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An aqueous one-pot route to gold/quantum rods heterostructured nanoparticles functionalized with DNA

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Supplementary Information

Materials and Methods

Materials and methods

All chemicals were purchased from Sigma-Aldrich unless otherwise specified. Peptide ligands were synthetized by Polypeptide (Strasbourg, France). Chloro-(diphenyl)-(3-sulfonato-phenyl) gold (I) sodium salt was purchased from Strem Chemicals. The two complementary single-stranded DNA functionalized with a thiol group were purchased from Sigma, their sequence being, in the 5' to 3' order:
DNA_1 = HS-T_{12}ACCTGGGGGAGTATTGCGGAGGAAGGT
DNA_2 = HS-T_{12}ACCTTCCTCCGCAATACTCCCCCAGGT.

Ultra-pure water (Milli-Q) with a conductivity of 18.2 MΩ was used to prepare aqueous ssDNA and buffer solutions.

***CdSe@CdS quantum rods synthesis*.**
The quantum rods were synthesized according to Carbone et al.[1] The synthesis consists in two steps: (1) synthesis of the CdSe seeds and (2) the synthesis of the CdS shell.
1/ Synthesis of the CdSe seeds: TOPO (TriOctylPhosphine oxide, 3.0 g), ODPA (OctaDecylPhosphonic Acid, 0.280 g), and CdO (Cadmium oxide, 0.060 g) were mixed in a 50 mL trinecked flask and heated to 150 °C under vacuum for 1 hour. Then, under argon, the mixture temperature was increased to 320 °C in order to dissociate CdO. When the reddish solution turned clear and colorless, TOP (TriOctylPhosphine, 1.5 g) was injected in the flask and the temperature allowed reaching 380 °C. Then 0.058 g of Se dissolved in 0.360 g of TOP were added. The heating mantel was removed immediately to allow the flask to cool down to room temperature. The nanocrystals were next extensively washed with a mixture of toluene and methanol (ratio v/v 1:4). The size and the concentration of the seeds were determined according to a previously published protocol, which determines their size and extinction coefficient by measuring the optical density at 350 nm as well as the position of their excitonic peak.[2] Thus, the CdSe seeds have a mean diameter of 2.7 nm ($\lambda_{excitonic}$ = 535 nm) and are finally dispersed in TOP at a concentration of 400 µM.

2/ Synthesis of QR: The appropriated amount of CdO was mixed in a 50 mL trinecked flask together with TOPO (3 g), ODPA (0.29 g) and HPA (HexylPhosphonic Acid, 0.08 g) and heated to 150 °C under vacuum. Then, temperature was increased to 320 °C under argon in order to dissociate CdO. When the reddish solution turned clear and colorless, 1.5 g of TOP (TriOctylPhosphine) was injected in the flask and the temperature allowed to reach 350 °C for the injection of the solution of sulphur precursor (0.120 g) + CdSe seeds (200 µL at a concentration of 400 µM) dissolved in TOP (1.5 g). The nanocrystals were then allowed to grow for 8 minutes after a thermal quenching by immersing the tri-necked flask in a water bath (60°C). Subsequently, the rods then extensively washed with a mixture of toluene and methanol (ratio v/v 1:4), dispersed in toluene, and stored at 4 °C. Two batches of QR of similar size (defined by length x diameter) were used for all the experiments: QR_1 (62, 8 x 4,6 nm) and QR_2 (71 x 5 nm). Nevertheless all the comparisons were performed on the same batch of QR.

Quantum rods functionalization in water.
Quantum rods were functionalized with C_3E_6D ligands in water relying on our procedure previously reported.[3] The quantum rods concentration was determined using an extinction coefficient at 350 nm of 4.58 10^{-7} $M^{-1}.cm^{-1}$.[4] The QRs in suspension in toluene were precipitated by addition of methanol and finally dissolved in chloroform at a concentration of 10 $mg.mL^{-1}$. 10k molar excess peptide/QR of an aqueous suspension of peptide (50 mM) was then added to the organic phase containing the QR. The addition of the aqueous peptide solution was followed by the introduction in the organic phase of a small amount (1-5 µL) of a phase transfer agent: tetramethylammonium hydroxide (TMAOH, 25% w/w in methanol). Then, the mixture was shaken vigorously and the nanoparticles transferred from the organic to the aqueous phase. The organic phase was discarded and chloroform residues present in the aqueous phase were evaporated under vacuum (300 mbar) for at least 30 min. Until further use, the hydrophilic nanocrystals were stored at 4 °C.

