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

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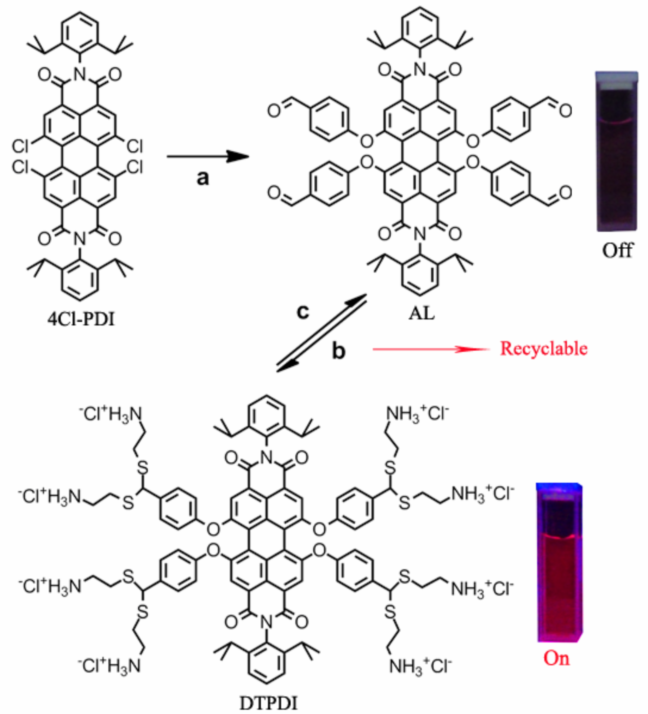
Multifunctional dithioacetal-modified perylenediimide (DTPDI) is synthesized as a highly sensitive and selective fluorescent chemosensor for recyclable Hg^{2+} detection and an effective DNA carrier. The central PDI chromophore allows the tracing of cell uptake by fluorescence microscopy, the dithioacetals enable the detection of Hg^{2+}, and the peripheral amine hydrochloride salts increase the water solubility and also serve as positive charges for noncovalent binding of negatively charged DNA. In addition to serve as a recyclable fluorescent probe for Hg^{2+} detection, DTPDI can be rapidly internalized into live cells with low cytotoxicity and high DNA delivery efficacy.

Mercury is an indispensable element in the chemical industry, yet the poisonous nature of mercury cannot be ignored. Accumulation of mercury (II) ion (Hg^{2+}) in the human body often leads to severe disease. It is a very important goal to obtain cost-effective, rapid detection and monitoring tools applicable to the environment and living species.[1] Therefore, various excellent sensors have been developed for Hg^{2+} detection, relying on biomolecules and materials.[2] Because of their highly selective, sensitive, and easy-to-use features, fluorescent probes have gained significant attention. Many excellent sensors have been developed for detection of Hg^{2+}.[3-7] However, many of these molecules have problems in actual applications due to their lack of water solubility and photochemical stability. Perylenediimides (PDIs) are an attractive class of fluorophores that display exceptional photochemical stability and high fluorescence quantum yield (>99%).[8] The emission maxima of PDIs are higher than 500 nm; thus, the cellular autofluorescence is minimized.[9] Up to now, PDIs are rarely developed as fluorescent chemosensors, especially for Hg^{2+} detection in water and cells.[1a,10] Due to the easy aggregation of perylene chromophores, PDIs exhibit low water solubility and fluorescence in water,[11] both of which pose major challenges in biological applications or use of these materials as fluorescent chemosensors in aqueous solution. To the best of our knowledge, current probes for the detection of Hg^{2+} are unifunctional molecules, which have no other functions in biological applications than Hg^{2+} detection. Therefore, it would be of high interest to develop a difunctional PDI with high water solubility and biocompatibility and explore its applications in the fields of chemosensor and DNA carrier in live cells.



Scheme 1 Synthesis approach for dithioacetal-functionalized perylenediimide (**DTPDI**) and intermediate (**AL**); (a) 4-hydroxybenzaldehyde, K_2CO_3, NMP, 80 °C; (b) **AL**, 2-aminoethanethiol hydrochloride, $BF_3{\cdot}Et_2O$, DMC, DMF, 1 day at 0 °C and 4 days at 37 °C; (c) $HgCl_2$, r. t., 3 min.

