COMMUNICATION

Spectroscopic differentiation between monomeric and aggregated forms of BODIPY dyes: effect of 1,1-dichloroethane†

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1,1-Dichloroethane induces a monomer–aggregate equilibrium of common BODIPY dyes in organic solvents at low μM dye concentrations, thus providing an opportunity to study monomeric, aggregate-free forms of BODIPY dyes.

Fluorophore assemblies are of significance due to their unique photophysical properties related to their optical and electronic responses,[1–4] as well as their applications in analytical and material sciences.[5–13]

BODIPY dyes are a widely used class of fluorescent probes, which attract continuous interest because their photophysical properties could be tuned *via* fairly straightforward synthetic transformations.[14–17] J- and H-aggregates of BODIPY dyes were noted in a number of studies; however, these cases primarily involved aggregation in the solid or gel states or in organic solvent-water mixtures.[18–24] The aggregated forms of BODIPY dyes, especially in aqueous environments, tend to exhibit a red-shifted transition at *ca.* 600 nm. A few cases of BODIPY H-aggregates were reported, and in some instances the H-aggregates of BODIPY dyes were found to be non-emissive.[18–20] Notably, in these cases, the BODIPY dyes possessed specific moieties that induced a particular aggregation state.[25] On the other hand, in organic solvents, BODIPY dyes are known to be monomeric, especially at low μM concentration ranges, as confirmed by the presence of a single peak in the emission spectra.[18,24,26,27] Here, we present an unusual case of spectroscopically distinct monomeric and aggregated forms of BODIPY in a chlorinated organic solvent.

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Building on our interest in developing molecular viscometers based on BODIPY scaffolds,[28–30] we examined the spectroscopic properties of BODIPY dimer **1** (Fig. 1) in various organic solvents, and found that both absorption and emission spectra of **1** in 1,2-DCE were distinctly different from those in other solvents, such as EtOH, DMSO, THF, dioxane, CH_3CN, and $CHCl_3$ (Fig. 1A). Specifically, notable hypsochromic shifts were observed in both the absorption (from *ca.* 540 to 515 nm) and emission (from *ca.* 560 to 535 nm) spectra (Fig. 1B and C).

To verify the aggregate formation, we measured fluorescence lifetime and anisotropy as a function of dye **1** concentration (Tables S2 and S3, Fig. S1†). A decrease in the average fluorescence lifetime was accompanied by an increase in the steady state anisotropy over 0.1–10 μM. In addition, the anisotropy data showed that at low concentrations (*i.e.*, 0.1 and 0.2 μM), only the monomeric form of **1** was present, as two components were sufficient to fit the anisotropy decay. The fast correlation time (93 ps) was attributed to the rotation of the BODIPY units around the diyne moiety, while the slower correlation time (541 ps) was attributed to tumbling of dye **1** in solution. At higher concentrations (≥0.5 μM) two components were no longer sufficient to fit the anisotropy decay, and the appearance of a third component (r_{inf}) was consistent with the presence of large (*i.e.*, aggregated) species, whose correlation time was much longer than the lifetime of the probe, thus requiring the use of r_{inf} (Table S3†).[31]

Next, in order to determine whether the observed monomer-aggregate behavior was specific to **1**, we evaluated the concentration dependent spectroscopic responses of several common BODIPY dyes (see ESI†), which did not possess any aggregation-inducing moieties (Fig. 2 and S2†).[32] In all cases, hypsochromic shifts in both absorption and emission spectra were observed. Because substantial changes in both absorbance and emission spectra were noted for **2**, this dye was chosen for all subsequent studies as a model compound.

