Electronic Supplementary Information (ESI) for Chemical Communications.

Pyrene-pyridyl nanooligomer as a methoxy-triggered reactive probe for highly specific fluorescence assaying of hypochlorite

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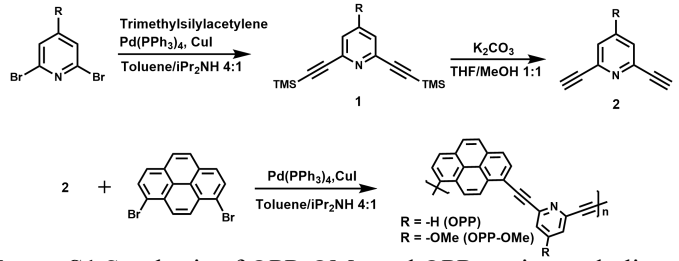
EXPERIMENTAL SECTION

Materials and instruments. 1,8-Dibromopyrene was purchased from Tokyo Chemical Industry (TCI). 2,6-Dibromo-4-methoxypyridine and 2,6-dibromopyridine were obtained from Aladdin (Shanghai, China) and J&K Scientific (Beijing), respectively. Monocarboxyl-terminated poly(ethylene glycol) (PEG-350-COOH) was bought from Shanghai Jinpan Biotech Co., Ltd. Other reagents were commercially obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China) and used without further treatment. The phosphate buffer solutions (PBS, pH 5-8.45) were prepared using 0.02 M Na_2HPO_4 and 0.02 M NaH_2PO_4. The pH of PBS buffer solution was adjusted to 2.5-5 and 8.45-11 using hydrochloric acid (0.1 M) or sodium hydroxide (0.1 M). Ultrapure water used in our work was produced by a Milli-Q system.

The UV-vis absorption spectra were obtained by a UV-2450 spectrophotometer (Shimadzu Co., Japan). The fluorescence spectra were measured using a Hitachi F-7000 fluorometer (Japan). Transmission electron microscope images were obtained on a JEM-2010 (JEOL, Japan) operating at 200 kV. ^{1}H NMR spectra were acquired on a Bruker DRX 500 spectrometer. Fourier transformed infrared (FTIR) spectra were collected on a NEXUS spectrometer. Zeta potentials were measured on a Zetasizer Nano ZS system (Malvern, UK). All pH values were obtained using a PHS-3C (Shanghai Pengshun Scientific Instrument Co., China) pH meter with a combined glass-calomel electrode. The LC-MS analyses were performed using LCMS 8050 mass spectrometer (Shimadzu). High resolution MALDI-TOF mass spectrum was obtained using a Bruker ultrafle Xtreme MALDI-TOF/TOF spectrometer. The confocal microscopy was operated using a Leica TCS SP8 confocal laser scanning fluorescence microscope.



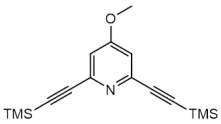
Scheme S1 Synthesis of OPP-OMe and OPP conjugated oligomers.

Preparation of conjugated oligomers (OPP-OMe and OPP). Firstly, compound 1 was prepared according to published literature with minor adjustment.[1] 2,6-Dibromo-4-methoxypyridine (2.0 mmol, 0.5338 g), $Pd(PPh_3)_4$ (0.15 mmol, 0.174 g) and CuI (0.1 mmol, 19.05 mg) were added to a 50 mL three-necked flask under N_2 atmosphere. 10 mL of toluene/diisopropylamine (iPr$_2$NH) (v/v, 4/1) and 2.2 mL trimethylsilylacetylene were then added slowly by a syringe. After stirring at room temperature for 24 h, the mixture was brownish in color. After removing the insoluble matter by centrifugation, the filtrate was concentrated and then purified by column chromatography with petroleum ether/ethyl acetate (PE/EA, v/v, 5/1) to obtain an orange compound 1a. ^{1}H NMR (500 MHz, Chloroform-*d*) δ (ppm): 6.91 (s, 2H), 3.82 (s, 3H), 0.23 (s, 18H). Then, compound 1a was dissolved in a mixed solvent of tetrahydrofuran/methanol (THF/MeOH, v/v, 1/1), anhydrous potassium carbonate (0.0128 mol, 1.77 g) was added, and stirred at room temperature for 12 h. After filtration, the residue was purified by column chromatography (PE/EA, v/v, 5/1) and compound 2a was obtained. ^{1}H NMR (500 MHz, Chloroform-*d*) δ (ppm): 6.99 (s, 2H), 3.86 (s, 3H), 3.12 (s, 2H). Conjugated oligomer (OPP-OMe) was synthesized by a modified Sonogashira coupling reaction reported in the literature.[2] In brief, 1,8-dibromopyrene (0.25 mmol, 90.01 mg), 2,6-bis(ethynyl)-4-methoxypyridine (compound 2a, 0.3 mmol, 47.1 mg), $Pd(PPh_3)_4$ (25.0 μmol, 28.6 mg) and CuI (25.0 μmol, 4.86 mg) were placed in a round-bottom flask. Toluene/iPr$_2$NH (v/v, 4/1) were added under Ar atmosphere. The mixture was stirred at 40 ℃ for 48 h. After cooling, the obtained mixture was filtered and the solvent was removed under reduced pressure. The crude product was purified by column chromatography using DCM/methanol (v/v, 20/1) as the eluent. A brownish black solid was obtained.

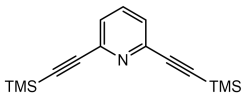
Compounds **1b** and **2b** were prepared according to our published literature.[1] Conjugated oligomer (OPP) was synthesized according to the similar procedure reported above and a brownish-black solid was obtained.

