

Supporting Information for

Aqueous Self-Assembly of a Charged BODIPY Amphiphile via Nucleation-Growth Mechanism

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1. General Methods

Chemicals and Reagents: All the chemicals used were analytical grade and were used without further purification unless otherwise stated. Ethyl magnesium bromide, iodomethane, 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone (DDQ), 1-dimethylamino-2-propyne, trifluoroacetic acid (TFA), $\text{BF}_3 \cdot \text{Et}_2\text{O}$, 2,4-Dimethylpyrrole, and were purchased from commercial source. Dichloromethane (DCM) and triethylamine were distilled over CaH_2 and stored under Ar. Silica gel (200-300 mesh) was used for column chromatography. 3,4,5-Tris(*n*-dodecyloxy)benzaldehyde¹ (compound **4**, Scheme S1) and BODIPY precursor **3**² were synthesized according to previously reported procedure.

NMR spectroscopy: ¹H and ¹³C NMR spectra were recorded at 300 K on a Bruker (400 MHz or 500MHz) spectrometer using tetramethylsilane (TMS) as internal standard. Multiplicities for proton signals are abbreviated as *s*, *t*, and *m* for singlet, triplet, and multiplet, respectively.

Mass spectrometry: ESI mass spectra were measured with a Bruker Daltonics micrOTOF-QII LC-MS system.

UV/Vis spectroscopy: UV/Vis absorption spectra were recorded on an Agilent Technologies Cary 300 UV/Vis spectrophotometer equipped with a SPV 1×1 temperature controller. The solvents for spectroscopic studies were spectroscopic grade and used as received. The spectra were recorded in quartz glass cuvettes and the extinction coefficients ϵ were calculated according to Lambert-Beer's law.

Steady-State Fluorescence spectroscopy: Fluorescence spectroscopic measurements were performed under ambient conditions on a calibrated FluoroLog-3 spectrofluorometer. All the fluorescence spectra were corrected. For the samples of dye aggregates with high optical densities, a front-face setup was used to minimize reabsorption. The fluorescence quantum yields were

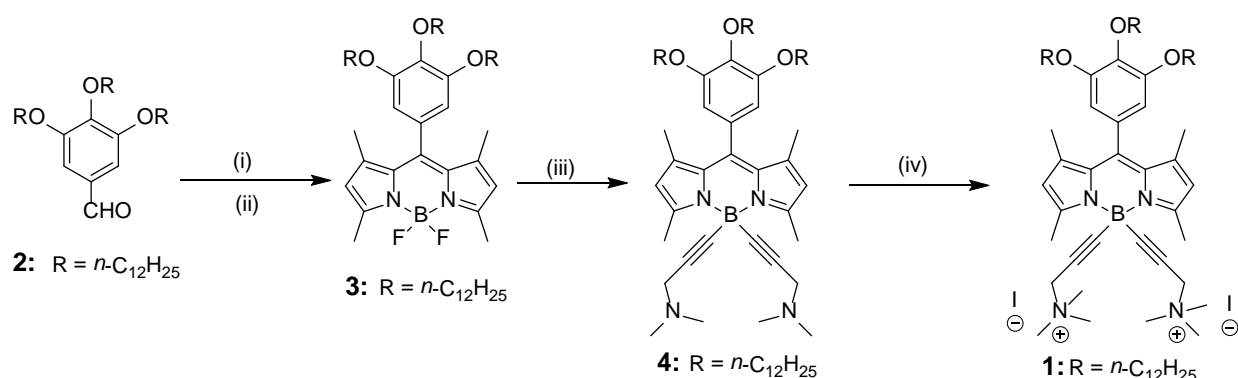
determined using following equation according to the literature³: $\Phi_{F(x)} = (A_s/A_x)(F_x/F_s)(n_x/n_s)^2\Phi_{F(s)}$, where $\Phi_{F(x)}$ is the fluorescence quantum yield, A is the absorbance at the excitation wavelength, F is the area under the corrected emission spectrum, and n is the refractive index of the solvents used. Subscripts “s” and “x” refer to the standard and to the unknown, respectively. Fluorescein in 0.1 M NaOH solution ($\Phi_{F(s)} = 0.92$) was used as standard.

Time-Resolved Fluorescence spectroscopy: Fluorescence lifetimes were measured with a Horiba DeltaFlex TCSPC system equipped a NanoLED laser (445 nm, pulse width <200 ps) excitation source and a TBX picosecond photon detection module. The instrument response function was collected by scattering the exciting light of a dilute, aqueous suspension of Silica (LUDOX). Decay curves were evaluated using the software supplied with the instrument applying least square regression analysis. The quality of the fit was evaluated by analysis of χ^2 (0.9 – 1.1), as well as by inspection of residuals and autocorrelation function.

Electron microscopy: Transmission electron microscopy (TEM) measurements were performed on a Tecnai G² 20 S-TWIN Transmission Electron Microscope, operating at an acceleration voltage of 200 kV. For the observation of aggregates, a drop of sample solution (0.1-0.5 mg/mL) was placed on 400-mesh formvar copper grids coated with carbon. About 2 min after the deposition, the grid was tapped with filter paper to remove surface water. Staining was performed by addition of a drop of uranyl acetate aqueous solution (0.5 %) onto the copper grid. After 1 min, the surface liquid on the grid was removed by tapping with filter paper.

Confocal laser scanning microscopy: Confocal fluorescence images were recorded on a Zeiss LSM710 confocal laser scanning microscope (CLSM) (Jena, Germany). The excitation was performed with Ar lase at wavelengths of 488 nm, 543 nm, 594 nm, and the emission was monitored at 500-550 nm, 550-600 nm or 600-650 nm respectively. For the sample preparation, a method reported in literature was used.⁴ An aqueous solution of gelatine (1mg/mL) was added into the dye aggregates at 40 °C and cooled to room temperature, and then the resultant viscous mixture was dropcast on untreated glass substrate for observation.

