

## Electronic Supplementary Information

# Eggshell Membrane as a Multimodal Solid State Platform for Generating Fluorescent Metal Nanoclusters

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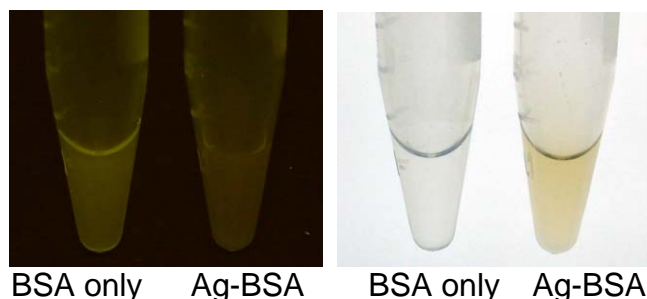
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## Experimental Section

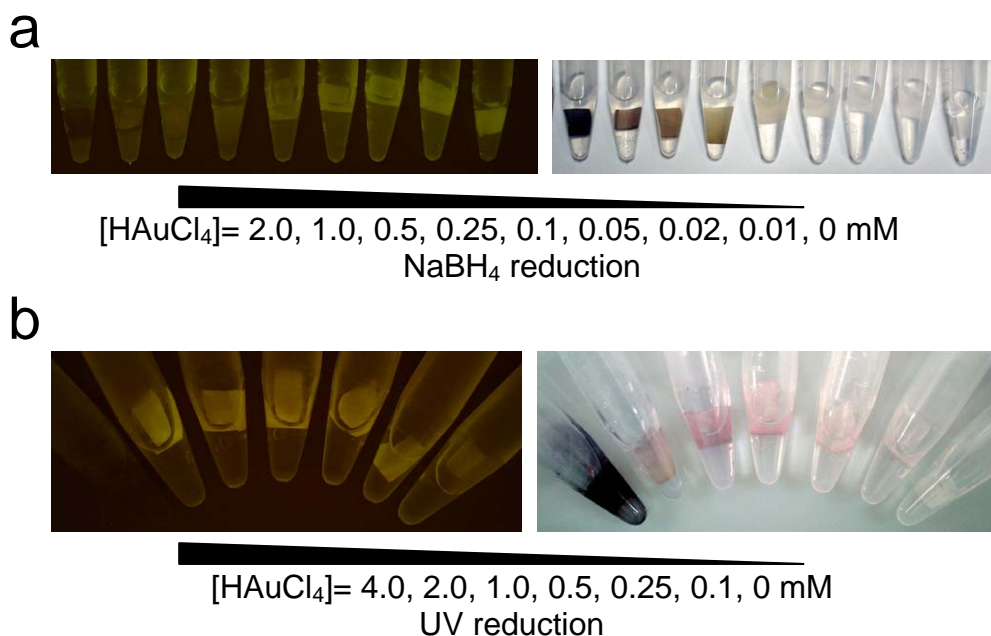
**Synthesis of Au NCs embedded in ESM:** Natural hen eggs were purchased from a local market. Eggshell membrane (ESM) was manually stripped from the inside of an eggshell. The ESM was rinsed with copious amount of doubly distilled water (ddH<sub>2</sub>O) and then cut into pieces of proper size and soaked in ddH<sub>2</sub>O at 4 °C before use. During the synthesis of fluorescent Au-ESM, a piece of ESM was firstly dipped in an aqueous HAuCl<sub>4</sub> solution (500 μL, 1 mM) for five minutes. After that, excessive HAuCl<sub>4</sub> was removed by rinsing with ddH<sub>2</sub>O, and the [AuCl<sub>4</sub>]<sup>-</sup> impregnated ESM was transferred into 500 μL 90 mM NaOH solution to initialize a chemical reduction of the adsorbed [AuCl<sub>4</sub>]<sup>-</sup> into gold NCs. The reaction typically took 12-22 hours at ambient temperature to give strong red fluorescence of an Au-ESM.

**Syntheses of Ag NCs embedded in ESM:** A piece of ESM was briefly incubated in an aqueous AgNO<sub>3</sub> solution (100 mM or 0.2 mM) for five minutes. Then, the Ag<sup>+</sup> impregnated ESM was transferred into 500 μL of 2 M NaOH or 1 mM NaBH<sub>4</sub> solution to convert the adsorbed Ag<sup>+</sup> into silver NCs. The reaction took 30 minutes (NaOH) or 12-24 hours (NaBH<sub>4</sub>) to generate significant fluorescence of Ag-ESMs at room temperature. In the case of a UV-induced reduction, the Ag<sup>+</sup> solution (100 mM) soaked ESM was irradiated by 365 nm UV light under a 6 W mercury lamp at a distance of about 1 cm. Strong fluorescence of Ag NCs were observed after about 6 hours.

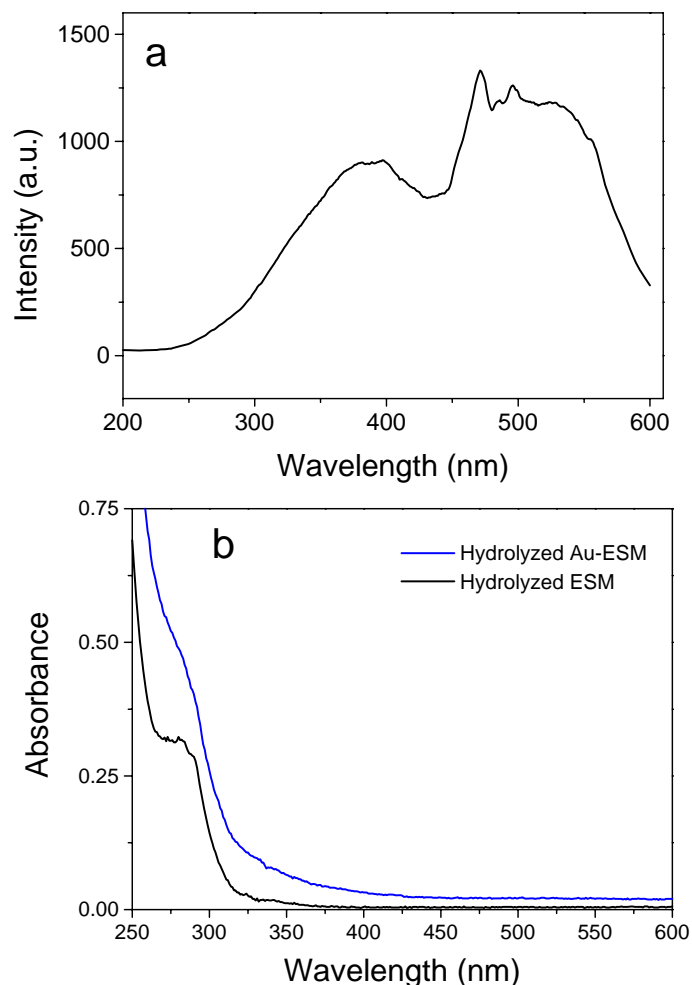
**Spectroscopic and TEM characterizations:** Fluorescence spectra of Au-ESMs and Ag-ESMs were recorded on a Hitachi 4600 fluorescence spectrophotometer. A 450 nm long-pass filter was inserted in the emission light path when necessary to get rid of the scattered 365 nm incident light. The fluorescent photos of Au/Ag-ESMs were taken with a Sony W5 digital camera equipped with a long-pass filter with a cutoff wavelength of 550 nm. Surface morphology of a fluorescent Au-ESM was observed under an Olympus IX71 fluorescence microscope. XPS data were acquired on an ESCALAB 250 X-ray photoelectron spectrometer. Quantitative analyses of the metal contents in Au-ESM and Ag-ESM samples were performed on a Perkin Elmer Optima 7300 DV ICP-AES (inductively coupled plasma atomic emission spectrometer). TEM images (see Supporting Information) were obtained on a JEOL-2010 transmission electron microscope operated at an accelerating voltage of 200 kV.



