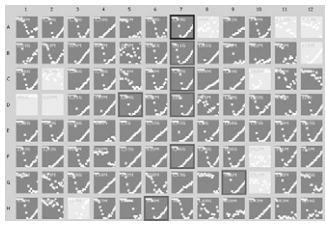
3683-Pos
Voltage Gated Trapping of fcAMP in HCN2 Channels
Jana Kusch[1], Susanne Thon[1], Christoph Biskup[1], Eckhard Schulz[2], Thomas Zimmer[1], Klaus Benndorf[1].
[1]Friedrich-Schiller-University, Jena, Germany, [2]Fachhochschule Schmalkalden, Schmalkalden, Germany.
HCN channels are nonselective tetrameric cation channels that are activated by hyperpolarizing voltages and modulated by the ligand cAMP. They generate spontaneous rhythmic activity in the heart and brain. Ligand binding to the intracellular cyclic nucleotide-binding site accelerates the activation kinetics, shifts the steady-state activation to more positive voltages and increases the open probability. We expressed homotetrameric HCN2 channels in *Xenopus* oocytes. Using fast solution exchange by a piezoelectric system we observed that removal of cAMP from the patch induces significant current deactivation if the channels are weakly activated by voltage but only minor deactivation if the channels are strongly activated by voltage. To visualize ligand unbinding directly, simultaneous measurement of activation and ligand binding was performed in excised inside-out macropatches by means of patch-clamp fluorometry with confocal resolution, using a fluorescent cAMP derivate (fcAMP) that activates the channels closely similar to cAMP. As a result, fast removal of saturating fcAMP (7.5 μM) from maximally activated HCN2 channels (−130 mV) caused a fluorescence decay with a fast and a slow component. Assuming four bound ligands per channel at a saturating ligand concentration and at saturating hyperpolarization, the amplitudes of the two exponentials of ~50% each suggest that two of the for bound ligands unbind fast, whereas the remaining two are trapped. This trapping can be shown to depend on channel activation. Comparing the time courses of deactivation and unbinding following a fast removal of the ligand, the results make it likely that only two of the four binding sites have to be occupied to cause maximal ligand induced modulation, resembling the situation in related CNGA2 channels, where two of the four ligands are sufficient to open the channel maximally.

Ion Channels, Other II

3684-Pos
Descent of the Prion Protein Gene Family from the Extracellular Domain of an Ancestral Zip Metal Ion Transporter
Holger Wille[1], Sepehr Ehsani[2], Joel C. Watts[1], David Westaway[3], Gerold Schmitt-Ulms[2].
[1]University of California, San Francisco, San Francisco, CA, USA, [2]University of Toronto, Toronto, ON, Canada, [3]University of Alberta, Edmonton, AB, Canada.
Since its discovery over twenty years ago, both the phylogenetic origin and the cellular function of the prion protein (PrP) have remained enigmatic. The subsequent discovery of the PrP paralog Doppel (Dpl) has strengthened the argument that the copper-binding ability of PrP and Dpl may be part of their cellular function.
Chemical crosslinking provides insights into a possible function of PrP through the characterization of its molecular neighborhood on the cell surface. Quantitative interactome data demonstrated the spatial proximity of two putative zinc ion transporters of the ZIP family, ZIP6 (Slc39a6) and ZIP10 (Slc39a10), to mammalian prion proteins in vivo. A subsequent bioinformatic analysis revealed the unexpected presence of a PrP-like amino acid sequence within the N-terminal, extracellular domain of a distinct sub-branch of the ZIP protein family, which includes ZIP5, ZIP6 and ZIP10. Structural threading and orthologous sequence alignment analyses argue that the prion protein gene family is phylogenetically derived from a ZIP-like ancestral molecule. The level of sequence homology and the presence of prion protein genes in most chordate species place the split from the ZIP-like ancestor gene at the base of the chordate lineage. This relationship explains structural and functional features found within mammalian prion proteins as elements of an ancient involvement in the transmembrane transport of divalent cations, presumably zinc and/or copper. The phylogenetic and spatial connection to ZIP proteins is expected to open new and thus unexplored avenues of research to elucidate the biology of the prion protein in health and disease.

