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THEMED ISSUE REVIEW

Advancements in G protein-coupled receptor biosensors to study GPCR-G protein coupling

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Enzymatic and cellular signalling biosensors are used to decipher the activities of complex biological systems. Biosensors for monitoring G protein-coupled receptors (GPCRs), the most drugged class of proteins in the human body, are plentiful and vary in utility, form and function. Their applications have continually expanded our understanding of this important protein class. Here, we briefly summarize a subset of this field with accelerating importance: transducer biosensors measuring receptor-coupling and selectivity, with an emphasis on sensors measuring receptor association and activation of heterotrimeric signalling complexes.

KEYWORDS
BERKY, biosensor, BRET, FRET, functional selectivity, G protein coupled receptors, G proteins, GEMTA, GPCR, signalling bias, SPASM, transducerome, TRUPATH

1 | INTRODUCTION

As cell surface receptors that initiate signal transduction cascades, G protein-coupled receptors (GPCRs) act as information conduits that allow cells to adapt to their dynamic local environment (Hilger et al., 2018). Extracellular signals are transmitted via recruitment and activation of transduction partners, which include arrestin proteins and multiple heterotrimeric G protein complexes (16 different non-visual Gα subunits from four major families, and a Gβγ dimer made up of any 4 Gβ and 12 Gγ subunits) (Olsen et al., 2020; Weis & Kobilka, 2018). Their diversity as a protein superfamily and that of their signalling partners, called transducers, combine to make GPCRs master regulators of cellular physiology.

Ontological features of GPCRs have included their endogenous ligands (inputs) and signalling pathways (outputs), and the goal of identifying and characterizing GPCRs has been dominated by establishing these relationships (Kenakin, 2017; Wacker et al., 2017). As GPCRs have been one of the most fruitful targets in drug discovery, expanding the number of druggable GPCRs by contextualizing these relationships holds significant promise for developing novel therapeutic strategies (Hauser et al., 2017; Insel et al., 2019). Additionally, it is now accepted that these features are interrelated rather than independent, with the study of multiple receptor–ligand–pathway relationships forming a subdomain within GPCR pharmacology called "biased-signalling" or "functional-selectivity" (Kenakin, 2012; Wingler & Lefkowitz, 2020). There are substantial efforts to exploit functional-selectivity to discover and design drugs that act through the same receptor, but produce divergent physiological effects, with the goal of producing drugs with increased safety and efficacy profiles (Singla et al., 2019).

Historically, GPCR activity has been measured in cellular contexts where enzymatic transduction cascades can be used to amplify and detect signals, such as accumulation of second messengers or persistent changes in cellular activity such as gene transcription (Cheng et al., 2010; Fan et al., 2008). Traditional second-messenger and transcriptional reporter assays can encompass a broad array of functional readouts of GPCR activity. However, their design and their position in intermediary or terminal stages of the transduction cascade can contribute interference in the form of feedback loops, kinetic considerations, and interactions with other cellular machinery (Purvis & Lahav, 2013). Methods for more direct measurement of GPCR-signalling activities have recently undergone substantial development in quality and variation, improving the confidence with which researchers map the transduction-coupling preference of GPCRs and ligands in their active states.

Abbreviations: BERKY, BRET biosensor with ER/K linker and yellow fluorescent protein (YFP); BRET, bioluminescence resonance energy transfer; EMTA, effector membrane translocation assay; FRET, fluorescent resonance energy transfer; GEMTA, G protein effector membrane translocation assay; RLuc, Renilla luciferase; SPASM, systematic protein affinity strength modulation; TRUPATH, TRansdUcer PATHways.

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Biosensors designed to measure the activity of signal transduction partners such as arrestins or specific Gα proteins offer a compelling alternative as molecular pharmacology tools. Because of their position in the signalling cascade, observations such as association with a GPCR or conformational changes associated with their activation can be interpreted with less ambiguity. To this end, multiple groups have put forth biosensors of different designs to better capture the breadth of GPCR-signalling capabilities.

