Electronic Supplementary Information (ESI)

Fluorescent Poly(β-amino ester)s Containing Aza-BODIPYs as Theranostic Agents for Bioimaging and Photodynamic Therapy

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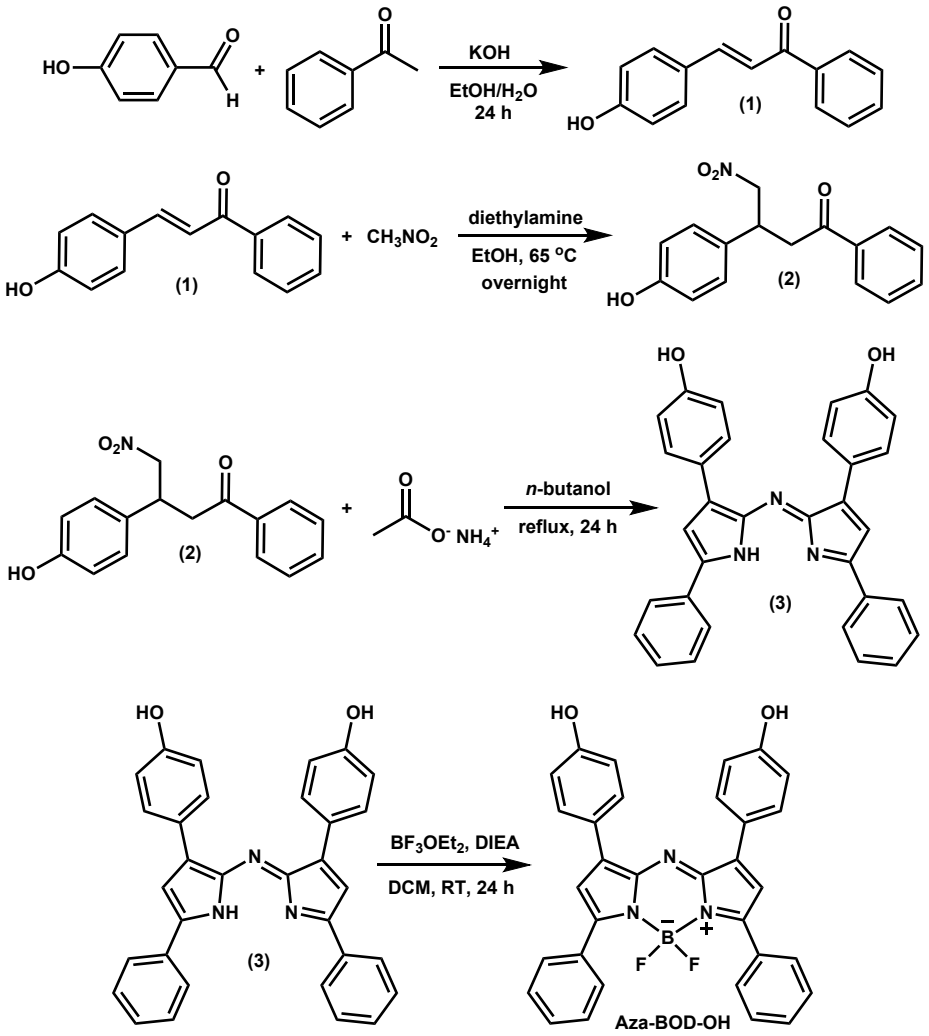
Synthesis of Hydroxyl Derivative of Aza-BODIPY (Aza-BOD-OH)

Aza-BOD-OH was synthesized in four steps (Scheme S1).[1] The first step involves the preparation of the precursor 3-(4-hydroxyphenyl)-1-phenylprop-2-en-1-one **(1)**. 12.5 g of potassium hydroxide (KOH) (0.223 mol, 5.7 eq.) was placed in a 250 mL beaker and dissolved in 100 mL of ethanol and placed in an ice bath. 4.60 mL of acetophenone (0.039 mol, 1 eq.) was added into the solution and was left to stir for 30 minutes. 5.0 g of 4-hydroxybenzaldehyde (0.041 mol, 1.05 eq.), was added to the reaction mixture as portions and the reaction mixture was removed from the ice bath and stirred for 24 hours at room temperature. Then, the reaction mixture was cooled in an ice bath again, and pH of the reaction mixture was adjusted to 2-3 with 4 M HCl (aq). The precipitate was washed sequentially with 20 mL of cold water and 20 mL of cold ethanol, filtered and left to dry under high vacuum. The desired product, 3-(4-hydroxyphenyl)-1-phenylprop-2-en-1-one **(1)**, was obtained as a yellow solid (Yield: 95%).

^{1}H-NMR (500 MHz, DMSO-d_6) δ 10.09 (s, 1H), 8.11 (d, 2H), 7.74 (d, 2H), 7.70 (d, 2H), 7.64 (t, 1H), 7.55 (t, 2H), 6.83 (d, 2H). FT-IR: 3165 cm^{-1} (–OH), 1646 cm^{-1} (–C=O–), 1600 cm^{-1} (–C=C–.)

In the second step, 3-(4-hydroxyphenyl)-4-nitro-1-phenyl-butan-1-one **(2)** was prepared. For its synthesis, 3.5 mL of diethylamine (DEA) (0.034 mol, 3.1 eq.) was added to the solution of of 3-(4-ydroxyphenyl)-1-phenylprop-2-en-1-one (**1**) (2.5 g, 0.011 mol, 1.0 eq.) in 50 mL ethanol. After stirring for 1 hour, 3.60 mL of nitromethane (0.067 mol, 6.1 eq.) was added to the reaction mixture and heated at reflux overnight. The reaction mixture was then cooled to room temperature and 4 M HCl (aq) was added to adjust the pH to 2-3. Afterwards, the product was extracted with ethyl acetate/water (50:30); and the organic phases were collected and dried over sodium sulfate (Na_2SO_4). The solvent was removed by rotary evaporation and resulting brown colored product, 3-(4-hydroxyphenyl)-4-nitro-1-phenyl-butan-1-one **(2),** was left to dry at room conditions (Yield: 90%). The obtained product (**2**) was used in the next step without further purification.



Scheme S1. Synthesis of Aza-BOD-OH.

For the preparation of aza-dipyrromethene derivative **(3)**, 2.65 g 3-(4-hydroxyphenyl)-4-nitro-1-phenyl-butan-1-one (2) (9.29×10^{-3} mol) was dissolved in 10 mL of *n*-butanol. 55 g of ammonium acetate (0.714 mol) was added, and the reaction mixture was stirred for 24 hours at reflux. After this period of time, the reaction mixture was cooled to room temperature and the product was precipitated from the reaction mixture. The solid was filtered and washed first with 30 mL of cold water and then with 30 mL of cold ethanol and with 5 mL of cold ether. The desired aza-dipyrromethene derivative was dried at room conditions (Yield: 65%).

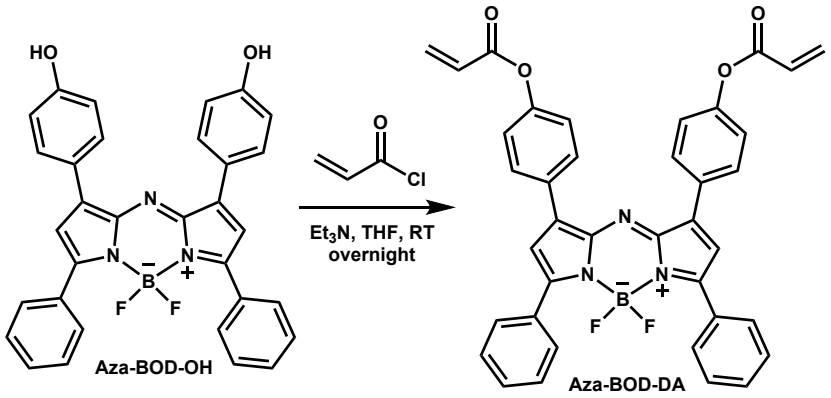
^{1}H-NMR (500 MHz, DMSO-d_6) δ 8.00 (dd, J = 51.1, 6.8 Hz, 8H), 7.67 – 7.43 (m, 8H), 6.87 (d, J = 7.4 Hz, 4H). FT-IR 3309 cm^{-1} (–OH), 1589, 1536 and 1493 cm^{-1} (–C=C– and –C=N–).

In the last step, the target aza-BODIPY derivative, aza-BOD-OH, was prepared. 0.5 g of aza-dipyrromethene derivative (**3**) (1.038×10^{-3} mol, 1 eq.) was dissolved in 40 mL of dichloromethane (DCM) under nitrogen atmosphere. Then, 4.8 mL of *N,N*-

diisopropylethylamine (DIEA) (0.028 mol, 26 eq.) was added slowly to the reaction mixture. After cooling the reaction mixture in an ice bath, 4.8 mL of boron trifluoride diethyl etherate ($BF_3.OEt_2$) (0.038 mol) was added dropwise. After 30 minutes of stirring, the reaction mixture was removed from the ice bath and allowed to stir for 24 hours at room temperature. The progression of the reaction was followed by thin layer chromatography (TLC). After the reaction was completed, 20 mL of ethanol was slowly added into the reaction mixture, and all organic solvents were removed on a rotary evaporator. The crude product was extracted with ethyl acetate (40 mL) and water (30 mL), and the organic layers were collected and dried over $MgSO_4$ and filtered. The solvent was removed under low pressure and the crude product was dried under high vacuum. The target **aza-BOD-OH** was obtained as a blue colored solid after a column chromatography using hexane/ethyl acetate (1:1 by volume) as an eluent (Yield: 75%).

^{1}H-NMR (500 MHz, DMSO-d_6) δ 10.15 (s, 2H, –O*H*), 8.07 (d, 4H), 8.05 (m, 4H), 7.54 (m, 6H), 7.36 (s, 2H), 6.92 (d, *J* = 8.7 Hz, 4H). FT-IR: 3501 and 3391 cm^{-1} (–OH), 3062 cm^{-1} (–CH=), 2921-2851 cm^{-1} (–C-H), 1603 cm^{-1} (–C=C– and –C=N–), 1476 and 1452 cm^{-1} (–B-N, –C=C– or –C=N–), 1087 cm^{-1} (–B-F).

Synthesis of Aza-BODIPY Diacrylate (Aza-BOD-DA)



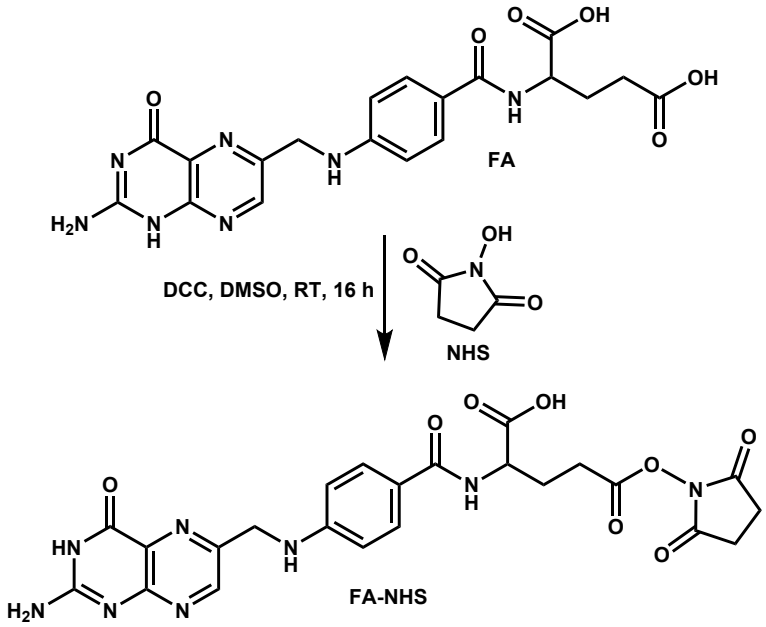
Scheme S2. Synthesis of Aza-BOD-DA.

