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Surfactant-regulated acetylpyrene assemblies as fluorescent probes for identifying heme proteins in an aqueous solution

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ABSTRACT

Heme proteins play various important roles in a variety of physiological and pathological processes. Surfactant assemblies have drawn great attention in fabricating fluorescent sensors to detect and identify proteins. In this study, an acetylpyrene fluorophore containing imidazole **HP-1** was synthesized, and it could be well modulated by an anionic surfactant sodium dodecyl sulfate (SDS). The selected ensemble based on **HP-1**/SDS assemblies exhibited selective fluorescence sensing performance towards the heme proteins, including neuroglobin (Ngb), myoglobin (Mb) and cytochrome *c* (Cyt *c*). Besides, phospholipid DMPC vesicles as membrane models were particularly explored the association process between the heme protein Mb and membrane. The present work showed that Mb induced the lysis of DMPC liposomes visualized by transmission electron microscopy and optical microscope.

The precise and specific recognition of proteins is of great significance because proteins are involved in the diagnosis of various affiliated diseases and physiological disorders [1–3]. Heme proteins are a class of the most widely distributed metallic proteins containing a heme group (an iron phorphyrin), which perform diverse biological functions [4]. Hemoglobin (Hb) and myoglobin (Mb) are two main globins with the heme group and are responsible for oxygen binding and transport [5]. Moreover, Mb is an important biomarker of early acute myocardial infarction [6]. Neuroglobin (Ngb) is a stress-inducible globin, which produces a protective effect against hypoxic/ischemic damaging insults by promoting oxygen availability within the mitochondria [7]. Another heme protein, cytochrome *c* (Cyt *c*) transfers electrons between Cyt *c* and Cyt *c* oxidase in the respiratory chain, which also participates in an early stage of the intrinsic apoptosis pathway [8,9]. Hence, the development of rapid and convenient recognition methods for different heme proteins is of critical importance in the diagnosis of clinical diseases, and in the monitoring of metabolic pathways as well.

The direct detection method of heme proteins was based on its absorption spectrum around 405 nm [10], which, however, it is easily influenced by scattered light and coexisting interferences. Other methods based on electrochemistry [11], electrophoresis [12], or chemiluminescence [13] are limited by the complexity, matrix interference or reagent instability. Recently, fluorescent sensor arrays composed of a number of cross-reactive sensor elements have been developed to obtain a specific response to the addition of heme proteins [14,15]. Meanwhile, the general drawback of these systems is the fact that the sensing process is susceptible to exterior parameters such as the concentration of any individual component. Therefore, the development of a simpler fluorescent sensor with high sensitivity and selectivity for heme proteins is highly desirable.

Surfactants are well-known amphiphilic molecules and usually self-assemble into supramolecular assemblies such as micelles and vesicles in aqueous solutions. These assemblies possess a hydrophobic core that could encapsulate hydrophobic fluorophores and tune the photophysical properties (fluorescence stability, fluorescence quantum yield, and fluorescence emission) and behaviors of the encapsulated fluorescent probe [16,17]. Therefore, the strategy of using surfactant assemblies encapsulating fluorophores has been widely used to develop fluorescent sensors in term of simplicity and modulating ability. Up to now, this method has been explored in developing label-free fluorescent sensors to detect proteins for the noncovalent interactions between proteins and surfactant assemblies [18–20]. Moreover, surfactant micelles could also be utilized as membrane mimetics for structure determination and functional analysis of proteins [21,22].

