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Review
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Platform Technologies for Research on the G Protein Coupled Receptor: Applications to Drug Discovery Research

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Abstract

G-protein coupled receptors (GPCRs) constitute an important class of drug targets and are involved in every aspect of human physiology including sleep regulation, blood pressure, mood, food intake, perception of pain, control of cancer growth, and immune response. Radiometric assays have been the classic method used during the search for potential therapeutics acting at various GPCRs for most GPCR-based drug discovery research programs. An increasing number of diverse small molecules, together with novel GPCR targets identified from genomics efforts, necessitates the use of high-throughput assays with a good sensitivity and specificity. Currently, a wide array of high-throughput tools for research on GPCRs is available and can be used to study receptor-ligand interaction, receptor driven functional response, receptor-receptor interaction,and receptor internalization. Many of the assay technologies are based on luminescence or fluorescence and can be easily applied in cell based models to reduce gaps between *in vitro* and *in vivo* studies for drug discovery processes. Especially, cell based models for GPCR can be efficiently employed to deconvolute the integrated information concerning the ligand-receptor-function axis obtained from label-free detection technology. This review covers various platform technologies used for the research of GPCRs, concentrating on the principal, non-radiometric homogeneous assay technologies. As current technology is rapidly advancing, the combination of probe chemistry, optical instruments, and GPCR biology will provide us with many new technologies to apply in the future.

Key Words: G protein coupled receptor, Calcium mobilization, Drug discovery, Receptor interaction, Time resolved fluorescence, Luminescence

INTRODUCTION

G protein coupled receptors (GPCRs) constitute the most important drug target classes (Schroder *et al.*, 2010),accounting for ~40% of current drugs on the market and are involved in various physiological processes (Rozenfeld and Devi, 2010). Drug development efforts have focused on this molecular class, as these receptors are located on the cell surface making them easier to target for modulation of cellular activities. According to a recent market analysis by Insight Pharma Reports (Rubenstein, 2008), GPCRs are targeted for approximately one-third of approved drugs and for hundreds of drugs currently in development.

GPCRs are a family of 7-transmembrane domain receptors that transduce a wide range of physiological functions. Theycan be classified by virtue of their coupling to second messenger signaling systems (Lefkowitz, 2004). GPCRs coupling to, and signaling through,the Gαs family of G proteins result in an increase of intracellular cyclic AMP (cAMP), while coupling to, and signaling through the Gαi/o family of G proteins results in a reduction in intracellular cAMP. Finally, GPCRs which couple to, and signal through, the Gαq family of G proteins produce an increase of cytoplasmic calcium (Berridge, 1993). These changes can be measured and used as surrogates for receptor activity. In addition to the G protein coupling and signaling, receptor-ligand interaction is another important target mechanism to detect and evaluate the affinity of each ligand against specific receptors, providing essential information for chemoinformatic approaches used in structure/activity relationship studies.

During the last decade, several mechanisms that regulate the function and properties of GPCRs have been identified,such as the crosstalk between distinct GPCRs (Alfaras-Melainis *et al.*, 2009). This often occurs as the result of protein-protein interaction between receptor types (Ferre *et al.*, 2009). Receptor-receptor interactions including heteromerization are emerging as an important process involved in the specialization of receptor function. Reflecting those research needs, vendors are launching technological schemes to test receptor-receptor interactions. Other noteworthy applications

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Table 1. Current technologies for the research of GPCRs and their detection methods