2. Synthesis and characterization



Scheme S1. Synthesis of BODIPY dye **1**. Reagents and conditions: (i) CF₃COOH, CH₂Cl₂, DDQ, r.t., 2 h; (ii) BF₃·Et₂O, triethylamine, r.t., 30%; (iii) 1-dimethylamino-2-propyne, EtMgBr, THF, 60 °C, 12 h, 75%; (iv) CH₃I, diethyl ether, 30 °C, 24 h, 94%.

4,4-difluoro-1,3,5,7-tetramethyl-8-(3,4,5-tris(dodecyloxy))-4-bora-3a,4a-diaza-s-indacene (**3**)²

3,4,5-Tris(dodecyloxy)benzaldehyde¹ (compound **2**) (1.3 g, 2.0 mmol) and 2,4-dimethylpyrrole (0.4 mL, 4.0 mmol) were dissolved in dry CH₂Cl₂ (30 mL) under Ar. After stirring for 15 min, 0.036 mL TFA was added and the reaction was continued at room temperature for about 2 h. After the complete consumption of compound **2** (monitored by TLC), DDQ (8.5 mmol, 2.0 g) was added into the reaction mixture. After stirring for 2 h, triethylamine (10.0 mL, 57 mmol) and BF₃·Et₂O (10.0 mL, 79 mmol) were added subsequently. The reaction mixture was stirred at room temperature for 10 h. Then the solvent was evaporated under reduced pressure and the residue was purified by column chromatography (silica gel, CH₂Cl₂/*n*-Hexane = 1/1, v/v) to give an orange-colored viscous substance (0.53 g, 30%). ¹H NMR (400 MHz, CDCl₃, 300K, TMS): δ = 6.47 (*s*, 2H), 5.99 (*s*, 2H), 3.96 (*qd*, *J* = 19.54), 2.55 (*s*, 6H), 1.87-1.70 (*m*, 6H), 1.53 (*s*, 6H), 1.49-1.39 (*m*, 6H), 1.26 (*m*, 48H), 0.89 (*m*, 9H).

4,4-bis-(3-dimethylamino-1-propynyl)-1,3,5,7-tetramethyl-8-(3,4,5-tris(dodecyloxy))-4-bora-3a,4a-diaza-s-indacene (**4**)

To a solution of 1-dimethylamino-2-propyne (0.14 g, 2.0 mmol) in dry THF (5 mL) under Ar in a flask, was added EtMgBr (1.0 M in THF, 1.7 mL) and the mixture was stirred at 60 °C for 2 h. Compound **3** (0.70 g, 0.79 mmol) was dissolved in a separate flask in dry THF (3 mL) under Ar, and then the solution was transferred via a cannula to the flask containing the Grignard reagent. The mixture was stirred at 60 °C for 2 h and then water (3 mL) was added. The mixture was extracted with CH₂Cl₂ (20 mL × 3). After the solvent was evaporated under reduced pressure, the residue was purified by column chromatography (silica gel, CH₂Cl₂ first, then CH₂Cl₂/MeOH = 3/1, v/v) to give a dark-orange solid (0.60 g, 75%). ¹H NMR (400 MHz, CDCl₃, 300K, TMS): δ = 6.50 (*s*, 2H), 6.01 (*s*, 2H), 4.02 (*d*, *J* = 6.36 Hz, 2H), 3.91 (*t*, *J* = 6.53 Hz, 4H), 3.22 (*s*, 4H), 2.78 (*s*, 6H), 2.30 (*s*, 12H), 1.77 (*dd*, *J* = 14.22, 7.01 Hz, 6H), 1.52 (*s*, 6H), 1.48-1.39 (*m*, 6H), 1.25 (*m*, 48H), 0.88 (*m*, 9H). ¹³C NMR (126 MHz, CDCl₃): δ = 154.79, 154.14, 142.12, 141.65, 138.57, 129.88, 129.60, 121.55, 106.48, 86.60, 73.77, 69.48, 48.60, 43.30, 32.05-32.03, 29.87-29.72, 29.50-29.41, 26.24, 26.11, 22.81, 22.81-22.80, 16.27, 14.57, 14.24. MS (ESI, positive mode): calculated for C₆₅H₁₀₇BN₄O₃ 1002.84, found: *m/z* [M+H]⁺ 1003.86.

4,4-bis-(3-trimethylammonioiodine-1-propynyl)-1,3,5,7-tetramethyl-8-(3,4,5-tris(dodecyloxy))-4-bora-3a,4a-diaza-s-indacene (**1**)

Compound **4** (0.10 g) was stirred with iodomethane (3-5 equiv.) in dry diethyl ether at 30 °C for 24 h.

After cooling to room temperature, the precipitated solid was isolated by filtration, and then another time precipitated from CH₂Cl₂ by the addition of Et₂O to give an orange colored powder (0.12 g, 94%). ¹H NMR (400 MHz, CDCl₃, 300K, TMS): δ = 6.50 (s, 2H), 6.12 (s, 2H), 4.71 (s, 4H), 4.02 (s, 2H), 3.94 (t, *J* = 5.70 Hz, 4H), 3.53 (s, 18H), 2.72 (s, 6H), 1.80-1.75 (m, 6H), 1.57 (s, 6H), 1.48-1.44 (m, 6H), 1.25 (m, 48H), 0.92-0.84 (m, 9H). ¹³C NMR (126 MHz, CD₃OD): δ = 156.21, 144.18, 143.71, 140.68, 133.16, 131.12, 129.03, 122.96, 111.12, 84.32, 66.87, 58.51, 53.37, 21.43, 16.72, 14.88. HRMS (ESI, positive mode): calculated for C₆₇H₁₁₃BN₄O₃²⁺ 1032.8895, found: m/z [M]²⁺ 516.4491. Elemental analysis: Anal. calcd for C₆₇H₁₁₃BN₄O₃I₂: C 62.51%, H 8.85%, N 4.35%; found: C 62.41%, H 8.81%, N 4.19%. UV/Vis (DMSO): λ_{max}(ε) = 365 (6120 mol⁻¹ L cm⁻¹), 471 (17400 mol⁻¹ L cm⁻¹), 501 (89300 mol⁻¹ L cm⁻¹). Fluorescence (DMSO): λ_{max} = 510 nm; quantum yield: Φ_{fl} = 0.17.

