providing recognition sequences. Here we report that for two proteins bound on DNA at a separation of tens of base pairs, their DNA binding affinities can be significantly altered. This coupling effect oscillates between positive and negative cooperativity, depending on the separation distance between the two proteins on the DNA. With a DNA hairpin experiment, we provide definitive evidence for the structural basis of DNA allostery. We prove this effect is not due to protein-protein interactions but originates from the distortion of the inter-helical distance along the linker DNA. The oscillation has a periodicity of ~10 base pairs, the helical pitch of the B-form DNA, and a characteristic decay length of ~15 base pairs. In the theoretical analysis, we elucidate the relation between the mechanical structural distortion of DNA induced by protein-binding and the free energy coupling measured thermodynamically, providing a complete picture for the origin of DNA allostery. The allosteric coupling between two DNA-bound proteins is found to be ubiquitous, regardless of proteins’ properties, implying its general roles in gene regulation. We demonstrate such DNA allostery affects gene expression levels in live E.coli cells. Pertinent to eukaryotic gene expression, we show that the binding affinity of a transcription factor depends on its separation from nearby nucleosomes. This work provides the first comprehensive study of allostery through DNA, with the understanding of its physical underpinning and ubiquity and biological relevance.

1020-Plat
In Silico Insight into Transitional Structures and Barrier-Crossing Dynamics of DNA and RNA Oligomers
Jeremy T. Copperman, Marina G. Guenza.
University of Oregon, Eugene, OR, USA.

In aqueous solution under physiological conditions DNA and RNA molecules fluctuate between various non-equilibrium free energy minima on a very complicated energy surface. Due to their dynamic nature, important sequence-specific transition structures such as partial fork formations, flipped open base-pairs, and unstacked/exposed bases, are difficult to identify using experimental techniques alone. In this talk, results of molecular dynamic simulations are presented which provide insight into the structural ensembles of free DNA and RNA molecules in solution, and the conformational selection upon binding to protein partners. Complicated 5’ to 3’ symmetric structures observed in simulation of single-stranded DNA (ssDNA) oligomers are restricted to a subset of the available conformational space upon binding of T4 gp32 ssDNA binding protein. Sequence-specific non-equilibrium structures which involve increased exposure of the bases are shown to be crucial in the recognition and specific binding of (CUG)n repeat RNA and the MBNL1 alternative splicing regulator, one of the molecular origins of muscular dystrophy. Insight gained from these in silico simulations are used to help interpret biochemical and biological measurements obtained by our collaborators at the University of Oregon, and to inform the development of analytical techniques to model the important barrier-crossing dynamics in these systems.

Platform: Membrane Transporters & Exchangers II

1021-Plat
Using DEER, Modelling and Simulations to Investigate the Dynamics of PepTso, a Major Facilitator Superfamily Transporter of Biomedical Importance
Philip W. Fowler1, Marcella Orwick-Rydmark1, Nicolae Solcan1, Sebastian Radestock2, Patricia Dijkman1, Jane Kwok1, Firdaus Samsudin1, Anthony Watts1, Lucy Forrest2, Simon Newsstead2.

1University of Oxford, Oxford, United Kingdom, 2Max Planck Institute of Biophysics, Frankfurt, Germany.

PepT1 is a Major Facilitator Superfamily (MFS) transporter expressed in the gastrointestinal tract and transports not only di- and tri-peptides across cell membranes but also a wide-range of hydrophilic drugs, including the beta-lactam antibiotics. Crystal structures of two homologous bacterial MFS transporters, PepTso [1] and PepTst [2], have recently been determined in two conformational states. In this study we examine the dynamics of PepTso using Double Electron-Electron Resonance (DEER) measurements, modelling and computer simulation. We first generated models of the outward facing state using the repeat-swapping method [3]. The DEER residue pairs were carefully chosen so our results can also be compared to a previous study of LacY, another MFS transporter [4]. We also ran extensive molecular dynamics simulations during which PepTst partially changes conformation. Taken together the results allow us to (i) examine the universality of MFS dynamics, (ii) tentatively assign some of the DEER peaks to known conformations and (iii) validate our outward-facing models of PepTso. Comparison between the outward-facing model and inward-facing crystal structure suggest that kinking of the helices at conserved proline residues may be vital for conformational changes between the two states. Biochemical and DEER data reveal that mutating these proline residues abolishes transport activity, and significantly alters the protein dynamics.


