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Optical biosensors: where next and how soon?

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From a direct comparison of the technical benefits of labelled reporter assays with the benefits of label-free assays, label-free appears to have significant advantages. Faster assay development times; accurate, high information content data; and less interference from labels. However, optical label-free platforms have not yet made a major impact in the drug discovery technology markets; are often viewed as having poor throughput, limited application; and are difficult to learn and use effectively.

Introduction

From the earliest days of screening and molecular profiling in drug discovery, assay development has exploited a variety of labelled assays to report an interaction of a drug candidate with a receptor or cell. These include ELISAs, radiolabelled pull-down assays, scintillation proximity assays (SPA) and an ever-expanding suite of intensity and time-resolved fluorescence assays [-intensity, -lifetime, -polarization, -fluorescence resonance energy transfer (FRET) and so on]. Such assays are used extensively in most stages of preclinical drug discovery and form the basis for dedicated high-throughput screening (HTS) instrumentation developed by the major technology suppliers to the pharmaceutical industry. Formats cover pathway-based screens (e.g. cellular Ca^{2+} flux), activity-based screens (e.g. phosphorylation) and interaction-based screens (e.g. AlphaScreen™, enhanced chemiluminescence, fluorometric microvolume assay technology (FMAT™), LeadSeeker™, SPA). The development of a label-based assay requires additional time and cost allocation, a component of which is not required for true label-free assays.

Following a decade of major investment in compound generation, storage and characterization, and the industrialization of assay development, implementation and data handling, major Pharma companies can now run several major screening campaigns each quarter, with each encompassing over a million drug candidates. Unfortunately, most HTS platforms still give high hit rates and do not always discriminate causal perturbation of a biological pathway from non-specific or concomitant activation of non-relevant cellular processes. More importantly, the label can, in some cases, interfere with the molecular interaction by occluding a binding site, leading to false negatives. For many fluorescent and chemiluminescent reporter compounds, background binding can be a significant problem, leading to false positives. There are also a large number of other artefacts inherent with label-based assays that originate from the screened compounds themselves – in particular, autofluorescence. These artefacts can be offset by the use of multicomponent fitting (e.g. fluorescence lifetime and fluorescence intensity) and other proprietary software algorithms; however, these approaches all add to the complexity of the screening assay. These effects and their impact on the data quality originating from HTS have been reviewed in detail elsewhere [1]. For applications of emergent technologies for multiplexed optical arrays and higher information content application of dual polarization interferometry, see Refs [2–5].

The label-free technology landscape

Although labelled assays require significant initial effort to develop the assay platform, it is important to note that the end user is only exposed to these additional R&D requirements through higher instrumentation pricing. The technology supplier usually carries out the development work that provides the foundation for most assay platforms. In other words, many tools companies put a lot of effort into developing robust assays tailored specifically to a particular assay class, a signalling event or a specific cellular process. A recent report from HTStec [1] has analysed responses and opinions to label-free technologies from

