**Visualizing membrane binding/unbinding event of Src homology 2 (SH2) modules**

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Many biochemical reactions are facilitated by modular protein-protein interaction domains. A proto-type modular protein domain, SH2 for example, specifically binds to phosphotyrosine (pY) peptide motifs with varied affinities, mediating protein-protein interactions [[1](#_ENREF_1)]. Upon activation, receptor tyrosine kinases (RTKs) phosphorylate tyrosine resides on themselves through conformational change [[2](#_ENREF_2)] or by enzymes, which serve as binding site for SH2 domains found within downstream signaling proteins. This earlier signaling complex formed on the cell membrane initiates multiple subsequent downstream signaling cascades, functioning for a diverse set of biological processes including proliferation, differentiation, and cell migration [[3](#_ENREF_3)]. For the last several decades, in vitro studies of pY-SH2 interactions have been extensively quantified [[4](#_ENREF_4)], less is known about the role kinetic and dynamic variations of pY-SH2 interactions play in signal transduction in living cells.

Using single molecule imaging and particle tracking methods, we visualized SH2 binding kinetics following activation of RTKs (EGF and Ephrin receptor) in the continuous time window (time bin, Δt ~ ms to hr) in living cells. We found that ligand-receptor interaction induced receptor-SH2 clustering on the plasma membrane which changed membrane recruitment and dwell time of SH2 modules [[5](#_ENREF_5)]. Fast membrane reactions (fast association and dissociation) and diffusion of SH2 modules became slower and reached to the equilibrium in several tens of minutes. These fluctuations in kinetic and dynamic curves were correlated each other and with clustering of SH2 modules as well, which was well explained with diffusion-controlled rebinding model. Our data suggest a mechanism whereby signal output can be regulated through the spatial organization of receptors.

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