Electronic Supplementary Material (ESI) for Materials Chemistry Frontiers.

Supporting Information

Luminescent supramolecular polymer nanoparticles for ratiometric hypoxia sensing, imaging and therapy

1. Materials and methods

Unless otherwise noted, materials were obtained from commercial suppliers and used without further purification. 2,3,4,5,6-pentafluorobenzaldehyde (> 96.0%), pyrrole (> 99%), 4-hydroxybenzaldehyde (> 98.0%) and acryloyl chloride (> 98%) were purchased from TCI. $PtCl_2$ (> 73%) was purchased from Acros. UPy-SH was synthesized according to the published procedure.[1]

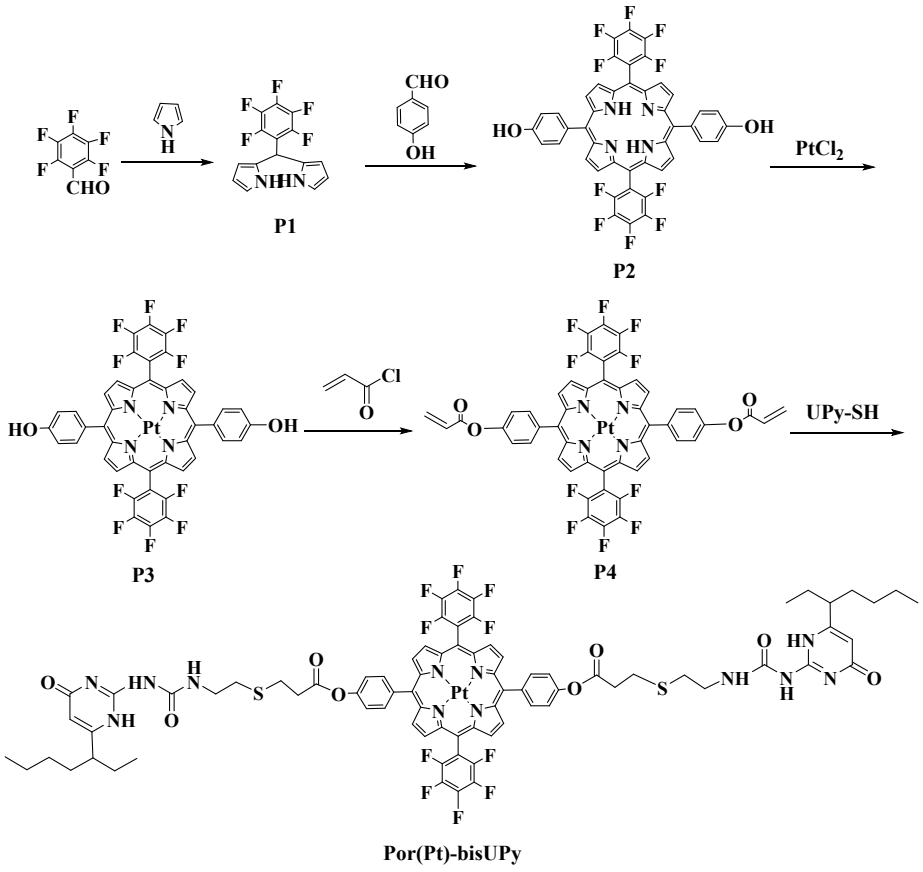
NMR spectra were recorded with a Bruker Avance 400 spectrometer. High-resolution mass spectra were recorded using a Bruker Daltonics Apex IV Fourier Transform Mass Spectrometer. Absorption spectra were recorded on a Hitachi U-3900 UV-visible spectrophotometer. Emission spectra were measured on a Hitachi F-4500 spectrophotometer. Phosphorescence decays were recorded by exciting the sample at 405 nm using a time-correlated single photon counting Edinburgh instrument FLS-920. The values of lifetime were estimated by exponential function fitting with luminescence spectrometer software F900. SEM and TEM images were obtained using a Hitachi S-4800 instrument and a JEOL-2100 microscope with an accelerating voltage of 200 kV, respectively. All SEM samples were coated on silica wafers, and conductivity was enhanced by sputtering thin gold film (3~5 nm) onto their surfaces. TEM samples were prepared by placing several drops of the water-dispersible nanoparticles onto a carbon-coated copper grid. DLS data were collected using Wyatt DynaPro NanoStar (Wyatt Technology) equipped with a gallium-arsenide diode laser (658 nm emission). Hydrodynamic diameters of the nanoparticles were determined by DLS at least 3 times for each sample. Z-potential was measured with Malvern Zetasizer 3000HS. The photostability of the nanoparticles was evaluated by irradiating with a medium pressure Hg lamp (300 W) with glass filter (cutoff < 360 nm) at 6 cm distance. A microplate reader was used for CCK-8 analysis using the absorbance at 450 nm. Confocal fluorescence imaging was recorded with Nikon A1R Eclipse Ti confocal laser scanning microscope (CLSM) with a 60×oil-immersion objective lens and a TDKAI HIT live cell imaging system. The sample was excited with a Si laser at 405 nm. ESR spectra were performed using a Bruker E-500 spectrometer at 9.8 GHz, X-band, with 100 Hz field modulation at room temperature. ESR samples are prepared by injecting the mixture of SPNPs in water

(100 μL, 4.1×10^{-4} M) and TEMP (3 μL) into specially made quartz capillaries, and illuminated by a Xe lamp at > 400 nm.