The structure of OPP-OMe and OPP was further confirmed by ^{1}H NMR. OPP-OMe: ^{1}H NMR (500 MHz, Chloroform-*d*) δ (ppm): 7.73 – 7.62 (m, 4H), 7.59 – 7.52 (m, 2H), 7.47 (td, J = 7.7, 2.8 Hz, 4H), 4.75 (s, 3H). OPP: ^{1}H NMR (500 MHz, Chloroform-*d*) δ (ppm): 7.74 – 7.58 (m, 5H), 7.56 – 7.52 (m, 2H), 7.46 (td, J = 7.6, 2.9 Hz, 4H). The molecular weights and possible structures of OPP-OMe were verified by GPC (Figure S2) and LC-MS (Figure S4) analyses. MS (m/z):

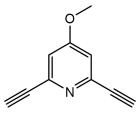
Calcd OPP-OMe $[M+H]^+$, 437.31; found, 437.35. $[M-H]^-$,714.44; found, 714.60. The molecular weights of OPP were also obtained by GPC (Figure S3).



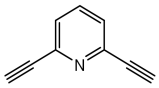
compound **1a**



compound **1b**



compound **2a**



compound **2b**

Preparation and characterization of small-sized OPP-OMe NPs and OPP NPs. OPP-OMe and OPP were encapsulated with an amphiphilic molecule (namely, monocarboxyl-terminated poly(ethylene glycol) via a modified microemulsion method to give the conjugated oligomer nanoparticles (OPP-OMe NPs or OPP NPs) with good water-solubility. Briefly, 0.020 g of PEG-350-COOH was dissolved in ultrapure water (20 mL), and 1.0 mg of OPP-OMe or OPP in CH_2Cl_2 was then added after stirring for 10 minutes under continuous sonication (KQ-250E water bath sonicator, 250 W in Power, Kunshan Ultrasonic Instrument Co., Ltd., China). After sonication for 12 h, the CH_2Cl_2 was removed under reduced pressure at 45 ℃. The aqueous solution was filtered through a filter (Millipore, 0.22 μm), then 50 μg/mL of OPP-OMe NPs or OPP NPs was obtained and stored at 4 ℃.

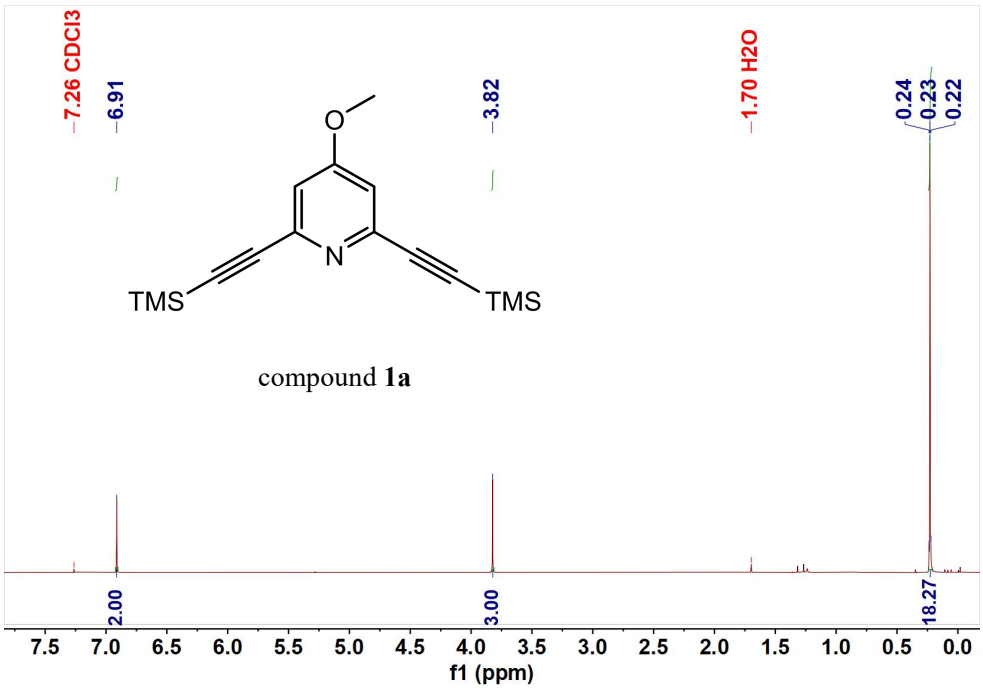
General procedures for spectra measurements. Typically, a freshly prepared OPP-OMe NPs and buffer solution were placed in a quartz cell (10.0 mm width). After introducing analyte, the spectral data of mixture were recorded after ~ 30 s. The concentration of the stock solution of ClO^- or other analyte is 0.01 M. Reactive oxygen and nitrogen species (ROS/RNS) with a concentration of 0.01 M were prepared according to the methods reported in the literature.[3,4]

Cell viability assay. HeLa cells were incubated in DMEM (Dulbecco's modified eagle's medium) containing 10% (v/v) FBS at 37 °C for 24 h in 5% CO_2 atmosphere. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was used to assess the viability of HeLa cells. Firstly, HeLa cells were incubated in a 96-well plate overnight. Then, the cells were washed three times with D-Hank's solution and incubated in DMEM containing OPP-OMe NPs with various concentrations (0-60 μg/mL) for 24 h. Next, the cultured solution was poured out, and 20 μL of MTT (5 mg/mL) solution was added. After 4 h, MTT solution was

