

## Photothermally responsive gold nanoparticles conjugated polymer-grafted porous hollow silica nanocapsules

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### Supplementary Information

#### Materials

Hollow silica nanoparticles (HSi) with an average diameter of 150 nm were synthesised according to the procedure described in literature.<sup>1, 2</sup> The RAFT agent 3-benzylsulfanylthiocarbonylsulfanyl-propionic acid (BSPA) was synthesised according to a previously reported protocol.<sup>3</sup> Inhibitor was removed from all monomers by passing it over a short layer of basic alumina. Gold nanoparticles of 5nm diameter stabilised in citrate buffer purchased from British Biocell (BBInternational Ltd., UK) were concentrated with NanoSep filters 10 KDa to about 0.5  $\mu$ M of nanoparticles. Fetal bovine serum (FBS) and Dulbecco's modified eagle medium (DMEM) were purchased from Thermo Fisher Scientific Inc.. All other chemicals were used as received from the manufacturers unless otherwise stated.

#### Instruments & Characterisation

Transmission electron micrographs (TEM) were obtained with a Philips CM300 FEGTEM instrument at an accelerating voltage of 300 kV. The sample was prepared by placing a drop of the sample dispersed in deionised water on holey carbon coated 200 mesh copper grids for 30 seconds, dried with filter paper, then left to completely dry in a desiccator overnight.

Thermogravimetric analyses (TGA) were performed on a TA Instruments Q500 under N<sub>2</sub> gas with a flowrate of 60 mL.min<sup>-1</sup>, a heating rate of 20 °C.min<sup>-1</sup> from 100 °C to 600 °C.

Dynamic light scattering (DLS) was performed using Malvern ZetasizerNano ZS with a HeNe laser (633 nm) to measure the particle size and distribution in solution. The zeta potential of the water dispersible nanoparticles was performed by the same instrument through application of voltage stimulus ranging from -100 to 100 mV between the electrodes of the dual purpose cuvette. A statistical average of three readings per sample was taken for the DLS and zeta potential measurements.

UV spectra were acquired using the Spectra Max Plus spectrophotometer (Molecular Devices, Wokingham, UK) and 384 wells plates from Corning (Lowell, US).

Fluorescence emission spectra were recorded on a Perkin–Elmer LS55 Fluorescence Spectroscopy instrument fitted with a R928-sensitive sample photomultiplier. Laser experiments were conducted with a 532 nm AOTK Superd-M DPSS laser with 70mW output power in quartz cuvettes. The laser beam diameter was 1cm at the centre of the cuvette using a defocusing lens (focal length 30cm) while the intensity of the laser light incident to the front wall of the cuvette was reduced to about 10mW/cm<sup>2</sup> using an ND filter with OD=1. *In situ* fluorescence measurements were performed with using an Avantes ULS3648RS-USB2 spectrometer placed at 90° to the laser beam.

The molecular weight of etched polymer chains was determined by size exclusion chromatography with a Waters 2690 apparatus with a refractive index detector; mobile phase: 0.05 M sodium nitrate with 0.02% sodium azide in water, calibrated with poly(ethylene oxide) standards.

***i) Synthesis of BSPA functionalized porous hollow silica nanoparticles***

The epoxide functionalized porous hollow silica nanoparticles were made by first mixing 0.2 g of HSi into 10 mL of p-xylene and dispersed in solution by sonication for 30 min. The solution was then heated to 90 °C under argon and 0.3 mL of (3-glycidyloxypropyl)trimethoxy silane was added. The mixture was allowed to react for 24 h. The mixture was cooled to room temperature, filtered and washed thoroughly with diethyl ether. The epoxide functionalised porous hollow silica nanoparticles were dried under vacuum then redispersed with an equal mass of BSPA (0.2 g, 0.77 mmol) in 10 mL of toluene. Triethylamine (107 µL, 0.77 mmol) was then added and the mixture was allowed to react for 24 hours at room temperature. The product was separated by filtration and washed with acetone to yield a pale yellow powder. The particles were analysed by thermogravimetric analysis (TGA) for their weight percent content (13%). The sulfur content of the product, found by elemental analysis is 1.49%.

