Supporting Information

Silver Nanocoral Structures on Electrodes. A Suitable Platform for Protein-Based Bioelectronic Devices

Jiu-Ju Feng,[†] *Peter Hildebrandt*[†] *and Daniel H. Murgida*[‡]*

[†]Institut für Chemie, Technische Universität Berlin, Str. des 17. Juni 135, Sekr. PC14, D10623-Berlin, Germany. [‡]Departamento de Química Inorgánica, Analítica y Química Física / INQUIMAE-CONICET, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires. Ciudad Universitaria, Pab. 2, piso 1, C1428EHA – Buenos Aires, Argentina.

*CORRESPONDING AUTHOR. E-mail: dhmurgida@qi.fcen.uba.ar. Fax: +54 (11) 4576-3341

Experimental Details

Reagents. Horse heart cytochrome *c* (MW 13,000, Sigma) was purified to remove deamidated forms of the protein by passing it through a cation exchange column (CM-52, carboxymethyl-cellulose, Whatman). 6-Mercaptohexanoic acid (MHA) was purchased from DOJINDO Laboratories. Hydrogen peroxide (H_2O_2, 30% (w/v)) solution and sodium dodecyl sulfate (SDS) was purchased from Sigma and used without further purification. Solutions were prepared with type I ultrapure water from a Direct-Q3 Millipore system (18.2 MΩ cm resistivity; <10 ppb TOC).

Preparation of Ag nanocoral films. The graphite working electrodes were thoroughly polished with 1.0, 0.3 and 0.05 μm alumina slurry, respectively, rinsed thoroughly with distilled water between each polishing step, then sonicated in acetone, ethanol and water successively and allowed to dry at room temperature. Electrodeposition of Ag was performed potentiostatically at -0.5 V (*vs.*Ag/AgCl) for 240s from solution containing 5.0 mM $AgNO_3$ and 0.1 wt.% SDS in the presence of 0.1 M KNO_3 at room temperature. SDS was removed from the electrode surface by washing with ethanol and water thoroughly. After being dried at room temperature, the electrode was immersed into 1.0 mM MHA in ethanol solution overnight to allow for the formation of a self-assembled monolayer. After thoroughly washing with ethanol and deionized water, the SAM-coated electrodes were incubated in 0.4 μM Cyt-c solution (in 10 mM PBS, pH 7.0) for 40 min.

Instrumental methods. The morphologies of coral-like-Ag were characterized with a LEO-1530VP field-emission scanning electron microscope. X-ray diffraction patterns were recorded with a Philips PW 1830 diffractometer, using a monochromatized X-ray beam with nickel-filtered Cu K_α radiation. Resonance Raman (RR) and surface-enhanced resonance Raman (SERR) spectra were measured at ambient temperature with 413.138 nm excitation (cw krypton ion laser; Coherent Innova 302) using a confocal Raman microscope (Horiba Jobin Yvon XY800) equipped with a liquid nitrogen cooled CCD detector. The spectral resolution was ca. 2 cm^{-1}. The working electrode, a graphite disk of ca. 0.9 cm^2, was placed at the bottom of a Delrin-machined electrochemical cell which includes a Pt wire and an Ag/AgCl (3 M KCl) electrode as counter and reference electrodes, respectively. The geometric area of the working electrode was controlled by a Viton O-ring and determined to be 0.40 cm^{-2}. The real area of the working electrode was ca. 1.78 cm^2, as determined electrochemically in a 0.1 M KCl solution containing 2.0 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (molar ratio 1:1). For SERR measurements, the laser beam was focused on the surface of a disk carbon working electrode (ca. 0.90 cm^2) by means of a long working distance objective (20×; N.A.0.35). The electrochemical cell body was mounted on an eccentrically rotating device such that the electrode surface is continuously moved with respect to the laser beam. Measurements were performed using laser powers at sample of ca. 0.5 mW and

accumulation times of 30 s. For all SERRS experiments, the electrochemical cell was filled with 10 mM PBS buffer (pH 7.0). RR measurements in solution were performed under similar conditions but placing the cytochrome c solution in a quartz rotating cuvette. Full reduction of the protein in solution was achieved by addition of sodium dithionite.

