Supporting Information

Simultaneous regulation of optical properties and cellular behaviors of gold nanoclusters by pre-engineering the biotemplates

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1 Experimental

1.1 Reagents

All chemicals used were of analytical grade without further purification. All stock and buffer solutions were prepared using water purified with a Molecular 1010b filtration system (Shanghai, China). Gold (III) chloride trihydrate ($HAuCl_4 \cdot 3H_2O$), 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and bovine serum albumin (BSA) were purchased from Sigma-Aldrich. Succinic anhydride, ethylenediamine dihydrochloride and sodium hydroxide were purchased from Aladdin. All other reagents were purchased from Sinopharm.

1.2 Modification and characterization of BSA

Proteins with different surface charges were prepared according to previous reports.[1] The solvent used in the experiment was PBS (phosphate buffered saline) unless otherwise specified. In general, to prepare cBSA, 1 mL ethylenediamine (0.5 M) was added into 1 mL native BSA (nBSA, 50 mg/mL), followed by 4 mg EDC. The reaction is completed after stirring for 2 h at room temperature. For the preparation of aBSA, 100 μL succinic anhydride solution (0.8 M) in 1,4-dioxane was added into 2 mL nBSA (0.8 mM) solution in PBS (10 mM, pH = 8.5), followed by stirring at room temperature for 2 h. Derivatives of BSA were purified by using a ultrafiltration tube (30 KDa, Sartorius, Germany) with ultrapure water as the elution solvent. The molar concentration of BSA was measured with a U-3900H spectrophotometer (Hitachi, Japan), using an extinction coefficient of $4.4\times10^{4}\ M^{-1}\cdot cm^{-1}$ at 278 nm. Number of free amino groups were obtained by a 2,4,6-Trinitrobenzenesulfonic acid (TNBS) - based method.[2] Through reacting with the free amino groups of proteins, TNBS will be converted to trinitrophenyl (TNP) derivatives with a strong adsorption band at 420 nm. With the comparison of nBSA (59 free amino groups), the number of free amino groups of proteins can be calculated from this absorption peak. The zeta potentials and hydrodynamic diameter of BSA and its variants in PBS (pH = 7.45, 10 mM) were measured with a Zetasizer (Malvern, UK). Circular dichroism (CD) spectra of BSA were taken by a Chirascan CD spectrometer (Applied Photophysics, UK).

1.3 Synthesis and characterization of different BSA-AuNCs

BSA-AuNCs were synthesized as reported previously with slight modification.[3,4] Briefly, 1 mL of 11.6 mM aqueous $HAuCl_4$ was added into 1 mL of 0.53 mM BSA (or its derivatives), followed by 22 μL NaOH (5 M, aqueous solution). The mixture was stirred for 15 min at 100 °C. As-prepared BSA-AuNCs were further purified by centrifugation filtration, using ultrafiltration tube (30 KDa, Sartorius, Germany) to remove impurities. Purified AuNCs were diluted with ultrapure water and stored at 4 °C for later use.

Fluorescence spectra were obtained with a FLS 980 spectrofluorometer (Edinburgh Instruments, UK). Transmission electron microscopy (TEM) images were taken using a Tecnai G2 F20 S-TWIN transmission electron microscope (FEI, USA) operated at 200 kV. X-ray photoelectron spectroscopy (XPS) was measured on an Axis Ultra DLD XPS spectrometer (Kratos, Manchester, UK), using Al Kα X-ray radiation (1486.6 eV) for excitation. All spectra were corrected by the C 1s peak at 284.8 eV. PXRD of BSA, cBSA-AuNCs, nBSA-AuNCs and aBSA-AuNCs were measured by Bruker D8 ADVANCE (Germany)

1.4 Cell culture and cytotoxicity assay

All operations were performed in a sterile environment. BSA-AuNCs were purified through a 200 nm filter before the cellular experiment. HeLa and RAW 264.7 cells were cultured in Dulbecco's modified eagle medium (DMEM), including 10% fetal bovine serum (FBS), 60 μg/mL of penicillin, and 100 μg/mL streptomycin in a humidified incubator (HF90, Heal Force, China) at 37 °C and 5% CO_2. The cell viability was evaluated by measuring the metabolic activity of HeLa and RAW 264.7 cells using a CCK-8 assay. HeLa and RAW 264.7 cells were seeded in a 96-well plate in cell medium (5×10^3 per well) overnight and then incubated in pure DMEM (without FBS) containing 0 μg/mL, 250 μg/mL, 500 μg/mL and 750 μg/mL BSA-AuNCs for 4 h at 37 °C and 5% CO_2. After washing thoroughly with PBS, cells were further incubated in cell medium for 24 h, followed by adding 100 μL fresh medium plus 6 μL CCK-8 solution (Beyotime, China). Upon 2 h incubation, the absorbance of the solution at 450 nm was measured.

