Fluorescence Enhancement on Silver Nanoplate at the Single- and Sub-Nanoparticle Level

Yangbin Shen,[†,‡] *Ting He,*[†,§] *Wenhui Wang,*[†,§] *Yulu Zhan,*[†,§] *Xin Hu,*[†,‡] *Binfang Yuan,*[†] *and Xiaochun Zhou*[*,†]

[†] Division of Advanced Nanomaterials, Suzhou Institute of Nano-tech and Nano-bionics, Chinese Academy of Sciences, Suzhou 215125, China

[‡] University of Chinese Academy of Sciences, Beijing 100049, China

[§] Shanghai University, Shanghai 200444, China

Table of Contents

SI-1. Materials and characterizations.

All commercial materials were used as received unless specified. The nanostructure and composition of silver nanoplates were characterized by TEM (200kV, Tecnai G2 F20 S-TWIN; FEI), and SEM (Quanta 400 FEG; FEI). Image analyses were done using home-written MATLAB codes. The UV-vis spectrum was done in PerkinElmer Lambda750.

SI-2. Information about alcohol dehydrogenase (ADH).

Alcohol dehydrogenase (Sigma-Aldrich) with a molecular weight 141 kDa is extracted from Saccharomyces cerevisiae. The ADH in this paper is a kind of tetramer, which has four same subunits as displayed with different colors in **Figure S 1a1-c1**. The dimension of ADH is ~10 nm × ~9 nm × ~6 nm. The isoelectric point is 5.4~5.8. **Figure S 1a2-c2** show the charge distribution on the ADH in three different views. The domains with negative charges, positive charges, no charge but have polarity, no charge and no polarity are drawn by different colors[1].

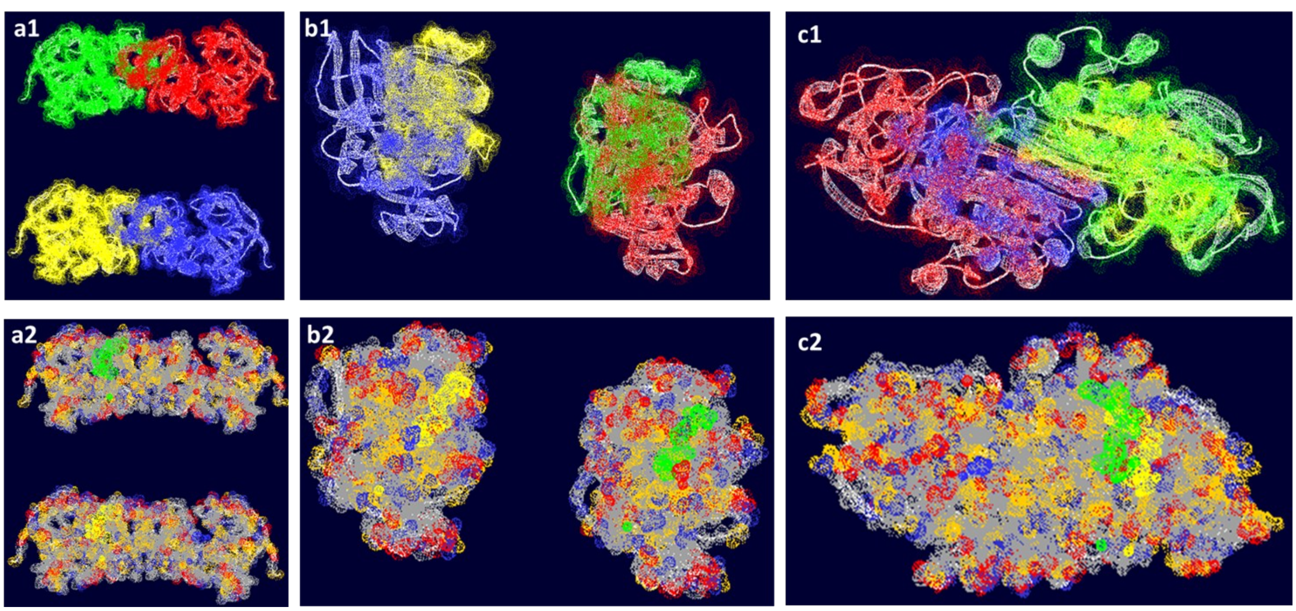

Figure S 1. Structure and charge distribution of ADH**.** (a1), (b1), (c1) Structure of ADH with four chains in different views of top (**a1**), front (**b1**) and side (**c1**). Four colors represent four different subunits. (a2), (b2), (c2) Domains with different types of charges on ADH in different views of top (**a2**), front (**b2**) and side (**c2**). Red color represents negative charge; blue color represents positive charge; yellow represents no charge but have polarity; gray color represents no charge and no polarity.

