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Perspectives on optical biosensor utility in small-molecule screening

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1. Introduction

Biophysical technologies appeared on the drug discovery scene more than two decades ago and have since provided drug discovery projects with enriched information about compound binding, thereby greatly impacting drug discovery.[1] Amongst the well-established and recently developed biophysical technologies that have been all highlighted in detail by Renaud et al. [1], optical biosensors have contributed significantly to the overall success of biophysics in drug discovery. The launch of the first commercial optical biosensor system based on surface plasmon resonance (SPR) in the early 1990s paved the way for a wide range of complementing optical biosensor platforms that shortly followed,[2] with optical waveguide grating (OWG) and biolayer interferometry (BLI) being currently the most prominent and established ones alongside SPR.[3, 4] In this context, either fluidics- or plate-based optical biosensor platforms find frequent applications as primary screening tools using both biochemical (SPR & BLI) and cellular (OWG) hit finding approaches. Besides providing a label-free readout of compound interaction thereby reducing costs and increasing assay relevance particularly for cell-based assays, their great versatility makes them impactful tools in various other stages of small molecule drug discovery

campaigns. This can include, but is not limited to support assay development prior to high-throughput screening (HTS) [5] or post-HTS for the validation of primary hits. It is often followed by extending our understanding of the molecular mode of action (MoA) and can include the assessment of compound binding kinetics and thermodynamics. Particularly the application of kinetic data appears to be a crucial factor when trying to improve drug efficacy and selectivity.[6] In this context, microfluidics-based SPR has further promoted the enhanced use of kinetic data within drug discovery, as it provides data with high precision and accuracy that goes along with good throughput.

3. Basic principles of optical biosensors

All optical biosensor platforms follow the same guiding principle by typically attaching the drug target protein or even entire cells to a biosensor surface [7]. The modified surface is subsequently challenged with solutions containing compounds in order to obtain direct binding information or to study the consequences of binding when working with cellular systems [8]. SPR and OWG make use of the evanescent-wave phenomenon and thus are able to measure changes in the refractive index that are proportional to mass-changes at the sensor surface [2, 3]. In contrast, BLI operates through the analysis of interference patterns that enables to monitor changes in the thickness of the layer that is in intimate contact with the sensor [4]. Common for all platforms is the capability for real-time measurements particularly when using microfluidics-based systems. Whilst this appears to be obvious for SPR, even the collection of real-time data when interrogating slow-responding cellular systems with OWG can result in distinct and characteristic kinetic signatures that enable to classify and understand the cellular MoA of novel compounds [9]. As optical biosensor systems do not require any labelling of the used reagents, they are often referred to as label-free technologies aiming to reduce the number of assay artefacts that can be

eventually introduced by the label itself. This, besides the ability for direct and real-time detection of compound binding has largely fuelled the success story of optical biosensors in the past.

4. Key success factors for the application of optical biosensors – the 3R's of biosensing

The successful application of optical biosensor platforms in small molecule drug discovery can be condensed down to three essential factors that should meet drug discovery requirements: right throughput, right sensitivity and right reagents (Figure 1) [10]. Lately, manufacturers have actually made remarkable improvements in the technical aspects of throughput and sensitivity. There is an apparent development towards parallel measurements as for example seen with the newest SPR- and BLI-platforms in order to move towards the throughput performance of plate-based OWG systems. This proves to be very powerful, as those platforms offer high sensitivity that nowadays enables detection of compounds with a molecular weight below 100 Da. This also explains, why localised SPR employing nanoparticles in a plate-based configuration have only found limited applications, as the sensitivity is simply not sufficiently high for small-molecule drug discovery [11].

A frequently neglected but very important factor is the access to fit-for-purpose reagents which can often compromise throughput and sensitivity. This becomes particularly apparent when performing traditional direct binding assays that employ tethering of the target protein to the sensor surface. Only ligand-binding competent proteins that preserve the majority of that competency after tethering are suitable, as the attainable signal scales directly with the surface activity. Obviously, this approach will only be successful, if the protein can be initially expressed and purified in an active and stable form, which can eventually present a challenge for some

soluble proteins, but is frequently experienced as a significant hurdle for most membrane protein systems due to their lability. Only specific protein engineering strategies can in some instances help to overcome those limitations, but these can be fairly resource intensive and are thus not compatible with typical drug discovery timelines [12]. In these cases it can be useful to study the consequences of the binding in a cellular assay instead of detecting the binding to the target directly, as for example provided by OWG. [8, 9]