1.5 Inductively coupled plasma - optical emission spectrometry (ICP-OES)

measurement

HeLa cells (200 per μL) were seeded overnight in a petri dish. After 4 h incubation in media containing 500 μg/mL BSA-AuNCs in pure DMEM without FBS at 37 °C and 5% CO_2, cells were washed thoroughly with PBS for 3 times. Afterwards, cells were harvested by treating with 4% trypsin and further concentrated by centrifugation (× 2000 g) for 2 min. 0.5 mL freshly prepared aqua regia (Caution! Aqua regia is a strong acid) was then added to the collected cells and incubated at room temperature for 2 h. Then 0.4 mL of dissolved samples were mixed with 2.6 mL deionized water and used for the further measurement. Concentration of gold in the samples was then determined by using a Perkin Elmer Optima 8300 DV ICP-OES (Shelton, USA) with a plasma flow of 15 L/min.

1.6 Fluorescence imaging

Cells were seeded in eight-well LabTekTM chambers (Nunc, Langenselbold, Germany) and allowed to adhere overnight in a humidified incubator at 37 °C and 5% CO_2 before experiments. Cells were then incubated with 500 μg/mL cBSA-AuNCs in pure DMEM without FBS for 4 h at 37 °C and 5% CO_2 before washing thoroughly with PBS. Cell imaging was performed by using an TCS SP8 Confocal Laser Scanning Microscope (Leica, Wetzlar, Germany). BSA-AuNCs were excited at 405 nm and the emission was collected in the range of 700 nm to 800 nm.

Reference

1 L. Shang, L. Yang, J. Seiter, M. Heinle, G. Brenner-Weiss, D. Gerthsen and G. U. Nienhaus, *Adv. Mater. Interfaces*, 2014, **1**, 1300079.