2 | CHIMERIC G PROTEIN BIOSENSORS

Chimeric G protein biosensors exploit putative determinants of receptor-Gα coupling to construct chimeric Gα subunits that retain the selectivity of their parent class but share a common output. The earliest versions of this sensor class exchanged the native Gαq and Gα15 C-termini with the C-termini of other Gα (Conklin et al., 1993; Gomeza et al., 1996). This approach was widely employed to uniformly measure protein kinase C-mediated phosphotidylinositol production and high-throughput intracellular calcium release across Gα classes (Liu et al., 1995). More recently, **TGFα** shedding, a non-canonical behaviour attributed to Gα12/13/q class subunits (Inoue et al., 2011; Kawakami et al., 2022), has been used in conjunction with C-terminal chimeras to measure Gα subunit coupling. The most extensive study of this method swapped the C-terminus of each Gα onto Gα12 and expressed this chimera in cells lacking Gα12/13/q proteins to measure TGFα shedding following receptor activation, which was interpreted as positive identification of a coupling preference for the Gα from which the C-termini was taken (Inoue et al., 2012) (Figure 1a, Table 1). While this approach provides an adaptable and high-throughput method for generic profiling, this method does not directly measure Gα/receptor engagement. TGFα-shedding relies on a cascade of effectors downstream of G protein activation and this method uses chimeric Gα proteins that do not entirely reconstitute the specificity of their endogenous counterparts (Okashah et al., 2019). For example, correlation of prostaglandin-receptor coupling preferences between this technology and an orthogonal Gα-activation sensor is modest, particularly for Gαs-coupling events (Inoue et al., 2012). This may be due to the insufficient understanding of coupling mechanisms underlying the engagement between receptors and their transducers. For example, the occupancy of the Gα C-terminus in the receptor has been demonstrated to be insufficient to fully confer Gα selectivity (Okashah et al., 2019). Recently, a high-throughput recruitment based Gα biosensor system was used to profile many of the same receptors (Avet et al., 2022). The overlapping identity of coupling between these methods has been scrutinized, and a number of discrepancies and agreements between the two methods have been noted (Hauser et al., 2022).

3 | RECRUITMENT-BASED BIOSENSORS

Advancing in popularity are sensors integrated directly into the transducer protein. While these sensors represent the most direct and accurate measurement of receptor enzymology, their designs have required time to fully mature. Other, more modular, GPCR/transducer biosensors were contemporaneously developed and adopted, using protein–protein interaction assay formats (Figure 1b) to measure recruitment of arrestins to GPCRs (Bertrand et al., 2002) (Figure 1c, Table 1). Most commonly this took the form of Förster Resonance Energy Transfer, labelling the receptor and arrestin with complementary fluorescent (FRET) or bioluminescent (BRET) proteins. Because G protein/receptor interactions are shorter lived, the extension of this methodology to Gα proteins has been limited in their development and application relative to other platforms (Laschet et al., 2019). Recently, "Mini" Gα proteins consisting only of the GTPase domain of the Gα were developed to overcome this kinetic limitation (Carpenter & Tate, 2016). These subunits associate with an active GPCR but are unable to load **GTP** and thus cannot dissociate. While generating fluorescently labelled chimeras (or using enzymatic complementation technology) have permitted the measurement of Gα-subunit recruitment to GPCRs (Figure 1d, Table 1), mini-G technology was originally developed with the requirement that the protein be catalytically inert, soluble and expressible in *E. coli*, properties which are non-native to most wild-type Gα subunits. Most variants are predicated on a chimera with the original mini-Gαs subunit (Wan et al., 2018). The effects of truncation and chimerization may permit coupling events, affinity or other behaviours uncharacteristic of the wildtype Gα. At present, no complete set of mini-G biosensors have been compiled and their full development and characterization remains outstanding. Nevertheless, mini-Gα technology represents an important complementary biosensor technology for both studying the molecular pharmacology of GPCRs but also in facilitating purification and structural biology efforts.

Another technology, Systematic Protein Affinity Strength Modulation (SPASM) (Malik et al., 2017), straddles the chimeric G protein biosensor and recruitment-based biosensor categories. Originally, SPASM utilized a GPCR fused to a fluorescent resonance energy transfer (FRET) acceptor, which was additionally linked to a C-terminal fragment of a FRET donor-labelled Gα. Association of the fused Gα fragment to its cognate GPCR resulted in increased FRET signal (Malik et al., 2013). The size and design of the helical linker spacing the sensor reduces stochastic interaction of the Gα fragment when the system is at rest but does not prevent agonist-induced formation of receptor/Gα fragment complexes and thus, FRET signal is a reliable readout of agonist-induced receptor activation. SPASM has been applied by some groups to study heterotrimeric complex behaviours such as the kinetics of transducer-engagement (Gupte et al., 2019) and allostery (Culhane et al., 2022). Many of the same caveats of the chimeric Gα sensory systems apply to these data, such as the question of whether a C-terminal fragment is sufficient to impart the selectivity profile of a full heterotrimeric complex, with additional consideration that receptor/Gα complex dynamics and kinetic data generated lack the influence of the full-length Gα/Gβγ complex or native receptor sequence. Towards addressing this, SPASM has undergone additional development to utilize full-length Gα subunits in place of the minimal C-terminal fragment

