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Current Opinion in Chemical Biology

Novel uses of fluorescent proteins

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The field of genetically encoded fluorescent probes is developing rapidly. New chromophore structures were characterized in proteins of green fluorescent protein (GFP) family. A number of red fluorescent sensors, for example, for pH, Ca^{2+} and H_2O_2, were engineered for multiparameter imaging. Progress in development of microscopy hardware and software together with specially designed FPs pushed superresolution fluorescence microscopy towards fast live-cell imaging. Deeper understanding of FPs structure and photophysics led to further development of imaging techniques. In addition to commonly used GFP-like proteins, unrelated types of FPs on the base of flavin-binding domains, bilirubin-binding domains or biliverdin-binding domains were designed. Their distinct biochemical and photophysical properties opened previously unexplored niches of FP uses such as labeling under anaerobic conditions, deep tissue imaging and even patients' blood analysis.

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Current Opinion in Chemical Biology 2015, **27**:1–9

This review comes from a themed issue on **Molecular imaging**

Edited by **Samie Jaffrey** and **Atsushi Miyawaki**

http://dx.doi.org/10.1016/j.cbpa.2015.05.002

Introduction

In the broad instrumentarium of modern biology, fluorescent proteins occupy an important and unique niche enabling direct observation of molecular processes in live systems [1].

This technology began from a single known member, green fluorescent protein (GFP) from jellyfish *Aequorea victoria*. In 1990s, a number of its improved and color-shifted variants from blue to yellow were created. Since the discovery of GFP-like proteins of different colors (from cyan to red) in corals in 1999, great efforts were directed towards characterization of natural diversity of this protein family. As a result, GFP-like proteins were found not only in coelenterates, but also in combjellies, crustaceans, and lower chordates — lancelets [1]. Proteins of diverse spectral, biochemical and biophysical properties were found in nature leading to development of a multitude of FP-based techniques.

The interest in novel natural GFP-like proteins has diminished by now. The last notable finding in this field was a discovery of multidomain FPs in Hydrozoa in 2012 [2]. In these FPs, two or four GFP-like domains are repeated within the same polypeptide chain. Curiously, a linker between these domains contains amino acid sequence VAMPRIVET that means 'hello to you' in Russian. This 'best regards' from Nature should encourage scientists to further study biological functions and evolution diversity of this amazing protein family.

