to more negative potentials. These effects result from a voltage sensor trapping mechanism, in which toxins trap the voltage sensor in its activated conformation. Determinants of β-scorpion toxin (CssIV) binding and action on sodium channel (Nav1.2) are located in the S1-S2 and S3-S4 extracellular linkers in the voltage-sensing module in domain II. To completely map these regions, we made substitutions for previously unstudied amino acid residues and examined modulation by $CssIV^{E15A}$, a highly active toxin derivative. Of 11 positions studied in IIS1-S2, only one significantly altered the toxin effect from wild-type by reducing binding to the resting state and almost abolishing trapping activity. In IIS3-S4, five positions surrounding a previously identified key binding determinant, G845, define a hotspot of high impact residues. Three of these substitutions reduced toxin binding and voltage-sensor trapping. The other two, V843A and E844N, increased voltage-sensor trapping approximately 4-fold and decreased apparent $EC_{50}$. The rate of voltage sensor trapping upon depolarization was unchanged for V843A and increased approximately 2.5-fold for E844N. The rate at which the toxin releases the voltage sensor upon repolarization was increased 2.2-fold for the V843A but was unchanged for E844N. Thus $CssIV^{E15A}$ interacts with a short segment of IIS1-S2 and a broader region of DIIS3-S4. The bidirectional effects of mutations on toxin efficacy suggest that native residues make both positive and negative interactions with the toxin. Substitutions that increase toxin effects do so by increasing affinity of resting channels for the toxin and further increasing the relative affinity of the activated voltage-sensor for the toxin. These results provide further support for the voltage sensor-trapping model.

**570-Pos**

**A Cytotoxic Peptide from a Marine Sponge, Polytheonamide B; II. Properties for Ion Conduction and Voltage Dependent Gating**

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A 48-mer peptide from a marine sponge *Theonella swinhoei*, polytheonamide B (pTB), is highly cytotoxic against eukaryotic cells. Alternating D- and L-chirals throughout the molecule suggest formation of β-helix, which was supported by channel activity having high permeability to $Cs^+$. The 48-mer β-helix is long enough to span the membrane and was shown to form the channel by single peptide. In this study we evaluated the ion conduction and gating properties of pTB channel by use of planar lipid bilayer technique. Single-channel *I-V* curve in CsCl solution exhibited weak outward rectification. Concentration-dependency of single-channel conductance of pTB channel was examined. For $Cs^+$ permeation, clear saturation in unitary conductance was observed in the concentration range up to 3.0 M, which was contrast to gramicidin A (gA) channel. Gating manner of pTB channel was characteristic. Fast transition between open and closed state was observed, suggesting that the structural changes of single pTB molecule directly link to the gating. In addition to asymmetrical single-channel conductance, distinctive voltage-dependent gating was observed in single-channel and macroscopic current traces of pTB channels. On the basis of the structure of pTB channel, mechanism of ion conduction and voltage-dependent gating will be discussed.

**571-Pos**

**Physalia physalis Poison Depolarizes Beta Cell Membrane and Increases Insulin Secretion**

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Peptide toxins isolated from marine cnidarians are considered as useful tools for studying ionic channels and promising drugs for therapeutics. Hemolytic and cardiotoxic activities have been described in Physalia physalis venom, and two toxins with anticholinergic and antiglutamatergic effects have been isolated in its high molecular weight fraction.
In this work, we explored some crude extract of P. physalis and its low molecular weight fractions on ionic currents and insulin secretion of pancreatic rat beta-cells. P. physalis specimens were collected at the north littoral of La Habana, Cuba. Crude extract were purified by a gel filtration (Superdex 200). Mass spectrometry MALDI-TOF and RP-HPLC (C18 column) were performed on each fraction collected from gel filtration. The biological activity on insulin secretion was tested in a reverse hemolytic plaque assay on primary cultures of pancreatic beta cells from male Wistar rats.
In basal glucose (5.6 mM), the crude extract (25 protein ug/mL) increased by 77% the average immunoplaque area, which is directly proportional to insulin secreted by isolated cells, without disrupting of beta cells or erythrocytes integrity. Low molecular fractions from gel filtration did not exceed of 20 kDa, and most of them eluted before the 20 % of acetonitrile on RP-HPLC. These fractions increased both, the percentage of insulin-secreting cells and the average immunoplaque area at a basal glucose level, suggesting a direct effect on TRP-type channels that are responsible for beta cell depolarization.
Physalia physalis poison contains polar bioactive compounds capable of enhance the secretory behavior of pancreatic beta cells from male Wistar rats by depolarizing the membrane, in non stimulant glucose conditions.
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**572-Pos**