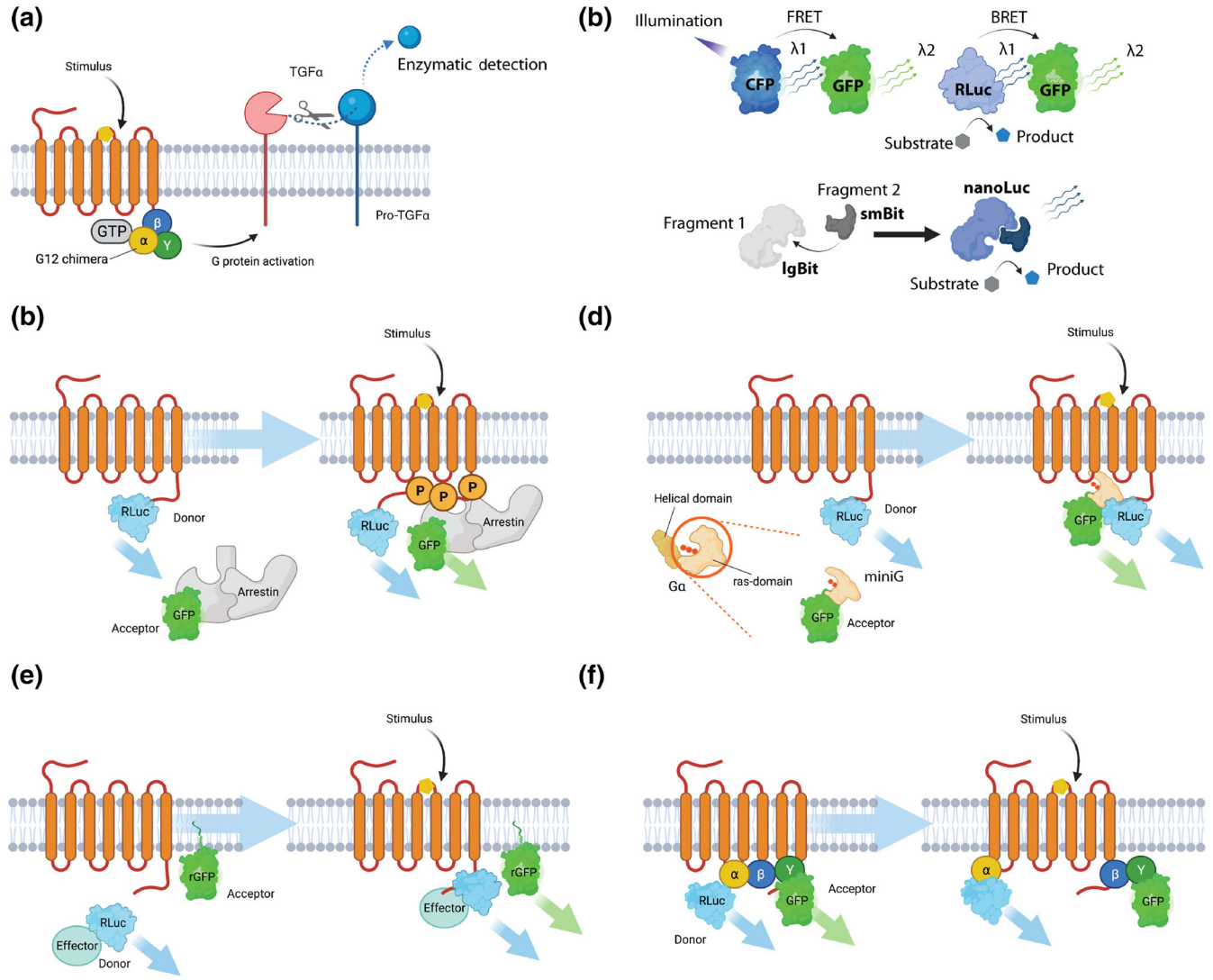

FIGURE 1 (a) GPCR-stimulation leads to activation of a heterotrimeric G-protein complex consisting of a chimeric Gα12 with a C-termini of another Gα. Downstream of Gα12 activation is the cleavage of Pro-TGFα and the release of TGFα which can be detected enzymatically (Inoue et al., 2012). (b) Different formats for detection of protein–protein interaction. Fluorescence resonance energy transfer (FRET) where external illumination of one fluorophore in sufficient proximity to a second fluorophore with complementary excitation and emission spectra produces two signals, a change in ratio of the second to the first can be interpreted as a change in proximity of the two fluorophores. Bioluminescent resonance energy transfer (BRET) wherein the first fluorophore is replaced with a luciferase (RLuc) as the light-emitting donor. Enzyme complementation, specifically NanoLuc luciferase complementation, where two fragments of the enzyme in close enough proximity reconstitute a functional luciferase capable of catalysing a light-emitting reaction with its substrate. (c) Intracellular labelling of a GPCR with a luciferase (or another donor) and an arrestin with a complementary fluorophore (Bertrand et al., 2002). When the GPCR is activated, Arrestin may be recruited and an increase in BRET can be detected. (d) Recruitment BRET assay with mini-G proteins (Wan et al., 2018). Labelling of the mini-G protein with a fluorophore allows for the detection of activity-induced BRET between the mini-G and a labelled GPCR. (e) Instead of labelling a receptor, a fluorescent protein can be labelled with a membrane localization/anchoring motif (Avet et al., 2022; Namkung et al., 2018). Recruitment of transducers to the receptor increases proximity and density at the membrane, producing an increase in BRET that avoids the need for modification of the GPCR. (f) Heterotrimeric G protein biosensors measure GPCR signalling by measuring a change in BRET between a labelled Gα and a labelled Gβγ dimer, which avoids the need for modification of the GPCR (Adjobo-Hermans et al., 2011; Galés et al., 2005; Janetopoulos et al., 2001; Mastop et al., 2018; Olsen et al., 2020; Saulière et al., 2012; van Unen et al., 2016). Notable examples of these technologies are referenced in Table 1.

