(Supporting Information)

Hydrothermal Synthesis of Novel Photosensitive Gold and Silver Bimetallic Nanoclusters Protected by Adenosine Monophosphate (AMP)

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Chemical

Adenosine 5'-monophosphate (AMP) was purchased from TCI (Shanghai) Development Co., Ltd. and its purity was higher than 99%. $HAuCl_4 \cdot 3H_2O$, $AgNO_3$, sodium citrate, NaH_2PO_4 and Na_2HPO_4 were bought from Beijing Chemical Factory (Beijing, China) and the purities of them were higher than 99% either. Distill water (ρ = 18.2 MΩ·cm, 25 °C) was obtained from a Millipore Milli-Q water purification system. The stock solution of AMP (20 mM) was prepared with distill water and stored in dark at 4 °C. And 20 mM phosphate buffer solution (PBS, pH=7.4) was prepared with 20 mM NaH_2PO_4 and Na_2HPO_4 in aqueous solution.

Luminescence spectra

Luminescence spectra were recorded on a Shimadzu (Japan) RF-5301PC spectrophotometer. To reduce the fluctuation of excitation intensity during measurement, the lamp was kept on for 0.5 h before the measurement. The growth process of the Au-AgNCs@AMP was monitored by using luminescence spectra, for that each extracted sample was ten-times diluted with 20.0 mM PBS (pH = 7.4). That was, after mixing 100 μL reaction solution with 900 μL PBS the luminescence spectrum of it was measured by the fluorescence spectrophotometer. All other spectroscopic measurements were performed in 20.0 mM PBS (pH = 7.4), and an excitation wavelength at 354 nm was fixed.

Time-resolved luminescence spectra were obtained by using an FLS980-Edinburgh instrument. A quartz cuvette of 1.0 × 1.0 cm was used to contain the sample. The excitation wavelength was selected at 375 nm for AuNCs@AMP and Au-AgNCs@AMP, and the intensity at 480 and 550 nm

were collected, respectively. The lifetimes and percentage of them were calculated by exponentially fitting the decay curve with the software including in the FLS980 Edinburgh Instruments.

Uv-vis absorption spectra

The Uv-vis absorption spectra were recorded on a Shimadzu UV-3600 spectrophotometer, and all measurements were performed in 1 cm × 1 cm quartz cuvettes (4 mL volume).

Transmission electron microscopy (TEM)

Transmission electron microscopy (TEM) images were performed to observe the constitutive nanocrystals of Au-AgNCs by using JEM-2200FS (Jeol Ltd. Japan) at an accelerating voltage of 200 kV. For allowing TEM observations, the sample was suspended in aqueous solution under ultrasonic treatment prior to direct deposition on a copper grid and air-drying.

Preparation of the gold and silver bimetallic nanoclusters (Au-AgNCs@AMP).

A) The preparation of Au-AgNCs@AMP with three methods.

Firstly, the Au-AgNCs@AMP were prepared by employing the hydrothermal synthesis method. To a 20 mL autoclave (Teflon-lined steel-stainless autoclave), 17.4 mg of AMP solid sample, 8.4 mL of deionized water, 200 μL, 10 mM $HAuCl_4$ solution, 1.0 mL, 10 mM $AgNO_3$ solution, 400 μL, 0.50 M sodium citrate solution were added and mixed well. The final ratio of Au: Ag: AMP was 0.2: 1: 5. After heating at 120 °C for 30 minutes, the autoclave was removed out and cooled down at room temperature. In addition, the products were improved through regulating the concentrations of AMP, $HAuCl_4$ and $AgNO_3$ and the heating temperature and time, and finally they were monitored by luminescence spectra.

Secondly, the Au-AgNCs@AMP were prepared by using heating and stirring method either. To a 50 mL round bottom flask wrapped by foil, 34.8 mg of AMP solid sample, 16.8 mL of deionized water, 400 μL, 10 mM $HAuCl_4$ solution, 2.0 mL, 10 mM $AgNO_3$ solution were added, and after stirring for 2 minutes, 800 μL, 0.50 M sodium citrate solution was introduced to the system. Then it was heating at 80 °C for 6 hours by using a heating jacket, then the reaction was stopped and cooled down at room temperature.