**Insights on Channel-Like Activity of Membrane Bound Alpha-Synuclein**

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Alpha-synuclein (syn) is a natively unfolded protein with the ability to acquire secondary structure upon interaction with membranes or with itself. It is linked to Parkinson's disease (PD) by two evidences: the accumulation of amyloid fibrils of the protein and the autosomal dominant forms of the disease (A53T, A30P and E46K mutants). Both the biological role of this protein as the mechanisms involved in the ethiopathogenesis of PD are still unknown. The protein is located at the presynaptic terminal of neurons in all the Central Nervous System, where it exists free in the citosol or bound to synaptic vesicles. The membrane binding causes the formation of an amphipatic alpha-helix in the first part of the protein, which lies at the membrane surface without crossing the bilayer. A recent paper by Zakharov et al. (Biochemistry, 2007) reports that upon the application of a potential a channel like activity of syn can be recorded. Authors suppose that the helices of the first hundred amino acid of syn can pass the membrane bilayer to compose a pore only upon the application of a potential. Both the mechanism and the biological implication of this behaviour are still unknown and potentially of interest for the role that syn channel may play. In this study we extended this approach to syn deletion mutants. Furthermore, the effect of monomer topology in the construction of the purported channels have been explored thought the design of several types of syn dimers. A comparative analysis of the electrophysiological properties of these constructs will be presented and discussed.

**573-Pos**

**Developing a Functional Screening Assay of Small Molecules That Can Reduce Leakage of Liposomes Induced by Amyloid-Beta Peptides**

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Alzheimer's disease (AD), an ultimately fatal neurodegenerative disorder, is characterized by the presence of plaques containing fibrillar aggregates of amyloid-beta (Aβ) peptides. These peptides, with 39-43 amino acids, especially Aβ(1-40) and Aβ(1-42), are the major components of plaques formed in the brain of patients with AD and are considered to be pathologically important. Both peptides aggregate rapidly in aqueous solution to form Aβ oligomers as well as Aβ fibrils over time. Increasing evidence indicates that Aβ peptides, especially in their oligomeric state, play an important role in pathogenesis of AD. One possible pathogenic mechanism of these Aβ oligomers is the formation of pores through neuronal membranes, resulting in cell death. This research examined the effect of Aβ on permeabilization of liposomes by monitoring the change of fluorescence intensity of pH-sensitive dye which was encapsulated in the liposomes due to the leakage of protons. These experiments showed that the effect of Aβ depended on the lipid composition and on the aggregation state of Aβ. The developed liposome leakage assay makes it possible to screen several potential drug candidates for their ability to inhibit or reduce the leakage of liposomes.

