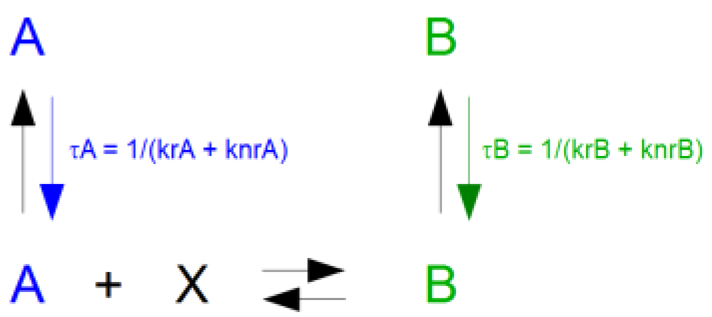
Some origins of multiexponetial decays for pure dyes

The fluorescence lifetime of a dye measured with a TCSPC spectrometer can be multiexponential due to many reasons. The most obvious cases are due to scattering or presence of impurities. Although less obvious, it is also widely known that in an inhomogeneous media a pure dye will also exhibit a multiexponential decay. Advanced users know that if the decay is not measured with polarizers at magic angle, the rotation correlation time shows up as a second exponential in the decay (the second exponential is in fact the product of the rotation correlation time by the fluorescence lifetime, divided by their sum). And they also know that measuring without polarizers is not equivalent to measure at magic angle… But suspicion may arise when even a pure dye measured at magic angle in a homogeneous media exhibits a multiexponential decay. Is the spectrometer properly adjusted? Are the polarizers properly calibrated? A typical mistake is to measure the IRF at the nominal laser wavelength instead of measuring at the ideal wavelength for that specific laser head. Note that all diode lasers heads emit at slightly different wavelengths and each of them have an optimum at which the IRF should be measured. As little as 0.5 nm displacement from their optimum may induce a “non perfect” deconvolution fit.

But it is worth noting that even at magic angle in a perfectly aligned spectrometer pure dyes in homogeneous media may exhibit a multiexponential decay. The origin may be physical, like solvent relaxation, or chemical, when the fluorescent molecule undergoes a ground or excited state reaction. In this brief article a few examples are described.

1) Ground-state reactions

The fluorophore (A) may be reacting with the medium or with diffusing molecules dissolved in it (X) to form a product (B). Typical cases are dissociation reactions. These reactions can be followed by Absorption spectroscopy, where the different bands can be identified and assigned. Excitation in regions where the two species (A and B) absorb may lead to biexponential decays if the fluorescence of both compounds is observed at the same wavelength.



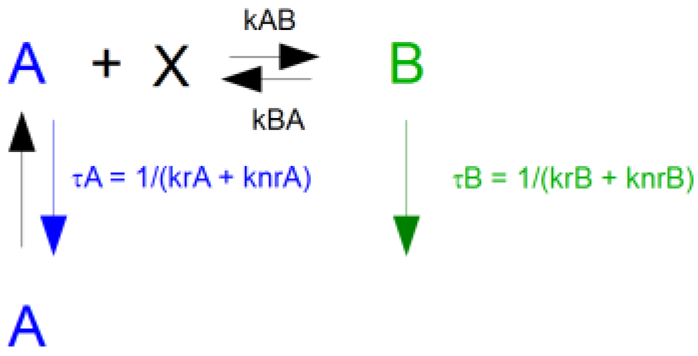
Scheme 1

Note that this situation corresponds to the typical case of Static-Quenching in which the intensity of A decreases with the concentration of X, but where the lifetime τ_A remains constant.

2) Excited-state reactions

The electronic redistribution of electrons due to optical excitation leads in many cases to a different reactivity in the ground and excited states. In other words, a fluorescent molecule which is boring under the dark may become reactive upon excitation. Common excited state reactions are redox (electron transfer) and acid-base (proton transfer) reactions. Depending on the rate of the excited-state rection relative the the original fluorescence lifetime, the observed decay time measured with a TCSPC spectrometer may be single- or multiexponential.

Let us consider the reaction in Scheme 2 in different situations:



Scheme 2

Starting point: The molecule A is prompted to te excited-state where it can react with a molecule X to form the compound B, through a rate constant kAB. Once the compound B is formed the back-reaction can occur, with a rate constant kBA. Compounds A and B are fluorescent with original fluorescence lifetimes τ_A and τ_B. Once B decays to the ground state the back-reaction takes place. Hence the system is always in its starting position (A + X) prior to any excitation pulse.

