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Electronic Supplementary Information

Aggregation Enhances Luminescence and Photosensitization Properties of a Hexaiodo-BODIPY

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S1.1. General Techniques

All experiments were carried out at room temperature (25 ± 1 °C) unless otherwise mentioned. Absorption spectra were recorded on a Shimadzu UV-Vis spectrophotometer in 3 mL quartz cuvettes having a path length of 1 cm. Fluorescence spectra were recorded on an Edinburgh FS5 spectrofluorometer. NMR spectra were measured on a 500 MHz Bruker Avance II 500 NMR spectrometer. ^{1}H NMR chemical shifts are reported in parts per million (δ) calibrated using tetramethylsilane as an internal standard for samples in $CDCl_3$. ^{19}F NMR spectra were referenced by inserting a sealed capillary containing 5% trifluroacetic acid (s, δ –76.55 ppm) into the NMR tube.[1] Mass spectra were measured on a waters Q-Toff, Micromass (ESI-MS). HR-TEM images were acquired on a Jeol 2100 HR operating at 120 kV. Samples were prepared by depositing a drop of diluted nanoaggregate suspension on 300 mesh TEM grid and dried under vacuum for 2 hours. Dynamic light scattering experiment was performed using Malvern Zetasizer 2000 DLS spectrometer with 633 nm CW laser. The particles were dispersed in Milli Q water before analysis. Photosensitization experiments were carried out using a 400 W Xenon arc lamp (Oriel instruments) with a 475 nm cut-off filter (Newport Corporation). Fluorescence lifetimes were measured by time correlated single-photon counting (TCSPC) using a Deltaflex modular fluorescence life time system from HORIBA Scientific using a nano-LED pulse diode light source. The instrument response function (IRF) of the setup was 200 ps and measured using 1% ludox (colloidal silica) solution. The radiative and non-radiative decays constants were calculated as described in ref. 2. For recording luminescence from singlet oxygen, samples were excited using 377 nm pulsed laser diode. Confocal microscopy was performed a Carl ZEISS (Germany) LSM 880 confocal microscope.

S1.2. Materials

Pyrrole, boron trifluoride diethyletherate, 1,3,5-benzenetricarbonyl trichloride, *N*-iodoscuccinimide, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, 1,3-diphenylisobenzofuran (DPBF) and rhodamine 6G were purchased from Sigma-Aldrich and used as received. Phalloidin tetramethyl rhodamine B isothiocyanate (TRITC), Hoechst 33258 and 4',6-diamidino-2-phenylindole (DAPI) were purchased from Invitrogen. Paraformaldehyde, Dulbecco's modified eagle medium (DMEM), fetal bovine serum (FBS) and 1% antibiotics antimycotic solution were purchased from Himedia. Silica gel (60-120 mesh), dichloromethane (DCM), hexane and triethylamine were purchased locally. Solvents were distilled before use. Thin layer chromatography (TLC) was used to monitor the progress of the reactions using Silica gel G (Merck).

S2. Synthesis

S2.1. Synthesis of B_3.[3] 1,3,5-Benzenetricarbonyl trichloride (2.55 mmol, 660 mg) was dissolved in dry DCM (100 mL) and stirred for 10 minutes. 2,4-Dimethylpyrrole (15 mmol, 1.42 mg) was added to the solution and stirred for 12 hours under an atmosphere of nitrogen at room temperature. The reaction mixture was cooled to 0 °C, triethylamine (7 mL) added and stirred for another 5 minutes. To this ice cooled solution, $BF_3.OEt_2$ (7.5 mL) was added and reaction mixture was stirred for another 12 hours at room temperature. The reaction mixture was washed with brine solution (3 × 50 mL) and the organic layer was collected and dried over anhydrous sodium sulphate. Evaporation of the solvent yielded the crude product as a dark brown residue which was purified by column chromatography using a mixture of DCM and hexane as eluents to yield 60 mg (35%) of **B_3**. ^{1}H NMR ($CDCl_3$, 298K, 500 MHz) δ (ppm) 2.18 (s, 18H), 2.45 (s, 18H), 5.98 (s, 6H), 6.96 (s, 3H); HRMS calculated for $C_{45}H_{46}B_3F_6N_6$ (M+H), 817.3967, obtained 817.3960.

