Electronic Supplementary Material (ESI) for Chemical Communications.

Supporting Information

Plasmon-controlled shaping of gold nanostar photothermal therapy agents

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Experimental

Materials

N-methyl-D-glucamine (≥99.0%), gold(III) chloride trihydrate (≥99.9%), sodium hydroxide (≥98.0%), and hydrochloric acid (37%) were received from Sigma Aldrich, St. Louis, MO USA. Water used was obtained from an ultrapure ELGA Purelab flex water purifier, purified to a resistivity of 18.2 MΩ cm. Rebel 7 LED Round Modules were purchased from Luxeon StarLEDs by QUADICA, Lethbridge, Alberta, Canada. LEDs were controlled by a proprietary, custom-built control board. Blue (470 nm) LED – 490 lm @ 700 mA, Green (530 nm) LED – 1127 lm @ 700 mA, Amber (591 nm) – 539 lm @ 700 mA, Red (627 nm) – 742 lm @ 700 mA, Deep Red (655 nm) – 4480 mW @ 700mA. A Polymer Optics 7 LED Cluster Concentrator Optic (Par No. 263, LuxeonStar, Lethbridge, Alberta Canada) was utilized to focus output of the 7 Rebel LEDs into a single beam diameter that is 12 mm wide, 25 mm in front of the lens with an efficiency of 85%. A custom, ethylene glycol-cooled temperature control container was utilized to offset any heating effects from the LEDs themselves, in conjunction with 130x70 mm Rectangular 40 mm high Alpha Heat Sink – 1.8 ^{o}C/W (LuxeonStar). 20 mL borosilicate glass scintillation vials (Fisher) were washed with Aqua Regia (3:1 HCl:HNO_3) overnight followed by washing with ultrapure water and dried before use.

Characterization

The UV/Vis extinction spectra for the gold colloids were measured at room temperature with a Cary 60 UV/Vis spectrophotometer using a quartz cuvette. The transmission electron microscopy (TEM) images were conducted on a JEOL JEM-1400 instrument operating at 120 kV. The sample was drop-casted onto carbon-coated copper grids (Ted Paella) and allowed to dry for 24 h before analysis. The particle size distribution was calculated using ImageJ software. For each sample, 300 particles were measured to determine particle size distributions. Zeta potential measurements were conducted by a Malvern Zetasizer Nano-ZS with a He-Ne laser (λ = 633 nm).

Synthesis of Gold Nanoparticles – *R* value Study

The gold nanoparticles synthesized at various [NMGA]:[$HAuCl_4$] ratios, R_{NMGA}, were conducted in a one-pot method. Briefly, 3.9 mL of ultrapure deionized water was added to clean 20 mL scintillation vial. 1.0 mL of various concentrations of aqueous *N*-methyl-D-glucamine stock was added followed by 5 s of rapid vortex (0.2 – 4.8 mM final concentrations). Then, 0.1 mL of aqueous 10 mM $HAuCl_4$ (0.2 mM final concentration) was added by autopipette, during 5 s of rapid vortex. The particles were aged for 24 h in a light-free environment before analyzed by UV/Vis spectroscopy or dropcast onto a TEM grid.

Synthesis of Gold Nanoparticles – pH Study

The gold nanoparticles synthesized at R_{NMGA} = 12 at various pH values were conducted in a one-pot method. Briefly, 3.9 mL minus the amount of acid or base to be added, ultrapure deionized water was added to clean 20 mL scintillation vial. 1.0 mL of aqueous 12 mM *N*-methyl-D-glucamine stock was added followed by 5 s of rapid vortex (2.4 mM final concentration). An amount of aqueous 100 mM NaOH or 10 mM HCl were added, followed by 5 s of rapid vortex and an equilibration time of 10 min. Then, 0.1 mL of aqueous 10 mM $HAuCl_4$ (0.2 mM final concentration) was added by autopipette, during 5 s of rapid vortex. The particles were aged for 24 h in a light-free environment before analyzed by UV/Vis spectroscopy or dropcast onto a TEM grid.

Synthesis of Gold Nanoparticles – Temperature Study

The gold nanoparticles synthesized at R_{NMGA} = 12 at various temperatures were conducted in a one-pot method. Briefly, 3.9 mL of ultrapure deionized water was added to clean 20 mL scintillation vial. 1.0 mL of various concentrations of aqueous *N*-methyl-D-glucamine stock was added followed by 5 s of rapid vortex (0.2 – 4.8 mM final concentrations). The vial was then placed in an oil bath set to a determined temperature to ensure desired solution temperature. Then, 0.1 mL of aqueous 10 mM $HAuCl_4$ (0.2 mM final concentration), heated to desired temperature, was added by autopipette, during 5 s of rapid stirring but magnetic stirbar. The stirbar was subsequently removed and the solution was allowed to age at the desired temperature for 30 min. The particles were aged for 24 h in a light-free environment before analyzed by UV/Vis spectroscopy or dropcast onto a TEM grid.