Activation of Folic Acid (FA)

Folic acid (FA) was activated with *N*-hydroxysuccinimide (NHS) through a procedure by Butzbach *et al*. reporting the conjugation of FA to amino-end functionalized PBAEs.[2] Briefly,

0.20 g of folic acid (4.53×10^{-4} mol, 1 eq.) was dissolved in dimethyl sulfoxide (DMSO) (3 mL) under nitrogen atmosphere. Then, 0.11 g of NHS (9.56×10^{-4} mol, 2.1 eq.) and 0.20 g of *N,N'*-dicyclohexylcarbodiimide (DCC) (9.69×10^{-4} mol, 2.1 eq.) were added to the reaction mixture in the dark. After 16 hours of stirring at room temperature, the reaction mixture was filtered and the filtrate was precipitated dropwise into 50 mL of ethyl acetate. The resulting solid was dissolved in DMSO and further precipitated in ethyl acetate (×4) to yield succinimdyl ester of folic acid (FA-NHS) as an orange-yellow colored solid (50 mg, yield 20.5%) (Scheme S3).

^{1}H-NMR (500 MHz, DMSO-d_6) δ 8.64 (s, 1H), 8.12 (d, 1H), 7.69-7.61 (m, 2H), 6.95 (m, 1H), 6.64 (m, 2H), 4.48 (br, 2H), 4.33 (m, 1H), 2.80 (br, 4H), 2.31 (m, 2H), 2.08-1.88 (m, 2H). FT-IR: 3405 cm^{-1} (–OH), 2993 and 2907 cm^{-1} (–C-H), 1682 cm^{-1} (–C(=O)–).



Scheme S3. Activation of folic acid with NHS.

Spectroscopic Characterizations

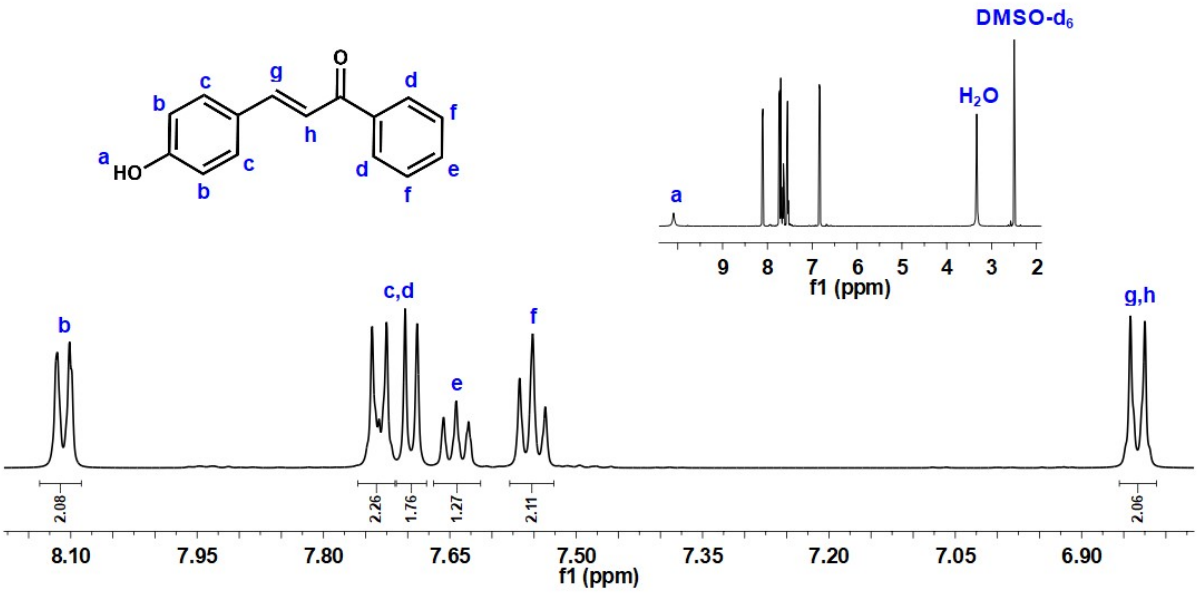


Figure S1. ^{1}H-NMR spectrum of 3-(4-hydroxyphenyl)-1-phenylprop-2-en-1-one **(1)** (DMSO-d_6).

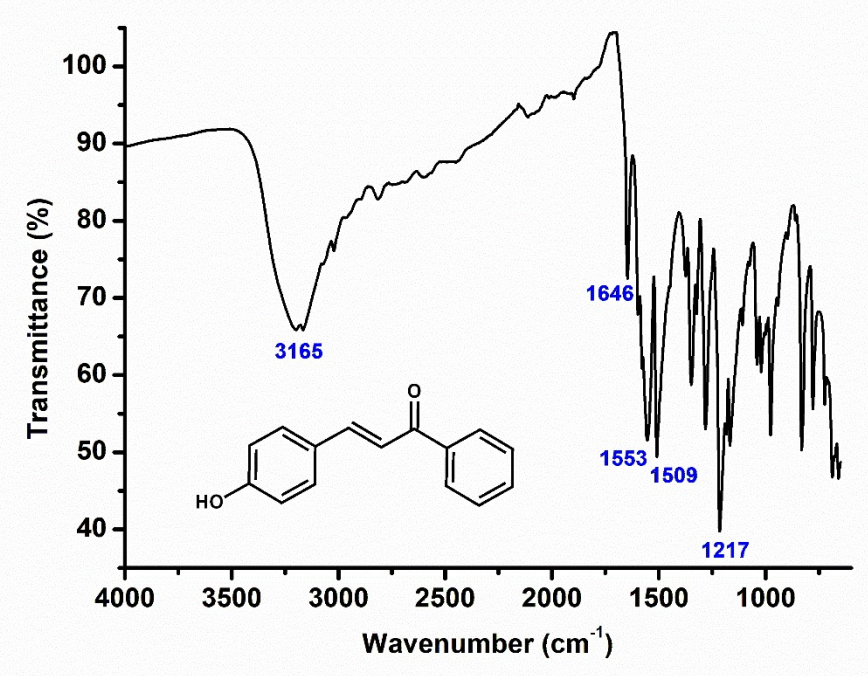


Figure S2. FT-IR spectrum of 3-(4-hydroxyphenyl)-1-phenylprop-2-en-1-one **(1)**.

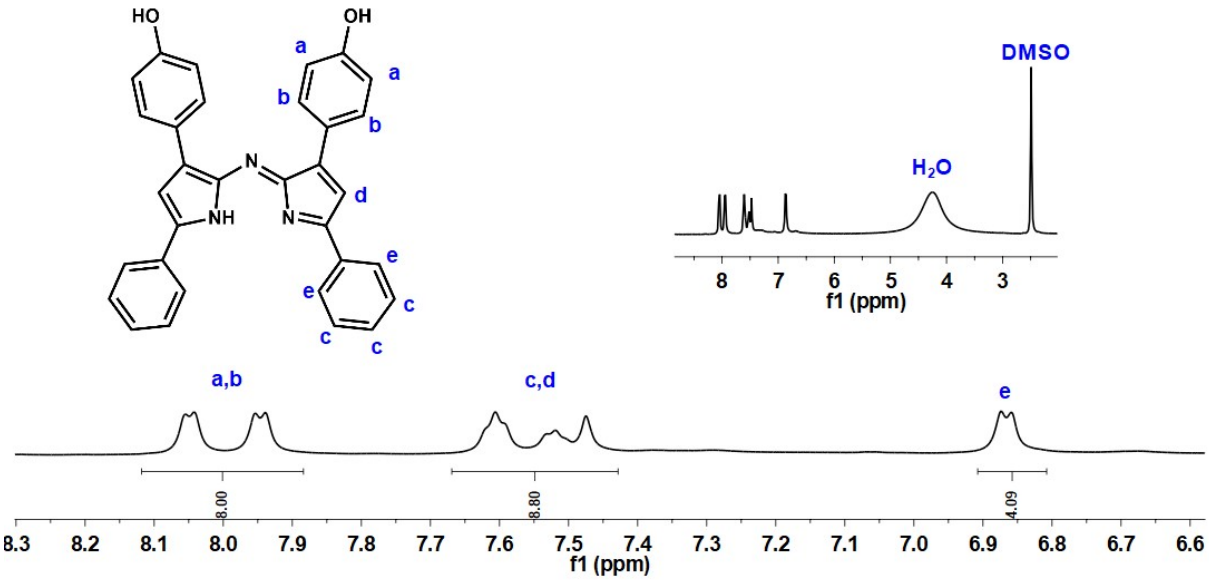


Figure S3. ^{1}H-NMR spectrum of aza-dipyrromethene derivative **(3)** (DMSO-d_6).

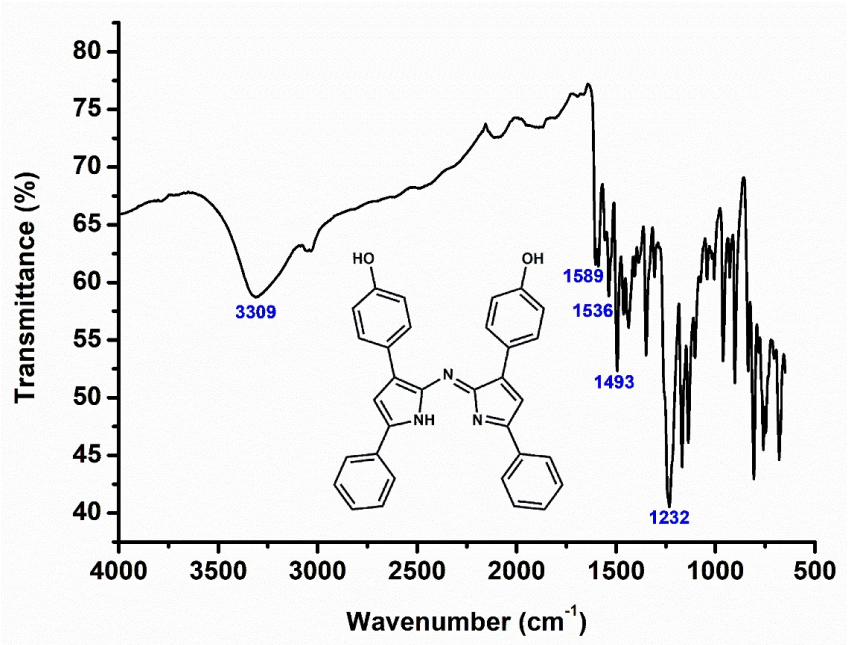


Figure S4. FT-IR spectrum of aza-dipyrromethene derivative **(3)**.

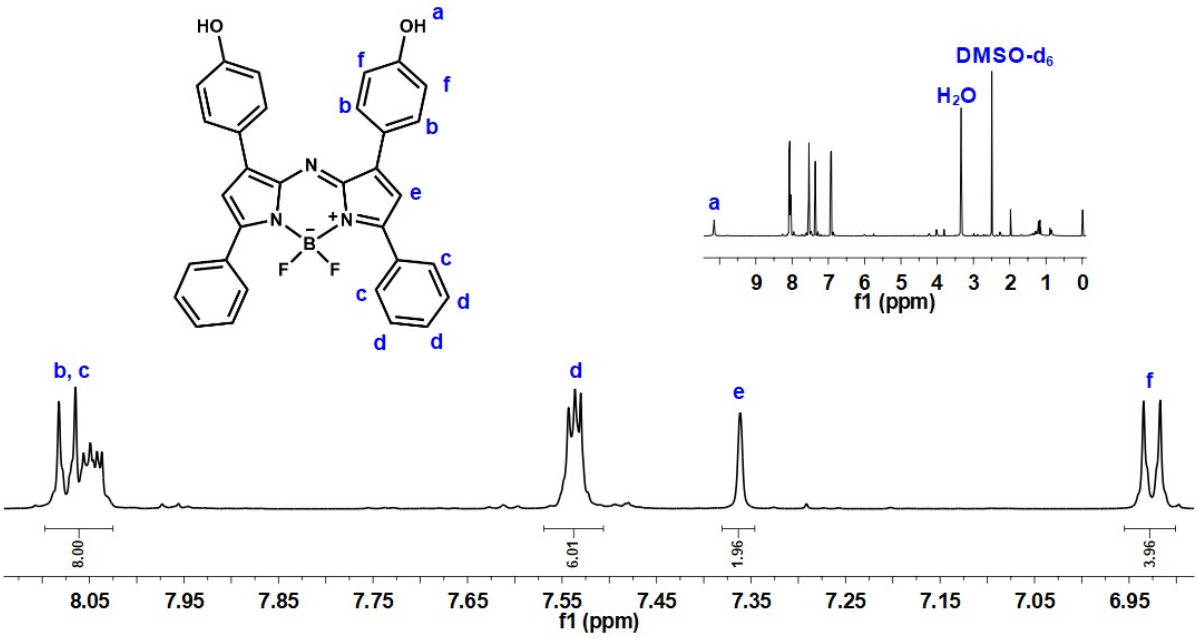


Figure S5. ^{1}H-NMR spectrum of aza-BOD-OH (DMSO-d_6).

