Optical Building Blocks Corp.
Technical Note: Time-Resolved FRET: Really Easy with the EasyLife™ V and the FRET Calculator!

EasyLife™ V - A Great Tool for a Variety of Applications

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 FRET Calculator, which comes standard with the new version of EasyLife™ V 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 a highly hydrophobic environment and will be localized exclusively inside TritonX micelles.

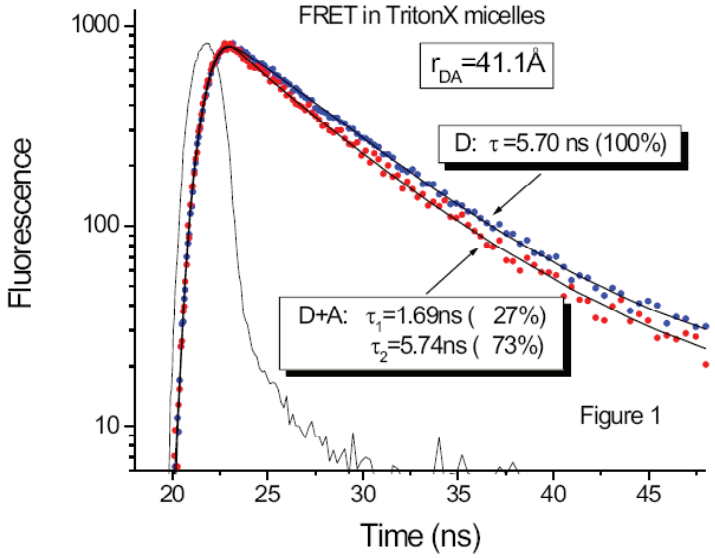

Figure 1

Its fluorescence decay (trace D in the plot) 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, energy transfer may occur. The perylene decay after the addition of Rhodamine B is shown as trace D+A in the plot. 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 inside the micelles is quite efficient, since the donor lifetime was quenched to 1.69 ns. 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 the technical note on the 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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