Comparing Label-Free Biosensors for Pharmacological Screening With Cell-Based Functional Assays

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ABSTRACT

The diversity and impact of label-free technologies continues to expand in drug discovery. Two classes of label-free instruments, using either an electrical impedance-based or an optical-based biosensor, are now available for investigating the effects of ligands on cellular targets. Studies of GPCR function have been especially prominent with these instruments due to the importance of this target class in drug discovery. Although both classes of biosensors share similar high sensitivity to changes in cell shape and structure, it is unknown whether these biosensors yield similar results when comparing the same GPCR response. Furthermore, since cell morphology changes induced by GPCRs differ depending on which G-protein is activated, there is potential for these instruments to have differential sensitivities to G-protein signaling. Here 1 impedance (CellKey™)- and 2 optical-based instruments (BIND® and Epic®) are compared using Gi-coupled (ACh M2), Gq-coupled (ACh M1), and Gs-coupled (CRF1) receptors. All 3 instruments were robust in agonist and antagonist modes yielding comparable potencies and assay variance. Both the impedance and optical biosensors showed similar high sensitivity for detecting an endogenous D1/D5 receptor response and a melanocortin-4 receptor inverse agonist (agouti-related protein). The impedance-based biosensor was uniquely able to qualitatively distinguish G-protein coupling and reveal dual signaling by CRF1. Finally, responses with a ligand-gated ion channel, TRPV1, were similarly detectable in each instrument. Thus, despite some differences, both impedance- and optical-based platforms offer robust live-cell, label-free assays well suited to drug discovery and typically yield similar pharmacological profiles for GPCR ligands.

INTRODUCTION

In recent years, there has been a significant expansion in the development of label-free biosensors for quantifying aspects of cell function. Initial applications included measuring cell proliferation and cytotoxicity, cell migration, and receptor activation.[1] Studying G-protein-coupled receptor (GPCR) pharmacology with label-free biosensors has emerged as highly valued based on the numerous advantages of label-free detection. (i) No labeling of the ligand or receptor is required, which simplifies assay design and minimizes artifacts or liabilities created by the labeling process. (ii) The limited number of variables to optimize shortens assay development time. (iii) A single detection system can quantify responses to GPCRs that couple to different signaling pathways. (iv) High sensitivity and label-free features enable detection of endogenously expressed GPCRs in cell lines and primary cells. (v) Noninvasive measurement enables one to acquire both real-time kinetic and endpoint measurements, which expands the types of studies that can be performed.

The application of label-free systems to GPCR screening has developed over the last several years and appears poised for wider implementation. Early instruments were designed for modest throughput whereas today's instruments accept 384-well or higher density plates, enabling high-throughput screening with live cells as a front-line approach to seek chemical leads. The lack of understanding of the molecular and cellular mechanisms that define the label-free response also may have hindered early adoption. The development of 2 independent technologies, impedance- or optical-based, may help to frame the cellular processes involved. A fundamental feature of both these technologies is exquisite sensitivity, which should resolve cellular processes

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previously undetectable. For example, impedance assays are capable of detecting actin-dependent cellular micromotion on the order of 1 nm or approximately one-third the thickness of the cell membrane.[2] As it happens, modulation of actin remodeling is a well-established but under-appreciated endpoint common to GPCRs.[3] Similarly, optical-based instruments have the sensitivity to detect mass changes of ~300 Da.[4] Certainly, the mass changes associated with cytoskeletal reorganization or receptor internalization would exceed this detection limit. The availability of 2 independent technologies should be valuable for unraveling the cellular mechanism of GPCR activation detected in each assay.

Several impedance-based biosensors are commercially available,[1] among which CellKey™ (MDS Analytical Technologies, Sunnyvale, CA) is specialized for detecting rapid responses observed with GPCRs. Impedance-based instruments utilize interdigitated electrodes embedded within each well of a microtiter plate. Cells plated on these electrodes resist the flow of a small, applied current. Thus, even subtle changes in cell shape, adhesion, and cell–cell interaction induced by GPCRs (through different signaling pathways) would be predicted to alter impedance.

The BIND® (SRU Biosystems, Woburn, MA) and Epic® (Corning Inc., Corning, NY) instruments are 2 examples of optical-based biosensors that detect changes in mass near the microtiter plate surface. These instruments have different design elements and data transformation processes, yet both utilize a light source, optical grating surface, and detector to quantify the index of refraction near the surface of the microtiter plate. The shift in the wavelength of reflected light is proportional to the change in mass near the sensor surface. The optical-based technology was originally developed for biochemical binding assays and subsequently shown to be capable of detecting GPCR-induced mass redistribution in cells.[5]

Both impedance- and optical-based biosensor instruments have been used to quantify GPCR pharmacology.[5–8] However, a comparative evaluation of the different instruments has not been published, and therefore, the relative strengths and limitations of these systems for quantifying cellular responses are unknown. For example, it is unclear whether the different biosensors reveal similar or different temporal responses to receptor activation, and whether these changes depend on the signaling pathways that are activated. The sensitivity and reproducibility of the instruments have not been compared, nor is it known whether the optical- and impedance-based biosensors yield comparable pharmacological profiles. To begin addressing these questions, we selected GPCRs with well-defined coupling through Gi, Gq, or Gs, and compared agonist and antagonist responses using the CellKey™ impedance-based biosensor and the BIND® and Epic® optical-based biosensors. We also evaluated the ability of these instruments to measure the response to agonists of TRPV1, a ligand-gated ion channel.

