The Fluorescent Protein family

Fluorescent proteins of the GFP family consist of ~220–240 amino acid residues (26 kDa) which fold into a barrel formed by 11 β-sheets that accommodates an internal distorted α-helix containing the covalently-bonded chromophore 4-(*p*-hydroxybenzylidene)imidazolidin-5-one (HBI) running through the centre. The chromophore is located within the stable β-barrel and therefore is well protected from denaturation or proteolysis. The beta barrel structure is a nearly perfect cylinder, 42 Å long and 24 Å in diameter, otherwise known as a "β-can" formation, which is unique to the GFP-like family.

Importantly, all GFP-like proteins have a more or less pronounced tendency to oligomerize. Even *Aequorea victoria* GFP, which is considered monomeric, forms dimers at high concentrations corresponding to physiological conditions in jellyfish. Notably, nearly all FPs from non-bioluminescent Anthozoa and other taxa form very stable homotetramers even at very low (nanomolar) concentrations. However, as a reporter for the labelling of most proteins, an FP *must* be monomeric. Otherwise, oligomerization of a chimeric construct would interfere with normal protein function and localization. For example, DsRed, an obligate tetramer, if attached to actin, would bring together four actin subunits creating a messy tangle inside the cell.

The emission of GFP-like chromophores cannot be red-shifted beyond 540 nm (yellow). The breakthrough in the red fluorescent field occurred only after the discovery of DsRed and other Kaede-like red fluorescent and chromo proteins in Anthozoa species. The evolutionary biologist Yulii Labas prompted Mikhail Matz to clone GFP-like homologues from the brightly colored tentacle tips of *Discosoma* sp. mushroom anemone and other Anthozoa. The story of this discovery stemming from the stock of a professional Moscow aquarium keeper is recounted here.

These discoveries opened the way for the development of orange, red, and far-red FPs with emission peaks located as far as 655 nm. Fluorescent proteins are currently known to exist in four phyla of multicellular animals: Cnidaria, Ctenophora, Arthropoda, and Chordata. Within Cnidaria, class Anthozoa contains the greatest diversity of FP colors. Those proteins with a peak emitting in the middle of the spectrum ~500–530 nm (green and yellow FPs) are brightest, decreasing towards the limit at each end of the visible spectrum (blue and far-red FPs).

Fluorescent proteins of lower brightness but higher photostability result better signal-to-noise ratios, particularly for especially for time-lapse experiments. Although important for fixed material, brightness should not be the only parameter considered while choosing an FP for a particular application. Most FPs in use have a maturation half-time from ~40 min to 1–2 h, which is sufficient to quantitatively label cells, organelles and proteins. However, for some applications, such as early detection of promoter activation, labeling proteins with a short lifetime or monitoring single translational events, FPs with very fast maturation are required. The high extinction coefficients of red FPs make them excellent FRET acceptors for yellow donors. Yellow/red FRET pairs may soon challenge traditional cyan/yellow pairs. Points to keep in mind when choosing a fluorescent protein.

Key choices for fluorescent proteins

1. mTagBFP2 (399/454) Brightest blue FP
2. mCerulean 3 (433/475) Very photostable & bright
3. mTFP1 (462/492) Brightest cyan FP
4. m eGFP (490/508) Gold standard FP – can dimerize
5. mNeon Green (506/518) Brightest green FP
6. mVenus (515/528)
7. wd yPET (517/530) Brightest yellow FP, FRET
8. mKO2 (551/565)
9. tdTomato (554/581) Brightest red FP, photostable
10. mTagRFP (555/584)
11. mFusion red (580/608) Very photostable, low toxicity
12. mCherry (587/610) Photostable
13. mKate2 (588/633) Brightest far-red FP