

Supporting Information:

**Fabrication, Characterization and Functionalization of Dual
Carbon Electrodes as Probes for Scanning Electrochemical
Microscopy (SECM)**

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S1. Materials and reagents

Solutions containing 1 mM FcTMA⁺ hexafluorophosphate (obtained from the metathesis of FcTMA⁺ iodide and silver hexafluorophosphate (Strem Chemicals)),⁴⁶ in Milli-Q water (Millipore Corp., resistivity ca. 18.2 MΩ cm at 25 °C) with 0.1 M potassium chloride (Sigma-Aldrich, AR grade), as supporting electrolyte, was used for cyclic voltammetry and IC-SECM approach curves measurements. The characterization of probes before and after Pt modification used 1 mM ferrocenemethanol (Sigma-Aldrich) in phosphate-buffered saline (PBS, Sigma-Aldrich). See Supporting Information for thylakoid preparation buffers, and thylakoid sample preparation.

S2. Thylakoid preparation buffers

1 mM ferrocenylmethyl trimethylammonium (FcTMA⁺) in aqueous HM buffer (10 mM HEPES (Sigma-Aldrich), 5 mM MgCl₂ (Sigma-Aldrich) and KOH (Sigma-Aldrich) to adjust the pH to 8.0) was used for the thylakoid membrane measurements. Thylakoid preparation used aqueous HS buffer (50 mM HEPES, 0.33 M Sorbitol (Sigma-Aldrich) and KOH to adjust the pH to 8.0), 5×HS buffer (250 mM HEPES, 1.65 M Sorbitol, KOH to adjust pH to 8.0) and Percoll pads (2 ml of 5×HS, 3.5 ml Percoll and 4.5 ml H₂O). In addition, poly-L-lysine (Sigma-Aldrich) coated on glass microscope slides was used to immobilize thylakoid membranes. 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) was used in some experiments to inhibit linear electron flow within immobilized thylakoid membranes.

S3. Thylakoid membrane sample preparation

Thylakoid membranes were prepared from the leaves of 8 to 9 day old peas (*Pisum sativum*, var. Kelvedon Wonder) using a mechanical disruption method.¹ First, chloroplasts

were prepared by homogenizing pea leaves using a Polytron blender (Kinematica GmbH) in HS buffer. This solution was then filtered through Microcloth (Calbiochem) and centrifuged (3300 g for 2 minutes). The chloroplast pellet was re-suspended in 2 ml of HS buffer and moved onto a Percoll pad, before being centrifuged (1400 g for 8 minutes). The supernatant was discarded and the pellet resuspended in 10 ml HS buffer before being centrifuged (3000 g for 2 minutes) and finally being re-suspended in HS buffer (0.5 ml). Thylakoid membranes were prepared from the chloroplasts by taking 0.4 ml of the solution, centrifuging (7000 rpm for 2 minutes) and then re-suspending in HM buffer on ice for 5 minutes to osmotically lyse the chloroplasts, and expose the thylakoid membranes. This solution was then washed twice in HS buffer and once in HM buffer by centrifuging (14,000 rpm for 2 minutes) and re-suspending. Thylakoid membranes were then re-suspended in 0.4 ml HM buffer and kept on ice. Finally, thylakoid membranes were deposited on PLL-covered glass bottomed petri dishes immediately before experiments.

S4. Probe Laser Pulling Parameters

Quartz theta pipets (O.D. 1.2 mm, I.D. 0.9 mm, Intracell) used for typical probes were pulled using a laser puller (P-2000, Sutter Instruments), with a two step program (step 1: HEAT = 750; FILAMENT = 4; VELOCITY = 20; DELAY = 140; PULL = 60. step 2: HEAT = 700; FILAMENT = 3; VELOCITY = 40; DELAY = 135; PULL = 90). The smaller probes, used for the Pt deposition experiment, used a single step program (HEAT = 760; FILAMENT = 3; VELOCITY = 45; DELAY = 130; PULL = 90). The larger DCEs were constructed by polishing smaller DCEs on diamond lapping disks (Buehler).

