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pH Responded Reversible Supramolecular Self-assembly of Water-soluble Amino-imidazole-armed Perylene Diimide Dye for Biological Application

Wei Zhang,[a,b] Shi-Yu Gan,[a] Feng-Hua Li,[a] Dong-Xue Han,[a] Qi-Xian Zhang,[a] Li Niu*[a]

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It is extremely important to design stimuli-responsive biomimetic supramolecular materials. Such type of materials requires the molecular monomers with multi-functionalities. Perylene diimide(PDI) has been considered as one of the most versatile building blocks units for supramolecular architecture. However, the PDI derivatives mostly reported work in organic media while a challenge for aqueous system due to their pronounced hydrophobicity of the perylene backbones. Here we report a water-soluble amino-imidazole-armed perylene diimide (AIA-PDI) dye that discloses reversible supramolecular structure and fluorescence emission conversion upon external pH-stimulation. Such characteristics offer a gap of PDI derivatives in fabrication of pH-responsive biomimetic system. Successful glucose detection application as a proof of concept further demonstrates this PDI derivative's biological suitability in pH-responsive system.

1. Introduction

Inspired by the stimulation response in biological tissue,[1] the design of a class of artificial smart materials that are able to perceive environmental variations is of importance for biomedical nanotechnology.[2, 3] Supramolecules among the various smart materials has been demonstrated as the most potential one because they can be designed on the molecular level depending on the requirements.[4] The supramolecule materials consisting of multi-functional molecule monomer have the potential to modulate their structures and functionalities[5, 6] upon external environmental changes, such as pH,[7] temperature[8] and mechanical forces.[9]

Supramolecule formation commonly favours by the self-assembly of its monomers based on the weak interactions, such as π-π interaction,[10] and hydrogen-bonding.[11] Among various monomers, perylene diimide (PDI) has been considered as one of the most versatile building blocks units for supramolecular architecture. PDI consists of five-connected benzene rings which are the intrinsic driving force for the self-assembly.[12] The rich grafting sites on PDI such as the bay and imide region[13] offer advantages for designing PDI derivatives monomer to enrich supramolecular materials with specific stimulation functionality such as mechanic-chromic[14] and thermo-chromic material.[15] However, the PDI derivatives mostly reported work in organic media while a challenge for aqueous system due to their pronounced hydrophobicity of the perylene backbones.[16] Many efforts were made to improve the hydrophilicity by grafting hydrophilic groups to expand the application field, e.g., Würthner's group proposed amphiphilic PDI derivatives and used them to form the vesicular nanocapsules as loader in aqueous system.[17]

Recently, a few water-soluble PDI derivatives molecules have been designed.[18-22] For example, Müllen's group proposed ionic PDI dyes with high fluorescence quantum yields and significant photostability and they were used as fluorescent labels in live cell.[23] Additionally, the water-soluble PDI derivatives with stimuli-responded functionality were also reported.[24, 25] For example, Malik's group designed a melamine-responded PDI molecule, which can form luminescent gel material in water.[26] It is extremely important to design PDI molecules for forming pH-responded supramolecular materials since pH is a critical biological stimulus.[27] Whereas there are very few pH-responded PDI molecules been synthesized.[28]

In this work, we design a new type of PDI derivatives, i.e., N, N-bis-(1-aminopropyl-3-propylimidazol)-3,4,9,10-perylene tetracarboxylic acid diimide (Fig. 1A). This molecule features two arm-like ionized amino-imidazole groups, which are symmetrically grafted on the imide-positions of PDI core (this amino-imidazole-armed PDI is denoted as AIA-PDI in the following description). Positively charged N-centres in each arm significantly improve its solubility in water. Meanwhile the π-conjugated PDI core reserves the ability of supramolecular self-assembly. Owing to introduced amino groups, the AIA-PDI molecule discloses reversible conversion in the fluorescence emission and supramolecular structure upon external pH-stimulation.

