemokine Receptor Antagonists.

Anny-Odile Colson, Ph.D.
Novartis Institutes for Biomedical Research
Global Discovery Chemistry – Computer Aided Drug Design Horsham, United Kingdom

Chemokine receptors are G protein coupled receptors which play a key role in the pathogenesis of inflammation, viral infection and autoimmune diseases. As such, they make an extremely attractive therapeutic target for the pharmaceutical industry. This talk will present some of our efforts in developing CCR3 and CCR9 antagonists in the treatment of asthma and IBD. Polypharmacology will also be discussed.

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MGMS Spring Meeting 2011

P44. Self-assembly of polyhedral clathrin cages simulated

W.K. den Otter W.J. Briels
Computational BioPhysics, University of Twente

Clathrin is a three-legged protein with the intriguing ability to self-assemble into polyhedral cages. This growth process enables living cells to 'swallow' external molecules (endocytosis), by wrapping the cell membrane around the cargo. Our computer simulations using a highly coarse-grained model indicate that the key to self-assembly is neither clathrin's characteristic triskelion shape, nor the alignment of four legs along evergy cage edge, but an asymmetric distribution of interaction sites. The simulations also answer the long-standing question of how flat patches of purely hexagonal clathrin lattice, which often decorate membranes in electron microscopy images, can produce cages with hexagonal and exactly twelve pentagonal faces.

Notes:

P45. MRAP - a GPCR accessory protein study

Barbara Sladek & Jason R. Schnell
Department of Biochemistry, Membrane Protein Interaction Laboratory, University of Oxford,

The G-Protein coupled receptor, Melanocortin-2-Receptor (MC2R), is the receptor for the pituitary hormone ACTH that is involved in stress responses. When activated, MC2R stimulates a cAMP pathway leading to the production of cortisol. For both trafficking from the endoplasmic reticulum to the cell surface, and receptor activity, MC2R requires the melanocortin receptor accessory protein (MRAP) - a single-transmembrane domain protein. A remarkable characteristic of MRAP is its native dual-topology: MRAP functions as an anti-parallel homodimer. With the goal of understanding the mechanism by which MC2R trafficking and signalling is activated by MRAP, we have solved the monomeric structure of MRAP in detergent micelles by solution NMR. Current structural studies are focussed now on a homodimeric form of MRAP. The structure will provide clues to the residues responsible for the interaction of MRAP with MC2R, and more generally help to understand the mechanisms of transmembrane protein insertion and dimerization.

Notes:

P46. Effects of the lipid bilayer phase state on the water membrane interface.

Stepniewski M, Bunker A, Pasenkiewicz-Gierula M, Karttunen M, Róg T.
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In this work we compared the properties of the water-membrane interfaces of two thermotropic bilayer phases using a 200ns MD simulations of phosphatidylcholine (PC) bilayers in the liquid crystalline (L(a)) and gel (L(ß)') states. The membrane phase was shown to determine the behavior of water, ions, and PC head groups. The gel phase was characterized by a partial dehydration (fewer PC-water interactions), particularly near the carbonyl groups region, as well as an almost complete lack of ionic penetration. On the other hand, the liquid-crystalline phase of the bilayer was characterized by an exchange of Na(+) ions between the water layer and the interfacial region. The main reason for this is the most stable binding of Na(+) in the liquid-crystalline bilayer to the carbonyl groups. The compact structure of the bilayer in the gel phase can explain the lack of Na(+) binding to the carbonyl groups. For more information about this work, please read: Stepniewski M, Bunker A, Pasenkiewicz-Gierula M, Karttunen M, Róg T. 'Effects of the lipid bilayer phase state on the water membrane interface.' J Phys Chem B. 2010 Sep 16;114(36):11784-92.

