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Supporting Information

Synthesis of tunable DNA-directed trepang-like Au nanocrystals for imaging application

Zhuomin Zhang*, Jiamin Gao, Zhongning Yu, Gongke Li*

*S*chool of Chemistry, Sun Yat-sen University, Guangzhou, 510275, China

* Corresponding author: Zhuomin Zhang, Gongke Li

Tel.: +86-20-84110922

Fax.: +86-20-84115107

Email: zzm@mail.sysu.edu.cn

(Zhuomin Zhang, ORCID: 0000-0003-1430-3996)

cesgkl@mail.sysu.edu.cn

(Gongke Li, ORCID: 0000-0003-3750-6358)

Tables

Table S1. Sequences of DNA strands used in the study

Name	Sequences (5'-3')
dsDNA1	SH C6-TGG TCC GAT GTC
dsDNA2	GAC ATC GGA CCA
hp1DNA	SH C6-TGGTCCGATGTCTTTTTTTTGACATCGGACCA
hp2DNA	SH C6-TGGTCCGATGTCTTTTTTTTTTTTTTTGACATCGGACCA
taDNA1	SH C6-TGG TCC GAT GTC CTG AGA AGC A
taDNA2	CCTGAGCACG GAC ATC GGA CCA
taDNA3	TGC TTC TCA G AA CGT GCT CAG G

Table S2. Statistical analysis of the bumpsizeof trepang-like AuNCs directed by different DNA strands

DNA structures	Bottom size/nm	Top size/nm	Length/nm
dsDNA	8.77±1.34	7.22±1.05	9.64±1.51
hp1DNA (small loop)	6.80±0.61	7.94±0.88	11.16±1.00
hp2DNA (big loop)	7.34±0.84	8.95±1.42	12.00±1.25
taDNA	7.16±0.81	8.50±1.19	11.50±1.23

Figures

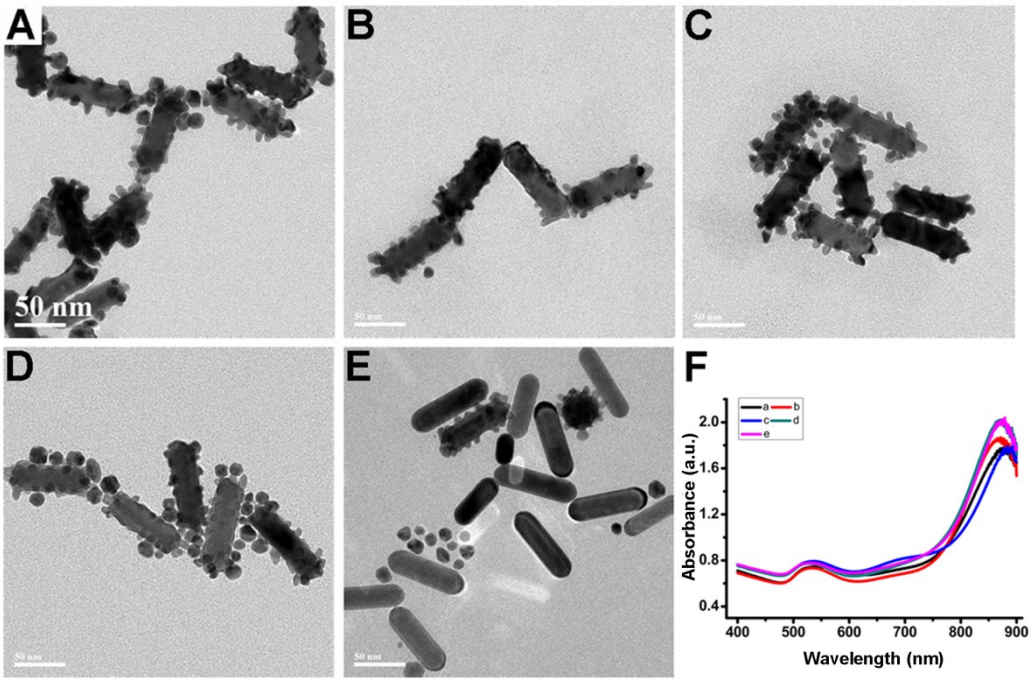

Figure S1. TEM images of AuNCs prepared by use of different amounts of AA solution including 10.0 (A), 20.0 (B), 30.0 (C), 40.0 (D) and 50.0 μL (E), respectively. The concentration of AA solution was 10.0 mM. (F) The corresponding UV-Vis spectra of these AuNCs.