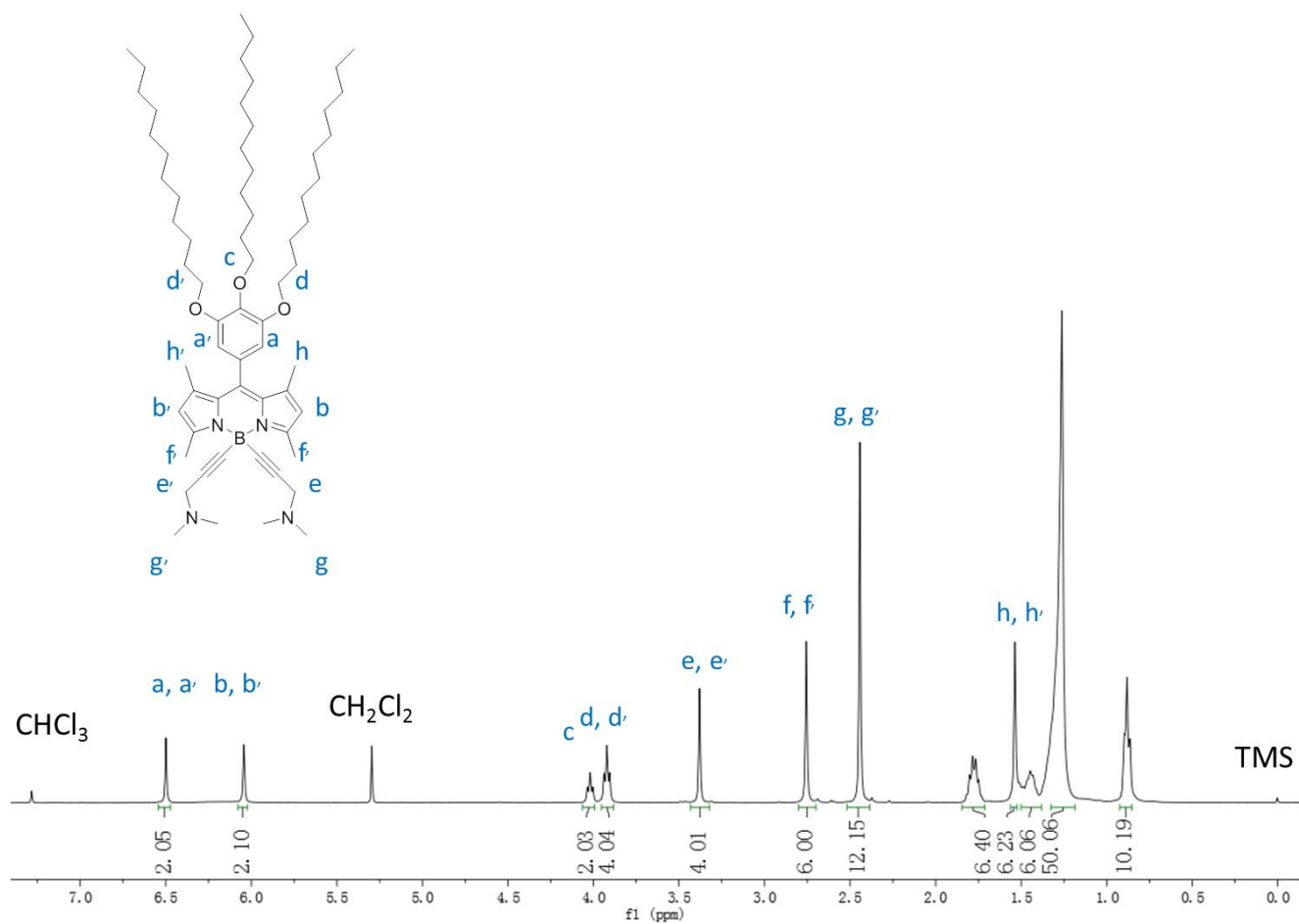


Figure S1. 400 MHz ¹H NMR spectrum with corresponding assignments and chemical structure of dye **4** in CDCl₃.

