

Seed-mediated biomineralization toward the high yield production of gold nanoprisms

Supporting information

Experimental

Expression of CTPR3 protein

CTPR proteins with 3 tandem repeating units were synthesized through recombinant bacterial expression technology.^{1, 2} Synthetic genes for the desired protein in pPROEx vector, coding for N-terminal (His)₆ tag and ampicillin resistance, were transformed into E.Coli BL21(DE3) cells and cultured overnight at 37°C on agar plates. One single colony was selected and incubated overnight in 50 mL of Luria–Bertani (LB) media containing 100 µg/mL of ampicillin. 10ml of overnight cultures were then dispensed into 1 L of LB media supplemented with 100 µg/mL ampicillin. The cells were grown in an incubator-shaker (250 rpm) at 37 °C until the optical density (OD₆₀₀) reached 0.6-0.8. Expression of CTPR3 was induced with 1mM isopropyl β-D-thiogalactoside (IPTG) followed by 5h expression at 37°C. The cells were harvested by centrifugation at 5,000rpm for 20min and the pellets were frozen at -80°C until purification.

Purification of CTPR3 protein

The cell pellets were resuspended in lysis buffer consisting of 50 mM Tris, 300mM sodium chloride and 0.1wt% Tween 20. After 2 min sonication at 30% power using a microtip and Mison sonicator, lysed cells were centrifuged at 16,000 rpm for 30 min and the protein supernatant was purified using standard Ni-NTA affinity purification protocol. The collected proteins were further purified by Akta Prime Plus size exclusion chromatography using Superdex 75 16/600 Prep Grade column in 150mM sodium chloride and 50mM sodium phosphate buffer at pH 8 with a flow rate of 0.5ml/min. As a final step, the aqueous solutions of CTPRs were dialyzed against 10mM phosphate buffer three times at 4 °C using a dialysis membrane with molecular weight cutoff of 3kDa.

Preparation of CTPR3-stabilized Ag seeds^{3,4}

Silver seeds were prepared by adding 5 ml 0.5 mM AgNO₃ at the rate of 2 mL/min using a syringe pump (KD Scientific) under vigorous agitation to the aqueous solutions of trisodium citrate (5 mL, 2.5 mM), NaBH₄ (0.3 mL, 10 mM) and CTPR proteins (0.125 mL 0.1-2 mg/ml) in a glass vial. The as-prepared Ag seeds were kept in the dark till further use to circumvent the interference such as homogeneous nucleation arising from the residual NaBH₄.

Preparation of Au nanoprisms

In a typical synthetic procedure, 0.1 ml CTPR3 (2 µM - 200 µM) was first mixed with 0.85 ml DI and 40 µl 10 mM HAuCl₄, followed by the addition of 0.2 ml 1M aqueous NaBr solution and

(2.5 – 50 μ l) CTPR3-stabilized Ag seed. Then, 0.8 ml 100 mM MOPS was injected into the solution. After 3s of gentle shaking, the Au(III) was quickly converted to Au(I), and the solution was left undisturbed overnight. The as-prepared Au colloidal solution was centrifuged at 3,000 rpm for 10 min and the resultant pellets were rinsed with DI water to remove the residue stabilizer and reducing agent. After several purification cycles, the Au NTs were re-dispersed into DI water for later TEM characterization.

