Supplement Information

Synthesis of silver nanoparticles within cross-linked lysozyme crystals as recyclable catalysts for 4-nitrophenol reduction

Miao Liang, Libing Wang, Rongxin Su, Wei Qi, Mengfan Wang, Yanjun Yu and Zhimin He*

Experimental Section

Materials: Ultrapure grade lysozyme was purchased from Amresco (Solon, OH, USA) and used without further purification. Silver nitrate ($AgNO_3$) and $NaBH_4$ were obtained from Aladdin Reagent Company (Shanghai, China). 4-nitrophenol (99%), glutaraldehyde (50 wt%), sodium hydroxide, sodium azide (NaN_3) and other chemicals were of analytical grade. Deionized water was used for all experiments.

Lysozyme crystal growth and cross-linking: Lysozyme crystals were grown by a batch method using reported conditions. Specifically, sodium acetate buffer (0.1M) at pH 4.6 was prepared with deionized water. Protein stock solution of 40 mg/mL and precipitant solution with a concentration of 6% (w/v) were prepared by dissolving lysozyme powder and sodium chloride in the buffer, respectively. All the solutions were passed through 0.22 μm sterile filters to remove foreign nucleation sources before use. In a typical experiment, equal volumes (500 μL) of protein solution and precipitant solution were mixed in a sealed 1.5 mL polypropylene microcentrifuge tube (Eppendorf, Germany). The samples were then incubated at 4 ^{o}C for 2 days to produce well-formed lysozyme crystals. Following crystallization, the

resulting lysozyme crystals were crosslinked by glutaraldehyde solution. The reservoir solution was removed carefully with syringe and replaced with 1 mL cross-linking solution which comprised 1 % glutaraldehyde, 6 % (w/v) NaCl and 0.05 % NaN_3 in the sodium acetate buffer. The lysozyme crystals were equilibrated with this cross-linking solution for 24 h. Subsequently, the solution was removed and the crystals were thoroughly washed with shaking for six times. The corss-linked lysozyme crystals (CLLC) were fianlly dried at room temperature for further use.

Preparation of Ag@CLLC composite materials: Washed and dried CLLC in each microcentrifuge tube were soaked in 1 mL $AgNO_3$ solution (2 mM) at 30 ^oC for 1 hour to allow metal ions diffuse into the solvent channels. The Ag^+ infiltrated CLLC was then collected, washed with water to remove unbound metal ions, and redispersed in 1 mL water. 50 μL $NaBH_4$ solution (0.1 M) was then introduced and the reduction was allowed to proceed for another 1 h at 30 ^oC under continuous stirring. The resulting crystals were again thoroughly rinsed and freeze-dried for further analysis.

Characterization: Optical microscopy imaging was performed using a polarized-light microscope (ShunYu XP, China) with an attached charge-coupled device video camera. After ultrasonication of the suspension which contained the Ag@CLLC samples, a drop containing thin fragments of Ag@CLLC was deposited onto carbon-coated copper grids. The size and morphology of supported AgNPs were determined using high resolution transmission electron microscopy (JEM-2100F, 200 kV) equipped with an energy-dispersive X-ray (EDX) analysis attachment. X-ray diffraction measurement was performed using D/max 2500 XRD spectrometer (Rigaku) with a Cu Kα X-ray source. Fourier transform-infrared (FTIR) spectra

were recorded using a Nicolet Nexus 470 FTIR spectrophotometer within the range from 400 cm^{-1} to 4000 cm^{-1}. Thermogravimetric analysis (TGA) was performed using a simultaneous TGA-DTA apparatus (PTC-10A, Rigaku, Japan) from room temperature to 700 ^{o}C at a heating rate of 10 ^{o}C /min in air.

Catalytic reduction of 4-nitrophenol: The catalytic reduction of 4-nitrophenol by $NaBH_4$ was chosen as a model reaction to investigate the catalytic activity and reusability of the Ag@CLLC composite catalyst. In a typical procedure, 3 mM 4-nitrophenol (750 μL) and Ag@CLLC (10 mg) were added into 7 mL deionized water first and then the solution was purged with N_2 for 10 min to remove the dissolved O_2. Subsequently, 1 mL fresh $NaBH_4$ solution (0.3 M) was injected to initiate the reduction reaction that conducted at 35^{o}C with continuous stirring. Part of the mixture was immediately transferred into a quartz cuvette at different times and UV-vis absorption spectra were recorded in the range of 250-500 nm to monitor the catalytic reaction. After the whole reduction process was completed, the catalysts were separated by brief centrifugation, washed with water, and dried in vacuo at temperature for the next cycle of catalysis. For comparison, the reaction was also carried out by using the same amount of CLLC and free AgNPs under the same experimental conditions.

