Electronic Supplementary Material (ESI) for Analyst.

Supplementary Information

Micro-thermography in millimeter-scale animals by orally-dosed fluorescent nanoparticle thermosensors

Satoshi Arai,[1,6] Ferdinandus,[2,6] Shinji Takeoka,[1,3,4] Shin'ichi Ishiwata,[1,4,5] Hirotaka Sato,[2,7] and Madoka Suzuki[1,4,7]

1) Waseda Bioscience Research Institute in Singapore (WABIOS), SINGAPORE
2) School of Mechanical and Aerospace Engineering, Nanyang Technological University, SINGAPORE
3) Department of Life Science & Medical Bioscience, Graduate School of Advanced Science & Engineering, Waseda University, JAPAN
4) Organization for University Research Initiatives, Waseda University, JAPAN
5) Department of Physics, Faculty of Science and Engineering, Waseda University, JAPAN
6) Equal contributions
7) Corresponding authors

Table S1. Screening results using different polymers

	PMMA	PMMA-MA	PS	PS-MA	PViCl-PAN
Size (nm)	ND	110 ± 40	ND	124 ± 45	69 ± 39
Temperature sensitivity (%/°C)	ND	-3.6	ND	-4.0	-3.3

Temperature sensitivity is calculated as relative to 36 °C.

*ND means that no particle formation was detected.

Table S2. The temperature sensitivity of PS-MA particles with different ratios of EuDT to $Ir(ppy)_3$

	Entry1	Entry2	Entry3
EuDT (mg)	1.5	1.5	1.5
$Ir(ppy)_3$ (mg)	0.01	0.05	0.15
Temperature sensitivity (%/°C)	-4.0	-3.9	-3.1

					Microscopic observation day ↓						
Particle (μl)	Food (μl)	State	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
0	50	Larva	10	10	7	2					
		Pupa			1	5	4	4	3	3	3
		Fly							1	1	1
		Total (survival)	10	10	8	7	4	4	4	4	4
1	50	Larva	10	10	5	3					
		Pupa			3	4	7	6	6	6	4
		Fly									2
		Total (survival)	10	10	8	7	7	6	6	6	6
10	50	Larva	10	10	8	4					
		Pupa			2	3	6	6	4	3	2
		Fly							1	1	2
		Total (survival)	10	10	10	7	6	6	5	4	4
*Selected condition		Larva	10	7	6	5	1				
50	50	Pupa			1	2	5	6	3	3	3
		Fly							1	1	
		Total (survival)	10	7	7	7	6	6	4	4	3
100	50	Larva	10	2	1						
		Pupa			1	1	1	1	1	1	1
		Fly									
		Total (survival)	10	2	2	1	1	1	1	1	1

Table S3. The toxicity test of PS-RNT in larvae (laboratory wild-type *D. melanogaster* Canton Special (CS) strain larvae). They were fed with mixtures with different ratios of fly food (Nutri-Fly BF) to the suspension of PS-RNT for 8 days at room temperature. For the microscopic experiments, the mixture with fly food (50 μl) and the suspension (50 μl) was used. In the third day, imaging experiments were performed. The toxicity of PS-RNT was appeared to be negligible except the mixture with the ratio of 100 to 50 μl. The toxicity should be evaluated in each animal of user's interest.

