

# Label-Free Optical Biosensor for Probing Receptor Biology: Theory, Modeling and Applications

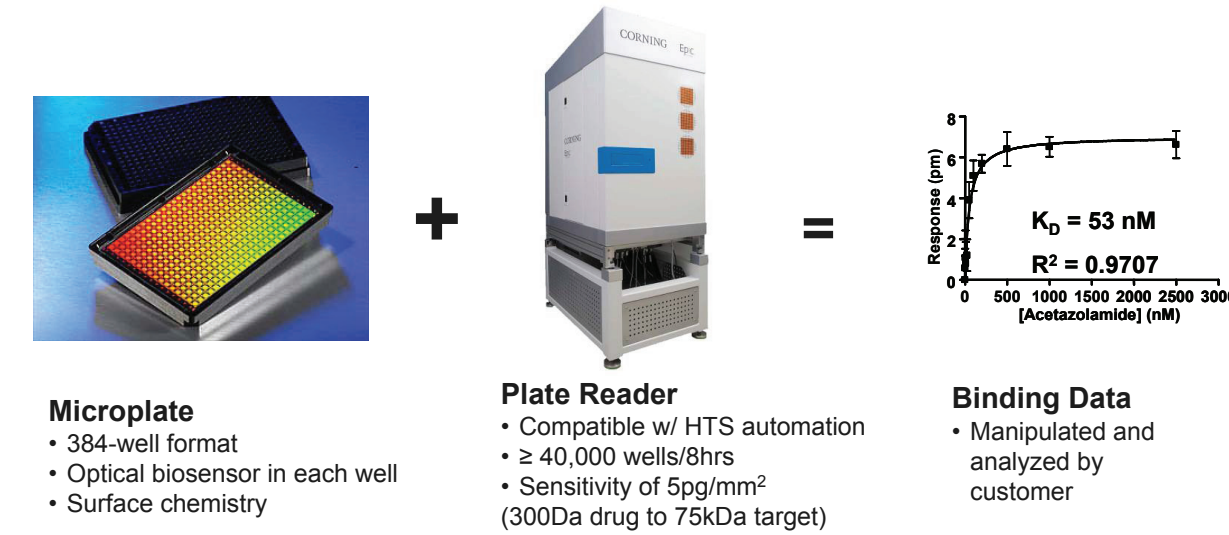
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## Abstract

Label-free optical biosensors have migrated from a tool solely for biomolecular interaction analysis to a universal platform for both biochemical and cell-based assays. This poster presents the theoretical analysis and experimental data for the use of resonant waveguide grating (RWG) biosensors to characterize stimulus-mediated cell responses including receptor signaling. The biosensor is capable of detecting redistribution of cellular contents in both directions that are perpendicular and parallel to the sensor surface. This capability relies on online monitoring cell responses with multiple optical output parameters, including the changes in incident angle and the shape of the resonant peaks. When the changes in the peak shape are mainly contributed to stimulation-modulated inhomogeneous redistribution of cellular contents parallel to the sensor surface, the shift in the incident angle primarily reflects the stimulation-triggered dynamic mass redistribution (DMR) perpendicular to the sensor surface. The optical signatures are obtained and used to characterize several cellular processes, particularly receptor signaling. A mathematical model is developed to link the bradykinin-mediated DMR signals to the dynamic relocation of intracellular proteins and the receptor internalization during B2 receptor signaling cycle. Chemical biology and cell biology analysis provides evidence linking specific cellular events to a ligand-induced DMR signal. Together with recent advancement in instruments for high throughput screening, the early discovered ability of optical biosensors for assaying living cells will accelerate wide adoption of label-free biosensors in both drug discovery processes and fundamental research.