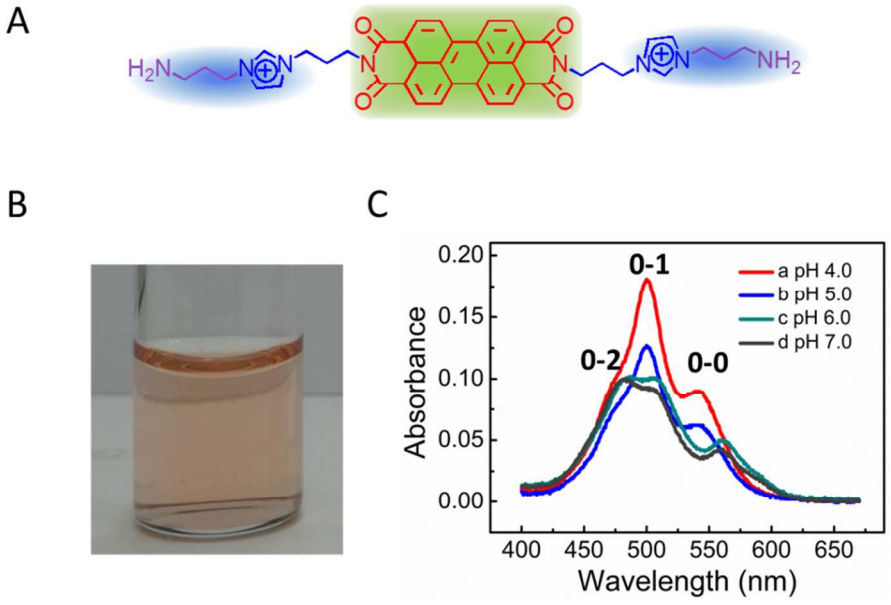

Figure 1 (A) The molecular structure of AIA-PDI; (B) The photograph of 0.5 mM AIA-PDI water solution; (C)The UV-Vis absorbance spectra of 1 μM AIA-PDI solution at pH 4.0 (a), 5.0 (b), 6.0 (c) and 7.0 (d).

2. Experimental

Chemicals

3,4,9,10-perylenetetracarboxylic dianhydride (PTCDA, 97%) was got from Sigma-Aldrich, USA. N-(3-Aminopropyl)-imidazole (98%) and 3-bromopropylamine hydrobromide (98%) were obtained from Alfa Aesar, USA. 3-Morpholinopropanesulfonic acid (MOPS), 99.5% was purchased from J&K Chemical Ltd. Glucose Oxidase (GOx) was obtained from Fluka. Other reagents were of analytical grade and used as received. All aqueous solutions were prepared with ultrapure water from a Millipore Milli-Q Plus (> 18 MΩ) system.

Instruments

Ultraviolet-Visible (UV-Vis) absorption spectra were recorded on a Hitachi U-3900 spectrophotometer. Fluorescence emission spectra were recorded using a Hitachi F-4600 fluorescence spectrophotometer with an excitation wavelength of 495 nm. Excitation and emission slit widths were both of 10 nm. Transmission electron microscopy (TEM) was conducted using a JEOL 2000 transmission electron microscope at an accelerating voltage of 200 kV. The pH measurement was performed on pH meter.

Synthesis and Preparation

AIA-PDI was made from 3,4,9,10-Perylenetetracarboxylic dianhydride according to our previous work.[29] The synthesis procedure and NMR characterizations of AIA-PDI were shown in **SI**. To prepare AIA-PDI solution with different pH, the AIA-PDI was first dispersed in 1 mM 3-Morpholinopropanesulfonic acid (MOPS) solution (pH 6.0, tuned by NaOH). Then 0.1 M HCl was added into this solution to tune the pH to 4.0. Finally, 0.1 M NaOH was added drop by drop to get the solution with different pH. And the prepared solutions were used to perform UV-Vis, fluorescence and TEM experiment. Successively, glucose detection application was used as a proof of concept. To perform glucose detection experiment, 40 μL GOx (4 mg/mL) was first added into 2 mM phosphate buffered (PB) solution (2 mL, pH 7.0) with different concentrations of glucose. Then the mixture was kept at 37 °C for 3 hours. After that, the solution was cooled into room temperature. Finally, 20 μL AIA-PDI solutions (100 μM, pH 7.0) were added. Few minutes later, fluorescence measurement was performed.

