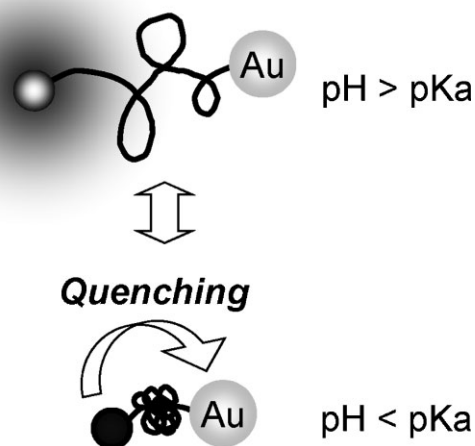


A New Polymeric pH Sensor Based on Photophysical Property of Gold Nanoparticle and pH Sensitivity of Poly(sulfadimethoxine methacrylate)^a

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A new pH sensor, which consists of a fluorophore and gold nanoparticle (AuNP) attached to each chain end of a pH-sensitive polysulfonamide, respectively, is synthesized, and its pH sensitivity is investigated in terms of the fluorescent quenching efficiency of AuNP. Since the pH-sensitive polymeric linker exhibits a pH-induced coil-globule transition that is rapid enough to show a typical two-state transition, it shows drastic on-and-off quenching efficiency with variation of pH due to the change in the distance between fluorophore and AuNP. This AuNP-based pH sensor exhibits a well-defined on-and-off behavior at a small change in pH, and therefore can be used for an ideal alarm-type sensor.



Introduction

An integration of nanotechnology with biology and medicine has been expected to provide a useful platform for developing novel nanosized functional materials that can be used for biological, medical, and environmental applications.^[1–12] In particular, there have been many attempts to use optical signals of the nanosized functional materials as a transducer for detecting many important

biological and environmental changes, since the change of optical signal has been regarded as a convenient and facile way to monitor those phenomena. For this reason, quantum dot and gold nanoparticle (AuNP) have been widely used as one of the promising candidates for the purpose.^[13–17] Traditionally, both quantum dot and AuNP have been mainly used for ratiometric measurement,^[18–22] since the ratiometric analysis has an important advantage of concentration independence and therefore it can accurately detect a delicate change in the biological and environmental phenomena being concerned. Moreover, it has been known that the sensor based on the ratiometric analysis is not affected by many factors in an aqueous solution such as pH, polarity, ionic strength, and so forth.^[23,24]

Contrary to quantum dot which emits the fluorescence, AuNP has a unique optical property that quenches the fluorescence emitting from a fluorescent compound,^[25,26]

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which results from coupling of oscillating electronic dipole of the fluorescent material with the surface of AuNP. When a fluorescent compound near AuNP is excited, its fluorescence is effectively quenched by an energy transfer from the fluorescent material to AuNP; this is generally termed as the nanosurface energy transfer.^[27] It should be noted that, owing to an intrinsically low intensity of background arising from this quenching characteristic of AuNP, it can ensure higher signal-to-noise ratio in the ratiometric analysis than quantum dot: this is one of the important advantages of AuNP over quantum dot. Furthermore, it has recently been recognized that quantum dot has a critical drawback of toxicity,^[28–31] which may prohibit extensive utilization of quantum dot for biological and environmental applications, whereas AuNP has been reported to exhibit no long-term toxicity and no other adverse effects in vivo.^[32,33] This is another advantage of AuNP over quantum dot.

Recently, several research groups have utilized AuNP for preparation of sensor in different ways through combination of the unique property of AuNP and the conformation change of macromolecule. Sugimoto and coworkers^[34] prepared the composite of AuNP and molecularly imprinted polymer and used as a sensing material. Dyadyusha et al.,^[35] Wang et al.,^[36] and Lee et al.^[37] have also utilized AuNP for preparation of the sensors for detecting DNA.