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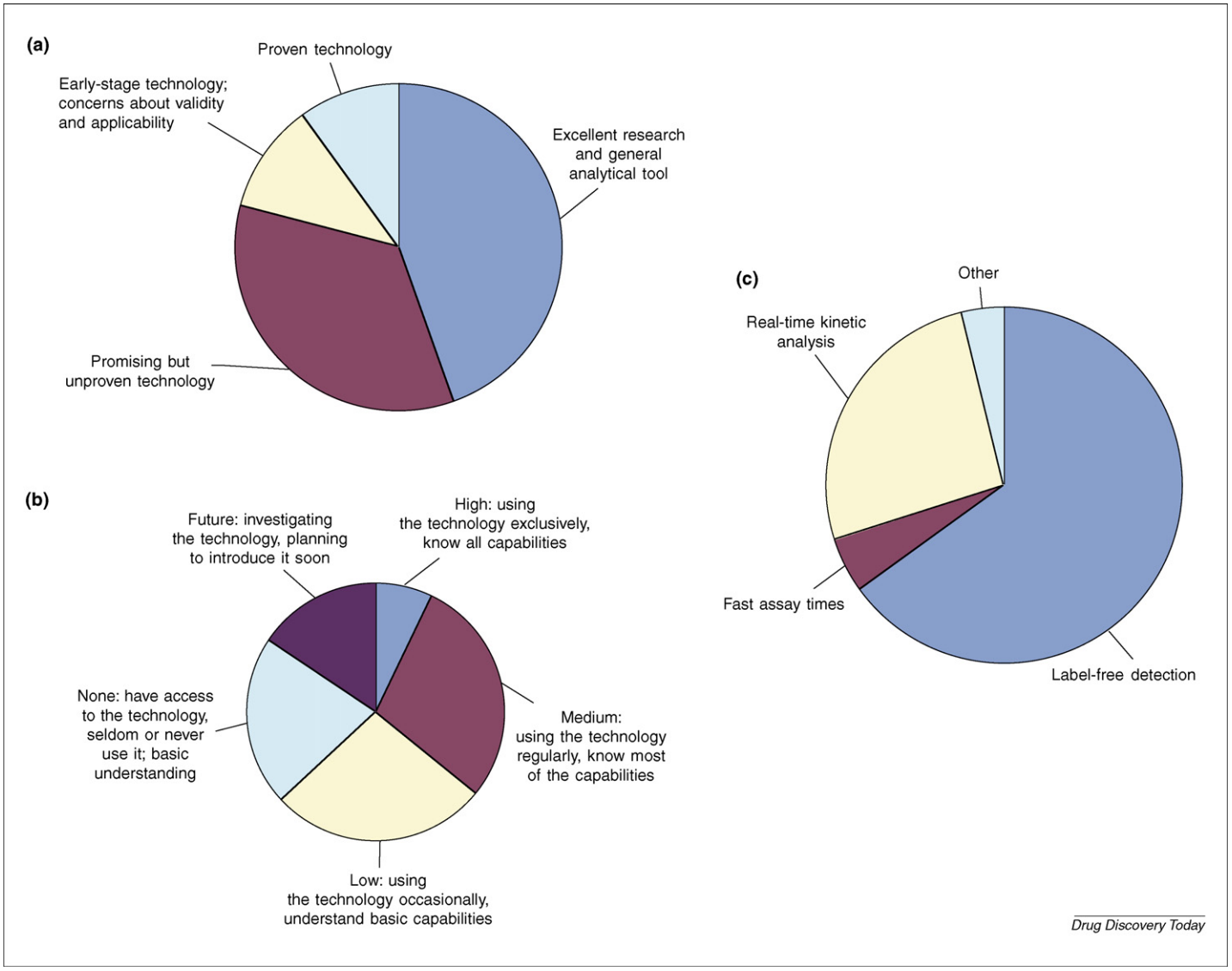

FIGURE 1

Market perceptions of label-free technologies. (a) Current perception of label-free detection; **(b)** current uptake of label-free detection; and **(c)** biggest perceived benefit of label-free detection. Data abstracted with permission from Ref. [1], a market report that summarizes the results of a comprehensive global Pharma & Biotech and Research web-based survey on label-free detection trends.

researchers and managers in 37 different large pharma laboratories, and 15 small pharma or biotech laboratories. Most respondents surveyed perceived label-free 'as a promising analytical tool that needs to mature' (Figure 1a), with most respondents having no access, or limited access, to label-free instrumentation (Figure 1b). Aside from the intrinsic benefits outlined above, most respondents felt that the real-time readout and ability to measure interaction kinetics was a principal benefit (Figure 1c), a view most likely predicated by the capabilities of the dominant player in the market today: Biacore (http://www.biacore.com). Biacore, a Swedish company spun out from Pharmacia Biotech in the 1990s, is the pioneer of commercial label-free systems and currently dominates the biosensor market in the life sciences. The late 1990s and early 2000s unfortunately witnessed the demise of several emergent competitors (e.g. Affinity Sensors, Prolinx, Signature Biosciences and BioTul), which slowed wider market penetration for label-free platforms. In addition, the rapid development of 1536-, 2080- and 3456-well plates has driven down screening consumable costs and potentially 'priced out' label-free technology for HTS.

