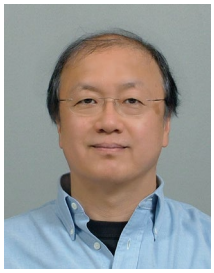
【Grant-in-Aid for Scientific Research (S)】

Biological Sciences (Medicine, Dentistry, and Pharmacy)



# Title of Project : Signal transduction by transient molecular complexes and its regulation by actin membrane skeleton: single-molecule tracking study

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Research Project Number : 16H06386 Researcher Number : 50169992
Research Area : Single-molecule cellular biophysics, Single-molecule medicinal chemistry
Keyword : Single-molecule tracking, living cells, plasma membrane, meso-scale domains

## 【Purpose and Background of the Research】

Recently, we have made two totally unanticipated discoveries about the signaling mechanisms common to all of the three experimental paradigms we study (CD59, a prototypical raft-associated receptor; Adrenergic receptor, a prototypical G-protein-coupled receptor; and Fc ε receptor, an immune receptor in mast cells, which is responsible for allergic reactions), which greatly surprised us. Based on these discoveries, we obtained the following two working hypotheses, which we aim to clarify in this project.

(1) Signaling complexes, when directly observed by single-molecule techniques in living cells, are extremely dynamic. Both signaling and scaffolding molecules are constantly exchanging with the dispersed molecules, and the complexes themselves are dynamically forming and dispersing continually, in the time scale often less than 0.1 s. Namely, most of the signaling complexes are extremely transient structures, and are much more dynamic than expected before.

(2) The part of the cortical actin cytoskeleton associated with the PM cytoplasmic surface, called membrane skeleton (MSK), works as the platform for signal transduction in many signaling pathways.

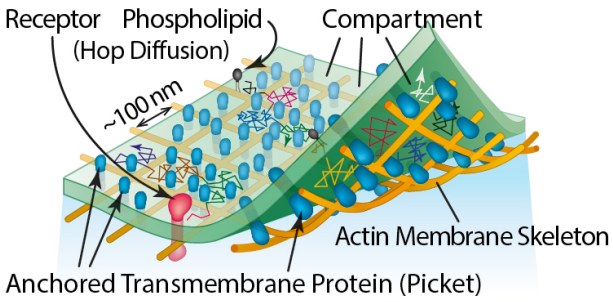


Figure 1. Actin-membrane-skeleton-induced PM compartments.

## 【Research Methods】

We will systematically examine our working hypothesis, i.e., the gradual minute-order binding/recruitment/activation events observed at the bulk level are induced as the sum of many pulse-like single-molecule events, using the three study paradigms (first in the CD59 signaling). We then reveal the pulse like single-molecule interactions and bulk signals lasting 100~1000 s.

## 【Expected Research Achievements and Scientific Significance】

We hope to induce a paradigm shift with regard to the mechanisms by which signal transduction in/on the PM is conducted.

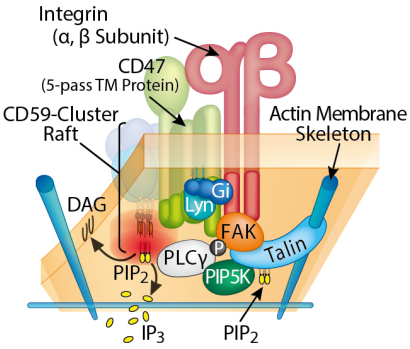


Figure 2. Transient recruitment of cellular signaling molecules to CD59-cluster rafts.

## 【Publications Relevant to the Project】

A. Kusumi et al. Tracking single molecules at work in living cells (review). Nat. Chem. Biol. 17, 524-532 (2014).

A. Kusumi et al. Organizing principles of the plasma membrane for signal transduction: Membrane mechanisms by the three-tiered hierarchical meso-scale domain architecture. Ann. Rev. Cell Dev. Biol. 28, 215-250 (2012).

## 【Term of Project】 FY2016-2020

## 【Budget Allocation】 145,500 Thousand Yen

## 【Homepage Address and Other Contact Information】

http://www.nanobio.frontier.kyoto-u.ac.jp/index.html