In this study, we reported a simple fluorescent ensemble sensor based on small fluorophore and surfactant assemblies for sensing

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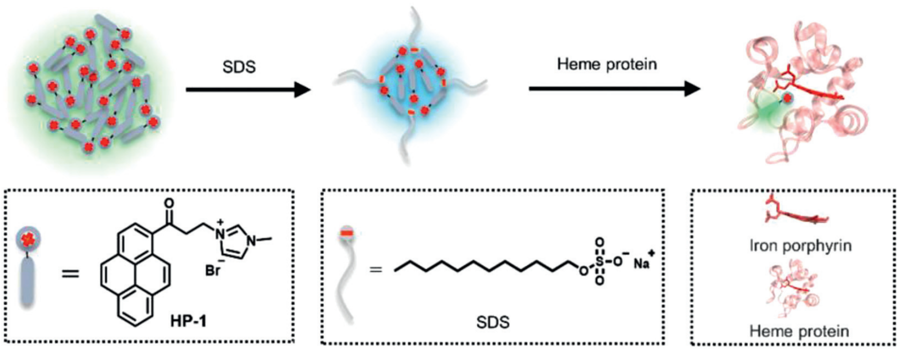

Scheme 1. Chemical structure of **HP-1** and its proposed mechanism for identification of heme proteins.

heme proteins. The structure of designed fluorophores **HP-1** was shown in Scheme 1. Imidazole has been reported that it can bind to a heme protein within a cavity formed in the proximal heme pocket from the interior of the protein to the surface [23–26]. Acetylpyrene was an important environment-sensitive pyrene derivative according to its different aggregate states as shown in our previous study [27]. Herein, **HP-1** was aggregated to form nanoparticles and displayed a long-wavelength peak at 475 nm in aqueous solutions. After the addition of sodium dodecyl sulfate (SDS), the anionic surfactant disassembled the fluorophore and induced fluorescence emission blue-shift of the encapsulated probe by interacting with imidazolium. The binding between imidazole and heme pocket provided a different microenvironment for the fluorophore, which resulted in the ensembles sensor **HP-1**/SDS, indicating the specific detection of heme proteins. Subsequently, based on the signals of heme proteins with **HP-1**/SDS micelle, 1,2-dimyristoyl-*sn*–glycero-3-phosphocholine (DMPC) could be established as a simple model for studying the heme protein with the cell membrane visualized by transmission electron microscopy (TEM) and optical microscope.

We firstly investigated the UV–vis absorption and fluorescence properties of fluorophore **HP-1** in different solvents. Compound **HP-1** demonstrated only one emission peak, which gradually shifts towards longer wavelengths as solvent polarity increases (Fig. S1 in Supporting information). In water solution, **HP-1** showed absorbance values at λ_{max}, 285 and 365, and emitted emission centered at 475 nm (Fig. 1a). Further study, the dynamic light scattering (DLS) experiments confirmed the formation of nanoparticles (Fig. 1b). The average diameter of the self-assembled nanoparticle was ~73 nm. These results show that the pyrene-pyrene stacking stabilized the self-assembling particles. Moreover, these environment-sensitive properties of the **HP-1** inspired us to apply them to discriminate different microenvironments such as surfactants.

The fluorophore **HP-1** aggregation was then used to interact with four types of surfactants, including anionic surfactants SDS, sodium dodecyl benzene sulfonate (SDBS) and sodium lauryl sulfate (SLS), cationic surfactant hexadecyl trimethyl ammonium bromide (DTAB), zwitterionic surfactant dodecyl dimethyl betaine (BS-12), and nonionic surfactant Triton X-100 (TX-100). To assess the fluorescence responses, fluorescence spectra were measured for the probe incubated with the same concentrations (400 µmol/L) of these surfactants, which were conducted in PBS buffer solution (20 mmol/L, pH 7.4). As shown in Fig. 2a, in SDS solutions, the fluorescence intensity of **HP-1** was quenched and the maximum emission was slightly blue-shifted to 450 nm, which indicates the slightly disassembled of the fluorophore.