Herein, we synthesized a water-soluble dithioacetal-functionalized perylenediimide (**DTPDI**, Scheme 1). The central PDI chromophore allows the tracing of cell uptake by fluorescence microscopy[12], the dithioacetals provide the compound with the ability for specific detection of Hg^{2+}, and the peripheral amine hydrochloride salts increase the water solubility of the compound and also serve as positive charges for noncovalent binding of negatively charged biomolecules such as DNA. The synthesis strategy started with tetrachloroperylenetetra carboxdiimide (**4Cl-PDI**, Scheme 1) which was prepared according to a literature procedure.[13] The four chlorine atoms in **4Cl-PDI** selectively reacted with the hydroxyl group in 4-hydroxybenzaldehyde, resulting in the intermediate product **AL** (Scheme 1(a)).[14] The aldehydes at the periphery of **AL** selectively reacted with the thiol group in 2-aminoethanethiol hydrochloride, resulting in **DTPDI**.[15] The mechanism for the conversion of **AL** to **DTPDI** is given in the Electronic Supplementary Information (**ESI†**, Scheme S1). The detailed synthesis procedures and structural characterizations can be found in **ESI†**.

DTPDI showed high water solubility (>20 mg/mL), which is desirable for biological applications. The optical properties of **DTPDI** in water were investigated. As shown in Fig. S1 (**ESI†**), **DTPDI** exhibits an absorption maximum at 581 nm and an emission maximum at 629 nm in UV and fluorescent spectra. The fluorescence quantum yield of **DTPDI** was 0.08, which is substantially higher than those of most chemosensors in neat aqueous media.[16]

As expected, the presence of Hg^{2+} led to the fluorescence quenching of **DTPDI**. **DTPDI** underwent a fast Hg^{2+}-promoted hydrolysis (Scheme S2, **ESI†**), generating **AL** within a few minutes in distilled water at room temperature (Fig. S2, **ESI†**). The fluorescence intensity of **DTPDI** decreases dramatically after the addition of Hg^{2+} ions. As shown in the inset of Fig. S2, in only 2.5 min the fluorescence intensity reaches a plateau because the dithioacetals in **DTPDI** were attacked by Hg^{2+} to yield the aldehyde **AL**,[17] with a significant decrease of fluorescence. The hydrolysis of **DTPDI** was monitored by ^{1}H NMR (Fig. 1). Fig. 1(A) and (B) show the ^{1}H NMR spectra of **DTPDI** and **AL**, respectively. Fig. 1(B) shows the appearance of the proton of the aldehyde at δ 10 ppm together with the concomitant disappearance of the methine proton at δ 5.4 ppm. Therefore, **PDT** can rapidly react with Hg^{2+}, resulting in **AL**.

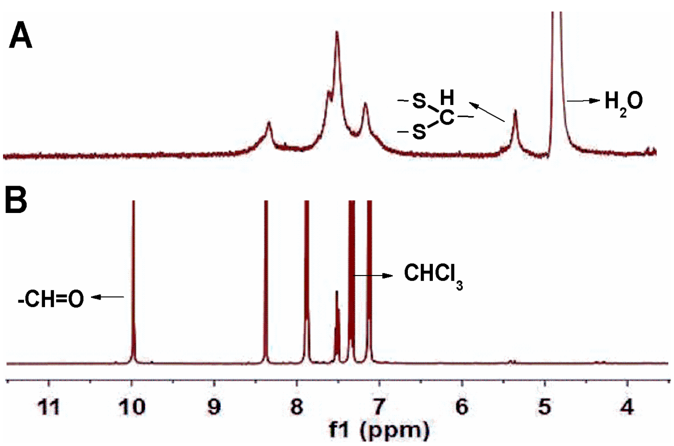


Fig. 1 ^{1}H NMR spectra of (A) **DTPDI** in D_2O and (B) **AL** in $CDCl_3$

Recyclable Hg^{2+} sensors are desirable for economical and environmental reasons. However, few studies have focused on them.[18] Because the solubilities of **DTPDI** and **AL** are different in organic solvents, these two compounds can be separated by simple washes with solvents. For instance, after probing for Hg^{2+}, **AL** could be extracted from the aqueous solution with dichloromethane in a 99% yield. And then **DTPDI** was obtained in a 93% yield by the protection step of **AL** (see Scheme S1, **ESI†**). Finally the cyclic utilization of **DTPDI** was realized in a convenient way.