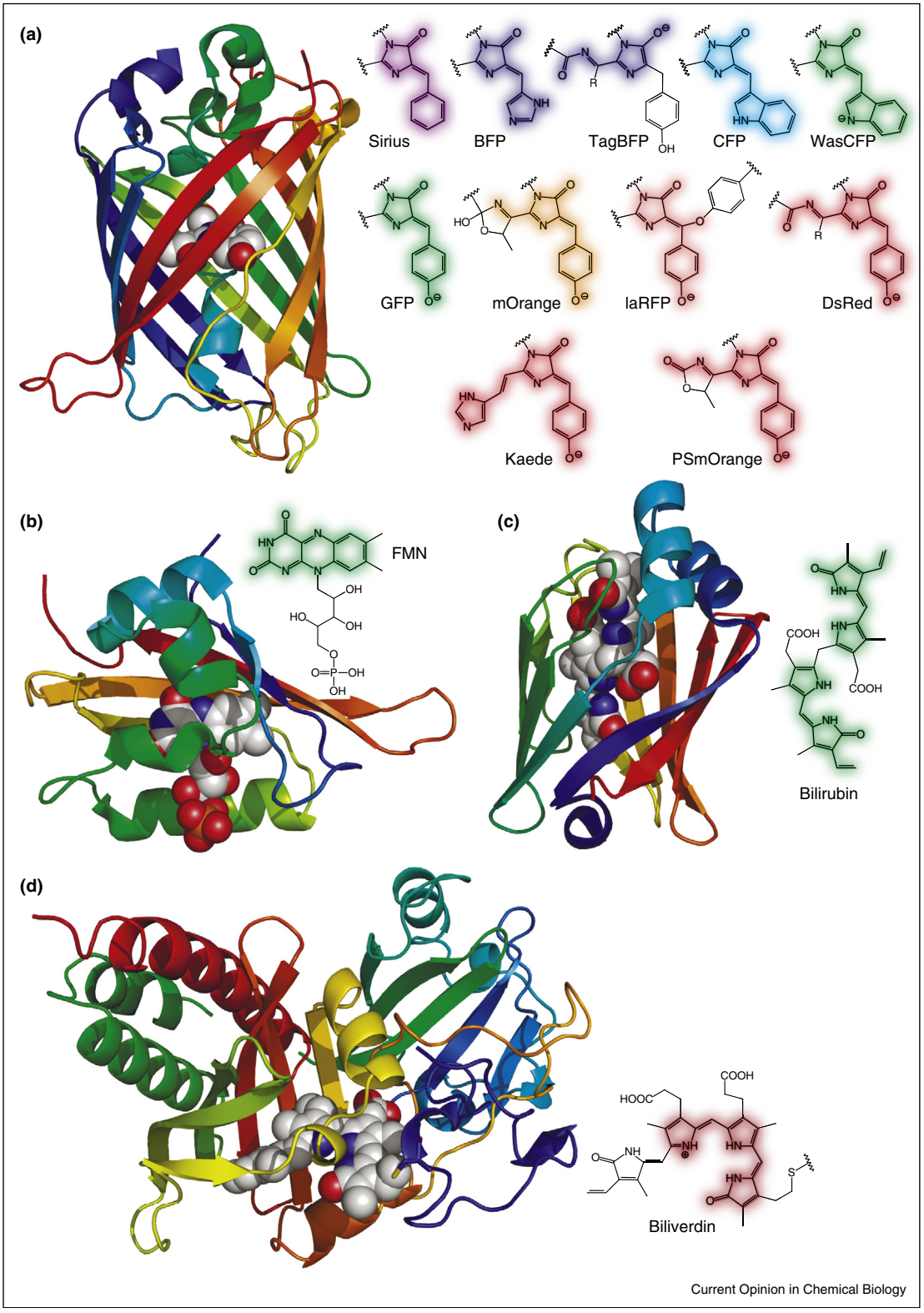
The focus of the GFP-like proteins research in the last few years has shifted towards deeper understanding of structure, photophysics and photochemistry of existing FPs and development of advanced imaging methods. An important new direction is engineering of FPs unrelated to GFP.

Applications of FPs are published in thousands of papers every year. In this short review, we focus only on a few topics, which in our opinion are of a special interest.

From novel fluorescent proteins to novel applications

By contrast to other natural proteinaceous pigments, which carry protein-bound cofactors, proteins of GFP family form chromophore by self-catalyzed posttranslational modifications of their own internal amino acids [1]. Different chromophores can be formed within the protein β-barrel (220–240 amino acids), and the list of known chromophore structures continues to expand (Figure 1a). For example, an unusual linkage of GFP-type chromophore with the hydroxyl of a nearby tyrosine was recently revealed in red laRFP from a lancelet [3]. Also, in engineered green WasCFP a possibility to deprotonate the tryptophan-based CFP-type chromophore was demonstrated [4]. WasCFP possesses a record high fluorescence lifetime that is advantageous for multiparameter fluorescence lifetime imaging (FLIM) and fluorescence resonance energy transfer (FRET). Potentially, these new types of chromophores

Figure 1



Structures of fluorescent proteins of different types. 3D protein structures are shown to the same scale in cartoon representation (rainbow colors from blue at N-termini to red at C-termini). Chromophore groups are shown in spacefill representation. Images were created using PyMol (DeLano Scientific) from PDB files 2Y0G, 4EET, 4I3B, and 1ZTU for (a–d), respectively. In chemical structures of chromophores the moieties responsible for fluorescence are highlighted by corresponding colors. **(a)** GFP-like proteins. Main types of chromophores formed by autocatalytic modifications of internal amino acids are shown. **(b)** FMN-binding fluorescent proteins (PpFbFP, iLOV, miniSOG, etc.). **(c)** Bilirubin-binding protein UnaG. **(d)** Biliverdin-binding fluorescent proteins (IFP1.4, iRFP, IFP2.0, etc.).