In this study we have proposed another prospective method that exploits AuNP as bio- or environmental-sensor, where AuNP is conjugated with a stimuli-sensitive polymer. Here the stimuli-sensitive polymer is generally defined as a smart polymer which shows a drastic change in its properties, responding to small variation in physical or chemical stimuli such as pH, temperature, and ionic species. Among various stimuli, detecting a specific pH in the physiological region is essentially needed for monitoring many programmed functions in biopharmaceutical systems.^[38–44] In our previous paper,^[12] we reported the design and synthesis of a polymeric pH sensor based on carbon nanotube that shows an excellent on-and-off characteristic in the quenching efficiency occurring at physiological pH. However, the pH sensor based on AuNP would be superior to the pH sensor based on carbon nanotube for medical and biological applications in terms of biocompatibility and biosafety.^[32,33] In this study, a polymeric pH sensor is prepared by attaching a fluorophore and AuNP to each chain end of pH-sensitive polymer, and its pH sensitivity is examined as a function of pH in terms of the quenching efficiency of AuNP.

Experimental Part

Materials

All materials were purchased from Sigma–Aldrich unless specified. Methylene chloride (Daejung Chemicals & Metals, 99.5%) was dried over calcium hydride and distilled under nitrogen. Sulfadimethox-

ine (SD), sodium hydroxide (Yakuri Pure Chemicals), methacryloyl chloride (97%), tris(2-aminoethyl)amine (97%, Lancaster Chem.), formaldehyde (Yakuri Pure Chemicals), formic acid (Yakuri Pure Chemicals), potassium hydroxide (Daejung Chemicals & Metals), 1-pyrenemethylamine hydrochloride (95%), 2-bromoisobutyl bromide (98%), triethylamine (99.5%), ethanol (Daejung Chemicals & Metals, 95%), copper (I) bromide (99.999%), *N,N*-dimethylformamide anhydrous (DMF) (99.8%), methanol (Daejung Chemicals & Metals, 99.5%), 2,2'-(ethylenedioxy)bis(ethylamine) (EDBEA) (98%), dimethyl sulfoxide anhydrous (DMSO) (99.9%), and pH 10 PBS buffer solution (Daejung Chemicals & Metals), and mono-sulfo-*N*-hydroxysuccinimido NANOGOLD[®] (AuNP-NHS) (Nanoprobes) with 1.4 nm diameter were used as received.

Synthesis of Pyrene-Poly(sulfadimethoxine methacrylamide)-NH₂ (Pyr-PSDM-NH₂)

Pyr-PSDM-NH₂ was synthesized according to the method previously reported.^[45] For all reactions, all the flasks were degassed and backfilled with Ar gas. DMF-*d*₇ was degassed for removal of oxygen by three freeze-and-thaw cycles, and distilled water was degassed by boiling for 48 h and bubbling with Ar gas for 12 h to completely remove dissolved oxygen. SD was conjugated with methacryloyl chloride to produce sulfadimethoxine methacrylamide (SDM). Then, SDM (20 g, 5.30×10^{-1} mol) and degassed DMF-*d*₇ (11.25 mL) were put in a flask, and 22.5 mL of an aqueous NaOH (2.12 g, 5.30×10^{-1} mol) solution was sequentially added. After addition of Me₆TREN (0.147 mL, 5.30×10^{-4} mol) and copper (I) bromide (0.0759 g, 5.30×10^{-4} mol), a solution of pyrene-based initiator (0.202 g, 5.30×10^{-4} mol) in DMF-*d*₇ (11.25 mL) was added to the reaction flask. The solution was stirred at 40 °C for 2 h to allow the atom transfer radical polymerization (ATRP). For purification of the product, the solution of crude product was precipitated in 1 N HCl solution. After filtration, the precipitate was washed with methanol at room temperature for 24 h to remove unreacted SDM. The final product (Pyr-PSDM) was collected by filtration and then dried in vacuum at 30 °C. \bar{M}_n (NMR): 49 000. Pyr-PSDM (1 g) was then placed in a flask and dissolved in DMSO-*d*₆ (5 mL), and reacted with DGEBEA (0.500 mL) in the presence of triethylamine (0.05 mL) to yield the product (Pyr-PSDM-NH₂). After 0.5 mL of distilled water was added, the solution was stirred at 40 °C for 24 h. The product was purified by dialyzing against methanol for 48 h using a cellulose dialysis membrane (molecular weight cut-off: 6 000–8 000, Membrane Filtration Products, Inc.). The solution was filtered, and the precipitate (Pyr-PSDM-NH₂) was dried in vacuum at 30 °C. Yield: 33%.

