Green Fluorescent Protein (GFP) and it's relatives

Biomolecular Structure 2005
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I (short) Introduction

Green fluorescent protein was discovered and purified from Aequorea Victoria. Its gene was isolated and can be expressed in E.coli and a variety of other species.
GFP and it's relatives are commonly used in molecular biology to study protein localisation (expressed fusion proteins) and in vivo protein-protein interactions (reconstitute GFP fluorescence by expressing two halves of GFP fusioned to different proteins; FRET).

II Jablonski diagram for fluorescence

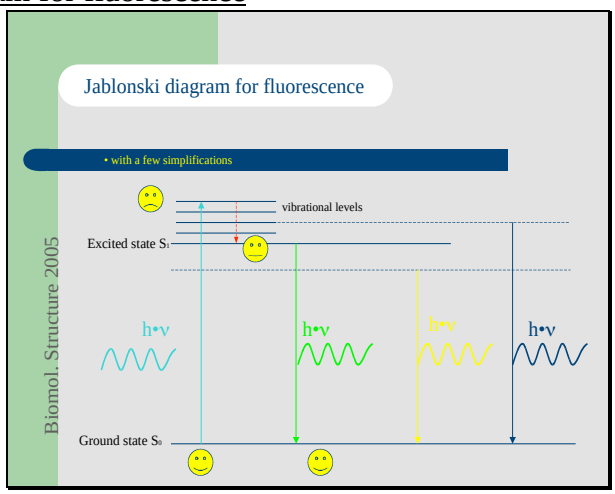

Figure 1: simplified Jablonski diagram

A very important property of fluorescence is the so-called Stokes shift, which is the difference between the excitation wavelength and the emitted wavelength. Remember what we normally refer to as color is only an electromagnetic wave with a wavelength, which is visible for us. Therefore each color we see emitted from a fluorescent molecule is a direct property of the energy difference between excited and ground state of the molecule.
In order to change the color of fluorescence of a given molecule, we have to change the energy of either the excited state (common) or the ground state (less common).

III The structure of GFP

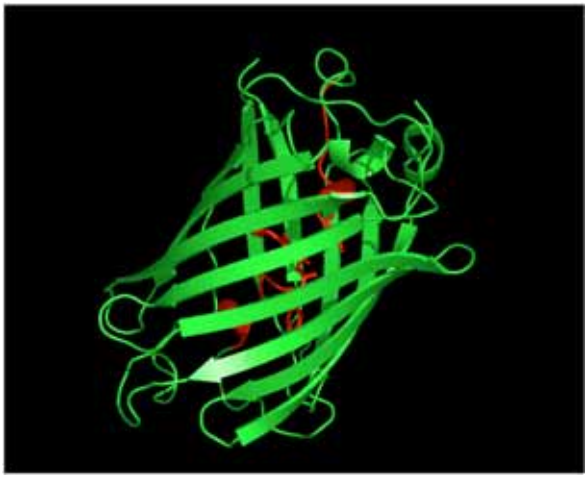

Figure 2: GFP overall structure – Pymol F2

The overall structure of GFP is dominated by 11 β-sheets forming an unusual β-barrel motif (3 antiparallel sheets- the helix- three antiparallel sheets and finally a greek key motif form that barrel) that contains an α-helix, spanning through the barrel. This α-helix also contains the fluorophore. The overall fold was considered a new structural motif and is called a β-can motif. The barrel together with the helix form a nearly perfect cylinder 42Å long and 24Å in diameter.
The top and the bottom of the cylinder are covered with short loop or distorted helical segments. The chromophore (see below) is

therefore completely protected from solvent (Pymol F3). This could have functional reasons, since in a cellular environment many potential fluorescent quenchers are readily available (e.g. O_2, reactive oxygen species, free thiol groups).

Of course the fluorophore and its interactions with the overall fold are of particular interest in GFP. The fluorophore consists of the residues 65-67 (Threonine-Tyrosine-Glycine) which auto-oxidize spontaneously to the p-hydroxybenzylidenimdazolidinone chromophore. Strikingly no enzyme is involved in this reaction pathway, it is completely spontaneous and occurs quite efficient once the protein is folded correctly.

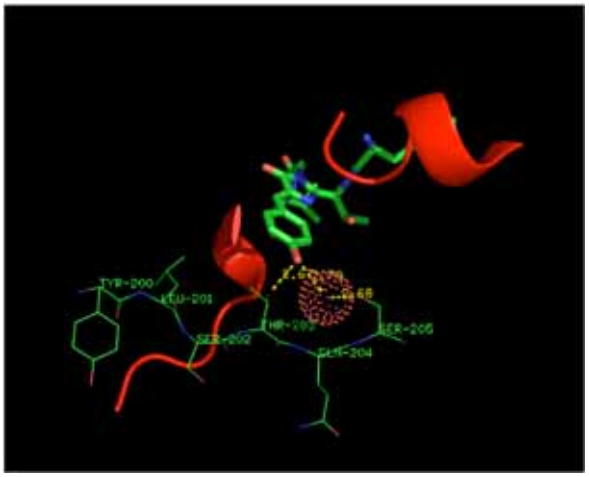

Figure 3: The Fluorophore and it's interactions- Pymol F5

The fluorophore is supposed to be stabilized and held in its orientation by contacts to a water molecule held in place by Serine 205 and a direct hydrogen bonding to Threonine 203.

As we will see these interactions also contribute to the color of the fluorescence.

