Supporting information

A multifunctional perylenediimide derivative as recyclable specific Hg$^{2+}$ ion sensor and efficient DNA delivery carrier

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Materials

1, 6, 7, 12-Tetrachloroperylene-3, 4, 9, 10-tetracarboxylic acid dianhydride was obtained from Beijing Wenhaiyang Perylene Chemistry (Beijing, China). 4-Hydroxybenzaldehyde (98%) and 2-aminoethanethiol hydrochloride (>95.0%) were purchased from Alfa Aesar and TCI, respectively. All other solvents and reagents were purchased from commercial suppliers and used as received. S2 cells were propagated in Schneider Drosophila Medium supplemented with 10% FBS, 100 U/mL penicillin and 100 µg/mL streptomycin at 25 oC without CO$_2$.

Instruments

Nuclear magnetic resonance (NMR) spectra were recorded on Bruker 400 (400 MHz $^1$H; 101 MHz $^{13}$C) spectrometer using D$_2$O, CDCl$_3$ and MeOD as solvent at room temperature. Chemical shifts were reported downfield from 0.00 ppm using TMS as internal reference. Matrix-assisted laser-desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) were determined on AXIMA-CFR plus MALDI-TOF mass spectrometer. The UV-Vis absorption spectra were recorded on a
spectrophotometer (Cintra 20, GBC, and Australia). The corrected Fluorescence spectroscopic studies were performed on a fluorescence spectrophotometer (Horiba Jobin Yvon FluoroMax-4 NIR, NJ, USA) at room temperature (25 °C). Fluorescence quantum yields (FQYs) were measured at room temperature by using cresyl violet in methanol as reference ($\Phi_f=0.54$).\(^1\)

**Cytotoxicity Assay**

Cell viability was monitored using Tali™ viability kit-Dead Cell Green (Invitrogen, Catalog A10787) that was a green-fluorescent nuclear and chromosome stain. It does not penetrate intact membranes, but easily penetrate compromised membranes characteristic of dead cells. The measurement was performed at 48 h post-incubation of DTPDI. Replace the fresh cell medium after 48 h of incubation and then add 1 µL Dead Cell Green into 100 µL cell medium for 0.5 h incubation.

**Cellular Hg\(^{2+}\) response**

HeLa cells were purchased from Gibco by Life Technologies. HeLa cells were incubated in Dulbecco’s Modified Eagle Medium (DMEM 1X) which was supplemented with 10% fetal bovine serum (FBS, GIBCO) and 1% penicillin streptomycin (PS). Cells were grown onto 35 mm × 35 mm cell culture dish at a density of 1×10^5 cells/well at 37 °C under 5% CO\(_2\). After 2 days, two groups of cells were incubated with DTPDI (2.5 µM) for 2 h and then washed three times with Phosphate Buffered Saline (PBS) to remove excess probe, and one group of cells were incubated with Hg\(^{2+}\) (7 µM) for 2 h and then washed with the PBS buffer to remove excess Hg\(^{2+}\). Then added the fresh cell culture media (DMEM 1X) and observed the cell imaging under the fluorescence microscopy.

**Cellular Uptake**

HeLa cells were purchased from Gibco by Life Technologies. HeLa cells were incubated in Dulbecco’s Modified Eagle Medium (DMEM 1X) which was supplemented with 10% fetal bovine serum (FBS, GIBCO) and 1% penicillin...
streptomycin (PS). Cellular uptake experiment was performed in 35 mm × 35 mm cell culture dish, 2.5×10^5 cells per well. After 8 h of cell seeding, making the HeLa cells adhere to the bottom of the dish, add the DTPDI to the cell culture dish. Cellular uptake was imaged by fluorescence microscope. The fluorescence intensity of DTPDI inside cells was calculated by Image-J Program.

**In vitro Transfection Efficiency**

*Drosophila* S2 cells were propagated in Schneider’s *Drosophila* Medium (Sigma) supplemented with 10% FBS, 100 U/mL penicillin and 100 μg/mL streptomycin at 25 °C without CO_2_. Single-strand DNA (20 bp, 100 μM) was labeled with 30 μM CXR Reference Dye (Promega, Catalog C5411) that could bind with DNA at a final concentration of 0.3-0.5 μM. DNA and DTPDI at different N/P ratios of 4:1, 8:1 and 16:1 were pre-incubated in culture medium at room temperature and then treated with cells. The fluorescence images of the distribution of DTPDI and DNA were obtained under a fluorescence microscope.