2 J. Su, S. C. Satchell, J. A. Wertheim and R. N. Shah, *Biomaterials*, 2019, **201**, 99-112.

3 J. Xie, Y. Zheng and J. Y. Ying, *J. Am. Chem. Soc.*, 2009, **131**, 888-889.

4 P. Zhang, X. X. Yang, Y. Wang, N. W. Zhao, Z. H. Xiong and C. Z. Huang, *Nanoscale*, 2014, **6**, 2261-2269.

2 Supporting figures

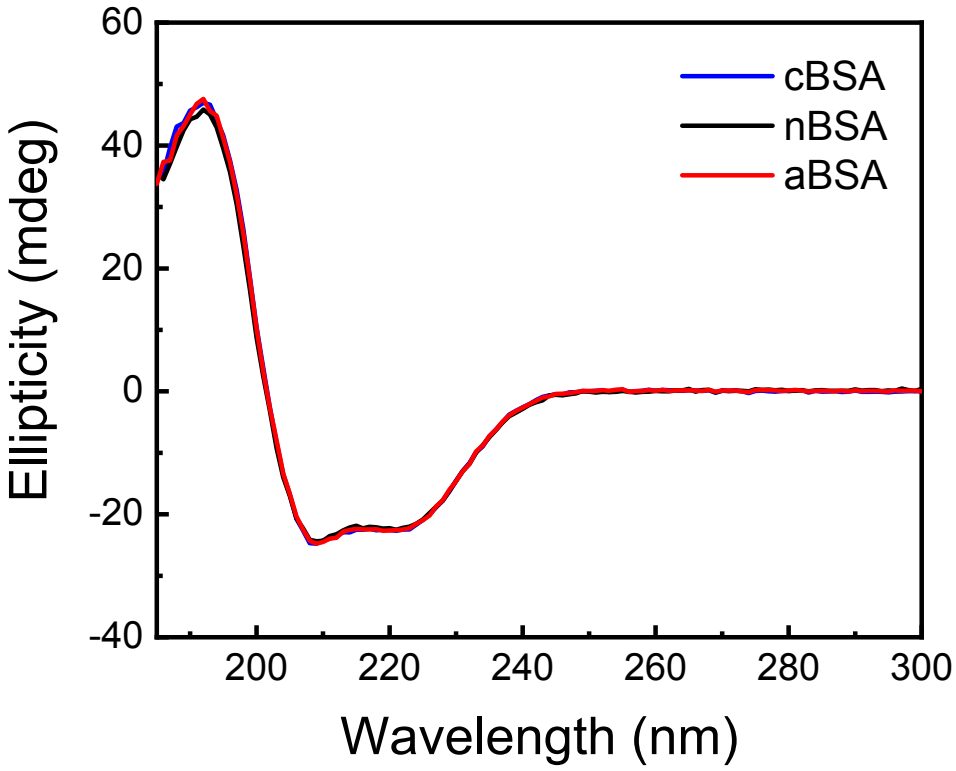

Figure S1 CD spectra of cBSA, nBSA and aBSA at a concentration of 0.0175 mg/mL.

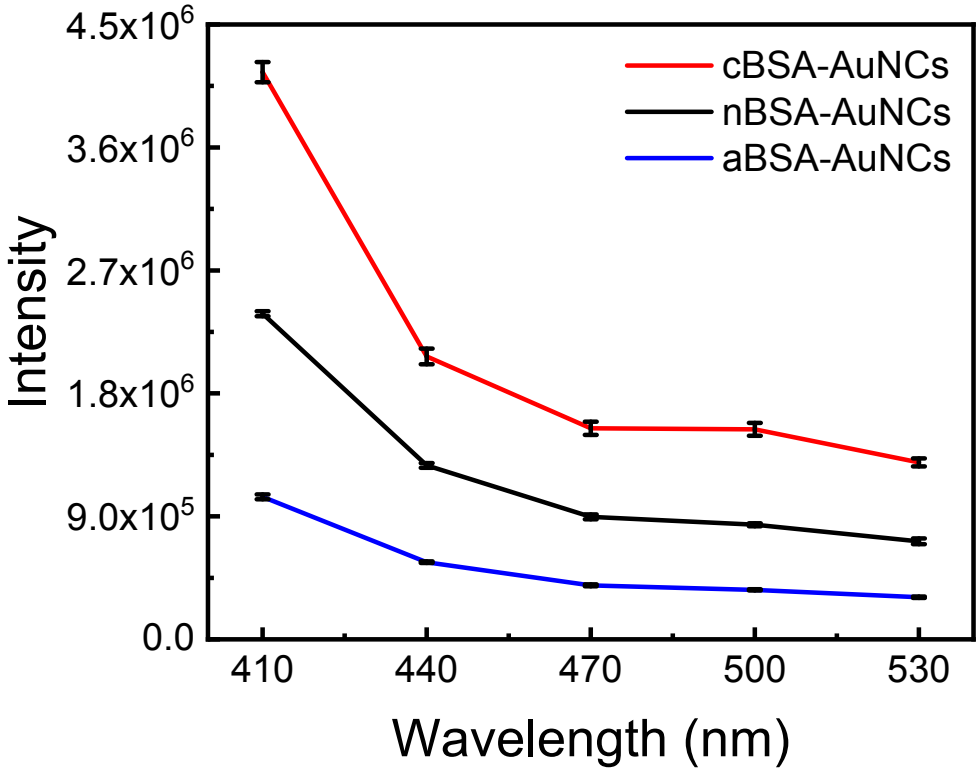

Figure S2 Change of fluorescence intensity of cBSA-AuNCs, nBSA-AuNCs and aBSA-AuNCs excited by different excitation wavelengths from 410 nm to 530 nm.

