

Label-free Optical Biosensor for Probing the Roles of Adenylate Cyclases in Signaling Integration of G Protein-Coupled Receptors

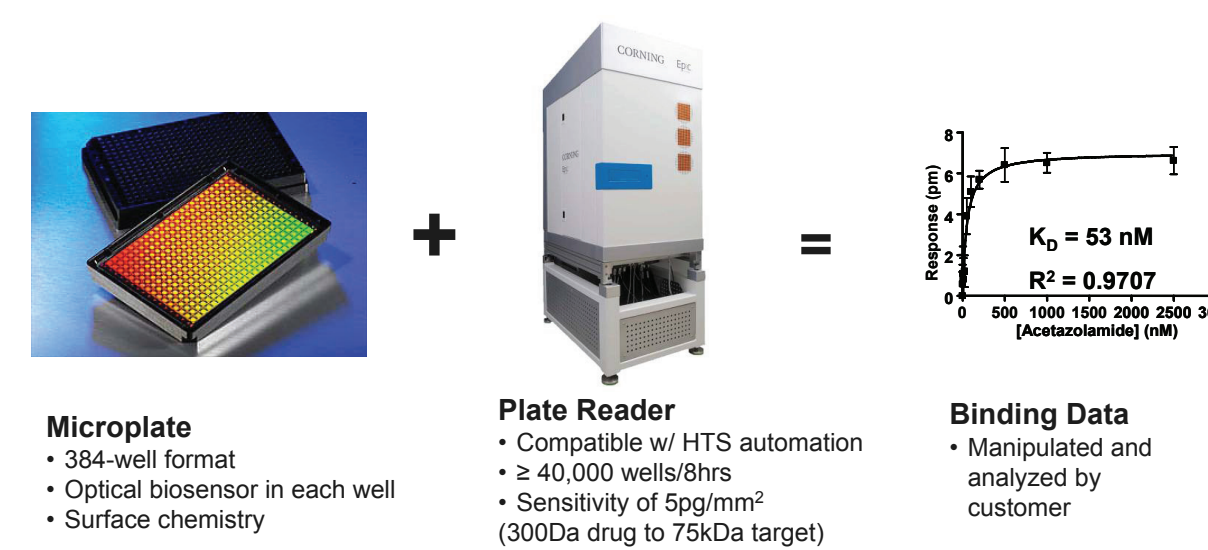
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Abstract

Adenylate cyclases, whose activation produces cyclic AMP in living cells, act as a signaling integrator, due to the ability of different isoforms to receive signals from a wide variety of inputs. Thus, cAMP levels in the cell are important in regulating numerous physiological events, and could serve as an indicator of the balance of signals between many pathways mediated through receptors, including G protein-coupled receptors (GPCRs). Here we applied a label-free and non-invasive optical biosensor to study the signaling integration of endogenous GPCRs at the adenylate cyclase-cAMP-PKA module. This study showed that the pre-activation of adenylate cyclases by forskolin causes the heterologous desensitization of Gs-coupled receptors, but leads to the heterologous sensitization of Gi-coupled receptors. For Gq-coupled receptors, the pre-activation of adenylate cyclases resulted in partial desensitization of the Gq-coupled receptors.