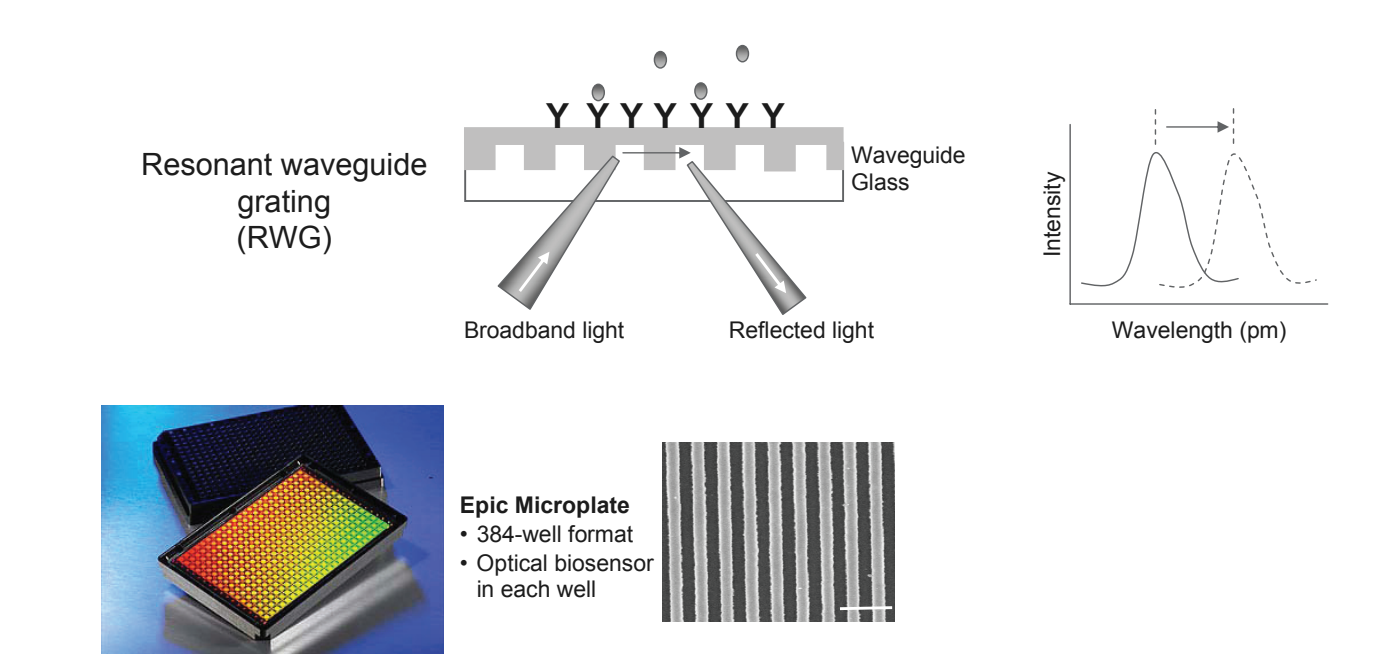
## Corning<sup>®</sup> Epic<sup>®</sup> System Concept

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.



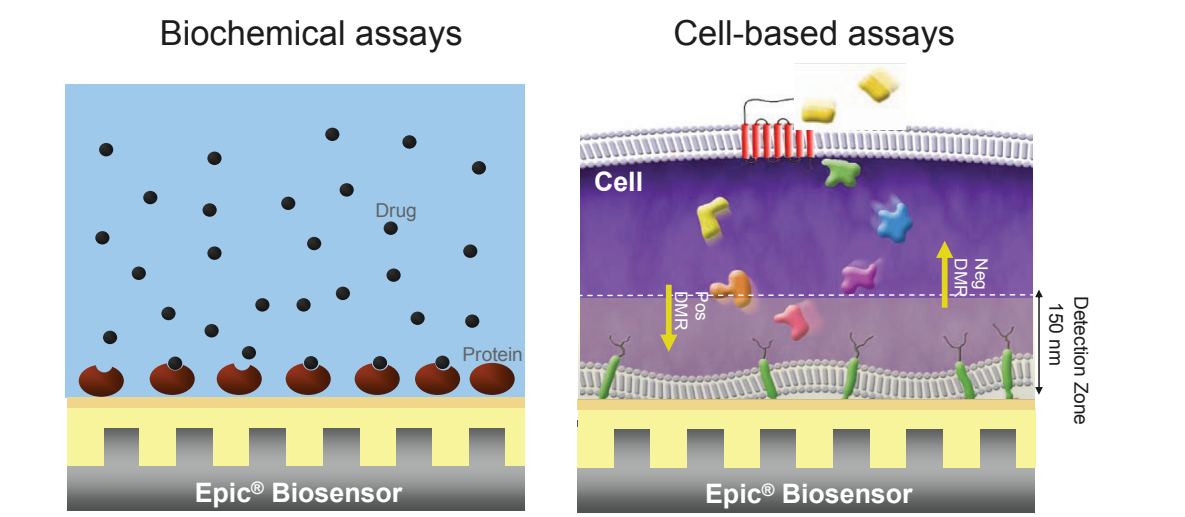
## Corning<sup>®</sup> Epic<sup>®</sup> System is Centered Around Resonant Waveguide Grating (RWG) Biosensor

