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Additionally, the photon-counting nature of the detector allows spatial and temporal correlations to be obtained very easily. Finally, the detector contains an integrated TCSPC electronics that makes it a perfect tool for fluorescence lifetime imaging. This and other types of detectors currently in development will transform the way single-molecule imaging and spectroscopy experiments are designed and performed.

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3963-Plat
Light Sheet Microscopy Optimized for Depth Penetration to Study Embryogenesis
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Fluorescence light sheet microscopy (FLSM) has gained widespread recognition in recent years, due to its distinct advantages for the 3-dimensional (3D) imaging of living biological samples. FLSM uses a planar sheet of light to illuminate a sample, generating fluorescence over an optical section of the sample that is collected by a wide-field microscope camera oriented orthogonal to the light sheet. The orthogonal geometry between the illumination and detection pathways enables massive parallelization in both illumination and detection; futhermore, it permits optical and physical access to samples (3D cell cultures or whole embryos) in ways that are impossible in the collinear geometry of standard microscopes. Because of these features, FLSM significantly outperforms standard laser-scanning confocal microscopy in imaging speed, phototoxicity, and signal to noise in many imaging applications. An important aspect of any 3D imaging technique is its imaging depth limit (how deep into a sample useful information can be collected). In this respect, standard FLSM fares only slightly better than confocal microscopy. To overcome this hurdle, we have optimized FLSM for imaging of live thick samples by minimizing the degradation of the light sheet due to scattering, while preserving acceptable axial resolution. Using this approach we have imaged whole, live fruit fly embryos and zebrafish embryos. We achieve higher depth penetration than standard FLSM, while maintaining sub-cellular resolution, at imaging speed of about ten times faster than standard confocal microscopy.

Platform BJ: Member-Organized Session: Kinetics, Mechanisms & Regulation of Ion-Transporting ATPases

3964-Plat
Characterization of Partial Reactions in the Catalytic Cycles of Calcium and Copper ATPases
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Calcium and copper P-type ATPases transduce ATP chemical energy to transmembrane osmotic energy, using a catalytic mechanism common to haloacid dehalogenases where phosphoryl transfer from substrate to a conserved aspartate yields a phosphoenzyme intermediate before hydrolytic cleavage of Pi. Favored by its native abundance, characterization of the calcium ATPase is by now quite detailed, including two transmembrane calcium binding sites, a phosphorylation site within the headpiece, a conserved TGES motif for catalytic assistance of hydrolytic cleavage, and domain movements permitting long range linkage of phosphorylation and calcium binding sites. Presently, mutational analysis is still yielding further details on the calcium ATPase. As for bacterial (CopA) and human (ATP7B) copper ATPases, due to low native abundance, heterologous expression in cultured cells is required. In addition to domains present in other P-type ATPases, copper ATPase sequences include an amino terminus extension (NMBD) with one (CopA) or six (ATP7B) copper binding sites. Using recombinant protein, it is possible to demonstrate formation of phosphoenzyme intermediate by utilization of ATP, undergoing rather slow turnover. In analogy to the calcium ATPase, phosphoenzyme intermediate is not formed following mutation of the conserved aspartate or the transmembrane copper site. In addition to the phosphorylated ATPase intermediate, copper dependent phosphorylation of various serine residues occurs in ATP7B. Interference with protein autophosphorylation, both of aspartate and serines, is observed following mutation of a histidine residue in the nucleotide binding domain, a mutation found in Wilson disease of humans. The effects of NMBD copper site mutations and deletions suggest that the NMBD sequence is involved in catalytic regulation as well as protein targeting. (Supported by 5 R01 HL069830-08).

