

Abstracts

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Plenary talks

O-001

View into the ribosomal exit tunnel

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Ribosomes, the universal polymerases for the translation of the genetic code into proteins, possess an elongated tunnel through which the nascent protein progress until they emerge into the cell. While traveling through the tunnel the nascent proteins can interact with specific tunnel wall components capable of imposing transient or a relatively long elongation arrest. Furthermore, internal architecture and flexibility allow response to cellular signals, thereby provides a mechanism for gene regulation.

O-002

The interplay between actin dynamics and membrane tension determines the shape of moving cells

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A central challenge in cell motility research is to quantitatively understand how numerous molecular building blocks self-organize to achieve coherent shape and movement on cellular scales. We focus on one of the classic examples of such self-organization, namely lamellipodial motility, in which forward translocation is driven by a treadmilling actin network. We combine detailed measurements of lamellipodial morphology, spatio-temporal actin dynamics and membrane tension, with mathematical modelling to explain how global shape and speed of the lamellipodium emerge from the

underlying assembly and disassembly dynamics of the actin network within an inextensible membrane bag.

O-003

Structure determination of dynamic macromolecular complexes by single particle cryo-EM

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Macromolecular complexes are at the heart of central regulatory processes of the cell including translation, transcription, splicing, RNA processing, silencing, cell cycle regulation and repair of genes. Detailed understanding of such processes at a molecular level requires structural insights into large macromolecular assemblies consisting of many components such as proteins, RNA and DNA. Single-particle electron cryomicroscopy is a powerful method for three-dimensional structure determination of macromolecular assemblies involved in these essential cellular processes. It is very often the only available technique to determine the 3D structure because of the challenges in purification of complexes in the amounts and quality required for X-ray crystallographic studies.

In recent years it was shown in a number of publications that it is possible to obtain near-atomic resolution structures of large and rigid macromolecules such as icosahedral viruses. Due to a number of methodological advances there are now also great perspectives for high-resolution single particle cryo-EM studies of large and dynamic macromolecules. Successful high-resolution structure determination of dynamic complexes requires new biochemical purification strategies and protocols as well as state of the art electron microscopes and high-performance computing. In the future cryo-EM will thus be able to provide structures at near-atomic

P-499

The Na⁺/H⁺ exchanger NHE7, biochemical features, intracellular localization and biological function

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Keywords: Acidification of intracellular compartments, Na⁺/H⁺ exchanger, somatic cell genetics, kinetic measurements, neurological diseases

The acidification of intracellular compartments is critical for a wide range of cellular processes. A recent candidate for pH regulation within early endosomes and TGN is the highly conserved intracellular Na⁺/H⁺ exchanger 7 isoform (NHE7), whose mutation leads to neurological syndromes in human patients. However, due to its intracellular localization, NHE7 biochemical features are still poorly characterized and its biological function remains elusive.

We have developed somatic cell genetic techniques that enable the selection of variant cells able to resist H⁺ killing through plasma membrane expression of H⁺ extruders. This enabled us to obtain stable cell lines with forced plasma membrane expression of NHE7. We used them to measure its functional and pharmacological parameters with high accuracy, using fast transport kinetics. To summarize, this exchanger displays unique features within the NHE family, especially with respect to its affinity for its substrates, lithium, sodium and protons and for its guanidine-derived inhibitors. Taken together with our results on the subcellular localization of the native NHE7, these unique biochemical features provide new insights on the biological function and pathological implications of this intracellular Na⁺/H⁺ exchanger.

P-500

Analysis of the collective behaviour of ion channels

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A novel approach to the analysis of the ion current recordings is proposed. The main goal of the standard patch clamp technique is to measure single channel activity (however the whole cell configuration is also used in various researches). In the presented study the ion channels time series recordings were several (up to four) ion channels were present and the collective behaviour of ion channels is investigated. The time current time series are converted into dwelltime series and the channel activity is analysed. The hypothesis of collective ion channels behaviour is verified and the influence of organolead compounds (Met3PbCl) on collective ion channel activity is measured. The analysis is performed on the SV cation channels of vacuolar membrane of *Beta vulgaris*.

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P-501

On motor and electrical oscillations in urinary tract: computer evaluation

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Introduction: Motor/electrical activity of urogenital tract is fundamental for patho-physiology (incontinence, reflux, etc. can lead to renal hypertonia).

Method: Parameters: *Motor patterns* (guinea-pig) – frequency/F, amplitudes/A (% init. length, isot. & intracell. rec.) of *spontaneous phasic/SPC & tonic/STC contractions*, also *electrical spikes/S, bursts/B, burst plateaus/BP* (Neu et al. Biophys.J. 125/6a/Jan2008; Eur.Biophys.J. 36/S1,S150/2007 & 34/6,765/2005; J.Physiol.Sci. 59/S1,249/2009; FASEB J. 19/4,139.4/2005).

Results: SPC-pyelon F:5.4±6.1/min, A:4.7±2.7%, SPC-ureter F:1.4±0.7, A:5.3±3.2, SPC-detrusor F:3.8±0.9, A:18.7±5.3, STC-trigone F:0.3±0.1, A:74.6±14.8; ureter-S: F:18.0±4.8/min, A:42.8±5.5mV, -BP: F:7.2±4.8, A:47.5±3.0, -BP-duration 1.3±0.9s; detrusor-S: F:15.0±7.8, A:47.6±7.3; -BP: F:1.1±0.9, A:52.3±9.0, -BP-duration 9.9±3.0s (n=327, p<0.01). Stretch (3-80mN), K⁺/Ca²⁺-influence induced specific changes in motor/electrical parameters. Special computer programme reflects exactly biophysical parameters.

