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Autofluorescent micelles self-assembled from an AIE-active luminogen containing an intrinsic unconventional fluorophore†

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Autofluorescent micelles were constructed *via* the self-assembly of amphiphilic molecules containing an intrinsic fluorophore. The amphiphilic molecule was an AIE-active luminogen without a conventional π–π conjugated structure. In this unconventional luminogenic system, the hydrogen-bonded amide groups were assigned as the emitting sources.

Interestingly, fluorescent micelles with core–shell structure self-assembled from amphiphilic block copolymers are of great fascination in the biomedical fields.[1–3] The hydrophobic core of the micelle facilitates the encapsulation of water-insoluble materials, such as anticancer drugs and contrast agents for imaging,[4] and the hydrophilic shell of the micelle ensures good dispersion in biological environments. The incorporation of fluorescent characteristics into polymeric micelles can been carried out either by physical encapsulation or chemical attachment of conjugated fluorophores.[5,6] However, most reported approaches include many lengthy operation procedures. In addition, the leakage of the conjugated fluorochromes is also a key consideration for potential biological applications. Up to now, it is still a great challenge to design and develop an appropriate method to assemble core–shell micelles with fluorescent characteristics. An ideal fluorescent nanomicelle should be autofluorescent without the need for conjugating an external fluorochrome. The term ''autofluorescence'' is used to distinguish the fluorescence emitted by intrinsic fluorophores in a system from that generated by the use of extrinsic fluorescent dyes. Unfortunately, autofluorescent micelles have not been widely studied.

In the past decade, AIE (aggregation-induced emission) luminogens, which emit more efficiently while in the aggregated state than in solutions, have attracted considerable attention, due to their totally different emissive behaviour against the notorious aggregation-caused quenching (ACQ) effect and their huge potential applications as solid-state emitters, chemical sensors, biological probes, and smart materials.[7,8] As a result of the enthusiastic research efforts, a variety of new AIE systems have been developed, a wealth of mechanistic information has been collected, and a number of practical applications have been explored. Several review papers have summarized the early progress of AIE materials.[9–11] However, these studies were focused mostly on small molecules and polymers with π–π conjugated structures, which brought a lot of complications for biological applications, such as poor aqueous solubility, high cytotoxicity, acute inflammation and serious immunogenicity. Therefore, the development of new AIE systems to overcome these shortcomings of conjugated compounds becomes imperative.

Fortunately, we accidentally discovered an amphiphilic molecule without π–π conjugated structures and its micellar structure in solution exhibited strong blue emission under 365 nm UV light irradiation. This discovery sparked tremendous interest in its emission mechanism. The amphiphilic molecule is a polyether amide (PEA) containing an intrinsic unconventional fluorophore, and it is AIE active. The PEA molecules in solution are non-emissive or feebly luminescent but exhibit remarkably enhanced blue emissions in micellar solution. Thus, autofluorescent micelles with enhanced blue emissions were formed by the self-assembly of the PEA amphiphilic molecules in water (Scheme 1). This exceptional blue emission was attributed to the formation of multiple hydrogen bonds among the amide groups and restriction of intramolecular motions proposed by Tang and co-workers.[9] The PEA molecule comprises a hydrophilic poly(ethylene oxide) (PEO) backbone and two hydrophobic chains on both ends prepared from commercially available polyetheramine (Jeffamine ED2003) and palmitic acid *via* a one-pot synthesis. PEO is chosen as the hydrophilic component because of its biocompatibility and non-toxicity when employed in biomedical applications such as fluorescence-based bioimaging and tissue engineering. The NMR spectra, IR spectra and mass spectra have shown the successful synthesis of the amphiphilic PEA (see Fig. S1, ESI†).