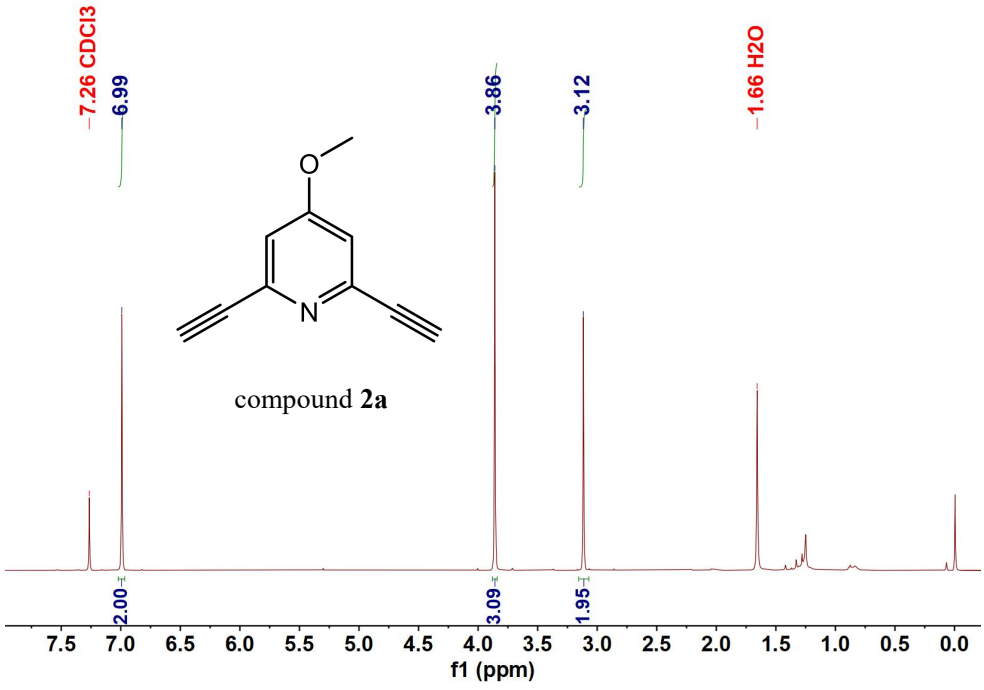
removed and DMSO was put into each well. Finally, the absorbance at 570 nm of each well was monitored in a spectrophotometer after being gently shaken for 10 min.

Cell imaging in vitro. First, three groups of HeLa cells were cultured with OPP-OMe NPs, OPP-OMe NPs/20 μM ClO^-, OPP-OMe NPs/100 μM ClO^- in DMEM (3 mL) for 45 min at 37 °C. Then the cells were washed three times with PBS solution, and the cellular images were acquired by a confocal laser scanning microscope.

Detection of endogenous hypochlorite.[4] Confocal fluorescence images of Hela cells incubated with OPP-OMe NPs and OPP-OMe NPs + LPS (5 μg/mL)/PMA (10 μg/mL) for 45 min at 37 ℃. Fluorescence images obtained via 405 nm excitation. [OPP-OMe NPs] = 22.5 μg/mL. LPS: lipopolysaccharide, PMA: phorbol 12-myristate 13-acetate.



compound **1a**



compound **2a**

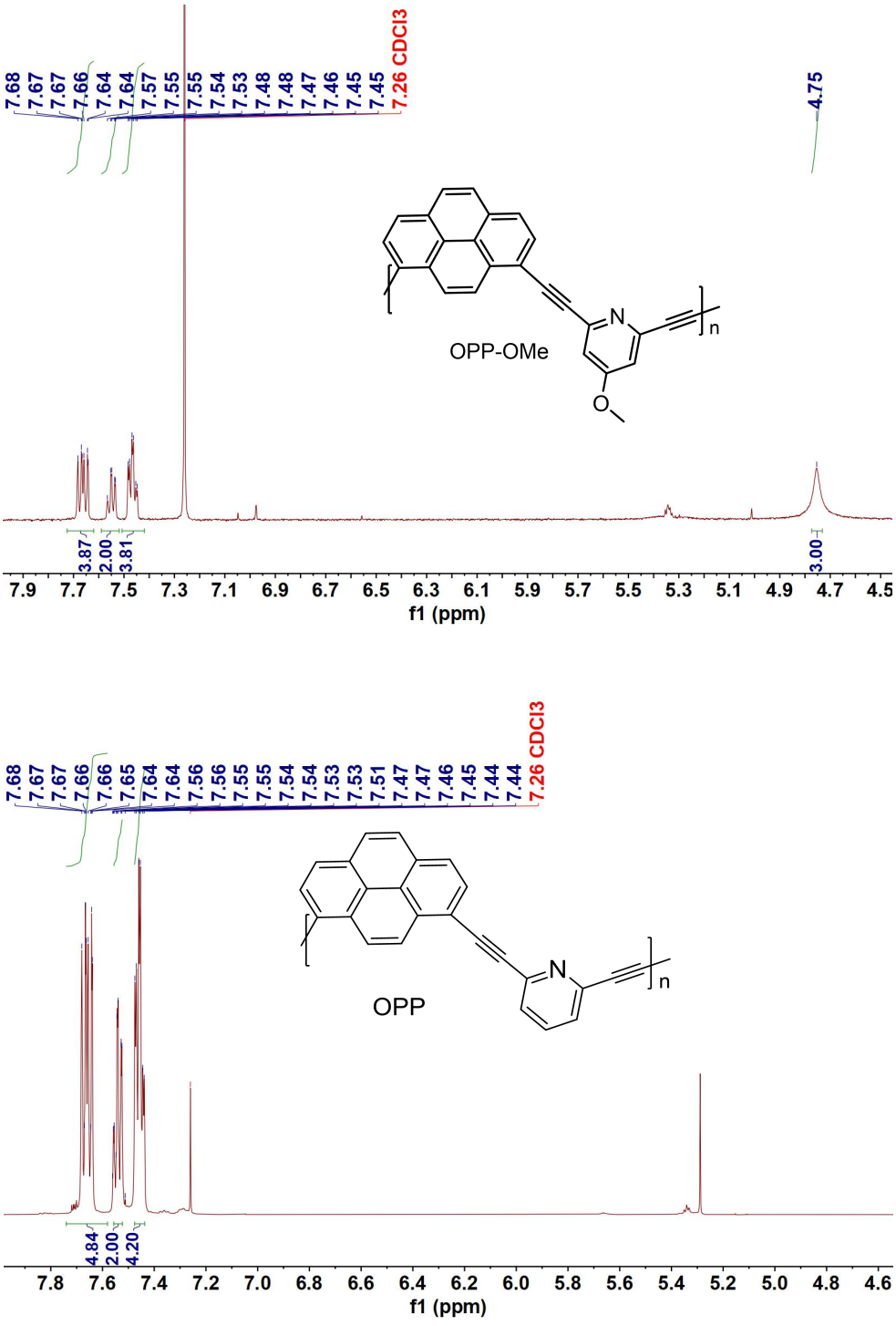
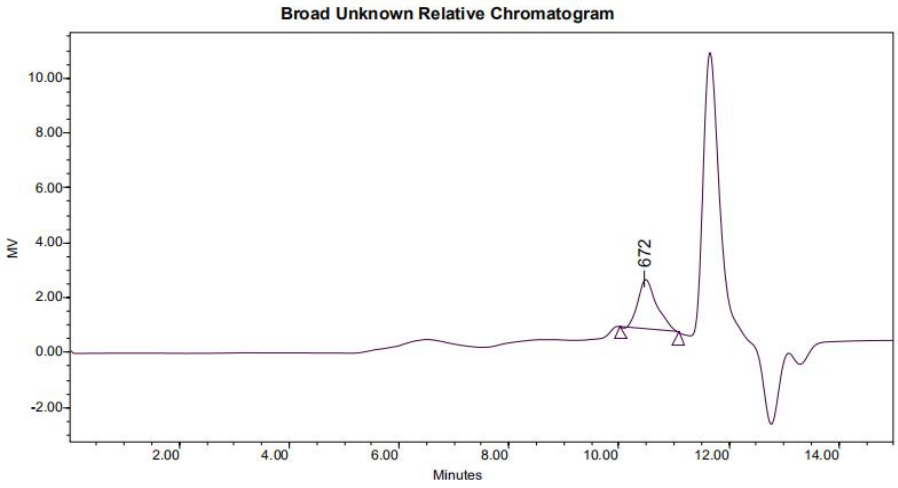