S5. SECM Instruments

Two SECM systems were used, and are based on a previously described

configurations.² Briefly, for the IC-SECM approach curve measurements a DCE was mounted on a bender piezoelectric positioner (P-871.112, Physik Instrumente), which was in turn mounted on a 3 axis piezoelectric positioner (611.3S Nanocube, Physik Instrumente). The bender piezoelectric positioner was used to oscillate the probe normal to the surface; and also measure the oscillation amplitude of the probe, through the inbuilt strain gauge sensor for the approach curves that used IC-SECM mode.² For the thylakoid membrane measurements a DCE was mounted directly on a 3 axis piezoelectric positioner (611.3S Nanocube, Physik Instrumente). This was situated on a confocal microscope (TCS SP5 MP, Leica). Within both configurations, the piezoelectric positioners were mounted on micropositioners (Newport Corp.) inside a Faraday cage with vibration isolation (granite slab or an optical table). The piezoelectric positioners were controlled and the currents recorded through either a DAQ card (E-671, National Instruments) or a FPGA card (PCIe-7852R, National Instruments), which was, in turn, controlled using custom code from a PC running LabVIEW 2010 or LabVIEW 2011 (National Instruments).

S6. Simulations and Theory

A steady-state three-dimensional FEM simulation of the local FcTMA⁺ and FcTMA²⁺ concentration at nanoscale (100 nm – 1000 nm) DCEs, based on the probe geometry observed in typical SEM images, was conducted.

A stationary diffusion equation describes the transport of FcTMA⁺ (redox species in bulk solution):

$$D\nabla^2 c = 0 \quad (1)$$

where D is the diffusion coefficient (which was $6.0 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$) and c is the concentration of FcTMA⁺.³ The concentration of FcTMA²⁺ is calculated from mass conservation by reasonably assuming equal diffusion coefficient as to FcTMA⁺.

Two steady-state configurations were simulated: (i) a single electrode oxidizing FcTMA^+ to FcTMA^{2+} at a diffusion-limited rate; and (ii) a G/C configuration where FcTMA^{2+} was generated from the oxidation of FcTMA^+ at one electrode, while being collected by reduction back to FcTMA^+ at the other electrode.

Based on the probe geometry and dimensions observed in SEM images of typical nanoscale DCEs, a FEM model of the probe geometry was developed. The geometry of the three-dimensional FEM model is summarized in Figure S1. Each electrode is semi-elliptical and the size is defined by major and minor axes. The major axis is the distance from the center of the electrode to the outside edge perpendicular to the septum separating the electrodes. The minor axis is the distance from the middle of the electrode to the edge of the electrode parallel to the septum. The size of the minor axis was set to the average of the two major axes, typical of representative SEM images of nanoscale DCEs. The septum width and width of the glass surround of the electrodes was set as a fifth of the minor axis size. These proportions were again based on typical SEM images of nanoscale DCEs. The outer wall of the probe has a typical taper angle of 10 degrees. Configuring the model in this way means that there are only two independent variables in the FEM model, i.e. the major axis size for each of the electrodes. Thus, in principle, only two current measurements are needed to determine the geometry of the probe. Recession, or protrusion, of the carbon from the probe is a possibility as we will discuss later; however this is not considered in this simple model as it would introduce a number of additional independent variables, which would complicate the basic geometry determination. It is also important to point out a further simplification: we have assumed that the carbon electrode has a uniform activity; although probes formed by pyrolytic carbon deposition have been shown to have a combination of conducting sp^2 (graphitic) and non-conducting sp^3 carbon,⁴ and this could complicate the response in certain situations, as we also consider later. Figure S1 A summarizes the forgoing description, with a

2D representation of the dimensions of the end of the probe, while the full 3D geometry of the model is shown in Figure S1 B with boundary colors used as an aid to identify the conditions applied.