Synthesis of Gold Nanoparticles – Plasmon-mediation Study

The gold nanoparticles synthesized at R_{NMGA} = 12 under five different visible wavelength LEDs were conducted in a one-pot method. Briefly, 3.9 mL of ultrapure deionized water was added to clean 20 mL scintillation vial. 1.0 mL of aqueous 12 mM *N*-methyl-D-glucamine stock was added followed by 5 s of rapid vortex (2.4 mM final concentration). Then, 0.1 mL of aqueous 10 mM $HAuCl_4$ (0.2 mM final concentration) was added by autopipette, during 5 s of rapid vortex. The vial was immediately placed into a custom built cooling chamber with circulating ethylene glycol set to maintain solution temperature at room temperature (20 °C) under with one of the five LEDs was on. The particles were grown under plasmon-mediation for 30 min before removal and subsequent aging for 24 h in a light-free environment before analyzed by UV/Vis spectroscopy or dropcast onto a TEM grid.

Cytotoxicity Studies

Cultivated human colon adenocarcinoma cells, HCT-15, were harvested through a typical method.[1] The cells were plated in shallow wells, with 100 µL of either saline control, 5, 10, 30 or 50 µg/mL of NMGA-reduced gold nanoparticles synthesized through the plasmon-mediated route. The concentration of AuNP sample is defined by the mass of Au, coming from the $HAuCl_4$ precursor, used in each synthetic method previous described, divided by total solvent volume. The cells were then incubated for 48 h at 30 °C. Cell viability was tested through a Sulforhodamine B (SRB) assay.[2]

Photothermal Conversion Efficiency Measurements

To evaluate the photothermal conversion efficiency of the synthesized gold nanoparticles, the temperature change of the aqueous colloids (0.5 mL, 80 µg/mL) was measured overtime as exposed to a near infrared laser (808 nm, 1.6 W cm^{-2} at a distance of 3 cm. The laser and cuvette containing the samples were contained in a custom 3D-printed holder to ensure precision. A thermocouple was threaded through the bottom of the cuvette, outside the reach of the laser's beam itself. Temperature readings were taken every 100 ms through three consecutive on-off cycles of the laser. Photothermal conversion efficiency was evaluated by measuring the temperature change of the aqueous cold colloids as the solutions cooled from a steady-state temperature during an "off" cycle of the laser. The photothermal conversion efficiency, η, was calculated using methods described previously in literature.[3, 4] Using Equation 1, where h is the heat transfer coefficient, A is the surface area of the container, T_{max} is the steady-state temperature, T_{surr} is the ambient room temperature, I is the incident laser power (1.6 W

cm^{-2}, E_λ is the extinction of the colloids at 808 nm, *Q*s is the heat associated with the absorbance of the water blank, 6.317 mW.

$$\eta = \frac{hA\Delta T_{max} - Q_s}{I(1 - 10^{-E_\lambda})} \quad (1)$$

In order to solve for *h*, the heat transfer coefficient, Equation 2 is used:

$$\tau_s = \frac{m_D C_D}{hA} \quad (2)$$

T_s is the sample system time constant, m_D is the mass of the deionized water blank (0.5 g) and C_D is the heat capacity (4.184 J g^{-} °C^{-}). To solve for *hA*, the constant Θ is introduced and *hA* can be found by applying the linear time data from the "off" cycle after the steady-state temperature is reached versus -lnΘ as seen in Figure S5.

$$\Theta = \frac{\Delta T}{\Delta T_{max}} \quad (3)$$

Photothermal Therapy of HCT-15 cells

To evaluate the photothermal therapy ability of the gold nanoparticles, 100 µL of selected particles at 10, 30 or 50 µg/mL were added to wells containing HCT-15 cells. The concentration of AuNP sample is defined by the mass of Au, coming from the $HAuCl_4$ precursor, used in each synthetic method previous described, divided by total solvent volume. Laser irradiation was preformed by attaching the laser 3 cm above the well and irradiating the cells for 5, 10 or 20 min. The cells were then incubated at 30 °C for 48 h and cell viability was measured using an SRB assay.

