Supporting Information

Thiol-containing hyperbranched polysiloxane for scavenging reactive oxygen species

Rui Wu,‡ Sixian Lian,‡ Yanyun He, Zheng Li, Weixu Feng, Yan Zhao and Hongxia Yan*

Key Laboratory of Special Functional and Smart Polymer Materials of Ministry of Industry and Information Technology, Shaanxi Key Laboratory of Macromolecular Science and Technology, Xi'an Key Laboratory of Hybrid Luminescent Materials and Photonic Device, School of Chemistry and Chemical Engineering, Northwestern Polytechnical University, Xi'an, 710129, China.

‡ These authors contributed equally to this work.
E-mail address: hongxiayan@nwpu.edu.cn

Table of Contents

Experimental section

Synthesis of hyperbranched polysiloxane (HE)

39.27 g (0.2 mol) of MPTMS and 28.97 g (0.47 mol) of EG were placed in a 250 ml three-neck flask. The reaction, stirring, condensation, and collection systems were then sequentially set up. The temperature was slowly heated to 120 ^{o}C in a N_2 atmosphere and maintained for 5 hours. Subsequently, the temperature was raised to 130 ^{o}C and kept for an additional 1 hours. Throughout the process, the distillation temperature was controlled within the range of 42 ^{o}C to 58 ^{o}C. After ceasing heating, the reaction device was allowed to cool to room temperature, the reaction product will be obtained.

Purification process: Same as above HP

Synthesis of hyperbranched polysiloxane (HB)

39.27 g (0.2 mol) of MPTMS and 42.05 g (0.47 mol) of 1,4-BDO were placed in a 250 ml three-neck flask. The reaction, stirring, condensation, and collection systems were then sequentially set up. The temperature was slowly heated to 130 ^{o}C in a N_2 atmosphere and maintained for 3 hours. Subsequently, the temperature was raised to 140 ^{o}C and kept for an additional 2 hours, followed by 2 hours at 150 ^{o}C. Throughout the process, the distillation temperature was controlled within the range of 42 ^{o}C to 58 ^{o}C. After ceasing heating, the reaction device was allowed to cool to room temperature, the reaction product will be obtained.

Purification process: Same as above HP

Determination of dissolved oxygen in water:

Weigh out 250 mg of HP and dissolve it in DMF to a final volume of 25 ml in a volumetric flask, yielding a HP solution at a concentration of 10 mg/ml. Prepare a blank control by pipetting 25 ml of DMF into a 25 ml volumetric flask and adjusting to volume. Measure 0.510 ml of a 30% hydrogen peroxide (H_2O_2) solution (with a molar concentration of 9.79 mol/L) and dilute it with deionized water to a final volume of 100 ml in a volumetric flask, achieving a H_2O_2 solution concentration of 50 mmol/L. For the reaction process: Mix the H_2O_2 solution with the HP solution and the blank control group in a volumetric ratio of 1 (5 ml) to 4 (20 ml). Under room temperature conditions, place the dissolved oxygen probe beneath the surface of the solution to be tested, rotate the probe at a constant rate in the same direction, and simultaneously begin recording the dissolved oxygen content in water in real time. Terminate the measurement when the readings stabilize and no longer change.

Cellular ROS Scavenging Evaluation.

Human Umbilical Vein Endothelial Cells (HUVECs) are cultured with Endothelial Cell Medium (ECM) containing LPS (1 μg/mL) under dark conditions for 24 hours to obtain LPS-pretreated HUVECs. Subsequently, these LPS-pretreated cells are seeded in a 6-well plate format and co-cultured with HP varying concentrations (0.01 mg/ml, 0.02 mg/ml, 0.05 mg/ml, 0.1 mg/ml, 0.5 mg/ml, 1 mg/ml, and 2 mg/ml)

for a period of 24 hours to generate the experimentally challenged cells. DCFH-DA, serving as an oxidative stress indicator, is utilized to assess intracellular ROS levels, diluted in serum-free medium to a final concentration of 10 μM/L with a dilution factor of 1:1000. The culture medium is aspirated, and the diluted DCFH-DA is applied to ensure complete coverage of the cells. Rosup is introduced into the positive control wells as a control benchmark, whereas the remaining wells omit Rosup supplementation.[1] The cells are incubated at 37°C within a cell culture chamber for 20 minutes. After incubation, the cells are washed three times with serum-free cell culture medium to remove any DCFH-DA that has not entered the cells. The ROS positive control is induced to significantly elevate ROS levels after a 25-minute stimulation period. The cells are subsequently harvested and analyzed using a multifunctional microplate reader.

NMR spectra of HE, HP, HB

In ^{1}H NMR spectra of EG (400 MHz, Chloroform-*d*), signals at 3.28 and 3.71 ppm come from protons of 1H(HOCH_2-) and 2H(-CH_2-) respectively. In ^{1}H NMR spectra of MPTMS (400 MHz, Chloroform-*d*), signals at 0.56~0.88, 1.32, 1.50~1.93, 2.36~2.68 and 3.56 ppm are attributed to the protons of 1H(-SiCH_2CH_2-), 2H(HSCH_2-), 3H(-$CH_2CH_2CH_2$-), 4H(-HSCH_2CH_2-), 5H(CH_3O-) respectively. In ^{1}H NMR spectra of HBPSi-EG (400 MHz, Chloroform-*d*), signals at 0.78, 1.09~1.49, 1.50~1.94, 2.36~2.68, 3.31~3.51 and 3.51~3.99 ppm are ascribed to 1H(-SiCH_2CH_2-), 2H(HSCH_2-), 3H(-$CH_2CH_2CH_2$-), 4H(-HSCH_2CH_2-), 6H(-SiOCH_2-) and 5H(HOCH_2-) respectively.

