

Multifunctional Ag@Fe₂O₃ Yolk-shell Nanoparticles for Simultaneous Capture, Kill, and Removal of Pathogen

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Supporting Information

Experimental Section

General. Oleylamine (tech. 70%), oleic acid (tech. 90%), 1-octadecene (tech. 90%), silver trifluoroacetate (99%), and 3-Hydroxytyramine hydrochloride were purchased from Acros. Iron pentacarbonyl and D-(+)-Glucosamine hydrochloride were purchased from Sigma-Aldrich, other solvents and chemicals were obtained from Sinopharm Chemical Reagent Co., Ltd. All chemicals were used as received without further purification.

Synthesis of Ag nanoparticles.^[1] Silver nanoparticles were prepared by thermal reduction of silver trifluoroacetate in 1-octadecene (ODE) in the presence of oleic acid. In a typical procedure for making Ag nanoparticles with diameters of ~8 nm, silver trifluoroacetate (0.22 g, 1 mmol), oleic acid (2 mL) and 1-octadecene (20 mL) were mixed in a 50 mL three-neck round-bottom flask under argon protection. The

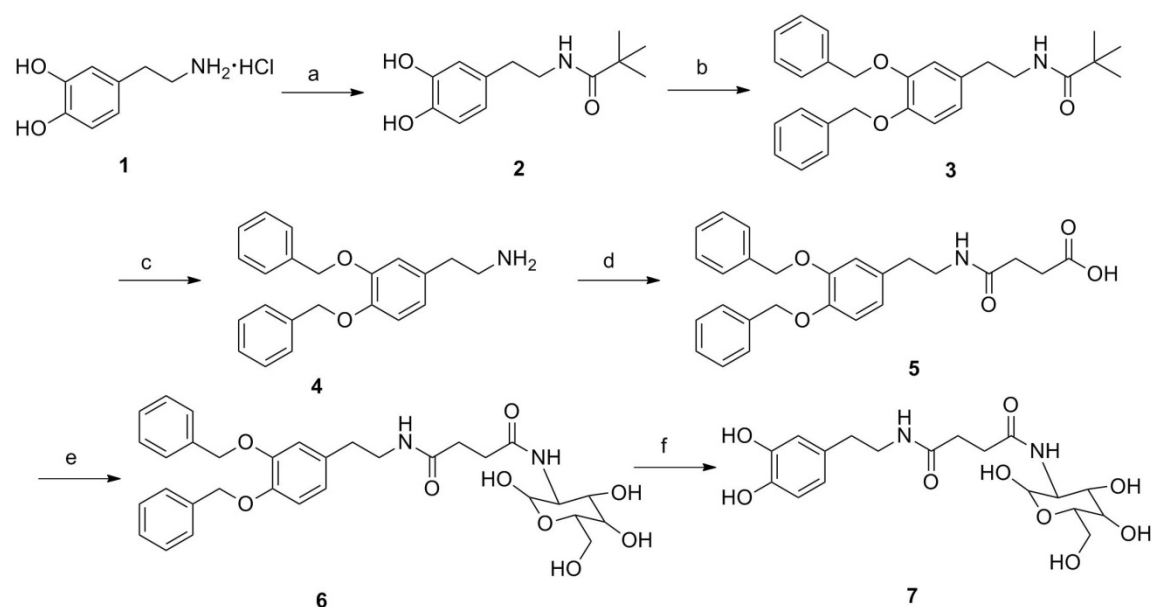
reaction mixture was heated to 180 °C and kept at this temperature for 1 h before being cooled down to room temperature. The addition of isopropanol (40 mL) to the reaction mixture afforded a brown precipitate by centrifuging. The silver nanoparticles were dispersed in hexane and then used as seeds.