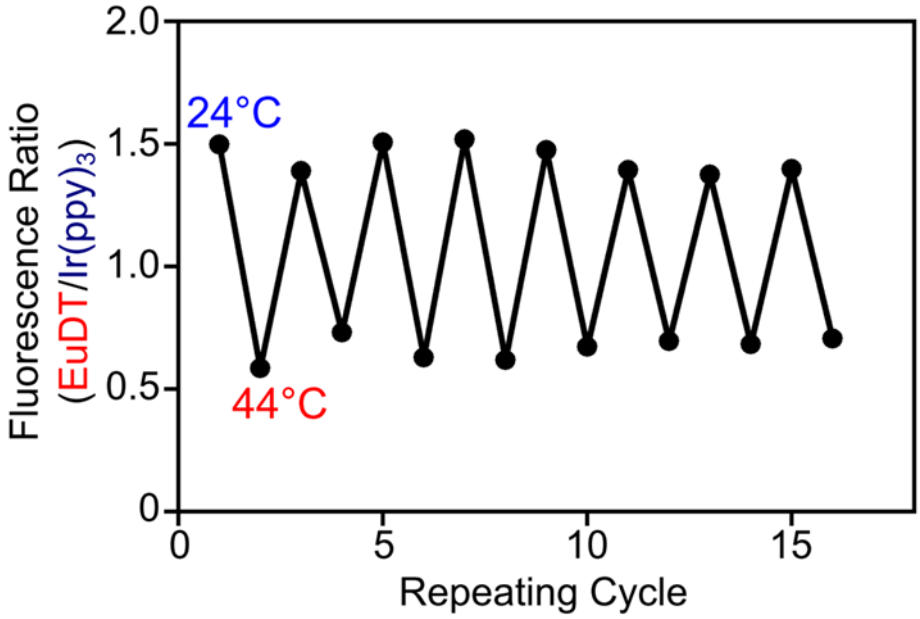

Figure S1. The reversibility test of PS-RNT in response to the change in temperature (24 and 44 °C). The excitation wavelength was 390 nm and the emission was recorded from 490 to 650 nm. The fluorescence ratio (I_{615}/I_{506}) was plotted versus number of measurement.

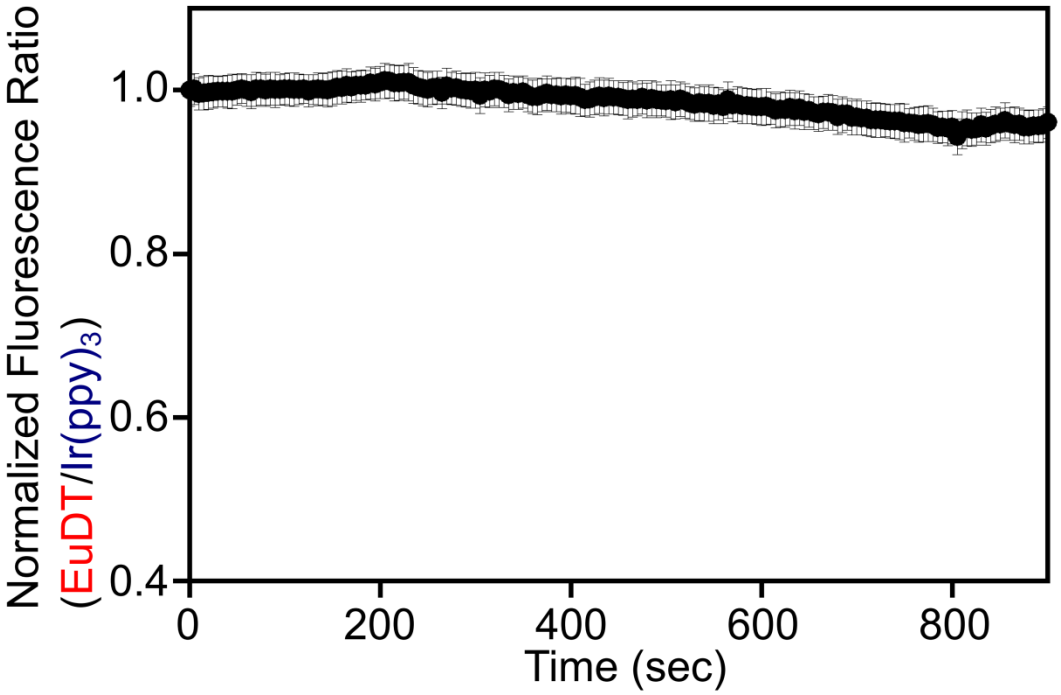

Figure S2. The photobleaching time-course of PS-RNT on the glass substrate at 24 °C. Bright dots of PS-RNT on the glass substrate were observed under a fluorescence stereo microscope (as can be seen in Fig. 2a). The normalized fluorescence ratio of PS-RNT was calculated from the average of ROIs covering a single dot (n = 20) and plotted versus time. Error bar, SD. The image was taken at every 5 sec. There was no notable photobleaching.

