In Situ Thermal Imaging and Absolute Temperature Monitoring by Luminescent Diphenylalanine Nanotubes

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ABSTRACT: The temperature sensing capability of diphenylalanine nanotubes is investigated. The materials can detect local rapid temperature changes and measure the absolute temperature in situ with a precision of 1 °C by monitoring the temperature-dependent photoluminescence (PL) intensity and lifetime, respectively. The PL lifetime is independent of ion concentrations in the medium as well as pH in the physiological range. This biocompatible thermal sensing platform has immense potential in the in situ mapping of microenvironmental temperature fluctuations in biological systems for disease diagnosis and therapeutics.

INTRODUCTION

Precise monitoring of intracellular temperature is crucial to the study of cell activities.1−6 It has been reported that pathological cells have a higher temperature than normal cells due to their enhanced metabolic activity.2,7,8 In fact, the temperature of living cells is affected by events such as cell division, gene expression, enzymatic reactions, and metabolism,2 and so accurate knowledge of the local cellular temperature is important to disease diagnosis2,7,8 as well as therapeutic treatment.5,7 The photothermal method has been developed for cancer therapy5,9,10 but hyperthermal treatments may damage healthy organs and tissues without precise temperature monitoring.5 Hence, a technique to map the local temperature in a microbiological environment such as microcircuits11,12 and microfluids with the submicrometer scale is necessary.13−16

The development of a luminescent temperature sensing platform that is noninvasive, accurate, and able to resist strong electromagnetic fields with micrometer and nanometer spatial precision is of both scientific and technical importance.7,8,17 The mechanism is based on the temperature-dependent photoluminescence (PL) properties such as intensity,2,5,18,19 peak shape,20−26 polarization,3 spectral shift,27,28 and lifetime1,13,15,16,29−31 of materials such as organic dyes13,14,23, quantum dots,18,19,21,24,28 and rare-earth-doped materials.17,22,30−32 In spite of recent progress, these materials suffer from drawbacks and limitations,2,6,16 such as the inability to determine the absolute temperature, toxicity in biological systems, poor solubility, photobleaching, and so on.2,5,7,18,19 Furthermore, luminescent measurements may suffer from fluctuations due to variations in the local sensor concentration, thereby rendering the measurement of the absolute temperature inaccurate. Ratiometric luminescent temperature sensors based on Cd-related binary or ternary semiconductors (e.g., CdSe, CdS, and CdSeTe) can measure the absolute temperature.21,24,26 A core−shell structure is normally preferred to isolate and protect the toxic Cd-based core, but release of toxic heavy metal ions Cd stemming from the degradation of QDs is inevitable,7,8,17 thus undermining the biocompatibility.

Much effort has been made to explore peptide-based nanostructures in biomedicine and nanotechnology since they are biocompatible, modifiable, and relatively simple in structure.34,35 Diphenylalanine (FF), which constitutes the core recognition motif of Alzheimer’s β-amyloid peptide, is a suitable bottom-up building block,34,36,37 and nanostructures composed of FF have high stability against proteolytic attacks and can work under harsh chemical conditions such as extreme pH and organic solvents with different polarities.38,39 FF nanostructures have been applied to drug delivery systems,35 supercapacitor electrodes,40 probing of water molecules,36,37 and second harmonic generation.41 In this work, the temperature-dependent PL and time-resolved PL (TRPL) spectra acquired from FF nanostructures are studied in detail to assess their role as a precise temperature sensor. Both the PL intensity and lifetime show a strong dependence on the temperature, and the intensity variation provides a convenient means to detect rapid temperature changes in situ while the lifetime conveys the absolute temperature.

MATERIALS AND METHODS

Details of the sample preparation have been described elsewhere.42,43 The FF microtubes were produced in a homemade cylindrical chamber containing a fixed volume of water, and a clean slide was placed in the chamber. The fresh FF solution was prepared by
dissolving FF powders in 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) to a concentration of 40 mg mL$^{-1}$ by sonication (150 W, 40 kHz). Afterward, 30 mL of the FF solution was dripped on the slide for 60 min. The FF microtubes were then formed through a self-assembly process. The formation mechanism of microtubes and more structural characterization (X-ray diffraction, Raman scattering and differential scanning calorimeter) can be found in our previous work.$^{36,37,42,43}$ The FF nanotubes were obtained by cleaving the microtubes. The microtubes were immersed in ethanol for 24 h, mechanically agitated (Vortex Shaker, Keer), and then allowed to settle for several minutes. Since the surface of microtube is hydrophobic, the large microtubes quickly sink down, while the FF nanotubes suspend in ethanol due to their small size. The precipitates (big microtubes) were removed, and a supernatant containing the FF nanotubes was acquired. Finally, powders of the FF nanotubes were obtained after evaporating the ethanol.

