Supplementary information

Electrochemistry of Mitochondrial Isolates on Ultrasonicated Graphene Oxide-Modified Electrodes

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1 Experimental

1.1 Physicochemical characterization of EGO-xh flakes and suspensions

Scanning electron microscopy (SEM) analysis was conducted using a TESCAN VEGA3 microscope, equipped with a tungsten-heated cathode and a field emission gun. The accelerating voltage was set to 20 keV and the images were taken in secondary electron mode.

X-ray photoelectron spectroscopy (XPS) analysis was performed using the Quantes instrument from PHI-ULVAC, equipped with a monochromatic aluminum (Al) source emitting X-rays at 1486.6 eV and a micro-focused beam with a diameter of 100 µm. Survey spectra were recorded from 0 eV to 1350 eV with a step size of 1 eV and a dwell time of 50 ms per step, over a total of 4 scans, with a pass energy of 280 eV. High-resolution spectra were acquired over energy ranges specific to each analyzed element, with a step size of 0.1 eV, a dwell time of 50 ms per step, and a number of scans adjusted to optimize the signal-to-noise ratio, using a pass energy of 55 eV.

UV-vis spectrophotometry measurements were carried out using a Cary 60 UV-vis spectrophotometer from Agilent Technologies in a wavelength range of 190-500 nm, at a scan rate of 600 nm min^{-1}.

High resolution transmission electron microscopy (HR-TEM) and scanning transmission electron microscopy (STEM) images were acquired using bright-field imaging at various magnifications using a Thermo Scientific Talos F200X G2 electron microscope operated at 200 keV, and located at the Facility for Electron Microscopy Research at McGill University. 10 µL of the corresponding EGO suspension were deposited onto a 300-mesh Cu grid with a SiOx substrate using the drop-casting method, followed by drying at room temperature.

1.2 Buffer composition for experiments with mitochondria

Mitochondrial Purification Solution (MPS): 250 mM sucrose (molecular biology grade, Sigma), 20 mM HEPES (Multicell), 10 mM KCl (99 % assay grade Fisher Bioreagents), 0.5 mM $MgCl_2$ (ThermoFisher), 1 mM EDTA (>99 %, Sigma Aldrich), 1 mM EGTA (Molecular Biology Grade, Millipore Sigma), protein inhibitor cocktail tablets containing: 15 ug ml^{-1} leupeptin, 5 ug ml^{-1} aprotonin, 1 mg mL^{-1} pepstatin A and 1 mM PMSF (Roche Diagnostics).

Mitochondrial Assay Solution (MAS): 70 mM sucrose (molecular biology grade, Sigma), 220 mM mannitol (Reagent grade 98 %, Bioshop), 10 mM KH_2PO_4 (Fisher Scientific), 5 mM $MgCl_2$ (Fisher), 2 mM HEPES (Multicell), 1 mM EGTA (Molecular Biology Grade, Millipore Sigma), 2 mM malic acid (reagent plus >99 %, Sigma Aldrich), 10 mM pyruvic acid (98 %, Sigma-Aldrich) and 10 mM succinic acid (assay grade, Fisher).

1.3 Transmission electron microscopy imaging of isolated mitochondria

For transmission electron microscopy (TEM) imaging, mitochondria were isolated as previously described and resuspended in MAS buffer with 0.17 µg of graphene oxide (EGO) flakes per µg of

mitochondrial protein. The suspensions were incubated for two hours and then pelleted by centrifugation at 8000 g for 20 minutes at 4° C.

The samples were fixed overnight in 2.5% glutaraldehyde prepared in 0.05 M cacodylate buffer (pH 7.4) at 4°C to preserve structural integrity. Following fixation, samples were washed three times for 5 minutes each in 0.05 M cacodylate buffer (pH 7.4) supplemented with 3% sucrose at 4°C. Samples were subjected to post-fixation using a 1:1 mixture of 2% osmium tetroxide and 2% potassium ferrocyanide, both prepared in distilled water. The mixture was applied to the samples for 1 hour at 4°C with constant agitation to enhance membrane contrast and fix lipids. Post-fixation was followed by three washes in distilled water, each lasting 5 minutes at room temperature with agitation, to remove residual fixatives. Samples were then stained with 1% uranyl acetate in distilled water for 30 minutes at room temperature, protected from light, with constant agitation. This step enhances electron density and provides contrast for cellular and subcellular structures. A final 5-minute wash in distilled water with agitation was performed to remove excess stain. Gradual dehydration was carried out through an ethanol series, with samples incubated for 30 minutes each in solutions of increasing concentrations: 25%, 50%, 75%, 95%, 100%, and a second 100% step. Dehydration was performed at room temperature with gentle agitation to avoid sample damage.

To prepare for embedding, samples were pre-infiltrated with SPURR resin using stepwise mixtures of acetone with SPURR resin in ratios of 3:1, 1:1, and 1:3. Each step lasted 2 hours at room temperature with constant agitation to ensure thorough resin penetration. Following pre-infiltration, samples were immersed in pure SPURR resin for two consecutive periods: 2 hours uncovered at room temperature and an additional 3 hours uncovered at room temperature with constant agitation. The infiltrated samples were transferred to BEEM capsules and polymerized at 60°C for 48 hours to create hardened blocks suitable for sectioning. Ultra-thin sections (~90 nm) were obtained using a Leica UC7 ultramicrotome and placed on 200-mesh copper grids to prepare for staining and imaging. The sections were stained with uranyl acetate in 50% ethanol for 15 minutes, followed by staining with lead citrate for 5 minutes to enhance contrast and electron density.

TEM imaging was performed using a Hitachi H-7100 transmission electron microscope operating at 75 kV. Images were captured using an AMT XR111 camera to visualize sample ultrastructure with high resolution.