**In vivo Transfection assay**

Immediately after hatching from eggs, Black Cutworm larvae were picked out and individually fed with an artificial diet mixed with dsRNA alone or complex of DTPDI/dsRNA. dsRNA targeting *wg* was synthesized using T7 RiboMAX expression RNAi system (Promega). For oral feeding, 4 μg of dsRNA was mixed with 4 μg of DTPDI, and then mixed with 16 mg of fresh artificial diet. The newly-hatched larvae were individually fed with above diet. The larvae fed with dsRNA alone were used as controls. After 6 days, dsRNA-contained food was replaced with fresh normal food. Then photos were taken for comparison.

The sequence of wg-dsRNA is:

TGACAACATTGGCTTTCCGATTTCAGAGAAATTTCGACACCCGGAGAAG
AGGCAAAACCTACGAGAAATGGAAATCAACAAACAGAAGCCGGCCGACG
ACGTGAAAAACGGAGATCGCCAGGAGTGCAGAAGTCGATGTCCATCGCTCGGCA
CAGTCAAGACCTCTGTTGGATGAGGCTGCCCAGCTCCGCTCGTGGCGACTCACTGA
AGGCCGGGTTTGCAGCGGCGGCCGCTCCGGGTCTGTCGACCTGGAACGGC
CAGCGCGAAGACGCACCCGCACTCAAGAGTGCGCGGAAGAGACCGATACCGG
The primers for dsRNA synthesis are:

FT7: TAATACGACTCAGATATGACAGGCTGG
RT7: TAATACGACTCACTATAGGCCGCGCGGCGGGAGCTGG

Synthesis Schemes

Scheme S1. The mechanism for the conversion of AL to DTPDI

Scheme S2 Hg^{2+}-promoted conversion mechanism of DTPDI to aldehyde AL in presence of HgCl_{2}
UV-Vis Absorption and Fluorescence Emission Properties

Fig. S1 UV-vis absorption and fluorescence ($\lambda_{ex} = 450$ nm) of DTPDI (5 µM) in distilled water.

Fig. S2 Fluorescence changes of DTPDI (5 µM) upon addition of Hg$^{2+}$ (10 eq) in distilled water from 0 min to 30 min ($\lambda_{ex} = 450$ nm). Inset: Plot of the intensity maximum of DTPDI versus Hg$^{2+}$ (10 eq) at different time.
Fig. S3 UV-vis absorption changes of DTPDI (5 μM) upon addition of Hg²⁺ (0 - 10 eq) in distilled water

Fig. S4 Fluorescence intensity ratio (I₀/I) of DTPDI (5 μM) in distilled water at 25 °C upon addition of 10 eq of different metal ions, measured after 2 min (λₑₓ = 450 nm): (A) Hg²⁺, (B) Fe²⁺, (C) Na⁺, (D) K⁺, (E) Zn²⁺, (F) Cu⁺, (G) Cu²⁺, (H) Fe³⁺, (I) Ca²⁺, (J) Mg²⁺, (K) Cr²⁺, (L) Mn²⁺, (M) Cd²⁺, (N) Pb²⁺, (O) Ni²⁺, (P) Ag⁺.
**Fig. S5** Fluorescence intensity ratio (I₀/I) of **DTPDI** (5 μM) of the competition experiments upon addition of each metal ion (200 μM, red bars) followed by Hg²⁺ (200 μM, black bars) in water at 25 °C, measured after 2 min (λ_ex = 450 nm): (A) Hg²⁺, (B) Fe²⁺, (C) Na⁺, (D) K⁺, (E) Zn²⁺, (F) Cu⁺, (G) Cu²⁺, (H) Fe³⁺, (I) Ca²⁺, (J) Mg²⁺, (K) Cr²⁺, (L) Mn²⁺, (M) Cd²⁺, (N) Pb²⁺, (O) Ni²⁺, (P) Ag⁺.