To access the oxygen sensitivity, the aqueous dispersion of the SPNPs was placed in screw-capped quartz cuvettes and the respective gas mixtures were bubbled through the cuvette for 15 min. Calibration mixtures were produced by mixing nitrogen (99.9999%, Huanyujinghui Gas, Beijing) and O_2 (99.999%, Jumingcheng Gas) using a computer-controlled gas mixing device (XMG Zhinengliuliangyi).

Cell Culture: HeLa cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM), supplemented with 10% fetal bovine serum, penicillin (100 units mL^{-1}), and streptomycin (100 mg mL^{-1}) at 37 °C in a humidified atmosphere of 5% CO_2 for 24 h. The cells were seeded in a glass-bottom cell culture dish (NEST Biotechnology Co. Ltd.) and incubated with SPNPs (1.8×10^{-6} M) in a humidified atmosphere of 5% CO_2 at 37 °C for 2 h. Then, the cells were incubated under 1% or 21% pO_2 for 1 h.

2. Synthesis



Scheme S1. Synthesis of Por(Pt)-bisUPy

Synthesis of **P1**: Pyrrole (5.5 mL, 79.4 mmol) and 2,3,4,5,6-pentafluorobenzaldehyde (2.5 g, 12.7 mmol) were added to a round-bottomed flask with 200 mL of acetonitrile and water (1:1,v/v). 8 drop of concentrated HCl (1.8 mmol) was then added, and the solution was stirred at room temperature for 2 h, the solvent was removed under vacuum. 100 mL of CH_2Cl_2 was then added, the organic phase was washed with water and dried over Na_2SO_4. The residue was purified by column chromatography using petroleum ether: dichloromethane (1:1.5, v/v) as eluent to afford the 1.9 g of product. Yield: 48%. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 5.90 (s, 1H), 6.04(s, 2H), 6.18(m, 2H), 6.72(m, 2H), 8.10(s, 2H).

Synthesis of **P2**[2]: 4-hydroxybenzaldehyde (0.39 g, 3.2 mmol) and **P1** (1.0 g, 3.2 mmol) were added to a dry round-bottomed flask with 300 mL of CH_2Cl_2 and degassed with a stream of N_2 for 10 min. Trifluoroacetic acid (TFA, 0.26 mL, 3.2 mmol) was then added, the solution was stirred under N_2 at room temperature for 24 h, and then 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ, 1.1 g, 4.8 mmol) was added. The mixture was stirred at room temperature for an additional 4 h, then the solvent was removed. The residue was purified by column chromatography using dichloromethane: methanol (100:5, v/v) as eluent to afford 406 mg of the product.Yield: 43%.^{1}H NMR (400MHz, $CDCl_3$): δ (ppm) = 2.82 (s, 2H), 7.232-7.252(d, 4H, J=8.0 Hz), 8.064-8.084(d, 4H, J=8.0 Hz), 8.788-8.799(d, 4H, J=4.4 Hz), 8.974-8.986(d, 4H, J=4.8 Hz).

Synthesis of **P3**:$PtCl_2$ (250 mg, 0.94 mmol) and **P2**(315 mg,0.38 mmol) were added to a 50 mL dry round-bottomed flask with 5 mL of anhydrous benzonitrile. The solution was stirred and refluxed under N_2 at 180 ºC for 12 h. The mixture was then cooled and added to petroleum ether. The precipitate was filtered and purified by column chromatography using dichloromethane: methanol (100:5, v/v) as eluent to afford 144 mg of the product. Yield: 37.2%. ^{1}H NMR (400MHz, $CDCl_3$): δ (ppm) = 7.215-7.235(d, 4H, J=8.0 Hz), 8.007-8.027(d, 4H, J=8.0 Hz), 8.690-8.702(d, 4H, J=4.8 Hz), 8.895-8.907(d, 4H, J=4.8 Hz).

Synthesis of **P4**: To a solution of P3(105 mg, 0.127 mmol) in CH_2Cl_2 (50 mL) was added Et_3N(62 μL, 0.45 mmol). After being stirred at room temperature for 15 min, acryloyl chloride (34.5 μL, 0.38 mmol) was added to the solution under ice bath. The solution was stirred at room temperature for 2 h, then washed with $NaHCO_3$ and

water.The solvents were removed. The residue was purified by column chromatography using petroleum ether and ethyl acetate (2.5:0.5, v/v) as eluent to afford 0.09 g of the product. Yield: 63%. ^{1}H NMR (400 MHz, $CDCl_3$): δ(ppm)=6.15-6.18(d, 2 H, J=11.2 Hz), 6.48-6.55(m, 2 H), 6.77-6.82(d, 2 H, J=18.0 Hz), 7.56-7.58(d, 4 H, J=8.4 Hz), 8.18-8.20(d, 4 H, J=8.4 Hz), 8.72-8.73(d, 4 H, J=5.2 Hz), 8.89-8.91(d, 4 H, J=4.8 Hz).