1.4 SEM imaging of mitochondria-modified electrodes

Model 110 screen-printed carbon electrodes (SPEs) from Metrohm were chosen for these observations because their geometry facilitates sample preparation which involves multiple solution immersion steps. EGO-60h, GOQD and mitochondria were drop-casted on the screen-printed electrodes and the following preparation protocol was followed:

The mitochondria-modified SPEs were first incubated overnight at 4°C in a solution containing 2.5% glutaraldehyde diluted in 0.2 M cacodylate buffer with sucrose, at pH 7.4 to stabilize the mitochondrial membranes. After fixation, they were washed at room temperature by immersion in a 0.2 M cacodylate buffer containing sucrose at pH 7.4 for 5 mins. This step was repeated three times. To enhance contrast and preserve ultrastructure, the samples were then post-fixed for 1 hour at room temperature in a solution of 1% osmium tetroxide prepared in a 0.2 M cacodylate buffer with sucrose at pH 7.4. Following post-fixation, the samples were quickly rinsed twice in distilled water before proceeding with dehydration.

Dehydration was carried out by immersing the samples in a series of ethanol solutions of increasing concentration: 25%, 50%, 75%, 95%, and finally twice in 100% ethanol for 5 minutes at each step. Samples were stored in 100% ethanol until further processing by critical point drying (Leica EM CPD300). The CPD process was performed to prevent structural collapse, using CO_2 exchange. The procedure included immersing the samples in 100% ethanol, setting a magnetic stirrer rotation at 50%, using a fast CO_2 inflow speed, performing 12 exchange cycles, and applying rapid gas-out heating and speed. Once completed, the chamber was vented, and dried samples were retrieved for coating.

Finally, to improve the quality of the SEM images, a 2 nm gold conductive layer was deposited on the samples using a sputter coater (Leica EM ACE600).

SEM images were acquired using a Hitachi Regulus8220 ultra high-resolution SEM microscope operated at 10keV.

1.5 Matlab code for baseline subtraction

```
% Define the variables for the datasets
xData1 = PGE1EGO60hmitosSWV2. ("PotentialAppliedV");
yData1 = PGE1EGO60hmitosSWV2. ("WE1CurrentA");

% Define the voltage range
voltageRange = [-0.8, 0.8];

% Remove NaN values and filter by voltage range for all datasets
validIndices1 = ~isnan(yData1) & xData1 >= voltageRange(1) & xData1 <= voltageRange(2);
cleanXData1 = xData1(validIndices1);
cleanYData1 = yData1(validIndices1);

% Normalize datasets
normalizedYData1 = normalizeData (cleanXData1, cleanYData1); % Use the function created below

% Extract new data points after baseline normalization
normalizedDataPoints1 = table (cleanXData1, normalizedYData1);

% Save the command window text of normalized data points to a file
diary ('normalizedDataPoint1.txt');
disp(normalizedDataPoints1);
diary off;

% Function to normalize data
function normalizedYData = normalizeData (cleanXData, cleanYData)
    % Find the lowest points for baseline calculation
    rangeA = cleanXData >= -0.65 & cleanXData <= -0.45;
    [~, minIndexA] = min (cleanYData (rangeA));
    xMinA = cleanXData (rangeA);
    yMinA = cleanYData (rangeA);
    lowestPointA_X = xMinA (minIndexA);
    lowestPointA_Y = yMinA (minIndexA);

    rangeB = cleanXData >= 0.35 & cleanXData <= 0.45;
    [~, minIndexB] = min (cleanYData (rangeB));
    xMinB = cleanXData (rangeB);
    yMinB = cleanYData (rangeB);
    lowestPointB_X = xMinB (minIndexB);
    lowestPointB_Y = yMinB (minIndexB);

    % Specify the potential ranges for baseline calculation
    range1 = cleanXData >= -0.8 & cleanXData <= lowestPointA_X;
```

```
    range 2 = cleanXData >= lowestPointB_X & cleanXData <= 0.8;

    % Perform spline fits to determine the baseline for the specified ranges
    splineFit1 = fit (cleanXData(range1), cleanYData(range1),
    'smoothingspline');
    splineFit2 = fit (cleanXData(range 2), cleanYData(range 2),
    'smoothingspline');

    % Evaluate the spline fits across the specified ranges
    baselineSpline1 = feval(splineFit1, cleanXData (range1));
    baselineSpline2 = feval(splineFit2, cleanXData (range 2));
    % Find the indices of the points at -0.6 and 0.2
    point1 = find (cleanXData >= lowestPointA_X, 1);
    point2 = find (cleanXData <= lowestPointB_X, 1, 'last');


    % Extract the coordinates of these points
    x1 = cleanXData (point1);
    y1 = cleanYData (point1);
    x2 = cleanXData (point2);
    y2 = cleanYData (point2);

    % Create a straight line connecting these two points
    straightLineIndices = cleanXData > x1 & cleanXData < x2;
    baselineStraightLine = interp1 ([x1, x2], [y1, y2],
    cleanXData (straightLineIndices));

    % Combine the spline fits and the straight line
    baseline = cleanYData; % initialize with original data size
    baseline (range1) = baselineSpline1;
    baseline (straightLineIndices) = baselineStraightLine;
    baseline (range 2) = baselineSpline2;

    % Subtract the baseline from the cleaned yData to normalize it
    normalizedYData = cleanYData - baseline;
end
```