Thirdly, the Au-AgNCs@AMP were prepared by using AuNCs@AMP as a seed and employing the hydrothermal synthesis method. For that, AuNCs@AMP were first obtained by heating and stirring method.[43] Then, 5.0 mL, 1.0 mM AuNCs@AMP stock solution, 1.0 mL, 10 mM $AgNO_3$ solution, 17.4 mg of AMP solid sample, and 4 mL deionized water were added to the 20 mL autoclave. After heating at 120 °C for different time, autoclave was removed out and cooled down at room temperature.

B) The purification of Au-AgNCs@AMP

After the reaction was ceased, the obtained solution was filtered with a 0.22 μm membrane to remove small amount of large particles. Then 20 mL acetone was added to the filtrate and stirred vigorously; after centrifuging at 4000 rpm for 30 min the precipitates were collected. The precipitates were dissolved in distill water and then added acetone and centrifuged for three times, which could remove all the residual AMP, citrate and other small molecules. The finally precipitates were dried with freeze-drying overnight.

The photosensitivity of Au-AgNCs@AMP

Firstly, 1.0 mL stored solution of Au-AgNCs@AMP was diluted with distill water to 5.0 mL and divided into 5 parts and removed to EP tubes wrapped by foil. The samples were exposed to sunlight for 0, 0.5, 1, 2 and 5 h, respectively. Then the luminescence and Uv-vis absorption spectra were detected to monitor the changes in the process, and the corresponding luminescence and visible light photos were taken.

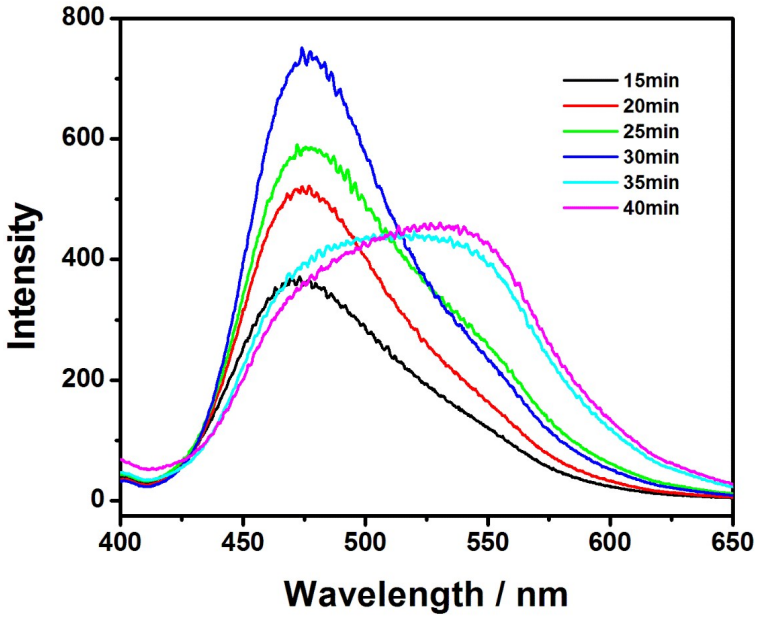

Fig. S1 The luminescence spectra of Au-AgNCs@AMP in monitoring the synthesis process by using AuNCs@AMP as a seed in the presence of 5.0 mM $AgNO_3$ and 25 mM AMP, at 120 °C.

