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Thermoresponsive Fluorescent Sensor Based on Core/Shell Nanocomposite Composed of Gold Nanoparticles and Poly(*N*-isopropylacrylamide)

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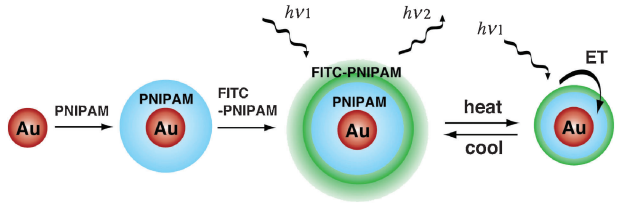
We developed a new thermometric fluorescent sensor based on core/shell nanocomposite composed of gold nanoparticles and thermoresponsive poly(*N*-isopropylacrylamide), which shows a reversible fluorescence change in response to a temperature change.

Detection of heat formation and transfer in a micrometer-sized small chamber such as a microreactor, microflow system, or a living cell is a critical issue to analyze the diverse chemical or biological events occurring inside them. To perform such thermometric analysis, a molecular nanodevise that can sense a slight change in temperature is needed. Despite the increasing requirements, there are few reports of thermometric devices available for this purpose.[1] We report herein the design of a new thermometric fluorescence sensor based on core/shell nanocomposite comprising gold nanoparticles (AuNPs) and poly(*N*-isopropylacrylamide) (PNIPAM), which shows a reversible fluorescence change in response to a temperature change.

PNIPAM is an important class of polymer because it undergoes a large-volume phase transition at around the lower critical solution temperature (LCST), typically in the range of 30–33 °C.[2] This behavior is accounted for a balance between hydrogen bonding and hydrophobic interaction. PNIPAM has a hydrophilic amide group and a hydrophobic isopropyl group. Below LCST, PNIPAM is in a swollen state since the chains are stabilized by the hydrogen bondings between the amide group and polar protic solvent such as water or alcohol.[2b,2c] Above LCST, the hydrogen bondings weaken and the hydrophobic interaction between the isopropyl groups instead becomes dominant, which induces a decrease of the polymer volume due to the collapse of the ordered PNIPAM chain array.

With biocompatible nature and unique optical properties, AuNPs are attractive scaffolds for PNIPAM-based thermoresponsive fluorescent sensors. It is well known that AuNPs show a distance-dependent energy transfer, which works effectively within 12 nm from their surfaces due to the phase-induced radiative rate suppression.[3] We envisioned that the assembly of the fluorophore-appended PNIPAM and AuNPs as core/shell nanocomposite would be a promising strategy to create a temperature-responsive fluorescence sensor (Scheme 1). In this system, the core/shell nanocomposite sensor emits strong fluorescence when the PNIPAM is in a swollen state below LCST. However, the emission would be quenched by AuNPs when the PNIPAM domain shrinks above LCST. These thermoresponse emission changes would provide a fluorescence sensor that could work in a reversible fashion.



Scheme 1. Thermoresponsive fluorescence sensor based on core/shell nanocomposite composed of gold nanoparticles (AuNPs) and poly(*N*-isopropylacrylamide) (PNIPAM).

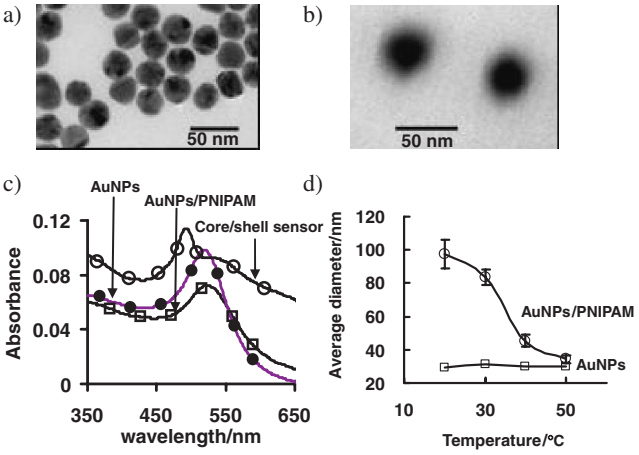


Figure 1. TEM images of the AuNPs (a) and AuNP/PNIPAM nanocomposite (b). (c) Visible absorption spectra of AuNPs (●), AuNP/PNIPAM nanocomposite before (□) and after the modification with FITC–PNIPAM (○). (d) Average diameters of the AuNPs (open squares) and AuNP/PNIPAM nanocomposite (open circles) at the different temperatures obtained by DLS analysis. The error bar indicates the standard deviation calculated from the measurement repeated 10 times.

