CHAPTER THREE

Mining the Potential of Label-Free Biosensors for Seven-Transmembrane Receptor Drug Discovery

Magalie Rocheville[*], Julio Martin[†], Jeff Jerman[‡], Evi Kostenis[§]
[*]GlaxoSmithKline, Medicines Research Centre, Stevenage, United Kingdom
[†]GlaxoSmithKline, Centro de Investigacion Basica, Parque Tecnologico de Madrid, Tres Cantos, Spain
[‡]MRC Technology, Centre for Therapeutics Discovery, London, United Kingdom
[§]Molecular-, Cellular-, and Pharmacobiology Section, Institute of Pharmaceutical Biology, University of Bonn, Bonn, Germany

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Abstract

Label-free is a broad term used to describe a number of cutting-edge biosensor technologies that have attracted considerable attention in the area of drug discovery for seven-transmembrane G protein-coupled receptors (GPCRs). Label-free biosensors resolve receptor-mediated responses noninvasively in real time and living cells and do so with high textural information and broad signaling-pathway coverage. They should facilitate studies of the receptor's integrated signal transduction biology intractable to classical assays with single pathway focus. Label-free occupies a privilege niche with respect to mechanistic studies in human native cells—healthy or disease-relevant—and the probing of context-dependent pharmacology in relation to whole

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biological system efficacy. It is expected that implementation of label-free approaches into the drug discovery process will improve clinical predictability of drug candidates at early stages of discovery research by their exquisite capability to sense whole cellular responses akin to tissue bioassays. Here, we present an overview of promises and challenges this rapidly evolving technology offers to drug screening and we also discuss the prospect of advancing drug discovery.

1. INTRODUCTION

G protein-coupled receptors (GPCRs) are membrane-bound integral proteins linked to a plethora of disease paradigms, both peripheral and centrally mediated, and have a rich history of therapeutic intervention for drug discovery. They have led to numerous breakthrough medicines, making them a highly tractable target class occupying ~50% market share of prescribed medicines.[1,2] Yet, recent business analysis shows a lack of return on investment in overall pharmaceutical research and forces a rethink of drug discovery models for GPCRs and other target classes.[3] GPCRs remain afflicted by a significant incidence of drug attrition at late stages of development and the issue is costly.[4] Accordingly, there is a strong impetus to improve preclinical predictability and particularly assay relevance at early stages of discovery research by exploiting paradigms amenable to a fuller interrogation of molecule activity. Currently, screening for GPCRs is commonly achieved by running high-capacity and homogenous assays of cellular signaling end points using reconstituted cellular systems overexpressing a receptor of interest.[5–7] In doing so, screening focuses on a receptor species typically isolated from its physiological context but thought to be involved in mechanisms of disease(s). The approach termed target-based discovery has proven valuable and replaced isolated tissues or *in vivo* animal models at the front-end of compound screening. However, it remains a surrogate and simplistic approach to native phenotypes and while powerful, presents some shortcomings by often lacking the desired predictability of intact physio- and/or pathological systems. An improved understanding of biological systems and disease paradigms will undoubtedly help resolving this as the etiology of diseases is complex, likely multifactorial and often patient-specific. A better fit of assay to end-game physiological system relevance as early as possible during screening may also be value-adding to triage molecules.

Understanding, controlling, and improving assay relevance is challenging for GPCRs. One needs to carefully choose assay readouts (that are being measured and how it is detected) and assay systems (cell reagent and species) to employ for screening at defined stages of drug discovery.[5–7] One also needs to consider the greater cellular signaling intricacy uncovered over recent years.[8–13] Signaling is thought to be collateral and intertwined with multiple cascades overlapping in both time and cellular sub-localization. Signaling is elicited via means dependently or independently of G proteins and relies on the characteristics of the system under study. Signaling can also be biased by compounds for a subset of features via receptor binding, a notion termed ligand-bias or functional selectivity. As a result, the observed receptor pharmacology will vary depending on the signaling end point being measured, the cellular context/reagent, and the compound used. Assays commonly employed in screening are focused on single functional end points and so provide a "reductionist" perspective with respect to the diversity of signaling. One cannot rule out the possibility that they may, when considered in isolation, be under-appreciative of compound activity and potentially mislead medicinal chemistry efforts. Multiple vantage points can be gathered by parallel testing of assays but the strategy holds several drawbacks, not least in terms of logistic, resource, and cost. Such effort is likely to be limiting as a simple collation of outputs will not necessarily capture the interplay between readouts (negative, positive, neutral).[5,9] To this end, label-free is of great interest as it captures whole cell responses noninvasively in a manner akin to tissue bioassays.[5,14,15] Its applicability is broad throughout drug discovery but raises several points for consideration that may limit it fulfilling a generic screening solution for GPCRs.

2. PROMISES OF LABEL-FREE SCREENING

Label-free is a broad term used to describe a series of cutting-edge assay technologies rapidly incorporating screening in industrial settings.[14–22] Label-free relies on biosensor-coated microplates to sense-integrated phenotypic changes that originate from application of pharmacological stimuli to living cells. It spans broad applicability across discovery research (Table 3.1). Numerous examples of pharmacological outputs have been reported across types of receptors and cell backgrounds,[23–29] and details of instrumentation have been recently reviewed elsewhere.[16] Here, we focus on aspects that may tackle assay relevance and throughput compatible with large-scale screening.

