Measuring Membrane Receptor Interactions Using Single Quantum Dot Tracking

Diane S. Lidke[1], Shalini T. Low-Nam[1], Patrick J. Cutler[1], Mara Steinkamp[1], Meghan McCabe[2], Jeremy Edwards[2], Bridget S. Wilson[1], and Keith A. Lidke[3]

***Short Abstract* — Imaging technologies and biological tools have developed to a point where many fundamental biological questions can now be addressed at the molecular level. In particular, single particle tracking (SPT) using bright and photostable quantum dots (QDs) provides information about protein dynamics with high spatial (~10 nm) and temporal (>30 Hz) resolution. Using these techniques, we have examined the interaction dynamics of the erbB receptors on living cells, revealing new insights into the roles of receptor dynamics and membrane partitioning in the regulation of receptor interactions.**

***Keywords* — EGFR, erbB, 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 [1, 2]. 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. ErbBI Dimerization

Ligand-induced signaling by the epidermal growth factor receptor (erbB) family drives cell growth and survival, with roles in normal development and disease pathogenesis. A wealth of structural knowledge supports a model of signal initiation through the formation of back-to-back erbB family homo and heterodimers. However, the specificity, ligand-occupancy status and lifetime of the dimerized signaling complexes remain controversial.

We used two-color QD tracking to directly visualize erbB family homo and hetero-dimerization on living cells. Our initial focus was on erbB1 (EGFR) homodimers, where kinetic parameters were extracted using a 3-state Hidden Markov Model to identify transition rates between free, co-confined, and dimerized. We report that erbB1 homodimers composed of two ligand-bound receptors are long-lived and their off rate (k_{off}) is independent of kinase activity. By comparison, unliganded erbB1 homodimers have >4-fold faster off rates. These results unequivocally link ligand occupancy to dimer stability. Transient co-confinement of receptors is shown to promote repeated encounters, enhancing dimer formation. Mobility decreases when ligand-bound receptors form dimers. Inhibition of erbB1 kinase activity with PD153035, or disruption of actin networks with Latrunculin B, results in faster diffusion of receptor dimers. These results implicate both signal propagation and the cortical cytoskeleton in reduced mobility of signaling-competent erbB1 dimers.

Our next focus was on two additional erbB family members, erbB2 and erbB3. We demonstrate that the lifetimes of erbB2/erbB2 homodimers and erbB2/erbB3 heterodimers are remarkably short, compared to long-lived erbB3 homodimers. The impact of the differential lifetimes of erbB complexes is explored using agent-based stochastic models, where contributions of relative erbB receptor density and expression can be considered.

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[1]Dept of Pathology and Cancer Research and Treatment Center, [2]Depts of Molecular Genetics/MicroBiology and Mechanical Engineering, and [3]Dept of Physics and Astronomy, Univ of New Mexico, Albuquerque, NM. E-mail: dlidke@salud.unm.edu

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