

FRET Assays

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Introduction

- FRET, Förster Resonance Energy Transfer, is a tool that can be used for proximity-based assays, since this phenomenon occurs when the emission of a fluorophore acting as the **energy donor (D)** and the absorption of another dye acting as the **energy acceptor (A)** overlap, and both dyes are close enough in the space. Thus, FRET-based detection techniques are popular in fluorescence immunoassays or DNA hybridization assays.¹
- Conditions for an efficient FRET are: A good spectral overlap (J) between the **D** emission and the **A** absorption, and a high quantum yield of fluorescence (Φ_f) of **D**.
- A strategy for increasing sensitivity on fluorescent assays is by using fluorescently doped nanoparticles since thousands of fluorophores can be encapsulated in a single particle. Additionally, the stability and fluorescence properties of the dyes can be improved in the beads.

Results

Molecular probes

- Spectroscopic properties. Absorption and emission bands of **1-4** are centered at $\lambda \geq 600$ nm, with high absorption coefficients (for example, $\epsilon_{605} = 7.6 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$ for **4** in Et_2O) and quantum yields of fluorescence between 50 and 100% (Table 1).
- FRET parameters. Spectral overlap integral $J > 10^{15} \text{ M}^{-1} \text{ cm}^{-1} \text{ nm}^4$ for all FRET pairs and a Förster radius $R_0 > 50 \text{ \AA}$ (with compound **1** always as the **D** and **2-4** as the **A**). For comparison: $J = 4.9 \times 10^{14} \text{ M}^{-1} \text{ cm}^{-1} \text{ nm}^4$ for the popular FITC-TRITC FRET pair, which is distinctly lower.

Table 1. Spectroscopic properties of **1-4** in diethyl ether at 298 K

	$\lambda_{\text{abs}}^a / \text{nm}$	$\lambda_{\text{em}}^a / \text{nm}$	Φ_f^a	$J / \text{M}^{-1} \text{ cm}^{-1} \text{ nm}^4$ (FRET pair)	$R_0 / \text{\AA}$
1	598	605	0.98	-	-
2	627	647	1.0	7.5×10^{15} (1-2)	71
3	594	638	0.83	7.3×10^{15} (1-3)	71
4	605	666	0.52	1.3×10^{15} (1-4)	53

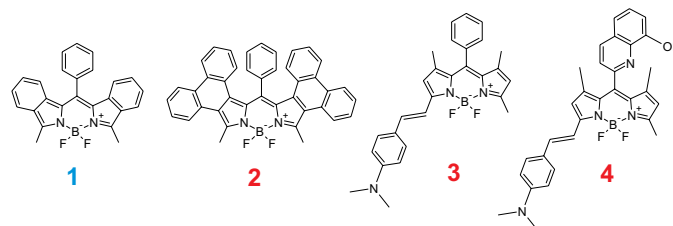
^aValues obtained from references 3a-d

Fluorescently doped polystyrene beads (PSBs)

- The dyes can be easily encapsulated into latex beads (neat, -COOH or -NH₂) simply by swelling of the particles in a mixture of water:THF. The distribution of the fluorophores in the polystyrene matrix is homogeneous, as shown in Figure 2 (large frame), and the particles are stable in water, no leaching of the dyes is observed.
- The beads are highly fluorescent in aqueous media (see Figure 2, inset, for 0.32 μm -COOH polystyrene particles doped with dye **3**, with Φ_f ca. 1).
- Particle sizes tested: 80 nm to 1.2 μm diameter.
- An efficient FRET process is observed for example, for dyes **1** and **2** co-encapsulated in the same particle.

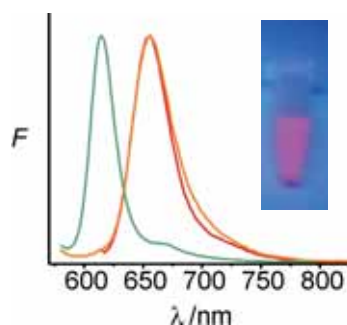
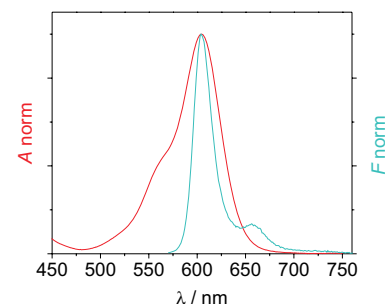
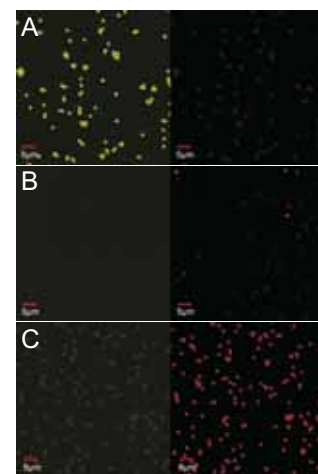
Approach

- Selection of appropriate **D-A** FRET pairs based on a series of BODIPY dyes (Chart 1), with an intense emission in the „biological window“ ($\lambda_{\text{em}} > 650$ nm).
- Encapsulation of the selected dyes into polystyrene (PS) particles containing surface functional groups for bioconjugation (-COOH and NH₂ groups).


Chart 1. Structures of the FRET donor (**1**) and acceptor (**2-4**) BODIPY dyes

 Where: $J = \int F_D(\lambda) A(\lambda) \lambda^4 d\lambda$

$$R_0^6 = 8.875 \cdot 10^5 \frac{2 \Phi_D J}{n^4}$$

Figure 1. Emission (blue) and absorption spectra (red) for the **1** (donor) and **4** (acceptor) FRET pair in diethyl ether.

Figure 2. Suspension of **PSB3** in water (inset) excited with UV lamp, emission spectra for PS particles, doped with: **1**, excited at 560 nm; **2**, excited at 600 nm; **1+2**, excited at 560 nm (right)

Figure 3. CLSM images for **PSB1**, **PSB2**, **PSB(1+2)**

Conclusions

- An easy procedure for doping of polystyrene particles with a new series of red-emitting BODIPY dyes was described. The doped particles are highly emissive and stable in aqueous solution.
- The PS beads are good candidates for FRET-based assays since the overlap integral of **1-2/3/4** pairs is very high, thus FRET is very efficient.
- Additionally, **3** and **4** doped particles are suitable as acceptors for other FRET assays in combination with rhodamine fluorophores, since their absorption spectra match perfectly the emission of popular rhodamines at λ_{em} of ca. 580–600 nm.

Acknowledgements



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Literature

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