3. Results and discussion

In contrast to water-insoluble PDI, AIA-PDI disperses well in water and forms a clear and stable pink solution (Fig. 1B). This solution can be stable even more than half a year without any sediment observed. UV-Vis absorption spectroscopy shows three absorption bands, i.e., A^{0-0} (541 nm), A^{0-1} (500 nm) and A^{0-2} (470 nm), which are consistent with the typical absorption characteristics of PDI derivatives. The intensity ratio between $A^{0\rightarrow0}$ and $A^{0\rightarrow1}$ is 0.5 smaller than 1.6, indicating a dominated aggregation-state of AIA-PDI molecules in this pH solution (pH 4.0).[30-32] Upon increasing the pH from 4.0 to 7.0, the absorption bands of A^{0-0} and A^{0-1} were redly shifted to 506 nm and 560 nm, respectively. Meanwhile, the ratios between A^{0-0} and A^{0-1} were decreased from 0.5 to 0.45. These variations indicate an increased aggregation-state of AIA-PDI molecules with increasing the pH.

Fluorescence measurements were further used to examine the above pH-dependent behaviour. In the case of pH 4.0, the fluorescence emission spectrum showed peaks at 549 and 590 nm, which represents typical fluorescence characteristics of AIA-PDI monomer in solutions (Fig. 2A, a). The fluorescence intensity decreases with increasing the pH (4.0-7.0). Finally it disappeared at pH 7.0, indicative of completely aggregated state

(Fig. 2A). PDI derivatives often disclose fluorescence ON in the monomer-state while OFF in the aggregation-state owing to the intramolecular fluorescence energy transfer.[33] The above pH-dependent fluorescence results thus imply a pH-sensitive molecular structure conversion between monomer and aggregation. Through further quantitative analysis, a good linear relationship between natural logarithm of fluorescence intensity (Ln(FL)) and pH (Fig. 2B) was observed, which indicates a tunable molecular state of AIA-PDI. In addition, we examined its fluorescent reversibility upon the pH stimulation. It was found that AIA-PDI switched reversibly on at pH 4.0 and off at pH 7.0 (Fig. 2C), in which the fluorescence intensity can maintain 80% after 10 cycles. Overall, this AIA-PDI molecule shows reversibly pH responded fluorescent conversion and can give sensitive pH information, which is suitable for continuous pH monitoring as fluorescent probes.