- An optical biosensor comprise an optical transducer for converting a molecular recognition event or a stimulus-induced cellular response into a quantifiable signal

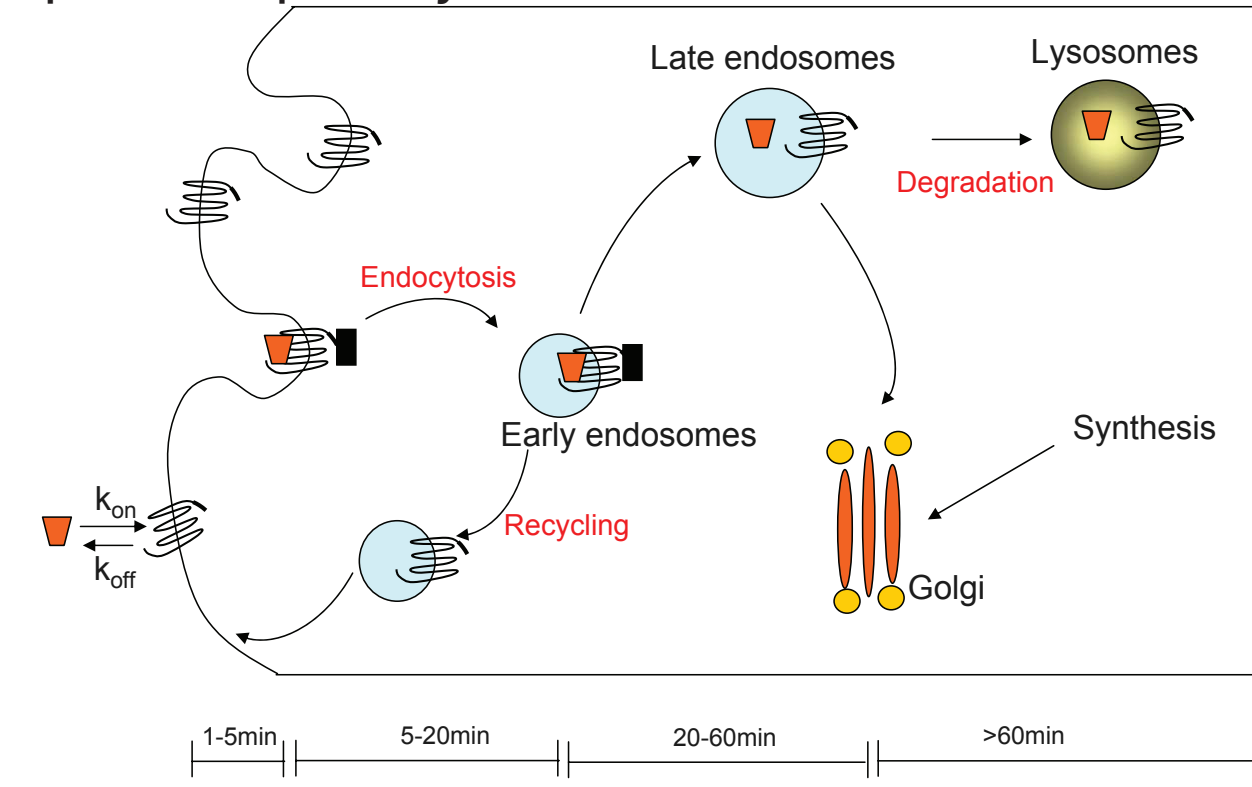


## Corning<sup>®</sup> Epic<sup>®</sup> System is Applicable to Both Biochemical and Cellbased Assays

- Corning<sup>®</sup> Epic<sup>®</sup> system is the first label-free and high throughput biosensor system for both biochemical and cell-based assays