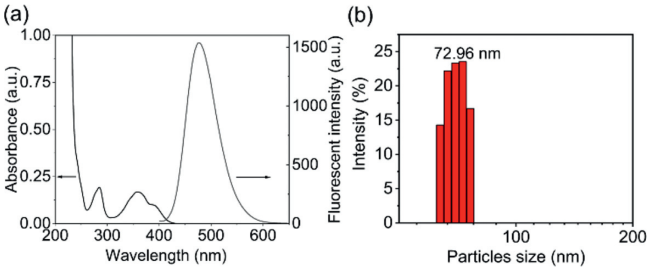

Fig. 1. (a) UV–vis absorption and steady-state fluorescence emission spectra of **HP-1** (10 µmol/L) in water (λ_{ex} = 365 nm); (b) DLS analysis of 10 µmol/L **HP-1**.

Meanwhile, the fluorescence spectra of **HP-1** with the other surfactants, including anionic surfactants SDBS and SLS, only displayed a fluorescence decrease. In addition, the fluorophore **HP-2** without a carbonyl group only exhibited a fluorescence decrease for all surfactants (Fig. S2 in Supporting information). Then, we titrated all surfactants to form micelles. As shown in Fig. 2b, for anionic surfactants SDS, concentration far below CMC values can cause a significant fluorescence decrease and blue-shifted. For other surfactants (Fig. S3 in Supporting information), with the addition of cationic surfactant DTAB and anionic surfactant SLS exceeding 6 mmol/L, the fluorescence blue-shifted to 450 nm. And, other micelle did not lead to obviously blue-shifted fluorescence. These observations suggested that the sulfonic of SDS interacted with imidazolium and disassembled the sensor to recover the fluorescence. Thus, SDS was selected to fabricate a binary sensor system and the detailed SDS modulation effect in identifying was further examined.

The ensemble sensor **HP-1**/SDS was then applied to detect proteins including heme proteins (Mb, Ngb, Cyt *c*) and non-heme proteins (pepsin, bovine trypsin, lysozyme). Firstly, titration experiments at a fixed sensor concentration of 10 µmol/L of **HP-1** and 400 µmol/L of SDS with different concentrations of six analyte proteins were systematically investigated. As shown in Fig. 3, the stepwise addition of proteins to the ensemble sensor resulted in a

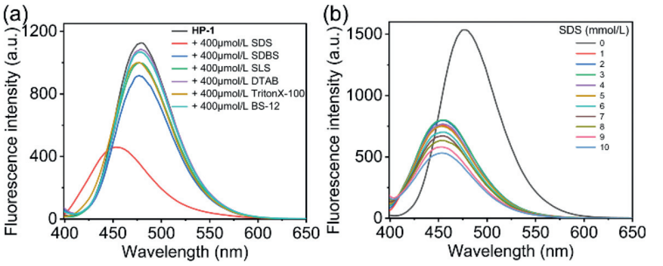

Fig. 2. (a) The fluorescence spectra of **HP-1** before and after incubating with SDS, SDBS, SLS, DTAB, TX-100, and BS-12, [**HP-1**] = 10 µmol/L; [surfactants] = 400 µmol/L; (b) Fluorescence emission spectra **HP-1** (10 µmol/L) in a series of different concentrations (0–10 mmol/L) of SDS buffer solutions (20 mmol/L PBS, pH 7.4).