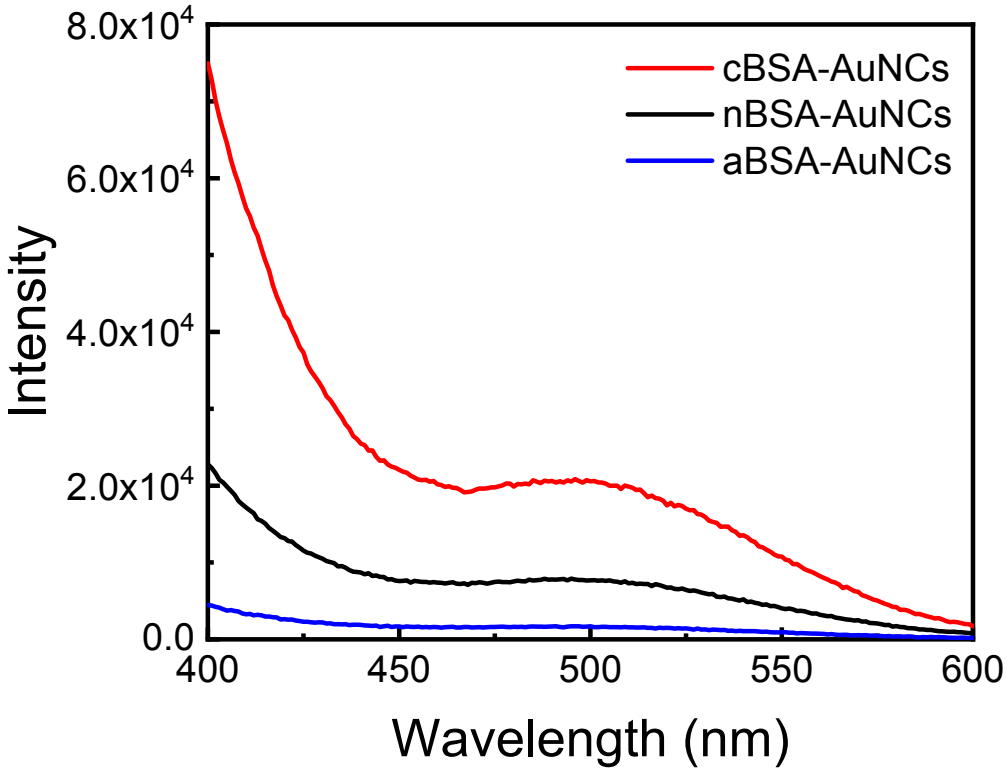

Figure S3 Excitation spectra of cBSA-AuNCs, nBSA-AuNCs and aBSA-AuNCs measured at the emission wavelength of 684 nm.

