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
Intracellular Delivery
Peptide Design
DNA/RNA Binding Protein
Membrane Curvature

Selected Publications

Akishiba, M.; Takeuchi, T.; Kawaguchi, Y.; Sakamoto, K.; Yu, H. H.; Nakase, I.; Takatani-Nakase, T.; Madani, F.; Graslund, A.; Futaki, S., Cytosolic Antibody Delivery by Lipid-Sensitive Endosomolytic Peptide, *Nat. Chem.*, **9,** 751-761 (2017).

Murayama, T.; Masuda, T.; Afonin, S.; Kawano, K.; Takatani-Nakase, T.; Ida, H.; Takahashi, Y.; Fukuma, T.; Ulrich, A. S.; Futaki, S., Loosening of Lipid Packing Promotes Oligoarginine Entry into Cells, *Angew. Chem. Int. Ed. Engl.*, **56,** 7644-7647 (2017).

Azuma, Y.; Imai, H.; Kawaguchi, Y.; Nakase, I.; Kimura, H.; Futaki, S., Modular Redesign of a Cationic Lytic Peptide to Promote the Endosomal Escape of Biomacromolecules, *Angew. Chem. Int. Ed. Engl.*, **57,** 12771-12774 (2018).

Shinoda, K.; Tsuji, S.; Futaki, S.; Imanishi, M., Nested PUF Proteins: Extending Target RNA Elements for Gene Regulation, *Chembiochem*, **19,** 171-176 (2018).

Imanishi, M.; Tsuji, S.; Suda, A.; Futaki, S., Detection of N6-methyladenosine Based on the Methyl-sensitivity of MazF RNA Endonuclease, *Chem. Commun.*, **53,** 12930-12933 (2017).

Importance of Net Hydrophobicity in Cellular Uptake of All-hydrocarbon Stapled Peptides

All-hydrocarbon stapled peptides are a promising class of protein-protein interaction regulators, targetable to intracellular molecules of therapeutic potentials with high binding affinity and specificity. Cell permeation efficacy of these peptides is a critical determinant to obtain their bioactivity. However, the factors stimulating their cellular uptake remain open to dispute. Using six types of known all-hydrocarbon stapled peptides, we evaluated the effect of staple (or cross-link) formation on their cellular uptake. We found that cellular uptake of unstapled peptides (i.e., bearing olefinic non-natural amino acids for staple formation by olefin metathesis (Figure 1)) was higher than those of the corresponding stapled peptides. Additionally, insertion of these olefinic non-natural amino acids into peptide sequences *per se* was suggested to significantly increase the cellular uptake of peptides. Judged from retention times in high performance liquid chromatography, overall hydrophobicity of all the unstapled peptides was higher than stapled peptides, followed by the original peptides. There was no tight correlation between the helical content and cellular uptake of these peptides. Therefore, as long as cell permeation abilities of the peptides concern, increase in overall hydrophobicity by the introduction of non-natural amino acids should be the key driver to promote cellular uptake, rather than structure stabilization by staple formation. Involvement of micropinocytosis, a form of fluid-phase endocytosis, was confirmed for the cellular uptake of all the six peptides.

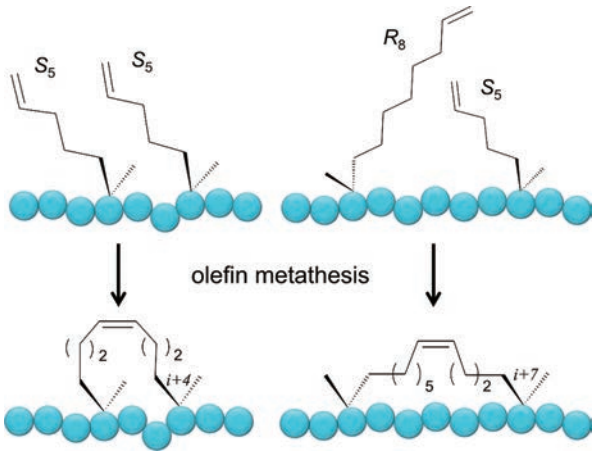

Figure 1. Strategy for stabilizing helical structure of peptides through ring-closing using olefin metathesis.

Development of a Simple Strategy to Detect Activities of N^6-methyladenosine Regulatory Enzymes

RNA methylation at the N6 position on adenine (m6A) is most prevalent internal RNA modification, which is mainly found at the RRACH sequence (R: G or A, H: U, A or C). The modification, which has been reported to regulate various physiological processes, is dynamic and reversible. It has been shown that FTO and ALKBH5 demethylate m6A and that METTL3 and METTL14 catalyze the methylation. To characterize these demethylases or methyltransferases and to find their inhibitors, it is required to develop new methods to easily detect their enzymatic activities. Here, we propose a convenient method to detect enzymatic activities of these demethylases and methyltransferases without using any specific apparatus or radioisotopes.

MazF is a bacterial toxin that plays an important role in growth regulation. MazF works as an endoribonuclease that specifically cleaves RNA at the 5'-end of ACA sequence. We found that MazF cleaved a single-stranded RNA fragment containing GGACA but not GG(m6A)CA by a conventional gel electrophoresis and a high-throughput FRET analysis (Figure 2). After being reacted with FTO or ALKBH5, the oligo RNA including an m6A was cleaved by MazF. The cleavage was inhibited by addition of known inhibitors of FTO or ALKBH5. In addition, the oligo RNA including a GGACA sequence was not cleaved by MazF after being reacted with the METTL3/METTL4 complex. These results indicate that the activities of both RNA demethylases and methylatransferases can be detected by this new method. Though the sequence is limited to "ACA", this method is easy to assay the enzymatic activities of RNA methylation/demethylation at the N6 position on adenine.

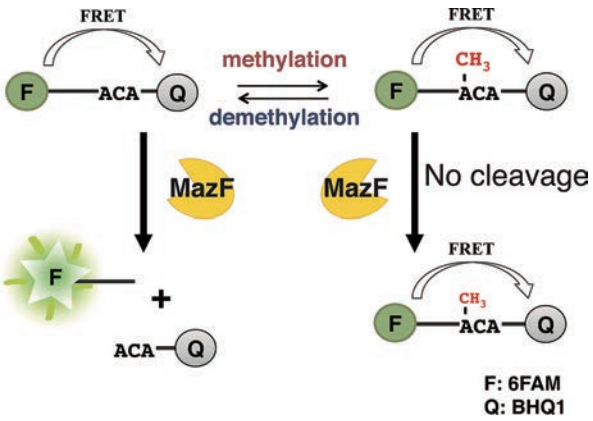

Figure 2. High-throughput detection of N^6-methyladenosine using an ACA-sequence specific MazF endoribonuclease.