MATERIALS AND METHODS

Compounds

Acetylcholine, carbachol, scopolamine, pirenzepine, forskolin, dihydrexidine, capsaicin, and cytochalasin D were obtained from Sigma (St. Louis, MO). CRF and sauvagine were purchased from Bachem (Torrance, CA) and Tocris Biosciences (Bristol, UK), respectively. Melanocyte-stimulating hormone (α-MSH) and agouti-related protein (AgRP) were obtained from Phoenix Pharmaceuticals (Burlingame, CA). The YP-20 peptide[9] and R121919 were synthesized in AstraZeneca chemistry labs.

Cell Culture

The base media for CHO and U-2 OS cells was Ham's F-12 and McCoy's 5A, respectively. Media was supplemented with 10% dialyzed FBS and 2 mM L-glutamine. CHO-K1 cells expressing human receptors as stable clones were cultured with appropriate selective agents. Cells were harvested with Cellstripper™ and plated at 150 µL in 96-well plates (CellKey), 50 µL in 384-well CA2 plates (BIND), or 50 µL in 384-well fibronectin-coated plates (Epic). Cell seeding density per well for each cell type was as follows: 20K for CHO in BIND, 7.5K for CHO-M1 and CHO-M2 in Epic, and 5K for CHO-CRF1 and CHO-TRPV1 in Epic. U-2 OS cells were plated at 30K per well. Cell density for CellKey experiments has been described previously.[7] Cells were allowed to adhere by culturing overnight prior to experiments. Expression of TRPV1 was induced by overnight incubation with tetracycline at 0.5 µg/mL.

BIND/CellKey/Epic Assays

For BIND experiments, media was replaced with 40 µL of Hank's balanced salt solution, 20 mM HEPES pH 7.4, 0.1% BSA (HBSS), supplemented with antagonist as indicated. After incubating at room temperature for 30 min, plates were loaded on the BIND™ (SRU Biosystems, Woburn, MA), and a baseline was established for 5 min. Data collection was paused for ~1 min while the cell plate was moved to a PlateMate™ and agonist (20 µL) was added from a 3× stock solution.

CellKey experiments were conducted as previously described,[7] with the exception of the following experiments: CRF concentration–response curve, U2-OS, CHO forskolin, and MC4R-CHO; where the initial volume of HBSS was reduced. This change allowed the use of a more dilute agonist stock solution (3× vs. 10×) thereby enabling a single agonist plate to be used in simultaneous CellKey and BIND experiments.

For Epic experiments on M1 and M2, media was replaced with 40 µL of Hank's balanced salt solution (HBSS), 20 mM HEPES

