Electronic Supplementary Information for

Sensing cisplatin-induced permeation of single live human bladder cancer cells by scanning electrochemical microscopy

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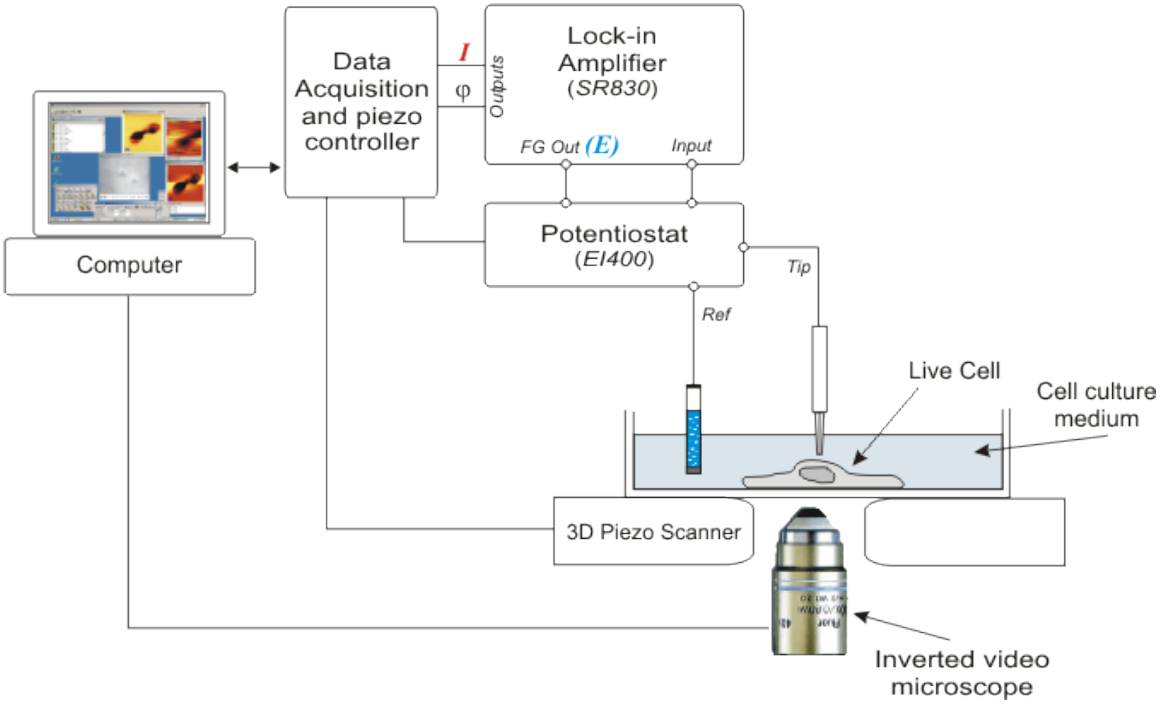
Running head: SECM of cancer-drug and cell interactions

Schematic of SECM apparatus



Simulation Model

In the simulation program, we assume a simple one electron transfer oxidation reaction at the UME surface

$$R \rightarrow O + e \qquad (1)$$

where R represents $FcCH_2OH$ and O represents Fc^+CH_2OH in the outer solution. Since the oxidation of $FcCH_2OH$ is diffusion-controlled process at 0.400 V vs. Ag/AgCl, the concentrations of $FcCH_2OH$ outside the cell, $c_R(r, z, t)$, and inside the cell, $c_{R1}(r, z, t)$, following Fick's second law of diffusion:

$$\frac{\partial c_R(r,z,t)}{\partial t} = D_R\left(\frac{\partial^2 c_R(r,z,t)}{\partial r^2} + \frac{1}{r}\frac{\partial c_R(r,z,t)}{\partial r} + \frac{\partial^2 c_R(r,z,t)}{\partial z^2}\right) \qquad (2)$$

$$\frac{\partial c_{R1}(r,z,t)}{\partial t} = D_{R1}\left(\frac{\partial^2 c_{R1}(r,z,t)}{\partial r^2} + \frac{1}{r}\frac{\partial c_{R1}(r,z,t)}{\partial r} + \frac{\partial^2 c_{R1}(r,z,t)}{\partial z^2}\right) \qquad (3)$$

where r and z are the axial symmetric coordinates shown in Figure S.1, t is the time and D is the diffusion coefficient of $FcCH_2OH$ which was assumed the same for outside and inside the cell with a value of 7.6×10^{-10} m^2/s (Guo and Amemiya 2005, Miao et al. 2002). In the simulation model the T24 cell is a nominal semi-oblate spheroid with a polar and equatorial length of 8 and 15 μm, respectively. Since the O concentration generated at the UME is very small outside the cell and is therefore negligible inside the cell.

