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RPPR Final Report
as of 21-Nov-2018

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Major Goals: The central objective of the proposed research was to demonstrate that plasmon resonating nanostructures can resonantly transfer charge carriers (electrons or holes) to biological components to drive redox reactions. The central objective will be achieved through the specific project aims listed below:
Aim 1: Synthesize and characterize metal-semiconductor core-shell structures composed of Ag/Au alloy cores and TiO2 or CuO2 shells. Solution-based approaches will be used to synthesize Ag:Au alloy nanoparticles with ~30 nm diameter and varied compositions from 100% Au to 100% Ag. This will allow a tuning of the plasmon resonance wavelength from 400-600 nm. Thin semiconducting TiO2 (n-type) and CuO2 (p-type) shells will be grown on top of the metal cores to control the transfer of electrons or holes to biological components. Pinhole free and porous semiconductor shells will be synthesized to allow for half reactions or complete redox reactions. The structures will be characterized using electro microscopy and optical spectrophotometry.
Aim 2: Demonstrate resonant control of cytochrome c oxidation and reduction mediated by the photoexcitation of plasmonic-semiconducting nanostructures. Photoexcitation wavelength dependent cytochrome c oxidation and reduction reactions will be executed. The plasmonic metal core composition will be varied, thus varying the plasmon resonance wavelength, to show the ability to create digital on/off switches for charge transfer. TiO2 coated metal will be used in cytochrome c reduction and CuO2 coated metal will be used for cytochrome c oxidation. Porous semiconductors coupled with appropriate hole/electron scavengers will be examined to study complete redox processes. Ag coated with TiO2 and Au coated with CuO2 will be explored simultaneously to demonstrate the independent control of cytochrome c oxidation and reduction in a cyclic manner in the same pot.
Aim 3: Demonstrate digital control of reductive and oxidative biocatalysis with cytochrome C mediated reactions. Photoexcitation wavelength dependent NADPH oxidation and O2 reduction will be executed. Oxidized cytochrome C species will be generated by photoexcitation of CuO2 coated metal nanoparticles, in turn the oxidized cytochrome C will drive the biocatalytic reaction of NADPH oxidation with the enzyme cytochrome C reductase. Separately, TiO2 coated metal nanoparticles will be used to generate reduced cytochrome C to drive O2 reduction with the enzyme cytochrome C oxidase. Reaction kinetics will be monitored by UV-Vis spectroscopy of cytochrome C and NADPH. Reaction conditions will be optimized by varying enzyme, cytochrome C, and nanoparticle concentrations.

Accomplishments: Note: Figures mentioned here are shown in the uploaded PDF.

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The broad goal in this proposal was to synthesize core-shell nanostructures consisting of a plasmonic Ag/Au core and a semiconducting shell. The core was meant to serve as a digital on/off switch based on the wavelength of photon excitation to trigger the transfer of electrons or holes through an n- or p-type semiconducting shell, respectively. It was proposed that this wavelength specific actuation of electron and hole localization on the semiconductor could be used to controllably actually redox enzymatic processes.

We successfully completed aim 1 via the controlled synthesis of core-shell nanostructures with Au cores (with tunable Ag-Au composition ratio) and shells with controllable thickness of TiO2 (n-type), Cu2O (p-type) ¬and NiO (p-type). During execution of aim 2 issues with the experimental design were identified. First, it was found that the p-type semiconductor shells were unstable under illumination, causing dynamic changes in the particles optical properties. Furthermore, the interaction of cytochrome C and the oxide shells in the dark induced charge transfer and caused oxidation of reduced cytochrome C and reduction of the oxidized Cu2O shells prior to photon activation. We tried to address this by using NiO and also by using thin SiO2 shells around the semiconducting oxides, but found significant stability issues. Based on these findings we used the synthetic protocols developed here to demonstrate controlled promotion of a heterogeneous catalytic processes, rather than digital control of the enzymatic process. While the result was not as exciting, the high-risk high-reward project yielded the necessary synthetic strategies to demonstrate important results in the field of plasmon-mediated catalysis.