HPLC Study of the catalytic reaction: The product of the catalytic reduction was analyzed by an Agilent 1200 HPLC system. Chromatographic separations were performed at room temperature using an Agilent Eclipse XDB-C18 column. The wavelength of UV detector was set as 254 nm and a mobile phase composed of 60% MeOH and 20% water at a flow rate of 0.6 mL min^{-1} was used.

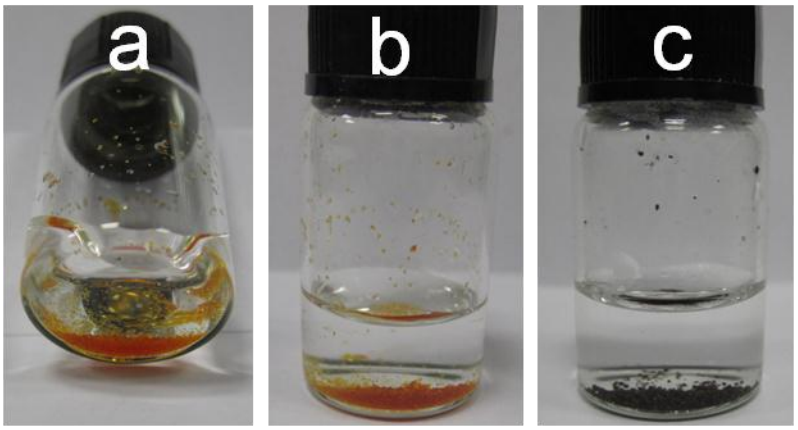

Figure S1. Images of a) CLLC dispersed in water; b) CLLC after coordination with Ag^+ in $AgNO_3$ solution; and c) Ag@CLLC after the reduction of Ag^+ by $NaBH_4$.

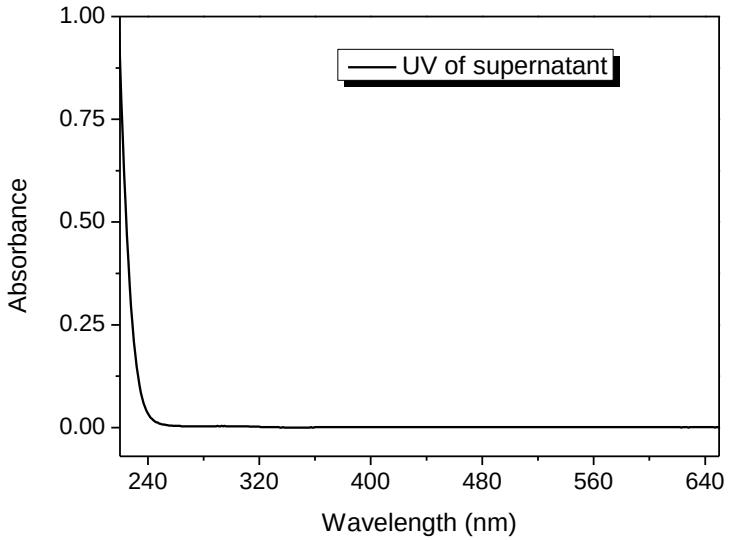

Figure S2. UV-vis spectrum of the supernatant after the formation of AgNPs within CLLC. No characteristic SPR peak for AgNPs was observed, showing the AgNPs were only formed within CLLC.