Mechanism	Technology	Detection method	HTS level[a]	Commercial vendor
Receptor-ligand interaction	Scintillation proximity assay	Radioactivity	+++	PerkinElmer
	Radioligand binding assay	Radioactivity	+	PerkinElmer
	Tag-lite fluorescent ligand	TR-FRET[b]	+++	Cisbio
	DELFIA[c] ligands	TRF[d]	+	PerkinElmer
	Fluorescent ligands	FP[e]	+++	Invitrogen (PanVera)
Receptor-functional responses	LANCE[f]-cAMP Detection	TR-FRET	+++	PerkinElmer
	AlphaScreen cAMP	ALPHA[g]	+++	PerkinElmer
	HitHunter cAMP	Luminescence	+++	DiscoveRx
	HitHunter IP_3	FP	+++	DiscoveRx
	Catchpoint cAMP/cGMP	FP	+++	Molecular Devices
	cAMP/IP-One HTRF	HTRF[h]	+++	Cisbio
	cAMP immunoassay	ELISA[i]	+	Abcam, BioVision
				Cayman Chemicals
				Cell Signaling Technology
				Enzo Life Sciences
				Promega Molecular Devices
	GloSensor cAMP assay	Luminescence	+++	Promega
	ACTOne cAMP Biosensor	FI[j]	++	BD Bioscience
	GeneBLAzer	FRET[k]	+++	Invitrogen
	PhotoScreen	Luminescence	++	PerkinElmer
	AequoScreen	Luminescence	++	PerkinElmer
	Calcium assay dye	FI	++	Molecular Devices
				Invitorgen, Biotium
				Enzo Life Sciences
				BD Biosciences, DiscoveRx
				ScienCell Research
	PathHunter β-arrestin	Luminescence	+++	DiscoveRx
	Tango GPCR assay	FRET	+++	Invitrogen
	Transflour assay	HCA[l]	++	Molecular Devices
Receptor-receptor interaction	PathHunter GPCR dimerization detection	Luminescence	+++	DiscoveRx
	Tag-lite GPCR dimer assay	TR-FRET	+++	Cisbio
	BRET[m]	Luminescence	+++	PerkinElmer
Receptor internalization	PathHunter GPCR endocytosis detection	Luminescence	+++	DiscoveRx
	Tag-lite GPCR internalization	TR-FRET	+++	Cisbio

+: low throughput because of operational procedure, ++: medium throughput as for the need of kinetic reading, +++: high throughput amenable assay. [a]HTS level was estimated in three levels based on detectors with photomultiplier tube, [b]TR-FRET: time-resolved fluorescence resonance energy transfer, [c]DELFIA: dissociation-enhanced lanthanide fluorescent immunoassay, [d]TRF: time-resolved fluorescence, [e]FP: fluorescence polarization, [f]LANCE: lanthanide chelate excite, [g]ALPHA: amplified luminescent proximity homogeneous assay, [h]HTRF: homogeneous time-resolved fluorescence, [i]ELISA: enzyme-linked immunosorbent assay, [j]FI: fluorescence intensity, [k]FRET: fluorescence resonance energy transfer, [l]HCA: high content analysis, [m]BRET: bioluminescence resonance energy transfer.

for GPCRs include platforms for the study of receptor internalization.

Currently, the increasing number of diverse small molecules is being generated through combinatorial chemistry (Pinilla *et al.*, 2003), and extensive genomic efforts have identified-numerous novel GPCR targets. Therefore, the use of high-throughput assays with good sensitivity and specificity is necessary for high throughput screening campaign (Burbaum and Sigal, 1997). Especially, for drug discovery research based on GPCR targets, the challenge is how to look for potential drug candidates using a predefined compound library.

In this review, a wide array of high-throughput tools for GPCR research are described, and their use for the development of diverse GPCR assessment platforms needed to study

physiological functions of GPCRs and to develop potential drug candidates, are classified into four different categories as summarized in Table 1: receptor-ligand interaction, receptor-functional responses, receptor-receptor interaction, and receptor internalization.

RECEPTOR-LIGAND INTERACTION

Radioactivity based detection

There are numerous methods to detect receptor-ligand interactions by means of radioactivity. As GPCR scientists are trying to find molecules that inhibit or modulate the action of a ligand on a receptor and thus influence the activity of the receptor itself, examining the binding of ligand to a receptor has been the classic means used to study the structure/activity relationship. Thousands of radiolabeled ligands containing ^{3}H, ^{125}I, and ^{14}C are available from PerkinElmer, Inc. The advantages of radiolabeled ligands are versatility, minimum size variation comparing to the original ligands, and cost-effectiveness, however, the use of those ligands are limited because of their intrinsic half-life and the fact that global environmental issues concerning radioactive materials are becoming more sensitive.

