Probing and perturbing receptor signalling with microscopy, nanotechnology and microfluidics

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Receptors are molecules usually found on the surface of cells that receive chemical signals from outside cells. When a specific extracellular substance, called a ligand, binds to a specific receptor, it ultimately triggers the cell to do something, such a divide, or die, or move. The classic model for receptor activation is ligand-induced dimerisation, wherein single receptor monomers pair-up to form a receptor dimer.

The Epidermal Growth Factor Receptor (EGFR) is a key receptor associated with normal physiological development and functioning. Except for the hair and skin, it is practically inactive in the adult human. When mutated or overproduced, however, it is linked to 30% of cancers. In this pathological setting, the receptor conveys an advantage to the cancer cells probably through sending (uncontrolled) growth and survival signals. Consequently, understanding how the EGFR is activated in physiological and pathological settings is a major goal for the field and has led to a 30 year race to solve the complete structure of the molecule.

Like many membrane proteins, the EGFR is insoluble in water and it is very difficult to crystallize or handle the complete molecule for use with traditional high-resolution methods such as x-ray crystallography or NMR. Fortunately, parts of the receptor that “poke” into the water have been solved by some researchers from around the globe. With these partial structures it is now possible to design experiments to test how the full length molecule might be assembled and activated on the cell surface.

Together with collaborators (Kozer *et al.*, 2014; Kozer *et al.*, 2013; Kozer *et al.*, 2011a; Kozer *et al.*, 2011b), my team has attempted to determine the structure (assembly and conformational states) of the EGFR on living cells using microscopy-based biophysical techniques. Spectroscopy plays a key role here. Forster resonance energy transfer experiments yield insight into structural changes that occur on the 2-10 nanometre scale. While fluorescence fluctuation techniques, using spatial image correlation spectroscopy, reveal the formation of receptor clusters that serve as activation and signalling “hotspots”. By combining different experimental approaches with models of reaction kinetics we can learn how receptor structure and receptor activation are correlated. Taken together our cell biophysics approaches suggest that receptor activation is more complex than previously recognized. In addition to the classic dimer, we obtain evidence for a tetrameric transactivation complex where receptor activation occurs in *trans* from one receptor dimer to another receptor dimer.

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