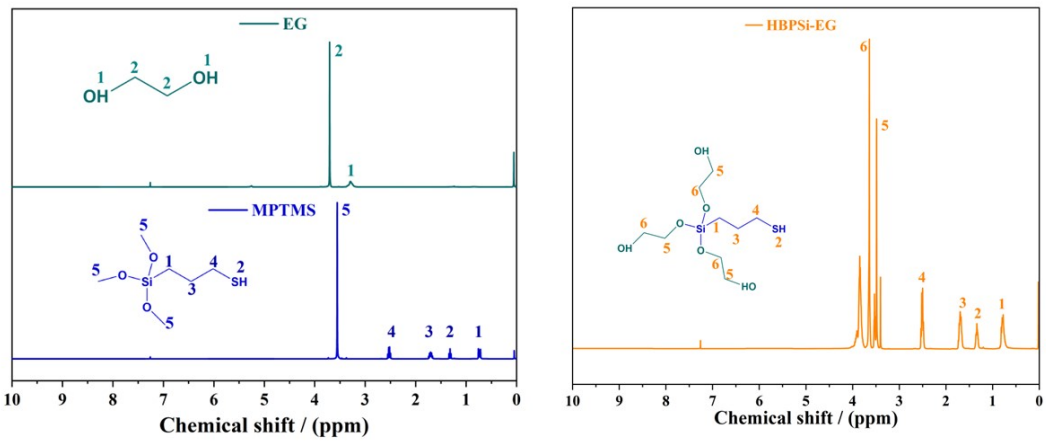

Figure S1 ^{1}H NMR spectra of EG, MPTMS and HE

In the ^{13}C spectra of EG (400 MHz, Chloroform-*d*), signal at 63.81 ppm comes from 1C (-CH_2-). In the ^{13}C spectra of MPTMS (400 MHz, Chloroform-*d*), signal at 8.62, 22.43, 27.50, 50.60 ppm m are attributed to 1C(-SiCH_2CH_2-), 2C(-$CH_2CH_2CH_2$-), 3C(HSCH_2-） and 4C(CH_3O-) respectively. In the ^{13}C spectra of HE (400 MHz, Chloroform-*d*), signal at 13.50, 18.49, 27.50, 58.50, 63.80 ppm are ascribed to 1C(-SiCH_2CH_2-), 2C(-$CH_2CH_2CH_2$-), 3C(HSCH_2-), 4C(-SiOCH_2-), 5C(HOCH_2-)

respectively. ^{1}H and ^{13}C NMR signals further confirmed the successful synthesis of the HE.

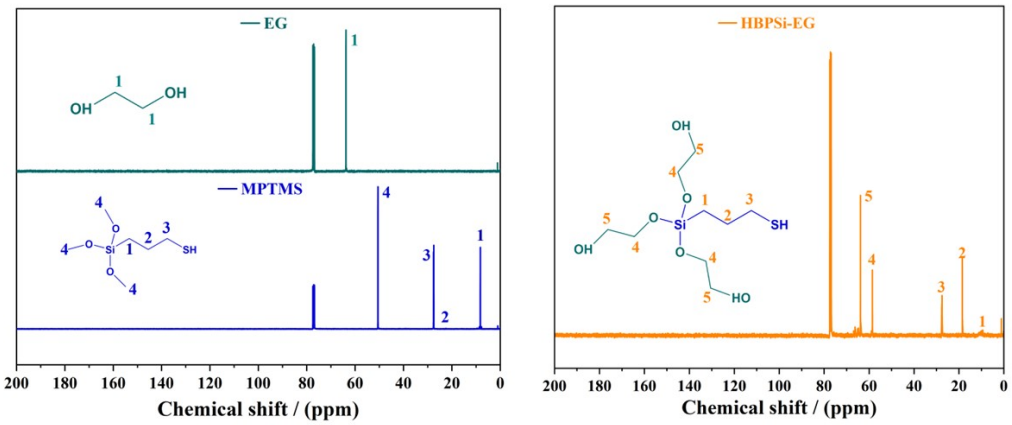

Figure S2 ^{13}C NMR spectra of EG, MPTMS and HE

In the ^{1}H spectra of PDO (400 MHz, Chloroform-*d*), signals at 1.49~1.59, 3.33~3.51, 4.27~4.34 ppm come from 1H(- $CH_2\underline{C}H_2CH_2$-), 2H(HO$\underline{C}H_2$-) and 3H($\underline{H}OCH_2$-) respectively. In the ^{1}H spectra of HP (400 MHz, Chloroform-*d*), signals at 0.77~0.90, 1.14~1.25, 1.58~1.66, 2.53~2.74, 3.67~3.76, 3.76~3.84, 1.77~1.83, 5.15 ppm are attributed to 1H(-SiC$\underline{H}_2CH_2$-), 2H($\underline{H}SCH_2$-), 3H(-$CH_2C\underline{H}_2CH_2SH$), 4H(-HSC$\underline{H}_2CH_2$-), 5H(HOC$\underline{H}_2$-), 6H(-SiOC$\underline{H}_2$-), 7H(-$OCH_2C\underline{H}_2CH_2O$-), 8H($\underline{H}OCH_2$-) respectively.

