ELECTRONIC SUPPLEMENTARY INFORMATION (ESI)

Carbon Dot Reduced Bimetallic Nanoparticles: Size and Surface Plasmon Resonance Tunability for Enhanced Catalytic Applications

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Experimental

Materials and Reagents

All experiments were carried out using Ultrapure Millipore water (18.2 M Ω ·cm). Anhydrous citric acid (791725, 99.5%), silver(I) nitrate (204390, 99.9999%), gold(III) chloride hydrate (254169, 99.999%), sodium hydroxide (306576, 99.99%), sodium borohydride (480886, 99.99%), 4-nitrophenol (241326, \geq 99%), 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS, 11557, 98%), and hydrogen peroxide (216763, 30 wt%) were purchased from Sigma-Aldrich. All chemicals were used as received. Dialysis tubing (132105, Spectra/Por 7, 1 kDa MWCO) was purchased from Spectrum Labs.

Characterization Techniques

Absorbance spectra and fluorescence spectra were collected using a Cary Bio 50 UV-Vis spectrophotometer and a Varian Cary Eclipse Fluorometer, respectively, using PMMA cuvettes. 4-NP catalytic studies and peroxidase activities were conducted in quartz micro-cuvettes (1 mL) and PMMA cuvettes, respectively, monitoring the reaction rates using the same Cary spectrophotometer. Transmission electron microscopy (TEM) studies were conducted on carbon coated copper grids (Ted Pella, Inc. 01822-F, support films, ultrathin carbon type-A, 400 mesh copper grid) using a FEI Tecnai (F30 G2, Twin) microscope operated at a 300 keV accelerating voltage.

Synthesis of Citric Acid-Derived C-Dots

In a typical synthesis, 50 g of solid anhydrous citric acid and a stir bar were added to a round bottom flask. The round bottom was placed into a metal BB bath on a hot plate and the citric acid was thermally decomposed at 250 °C (300 rpm) for 2 h. The resultant dark red product was dissolved in 50 mL of D.I. water and then centrifuged (5000 rpm, 30 min) to remove any larger particles. The supernatant was transferred to a cellulose membrane (1 kDa MWCO) and dialyzed for 48 h against 1.5L of D.I. water. After 24 h, the dialysate was replaced with a fresh 1.5L of D.I. water. Once the dialysis was complete, the dark orange solution was lyophilized and stored in a drawer until needed. A stock solution of the CA-derived C-dots (5 mg/mL) was made for collecting UV-Vis, fluorescence, and TEM.

Au@C-dot NP Synthesis

Initially, a broad range of C-dot to HAuCl₄ ratios were tested for Au reduction at various temperatures (100 °C, 60 °C, 40 °C, and room temperature). While AuNP formation appeared to occur at all tested temperatures and an increase in reaction temperature resulted in a more rapid reduction, the C-dots adequately reduced Au at room temperature over a 1 - 4 h period. Therefore, all subsequent Au reductions were carried out at room temperature. For the Au reductions, a 2 mg/mL sample of C-dots was made and two series of room temperature Au reductions were generated; one in which the Au concentration was kept constant with increasing C-dot additions and another in which the C-dot concentration was kept constant with increasing Au additions. For the constant Au series, the concentration of Au was held at 0.3 mM while increasing amounts of C-dots were added to generate concentrations of 0.05, 0.15, 0.30, 0.45, and 0.60 mg/mL. Conversely, for the other series, the C-dot concentration was held constant (0.30 mg/mL) and the Au concentration was increased (0.10 to 0.60 mM in 0.05 mM increments).