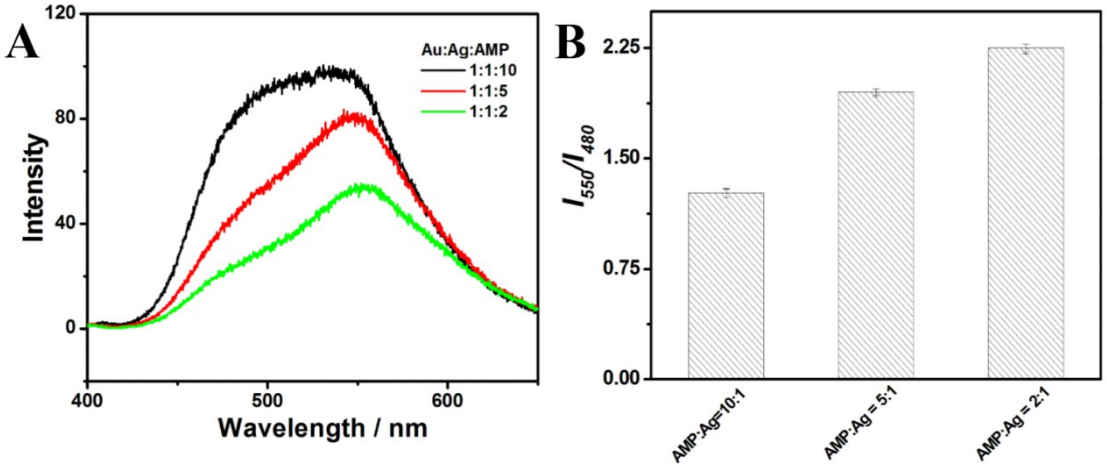

Fig. S2 (A) The luminescence spectra of Au-AgNCs@AMP prepared in the presence of different amount of AMP by one-pot hydrothermal method, where the initial concentrations of $HAuCl_4$, $AgNO_3$ and citrate is kept at 1.0, 1.0 and 50 mM, respectively; (B) The corresponding ratio of the peak intensities in changing with the amount of AMP (λ_{ex} = 354 nm).

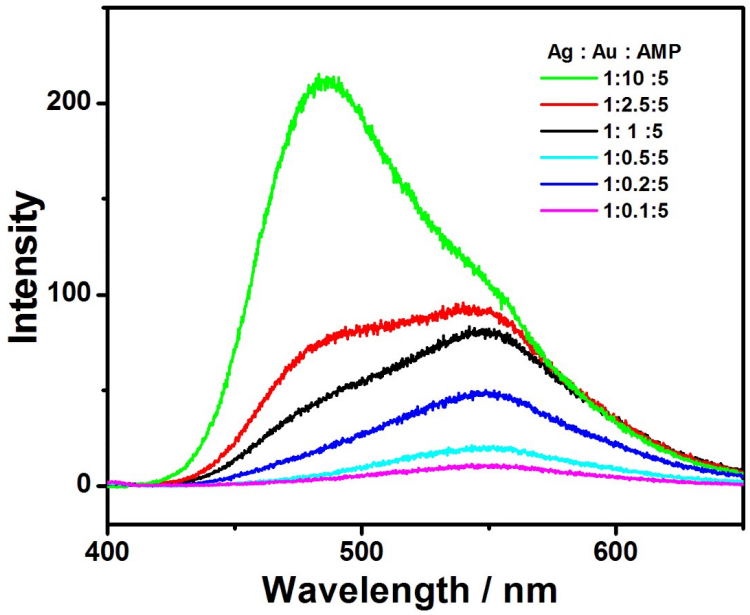

Fig. S3 The luminescence spectra of Au-AgNCs@AMP, which are synthesized by the one-pot hydrothermal method for 30 min. The concentrations of AMP and $AgNO_3$ are fixed at 5.0 and 1.0 mM, respectively; and that of $HAuCl_4$ are changed at 0.10, 0.20, 0.50, 1.0, 2.5 and 10 mM, respectively.