3. Characterization of SPNPs

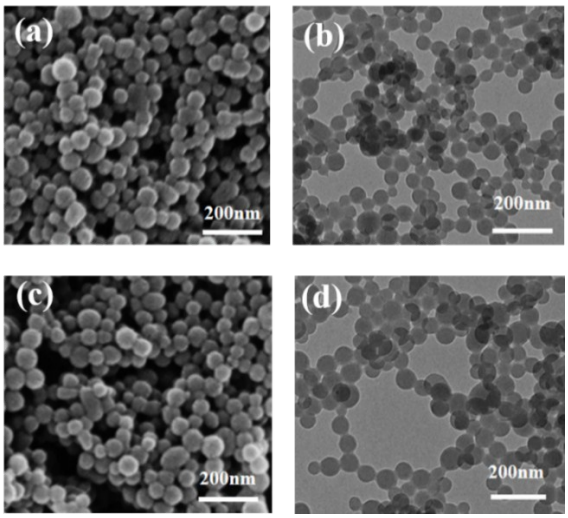


Figure S1. SEM and TEM images of SPNPs dispersed in water with different molar ratios between DPA-bisUPy (donor, D) and Por(Pt)-bisUPy (acceptor, A). [D] to [A] molar ratio is 30 : 1 (a, b) , 60 : 1 (c, d), respectively.

4. Photophysical parameters of the monomers and the SPNPs

Table S1. Photophysical parameters of the monomers and the SPNPs

	λ_{abs}[nm][a)]	ε[$M^{-1}cm^{-1}$][b)]	λ_{em}[nm]	Φ(%)	τ
DPA-bisUPy	375	4.8×10^4	450	90	5.4 ns [e)]
Por(Pt)-bisUPy	398, 510, 542	2.4×10^5 (398 nm)	660	0.4[c)]	36.4 μs[f)]
DPA-bisUPy Nanoparticle	378	9.1×10^3	440	11.9	1.6 ns [g)]
SPNPs in aqueous solution (D/A=40:1)	375, 400, 510, 542		440, 660	5.7[d)]	2.8 ns [h)] 70.3 μs[i)] 32.3 μs[j)] 15.7 μs[k)]

[a)]In chloroform (1.0×10^{-5}M); [b)]Molar extinction coefficient at the absorption maxima ε: $M^{-1}cm^{-1}$; [c)]In nitrogen saturated chloroform, with $[Ru(bpy)_3]Cl_2$ as reference; [d)]In deoxygenated aqueous solution, with $[Ru(bpy)_3]Cl_2$ as reference (Φ=0.094 in deoxygenated MeCN)[3]; [e)] Decay at 450 nm

in chloroform, quoted from reference 4; [f] Decay at 660 nm in nitrogen saturated chloroform; [g] Decay at 440 nm in chloroform; [h] Decay at 440 nm; [i] Decay at 660 nm in nitrogen saturated aqueous solution; [j] Decay at 660 nm in air saturated aqueous solution; [k] Decay at 660 nm in oxygen saturated aqueous solution.

5. Energy Transfer Calculations

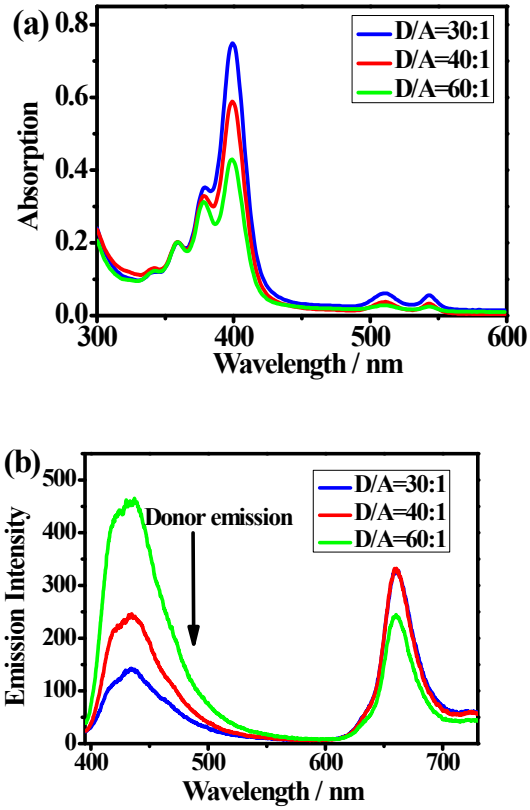


Figure S2. The absorption spectra (a) and emission spectra (b) of SPNPs-dispersed aqueous solution with different molar ratios between DPA-bisUPy (donor, D) and Por(Pt)-bisUPy (acceptor, A). [D] to [A] molar ratio is 30 : 1, 40 : 1, 60 : 1, respectively. (λ_{ex} = 375 nm) The concentration of the SPNPs based on DPA-bisUPy is 2.7×10^{-5} M.

The fluorinated phenyl of Pt(II) porphyrin was selected as phosphorescent indicator due to its good photostability and moderate phosphorescence quantum yield. DPA-bisUPy was employed due to the good spectral separation between its fluorescence and phosphorescence of Por(Pt)-bisUPy, and good emission overlap with the absorption of the phosphorescent indicator. These would facilitate ratiometric luminescence quantification and bright phosphorescence owing to the efficient energy transfer from the fluorescence part to the phosphorescence dye. SPNPs with different ratios of DPA-bisUPy/Por(Pt)-bisUPy were prepared to study the FRET process.

