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Rhodamine-Based Fluorescent Thermometer Exhibiting Selective Emission Enhancement at a Specific Temperature Range

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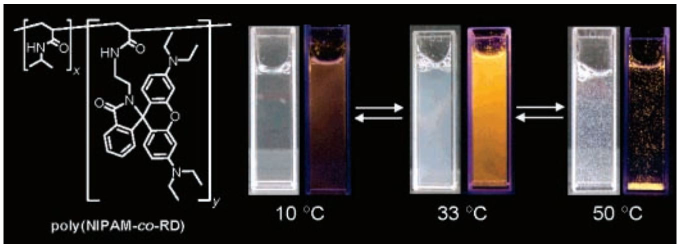
ABSTRACT



A simple copolymer, poly(NIPAM-*co*-RD), consisting of *N*-isopropylacrylamide (NIPAM) and rhodamine (RD) units, behaves as a fluorescent thermometer exhibiting selective emission enhancement at a specific temperature range in water.

Design of fluorescent chemosensors has attracted a great deal of attention because they allow a rapid detection of analyte in solution by simple emission enhancement or quenching without special instrumentation.[1] Various chemosensors detecting various types of analyte (pH,[2] cations,[3] anions,[4] gases,[5] and organic molecules[6]) have been proposed. Recently, fluorescent thermometers have also been studied extensively, since they allow simple monitoring of the solution temperature in terms of emission intensity. Various fluorescent thermometers have been proposed so far; however, they show a monotonous emission enhancement[7,8] or quenching[9] as temperature rises. To the best of our knowledge, there are no reports of fluorescent thermometers

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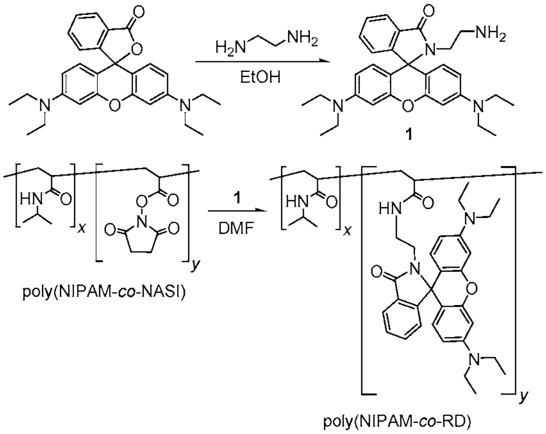
exhibiting selective emission enhancement at a specific temperature range.

Our system presented here is the first fluorescent thermometer exhibiting selective emission enhancement at a specific temperature range (25−35 °C). We use a simple copolymer, poly(NIPAM-*co*-RD), consisting of *N*-isopropylacrylamide (NIPAM) and rhodamine (RD) units 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 of the polymer chain by temperature.[10] PolyNIPAM is hydrophilic at low temperature (*coil*), but a rise in temperature leads to a formation of hydrophobic domain inside the polymer, along with an increase in the size of polymer particles (*globule*).[11] RD is a molecule extensively used as a fluorescent labeling reagent and a dye laser source due to its excellent properties, such as long-wavelength absorption and emission, high fluorescence quantum yield, and high stability against light.[12] It is well-known that the fluorescence quantum yield of RD increases with a hydrophobicity increase of the solution.[13] Our thermometer is based on the heat-induced self-assembly of the polymer, which cleverly controls the enhancement and quenching of the RD emission by heat-induced increases in the hydrophobicity and size of polymer particles. Various polymeric fluorescent thermometers have been proposed so far; however, none of the systems have achieved selective emission enhancement at a specific temperature range.[8]

Scheme 1. Synthesis of Poly(NIPAM$_x$-*co*-RD$_y$) ($x/y = 92/1$)[a]



[a] Each of the NIPAM and RD units within the polymer is randomly arranged along the chain.

