

Supporting Information

Reduced graphene-tungsten trioxide-based hybrid materials with peroxidase-like activity

Chenbo Dong,^{a#} Alixandra Wagner,^{a#} Valentina Dinca,^b and Cerasela Zoica Dinu^{a*}

^aDepartment of Chemical and Biomedical Engineering, West Virginia University,
Morgantown, WV, 26506

^bNational Institute for Lasers, Plasma and Radiation Physics, 409 Atomistilor Street,
Magurele, 077125, Romania

#The authors have contributed equally to this publication.

***Corresponding author:**

Cerasela Zoica Dinu, Ph.D.

Department of Chemical and Biomedical Engineering

West Virginia University

Benjamin M. Statler College of Engineering and Mineral Resources

PO Box 6102

Morgantown, WV, 26506, USA

E-mail: cerasela-zoica.dinu@mail.wvu.edu

Tel.: +1 304 293 9338

Fax: +1 304 293 4139

Experimental Section

Materials Preparation

Graphene oxide (GO) was synthesized using the established Hummer method and natural graphite powders.¹⁻² Specifically, 1g graphite powder (Acros Organics), 0.5 g sodium nitrate (Acros Organics, 99%) and 25 mL concentrated sulphuric acid (96.4%, Fisher Scientific) were mixed together into a 250 mL flask and placed in an ice bath. Subsequently, 3 g of potassium permanganate powder (Fisher Scientific) was slowly added into the mixture, under continuous stirring and cooling over a bath of fresh ice for 2 h. After time elapsed, the mixture was transferred to a water bath with a temperature of about 35°C and stirred again for 30 min. Next, 46 mL of deionized water (Di water) were added slowly and the flask was placed at 98°C for 15 min. Lastly, 140 mL of Di water followed by 10 mL of 30% hydrogen peroxide (H₂O₂; Fisher Scientific) aqueous solution were also added. The product was isolated by centrifugation, washed several times with 5 % hydrogen chloride (HCl) solution (diluted from 37.3%, Fisher Scientific), Di water and vacuum dried.

To create hybrids, photodeposition and mechanical mixing were used. Briefly, 500 mg of tungsten trioxide nanoparticles (WO₃; 99%, Sky Spring Nanomaterials Inc) were first decorated with Pt using a photodeposition method, visible light, a 500 W lamp and chloroplatinic acid (MP Biomedicals, LLC) in methanol solution (5 mL methanol, 99 % in 95 mL Di water). The weight of Pt was chosen to lead to 2 wt %.³ Subsequently, 10 mg of the previously synthesized GO were added to the solution and the mixture was stirred overnight and at room temperature. Upon time elapsed, the mixture was filtered and washed with 1M HCl solution containing 1% H₂O₂ in Di water.⁴ The resulting wet powder containing Pt-WO₃-GO was suspended in a solution containing 95 ml Di water and 5 ml methanol (99%, Fisher Scientific) and irradiated using a 500 W lamp for a 2 h period to produce reduced Pt-WO₃-r-GO.

Materials Characterization

Surface morphology of the samples (r-GO, WO₃, Pt-WO₃ and Pt-WO₃-r-GO) was investigated using a Hitachi S-4700 Field Emission Scanning Electron Microscope (SEM, Hitachi High-Technologies Corporation). For the analyses, dry individual powders were mounted on carbon tape and their surface morphology was examined at 5.0 kV. For such analysis, samples were also sputter coated for 10 s in vacuum injected with argon using a gold/palladium target. The argon atoms were ionized and collided with the gold/palladium target, causing the metal ions to deposit on the sample in a thin conductive layer of about 3 nm as calculated using the equation $d=KIVt$, where d is thickness, k is a constant value of 0.17, I is plasma current, V is voltage, and t is the time.

Samples were also characterized by Transmission Electron Microscope (TEM). For this, individual powders were dispersed in isopropanol (Sigma, 99.5%), dropped onto a grid (Ted Pella Inc.), dried overnight, at room temperature and subsequently analyzed on a JEM-2100F TEM.

Raman spectroscopy (Renishaw InVia Raman Spectrometer, CL532-100, 100 mW) was used to evaluate chemical characteristics of the individual samples. Briefly, samples were deposited on glass slides (Fisher, 25x75x1 mm) and excited through a 20X microscope objective using an Argon ion (Ar⁺) laser beam with a spot size of <0.01 mm² operating at 514.5

nm. Scans were performed in the 100 to 3200 cm^{-1} ranges, under a low laser energy (i.e., <0.5 mV) and short exposure times (10 s) chosen to prevent unexpected heating effects.

