Division of Biochemistry
– Biofunctional Design-Chemistry –

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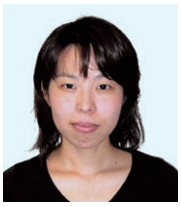

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Visitors

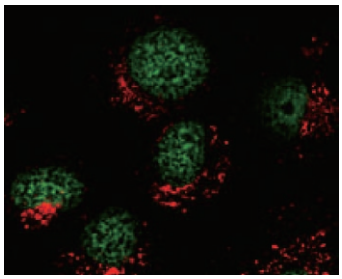
Prof GRÄSLUND, Astrid — Stockholm University, Sweden, 23 January
Prof NEUNDORF, Ines — University of Cologne, Germany, 15 September
Prof XIA, Jiang — The Chinese University of Hong Kong, China, P.R., 15 September

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

Nakase, I.; Okumura, S.; Katayama, S.; Hirose, H.; Pujals, S.; Yamaguchi, H.; Arakawa, S.; Shimizu, S.; Futaki, S., Transformation of an Antimicrobial Peptide into a Plasma Membrane-Permeable, Mitochondria-Targeted Peptide via the Substitution of Lysine with Arginine, *Chem. Commun.*, **48,** 11097-11099 (2012).

Nakase, I.; Okumura, S.; Tanaka, G.; Osaki, K.; Imanishi, M.; Futaki, S., Signal Transduction Using an Artificial Receptor System that Undergoes Dimerization upon Addition of a Bivalent Leucine-Zipper Ligand, *Angew. Chem. Int. Ed.*, **51,** 7464-7477 (2012).

Hirose, H.; Takeuchi, T.; Osakada, H.; Pujals, S.; Katayama, S.; Nakase, I.; Kobayashi, S.; Haraguchi, T.; Futaki, S., Transient Focal Membrane Deformation Induced by Arginine-rich Peptides Leads to Their Direct Penetration into Cells, *Mol. Ther.*, **20,** 984-993 (2012).

Noshiro, D.; Asami, K.; Futaki, S., Control of Leakage Activities of Alamethicin Analogs by Metals: Side Chain-Dependent Adverse Gating Response to Zn^{2+}, *Bioorg. Med. Chem.*, **20,** 6870-6876 (2012).

Imanishi, M.; Yamamoto, K.; Yamada, H.; Hirose, Y.; Okamura, H.; Futaki, S., Construction of a Rhythm Transfer System That Mimics the Cellular Clock, *ACS Chem. Biol.*, **7,** 1817-1821 (2012).

Creation of Artificial Epidermal Growth Factor Receptor Activated by Coiled-Coil Peptides

Epidermal growth factor receptor (EGFR) regulates various signaling pathways to induce cellular functions (e.g. proliferation, survival). If activation of this receptor could be controlled on demand, the techniques contribute to a lot of valuable aspects of cellular regulations. However, because overexpression and overactivation of the EGFR lead to cancer-like modality, the control is difficult using intact receptor and ligands. From this point of view, our research objectives are to create artificial EGFR on cell membranes, which can be specifically activated with artificial ligands.

Coiled-coil has been used in numerous applications such as affinity purification, miniaturized antibodies, and receptor imaging. Using advantage of the coiled-coil peculiarities, we designed artificial EGFR, of which the receptor dimerization can be controlled by coiled-coil interactions. Growth factor binding to the extracellular region of EGFR promotes dimerization of the receptor and increases the tyrosine kinase activity of its intracellular domain. To create artificial EGFR and ligands, coiled-coil peptides (E3 and K4) were used. We designed surface-exposed tag sequence E3 fused EGFR (E3-EGFR) lacking domains I~III and a portion of domain IV, which participate in dimerization of the EGFR after binding of natural ligand (e.g. EGF) to the receptor. To dimerize the E3-EGFR, we synthesized conjugates of two K4 peptides (K4-conjugates), and the linker lengths (~10 angstrom) mimic distance during dimerization of the EGFR (Figure 1).

To test receptor activation, E3-EGFR expressed CHO cells were treated with the K4-conjugates, and this was followed by western blot assay for detection of receptor phosphorylation. Treatment of the cells with the K4-conjugates increased level of the phosphorylation in ~5 min, and enhanced lamellipodia formations and cell migrations were observed. This model-receptor system should be applicable to design of various receptors and other membrane-associated proteins to attain control of cellular functions.

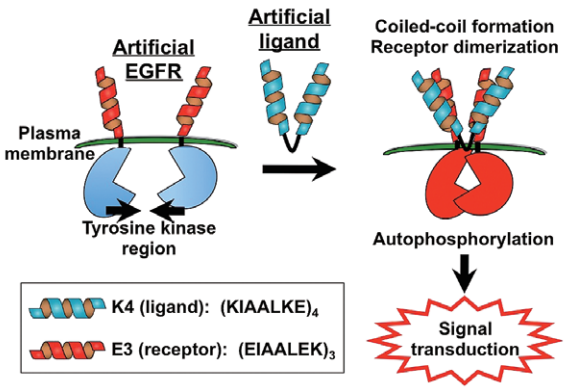


Figure 1. Concept of artificial EGFR activated by a helix peptide ligand via a coiled-coil formation.

CXCR4 is a Receptor Stimulating the Cellular Uptake of Arginine-rich Cell-penetrating Peptides: the Relevance to Macropinocytosis and HIV Infection

The CXCR4 chemokine receptor is a co-receptor of HIV-1 infection in host cells. It has been reported that ligand binding to CXCR4 leads to its internalization via clathrin-dependent endocytosis. Here, through the photo-cross-linking study to identify receptors involved in internalization of oligoarginine cell-penetrating peptides (CPPs), we found that CXCR4 serves as a receptor that stimulates internalization of the arginine 12-mer peptide (R12), in which actin-driven fluid-phase endocytosis (macropinocytosis) plays an important role in cellular uptake. Interestingly, CXCR4 was responsible for the uptake of R12 (Figure 2), but there was no significant effect for the uptake of the arginine 8-mer (R8) or the HIV-1 TAT derived CPP. We also found that stimulating CXCR4 with its intrinsic ligands, stromal cell-derived factor 1α (SDF-1α) and HIV-1 envelope glycoprotein 120 (gp120), induced macropinocytosis. R12 had activity to prevent viral infection for HIV-1_{IIIB}, a subtype of HIV-1 that uses CXCR4 as a co-receptor for entry into susceptible cells. On the other hand, the addition of macropinocytosis inhibitor, dimethylamiloride (DMA), resulted in enhancement of viral infection. Thus, the present study showed that CXCR4 triggers macropinocytosis, which may have implications for the cellular uptake of oligoarginine CPPs and internalization of HIV.

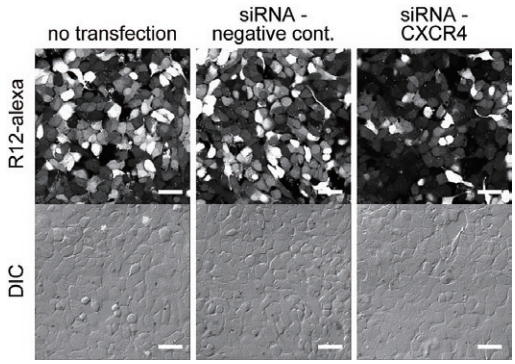


Figure 2. Down-regulation of CXCR4 led to diminished cellular uptake of R12-alexa. HeLa cells were transfected with 10 nM CXCR4-targeted siRNA for 24 h followed by treatment with 10 μM R12-alexa for 30 min at 37°C. Cellular internalization of R12-alexa was analyzed by confocal microscopy.