Preparation of Pyr-PSDM-AuNP

Pyr-PSDM-NH₂ (1 mg) was placed in a flask under Ar atmosphere, followed by addition of pH 10 buffer solution (1 mL) for dissolution of Pyr-PSDM-NH₂. A solution of AuNP-NHS (6 nmol) was successively added to the reaction flask, and the solution was stirred at room temperature for 3 h to yield Pyr-PSDM-AuNP. The crude product was purified by cautious removal of the supernatant after ultracentrifugation at 32 000 *g* several times. The removal of the supernatant containing free polymers is repeated until the peaks corresponding to pyrene are not observed in both UV-vis and fluorescence spectra, indicating that no Pyr-PSDM is present in the solution.

Characterization

The chemical structure of materials synthesized in this study were identified by ^1H NMR (Avance DPX-300 or Avance 500, Bruker). The modified AuNP was purified by ultracentrifugation (Beckman Coulter Ultracentrifuge, Beckman Coulter). All the polymer solutions were prepared by dissolving polymers in the commercially available pH 10.0 buffer solution, and the pH value was then adjusted by acid–base titration using 0.1, 0.05, and 0.01 M HCl aqueous solutions. UV-vis absorption and fluorescence spectra were recorded with a UV-vis spectrometer (HP 8452A, Hewlett Packard) and a fluorescence spectrometer (Felix32, Photon Technology International, Inc.), respectively.

Results and Discussion

The synthetic route for preparation of an AuNP-based pH sensor is graphically shown in Figure 1. Among many kinds of sulfonamides, SD was chosen for synthesis of pH-sensitive polymeric linker, since its pK_a value is in the range of physiological pH region.^[46,47] SD was then conjugated with methacryloyl chloride to yield SDM, which is used as the monomer for ATRP. A stronger ligand such as Me_6TREN is used for ATRP of SDM, because sulfonamide-type monomers are not effectively polymerized by the use of

conventional ATRP ligands.^[45] Pyrene was selected as a fluorophore in this study, because its photoluminescent property is hardly influenced by the change in pH.^[13] Pyrene was modified to be used as an initiator for ATRP of SDM, according to the same method described in our previous report.^[45] Since the pyrene-based initiator acts as not only an initiator for ATRP but also a fluorophore, the resulting polymer (Pyr-PSDM) intrinsically has a fluorescent pyrene at one end of the polymer chain. Since all the peaks in the ^1H NMR spectrum of Pyr-PSDM are appropriately assigned, as shown in Figure 2, it is assumed that Pyr-PSDM has successfully been synthesized. From the acid–base titration method, pK_a of Pyr-PSDM is determined to be 8.00. It should be noted that the end group of the growing polymeric chain formed by ATRP of SDM is intrinsically *tert*-alkyl bromide group, which can be easily transformed into various functional groups via simple nucleophilic displacement reaction.^[48] In this study, EDBEA (a diamine-type molecule) was reacted with *tert*-alkyl bromide group to yield the product (Pyr-PSDM- NH_2) having an amine group at the other chain end of polymer. Nynhydrin test qualitatively confirms that the amine group is successfully introduced to the chain end, as shown in Figure 3. Since an excess amount of EDBEA was used for this amination reaction, it is reasonable to assume that two

polymers are rarely linked with each other through the diamine molecule.^[49]