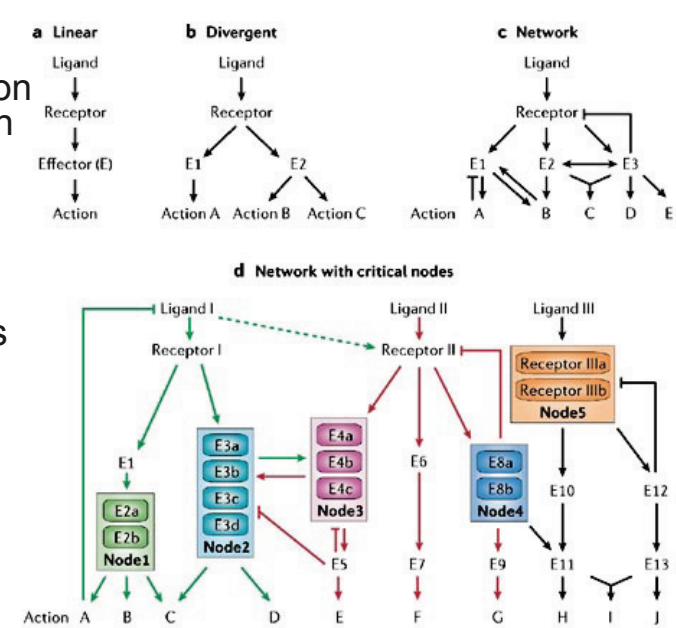


## G Protein-Coupled Receptor Signaling: Temporal and Spatial Dynamics



## Evolution of Receptor Signaling

- Linear signaling cascades
- Network interactions
- Cross-talks
  - Receptor dimerization/oligomerization
  - Transactivation and transinactivation
  - Networks with critical nodes that participate in the crosstalk between the signaling networks
- Signaling compartmentalization
  - Microdomains
  - Organization of signaling complexes
  - Restricted collision and diffusion
  - Spatial and temporal gradients
- Signaling integration
- Phenotypic receptor biology
  - Cellular context-dependent
  - Tissue-specific



## RWG Biosensor Detects Stimulus-Induced Dynamic Mass Redistribution (DMR) Within Cells

The effective refractive index of the sensor system is:

$$N = f_N(n_e, n_s, n_f, n_m, d_f, \lambda, m, \sigma)$$

A stimulus-induced change in the effective refractive index of the sensor system is:

$$\Delta N = f(\Delta n_e, d) = S(N)\Delta n_e$$

The refractive index of a given volume within cells is largely determined by the concentrations of bio-molecules, mainly proteins:

$$n_i = n_o + \alpha C_i$$

The stimulus-induced change in refractive index ( $\Delta n_i$ ) of the homogeneous layer  $i$  approximately form a piece-wise continuous function:

$$\Delta n_i = \alpha \Delta C_i$$

The stimulus-induced change in the effective index of the cell layer is:

$$\Delta n_c = \alpha \sum_i (\Delta C_i \left[ e^{-\frac{z_i}{L}} - e^{-\frac{z_{i+1}}{L}} \right])$$

Epic<sup>®</sup> signal =  $f(C, d, t)$

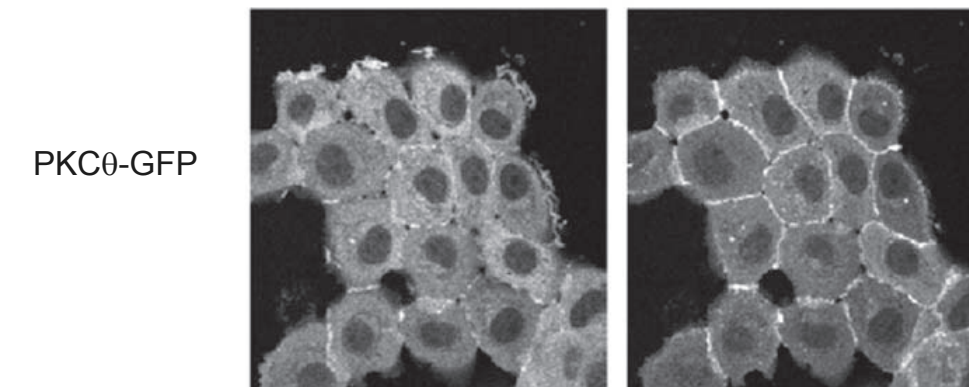
Fang, Y., et al. (2006) *Biophys. J.* 91, 1925-1940

## Dynamic Relocation of Cellular Context in Gq-Coupled Receptor Signaling: Basis for Numerical Modeling

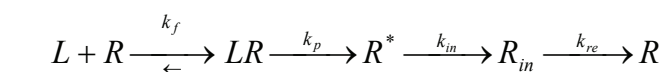
- Common to Gq-coupled receptor is the dynamic relocation of cellular targets:
  - Protein kinase C isoforms
  - GPCR kinases
  - Arrestins
  - PIP-binding proteins
  - Diacylglycerol-binding proteins

Sato, T.K., et al., *Science* 294, 1881-1885.