Electrochemical measurements were carried out at room temperature using a CH Instrument Electrochemical Analyzer 618B. The electrochemical cell was as described above for SERR experiments. Electrochemical impedance experiments were performed in 0.10 M KNO_3 containing 5.0 mM $Fe(CN)_6^{3-/4-}$ (1:1) and using an alternating current voltage of 5.0 mV. The impedance measurements were recorded at an open circuit potential of 216 mV within the frequency range of 10^{-2}-10^5 Hz.

Supporting Figures

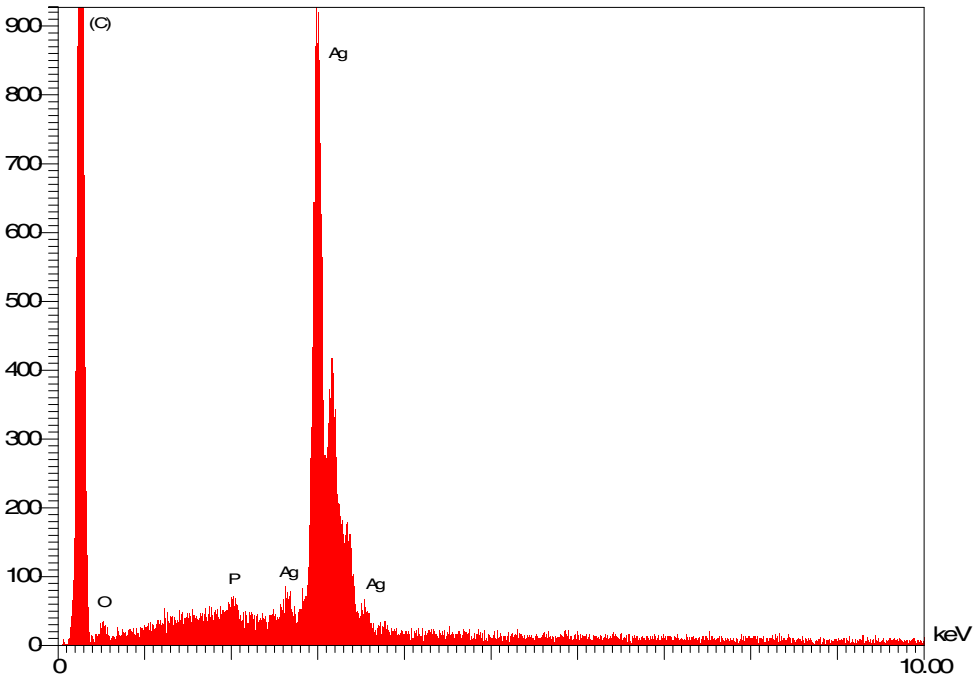

Figure SI1. Representative energy dispersive X-ray (EDX) analysis of Ag nanocoral deposited on a graphite disk electrode.

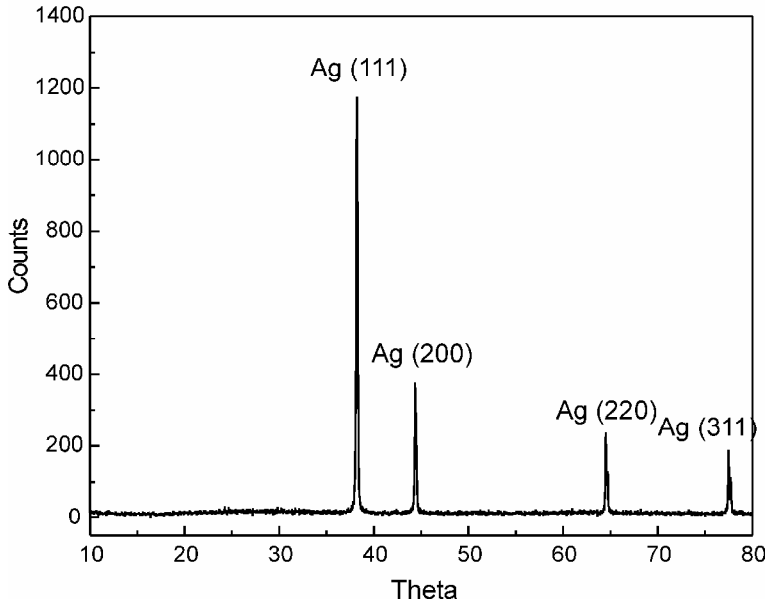

Figure SI2. Representative wide angle X-ray diffraction (XRD) pattern of Ag nanocoral deposited on a graphite disk electrode, after removal of SDS.

