

Chapter 17

Resonant Waveguide Grating Biosensor for Whole-Cell GPCR Assays

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Summary

Current drug discovery campaigns for G protein-coupled receptors (GPCRs) heavily rely on assay technologies that use artificial cell systems tailored to a point-of-contact readout and as a consequence are mostly pathway biased. Recently, we have developed label-free optical biosensor cellular assays that are capable of examining systems cell biology of endogenous receptors and systems cell pharmacology of GPCR ligands in both physiologically and disease relevant environments. We have shown that these biosensor assays enable high-throughput screening of pathway-biased ligands acting on endogenous β_2 -adrenergic receptor in cells. These biosensor cellular assays hold the potential to reduce attrition rates in drug discovery and development process.

Key words: G protein-coupled receptor, Optical biosensor, Resonant waveguide grating biosensor, Dynamic mass redistribution, Ligand-directed functional selectivity.

1. Introduction

Resonant waveguide grating (RWG) biosensor is an optical biosensor that is capable of detecting minute changes in local index of refraction near the sensor surface (1). Since local index of refraction within a cell is a function of density and its distribution of biomass (e.g., proteins, molecular complexes) (2), RWG biosensor non-invasively detects stimulus-induced dynamic mass redistribution (DMR) in native cells without the need for any labels and cellular manipulations (3–6). Common to current cell-based assays is the use of labels and cellular manipulations, which could require significant assay development efforts and often lead to many complications in receptor

biology and ligand pharmacology (7, 8). Instead of measuring single point of contacts within complex signaling pathways, RWG biosensor can follow the evolution of receptor signaling in time and space, leading to a kinetic and integrative response, termed DMR. The DMR measure is mostly pathway unbiased but pathway sensitive (9–13). With the recent advancements in biosensor design and instrumentation (5, 9), these biosensors are easily scalable and enable high-throughput screening of drug compounds for endogenous G protein-coupled receptors (GPCRs) in native cells (14).

For whole-cell sensing, a RWG biosensor is considered as a three-layer waveguide configuration: a substrate with an optical grating, a high index of refraction waveguide coating, and a cell layer (**Fig. 1a**). The biosensor employs the resonant coupling of light into the waveguide by means of a diffraction grating. When illuminated with broadband light at a fixed and nominally normal angle of incidence, these sensors reflect only a narrow band of wavelengths that is a sensitive function of the index of refraction of the biosensor and is governed by (1):

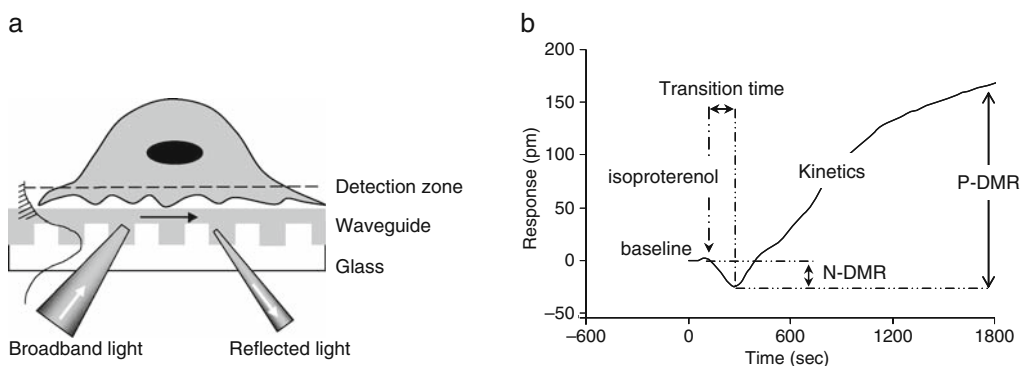


Fig. 1. RWG biosensor for whole-cell GPCR sensing. (a) A three-layer waveguide system for detecting ligand-induced DMR signals in living cells using RWG biosensor. Cells are cultured on the sensor surface. (b) The DMR signal of quiescent A431 cells induced by 0.1 nM isoproterenol. Isoproterenol is a full agonist of β_2 AR.

$$\sin(\theta) = n_{eff} - l \frac{\lambda}{\Lambda}$$

where θ is the maximum efficiency coupling angle, n_{eff} the effective index of refraction of the waveguide, l the diffraction order, λ the resonant wavelength, and Λ the grating period.