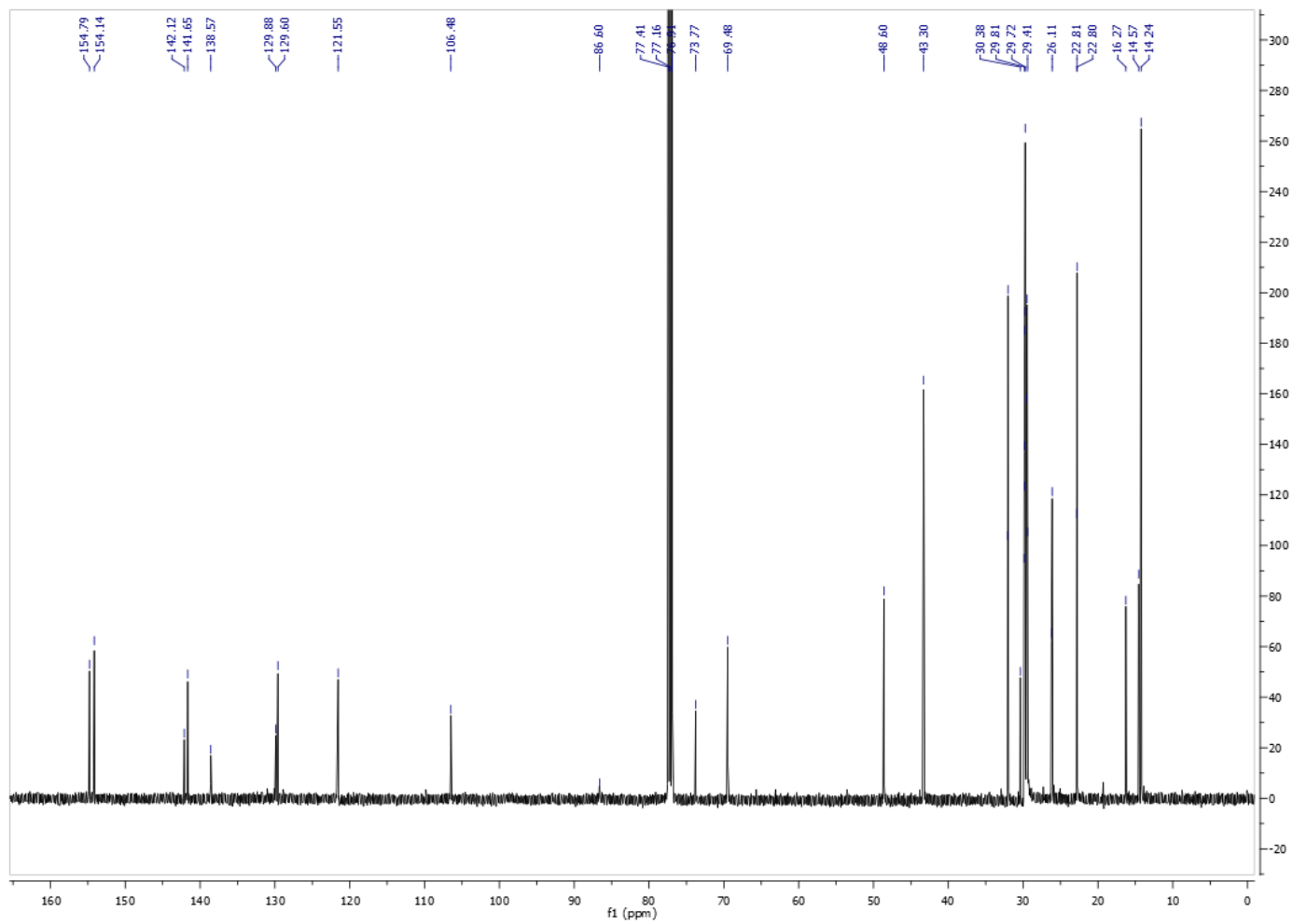


Figure S2. 126 MHz ^{13}C NMR spectrum of dye **4** in CDCl_3 .

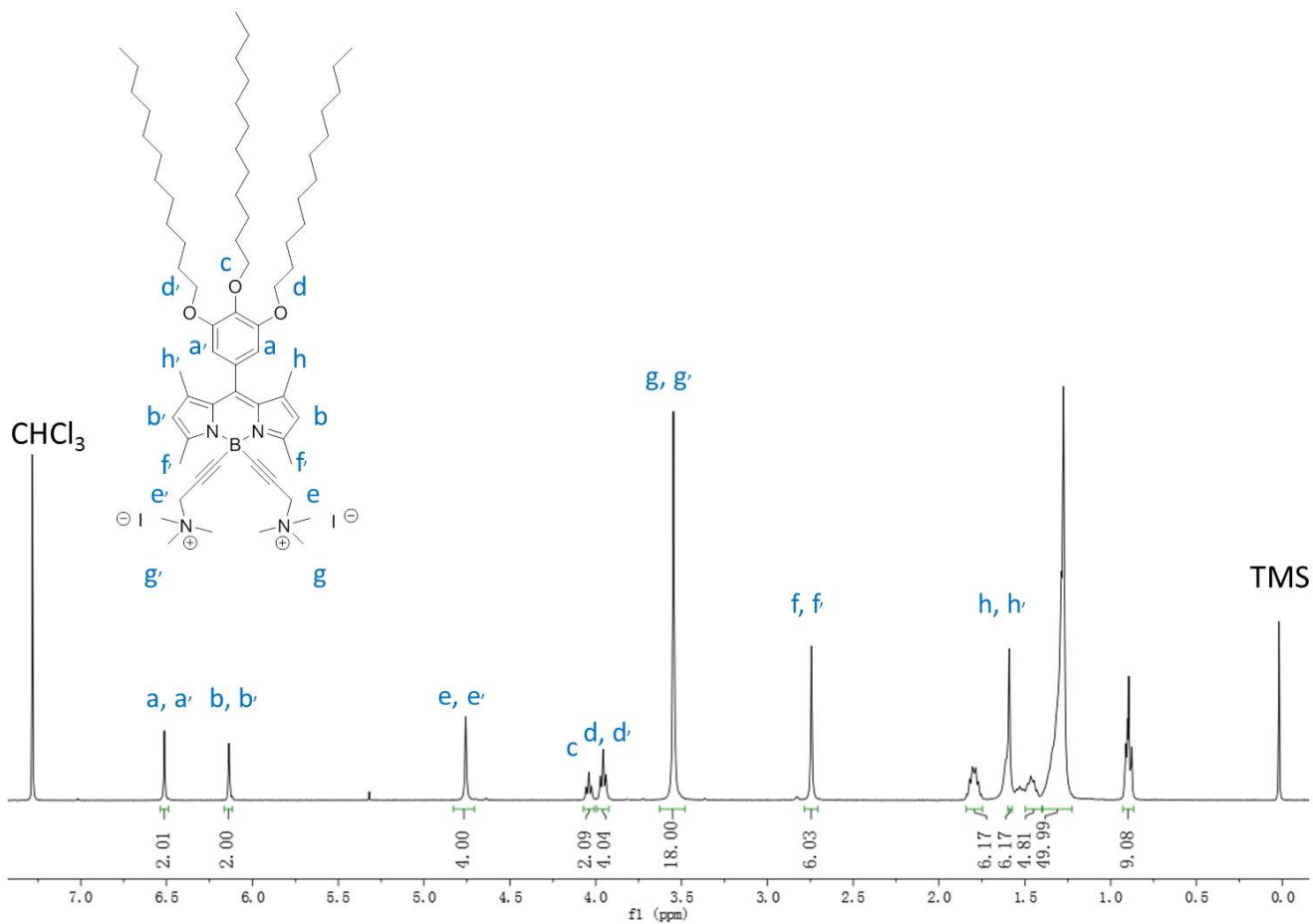


Figure S3. 400 MHz ^1H NMR spectrum with corresponding assignments and chemical structure of dye **1** in CDCl_3 .