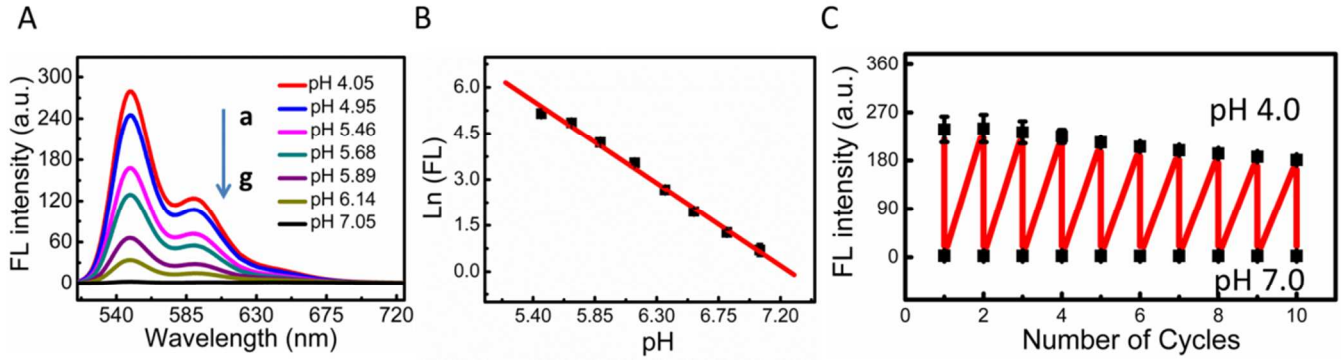

Figure 2 (A) The fluorescence emission spectra of 1 μM AIA-PDI solution obtained at different pH from 4.0 to 7.0: 4.05 (a), 4.95 (b), 5.46 (c), 5.68 (d), 5.89 (e), 6.14 (f), 7.05 (g). (B) The corresponding linear relationship between Ln (FL) and pH. (C) The Fluorescence intensity changes at pH 4.0 and 7.0 with number of cycles.

To better understand the mechanism behind the above fluorescence response under pH-stimulation, the corresponding transmittance electronic microscope (TEM) images at different pH were recorded. In the case of pH 4.0, AIA-PDI molecules self-assembled to short nanobelts with the length of 50 nm (Fig. 3, A). With the increase of pH, the nanobelts become long and hybranched structure (Fig. 3, B-D). Such structural variation is consistent with the fluorescence intensity change. From pH 4.0 and 5.0 (as well as pH 6.0 and 7.0), the structure change is small, so the change of fluorescence intensity is small. On the contrary, the change of structure and fluorescence intensity are large between pH 5.0 and 6.0 (Fig. 2A). And such pH-dependent structural and fluorescent variation can be explained by intermolecular electrostatic and dispersion interactions.[34] According to quantum chemical calculations the electrostatic and dispersion interactions can largely affected the strong π-π interaction among the AIA-PDI.[35, 36] The strong intramolecular repulsive interaction arisen from the protonation of amine groups at pH 4.0 effectively cuts down the π-π attractive interaction and results in short nanobelts. With the increase of pH, the decrease of intramolecular repulsive interaction because of the deprotonation leads to long hybranched nanobelts.[37]

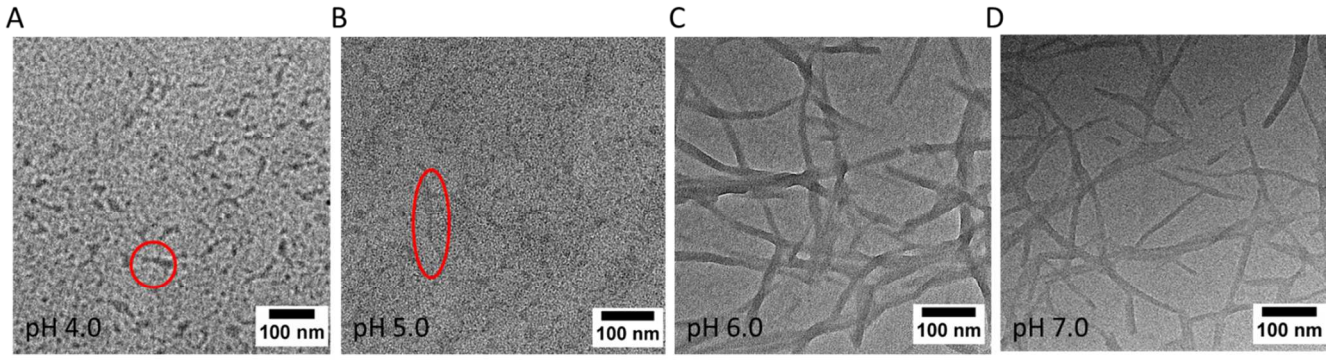

Figure 3 The TEM images of AIA-PDI solution in pH 4.0 (A), 5.0 (B), 6.0 (C), 7.0 (D).