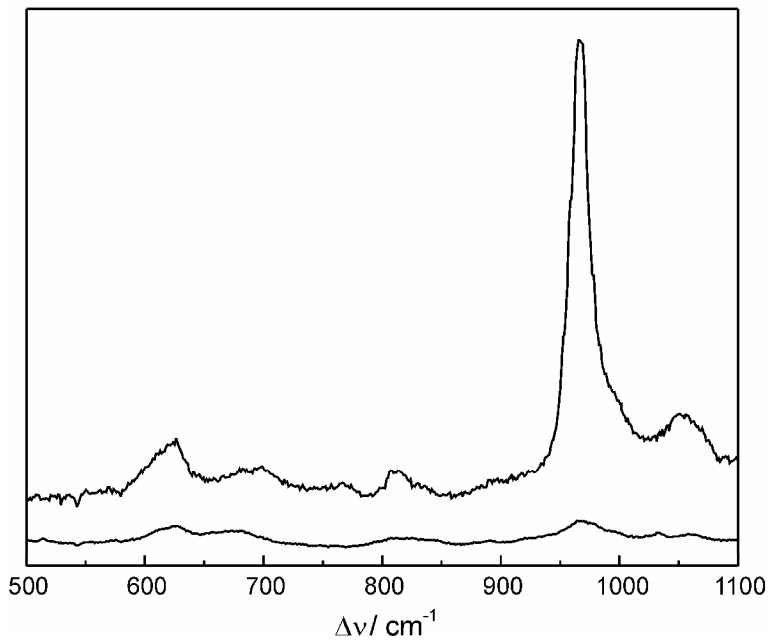

Figure SI3. Surface enhanced Raman (SER) spectra of Ag nanocoral deposited on a graphite disk electrode, before (top) and after (bottom) removal of SDS. Measurements were performed with 514 nm excitation under, otherwise, the same conditions described above.

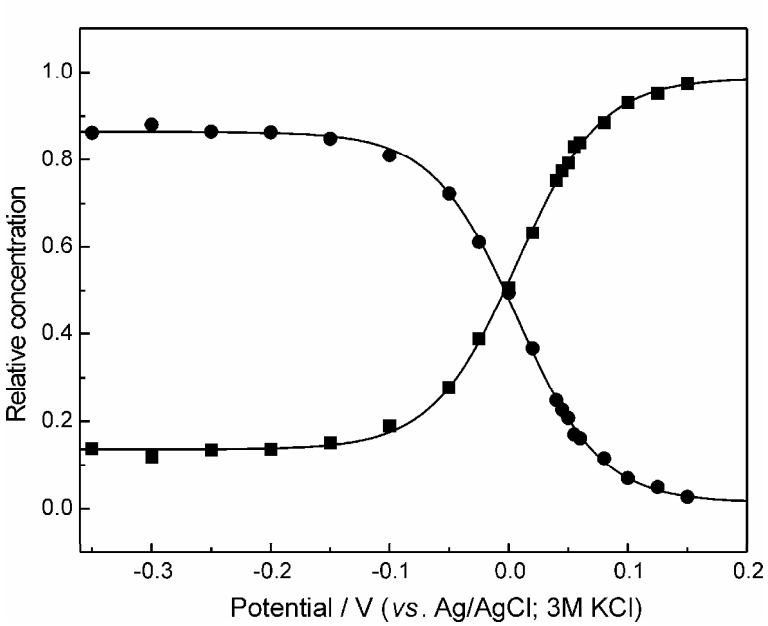

Figure SI4. Relative concentrations of ferrous (circles) and ferric (squares) cytochrome *c* as a function of the applied potential, as determined by quantitative fitting of the SERR spectra for a graphite/Ag nanocoral/SAM/Cyt electrode. After polynomial baseline subtraction, the measured SERR spectra were treated with home-made component analysis software. Series of SERR spectra measured at various potentials could be consistently described on the basis of only two spectral components, i.e. the ferrous and ferric native Cyt, using only their relative contributions as adjustable parameters. The spectral parameters and SERR relative cross sections for the two species were determined from RR measurements in solution under, otherwise, identical conditions. Relative intensities were converted to relative concentrations using a relative SERR cross section ferrous / ferric = 3.57.

