Biochemical Engineering Journal 133 (2018) 83–95

Contents lists available at ScienceDirect

Biochemical Engineering Journal

journal homepage: www.elsevier.com/locate/bej

Review

Nanoscale gizmos – the novel fluorescent probes for monitoring protein activity

Ovais Manzoor, Neha Soleja, Mohd. Mohsin*

Department of Biosciences, Jamia Millia Islamia, New Delhi, 110025, India

ARTICLE INFO

Article history:
Received 23 October 2017
Received in revised form
27 December 2017
Accepted 6 February 2018
Available online 7 February 2018

Keywords:
Fluorescent microscope
Real-time
Quantum dot
FRET
Nanosensor
Spatiotemporal resolution

ABSTRACT

Nanobiotechnology has emerged inherently as an interdisciplinary field, with collaborations from researchers belonging to diverse backgrounds like molecular biology, materials science and organic chemistry. Till the current times, researchers have been able to design numerous types of nanoscale fluorescent tool kits for monitoring protein–protein interactions through real time cellular imagery in a fluorescence microscope. It is apparent that supplementing any protein of interest with a fluorescence habit traces its function and regulation within a cell. Our review therefore highlights the application of several fluorescent probes such as molecular organic dyes, quantum dots (QD) and fluorescent proteins (FPs) to determine activity state, expression and localization of proteins in live and fixed cells. The focus is on Fluorescence Resonance Energy Transfer (FRET) based nanosensors that have been developed by researchers to visualize and monitor protein dynamics and quantify metabolites of diverse nature. FRET based toolkits permit the resolution of ambiguities that arise due to the rotation of sensor molecules and flexibility of the probe. Achievements of live cell imaging and efficient spatiotemporal resolution however have been possible only with the advent of fluorescence microscopic technology, equipped with precisely sensitive automated softwares.

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* Corresponding author.
E-mail address: mmohsin1@jmi.ac.in (Mohd. Mohsin).

https://doi.org/10.1016/j.bej.2018.02.003
1369-703X/

1. Introduction

Fluorescence is a luminescence process in which atoms and molecules absorb a specific lower wavelength light and after a brief gap of fluorescence lifetime emit a longer wavelength light. Over the past many years in the service of biology, fluorescence microscopy has emerged as the prime pillar of microscopy due to its inherent selectivity to expose only objects of interest against black background [1]. To investigate myriad of cellular processes, fluorescence imaging has been realistic for the visualization of molecules and whole organisms. It started with the attachment of organic dyes to proteins through antibodies and later sought the genetic tagging of target proteins with fluorescent proteins. However, the linking of antibodies has to be supplemented with the fixation and permeabilization of cells [2]. With the efficacy of fluorophores as direct recognition agents for various cellular molecules like nucleic acids, ions and other cell organelles, the technique of immuno-fluorescence became less valuable. Additionally, with the development of FRET based sensors, noninvasive behavior and live cell imaging in both prokaryotic and eukaryotic cells proved to be a novelty for researchers exploring fluorescent probes for real time cellular imaging. Originally, FRET stands for Forster Resonance Energy Transfer in the honour of Theodor Forster, physical chemist, who first discovered and understood it. The term "fluorescence resonance energy transfer" is often used, when both the chromophores are fluorescent. The latter enjoys common usage in scientific literature and has been in practice as such commonly.

However, It has been established that compared to other fluorophores, amplified brightness and photo-stability are the two critical parameters of semiconductor nanocrystals that makes them unique for fluorescent microscopic imaging (Fig. 1), although their targeting potential still endures many hindrances [3]. In recent years, using newly engineered photo-controllable fluorescent proteins; numerous super-resolution microscopic techniques became ready to use in order to get better visualization of objects having dimensions smaller than 500 nm and 200 nm in axial and lateral directions, respectively. In current times, researchers are now powerfully aided with more precisely optimized FPs. There are three groups of photo-controllable FPs- photo-convertible FPs (PCFPs), photoactivatable FPs (PAFPs), and reversibly photo-switchable FPs (rsFPs). PAFPs are activated from non-fluorescent (dark) to fluorescent state, whereas, rsFPs are reversibly photo-switched between inactive and active states, and PCFPs are made to convert from original color to another. The super-resolution microscopic imaging using these FPs is always carried out by controllably turning them on and off [4].