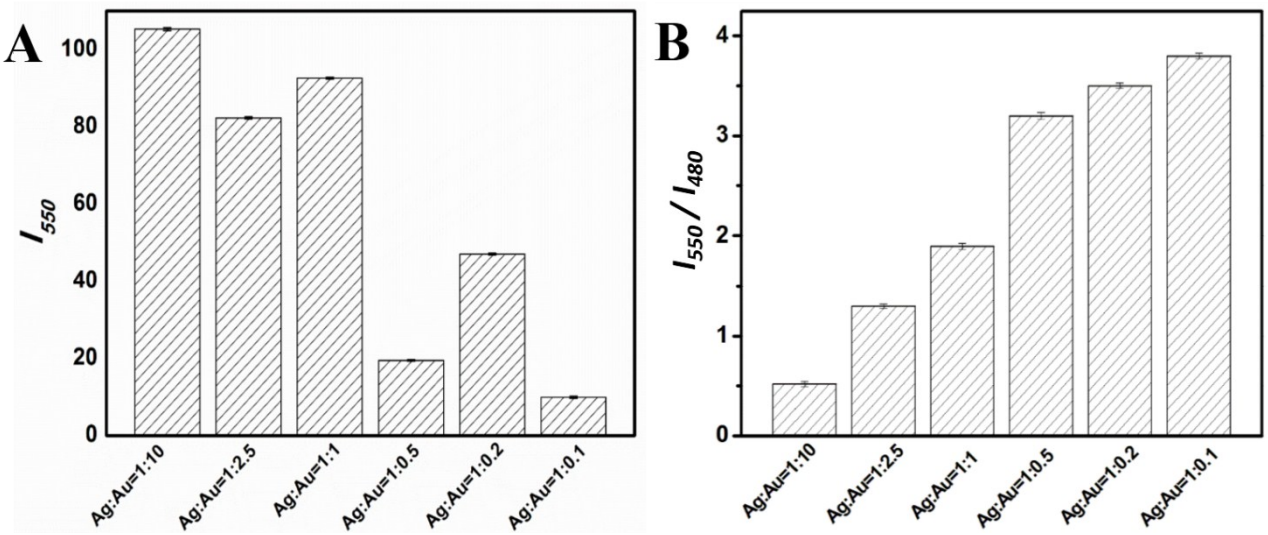

Fig. S4 (A) The corresponding peak intensities and (B) the intensities ratio in changing with the ratio of $AgNO_3$ to $HAuCl_4$ (λ_{ex} = 354 nm).

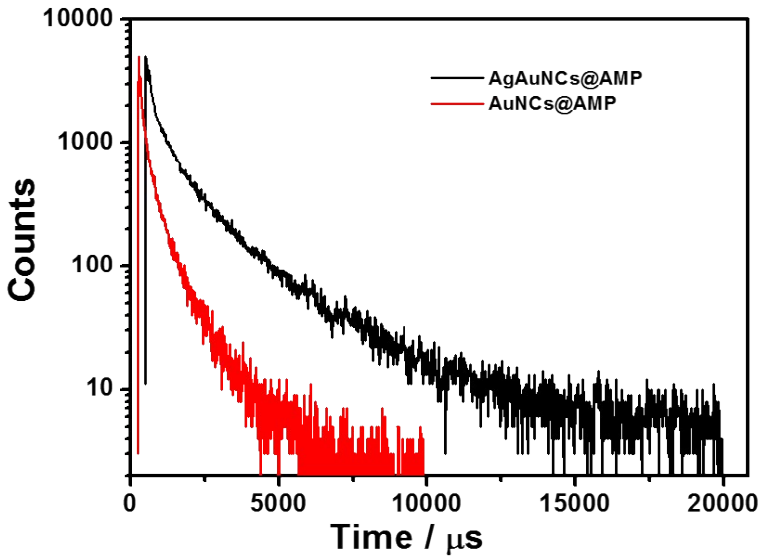

Fig. S5 The time-resolved luminescence decay curves of Au-AgNCs@AMP and AuNCs@AMP.

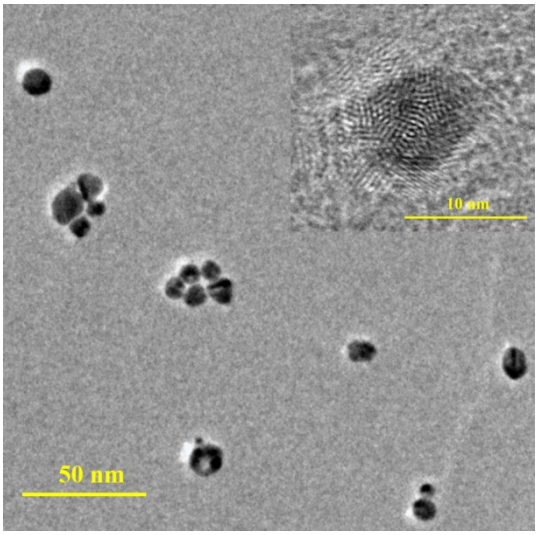

Fig. S6 Typical TEM image of Au-AgNCs@AMP prepared at 120 °C after six hours exposure under sunlight (Inset: crystal lattice).