Notes:

P47. Dynamics and gating of an ionotropic glutamate receptor

Ranjit Vijayan and Philip C. Biggin
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Ionotropic glutamate receptors are modular ligand-gated cation channels that modulate the vast majority of excitatory neurotransmission in the central nervous system. A series of X-ray crystal structures of the amino terminal domain (ATD), ligand binding domain (LBD) and finally the full-length receptor has provided atomic level insights into subunit organization and interfaces of the receptor. However, the open state of the channel is currently unknown. The free energy of ligand binding in the LBD is believed to drive the opening of the transmembrane pore. From the ligand-free (LBD open) and ligand-bound (LBD closed) structures of the LBD, we derived possible transition pathways using dynamic importance sampling. Simulations indicate that the LBD closing event is associated with the rearrangement of a salt bridge in the "hinge" region of the LBD. These transition pathways were then used to drive channel opening using restrained and equilibrium molecular dynamics simulations. In spite of near four-fold symmetry in the transmembrane domain (TMD), results suggest that the channel opens asymmetrically reflecting the dimer-of-dimer assembly of the extracellular domains and structural differences of the LBD-TMD linkers.

Notes:

P48. Molecular basis of Na+/H+ antiporter selectivity

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One of the most fundamental requirements of any living organism is to maintain and control its internal environment in the face of a constantly changing surrounding. This challenge is met by transporters and channels that rely on their ability to selectively discriminate between various solutes. Herein, we examine in detail the selectivity mechanism of one of the most important salt transporters: the Na+/H+ antiporter. Using both computational and experimental analyses synergistically, we show that binding per se is not a sufficient determinant of selectively. All alkali ions from Li+ to Cs+ were able competitively bind the antiporter, whether the protein was capable of pumping them or not. However, we noticed that only Na+ and Li+ retained their solvation shells upon binding the protein. Since the same two cations are also the only ones that the antiporter can pump, we propose that the hydration state of the bound ion plays a major role in determining selectivity. Taken together, the Na+/H+ antiporter is an example of a transport system in which hydration is a critical factor for selectivity, but surprisingly not for substrate binding.

Notes:

P49. The action of molecular switches in complexes of GPCRs with agonists and antagonists

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GPCRs (G-protein-coupled receptors) are flexible membrane receptors able to passing the signal to the cell interior and even to activate different cellular pathways depending on the ligands they bind. To explore how the alterations in a ligand structure affect the receptor structure and dynamics we investigated the final steps of ligand binding process (for agonists and antagonists) in the binding site of the opioid and cannabinoid receptors. These receptors belong to the class A (similar to rhodopsin) of GPCRs, however, the detailed activation mechanism is different mainly because of different construction of molecular switches and also the opposite properties of the ligands: hydrophilic and having charged amine group versus highly hydrophobic. In opioid receptors binding of an agonist induced breaking of the 3-7 lock (a hydrogen bond D3.32-Y7.43) between helices TM3 and TM7. We also observed an action of the rotamer toggle switch consisting of residues on the same transmembrane helix TM6, W6.48 and H6.52. In cannabinoid receptors the 3-7 lock is not present and the rotamer toggle switch is composed of two residues, F3.36 and W6.48, located on opposite transmembrane helices TM3 and TM6 in the central part of the membranous domain. Nearly simultaneous action of two molecular switches in opioid receptors can suggest strong interdependence between them and revealing temporal and spatial dependences between molecular switches in GPCRs is of highest importance for drug design.

Notes:

P50. Picking Hits From Zillion Scaffolds When No Protein Structure Is Available

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Several years ago, the concept of similarities has been extended to fuzzy scaffold alignments using Feature Trees.[1] Since then the method has developed to an industry standard helping to understand WHY something is considered similar or not. The nature of the algorithm in addition allows an assembling of fragment-based 'de novo' compounds from gigantic (>1013 molecules) fragment spaces (= fragments + assembly chemistry rules) within a few minutes. One of the application hurdles used to be that most fragment spaces are proprietary. We present a public domain fragment space created from real synthetic chemistry and a novel toolbox for a generation of one's own fragment space plus examples with proven success in big pharma. [2] [1] Rarey et al, JCAMD 1998, 12, 471 and JCAMD 2001, 15, 497 [2] Lessel et al, JCIM 2009, 49, 270 and Boehm et al, JMC 2008 51 2468

Notes:

P51. Lipid Packing Drives Transmembrane Helices into Disordered Lipid Domains in Model Membranes

Lars V. Schäfer, Djurre H. de Jong, Andrea Holt, Andrzej J. Rzepiela, Alex H. de Vries, Bert Poolman, J. Antoinette Killian, and Siewert J. Marrink

