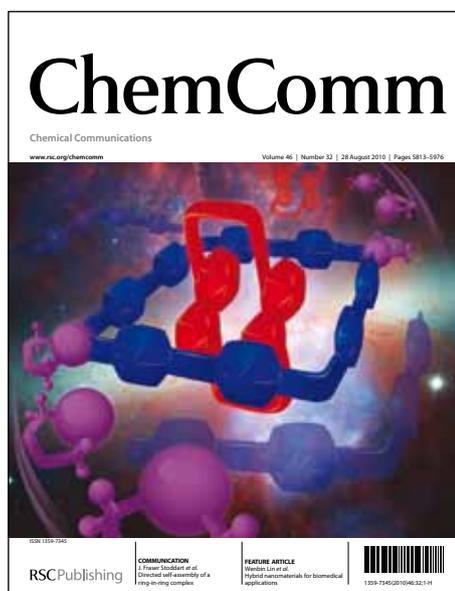


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COMMUNICATION

pH-Sensitive Unimolecular Fluorescent Polymeric Micelles: From Volume Phase Transition to Optical Response

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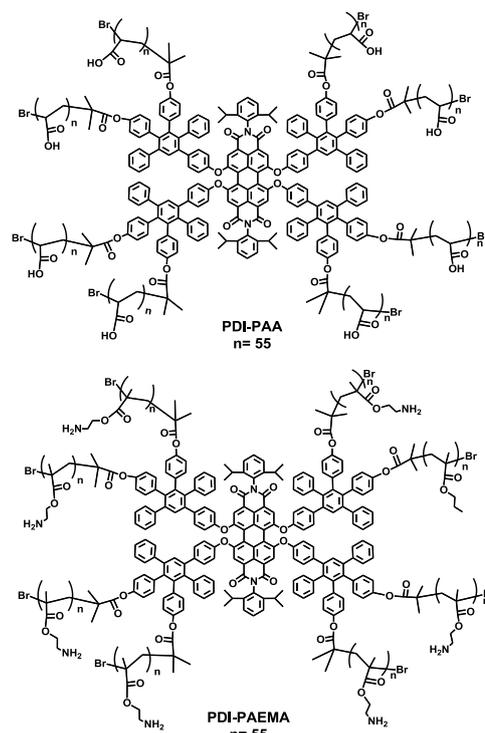
Unimolecular fluorescent micelles of star polyelectrolytes with a perylenediimide core are very sensitive to changes in pH values. The pH-responsive behavior relies on the ionization or deionization of the star polyelectrolytes which causes a reversible volume phase transition and optical response.

The pH value is an important and universal indicator in the fields of biology, chemistry and agriculture.¹ In order to readily detect the local pH, many pH probes based on small dye molecules have been designed and studied extensively.² In contrast, only a few reports have described polymeric fluorescent pH sensors. Gao's group introduced a series of pH-activatable micellar (pHAM) nanoparticles based on the supramolecular self-assembly of ionizable block copolymer micelles.³ The reported micelles exhibited an ultrasensitive pH-response. The amplification of optical signals over small-molecule analogues makes polymeric micelles good candidates for pH-sensor applications. In the last decades, unimolecular polymeric micelles (UPMs) have attracted much attention. Unlike self-assembled polymeric micelles (PMs), UPMs are stable in dilute solutions because of their inherent structures; thus, they have been applied in drug delivery systems.⁴

Perylenediimide (PDI) derivatives have been intensively studied⁵ because of their excellent chemical, thermal, and photochemical stability. Previously, water-soluble core-shell macromolecules based on the PDI chromophore as a core have been synthesized and used as bioimaging agents and gene carriers.⁶ Owing to the prevailing fluorescence, the relevant processes could be optically monitored. Until now, pH sensors based on unimolecular fluorescent polymeric micelles (UFPM) have not been reported. Therefore, it would be rewarding to investigate the pH-responsive behavior of PDI-cored UFPMs based on their optical and morphological properties.