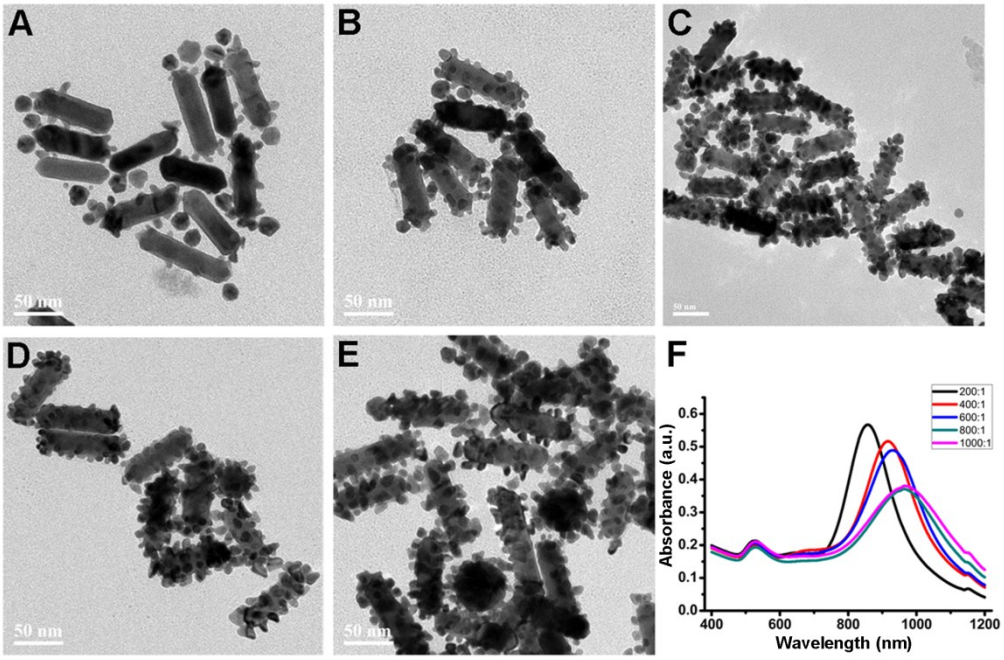

Figure S2. TEM images of AuNCs prepared with different molar ratios of DNA to AuNRs at 200:1 (A), 400:1 (B), 600:1 (C), 800:1 (D) and 1000:1 μL (E). (F) The corresponding UV-Vis spectra of these AuNCs.