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Cell membranes comprise of multi-component lipid and protein mixtures that exhibit a complex partitioning behavior. Regions of structural and compositional heterogeneity play a major role in the sorting and self-assembly of proteins, and their clustering into higher-order oligomers. We combined MD computer simulations and confocal microscopy to study the sorting of transmembrane (TM) helices into the liquid-disordered (Ld) domains of phase-separated model membranes, irrespective of peptide-lipid hydrophobic mismatch (PNAS 2011, 108, 1343). Free energy calculations show that the enthalpic contribution due to the packing of the lipids drives the lateral sorting of the helices. Hydrophobic mismatch regulates the clustering into either small dynamic or large static aggregates. These results reveal important molecular driving forces for the lateral organization and self-assembly of transmembrane helices in heterogeneous model membranes, with implications for the formation of functional protein complexes in real cells.

Notes:

P52. Native membrane patches containing Na+, K+-ATPase studied by AFM

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Sodium potassium ATPase is a transmembrane protein existing in all animal cells. It utilizes energy from ATP hydrolysis to transport Na+ and K+ across the cell membrane against their electrochemical gradients and thus maintain the resting potential. This transport process is accomplished by cyclic conformational changes of this protein. Atomic force microscopy (AFM), which does not require crystallization of the sample, can be used to image membrane proteins in native membranes at room temperature and in aqueous solutions, and can thus allow the detection of the functional conformational changes of the proteins. Thus we aim to investigate the conformational changes in the Na+, K+-ATPase in native membranes using AFM

Notes:

P53. Microsecond scale simulations of Cys-loop receptors: ethanol binding location and functional effects

Samuel Murail, Torben Broemstrup, Rebecca Howard, Bjorn Wallner, Edward Bertaccini, R. Adron Harris, James R. Trudell, Erik Lindahl

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Cys-loop receptors constitute a superfamily of ion channels gated by ligands such as acetylcholine, serotonin, Glycine and ?-aminobutyric acid. Recently, several structures of bacterial homologues have been solved and are of great help to understand the binding location of anesthetics molecules on Cys-loop receptors. At the same time, structural and functional effects of such molecules are still poorly understood, as little information is available to understand the opposite effects of anesthetics molecules on different pentameric receptors. We have used molecular simulations to study ethanol binding and equilibrium exchange for Gloeobacter violaceus pentameric ligand-gated (GLIC) and the homomeric a1 glycine receptor (GlyRa1), modeled on the structure of GLIC. Ethanol has well-known potentiating effect and can be used in high concentrations. By performing full 1 µs simulations of GlyR and GLIC with and without ethanol, we observe spontaneous binding in cavities as well as equilibrium ligand exchange. Interestingly, there appears to be binding sites both between and within subunits of the transmembrane domain, with different predominant binding site for GLIC and GlyR ; the inter-subunit having highest occupancy for GlyR, and the inter-subunit having highest occupancy for GLIC. Finally, ethanol appears to stabilize the open form of GlyR, which could help explain the allosteric effect of ligand binding on Cys-loop receptors.

Notes:

P54. Computational analysis of the binding modes of Palonosetron into the 5-HT3 Receptors

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The 5-hydroxytryptamine receptor (5-HT3R) belongs to the Cys-loop superfamily of Ligand-Gated Ion Channels (LGICs), and its inhibition has important antiemetic effects. Palonosetron, a second generation antagonist, demonstrates superior inhibition potency towards 5-HT3Rs compared to first generation antagonists, such as Granisetron, and Palonosetron is unique in exhibiting allosteric binding and positive cooperativity. Five different receptor monomers, the 5-HT3R A-E, have been identified although A and B subunits are the best understood; they form the homopentameric 5-HT3AR and the heteropentameric 5-HT3ABR. To understand the binding properties of agonists and antagonists and to translate Palonosetron functional characteristics at a molecular/atomic level, models of the extracellular portion of 5-HT3R A and B subunits were built and assembled into the receptor (homo- and hetero-) pentameric structure(1) on the basis of nAChR structure (PDB ID: 2BG9). The results of docking studies of the natural agonist serotonin and antagonists Palonosetron and Granisetron into the modelled homomeric and heteromeric 5-HT3R binding interfaces, provide a possible rationalisation both of the higher potency of Palonosetron with respect to other antagonists, and of its previously reported allosteric binding and positive cooperativity properties. We are currently using mutagenesis studies to probe the accuracy of the interactions. (1). Moura Barbosa, A. J.; De Rienzo, F.; Ramos, M. J.; Menziani, M. C. Eur. J. Med. Chem. 2010, 45(11), 4746-60

Notes:

P55. Sequence space of G-protein-coupled receptors: What does it tell us for molecular modeling?

