Electronic Supplementary Material (ESI) for ChemComm.


# Supporting Information

# Lifetime-based nanothermometry *in vivo* with ultra-long-lived luminescence

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## 1. Materials and Methods

### Materials.

Palladium(II) 1,4,8,11,15,18,22,25-octabutoxypthalacyanine ($PdPc(OBu)_8$)[1] and PCU[2] were prepared by the methods described in the literature and confirmed by $^1H$ NMR spectroscopy. All other not mentioned starting materials and solvents were used as received without further purification from the following suppliers (Sigma-Aldrich Co. LLC, Fisher Scientific, Alfa Aesar, Sinopharm Chemical Reagent Co., Ltd., J&K Scientific). Deionized water was used in the experiments throughout.

### Instrumentation.

Fluorescence spectra and lifetime were recorded on a fluorescence spectrometer (Edinburgh, FS5 or FLS1000). For the measurement of emission spectra and lifetime of nanothermometer, a 730 nm semiconductor laser (Changchun fs-optics Co., China) was used as the excitation source. The nuclear magnetic resonance spectra of $^1H$ NMR (400 MHz) were recorded on a Bruker spectrometer (Bruker, AVANCE III HD). Dynamic light scattering was measured at 25 °C on a Zetasizer Nano ZS-90 analyser (Malvern Instrument Ltd, UK). TEM images were taken on a transmission electron microscopy (Hitachi Limited, HT7700 Exalens) operated at an acceleration voltage of 120 kV by dropping solution onto a carbon-coated copper grid.

### Synthesis of photooxidation product of PCU (PCU').

PCU (0.3 g, 1 mmol) and $PdPc(OBu)_8$ (0.1 mL, 1 mM in toluene) were added in toluene (10 mL). Oxygen was bubbled through the solution while irradiating with 730 nm light for 30 min. The solution was concentrated by evaporation under reduced pressure. The crude product was purified by silica-gel flash column chromatography to give PCU' (290 mg, 88%). $^1$ H NMR (400 MHz, $CDCl_3$) δ: 8.07-8.05 (m, 2H), 7.91-7.87 (m, 2H), 7.58-7.54 (m, 1H), 7.46-7.42 (m, 2H), 6.67-6.63 (m, 2H), 4.51 (t, $J$ = 4.51 Hz, 2H), 3.44 (t, $J$ = 6.59 Hz, 2H), 3.06 (s, 6H). $^{13}C$ NMR (100 MHz, $CDCl_3$) δ: 188.48, 166.35, 153.83, 133.01, 130.05, 129.73, 129.46, 128.37, 124.26, 110.63, 63.82, 40.07, 27.32. MS (Maldi-TOF): m/z calculated for 330.11 ($M+H^+$), Found 330.12.

### Preparation of UL-NCs.

Perylene, PCU and $PdPc(OBu)_8$ were dissolved in nujol to get stock A (concentrations of Perylene, PCU and $PdPc(OBu)_8$ were $5 \times 10^{-3}$ mol $L^{-1}$, $5 \times 10^{-3}$ mol $L^{-1}$ and $2 \times 10^{-5}$ mol $L^{-1}$, respectively), and BSA was dissolved in deionized water to get stock B (concentration of BSA was 5 mg $mL^{-1}$). Then, 2.5 mL stock A was added into 100 mL stock B. The mixture was pre-emulsified at 0 °C using an ultrasonic (Sonics VC750, Sonics & Materials, Inc.) for 5 min, and then was emulsified using a high pressure nano homogenizer machine (FB-110Q, LiTu Mechanical equipment Engineering Co., Ltd.) at 800 bars for 30

min. The emulsion was heated at 90 °C for 1 h. Then, the emulsion was filtrated with 0.8 μm membrane to obtain sterile emulsion. Finally, the uniform nanoparticles were obtained after gradient centrifugation.

**Preparation of UL-NCs-1**.

The preparation of UL-NCs-1 is the same as UL-NCs, except that the stock A is obtained by dissolving PCU and $PdPc(OBu)_8$ at in nujol (concentrations of PCU and $PdPc(OBu)_8$ were $5 \times 10^{-3}$ mol $L^{-1}$ and $2 \times 10^{-5}$ mol $L^{-1}$, respectively).

**Preparation of FL-NCs**.

The preparation of FL-NCs is the same as UL-NCs, except that the stock A is obtained by dissolving perylene at in nujol (concentrations of perylene were $1 \times 10^{-5}$ mol $L^{-1}$).

**Luminescence lifetime imaging and temperature detection *in vivo***.

The animal procedures were in accordance with the guidelines of the Institutional Animal Care and Use Committee, Fudan University. Luminescence lifetime imaging were performed with an *in vivo* imaging system, using 730 nm laser or LED as the excitation source, and an electron-multiplying charge-coupled device (EMCCD, Andor DU897, 512×512 pixels) or a camera (Canon, EOS 6D, 5472×3648 pixels) were used as the signal collector. The laser or LED pulse frequency was precisely controlled with a DDS signal generator and a relay (just for LED). The mice were anesthetized with chloral hydrate. After anesthesia, 50 μL or 20 μL of UL-NCs (5 mg $mL^{-1}$) was injection into the nude mouse or Kunming mouse. At 10 min post-injection, luminescence lifetime imaging was performed under irradiation at 730 nm (0.05 Hz pulse frequency, 2 s irradiation duration).

**Data analysis of luminescence lifetime and temperature imaging**

The Luminescence lifetime was fitted by the equation**:**

$$I_t = I_0 \times e^{-\frac{t}{\tau}} + C$$

$I_0$ is the luminescence intensity at $t = 0$ and $\tau$ is the lifetime what we need.

Data analysis of luminescence lifetime and temperature is completed by MATLAB or manual analysis after luminescence lifetime imaging in vivo.

MATLAB analysis:

Data analysis of luminescence lifetime and temperature based on MATLAB is carried out according to literature.[3]

Manual analysis:

Firstly, The luminescence area of the luminescence decay image is divided into several small areas (2×2 or 5×5 pixels when EDCCM is the signal collector, 20×20 or 50×50 pixels when the camera is the signal collector ), and the average luminescence intensity of each small area is read using software (Andor SOLIS).

Then, the luminescence intensity of the corresponding position in the series of luminescence decay image is fitted according to the above equation to obtain the luminescence lifetime of each small area, so that the luminescence lifetime and temperature distribution of the entire luminescence area in the mouse is obtained.

Finally, the obtained luminescence lifetime and temperature distribution image was overlay on the bright-field image to obtain luminescence lifetime and temperature image.