The synthesis route of poly(NIPAM$_x$-*co*-RD$_y$) ($x/y = 92/1$) is depicted in Scheme 1. Reaction of an ethylenediamine-modified RD (**1**) and a copolymer, poly(NIPAM-*co*-NASI),[14] consisting of NIPAM and *N*-acryloxysuccinimide (NASI) in DMF affords a pink-white fluffy powder of poly(NIPAM-*co*-RD) ($M_n = 50694$; $M_w/M_n = 1.5$; Materials[15]). Absorption and emission spectra were measured on equipment with a digitally controlled heating/cooling cell holder and a sample stirring capability (Methods[15]). After stirring the solution for 30 min at the designated temperature, the measurements were carried out with continued stirring.

Figure 1 (inset) shows temperature-dependent change in emission spectra ($\lambda_{exc} = 530$ nm) of poly(NIPAM-*co*-RD) dissolved in an aqueous NaClO$_4$ (0.15 M) solution of pH 2, where the polymer amount is 0.2 g l^{-1} (containing 18.4 μM RD unit). A distinctive RD emission appears at 540−640 nm. The intensity of the emission monitored at 571 nm is plotted against temperature in Figure 1 (circle). The intensity at 10−25 °C is very weak but increases drastically at >25 °C with the strongest intensity at 33 °C. In contrast, at >33 °C, the intensity decreases drastically and becomes very weak at >40 °C, resulting in an *off*−*on*−*off* intensity profile against the temperature window. Figure 1 (square) shows the change in emission intensity of **1** (18.4 μM). Almost no emission enhancement is observed at any temperature, meaning that the emission enhancement and quenching of poly(NIPAM-*co*-RD) is due to the properties of the NIPAM units.

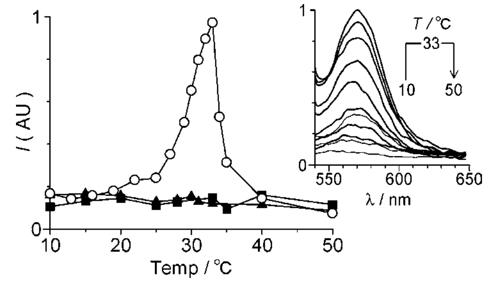


Figure 1. Temperature-dependent change in emission intensity ($\lambda_{exc} = 530$ nm; $\lambda_{em} = 571$ nm) of (circle) poly(NIPAM-*co*-RD) (0.2 g l^{-1} containing 18.4 μM RD unit), (square) **1** (18.4 μM), and (triangle) **1** (18.4 μM) with RD-free polyNIPAM (0.2 g l^{-1}) in an aqueous NaClO$_4$ (0.15 M) solution of pH 2. (Inset) Change in emission spectra of poly(NIPAM-*co*-RD). The measurements were done during heating sequence. The emission, absorption, and excitation spectra for the respective samples are shown in Figures S1−S3.[15]

The on/off emission response of the polymer is driven by a heat-induced phase transition of the polymer from *coil* to

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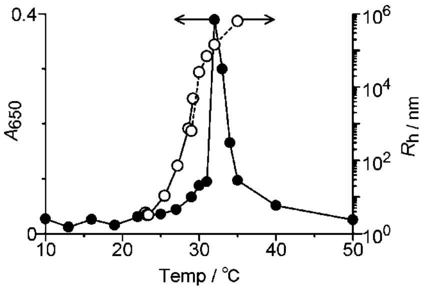


Figure 2. Temperature-dependent change in (black) turbidity (A_{650}) and (white) hydrodynamic radius (R_h) of poly(NIPAM-*co*-RD) in aqueous solution of pH 2. The measurements were done during the heating sequence. The R_h data for solid and dotted lines were obtained by dynamic and static (Mie) laser scattering methods, respectively. The R_h distribution data are shown in Figure S4.[15]