(Gupte et al., 2021; Mackenzie et al., 2019; Malik et al., 2017) though the earlier format continues to be used by some groups (Gupte et al., 2019). While earlier applications of SPASM often utilized confocal microscopes (Gupte et al., 2019) or cuvette readers (Semack et al., 2016) for detection, SPASM sensors have been incorporated into giant plasma membrane vesicles, which conferred improved dynamic range sufficient for miniaturization and use in conventional plate readers (Kim et al., 2021). Bioluminescence resonance energy transfer (BRET) formulations of both full-length Gα and C-terminal fragments have also been used to miniaturize the assay to a 96-well

TABLE 1 Examples of GPCR biosensor classes from Figure 1 and representative citations

Biosensor category	Example biosensor	Description	Representative citations
Chimeric G protein biosensors	Gαq/Gα15 C-terminal chimeras	Chimeric Gα proteins substituting subclass-specific C-terminal fragments onto Gαq or Gα15, leading to the activation of the production of IP3 and Ca^{2+} as detectable second messengers	Conklin et al., 1993; Gomeza et al., 1996
	Gα12 chimera	Chimeric Gα proteins substituting subclass-specific C-terminal fragments onto Gα12, activation of which leads to extracellular shedding and accumulation of TGFα	Inoue et al., 2012
Recruitment based biosensors	Labelled receptor/Arrestin complexes	Complementary labelling of a receptor and arrestin protein for detection of protein–protein interaction (e.g. via BRET or FRET)	Bertrand et al., 2002
	"Mini" Gα subunits	Labelled Gα proteins lacking the GTPase domain, unable to load nucleotide	Carpenter & Tate, 2016; Wan et al., 2018
	SPASM (systematic protein affinity strength modulation)	FRET, BRET or split-luciferase sensor consisting of a tethered receptor/Gα or Gα-fragment, separated by a flexible linker	Gupte et al., 2021; Kim et al., 2021; Mackenzie et al., 2019; Malik et al., 2013
	GEMTA (G protein effector membrane translocation assays)	BRET based sensors consisting of membrane localized acceptor proteins, and RLucII labelled effectors downstream of Gα activation. Activation of the receptor system culminates in translocation of pathway-selective effectors to the membrane, detected by BRET	Avet et al., 2022
	BERKY	BRET-based biosensors that detect the nucleotide state (e.g. GTP-loaded) of subsets of Gα proteins, as a proxy for receptor-activation	Maziarz et al., 2020
Heterotrimeric sensors	TRUPATH	Biosensors measuring the dissociation and association of Gα/Gβγ complexes, measured by FRET, BRET or enzyme-complementation methods	Adjobo-Hermans et al., 2011; Bünemann et al., 2003; Frank et al., 2005; Galés et al., 2006; Janetopoulos et al., 2001; Mastop et al., 2018; Olsen et al., 2020; Saulière et al., 2012; Schihada et al., 2021; van Unen et al., 2016

Abbreviations: BRET bioluminescence resonance energy transfer; FRET, fluorescent resonance energy transfer.

plate format suitable for conventional plate readers and thus higher throughput screening (Mackenzie et al., 2019) without these additional technically challenging steps.

Labelling a receptor as a component in a biosensor system can impart an additional degree of specificity of the signal. However, modification of the receptor itself may hinder typical or impart atypical behaviour to the receptor. More recent designs of recruitment sensors utilize a membrane targeted fluorophore to decorate the intracellular surface of the cell. By tagging G proteins, arrestins or other signalling machinery with a complementary fluorophore or luciferase, changes in RET signals are measured as receptor-mediated recruitment of these proteins (Avet et al., 2022; Namkung et al., 2018) (Figure 1e, Table 1), and specificity in the response is inferred via separate negative control experiments. For example, the G protein effector membrane translocation assay (GEMTA) method enables high-throughput profiling of receptor-effector profiling of 100 GPCRs. However, the agreement between these membrane association profiles and functional confirmation are not always identical (Jang et al., 2022; Hauser et al., 2022). These sensors demonstrate that substrate binding (recruitment) and activation can be unique and independent properties of GPCRs, as with most enzymes (Thoma & Koshland, 1960), a behaviour often unexplored in the GPCR field.