## 2. Supporting Tables and Figures

**Table S1**. Interference of experimental conditions on different thermometry methods[4, 5].

| | **Luminescence Intensity-based thermometry** | **Luminescence intensity ratiometric thermometry** | **Luminescence lifetime-based thermometry** |
|---|---|---|---|
| Thermometer concentration | yes | no | no |
| Illumination intensity | yes | partial | no |
| Light scattering and reflections | yes | partial | no |
| Color of sample | yes | partial | no |

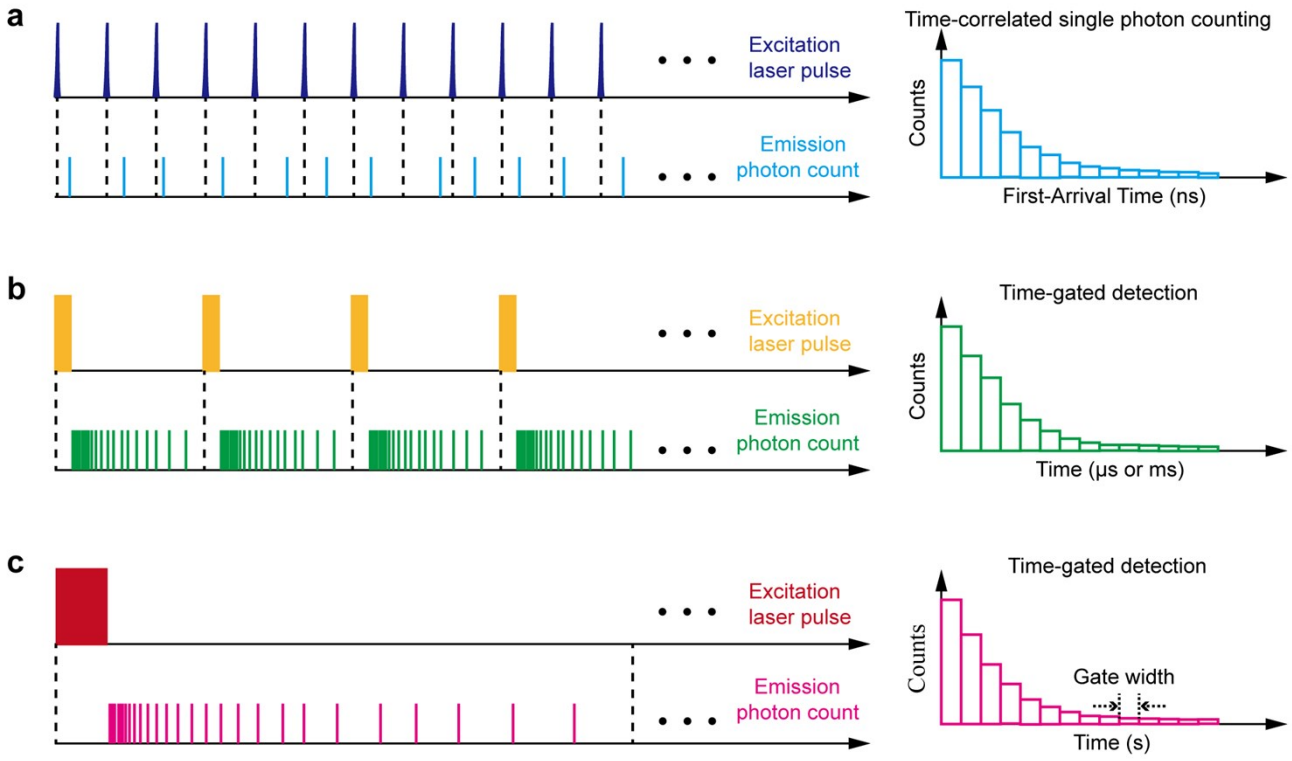


**Figure S1.** The scheme illustration the principle of luminescence lifetime measurement for different luminescence lifetime–based thermometers. (a) The luminescence lifetime measurement of the thermometers with nanosecond luminescence lifetime employs the time-correlated single photon counting (TCSPC) method, which relies on pulsed laser of ultra-high-frequency (10 MHz) as the excitation source over many cycles to obtain a single lifetime decay curve, is too slow to measure the luminescence decay lifetime in the microsecond, millisecond and second region. (b) Long-lived luminescence thermometers (μs-ms) for time-resolved imaging use the time-gated detection, their luminescence lifetime measurement by directly collecting and binning many emitted photons after a pulsed excitation. However, long-lived luminescence thermometers for time-resolved imaging require high-frequency pulsed laser as the excitation source and high-speed camera (Shutter speed in nanoseconds or microseconds) as the signal collector. (c) Ultra-long-lived (~ s) luminescence thermometers for time-resolved imaging also use the time-gated detection, which require relatively low instrumentation for time-resolved imaging requires, the typical imaging equipment (EMCCD, domestic camera) can be used as a signal collector, and the excitation source can be either a laser or an LED.

**Table S2.** Imaging experimental parameters of several typical luminescence lifetime-based thermometers.

| Luminescence lifetime of the thermometers | Luminescence lifetime measurement method | Pulse width | Pulse frequency | Signal collector | Gate width |
|---|---|---|---|---|---|
| ~ ns[6-8] | Time-correlated single photon counting | ~ ps | Ultrahigh (10 MHz) | Photomultiplier tube (PMT) | / |
| μs-ms[9] | Time-gated detection | ~ μs | High (1000 Hz) | Charge-coupled device (CCD) | 20 μs |
| ~ s (this work) | Time-gated detection | ~ s | Low (0.05 Hz) | Electron-multiplying Charge-coupled device (EMCCD) | 0.1 s |
| ~ s (this work) | Time-gated detection | ~ s | Low (0.05 Hz) | Consumer grade camera | 0.1 s |