Table 3.1 The applicability of label-free ranges from target inception to lead optimization and *in vitro* safety profiling

Drug discovery activity	Potential application
Target ID	Receptor panning against immortalized cell lines, primary cultured cells, stem cells
	Orphan screening across array of cell types; catch-all platform to screen for multiple signaling route(s)
Assay development	Direct measure of signaling route(s) traditionally deemed challenging such as Gα12/13 without forcing coupling via promiscuous or chimeric G proteins
	Use of endogenously expressed targets in immortalized cell lines or primary cultures for assay development
	Straight-forward reagent validation without requiring target tagging, including study of preferred coupling route(s) and/or pleiotropic coupling
	Selectivity testing at multiple receptor subtypes, enabling cross-validation of conventional technology outputs versus label-free for further reagent characterization
	Profiling of host cells prior to reagent generation. This aspect may be valuable for ubiquitously expressed receptor families for which identifying null-background cells has proven challenging, for example, lipid receptors
Primary screening	Robust single shot assay platform for hit identification, which may offer the potential of additional chemotypes versus the use of a single linear output
	Potential reduction in technology-based false positives typically associated with existing screening strategies, consequently lower hit rate and follow-up work
	New (Gα12/13) or complementary approach (i.e., non-radioactive Gαi/o) to commonly used HTS assay formats
	Enabler of HTS based on native cells, primary cells, stem cells, and cells isolated from disease patients
Secondary screening & MoA	Support of SAR and MoA using recombinant cell lines or native cell types aligned to disease interests (context-relevant)
	Optimization of lead molecules for subset of signaling pathways deemed physiologically relevant (functional selectivity)

Table 3.1 The applicability of label-free ranges from target inception to lead optimization and *in vitro* safety profiling—cont'd

Drug discovery activity	Potential application
	Additional texture on existing chemotypes, providing fingerprint to help define "desired" drug profiles in relation with *in vivo* testing in behavioral animal models
	Downstream native assay; confirmation of recombinant HTS/SAR data in native systems
	Integrated view into cell physiology, for example, cell–cell interactions, chemotaxis, adherence or polarization, membrane permeability, cell differentiation, proliferation, etc.
Safety profiling	Exploitation of native cells such as human stem cells to improve data predictability and physiological relevance

2.1. Sensitivity of detection and compatibility with native cells

Label-free is characterized by an exquisite sensitivity of detection to whole cellular responses achieved independently of target overexpression or tagging. Its ability to do so is impressive in terms of overall power of resolution and extends beyond the realm of light microscopy. Both optical and impedance-based technologies perform comparatively and quantify receptor activation robustly through signals elicited within an estimated 100–500 nm from the biosensor surface upon cycloskeletal changes, dynamic mass redistribution (DMR), or changes in cell adhesion.[16,30–32] Reader capabilities have progressed significantly over the last year, and according to vendors, signals can be resolved in milliseconds to observe rapid cellular events such as cardiomyocyte beating patterns (e.g., Roche xCELLIgence Cardio).

Signals can also be selectively measured for a subset of cells covering the well using a combination of label-free and imaging techniques, therein providing a powerful solution to screen sparsely represented cell types among heterogeneous populations or small cell clusters in nonconfluent wells (Fig. 3.1 and Ref. 33). Label-free embraces a large repertoire of fit-for-purpose cultured cell reagents expressing receptors of interest heterologously or endogenously.[15–22] In particular, the approach has already been applied successfully not only to various primary cell types, as exemplified in Fig. 3.2 for human keratinocytes, but also to muscle, cancer, blood, and neuronal cells.[18,19,23,34] The ability to test native-like cell types coupled with the

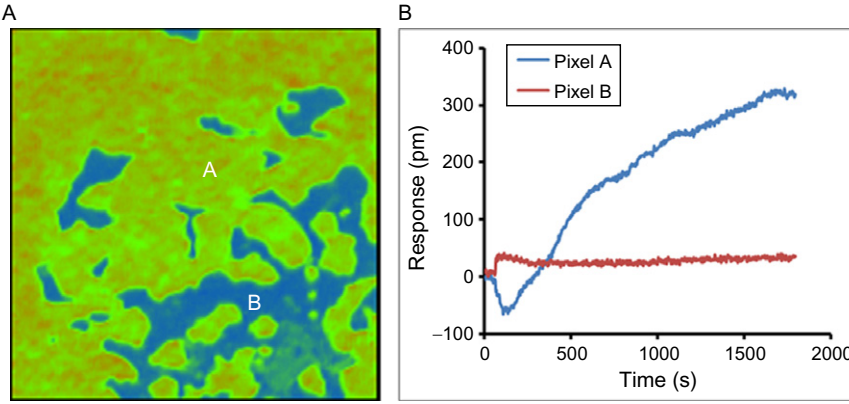

Figure 3.1 Resonant waveguide grating imager for real-time measurement of cell signaling in living cells. (a) False-colored basal resonant wavelength image of nonconfluent A431 cells. Cell adhesion results in higher basal resonant wavelength (λ_{br}) values (green area: adherent cells; blue area: cell-free). (b) Real-time DMR recording in A431 cells treated with 100 nM of the β-adrenergic agonist epinephrine at two distinct pixelated positions shown in (a). The imager used has a spatial resolution of 12 μm. (The Figure is a courtesy of Ye Fang, Corning Inc.; for details on the DMR imager, see Ref. 33)

