Division of Biochemistry
– Biofunctional Design-Chemistry –

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Scope of Research

The ultimate goal of our research is the regulation of cellular functions using 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-responsible artificial peptides and proteins.

KEYWORDS

Membrane-Permeable Peptides
Peptide Design
Membrane Curvature
Intracellular Delivery
DNA/RNA Binding Protein

Selected Publications

Takeuchi, T.; Suzuki, M.; Fujikake, N.; Popiel, H. A.; Kikuchi, H.; Futaki, S.; Wada, K.; Nagai, Y., Intercellular Chaperone Transmission via Exosomes Contributes to Maintenance of Protein Homeostasis at the Organismal Level. *Proc. Natl. Acad. Sci. U.S.A.*, **112,** E2497-E2509 (2015).

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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).

Futaki, S.; Noshiro, D.; Kiwada, T.; Asami, K., Extramembrane Control of Ion Channel Peptide Assemblies, Using Alamethicin as an Example, *Acc. Chem. Res.,* **46,** 2924-2933 (2013).

Intercellular Chaperone Transmission via Exosomes Contributes to Maintenance of Organismal Protein Homeostasis

Heat shock response (HSR) is a protective system necessary for cell survival in a stressful environment, to maintain protein homeostasis (proteostasis). Recent studies, however, have indicated that HSR is not ubiquitous at the organismal level, but depends highly on the cell types. Despite such imbalanced HSR upon stress, it is unclear as to how organismal proteostasis is maintained. We addressed this issue by analyzing cell culture and *Drosophila* models that mimic the imbalanced state of HSR by expressing molecular chaperones in limited groups of cells. We found that increased expression of molecular chaperones such as Hsp70 and Hsp40 in a group of cells improves proteostasis in other groups of cells in a cell-nonautonomous manner. We also found that exosome-mediated secretion and intercellular transmission of molecular chaperones are crucial for this cell-nonautonomous improvement of proteostasis. Our study reveals a novel cell-nonautonomous mechanism for organismal proteostasis that relies on cell-to-cell communication of molecular chaperones via exosomes, which could functionally compensate the imbalanced HSR between cells and tissues.

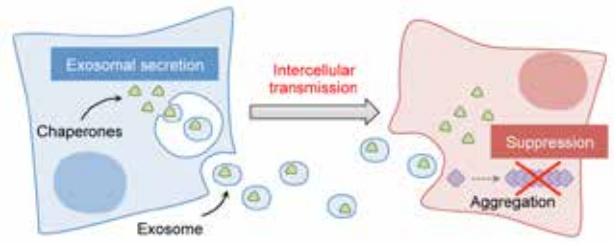

Figure 1. Molecular chaperones are secreted via exosomes and transmitted to the other cells, where they show chaperoning activity.

Effects of Amino Acid Substitution in the Hydrophobic Face of N-terminal Helix of Adenovirus Internal Protein VI on the Membrane Perturbation

Amphiphilic helices in membrane-interacting peptides and proteins often play crucial roles in their bioactivities. Understanding the roles of each amino acid in helical structural formation and membrane interaction is important since the functions of these peptides can be decided by the amino acid composition. We used the N-terminal helical segment of adenovirus internal protein VI positions 33–55 (WT) as a model amphipathic peptide to study the roles of hydrophobic amino acids. The leucine residue at position 40 has been suggested to be critical for viral infectious activity. The phenylalanine-substituted peptide (L40F) yielded less helicity on membrane binding and showed a shallower membrane-bound structure than WT. However, the liposomal leakage assay indicated that WT and L40F had similar degrees of membrane perturbation activity. Both peptides could induce positive membrane curvature and possessed higher affinities to membranes with high curvature, but no apparent curvature-sensitive membrane perturbation was observed.

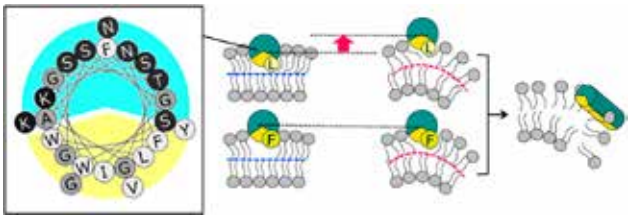

Figure 2. Helical wheel projection of N-terminal segment of adenovirus internal protein VI (left). WT and L40F peptide may have different membrane-bound states but the same extent of membrane perturbation activity (right).