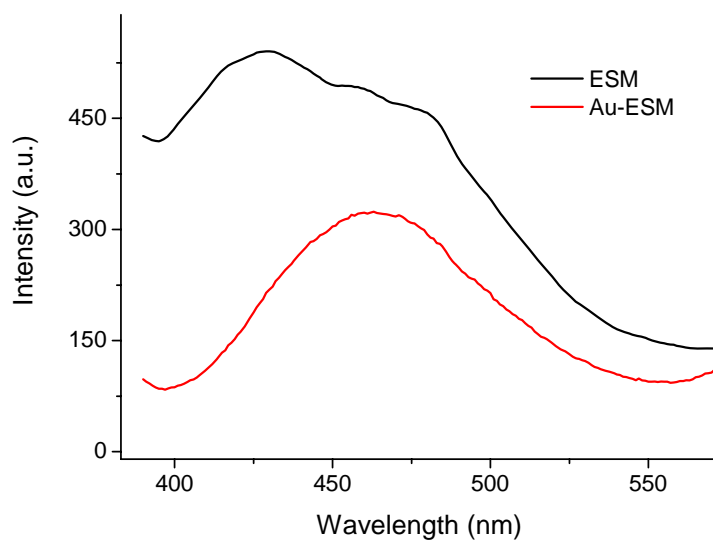
**Figure S1.** Photographs of BSA and Ag-BSA solutions taken under 365 nm UV (left) and room light (right). For the synthesis of Ag-BSA, an aqueous  $\text{AgNO}_3$  solution (500  $\mu\text{L}$ , 2 mM) was combined with a BSA solution (500  $\mu\text{L}$ , 50 mg/mL) at 37 °C under vigorous stirring, followed by addition of a NaOH solution (50  $\mu\text{L}$ , 1 M) in 2 min; the reaction was allowed to proceed under vigorous stirring at 37°C for 24 h.



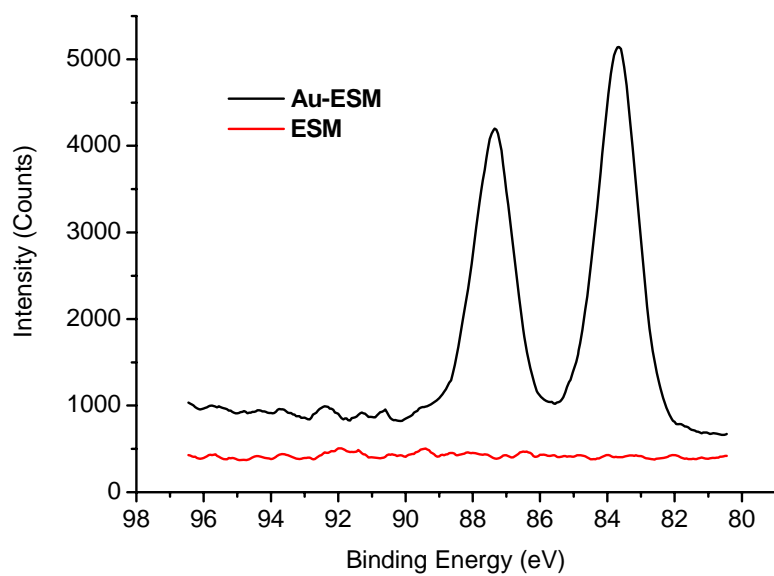
**Figure S2.** Control experiments demonstrating the reductions of  $[\text{AuCl}_4]^-$  on ESMs by  $\text{NaBH}_4$  or UV irradiation. (a) ESMs were pre-incubated in 2.0, 1.0, 0.5, 0.25, 0.1, 0.05, 0.02, 0.01 and 0 mM  $\text{HAuCl}_4$  solutions, respectively (from left to right tubes), for 5 min, and then transferred to 1 mM  $\text{NaBH}_4$  solutions to initialize the reduction process at room temperature. Photographs of the samples were taken under 365 nm UV illumination (left) and room light (right) after the reactions had proceeded for 3 h. (b) ESMs were pre-incubated with  $\text{HAuCl}_4$  at various concentrations (4.0, 2.0, 1.0, 0.5, 0.25, 0.1, 0 mM, from left to right tubes) for 5 min, and then subjected to UV irradiation at 365 nm to initialize the reduction process. Photographs of the samples were taken after the reactions had proceeded for 2.5 h.