sensitivity of signals from 384- or 1536-well microplates to minimize cell requirement is truly enabling and can extend beyond primary targets to the profiling of selectivity and liabilities.[35] Preliminary reports also suggest the ability to assay differentiated embryonic stem cells, an emerging area that may provide human-derived cell models and improve relevance during early *in vitro* toxicity screening.[36–38]

2.2. Derivation of signature response profiles

Label-free can qualitatively and temporally distinguish signal transduction pathway(s) involved in cellular responses through an examination of response profiles as recently reviewed by Schröder *et al.*[23,24] Response profiles are informative "signatures" as they hold shape characteristics pertaining to distinct pathways, allowing compounds to be classified and compared. Signatures have been reported for Gαs, Gαi/o, Gαq, and Gα12/13, but it is important to note that such observed profiles are assay system- and technology-dependent and so call for a rigorous approach of deconvolution if mechanistic information is to be extracted.[16,23,24] Figure 3.3 exemplifies that a mere inspection of response profiles is not sufficient to deduct the underlying signaling mechanisms even in a defined cellular background. Nevertheless,

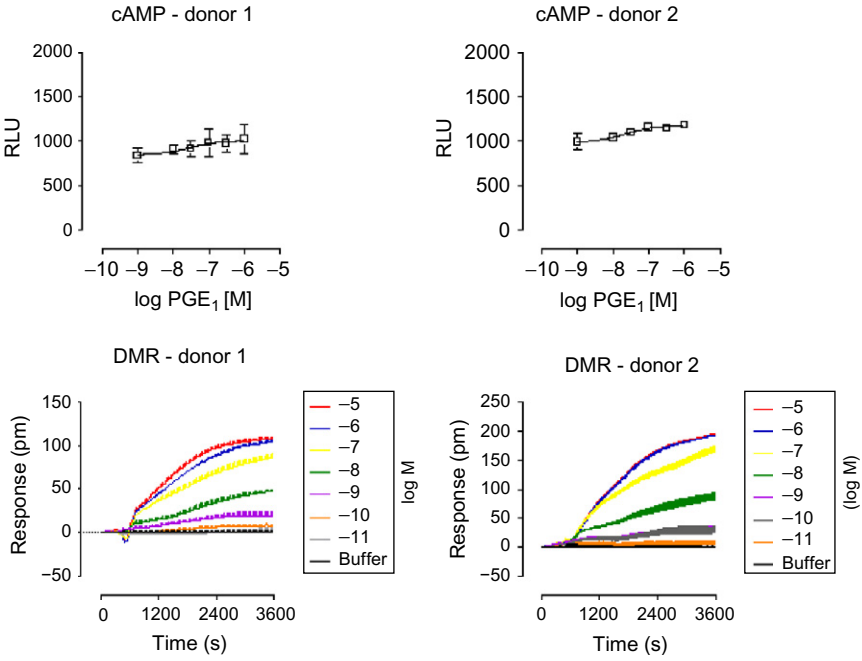

Figure 3.2 DMR enables analysis of GPCR function in primary human keratinocytes. Primary human keratinocytes were obtained from patients who underwent skin surgery (for details, see Ref. 23), and DMR was monitored upon application of prostaglandin E_1 (PGE_1), a lipid mediator known to affect growth and cytokine production of this cell type. Robust and concentration-dependent DMR recordings were observed over time in cells from individual donors. Signal-to-noise ratio and scatter in DMR recordings are clearly superior to classical end point assays, quantifying production of the second messenger cAMP with a competitive immunoassay. RLU, relative light units.

when response profiles are collected for a given receptor in a defined cellular context, signature profiles can be established with endogenous activator molecules or reference ligands and deviations from signature profiles of the reference ligand are then most likely indicative of altered cellular signaling.