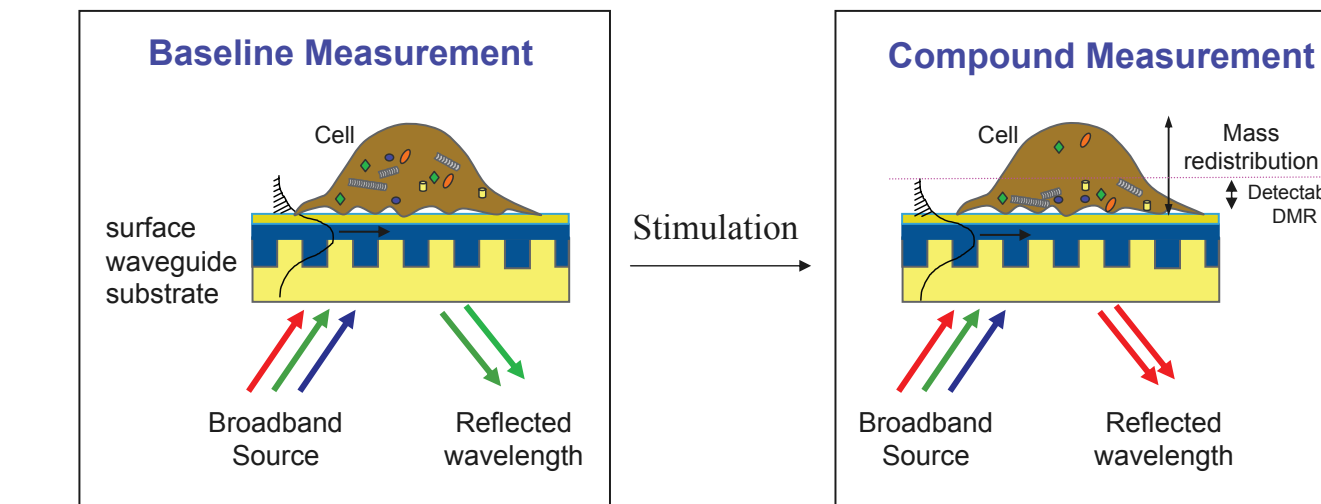
Corning® Epic® System Concept

The Corning Epic System is a high-throughput, label-free detection platform that consists of SBS-standard 384-well microplates with optical sensors inside each well, an HTS-compatible microplate reader and a set of label-independent assay protocols. The Epic System is applicable to both biochemical and cell-based assays, and enables high-throughput screening of "intractable" targets.



Operating Principle: Cell-based Assays

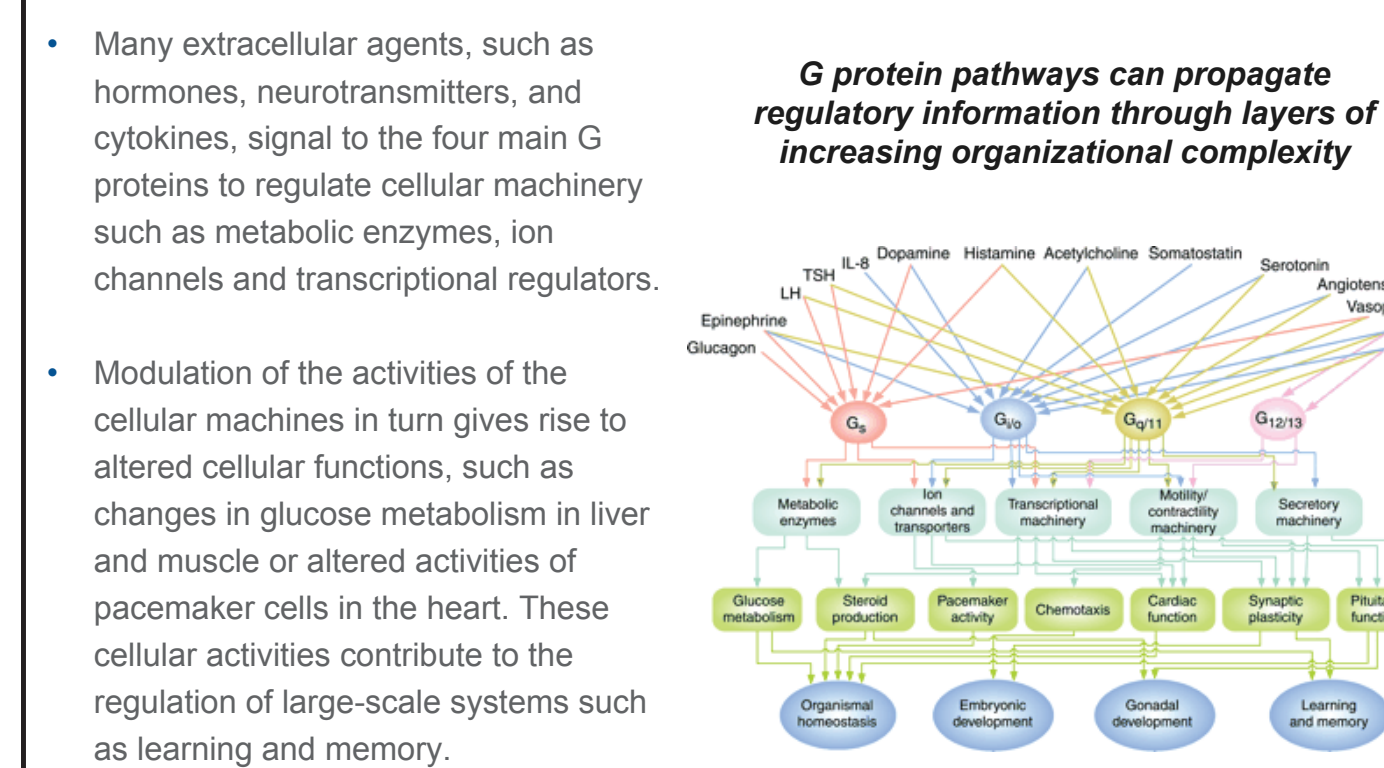
- Measures changes in local index of refraction resulting from the ligand-induced dynamic mass redistribution (DMR) within the bottom region (~150nm) of the cell monolayer.
- Change in index is manifested by a shift in resonant wavelength