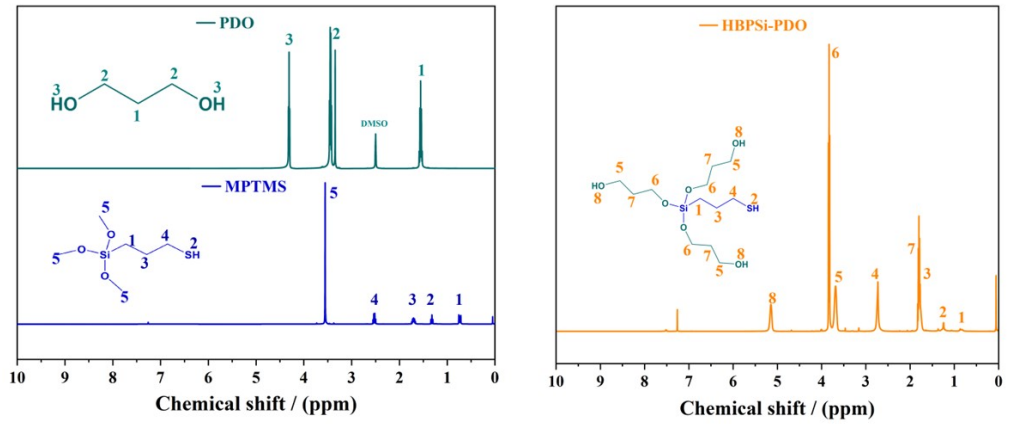

Fig. S3 ^{1}H NMR spectra of PDO, MPTMS and HP

In the ^{13}C spectra of PDO (400 MHz, Chloroform-*d*), signal at 34.17, 59.26~61.96 ppm come from 1C(-$CH_2\underline{C}H_2CH_2$-) and 2C(-$\underline{C}H_2CH_2CH_2$-). In the ^{13}C spectra of HP (400 MHz, Chloroform-*d*), signal at 11.01, 18.49, 27.50, 58.35~59.12, 34.15, 61.27 ppm are ascribed to 1C(-Si$\underline{C}H_2CH_2$-), 2C(-$CH_2\underline{C}H_2CH_2$-), 3C(HS$\underline{C}H_2$-), 4C(-SiO$\underline{C}H_2$-)， 5C(-$CH_2\underline{C}H_2CH_2OH$), 6C(HO$\underline{C}H_2$-) respectively. ^{1}H and ^{13}C NMR signals further confirmed the successful synthesis of the HP.

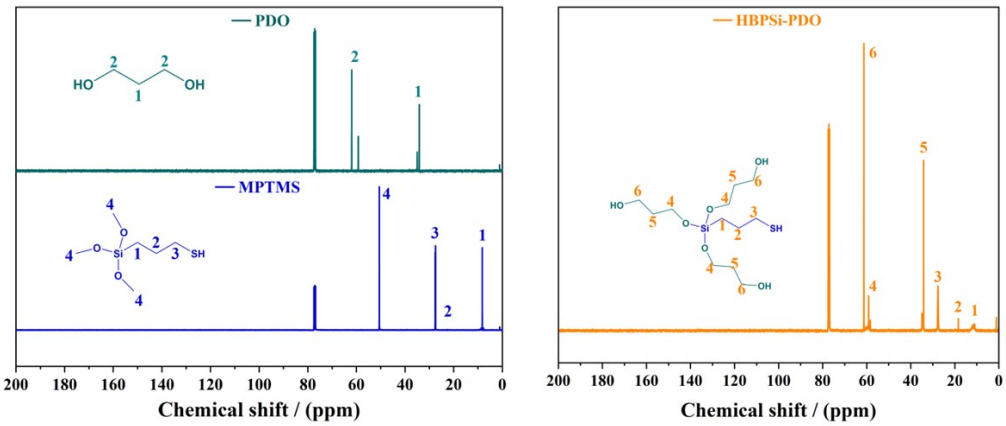

Fig S4 ^{13}C NMR spectra of PDO, MPTMS and HP

In the ^{1}H spectra of BDO (400 MHz, Chloroform-*d*), signals at 1.36~1.71, 3.35~3.54, 3.54~3.75 ppm come from 1H(-$CH_2CH_2\underline{C}H_2CH_2$-), 2H($\underline{H}OCH_2$-) and 3H($HOC\underline{H}_2$-) respectively. In the ^{1}H spectra of HB (400 MHz, Chloroform-*d*), signals at 0.56~0.78, 1.15~1.38, 1.51~1.60, 1.67~1.72, 1.72~1.81, 2.41~2.58, 2.94~3.15, 3.57~3.69, 3.69~3.99 ppm are attributed to 1H(-$SiC\underline{H}_2CH_2$-), 2H($\underline{H}SCH_2$-), 3H(-$CH_2C\underline{H}_2CH_2SH$）, 4H($HOCH_2CH_2C\underline{H}_2CH_2O$-), 5H($HOCH_2C\underline{H}_2CH_2CH_2O$-), 6H(-$HSC\underline{H}_2CH_2$-), 7H($\underline{H}OCH_2$-), 8H($HOC\underline{H}_2$-), 9H($HOCH_2CH_2CH_2C\underline{H}_2O$-) respectively.