RWG biosensor utilizes the evanescent wave to characterize ligand-induced alterations in the cells. The evanescent wave is an electromagnetic field created by the total internal reflection of resonant light in the waveguide thin film, whose intensity exponentially decays away from the sensor surface. The distance from the sensor surface at which the electric field strength has decreased

to $1/e$ of its initial value is the penetration depth or detection zone or sensing volume (typically ~ 150 nm). This feature indicates that the biosensor only samples the bottom portion of the cells contacting with the sensor surface (**Fig. 1a**).

Using a three-layer waveguide system, we have found that a ligand-induced change in effective refractive index (i.e., the detected signal) is governed by (3):

$$\Delta N = S(C)\alpha d \sum_i \Delta C_i(t) \left[e^{\frac{-z_i}{\Delta Z_c}} - e^{\frac{-z_{+1i}}{\Delta Z_c}} \right]$$

where $S(C)$ is the sensitivity of the biosensor to the cell layer, ΔZ_c the penetration depth into the cell layer, α the specific refraction increment (about 0.18/mL/g for proteins), $\Delta C_i(t)$ the change in local concentration of biomolecules at the given location at a specific time, z_i the distance where the mass redistribution occurs, and d an imaginary thickness of a slice within the cell layer. This theory predicates, as confirmed by experimental studies (3–6, 9–13), that the biosensor is able to monitor cell signaling in real time, a stimulus-induced optical response is dominated by the vertical DMR, and the resultant DMR signal is an integrated cellular response containing contributions from many cellular events mediated through a receptor. The DMR signals are often manifested by a shift in resonant wavelength of the biosensor (**Fig. 1b**).

The full activation of endogenous β_2 -adrenergic receptor (β_2 AR) in human epidermoid carcinoma A431 cells leads to a prototypical G_s -mediated signaling (13). The DMR signal mediated through the activation of β_2 AR offers multiple parameters to differentiate pathway-biased activity of many ligands acting on the receptor (13). Assays using a subset of Library of Pharmaceutically Active Compounds (Sigma Chemical Co.) show the ability of the biosensor cellular assay for screening pathway-biased ligands. Here using the endogenous β_2 AR in A431 cells as a model system we describe a three-step protocol for whole-cell GPCR sensing using RWG biosensors: (i) preparation of cells, (ii) detecting cellular responses upon stimulation using the biosensor, and (iii) extracting DMR parameters for analysis of ligand pharmacology and receptor biology.

2. Materials

2.1. Tissue Culture Medium and Cell Line

1. Dulbecco's modified Eagle's medium (DMEM; Invitrogen, Carlsbad, CA, USA).
2. Heat-inactivated fetal bovine serum (FBS; Invitrogen).

3. Penicillin-streptomycin liquid ($100\times$; Invitrogen).
4. Complete cell culture medium: DMEM supplemented to a final concentration of 10% with heat-inactivated FBS, 4.5 g/L of glucose, 2 mM of glutamine, 50 U/mL of penicillin, and 50 μ g/mL of streptomycin.
5. Serum-free starvation medium: DMEM supplemented to a final concentration of 4.5 g/L of glucose, 2 mM of glutamine, 50 U/mL of penicillin, and 50 μ g/mL of streptomycin.
6. Trypsin-EDTA: 0.25% Trypsin with EDTA.4Na⁺ (Invitrogen).
7. Human epidermoid carcinoma A431 cells (American Type Cell Culture, Manassas, VA, USA).

2.2. Microplates and Instruments

1. Corning 75 cm² tissue culture-treated polystyrene flask with vented cap (T-75) (Corning Inc., Corning, NY, USA).
2. Corning 384-well polypropylene compound storage plate (Corning Inc.).
3. Epic[®] 384-well microplate tissue culture treated (Corning Inc.) (*see Note 1*).
4. Matrix 16-channel electronic pipettor (Thermo Fisher Scientific, Hudson, NH, USA).
5. Epic[®] system (Corning Inc.; *see Note 2*).
6. BioTek cell plate washer ELx405 (BioTek Instruments Inc., Winooski, VT, USA).