***ii) Synthesis of (a) PDMAPS-co-PPEGMA<sub>1.1k</sub> and (b) PDMAPS grafted porous hollow silica nanoparticles***

BSPA functionalised porous hollow silica nanoparticles (0.1g) were dispersed in 10 mL of toluene in a Schlenk flask by sonication. Either (a) DMAEMA (2 g, 12.7 mmol) and PEGMA (0.2 g, 0.18 mmol,  $M_n \sim 1.1$  kDa); or (b) DMAEMA (2 g, 12.7 mmol) together with 4,4'-azobis(4-cyanovaleric acid) (3.4 mg, 0.012 mmol) was added and the Schlenk flask was sealed. The mixture was subjected to 3 freeze-pump-thaw cycles to remove all dissolved gases and the reaction was left at 60 °C for 18 hours. To terminate the polymerization, the Schlenk flask was unsealed and compressed air was blown into it. The particles were then separated by centrifugation, re-suspended and centrifuged 3 times in acetone to remove all unreacted monomer, initiator and ungrafted polymer. For the betanisation, the particles were dried under vacuum and dispersed in 10 mL of THF. To this, 0.2 g of 1,3-propanesultone (0.2 g, 1.64 mmol) were added and the mixture was left to react at 60 °C for 24 hours. The nanoparticles were separated by centrifugation, re-suspended and centrifuged 3 times in acetone to remove all excess reagents. The particles were dried under vacuum and analysed by TGA for their polymer content ((a) 39%; (b) 22%). These particles were further treated with HF to etch the silica, the polymer was recovered and analysed by size exclusion chromatography ((a)  $M_n$ : 810, PDI: 1.57; (b)  $M_n$ : 450, PDI: 1.42).

***iii) Thermo-stimulated release study of rhodamine B***

30 mg of particles (a) and (b) were each added to separate solutions of 5 mg of rhodamine B in deionized water. The mixture was heated to 50 °C and the dye was allowed to permeate the particles for 24 hours. The solutions were then cooled to 15 °C and centrifuged to wash away all excess dye until the supernatant remained clear. The two sets of rhodamine B loaded nanoparticles were then dispersed in 30 mL of deionized water and 1 mL samples were taken and filtered. The remaining solution was left at room temperature for 22 hours, after which samples were taken again. The solution containing PDMAPS-co-PPEGMA<sub>1.1k</sub> grafted porous hollow silica nanoparticles was heated to 40 °C and the solution containing PDAMPS grafted porous hollow silica nanoparticles was heated to 50 °C. 1 mL samples were taken immediately upon heating, and samples were then taken at predetermined intervals thereafter for up to 24 hours. All collected filtered samples were analysed by spectrofluorimetry at excitation wavelength 552 nm and emission wavelength 572 nm in triplicate.

***iv) Preparation of gold nanoparticles conjugated polymer grafted porous hollow silica nanoparticles***

Gold nanoparticles conjugated polymer-grafted porous hollow silica nanoparticles were obtained by first dispersing 10 mg of rhodamine B loaded (a) PDMAPS-co-PPEGMA<sub>1.1k</sub> or (b)

PDMAPS grafted porous hollow silica nanoparticles in 5 mL of deionized water. Concentrated 5 nm citrate coated gold colloid (1 mL) was added and the mixture was stirred for 30 minutes at room temperature. The mixture was then centrifuged at 6000 rpm for 5 minutes. The completion of the reaction was verified when no absorbance of the supernatant at 518 nm was observed, signifying that all gold attached to the pelleted hollow silica-polymer particles. Dynamic light scattering measurements were performed on bare hollow silica, PDMAPS grafted hollow silica and gold conjugated PDMAPS grafted hollow silica to determine changes in size with respect to functionalization and are reported in Table S1.

