ACS APPLIED MATERIALS & INTERFACES

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Reduction of CuO Butterfly Wing Scales Generates Cu SERS Substrates for DNA Base Detection

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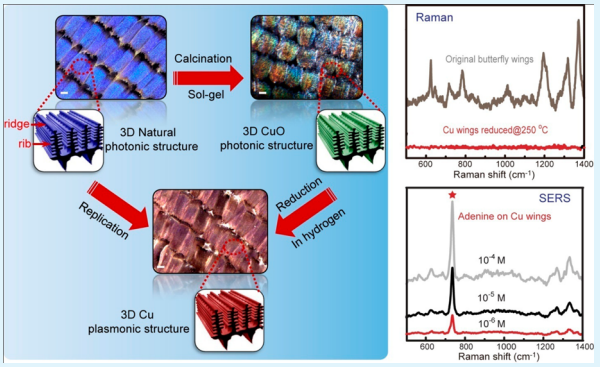
Supporting Information

ABSTRACT: We prepare three-dimensional Cu plasmonic structures via a reduction of CuO photonic crystals replicated from butterfly wing scales. These Cu superstructures with high purity provide surface-enhanced Raman scattering (SERS) substrates for the label-free detection of DNA bases down to a micromolar level, which is achieved for the first time on Cu and even comparable to the detection-sensitivity for DNA bases on some Ag substrates. The generation of such superstructures has provided a substantial step for the biotemplated SERS substrates with high sensitivity, high reproducibility, and ultra-low cost to detect biomolecules, and presented affordable high-quality routine SERS consumables for corresponding biolaboratories.



KEYWORDS: *biological templates, three-dimensional, Cu, surface-enhanced Raman scattering, DNA bases*

Three-dimensional (3D) sub-micrometer metallic structures have received enormous interest due to their broad applications in catalysis,[1] biosensors,[2] surface-enhanced Raman scattering (SERS),[3] and metal-enhanced fluorescence (MEF),[4] et al. Present fabrication methods for these structures are either complex and prohibitively expensive, or less of reproducibility for large surfaces, giving rise to technical bottle necks in generating metallic structures for research and for commercial applications. Recently, promising routes were brought forward to create such textures by a direct replication of hierarchical structures of natural species into metals.[5−10] For example, metals have been deposited onto butterfly wing scales via wet chemical methods to fabricate 3D metallic structures.[6,10] However, the bio-templates had to be removed therein through a post-treatment in H_3PO_4 up to 72 h.[6,10] Even so, the original chitin and protein based scales still could not be completely dissolved.[6] The remaining biopolymeric compounds brought the Raman signals of impurities and high autofluorescence especially when incident lasers with longer wavelengths (e.g., 785 nm) were used to excite biomolecules (e.g., DNA bases, see Figure 1).[11,12]

Herein, we report the generation of a new group of 3D Cu butterfly wing scales capable of detecting DNA bases in low concentrations. By reducing intermediate CuO butterfly wings in H_2 (Figure 2), we have successfully fabricated 3D Cu plasmonic structures (see extinction spectra in Figure S1 in the Supporting Information). An optimized reducing process (250 °C in H_2 for 1 h) yields Cu replicas with the best conformal morphologies of original wing scales, and most importantly, with a high purity. These Cu materials show a detection limit (label-free) in concentration down to the micromolar level for DNA base molecules. Such sensitivity is even comparable to that of some Ag SERS substrates. These "cleaner" Cu superstructures render the metallic butterfly wings as SERS substrates to detect biomolecules, which are one of the most important applications of SERS technologies.

The original scales were collected from the blue part on the dorsal wing surfaces of *Euploea mulciber*.[6] We first fabricated their CuO replicas using a sol-gel process followed by calcinations in air to remove the biotemplates.[13] Figure S2 in the Supporting Information compares the 3D sub-micrometer structures of original blue wing scales of *E. mulciber* with their CuO replicas. The whole scale was perfectly replicated in CuO (see Figure S2d in the Supporting Information) owing to a homogeneous Cu^{2+} adsorption across the scale surface.[13] Higher magnification image (see Figure S2e in the Supporting Information) shows that CuO replicas conformally inherited the features of natural templates down to a sub-micrometer level. Not only the microscaled parallel main ridges, but also the nanoscaled ribs (overlapped layers within the ridges, see Figure S2c in the Supporting Information) were preserved. These structures are essential to the colorization of CuO photonic crystals (see the iridescence phenomenon for the CuO scales in Figure 2). The successful fabrication of CuO scales promised the formation of Cu plasmonic structures via the subsequent reduction process. X-ray diffraction (XRD) results of CuO wing replicas (see Figure S3a in the Supporting Information) indicate that their average grain size was ∼10.2 nm (Scherrer formula). Additionally, energy-dispersive X-ray spectroscopy