To synthesize the core shell particles, we started by synthesizing Au nanoparticles of ~12 nm diameter using standard citrate reduction of HAuCl4 (chloroauric acid), which we previously developed. To deposit p-type semiconducting Cu2O shells around Au, which are meant to conduct holes to the liquid solid interface to drive cytochrome C oxidation, a 10 mL aqueous suspension of Au nanoparticles (as synthesized) was mixed with 3.2 mg/mL of polyvinylpyrrolidone (PVP), 0.25 mL of 0.1M CuCl2, 0.1 mL of 1 M NaOH, and 1 mL of 0.1 M Ascorbic Acid. These species acted to cap Au, nucleate Cu2O and continuously oxidize the shell. These procedure was optimized through analysis of the influence of reactant concentrations, addition order and addition time. Following mixture of the solution for ~10 minutes the reaction was quenched using an ice bath and the particles were centrifuged from the reaction mixture. The resulting structures when starting with ~ 10 nM Au solutions formed thin, 2 nm Cu2O shells as shown in Figure 1A. To increase the Cu2O shell thickness, the amount of Au nanoparticle seeds was decreased to ~ 3.75 mM, resulting in shells of 10 nm thickness (the reasoning for doing this will be discussed further below), see for example Figure 1B. We developed similar synthetic approaches for depositing controlled thickness NiO and TiO2 shells on Au nanoparticle cores with controlled thicknesses. Examples of the resulting structures are shown in Figure 2 and 3. Based on these results we successfully completed all of Aim 1 in the proposed research.

The next aim was to demonstrate that the synthesized nanostructures could function as digital (wavelength selective) on/off switches for the transfer of electrons or holes (chosen by p- or n-type semiconductor shells) to biological redox active enzymes. As a first test of this idea we examined the ability of these structures to drive the oxidation or reduction of cytochrome C, which can be followed using spectrophotometry based on the characteristic changes of absorption bands between 500-600 nm, see Figure 4A. Initial experiments were performed using a small 1 mL volume glass vial, with a 50 mM phosphate buffer, 0.1 mg/mL of reduced cytochrome C, 2.5 nM of Au@Cu2O (this was optimized over a few trials) and illumination via 20 mW of monochromated 523 nm light (this matched the LSPR peak of Au@Cu2O). The initial trials focused on the thin Cu2O shells (Figure 1A).

Initial trials of photo-exciting Au@Cu2O to provide holes to oxidized reduced cytochrome C proved successful. For example, Figure 4A shows the transformation of the absorption feature at ~550 nm from characteristics of the reduced form of cytochrome C to the oxidized form during illumination. The oxidation of cytochrome C driven by illumination followed first order kinetics in the short time limit, Figure 4B, and it was observed that the rate of conversion increased with increasing catalyst loadings. While the initial experiments were promising, some issues were also observed in the analysis including: (1) a steadily decreasing baseline in the UV-vis spectra as a function of illumination time and (2) onset of cytochrome C oxidation in the dark, once mixed with the catalyst.
To probe these issues further, control experiments were performed to examine the stability of the Au@Cu2O structures. An identical experiment as in Figure 4B, except in the absence of cytochrome C was performed, where it was observed that illumination of the catalyst alone caused a decrease in the intensity of the Au plasmon resonance and a red shift in the peak position, which together suggest that the Cu2O shell was reducing in response to illumination, see Figure 5A. We attempted to address this issue by growing thicker Cu2O shells (Figure 1B), however a similar phenomenon was observed (not shown here), suggesting that the proposed structures for digital control of oxidation reactions were not stable under reaction conditions. Furthermore, control experiments were executed to examine the potential for reactions between cytochrome C and the Au@Cu2O structures in the

