PTI Technical Note

Picosecond Lifetime Performance of the TimeMaster™ Fluorescence Lifetime Spectrometer

Need to find the distance between two fluorophores in a multi-phase system, such as micelles, vesicles, liposomes, or membranes? No problem, all you need is the new, a really simple and portable, but powerful fluorescence lifetime system and the dedicated, which comes standard with the new version of FeliX32 software. Forget your steady state fluorometer, you'll never get the DA distance right with FRET when the donor and acceptor are in a multi-phase environment! As an example, consider TritonX micellar solution in water with perylene added as the donor and Rhodamine B as the acceptor. Perylene, an aromatic hydrocarbon, virtually insoluble in water will seek highly hydrophobic environment and will be localized exclusively inside TritonX micelles.

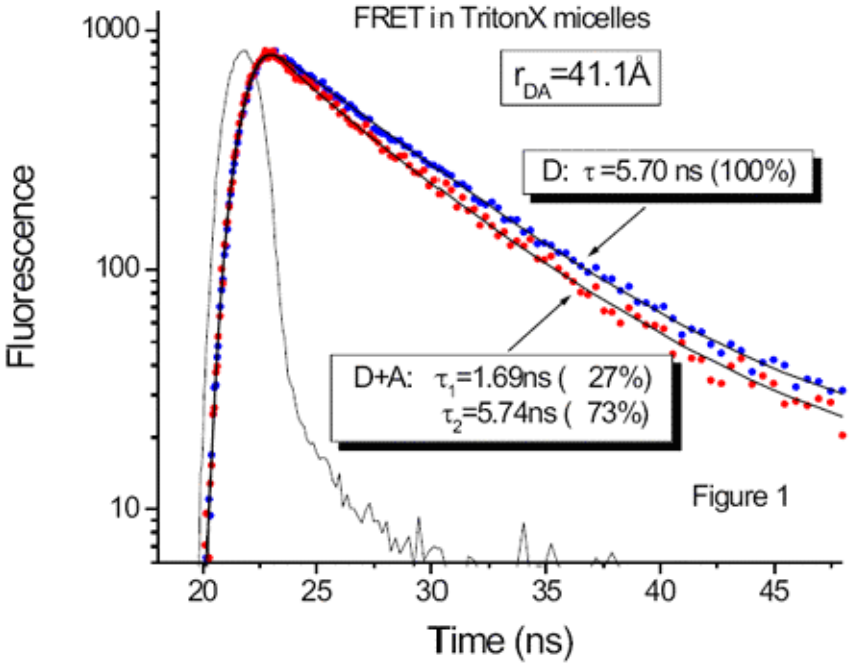

Figure 1

Its fluorescence decay as shown in Fig. 1 (trace D) is single exponential with the lifetime of 5.7 ns. The Rhodamine B is a hydrophilic molecule and will be mostly in the aqueous phase, but some fraction will also diffuse inside the micelles. When both D and A are confined to the same micelle, the energy transfer may occur. The perylene decay after the addition of Rhodamine B is shown in Fig. 1 (trace D+A). Now with the acceptor molecules added, the decay is double exponential with the short lifetime of 1.69 ns (27%) and the long lifetime of 5.74 ns (73%), the latter being the same as for the donor alone (trace D). The following conclusion can now be drawn: 27% of all micelles containing perylene also contain Rhodamine B acceptor and the FRET is quite efficient. For the Rhodamine B molecules, which are in the aqueous phase the average distance is too great to cause FRET with the micelle-embedded perylene. To find the average distance between the D and A in a micelle, just open the FRET Calculator, enter the lifetimes of D alone (5.7ns), D in the presence of A (1.69 ns) and Ro = 47.5 Å (determined in a separate experiment see PTI note on FRET Calculator), click Calculate and the distance is 41.1 Å. In addition you also get the FRET efficiency (70%) and the rate constant (4.2x108 s-1). It's that simple!

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