Emergent competitors also suffered from competing project and technology investment cycles in large pharma companies. Following a sustained period of technology spend in the 1990s, many pharmaceutical companies then reduced technology evaluation and acquisition budgets in favour of increased of project- and product-based expenditure. There has also been an element of 'novel platform fatigue', and young technologies seeking to ride on the back of the HTS and combichem 'wave' have found senior management more reluctant to invest significant time and money into less established discovery tools than was the case in the 1990s. However, the past five years have seen the emergence of several new players in the label-free development arena that are bringing new products to the market, and that will stimulate the development of new products from existing players. These companies are detailed in Table 1; several of these technologies and

TABLE 1

Selected optical label-free platform developers

Provider	Technology	Product	Website
Axela	Diffractive optics technology	DOT™	http://www.axelabiosensors.com
Bioanalytic Jena	SPR	BIAffinity®	http://www.analytik-jena.de
Biacore	SPR	A100, T100, S51, FLEXchip	http://www.biacore.com
BioRad	SPR	ProteOn™	http://www.bio-rad.com/proteininteraction
CSEM	Waveguide grating evanescence	WIOS	http://www.csem.ch
Corning	Resonant waveguide grating	Epic™	http://www.corning.com
EcoChemie	SPR	AutoLab Espirit	http://www.ecochemie.nl
Farfield Sensors	Dual polarisation interferometry	AnaLight®	http://www.farfield-sensors.com
FortéBio	Biolayer interferometry	Octet™	http://www.fortebio.com
GWC Technologies	SPR	SPRimager®II, FT-SPRi200	http://www.gwctechnologies.com
IBIS	SPR	IBIS-1, IBIS-2, IBIS-iSPR	http://www.ibis-spr.nl
Johnson & Johnson	ThermoFluor®	ThermoFluor®	http://www.jnjpharmarnd.com
Reichart	SPR	SR7000	http://www.reichertai.com/spr.html
Solus Biosystems	Isoelectric focusing/IR	Solus100™	http://www.solusbiosystems.com
SRU Biosystems	Colorimetric resonant reflection	BIND™	http://www.srubiosystems.com
TechElan	Thermal IR	–	http://www.techelan.com

the accompanying products are briefly reviewed in the following sections.

Biacore

For more than 15 years, Biacore systems have been used by scientists to profile the specificity, affinity and kinetics of protein interactions. Over the past 3–5 years, Biacore instruments, such as S51 and 3000, and more recently Biacore A100 and T100, have demonstrated the sensitivity required for typical drug candidates (Figure 2) and lower molecular weight (~150 Da) drug fragments or 'needles'. The response levels for drug 'needles' are lower than those for larger molecular weight drugs because surface plasmon resonance (SPR) measures changes in refractive index that are directly related to the molecular weight of the binding molecule. It is possible to get high-quality data from extremely low responses, provided that the assay is well-controlled and carefully performed. Example applications have been reported for profiling of interactions of low molecular weight binders to serine/

