Fluorescence detecting of paraquat using host-guest chemistry with cucurbit[8]uril

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Paraquat (PQ) is one of the most widely used herbicides in the world, which has a good occupational safety record when used properly. While, it presents high mortality index after intentional exposure. Accidental deaths and suicides from PQ ingestion are relatively common in developing countries with an estimated 300,000 deaths occurring in the Asia–Pacific region alone each year, and there are no specific antidotes. Good predictors of outcome and prognosis may be plasma and urine testing within the first 24 h of intoxication. A fluorescence enhancement of approximately 30 times was seen following addition of PQ to a solution of the supramolecular compound 2MB@CB[8], which comprised two methylene blue (MB) molecules within one cucurbit[8]uril (CB[8]) host molecule. The fluorescence intensity was linearly proportional to the amount of PQ added over the concentration range \(2.4 \times 10^{-10} \text{ M} - 2.5 \times 10^{-4} \text{ M}\). The reaction also occurred in living cells and within live mice.
was ejected from the CB[8] cavity. As these released MBs exist as single molecules in the solution, this results in a strong fluorescence enhancement which can be readily observed (the fluorescence is “on”), as illustrated in Fig. 1.

Results

As shown in Fig. 2a, following addition of PQ to 2MB@CB[8] in tris-HCl buffer solution (pH = 7.14), a strong fluorescence emission (up to 30-fold enhancement) which peaked at 682 nm was observed, when excited at 620 nm. The inset in Fig. 2a shows the appearance of the cuvettes when irradiated by a 625 nm laser pen. A pink line is seen in the cuvette containing 2MB@CB[8] + PQ whilst no fluorescence can be observed when only 2MB@CB[8] is present. The effect is further demonstrated by the UV-Vis spectra shown in Fig. 2b. As increasing amounts of PQ were added, the spectra of 2MB@CB[8] showed a significant hypochromic effect accompanied by the appearance of an isosbestic point at 630 nm and a new peak at 660 nm. This is the characteristic absorbance of free MB, demonstrating that 2MB@CB[8] had a very strong interaction with PQ, leading to the formation of PQ@CB[8] and free MB in solution.

To assess the selectivity of the detection method, the probe 2MB@CB[8] was exposed to 50 equivalents of potentially competing species — such as a mixture of inorganic salts Na⁺, K⁺, Mg²⁺, different herbicides such as aquacide, acetochlor, (2,4-dichlorophenoxy)acetic acid (2,4-D), dichlorovos, glyphosate, haloxlyfop, methomyl, omethoate and quizalofop-p-ethyl. Most of these potentially competing species gave little fluorescence enhancement even though present in large excess (Supporting Information Fig. S1). This indicates that the method has a high selectivity for PQ detection.

It was further noted that the fluorescence intensities were linearly proportional to the amount of PQ added, over the range 2.4 × 10⁻¹⁰ M–2.5 × 10⁻⁴ M (Supporting Information Fig. S2), and that a relative standard deviation (RSD) of less than 2% was found for 5 repeat observations, suggesting this method can be utilized as good predictors of outcome and prognosis within the first 24 h of intoxication²⁻¹¹. The detection limit was as sensitive as 2.4 × 10⁻¹⁰ M (0.06 µg/L), which is lower than the current PQ residue tolerance (no more than 0.05 mg/kg) for fruits, vegetables and drinking water in the EU and the US²⁰⁻²². As a result, the specific interaction between 2MB@CB[8] and PQ can be employed for the quantitative detection of trace levels of PQ.

To demonstrate the practical utility of the PQ detecting method, two peanut kernels were separately immersed into 5 × 10⁻⁴ M and 5 × 10⁻³ M PQ solutions. As a negative control, a third peanut kernel was immersed into drinking water. After 10 min, the peanut kernels were placed into the wells of a 96-well plate. Then 2MB@CB[8] (200 µL, 5 µM) was added to each well. Any resulting fluorescence was measured using a microplate reader, with fluorescence filters providing an excitation wavelength of 640 nm and an emission wavelength of 680 nm. As shown in Fig. 3, the higher the PQ concentration to which the peanut kernels were exposed, the stronger the fluorescence exhibited, with the control showing no fluorescence.

To provide another everyday example, fresh potato (50 g) purchased from a local market was immersed in 100 mL drinking water for 30 min. The mud was filtered off, and the filtrate used to dissolve 2MB@CB[8] and PQ can be employed for the quantitative detection of trace levels of PQ.

