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# An imidazole-appended *p*-phenylene-Cu(II) ensemble as a chemoprobe for histidine in biological samples†

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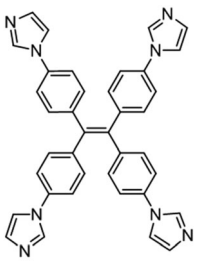
**A tetra-imidazole-appended tetrakis(*p*-phenylene)ethylene 1-$Cu^{2+}$ ensemble was found to enhance fluorescence upon addition of histidine, but not with any other amino acids. The 1-$Cu^{2+}$ ensemble also selectively detected proteins containing histidine residues in a mixture of water and methanol (90 : 10, v/v%). The 1-$Cu^{2+}$ ensemble-coated thin-layered chromatography (TLC) plate could also detect histidine quantitatively. Furthermore, the fluorescence emission recovery upon addition of five concentrations of His was ~80% with good linearity.**

Histidine (His) is an essential amino acid required for human growth.[1] It can also act as a neurotransmitter in the central nervous system of mammals.[2] A deficiency of histidine may result in the impaired nutritional state of patients with chronic kidney disease.[3] Moreover, an abnormal level of histidine-rich proteins in the urine could indicate a variety of diseases such as asthma[4] and advanced liver cirrhosis.[5] Accordingly, determination of His in biological samples is of great importance in biochemical analysis and a number of methods have been developed for its detection.[6] In particular, fluorimetric detection has recently attracted considerable attention because of its high sensitivity and spatial resolution.[7]

In chemical ensembles such as fluorogenic chemoprobes, the receptor is noncovalently attached to a fluorescence moiety which serves as an indicator.[8] The binding between the receptor and the analyte at the binding site results in either formation of a new complex or displacement of the fluorophore from the receptor, both of which would cause changes in fluorescence. These ensemble systems have several advantages for this method of signaling. First, the water solubility of an ensemble can be greatly improved, especially those containing a metal center. Second, an ensemble can usually be easily prepared, without the requirement for complicated synthesis procedures, which affords more practical and convenient applications. Third, sensing of various analytes can be accomplished by simply changing the receptor or the fluorescent indicator. There are a few examples of an ensemble as a "turn-on" chemoprobe for His and proteins containing His moieties.[9] Herein, we report a tetrakis(*p*-phenylene)ethylene-based-$Cu^{2+}$ ensemble-based chemoprobe for proteins containing His in aqueous solution and in biological fluids.

The tetra-imidazole-appended tetrakis(*p*-phenylene)ethylene (**1**) was prepared using a slightly modified version of a previously reported[10] procedure as shown in Scheme S1 (ESI†). The ligand structure, based on the phenylethylene central core, facilitated the conversion of the initial self-assembled **1** into a stabilized form through four part π–π stacking. The core was connected to the imidazole by a solid-state reaction using an Ullmann coupling protocol in 50% yield; the imidazole moiety enabled the coordination of specific metal ions and the concomitant formation of a higher-order self-assembled nanostructure.



**1**

Compound **1** exhibited a strong emission when dissolved in pure $H_2O$ (excitation wavelength = 345 nm). On the other hand, the addition of methanol into these solutions decreased the emission. As shown in Fig. 1, the luminescence properties of **1** were observed at different ratios of water and methanol in composite solutions (100 : 0–0 : 100 v/v%). When the water and methanol solution was 90 : 10 v/v%, **1** showed an intense fluorescence emission band at 470 nm when photoexcited at 345 nm; compared to the fluorescence spectrum acquired in pure water, the emission

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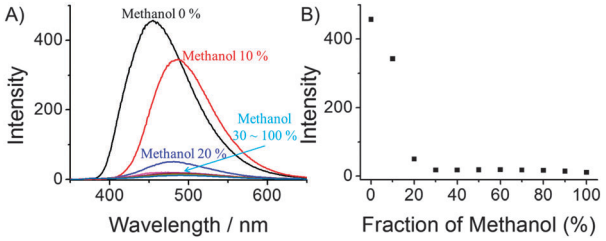


Fig. 1 (A) Photoluminescence spectra of **1** (1.0 μM) at different ratios of water and methanol in composite solutions (100 : 0–0 : 100 v/v%). (B) Plot of fluorescence intensity against different ratios of water and methanol in composite solutions (100 : 0–0 : 100 v/v%).

band was red shifted by 20 nm. On the other hand, the intensity of the strong emission band of **1** dramatically decreased when the content of water and methanol was changed to 60 : 40 v/v%, due to changes in monomeric species by the increase in the fraction of methanol. The mixture was transparent and homogeneous. Thus, the SEM images of **1** prepared in both pure water and a mixture of water/methanol (80 : 20 v/v%) were observed by scanning electron microscopy (SEM). The SEM images of **1** in pure water and in a mixture of water/methanol (80 : 20 v/v%) showed nanoparticles of ~20 nm diameter, which is homogeneous (Fig. S1, ESI†). In contrast, we did not observe the aggregated morphology of **1** in a mixture of water/methanol (40 : 60 v/v%), suggesting that molecules of **1** did not aggregate in an aqueous mixture with a higher methanol fraction.