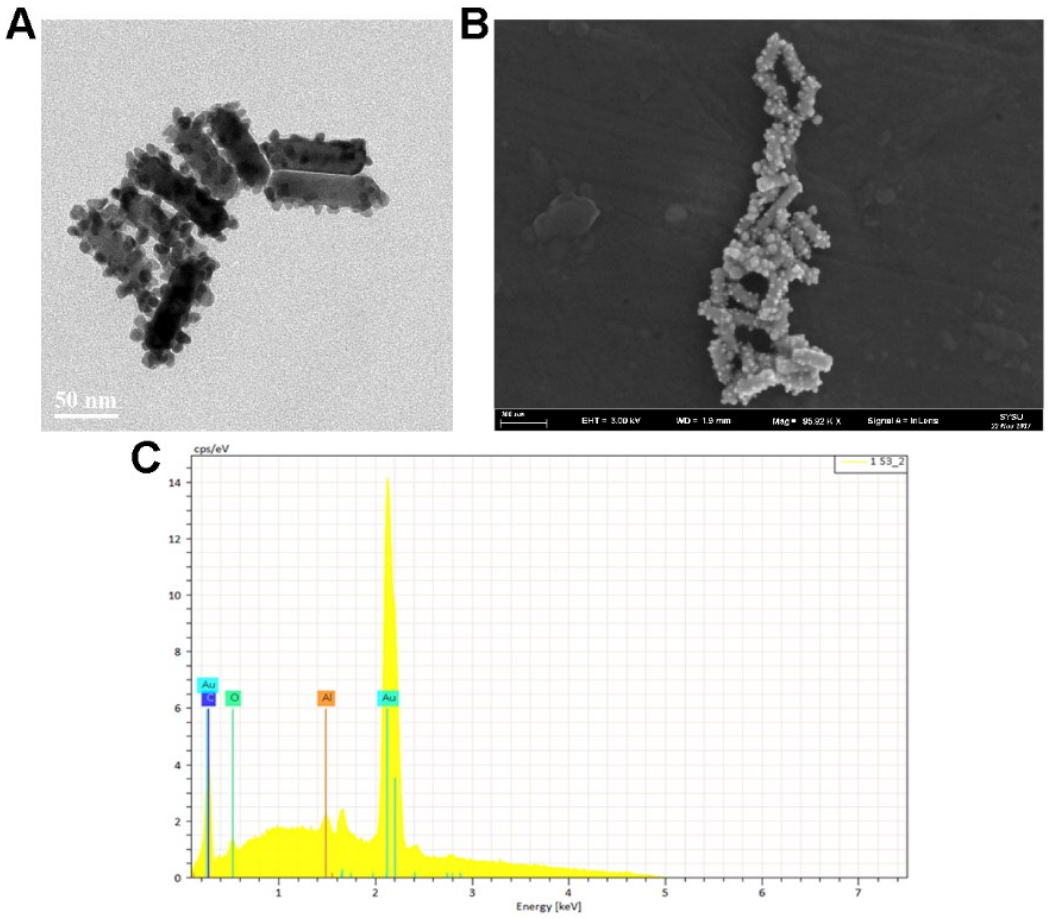

Figure S3. TEM (A) and SEM (B) image of trepang-like AuNCs under optimal preparation conditions. (C) EDX analysis of trepang-like AuNCs. AuNRs were incubated with hp2DNA with a molar ratio of 1:600. Then, 100 μL hp2DNA-conjugated AuNRs solution was mixed with 0.5 μL of 20% Tween 80 solution, and 45.0 μL of $HAuCl_4$ solution (pH=5.0) and 56.3 μL of AA solution (pH=5.0) were added for reduction reaction.

