

**RECENT ADVANCES IN LABEL FREE TECHNOLOGIES IN DRUG DISCOVERY AND DEVELOPMENT****T. M. Kalyankar*, Khadkutkar P.K., Chevale S.L**

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ABSTRACT

The drug discovery process has been hastily developed from the past few years due to the application of label free technologies in drug discovery process for new drugs as well as for existing drugs. To overcome the disadvantages with labelled techniques, a newer technology has been developed called label free technology to make the drug discovery process reliable. Label free technology has a key role in high throughput screening in drug discovery process. This review directs toward the advances in label free technology for their application in drug discovery process. Label free technology generally used in biochemical assays and cell based assays with various transducer systems. Newer devices have been launched in the market to minimise the cost of drug discovery tools. Due to the risk of many diseases to human life have been increases, the label free technology have great important in screening of new drug or entire drug discovery process.

Keywords: Label free technology, drug discovery and development, biochemical assays, Cell based (whole cell) assays, Cellkey, GPCRs.

INTRODUCTION

Drug discovery processes used in the earlier 1970s were mainly driven by limited random screening and serendipity and these processes were strengthened considerably during the 1980s to allow more rational approach to structure activity relationship studies. At the beginning of 21st century the drug discovery processes were accelerated significantly due to advanced technology implementation. However evolution of drug discovery processes ultimately led to process standardization across industry. The drug discovery process can be divided into four critical components including target ID/validation, hit identification, hit assessment and lead optimization.¹ Recently the Label-free technologies (LFT) have gained large endorsement not only in academic research settings but also in drug discovery laboratories or industries such as pharmaceutical and biotechnology industries, shows a essential role in drug discovery of small molecule, proteomic studies, biopharmaceutical development etc. studies and in manufacturing and quality control.² Biochemical assays and cell-based assays are suitable for drug discovery with the implementation of Label-free technology as the uses of usual labels are avoided.³ Label-free detection is proving to be a very sensitive method for measurement of number of targets in live cell assays, and there is no need to use tags, dyes or specialized reagents for assay development, simplification of assay design, since it minimizes liabilities created by the use of labels and enabling the use of native cells for greater biological importance.⁴ Thus, more accurate technologies are introduced by pharmaceutical industries uses very sensitive HTS cellular and biochemical assays in a micro plate format which is frequently possible to assist scientist to know fuller picture of their system for screening of compounds for drug discovery.⁵

NEED FOR LABEL FREE TECHNOLOGY ANALYSIS

As the advances in screening technologies, the use of labels in traditional drug discovery and screening assays has been criticized as they produce some liabilities created by the use of labels such as unfavourable and unanticipated interactions that may compromise the screening data and lead to false conclusions. In addition, the LFA are non-destructive and measure cell-based kinetics in real-time, while labelled assays provide only endpoint reads. According to Shoffner, the LFA allow researchers to use more "physiologically relevant" systems, improve sensitivity, reduce false positives or negatives sometimes caused by the steric effects of tags and increase throughput by eliminating the time and effort involved in developing labelling reagents.⁶ Label used in drug discovery have many undesirable artifacts in drug screening applications such as false positives create unnecessary work, false negatives are unknown, additional time and expense of implementing labelling strategy. This can overcome with the use of label free technologies having capacity to Screen orphan targets, identify compounds with novel mechanisms of action and orthogonal to many HTS assays.

LABEL FREE TECHNOLOGIES

Basically there are two types of techniques used to label free technology analysis.

Biochemical assays

The index of refraction is determined by the biochemical assays. As in cellular assays, the change is indicated by a shift in wavelength. These assays are most useful where target proteins are inactive or where the ligand binding partner is unknown. These assays allow testing of direct binding of compounds to the immobilized target.



Functional assays can also be performed anywhere as the target does not need be labelled.^{7,8}

Typical biochemical label-free assays include:

- Screening binding strength (K_D) assays that complement Surface Plasmon Resonance (SPR) technology
- Detection of direct biomolecular interactions (protein:protein interactions)
- Proteases
- Protein-oligo (DNA/RNA) interactions⁷.

Label-free biomolecular interaction analysis is an important technique to study the chemical binding between target molecules. The parameters obtained with this technique, such as the affinity, are important for drug development. Technologies used in biochemical assay are FP (fluorescent polarization), FI (fluorescent intensity), TR-FRET (time resolved fluorescence), wavelength absorbance, ELISA (enzyme-linked immunosorbent assay), and radiometric methods^{8,9}.

Cellular Assays

The cellular assay based on the changes in dynamic mass redistribution (change in wavelength) resulting from refraction within cell which will be observed as a response in activation or deactivation in cells monolayer.