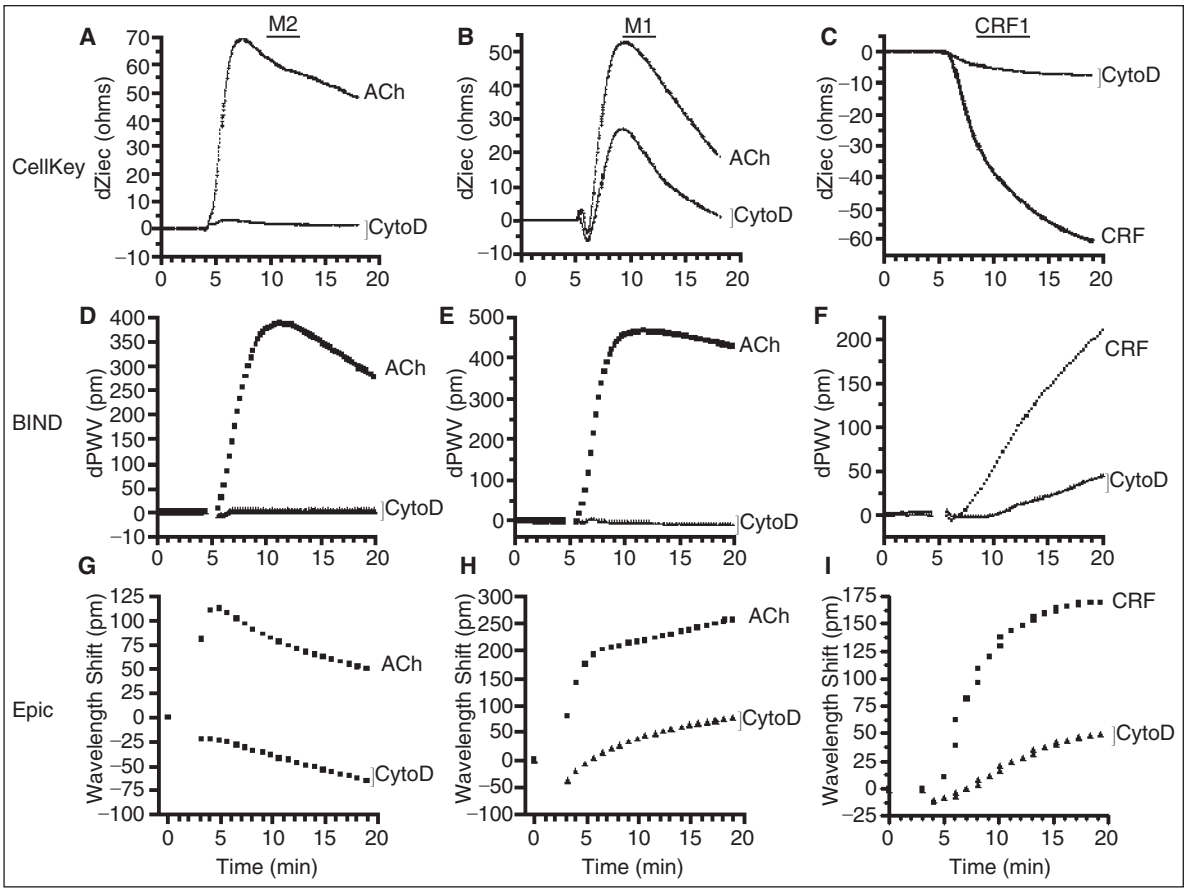

Fig. 1. Agonist response profiles for ACh M2, ACh M1, and CRF1 in CellKey, BIND, and Epic. Cells were stimulated with concentrations of acetylcholine (ACh) or corticotropin-releasing factor (CRF) that correspond to an approximate EC_{80}. Profiles are the net response obtained by subtracting vehicle or vehicle plus cytochalasin D. Data are the average of at least 3 wells.

pH 7.4, without BSA. After incubating at room temperature for 60 min, plates were loaded on the Epic® system (Corning, NY), and a baseline was established for 2 min. Data collection was paused for ~2 min while the agonist (10 µL) was added, and then results were recorded for 30 min with an interval of ~1 min. For antagonist experiments, cells were treated with the antagonist (10 µL) for 15 min before agonist addition. For Epic experiments on CRF1 and TRPV1, media was replaced with 25 µL HBSS, 0.1% BSA, with glucose (VR1) or without (CRF-R1), with magnesium (CRF1) and without magnesium (VR1). Antagonists (12.5 µL) were added and recorded for 15 min. On the second addition, agonist (12.5 µL) was added and data recorded for 20 min with an interval of ~1 min.

Actin dependence was assessed by pre-treating cells with cytochalasin D (10 µM) in HBSS for 30 min in BIND and CellKey experiments, or 15 min in Epic experiments prior to agonist addition.

Data Analysis

Concentration–response curves were generated by fitting data to a 4-parameter logistic equation using Prism 4.0 software (GraphPad, San Diego, CA). For CRF1, agonist EC_{50} calculations gave similar values whether using 10, 20, or 30 min data points. Thus for all responses, peak response values were utilized in data analysis. In the case of CRF1, peak responses were defined as the last data point. In Epic, a ~14 min read was used to determine EC_{50} and IC_{50} values of CRF-R1 and VR1. All data in concentration–response curves and bar graphs include variance plotted as SEM. Variance in the text is reported as standard deviation.

RESULTS

A key feature of CellKey, BIND, and Epic is the ability to detect GPCR signaling through each of the 3 major classes of G-proteins. Data suggests that CellKey can go beyond simple detection to