The ability to directly assay Gα12/13 or Gαi/o without either the presence of forskolin and phosphodiesterase inhibitors or the need of adding chimeric G proteins for forced G protein coupling is particularly enabling, as shown for the Gαi-linked prostaglandin D2 receptor CRTH2, the Gαi-sensitive dopamine receptor DRD2, the Gα12/13-sensitive GPR55, and other receptors.[16,23,24,39–41] Signal specificity can be tested using receptor antagonists, signaling-pathway blockers, or silent RNA delivery. However, careful validation of such tools is imperative as specificity may not translate

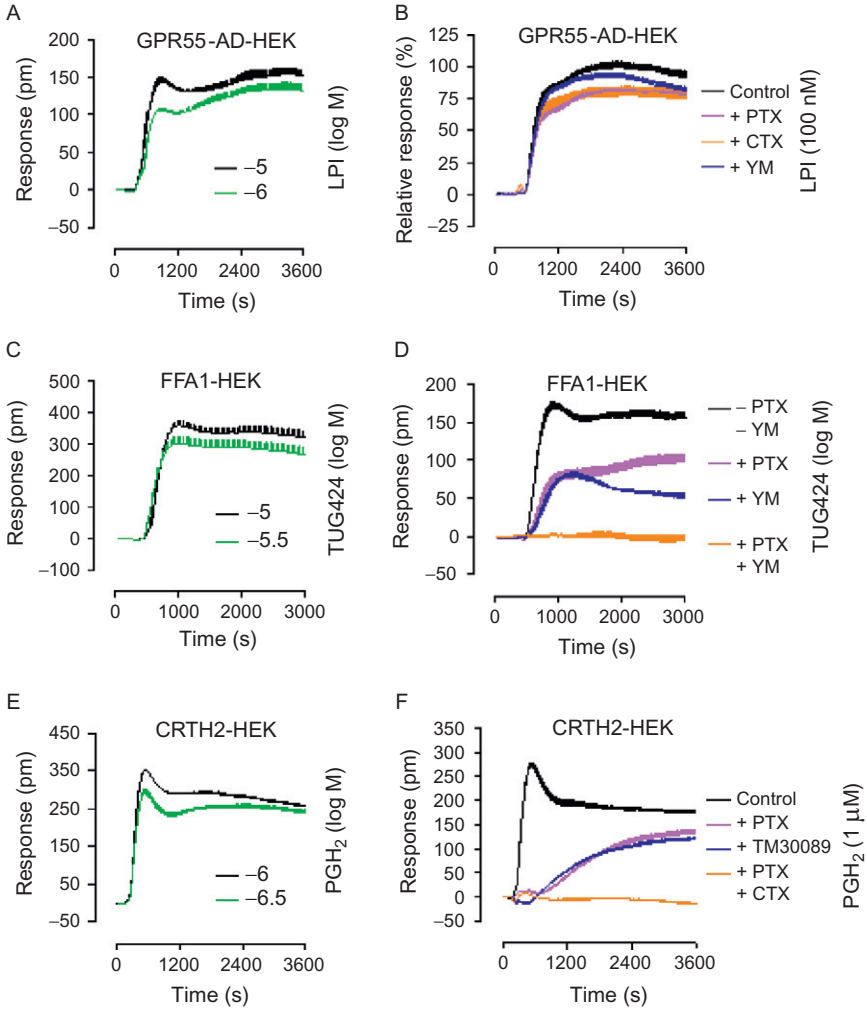

Figure 3.3 Cellular response profiles cannot be linked automatically to defined signaling routes. (A) GPR55-HEK cells were stimulated with the indicated concentrations of lyso-phosphatidylinositol (LPI) and DMR is recorded over time. (B) LPI-mediated GPR55 activation is insensitive to pretreatment of cells with choleratoxin (CTX) to mask Gαs signaling, pertussis toxin (PTX) to abolish Gαi signaling, and YM to inhibit Gαq signaling. (C) FFA1-HEK cells were stimulated with the small-molecule FFA1 agonist TUG424 and DMR was monitored over time. (D) TUG424-mediated FFA1 activation is sensitive to pretreatment of cells with PTX and YM. (E) CRTH2-HEK cells were stimulated with the lipid mediator prostaglandin H_2 (PGH_2) and cell response was monitored over time. (F) PGH_2-mediated signals are sensitive to pretreatment of cells with PTX and CTX. Note that PGH_2-induced DMR in a HEK cell background is triggered by the Gαi-linked CRTH2 receptor and endogenous Gαs-coupled EP2/4 receptors.

from other test systems,[16,23,24] and pathway-perturbing agents frequently used in other assays often display prominent effects on cellular function on their own making it hard to interpret sensitivity to the GPCR agonist response.

2.3. Integration of signaling responses

Label-free resolves signaling in a cumulative manner into a single measurable function and does so without any bias or exclusion. Consequently, it holds a privilege and arguably unique position from where to observe pleiotropic signaling and functional selectivity in plate-based assays.[5,15,16,23,24,29,30] These areas have benefited from increasing therapeutic interest and are of theoretically high value to drug discovery and the design of safer drugs.[11–13] In practical terms, however, they are very difficult to prosecute at the early end of drug discovery. This may be attributed, at least in part, to the reliance of multiple distinct "reductionist" assays to witness it. Such cross-analysis is sometimes done using assays configured in different cellular backgrounds or receptor expression levels, all of which may lead to confounding pharmacologies. Moreover, once/if observed, understanding the physiological consequence of functional selectivity from a perspective of a theoretical reintegration of reductionist assay data is extremely challenging. Functional selectivity can manifest itself in different ways, as the underlying responses may be temporally distinct.[42,43] Pathways involved may also operate independently or dependently,[42] emphasizing the need to focus on integrated system responses. Studying the same system through the "eyes" of label-free may therefore be an interesting catch-all strategy, whether for high-throughput screening (HTS), structure–activity relationship (SAR), or mode-of-action (MoA) studies. This is illustrated by GPR55, which engages in multiple signaling routes, differentially so in response to distinct ligands as shown by reductionist and integrated readouts.[39]