Label-free cell-based assays generally employ a biosensor to monitor ligand-induced responses in living cells. A biosensor typically utilizes a transducer such as an optical, electrical, calorimetric, acoustic, or magnetic transducer, to convert a molecular recognition event or a ligand-induced change in a cell layer into a quantifiable signal.¹⁰

By label-free detection technology high sensitivity at slight expression levels and the integrated response profile allows for determination of Gi, Gq, and Gs pathway determination in a single assay.

Cell-based assays have an important role in drug discovery. Designed appropriately, these in vitro tests can help predict the effect of chemical agents in vivo and can provide relevant biochemical and pharmacological insight that is not possible in a whole animal study¹¹. Cell based allows for examination of targets and pathways in a more physiological setting compared to biochemical assays. One of the main hurdles in the cell-based assay field is to develop sufficiently robust assays with adequate signal to noise parameters while maintaining the inherent physiology of the pathway or target being investigated. Even though some of the label-free technologies discussed in this review have existed for at least a couple of decades, the essence of label-free technology as applied to cell biology and drug discovery is increasingly being realized.¹²

Most of cellular assay use the transducer system such as Impedance-based label-free technologies,¹³⁻¹⁵ Refractive index-based technologies,¹⁶⁻¹⁸ Acoustic technology,¹⁹⁻²³

and Fiber optic waveguide measurement of extracellular flux.²⁴⁻²⁶

RECENT ADVANCES IN LABEL FREE TECHNOLOGY

In drug discovery lab interest in use of label-free technology has been increased in the past as compared to previously reported in 2007 study, 42% vs. 31% of the respondents are allowing for adopting the technology now than in 2007. Label-free technologies have been employed to the entire drug discovery process. The use of robotic systems not only increases sensitivity but also facilitate the expansion of recent technologies that have been recently widen the application of label free in lead optimisation starting from primary screening of chemical compound to mechanism of action studies in the lead optimisation. Recently the label-free methods are commonly adopted process of drug discovery in order that compounds are to be tested against original protein or cells which results in enhanced biological evaluation of compounds more prognostic of therapeutic effect. Label-free technology analysis covers variety of assays this further leads number of different aspects of molecular and cellular biology as in the assessment of binding affinity, rate constants, activation of signalling pathway, cell growth or proliferation of cell, receptor pharmacology, cell adhesion, viral replication, GPCR functional selectivity etc.

Label-free technology is attaining acceptance and providing new opportunity for drug discovery new compounds or drugs which is relatively new in the established world of high throughput screening (HTS), for searching biomolecular interactions within new or existing drug molecule. According to the latest report of 2010, Effective Strategies, inventive Technologies and government-sponsored institutes, label-free technology acceptance is reported as one essential tool which will affect the high throughput screening in future by the way of variety of label free instruments which are using either biomolecular interactions or whole cells based assays. Improved cost, throughput, improved sensitivity and additional sophisticated data analysis are some of advances of label free instruments. Some examples of these label-free instruments are described below.²⁷

Recently some other techniques are used in label free analysis such as LFT for soluble enzymes (measurement of binding affinity with the native molecular target without the need for tagging or labelling), orthogonal screening (ensures the compounds that appear active are binding to the molecular target of interest and not interfering with detection reagents), fragment based lead generation (screen a small but diverse collection of low molecular weight highly soluble ligands to identify relatively weak compounds with high ligand efficiency), binding kinetics and binding thermodynamics.²⁸

Roche Diagnostics

The Roche diagnostic system includes the xCELLingence™ system by a Roche Diagnostic Corporation in 2011 as



RTCA HT instrument with a four plate stationed well format of model 384 may be run on a robotic platform. Secondary screening in drug discovery is to be carried out by this system because of its capacity to carry out short-term GPCR assays and longer-term cytotoxicity applications in accumulation for use as in primary cells and also helps in assessment of endogenous receptor for physiologically pertinent informational data. It is introduced in 2011 as one more instrument used in preclinical in drug discovery as RTCA cardio instrument for testing of lead compound.²⁷

Molecular Devices

In late 2008, Molecular Devices launched the CellKey® cell based 384 System. By means of this instrument assays for measuring completely large range of targets essential for drug discovery. It has integrated fluidics and thermal control. The system has well confirmed capabilities to measure G-protein coupled receptor, activation of Tyrosine Kinase Receptor and ligand gated ion channels measurement between other targets.²⁷