Heterostructures (HNP) synthesis (samples 1 to 6 without DNA)

The QR suspension in water was purified by a size exclusion chromatography (NAP-5, GE Healthcare) and finally dispersed in a 10 mM borate buffer, pH = 8.5, containing 10 mM of potassium chloride. The nanoparticle concentration was 2.5 x 10^{-8} M. Reactants were mixed in a quartz cell according to the concentrations given in the Main Text Table 1. A LED (M405L2**,** Thor Labs, λ = 405nm) was placed at 0.5 cm of the cell. After irradiation, the hetererostructures were submitted to at least 4 purification steps on centrifugal filters (Amicon Ultra 0.5 mL, MWCO = 100 kDa). Each step consists in a concentration at a low speed (2800 g, 5 min) and a redispersion in water .

Various conditions following the previous procedure were also tested to evaluate the role of each reactant as summarized in the table 1.

Sample	QR (nM)	AA (mM)	TEA (M)	Chloro-Au(I) (mM)
1	25	10	0.8	1
2	25	10	0.8	10
3	25	10	0.8	0.3
4	25	10	-	1
5	25	-	0.8	1
6	25	-	-	1

Table S1. Chemical conditions for the photo-induced synthesis of HNPs.

DNA-grafted Heterostructures (DNA-HNP) synthesis.

The QR suspension in water was purified by a size exclusion chromatography (NAP-5, GE Healthcare) and finally dispersed in a 10 mM borate buffer, pH = 8.5, containing 10 mM of potassium chloride. The nanoparticle concentration of the stock solution was 2.5 x 10^{-7} M.

The Au(I)-DNA complex was prepared extemporaneously by incubing an excess of chloro-(diphenyl)-(3-sulfonato-phenyl) phosphine gold (I) (denoted Au(I)-Cl) salt with Thiol-DNA (DNA_1 or DNA_2) at various gold/DNA ratios (1:1, 3:1, 30:1) in 10 mM borate buffer, pH = 10.5. The best conditions of exchange were found by using a 1:1 DNA/ Au(I)-Cl ratio during 2 hours prior to the heterostructure synthesis. The corresponding heterostructured particles were next synthesized. A mixture composed of a QR suspension (25 nM), triethanolamine (TEA, 0.8 M), ascorbic acid (AA, 10 mM) was added to a 4:1 molar mixture of Au(I)-Cl /Au(I)DNA (1 mM). The final volume was adjusted to 1 mL with 10 mM borate buffer at pH 8.5 to obtain the mentionned final concentrations of the various reactants. The mixture is then put into a quartz cell under magnetic stirring. A LED (M405L2**,** Thor Labs, λ = 405nm) was placed at a 0.5 cm distance of the cell for 20 min. At the end of the irradiation duration, the HNPs modified with DNA were washed using centrifugal filter (Amicon Ultra 0.5 mL, MWCO = 100 kDa). The concentration of the colloids was done at a low speed (2800 g) during 5 min before redispersion in 25 mM Tris buffer, containing 0.3 M NaCl. This procedure was repeated 4 times.

Assembly with gold NPs directed by DNA.

The gold nanoparticles (ø ≈ 15 nm) were synthesized according to the Turkevitch procedure[5] and functionalized with 500 k molar excess of thiol-DNA during 24 h. The gold colloids were washed using centrifugal filter (Amicon Ultra 0.5 mL, MWCO = 100 kDa). The concentration of the colloids was

performed at a low speed (2800 g) during 5 min before redispersion in 25 mM Tris buffer containing 0.3 M NaCl. This procedure was repeated 4 times. The gold NP concentration was determined using an extinction coefficient of 3.9 10^8 $M^{-1}.cm^{-1}$.
The assembly was triggered by mixing HNPs (5 nM) and gold NPs (7 nM) bearing complementary DNA at different molar ratio and the resulting suspension was incubated during 10 min at 90°C and then during at least 48 h at room temperature. Thereafter, the sample were either precipitated at a low speed (664 g, 10 min) and redispersed in pure water or directly aliquoted for TEM characterization without further purification. The desalting procedure by precipitation was repeated twice to eliminate salt and buffer prior to cryoTEM characterization.

Transmission electron microscopy (TEM).

