

Preparation of Reversible Colorimetric Temperature Nanosensors and Their Application in Quantitative Two-Dimensional Thermo-Imaging

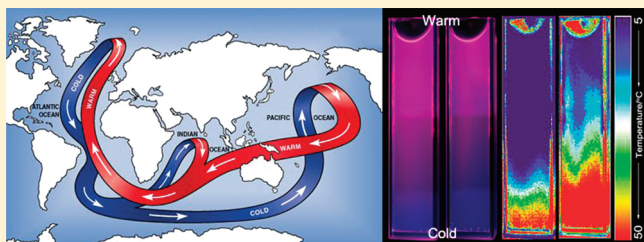
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S Supporting Information

ABSTRACT: Reversible colorimetric temperature nanosensors were prepared using a very simple precipitation method to encapsulate two color luminescent dyes. These nanosensors presented obvious reversible temperature response and enabled both rapid colorimetric temperature estimation using the eyes and quantitative two-dimensional thermo-imaging. Heat-exchange induced fluid motion was, for the first time, rapidly, precisely, and quantitatively imaged by just taking color pictures, and this presented good temporal and spatial resolution for studying heat-driven hydrodynamics. These nanosensors should have great application in micro/nanoscale research and also fabrication into films for macroscopic study.



The temperature sensor, as one of the earliest developed and most abundant devices (shared 75–80% of the world sensor market¹), has countless applications in daily usage: meteorology, aerodynamics, scientific research, industry, military technology, medical care, etc. As a result of safety and environmental contamination considerations, the uses of traditional liquid mercury- or kerosene-filled temperature sensors have been gradually reduced. Thermocouples and infrared thermometers have, thus, become the main products and dominate the mainstream of the temperature sensor market. However, these sensors are hard to miniaturize and unsuitable for temperature sensing on the micro/nanoscale.² Conversely, since they are easily miniaturized, noninvasive, accurate, and enable remote micro/nanoscale imaging, optical thermo-responsive sensors^{3–7} have recently attracted much attention. Several attempts have been made to prepare thermo-responsive micro/nanoparticles employing quantum dots,^{8,9} upconverting nanoparticles,¹⁰ rhodamine,¹¹ ruthenium complex,^{7,12} lanthanide complex,^{2,13–16} nanogel,¹⁷ and conjugated polymer.¹⁸ However, these sensors all suffer from cytotoxicity,¹⁹ irreversibility,¹⁸ cross-sensitivity to oxygen, or their large size.¹² More importantly, these sensors lack the ability for real-time quantitative imaging, which is, however, essential for monitoring in microfluidic research, microreactor reaction, and hyperthermal tumor treatment²⁰ and studying heat-driven hydrodynamics. Thus, achieving real-time quantitative imaging is a great challenge. Herein, we fabricated reversible colorimetric temperature nanosensors (CTNSs) by encapsulating two color luminescent dyes in gas-impermeable polymer nanoparticles.

These CTNSs present obvious and full reversible temperature response and enable both rapid colorimetric temperature estimation using the eyes and quantitative two-dimensional (2-D) thermo-imaging. Heat-exchange induced fluid motion was, for the first time, rapidly, precisely, and quantitatively imaged using a digital camera, which provided good temporal and spatial resolution.

EXPERIMENTAL SECTION

Materials. 4,4'-Bis(2-benzoxazolyl)stilbene, tris(benzoylacetato) mono(phenanthroline)europium(III), and poly(vinylidene chloride-co-acrylonitrile) [P(VDC-co-AN), $M_w \sim 150\,000$] were purchased from Sigma-Aldrich (www.sigmaaldrich.com). All reagents were analytical grade and used without further purification.

Preparation of CTNSs. The CTNSs were prepared following the precipitation method.²¹ Typically, 40 mg of P(VDC-co-AN), 0.5 mL of 4,4'-bis(2-benzoxazolyl)stilbene saturated acetone solution, and 4.0 mg of tris(benzoylacetato)mono(phenanthroline)europium(III) were dissolved in 20 mL of acetone. With rigorous stirring, 60 mL of double distilled water was poured into the solution, and the acetone was removed at reduced pressure. The prepared CTNSs were stored at 4 °C in the dark.

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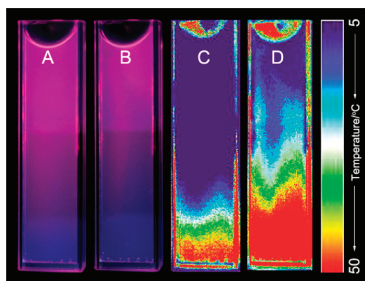


Figure 1. Temperature gradient and quantitative imaging of temperature using the photographic method. (B was filmed 60 s later than A; the concentration of the CTNSs was 0.67 mg/mL, and they were excited using a 365 nm UV lamp.)

