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ARTICLE TYPE

Supplementary Information for

Application of PARAFAC to a two-component system exhibiting Fluorescence Resonance Energy Transfer: from theoretical prediction to experimental validation

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1. Verification of assumption of weak excitation in theoretical derivation

An assumption was made for deriving equations (7) and (8), i.e., $I(\lambda_i)k_{ex}^D(\lambda_i) \ll k_f^A + k_{nf}^A$, which should be verified. Time-resolved experiments revealed that the decay time τ =1.8 ns for the acceptor, and $k_f^A + k_{nf}^A = 1/\tau = 5.5 \times 10^8\ s^{-1}$. Extensive measurements of excitation photon flux $I(\lambda_i)$ on various conventional fluorometers are beyond the scope of this work. The photon flux is of the comparable order of magnitude for various conventional fluorometers with Xenon lamps. We previously used a commercial fluorometer (SLM) and found[1] that an excitation light of 313 nm wavelength with 5 nm bandwidth passing through a 50-µm pinhole showed a photon flux ~100 nJ sec^{-1}. Provided the molar absorption rate constant $k_{ex}^D(\lambda_i)$ is 5×10^4 M^{-1} cm^{-1} which has the same order of magnitude for many highly absorbing chromophores, calculations gave 7648 sec^{-1} for $I(\lambda_i)k_{ex}^D(\lambda_i)$, a number far less than 10^8 sec^{-1} that corresponds to a typical 10 ns decay time τ. Therefore, the above approximation will also hold valid for shorter-decayed fluorophores like A, with τ=1.8 ns.

2. Appropriateness of the selected D-A pair

a. Estimating the Förster distance

The Förster distance R_0 can be evaluated using equation (S1),

$$R_0 = 0.211(\kappa^2 n^{-4} \Phi_F^D J)^{1/6}\ , \qquad \text{(S1)}$$

where $\kappa^2$is the mean transition dipole-dipole orientations between D and A. As stated in early work,[2,3] κ^2 lies in a range between limiting values 2/3 and 0.475. Detailed knowledge about the orientation of D and A dipoles in SDS micelles is not available. R_0 also depends on *n*, the refractive index of the microenvironment around D/A pair. Again, it is difficult to know the refractive index. Here it is assumed 1.4, a typical value assumed for a hydrocarbon environment.[4] As stated in early studies,[5] the solute may be preferably to stay close to or at the micelle-water inter-surface. The inter-phase surface refractive index may be different from that of hydrocarbon core. Therefore, the true refractive index may be intermediate between the indices for pure water and for pure hydrocarbon. Due to the narrow range of refractive index and the fact that R_0 depends on $n^{-2/3}$, it can be expected that refractive index does not affect the value of R_0 very much. Φ_F^D is the quantum yield of D in the absence of A. For biphenyl confined in micelles SDS, Φ_F^D is not reported in the literature. Biphenyl (D) in cyclohexane in the absence of O_2 has Φ_F^D=0.18. This value is borrowed herein based on the fact that O_2 has low occupancy in the micelles under moderate to low gas pressure.[6,7] The low occupancy of O_2 does not give rise to considerable fluorescence quenching of the fluorophores. *J* is the spectral overlap of D's emission and A's absorption spectrum in micelles, as defined by equation (S1).[8]

$$J = \int_0^\infty F_D(\lambda)\varepsilon_A(\lambda)\lambda^4 d\lambda \qquad \text{(S2)}$$

$F_D(\lambda)\mathrm{F_D}(\lambda)$ is the normalized emission spectrum of D that has total under-curve area equal to unity. $\varepsilon_A(\lambda)$ is molar extinction coefficient of A at wavelength λ. For efficient FRET process in practice, the emission spectrum of D and the absorption spectrum of A in micelles should overlap to a significant extent; this requirement is met for our D and A as shown in Figure S1 The pure spectra of solutes in micelles are not readily obtained, because the solutes are always distributed in two phases in the presence of micelles. However, we can estimate reliably the pure spectra in micelles by means of PARAFAC, as reported in our previous study, *CITATION Che101 \l 1033* [6] and these spectra are shown in Figure S1. The spectral overlap was assessed with numerical integration in Matlab. Calculations gave R_0=27.9 Å and R_0=26.3 Å provided $\kappa^2 = 2/3$ and $\kappa^2 = 0.475$, respectively. This range is comparable to the literature value of 28.88 Å for this D-A pair in degassed cyclohexane.[10]

b. Absence of ground state association

Figure S2 shows the linear plots of absorbance cited in the main text.