2.3. The RWG Biosensor Cellular Assays

1. Betaxolol, dopamine, ICI118551, (-)-isoproterenol, norepinephrine, pindolol, propranolol, and salbutamol (Tocris Chemical Co., St. Louis, MO).
2. Catechol, halostachine, and S(-)-epinephrine (Sigma Chemical Co., St. Louis, MO, USA).
3. Hank's balanced salt solution (HBSS), $1\times$ with calcium and magnesium, but no phenol red (Invitrogen).
4. 1 M HEPES buffer solution, pH7.1 (Invitrogen).
5. Assay buffered solution ($1\times$ HBSS, 10 mM HEPES, pH 7.1).
6. Library of Pharmaceutically Active Compounds (Sigma Chemical Co.).

2.4. Data Analysis Software

1. Epic[®] Offline Viewer (Corning Inc.).
2. Microsoft Excel (Microsoft Inc., Seattle, WA, USA).
3. Prism Software (GraphPad Software Inc., La Jolla, CA, USA).

3. Methods

3.1. Preparations of Cells for Assaying Endogenous β_2 AR by RWG Biosensor

This protocol employs standard cell culture and starvation techniques to prepare cells for assaying the endogenous β_2 AR in A431 cells (*see Note 3*). Simple modifications of this protocol can be used for assaying receptors in different types of native or engineered cells, including suspension cells (*see Note 4*). These modifications include the cell culture medium and time, initial seeding numbers of cells, cell synchronization, and types of biosensor surfaces.

1. The wild-type human epidermoid carcinoma A431 cells received from ATCC are passed in T-75 flasks using the recommended protocol. The cells up to at least 25 passages after received from ATCC are appropriated for the biosensor cellular assays in our laboratory. The native A431 cells are passed when approaching confluence with trypsin/EDTA to provide new maintenance cultures on T-75 flasks and experimental cultures on the Epic 384-well tissue culture-treated microplate. A 1:10 split of the native cells will provide maintenance cultures on T-75 flasks that are approaching confluence after 5 days. Cells on a T-75 flask are sufficient for experimental cultures on at least one biosensor microplate.
2. Once the cells reach confluence in the T-75 flask, the cells are harvested using $1 \times$ trypsin-EDTA solution. After centrifuging the cells down, the supernatant is removed and the cell pellet is resuspended in freshly prepared complete cell culture medium.
3. Approximately 1.8×10^4 cells at passages 3–25 suspended in 50 μ L of the complete medium are added into each well of an Epic 384-well biosensor microplate (*see Note 5*). The cell plating is carried out using a 16-channel electronic pipettor. Air bubbles trapped in the bottoms of the wells at this stage, if any, are manually removed by the pipettor.
4. After incubation in the laminar flow hood for ~ 20 min, the cells are then cultured for 24 h at 37°C under air/5% CO₂. At this point the cells usually reach approximately 80–90% confluency. After rinsing twice with the serum-free starvation medium, the cells are incubated for a further 24 h in the serum-free DMEM at 37°C under air/5% CO₂. During the starvation, the cells continue to grow until approaching 100% confluence and reaching a quiescent state through serum withdrawal and contact inhibition (15) (*see Note 6*).

3.2. Biosensor Cellular Assays for Kinetic Measurements of Receptor Signaling and Ligand Pharmacology

This protocol describes both one-step and sequential two-step kinetic assays. The one-step assay is useful for determining agonism of individual ligands, determining competitive antagonism of a ligand against an agonist under non-equilibrium condition, and examining cellular responses upon costimulation. The two-step assay is useful for assaying antagonism of ligands, determining competitive antagonism of a ligand against an agonist under equilibrium condition, and analyzing systems cell biology of an agonist-induced signaling. The systems cell biology analysis is assessed based on the impact of chemical and biochemical (e.g., interference RNA) intervention of specific cellular signaling cascades on the agonist-induced DMR signal (4–6, 9, 10). For the two-step assays, the separation time between the two stimulations are typically within tens of minutes.