The reason for the use of the diamine-type molecule as a linker is because amine group is a strong nucleophile, which can easily conjugate with *tert*-alkyl bromide via $\text{S}_\text{N}2$ reaction. Moreover, when an amine group is reacted with a carboxyl acid group, the resulting amide linkage exhibits an excellent stability against hydrolysis. Since mono-AuNP-NHS contains a reactive hydroxysuccinimidyl group on the surface of AuNP, it can be covalently linked to the amine group of Pyr-PSDM- NH_2 to yield Pyr-PSDM-AuNP, as shown in Figure 1. The Pyr-PSDM-AuNP is dissolved in pH 10 buffer solution before UV-vis and fluorescence measurements. Figure 4 shows the UV-vis spectra of Pyr-PSDM, Pyr-PSDM-AuNP, and AuNP. Since the UV-vis absorbance of Pyr-PSDM-AuNP substantially increases in the spectral range from 360 to 500 nm as compared with that of Pyr-PSDM, it is concluded that AuNP is successfully introduced into Pyr-PSDM. The gold content and the concentration of final solution can also be calculated from the Beer–Lambert law ($A = \epsilon cl$), where A is obtained from UV-vis

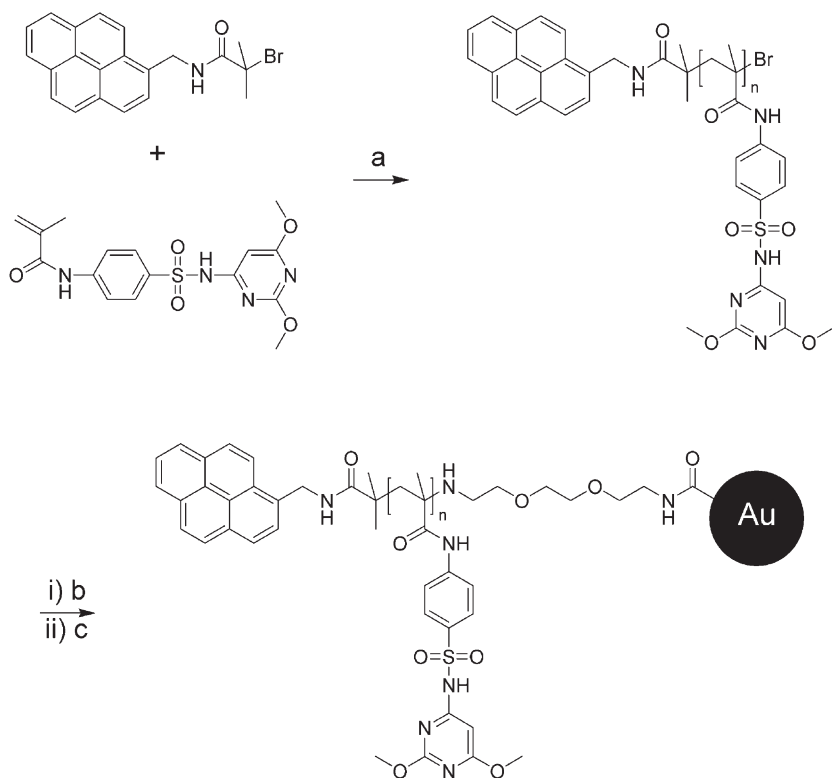


Figure 1. Overall scheme for synthesis of Pyr-PSDM-AuNP: (a) copper (I) bromide, Me_6TREN , DMF-d_7 , $\text{NaOH}/\text{H}_2\text{O}$, 40°C , 2 h; (b) EDBEA, triethylamine, DMSO-d_6 , H_2O 40°C , 24 h; (c) AuNP-NHS, pH 10 buffer solution, room temperature, 3 h.