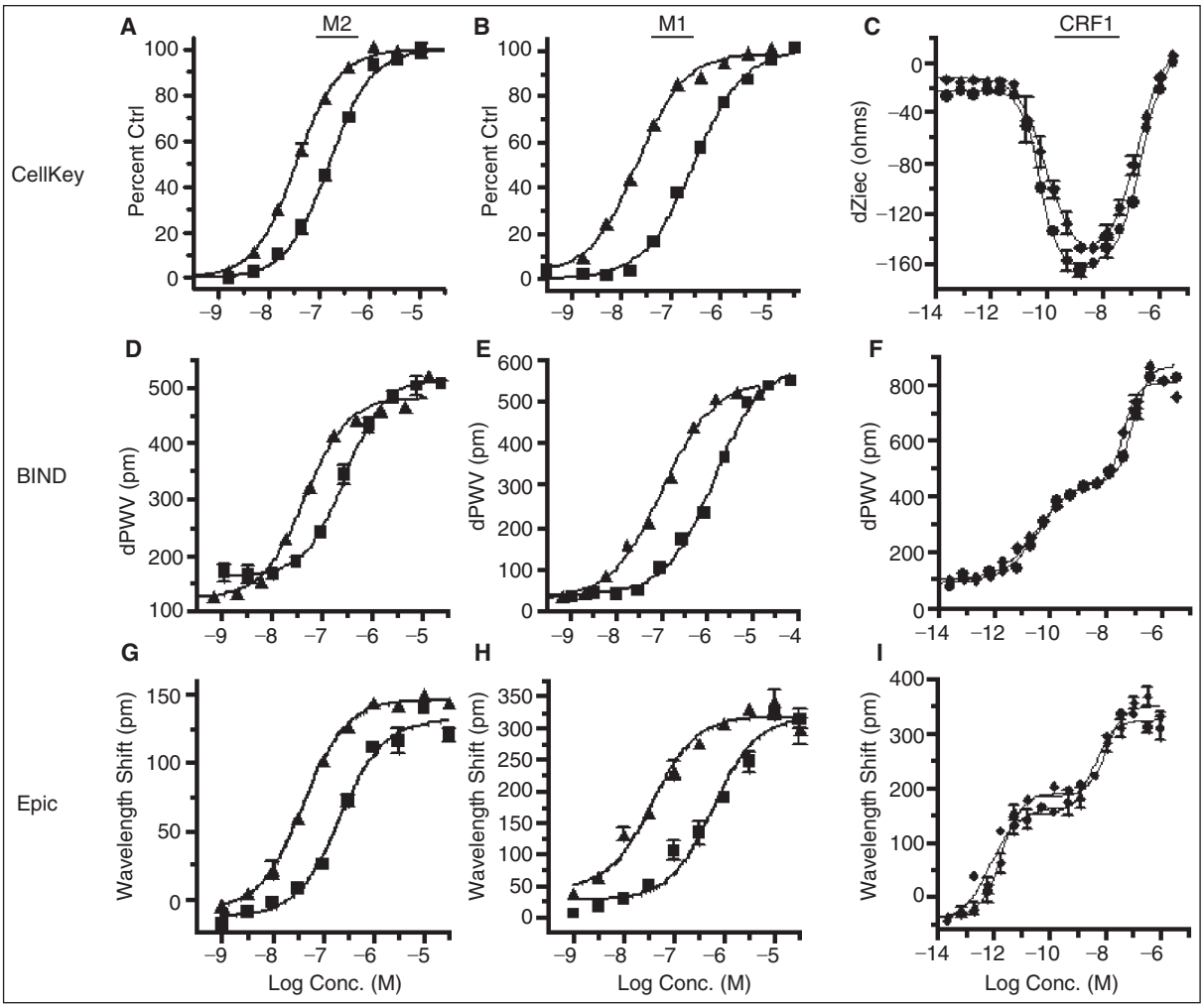

Fig. 2. Representative concentration–response curves for ACh M2, ACh M1, and CRF1 in CellKey, BIND, and Epic. Cells were stimulated with acetylcholine (ACh) (▲), carbachol (■), corticotropin-releasing factor (CRF) (♦), or sauvagine (●). Data are the average plus standard error of the mean of 4 wells (CellKey and BIND) or 2 wells (Epic).

qualitatively distinguish which G-protein is activated based on time-dependent response profiles.[8,10] To compare the response profiles, we selected receptors known to activate Gi (ACh M2), Gq (ACh M1), and Gs (CRF1). Assays were performed under similar conditions for all 3 instruments. A baseline read was taken for ~5 min before ligand addition. Response data were collected at the maximum update frequency of 2 s for CellKey, 10 s for BIND, and 50 s for Epic. With each GPCR studied and on each instrument, responses were detected within 1–2 min of agonist addition. A liquid addition artifact was often observed on each instrument, but this did not affect qualitative or quantitative aspects of data analysis.

As shown in *Fig. 1*, agonist stimulation of M2-CHO cells (Gi activation) resulted in a rapid increase in signal in all 3 instruments: the signal peaked within ~5 min and then slowly declined. Activating M1-CHO (Gq signaling) also induced an increase in signal that peaked within ~5 min. This response remained elevated in BIND (*Fig. 1E*) and Epic (*Fig. 1H*) but slowly declined in CellKey (*Fig. 1B*). Furthermore, the CellKey response showed a transient dip before the increase in signal; this was consistently larger than the fluid addition artifact. Treating the CRF1-CHO cells (Gs activation) with agonist caused a slightly slower response that was typically sustained for the 15-min assay window on each instrument. Importantly, the CellKey profile showed a negative