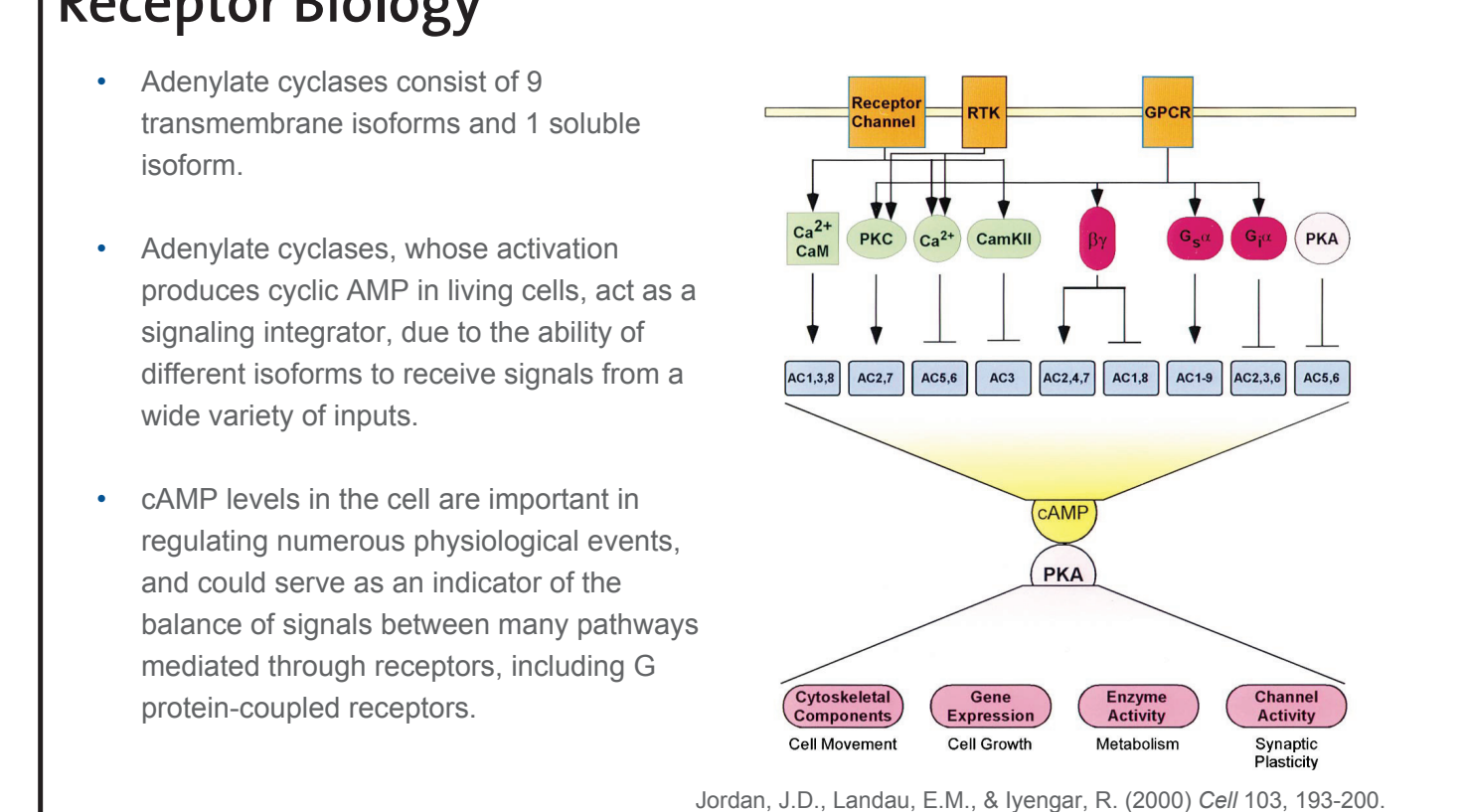
Importance of GPCRs

- GPCRs are "switch proteins"
 - They sense molecules outside a cell and activate signal transduction pathways
 - Signal pathways trigger cell responses
- There are several GPCR pathways
 - G_q, G_s, G_i are most common
 - G_{12/13} is a recently discovered fourth class
- Signaling molecules that bind to GPCRs include hormones, neurotransmitters, growth factors, and cytokines
- 50% of all human medicines target GPCRs directly or indirectly
- GPCR screens account for ~30% of all drug screening markets