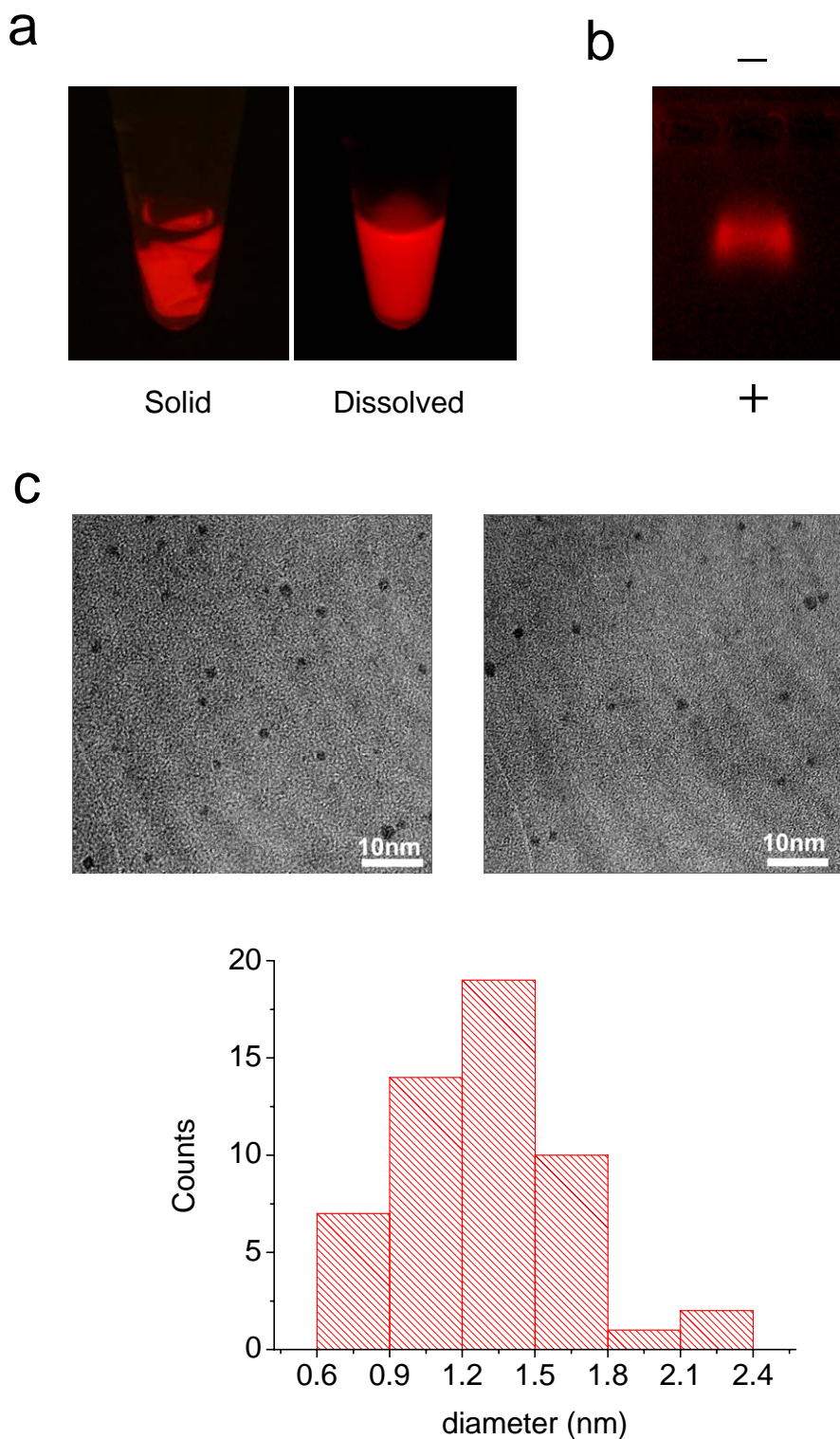
**Figure S3.** Fluorescence excitation spectrum and optical absorbance curves of a solid state Au-ESM and its dissolved form. (a) Fluorescence excitation scan of a solid Au-ESM prepared by chemical reduction in a NaOH solution. The emission wavelength was set at 671 nm. (b) Absorbance curves of ESM and Au-ESM (both were hydrolyzed in concentrated NaOH solutions to give homogeneous solutions).



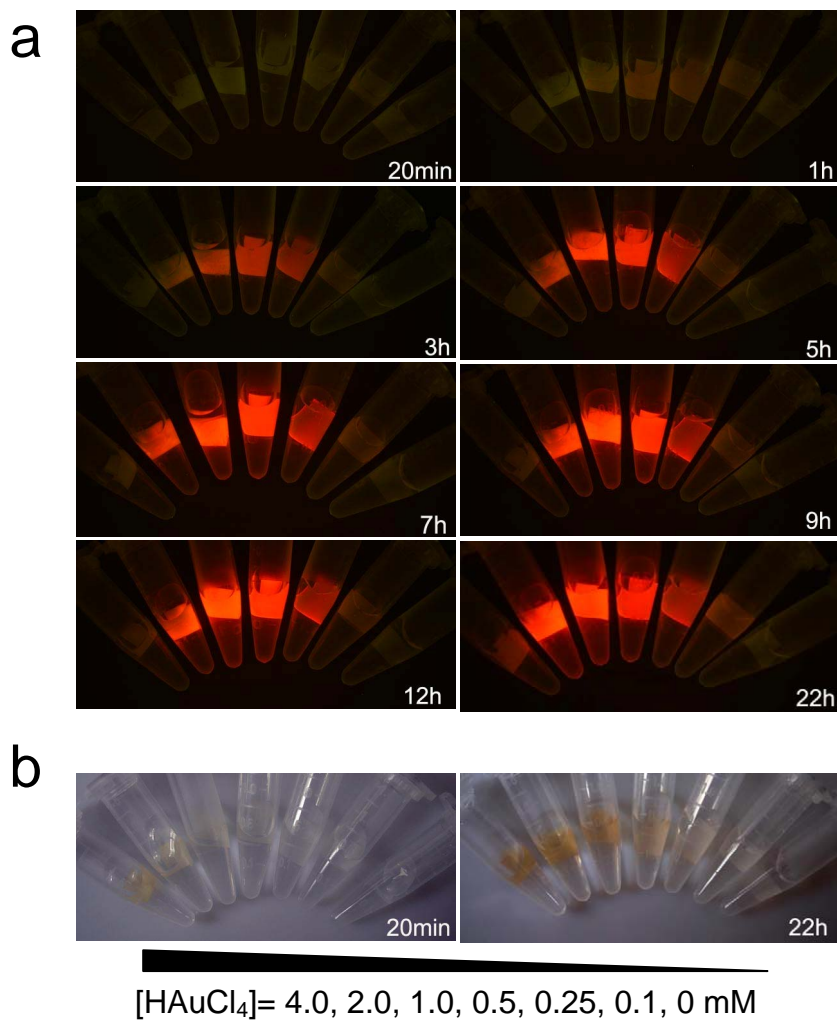
**Figure S4.** Fluorescence emission spectra ( $\lambda_{\text{ex}} = 365 \text{ nm}$ ) of an ESM before (black line) and after (red line) the generation of fluorescent Au NCs.