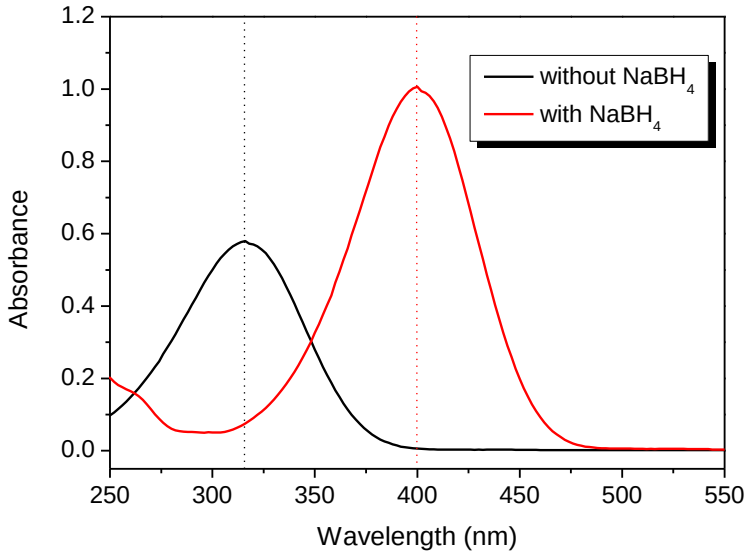

Figure S3. UV-vis detection of a solution of 4-NP with and without $NaBH_4$.

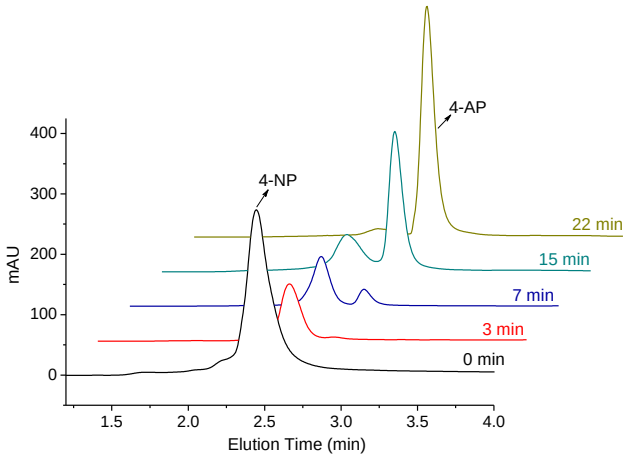

Figure S4. Catalysis of 4-NP to 4-AP using Ag@CLLC followed by HPLC. The reaction products were taken out at different times.

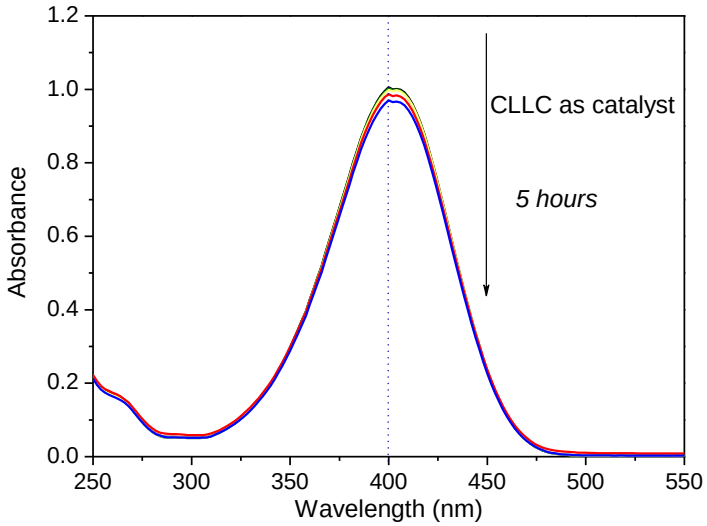

Figure S5. The reduction of 4-NP by $NaBH_4$ using CLLC as catalyst for comparison.

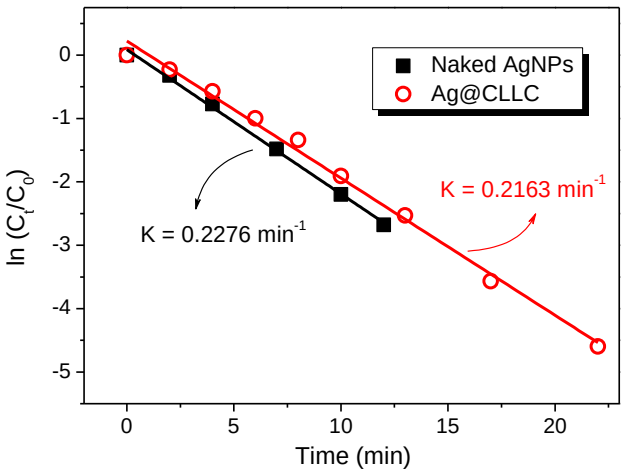

Figure S6. Comparison of the $\ln(C_t/C_0)$ versus reaction time between Ag@CLLC and naked AgNPs.