In order to estimate the Förster radius of donor-accepter, the spectral overlap integral was calculated using the following equation[5]:

$$J = \int_0^\infty F_D(\lambda)\varepsilon_A(\lambda)\lambda^4 d\lambda \quad (1s)$$

Where λ is the wavelength of light (nm), $\varepsilon_A(\lambda)$ is the molar absorptivity of the acceptor at that wavelength (2.4×10^5 $M^{-1}cm^{-1}$), and $F_D(\lambda)$ is the donor fluorescence spectrum normalized on the wavelength scale according to

$$1 = \int_0^\infty F_D(\lambda)d\lambda \quad (2s)$$

The overlap integral was estimated to be 7.398×10^{14} $M^{-1}cm^{-1}nm^4$ for transfer between DPA-bisUPy and Por(Pt)-bisUPy. The Förster radius can be calculated using the following equation:

$$r_0 = 0.211[J\Phi K^2\eta^{-4}]^{1/6} \quad (3s)$$

k^2 as the orientation value was assumed to be 2/3, the refractive index of water η= 1.333 and fluorescence quantum yield of donor (Φ) in the nanoparticles is 0.119. The Förster radius was thus estimated to be 3.4 nm for transfer between donor and accepter.

The energy transfer efficiency (Φ_{ET}) was calculated by using the following equation:

$$\Phi_{ET} = 1 - I_{DA}/I_D \quad (4s)$$

Where I_D and I_{DA} are the emission intensities of donor in the absence and presence of the acceptor, respectively.

Table S2. Energy transfer efficiency of the SPNPs with different molar ratios

Molar ratio between D: A	Φ_{ET}（λ_{ex}= 375 nm）
D/A=30:1	96.8%
D/A=40:1	94.4%
D/A=60:1	89.3%

6. Oxygen sensing of SPNPs

The sensitivity of the SPNPs toward oxygen can be expressed by the overall quenching response to oxygen

$$Q = \frac{R_{N2} - R_{O2}}{R_{N2}} \times 100\% \qquad (5s)$$

where Q is the quenching efficiency, and R_{N2} and $R_{O2}$are the emission intensity ratios between phosphorescence at 660 nm and fluorescence at 440 nm for the oxygen nanoprobe saturated with N_2 and O_2. The measured Q value for the SPNPs, 82.6%, indicates that this nanoprobe is sensitive to oxygen.

7. The determination of singlet oxygen quantum yield

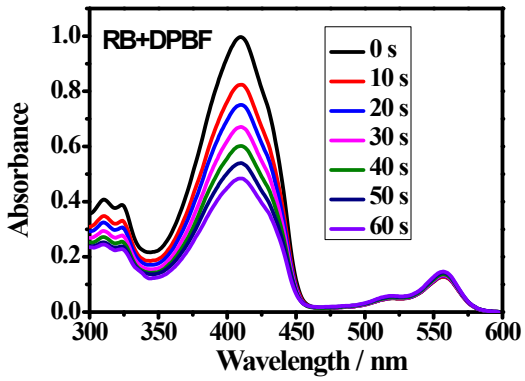


Figure S3. UV−vis absorption spectra of DPBF and RB upon irradiation by a 532 nm laser.

On the other hand, our SPNPs stock solution remained stable over several months of storage at 4 ℃, in which agent leaching was prohibited by the strong quadruple hydrogen-bonding interaction.

8. Cell morphology changes upon irradiation

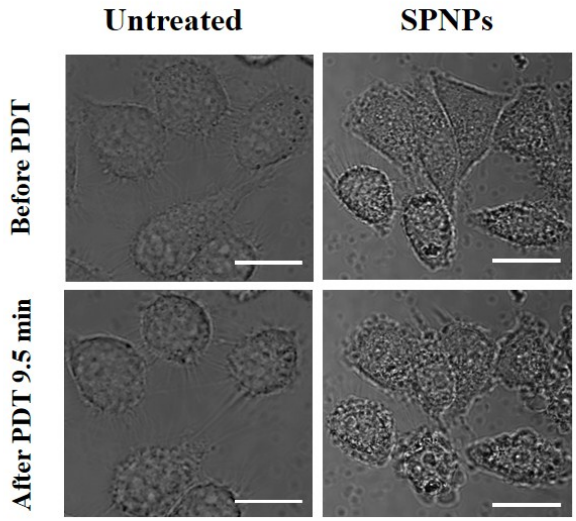


Figure S4. Confocal images of HeLa cells, HeLa cells untreated and HeLa cells incubated with SPNPs irradiated with 514 nm laser for 9.5 min. The incubated concentration based on DPA-bisUPy was 1.6×10^{-6} M in water. Scale bar：20μm.

9. Stability of the SPNPs

The photostability of the SPNPs has been investigated. A water-dispersible SPNPs solution was placed in a cuvette, deoxygenated by bubbling with nitrogen for at least 20 min and then sealed. The samples were continuously illuminated by a mercury lamp (300 W) with a glass filter (cutoff < 360 nm) at 6 cm distance. Emission intensities of the samples at 440 and 660 nm were monitored with the fluorimeter.

