Supplementary Information

Development of a conjugated polymer-based fluorescent probe for selective detection of HOCl

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General Methods

All reagents were purchased from commercial supplies and used without further purification. $^1$H NMR was measured on Bruker Avance 400M. UV-Vis absorption spectra were recorded on HitachiU-3010 spectrophotometer. Polymer molecular weights were determined using a Varian PL-GPC 50 (THF, 40°C) with narrow weight distribution polystyrene standards. FluoroMax 4 spectrofluorometer (Horiba JobinYvon) was used to measure fluorescence spectra. The pH adjustments were confirmed by a pH meter (METTLER TOLEDO FE20K). FT-IR data was obtained as KBr pellets using Spectrum One (Perkin Elmer Instruments Co. Ltd). Scanning Electron Microscope data was measured on Hitachi S4800. Transmission Electron Microscope date was recorded on Tecnai G2 20(S-TWIN). Dynamic Light Scanning data was recorded on Zetasizer (Nano ZS).

Synthesis of Poly [2, 7-(9, 9'-dioctylfluorene)-co-alt-2, 5-phenylamine]

$K_2$CO$_3$ (160 mg, 0.3 mmol) was dissolved in the mixture of 2.0 mL water and 3.0 mL toluene, and then added a few droplets of Aliquat® 336. Subsequently, the mixture degassed by three freeze-pump-thaw cycles and backfilled with argon. The degassed solution was added to a mixture of 9, 9-Dioctylfluorene-2, 7-diboronic acid bis (1, 3-propanediol) ester (167.524 mg, 0.3 mmol), 2, 5-dibromoaniline (75.276 mg, 0.3 mmol) and Tetrakis(triphenylphosphine)palladium(0) [Pd(PPh$_3$)$_4$] that was prepared in the glove box. The mixture was vigorously stirred at 80°C for 48 h under argon atmosphere. After the mixture was cooled to room temperature, it was slowly dropped into the 200 mL mixture of methanol and deionized water (10:1). The solid was collected by filtration, and then washed with methanol, acetone and chloroform to remove oligomers and catalyst residues. The resulting polymers were collected and dried under vacuum, and a brown solid was obtained (155mg, 64%). $^1$H NMR (CDCl$_3$, 400MHz, ppm): δ 7-8 (m, 9H), 3.94 (b, 2H), 2.07 (b, 4H), 1.5-0.5 (m, 24H). GPC (THF, polystyrene standard): $M_n$=10976, $M_w$=21513, PDI=1.96.

Preparation of conjugated polymer nanoparticles

The nanoparticles were prepared by using a precipitation method according to the literature. 10 mg of as-synthesized polymer was dissolved in 10 mL tetrahydrofuran (THF) by stirring under inert atmosphere and filtered through a 1.6 micron filter. A 2 mL of the dilute polymer/THF solution was added quickly to 8 mL of deionized water, and then sonicated for 5 min. The THF was removed by evaporation under vacuum, followed by an additional filtration step.

Preparation of ROS and RNS

NaClO was obtained from dilution of 10% solution in water. $H_2O_2$ was acquired from dilution of 30% solution in water. Tert-butyl hydroperoxide (TBHP) was obtained from dilution of 70% solution in water. NO$•$ was produced by sodium nitroprussidehydrate (SNP). $O_2$ was generated by mixing xanthine and xanthine oxidase.
Confocal laser scanning microscope image of cell
Conjugated polymer nanoparticles were prepared by the above described method. The Hela cells were cultured in medium (DMEM+10%FBS+1%P/S) at 37°C in an atmosphere of 5% carbon dioxide for two days on a confocal dish (105 cells/mL), then the cells were incubated with 1 μM nanoparticles for 2 h at 37°C. After washing the cells several times with PBS buffer, the cells were imaged in PBS buffer with fluorescence confocal microscope. Then the cells were filled with HOCl (15 μM) at room temperature for 30 min. The cells were imaged in PBS buffer with fluorescence confocal microscope.

Cell Viability Assays
The Hela cells were seeded in 96-well plates at a density of 5000 cells/200ul per well and were incubated at 37°C in a 5% CO₂/95% air humidified incubator for one day, then we added the probe 1uM and 2uM separately. Cell viability was measured using the Cell Counting Kit-8(CCK-8; WST-8) assay (DOJINDO, Japan) by taking media from the following wells: the experimental group (containing different concentrations of the probe), the control group (media incubated with normal cells), and the blank group(media without any cells).Before measuring the absorbance (A), 100ul DMEM (including 10ul CCK-8) was added to every well and the samples were incubated for 2h at 37°C before reading their optical density at 450nm in a multimode microplate reader. The cell viability was calculated using the equation below:
Cell viability (%) = (A_{experimental group} - A_{blank group})/(A_{control group} - A_{blank group})*100%

CalceinAcetoxymethyl Ester (Calcein AM)/ Propidium Iodide (PI) Assay
We seeded cells (5×105 cells/well) in confocal dishes with 1 mL cell sap and incubated them for 1 days at 37°C and 5% CO₂ humidified atmosphere, then add 1μM and 2μM probe in different dishes ,and the control group was add no samples. After culturing 24h at 37°C and 5% CO₂ humidified atmosphere, we removed the medium from the dishes and washed the samples by PBS two times followed by addition of 500 mL Calcein AM/PI mixed solution (2 mL PBS: 4 mL Calcein AM (1 mmol/L, DMSO): 6 mL PI (1.5 mmol/L, H₂O)) to each well and incubated the mixture for 20 min at 37°C and 5% CO₂ humidified atmosphere. We removed Calcein AM/PI mixture from the dish followed by washing with PBS once and added 500 mL PBS before observing by confocal laser scanning microscope (CLSM) (LSM710, Carl Zeiss).

Reference
Figures

Fig. S1 $^1$H NMR Spectra of the conjugate polymer
Fig. S2 GPC spectrum of the conjugated polymer
Fig. S3 Absorption and PL Spectra of the conjugated polymer in THF
Fig. S4 The fluorescence decay trace of the conjugated polymer nanoparticles
Fig. S5 The absorption spectra of SCP nanoparticles in water and SCP in THF after HOCl addition.
Fig. S6 Size distribution of SCP nanoparticles after oxidation by HOCl
Fig. S7 PL Spectra of the SCP nanoparticles under different pH value
Fig. S8 Photo-stability of SCP nanoparticles
Fig. S9 Hela Cell viability assay after adding 1 and 2 μM of SCP nanoparticles
Fig. S10 FT-IR Spectroscopy of (a) fluorene and (b) pyridine monomers, (c) parent polymers and polymers after oxidation by (d) 1 and (e) 5 equivalent of HOCl.
Fig. S11 HPLC/MS Spectroscopy of the oxidation products from aniline oxidized by HOCl.