The absorption spectra for the samples were obtained in the 200–800 nm range using a Shimadzu UV-Vis Spectrophotometer (UV-2600; Shimadzu Scientific Instruments) with the powder sample holder for integrating sphere attachment. Specifically, each of the samples was placed in a thin layer in the powder sample loading space and inserted in the respective powder sample holder. Absorbance was read at an interval of 1 nm from 200 to 800 nm. Three different areas were considered for each sample being analyzed with such areas being averaged to form the final spectrum. Barium sulfate was used as the reference materials to create the baseline.

Nanozyme Evaluation

Synthesized Pt-WO₃-r-GO hybrids were dispersed in a solution of reagent 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS; 250 $\mu\text{g}/\text{mL}$, Sigma Aldrich) to a final concentration of 2 mg/mL. The mixture was stirred continuously for 30 min and subsequently exposed to a visible light lamp (500 W) at room temperature for 360 min, while under shaking. At given time intervals (10, 30, 60, 180, or 360 min respectively), 20 mL of the ABTS solution containing hybrids was isolated and centrifuged for 1 min at 5000 rpm. Solution's supernatant was removed and monitored on an Evolution 300 UV-VIS spectrophotometer operating at 412 nm. A blank group (250 $\mu\text{g}/\text{mL}$ ABTS solution without hybrids however kept in the same conditions of irradiation) was used as control for each of the time points being recorded. The ABTS oxidation concentration was obtained by converting the absorption data using the Beer–Lambert law.⁵

Reactive Oxygen Species Detection

The reactive oxygen species generation was determined by a spectroscopical assay. Briefly, the absorbance spectrum of Pt-WO₃-r-GO hybrids (2 mg/mL) dispersed in Di water and irradiated under a visible light lamp (500 W) was recorded in the scan range of 190 to 400 nm and at different time points, with blanks of Di water. Reactive oxygen species generation was quantified by converting the absorption data of series diluted hydrogen peroxide (H₂O₂) using the Beer–Lambert law.

Statistical Analysis

The tests were repeated 3 times and the analysis are expressed as mean \pm standard deviation.

Additional Results Section

Pt-WO₃-r-GO showed an increase in adsorption in the visible region (Figure 1). Specifically, the effect was significant (*p<0.05) for the data in the visible range (400-700 nm). This could be presumably due to the improved photoactivity of the hybrid as resulted from the incorporation of r-GO. In particular, previous research has showed that r-GO could increase the electron transfer from the conduction band of WO₃ to contribute to enhanced photoactivity.⁶

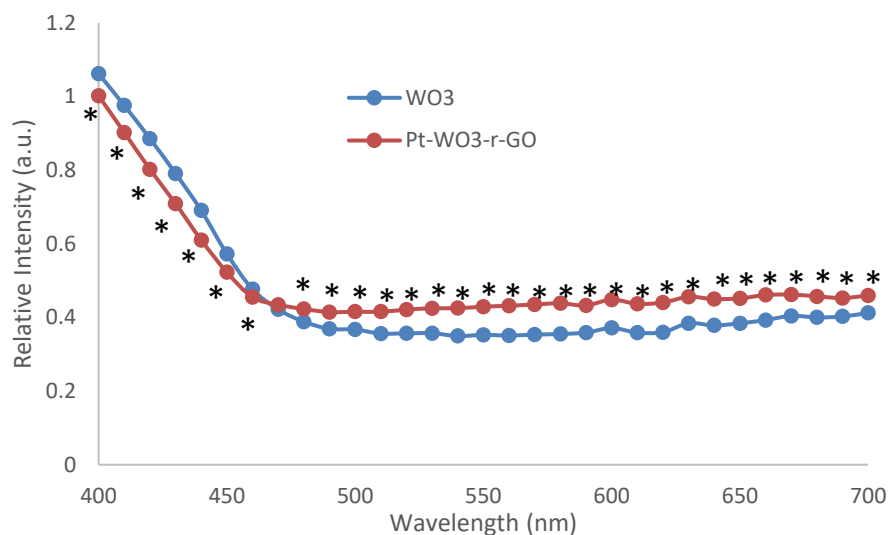


Figure 1: Absorbance of WO₃ and Pt-WO₃-r-GO in the visible range (400-700 nm) (*p<0.05) (n=3).

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