dark. Control experiments with mixture of reduced cytochrome C and bare Au nanoparticles showed no evidence of reactions, Figure 5B. However, a mixture of Au@Cu2O and cytochrome C that was left in the dark for 15 minutes showed significant reduction of cytochrome C, suggesting that reactions can also occur in the dark.
We attempted to further address these issues by trying a different p-type semiconducting shell (NiO) as well as an n-type shell (TiO2) to drive cytochrome C reduction. Unfortunately, we saw similar issues of stability under illumination in the case of NiO shells and reactivity under dark conditions in the case of TiO2 shells (data not shown here). As a final mitigation strategy for these issues we attempted to wrap the Au@Cu2O structures with a very thin layer of SiO2 to stabilize the materials, minimize reactivity in the dark, but still enable charge transfer to the redox active biological molecules. This approach also failed as we either deposited so little SiO2 that we did not address issues with the native structure or deposited so much SiO2 that we rendered the structures inactive. While there are other approaches that could be used to achieve the proposed objectives, the very short time line of this project made further analysis infeasible.

We used the developed synthetic protocols to demonstrate plasmon-mediated control over a heterogeneously catalyzed reaction, rather than a biological redox process. Structures were created with plasmonic Ag cores of varying diameter (12, 25, 50, 100 nm), SiO2 shells with constant 10 nm thickness and 5 nm Pt nanoparticles deposited on the shells, see Figure 6A and C-G. The influence of Ag core size on the photocatalytic reactivity of the exposed Pt particles was analyzed where it was identified that an optimum Ag nanoparticle core size exists that maximized field localization at the active Pt surface and minimized deleterious photon loss mechanisms, Figure 6B. This is an important finding for the design of optimal plasmon mediated photocatalysts.

Training Opportunities: This was a short term, 9 month, project that funded a single post doctoral researcher. During this period the postdoc was trained in the synthesis of core-shell nanostructures, structural and optical characterization of the materials and analysis of the stability of the materials. Furthermore, the postdoc was trained in making kinetic measurements of photon driven oxidation and reduction of cytochrome C as a function of catalyst loading, illumination characteristics and analysis of the mechanism of reactivity.

Results Dissemination: The results from this work were published in Nano Letters in 2017. Furthermore, talks at national conferences (American Chemical Soceity) and Gordon Research Conferences (Nanophotonics and Plasmonics) highlighted work and funding from this research.

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Technology Transfer: Nothing to Report

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Person Months Worked: 1.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

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Other Collaborators:

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Participant Type: Postdoctoral (scholar, fellow or other postdoctoral position)
Participant: Kun Li
Person Months Worked: 9.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

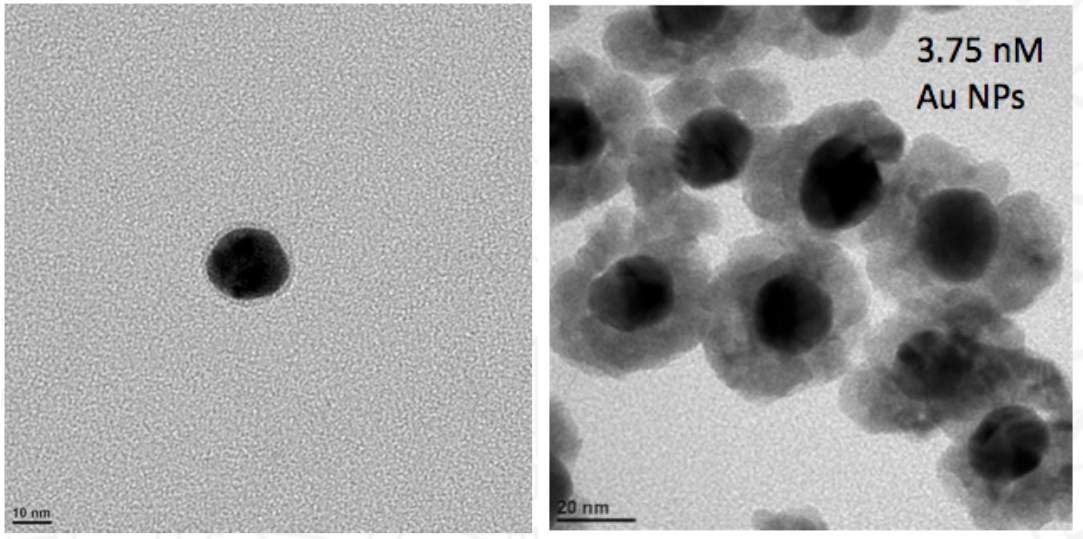

Figure 1. TEM images of ~15 nm diameter Au nanoparticles wrapped in ~ 2 nm thick and ~10 nm thick Cu_2O shells. The "darker" core is Au and the "lighter" shell is Cu_2O.