Bradykinin stimulation causes relocation of PKC $\beta$ -GFP to cell membrane in A431



## Numerical Analysis Predicts an Optical Signal That is Similar to Those Obtained Using RWG Biosensor

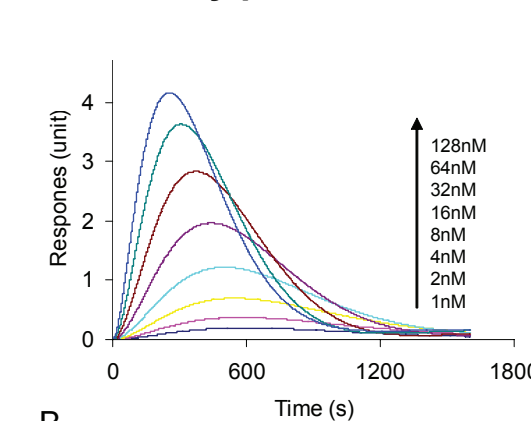


For the net change in protein concentration adjacent to the cell surface:

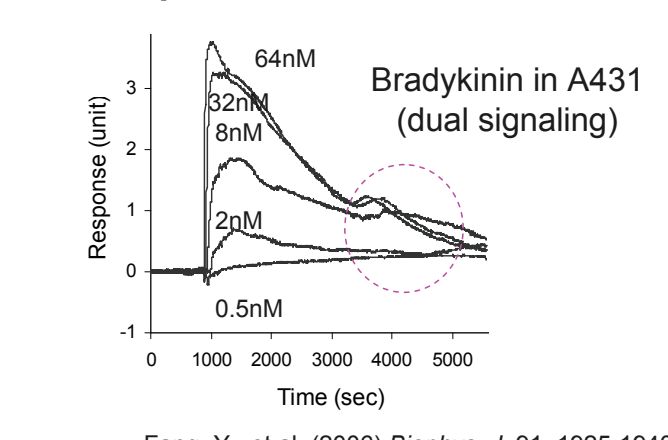
$$\frac{dC_i}{dt} = ak_p[LR] - bk_{in}[R^*]$$