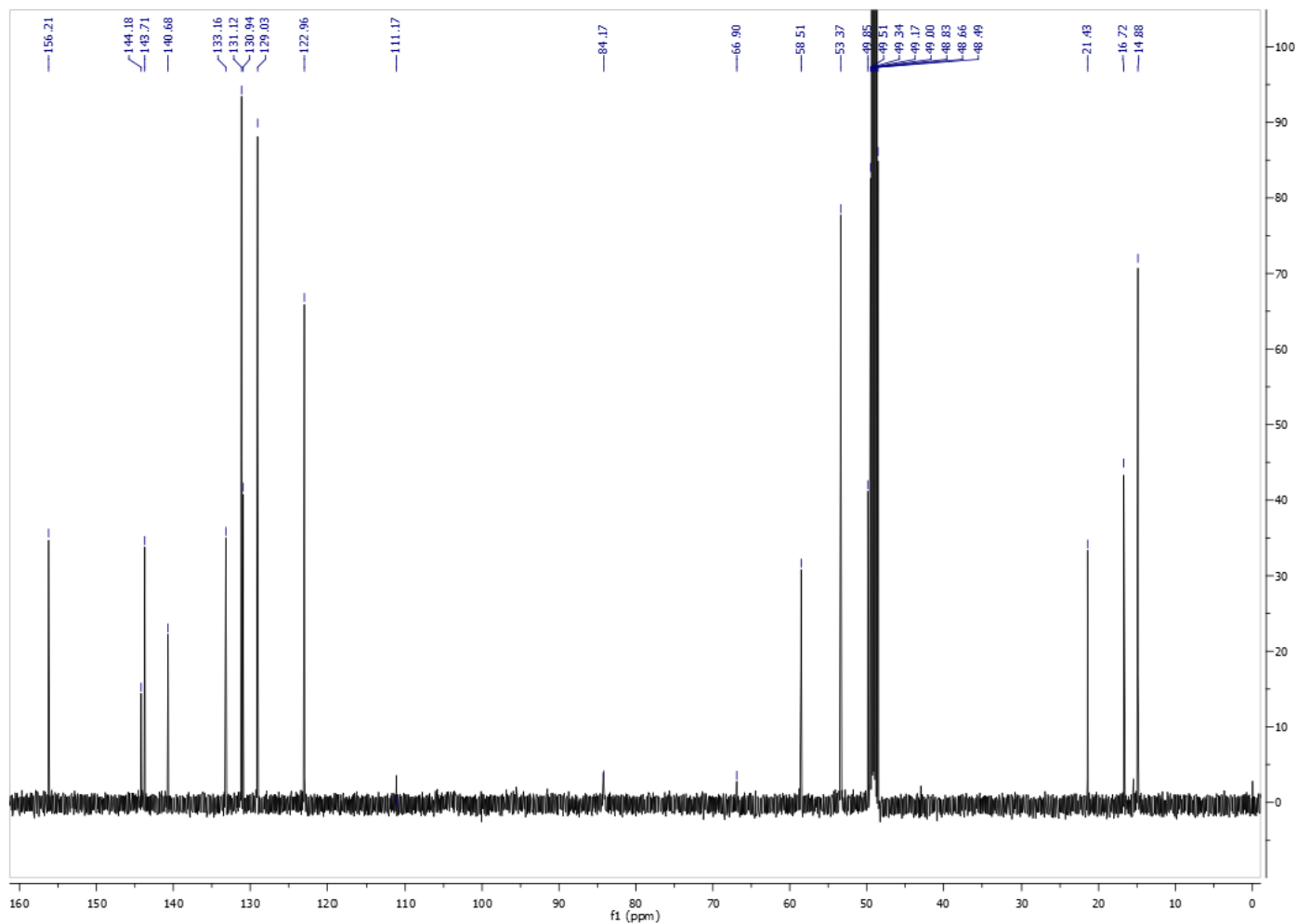


Figure S4. 126 MHz ^{13}C NMR spectrum of dye **1** in CD_3OD .