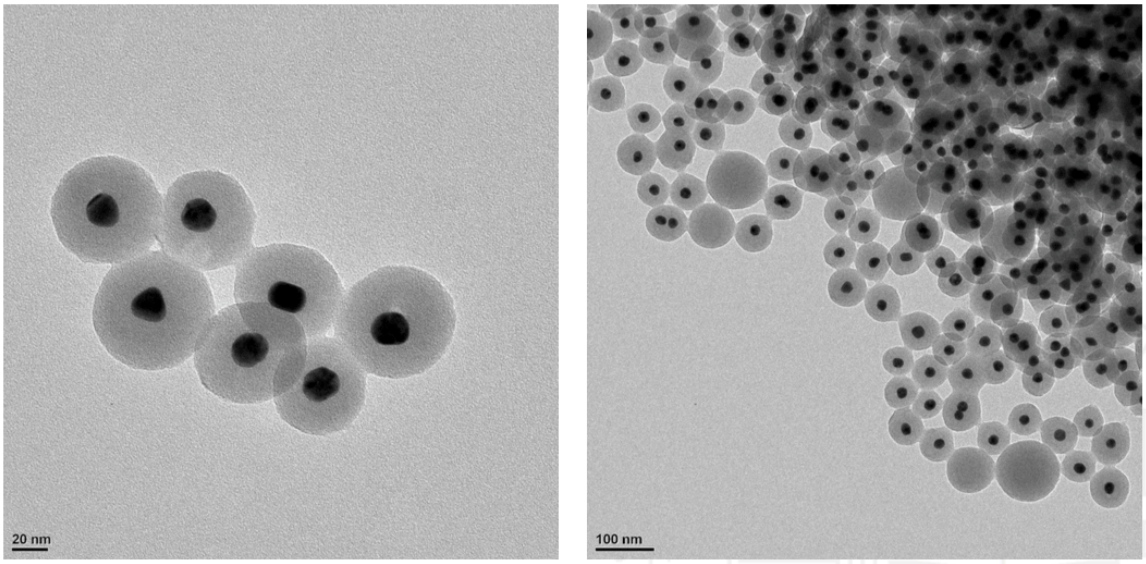

Figure 2. Figure 2. TEM images of ~15 nm diameter Au nanoparticles wrapped in ~ 10 nm thick NiO shells. The "darker" core is Au and the "lighter" shell is NiO. In a majority of the cases the shell only encapsulates a single Au particle.