Spectroscopic and morphological characterization

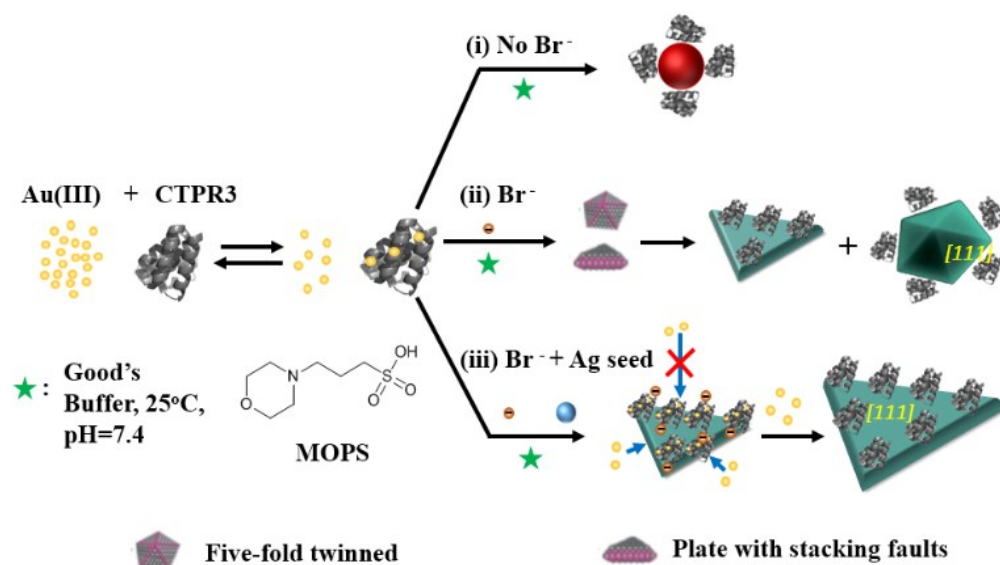
TEM and High resolution TEM (HR-TEM) analysis was performed on a Philips EM420 at an accelerating voltage of 120 kV and JEOL 2100 at 200 kV, respectively. TEM samples were prepared by applying a 7 μ l sample solution on 300 mesh ultrathin carbon-coated Cu grids (EM Science), followed by drying overnight before observation. The thickness, as well as the size distribution of Au NTs, was measured from [atomic force microscope \(AFM, Veeco BioScope II\)](#) and TEM images using Image J. The UV-Vis spectra of Au NPs were monitored using Agilent Cary 100 Bio and UV-Vis-NIR spectrophotometer. All UV-Vis measurements were conducted in 1cm path length PMMA or quartz cuvette at room temperature.

Dynamic light scattering (DLS) measurement

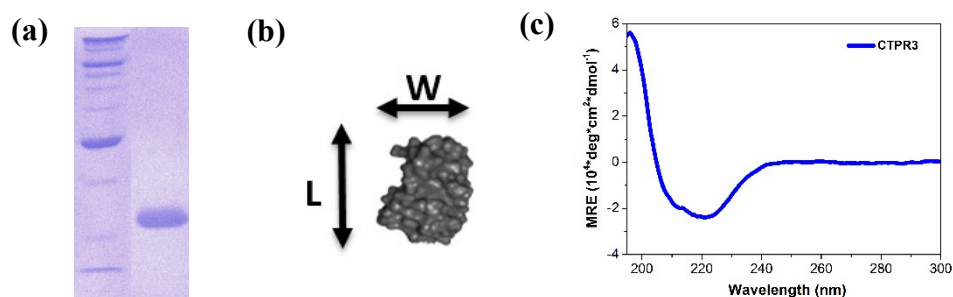
The Au NTs colloidal solution (40 μ g/mL) was incubated with 500 μ L undiluted Fetal bovine serum (FBS, Life Technologies) for 2 hours at 37°C under constant agitation. The Au NTs were centrifuged at 14,000 rpm for 20 minutes. The pellet was resuspended in water and washed two times by centrifuging at 14,000 rpm for 20 min. The final pellet was resuspended in 1 mL of 10mM NaBr. The average particle size and size distribution of the NPs was measured using a Malvern Zetasizer Nano-ZS, with five measurements taken for each sample. Zeta potential measurements were performed in a 10mM NaBr solution.

MTT (3-(4, 5-dimethylthiazol-2-yl) 2, 5-diphenyl tetrazolium bromide) assay

Mouse brain endothelial cells (MBEC) were seeded at 5,000 cells per well in a 96-well plate and incubated for 24 hours at 37°C in a 5% CO₂ incubator. Series of dilutions (40, 30, 20, 10, 5 μ g/mL) of Au NTs in cell growth medium was added to the wells. A 1% Triton-X solution was used as a positive control. After 6 hours of incubation the nanoparticle solutions were removed and the cells washed with PBS. Then 10 μ L of a 5 mg/mL MTT solution and 90 μ L growth media were added to each well and further incubated for 4 hours. After 4 hours the media was removed and the formazan crystals were dissolved in 100 μ L DMSO. The absorbance at 490 nm was measured using a microplate reader. Percent viability of the cells was calculated as the ratio of mean absorbance of triplicate readings with respect to mean absorbance of control wells.