2 Results and discussion

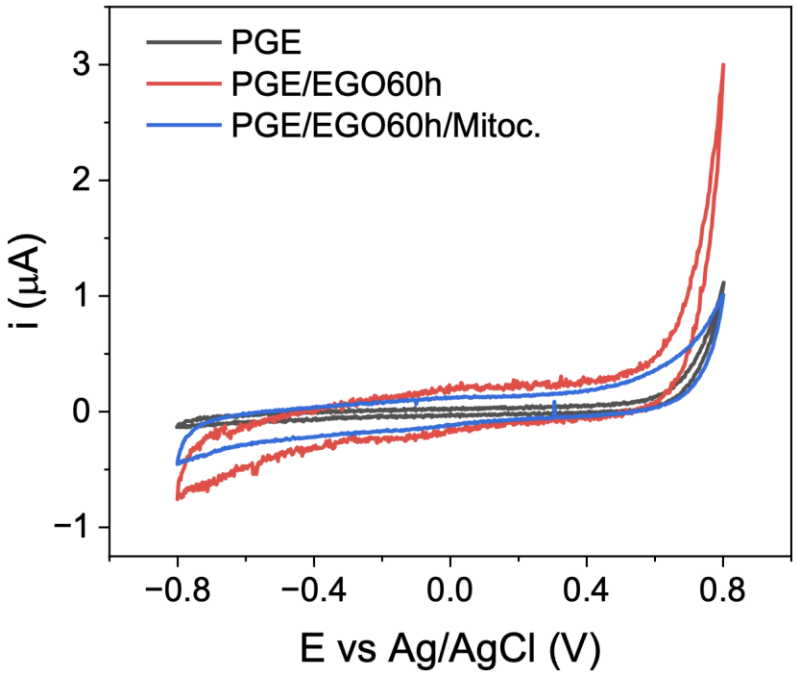

Figure SI1. Cyclic voltammograms at 2mV s^{-1} of bare PGE, PGE/EGO-60h electrode, and PGE/EGO-60h/Mitoc. electrodes in MAS electrolyte.

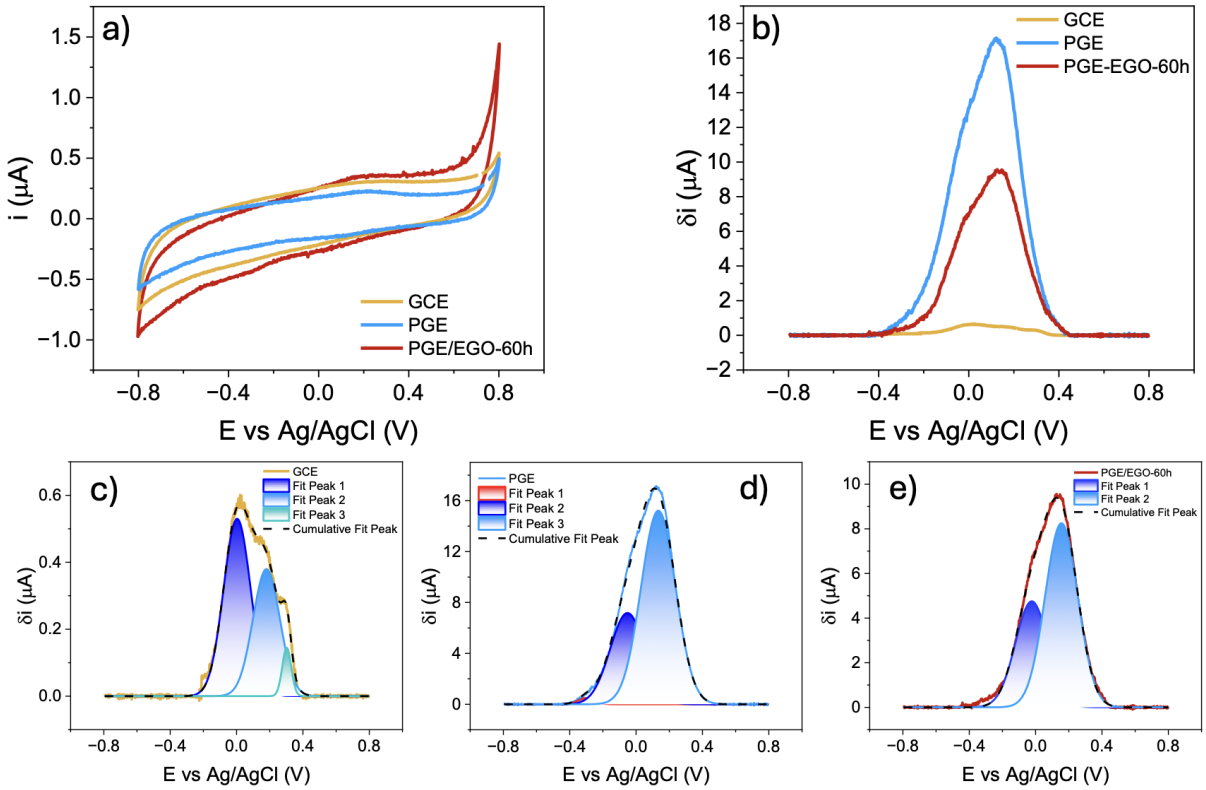

Figure SI2. a) Cyclic voltammograms and b) baseline-subtracted square wave voltammograms of glassy carbon electrodes (GCE), pyrolytic graphite electrodes (PGE) and PGE modified with graphene oxide sonicated for 60 h (PGE/EGO-60h) in MAS buffer. The signal deconvolution is shown in c) for GCE, d) for PGE and e) for PGE/EGO-60h.

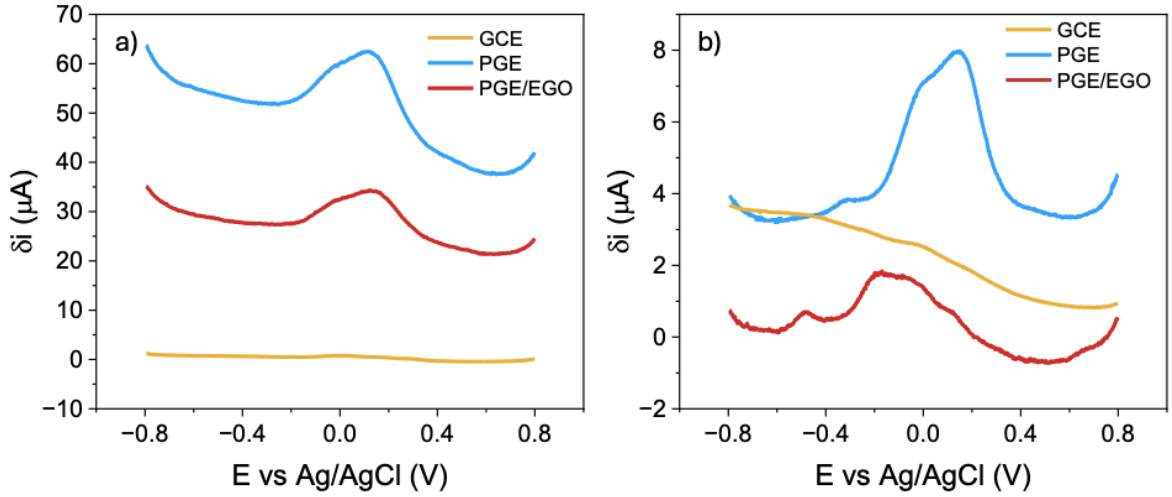

Figure SI3. Square wave voltammograms of a) bare GCE, PGE and PGE/EGO-60h electrodes in MAS buffer, and b) drop-casted mitochondria on GCE, PGE and PGE/EGO-60h in MAS buffer.