In classic binding assays, membrane preparations containing GPCRs of interest are incubated with radiolabeled ligands in the presence of test compounds to assess the chemical's ability to disrupt receptor-ligand interactions in a heterogeneous mode. However, scintillation proximity assays (SPAs) can avoid washing steps needed to remove unbound ligands prior to reading, and provide a homogeneous assay (Wu and Liu, 2005; Glickman *et al.*, 2008). SPAs uses scintillation beads that can be read on conventional scintillation counters and are suitable for the detection of various isotopes including ^{3}H, ^{125}I, ^{33}P, and ^{14}C. In addition to the ligand and assay characteristics, membrane preparations for target GPCRs can be secured by in-house development or can be supplied from vendors such as PerkinElmer Life Science, Multi span, Chan Test, and Caliper Life Sciences.

Fluorescence based detection

To overcome numerous limitations related to health and waste disposal in the use of radioactivity based experiments, a growing interest in alternative, nonradioactive technologies (Liu *et al.*, 1998) has become more evident. One traditional fluorescence application for receptor-ligand binding is the fluorescence polarization (FP) based approach (Gagne *et al.*, 2002). Although the FP based approaches are still applied to various enzyme activity assessments, their use in GPCR research is not as wide-spread because of the limited signal to noise ratio. However, the FP assay itself is a very simple, homogeneous, and cost-effective platform.

Fluorescent ligands have been used for decades and offer several advantages over traditional radioligand binding techniques (McGrath *et al.*, 1996). The use of fluorescent ligands is steadily growing within the pharmacological community and other disciplines (Daly and McGrath, 2003), although such ligands have multiple drawbacks including a large fluorescent background of reagents, which decreases the assay window with plastics and biological samples, leading to reduced-dynamic range and sensitivity of fluorescent tags. Recently, lanthanide labels were introduced and a new caliber of fluorescence applications was made possible. One of major benefits of lanthanide labels is that the long lifetime of the excited state allows the specific signal from lanthanide to be temporally separated from the non-specific signal (Handl and Gillies, 2005), and this phenomenon enables the time resolved fluorescence (TRF) detection. Recent reports (Inglese *et al.*, 1998; Gao *et al.*, 2004; Handl *et al.*, 2004, 2005; Lee *et al.*, 2006) have described the use of lanthanide labeled ligands in a traditional competitive binding assay format, and those ligands are similar to the radiolabeled ligands in potency and efficacy .Several dissociation-enhanced lanthanide fluorescent immunoassays (DELFIAs) are now available.

Current developments in TRF applications include the time resolved fluorescence resonance energy transfer (TR-FRET) technology. To construct a homogeneous assay, TR-FRET employs a second acceptor component to emit a light when the lanthanide labeled ligand is bound to its receptor. The lanthanide chelate excite (LANCE) TR-FRET uses alexa647 labeled lectins or biotinylated lectins in combination with allophycocyanin labeled streptavidin (Hemmila, 1999; Jansson *et al.*, 1999; Branchek *et al.*, 2000). Most recently, Tag-lite™, a homogeneous assay technology based on TR-FRET, was announced as a versatile platform technology for the study of receptor-ligand interactions (Maurel *et al.*, 2004). This assay employs cells genetically modified to express GPCRs that are coupled to a small suicide enzyme (SNAP tag). In the presence of an appropriate fluorescent substrate, an enzyme covalently conjugates part of its substrate onto itself, providing a way to label GPCR. To detect ligand binding, the labeled cells are incubated with a ligand that is labeled with another fluorophore, and if there is an interaction with a receptor-ligand, the receptor produces a FRET signal in a time resolved mode.