Synthesis of Ag@Fe₂O₃ yolk-shell nanoparticles. Under argon protection, 20 mL of 1-octadecene containing 0.1 mL of oleylamine and the as-synthesized 8-nm silver nanoparticles (100 mg) was heated at 120 °C for 30 minutes to remove moisture and oxygen. 0.2 mL of iron pentacarbonyl (Fe(CO)₅) was injected into the hot solution, then the solution was kept at 180 °C for 30 minutes. Without any centrifugation and purification, the dispersion of mixture was moved to ambient atmosphere and heated up to 250 °C with compressed air flow at 1 m³/h for about 2 hour. The Fe nanoshells were completely oxidized to produce Fe₂O₃ nanoshells by Kirkendall effect during the oxidization. After cooling to the room temperature, 0.1 mL of oleylamine and 0.2 mL of oleic acid were injected into the dispersion, then it was heated to reflux and kept for 1 hour before being cooled down to room temperature. The addition of isopropanol (40 mL) to the reaction mixture afforded a blackish purple or brown product by centrifuging. The product could be further washed three times by repetition of dispersion in hexane (5 mL) and precipitation with ethanol (20 mL) and centrifugation. Finally, the porous Ag@Fe₂O₃ yolk-shell nanoparticles were dispersed in hexane.

Synthesis of γ -Fe₂O₃ hollow nanoparticles. Under argon protection, 20 mL of 1-octadecene containing 0.15 mL of oleylamine was heated at 120 °C for 30 minutes to remove moisture and oxygen. 0.35 mL of Fe(CO)₅ was injected into the hot solution, then the solution was kept at 180 °C for 30 minutes. Without any centrifugation and purification, the dispersion of mixture was moved to ambient atmosphere and heated up to 250 °C with compressed air flow at 1 m³/h for about another 2 hour. Centrifugation and purification procedures were the same to the procedures of Ag@Fe₂O₃ yolk-shell nanoparticles.

Synthesis of DA-Glu. The processes for the synthesis of compound **5** from compound **1** were followed as the previous reported article.^[2]

Scheme 1^a Synthesis route of DA-Glu



^a Reagents and Conditions: (a) NaOH, *tert*-butyl dicarbonate, dioxane, H₂O, 24 hrs; (b) BnBr, K₂CO₃, DMF, rt, 24 h; (c) 10% CF₃COOH, CH₂Cl₂, rt, 5h; (d) succinic anhydride, pyridine, rt, 3h; (e) EDC, NHS, Et₃N, CH₃OH, H₂O, D-(+)-Glucosamine hydrochloride, rt, 5h; (f) Pd/C, CH₃OH, H₂, 24h.

Synthesis of N-[2-(3,4-Bis-benzyloxy-phenyl)-ethyl]-(gluco-1-yl)succinamide (compound 6).

Compound 5 (217 mg, 0.5 mmol) was dissolved in 20 mL of CH₃OH, EDC Hydrochloride (96 mg, 0.5 mmol) and NHS (58 mg, 0.5 mmol) were added sequentially. 5 mL of H₂O containing Glucosamine Hydrochloride (108 mg, 0.5 mmol) was added to it. Reaction was allowed to perform at room temperature for 3 h, the result solution was filtered and dried over reduced pressure to obtain a slightly yellow solid. Product clarified by LC-MS: calc. M⁺= 594, obsvd. (M+1)⁺=594.8, and used directly without any further purification.

Synthesis of N-[2-(3,4-dihydroxyl-phenyl)-ethyl]-(gluco-1-yl)succinamide (compound 7, denoted as DA-Glu). Compound 6 (297 mg, 0.5 mmol) was dissolved in 20 mL CH₃OH with 10% Pd/C (53 mg, 0.05 mmol). Reaction system was degassed with H₂ and stirred vigorously for 24 h. Then, Pd/C was removed by filtration. The filtrate was dried over reduced pressure to obtain a slightly yellow solid. Product clarified by LC-MS: calc. M⁺= 414, obsvd. (M+1)⁺=414.8.

Surface modification of Ag@Fe₂O₃ yolk-shell nanoparticles using DA-Glu. In ambient environment, about 30 mg of oleate coated nanoparticles was dispersed in 5 mL of toluene, 30 mg of DA-Glu in 5 mL dimethyl sulfoxide (DMSO) was added into the solution, and the mixture was stirred to form a homogeneous solution. After heating at 70 °C for 1h, the mixture was cooled down to room temperature. 10 mL of

hexane, 10 mL of ethanol, and 20 mL of acetone was added into the mixture to precipitate the product, followed by centrifuging at 10000 rpm for 10 min. The black product could be washed again by repetition of dispersion in water and precipitation with acetone and centrifugation in order to remove redundant DA-Glu. Subsequently, the particles were redispersed in deionized water with the aid of sonication. The surface modification procedure of Fe₂O₃ hollow nanoparticles is similar to that of Ag@Fe₂O₃ nanoparticles.