Due to the unprecedented ability of 1,2-DCE to induce H-aggregate formation, we screened several samples of 1,2-DCE from various vendors (Fig. S3†), and unexpectedly found that
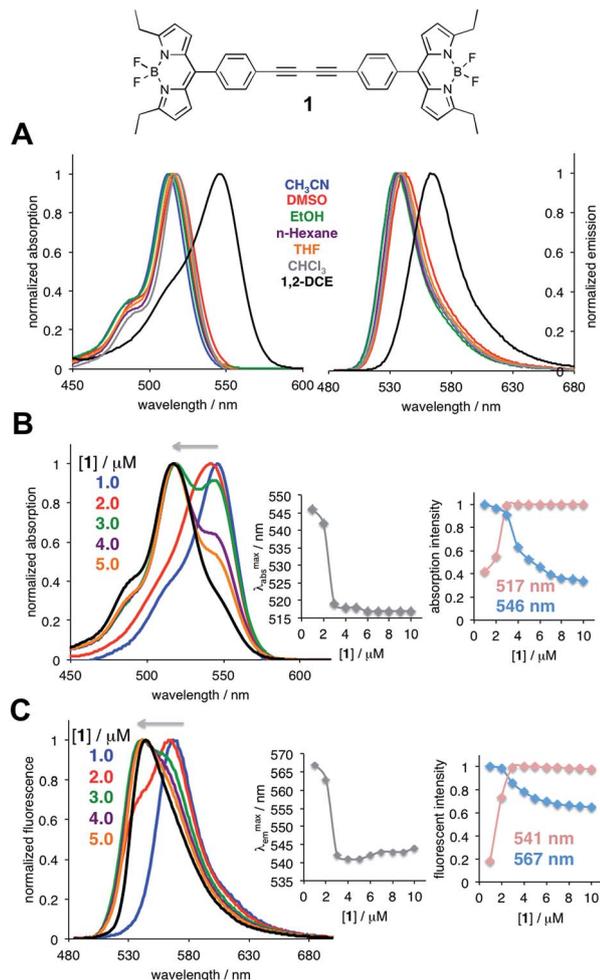
Fig. 1 Structure of dye **1**; (A) absorption (left) and emission (right) spectra of [**1**] = 1.0 μM in various organic solvents; concentration dependent absorption (B) and emission (C) spectra of dye **1** in 1,2-DCE (ACROS/for spectroscopy; Table S1†), arrows above the spectra show the direction of the spectral shift upon increasing dye concentration. Insets: changes in the absorption and emission maxima, and changes in the absorption an emission intensity at specified wavelengths as a function of dye concentration.

Fig. 2 Concentration dependent absorption (A) and emission (B) spectra of dye **2** in 1,2-DCE (ACROS/for spectroscopy; Table S1†); arrows above the spectra show the direction of the spectral shift upon increasing dye concentration. Insets: changes in the absorption and emission maxima, and changes in the absorption an emission intensity at specified wavelengths as a function of dye concentration.

the differentiation between monomeric and aggregated forms of BODIPY **2** was only observed in 1,2-DCE from ACROS (ACROS/for spectroscopy, Table S1†). Subjecting 1,2-DCE samples (ACROS/ACS-spectrograde and ACROS/for spectroscopy, Table S1†) to various treatments (purging with nitrogen, washing with water and 1 N HCl) did not change the initial observation with this sample of 1,2-DCE, *i.e.*, no differentiation between the monomeric and aggregated forms of BODIPY was observed. In an attempt to clarify this discrepancy, we subjected several samples to GC-VUV (gas chromatography with vacuum ultraviolet detector) analysis,[33] which established that 1,1-DCE was the only impurity present in ACROS/for spectroscopy and not ACROS/ACS-spectrograde 1,2-DCE (Fig. S4;† vinyl chloride was not considered due to high volatility).