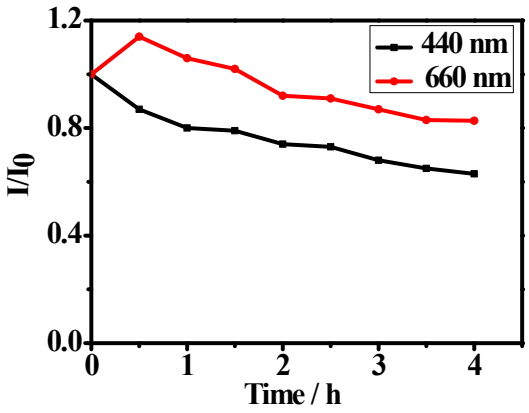
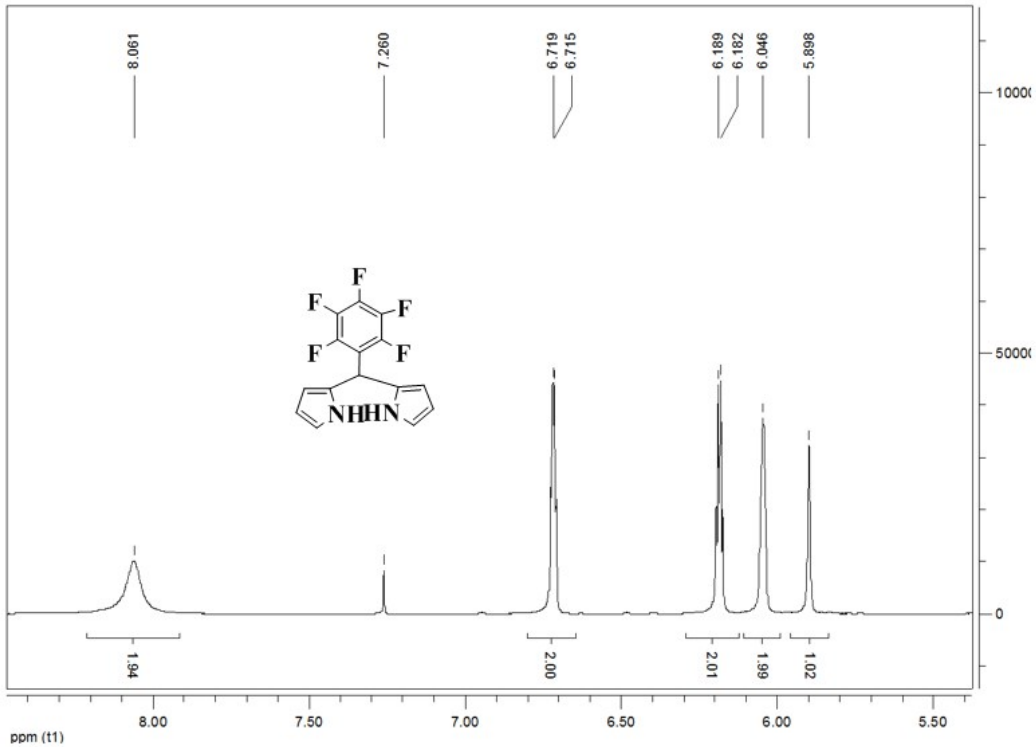


Figure S5. The photostabilities of the SPNPs-dispersed aqueous solution. The samples were continuously irradiated using a 300-W xenon lamp at > 360 nm under

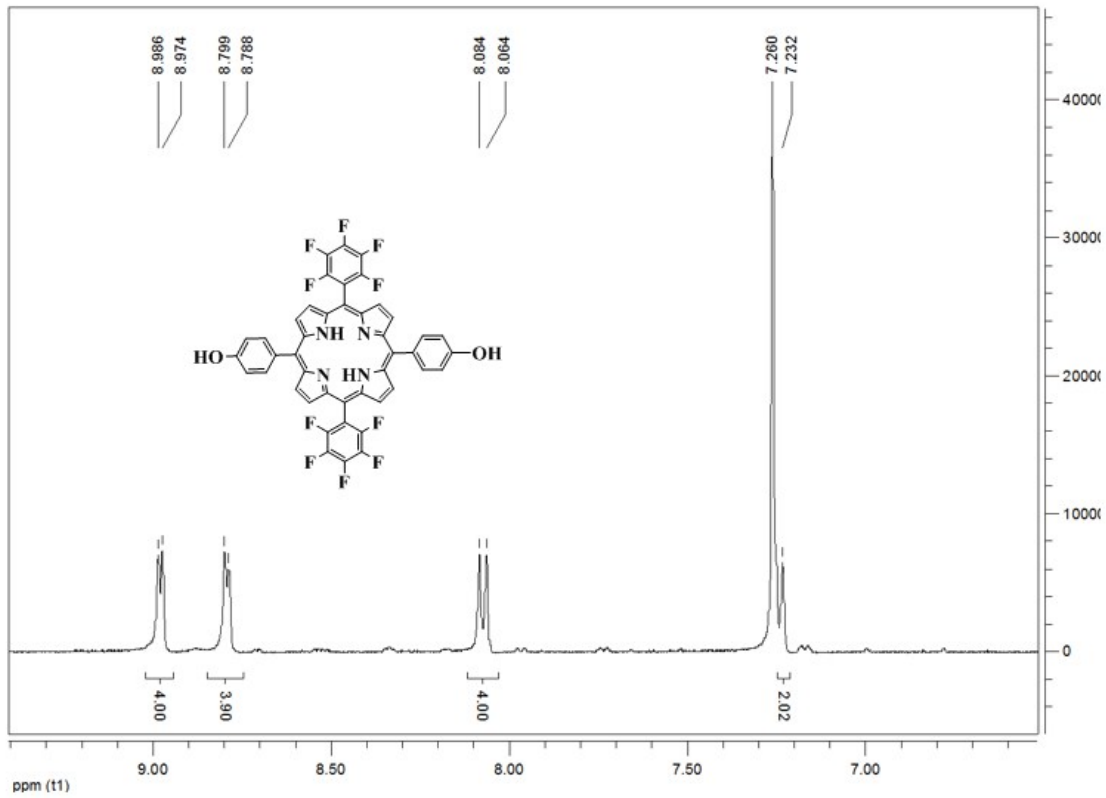
nitrogen atmosphere. I_0 and I are the luminescence intensity of the samples at 440 nm and 660 nm before and after irradiation, respectively.