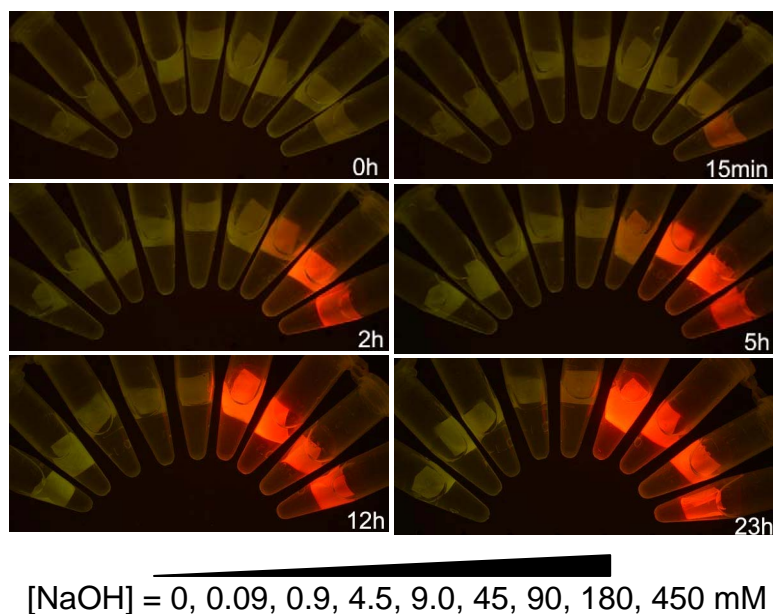
**Figure S5.** XPS Au 4f spectra of an ESM with (black line) and without (red line) Au NCs. The Au 4f<sub>7/2</sub> peak centered at 83.7eV indicated the existence of Au (0).



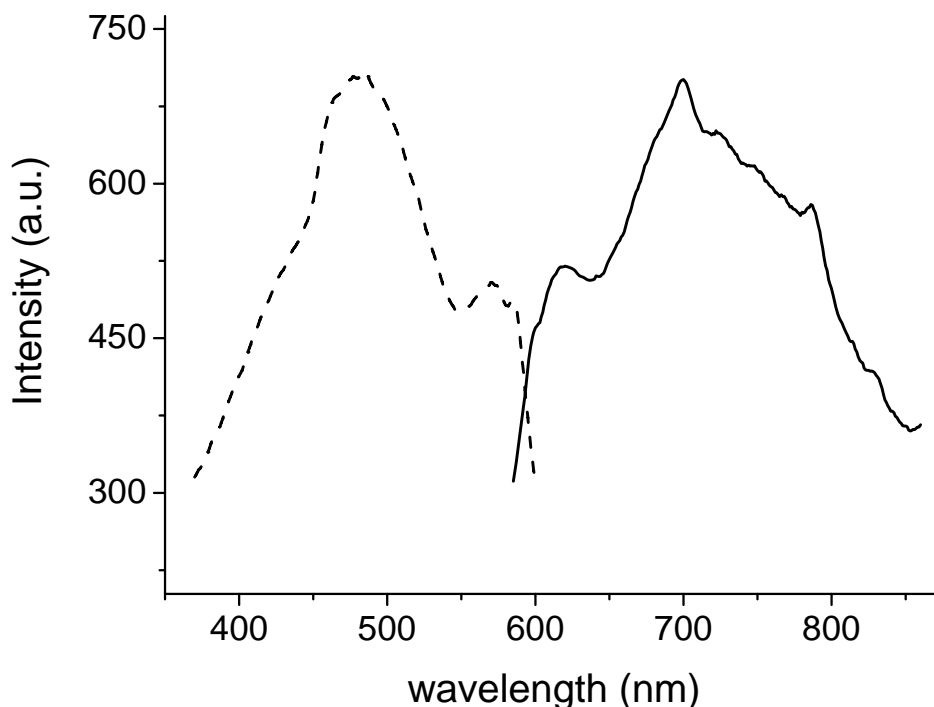
**Figure S6.** (a) Fluorescent pictures of an Au-ESM and its dissolved form in a 4.5 M NaOH solution. (b) 1% agarose gel electrophoresis of the dissolved Au-ESM sample in (a). Gel picture was taken under 365 nm UV illumination. A clear and sharp gel band indicated that the Au NCs were relatively uniform in size. “+” and “-” in (b) indicate the polarity of applied electric field during electrophoresis. (c) Typical TEM images of the as-synthesized Au NCs on an ESM template along with a statistical analysis of their size distribution based on the TEM data.



**Figure S7.** (a) Fluorescent pictures of the samples under UV light corresponding to different reaction times and a series of HAuCl<sub>4</sub> concentrations. ESMs were pre-incubated in HAuCl<sub>4</sub> solutions of various concentrations: 4.0, 2.0, 1.0, 0.5, 0.25, 0.1 and 0 mM (from left to right) for 5 min, and then transferred to 90 mM NaOH solutions to initialize the reduction reactions. (b) Room light photographs of the samples after reactions of 20 min and 22 hours.