The powders of FF nanotubes (4 mg) were redispersed in deionized water (2 mL) and encapsulated in a quartz cuvette. In the temperature-dependent PL measurements, the cuvette with the specimen was placed in a holder while water was pumped around the holder from an external circulating temperature bath. The PL and fluorescence lifetime were measured on an Edinburgh FLS-920 PL spectrometer. The excitation and monitoring emission wavelengths were 266 and 282 nm, respectively, and the instrumental response of the system was measured using a standard scattering. In the pH-dependent TRP measurements, the FF nanotubes were dispersed in PBS with different pH. The pH value was adjusted by the 0.1 M KH$_2$PO$_4$ and K$_2$HPO$_4$ solution and the K$^+$ concentration was 150 mM. The SEM images were acquired on a Hitachi S-3400N II scanning electron microscope (SEM).

## RESULTS AND DISCUSSION

The FF microtubes are synthesized using the protocols reported previously.$^{36,37,42,43}$ As shown in Figure 1a, the microtubes have lengths of up to several hundred micrometers and diameters of about 20 μm. The large size limits its application in microenvironmental temperature sensing, and in order to improve the spatial resolution and solubility, the microtubes are cleaved to form nanotubes by immersing them in ethanol under vigorous stirring. Afterward, the suspension can be separated into two parts: big microtubes with a step-like morphology (precipitates, Figure 1b) and small nanotubes (supernatant, Figure 1c) by stilling for several minutes. The lengths of the nanotubes depend on physical agitation and some short nanotubes can be obtained to have lengths less than 300 nm (the inset of Figure 1c). Our previous investigation has indicated that the PL peak positions and lifetimes mainly depend on the content of water molecules in nanochannels.$^{36}$ The size effect of the nanotubes is negligible. The dimensions of the nanotubes are below the thickness of microchips and size of microfluidic devices (hundreds of micrometers)$^{13-16}$ rendering them feasible in in situ thermal imaging. More importantly, common cancer cells have dimensions of several to dozens of micrometers, and these small fragments can serve as intracellular temperature sensors due to cellular uptake of nanostructures.$^{45,46}$

Figure 2 depicts the temperature-dependent PL and TRPL spectra of the FF nanotubes. As shown in Figure 2a, the PL intensity decreases dramatically as the suspension temperature is increased from 5 to 65 °C. By setting the intensity of the strongest PL peaks at the lowest temperature as 1, the intensity at a higher temperature exhibits a proportional trend. The circles in Figure 2c show the relationship between the PL intensity and temperature. In the physiological temperature range between 25 and 45 °C, the PL intensity decays by about 39.2%, corresponding to a decreases 1.96% per °C. Assuming that it is difficult to distinguish a 1% variation in the spectrum, the FF nanotubes are capable of detecting temperature changes with a sensitivity of about 0.5 °C, which is adequate in monitoring intracellular temperature variations in biological processes that require a temperature resolution of about 0.5 °C.$^{4,-6}$ and also competitive compared to other reported luminescent thermal sensors.$^{2,7,8,14,19,24,30,32}$ For example, the typical temperature changes in opto-fluidic devices caused by laser radiation are on the order of several degrees, and the cancer cell temperature increases by more than 10 °C under laser radiation during photothermal therapy.$^{5,6,9}$ Figure 2b shows that with increasing temperature, the PL decay becomes faster, and the PL lifetime also diminishes monotonically. The lifetime is obtained by fitting the TRP curves with a single exponential decay function. It should be mentioned that the current room-temperature PL lifetime is shorter than that reported previously (17.2 ns).$^{43}$ Previously, the time-resolved PL decay curve was obtained from the powder-like FF microtubes, while currently all the time-resolved PL decay curves were measured from the FF nanotubes dispersed in deionized water. The recombination of electron–hole pairs is easily affected by the polarization field,$^{42}$ and the polarity of the solvent may be responsible for the lifetime difference in different environments. The relationship between the PL lifetime and temperature is plotted in Figure 2c (squares), and it can also be fitted approximately by an exponential decay function as follows:

\[
\tau_i(T) = 15.17 \exp \left(-\frac{T}{43.94}\right) - 1.84
\]

According to ref 8, the ability of a luminescent system is usually gauged by the normalized lifetime thermal coefficient, $\alpha_T(T)$ defined as $\alpha_T(T) = \frac{d\tau_{\text{norm}}(T)/dT}{\tau_i(25^\circ C)}$, where $\tau_{\text{norm}}(T) = \tau(T)/\tau_i(25^\circ C)$ is the normalized PL lifetime at temperature $T$ relative to room temperature. As a result, $\alpha_T(T)$ of the FF nanotubes can be calculated as

\[
\alpha_T(T) = \frac{d\tau_{\text{norm}}(T)/dT}{0.0515 \exp \left(-\frac{T}{43.94}\right)} = 0.0515 \exp \left(-\frac{T}{43.94}\right)
\]

At 0 °C, $\alpha_T(T)$ is as large as 0.0515/°C. When the temperature is increased to 35 °C, $\alpha_T(T)$ is about 0.0232/°C, which is still a competitive value.$^{7,8}$ Although organic dyes embedded in polymers and layer double hydroxides exhibit higher relative sensitivity, they function in a narrower temperature range ($\sim$10 °C) than these luminescent nanomaterials$^2$ and also cannot cover the entire physiological temperature range (several dozen degrees) in photothermal therapy.$^{5,9,10}$ In addition to monitoring the local temperature...
changes by measuring the PL intensity, the absolute temperature can be determined by the PL lifetime, and so this sensing platform serves two important purposes simultaneously.