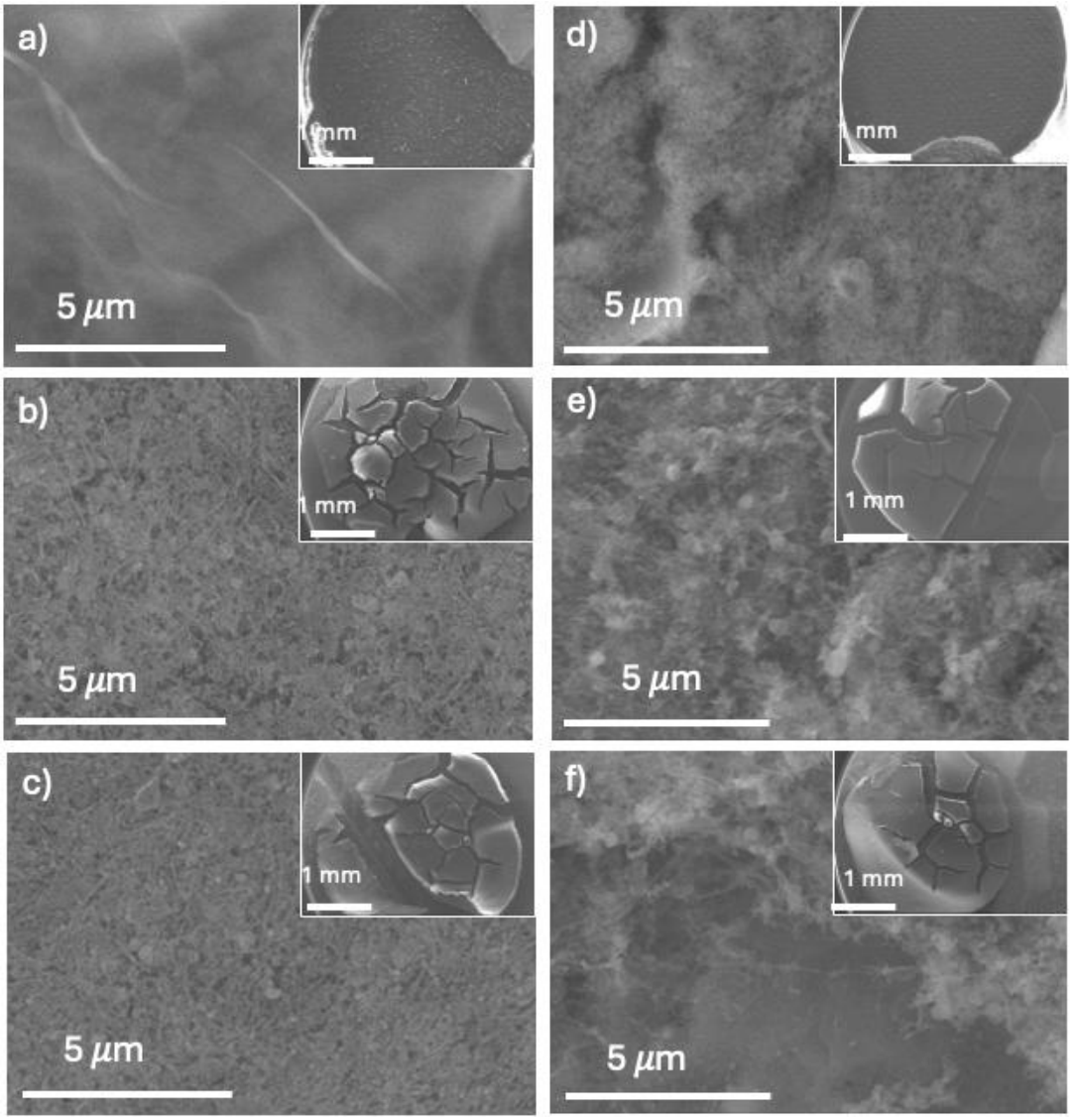

Figure SI4. SEM images of (a–c) screen-printed carbon electrodes modified with EGO-60h: (a) before mitochondrial addition, showing EGO flakes on the surface; (b) after mitochondria were added and dried; (c) after immersion in the working electrolyte for 20 minutes. The insets show low-magnification images highlighting the thick biological membrane formed on the electrode. (d–f) Corresponding images for electrodes modified with GOQD instead of EGO-60h, where a biological matrix is also observed, though less dense.

