NUMBER AND BRIGHTNESS ANALYSIS TO INVESTIGATE EPIDERMAL GROWTH FACTOR RECEPTOR OLIGOMERIZATION IN LIVE CELLS

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The epidermal growth factor receptor (EGFR) is a transmembrane protein, a member of the ErbB family of receptor tyrosine kinases. In the traditional model of EGFR activation, the receptor exists in an inactive monomeric form and is activated by the extracellular domain binding of its ligand, the epidermal growth factor (EGF). The receptor adopts an open configuration upon ligand binding and two EGFR monomers dimerize back-to-back. Receptor dimerization is responsible for the autophosphorylation of tyrosine residues in the intracellular domain of EGFR, which leads to the initiation of downstream signalling.

But new evidence continues to emerge that indicates the presence of pre-formed EGFR dimers and oligomers in the absence of bound ligand. This suggests that EGFR signalling regulation is more complex than implied by the traditional ligand-dependent model. Because EGFR has been implicated in many cancers due to mutations and dysregulated signalling, it is important to understand EGFR oligomerization and dynamics in the plasma membrane and its role in the regulation of EGFR signalling. This can help elucidate its role in cancer progression and help in the development of new anti-cancer treatments [1,2].

We have investigated EGFR oligomerization in live cells using the number and brightness (N&B) analysis. Using electron-multiplying charge-coupled device (EMCCD) cameras as detectors in total internal reflection fluorescence (TIRF) microscopy, the apparent number of particles and the apparent brightness can be obtained from the average intensity and variance values recorded by the camera. The true number and brightness values can then be calculated from the slope S of the average intensity vs variance plot [3]. The S parameter can be determined by calibrating the camera with two measurement files, one of which is from a probe that is higher in brightness than the other.

In our study we have calibrated the camera using the monomeric and dimeric forms of the fluorescent proteins mEGFP and mApple. Then EGFR tagged with either mEGFP or mApple was measured under the same experimental conditions. By comparing the obtained brightness values of the EGFR files with the calibration files, we could estimate the fraction of EGFR molecules present in monomeric and dimeric forms. The experiments were performed in resting cells and also with EGF stimulation, actin cytoskeleton disruption by latrunculin A (LAT-A), and cholesterol depletion by methyl-β-cyclodextrin (mβCD).

[1] N. Bag, S. Huang and T. Wohland, “Plasma Membrane Organization of Epidermal Growth Factor Receptor in Resting and Ligand-Bound States”, *Biophysical Journal*, **109(9)**, 1925-1936 (2015).

[2] S. Yavas, R. Macháň and T. Wohland, "The Epidermal Growth Factor Receptor Forms Location-Dependent Complexes in Resting Cells", *Biophysical Journal*, **111(10)**, 2241-2254 (2016).

[3] J. R. Unruh and E. Gratton, "Analysis of Molecular Concentration and Brightness from Fluorescence Fluctuation Data with an Electron Multiplied CCD Camera", *Biophysical Journal*, **95(11)**, 5385-5398 (2008).