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Spotlight on Fluorescent Biosensors—Tools for Diagnostics and Drug Discovery

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ABSTRACT: Fluorescent biosensors constitute potent tools for probing biomolecules in their natural environment and for visualizing dynamic processes in complex biological samples, living cells, and organisms. They are well suited for highlighting molecular alterations associated with pathological disorders, thereby offering means of implementing sensitive and alternative technologies for diagnostic purposes. They constitute attractive tools for drug discovery programs, from high throughput screening assays to preclinical studies.

Although we have entered an era that beholds a wealth of genomic and proteomic information, it remains a major challenge to visualize biomolecules of interest and biomarkers of disease in their native environment. The development of an attractive class of imaging tools, known as fluorescent biosensors, has offered means to address this issue, thereby providing scientists with a powerful and exciting means of detecting their favorite protein, enzyme, biomarker, or target and monitoring its dynamic behavior in living cells.

Fluorescent biosensors come in all sizes, shapes, and flavors, but all share in common their ability to respond to the presence, activity, or conformation of the target through sensitive changes in fluorescence of one or several fluorescent probes coupled to a complementary receptor moiety (typically a substrate or protein binding domain). They may be genetically encoded protein constructs expressed in fusion with autofluorescent proteins or peptide/protein scaffolds coupled or conjugated to small synthetic probes and respond through changes in their subcellular localization or in their spectral properties (fluorescence intensity, lifetime, or wavelength excitation or emission maxima) (Figure 1).[1−6] Fluorescence lends itself to nonradiative, nondestructive imaging and offers a means to identify intrinsically fluorescent or labeled compounds with a high level of sensitivity within a complex mixture of biomolecules. Moreover, fluorescence imaging provides information with high spatial and temporal resolution, and further allows monitoring of dynamic changes in space and in time in a continuous fashion. Fluorescent biosensors are therefore very well suited to report on soluble or secreted biomarkers, cell surface antigens, or intracellular targets both in vitro and in living cells, thereby providing scientists with the power to detect their favorite marker in a complex environment and follow dynamic changes in its subcellular localization, activity, and/or conformation in real time and in a reversible fashion. In fact, fluorescent biosensors are ideally suited for dynamic measurements and real-time kinetic studies, thereby providing precious information on biomolecular function, which complements static biochemical, mechanistic, and structural studies. As such, these tools offer countless opportunities and alternative means of addressing fundamental questions which cannot be investigated through traditional approaches, while also contributing to development of modern diagnostics, biomedical imaging technologies, and drug discovery programs.[7−10]

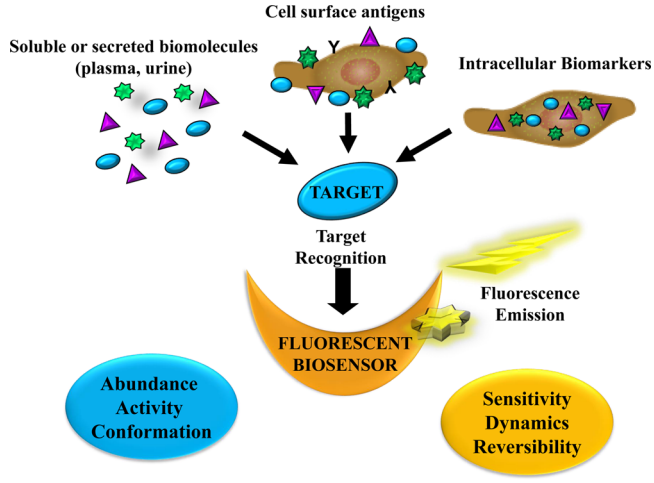

Figure 1. Fluorescent Biosensors—detecting biomolecules in their native environment and monitoring dynamic processes in complex biological samples. Fluorescent biosensors respond to the presence, activity, or conformation of a soluble or secreted target or biomarker, a cell surface antigen, or an intracellular biomolecule, through sensitive, dynamic, and reversible changes in fluorescence of a (or several) fluorescent probe.

Whereas conventional diagnostic approaches rely on antibody-based recognition of cell surface antigens or seric biomarkers, biosensor technology allows implementation of alternative detection strategies to highlight molecular alterations associated with pathological disorders. Biosensor response associated with target recognition is generally proportional to the abundance or activity of the target. Hence, and thanks to their inherent sensitivity, fluorescent biosensors are particularly useful to develop quantification strategies and can therefore serve for early stage diagnostics, to determine the origin, stage, and grade of disease, and for monitoring disease progression, as well as response to drugs, therapeutic benefit, and emergence of resistance. In this respect, and to name but a few, fluorescent

