thermodynamics as well as in the introduction of the small molecule ligands on fast time scales. An attractive strategy is to 'cage' the protein ligand with a photochemically cleavable protecting grou which, upon photolysis, releases the ligand on nanosecond timescales allowing for the examination of fast time dynamics associated with ligand-protein interactions. Here, a new class of photocage is presented based upon the photophysics of Ru(II)bis(2,2′bipyridine)L_2 type complexes. Specifically, we present the thermodynamics of ligand photodetachment from both Ru(II)bis(2,2′-bipyridine)(acetonitrile)$_2$, Ru(II)bis(2,2′-bipyridine)(6,6′-dimethyl-2,2′-bipyridine), Ru(II)bis(2,2′-bipyridine)(seratonin)$_2$ and Ru(II)bis(2,2′-bipyridine)(tyramine)$_2$ complexes in aqueous solution using photoacousctic calorimetry (PAC) and density functional theory. Our results are consistent with N donation form the ligands to the metal dominating the interaction energies and positive reaction entahlpies. In addition, the relative small changes in molar volume change suggest the each complex is highly solvated in aqueous solutions.

2415-Pos Board B107

"*The Whole is Greater than the Sum of Its Parts*-" Hit Selection and the Power of Orthogonality

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Biophysical methods are essential tools in current front line drug discovery processes. They can be used for optimization of protein constructs and verification of proper and consistent protein folding, identification of optimal assay buffer, screening of fragments and the confirmation of binding to the right or allosteric binding site(s) of the target molecule- each approach providing a wealth of information. With a growing industry need to reduce project attrition (and therefore cost) by providing more physiological mechanistic data to aid in the identification of compounds that translate in vitro activity into in vivo efficacy. Application of panels of biophysical techniques allow cross correlation of data and better control of experimental parameters - increasing reliability and confidence in results whilst eliminating artifacts. In this work we show how SPR, DSC and ITC can be used in the selection and verification of binders in a Fragment Based Drug Discovery campaign by providing quantitative binding information to advance true hits for chemistry and later stage biology in hit to lead development.

2416-Pos Board B108

'Clickable'-Photoactive Propofol Analogue for the Identification of Anesthetic Targets

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Propofol, a dialkylphenol intravenous anesthetic, has been widely administered for over a quarter century in general anesthesia and for rapid sedation. Regardless, the drug's mechanism of action remains elusive with mounting evidence suggesting contributions from multiple pathways. Most proposed targets, such as ion channels and ligand gated receptors, are low abundance but functionally important proteins. To uncover these significant targets, we synthesized a photoactive- and 'clickable'- propofol analogue with *meta*-trifluoromethyldiazirinyl and *ortho*-(propynyloxy)methyl substitutions in place of the two isopropyl groups. This novel *o*-alkynyl-*m*-azipropofol maintains the physiochemical, biochemical and *in vivo* properties of propofol. It also photoadducts to known propofol binding sites in model proteins. Once adducted, the alkyne click moiety successfully undergoes copper catalyzed 1,3-dipolar cycloaddition reactions with azide-rodamine and -biotin tags allowing for fluorescent labeling and avidin resin enrichment of photolabeled targets within *ex vivo* systems. Using fluorescent microscopy or mass spectroscopy, the chemically 'dual-active' propofol analogue allows localization and identification of key targets in the biochemical pathways that underlie alkylphenol induced anesthesia.

2417-Pos Board B109

Exploring Anesthetic Binding on Voltage-Gated Cation Channels

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A multi-dimensional docking analysis was applied to investigate the binding of general anesthetics against distinct channel isoforms and conformations. Specifically, MD-generated ensembles of the channel isoforms K-Shaw2, Kv1.2 and Kv1.2-G329T in the activated-open and resting-closed conformations were docked against a family of anesthetic chemotypes, composed by halothane, isoflurane, sevoflurane and propofol. The total 240,000 docking solutions bind the channel structures at multiple sites split over four major regions (voltage-sensor, S4-S5 linker, selectivity filter and central cavity) within a diverse range of binding affinities Kb, ie., 2-50 mM. We followed a subtraction analysis to resolve channel and conformation dependency of ligand affinity on each binding site. From the analysis, sites placed at the S4-S5 linker and at the selectivity filter arise for every ligand as putative regions accounting for differential binding over channel variants and conformations. Although useful to identify sites potentially associated with differential binding of anesthetics across distinct conformations and isoforms, the present estimates for binding affinities as provided by Autodock Vina are largely approximative, thereby limiting concrete connections between the data and experimental measurements. To circumvent this difficulty, we have started a long series of FEP calculations to evaluate the binding affinity of sevoflurane and isoflurane against K-Shaw2 and Kv1.2 in both their activated-open and resting-closed states. The FEP computations carried so far have confirmed the differences between conformation affinities on the S4-S5 linker region for the Kv1.2 isoform. Taken together, our results reveal binding sites that may underlie channel specific effects of general anesthetics providing thus new directions for further experimental investigation of this topic.