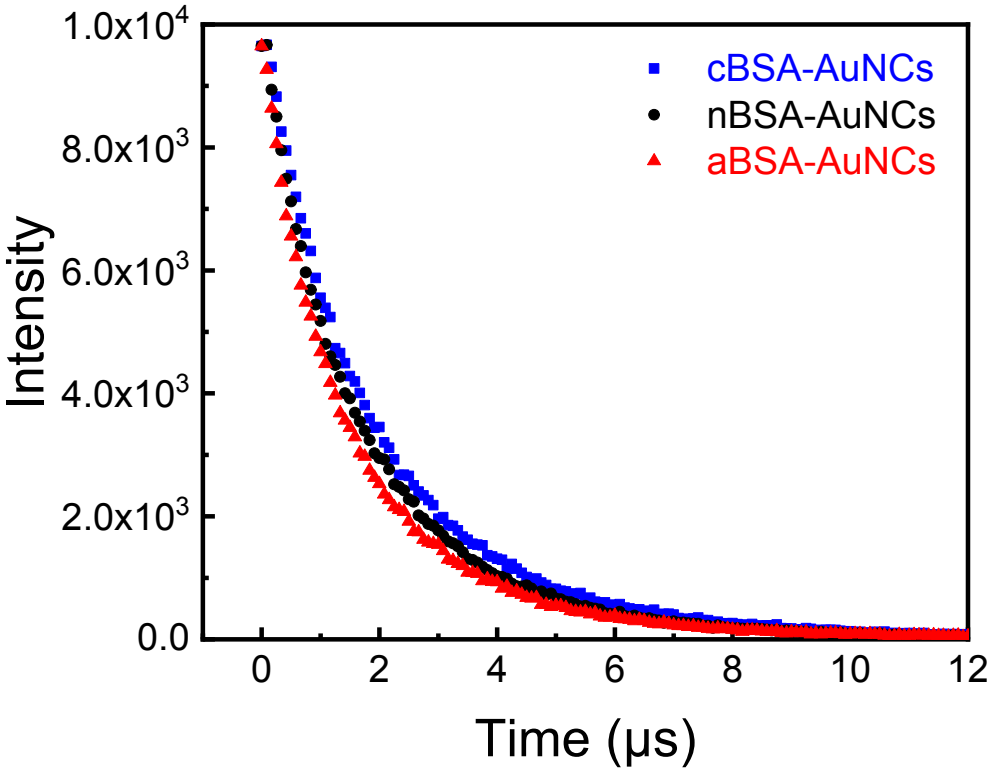

Figure S4 Fluorescence decay of cBSA-AuNCs, nBSA-AuNCs and aBSA-AuNCs measured at the excitation wavelength of 405 nm.

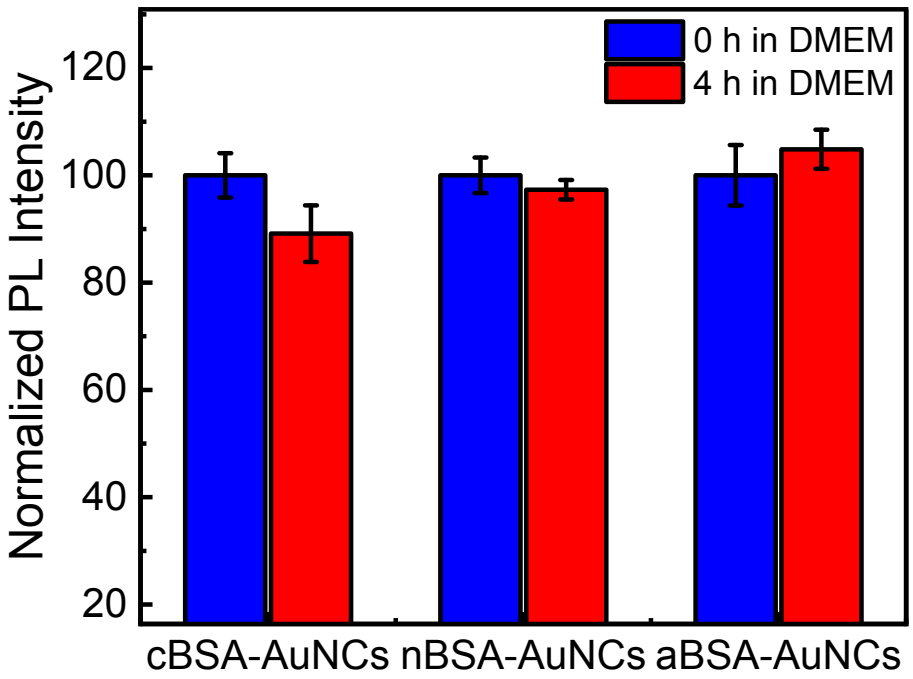

Figure S5 Stability of different BSA-AuNCs in DMEM for 4 h at 37 °C as monitored by their fluorescence intensity.