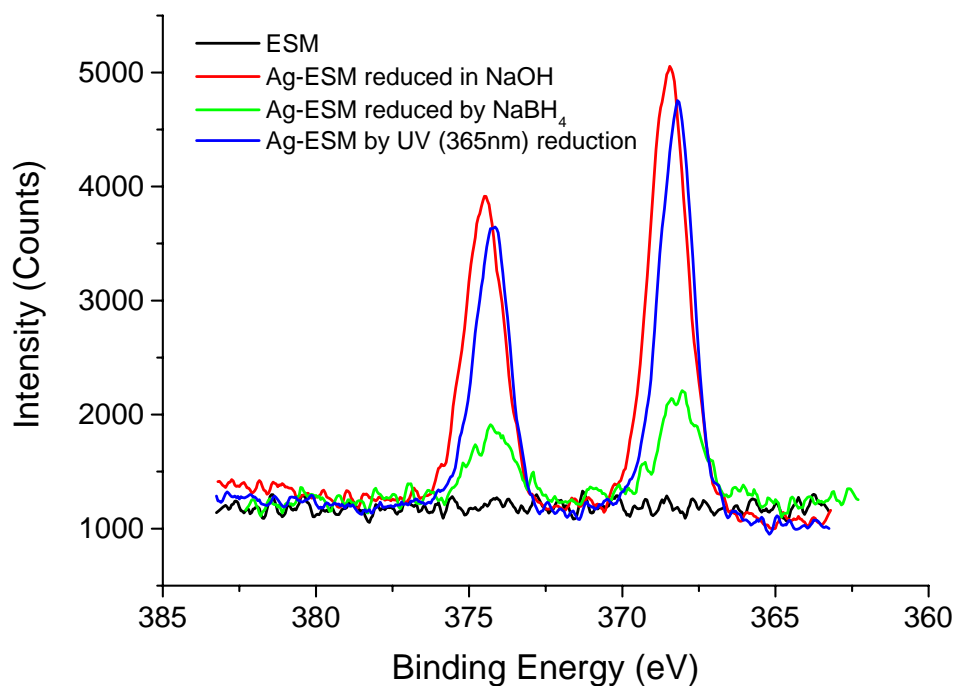


**Figure S8.** Influence of NaOH concentration on the fluorescence of the product after different reaction times. ESMs were incubated with  $\text{HAuCl}_4$  (1 mM) for 5 min and then transferred to NaOH solutions of different concentrations (0, 0.09, 0.9, 4.5, 9.0, 45, 90, 180, 450 mM, from left to right) to initialize the nucleations. No fluorescence was observed for a NaOH concentration lower than 4.5 mM even after a 23 h reaction. The sample soaked in 9.0 mM NaOH generated fluorescence after a prolonged reaction of several days (data not shown).

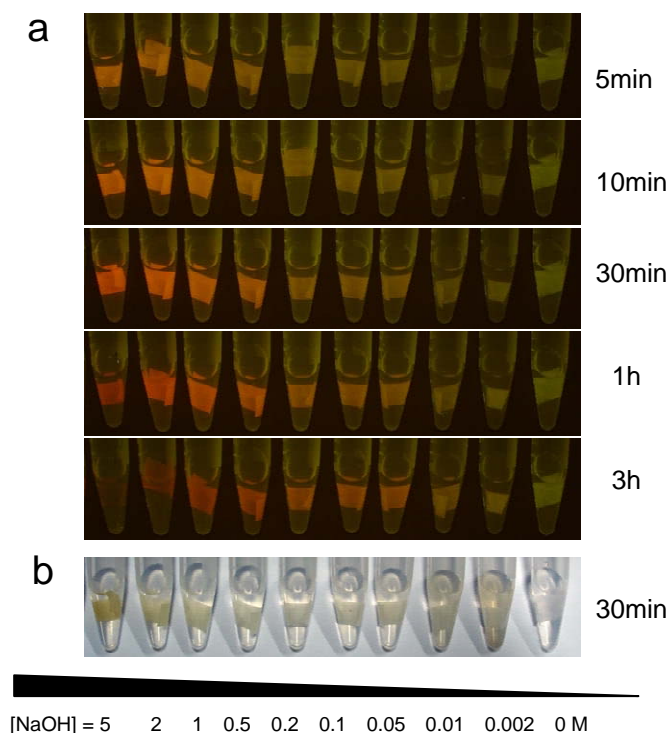


**Figure S9.** Fluorescence excitation and emission spectra of an Ag-ESM reduced in a NaOH solution. The emission spectrum (solid line) was measured under 480 nm excitation, and the excitation spectrum (dashed line) was recorded at an emission wavelength of 702 nm.

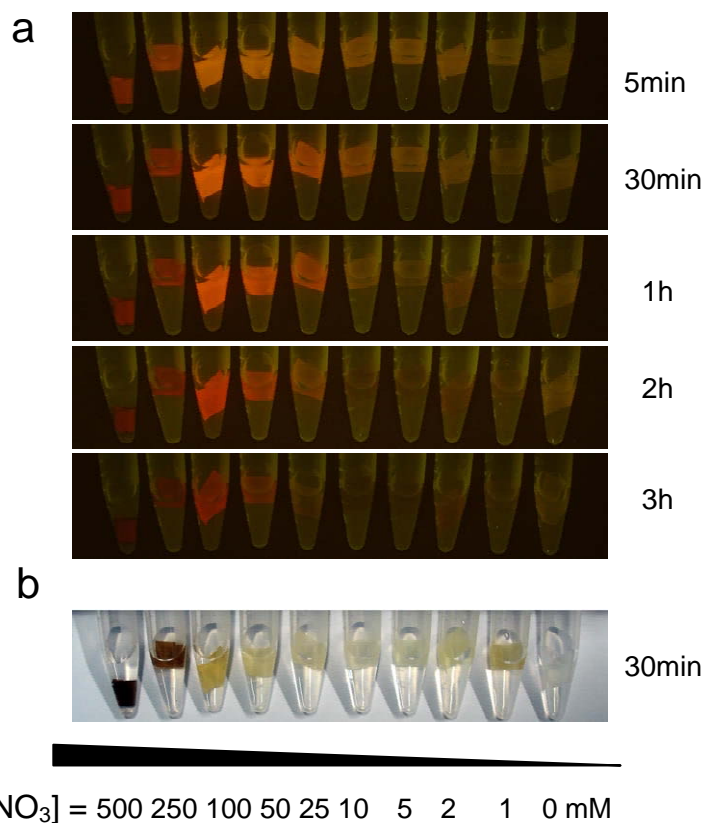




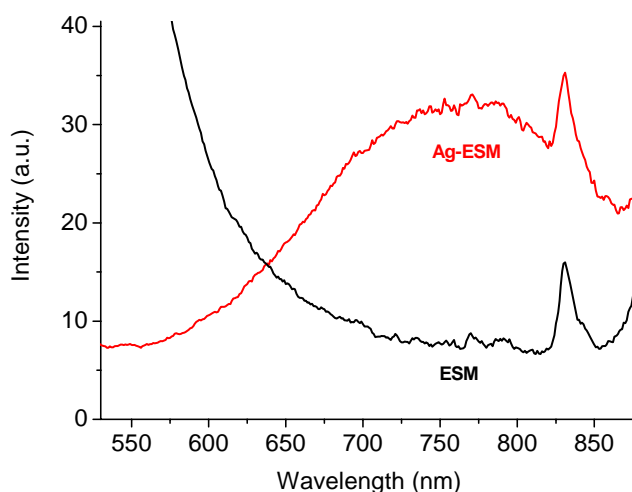
**Figure S10.** XPS Ag 3d spectra of an ESM and an Ag-ESM synthesized by reductions in NaOH and NaBH<sub>4</sub> solutions, as well as by UV irradiation. The Ag 3d<sub>5/2</sub> peaks centered at 368.4, 368.0 and 368.2 eV, respectively, indicated the existence of Ag(0).