***v) In situ laser mediated release of rhodamine B***

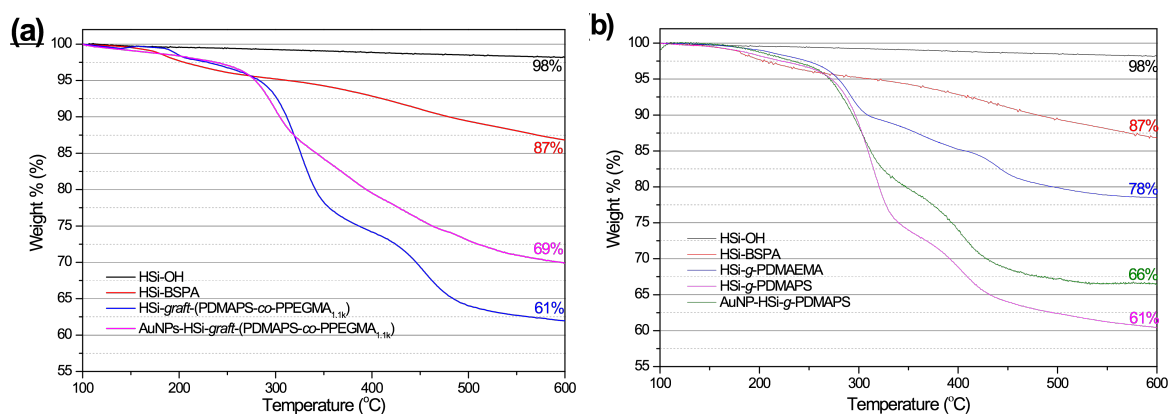
Rhodamine B loaded gold nanoparticles conjugated polymer-grafted porous hollow silica nanoparticles and rhodamine b loaded particles without gold nanoparticles were dispersed in cold deionized water at a concentration of 1 mg/mL. These samples were irradiated with a 532 nm laser and continuously stirred using a magnetic stirrer. Fluorescence was measured in situ at 10 minute intervals between 400 and 800 nm with a 10ms integration time period.

***vi) Stability studies with 10% FBS in DMEM***

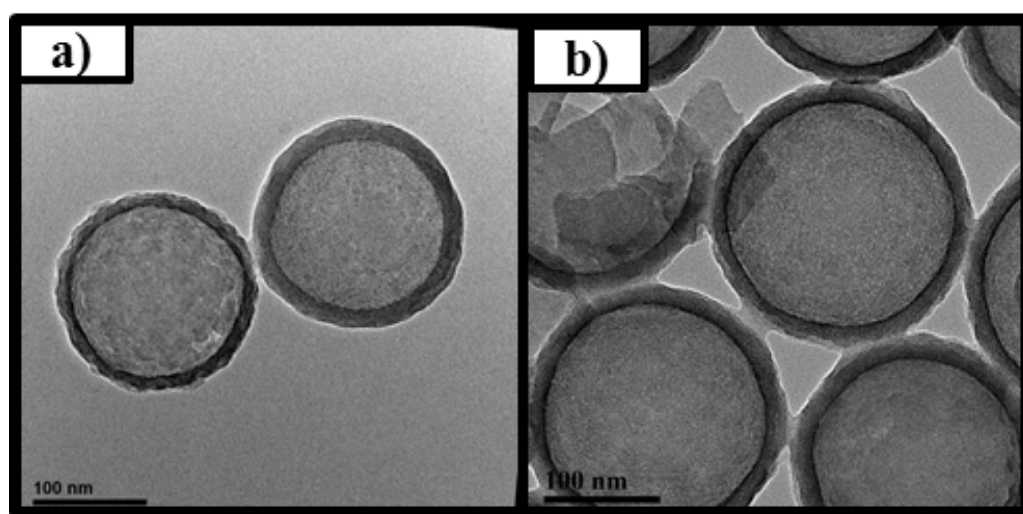
To evaluate the stability of the gold nanoparticles conjugated PDMAPS grafted silica nanoparticles in physiological environment, 10 mg of nanoparticles are dispersed in 10 % FBS in DMEM. The samples are left in the solution over different period of time at room temperature and 37°C. The UV-Visible absorbance of the whole sample and the supernatant (after centrifugation at 1000 rpm for 1 minute) are recorded. The spectra are compared to water and to the solution of 10% FBS in DMEM and are reported in Fig. S8. The spectra of the nanoparticles did not change over time (Fig. S8). Moreover, no gold nanoparticles were observed in the supernatant after quick centrifugation of the samples and the spectra are similar to the control spectrum of the solution of 10% FBS in DMEM (Fig. S9). A very light increase of absorbance at around 560 nm is observed after 2 days that could be attributed to some gold nanoparticles conjugated material.

