ESI - ELECTRONIC SUPPLEMENTARY INFORMATIONS

Monolayers of gold nanostars with two Near-IR LSPR capable of additive photothermal response

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S1. INSTRUMENTATION.

S1.1Thermograms. Thermograms were recorded using a FLIR (FSI Thermovision ALERT-PAL) thermocamera interfaced to the acquisition software Thermacam Researcher 2001.

S1.2 Laser sources. As laser sources we employed Thorlabs (Thorlabs, USA) He-Ne laser tuned at 633 nm (model HNL210L, working at the power = 30 mW, beam diameter 0.5 mm, 15.3 W/cm^2 irradiance), and Thorlabs 1064 nm diode laser (model M9-A64-0300, 50 mW power, beam diameter 1.2 mm, 4.4 W/cm^2 irradiance). The slides were irradiated by both laser simultaneously by letting the laser beam impinge on the slide at 45° with respect to the slide surface. The He-Ne laser was used without any beam expander. The shape of the cross-section of the laser diode was circularized at best by means of a pair of anamorphic prisms and the beam was focused on the slide surface in order to obtain an average beam waist of 1.2 mm.

S1.3 Absorption spectra. Absorbance spectra of colloidal suspensions were taken with a Varian Cary 100 spectrophotometer in the 200–1000 nm range or with a Cary 6000 spectrophotometer in the 300-1800 nm range. Spectra of GNS-functionalized glass slides were obtained placing the glasses on the same apparatus equipped with a dedicated Varian solid sample holder, or directly using the modified cuvettes, in this latter case for the determination of the local refractive index as a function of the solvent refractive index.

S1.4 Transmission Electron Microscopy. TEM images were obtained on colloidal solutions of GNS prepared as described in S 2.2, coated with excess $PEG_{2000}SH$ (polyethyleneglycol thiol, mw 2000, concetration 2×10^{-5}M,) and ultracentrifuged. The pellet of coated GNS was separated from the supernatant and redissolved in bidistilled water using a 10-fold larger volume with respect to the starting one (this process is necessary to decrease the TritonX-100 content of the solution, that otherwise would decompose the Parlodion membrane). A 10 μL drop of this colloidal solution was deposited on Nickel grids (300 mesh) covered with a Parlodion membrane and observed with a Jeol JEM-1200 EX II instrument.

S1.4 Scanning Electron Microscopy. The SEM-BSE images reported in Figure 1 of the main text and in Figures SI6.1-6.6, were acquired by a Jeol JSM 7500 FA scanning electron microscope, equipped with a cold field emission gun, and operating at an acceleration voltage of 5 kV. Due to the concomitant effect of the not conductive nature of the glass substrates and the low density of GNS deposed on them, the samples showed a strong tendency to be charged by the electron beam. To overwhelm this effect, a 10 nm-thin layer of Chromium was deposed by a high-resolution sputter coater (Cressington 208 HR) on the samples. In order to collect the image of the actual GNS covered by Cr, the compositional contrast

provided by the backscattered electron (BSE) signal was exploited, then allowing to image only the gold part of the Cr-covered GNS. An in-chamber solid state multiple quadrant detector was used to acquire the SEM-BSE images.

The morphologies of GNS-coated slides of Figure S31A-C were observed under Tescan Mira XMU variable pressure Field Emission Scanning Electron Microscope – FEG SEM (Tescan USA Inc., USA). Slides were mounted onto Aluminum stubs using double sided carbon adhesive tape and were then made electrically conductive by coating in vacuum with a thin layer of graphite (few nm). Observations were made in backscattered electrons mode (BSE) at 30 kV and with InBeam secondary electron detector for higher spatial resolution.

S2. SYNTHETIC METHODS

S2.1 Materials. Trimethoxysilylpropyl(polyethylenimine) (50% in isopropanol, mw 2000-4000) was purchased from Gelest Inc. TritonX-100, Tetrachloroauric acid, Sodium borohydride, Ascorbic acid, Silver Nitrate, were all purchased from Sigma-Aldrich and used without further purification. PEG_{2000}-SH (α-Methoxy- ω - Mercapto poly(ethylene glycol) for preparing the coated samples for TEM imaging) was purchased from Iris Biotech.

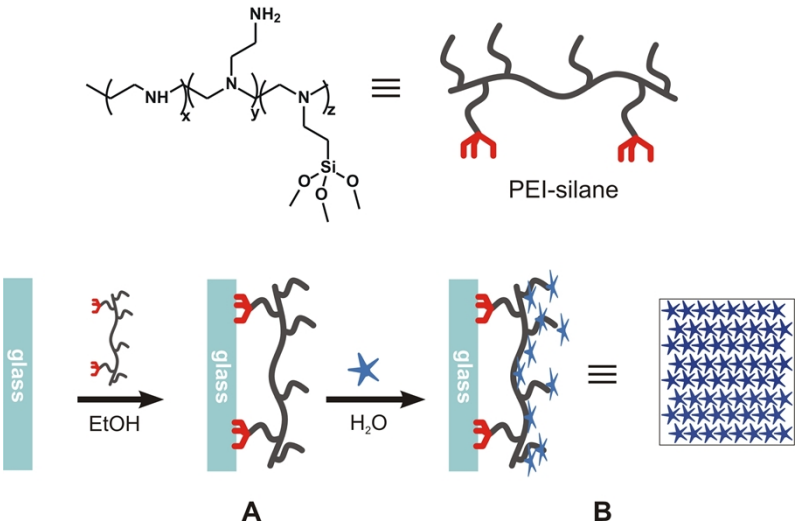
Microscopy cover glass slides (2.6 x 2.1 cm) were purchased from Forlab (Carlo Erba).

