Investigating FcεRI Complex Stability using Fluorogen Activating Peptides

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***Short Abstract* — Regulation of signal transduction from the cell surface to the cytoplasm and nucleus is crucial for cell survival. Signaling across the cell membrane boundary is typically mediated through ligand bound transmembrane receptors whose activation is commonly associated with changes in receptor dynamics. Thus, characterizing the spatio-temporal organization of these receptors is crucial for understanding cell signal transduction. In this study, we investigate the high affinity IgE receptor, FcεRI, a multi-subunit receptor complex involved in initiation of the allergenic response. We follow the integrity of the receptor complex throughout signal transduction using a fluorogen activating peptide tag to observe individual receptor subunit dynamics.**

***Keywords* — FcεRI, IgE, fluorogen activating peptide, single particle tracking, superresolution imaging, RBL cells.**

THE FcεRI is the primary multi-subunit immune receptor on mast cells and basophils, a class of white blood cells central to the allergenic response. Allergen-specific IgE molecules bind to FcεRI on the cell surface. Circulating multi-valent allergen in the bloodstream or tissue crosslinks multiple IgE-bound receptors. This initiates a complex signaling pathway, ultimately resulting in cell degranulation and the release of key mediators of allergic inflammation including histamine[1]. FcεRI is comprised of three distinct subunits: α,β,γ. The α subunit binds IgE and the β,γ subunits contain immunoreceptor tyrosine activation motifs (ITAMs), which are phosphorylated following FcεRI crosslinking and perpetuate signaling through interactions with intracellular adaptor proteins. The stability of the FcεRI complex and how this regulates mast cell response is still not completely understood.

Here, we investigate the stability of the FcεRI complex using a range of fluorescent tools to characterize the behavior of the α- and γ-subunits. We have previously characterized the dynamics of the FcεRI by tracking of fluorescently-labeled IgE bound to FcεRIα[2,3]. To compare behavior of the α- and γ-subunits, we use a fluorogen activating peptide tag to track the FcεRI γ-subunit.

Fluorogen activating peptides (FAPs) are genetically expressible tags that increase the fluorescence excitation cross-section of dye binding partners by up to four orders of magnitude. The binding of FAPs with corresponding fluorogen is characterized by nanomolar affinity, with bound lifetimes of up to ten seconds. FAPs exhibit resistance to photobleaching, with single peptides repeatedly binding and activating new dye molecules[4].

We have generated an FAP-tagged FcεRIγ that binds the Mg2p-NH2 fluorogen and is successfully expressed on the surface of rat basophilic leukemia cells (RBL-2H3).

We use Alexa488-IgE, in conjunction with Mg-2p bound FAP-FcεRIγ, for two-color single particle tracking to observe relative changes in diffusional dynamics of the α- and γ-subunits during crosslinking. The long observation lifetimes for single FAP peptides make the FAP system a convenient, expressible probe for single particle tracking on live cells. We observe similar mobility for the α- and γ-subunits in the resting state, suggesting the subunits diffuse together as an intact receptor complex.

Subunit co-clustering in the presence of multi-valent antigen is also observed. Using a newly developed FAP based superresolution technique, equilibrium localization microscopy (ELM), we will compare FAP-FcεRIγ and Alexa488-IgE clustering at the molecular scale. The intermittency due to the equilibrium of fluorogen binding and unbinding can be used for localization-based superresolution and serves as the basis for this technique. A multi-fluorophore fitting algorithm is used to identify individual point emitters within the densely labeled image field.

We quantify co-internalization of the subunits after activation via flow cytometry to determine if both FcεRIα- and γ-subunits internalize as a mechanism for signal termination.

In summary, this study applies FAP technology to observe localization and dynamics of the FcεRI subunits throughout signal transduction to determine how receptor complex stability influences cellular signaling.

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