***vii) Control experiment of photothermal-induced controlled release with gold nanoparticles conjugated pH-responsive polymer-grafted porous hollow silica nanoparticles***

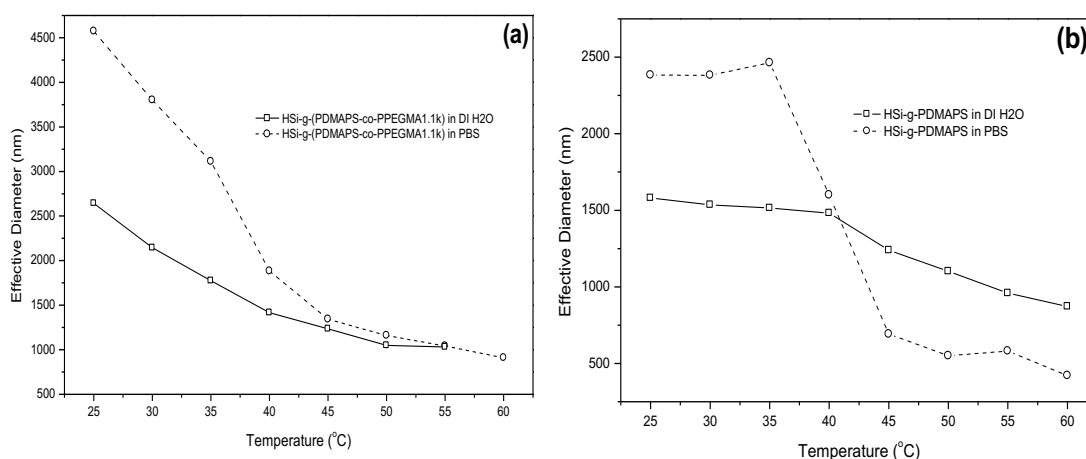
A negative control experiment was performed with gold nanoparticle conjugated polymer-grafted silica nanoparticles in order to confirm the photothermal activity of the gold nanoparticles. Methacrylic acid (MAA) was grafted on the porous hollow silica nanoparticles with the same reaction conditions and reagent ratios as reported in part (ii). The resultant PMAA grafted silica nanoparticles exhibit no thermo-response but a pH triggered encapsulation and release mechanism as reported by Lay and co-workers.<sup>1</sup> The PMAA grafted silica nanoparticles were loaded with rhodamine B by adding 30 mg of the particles to 5 mg of rhodamine B in 10mL of water at pH 8 and the dye was allowed to permeate the particles for 24 hours. The pH of the solution was then reduced to 2 with HCl and the nanoparticles were centrifuged to wash away all excess dye until the supernatant (0.01M HCl) remained clear. To this, gold nanoparticles were conjugated using the method described in part (iv). Laser studies were performed on the gold nanoparticle conjugated PMAA grafted silica particles following the exact method outlined in part (v). No release of the encapsulated RhB was observed (Fig. S10). No significant change was observed in the relative change in fluorescence at 583 nm for the particles over time. This thus confirms the hypothesis that a thermally responsive grafted polymer is essential to trigger a photothermal release of encapsulated compounds from this strategy.