In order to further illustrate the sensitivity of **DTPDI** for the detection of Hg^{2+}, different concentrations of Hg^{2+} (0-200 μM) were tested. A decreasing trend in the UV-vis absorption spectra of **DTPDI** (5 μM) is observed by increasing the Hg^{2+} concentration from 0 to 200 μM (Fig. S3). At sufficiently high Hg^{2+} concentrations, the absorption maximum of **DTPDI** approaches that of the aldehyde **AL**. Fluorescence titration of **DTPDI** (5 μM) with various amounts of Hg^{2+} (0-200 μM) in distilled water was also performed, and the fluorescence peak of **DTPDI** decreases rapidly to the level of the aldehyde **AL** (Fig. 2). With the addition of 20 μM of Hg^{2+}, the fluorescence of **DTPDI** (5 μM) is quenched completely (Fig. 2 inset). The detection limit of Hg^{2+} ions is 0.1 nM, which is lower than those of many turn-off sensors.[3,16a] It demonstrates a highly sensitive detection of Hg^{2+} ions.

To evaluate the Hg^{2+} selectivity of **DTPDI**, the effects of other metal ions were also investigated. As shown in Fig. S4, **ESI†**, the addition of other metal ions, namely, Fe^{2+}, Na^+, K^+, Zn^{2+}, Cu^+, Cu^{2+}, Fe^{3+}, Ca^{2+}, Mg^{2+}, Cr^{2+}, Mn^{2+}, Cd^{2+}, Pb^{2+}, Ni^{2+} and Ag^+, does not lead to a significant decrease of the fluorescence intensity of **DTPDI.** While previously reported sensors were easily interfered by Cd^{2+}, Cu^{2+}, Zn^{2+} and Ag^+ during the detection of Hg^{2+}.[3,19] Then cross-contamination experiments were conducted in the presence of Hg^{2+} mixed with other metal ions, such as Fe^{2+}, Na^+, K^+, Zn^{2+}, Cu^+, Cu^{2+}, Fe^{3+}, Ca^{2+}, Mg^{2+}, Cr^{2+}, Mn^{2+}, Cd^{2+}, Pb^{2+}, Ni^{2+}, and Ag^+. As shown in Fig. S5 (**ESI†**), the fluorescence of **DTPDI** is quenched selectively by Hg^{2+}, but is not affected by other competitive ions. Thus, **DTPDI** shows high selectivity for Hg^{2+} detection and can be used as an ion-selective fluorescence chemosensor for Hg^{2+}.

To further explore the applications of **DTPDI** as a Hg^{2+} sensor, cellular experiments with Hg^{2+} contamination were performed. The cytotoxicity of **DTPDI** was first assessed by the Tali^{TM} viability assay. As shown in Fig. S6 (**ESI†**), the cell viability of **DTPDI** is higher than 92% at all concentrations studied. The cell viability is significantly higher than those of oil-soluble probes, indicating that **DTPDI** can be developed as a new sensor for the following biological application.

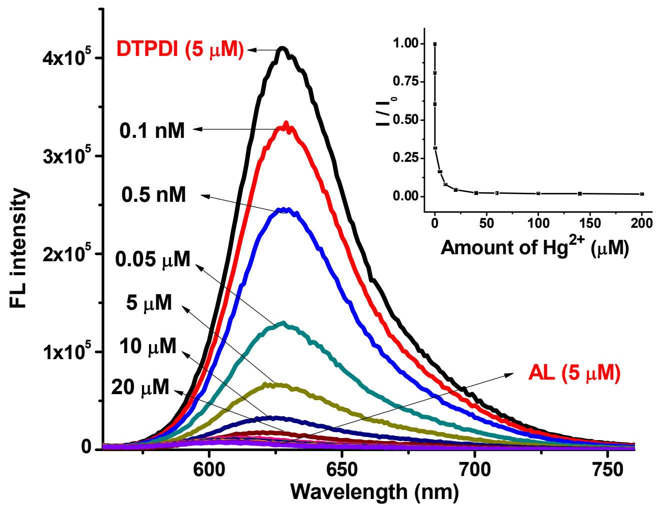


Fig. 2 Fluorescence changes of **DTPDI** (5 μM) upon addition of Hg^{2+} (0 - 200 μM) in distilled water, measured at λ_{ex} = 450 nm. Inset: Plot of intensity maximum (I) of **DTPDI** versus amount of Hg^{2+}.