Case A) The constant kAB is too slow with respect to τ_A and τ_B. In this case the compound A would decay to the ground-sate before the excited-state reaction could take place. The decay measured would be single exponential and coincident with τ_A.

Case B) The forward reaction constant kAB is fast, but the back-reaction constant kBA is too slow in comparison to τ_A and τ_B. In this case the decay time measured in the spectral region of A would be single exponential, with decay time τ_1. However τ_1 would be shorter than τ_A, and it would be dependent on the concentration of X (τ_1 = 1/ (krA+knrA + kAB[x]), where [x] denotes the concentration of X and kr and knr the intrinsic radiative and non-radiative rate constants of A, respectively) . The lifetime measured in the spectral region of B would be biexponential with times τ_1 and τ_2. τ_1 would have a negative pre-exponential factor (rising component) and it would be coincident with the decay time measured in the spectral region of A. The decaying component τ_2 would be coincident with the original lifetime of compound B, τ_B.

Note that this situation is the typical case of dynamic quenching with the particular case that the product being formed is fluorescent.

Case C) The interconversion rate constants kAB and kBA are in the same order of τ_A and τ_B. In this case, the decay curves measured for species A and B would be biexponential for both, with common lifetimes τ_1 and τ_2. However, τ_1 and τ_2 would not correspond to τ_A or τ_B , but would be a function of both and of their interconversion rate constants kAB and kBA, as well as of the concentration of X. The system would have to be solved mathematically. For a system like in Scheme 2 the time evolution of species A and B would be:

A(t) = A1 e^{-t/τ_1} + A2 e^{-t/τ_2}

B(t) = B1 e^{-t/τ_1} + B2 e^{-t/τ_2}

where:

τ_1= 2/(M+Y-Z)

τ_2 = 2/(M+Y+Z)

being M = kAB[x] + kA (summation of disappearance constant of compound A; kA=1/τ_A)

being Y = kBA + kB (summation of disappearance constant of compound B; kB=1/τ_B)

being Z = [(M-Y)^2 + 4 kAB kBA[x]]^(1/2)

A1= A0 [M- (1/τ_2)] / [(1/τ_1 – 1/τ_2]

A2= A0 [(1/τ_1)- M] / [(1/τ_1 – 1/τ_2]

B1= A0 kAB [x] / [(1/τ_1 – 1/τ_2]

B2= -A0 kAB [x] / [(1/τ_1 – 1/τ_2]

being A0 the concentration of A at t=0.

Note from A(t) that, even if B would not be fluorescent, the decay of A will still be biexponential!

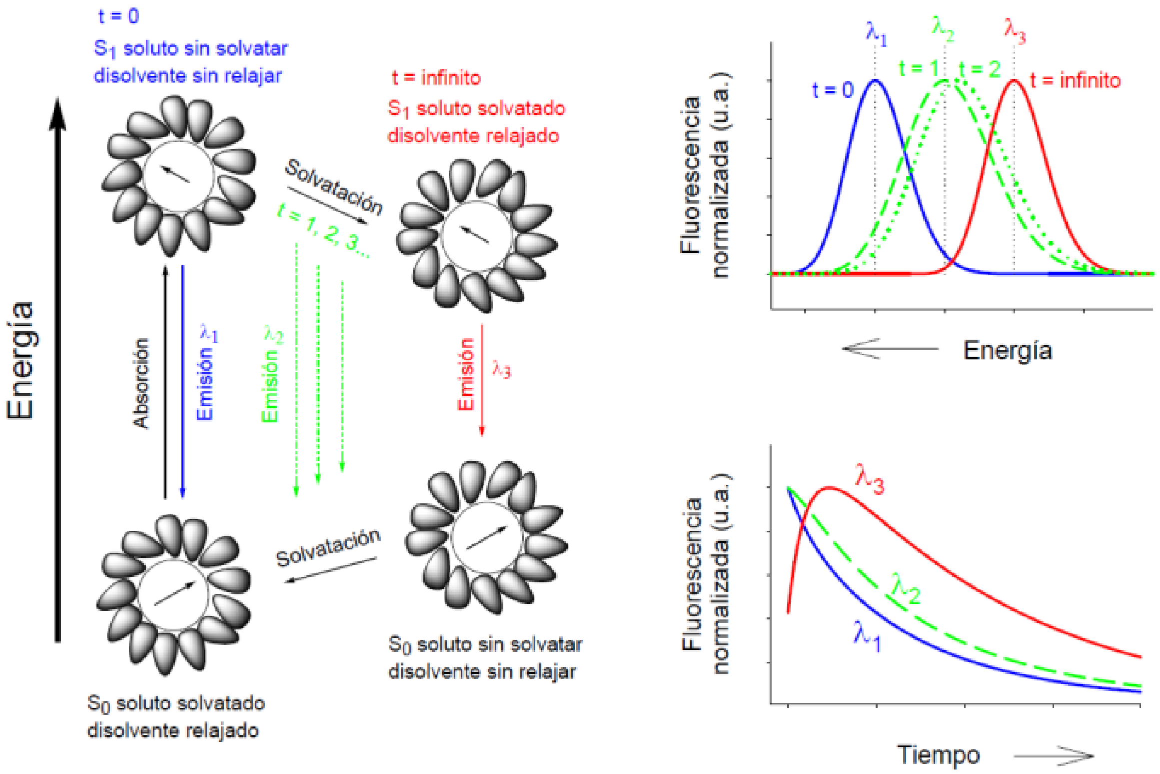
A situation like this may occur with molecules dissolved in an aprotic but hygroscopic media, like acetonirile. Water molecules may diffuse (diffusion happens in the nanosecond time scale) and react with fluorophores bearing proton-transfer groups like -OH or -NH2.

