ethanolamine (PE18:1/16:0) and phosphatidyl serine (PS18:0/18:1). Since this membrane is thicker than the estimated length of tolaasin channel, mismatch in thickness may make the channel unstable. Phospholipids composed of medium or short-chain fatty acids may be helpful to the stability of tolaasin channel by making the membrane thinner. When phosphatidyl ethanolamines made with decanoic acids (capric acid, DDPE), myristic acids (DMPE), and stearic acids (DSPE) were added, DDPE (200 nM) facilitated tolaasin-induced hemolysis. When the concentration of DDPE was adjusted from 0.2-200 nM, the hemolysis was stimulated at the concentrations above 2 nM. Ks value of DDPE effect was obtained at 6 μM DDPE. When the preincubation effect of tolaasin and DDPE was measured, binding of tolaasin to DDPE was completed within 5 min. In the lipid bilayer recording, the addition of DDPE increased tolaasin channel activity by increasing open probability. Therefore, tolaasin molecules make more stable channels with phospholipids composed of medium-chain fatty acids.

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Competition Effect of DDPE and Zn^{2+} on the Hemolysis Induced by Tolaasin, a Pore-Forming Peptide Toxin

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Tolaasin is an antimicrobial peptide produced by *Pseudomonas tolaasii* and causes a brown blotch disease by disrupting membrane structures of cultivated mushrooms. The pore-formation of tolaasin was demonstrated in an artificial lipid bilayer. The length of tolaasin channel is not enough to penetrate through the membrane. The additions of phospholipids composed of two medium-chain decanoic acids (DDPE) facilitated tolaasin-induced cytotoxicity. When tolaasin was added to RBCs, its hemolytic activity was increased by the addition of DDPE at the concentrations above 2 nM. Although various tolaasin inhibitors have been isolated and characterized, no activators are identified up to date. Therefore, working mechanism of DDPE will tell us about very useful information how tolaasin works on the membrane. In the presence of DDPE, the complete hemolysis occurred very fast, within 5 min, compared to the control experiment of 25 min. Competition effect of Zn^{2+}, a potent tolaasin inhibitor, and DDPE on tolaasin-induced hemolysis was investigated. When Zn^{2+} and DDPE were added simultaneously, the stimulatory effects of DDPE observed at low concentrations of Zn^{2+}. Zn^{2+} at 0.5 mM inhibited the tolaasin-induced hemolysis by 70%; however, it was reduced to 30% in the presence of DDPE at 1 μM. At the concentrations above 1 mM, Zn^{2+} completely blocked the tolaasin activity and no effect of DDPE was measured. In these experiments, the effect on the tolaasin-induced hemolytic activity was dominated by Zn^{2+} rather than DDPE at Zn^{2+} concentrations above 1 mM. Mixed effect of these two modulators was observed at 0.5 mM Zn^{2+}. Based on these results, we suggest that DDPE stabilizes the tolaasin channel but it does not inhibit the binding of Zn^{2+}, representing their binding sites or working mechanisms are different.

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Protein Translocation through Mitochondria Channel: Peptide Interactions with TOM40 Channel

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TOM is high molecular mass protein complex that facilitates the transfer of nearly all mitochondrial preproteins across outer mitochondrial membrane. Preproteins bound by different receptor subunits travel via a pore formed by a specific subunit that constitutes an ion-conducting channel into mitochondria. High resolution ion conductance measurements through mitochondrial TOM40 channel in the presence of peptide revealed binding kinetics. More specifically, we have investigated the voltage dependence of the membrane transport of the peptide pF1β through single TOM40 channel. It is shown that association rate $k$on and dissociation rate k_{off} strongly depends on the applied transmembrane voltage and kinetic constants increase with increase in the applied voltage. This model involves a binding site inside the channel and attractive interactions between the peptide and binding site in the channel facilitates the peptide translocation at increasing voltage. Our analysis of the data provides a full quantitative description of all the relevant thermodynamic, kinetic and electric parameters including a detailed formulation of the peptide partition through the channel at single molecule level.