A small drop of sample in aqueous solution was deposited on carbon-coated copper grids (400 mesh). After 3 minutes, the excess liquid was blotted with filter paper (Whatman). TEM was performed at room temperature using a JEOL 1011 electron microscope operating at 100 kV equipped with a Gatan Orius CCD camera.

Cryo-TEM.

A small drop of sample in aqueous solution was deposited on plasma treated Quantifoil grids before vitrification: excess sample was removed by blotting with filter paper (Whatman) to obtain a thin film before plunging the grid in liquid ethane cooled by liquid nitrogen. CryoTEM was then performed using a FEI Technai Spirit G2 operating at 100 kV equipped with a a Gatan Orius CCD camera and a Gatan cryo-holder operating at -180 °C.

Dynamic light scattering (DLS).

DLS was performed on a Zetasizer Nano-ZS ZEN3600 (Malvern Instrument, UK). The autocorrelation functions were recorded at a scattering angle of 173°. The evolution of the hydrodynamic diameter of the mixture colloids bearing complementary DNA was recorded just after mixing and after at least 15 h. Only an analysis for spherical nanoparticles can be implemented on this apparatus; therefore, the extracted hydrodynamic diameter is just a rough estimate giving information on colloidal stability but not providing fine details on object shape.

Fluorescence spectroscopy.

Fluorescence spectroscopy was performed on a Fluorolog-3 (FL3-22, Horiba Jobin Yvon, Japan). The light source consisted in a 450 W Xenon lamp and intensity was recorded at 90° from the source (right angle mode). Samples were excited at 350 nm and emission was recorded between 400 and 690 nm.

UV spectroscopy. UV spectroscopy was performed on a Cary 100 SCAN UV-visible spectrophotometer (Varian, Australia). Absorbance was recorded between 200 and 700 nm using 120 µL cuvette (Suprasil, 105.250-QS, Hellma, France) with a path length of 1 cm.

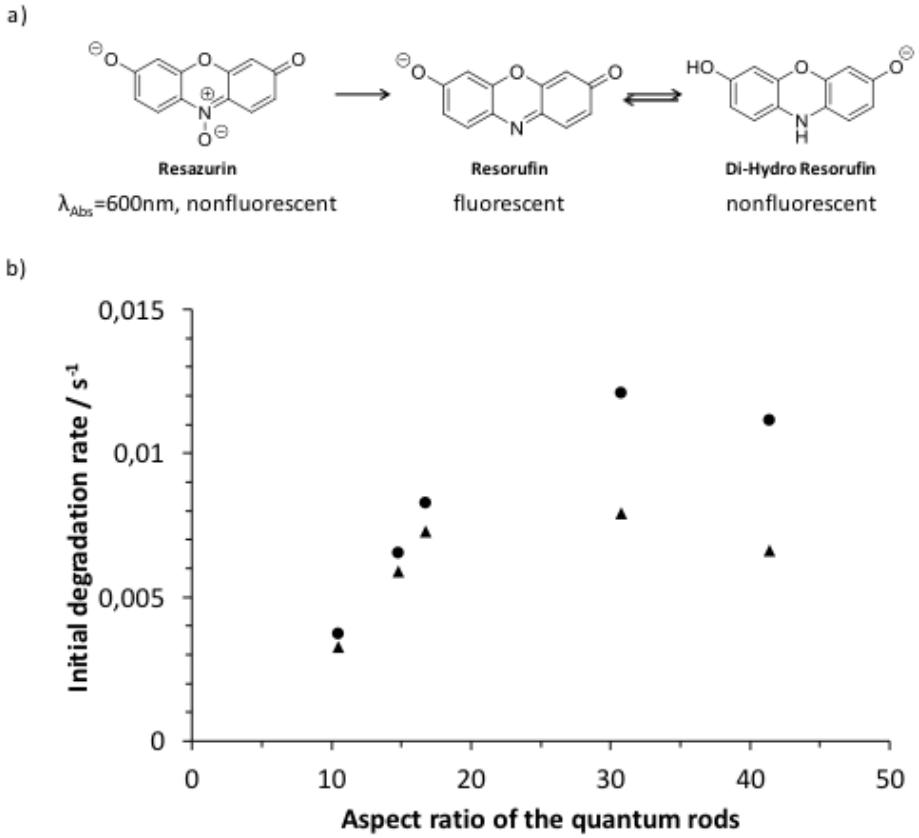

Figure S1. Effect of the QR aspect ratio and of the peptide coating on photoreduction kinetics. **(a)** Redox chemistry of resazurin in aqueous medium. **(b)** Effect of the QRs aspect ratio on the kinetics of resazurin photo-reduction at pH 9. The initial disappearance rates of the resazurin were extracted from the absorbance spectra under the same conditions for each population of nanoparticles, either grafted with the C_3E_6D short ligand (circle) or the $C_3A_{11}E_4CO_2H$ long one (triangle).