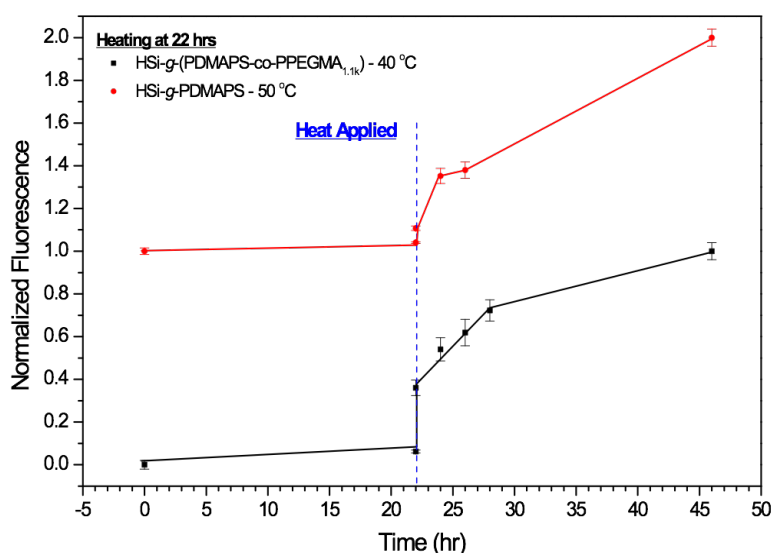
**Fig. S1** (a) Thermogravimetric analysis of PDMAES-co-PPEGMA<sub>1.1k</sub> grafted nanoparticles at various stages of synthesis and functionalisation. At 600 °C, the residual material corresponds to silica and/or gold and therefore all weight loss corresponds to the functional organic moieties and polymer on the nanoparticles. For bare porous hollow silica nanoparticles, there is a marginal 2% loss. The BSPA moieties in BSPA functionalised porous hollow silica nanoparticles make up 13% of the total particle weight. PDMAES-co-PPEGMA on PDMAES-co-PPEGMA<sub>1.1k</sub> grafted nanoparticles is approximately 39% and the weight fraction on the gold nanoparticles conjugated PDMAES-co-PPEGMA<sub>1.1k</sub> grafted nanoparticles is 31%. By back calculating with known silica to polymer ratio, the gold content for the gold nanoparticles conjugated PDMAES-co-PPEGMA<sub>1.1k</sub> grafted nanoparticles is 21%; (b) Thermogravimetric analysis of the PDMAES-grafted nanoparticles at various stages of synthesis and functionalisation. At 600 °C, the residual material corresponds to silica and/or gold and therefore all weight loss corresponds to the functional organic moieties and polymer on the nanoparticles. PDMAEMA on PDMAEMA grafted nanoparticles is approximately 22% and the betanisation increases polymer percentage to 39%. The weight fraction on the gold nanoparticles conjugated PDMAEMA grafted nanoparticles is 34%. By back calculating with known silica to polymer ratio, the gold content for the gold nanoparticles conjugated PDMAEMA grafted nanoparticles is 15%.



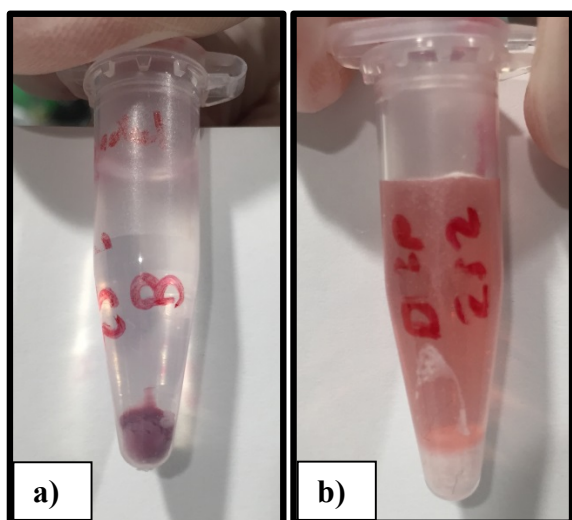
**Fig. S2** Electron microscopy imaging of (a) bare hollow silica nanoparticles and (b) PDMAES-co-PPEGMA<sub>1.1k</sub> grafted hollow silica nanoparticles.



