Cellular Informatics Laboratory
Chief Scientist: Yasushi Sako (D.Sci.)

(0) Research field

CPR Subcommittee**:** Biology

Keywords:

Biophysics, Biomembranes, Cell signaling, Membrane receptors, Protein dynamics

(1) Long-term goal of laboratory and research background

The aim of us is to understand the principles of signal processing carried out by biological systems in the classes of proteins, protein networks, and cells. We are studying how biomolecules assemble to process the intra- and extra-cellular information and express flexible higher-order cellular responses. In these studies, we develop and use techniques of single-molecule measurements, optical microscopy, cell engineering, reconstruction of biosignaling systems, as well as mathematical analysis and computer simulations. The recent main target of us is an intracellular protein reaction network called the ERBB-RAS-MAPK system. This system is responsible for cell fate decisions including cell proliferation, differentiation, and apoptosis. In addition, we are investigating the functions and dynamics of proteins, including GPCRs, which is also involved in cell signaling and fate decision. We are analyzing how diverse dynamics of reaction systems, which lead to higher-order biological function, emerged from the accumulations of elemental protein reactions.

(2) Current research activities (FY2024) and plan

Bilateral regulation of EGFR activity and local PI dynamics observed with superresolution microscopy

Anionic lipid molecules, including phosphatidylinositol-4,5-bisphosphate ($PI(4,5)P_2$), are implicated in the regulation of epidermal growth factor receptor (EGFR). However, the role of the spatiotemporal dynamics of $PI(4,5)P_2$ in the regulation of EGFR activity in living cells is not fully understood, as it is difficult to visualize the local lipid domains around EGFR. Here, we visualized both EGFR and $PI(4,5)P_2$ nanodomains in the plasma membrane of mammalian cells using super-resolution single-molecule microscopy. The EGFR and $PI(4,5)P_2$ nanodomains aggregated before stimulation with epidermal growth factor (EGF) through transient visits of EGFR to the $PI(4,5)P_2$ nanodomains. The degree of coaggregation decreased after EGF stimulation and depended on phospholipase Cγ, the EGFR effector hydrolyzing $PI(4,5)P_2$. Artificial reduction in the $PI(4,5)P_2$ content of the plasma membrane reduced both the dimerization and autophosphorylation of EGFR after stimulation with EGF. Inhibition of $PI(4,5)P_2$ hydrolysis after EGF stimulation decreased phosphorylation of EGFR-Thr654. Thus, EGFR kinase activity and the density of $PI(4,5)P_2$ around EGFR molecules were found to be mutually regulated.

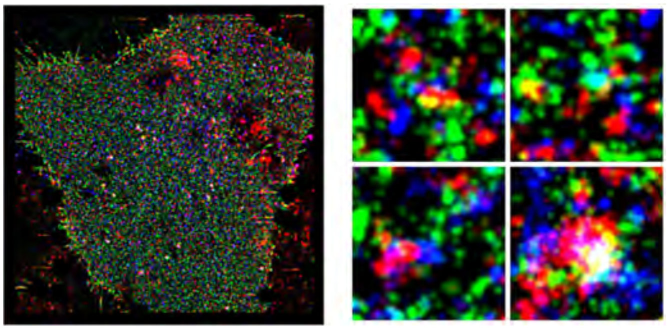

Fig.1. Superlocalization microscopy of a membrane protein and membrane lipids.
green: EGFR, red: PIP_2, blue: PS. The size of right panels is 1 μm square.

Evaluation of information flows in the RAS-MAPK system using transfer entropy measurements