MRI imaging. Different concentrations (12.5, 25, 50, 100, 200, 400 and 800 μM of Fe, respectively) of Ag@Fe₂O₃ yolk-shell nanoparticles were embedded in 1% agarose gel for MRI scanning. The MRI experiment was performed on a 7 Tesla MRI scanner (Varian 7T micro MRI System, America). These samples were scanned using a multi-echo T₂-weighted fast spin echo imaging sequence to collect a series of echo time (TE) dependent data points simultaneously.

Preparation of microbiological experiments. Stock culture of *Escherichia coli* (*E. coli* O157:H7 and *E. coli* ER2566) and *B. subtilis* were kind gifts from Professor Xiaomei Yan's group (Xiamen University). For safety considerations, all of the bacterial samples were placed in an autoclave at 121 °C for 20 min to kill bacteria before disposal and all glassware in contact with bacteria was sterilized before and after use. In the meantime, the Ag@Fe₂O₃-Glu yolk-shell Nanoparticles and γ-Fe₂O₃-Glu hollow Nanoparticles water solution were sterilized under the UV light

for a minimum of 10 h. The tested bacteria, *E. coli* ER2566, was firstly constructed of the recombinant plasmids encoding the Enhanced Green Fluorescent Protein (EGFP); Inoculate 30 μ L of freshly grown transformed *E. coli* ER2566 cells into 3 mL of LB medium supplement with 50 μ g/mL kanamycin, add IPTG (β -D-thiogalactopyranoside) to a final concentration of 0.4 mM to induce Enhanced Green Fluorescent Protein (EGFP), followed by additional incubation at 30 °C for 6 h.

Preparation of bacteria samples for TEM. The morphological changes of bacteria by Ag@Fe₂O₃-Glu yolk-shell nanoparticles were observed with a TEM (JEM-2100) at an accelerating voltage of 120 kV. Samples (aggregates of nanoparticles and bacterial after magnetic separation) were firstly fixed for a minimum of 3 h in 2.5% (v/v) glutaraldehyde (100mM phosphate buffer solution, pH 7.2), then washed three times by centrifugation and redispersion in PBS. The aggregates were dehydrated through a graded series of ethanol (30, 50, 70, 80, 90 and 100%; each level was applied once for 15 min each time) and ethanol:acetone (1:1 and 100%; each level was applied once for 30 min each time) by centrifugation and redispersion. TEM samples were prepared by depositing the particle dispersion onto 400 mesh carbon-coated copper grids. A drop of 1% molybdophosphoric acid was then used to negatively stain the bacteria aggregates for 1minute.

Measurements of antibacterial properties of Ag@Fe₂O₃-Glu yolk-shell Nanoparticles and γ -Fe₂O₃-Glu hollow Nanoparticles. The minimum inhibitory

concentration (MIC) values for *E. coli* O157:H7 and *B. subtilis* were determined. The inoculation of these kinds of bacteria were prepared by growing strains in Luria-Bertani (LB) liquid medium at 37 °C until a level of approximately 10^8 - 10^9 CFU/mL of bacteria was reached. The in vitro antimicrobial activity was evaluated by the serial two-fold agar dilution method. Different concentrations of Nanoparticles in 10 mL LB solid medium were added to Petri dishes and solidify, with an applicator, 100 μ L of LB liquid medium containing the tested bacteria at a concentration of approximately 10^4 CFU/mL was added and imprinted on the surface of agar. The minimum inhibitory concentration (MIC) was read after 12 h of incubation at 37 °C as the MIC of the tested substance that inhibited the growth of the bacterial strain. All assays were carried out in three duplicates.

Characterization. TEM were prepared by placing a drop of the colloidal dispersion onto an amorphous carbon-coated copper grid. TEM and HRTEM images were recorded on JEM-2100 microscope at an accelerating voltage of 200 kV, HAADF-STEM and element mapping images were recorded on Tecnai F30 at an accelerating voltage of 300 kV. UV-vis spectra were recorded using Shimadzu UV 2550, while fluorescence spectra were recorded on Hitachi F-7000. The hysteresis loop (at 300 K) and temperature dependence of the ZFC/FC magnetization (at a magnetic field of 100 Oe) was recorded on Quantum Design MPMS-XL-7 system. The element analysis of Fe and Ag in the samples was carried out by inductively coupled plasma atomic emission spectroscopy (ICP-AES).