AuNPs were prepared by a conventional technique using citrate anion as a reducing agent.[4,9] Transmission electron microscope (TEM) analysis revealed that the synthesized AuNPs were monodispersed in a narrow size distribution with average size of 28.5 ± 1.9 nm ($n = 50$) (Figure 1a). By dynamic light scattering (DLS) analysis, the average diameter of AuNPs was 29.8 ± 0.86 nm, in good agreement with the TEM data (Figure 1d). We selected the covalent "graft-to" strategy to coat AuNPs with HOOC–PNIPAM–SH.[5] A salt aggregation assay was performed to determine the requisite amount of HOOC–

PNIPAM–SH to cover the surface of AuNPs, according to the Singh and Lyon procedure.[6] Then, the freshly prepared AuNPs were mixed with a theoretical amount of thiol-terminated PNIPAM HOOC–PNIPAM–SH ($M_w \approx 32000$) in aqueous ethanol solution (EtOH:water = 3:1) to yield the AuNP/PNIPAM nanocomposite.[9]

Encapsulation of AuNPs with the PNIPAM was confirmed by TEM (Figure 1b), in which the PNIPAM phase was observed as the thin gray layer around the dark AuNP cores. In UV–vis spectroscopy, the absorbance due to the surface plasmon resonance of the AuNP core (λ_{max}) was slightly shifted from 519 to 524 nm after the treatment with PNIPAM (Figure 1c), indicative of the microenvironment change of the AuNPs by the encapsulation. In DLS analysis in an aqueous EtOH solution (EtOH:water = 3:1), the average diameters of the AuNP/PNIPAM nanocomposites were evaluated to be 97.4 ± 8.5 and 83.5 ± 4.5 nm at 20 and 30 °C, respectively. When increasing the temperature above LCST of PNIPAM, at 40 °C, the diameter decreased sharply to about 45.5 ± 3.4 at 40 °C and 34.4 ± 2.2 nm at 50 °C (Figure 1d). This temperature-dependent volume change is reasonably attributable to the shrinking behavior of the PNIPAM shell at LCST, typically around 32 °C.[1a,2]

The modification of the AuNP/PNIPAM nanocomposites with fluorescent FITC (fluorescein-5′-isothiocyanate) was conducted by layer-by-layer (LbL) technique (Scheme 1).[9] We employed the PNIPAM–FITC conjugate having a low average molecular weight ($M_w \approx 2000$) to facilitate its interpenetration to the PNIPAM phase in the LbL assembly. The formation of the nanocomposite sensor was confirmed by the UV measurement, in which the new peak (λ_{max}) at 492 nm due to the conjugated FITC was observed together with the peak of AuNPs at 524 nm[7] in an aqueous EtOH solution (EtOH:water = 3:1) (Figure 1c).

The thermoresponsive fluorescence properties of the nanocomposite sensor was evaluated in an aqueous EtOH solution (EtOH:water = 3:1).[8] When the nanocomposite was excited at 492 nm, a bright fluorescence emission at 514 nm ($\lambda_{em,max}$) was observed. This fluorescence gradually decreased when the solution temperature increased from 20 to 50 °C and decreased to ca. 30% of its initial value over this temperature range (Figure 2). It is noteworthy that the sharp fluorescence change was observed in the range from 30 to 40 °C with an inflection point at 34 °C, which is coincident with the LCST of PNIPAM. Such fluorescence change was not observed with the PNIPAM–FITC conjugate alone over this temperature range. These results strongly suggest that the thermoresponsive volume transition of the PNIPAM shell induces the temperature-dependent fluorescence change as the result of the quenching effect of the AuNPs above LCST. The reversibility of the fluorescence change was also examined. As shown in Figure 2b, the decreased fluorescence intensity at 50 °C gradually recovered with decreasing temperature to 20 °C, although the intensity at 20 °C after the reverse change was almost half of its initial value. This is probably due to the relatively large fluidity of the PNIPAM phase, which may prevent the complete recovery of the orientation of FITC in the PNIPAM phase after the swelling/deswelling processes.

In conclusion, we have successfully developed a thermoresponsive fluorescence sensor based on core/shell nanocomposite, which combines the temperature-dependent volume change

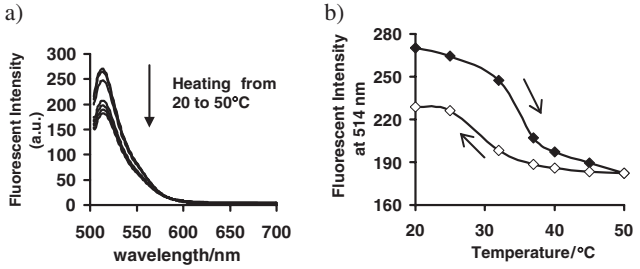


Figure 2. (a) Thermoresponsive fluorescence change of the core/shell nanocomposite sensor. $\lambda_{ex} = 492$ nm. (b) Fluorescence emission ($\lambda_{em,max} = 514$ nm) of the core/shell nanocomposite sensor upon increase (black dot) and decrease (white dot) temperature between 20 and 50 °C.

of PNIPAM and the distance-dependent fluorescence quenching of AuNPs. The sensor showed a reversible fluorescence change in the range from 20 to 50 °C, demonstrating the validity of our design strategy for the thermoresponsive core/shell nanocomposite sensor. Further improvement of the incomplete fluorescence reversibility and low water solubility of the present system would provide a more sophisticated sensor that could be applied to thermometric analysis of aqueous systems such as living cells. Our research is now in progress along this line.

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5 HOOC–PNIPAM–SH was commercially available from Polymersource Inc., Canada. The M_n of the polymer was about 32000 as determined from GPC.

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7 Some aggregation occurred after FITC-modification as observed by broad surface plasmon resonance of AuNPs.

8 In a complete water solution, the nanocomposite sensor formed an aggregation due to its low water solubility, which reduced the thermoresponsive fluorescence change in approx. 10% compared to that in aqueous EtOH solution.

9 See the Supporting Information for the experimental procedures in details. Supporting Information is available electronically on the CSJ-Journal Web site, http://www.csj.jp/journals/chem-lett/index.html.