S2.2 GNS preparatation. All the glassware used for seed-growth methods was always pretreated before use. It was washed in aqua regia for 30 min, then washed and filled with bidistilled water and ultrasonicated for 3 minutes before discarding water. The bidistilled water/ultrasound treatment was repeated 3 times.

S2.2.1 – GNS with $LSPR_A$ at 680nm and $LSPR_B$ at 1320 nm (these are the GNS used in the paper). A seed solution was prepared in a 20mL vial. 5mL of $HAuCl_4$ $5*10^{-4}$M in water were added to 5mL of an aqueous solution of TritonX-100 0.1M (or 0.2 or 0.3 M). The mixture is gently hand-shaken and a pale yellow colour appeared. Then, 0.6mL of an ice-cooled solution of $NaBH_4$ 0.01M in water were added. The mixture was gently hand-shaken and a reddish-brown colour appeared. The seed solution wss kept in ice and used within 2 hours. The growth solution was prepared in a 20mL vial. 250µL of $AgNO_3$ 0.004M in water and 5mL of $HAuCl_4$ 0.001M in water were added in this order to 5mL of an aqueous solution of TritonX-100 0.100M. Then, 140µL of an aqueous solution of ascorbic acid 0.0788M were added. The solution, after gentle mixing, became colourless. After this, 12µL of the seed solution were added. The solution was gently hand-shaken and a grey colour appeared and quickly changing to more intense and blue-black colors. The sample was allowed to equilibrate for 1h at room temperature before using it for the coating procedurs (S2.4). ICP-OES analysis (on ultracentrifuged pellets oxidized with aqua regia) allowed to determine the concentration of Au (~60 µg/mL, corresponding to 61 % yield)

S2.2.2 – GNS with $LSPR_A$ at 835 nm and $LSPR_B$ at 1530 nm. The colloidal solution of these GNS has been prepared with an identical procedure as that described in the previous paragraph (S2.2.1). The only difference is in the volume of 0.0788M ascorbic acid added to the growth solution, that in this case was 400 µL. Both procedures (S2.2.1 and S2.2.2) follow exactly what we have already published (ref 10)

S2.3 Glass slides coating with PEI. Glass substrates were cleaned for 30 min in freshly prepared Piranha solution (3 : 1 v/vH_2SO_4:H_2O_2 (30%). *Caution!* Piranha solution is a strong oxidizing agent and should be handled with care.), washed three times with bidistilled water in a sonic bath and oven-dried. The slides were then immersed for 6 min in a 2%(v/v) solution of PEI-silane in ethanol at room temperature, to obtain PEI-coated slides, species A in Scheme S2.1. In a typical preparation, 5 glass slides were prepared at the same time, i.e. reacting in the same silane solution inside a 5-place holder (a microscope glass slides staining jar). After this, the slides were washed three times with ethanol and one time with ultrapure water in a sonic bath and blow-dried with nitrogen. Functionalized glasses were left to cure overnight at room temperature before use.



Scheme S2.1: sketch of the preparative steps to obtain the GNS-coated slides used in this paper

S2.4 GNS grafting on PEI-coated slides. Species B in Scheme S2.1 were obtained by using PEI-coated slides (species A in scheme 2.1, prepared as described in S2.3). In a typical preparation, 5 slides were prepared at the same time, kept in vertical position inside a 5-place holder (a microscope glass slides staining jar) filled with a colloidal solutions of GNS, prepared as described in S2.2.1 and S2.2.2, at RT (20-25 °C). Slides were taken off from the colloidal solutions after a chosen time (typically 7, 14 or 18 h) and appeared of a pale blue color due to the grafted GNS monolayer (Figure S2.4A and S2.4B). The GNS-coated slides were then washed twice with 5 mL bidistilled water and blow dried with nitrogen. They were kept in air, at room temperature, in a vertical position inside empty staining jars, under which conditions they were stable for weeks (checked by UV-Vis absorption spectra).

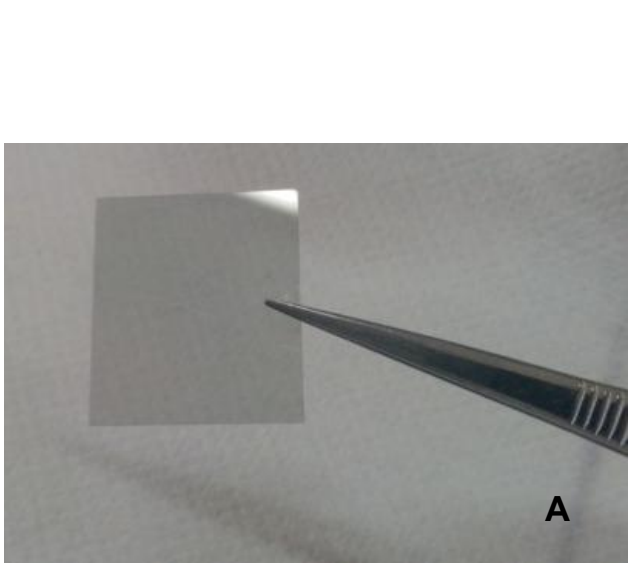
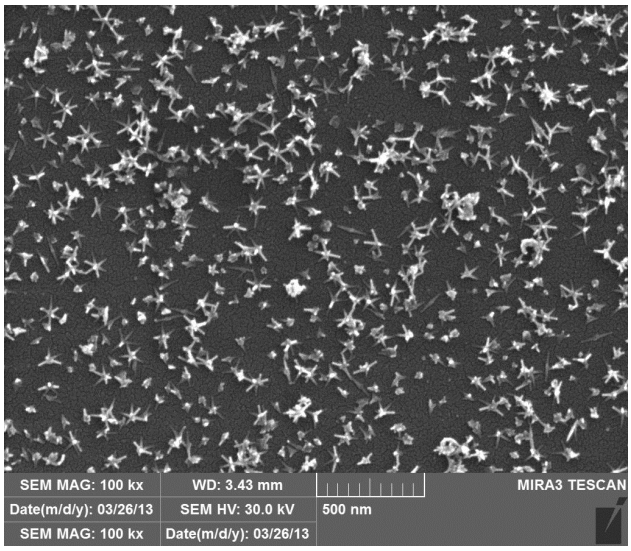
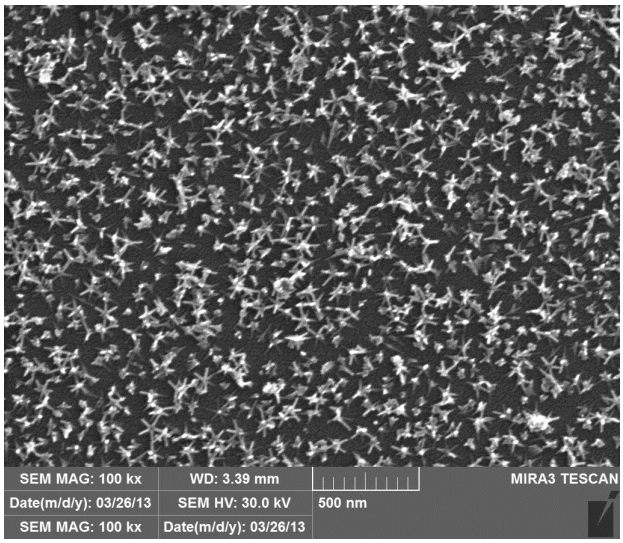


