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ARTICLE TYPE

Acidic microenvironment triggered release of a Cys probe from the cavity of a water-soluble pillar[5]arene

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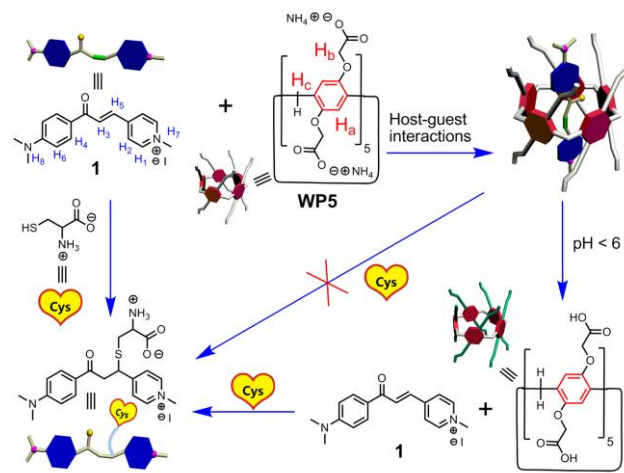
The release of a Cys probe from the cavity of a water-soluble pillar[5]arene can be realized in acidic microenvironment.

Biothiols, such as cysteine (Cys), homocysteine (Hcy), and glutathione (GSH) play crucial roles in maintaining biological systems, and are significantly associated with a wide variety of diseases, including cardiovascular disease, Alzheimer's disease, leucocyte loss, psoriasis, liver damage, cancer, and AIDS.¹ Therefore, it is important to develop efficient methods for the detection and quantification of biothiols in physiological media. Indeed, numbers of thiol-specific probes have been developed on the basis of various strategies, for example, Michael addition, cleavage reactions by thiols, metal coordination, cyclization with aldehydes, and so on.² Currently, it is well-known that, in the thiol-specific probes systems reported previously, thiols will interact with their corresponding probe as soon as they met each other, making it difficult to develop a thiol-specific probe system that can detect thiol in specific regions as we expected, in other words, the interaction between thiol and its probe only exist in the targeted area but not others.³

It is generally known that the contents of thiols in tumor cells are different from those in normal cells, thus the quantitative detection of thiols can be helpful to find tumor cells and further benefit the early identification of diseases and assessment of disease development.⁴ Therefore, it is significant to develop an specific targeted probe with the ability to detect thiols only in tumor cells. Inspired by the concept of drug delivery system,⁵ we assumed that if a probe was placed into the cavity of a macrocyclic host, the captured probe would not interact with thiols, while the release of the thiol-specific probe could be realized in tumor cells. Due to acidic microenvironment of the intracellular and extracellular compartments in tumor cells, the release should be triggered by acidic microenvironment. To achieve this goal, it is necessary to develop a macrocyclic host with the ability to bind the probe much stronger than thiol, and the complex of the macrocyclic host/probe can be destroyed by the addition of acid.

Pillararenes^{6,7} are a new generation of macrocyclic hosts for supramolecular chemistry after crown ethers, cyclodextrins, calixarenes, and cucurbiturils.^{8,9} Their repeating units are connected by methylene bridges at the *para*-positions, forming a special rigid pillar-like architecture. The unique structures and easy functionalization of pillararenes have endowed them with outstanding abilities to selectively bind different kinds of guests

and provided a useful platform for the construction of various interesting supramolecular systems. Especially, water-soluble pillar[5]arene **WP5** (Scheme 1) has been demonstrated to be an excellent host for molecules of various sizes and shapes in water.^{6f,7e,7m} Moreover, **WP5** can be neutralized by adding acid into water, disassembling the **WP5**-based host-guest complexes.^{7m} Consequently, **WP5**-based molecular recognition has been utilized to construct many pH-responsive supramolecular materials in water.^{5a,7e,7i,7m}



Scheme 1. Chemical structures of **WP5**, **1**, and Cys and the cartoon representation of the controlled release of Cys probe **1** from the cavity of **WP5** by changing pH.