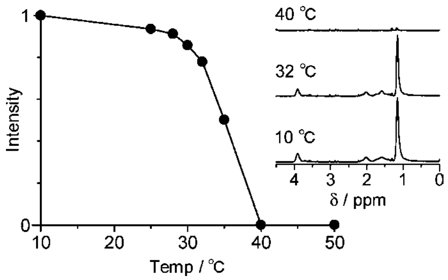
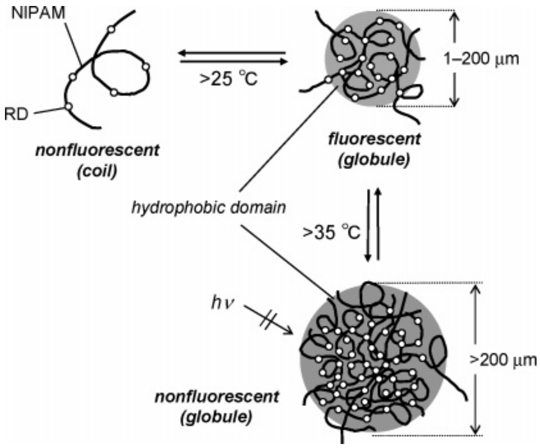


Figure 3. (Inset) Temperature-dependent change in ^{1}H NMR spectra of poly(NIPAM-*co*-RD) in D_2O. Change in the integrated proton intensity of C*H* resonances of the polymer chain and NIPAM unit as a function of temperature. The C*H* intensity of poly(NIPAM-*co*-RD) obtained in D_2O at 10 °C is set as 1.

globule. The emission enhancement at 25−33 °C is due to the formation of hydrophobic domain within the globule-state polymer. Figure 2 (black) shows change in turbidity (A_{650}) of an aqueous solution (pH 2) containing poly(NIPAM-*co*-RD). At 10−25 °C, turbidity is almost zero, indicating that the polymer exists as a coil state. At >25 °C, turbidity increases drastically, suggesting that the polymer aggregation (globule formation) occurs at >25 °C.[16] This turbidity increase is consistent with the emission intensity increase (Figure 1, circle), implying that the hydrophobic domain formed inside the polymer leads to an emission enhancement. This is confirmed by ^{1}H NMR analysis. Figure 3 (inset) shows change in ^{1}H NMR spectra of poly(NIPAM-*co*-RD) dissolved in D_2O (pH 2). Temperature-dependent change in the integrated proton intensity of the C*H* resonances of the polymer chain and the NIPAM unit is also shown in the figure. The gradual decrease of the proton intensity at 10−25 °C indicates that the hydrophobicity of the domain within the polymer increase with a rise in temperature.[11,17] At >25 °C, the intensity decrease is more apparent, which is consistent with the drastic emission enhancement at >25 °C (Figure 1). These findings clearly suggest that the hydrophobicity increase of the domain within the polymer enhances the emission intensity at 25−33 °C (Scheme 2).

Scheme 2. Schematic Representation of the Temperature-Dependent Changes in the Structure of Poly(NIPAM-*co*-RD)



As shown in Figure 2 (white), light scattering analysis of the polymer solution detects scattered light at >23 °C (detection limit, 3 nm),[18] meaning that, at this temperature range, polymer particles form by aggregation. At 33 °C, the size of the polymer particles is estimated to be 143 μm.

As shown in Figure 3, the integrated proton intensity of the polymer decreases drastically at >33 °C. This means that the hydrophobicity within the polymer is much higher at >33 °C; however, the emission intensity at >33 °C decreases significantly (Figure 1). This is due to the decrease in incident light absorption of the RD unit by the strong polymer aggregation. As shown in Figure 2 (black), turbidity of the polymer solution decreases at >33 °C. Accordingly, the particle size of the polymer increases (white); the size at 35 °C reaches 630 μm. At this temperature range, formation of huge polymer particles is confirmed even with the naked eye (see graphical abstract picture). The turbidity decrease at >33 °C (Figure 3, black) is therefore due to the strong interpolymer aggregation (formation of huge polymer particles), leading to the increase in solution transparency. Within the huge particles, the RD units scarcely absorb the incident light (Scheme 2), thus resulting in strong emission quenching.

It must be noted that copolymerization of the RD units with the NIPAM units is necessary for onset of the on/off emission response. Figure 1 (triangle) shows temperature-dependent change in emission intensity of **1** (18.4 μM) measured together with RB-free polyNIPAM (0.2 g l^{-1}; M_n

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= 56742).[19] Almost no emission appears at any temperature, as is also the case for **1** without polyNIPAM. This is because bulky RD molecule is scarcely encapsulated within the hydrophobic domain of the globule polymer.