Measuring GPCR activity via monitoring Gα catalytic activity is a consistently popular format. Most often this is done via inference

(e.g. effector translocation in GEMTA or Gα interactions with other signalling partners). However, detection of GTP-Gα species (e.g. GDP or GTP loaded states) has recently been enabled by BRET sensors with ER/K linker and yellow fluorescent protein (YFP), which is abbreviated to BERKY (Maziarz et al., 2020). BERKY sensors use N-terminally membrane anchored NanoLuc luciferase (NLuc) with a long C-terminal linker into the cytoplasm. The tip of the linker contains a YFP-detector protein fusion, where the detector protein fragment is exchanged to enable selective engagement with active membrane-bound proteins. When the detector binds to a membrane bound target the linker bends and the YFP is brought into proximity of the membrane-bound Nluc on the sensor, increasing observed BRET ratios as GTP-Gα accumulates at the plasma membrane. Currently there are a number BERKY sensors with unique detector proteins for monitoring GTP-Gαq, 13, and i3 with the potential for many more. The ability to measure endogenous GTP-Gα is a unique feature of this sensor and of particular value for applications *in vivo* and in complex tissue samples where over-expression of signalling complex components can directly interfere with endogenous signalling activity.

As G proteins are generally considered to be natively membrane associated, it remains to be determined whether recruitment activity is necessarily detecting transduction or some other behaviour, especially for novel findings. For example, it has been postulated that GPCRs can form inactive complexes with G proteins in the absence of agonists (Jang et al., 2020) or in response to stimulation by known agonists (Okashah et al., 2020), the significance of which remains underexplored. Some receptors are thought to function as decoy receptors in certain cases, and so, effector translocation or association may imply a functional event that never takes place (Graham, 2009). A recent study utilized nucleotide-deficient G proteins (Jang et al., 2022), similar to the concept employed in creating mini-G probes, to demonstrate that the high-affinity state of these mutants decreased the receptor selectivity seen for wild-type G proteins and in other technologies. The authors suggest that the transition-states associated with receptor-mediated G protein activation are critical components of selectivity that are lost in probes that lack these structural and biochemical states. Nevertheless, these sensors recapitulate many well-established signalling profiles emphasizing their utility as a novel screening technology. Extension of these approaches to secondary effectors or scaffold proteins associated with GPCR signalling (e.g. EMTA) further promises to extend this technology into more complex layers of signal transduction, while orthogonal technologies that provide a confirmatory measure of Gα state (e.g. BERKY and others described below) could provide additional context for these recruitment profiles and strengthen the quality of these findings.

4 | HETEROTRIMERIC SENSORS

Heterotrimeric G protein biosensor formats measure GPCR activation by reporting the conformational status of heterotrimeric G protein complexes. A hallmark of heterotrimeric G protein activation by GPCRs is the rearrangement or dissociation of the Gα and Gβγ subunits. The most consistent design format for Gα/Gβγ-based GPCR biosensors utilizes FRET or BRET components inserted or fused to some part of the Gα and to either the Gβ or Gγ2 (Figure 1f, Table 1, Figure 2). High-resolution crystal structures of G proteins have informed chimera design, and most probes have used internal fusion sites for the Gα with N-terminally fused Gβ or Gy proteins. The legacy of the earliest of these probes, a *Dictyostelium discoideum* Gα/Gβγ FRET sensor (Janetopoulos et al., 2001), has been long-lasting and led to a large number of variations on its theme. Subsequent modifications and advancements have been made on this design, replacing a fluorescent donor with a luminescent enzyme (BRET) (Galés et al., 2005; Galés et al., 2006; Olsen et al., 2020; Saulière et al., 2012; Schihada et al., 2021), employing the NanoLuc complementation system (Inoue et al., 2019) and targeting new regions within the Gα (Figure 2b) (Olsen et al., 2020; Yano et al., 2017). Heterotrimeric sensors of similar design but differing detection methodologies have occasionally produced inconsistent or contradictory results (detailed