RECEPTOR-FUNCTIONAL RESPONSES

Measurements of cAMP and IP_3 (IP_1)

After a receptor-ligand interaction, ligands are bound to their specific receptors, and a series of intracellular events are started based on the types of receptor coupled G proteins involved. Those second messengers include cAMP, inositol triphosphate (IP_3), and calcium released from intracellular storage. Ligand binding analyses conducted in membrane based assays don't always directly translate to the functions of live cells, and most GPCR assays focus on downstream signaling events, especially those involving second messengers. Some radioactivity based assays are available for this purpose. Additionally, the enzyme-linked immunosorbent assay (ELISA) has been widely employed to detect cAMP. The ELISA method, while reliable and accessible by any laboratory, is heterogeneous and involves a washing step, making it slow and not cost-effective. Recently, amplified luminescent proximity homogeneous assay (ALPHA) technology was introduced as substitute for various ELISA methodologies to improve the assay quality and throughput.

For the measurements of cAMP and IP_3/IP_1, recent developments include LANCE (TR-FRET), the β-lactamase reporter assay (FRET), fluorescence polarization, and homogeneous time resolved fluorescence (HTRF) based detection. Unlike ELISA methods, these assays are homogeneous, can be performed using a high-throughput format and allow for scale-up or miniaturization, thereby reducing assay development time

and costs. Especially, several metabolites in the inositol phosphate cascade have extremely short half-lives, making them difficult to accurately quantify. IP_1, a downstream metabolite of IP_3, accumulates in cells following Gq receptor activation and is stable in the presence of LiCl, making it a reasonable readout of receptor activation. Also, cAMP can be detected with the live cell luminescent assays by fusing a cAMP binding domain from human protein kinase A to the wild type N- and C-termini of firefly luciferase, with new termini created in the larger N-terminal domain (Fan *et al.*, 2008).

Most recently, enzyme fragment complementation (EFC) technology was introduced as a robust and reliable tool for the assay of biochemical components. EFC is a homogeneous, non-radioactive detection technology based on two genetically engineered β-galactosidase (β-gal) fragments: a large protein fragment (enzyme acceptor, EA), and a small peptide fragment (enzyme donor, ED). Separately, the β-gal fragments are inactive, but in solution, they rapidly recombine to form the active β-gal enzyme that hydrolyzes substrate, producing an easily detectable chemiluminescent or fluorescent signal. Using this technology, cAMP (luminescence detection) and IP_3 (FP) have been investigated in various biological systems (Sanger *et al.*, 2009; Chen *et al.*, 2010; Eapen *et al.*, 2010; Hampton and Kinnaird, 2010).

Measurement of intracellular calcium

The central role of Ca^{2+} in intracellular signaling makes it a very attractive reporter for the process of drug discovery. Many of the most interesting target classes for the pharmaceutical industry, such as GPCRs, ion channels, and transporters, trigger a Ca^{2+} mobilization upon activation (Rink, 1990). Determination of the activity of targets by measurement of increased intracellular Ca^{2+} concentration is now a very common approach, and the development of more sensitive technologies for the measurement of Ca^{2+} concentration has long been a matter of interest. The first Ca^{2+} mobilization assays were developed using fluorescence intensity produced by formation of a complex with Ca^{2+}, but their use for HTS was limited until the development of the fluorometric imaging plate reader (FLIPR). Although the use of fluorescent probes in a homogeneous no-wash format has become very common in HTS, its use is complicated by the inherent fluorescence of dye loaded cells and interference by the compounds themselves.

Photoproteins have been used to more effectively sense intracellular Ca^{2+} concentrations and overcome current limitations of fluorescence applications (Hedley *et al.*, 1996; Stables *et al.*, 1997; Dupriez *et al.*, 2002; Le Poul *et al.*, 2002; Bovolenta *et al.*, 2007). The most commonly studied photoproteins are aequorin, isolated from *Aequorea victoria*, and obelin, isolated from *Obelia longissima*. Upon calcium binding, the photoprotein oxidizes coelenterazine into colenteramide, with the production of CO_2 and the emission of luminescentlight. Aequorin assays have been validated for many GPCRs and some Ca^{2+} channels, and the results obtained are comparable to those obtained with the use of fluorescent dyes (Maeda *et al.*, 1996).