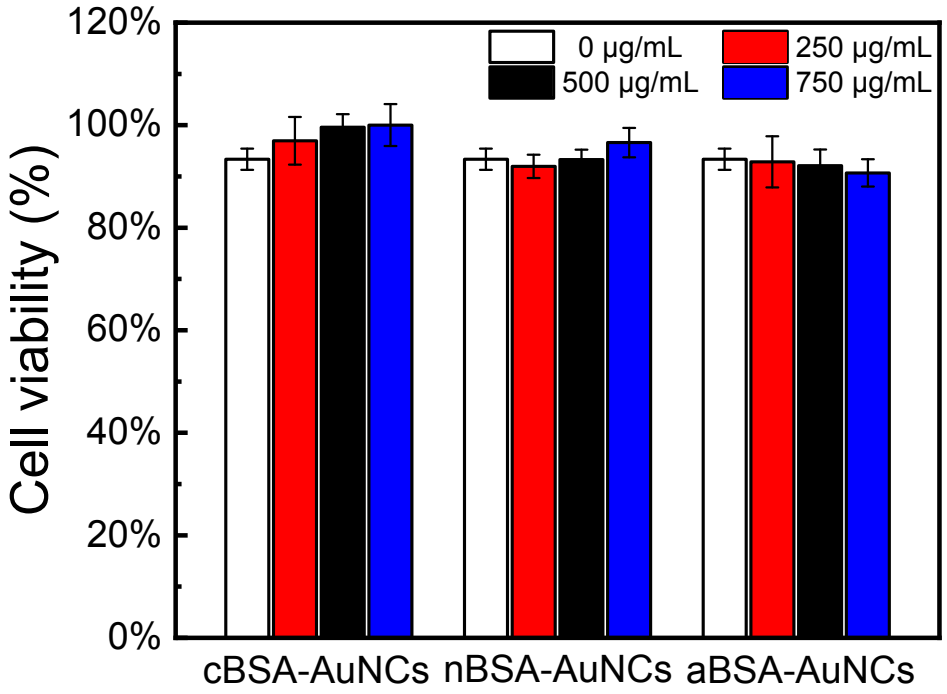

Figure S6 Cell viability of RAW 264.7 cells after incubation with (0 μg/mL, 250 μg/mL, 500 μg/mL and 750 μg/mL) BSA-AuNCs for 4 h.

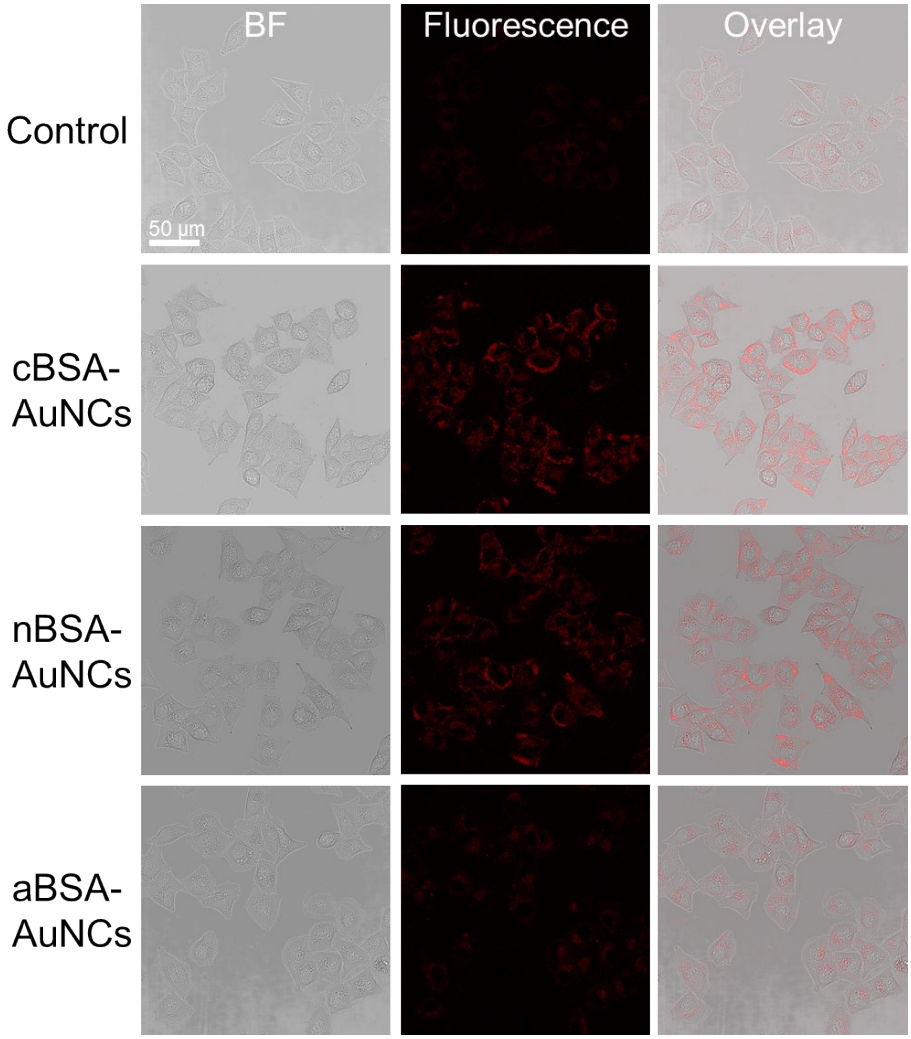

Figure S7 Brightfield (BF, left), confocal fluorescence (middle) and overlay (right) images of HeLa cells before (control) and after co-culture with different BSA-AuNCs for 4 h.