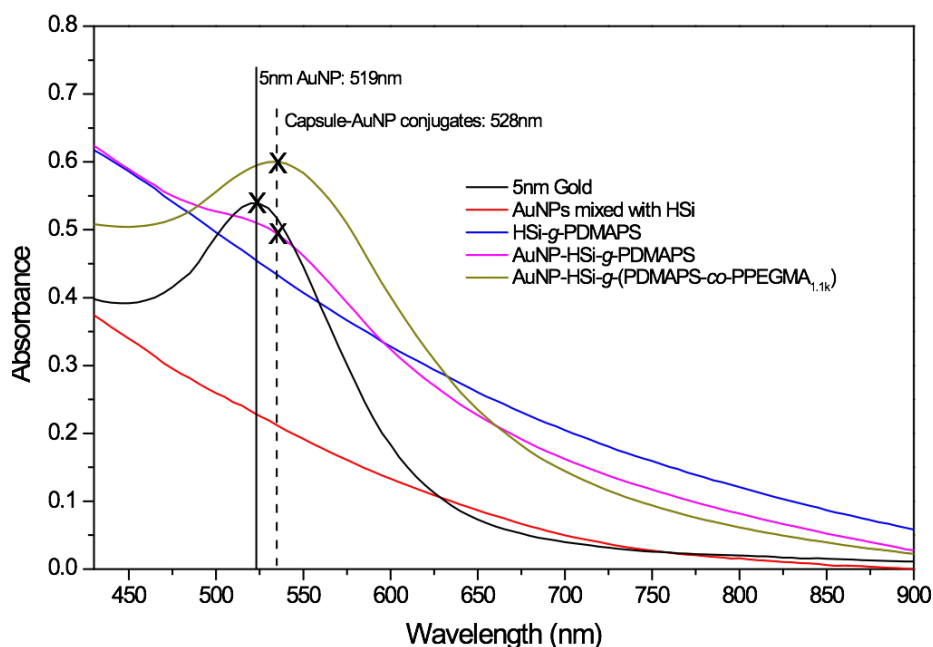
**Fig. S3** Dynamic light scattering analysis of particle size against temperature for **(a)** PDMAPS-*co*-PPEGMA<sub>1.1k</sub> grafted porous hollow silica nanoparticles and **(b)** PDMAPS grafted porous hollow silica nanoparticles in both deionized water and phosphate buffered saline solution at pH 7.



**Fig. S4** Release profile of rhodamine B loaded PDMAPS-*co*-PPEGMA<sub>1.1k</sub> grafted silica nanoparticles (red line) and PDMAPS grafted silica nanoparticles (black line) as determined by fluorescence. The particles were left at room temperature for the first 22 hours, and then heated to 40 °C and 50 °C respectively. Samples were taken at 0, 2, 4 and 24 hours after raising the temperature.



**Fig. S5** Photographs of (a) centrifuged PDMAAPS grafted porous hollow silica nanoparticles mixed with gold nanoparticles – the supernatant is colourless while the particles have taken on a deep burgundy hue, signifying that the gold nanoparticles have conjugated with the polymer-grafted silica nanoparticles; (b) centrifuged porous hollow silica nanoparticles mixed with gold nanoparticles – the supernatant remained red while the particles remained white, signifying that the gold nanoparticles did not conjugate with the polymer-grafted silica nanoparticles.