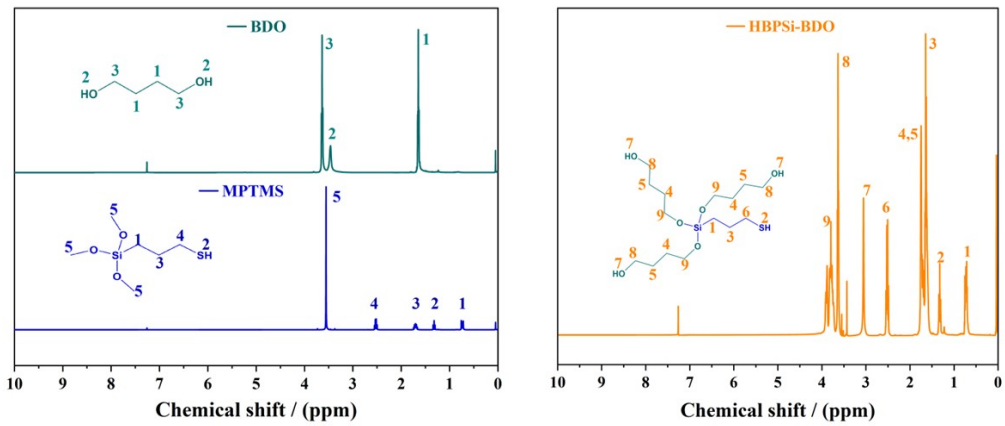

Fig. S5 ^{1}H NMR spectra of BDO, MPTMS and HB

In the ^{13}C spectra of BDO (400 MHz, Chloroform-*d*), signal at 29.95, 62.66 ppm come from， 1C(-$CH_2CH_2\underline{C}H_2CH_2$-) and 2C(-$\underline{C}H_2CH_2CH_2CH_2$-). In the ^{13}C spectra of HB (400 MHz, Chloroform-*d*), signal at 9.04~10.41, 22.76, 27.76, 29.96, 32.50, 62.68, 62.68~64.92 ppm are ascribed to1C(-$Si\underline{C}H_2CH_2CH_2$-), 2C(-$SiCH_2\underline{C}H_2CH_2$-), 3C($HS\underline{C}H_2$-), 4C(-$CH_2CH_2\underline{C}H_2CH_2OH$)， 5C(-$CH_2\underline{C}H_2CH_2CH_2OH$), 6C(-$SiO\underline{C}H_2$-), 7C(-$CH_2CH_2CH_2\underline{C}H_2OH$) respectively. ^{1}H and ^{13}C NMR signals further confirmed the successful synthesis of the HB.

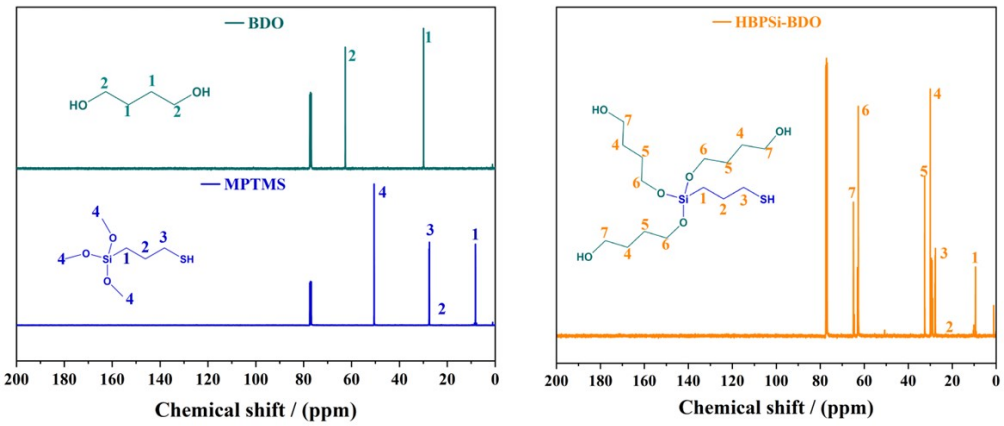

Fig. S6 ^{13}C NMR spectra of BDO, MPTMS and HB

FT-IR of HE, HP, HB

In the FTIR spectrum of MPTMS, EG and HE (Fig. S7a), The absorption peak at 2560 and 460 cm^{-1} belong to the stretching vibration of S-H and asymmetric Si-O-C (for MPTMS). The absorption peak at 3380 and 517 cm^{-1} belongs to the stretching vibration of O-H and the skeleton vibration of C-C (for EG). The absorption peak at 3385, 2560 and 514 cm^{-1} belong to the stretching vibration of O-H, S-H and skeleton vibration of C-C. The absorption peak at 460 cm^{-1} of original asymmetric stretching vibration of Si-O-C on MPTMS weakened and almost disappeared, indicating that MPTMS and EG formed a polymer containing hydroxyl groups. (for HE).

In the FTIR spectrum of MPTMS, PDO and HP (Fig. S7b), the absorption peak at 3320 and 528.54 cm^{-1} belongs to the stretching vibration of O-H and the skeleton vibration of C-C (for PDO). The absorption peak at 3140~3440, 2564 and 524 cm^{-1} belong to the stretching vibration of O-H, S-H and skeleton vibration of C-C. The absorption peak of original asymmetric stretching vibration of Si-O-C on MPTMS weakened and almost disappeared, indicating that the HP was successfully synthesized.