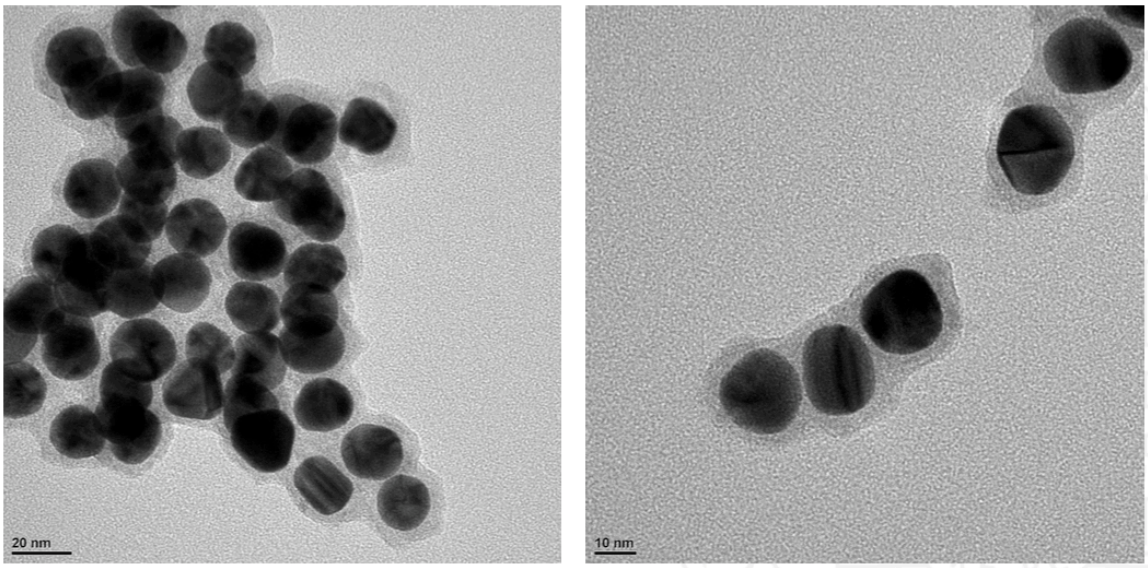

Figure 3. TEM images of ~15 nm diameter Au nanoparticles wrapped in ~ 3 nm thick TiO_2 shells. The "darker" core is Au and the "lighter" shell is TiO_2. In a majority of the cases the shells .

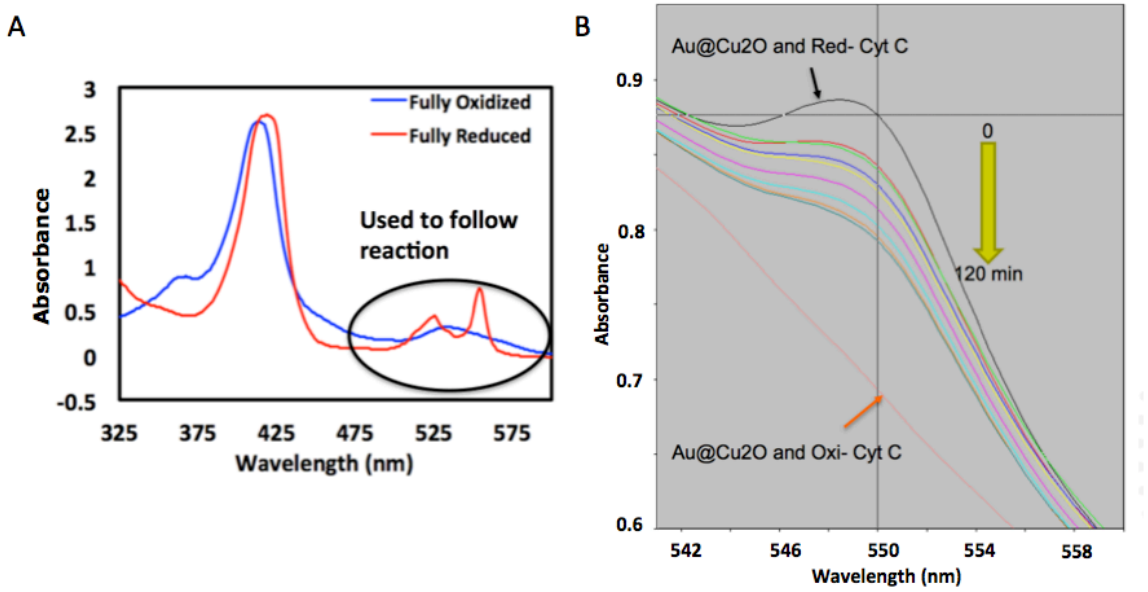

Figure 4. (A) UV-Vis absorbance spectra of fully oxidized and fully reduced cytochrome c. The features at 500-600 nm that are distinct between the two states will be used to follow the oxidation or reduction of this species. (B) UV-Vis absorbance spectra in the 540-560 nm region during an experiment where Au@Cu_2O particles shown in Figure 1(A) were mixed with cytochrome c and exposed to monochromatic 532 nm illumination (in resonance with the Au plasmon). The spectra are shown as a function of illumination time where simultaneous decrease in the feature at 550 nm and decrease in the baseline intensity were observed.