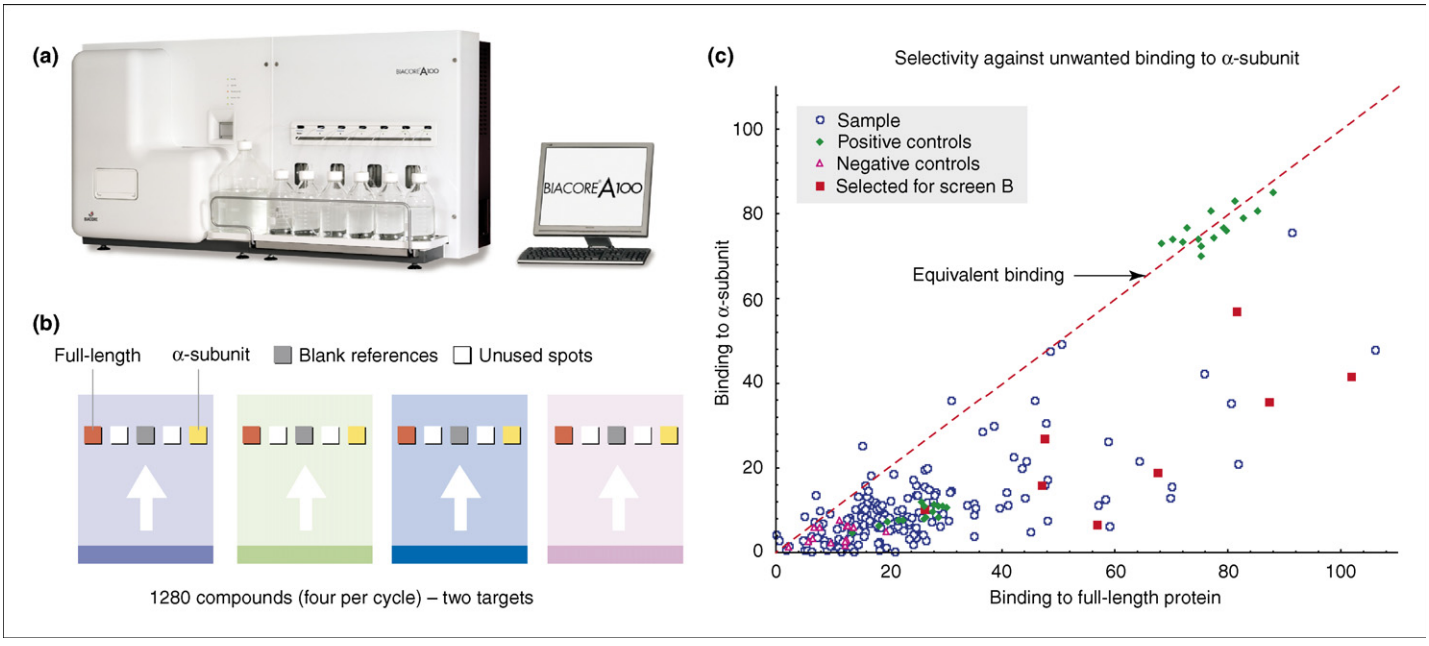

FIGURE 2

Selectivity-based screening of 1280 compounds using Biacore A100. (a) The Biacore A100 system; **(b)** Assay setup for optimal sample throughput. Full-length and subunit variants of a target protein were immobilized using spots 1 and 5 in each flow cell; four different compounds were then analyzed in parallel per cycle; **(c)** Rapid identification of selective binders to the target subunit. Data courtesy of W. Huber, Hoffman La-Roch, Basel.

threonine phosphatases [6], protein kinases [7], cyclophilins [8], thrombin [9] and solubilized and purified chemokine receptors CXCR4 and CCR5 [10].

There is a general issue with data quality and fragment libraries because these compounds tend to be of low affinity and are screened at high concentrations, with all of the associated problems of solubility and sensitivity thresholds and non-specific binding. After more than 20 years of R&D, SPR sensitivity might be approaching theoretical limits in terms of the detection interface sensitivity; however, there is still significant scope for improving usability, increasing throughput and integrating better with existing liquid-handling capabilities in HTS, and with industry standard data archiving and data-mining software. A detailed review of the strengths, weaknesses and key applications of the technology developed by Biacore for drug discovery and development is available elsewhere [11–16].

Stefan Löfås (CSO, Biacore) has commented:

> Over the past 15 years recombinant proteins including antibodies have moved from molecules seen to have great potential in research and medicine, to the status of therapeutic agents. Biacore systems appeared shortly after the introduction of the monoclonal antibodies, enabling researchers to characterize the molecules in terms of their specificity of interaction with other molecules, the rates at which they interact (binding and dissociating), their concentration and their affinity (how tightly they bind to another molecule). Today all of the 20 biggest pharma companies have multiple units of the Biacore instruments. Flexible systems such as Biacore T100 and Biacore A100 are used in drug discovery and development and in manufacturing, enabling users to make faster, more confident decisions, when armed with the high quality, comprehensive information obtained from an interaction analysis. The best possible decisions are extremely important in the critical path of pharmaceutical development.

