Correlating FcεRI Signaling with Dynamics using Single Quantum Dot Imaging

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***Short Abstract* — Examining protein distributions and dynamics in live cells using high-resolution bio-imaging methods and biophysical techniques can reveal information about the real-time activities of cell signaling networks. Here, we describe the use of quantum dot labels to track the dynamics of membrane receptors and correlate these dynamics to signaling state.**

***Keywords* — IgE Receptor, FcεRI, quantum dots, single particle tracking**

I. Imaging Protein Dynamics

THE responses of a cell to its surrounding environment result largely from the transduction of signals from the outer cell surface to the cytoplasm and nucleus. Strict regulation of signal transduction is crucial for cell survival, differentiation and proliferation. However, many aspects of how the cell maintains spatio-temporal control of signaling pathways remain unclear.

Correlating protein activity with spatial distribution and dynamics is essential for understanding cell function. Single particle tracking (SPT) is a powerful tool for monitoring protein dynamics. Until recently, SPT has been limited to very short times due to the rapid photobleaching of conventional fluorophores or has involved large, multi-valent gold particles that may perturb the system. Semiconductor nanocrystals, or quantum dots (QDs), have emerged as new tools in cellular imaging, providing the photostability and high brightness needed for long-term and single particle tracking. Another advantage of QDs is their wide excitation but very narrow emission bands, which permit simultaneous excitation of different colored QDs with relatively easy discrimination between their emissions, such that multiple color labeling can be achieved.

II. FcεRI Dynamics

The high affinity IgE receptor, FcεRI, is the principal multi-subunit immunoreceptor on the surface of mast cells and basophils. These receptors bind circulating IgE with high affinity and are activated when multivalent allergen crosslinks IgE-bound receptors. Crosslinking initiates a complex signaling pathway that ultimately leads to degranulation and release of key mediators of allergic inflammation. To study the dynamic events that induce FcεRI signaling, we generated two novel quantum dot probes for SPT: monovalent QD-IgE that binds FcεRI without crosslinking and multivalent DNP-QD that mimics allergen by crosslinking DNP-specific IgE.

Previously, we provided direct evidence that actin filaments "corral" resting receptor motion and are involved in receptor immobilization [1]. Simultaneous imaging of single QD-IgE-FcεRI complexes and GFP-tagged actin revealed that membrane-proximal actin bundles form a dynamic labyrinth that restricts receptor diffusion. Real-time imaging revealed that receptors become immobilized within seconds of crosslinking by high doses (1 μg/ml) of multivalent antigen. Interestingly, disruption of the actin cytoskeleton results in delayed immobilization kinetics and increases the diffusion of crosslinked aggregates. These results implicate actin in membrane partitioning that not only restricts diffusion of membrane proteins, but also dynamically influences their long-range mobility and response to ligand binding.

The influence of actin on receptor immobilization prompted us to examine further the relationship between dynamics and signaling [2]. We find that the degree of immobilization is also dependent on antigen dose. In fact, receptors remain mobile at antigen doses corresponding to maximal degranulation (0.001-0.01 μg/ml), suggesting that small, mobile receptor aggregates are signaling competent. Using a novel hyperspectral microscope with ~1 nm spectral resolution, we tracked up to five spectrally distinct QDs simultaneously. Multi-color tracking of QD-IgE-FcεRI complexes revealed that small crosslinked clusters (2-4 receptors) remain mobile under activation conditions. In addition, DNP-QDs were shown to induce degranulation, yet DNP-QD-crosslinked receptors continue to diffuse. We found that immobilization is not dependent on receptor phosphorylation, but is correlated with increases in crosslink-induced aggregate size and receptor internalization. These results indicate that receptor immobilization is a result of highly cross-linked aggregates and is a trigger for receptor internalization.

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