2.4. Application of label-free to study complex cellular phenomena

Recently, label-free DMR has been applied in an elegant experimental approach to study assembly and disassembly of purinosomes, a multienzyme complex required for biosynthesis of the purine nucleosides adenosine and guanosine and important for cell replication.[28,44] Purinosome formation has so far been studied with methods such as fluorescence live-cell imaging. By linking purinosome assembly and disassembly to discrete alterations of cellular DMR, Verrier *et al.* examined a set of GPCR modulators for their effects on purinosome formation in HeLa cells and identified the adrenergic

α2a receptor and its associated Gαi signaling pathway as the specific receptor mediating the cellular response.[28,44] Although the molecular details linking Gαi signaling with purinosome formation still remain to be unraveled, these data offer the intriguing possibility to use label-free DMR technology to search for new therapeutics to regulate mitogenic GPCR signaling.

3. CHALLENGES OF LABEL-FREE SCREENING

The use of label-free in screening is associated with perceived limitations that are briefly summarized here below. But first, it is important to qualify the emergence of label-free in context of the increasing demands of screening throughput for drug discovery over the past decade. While improving assay relevance is undoubtedly an area of intense focus, the cultural aspect of running signaling end point assays at the stages of HTS and primary SAR is still deeply engrained. Label-free is a young field of investigation for GPCRs and yet unproven in terms of exploitability of outputs to discover "better drugs" and so comes at a short-term cost for screening organizations, even if it may ultimately lead to longer-term gain, for example, reduced attrition.

3.1. Data analysis

Label-free responses are qualitatively informative but analysis of outputs can be highly challenging (Fig. 3.4). They entail a significant degree of complexity linked to the dynamics of cell signaling, which can be observationally differentiated by response magnitude and signal orientation over the defined reading time. While some responses are simple and monophasic, others appear multiphasic (negative and/or positive), and this bears significance for screening.[16,23,24,30,33] Depending on the part of the response chosen, a compound may display differential potency and/or efficacy values, making it hard to define what is most meaningful to drive medicinal chemistry. Kinetic profiles may also be unique to distinct compound concentrations, even for closely related ligands acting via the same receptor, which complicates fitting of concentration–effect curves further (Fig. 3.5). When profiling related chemotypes during SAR, it is also possible to witness differential modulation in aspects of response signatures as a consequence of ligand-bias or multiple signaling pathways, each of which may be distinct from responses of native ligand(s) (Fig. 3.6). For example, appreciative yet modest changes to response profiles were picked up across closely related compounds as seen for A431 cells and β2-ligands.[46] Label-free can also detect additional pharmacologies from classical single end point readouts,[16,23,24] whether desirable

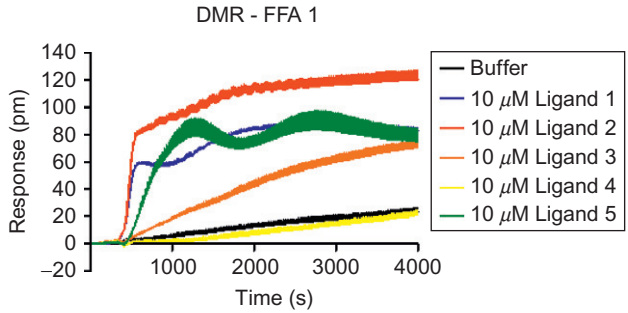

Figure 3.4 Small-molecule ligands targeting the long-chain fatty acid receptor FFA1 do not trigger uniform signature profiles. FFA1-HEK cells were stimulated with the indicated small-molecule agonists, each applied at a maximally effective concentration and DMR was recorded over time as a measure of FFA1 activity. If DMR assays are performed in an end point mode, throughput and screening logistics may benefit; however, mechanistic differences between individual chemotypes may be lost. Ligand 2 is identical to GW9508, Ligand 1 corresponds to TUG20 in Ref. 45, and Ligands 3–5 are unpublished so far. Ligand 4 appears inactive in DMR assays but stimulates inositolphosphate production in IP1 accumulation assays in a FFA1-dependent manner.