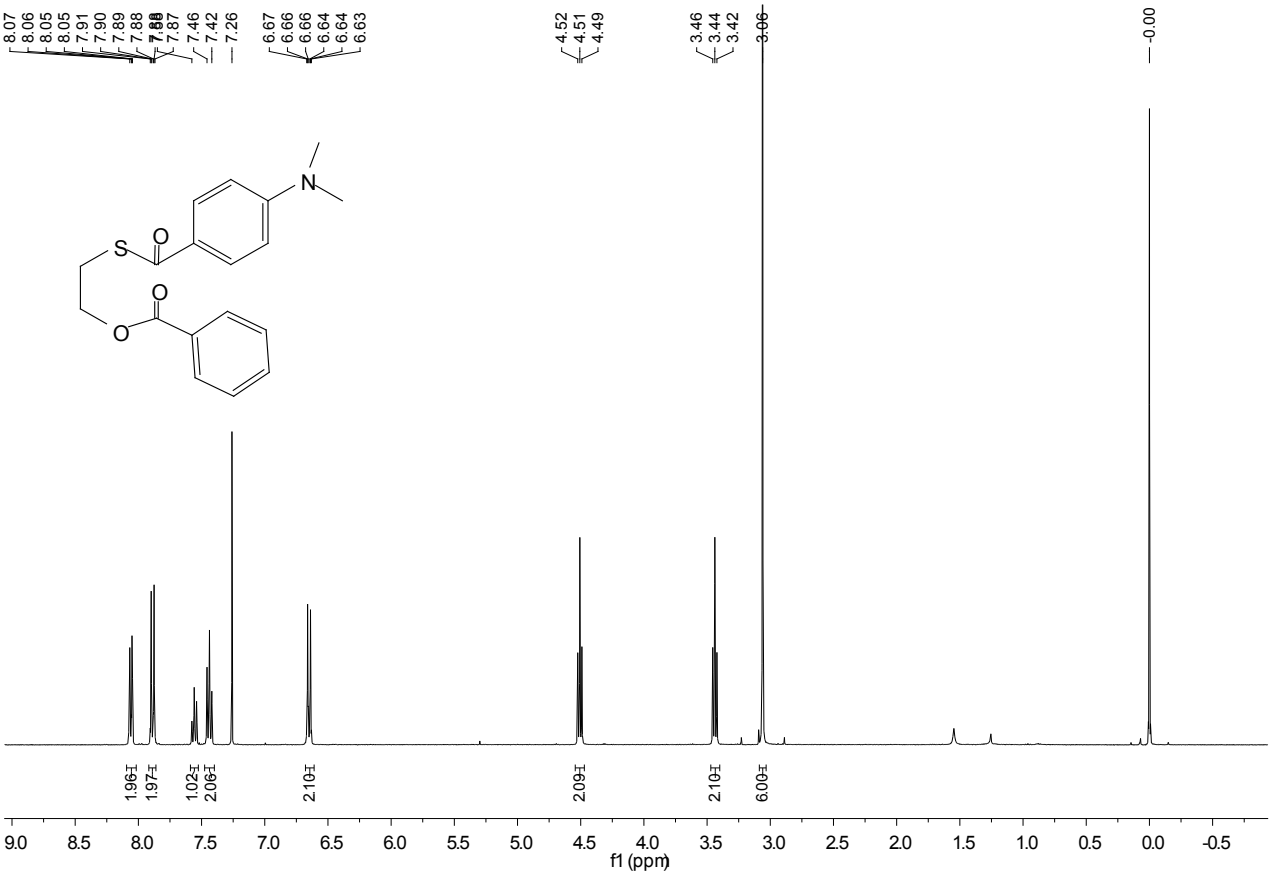


**Figure S2**. $^{1}H$ NMR spectra of PCU' in $CDCl_3$.

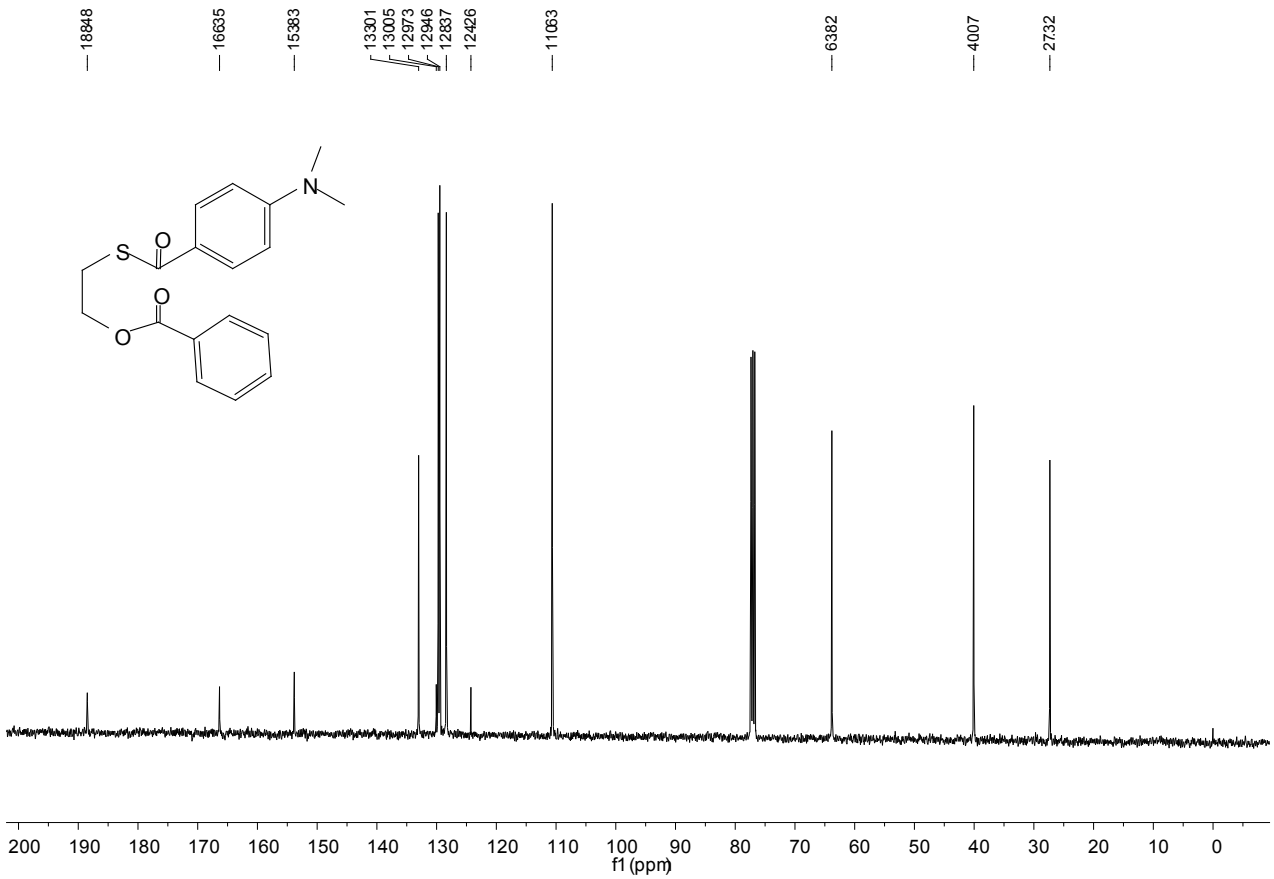


**Figure S3**. $^{13}C$ NMR spectra of PCU' in $CDCl_3$.

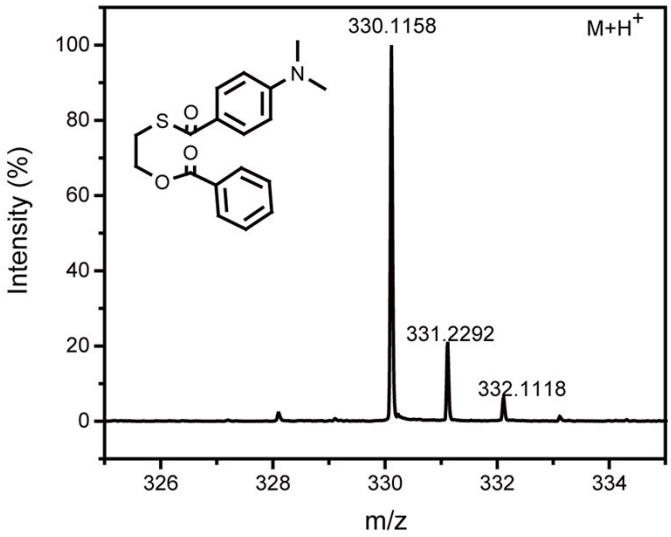


**Figure S4**. Mass spectra of PCU'.

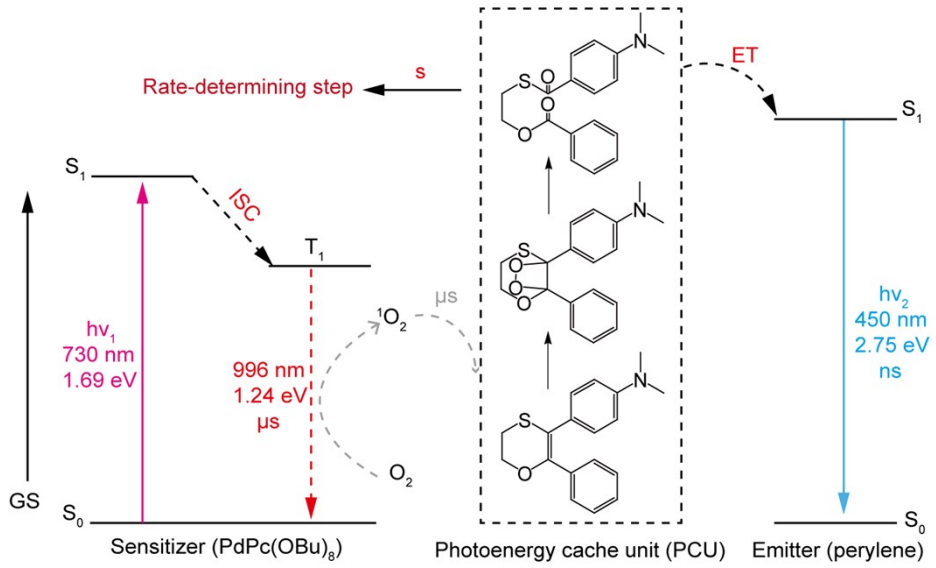


**Figure S5.** Schematic illustration of detailed photochemical energy cache and transfer processes leading to ultra-long-lived luminescence in nanothermometer. (1) The sensitizer ($PdPc(OBu)_8$) is excited to transition from the ground state ($S_0$) to the singlet-excited state ($S_1$), which can transform to the triplet-excited state ($T_1$) by intersystem crossing. This excited molecule at $T_1$ transfer the energy to oxygen to form singlet oxygen ($^1O_2$); (2) PCU reacts with $^1O_2$ to generate 1,2-dioxetane intermediates, which can store energy in chemical bonds; (3) then