Figure S2.4. **A**: slide coated with GNS. **B**: five slides coated with GNS, stored in the slide-holder microscopy jars used also for synthesis.

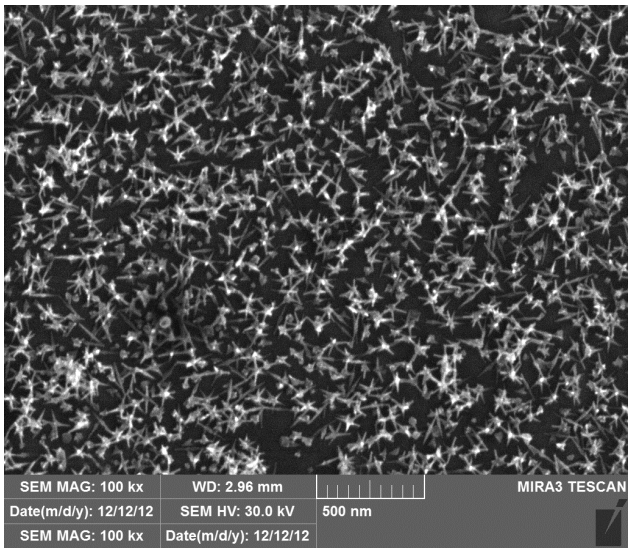
S.3 SLIDES PREPARED WITH GNS FEATURING DIFFERENT ASPECT RATIO ($LSPR_A$ 835 nm AND $LSPR_B$ 1560 nm). TEM AND SEM IMAGES OF THE GNS AND ABSORPTION SPECTRA



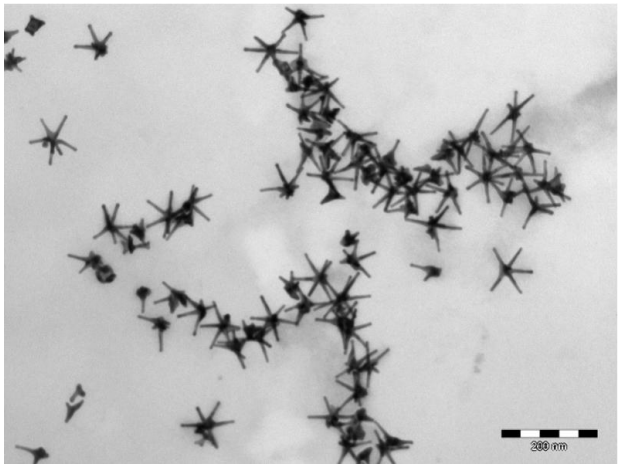
A (SEM, 7 h)



B (SEM, 14 h)



C (SEM, 18 h)



D (TEM image)

Figure S3.1. **A-C**: SEM images obtained on slides coated with GNS ($LSPR_A$ 835 nm; $LSPR_B$ 1560 nm), on PEI-coated slides with 7h, 14h and 18h immersion times. **D**: TEM image from the colloidal solution, prepared as in S2.2.2

