

# EGF Receptor in the Lime Light

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CELL surface EGF receptor exists in monomeric, dimeric and higher order forms. Understanding which form(s) are active or inactive is an important aspect of the regulation of signal transduction and antagonist design. For many years it has been believed that receptor activation occurs via a monomer:dimer transition which is associated with a conformational change which activates the receptor kinase. An alternative model, the rotational twist model, has been proposed based on the observation that a significant fraction of receptors are in a ligand-free dimerized state. However, little is known about the quaternary state of the EGF receptor at normal levels of expression (<105 receptors/cell), in the absence of other EGFR family members and in the absence of ligands. We have employed multi-dimensional microscopy techniques to gain insight into the state of association of the human EGFR, in the absence and presence of ligand, on the surface of intact BaF/3 cells (50,000 receptors/cell). Image correlation microscopy of an EGFR-enhanced Green Fluorescent Protein (eGFP) chimera was used to establish that the average degree of aggregation on the sub-micron scale is 2.2 receptors/cluster. In the presence of ligand the degree of aggregation increase to 3.7 receptors/cluster. Energy transfer between mixtures of FITC-EGF and Alexa555-EGF on the surface of EGFR-Baf/3 cells can be detected using fluorescence lifetime imaging microscopy (FLIM). By varying the donor:acceptor labeling ratio it is possible to gain insight into the spatial disposition of EGFR ligand binding sites on the nanometre scale. This microscopy data can be related to the 3D structure of the ligated EGFR extracellular domain. In the context of a monomer-dimer-oligomer model, our biophysical measurements are consistent with a population of ligated EGFR tetramers comprising two dimers juxtaposed in a side-by-side arrangement. We have explored the relationship between association state and EGF receptor kinase phosphorylation (activation) using a novel hybrid microscopic method. Contrary to the prevailing view that the EGFR dimer is the predominant, active form, our data determine that higher-order EGFR oligomers are the dominant species associated with the ligand activated EGFR tyrosine kinase.