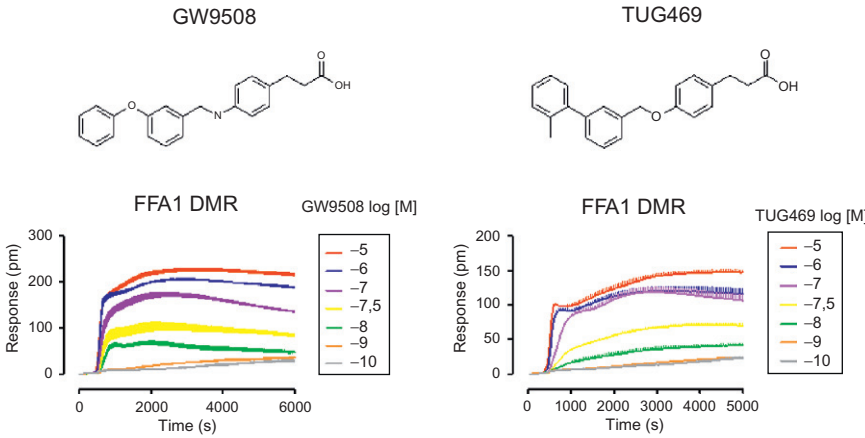

Figure 3.5 Label-free DMR recordings of small-molecule FFA1 agonists are concentration-dependent and chemotype-specific. FFA1-HEK cells were stimulated with the indicated concentrations of the small-molecule agonists GW9508 and TUG469,[45] and DMR was monitored in real time as a measure of receptor activation. Please note that agonist traces are concentration-dependent, chemotype-dependent, and additionally differ kinetically in the time-to-peak profile. By measuring a set time point, "profile-distinct" hits or changes in the subset of the profiles may be missed or overlooked, which is not desirable.

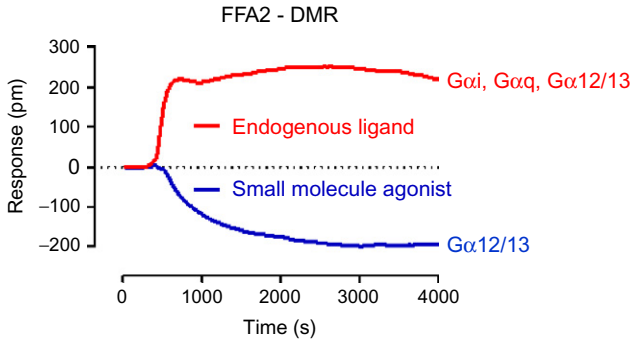

Figure 3.6 Label-free DMR recordings of FFA2 modulators differ depending on signaling pathway usage. FFA2-HEK cells were stimulated with 300 μM of the endogenous agonist propionic acid or 100 μM of a small-molecule activator and DMR was monitored over time. The signature profile of the endogenous agonist propionic acid—applied in a maximally effective concentration—is totally distinct from that observed with a small-molecule ligand biased toward Gα12/13 signaling (J. Schmidt and E. Kostenis, unpublished observations).

or not for therapeutic efficacy, and analysis methods need to evolve to allow on-demand detailed analysis and pattern recognition to be performed when necessary. In fact, elegant examples for pattern recognition using DMR analysis have recently emerged for drugs acting via the adrenergic β2 receptor and the mu opioid receptor.[47,48]

3.2. Data interpretation

An area of challenge pertains to the cumulative overlay of distinct pathways. The integrated trace alone may be insufficient to drive decision making on compounds if the changes observed through SAR are subtle or possibly masked by dominant coupling events. Conventional assays may need to be used in parallel to independently corroborate findings of individual pathways. However, one needs to be aware that assay formats may not be equivalent in their pharmacological outputs as they measure distinct signaling end points. This is exemplified by CRTH2 and a receptor truncate profiled in label-free versus GTPγS, inositol phosphate, and cAMP assays.[40] Studies using pathway blockers can be useful; but in other cases, may lead to the uncovering of novel features of signaling that were otherwise not dominant, making outputs hard to interpret.[16] Assessing compound selectivity can prove difficult as label-free captures cellular signaling beyond the target of interest and more often than not, compound optimization stages involve

chemotypes with some degree of panactivity.[23] Screening with compounds that have unappreciated activities at related or unrelated receptors may result in conflicting pharmacologies or inappropriate conclusions.

3.3. Signal specificity

Label-free is a "black-box" assay readout that may not be easily amenable to simple deconvolution. This is particularly true for endogenously expressed targets when selective pharmacological tools are not necessarily available to probe for receptor specificity. Signals have been modeled in terms of output parameters and are likely dependent on actin re-arrangement although the exact details underpinning label-free responses are not yet resolved.[16] The relevance of such changes to cell physiology is not fully understood and may vary upon cell types used. Label-free works on the assumption that it offers a "catch-all" approach to cell signaling, but how universal sensitivity may be across GPCR types, cellular systems, and signaling events is currently unknown. Also unknown is the capability of label-free to detect G protein-independent but arrestin-dependent events, although the relevance of arrestin as signal transducer in its own right has recently been questioned at least for the angiotensin II AT1a receptor.[42] Cellular adherence is a parameter of prime importance believed to dictate on signal magnitude and several surface chemistries and coating agents have been explored (including cell–cell matrix) in which to favor or limit cellular attachment in a cellular background-specific manner. However, this can theoretically introduce some level of bias on cellular signaling, especially considering functional selectivity for distinct route(s) over others (Fig. 3.7).