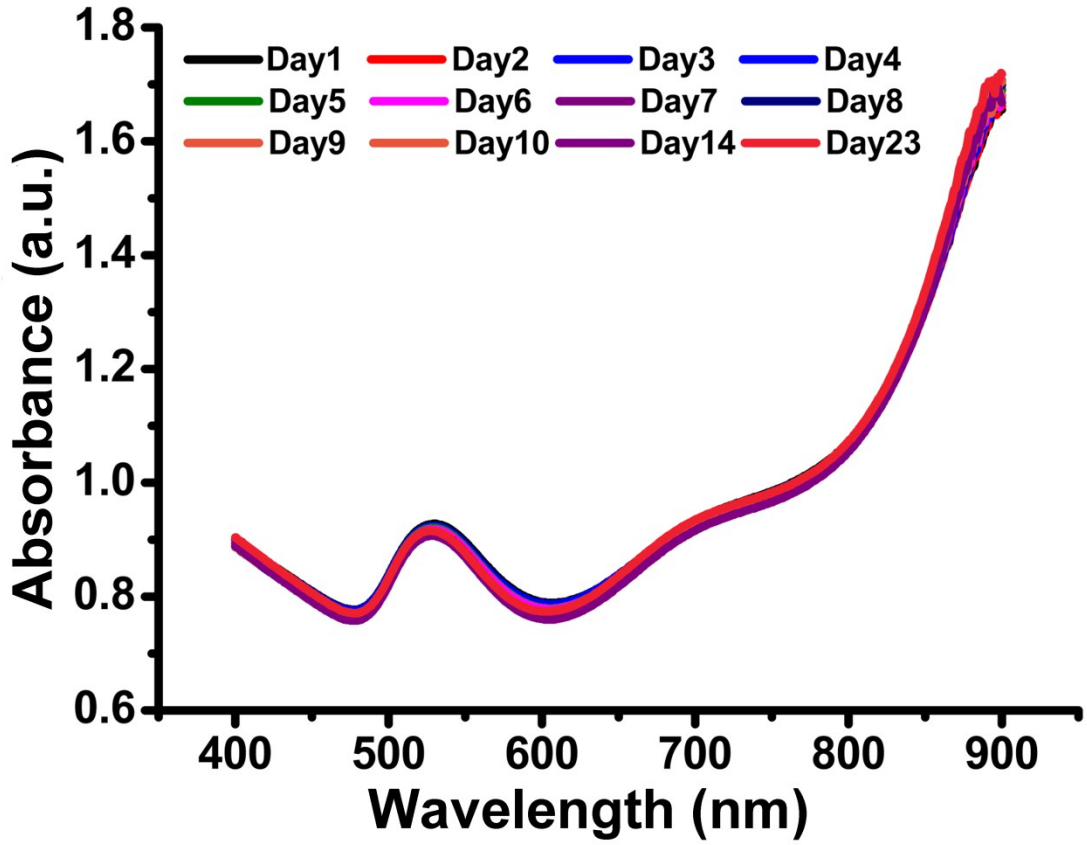

Figure S4. UV-Vis absorption spectra of trepang-like AuNCs solutions during a 23-day testing period.

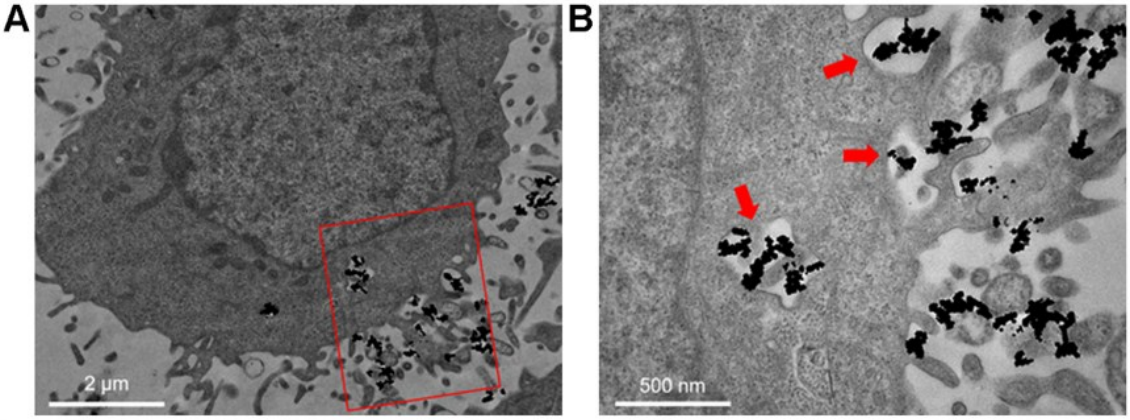

Figure S5. TEM images of Hela cells slicesafter incubation with trepang-like AuNCs for 12 h; (B) The magnification of the area marked with red lines in (A).

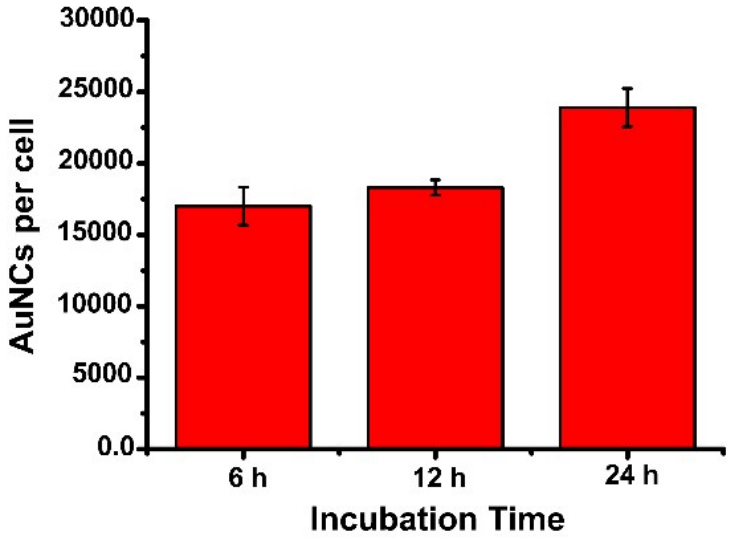

Figure S6. Uptake amounts of trepang-like AuNCs by Hela cells for different incubation time.