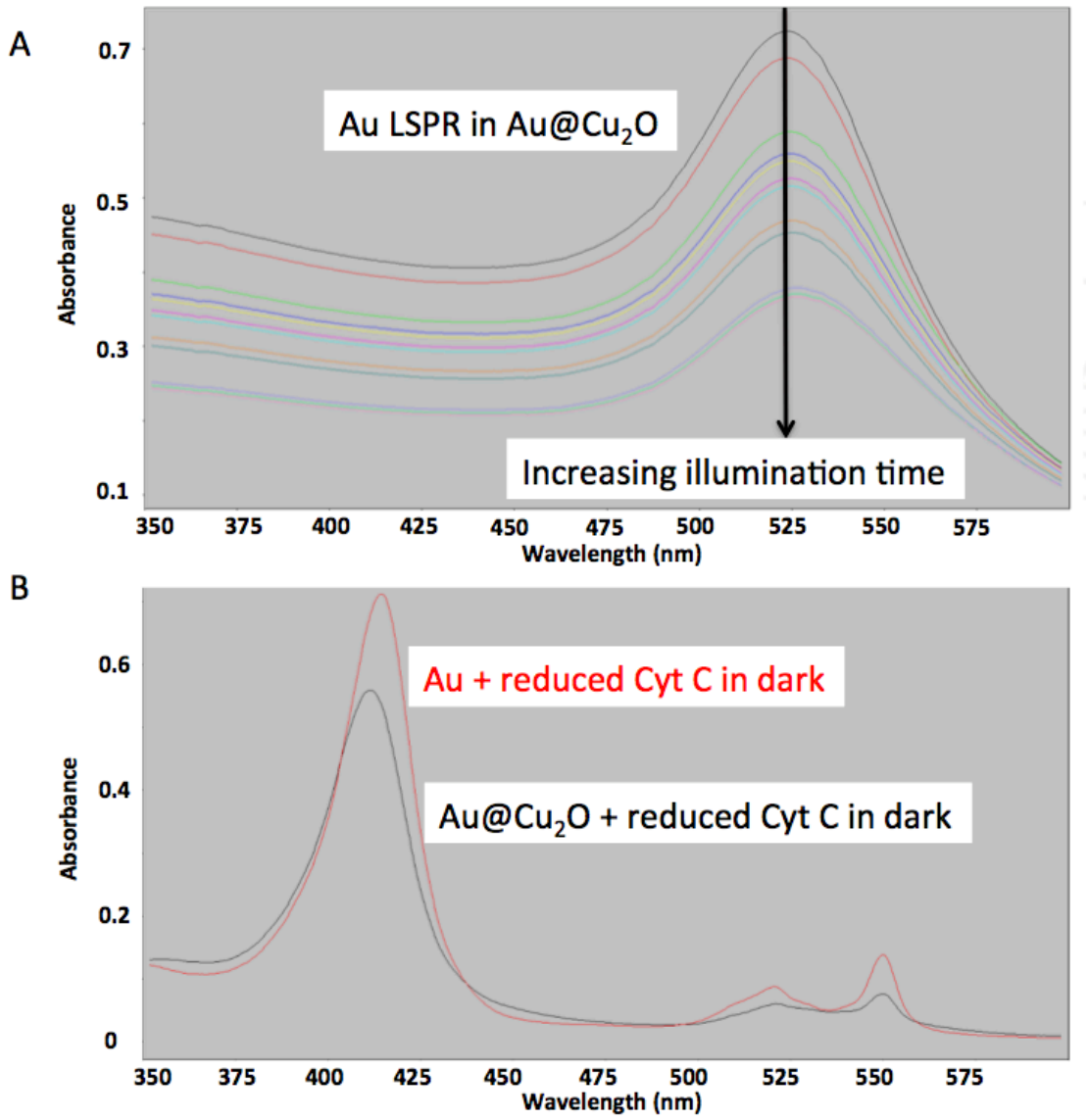

Figure 5. (A) UV-Vis absorbance spectra of Au@Cu_2O nanoparticles with a 2 nm thick Cu_2O shell in the solution used for cytochrome C reactivity measurements, but without cytochrome C. The measurements were collected as a function of time during illumination by 10 mW of monochromatic 532 nm light, where is it seen that the Au localized surface plasmon resonance (LSPR) peak decreased in intensity and shifted as a function of time, showing degradation of the particles. (B) UV-Vis absorbance spectra for mixtures of Au or Au@Cu_2O with reduced cytochrome c (Cyt C) after 15 minutes in the dark, where it is clearly seen from the peaks between 500-600 nm that the Au@Cu_2O particles can induce oxidation of cytochrome C, even in the dark.