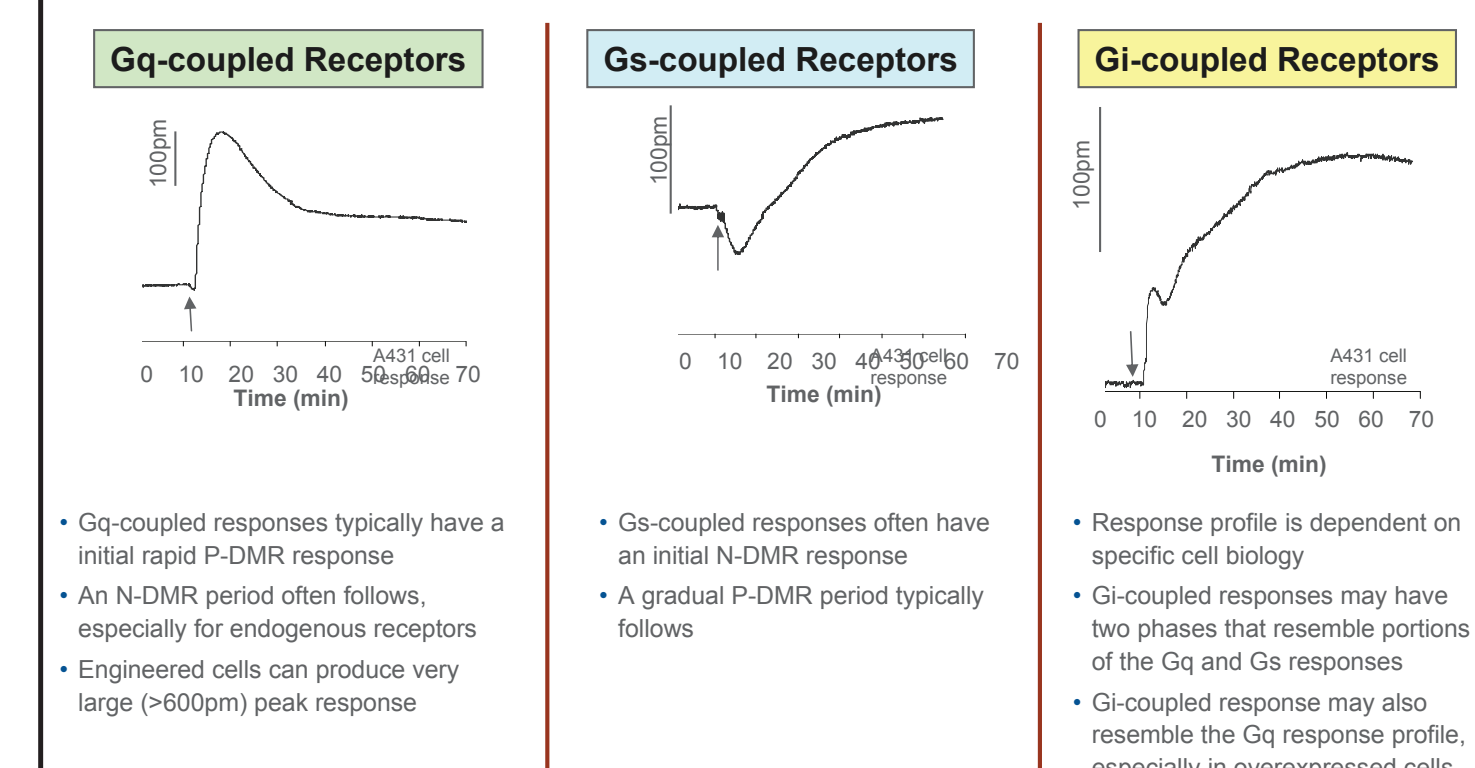
Signaling Networks: The Origin of Cellular Multitasking