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Class A G-protein-coupled receptors (GPCRs) constitute the largest family of transmembrane receptors in the human genome. The recent availability of several GPCR structures is of great help for homology modeling. However, this implies to correctly select the best template(s) for the modeling procedure. To gain information on class A GPCRs, we explored their sequence space by metric multidimensional scaling analysis (MDS). Tri-dimensional mapping of human sequences shows a non-uniform distribution of GPCRs, organized in four clusters. These clusters provide an intermediate classification between the class and the sub-family levels and are characterized by specific sequence determinants. They are interpretable in terms of three main evolutionary pathways from a central node formed by peptide receptors. The first pathway corresponds to the differentiation of the amine receptors. The second pathway was initiated by a deletion in transmembrane helix 2 (TM2) and led to three sub-families by divergent evolution. The third pathway corresponds to parallel evolution of several sub-families in relation with a covarion process involving proline residues in TM2 and TM5. The sequence space obtained by MDS emphasizes the importance of the proline residues in the evolution of GPCRs, with possible structural and functional consequences. The four clusters obtained by MDS provide a useful help in template selection for homology modeling.

Notes:

P56. Multiscale Modeling and Simulations of Transmembrane a-helix Dimers

Khairul B. Abd Halim, Benjamin A Hall, Beatrice Nikolaidi, & Mark S.P. Sansom
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Dimerization of transmembrane (TM) a-helices is a key step in receptor-mediated cell signalling. Previous studies suggested that dimerization is driven by short sequence motifs, and that small variations in the motifs may lead to destabilization of helix packing. Coarse grain molecular dynamics (CG-MD) have been combined with all atom MD simulations to provide a multi-scale approach to study TM helix interactions. CG-MD simulations were used to study dimerization of four different TM a-helices which contain different potential helix packing motifs. The GxxxG related motifs (FLRT2 and MUSK), a single Gly residue motif, either adjacent to a Pro (LAR) or to an Asp residue (CD3?) have been studied. We compared similar small ensembles with the results of high throughput CG-MD methods which yielded larger (ca. 100) ensembles of simulations. In this study, we found that all the TM helices formed a dimer within the bilayer, albeit with some variation in terms of handedness and tightness of helix packing. Interestingly, the improved sampling in the larger ensembles revealed greater variation in helix packing than had previously been observed, e.g. for FLRT2 and LAR for which bimodal crossing angle distributions were observed. Further refinement of representative structures from CG-MD was performed using atomistic simulations. Overall, this multi-scale approach allows a more detailed understanding between sequence motifs and the mode of packing of TM helix dimers.

Notes:

P57. Role of membrane cholesterol in hydrophobic matching and the resulting redistribution of proteins and lipids

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One of the physical mechanisms leading to lateral self-organization of cell membranes is the hydrophobic mismatch between a lipid membrane and the transmembrane part of a membrane protein. Meanwhile, cholesterol is in many ways a unique molecule with regard to its capability to promote membrane order and control the physical properties of lipids around it. In this spirit, it is tempting to consider how cholesterol could contribute to hydrophobic mismatch. The topic is particularly exciting given that there is a gradient of cholesterol along the secretory pathway, implying that the changes in membrane properties due to varying concentration of cholesterol can be an important factor for the sorting of non-matched Golgi transmembrane proteins. We have combined atomistic simulations with a major arsenal of experimental techniques to study the role of cholesterol in hydrophobic mismatch as well as its biological consequences. We have observed cholesterol to play a central role in controlling structural adaptations at the protein-lipid interface under mismatch. This is shown to result in a sorting potential that leads to selective segregation of proteins and lipids according to their hydrophobic length. The results allow us to provide a mechanistic framework for a better description of the organizing role of cholesterol in eukaryotic membranes

