

Featured Publication Note

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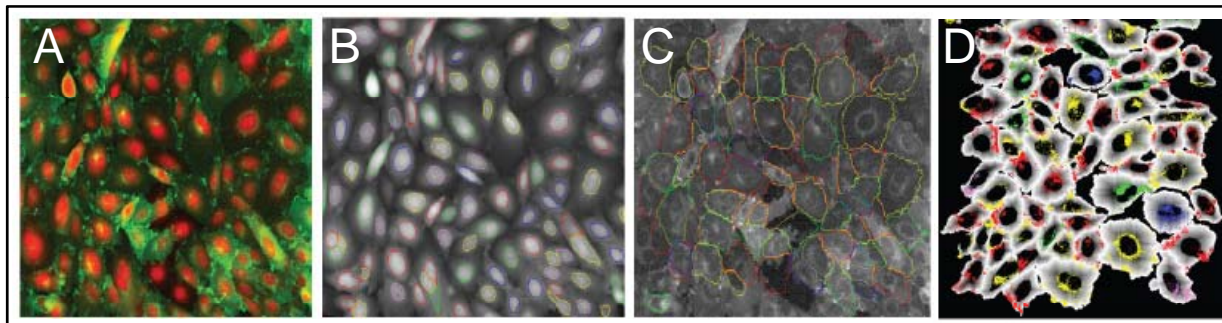
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Studying GPCR Trafficking Using High Content Screening (HCS)

G-protein-coupled receptors (GPCRs) are an important class of drug target. Many GPCRs undergo agonist-induced endocytosis and are directed into either a lysosomal degradation pathway or an endosomal recycling pathway, resulting in differentially regulated GPCR-mediated signaling. There is evidence to suggest that different drugs can evoke different receptor endocytosis and trafficking profiles, which ultimately serve to determine the drug's *in vivo* efficacy.

Investigating GPCR trafficking has traditionally been confined to lower throughput assays. In this study, researchers have developed a flexible HCS platform, which includes the **Opera**[®] HCS system, to measure GPCR internalization and recycling kinetics from a large number of compounds at multiple concentrations. Quantitative analyses were performed using **Acapella**[®] image analysis software.

Figure 1. Acapella Membrane Fluorescence Scoring Algorithm: (A) Composite Image. (B-D) An algorithm measures GPCR-GFP signal on cell membrane by identification of nuclear (B) and cytoplasmic (C) regions. GPCR-GFP signal was scored based on the spot area of the GFP signal and the closeness of GFP signal to cell membrane (D). The closer the GFP signal is to cell membrane, the higher the score. The score was normalized on a per cell basis and the average score from each well was used to calculate the loss (%) of GFP signal from cell membrane to cytoplasm.



CHO cells over-expressing GPCR-GFP were seeded in 384-well plates and GPCR internalization was induced. Internalization was monitored by measuring the translocation of GPCR-EGFP from plasma membrane to cytoplasm. The recycling of receptor back to the cell membrane was then also measured. Images from each plate were analyzed during the acquisition process using a custom membrane fluorescence scoring algorithm in **Acapella** (figure 1). Concentration-response curves obtained from two compounds are shown in figure 2. The different receptor recycling profiles suggest different intracellular trafficking fates of GPCRs stimulated by different compounds.

This novel, integrated approach to drug screening will enable the differentiation of ligands based on their effects on GPCR internalization and trafficking. This will lead to a better understanding of the mode of action of different compounds, and may aid the discovery of more efficacious drugs.

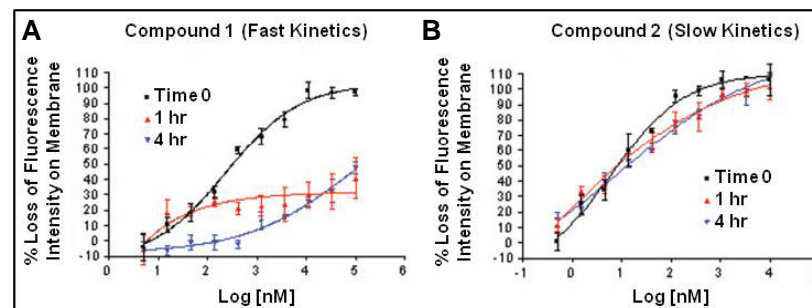


Figure 2. GPCR recycling kinetics: Cells were incubated with compounds at different concentrations, for various durations, to allow for receptor recycling. The figure shows examples of GPCRs displaying fast (A) and slow (B) recycling kinetics.