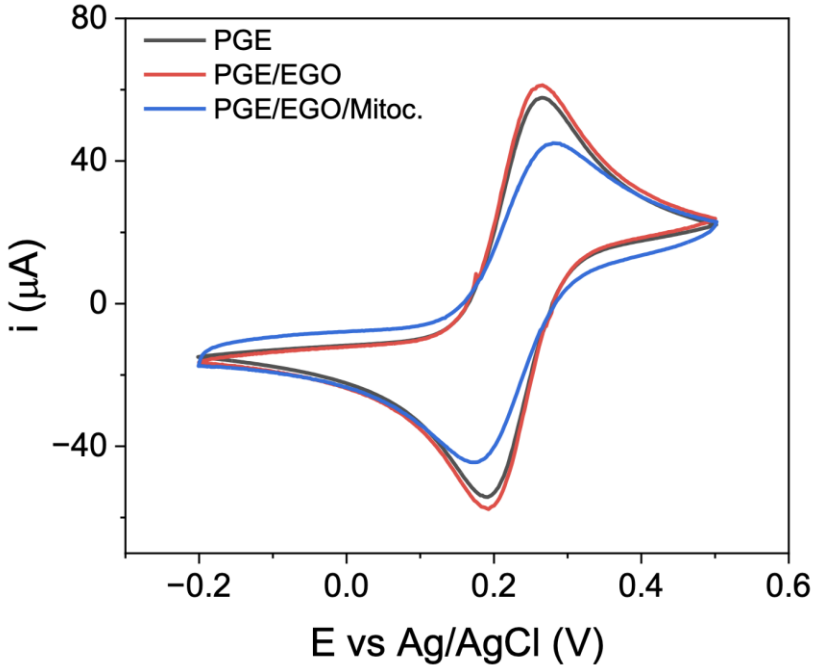

Figure SI5. Cyclic voltammograms of a bare pyrolytic graphite electrode (PGE, black curve), PGE modified with graphene oxide (PGE/EGO, red curve), and PGE further modified with EGO and mitochondria (PPGE/EGO/Mitoc, blue curve).

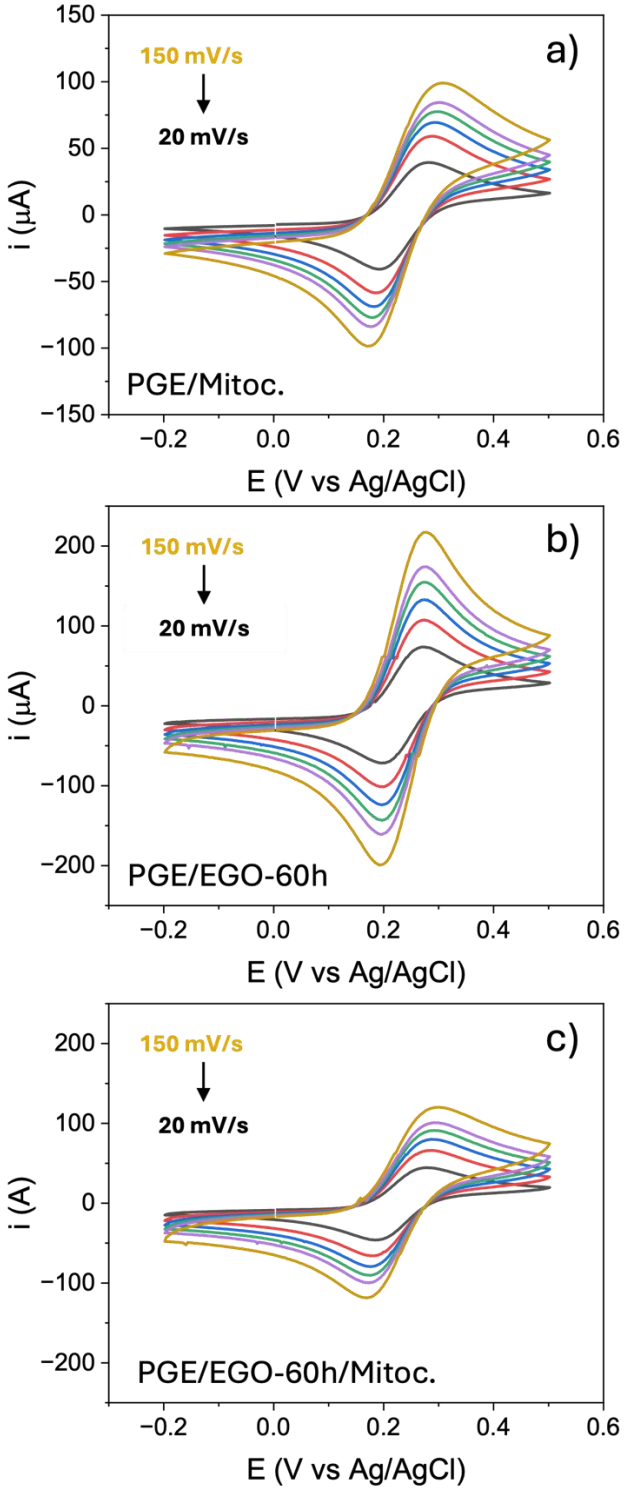

Figure SI6. Cyclic voltammograms of 5 mM Ferri-Ferrocyanide in 0.5 M KCl at different scan rates on a) PGE/Mitoc., b) PGE/EGO-60h, and c) PGE/EGO-60h/Mitoc.

The k^0 was calculated by the Nicholson method from the peak-to-peak separation valued in the cyclic voltammograms in **Figure SI5**, following the equation:

$$k^0 = \left[\frac{\pi D n \nu F}{RT}\right]^{1/2} \psi$$

where k^0 is the standard rate constant in cm s^{-1}; π is the mathematical constant; D is the diffusion coefficient of $[Fe(CN)_6]^{3-/4-}$ (7.2 × 10-6 cm^2/s); n is the number of electrons transferred in the redox event (1 in the case of $[Fe(CN)_6]^{3-/4-}$ redox couple); ν is the scan rate in V s^{-1}; F is the Faraday constant (96485C mol−1); R is the gas constant (8.314 J K^{-1} mol^{-1}); T is the temperature in K; and ψ is the Nicholson dimensionless number which is a function of the peak-to-peak separation (ΔEp). The Nicholson dimensionless number can be calculated using the following function:

$$\psi = \frac{-0.6288 + 0.0021X}{1 - 0.017X}$$

where the X indicates ΔEp × n expressed in mV.

For each electrode, the k^0 values were calculated for the different scan rates and the average value ± standard deviation is reported in the manuscript.

