Electronic Supplementary Information (ESI)

Photosensitizer Anchored Gold Nanorods for Targeted Combinational Photothermal and Photodynamic Therapy

Huijun Phoebe Tham,[a,b,c] Hongzhong Chen,[b] Yu Hui Tan,[b] Qiuyu Qu,[b] Sivaramapanicker Sreejith,[b] Lingzhi Zhao,[b] Subbu S Venkatraman*,[c] and Yanli Zhao*,[b,c]

[a] Interdisciplinary Graduate School (IGS), 50 Nanyang Avenue, Nanyang Technological University, 639798, Singapore.

[b] Division of Chemistry and Biological Chemistry, School of Physical and Mathematical Sciences, Nanyang Technological University, 21 Nanyang Link, 637371, Singapore. Email: zhaoyanli@ntu.edu.sg

[c] School of Materials Science and Engineering, Nanyang Technological University, 50 Nanyang Avenue, 639798, Singapore. Email: assubbu@ntu.edu.sg

1. Experimental methods

1.1 Materials and instruments

All chemicals were purchased from Sigma-Aldrich and used without further purification. Transmission electron microscopy (TEM) images were observed using a JEOL 1400. UV-vis spectra were obtained on a Shimadzu UV-3600 UV-Vis-NIR spectrophotometer (1-mm quartz cell used). Fluorescent spectra were recorded on a Shimadzu RF-5301 spectrofluorophotometer. Cell viability assay was obtained using a Tecan Infinte M200.

1.2 Synthesis of gold nanorods

Gold nanorods (AuNR) were synthesized using an established protocol with minor modifications.[S1] A seed solution was first prepared by mixing hexadecyltrimethylammonium bromide (CTAB) solution (0.5 mL, 0.20 M) with gold (III) chloride trihydrate ($HAuCl_4 \bullet 3H_2O$) solution (0.5 mL, 0.50 mM). Then, freshly prepared ice-cold sodium borohydride ($NaBH_4$) solution (60 μL, 0.010 M) was added under vigorous stirring. Immediately, the mixture turned from a bright yellow to a brownish yellow solution. The seed solution was stirred for 2 minutes and left to stand at 25 ^oC for 30 - 45 minutes before use.

The growth solution was prepared by adding silver nitrate ($AgNO_3$) solution (6.8 mL, 4.00 mM) to CTAB solution (100 mL, 0.20 M). $HAuCl_4$ solution (100 mL, 1.00 mM) was then added to this solution. Ascorbic acid (1.4 mL, 78.8 mM) was slowly added. The mixture was stirred continuously at 800 rpm and the temperature was kept at 27 – 30 ^oC. Upon addition of ascorbic acid, the solution turned from bright orange to colorless. Above seed solution (240 μL) was injected directly into the growth solution. The color of the solution was changed to wine red within 10 – 20 minutes. The reaction was continued for 6 hours.

The AuNR (700 nm absorption) was collected by centrifuging at 9000 rpm for 45 minutes, washed once with deionized water to remove excess CTAB, and centrifuged to get a saturated solution. The concentration of gold was determined using ICP.

For the preparation of 621 nm absorbing AuNR, $AgNO_3$ (2 mL, 4.00 mM) was added instead.

1.3 Synthesis of zinc phthalocyanine modified silica precursor (ZnPc-Si)

ZnPc-$4NH_2$ (9.0 mg) was weighed and transferred to a three-necked flask. Anhydrous DMF (5 mL) was added to dissolve ZnPc-$4NH_2$. 3-(Triethoxysilyl)propyl isocyanate (13.8 μL) was dissolved in anhydrous DMF (0.1 mL) and then injected into the flask. The reaction was refluxed at 120 ^oC under nitrogen protection overnight. This procedure is similar to a previous protocol.[S2]

1.4 Encapsulating AuNR with a layer of silica

The saturated AuNR solution (0.8 mL) was mixed with deionized water (1 mL). A 11-mercaptoundecanoic acid (11-MUA) stock solution (10 mM) was prepared in ethanol. The MUA solution (160 μL) was added to ethanol (2 mL), 1 mL of which was added dropwise to the AuNR over a period of 30 minutes. After which, it was stirred for an additional 30 minutes. Tetraethyl orthosilicate (TEOS, 2 μL) was then directly added into the solution and stirred for 10 minutes. Ammonia solution (60 μL, 25%) was added. The mixture was allowed to stir for 4 hours and the obtained product was denoted as AuNR-Si.

1.5 Encapsulating AuNR-Si with ZnPc

To load ZnPc onto AuNR-Si, the as-synthesized ZnPc-Si in DMF solution (20 μL) was dissolved in ethanol (6 mL) with NH_3 (25 μL) added, which was added into AuNR-Si. The mixture was stirred at 500 rpm for 3 hours. After which, the functionalized AuNR was centrifuged down and the supernatant was kept for further analysis. The obtained product, AuNR-Si-ZnPc, was washed several times.

The ZnPc loading percentage was tracked by measuring the difference between the absorbance of the ZnPc-Si DMF solution (20 μL) in ethanol (6 mL) before mixing with AuNR-Si, and the supernatant obtained after centrifuging for 3 hours. The concentration of ZnPc loaded was calculated using the ZnPc-Si calibration curve.

1.6 Wrapping AuNR-Si-ZnPc with hyaluronic acid

Amine groups were grafted on the external surface by stirring the synthesized AuNR-Si-ZnPc with (3-aminopropyl)triethoxysilane solution (50 μL, 0.2 mM) in ethanol / water (1:1, 5 mL) for 4 hours. The product was centrifuged down to get rid of excess (3-aminopropyl)triethoxysilane. The product was denoted as AuNR-Si-ZnPc-NH_2. A hyaluronic acid (HA) solution was prepared by dissolving HA powder (1.3 mg) in deionized water (6 mL).

The HA solution (800 μL) was added to the AuNR-Si-ZnPc-NH_2 solution and stirred at 500 rpm overnight to completely wrap the AuNR-Si-ZnPc. After which, the solution was centrifuged and washed with water to remove the excess HA. The final product was denoted as 711-AuNR-Si-ZnPc-HA for the 711 nm AuNR batch and 621-AuNR-Si-ZnPc-HA for the 621 nm AuNR batch.