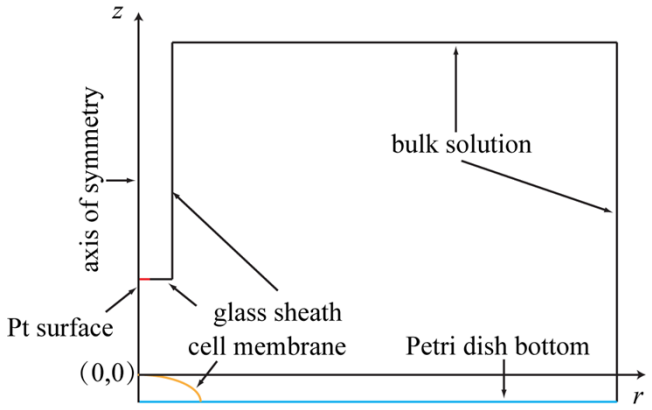


Figure S.1. Geometry for SECM simulations.

Boundary conditions are described as following:

When t = 0, E = 0.000 V at the UME, the concentration of $FcCH_2OH$ inside the cell is

$$c_{R1} = 0 \text{ mol/m}^3 \quad (4)$$

The concentration of $FcCH_2OH$ in the outer solution is

$$c_R = 0.5 \text{ mol/m}^3 \quad (5)$$

When $0 < t \leq 10$ min, E = 0.000 V, the inward and outward fluxes of $FcCH_2OH$ across the cell membrane (Koley and Bard 2010, Yasukawa et al. 1998) are

$$f_{in} = P_m (c_R - c_{R1}) \quad (6)$$

$$f_{out} = P_m(c_{R1} - c_R) \quad (7)$$

where P_m is the permeability, f_{in} is the inward flux of $FcCH_2OH$, f_{out} is the outward flux of $FcCH_2OH$, that approached toward an equilibrium.

Since no potential is applied to the UME at this moment, the Pt surface is equivalent to insulator. The concentration gradient of $FcCH_2OH$ at the Pt surface is

$$\frac{\partial c_R(r,z,t)}{\partial z} = \frac{\partial c_R(r,z,t)}{\partial r} = 0 \quad (8)$$

The concentration gradients of $FcCH_2OH$ at glass sheath and Petri dish bottom are the same as Equation 8.

The concentration gradient of $FcCH_2OH$ at the axis of symmetry is

$$\left[\frac{\partial c_R(r,z,t)}{\partial r}\right]_{r=0} = 0 \quad (9)$$

When $t > 10$ min, E = 0.400 V, the inward and outward fluxes of $FcCH_2OH$ across the cell membrane follows Equation 6 and Equation 7, respectively.

Under the applied potential of 0.400 V, $FcCH_2OH$ is oxidized at diffusion-controlled rate. Therefore the concentration of $FcCH_2OH$ at the Pt surface is

$$c_R(r, z, t) = 0 \quad (10)$$

The concentration gradients of $FcCH_2OH$ at glass sheath and Petri dish bottom follows Equation 8.

The concentration gradient of $FcCH_2OH$ at the axis of symmetry follows Equation 9.

The tip current, *i*, can be obtained by integrating the flux to the Pt surface

$$i = 2\pi nDF \int_0^a r \left[\frac{\partial c_{\mathrm{R}}(r,z,t)}{\partial z}\right] dr \tag{11}$$

where n is the number of electrons transferred in the oxidation of each $FcCH_2OH$ molecule, F is Faraday constant, and a is the radius of the Pt surface.

