Supporting Information for

Near-Infrared Photothermal Release of siRNA from the Surface of Colloidal Gold-Silver-Gold Core-Shell-Shell Nanoparticles Studied with Second Harmonic Generation

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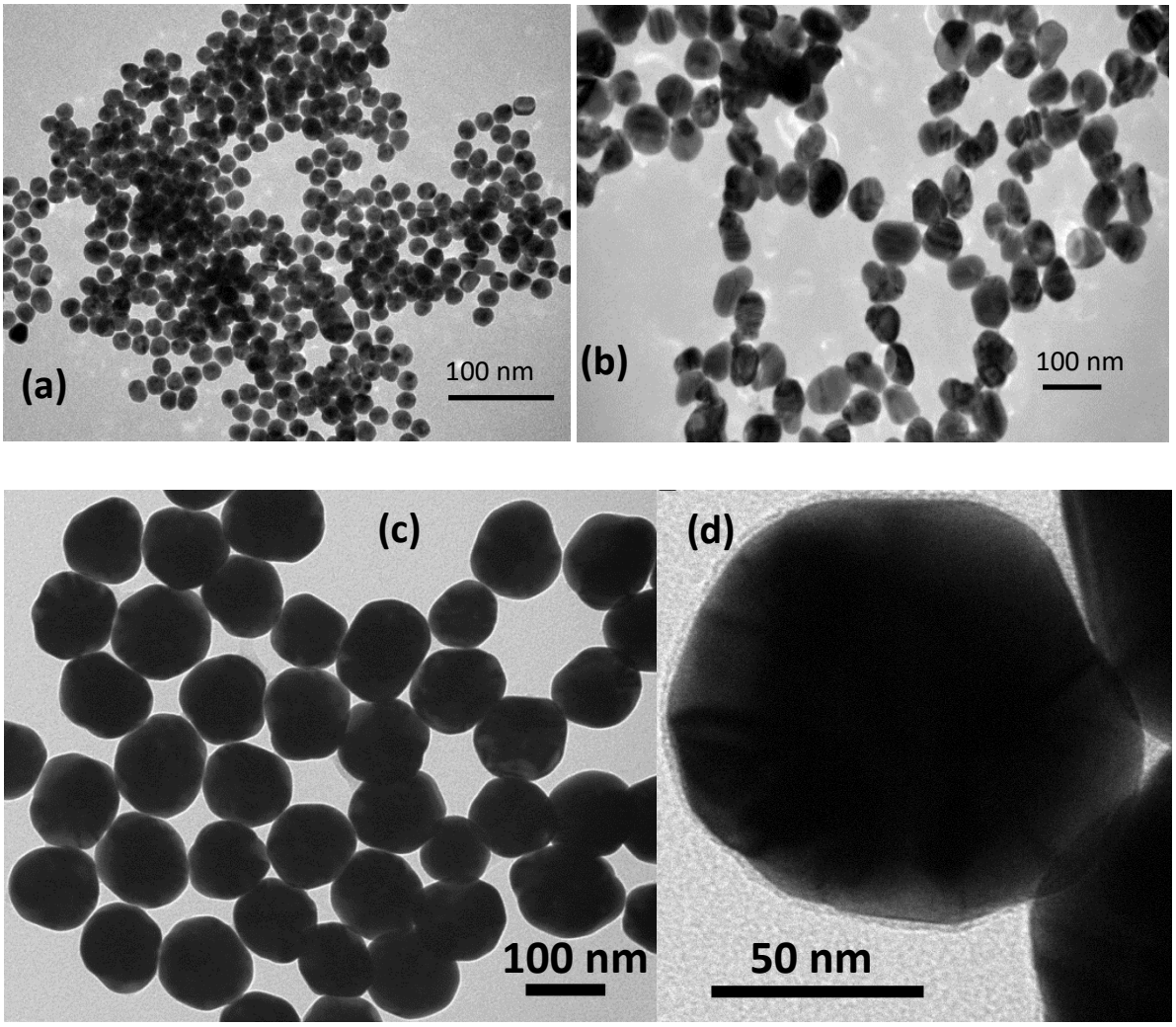
Gold and gold-silver-gold core-shell-shell nanoparticles are prepared using a procedure which has been described previously.[1-3] In the first step, gold nanoparticle seeds are synthesized by adding 900 μL of 34 mM sodium citrate to 30 mL of 290 μM gold chloride in ultrapure water under boiling conditions and vigorous stirring. The solution changes color to a bright red after 10 min and the reaction is cooled to room temperature. For the synthesis of larger gold nanoparticles, 50 μL of the seed solution is added to 3.9 mM of gold chloride in 9.8 mL of ultrapure water, followed by the addition of 100 μL of 0.03 M hydroquinone and 22 μL of 34 mM sodium citrate. The solution is left to stir at room temperature for 60 min to produce the 105 nm colloidal gold nanoparticles. For the growth of the silver shell, 200 μl of the gold nanoparticle seeds is added to 10 mL ultrapure water followed by the addition of 60 μL of 100 mM ascorbic acid, 15 μL of 100 mM silver nitrate, and 75 μL of 100 mM sodium hydroxide. The obtained gold-silver core-shell nanoparticle is then centrifuged and resuspended in 10 mL ultrapure water. The outer gold shell is grown by the addition of 100 μL of 29 mM chloroauric acid, 100 μL of 0.03 M hydroquinone and 25 μL of 34 mM sodium citrate in 10 mL of the gold-silver core-shell nanoparticle sample. The