S2.2. Synthesis of I_6. To a well-stirred solution of **B_3** (0.049 mmol, 40 mg) in dry DCM (10 mL), *N*-iodosuccinimide (0.32 mmol, 73 mg) was added and the reaction was stirred for 6 hours at 25 °C. The reaction mixture was then washed with water (3 × 50 mL), and the organic layer was collected and dried over anhydrous sodium sulphate. Evaporation of the solvent yielded the crude product as a red residue which was purified by column chromatography using a mixture of DCM and hexane as eluents to yield 68 mg (88%) of **I_6**. ^{1}H NMR ($CDCl_3$, 298K, 500 MHz) δ (ppm) 2.15 (s, 18H), 2.53 (s, 18H), 7.03 (s, 3H); ^{13}C NMR ($CDCl_3$, 298K, 125 MHz) δ (ppm) 11.26, 14.65, 29.69, 118.98, 120.05, 133.4, 141.17, 156.72; HRMS calculated for $C_{45}H_{40}B_3F_6N_6I_6$ (M+H) 1572.7766, obtained 1572.7761.

S2.3. Synthesis of I_6-NA. A solution of **I_6** (0.3 mg/mL) in 4 mL acetone was added drop wise to distilled water (10 mL) and stirred vigorously for 12 hours. Acetone was then evaporated off in a rotary evaporator and the reaction mixture was centrifuged at 6000 rpm for 15 minutes. The residue was collected and redispersed in water. The size and morphology of the nanoaggregates were characterised by dynamic light scattering (DLS) and high-resolution transmission electron microscopy (HR-TEM).

S3. Investigation of singlet oxygen generation

Stock solutions of **I_6** (4.5 μM) in acetonitrile and **I_6-NA** (10 μg/mL) and the reference standard methylene blue (MB) were prepared in water whereas a stock solution of 1,3-diphenylisobenzofuran (DPBF) was prepared in ethanol. 2.5 mL of an aqueous solution of the photosensitizer was taken in a

cuvette to which an aliquot of DPBF stock solution in ethanol was added. The absorbance of the solution was recorded, and the solution was then irradiated using a Xenon lamp with a 475 nm band filter. To monitor the progress of the reaction, the decrease in absorbance of DPBF at 420 nm was recorded at regular intervals.

S4. Determination of singlet oxygen quantum yield[4]

Singlet oxygen quantum yield was determined by following a reported procedure. The quantum yield was calculated with reference to MB in water which is reported to have a quantum yield of 0.52. Singlet oxygen quantum yield was calculated according to the equation:

$$\Phi_{\Delta}(\text{sample}) = \Phi_{\Delta}(\text{ref}) \times \frac{\text{m(sample)} \times \text{F(ref)}}{\text{m(ref)} \times \text{F(sample)}}$$

where m is the slope of difference in change in absorbance of DPBF (at 420 nm) with the irradiation time, and F is the absorption correction factor, which is given by $F = 1 - 10^{-OD}$.

S5. Determination of fluorescence quantum yield

The fluorescence quantum yield for **I_6** and **I_6-NAs** were calculated using the following formula.

$$\Phi_f(\text{sample}) = \Phi_f(\text{ref}) \times \frac{\text{I(sample)} \times \text{OD(ref)} \times n^2(\text{sample})}{\text{I(ref)} \times \text{OD(sample)} \times n^2(\text{ref})}$$

where I is the integrated intensity, OD is the optical density at the excitation wavelength, n is the refractive index of the solvent used. Rhodamine 6G ($\phi_f = 0.95$) was used as the reference standard.[5]

S6. Molecular Simulations

B3LYP hybrid functional was used as implemented in ORCA code to optimize the structures of both the molecules. Def2-TZVPP basis set was used with an auxiliary basis set Def2/J. RIJCOSX approximation was used to accelerate the SCF cycles.

S7. Cell culture

A rat origin glioblastoma cell line, C6 cells obtained from National Centre for Cell Sciences, Pune, India. The cells were cultured and maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% antibiotics antimycotic solution at 37 °C in a humidified incubator containing 5% CO_2.

S8. *In vitro* cytotoxicity and cell viability study

The in vitro cytotoxicity of different concentrations of **I_6-NA** was assessed against C6 cells using the MTT method. Specifically, cells were seeded in a 96-well flat culture plate at a density of 2.5×10^4 cells per well and incubated at 37 °C under 5% CO_2 for 24 hours. Next day, different concentrations of **I_6-NA** was prepared in media and added to each group. After 6 hours of incubation, the cells were exposed to white light for 10 minutes followed by incubation for 48 hours in the CO_2 humidified incubator. The cells were washed with pre-warmed PBS buffer thrice to remove traces of sample. 20 µL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution (5 mg/mL in PBS buffer) diluted with 180 µL media was added to the wells and incubated for 4 hours. After 4 hours, the plates were centrifuged at 1500 rpm for 5 minutes at room temperature. 150 µL DMSO was added to each well to dissolve the formazan crystals and all the wells were aspirated well before taking absorbance. The absorbance of the suspension was measured at 570 nm on an ELISA reader.

