Electronic Supplementary Information (ESI)

Super-Resolution Imaging for Self-Assembly of Amphiphilic Photoswitchable Macrocycles

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1. Materials, Reagents and Instruments

Materials and Reagents

2, 4-dimethylpyrrole, boron trifluoride etherate ($BF_3 \cdot OEt_2$), tetraethyleneglycol monoethylether, 4, 4'-dimethoxybenzil were purchased from Energy Chemical. 2, 3-Dichloro-5, 6-dicyano-p-benzoquinone (DDQ), Pyridine hydrochloride, trifluoroacetic acid (TFA), p-toluenesulfonyl chloride and polystyrene were purchased from Aladdin. DMF was dried over CaH_2 with stirring overnight followed by distillation under reduced pressure. Toluene and THF were dried using sodium wire and benzophenone as indicator. DCM was dried over calcium sulfate and distilled. Reported yields are isolated yields. Purification of most intermediates and all final products was accomplished in most cases by gravity column chromatography using silica gel (200-300) or Al_2O_3 (200-300).

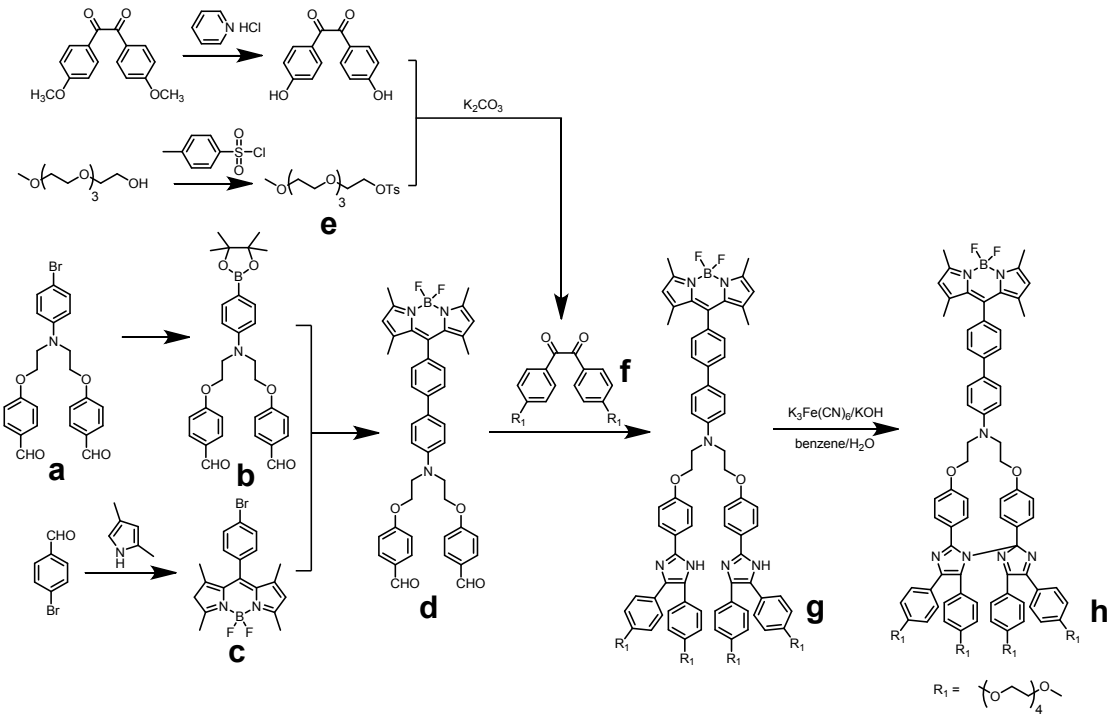
Instruments

UV-VIS: Shimadzu UV-VIS-NIR Spectrophotometer (UV-3600) PL: Edinburgh instruments (FLSP920 spectrometers) ^{1} HNMR: (Bruker AV600). Mass Spectrometry: Agilent (1100 LC/MSD Trap), MALDI-TOF Elemental Analysis: Elementar (Vario Micro-cube). Atomic Force Microscopy: Bruker (Veeco DimensionTM 3100 SPM). HPLC: Waters 1525 Binary HPLC Pump with 2489 UV-VIS Detector. Heidolph rotary evaporator (German, Advantage HL G6).

Preparation of the PS solution and film

5 g of PS was dissolved in 50 ml distilled DCM and this solution was stirred at room temperature over night to ensure the completely dissolved of the polymer. To this transparent polymer solution (1 ml), m-TEG-HABI-BODIPY (1 mg) is dissolved and then dropped onto clean quartz carefully in darkness. The solvent was evaporated in open air and further dried in vacuum for 3 h (in darkness).

2. Synthetic scheme of mTEG-HABI-BODIPY

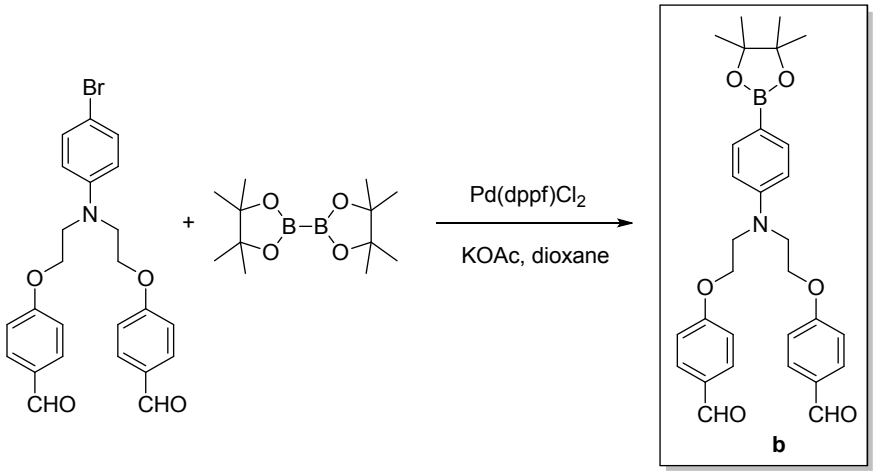


Scheme S1. Synthetic procedures of mTEG-HABI-BODIPY

3. The synthetic procedures of mTEG-HABI-BODIPY and the corresponding intermediates

The preparation procedures of 4, 4'-dihydroxybenzil[1], **a**[2] and **e**[3] are according to previous literatures and our recent work.

Synthetic procedure of **b:**

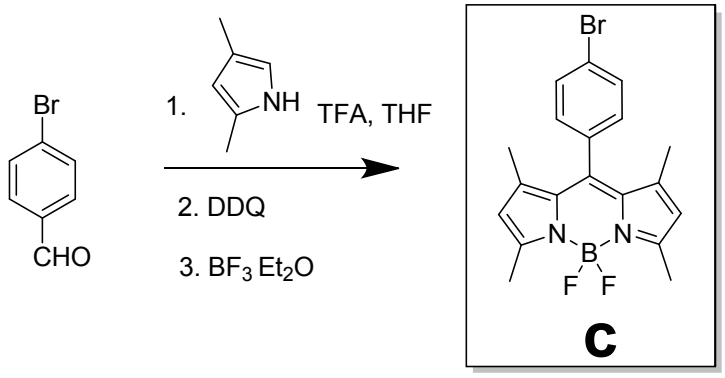


Into a 250 ml round-bottomed flask, **a** (3.35 g, 7.153 mmol), bis(pacolato)diboron (2.72 g, 10.73 mmol), potassium acetate (2.81 g , 28.61 mmol) and Pd(dppf) Cl_2 (0.26 g, 0.36 mmol) were added and dispersed in 100 ml dry dioxane under N_2 atmosphere. The solution was heated to 90 ℃and stirred for 24 h. After the reaction was finished, the liquid was poured into water (100 ml) and then extracted with DCM (3 × 100 ml). The organic layer was then washed with brine for five times, dried over $MgSO_4$, filtered and evaporated. The residue was further purified by column chromatography on silica gel using ethyl acetate and dichloromethane as eluent (3:100, v-v). 1.88 g white solid was obtained, yield 51%.

^{1}H NMR (600 MHz, $CDCl_3$) δ 10.24–9.62 (m, 2 H), 8.00–7.77 (m, 4 H), 7.80–7.65 (m, 2 H), 7.10–6.89 (m, 4 H), 6.81 (t, J=7.0 Hz, 2 H), 4.53–4.17 (m, 4 H), 4.09–3.78 (m, 4 H), 1.33 (d, J=12.6 Hz, 12 H).

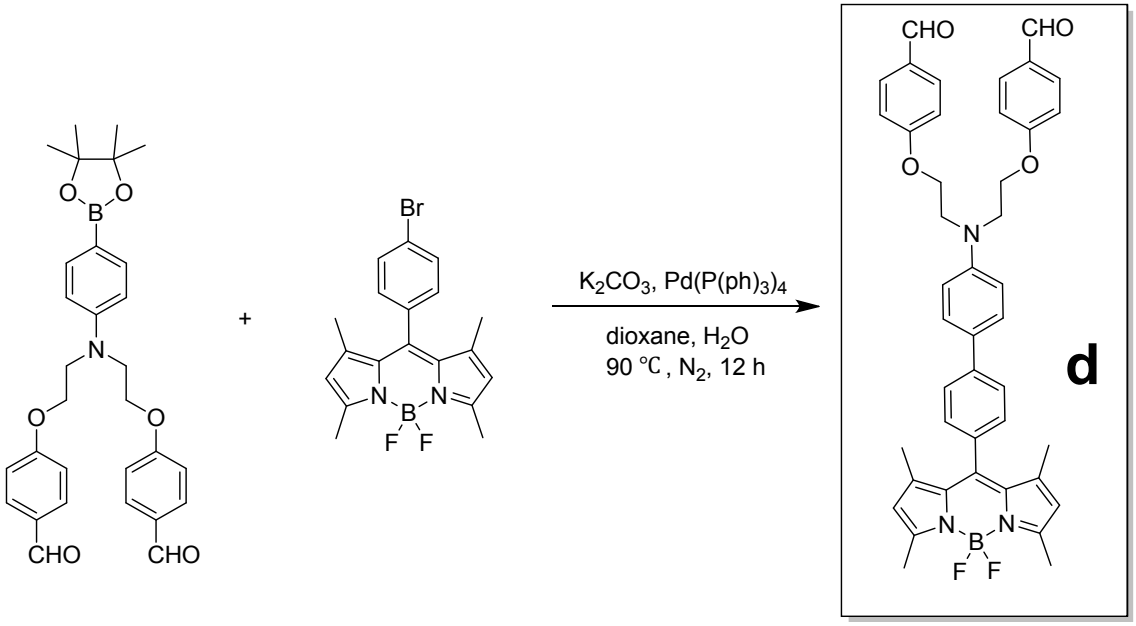
LC-MS (APCI): calculated: 515.2, found: 516.3, [M+H$^+$]