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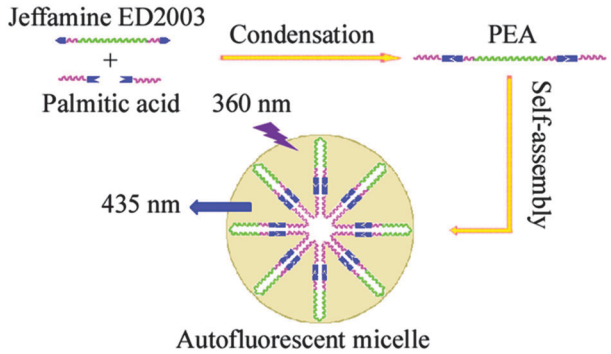
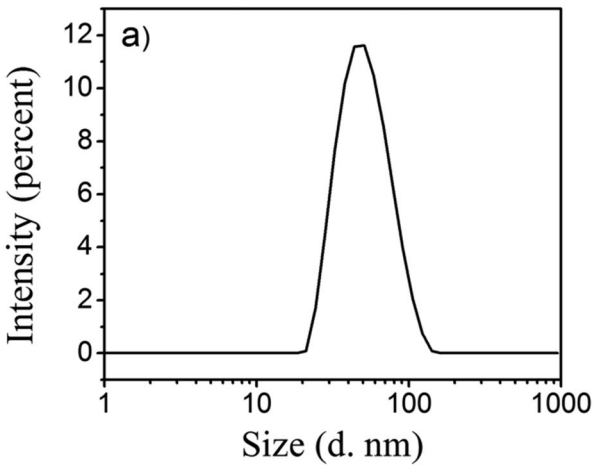
† Electronic supplementary information (ESI) available: Detailed information about materials and measurements; synthesis of PEA; NMR spectra and mass spectra of PEA; and IR spectra of PEA are provided. See DOI: 10.1039/c5sm03048j



Scheme 1 Schematic illustration for the preparation of autofluorescent micelles.



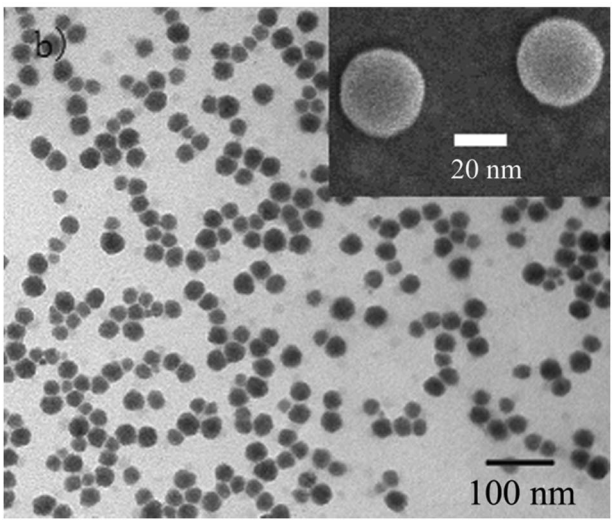


Fig. 1 (a) Size distribution of the PEA micelles in aqueous solution; (b) TEM image of the PEA micelles, the inset is their SEM image.

The critical micelle concentration (CMC) is an important factor not only for determination of micelle formation but also for evaluating their potential in biomedical applications. The CMC of amphiphilic PEA was determined from the plot of the surface tension *versus* the concentration of PEA in water. The result showed that the CMC was 0.47 mg mL^{-1} (see Fig. S2, ESI†). The PEA autofluorescent micelles were successfully constructed by the self-assembly process when the concentration of PEA in aqueous solution is above the CMC. The nanostructures of the PEA micelles were explored by dynamic light scattering (DLS), TEM and SEM techniques as shown in Fig. 1. DLS measurements at room temperature showed that the amphiphilic PEA in aqueous solution (2 mg mL^{-1}) formed monodisperse micelles with a hydrodynamic size of 54 nm and polydispersities of 0.21 (Fig. 1a). TEM images of PEA micelles also suggested the formation of uniform micelles with an average size of 25 nm (Fig. 1b). The size estimated using TEM is smaller than that using DLS due to the dehydration of micelles when preparing the TEM sample on a copper grid. In addition, the SEM image (see inset of Fig. 1b) also showed that a single nanomicelle with a diameter of about 30 nm was of regular spherical morphology, consistent with the result of TEM. Fig. 2 shows the absorption and fluorescence (FL) spectra of the PEA micellar solution. The UV-vis spectrum shows a strong absorption at a wavelength of 229 nm (Fig. 2a), which is a typical absorption peak for an amide group. Fig. 2b shows the fluorescence excitation (Ex) and emission (Em) spectra of the PEA micellar solution. The maximum emission wavelength was located at around 435 nm, while the fluorescence excitation wavelength was located at 360 nm. The PEA micellar solution showed intense blue emission under 365 nm UV light illumination (see inset of Fig. 2b).