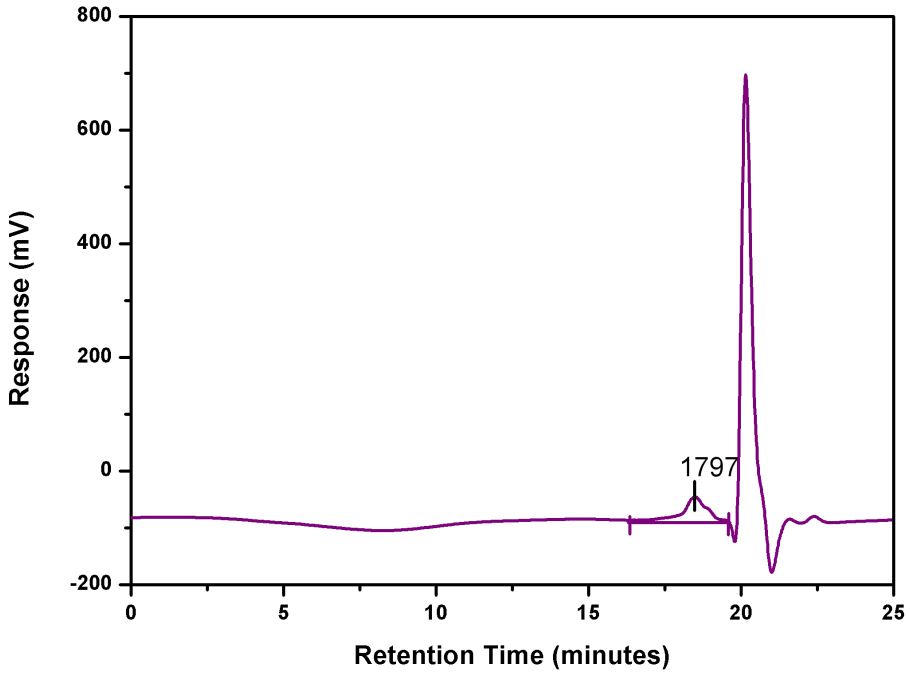
Fig. S1 ^{1}H NMR spectra of compounds **1**, **2**, and conjugated oligomers **OPP-OMe** and **OPP** in chloroform-*d*.



Broad Unknown Relative Peak Table

	Distribution Name	Mn (Daltons)	Mw (Daltons)	MP (Daltons)	Mz (Daltons)	Mz+1 (Daltons)	Polydispersity	Mz/Mw	Mz+1/Mw
1		590	638	672	685	729	1.081496	1.072984	1.142079

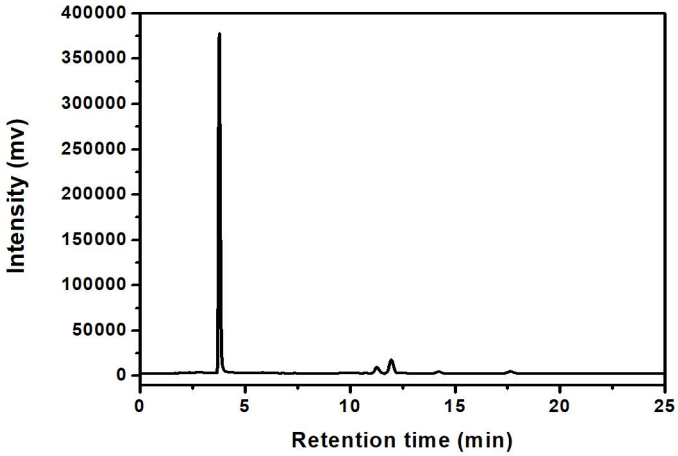
Fig. S2 Molecular weights of OPP-OMe indicated by GPC.