the intermediates slowly decompose to form PCU' and transfer energy to perylene; (4) at last, perylene releases the energy by emitting the photon.

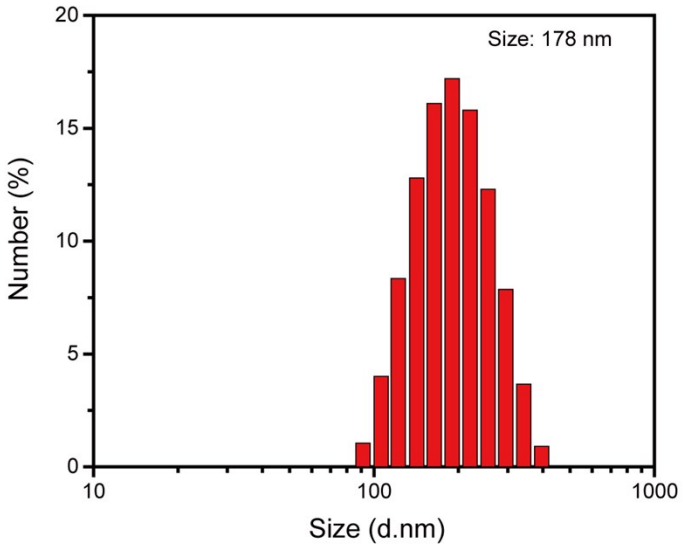


**Figure S6.** Dynamic light scattering (DLS) of UL-NCs.

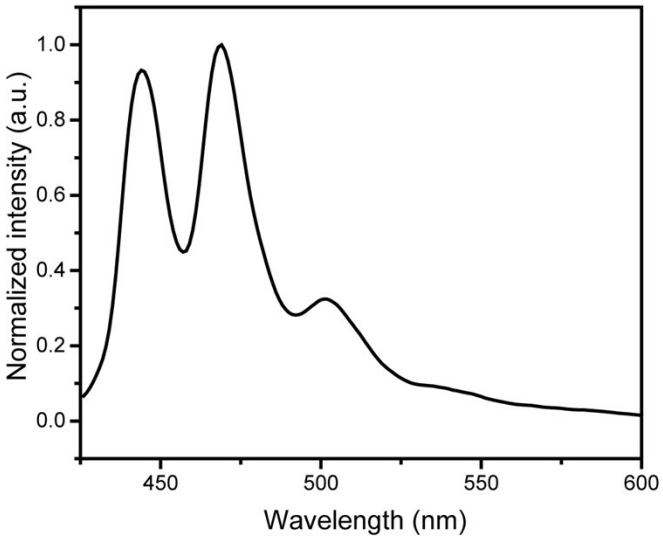


**Figure S7.** Fluorescence spectra of UL-NCs with the excitation of 375 nm.

**Table S3.** Luminescence lifetime of UL-NCs at 25.0 $^{o}$C under different fitting ranges by single-exponential fitting.

| Fitting range / s | 0-20 | 0-5 | 0-3 | 0.5-20 | 1-20 |
|---|---|---|---|---|---|
| Lifetime / s | 1.08 | 1.08 | 1.07 | 1.09 | 1.09 |
| Temperature/ $^{o}$C | 25.0 | 25.0 | 25.1 | 24.9 | 24.9 |
| $R^2$ | 0.9996 | 0.9995 | 0.9993 | 0.9996 | 0.9994 |

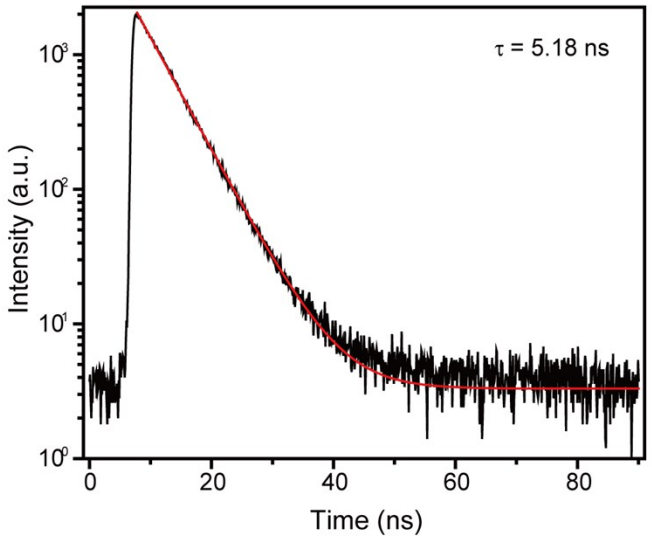


**Figure S8.** Fluorescence decay profile of perylene in fluorescence nanocapsules (FL-NCs) with the excitation of 375 nm.