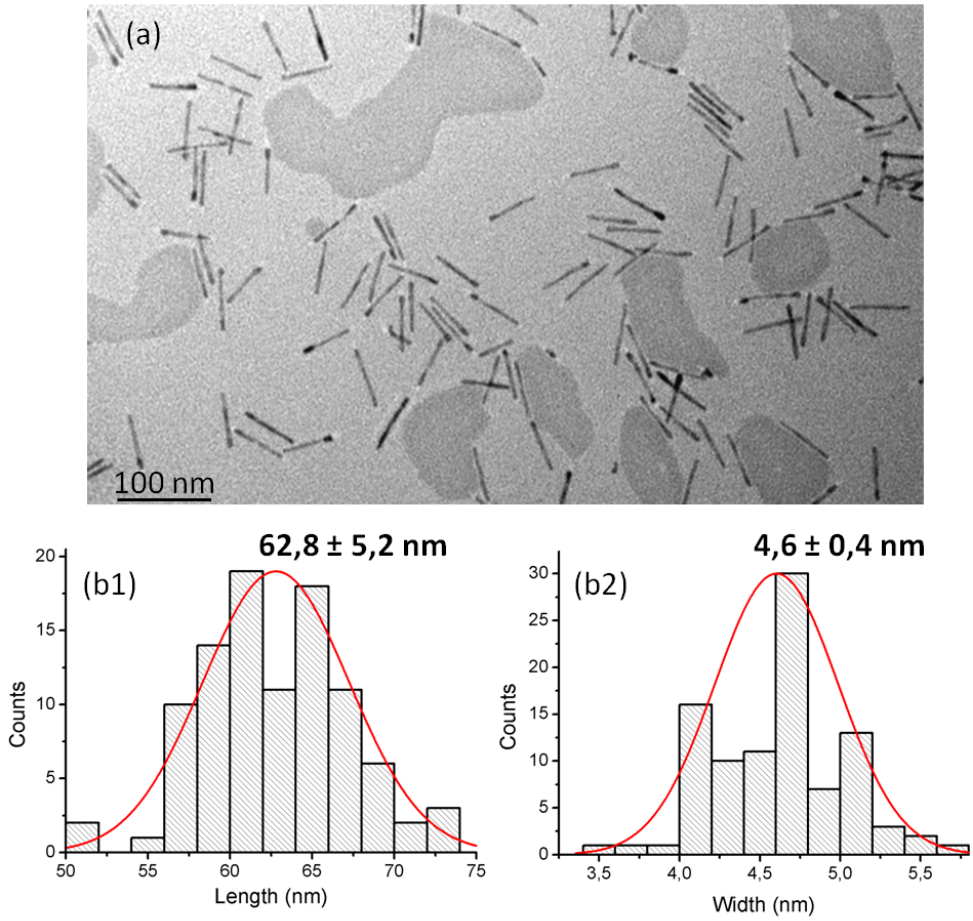

Figure S2. Characterization of the starting QR_1 used for photo-reduction. **(a)** Typical TEM image and **(b1-2)** corresponding length and width histograms, respectively (analysis performed over 100 particles).