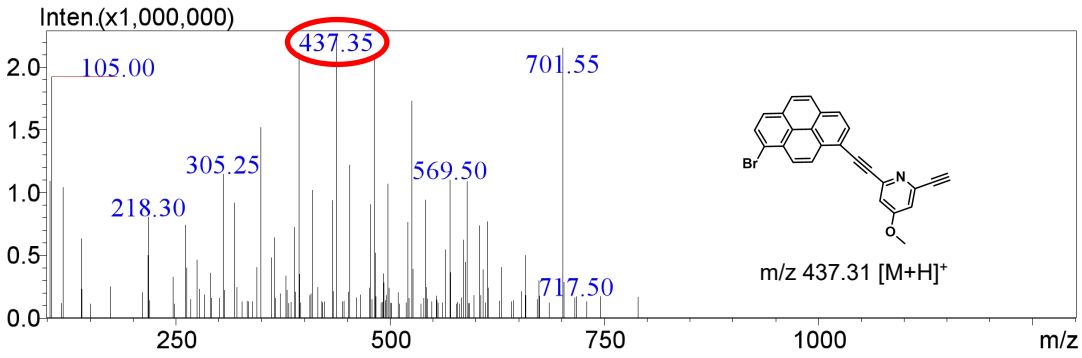


Broad Unknown Relative Peak Table

Distribution Name	Mn (Daltons)	Mw (Daltons)	Mp (Daltons)	Mz (Daltons)	Mz+1 (Daltons)	Mv (Daltons)	Polydispersity	Mz/Mw	Mz+1/Mw
1	1711	2061	1797	2642	3532	1995	1.20456	1.2819	1.7137

Fig. S3 Molecular weights of OPP indicated by GPC.





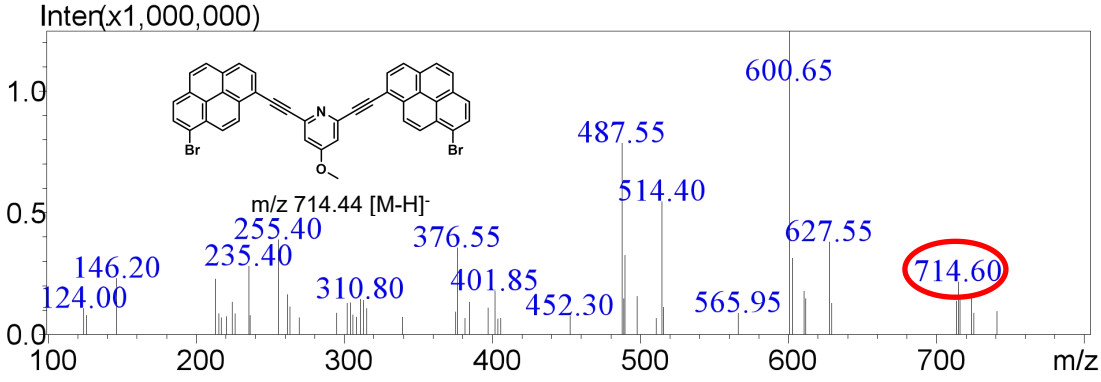


Fig. S4 LC-MS analyses of OPP-OMe.

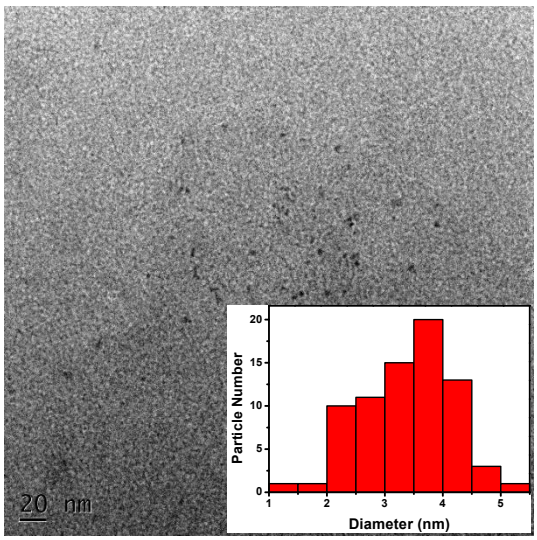


Fig. S5 TEM image of the conjugated oligomer nanoparticles (OPP NPs).