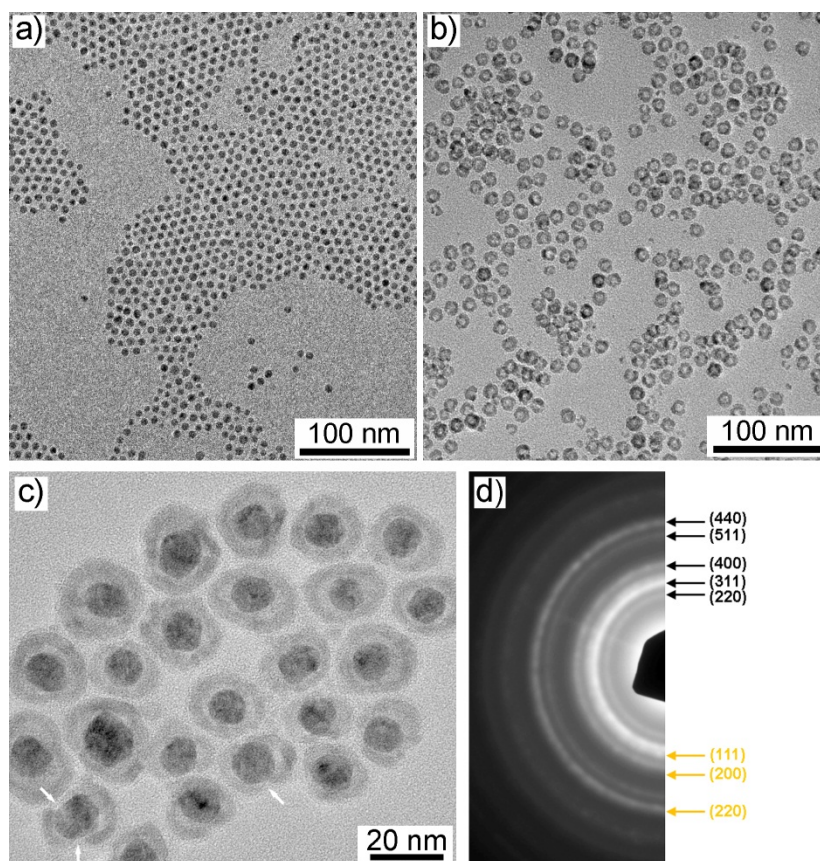


Figure S1. TEM images of (a) Ag nanoparticles and (b) γ -Fe₂O₃ hollow nanoparticles. (c) TEM image (arrows indicate the iron oxide nanoshells are partially broken) and (d) SAED pattern of Ag@Fe₂O₃ yolk-shell nanoparticles, showing the significantly discriminable structures between face center cubic (fcc) phase of γ -Fe₂O₃ ((220), (311), (400), (511), and (440) planes) and Ag ((111), (200), and (220) planes).

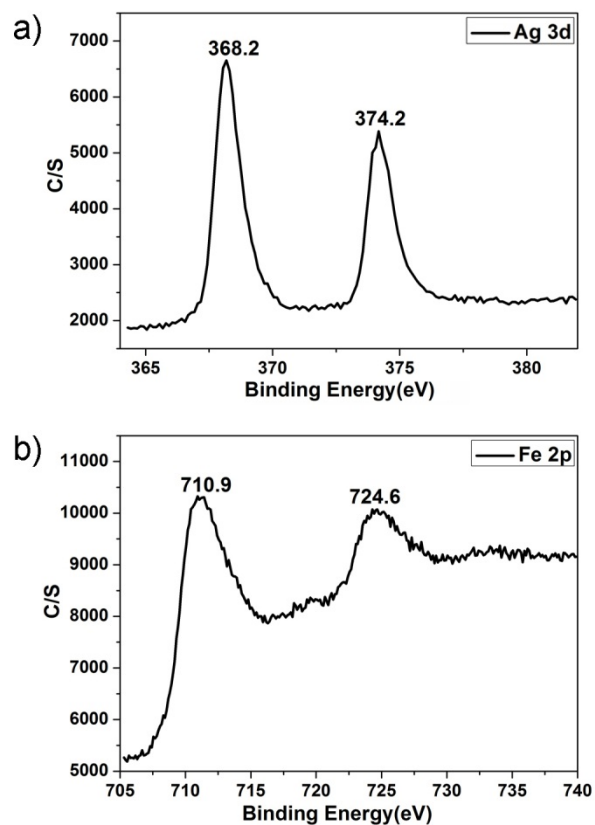


Figure S2. XPS analysis of Ag@Fe₂O₃ yolk-shell nanoparticles. The Ag 3d doublet at 368.2 eV (Ag 3d_{5/2}) and 374.2 eV (Ag 3d_{3/2}) corresponds to metallic silver.^[3] The Fe 2p doublet at 710.9 eV (Fe 2p_{3/2}) and 724.6 eV (Fe 2p_{1/2}) corresponds to Fe₂O₃.^[3]