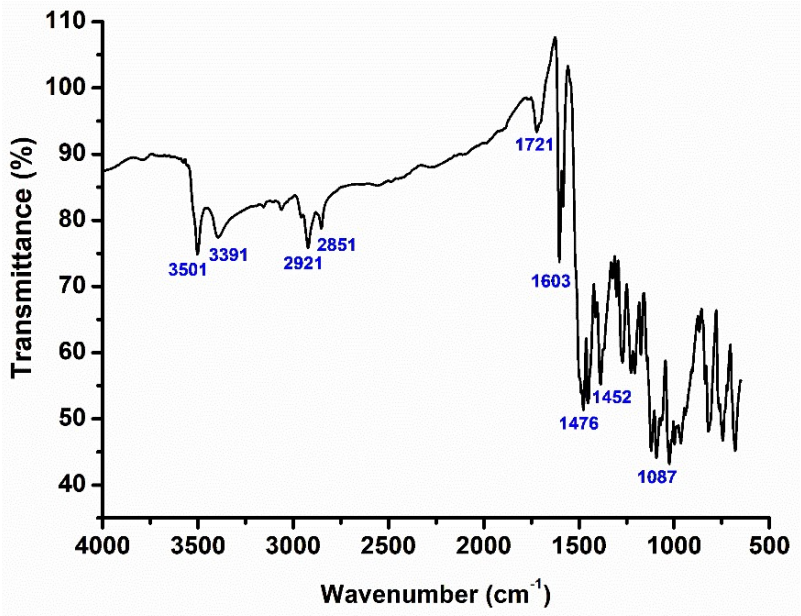


Figure S6. FT-IR spectrum of aza-BOD-OH.

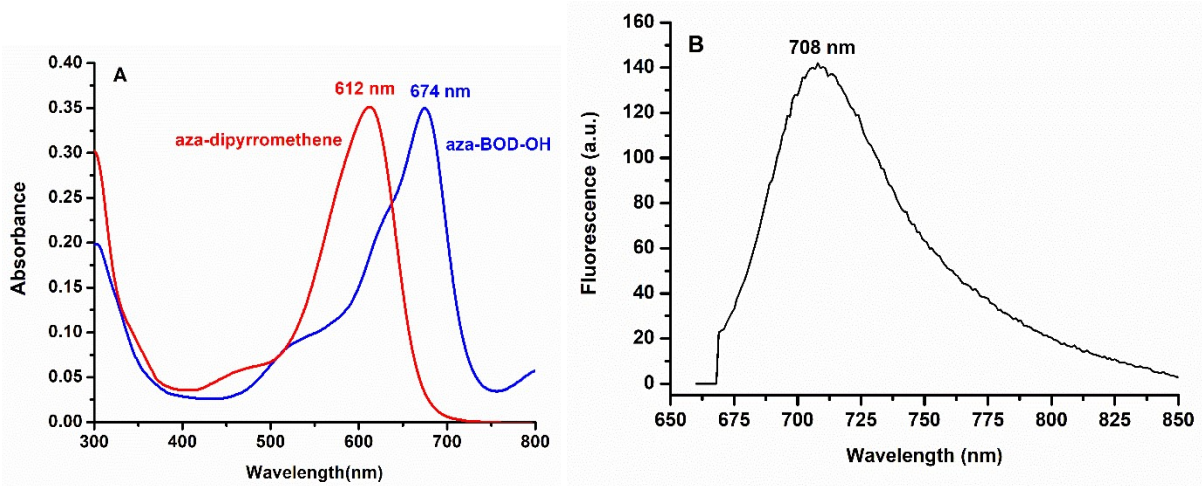


Figure S7. UV-Vis spectra of Aza-BOD-OH (1×10^{-5} mg/mL in DMF) and aza-dipyrromethene derivative **(3)** (**A**); and fluorescence spectrum of aza-BOD-OH in THF (4×10^{-3} mg/mL; $\lambda_{excitation}$: 660 nm) (**B**).

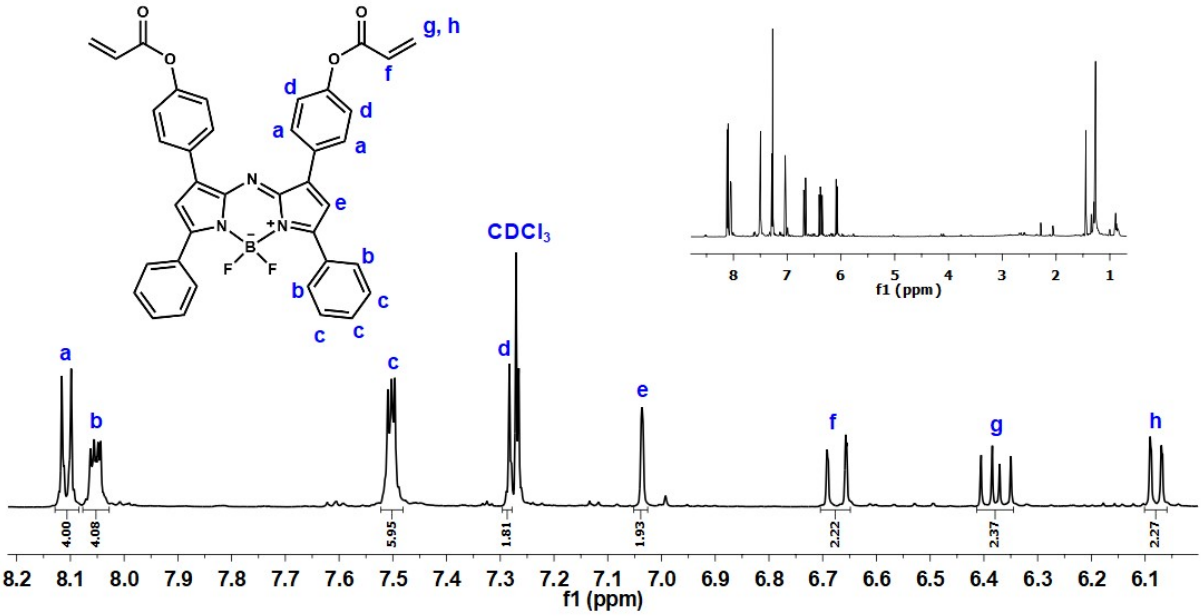


Figure S8. ^{1}H-NMR spectrum of aza-BOD-DA ($CDCl_3$).

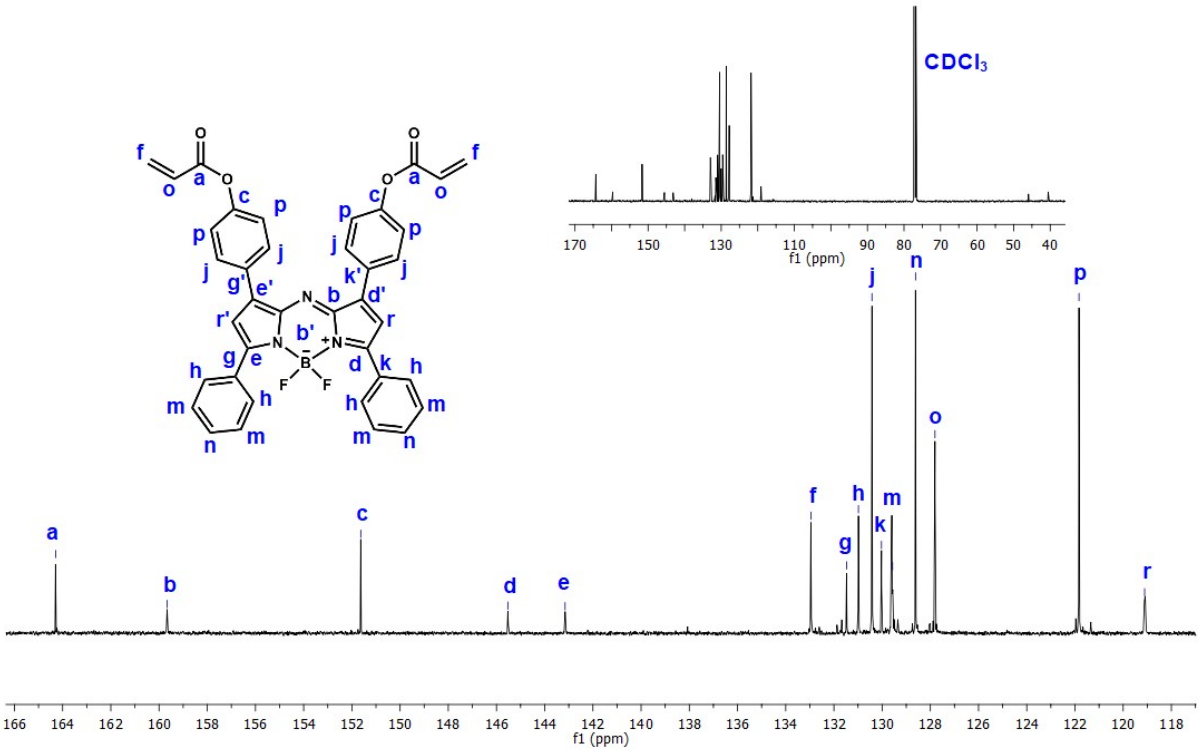


Figure S9. ^{13}C-NMR spectrum of aza-BOD-DA ($CDCl_3$).

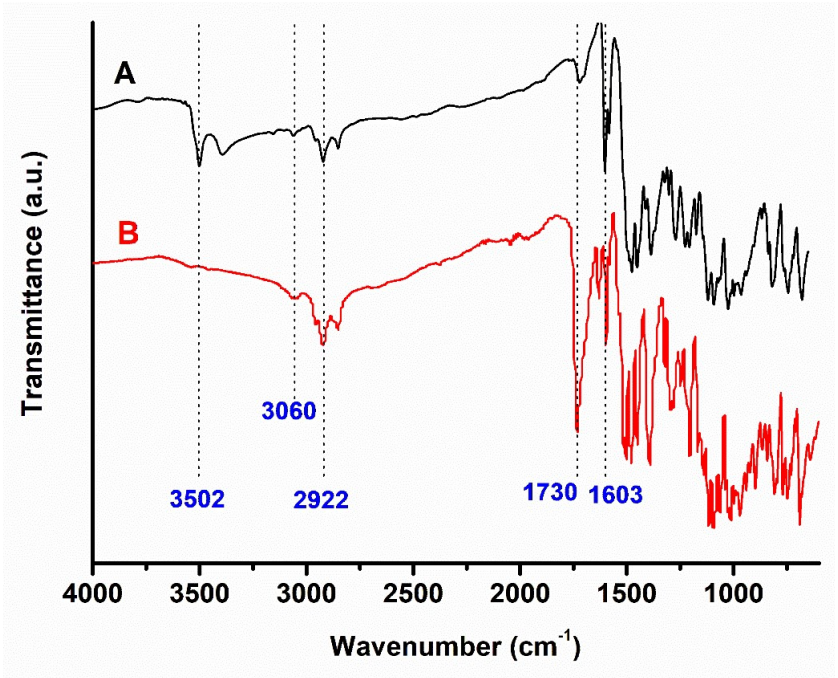


Figure S10. FT-IR spectra of aza-BOD-OH (A) and aza-BOD-DA (B).