The simulation was solved by running finite elemental analysis with COMSOL version 3.5 (COMSOL Inc., Burlington, MA). In general practice, for each permeability value, P_m, a theoretical PAC was obtained by plotting the calculated normalized currents (actual probe current (Equation 11) divided by the current when the probe is far away from the cell) versus 20 corresponding normalized distances which were values of probe-cell distances divided by Pt electrode radius. For each theoretical PAC, no $FcCH_2OH$ was inside the cell at t = 0. The concentrations of $FcCH_2OH$ inside and outside cells were first calculated for 10 min with flux of $FcCH_2OH$ across the cell membrane assuming the UME is far away from the cell and no potential was applied to the UME *(Koley and Bard 2010)*. At t = 10 min, the calculated concentration of FcCH2OH inside the cell is c_{R1}, which is used as the initial concentration of $FcCH_2OH$, and the calculated concentration profile in the entire domain at 10 min was used as the initial concentration profile of $FcCH_2OH$, for the sequential simulations with different normalized probe-cell distances and applied potential of 0.400 V *(Koley and Bard 2010)*.

Step-by-step instruction of the simulation using COMSOL software.

1. Beginning the Modeling Process

1.1. Launch the COMSOL software. COMSOL's "Model Navigator" window will appear.

1.2. Choose "Axial symmetry (2D)" for the "Space dimension".

1.3. In the "Application Modes", choose "Chemical Engineering Module" → "Mass transport" → "Diffusion" → "Transient analysis".

1.4. Input "dependent variables", R. Click "add" and input another "dependent variables", R1. Click "OK".

2. Draw Mode

2.1. Click "Draw" → "Draw Objects" → "Line". Click at (0, 0), (1, 0), (5, 0), (5, 20) (50, 20), (50, −20), (0, −20), and right click at (0, 0). The line between (0, 0) and (1, 0) represents the electrode surface; the line between (1, 0) and (5, 0) and the line between (5, 0) and (5, 20) represent the glass sheath of the electrode; the line between (5, 20) and (50, 20) and the line between (50, 20) and (50, −20) represent the bulk solution; the line between (50, −20) and (0, −20) represents the bottom of the Petri dish; the line between (0, −20) and (0, 0) represent the distance between the electrode surface and the Petri dish bottom.

2.2 Click "Draw" → "Draw Objects" → "Ellipse/Circle (Centered)". Click at (0, 0), keep the left mouse button down and drag to draw an ellipse with a = 8 and b = 15. Drag this circle out of the domain drawn in step 2.1. Click "Draw" → "Draw Objects" → "Rectangle/Square". Draw a square with a = 8 and b = 15. Drag this square to overlap the upper right quarter of the ellipse. Choose both the square and the ellipse,

click “Intersection” button on the left side of the drawing area. This action will lead to a quarter-ellipse representing the T24 cell.

2.3. Copy and paste the quarter-ellipse. Move the center of one of the duplicates to (0, −20). Choose both the quarter-ellipse and the SECM experiment domain drawn in step 2.1, click “Difference” button on the left side of the drawing area. Then move the center of the other quarter-ellipse to (0, −20). This action will lead to SECM experiment geometry with a T24 cell under the UME. The subdomains representing the outer solution and intracellular space are independent.

2.4. This step will finalize the geometry drawn in step 2.1 to step 2.3 into actual geometry size in the SECM experiments. Choose the outer solution domain and click “Draw” → “Draw Objects” → “Object Properties”. The lines in the geometry will be numbered and the coordinates of the beginning and ending points of each line are listed in a box. Change the coordinates from TaleS.1 to Table S.2, in which point 1 corresponds to the beginning point of each line while point 2 corresponds to the ending point of each line. Click OK.

2.5. Choose intracellular domain and click “Draw” → “Draw Objects” → “Object Properties”. Change the coordinates from Tale S.3 to Table S.4. Click “OK”. Click the “Zoom Extents” button. The geometry will present the SECM experiments in real size (Figure S.2).

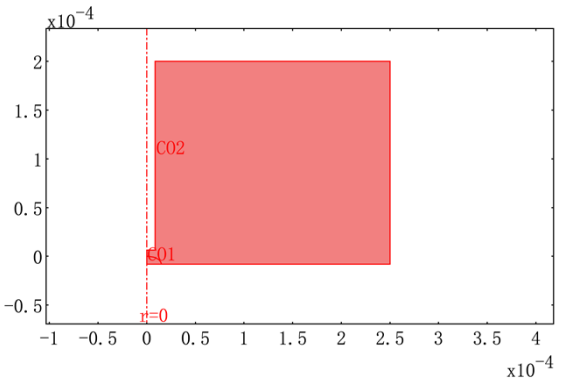


Figure S. 2. Simulation domain in COMSOL software.