2418-Pos Board B110

Nudt9H Interactions in the Tetrameric TRPM2 Ion Channel

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TRPM2 is a Ca^{2+}-permeable cation channel that belongs to the M subfamily of Transient Receptor Potential (TRP) channel proteins. TRPM2 is a homotetramer, each monomer contains a cytosolic N-terminal TRPM-homology region, six transmembrane helices with an architecture typical to that of voltage-gated cation channels, and a cytosolic coiled-coil region followed by a NUDT9-Homology (NUDT9H) domain at the C-terminus.

TRPM2 activates in the presence of Ca^{2+}, ADP-Ribose (ADPR), and PIP2 (Toth and Csanady, 2012). ADPR binds to the NUDT9H-domain which displays ~35% identity with NUDT9, a monomeric mitochondrial ADPR-pyrophosphatase with available crystal structure (Shen et al., 2003). At present, it is unclear whether NUDT9H is an active enzyme itself, or just an ADPR-binding domain. In contrast to the extremely high solubility of NUDT9, NUDT9H solubility is very low: when overexpressed in bacterial systems it forms inclusion bodies, and reprecipitates upon removal of chaotropic agents. This might be a consequence of the tetrameric nature of TRPM2, in the context of which it is unclear how the four N-terminal domains and the four NUDT9H domains interact with each other and/or with the transmembrane segments. The fact that ADPR binding to NUDT9H is necessary for channel activation suggests an extended interface between NUDT9H and some partnering segment within the complex. Surface exposure of this interface region may account for the reduced solubility of NUDT9H when expressed in isolation.

Here we explore this hypothesis by expressing soluble NUDT9, insoluble NUDT9H, as well as different NUDT9/NUDT9H chimeras. By monitoring the solubility of the chimeras, we attempt to narrow down the search for the functional interface of NUDT9H. In addition, we try to identify the minimal substitutions required for increasing NUDT9H solubility. Finally, we use soluble NUDT9 to test hydrolysis of various substrates/ligands that activate the TRPM2 channel.

2419-Pos Board B111

Mapping of Netrin-1 Binding to its Dependence Receptors

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Netrin-1 is a bifunctional guidance cue for migrating axons in vertebrates. Depending on the target receptor family netrin-1 interacts with, it either causes attraction or repulsion of axonal growth cones. Chemoattraction is mediated by adhering to DCC or its homologue neogenin, whereas chemorepulsion

results upon binding to the closely related UNC5A, UNC5B, UNC5C or UNC5D receptors. In addition to the nervous system, netrin-1 and its receptors have been detected in developing heart, lung, mammary gland, intestine and pancreas and are believed to be expressed ubiquitously. Both families of receptors have been implicated as dependence receptors, that is receptors that induce apoptosis if they are not bound to their ligand such as netrin-1. Thus they play a pivotal role in the development of the organism and in tumour suppression.

Here, we present the 2.5 Å high resolution structure determined by X-ray crystallography that comprises four domains of netrin-1. We mapped the netrin-1/neogenin and the netrin-1/UNC5 binding sites by a series of domain swap experiments where we exchanged individual domains of netrin-1 with a structurally closely related -but non-functional- domain of the laminin γ1 short arm. We probed the binding behaviour of the chimeras by surface plasmon resonance and solid phase binding assays. Once the binding domain had been unambiguously identified, we determined the binding epitopes on netrin-1 by mutational analysis, using structural information and phylogenetic conservation as guides. We found that the DCC family and the UNC5 family of receptors bind to spatially distinct sites within the same domain. Reciprocally, we confirmed that the 5th fibronectin domain of DCC harbours the binding site for netrin-1 and determined the binding epitopes. Finally, we show the conformation of the netrin-1/neogenin complex in solution using SAXS data and present a map of the binding sites.

2420-Pos Board B112

Molecular Interaction Between PPTI and Shakv1.1 Potassium Channel Explored by Docking and Molecular Dynamics Simulation

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PPTI, proposed as a potential dendrotoxin, which isolated from the venom of Pseudocerastes persicus has 58 amino acid residues and cross-linked by three disulfide bonds. We explored the interaction between PPTI and ShaKv1.1 potassium channel by successive application of protein-protein docking and molecular dynamic simulation. The analysis of the complexes revealed that the Lys5 residue of PPTI plugged its side chain into the channel selectivity filter. Molecular dynamic simulation in membrane environment of the complex structure resulted in docking stage confirmed the stability of the complex.

2421-Pos Board B113

Elucidating Time Course of Structural Changes Leading to Receptor-Ligand Complex Formation with Transient-EPR

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Protein functions rely on protein motions. Protein structures, obtained with X-ray diffraction or NMR, reveal mostly static pictures of structure-function relation. To connect structural changes of proteins with function, we need to look at events happening in real time, i.e. the dynamics of the processes.