In addition, in accordance to the fluorescence reversibility, the following optical images and TEM images powerfully demonstrate the reversible supramolecular structure conversion upon the pH stimuli. In the pH modulation cycles, the AIA-PDI solution was always clear at low pH (4.0) and aggregated at high pH (8.0) as shown in Fig. 4A. And the short nanobelts observed at pH 4.0 and long hybranched nanobelts for pH 8.0 (Fig. 4B left and right). That means the short nanobelts can self-assemble to form long hybranched nanobelts by modulating pH to basic pH; conversely, the long hybranched nanobelts can disassemble to short nanobelts. The optical

photographs and TEM images clearly disclosed the reversible molecular self-assembly upon pH (Fig. 4C).

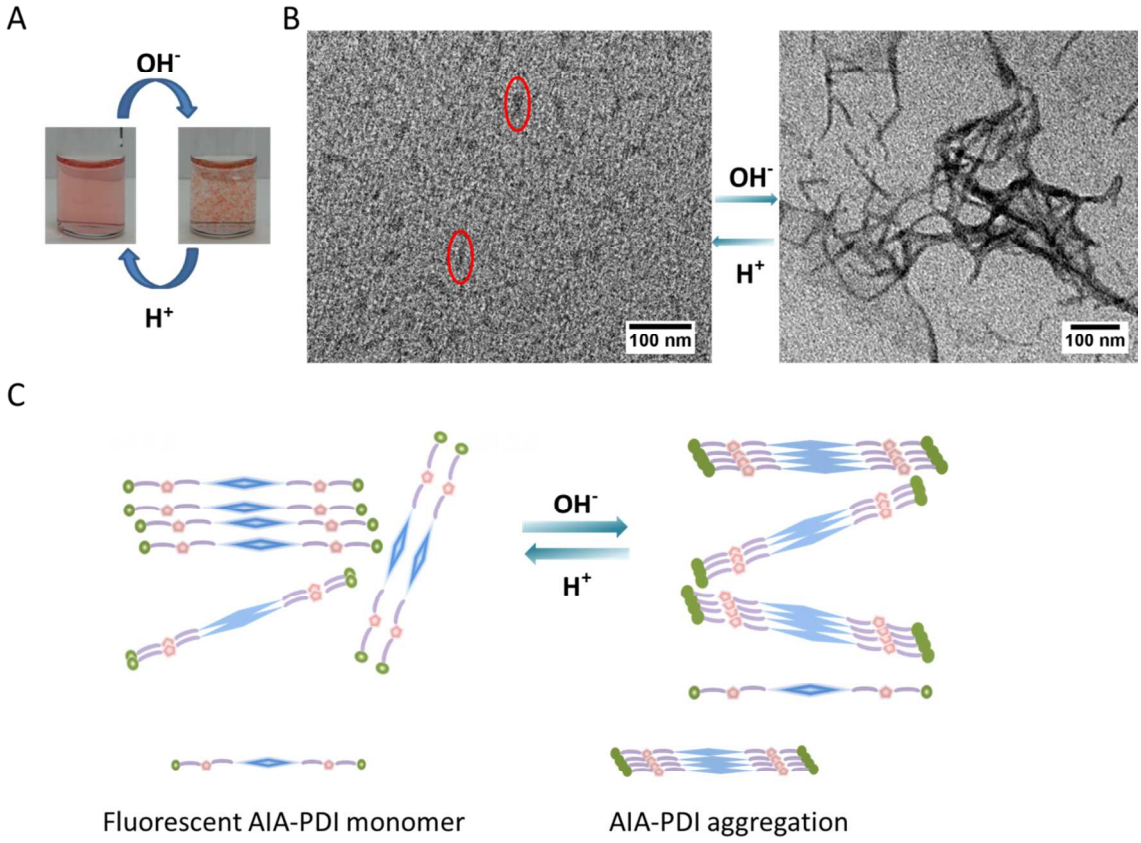

Figure 4 (A) The photographs of 0.5 mM AIA-PDI water solution at low pH (4.0, left) and high pH (8.0, right); (B) the TEM images of AIA-PDI at pH 4.0 and pH 7.0; (C) The scheme presentation of AIA-PDI molecules reversible self-assembly upon pH.