3. Constants

Choose “Options” → “Constants”. Type in constants as Table S.5.

Press OK. In TableS.5 n is the number of electrons involved in the oxidation of ferrocene methanol, F is Faraday constant, D is the diffusion coefficient of ferrocene methanol (m^2/s) and P is the permeability coefficient of T24 cells (m/s).

4. Subdomain Settings

4.1 Click “Multiphysics”, choose “1 Diffusion (chdi)”. Choose “Physics” → “Subdomain Settings”. Choose subdomain 2, which represent the outer solution domain, and check “Active in this domain”. Meanwhile choose subdomain 1, which represent the intracellular domain, and decheck “Active in this domain”. Choose

“R” tab, select “D (isotropic)” and input “D”. Choose “Init” tab, input 0.5 as the initial value of concentration $R(t_0)$.

4.2. Click “Multiphysics”, choose “2 Diffusion (chdi2)”. Choose “Physics” → “Subdomain Settings”. Choose subdomain 1, which represent the intracellular domain, and check “Active in this domain”. Meanwhile choose subdomain 2, which represent the outer solution domain, and decheck “Active in this domain”. Choose “R1” tab, select “D (isotropic)” and input “D”. Choose “Init” tab, input 0 as the initial value of concentration $R(t_0)$. Press “OK”.

5. Boundary Mode

5.1 Click “Multiphysics”, choose “1 Diffusion (chdi)”. Choose “Physics” → “Boundary Settings” Set the boundary conditions according to Table S.6. Press “OK”.

5.2 Click “Multiphysics”, choose “2 Diffusion (chdi2)”. Choose “Physics” → “Boundary Settings” Set the boundary conditions as Table S.7. Press “OK”.

6. Mesh Mode

Choose “Mesh” → “Free Mesh Parameters” and choose “General” tab. Input 1.2 in the “element growth rate”. Choose “Boundary” tab, choose the boundary 10 (cellular membrane) and input 1e-7 in the “Maximum element size”. Press “Point” tab, choose point 4 (the edge between the Pt surface and the glass sheath) and input 1e-8 in the “Maximum element size”. Press “OK”.

7. Solving the Model

7.1. Click “Solve” → “Solver Parameters”. Choose “Transient” for the “Analysis types” for both “Diffusion (chdi)” and “Diffusion (chdi2)”. Choose “General” tab, and input in the “Time stepping” box as following:

Time stepping: 0:1:600

Relative tolerance: 1e−9

Absolute tolerance: 1e−10.

Click “OK”.

7.2. Click Solve Problem. After the model is solved, click “Save”. NOTICE that saving the file in this step is very important since only in such way the concentration profile of ferrocenemethanol that has been calculated for 10 min when the UME is far away from the cell and no potential was applied to the UME could be used as the initial value in later simulations.

7.3. Click “Physics” → “Boundary Settings”. Switch the boundary condition of Boundary 4 (electrode Pt surface) into “Concentration”, and input $R_0 = 0$. This boundary condition represents when the UME is biased at 0.400 V, ferrocene methanol is oxidized at the diffusion-controlled rate at the Pt surface. Press “OK”.

7.4. Click “Solve” → “Solver Parameters”. Switch the “Analysis types” of both “Diffusion (chdi)” and “Diffusion (chdi2)” into “Stationary”. Press “OK”.

7.5. Choose “Solve” → “Solver Manager” → “Initial Value” tab. Choose “Stored solution” and “Solution at time: 600” in both “Initial value” box and “Values of variables not solved and linearization point” box.

7.6. Click “Solve”.

8. Postprocessing the Model

8.1. Click “Postprocessing” → “Boundary integration”. Choose the Boundary 4 (electrode Pt surface), input n*F*dflux_R_chdi in the “Expression” box. Tick “Compute surface integral”. Click “Ok”. The simulated current value at a certain distance with P_m = 1.0 ×10^{-3} m/s appears under the geometry image. Do NOT SAVE the file.

8.2. This step allows one to change the geometry and simulate the current value with a closer probe-to-cell distance. Choose the outer solution domain. Click “Draw” → “Object Properties”. Decrease the probe-cell distance by changing the coordinate value of “r” of Line 1, 2, 3, and 4 in Table S.2. Repeat step 8.1 and 8.2 WITHOUT saving the program UNTIL the simulation of one PAC is done.