1.7 Synthesis of Si-ZnPc-HA

Silica nanoparticles with a diameter of 45.6 nm were first synthesized using the established Stöber method.[S3] Briefly, ammonia solution (3 mL) and TEOS (5 mL) were added to a mixture of ethanol/water (216

mL/30 mL, v/v), which was stirred gently at 30 ^{o}C for 2 hours. After centrifuging, the obtained silica nanoparticles were rinsed with ethanol and water several times. The silica nanoparticles were loaded with ZnPc by mixing silica nanoparticles (5 mg) with ZnPc-Si DMF solution (20 μL) in ethanol (6 mL) added with NH_3 (25 μL). The loading percentage of ZnPc per mg of silica nanoparticles was ensured to be similar to that of AuNR-Si. After which, the product (Si-ZnPc) was washed repeatedly to remove excess ZnPc, and amine groups were grafted in a way similar to section 1.6 to yield Si-ZnPc-NH_2. The HA solution (800 μL) was added to the Si-ZnPc-NH_2 solution (3 mL) and stirred overnight. The product (Si-ZnPc-HA) was then washed with water to remove excess HA.

1.8 Synthesis of AuNR-Si-HA

AuNR without ZnPc was synthesized as a control. Briefly, the AuNR-Si as obtained in section 1.4 was grafted with amine groups to obtain AuNR-Si-NH_2. The HA solution (800 μL) was added and stirred overnight before centrifuging and washing several times with water. The obtained product was denoted as 711-AuNR-Si-HA and 621-AuNR-Si-HA for the 711 nm and 621 nm AuNR, respectively.

1.9 In vitro experiments

All in vitro experiments were carried out by seeding HeLa and/or MCF-7 cells on 96-well plates at a cell density of 10,000 cells per well. MTT assays were carried out by measuring the absorbance of the metabolized formazan products at 570 nm.

2. Experimental results

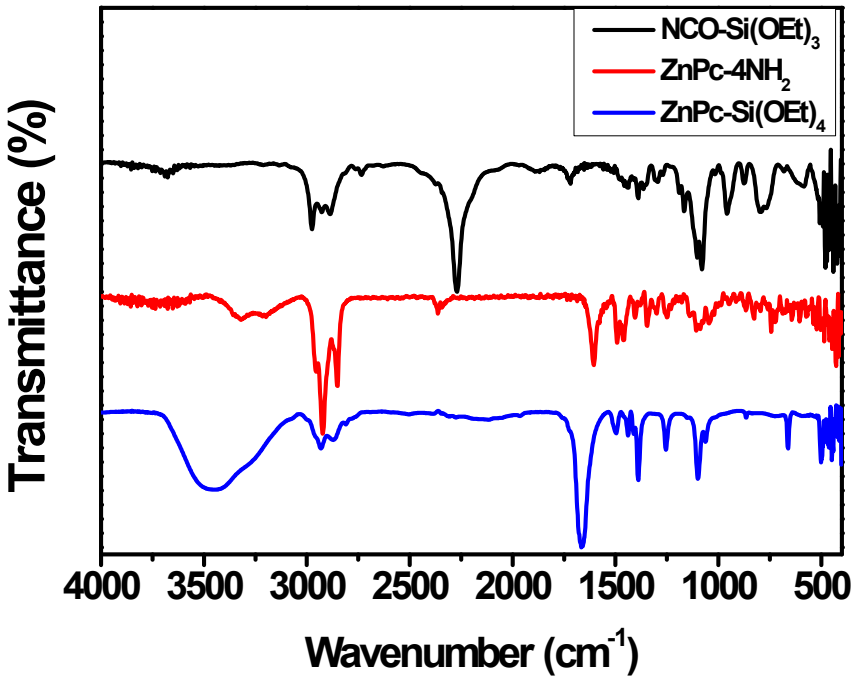

Figure S1: FTIR spectra of (a) 3-(triethoxysilyl)propyl isocyanate (NCO-Si(OEt)$_3$), (b) ZnPc-4NH$_2$ and (c) ZnPc-Si.

