Division of Biochemistry
– Biofunctional Design-Chemistry –

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

Prof
FUTAKI, Shiroh
(D Pharm Sc)

Assoc Prof
IMANISHI, Miki
(D Pharm Sc)

Assist Prof
KAWAGUCHI, Yoshimasa
(D Pharm Sc)

Program-Specific Assoc Prof
HIROSE, Hisaaki
(D Pharm Sc)

Students

SAKAI, Takayuki (D3)
HIRAI, Yusuke (D3)
IWATA, Takahiro (D1)
KURIYAMA, Masashi (M2)
MICHIBATA, Junya (M2)
OKANO, Syusuke (M2)
OTONARI, Kenko (M2)
IKITOU, Asuka (M1)
IMAO, Keisuke (M1)
KAWAMURA, Yuki (M1)
NAKAGAWA, Yuna (M1)
TANAKA, Kamui (M1)
ASAMI, Yuri (UG)
TAMUKAI, Kento (UG)
YOSHIE, Shunsuke (UG)

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 or RNA binding modes of nucleic acid binding proteins, and design of artificial regulators of gene expression, (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

Recent Selected Publications

Iwata, T.; Hirose, H.; Sakamoto, K.; Hirai, Y.; Arafiles, J. V. V.; Akishiba, M.; Imanishi, M.; Futaki, S., Liquid Droplet Formation and Facile Cytosolic Translocation of IgG in the Presence of Attenuated Cationic Amphiphilic Lytic Peptides, *Angew. Chem. Int. Ed.*, **60,** 19804-19812 (2021).

Hirai, Y.; Hirose, H.; Imanishi, M.; Asai, T.; Futaki, S., Cytosolic Protein Delivery Using pH-Responsive, Charge-Reversible Lipid Nanoparticles, *Sci. Rep.*, **11,** 19896 (2021).

Sakamoto, K.; Michibata, J.; Hirai, Y.; Ide, A.; Ikitoh, A.; Takatani-Nakase, T.; Futaki, S., Potentiating the Membrane Interaction of an Attenuated Cationic Amphiphilic Lytic Peptide for Intracellular Protein Delivery by Anchoring with Pyrene Moiety, *Bioconjug. Chem.*, **32,** 950-957 (2021).

Arafiles, J. V. V.; Hirose, H.; Hirai, Y.; Kuriyama, M.; Sakyiamah, M. M.; Nomura, W.; Sonomura, K.; Imanishi, M.; Otaka, A.; Tamamura, H.; Futaki, S., Discovery of a Macropinocytosis-Inducing Peptide Potentiated by Medium-Mediated Intramolecular Disulfide Formation, *Angew. Chem. Int. Ed.*, **60,** 11928-11936 (2021).

Sakamoto, K.; Akishiba, M.; Iwata, T.; Murata, K.; Mizuno, S.; Kawano, K.; Imanishi, M.; Sugiyama, F.; Futaki, S., Optimizing Charge Switching in Membrane Lytic Peptides for Endosomal Release of Biomacromolecules, *Angew. Chem. Int. Ed.*, **59**, 19990-19998 (2020).

Liquid Droplet Formation and Facile Cytosolic Translocation of IgG in the Presence of Attenuated Cationic Amphiphilic Lytic Peptides

Antibodies (IgGs) have excellent antigen recognition ability and are not only used as a tool for molecular analysis in the life science field, but are also being developed as molecular targeting drugs. However, due to their large size (approximately 150 kDa) and hydrophilicity, IgGs are unable to penetrate cell membranes, making it difficult to target intracellular molecules. In our laboratory, we have developed L17E, a macromolecule delivery peptide [1]. L17E enables efficient intracellular delivery of macromolecules, including IgGs. However, to achieve effective intracellular delivery of IgGs by L17E and recognition of subcellular targets by IgGs, L17E and IgGs must be administered at high concentrations (L17E 40 μM, IgG 0.5–1 mg/mL). In view of the application to basic research such as cell biology and clinical application, it is necessary to develop a method that enables efficient intracellular delivery of L17E and IgG at lower concentrations.