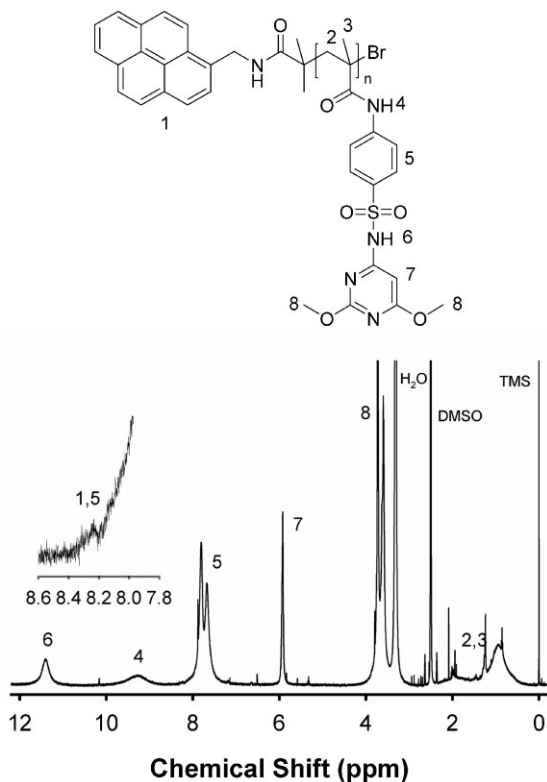


Figure 2. ^1H NMR spectra of Pyr-PSDM in $\text{DMSO}-d_6$.

spectra, l is 1 cm, the extinction coefficient of pyrene is $1\,022\,860\ \text{M}^{-1}\cdot\text{cm}^{-1}$ (at 346 nm), and the extinction coefficients of AuNP are $155\,000\ \text{M}^{-1}\cdot\text{cm}^{-1}$ (at 420 nm) and $182\,289\ \text{M}^{-1}\cdot\text{cm}^{-1}$ (at 346 nm). From the calculated values of c_{pyrene} and c_{AuNP} , it is estimated that one AuNP is attached to every sensor molecule and the concentration of final solution containing Pyr-PSDM-AuNP is $6.04 \times 10^{-7}\ \text{M}$ (see Supporting Information for more detailed procedure). We have used this extremely low concentration condition for fluorescence study in order to eliminate the scattering effect on the fluorescent quenching induced by inter-chain coagulation.

Figure 5 illustrates the sensing mechanism of Pyr-PSDM-AuNP. When pH is higher than pK_a of PSDM, PSDM chain is extended, and as a result the distance between the

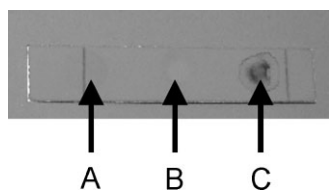


Figure 3. The result of Nynhidrin test. The original color-version image was converted into the gray-scale-version image. (A) Nynhidrin only; (B) Nynhidrin with Pyr-PSDM; (C) Nynhidrin with Pyr-PSDM- NH_2 .

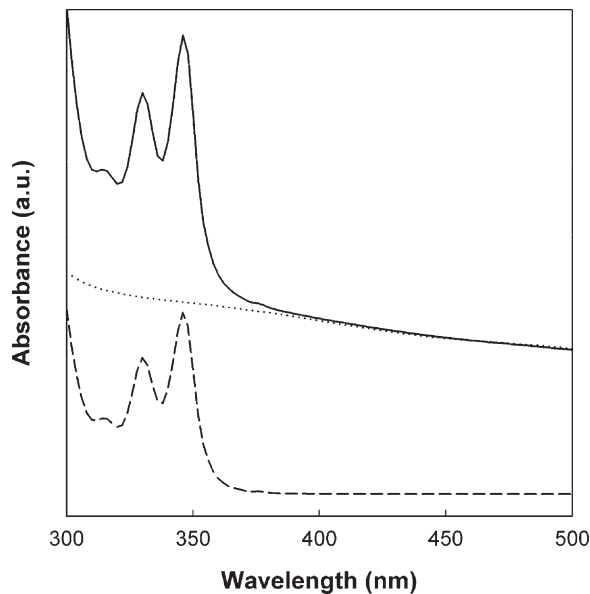


Figure 4. UV-vis spectra of Pyr-PSDM (dashed), AuNP (dotted), and Pyr-PSDM-AuNP (solid) in pH 10.0 buffer solution.