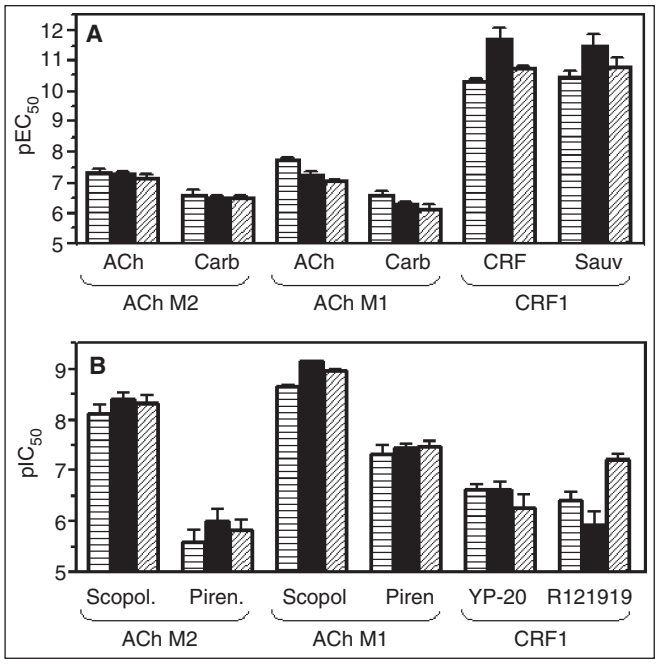

Fig. 3. The relative potency and variance of corticotropin-releasing factor (CRF)1, acetylcholine (ACh) M1, and ACh M2 agonists (**A**) and antagonists (**B**) in CellKey (horizontal bars), Epic (solid bars), and BIND (angled bars). Data are the average of at least 3 experiments plus standard deviation with the exception of M1 Epic antagonist data, which is the average plus range of 2 experiments. The pIC_{50} values were generated by inhibiting either CRF or ACh at assay-specific EC_{80} concentrations.

response (*Fig. 1A*) whereas BIND (*Fig. 1F*) and Epic (*Fig. 1I*) were positive. On each instrument, pre-treating the cells with cytochalasin D, which disrupts actin microfilaments, reduced the agonist response. This was true for all 3 GPCRs. Although full inhibition was not seen at 10 μM in all assays, qualitative sensitivity was clearly observed. (Higher cytochalasin D concentrations affect the baseline signal thereby preventing a clear assessment of the sensitivity to GPCR agonist response.)

Relative potency and precision of each instrument was evaluated under routine screening conditions using 2 agonists and 2 antagonists for each receptor. This matrix of receptors, ligands, and instruments necessitated restricting assay conditions to uniform buffers and standard plate types recommended by the manufacturers. Even under these optimization constraints, assay development was straightforward and yielded acceptable *Z′* values (*Table 1*).

In agonist mode, M2 and M1 were activated with acetylcholine (ACh) and carbachol while CRF1 was stimulated with CRF and sauvagine. Similar high levels of precision were observed for all receptors and in all assay platforms in individual concentration–response

Table 1. *Z′* Values Obtained With EC_{80} Concentrations of corticotropin-releasing factor (CRF) or acetylcholine (ACh)

	CellKey	BIND	Epic
CRF1	0.6	0.8	0.5
M1	0.8	0.8	0.5
M2	0.7	0.7	0.6

curves (*Fig. 2*). For CRF1, biphasic concentration–response curves were observed with both agonists and on each instrument. Both phases showed similar potency in each instrument (high potency data shown in *Figure 3*, low potency pEC_{50} values for CRF: 6.8 ± 0.2, 7.3 ± 0.2, 7.8 ± 0.6 for CellKey, BIND, and Epic, respectively). The CellKey response profile showed a negative dZiec response at low agonist concentrations, switching to a positive dZiec response at higher agonist concentrations. These CellKey data indicate that only the initial high potency phase was Gs-mediated and is consistent with published data demonstrating Gs and Gi coupling at low and high agonist concentrations, respectively.[11] In contrast, the response profiles from the optical-based instruments did not provide distinguishing features to discern a shift in CRF1 coupling at higher agonist concentrations.

Results from multiple agonist experiments revealed comparable potency and assay variance across all 3 instruments (*Fig. 3A*). In antagonist mode, potency values and assay variance were similar across the instrument platforms for the M2 and M1 antagonists pirenzepine and scopolamine as well as for the CRF1 antagonists YP-20 and R121919 (*Fig. 3B*).

Due to the dual response observed for CRF1 (*Fig. 2*), further comparison of Gs-coupled responses was warranted. This comparison was streamlined by focusing on CellKey and one optical-based platform, BIND. In selecting a second GsPCR, the challenge of detecting an endogenous receptor was added. U-2 OS cells express an endogenous response that matches the pharmacology profile for Gs-coupled dopamine D1/D5 receptors.[8,10,12] The D1/D5 selective agonist dihydrexidine (DHX) showed the expected response profiles (*Fig. 4A and 4D*) and similar potencies in both instruments (pEC_{50} values of 8.8 ± 0.1 and 9.1 ± 0.4 in CellKey and BIND, respectively). Both responses were specifically inhibited with the D1/D5-antagonist SCH23990 (ref. 8 and data not shown). Next, to bypass receptor-specific effects, untransfected CHO cells were treated with forskolin to activate adenylate cyclase and induce a Gs-like response (*Fig. 4B and 4E*). No significant difference in potency was detected between CellKey and BIND (pEC_{50} values of 6.9 ± 0.2 and 6.8 ± 0.3, respectively). We conclude that like Gi and Gq, detection of Gs response is generally similar across the platforms.