Cell viability was calculated by means of the following formula:

$$\text{Cell viability (\%)} = \frac{OD_{570\,(sample)} - OD_{570\,(blank)}}{OD_{570\,(control)} - OD_{570\,(blank)}} \times 100\ \%$$

S9. Cellular uptake studies

The intracellular localization of **I_6-NA** was investigated by confocal microscopy. C6 cells were incubated with **I_6-NA** (10 µg/mL in DMEM) in the dark for 4 hours. Cells were fixed with 4% paraformaldehyde followed by staining with Hoechst 33342 (200 µg/mL) for 10 minutes. The cytoskeleton was stained using Phalloidin tetramethyl rhodamine B isothiocyanate (TRITC) for 20 minutes.

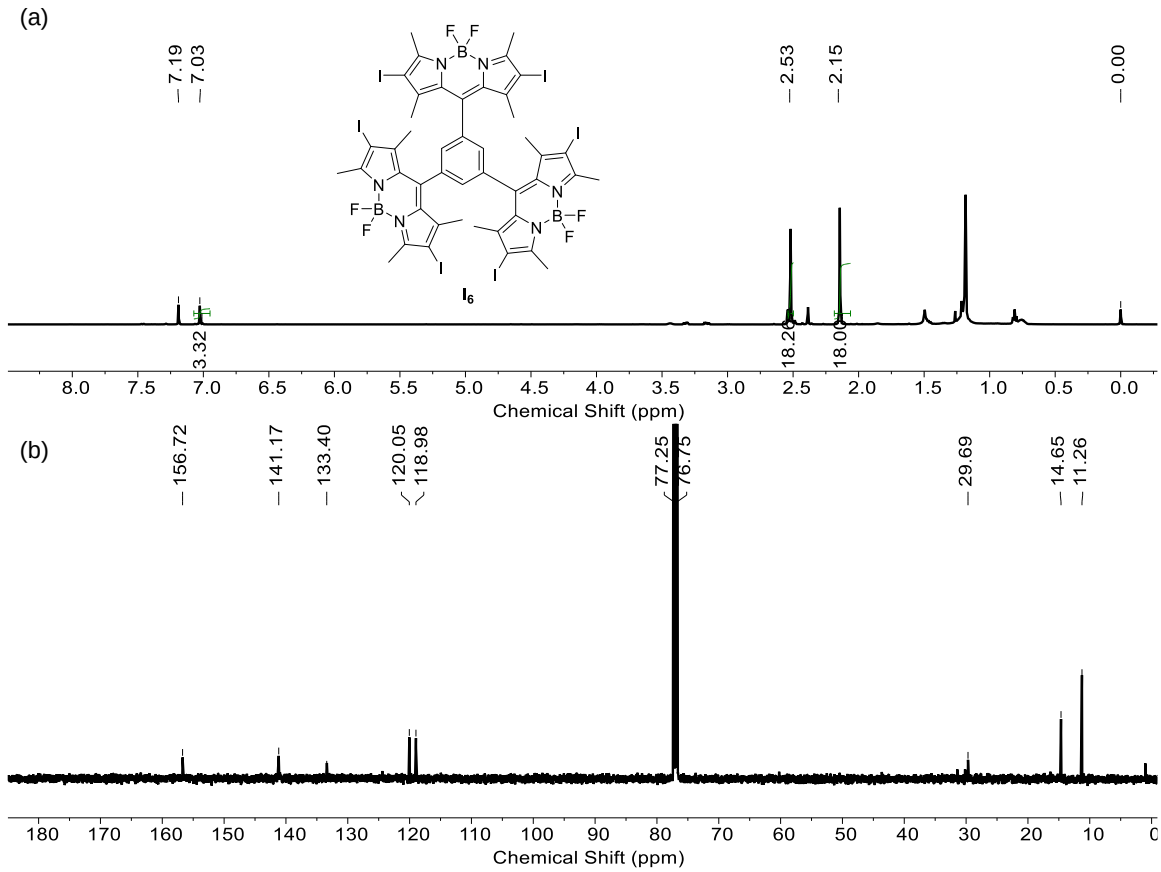

Fig. S1. (a) ^{1}H and (b) ^{13}C NMR spectra of **I_6** in $CDCl_3$ at 298 K.