3. Rejection of the 4- and 5-component PARAFAC models

The scores and loadings of the 4- and 5-component models are shown in Figure S3.

The 4-component and 5-component models did not give significantly smaller SSR compared to the 3-component model. In the 4-component model, a scattering-like component appeared which was not observed in the 3-component model, and the additional variation explained by this component was very low, as indicted by the low score values and little change in RELFIT and

SSR compared to the 3-component model. The emission spectra of the other three components remain similar to those in the 3-component model, but the excitation spectra showed different profiles.

The 5-component model still gave a scattering-like component. We limited our discussion to the remaining four non-scattering components thereafter. Three of them showed almost identical emission spectra similar to those of pure A, whereas one of them showed an emission spectrum almost identical to that of pure D. However, two of the components show pure D excitation spectra, whereas one shows an excitation spectrum of pure A, and one shows an excitation spectrum that is neither identical to pure D nor pure A. The spectra loading and the concentration scores of these components are difficult to interpret. The 4-component and 5-component models obviously give incorrect results, and only the 3-component model result is appropriate under our experimental conditions.

4. The PARAFAC spectra for directly excited D and A

It may be noted that the presence of FRET in micelles does not break the co-varying ***concentration*** relationship between the same solute in the two phases, but does break the co-varying ***fluorescence*** relationship between the same solute in the two phases. The latter is true because a fraction of solute (D or A) in the micelles participates in FRET, and this fraction has altered fluorescence intensities. However, if the D or A's fluorescence alteration induced by FRET is not occurring to great extent compared to the total fluorescence intensities of the whole micellar solution, the co-varying fluorescence relationship between the same solute in the two phases may still be approximately true. For example, we showed that there is some D fluorescence from type 1 micelles, and its yield is significantly lower than that of D in type 2 micelles or in bulk water. But since only about 2.6% of D are in type 1 micelles, the D fluorescence from these micelles was not different enough in relative intensity to be broken out as a separate component by PARAFAC.

PARAFAC was not able to distinguish two covarying components; it instead appeared to combine them using proper weights to give a weighted component. Suppose the mean ratio of micelle to water phase is r for a solute (D or A). The weight for the spectrum in the micelles and water is r/(r+1) and 1/(r+1), respectively. We constructed the weighted spectra for D and A, respectively, and compared the spectra with the PARAFAC-recovered spectra. As illustrated in Figure S5 and Figure S6, the constructed and PARAFAC-given emission and excitation spectra matched well for both D and A.

Single-phase spectra and two-phase weighted spectra, as discussed in the main text, are shown in Figure S7-Figure S9.

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Figures

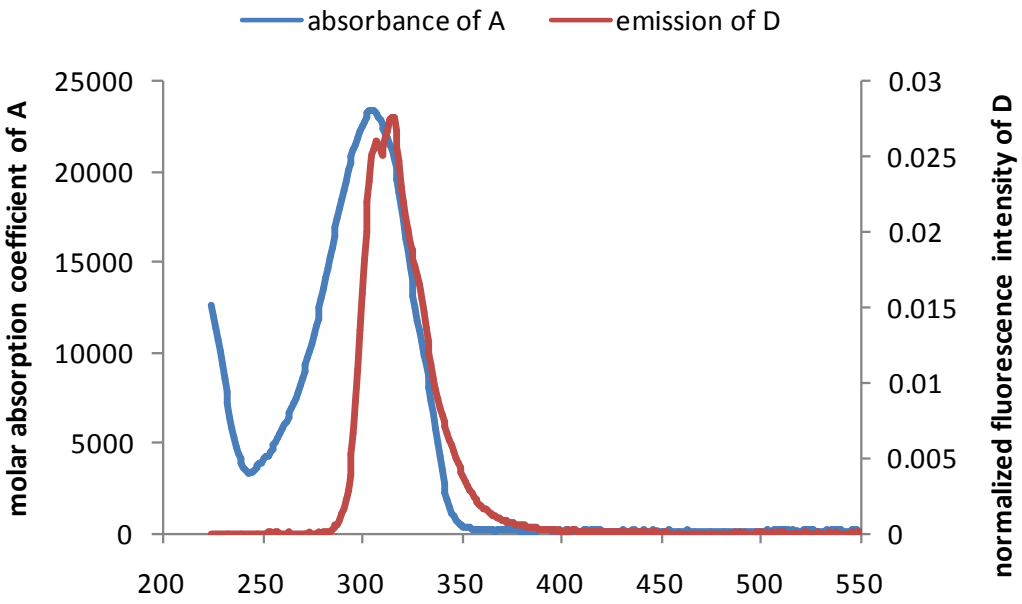

Figure S1 Overlapping absorption spectrum of acceptor (A) and emission spectrum of donor (D) in micelles.