Herein, we synthesized two types of PDI-cored UFPMs (Scheme 1), i.e. PDI-*star*-poly(acrylic acid) (PDI-PAA) and PDI-*star*-poly(aminoethyl methacrylate) (PDI-PAEMA). The core was composed of a rigid polyphenylene dendrimer and capable of preventing the central PDI chromophore from aggregation in water. The outer shell was formed from flexible cationic or anionic polyelectrolytes that contribute to the water solubility and pH sensitivity. The detailed synthesis is given in the ESI†. The final products PDI-PAA and PDI-PAEMA have high water solubility (>10 g/L) and high fluorescence quantum yields in water ($\Phi_f = 0.11$ for PDI-PAA and $\Phi_f = 0.13$ for PDI-PAEMA).^{5a}

^{5b} Owing to the polyelectrolytic nature of the outer polymer shells, these PDI-cored UFPMs are sensitive to pH changes. Thereby, both the volume and the fluorescence response of the core-shell system undergo remarkable changes.



Scheme 1 Chemical structures of PDI-PAA and PDI-PAEMA.

In order to obtain the shape and size of the PDI-cored UFPMs in aqueous solution (pH=7), small angle X-ray scattering (SAXS) was applied (Fig. S2, ESI†). SAXS is a powerful means for the structural investigation of micelles.⁷ The shape and size of the UFPMs in aqueous solution are calculated by using the generalized indirect Fourier transformation (GIFT) method.⁸ As shown in Fig. 1, both PDI-PAA and PDI-PAEMA are globular micelles with an average diameter of about 20 nm.

The pH dependent sizes of PDI-PAA and PDI-PAEMA in aqueous solution were measured by dynamic light scattering (DLS). The concentration of each UFPM was 1 mg/mL. The DLS results are given in Table 1. The size of PDI-PAA increases with increasing pH value. On the other hand, the size of PDI-PAEMA decreases with the increase of pH value. In neutral solution (pH=

7), the size of both PDI-PAA and PDI-PAEMA appears to be consistent with the results obtained by SAXS.

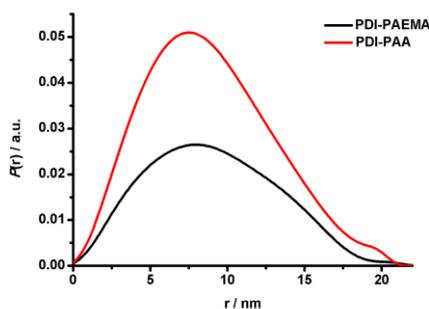


Fig.1 Pair distance distribution functions (PDDFs) of PDI-PAEMA and PDI-PAEMA in aqueous solution (pH= 7) at room temperature in real space by using GIFT algorithm (r : radius of the particles).

Table 1 DLS results of PDI-PAA and PDI-PAEMA at different pH values adjusted by NaOH (0.1 mol/L) and HCl (0.1 mol/L).

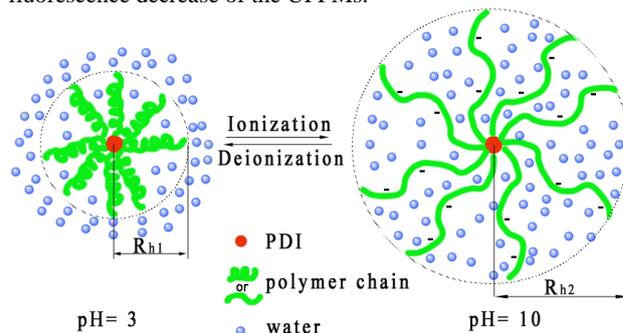
D_h^a (nm)	pH= 3	pH= 5	pH= 7	pH= 8.5	pH= 10
PDI-PAA	16.8±1.1	17.3±0.8	19.5±0.9	20.0±1.2	24.5±1.6
PDI-PAEMA	26.2±0.9	25.6±1.2	20.5±0.2	18.5±1.3	17.5±1.5