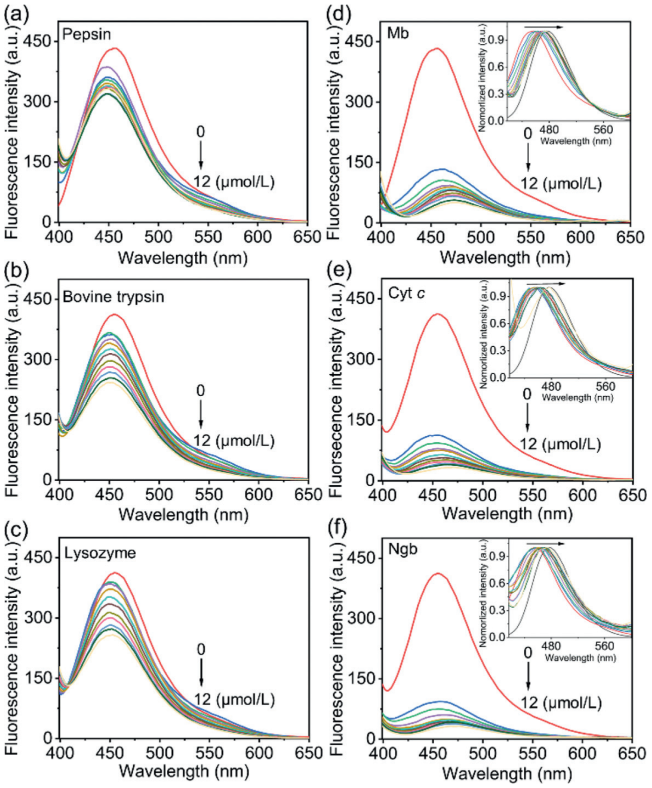

Fig. 3. Fluorescence spectra of **HP-1**/SDS (10 μmol/L/400 μmol/L) in aqueous buffer solution (20 mmol/L PBS, pH 7.4) upon titration of proteins (0–12 μmol/L): (a) pepsin, (b) bovine trypsin, (c) lysozyme, (d) Mb, (e) Cyt *c* and (f) Ngb. Inset: Amplification of the fluorescence intensity at 400–600 nm shown in panel d, e, f.

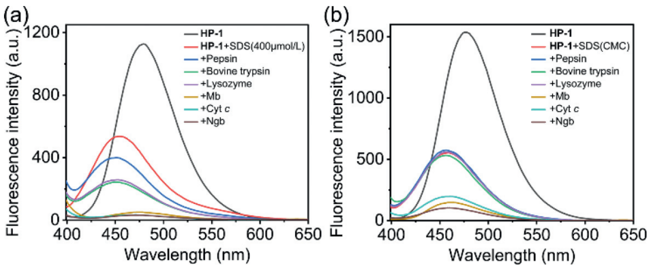

Fig. 4. (a) Fluorescence spectra of **HP-1**/SDS (10 μmol/L/400 μmol/L) treated with 6 species of proteins (12 μmol/L); (b) Fluorescence spectra of **HP-1**/SDS (10 μmol/L/CMC) treated with 6 species of proteins (12 μmol/L).

continuous decrease in the fluorescence intensity for all proteins. Meanwhile, only the wavelength for Mb, Ngb and Cyt *c* was gradually red-shifted. By comparing the concentration dependence of the absolute fluorescence change, a protein concentration of 12 μmol/L was identified as the most suitable for the specific discrimination of the analyzed proteins.

The selectivity of the sensor was also investigated. As shown in Fig. 4a, heme proteins lead to obvious fluorescence quenching of the sensor, and the wavelength red-shifted back to 475 nm, while other proteins just showed a quenching response at 450 nm. To explore the importance of surfactant for providing the environment, the probe itself for sensing proteins was observed. All of the proteins showed a quenching response at 475 nm (Fig. S4 in Supporting information). These results suggest that **HP-1** was not selective for heme proteins. All these results demonstrated the selectively sensing performances of **HP-1**/SDS for heme proteins. A possible reason was that imidazole bound to the cavity of the heme pocket leads to an environmental change for the acetylpyrene group from SDS atmosphere.

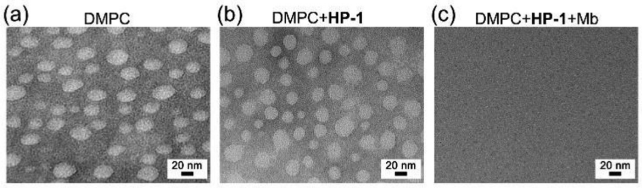

Fig. 5. TEM images of DMPC vesicles in aqueous solution (a), treated with **HP-1** (10 μmol/L) (b) and then incubated with 5 μmol/L Mb (c). Scale bar: 20 nm.