1022-Plat
The Molecular Determinants of the Zinc Transporter, hZIP4
Robert Dempski, Sagar Antala, Kumari Alka.
Worcester Polytechnic Institute, Worcester, MA, USA.

Zinc is an essential micronutrient which is required for the function of hundreds of cellular enzymes. In addition, zinc is the second most abundant transition metal found in biological systems (iron is most abundant). However, the concentration of free zinc is nano to picomolar since most zinc is bound to proteins. This makes investigating the mechanism of zinc transport across the plasma membrane a challenge. Our interest has been to elucidate the mechanism of zinc transport mediated by one member of the ZIP family of proteins, hZIP4. hZIP4 is located at the primary location of zinc uptake in humans and has been directly implicated in multiple disease states including Acridermatitis enteropathica and pancreatic cancer. However the mechanism of transport is not yet known. We have previously shown that Zn²⁺, Ni²⁺ and Cu²⁺ can be transported by hZIP4, following heterologous expression in X. laevis oocytes, where these metal ions are not naturally found (in the nM and µM range of biological interest). Current research interests are to investigate the mechanism of ion translocation using a mixture of biochemical and biophysical techniques.

1023-Plat
Structure-Enhanced Ligand Discovery for Solute Carrier Transporters
Avner Schlessinger.
Mount Sinai School of Medicine, New York, NY, USA.

Solute Carrier (SLC) Transporters are membrane proteins that control the uptake and efflux of various solutes such as amino acids, sugars, and drugs. In humans, SLCs can be drug targets themselves or be responsible for absorption, targeting, and disposition of drugs. We first perform a comprehensive comparison of SLCs to inform attempts to model their structures, a prerequisite for structure-based ligand discovery. We then describe an integrated computational and experimental approach for identifying transporter-small molecule interactions. Particularly, we use comparative modeling and virtual screening, followed by experimental validation by measuring uptake kinetics, to identify interactions between SLC transporters and small molecules ligands, including prescription drugs, metabolites, and fragment-like compounds. For example, we discovered that several existing prescription drugs interact with the norepinephrine transporter, NET, which may explain some of the pharmacological effects (i.e., efficacy and/or side effects) of these drugs via polypharmacology. We also applied our approach to related transporters, to identify rules for substrate specificity in a key membrane transporter family of the nervous system (i.e., the SLC6 family). Our combined theoretical and experimental approach is generally applicable to structural characterization of protein families other than SLCs, including receptors, ion-channels, and enzymes, as well as their interactions with small molecule ligands.

1024-Plat
Mechanistic Insights from Modeling the Substrate Translocation Path of the Bacterial Glutamate Transporter Homologue GltPh
Sebastian Stolzenberg1, Olga Bobkov2, Harald Weinmaster1.

1Weill Cornell Medical College of Cornell University, New York, NY, USA, 2Cornell University, Ithaca, NY, USA.

The membrane protein GltPh, a bacterial homologue of eukaryotic Excitatory Amino Acid Transporters (EAATs), is an important prototype in the study of glutamate reuptake mechanisms that terminate synaptic transmission and prevent excitotoxicity in the brain. Crystal structures of GltPh in various function-related states have provided key insights into conformational changes associated with the translocation path of substrate reuptake, and have enabled the computational modeling of intermediate GltPh structures connecting these states. Still, many details of these molecular changes, which could help integrate results from various experimental approaches, have remained unexplored. To investigate such detailed changes, we have modeled the translocation path of GltPh with a variant of targeted Molecular Dynamics (sTMD-MD) that...
combines stepwise targeted motion and long, unrestrained equilibrations. The structural inferences regarding intermediate steps in the modeled translocation path agree well with those from MP-MD and sTMD computations, including the observation of intermediate trimer asymmetry (average monomer-monomer RMSD<4Å), relative domain movements, and protein-protein interface changes. A new finding is that the TM3-4 loop, previously suggested to play an essential role in GlnPh’s substrate transport, undergoes remarkable changes in both conformational and dynamic properties during transition from the outward- to inward-facing end states. Some structural differences between results from the present path calculation and findings from MP-MD are identified in the TM1-TM5 and HPI-2/TM7 region (<5Å), TM3 (<3Å) and TM3-4 loop (<8Å). Together, these computational modeling studies have produced specific predictions amenable to experimental testing, e.g. with FRET/EPR and cross-linking experiments, in the form of predicted residue-specific proximity and accessibility along the translocation path.