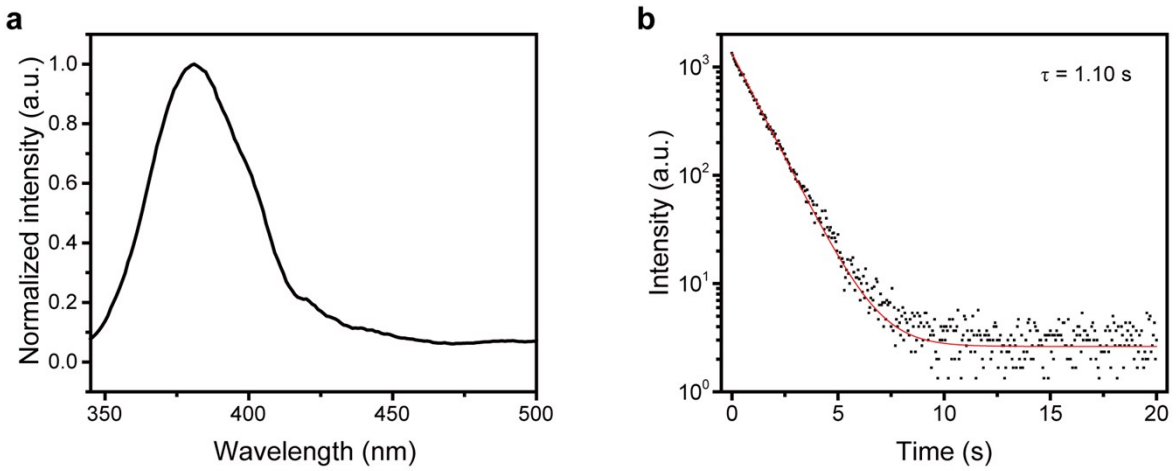


**Figure S9.** The luminescence spectra (a) decay profile (b) of UL-NCs-1 after irradiation with a 730 nm laser at 25.0 $^{o}$C.

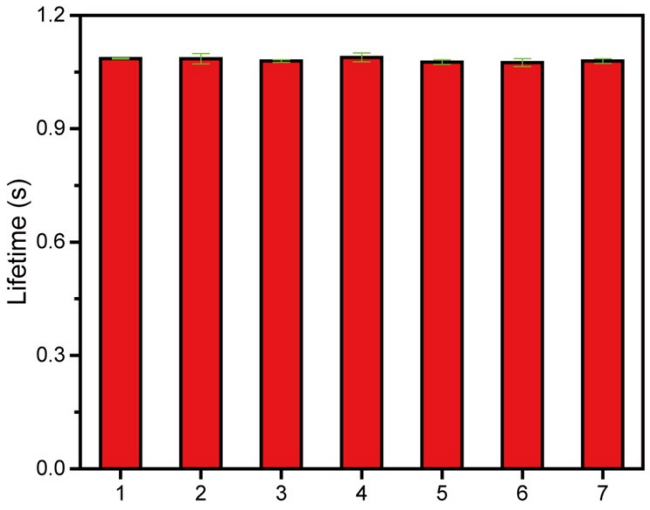


**Figure S10**. Luminescence lifetime of nanocapsules coated with different concentrations of $PdPc(OBu)_8$, PCU and perylene (concentration ratios of $PdPc(OBu)_8$, PCU and perylene**: 1**, **2** and **3** are 1:50:50, 1:25:25 and 1:10:10 with PCU and perylene fixed at $5 \times 10^{-3}$ mol $L^{-1}$ and $5 \times 10^{-3}$ mol $L^{-1}$; **4** and **5** are 1:10:25 and 1:50:25 with $PdPc(OBu)_8$ and perylene fixed at $2 \times 10^{-5}$ mol $L^{-1}$ and $5 \times 10^{-3}$ mol $L^{-1}$; **6** and **7** are 1:25:15 and 1:25:30 with $PdPc(OBu)_8$ and PCU fixed at $2 \times 10^{-5}$ mol $L^{-1}$ and $5 \times 10^{-3}$ mol $L^{-1}$) at 25.0 $^{o}$C with the irradiation of 730 nm (0.05 Hz pulse frequency, 2 s irradiation duration and 100 mW $cm^{-2}$ irradiation power density) (n = 3).