To further investigate the aggregation-induced properties of PEA, PEA aqueous solutions with various concentrations were optically characterized (Fig. 3). Note that at low concentration of PEA in aqueous solution where micelles were not formed, no emissions or very weak emissions with the sharp Raman scattering peaks at 412 nm were observed (see inset of Fig. 3a). After the formation of micelles with increasing PEA concentration, fluorophore moieties aggregated in the hydrophobic core and showed intense fluorescence. The lifetime and quantum yield of the PEA micellar solution of 0.5 mg mL^{-1} were 4.2 ns and 10.7%, respectively (see Fig. S3 and S4, ESI†). Above the CMC, quantum yields gradually decreased with increasing concentration due to reabsorption. This property is known as the ''aggregation-induced emission'' (AIE) effect. The inset of Fig. 3b shows the emission color of PEA in solution (0.2 mg mL^{-1}) and the aggregated PEA micellar solution (2 mg mL^{-1}) under 365 nm UV light illumination. No visual detection of emission color for PEA in solution indicates that the emission intensity was too weak to be observed with the naked eye. The intense blue emission from the aggregated PEA micellar solution suggests that AIE occurred in our present case.

The CMC of PEA in aqueous solution was also estimated from the plot of the FL intensity *versus* the solution concentration.[12–15] Fig. 3b shows the plot of the FL intensity as a function of the logarithm of PEA solution concentration. At low concentrations of PEA (below 0.2 mg mL^{-1}), negligible changes in the FL intensity were observed. It was quite clear that the FL intensity started to increase at 0.5 mg mL^{-1}. However, the fluorescence of PEA was slightly distorted by reabsorption at concentrations at and above the CMC, with no strictly linear region, so the CMC couldn't be estimated as the intersection of

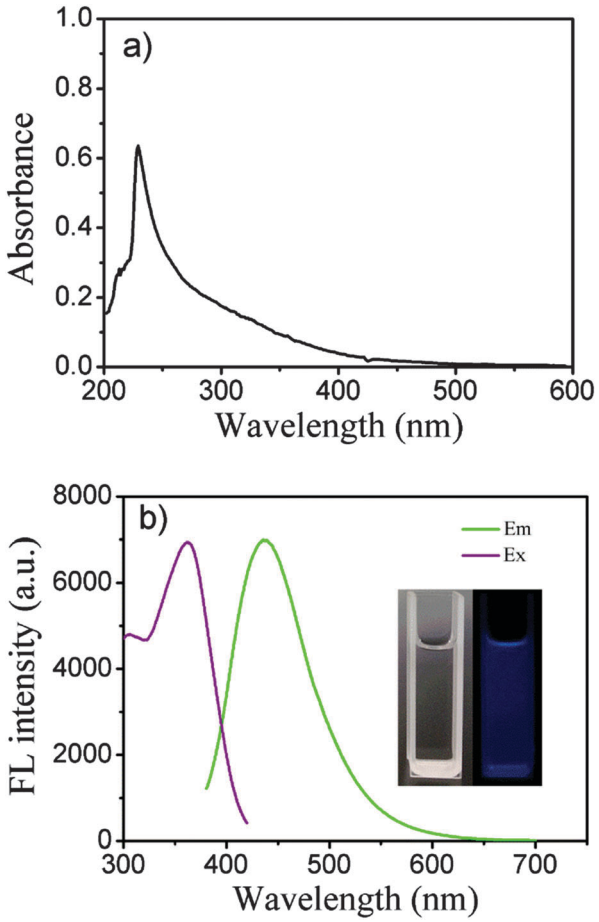


Fig. 2 (a) UV-vis absorption spectrum and (b) fluorescence excitation (Ex) and emission (Em) spectra of the PEA micellar solution at room temperature. Inset is the photographs of the PEA micellar solution under ambient light (left) and 365 nm UV light (right) illumination.