1025-Plat
A Sodium-Sensitive Salt Bridge in the Na\(^+\)/H\(^+\) Antiporter NhaA
Olive Beckett\(^1\), David Drey\(^2\), Chiara Lee\(^3\), Shoko Yashiro\(^2,3\),
Mark S.P. Sansom\(^4\), So Iwata\(^2,5\), Alexander D. Cameron\(^2,3\),
\(^1\)Arizona State University, Tempe, AZ, USA, \(^2\)Imperial College, London, United Kingdom, \(^3\)Diamond Light Source, Harwell Science and Innovation Campus, Didcot, United Kingdom, \(^4\)University of Oxford, Oxford, United Kingdom, \(^5\)Japan Science and Technology Agency, ERATO, Human Crystallography Project, Kyoto, Japan.

The transmembrane protein NhaA from Escherichia coli is a prototypical sodium/proton antiporter. It enables the bacterium to grow under high salt conditions while homologous proteins in eukaryotes are involved in pH and cell volume regulation. A number of acidic and basic residues have been shown to be essential for the transport of one sodium ion for two protons but the mechanistic details of their involvement have not been fully determined. In particular, a highly conserved lysine residue (Lys300) near the center of the membrane had so far been only given a possible indirect role in the transport mechanism. We present a new atomic resolution structure of the inward facing conformation that shows a novel salt bridge between Lys300 and the conserved Asp163. Microsecond molecular dynamics simulations indicate that the salt bridge is sensitive to the presence of a sodium ion that spontaneously binds to the conserved aspartate residue 164. The simulations show how binding of sodium ion can be coupled to a structural change which might trigger a conformational change to an outward facing conformation. Taken together, the structural and simulation data generate a new hypothesis for how Lys300 could be directly involved in proton transport.

1026-Plat
Identification of Amprenavir, Quinidine and Loperamide Kinetic Parameters for P-gp Transporter in Caco-2 Confluent Cell Monolayer
Zhou Meng
Drexel University, Philadelphia, PA, USA.

P-glycoprotein (P-gp) is a member of the ATP binding cassette (ABC) family of proteins that has been extensively studied due to its ability of multidrug resistance and for causing clinically important drug-drug interaction (DDI). Structural knowledge and functional knowledge of transport kinetics in physiological relevant system have been intensively studied for a molecular understanding of P-gp activity. Using the mass action kinetic model without imposing the steady-state assumptions, previous studies have identified the kinetic parameters for a series of P-gp substrates in MDCKII-hMDR1 confluent cell monolayer. However, little is known whether this model can be extended to other cell lines to study P-gp. Here, we applied our model to another widely-used P-gp expressing cell line, human colon adenocarcinoma (Caco-2), and successfully fitted the elementary rate constants of P-gp for substrates amprenavir, quinidine and loperamide as well as P-gp surface density. Furthermore, the fitted rate constants of above drugs in Caco-2 cells are similar to those in MDCKII-hMDR1 cells. Our results suggest that the mass action kinetic model can identify P-gp rate constants in human cell line, Caco-2 cells and that this model can be used to predict and characterize P-gp pharmacokinetics in vivo.