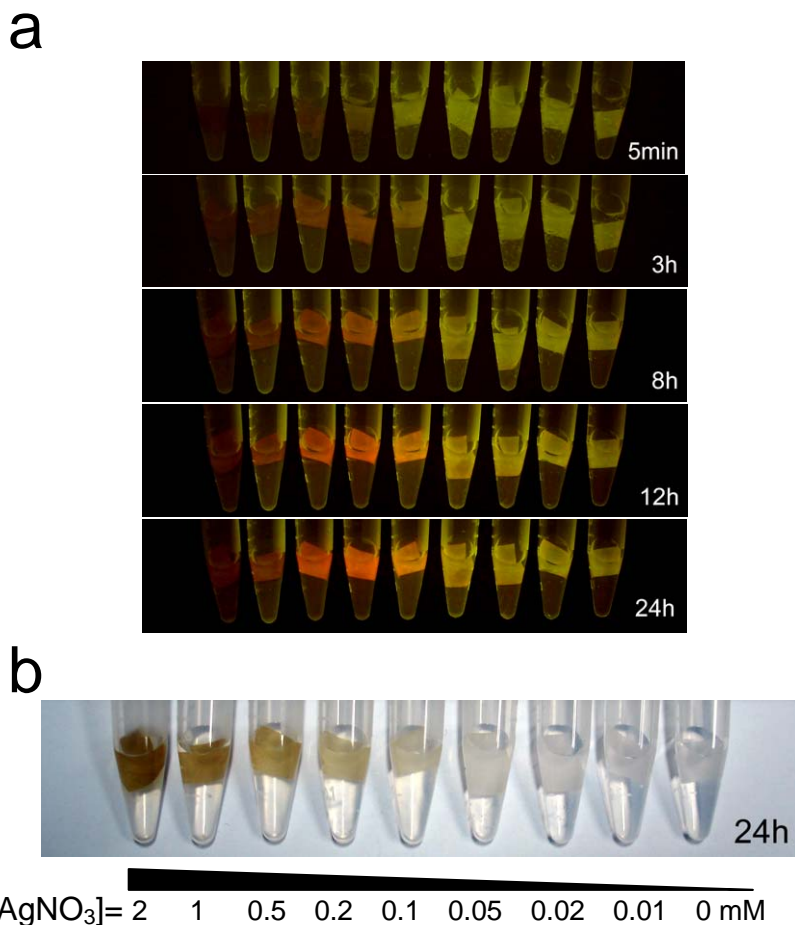
**Figure S11.** (a) Influence of NaOH concentration on the fluorescence of as-prepared Ag-ESMs. An ESM was incubated with 50 mM AgNO<sub>3</sub> for 5 min and then transferred to a NaOH solution at a series of concentrations (5, 2, 1, 0.5, 0.2, 0.1, 0.05, 0.01, 0.002, 0 M, from left to right tubes) to initialize metal nucleations. (b) A photograph of the samples under room light.



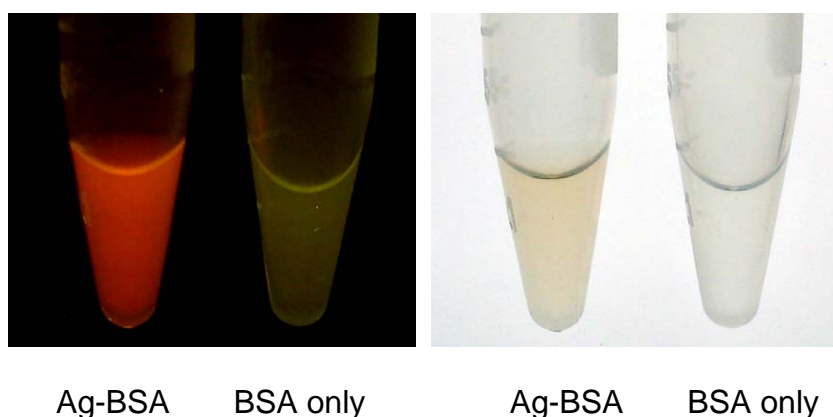
**Figure S12.** (a) Fluorescence evolution of Ag-ESMs during chemical reductions in 2.0 M NaOH solutions at room temperature. ESMs were pre-incubated with AgNO<sub>3</sub> solutions at various concentrations: 500, 250, 100, 50, 25, 10, 5, 2, 1, 0 mM (from left to right tubes) for 5 min, and then transferred to 2.0 M NaOH solutions to initialize corresponding reduction reactions. (b) Photograph of the samples under room light.



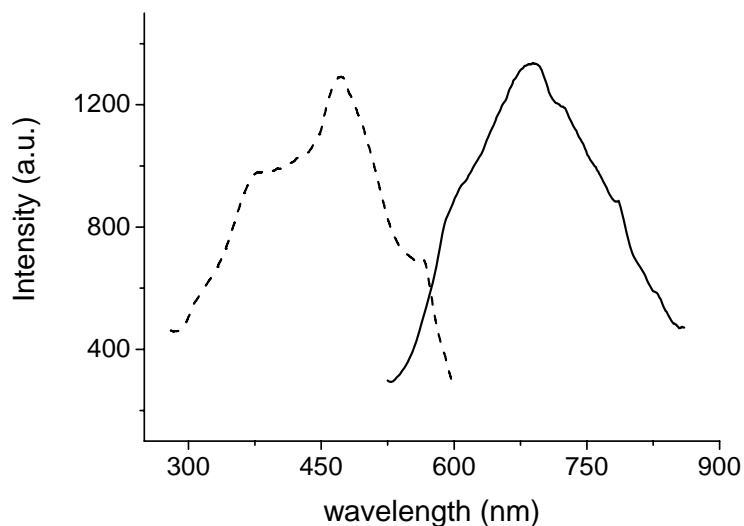
**Figure S13.** Fluorescence emission spectra ( $\lambda_{\text{ex}} = 365\text{nm}$ ) of an Ag-ESM composite (in comparison with an ESM) produced by chemical reduction of an Ag<sup>+</sup> impregnated ESM in a NaOH solution for a relatively long reaction time (2 hours), which induced a red-shift of the fluorescence emission as compared to Figure 3a. The fluorescence emission gradually shifted to near-infrared for a prolonged reaction.



