

# Temperature-driven on/off fluorescent indicator of pH window: an anthracene-conjugated thermoresponsive polymer

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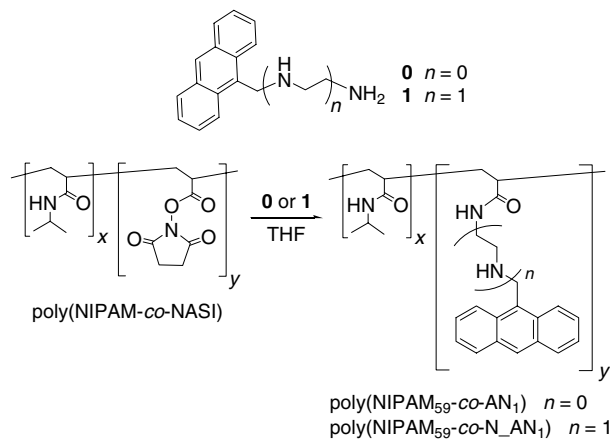
**Abstract**—A simple copolymer, poly(NIPAM-co-N<sub>9</sub>AN), consisting of *N*-isopropylacrylamide (NIPAM) and 9-aminomethylantracene (N<sub>9</sub>AN) units behaves as a temperature-driven on/off fluorescence indicator of pH window (2–12): showing an ‘on–off’ fluorescence intensity profile against the pH window at 15 °C, while showing an ‘off–on–off’ profile at 34 °C.

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Design of molecular systems performing as elementary electronic devices is an area of intense research activity and of tremendous significance to the development of miniaturized device components.<sup>1</sup> Potential application of these components in optical and electronic molecular-scale computational devices has attracted considerable effort.<sup>2</sup> Among the research, design of fluorescent signaling molecules has attracted a great deal of attention.<sup>3</sup> So far, various molecules whose emission properties can be modulated by external inputs such as light,<sup>4</sup> redox potential,<sup>5</sup> and metal ions<sup>6</sup> have been proposed. In particular, molecular systems behaving as a fluorescent indicator of pH window have attracted much attention, because the proton (H<sup>+</sup>) concentration is simply controlled (high operability).<sup>7–10</sup> However, most of the pH indicators proposed so far show simple ‘on–off’<sup>7</sup> or ‘off–on’<sup>8</sup> fluorescence intensity profile against the pH window. For creation of more sophisticated device components, development of pH indicators capable of detecting even small pH change is necessary. Recent interest is, therefore, focused on more integrated systems showing ‘on–off–on’<sup>9</sup> and ‘off–on–off’<sup>10</sup> profiles. In addition, most of the pH indicators proposed so far show single pH–fluorescence intensity profiles; in other words, one molecular system shows only one profile type. To the best of our knowledge, there are two reports of molecular systems behaving as a ‘configurable’ fluorescent pH indicator, whose pH–fluorescence intensity profile can be modulated by external stimuli.<sup>11</sup> However,

both systems require addition of metal cation or organic solvent for the profile configuration, leading to an irreversible profile change.

Our system presented here is the first reversibly-configurable fluorescent pH indicator, which controls the ‘on–off’ and ‘off–on–off’ pH–fluorescence intensity profiles by temperature as the external stimulus. We employ a simple-structured copolymer, poly(NIPAM-co-N<sub>9</sub>AN), consisting of *N*-isopropylacrylamide (NIPAM) and 9-aminomethylantracene (N<sub>9</sub>AN) as the thermoresponsive and emitting parts (Scheme 1). It is well-known that polyNIPAM in water shows a reversible *coil-to-globule* phase transition associated with hydration/dehydration



