Division of Biochemistry
– Biofunctional Design-Chemistry –

http://www.scl.kyoto-u.ac.jp/~bfdc/index.html

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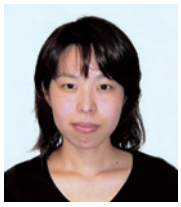

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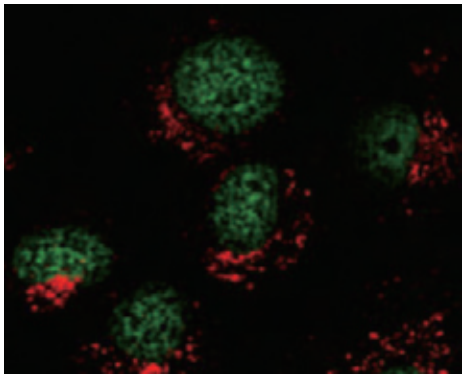
Visitors

Prof GARIÉPY, Jean	Department of Medical Biophysics, University of Toronto, Princess Margaret Hospital, Ontario Cancer Institute, Canada, 24 August
Prof GRÄSLUND, Astrid	Department of Biochemistry and Biophysics, Stockholm University, Sweden, 2 November
FATEMEH, Madani	Department of Biochemistry and Biophysics, Stockholm University, Sweden, 2 November
Dr LINDBERG, Staffan	Department of Neurochemistry, Stockholm University, Sweden, 2 November
Prof GIRALT, Ernest	Institute for Research in Biomedicine Parc Cientific de Barcelona, Spain, 2–13 December

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 and recognition modes of C2H2-type zinc finger proteins and design of artificial transcription factors with various DNA binding specificities, and (3) design of stimulation-responsible artificial peptides and proteins.



KEYWORDS

Membrane-Permeable Peptides
Intracellular Delivery
Peptide Design
Zinc Finger Protein

Selected Publications

Yu HH, Nakase I, Pujals S, Hirose H, Tanaka G, Katayama S, Imanishi M, Futaki S: Expressed Protein Ligation for the Preparation of Fusion Proteins with Cell Penetrating Peptides for Endotoxin Removal and Intracellular Delivery, *Biochim Biophys Acta*, **1798,** 2249-2257 (2010).

Noshiro D, Asami K, Futaki S: Metal-assisted Channel Stabilization: Disposition of a Single Histidine on the N-terminus of Alamethicin Yields Channels with Extraordinarily Long Lifetimes, *Biophys J*, **98,** 1801-1808 (2010).

Nakase I, Kobayashi S, Futaki S: Endosome-disruptive Peptides for Improving Cytosolic Delivery of Bioactive Macromolecules, *Biopolymers*, **94,** 763-770 (2010).

Imanishi M, Nakaya T, Morisaki T, Noshiro D, Futaki S, Sugiura Y: Metal-stimulated Regulation of Transcription by an Artificial Zinc-finger Protein, *Chembiochem*, **11,** 1653-1655 (2010).

Metal-Assisted Channel Stabilization: Disposition of a Single Histidine on the N-terminus of Alamethicin Yields Channels with Extraordinarily Long Lifetimes

Alamethicin, a member of the peptaibol family of antibiotics, is a typical channel-forming peptide with a helical structure. The self-assembly of the peptide in the membranes yields voltage-dependent channels. The channels are characterized by frequent fluctuation of several current levels due to spontaneous uptake and release of alamethicin molecules into/from the channel assembly. Because certain classes of biological ion channels, including the nicotinic acetylcholine receptor (nAChR), have parallel bundles of amphipathic α-helices around the channel pores, alamethicin has been studied as a simplified model of such channel proteins and has been employed as an appropriate framework for creating artificial ion channels. In this study, three alamethicin analogs possessing a charged residue (His, Lys or Glu) on their N-termini were designed with the expectation of stabilizing the transmembrane structure (Figure 1). A slight elongation of channel lifetime was observed for the Lys and Glu analogs. On the other hand, extensive stabilization of certain channel open states was observed for the His analog. This stabilization was predominantly observed in the presence of metal ions such as Zn^{II}, suggesting that metal coordination with His facilitates the formation of a supramolecular assembly in the membranes. Channel stability was greatly diminished by acetylation of the N-terminal amino group, indicating that the N-terminal amino group also plays an important role in metal coordination. In addition, the potential applicability of the His analog channel to metal sensing was also demonstrated.

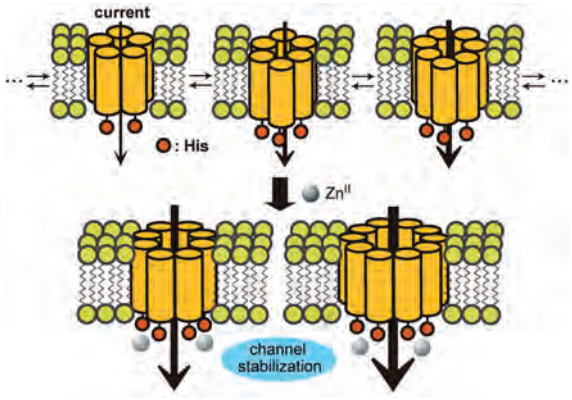


Figure 1. Schematic representation of channel formation of the His analog of alamethicin in the membrane.

Metal-Stimulated Regulation of Transcription by an Artificial Zinc-Finger Protein

The regulation of the gene expression of specific genes at a desired time opens attractive avenues for research in chemical biology, cell biology, and future gene therapy. A C2H2-type zinc finger motif, one of the most ubiquitous DNA binding motifs, binds a zinc ion with two conserved cysteine (Cys: C) and two histidine (His: H) residues. Zn^{II} binding is necessary for the proper folding of the peptides into the globular $\beta\beta\alpha$ structure and for the DNA binding ability. The binding between a typical C2H2-type zinc finger peptide and Zn^{II} is extremely stable. Such C2H2-type zinc fingers always preserve the Zn^{II} ions inside the cells and thus, it is difficult to control their DNA binding ability by changing the Zn^{II} concentration. By substituting zinc-ligating residue(s) of a C2H2-type zinc finger motif, we expected that the binding affinity to Zn^{II} would decrease and that the DNA binding ability of the peptide can eventually be controlled by changes in the Zn^{II} concentration (Figure 2). By substituting zinc-ligating residue(s) to CDH2-type, we succeeded in controlling the DNA binding and transcriptional ability of the peptide in response to changes in the Zn^{II} concentration. This zinc finger-based system has the bright prospect of an important prototype for the design of metal-responsive artificial transcriptional switches.

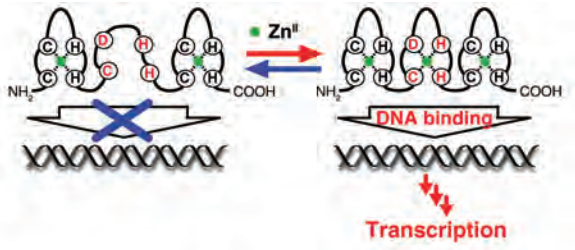


Figure 2. Zn(II) concentration responsive DNA binding of a ligand substituted zinc finger protein, CDH2-ZF3.