Concentrations dominated membrane permeability variation by fullerol nanoparticles on a single living HeLa cell

(Supporting information)

Fig. S1 AFM image and height profile of the C$_{60}$(OH)$_n$ nanoparticles.

Fig. S2 TGA trace of C$_{60}$(OH)$_n$ nanoparticles (the heating rate was 10 °C/min under N$_2$ flow).
**Fig. S3** The optical microscopy image of HeLa cells after being incubated $25 \times 10^{-3} \text{ mg mL}^{-1} C_{60}(\text{OH})_n$. The PBS aqueous solution was mixed with Live/Dead cell staining solution (see Methods).

**Fig. S4** The experiment x-scan line above a single living cell contrast to the simulated x-scan line.

**Fig. S5** Diagram of the scanning electrochemical microscopy for measuring a HeLa cell.
**Fig. S6** The tip was positioned above the cell membrane about 10 μm and the current changing was measured.

**Fig. S7** The x-scan lines against distance of a single cell in present of (a) $0.5 \times 10^{-3}$ mg·mL$^{-1}$ and (b) $2 \times 10^{-3}$ mg·mL$^{-1}$ C$_{60}$(OH)$_n$.

**Fig. S8** The variation in cell membrane permeability with (a) increasing or (b) decreasing concentrations of C$_{60}$(OH)$_n$ solution step by step.

**Simulation**

In the experiment, the tip was held at diffusion controlled potential to avoid any kinetics complications and we assumed that the ferrocyanide present in the solution underwent one electron transfer as shown below:

$$R\text{(solution)} - ne \rightarrow O\text{(solution)}$$

where, $R$ represents ferrocyanide and $O$ represents ferricyanide. For ferrocyanide, $n=1$.

Because the redox species $R$ and $O$ moved toward and away from the electrode surface only by concentration gradient Fick’s second law of diffusion was used in the simulation. The concentration of species $R$ is given as

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

where $r$ and $z$ are the coordinates (Figure S5), $t$ represents time, $c$ and $D$ represents the concentration and the diffusion coefficient, respectively.
diffusion coefficient of \( R \).

**The boundary conditions at \( t > 0 \)**

At the tip: \( 0 < r < a, z = h_1 \)
\[
c_p(r, h_1) = 0
\]
\[
c_o(r, h_1) = 4
\]
At the substrate: \( 0 < r < r_m, z = h_2 \)
\[
\frac{\partial c}{\partial z} = 0
\]
At the cell membrane: \( \text{arc} 1, d < z < h_2 \)

Flux of \( R \) across the \( \text{arc} 1 = P(R-R_1) \)

where \( P \) (m/s) represents the permeability of \( R \) across cell membrane in the simulation. \( R \) and \( R_1 \) is the mediator outside and inside the cell, respectively. The glass sheath surrounding the electrode was considered as an insulator.

At first, the concentration of ferrocyanide inside the cell was zero and the concentration in the bulk solution was 4 mM. Depending on the permeability of cell membrane, the ferrocyanide molecular was consumed by the cell. And the current can be determined by

\[
I_{\text{tip}} = \int_{r=0}^{r=a} 2\pi n F D \frac{\partial c_p(r, h_2)}{\partial z} dr
\]

where \( n = 1, F = 96485 \text{ C/mol} \) and \( D_R = 1 \times 10^{-9} \text{ m}^2/\text{s} \).

The simulation model described above was solved by finite element method where the mesh was increased in exponential grid fashion to generate two-dimensional grid at the regions where sharp change in the concentration gradients were noticed.

**X-Scan Simulation.** As showed in Figure S5, the HeLa cell was assumed to be semielliptical shape with symmetry along \( z \)-axis. In this model, since topography was the subject of interest here, permeability was assumed to be zero along cell membrane or \( \text{arc} 1 \). The 25 \( \mu \text{m} \) tip (RG = 10) was also considered symmetrical along \( z \)-axis.

In the experiment, the tip was held at diffusion controlled potential at all times over the cell or \( \text{arc} 1 \) and the model was solved in steady state solver condition with the aid of Comsol Multiphysics software. The tip to dish distance was maintained at 20 \( \mu \text{m} \) at all times. To measure the tip current at different position over the cell, the \( \text{arc} 1 \) was moved toward left by a distance of 1 \( \mu \text{m} \) out of the active simulation sub domain instead of tip moving over the \( \text{arc} 1 \). This imitates the same condition such as a tip was moving over a single cell in \( x \)-direction. Due to symmetry of cell along \( z \)-axis, scanning along \( \text{arc} 1 \) was adequate to obtain the full simulated x-scan over the cell. Both height and radius of cell were considered as adjustable simulation parameters and were fitted with experimental data.

Simulations were done first with \( P = 0 \) to determine the current at the tip for the certain fixed height of the cell. After that, to fit the experiment data different value of \( P \) was used in the simulation. The tip current was always calculated with the tip located right above the highest point of the cell height. For example, when \( P = P_1 \) the concentration inside and outside the cell was calculated until \( t = t_1 \) and then the tip was brought close to the cell top 20 \( \mu \text{m} \) away from the dish and held there for 0.1 s (because the x-scan speed was 1 \( \mu \text{m}/0.1 \text{ s} \)) to record the current at \( t = t_1 + 0.1 \text{ s} \). The tip was then withdrawn from the top of the cell and the concentration gradient across the cell was again calculated with new value of parameter of \( P_2 \). Then the steps were repeated until this experiment finished. In this system, \( P = KD/L \), where \( P \) is the velocity of ferrocyanide across cell membrane, \( K \) is a constant, \( D \) is the diffusion coefficient of ferrocyanide and \( L \) is the thickness of cell membrane. Base on the formula, the average value of \( P \) was about \( 6.35 \times 10^{-6} \text{ m/s} \).
Fig. S9 The schematic of simulation model.