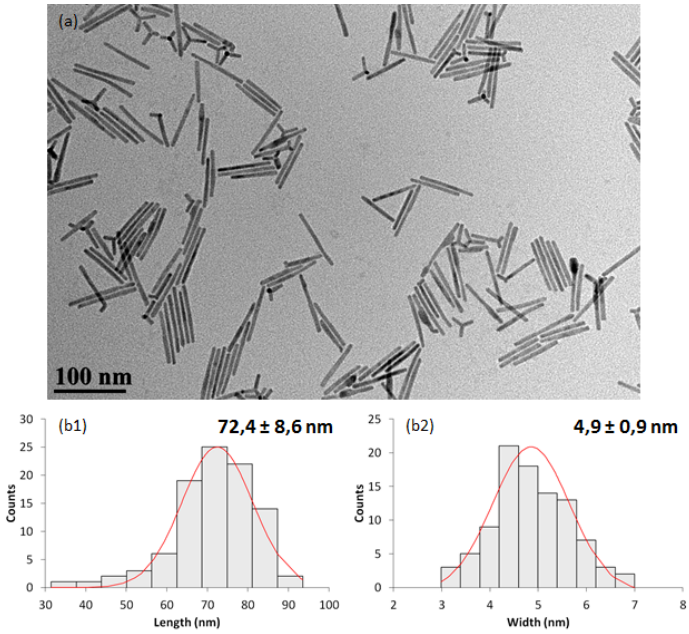

Figure S3. Characterization of the starting QR_2 used for photo-reduction. **(a)** Typical TEM image and **(b1-2)** corresponding length and width histograms, respectively (analysis performed over 100 particles).

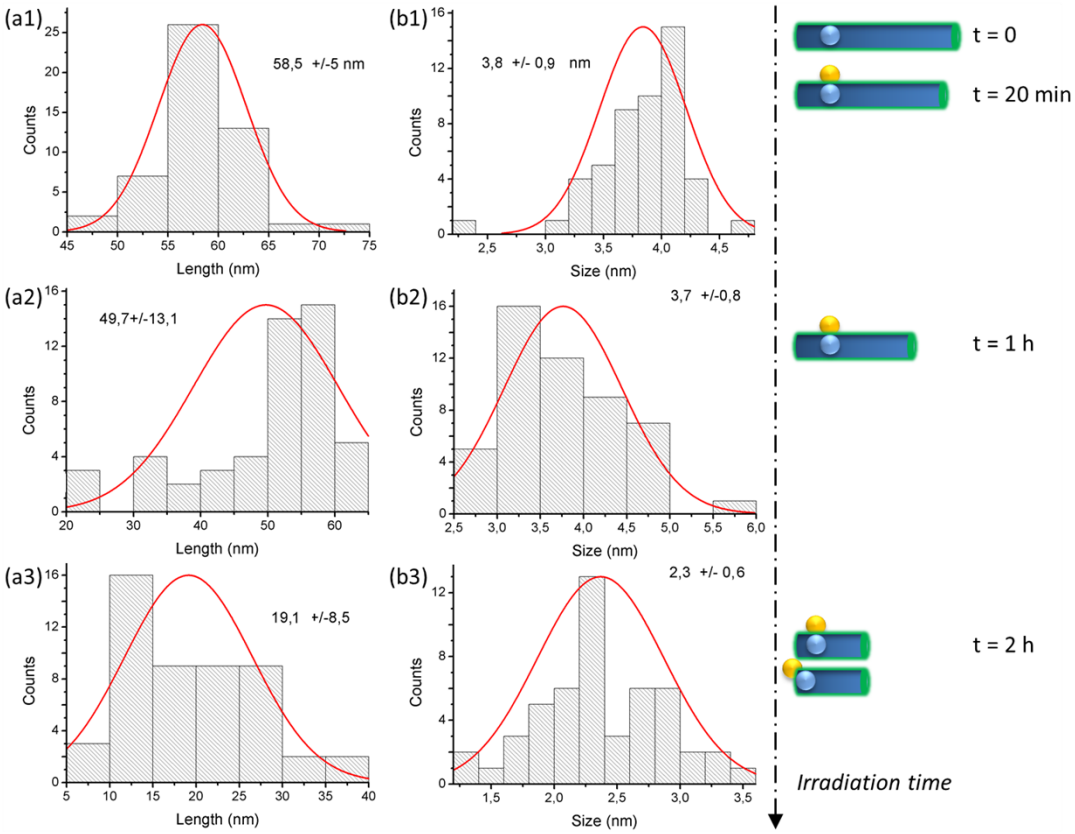

Figure S4. Kinetics of the degradation of the HNPs with increasing photo-irradiation time. Evolution of **(a)** the length of the QR_1, and **(b)** the size of the gold domain after **(1)** 20 min, **(2)** 1 h, and **(3)** 2 h of photo-irradiation.