^a D_h : hydrodynamic diameter

10 The pH-responsive behavior is attributed to the phase transition of the UFPMs. Both PDI-PAA and PDI-PAEMA are weak polyelectrolytes because of the existence of carboxyl or amino groups in the polymer side chains. Thus the surface charges of these two UFPMs can be easily tuned by adjusting the pH value. In general, the polymer chains will experience phase transition around their pKa values. The pKa values of PDI-PAA and PDI-PAEMA were determined by potentiometric titration as 6.34 and 8.05, respectively (**Table S1**). For example, **Scheme 2** illustrates the size changes of PDI-PAA in buffer solutions. At pH 3 (\ll pKa), the peripheral PAA chains are totally uncharged and collapsed to encapsulate the hydrophobic core. At pH 10 (\gg pKa), the PAA chains are fully ionized and fully extended because of electrostatic repulsion. In neutral solution, the PDI-PAA is partly ionized and the polymer chains are partially extended along the periphery. The size of the PDI-PAA increases with increasing pH value because of the ionization of the polymer shells. For the same reason, the PAEMA chains are fully ionized at pH 3 and totally uncharged at pH 10. Thus, the size of PDI-PAEMA in acidic solution is larger than that in basic solution. In the above process, the pH variation causes the changes in UFPM size because of the ionization of the outer polymer electrolytes. The electrostatic repulsion and the resulting polymer-chain stretching lead to the increase in the volumes of the charged UFPMs. Generally, we call the process as volume phase transition.⁹

During the process, as the polymer shells extend to the stretch mode, the environment around the central PDI chromophore becomes more hydrophilic, and the surrounding water can readily access the center of the UFPMs. Henry reported that the fluorescence of hydrophobic core can be quenched by polar solvents such as water.¹⁰ To evaluate the effect of water on the fluorescence property, PDI-PrBA, the precursor of PDI-PAA, was chosen as the model polymer for fluorescence measurements.

PDI-PrBA was dissolved in 2 mL of THF at a concentration of 0.01 mg/mL. Then 20 μ L of deionized water was added gradually into the solution. As shown in **Fig. S6(A)**, the fluorescence intensity of PDI-PrBA decreases by 30% at a water content of 10%. For comparison, blank experiments were performed by the addition of the same volume (20 μ L) of THF instead of deionized water, and the fluorescence intensity of PDI-PrBA was almost unchanged. Thus the effect of dilution on the fluorescence intensity is negligible, and water is the key factor in the fluorescence decrease of the UFPMs.



Scheme 2 Schematic representation of volume phase transition of unimolecular micelle (PDI-PAA).

Because the PDI chromophore is located in the interior of the UFPMs, its optical properties can be strongly influenced by the volume phase transition. In other words, the volume phase transition can be converted into optical transition. By using UV-vis and fluorescence measurements, the pH-dependent optical properties of the UFPMs were investigated in Tris-HCl buffer. The UV-vis spectra of PDI-PAA and PDI-PAEMA at different concentrations are shown in **Fig. S4**. The absence of red shifts in the UV-vis spectra indicates that PDI-PAA and PDI-PAEMA are UFPMs at the measured concentration (≤ 1 mg/mL). At a concentration of 1 mg/mL, the absorption intensities of the two UFPMs do not depend on the pH (**Fig. S5, ESI†**). In contrast, the fluorescence intensities of the UFPMs are sensitive to pH changes (**Fig. 2**). At pH values lower than pKa (pH < 6.34), PDI-PAA has a relatively high fluorescence intensity. As the pH approaches pKa, the fluorescence intensity decreases sharply and subsequently enters the second plateau region (**Fig. 2(C)**). The change of fluorescence intensity of PDI-PAEMA with pH shows a trend opposite to that of PDI-PAA, consistent with DLS results. Therefore, the pH-sensitive volume phase transitions indeed lead to fluorescence changes of the UFPMs, especially the dramatic changes around the pKa values.