solution is allowed to stir at room temperature for another 1 h. The obtained CSS nanoparticles are centrifuged and resuspended in 10 mL ultrapure water. Figure S1 shows the representative TEM images of gold nanoparticle seeds of size 18 ± 1 nm, (b) gold-silver core-shell nanoparticles of size 60 ± 10 nm, (c) gold nanoparticles of size 105 ± 10 nm, and (d) the 105 nm siRNA-functionalized gold nanoparticles.



Figures S1. Representative TEM images of (a) 18 nm gold nanoparticle seeds, (b) 60 nm gold-silver core-shell nanoparticles, (c) 105 nm gold nanoparticles, and (d) 105 nm siRNA-functionalized gold nanoparticles.

Retro-Diels-Alder based chemistry is used to tether the 5' fluorophore, 3' amine-modified miRNA-148b mimics (siRNA) to the CSS and gold nanoparticle surface by the thermally-cleavable furan and maleimide cycloadduct groups as described previously, with minor modifications.[4-5] Briefly, 4.19 g of 6-maleimide hexanoic acid is mixed with 1 mL of furfuryl mercaptan in 20 mL 1:1 (v/v) dichloromethane (99.8%) : methanol (DCM:MeOH) solvent. The reaction mixture is allowed to react for 7 days under agitating conditions at room temperature in a sealed container. Excess solvent is removed by gentle nitrogen flow for approximately 30 min until a viscous product remains. 1 mL aliquots of nanoparticles are separately centrifuged and resuspended in 0.5 mL of the Diels-Alder solution. After 24 h rocking in ambient conditions, the particles are centrifuged three times and cleaned with a 70%-30% ethanol-water solution. The 3' amine miRNA-148b mimic is linked to the functionalized nanoparticles via 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride)/N-hydroxysuccinimide (EDC/NHS) coupling and agitated for 24 h at room temperature. The final product is centrifuged, cleaned again, and then finally resuspended in ultrapure water.

Figure S2 shows the experimental extinction spectrum of the 105 nm GNPs in water (red line) with a localized surface plasmon peak centered on 573 nm, compared to the best fit from Mie theory (dashed black line) at a concentration of 3.0×10^8 nanoparticles/mL. Dynamic light scattering measurements determine the hydrodynamic diameter of the GNP sample to be 115 ± 39 nm with a polydispersity index of 0.1. The electrophoretic mobility for the CSS and the CSS functionalized with siRNA is shown in Figure S3. The zeta potential of the colloidal CSS nanoparticle before and after functionalization with siRNA is obtained from electrophoretic mobility measurements using the Huckel approximation.[6] The electrophoretic mobility of siRNA-

CSS sample is higher than the CSS nanoparticle. The increase in the zeta potential is due to the increase in surface charge density of the CSS nanoparticles after siRNA functionalization.