Supplementary Figures

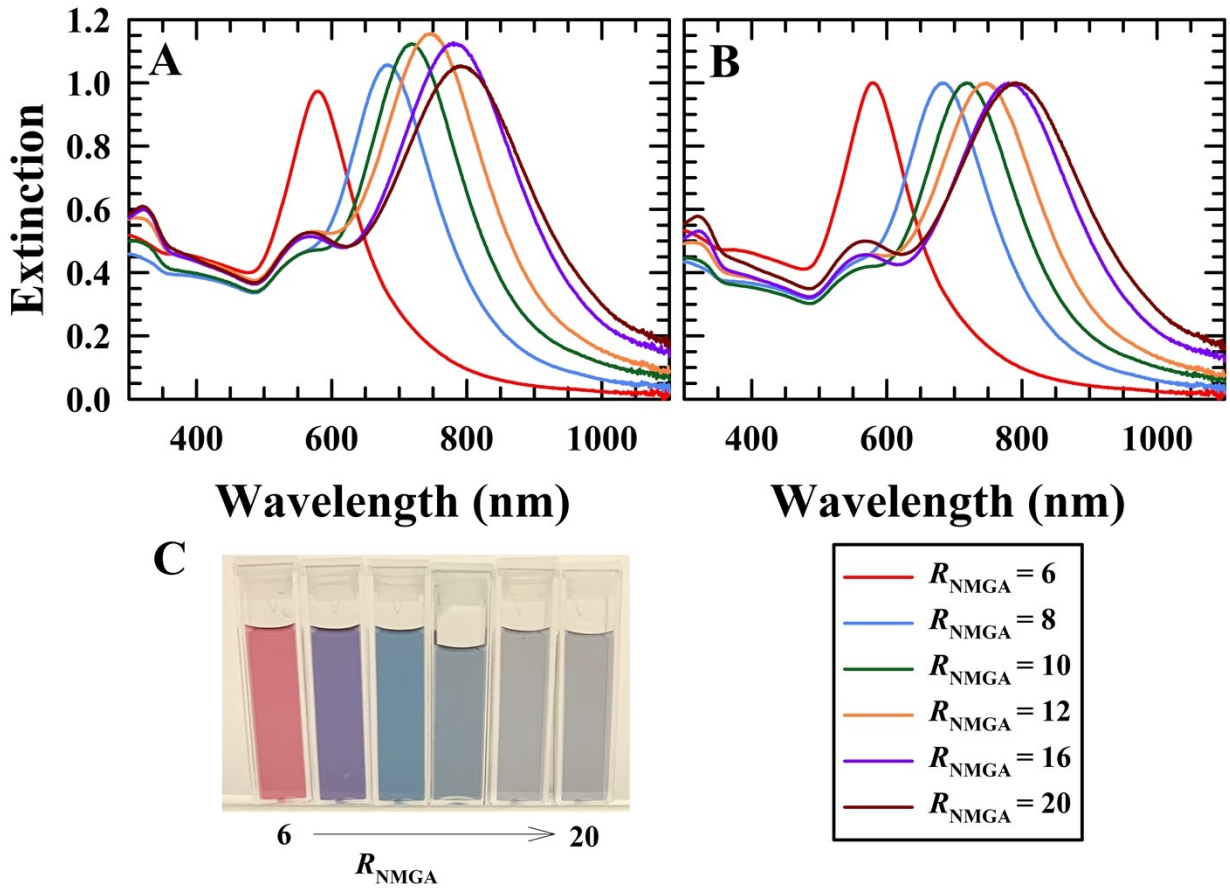

Fig. S1. a) Selected UV-vis extinction spectra and b) normalized spectra of *N*-methyl-D-glucamine-reduced gold nanosystems containing 0.2 mM Au(III) and various concentrations of NMGA at a constant pH of 11. Spectra and c) photograph of colloids were collected after 24 h of aging in the dark.

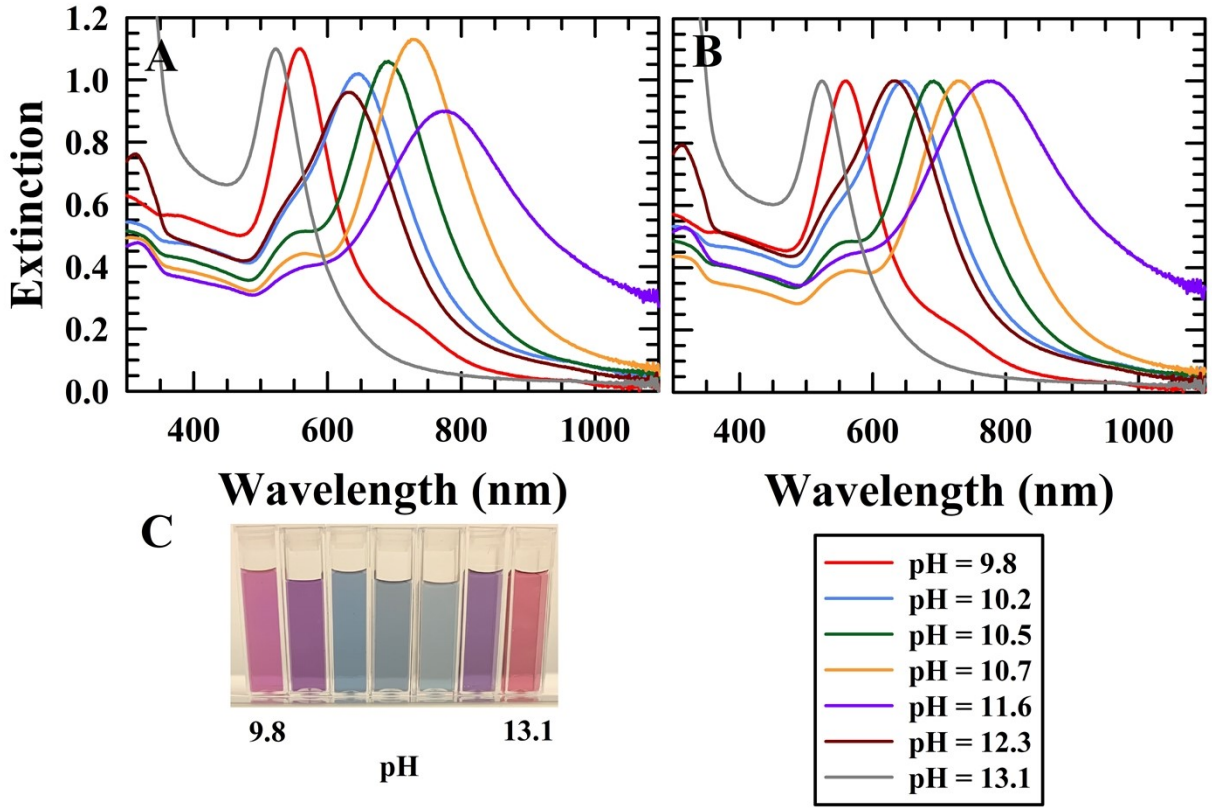

Fig. S2. a) UV-vis extinction spectra and b) normalized spectra of *N*-methyl-D-glucamine-reduced gold nanosystems containing 0.2 mM Au(III) and 2.4 mM NMGA adjusted to various pH values using either dilute HCl or NaOH. Spectra and c) photograph of colloids were collected after 24 h in the dark.