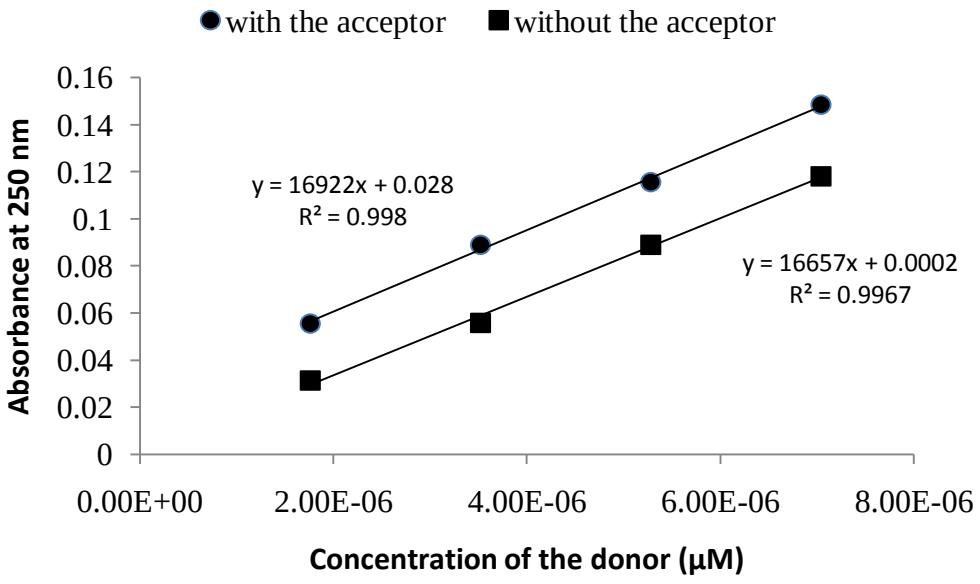

Figure S2 Absorbance of the donor (7.04 µM) at 250 nm in the presence and absence of the acceptor (7.04 µM).