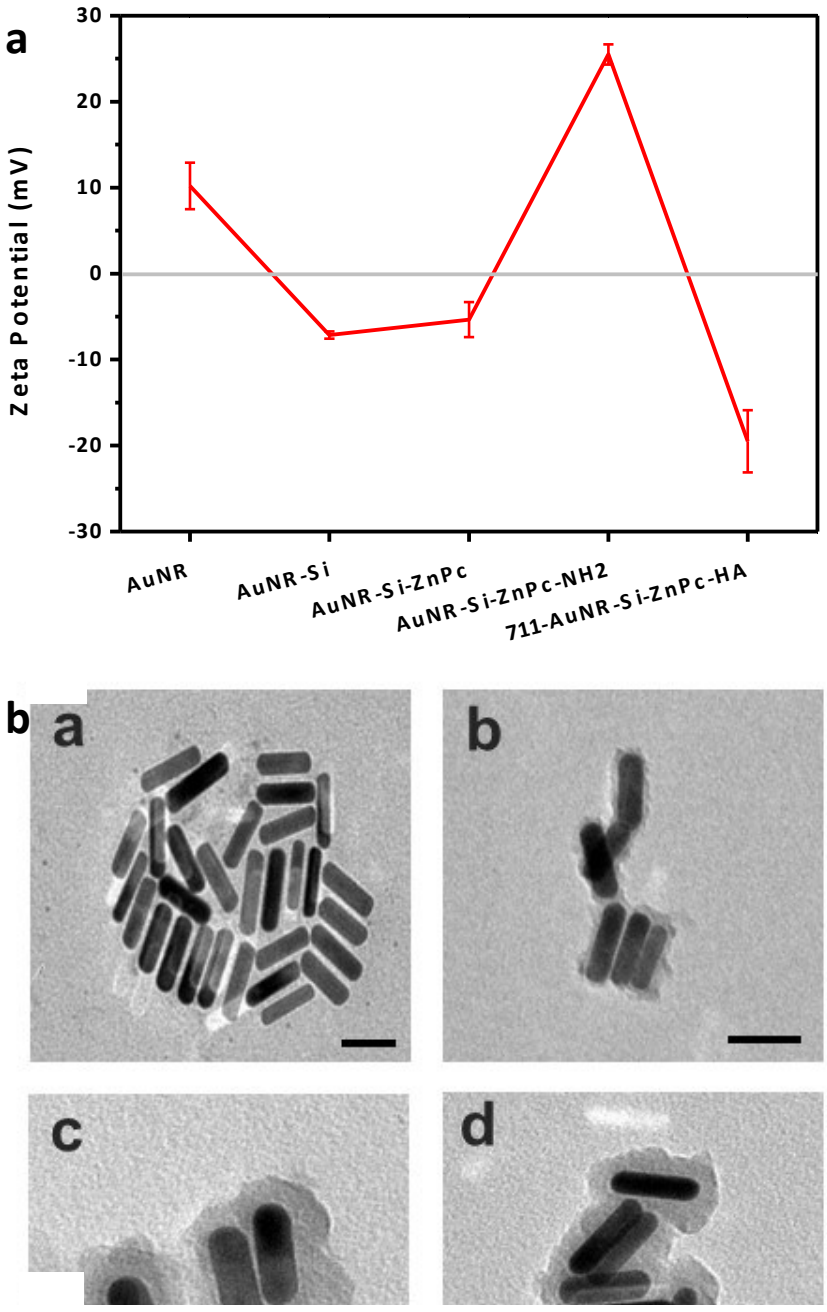

Figure S2: (a) Zeta potential changes with successive coatings on AuNR. (b) Absorbance spectra of AuNR, AuNR-Si, AuNR-Si-ZnPc in EtOH, 711-AuNR-Si-ZnPc-HA in H_2O, and ZnPc-Si in EtOH. Upon coating with a layer of silica and dispersing in ethanol, the absorption peak of AuNR red-shifted from 700 nm to 717 nm. When ZnPc was anchored onto AuNR-Si, the absorption peak red-shifted again by 1 nm to 718 nm. Finally, when HA was wrapped around the nanorods and the hybrid was dispersed in water, the absorption peak blue-shifted by 7 nm to 711 nm. The peaks appeared to broaden with successive coatings possibly due to the variations in the local dielectric field.

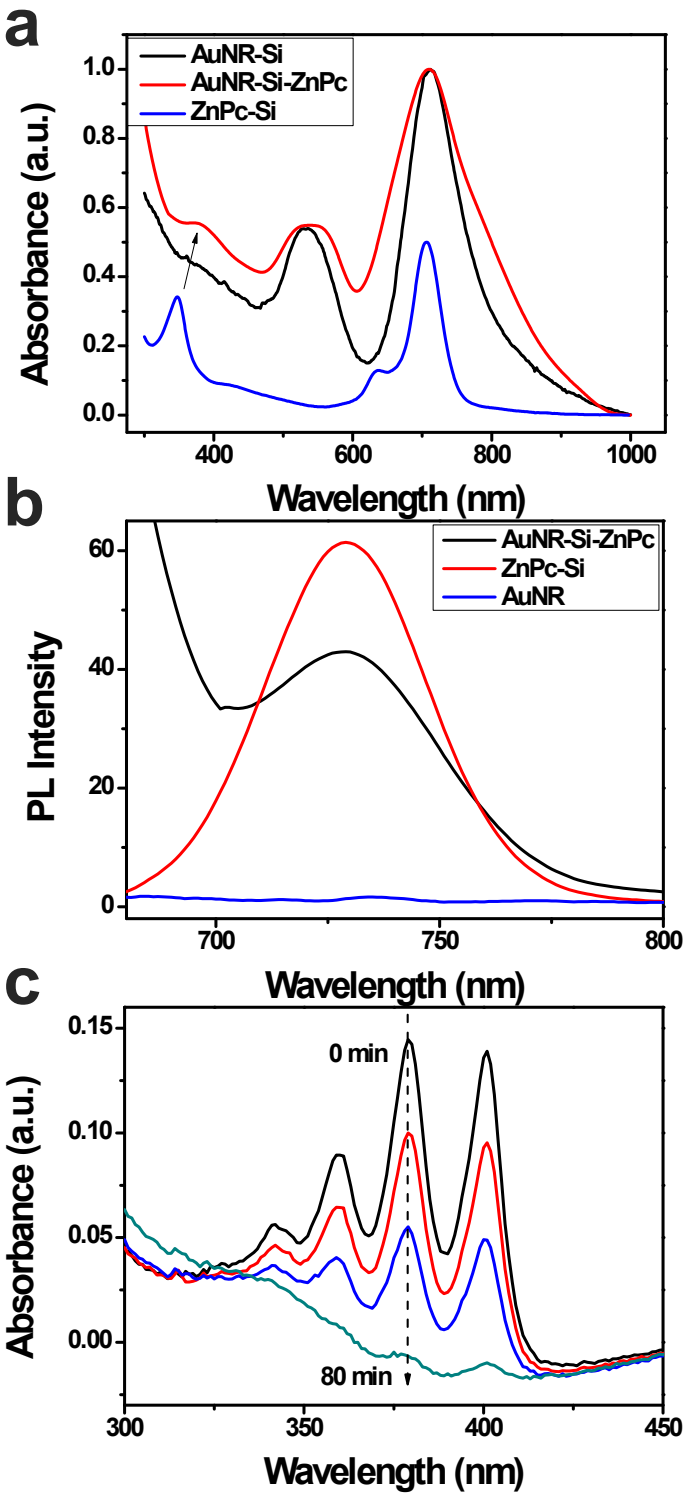

Figure S3: Absorbance spectra of (a) AuNR-Si, AuNR-Si-ZnPc and ZnPc-Si showing successful grafting of ZnPc, (b) Fluorescent spectra of AuNR-Si-ZnPc, and (c) UV-Vis absorbance changes upon quenching of ABDA with singlet oxygen generated by 711-AuNR-Si-ZnPc-HA (20 µg/mL) under light irradiation. A solution (10^{-4} M) of ABDA was added to a solution of 711-AuNR-Si-ZnPc-HA with a similar optical density (1 : 1 v/v). This mixture was irradiated at 730 nm, and the absorbance of the mixture was measured periodically.