Table S1. Size analysis of the pH adjusted R_{NMGA} = 12 gold nanoparticles, analyzed using ImageJ software from TEM images (n = 300)

Growth Solution pH	Core Size (nm)	Tip Size (nm)	Total Size (nm)
10.5	77 ± 11	34 ± 6	111 ± 13
10.7	72 ± 12	41 ± 11	113 ± 16
10.8	89 ± 28	29 ± 10	118 ± 30
10.9	82 ± 13	32 ± 12	114 ± 18
11.6	85 ± 40	20 ± 6	105 ± 40
12.3	68 ± 12	14 ± 7	82 ± 14
12.7	14 ± 11	-	14 ± 11
12.8	10 ± 4	-	10 ± 4

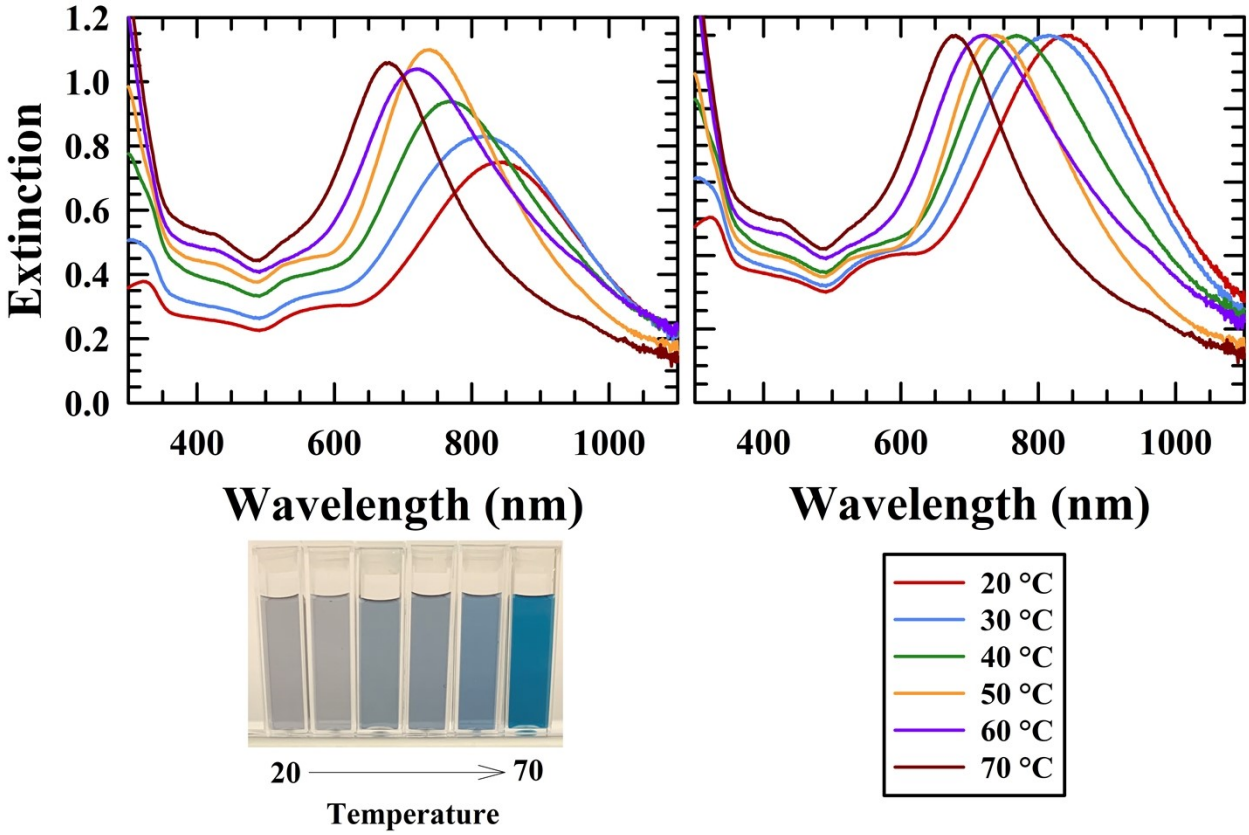

Fig. S3. A) UV-Vis extinction spectra and B) normalized UV-Vis spectra of gold nanoparticles synthesized through the reduction of Au(III) by *N*-methyl-D-glucamine at an R_{NMGA} = 12 at various reaction temperatures. Final Au(III) concentration = 0.2 mM, final NMGA concentration = 2.4 mM. Particles synthesized in 20 mL glass scintillation vials in oil bath for 30 min under specified conditions. Spectra and C) photograph of colored colloids taken after 24 h aging in dark.