**Fig. S6** Cell viability of cells treated with **DTPDI** at various concentrations for 48 h. The data were mean±SEM.
The DNA-binding affinity of “DTPDI” in water was determined using an isothermal titration calorimetry (ITC) as well as agarose gel electrophoresis. The association constant (Ka of “DTPDI” / DNA) is $1.153 \times 10^6 \text{ M}^{-1}$ (Fig. S7). “DTPDI” could compact DNA completely at a N : P ratio of above 1 (Fig. S8). These data demonstrate the interactions between “DTPDI” and DNA.

**Fig. S7** Agarose gel Electrophoresis of pDNA/DTPDI complexes at various N : P ratios.
Synthesis and characterizations

1. Synthesis of AL

Under an atmosphere of argon, 4Cl-PDI (0.1698 g, 0.2 mmol), 4-hydroxybenzaldehyde (0.244 g, 2 mmol) and K₂CO₃ (0.11 g, 0.8 mmol) were added into NMP (20 mL) with stirring. The reaction was carried out 80°C under stirring for 36 h. After cooled down to room temperature, the mixture solution was treated with diluted hydrochloric acid and filtered to remove excess 4-hydroxy benzaldehyde. The residue was dissolved in dichloromethane and then washed with distilled water until the solution was neutral. The solvent was removed under reduced pressure, then the solid was purified by column chromatography with dichloromethane as the eluting solvent to give a reddish black solid AL (0.119 g, 50%). ¹H NMR (400 MHz, CDCl₃) δ 9.95 (s, 4H), 8.35 (s, 4H), 7.85 (d, J = 8.7 Hz, 8H), 7.48 (t, J = 7.8 Hz, 2H), 7.32 (d, J = 7.8 Hz, 4H), 7.09 (d, J = 8.6 Hz, 8H), 2.70 (dt, J = 13.6, 6.8 Hz, 4H), 1.15 (d, J = 6.8 Hz, 24H). ¹³C NMR (101 MHz, CDCl₃) δ 190.37, 162.66, 160.29, 154.91, 145.49, 132.97, 132.07, 130.02, 129.81, 124.10, 123.74, 121.76, 121.35, 119.66, 29.44, 24.02. MS (MALDI-TOF, m/z) Calc. for C₇₆H₅₈N₂O₁₂: 1191.28, found: 1192.547 (M+H⁺).
**Fig. S8** $^1$H NMR spectrum of AL in CDCl$_3$

**Fig. S9** $^{13}$C NMR spectrum of AL in CDCl$_3$
2. Synthesis of DTPDI

![Diagram of AL and DTPDI]

Scheme S4 Synthesis approach for DTPDI

A solution of BF$_3$·Et$_2$O (102 mg, 0.72 mmol) in dry dichloromethane (3 mL) was slowly added into a mixture solution of AL (39 mg, 0.033 mmol) and 2-aminoethanethiol hydrochloride (63 mg, 0.55 mmol) in DMF (3 mL) under nitrogen atmosphere at 0 °C. After one day, the temperature of reaction was raised to 37 °C. After stirred for 4 days at 37 °C, the solution was redissolved in deionized water (0.5 mL) and washed with diethyl ether for several times. The further dialysis in 1 mM HCl and lyophilization afforded a purple solid AL. Yield: 96.5 %. $^1$H NMR (400 MHz, D$_2$O) δ 8.21 (s, 4H), 7.43 (d, $J = 40.5$ Hz, 16H), 7.02 (s, 6H), 5.19 (s, 4H), 2.86 (dd, $J = 116.4$, 65.5 Hz, 36H), 1.01 (s, 24H). $^{13}$C NMR (101 MHz, MeOD) δ 165.01, 157.38, 156.64, 147.20, 137.90, 134.48, 131.77, 131.15, 130.81, 125.18, 123.96, 122.27, 121.60, 121.20, 52.87, 40.31, 30.72, 30.36, 24.33. MS (MALDI-TOF, m/z) Calc. for C$_{92}$H$_{106}$N$_{10}$O$_8$S$_8$: 1736.41, found: 1736.91 (M+H$^+$).
Fig. S10 $^1$H NMR spectrum of DTPDI in D$_2$O

Fig. S11 $^{13}$C NMR spectrum of DTPDI in MeOD