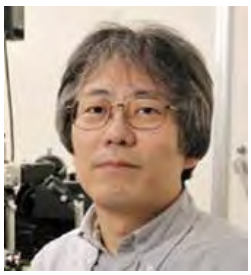
Cellular Informatics Laboratory
Chief Scientist: Yasushi Sako (D.Sci.)



(0) Research field

CPR Subcommittee**:** Biology

Keywords:

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

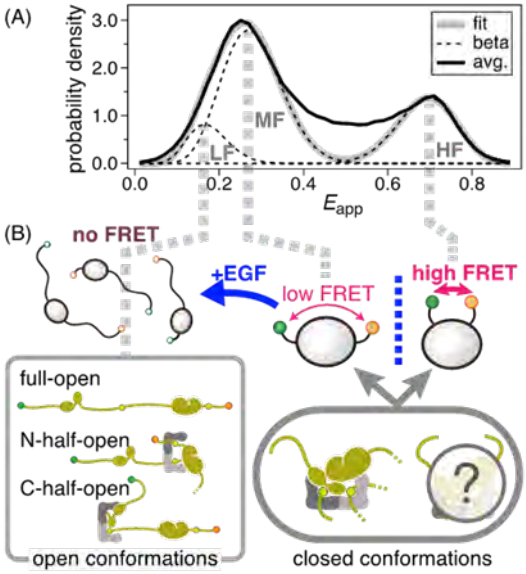
(1) Long-term goal of laboratory and research background

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 (FY2022) and plan

Structure distribution of RAF kinase in the cytoplasm

RAF is a kinase downstream of the membrane protein RAS in the cellular signal transduction system. In the structure of RAF, the N- and C-terminus domains are connected with a flexible linker. The open/close dynamics and dimerization of RAF are thought to regulate its activity, although the details of these conformations are unknown, especially in live cells. In this work, we used alternating laser excitation to measure cytosolic RAF in live HeLa cells and obtained single-molecule Förster resonance energy transfer (smFRET) distributions of the structural states. We compared the results for wild-type (WT)-RAF before and after epidermal growth factor (EGF) stimulation, with mutations of the 14-3-3 binding sites and cysteine-rich domain, and an N-terminus truncation. The smFRET distributions of full-length RAFs were analyzed by global fitting with three beta distributions. Our results suggested that a 14-3-3 dimer bound to two sites on a single RAF molecule and induced the formation of the autoinhibitory closed conformation. There were two closed conformations, which the majority of WT-RAF adopted. These two conformations showed different responsiveness to EGF stimulation. (Okamoto and Sako 2022)

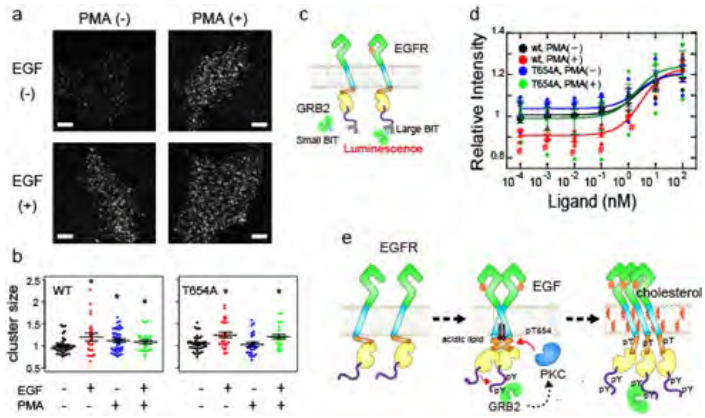


Distribution of FRET efficiencies (**A**) and expected structures (**B**) of RAF in the cytoplasm.

Membrane lipids and threonine phosphorylation regulate assembly and function of EGFR

The cytoplasmic domain of the receptor tyrosine kinases (RTKs) plays roles as a phosphorylation enzyme and a protein scaffold but the regulation of these two functions is not fully understood. We here analyzed assembly of the transmembrane (TM)-juxtamembrane (JM) region of EGFR, one of the best studied species of RTKs, by combining single-pair FRET imaging and a nanodisc technique. The JM domain of EGFR contains a threonine residue that is phosphorylated after ligand association. We observed that the TM-JM peptides of EGFR form anionic lipid-induced dimers and cholesterol-induced oligomers. The two forms involve distinct molecular interactions, with a bias towards oligomer formation upon threonine phosphorylation. We further analyzed the functions of whole EGFR molecules, with or without a threonine to alanine substitution in the JM domain, in living cells. The results suggested an autoregulatory mechanism in which threonine phosphorylation of the JM domain causes a switch from kinase activation dimers to scaffolding

oligomers. (Maeda et al. 2022)



a. Single-molecule imaging of EGFR-GFP on the cell surface. **b.** Increases in the oligomerization degree after EGF stimulation was inhibited by pT654. **c.** Nano-bit assay of EGFR/GRB2 interaction. **d.** EGF-dose dependency of EGFR/GRB2 association. **e.** A model for EGFR kinase activation and scaffolding.

In addition, this year, we have revealed that the insulin signaling regulates the multiple fractality in the *C. elegans* behavior by analyzing movements of single individuals for a long timerange in high temporal resolution (Arata et al. 2022). We also performed collaborative research with Osaka University to report enhanced transcriptional heterogeneity mediated by NF-κB super enhancers (Wibisata et al. 2022), and with Strasburg University to reveal heterogeneous cholesterol distribution in cells by using a novel sterol binding protein as the probe (Yamaji-Hasegawa et al. 2022). We will continue studies on the cell signaling protein dynamics in relation to the cell structure.

(3) Members

as of March, 2023

(Chief Scientist)
Yasushi Sako

(Senior research scientist)
Akihiro Yamamoto

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

(Technical Staff)
Dong Kese, Maiko Minatohara, Mutsumi Nakanishi, Hiromi Sato, Miyoshi Suga

(Student Trainee)
Yutaro Kuwashima

(Part-time Worker)
Itsumi Ota, Hiroaki Toyoda

(4) Representative research achievements

1. “Two closed conformations of CRAF require the 14-3-3 binding motifs and cysteine-rich domain to be intact in live cells”, Okamoto, K. and Sako, Y. **J. Mol. Biol.** 435, 167989 (2022) .
2. “Threonine phosphorylation regulates the molecular assembly and signaling of EGFR in cooperation with membrane lipids”, Maeda, R., Tamakgaki-Asahina, H., Sato, T., Yanagawa, M., and Sako, Y. **J. Cell Sci.** 135, jcs260355 (1-12) (2022).
3. “Enhanced transcriptional heterogeneity mediated by NF-κB super-enhancers”, Wibisana, J N., Inaba, T., Shinohara, H., Yumoto, N., Hayashi, T., Umeda, M., Ebisawa, M., Nikaido, I., Sako, Y., and Okada, M. **PLoS Genetics**, 18, e1010235 (1-25) (2022).
4. “Insulin signaling shapes fractal scaling of *C. elegans* behavior”, Arata, Y., Shiga, I., Ikeda, Y., Jurica, P., Kimura, H., Kiyono, K., and Sako, Y. **Sci. Rep.** 12, 10481 (1-10) (2022).
5. “A novel sterol binding protein reveals heterogeneous cholesterol distribution in neurite outgrowth and in late endosome/lysosomes”, Yamaji-Hasegawa, A., Murate, M., Inaba, T., Dohmae, N., Sato, M., Fujimori, F., Sako, Y., Grimel, P., and Kobayashi, T. **Cell. Mol. Life Sci.** 79, 324 (1-19) (2022).

Laboratory Homepage
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