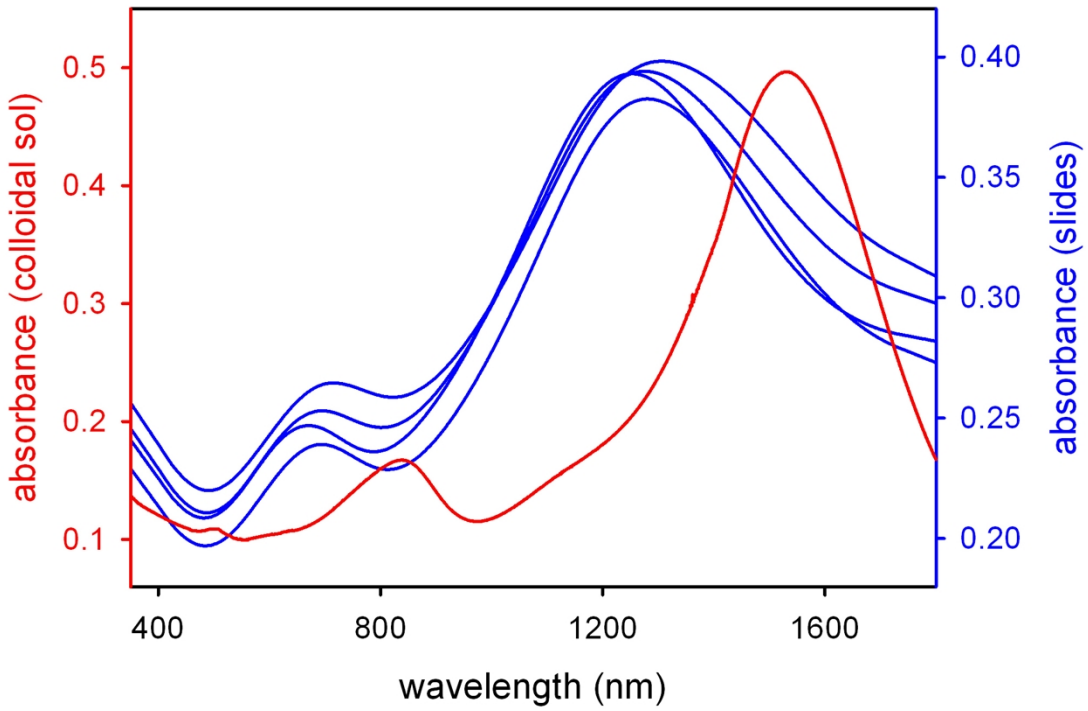


Figure S3.2. Absorption spectra of the GNS with $LSPR_A$ at 830 nm and $LSPR_B$ at 1560 nm in their colloidal solution (red spectrum, left vertical axis for absorbance, ~ fold diluted solution) and for a series of 4 slides prepared at 18 h coating time (blue spectra, right vertical axis. Absorbance values are not normalized in this case)

S.4 DETERMINATION OF THE LSPR WAVELENGTH DEPENDENCE ON THE SOLVENT REFRACTIVE INDEX. Coating procedures were carried out on the internal walls of a glass spectroscopy cuvettes, as described for glass slides in S2.3 and S2.4. After this, absorption spectra were recorded with empty cuvette or with the cuvette filled with solvents with a known refractive index. A step-decreasing gradient of solvent polarity was followed in order to achieve the total removal of the previous solvent,i.e. the solvent was discarded, the cuvette was gently dried in a nitrogen flux and then washed three times with the next solvent before refilling. The following sequence of solvents was used: water,acetonitrile, DMF, n-butanol, ethyl acetate, toluene, n-hexane.

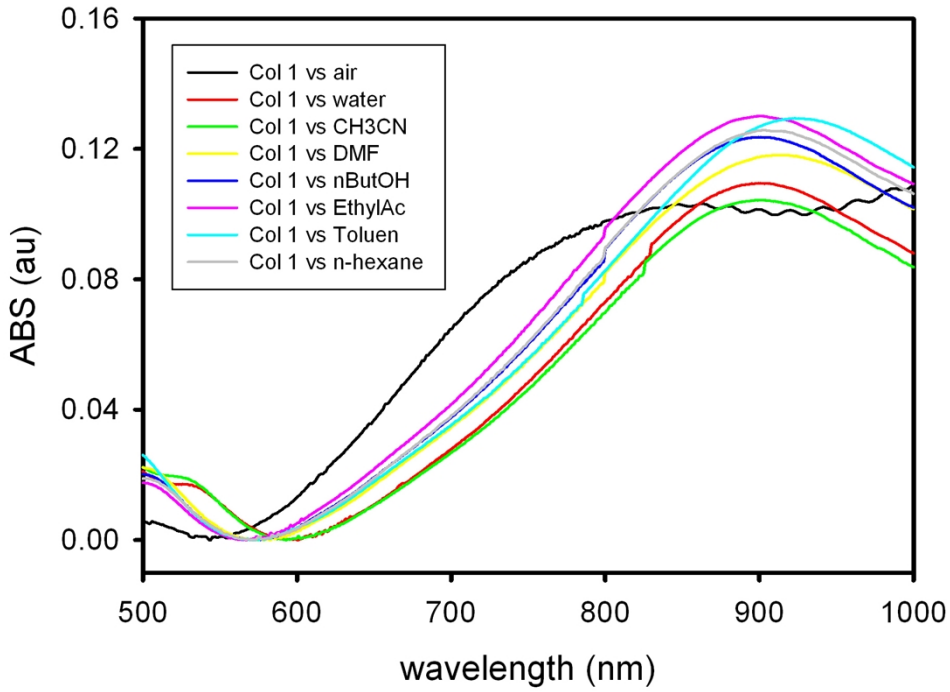


Figure S4.1 Series of absorption spectra with the cuvette filled with different solvents. The GSN used for coating were prepared as in S2.2.2

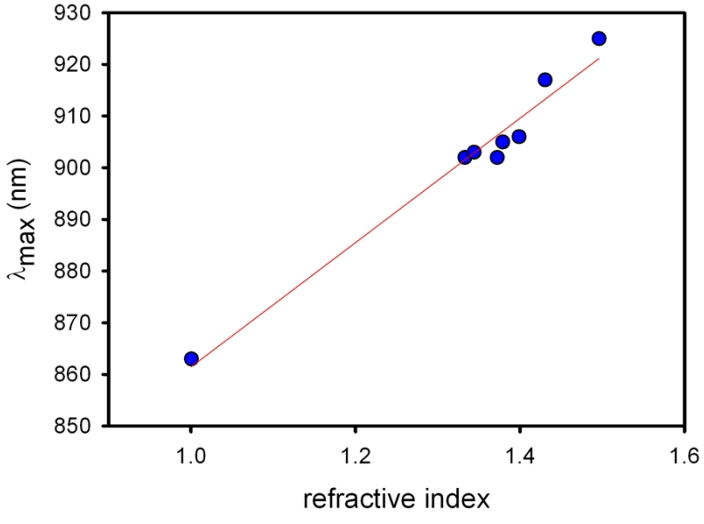


Figure S4.2 Plot of the LSPR maximum position vs solvent r.i. (taken from the spectra of Figure S4.1). Red line: linear regression on the data (r = 0.986; slope = 120.3 nm/refractive index unit)

S5. KINETICS OF COATING WITH GNS.