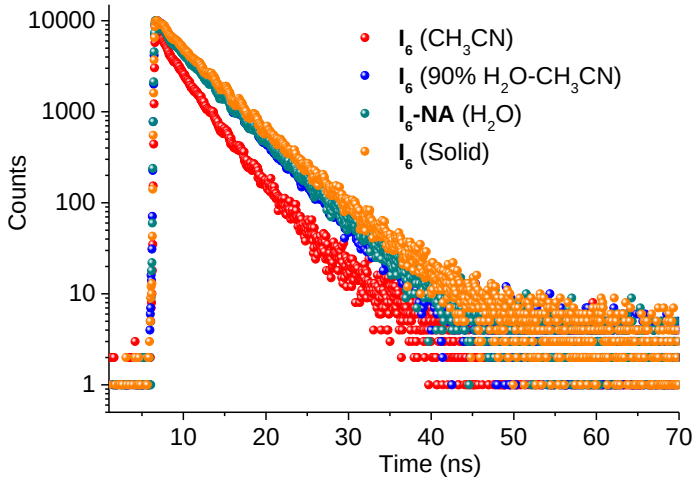

Fig. S2. Fluorescence decay profiles of **I_6** (4.5 μM) in acetonitrile, solid state and that of **I_6-NA** (10 μg/mL) in water. Excitation wavelength 402 nm.

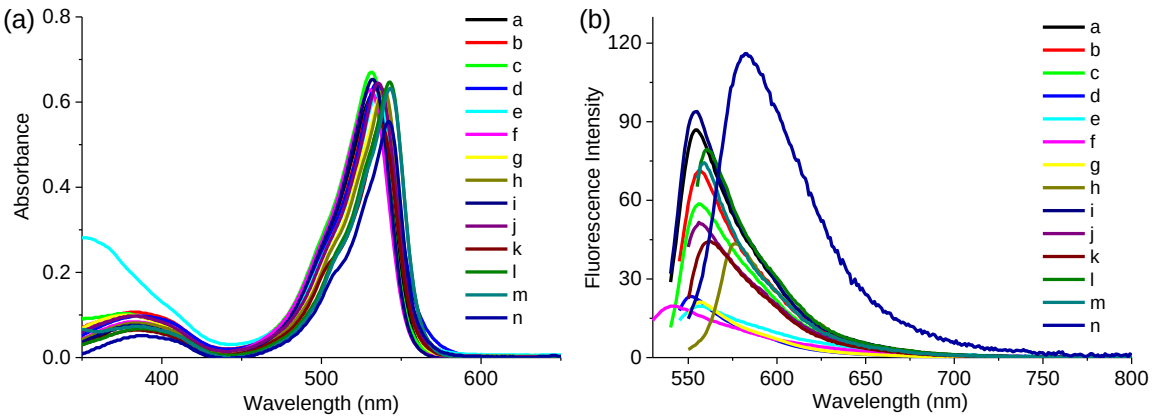

Fig. S3. (a) Absorption and (b) emission spectra of $\mathbf{I_6}$ (4.5 μM) in (a) methanol, (b) isopropanol, (c) acetonitrile, (d) dimethyl sulfoxide, (e) dimethyl formamide, (f) acetone, (g) dichloroethane, (h) chloroform, (i) ethyl acetate, (j) tetrahydrofuran, (k) 1,4-dioxane, (l) benzene, (m) toluene, and (n) cyclohexane. Emission spectra were recorded by exciting the sample at the respective absorption maximum in each solvent.