Table S1. Peak potential from the square wave voltammograms recorded in presence and in absence of mitochondria on different electrodes

	GCE		PGE		PGE/EGO-60h	
Peak	Bare	Mitochondria	Bare	Mitochondria	Bare	Mitochondria
Peak I	-	-	-	-0.28 V	-	-0.48 V
Peak II	0 V	0 V	-0.05 V	-0.02 V	-0.02 V	-0.19 V
Peak III	0.18 V	0.2 V	0.14 v	0.16 v	0.16 V	-0.02 V
Peak IV	0.30 V	-	-	-	-	0.16 V

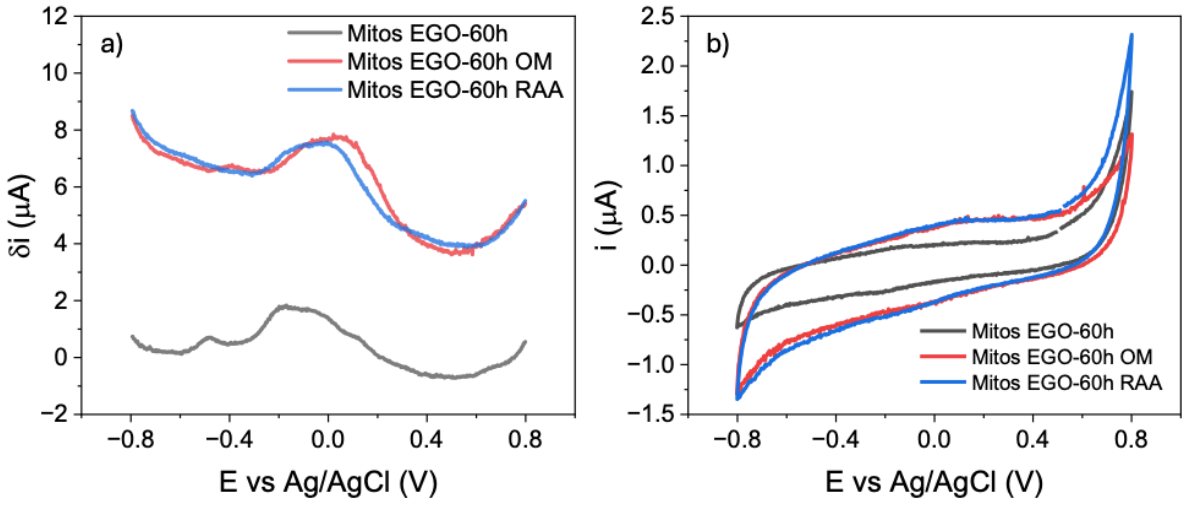

Figure SI7. a) Square wave voltammograms and b) Cyclic voltammograms at 20 mV s^{-1} of drop-casted mitochondria on EGO-60h alone (black curve), in MAS buffer, in presence of RAA (blue curve) and in presence of OM (red curve).

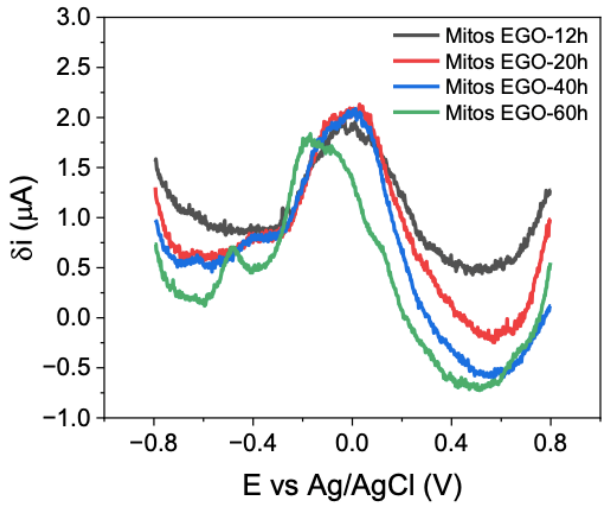

Figure SI8. Square wave voltammograms of drop-casted mitochondria on PGE /EGO sonicated for different time in MAS buffer.