Herein we report that **WP5** can interact with probe **1** strongly in water to form an inclusion complex. The formation of this inclusion complex inhibits the interaction between probe **1** and Cys. The encapsulated **1** can be released from the cavity of **WP5** upon the addition of acid into water, leading to the formation of **1**-Cys adduct.

WP5 was prepared according to the previous work.^{7e} **1** is a new compound composed of 1-(4-Dimethylamino-phenyl)-ethanone conjugated with a substituted methyl pyridinium group through an unsaturated ketone unit. It was confirmed by ¹H NMR, ¹³C NMR, and ESI-MS spectroscopy (Figs. S4–S6, ESI†).

The complexation of **WP5** with **1** was first studied by ¹H NMR experiments (Fig. 1). When 1.00 equiv of **WP5** was added into a D₂O solution of **1** (3.00 mM), all the signals related to the

protons on **1** shifted upfield obviously except H₈. Additionally, extensive broadening occurred due to the complexation dynamics. This phenomenon was the result of the formation of a threaded structure between **WP5** and **1**.^{7j} On the other hand, the protons on **WP5** also exhibited slight chemical shift changes. From the 2D NOESY spectrum (Fig. S7, ESI†) of a mixture of 10.0 mM **WP5** and **1** in D₂O, correlations were observed between protons H₁–H₈ of **1** and protons H_a–H_c on **WP5**, suggesting that **1** was threaded into the cavity of **WP5**. Therefore, we concluded that when **1** was mixed with **WP5**, it penetrated through the cavity of **WP5**. We speculated that the formation of the complex between **WP5** and **1** was mainly driven by multiple electrostatic interactions between the carboxylate anionic groups on **WP5** and the cationic pyridinium unit of the **1**, hydrophobic interactions, and π – π stacking interactions between the benzene rings on the host **WP5** and the pyridinium ring on the guest **1** in aqueous solution. The binding affinity of this host–guest system can be attributed to the cooperativity of these noncovalent interactions. It means that **1** was placed inside the cavity of **WP5** successfully.

The isothermal titration calorimetry (ITC) experiments were performed under neutral conditions, and the results suggested: the complex stability constant $K_s = (1.15 \pm 0.0300) \times 10^5 \text{ M}^{-1}$, enthalpy change $\Delta H^\circ = -39.8 \pm 0.321 \text{ kJ mol}^{-1}$, entropy change $\Delta S^\circ = -0.0367 \text{ kJ mol}^{-1}$ and the stoichiometry of the complex of **WP5** with **1** was 1:1 (Fig. S8, ESI†).

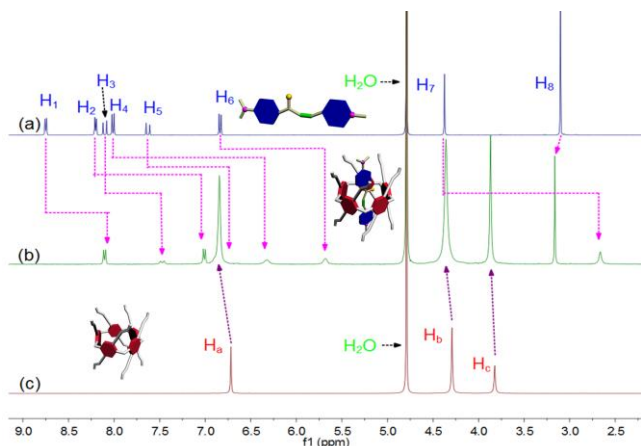


Fig. 1 Partial ¹H NMR spectra (500 MHz, D₂O, 298 K): (a) 3.00 mM **1**; (b) 3.00 mM **1** and **WP5**; (c) 3.00 mM **WP5**.