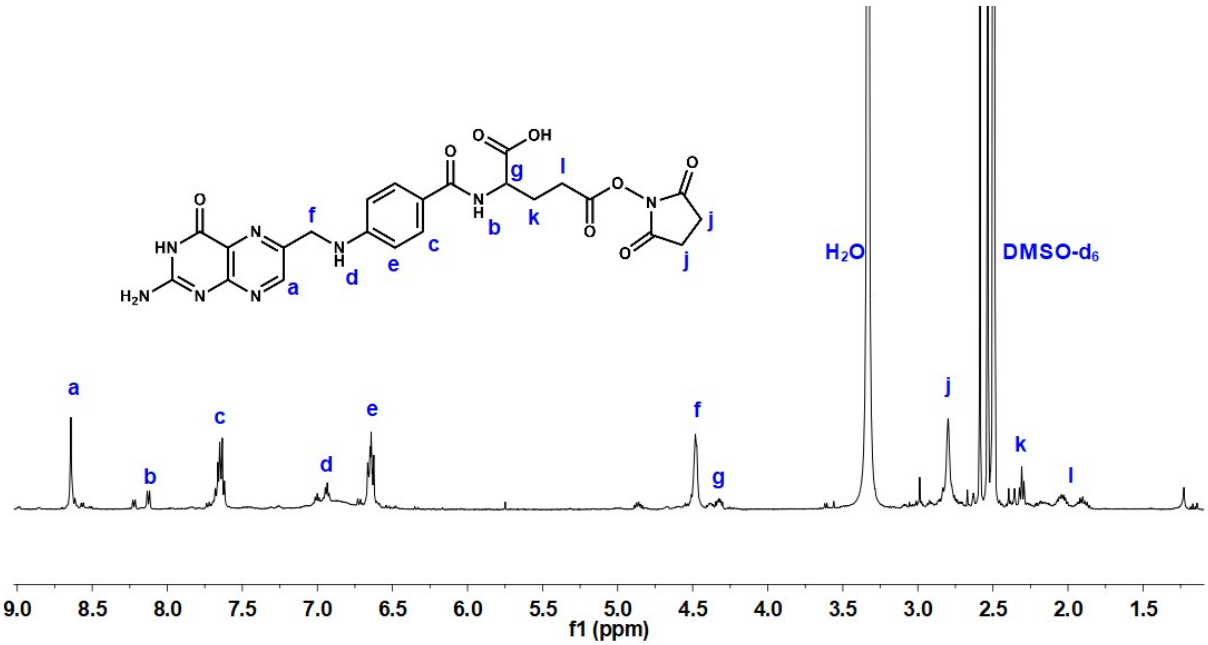


Figure S11. ^{1}H-NMR spectrum of FA-NHS (DMSO-d_6).

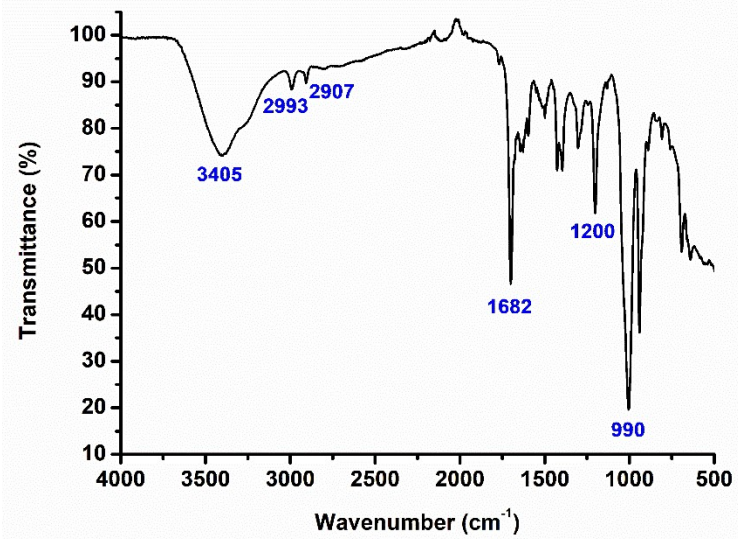


Figure S12. FT-IR spectrum of FA-NHS.

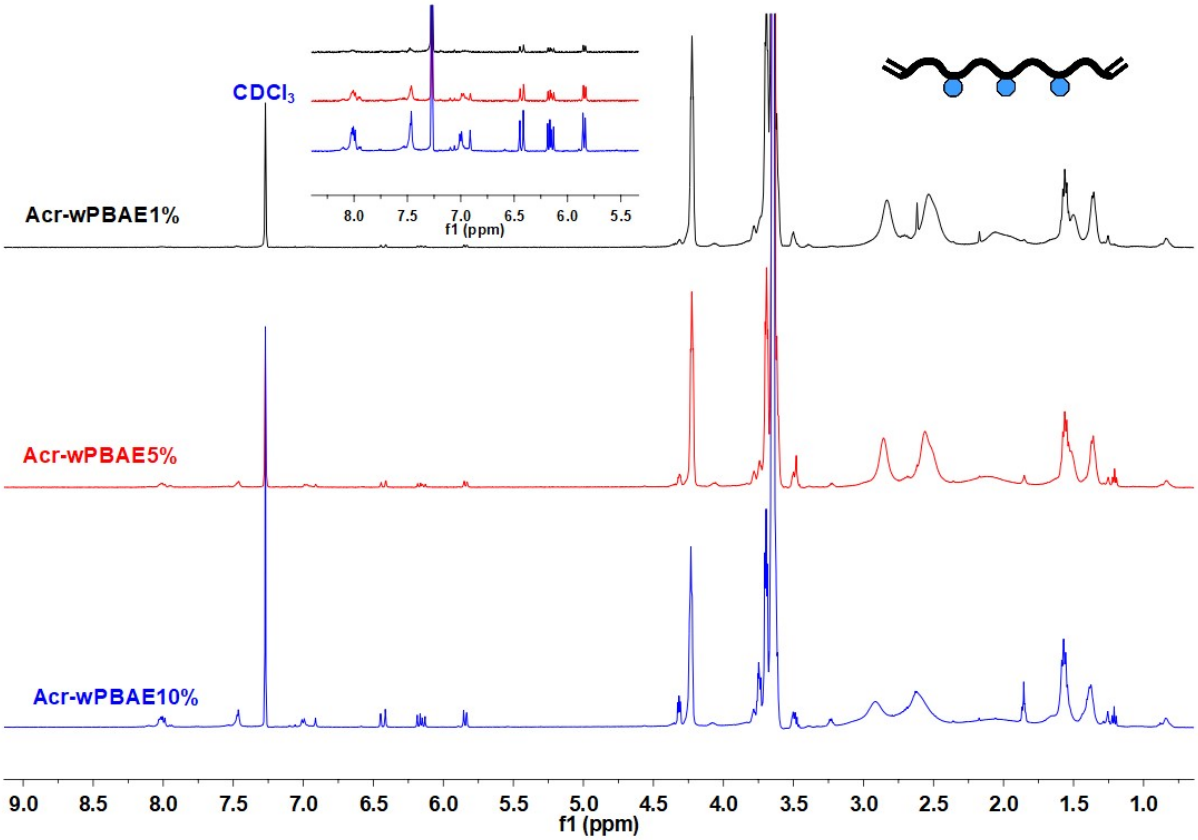


Figure S13. ^{1}H-NMR spectra of acrylate end-functional PBAE with different aza-BODIPY contents ($CDCl_3$).

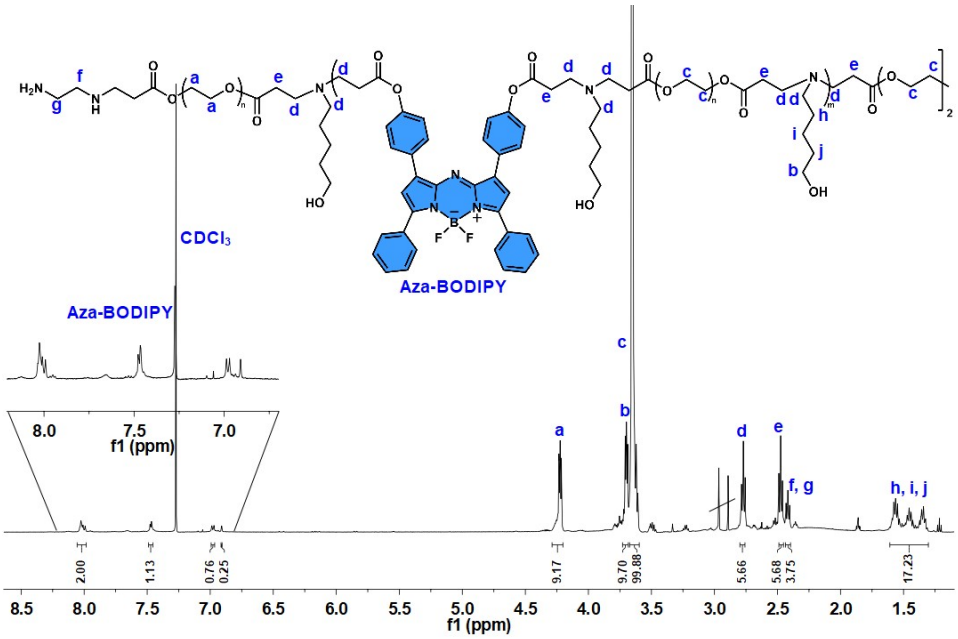


Figure S14. ^{1}H-NMR spectrum of amine end-functional PBAE synthesized with aza-BODIPY (10% feed ratio) (amine-wPBAE10%) ($CDCl_3$).

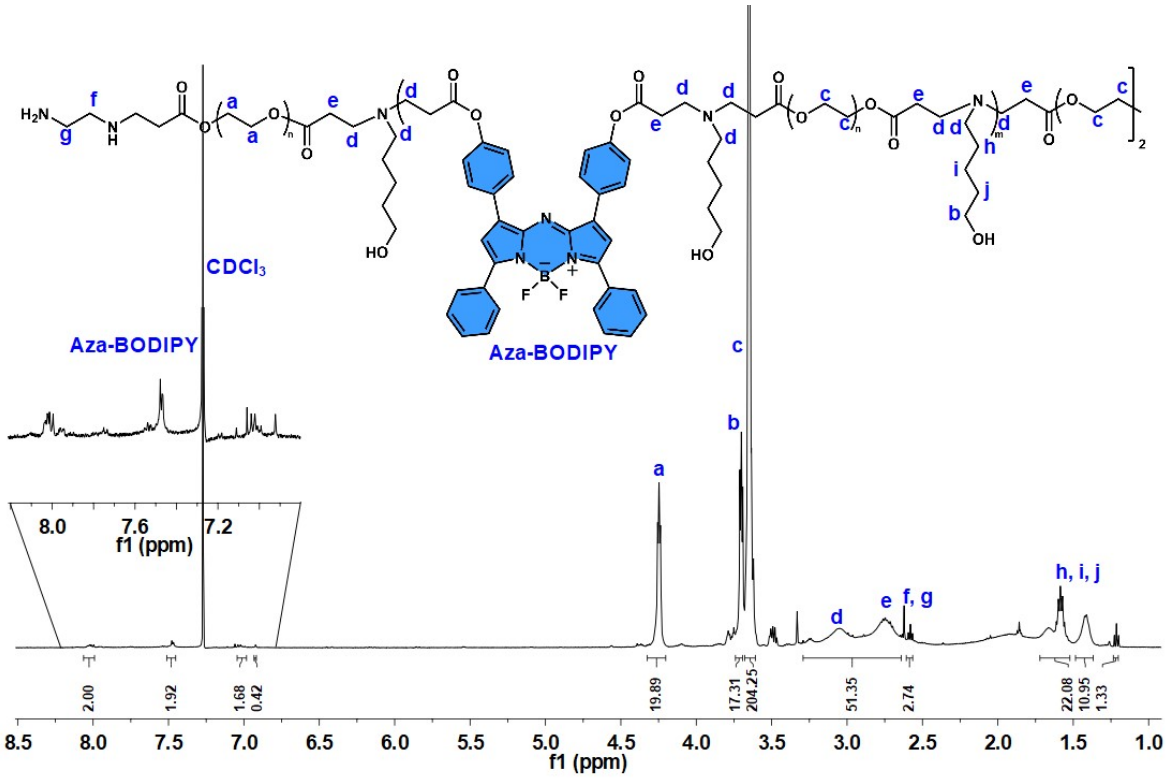


Figure S15. ^{1}H-NMR spectrum of amine end-functional PBAE synthesized with aza-BODIPY (5% feed ratio) (amine-wPBAE5%) ($CDCl_3$).

