Investigating membrane protein interactions by single-protein tracking in a single living cell

Nam Ki Lee

Department of Chemistry, Seoul National University, Republic of Korea.

(namkilee@snu.ac.kr).

Abstract

Various membrane protein interactions determine cellular responses to the diverse environment around cells dynamically in space and time. Current assays are limited in their ability to unravel the membrane protein interactions under physiological conditions in a single cell. Here, we present a single-molecule diffusional mobility shift assay that is sufficiently sensitive to measure the interactions of a membrane protein with soluble proteins in a living cell membrane. Interactions involving the extracellular domains of membrane proteins are thought to contribute negligibly to the lateral diffusion of membrane protein on a plasma membrane, limiting the applicability of current diffusion-based imaging methods for investigating membrane protein interactions. However, we found that the diffusional mobility of ErbB receptors and beta-adrenergic receptors, as determined by single-particle tracking photoactivated localization microscopy, was decreased by the interactions with specific monoclonal antibodies and their Fab fragments. The shift of diffusional mobility was sensitive to the size of water-soluble binders ranging from a few ten to several hundred kD. We measured the dissociation constant and the cooperativity of cetuximab interactions with EGFR and its mutants on a plasma membrane. Our new approach is a simple and efficient tool for investigating the interactions of membrane proteins, including ligand-receptor interactions, in living cells.