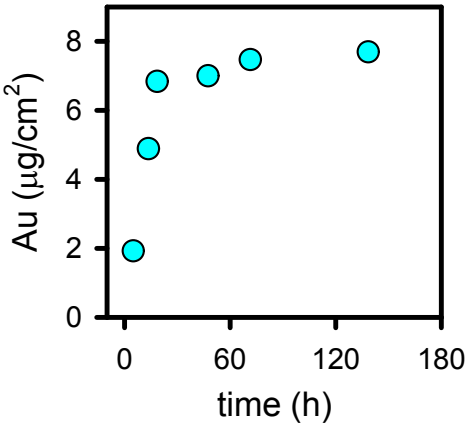


Figure S5.1 surface concentration (μg Au/cm^2) for slides coated with GNS colloidal solutions prepared as in S2.2.2 (larger branches aspect ratio, $LSPR_A$ and $LSPR_B$ on slides = 820 and 1350 nm, respectively).

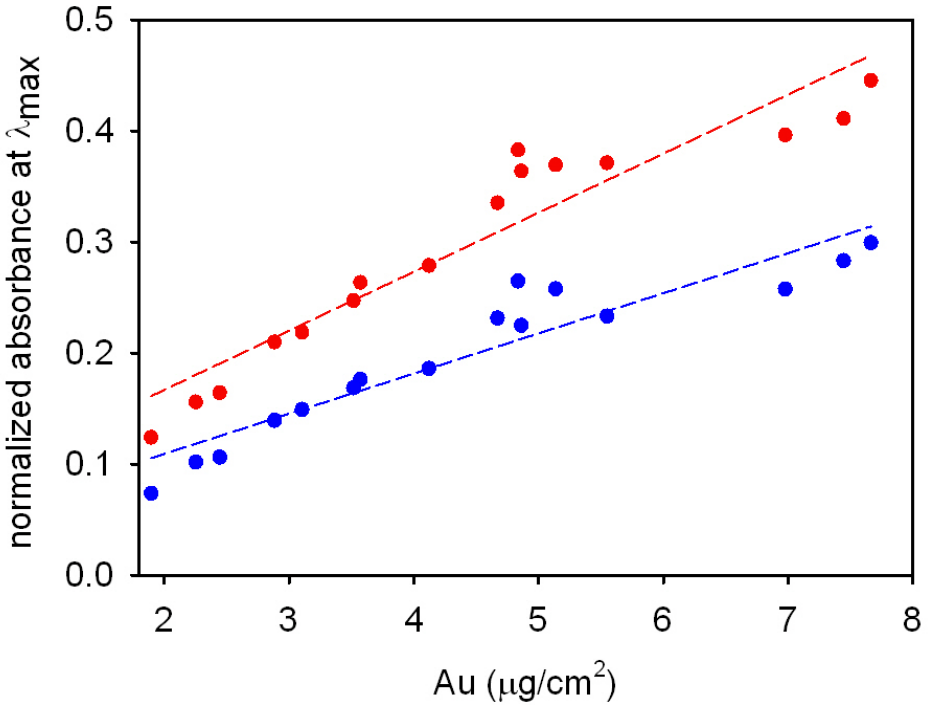


Figure S5.2 Linear relation between absorbance of glass slides at λ_{max} (after normalization by settimg Abs = 0 at 486 nm) and gold surface density (μg/cm^2) determined by ICP-OES. Red point refer to $LSPR_B$ (λ_{max} at 1350-1400 nm on glass slides) and blue point to $LSPR_A$ (λ_{max} at 820-830 nm on glass slides). The GNS colloidal solution used for coating was prepared as in S2.2.2

S6. LARGER SEM IMAGES OF THE GLASS SLIDES PREPARED WITH 7H AND 14H IMMERSION TIME (AND USED FOR THE PHOTOTHERMAL EXPERIMENTS).

GNS colloidal solutions are those prepared as in S2.2.1 ($LSPR_A$ at 680nm and $LSPR_B$ at 1320 nm), and the obtained glass slides have absorption maxima as shown in Figure 1D, main text. The SEM images of Figure 1B and 1C of the text are details of those shown here below.