Received: July 7, 2013
Accepted: September 30, 2013
Published: September 30, 2013

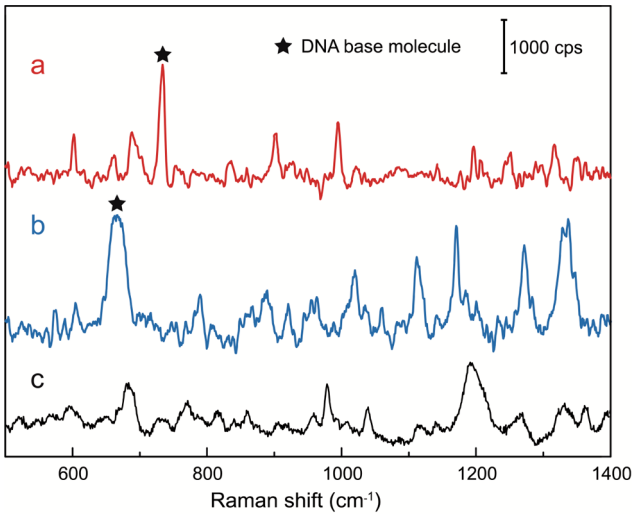


Figure 1. SERS detection of DNA base molecules with 785 nm excitation on Cu butterfly wings fabricated via a wet chemical method.[6,10] (a, b) Detection of DNA bases (1×10^{-5} M) on such Cu wings with (a) adenine and (b) guanine. (c) is the Raman signals from the Cu wings themselves. Under a 785 nm excitation usually adopted for biodetection, strong impurity peaks and backgrounds originating from the remains of biotemplates interfered with the analyses. Only the main peaks of the DNA bases can be indexed. This serious problem drove us to seek new ways toward "much cleaner" Cu wing substrates in this work.

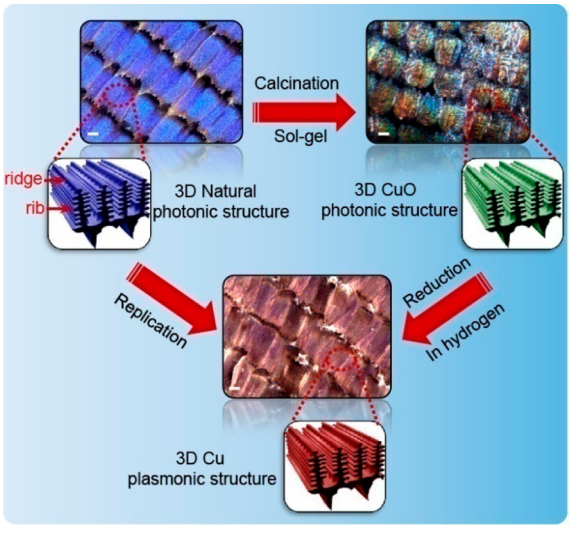


Figure 2. Route to "purer" 3D Cu plasmonic structures. The successful fabrication of CuO photonic structures (see the iridescence phenomenon for CuO scales and Figure S1 in the Supporting Information) allows the subsequent conformal replication of Cu structures via reduction.

(EDS) results of CuO replicas suggest the successful replication in composition as well (see Figure S3b in the Supporting Information).