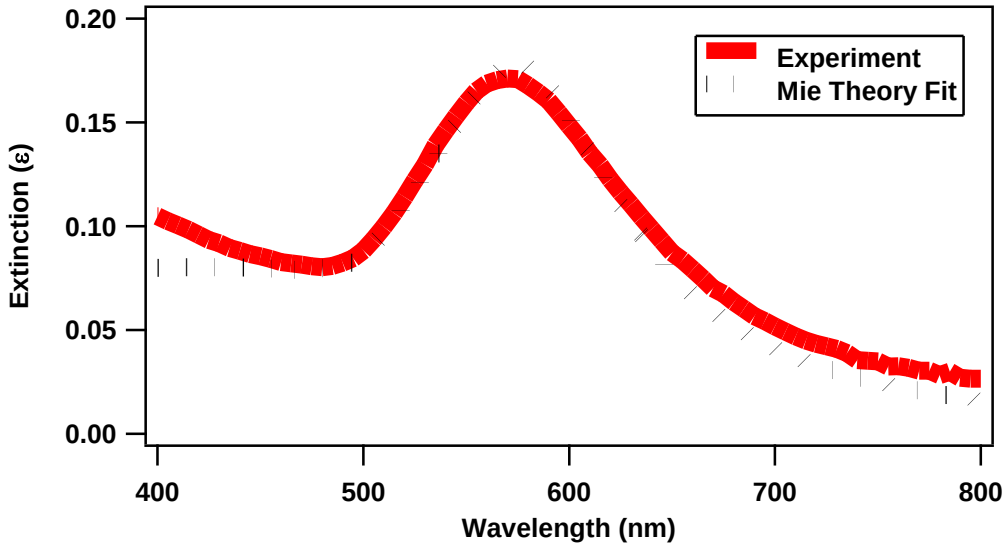


Figure S2: Extinction spectrum of the 105 nm colloidal gold nanoparticle sample in water (red line) compared with the best fit from Mie theory (dotted black line).

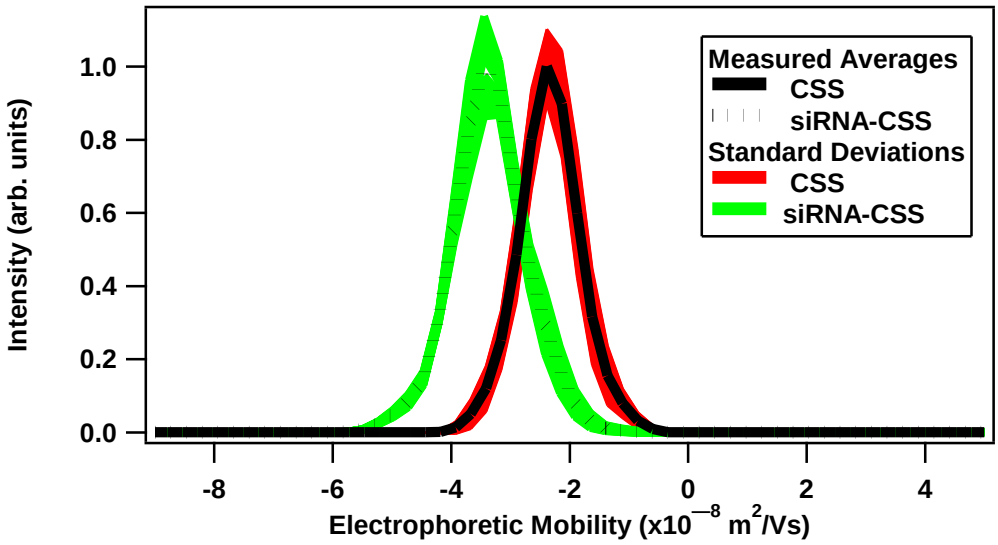


Figure S3. Electrophoretic mobility plot of CSS nanoparticles and siRNA-functionalized CSS nanoparticles showing the measured averages (solid black and dashed lines respectively) and corresponding standard deviations (red and green areas, respectively).

Figure S4 (a) shows the power-dependent analysis of the photothermal release of siRNA from the surface of the siRNA-functionalized GNP sample at a concentration of approximately 1.1 x 10^8 nanoparticles/mL. Here, the siRNA surface coverage is determined to be 370 ± 10 oligo/nanoparticle using fluorescence quantification. The sample is irradiated with different laser powers of 0 mW, 20 mW, 50 mW, 100 mW, and 200 mW centered at 800 nm using the amplifier laser. The time profile using no NIR irradiation from the amplifier laser (0 mW) shows only a slight decrease in SHG intensity of approximately 1% over time which is attributed to a small amount of photothermal cleaving by the oscillator probe laser. The kinetics of the photothermal release of siRNA from the GNPs under NIR irradiation is also analyzed by fitting the SHG time traces using a pseudo first-order exponential equation given by $E_{SHG} = A + B\, e^{-kt}$, where A and B are constants. The obtained rate constants k are (0.03 ± 0.18) × 10^{-2} s^{-1}, (0.28 ± 0.17) × 10^{-2} s^{-1}, (1.03 ± 0.16) × 10^{-2} s^{-1}, (1.59 ± 0.26) × 10^{-2} s^{-1} and (1.68 ± 0.18) × 10^{-2} s^{-1} for 0 mW, 20 mW, 50 mW, 100 mW, and 200 mW irradiation laser powers, respectively. The obtained rate constants are plotted as a function of laser power, as shown in Figure S4 (b).