10. Characterization spectra of key compounds

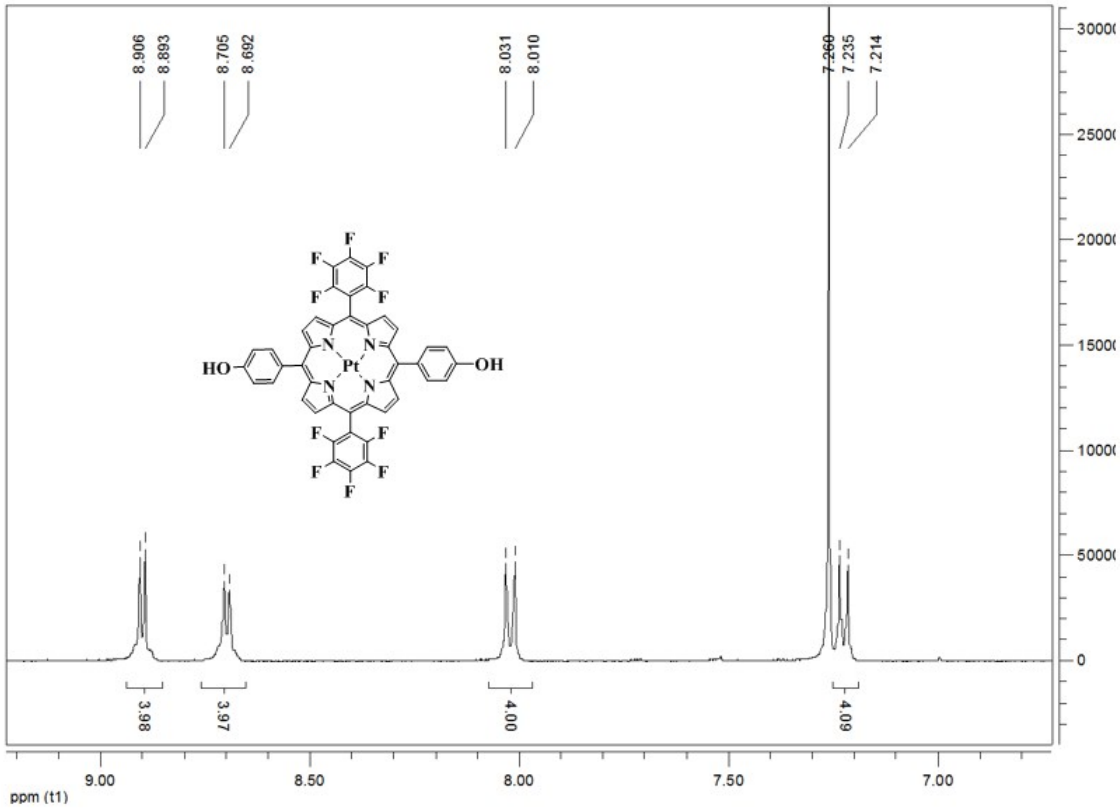
^{1}H NMR of P1



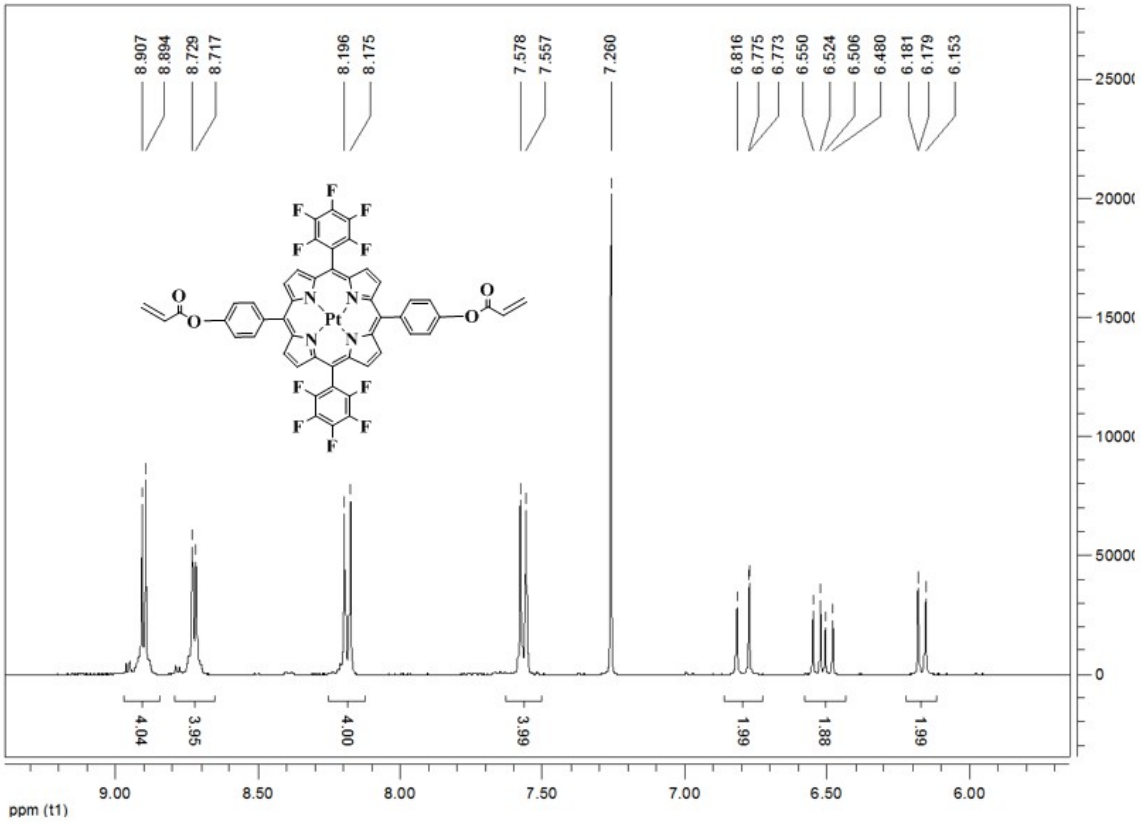
^{1}H NMR of P2



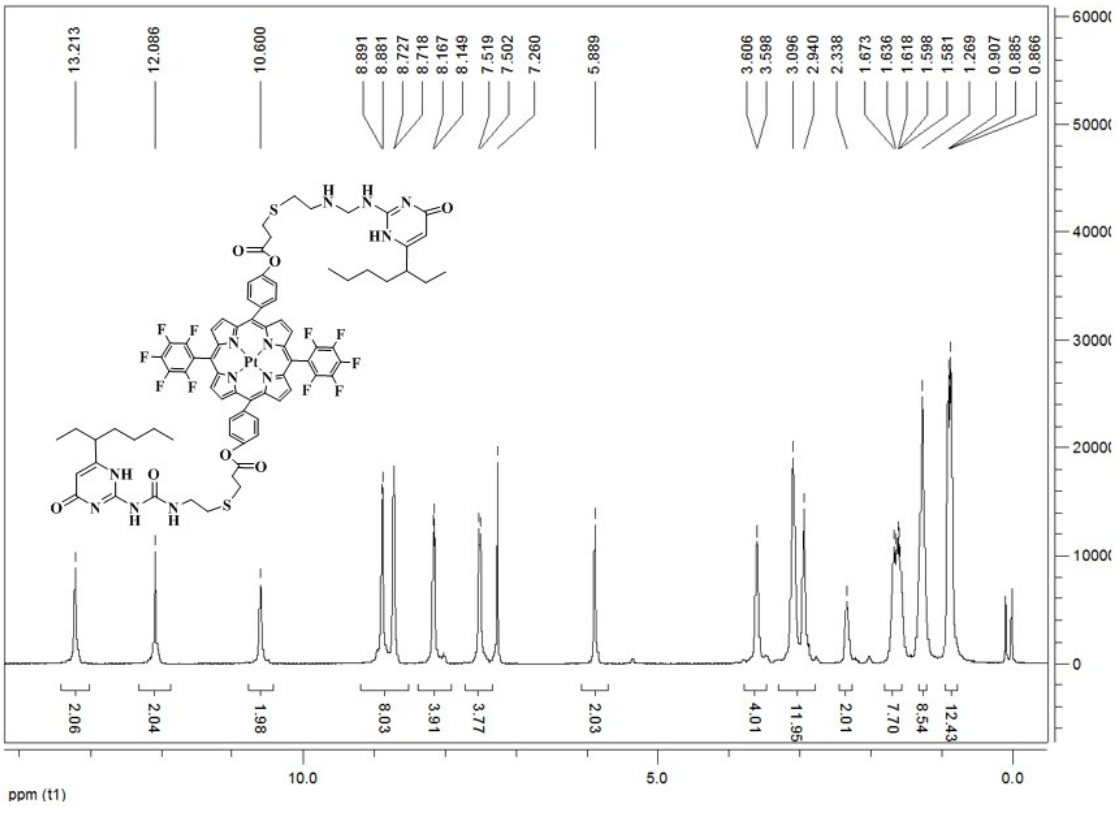
^{1}H NMR of P3

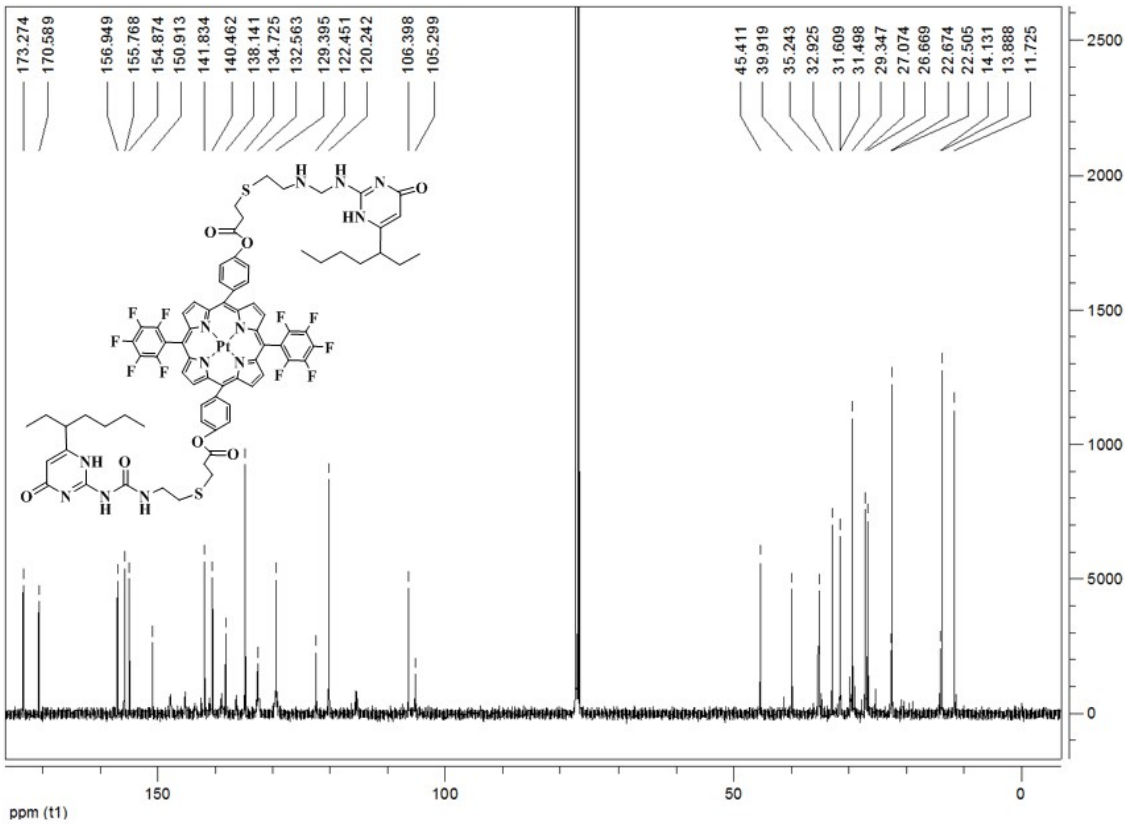


^{1}H NMR of P4



^{1}H NMR, ^{13}C NMR and HRMS of Por(Pt)-bisUPy