To prove that 1,1-DCE was capable of inducing the formation of monomeric **2**, a small amount of 1,1-DCE was added to several organic solvents (Fig. 3). In the case of EtOH and CH₃CN
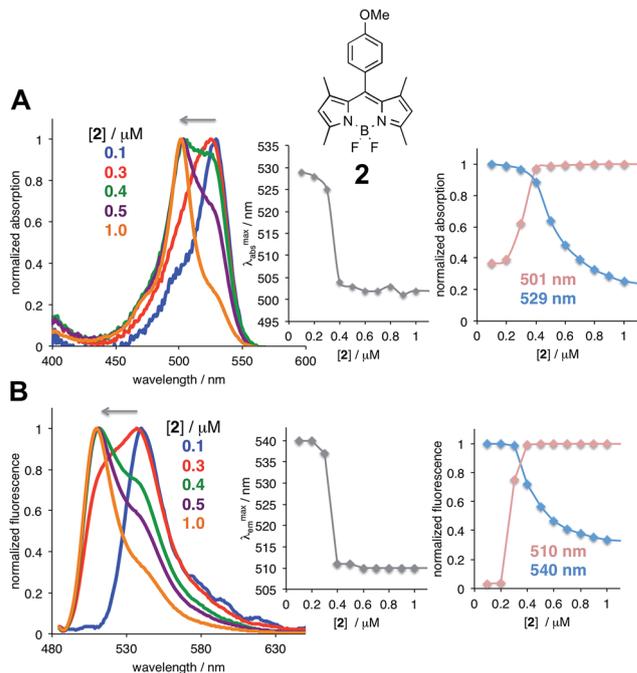
(solvents in which no spectroscopic differentiation between monomeric and aggregated forms of BODIPY dyes was noted) a bathochromic shift was observed upon addition of 1,1-DCE (Fig. 3A and C). This confirmed that 1,1-DCE was responsible for providing the spectroscopic distinction between the monomeric and aggregated forms of BODIPY. Arguably, the inability of 1,1-DCE to induce a spectral shift in CHCl₃ and DMSO (Fig. 3B and
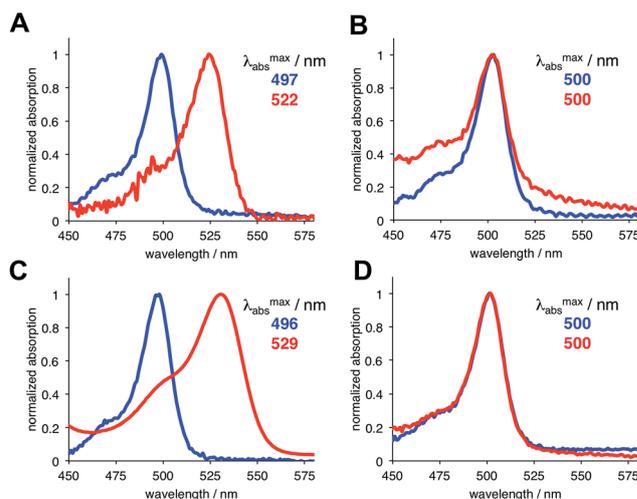

Fig. 3 Normalized absorption spectra of dye **2** (0.1 μM) in various solvents ((A) EtOH; (B) CHCl₃; (C) CH₃CN; (D) DMSO) in the absence (blue) and in the presence (red) of 5% (v/v) of 1,1-DCE.
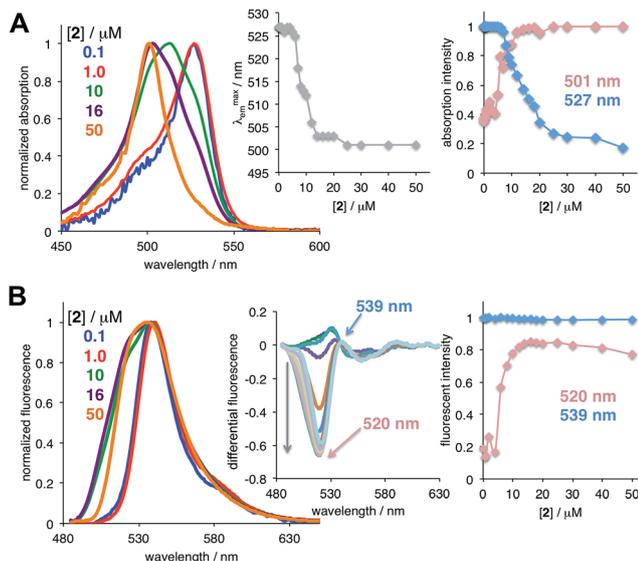
Fig. 4 Absorption (A) and emission (B) spectra of dye **2** in 1,1-DCE. Insets: changes in the absorption and emission maxima as a function of concentration, and changes in the absorption and emission intensities at the maxima of the monomer and aggregate as a function of dye concentration. Differential emission spectra (bottom right inset) show the increasing intensity of the aggregated form of the dye at 520 nm (spectra were obtained as follows: $I_F^n - I_F^{50}$, where I_F^{50} is the normalized emission spectra of [**2**] = 50 μM, and I_F^n is the normalized emission spectra of [**2**] = n μM, with n = 0.1, 0.2, ... 8.0, 9.0, etc. μM).