3685-Pos
96 Parallel Gigaseal Patch Clamp Recordings
Andrea Bruggemann, Cecilia Farre, Timo Stengel, Claudia Haarmann, Christian Patzig, Christian Grad, Corinna Voll, Bertholt Heintzel, Stephanie Simmel, Juergen Steindl, Niels Fertig, Michael George.
Nanion Technologies, Munich, Germany.
Over the last several years ion channels have received more and more interest as potential drug targets, because of their known involvement in chronic and acute disease. Patch clamp is the gold standard for obtaining highly relevant information about ion channels and their effectors but it is a notoriously laborious technique. To meet the ever increasing demand for higher throughput in ion channel screening and safety testing we have developed a highly parallel patch clamp platform, the SyncroPatch 96. The platform supports giga-seal recordings, continuous recording during compound application and addition of multiple compounds at each of the 96 cells recorded from at a time.
The figure shows 96 individually recorded IV's of the sodium channel hNav1.5 expressed in HEK 293 cells.
Here we present high quality data of voltage gated and ligand gated channels expressed in various cell lines. Whole cell access was achieved using either pore forming agents such as Nystatin, or by short applications of suction pulses.
The SyncroPatch 96 offers high throughput without compromising data quality.



3686-Pos
Acid Extrusion from Human Spermatozoa is Mediated by Flagellar Hv1 Proton Channel
Polina V. Lishko, Andriy Fedorenko, Inna L. Botchkina, Yuriy Kirichok.
UCSF, San Francisco, CA, USA.
Human spermatozoa are quiescent in the male reproductive system and must undergo activation once introduced into the female reproductive tract. After ejaculation, and during their transit through the female reproductive tract, sperm motility is initiated and then hyperactivated to allow the spermatozoa to penetrate through the viscous oviductal mucus and the egg's protective vestments. These processes are known to require alkalinization of sperm cytoplasm, but the mechanism responsible for transmembrane proton extrusion has remained unknown due to the inability to measure membrane conductances in human sperm. Here, by successfully patch clamping human spermatozoa, we show that proton channel Hv1 is their dominant proton conductance. Hv1 is confined to the principal piece of the sperm flagellum, where it is expressed at an unusually high density. Robust flagellar Hv1-dependent proton conductance is activated by membrane depolarization, an alkaline extracellular environment, and removal of extracellular zinc, a potent Hv1 blocker.
Surprisingly, endocannabinoid anandamide was also found to modulate Hv1 activity in the sperm cells as well as in the heterologously expression system. Hv1 allows only outward transport of protons and is therefore dedicated to inducing intracellular alkalinization and activating spermatozoa. In contrast to the large Hv1 current in human sperm cells, the amplitude of the outward current recorded under similar conditions from mouse spermatozoa was 30 times smaller; therefore, mouse sperms seem to have a different mechanism for acid extrusion.
In human sperm, Hv1-induced intracellular alkalinisation should activate pH-dependent Ca2+ channel CatSper and control intracellular Ca2+ concentration. Finally, since Hv1 lies upstream in the signalling cascades leading to sperm activation, hyperactivation, and capacitation, Hv1 is an attractive target for the control of male fertility.

3687-Pos
Ion Channel Reconstitution on a Pore-Suspending Membrane on Microstructured Glass Chip: Towards Artificial Gap Junction Formation Between Bilayers and Cells
Mohamed Kreir[1], Christoph Methfessel[2], Michael Habig[2], Mathias Winterhalter[3], Niels Fertig[1].
[1]Nanion Technologies GmbH, Munich, Germany, [2]Bayer Technology Services, Leverkusen, Germany, [3]Jacobs University Bremen, Bremen, Germany.
Chip based automated patch clamping is an attractive biophysical tool for studying ion channel proteins. Solvent-free planar lipid bilayers can be formed in an automated fashion by positioning and subsequent bursting of giant unilamellar lipid vesicles containing membrane proteins on micron-sized apertures in a borosilicate glass substrate. The use of proteoliposomes for bilayer formation on such chips allows for the direct recording of single channel activity without need for commonly difficult reconstitution of membrane protein after bilayer formation. This approach is specifically attractive for investigations of membrane proteins not accessible to patch clamp analysis, like e.g. proteins from organelles or proteins from bacteria.
Here, the biophysical and pharmacological characterization of different membrane proteins was performed. A wide variety of ion channels have been studied with this technique, for example potassium channels (KcsA, Kv1.2), sodium channels (NachBac) as well as other ligand-dependent (IP3 receptor) or mechanosensitive channels (MscL, TRP channels). Also, screening for influx

of antibiotics through porins (OmpF, OmpC) was done to elucidate the uptake kinetics of antibiotics through porins.
One main set of experimental data to be presented is on connexins proteins. Connexins are widely distributed in mammalian tissues and serve to join cells together into larger, functional units. We investigated the properties of hemichannels from Cx26 and Cx43 which were isolated biochemically and reconstituted into synthetic lipid membranes. In this study, preliminary data suggest the formation of gap junctions between cells and synthetic bilayer membranes. This opens possibilities to access the cytoplasm of living cells for biochemical or electrical studies, and especially to develop novel automated techniques for electrophysiological studies.