To further understand the SDS modulation effect on the heme protein detection of **HP-1**, the protein experiments were measured at the critical micellar concentration (CMC, 8 mmol/L) of SDS solutions. The results are illustrated in Fig. 4b. The fluorescence emission profiles of the ensemble sensor were slightly decreased for pepsin, bovine trypsin and lysozyme. The fluorescence emission was sharply quenched at 450 nm by increasing the concentration of these heme proteins. Thus, **HP-1**/SDS micelles were also used to further examine the sensitivity and selectivity of heme proteins. Moreover, surfactant micelles were often utilized as membrane mimetics for structural determination and functional analysis of proteins.

Heme protein-membrane interactions can influence the effectiveness of some membrane-related physiological processes. For example, Cyt *c* plays a key role in apoptosis by acting as a signaling molecule that is released from the mitochondrial membrane [28]. Interactions of Mb with membranes promote conformational changes in the protein and lead to a decrease in the affinity of Mb for O_2 [29]. DMPC (1,2-dimyristoyl-*sn*–glycero-3-phosphocholine) is one of many lipids within the phosphatidylcholine lipid family that is highly abundant in biological membranes and has drawn much attention as model membranes [30]. Thus, we examined the fluorescence response of **HP-1** to the lipid membrane of DMPC. **HP-1** exhibited a different fluorescence in SDS micellar, and the emission spectrum of the probe in DMPC showed a significant decrease at 475 nm (Fig. S5 in Supporting information). The selectivity of the **HP-1**/DMPC was also investigated and shown in Fig. S5. Heme proteins lead to fluorescence quenching of the sensor, while no significant quenching response was observed for other proteins.

Furthermore, to explore the interaction between heme protein and DMPC liposomes, TEM images and optical microscope were applied to obtain information on the structure of the DMPC aggregates. As seen from TEM in Fig. 5, the liposomes after incubation with **HP-1** led to dispersive signals of the DMPC. With further addition of Mb in the mixed solvent, the protein can induce the lysis of liposomes at a low concentration. Similarly, confocal microscopy experiments suggested that the protein can induce the lysis of DMPC liposomes (Fig. S6 in Supporting information).

Finally, **HP-1** was tested as a bioimaging probe in live cells. HeLa cells were incubated with 10 μmol/L **HP-1** for 60 min. As shown in Fig. 6, the blue fluorescent dye **HP-1** was found most concentrated at the membranes of the cells, thus pointing firmly to its affinity for the plasma membrane.

In summary, we developed a simple assembled sensor system for identifying heme proteins using the strategy of surfactant assemblies encapsulating an acetylpyrene fluorophores. Fluorescence measurements found that the fluorescence emission of the fluorophore **HP-1** aggregates could be selectively disassembled by SDS assemblies. **HP-1**/SDS system sensor showed sensitive fluorescence quenching and red-shift response to Ngb, Mb, and Cyt *c*, attributed to the microenvironment change of **HP-1** between surfactants and proteins. As visualized by TEM and optical microscope, the studies of Mb with the DMPC liposomes aggregates revealed that heme

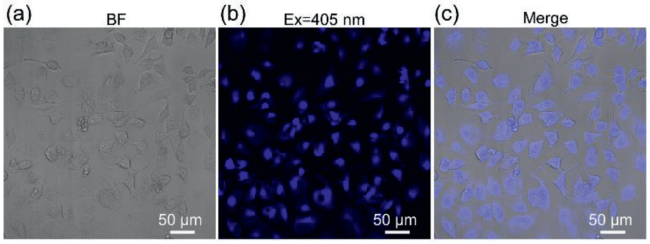

Fig. 6. Confocal fluorescence images of HeLa cell incubated with **HP-1** (10 µmol/L). (a) Bright fields of HeLa cell; (b) Fluorescence imaging of HeLa cell from blue channel, collected at 410–585 nm; (c) Merged. Scale bar: 50 µm.

proteins could induce the lysis of DMPC liposomes. Compared with those reported methods, the current sensor is simple and straightforward, which avoids the complex sample pretreatment or the use of unstable reagents.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.cclet.2022.107802.

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