The plot shows that ca. 85 % of the protein is electrochemically active. No spectral indication of non-native species was observed.

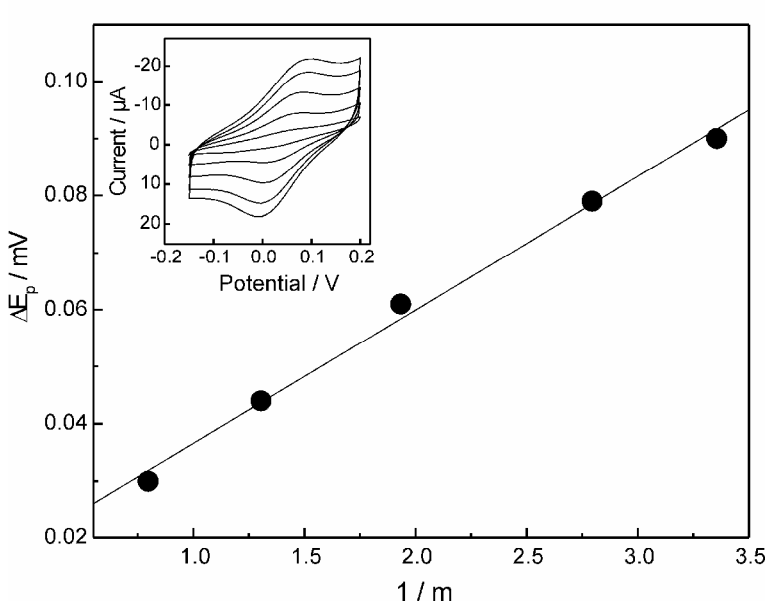

Figure SI5. Laviron´s plot (Laviron, E. *J. Electroanal. Chem*. **1979**, *101*, 19-28) of the peak separation in the cyclic voltammograms of a graphite/Ag-nanocoral/SAM/Cyt electrode recorded different scan rates. The inset shows the voltamograms used for the analysis. The average apparent electron transfer rate constant that results from the analysis of several preparations is ca. 10 s^{-1}.

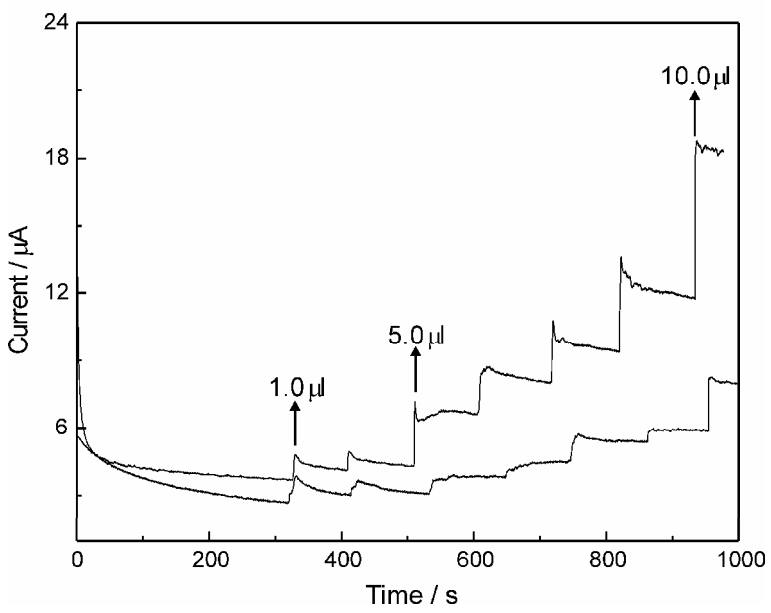

Figure SI6. Comparison of catalytic currents obtained from graphite/Ag-nanocoral/SAM (lower trace) and graphite/Ag-nanocoral/SAM/Cyt (upper trace) electrodes, upon successive additions of different volumes of 0.4 mM H_2O_2 to 5.0 ml PBS (10mM, pH 7.0).