SI-3. Synthesis and characterizations of silver triangle nanoplates

Silver triangular nanoplates were synthesized by the method presented by Zhang[2]. The UV-vis spectrum of the as-synthesized nanoplates is shown in **Figure S 2**. There is a very broad peak in the spectrum indicating the size of nanoplates is very uneven. The peak wavelength λ_{peak} is at 600 nm, which means most of the nanoplates are about tens nanometers. The TEM images in **Figure S 3** show that most of the nanoplates are equilateral triangle with round tips, 30-150 nm size and 7±2 nm thickness. The spectrum is very sensitive to the sharpness of the corner. If the tips become rounded, the spectrum peak position will blue shift[3]. When we simulate the electric-field on nanoplates by FDTD method, the thickness and size are all taken into account.

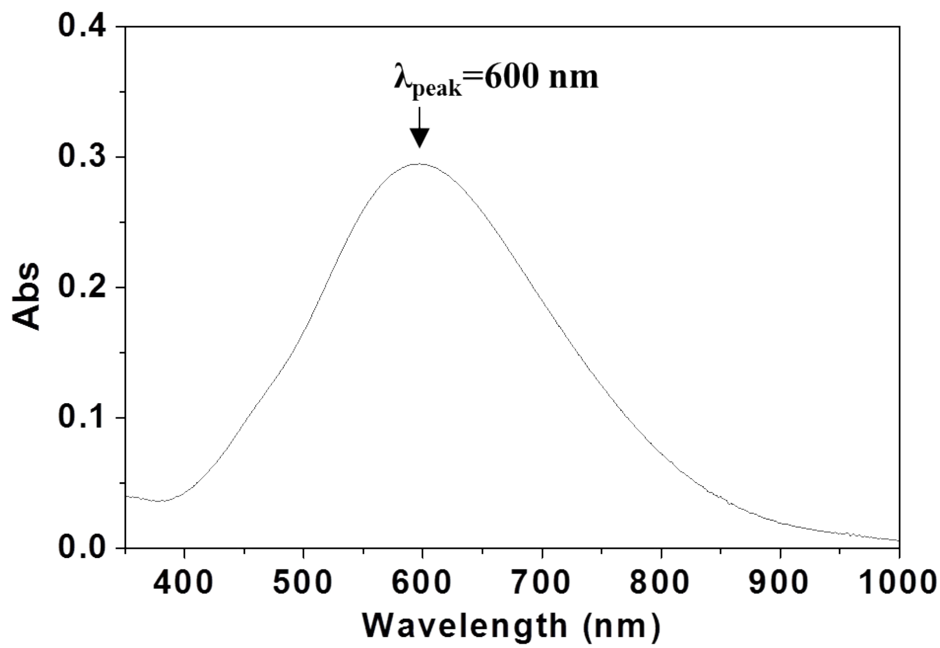

Figure S 2. UV-vis spectrum of the as-synthesized nanoplates.