Ag@C-dot NP Synthesis

Similarly to the Au reductions, a broad range of C-dot to AgNO₃ ratios were tested for Ag reduction at various temperatures (100 °C, 90 °C, 70 °C, 50 °C, 30 °C and room temperature). No apparent AgNP formation occured at any of the tested temperatures. Based of related literature¹, the addition of NaOH at concentrations of 1, 10, and 20 mM was tested at all of the above temperatures. While the addition of NaOH promoted the reduction of Ag at all tested temperatures, it was found that temperatures >80 °C and NaOH concentrations of 20 mM resulted in the optimum formation of AgNPs. For all subsequent Ag reductions, the NaOH concentration was kept constant at 20 mM and a reduction temperature of 100 °C was used. To generate the Ag@C-dot NPs, the C-dot solution was heated at 100 °C for 15 min and then various aliquots of Ag were added, heating the samples for an additional 15 min. Again similar to the Au reductions, two series of Ag reductions were generated; one in which the Ag concentration was kept constant with increasing C-dot additions and another in which the C-dot concentration was kept constant with increasing Ag additions. For the constant Ag series, the concentration of Ag was held at 1 mM while increasing amounts of C-dots were added to generate concentrations of 0.05, 0.15, 0.30, 0.45, and 0.60 mg/mL. For the constant C-dot series, the concentration of C-dots was held at 0.3 mg/mL while increasing amounts of 0.15, 0.30, 1.0, 1.5, and 3.0 mM.

Au_xAg_y@C-dot NP Synthesis

First, the C-dot solution (0.43 mg/mL) was heated at 100 °C for 15 min to allow for thermal equilibration. NaOH was then added to HAuCl₄ to produce gold hydroxide, Au(OH)₄⁻. The Au(OH)₄⁻ solution and AgNO₃ were simultaneously added to the hot C-dot solution and allowed to react for 15 min. In all instances, the C-dot concentration and NaOH concentration were held constant at 0.3 mg/mL and 20 mM, respectively. Aliquots of 10 mM Au(OH)₄⁻ and 10 mM AgNO₃ were mixed (totaling 1 mM of metal salt) to generate Au : Ag ratios of 90 : 10, 80 : 20, 70 : 30, 60 : 40, 50 : 50, 40 : 60, 30 : 70, 20 : 80, and 10 : 90. For example, to generate a 50 : 50 Au : Ag sample, 0.5 mL of 10 mM HAuCl₄ was added to 2 mL of 100 mM NaOH which was simultaneously mixed with the

 $0.5 \text{ mL of } 10 \text{ mM AgNO}_3$ into the hot C-dot solution (7 mL of 0.43 mg/mL). In addition, samples containing 100% Au and 100% Ag were also generated following the approaches described above for the mono-metallic NPs.

4-Nitrophenol (4-NP) Catalytic Studies

For the catalytic rate studies, 400 μ L of 0.2 M NaBH₄, 400 μ L of 0.2 mM 4-NP (1000 : 1 NaBH₄: 4-NP), and 20 μ L of the catalysts (Au/Ag @C-dots and Au_xAg_y@C-dots; 0.2 mM metal concentration) were combined in a 1 mL micro-cuvette and UV-Vis spectra were collected every 5 s until the characteristic 4-NP peak at 400 nm disappeared. More specifically, the as-synthesized Ag@C-dot NP and Au_xAg_y@C-dot BMNP samples were diluted 5-fold using D.I. water. Since the Au@C-dot NPs did not contain NaOH and were at a lower overall metal concentration then the other samples, these catalysts were only diluted 1.25-fold using 20 mM NaOH to keep the base (4 mM in MNP solutions; 97.6 μ M in cuvettes) and metal concentration (0.2 mM stocks; 4.9 μ M in cuvettes) similar in all samples. Within these diluted samples, the highest C-dot concentration (0.24 mg/mL) was present within the Au@C-dot NPs due to the smaller dilution factor. Therefore, for the C-dot control studies, a 2 mg/mL stock solution of C-dots was diluted 8.3-fold to generate a 0.24 mg/mL solution and 20 μ L of this solution was used in place of the Au/Ag @C-dot NPs. Prior to use, the 0.2 mM stock solution of 4-NP was degassed for 20 minutes to remove any dissolved gases, minimizing the induction time. In addition, freshly prepared (less than 3 h old) NaBH₄ was used in all catalytic studies.