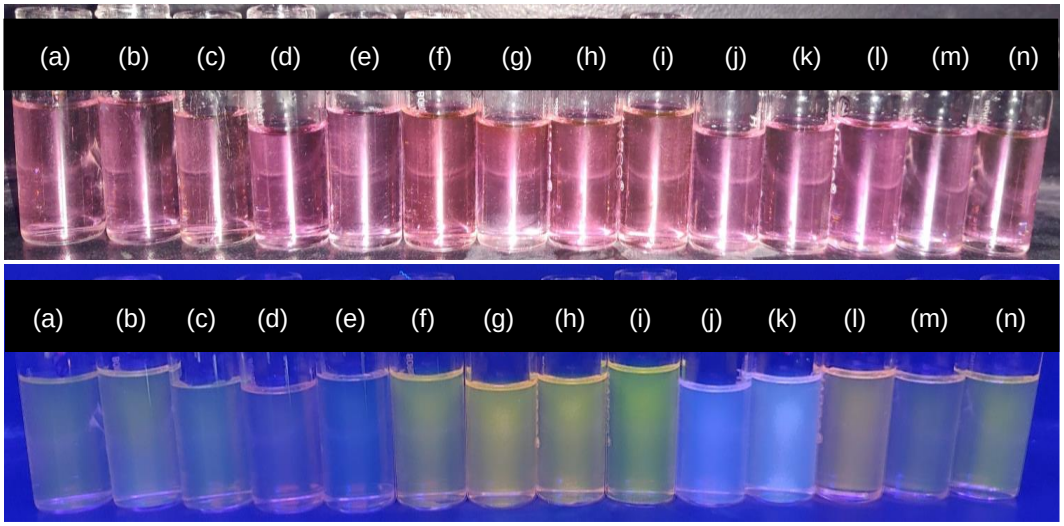

Fig. S4. Photographs of $\mathbf{I_6}$ under ambient (top panel) and UV light (bottom panel) in (a) methanol, (b) isopropanol, (c) acetonitrile, (d) dimethyl sulfoxide, (e) dimethyl formamide, (f) acetone, (g) dichloroethane, (h) chloroform, (i) ethyl acetate, (j) tetrahydrofuran, (k) 1,4-dioxane, (l) benzene, (m) toluene, and (n) cyclohexane.

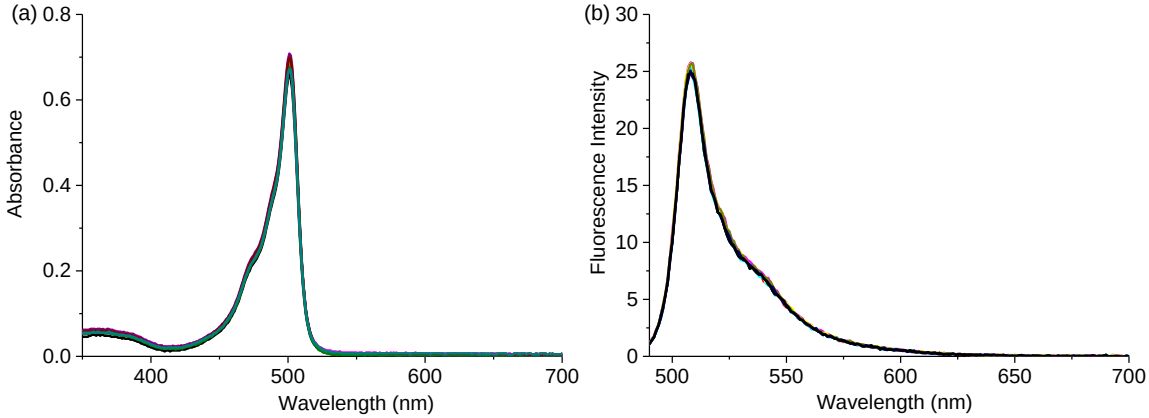

Fig. S5. (a) Absorption and (b) emission spectra of **B_3** (6 μM) in acetonitrile-water mixtures. Excitation wavelength, 490 nm.