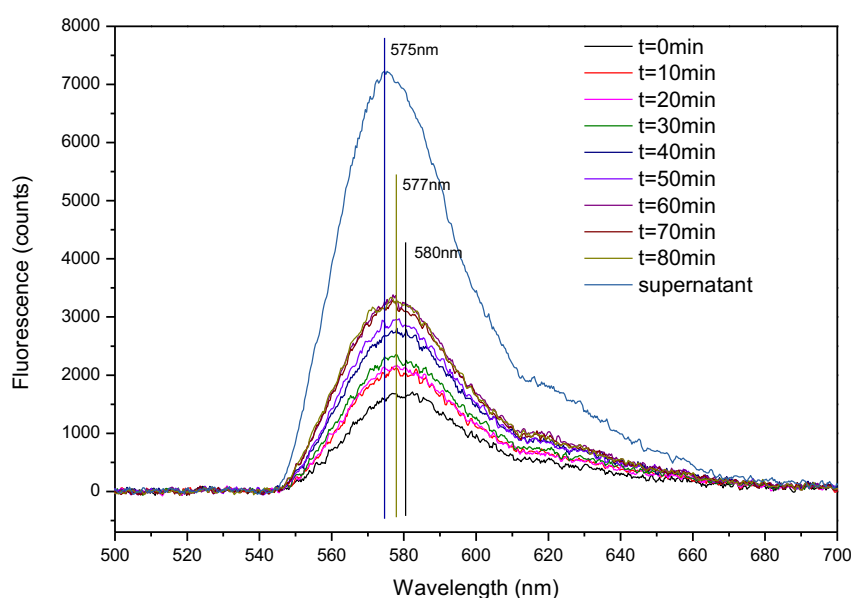


**Fig. S6** UV/Vis absorbance curves of 5 nm gold nanoparticles (black line), porous hollow silica nanoparticles that was mixed with gold nanoparticles (red line), PDMAAPS grafted porous hollow silica nanoparticles (blue line), gold nanoparticles conjugated PDMAAPS grafted porous hollow silica nanoparticles (purple line), gold nanoparticles conjugated PDMAAPS-co-PPEGMA<sub>1.1k</sub> grafted porous hollow silica nanoparticles (green line). The recorded peak absorbance of the 5 nm gold nanoparticles is at 518 nm, in agreement with the supplier's speci-

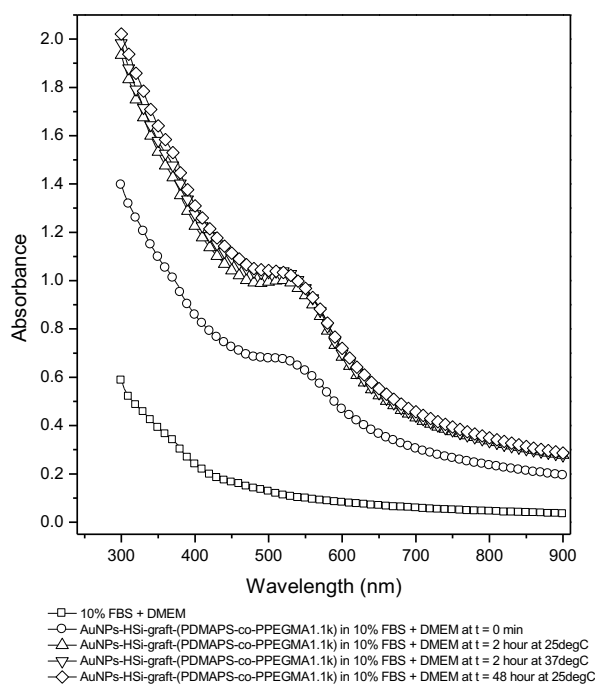
fications. Once gold nanoparticles were conjugated to the polymer-grafted porous hollow silica nanoparticles, a shift in peak absorbance to 528 nm suggested a change in plasmon resonance due to the closer proximity of the gold nanoparticles.

**Table S1:** Particle size determined by dynamic light scattering of bare silica nanoparticles, PDMAPS grafted silica nanoparticles and gold conjugated PDMAPS grafted silica nanoparticles.

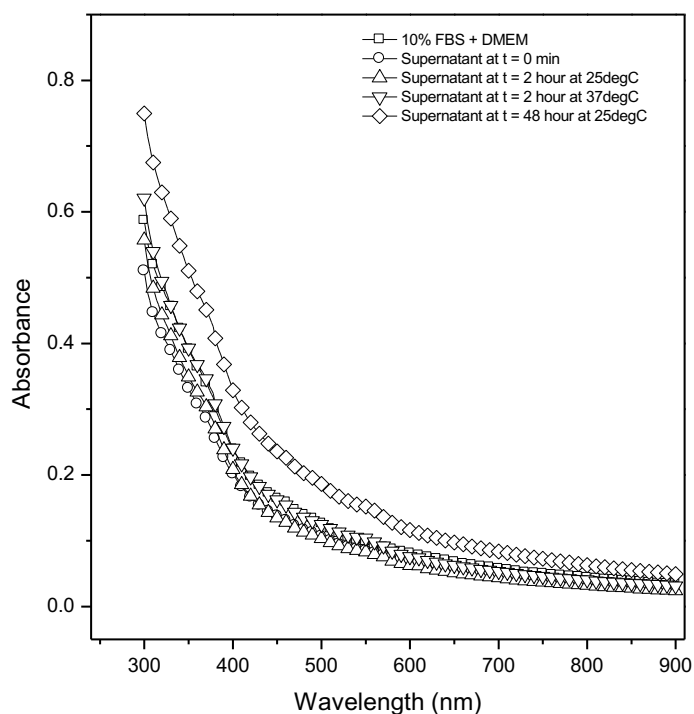
Sample	Particle diameter (nm)	PDI
Bare silica nanoparticles	620	0.350
PDMAPS grafted silica nanoparticles	1022	0.261
Gold nanoparticles conjugated PDMAPS grafted silica nanoparticles	1047	0.710