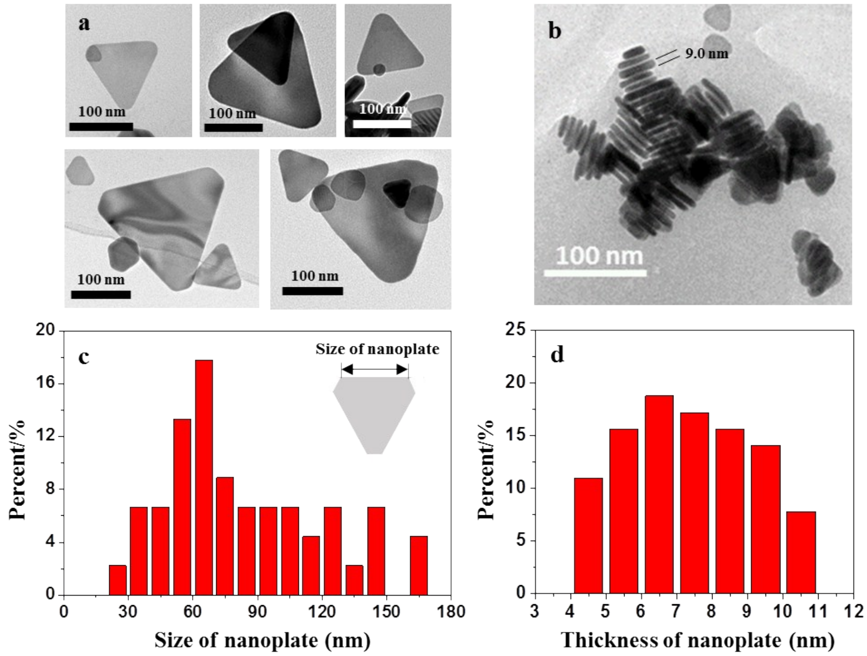

Figure S 3. TEM measurement of nanoplates. **(a)**, **(b)** TEM images about size and thickness of nanoplates. **(c)**, **(d)** Size (edge length is used) and thickness distribution of nanoplates.

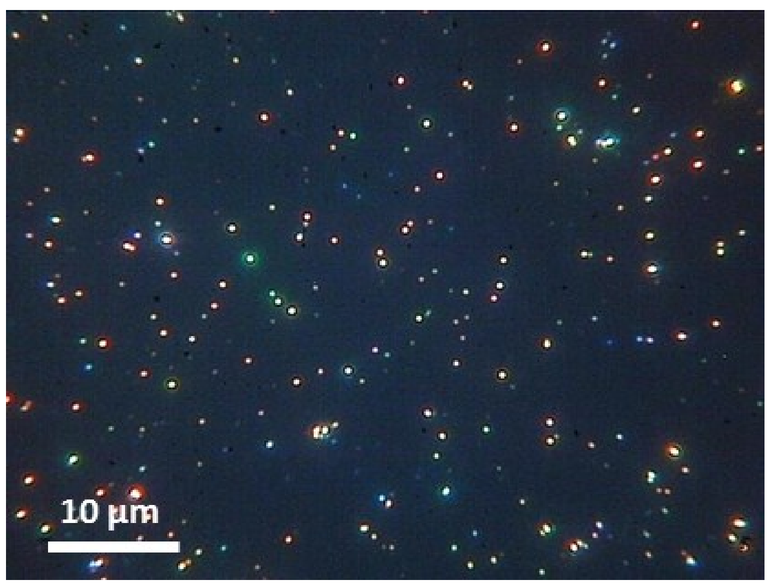

Figure S 4. Dark-field image of sliver nanoplates and markers (gold nanoparticles). This image was taken by a CMOS color camera. Red dots are mainly gold nanoparticles [4] and other color dots are sliver nanoplates with different sizes. The nanoparticles are dispersed well, and every nanoparticle is several micron meters away from each other.