The company has also released dedicated systems for specific applications in pharma companies, such as Biacore C. Löfås adds:

> Biacore C is an example of a dedicated system designed specifically to provide rapid concentration measurements required during manufacture and quality control of a biotherapeutic drug, for example, to check process control, to make potency assessments and for batch release assays. Biacore C is supplied as a fully compliant with GLP/GMP (Good Laboratory Practice/Good Manufacturing Practice) regulations to help the customer fulfil demanding regulatory requirements. The ability to follow the interaction of small molecules (such as drug candidates) with a target protein is extremely important during the later stages of drug development. Scientists are faced with hundreds of potential ''lead compounds'' and must be able to make fast, confident decisions as to which compounds should move forward into the development process and which should be discarded. Again the unique information from a protein interaction analysis by Biacore enables them to have a clear view of how each potential candidate is interacting, facilitating rapid decisions. Biacore A100 and Biacore T100 are flexible, GxP-compliant systems that are ideally suited for drug discovery and development applications. Biacore A100 also offers a new level of productivity for screening assays, with parallel processing and multiplex assay possibilities enabling up to 3800 interactions/24 h to be analyzed.

SRU Biosystems

The SRU BIND™ system is comprised of SBS-standard 96, 384, and 1536-well microplates and a selection of two types of detection instruments [17,18]. Photonic crystal optical biosensors are incorporated into the bottom surface of the microplate wells, and are designed to reflect only a very narrow band of wavelengths when illuminated with a broad band of incident wavelengths. The photonic crystal tightly confines resonantly coupled light to the device surface, resulting in a shift of the reflected wavelength when biomolecules, small molecules, or cells are adsorbed. Mass density resolution of < 0.1 pg/mm^2 is obtained, resulting in the capability to observe small molecule binding (<70 Da) to immobilized protein targets with high signal-to-noise ratio. The BIND™ Reader detection instrument was launched in May 2005 after more than four years of testing and assay development at several academic and pharmaceutical research sites. Demonstrated applications include hybridoma screening, small molecule direct binding, small molecule inhibition, epitope binning, protein-protein affinity ranking, and cell-based assays.

The BIND™ Reader contains eight parallel detection heads, and reads a 96-well plate in 15 seconds. The system has a small footprint for compatibility with robotic plate handling systems or single-user operation. The BIND™ Scanner is a high resolution imaging detection instrument that can scan any BIND™ microplate with user-selectable pixel resolution down to 7×7 μm^2. The BIND™ Scanner further enhances detection resolution, and is compatible with 1536-well BIND™ microplates, assays with multiple spots/well, microarrays and microfluidic formats (under development). The Scanner is capable of single-cell detection resolution while imaging large populations of cells in the microplate culture environment for rapid label-free apoptosis and cell-protein interaction assays. BIND™ microplates are available with streptavidin and aldehyde-activated surface chemistries for high density, highly functional covalent attachment of a wide range of analytes without non-specific binding.

Corning

Corning has also developed a label-free detection platform that utilizes resonant waveguide grating (RWG) sensors [19]. The Corning® Epic™ system consists of a Society for Biomolecular Sciences (SBS; http://www.sbsonline.org) standard 384-well microplate with RWG sensors and attachment surface chemistry within each well (Figure 3) and an HTS-compatible microplate reader capable of reading up to 40 000 wells in an eight-hour period. The optical reader head inside the Epic™ instrument reads 16 wells simultaneously (one complete column) as the microplate is scanned over the reader head to perform a complete microplate measurement in ~2 minutes. When illuminated with broadband light, the RWG sensors inside each well of the Epic™ microplate reflect only a specific wavelength that is a sensitive function of the index of refraction close to the sensor surface. The sensors are chemically modified with a surface layer that enables covalent attachment of protein targets via a primary amine group. The surface chemistry

