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# 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

Kawaguchi, Y.; Takeuchi, T.; Kuwata, K.; Chiba, J.; Hatanaka, Y.; Nakase, I.; Futaki, S., Syndecan-4 Is a Receptor for Clathrin-Mediated Endocytosis of Arginine-Rich Cell-Penetrating Peptides, *Bioconjug. Chem.*, **27**, 1119-1130 (2016).

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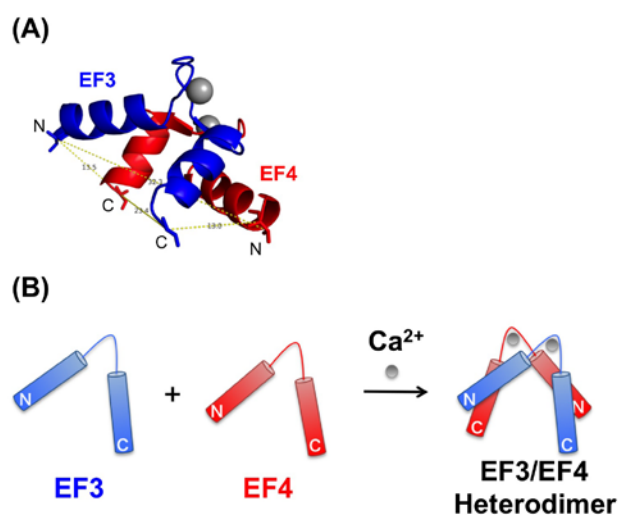
Backlund, C. M.; Takeuchi, T.; Futaki, S.; Tew, G. N., Relating Structure and Internalization for ROMP-Based Protein Mimics, *Biochim. Biophys. Acta*, **1558**, 1443-1450 (2016).

Nakase, I.; Noguchi, K.; Fujii, I.; Futaki, S., Vectorization of Biomacromolecules into Cells Using Extracellular Vesicles with Enhanced Internalization Induced by Macropinocytosis, *Sci. Rep.*, **6**, 34397 (2016).

Azuma, Y.; Kükenshöner, T.; Ma, G.; Yasunaga, J.; Imanishi, M.; Tanaka, G.; Nakase, I.; Maruno, T.; Kobayashi, Y.; Arndt, K. M.; Matsuoka, M.; Futaki, S., Controlling Leucine-Zipper Partner Recognition in Cells through Modification of  $\alpha$ -g Interactions, *Chem. Commun.*, **50**, 6364-6367 (2014).

## Use of Calmodulin EF-Hand Peptides as Ca<sup>2+</sup>-Switchable Recognition Tags

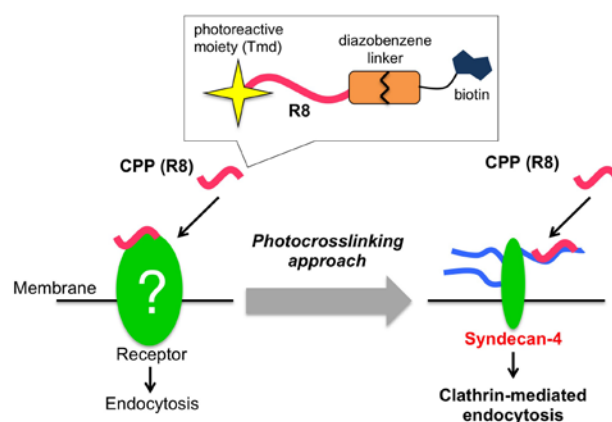
Peptide motifs specifically recognizing each other are useful molecular tags to detect specific proteins and to control protein interactions. Calmodulin is a representative calcium-binding protein composed of four Ca<sup>2+</sup>-binding motifs with a helix-loop-helix structure called EF-hands, in which two helices pack together at an angle of approximately 90 degrees. The two helices are separated by a calcium-binding loop region. Here, we revealed the potential of peptide segments derived from the third and fourth EF-hands (EF3 and EF4) to act as recognition tags. An analysis of the disulfide formation mode among cysteines inserted at the N- or C-terminus of these peptide segments suggested that EF3 and EF4 peptides form a heterodimer with a topology similar to that in the wild-type protein. Heterodimer formation was shown to be a function of the Ca<sup>2+</sup> concentration, indicating that these structures can be used as Ca<sup>2+</sup>-switchable recognition tags. Using this EF-tag system, membrane fusion of liposomes decorated with EF3 and EF4 peptides was successfully induced. Because the EF-tags have different modes of mutual recognition and dimer formation from conventional peptide tags, this system would be used as an orthogonal recognition system to conventional ones.



**Figure 1.** (A) A possible structure of EF3 and EF4 dimers in the presence of Ca<sup>2+</sup>. (B) Schematic representation of complex formation by EF3 with EF4 dependent on Ca<sup>2+</sup>.

## Identifying Membrane Proteins Responsible for Cellular Uptake of Octaarginine by Photocrosslinking

Arginine-rich cell-penetrating peptides (CPPs) have been widely used as carriers for intracellular delivery of bioactive molecules. In spite of increasing evidence for involvement of endocytosis in the cellular uptake of arginine-rich CPPs, the primary cell-surface receptors for these peptide carriers that would initiate endocytic processes have remained unknown. Our previous effort to identify membrane receptors for octa-arginine (R8) peptide using the photo-cross-linking probe bearing a photoreactive diazirine was unsuccessful due to substantial amounts of cellular proteins nonspecifically bound to the affinity beads. To address this issue, here a photo-cross-linking probe was developed, in which a cleavable linker of a diazobenzene moiety was employed to selectively elute cross-linked proteins by reducing agent-mediated cleavage. It was demonstrated that introduction of the diazobenzene moiety into the photoaffinity probe results in efficient purification of cross-linked proteins with remarkable reduction of nonspecific binding proteins, leading to successful identification of 17 membrane-associated proteins that would interact with R8 peptide. Among the proteins identified, syndecan-4, one of the heparan sulfate proteoglycans, was revealed to be an endogenous membrane-associated receptor for the cellular uptake of R8 peptide via clathrin-mediated endocytosis. It was also clarified that the intracellular delivery of bioactive proteins mediated by R8 peptide this syndecan-4-dependent pathway was also involved in. These results reveal that syndecan-4 is a primary cell-surface target for R8 peptide that allows intracellular delivery of bioactive cargo molecules via clathrin-mediated endocytosis.



**Figure 2.** Identification of cell-surface receptors bound to R8 using photo-cross-linking reaction.