1. The assay-buffered solution is made ready and stored at room temperature. Compounds are generally dissolved in dimethyl sulfoxide (DMSO) and stored at high concentrations (10–100 mM) at -20°C .
2. Compound solutions are made by diluting the stored concentrated solutions with the assay-buffered solution at room temperature and transferred into a 384-well polypropylene compound storage plate using a pipettor to prepare a compound source plate. Two separate compound source plates are applied when a two-step assay is performed.
3. The starved cells are washed twice with the assay-buffered solution using the BioTek washer and maintained in 30 μL of the assay-buffered solution to prepare a cell assay plate.
4. Both the cell assay plate and the compound source plate(s) are incubated in the hotel of the Epic system for at least 1 h (*see Note 7*). When a compound solution contains greater than 0.1% DMSO, the DMSO in the corresponding well of the cell assay plate is carefully matched (*see Note 8*).
5. After approximately 1 h of incubation in the reader system, the baseline wavelengths of all biosensors in the cell assay microplate are recorded and normalized to zero. Afterwards, a 2–10 min continuous recording is carried out to establish a baseline. At this point, the baseline should reach a steady state, leading to a net-zero response (**Fig. 1b**) (*see Note 9*).
6. Cellular responses are triggered by transferring 10 μL of the compound solutions from the compound source plate into the cell assay plate using the on-board liquid handler. Afterwards, the cellular responses are continuously monitored for a period of time (typically 1 h for GPCR signaling) (*see Note 10*).
7. For studying the influence of chemical or biochemical interventions, a second stimulation with an agonist at a fixed dose (typically a dose at EC_{80} or EC_{100}) is applied. The cellular

responses are continuously recorded. The resonant wavelengths of all biosensors in the microplate can be normalized again to establish a second baseline, right before the second stimulation.

3.3. Biosensor Cellular Assays for High-Throughput Screening

Once the DMR signal is known for a receptor–agonist pair in a cell type, a two or multiple end-point assay can be developed in order to screen compounds in high-throughput manner. Since the biosensor cellular assays are non-invasive in nature, both agonism and antagonism screening modes can be incorporated in a single screening campaign. The following is an example of high-throughput screening of agonists for endogenous G_s -coupled receptors in A431 cells using a subset of LOPAC library of compounds.

1. The Sigma-Aldrich LOPAC 1280TM library of compounds includes 1280 bioactive small organic molecules against many target classes including several GPCRs. The compounds received are stored in barcoded 96-well microplates as 10 mM solution in neat DMSO. Four of these compound microplates are aliquoted and reconfigured into a single 384-well compound source microplate. After appropriate dilution with the assay-buffered solution, a 384-well compound source plate having 320 compounds in wells from columns 3–2 is made ready, such that the final concentration of each ligand is 4 μM . The DMSO concentration is 0.4%. The wells in the columns 1 and 2 only contain the assay-buffered solution having 0.4% DMSO and are used as a negative control. The wells in the columns 23 and 24 only contain 2 nM epinephrine in 0.4% DMSO and are used as a positive control.
2. The starved A431 cells in the biosensor microplate are washed with the assay-buffered solution twice and maintained in 30 μL of the buffered solution containing 0.4% DMSO.
3. Both the compound source plate and the cell assay plate are incubated in the hotel of the reader system for 1 h. After a baseline read, 10 μL 4 $\times$ compound solutions are transferred into the cell assay plate. After 50 min of stimulation, a second end-point read is recorded. The shift in resonant wavelength in each well is calculated and plotted as a function of compounds.

3.4. Data Analysis

The practical applications of these biosensor cellular assays are numerous. This section presents a brief description of some of these assays to study receptor signaling and ligand pharmacology.

3.4.1. Extracting Kinetic Information from Ligand-Induced DMR Signals

RWG biosensors measure ligand-induced DMR signals in living cells. The resultant DMR signal is a real-time kinetic response and contains high information contents. As exemplified in the isoproterenol response in quiescent A431 cells, following the steady

baseline is an initial decrease in signal (termed negative-DMR, N-DMR) and a subsequent increase in signal (positive-DMR, P-DMR) (**Fig. 1b**). Multiple parameters can be extracted from the DMR signal, each of which can be used to analyze ligand pharmacology and receptor biology. These parameters include overall dynamics, number of phases, the transition time from one phase to another, and amplitudes, duration, and kinetics of each phase.

1. After a 2 min baseline recording, the quiescent A431 cells are stimulated with isoproterenol at different doses. The cellular response is continuously monitored for 1 h until reaching an elevated plateau.
2. The amplitudes of both N-DMR and P-DMR events are calculated using the Epic offline viewer. The transition time for the P-DMR event to occur is calculated using Microsoft Excel, whereas the kinetics of the P-DMR event is determined by fitting it with a one-phase association non-linear regression process using the Prism software.