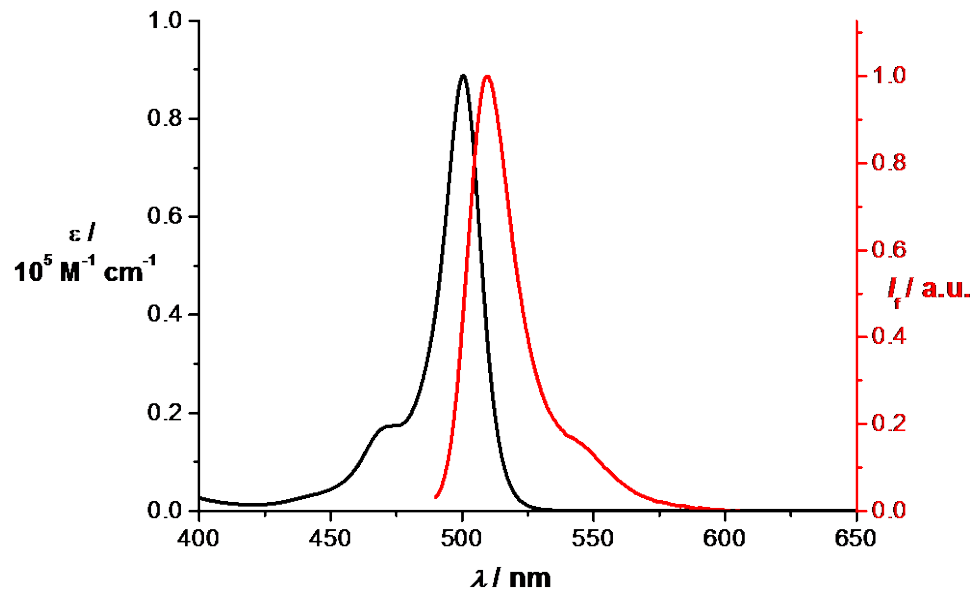


Figure S5. UV/Vis absorption and fluorescence spectra of **1** in DMSO at concentrations of $2.0 \times 10^{-6} \text{ M}$ ($\lambda_{\text{ex}} = 470 \text{ nm}$).

3. Aggregation studies

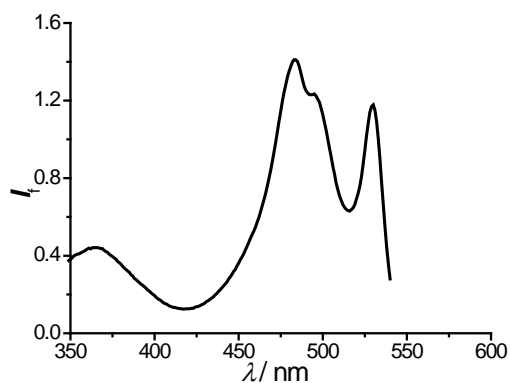


Figure S6. Fluorescence excitation spectrum of the aggregates of dye **1** (1.0×10^{-5} M) in water monitored at 550 nm.

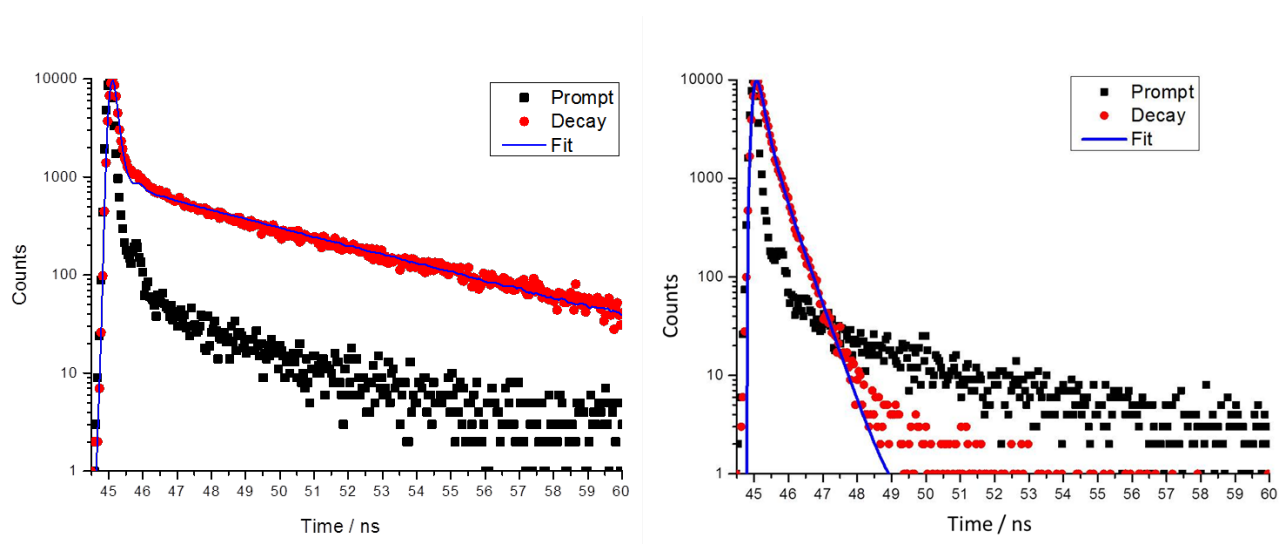


Figure S7. The fluorescence decay curves of the monomer ($\lambda_{\text{detection}} = 580$ nm) and aggregates ($\lambda_{\text{detection}} = 580$ nm) of dye **1** in water. The curves were fitted with two-exponential decay function and the lifetime τ was calculated as an amplitude-averaged decay time. For the monomer, $\tau_{\text{mono}} = (51.87\% \times 0.1 \text{ ns}) + (48.13\% \times 5.0 \text{ ns}) = 2.5 \text{ ns}$, $\chi^2 = 1.06$. For the aggregates, $\tau_{\text{agg}} = (53.61\% \times 0.2 \text{ ns}) + (46.39\% \times 0.5 \text{ ns}) = 0.3 \text{ ns}$, $\chi^2 = 0.99$.

