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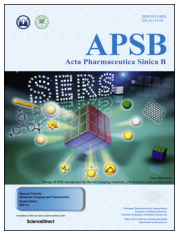

REVIEW

Fluorogen-activating proteins: beyond classical fluorescent proteins

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Abstract Fluorescence imaging is a powerful technique for the real-time noninvasive monitoring of protein dynamics. Recently, fluorogen activating proteins (FAPs)/fluorogen probes for protein imaging were developed. Unlike the traditional fluorescent proteins (FPs), FAPs do not fluoresce unless bound to their specific small-molecule fluorogens. When using FAPs/fluorogen probes, a washing step is not required for the removal of free probes from the cells, thus allowing rapid and specific detection of proteins in living cells with high signal-to-noise ratio. Furthermore, with different fluorogens, living cell multi-color proteins labeling system was developed. In this review, we describe about the discovery of FAPs, the design strategy of FAP fluorogens, the application of the FAP technology and the advances of FAP technology in protein labeling systems.

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1. Introduction

Fluorescence imaging is one of the most powerful techniques to observe biomolecules in real-time with high spatial and temporal resolution, which reveals the fundamental insights into the production, localization, trafficking, and biological functions of biomolecules in living systems[1–4]. As the biological objects are poorly fluorescent, fluorescent probes including fluorescent proteins and organic fluorescent dyes are essential molecular tools for bio-imaging[5,6]. Among them, a diverse set of genetically encodable fluorescent biosensors have been designed to probe dynamic cellular events. These sensors that generally involve the incorporation of a fluorescent tag into a protein or a selected protein domain, have enabled researchers to track various components of intracellular signaling networks in real time within the native cellular environment. In the past several decades, two approaches have been developed to construct genetically encodable biosensors for live cell studies (Fig. 1): 1) fluorescent protein-based reporters: chimeric genetic fusions of fluorescent proteins (*e.g.*, GFP and its variants) with a protein (or RNA) domain[7]; 2) fluorogen-based reporters: a genetically encodable tag binds a fluorogenic ligand (endogenously present or exogenously applied) and activates its fluorescence. As the fluorogenic chromophore is non-fluorescent by its own and becomes strongly fluorescent only upon binding its target, unspecific fluorescence background in cells remains minimal even in the presence of an excess of dye, thus ensuring high imaging contrast[8]. Labeling with fluorogenic probes can be covalent, relying on chemical or enzymatic reaction, or non-covalent, relying on binding equilibrium. In the past 20 years, great efforts have been dedicated to exploring covalence-based self-labeling tags, such as the commercially available SNAP-tag[9–11], CLIP-tag[12] and Halo Tag[13–15]. Parallel to the development of covalent fluorogenic protein labeling strategies, methods based on the non-covalent interaction between a protein tag and a fluorogenic dye have emerged[16,17]. Unlike the covalent labeling strategies, non-covalent labeling can be very fast since no covalent bond has to be created. Moreover, systems based on reversible non-covalent binding could provide an additional degree of control as fluorescence could also be switched off by washing away the fluorogenic ligand, given that the off-rate is fast enough. In this review, we describe the discovery of one of the non-covalence-based fluorogenic probes based on fluorogen activating proteins (FAPs), the design strategy of FAP fluorogens, the application of the FAP technology and the advances of FAP technology in protein labeling systems.

1.1. The discovery of the FAPs

FAP technology was first introduced in 2008 by Szent-Gyorgyi et al.[18] by using single-chain antibodies as genetically encodable FAP. FAPs binding modified thiazole orange (TO) and malachite green (MG) were first generated by screening a yeast surface-displayed library of human single-chain antibodies (scFvs) using fluorescence-activating cell sorting (FACS). Eight unique FAPs were isolated from the library, among which six proteins specifically activate modified MG. scFvs are engineered proteins composed of Immunoglobulin (IgG) variable heavy (V_H) and variable light (V_L) domains tethered together *via* a short flexible peptide linker, which retain the wide range of antigen recognition capabilities of the full-length antibodies, and are also conformable to be used as recombinant tags in diverse fusion proteins[19].

The FAP technology is a fluorogenic tagging approach that utilizes molecular recognition to directly activate the fluorescence of otherwise nonfluorescent small-molecule dyes (fluorogens). Selected FAPs bind TO and MG with nanomolar affinity and increase their respective green and red fluorescence by as much as thousands of fold. The fluorescence enhancement results from FAPs constraining the rapid rotation around a single bond within the chromophore (Fig. 2)[6]. The non-covalent interactions between the fluorogens and FAPs are like those of ligands and their receptors, mainly including van der Waals forces, π-effects and hydrogen bonds. Molecular recognition capabilities are largely determined by these loops of FAPs, termed complementarity determining regions (CDRs), which undergo somatic hypermutation during the immune response to generate specific high affinity binding to the antigen (Fig. 3)[20,21]. FAPs represent a new class of fluorogen-based reporters, which provide a fluorescent tool for imaging fusion protein's location and abundance in time and space. FAP-fluorogen imaging system offers a number of distinct advantages in bio-applications: 1) unbound dye remains nonfluorescent in solution, allowing for the simple addition of dyes to the cellular media without any need for fixation or washout, a property that will enable imaging in more complicated tissue environments and live-cell imaging; 2) fluorogen binding to most FAPs occurs within seconds of addition, and can be carried out in a near physiological buffer or medium of choice. The interaction between the fluorogen and FAP is highly specific, with some FAP clones exhibiting subnanomolar affinity; 3) since FAPs are small in size (<30 kDa), they are easy to genetically engineer. The FAP technology thus allows specific fluorescent labeling of fusion proteins of interest in both living or chemically fixed cells; 4) the possibility offered to completely control the concentration of fluorogens paves the way for on-demand applications wherein

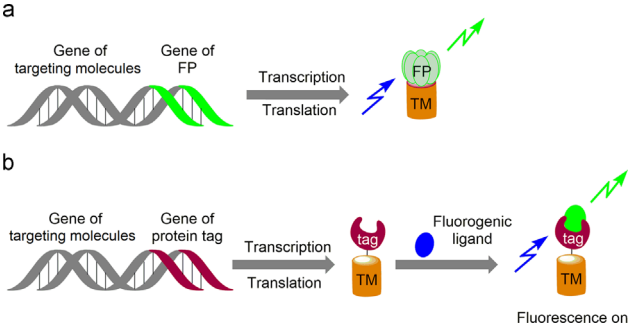

Figure 1 (a) Fluorescent protein-based reporters and (b) fluorogen-based reporters for fluorescence imaging, TM: targeting molecules.