The cell-internalization ability of **DTPDI** was assayed in live cells by fluorescent tracing of the distribution of **DTPDI**. *HeLa* cells were incubated with **DTPDI** at various concentrations. After 45 min of incubation with 2.5 μM **DTPDI**, strong red fluorescence was detected in the live cells by fluorescence microscopy, demonstrating the efficient and rapid cellular internalization of **DTPDI**. The fluorescent changes of **DTPDI** were then assayed in live cells in the presence of Hg^{2+}. Then the above cell medium was removed and rinsed with PBS buffer. Subsequently 7 μM $HgCl_2$ was added into the fresh medium for further incubation. The fluorescence intensity significantly decreases inside the cells after 2 h of incubation with Hg^{2+}, as shown in Fig. 3(B). But in the control treatment without $HgCl_2$, the intracellular fluorescence intensity is much higher than that in the treatment with $HgCl_2$ (Fig. 3(A)). To quantify the changes in fluorescence intensity inside the cells, the Image-J program was used to plot the fluorescence intensities, indicating a large decrease in intensity upon $HgCl_2$ addition (Fig. 3(C)). These results demonstrate the efficient Hg^{2+}-sensing capability of **DTPDI** in live cells.

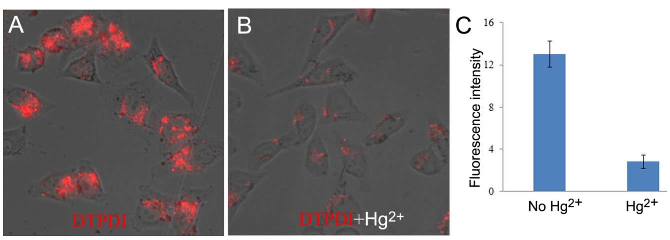


Fig. 3 Fluorescence microscopy assays: (A) Live cells are incubated with 2.5 μM **DTPDI** (red) for 2 h, (B) **DTPDI**-internalized cells from (A) are treated with 7 μM $HgCl_2$ cell medium for 2 h, and (C) quantified fluorescence intensities of **DTPDI** inside cells with and without Hg^{2+}.

The above results also demonstrate that **DTPDI** can be rapidly internalized into cells within a short incubation time. The positive charges of the peripheral amine hydrochlorides can interact with negatively charged macromolecules such as DNA through electrostatic forces.[20] To date, no Hg^{2+} probes have been explored as DNA carrier according to the literature.[1-7] The positive charges in **DTPDI** can bind DNA. In order to assess whether **DTPDI** could act as a carrier to deliver DNA into cells, *Drosophila* S2 live cells were incubated with a buffer containing **DTPDI** -DNA complexes at N/P ratios of 4:1, 8:1, and 16:1. N/P ratios are expressed as molar ratios of nitrogen (N) in **DTPDI** to phosphate (P) in DNA. The DNA delivery efficacies of **DTPDI**-DNA complexes were visualized by fluorescent tracing of the cellular distribution of **DTPDI** and DNA labeled with CXR Reference Dye. Both **DTPDI** and DNA exhibit effective cellular internalization at all N/P ratios (see Fig. 4(A), (A′), and (A″)), as confirmed by the quantified fluorescence intensity of DNA labeled with CXR Reference Dye inside the cells (Fig. 4(C)). The results clearly highlight the remarkable DNA delivery efficacy of **DTPDI** *in vitro*. In addition to human cancer (*HeLa*) and insect (S2) cell lines, other types of cell lines including human non-cancer cells were also tested for cytotoxicity and cell uptake and showed no obvious differences. Therefore, **DTPDI** is suitable for different cell types. To our knowledge, this is the first case of a multifunctional perylenediimide derivative for selective Hg^{2+} detection and DNA delivery.[1-7]