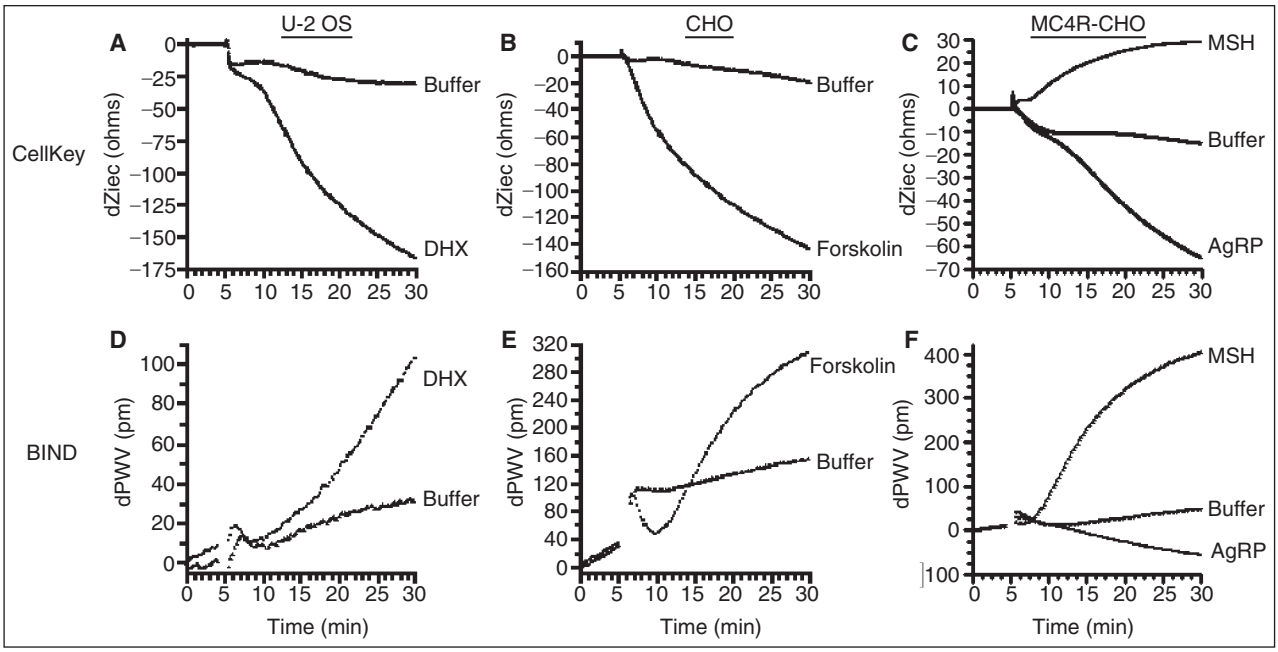

Fig. 4. Comparing Gs and inverse agonist response profiles in CellKey and BIND. Gs response to endogenous dopamine receptor was evaluated in U-2 OS cells (**A**, **D**) treated with D1/5 agonist dihydrexidine (1 μM) and CHO cells (**B**, **E**) treated with forskolin (1 μM). Inverse agonist response was evaluated using MC4R-CHO cells (**C**, **F**) treated with agonist MSH (80 nM) or inverse agonist agouti-related protein (AgRP; 80 nM). CellKey data (**A**–**C**) and BIND data (**D**–**F**).

Compared to Gi, Gs-coupled receptors have an inverse effect on cAMP yet the resulting response profiles are inverted in CellKey only. The lack of a negative response profile for the light-based platforms in agonist mode raises questions about dynamic range. A lack of negative dynamic range could be an important limitation particularly for detecting inverse agonism. To explore inverse agonism, agouti-related protein (AgRP) was selected as a well-established endogenous inverse agonist for melanocortin-4 receptor (MC4R).[13] In CHO cells, the MC4R response is consistent with Gq coupling.[8] In both CellKey and BIND the agonist melanocyte-stimulating hormone (MSH) induced positive response profiles whereas the inverse agonist AgRP produced negative response profiles (*Fig. 4C and 4F*). The potency of AgRP was similar in both assays (pEC_{50} values of 7.3 ± 0.02 and 7.6 ± 0.4 in CellKey and BIND, respectively). These data indicate that the dynamic range for BIND includes negative response profiles, even though this is not observed with agonists that differentially affect cAMP levels.

Success using these label-free whole cell technologies to detect ion channel responses has been limited. One recent exception is the ligand-gated transient receptor potential cation channel, subfamily V, member 1 (TRPV1), which was detected using BIND.[14] In the present study, all 3 instruments detected capsaicin (agonist) responses in TRPV1-CHO cells (*Fig. 5*). These responses were judged to be specific as they were: (i) replicated with the selective agonist resiniferatoxin, (ii) blocked with TRPV1 antagonists (capsazepine and AMG9810), and (iii) not observed in untransfected cells (data not shown). The TRPV1 responses were sensitive to cytochalasin D in each assay. With each instrument, a biphasic concentration–response curve was observed. The optical-based instruments showed an initial positive response with an inflection point at ~10 nM followed by a robust negative response. In contrast, CellKey did not show an inflection point at low agonist concentrations, but rather had a concentration-dependent negative response with an inflection point at 500 nM. The biochemical basis for the distinct phases is unknown and raises the potential for differential sensitivity of these phases. Nonetheless, the data clearly suggest applications for these instruments may extend to ligand-gated ion channels.