UV-vis spectroscopy characterization of the complexation between **WP5** and **1** was also carried out at room temperature in water (Fig. S9, ESI†). Upon gradual addition of 0.00–1.50 equiv. of **WP5** (20 mM) to **1**, a broad absorption band at 540 nm corresponding to the charge-transfer interaction between electron-rich **WP5** and electron-deficient **1** was observed. A notable red shift occurred, indicating the formation of a typical charge-transfer complex.^{7g}

Fig. 2 (a) shows the changes in the UV-vis spectra of the PBS (0.100 M, pH = 7.4) solution of **1** (20.0 μM) upon the gradual addition of Cys. As the concentration of Cys (0.00–3.00 equiv.) increased, the absorption peaks at 435 nm gradually decreased and a new peak appeared at 350 nm (blue shifted 85 nm). A well-defined isosbestic point was noted at 380 nm, indicating the formation of a new kind of species.² The hypsochromic shift in absorption suggested that the intramolecular charge transfer (ICT)

from the electron-rich dimethylamino moiety to the electron-poor pyridinium moiety was turned off in the adduct.⁴ In addition, the formation of the **1**–Cys adduct was confirmed by mass spectrometric analysis (Fig. S10, ESI†). Moreover, **1** was stable and displayed the best response for Cys in the pH region of 4–11 (Fig. S12, ESI†). These results indicated that **1** reacted with Cys and allowed the Cys detection in a wide range of pH values.

We recorded the UV-vis spectrum changes of **WP5** \supset **1** upon the addition of 3.00 and 9.00 equiv (Fig. 2b) of Cys in water. It can be seen that the inclusion compound exhibited almost no response with the addition of Cys. For this system, **1** resided in the cavity of **WP5** and Cys could not pass through the **WP5** carrier. Therefore, **1** showed no responsiveness to Cys as we expected.

From the above results, it was seen that the absorbance intensity of **1** induced by 3 equiv. of Cys increased much faster and then leveled off within 3 min (Fig. 2a). However, for **WP5** \supset **1**, with the presence of 3 equiv. or even 9 equiv. of Cys, it exhibited almost no change in about 5 min (Fig. 2b). These results indicated that **WP5** played a crucial role in the inhibition for the interaction of **1** with Cys.

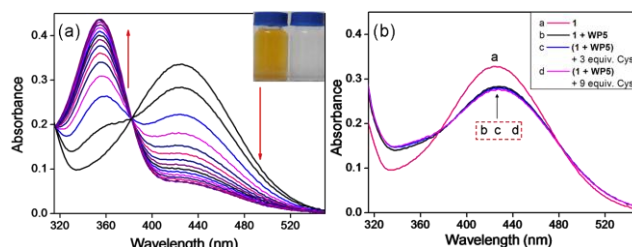


Fig. 2 (a) UV-vis spectra of **1** (20.0 μM) in PBS buffer (0.1 M, pH = 7.4) upon addition of various concentrations of Cys (0–3 equiv.). Inset: a color change photographs for **1** (left) and **1** + Cys (right). (b) Absorption intensity of **1** (20.0 μM) upon addition of **WP5** (20 mM) and Cys in water.

It is well-known that anionic carboxylate groups and neutral carboxylic groups can be interconverted by changing the solution pH. We envisioned that the present inclusion complex **WP5** \supset **1** could show pH-sensitivity. Thus we then tested the release behavior of **1** from the **WP5** \supset **1** complex by changing the solution pH. The release of **1** was easily observed by the naked eye, a white precipitate appeared after the aqueous HCl solution was added and the solution pH was 6.0~4.0. ¹H NMR provided convincing evidence for the pH-responsive complexation between **WP5** and **1** (Fig. S11, ESI†). When the solution pH was adjusted from 7.4 to 4.0, the chemical shifts corresponding to protons of **1** almost returned to their uncomplexed values and the signals of the protons of **WP5** disappeared (Fig. 11c, ESI†), indicating that the complexation of **1** and **WP5** was totally quenched.