It must also be noted that the on/off emission response of the polymer occurs only in acidic media. As shown in Figure 4, the emission intensity of the polymer measured at 33 °C decreases with a pH increase. This is because, as shown in inset, the RD moiety within the polymer exists as a nonfluorescent spirocyclic form at neutral−basic pH.[20] This indicates that the open-ring form of the RD unit is the emitting component and the polymer behaves as a fluorescent thermometer at acidic media (pH < 5).

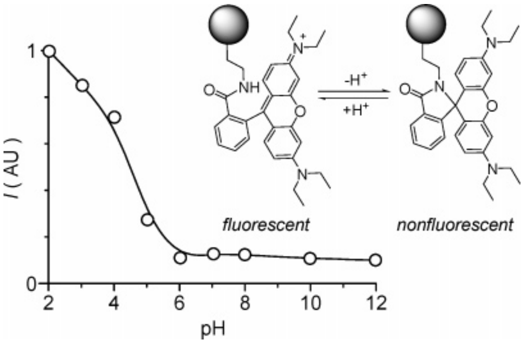

Figure 4. pH-dependent change in emission intensity (λ_{exc} = 530 nm; λ_{em} = 571 nm) of poly(NIPAM-*co*-RD) in aqueous solution.

The emission enhancement/quenching of the polymer occurs reversibly regardless of the heating or cooling process. Figure 5A shows change in emission intensity of the polymer, where the temperature is changed repeatedly; the data clearly show that the polymer can change the intensity *reversibly* by temperature. Another notable feature of the polymer is the high reusability with a simple recovery process; heating the solution to 50 °C followed by centrifugation (5 min, 2.5 × 10^4 rpm) affords >98% polymer recovery, and the recovered polymer (Figure 5B, black) shows similar emission response as does the virgin polymer (white).

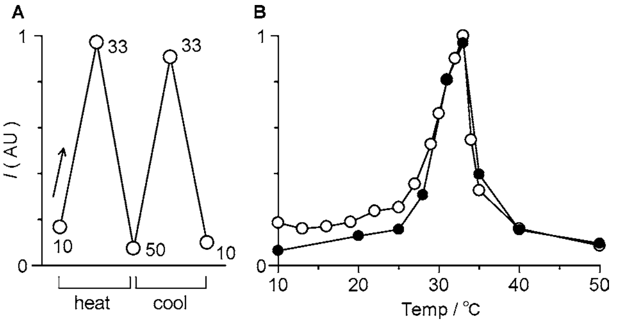

Figure 5. (A) Change in emission intensity (λ_{exc} = 530 nm; λ_{em} = 571 nm) of poly(NIPAM-*co*-RD) in aqueous solution (pH 2) during heating or cooling processes. (B) Temperature-dependent change in emission intensity of (white) virgin and (black) recovered polymers measured during heating sequence. The recovery process is heating the sample to 50 °C, followed by centrifugation (5 min, 2.5 × 10^4 rpm).

In summary, we have synthesized a simple-structured polymeric fluorescent thermometer, poly(NIPAM-*co*-RD), exhibiting selective emission enhancement at a specific temperature range. This property is driven by a heat-induced self-assembly of the polymer, controlling the inner hydrophobicity and incident light absorption by RD unit. The simple polymer design presented here may contribute to the development of more efficient fluorescent thermometers. It is well-known that the aggregation temperature of the polymer can be tuned by changing the chemical structure of the *N*-alkylacrylamide component.[21] The fluorescent thermometers acting at different temperature ranges may easily be synthesized by this strategy. The work is in progress in our laboratory.

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Supporting Information Available: Materials, methods, and supplementary data (Figures S1−S8). This material is available free of charge via the Internet at http://pubs.acs.org.

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[19] The temperature-dependent changes in turbidity and R_h of RD-free polyNIPAM are shown in Figure S5.[15]

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