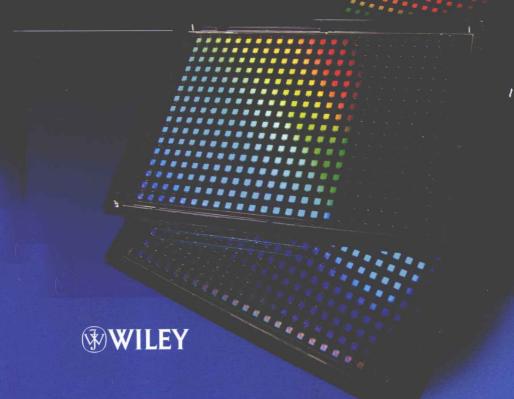
Editors MATTHEW COOPER LORENZ M. MAYR

# Label-Free Technologies

FOR DRUG DISCOVERY



# Label-Free Technologies For Drug Discovery

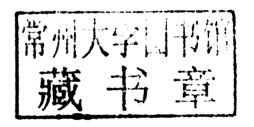
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# **Preface**

In the 1980s, surface plasmon resonance (SPR) and related techniques exploiting evanescent waves were first applied to the interrogation of biological and chemical interactions. These techniques allowed us to study the interaction between immobilized receptors and analytes in real time and without labelling of the analyte; leading to the term 'label-free'. While initially intended as a method of determining affinities, the use of a microfluidic delivery system to the sensor interface allowed kinetics (on and off rates of binding) to be measured. This, in turn, allowed new questions on compound action to be addressed and new compound optimization strategies to be explored. Today it is generally accepted that observed binding rates and binding levels can be interpreted to provide information on the specificity, kinetics and affinity of a drug-receptor interaction that relate to compound mode of action. This builds on the most often quoted maxim used in selecting bioactive compounds; 'Corpora non agunt nisi fixate': a drug will not work unless it is bound (Paul Ehrlich; 1854–1915). This now axiomatic statement guided Ehrlich through many scientific discoveries covering haematology, immunology, bacteriology and early chemotherapy. In the drug discovery process, we are now having to consider not just equilibrium-based, static descriptors of drug-receptor interactions (e.g. IC<sub>50</sub> and EC<sub>50</sub>), but also descriptors of the dynamic nature of drug action. For example, similarly structured molecules can bind to a target with similar affinity. However, only one may have a slow enough off rate to effectively block action of an endogenous ligand; only one may bind in an orientation suitable for a catalytic reaction; only one may induce a conformational change in the receptor. At the extreme, two similarly structured molecules may bind with similar affinity, but one may initiate the receptor response (an agonist), whereas the other may block the response (an antagonist). Optimal binding xvi PREFACE

mechanisms can thus define the therapeutic index and the utility of a drug. Label-free techniques can hence help us understand and optimize these parameters, particularly with respect to predictive pharmacodynamics, competition/interaction with endogenous ligands, binding to side effect profiling targets and metabolic enzyme, and many other attributes that lead to differentiation of a drug candidate from competitor compounds.

Since the development of the first commercial label-free biosensors in the late 1980s, their use in research and development has been described in over 5000 scientific publications covering most disciplines found in the pharmaceutical and diagnostic industries. Traditional solutionbased thermodynamic techniques, such as isothermal titration calorimetry (ITC), have evolved from cumbersome, labour-intensive techniques to automated systems with much lower requirements for reagents and reduced (200 µl) sample volume. Nonfluorescent (white light) high content cell-based assays have been developed together with automated microscope systems combining rapid auto-focusing, automated stage movement and dedicated analysis software capable of batch processing large numbers of images from 96 and 384 well plates. Mass spectrometry combined with high throughput size exclusion and solid phase extraction methods now allows quantification of free and bound species in minutes. Mass spectrometry is thus now a powerful label-free technique that has transitioned from an analytical quality control tool to a mainstream compound profiling and screening platform. In a similar manner, nuclear magnetic resonance (NMR) has evolved from a method to confirm compound structure to a powerful screening tool for identifying low molecular weight drug 'fragment' binders, and even elucidate the specific target binding site of a fragment or lead compound. This approach was pioneered by scientists at Abbott Laboratories, who identified hits from changes in NMR chemical shifts (15N-1H HSQC) and Vertex, who relied on detecting changes in the NMR relaxation properties of the fragments themselves when bound to a protein target.

Finally, in the last five years, the advent of 384- and 1536-well screening systems based on resonant waveguide patterned microtitre plates and electrical impedance 96- and 384-plates has led to an explosion in the application of label-free to GPCR screening. This is highly significant for drug discovery, as at least 800 distinctive human G-protein coupled receptors (GPCRs) are known, with  $\sim\!350$  being estimated to be useful drug targets. Although only  $\sim\!7\%$  of GPCRs are currently targeted by drugs, this accounts for  $\sim\!35\%$  of blockbuster pharmaceuticals. Here label-free can really challenge current screening paradigms. It has emerged that

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different ligands (agonists or antagonists) that bind to the same GPCR, even the same subtype, can display profoundly different biological properties (ligand directed signalling) arising through different regulation of intracellular pathways (e.g. IP3 flux, ERK 1/2 phosphorylation, cAMP activation,  $Ca^{2+}$  release,  $\beta$ -arrestins, etc.). GPCR-mediated pathways, initially thought to be independent, are now known to cross-communicate with other activation paths. For instance, GPCR stimulation can lead to activation of 'traditional' tyrosine kinase pathway components such as Raf, MEK and ERK.

Drug candidate screening paradigms typically involve selection of a transfected cell line, over-expressing the target GPCR. This cell line is then used with one, or a variety, of downstream markers of receptor activation, such as Ca<sup>2+</sup>, cAMP, inositol phosphate and diacylglyercol flux. Standard assay development can be summarized as: (i) compound selection and synthesis, (ii) cell line selection and (iii) downstream reporter assay selection, all of which lead to a data set predicated by cell line and assay format chosen in the first instance. Although this standard approach has become well-accepted for compound screening and pharmacological characterization, it is fundamentally limited in scope in profiling target-related response outcomes. An 'agonist' or 'antagonist' may only be so in the specific screen used; a response in a transfected or transduced cell line may not be the same as that found in the disease relevant endogenous cell. In contrast, label-free screening, which can be carried out using parental cell lines, is thought to be indicative of ligand binding induced changes in cell morphology and holistic behaviour. The readout is noninvasive, temporal, cumulative and most importantly, signalling pathway independent. Kinetic responses or 'fingerprints' elicited by a compound are mechanistically informative, and profiles for particular G-protein coupling can be determined. Hence, the combination of label-free, pathway independent receptor and whole cell profiling with standard reporter and pathway dependent screening should provide new insight into compound mode of action, in addition to identifying new hits that could be missed by traditional assays.

Label-free continues to grow from a niche technology with a user base comprised of early adopters, towards a mainstream, easy to use (but sometimes not easy to understand) technology. We hope the reader finds this compendium of chapters describing label-free technologies and case studies both useful and thought provoking.

Matt Cooper and Lorenz M. Mayr Brisbane, Australia and Basel, Switzerland, July 2010

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