To gain insights into the forces that drive the assembly of **1**, we observed the $^1$H NMR spectra of **1** at different compositions of solvents (Fig. S2, ESI†). In pure water, the aromatic protons of **1** were shifted to the high field region and were broadened whereas in mixtures of methanol and water, the aromatic protons of **1** were shifted to the low field and sharpened as the concentration of methanol increased. These results suggest that molecules of **1** form an aggregate by π–π stacking in pure $H_2O$, but they exist as monomeric species in higher compositions of methanol in mixtures of methanol and water. Blue shift of **1** with strong emission in pure water can be explained as follows. The first is due to restriction of intermolecular rotations of monomeric **1** by solvent. The second is due to π–π stacking between molecules **1**. Thus, π–π stacking of **1** definitely hinders intermolecular rotation, as observed by the NMR experiment, so the strong emission of **1** would be induced by restriction of intermolecular rotations with π–π stacking between molecules **1** in pure water.

We then examined the fluorescence response of **1** (1.0 μM) upon addition of $Cu^{2+}$ (ranging from 0–1.0 equivalent) in a mixed water and methanol (90 : 10 v/v%). The fluorescence of **1** at 470 nm dramatically decreased when 0.2 equivalents of $Cu^{2+}$ were added and became quenched completely when *ca.* 0.66 equivalents of $Cu^{2+}$ were added, indicative of formation of a 2 : 3 complex between $Cu^{2+}$ and **1** (Fig. 2). The π–π stacking between the phenylene groups of **1** with $Cu^{2+}$ induced a quenching effect by H-aggregation mode. A Job plot for complexation also gave a 2 : 3 stoichiometry (Fig. S3, ESI†). On the other hand, we also observed the fluorescence changes of **1** upon addition of other

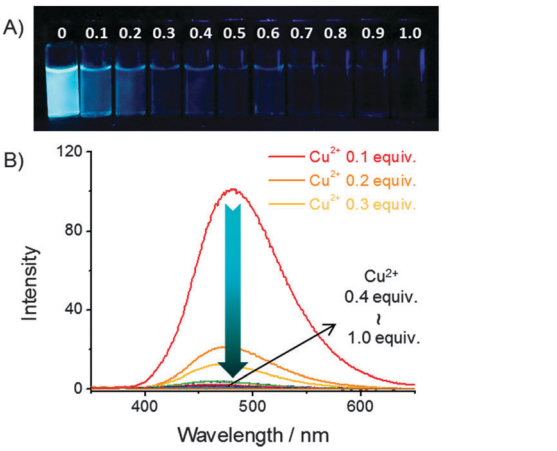


Fig. 2 (A) Photograph of **1** (1.0 μM) upon addition of various concentrations of $Cu^{2+}$ (0–1.0 equivalent) under UV lamp. (B) Photoluminescence spectra of **1** (1.0 μM) with various concentrations of $Cu^{2+}$ (0–1.0 equivalent) in a mixed water and methanol (90 : 10 v/v%).

metal ions such as $Hg^{2+}$, $Co^{2+}$, $Ni^{2+}$, $Zn^{2+}$ and $Mn^{2+}$ (Fig. S4, ESI†). No significant fluorescence changes of **1** were observed upon addition of metal ions, because **1** did not form strong complexes with other metal ions.

To investigate the selectivity for amino acids, the fluorescence intensity change of **1**-$Cu^{2+}$ ensemble was observed upon addition of various amino acids (1.5 equivalents). As shown in Fig. 3A, **1**-$Cu^{2+}$ exhibited a strong emission with addition of histidine (His) when the mixture was viewed under a UV lamp. The addition of other amino acids, in contrast, had no effect on the fluorescence changes of **1**-$Cu^{2+}$. Thus, **1**-$Cu^{2+}$ responded selectively to histidine, a response which can be attributed to the displacement of $Cu^{2+}$ from **1** by His. The fluorescence of **1**-$Cu^{2+}$ increased significantly upon addition of His, with a fluorescence enhancement of up to ~100-fold with the addition of 1.5 equivalents of His (Fig. 3B). The addition of His resulted in a linear increase in the fluorescence intensities of the **1**-$Cu^{2+}$ ensemble (Fig. 3C and Fig. S5, ESI†), indicating that $Cu^{2+}$ became dissociated from the **1**-$Cu^{2+}$ complex, and then the His molecule became bound to $Cu^{2+}$ (Fig. 3D). The **1**-$Cu^{2+}$ complex also showed excellent sensitivity with 20 ppb of histidine fluids (Fig. S6, ESI†), which is sensitive enough for its practical applications in measurements of His in biological samples.