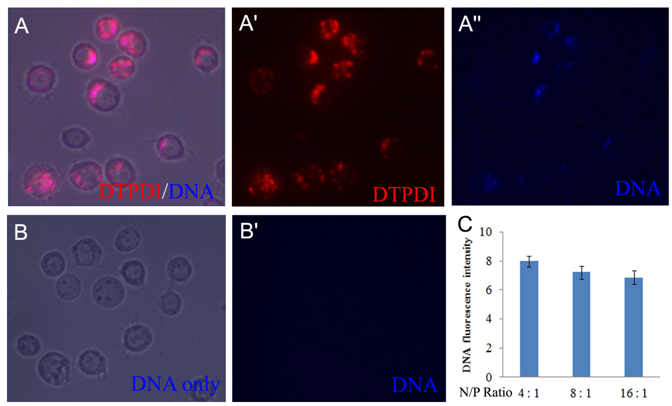


Fig. 4 Fluorescence images show that **DTPDI** delivers DNA into cells. (A) Cells incubated with **DTPDI**-DNA complexes (1.5 μM **DTPDI**, 100 μM DNA, N/P = 4:1, **DTPDI** (red), DNA labelled with CXR Reference Dye (blue)). Separated channels for **DTPDI** (red) and DNA (blue) are shown in (A′) and (A″), respectively. (B) Cells incubated with DNA only. Separated channel for DNA (blue) is shown in (B′). (C) Quantified fluorescence intensity of CXR-labelled DNA inside cells at various N/P ratios.

Subsequently, we determined whether **DTPDI** delivered dsRNA targeting a key gene of insect pest can induce apparent phenotype of growth defects in an *in vivo* application. The dsRNA against a key developmental gene *wingless* (*wg*) of Black Cutworm was synthesized. **DTPDI** was mixed with dsRNA in solution, and the mixture was added to the insect's diet, which was then fed to the newly-hatched larvae. After six days of feeding, the **DTPDI**/dsRNA-fed larvae clearly showed a smaller body size than that of the control (Fig. 5(A)). Thus, the **DTPDI**-carried dsRNA interfered with normal development of the insect larvae, suggesting that **DTPDI** is a good gene carrier with high gene transfection efficacy in *in vivo* manipulation.

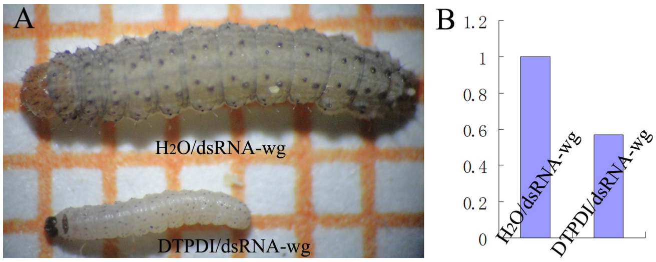


Fig. 5 *In vivo* gene transfection assay of **DTPDI**. (A) After oral feeding with artificial diet containing **DTPDI**-delivered dsRNA targeting a key developmental gene *wingless* (*wg*), larvae show apparent growth defects compared with the control. (B) qRT-PCR assay shows that expression level of target gene *wg* is apparently lower in larvae fed with artificial diet containing **DTPDI**-delivered dsRNA targeting *wg* than in the control.

In summary, the synthesis, optical properties, and living cell imaging applications of the difunctional perylenediimide derivative **DTPDI** have been reported. **DTPDI** can serve as a recyclable fluorescent probe for Hg^{2+} detection and as an effective gene carrier. To the best of our knowledge, a synthetic water-soluble fluorescent probe based on a PDI-core for Hg^{2+} sensing has not been reported. Based on its excellent water solubility, highly selective and sensitive responses to Hg^{2+}, and low cytotoxicity, this probe can be used to determine Hg^{2+} in water as well as in live cells. Moreover, **DTPDI** can be rapidly internalized into live cells with low cytotoxicity and remarkable gene transfection efficacy *in vitro*. **DTPDI** is successfully applied *in vivo* to live insect larvae for gene interference, leading to apparent phenotype of growth defects. With high cyclic utilization and low cytotoxicity, **DTPDI** can be developed into a general tool for biological applications in an environment-friendly way.

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Notes and references

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† Electronic Supplementary Information (ESI) available: Synthesis procedures and material characterizations. See DOI: 10.1039/c000000x/

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