Scheme 1. Schematic illustration of synthetic routes toward quasi-globular Au NPs(i), Au NPs bounded with (111) facets (ii) and Au triangular nanoprisms (iii).



Recombinant CTPRs	# of amino acid residues	Mw (kDa)	Aspect ratio(L/W)
n=3	107	12.5	1.1

CTPR3 is composed of the 3 tandem repeating motifs where each individual repeat is a 34 amino acids helix-turn-helix structure. CTPR3 protein is ~ 12kDa in size and carries overall negative charge. The 34 amino acid sequence is shown as follows:

AEAWYNLGNAYYKQGDYQKAIEYYQKALELDPNN

Fig. S1. (a) 15% SDS-PAGE gel of Protein marker (left lane) and CTPR3 stained with coomassie blue (right lane). (b) Space-filling representation of the structure of CTPR3 protein drawn in Pymol. (c) Circular dichroism (CD) spectrum of CTPR3 protein (MRE: The mean residue ellipticity). Bottom: single letter amino acid sequence of the CTPR repeat. CTPRn proteins are created by repeating this sequence n times (n=3).

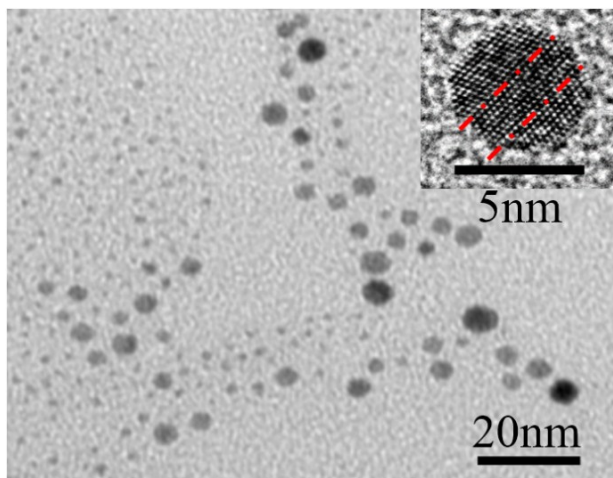


Fig. S2 TEM image of CTPR3-stabilized Ag seeds (inset: HR-TEM of planar twinning nanocrystal).

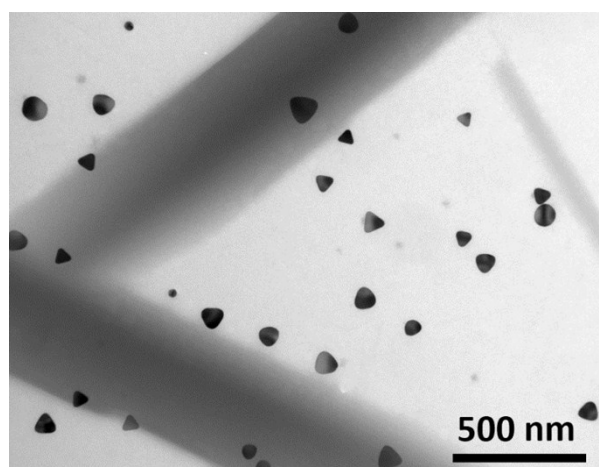


Fig. S3 (a) TEM image of Au NTs prepared in the presence of 5 mM NaBr, 10 μ M CTPR3, 40 mM HEPES and 5 μ l Ag without any purification.