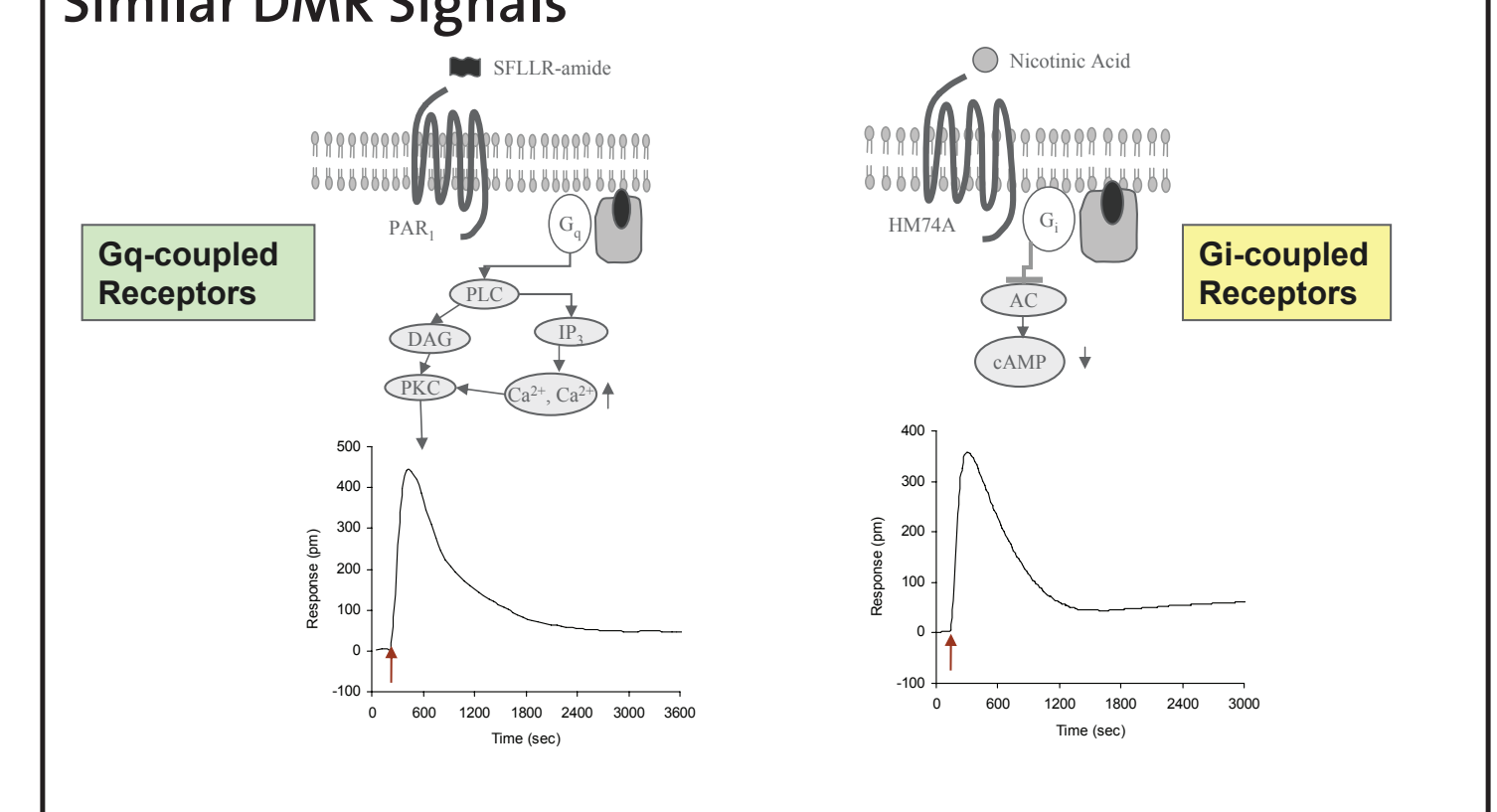
Adenylate Cyclases as a Signaling Integrator for Receptor Biology



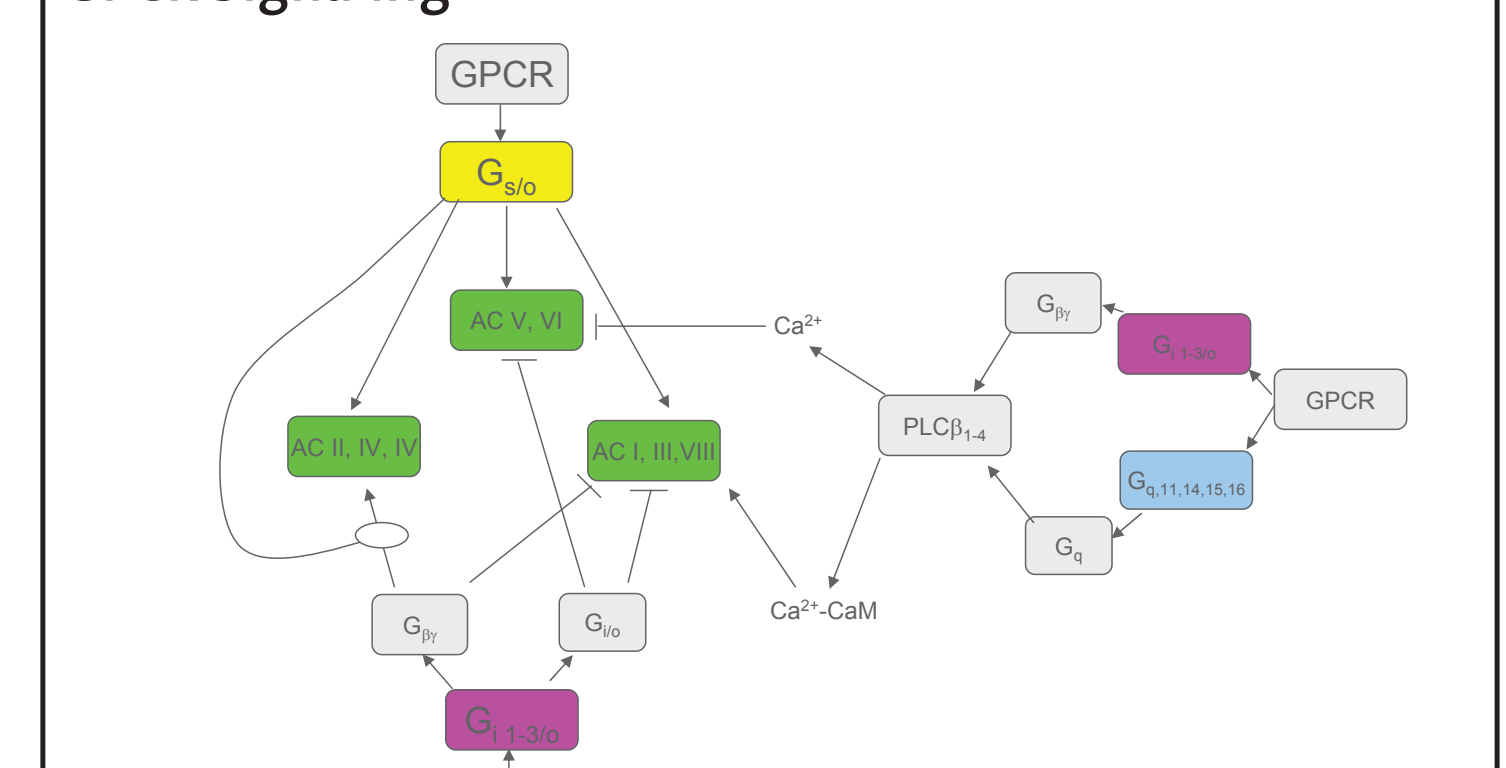
We Have Seen Variations in GPCRs DMR Signals