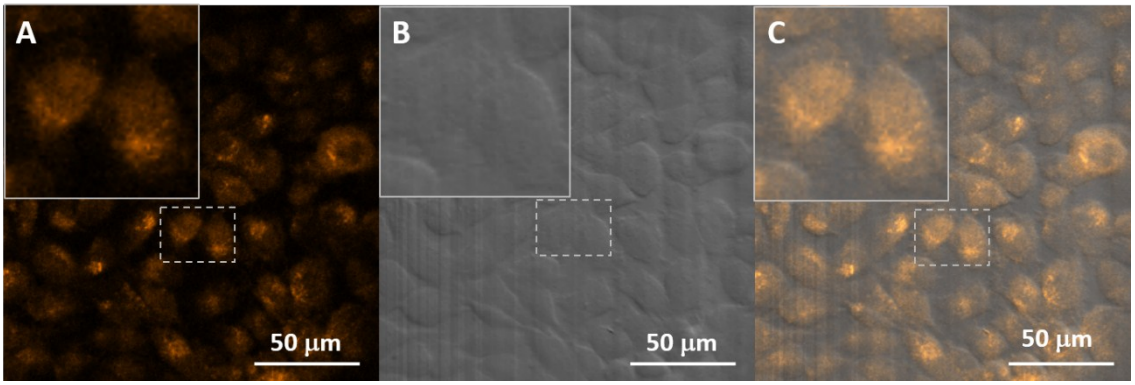

Fig. S7 The confocal luminescence (A), bright field (B) and overlap field (C) microphotographs of

live Hela cells in the absence and presence of Au-AgNCs@AMP (60 mg/L) and incubated for 30 min, respectively.

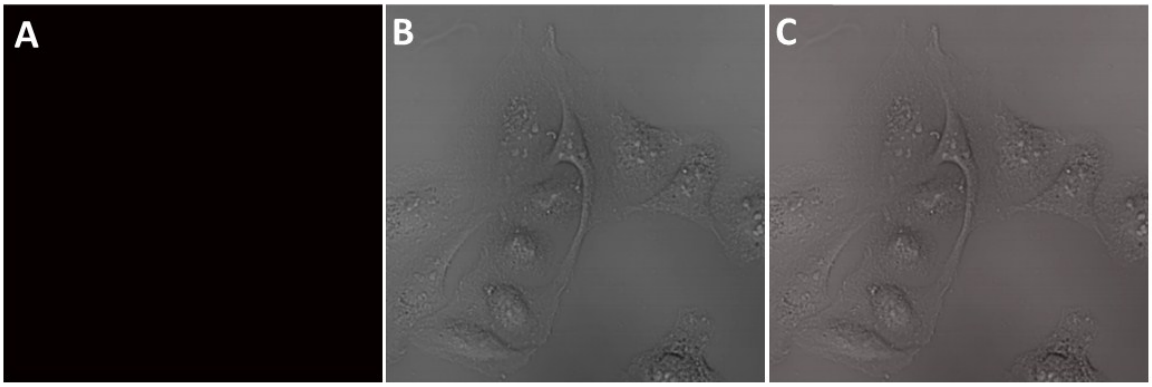

Fig. S8 The confocal luminescence (A), bright field (B) and overlap field (C) microphotographs of live Hela cells in the absence and presence of AuNCs@AMP (60 mg/L), which were measured after 30 min incubation, respectively.

Table S1 The luminescence life-times and percentages of AuNCs@AMP and Au-AgNCs@AMP prepared by one-pot hydrothermal method.

	τ_1	(Rel %)	τ_2	(Rel %)
Au-AgNCs	**4.278E-007 s**	**35.28**	**2.076E-006 s**	**64.72**
AuNCs	**1.298E-007 s**	**41.23**	**6.350E-007 s**	**58.77**