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A supramolecular red to near-infrared fluorescent probe for the detection of drugs in urine†

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Water-soluble and fluorescent perylene dyes **PMI1** and **PMI2** with red to near-infrared (red-NIR) emission and a large Stokes shift were designed and synthesized. These dyes were designed to have two binding units (an aromatic perylene-core and a cationic side group) that allow **PMI1** and **PMI2** to form strong host–guest complexes with cucurbit[8]uril (CB8) through hydrophobic and electrostatic interactions. As a result, the binding constant of the resulting complexes was determined to be in the range of 10^6 M^{-1} which increased about 2 orders of magnitude compared to the previously reported perylene dye with only one binding unit. The results also revealed that the fluorescence emission of **PMI1** or **PMI2** only in aqueous solution is very low due to the aggregation effect. Upon complexation with CB8, the fluorescence intensity increased about 10-fold while the red-NIR emission and the large Stokes shift were preserved. These host–guest complexes can serve as red-NIR fluorescent displacement probes for the detection of CB8 binding guests. The successful detection of addictive drugs in urine was further demonstrated using the host–guest CB8·**PMI1** complex and the interference of autofluorescence from the urine sample was successfully eliminated.

Introduction

Fluorescent probes are widely used in biological and biomedical research and life sciences.[1] Among the existing fluorescent probes, organic dyes absorbing long-wavelength and emitting red to near-infrared (red-NIR) light (600–900 nm) are particularly useful in biological applications due to low background interference by the autofluorescence of biological structures, low light scattering of tissue samples, and the use of inexpensive excitation light sources.[2] Commercially available red-NIR fluorescent dyes (*e.g.*, cyanines and boron–dipyrromethene dyes) usually suffer from certain insufficient optical characteristics such as poor water solubility and photostability, low quantum yield, and narrow Stokes shift.[3] Therefore, new red-NIR fluorescent dyes with improved optical characteristics are still highly desirable in fluorescence-based applications. Perylene dyes have found widespread applications in organic materials due to their unique optoelectronic properties, excellent thermo- and photo-stability, and low toxicity.[4] However, the application of water-soluble perylene dyes as fluorescent probes has only been minimally explored due to their strong tendency to aggregate in aqueous solutions.[5] Therefore, considerable efforts have been devoted to preventing the aggregation of perylene dyes by structural modifications.[6] Host–guest complexes have recently drawn great attention because of their potential applications in drug delivery and chemosensors.[7] Several studies reported simple supramolecular approaches to prevent the aggregation of perylene dyes in water using large host molecules such as cyclodextrin (CD), cucurbit[n]uril (CBn), and ExBox that form reversible host–guest complexes.[8] Among them, the large macrocyclic host cucurbit[8]uril (CB8) shows very efficient encapsulation of perylene dyes through hydrophobic interactions between the perylene-core and the CB8 cavity, resulting in dramatic fluorescence enhancement with moderate binding affinities in the range of 10^4–10^5 M^{-1}.[8d,e] To improve the binding affinity, we recently reported a perylene dye with two hydrophobic binding units that forms a high-affinity complex with CB8 through intracavity folding.[9] Herein, we present the design and synthesis of a new water-soluble cationic perylene dye **PMI1** with an amino-substituent at the 9-position of the perylene-core, in which the 2nd quaternary ammonium cation can serve as a secondary binding motif to CB8 through electrostatic interactions with the CB8 carbonyl portal in addition to the hydrophobic binding unit (the perylene-core) (Fig. 1A). The experimental results indicated that the introduction of the secondary binding motif not only increases the solubility of the resulting complex significantly, but also enhances the binding affinity by about 2 orders of magnitude compared to the previously