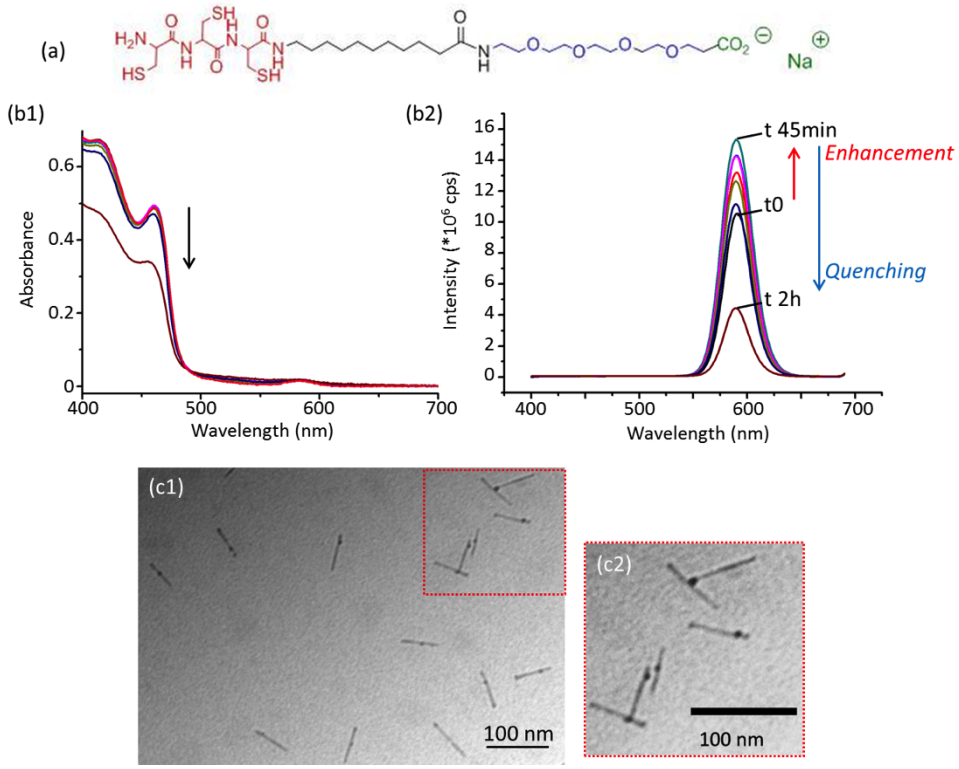

Figure S5. Optical and TEM characterization of the HNPs obtained from QR_1s coated with the $C_3A_{11}E_4CO_2H$ peptide . **(a)** $C_3A_{11}E_4CO_2H$ structure. **(b1-2)** UV-visible and fluorescence spectra respectively, recorded every 15 min in the course of a 2 h irradiation. **(c1-2)** TEM images of the HNPs obtained at the end of the process.