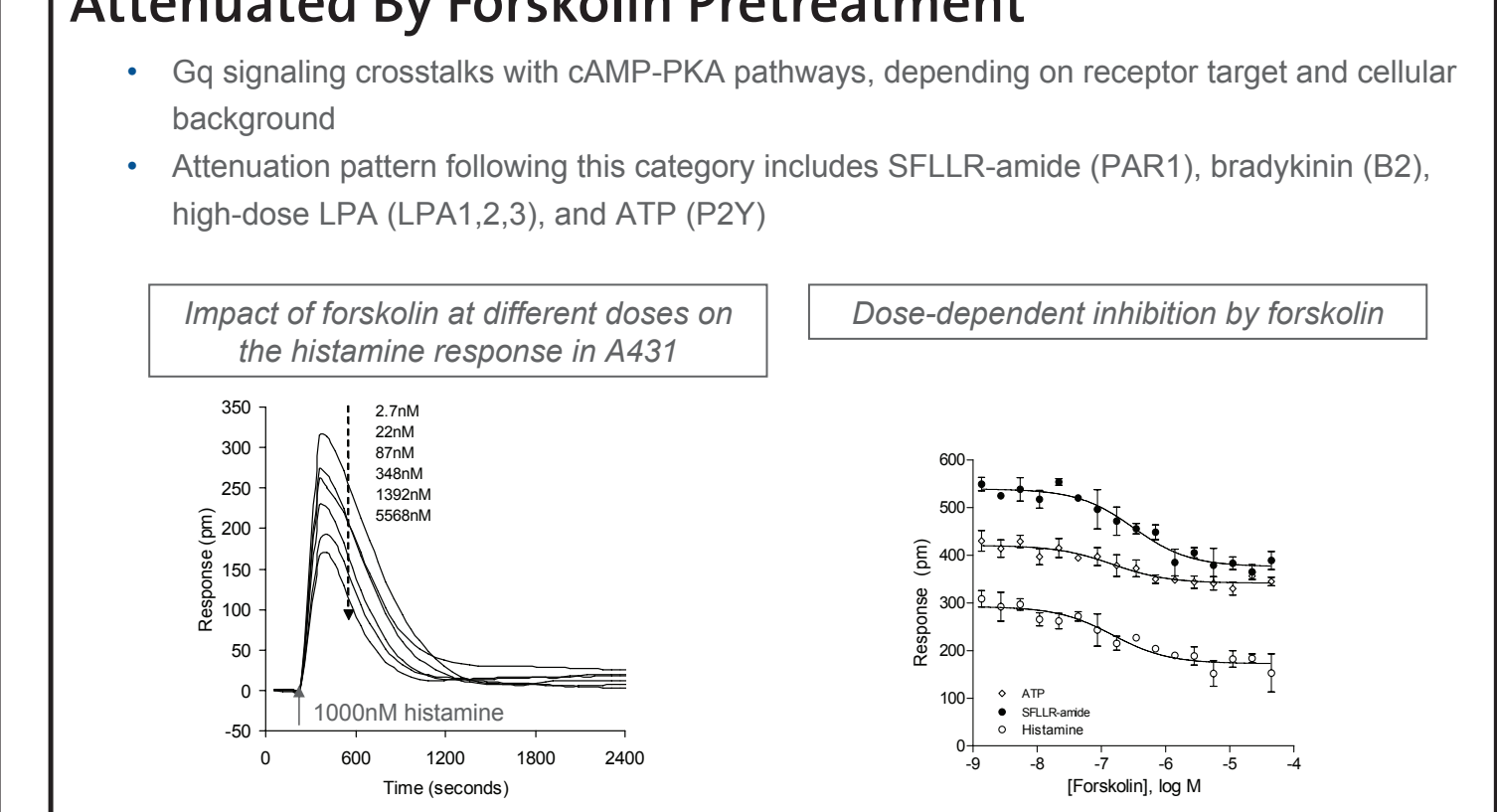
We Realize That Distinct Classes of GPCRs May Lead to Similar DMR Signals