Characterization of the Prepared CTNSs. Fluorescence profiles were obtained from a Hitachi F-4500 fluorometer (Hitachi Co. Ltd., Japan). A 365 nm UV lamp was used to excite the CTNSs. A Hitachi S-4800 scanning electron microscope (SEM, Hitachi, Japan,) was used to characterize the particle morphology. A Nikon D300 CMOS digital camera (Nikon Co. Ltd., Japan) was used for recording color images. Temperature was controlled using a Julabo F12-ED (Julabo Inc., Germany) refrigerated/heating circulator. Precise solution temperatures were calibrated using a thermocouple based temperature meter (Yudian AI-5600. <http://www.yudian.com/>).

RESULTS AND DISCUSSION

Figure 1 shows the real apparent (Figure 1A,B) and pseudocolor pictures (Figure 1C,D) of the CTNSs in a 2-D temperature gradient solution. The temperature gradient was created by putting a cuvette containing the thermo-sensitive CTNSs suspension onto two home-built hollow copper plates with circulated water for maintaining certain temperatures. One copper plate was set at 5 °C, and the other was at 50 °C. Because of the temperature difference between the two plates, the CTNS suspension presented obvious color distribution under the UV excitation selected, from red to blue, as shown in Figure 1A,B. Using the photographic readout which is recently introduced for quantitative imaging based on dual-color emission,²² the exact and precise temperature distribution was imaged and depicted in pseudocolor (Figure 1C,D). The heat-exchange induced fluid motion could be rapidly and quantitatively imaged by just taking color pictures using a digital camera, which presented good temporal and spatial resolution. Figure 1A,B was recorded at different times (Figure 1B was filmed 60 s later than Figure 1A), and they showed only a slight color-distribution difference, while, on applying the photographic readout,²² the pseudocolor pictures (Figure 1C,D) present more details and a remarkable difference. In Figure 1C, it seemed that the CTNSs suspension had just begun convection. After 60 s, the convection became more serious and deeper as shown in Figure 1D. It should be mentioned that the photographic readout can be used for analyzing not only separate pictures but also possibly movies, which is essential for real-time quantitative imaging.

This quantitative imaging relied on the CTNSs. They were prepared by simultaneously encapsulating a blue stilbene dye and a temperature sensitive dye in ~85 nm P(VDC-co-AN) nanoparticles, which are well dispersed in water. These CTNSs presented bright luminescence and vivid color under a 365 nm

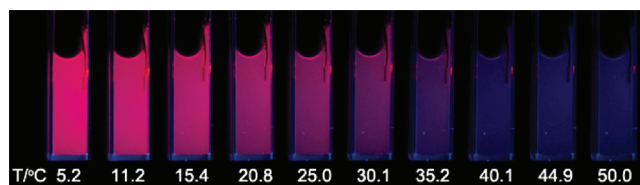


Figure 2. Apparent colors of the reversible CTNSs at different temperatures.

UV-lamp excitation. The color gradually changed from red to purple to blue with increasing temperature, as shown in Figure 2, and displayed distinguishable color changes. Using the eyes, the temperature detection range was 0–50 °C, which is very suitable for temperature sensing in the physiological range.

The apparent color change was induced by the dual-emission intensity change (Figure 3). The blue luminescent stilbene dye possesses a good thermo-stability, and its intensity remained constant (relative standard deviation, RSD = 0.78% at 412 nm) in the temperature range of 5–55 °C. However, the europium complex is very sensitive to temperature change both in dissolved form (Figure S1, Supporting Information) and inside polymer nanoparticles, and its red emission decreased gradually with increasing temperature. A mixture of the two colors at different intensity ratios produced new colors and resulted in the CTNSs presenting different distinguishable colors at various temperatures. Since most luminescent dyes present thermo-quenching, we further studied the thermo-responses of stilbene dye in ethanol and in polymer nanoparticles. The experimental results revealed that the stilbene dye dissolved in ethanol presented obvious thermo-quenching as shown in Figure S2, Supporting Information. The abnormal thermo-stability (5–55 °C) could be attributed to polymer encapsulation, which provided a good environment to firmly host the dyes in its net structure and prevented collision quenching. However, the thermo-induced nonradioactive transition, which was remarkable at high temperature, could not be avoided. Experimental results confirmed that the blue luminescence presented obvious thermo-quenching when the temperature exceeded 55 °C. It should be mentioned that the CTNSs would aggregate together if the solution was suddenly cooled after use at a temperature higher than 70 °C. By studying the excitation and emission spectra of the two dyes in acetone (Figure S3, Supporting Information), we found that both dyes could be effectively excited using the 365 nm UV lamp. In this sensing process, no additional optical filters were needed for colorimetric measurement, since the UV light was invisible and the dual emissions from the two dyes were visible to the eyes. Thus, temperature could be easily and directly distinguished using the eyes.