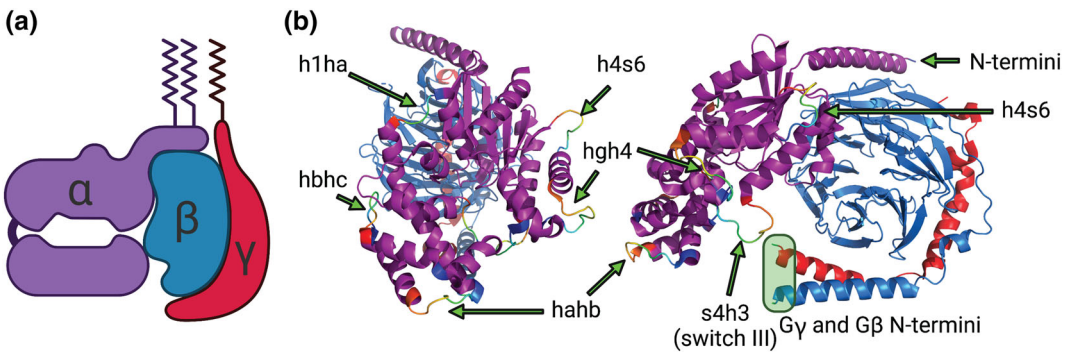

FIGURE 2 (a) Cartoon of inactive heterotrimeric G protein complex. (b) Cartoon from protein data bank (PDB) 5KDO of the inactive GDP bound Gαi1Gβ1γ1 complex. Documented insertion sites (Bertrand et al., 2002; Carpenter & Tate, 2016; Culhane et al., 2022; Gupte et al., 2019; Laschet et al., 2019; Malik et al., 2013; Malik et al., 2017; Olsen et al., 2020; Wan et al., 2018) for donor and acceptor proteins (e.g. luciferases and fluorescent proteins) in heterotrimeric BRET sensors are indicated with rainbow colouring and arrows. Helical nomenclature annotations (hxxx) are from the common Gα numbering (CGN) system (Flock et al., 2015).

below), which highlights the importance of understanding the limits of all detection technologies as well as characterizing the functionality of the modified Gα protein itself.

There are strengths and caveats to each detection methodology within this biosensor class. Enzyme complementation assays can produce high signal depending on the reconstituted enzyme but are less robust to intra-assay variation relative to ratiometric assays such as FRET or BRET. FRET offers perhaps the greatest diversity in signal detection as there are substantially more fluorophores than luciferases that may be employed in BRET. Conversely, the lack of a need for an external light-source and considerations of photo-bleaching are strengths of BRET-based approaches. Fluorescent proteins used for FRET experiments can sometimes result in several undesirable artefacts including oligomerization of putatively monomeric fluorophores. Fluorophore oligomerization can disturb protein–protein interactions, facilitate non-specific protein–protein interactions, stabilize unnatural states and otherwise complicate the design of biosensors. For example, some of the earliest iterations of the Gα/Gβγ biosensors occasionally showed increased FRET following receptor stimulation, and this was interpreted to mean that different Gα species underwent unique patterns of rearrangement during activation rather than dissociation (Bünemann et al., 2003; Frank et al., 2005). However, donor (Olsen et al., 2020) and positional substitutions (van Unen et al., 2016) within the same domain failed to reproduce these findings, suggesting that the FRET pair rather than the large insertion alone may have altered the conformational dynamics of the heterotrimeric complex, perhaps stabilizing an intermediate state before Gα/Gβγ dissociation due to weak dimeric properties of the FRET pairs used at the time (Zacharias et al., 2002).

The structural design of the Gα/Gβγ sensors can also significantly impact the functionality of the sensor, in terms of both the signal strength and the trafficking and wild-type functionality of the G protein. While the alpha-helical domain of the Gα has been the dominant region targeted for most Gα/Gβγ biosensor designs, alternate sites for insertion, such as those in the Ras domain or within the N-terminal alpha-helix, have been produced but with inconsistent or mixed results in terms of their robustness, reproducibility and measurements of agonist potency relative to other designs (Mastop et al., 2018; Olsen et al., 2020; Yano et al., 2017). This is perhaps unsurprising, given that these regions are well studied for their role in trafficking, receptor coupling, and activation/deactivation mechanisms. Nevertheless, sensor engineering even within the alpha-helical domain has historically presented its own problems.

Some early heterotrimer Gα sensor designs exhibited aberrant FRET responses. For example, one sensor that demonstrated an agonist-induced increase in FRET was later shown to possess impaired nucleotide exchange properties (Gibson & Gilman, 2006). Moving the sensor insertion site from the first loop in the alpha-helical domain to the second generated a probe with native-like exchange properties and an agonist-induced decrease in FRET (Gibson & Gilman, 2006), consistent with most other heterotrimeric G protein sensors targeting this domain (Adjobo-Hermans et al., 2011; Galés et al., 2006; Mastop et al., 2018; Olsen et al., 2020; Saulière et al., 2012; Schihada et al., 2021; van Unen et al., 2016). It is unknown whether the aberrant nucleotide exchange phenotype was related to the inverted FRET response but it is clear that the choice of insertion site and type (fluorophore type or luciferases) within the same Gα subdomain, or even insertion sites within the subdomain, can exert significant effect on probe functionality.