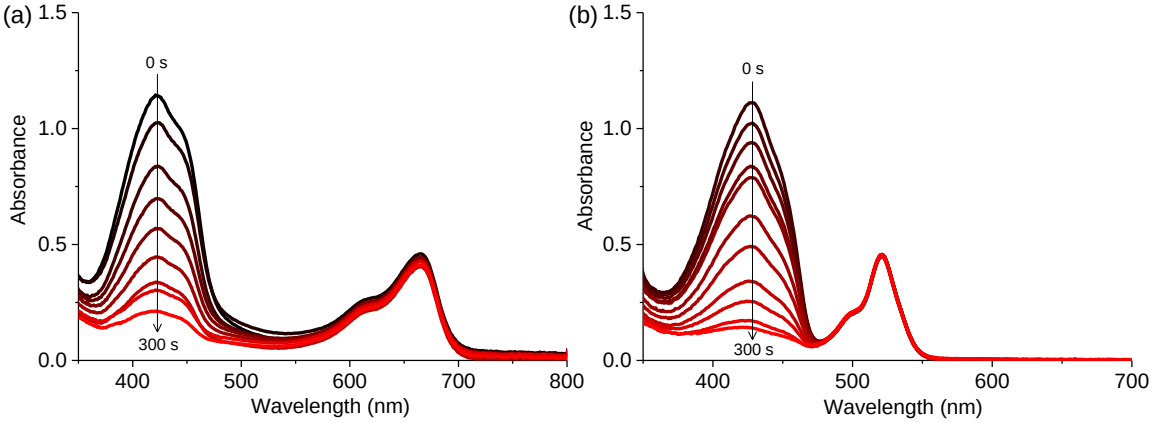

Fig. S6. Changes in the absorption spectrum of a mixture of 1,3-diphenylisobenzofuran (DPBF, 90 μM) and (a) methylene blue (12 μM) in water and (b) **I_6** (4.5 μM) in acetonitrile.

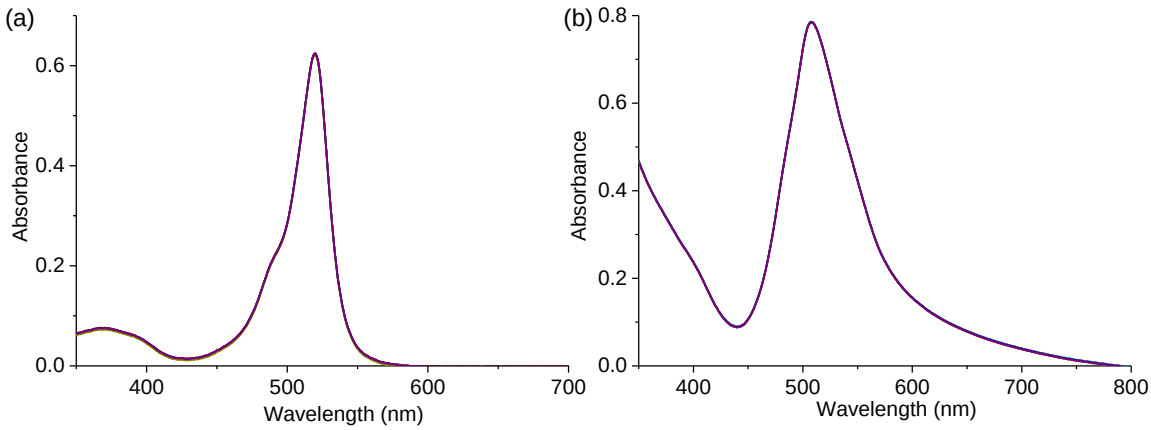

Fig. S7. Changes in the absorption spectrum of (A) **I_6** (4.5 μM) in acetonitrile and (B) **I_6-NA** (10 μg/mL) in water after irradiation for 10 minutes with a Xenon lamp using a 475 nm band filter.

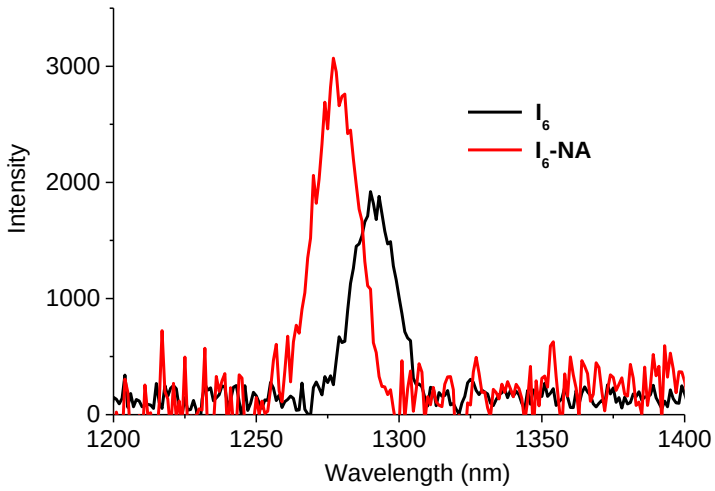

Fig. S8. Luminescence spectra of **I_6** (4.5 μM) in acetonitrile and **I_6-NA** (10 μg/mL) in water. Excitation wavelength 377 nm.