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biosensors have been developed to probe biomarkers associated with viral infection, inflammatory, cardiovascular, and neurodegenerative diseases. Several noteworthy examples of biosensors have been described for detection of enzyme biomarkers, most notably protein kinases and proteases, whose activities are deregulated in disease.[7,8] For instance, genetically encoded FRET biosensors of Bcr-Abl have been developed and employed to monitor drug-resistant phenotypes of chronic myelogenous leukemia, and fluorescent peptide biosensors have been applied to monitor both Bcr-Abl and Lyn protein tyrosine kinase activities simultaneously. Further, a genetically encoded fluorescent biosensor of Src has been applied to image kinase activity and response to dasatinib treatment in pancreatic tumor models and a matrix metalloproteinase biosensor. More recent developments in biomedical imaging involve application of fluorescent biosensors to image-guided surgery, since they allow identification of structures and tissues in which disease biomarkers are overexpressed or hyperactive.[9]

Since the initial development of fluorescent biosensors to study enzymatic activities and understand their role in biological signaling pathways, these tools have now become central and widely used in drug discovery programs. Indeed, the inherent sensitivity of fluorescence and the simplicity of signal readout makes fluorescent biosensors attractive and powerful tools for high throughput screening in multiwell formats, and has enabled the development of high content cell- and image-based screens, thereby allowing identification of candidate inhibitors of molecular targets directly within their physiological environment[10]

Although the larger part of drug discovery programs have relied on activity-based assays over the past 20 years, with the development of fluorescent biosensor technology, novel strategies have been devised to identify drug candidates, based on features other than inhibition of catalytic activity. Different strategies have been designed to screen complex libraries of natural or synthetic compounds in vitro or in cellular formats in view of identifying drug candidates that affect target expression, activity, conformation, or subcellular localization (Figure 2). Positional biosensors provide very simple means of screening for compounds that can modulate the subcellular localization of a target or substrate. FRET—or intensity-based activity biosensors—allow identification of modulators of enzymatic activities through changes in probe fluorescence. Conformational assays allow identification of ligands that are capable of inducing conformational changes which affect target function, thereby providing an attractive means of identifying allosteric ligands.

Although biosensor technology has allowed us to overcome some of the major hurdles in the field of drug discovery, well-defined criteria must be met upon implementation of biosensors for the successful outcome of a screen, with respect to their sensitivity and selectivity, and robustness and reproducibility of signals. One of the keys to success lies in the "clever design" of biosensors for well-defined purposes, so they will respond in a robust yet specific fashion to candidate drugs with high selectivity and desired inhibitory profiles. For instance, structure-guided approaches allow design of inhibitors that bind specific pockets or protein/protein interfaces, but it remains much harder to develop inhibitors that target mechanistic intermediates and/or conformational transitions, let alone screen for such compounds. In this respect, the design of fluorescent biosensor technologies, to probe specific

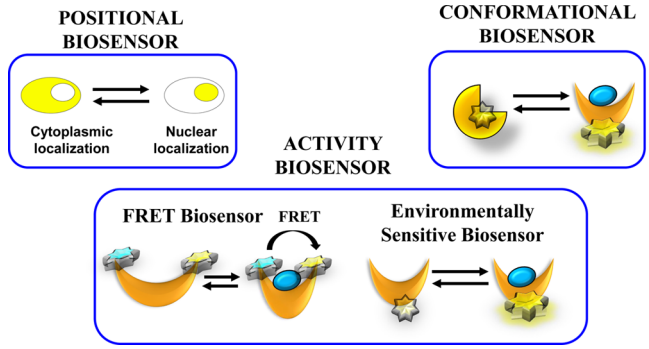

Figure 2. Fluorescent Biosensors for high throughput screening. Three strategies have been employed to establish high throughput or high content screening programs. Positional Biosensors respond to target recognition through changes in their subcellular localization. Activity Biosensors respond to changes in enzymatic activity. FRET biosensors undergo changes in fluorescence intensity and lifetime of a FRET pair of acceptor and donor probes, due to an intramolecular conformational change which alters the proximity of the probes in response to target activity. Environmentally sensitive biosensors transduce target activity through changes in fluorescence intensity, lifetime or wavelength excitation, or emission maxima of an environmentally sensitive probe. Conformational Biosensors respond to ligands that induce conformational changes.

enzymatic conformations, such as FLIK assays (fluorescent labels in kinases), provides an opportunity to screen for inhibitors that target enzymes in an unconventional fashion, such as allosteric inhibitors, which are expected to offer greater selectivity.

Beyond primary screens, fluorescent biosensors are further employed for postscreen validation and characterization of hits, to evaluate their inhibitory potential and characterize their mechanism of action in vitro and in cellulo. Finally, molecular imaging with fluorescent biosensors provides means of characterizing candidate drugs in animal models of disease and of assessing their therapeutic value in preclinical studies.[10] Fluorescent Biosensors constitute sensitive tools for detection of target analytes and biomarkers. Needless to say, the future of these tools looks very bright, offering new avenues of exploration in basic life sciences, and countless and tantalizing opportunities for development of biomedical applications and drug discovery programs. Fluorescent biosensors can be expected to pave the way for development of personalized medicine by providing alternative technologies for clinical diagnostics and theragnostics. Last but not least, they offer promising perspectives for identifying new classes of pharmacological inhibitors and providing complete studies of candidate drugs at the preclinical level by molecular imaging.

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Notes

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