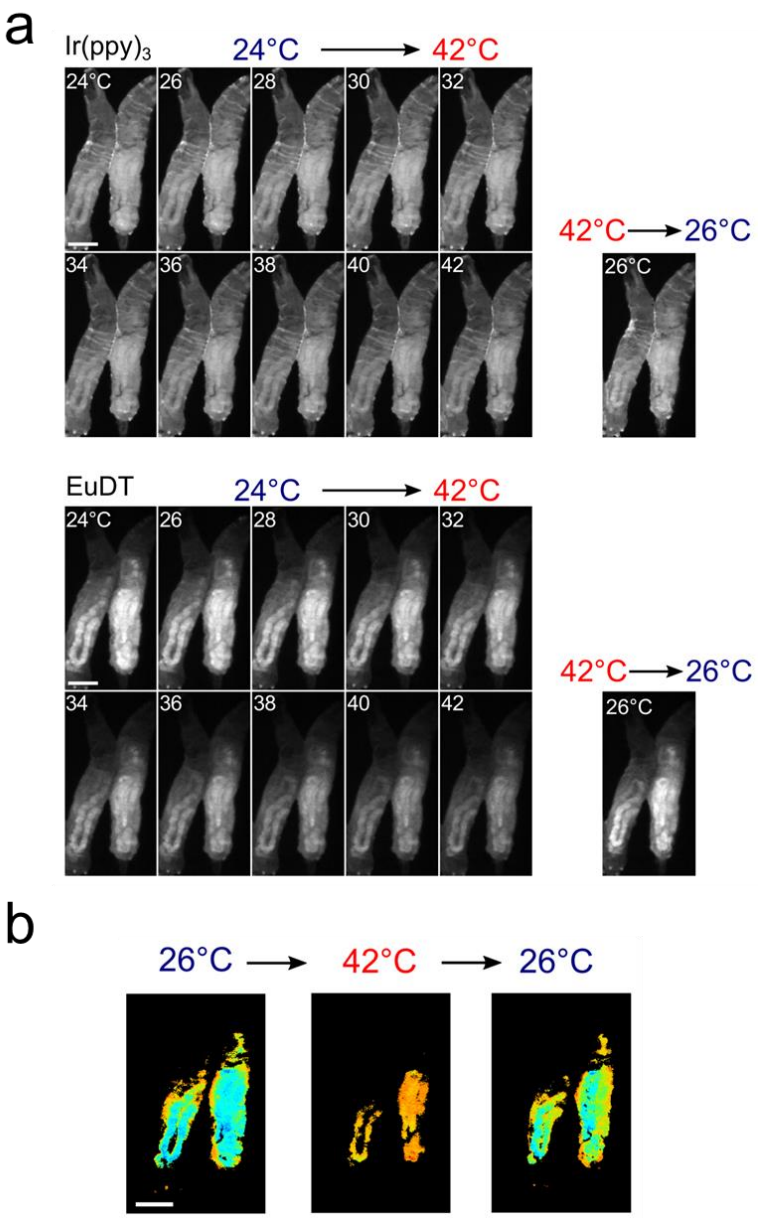

Figure S3. Fluorescence images in the fluorescence micro-thermography of the larvae which were orally dosed with PS-RNTs. These data were relevant to Fig.3. a) As temperature was increased from 24 to 42 °C, the fluorescence intensity in EuDT channel (lower panels) decreased, while that in $Ir(ppy)_3$ channel decreased much less (upper panel). b) Reversibility was tested in cooling process. The shape of the larva changed slightly upon heating and cooling processes, but the ratio value returned to the basal level at 26 °C before heating. In cooling, it was difficult to control the temperature at 24 °C that was close to the room temperature. Thus, the image at 26 °C was used as a reference. Scale bar, 500 μm.