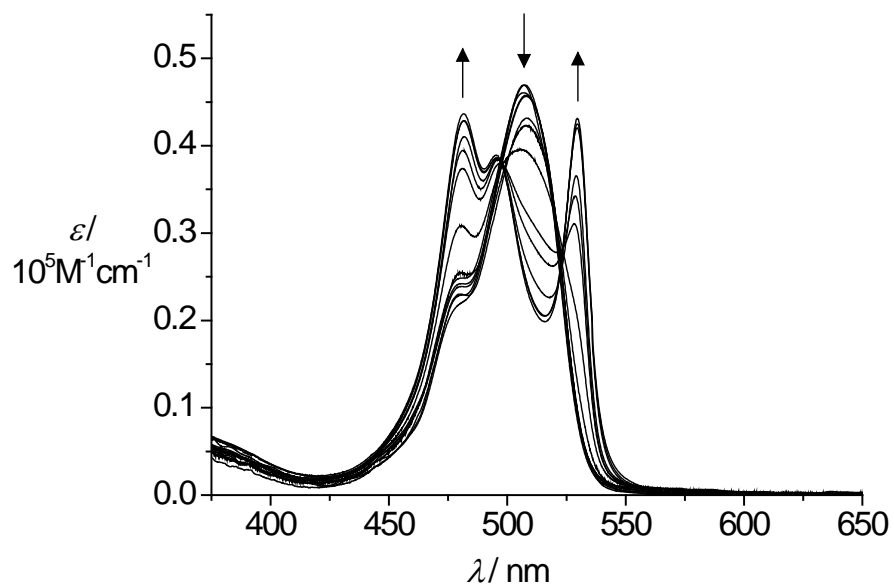


Figure S8. Concentration-dependent UV/Vis absorption spectra of dye **1** in water with concentration increasing from 2×10^{-7} to 5×10^{-4} M.

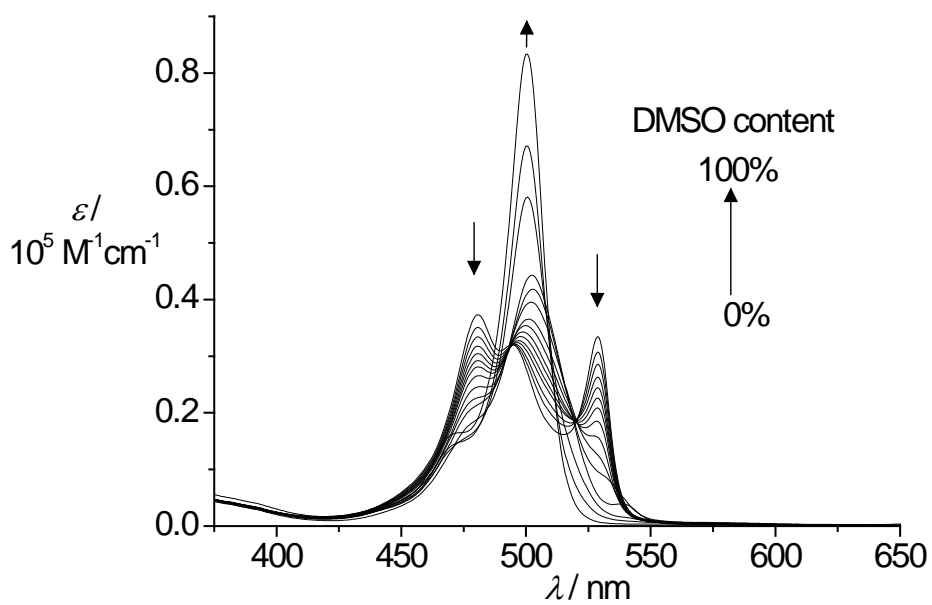


Figure S9. UV/Vis absorption spectra of **1** (1.0×10^{-5} M) in DMSO-containing water with increasing DMSO content (0% to 100 %, v/v).

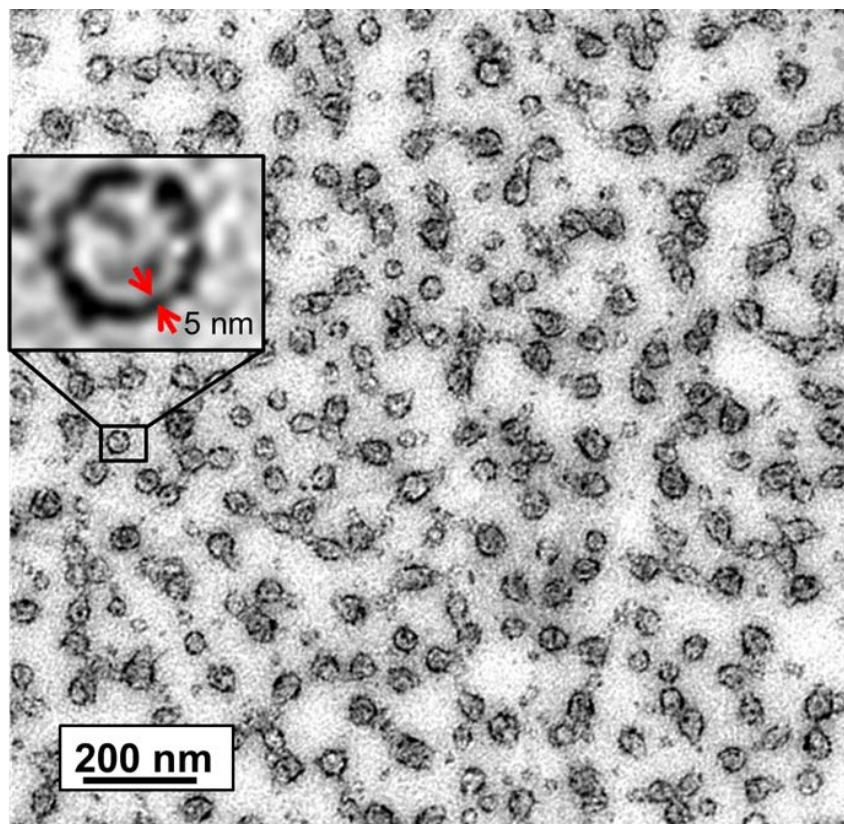


Figure S10. TEM image of the aggregates of dye **1** in DMSO-containing water ($[1] = 0.1$ mg/mL, DMSO content 0.5%, v/v).