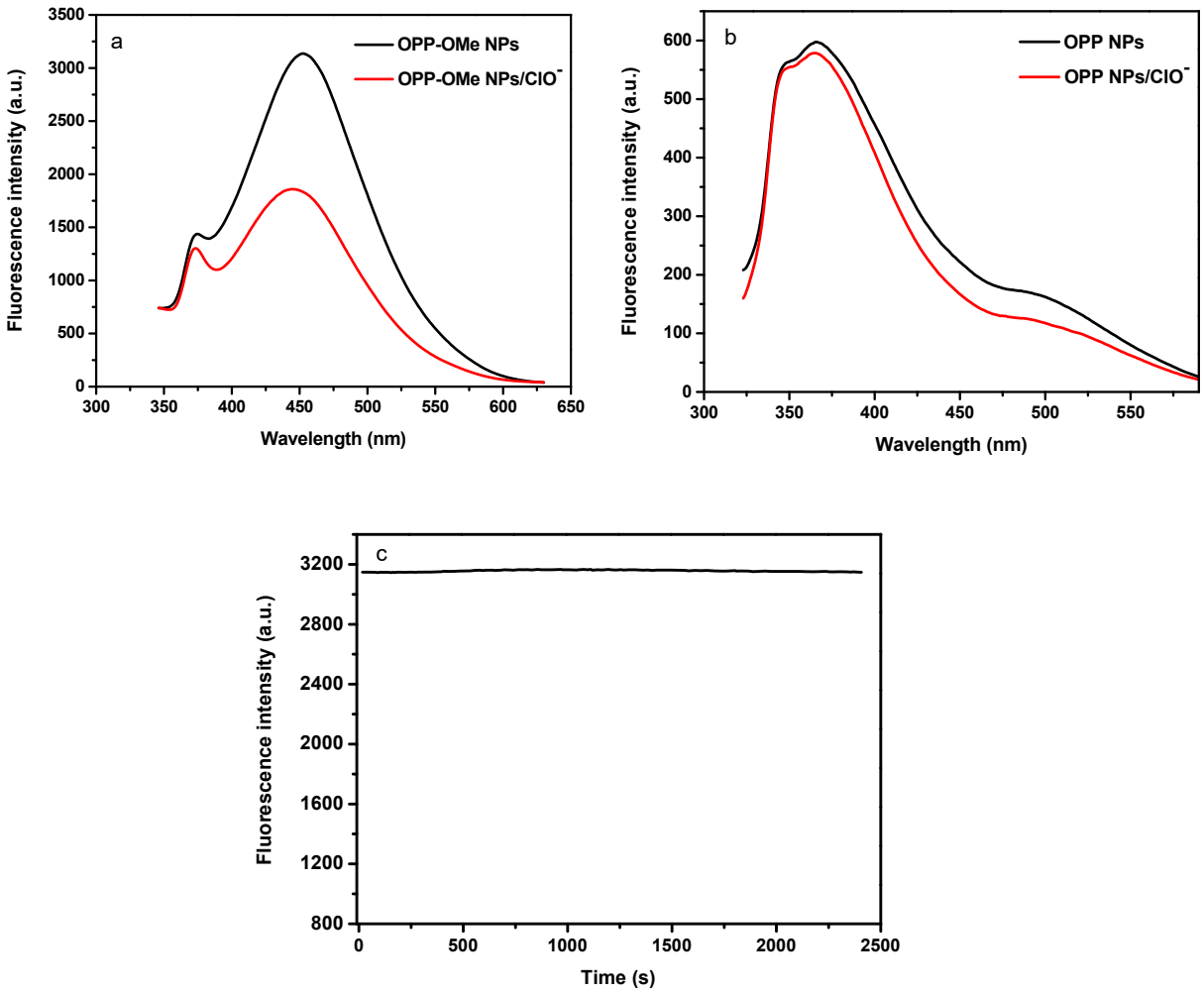


Fig. S6 Fluorescence spectra of OPP-OMe NPs (a) and OPP NPs (b) in the absence and presence of ClO^-. (c) Photostability of OPP-OMe NPs in PBS buffer (pH = 7.4) upon irradiation at 327 nm for 40 min.

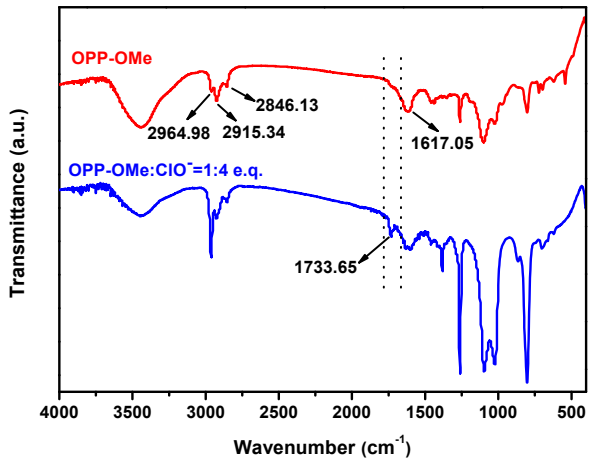


Fig. S7 FTIR spectra of OPP-OMe before and after the addition of ClO^-.

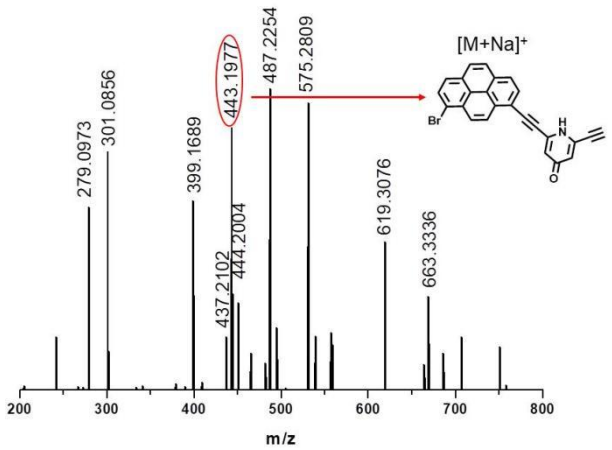


Fig. S8 HRMS data of reaction mixture of OPP-OMe and ClO^-.