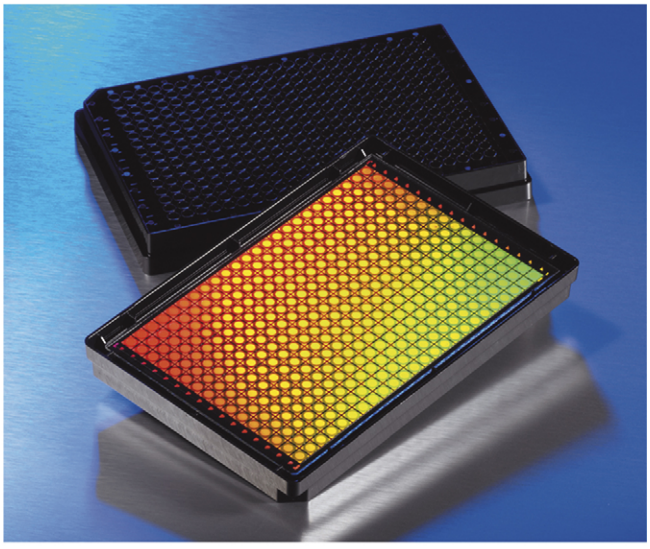

FIGURE 3

A Corning Epic™ system 384-well SBS format microtitre plate with bonded resonant waveguide grating.

provides a high-binding-capacity surface, with low levels of non-specific binding; users can choose to immobilize proteins, peptides, small molecules or DNA. After a target is immobilized, the reader obtains a baseline measurement. During the subsequent binding assay, if analyte molecules bind to the immobilized target, a change in the local refractive index is induced, and this results in a shift in the wavelength of light that is reflected from the sensor. The magnitude of this wavelength shift is proportional to the amount of analyte that binds to the immobilized target.

The Epic™ system determines the binding signal strength by subtracting a reference signal from the sample signal to determine the net response, measured in picometres of wavelength shift. The platform has a sensitivity of 5 pg/mm^2, which enables the detection of the binding of a 300 Da compound to a 70 kDa immobilized target with coefficients of variation (%CV) of 10% or less (depending on assay type). If the immobilized target is smaller (e.g. 25 kDa), it is possible to detect the binding of a smaller compound (e.g. 150 Da). To demonstrate the sensitivity of the Epic™ system, a model assay system was developed based on the binding of benzenesulfonamides to the enzyme carbonic anhydrase (~30 kDa). In these experiments, carbonic anhydrase was immobilized in the wells of an Epic™ microplate and was assayed with a titration series of the drugs dansylamide (250 Da) and acetazolamide (222 Da). The binding signal of both drugs was dose dependent, with estimated affinities that were in general agreement with literature values (Figure 4).

Numerous applications for this platform have been demonstrated, including protein–DNA interactions, antibody–antibody interactions, kinase direct binding and functional assays, cytokine–cytokine receptor assays, and protease direct binding and functional assays, to name a few. According to Dana Moss (Corning Life Sciences): 'The label-free, direct binding features enable the screening of ''intractable'' targets and pathway interactions that cannot be screened today because of labels, license fees, or a lack or adequate methods'. Beta-testing evaluations of Corning's Epic™ system are currently being conducted at select pharmaceutical, biotechnology and academic institutions, with full commercial launch of Corning's Epic™ in September 2006.

In addition to biochemical assays, the Corning® Epic™ system has found applicability in cell-based assays [20,21]. The detection principles for performing whole-cell assays are similar to those for biochemical assays: changes in local refractive indices are manifest by a shift in response of the sensor. The sensors in each well detect the index of refraction changes that occur within the first ~200 nm from the sensor surface. This surface sensitivity means that only the bottom portion of whole cells cultured on the sensor is monitored during an assay. Because the amplitude of the evanescent wave decays exponentially from the sensor surface, a target or complex contributes more to the overall response when the target or complex is closer to the sensor surface compared with when it is farther from the sensor surface. Thus, when endogenous macromolecules within the cytoplasm of mammalian cells move into or out of the sensing volume, a change in the local refractive index is induced, leading to a shift in sensor response [21]. More-