3.4.2. Determining Agonist Potency and Efficacy

To determine the efficacy and potency of an agonist, the cells are stimulated with a concentration series of the agonist. The kinetic responses of cells are recorded and subsequently analyzed for the dose dependency of the cellular responses. **Figure 2a** shows the dose-dependent DMR signals of quiescent A431 cells induced by isoproterenol. **Figure 2b** shows the time-dependent amplitudes of isoproterenol-induced DMR signals in A431 cells. The isoproterenol response displays classical dose dependency, that is, both the amplitudes and kinetics increase until reaching a maximal level at saturating concentrations. The potency and efficacy of the agonist can be determined in many different ways. Typically the amplitude of a DMR event (e.g., the P-DMR event for the isoproterenol response) at a specific time point is plotted as a function of agonist doses to determine the potency and efficacy of the agonists (**Fig. 2b**).

3.4.3. Determining Relative Potency of Antagonists

To determine relative potency of antagonists, the potency of a β_2 AR agonist such as epinephrine is determined first using the above-mentioned method (**Section 3.4.2**). Epinephrine at EC_{80} is chosen to determine the relative potency of β_2 AR antagonists, such as betaxolol, ICI 118551, and propranolol. The quiescent A431 cells are pretreated with each antagonist at different doses for about 5 min to 1 h. Afterward a baseline read, and the agonist responses are triggered by transferring 10 μ L of the $5 \times$ solution of the agonist into the cell assay plates. Later, the amplitudes of the agonist P-DMR event in the presence of an antagonist at different doses are calculated based on the shift in resonant wavelength before and 50 min after stimulation. Non-linear regression analysis

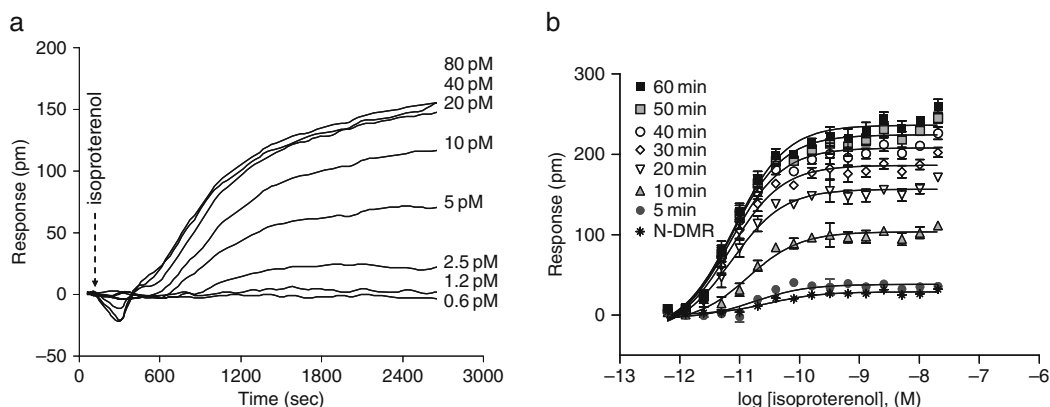


Fig. 2. The dose-dependent isoproterenol responses of quiescent A431 cells. (a) Real-time kinetic responses. (b) The time-dependent amplitudes. A431 cells were cultured on the Epic 384-well tissue culture-treated microplate and grown to confluence. After starvation, the cells were stimulated with isoproterenol at different doses as indicated. The amplitudes were calculated based on the difference of resonant wavelength between before and a specific time after stimulation. The broken arrow indicated the time when isoproterenol is introduced.

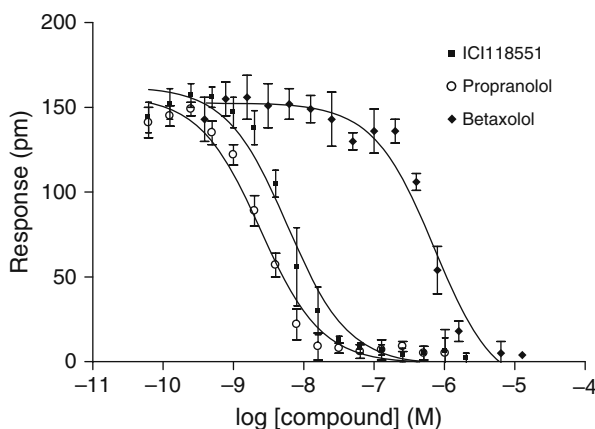


Fig. 3. The dose-dependent inhibition of the 2 nM (-)-epinephrine response of quiescent A431 cells by three β_2 AR antagonists, ICI118551, propranolol, and betaxolol. The amplitudes of the P-DMR event of the epinephrine DMR signals were plotted as a function of antagonist concentration. The P-DMR amplitudes were calculated based on the shift in resonant wavelength before and 50 min after the epinephrine stimulation.