Peroxidase Activity Studies

For conducting peroxidase activity studies, the desired volume of MNP@C-dot solution (ranging from 15.6 to 500 μ L) plus 100 μ L of 25 mM ABTS were mixed in disposable PMMA cuvettes and diluted with deionized water to bring the total volume to 3.1 mL. To initiate the reaction, a 5 μ L droplet of 30 wt% H₂O₂ was carefully pipetted onto the side of the cuvette and gently mixed by manually inverting the cuvette twice. This procedure resulted in concentrations of 0.805 mM ABTS and 5.4 mM H₂O₂. The peroxidase activity was assessed by monitoring the absorbance at 418 nm over a 45 min period collecting data points every 30 s. A control experiment was carried out using neat C-dots at a concentration of 9.7 μ g mL⁻¹. While no activity was observed on this timescale for unmodified C-dots, Au@C-dots clearly showed pronounced activities which increase with nanocatalyst concentrations given in Fig. S12 denote the C-dot concentrations employed to generate the corresponding Au@C-dots.

SUPPORTING FIGURES

CA-derived Carbon Nanodots



Fig. S1 (A) Photophysical properties of CA-derived C-dots. Akin to other reported dots, these C-dots displayed excitation wavelength-dependent emission. The dots also showed excitation wavelength-dependent quantum yields (inset). (B) Representative TEM image confirming that C-dots, 30–60 nm (up to 100 nm), were in fact produced. (C) FTIR spectrum of the CA-derived C-dots indicating that the C-dots possessed alcohol, carboxyl, epoxy, ester, and ether functional moieties accounting for their high aqueous solubility. The FTIR peaks were assigned as such: stretching vibrations of O–H (v_{O-H} , 3600–3000 cm⁻¹), stretching vibrations of C=O ($v_{C=0}$, 1800–1600 cm⁻¹), skeletal vibrations of aromatic groups (v_{C-C} , 1800–1600 cm⁻¹), stretching vibrations of C-O moieties (v_{C-O-C} and v_{C-OH} , 1300–1000 cm⁻¹) and aliphatic carbon stretches (v_{C-H} and $v_{=C-H}$, 2900, 1400, and 915 cm⁻¹). The outset plot represents an expansion of the area within the boxed region of panel (C).



Fig. S2 UV-Vis spectra of the resultant Au@C-dot NP solutions when the concentration of Au salt was held constant at 0.3 mM and the C-dot concentration increased from 0.05–0.60 mg/mL. As the C-dot concentration increased up to 0.30 mg/mL, the AuNP SPR appeared and hypsochromically shifted. For the three highest concentrations of C-dots, the AuNP SPR remained relatively constant in intensity and peak position.



Fig. S3 Representative TEM images of the resultant Au@C-dot NPs when the C-dot concentration was held at 0.3 mg/mL and the HAuCl₄ concentration was (A) 0.20 mM, (B) 0.35 mM, (C) 0.45 mM, and (D) 0.60 mM. The following C-dot : Au ratios resulted in average quasi-spherical particle sizes of (A) 17.8 ± 6.4 , (B) 26.7 ± 10.0 , (C) 47.8 ± 20.7 , and (D) 96.7 ± 29.9 nm. For the higher Au : C-dot ratios, large hexagonal and trigonal Au plates formed with average particle sizes of 266.7 ± 99.3 and 528.8 ± 214.3 nm for the 0.45 and 0.60 mM Au concentrations, respectively. The higher Au : C-dot ratios also produced much smaller quasi-spherical particles in the 5–20 nm regime as evidenced by the insets of (C) and (D) but these particles were less concentrated than the larger quasi-spherical particles and platelets.



Fig. S4 (A) Time-dependent UV-Vis spectra of the room temperature Au reduction (0.3 mg/mL C-dots : 0.35 mM HAuCl₄) showing that after 3 h the reduction was near completion. (B) Time-dependent fluorescence spectra ($\lambda_{ex} = 350$ nm) of the room temperature Au reduction (0.3 mg/mL C-dots : 0.35 mM HAuCl₄). After the reduction, the Au@C-dot NPs still retained approximately 25% of the original C-dot fluorescence. Since HAuCl₄ did not show any absorbance or fluorescence features within the wavelength range of interest, the increase in absorbance and decrease in fluorescence intensity was solely attributed to the growth of AuNPs.



