Supporting Information

Thermo-optical characterization of fluorescent rhodamine B based temperature-sensitive nanosensors using a CMOS MEMS micro-hotplate

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Supporting Methods

Movement of MEMS Hotplate with Changes in Temperature: The MEMS hotplate was placed in a fluorescence confocal microscope, and was imaged using a 50x 0.80 NA (air) objective. A saturation lookup table was initially visualised the centre of the hot plate at 25 °C, such that the centre plate was completely saturated. The temperature of the hot plate was gradually increased to 605 °C, in 20 °C steps. The change in μm of the focal plane was recorded at each temperature (n = 3).

Temperature cycling: Nanoparticles were suspended in deionised water (50 μL, 1mg/mL) and deposited and dried of the surface of a quartz heater plate of a Linkam Scientific DSC600 stage. For thermal cycles samples were heated at a rate of 30 °C/min, controlled by Linksys 32 software, to well-defined temperature points between 0 and 200°C and allowed to thermally stabilize for 2 minutes after attaining each new temperature.

Environmental Scanning Electron Microscopy (ESEM): TetraSpeck 500 nm (TS500) nanoparticles (Invitrogen™, Paisley, United Kingdom) were spread and dried on 400 mm² mica placed on an aluminium scanning electron microscope stub (Agar Scientific). The sample was platinum coated (90 seconds, 2.2 kV, 20 mA plasma current and 4x10⁻² mbar vacuum) using a Polaron SC7640 sputter coater. A Philips XL30 ESEM-FEG scanning electron microscope was used to image dry nanoparticles (20 kV, 10.1 mm working distance). The measured diameters were validated using dynamic light scattering.

Dynamic light scattering: Dynamic light scattering was performed using a Viscotek (802) system. The system is equipped with a 50 mW laser source (830 nm), operating at an angle of 90°. TS500 nanoparticles (10 μL) were suspended in 1 mL of deionised water. Measurements (10 runs, 25 °C) were made using a Hellma® Analytics quartz cuvette (1.5 mm diameter). The mean hydrodynamic diameter of the particles was computed from the intensity of the scattered light using the OmniSize 3.0 software.
Full width half max (FWHM) analysis of TetraSpeck 500 nm (TS500) nanoparticles: TS500 nanoparticles (5 μL) were spread on the surface of a glass slide and allowed to dry. A Leica SP2 confocal fluorescence microscope coupled with a Leica HC PL FLUOTAR 50x 0.8 NA (air) (pixel size 0.39 x 0.39 μm, pinhole 0.119 mm (1 Airy)) was used to image TS500 nanoparticles, at 25 °C. A He-Ne 568 nm laser was used as excitation source. A photomultiplier tube (1225 HV, 100 offset) was used to collect fluorescence between 580 nm and 625 nm. FWHM values were calculated by fitting a Gaussian distribution curve, which was solved for half the maximal intensities of a line profile of single TS500 nanoparticle (n = 6). Student’s t test was used to identify significant differences FWHM of temperature-sensitive nanosensors and TS500 nanoparticles.

Supporting Results

Movement of MEMS Hotplate with Changes in Temperature: Fig. S1 shows the MEMS hotplate buckles by 9.39 ± 5.4 μm when the temperature is increased from 25 to 605 °C. By use of confocal microscope, rather than a conventional fluorescence microscope, the temperature sensitive nanoparticles deposited on the surface of the MEMS hotplate can be kept in focus.

Temperature cycling: Fig. S2 shows fluorescence intensity levels recorded during thermal cycling of the aggregated sensors from 200 °C to 0 °C. The response of the sensors was reversible, although some overall reduction in peak intensity of 18% was observed over the 70 minute time period of the experiment.

Size characterisation of TetraSpeck 500 nm (TS500) nanoparticles: ESEM of TS500 show nanoparticles with an average diameter of 485 ± 20 nm, Fig. S3A. These measurements were validated with DLS which show nanoparticles with diameters ranging from 300 to 1000 nm, with an intensity distribution centred at 531 nm, Fig. S3B.

Full width half max (FWHM) analysis of TetraSpeck 500 nm (TS500) nanoparticles: Analysis of TS500 nanoparticles shows particles with a FWHM of 1.04 ± 0.44 μm, Fig. S4. The FWHM of TS500 is not statistically different to temperature-sensitive nanoparticles (p < 0.05).

Fig. S1. Temperature induced changes in focal plane, as measured used saturation of reflected light from the surface of the MEMS hot plate, using confocal microscopy.
**Fig. S2.** Fluorescence intensity measured for temperature-sensitive nanosensors when cycled between 0 and 200 °C.

**Fig. S3.** (A) Environmental scanning electron microscopy image TetraSpeck 500 nm (TS500) nanoparticles, with an average nanoparticle diameter and (B) dynamic light scattering intensity distribution for TS500 nanoparticles.

**Fig. S4.** (A) Image and (B) full width half max (FWHM) analysis of TetraSpeck 500 nm (TS500) nanoparticles ($n = 6$).