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 comformational selection upon binding to protein partners. Complicated 5' to 3' asymmetric structures observed in simulation of single-stranded DNA (ssDNA) oligomers are restricted to a subset of the available comformational 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 biophysical and biochemical 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. Fowler**[1], Marcella Orwick-Rydmark[1], Nicolae Solcan[1], Sebastian Radestock[2], Patricia Dijkman[1], Jane Kwok[1], Firdaus Samsudin[1], Anthony Watts[1], Lucy Forrest[2], Simon Newstead[1].
[1]University of Oxford, Oxford, United Kingdom, [2]Max Planck Institute of Biophysics, Frankfurt, Germany.
hPepT1 is an 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 PepTSo 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.
1. Newstead S, Drew D, Cameron AD et al (2011) EMBO J 30:417
2. Solcan N, Kwok J, Fowler PW, Cameron AD, Drew D, Iwata S, Newstead S (2012) EMBO J 31:3411
3. Radestock S, Forrest LR (2011) J Mol Biol 407:698
4. Smirnova I, Kasho V, Choe JY, et al (2007) PNAS 104:16504

**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 Acrodermatitis enteropathica and pancreatic cancer. However the mechanism of transport is not known. We have previously shown that $Zn^{2+}$, $Ni^{2+}$ and $Cu^{2+}$ can be transported by hZIP4, following heterologous expression in X. laevis oocytes, where there are two binding affinities (in the nM and μM range of biometal). Currently, our research interests are to investigate the mechanism of ion translocation using a mixture of biochemical and biophysical techniques.

**1023-Plat**
**Structure-Based 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 Stolzenberg**[1,2], Olga Boudker[1], Harel Weinstein[1].
[1]Weill Cornell Medical College of Cornell University, New York, NY, USA, [2]Cornell 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