Reference

Romero-Ruiz M, Mahendran KR, Eckert R, Winterhalter M, Nussberger S. Interactions of mitochondrial presequence peptides with the mitochondrial outer membrane preprotein translocase TOM. *Biophys J.* 99 (2010) 774-81.

3332-Pos Board B193

Electrical Aspects of Membrane Permeabilization by New Polycationic Peptides Derived from the Cry11Bb Protoxin

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The permeabilization of mitochondrial and plasma membranes by synthetic polycationic peptides derived from the Cry11Bb protoxin was studied. The peptides were designed with the aim to further study of their antimicrobial and anticancer activities. It was observed that the membrane permeabilizing activity of these polycationic peptides strongly increased by the transmembrane potential (minus inside). This phenomenon was confirmed by the study of the artificial planar membrane permeabilization: applying 50 mV to the planar membrane (minus to the trans side) during 30 sec induced time-dependent increase in the transmembrane current in the presence of a peptide added to the cis side, while subsequent application of the opposite potential caused its decrease. We also observed that the activation of the cell suicide mechanism, which partially revealed in phosphatidylserine exposure at the cell surface, significantly increased the plasma membrane permeabilization by polycationic peptides. Some peptide topology characteristics, such as the value and the orientation of the electrical dipole moment(s) interacting with the membrane dipole potential seem to also be important factors influencing the membrane-permeabilizing activity of polycationic peptides. In general, our data are consistent with the concept that various electrical properties of biomembranes (the transmembrane potential, membrane dipole potential and the surface charge) might explain at least partially certain selectivity of antimicrobial and anticancer activities of many natural and synthetic polycationic peptides. (Financial support: Colciencias (Colombia) research grant #111840820380).

3333-Pos Board B194

Helix N-Cap Asp are the pH Trigger for Colicin a Membrane Insertion

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Colicins are antibacterial toxins produced by *Escherichia coli* cells to kill closely related competing bacteria. They are large proteins (>40kDa) composed of three domains. The N terminal and central domains are required to cross the gram negative outer membrane whilst the C-terminal domain carries a toxic activity such as a nuclease or pore-forming function. The pore forming domains are all homologous, ten helix bundles with a buried hydrophobic hairpin which is exposed in the membrane bound state. As a result, unfolding of the domain is required for function and, for some colicins such as N, this is a significant barrier to in vitro study. Two colicins, A and B, show significantly enhanced membrane insertion at low pH and this has been correlated with the formation of an acidic molten globule. Furthermore colicin A requires acidic lipids in the inner membrane of target cells whilst colicin N, which shows no pH sensitivity, does not. The mechanism for this pH sensitivity has been unclear. Here we show that helix N-Cap residues are the critical pH switch. At several sites in Colicin A, surface Asp residues replace the Asn found in colicin N. Surprisingly mutation of these surface Asp to Ala leads to a molten globule phenotype whereas replacement by Asn stabilises the domain. HSQC NMR shows that the effects of the Ala mutations are not localised. Examination of the structure at each site shows that each critical Asp is a helix N-capping residue. Protonation of each Asp destabilises the colicin and allows membrane insertion. This not only solves the riddle of colicin A pH dependence but also reveals a generic method for pH regulation of protein stability.

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On Channel Activity of Synthetic Peptides Derived from Severe and Acute Respiratory Syndrome Coronavirus (SARS-CoV) E Protein

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Members of the *Coronaviridae* family are enveloped viruses causing in humans from common colds to acute respiratory syndrome and, in animals, a variety of lethal diseases of economical relevance. The envelope (E) protein from coronaviruses (CoVs) is a small polypeptide that modulates coronavirus morphogenesis, tropism and virulence [1]. SARS-CoV E protein forms an amphipatic alpha-helix that expands the viral membrane once and displays