3.4. Assay throughput and cost

Cellular responses are resolved in real time, and each plate can typically take minutes up to an hour to read depending on the type of experiment. The capture of full temporal profiles therefore comes at a significant cost in throughput and resource, which may be prohibitive to fulfill large screening demands such as HTS. To circumvent this issue, data acquisition can be reduced to one or more discrete time points using a screen configured with known native or standard tool ligand(s) as exemplified for the β2 receptor.[48] However, such an approach does not preclude the possibility that hits may be missed or classified inappropriately, especially so for agonists or positive allosteric modulators. For SAR, the implication around throughput is less critical as it typically involves fewer plates so may withstand more flexibility

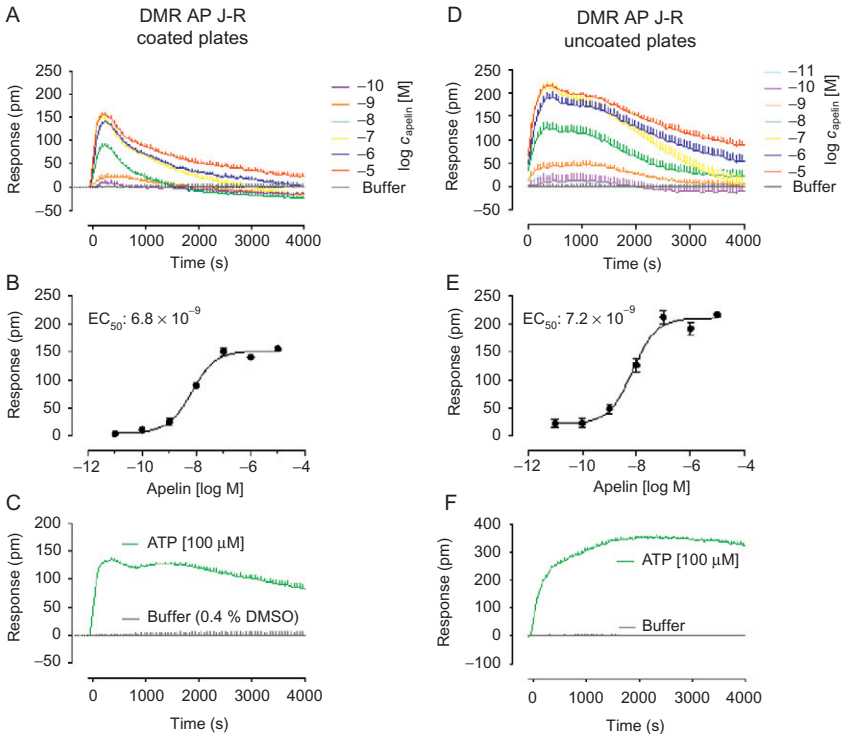

Figure 3.7 Label-free DMR recordings of apelin activity on the APJ receptor. (A, B) CHO-K1 cells stably expressing the apelin receptor APJ were stimulated with the indicated concentrations of apelin, and DMR was monitored over time as a measure of receptor activity on coated DMR plates (left panels) and uncoated DMR plates (right panels). (C, D) concentration–effect curves derived from the data in A and B. (E, F) ATP traces obtained on CHO-APJ cells using coated (E) and uncoated (F) plates, respectively. Surface coating affects the signaling profiles of apelin acting via the APJ receptor and ATP-stimulating endogenous nucleotide receptors (H. Dargatz and E. Kostenis, unpublished data).

in screening run times. In addition to throughput, label-free's operating costs per well are significantly higher than several other commonly employed GPCR assay technologies, prompting users to be more critical as to what level of screening support can be sustained with respect to budget limitations, for example, weekly, monthly, or spot-checks.

4. THE VALUE PROPOSITION OF LABEL-FREE TO DRUG DISCOVERY

Selection of the "right" molecules to progress through the life of a drug discovery program is a major challenge faced by the pharmaceutical industry. Attention is not solely focused on developing "good" assays,

so-called fit for purpose, but also "relevant" in terms of biological/disease output. This prompts questions such as which system(s)/reagent(s) should be used and what output should be measured to best capitalize and integrate with screening strategies. In this context, the advent of assay technologies that enable phenotypic pharmacology is very appealing. First, label-free presents a means to *interrogate what molecules do (or do not do)* by exploiting the enhanced biological texture of outputs. It infers complementary information on the dynamic nature of cell signaling beyond the quantification of receptor activation *per se* at a single point in time. The response profile lends itself to classification of compound responses (same or distinct), which may be subsequently followed up for signal deconvolution. It offers "activity fingerprints" as integrated responses are captured irrespectively of pathway or target class (on- and off-target effects), akin to tissue bioassays. Old drugs and failed candidates can be screened in order to build an understanding of assay relevance to suit desired therapeutic profiles and can potentially lead to repositioning within clinical settings. Examples of biological texture include receptor internalization, kinetic of binding (on/off rates), persistent agonism, surmountable versus insurmountable antagonism or else and thought to be key to drug efficacy in preclinical animal models or even clinical settings.[5,6,49–51] Label-free may offer a tangible opportunity to fill gaps in current screening paradigms. Late-stage drug attrition calls for discovery research to evolve toward a more comprehensive assessment of compound action. Conceptually, this endeavor is very challenging as existing screening strategies are mostly implemented on the basis of historical knowledge and are less geared toward exploring the unknown.[52] Also, rarely are conventional screenings resolved kinetically. Hence, screening does not typically lend itself to incorporation of assay systems perceived to be "black boxes" when this is arguably what makes label-free most appealing.