Figure S5 shows the SHG time trace during thermal release of siRNA from the surface of gold nanoparticle at different temperatures. The sample is allowed to run for 20 min under slow stirring for each measurement using temperatures of 25 °C, 40 °C, 60 °C, and 80 °C while measuring the SHG signal as a function of time. There is no thermal release of siRNA at 25 °C and 40 °C indicating that the oligonucleotides do not release at room temperature or at normal body temperature, in agreement with the results from the siRNA-functionalized CSS nanoparticles.

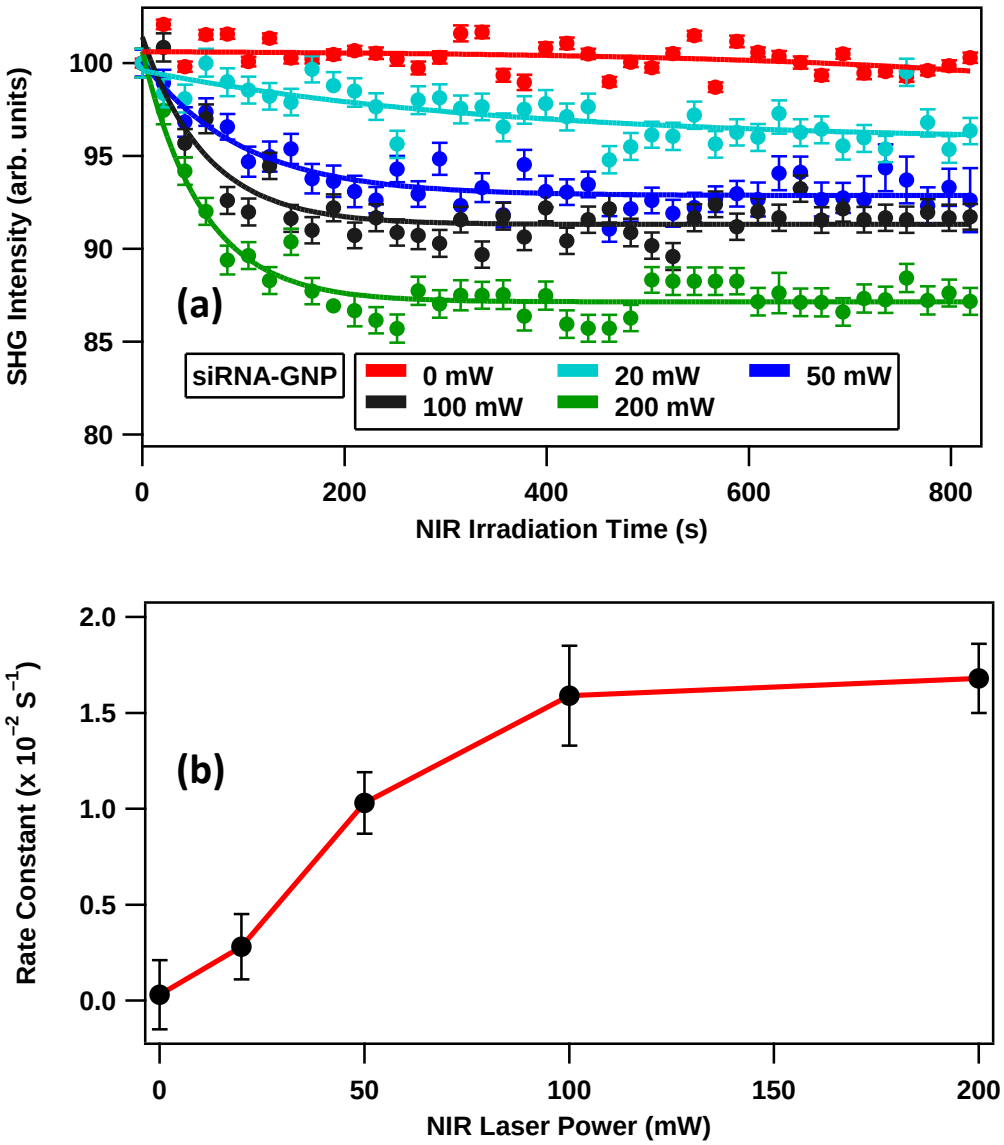


Figure S4. (a) SHG intensity of the siRNA-functionalized gold nanoparticles as a function of time under varying NIR irradiation powers. (b) The obtained photothermal cleaving rate constants as a function of laser power.