We also tested the responsiveness of **1** to Cys after released from acidic microenvironment. As shown in Fig 3, the absorption peaks at 435 nm of the UV-vis curve of **1** + **WP5** + HCl was higher than that of **1** + **WP5** when the solution pH was adjusted from 7.4 to 5.0, proving the pH-responsiveness of the **WP5** \supset **1** complex. Moreover, the absorption peaks at 435 nm decreased and a new peak appeared at 350 nm upon the subsequent addition of 3 equiv. of Cys, implying that the interaction between **1** and Cys recovered after the removal of **WP5**.

This pH-responsive rapid release of **1** can be explained by

introducing a pH-triggered inclusion complex. This rapid release was triggered in a physiologically relevant pH region (4.0–6.0), namely acidic microenvironment, making the **WP5**→**1** complex as an ideal candidate for probe release. However, the UV-vis spectrum did not recover to its original level. Three possible reasons are: (1) the solution was diluted; (2) the ionic strength of the solution increased; (3) different counterions were introduced, influencing ion pairing.¹⁰

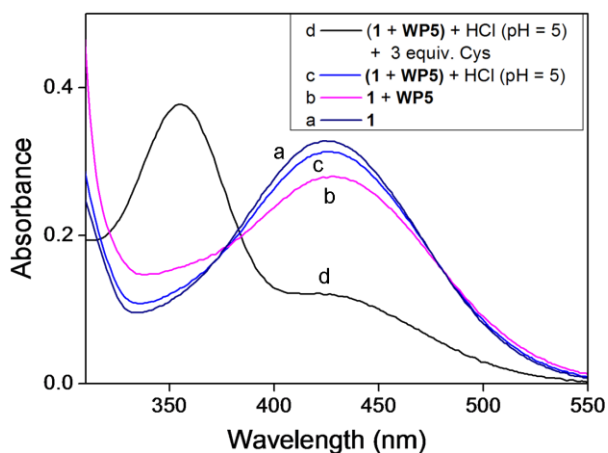


Fig. 3 Absorption intensity of **1** (20 μ M) upon addition of **WP5** (20 mM), HCl and Cys in water.

In conclusion, we have successfully constructed a novel and strong inclusion complex between water-soluble pillar[5]arene **WP5** and Cys probe **1** in water. This complex exhibited no responsiveness to Cys under neutral condition. The reason was that the formation of the stable inclusion complex inhibited the interaction between probe **1** and Cys. The encapsulated **1** could be released from the cavity of **WP5** upon the addition of acid into water, recovering the formation of **1**–Cys adduct. This acidic microenvironment triggered release behavior has potential applications in the quantitative detection of thiols in tumor cells, and the novel supramolecular inclusion complex of **WP5**→**1** will provide an important pathway for guiding the design of functional supramolecular biomaterials.

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Notes and references

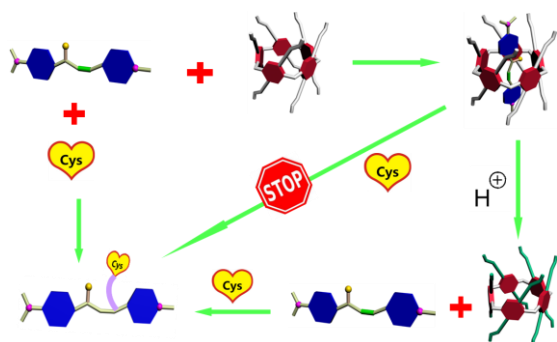
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† Electronic Supplementary Information (ESI) available: Synthetic procedures, characterizations, association constant determination, and other materials. See DOI: 10.1039/c0xx00000x.

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Toc Graphic:**Text:**

10 A Cys probe is prepared. It can be included into the cavity of a
 water-soluble pillar[5]arene. This **WP5** probe complex shows no
 responsiveness toward Cys under neutral condition in water,
 while the release of Cys probe can be realized in acidic
 microenvironment.

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