Division of Biochemistry
– Biofunctional Design-Chemistry –

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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)

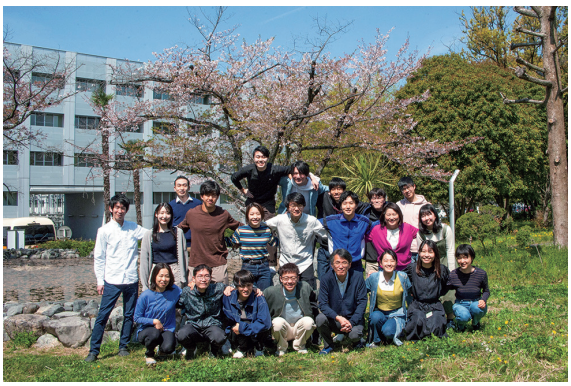
Program-Specific Assist Prof
KIMURA, Seigo
(D Pharm Sc)

Students

IWATA, Takahiro (D3)
KURIYAMA, Masashi (D2)
MICHIBATA, Junya (D2)
OTONARI, Kenko (D2)
IDA, Naka (D1)
NAKAGAWA, Yuna (D1)
TANAKA, Kamui (D1)
ASAMI, Yuri (M2)
IMAO, Keisuke (M2)
TERADA, Sakahiro (M2)
YOSHIE, Shunsuke (M2)
BABA, Nozomi (M1)
KASAHARA, Chisato (M1)
TAKEUCHI, Kosuke (M1)
UEHATA, Yusuke (M1)
YAMASAKI, Daisuke (M1)
KIKKAWA, Ayumi (UG)
NISHIJIMA, Hiroto (UG)
OBATA, Keito (UG)
KIYOKAWA, Megumi (RS)

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

Hirose H.; Nakata E.; Zhang Z.; Shibano Y.; Maekawa M.; Morii T.; Futaki, S., Macropinoscope: Real-Time Simultaneous Tracking of pH and Cathepsin B Activity in Individual Macropinosomes, *Anal. Chem*., **95,** 11410-11419 (2023).

Tanaka K.; Suda A.; Uesugi M.; Futaki S.; Imanishi M., Xanthine Derivatives Inhibit FTO in an L-ascorbic Acid-Dependent Manner, *Chem. Commun*., **59,** 10809-10812 (2023).

Omura M.; Morimoto K.; Araki Y.; Hirose H.; Kawaguchi Y.; Kitayama Y.; Goto Y.; Harada A.; Fujii I.; Takatani-Nakase T.; Futaki S.; Nakase I., Inkjet-Based Intracellular Delivery System that Effectively Utilizes Cell-Penetrating Peptides for Cytosolic Introduction of Biomacromolecules through the Cell Membrane, *ACS Appl. Mater Interfaces*, **15,** 47855-47865 (2023).

Nishimura M.; Kawaguchi Y.; Kuroki K.; Nakagawa Y.; Masuda T.; Sakai T.; Kawano K.; Hirose H.; Imanishi M.; Takatani-Nakase T.; Afonin S.; Ulrich A. S.; Futaki S., Structural Dissection of Epsin-1 N-Terminal Helical Peptide: The Role of Hydrophobic Residues in Modulating Membrane Curvature, *Chemistry*, **29,** e202300129 (2023).

Hirose H.; Maekawa M.; Ida H.; Kuriyama M.; Takahashi Y.; Futaki S., A Noncanonical Endocytic Pathway is Involved in the Internalization of 3 μm Polystyrene Beads into HeLa Cells, *Bioconjug. Chem*., **33,** 1851-1859 (2022).

Development of a Dual Sensor to Track pH and an Enzyme Activity in Individual Macropinosomes

Macropinocytosis is an endocytic pathway that results in the formation of macropinosomes through massive uptake of extracellular fluids. For instance, certain cancer cell types use macropinocytosis to obtain extracellular nutrients, such as amino acids, to support their proliferation. Furthermore, macropinocytosis represents a promising route for the effective delivery of biological macromolecules including proteins and antibodies. Nonetheless, a comprehensive understanding of the intracellular dynamics of macropinosomes has yet to be attained. Increased knowledge of individual macropinosomes behavior in living cells could significantly impact the design concept of drug delivery tools and development of drugs against cancer.