**Scheme 1.** Synthesis of the thermoresponsive polymer.

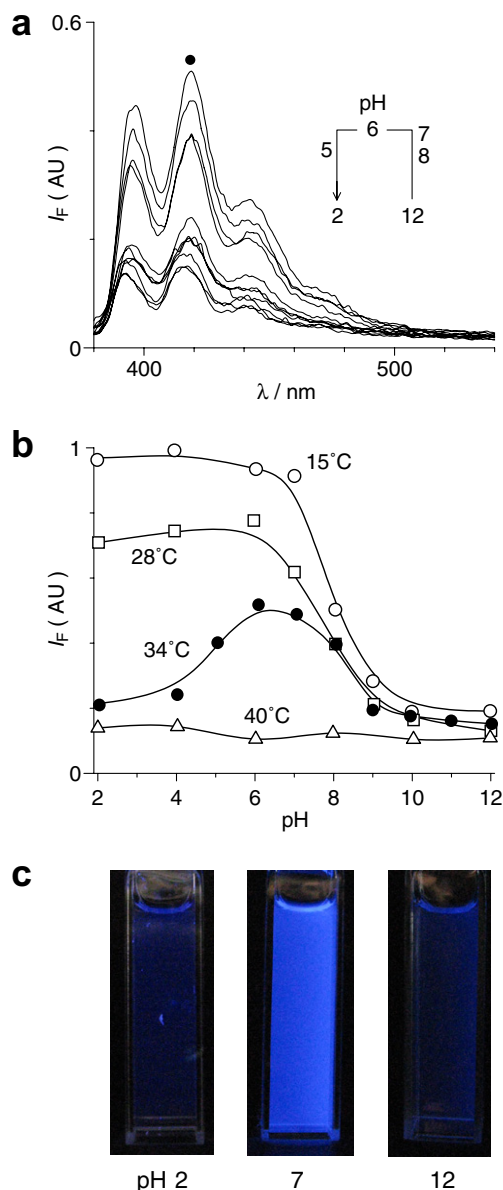
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of the polymer chain by temperature.<sup>12</sup> Our pH indicator is based on the temperature-induced self-assembly of the polymer, which cleverly controls the on/off emission switching. Temperature-induced on/off emission switching has already been achieved by some fluorophore-conjugated polyNIPAM systems.<sup>13</sup> We describe here that the change in the pH–fluorescence intensity profiles of our polymer is driven by the cation– $\pi$  interaction between proton ( $H^+$ ) and  $\pi$ -electron of the AN moieties, leading to strong polymer aggregation at acidic pH. This is the first report of the aggregation of polyNIPAM system controlled by proton– $\pi$  interaction.

The synthesis of poly(NIPAM-*co*-N<sub>2</sub>AN<sub>y</sub>) ( $x/y = 59:1$ ) is depicted in Scheme 1.<sup>14</sup> Reaction of an ethylenediamine-modified AN (1)<sup>15</sup> and a copolymer, poly(NIPAM-*co*-NASI),<sup>16</sup> consisting of NIPAM and *N*-acryloxysuccinimide (NASI) in THF with triethylamine affords a fluffy white powder of poly(NIPAM-*co*-N<sub>2</sub>AN) ( $M_n = 10,468$ ,  $M_w/M_n = 2.2$ ). Absorption and emission spectra were measured on equipments with a digitally-controlled heating/cooling cell holder and a sample stirring capability. After stirring the solution for 30 min at the designated temperature or pH, the measurements were carried out with continued stirring.

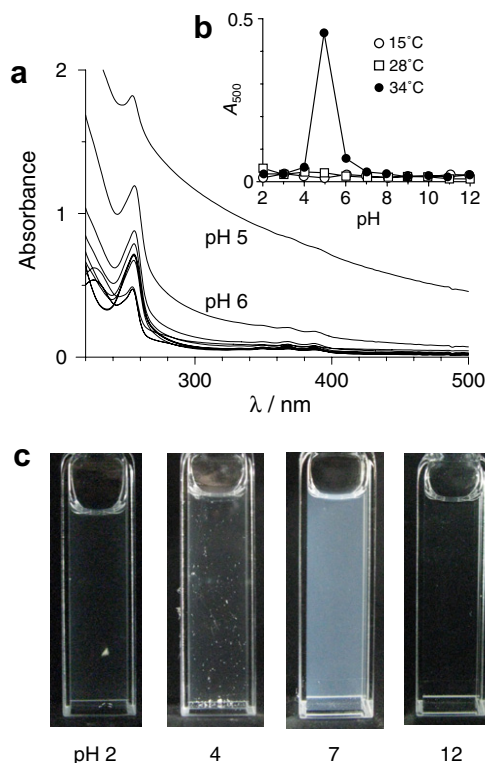
Poly(NIPAM-*co*-N<sub>2</sub>AN) dissolved in water shows a distinctive fluorescence at 380–500 nm ( $\lambda_{exc} = 368$  nm), assigned to a monomer emission from the locally excited AN fragment (Fig. 1a). Figure 1b summarizes the pH-dependent change in the emission intensity monitored at 416 nm. At 15 °C, the intensity is strong at acidic pH (<6), but decreases with a pH increase and becomes very weak at pH > 10, showing a typical ‘on–off’ fluorescence intensity profile against the pH window.<sup>7</sup> As is also observed for related AN-conjugated polyamines,<sup>17</sup> this intensity decrease associated with a pH increase is due to the deprotonation of the benzylic amine of the polymer, leading to an electron transfer from the unprotonated amine to the photoexcited AN fragment. Potentiometrical titration of the polymer reveals that the protonation constant of the benzylic amine is 6.68, which is consistent with decrease in the fluorescence intensity. In contrast, at 34 °C, pH–fluorescence intensity profile differs completely from that obtained at 15 °C: the intensity at acidic and basic pH (<5 and >8) is weak, but is relatively strong at neutral pH (5–8), showing an ‘off–on–off’ profile.<sup>10</sup> These indicate that the present polymer actually behaves as a configurable fluorescent indicator of the pH window driven by temperature.

