derived from tarantula venom allosterically modulate voltage sensors of ion channels. Although tarantula peptides have varying affinities for different channels, all affect multiple subtypes. Theoretically, a peptide's selectivity between channel types could be amplified by summing binding energies, if a multimeric version of the peptide could bind multiple subunits simultaneously. We have amplified the inherent specificity of a voltage sensor peptide, guangxitoxin-1E (GxTX), by tethering two peptides together to form a dimer coupled by a flexible polyethylene glycol linker. We synthesized GxTX mutants functionalized with artificial amino acids for chemoselective coupling and tethered them with homobifunctional linkers using azide-alkyne cycloaddition chemistry. The GxTX dimers inhibited Kv2.1 with higher affinity than GxTX monomers. Similar to GxTX monomers, these tethered dimers shifted channel opening to positive voltages. Association rates were reduced for the GxTX dimers versus monomers, indicating that increased affinity for Kv2.1 channels was entirely due to the extremely slow dissociation of GxTX dimers. The strong voltage dependence of dimer dissociation was consistent with a two step binding model, where the kinetics of the second GxTX binding domain determines the amplification of affinity by the dimer. This mechanism predicts that dimerization will selectively amplify the affinity for channels with slow toxin dissociation rates. Experimentally, dimerization amplified GxTX specificity for Kv2.1 over other channel subtypes. We conclude that tethered multimers can increase the pharmacological selectivity of voltage sensor modulators.

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An Ion Channel Platform for Detection of Small Molecule Analytes

Young Hun Kim[1], Leibniz Hang[1], Michael Mayer[2], Jerry Yang[1].
[1]UC, San Diego, La Jolla, CA, USA, [2]University of Michigan, Ann Arbor, An Arbor, MI, USA.

The development of detection assays for small molecule analytes based on ion channel proteins makes it possible to exploit the amplification properties of membrane pores in response to specific external stimuli. Here, we used C-terminal derivatives of an ion channel-forming peptide, gramicidin A (gA), to explore an ion channel-based method for monitoring environmental contamination such as toxins or lethal microorganisms. Our initial results suggest that the modified ion channel-forming peptides can respond to a range of tailored, external, small molecule stimuli to produce detectable changes in electrophysiological properties.

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Conformational Analysis of the Frog Skin Peptide, Plasticin-L1 and its Effects on the Production of Proinflammatory Cytokines by Macrophages

Andrea C. Rinaldi[1], Giorgia Manzo[2], Roberta Sanna[2], Mariano Casu[2], Jelena M. Pantic[3], Miodrag L. Lukic[3], J. Michael Conlon[4], Mariano A. Scorciapino[2].
[1]Department of Biomedical Sciences, University of Cagliari, Monserrato, Italy, [2]Department of Chemical and Geological Sciences, University of Cagliari, Monserrato, Italy, [3]Center for Molecular Medicine, University of Kragujevac, Kragujevac, Serbia, [4]Department of Biochemistry, United Arab Emirates University, Al Ain, United Arab Emirates.

Plasticin-L1 (GLVNGLLSSVLGGGQGGGGLLGGIL) is a conformationally flexible glycine/leucine-rich peptide originally isolated from norepinephrine-stimulated skin secretions of the South-American Santa Fe frog Leptodactylus laticeps (Leptodactylidae). A nuclear magnetic resonance (NMR)/molecular dynamics (MD) characterization of plasticin-L1 in the presence of dodecylphosphocholine (DPC) and DPC/sodium dodecylsulphate (SDS) micelles as membrane-mimetic models showed that the peptide has affinity for both neutral and anionic membranes. The peptide adopts a stable helical conformation at the N-terminal region and a more disordered helix at the C-terminal region, separated by an unstructured loop wherein the highest number of glycines is localized. In both micelle environments, plasticin-L1 slowly inserts between the detergent head groups, but always remains localized at the micelle/water interface. Plasticin-L1 lacks direct antimicrobial activity but stimulates cytokine production by macrophages. Incubation with plasticin-L1 (20 μg/mL) significantly ($P < 0.05$) increased the production of the proinflammatory cytokines IL-1β, IL-12 IL-23 and TNF-α from unstimulated peritoneal macrophages from both C57BL/6 and BALB/C mice. The peptide also increased IL-6 production by unstimulated ($P < 0.01$) and lipopolysaccharide-stimulated ($P < 0.01$) macrophages, while the effects on production of the anti-inflammatory cytokine IL-10 were not significant. These findings suggest that plasticin-L1 may play an immunomodulatory role in vivo by stimulating cytokine production from frog skin macrophages in response to microbial pathogens. This peptide may represent a template for the design of peptides with therapeutic applications as immunostimulatory agents.