DISCUSSION

Relative to traditional GPCR assays, label-free cellular assays offer compelling advantages including: direct detection of Gi-coupled receptors, sensitivity to detect endogenous receptors, detection of orphan receptors regardless of G-protein coupling,

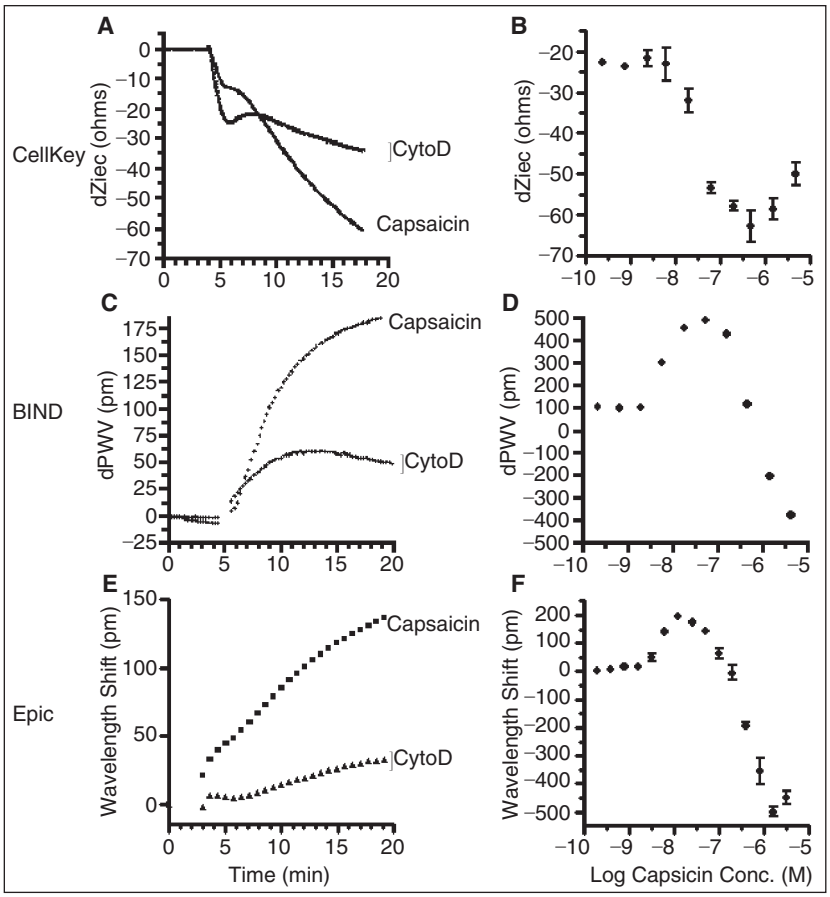

Fig. 5. Responses for the ion channel TRPV1 in CellKey, BIND, and Epic. TRPV1-CHO cells were pre-treated for 30 min (CellKey, BIND) or 15 min (Epic) with buffer or 10 μM cytochalasin D then incubated with capsaicin (100, 50, and 10 nM in **A**, **C**, and **E**, respectively). Profiles are the net response obtained by subtracting vehicle or vehicle plus cytochalasin D. Representative concentration–response curves are shown for capsaicin (**B**, **D**, and **F**). Data are the average of at least 4 wells.

and the ability to discern differential G-protein coupling. Despite these advantages, the technology has been adopted with a cautious recognition that these are essentially phenotypic screens that detect the downstream and summed effects on cell morphology and adhesion. While there is ample evidence supporting numerous connections between GPCRs and morphology,[3] the precise mechanisms detected in each label-free system are not yet established. Thus, it is conceivable that different biosensor technologies could detect different cellular mechanisms and thus different pharmacological responses. Examining the relationship between these technologies is an important first step toward wider application. For this reason, we performed a series of comparative studies with impedance- and optical-based biosensor instruments using well-established targets and ligands.

Developing assays with the BIND, CellKey, and Epic instruments was straightforward. There are limited variables to optimize with label-free detection, resulting in rather short assay development times. All 3 instruments gave similar time-course responses to GPCR agonists, with peak responses of ~5 min for ligands known to activate Gi and Gq pathways. A somewhat slower response was observed for Gs activation, which was typically sustained over the 15-min response period.

In terms of quantitative output, each instrument produced a strong agonist signal with good precision as demonstrated with (i) Z' values ≥0.5 for all assays (*Table 1*) and (ii) relatively small SEM values in concentration–response data for agonists and antagonists (*Figs. 2 and 3*; ref. 5,7,8). Both agonist and antagonist potency values were comparable across all 3 instruments (*Fig. 3*; ref. 5,7,8). Furthermore, the relative potency for the agonist and antagonist pairs are consistent with published results (ACh>CCh for M1[15,16] and M2[17]; scopolamine>pirenzepine for M1[16,18] and M2[18]; YP-20 ~R121919[19]). This suggests that more extensive rank-order potency testing of agonists and antagonists would likely translate across the different instruments; meaning receptor pharmacology for these targets is likely independent of the label-free detection system. This is further supported by the observation that both impedance and optical biosensors detected inverse agonism and gave similar potency values (*Fig. 4C and 4F*).