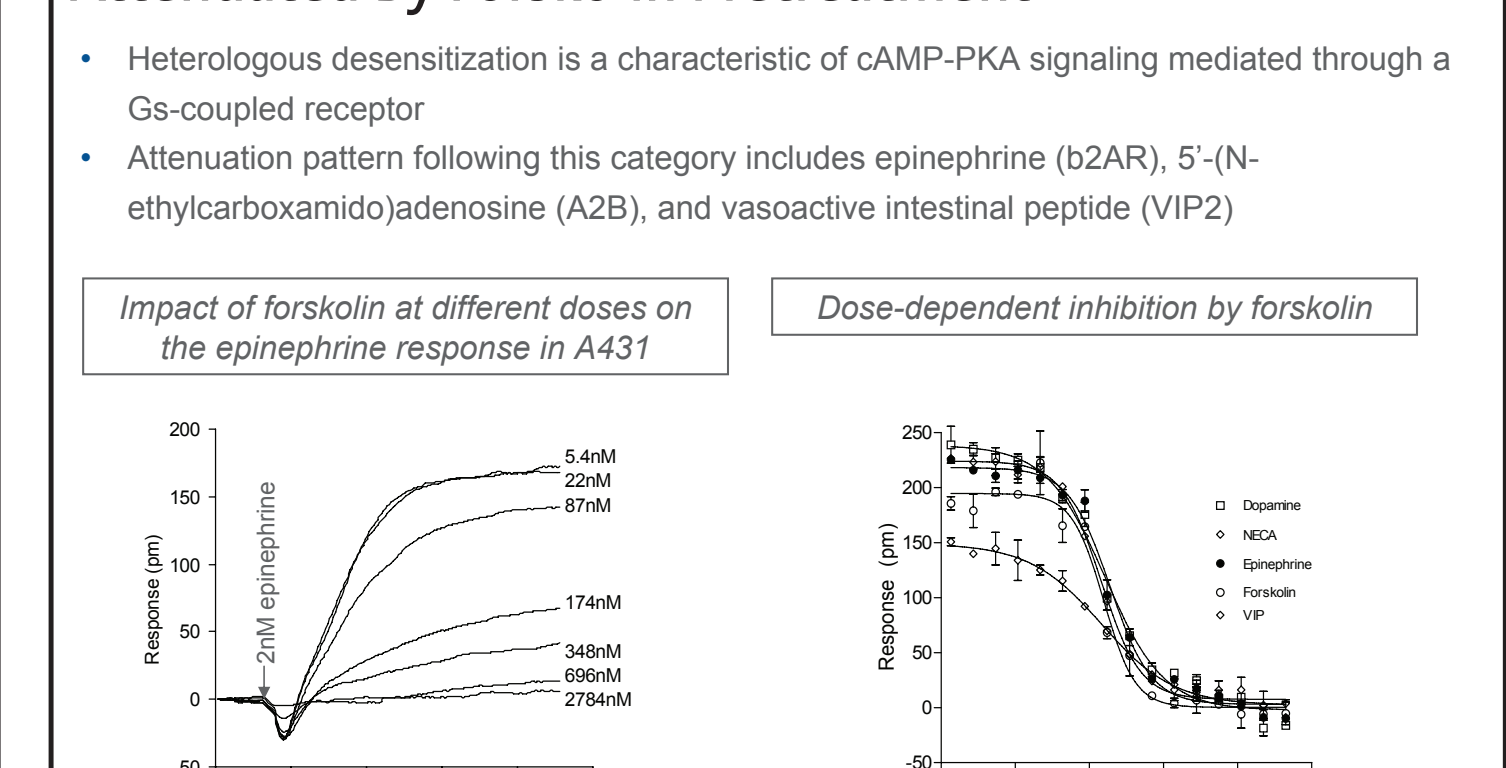
Forskolin as a Chemical Biology Tool to Deconvolute GPCR Signaling