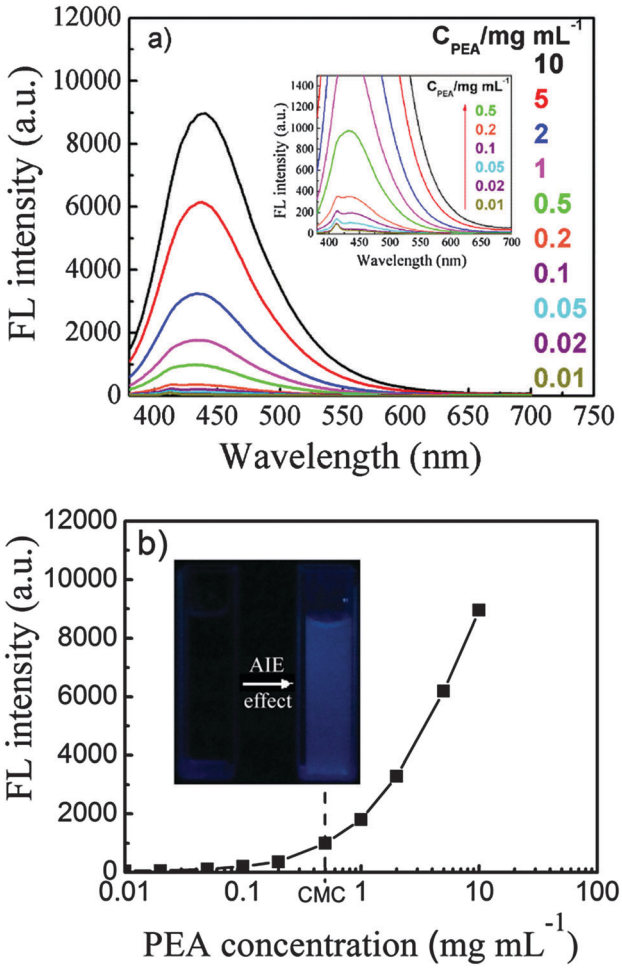
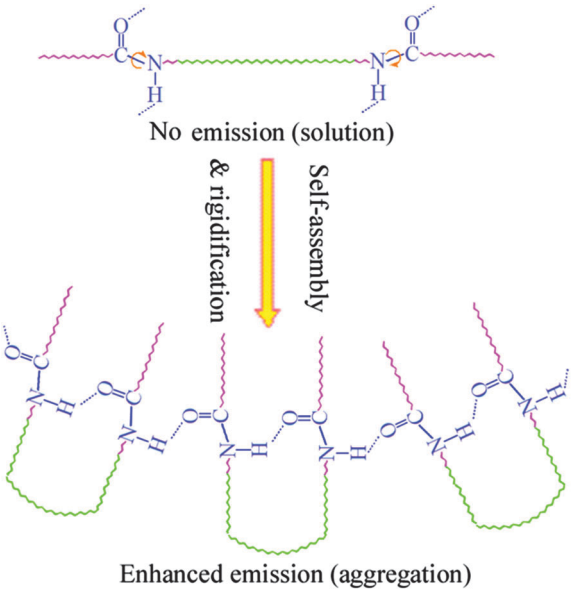


Fig. 3 (a) Fluorescence emission spectra of PEA aqueous solutions with various concentrations at room temperature, the inset is the same with a reduced *Y*-axis scale; (b) FL intensity of PEA in water as a function of the logarithm of PEA solution concentration for CMC determination, inset is photographs of PEA in solution (dark) and in the aggregated state (blue) under 365 nm UV light illumination.

two linear regions. The global fit of the FL intensity–concentration was excellent. The value of CMC = 0.5 mg mL^{-1} coincides with that determined above from surface tensiometry measurements.