is used to determine the relative potency of the antagonists against the agonist. **Figure 3** shows the dose-dependent inhibition of the epinephrine response by the three β_2 AR antagonists.

3.4.4. Determining Ligand-Directed Functional Selectivity

GPCRs display rich behaviors in cells and many ligands can induce operative bias to favor specific portions of the cell machinery and exhibit pathway-biased efficacies (16, 17). To determine ligand-directed functional selectivity with the biosensor cellular assays, panels of structurally similar β_2 AR ligands that have a wide

spectrum of efficacies are chosen and used to stimulate the quiescent A431 cells. The DMR signals are recorded, and multiparameter analysis is carried out to determine the correlation between the structures of ligands and the characteristics of their optical signals (13). **Figure 4** graphically depicts the chemical structures of agonists examined and their corresponding DMR signals in quiescent A431 cells.

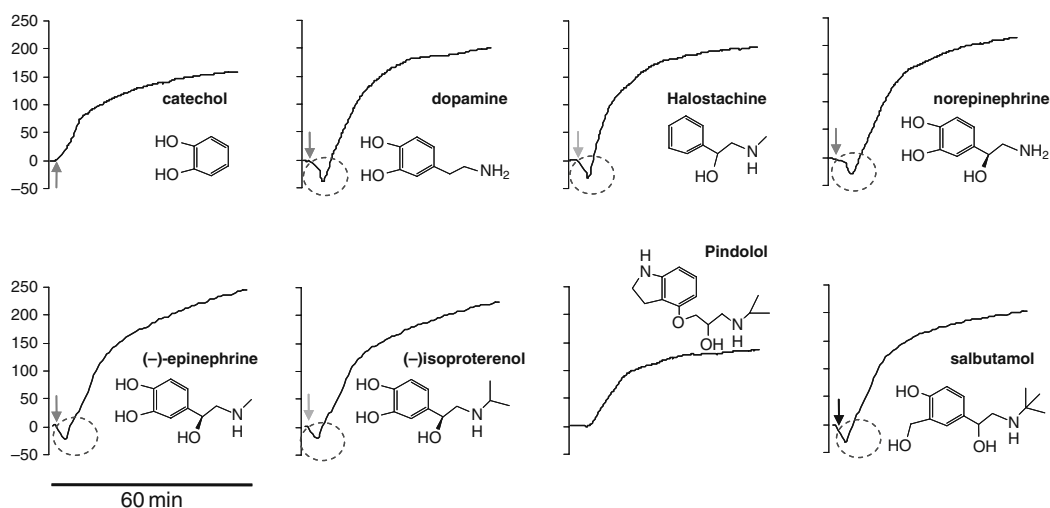


Fig. 4. The structures of β_2 AR ligands and their DMR in quiescent A431 cells. The ligands included (–)epinephrine (8 nM), (–)isoproterenol (10 nM), norepinephrine (100 nM), dopamine (32 μ M), halostachine (500 μ M), catechol (500 μ M), salbutamol (164 nM), and pindolol (8 μ M). The gray arrows indicated the time when the agonist is introduced. The y-axis is the response in picometer (pm). The inhibition profiles by propranolol suggest that these ligand-induced DMR signals are specific to the β_2 AR (13). (Reproduced with permission from (13)).

3.4.5. Determining Competitive Antagonism

The competitive antagonism can be assessed under two distinct experimental conditions: equilibrium and non-equilibrium conditions. The equilibrium competitive assay is performed using a two-step stimulation procedure, wherein the cells are pretreated with an antagonist at a fixed dose, followed by stimulation with an agonist at different doses. The non-equilibrium assay is performed using costimulation of cells with an agonist in several independent dose series, each series in the presence of an antagonist at a fixed dose. The Schild analysis is then carried out. **Figure 5** shows an example of the non-equilibrium assay to determine the competitive antagonism of propranolol against epinephrine acting on the β_2 AR in A431 cells.