The first effort at large-scale expansion of Gα/Gβγ BRET sensors targeted homologous insertion sites of the same position in the alpha-helical domain of the original *Dictyostelium* Gα FRET biosensor (Saulière et al., 2012) for a large set of human Gα/Gβγ heterotrimeric complexes (Galés et al., 2006). Later and more detailed analyses discovered that some of these insertion sites could impair membrane trafficking as well as effector activation such as in the case of Gα13 (Mastop et al., 2018). Targeting novel insertion sites within the alpha-helical domain restored trafficking and effector coupling, as well as improved agonist-induced FRET responses. During development of a subsequent Gα12 BRET probe, no quantifiable responses were observed using this early design. Through exhaustive experimental refinement, strong coupling responses were observed for a number of receptors including **angiotensin AT_1 receptor** (Olsen et al., 2020), which had been previously categorized as exhibiting selectivity for Gα13 over Gα12 (Saulière et al., 2012) due to a lack of observed agonist-induced BRET response using the *Dictyostelium* Gα-insertion site probe. As such, caution is urged when developing novel heterotrimeric sensors using even previously validated design principles.

At present, one of the broadest sets of Gα/Gβγ biosensors are the TRansdUcer PATHways (TRUPATH) collection (DiBerto et al., 2022; Olsen et al., 2020), the result of an effort to produce a standardized and near-complete toolkit to study GPCR Gα transducer signalling (Olsen et al., 2020). Thus potentially minimizing experimental differences that may be due to the application of probes with unique caveats or of uneven characterization. Using exhaustive exploration of insertion sites within the alpha-helical domain and a previously unexplored region (switch III), direct comparisons for all Gα donor variants and each Gβγ-acceptor pairing could be made, and patterns of behaviour and inconsistencies in probe function could be tracked in parallel rather than assembled post hoc from separate biosensor-generation campaigns. While TRUPATH extended the library of available heterotrimeric biosensors to include GαGustducin and Gα15, no Gα14 or GαOlf sensor construct, regardless of insertion region, site or Gβγ pair was found to be functional. All Gα14 sensor designs lacked measurable basal BRET and all GaOlf sensor designs could not be disassociated by a panel of presumed GαOlf coupled receptors (Gs designer receptor exclusively activated by designer drugs [GsDREADD] which was previously shown to couple to GαOlf, Farrell et al., 2013, the **β_2-adrenoceptor** and the **adenosine A_{2A} receptor**). Aside from this, no significant aberrant BRET responses were noted during the development of TRUPATH (e.g. increase in BRET upon agonist stimulation) and in general, potency values for reference receptor-agonist pairings were relatively consistent within Gα subunits regardless of insertion site or Gβγ-pairing, with the exception of those that exhibited little or no measurable responses. These observations suggested that, from a recombinantly expressed screening-tool perspective, the only consistent difference among the

TABLE 2 Table of referenced GPCR transducer biosensors available in Addgene

Biosensor	Publication	Detection methodology	Addgene accession
G-CASE: single-plasmid NanoBRET based heterotrimeric G protein biosensors (Gα13, Gα15, Gαq, GαsS, GαoA, Gαi3, Gαi2, Gαi1)	Schihada et al., 2021: Quantitative assessment of constitutive G protein-coupled receptor activity with BRET-based G protein biosensors.	NanoBRET	Article ID 28216239
Single-plasmid heterotrimeric FRET sensor for measuring Gαq activation	Goedhart et al., 2011: Quantitative co-expression of proteins at the single cell level—Application to a multimeric FRET sensor	FRET	Article ID 28207467
Single-plasmid heterotrimeric FRET sensor for measuring Gαi1, Gαi2 and Gαi3 activation	van Unen et al., 2016: A New Generation of FRET Sensors for Robust Measurement of Gαi1, Gαi2 and Gαi3 Activation Kinetics in Single Cells	FRET	Article ID 16545
Single-plasmid heterotrimeric FRET sensor for measuring Gα13 activation	Mastop et al., 2018: A FRET-based biosensor for measuring Galpha13 activation in single cells	FRET	Article ID 28196059
BERKY sensors for measuring GTP-bound Gα subunits (Gαq, Gαi, Gα13)	Maziarz et al., 2020: Revealing the Activity of Trimeric G-proteins in Live Cells with a Versatile Biosensor Design	NanoBRET	Article ID 28211737
TRUPATH biosensor platform (Gαi1, Gαi2, Gαi3, GαoA, GαoB, GαGustducin, GαsS, GαsL, Gαq, Gα11, Gα12, Gα13, Gα15)	Olsen et al., 2020: TRUPATH, an open-source biosensor platform for interrogating the GPCR transducerome	BRET2	Addgene Kit #1000000163
LgBit/Gα-fusion plasmids for Gα12, Gα13, Gαs, Gα11, Gαq, Gαi1, Gαi2, Gαi3	Laschet et al., 2019: A dynamic and screening-compatible nanoluciferase-based complementation assay enables profiling of individual GPCR–G protein interactions	NanoLuc complementation	Article ID 28207007

Abbreviations: BRET bioluminescence resonance energy transfer; FRET, fluorescent resonance energy transfer; G-CASE, G protein-based, triCistronic Activity Sensors.