In the FTIR spectrum of MPTMS, PDO and HP (Fig. S7c), the absorption peak at 3315 and 450 cm^{-1} belongs to the stretching vibration of O-H and the skeleton vibration of C-C (for BDO). The absorption peak at 3361, 2560 and 524 cm^{-1} belong to the stretching vibration of O-H, S-H and skeleton vibration of C-C. The absorption peak of the peak at 811cm^{-1} weakens or even disappears, indicating that Si-O-C in MPTMS is replaced by BDO, and the HP was successfully synthesized.

Fig. S7d shows the comparison of the distillates and methanol, the absorption peak positions of each distillate and methanol are almost the same, the peak around 3350 cm^{-1} is the stretching vibration peak of O-H in methanol, the peak at 2940 cm^{-1} is the asymmetric stretching vibration of methyl group, the peak at 2830 cm^{-1} is the symmetric stretching vibration of methyl group, and the peak at 1450 cm^{-1} is the stretching vibration peak of methyl group. Asymmetric deformation of the base, the peak at 1030 cm^{-1} is the C-C stretching vibration, so it can be judged that the distillate is methanol.

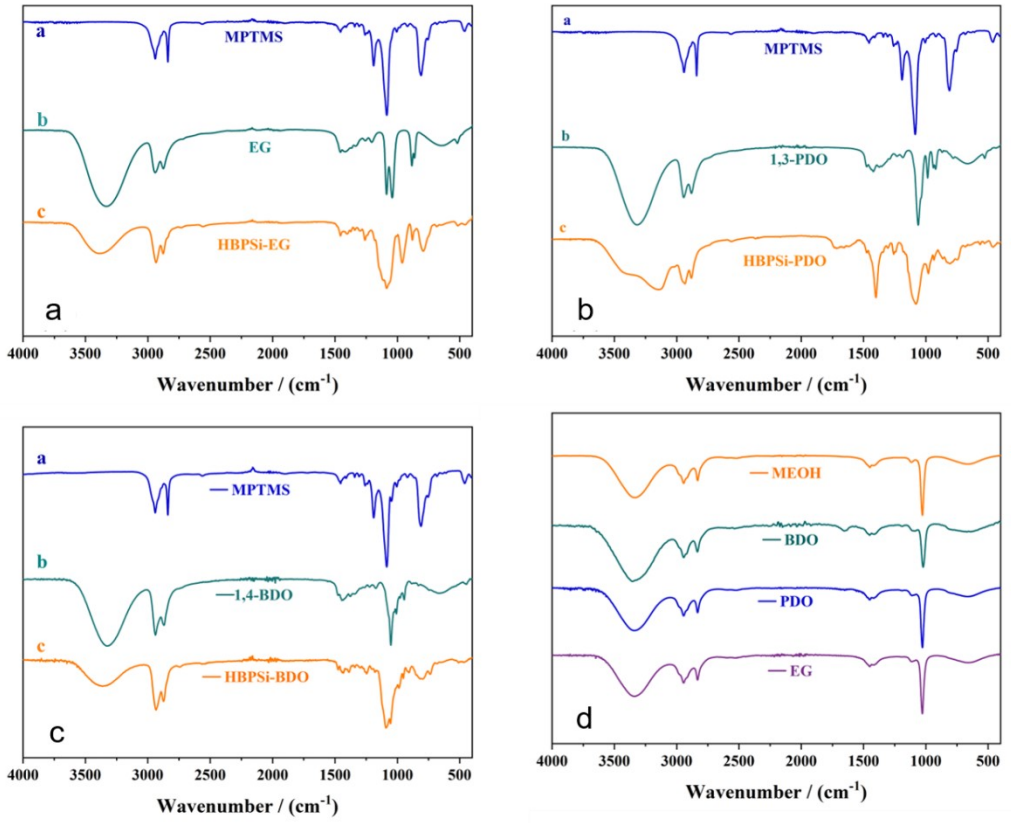

Fig. S7 FT-IR spectra of HE (a), HP (b), HB (c), FT-IR spectra of distillate and standard methanol(d).

GPC curves

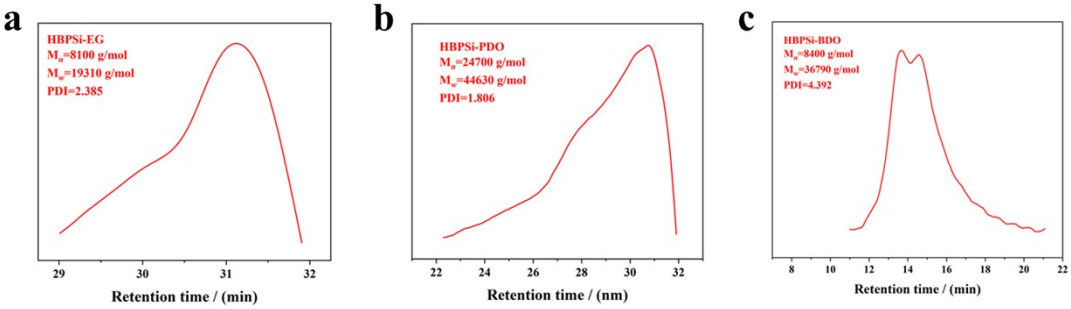

Fig. S8 GPC curves of HE(a), HP(b) and HB(c).