Case D) Interconversion rate constants kAB and kBA are very quick compared to the intrinsic lifetimes $\tau_A and $\tau_B. In this case the equations of case C would still apply. But in practice, a quick equilibrium between reactants and product would be established. This means that the concentrations of A and B with respect to each other would always be constant prior to their decay, and hence the whole system could be treated as a single dye. The decay would be single exponential, being an average of τ_A and τ_B weighted by their fraction in the equilibrium.

This situation may happen if compounds A and X were directly in contact prior to excitation, for example throgh ground-state interactions.

3) Solvation dynamics

Even pure fluorophores in pure solvents may lead to multiexponential decays. This is the case of molecules with strong charge transfer character in polar solvents when undergoing solvation dynamics. In such cases the fluorescence spectra shifts to longer wavelengths in time. Measuring the decay in the blue edge of the steady-state spectrum will lead to a multiexponential decay. The ns lifetimes corresponds to the intrinsic fluorescence lifetime of the fluorophore, whereas the ultrafast components (ps) are related to the rate of the shift. Measuring in the red-flank of the steady-sate spectrum leads to multiexponential decays with positive (intrinsic lifetime, ns) an negative (rate of shift, ps) pre-exponential factors. This is depicted in Scheme 3.



Scheme 3. Left: energetic representation of solvation dynamics. The fluorophore is represented as a sphere with a pointing dipole. Solvent molecules are represented in gray around the fluorophore. Right up : Spectral consequence of solvation dynamics. The fluorescence spectrum shifts in time to lower energies. Right bottom: Decay traces measured in different spectral regions. Blue flanks are multiexponential with positive pre-exponentila factors, red flanks are multiexponential with rising components.

Before the excitation, the fluorophore is in the ground state S0 , which has a characteristic dipole moment. Solvent molecules, which also have their characteristic dipole moment are oriented in such a way that the interactions dipole-dipole with the fluorophore are as favorable possible. When the fluorophore is prompted to the excited state, its electronic distribution switches almost instantly. At time zero after excitation the solvent molecules remain in their “original” orientation to solvate ground state . The resulting dipole -dipole interactions with the fluorophore are hence less favorable. As a result , the solvent begins to relax to solvate the S1 state and brings the system to a more favorable position. Spectroscopically, this is manifested by the time-shift of the emission spectrum to longer wavelengths. Consider that the Steady-State spectrum is the time integral of all those shifting spectra. Measuring in the blue flank (λ1) will lead to a multiexponential decay: the signal decreases because of the shift and the intrinsic decay. Measuring in the red flank (λ3) will lead to a multiexponential decay with a negative pre-exponential factor: the signal first rises due to the increase in signal due to the displacement, and then decays due to the fluorescence lifetime.

In fluid media, solvation dynamics can be described with a multiexponential function spanning from the femtosecond time-scale to tens of picoseconds. Hence, the tail of this process can be monitored with a TCSPC spectrometer equipped with fast detectors such as a MCP or a Hybrid-PMT. In viscous media or at low temperatures, the ps tail component slows down to ns, and the process can be monitored with slower detectors, such as standard PMT.

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