Alternative strategies are building on providing fresh target protein for each new binding experiment, which obviously impacts negatively on reagent consumption, thereby taking away one of the major advantages of the iterative use of the same biosensor surface like in SPR. BLI employs for example disposable biosensor tips to provide fresh target protein for subsequent experiments. Changing the assay configuration to an inhibition in solution assay (ISA) format can serve a similar purpose, as a so-called target definition compound rather than the target protein itself is tethered to the biosensor [13]. Such tactics can in fact help to significantly increase the assay sensitivity due to the large change in mass caused by protein binding [14]. Another recent approach for sensitivity improvement whilst maintaining throughput employs label-enhanced SPR, but this requires the utilisation of dyes and special software that can analyse the full shape of the SPR curve which both can be perceived as a shortcoming [15].

Something that in our opinion should be added to the 3 R's of biosensing is the right information content. Whilst initially the value of kinetic information from fluidics-based biosensors was largely underestimated, this has nowadays transformed into a key decision parameter when selecting lead- or drug candidates. This has been mainly driven by the strong desire to consider affinity-independent parameters that may provide a better handle to select molecules that will display a distinctive and differentiating behaviour *in vivo*. This is for example realised on OWG-

platforms, where the complex signals originating from cellular responses upon compound challenge can be used as a compound discriminator and predictor of *in vivo* responses.

5. Conclusions

The successful application of biophysical methods and in particular optical biosensor technologies in small molecule screening have positioned them as key components in drug discovery campaigns. The last two decades have witnessed an increasing spectrum of optical biosensor platforms with a strong focus on improving sensitivity and throughput but also information content. Whilst optimising the technical specifications there has been much less focus on the requirements of the reagents that need to display compatibility with the platform architecture, the purpose of the assay as well as the required information content. But only this will ensure that the technical improvements of increased sensitivity and throughput can be fully realised with the respective drug targets in mind. Currently, a combination of resource-intensive reagent optimisation and innovative assay configurations is typically applied to increase the efficiency of optical biosensor screening. This calls for more innovative technical solutions that directly address reagent shortcomings, but may also provide additional information content to be used for a more successful lead- and drug selection process.

6. Expert opinion

Bigger focus on cost reduction and efficiency improvements within drug discovery play nowadays an important role for the application of optical biosensors for small molecule screening. An adequate response to that challenge has been for example the introduction of short-cutting procedures that allow for screening of unpurified reaction mixtures to quickly identify compounds with improved residence time [16]. This serves also as a good example for moving

away from an affinity-centric mind-set, as in this example project decisions are in fact based on kinetic data and not affinity. In our opinion, these aspects of generating and considering secondary information will need to gain greater momentum, as compounds are nowadays expected to show a differentiating behaviour to provide a therapeutic advantage. Thus, improved optical biosensor platforms or approaches need to enable researchers to generate such information in order to provide a handle to discriminate compounds based on novel data that eventually reflect a difference in the MoA.

This has been recently realised through a novel biosensing platform that is based on second harmonic generation (SHG). As it is plate-based it offers a similar throughput as OWG-platforms, but in contrast to all other discussed platforms it is not label-free, as the target protein requires labelling with dyes that can generate second harmonic light upon excitation with a laser. The light intensity is dependent on the angular orientation of the dye and thus serves as an effective reporter for the magnitude and direction of conformational changes induced by compound binding [17]. Since conformational changes are often causing alterations in protein function, this can be used to discriminate compounds that will display more desirable functional consequences. Even though this presents a very promising concept, it does not in our opinion address general sensitivity issues relating to the detection of binding events and also puts some additional demand on producing tailored reagents due to the requirements for protein labelling and tethering. But the extra information gain might be indeed of great additional value for drug discovery, and particular methods that are highly sensitive towards conformational changes will likely see a great utility moving forward.

As fluidics-based biosensors are currently unable to achieve similar throughput as plate-based platforms, it will be essential to combine them with automated self-learning approaches (smart

biosensing). Current state-of-the-art solutions of controlling laboratory equipment are already providing a blueprint of what could be achieved with fluidics-based optical biosensors in the short-term. For example, triggering surface regeneration or sensor tip change event-based instead of using a fixed time for the dissociation phase would be an interesting feature allowing to increase throughput in many instances. Many laboratory devices provide already the ability to design smarter and faster experiments by scripting and triggering certain events based on the collected data, for example automated peak collection used in chromatography. In our opinion, the design of smarter, feedback-driven experiments will be a required first step towards improving throughput and paving the way for smarter biosensing in the long-term.