Measurements of β-arrestin and extracellular signal regulated kinase (ERK)

The arrestins constitute a family of proteins that are capable of interacting with GPCRs following their activation by agonists and subsequent phosphorylation by G protein coupled receptor kinases (GRKs)(Perry and Lefkowitz, 2002). Arrestin binding, recognized by both the GRK phosphorylation site and the agonist-activated conformation of receptors (Luttrell and Lefkowitz, 2002), leads to uncoupling of the receptor from its cognate G protein causing desensitization of G protein signaling via downstream second messenger molecules (Lohse *et al.*, 1990; Perry and Lefkowitz, 2002; Gainetdinov *et al.*, 2004; Lefkowitz, 2004). Using this theoretical background, a β-arrestin-based assay can rule out false positive hits from a calcium or cAMP primary screen, or validate second messenger hits as an alternative, non-G-protein mediated signaling pathway.

The assay monitors GPCR activity by detecting the interaction of arrestin with the activated GPCR using EFC (Zhao *et al.*, 2008; McGuinness *et al.*, 2009). In this system, the β-galactosidase (β-gal) enzyme is split into two inactive fragments. The larger portion of β-gal, termed EA for enzyme acceptor, is fused to the C-terminus of β-arrestin. The smaller, 4 kDa complementing fragment of β-gal, the ProLink tag, is expressed as a fusion to the C-terminus of the GPCR of interest. Upon activation, the interaction of β-arrestin and the GPCR forces the interaction of ProLink and EA, resulting in complementation of the two fragments of β-gal and the formation of a functional enzyme capable of hydrolyzing substrate and generating a chemiluminescent signal.

Other assay platforms use the protease tagged arrestin and a non-native transcription factor fused to the C-terminus of the GPCR. As the recruited protease tagged arrestin cleaves the transcription factor by its fused protease site, the transcription factor directly regulates transcription of a β-lactamase reporter construct, which is measured upon addition of the substrate by FRET. Furthermore, by attaching a fluorescent label to β-arrestin (Oakley *et al.*, 2002), the location of the receptor-arrestin complex can be monitored during receptor activation by high content analysis (HCA).

Upon stimulation of GPCRs by their ligands, receptors activate effectors such as adenylate cyclase and phospholipase C, which influence intracellular concentrations of second messengers,and also mediate the phosphorylation of extracellular signal regulated kinase (ERK1/2). GPCRs have also been shown to mediate ERK1/2 activation in a G protein-independent, but β-arrestin dependent manner (Lin *et al.*, 1999; Luttrell and Lefkowitz, 2002; Shenoy and Lefkowitz, 2003). Based upon this background, measurements of phosphorylated ERK by TR-FRET and ALPHA can be deployed as a secondary screening platform.

RECEPTOR-RECEPTOR INTERACTION

GPCRs can interact with each other at the plasma membrane to form homodimeric and heterodimeric complexes that can profoundly impact GPCR pharmacology and signaling (Franco *et al.*, 2008; Ferre *et al.*, 2010). Compounds that specifically target GPCR heterodimers have the potential to achieve higher specificity with reduced side effects, define new drug targets, or behave as modulators of GPCR activity in specific tissues (Rozenfeld and Devi, 2010). As a result, there is considerable interest in the design of drugs that target GPCR heterodimers (Bhushan *et al.*, 2004; Daniels *et al.*, 2005; Waldhoer *et al.*, 2005; Xie *et al.*, 2005).