Synthetic procedure of **c:**



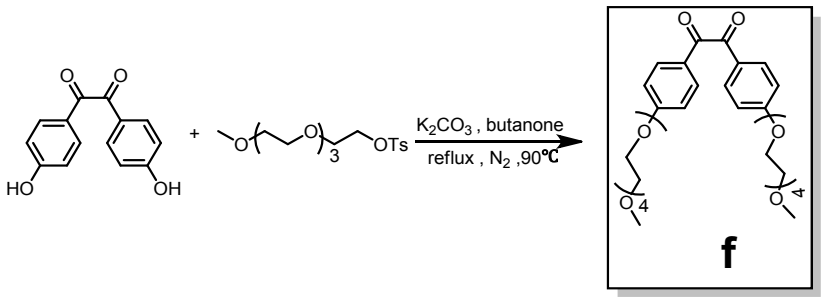
Under N_2 atmosphere, 4-bromobenzaldehyde (2.2 g, 12 mmol) and 2,4-dimethylpyrrole (2.38 g, 25 mmol) were dissolved in dry THF (350 mL), when the mixture were completely dissolved, a few drops of trifluoroacetic acid (30 uL) was added to the solution and reaction mixture was stirred at RT overnight. A solution of DDQ (2.9 g, 12 mmol) in dry THF was added and stirring continued for 4 h. Absolute triethylamine (12 mL) was then added to the mixture. After stirring for 15 min, $BF_3 \cdot OEt_2$ (12 mL) was added dropwise with ice bath cooling. After stirring for another 2h, the reaction mixture was washed with water several times and extracted with dichloromethane. The organic phase was dried over Na_2SO_4. The solvent was evaporated and the residue was purified by silica gel column chromatography (dichloromethane as eluent) to obtain red solid **c** in 23 % yield.^{1}H NMR (600 MHz, $CDCl_3$) δ 7.64 (2 H, d, J = 8.4), 7.18 (2 H, d, J = 8.4 Hz), 6.02 (2 H, s), 2.55 (6 H, s), 1.41 (6 H, s).
LC-MS (APCI): calculated: 404.1, found: 405.3, [M+H$^+$]

Synthetic procedure of **d**:



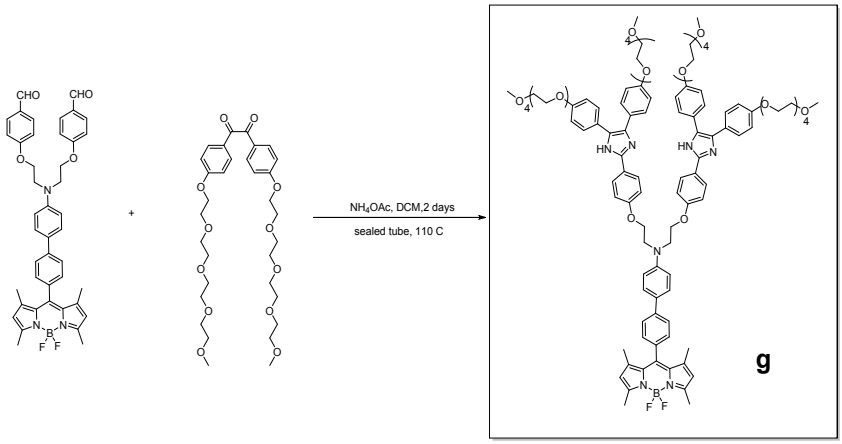
In a 100ml two-neck flask, BODIPY-Br (0.26 g, 0.646 mmol) and **b** (0.4 g, 0.776 mmol) were dispersed into 20 ml dioxane and then 6 ml K_2CO_3 aqueous (0.5 mol/L) was added. The reaction system was pumpped to low pressure and then injected with N_2. Then added **$Pd(P(ph)_3)_4$** ,Again ,the protect process was repeated for 3 times to ensure N_2 atmosphere of the system. Then the reaction was stirred and heated to 90 ℃ for 1 day. After the reaction was finished, the organic layer was separated and washed with brine (3 × 50 ml). Then it was dried over $MgSO_4$, filtered, evaporated under reduced pressure and purified by column chromatography on silica gel (200-300) using dichloromethane as eluent. 0.27 g red solid was obtained, yield 60%.
^{1}H NMR(600 MHz, $CDCl_3$) δ 9.88 (2 H, s), 7.82 (4 H, d, J=8.6), 7.69 (2 H, d, J=8.1), 7.62 (2 H, d, J=8.6), 7.29 (2 H, d, J=8.1), 6.98 (4 H, d, J=8.6), 6.91 (2 H, s), 5.98 (2 H, s), 4.31 (4 H, s), 4.00 (4 H, t, J 5.5), 2.56 (6 H, s), 1.45 (6 H, s).
LC-MS (APCI): calculated: 712.3, found: 713.3, [M+H$^+$]

Synthetic procedure of **f:**



Into a two-neck flask (100 ml) equipped with a constant-voltage funnel, 4, 4-dihydroxybenzil (0.4 g, 1.65 mmol) and K_2CO_3 (1.14g, 8.26 mmol) were dispersed in 30 ml butanone. In the constant-voltage funnel, 3, 6, 9, 12-tetraoxatridecan-1-ol, 1-(4-methylbenzenesulfonate) (**mTEG-OTs)** (1.5g, 4.13 mmol) was dissolved in 10 ml butanone. The system was pumped and injected with N_2 for three cycles to ensure the N_2 reaction atmosphere. The mixture was then stirred and heated to reflux. When dihydroxybenzil was completely dissolved, the **mTEG-OTs** (1.5g, 4.13 mmol) solution was then added into this stirring solution dropwise. The mixture was stirred and heated to 95 ℃ for 3 hours to afford a faint yellow emulsion and the reaction was checked by TLC plate until the fully consuming of 4, 4-dihydroxybenzil. After it was finished, the reaction mixture was cooled to room temperature, diluted in dichloromethane (200 mL) and washed three times with brine. The organic layer was dried over $MgSO_4$ and filtrated. The solvent was then evaporated and the crude product was purified by column chromatography on silica gel (200-300) with dichloromethane/methanol (from 100: 1 to100:2, v-v) to afford 4, 4-di-mTEG-benzil (**f**) as a yellow oil in 53% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.92 (4 H, d, J=8.8), 6.97 (4 H, d, J=8.9), 4.24–4.16 (4 H, m), 3.91–3.84 (4 H, m), 3.74–3.69 (4 H, m), 3.65 (16 H, ddd, J= 9.3, 5.5, 2.9), 3.53 (4 H, dd, J = 5.6, 3.5), 3.37 (6 H, d, J = 4.6).
LC-MS (APCI): calculated: 622.3, found: 623.7, [M+H$^+$]

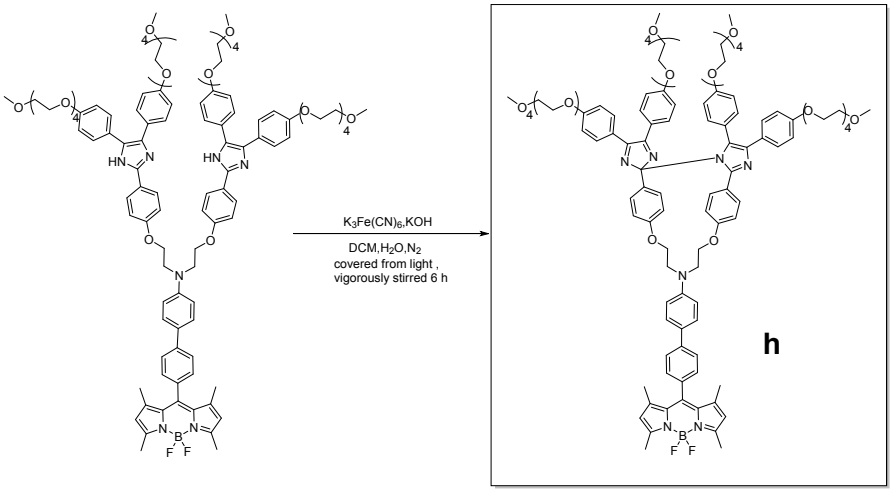
Synthetic procedure of **g:**



Into a sealed tube (100 ml), **d** (0.2 g, 0.281mmol), TEG-benzil (0.39 g, 0.62 mmol) and ammonium acetate (0.65 g, 8.4 mmol) were dissolved in 30 ml DCM with 0.1 ml acetic acid as catalyst. The solution was subjected to 4 × freeze-pump-thaw cycles to a low pressure. The

reaction was stirred and heated to 110 ℃ for 2 days. After the reaction was finished, the reaction mixture was diluted in dichloromethane and washed twice with brine. After drying over $MgSO_4$, filtration and evaporation of the solvent, the crude product was used for purification by column chromatography on silica gel (200 - 300) using DCM and Methanol as eluent. The volume of methanol was increased from 1 % to 2 % and finally 0.27 g deep red solid was obtained, yield 50%.^{1}H NMR(600 MHz, DMSO) δ 12.37 (2 H, s), 7.98 (4 H, d, *J*=8.5), 7.82 (2 H, d, *J*=8.1), 7.68 (2 H, d, *J*= 8.6), 7.38 (10 H, dd, *J*=21.0, 7.6), 7.15 – 6.76 (14 H, m), 6.18 (2 H, s), 4.27 (4 H, s), 4.09 (8 H, s), 3.95 (4 H, s), 3.74 (8H, s), 3.62 – 3.56 (8 H, m), 3.53 (8 H, dd, *J* = 9.3, 5.2), 3.52 – 3.45 (24 H, m), 3.44 – 3.39 (9 H, m), 3.22 (11 H, d, *J* = 6.1), 2.45 (6 H, s), 1.43 (6 H, s).
MS (LC-MS): calculated: 1917.96, found: 1918.3, [M+H$^+$]