Because the volume phase transition is a physical process, and no chemical reaction occurs, the fluorescence change induced by the volume phase transition is reversible. **Fig. 3** shows the cycling of fluorescence maxima between the pH values of 3 and 10. The fluorescence transition of the UFPMs is very rapid (within 1 minute). At pH = 10 (\gg pKa), the carboxylic groups of PDI-PAA are fully ionized (**Fig. 3(A)**). Because of electrostatic repulsion, the outer polymer shells in the PDI-PAA UFPMs become extended and more water can access the fluorescent core. The UFPM size increases and the fluorescence intensity decreases correspondingly. When the pH returns to 3 (\ll pKa), the carboxylic groups are completely deionized, and the polymer chains shrink to exclude water. The UFPM size decreases and the fluorescence intensity increases. The cycling can be repeated

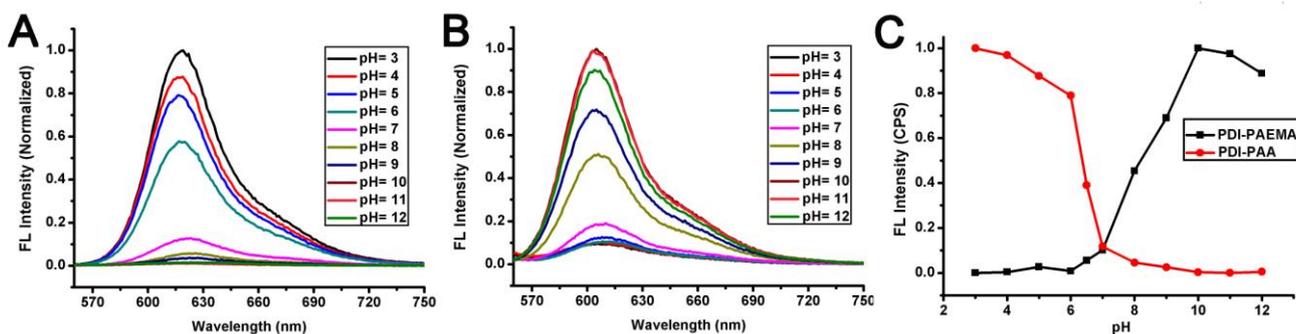


Fig. 2 Normalized fluorescence spectra of (A) PDI-PAA (0.01 mg/mL) and (B) PDI-PAEMA (0.01 mg/mL) in pH buffer (pH ranging from 3 to 12); (C) normalized peak fluorescence intensities (at ~620 nm for PDI-PAA and ~607 nm for PDI-PAEMA) in Tris-HCl buffer.

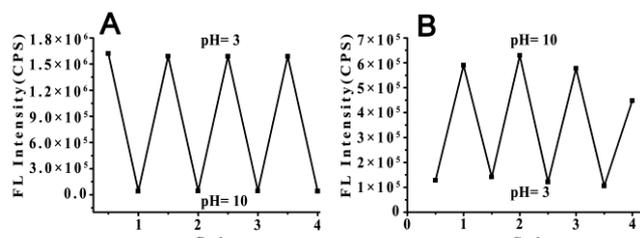


Fig. 3 Cycling of fluorescence maxima of (A) PDI-PAA (617 nm, 0.01 mg/mL) and (B) PDI-PAEMA (605 nm, 0.01 mg/mL) between pH=3 and pH=10.

several times (**Fig. 3(A)**). Similarly, the behavior of PDI-PAEMA is fully reversible during pH cycling (**Fig. 3(B)**). Because of these reversible responses to pH, both UFPMs can be used as recyclable pH sensors.

In conclusion, two types of fluorescent PDI-cored star polyelectrolytes were prepared and characterized. According to SAXS and DLS results, the polyelectrolytes formed pH-responsive globular UFPMs in aqueous solution. Because of the ionization or deionization of the polymer chains induced by pH variation, the UFPMs displayed volume phase transition around their pKa values. Both the size and the fluorescence intensity of the two UFPMs showed pH-sensitive behavior between the fully charged and totally uncharged states. Because of electrostatic repulsion (in the fully charged state), the UFPM increased in size and the fluorescence intensity decreased. When PDI was encapsulated by the shrinking polymer chains (in the totally uncharged state), the UFPM size decreased and the fluorescence intensity increased. In this way, volume phase transition was converted into optical signals. We have presented the first examples of UFPMs used as candidates for pH sensors owing to the volume phase transition and the fluorescence response. The proposed new method for designing pH sensors provides an avenue to precise, rapid, and recycled environmental sensors.

Notes and references

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[†] Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/b000000x/

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