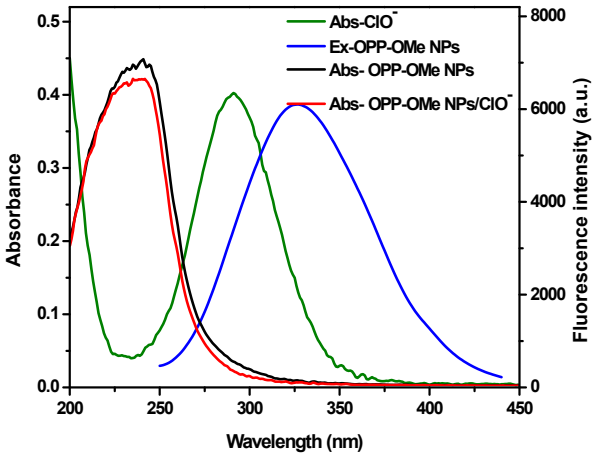


Fig. S9 Absorption spectra of ClO^- and OPP-OMe NPs before and after the addition of ClO^-, and fluorescence excitation spectrum of OPP-OMe NPs.

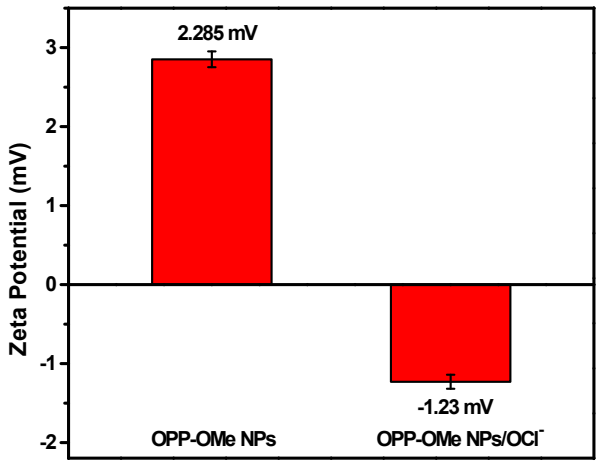


Fig. S10 Zeta potentials of OPP-OMe NPs under different conditions. [OPP-OMe NPs] = 22.5 μg/mL, $[ClO^-] = 1.0 \times 10^{-4}$ M.

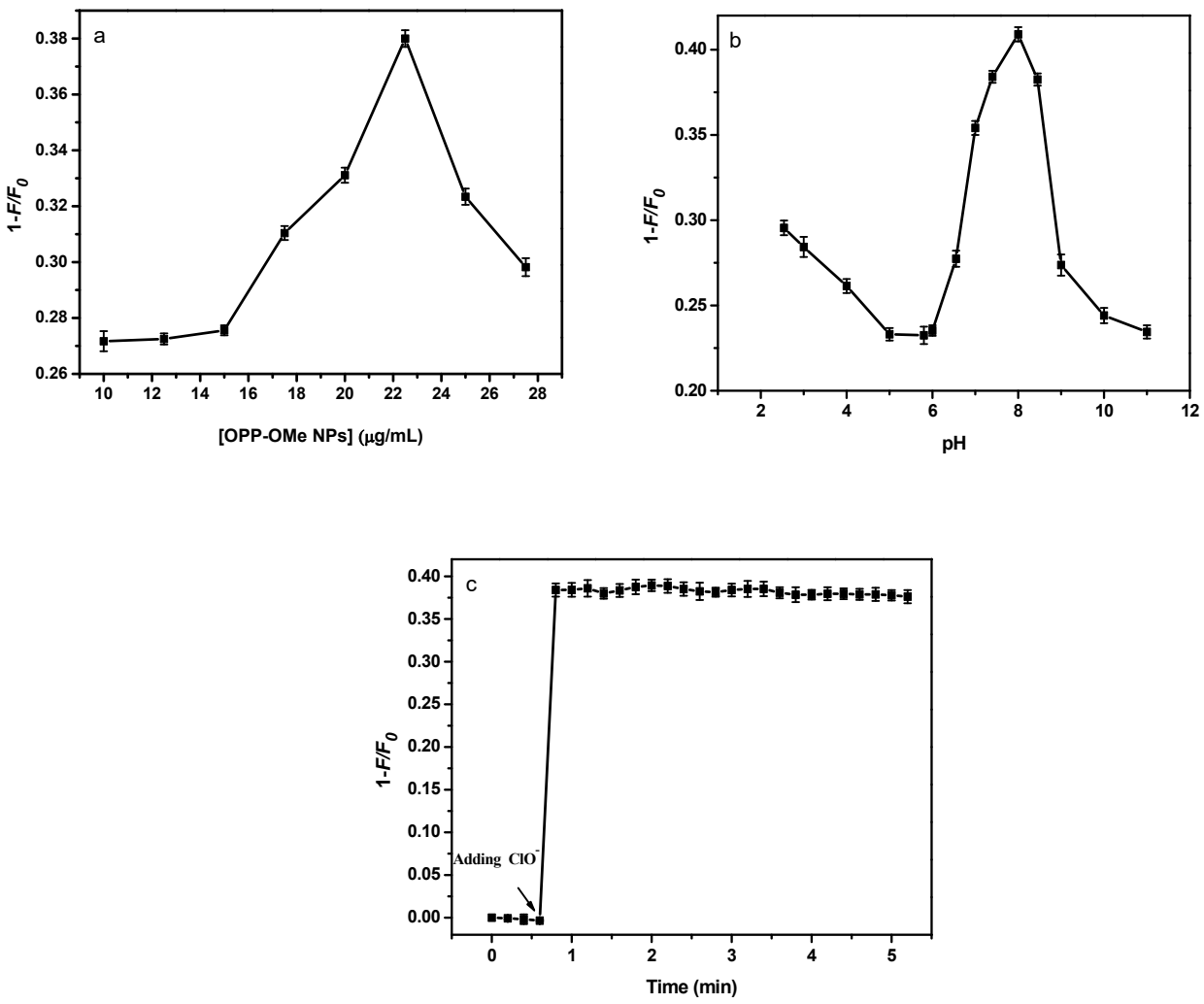


Fig. S11 The influence of nanoprobe concentration (a), pH (b) and response time (c) on the fluorescence quenching efficiency. [ClO^-] = 5.0 × 10^{-5} M. F_0 and F are the fluorescence intensity of OPP-OMe NPs before or after adding ClO^-, respectively. λ_{ex} = 327 nm.