Synthetic procedure of **mTEG-HABI-BODIPY (h):**



Into a 150 ml two-neck flask, **e** (0.27 g, 0.14 mmol) was dispersed in 30 ml DCM. This solution was vigorously stirred and covered from daylight by aluminum foil. Consequently, an aqueous solution (25 ml) of $K_3Fe(CN)_6$ (2.32 g, 7 mmol) and KOH (0.79 g, 14 mmol) was added into dropwise in 20 min. The reaction was detected by TLC plate until the starting material was totally converted. After the reaction was finished, the organic layer was collected, washed with brine, dried over Na_2SO_4 and filtered. The resulting solution was then evaporated under reduced pressure and further purified by flash column chromatography on Al_2O_3(200-300) using DCM-Methanol as eluent (methanol 1%, v) to obtain black red solid (150 mg yield 56 %).^{1}H NMR(600 MHz, CD_2Cl_2) δ 7.74 (4 H, d, *J* = 7.7), 7.64 (2 H, d, *J* = 8.1), 7.52 (2 H, d, *J* = 8.0), 7.30 (8 H, dt, *J* = 20.6, 10.2), 7.17 (2 H, d, *J* = 8.1), 6.86 (4 H, d, *J* = 8.3), 6.80 (2 H, d, *J* = 8.3), 6.69 (4 H, d, *J* = 7.5), 6.63 (2 H, d, *J* = 7.9), 6.52 (2 H, d, *J* = 8.3), 6.03 (2 H, s), 4.41 (2 H, s), 4.28 (2 H, s), 4.16 (4 H, s), 4.03 (2 H, s), 3.88 (2 H, s), 3.84 (6 H, s), 3.76 (4 H, s), 3.71 – 3.55 (41 H, m), 3.49 (9 H, d, *J* = 3.2), 3.32 (12 H, d, *J* = 5.4), 2.52 (6 H, s), 1.50 (6 H, s); ^{13}C NMR(151 MHz, CD_2Cl_2) δ133.33–132.87 (m), 132.45-132.12 (m), 131.26 (s), 130.78–130.49 (m), 128.40 (s), 127.91 (s), 126.57-126.11 (m), 124.31-123.69 (m), 121.60-120.68 (m), 115.42-114.50 (m), 113.83 (s), 111.62-111.04 (m), 71.89 (s), 70.96-70.21 (m), 69.47 (s), 67.63 (s), 58.59 (s), 53.43 (dt, J 54.4, 27.2), 32.21-31.59 (m), 31.29-30.84 (m), 29.68 (s), 23.03-22.28 (m), 14.37 (s), 14.14-13.38 (m).
MS (MALDI-TOF): calculated: 1915.019, found: 1916.040, [M+H$^+$]

4. Optical spectra of mTEG-HABI-BODIPY in various organic solvents

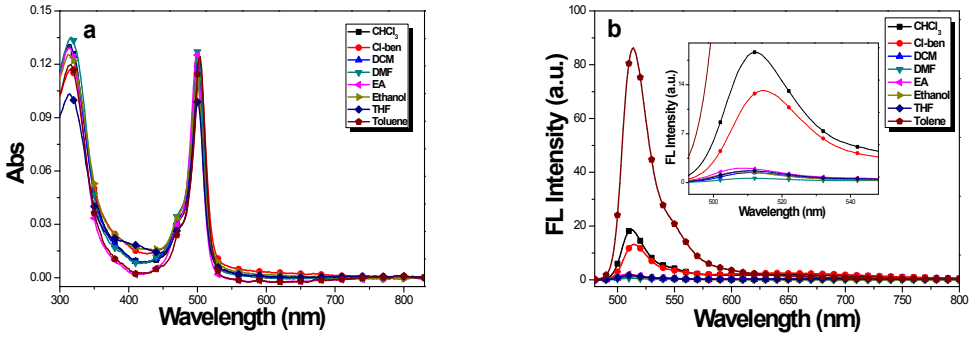


Figure S1. (**a**) UV-vis and (**b**) FL spectra of mTEG-HABI-BODIPY in various organic solvents. The excitation wavelength in FL measurement is 470 nm with excitation bandwidth and emission bandwidth 2-2. The corresponding organic solvents are chloroform, chloro-benzene, DCM, DMF, ethyl acetate, ethanol, THF and toluene.

Table S1 Optical properties of mTEG-HABI-BODIPY in various organic solvents

solvent	$\lambda_{abs,\ max}$ (nm)	$\lambda_{em,\ max}$ (nm) [a]	Stokes shift (nm)[b]	FLQY (Φ_F, %)[c]
Ethanol	500	508	8	0.38
DMF	500	516	16	0.63
DCM	501	510	9	0.88
THF	500	514	14	0.82
EA	499	508	9	2.0
$CHCl_3$	502	512	10	8.2
Toluene	504	514	10	39
Cl-benzene	504	514	10	5.8

a: The $\boldsymbol{\lambda_{em,\ max}}$ and $\boldsymbol{\lambda_{em,\ max}}$ were calculated from the UV-vis absorption and emission spectra

b: The stokes shift value are calculated by ($\boldsymbol{\lambda_{em,\ max}}$ - $\boldsymbol{\lambda_{abs,\ max}}$)

c: The fluorescence quantum yield was calculated using DCM as standard (in ethanol, 43.5%);

5. Photostability of compound d in PMMA film

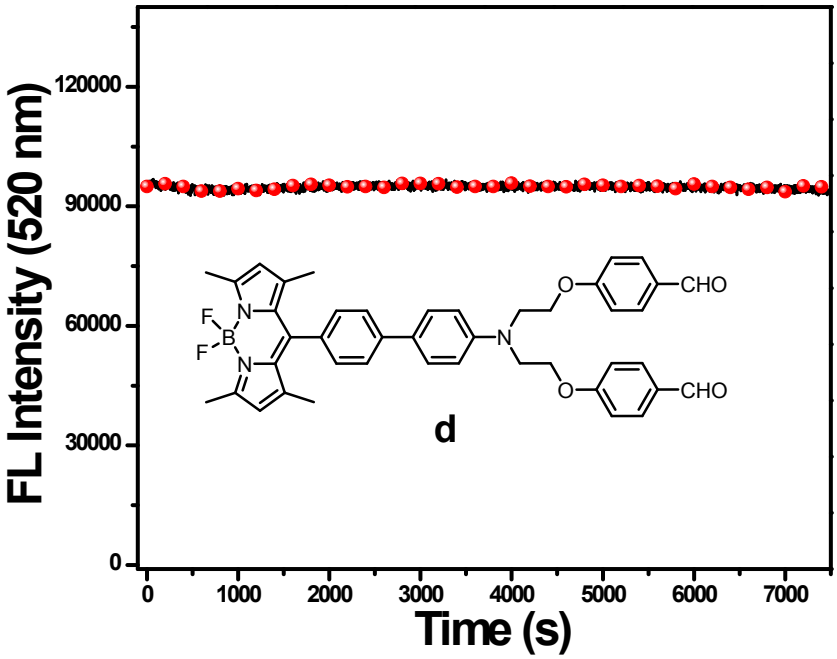


Figure S2. Photostability of **d** in PS film upon continuous excitation of 405 nm light by monitor the fluorescence change at 520 nm. 5 g of PS was dissolved in 50 ml distilled DCM and 1 mg compound **d** was added to this polymer solution (1 mL). Condition of spin-coating: 3000 rad/s, 1 min. The power of light at 405 nm from PL machine is ~1 mW/cm^2. The excitation and emission bandwidth are 3 and 3 nm.

6. The self-assemblies procedures and determination of critical aggregating concentration (CGC) of mTEG-HABI-BODIPY

General procedures: A certain amount of mTEG-BODIPY-HABI was natural dissolved in 5 ml of ethanol as stock solution and half of the supernatant was then transferred into a bottom flask (25 ml). To this solution, 5 ml ethanol and 2 ml distilled water was added dropwise while shaking by hand. And then the solution was evaporated under a rotary evaporator (water pump) at 25-30℃ for some time in darkness. Then the flask covered by aluminum foil was then held in normal pressure in oven at 35 ℃ overnight. The remaining opaque solution diluted two times was dropped into the corresponding substrates. All these procedures were performed in darkness due to the photosensitive nature of mTEG-HABI-BODIPY. It is found that the concentration of stock solution ([**c**]) and the evaporating time (**t**) are the key factors for the controlling of the size and morphology of the nanospheres. When [**c**] = 0.2 mg/ml, **t** = 0.5 h, uniform nanospheres with diameter ~80 nm were obtained; [**c**] = 0.25 mg/ml, **t** = 1 h, nanospheres with diameter ~200 nm were obtained; [**c**] = 0.3 mg/ml, **t** = 2 h, nanospheres with diameter ~500 nm were obtained; [**c**] = 0.5 mg/ml, **t** = 3 h, large but much more nonuniform microvesicles with diameter several microns were obtained.