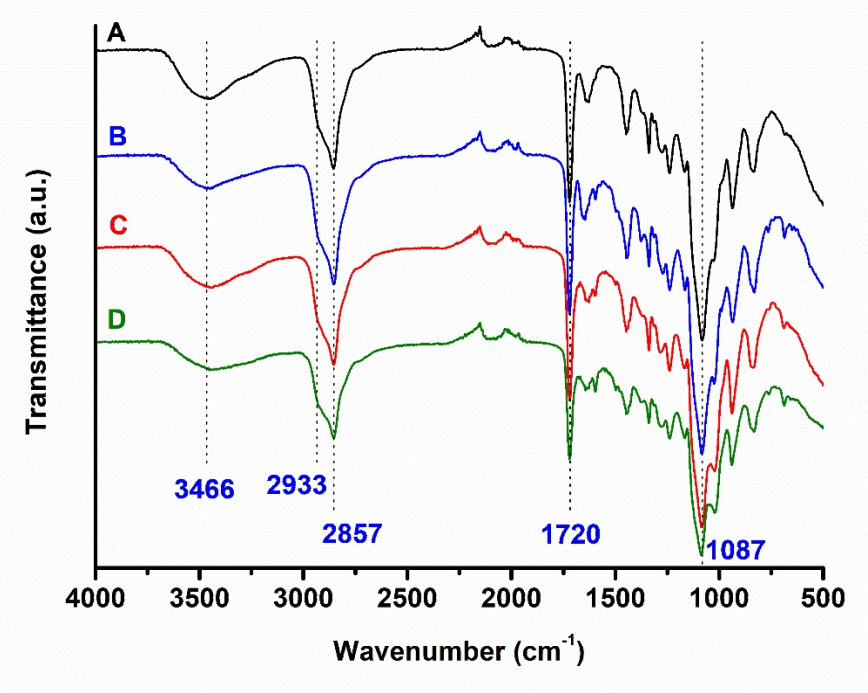


Figure S16. FT-IR spectra of the PBAEs (A: amine-wPBAE5%; B: amine-wPBAE10%; C: FA-wPBAE5%; D: FA-wPBAE10%).

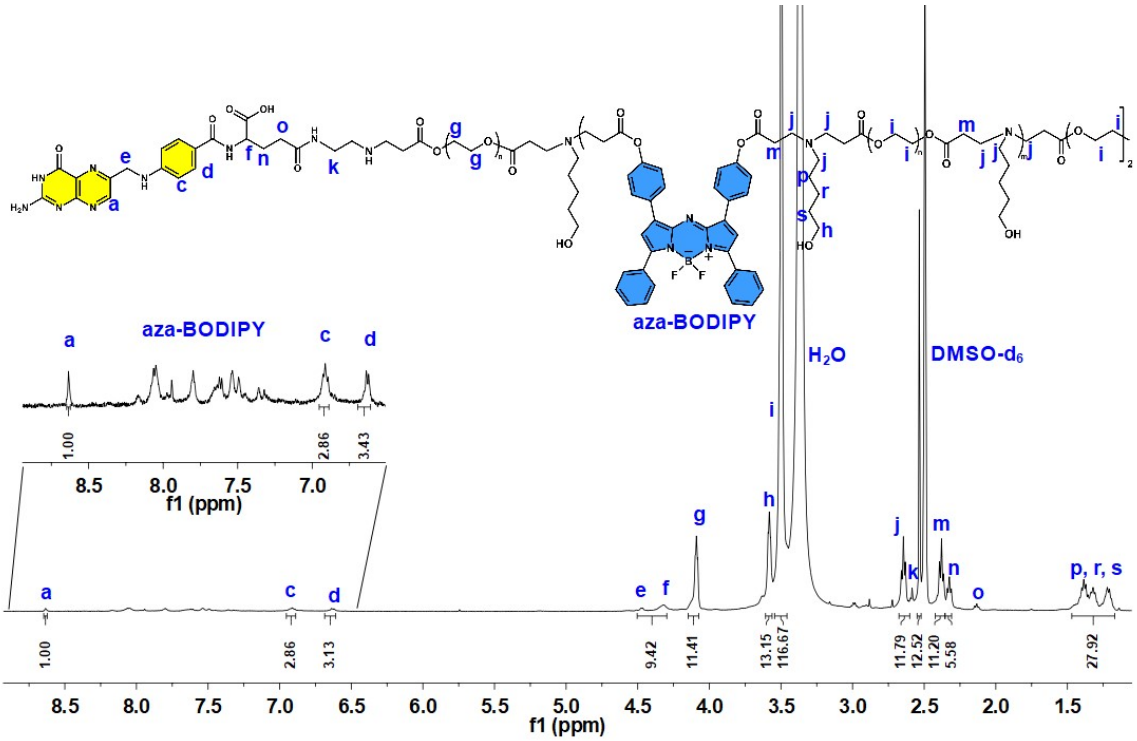


Figure S17. ^{1}H-NMR spectrum of FA-wPBAE10% (DMSO-d_6).

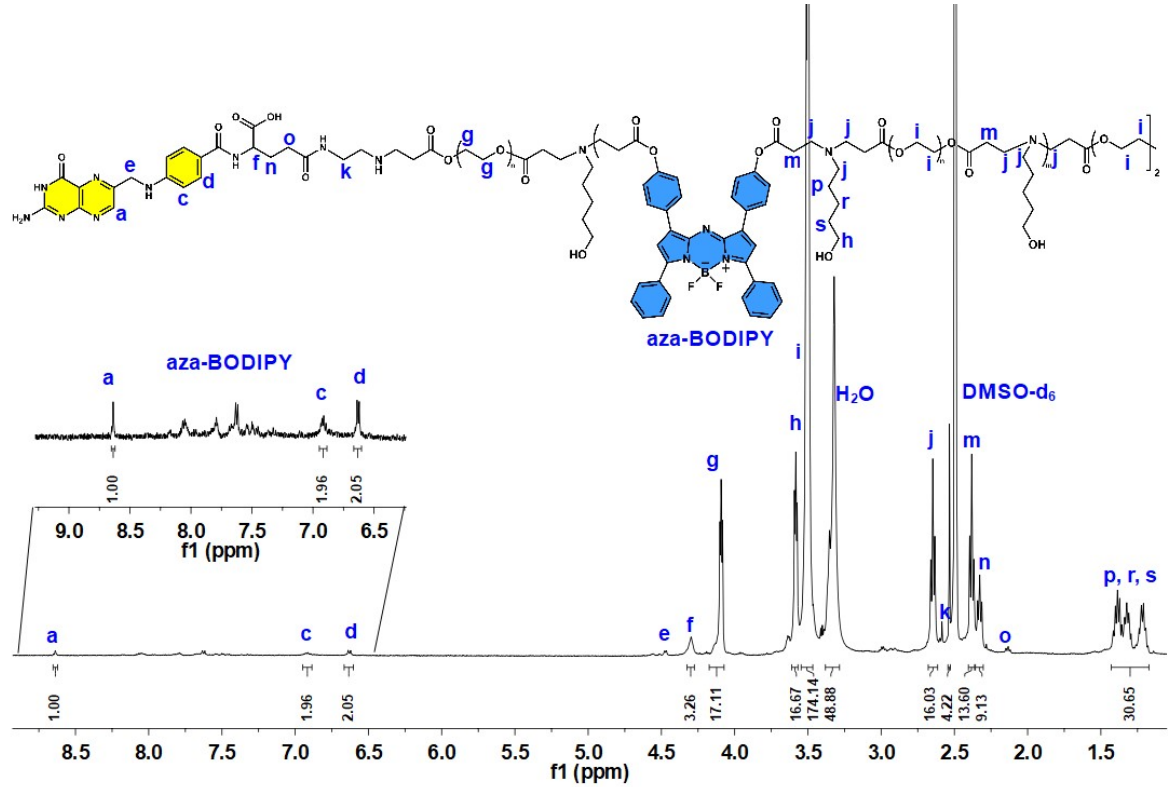


Figure S18. ^{1}H-NMR spectrum of FA-wPBAE5% (DMSO-d_6).

Table S1. Average particle sizes and particle size distributions (PDIs) of the polymers determined on DLS.

Polymer	Average Size (d.nm)	Average PDI
Acr-wPBAE10%	177.4	0.22
Acr-wPBAE5%	85.1	0.24
Acr-wPBAE1%	472.7	0.68
Amine-wPBAE10%	92.0	0.09
Amine-wPBAE5%	204.8	0.27
FA-wPBAE10%	108.8	0.13
FA-wPBAE5%	98.1	0.30

