

Division of Biochemistry – Biofunctional Design-Chemistry –

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Dr. MADANI, Fatemeh Stockholm University, Sweden, 1 December 2012–31 March 2013

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Scope of Research

The ultimate goal of our research is the regulation of cellular functions by designed peptides and proteins. Current research subjects include (1) development of novel intracellular delivery systems aiming at elucidation and control of cellular functions using designed membrane permeable peptide vectors, (2) elucidation of the DNA binding modes of zinc finger proteins and TALEs, and design of artificial transcription factors with various DNA binding specificities, (3) elucidation and control of membrane curvature, and (4) design of stimulation-responsive artificial peptides and proteins.

KEYWORDS

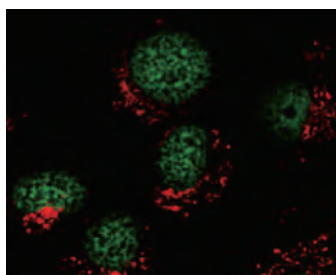
Membrane-Permeable Peptides

Intracellular Delivery

Peptide Design

DNA Binding Protein

Membrane Curvature



Selected Publications

Pujals, S.; Miyamae, H.; Afonin, S.; Murayama, T.; Hirose, H.; Nakase, I.; Taniuchi, K.; Umeda, M.; Sakamoto, K.; Ulrich, A. S.; Futaki, S., Curvature Engineering: Positive Membrane Curvature Induced by Epsin N-Terminal Peptide Boosts Internalization of Octaarginine, *ACS Chem. Biol.*, **8**, 1894-1899 (2013).

Kawaguchi, Y.; Tanaka, G.; Nakase, I.; Imanishi, M.; Chiba, J.; Hatanaka, Y.; Futaki, S., Identification of Cellular Proteins Interacting with Octaarginine (R8) Cell-Penetrating Peptide by Photo-Crosslinking, *Bioorg. Med. Chem. Lett.*, **23**, 3738-3740 (2013).

Tsuji, S.; Futaki, S.; Imanishi, M., Creating a TALE Protein with Unbiased 5'-T Binding, *Biochem. Biophys. Res. Commun.*, **441**, 262-265 (2013).

Katayama, S.; Nakase, I.; Yano, Y.; Murayama, T.; Nakata, Y.; Matsuzaki, K.; Futaki, S., Effects of Pyrenebutyrate on the Translocation of Arginine-Rich Cell-Penetrating Peptides through Artificial Membranes: Recruiting Peptides to the Membranes, Dissipating Liquid-Ordered Phases, and Inducing Curvature, *Biochim. Biophys. Acta*, **1828**, 2134-2142 (2013).

Noshiro, D.; Sonomura, K.; Yu, H. H.; Imanishi, M.; Asami, K.; Futaki, S., Construction of a Ca²⁺-Gated Artificial Channel by Fusing Alamethicin with a Calmodulin-Derived Extramembrane Segment, *Bioconjug. Chem.*, **24**, 188-195 (2013).

Curvature Engineering: Positive Membrane Curvature Induced by Epsin N-Terminal Peptide Boosts Internalization of Octaarginine

Epsin-1 is a representative protein for inducing the positive curvature necessary for the formation of clathrin-coated pits. We demonstrated that the N-terminus 18-residue peptide of epsin-1 (EpN18) has this ability *per se*, as proved by differential scanning calorimetry and solid-state NMR. Moreover, it was shown how this positive curvature promotion can be exploited for promoting the direct penetration of a representative cell-penetrating peptide (CPP), octaarginine (R_8), through artificial and plasma membranes (Figure 1). This synergistic effect has been used for the efficient delivery of a proapoptotic domain peptide (PAD), which induced high level of apoptosis only when coadministered with R_8 and EpN18, thus emphasizing the importance of positive curvature induction for achieving the desired ultimate cargo bioavailability.

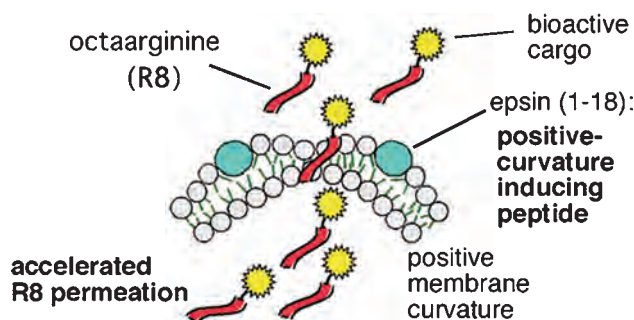


Figure 1. The synergistic effect of positive curvature induction by the epsin-1 N terminal peptide and permeation of R_8 peptides.

Effects of Pyrenebutyrate on the Translocation of Arginine-Rich Cell-Penetrating Peptides (CPPs)

Arginine-rich CPPs have the ability to translocate through cell membranes. Hydrophobic counteranions such as pyrenebutyrate (PyB) have been reported to markedly promote the membrane translocation of these peptides. Using model membranes having liquid-ordered (Lo) and liquid-disordered (Ld) phases, we explored the effects of PyB on the promotion of R_8 translocation. Confocal microscopic observations of giant unilamellar vesicles (GUVs)

(Figure 2) showed that PyB significantly accelerated the accumulation of R_8 on membranes containing negatively charged lipids, leading to the internalization of R_8 without collapse of the GUV structures. PyB displayed an alternative activity, increasing the fluidity of the negatively charged membranes, which diminished the distinct Lo/Ld phase separation on GUVs. Additionally, PyB induced membrane curvature, which has been suggested as a possible mechanism of membrane translocation for R_8 . Our results indicate that PyB may have multiple effects that promote R_8 translocation through cell membranes.

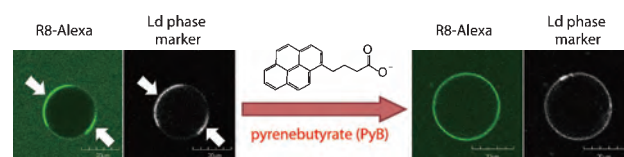


Figure 2. Confocal microscopic observations of giant unilamellar vesicles.

Construction of a Ca^{2+} -Gated Artificial Channel by Fusing Alamethicin with a Calmodulin-Derived Extramembrane Segment

Using native chemical ligation, we constructed a Ca^{2+} -gated fusion channel protein consisting of alamethicin and the C-terminal domain of calmodulin (Figure 3). At pH 5.4 and in the absence of Ca^{2+} , this fusion protein yielded a burst-like channel current with no discrete channel conductance levels. However, Ca^{2+} significantly lengthened the specific channel open state and increased the mean channel current, while Mg^{2+} produced no significant changes in the channel current. On the basis of 8-anilino-1-naphthalene-1-sulfonic acid (ANS) fluorescent measurement, Ca^{2+} -stimulated gating may be related to an increased surface hydrophobicity of the extramembrane segment of the fusion protein.

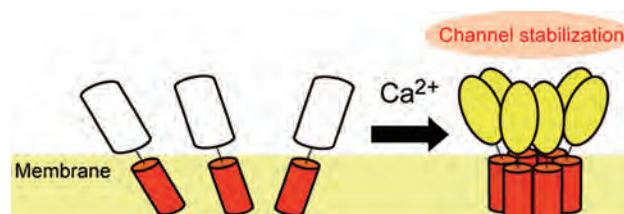


Figure 3. Construction of a Ca^{2+} -gated artificial channel protein.