3.4.6. Screening Pathway-Biased Agonists

The pathway-biased agonists can be assessed using two different types of assays: kinetic and end-point assays. The quiescent A431 cells are stimulated with a subset of the LOPAC compounds at a fixed dose (each at 1 μ M). The cellular response of each biosensor

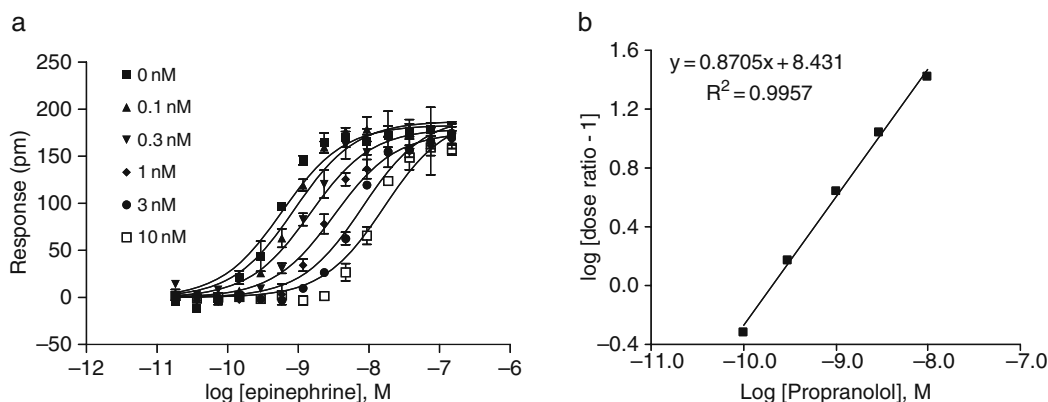


Fig. 5. Schild analysis for the competitive antagonism of propranolol against epinephrine under non-equilibrium condition. (a) The amplitudes of the epinephrine responses in the presence of propranolol were plotted as a function of epinephrine concentration. The amplitudes were calculated based on the shift in resonant wavelength before and 50 min after stimulation. The cells were stimulated with epinephrine at different doses. Propranolol at different fixed doses was added into each epinephrine dose series. (b) The Schild plot analysis. The results indicate that propranolol is a competitive antagonist against epinephrine under the non-equilibrium condition.

well is calculated based on the shift in resonant wavelength before and 50 min after stimulation. The difference between the positive and negative controls is used to calculate assay robustness. The relative ratio of an agonist response to the positive control is an