**574-Pos**

**Efficacious in vivo Electrophysiological Screening of Neuromodulatory Compounds: Using Drosophila to Evaluate the Activity of Conotoxins**

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Finding compounds that affect neuronal function is central for the development of probes or potential therapeutic agents. We have devised an efficacious

approach that allows the ''unbiased'' screening for biological activity of compounds *in vivo* against molecular targets on various types of neurons with cholinergic, glutamatergic or electrical synapses and muscles. For this, we use the Giant Fiber System, which is a simple neuronal circuit that mediates the escape response in the fly. The giant fiber cell bodies and dendrites are localized in the brain and each extends a single axon into the second thoracic neuromere, where it makes a mixed electrical (GAP junctions) and chemical (ACh neurotransmitter) synapse on the tergo trochanteral motorneuron, which further innervates the jump muscle. The GF also connects to a peripheral synapsing interneuron (PSI), which makes a cholinergic synapse onto the dorsal longitudinal motorneurons (DLM). Both the TTM and the DLM neuromuscular junctions are using glutamate as the neurotransmitter. Here, we show that we are able to routinely screen components of the venom of cone snails by injecting then into the fly while continuing the recordings from GF circuit allowing us to instantly determine whether a compound has an effect on neurons or muscles of this neuronal circuit. Components of the venom of cone snails have been shown to elicit a wide range of physiological effects and are well-established neuronal probes or drug-lead candidates. The use of the tiny drosophila (a model organism) to evaluate the activity of conotoxins represents an efficacious in vivo assay that can be expanded to evaluate other compounds.

**575-Pos**

**Probing Interactions Within Anthrax Toxin by Electron Paramagnetic Resonance**

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Anthrax toxin, besides its role in the pathogenesis of *Bacillus anthracis*, is also an important model system in understanding how proteins cross cellular membranes. Anthrax toxin consists of three proteins: two enzymes, edema factor (EF) and lethal factor (LF), and a pore-forming protein called protective antigen (PA) that acts as a delivery vehicle for the two enzymes. The toxin enters the cell through endocytosis and is trafficked to the endosome where, upon a decrease in pH, PA inserts into the membrane forming a pore through which LF and/or EF are subsequently translocated. Although the details of PA-assisted translocation are still unclear, biochemical studies indicate that LF binds to the surface of PA with its unstructured N-terminal region (residues 1-26) poised above the entryway of the pore, suggesting that these residues may extend into and bind within the lumen of PA, thus initiating translocation. To probe such putative interactions, we attached a nitroxide spin label to the N-terminal, PA-binding domain of LF (LFn) at a number of positions within the N-terminal region. We then used electron paramagnetic resonance to measure the mobility of these spin labels with LFn alone and in complex with PA. We found that for LFn spin labeled at position 2 or 5 the mobility of the label significantly decreases when LFn is in complex with the PA pore, indicating a binding interaction between these N-terminal residues and the pore. Additionally, translocation-compromising mutations within the PA phenylalanine clamp eliminate the observed interaction between the LFn N-terminus and PA. These results suggest that the LFn N-terminus binds within the lumen of the PA pore, likely at or near the phenylalanine clamp, initiating translocation.

**576-Pos**

**A Cytotoxic Peptide from a Marine Sponge, Polytheonamide B: I. Channel Activity and Vectorial-Insertion Into the Membrane**

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A peptide from marine sponge *Theonella swinhoei*, polytheonamide B (pTB), shows potent cytotoxic activity. The cytotoxic activity to various types of cells was examined and found that pTB was most effective to eukaryotic cells. We examined mechanisms underlying the cytotoxic activities of pTB. The amino acid sequence of pTB is unprecedented, having alternative D- and L-amino acid residues throughout the 48-mer peptide. The alternative chiral sequence suggests the formation of a β-helix similar to gramicidin channels, and planar bilayer experiments were performed. pTB forms monovalent cation-selective channels (the selectivity sequence: $Cs^+ > Rb^+ > K^+ > Na^+ > Li^+$), which is compatible with the inner pore diameter of ~4 Å for a β-helical structure. The single channel current-voltage curve showed slightly outward-rectifying. Single-channel conductance was 18 pS for symmetrical 1 M CsCl solution. Concentration-dependent macroscopic current amplitude exhibited the Hill coefficient of one, suggesting that the channel is formed by monomer. We found a periodic pattern of unusual amino acids which align on one side of the β-helix and may form a hydrogen-bonded chain through those side-chains. This novel motif may reinforce the long pore structure. pTB penetrated vectorially into the membrane, formed a channel by means of a single molecule and was retained in the membrane. A hydrophobic lead of the pTB molecule may drive a wedge into membrane. Retaining pTB in the first membrane prevents further access to the next membrane in cells with outer membranes, suggesting alleviated cytotoxic activity towards cells of this type.