UV-vis absorption

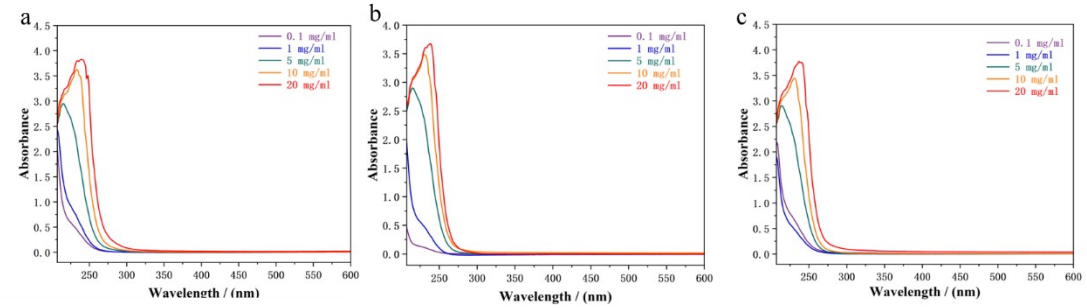

Fig. S9 UV-vis absorption of HBPSi ethanol solutions at different concentrations HE (a), HP (b), HB (c).

Quantum yield and Fluorescence lifetime

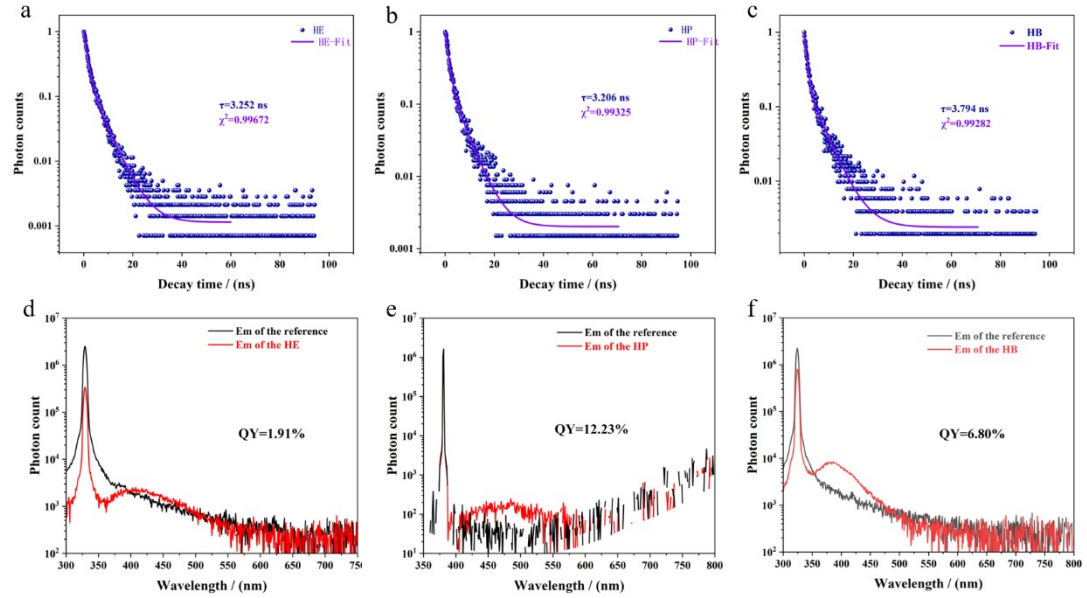

Fig. S10 Absolute quantum yield of pure HE (a), HP (b), HB (c). Fluorescence lifetime of HE (d), HP (e), HB (f).

DLS

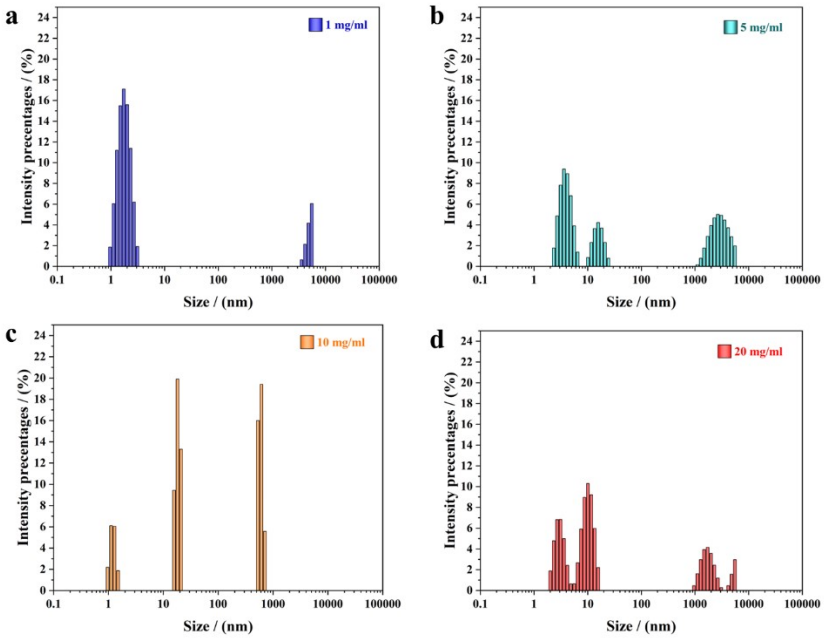

Fig. S11 DLS of HP in different concentrations, 1 mg/ml (a), 5 mg/ml (b), 10 mg/ml (c), 20 mg/ml (d).