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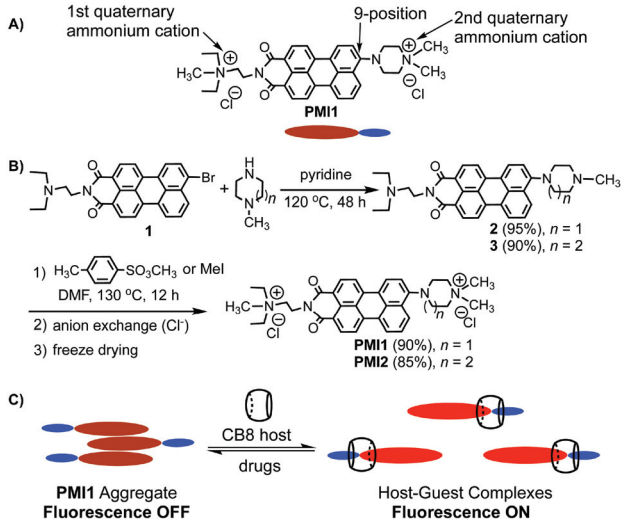

Fig. 1 (A) The chemical and cartoon structures of **PMI1**. (B) Synthesis of **PMI1** and **PMI2**. (C) Schematic illustration of the formation of the host–guest CB8·**PMI1** complex.

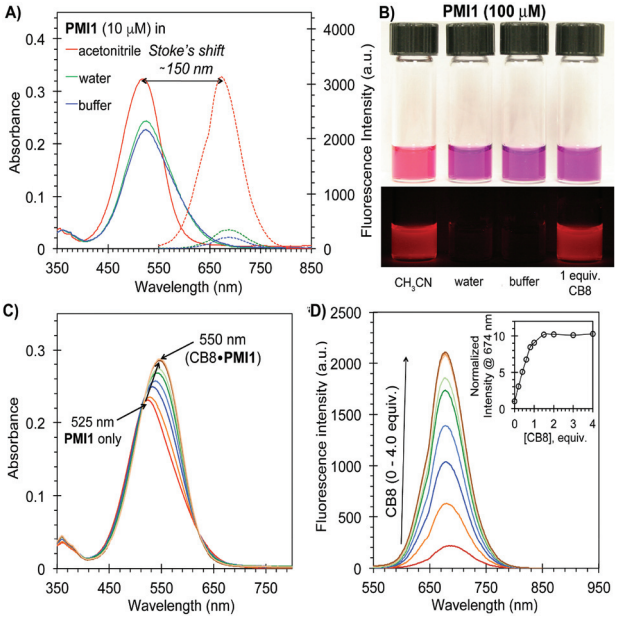

Fig. 2 (A) UV-Vis absorbance of **PMI1** (10 μM) in water, Tris-HCl (10 mM Tris, pH 7.4) buffer, and acetonitrile (CH_3CN). (B) Optical images of **PMI1** (100 μM) and the CB8·**PMI1** complex (1 : 1) under room light (top), and green LED flashlight, and a 2″ square RG665 color filter (bottom). (C) UV-Vis absorbance and (D) fluorescence emission spectra of **PMI1** (10 μM) in Tris-HCl buffer and in the presence of CB8 (0–4.0 equiv.) (inset: normalized fluorescence intensity at 674 nm *vs.* the molar equivalent of CB8. The fluorescence intensity of **PMI1** at 674 nm was normalized to 1.) The fluorescence spectra were collected when the samples were excited at 540 nm.

reported complex.[8e] More importantly, the amino-substitution was able to tune both absorption and fluorescence emission towards the red spectral range through internal charge transfer (ICT),[1d,10] resulting in red-NIR emission at 600–800 nm and a large Stokes shift of ~150 nm. Such a large Stokes shift allows the emission light to be easily separated from the excitation light and be detected readily.[11] The application of the CB8·**PMI1** complex as a displacement probe was successfully demonstrated to detect well-known addictive drugs (*e.g.*, cocaine and angel dust) in urine with low background interference by the autofluorescence of the urine sample.