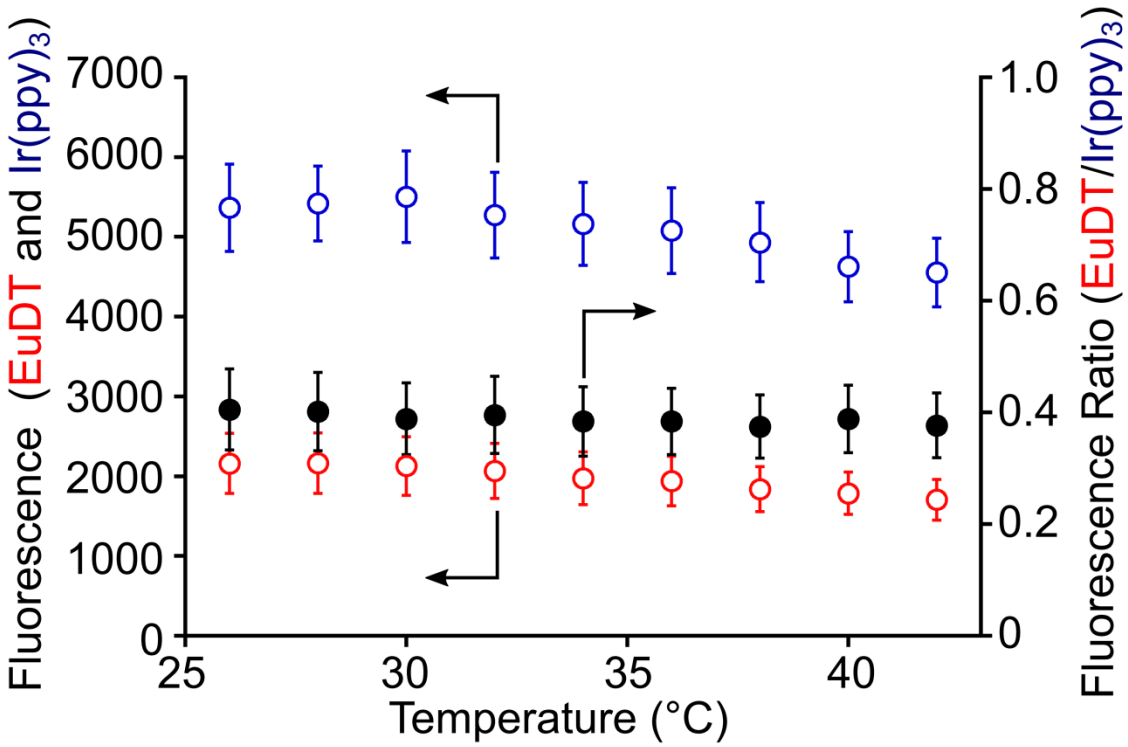

Figure S4. The temperature dependency of autofluorescence of non-dosed larva in EuDT and $Ir(ppy)_3$ channels. The average values of fluorescence intensity at the region of interest (ROI) was plotted against varying temperature of the control larva (as shown in Fig. 3a) in both $Ir(ppy)_3$ (blue open circle) and EuDT (red open circle) channels. The average of fluorescence ratio (EuDT/$Ir(ppy)_3$) of different ROIs (14 ROIs) calculated from these values was plotted as black closed circle. The calibration slope for the ratio obtained from heating is -0.0016/°C ($y = -0.0016x + 0.44$, $R^2 = 0.71$).