8.3. From this step we start the simulation of a new PAC representing another P_m value. Choose the outer solution domain. Click “Draw” → “Object Properties”. Move the UME back to its initial position by setting the coordinates of every line according to TableS.2.

8.4. Click “Options” → “Constants”. Change the P (permeability coefficient) value as desired. Repeat from step 7.1 to step 8.4.

Table S.1. Coordinates of the original geometry in the outer solution domain drawn in step 2.1.

Line No./Property	Points	r	z	Line No./Property	Points	r	z
1/Probe-cell distance	1	0	−15	5/Bulk solution	1	5	20
	2	0	0		2	50	20
2/Electrode Pt surface	1	0	0	6/Petri dish bottom	1	5	−20
	2	1	0		2	50	−20
3/Electrode glass surface	1	1	0	7/Bulk solution	1	50	20
	2	5	0		2	50	−20
4/Electrode glass sheath	1	5	0	8/Cellular membrane	1	0	−15
	2	5	20		2	5	−15
					3	5	−20

Table S.2. Coordinates of the real size geometry of the outer solution domain in SECM experiment.

Line No./Property	Points	r	z	Line No./Property	Points	r	z
1/Probe-cell distance	1	0	0	5/Bulk solution	1	8.75E−6	2.0E−4
	2	0	5.0E−5		2	2.5E−4	2.0E−4
2/Electrode Pt surface	1	0	5.0E−5	6/Petri dish bottom	1	1.5E−5	−8.0E−6
	2	2.5E−6	5.0E−5		2	2.5E−4	−8.0E−6
3/Electrode glass surface	1	2.5E−6	5.0E−5	7/Bulk solution	1	2.5E−4	−8.0E−6
	2	8.75E−6	5.0E−5		2	2.5E−4	2.0E−4
4/Electrode glass sheath	1	8.75E−6	5.0E−5	8/Cellular membrane	1	0	0
	2	8.75E−6	2.0E−4		2	1.5E−5	0
					3	1.5E−5	−8.0E−6

Table S.3. Coordinates of the original geometry in the intracellular domain drawn in step 2.1.

Line No./Property	Points	r	z
1/Cell height	1	0	−20
	2	0	−15
2/Cell radius	1	0	−20
	2	5	−20
3/Cellular membrane	1	0	−15
	2	5	−15
	3	5	−20

Table S.4. Coordinates of the real size geometry of the intracellular domain in SECM experiment.

Line No./Property	Points	r	z
1/Cell height	1	0	−8.0E−6
	2	0	0
2/Cell radius	1	0	−8.0E−6
	2	1.5E−5	−8.0E−6
3/Cellular membrane	1	0	0
	2	1.5E−5	0
	3	1.5E−5	−8.0E−6

Table S.5. Constants in the simulation of PACs with different membrane permeability in COMSOL.

Name	Expression	Value
n	1	1
F	96485	96485
D	7.6e−10	7.6e−10
P	1e−3	1e−3

Table S.6. Boundary settings for the outer solution domain in the simulation of PACs with different membrane permeability in COMSOL.

Boundary No.	3	4	5	6	7	8	9	10
Property	Probe-cell distance	Electrode Pt surface	Electrode glass surface	Electrode glass sheath	Bulk solution	Petri dish bottom	Bulk solution	Cellular membrane
Boundary Conditions	Axial Symmetry	Insulation/ Symmetry (No potential is applied)	Insulation/ Symmetry	Insulation/ Symmetry	Concentration $R_0 = 0.5$	Insulation/ Symmetry	Concentration $R_0 = 0.5$	Flux Inward flux: $N_0 = 0$ Mass transfer coefficient: $k_c = P$ Bulk concentration: $c_b = R1$

Table S.7. Boundary settings for the outer solution domain in the simulation of PACs with different membrane permeability in COMSOL.

Boundary No.	1	4	10
Property	Cell height	Petri dish bottom	Cellular membrane
Boundary Conditions	Axial Symmetry	Insulation/ Symmetry	Flux Inward flux: $N_0 = 0$ Mass transfer coefficient: $k_c = P$ Bulk concentration: $c_b = R$

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