

## Reporter Fluorescent Molecules in Biological Systems: The Current Overview

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Labeling molecules without losing its activity has a long track in science. The first labeling method for detection of the biomolecules was the use of radioisotope entities like  $^{32}\text{P}$ , which have been successfully employed to study protein-protein, protein-DNA, protein-RNA and protein-ligand interactions. In the past years however, radioisotope utilization as label has declined due to safety and health concerns [1]. Various kind of fluorophore moieties as suitable alternative has been developed and widely accepted. Dyes such as rhodamine, fluorescein, phycobiliproteins, nitrobenzoxadiazole, acridines, BODIPY and cyanine compounds or their derivatives are most commonly used as a reporter molecule. The choice of fluorophore molecule to be used for detection depends on the sample type, substrate, the number of analyzed molecules in the experiments and light emission spectra characteristics [2]. Cyanine dyes, Cy3 and Cy5, are among are a good example of dyes used due to their brightness and ability to easily label proteins with  $\epsilon$ -amino group of lysine residues [3]. Detection using the fluorescence labeling can be performed in two ways: by direct labeling (one antibody assay) or indirect labeling [4]. In the direct labeling method, the selected protein is labeled directly with a fluorophore (Cy3 or Cy5), which interacts with, immobilized on the surface of the chip, antibodies. This method allows simultaneous incubation of a reference sample with tested sample, both having different dyes attached [5].

Bodipy that is acronym of *4,4-difluoro-4-bora-3a,4a-diaza-s-indacene* are the highly fluorescent molecule that is alternative for Cy dyes. Pei and coworkers [6] constructed chip that performance was evaluated by performing assays for G protein GTPase activity on chip using BODIPY-GTP as enzyme substrate.

### Nanomolecules

Quantum dots (Qdot) and gold nanoparticles [7,8] are another group of fluorescence emitting molecules. The Qdot is a nano-metal fluorophore with bright and linear signal of emitted light. One advantage of using Qdot is that it has no photo-bleaching effect that often occurs while using organic fluorophores which combined with narrow emission spectra and greater quantum yield [9] makes the quantum dots an excellent alternative for conventional fluorophores. Indeed Qdots find a diverse range of applications in biological sciences such as diagnostic imaging [10], detection of cancer biomarkers [11,12], and probing of human serum [13]. However the low stability and short lifetime of Qdots due to their susceptibility to oxidation are the main shortcoming of these methods.

### Rare Metal Chelates

Rare metal (lanthanide) complexes are found to be highly fluorescent and long lasting fluorophore species [14]. In late 80's of the last century, intrinsically fluorescent lanthanide chelates were developed, which made them viable donors in FRET applications. Lanthanide chelates have exclusive properties that make them excellent donors compared to organic dyes. Lanthanide chelates have long lifetimes that enable time-resolved measurement of acceptor emission, which significantly decreases the effect of autofluorescence and possible direct acceptor excitation.

### Bioluminescence

Light emission (luminescence) is another method to visualize the target/captured protein. Biotin market proteins or protein with attached Strep tag that is peptide that reveal high affinity for streptavidin, are applied on microarray system. Light intensity boost is observed as positive output after incubation with streptavidin conjugated with fluorophore [15] or horse radish peroxidase in presence of appropriate substrate [16]. In the last paper using this biotin label-based antibody array technology, the expression levels of 507 human, 308 mouse and 90 rat target proteins can be simultaneously detected, including chemokines, growth factors, cytokines angiogenic factors, proteases, soluble receptors, soluble adhesion molecules, and other proteins in a variety of samples. Most proteins can be detected at pg/ml and ng/ml levels.

Another strategy important to evaluate bimolecular interactions is a FRET based compounds (Fluorescence Resonance Energy Transfer) [17,18]. Since the efficiency of FRET depends on distance between target molecules (containing donor and acceptor of fluorescence) this effect is easily adapted for two labeled molecules. The system with two labeled interacting molecules display upon excitation low fluorescence intensity due to high FRET occurrence. Single molecules display high fluorescence intensity. Another important filed utilisation of fluorescent dyes is to follow the activity of proteolytical enzymes by FRET displaying molecules. This issue has been generally recognize as a method of choice in construction of fluorescent substrates [19,20] or molecular probes [21].

### Closing remarks

The analysis of biomolecule is a demanding issue due to complexity of the origin sample (blood, serum, tissue). Therefore there is an ongoing need to improve the final read out in sense of the sensitivity and stability of the fluorophores. Novel fluorescent materials like quantum dots or new organic dyes have proven to be a progress in this area. Luminescence-based detection or its combination with fluorescent one is a good example of upcoming systems.

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