Results and discussion

The synthesis of **PMI1** starts from **1**, which was prepared as described previously.[12] Compound **2** was obtained with a high product yield of 95% after substitution of **1** with 1-methylpiperazine in pyridine without using a catalyst (*e.g.*, nickel catalysts and Pd_2dba_3).[13] **PMI1** was obtained after quaternization of two tertiary amino groups followed by anion exchange with Cl^- from **2** (Fig. 1B). **PMI1** is very soluble in polar organic solvents (*e.g.*, acetonitrile) and aqueous solvents (*e.g.*, water and buffers), and the solubility of **PMI1** in water is higher than 5 wt% (~0.08 M), owing to two permanent positive charges. In water or Tris-HCl buffer (pH 7.4), **PMI1** (10 μM) exhibits an absorption spectrum with a λ_{max} at ~525 nm and low fluorescence with a λ_{max} at ~680 nm (Fig. 2A), owing to the self-quenching of **PMI1** aggregates in water.[8e,9] In acetonitrile (CH_3CN), the absorption λ_{max} of **PMI1** (10 μM) is at ~520 nm with an increased absorptivity while the fluorescence intensity at λ_{max} increased about 14 times. The Stokes shift was found to be about 150 nm. In addition, **PMI1** (100 μM) in CH_3CN shows bright red emission through a RG665 color filter while it is dark in water and Tris-HCl buffer when the samples were excited with a green LED flashlight (Fig. 2B). These results suggest the formation of non-fluorescent aggregates of **PMI1** in aqueous solution and the presence of fluorescent monomers in CH_3CN.

The host–guest complexation between **PMI1** and CB8 was investigated in detail using UV-Vis and fluorescence spectroscopy. The addition of CB8 to **PMI1** (10 μM) in Tris-HCl buffer (pH 7.4) caused a gradual increase of the absorption peak with a spectral shift from 525 to 550 nm (Fig. 2C). The fluorescence intensity of **PMI1** at λ_{max} increases gradually as the concentration of CB8 increases and a maximum enhancement (~10 times) was reached with about 1.5 equivalents of CB8 (Fig. 2D). The Job plot shows the maximum intensity at a molar fraction of the guest equal to 0.5, indicating the formation of a 1 : 1 host–guest complex for **PMI1** with CB8 (Fig. 3A). The binding affinity (K_a) values of CB8·**PMI1** in Tris-HCl buffer (pH 7.4) and water as determined by fluorescence titrations at a lower concentration (1.0 μM) were calculated to be 1.32×10^6 M^{-1} and 1.63×10^6 M^{-1} using a 1 : 1 binding model with the Origin program (Fig. 3B, Fig. S1, and Table S1†).[14] These values are about 100 times larger than that of the previously reported complex with one hydrophobic binding unit. The binding of **PMI1** to CB8 was further investigated by ^{1}H-NMR spectroscopy and the key peaks on the

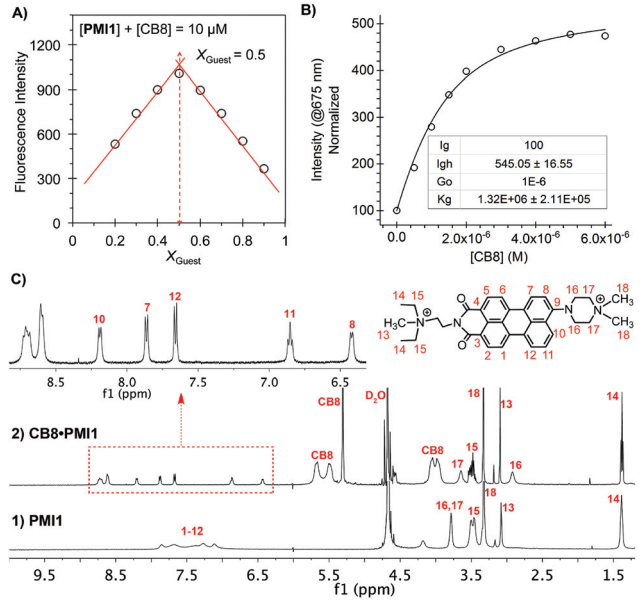

Fig. 3 (A) A Job plot for CB8·**PMI1** (the total concentration of guest **PMI1** and host CB8 was fixed at 10 μM and the fluorescence intensity was used in the calculation). (B) The binding curve for CB8·**PMI1**. (Fluorescence titrations of **PMI1** (1.0 μM) in the presence of CB8 and the fluorescence intensity at 675 nm was plotted against the concentration of CB8. The solid line represents the best fitting of the data to a 1 : 1 binding. The fluorescence intensity of **PMI1** at 675 nm was normalized to 100.) (C) ^{1}H-NMR spectra of **PMI1** (1.0 mM) and CB8·**PMI1** (1.0 mM, 1.0 mM) in D_2O.