SI-4. Control experiments

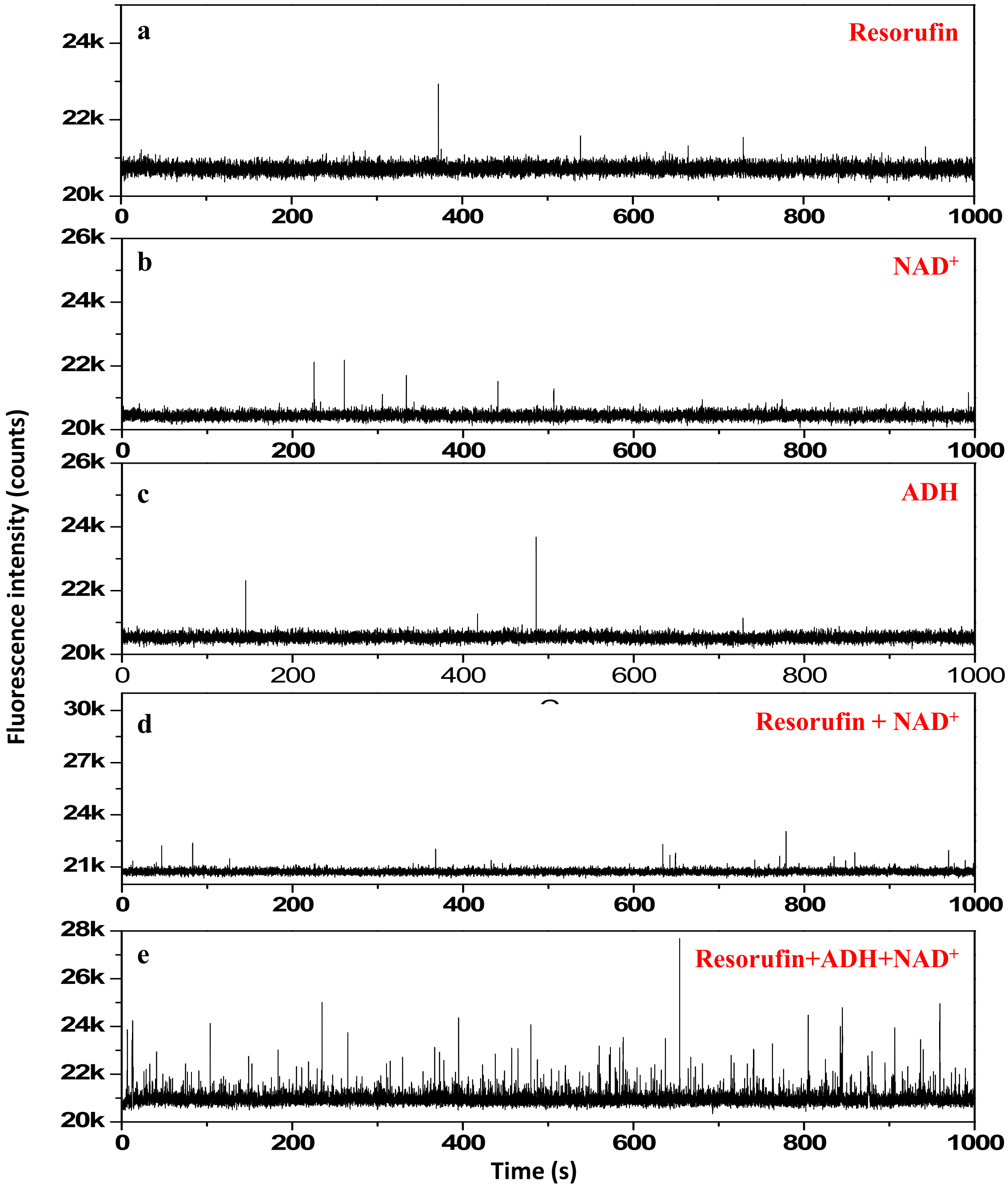

Figure S 5. Typical fluorescence intensity versus time trajectories for single nanoplate when the microfluidic reactor was fed with different species. **(a)** Resorufin. (**b)** NAD^+. (**c)** ADH. **(d)** Resorufin and NAD^+. (**e)** Resorufin, ADH and NAD^+. 50 mM pH 6.8 phosphate buffer was always used.