Table S2. Size analysis of the plasmon-mediated R_{NMGA} = 12 gold nanoparticles, analyzed using ImageJ software from TEM images (n = 300)

LED Wavelength (nm)	Core Size (nm)	Tip Size (nm)	Total Size (nm)
470	16 ± 3	-	16 ± 3
530	34 ± 24	-	34 ± 24
591	63 ± 9	26 ± 13	89 ± 16
627	88 ± 15	33 ± 10	121 ± 18
655	113 ± 20	42 ± 9	155 ± 22
No LED	82 ± 13	32 ± 12	114 ± 18

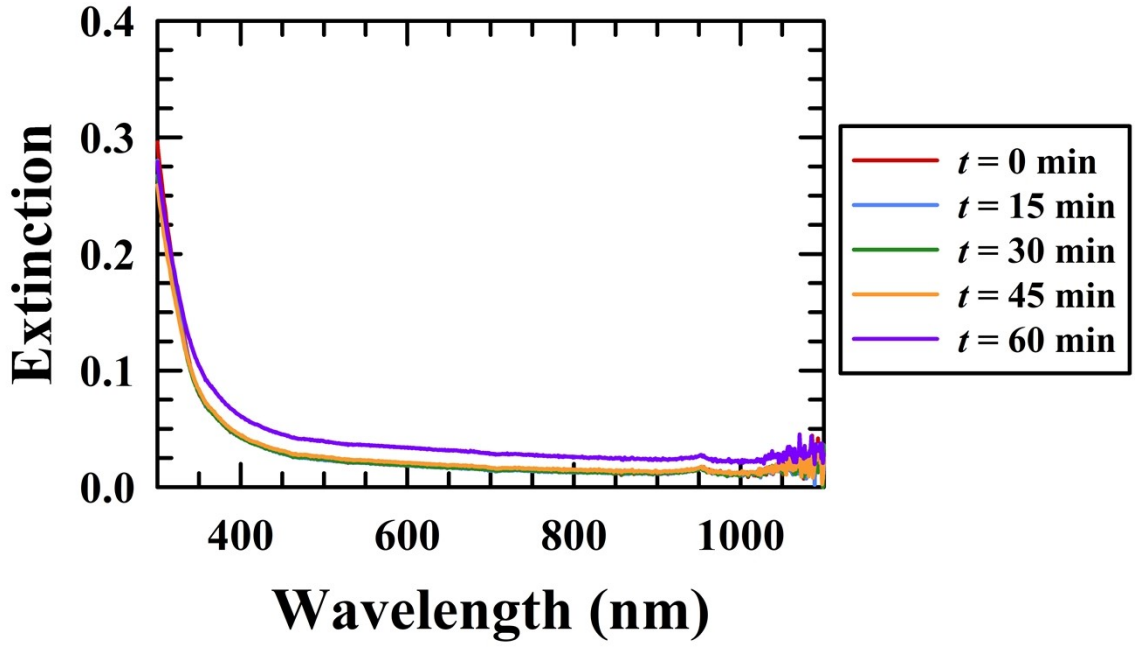

Fig. S4. UV-Vis extinction spectra of the first hour of nanoparticle growth of gold nanoparticles synthesized through the reduction of Au(III) by *N*-methyl-D-glucamine at an R_{NMGA} = 12 at room temperature. Final Au(III) concentration = 0.2 mM, final NMGA concentration = 2.4 mM.