Then, we reduced the CuO butterfly wings in H_2 to obtain Cu plasmonic structures. Because of their high surface energies, metallic nanoparticles (NPs) have lower melting temperatures than corresponding bulk metals and are prone to deformation and coalescence when heated.[14−19] So the key point here is to find out a suitable reduction temperature, under which one can simultaneously acquire Cu products with high-purity (low SERS backgrounds) and the conformal microstructures of the original butterfly scales critical to optimized SERS properties.[6,7,10] In this respect, we prepared a series of Cu replicas by treating CuO replicas in H_2 for 1 h under 200, 250, and 300 °C, respectively. When heated up to 200 °C, CuO replicas showed no obvious changes in morphology (Figure 3b) but could not

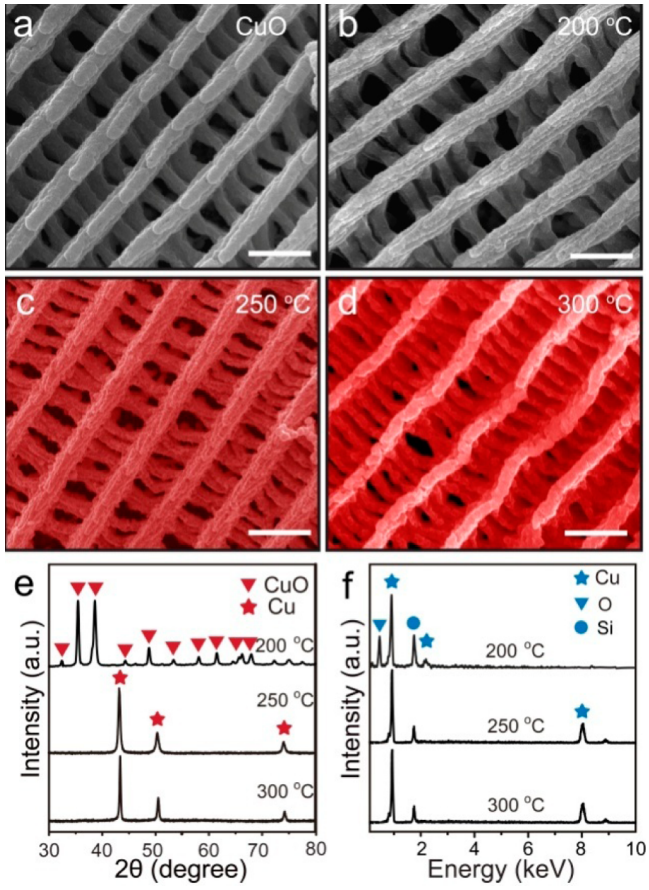


Figure 3. Influence of reduction temperatures on the periodic structure of Cu replicas. (a−d) Field emission scanning electron microscopy (FESEM) images of (a) CuO butterfly scale; (b−d) Cu replicas fabricated via the reduction of a by H_2 under (b) 200, (c) 250, and (d) 300 °C. Scale bars: 1 μm. (e) XRD results of Cu replicas prepared at different reduction temperatures. The grain sizes are ~22.6 nm and ~30.2 nm for Cu generated under 250 and 300 °C (Scherrer formula), respectively, consistent with the results of transmission electron microscopy (TEM) analysis (see Figure S4 in the Supporting Information). (f) EDS results of b−d. Silicon signals originated from the Si wafer as substrates.

be reduced (see XRD results in Figure 3e). In comparison, the CuO replicas can be reduced at 250 °C (Figure 3c, e). However, when overheated to 300 °C, the Cu replicas obviously deformed and underwent particle-coalescence. Larger particles were formed on the surfaces of Cu replicas,[14,18,19] and one can observe the deformation of ridges and the collapse of ribs (Figure 3d). Thus, we take the reduction under 250 °C for 1 h as an optimized condition for the conformal generation of Cu wing scales.

The X-ray photoelectron spectroscopy (XPS) spectra (Figure 4) further reveal that the Cu replicas prepared via the heat-treating are much purer in composition than those synthesized via previous wet chemical method conducted at mild temperatures.[6,10] The original butterfly wings are mainly