Notes:

P58. Structural analysis and thermodynamics of the ionotropic glutamate receptor GluA2 modulator BPAM-97

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Ionotropic glutamate receptors are tetrameric ligand gated ion channels that mediate influx and efflux of metal ions in response to glutamate. Positive allosteric modulators of the ionotropic glutamate receptor 2 (GluA2) are promising lead compounds for drugs against cognitive disorders. These compounds bind within the dimeric interface formed by the receptor ligand binding domains (LBDs) attenuating deactivation and desensitisation. In this study we determined the structure of the complex formed between a dimeric GluA2 LBD-L483Y-N754S mutant and the potent novel modulator BPAM-97 by X-ray crystallography. We provide a molecular explanation for the 200 fold increased potency of BPAM-97 compared to its parent compound IDRA-21. We also utilized isothermal titration calorimetry to measure the binding affinity and thermodynamics of the LBD-L483Y-N754S:BPAM-97 complex formation as well as that for the non-dimeric LBD-N754S:BPAM-97.

Notes:

P59. In silico Studies on Partial Agonism of GluN1 Ligand-Binding Domain

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N-methyl-D-aspartate (NMDA) receptors belong to a family of ionotropic glutamate receptors (iGluRs) that contribute to the signal transmission in the central nervous system. Binding of full agonists glycine and glutamate to the extracellular ligand-binding domains NR1 and NR2, respectively, is linked to NMDA receptor activation. Partial agonists are able to activate NR1 sub-maximally, but the accurate mechanism and interactions contributing to the closure of the ligand-binding cleft (NR1-LBC) is intricate to study experimentally. We have studied the NR1-D-cycloserine complex with various in silico techniques. With molecular dynamics (MD) and other force field-based computational methods we were able to clarify the crucial stages and interactions between NR1-LBC and D-cycloserine in receptor closure. For example, we show for the first time the closing of an open-cleft conformation of NR1 structure with bound ligand in MD simulations. Because iGluRs have been implicated in many neuropathologies, the understanding of the exact mechanism of ligand-binding to the receptors is vital for rational drug discovery.

Notes:

P60. Kv channels are frustrated

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When the voltage sensor of a Kv channel moves in response to changes in the transmembrane potential, it pulls on the S4-S5 linker, moving this also, which in turn presses on the S6 helix and closes the channel. What remains unclear in this mechanical model is how the channel then opens. We shall show using free energy calculations that the S6 helices of the paddle chimera prefer to be kinked at 0 V. These calculations include the pore region of the paddle chimera, a lipid bilayer and explicit water and therefore include the steric and cooperative effects introduced by moving all four S6 helices simultaneously. We conclude that when closed the paddle chimera is frustrated; the S6 helices are only straight because the voltage sensor, via the S4-S5 linker, is held in the down position by the transmembrane voltage. Through further free energy simulations we shall also show that, in order to produce a barrier of 10-15kT to the passage of potassium ions, the S6 helices do not need to straighten as much as the inner helices of KcsA. Lastly we shall demonstrate that the S4 helix must be displaced by ~6 Å to form a barrier of this height.

Notes:

P61. Structure-guided optimisation of positive modulators of the AMPA receptor

John K.F. Maclean, Stephanie Basten, Robert A. Campbell, Kevin J. Gillen, Jonathan Gillespie, Craig Jamieson ,Bert Kazemier, Michael Kiczun, Yvonne Lamont, Amanda J. Lyons, Elizabeth M. Moir, John A. Morrow, John Pantling, Zoran Rankovic and Lynn Smith

Chemistry, Modelling & Informatics / Merck

The a-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA) subtype of glutamate receptors mediate fast excitatory neurotransmission throughout the mammalian nervous system and have been implicated in learning and memory. Positive allosteric modulators (PAMs) of AMPA receptors (sometimes termed AMPAkines) are of pharmacological interest because of their emerging therapeutic potential in a range of psychiatric and neurological disorders, including schizophrenia and Alzheimer's disease. There is pre-clinical evidence that AMPAkines have pro-cognitive effects in rodents and primates, suggesting such compounds could treat cognition-related disorders in the human population. Numerous crystal structures of AMPA receptor domains have probed the mechanism of action of these receptors. Here, we demonstrate how crystal structures and computational methods have supported the design of novel AMPA PAMs, from HTS through to compounds with robust in vivo activity.