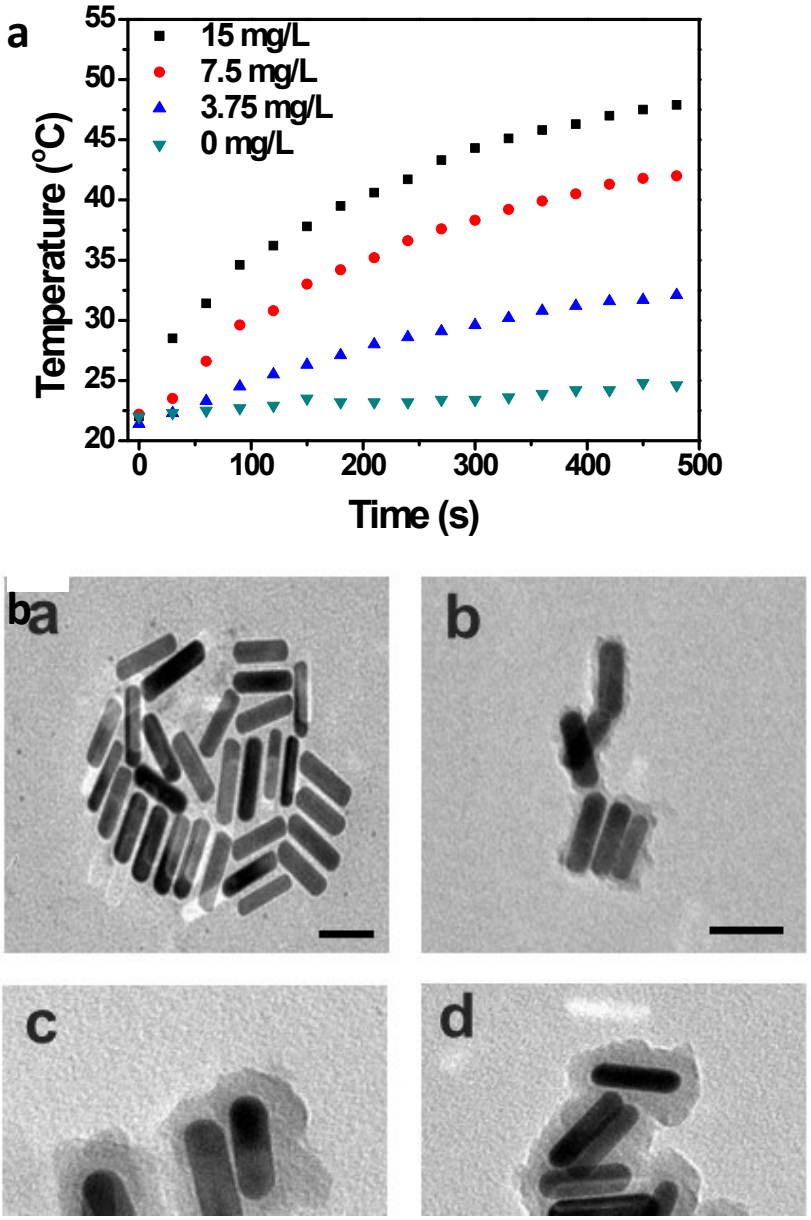

Figure S4: (a) Concentration dependent temperature change of 711-AuNR-Si-ZnPc-HA. 730 nm, 2 W/cm^2. (b) Photothermal performance of 711-AuNR-Si-ZnPc-HA as compared with bare AuNR and water upon laser irradiation (730 nm, 2 W cm^{-2}). Both AuNR and 711-AuNR-Si-ZnPc-HA solutions were fixed at the same concentration of gold (10.6 mg L^{-1}). Water was used as a control.

