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Snake Venom-Derived Peptides as Tools for Intracellular Delivery
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Nucleolar-targeting peptides (NrTPs) are a new familiy of cell penentrating peptides (CPP). These peptides are derived from crotamine, a toxin from the venom of a South-American rattlesnake. The aim of this work was to study NrTPs' molecular mechanism for translocation, as well as to determine their ability to deliver large molecules into cells. The biophysical characterization of these peptides revealed high partition coefficients for lipid vesicles of different compositions. Fluorescence quenching studies highlighted the role of anionic lipids (POPG) on the degree of peptide penetration into the membranes. Rhodamine B-labeled NrTPs were used to test translocation into giant multilamellar vesicles. Confocal microscopy results show that there is an efficient translocation across model membranes. NrTPs are able to penetrate different cell types, such as PBMC, HeLa, Bv173, MOLT4 and BHK21 cell lines. Furthermore, a conjugate of NrTP (NrTP6C) bound to β-galactosidase was prepared by chemical synthesis and tested in HeLa cells. Successfully cellular delivery of β-galactosidase was observed and quantified. In order to expand the range of application of these peptides, we tested the delivery of nucleic acids mediated by NrTPs. Positive results suggest that they can be used as transfection reagents. The work done so far, with this new family of CPP, revealed strong interaction and translocation with lipid model systems, as well as efficient cellular delivery of biomolecules. Together, these results point towards a potential application of NrTPs in therapeutics.

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Bacterial Uptake of the Cell-Penetrating Peptide pVEC
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The discovery of cell-penetrating peptides (CPPs), which can translocate through the cell membrane without the need for a receptor, has led to an increased interest in the design and development of peptide based drug delivery methods. CPPs are peptides of fewer than 30 residues derived from natural or unnatural proteins or chimeric sequences. The 18 amino acid long pVEC peptide is derived from murine vascular endothelial cadherin (VE-cadherin) protein, which mediates physical contact between adjacent cells by homophilic dimerization. The pVEC peptide has been shown to cross the cell membrane. The pVEC sequence, LLIILRRRIRKQAHAHSK, has a hydrophobic N-terminus and a positively charged midsection. The residues contributing to the uptake of pVEC across the membrane were previously examined by alanine mutagenesis studies (Elmquist, A., M. Hansen, et al., 2006, Biochimica et biophysica acta, 1758(6): 721-729) and the N-terminal residues were shown to be crucial in cellular uptake. Here, we use simulations to study the bacterial uptake of pVEC at atomic detail. Steered molecular dynamics simulations on the original pVEC and its various mutants are performed in an effort to determine the important peptide - membrane interactions on a residue basis and to elucidate the mechanism whereby pVEC passes through the membrane.

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Investigation of the Self-Association of δ-Lysin Variants via Fluorescence Anisotropy
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The self-association processes of three synthetic variants of the S. aureus peptide delta-lysin were studied in aqueous solution using fluorescence anisotropy. The goal was to determine if any of these variants were capable of oligomerization, as delta-lysin is known to form tetramers in aqueous solutions at concentrations higher than 1 microM. The three analogues studied were delta-lysette, delta-lysette 26 and DL-2A. delta-Lysette and delta-lysette 26 both have a net charge of +2 at pH 7.5, whereas DL-2A and delta-lysin have a net charge of zero. It was expected that the two charged variants would each begin aggregating at higher concentrations in aqueous solution, while DL-2A would aggregate in a manner similar to delta-lysin. The analysis of the self-association of each peptide was conducted in two stages. First, the Trp residues were excited and the anisotropy of the resulting emission was measured as a function of peptide concentration. Second, to determine the degree of oligomerization at given peptide concentrations, Perrin plots were generated from data obtained at peptide concentrations that showed either low or high anisotropy measurements. Initial results indicated that delta-lysette 26 oligomerizes at concentrations near 20 microM, whereas DL-2A already oligomerizes starting at 0.05 microM. This research was supported by NIH Grant GM072507.

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Self-Assembly of the Membrane-Bound β-Stranded Peptide $(KIGAKI)_3$ into Immobilized Amyloid Fibrils Observed by Solid-State ^{19}F-NMR
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The structure and membrane alignmentof β-stranded antimicrobial peptide KIGAKI [$(KIGAKI)_3$-NH_2] has been determined in oriented DMPC bilayers using circular dichroism and solid state ^{19}F-NMR spectroscopy. CF_3-Bpg was used as a reporter group to label the peptide, where several IIe or Ala residues were individually replaced one by one. At high peptide-to-lipid molar ratios (P/L) of 1:200 or above, the ^{19}F dipolar coupling of all five labels exhibits the maximum possible value, which is indicative of a parallel alignment of the Cα-CF_3 bond vector with respect to the bilayer normal. This finding suggests that at high concentration KIGAKI self-assembles into immobilized -sheets, which lie flat on the membrane surface. Transmission electron microscopy images reveal that the aggregated KIGAKI forms amyloid like fibrils. At low peptide concentrations, on the other hand, the dipolar couplings of the CF_3-groups indicate that their time-averaged alignment is still parallel to the bilayer normal, but the mobility of the peptides increases drastically and reflects a monomeric state. Correspondingly, the flexible β-strands float on the membrane surface and undergo motional averaging in the 2-dimensional membrane plane, similar to intrinsically unstructured proteins in solution. This is the first example of concentration dependent transition of a flexible β-strand to an amyloid like fibril in membranes that is directly observed by solid state NMR.

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Phosphatidylethanolamine Enhances Amyloid Fiber Dependent Membrane Fragmentation
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The toxicity of amyloid-forming peptides has been hypothesized to reside in the ability of protein oligomers to interact with and disrupt the cell membrane. Much of the evidence for this hypothesis comes from in vitro experiments using model membranes. However, the accuracy of this approach depends on the ability of the model membrane to accurately mimic the cell membrane. The effect of membrane composition has been overlooked in many studies of amyloid toxicity in model systems. We show here that PE (phosphatidylethanolamine), an abundant lipid in mitochondrial and plasma membranes, strongly modulates the membrane disruption by IAPP, an amyloidogenic protein involved in type II diabetes.
Using insulin as an inhibitor to block fiber formation, we have previously shown that membrane disruption by IAPP is a two-stage process with a different mechanism occurring in each phase The first phase of membrane disruption occurs within the lag-time of fiber formation, while the second phase closely follows fiber formation and results in total disruption of the membrane by fragmentation into small micelle like structures. While PE initally surpresses the membrane disruption in the first phase, it results in greater membrane fragmentation during fiber formation. The origins of this behavior result from the relative affinities of different oligomeric species of IAPP. While monomeric IAPP has weaker affinity for membranes containing PE, solid-state NMR shows that amyloid fibers of IAPP interact strongly and specifically with PE in mixed bilayers. Since membrane disruption through fiber growth has been detected for other proteins and reduction of PE levels is correlated with a decrease in cell toxicity, it is likely that a similar mechanism is operative for other toxic amyloidogenic proteins.

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Copper Modulates Aβ42 Aggregation in Model Membranes
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The amyloid-beta (Aβ) peptide is associated with Alzheimer's disease (AD). Evidence suggests that Aβ interaction with neuronal cell membranes correlates strongly with neurodegeneration but understanding the molecular mechanism remains a challenge. The role of heavy metals often found in amyloid plaques from AD brain patients is another path for investigation. Our recent findings support the role of lipid membrane composition as crucial in many biological mechanisms. We observed that different lipids promote different fibril