*Commentary*

# Green fluorescent protein: a molecular lantern that illuminates the cellular interior

The green fluorescent protein (GFP) has become an extremely popular reporter molecule in contemporary cell biology research in a relatively short span of time (figure 1). GFP fluorescence is extensively used as a tool for monitoring gene expression, localisation, mobility, traffic, and interaction of various membrane and cytoplasmic proteins. The major advantage of GFP as a fluorophore is its intrinsic, cofactor-independent fluorescence, which exhibits remarkable stability in the presence of denaturants and over a wide range of pH (Tsien 1998). In addition to visualisation by fluorescence microscopy, GFP-tagged proteins can be monitored by a variety of techniques such as fluorescence resonance energy transfer (FRET), fluorescence recovery after photobleaching (FRAP) and fluorescence correlation spectroscopy (FCS).

The Nobel Prize in chemistry in 2008 was awarded for the discovery and development of GFP to Osamu Shimomura, Martin Chalfie and Roger Tsien. GFP was discovered by Shimomura in the jellyfish *Aequorea victoria* (figure 2) in the early 1960s (Shimomura *et al.* 1962). It took almost three decades for the gene that encodes GFP to be cloned (Prasher *et al.* 1992). Subsequently, it was shown by Martin Chalfie and co-workers that GFP could be expressed in heterologous systems (Chalfie *et al.* 1994). This

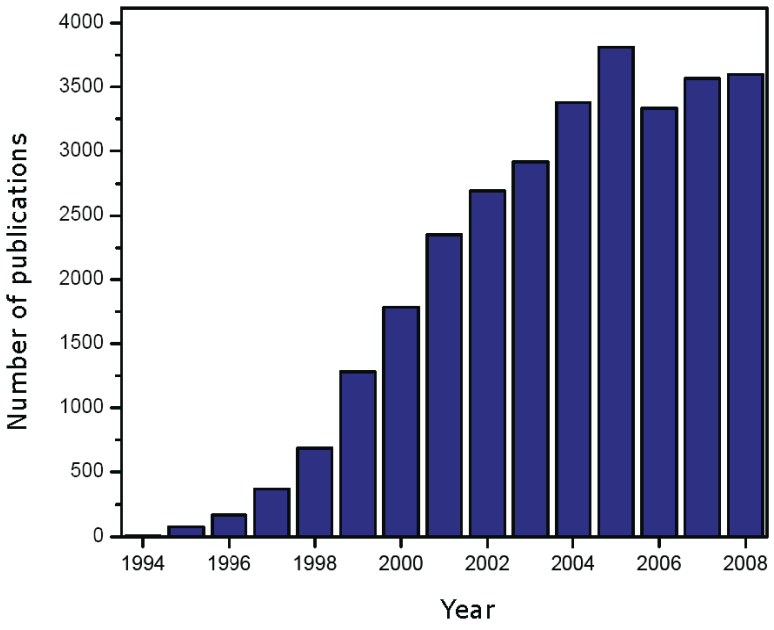


**Figure 1.** Number of publications (year-wise) in which the words *green fluorescent protein (GFP)* appear either in the title or abstract (data taken from PubMed). This list is not exhaustive.

**Keywords.** Bimolecular fluoroscence complementation; green fluorescent protein; molecular lantern; Noble Prize

Abbreviations used: BFP, blue fluorescent protein; BiFC, bimolecular fluorescence complementation; CFP, cyan fluorescent protein; ESPT, excited state proton transfer; EYFP, enhanced yellow fluorescent protein; FCS, fluorescence correlation spectroscopy; FRAP, fluorescence recovery after photobleaching; FRET, fluorescence resonance energy transfer; GFP, green fluorescent protein

was the crucial development that led to the popularity of GFP (figure 1). Roger Tsien and co-workers later provided the structural and molecular details of GFP (Ormö *et al.* 1996). GFP is a compact, barrel-shaped protein of 238 amino acids made up of 11 $\beta$ strands with an $\alpha$-helix running through the central axis of the cylindrical structure, giving it a *monolithic cylindrical symmetry* that can be viewed as a *molecular lantern* (figure 2). The fluorophore of GFP (*p*-hydroxybenzylideneimidozolidinone), responsible for its green fluorescence, lies at the centre of the $\beta$ barrel structure. It is formed spontaneously (as a result of post-translational modification) upon folding of the polypeptide chain by internal cyclisation followed by oxidation of the residues Ser65-Tyr66-Gly67 (figure 3). The uniqueness of GFP stems from the fact that its fluorescence does not depend on the idiosyncrasies (any cofactor or specific enzyme) of the jellyfish *A. victoria*; it only requires molecular oxygen to glow! Interestingly, the fluorophore is in a highly constrained environment, protected from the bulk solvent by the surrounding rigid $\beta$ strands, as shown by dipolar relaxation measurements (Haldar and Chattopadhyay 2007).