To solve the mass transport problem and calculate the currents at the DCEs, we applied the following boundary conditions (where n is the surface normal and N is the flux of FcTMA^+): insulating boundaries ($n \cdot N = 0$) which are shown in gray in Figure S1 B; symmetry boundaries ($n \cdot N = 0$) which are shown in green on Figure S1 B; generator boundaries ($c = 0$) which are shown in pink on Figure S1 B; collector boundaries ($c = 1 \text{ mM}$) which are shown in light blue; bulk concentration boundaries ($c = 1 \text{ mM}$) which are shown in dark blue; and a surface/substrate boundary, which is either insulating ($n \cdot N = 0$) or conducting ($c = 1 \text{ mM}$), and is shown in red on Figure S1 B. The initial conditions for the simulations were 1 mM of FcTMA^+ . The current at an electrode was calculated from the integral of the normal flux of active species to the electrode surface multiplied by the Faraday constant. Comsol 4.2a (Comsol) was used on a PC running WindowsXP 64 (Microsoft).

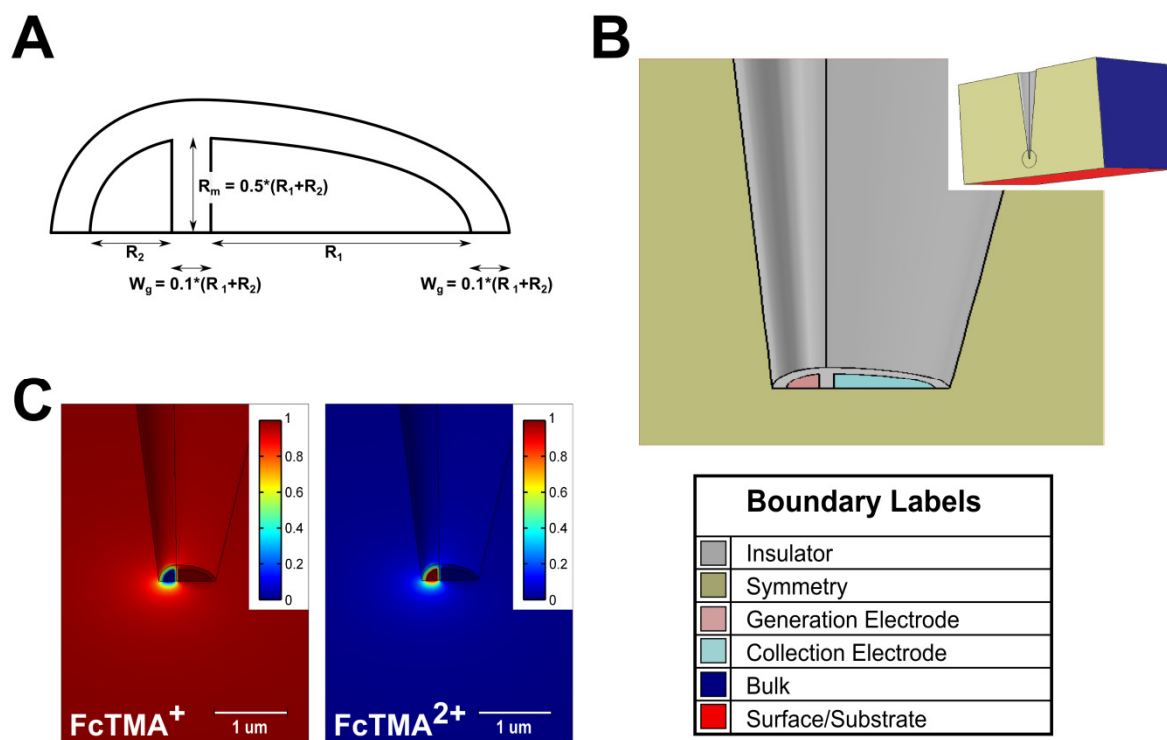


Figure S1. A. Sketch of the end of a DCE probe showing dimensions used in the FEM model. R_1 is the major axis size of electrode 1 and R_2 the major axis size for electrode 2. W_g is the width of the glass surround and is set to $0.1 \cdot (R_1 + R_2)$. R_m is the size of the minor axis of both electrodes, and is set to $0.5 \cdot (R_1 + R_2)$. **B.** 3D geometry of the FEM model, with color coded boundaries to aid understanding of boundary conditions (see text). **C.** Typical concentration profiles for FcTMA^+ and FcTMA^{2+} (normalized with respect to the bulk concentration of FcTMA^+), when FcTMA^{2+} is generated at the left electrode (by oxidation of FcTMA^+) and collected at the right electrode.