**Fig. S7** Fluorescence spectra for the 5 mg/mL rhodamine B loaded gold nanoparticles conjugated PDMAPS grafted porous hollow silica nanoparticles solution between 400 and 800 nm, taken at 10 minute intervals while being irradiated with a 532 nm laser. Spectra at  $t = 0, 10, 20$  and 30 mins overlap and thus the first 3 have been omitted. At the end of the experiment, the solution is centrifuged and the supernatant shows a doubling of the fluorescence due to the absence of light scattering particles. Fluorescence maxima is seen to shift from 580nm to 575nm over the course of the experiment most likely due to the fact that the encapsulated dye is significantly more concentrated and therefore is affected by self-absorption than the released dye in solution.

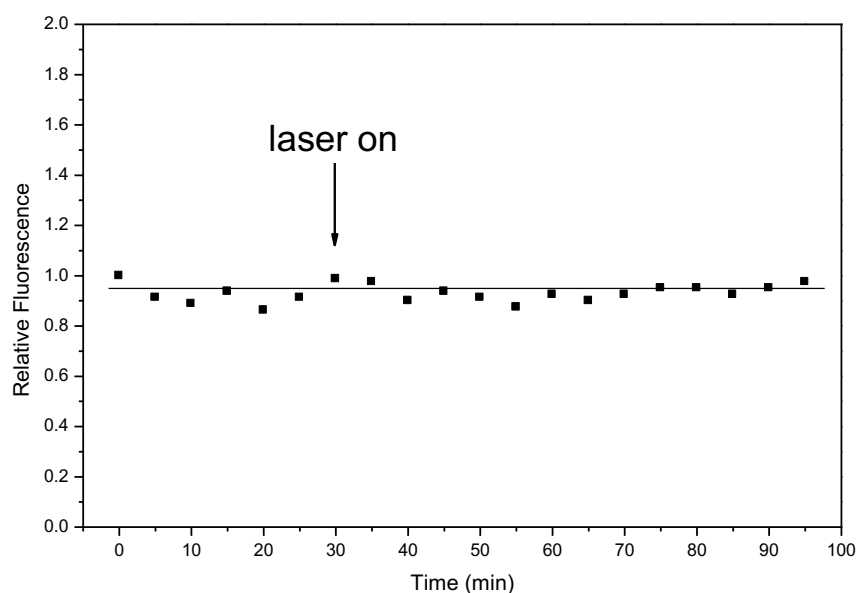


**Fig. S8** UV/Vis absorbance curves of 10% FBS in DMEM and gold nanoparticles conjugated PDMAPS grafted porous hollow silica nanoparticles dispersed in 10% FBS in DMEM at room temperature and 37°C for two hours and 2 days. The presence of the 5 nm gold nanoparticles is validated by the plasmon band absorbance at around 520 nm.



**Fig. S9** UV/Vis absorbance curves of 10% FBS in DMEM and supernatant of the solutions of gold nanoparticles conjugated PDMAPS grafted porous hollow silica nanoparticles dispersed in 10% FBS in DMEM at room temperature and 37°C for two hours and 2 days, and centrifuged at 1000 rpm for 1 min. No gold nanoparticles in the supernatants are observable.





**Fig. S10** Photothermal-induced controlled release of RhB encapsulated in gold nanoparticles conjugated PMAA-grafted porous hollow silica nanoparticles. Relative fluorescence as a function of time of release of RhB from gold nanoparticles conjugated PMAA-grafted porous hollow silica nanoparticles. The particles were kept at 37°C and irradiation commenced at 30 minutes.

## References

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2. C. L. Lay, H. R. Tan, X. Lu and Y. Liu, *Chem. Eur. J.*, 2011, **17**, 2504-2509.
3. M. H. Stenzel and T. P. Davis, *J. Polym. Sci., Part A: Polym. Chem.*, 2002, **40**, 4498-4512.