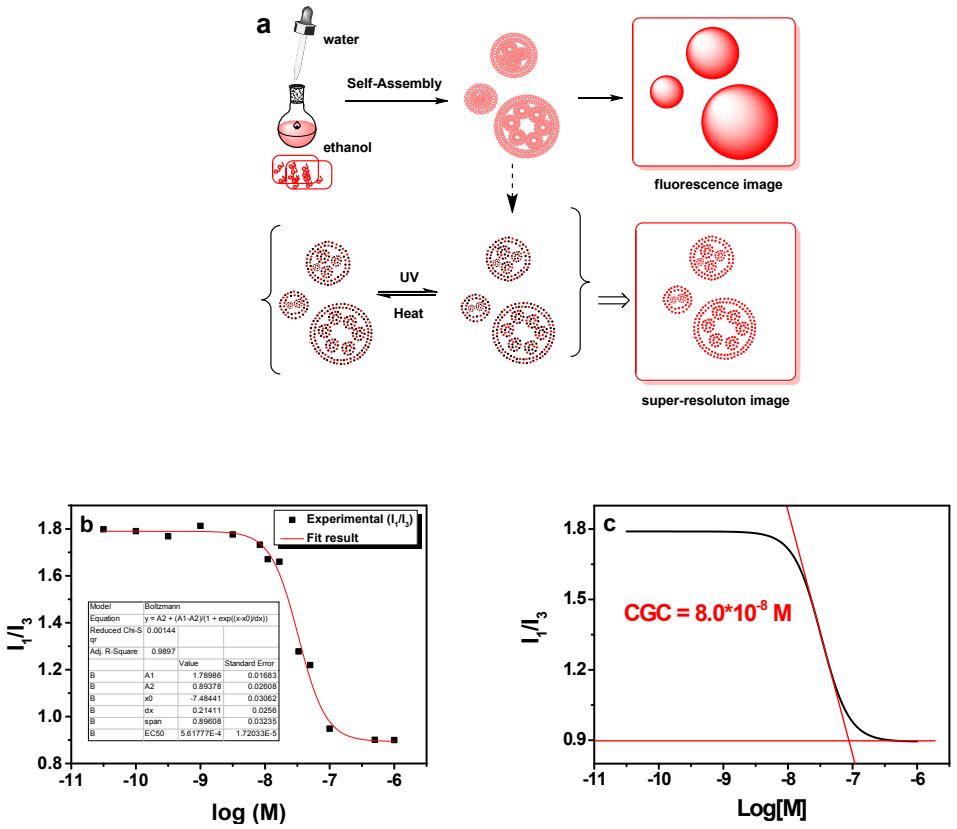


Figure S3. (a) A schematic diagram on self-assembly of mTEG-HABI-BODIPY in ethanol / water, **(b)** experimental and fitted results and **(c)** determination of critical aggregating concentration (CGC) of mTEG-HABI-BODIPY in water.

7. SEM and Fluorescence microscopy images of m-TEG-HABI-BODIPY self-assemblies

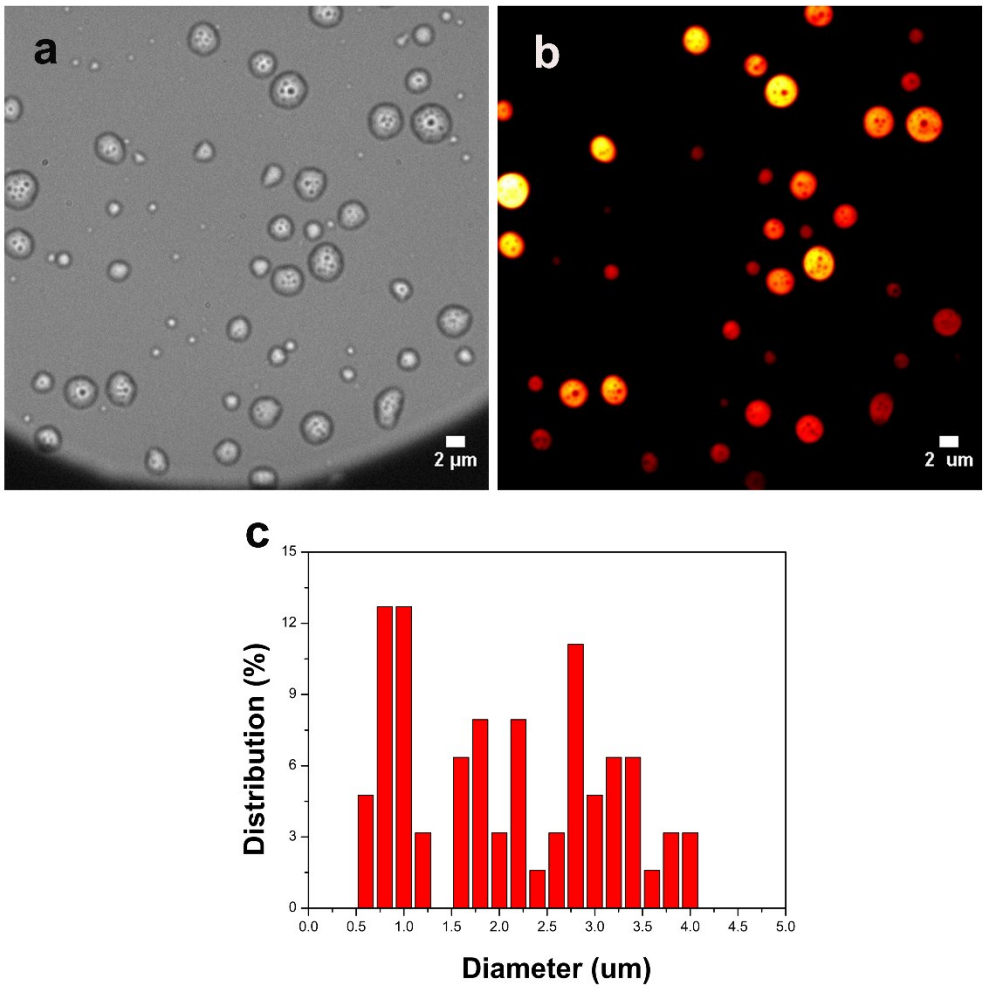


Figure S4. Fluorescence microscopy images of microvesicles (diameter larger than 2 µm) prepared with stock concentration [**c**] = 0.5 mg/ml and evaporation time **t** = 3 h. **a**) wide field image, **b**) fluorescence image, **c**) diameter distribution diagram

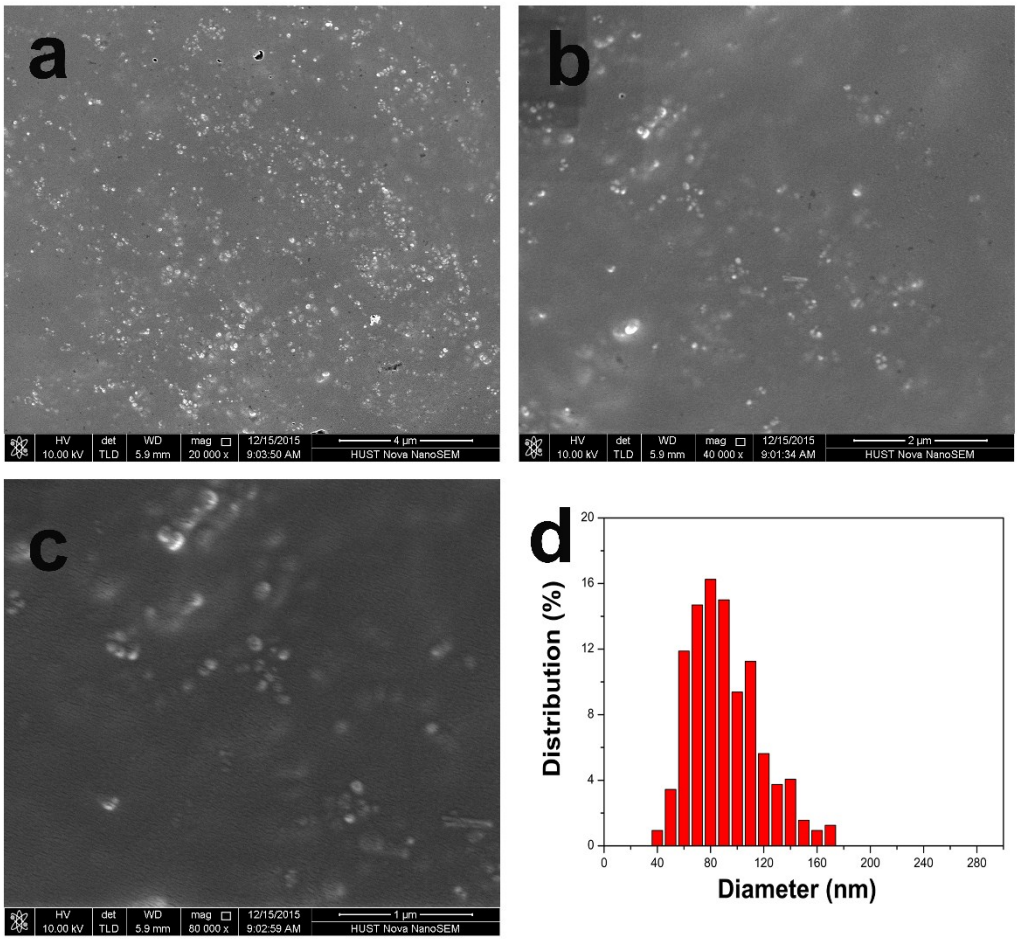


Figure S5. **a**) SEM images of nanosphere (diameter ~ 80 nm) prepared with stock concentration [**c**] = 0.2 mg/ml and evaporation time **t** = 0.5 h, **b**) and **c**) are expansion in **a**), **d**) diameter distribution diagram