dx.doi.org/10.1021/am402699c | ACS Appl. Mater. Interfaces 2013, 5, 9878−9882

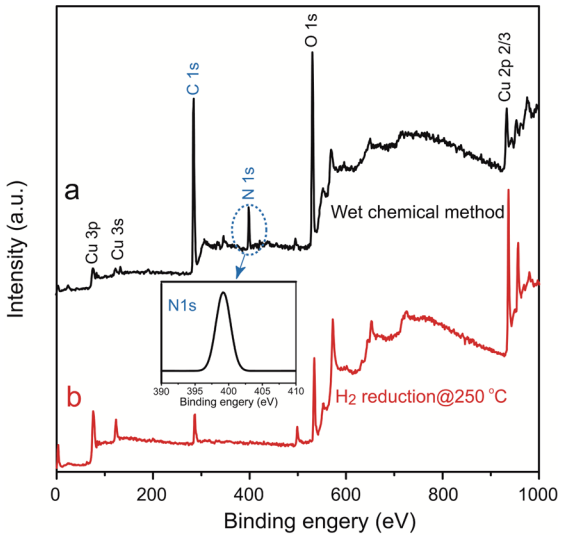


Figure 4. XPS spectra from Cu replicas (a) fabricated via the previous method of electroless plating plus selective dissolution of biotemplates;[6,10] (b) synthesized by the reduction process reported herein. Note that the C, N signals from the bio-templates significantly decrease or even disappear in (b) after the calcinations in air and H_2. The peak at ~531.7 eV is attributed to the oxygen in the form of hydroxide due to the oxidation of the Cu surface.

composed of chitin and protein, including the elements of C, N, O, and H, et al. For Cu replicas, the Cu present is indicated by the peaks of Cu $2p_{2/3}$ (932.6 eV), Cu 3s (122.1 eV), and Cu 3p (83.8 eV). Although C present can be observed in samples either reduced at 250 °C or dissolved in H_3PO_4 at room temperature,[6,10] its intensity significantly decreased for the samples treated via the heat-treating. The lost of N signal in the as-reduced sample further proves that the organic matter of butterfly wings was almost fully removed after the calcinations in air and in H_2.

To investigate whether these 3D periodic Cu structures can be used as SERS substrates or not, we first compared the Raman spectra of 10^{-5} M Rhodamine 6G (R6G) absorbed on various SERS substrates (see Figure S5 in the Supporting Information). Two substrates covered by Cu NPs manually ground from Cu scale replicas in liquid N_2 (see Figure S6 in the Supoprting Inforamtion) were also used there for comparison. The CuO replicas and their products reduced under 200 °C could not enhance the Raman signals of R6G molecules as CuO is an inert SERS material (see Figure S5 in the Supporting Information). In comparison, the R6G peak at 1650 cm^{-1} increased 8 and 3 times of magnitude on samples reduced under 250 and 300 °C, respectively, as compared with that on the Cu NPs ground from the corresponding Cu replicas. Moreover, the Raman intensity at 1650 cm^{-1} for R6G on the sample reduced under 250 °C was about twice of magnitude that on 300 °C-reduced substrates. The decrease in SERS with the periodicity-break in Cu replicas reveals that the SERS is structurally dominated in Cu replicas. Because the Cu grain size increased with the reduction temperature, approaching to the reported optimal value of 50 nm for SERS,[20] the reduction under 300 °C should render large particles helpful to SERS. However, the periodicities of microstructures were destroyed under 300 °C. The gain from the particle size could not compensate the loss of the localized electromagnetic fields contributed by the 3D structures (see Figure S2b in the Supporting Information),[21−23] giving rise to the lower SERS properties for B than A as shown in Figure S5 in the Supporting Information. Because the laser excitation of 514.5 nm for R6G detection would also induce resonance Raman scattering, as such a wavelength falls within the electronic absorption band of R6G molecules (524 nm),[24,25] we measured the N_2 adsorption isotherms at 77 K on the Cu scale replicas reduced under 250 °C and on their as-ground NPs, respectively, to rule out the influence exerted by the number difference in the molecules absorbed on various surfaces. The Brunauer−Emmett−Teller (BET) surface areas of the Cu replicas and Cu NPs were caculated to be 56.6 and 202.5 m^2/g, respectively (see Figure S7 in the Supporting Information). Therefore, the molecules absorbed on the Cu NPs could be more than those on the Cu replicas within the laser beam area. Considering that the Raman signals were significantly enhanced on Cu wing scales as compared with on Cu NPs, this result indicates that the structure-effect rather than the molecule-number-effect is dominant in the observed Raman enhancement as well.

Additionaly, it should be noted that the samples reduced at 250 °C exhibited excellent reproducibility for detecting R6G molecules. Considering the Raman spectra randomly collected at 20 spots on 5 different Cu scales fabricated herein (see Figure S5c in the Supporting Information), the relative standard deviation (RSD) of Raman intensity at 1650 cm^{-1} is as low as 10.8%. Such reproducibility is comparable to that of Klarite, the famous commercial SERS substrates.