ForteBio ForteBio

Bio-Layer Interferometry (BLI) is a technology from ForteBio for measuring biomolecular interactions.²⁹ Inc. in last two years has launched two next-generation instruments, as the Octet® RED384 for protein, small molecule, peptide, and fragment screening and the Octet® QK384 is for protein, peptide, fragment screening and antibody assays respectively. The Octet® RED384 and the Octet® QK384 will enable 384 well detection capabilities for pharmaceutical and biotherapeutic drug discovery assays.²⁷

Corning Epic®

The first bench-top multimode reader using Corning Epic® based on optical label-free technology has been launched by PerkinElmer in a year 2011, it is a novel system which includes the number of technologies as absorbance, alpha screen luminescence readout etc with an advantages such as lower cost, compact platform along with label free detection.²⁷ The Epic® System uses proven optical biosensor technology to perform label-free measurements for drug discovery applications.²⁹ Their technology is based on changes in the index of refraction off the sensor/plate. Like SRU's, Corning's system can perform both biochemical and cellular assays. Most competing label-free technologies are capable of performing either biochemical or cell-based analysis in low to medium throughputs, while Epic is able to screen either target class at throughputs up to 40,000 wells in eight hours.^{30, 31}

Using the Corning Epic® high throughput screen has been completed for a muscarinic antagonist using a recombinant cell line15 and compared to data for the same compounds in a fluorescent calcium mobilisation assay. Approximately 70 compounds identified from the label free assay were not active in the calcium assay, around 50 of these inhibited binding in a radio ligand

assay. The mechanism of these compounds is yet to be determined but this demonstrates that label-free assays open up the possibility of identifying novel compound series that may be missed with other assay formats.²⁸

Label-free imaging

By means of assay with high density formats by using the primary cells or stem cells may be limited until new methods have been developed to the decrease cell numbers. SRU Biosystems has launched an image reader which is small in size to robust into an incubator. It is able to differentiate human mesenchymal stems cells into osteoblasts and demonstrated this differentiation can be expedited in the existence of a GSK3 inhibitor. In future the combination of some new technologies can be it possible to use induced pluripotent stem (iPS) cells for HTS after that next step may be joining of label-free imaging with high content imaging. Incucyte™ (Essen Bioscience; or Cell-IQ®) phase contrast instrument which may employ for to scrutinize cell growth in a non-invasive manner as like microplates and flasks. Such technologies are employed in expanding new application as CellPlayer™ kinetic assays by Essen Bioscience.²⁸

SRU Biosystems BIND® technology

BIND is a powerful assay development tool that enables qualify and quantify binding [29]. It uses optical resonance in their BIND® technology based on BIND® readers and a range of biosensors. "Our biosensors contain an optical grating, so that when the biosensor is illuminated with multi-wavelength light, only a very narrow range of wavelengths is reflected," says Steger. "The peak wavelength that is reflected changes when things bind to the biosensor surface." Their systems are suitable for both biochemical and cell-based assays, and for lower (16-well) and higher (1536-well) throughputs. SRU is hoping to use their optical resonance technology for label-free cellular imaging and other applications in the area between high-throughput screening and high-content screening, according to Steger.³¹

Cellular Dielectric Spectroscopy

Cellular dielectric spectroscopy (CDS) is a novel technology that able comprehensive pharmacological evaluation of endogenous cell surface receptors by using a label-free, real-time, kinetic cell-based assay approach. In addition, CDS is universal, allowing measurement of multiple types of receptors, including G protein - couple receptors (GPCRs), , nuclear receptors and tyrosine kinase receptors (TKRs) using the same platform, without the need for any modification of the cell.³²

Emerging Label-Free Technologies

There are of the many reasons for increasing clinical costs, failure due to toxicity holds the promise of being successfully addressed with new discovery stage tools. The technologies such as high content and toxicogenomics imaging have the potential to hold down cost in long run predictive assays in clinical trials and lower the costs in



much more shorter frame. According to her toxicity is a chronic and growing problem in drug development such drugs includes antibiotics trovafloxacin which was withdrawn from market because of its association with hepatotoxicity.³⁵

CONCLUSION

Label-free detection is proving to be a very high sensitive method for target and live cell assays measurement and also does not require dyes, specialized reagent and tags. A broad variety of transducer principles different devices have been introduced as CellKey®, Corning Epic®, xCELLingence™ etc. These have different working principles that result in different properties like sensitivity, sample consumption or the ability for multiplexed sensing. For this reason, in addition to sensitivity, also usability, easy handling, reproducibility and robustness play an important role assays processes. All these key parameters are an integral part of the pre-clinical drug discovery process. The label free technology approach can be very powerful in identifying and characterizing potential lead compounds and biologics.

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