the amino-terminus to the virus surface and the carboxy-terminus inside of the viral particle. In vitro studies have shown that synthetic coronavirus envelope proteins oligomerize forming pentameric structures with ion channel activity [2].
We have evaluated the ion channel activity of several synthetic peptides derived from the transmembrane domain of SARS-CoV and HCoV-229E E proteins by using planar bilayer electrophysiology of peptide oligomers in DPhPC membranes. The conditions under which some of these peptides form ion channels and their properties at the single channel level were determined. The ion channel activity of a collection of SARS-CoV E protein transmembrane domain derived peptides with point mutations showed that specific residues, mapping at the internal side of the pore, abolished the conductivity. The conductance of the wild type SARS-CoV and HCoV-229E E protein derived peptides was studied under different salt concentrations. At 1M solutions of monovalent ion concentration conductance was lower than 1 nS, suggesting that these peptides form relatively large channels. In contrast to previously published data, selectivity measurements did not show a marked preference between cations and anions in our system. Possible causes of this divergence will be analyzed.
[1] M. L. DeDiego *et al.* (2008) Virology 376:379-89.
[2] L. Wilson *et al.* (2004) Virology 330:322-31.

3335-Pos Board B196

Phosphatidyl-Serine-Positive Cells with High Sensitivity to the Alzheimer's Disease Aβ Peptides Display Distinctive Mitochondrial Characteristics

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The Alzheimer's Aβ peptides exhibit cell-selective toxic effects in cultured cells and in neurons of Alzheimer's disease (AD) brains. As we previously showed *ex vivo* neuronal cells and cell lines consisted of subpopulations of cells characterized by differential levels of a common surface membrane target for the phosphatidyl-serine (PtdSer) specific binder annexin V and for the Alzheimer's Aβ peptidesbinding. These findings enable us to efficiently separate by flow cytometry sorting PtdSer positive cells on a variety of neuronal cell lines and ex vivo neurons and analyze their Aβ-binding affinity. We collected compelling data confirming the involvement of PtdSer as one of the surface membrane signal molecules for Aβ. This investigation used the persistent presence of PtdSer on the outer leaflet of the plasma membrane of PC12 cells to identify, sort and further culture the subpopulation of cells that are PtdSer positive ($PtdSer^{+}$) and have high affinity to bind Aβ, and consequently are more likely to be harmed by Aβ. Various viability tests showed that the group of cells sorted as $PtdSer^{+}$ was the most sensitive to Aβ toxic effects. The caspase3/7 activation level of the $PtdSer^{+}$ subpopulation of cells was similar to one of the PtdSer negative ($PtdSer^{-}$) subpopulation and the control cells. Therefore we assume, $PtdSer^{+}$ cells are not undergoing apoptosis. Additionally, $PtdSer^{+}$ cells have persistent higher levels of PtdSer on the outer leaflet of the plasma membrane, significantly lower levels of cytosolic ATP, lower mitochondrial mass and mitochondrial membrane potential, and slightly higher production of reactive oxidative species compared to $PtdSer^{-}$ and control cells. We hypothesize that all of these distinctive cellular and mitochondrial conditions are the basis for the high sensitivity to Aβ displayed by some selective cell lines and neurons.

3336-Pos Board B197

Sublytic Concentrations of Staphylococcus Aureus α-Toxin Trigger Na+/K+ Atpase Mediated Cell Shrinkage

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Cell lysis can occur through the pathologic action of various membrane-targeting toxins, of which the pore-forming toxin Staphylococcus aureus α-toxin is a prominent example. Paradoxically, S. aureus α-toxin, which forms monovalent cation permeable channels in the host cell plasma membrane, has been observed to cause significant volume shrinkage in many cell types. In HeLa cells we note a ≈45% decrease in cell volume 30-60 mins post α-toxin treatment in both interphase and mitotic cells. We show that inhibition of the Na+/K+ ATPase pump with ouabain not only prevents the cell shrinkage, but leads to cell volume expansion after exposure to α-toxin. This suggests α-toxin mediated volume decrease occurs through the upregulation of Na+/K+ ATPase activity because the 3:2 export:import ratio of Na+:K+ leads to the loss of one osmolyte per cycle. We therefore conclude that α-toxin induced cell shrinkage is an active cellular process driven by the Na+/K+ ATPase and elucidate sub-lytic mechanisms of pore-forming toxin assault.