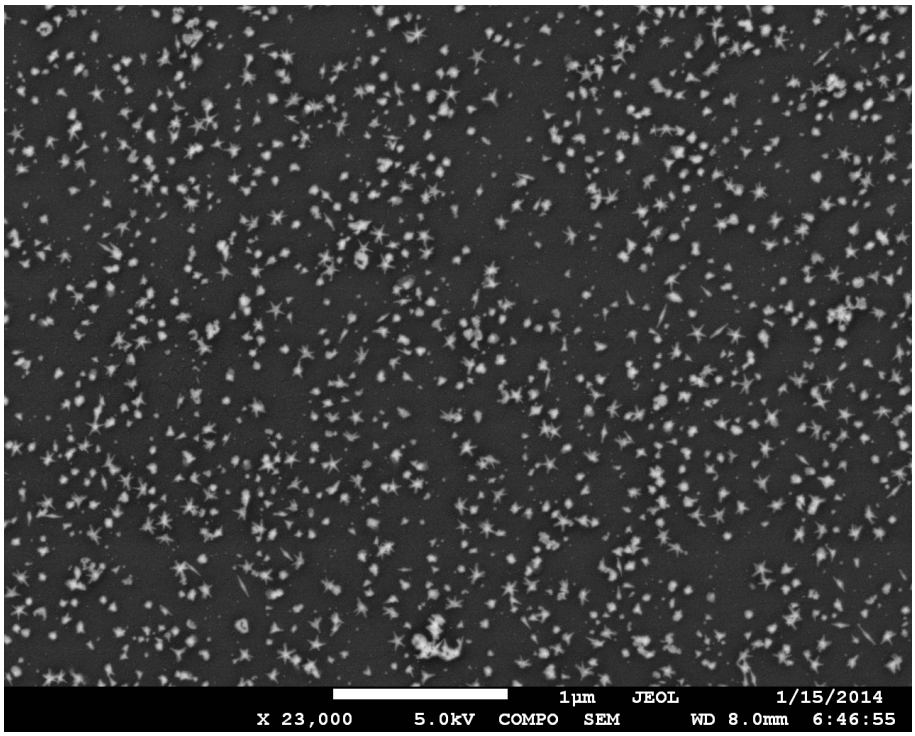


Figure S6.1 – 7 h immersion time, 23000x magnification

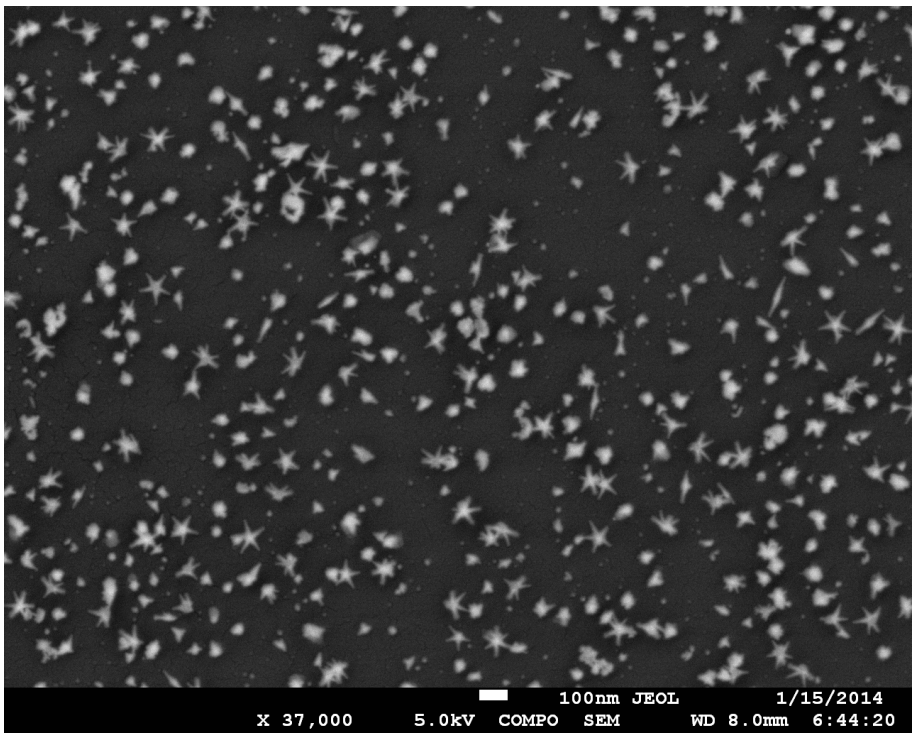


Figure S6.2 – 7 h immersion time, 37000x magnification

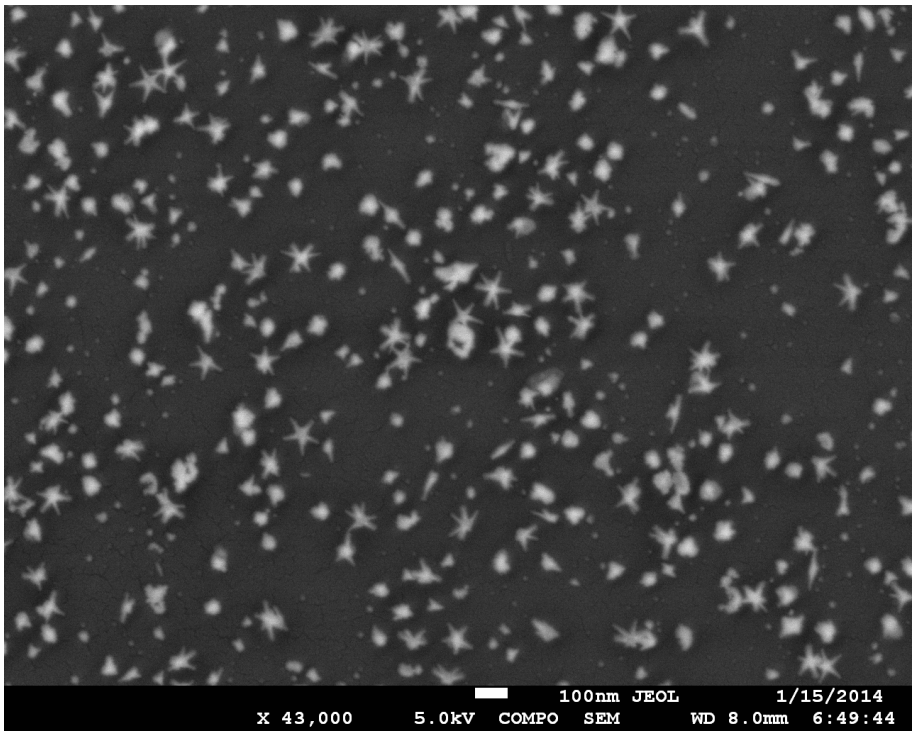


Figure S6.3 – 7 h immersion time, 43000x magnification

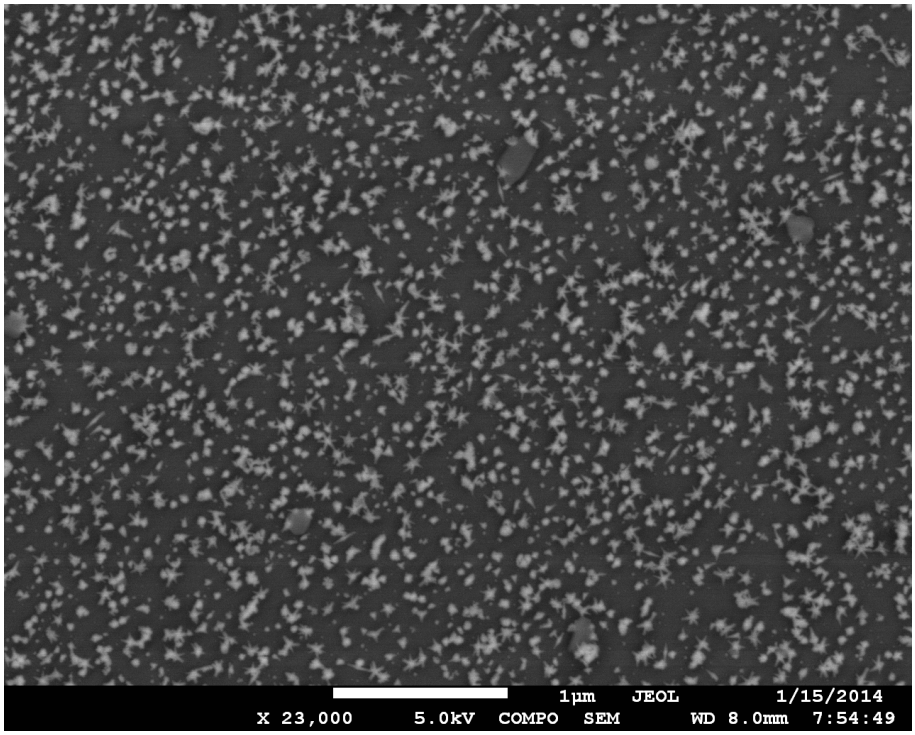


Figure S6.4 – 14 h immersion time, 23000x magnification

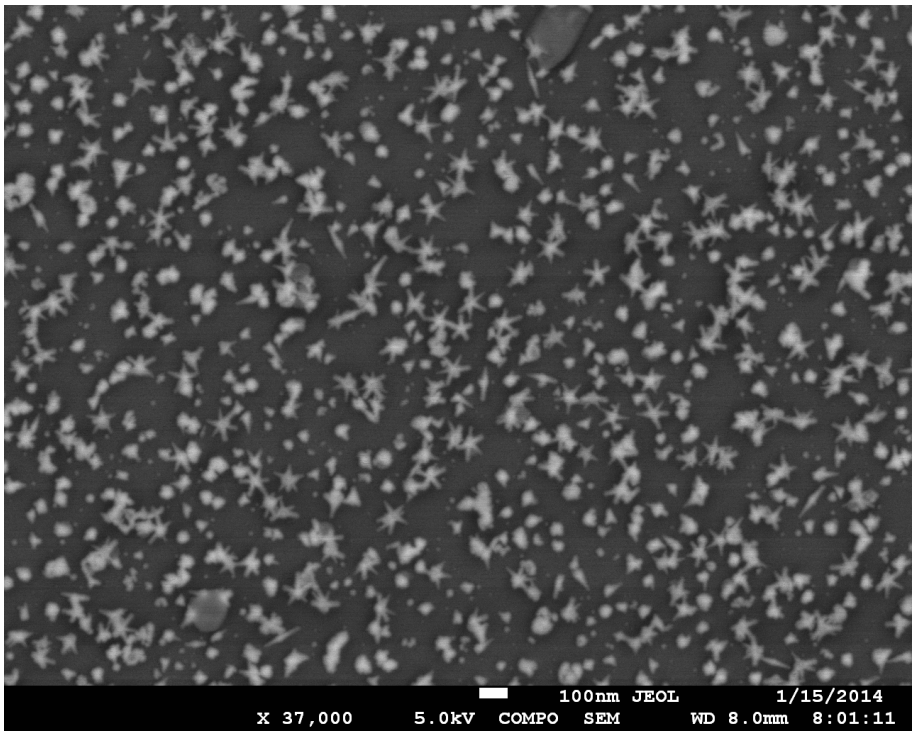


Figure S6.5 14 h immersion time, 37000x magnification

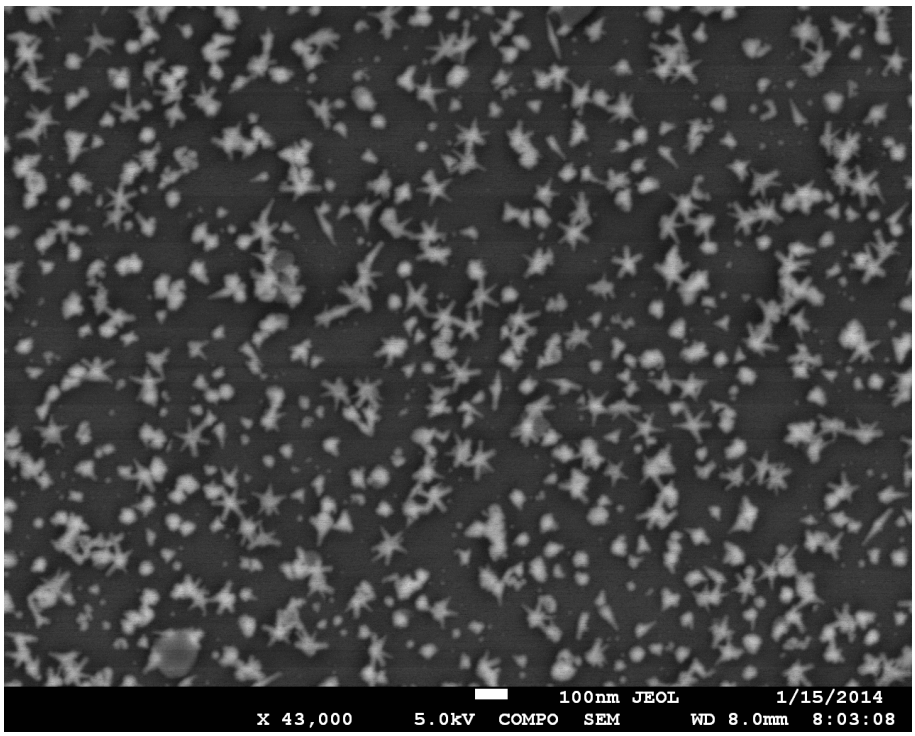


Figure S6.6 14 h immersion time, 43000x magnification

S7. DOUBLE EXPONENTIAL FIT OF THE THERMOGRAMS

The temperature was monitored by acquiring thermogram movies and measuring the average temperature on a ROI that encompassed the irradiation area on the monolayers. The typical temperature kinetics is shown in Fig. ESI1 for a monolayer treated with GNSs for 7 hours. The growth could always be fit to the sum of two exponential functions according to the trial function:

$$T(t) = T_0 + \Delta_1\left[1 - \exp\left(-t/\tau_1\right)\right] + \Delta_2\left[1 - \exp\left(-t/\tau_2\right)\right] \qquad \text{(SI1)}$$

The best fit parameters of the growth observed for the monolayers obtained by incubation for 7, 14 and 18 hours (two different preparations) are reported in the **Table I**.

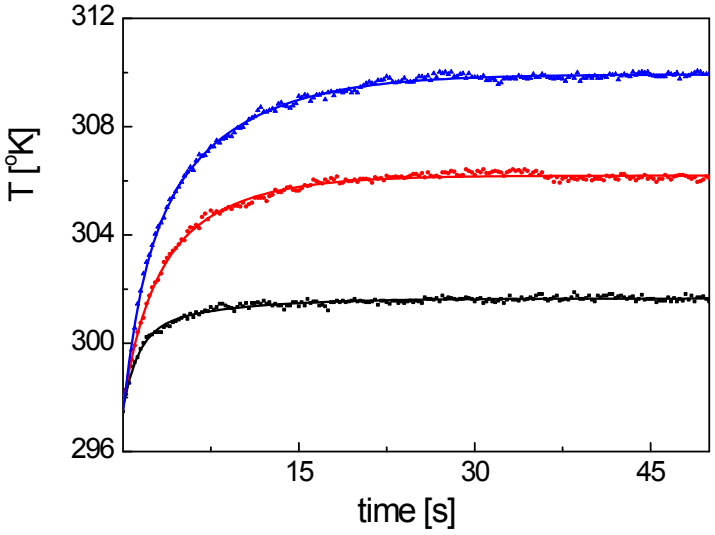


Figure S7.1. Temperature increase measured as the average of the temperature over the Region of interest that encompassed the irradiation spot. The black and red symbols refer to the data collected under single laser irradiation at λ=633 nm and at λ=1064 nm, respectively. The blue symbols refer to the data collected under double laser irradiation at λ=633 nm and at λ=1064 nm. The solid lines are the best fit curve to **Eq.SI1**.

Table S7.1

	τ_1 [s]	τ_2 [s]	$\Delta T_1+\Delta T_2$	W_2
	Single irradiation, λ =633 [nm]			
7 h	1.1 ± 0.2	6.8±1.1	4.3±0.3	7±3
14 h	1.0±0.3	6.2±1.8	8.8±1.6	12±5
18 h	1.1±0.9	9.2±2.8	11.4±1.5	4.0±2