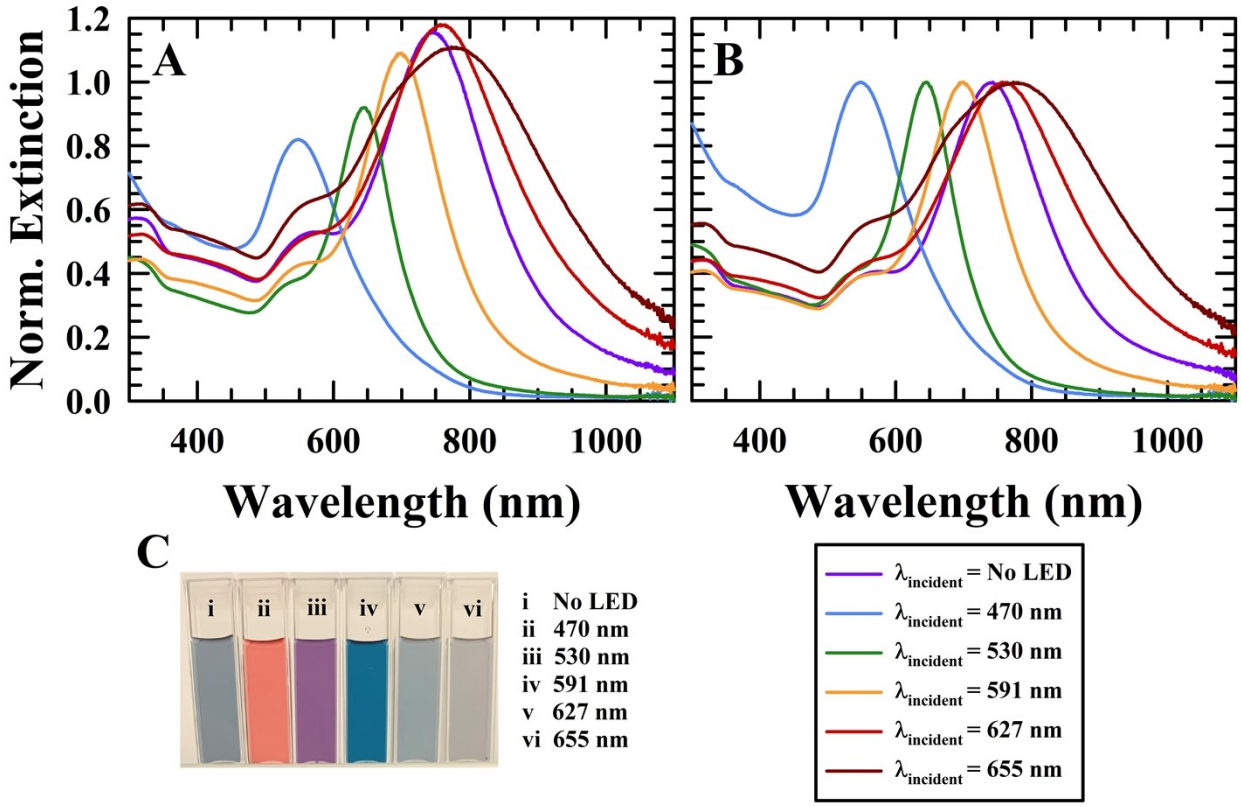

Fig. S5. A) UV-Vis extinction spectra and B) normalized UV-Vis extinction spectraof gold nanoparticles synthesized through the reduction of Au(III) by *N*-methyl-D-glucamine at an R_{NMGA} = 12 under various high-powered visible LEDs for 30 min. Final Au(III) concentration = 0.2 mM, final NMGA concentration = 2.4 mM. Particles synthesized in 20 mL glass scintillation vials at room temperature, maintained by a custom cooling device. Spectra and c) photograph of colored colloids taken after 24 h aging in dark.

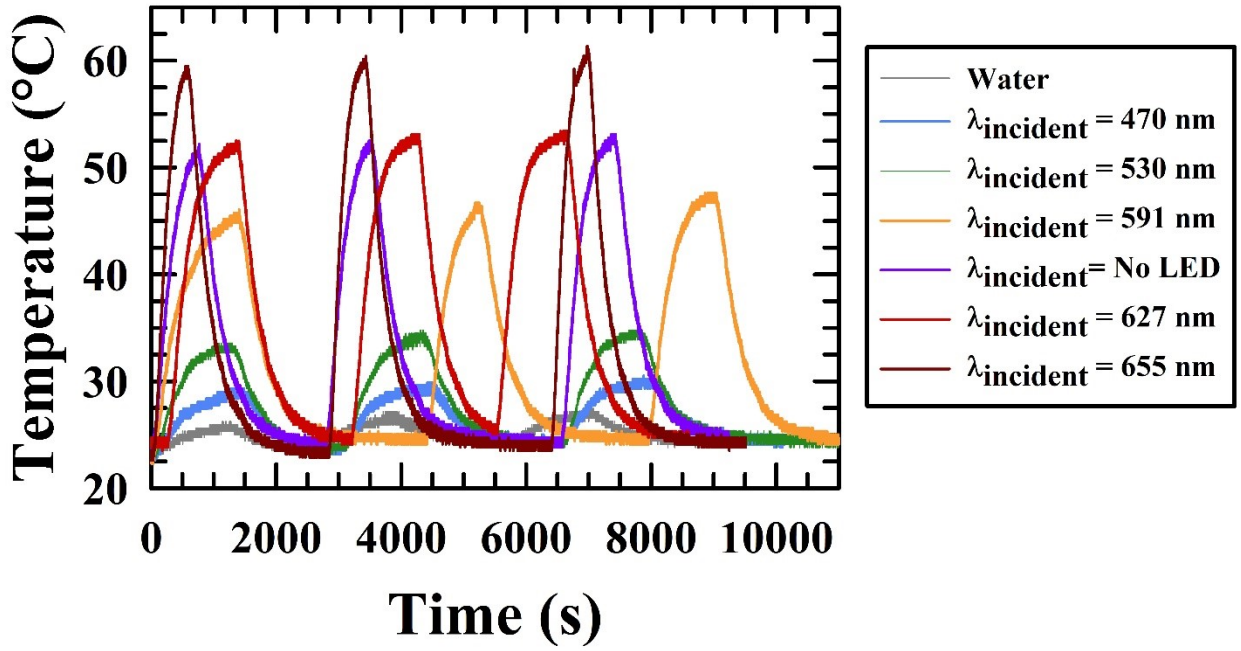

Fig. S6. Photothermal response of the plasmon-mediated gold nanoparticles (0.5 mL, 80 µg/mL), irradiated with a NIR laser (808 nm, 1.6 W cm^{-}) until temperature maxima reached. Heating followed by cooling by turning off of laser and subsequent two cycles of irradiation and cooling.