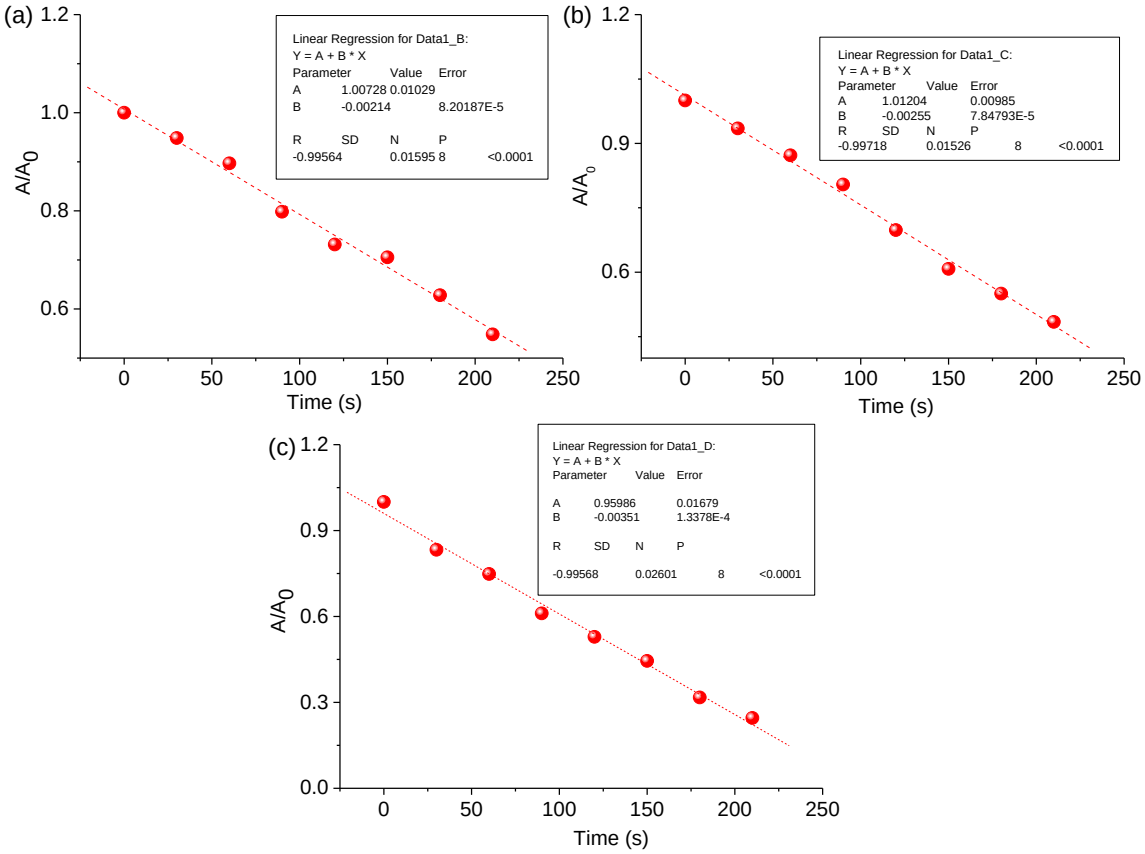

Fig. S9. Curve fitting data for the decrease in absorbance of DPBF at 420 nm in the presence of (a) methylene blue in water, (b) **I_6** in acetonitrile and (c) **I_6-NA** in water.

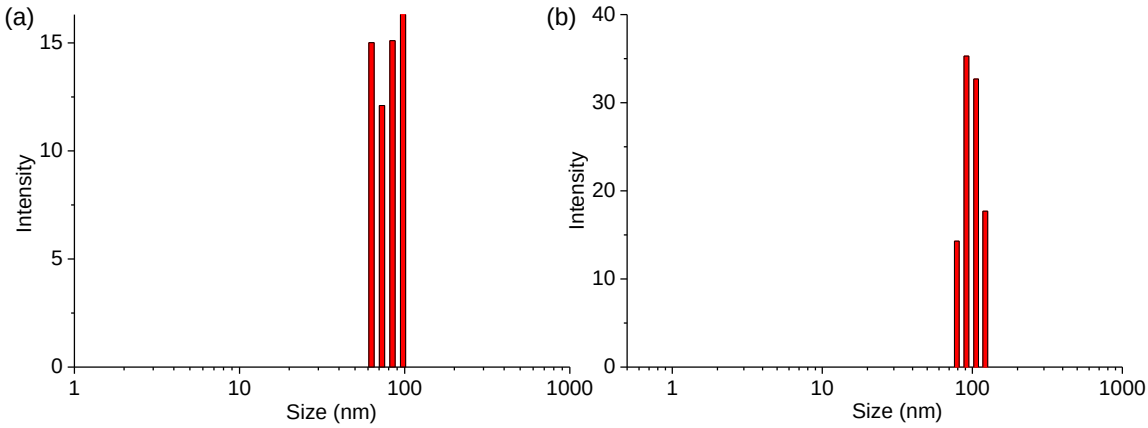

Fig. S10. Dynamic light scattering profile of (a) **B_3-NA** and (b) **I_6-NA** in water at 25 °C.