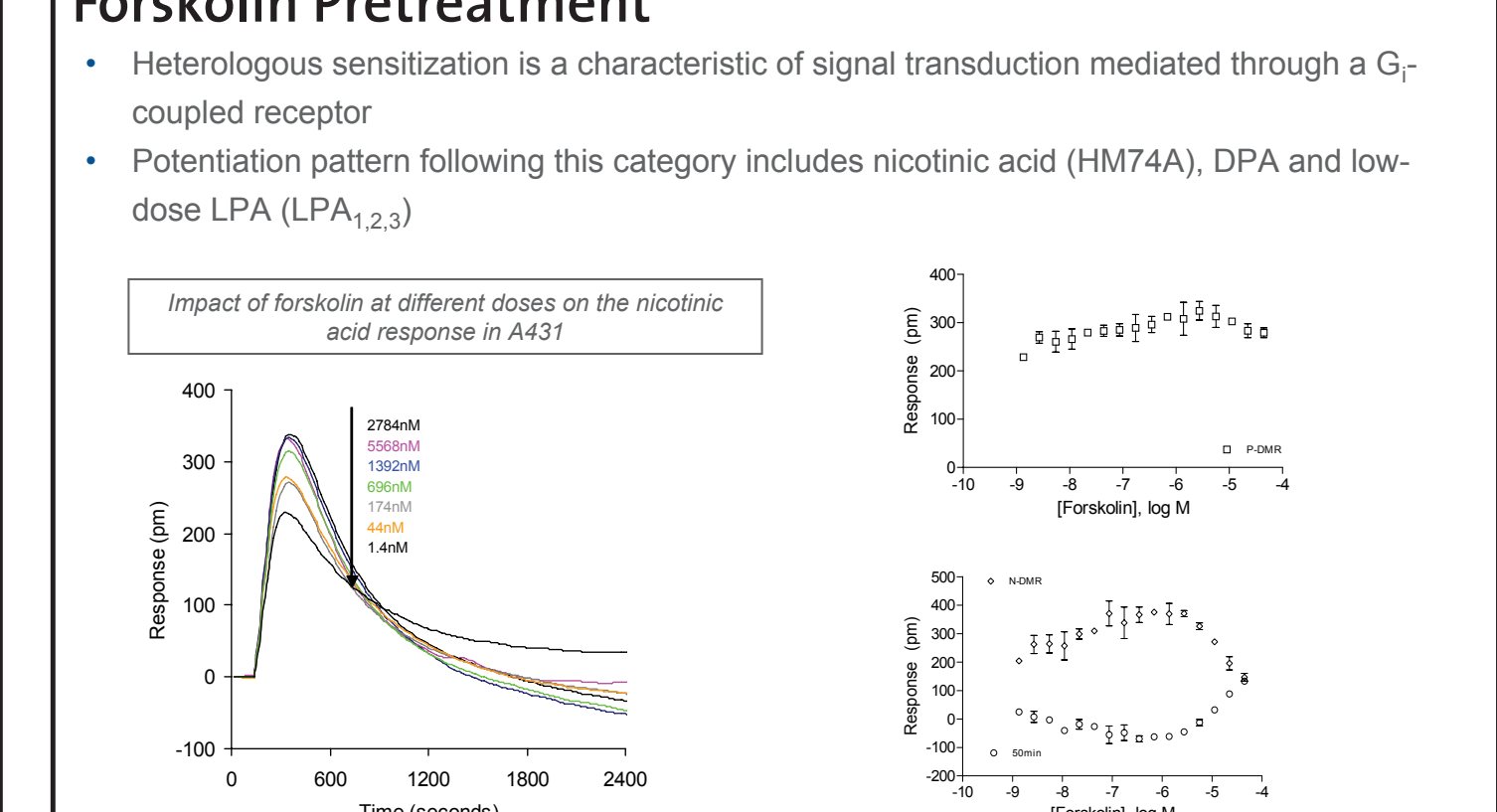
Gq-Coupled Receptor DMR Signal Can Be Partially Attenuated By Forskolol Pretreatment



Gs-Coupled Receptor DMR Signal Can Be Fully Attenuated By Forskolol Pretreatment



Gi-Coupled Receptor DMR Signal Can Be Modulated By Forskolol Pretreatment



Summary

- Adenylate cyclases act as an integrator in GPCR signaling
- Forskolol is an effective tool to deconvolute GPCR signaling pathways
- Forskolol dose-dependently
 - inhibits G_s signaling
 - partially attenuates G_q signaling
 - potentiates G_i signaling
- This simple chemical biology approach provides an additional tool for G-protein coupled receptor biology analysis, along with the dynamics and related parameters defining a DMR signal