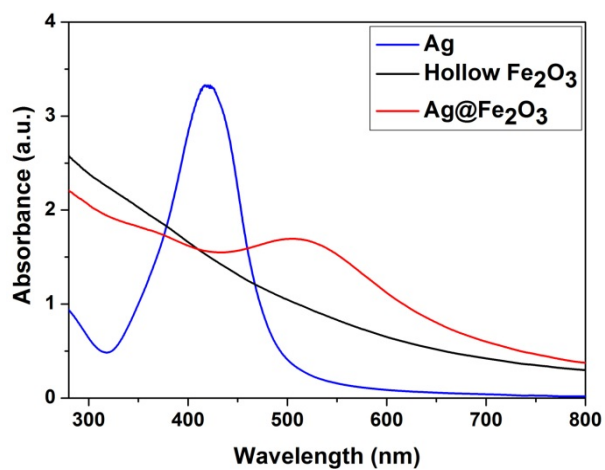


Figure S3. UV-vis analysis of Ag nanoparticles, γ -Fe₂O₃ hollow nanoparticles, and Ag@Fe₂O₃ yolk-shell nanoparticles in hexane.

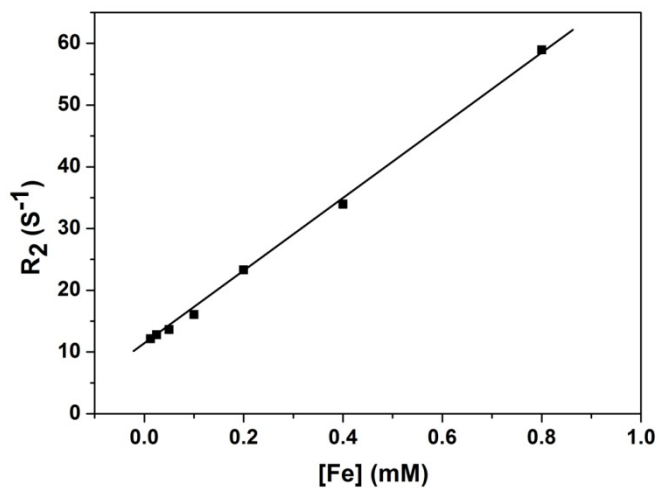


Figure S4. The analysis of relaxation rate R_2 ($1/T_2$) vs Fe concentration for Ag@Fe₂O₃-Glu yolk-shell nanoparticles. The relaxivity value r_2 was obtained from the slope of linear fit of experimental data.