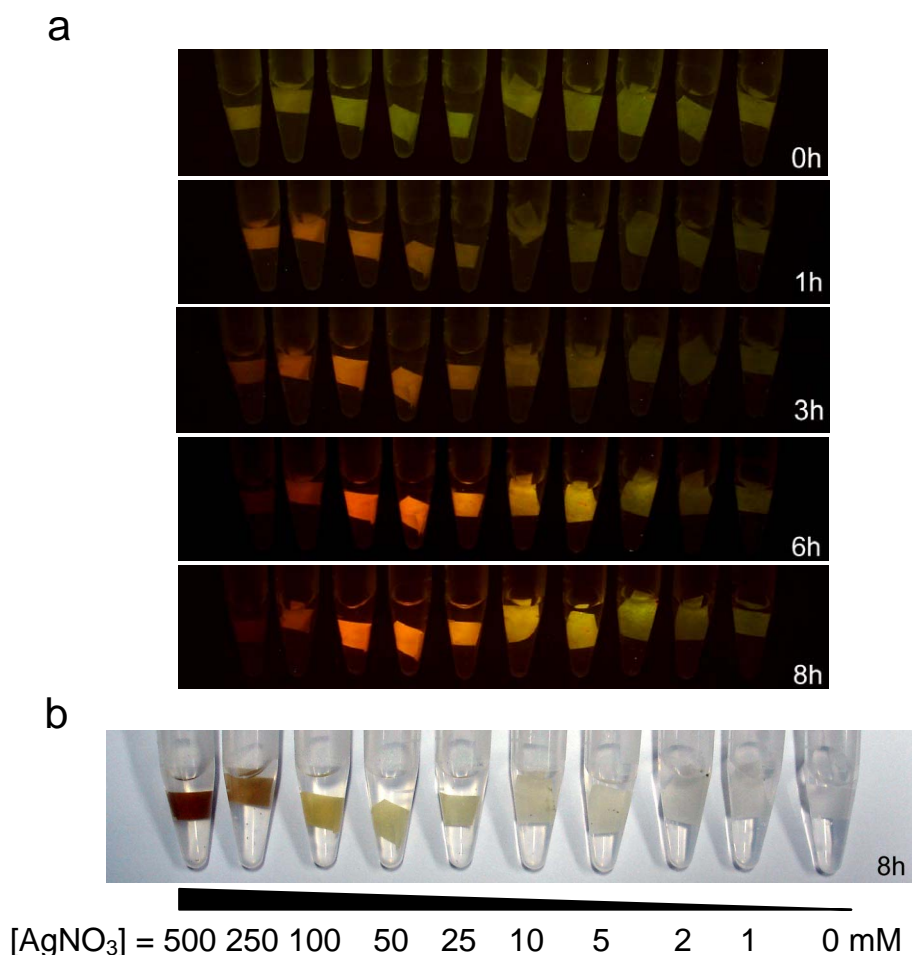
**Figure S14.** (a) Evolution of fluorescence during chemical reductions of Ag<sup>+</sup> adsorbed ESMs by NaBH<sub>4</sub> solutions at room temperature. ESMs were pre-incubated with AgNO<sub>3</sub> at various concentrations: 2, 1, 0.5, 0.2, 0.1, 0.05, 0.02, 0.01, 0 mM (from left to right tubes) for 5 min, and then transferred to 1 mM NaBH<sub>4</sub> solutions to initialize the reduction reactions. (b) Photograph of corresponding Ag-ESM and ESM samples under room light.



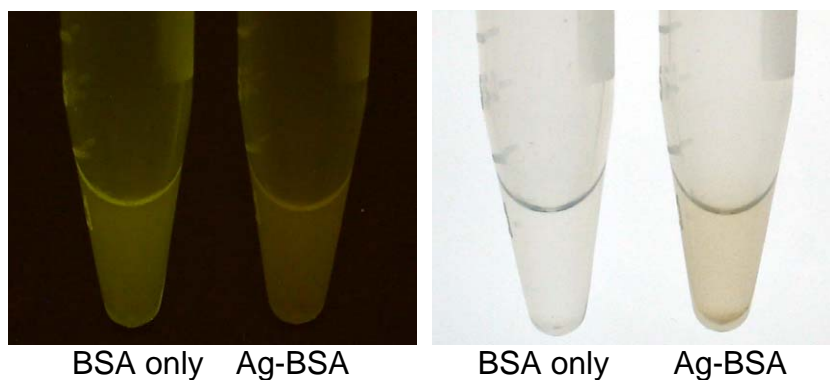
**Figure S15.** Photographs of BSA and Ag-BSA solutions under 365 nm UV excitation (left) and room light (right). Synthesis procedure for fluorescent Ag-BSA solution: an aqueous AgNO<sub>3</sub> solution (500  $\mu$ L, 2 mM) was combined with a BSA solution (500  $\mu$ L, 50 mg/mL) at 37  $^{\circ}$ C under vigorous stirring; fresh NaBH<sub>4</sub> solution (100  $\mu$ L, 0.1 M) was then introduced to the above mixture 2 min later, the reaction was allowed to proceed under vigorous stirring at 37 $^{\circ}$ C for 24 h.



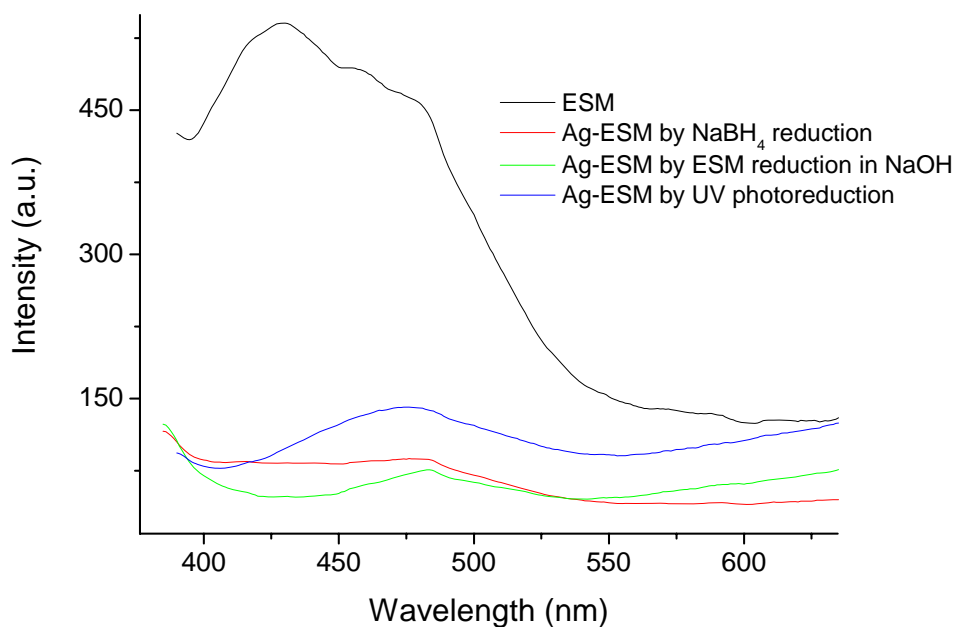
**Figure S16.** Fluorescence emission and excitation spectra of as-produced Ag-ESM via UV irradiation of an  $\text{Ag}^+$  impregnated ESM. Excitation and emission wavelengths were 472 nm and 680 nm, respectively.