Fig. S5 UV-Vis spectra of the resultant Ag@C-dot NP solutions when the concentration of Ag salt was held constant at 1.0 mM and the C-dot concentration increased from 0.05 - 0.60 mg/mL. In general, as the C-dot concentration increased, the AgNP SPR displayed a slight bathochromic shift. Interestingly, at the lowest concentration of C-dots (0.05 mg/mL), a broad shoulder centered at approximately 535 nm appeared in addition to the SPR at 396 nm which is indicative of larger Ag nanostructures with high polydispersity. The origin of this shoulder is currently under exploration.



Fig. S6 Representative TEM images of the resultant Ag@C-dot NPs when the C-dot concentration was held at 0.3 mg/mL and the AgNO₃ concentration was (A) 0.30 mM, (B) 1.00 mM, and (C) 3.00 mM. The following C-dot : Ag ratios resulted in average quasi-spherical particle sizes of (A) 11.1 ± 9.9 , (B) 6.9 ± 3.4 , and (C) 6.0 ± 4.0 nm.



Fig. S7 Au@C-dot NP SPR *versus* the concentration of Au salt added. For these samples, varying concentrations (0.125 - 1.0 mM) of HAuCl₄ were added to a 100 °C solution of C-dots (0.3 mg/mL) and were heated for an additional 15 min. Similarly to the room temperature reduction of Au using C-dots, the 100 °C reduction resulted in a bathochromic shift of the SPR with increasing Au concentration, indicative of the formation of larger AuNPs and further highlighting the SPR tunability even when using elevated temperatures.



Fig. S8 UV-Vis absorbance of a 50 : 50 ratio of Au : Ag heated at 100 °C in the absence of C-dots providing further evidence that the C-dots acted as reducing agents towards Au_xAg_y NP formation.



Fig. S9 Representative TEM images of the (A) $Au_{0.9}Ag_{0.1}@C-dot$, (B) $Au_{0.7}Ag_{0.3}@C-dot$, (C) $Au_{0.3}Ag_{0.7}@C-dot$, and (D) $Au_{0.1}Ag_{0.9}@C-dot$ BMNPs. The size of the BMNPs decreased slightly with increasing Ag content. Size analysis resulted in average particle sizes of 4.1 ± 1.4, 3.4 ± 0.9, 2.4 ± 0.8, and 1.9 ± 0.7 nm for the $Au_{0.9}Ag_{0.1}@C-dot$, $Au_{0.7}Ag_{0.3}@C-dot$, $Au_{0.7}Ag_{0.9}@C-dot$, $Au_{0.1}Ag_{0.9}@C-dot$ BMNPs, respectively.



Fig. S10 Time-dependent UV-Vis absorption spectra of the NaBH₄-assisted reduction of 4-NP catalyzed by (A) $Au_{0.5}Ag_{0.5}@C-$ dot NPs and (B) Ag@C-dot NPs. (C) Plots of $ln(A_t/A_0)$ of 4-NP absorbance at 400 nm *versus* time for various 'aged' MNP@C- dot catalysts.



Fig. S11 UV-Vis absorption spectra of freshly prepared and 'aged' (for 5 months in a lab drawer) MNP@C-dot NPs, highlighting the stability of the (A) Au@C-dot, (B) $Au_{0.5}Ag_{0.5}$ @C-dot, and (C) Ag@C-dot NPs. Based on their UV-Vis profiles, the Au@C-dot NPs remained unchanged after 5 months while the 'aged' $Au_{0.5}Ag_{0.5}$ @C-dot and Ag@C-dot NPs displayed an increased absorbance at longer wavelengths, an effect likely originating in some small degree of particle aggregation. We note that this change did not adversely affect the catalytic properties of these colloids.



Fig. S12 The peroxidase activity of different concentrations of Au@C-dots followed by monitoring the absorbance change of ABTS at 418 nm. Results of a control experiment using 9.7 μ g mL⁻¹ unmodified C-dots alone are provided by the black symbols. While no activity was observed on this timescale for 'naked' C-dots, Au@C-dots clearly show activities which increase with nanocatalyst concentration. The concentrations of ABTS and H₂O₂ were held constant at 0.805 mM and 5.4 mM, respectively. The reported concentrations denote the C-dot concentrations employed to generate the Au@C-dots.

References

1 L.-M. Shen, M.-L. Chen, L.-L. Hu, X.-W. Chen and J.-H. Wang, Langmuir, 2013, 29, 16135-16140.