^{1}H-NMR spectrum were assigned to corresponding protons with the aid of 2D NMR correlation spectroscopy (COSY) (Fig. S2†). Similar to previously reported perylene dyes, the ^{1}H-NMR spectrum of **PMI1** (1.0 mM) in D_2O shows a set of broad aromatic peaks at 7.0–8.0 ppm for protons of the perylene-core due to **PMI1** aggregates (Fig. 3C).[8e,9] The addition of 1.0 equivalent of CB8 resulted in a sharp one-proton doublet at ~6.4 ppm and a sharp one-proton triplet at ~6.8 ppm for aromatic protons 8 and 11, respectively. Additionally, the peaks of protons 16 and 17 show a substantial upfield shift. These observations suggest the encapsulation and inclusion of part of the perylene-core (8–11) and the piperazinyl ring (16 and 17) by the cavity of CB8.[15] To our knowledge, this is the first observation of a dramatic upfield shift for protons of the perylene-core upon encapsulation with CB8.[8e,9] Interestingly, by considering the chemical structure of **PMI1**, proton 10 must be encapsulated in the cavity of CB8 together with protons 8 and 11. However, the peak of proton 10 shows an opposite downfield shift instead probably due to the deaggregation effect and a weak interaction with CB8. Additionally, no significant changes were observed for the chemical shift of protons 13, 14, and 15 of the quaternary ammonium alkyl side-chain, further confirming the encapsulation of CB8 on the amino-substituted side and not the alkyl side-chain. The formation of a CB8·**PMI1** 1 : 1 complex was also supported by mass spectrometry (Fig. S3†). Furthermore, the stability of the CB8·**PMI1** complex with temperature and pH was further investigated by fluorescence spectroscopy. No significant changes were observed in the fluorescence emission of the CB8·**PMI1** complex (1 : 1, 10 μM) during a temperature course study from 25 to 75 °C and a pH study from 5 to 9, suggesting high stability of the CB8·**PMI1** complex (Fig. S4 and S5†). As expected, the binding affinities of the complex at different pH values were determined to be about 1.2–1.4 × 10^6 M^{-1} (Fig. S6–S8 and Table S1†). The high affinity and stability allow the CB8·**PMI1** complex to serve as an excellent probe for displacement applications.

Previous studies suggest that the structural modification of the guest molecules can afford differences in binding, self-assembly, and optical properties of the corresponding host–guest complexes.[16] Therefore, we further synthesized **PMI2** by replacing the six-membered cyclic diamine (1-methyl piperazine) with a seven-membered cyclic diamine (1-methyl homopiperazine) by using the same synthetic route (Fig. 1B). We expected that this minor one-carbon modification would improve the binding affinity further. The optical properties and the binding of **PMI2** were investigated by UV-Vis, fluorescence, and ^{1}H-NMR spectroscopy. The results revealed that **PMI2** forms a 1 : 1 host–guest complex with CB8 and the binding affinity was determined to be 6.97 × 10^6 M^{-1} in Tris-HCl buffer (pH 7.4), which is about 5 times greater than that of the CB8·**PMI1** complex (Fig. S9–S11†).