TEM

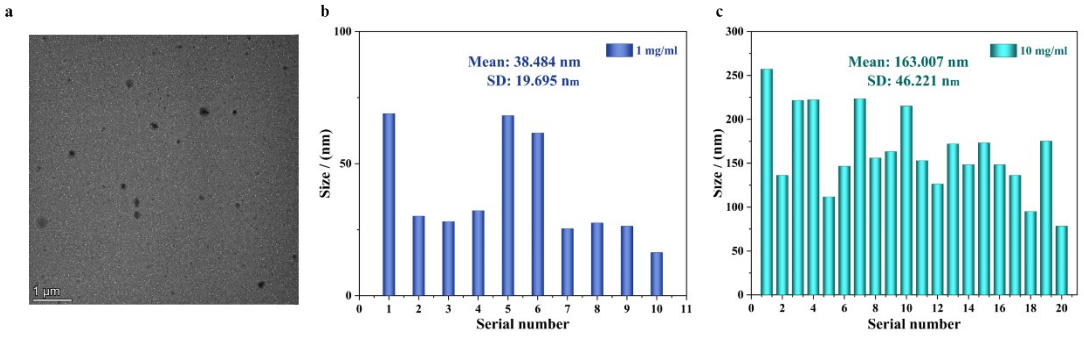

Fig. S12 (a)TEM image of HP aggregates (1mg/mL), Particle size measured by ImageJ 1mg ml^{-1}(b) and 10 mg ml^{-1} (c).

Table S1 Particle size of HP at 1 mg/ml.

Label	Area	Mean	Min	Max	Angle	Length
1	234.95	73.88	10.73	111.00	-58.57	68.94
2	106.79	78.78	26.07	149.59	-40.60	30.13

3	106.79	61.85	33.00	116.00	-35.54	28.11
4	117.48	88.58	57.58	113.00	-66.04	32.19
5	234.95	43.08	2.64	91.01	-64.65	68.20
6	213.59	64.75	0	137.55	-45.00	61.62
7	96.12	79.10	33.86	164.00	-29.75	25.41
8	96.12	58.17	0	121.11	-69.44	27.56
9	96.12	55.67	0	131.00	-60.26	26.35
10	64.08	32.50	0	102.00	-90.00	16.34
Mean	136.70	63.64	16.39	123.63	-55.98	38.48
SD	64.63	17.27	20.19	22.16	18.27	19.69
Min	64.08	32.50	0	91.01	-90.00	16.34
Max	234.95	88.58	57.58	164.00	-29.75	68.94

Table S2 Particle size of HP at 10 mg/ml.

Label	Area	Mean	Min	Max	Angle	Length
1	1060.44	75.13	3.48	131.19	-7.35	257.19
2	563.36	68.67	15.21	146.24	-3.37	136.23
3	911.32	62.32	0.21	110.59	-4.24	221.60
4	927.89	64.63	4.14	139.17	-7.39	222.41
5	463.94	59.29	8.03	101.83	-10.49	111.77
6	613.07	83.39	45.62	126.93	-9.46	146.70
7	927.89	82.19	8.94	137.55	-8.43	223.55
8	646.21	90.82	27.72	145.15	-29.19	156.00
9	679.35	91.49	42.06	136.19	-52.00	163.24
10	894.75	102.01	11.29	142.66	-8.58	215.15
11	646.21	92.65	31.58	139.91	0	152.96
12	530.22	104.53	57.07	150.06	-19.03	126.33
13	712.49	100.76	50.12	135.93	-20.56	172.07
14	613.07	109.19	63.09	161.79	-23.81	148.41
15	729.06	107.87	37.33	179.57	-12.09	173.33
16	613.07	108.28	45.53	159.72	-14.42	148.41
17	563.36	107.27	31.75	185.39	-3.47	136.23
18	397.67	99.18	42.67	147.36	-25.46	95.01
19	729.06	96.80	0.54	195.93	-13.39	175.19
20	331.39	92.25	52.12	146.83	-130.60	78.35
Mean	677.69	89.94	28.93	146.00	-20.17	163.01
SD	190.06	16.34	20.75	22.69	28.57	46.22
Min	331.39	59.29	0.21	101.83	-130.60	78.35
Max	1060.44	109.19	63.09	195.93	0	257.19

Fluorescence inverted microscopy

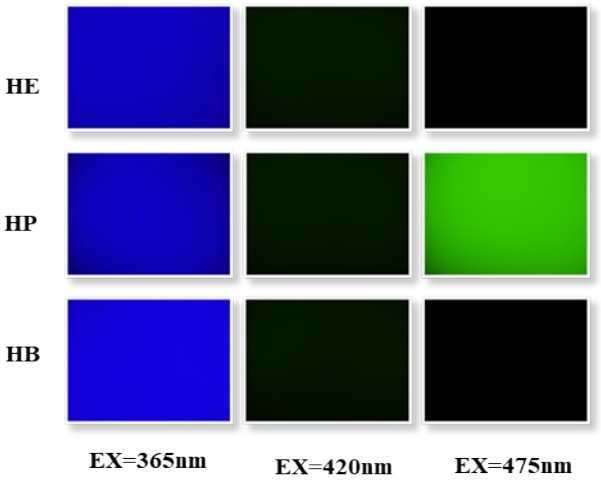

Fig. S13 Fluorescence inverted microscope images of pure HE, HP, and HB.