**577-Pos**

**Real-Time Afm Imaging of Surface-Induced Oligomerization of the Non-Amyloidogenic P3 Peptide: Implications for Membrane Insertion and Ion Channel Formation**

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The non-amyloidogenic p3 ($A\beta_{17-42}$) peptide is produced by cleavage of amyloid precursor protein (APP) by α and β secretases. The p3 peptide is present in amyloid plaques and is a main component of cerebellar preamyloid lesions in Down's Syndrome (DS). Its pathogenic potential is just beginning to emerge. Like other amyloidogenic peptides, interaction of p3 with cell membrane surfaces will be a critical determinant in its pathogenicity. This study aims to examine biophysical properties and structures of p3 on different surfaces. Using atomic force microscopy (AFM) and molecular dynamics (MD) simulations, we have studied the adsorption properties of p3 peptides on surfaces with varying degree of hydrophobicity. On hydrophobic graphite surfaces, low peptide concentrations produce parallel fibrils of ~ 5 nm diameter and ~ 1 nm height oriented along graphite superstructures over extended periods of time (~ 5 hr). At higher concentrations, peptides reorient on the surface over time and form a more disordered pattern. The observed structures are modeled as hydrophobic C-terminal β-strands in contact with the graphite surface by MD simulations. Mature fibers were not observed in our study. Because of their hydrophobic nature, p3 peptides either did not adsorb on hydrophilic mica surfaces or adsorbed too weakly to be imaged. Preliminary AFM data suggest an adsorption stage where p3 peptides form small agglomerates on lipid bilayers. These results agree with MD simulations that predict peptide adsorption as a preliminary step to subsequent insertion into the lipid bilayer. The insertion of p3 into the lipidic bilayers is a prerequisite for p3 peptide to form toxic ion channels that we have described previously.

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**578-Pos**

**Designer Ligands Specific for Kv1.3 Channels from a Scorpion Neurotoxin-Based Library**

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Venomous animals immobilize prey using protein toxins that act on ion channels and other targets of biological importance. Broad use of toxins for biomedical research, diagnosis and therapy has been limited by inadequate target discrimination, for example, among ion channel subtypes. Here, a synthetic toxin is produced by a new strategy to be specific for human Kv1.3 channels, critical regulators of immune T-cells. A phage-display library of 11,200 novel proteins is designed using the α-KTx scaffold found in 31 scorpion toxins that bind to potassium channels and mokatoxin-1 (moka1) isolated by sorting on purified target. Moka1 blocks Kv1.3 at nanomolar levels that do not impact Kv1.1, Kv1.2 or KCa1.1. Thus, moka1 suppresses CD3/28-induced cytokine secretion by T-cells without cross-reactive gastrointestinal hyperactivity. The 3D structure of moka1 rationalizes its specificity and validates the engineering approach revealing a unique interaction surface supported on an α-KTx scaffold. This scaffold-based/target-biased strategy overcomes many obstacles to production of selective toxins. Success with other toxin scaffolds and sorting with cell-surface targets has extended utility of the approach.

**579-Pos**

**Gramicidin Pores Report the Activity of Membrane-Active Enzymes**

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Phospholipases constitute a ubiquitous class of membrane-active enzymes that play a key role in cellular signaling, proliferation, and membrane trafficking. Aberrant phospholipase activity is implicated in a range of diseases including cancer, inflammation, and myocardial disease. Characterization of these enzymes is therefore important, both for improving the understanding of phospholipase catalysis, and for accelerating pharmaceutical and biotechnological