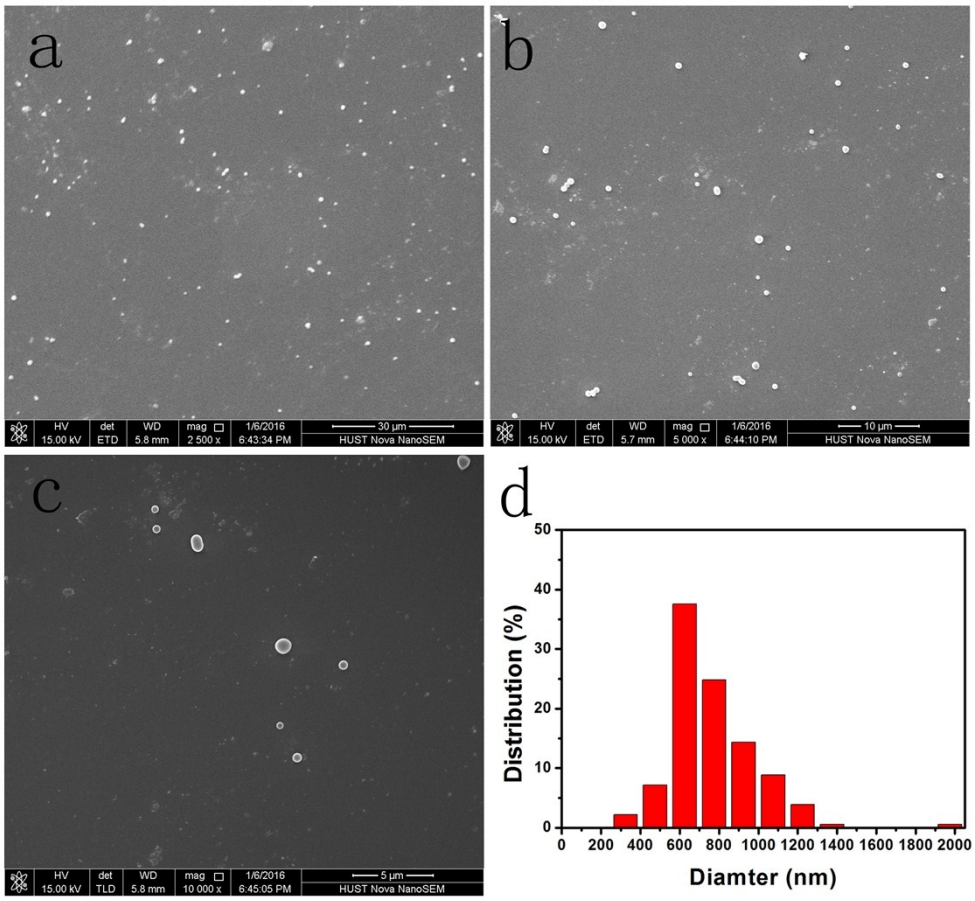


Figure S6. **a**) SEM images of nanosphere (diameter ~ 600 nm) prepared with stock concentration [**c**] = 0.3 mg/ml and evaporation time **t** = 2 h, **b**) and **c**) are expansion in **a**), **d**) diameter distribution diagram

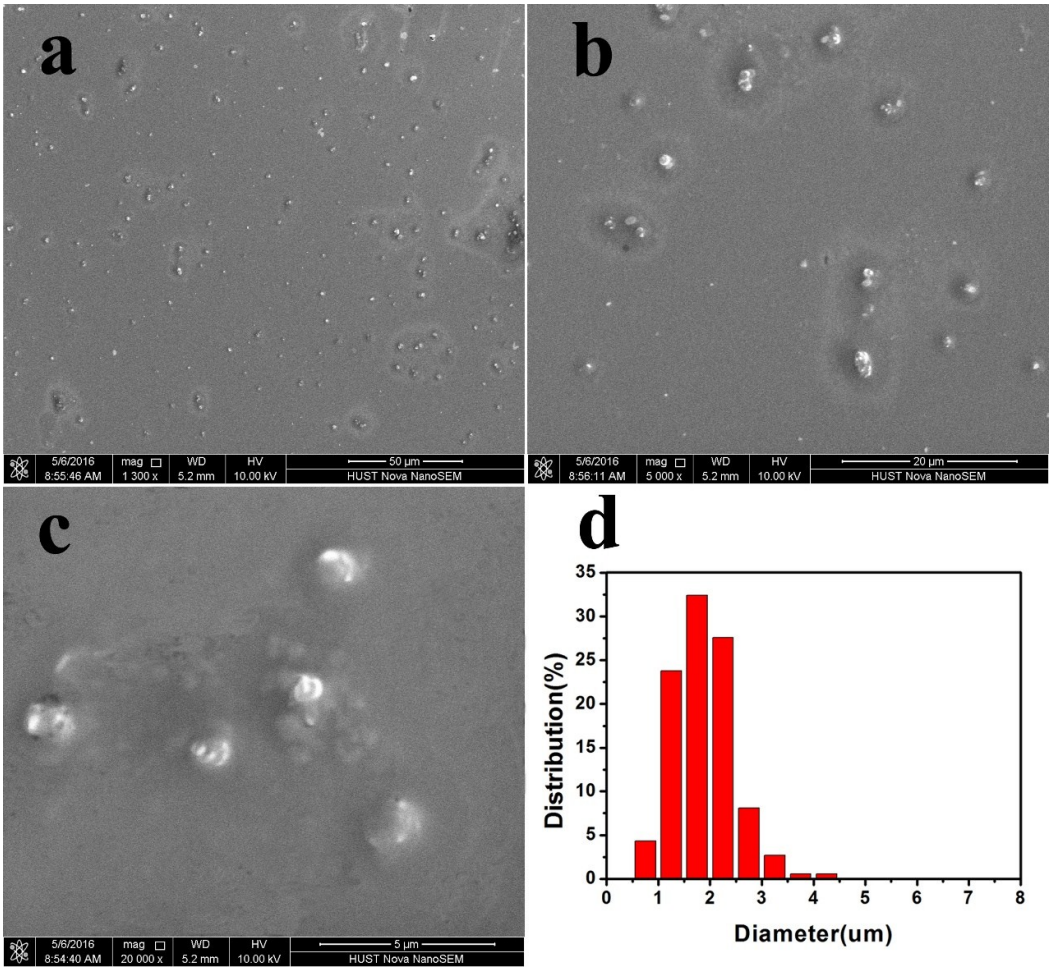


Figure S7. **a**) SEM images of nanosphere (diameter ~2 um) prepared with stock concentration [**c**] = 0.5 mg/ml and evaporation time **t** = 3 h, **b**) and **c**) are expansion in **a**), **d**) diameter distribution diagram

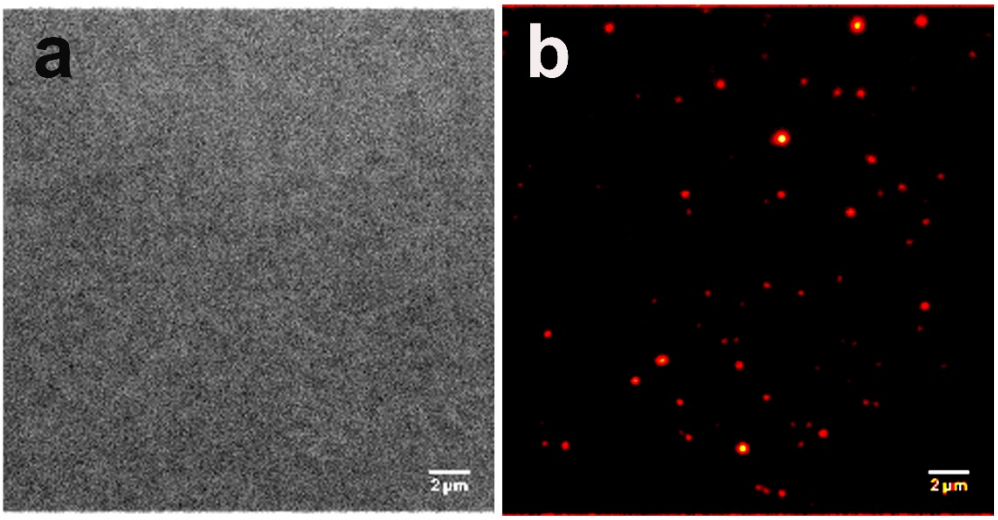


Figure S8. Large scale images of ~80 nm nanospheres in **Fig 3(a-c)** under microscope. Wide field (a) and fluorescence images of ~80 nm mTEG-HABI-BODIPY self-assemblies.

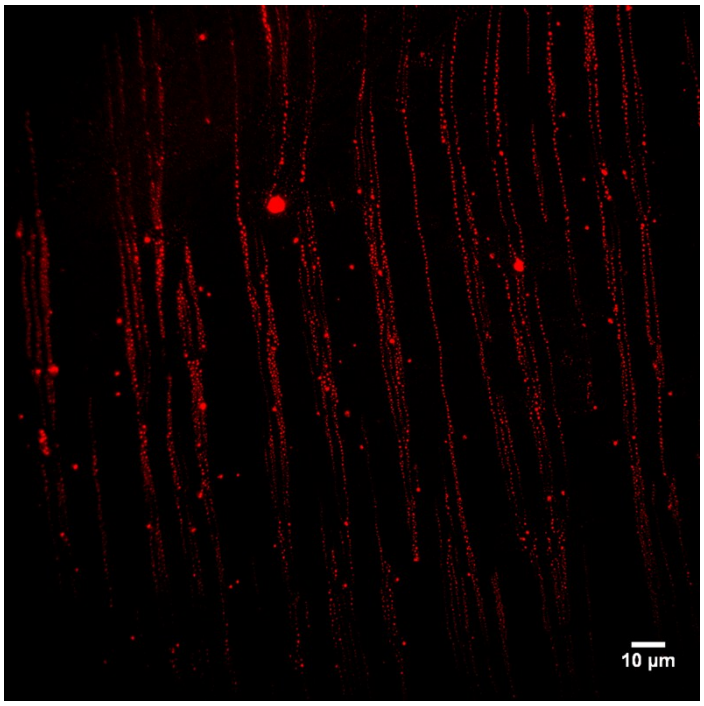


Figure S9. Large scale fluorescence microscopy images of mTEG-HABI-BODIPY nanospheres in **Fig 3(f-h)** (D = 200-500 nm)

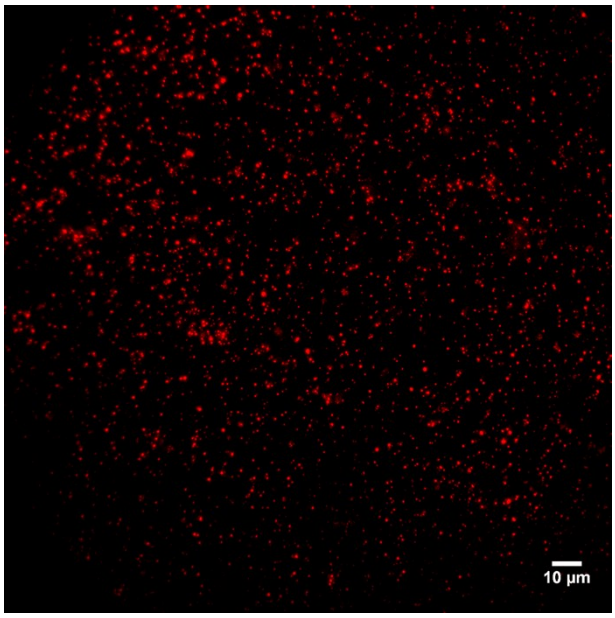


Figure S10. Large scale fluorescence microscopy images of mTEG-HABI-BODIPY nanospheres (**D** = 500-1000 nm).

