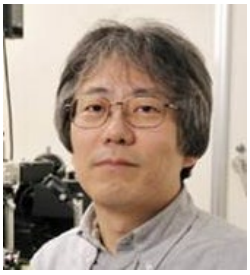
Cellular Informatics Laboratory
Chief Scientist: Yasushi Sako (D.Sci.)



(0) Research field

CPR Subcommittee: Biology

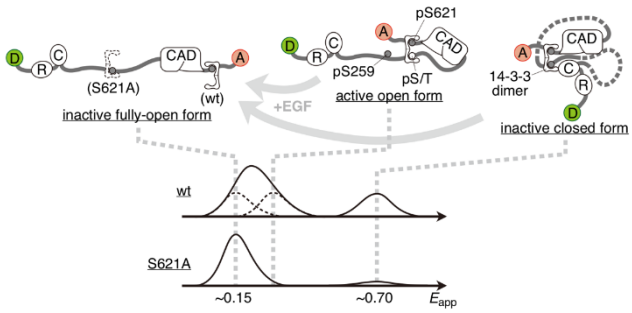
Keywords:
Biophysics, Biomembranes, Cell signaling, Membrane receptors, Protein dynamics

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

The aim of us is to understand 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 that called ERBB-RAS-MAPK system. This system is responsible for cell fate decisions including cell proliferation, differentiation, and apoptosis. In addition, we are investigating 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 (FY2019) and plan (until Mar. 2025)

RAF is a serine/threonine kinase in the cytoplasm and activated downstream of RTKs and GPCRs. The major role of RAF is to phosphorylate various cell signaling proteins for regulation of their activity. The activity of RAF itself is regulated through multiple phosphorylations, open/close structural dynamics, and homo- and hetero-dimerizations. RAF is involved in various input and output pathways, and the complicated regulation mechanism of RAF is thought to be used for its multimodal functions. However, details of which is still unknown. This year, we investigated structural dynamics of RAF in the cytoplasm of living cells by using single-pair FRET measurements. We found that RAF adopts at least three conformations in the cytoplasm (Figure). The closed state which showed the highest FRET efficiency is the basal inactive state, and phosphorylation to induce RAF activation after cell stimulation with a signaling molecule like EGF shifts the conformation to the active open state with the medium FRET efficiency. In addition, RAF has inactive fully open state, which seems to be important for adaptation. Detection of the conformational variation of cytoplasmic proteins of RAF in live cell condition was first done in our experiment as far as we know (Okamoto et al). We also found, in collaboration with Yamagata Univ., anti-inflammation effect of a lipid derivative, resolvin E3 is its function as a ligand of a GPCR, lukotoriene B4 receptor. The highest activity of resolvin E3 than those of E1 or E2 suggests a biased ligand function of E3 to the arrestin pathway downstream of the receptor (Sato et al). We will continue studies on cell signaling.



A model of structural dynamics of RAF: Multiple phosphorylation regulates conformation and activity of RAF through interaction with a cytoplasmic protein 14:3:3.

As parts of the collaboration project in RIKEN on lipid biology (Glyco-lipidoloque Initiative Project), we studied lipid accumulation for the formation of baculovirus envelope by a viral protein BNEMP (Nagamine et al), and found that CPE, a major lipid in invertebrate, forms specific tubular and ribbon structure in solution (Inaba et al). These

studies on lipids relate with the study of biomembranes in which the receptor molecules are embedded. We also revealed that the K336I mutant of actin incorporated in a filamentous structure induces a conformational change of neighboring wild-type actin molecules to affect the interaction with an actin binding protein (Umeki et al). This study has suggested that polymorphology in protein structure, as observed in RAF protein, is a common basis of the sophisticated biological function of proteins.

(3) Members

as of March, 2020

(Chief Scientist)
Yasushi Sako

(Senior research scientist)
Akihiro Yamamoto

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

(Technical Staff)
Hiromi Sato

(Student Trainee)
Ryo Yoshizawa, Yusaku Ikeda
Momoko Akiyama, Yutaro Kuwashima
Ryota Nakazato, Hiroaki Toyoda

(Visiting Scientist)
Jurica Peter, Pack Chan-Gi
Takeshi Sato, Yoshio Hirabayashi
Toshihide Kobayashi

(Part-time Worker)
Mio Tokuda, Mitsuko Ide, Itsumi Ota
Mutsumi Nakanishi, Miyoshi Suga

(4) Representative research achievements

1. "Resolvin E3 attenuates allergic airway inflammation via the interleukin-23/interleukin-17A pathway", Sato, M., Aoki-Saito, H., Fukuda, H., Ikeda, H., Koga, Y., Yatomi, M., Tsurumaki, H., Maeno, T., Saito, T., Nakakura, T., Mori, T., Yanagawa, M., Abe, M., Sako, Y., Dobashi, K., Ishizuka, T., Yamada, M., Shuto, S., Hisada, T., **FASEB J.** 33, 12750-12759 (2019).
2. "A nuclear envelop-associated baculovirus protein promotes intranuclear lipid accumulation during infection", Nagamine, T., Inaba, T., and Sako Y., **Virology** 532, 108-117 (2019). (cover article)
3. "In-cell single-molecule FRET measurements reveal three conformational state changes in RAF protein", Okamoto, K., Hibino, K., and Sako Y., **Biochim. Biophys. Acta Gen. Subj.** 1864, 129358 (2019).
4. "Formation of tubules and helical ribbons by ceramide phosphoethanolamine-containing membranes", Inaba, T., Murate, M., Tomishige, N., Lee, Y.-F., Hullin-Matsuda, F., Pollet, B., Humbert, N., Mely, Y., Sako, Y., Greimel, P., and Kobayashi, T., **Sci. Rep.** 9, 5812 (2019).
5. "K336I mutant actin alters the structure of neighboring proteins in filaments and reduces affinity for actin-binding proteins", Umeki, N., Shibata, K., Noguchi, T. Q. P., Hirose, K., Sako, Y., and Uyeda, T. Q. P., **Sci. Rep.** 9, 5353 (2019).

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