IV Comparison with Cyan/Blue Fluorescent Protein (BFP) and Yellow Fluorescent Protein (YFP)

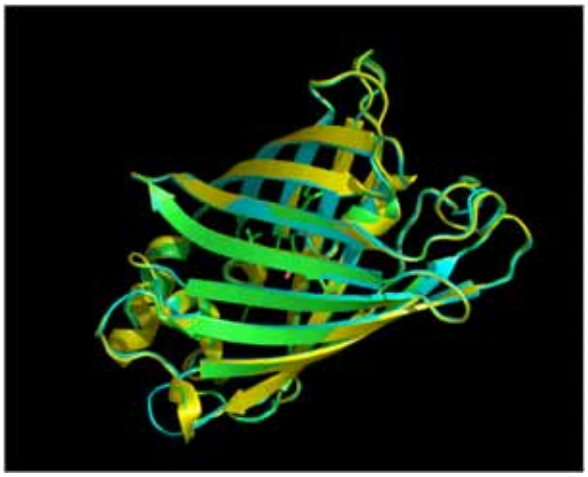

Figure 4: Comparison GFP (green), YFP (yellow), BFP (blue) – Pymol F6

BFP and YFP are both designed proteins and the design of these variants of GFP only became possible by the analysis of the crystal structure of GFP. Therefore BFP and YFP are very early examples of one of the "holy grails" of the field, namely designing a protein with a different function by analysing the crystal structure.

An alignment of all three protein reveals, that all three have essentially exactly the same fold. What really is surprising is the fact that even the loops at the bottom and top of the barrel overlay pretty well. This fact and the fact that all these loops have small b-values indicates, that they are not very flexible and highly stable.

An interesting question is, when the three proteins share the same structure, what makes the difference in terms of fluorescence?

A closer look at the fluorophores and their interactions in BFP and YFP gives the answer.

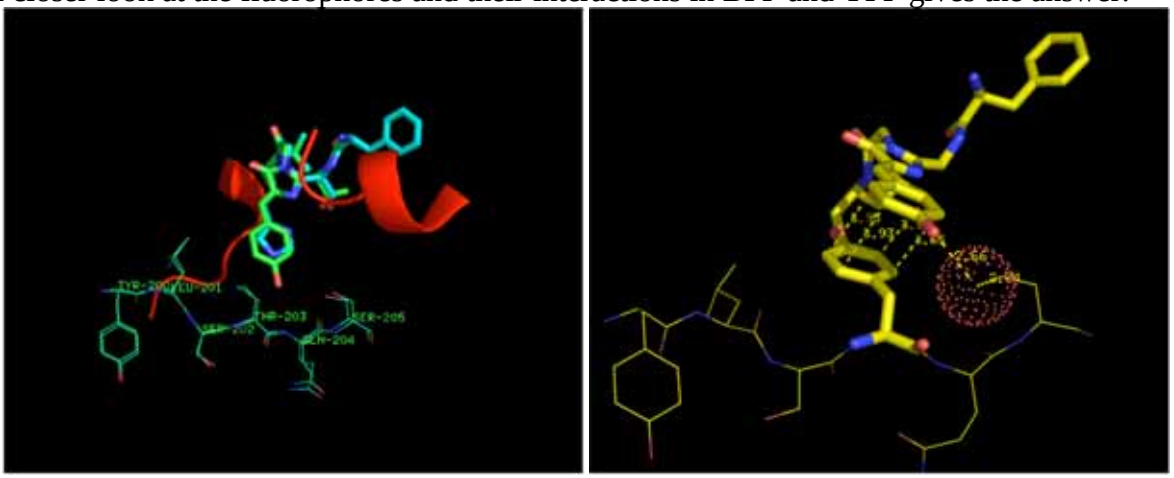

Figure 5: Comparison of the fluorophores of GFP (green) and BFP (blue) [left-Pymol F8]
The interactions of the fluorphore with the T203Y mutation in GFP [right - Pymol F12]

As you can clearly see in the left picture the fluorophore of BFP is altered in comparison to GFP. Tyrosin 66 is changed to Histidine. Consequently the interaction between Ser 205 , Thr 203 and the fluorophore are weakened. This change in the fluorophore and the weakened interactions lead to a destabilization of the excited state and therefore to a shift in fluorescence to shorter wavelengths (higher energy between ground and excited state = light of shorter wavelength = blue shift).

Surprisingly there is no change in the fluorophore itself in YFP. The only residue that differs between GFP and YFP is a Thr 203 → Tyr mutation. This new introduced Tyrosin shows a stacking interaction with Tyr 66 of the fluorophore (see Figure 5), therefore the direct environment of the fluorophore becomes more polarisable and this stabilizes the excited state. This leads subsequently to a lower fluorescent wavelength (lower energy between ground and excited state = light of longer wavelength = red shift).

V References

[1] Rebekka M Wachter, Marc-André Elsliger, Karen Kallio , George T Hanson and S James Remington ; “Structural basis of spectral shifts in the yellow-emission variantsof green fluorescent protein” ; Structure 15 October 1998, 6:1267–1277

[2] Mats Ormo, Andrew B. Cubitt, Karen Kallio, Larry A. Gross, Roger Y. Tsien, S. James Remington; “Crystal Structure of the Aequorea Victoria Green Fluorescent Protein” , Science (273) Sep 1996 5280, 1392-1396

[3] Brian G. Reid and Gregory C. Flynn ; “Chromophore Formation in Green Fluorescent Protein” ; Biochemistry 1997**,** *36*, 6786-6791

[4] Wachter, King, Heim, Kallio, Tsien, Boxer and Remington; “Crystal Structure and Photodynamic behaviour of the blue emission variant Y66H / Y145F of Green Fluorescent protein” ; Biochemistry 1997, 36, 9759-9765

[5] Crystal structures: 1EMA , 1BFP, 1HUY