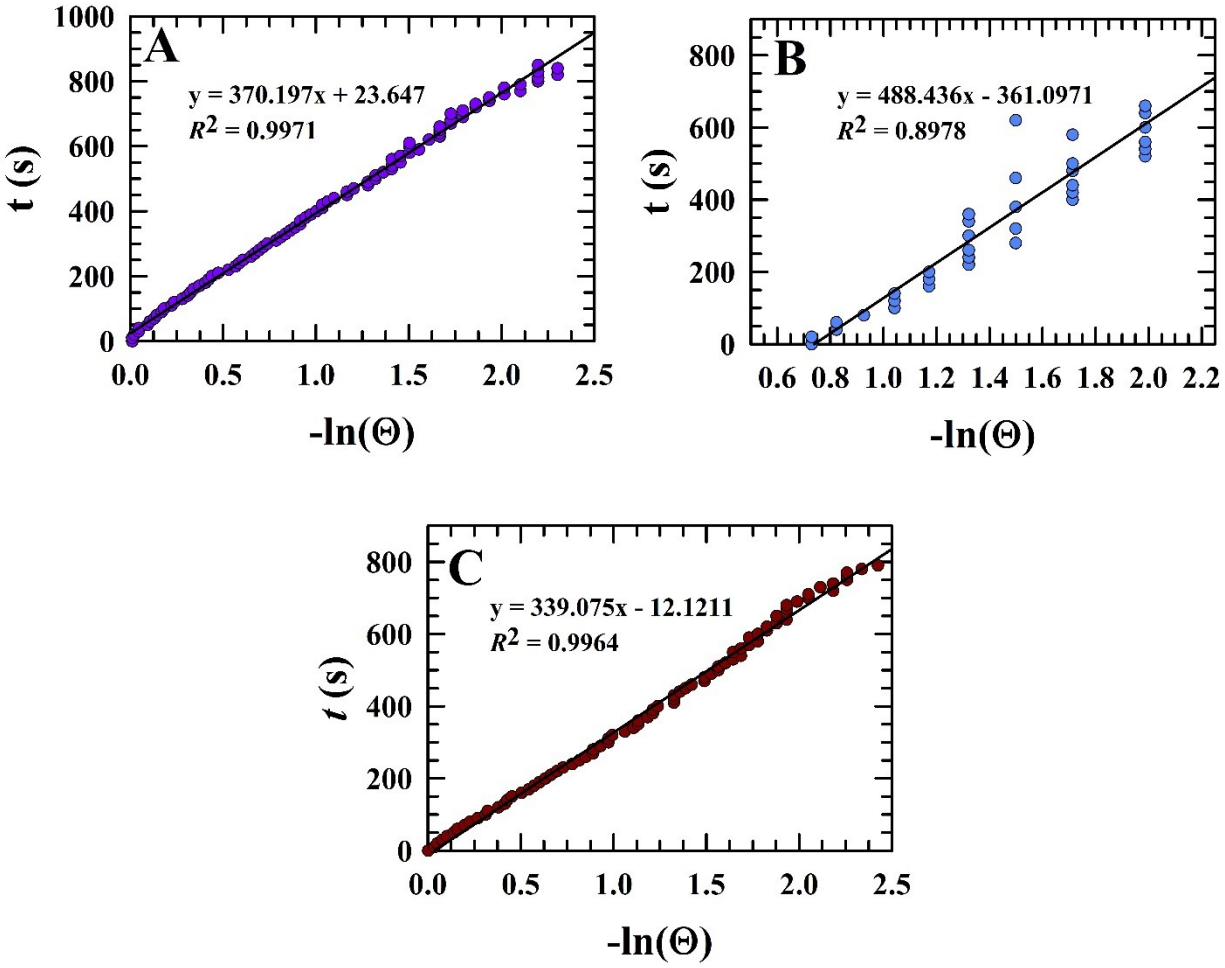

Fig. S7. Linear time data versus -lnΘ obtained from the cooling periods of the three NMGA-reduced gold nanoparticles tested for photothermal therapy effects on the HCT-15 cells: those synthesized with R_{NMGA} = 12 and A) No LED, B) $\lambda_{incident}$ = 470 nm, and C) $\lambda_{incident}$ = 655 nm