The EFC based GPCR dimerization system is designed to identify novel compounds that act through GPCR heterodimer

pairs. In this system, arrestin is fused to the larger portion of β-gal, termed enzyme acceptor of EA, and the smaller, 42 amino acid ProLink tag is localized to one of the GPCR targets of interest. Using these arrestin clones as the starting material, a second untagged GPCR receptor can be stably introduced into the cell line and the effects of the untagged GPCR on the ProLink-tagged GPCR can be measured using chemiluminescence detection reagents (Vidi and Watts, 2009). In addition to the EFC technology, the bioluminescence resonance energy transfer (BRET) has been applied for research on receptor-receptor interactions (Hamdan *et al.*, 2006). One candidate interacting GPCR is fused to a luminescent energy donor, such as Renilla luciferase, and the other to a fluorescent energy acceptor, such the green or yellow fluorescent protein (GFP/YFP), and the two are then coexpressed in the same cells. If the two GPCRs interact, their close proximity allows nonradiative energy transfer (BRET) between the luciferase and the GFP/YFP. BRET does not occur if the two GPCRs are separated by more than 100 Å, making the technique ideal for monitoring receptor-receptor interactions in biological systems.

Most recently, a methodology combining TR-FRET with SNAP technology to quantitatively analyze receptor-receptor interactions at the surface of living cells was investigated (Albizu *et al.*, 2010; Maurel *et al.*, 2008). A newer version of the Tag-lite platform, with a dual tag (SNAP/CLIP tag), is under development to label with two different substrates. Some of the known receptor-receptor interactions in various GPCR targets are listed in Table 2.

RECEPTOR INTERNALIZATION

GPCRs are thought to become separated from their cognate G proteins and effectors as they start to internalize. Endocytosed receptors either traffic to lysosomes for degradation or follow a recycling route. In this way, receptor internalization controls receptor density at the cell surface and serves as a mechanism to attenuate signal duration and strength, although it has been known that endocytic trafficking does not necessarily lead to signal termination (Calebiro *et al.*, 2010; Jalink and Moolenaar, 2010). Therefore, GPCR platforms have been developed that are not based on specific signaling mechanisms to confirm and expand effects of an agonist or antagonist.

Most recently, an internalization assay based on a reduction of the HTRF was introduced. This Tag-lite™ assay is G protein and β-arrestin independent, and applicable to perform GPCR kinetic internalization assays. The detection reagent used is non-permeable and contains the HTRF acceptors. When the receptor is at the cell surface, HTRF occurs between the SNAP-Lumi4-Tb labeled GPCR and the detection reagent in the medium causing the FRET ratio to be decreased. When the receptor is internalized, energy transfer can no longer occur, causing an increased FRET ratio.In the presence of a specific agonist, the GPCR desensitization induces receptor internalization which causes low dynamic FRET to have a high ratio signal.

Another assay was designed using low-affinity EFC technology, which relies on protein proximity to yield complementation of two partial fragments of β-galactosidase. One enzyme fragment is localized exclusively to the surface of cellular endosomes and the complementing enzyme fragment is fused to the GPCR of interest. Stimulation of the target receptor results in internalization of the receptor and trafficking to cellular endosomes. This action forces the complementation of the two enzyme fragments, resulting in an increase of enzyme activity that is easily measured using chemiluminescent detection.

CONCLUSION AND FUTURE PERSPECTIVES

GPCRs are transmembrane receptors that transmit signals from the outside of a cell to the inside. These receptors are involved in every aspect of human physiology, including sleep regulation, blood pressure, mood, food intake, perception of pain, control of cancer growth, and immune response (Lefkowitz, 2004). With their complex signaling network pathways, GPCRs constitute one of the most important target