biosensor variants that produced a response was dynamic range, which became the criteria for selecting and finalizing the TRUPATH probe set. To provide support that the selection of probes by dynamic range was unlikely to have significantly disrupted Gα function, subsets of finalized TRUPATH sensors representative of the major Gα classes were screened in classical second-messenger assays and found to perform equivalent to their wildtype counterparts whether targeting different loops within the alpha-helical domain (GαS, Gαi3, Gαz, Gαq) or the switch III region (Gα11, and Gα15). While additional validation efforts could be applied to bolster this assumption (e.g. nucleotide exchange, trafficking and localization and further orthogonal comparisons), the entire collection has been deposited into Addgene as a kit (#1000000163) for unrestricted academic use, including such further validation and development. For instance, the TRUPATH system has been distilled into a single plasmid format (Olsen, 2019; Schihada et al., 2021), adapted for 384-well applications (DiBerto et al., 2022), and engineered to use key nanoBRET donor/acceptors, highlighting the future potential for the TRUPATH platform and the creativity of the GPCR community.

There are important assumptions to note for the heterotrimeric biosensor systems. Notably, that the constituents of the Gβγ dimer are suitable for all receptor/Gα pairs. Early heterotrimeric sensors often defaulted to a single Gβ-Gγ pair for all Gα (Saulière et al., 2012) likely due to the resource and time-intensiveness of developing these tools with the common molecular biology methods in use at the time. In contrast, the TRUPATH system optimized Gβ and Gγ pairings with Gα proteins using **neurotensin receptors**, **kappa (κ) opioid receptor** and β_2-adrenoceptors as presumptive models and chose heterotrimeric compositions with the greatest span (efficacy) for those systems. While no Gα sensor failed to couple with any of the Gβγ dimers used in the study, subtleties imparted by the trimer composition in a native system may not be captured in the recombinant environment. Summarizing coupling to a single heterotrimer for each alpha, from the 48 potential pairings (4 Gβ and 12 Gγ), could lead to overlooked coupling identities across the GPCRome or over-emphasizing weak or irrelevant coupling events. These types of systems also fail to capture "non-productive" complex formation, or transducer coupling without activation, and thus represent the opposite set of caveats to some of the recruitment-based biosensors.

5 | CONCLUSION

Major initiatives are being undertaken to expand our knowledge of understudied GPCRs, as well as novel behaviours of "well-studied" receptors. These efforts have led to the development of cell-based genetically encoded screening technologies that have enabled broad investigation of GPCR signalling, most often targeting aspects of

G protein signalling. This review has focused on sensors detailing Gα coupling behaviours, however many sensors have been developed to profile interactions of other GPCR signalling complexes and downstream signalling events (Allen et al., 2006; Asher et al., 2021; Barak et al., 2008; Che et al., 2020; Kroeze et al., 2015; Laschet et al., 2019; van der Linden et al., 2021). Biosensor development programs are trending towards larger scale implementations with platforms capable of broad transducerome analysis. As each method, possessing unique forms and functions, accumulates, it is critical that users understand the assumptions inherent in the design of each system and the caveats these present in contextualizing divergent findings. Because a major application of these technologies is the dissection and classification of receptor signalling profiles, experimenters should be circumspect when asserting their findings given that the recombinant systems required here may fail to mirror native physiology.

All of the technologies reviewed here run the risk of false positives and negatives due to assumptions in their design and limits of sensitivity. Rather than competitive, these technologies should be viewed as complementary assets that extend the utility of the others in forming more complex models of GPCR pharmacology and physiology. Unfortunately, not all tools outlined are equally accessible. Those that are available in Addgene have been cited in Table 2. We hope future toolkits and GPCR sensor platforms will be made more easily accessible to the community through Addgene, which will allow investigators to compare and evaluate these sensors in parallel. In addition, Addgene submission will preserve the continuity and accessibility of these sensors past the lifetime of their originating labs.

As the field adopts and builds on these tools and strategies, settling on a set of consensus technologies or "standards" will enable the interconvertibility of data from different groups and allow for more efficient analysis of aggregate datasets. The openness with which design and optimization data are disclosed, and the extent to which these reagents are made available to the community whether for open academic use or commercial licence, will accelerate the collection of complementary data needed to understand ternary complex behaviour such as signalling profiles and even functional selectivity.

5.1 | Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in the IUPHAR/BPS Guide to PHARMACOLOGY http://www.guidetopharmacology.org and are permanently archived in the Concise Guide to PHARMACOLOGY 2021/22 (Alexander et al., 2021).

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AUTHOR CONTRIBUTIONS

Reid Olsen: Conceptualization; project administration. **Justin G English:** Conceptualization; project administration.

CONFLICT OF INTEREST

The authors are listed as inventors on a provisional patent describing the TRUPATH platform which is held by the University of North Carolina at Chapel Hill.

DATA AVAILABILITY STATEMENT

No original data are contained within this review.

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