Further studies were performed to explore the fluorescence assay for drug detection in aqueous buffer utilizing the CB8·**PMI1** complex as a fluorescence displacement probe. Several commonly used addictive drugs were examined as examples including phencyclidine (PCP, an illegal street drug, commonly referred to as "angel dust"), cocaine (COC, an illicit addictive stimulant drug), methadone (MTD, an opioid used to treat pain), and phenazopyridine (Pyridium, a prescribed drug for local analgesic effects on the urinary tract).[17] As shown in Fig. 4A, the presence of 10 μM (1 eq.) of PCP caused a significant drop (~85%) in the fluorescence intensity of the CB8·**PMI1** complex (1 : 1, 10 μM) in Tris-HCl buffer, suggesting the strong binding of PCP to CB8. The study also revealed that the displacement process is very rapid and a stable fluorescence intensity can be achieved after mixing PCP in the complex solution. The detection limit of PCP was determined to be about 0.6 μM (Fig. S15†). In addition, the ^{1}H-NMR spectroscopy study suggests the complete displacement of the CB8·**PMI1** complex (1 : 1, 1.0 mM) upon the addition of 1.0 equiv. of PCP (Fig. S12†). In comparison, the fluorescence intensity of the CB8·**PMI1** complex dropped 55% and 20% in the presence of 10 μM of Pyridium and COC, respectively. The binding affinity of CB8·Pyridium and CB8·COC was calculated to be 5.09 × 10^6 M^{-1} and 6.05 × 10^5 M^{-1}, respectively, using a fluorescence displacement model (Fig. S13, S14, and Table S1†).[18] In contrast, the presence of excess MTD did not cause a significant change in the fluorescence emission of the CB8·**PMI1** complex, indicating very weak binding of MTD to CB8 (Fig. 4D). Additionally, the titration curves clearly indicate that the binding affinities of all four drugs with CB8 follow the order of PCP > Pyridium > COC > MTD (Fig. 4E). These results

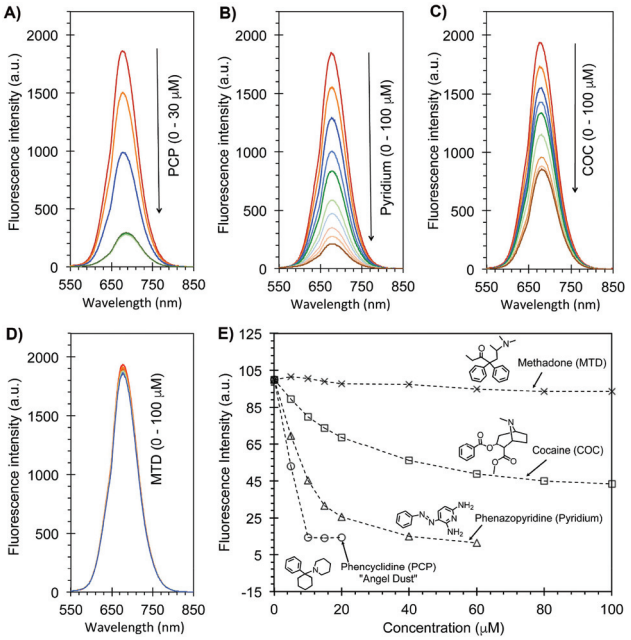

Fig. 4 Fluorescence spectra of the CB8·**PMI1** (1 : 1) complex (10 μM) in Tris-HCl buffer (pH 7.4) in the presence of (A) PCP, (B) Pyridium, (C) COC, and (D) MTD. (E) Fluorescence titration curves of the CB8·**PMI1** (1 : 1) complex (10 μM) in Tris-HCl buffer (pH 7.4) in the presence of PCP, Pyridium, COC, and MTD.