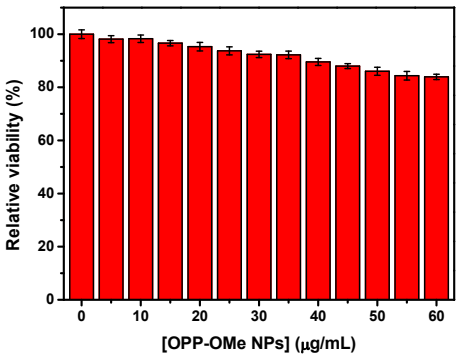


Fig. S12 Cell viability of HeLa cells after incubation with different concentrations of OPP-OMe NPs.

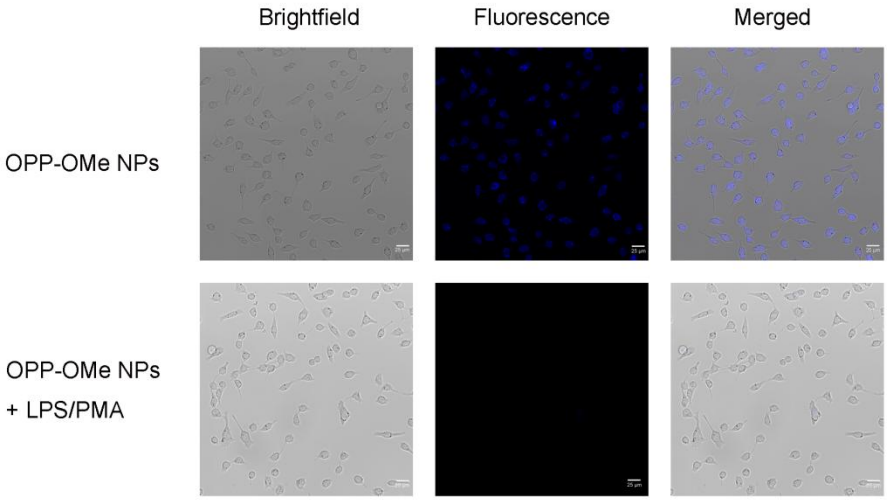


Fig. S13 Imaging of endogenous ClO^-. Confocal fluorescence images of Hela cells incubated with OPP-OMe NPs and OPP-OMe NPs + LPS (5 μg/mL)/PMA (10 μg/mL) for 45 min at 37 °C. Fluorescence images obtained via 405 nm excitation. [OPP-OMe NPs] = 22.5 μg/mL. Scale: 25 μm. LPS: lipopolysaccharide, PMA: phorbol 12-myristate 13-acetate.

Table S1

Comparison of representative small molecules and conjugated oligomer or polymer nanoprobes for ClO^- detection.

Probe	Linear range	LOD	Response time	Ref.
TP-HOCl 1	0-200 nM	16.6 nM	---	[7b]
NI-Se	0-15 μM	586 nM	---	[7c]
Naphthalimide derivative	0.1-50 μM	20 nM	Within 2 min	[7d]
RSPN	0-5 μM	50 nM	---	[9]
CPN-PFV-*co*-MEHPV	2-30 μM	0.47 μM	---	[10]
SPNP25	0-40 μM	0.68 μM	Within 6 min	[11a]
PFPT Pdots	50 nM-2 μM	37 nM	Within 30 s	[11b]
$PFOBT_{36}SeTBT_5$ Pdots	0-50 μM, 50-250 μM	0.5 μM	Within 2 min	[12]
SOA-based nanoprobe	0-10 μM	1.3 μM	---	[13b]
OPP-OMe NPs	0.5 nM-8.0 μM, 8.0-500 μM	0.3 nM	Within 30 s	This work

Table S2

Detection of ClO^- in real water samples. [OPP-OMe NPs] = 22.5 μg/mL. All measurements were performed in PBS buffer solution (pH = 7.4).

Sample	Spiked (μM)	Detected (μM)	Recovery (%)	RSD (%, n = 3)
Xiangjiang River water	0	None	-	-
	5	5.06	101.2	1.02
	20	19.19	96.0	2.08
	50	47.86	95.7	2.49
Tap water	0	1.82	-	1.56
	5	6.96	102.1	2.46
	10	12.17	103.0	3.32
	20	22.74	104.2	1.72

Table S3

The quantum yields of pyrene-pyridyl oligomers and their corresponding NPs were obtained in pH = 7.4 PBS buffer solution.

Sample	Φ
Quinine sulfate	0.55
OPP-OMe	0.022
OPP-OMe NPs	0.031
OPP-OMe NPs/ClO^-	0.0049
OPP	0.021
OPP NPs	0.025
OPP NPs/ClO^-	0.022

The quantum yield was estimated with the equation:

$$\Phi_x = \Phi_{std}(F_x A_{std} \eta_x)/(F_{std} A_x \eta_{std})$$

Where Φ, F, A, and η are the quantum yield of the standard sample, integrated fluorescence intensity, absorbance, and refractive index, respectively. The subscript "std" refers to the standard fluorophore (quinine sulfate) of known quantum yield.

References

[1] H. Huang, K. Wang, W. Tan, D. An, X. Yang, S. Huang, Q. Zhai, L. Zhou, Y. Jin, *Angew. Chem. Int. Ed.* **2004**, *43*, 5635-5638.

[2] K. D. Demir, B. Kiskan, Y. Yagci, *Macromolecules* **2011**, *44*, 1801-1807.

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[4] Z. Lou, P. Li, Q. Pan and K. Han, *Chem. Commun.* **2013**, *49*, 2445-2447.