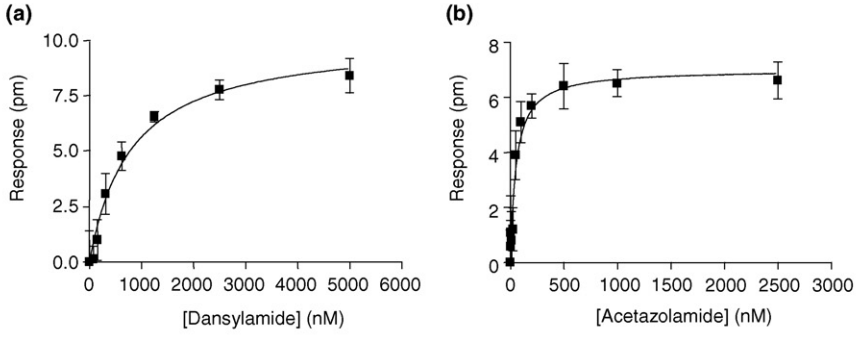

FIGURE 4

Binding response of two different small molecules to immobilized human carbonic anhydrase isozyme II. In **(a)**, K_D(Epic™) = 795 nM, whereas K_D (literature) = 760 nM. In **(b)**, K_D(Epic™) = 53 nM, whereas K_D (literature) = 19 nM. Error bars are standard deviations for $n = 4$. Reproduced, with permission, from the poster 'Analysis of small molecule/protein interactions using Corning® Epic™ system', Fang, J., Xie, X., Piech, G., Frutos, A., Pharma Discovery, May 2006, http://www.corning.com/lifesciences/US-Canada/en/.