Conclusion: Acc. to earlier/recent results mechano-sensitive Ca²⁺-activated K⁺-channels participate in electrical oscillations of detrusor/ureteral myocytes. Further experiments/evaluations incl. computer analysis are necessary to prove correlation with motor patterns.



O-502

Molecular rearrangements during slow inactivation of the Shaker-IR potassium channel

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Crosstalk between the activation and slow inactivation gates in *Shaker* potassium channels is now well-established. The activation gate perceives the conformation of the inactivation gate (Panyi and Deutsch, 2006, 2007). Closure of the inactivation gate speeds opening and slows closing of the activation gate, i.e., stabilizing the gate in the open configuration. If this coupling involves movement of the S6 transmembrane segment, then we predict state-dependent changes in accessibility of residues lining the channel cavity. We engineered cysteines, one at a time, at positions 470,

471, 472, 473, and 474 in a T449A *Shaker*-IR background and determined modification rates for the cysteine modifying reagents, MTSET and MTSEA, in the open, closed, and inactivated state of the channel. Neither reagent, applied from the intracellular side, modifies cysteines at 470–474 in the closed state. Both 470C and 474C are rapidly modified in the open state and at approximately one-tenth this rate in the inactivated state. In contrast, 471C is not modified in the open state but can be modified by MTSEA but not MTSET in the inactivated state. Residue 472C cannot be modified in any of the three states. Mutant 473C did not express current. Our findings are consistent with a rotation of S6 in the inactivated state, which increases the accessibility of residue 471 while simultaneously decreasing accessibility of residues 470 and 474. Any model of C-type inactivation in the *Shaker* Kv channel must conform to these experimental observations. [Supported by NIH grant GM 069837 (CD) and OTKA K 75904 (GP)].

P-503

Mapping of an I_{KS} channel opener reveals interactions between KCNE1 and the Kv7.1 voltage sensor

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In the heart, assembly of Kv7.1 with KCNE1 generates the repolarizing K^+ current I_{KS} . We and others recently suggested a strategic location of KCNE1 wedged between two adjacent Kv7.1 voltage sensing domains (VSD) and nearby helix S6 of another Kv7.1 subunit. Here we show that the I_{KS} channel opener, diisothiocyanostilbene-2',2'-disulfonic acid (DIDS) acts on I_{KS} to convert the time- and voltage-dependent channels into nearly voltage- and time-independent currents. The two isothiocyanate functionalities are crucial for the potent activating effect of DIDS on I_{KS} , since SITS that has only one of these groups and DNDS, which lacks isothiocyanate groups do not activate I_{KS} currents. DIDS does not affect Kv7.2 and has a weaker effect when KCNE1 is co-expressed with a chimeric Kv7.1 bearing a Kv7.2 VSD paddle. Mutagenesis data suggest that DIDS may activate I_{KS} by interacting to an external pocket, formed at the interface of the superficial boundary of the KCNE1 transmembrane segment and the VSD of Kv7.1. DIDS interaction at the Kv7.1 VSD-KCNE1 interface reveals the importance of two lysine residues, K41 in KCNE1 and K218 in Kv7.1 S3-S4 linker and underscores the proximity of KCNE1 to the VSD of Kv7.1.

P-504

An in-silico approach to primaquine binding to Trp756 in the external vestibule of sodium channel Nav 1.4

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The aim of our computed study was to examine the possible binding site of primaquine (PQ) using a combined

homology protein modeling, automated docking and experimental approach. The target models of wild-type and mutant-types of the voltage-dependent sodium channel in rat skeletal muscle (rNav_v 1.4) were based on previous work by Tikhonov and Zhorov. Docking was carried out on the P-loop into the structure model of rNav_v 1.4 channel, in the open state configuration, to identify those amino acidic residues important for primaquine binding. The three-dimensional models of the P-loop segment of wild types and mutant types (W402, W756C, W1239C and W1531A at the outer tryptophan-rich lip, as well as D400C, E755C, K1237C and A1529C of the DEKA motif) helped us to identify residues playing a key role in aminoquinoline binding. In good agreement with experimental results, a 1000-fold inhibition loss was observed, tryptophan 756 is crucial for the reversible blocking effects of PQ. As a result, W756C abolished the blocking effect of primaquine in voltage-clamp assays.

P-505

Hydrogen bond formation accelerates channel opening of the bacterial mechanosensitive channel MscL

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The bacterial mechanosensitive channel MscL is constituted of homopentamer of a subunit with two transmembrane inner and outer (TM1, TM2) α -helices, and its 3D structure of the closed state has been resolved. The major issue of MscL is to understand the gating mechanism driven by tension in the membrane. To address this question, we performed molecular dynamics (MD) simulations for the opening of MscL embedded in the lipid bilayer. In the closed state of MscL, neighboring TM1 inner helices are crossed each other near the cytoplasmic side and Leu19 and Val23 in the constricted part form a stable hydrophobic environment called gate. Upon membrane stretch, Phe78 in TM2 outer helices was dragged by lipids, leading to an opening of MscL. Thus Phe78 was concluded to be the major tension sensor. During opening, TM1 inner helices were also dragged and tilted, accompanied by the outward sliding of the crossings. This led to a slight expansion of the gate associated with an exposure of oxygen atoms of the backbone to the inner surface of the gate. This allows water penetration in the gate and formation of hydrogen bonds between water and the exposed oxygen, which in turn weakened the hydrophobic interaction at the crossings, causing a further opening of the gate and water permeation.

O-506

Molecular characterization of BK_{Ca} channel in cardiac mitochondria

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Mitochondrial BK_{Ca}, mitoBK_{Ca} has been proposed to be cardioprotective and formed by proteins of ~50 to ~125