3688-Pos
Viral and Host Channels: A Comparison
Wolfgang B. Fischer.
National Yang-Ming University, Taipei, Taiwan.
Viruses and their host cells have something in common they both need and encode ion channels. Whilst for the host the role and the mechanism of function of these membrane proteins is straight forward, knowledge about the viral channels seems just to unroll on the molecular level. Experimental tools are gradually delivering low and high resolution structures with computational methods as another source for structural information on the atomic level.
The viral membrane proteins identified as channels are becoming increasingly more complex in respect to their topology. Most of the channels are still very much smaller than the channels of the host. This triggers an intrigued discussion about (i) when is a membrane protein a channel and (ii) what do the smaller viral channels have in common with their bigger class mates, the host channels. Data from computational modeling will be presented along these lines.

3689-Pos
Channelrhodopsin-2 Variants with Accelerated and Decelerated Channel Kinetics
Andre Berndt[1], Katja Stehfest[1], Dietrich Gradmann[2], Peter Hegemann[1].
[1]Humboldt Universität zu Berlin, Berlin, Germany, [2]Georg August Universität, Göttingen, Germany.
The light-activated cation channel Channelrhodopsin-2 (ChR2) is a powerful tool for controlling neuronal activity. Its genetic information is carried into neurons which express the protein. Channel activation by blue light exposure causes membrane depolarizations that immediately trigger action potentials. We genetically modified ChR2 wildtype and created variants with decelerated and accelerated channel kinetics as well as changed ion selectivities. ChR2 mutations with new features broaden the toolbox for neuroscientists but the mechanisms of channel activation and ion translocation are still unclear. We use a combination of theoretical approaches like molecular and mathematical modeling as well as experimental techniques like UV/vis spectroscopy, flashlight photolysis and two electrode voltage clamp to reveal how mutations affect the channel properties.

3690-Pos
VSOP/Hv1 Proton Channels Sustain Superoxide Production, Calcium Entry, and Cell Migration by Limiting the Depolarization and Acidification of Activated Neutrophils
Antoun El Chemaly[1], Yoshifumi Okochi[2], Mari Sasaki[2], Serge Arnaudeau[1], Yasushi Okamura[2], **Nicolas Demaurex**[1].
[1]University of Geneva, Geneva, Switzerland, [2]University of Osaka, Osaka, Japan.
Neutrophils kill microbes with superoxide radicals generated by the NADPH oxidase, an enzyme that moves electrons across membranes. Voltage-gated proton channels (VSOP/Hv1) are required for high-level superoxide production by phagocytes, but the mechanism of this effect is not clear. Using mice bearing a targeted disruption in the VSOP/Hv1 gene (VSOP/Hv1-/-), we show that neutrophils devoid of VSOP/Hv1 lack proton currents but have normal electron currents, indicating that these cells have a fully functional oxidase that cannot conduct protons. VSOP/Hv1-/- neutrophils were more acidic and more depolarized than neutrophils from wild-type mice, and consequently produced less superoxide. Loss of VSOP/Hv1 also aborted calcium responses to chemoattractants, increased neutrophil spreading, and decreased chemokinesis. Our findings indicate that proton channels extrude the acid and compensate the charge generated by the oxidase, thereby sustaining calcium entry signals that control the adhesion and motility of neutrophils. Loss of proton channels thus aborts superoxide production and causes a severe signalling defect in neutrophils.