A fluorescent sensor that enables simultaneous analysis of multiple parameters in limited or specific cellular space is useful for comprehending molecular dynamics and biological responses in living cells. In this study, we aimed to develop a macropinoscope, a fluorescent sensor that detects both pH and cathepsin B activity in individual macropinosomes through fluorescence microscopy. Dextran (70 kDa) was utilized as a macropinosome-specific marker platform, onto which three fluorophores (fluorescein, Oregon Green, and tetramethylrhodamine) were loaded for ratiometric pH sensing and imaging. Additionally, a cathepsin-B-cleavable peptide sequence, bearing sulfo-Cy5 fluorophore and the BHQ-3 quencher, was loaded onto the platform. The cathepsin B activity can be detected by an increase in sulfo-Cy5 fluorescence when the peptide sequence is cleaved. The macropinoscope was utilized to investigate the behavior of individual macropinosomes produced by constitutive macropinocytosis in HT1080 cells. We discovered that a sharp decrease in pH occurred close to the cell nucleus, 5-10 minutes after macropinosome formation. We also observed an immediate rise in cathepsin B activity as the pH of the macropinosome reached around 6.

In the future, the sensor is expected to enable thorough examination of macropinosome behavior under other conditions and in different cell types. Additionally, our design concept will pave the way for the development of various macropinoscopes tailored to other parameters in individual macropinosomes.

Reference

[1] Hirose, H. *et al., Anal. Chem.* **95**, 11410-11419 (2023).

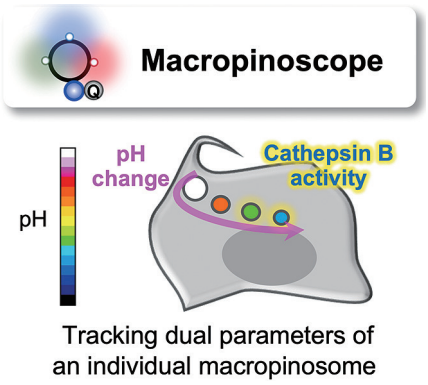


Figure 1. Macropinoscope can track dual parameters (pH and cathepsin B activity) of individual macropinosomes by fluorescence microscopy. Reprinted from [1].

Inhibition of m^{6}A Demethylation Activity of FTO by Xanthine Derivatives with L-Ascorbic Acid-Related Manner

Chemical modifications of RNA play an important role in gene regulation. In particular, N^6-methyladenosine (m^{6}A), which is present in transcripts at high frequency, is involved in development and differentiation as well as various diseases. Despite the increasing evidence of the importance of m^{6}A RNA methylation, convenient m^{6}A detection methods for measuring the activity of RNA methyltransferases and demethylases and for screening their inhibitors have been lacking. Here, a simple method to detect the methylation states of RNA was constructed using the *Escherichia coli* RNA endonuclease MazF, which we found to be m^{6}A-sensitive, in combination with the FRET probe containing an m^{6}A consensus RNA sequence. We searched for inhibitors of the m^{6}A demethylase FTO, one of the member of Fe(II) and 2-oxoglutarate-dependent oxygenases. Xanthine derivatives were identified as specific inhibitors of the m^{6}A demethylase activity of FTO. These inhibitors exhibited L-ascorbic acid concentration-dependent inhibitory activity against FTO, an unprecedented mode of inhibition. Our results have significant implications for understanding the regulatory mechanisms of FTO and the design of FTO-specific inhibitors.

Reference

[2] Tanaka, K. *et al.*, *Chem. Commun*. **59**, 10809-10812 (2023).

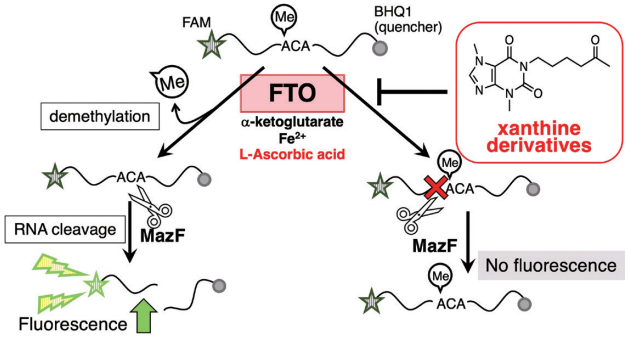


Figure 2. An activity-based screening for FTO inhibitor identified xanthine derivatives as specific inhibitor with an L-ascorbic acid-dependent mode of inhibition. Reprinted from [2].