SI-5. Effect of pH value on the number of events

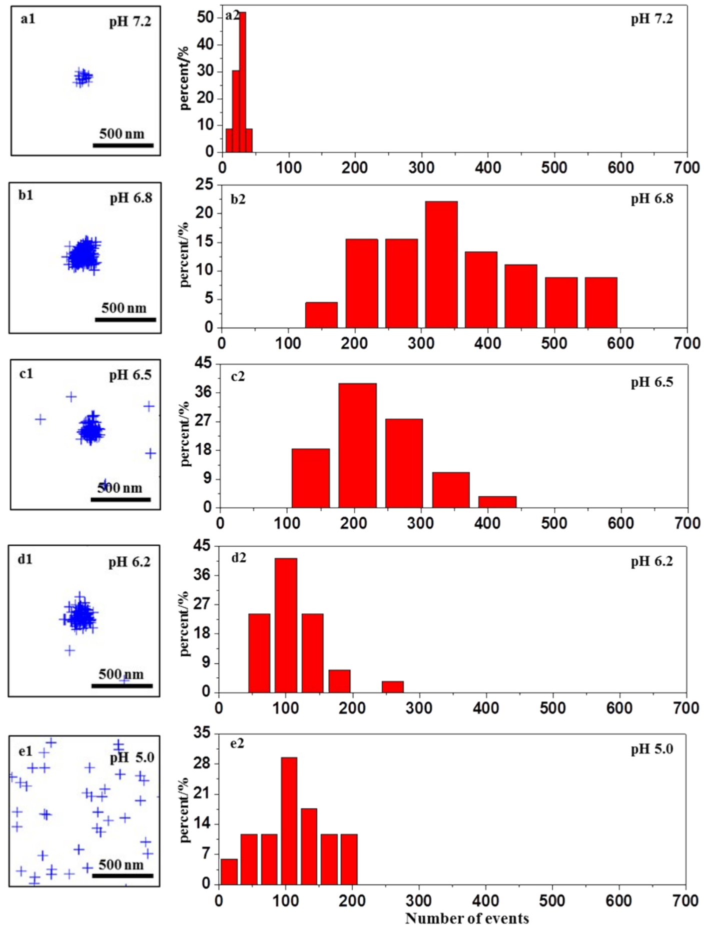

Figure S 6. Effect of pH value on the number of events under pH 7.2, 6.8, 6.5, 6.2, 5 phosphate buffer, respectively. **(a1)-(e1)** Representative scattered plots of the positions of single fluorescent molecules on single Ag nanoplates. **(a2)-(e2)** Distribution of the number of events on single Ag nanoplates.

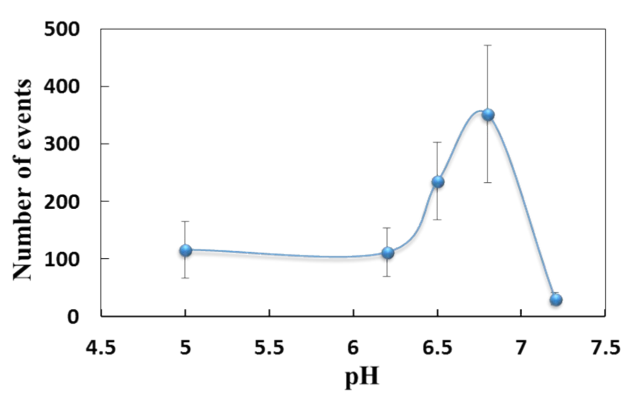

Figure S 7. Number of events on single nanoplates at different pH values. The maximum value appears at pH 6.8, which will be used in our research.

SI-6. Combination of single molecule TIRFM and single particle spectrometer

1. Total internal reflection fluorescence microscope (TIRFM)

In this research, we combined the TIRFM and the spectrometer for single particle together to study the size dependent fluorescence enhancement. In **Figure S 8a,** a super-resolution imaging was performed using an Olympus IX71 microscope. The single Ag nanoplates were illuminated by a 532 nm laser by total internal reflection fluorescence microscope (TIRFM). The fluorescence signal was collected by a 60X NA1.2 water-immersion objective, and detected by an ANDOR Ixon DU-897D-CS0-#BV EMCCD camera operated at 25 ms frame rate. More than 1.5 million frames were recorded in order to detect enough number of single fluorescence molecules. **Figure S 8b** and **c** are the wide-field image of nanoplates under TIRFM and dark-field microscope respectively. The bright spots A, B, C, D in the TIRFM field have the same locations as those in the dark-field.

2. Homebuilt spectrometer for single particle spectrum measurement

In order to massively screen the spectrum of the single Ag nanoplates on quartz slide, we made a homebuilt spectrometer based on the dark-field microscopy as shown in **Figure S 8a**. First of all, the silver nanoplate and gold nanoparticle was well dispersed on the quartz slide (Jinzhou Best Quartz Glass CO.LTD) with the thickness 0.5 mm. The slide was assembled into a microfluidic reactor. Second, the silver nanoplates on the slide were irradiated by the 25 narrow band lights, which were obtained by 25 narrow band filters as shown in **Figure S 8a**. The central wavelength of 25 filters is 400, 420, 436, 451, 470, 489, 510, 529, 551, 571, 590, 614, 630, 651, 669, 690, 713, 733, 753, 771, 788, 808, 829, 851, 880 nm, respectively. Third, the scattering signal of nanoplates under different lights was recorded by an EMCCD camera. Fourth, the scattering signal was analyzed and fitted by home-written MATLAB codes. The spectrums of the nanoplates at the locations A, B, C, D are shown in **Figure S 9a1-d1**. The fluorescence intensity distributions on the corresponding nanoplates are shown **Figure S 9a2-d2**.