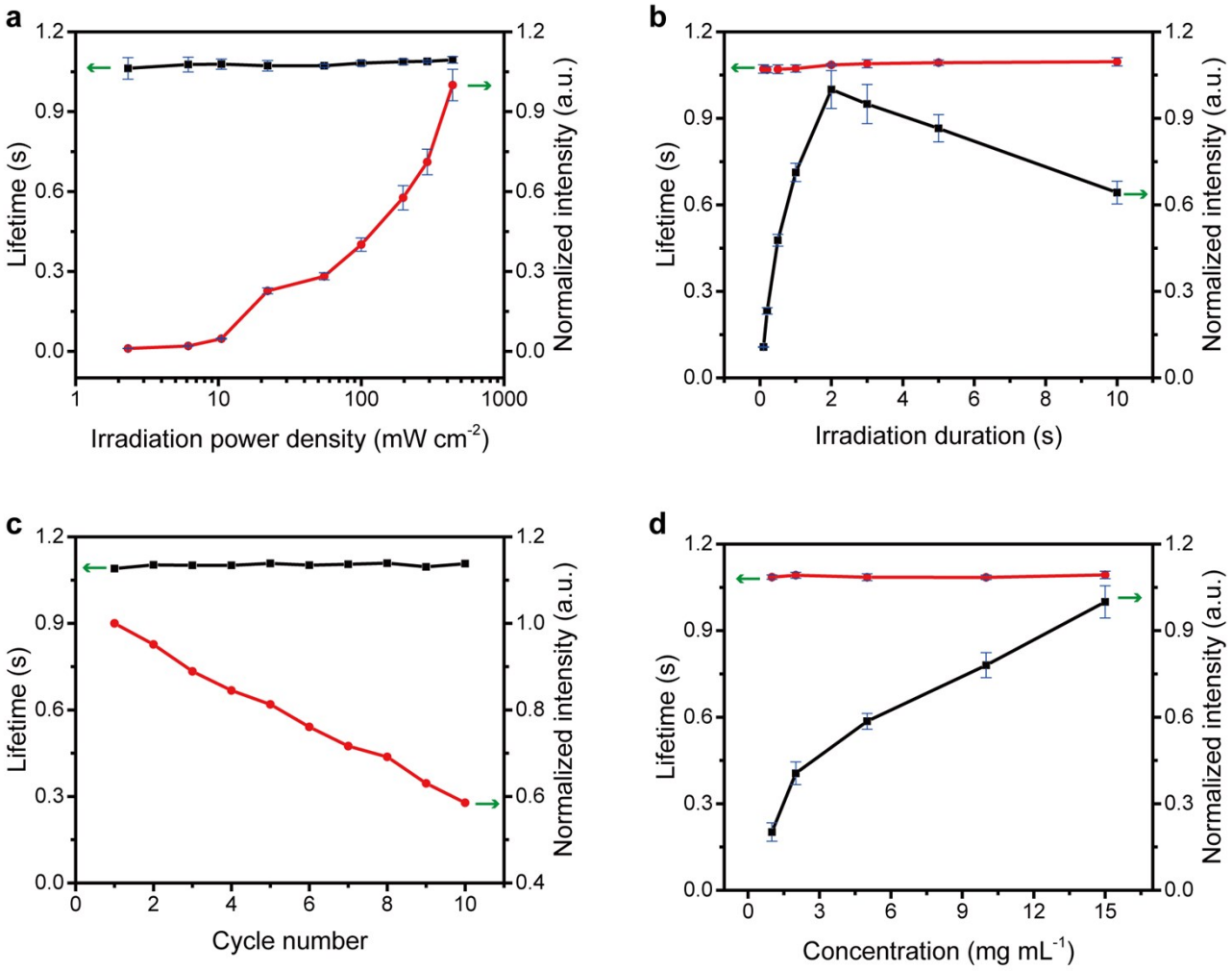


**Figure S11.** (a) Luminescence lifetime and intensity of UL-NCs (5 mg $mL^{-1}$) at 25.0 °C under different irradiation power density. (b) Luminescence lifetime and intensity of UL-NCs (5 mg $mL^{-1}$) at 25.0 °C under different irradiation duration. (c) Luminescence lifetime and intensity of UL-NCs (5 mg $mL^{-1}$) at 25.0 °C under different cycle number. (d) Luminescence lifetime and intensity of UL-NCs at 25.0 °C under different concentrations of UL-NCs. A 730 nm laser was used as the excitation source. The laser pulse frequency, irradiation duration and power density were 0.05 Hz (a, b, c, d), 2 s (a, c, d) and 100 mW $cm^{-2}$ (b, c, d). Error bars indicated standard deviations of three parallel measurements (n = 3).

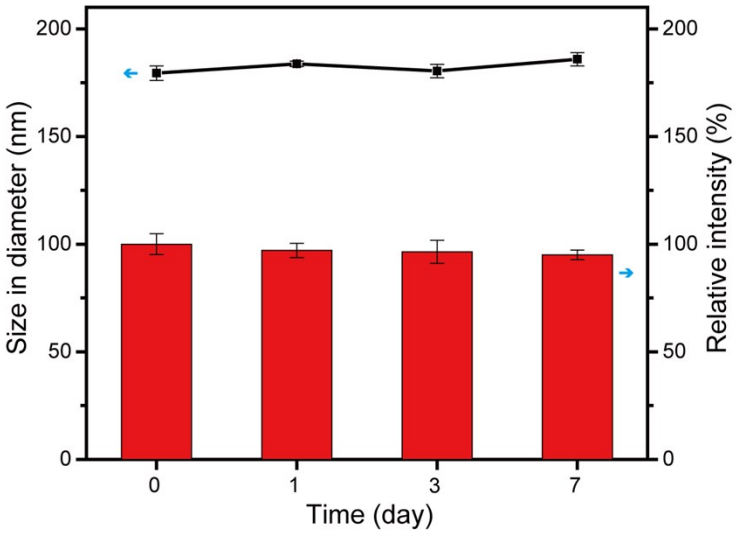


**Figure S12.** Particle size measured by DLS and fluorescence intensity of UL-NCs under different storage time duration in dark (n = 3).

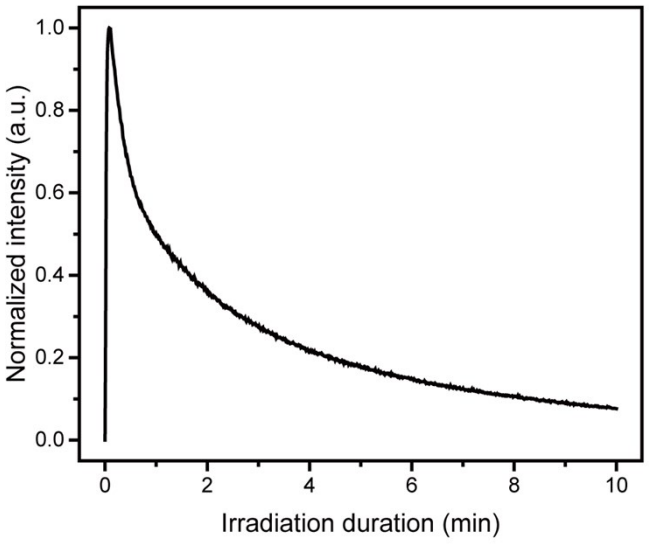


**Figure S13.** Luminescence intensity of UL-NCs (5 mg $mL^{-1}$) at 450 nm under different irradiation duration. A 730 nm laser (100 mW $cm^{-2}$) was used as the excitation source, and a 650 nm shortpass filter was used to eliminate the effects of light scattering.

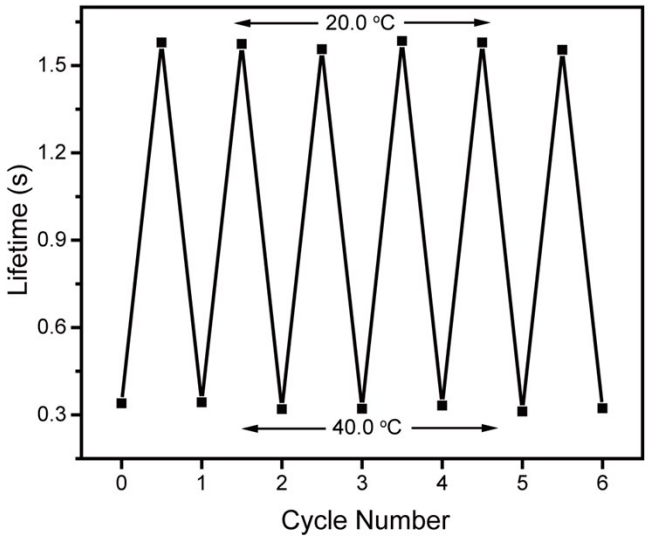


**Figure S14.** Luminescence lifetime of UL-NCs (5 mg $mL^{-1}$) at corresponding temperatures after repeated changes in temperature with the irradiation of 730 nm (0.05 Hz pulse frequency, 2 s irradiation duration and 100 mW $cm^{-2}$ irradiation power density) (n = 3).