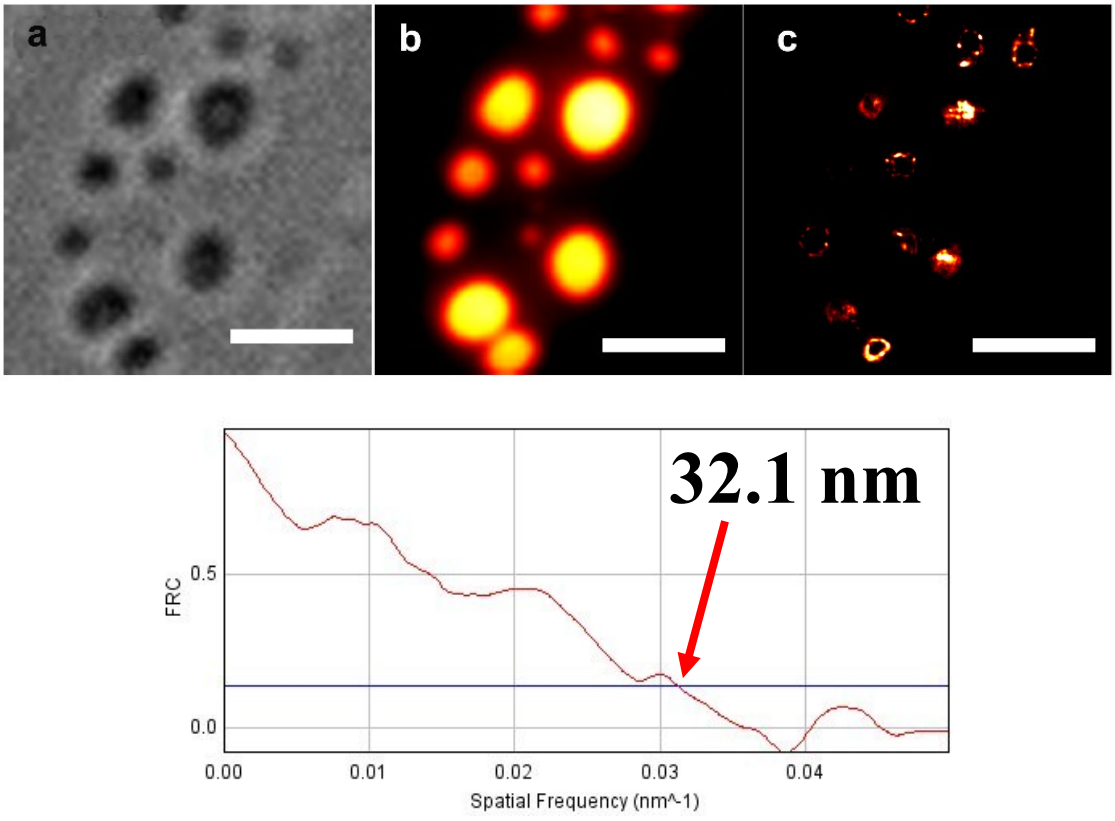


Figure S11. Super-resolution imaging of selected area in **Figure S9**. Wide filed image (**a**), fluorescence image (**b**) and super resolution imaging (**c**). The scale bar is 2 µm for all of these images. (**d**) Fourier ring correlation curve calculated from fluorescence image. The resolution in optical nanoscopy was calculated at about 32.1 nm using Fourier ring correlation.

8. DLS data

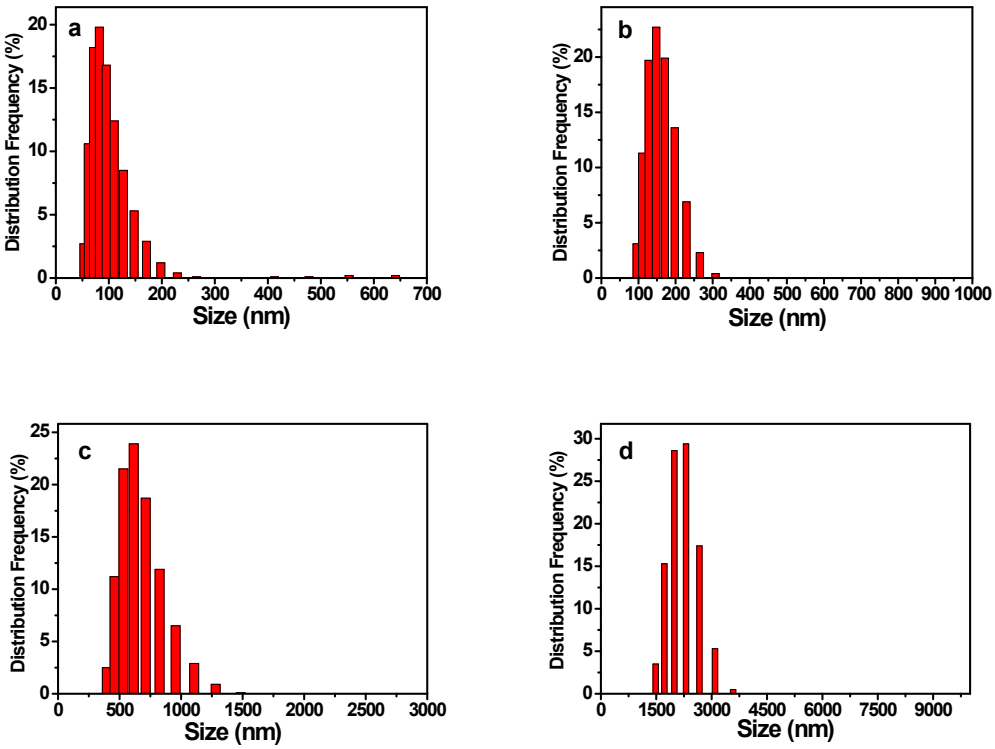


Figure S12. DLS data of the m-TEG-HABI-BODIPY self-assemblies with different stock solution concentration ([**c**]) and evaporating time (**t**). **a)** [**c**] = 0.2 mg/ml, **t** = 0.5 h; **b**) [**c**] = 0.25 mg/ml, **t** = 1 h; **c**) [**c**] = 0.3 mg/ml, **t** = 2 h and **d**) [**c**] = 0.5 mg/ml, **t** = 3 h.

