Membrane Transporters & Exchangers II

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Quaternary Structure of the NBD Subunit Wzt of a Bacterial ABC Transporter in the Absence and Presence of TMD Subunit Wzm using Pixel-Level FRET

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The pathogenic Pseudomonas aeruginosa is a Gram-negative bacterium coexpressing two unique forms of lipopolysaccharides on its surface, the A and B bands. The A-band polysaccharides are thought to be translocated into the periplasm through an ATP-binding-cassette (ABC) transporter consisting of two subunits: a transmembranar protein, Wzm, and a nucleotide-binding protein, Wzt. Since Wzm and Wzt proteins possess no domains or motifs that are targeted for modifications by eukaryotic cellular machineries or exhibit any signal for organelles localization, we used Chinese hamster ovary (CHO) cells as a model system to study the protein complex structure and stoichiometry of Wzt protein in the absence or the presence of Wzm. We transfected the CHO cells with Wzt proteins fused to the green fluorescent protein (GFP2) or its variant yellow fluorescent protein (YFP) to study the protein complex structure of Wzt. A spectrally resolved two-photon microscope was used to obtain fluorescent images from transfected cells, and a novel spectral Fluorescence Resonance Energy Transfer (FRET) provided apparent FRET efficiency (Eapp) for each image pixel (Raicu et al., Nature Photonics, 2009). Distributions of FRET efficiencies were obtained from several cells, and the peak positions for the cells whose Eapp histograms showed single peak were binned to obtain a ''meta-histogram''. The meta-histograms were fitted to simulated distributions obtained from various theoretical models (Raicu et. al., Nature Photonics, 2009), to determine the stoichiometry and geometry of Wzt oligomers. Wzt formed rhombus-shaped homo-tertamers, which became square shaped upon co-expression of untagged Wzm.

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Microfluidic Analysis of ATP-Binding Cassette Transporters at Single-Vesicle Level

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ATP-binding cassette (ABC) transporters, a protein superfamily that ranges from prokaryotes to eukaryotes, transport a wide variety of substrates across membranes, including metabolic products, lipids, and drugs. They utilize the energy of ATP hydrolysis for transport, and are involved in various human diseases, such as cancer and cystic fibrosis. Much attention has been paid in recent years to ABC-transporters as potential drug targets, and high-throughput drug-screening systems for ABC-transporters are in great demand. We have succeeded in detecting ATP-dependent transport by the immobilized P-glycoprotein, an ABC transporter, in polydimethylsiloxane (PDMS) microfluidic channels. We here present a first report of the on-chip accurate analyses of the inhibition of P-glycoproteins with several drugs, digoxin, verapamil, cyclosporine A and quinidine. The half-maximal inhibitory concentration (IC50) values for the Rh123 transport, determined by the microchip were 1.82 $\pm$ 0.36 μM (digoxin), 1.91 $\pm$ 0.28 μM (verapamil), 0.45 $\pm$ 0.01 μM (cyclosporine A) and 0.66 $\pm$ 0.04 μM (quinidine). Because our procedure detects the fluorescence of immobilized ABC transporter-containing vesicles and their size and intensity can be directly measured from the images, we could estimate the substrate transport at the single-transporter level.

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Molecular Mechanics Investigation on the Mechanism of Multidrug Resistance

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Multi-drug resistance is a fascinating phenomenon from both the physiological and biophysical perspectives. While a plethora of functional and structural studies provide a general view of this mechanism, the underlying microscopic details remain elusive. To advance our understanding of the key molecular interactions giving rise to the function of the multi-drug transporters, we pursued a comparative study of the P-glycoprotein (Pgp) and Sav1866 (Sav) transporters using molecular dynamics simulations. Crystal structures of these proteins reveal two alternative conformations: The Sav structure shows that binding of ADP/ATP to the nucleotide binding domains (NBD) favors their interactions and forces the opening of the transporter on the extracellular side. Unbinding of ADP leads the NBDs to dissociate and subsequently to the formation of a putative allocrit-binding cavity on the intra-cellular leaflet of the membrane, as illustrated by the Pgp structure. Before addressing how allocrits are selected and transported, it is important to characterize the key states of the transporter and, if possible, the ATP-driven transitions between these states. Simulations were thus performed under different conditions for both proteins: apo, with one ADP/ATP bound and with two ADPs/ATPs bound. The possibility that lipids present in the cavity could impact on the dynamics and conformation of the transporters was also investigated.

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

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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 movement of many therapeutically useful drugs from the blood into the brain. A major factor contributing to CNS penetration of a drug is its permeability through the BBB membranes. We have examined the dynamics and localisation of several CNS-acting drugs in model lipid bilayers using both atomistic molecular dynamics (MD) simulations and NMR techniques. The localisation and orientation of these drugs were shown to be dependent on both protonation state and lipid composition of the bilayer.

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

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The Amphiphilicity of Allorcits and the Rate of ATP Hydrolysis and Flopping by P-Glycoprotein

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We investigated how the amphiphilicity of detergents influences the rate of ATP hydrolysis by P-glycoprotein. To this purpose we used 34 electrically neutral and cationic detergents with different head groups and alkyl chain lengths and thus varied amphiphilicity. The free energy of detergent partitioning into the lipid membrane (dominated by the alkyl chain) and the free energy of detergent binding from the membrane to the transporter (due to the polar head group) were determined. For seven of these detergents the rate of ATP hydrolysis (Log scale) decreased linearly with increasing affinity from water to the transporter as observed previously for typical P-glycoprotein allocrits. Although, structurally closely related the remaining 27 detergents significantly reduced the rate of ATP hydrolysis. Inhibition of the P-glycoprotein-ATPase due to membrane disordering could be excluded based on order parameter measurements of deuterated lipid bilayers in the presence of relevant detergent concentrations using D-NMR spectroscopy. An optimal rate of ATP hydrolysis was observed only if the two free energies of binding were in appropriate proportion to each other, i.e., if the ratio of the two free energies of binding was slightly above three for cationic, and somewhat below three for neutral detergents. Lower or higher ratios inhibited the P-glycoprotein-ATPase activity and effective allocrit flopping.

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Fundamental Difference of Transport Mechanisms Between ABC Importers and Exporters

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ABC transporters use the energy of ATP to drive the active transport of various substrates across the membrane. All members of this superfamily share a common machinery of two nucleotide binding domains (NBDs) at their cytoplasmic side, which close and open in response to ATP binding and hydrolysis. Despite highly homologous NBDs, substrates can be either imported or exported in different subfamilies. Although crystal structures of ABC transporters have been resolved in different conformational/functional states, it is still puzzling how a common set of motor domains can drive transport in opposite directions. We use comparative molecular dynamics simulations of an ABC-importer (maltose transporter) and an ABC-exporter (P-glycoprotein), to investigate functionally relevant dynamical differences. The two transporters exhibit