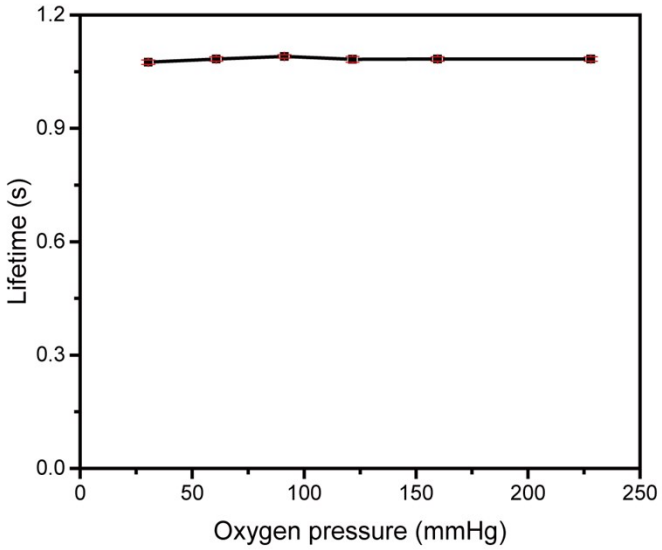


**Figure S15.** Luminescence lifetime of UL-NCs (5 mg $mL^{-1}$) at 25.0 $^{o}C$ under different concentrations of oxygen with the irradiation of 730 nm (0.05 Hz pulse frequency, 2 s irradiation duration and 100 mW $cm^{-2}$ irradiation power density) (n = 3).

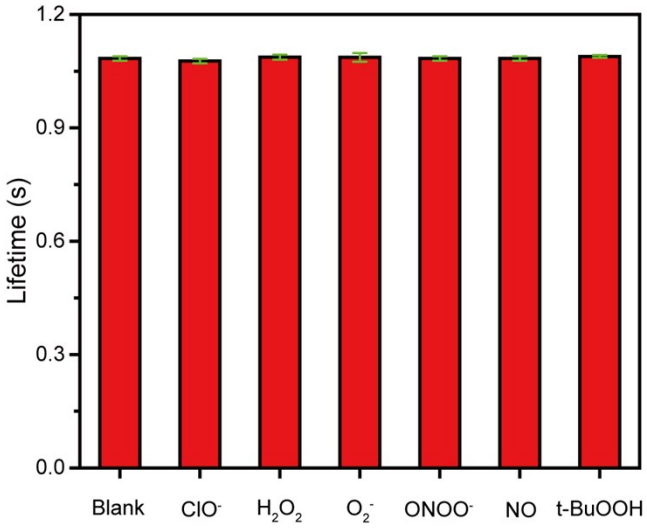


**Figure S16.** Luminescence lifetime of UL-NCs (5 mg $mL^{-1}$) at 25.0 $^{o}$C in the presence of various reactive oxygen species (50 μM) with the irradiation of 730 nm (0.05 Hz pulse frequency, 2 s irradiation duration and 100 mW $cm^{-2}$ irradiation power density) (n = 3).

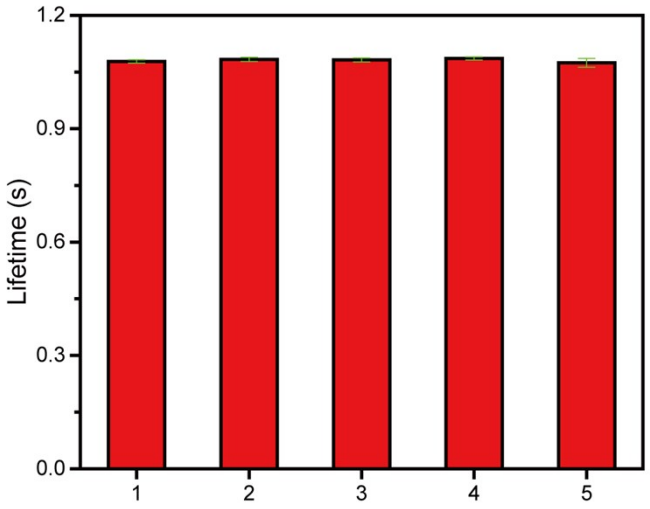


**Figure S17**. The luminescence lifetime of nanocapsules prepared in different batches under the same conditions at 25.0 $^{o}$C with the irradiation of 730 nm (0.05 Hz pulse frequency, 2 s irradiation duration and 100 mW cm$^{-2}$ irradiation power density) (n = 3).

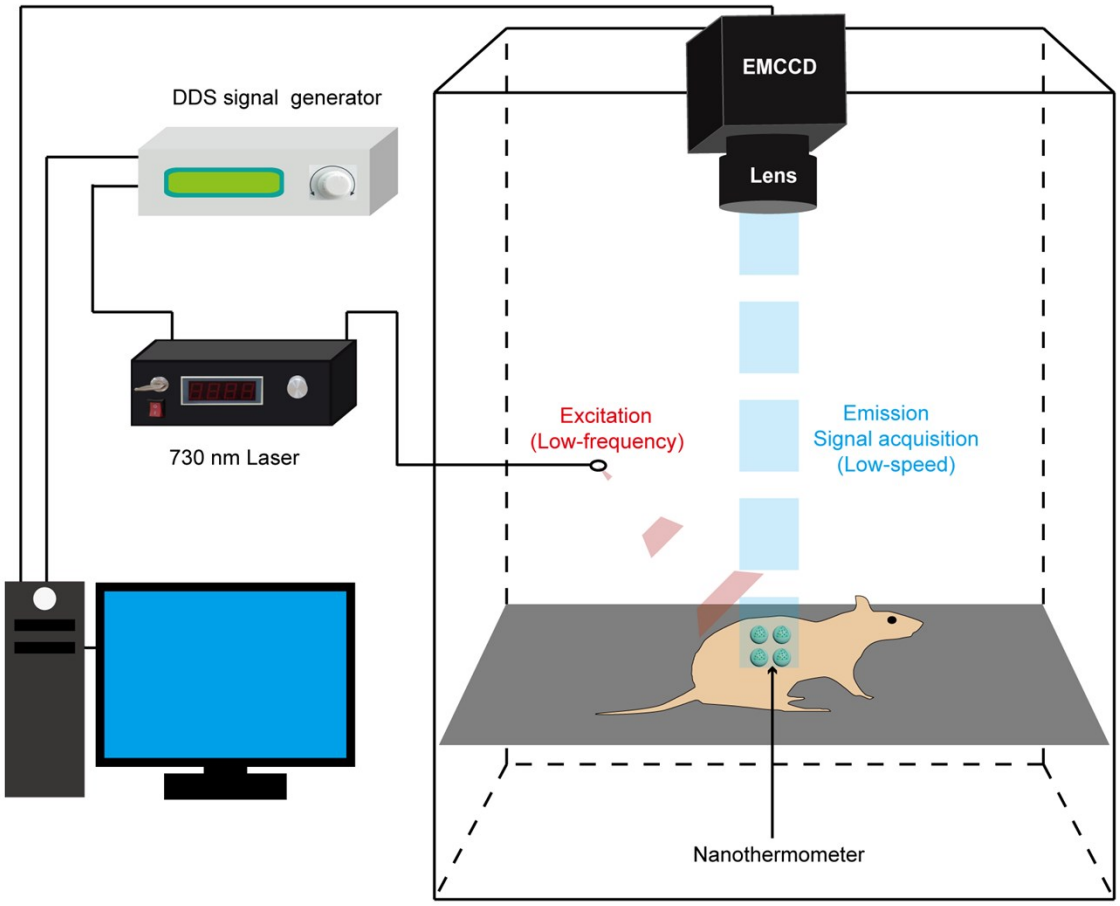


**Figure S18.** Schematic illustration of the setup for luminescence lifetime imaging of temperature *in vivo*. A laser at 730 nm was used as the excitation source. The laser pulse frequency was precisely controlled with a DDS signal generator. An Andor DU897 electron-multiplying charge-coupled device (EMCCD) as the signal collector.