Recruitment Internalization

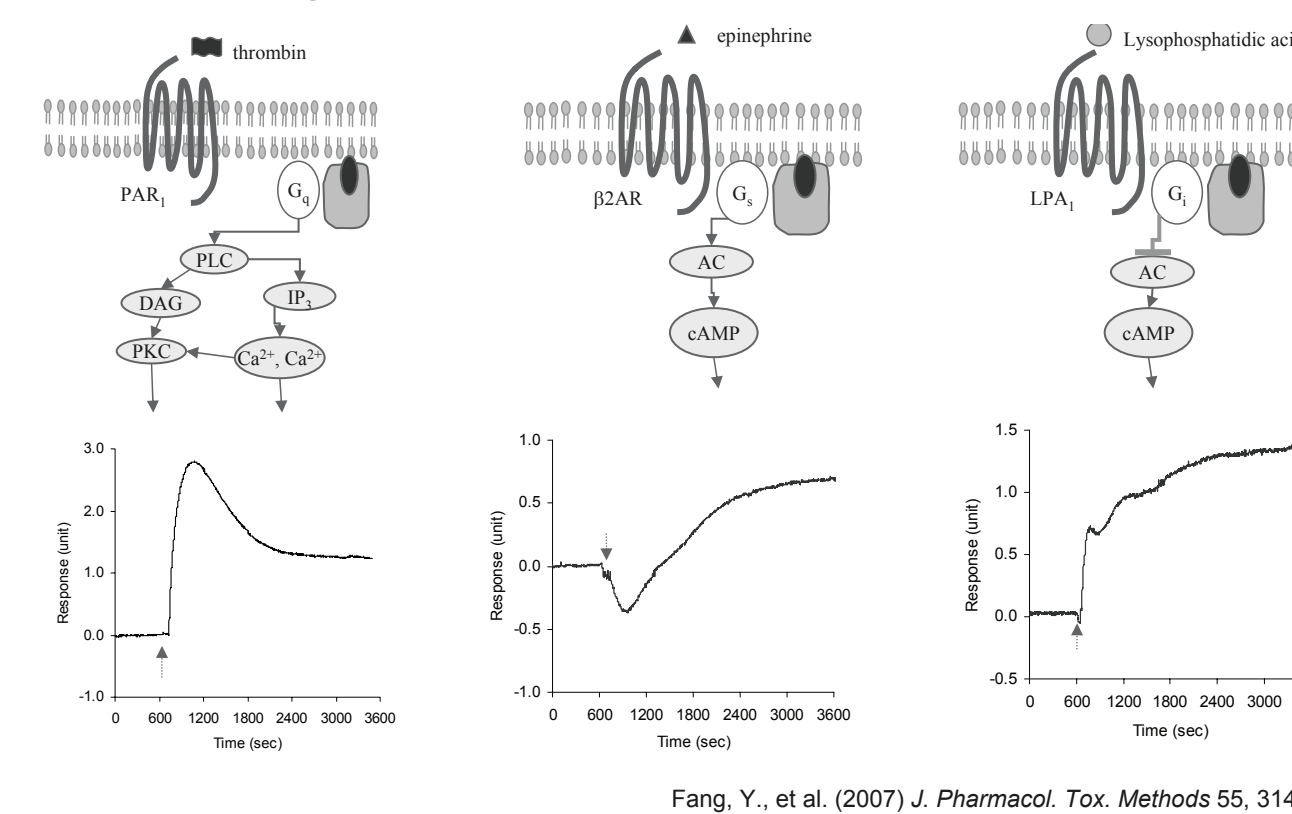
### Theory predicted



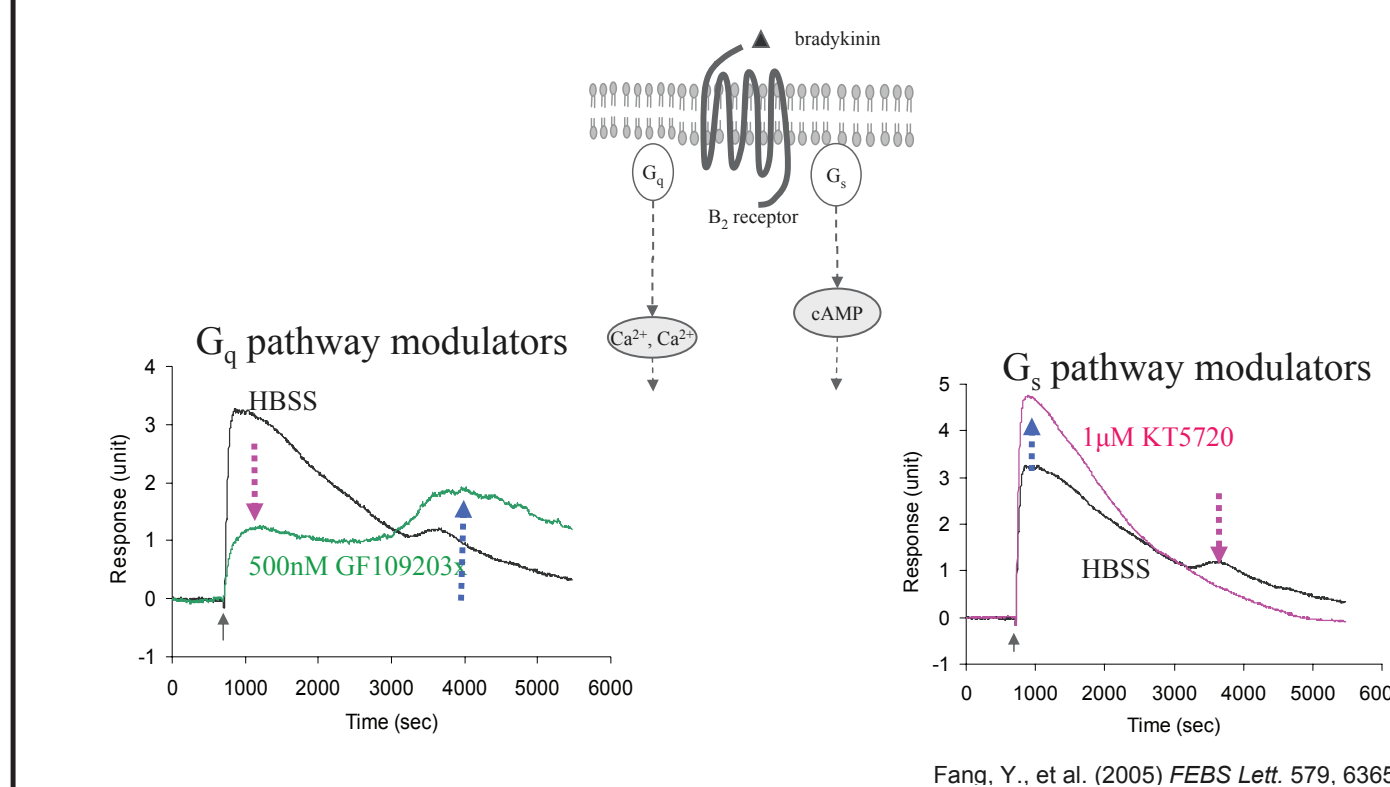