Tang *et al.* first discovered the AIE phenomenon in 2001.[7] Most of the reported AIE-active molecules were propeller-like shapes with π–π conjugated structures. The restriction of intramolecular rotation processes was proposed as the main mechanism for AIE. Recently, some non-conjugated nitrogen/oxygen-rich polymers with multiple lone pair electrons were also found to be AIE-active, and the highly dense clusters of heteroatoms with lone pair electrons in these systems may serve as the chromophore.[16–18] In order to investigate whether or not the clusters of oxygen atoms with lone pair electrons from PEO segments in our system serve as the chromophore, a commercially available PEO–PPO–PEO triblock copolymer of Pluronic F127 was selected. It was found that the aggregations of the triblock copolymer had no luminescent property. Thus, in our new luminogenic system, the amide groups with hydrogen bonding (H-bonding) linkage were assigned as the emitting sources. The AIE mechanism was attributed to the structural rigidification resulting from the restriction of intramolecular rotation by multiple H-bonding interactions among the amide groups. Due to its amphiphilic property, when PEA was dissolved in water at the concentration above the CMC, it tended to self-assemble into micelles with surfaces covered with hydrophilic PEO segments and adopted a loop shape, while the hydrophobic PPO and alkyl chains with the amide groups aggregated into hydrophobic cores by cooperative effects of intra- and intermolecular hydrogen bonds among the amide groups and hydrophobic interactions between hydrophobic chains. The cooperative effects and the artful structure of PEA in water are somewhat reminiscent of the tertiary structure of globular proteins. In bulk solution, the amphiphilic polyether amide dissolves in water as flexible individual molecules at low concentrations. The PEA molecules in solution are feebly luminescent due to the free twisting of the C–N single bonds after photoexcitation, and as a consequence the PEA molecules waste a major part of their excitation energy through non-radiative pathways. While the PEA molecular aggregation will form spherical micelles of core–shell structure with increasing concentration.



Scheme 2 Schematic illustration of the H-bonding aided RIR mechanism for this kind of non-conventional luminescence process.

An increase of the rigidity of the system by multiple H-bonding interactions among the amide groups in the micellar solution may therefore be an explanation for the fluorescence enhancement. The structural stiffening in the PEA micelles impedes rotational motions of C–N single bonds, leading to the blockage of the non-radiative pathway. As a result, the AIE effect with a strong blue emission is shown in the PEA micellar solutions. The presence of hydrogen bonding among the amide groups in the PEA micellar solution was confirmed by FT-IR experiments (see Fig. S1, ESI†). It can be seen clearly that the infrared bands at 3504 cm^{-1} and 3282 cm^{-1} can be assigned to the "free" (higher than 3400 cm^{-1}) and hydrogen-bonded N–H stretching peaks, respectively.[19,20] This, together with the rather low-frequency peak of the amide I band (1658 cm^{-1}, C═O stretching), clearly demonstrates that most N–H groups are associated with C═O groups through hydrogen bonding in the PEA micellar solution. Thus, the self-assembled nanostructures of PEA were stiffened by intra- and intermolecular multiple hydrogen bonds in the aggregate state, and the H-bonding aided restriction of intramolecular rotation (RIR). These characteristics were proposed to explain the unconventional AIE phenomenon of PEA (Scheme 2).

In summary, a novel autofluorescent nanomicelle has been developed *via* an amphiphilic molecular self-assembly strategy. The PEA amphiphilic molecules are AIE active and have no highly hydrophobic π–π conjugated structure. The AIE mechanism of the unconventional luminogenic system is attributed to the structural rigidification resulting from the restriction of intramolecular rotation by multiple H-bonding interactions among the amide groups. The understanding of the emission mechanisms of unconventional luminogenic systems without π–π conjugated structures will surely lead to structural design strategies for the development of novel luminescent materials. The PEA autofluorescent micelles are non-toxic, reliable, biocompatible, and are promising for various biomedical applications, such as biological imaging and drug transport. Compared with the previous fluorescent polymeric micelles obtained by physical encapsulation or chemical attachment of π–π conjugated fluorophores, these novel fluorescent micelles are richly endowed by nature with autofluorescence effects and artful structures. These findings not only extend the AIE systems from π–π conjugated compounds to non-conjugated materials, but also open up a new avenue for the design and development of new autofluorescent micelles. Further studies on the development of new AIE materials without π–π conjugated structures and autofluorescent micellar-based biomedical applications, are currently underway.

Acknowledgements

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