3691-Pos
Design of a Potent and Selective Small Molecule Kv1.5 Blocker
Ananthakrishnan Sankaranarayanan.
University of New Orleans, New Orleans, LA, USA.
The voltage-gated potassium channel Kv1.5 is being studied extensively as a potential target for treating atrial fibrillation and other life-threatening arrhythmias. Since Kv1.5 is expressed selectively in the human atrium and not in the ventricle, a potent and selective Kv1.5 blocker should therefore significantly increase the action potential duration (APD) of the atrium without affecting that of the ventricle. Unlike the existing anti-arrhythmic drugs such as amiodarone, sotalol etc. that block the Kv11.1 channel (hERG), a potent and selective Kv1.5 blocker should not induce dangerous and fatal proventricular arrhythmia.
Phenoxyalkoxypsoralens (PAPs) are a class of compounds that has previously been described to block both the lymphocyte Kv1.3 and the cardiac Kv1.5 channel (*Mol. Pharmacol. 2005*). Through a combination of classical medicinal chemistry and traditional electrophysiology, we now studied the structure-activity relationship of PAPs with the aim of generating more selective Kv1.5 blockers. When the side chain phenyl ring of PAPs were decorated with a combination of electron-donating (methyl) and electron-withdrawing (nitro) groups as in PAP-22 {5-[3-(4-methyl-2-nitrophenoxy)-propoxy]psoralen}, the compounds exhibited a four to five fold increase in selectivity for Kv1.5 over Kv1.3. However, when we substituted the nitro group with a chloro group, as in PAP-25 {5-[2-chloro-4-methylphenoxy)propoxy]-psoralen}, more selective Kv1.3 blockers were generated. We are currently further investigating the effect of other strongly electron-withdrawing groups instead of the nitro group in order to increase the potency and selectivity of PAPs for Kv1.5 over Kv1.3. Other fused tricyclic rings containing 2-aminobenzothiazole are also being explored as a potential pharmacophore to design and develop Kv1.5 blockers.
Supported by The Board of Regents Support Fund

3692-Pos
Using Domain Based Discovery Methods to Identify Prokaryotic Counterparts to Eukaryotic Protein Ion Channels
Eric Jakobsson[1], Gloria Rendon[1], Ruth Kantorovitz[1], Jeffrey Tilson[2], Mao-Feng Ger[1].
[1]University of Illinois, Urbana, IL, USA, [2]Renaissance Computing Institute, Chapel Hill, NC, USA.
Our lab, in collaboration with the laboratory of I. Aravind, used domain based methods to discover the prokaryotic counterparts of Ach receptor channels (Tasneem et al), after whole-protein approaches such as BLAST had failed. Our identification was verified by functional studies and by x-ray crystallization of targets we identified. We have subsequently streamlined and formalized the domain search methods and applied them to a variety of ion channels and other membrane proteins. It has become clear that domain based methods are more powerful than whole-protein approaches, provided one has good domain definitions to start the search. The use of domain-based methods depends on a somewhat different model of evolution from BLAST. In BLAST, the operational model is the substitution of one amino acid for another, with gaps being treated as a particular type of substitution. Domain-based methods deal readily and directly with the phenomenon of large scale reorganization of domains, which is now recognized as an essential process for innovation in evolution. In this presentation we will provide an update on prokaryotic Ach receptor channels, a survey of prokaryotic glutamate receptor channels and their relationship to their eukaryotic counterparts, the discovery of a prokaryotic counterpart to HCN and CNG channels which appears likely to be closely related to their common ancestor, and searches on other families that are under way at the time of preparation of this abstract. We gratefully acknowledge support from NSF grants 0835718 and 0235792, from NIH grants 5PN2EY016570-06 and 5R01NS063405-02 from the Beckman Institute for Advanced Science and Technology, the National Center for Supercomputing Applications, and the Renaissance Computing Institute.

3693-Pos
A Novel Fluorescence Assay for Voltage-Gated Ion Channels Based upon Light Induced Voltage Clamp
Ulrich Pehl[1], Sonja Kleinlogel[2], Ingo Janausch[3], **Bela Kelety**[1], Ernst Bamberg[2].
[1]LIVC Technologies GmbH, Frankfurt Main, Germany, [2]Max-Planck-Institute of Biophysics, Frankfurt Main, Germany, [3]IonGate GmbH, Frankfurt Main, Germany.
Ion channels are a key target class with a high therapeutic potential in virtually all possible disease indications. In addition, a potential side effect of pharmaceutical compounds is the blocking of hERG channels in heart cells making easy and cost effective hERG safety screening necessary for drug development today. Conventional screening techniques yield insufficient data quality particularly when assessing voltage-gated ion channels. Thus, the development of new reliable technologies is desirable to integrate ion channel screening into early lead generation stages of drug discovery.