### Experimental results



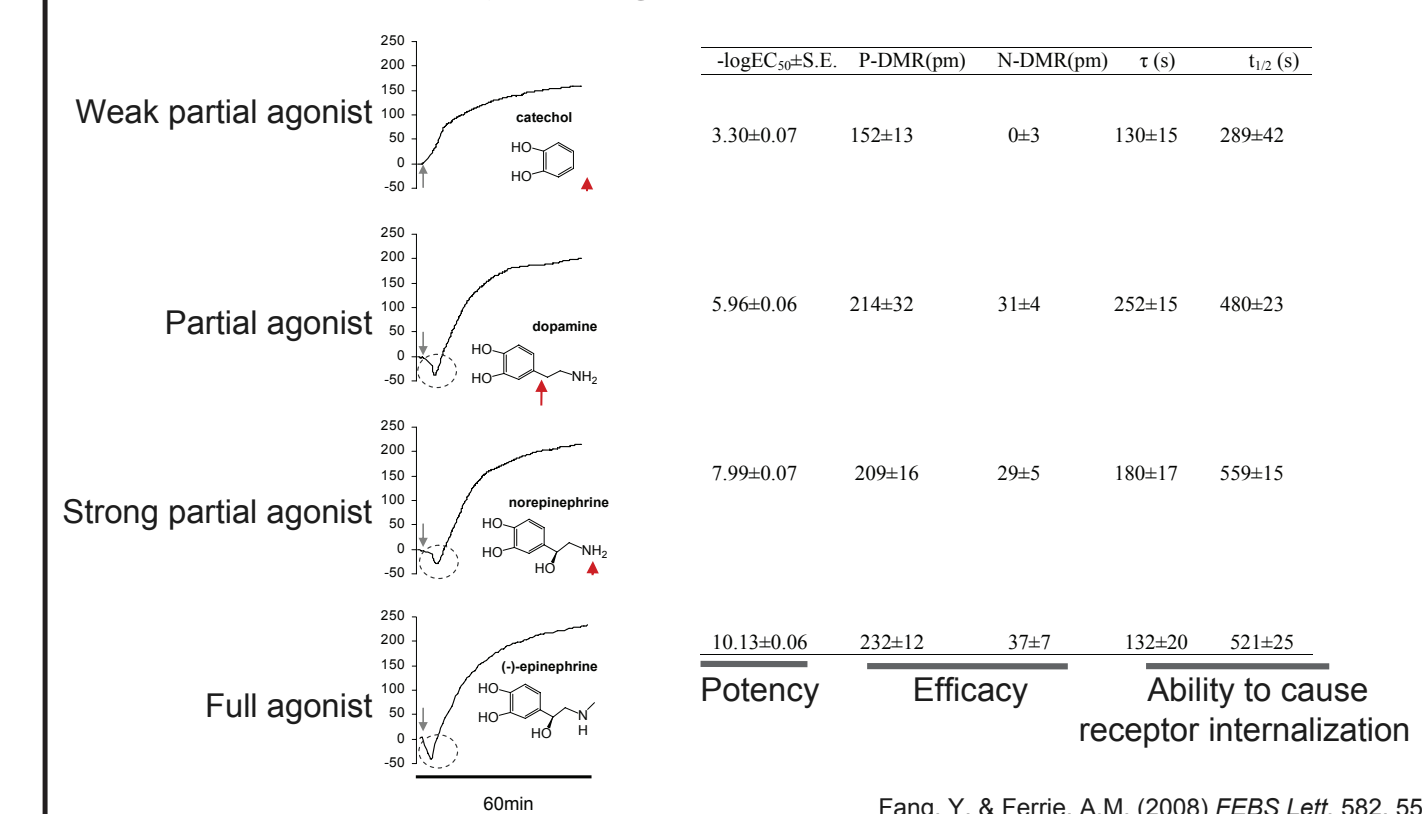
## RWG Biosensor Differentiates Cellular Responses Mediated Through Distinct Classes of GPCRs