Although this will help to better exploit existing infrastructure, there are still limitations that are intrinsic to the technology and cannot be overcome without major advances in existing biosensor technologies or the introduction of new ones. One of the fundamental limitations is based on detection of net changes in the signal response. But the extracted signal from the sensor surface is in fact a result of the convoluted association and dissociation reaction to the biosensor. The ability to simultaneously quantify the association and dissociation reaction, as for example provided by single molecule biosensing technologies, will in our opinion change this accepted paradigm of small-molecule screening fundamentally.

Examples of how single molecule biosensor platforms can in fact help to overcome those fundamental limitations have been recently provided [18]. The experimental configuration allows to determine the association and dissociation kinetics within a single experiment. As much lower quantities of functional material is required (typically nanogram quantities are sufficient), it enables even the assessment of very challenging systems like membrane-bound targets [19]. Additionally, the literal visibility of the binding dynamics in equilibrium will have surprising effects on the lower limit of detection and thus the sensitivity. At the single molecule level, each

binding event is observed. Increasing the measurement time simply allows to observe more binding events, which consequently will lead to a decrease in the lower limit of detection. In addition, the observation of single molecules instead of entire molecular ensembles provides additional information, as it enables to detect heterogeneities within a population. In our opinion it remains to be seen if this type of information can be indeed beneficial for drug discovery, but compounds might eventually act differently on particular subpopulations of a receptor, which can be revealed through single molecule biosensing. As this can be, upon modification of existing optical microscopes, also performed in a fully automated, plate-based format, it will not only help to address the the 3 R's of biosensing but also adds another interesting level of information that is required to discriminate compounds in an affinity-agnostic fashion. Thus it is our view, that in particular single molecule biosensors will see enhanced utility in small molecule screening in the future. Their application will enable to unlock difficult target classes and provide a novel information level for the benefit of small-molecule drug discovery.

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Declaration of interest

Tim Kaminski and Stefan Geschwindner are both employees of AstraZeneca. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

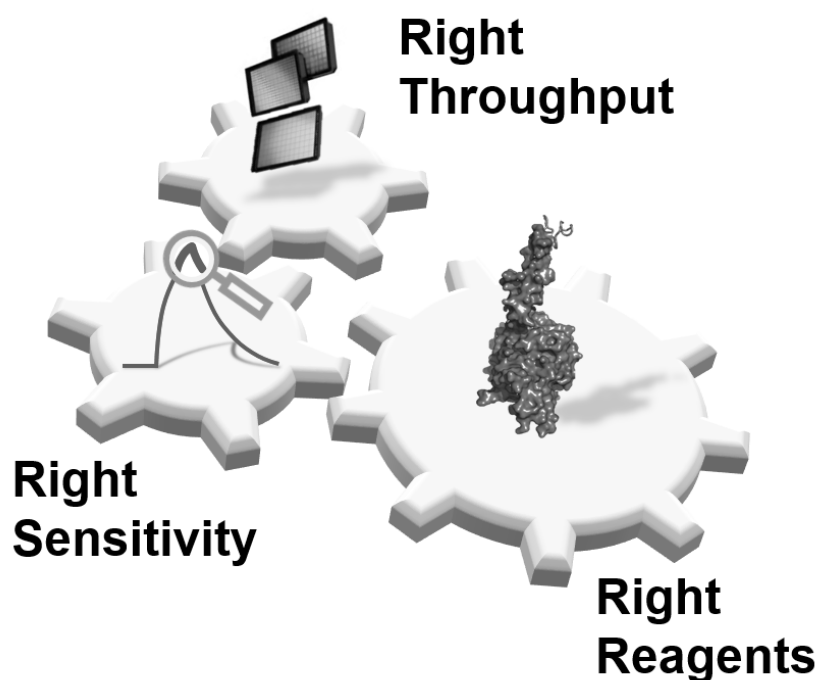


Figure 1. The 3 R's of biosensing. These represent key factors for the successful delivery of biosensor data to small-molecule drug discovery projects. Modified and reprinted with permission from [10]. Copyright 2017 American Chemical Society.

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