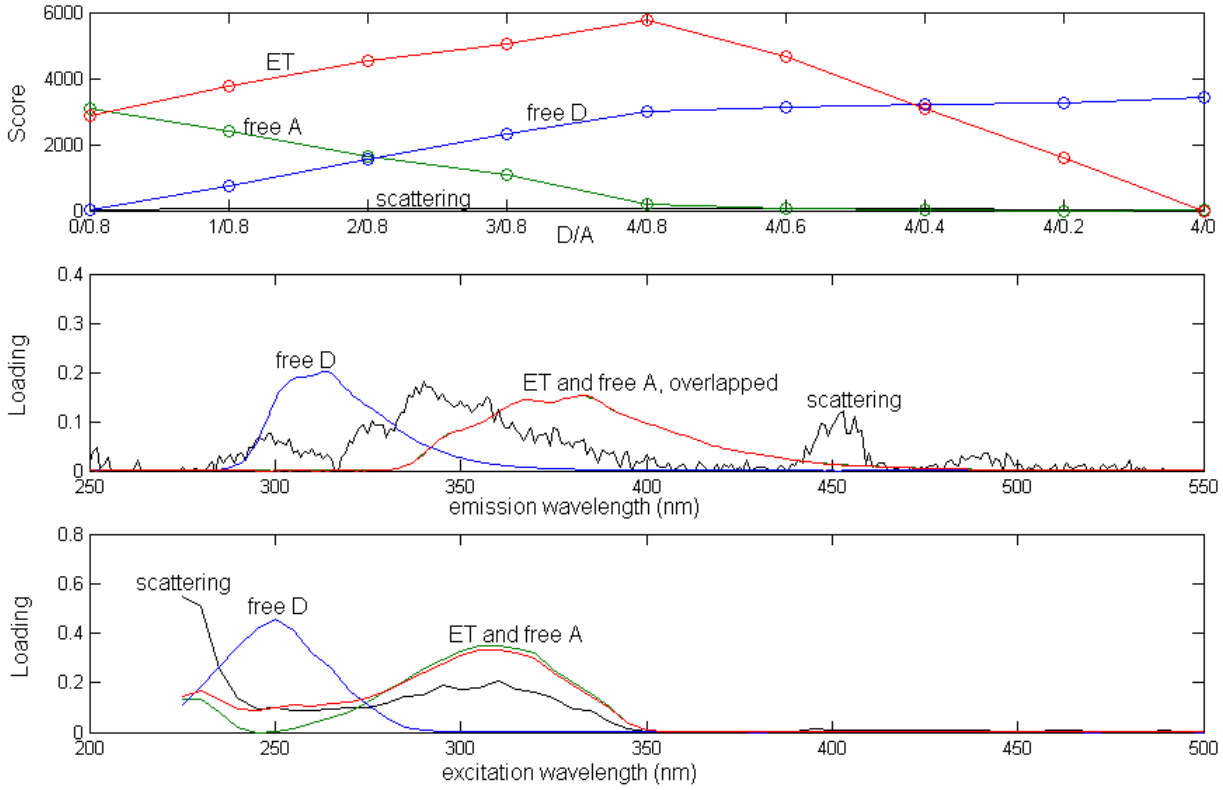

Figure S3 Relative concentrations and spectral profiles of the components recovered by a 4-component PARAFAC model fitted to the micellar EEMs.

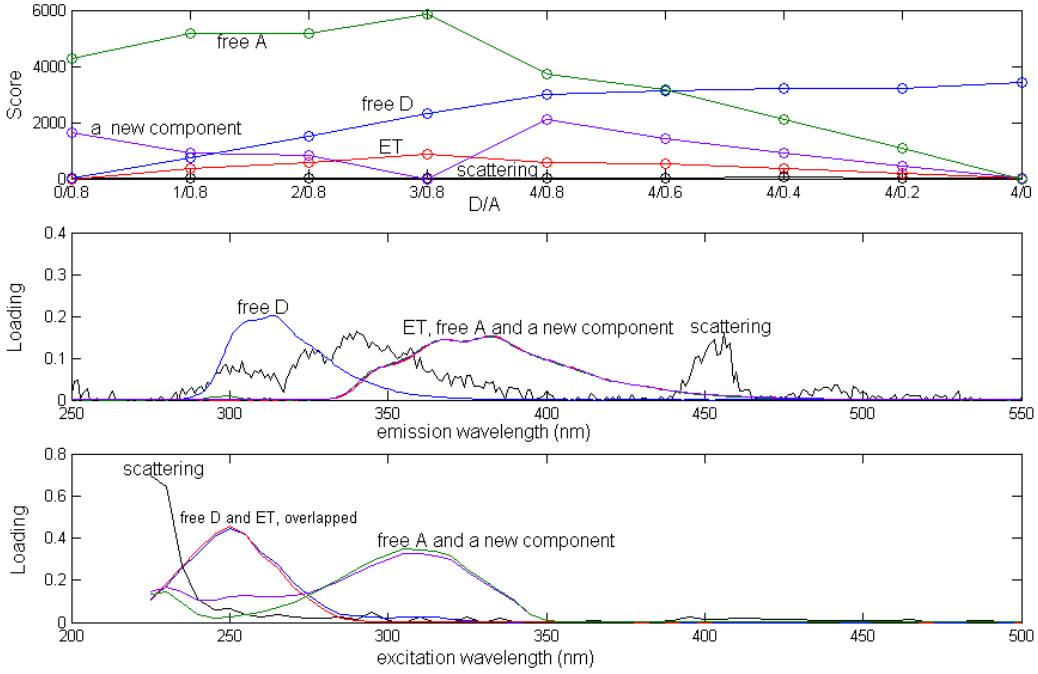

Figure S4 5-component model. A new component shows up in addition to those four components in 4-component model.