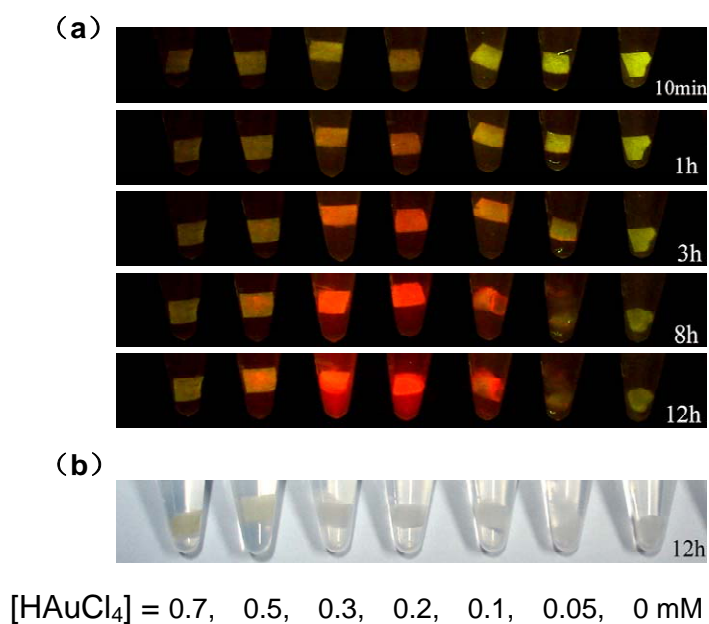
**Figure S17.** (a) Fluorescence evolution of Ag-ESMs prepared by UV light (365 nm) induced reduction of  $\text{Ag}^+$  at room temperature. ESMs were pre-incubated with  $\text{AgNO}_3$  at various concentrations: 500, 250, 100, 50, 25, 10, 5, 2, 1, 0 mM (from left to right tubes) for 5 min, and then subjected to UV irradiation at 365 nm to initialize the reduction process. (b) Photograph of the samples taken under room light after an 8 h reaction.



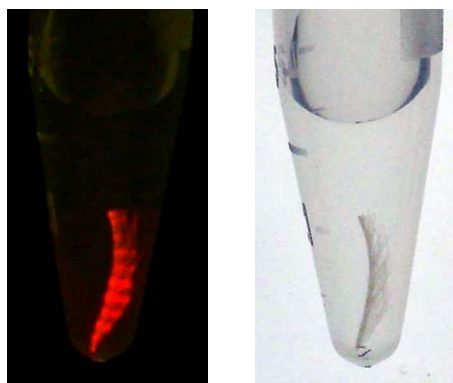
**Figure S18.** Photographs of BSA and Ag-BSA solutions under 365 nm UV excitation (left) and room light (right). Aqueous  $\text{AgNO}_3$  solution (100  $\mu\text{L}$ , 2 mM) and BSA solution (100  $\mu\text{L}$ , 50 mg/mL) was combined and incubated at room temperature for ca. 30 min. The resulting mixture was then subjected to UV irradiation at 365 nm for 6 h.



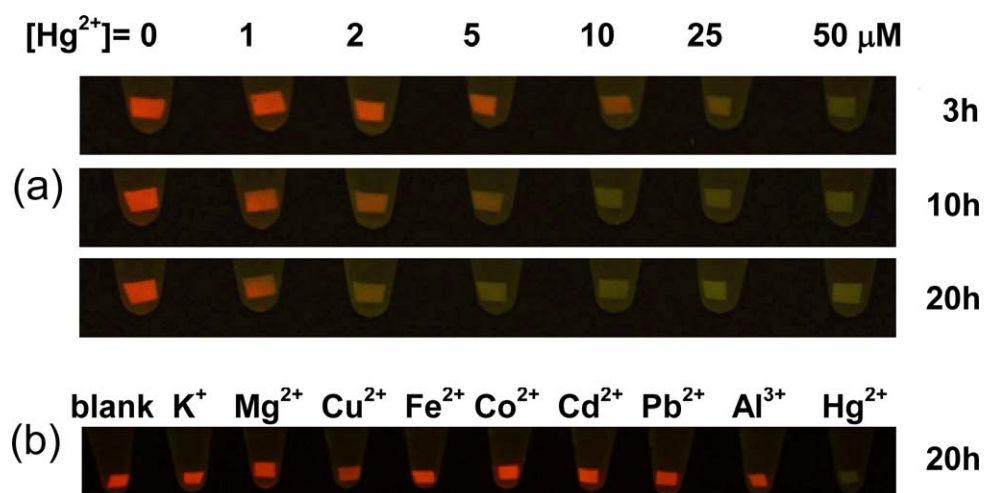
**Figure S19.** Fluorescence emissions ( $\lambda_{\text{ex}} = 365 \text{ nm}$ ) of ESMs from different samples, indicating possible FRET processes between ESM and silver NCs.



**Figure S20.** Synthesis of fluorescent Au NCs on a mulberry silkworm cocoon. (a) Fluorescence evolution of the Au NCs during the reduction process. Pieces of cocoon were pre-incubated with H AuCl<sub>4</sub> at various concentrations: 0.7, 0.5, 0.3, 0.2, 0.1, 0.05, 0mM (from left to right tubes) for 1h, and then transferred to 0.1 M NaOH solution to initialize the reduction reactions. (b) Photograph of the samples taken under room light.



**Figure S21.** A small duck feather embedded with fluorescent gold nanoclusters under 365 nm UV (left) and room light illumination (right). 1 mM H AuCl<sub>4</sub> and 90 mM NaOH were used for the synthesis.



**Figure S22.** Au-ESM membrane as an Hg<sup>2+</sup> sensing paper. (a) Visual detection of Hg<sup>2+</sup>. (b) Specificity test of the Hg<sup>2+</sup> sensor against various other metal ions at 2 mM concentrations (Note: Hg<sup>2+</sup> concentration was 10 μM).