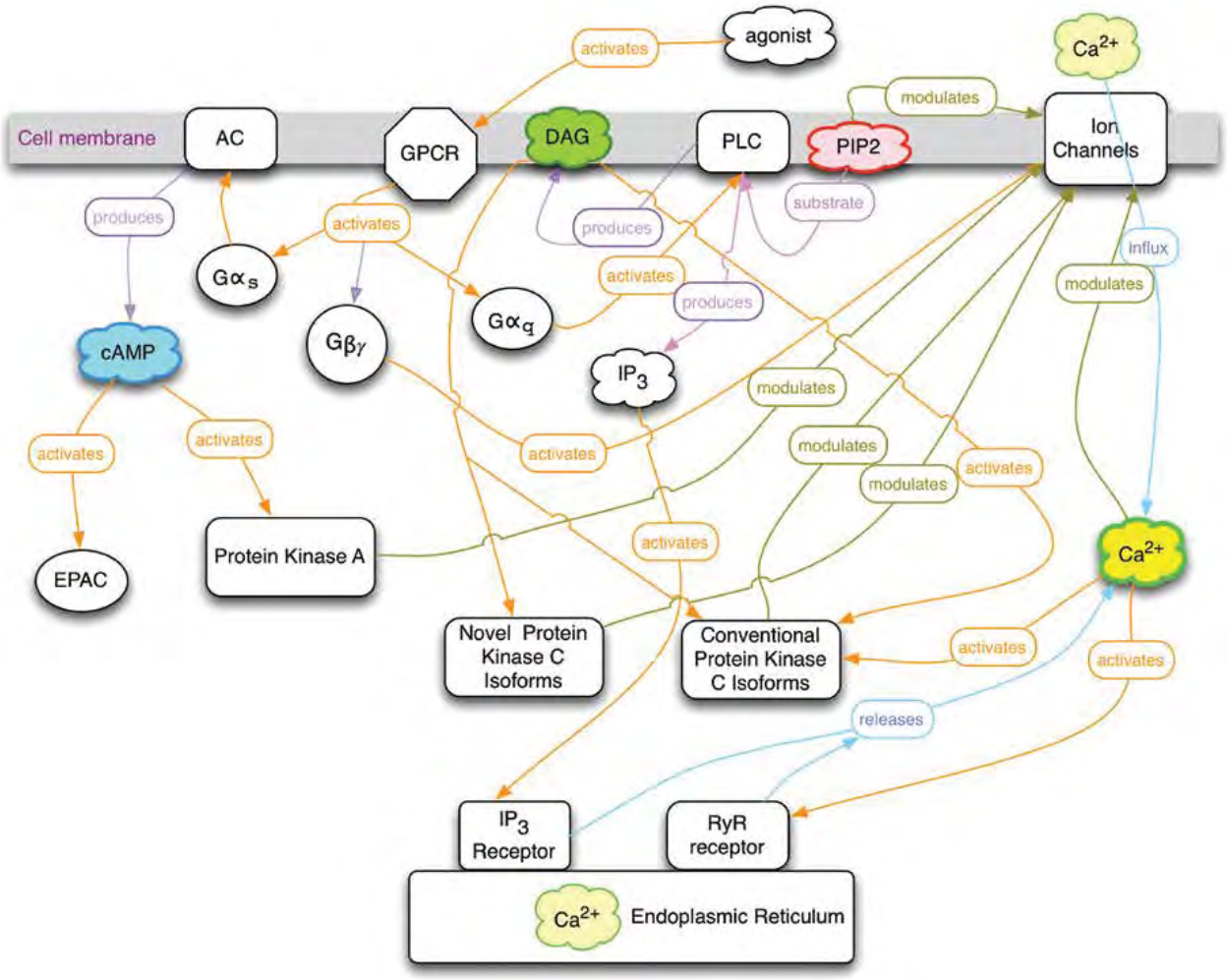
Characterizing GPCR Activation Using Automated Live Cell Imaging

Joe Clayton and Peter Banks
BioTek Instruments, Inc. | Winooski, VT | USA

Introduction

G protein coupled receptor (GPCR)-mediated pathways are critical for cells to respond to intercellular and environmental cues, and are a major focus of drug discovery efforts, particularly for cancer treatment. The molecules that activate GPCRs, and the resulting signaling cascades triggered by associated G proteins, are diverse. Fluorescent dyes and biosensors can be used to monitor changes in second messenger levels, including Ca^{2+} and cyclic AMP (cAMP), in response to GPCR activation. Here we describe a live cell imaging based approach to detect GPCR activation using the Lionheart™ FX Automated Live Cell Imager and Gen5™ Microplate Reader and Imager Software. This method provides a large assay window and improved sensitivity over methods relying on total fluorescence intensity measurements. Dual in-line dispense tips enable addition of GPCR agonists with continuous monitoring of cellular response. Additionally, an image capture rate of up 20 frames per second enables characterization of rapid GPCR kinetics.



Schematic diagram of G-Protein Coupled Receptor (GPCR) Signaling Pathways.