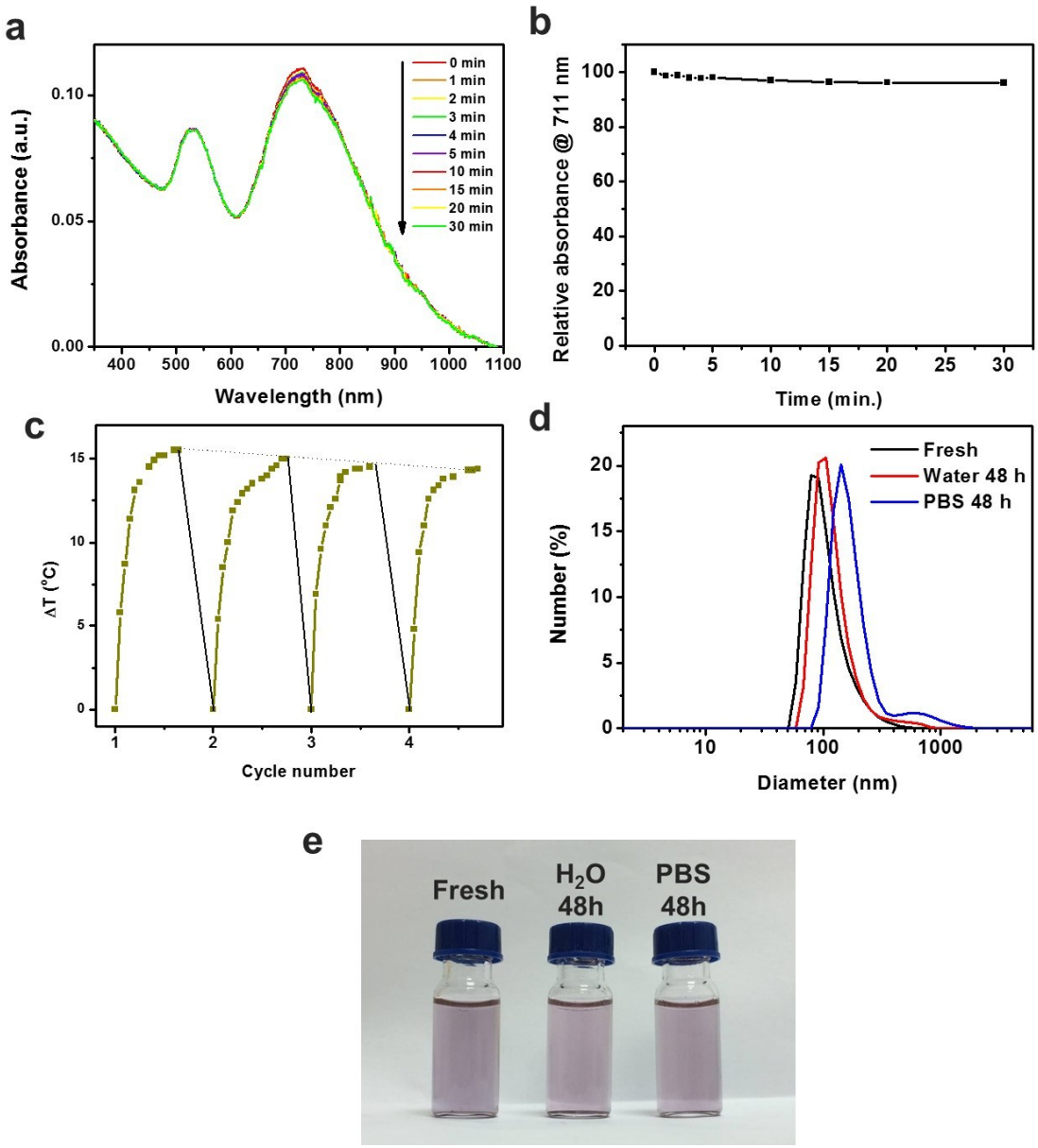

Figure S5: Photostability of 711-AuNR-Si-ZnPc-HA. (a): Absorbance spectra upon 1.5 W/cm^2 laser irradiation for different duration. (b): Relative absorbance at 711 nm. (c) Photothermal performance after 4 cycles of 5 min irradiation each at 2W/cm^2. (d) Stability determined by hydrodynamic size of fresh 711-AuNR-Si-ZnPc-HA in water and PBS for 48 hours. (e): Photographs of fresh 711-AuNR-Si-ZnPc-HA, and those in water and PBS for 48 hours.

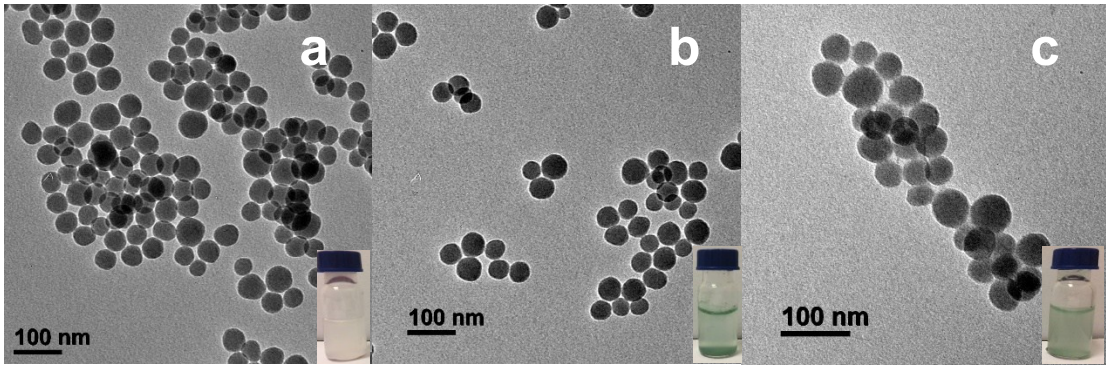

Figure S6: TEM images of (a) silica nanoparticles, (b) Si-ZnPc, and (c) Si-ZnPc-HA. The diameter of silica nanoparticles was measured to be 45.6 ± 4.1 nm. After grafting with ZnPc-Si, this diameter increased to 52.0 ± 4.2 nm. This increase corresponds to about 2 layers of ZnPc-Si grafted around silica nanoparticles. This intermediate product was denoted as Si-ZnPc. The HA coating can be slightly seen from very thin translucent layer surrounding the silica nanoparticles. Insets: corresponding photographs. Measurements were based on 20 silica nanoparticles.

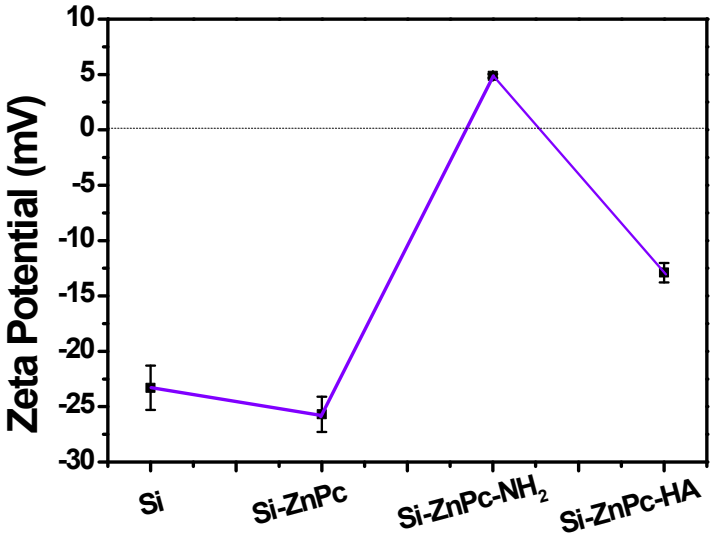

Figure S7: Zeta potential changes with each successive layer of coating on silica nanoparticles.

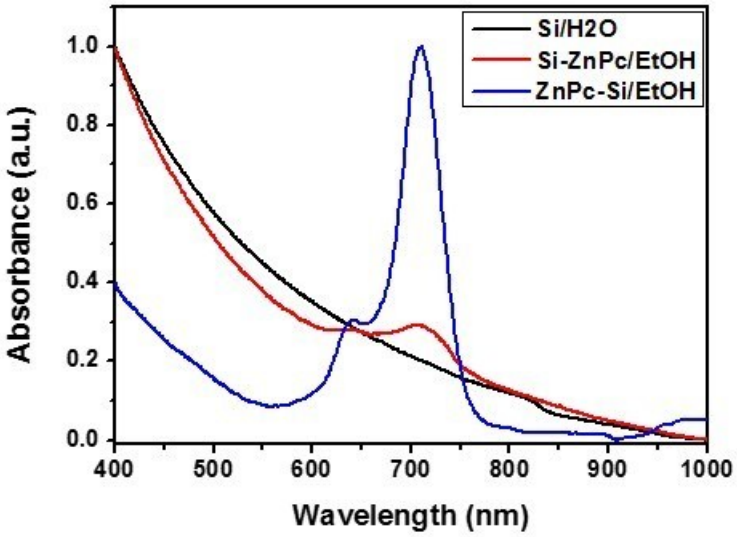

Figure S8: Absorbance spectra of silica nanoparticles (Si) and Si-ZnPc.