The relationships of the PL intensity and PL lifetime versus temperature are nearly the same (Figure 2c) because the intrinsic mechanisms of these two effects are similar. At a higher temperature, inelastic scattering of excitons by optical or acoustic phonons reduces the exciton lifetime, resulting in the corresponding reduction in the radiative state population and consequent emission.47 The temperature dependence of the experimental lifetime may be approximated by the Mott−Seitz model:7,17,18,29,31,32

$$\tau^{-1} = \tau_r^{-1} + k \exp(-\Delta E/k_B T)$$

(3)

where $\tau_r$ is the radiative lifetime, $k$ is the pre-exponential factor, $\Delta E$ is the energy gap between the emitting level (EL) and the higher excited-state level (HESL), and $k_B$ is Boltzmann’s constant. As shown in Figure 3, a high temperature activates the phonon-assisted process. The thermally assisted energy transfer process accelerates the nonradiative events, increases the net de-excitation probability of the emitting level, and eventually reduces the PL lifetime and intensity.7

The experimentally obtained lifetime values are not affected by the FF concentrations. As shown in Figure 2d, when the concentration of FF nanotubes is varied from ∼2 to 1 and 0.5 mgmL$^{-1}$, the three PL decay curves are almost identical. It has been reported that the blinking properties of fluorescent protein are sensitive to pH and buffer composition22 and therefore, a specific calibration curve must be obtained for each buffer.2,22 However, our experiments indicate (Figure 4a) that the fluorescence decay process inherent to FF nanotubes is almost independent of the environmental pH in the physiological range because the 282 nm emission peak stems from band-to-band recombination in the FF nanotubes, which have a band gap depending on the amount of water molecules in the nanochannels.36 Since the FF nanotubes are in an aqueous environment, no addition or loss of water takes place in the nanochannels, and the PL properties do not vary. This is a unique advantage of this sensing platform because the local pH in living cells is frequently affected by neighboring structures and can vary by as much as ±1.3 The total ion concentration in cytoplasm is 150 mM (K$^+$ is the most abundant), and the phosphate buffered saline (PBS) used here for the lifetime measurements also contains about 150 mM K$^+$. However, the lifetime derived from the PBS is almost the same as that determined from FF nanotubes dispersed in deionized water showing unambiguously that the lifetime at the same temperature is constant regardless of ionic concentrations. However, it should be noted that the PL decay curves obtained from FF nanotubes in PBS with a pH of less than 5.5 show slightly longer PL lifetime (Figure 4b), possibly due to protonation of oxygen-containing functional groups.48 The FF nanotube thermal sensing platform thus works best in the physiological pH range between 6.0 and 8.0.

The FF nanotubes are used to monitor random temperature variations controlled by an external circulating bath. The
temperature at six points of A-F is set randomly, and Figure 4c displays the PL decay curves at these six points. By fitting the lifetime $\tau$ and inserting $\tau$ to the PL lifetime versus temperature function, the temperature is determined and shown in Figure 4d. Compared to the temperature detected by a K-type thermocouple, the discrepancy is less than 1 °C. The temperature fluctuation caused by the thermocouple can be ignored since its size is quite small, and so this discrepancy can be attributed to two factors. The first one is that it takes a finite time to acquire the PL decay curve, and during this period of time, the temperature is not strictly constant. In comparison, the temperature measured by the thermocouple is instantaneous. The second factor is the sum of the normal errors of both a thermocouple (an error of at least 0.5 °C) and our sensing materials. All in all, the temperature accuracy of the FF nanotube-based platform should be better than 1 °C, which is also comparable to that reported previously.49

### CONCLUSION

An in situ thermal imaging and absolute temperature monitoring platform comprising bioinspired FF nanotubes is described. The absolute temperature can be determined from the PL lifetime, and rapid thermal fluctuations can be monitored by the PL intensity simultaneously. The temperature sensing capability is independent of ionic concentrations and environmental pH in the physiological range. Hence, accurate mapping of local temperature in microenvironments such as microcircuits and microfluids is possible by these biocompatible FF nanotubes, which offer excellent spatial resolution, sensitivity, and accuracy.

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**Notes**

The authors declare no competing financial interest.

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### REFERENCES