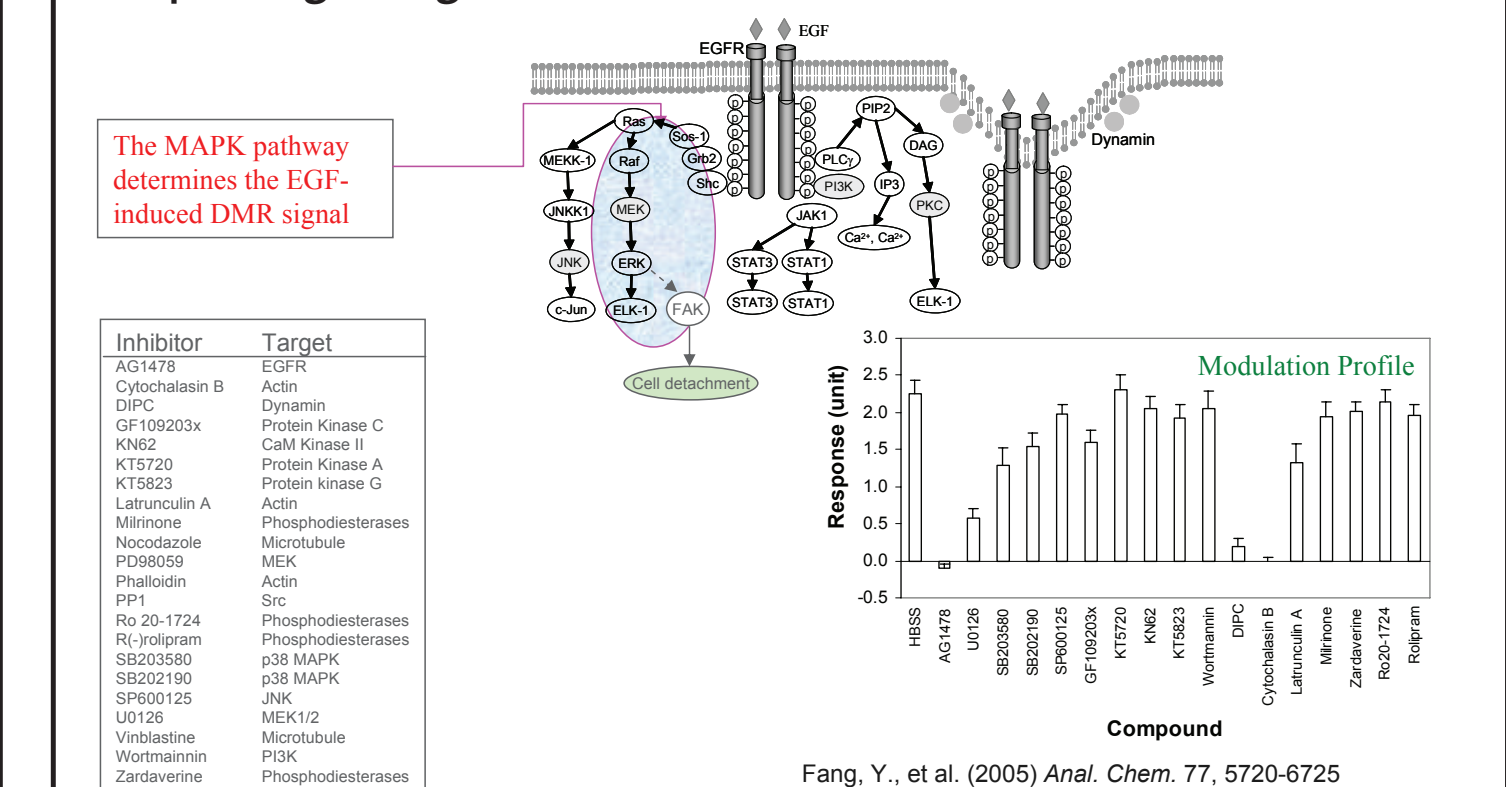
## RWG Biosensor Assays Detect Complex GPCR Signaling: Dual Signaling Pathways of Endogenous Bradykinin B2 Receptor in A431



## RWG Biosensor Cell Assays Differentiate Ligand-Directed Functional Selectivity Acting on $\beta$ 2AR



## RWG Biosensor Cell Assays Enable Systems Cell Biology Analysis of Receptor Signaling



## Summary

- Epic<sup>®</sup> cell assays employ label-free optical biosensor to measure stimulus-induced dynamic mass redistribution in cells within the detection zone of the biosensor
- The DMR signal is a novel physiologically relevant and kinetic response of living cells
- The DMR signal is a global representation of receptor biology and ligand pharmacology
- Epic cell assays have broad applicability in many classes of targets, and have potentials in drug discovery and development process as well as others