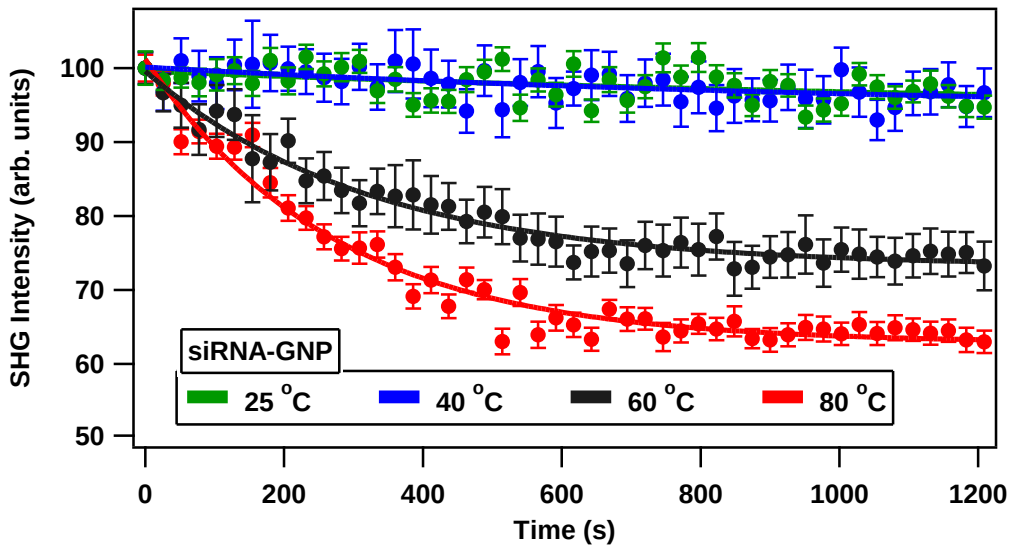


Figure S5: SHG intensity as a function of time from the siRNA-functionalized gold nanoparticles showing the thermal release of siRNA from the nanoparticle surface under different temperatures.

Table S1: Fit parameters obtained for siRNA-CSS nanoparticle system at different laser powers

Power (mW)	Fit Parameters (siRNA-CSS)		
	y_0	A	τ^{-1} (10^{-2} S^{-1})
0	96.4 ± 2.3	4.3 ± 2.0	0.03 ± 0.15
20	65.4 ± 0.7	35.8 ± 1.1	0.78 ± 0.06
50	60.4 ± 0.5	41.9 ± 1.1	1.12 ± 0.06
100	52.2 ± 0.2	47.9 ± 0.6	1.77 ± 0.04
200	46.6 ± 0.3	54.0 ± 1.0	1.85 ± 0.06

Table S2: Fit parameters obtained for siRNA-GNP system at different laser powers

Power (mW)	Fit Parameters (siRNA-GNP)		
	y_0	A	τ^{-1} (10^{-2} S^{-1})
0	100.7 ± 0.5	0.07 ± 0.28	0.03 ± 0.18
20	95.8 ± 0.8	3.8 ± 0.7	0.3 ± 0.2
50	92.9 ± 0.2	7.3 ± 0.6	1.0 ± 0.2
100	91.3 ± 0.2	10.1 ± 0.9	1.6 ± 0.3
200	87.2 ± 0.2	13.5 ± 0.8	1.7 ± 0.2

Table S3: Fit parameters obtained for siRNA-CSS system at different temperatures

Temperature (°C)	Fit Parameters (siRNA-CSS)		
	y_0	A	τ^{-1} (10^{-2} S^{-1})
25	97.3 ± 0.3	3.5 ± 0.7	0.08 ± 0.04
40	96.6 ± 0.7	3.6 ± 0.7	0.13 ± 0.08
60	73.4 ± 0.2	28.4 ± 1.0	1.12 ± 0.07
80	59.7 ± 0.2	41.6 ± 1.0	1.09 ± 0.05

Table S4: Fit parameters obtained for siRNA-GNP system at different temperatures

Temperature (°C)	Fit Parameters (siRNA-GNP)		
	y_0	A	τ^{-1} (10^{-2} S^{-1})
25	94.5 ± 14.1	6.1 ± 13.5	0.06 ± 0.05
40	94.1 ± 5.4	6.1 ± 5.0	0.09 ± 0.08
60	73.3 ± 0.7	26.5 ± 0.8	0.31 ± 0.02
80	62.8 ± 0.6	38.4 ± 1.1	0.37 ± 0.03

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