Average	*1.1±0.1*	*7.4±1.5*		*7.4±3*
	Single irradiation, λ =1064 [nm]			
7 h	2.5±1	6.5±0.7	9 ± 1	22±14
14 h	2.14±0.08	7.7±0.3	15±0.4	22±4
18 h	1.9±0.7	9.4±1.6	20±1.8	11±4
Average	*2.2±0.3*	*7.8±1.5*		*18±6*
	Double irradiation, λ = 633 nm ; 1064 [nm]			
7 h	1.0±0.8	7.2±0.2	12.6±0.1	14±7
14 h	1.3±0.3	6.3±0.7	23±1	20±3
18 h	1.2±0.2	6.1±0.3	29±1	20±4
Average	*1.2±0.2*	*6.5±0.5*		*18±3*

Table S7.1: best fit parameters of the analysis of the temperature growth measured on the gold nanostars monolayers prepared with different incubation times (left most column): 7, 14 and 18 hours. The best fit function and the definition of the parameters are given in **Eq. SI1**. The right most column reports the weight of the slow component defined as the fractional intensity:
$W_2 = 100 * (\Delta T_2 / \tau_2) / (\Delta T_1 / \tau_1 + \Delta T_2 / \tau_2)$.

From Table S7.1 is quite clear that the relaxation times are ≈ 1.5 s and 7 s with a relative ratio ≈ 5, much less than that found for the GNSs suspensions [ref 15, main text].