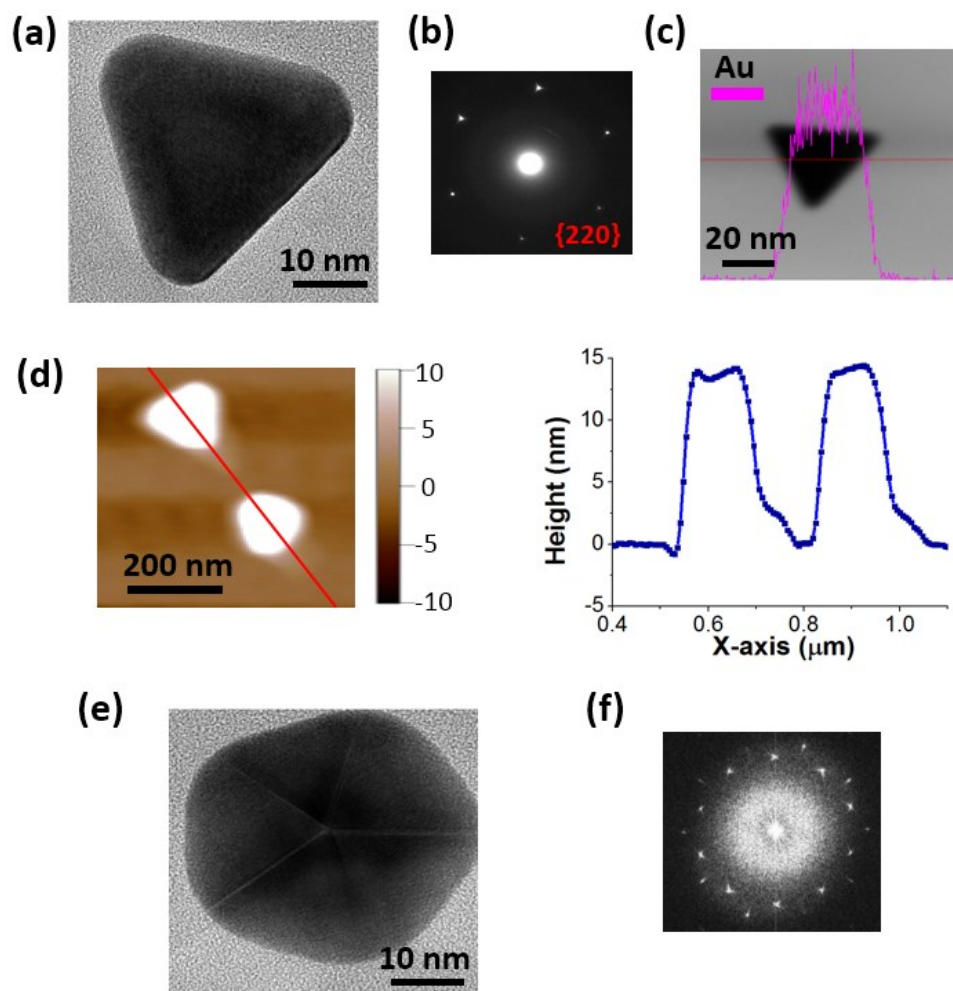


Fig. S4 (a) HR-TEM images (b) SAED diffraction pattern (c) line scanning EDS characterization of Au NTs (d) AFM profile of Au NTs (e) HR-TEM image and (f) FFT diffraction pattern of Au decahedron.

