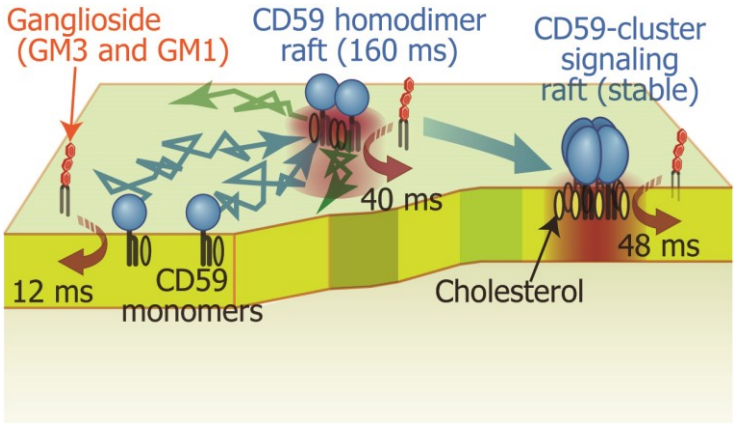
**Principal Investigator: Kenichi Suzuki**
**Grant Title: Unraveling of regulation mechanisms of receptor activity by gangliosides**

Abstract

Raft domains have been drawing extensive attention as a signaling platform. However, raft structure and function are still very controversial. This is partially due to lack of raft lipid probes which behave like the parental molecules in cell membranes. Here, we developed methods for systematically synthesizing probes of raft lipids such as gangliosides, which behave like the parental molecules in terms of partitioning into raft-related membrane domains/preparations for the first time ever in collaboration with Kiso and Ando group of Gifu University[1].

Surprisingly, single-molecule observations in live cell plasma membrane revealed that all the fluorescent ganglioside probes (GM1, GM2, GM3, GalNacGD1a, GD1b, GD3, GT1b, GQ1b) we developed, underwent fast diffusion in steady-state cell plasma membranes. High-speed single-molecule observation at 2000 frames/sec showed that these probes were not confined in small domains (< 100 nm in diameter) for longer than 5 ms. These results suggest that the ganglioside probes are always mobile in steady-state cell membranes, and there are no domains which trap the ganglioside probes for prolonged periods.

Single-molecule observations in live cell plasma membrane also revealed the clear but transient colocalization-codiffusion of fluorescent ganglioside analogues with a fluorescently-labeled GPI-anchored protein, human CD59 with lifetimes of 12 ms for CD59 monomers, 40 ms for CD59's transient homodimer rafts in steady state cell plasma membranes, and 48 ms for engaged- CD59-cluster rafts, in cholesterol and GPI-anchoring dependent manners (Figure). These results suggest that ganglioside molecules continually and dynamically exchange between raft domain and the bulk domain, indicating that raft domain are dynamic entities.




Ganglioside probes were frequently and transiently recruited to CD59 monomers, homodimers and cluster rafts.

Reference

1. Komura, N., et al., Nat. Chem. Biol. 12, 402-410 (2016).
2. Suzuki, K. G. N. et al., Nat. Chem. Biol. 8, 774-783 (2012).