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– Biofunctional Design-Chemistry –

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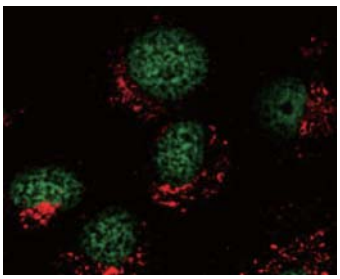
Scope of Research

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-responsible artificial peptides and proteins.

KEYWORDS

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



Selected Publications

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 *a-g* Interactions, *Chem. Commun*., **50,** 6364-6367 (2014).

Nakase, I.; Osaki, K.; Tanaka, G.; Utani, A.; Futaki, S., Molecular Interplays Involved in the Cellular Uptake of Octaarginine on Cell Surfaces and the Importance of Syndecan-4 Cytoplasmic V Domain for the Activation of Protein Kinase Cα, *Biochem. Biophys. Res. Commun*., **446,** 857-862 (2014).

Takeuchi, T.; Popiel, H. A.; Futaki, S.; Wada, K.; Nagai, Y., Peptide-Based Therapeutic Approaches for Treatment of the Polyglutamine Diseases, *Curr. Med. Chem*., **21,** 2575-2582 (2014).

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).

Nakase, I.; Tanaka, G.; Futaki, S., Cell-Penetrating Peptides (CPPs) as a Vector for the Delivery of siRNAs into Cells, *Mol Biosyst*., **9,** 855-861 (2013).

Controlling Leucine-Zipper Partner Recognition in Cells through Modification of *a–g* Interactions

The establishment of peptides that specifically inhibit protein interactions has been one of the major challenges in chemical biology. Selection of peptides from sophisticatedly designed libraries is one of the promising approaches in order to obtain peptides that strongly bind the target proteins. We have focused on the inhibition of a protein from the human T-lymphotropic virus type I (HTLV-1) known to cause adult T-cell leukemia (ATL). ATL is a peripheral T-cell neoplasm associated with infection by HTLV-1. The viral genome encodes the HTLV-1 bZIP factor (HBZ) known to promote T-cell proliferation and systemic inflammation. HBZ has a cFos-like leucine-zipper segment, which confers interaction of HBZ with cJun and related leucine-zipper proteins. Peptides that specifically block the interaction of HBZ with Jun related proteins could be powerful tools to elucidate the role of the interaction in the onset of ATL. By focusing on the *a–g* interactions, we successfully obtained a leucine-zipper segment that discriminates the appropriate partner over another that provides very similar patterns of electrostatic interactions (Figure 1).

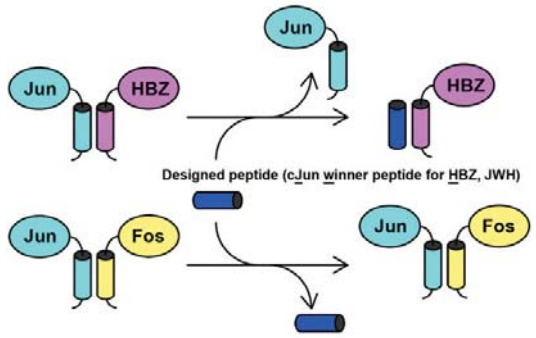


Figure 1. Designing an HBZ specific peptide without interfering with Jun–Fos recognition in cell.

Creating a TALE Protein with Unbiased 5'-T Binding

Artificial DNA binding proteins binding to desired DNA sequences are useful to control transcription of various genes and/or gene editing. So, they can be powerful tools for synthetic biology. Transcription activator-like effectors (TALEs) are sequence-specific DNA binding proteins secreted by the bacterial pathogen *Xanthomonas*. Though TALEs have the target sequence versatility determined by tandem repeats regions, almost all TALE binding sites are preceded by a highly conserved 5' terminal T nucleotide. An N-terminal noncanonical repeat (Repeat -1) has been thought to interact specifically with the 5'-T. We performed directed evolution of the repeat to bind to non 5'-T sequences using a bacterial 1-hybrid assay (Figure 2). The selected mutants showed significantly strong DNA binding activity to the binding sites starting from non 5'-T.

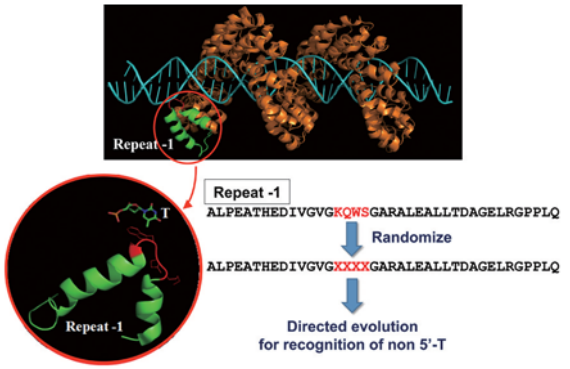


Figure2. Directed evolution of TALE repeat-1 for recognition of non 5'-T.

Molecular Interplays Involved in the Cellular Uptake of Octaarginine on Cell Surfaces and the Importance of Syndecan-4 Cytoplasmic V Domain for the Activation of Protein Kinase Cα

Arginine-rich cell-penetrating peptides (CPPs) are promising carriers for the intracellular delivery of various bioactive molecules. However, many ambiguities remain about the molecular interplays on cell surfaces that ultimately lead to endocytic uptake of CPPs. By treatment of cells with octaarginine (R8), enhanced clustering of syndecan-4 on plasma membranes and binding of protein kinase Cα (PKCα) to the cytoplasmic domain of syndecan-4 were observed; these events potentially lead to the macropinocytic uptake of R8. The cytoplasmic V domain of syndecan-4 made a significant contribution to the cellular uptake of R8, whereas the cytoplasmic C1 and C2 domains were not involved in the process.

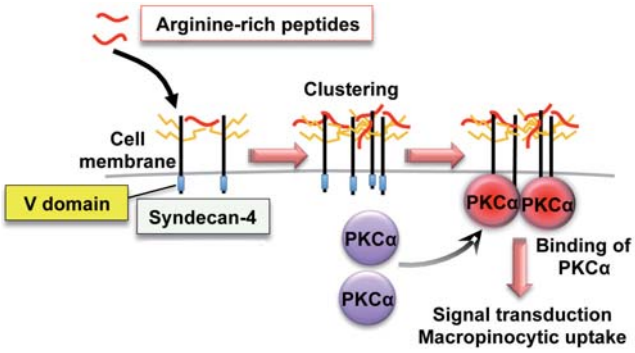


Figure 3. Schematic diagram of Syn-4 clustering and binding to PKCα inside cells induced by arginine-rich peptides.