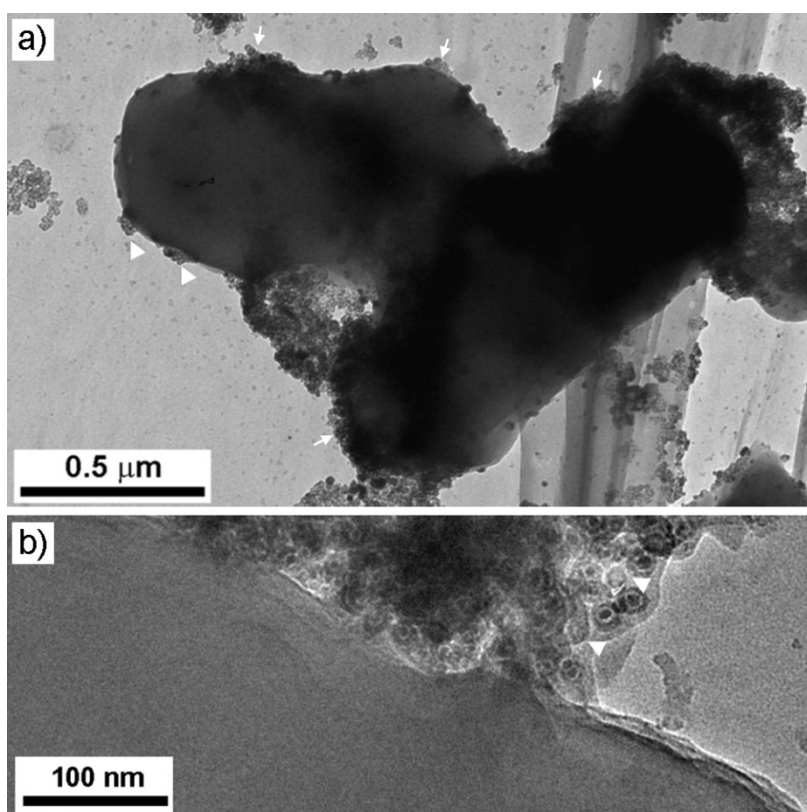


Figure S5. The representative TEM images of the Ag@Fe₂O₃-Glu conjugates captured bacteria (*E. coli*). The arrows indicated the aggregations of nanoparticles interact with the surface of bacteria. It is noted that there are some of hollow nanoparticles without Ag cores (arrowheads).

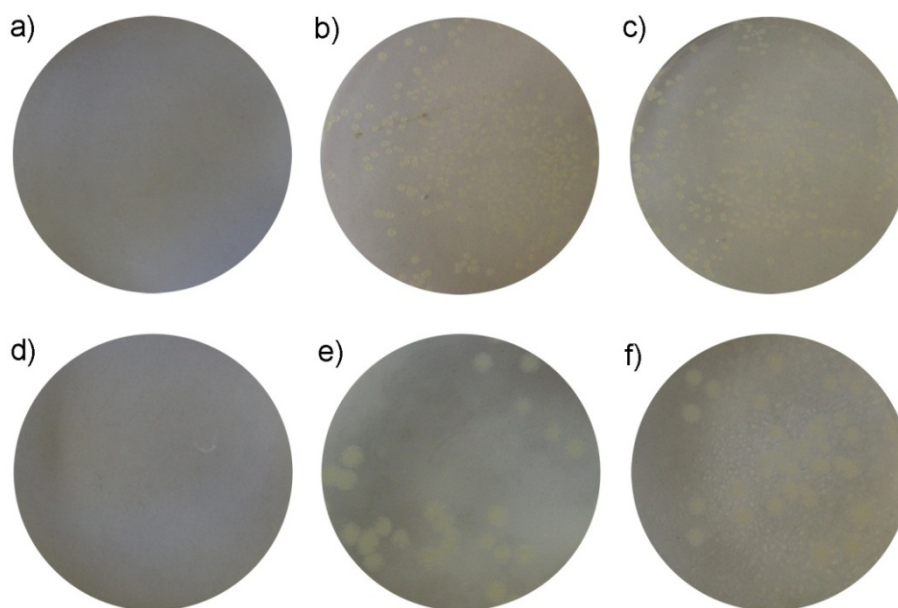


Figure S6. The representative antibacterial results on agar. The images of *E. coli* O157:H7 (10^4 CFU/mL) cultivated with (a) 14.8 $\mu\text{g/mL}$ of Ag@Fe₂O₃-Glu conjugates, (b) 150 $\mu\text{g/mL}$ of glucose-modified γ -Fe₂O₃ hollow nanoparticles, and (c) PBS after 12 h. The images of *B. Subtilis* (10^4 CFU/mL) cultivated with (d) 21.2 $\mu\text{g/mL}$ of Ag@Fe₂O₃-Glu conjugates, (e) 150 $\mu\text{g/mL}$ of glucose-modified γ -Fe₂O₃ hollow nanoparticles, and (f) PBS after 12 h.

Reference:

- [1] X. Z. Lin, X. Teng, H. Yang, *Langmuir* **2003**, *19*, 10081-10085.
- [2] C. Xu, K. Xu, H. Gu, R. Zheng, H. Liu, X. Zhang, Z. Guo, B. Xu, *J. Am. Chem. Soc.* **2004**, *126*, 9938-9939.
- [3] J. F. Moulder, W. F. Stickle, P. E. Sobol, K. D. Bomben, *Handbook of X-ray photoelectron spectroscopy*, Perkin-Elmer Corporation, Physical Electronics Division, Eden Prairie, MN, USA, **1992**.