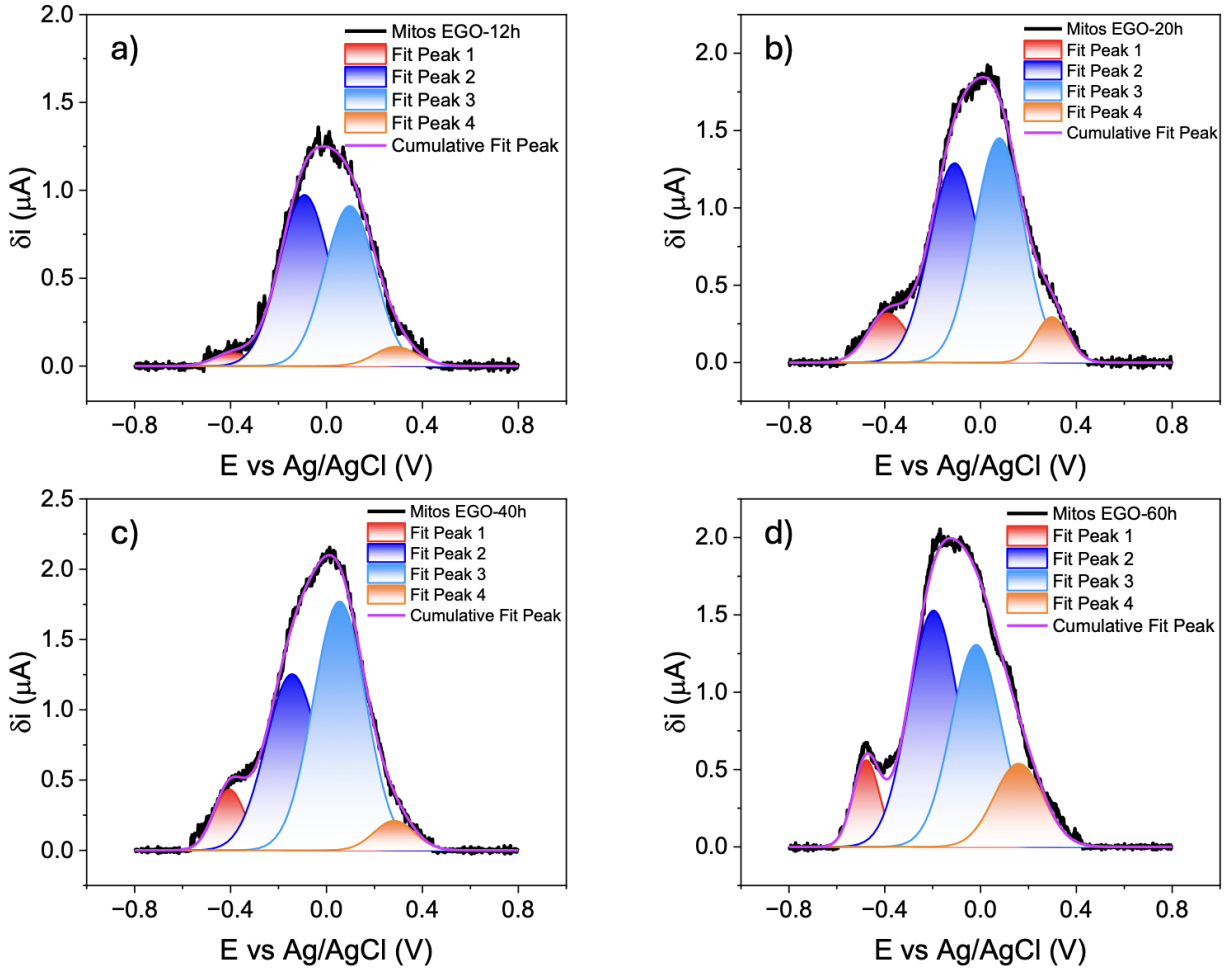

Figure SI9. Peak deconvolution and fitting of the SWV of drop-casted mitochondria on PGE modified with EGO sonicated for a) 12 h, b) 20 h, c) 40 h, and d) 60 h. MAS buffer was used as the working electrolyte.

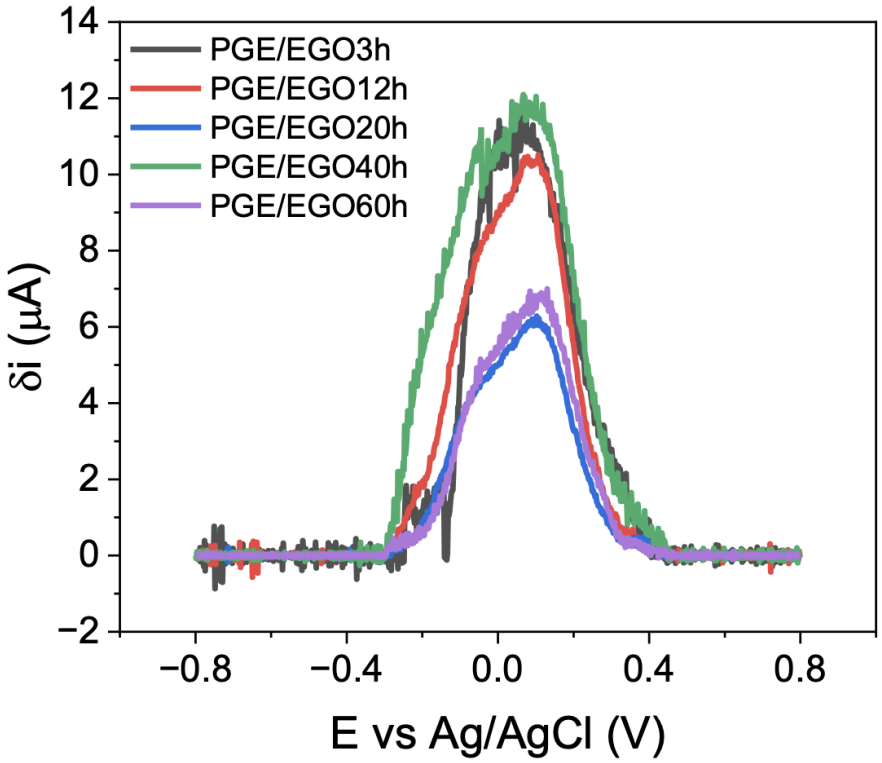

Figure SI10. Square wave voltammograms of drop-casted EGOs sonicated for different times in MAS buffer.

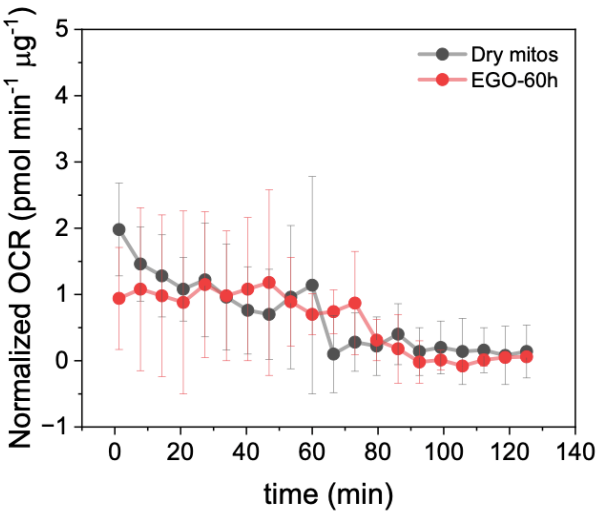

Figure SI11. SeaHorse analysis of isolated mitochondria dried at 37 °C alone and in presence of EGO-60h in MAS buffer. Once dried, mitochondria lose their respiration activity.