1027-Plat
Chemical Flexibility of P-Glycoprotein for Interaction with Substrates and Modulators
Eduardo E. Chufan, Khyti Kapoor, Hong-May Sim, Stewart Durrell, Suresh V. Ambudkar.
National Institutes of Health, Bethesda, MD, USA.
P-glycoprotein (Pgp, ABCB1) is an ATP-Binding Cassette (ABC) transporter, which plays a major role in the development of multidrug resistance in cancer cells. This transporter pumps out from cells a variety of anti-cancer drugs using the energy from ATP hydrolysis. One of the most important features of Pgp is its ability to recognize multiple chemically dissimilar compounds. Although the highly flexible structure of Pgp can explain, in part, this broad substrate specificity, this phenomenon is poorly understood. With the aim to get insight into the mechanism and molecular basis of the polypspecificity, we have modeled the structure of human Pgp using the X-ray structure of mouse Pgp as a template, and docked substrates and modulators in the pocket where the QZ59-peptide inhibitors bind mouse Pgp. Single, double and triple mutants with substitutions of residues at the drug-binding site, Y307, F343, Q725, F728, M949, Y953, F978, V982 and A985, with Cys in a cys-less Pgp, were expressed in insect and mammalian cells using baculovirus expression system. All the mutant proteins were expressed at cell surface to same extent as the cys-less wild-type Pgp. Several compounds including cyclosporine A, tariquidar, valinomycin and nilotinib lose partially or completely the ability to inhibit the photolabeling of mutant Pgps with the transport substrate \[^{125}\text{I}]-Iodoazidoprazosin, indicating these drugs cannot bind at their primary binding site. However, the drugs can still modulate the ATP hydrolysis of the mutant Pgps, indicating that they bind at alternate sites. The transport of fluorescent substrates in HeLa cells overexpressing these mutants is also not significantly altered, showing that the alternate sites are active for transport. These results are thus revealing an exceptional chemical flexibility of Pgp, in addition of its structural flexibility, for interaction with substrates and modulators.

1028-Plat
Effect of Internal ATP-Mg\(^{2+}\) Concentration on the Human Red Blood Cell K\(^{+}\)/Ca\(^{2+}\) Exchanger
Almary Guerra, Jesus G. Romero,
Universidad Central de Venezuela, IBE, Lab. Fisiologia Molecular y Biofisica, Caracas, Venezuela, Bolivarian Republic of.

Little is known about the process of senescence of human erythrocytes, however it is accepted that the \([\text{Ca}^{2+}]_{i}\) increases with age. We have previously presented evidence of the existence of a novel transporter capable to account with the \([\text{Ca}^{2+}]_{i}\) intake during the ageing process, the K\(^{+}\)/Ca\(^{2+}\) Exchanger (1)(2). The Activation process of the K\(^{+}\)/Ca\(^{2+}\) Exchanger has a sigmoidal voltage dependence and a permeability sequence: K\(^{+}\)>>Rb\(^{+}\)>>>Ca\(^{2+}\) and Ca\(^{2+}\)>>Ba\(^{2+}\)>>>>Mg\(^{2+}\). The ATP-Mg\(^{2+}\) complex is known to regulate the activity of different transporters. In order to determine the effect of the internal ATP-Mg\(^{2+}\) concentration on the K\(^{+}\)/Ca\(^{2+}\) Exchanger’s permeability, activation and deactivation, we used Patch-clamp, changing the ATP concentration in the internal solution from 0 to 4mM. We found that exchanger currents increase in the presence of ATP-Mg\(^{2+}\). Interestingly, in the Ca\(^{2+}\) output mode the effect is dose-dependent, and no saturation is observed even at 4mM ATP-Mg\(^{2+}\), increasing from 48% to 200% (500\(\mu\)M to 4mM). On the other hand, in the Ca\(^{2+}\) entry mode, the permeability increases by 36% and this effect is already saturated at 500\(\mu\)M. Also, in this mode there is an increment of 20% in the time course of the deactivation, saturated at 500\(\mu\)M, whereas no such effect on the Ca\(^{2+}\) output mode was observed. These results suggest the existence of either two different binding sites for ATP, or one binding site that changes its affinity with the mode of exchange: Site A with low affinity in the order of mM, affect-