The temperature-driven switching of the pH–fluorescence intensity profile is due to the heat-induced phase transition of the polymer from *coil* to *globule*. Figure 2b shows pH-dependent change in turbidity ( $A_{500}$ ) of an aqueous solution containing poly(NIPAM-*co*-N<sub>2</sub>AN). At 15 °C, turbidity does not change at any pH, meaning that the polymer is fully dissolved in solution and exists as a coil state.<sup>18</sup> At 34 °C, turbidity also scarcely changes at pH > 7, but increases at pH < 7. As shown in Figure 2c, the solution becomes clouded at pH 7, indicative of the occurrence of polymer aggregation



**Figure 1.** (a) pH-dependent change in fluorescence spectra ( $\lambda_{exc} = 368$  nm) of poly(NIPAM-*co*-N<sub>2</sub>AN) (0.1 g L<sup>-1</sup> containing 15.1  $\mu$ mol L<sup>-1</sup> N<sub>2</sub>AN unit) dissolved in an aqueous NaClO<sub>4</sub> (0.15 mol L<sup>-1</sup>) solution at 34 °C. (b) pH-dependent changes in the fluorescence intensity monitored at 416 nm at different temperature. The measurements were carried out by addition of HClO<sub>4</sub> from basic pH. The respective fluorescence spectra measured at 15, 28, and 40 °C are shown in Figure S1.<sup>14</sup> (c) Change in fluorescence color of the solution with pH at 34 °C.

(globule state formation).<sup>18</sup> The turbidity, however, decreases at pH < 5, where large polymer particles with >100  $\mu$ m diameter appear in solution (Fig. 2c, pH 4). Notably, at pH 2 (Fig. 2c), single huge polymer particle with >1 mm diameter appears. The turbidity decrease at pH < 5 (Fig. 2b) is therefore due to the strong interpolymer aggregation, leading to an increase in the solution transparency. As shown in Figure 2a, at acidic pH, absorption band for the AN moieties at 340–400 nm is overlapped by the absorption of the aggregated polymer. The above findings imply that the decrease in the