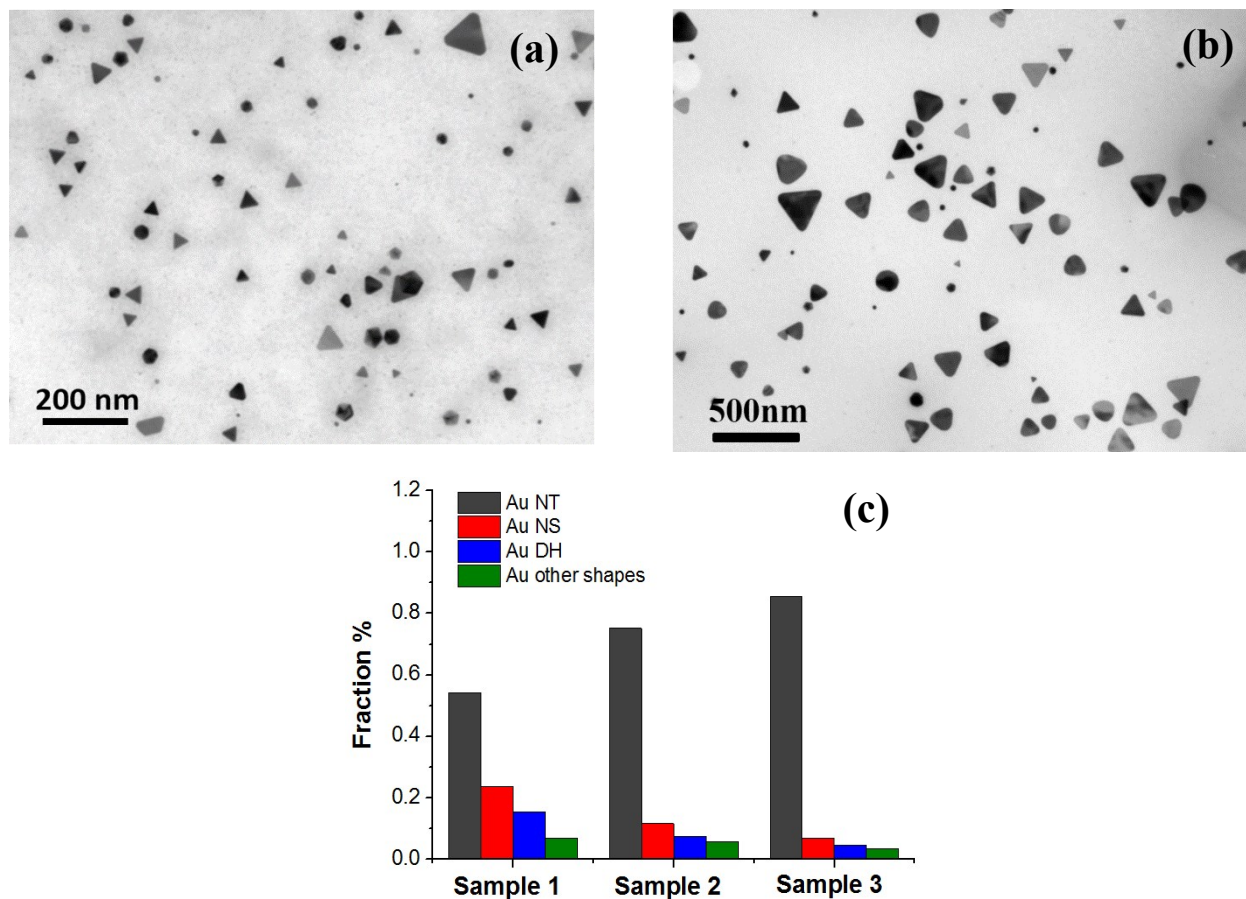


Fig. S5 (a) TEM image of Au NTs prepared in the presence of 5 mM NaBr, 10 μ M CTPR3, 40 mM MOPS and 2.5 μ l Ag seed without any purification. (b) Histogram representing the distribution of Au NPs with different shapes. Sample 1 is prepared in the absence of Ag seed, Sample 2 and 3 is prepared using 2.5 μ l Ag seed before and after purification, respectively. NTs: nanoprisms (dark gray); NS: nanospheres (red); DH: decahedrons (blue); Au with other shapes (green)

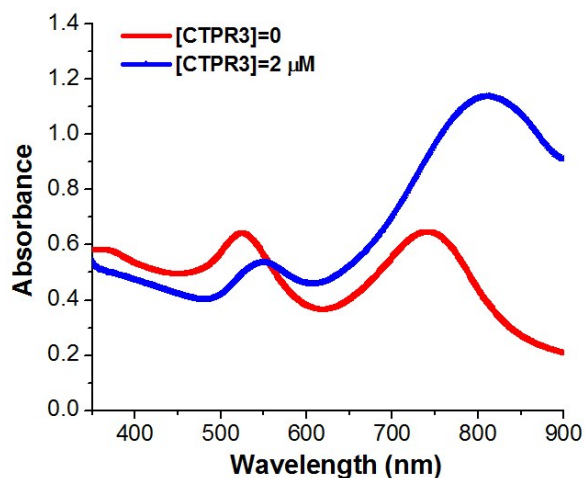


Fig. S6 UV-Vis spectrum of Au NPs prepared in the absence of CTPR3(red) and in the presence of 2 μ M CTPR3(blue) (NaBr=5 mM and Ag seed = 5 μ l).

