

Supporting Results

I. Purity of green fluorescence protein (GFP) crystal (courtesy R. E. Stephens). Fig. 5 shows an SDS acrylamide gel, stained with Coomassie blue, of the GFP crystals used for our optical studies and the deionized water-based mother liquor in which the crystals were formed. The major band at 28 kDa for both the crystal and mother liquor is due to the GFP protein. As seen, there is virtually no material at the dye front and there are very faint auxiliary bands. The GFP protein is >98% pure, as shown by the gel analysis.

II. X-ray diffraction pattern of GFP crystal at ≈ 2.7 -Å resolution (courtesy T. Senda). Fig. 6 shows an x-ray diffraction pattern of a 15- μ m-wide crystal formed from the native GFP protein used in our optical studies. The larger crystals were formed more slowly by diluting the GFP solution by three times before dialysis against deionized water. The pattern shows the crystal system to be orthorhombic with a space group of $P2_12_12_1$ and with lattice constants of $a = 51.4$ Å, $b = 62.7$ Å, $c = 69.0$ Å, $\alpha = \beta = \gamma = 90^\circ$. The unit cell in this crystal is made up of four molecules of GFP. The values for the lattice constants obtained on our crystal of native GFP are virtually identical to those obtained on wild-type recombinant GFP by Brejc *et al.* (1).

III. Derivation of equations (by MS). Let us assume that each polarization component of excitation illumination excites two orthogonal linearly polarized noncoherent components of fluorescence light. A component of absorbed illumination that is polarized parallel to the long crystal axis I_{p1} transforms into two components of emitted fluorescence with parallel and perpendicular polarizations I_{p2} and I_{s2} respectively:

$$I_{p2} = \alpha_{pp} I_{p1} \text{ and } I_{s2} = \alpha_{sp} I_{p1}.$$

In the same manner, a component of absorbed light with perpendicular polarization I_{s1} creates two components of emitted light:

$$I_{p2} = \alpha_{ps} I_{s1} \text{ and } I_{s2} = \alpha_{ss} I_{s1}.$$

When two components of excitation radiation are present, then

$$\begin{aligned} I_{p2} &= \alpha_{pp} I_{p1} + \alpha_{ps} I_{s1}, \\ I_{s2} &= \alpha_{sp} I_{p1} + \alpha_{ss} I_{s1}. \end{aligned}$$

Here α_{pp} , α_{sp} , α_{ps} and α_{ss} are proportionality coefficients.

(i) In the case when the polarizer is oriented to the GFP crystal at an angle ϕ and the analyzer is absent, the intensity of fluorescence I is:

$$I = 0.5(\alpha_{ps} + \alpha_{ss}) I_0 + 0.5((\alpha_{pp} + \alpha_{sp}) - (\alpha_{ps} + \alpha_{ss})) I_0 \cos^2 \varphi, \quad [1]$$

where I_0 is intensity that falls on the polarizer.

The fluorescence becomes maximum at I_{\max} and minimum at I_{\min} , where

$$I_{\max} = 0.5(\alpha_{pp} + \alpha_{sp}) I_0, \text{ when } \varphi = 0^\circ \text{ and } 180^\circ, \text{ and} \\ I_{\min} = 0.5(\alpha_{ps} + \alpha_{ss}) I_0, \text{ when } \varphi = 90^\circ \text{ and } 270^\circ.$$

Thus, the intensity ratio of the fluorescence ξ_1 is

$$\xi_1 = \frac{\alpha_{pp} + \alpha_{sp}}{\alpha_{ps} + \alpha_{ss}}.$$

(ii) When the polarizer is absent and the analyzer is oriented at an angle φ to the GFP crystal, the intensity of fluorescence I is

$$I = 0.5 I_0 (\alpha_{sp} + \alpha_{ss}) + 0.5 I_0 ((\alpha_{pp} + \alpha_{ps}) - (\alpha_{sp} + \alpha_{ss})) \cos^2 \varphi, \quad [2]$$

where I_0 is intensity that falls on the GFP crystal.