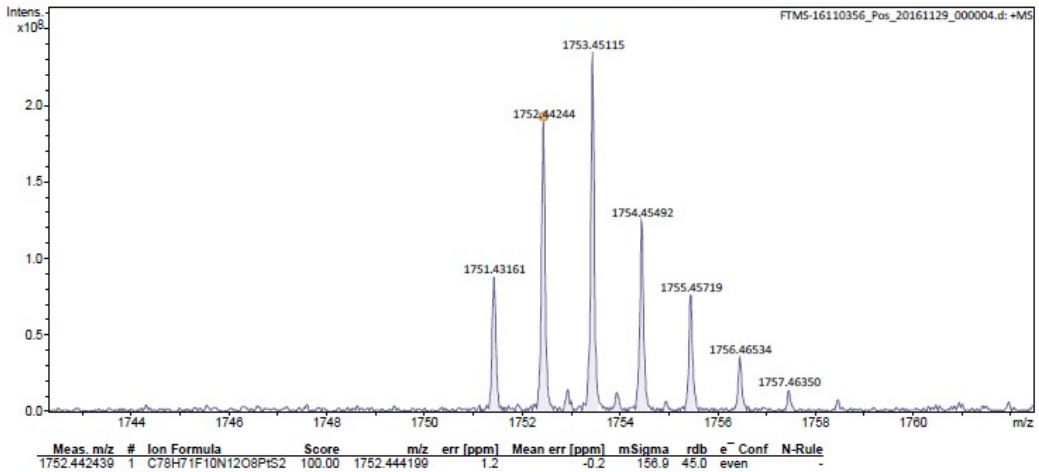




Peking University Mass Spectrometry Sample Analysis Report

Analysis Info

Analysis Name	FTMS-16110356_Pos_20161129_000004.d	Acquisition Date	11/29/2016 10:14:47 AM
Sample	HYY-2016-11-29	Instrument	Bruker Solarix XR FTMS
Comment		Operator	Peking University



Meas. m/z	#	Ion Formula	Score	m/z	err [ppm]	Mean err [ppm]	mSigma	rdb	e⁻ Conf	N-Rule
1752.442439	1	C78H71F10N12O8PtS2	100.00	1752.444199	1.2	-0.2	156.9	45.0	even	-

Bruker Compass DataAnalysis 4.4 printed: 11/29/2016 10:20:22 AM Page 1 of 1

11. References:

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