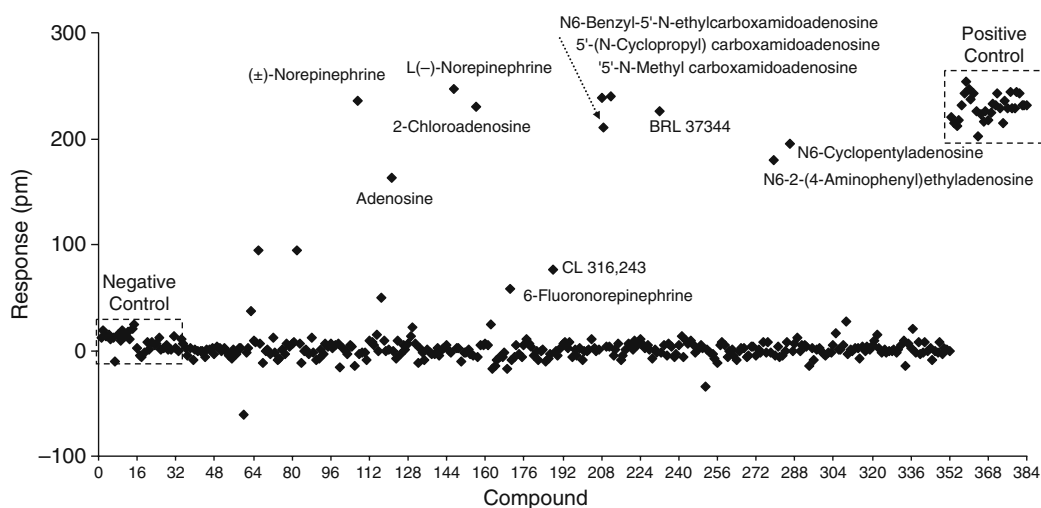


Fig. 6. The response of quiescent A431 cells as a function of compound. The quiescent A431 cells were stimulated with a subset of LOPAC library of compounds. The shift in resonant wavelength before and 50 min after stimulation was calculated for each well. The negative controls (the vehicle only) led to a response of 8 ± 7 pm ($n=32$), whereas the positive controls (2 nM (-)-epinephrine) led to a response of 230 ± 12 pm ($n=32$), indicating a robust assay with a Z' factor of 0.74. This end-point screening identified all agonists in this subset of compounds for both the endogenous β_2 AR and adenosine receptors in A431. Several other compounds whose mechanisms to trigger the DMR responses are unknown were also identified.

indicator of the ability of the agonist to trigger the receptor signaling; a ratio of less than 1 indicates that the agonist is a partial agonist. **Figure 6** shows an example of screening pathway-biased agonists using the end-point assays.

4. Notes

1. Two types of biosensor microplates are commercially available and are ready to culture. They are Epic 384-well tissue culture treated and Epic 384-well fibronectin coated. Within each well of either type of microplates, there is an RWG biosensor.
2. The Epic system is the first high-throughput screening biosensor system for both biochemical and cell-based assays (4–6, 14). The system consists of two components: a disposable SBS (Society for Biomolecular Sciences) standard 384-well biosensor microplate in which each well contains an RWG biosensor and an optical reader that utilizes a linear array of fiber optic heads for recording cellular responses in the biosensor microplate. The commercially available Epic system is a standalone optical reader. An external liquid handling accessory can be directly attached to the reader system. This system is capable of measuring as many as 40,000 wells in an 8 h period, based on end-point measurements. For kinetic assays, the temporal resolution is approximately 7 or 15 s, depending on the scanning mode selected. Here a beta version of the Epic system is used, in which an on-board liquid handler is available for onsite compound addition. The entire reader system is maintained at 26°C.
3. A431 cell line is chosen because it endogenously expresses a large number of the β_2 AR but no β_1 AR or β_3 AR (18). The endogenous β_2 AR is a prototypical G_s -coupled receptor and has served as a model system for studying GPCR signaling cycle, including receptor trafficking (19, 20).
4. The biosensor cellular assays are applicable to both adherent and suspension cells. For adherent cells such as A431, the cells are directly cultured on the surfaces of the 384-well Epic microplate tissue culture treated. For weakly adherent cells such as human embryonic kidney 293, the cells are directly cultured on the surfaces of the 384-well Epic microplate fibronectin coated. For suspension cells, the cells are brought to contact with the surface of a biosensor microplate through either gravity or chemical interactions (e.g., covalent coupling or specific biomolecular interactions of the cell surface molecules).

5. The seeding number of cells on the biosensor microplates is usually optimized first. Since the biosensor measures an averaged response of a population of cells located in the area illuminated by the incident light, a cell layer of high confluency is usually used to achieve optimal results.
6. Synchronizing cells into a specific state is not necessary. However, it can be, in some cases, beneficial to receptor biology analysis, assay reproducibility, and robustness. This is because the biosensor assays are sensitive to cellular context (e.g., passages, proliferating and quiescent states, and confluency) due to the integrative nature of the DMR signals measured with the biosensor. The cellular environment is known to modulate receptor pharmacology, rendering many GPCR ligands to often display cellular context-dependent pharmacological profiles (21).
7. Preincubation ($\sim$ tens of minutes) of both the cell assay plates and the compound source plates can significantly reduce the environmental effects on cellular response. A temperature-controlling unit is built in the reader system to minimize any temperature fluctuation.
8. DMSO is a high index of refraction solvent. Also, DMSO is often considered a cytotoxic agent and has significant impact on cell biology.
9. Although cells are dynamic and constantly undergo micro-motion (i.e., a dynamic movement and remodeling of cellular structure under unstimulated conditions), the cells generally give rise to an almost net-zero DMR response, following the cell preparation and assay protocol described in **Sections 3.2** and **3.3**. This is partly because of the low spatial resolution of the biosensors, also partly due to highly ordered structure of intracellular macromolecules in cells, most of which have just completed a single cycle of division at the time of assay.
10. GPCR agonist-induced optical responses in living cells typically last for about 1 h until they reach a plateau.

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