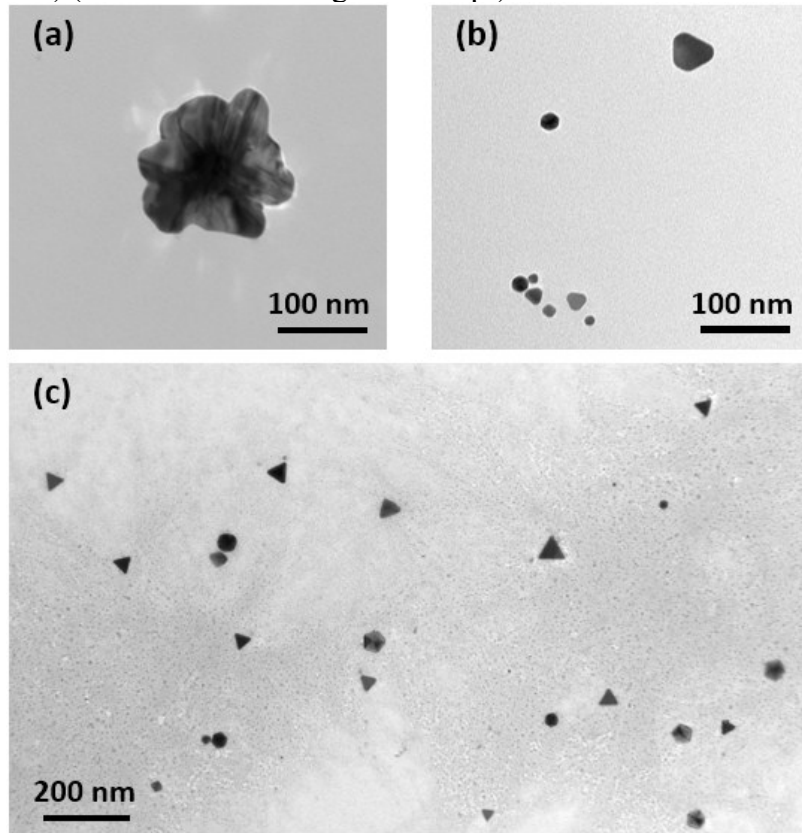


Fig. S7 TEM images of Au NPs prepared in the presence of (a) 0 μ M (b) 0.8 μ M and (c) 4 μ M CTPR3 ([NaBr] =100 mM).

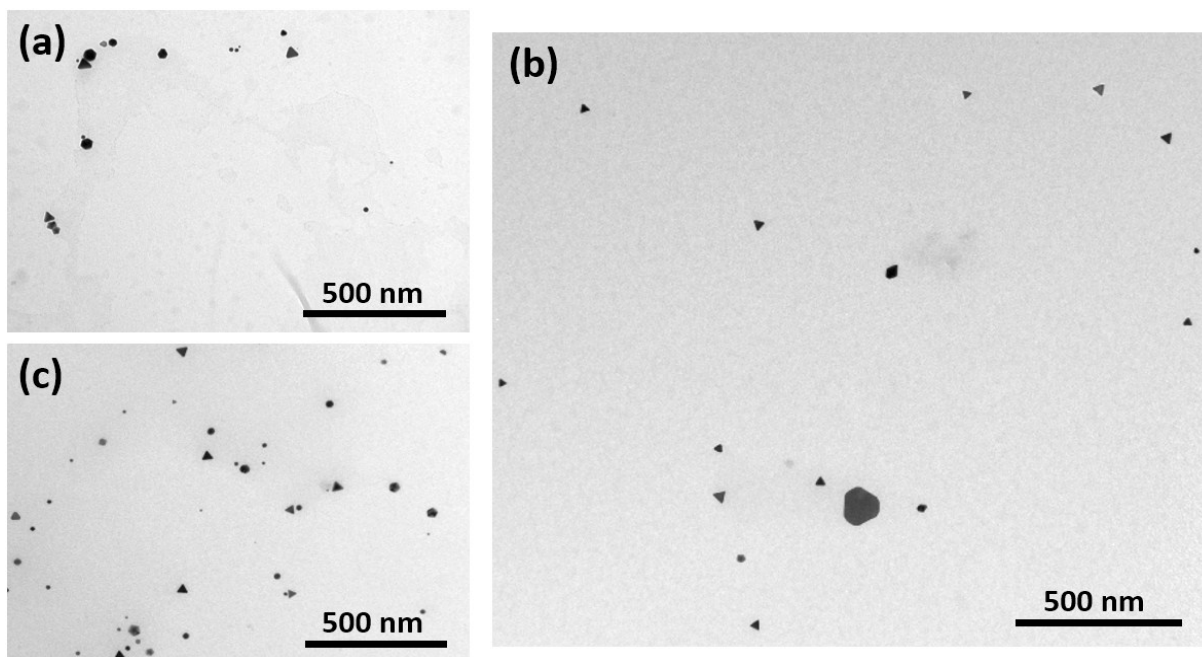


Fig. S8 TEM images of Au NPs prepared in the presence (a) 10 mM, (b) 100 mM, and (c) 300 mM NaBr ([CTPR3]= 10 μ M).