3337-Pos Board B198

Evidence for a TH6-TH7 Transmembrane Hairpin in the Diphtheria Toxin T-Domain Open Channel State

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Diphtheria toxin has three domains: a C-terminal receptor-binding (R) domain, a central translocation (T) domain, and an N-terminal catalytic (C) domain. After the toxin binds to the target cell and undergoes endocytosis, it reaches an acidic vesicle compartment. Here the T-domain translocates the C-domain across the endosomal membrane to the cytosol, where it acts as a lethal enzyme. T-domain added to the *cis* side of a planar lipid bilayer forms conducting channels in the presence of a pH gradient (e.g., 5.3 *cis*, 7.2 *trans*). Previous work has identified three transmembrane segments of T-domain in the open channel state, corresponding to TH5, TH8 and TH9 in the crystal structure. Residues near either end of the TH6-TH7 segment are located on the *cis* side of the membrane, based on the polarity of voltage-dependent channel block induced by an attached peptide tag, as well as the effect of streptavidin binding to a biotinylated residue. The TH6-TH7 segment (19 residues) is too short to span the bilayer as an alpha-helical hairpin, but it could as an extended hairpin structure. We have constructed a series of mutant T-domains with a single cysteine residue at positions in TH6-TH7, and probed for an effect of sulfhydryl-specific methanethiosulfonate (MTS) reagents on the channel conductance. Interestingly, at positions in the TH6-TH7 loop, MTS-ET reacts much more quickly from the *trans* side than from the *cis* side. (This is despite the *cis*-positive voltage, which drives the cationic MTS-ET into the channel from the *cis* side.) This suggests that these residues are located near the *trans* side, consistent with a hairpin structure. We are comparing the *cis*-side *vs*. *trans*-side reaction rates of an uncharged reagent (MTS-glucose) to more systematically determine the transmembrane positions of TH6-TH7 residues.

3338-Pos Board B199

Development of the Periss Method to Generate GPCR Ligands/Binders from a Random Peptide Library with a Spider Neurotoxin Scaffold

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The *in vitro* evolution technology has been effectively applied to generate peptides specifically bound to target molecule(s). In conventional methods, however, soluble proteins are mostly the target. We previously succeeded in generating IL6 receptor agonists and antagonists by *in vitro* evolution from a random peptide library with a three-finger type neurotoxin scaffold (Naimuddin *et al*, 2011). The candidates were initially selected by binding to soluble portion of IL6 receptor, and then confirmed by cell-based assay.
To apply *in vitro* evolution techniques to membrane proteins, however, optimal solubilization/reconstitution is critical and is a major bottleneck in the process. To overcome the problems, we have developed a new technique named PERISS (intra periplasm secretion and selection) method. In this method, the peptides and the target membrane protein are expressed in the periplasm and in the inner membrane of *E. coli*, respectively. Interaction between the peptide and the target, and the following selection are achieved in the periplasmic space. The peptides interacting with the target proteins are collected as binding complexes [peptide-target-*E. coli*]. The selected peptide sequence is encoded in the plasmid in *E. coli*, and the corresponding DNA is amplified by PCR to prepare the second round library.
To evaluate the PERISS method, we have expressed the muscarinic acetylcholine receptor m2 subtype in the inner membrane. The peptide library was constructed based on a neurotoxin GTx1-15 (34 aa) with an ICK motif, which we originally isolated from the spider venom and characterized as a T-type calcium channel blocker (Ono *et al*, 2011). After six round selections by the PERISS method, selected peptides showed convergence in sequences. One of the peptides showed moderate affinity (K_d^{app} ~300 nM) and subtype selectivity to m2 receptor.

3339-Pos Board B200

Peptide-Gated DEG/ENaC Channels from Hydra Magnipapillata are Highly Permeable for Calcium

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DEG/ENaC ion channels are trimeric proteins, forming a heterogeneous group of sodium-selective ion channels sensitive to the inhibitor amiloride. Most family members known so far are impermeable to calcium. Only ASIC 1a (and possibly MEC-4) conduct calcium to a minor extent. To identify ancient properties of this ion channel family, we have recently cloned five new DEG/ENaC