We have chosen mlCNBD as a model protein to study the dynamics of receptor-ligand complex formation. mlCNBD is a cytosolic cyclic nucleotide-binding domain of a bacterial potassium channel (MloK1). mlCNBD binds to cyclic adenosine monophosphate (cAMP) and undergoes conformational changes as evident from the NMR and X-ray structures of the *apo* and *holo* conformational states of the protein. Recent kinetic and NMR studies indicate that these structural transitions follow the "induced-fit" mechanism, i.e. these are a direct consequence of ligand binding. However, the detailed mechanism of these structural rearrangements leading to receptor activation remains elusive.

We use transient Electron Paramagnetic Resonance (tr-EPR) spectroscopy in conjunction with Site- Directed Spin Labelling (SDSL) to resolve the dynamics of mlCNBD-cAMP complex formation. We introduce single cysteine residues at different sites in the protein. The mutants are labelled with Methane Thio Sulphonate Spin Label (MTSSL). Binding of cAMP to the mutants is rapidly initiated either via a caged-cAMP approach or through a micro-mixer. The time-resolved EPR data reveals the progression of structural changes taking place at a particular site on millisecond time scale. Collating data across the whole protein will enable us to reconstruct the steps from the *apo* to the *holo* state of the protein. It will also provide the answer to the question whether the "induced-fit" mechanism follows a concerted (single step) or sequential (multi-step) path from the *apo* to the *holo* conformation.

2422-Pos Board B114

Exploring the Binding Site of the G Protein-Coupled Receptor GPR119 Model using a Pair of Diastereomers with Opposing Action

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GPR119 is a Class A (rhodopsin-like) G-protein coupled receptor that has insulin-regulating activity. GPR119 agonists stimulate glucose-dependent insulin secretion in vitro and lower elevated blood glucose in vivo. Hence, the GPR119 receptor has emerged as an important target for the treatment of type 2 diabetes. An initial GPR119 homology model was constructed using the adenosine A2A receptor crystal structure bound to an antagonist at 2.6Å resolution (PDB id: 3EML) as the template. The preliminary model was refined by exploring the flexibility of individual helices using the Monte Carlo/simulated annealing method, Conformational Memories (CM). CM employs multiple Monte Carlo/simulated annealing random walks, exploring peptide and protein dihedral conformational space. A pair of previously reported diastereomers, one of which acts as a GPR119 receptor agonist and the other as an antagonist, were docked into the active (R*) and inactive (R) receptor models. The putative binding site of the preliminary R model starts between the top of transmembrane helices (TMHs) 2 and 7 and spans the bundle in a shallow diagonal direction, almost perpendicular to the TMH region. The floor of the binding site is between TMHs 3, 5 and 6 at the level of F234(6.44) (middle of the TMH bundle). W238(6.48) ($\chi1 = g+$) places its bulk between TMHs 6 and 7. The only charged amino acid that is accessible to the binding site, R81(3.28), was used as the primary interaction site for both compounds. The GPR119 R* state binding site is more vertical compared to the R state and somewhat deeper because of a $\chi1$ rotamer change of W238(6.48) to a trans conformation in the R* state, which places its bulk between TMHs 5 and 6.

2423-Pos Board B115

A Comparison of 3D Conformations of Endothelin-1 Analogs to Find the Pharmacophore Model Required for Endothelin Receptor Ligand Activity

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Endothelin receptors (ETRs) are GPCRs whose activation results in the increase of intracellular calcium levels, and are ultimately involved in vasoconstriction and vasodilation. Because control of ETR activity has important medical implications, it is beneficial to rapidly discover effective synthetic ETR ligands by virtual drug screening methods. Despite its biological importance, little is known about ETR structure, since there are no reported X-ray structures to date. However, structural information is available for Endothelin-1, the natural ligand to ETR and the most potent vasoconstrictor yet identified. Thus, our work takes the ligand-based approach instead of receptor-based approach to ETR ligand pharmacophore discovery. Two well-studied ETR ligands, BQ-123 and bosentan, were chosen to be reference compounds in addition to Endothelin-1, and 30 known synthetic ligands were defined as the sample active compounds set. Conformers of the sample set were prepared by genetic algorithm-based approach using OpenBabel, while conformers of the reference set were prepared by molecular dynamics, using myPresto/cosgene. For molecular superimpositions, we built and used a program called 'BMSIP,' which is an algorithmic extension of the ROCS program. While it performs rigid-body superimpositions using a volume overlap score function based on spherical Gaussian descriptions of atomic shape, BMSIP also introduces a scores-weighting matrix to bias atom-pairings by atom-type correspondence. Superimposition of the sample set against the reference set was performed to obtain a density map that shows the frequency of atom overlaps between the superimposed compounds and reference compounds. This map can indicate the essential chemical features (the pharmacophore) required for interaction with ETRs. The results suggest that nonpolar groups alone, in particular aromatic groups, are important for ETR binding affinity and provide a good initial guess for directed ETR ligand design.