The complex yet fascinating photophysics of GFP represents a challenging area of research (Chattoraj *et al.* 1996; Prendergast 1999). An interesting photochemical feature of wild-type GFP is the existence of an excited state proton transfer (ESPT) mechanism. Although wild-type GFP has two absorption/excitation maxima (at 398 and 478 nm at 25°C), it has only one emission maximum. This puzzle was solved by invoking an intramolecular ESPT mechanism involving the phenol group (in tyrosine) (Chattoraj *et al.* 1996). GFP thus provides a platform to study fundamental photophysical processes such as proton transfer and solvent relaxation. It later became apparent that mutations in the fluorophore region as well as in the surrounding $\beta$ barrel structure can alter the photophysical and spectral properties of wild-type GFP. By employing a protein engineering approach (random as well as site-directed mutagenesis), Roger Tsien generated a number of variants of GFP such as the blue fluorescent protein (BFP), cyan fluorescent protein (CFP) and enhanced yellow fluorescent protein (EYFP) (Tsien 1998; Patterson 2004). These variant fluorescent proteins have better spectral properties and photostability. GFP therefore represents an ideal system for studying directed protein evolution.

Interestingly, GFP has turned out to be a good model for protein folding since secondary structure is necessary for its fluorescence. Unlike tryptophans in proteins, GFP does not fluoresce when denatured. The green fluorescence is regained only when the protein refolds (Prendergast 1999). Since GFP has

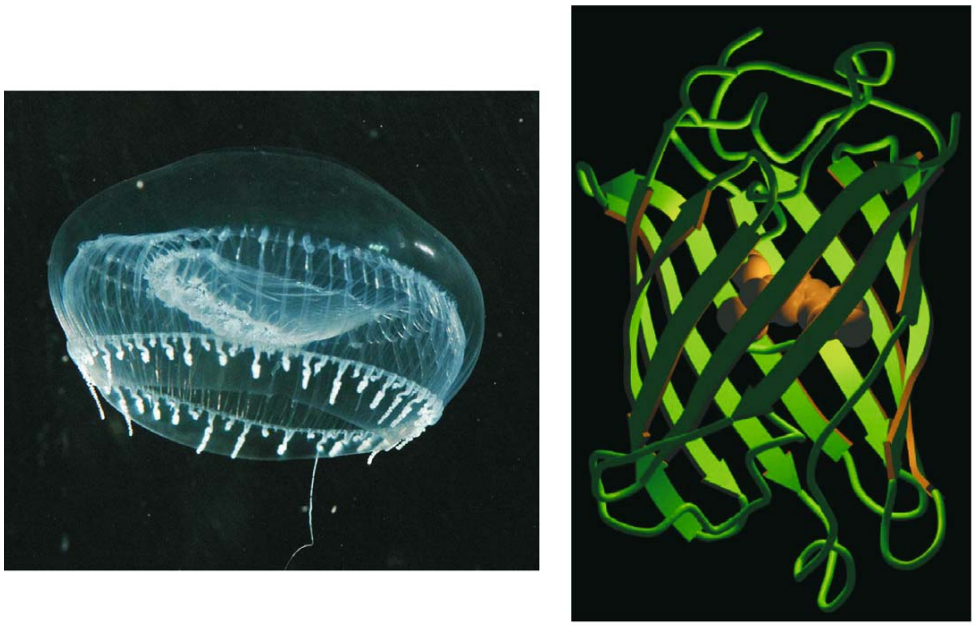

**Figure 2.** The jellyfish *Aequorea victoria* (left) from which GFP (right) was isolated.

a $\beta$-barrel structure, and the majority of protein folding studies are carried out with $\alpha$-helical globular proteins, GFP could be a valuable tool in contributing to our knowledge on the folding of $\beta$ sheets/barrel proteins. Another remarkable aspect of GFP is that in spite of its large size (27 kDa), most (but not all, *see* Fucile *et al.* 2002) proteins maintain their biochemical characteristics upon fusion with GFP (e.g.