S7. Working surfaces

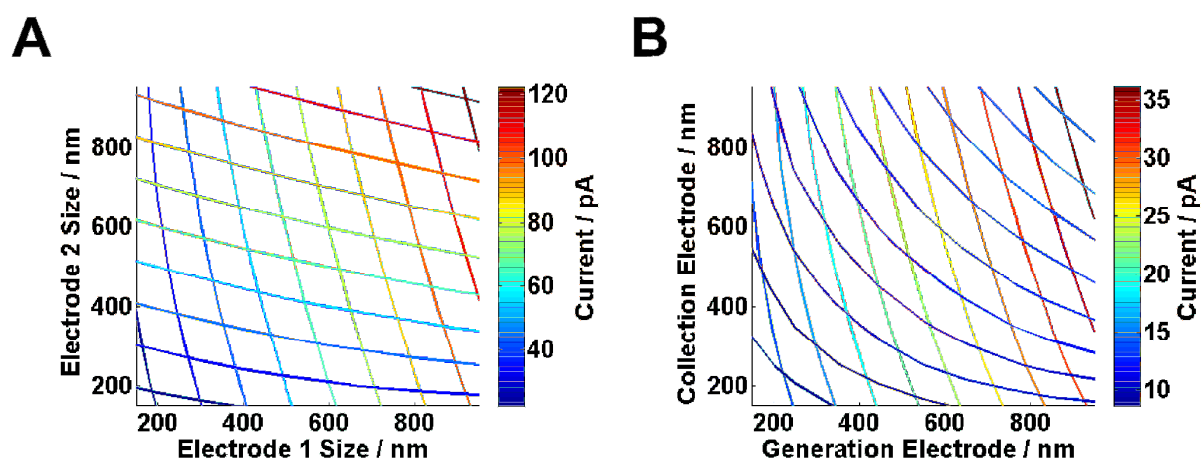


Figure S2. **A.** Working surface for the single barrel generation current. **B.** Working surface for the G/C currents.

The simulations outlined in section S6 were used to produce the working surface shown in Figure S2, which could be used as a guide to the size of individual electrodes in a DCE probe.

S8. Selective Pt Deposition

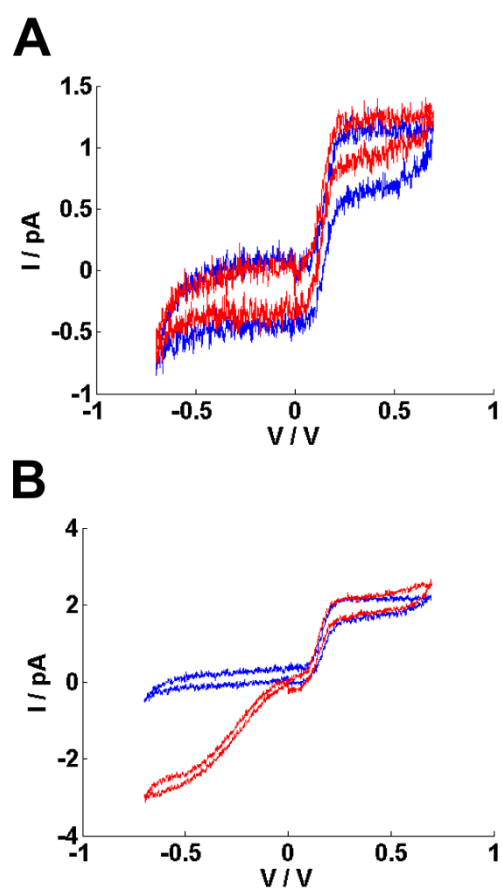


Figure S3. A. CV for the oxidation of ferrocenemethanol at the first electrode in a DCE before (blue) and after (red) deposition of Pt on the second electrode. **B.** CV at the second electrode in a DCE before (blue) and after (red) deposition of platinum on this electrode. Only the oxidation of ferrocenemethanol occurs on the carbon electrode, whereas an additional oxygen reduction wave is seen after Pt electrodeposition.

References

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