According to the above discussion, we have demonstrated that AIA-PDI molecule showed reversible pH responded fluorescence emission and self-assembly in aqueous system. Based on the ultrasensitive pH response, we provide a proof of concept using glucose detection application. In this experiment, AIA-PDI molecule acted as a fluorescence probe. Gluconic acid, one of generations of the glucose and glucose oxide (GOx) reaction mixture, could cause the pH changes in situ[38, 39] thus induce fluorescence change of AIA-PDI. The detection mechanism was shown in Fig. 5A. The pH values of glucose with different concentrations and GOx reacted mixture were shown in Table S1, which displayed the pH value decreased with the increase of glucose concentration. The fluorescence emission spectra of AIA-PDI in these solutions were also conducted in Fig. 5B. As expected, with the increase of glucose concentration, the fluorescence intensity increased (Fig. 5B and C) indicating some aggregated AIA-PDI molecules disassemble to monomers as shown in Fig. 5A. And a good linear relationship between Ln (FL) and the glucose concentration was also observed (Fig. 5C, inset). Despite that, we also perform the control experiment using the interference agents such as fructose, saccharose, as well as dopamine (DA), ascorbic acid (AA) and uric acid (UA). The results show that there is little fluorescent response to these interference agents (Fig. S1). The stability of AIA-PDI molecule is an important parameter for evaluating the glucose biosensor. To investigate the stability of AIA-PDI in glucose detection, the fluorescence stability of AIA-PDI molecule incubated in the mixtures of GOx and glucose for 20 minutes was examined. There is no obvious fluorescence change (Fig. S2). The reproducibility of glucose detection was examined by thrice parallel measurements. The small error bars in Figure 5c indicate a good reproducibility for the glucose sensing. Here 1 mM glucose detection was taken as an example to calculate the relative standard error. Fluorescence spectra of the three parallel experiments showed that intensity values are 2.77, 2.57 and 2.66, respectively. A relative standard deviation (RSD) is 3.75%. Such small value demonstrates high reproducibility for the glucose detection. The glucose detection experiment further demonstrates this PDI derivative's biological suitability in pH-responsive system.