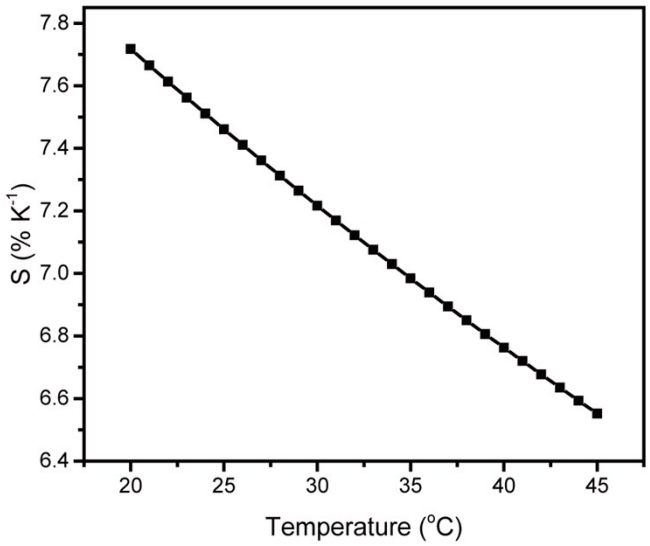


**Figure S19**. Thermal sensitivity curve of nanothermometer. A laser at 730 nm was used as the excitation source. An Andor DU897 EMCCD as the signal collector.

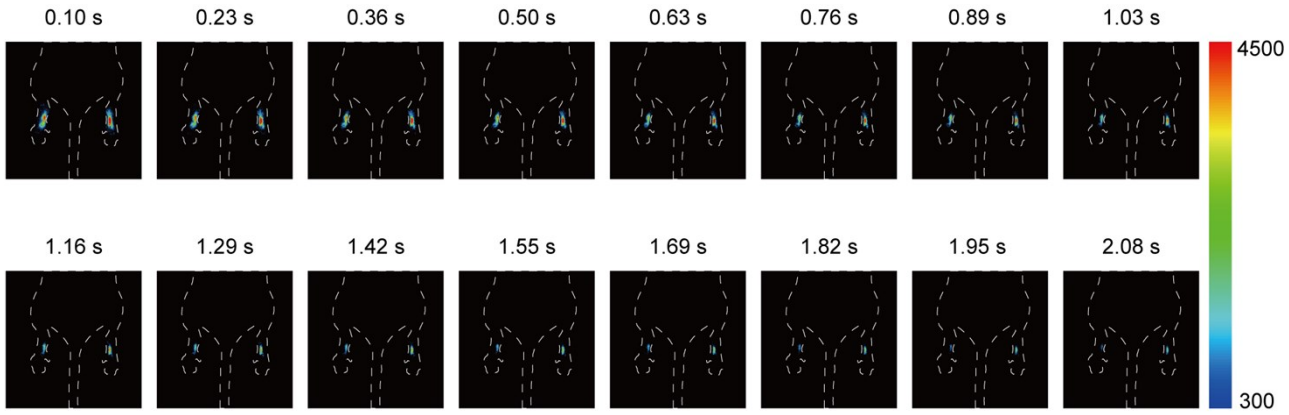


**Figure S20**. The luminescence decay image (exposure time, 0.1 s) of UL-NCs in an inflammatory mode after injection of carrageenan for 12 h (1 wt %, 100 μL) with a 730 nm laser (0.05 Hz pulse frequency, 2 s irradiation duration and 100 mW $cm^{-2}$ irradiation power density) as the excitation source and an EMCCD as the signal collector. The UL-NCs (20 μL of left and right legs, respectively) was injected into the Kunming mouse via the footpad. In the inflammation model, the left leg of a Kuming mouse was inflamed while the right leg as control was normal.

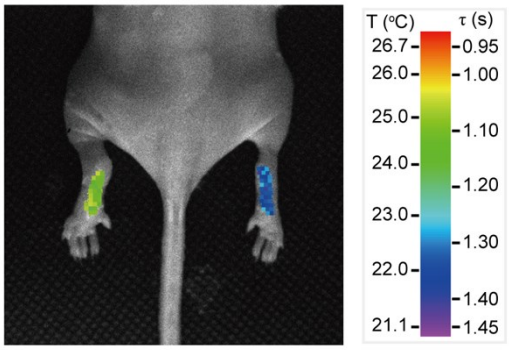


**Figure S21.** Luminescence lifetime-based thermometry based on UL-NCs in an inflammatory mode after injection of carrageenan for 12 h (1 wt %, 100 μL) with a 730 nm laser (0.05 Hz pulse frequency, 2 s irradiation duration and 100 mW $cm^{-2}$ irradiation power density) as the excitation source and an EMCCD as the signal collector. The data analysis of luminescence lifetime and temperature is completed by manual analysis.

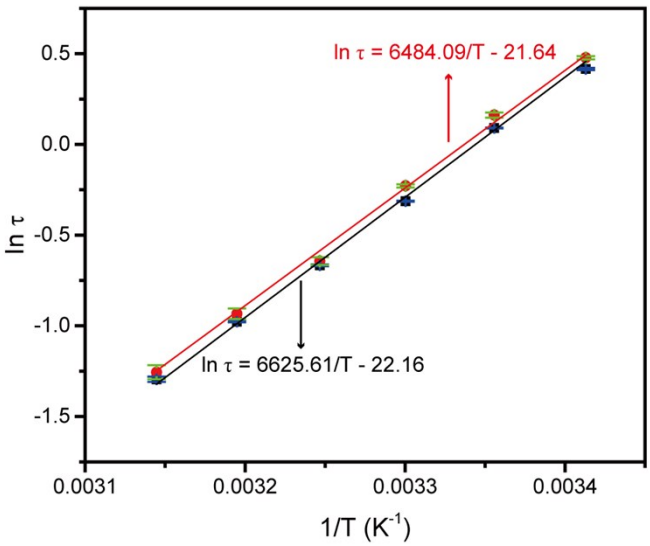


**Figure S22.** Comparison of two standard curves of *in vivo* imaging system (Black line, a 730 nm laser as the excitation source and an EMCCD as the signal collector) and low-budget luminescence lifetime imaging system (Red line, a 730 nm LED as the excited source and a camera (Canon, EOS 6D) as the signal collector). The temperature deviations from the two standard curves are not significant, for example, at a specific temperature of 25.0 $^{o}$C, the temperature obtained from standard curves fitting are 24.8 ± 0.3 $^{o}$C (EMCCD) and 24.9 ± 0.5 $^{o}$C (domestic camera), respectively.

## 3. Reference


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