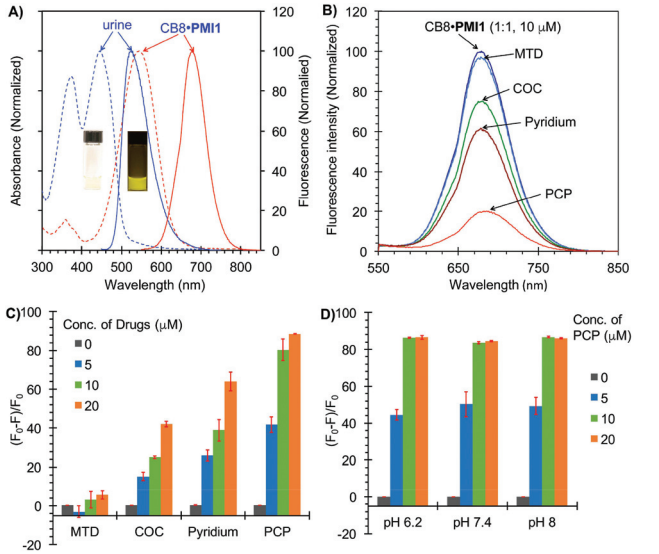

Fig. 5 (A) Absorbance and fluorescence spectra of synthetic urine and the CB8·**PMI1** (1 : 1) complex (10 μM, in pH 7.4 Tris-HCl buffer). (B) Fluorescence spectra of the CB8·**PMI1** (1 : 1) complex (10 μM, in synthetic urine) in the presence of 10 μM of PCP, Pyridium, COC, or MTD. (C) Fluorescence changes of the CB8·**PMI1** (1 : 1) complex (10 μM, in synthetic urine) in the presence of PCP, Pyridium, COC, and MTD with concentrations of 0, 5, 10, and 20 μM. The error bars were obtained from three independent experiments. (D) Fluorescence changes of the CB8·**PMI1** (1 : 1) complex (10 μM, in synthetic urine) in the presence of PCP at pH 6.2, 7.4 and 8.0. Fluorescence intensity of CB8·**PMI1** at λ_{max} was normalized to 100.

suggest that MTD cannot displace **PMI1** from the CB8·**PMI1** complex presumably due to the steric hindrance of MTD. Complete displacement of **PMI1** with 1 equiv. of PCP suggests strong binding of PCP with CB8 probably due to the inclusion of the phenyl group and the pyridine ring in the CB8 cavity. In the case of Pyridium and COC, the relatively weak binding compared to CB8·PCP is presumably due to the loose fitting of Pyridium and the carbonyl group effect of COC, respectively. This study suggests that the CB8·**PMI1** complex may serve as an efficient displacement probe for the detection of drugs in aqueous solutions and the binding affinity of certain drugs with CB8 can be determined.

To further explore the feasibility of the CB8·**PMI1** complex as a red-NIR fluorescence displacement probe in a real sample, we demonstrated the detection of drugs in synthetic urine. Interestingly, the study revealed that this synthetic urine sample itself absorbs UV-Vis light at 300–500 nm and generates very strong autofluorescence in the range of 500 to 650 nm when it is excited at 450 nm (Fig. 5A). However, the red-NIR emission and large Stokes shift minimize the background interference from autofluorescence and allow the CB8·**PMI1** complex to serve as a probe for detecting drugs in synthetic urine. As expected, the presence of different drugs (PCP, Pyridium, COC, and MTD) in urine showed similar results compared to those in aqueous buffer (Tris-HCl, pH 7.4) (Fig. 5B and 5C). The above study suggests that the binding affinities of the CB8·**PMI1** complex are similar in the pH range of 5 to 9. We further investigated the pH effect on the fluorescence displacement of the CB8·**PMI1** complex using PCP in urine samples (Fig. 5d). As expected, similar displacement profiles were obtained for pH 6.2, 7.4, and 8.0, indicating the pH independence of the fluorescence displacement of the CB8·**PMI1** complex (Fig. S6–S8†).

Conclusions

In summary, we have designed and synthesized water-soluble fluorescent perylene dyes **PMI1** and **PMI2** with two binding units for strong binding with CB8 through hydrophobic and electrostatic interactions with CB8. The binding affinity of CB8·**PMI1** and CB8·**PMI2** complexes was determined to be 1.32×10^6 M^{-1} and 6.97×10^6 M^{-1}, respectively, which increased by 2 orders of magnitude compared to the dye with only one hydrophobic binding unit. These results suggested that the binding affinities of host–guest complexes can be tuned by rational design of guest molecules. More importantly, the red-NIR emission, high affinity and stability, and large Stokes shift allow the CB8·**PMI1** complex to serve as a useful displacement probe for the detection of addictive drugs in urine and the interference of autofluorescence from the urine sample can be minimized.

Conflicts of interest

There are no conflicts to declare.

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