9. NMR and MS spectra

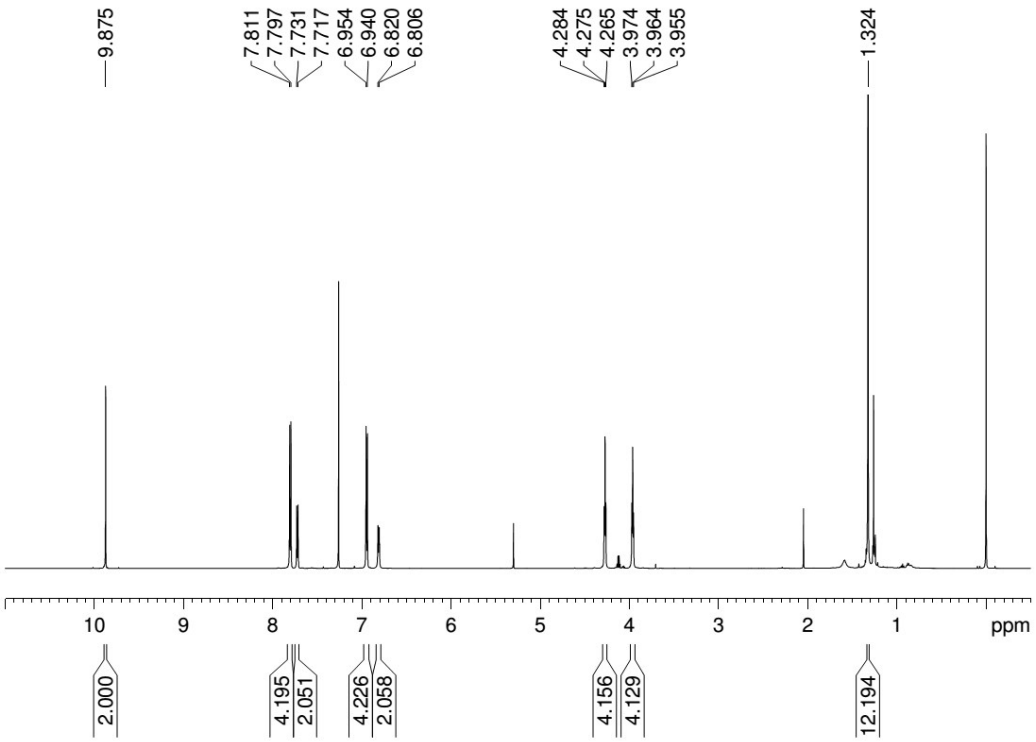


Figure S13. ^{1}H NMR spectrum of compound **b** in $CDCl_3$

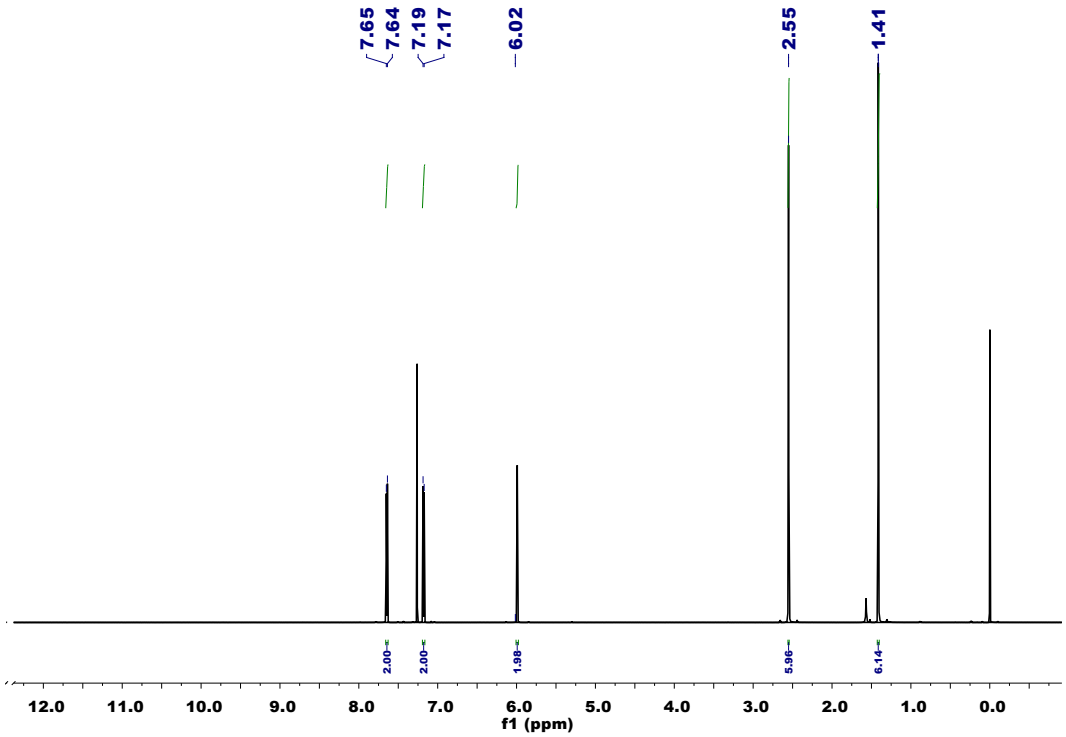


Figure S14. ^{1}H NMR spectrum of BODIPY-Br in $CDCl_3$

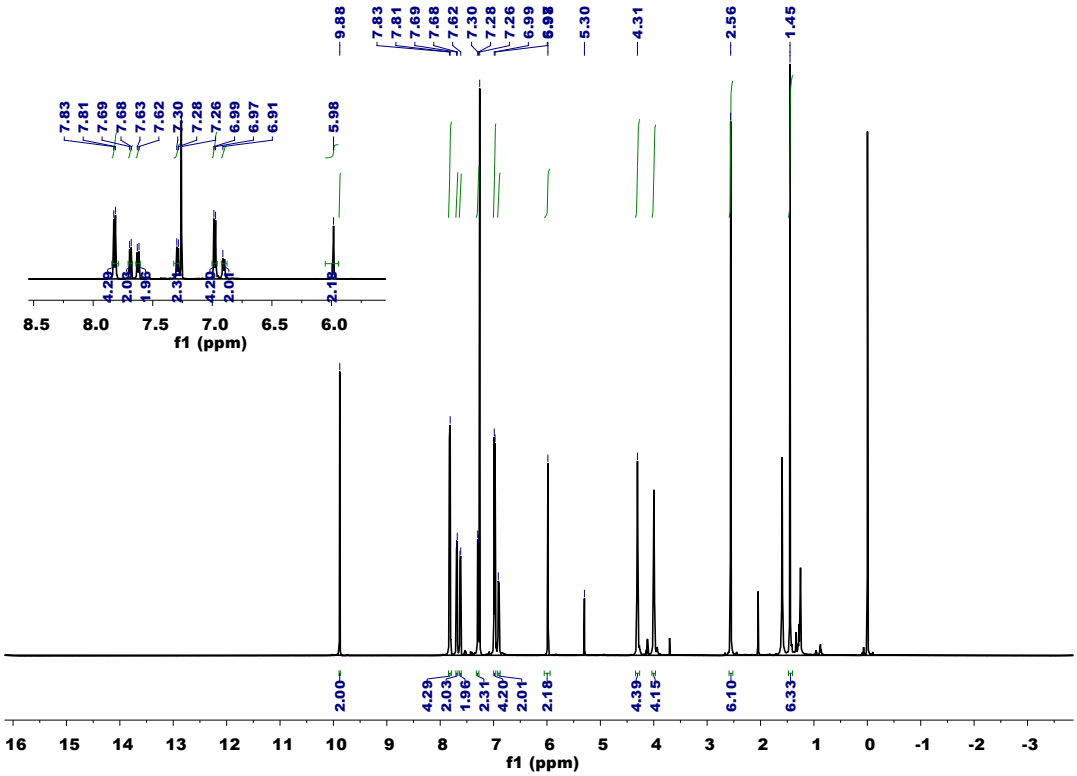


Figure S15. ^{1}H NMR spectrum of compound **d** in $CDCl_3$

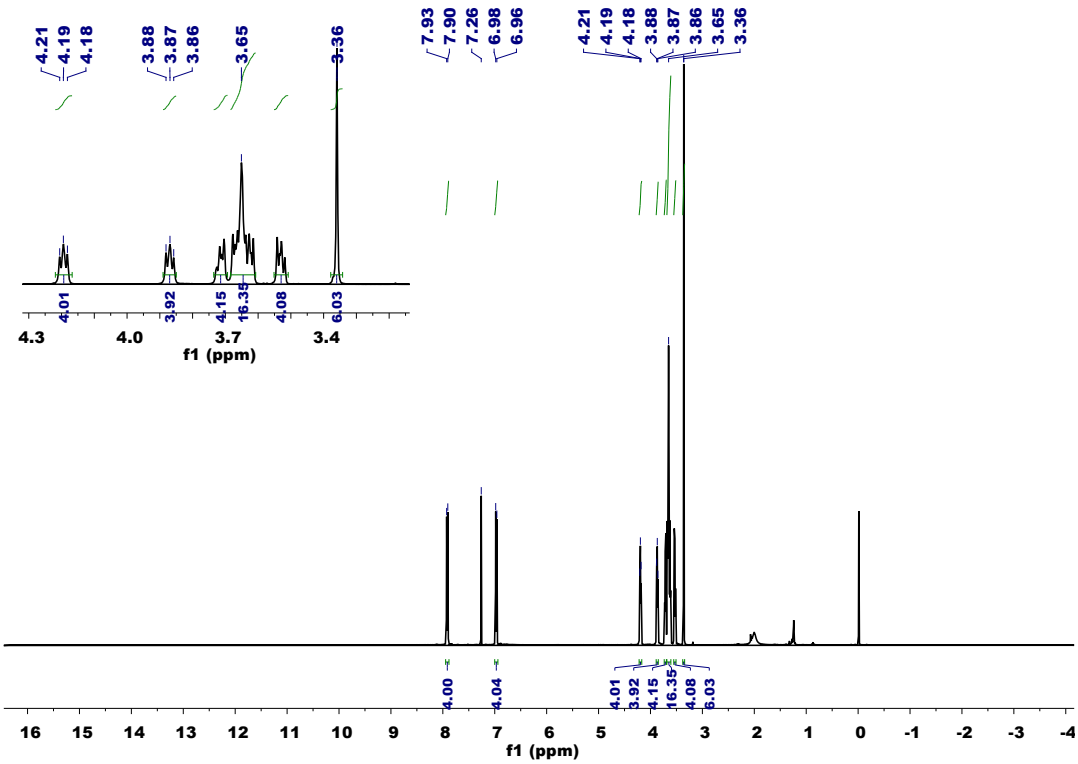


Figure S16. ^{1}H NMR spectrum of compound **f** in $CDCl_3$

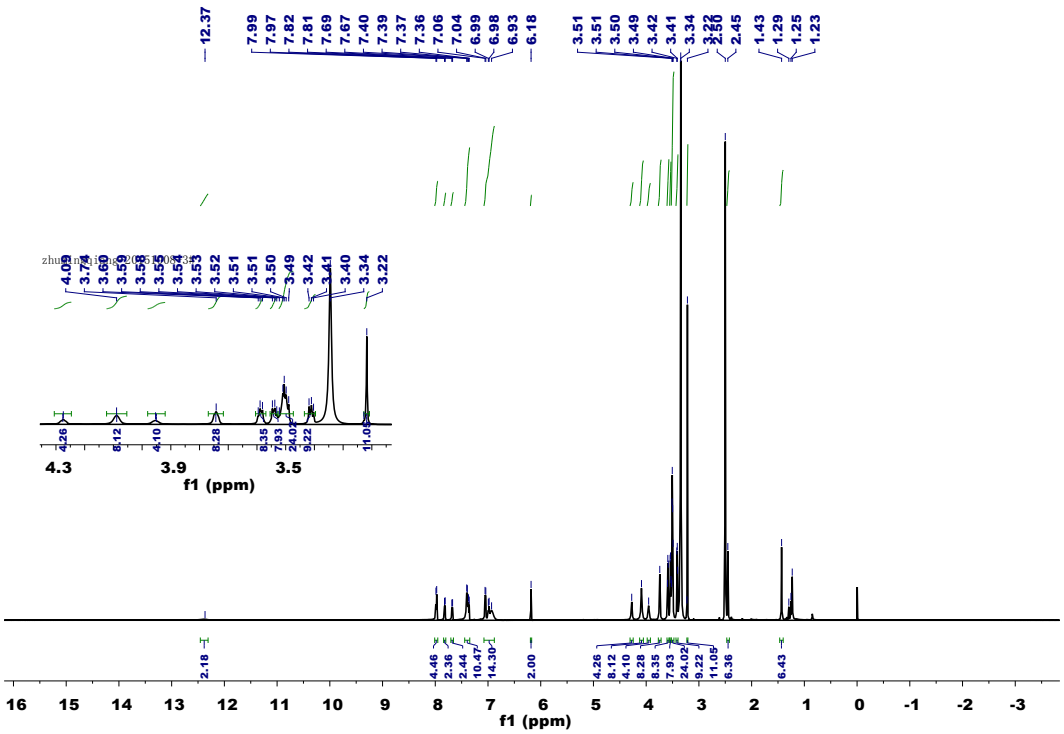


Figure S17. ^{1}H NMR spectrum of compound **g** in d^{6}-DMSO