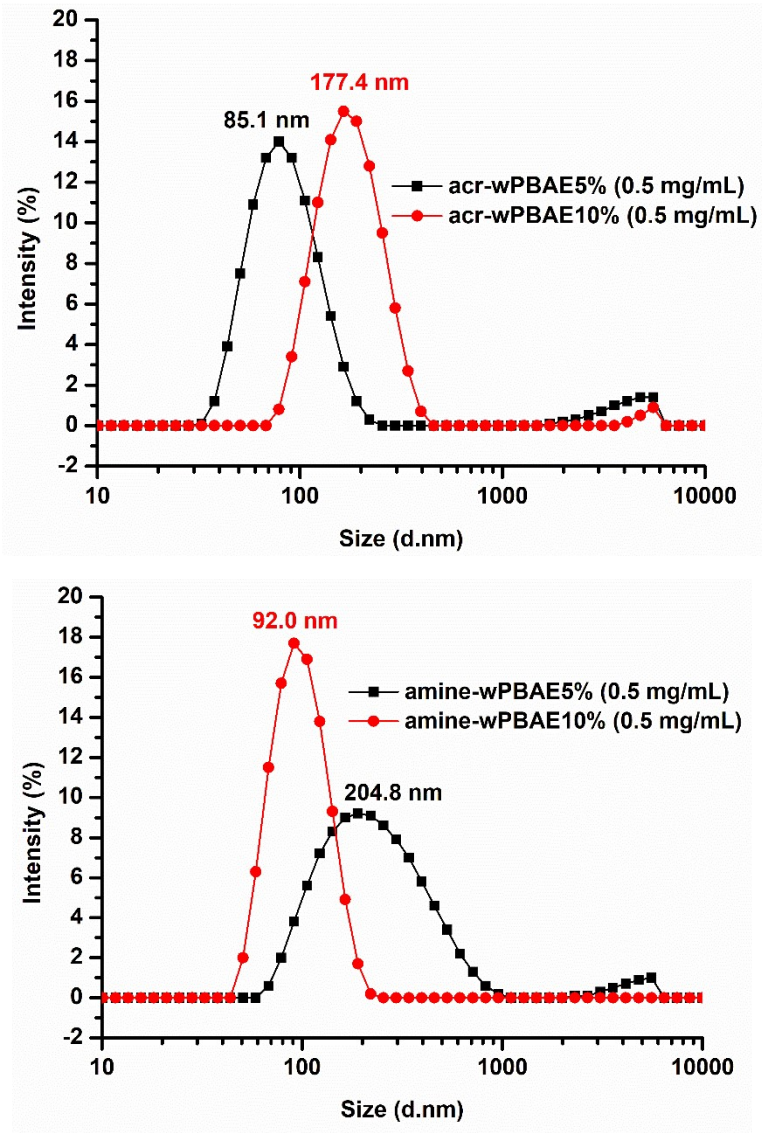


Figure S19. Average particle sizes and PDIs of the polymers in water by DLS

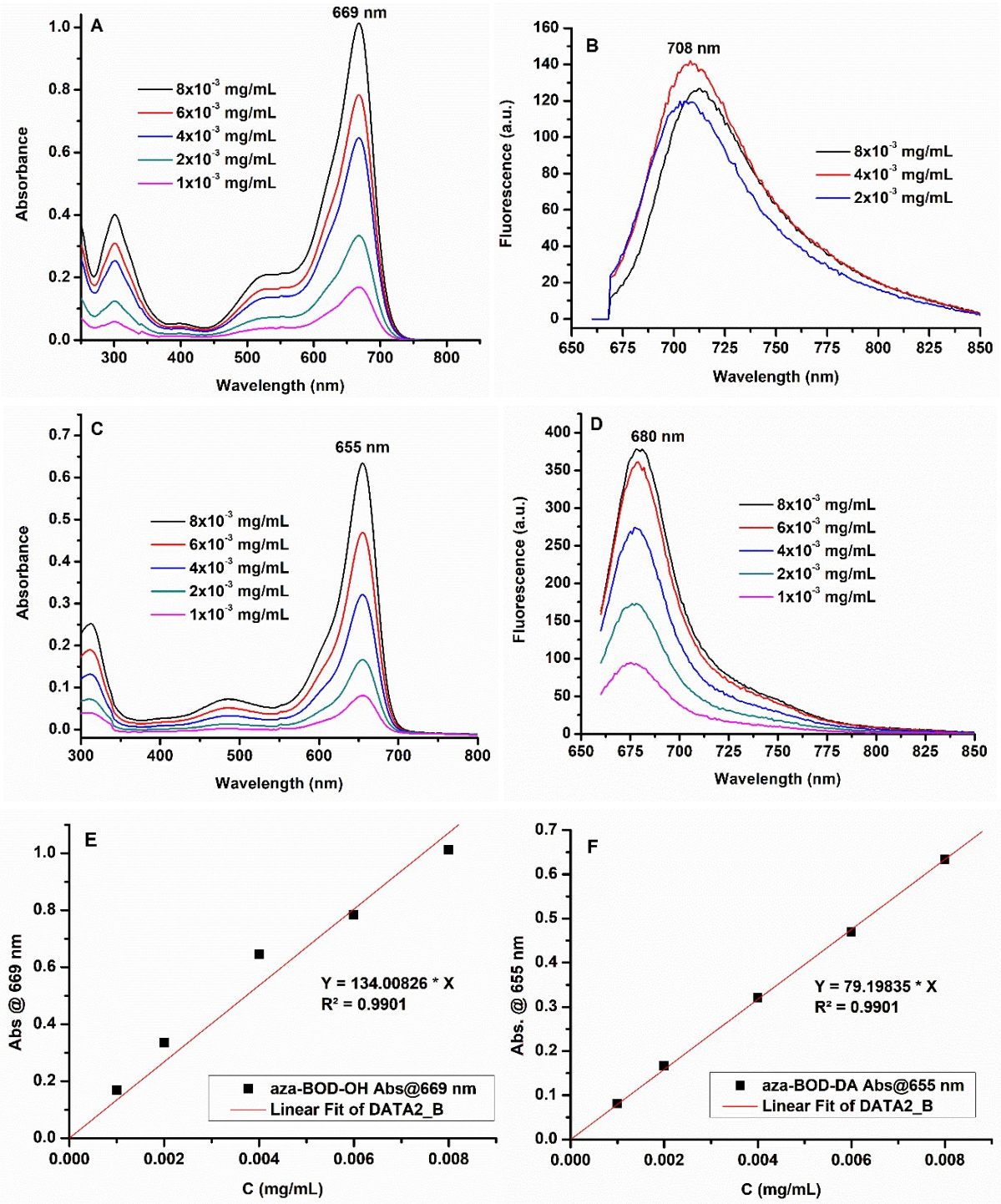


Figure S20. UV-Vis (**A**) and fluorescence ($\lambda_{excitation}$: 660 nm) (**B**) spectra of aza-BOD-OH at various concentration in THF. UV-Vis (**C**) and fluorescence ($\lambda_{excitation}$: 650 nm) (**D**) spectra of aza-BOD-DA at various concentration in THF. The linear fit of absorbance values against concentration (mg/mL) (**E** and **F**).

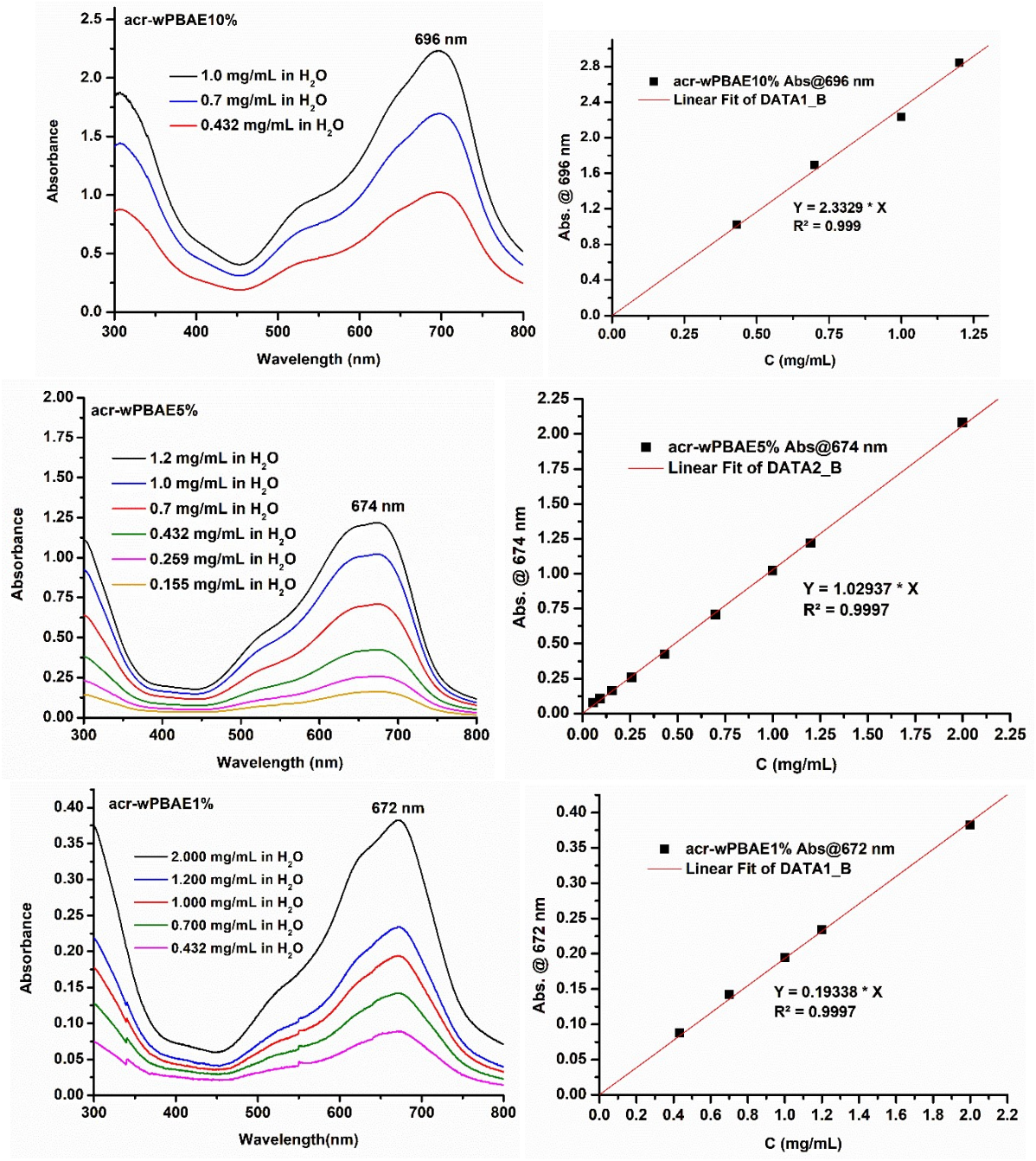


Figure S21. UV-Vis spectra of acrylate end-functional PBAEs in water, and linear fit of maximum absorbance values against concentration (mg/mL).

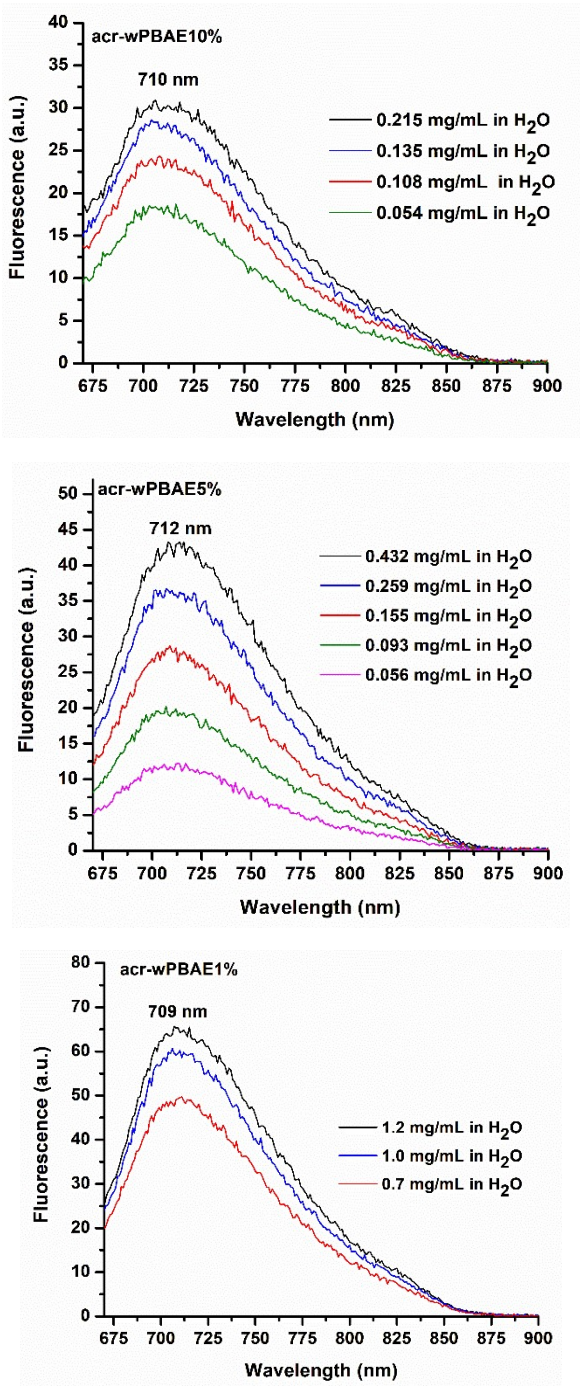


Figure S22. Fluorescence ($\lambda_{excitation}$: 650 nm) spectra of acrylate end-functional PBAEs in water.

