Theoretical background

Extrinsic fluorophores have been widely used in recent years to study biological macromolecules or structures because their optical properties are often affected unequivocally by the physical or chemical properties of the biomolecules to which they bind [1]. These studies add to those utilizing fluorophores as labels of specific biomolecules for e.g. cytometric quantitations of biomolecules or membrane permeation measurements. In our perspective the specific affinity of the dye for the biomolecule to be studied is a pre-requisite, whereas the main point of our investigations will be the interaction of the fluorophore with the biosubstrate which can influence either the radiative or the radiationless decay pathways from its excited singlet state $S_1$. For the sake of simplicity we will first consider the case of a dye exhibiting a first-order kinetics for its decay from the $S_1$ state when it is free in solution. In other words, we disregard complicating effects such as self-association or the coexistence of various ground-state configurations which can cause the chromophore to depart from simple kinetics even in solution. Thus we write the overall decay rate $k_F$ of the $S_1$ state as

$$k_F = k_F^0 + k_{IC} + k_{ISC}$$

(1)
in which $k_F^0$ is the radiative decay rate and $k_{\text{IC}}$ and $k_{\text{ISC}}$ are the rates of the most relevant non-radiative decay mechanisms, i.e. internal conversion to the ground state $S_0$ and intersystem crossing to the triplet $T_{1}$ state [2]. So, a chromophore excited-state population $N_{1}(0)$, which can be excited into $S_{1}$ at time $t = 0$ by a δ-function light pulse at any frequency $\nu$ within the ground-state absorption spectrum, will decay exponentially with time with the time constant $k_F$ according to the law

$$N_{1}(t) = N_{1}(0) \exp(-k_F t)$$

(2)

The decay of the $S_{1}$ population is accompanied by the emission of a fluorescence pulse having the same time dependance; it is convenient to describe it by means of the molecular fluorescence response function $i(t)$ that represents the number of photons emitted per unit time relative to the $N_{1}(0)$ population [2], that is

$$i(t) = k_F^0 \exp(-k_F t)$$

(3)

Note that the time integral of $i(t)$ gives the number of photons emitted per photon absorbed, i.e. per unit $N_{1}(0)$; thus it is by definition the fluorescence quantum yield $\phi_F$:

$$\phi_F = k_F^0 / k_F$$

(4)

The radiative decay rate $k_F^0$ is equal to the Einstein A coefficient summed over the complete fluorescence spectrum. In turn, $A$ is related to the Einstein B coefficient, which determines the probability of absorption at the same frequency $\nu$, by the well known quantum-mechanical relation

$$A = 8\pi \left( \frac{h\nu^3}{v^3} \right) B$$

(5)

where $h$ is the Planck constant and $v$ is the velocity of light in the medium considered. Taking into account that $B$ is related to the absorption cross-section $\sigma(\nu)$ by the equation:

$$B = (\nu/h) \int_{\text{ABS}} [\sigma(\nu)/\nu] \, d\nu$$

(6)

where the integral is over the whole absorption spectrum, we are led to the final relation:

$$k_F^0 = \left[8\pi/(\nu^2 <v^{-3}>_{\text{EM}})\right] \int_{\text{ABS}} [\sigma(\nu)/\nu] \, d\nu$$

(7)