We also observed the fluorescence intensity changes of the **1**-$Cu^{2+}$ ensemble upon addition of various amino acids. As shown in Fig. S7 (ESI†), the addition of 100 equivalents of other amino acids such as valine (Val), serine (Ser), phenylalanine (Phe), alanine (Ala), cysteine (Cys), lysine (Lys), leucine (Leu), asparagine (Asp), glutamic acid (Glu), glycine (Gly), methionine (Met) and arginine (Arg) did not cause any obvious fluorescence enhancement. These findings suggest that the highly selective nature of the fluorescence change of the **1**-$Cu^{2+}$ ensemble is due to the high selectivity of the assay for His.

We further investigated the interaction between His and **1**-$Cu^{2+}$ by using ESI spectroscopy (Fig. S8, ESI†). The ESI spectrum of the **1**-$Cu^{2+}$ ensemble upon addition of His corresponded to [2His + $Cu^{2+}$] at $m/z$ ~ 373.08 while the peak at $m/z$ ~ 597.50 was related to **1**,

 

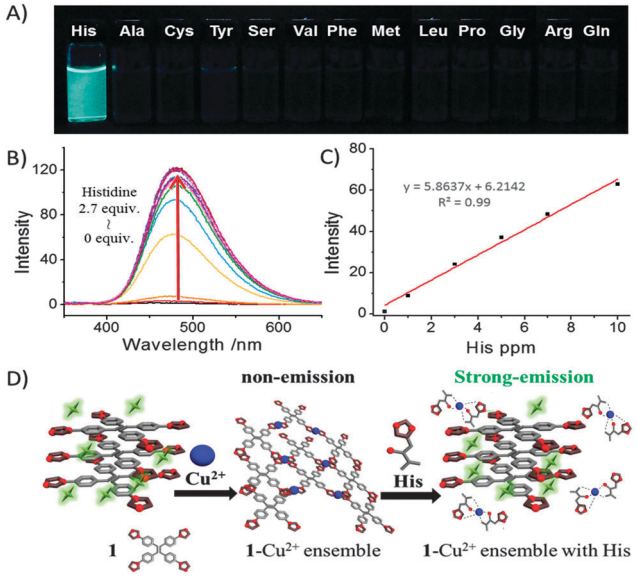


Fig. 3 (A) Photograph of **1** (1.0 μM) upon addition of various amino acids (2.0 equivalent) under UV lamp. (B) Photoluminescence spectra of **1** (1.0 μM) with various concentration of His (0–2.7 equivalent) in a mixed water and methanol (90 : 10 v/v%). (C) Plot of fluorescence intensity at 470 nm against the concentration of histidine (0–10 ppm). (D) Schematic illustration of the detection process for histidine by the **1**-$Cu^{2+}$ ensemble.

indicating that the binding between His and $Cu^{2+}$ led to the dissociation of the **1**-$Cu^{2+}$ ensemble. Thus, the selective detection of His by the **1**-$Cu^{2+}$ ensemble was based on a displacement approach. Furthermore, the appearance of a peak at 373.08 evidenced the formation of a typical complex for [2His + $Cu^{2+}$].

To determine if the specificity of detection for His was still functional in the presence of an excess of other amino acids, we performed competition experiments to investigate the further utility of the **1**-$Cu^{2+}$ ensemble (Fig. S9, ESI†). The addition of various amino acids (100 equivalents) in water and methanol (90 : 10 v/v%) to the solution of the **1**-$Cu^{2+}$ ensemble containing 1.5 equivalents of His did not induce any significant changes. Hence, the **1**-$Cu^{2+}$ ensemble may serve as a selective chemoprobe for His even in the presence of other relevant amino acids.

We also observed the microscopic image of the **1**-$Cu^{2+}$ ensemble in the absence and the presence of His (Fig. S10, ESI†). Interestingly, the microscopic image of **1**-$Cu^{2+}$ in the absence of His revealed a cubic shape structure. In contrast, the microscopic image of **1**-$Cu^{2+}$ in the presence of His showed a linear fibrillar structure of 100 nm diameter. The fluorescence microscope image of the **1**-$Cu^{2+}$ ensemble with His also showed a strong luminescence that emitted blue light (Fig. S11, ESI†).