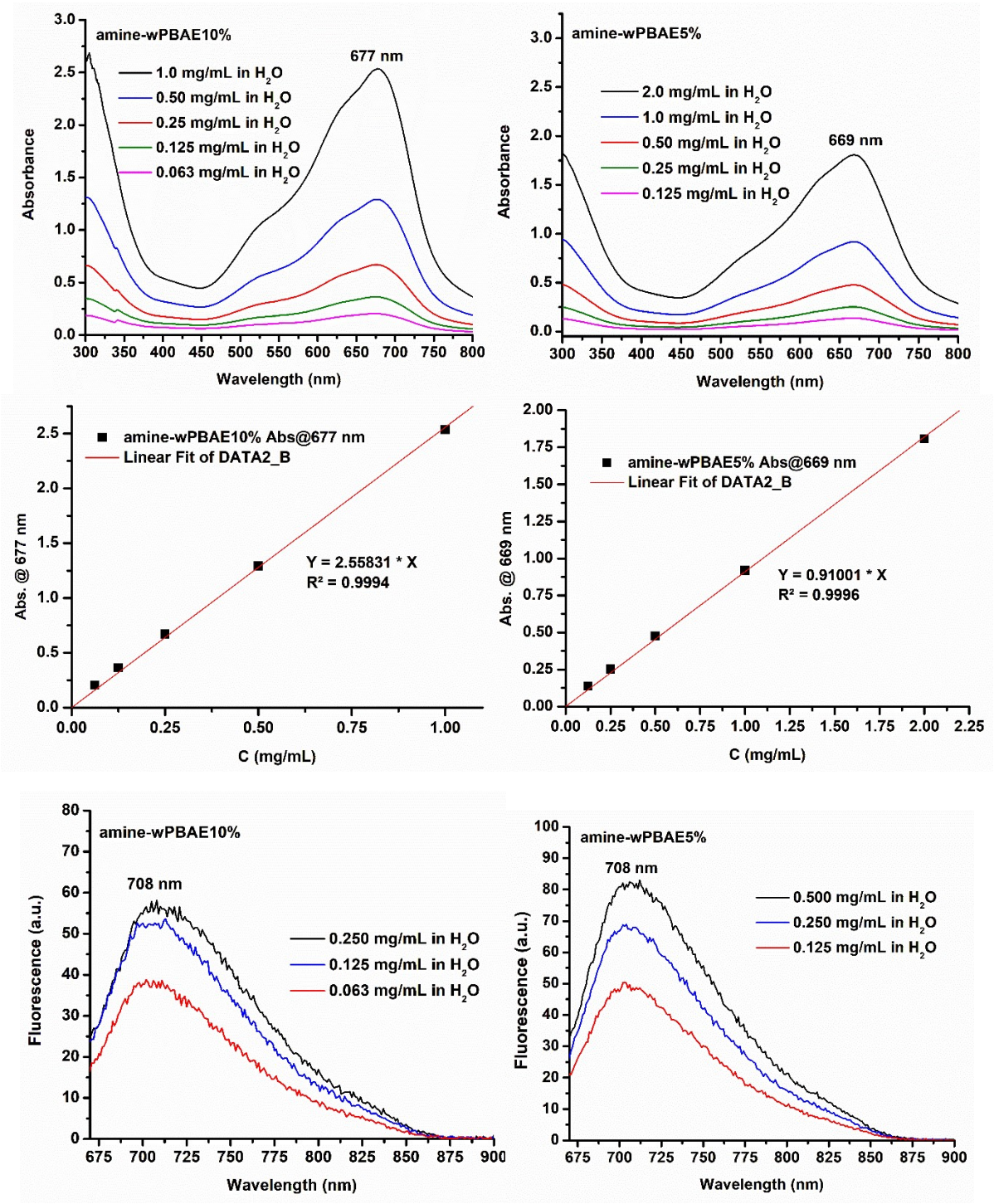


Figure S23. UV-Vis spectra, linear fit of maximum absorbance values against concentration (mg/mL), and fluorescence ($\lambda_{excitation}$: 650 nm) spectra of amine end-functional PBAEs in water.

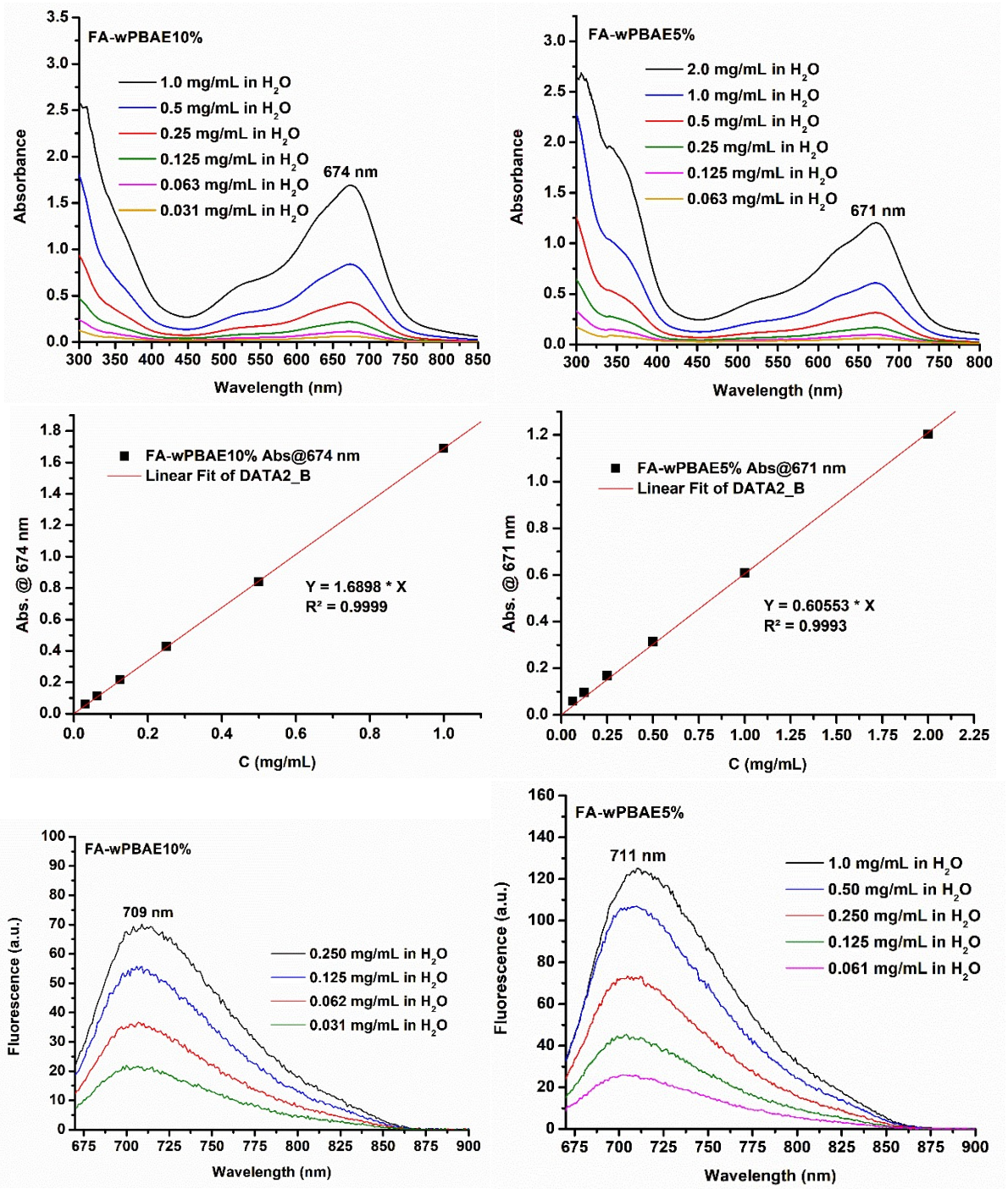


Figure S24. UV-Vis spectra, linear fit of maximum absorbance values against concentration (mg/mL), and fluorescence ($\lambda_{excitation}$: 650 nm) spectra of FA end-functional PBAEs in water.

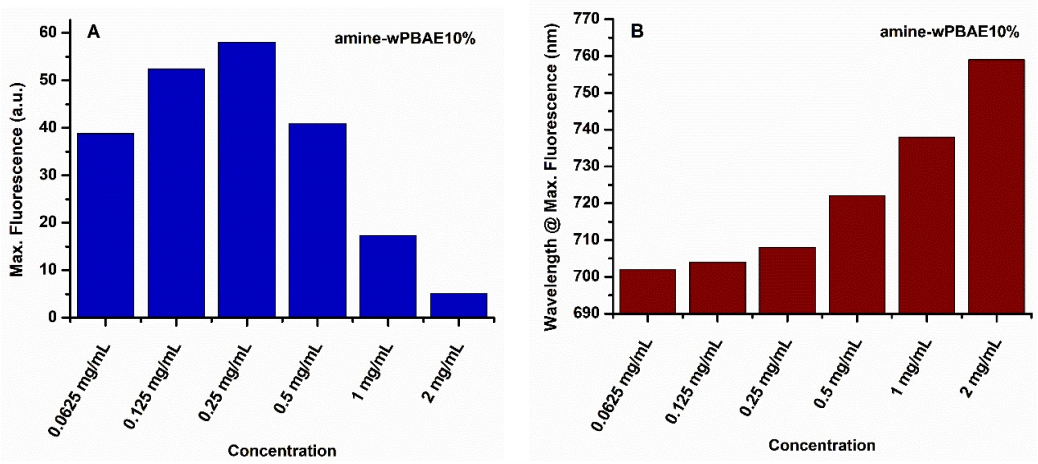


Figure S25. Maximum fluorescence (A), and the maximum emission wavelengths (B) of the aqueous solution of amine-wPBAE10% at various concentration (mg/mL).

Singlet Oxygen ($^1O_2$) Quantum Yield (φ_Δ)

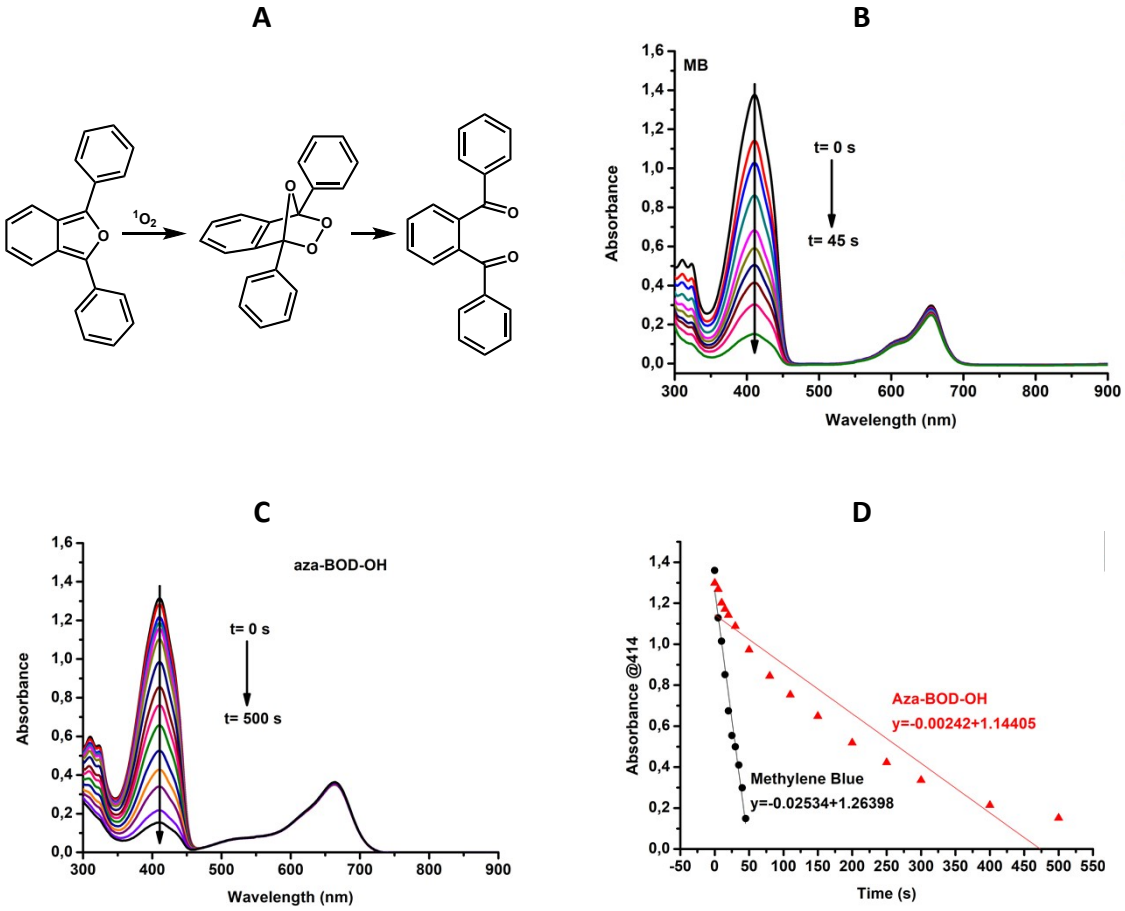


Figure S26. The mechanism of decomposition of DPBF via singlet oxygen scavenging (A). Singlet oxygen mediated photobleaching of DPBF in the presence of Methylene Blue (B) and aza-BOD-OH (C) in air saturated ethanol at 25 ^oC. The kinetics of decomposition of DPBF via singlet oxygen scavenging (D).