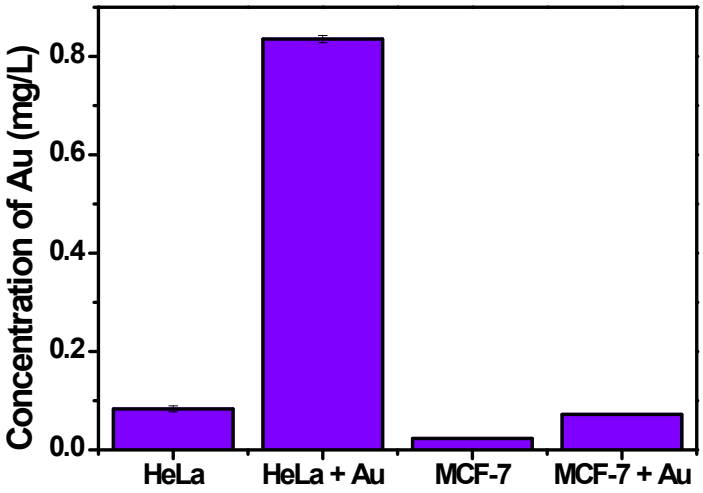

Figure S9: ICP-OES analysis of HeLa and MCF-7 cells after incubation with 711-AuNR-Si-ZnPc-HA at a concentration of 25 μg/mL for 24 hours.

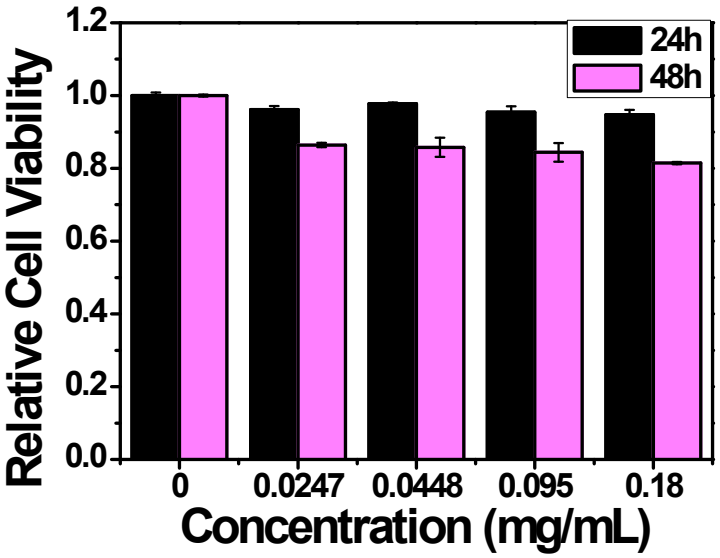

Figure S10: Cytotoxicity of 711-AuNR-Si-ZnPc-HA at various concentrations on HeLa cells for 24 and 48 h.

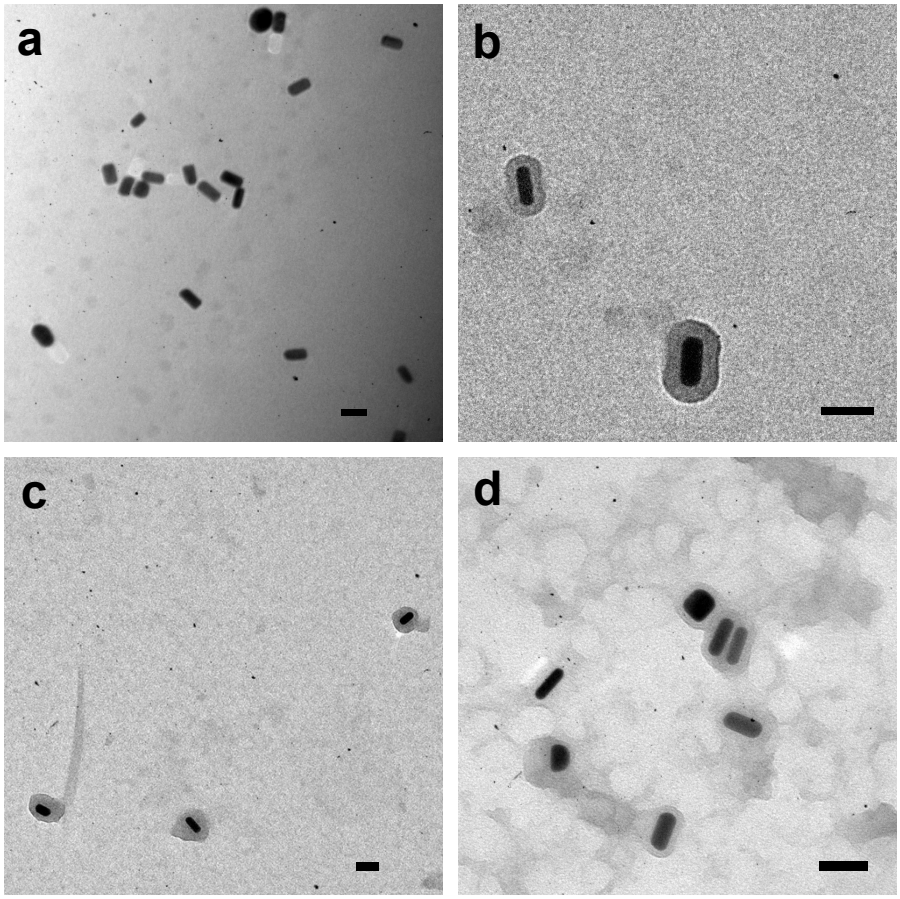

Figure S11: TEM images of (a) 621-AuNR, (b) 622-AuNR-Si, (c) 625-AuNR-Si-ZnPc and (d) 621-AuNR-Si-ZnPc-HA. Scale bar = 50 nm.