To confirm the ability of those 3D Cu plasmonic structures to detect biomolecules, we chose the same DNA bases used in Figure 1 as analytes. Once more, we applied the 785 nm excitation laser with which DNA base molecules are optically nonresonant.[11,26,27] Because of the success in removing the original chitin-based bio-templates by heat-treating, all the exhibited Raman peaks of analytes have no background contributions from the original butterfly wing scales (see Figure 5a).[6,10] Panels b and c in Figure 5 show the SERS spectra of adenine and guanine aqueous solution with different concentrations. The SERS signature peak at 738 cm^{-1} from the ring breathing mode of adenine (1 μM), and the peak at 662 cm^{-1} from the ring breathing mode of guanine (1 μM) is clearly distinguishable.[12,26,28,29] Such a detection sensibility is even comparable to that of some Ag SERS substrates,[30−32] while Au and Ag are ~5000 and ~100 times more expensive than Cu, respectively.[33]

CONCLUSIONS

We have fabricated Cu plasmonic structures with ordered morphological features by reducing the CuO photonic crystals replicated from natural butterfly wing scales. A reduction process in H_2 under 250 °C for 1 h yields the Cu replicas with low SERS backgrounds and the best morphology in terms of the conformality of replication, giving rise to the best SERS properties including high detection-sensitivity and high signal-reproducibility. Most importantly, the DNA base molecules are detectable (label-free) at a micromolar level on Cu replicas, which is achieved for the first time on Cu SERS substrates and even comparable to the detection-sensitivity for DNA bases on some Ag SERS substrates. The generation of such "cleaner" Cu superstructures has provided a substantial step for the novel butterfly SERS substrates to detect biomolecules, where excitation lasers with longer wavelengths are needed.[11,12] Additionally, as one of the three common SERS-active metals, the ultra-low price of Cu against Au and Ag (~1/5000 and ~1/

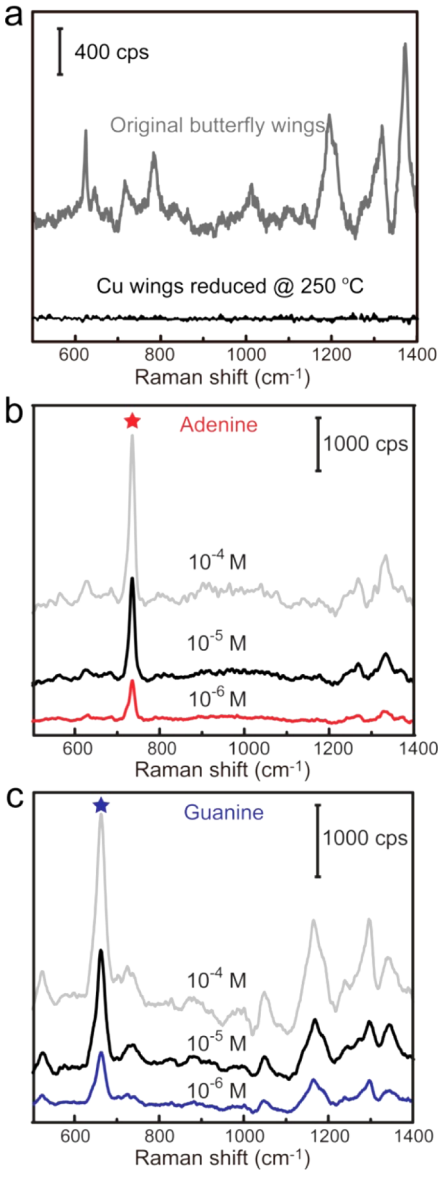


Figure 5. (a) Comparison of Raman signals of the 3D Cu scale replicas (reduced at 250 °C) and the original butterfly wings (785 nm excitation). Note the difference between these results and that shown in Figure 1c. (b, c) SERS spectra of DNA base molecules acquired on these new Cu wings: (b) adenine and (c) guanine. A detection sensitivity down to 1 μM is achievable on these Cu scales, even comparable to that on some Ag substrates.[30−32]

100, respectively) as well as the simple and mature technologies adopted here makes these Cu butterfly scales suitable as routine consumables for a broad range of biolaboratories.