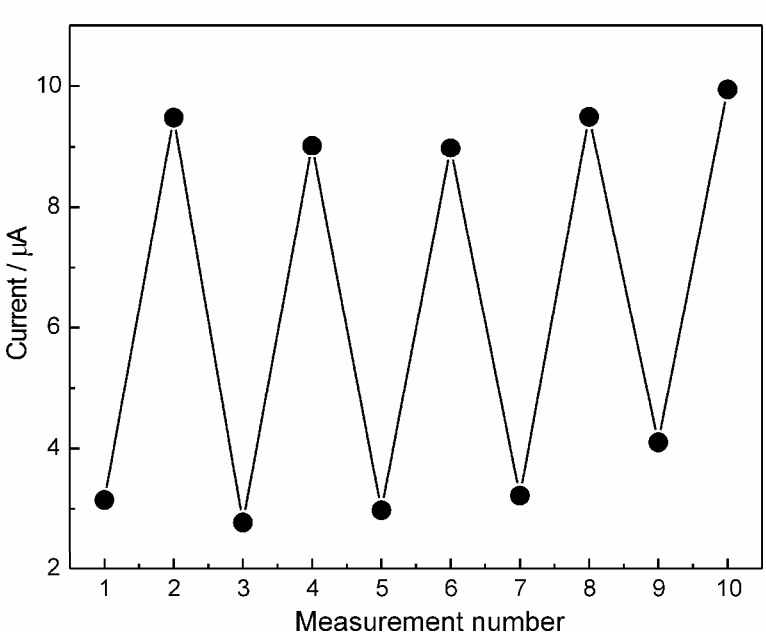

Figure SI7. Amperometric response of a single graphite/Ag-nanocoral/SAM/Cyt electrode upon successive immersions in pure water and in 0.5 mM H_2O_2 solutions. Measurements were performed at -100mV.

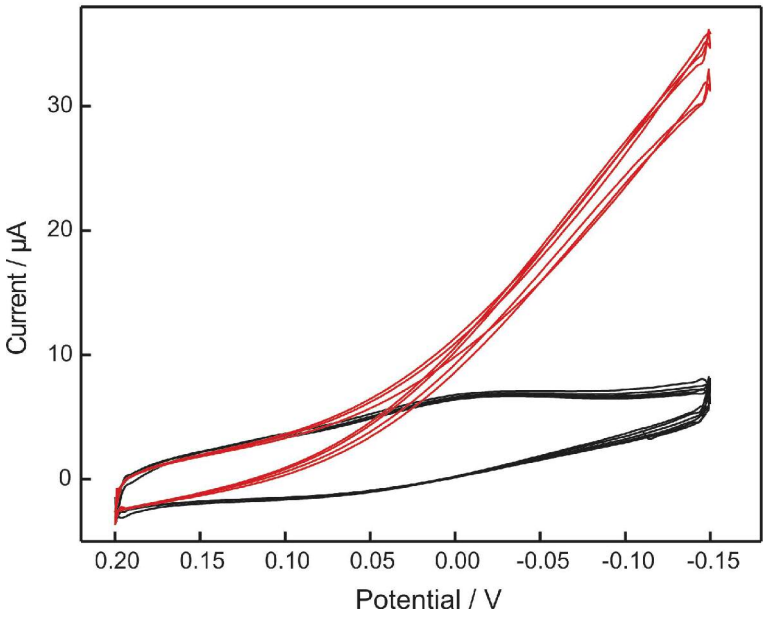

Figure SI8. Cyclic voltammetry of a graphite/Ag-nanocoral/SAM/Cyt electrode measured in the absence (black) and in the presence (red) of H_2O_2 9.7 µM.