We hypothesized that increasing the local concentration of IgG and L17E in the vicinity of the membrane would be important for efficient intracellular delivery of IgG. To increase the local concentration of L17E, we prepared an L17E analogue [FcB(L17E)$_3$] comprised of trimerized L17E tethered with a peptide sequence having binding ability to IgG. Marked intracellular delivery of IgGs was achieved using FcB(L17E)$_3$ at lower peptide and IgG concentrations than with L17E. Different from our original expectation, liquid-liquid phase separation, caused by the electrostatic interaction between the FcB(L17E)$_3$ and the fluorescently labeled IgG, was found to be the driving force to attain the facile antibody delivery into cells (Figure 1) [2]. The results of this research are expected to serve as a basis for the development of new drug carriers based on liquid-liquid phase separation in the future.

References

[1] Akishiba, M. *et al*., *Nat. Chem*., **9,** 751-761 (2017).
[2] Iwata, T. *et al.*, *Angew. Chem. Int. Ed*., **60,** 19804-19812 (2021).

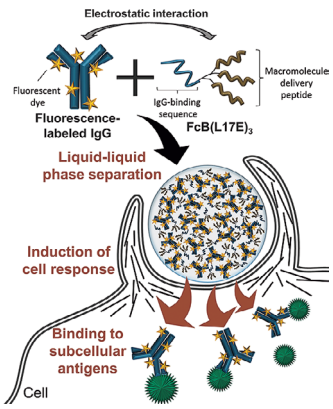

Figure 1. Proposed mechanism of action of FcB(L17E)$_3$.

Cytosolic Delivery of Negatively Charged Protein Using pH-responsive, Charge-reversible Lipid Nanoparticles

Proteins have attractive features as biopharmaceuticals because of their specific effects to target molecules. However, their applicability is limited to only extracellular environment because it is difficult to deliver them into the cell interior. Lipid nanoparticles (LNPs) composed with pH-responsive lipids are a promising class of intracellular delivery vehicles of negatively charged molecules like nucleic acids. In using LNPs system for protein delivery, the major challenges include: (i) formulation of LNPs with appropriate particle sizes and dispersity, (ii) efficient encapsulation of proteins into LNPs, and (iii) effective cellular internalization and cytosolic release of proteins. To develop new carrier for protein delivery, we used dioleoylglycerophosphate-diethylenediamine (DOP-DEDA), a pH-responsive, charge-reversible lipid for delivery of nucleic acids into human cancer cells [1]. A negatively charged green fluorescent protein analog with nuclear localization signal (NLS-(−30)GFP) was successfully encapsulated into DOP-DEDA-based LNPs (protein encapsulation efficiency, ~ 80 %) to yield particle diameters and polydispersity index of < 200 nm and < 0.2, respectively. Intracellular distribution of fluorescent signals of the protein was observed for up to ~ 90 % of cells treated with DOP-DEDA-based LNPs. Overall, our results indicate the facilitated endocytic uptake and endosomal escape of a negatively charged protein attained using DOP-DEDA-based LNP system.

Reference

[1] Hirai. Y., *et al*., *Int. J. Pharm.* **585,** 119479. (2020).

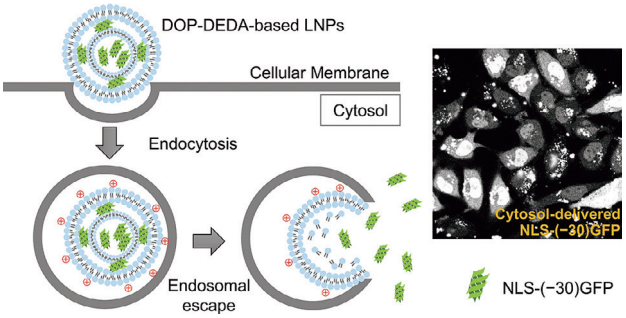

Figure 2. DOP-DEDA-based LNPs enhance intracellular and cytosolic delivery of negatively charged protein.