REAL-TIME G-PROTEIN-COUPLED RECEPTOR IMAGING TO UNDERSTAND AND QUANTIFY RECEPTOR DYNAMICS

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G-protein-coupled receptors (GPCRs) trafficking and its regulation by agonists and antagonists is fundamental to understand their exact physiological function and to develop more effective drugs. Optical methods using fluorescent-tagged receptors are of election to investigate membrane receptor dynamics. However, convenient tools to qualitatively and quantitatively address GPCR dynamics in living cells are still required.

In this report, a method to measure receptor dynamics using in vivo confocal microscopy in living cells is provided. The methodology includes development of tools to correct the photobleaching effect and the subsequent image analysis. Using this strategy it has been possible to calculate the rate at which GPCRs move at the cell surface. Whereas the constitutive lateral diffusion of dopamine D2 receptors in HEK-293 cells was negligible, as early as 70 s after agonist addition lateral movement of the receptors was triggered at the notable rate of 0.083µm/s. The receptor mobile fraction ranged between 13-66%. Internalization of D2 receptors was independent of the rate of lateral diffusion and required coexpression of G protein-coupled receptor kinase 2 (GRK2). The methodology also includes a way to measure internalization by means of a parameter reflecting the disappearance of the receptor from the cell surface. The main advantage of this method to study protein dynamics is the lack of cell perturbation during the time course of the experiment.