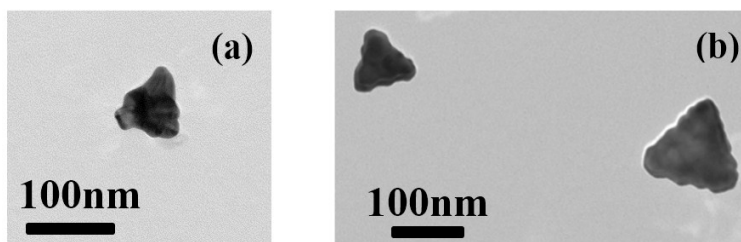


Fig. S9 TEM image of Au NTs with corrugated and stepped edges, prepared in the presence of 100 mM NaBr and 10 μ M CTPR3 (a) after 12 hr and (b) after 1 day.

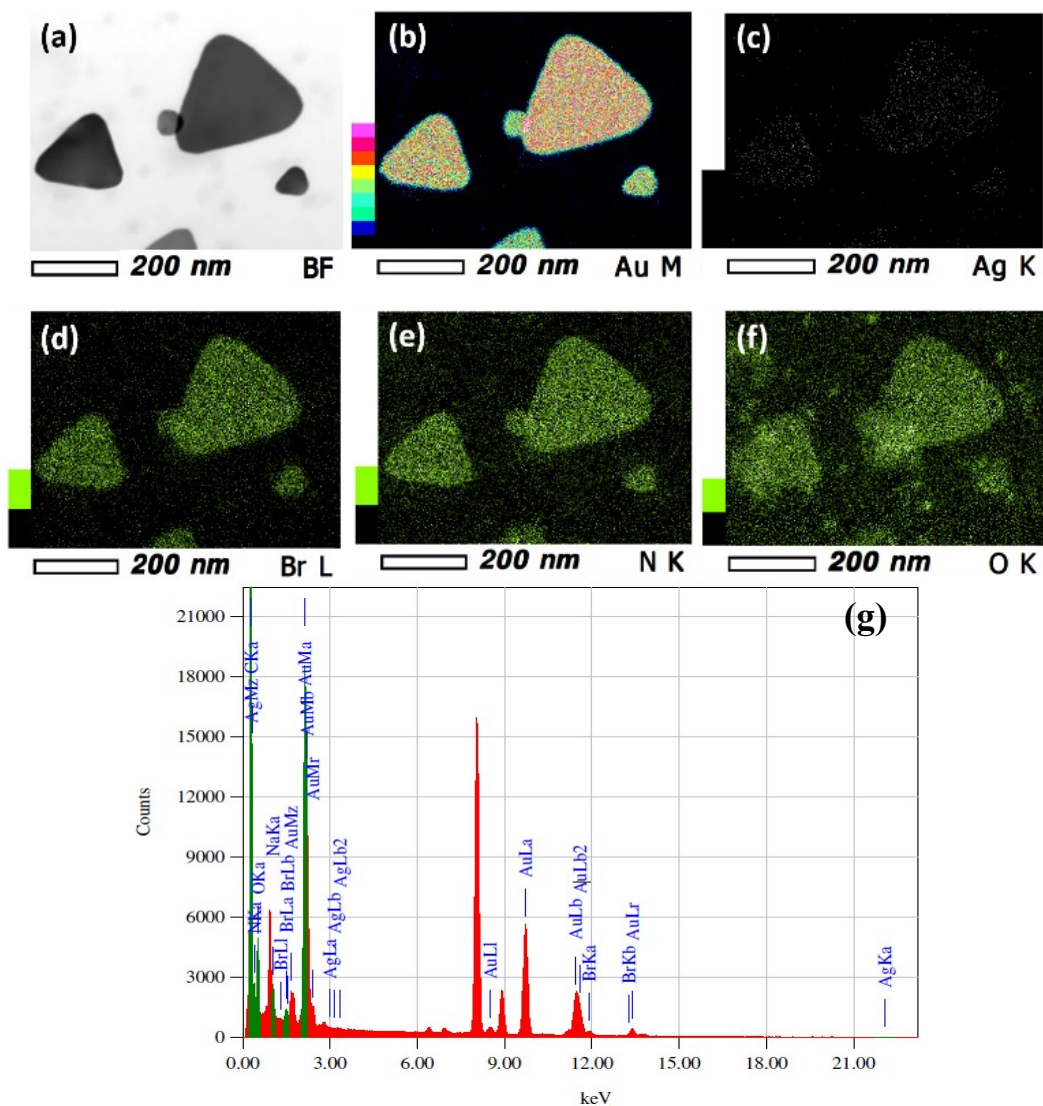


Fig. S10 (a) TEM images of Au NTs prepared in the presence of 2.5 μ l Ag seed. (b-f) Scanning TEM (STEM)-EDS mapping of Au, Ag, Br, N, O, respectively. (Warm color bands indicate higher intensity) (g) EDS spectrum corresponding to Fig.10a.

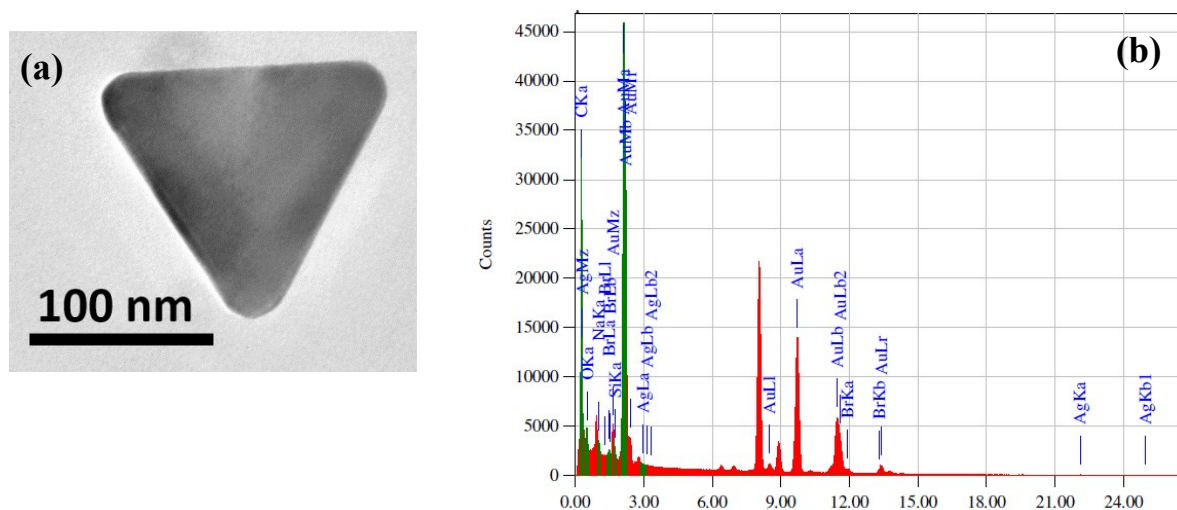


Fig. S11 (a, b)TEM image and STEM-EDS spectrum for single Au NT prepared in the presence of 5 mM NaBr, 10 μ M CTPR3, 40 mM MOPS and 2.5 μ l Ag.

Table S1. The hydrodynamic size and zeta potential values of Au NTs before and after incubating with Fetal bovine serum (FBS).

	Peak 1 Diameter		Peak 2 Diameter		ζ -Potential	
	before (nm)	after FBS (nm)	before (nm)	after FBS (nm)	before (mV)	after FBS (mV)
Au NTs	6.05 \pm 0.14	14.23 \pm 1.37	59.87 \pm 1.17	89.87 \pm 3.01	-27.02	-17.90

Reference

- 1 T. Kajander, A. L. Cortajarena, E. R. G. Main, S. G. J. Mochrie and L. Regan, *J. Am. Chem. Soc.*, 2005, **127**, 10188-10190.
- 2 A. L. Cortajarena, F. Yi and L. Regan, *ACS Chem. Biol.*, 2008, **3**, 161-166.
- 3 D. Aherne, D. M. Ledwith, M. Gara and J. M. Kelly, *Adv. Funct. Mater.*, 2008, **18**, 2005-2016.
- 4 X. Geng, W. Leng, N. A. Carter, P. Vikesland and T. Z. Grove, *J. Chem. Mater. B*, 2016, **4**, 4182-4190.