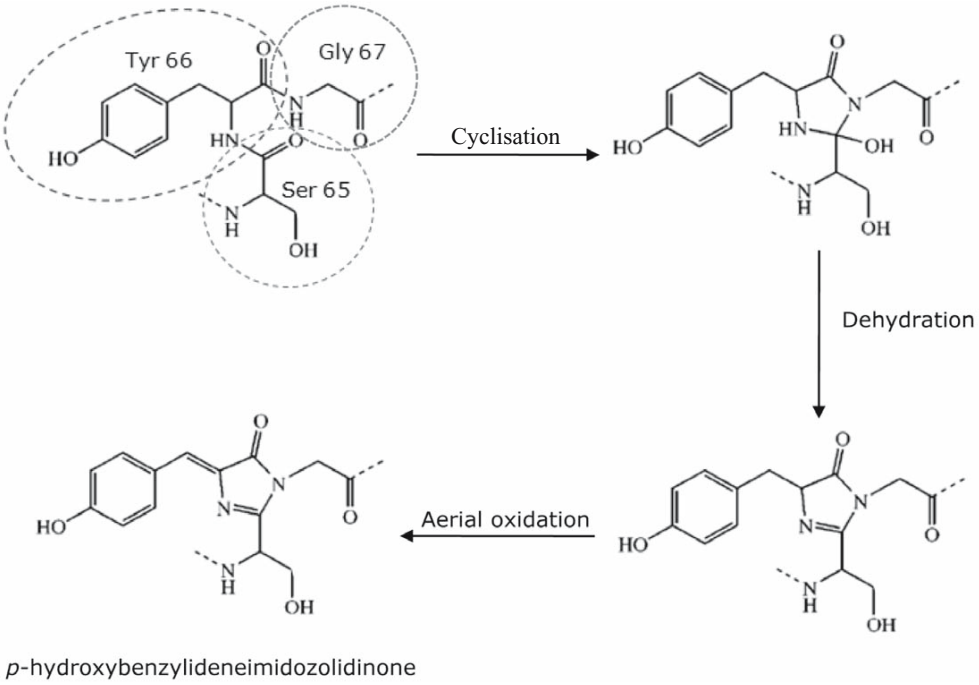


**Figure 3.** Mechanism for the formation of the green fluorescent protein (GFP) fluorophore, *p*-hydroxybenzylidene-imidozolidinone, by intramolecular cyclisation involving Ser65-Tyr66-Gly67 residues followed by oxidation. Adapted and modified from Tsien (1998).

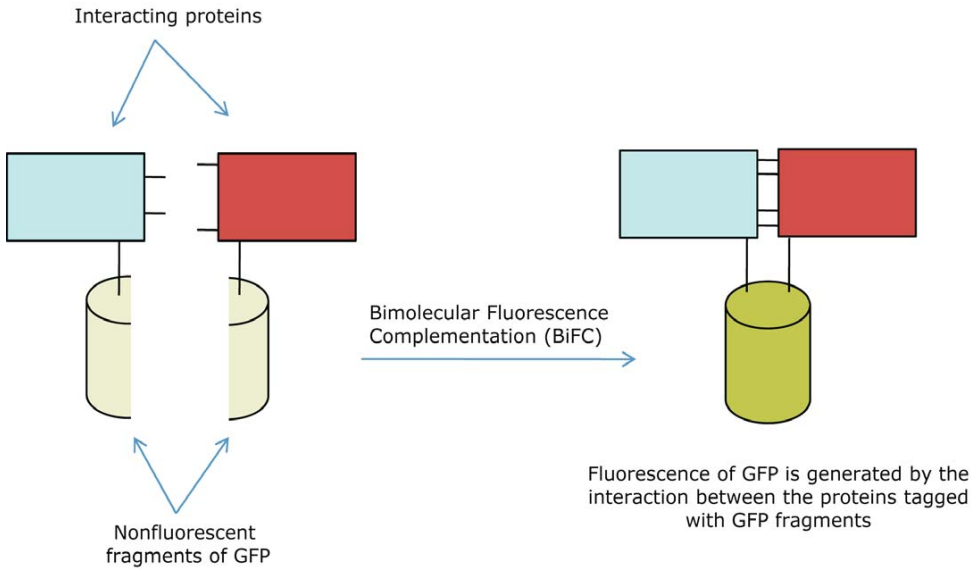


**Figure 4.** The bimolecular fluorescence complementation (BiFC) approach. Adapted and modified from Hu *et al.* (2002).

Pucadyil *et al.* 2004). Interestingly, GFP can be dissected into two non-fluorescent fragments that can be brought together by interactions between proteins fused to each fragment, giving rise to GFP fluorescence (figure 4). This approach, known as bimolecular fluorescence complementation (BiFC), is used for monitoring protein–protein interactions in live cells (Hu *et al.* 2002).

From a modest beginning, GFP has come a long way. It has made life visible and continues to be a bright hope amid the dark mysteries of the cellular interior.

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