Table 2. Receptor-receptor interactions in various GPCR targets

Receptor homodimerization or heteromerization	Methods deployed	Reference
α2A/α2C adrenergic receptor	BRET, coimmunoprecipitation	(Small *et al.*, 2006)
AT_1/B_2 receptor	Colocalization, coimmunoprecipitation	(AbdAlla *et al.*, 2001)
D1/D3 dopamine receptor	FRET, BRET	(Fiorentini *et al.*, 2008; Marcellino *et al.*, 2008)
$GABA_B$ receptor	TR-FRET, HTRF orantibodies	(Maurel *et al.*, 2008)
$GABA_B$/CaS receptor	Colocalization, coimmunoprecipitation	(Cheng *et al.*, 2007; Tamayama *et al.*, 2005)
$GABA_B$/MGlu1a receptor	BRET, HTRF, colocalization	(Hirono *et al.*, 2001; Kamikubo *et al.*, 2007; Rives *et al.*, 2009; Tabata *et al.*, 2004)
MGlu1a/A1 adenosine receptor	Colocalization, coimmunoprecipitation	(Ciruela *et al.*, 2001; Toms and Roberts, 1999)
MGlu5/A2A adenosine / D2 dopamine receptor	Colocalization, coimmunoprecipitation, pull-down, bimolecularfluorescence complementation, BRET	(Cabello *et al.*, 2009; Ferre *et al.*, 2002; Ferre *et al.*, 2010; Soriano *et al.*, 2009)
MGlu2a/5-HT2A serotonin receptor	BRET, colocalization	(Gonzalez-Maeso *et al.*, 2008)
δ/μ opioid receptor	Colocalization, coimmunoprecipitation	(Decaillot *et al.*, 2008; Wang *et al.*, 2008)
μ opioid/α2A adrenergic receptor	FRET microscopy	(Vilardaga *et al.*, 2008)

classes studied in the pharmaceutical industry. Currently, a wide array of high-throughput tools is available for research on GPCRs, and these tools are applicable for detecting the receptor-ligand interaction, receptor driven functional response, receptor-receptor interaction and receptor internalization. The main focus in these research areas includes development of non-radiometric technologies in the homogeneous assay format, permitting the newly developed assay to be applied in a HTS campaign for drug discovery efforts.

As one of important emerging technologies, label-free platforms such as EPIC (Dodgson *et al.*, 2009; Schroder *et al.*, 2010), an optical-based biosensor, and Cellkey (Verdonk *et al.*, 2006; Peters *et al.*, 2007), an electrical impedance-based biosensor, are receiving attention because there is no need to modify target cells. These label-free platforms may provide a broader cellular concept of GPCR functionality. For example, EPIC uses changes in the refractive index of light on a sensor surface to detect subtle fluctuations in mass distribution such as those caused by cell movement or vesicle trafficking. However, the resulting label-free data information is complex and difficult to analyze, therefore, established *in vitro* assays should be integrated to deconvolute the holistic information.

In the same sense, multiple readouts in the mechanistic studies of GPCRs are receiving more attention because of their complicated signaling mechanisms and intracellular interactions. Recently, a possible flaw in a luminescence reporter assay was reported (Auld *et al.*, 2009). In this case, compounds could directly affect the reporter, leading to nonspecific, but highly reproducible assay signal modulation. Therefore, the consideration of counter-screening is recommended in most uses of the assay platform.

In addition to luminescence technologies, homogeneous fluorescence methodologies provide highly sensitive and robust technology for the detection of molecular interactions during the HTS stage of drug development. New fluorescent ligands and photoproteins have been developed to address the need for cell based models that can reduce gaps between *in vitro* and *in vivo* studies conducted for drug discovery programs. However, because of the technology barrier presented by intellectual property issues, such as licensing costs, the use of those technologies is very limited in the academic sector comparing to the pharmaceutical industries. Fortunately, some of those assay kits are provided in the form of growth arrested frozen cells, so that one can conveniently use commercial kits without the need for assay validation or culture maintenance.

In conclusion, the availability of research tools for GPCR studies has been very limited compared to other biochemical targets. Recently, various platform technologies for research on GPCRs have been developed and efforts are being made to more closely mimic physiological conditions and obtain a higher screening throughput. Assay technology is rapidly advancing and the combination of probe chemistry, optical instruments, and GPCR biology will provide us with many new technologies to apply in the future.

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