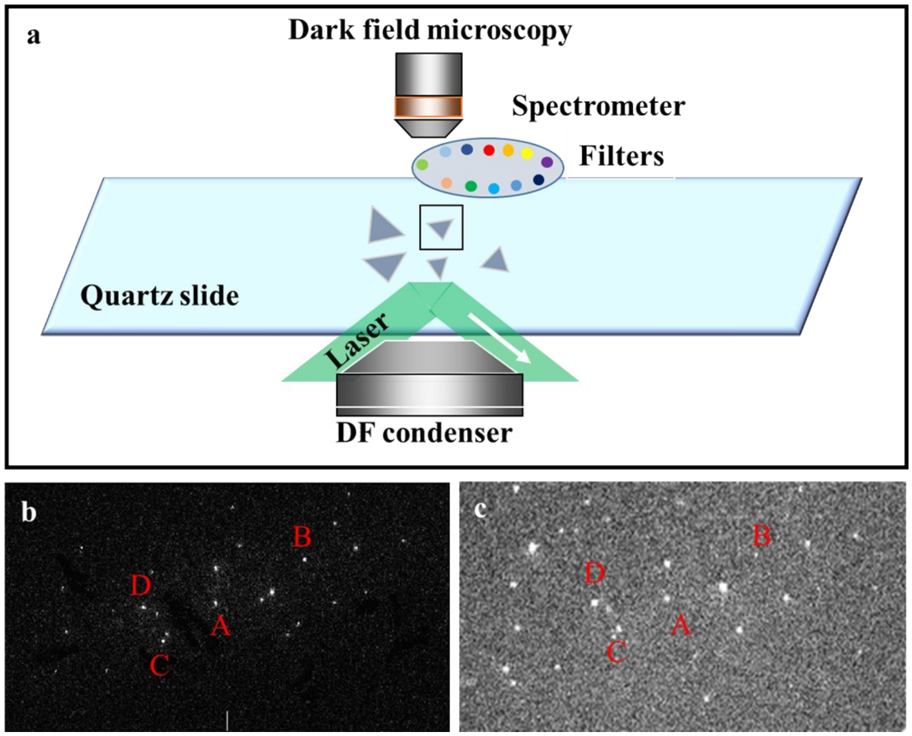

Figure S 8. Method and procedure for the experiments (**a**) Experimental design of dark-field microscope equipped with a homebuilt spectrometer. (**b**) Wide-field image of nanoplates in TIRFM. (**c**) Wide-field image of the same nanoplates in the homebuilt spectrometer.