The qualitative response profiles from the 3 instruments are more similar than different, and are consistent with each technology likely detecting similar underlying cellular mechanisms that involve cytoskeletal reorganization that ultimately influences cell morphology.[2] GPCR activation is known to induce cytoskeletal changes[3] including those associated with receptor internalization.[20,21] Such changes are proposed to influence properties measured with the biosensors used in these studies, namely the index of refraction at the base of the microtiter plate and the resistance to current flow across the cell monolayer. The sensitivity of these measurements to the actin polymerization inhibitor cytochalasin D is consistent with this hypothesis.

Although the 3 instruments gave very similar pharmacology, 2 important differences between the impedance- and optical-based instruments are worth noting. First, the optical-based instruments

can also be used in biochemical assays to quantify binding affinity of ligands to purified proteins (not tested here). Second, the cell-response profiles generated by CellKey qualitatively distinguished Gs, Gi, and Gq activation. Gs signaling induced a negative response; Gi activators induced a positive response; and Gq activation caused a small, transient downward deflection followed by a positive response. These distinct CellKey profiles have been reported previously with other GPCRs.[8,10,22] Our experience agrees with the finding that CellKey profiles are consistent across a range of receptors and cell types. This is noteworthy for its contrast with the optical-based profiles, which are less distinctive (*Fig. 1*). Thus, although CellKey profiles are not definitive (confirmation with pathway-specific agents is required), their apparent robustness is an important distinguishing feature, one that will need to be monitoring as more labs adopt the technology.

Interestingly, another impedance-based instrument, RT-CES, does not distinguish G-protein activation,[6] suggesting that CellKey's scanning of an extended spectrum of wavelengths and its proprietary data transformation process may combine to distinguish signaling. Although subtle differences in response profiles have been reported by Corning on the Epic instrument,[23] it remains to be seen whether such distinctions will be characteristic of those signaling pathways when evaluated on a larger number of GPCRs and by multiple labs. This leads to speculation that future versions of optical-based instruments might employ refined data transformation thereby enhancing the pathway distinguishing capability. Currently, CellKey's unique ability to qualitatively distinguish G-protein pathways has numerous applications including studying the coupling of orphan GPCRs, assessing GPCR pleiotropic signaling and detecting ligands with biased signaling.

Each of the label-free instruments detected biphasic concentration–response curves with the CRF agonists. However, only CellKey was able to provide additional insight on the functional coupling of the receptor relative to the 2 affinity states. The negative impedance response at low agonist concentration and positive response at high concentration was indicative of Gs and Gi coupling, respectively. These results are consistent with published literature measuring cAMP levels that demonstrate high-affinity Gs and low-affinity Gi coupling of CRF1.[11]

Dual coupling is increasingly being recognized as a common feature in GPCR signaling.[24,25] Each of these label-free instruments tested here offers the ability to detect multiple responses yet they differ in ability to distinguishing pathways, which leads to distinct advantages depending on the application. For example, CellKey offers clear advantages for comparing pathway bias of established ligands during secondary screening of HTS hits (CRF in *Fig. 3* and CB1 in ref. 8). Conversely in primary screening mode, nondistinguishing assays can have an advantage for single concentration, single time point screening. For example, due to the similar levels of Gi and Gs signaling with 1 μM CRF and sauvagine, one would not see a signal above background in CellKey at 15 min whereas a signal would be detected in BIND and Epic. Thus, each platform offers distinct features for dual signaling. This requires careful consideration during assay development including fully profiling the target GPCR with reference ligands (if they exist) to ensure the ability to detect the desired pharmacology with a label-free instrument.

Commercial instruments using impedance-based biosensors in 96-well format were introduced in 2004[22] and optical-based biosensors came to market several years later. Thus, these technologies are still relatively new and their full impact on biological and pharmaceutical discovery remain to be fully vetted. In addition to GPCR signaling, label-free biosensors also have been used to quantify cell proliferation, migration, and cytotoxicity.[1] Other applications will emerge as the technology is applied to different biological systems that regulate cell behavior. Additional opportunities under evaluation for label-free detection include studying the regulation of ion channel, nuclear receptor, adhesion molecules, and intracellular enzyme functions. Our initial studies with the TRPV1 ion channel indicate that the label-free biosensors can measure an agonist-specific and concentration-dependent response for this target class. However, the biphasic concentration–response profiles were different between the optical and impedance measurements for reasons that are presently unclear, further demonstrating the complexity of cell-response profiles that can occur. Future studies with TRV1 and other ligand-gated ion channels will hopefully clarify the biochemical properties of these pharmacological targets that can be discerned with label-free biosensors.

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