fluorescent pyrene and AuNP becomes so far that AuNP hardly quenches the fluorescence emitting from pyrene. On the other hand, when pH is lower than pK_a , PSDM chain becomes collapsed and consequently the distance between pyrene and AuNP becomes close enough to induce the effective quenching of the fluorescence of pyrene by AuNP. For the proposed mechanism described above to be realized, it is important to determine the optimum length of the polymeric linker (PSDM in this study), because the rate of energy transfer is dependent on d^{-4} , where d represents the distance between a dye molecule and AuNP.^[31] If the polymeric linker connecting pyrene and AuNP is too close, the fluorescence emitting from pyrene is always quenched even though the PSDM chain is fully extended. On the other

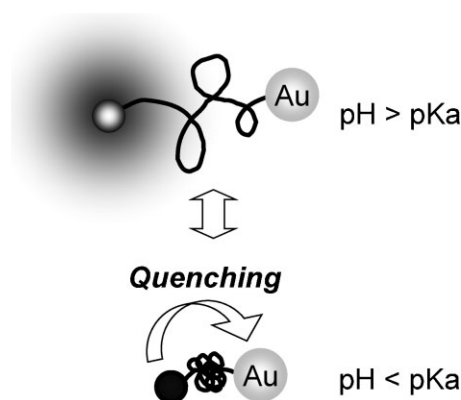


Figure 5. Diagrammatic representation of the induction of fluorescence quenching under change of pH.

hand, if the chain length of polymer linker is too long, pyrene may always emit the fluorescence even though the PSDM chain is completely collapsed. In this study, several PSDMs with different molecular weights were synthesized and the fluorescence quenching was examined as a function of molecular weight of PSDM in order to determine an optimum molecular weight of PSDM. We found that the molecular weight of $49\,000\text{ g}\cdot\text{mol}^{-1}$ was the optimum. We prepared several sensors with \bar{M}_w lower than 49 000 (4 100, 10 000, and 22 000) and observed that each quenching efficiency is much lower than 48%, indicating that the fluorescence emitting from pyrene is always quenched by AuNP regardless the pH change. On the other hand, we did not prepare a polymer with \bar{M}_w higher than 49 000, because we already observed that the calculated quenching efficiency at \bar{M}_w 49 000 was 48%, which is close to the value reported by the previous study,^[50] where pyrene is directly attached to the surface of AuNP. It should be noted that the charge and energy transfer between a dye molecule and AuNP is dependent not only on the distance between them but also on the conductivity of the molecule in different states. However, the latter effect might be small in this work since pyrene is an insulating material which has low conductivity. Therefore, it is reasonable to conclude that the distance between pyrene and AuNP is the major factor to be considered in this quenching mechanism.

Figure 6 shows the change of the fluorescence emitting from Pyr-PSDM-AuNP with pH, where all of the emission spectra are obtained after irradiating the solution at 334 nm corresponding to the absorption wavelength of pyrene. It