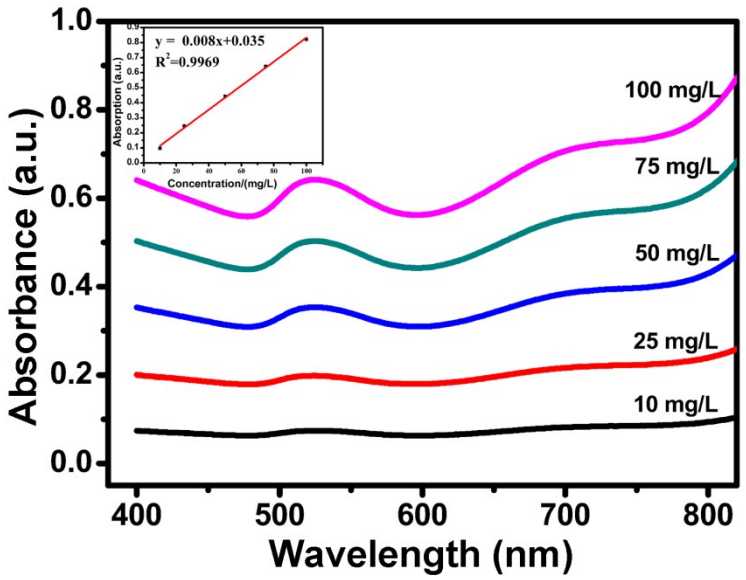

Figure S7. UV-vis absorption spectra of trepang-like AuNCs solution with different concentrations. The inset figure showedthe relation between the absorption intensity of trepang-like AuNCs at 808 nm and the corresponding concentration.

FDTD simulation

FDTD calculations were performed using commercial software FDTD (FDTD solutions 6.0, Lumerical Solutions). A mesh size of 0.5 nm was used for all calculations. For FDTD calculation, AuNR was modelized with a cylinder capped with two half spheres at both ends whose average values were 76 × 24 nm. For four different trepang-like AuNCs directed by different DNA structures, the protrusions on the surface of AuNCs were modelized with trapezoidal cylinder and hemisphere based on the modelized AuNRs, whose sizes were referred to the relative data shown in Table S2. The light source with a wavelength of 400-1200 nm was performed for the simulation of absorption mode.

Cell culture

A human cervical cancer cell line (HeLa) was maintained as a monolayer culture in DMEM (high glucose, GIBCO, Invitrogen) medium supplemented with 10% fetal bovine serum (Atlanta Biologicals, Lawrenceville, GA, USA) and 1% penicillin–streptomycin (Gibco BRL, Grand Island, NY, USA) at 37 °C in a humidified 5% CO_2 incubator.

Cellular uptake

HeLa cells were seeded into a 6-well plate at a density of 2×10^5 cells/well. After incubation for 24 h, the culture medium was replaced with 3 ml of fresh medium containing trepang-like AuNCs (100.0 $mg \cdot L^{-1}$ of Au) and then incubated in for another 12 h. After that, the cells were washed three times with PBS and then trypsinized and collected by centrifugation at 2000 rpm for 15 min. Finally, the cells were fixed with 2.5% glutaraldehyde fixative at 4 ℃ for 12 h before TEM observation. As shown in **Fig. S5**, it was indicated that with endocytic uptake AuNCs were transferred by endosome and located in the lysosome.

In order to quantify the amount of gold uptaken in cancer cells, inductively coupled plasma-atomic emission spectrometry (ICP-AES) analysis was performed in Hela cells. Hela cells were cultured in a 6-well plate with a density of 2×10^5 cells per well and incubated at 37 ℃ for 24 h. Then, the medium was removed and fresh medium containing 100.0 $mg \cdot L^{-1}$ trepang-like AuNCs was added to each well for the cellular uptake experiment. The cells were incubated for 6 h, 12 h and 24 h respectively with three dishes of cells being used at each time point. Thereafter, the