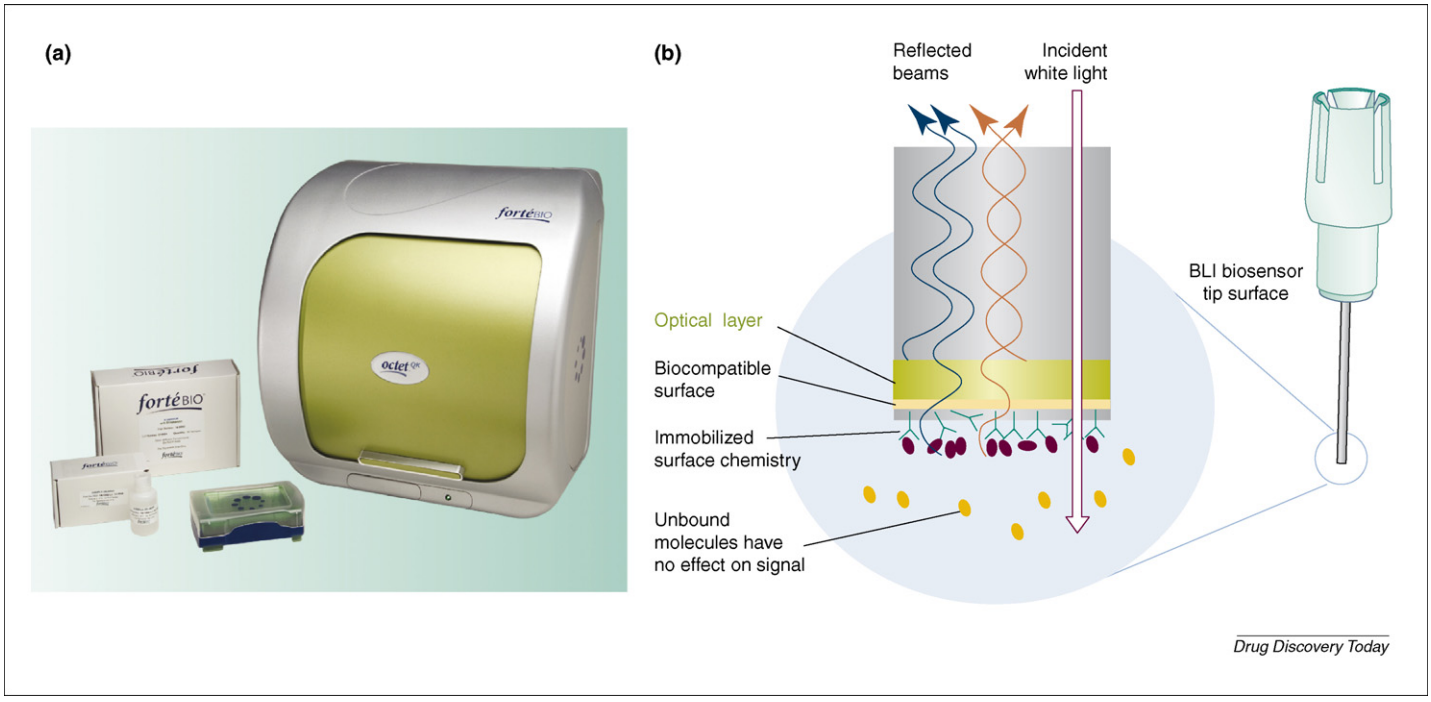

FIGURE 5

The ForteBio Octect system: (a) the reader with integrated sensor tip handling for application to 96 well plates; and **(b)** a schematic of a disposable fibre optic biosensor tip with capture antibodies.

over, if, in response to a stimulus, the cell changes shape, or the endogenous material within the cell that is in close proximity to the sensor reorganizes, a shift in sensor response is induced. Hence, the Epic™ system is claimed to be sensitive to 'whole-cell movement' and 'mass redistribution' within a cell.

ForteBio

In 2006, ForteBio (http://www.fortebio.com) released the Octet system, based on a proprietary technique called BioLayer Interferometry (BLI) [22]. The Octet system uses disposable sensors with an optical coating layer at the tip of each sensor (Figure 5). This optical surface is coated with a biocompatible matrix that can interact with molecules from a surrounding solution. A minimum sample volume of 80 μL should be used in low-volume microplate wells to make accurate measurements because smaller volumes than this can generate measurement artefacts due to internal reflections during measurement. To overcome the effects of diffusion on kinetic measurements, the sample plate is subject to orbital motion relative to the biosensor. Experiments can be performed with static samples (for binding steps), or with motion ranging from 100 to 1500 rpm.

The Octet instrument then shines white light down the biosensor and collects the light reflected back. Interference patterns in the reflected light are captured by a spectrometer as a characteristic profile of wavelength peaks and troughs. When biological molecules bind to the biosensor surface, its thickness increases and the binding can be monitored by analysing changes in the interference pattern at the spectrometer. Unbound molecules and changes to the matrix do not change the interference pattern, which enables the use of crude cell lysates or periplasmic samples. Samples can be presented in a variety of buffers or diluents, including common cell culture media, serum–containing media (up to 25%) and DMSO-containing buffers (up to 10%). Joy Concepcion (Product Manager, ForteBio) explains: 'BLI differs from [SPR] in that the technique does not involve measurement of dielectric constant or refractive index of the solution using an evanescent sensing field, and is hence only minimally perturbed by changes in the medium such as bulk refractive index shifts'. Stephen Oldfield (VP Sales and Marketing, ForteBio) elaborates:

> 'We shine white light down an optical fibre onto a proprietary optical coating with biomolecules attached. Most of the light passes through the coating, however ~4% is reflected back through the optical fibre to a spectrometer. Some wavelengths of light are subjected to constructive interference, and others to destructive interference, which give rise to an interference pattern across the visible spectrum. This pattern is sensitive to the thickness of the biological layer at the surface of the coating. As the surface layer thickness increases, the interference pattern is red-shifted, which can be accurately measured in real time. Since the binding measurements are made from the ''clean'' side of the fibre, refractive index changes and even particulates in the surrounding solution have minimal effect on the signal.'

Conclusion

The past five years have witnessed the emergence of an increasing number of commercially available technology platforms that are driving the development of novel label-free assays. Several new platform technologies have been developed and were launched during 2006. These have come from both small companies and from more established players in the drug discovery tools market. In theory, a label-free screening system

imparts additional flexibility and efficiency to the process of assay design, with potentially fewer artefacts. Although scientists in both academia and industry are using biosensors in areas that encompass almost all stages of the drug discovery process, the technology remains some way from being accepted as mainstream. However, the wider availability of novel sensor platforms that exploit optical, electrical and acoustic interrogation of a sample should finally begin to break down the resistance to uptake of label-free assays as a valuable and easy-to-use tool in drug discovery.

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