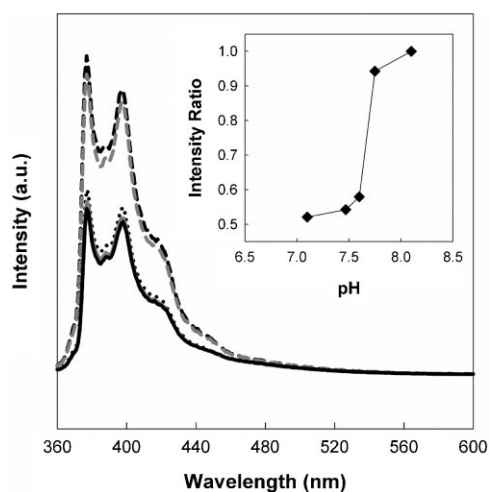


Figure 6. Fluorescence emission spectra of Pyr-PSDM-AuNP at pH 7.10 (black solid), 7.47 (gray solid), 7.60 (black dotted), 7.75 (gray dashed), and 8.10 (black dashed) when the excitation wavelength of 334 nm is irradiated. All the fluorescence emission spectra are measured at the concentration below $0.46 \times 10^{-6}\text{ M}$. The inset is the plot of the normalized fluorescence emission intensity of Pyr-PSDM-AuNP at 397 nm against pH.

should be noted that the emission peak (480 nm) from pyrene excimer is not observed, indicating that there is no aggregation between sensor molecules. Therefore, it is concluded that the fluorescence quenching is induced solely by the conformational change (coil-globule transition) of the sensor molecule. According to the magnitude of intensity of fluorescence emission, the emission spectra are divided into two groups: one is spectra at $\text{pH} < 7.60$ and the other is spectra at $\text{pH} > 7.75$. When the intensity is plotted as a function of pH after the fluorescent intensity of Pyr-PSDM-AuNP is normalized with respect to the intensity at 397 nm at pH 8.1 for quantitative comparison, as shown in the inset of Figure 6, it reveals that the normalized intensity is abruptly decreased at pH 7.6 as the pH value is decreased. This is easily explained by considering the change in the end-to-end distance of PSDM, corresponding to the distance between pyrene and AuNP, with variation of pH. When pH is higher than pK_a of PSDM, the sulfonamide group in PSDM is ionized and PSDM becomes water soluble with an expanded coiled structure, which makes pyrene and AuNP far apart from each other, as shown in Figure 5. However, as pH is lower than pK_a of PSDM, the sulfonamide group of PSDM is protonated and PSDM becomes insoluble in water with a collapsed globular structure (see Supporting Information for graphical interpretation). This phenomenon is well consistent with the previous report, which explains the conformational transition of oligo-sulfonamide upon pH change.^[51] Therefore, PSDM undergoes the transition from coiled structure to globular structure at pH 7.6 as pH is decreased. As a result, the distance between pyrene and AuNP becomes close enough for AuNP to quench the fluorescence emitting from pyrene, as demonstrated in Figure 5. It is noted that the calculated quenching efficiency is 48%, which is close to the value reported by the previous study,^[50] where pyrene is directly attached to the surface of AuNP. Therefore, it is reasonable to conclude that the molecular weight of PSDM used in this study is appropriate for effective sensing of pH. Another interesting feature from the inset of Figure 6 is that Pyr-PSDM-AuNP responds to the relatively narrow change in pH with well-defined on-and-off behavior, which strongly demonstrates that our AuNP-based pH sensor can be used as an alarm-type probe for detecting a specific pH. It should also be noted that the selection of a specific sulfonamide allows us to prepare the pH sensor for detecting the specific pH range as one wish, because different types of sulfonamide exhibits different pH transitions.^[49]

Conclusion

In this work, a new pH sensor is prepared by using the photophysical property of AuNP and pH sensitivity of

polysulfonamide and its photophysical behavior is examined as a function of pH. Since the polymeric linker (PSDM) containing pH-sensitive SD moiety undergoes the coil-globule transition upon pH change, the distance between pyrene and AuNP attached to each end of PSDM is changed with pH variation, which controls the fluorescence quenching efficiency of AuNP. As the pH value is lower than pK_a of PSDM, the fluorescence emitting from pyrene decreases, indicating that the conformation of PSDM changes from coil to globule so as to induce a dramatic on-and-off quenching efficiency. Since this AuNP-based polymeric pH sensor responds well to a small change in pH, it would be an ideal alarm-type sensor for sensing pH-induced changes in many important bio- and environmental phenomena.

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