

CP9: Protein motion in Cancer Signaling: Matthew Coleman, LLNL/UC Davis Med School

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Associated TRDs: TRD1, TRD4

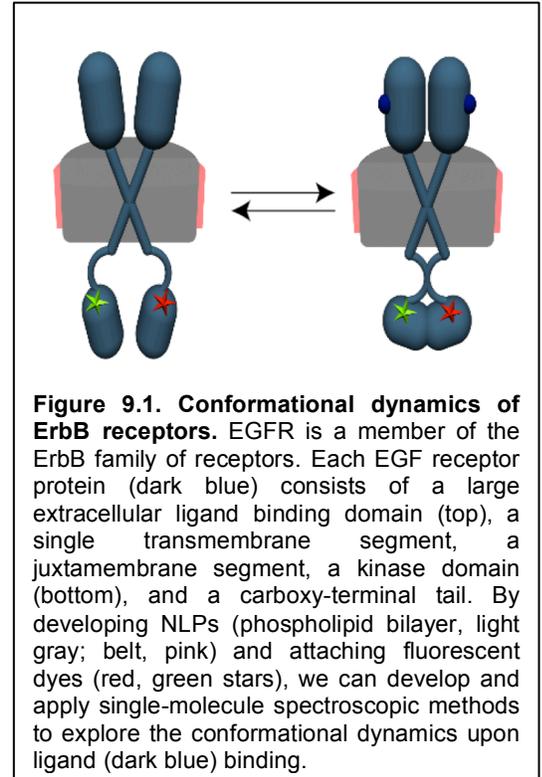
Significance: We propose to develop membrane systems and spectroscopic tools to study the mechanism behind receptor function. The four members of the ErbB family of mammalian receptor tyrosine kinases promote cellular proliferation, survival, transformation, invasion and migration. Overexpression has been observed in tumors and they are associated with worsened prognosis (1-3). The extracellular domain is a target for therapeutics, yet often resistance to treatments develops (4, 5). This suggests more specific and efficient methods are required to target the receptors, requiring a mechanistic understanding of their function.

Studying the biochemical and structural changes upon activation of the ErbB family of receptor is an important focus of Dr. Coleman's laboratory (6). In ErbB activation, dimerization and ligand binding in the extracellular domain induces conformational changes in the intracellular kinase domain (7, 8). However, the structure and dynamics of these changes remain poorly understood. A major barrier to developing a mechanistic picture has been the inability to perform *in vitro* and high-resolution studies on the membrane-bound receptor without a background of extraneous cellular processes. Here, we overcome this barrier using a mixed biochemical (TRD4) and spectroscopic (TRD1) approach.

Approach: Studies of the mechanism behind receptor protein function require a near native environment to access the intrinsic behavior. Dr. Coleman's laboratory has developed novel nanolipoprotein particles (NLPs) that allow reconstitution of membrane proteins into a near native phospholipid bilayer with an amphiphilic belt (9). The LBRC will adopt this platform and will attempt to increase the membrane area two-fold and add easily functionalizable handles. Under native conditions, multiple proteins are inserted within the membrane and at least a dimer is required for activation (8). By extending this technology, multiple proteins can be inserted, replicating native conditions. Dr. Coleman has pioneered an approach to integrate polymer into the belt, improving the size tunability. By synthesizing a range of polymers (dendrimers), the membrane area and size homogeneity will be increased. Furthermore, the dendrimers will be functionalized to enable attachment of a quantum dot as an additional, spectrally separate fluorescent reporter. The quantum dot will monitor motion of the protein structure relative to the membrane.

In addition, Dr. Coleman requires methods that monitor the receptor conformational changes, and the dynamics of these changes, in a physiological environment. To address this need, the LBRC will use single-molecule Förster Resonance Energy Transfer (smFRET) and develop new single-molecule methods (TRD1) to monitor the protein-protein association and large-scale conformational motions during the signaling process. Spectroscopic reporters will be attached to key points on the protein structure, as illustrated in Fig. 9.1. These experiments will read out the spatiotemporal dynamics, enabling an understanding of the receptor activation and drug interactions, which will, in turn, facilitate the development of targeted cancer therapeutics.

Push-Pull Relationship: The LBRC will **push** smFRET to monitor the conformational dynamics of the ErbB receptors. The Coleman lab will **pull** us towards increasing the temporal and spatial resolution of smFRET because of the wide range of dynamics exhibited by the proteins (TRD1.3). Upon resolution of a broader range of temporal and spatial dynamics, we further envision that the Coleman lab will **pull** us towards adding a third spectroscopic channel. The LBRC will **push** our model membrane systems to enable *in vitro* studies of the receptors. This Coleman lab **pulls** the LBRC to increase the size of the membrane area to enable exploration of the mechanisms behind dimerization. With the development of improved, robust NLPs, we will **push** the project to explore the role of the plasma membrane (10, 11), addressing questions such as the role of lipid composition.



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