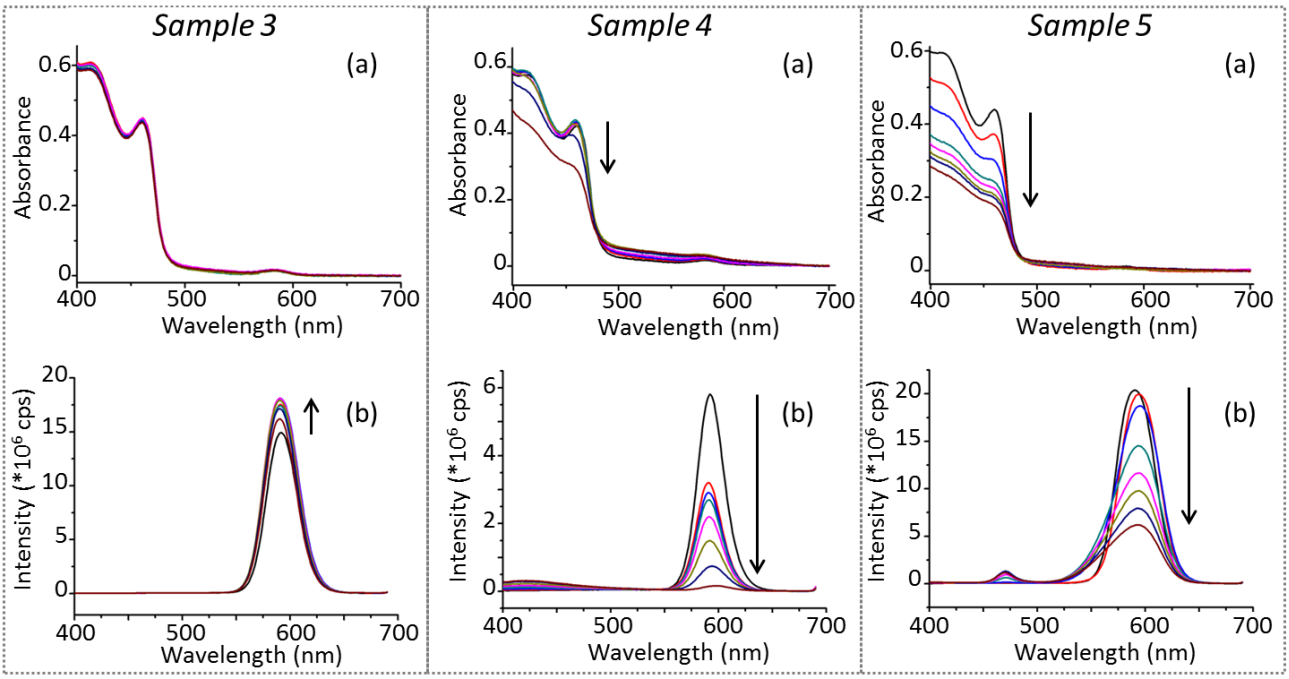

Figure S6: Optical characterization of HNP syntheses performed in different chemical conditions. **Sample 3, 4, and 5** compositions are described in the Table S1. **(a)** UV-visible and **(b)** fluorescence spectra recorded every 15 min in the course of a 2 h irradiation.

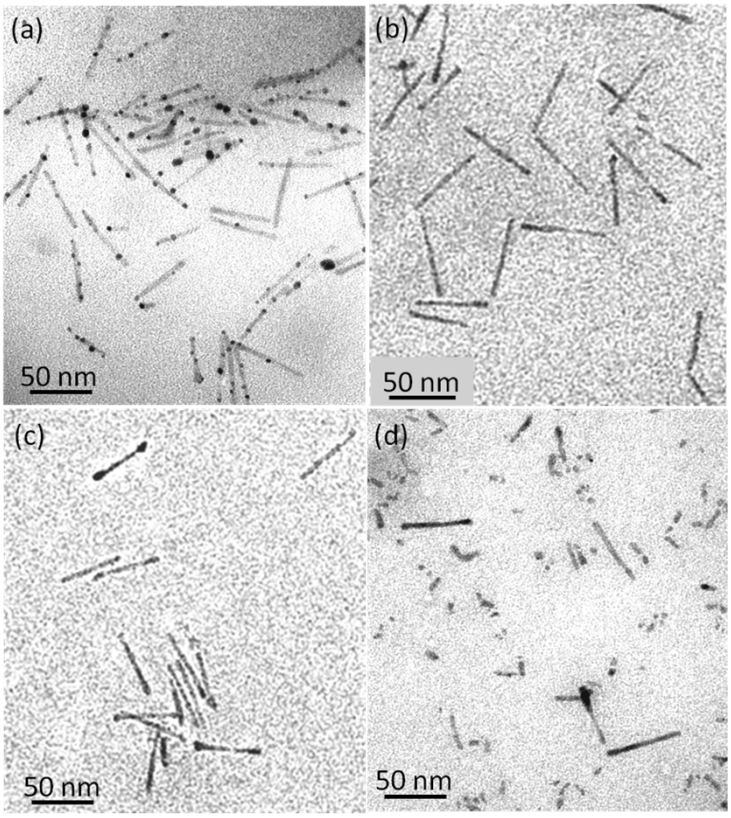

Figure S7. TEM characterization of HNPs synthesized in different chemical conditions and with a 30 min photo-irradiation at room temperature. The following composition must be understood in reference to Sample 1- see Table 1 in the Main Text for details: **(a)** with 10 times more chloro-Au(I) (sample 2), **(b)** without TEA (sample 3), **(c)** without AA (sample 4), and **(d)** without both AA and TEA (sample 5).