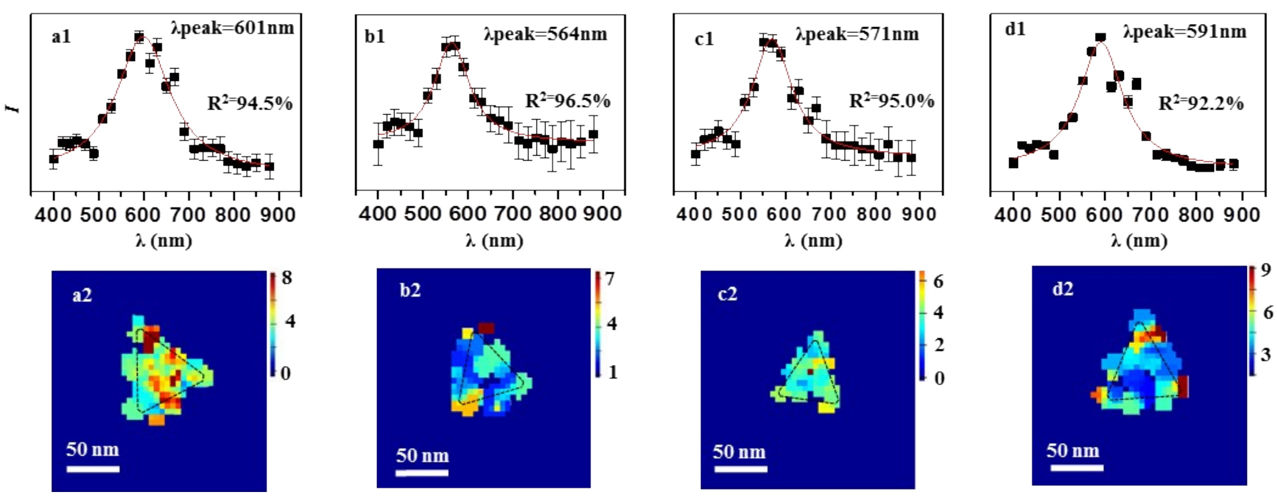

Figure S 9. Spectra and corresponding super-solution two-dimensional histogram of nanoplates in A, B, C, D spots in **Figure S 8 b**.

SI-7. Enhancement at different angles and sizes

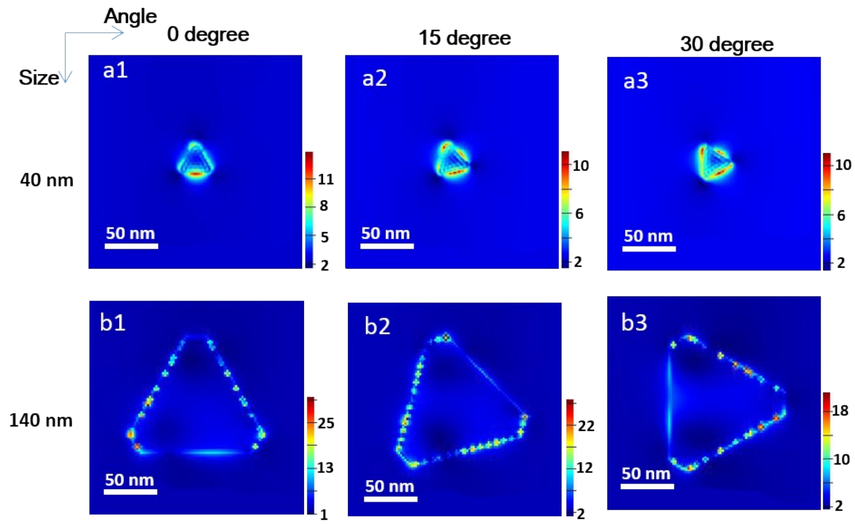

Figure S 10. Electric-field distribution of nanoplates when the sizes are 40 nm and 140 nm respectively, and angles between nanoplate and incident light are 0°,15°,30°respectively.