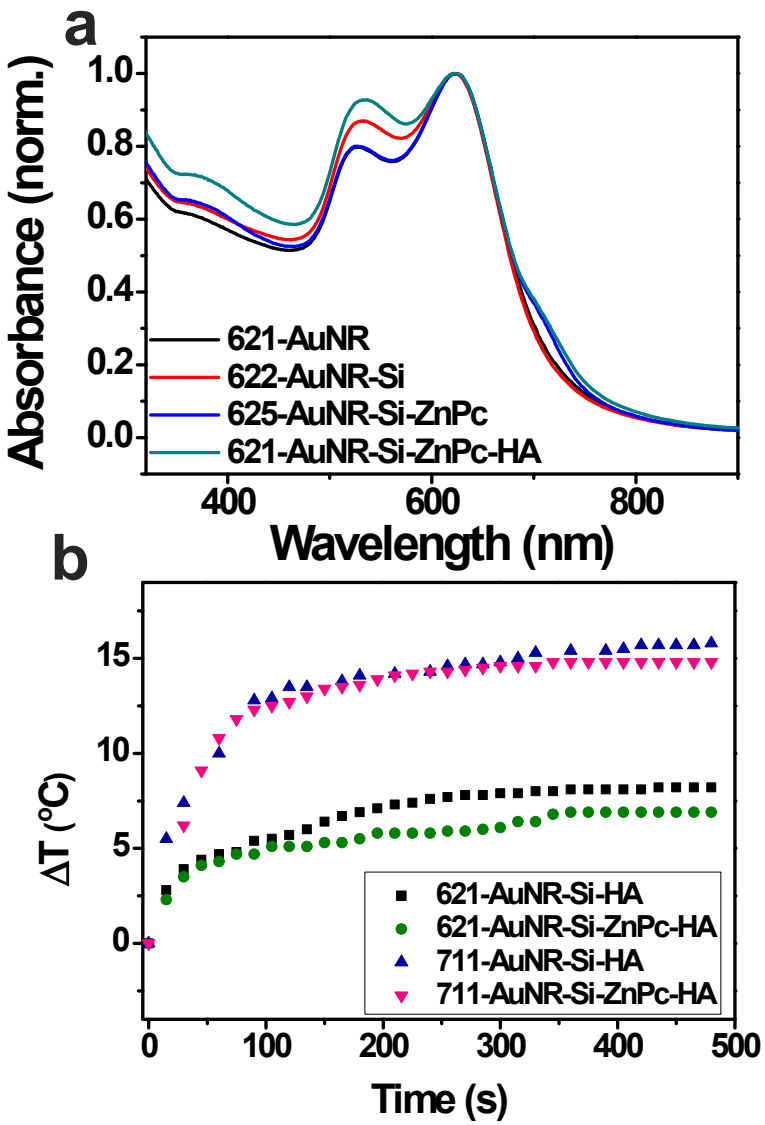

Figure S12: (a) UV-Vis spectra of 621-AuNR-Si-ZnPc-HA. Bare AuNR absorbs maximally at 621 nm. Upon coating with silica, it red-shifted by 1 nm to 622 nm. After grafting with ZnPc-Si, it red-shifted to 625 nm. After encapsulating with HA, it blue-shifted back to 621 nm. (b) Photothermal effect with time for 621-AuNR-Si-HA and 711-AuNR-Si-ZnPc-HA. Optical density at absorption maximum for 621-AuNR-Si-ZnPc-HA and 711-AuNR-Si-ZnPc-HA was fixed to be similar. Irradiation intensity was 2 W/cm^2.

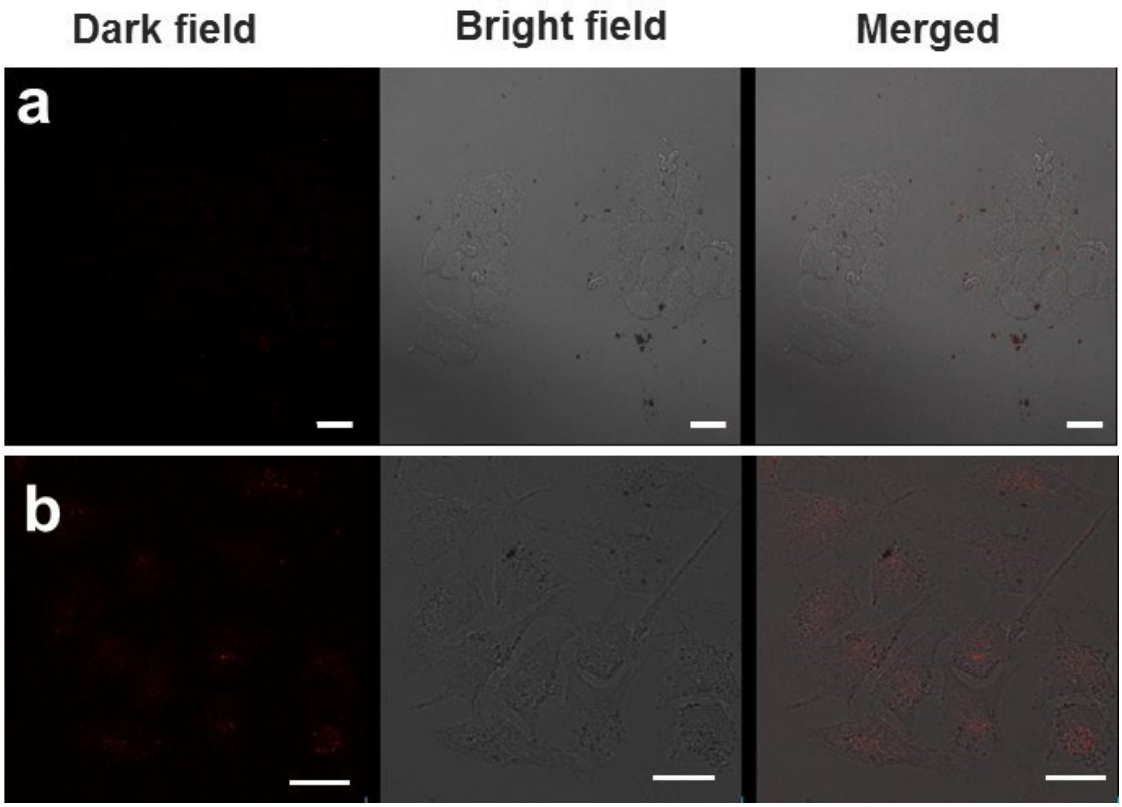

Figure S13: Targeting ability of 711-AuNR-Si-ZnPc-HA. (a,b) MCF-7 and HeLa cells after 6 h of incubation, respectively. Cells were incubated at 12.5 µg/mL of 711-AuNR-Si-ZnPc-HA. Scale bar = 20 µm.

Table S1: Temperature rise and cell viability at different time points for (Upper): 711 nm nanorod pair and (Lower): 621 nm nanorod pair.