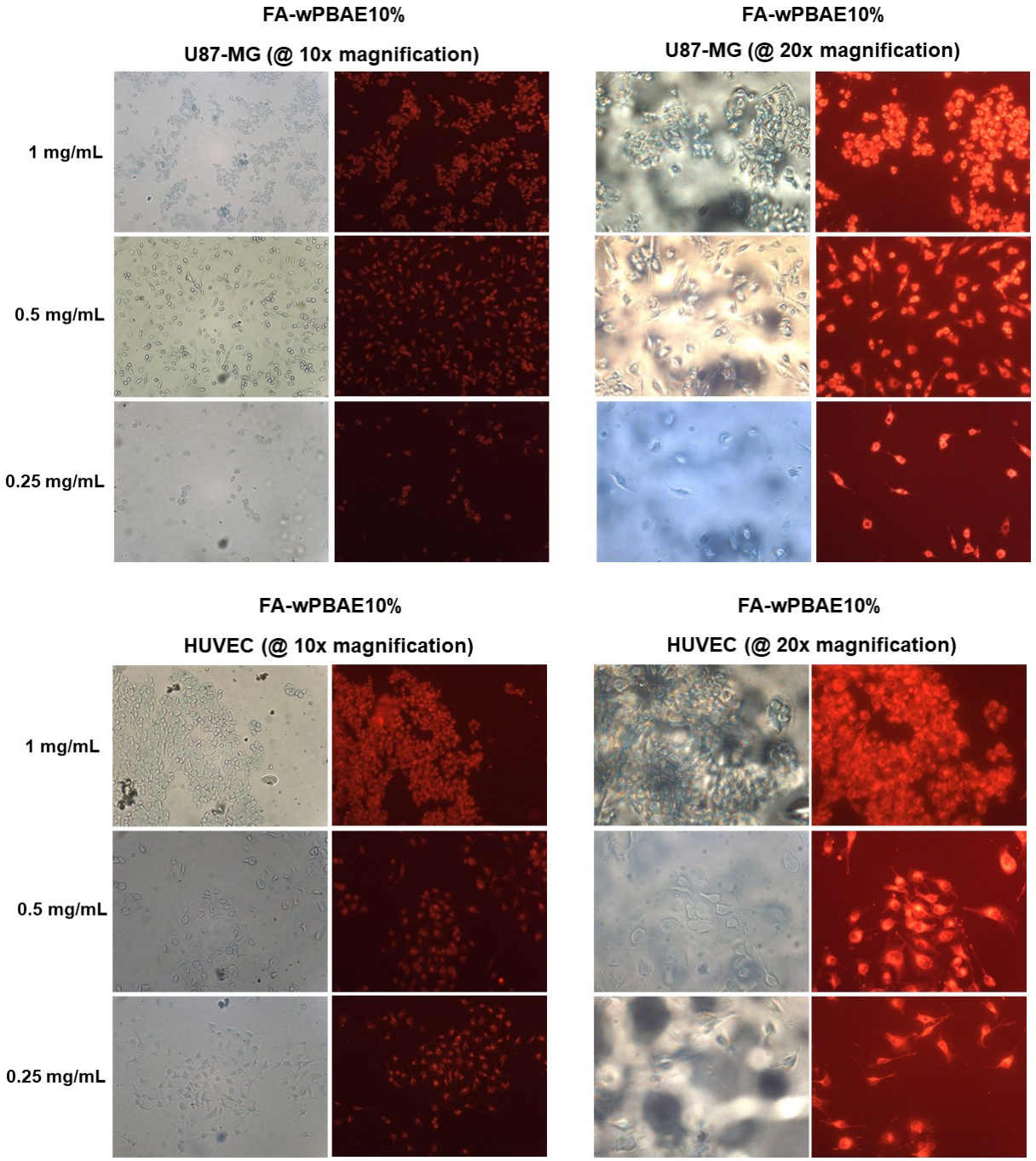


Figure S27. Day-light and fluorescence microscopy images of U87-MG and HUVEC cells incubated with FA-wPBAE10%.

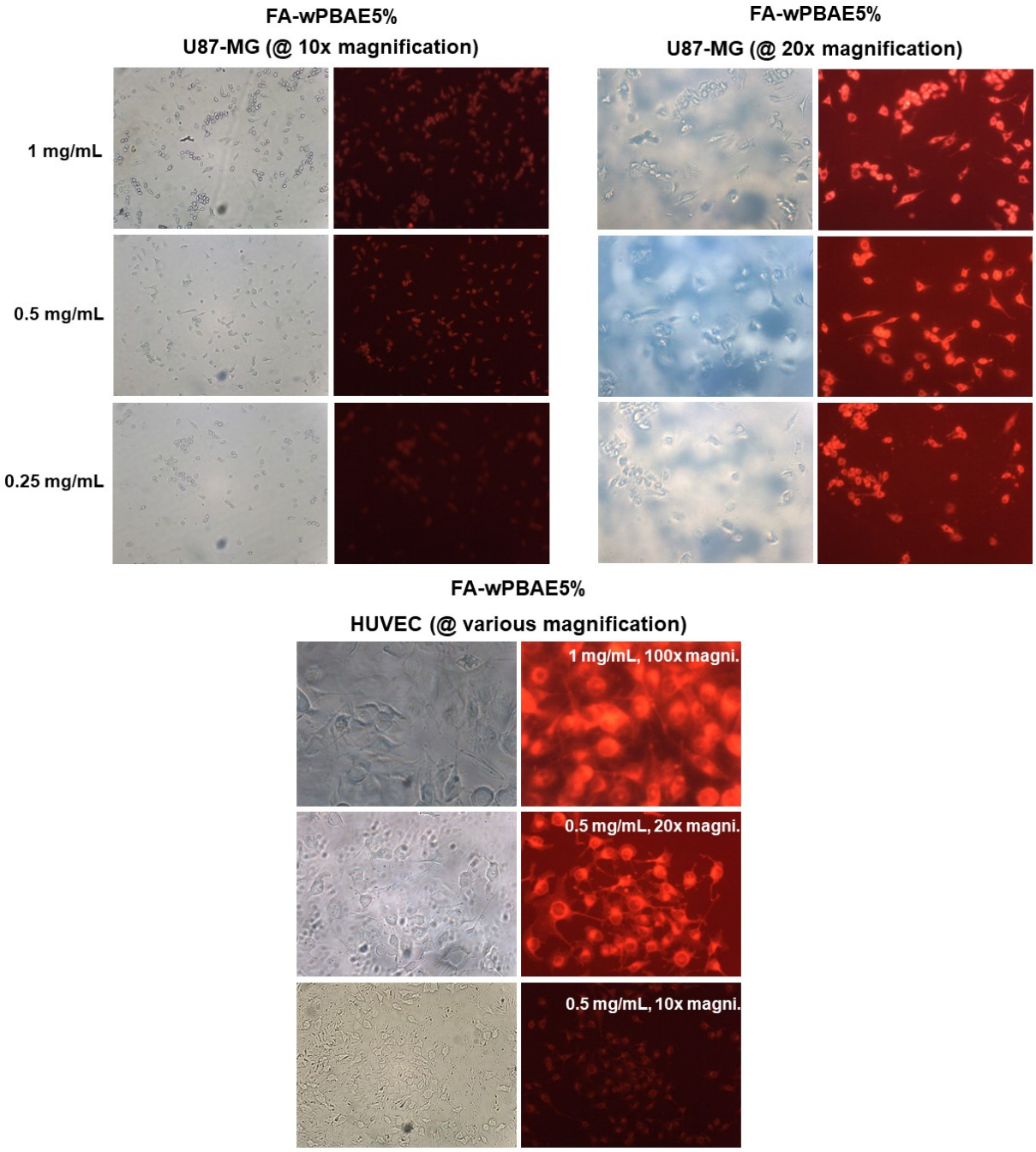


Figure S28. Day-light and fluorescence microscopy images of U87-MG and HUVEC cells incubated with FA-wPBAE5%.

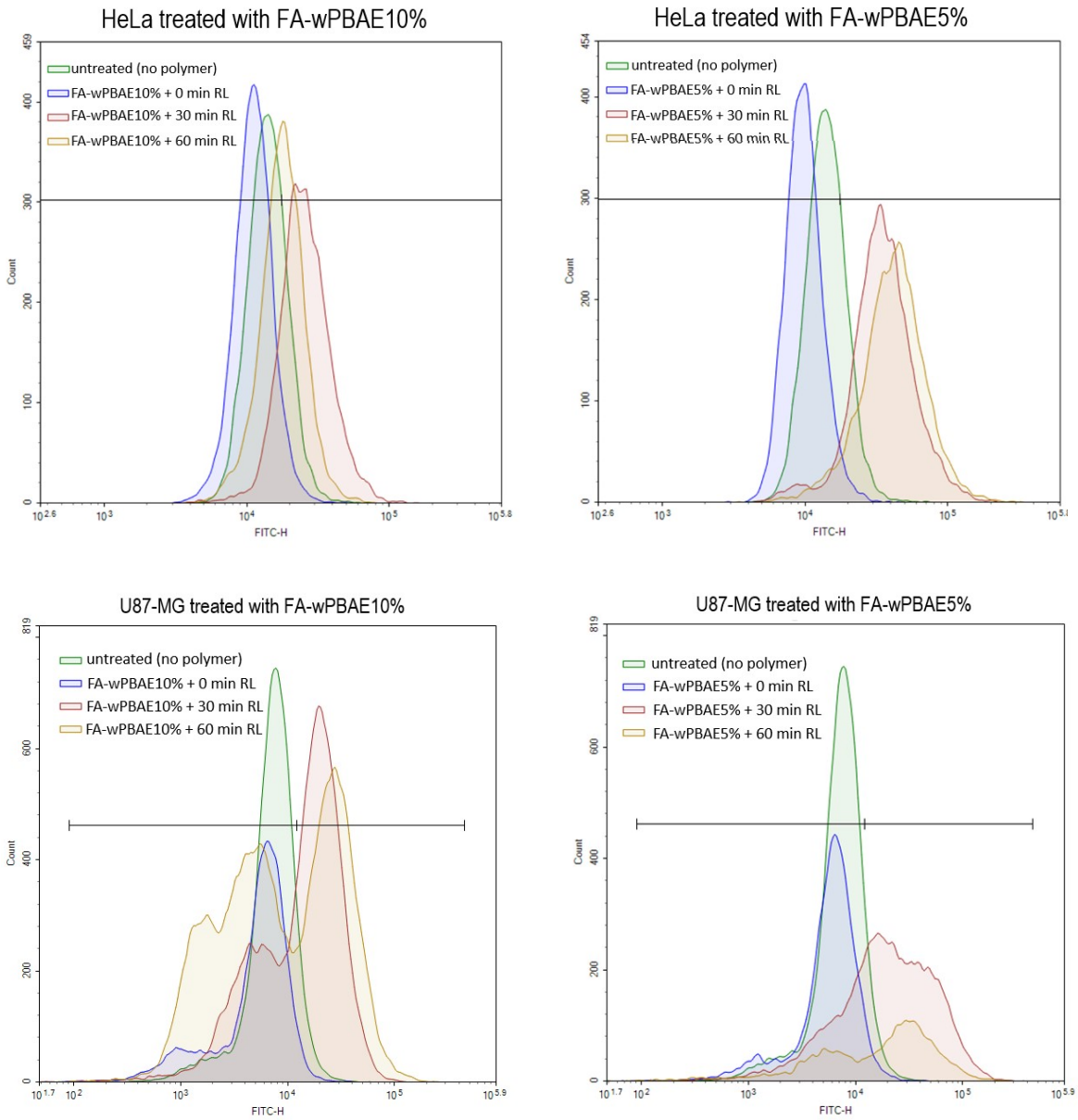


Figure S29. Flow cytometric ROS activity analysis of HeLa and U87-MG cells by DCFH-DA staining, treated with FA-wPBAE10% and FA-wPBAE5% and red light (RL) exposure.

References

[1] J. Murtagh, D. O. Frimannsson, D. F. O'Shea, *Organic Letters* **2009**, 11, 5386.
[2] K. Butzbach, M. Konhäuser, M. Fach, D. N. Bamberger, B. Breitenbach, B. Epe, P. R. Wich, *Polymers* **2019**, 11, 896.