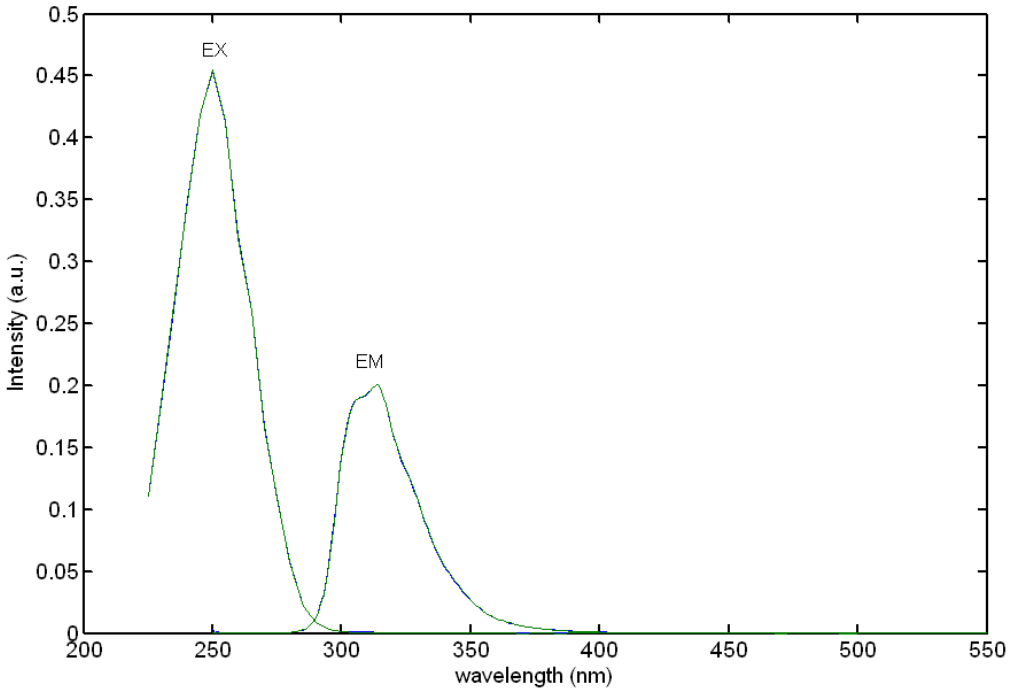

Figure S5 Comparison between the weighted spectra (green) and PARAFAC recovered spectra (blue) for the donor D in the micellar solutions in the presence of FRET. The weighted spectra are the combinations of the spectra in micelles and in water phase.

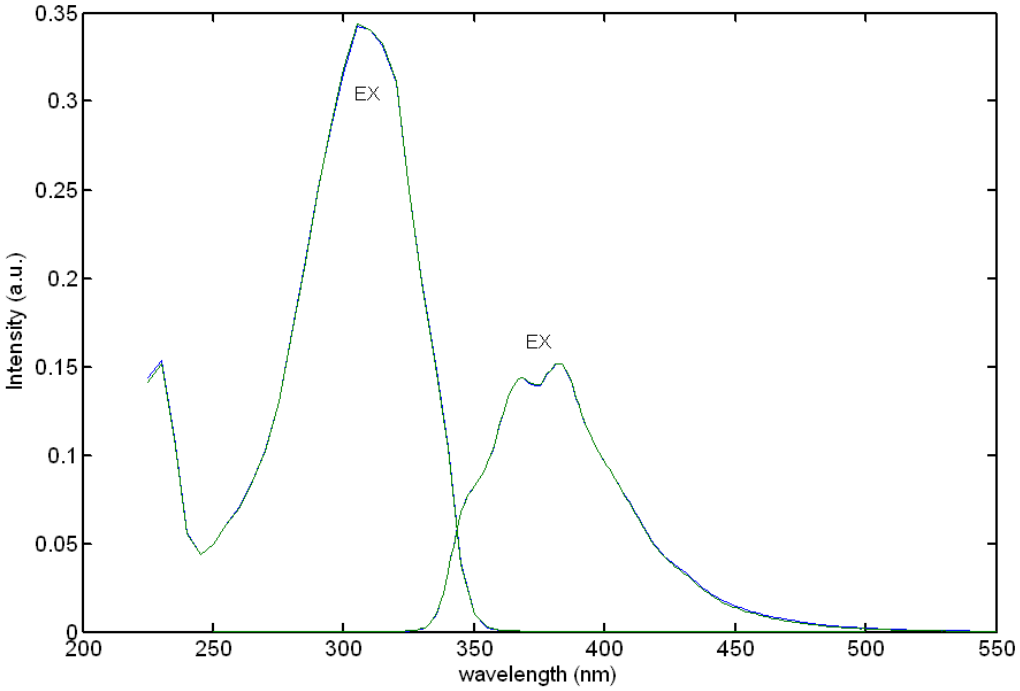

Figure S6 Comparison between the weighted spectra (green) and PARAFAC recovered spectra (blue) for the acceptor A in the micellar solutions in the presence of FRET. The weighted spectra are the combinations of the spectra in micelles and in water phase.