The fluorescence then has a maximum at I_{\max} and minimum at I_{\min} , where

$$I_{\max} = 0.5(\alpha_{pp} + \alpha_{ps}) I_0, \text{ when } \varphi = 0^\circ \text{ and } 180^\circ, \text{ and} \\ I_{\min} = 0.5(\alpha_{sp} + \alpha_{ss}) I_0, \text{ when } \varphi = 90^\circ \text{ and } 270^\circ.$$

Thus, the polarization ratio ξ_2 in fluorescence beam is

$$\xi_2 = \frac{\alpha_{pp} + \alpha_{ps}}{\alpha_{sp} + \alpha_{ss}}.$$

(iii) In the case when the polarizer is oriented at an angle φ to the GFP crystal and the analyzer is oriented parallel to the polarizer, the intensity of fluorescence I is described by a formula containing terms $\cos^2 \varphi$ and $\cos^4 \varphi$:

$$I = 0.5(\alpha_{ss} + (\alpha_{ps} + \alpha_{sp} - 2\alpha_{ss}) \cos^2 \varphi + (\alpha_{pp} + \alpha_{ss} - (\alpha_{ps} + \alpha_{sp})) \cos^4 \varphi) I_0, \quad [3]$$

where I_0 is intensity that falls on the polarizer.

The fluorescence becomes maximum at I_{\max} , and minimum at I_{\min}

$$I_{\max} = 0.5\alpha_{pp} I_0, \text{ when } \varphi = 0^\circ \text{ and } 180^\circ,$$

$$I_{\min} = 0.5\alpha_{ss} I_0, \text{ when } \varphi = 90^\circ \text{ and } 270^\circ.$$

Thus, the fluorescence intensity ratio ξ_3 is

$$\xi_3 = \frac{\alpha_{pp}}{\alpha_{ss}}.$$

(iv) When the polarizer is oriented at an angle φ to the GFP crystal and the analyzer is oriented perpendicular to the polarizer, the fluorescence intensity becomes:

$$I = 0.5(\alpha_{ps} + (\alpha_{pp} + \alpha_{ss} - 2\alpha_{ps})\cos^2\varphi - (\alpha_{pp} + \alpha_{ss} - (\alpha_{ps} + \alpha_{sp}))\cos^4\varphi)I_0, \quad [4]$$

where I_0 is intensity that falls on the polarizer.

The fluorescence has two sets of minima I_{\min}' for $\varphi = 0^\circ, 180^\circ$ and I_{\min}'' for $\varphi = 90^\circ, 270^\circ$. Moreover, $I_{\min}' = 0.5\alpha_{sp} I_0$ and $I_{\min}'' = 0.5\alpha_{ps} I_0$.

Thus, we get a ratio: $\xi_4 = \frac{I_{\min}'}{I_{\min}''} = \frac{\alpha_{ps}}{\alpha_{sp}}$.

Maxima will occur at the following points:

$$\varphi = \tan^{-1} \left(\sqrt{\frac{\alpha_{pp} + \alpha_{ss} - 2\alpha_{sp}}{\alpha_{pp} + \alpha_{ss} - 2\alpha_{ps}}} \right).$$

The position of the maximum corresponds to 45° when $I_{\min}' = I_{\min}''$ and $\alpha_{ps} = \alpha_{sp}$ only.

From three of the four measured values ξ_1, ξ_2, ξ_3 and ξ_4 , one can determine the relative values $\frac{\alpha_{pp}}{\alpha_{ss}}, \frac{\alpha_{ps}}{\alpha_{ss}}$ and $\frac{\alpha_{sp}}{\alpha_{ss}}$ and then plot the normalized curve $I(\varphi)$. For example,

because the values ξ_1, ξ_3 and ξ_4 are known, we find: $\frac{\alpha_{pp}}{\alpha_{ss}} = \xi_3, \frac{\alpha_{ps}}{\alpha_{ss}} = \frac{\xi_3 - \xi_1}{\xi_1 - \xi_4}$ and

$$\frac{\alpha_{sp}}{\alpha_{ss}} = \frac{\xi_3 - \xi_1}{\xi_4 (\xi_1 - \xi_4)}.$$

Reference:

1. Brejc, K., Sixma, T. K., Kitts, P. A., Kain, S. R., Tsien, R. Y., Ormö, M. & Remington, S. J. (1997) *Proc. Natl. Acad. Sci. USA* **94**, 2306–2311.