Estimation of Critical Packing Parameter (P_c) for Dye **1**

According to Israelachvili,⁵ P_c can be estimated from the equation (1),

$$P_c = \frac{v}{a_0 \times l_c} \quad (1)$$

where v is the volume of an individual molecule, a_0 stands for the optimal area occupied by the hydrophilic part, and l_c stands for the length of the molecule. For dye **1**, $a_0 = 2 \times 0.47$ nm² (for one quaternary ammonium groups,⁶ $a_0 = 0.47$ nm²), $l_c = 2.5$ nm (based on molecular modeling). The volume of dye **1** v can be considered as the sum of two parts, i.e. the three dodecyl chains (v_1) and the rest of the molecule (v_2). The value of v_1 can be obtained from equation (2),⁵

$$v_1 = 3 \times (27.4 + 26.9n) \times 10^{-3} \text{ nm}^3 \quad (2)$$

where n is the number of carbon atoms. For $n = 12$, $v_1 = 1.05$ nm³ can be calculated.

The value of v_2 can be estimated by $v_2 = m / \rho$, where m is the mass of this part and ρ is the density obtained from the crystallographic data of related BODIPYs.⁷ Accordingly, $v_2 = (1.29 \times 10^{-21} \text{ g}) / (1.4 \text{ g/cm}^3) = 0.92$ nm³.

The critical packing parameter of dye **1** can be calculated as:

$$P_c = \frac{v}{a_0 \times l_c} = \frac{1.05 + 0.92}{0.94 \times 2.5} = 0.84$$

Aggregation mechanism studies

Studies based on the temperature-dependent UV/Vis spectroscopic data according to isodesmic and nucleation-growth model⁸

The fraction of aggregated molecules α_{agg} at a certain concentration and temperature can be estimated according to equation (3) based on the assumption that the dye molecules aggregate fully ($\alpha_{agg} = 1$) at lowest temperature ($T = 0^\circ\text{C}$) or highest concentration ($c = 5 \times 10^{-4}$ M) and stay in monomers ($\alpha_{agg} = 0$) at highest temperature ($T = 60^\circ\text{C}$) or lowest concentration ($c = 2 \times 10^{-7}$ M) in water, ε_{mon} and ε_{agg} stands for absorption coefficients of the monomer and fully aggregated state respectively.

$$\alpha_{agg}(T) = 1 - \frac{\varepsilon(T) - \varepsilon_{agg}}{\varepsilon_{mon} - \varepsilon_{agg}} \quad (3)$$

For isodesmic model, the Gibbs free energy change of each step of adding a new monomer to the aggregate is the same. Accordingly, the fraction of aggregated species (α_{agg}) in temperature-dependent experiments can be described as equation (4):

$$\alpha_{agg} = \frac{1}{1 + \exp(-0.908\Delta H \frac{T - T_m}{RT_m})} \quad (4)$$

In this equation, ΔH is the enthalpy release, R is the ideal gas constant. T_m is the “melting temperature” of the aggregates (when $T = T_m$, $\alpha_{agg} = 0.5$).

For the nucleation-growth model, the cooperative aggregation process can be described as two steps: nucleation and elongation. In the elongation regime, the molar fraction of aggregated dye (α_{agg}) can be expressed as equation (5)

$$\alpha_{agg} = 1 - \exp[\frac{-\Delta H_e}{RT_e^2} (T - T_e)] \quad (5)$$

Here, ΔH_e is the enthalpy corresponding to elongation regime, T is the temperature, T_e is the elongation temperature, R is the ideal gas constant and α_{SAT} is a parameter introduced to ensure that $\alpha_{agg} / \alpha_{SAT}$ does not exceed unity. At the temperature above T_e (nucleation regime), the fraction of aggregated molecules in the nucleation regime can be described as equation (6):

$$\alpha_{agg} = K_a^{1/3} \exp[(2/3K_a^{-1/3} - 1) \frac{\Delta H_e}{RT_e^2} (T - T_e)] \quad (6)$$

In this equation, ΔH_e , T and T_e are the same as equation (6) and K_a is the dimensionless equilibrium constant of the activation.

The average length of the stack $\langle N_n \rangle$ at the T_e is given by Equation (7).

$$\langle N_n(T_e) \rangle = \frac{1}{K_a^{1/3}} \quad (7)$$

Studies based on concentration-dependent UV/Vis spectroscopic data according to the Goldstein-Stryer model⁹

In this model, a nucleus of size s is formed in the nucleation regime through a isodemic process with an equilibrium constant K_s while further steps of adding more molecules to the nucleus take place with equal equilibrium constant K ($K > K_s$), i.e. $K_1 = K_2 = \dots = K_s$ and $K_{s+1} = K_{s+2} = \dots = K$. The cooperativity is reflected by the parameter σ defined as $\sigma = K_s/K$. The relation between Kc_T and Kc_1 can be described as the equation (8), where c_1 is the concentration of the monomer species and c_T is the total concentration of the molecules:

$$Kc_T = \sum_{n=1}^s n\sigma^{n-1}(Kc_1)^n + \sum_{n=s+1}^{\infty} n\sigma^{s-1}(Kc_1)^n$$
$$= \frac{s(Kc_1)^s \sigma^{s-1}}{1 - Kc_1} + \frac{(Kc_1)^{s+1} \sigma^{s-1}}{(1 - Kc_1)^2} + \frac{Kc_1(s(\sigma Kc_1)^{s-1} - 1)}{\sigma Kc_1 - 1} - \frac{\sigma(Kc_1)^2((\sigma Kc_1)^{s-1} - 1)}{(\sigma Kc_1 - 1)^2} \quad (8)$$

In the meantime, α_{agg} can be calculated from equation (9):

$$\alpha_{agg} = 1 - \alpha_{mon} = 1 - \frac{Kc_1}{Kc_T} \quad (9)$$

Both α_{agg} and Kc_T can be obtained from the data of Kc_1 and the curve of α_{agg} against Kc_T can be drawn. The experiment data from three different wavelengths were collected and manually fitted into the curve for the best match, the result presented in the paper was the average value.

4. References

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