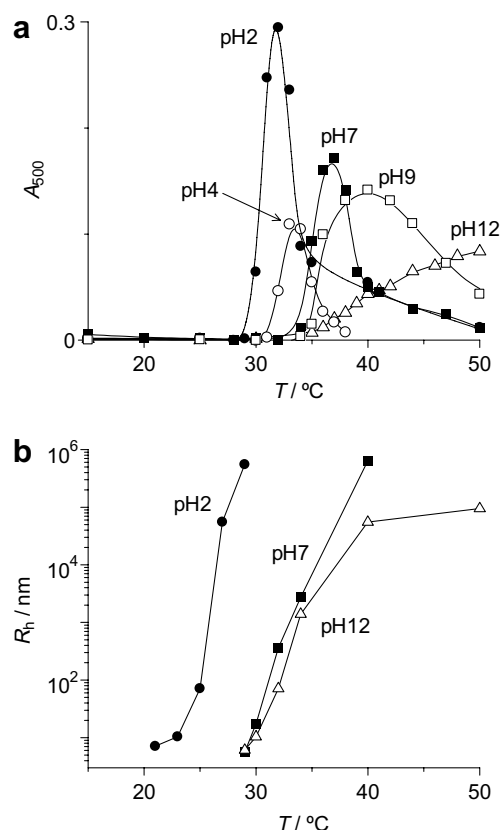


**Figure 2.** (a) pH-dependent change in absorption spectra of poly(NIPAM-*co*-N<sub>AN</sub>) measured at 34 °C. (b) pH-dependent change in turbidity ( $A_{500}$ ) of an aqueous solution containing poly(NIPAM-*co*-N<sub>AN</sub>) measured at 15, 28, and 34 °C. The measurements were carried out by the addition of HClO<sub>4</sub> from basic pH. The absorption spectra measured at 15, 28, and 34 °C are shown in Figure S2.<sup>14</sup> (c) Change in solution with pH at 34 °C.

emission intensity at pH 2–5 (34 °C; Fig. 1b, closed circle) is due to the decrease in incident light absorption of the AN units by the strong polymer aggregation. These findings also reveal that the polymer aggregation takes place more significantly at acidic pH; this leads to an expression of the ‘off-on-off’ profile.

The aggregation behavior of the polymer was studied in detail. Figure 3a shows temperature-dependent change in turbidity of the polymer solution at different pH. At pH 12, the lower critical solution temperature (LCST) of the polymer is estimated to be 34 °C, but decreases with a pH decrease: LCST at pH 2 is 27 °C. This suggests that the polymer aggregates more easily at acidic pH. Figure 3b shows temperature-dependent change in hydrodynamic radius of the polymer particles at different pH. At pH 2, particles form at >21 °C (detection limit, 3 nm) and the size increases exponentially with a rise in temperature: huge polymer particle with >1 mm diameter appears at >28 °C. In contrast, at pH > 7, particles form at higher temperature (>29 °C) and the sizes are much smaller than that obtained at pH 2. These findings clearly indicate that the polymer aggregates more easily and strongly at acidic pH.

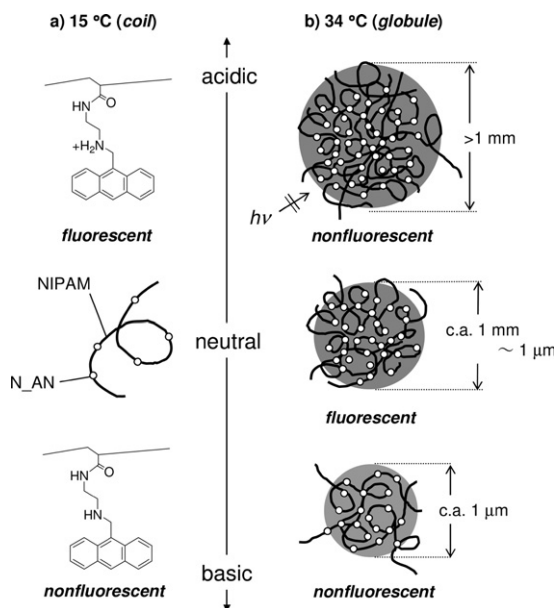
It is well-known that LCST of the unfunctionalized polyNIPAM is not pH dependent (32 °C; Fig. S4<sup>14</sup>).<sup>19</sup> This means that the pH-dependent aggregation of poly-



**Figure 3.** Temperature-dependent change in (a) turbidity ( $A_{500}$ ) and (b) hydrodynamic radius ( $R_h$ ) of poly(NIPAM-*co*-N<sub>AN</sub>) dissolved in an aqueous solution of different pH. These measurements were carried out during heating sequence. The detailed  $R_h$  distribution data are summarized in Figure S3.<sup>14</sup>

(NIPAM-*co*-N<sub>AN</sub>) is due to the presence of benzylic amines or AN moieties. In the case of poly(NIPAM-*co*-AN) without benzylic amines, LCST decreases with a pH decrease (Fig. S4<sup>14</sup>), as is also the case for poly(NIPAM-*co*-N<sub>AN</sub>). This clearly suggests that the AN moieties trigger the pH-dependent aggregation of poly(NIPAM-*co*-N<sub>AN</sub>). The enhancement of the polymer aggregation at acidic pH may probably be due to the cation- $\pi$  interaction between H<sup>+</sup> and  $\pi$ -electron of the AN moiety,<sup>20</sup> where the  $\pi$ -electron of the AN moiety is strongly withdrawn by the proton- $\pi$  interaction, leading to polarity decrease of the AN moiety.<sup>21</sup> As reported,<sup>22</sup> aggregation behavior of polyNIPAM depends strongly on the polarity of the grafted moieties: polyNIPAM with less polar moieties aggregates at lower temperature. The enhancement of the polymer aggregation at acidic pH is therefore due to the polarity decrease of the AN moieties by the proton- $\pi$  interaction.<sup>23</sup>