Membrane Structure I

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Detergent-Free Extraction of Membrane Proteins into Native Nanodiscs. Application to the Reaction center of *Rhodobacter Sphaeroides*

Stefan Scheidelaar[1], Martijn Koorengevel[1], David Swainsbury[2], Hans Meeldijk[1], Eefjan Breukink[1], Michael Jones[2], Rienk van Grondelle[3], Antoinette Killian[1].
[1]Utrecht University, Utrecht, Netherlands, [2]Bristol University, Bristol, United Kingdom, [3]VU University, Amsterdam, Netherlands.

Recently, it has been discovered that styrene-maleic acid (SMA) copolymers are able to solubilize membrane proteins in the form of nanodiscs (SMALPs), also called 'native nanodiscs', directly from their native membrane [1,2]. To understand what physical properties of membranes and SMA modulate this unique property of SMA, we first studied the solubilization of vesicles of synthetic phospholipids by SMA using transmission experiments. We found that SMA is an excellent membrane solubilizer, able to solubilize different types of membranes below, at and above the gel to crystalline-liquid phase temperature. Based on the kinetics of solubilization under different experimental conditions we developed a model for the mode of action of SMA that will also explain why SMA is an efficient solubilizer, whereas membrane scaffold proteins (MSPs) and amphipols are not.

Next we used the SMA technology to purify and characterize reaction centers (RCs) from the purple bacterium *Rhodobacter sphaeroides* in the form of native nanodiscs. Monitoring of heat stability and recombination kinetics of $P^+Q_B^-$ in photo-excited RCs in native nanodiscs showed that (1) RCs are much more stable in native nanodiscs than in detergent, and (2) the charge recombination kinetics display native-membrane like behavior.

Our study contributes fundamental knowledge about the mode of action of SMA that is essential for optimizing methods to extract membrane proteins from different organisms and it promotes the general applicability of SMALPs as host for membrane proteins in studies on interactions of proteins with native lipids and in protein structure determination studies.

1. Knowles et al., 2009, JACS, 131, 7484-7485.
2. Long et al., 2013, BMC Biotechnology, 13:41.

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Pyridinium Salts Influence on Lipid Bilayers

Sergio S. Funari[1], Claudio Di Vitta[2], Liliana Marzorati[3].
[1]HASYLAB, Hamburg, Germany, [2]Inst. Chemistry, University of Sao Paulo, Brazil, [3]Inst Chemistry, University of Sao Paulo, Brazil.

In this study, we continue the investigation of different functional groups of molecules having a side long alkyl chain (C14 or C16) [Langmuir; 2011, 27(13), 8257 and J. Lipids, 2013, Article ID 592318]. Thioquinones and similar naphtothioquinones showed strong influence on the structural behavior of POPC and POPE model membranes bilayers. Such effects were evidenced further upon thermal evolution. We identified the structures formed by Small-Angle X-rays scattering, and more recently also by 2H NMR from D2O, with measurements at different temperatures, either fixed or with continuous heating/cooling of the samples.

In all systems, one sees strong effects. The temperature of the phase transitions lower considerable and the sequence of phase are strongly affected. The formation of two phase systems over unusually large temperature spans or the coexistence of one structure, e.g. lamellar, showing different lattice parameters are strong evidences of inhomogeneous systems. Cubic phases, Im3m, Pn3m, Pm3m and P4332, have also been identified at high temperatures. The cubic structures have been seen either as a single phase or in many cases in coexistence with others. Their unique identification is hindered by the limited resolution of the X-rays scattering patterns obtained. Interestingly, this seems to be a feature of the system rather than a dynamic effect that would vanish with time. Moreover, it seems that these systems are able to form both micellar and bicontinuous cubic phases and even convert one into the other. Their unique assignment has not been possible with die to the limited resolution obtained. A simplified view of the results obtained indicates that the additives have a strong influence on the curvature of the bilayer formed by the lipid matrix. Data from different mixtures between these pyridinium salts and POPC, or POPE, at different temperatures will be shown.