Microscopy imaging studies were carried out to examine application of the method to living cells. MCF7 cells were incubated with 10 µM 2MB@CB[8] for 30 min, and then washed with phosphate-buffered saline (PBS) three times. Following this, cells were further incubated for 90 min with 25 µM PQ. Fluorescence imaging was carried out using a spectral confocal microscope (Olympus, FV1000). As seen in Fig. 4, staining results indicate that both 2MB@CB[8] and PQ enter the cells, where they react: the difference between cells with and without PQ treatment being dramatic.
Real-time in vivo fluorescence imaging of PQ in the living mice was also carried out, using a NightOWL II LB 983 system equipped with a NC 100 CCD deep-cooled camera (Berthold Technologies, Bad Wildbad, Germany). Before the study, the mice were sedated with isoflurane (2% in air) and shaved. PQ was introduced by thoracic (Fig. 5 left) or intraperitoneal injection (Fig. 5 right). Three minutes later, 2MB@CB[8] was injected at the same sites and fluorescence images obtained with the animals in a prone position. It is apparent from Fig. 5 that PQ can be readily tracked by using 2MB@CB[8] in living mice.

**Discussion**

To further confirm the above mentioned proposed reaction process, electrospray ionization mass spectrometry (ESI-MS) was carried out on the system (Supporting Information Fig. S4). The MS spectrum gave one positively charged peak at m/z 948.4 (calc. for [2MB + CB[8]]^{2+}, 948.3) for 2MB@CB[8] alone. To avoid any influence of free CB[8] on the ESI-MS, 3 equiv. of MB were used in the detection. After addition of PQ, an additional peak at m/z 756.96 (calc. for [PQ + CB[8]-2Cl]^{2+}, 757.2) was observed, providing strong evidence for the formation of a host–guest complex PQ@CB[8]. The supramolecular interaction of PQ with 2MB@CB[8] was further studied by 1H NMR experiments (Supporting Information Fig. S5). Upon introduction of PQ into an aqueous solution of 2MB@CB[8], typical peaks of the free MB molecule appeared. This result indicates that the MB molecules were ejected from the cavity of CB[8] as illustrated in Fig. 1.

Meanwhile, the phenomenon appeared robust during MCF7 cell staining, since if MCF7 cells were first incubated with PQ (25 μM for 30 min), washed with PBS three times, then incubated with 2MB@
CB[8] (10 μM for 90 min), similar results were obtained (Supporting Information Fig. S6). In particular, 2MB@CB[8] sensor showed a good selectivity in living cells towards other competitive binding analytes like aquacide and glyphosate etc (Supporting Information Fig. S7). The staining results thus demonstrated that 2MB@CB[8] could enter living cells and selectively demonstrate PQ in situ.

According to the literature, no significant cytotoxicity was observed for CB[8] alone within its solubility range. To confirm the cytotoxicity of the 2MB@CB[8] sensor to living cells, MTT assays were carried out and low toxicity of 2MB@CB[8] in vivo was acquired for the 12 h measurement (Supporting Information Fig. S8), suggesting that this probe would function in a biological environment.

Methods

All the solvents were of analytic grade, and the water used was double distilled. Cucurbit[8]uril was synthesized by a published method. Paraquat (PQ) and melamine blue were purchased from Sigma Aldrich. All NMR spectra were recorded on a Varian Inova-400 spectrometer with chemical shifts reported as ppm. Mass spectrometric data were obtained on a Q-ToF MS spectrometer (Micromass, Manchester, England). Absorption spectra were measured on a Perkin-Elmer Lambda 35 UV-Vis spectrophotometer. Fluorescence measurements were performed on a Varian Cary Eclipse Fluorescence Spectrophotometer. Cell imaging measurements were obtained with a spectral confocal microscope, Olympus, FV1000. Real-time in vivo fluorescence imaging of PQ in the living mice were acquired using a NightOWL II LB 983 system equipped with a NC 100 CCD deep-cooled camera (Berthold Technologies, Bad Wildbad, Germany). Mouse experiments were performed in compliance with the relevant laws and institutional guidelines, and were approved by Dalian University of Technology.

MCF7 cells were cultured in DEME (Invitrogen) supplemented with 10% FCS. Prior to staining experiments, cultured cells grown on a special confocal microscope dish were fixed by precooled methanol (−20 °C) for 15 min, and washed twice with PBS for 5 min. Then the pretreated MCF7 cells were incubated with 10 μM 2MB@CB[8] for 30 min, then PBS washed. Some cells were further incubated with 25 μM PQ for 90 min before imaging. For cells without any PQ treatment were taken as a staining control.

**Figure 5** | Fluorescence imaging of PQ in living mice. Excitation wavelengths of 580 ± 20 nm were used and the fluorescence emission was detected at 680 ± 30 nm in a Berthold chamber. Injection sites were thoracic (left) or intraperitoneal (right).

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Author contributions
S.G.S. supervised and interpreted the research and wrote the manuscript. F.S.L. and F.Y.L. performed the measurements. J.T.W. performed cell staining. X.J.P. helped withinterpreted data and wrote the manuscript. All authors discussed the results and commented on the manuscript.

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