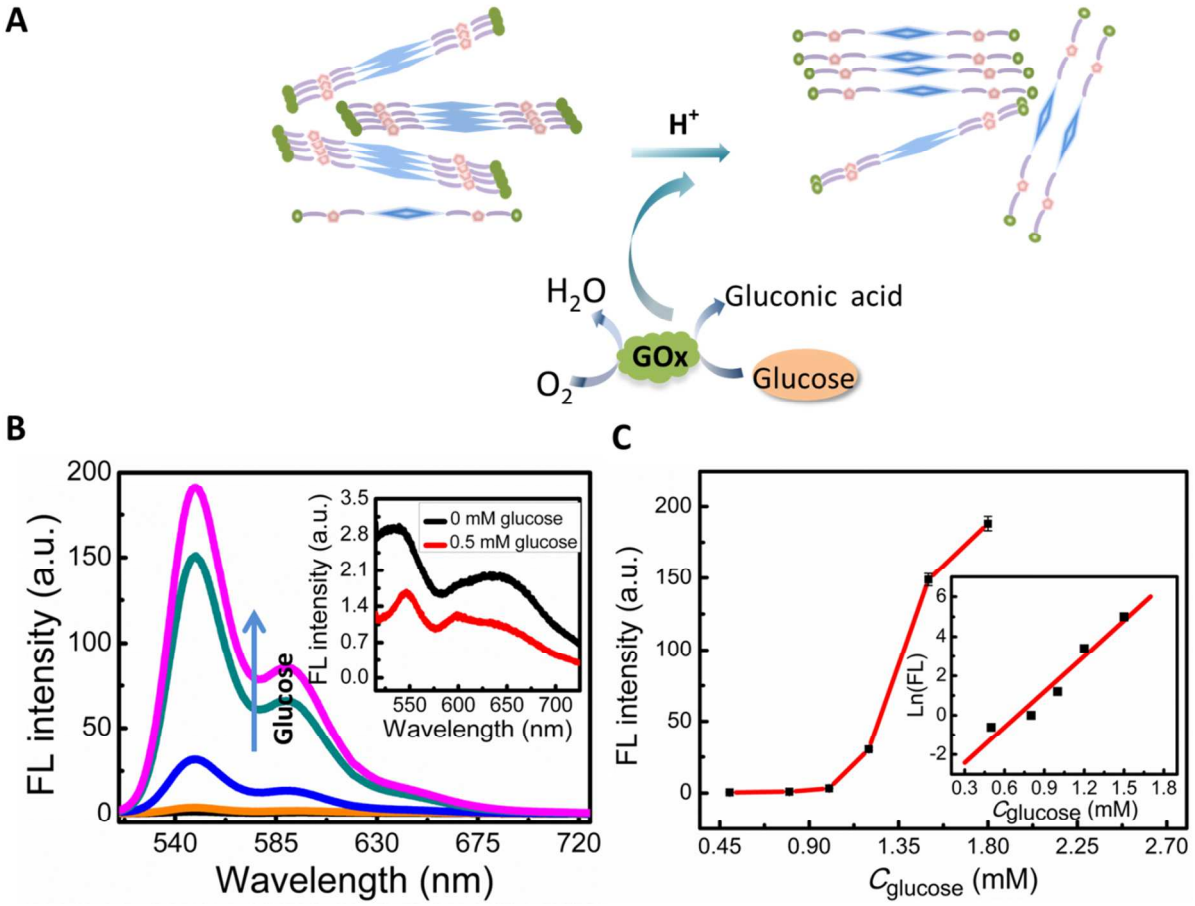

Figure 5 (A) The scheme of glucose detection using AIA-PDI molecules as fluorescence probes. (B) The corresponding fluorescence spectra of AIA-PDI incubated in reaction mixture of GOx and glucose with different concentration. 0.8 mM (black), 1.0 mM (orange), 1.2 mM (blue), 1.5 mM (dark cyan), 1.8 mM (magenta) glucose and (inset) 0 mM (black), 0.5 mM (red) glucose. (C) Fluorescence intensity plotted as a function of glucose concentration and the linear region from 0.5 to 1.5 mM glucose (inset).

4. Conclusions

In this work, we design a new type of PDI derivatives, featured two arm-like ionized amino-imidazole groups that significantly improve its solubility in water. Meanwhile the π-conjugated PDI core reserves the ability of supramolecular self-assembly. Owing to the introduced amino groups, this PDI derivative discloses reversible conversions in the fluorescence emission and supramolecular structures upon external pH-stimulation. Based on the ultrasensitive pH response, we provide a proof of concept using glucose detection application, which further demonstrates this PDI derivative's biological suitability in pH-responsive system. We believe this PDI derivative has a great potential for application in pH region of biological interest such as imaging pH gradients within live tumour models and probing intracellular microenvironments.[40]

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References

[a] Engineering Laboratory for Modern Analytical Techniques, c/o State Key Laboratory of Electroanalytical Chemistry, Changchun Institute of Applied Chemistry Chinese Academy of Sciences Changchun 130022, PR China. Fax: +8643185262800; Tel: +8643185262425; E-mail: lniu@ciac.ac.cn
[b] University of Chinese Academy of Sciences Beijing 100039, P. R. China

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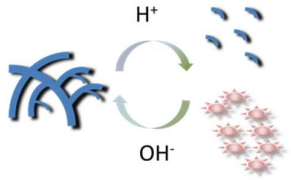

A water-soluble amino-imidazole-armed perylene diimide dye discloses reversible supramolecular structure and fluorescence emission conversion upon external pH-stimulation.