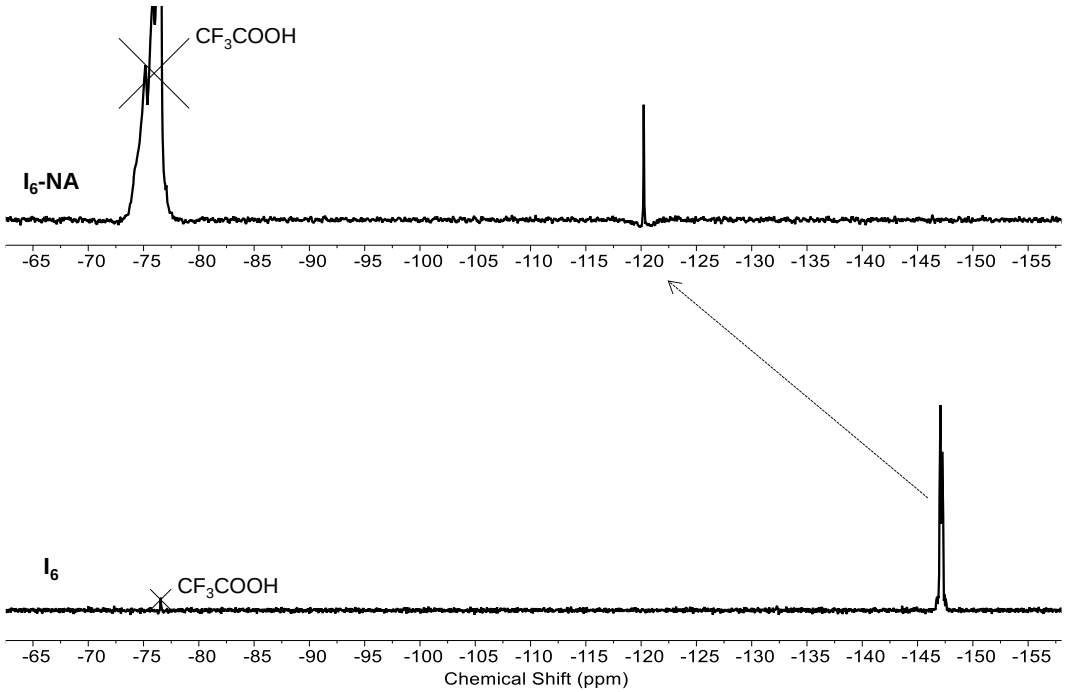

Fig. S11. ^{19}F NMR spectrum of $\mathbf{I_6}$ in $CDCl_3$ and $\mathbf{I_6}$**-NA** in D_2O at 298 K.

References

(1) Jiang, Z.-X.; Feng, Y.; Yu, Y. B. Fluorinated Paramagnetic Chelates as Potential Multi-Chromic ^{19}F Tracer Agents. *Chem. Commun.* 2011, **47**, 7233–7235.

(2) Ghosh, S.; Mandal, S.; Banerjee, C.; Rao, V. G.; Sarkar, N. Photophysics of 3,3'-Diethyloxadicarbocyanine Iodide (DODCI) in Ionic Liquid Micelle and Binary Mixtures of Ionic Liquids: Effect of Confinement and Viscosity on Photoisomerization Rate. *J. Phys. Chem. B* 2012, **116**, 9482–9491.

(3) Liu, Y.; Song, N.; Chen, L.; Xie, Z. Triple-BODIPY Organic Nanoparticles with Particular Fluorescence Emission. *Dyes Pigm.* 2017, **147**, 241–245.

(4) Adarsh, N.; Shanmugasundaram, M.; Avirah, R. R.; Ramaiah, D. Aza-BODIPY Derivatives: Enhanced Quantum Yields of Triplet Excited States and the Generation of Singlet Oxygen and Their Role as Facile Sustainable Photooxygenation Catalysts. *Chem. Eur. J.* 2012, **18**, 12655–12662.

(5) Magde, D.; Rojas, G. E.; Seybold, P. G. Solvent Dependence of the Fluorescence Lifetimes of Xanthene Dyes. *Photochem. Photobiol.* 1999, **70**, 737–744.