We evaluated the sensing abilities of **1**-$Cu^{2+}$ ensemble in the solid state when the ensemble was coated onto TLC plate for use as a portable chemoprobe under neutral conditions (pH 7) to detect proteins containing His (Fig. 4). For the protein sensing experiments, the **1**-$Cu^{2+}$ ensemble coated-TLC plates were dropped into protein solutions (SPL 12), and then dried in air. Fluorescence changes were monitored by measurement of the solid fluorescence spectra. As expected, the TLC plates coated with only the **1**-$Cu^{2+}$ ensemble were non-fluorescent in the apo state, due to the ACQ effect. When the

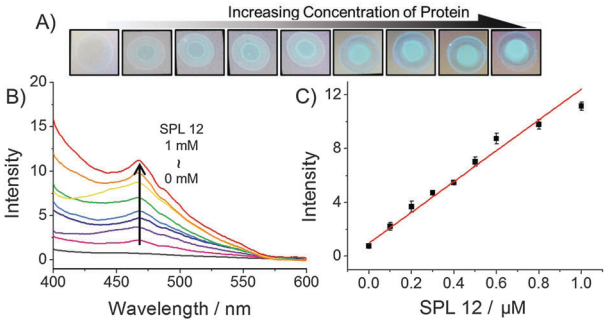


Fig. 4 (A) Photograph of the TLC plate coated with the **1**-$Cu^{2+}$ ensemble (1.0 μM) after addition of various concentrations of SPL 12 (0–1.0 μM) under a UV lamp. (B) Photoluminescence spectra of **1** (1.0 μM) with various concentrations of SPL 12 (0–1.0 μM) in a mixed water and methanol (90 : 10 v/v%). (C) Plot of fluorescence intensity at 470 nm against the concentration of SPL 12 (0–1.0 μM).

coated TLC plates were sprayed with solutions of proteins containing His, the fluorescence intensity of the immobilized **1**-$Cu^{2+}$ ensemble on TLC plates gradually increased in a linear manner with respect to the increasing concentration of the protein. This result was attributed to the displacement of $Cu^{2+}$ from the receptor **1**. The results are consistent with the view that the **1**-$Cu^{2+}$ ensemble coated-TLC plates not only detected His in a quantitative manner in a solid state system, but also acts as a portable chemoprobe.

To apply this new material to a biological sample, we also tested the ability of the **1**-$Cu^{2+}$ ensemble to detect His in urine samples.[7a] The urine samples were collected from a single subject in our University. Taking into account the possible interferences in the determination of His in urine, we undertook the quantification of His and the fluorescence emission recovery of His by spiking samples with His over the range of 10 μM–50 μM. As shown in Fig. 5, we recovered ~80% of the His as determined by fluorescence emission when we tested five concentrations of His and the response was linear with respect to concentration. The percentage of recovery illustrates the validity of the developed method. Thus, this **1**-$Cu^{2+}$ ensemble would be useful as a quantitative detection method for His in the biological samples.

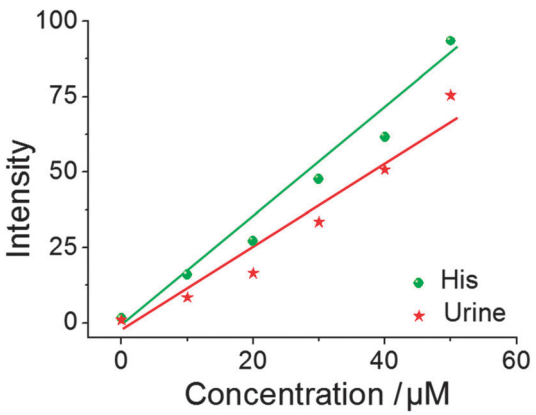


Fig. 5 Plot of fluorescence intensity upon addition of various concentrations of histidine (green) and urine (red).

In conclusion, an imidazole-appended tetrakis(*p*-phenylene)-ethylene derivative has been synthesized and its fluorogenic sensing properties have been investigated. The **1**-$Cu^{2+}$ ensemble alone was almost non-emissive, which is attributed to the aggregation-caused-quenching (ACQ) effect. In contrast, the **1**-$Cu^{2+}$ ensemble exhibited a strong emission upon addition of His and also with protein containing His moieties, due to displacement of receptor **1**. Thus, the **1**-$Cu^{2+}$ ensemble can function as a "turn-on" fluorescent chemoprobe for His and proteins containing His moieties. The **1**-$Cu^{2+}$ ensemble coated-TLC plates can also serve as a chemoprobe for the selective detection of His and proteins containing His moieties. Furthermore, this advanced method was found to be valid up to 80% for His detection over the range of 10–50 μM. Thus, this bioassay offers a promising approach for clinical diagnosis because it allows the detection of His in urine samples without requirement of any pre-treatment of the sample.

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