The RAS-MAPK system plays an important role in regulating various cellular processes, including growth, differentiation, apoptosis, and transformation. Dysregulation of this system has been implicated in genetic diseases and cancers affecting diverse tissues. To better understand the regulation of this system, we employed information flow analysis based on transfer entropy (TE) between the activation dynamics of two key elements in cells stimulated with EGF**:** SOS, a guanine nucleotide exchanger for the small GTPase RAS, and RAF, a RAS effector serine/threonine kinase. TE analysis allows for model-free assessment of the timing, direction, and strength of the information flow regulating the system response. We detected significant amounts of TE in both directions between SOS and RAF, indicating feedback regulation. Importantly, the amount of TE did not simply follow the input dose or the intensity of the causal reaction, demonstrating the uniqueness of TE. TE analysis proposed regulatory networks containing multiple tracks and feedback loops and revealed temporal switching in the reaction pathway primarily responsible for reaction control. This proposal was confirmed by the effects of a MEK inhibitor on TE. Furthermore, TE analysis identified the functional disorder of a SOS mutation associated with Noonan syndrome, a human genetic disease, of which the pathogenic mechanism has not been precisely known yet. TE assessment holds significant promise as a model-free analysis method of reaction networks in molecular pharmacology and pathology.

In addition, this year, we have developed novel method for probing membrane proteins for single-molecule imaging (Yoda et al. 2024), and performed comprehensive analysis of the transposon genes in Nematoda (Arata et al. 2024).
We will continue studies on the cell signaling protein dynamics in relation to the cell structure.

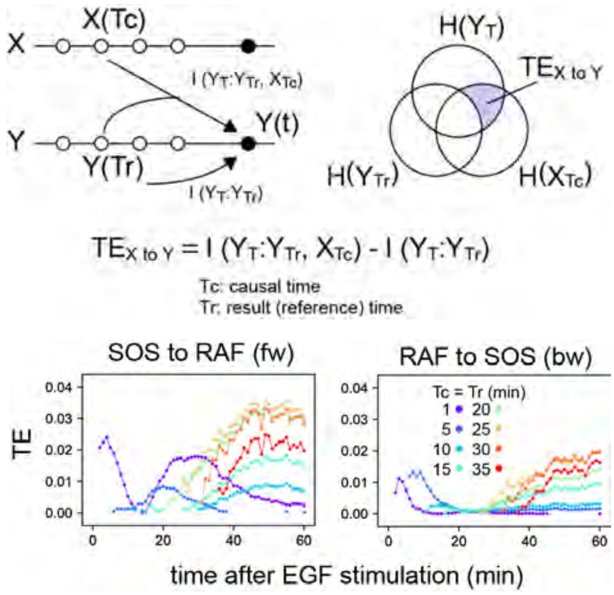

Fig. 2. upper: Concept of TE assessment between two reaction timeserieses, X and Y. lower: Time profiles of TE from a single time point (Tc) in the upstream reaction (SOS or RAF) to the downstream reaction (RAF or SOS).

(3) Members as of March, 2024

(Chief Scientist)
Yasushi Sako

(Senior research scientist)
Akihiro Yamamoto

(Research scientist)
Mitsuhiro Abe, Yukinobu Arata, Kenji Okamoto, Toshihiro Nagamine, Nobuhisa Umeki, Ryo Yoshizawa

(Technical Staff)
Hiromi Sato

(Part-time Worker)
Itsumi Ota

(4) Representative research achievements

1. T. Yoda, Y. Sako, A. Inoue, and M. Yanagawa, “Four-color single-molecule imaging system for tracking GPCR dynamics with fluorescent HiBiT peptide”, **Biophys. Physicobiol.** 21, e210020 (1-16), (2024). *cover article, Editors’ Choice Award*
2. M. Abe, M. Yanagawa, M. Hiroshima, T. Kobayashi, and Y. Sako, “Bilateral regulation of EGFR activity and local PI dynamics observed with superresolution microscopy”, **eLife** 2024:e101652 (1-27), doi: 10.7554/eLife.101652, (2024). *selected for eLife digest*
3. Y. Arata, P. Jurica, N. F. Parrish, and Y. Sako, “Bioinformatic annotation of transposon DNA processing genes on the long-read genome assembly of *Caenohabiditis elegans*”, **Bioinformatics and Biology Insights**, doi: 10.1177/11779322241304668 (2024).
4. N. Umeki, Y. Kabashima, and Y. Sako, “Evaluation of information flows in the RAS-MAPK system using transfer entropy measurements”, **eLife** 2025, 14:e104432 (1-32) (2025)..

Laboratory Homepage
https://www.riken.jp/en/research/labs/chief/cell_inf/index.html
http://www2.riken.go.jp/cell-info/en/