D) could be attributed to the stronger solvation of the dye in these solvents. Notably, 1,1-DCE samples were purchased from different vendors (Aldrich and TCI,‡ Table S1†) and similar results were observed. Importantly, when concentration dependent absorption spectra were acquired in 1,1-DCE and two spectroscopic grades of 1,2-DCE (Fig. S5†), a hypsochromic shift from 529 nm to 502 nm was only noted in 1,2-DCE (ACROS/for spectroscopy), a single absorption maximum was noted at 502 nm and at *ca.* 528 nm in 1,2-DCE (ACROS/ACS-spectrograde) and 1,1-DCE, respectively. Furthermore, the anisotropy measurements confirmed the absence of the aggregated form of dye **2** in 1,1-DCE upto 10 μM, whereas in 1,2-DCE the aggregated form of **2** was noted even at 1.0 μM (Fig. S6, Table S3†).

Next, to further characterize the behavior of **2** in 1,1-DCE (Fig. 4, S7 and S8, and Table S4†), more detailed spectroscopic measurements were carried out, even though due to a relatively high cost,§ 1,1-DCE is more likely to be used as an additive rather than a solvent.¶ The concentration dependent spectra only started to exhibit the apparent hypsochromic shift above 8 μM (Fig. 4A). Emission spectra on the other hand did not exhibit significant changes upon increasing the concentration of dye **2** (Fig. 4B). However, differential spectra (Fig. 4B, inset) unambiguously demonstrated that the emission of the higher energy species increased with increasing concentrations of the dye.

Furthermore, the full width half maxima (FWHM) as a function of increasing dye's concentration (Fig. S7†) indicated a gradual transition from a monomeric species to an aggregate.

In conclusion, we showed that it is possible to spectroscopically distinguish the monomeric and aggregated forms of BODIPY dyes in 1,1-DCE. Notably, small quantities of 1,1-DCE could be used to modify the aggregate–monomer equilibrium in several organic solvents. These results could provide a novel approach toward the superior evaluation and characterization of fluorophores and their assemblies, in general, and BODIPY-based dyes, in particular. Therefore, additive-induced disaggregation could be a valuable approach towards modulating the supramolecular structure of fluorophore assemblies, which could have far reaching applications in a variety of fields. Further studies on the behavior of other BODIPY dyes in 1,1-DCE as well as the mechanism behind the 1,1-DCE-induced differentiation between the monomeric and aggregated forms of BODIPY dyes are in progress in our laboratories and will be reported in due course.

Acknowledgements

Notes and references

‡ 1,1-DCE sample from TCI is stabilized with CH_3NO_2 (Table S3). To rule out the effect of CH_3NO_2, both absorption and emission of dye **2** were acquired ([**2**] = 0.1–10 μM); no concentration-dependent hypsochromic shift was observed; thus the behaviour of dye **2** in CH_3NO_2 was similar to that found for other organic solvents, such as DMSO, THF, *etc.*, (see Fig. 1A).

§ Aldrich (order #: 36967): $35.40/1 g; TCI (order #: D0363): $81.00/25 g.

¶ For a preliminary spectroscopic characterization of other BODIPY dyes in 1,1-DCE see Table S6.

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