The prepared CTNSs could be used not only for rapid temperature estimation but also for quantitative measurement and imaging. The blue dye has no thermo-quenching in the temperature range of 5–55 °C, which acts as an internal reference signal for quantitative ratiometric temperature measurement. The europium complex was very sensitive to temperature change and, with increasing temperature, its luminescence intensity decreased gradually (Figure 3). The unique characteristics of the two dyes created self-referencing CTNSs, and system fluctuation and inaccuracy could be corrected. The CTNSs presented a good linear relationship between the luminescence intensity ratio and the temperature in the range of 5–50 °C (Figure 3 inset, and Figure S4, Supporting Information).

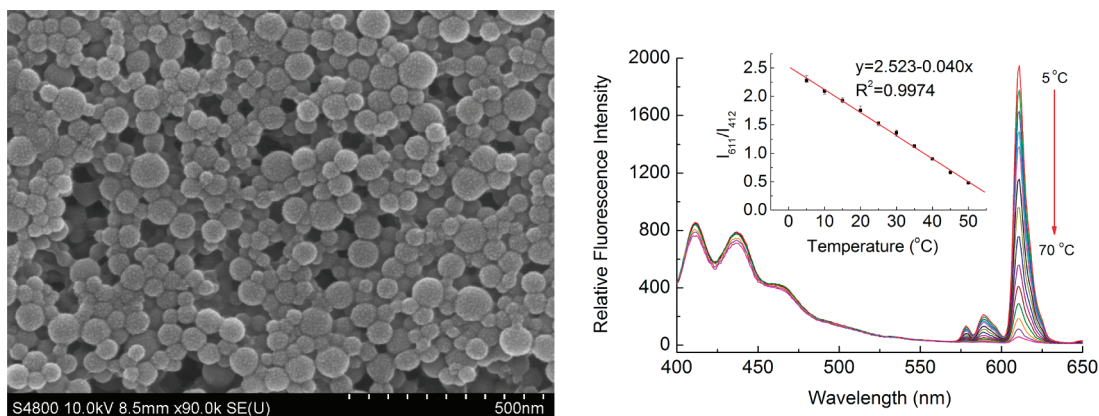


Figure 3. Scanning electron microscope images of the CTNS and fluorescence response of the prepared CTNSs at different temperatures (inset picture: linear relationship between fluorescence intensity ratio and temperature).

Furthermore, the emissions of the selected dyes totally matched the requirement for photographic readout.²² As discussed above, this approach will provide good temporal and spatial resolution for studying fluidic dynamics. Compared with high cost, time-consuming lifetime based measurement,^{14,16} the photographic readout presents obvious advantages in temporal resolution.

Results from dynamic light scattering (Figure S5, Supporting Information) and SEM (Figure 3) indicated that the prepared CTNSs had an average size around 85 nm with a relatively narrow size distribution. To verify that both dyes were encapsulated inside the P(VDC-co-AN) polymer nanoparticles, the prepared CTNSs were isolated from water using centrifugation at 10 000 rpm for 10 min. Under UV excitation, the gathered polymer nanoparticles presented bright luminescence; however, the supernatant had no fluorescence (Figure S6, Supporting Information). This result further proved that both the dyes were completely encapsulated inside the polymer nanoparticles. Since most current optical temperature sensors are cross-sensitive to oxygen, we studied the influence of oxygen on the performance of the CTNSs. The results revealed that the P(VDC-co-AN) polymer was a very good gas-blocking material, and there was no oxygen-quenching influence on the dual-emission at different oxygen concentrations (Figure S7, Supporting Information). For achieving continuous thermo-imaging, the reversibility of the prepared CTNSs is critical. We systematically studied this parameter at alternately 5 and 50 °C for 10 cycles, and the CTNSs presented excellent full reversibility (Figure S8, Supporting Information). The preparation of a thermo-responsive nanosensor was very simple and environmentally friendly. The prepared CTNSs presented good long-term stability while stored at 4 °C in the dark for 3 months, and no obvious aggregation or color change was found. Their convenience and fast quantitative imaging characteristics provide these CTNSs with great applications in micro/nanoscale research. CTNSs will be further developed for sensing temperature *in vivo*, since the current CTNSs could only be excited under UV light, which is unsuitable for biospecies and also generates strong tissue autofluorescence background. Our future research will focus on searching for new dyes that can be excited around the biological window.

CONCLUSIONS

In summary, reversible CTNSs were prepared using a very simple precipitation method to encapsulate two color luminescent

dyes. These CTNSs presented full reversible temperature responses and enabled rapid colorimetric temperature estimation. With the combination of photographic readout, quantitative two-dimensional thermo-imaging was successfully achieved, which provided good temporal and spatial resolution. Heat-exchange induced fluid motion was employed as a model system in order to prove the ability of quantitative two-dimensional thermo-imaging, which was rapidly, precisely, and quantitatively imaged by taking color pictures using a digital camera. On the basis of our research, these CTNSs should have great application in micro/nanoscale research and, also, for fabrication into films for macroscopic study.

ASSOCIATED CONTENT

S Supporting Information. Additional information as noted in text. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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