Moreover, proteases have emerged favorable enzymes for synthesizing their inhibitory compounds as potential drugs for some major human diseases such as cancer, AIDS and other neurodegenerative infections. Because of the latest coherent advancements between genomic, proteomic and biophysical techniques, peptide substrates for several proteases have been ascertained, that paved a way to trace and analyze the functioning of their equivalent proteases through a simplistic and speedy mode. By combining such peptide substrates with appropriate FPs, researchers develop a chimeric protein and then sequentially analyze many protease inhibitors through FRET disruption. Present review will focus on latest advances in major fluorophores and diverse fluorescence strategies that are in current use of fluorescence microscopy to visualize protein dynamics involved in their location and function within a cell.

2. Variety of fluorescent probes

Varieties of fluorophores that are beyond the scope of this review are now available for evaluating the protein activity. Regarding fluorescent probes, two critical considerations must be acknowledged first the fact that spectral properties of fluorescent probes determine the basic settings of time resolution and wavelength of instruments, and secondly certain fluorescent probes are used to monitor specific activities. For example, fluorophores that are sensitive to pH can only be explored to measure pH, and rotational diffusion can be tracked by only those probes having non-zero anisotropies. For histological studies, probes possessing long excitation and emission wavelengths are utilized to display auto-fluorescence at short excitation wavelengths. We are elaborating here some basic fluorophores which are being exploited through various bioengineering approaches to visualize the sea of protein dynamics, so as to come up with innovative nanoscale toolkits for monitoring molecular interactions operating within a living cell in real time.

2.1. Molecular organic dyes

In the synthesis of organic fluorophores, many molecular strategies have been adapted. For this purpose, addition of electrophiles or other charged substituents like sulfonates, conjugation of double bonds, extra ring rigidification by locking rotatable rings into parent ring structures, etc. are some of the most important assignments. Though the dyes made by such strategies are now available commercially (Haugland, 2005), however, such dyes are handicapped by being non-specific to all proteins. Therefore, their use in permeabilized and fixed cells has to be supplemented with antibodies (Fig. 2A). These organic fluorophores are usually of low magnitude (<1 kD) and their important etiquettes like reduction in self-quenching, good brightness and appropriate wavelength have made them industrially optimized.

For a successful delivery of fluorophore into a cell, broadly two types of cellular environments- intracellular and extracellular are available. The major confrontations to overcome lies in optimized intracellular delivery of fluorophores, the perfect labeling of target biomolecule and negligible cytotoxicity. Due to smaller size of organic fluorophores there is least spatial hindrance to impede with functioning of target biomolecule. Therefore, this opportunity has been explored by attaching many fluorescent probes with a single target biomolecule in order to gather maximum fluorescence signal. Because of high label densities the strong electrostatic repulsion between neighboring molecules, the dye structural conformation and hydrophilicity altogether can cause fluorescence quenching [5–7] and may also affect functioning of the biomolecule [8].

For the selective detection of enzymatic and non-enzymatic proteins, fluorescent chemical turn-on probes are synthesized by fusing an environment sensitive fluorophore with a protein-specific small molecule [9]. In this scenario, localized deposition of proteins in proteostasis has been elaborately exploited to develop sensors for understanding the mechanism of protein deposition in neurodegenerative disease progression [10]. The process of proteostatis involves biogenesis, traffic and protein degradation within and outside a cell, involving different integrated biological pathways. The basis of such a designed fluorescent probe is that most of the ligand binding sites in proteins are hydrophobic, that constitute the prime thermodynamic driving force for the binding of small molecule ligands to their respective proteins. This approach has been exploited to develop a precious turn on folding sensor for effective live cell monitoring of proteostatis [11]. This small fluorogenic molecule became fluorescent when it binds and reacts with folded and functional retroaldolase enzyme. Recently, in a similar approach, dual signal fluorescence-enhanced sensor, based on Cu^{2+} mediated fluorescence switchable strategy has been designed to detect Cysteine (Cys) in a simple and fast way. It was observed that two fluorescence emissions of ultrathin films (calcein@NFR/LDHs UTFs) are effec-