Notes:

P62. Mass Spectrometry of Membrane Proteins

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Membrane proteins are notoriously difficult to study as detergents are required to maintain solubility. Recently, however, we have shown that when membrane proteins are introduced in detergent micelles, they can break away from the electrospray droplet into the gas phase1. Activation of this micelle complex allows us to study the detergent free complex, allowing a more accurate mass measurement to aid determination of subunit stoichiometry and small molecule binding. Mass spectrometry of membrane proteins is of particular interest since it is the only analytical technique that allows us to study the naked membrane in the absence of detergents. Currently we are looking at the effects of different detergents upon the stability of BtuC2D2 in the gas phase. BtuC2D2 is an ATP-binding cassette transporter which mediates vitamin B12 uptake. We have seen the tetrameric structure in the gas phase, composed of two ATP-binding cassettes (BtuD2) and two transmembrane subunits (BtuC2). We have found that BtuC2D2 is relatively stable in the non-ionic detergent, ß-D-dodecyl-maltoside (DDM)2, however with other transporter membrane proteins, there is the risk of unfolding by the gas phase collisions needed to remove the detergent. We are using nano-electrospray ionisation mass spectrometry to investigate the stability of BtuCD in a range of detergents in an effort to better understand the gas phase properties of membrane proteins. 1N P Barrera, et al. Science, 321, 243 (2008) 2S Wang, et al. JACS, 132, 15468 (2010)

Notes:

P63. Breaching the Blood Brain Barrier

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The ability of drugs acting within the central nervous system (CNS) to cross the blood-brain- barrier (BBB) is crucial to their effectiveness. The BBB lies at the interface between the blood and brain and very effectively restricts the movment of many therapeutically useful drugs from the blood into the brain. A major factor contributing to BBB permeation of a drug is its pemeability through the BBB membrane. We have recently examined the dynamics and localisation of amitriptyline, a CNS-acting drug, in model lipid bilayer membranes using both atomistic molecular dynamics (MD) simulations and NMR techniques. The localisation of amitriptyline was shown to be dependent on both its protonation state as well as the lipid composition of the membrane.

Another important factor influencing whether a drug can cross the BBB is whether it will be a substrate for efflux transporter proteins such as P-glycoprotein (P-gp), which is able to actively expel drugs from the cell. There is growing evidence that P-gp is able to bind drugs while they are still in the membrane rather than via the cytoplasm. We have been using atomistic MD to compare the dynamics of the recently published X-ray structure of ligand bound Pgp and apo P-gp in order to explore potential routes by which drugs enter P-gp.

Notes:

P64. The role and mechanism of proton transfer reactions along the activation pathway of GPCRs

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G protein coupled receptors (GPCRs), a large family of integral membrane proteins involved in many signal transduction pathways, have recently been the subject of an extraordinarily successful research effort that has led, in the last few years, to substantial progress in the understanding of their mechanism. Crystallographic data, often complemented with force field based molecular dynamics (MD) simulations, have led to an atomistic description of many important steps of their activation pathway, from agonist binding to downstream signaling. Although infrared spectroscopy and NMR have provided some evidence of chemical reactions taking place inside the binding pocket of these receptors along their activation mechanism, very little structural information concerning these processes is available. Here we study these reactions in two prototypical GPCRs, rhodopsin and beta2 adrenergic receptor, using a hybrid quantum mechanics/molecular mechanics (QM/MM) approach based on Density Functional Theory (DFT). Our results elucidate the role and the mechanism of proton transfer reactions in GPCRs. In rhodopsin, our approach allows to characterize the optical properties of the different intermediates along the activation pathway and to estimate the barrier for the reaction that leads to Metarhodopsin II formation. In adrenergic receptors, our studies shed light into the electronic details of the interaction between the receptor and, respectively, agonists and antagonists.

Notes:

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