cells in each well were washed for three times with cold PBS to remove unbound particles. Cells were trypsinized by adding 500 μL of trypsin EDTA and cell numbers were counted. These trypsinized cells containing nanocrystals were collected by centrifugation and then digested completely by concentrated nitric acid with 90 ℃ water bath and hydrochloric acid. Finally, they were made up to 20 ml by diluting with pure water and subjected to ICP-AES analysis to measure the amount of gold uptaken by cancer cells. It was seen from **Fig. S6** that uptake amount of trepang-like AuNCs by Hela cell was increased from 1.70×10^4 to 2.39×10^4 AuNCs/cellwith the increasing incubation time from 6 to 24 h. Thus, 24 h was chosen as the optimized incubation time of trepang-like AuNCs with cells.

Calculation of Photothermal Conversion Efficiency

According to the previous report,[1,2] the total energy input and output from a system was calculated as follows:

$$\sum_i m_i C_{p,i} \frac{dT}{dt} = Q_{in,\ AuNCs} + Q_{in,\ surr} - Q_{out} \quad (1)$$

in which $m_i$and $C_{p,i}$were the mass and heat capacity of solvent (water), T was the solution temperature, $Q_{in,\ AuNCs}$was the energy inputted by AuNCs, $Q_{in,\ surr}$was the energy inputted by the sample cell, and $Q_{out}$was heat conduction away from the system surface by air.

The NIR laser induced source term, $Q_{in,\ AuNCs}$, represented heat dissipated by electron-phonon relaxation of the plasmon on the surface of AuNCs under the laser irradiation at 808 nm

$$Q_{in,\ AuNCs} = I\left(1 - 10^{-A_{808}}\right)\eta \quad (2)$$

in which I was the incident laser power (in unit of mW), A_{808} was the absorbance of trepang-like AuNCs at 808 nm, and η was the photothermal conversion efficiency from incident laser energy to thermal energy.

In addition, $Q_{in,\ surr}$ that expressed heat dissipated from light absorbed by the eppendorf tube itself was measured independently to be 15.4 mW using a sample cell containing pure water without AuNCs.

The heat output, Q_{out}, could be calculated as follows

$$Q_{out} = hS(T - T_{Surr}) \quad (3)$$

where h was heat transfer coefficient, S was the surface area of the container, and T_{Surr} was ambient temperature.

When the heat input was equal to heat output, the equation (1) could be transferred to be

$$Q_{in,\ AuNCs} + Q_{in,\ surr} = Q_{out} = hS(T_{Max} - T_{Surr}) \quad (4)$$

where T_{Max} was the equilibrium temperature. The laser photothermal conversion efficiency (η) at 808 nm could be determined by substituting equation (2) for AuNCs into equation (4) and rearranged as

$$\eta = \frac{hS(T_{Max} - T_{Surr}) - Q_{in,\ surr}}{I\left(1 - 10^{-A_{808}}\right)} \quad (5)$$

In the above equation, $(T_{Max} - T_{Surr})$ was 52.95 °C, $Q_{in,\ surr}$ was 15.4 mW, I was 2000.0 mW, and A_{808} was 0.82 at 808 nm. Thus, only the item of hS was unknown for calculating η.

In order to get hS, a sample system time constant $\tau_s$was expressed. And when the light source was turned off, the equation (1) could be transferred to be

$$Q_{in,\ AuNCs} + Q_{in,\ surr} = 0 \quad (6)$$

Thus, the equation (7) was obtained after integration as follows.

$$t = -\tau_s ln\frac{T - T_{amb}}{T_{Max} - T_{amb}} \quad (7)$$

Therefore, τ_s was determined to be 390.91 s. As the m and C were referred to the mass (1 g) and heat capacity (4.2 J/g) of deionized water used as solvent, the hScould be calculated to be 10.74 mW/°C. Finally, the photothermal conversion efficiency (η) of trepang-like AuNCs could be calculated to be 36.2%.

Reference

1. H. Chen, L. Shao, T. Ming, Z. Sun, C. Zhao, B. Yang and J. Wang, Small, 2010, 6, 2272-2280.
2. Q. Tian, F. Jiang, R. Zou, Q. Liu, Z. Chen, M. Zhu, S. Yang, J. Wang, J. Wang and J. Hu, ACS nano, 2011, 5, 9761–9771.