METHODS

Wings of *E. mulciber* were chosen as biotemplates (purchased as specimens from the Shanghai Wild Animal Park). Analytic grade reagents of HNO_3, NaOH, $CuSO_4{\cdot}5H_2O$, and ethanol were purchased from the Shanghai Chemical Company. R6G, adenine, and guanine were purchased from Sigma-Aldrich.

The original butterfly wing were pretreated by dipping in ethanol solution for 0.5 h, and were then subsequently immersed in an 8% HNO_3 aqueous solution and an 8% NaOH solution aqueous solution for 3 h, respectively. Next, the wings were carefully dipped into a closed vessel containing $CuSO_4$ aqueous solution (0.5 g/mL) for 12 h at room temperature, rinsed in deionized water, and dried in air at 40 °C between a pair of clamped silicon slides. The dried wing scales, clamped by the slides, were then moved into a sintering furnace. To ensure the complete decomposition of organic templates and the full crystallization of CuO, we sintered the wing scales in air at 500 °C for 5 h.

To obtain conformal Cu plasmonic structures, we carried out the reduction of CuO scales in H_2 at different temperatures in a controlled atmosphere furnace. During this process, the flow rate of H_2 were kept at 300 mL/min. Different samples were held at 200, 250, and 300 °C for 1 h, respectively.

The synthesized samples were examined using XRD on a Rigaku D-max/2550 X-ray diffractometer system (Cu Kα radiation) operated at 35 kV, 20 mA, with a scan rate of 4° min^{-1} and a step size of 0.05° 2θ. FESEM observations were carried out using a field emission SEM (Quanta 250 from FEI, 20 kV) equipped with EDS analysis capability (Oxford Instruments, 80 mm^2 detector). TEM analyses were conducted on a JEOL JEM-2100F (200 kV) TEM. Optical microscopy was carried out using a stereomicroscope of VHX-1000, Keyence. XPS spectra were recorded on an AXIS UltraDLD system using the Al K_α radiation as X-ray source. Extinction spectra were acquired using a Lambda 950 UV/Vis/NIR spectrophotometer (PerkinElmer).

Raman spectra were acquired using a Renishaw inVia Raman microscope. The Raman signals were collected through a confocal pinhole of 25 μm in diameter by using a dry objective (50×, 0.75 NA). The Cu substrates were placed in a chamber vacuumed by a rotary pump for two days before testing. For R6G detection, the equipment was operated with a 514.5 nm Ar ion laser (beam size ~2 μm). Approximately 2 mW (10% of the laser power) of laser irradiation was used to excite the samples. The signal collection time was 20 s. R6G reagents were dissolved into the ethyl alcohol for detection. The Cu butterfly wing scale replicas as substrates were immersed in the corresponding analyte solutions for 2 h towards a sufficient molecule adsorption, and were dried at room temperature before SERS measurement. For DNA base detection, adenine and guanine were dissolved into the deionized water with different concentrations for SERS detection, respectively. The Cu butterfly wing scale replicas as substrates were immersed in the corresponding analyte solutions for 3 h, respectively. Then the substrates were taken out, rinsed with deionized water, and dried in a vacuum chamber. A 785 nm semiconductor laser with an very low output power of 0.25 mW was adopted to avoid possible damages exerted by the laser irritation. The accumulation time of each spectrum is 40 s.

ASSOCIATED CONTENT

Supporting Information

Extinction spectrum of Cu wing replicas; FESEM images of CuO butterfly wing scales; XRD and EDS results of CuO butterfly wings; TEM images of 3D Cu wing scales reduced under 250 °C in H_2; SERS detections of R6G on Cu replicas prepared under various reduction temperatures; SEM and XRD results of Cu NPs ground from 3D Cu replicas; N_2 adsorption isotherms (77 K) on Cu replicas and Cu nanoparticles. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work was supported by the National Natural Science Foundation of China (Grants 51271116, 51171110), the National Basic Research Program of China (973 Program, Grant 2012CB619600), and the Shanghai Science and Technology Committee (Grant 10JC1407600), and the Cultivation Fund for Excellent Doctoral Thesis of Shanghai Jiao Tong University.

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