Time (min)	Temperature rise (°C)			Cell viability (%)		
	711-AuNR-Si-ZnPc-HA	711-AuNR-Si-HA	Difference	711-AuNR-Si-ZnPc-HA	711-AuNR-Si-HA	Difference
0	0	0	0	100	100	0
1	10.8	10	**0.8**	97.18	100.88	**3.70**
2	12.7	13.5	**0.8**	89.99	93.14	**3.15**
3	13.6	14.1	**0.5**	79.80	87.39	**7.60**

Time (min)	Temperature rise (°C)			Cell viability (%)		
	621-AuNR-Si-ZnPc-HA	621-AuNR-Si-HA	Difference	621-AuNR-Si-ZnPc-HA	621-AuNR-Si-HA	Difference
0	0	0	0	100	100	0
1	4.5	4.6	**0.1**	99.60	100.19	**0.59**
2	5.1	5.4	**0.3**	96.36	98.57	**2.21**
3	5.3	6.8	1.5	91.00	97.16	6.16

Tables S2-S4 are detailed analyses to obtain Table S1

Table S2: Cell viability analysis of 711-AuNR-Si-ZnPc-HA, 711-AuNR-Si-HA, 621-AuNR-Si-ZnPc-HA and 621-AuNR-Si-HA.

Time (min)	Cell Viability (%)					
	711-AuNR-Si-ZnPc-HA	711-AuNR-Si-HA	Difference	621-AuNR-Si-ZnPc-HA	621-AuNR-Si-HA	Difference
0	100	100	100-100 = 0	100	100	100-100= 0
1	97.18	100.88	100.88-97.18 = 3.70	99.60	100.18	100.18-99.60 = 0.59
2	89.99	93.14	93.14-89.99 = 3.15	96.36	98.57	98.57-96.36 = 2.21
3	79.80	87.39	87.39-79.80 = 7.60	91.00	97.16	97.16-91.00 = 6.16

Table S3: Photothermal efficiency analysis of (top) 711-AuNR-Si-ZnPc-HA and 711-AuNR-Si-HA, and (botom) 621-AuNR-SI-ZnPc-HA and 621-AuNR-Si-HA.

Time (min)	Temperature (°C)				
	711-AuNR-Si-HA	Increase wrt t=0	711-AuNR-Si-ZnPc-HA	Increase wrt t=0	Difference in temperature rise
0	22	22-22 = 0	26	26-26 = 0	0
1	32	32-22 = 10	36.8	36.8-26 = 10.8	10.8-10 = 0.8
2	35.5	35.5-22 = 13.5	38.7	38.7-26 = 12.7	13.5-12.7 = 0.8
3	36.1	36.1-22 = 14.1	39.6	39.6-26 = 13.6	14.1-13.6 = 0.5
Time (min)	Temperature (°C)				
	621-AuNR-Si-HA	Increase wrt t=0	621-AuNR-Si-ZnPc-HA	Increase wrt t=0	Difference in temperature rise
0	21.4	21.4-21.4 = 0	25	25-25 = 0	0
1	26	26-21.4 = 4.6	29.5	29.5-25 = 4.5	4.6-4.5 = 0.1
2	26.8	26.8-21.4 = 5.4	30.1	30.1-25 = 5.1	5.4-5.1 = 0.3
3	28.2	28.2-21.4 = 6.8	30.3	30.3-25 = 5.3	6.8-5.3 = 1.5

Table S4: Derivation summary of Figure S1 for (top) 711-AuNR-Si-ZnPc-HA and 711-AuNR-Si-HA, and (bottom) 621-AuNR-Si-ZnPc-HA and 621-AuNR-Si-HA.

Time (min)	Temperature rise (°C) *(as quoted from Table S3)*			Cell viability (%) *(as quoted from Table S2)*		
	711-AuNR-Si-ZnPc-HA	711-AuNR-Si-HA	Difference	711-AuNR-Si-ZnPc-HA	711-AuNR-Si-HA	Difference
0	0	0	0	100	100	0
1	10.8	10	**0.8**	97.18	100.88	**3.70**
2	12.7	13.5	**0.8**	89.99	93.14	**3.15**
3	13.6	14.1	**0.5**	79.80	87.39	**7.60**

Time (min)	Temperature rise (°C) *(as quoted from Table S3)*			Cell viability (%) *(as quoted from Table S2)*		
	621-AuNR-Si-ZnPc-HA	621-AuNR-Si-HA	Difference	621-AuNR-Si-ZnPc-HA	621-AuNR-Si-HA	Difference
0	0	0	0	100	100	0
1	4.5	4.6	**0.1**	99.60	100.19	**0.59**
2	5.1	5.4	**0.3**	96.36	98.57	**2.21**
3	5.3	6.8	1.5	91.00	97.16	6.16

Table S5: Summary of materials used (top) and *in vitro* experiments conducted (bottom).

	Overlapping with LSPR	**Not overlapping with LSPR**
PTT	711-AuNR-Si-HA	621-AuNR-Si-HA
PDT	Si-ZnPc-HA	
PTT + PDT	711-AuNR-SI-ZnPc-HA	621-AuNR-Si-ZnPc-HA

	Cell line used	**Sample name**	**Controls**	**Figure number**
Biocompatibility studies	HeLa	711-AuNR-Si-ZnPc-HA	-	Fig. S10
Targeting & phototoxicity studies	HeLa	711-AuNR-Si-ZnPc-HA	711-AuNR-Si-HA Si-ZnPc-HA	Fig. 4b
	MCF-7	711-AuNR-Si-ZnPc-HA	711-AuNR-Si-HA Si-ZnPc-HA	Fig. 4c
LSPR effect	HeLa	711-AuNR-Si-ZnPc-HA	711-AuNR-Si-HA Si-ZnPc-HA	Fig. 4d Table S1
	HeLa	621-AuNR-Si-ZnPc-HA	621-AuNR-Si-HA Si-ZnPc-HA	

References

S1. B. Nikoobakht and M. a. El-Sayed, *Chem. Mater.*, 2003, **15**, 1957–1962.

S2. M. Fujiwara, M. Akiyama, M. Hata, K. Shiokawa and R. Nomura, *ACS Nano*, 2008, **2**, 1671–1681.

S3. W. Stöber, A. Fink and E. Bohn, *J. Colloid Interface Sci.*, 1968, **26**, 62–69.