DFT theoretical calculations

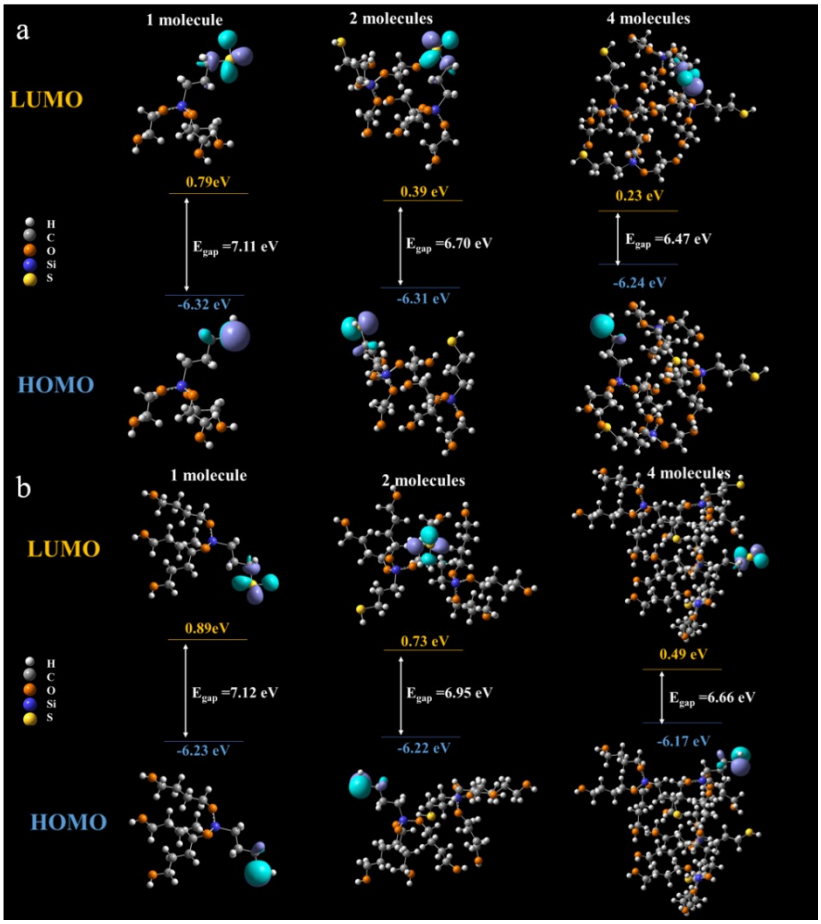

Fig. S14 DFT theoretical calculations of HE(a), HB(b).[2]

Table S3 DFT calculation results of HOMO–LUMO energy levels of the conformations with various molecules.

HE

Molecule number	E(HOMO) / a.u.	E(LUMO) / a.u.	Energy gap / a.u.	Energy gap / eV
1	-0.23217	0.02919	0.26136	7.11197
2	-0.23184	0.01460	0.24644	6.70597
4	-0.22402	0.00025	0.22427	6.46815

HP

Molecule number	E(HOMO) / a.u.	E(LUMO) / a.u.	Energy gap / a.u.	Energy gap / eV
1	-0.22865	0.03263	0.26128	7.10979
2	-0.21516	0.02887	0.24403	6.64039
4	-0.21880	0.00671	0.22551	6.136442

HB

Molecule number	E(HOMO) / a.u.	E(LUMO) / a.u.	Energy gap / a.u.	Energy gap / eV
1	-0.22886	0.03279	0.26165	7.11986281
2	-0.22868	0.02674	0.25542	6.950335788
4	-0.22676	0.01828	0.24504	6.667881456

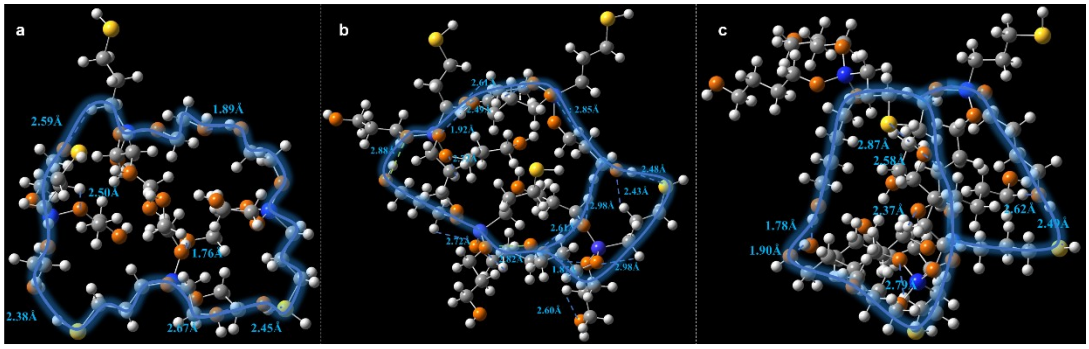

Fig.S15 The spatial conjugated rings formed by HE(a), HP(b), and HB(c).

References

1 P Sun, H-C Chen, W Guo, Z Zhang, S Sun, N Gao, Y-H Jing and B Wang, *J. Mater. Chem. B,* 2024, 12, 5619.

2 Frisch, M. J. et al. Gaussian 09, Revision D.01. (Gaussian Inc., Wallingford CT, 2013).