Second, label-free offers *a discovery platform to identify hits independently of any a priori bias of receptor signaling pathways* described by traditional means. Label-free can be described as a "catch-all" approach that may uncover novelties of ligands within the confined chemical space coverage of existing compound libraries.[23,24] A recent publication from Dodgson *et al.* suggests that the hit rate and incidence of technology false-positives in HTS mode are both low for label-free versus conventional detection technologies and proves successful at identifying multiple types of compounds for a given receptor, including some that are putatively novel.[53] Diversity in molecule MoAs can be of high value to drug discovery programs as it enables the testing of biological hypotheses, therein gaining a better understanding of disease paradigms. Moreover, biased signaling can be assayed during lead

optimization, thereby offering an all-encompassing screening readout of cellular phenotype.[14–16,23,30] The concept of functional selectivity is a very attractive one for drug discovery, as it indicates that compounds may be designed to retain desirable functionalities at the expense of putative side-effect or undesirable features.[11–13] It is therefore imperative to consider the need for a fuller interrogation of signaling during early phase of drug discovery. It can be argued that if a screen is thought to be predictive of *in vivo* efficacy, then subcellular signaling resolution may be achieved subsequently. While this has merit, it is also true that distinction of profiles at early phases can allow grouping of preferred characteristics and aid elucidation of cause (desired or undesired activities) at later stage.

Third, label-free enables *the integration of native biological systems at a higher level of throughput* than previously possible. It therefore supports translational pharmacology across discovery research and preclinical development. It pushes screening a step closer to assaying intact systems, a strategy that has in the past been fruitful to the discovery of drugs at GPCRs. The idea of probing for cell phenotypes as a screening approach is far from new. Indeed, this has formed the bases of most pregenetically engineered target initiatives of drug discovery. The prospect of profiling compounds using healthy or therapeutically relevant cell phenotypes has always been very attractive. Examples include the release of cytokines or chemotaxis from immune cells, the propagation of action potentials from cardiomyocytes or membrane permeability from endothelial cells. However, this is rarely feasible at scale, especially when considering human-derived tissues (healthy or disease-relevant donors) due to the limited nature of cell supply, assay costs, or lack of suitable detection methodology. Studying cells unaltered by label-free offers clear value in this arena. Questions relating to cell physiology can be probed by a holistic assessment of drug activity, as label-free captures complicated biochemical networks and processes influenced or controlled by receptor modulation. Where this may be important to drug discovery is that such network interactions can be partially or fully redundant within the cells and play a role in the overall efficacy profile of a drug; conversely, the engagement of multiple targets may be required for desired drug activity.[54–56] Consequently, a system-based approach to efficacy and safety may be worthwhile as it embraces the dynamic interplay of macromolecular targets. Such paradigm contrasts significantly from single-analyte screens typically used in the recent past. It can be postulated that systems "as a whole" may help delivering the next generation of therapeutics. In support of this, recent evidence highlights the benefit of agents acting via multiple points of intervention within biological mechanism(s)

underlying the onset and development of diseases. This is exemplified by an analysis of clinical antipsychotics for schizophrenia and mood disorders, which links drug effectiveness to receptor signatures or poly-pharmacology.[57]

5. CONCLUDING REMARKS

As any new technology unfolds such as label-free detection, it raises fundamental questions on the potential benefits it will provide. While the applicability of label-free is broad and holds tantalizing prospects to transform drug discovery, its uptake in the "real world" appears slow. This may be a reflection, at least in part, of the current economical situation limiting research budgets globally, but it may also be cultural as label-free is a black-box approach. It is therefore not a replacement technology, a gain in process efficiency, or a reduction in operational costs for GPCR screening. The introduction of label-free gives new opportunities to screen whole cellular systems as it integrates multiple independent activities to give a holistic assessment of drug activity. In many ways, it is reminiscent of tissue/organ-based experimentation. As it is of relatively large throughput, label-free may improve focus of precious resource on molecules ultimately successful toward the clinic. This is, however, aspirational and only time will tell if label-free can deliver. An area of immediate impact for label-free relates to functional selectivity using human native cells. The cost incurred by functional selectivity of chemotypes progressed through the discovery phase is currently unknown, and addressing this issue requires the ability to interrogate it and modify screening cascades accordingly.[8,10] In the years to come, the exploration of the spatiotemporal dynamics of cell signaling with other biophysical techniques may be powerful to complement label-free and kinetically resolve discrete events underlying receptor activation.[58] In addition, advances in label-free will likely include the deciphering of kinetic traces to a subset of distinct signaling event(s) in order to maximize the exploitability of label-free's high mechanistic content. In this regard, a recent study presents a compelling approach to tackle response complexity.[23] Safety profiling and assessment of cardiomyocyte safety/toxicity and function with human cells is yet another emerging area of great promise that may be transformational. As drug discovery takes on label-free methods, it raises the question of whether it is advancing or more simply moving "back to the future" in the days of whole systems research.

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