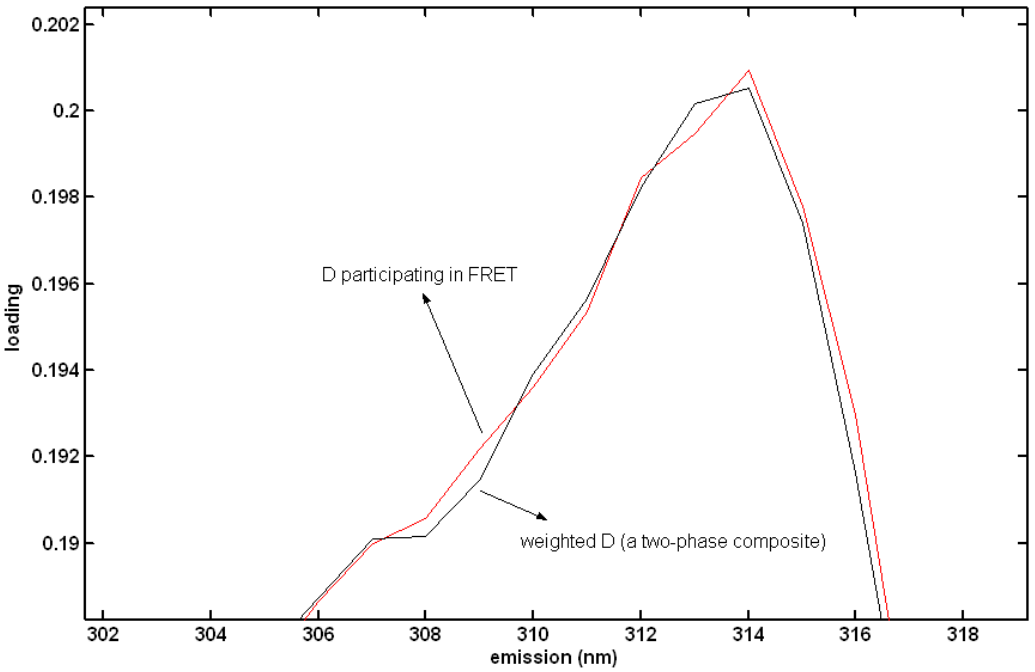

Figure S7 Comparison between the emission spectra of weighted D and D participating in FRET.

