Application of Fluorescent Protein Exchange (FPX) on Visualization of G-protein Coupled Receptor Activation

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POSTER

G-protein coupled receptors (GPCRs) are widely expressed in cells and respond to a wide variety of ligands, and are therefore the primary targets of drug design. This experiment was conducted to utilize dimerization dependent fluorescent proteins (ddFPs) and the Fluorescent Protein Exchange (FPX) technology to visualize GPCR activation. The prototypical GPCR β-2-adrenergic receptor (β2AR) and its downstream interacting partner β-Arrestin-2 (βArr2) were chosen for investigation. Two pcDNA vectors, β2AR-ddRFP(A) and βArr2-ddFP(B)-P2A-ddGFP(A), were created, and were used to double-transfect HeLa cells. The transfected cells were then imaged using fluorescence microscopy, both before and after isoproterenol stimulation, to determine fluorescence change. Before adding isoproterenol, there was dim but visible red fluorescence localized to the cellular membrane, and bright cytoplasmic green fluorescence. After adding isoproterenol, membrane red fluorescence starts increasing within minutes, and reaches 250% within 15 minutes, while the cytoplasmic green fluorescence decreased 20%. Taken together, the ratiometric fluorescence change was around 310%. The experiment was successful in detecting β2AR-βArr2 interaction, and the process could be easily monitored by eye. Since GPCRs are structurally and functionally related, the FPX assay could be employed in receptor activation detection of other GPCRs and interacting protein partners. This assay could also be utilized in large scale screening of potential drug candidates.