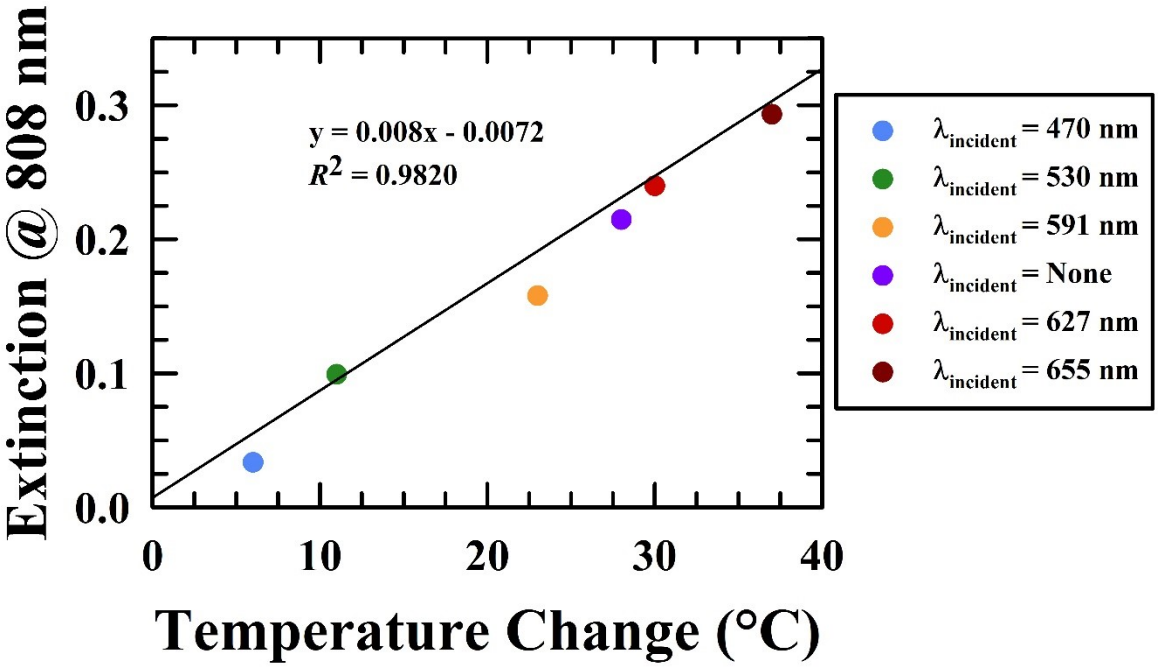

Fig. S8. Maximum temperature change of the five plasmon-mediated NMGA gold nanoparticles and one no LED standard NMGA gold nanoparticle, all at R_{NMGA} = 12, while irradiated by NIR laser (0.5 mL, 80 µg/mL, 1.6 W cm^{-2}) plotted versus the blank-subtracted extinction at 808 nm of the respective samples.

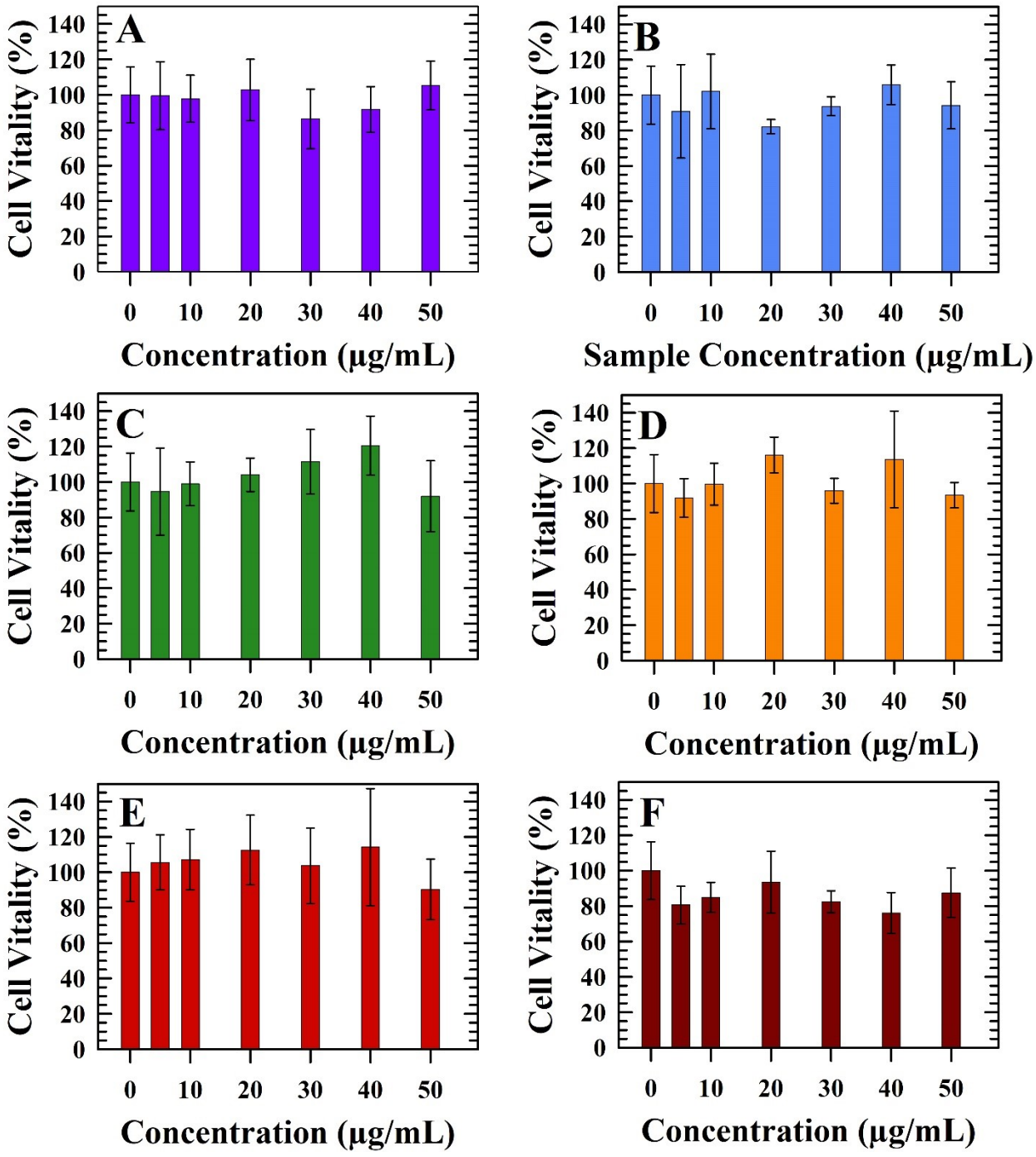

Fig. S9. Cell viability of HCT-15 cells measured by SRBA assay after 48 h incubation with various concentrations of NMGA-reduced AuNPS synthesized at an RNMGA = 12 under A) No LED or 30 min B) 470 nm, C) 530 nm, D) 591 nm, E) 627 nm, or F) 655 nm incident light irradiation. Each sample analyzed in triplicate.

References

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2. V. Vichai and K. Kirtikara, *Nat. Protoc.*, 2006, **1**, 1112-1116.
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