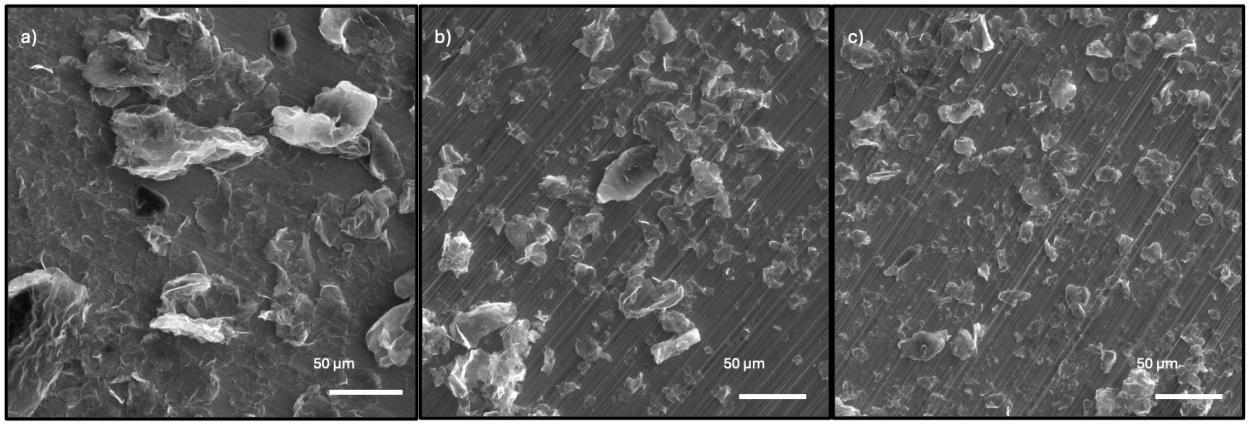

Figure SI12. Scanning electron microscopy images of EGO sonicated for different times. a-c) SEM images of EGO-3h, EGO-20h and EGO-60h, respectively.

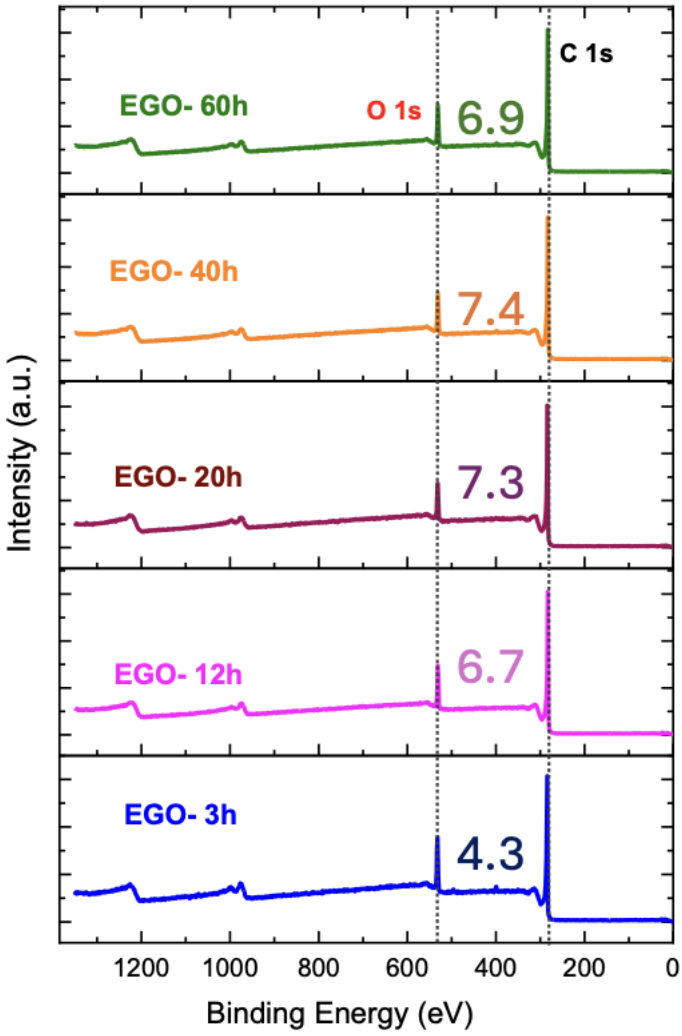

Figure SI13. XPS survey spectra of EGOs sonicated for a) 3 h, b) 20 h, c) 40 h, and d) 60 h. The C/O at% ratio value for each EGO is written next to the corresponding spectrum.

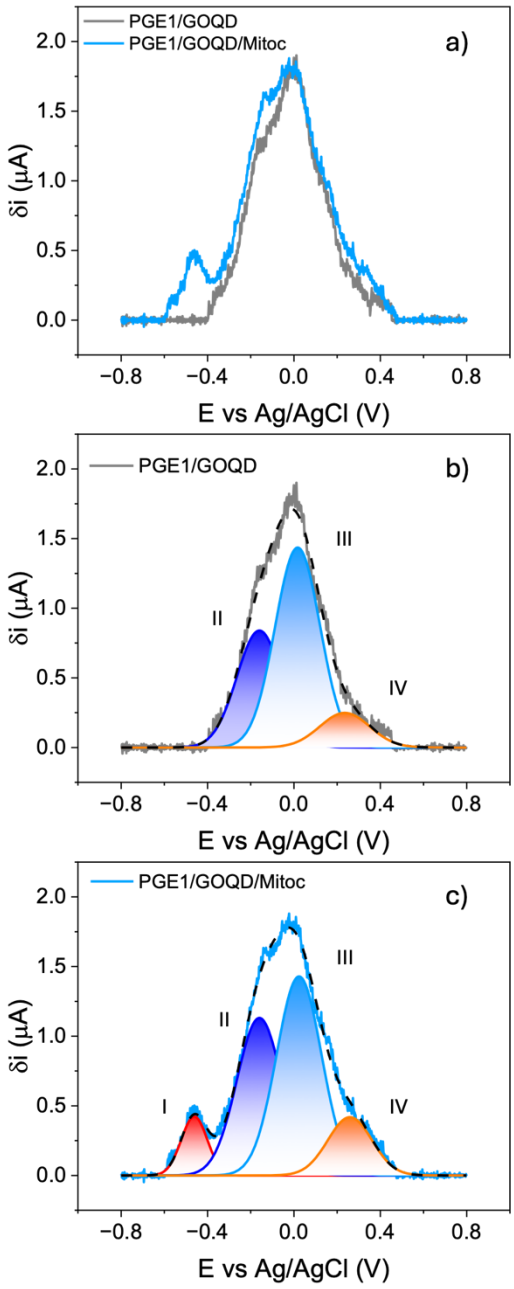

Figure SI14. a) SWV of PGE modified with graphene oxide quantum dots (PGE/GOQD) both in the absence and presence of mitochondria; b) and c) deconvoluted SWV of PGE/GOQD, with and without mitochondria. All measurements were conducted using MAS buffer as the electrolyte.