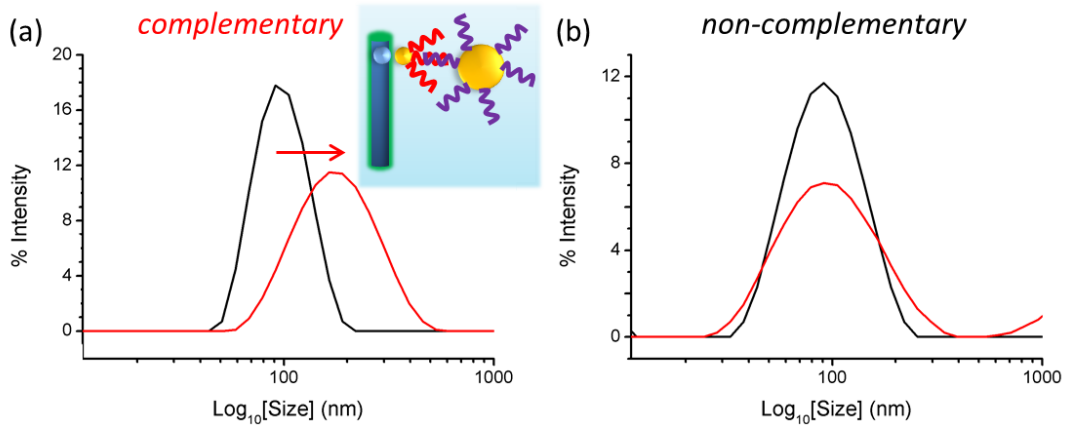

Figure S8. Evolution of the mean size of DNA_1-HNPs obtained by dynamic light scattering upon addition of (a) complementary DNA_2-AuNPs (1:1), and (b) non-complementary DNA_1-AuNPs (1:1).

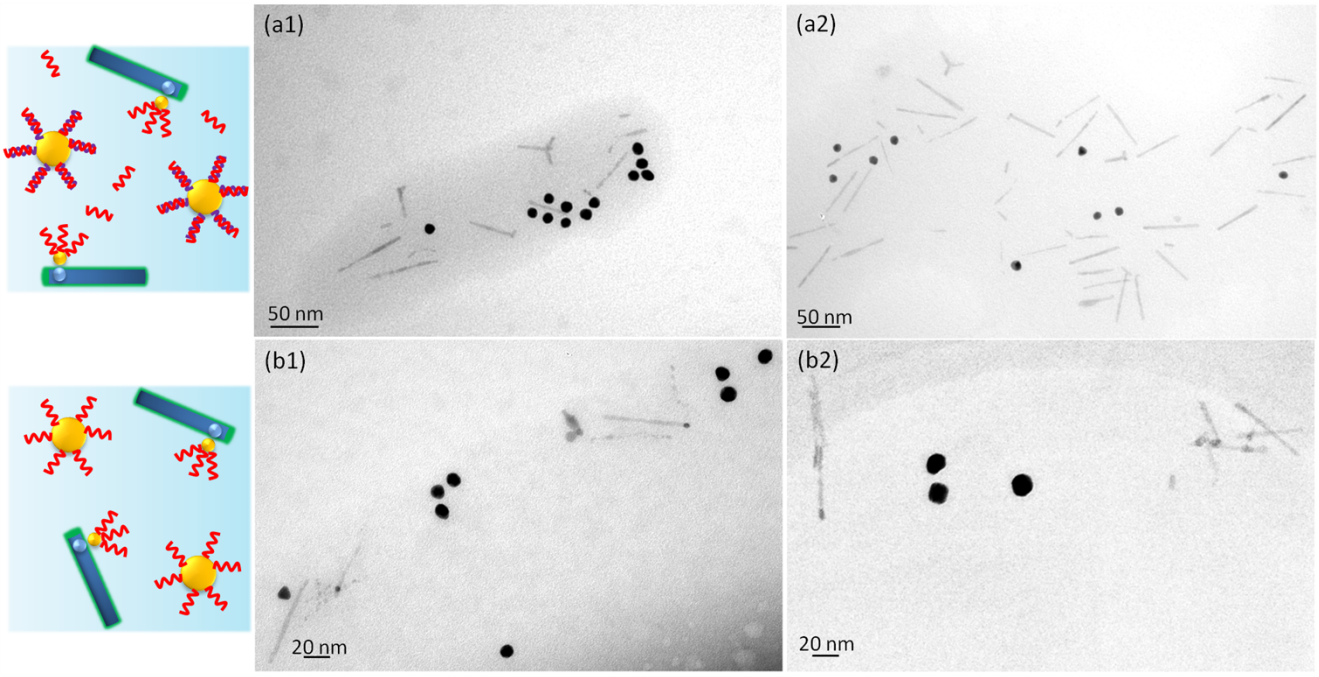

Figure S9. TEM images corresponding to molar mixtures (3/1) of (a) complementary DNA_1-HNPs and DNA_2-AuNPs in the presence of a 100-fold excess of free DNA_1, and (b) of non-complementary DNA_1-HNPs and DNA_1-AuNPs.

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