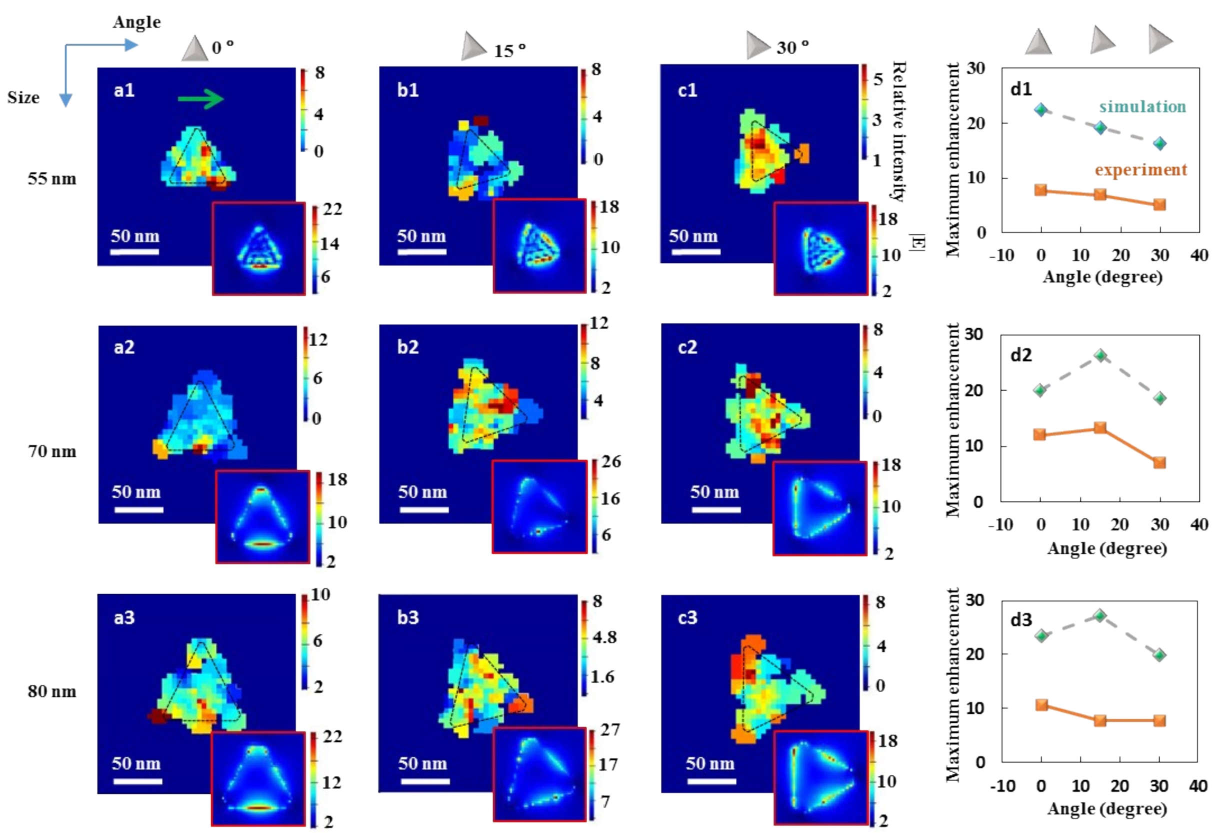

Figure S 11. Distributions of fluorescence intensity and simulated electric-field intensity for the single Ag nanoplates with different angles between nanoplate and incident light. (a-c) Distributions of fluorescence intensity (big panel) and simulated electric-field intensity (small panel with red frame) for the single Ag nanoplates with different angles (i.e. 0°, 15°, 30°) between nanoplate and incident light at the same size ~55 nm, ~70nm and ~80nm respectively. (d) IPFEF for the Ag nanoplates in **a**, **b**, **c**. The orange squares and curve are for the IPFEF in experiment, while the green diamonds and curve are for the IPFEF in theory.

SI-8. Size and Spectrum of Single Ag Nanoplates.

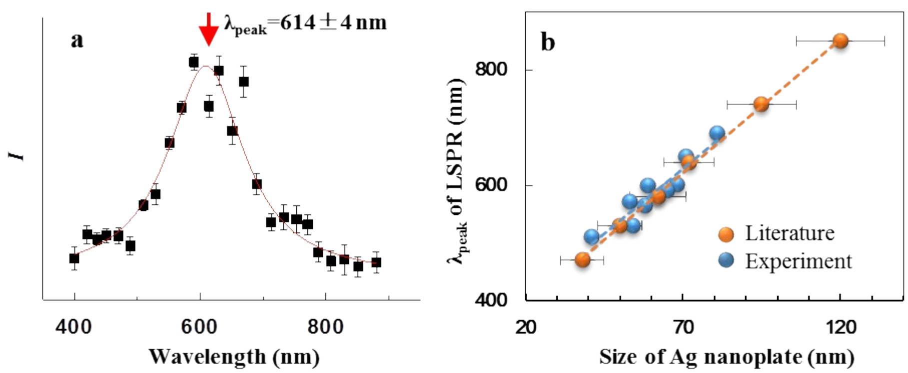

Figure S 12. Relationship between the peak of spectrum (λ_{peak}) and size of single Ag nanoplate. (a) A representative spectrum of a single Ag nanoplate. The peak wavelength of the spectrum is 614±4 nm, which is from a fitting of Lorentz curve, and the size of this single Ag nanoplate is ~68 nm (edge length). Error bars are standard deviation. (b) Correlation of the λ_{peak} and the size of single Ag nanoplates. The blue dots are from our experiment, while the yellow dots and dash line are from Mirkin's work.[5]

We obtain the correlation relationship between the peak of spectrum (λ_{peak}) and size of single Ag nanoplate. **Figure S 12**b shows that the λ_{peak} and the size have a high correlation coefficient up to 0.940. This result is consistent with the reported result by Mirkin (orange dots and dash curve in **Figure S 12**b).[5] That is to say the λ_{peak} is sensitive enough to the size variation,[3] and can be used in fluorescence enhancement as well as the size.

SI-9. Acknowledgment

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SI-10. References

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