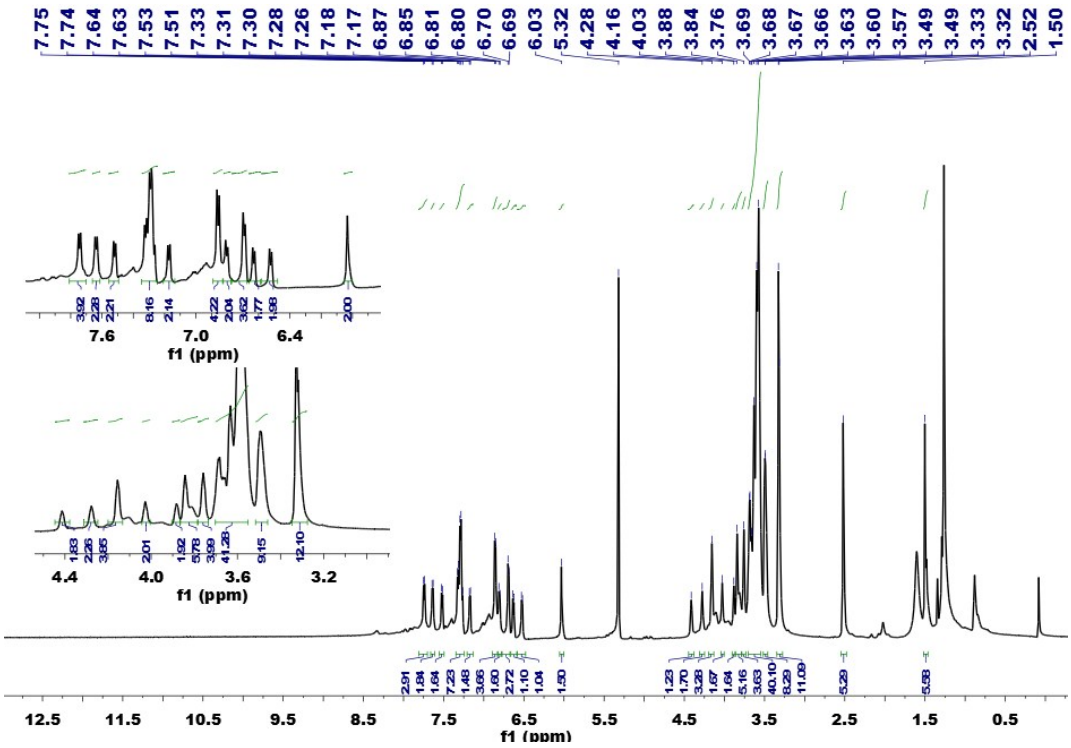


Figure S18. ^{1}H NMR spectrum of **mTEG-HABI-BODIPY** in CD_2Cl_2

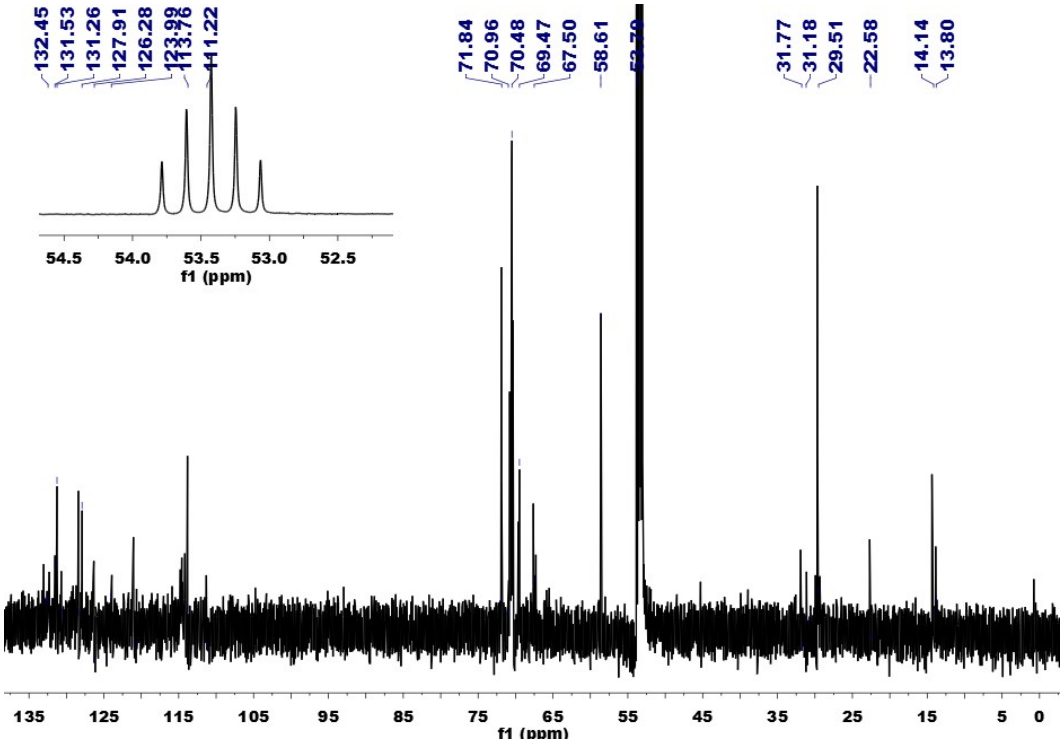


Figure S19. ^{13}C NMR spectrum of **mTEG-HABI-BODIPY** in CD_2Cl_2

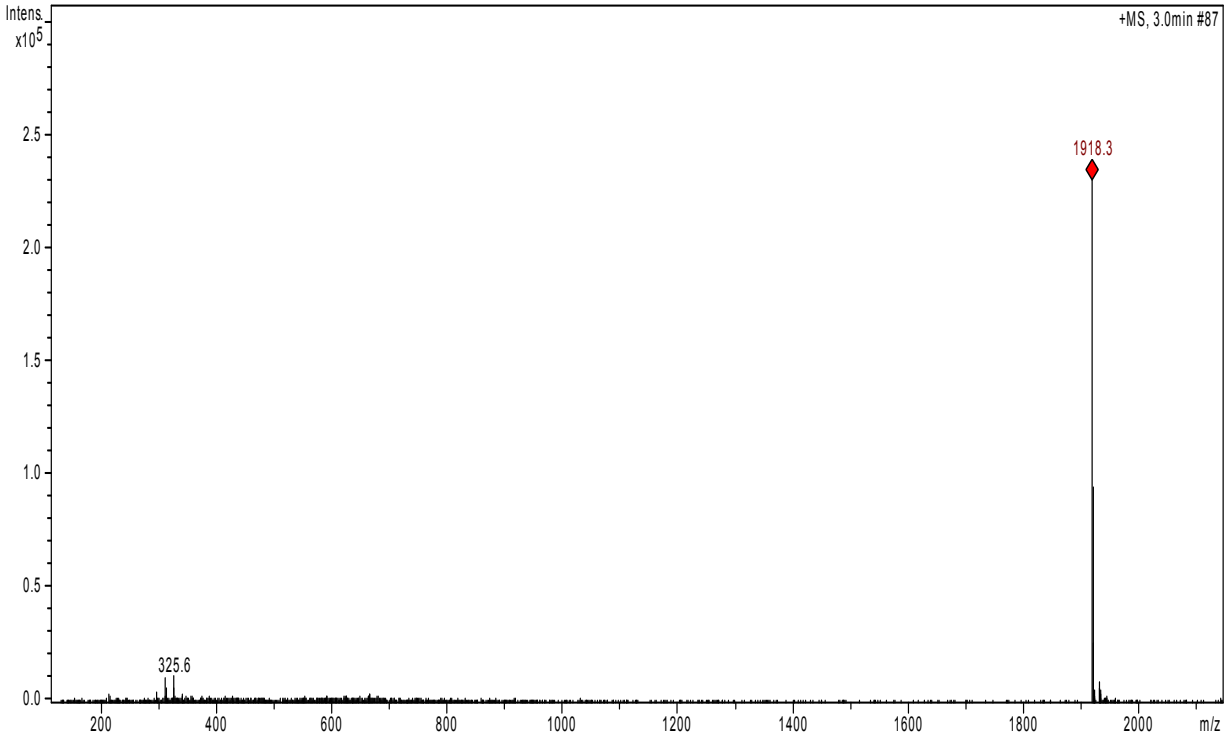


Figure S20. LC-MS spectra of **mTEG-2TPI-BODIPY**

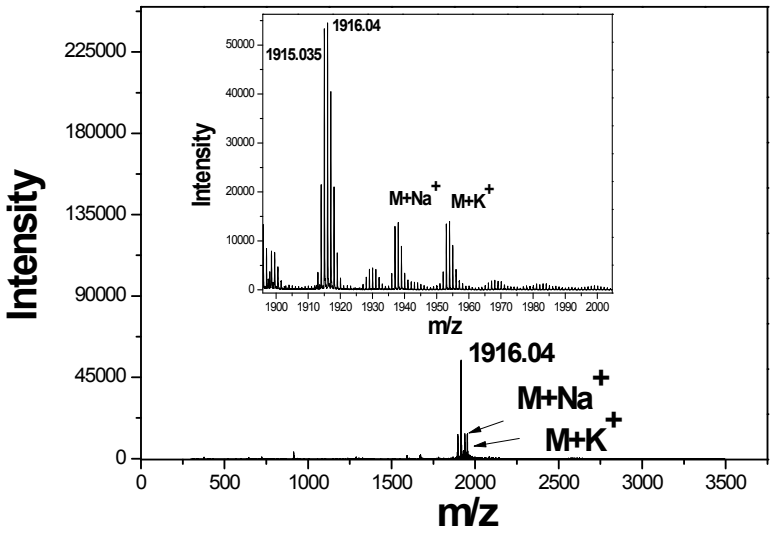


Figure S21. MALDI-TOF spectrum of **mTEG-HABI-BODIPY**

10. References

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