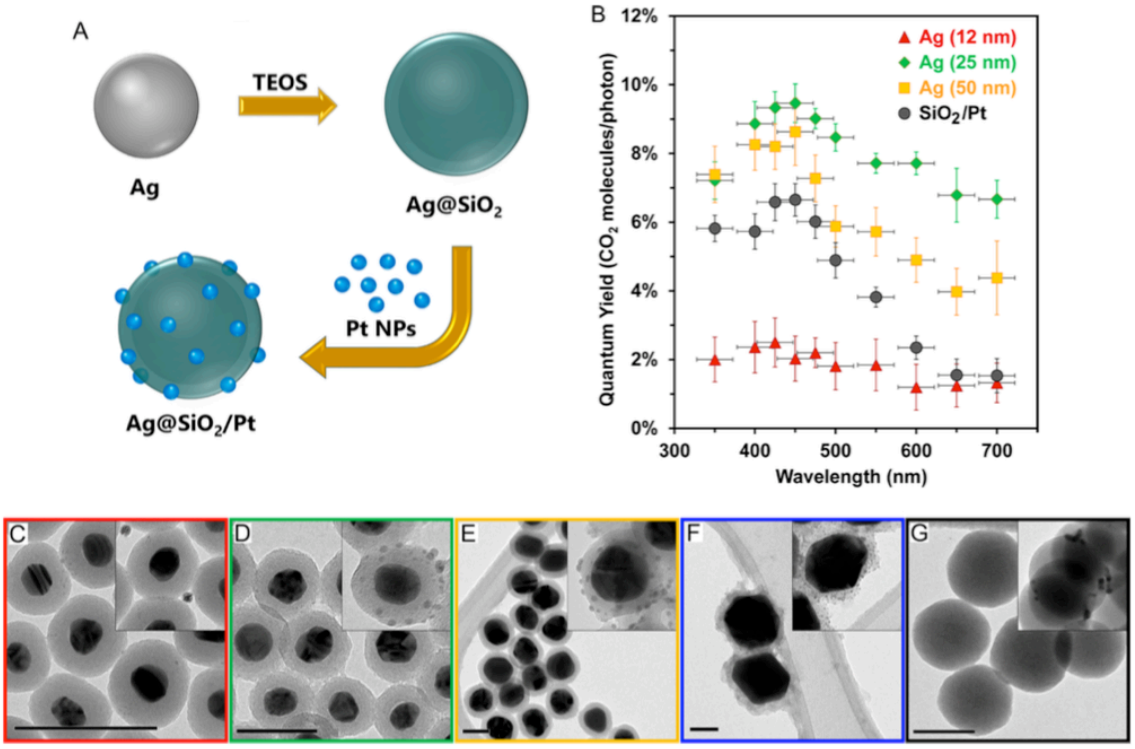

Figure 6. (A) Synthesis scheme for the Pt decorated core-shell Ag@SiO_2 particles. (B) Quantum yield for CO_2 production as a function of wavelength for core-shell structures with varying Ag particle size and constant SiO_2 thickness and Pt particle loading. The results demonstrate the clear ability of Ag localized surface plasmon resonance to promote photocatalysis by nearby Pt particles. (C)-(G) TEM images of Ag@SiO_2/Pt particles with 12 nm (C), 25 nm (D), 50 nm (E) and100 nm (F) Ag cores and constant 10 nm thick shells. (G) shows a TEM image of the control sample with Pt deposited on 50 nm SiO_2 particles.