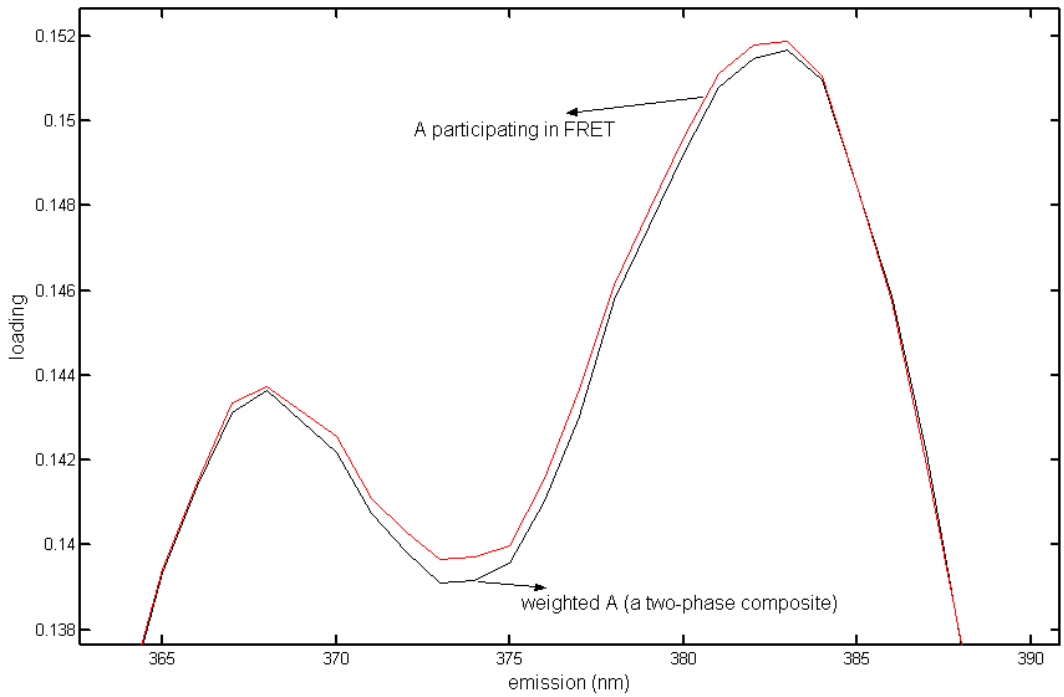

Figure S8 Comparison between the emission spectra of weighted A and A participating in FRET.

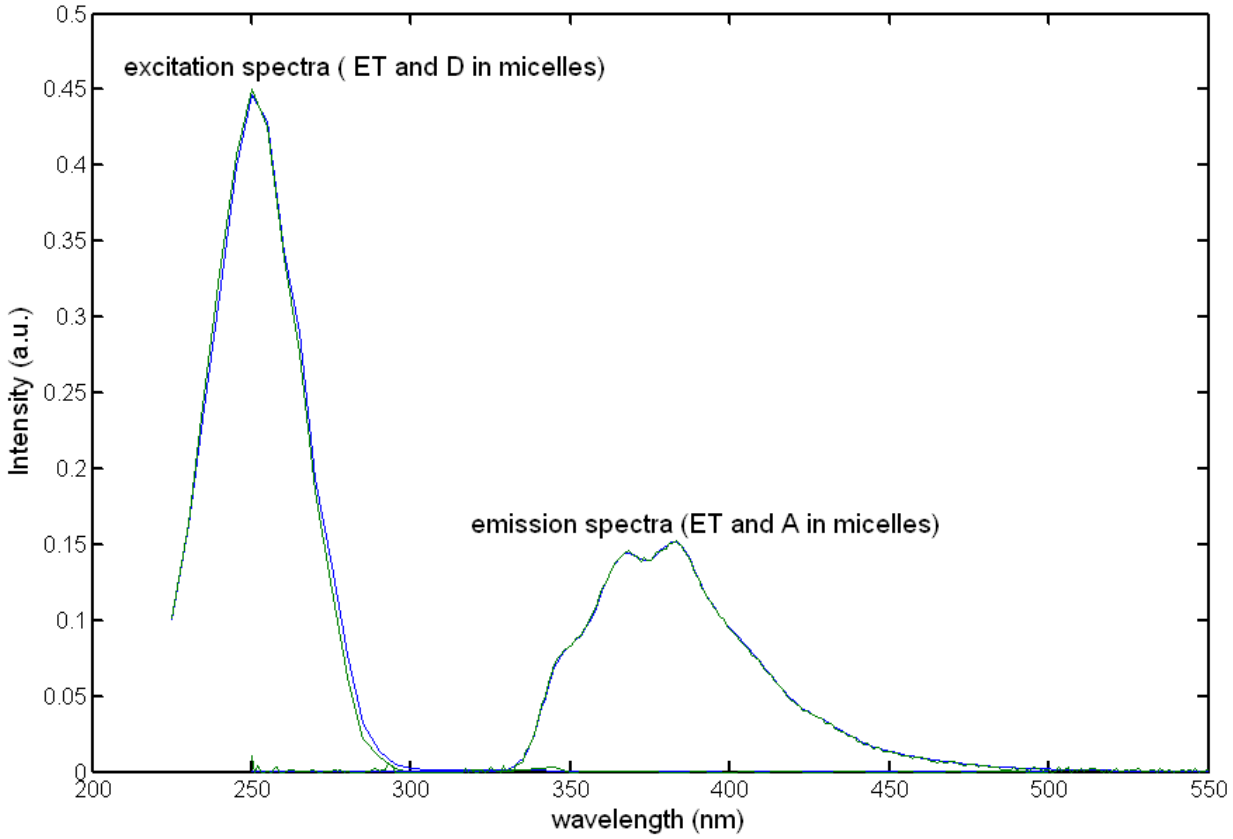

Figure S9 Comparison between the PARAFAC-recovered spectra of the FRET component and the pure excitation spectrum of D and emission spectrum of A in micelles.

Notes and references

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