The mechanism of the temperature-driven switching of the pH-fluorescence intensity profiles is summarized in Scheme 2. At 15 °C, the polymer exists as a coil state at any pH, where the on/off emission switching is just controlled by the protonation states of the benzylic amines; the emission intensity decreases with a pH increase, showing an ‘on-off’ pH-fluorescence intensity profile (Fig. 1b). In contrast, at 34 °C, strong polymer



**Scheme 2.** Proposed emission switching mechanism of poly(NIPAM-co-N\_AN) at (a) 15 and (b) 34 °C.

aggregation takes place at acidic pH, forming a huge polymer particle. Within the huge particle, the AN moieties scarcely absorb the incident light, thus resulting in very weak emission intensity. However, at neutral pH, the size of the polymer particle is relatively low, thus allowing the incident light absorption by the AN moieties and showing relatively strong emission intensity. This leads to an expression of ‘off–on–off’ profile (Fig. 1b).<sup>24</sup>

The ‘on–off’ and ‘off–on–off’ pH–fluorescence intensity profiles of poly(NIPAM-co-N\_AN) can be obtained by addition of either  $H^+$  or  $OH^-$  (Fig. S7<sup>14</sup>). This indicates that the polymer can change the protonation and aggregation states reversibly by pH. In addition, the pH–fluorescence intensity profiles are reversibly changed by temperature: the polymer, after the measurements at 34 °C, demonstrates ‘on–off’ profile at 15 °C (Fig. S7<sup>14</sup>), meaning that the aggregation states of the polymer are reversibly changed by temperature. Another notable feature of the polymer is the high reusability with a simple recovery process; heating the solution to 50 °C (at any pH) followed by centrifugation (5 min,  $2.5 \times 10^4$  rpm) affords >98% polymer recovery, and the recovered polymer shows similar emission response as does the virgin polymer.

In conclusion, we have synthesized a simple-structured copolymer, poly(NIPAM-co-N\_AN), behaving as a temperature-driven on/off fluorescent pH indicator. The unprecedented emission switching is driven by a heat-induced phase transition of the polymer by pH and temperature. The concept presented here, which controls the on/off emission switching by a simple copolymer and simple inputs (pH and temperature), may contribute to the design of more sophisticated fluorescent pH indicator and to the development of fluorescent signaling materials with multiple functions.

## Acknowledgments

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## Supplementary data

Supplementary data (materials, methods, and Figs. S1–S6) associated with this article can be found, in the online version, at [doi:10.1016/j.tetlet.2007.07.124](https://doi.org/10.1016/j.tetlet.2007.07.124).

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23. As shown in [Figure S5](#),<sup>14</sup> poly(NIPAM-co-AN) (without benzylic amines) dissolved in aqueous solution of 15 °C shows a strong and constant fluorescence intensity at any pH. This is due to the lack of the benzylic amines (lack of the electron transfer quenching of the excited AN moieties). However, the fluorescence intensity at acidic pH decreases as a rise in temperature, showing an ‘off-on’ pH–fluorescence intensity profile. This is due to the strong polymer aggregation at acidic pH, as is also the case for poly(NIPAM-co-N<sub>2</sub>AN). This suggests that the proton– $\pi$  interaction between H<sup>+</sup> and AN moieties actually triggers the strong aggregation of poly(NIPAM-co-N<sub>2</sub>AN) at acidic pH and leads to the fluorescence intensity decrease at acidic pH associated with a rise in temperature.
24. The ‘off-on-off’ profile is expressed most clearly at 34 °C: as shown in [Figure S6](#),<sup>14</sup> the emission intensity of the poly(NIPAM-co-N<sub>2</sub>AN) solution monitored at pH 2 decreases with a rise in temperature and the decrease almost stops at >34 °C. Although the emission intensity monitored at pH 7 also decreases as the temperature rises, the intensity at 34 °C is still strong.