3965-Plat
The Involvement of Protein-Protein Interactions in the Mechanism of the Na^+,K^+-ATPase
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The Na^+,K^+-ATPase (or sodium pump) was the first ion pump to be discovered (Skou, 1957) and it is one of the most fundametally important enzymes of animal physiology. The electrochemical potential for Na^+, which the enzyme maintains, is used as the driving force for numerous secondary transport systems, e.g. voltage-sensitive Na^+ channels in nerve. ATP provides the energy source to drive ion pumping. However, it also plays a crucial allosteric role, accelerating significantly the enzyme's rate determining E2-E1 transition and the associated release of K^+ ions to the cytoplasm. Based on the results of stopped-flow kinetic experiments and recently published crystal structural data for the related enzyme, the sarcoplasmic reticulum Ca^{2+}-ATPase, it is suggested that the allosteric role of ATP in the mechanism of the Na^+,K^+-ATPase can be explained by an ATP-induced closing of the cytoplasmic domains of the enzyme which relieves steric hindrance arising from interactions between neighbouring pump molecules within the native membrane environment and hence an acceleration of the E2-E1 conformational change (Clarke, 2009). In the presence of millimolar concentrations of ATP, therefore, it is proposed that the enzyme functions as a monomer (alpha-beta protomer), whereas at low ATP concentrations it functions as a dimer ($(alpha\text{-}beta)_2$ diprotomer) or higher aggregate. The physiological advantage of protein-protein interactions is still unclear, but a possibility is that they may lead to an enhancement of the enzyme's ATP affinity and allow it to continue functioning even under hypoxic conditions.

Skou JC. (1957) *Biochimica et Biophysica Acta*, **23**: 394-401.
Clarke RJ (2009) *European Biophysics Journal*, in press.

3966-Plat
The Na/K-ATPase/Src Interaction and the E1/E2 Transition
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In our previous studies, we have shown that Na^+/K^+-ATPase interacts directly with Src to form a signaling receptor complex. This complex is involved in control of basal Src activity and ouabain-induced signal transduction. The aim of this work is to demonstrate that Na^+/K^+-ATPase also regulates Src activity through its conformation-dependent domain movements during pumping cycles. It is known that the Na^+/K^+-ATPase transits from E1 to E2 conformation during an ion pumping cycle. Based on the known crystal structures of Na/K-ATPase, this conformational transition results in a 110 degree turn of the A domain of Na/K-ATPase where Src SH2 domain binds. Computational modeling suggests that the movement of A domain during the pump cycle is likely to release Src from the Na/K-ATPase, resulting in the activation of Src kinase. Indeed, in vitro kinase assays show that while stabilization of Na/K-ATPase by N-ethylmaleimide and AMPPNP in E1P conformation keeps Src in an inactive state, converting the pump into an E2P state by fluoride compounds stimulates Src. Consistently, the Na^+-liganded E1 form of Na^+/K^+-ATPase inhibits Src whereas the K^+-liganded E2 form releases Src from the Na^+/K^+-ATPase and re-activated Src. Finally, Src is completely inactivated by the Na/K-ATPase in the presence of physiological concentrations of Na^+ (150 mM) and K^+ (5 mM). Reduction of K^+ results in an accumulation of E2P Na/K-ATPase and consequently an increase in active Src. Taken together, these new findings suggest that the Na^+/K^+-ATPase/Src complex may function as a pumping receptor, capable of coordinating ion pumping at the plasma membrane and other cellular activities by activating/inhibiting Src kinase.

3967-Plat
Oligomer Structure Detected in Na/K- and H/K-ATPase
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Since Repke's proposal of Flip-Flop model of Na/K-ATPase in 1973, Askari, Schoner, Hayashi, Taniguchi, Froehlich, Clarke and their coworkers have indicated the oligomericity of the enzyme from reactivity to various ligand (2006, J. Biochem. 140, 599 and also see review 2001, J.Biochem.129, 335). One of the most compelling pieces of evidence in favor of the oligomeric nature of Na/K-ATPase and gastric H/K-ATPase is the simultaneous presence of EP:EATP with half site phosphorylation and nearly half site ATP binding (1999, J. Biol. Chem.274,31792, 2002, Biochemistry 41, 2438). Recently