Supporting Information

Inhibition of the MRP1-Mediated Transport of the Menadione-Glutathione Conjugate (Thiodione) in HeLa Cells as Studied by Scanning Electrochemical Microscopy

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Viability Protocols:

Trypan Blue Based Viability Test: When an appropriate coverage of cells was achieved, the Petri dish was taken out of the incubator and HEPES buffer was added for one hour at room temperature. The buffer was then replaced by an appropriate concentration of menadione solution in buffer for varying periods of time. The menadione solution was then replaced, the cells were washed with only buffer twice, and then trypsin was added to dish for five minutes. The cell suspension was then centrifuged and washed with buffer before dispensing the cell suspension on the Coulter counter. If the cell membrane collapses, the blue dye (trypan blue) can pass through the membrane, and the cell will be stained with blue color. The blue dye cannot pass through the intact membrane of a living cell, and so the living cells will look transparent under microscope. The blue-color-stained and non-stained cells were then counted under optical microscope.

Fluorescent Based Viability Test: The fluorescent based viability assay kit (Biotium Inc., USA) was used to detect living and dead cells simultaneously in the sample. This assay employs two dyes, calcein AM (green dye) and EthD-III (red dye). In this work, 1 mL of 2 μM calcein AM and 4 μM EthD-III was used to detect the viability of the cells. Calcein AM has the ability to pass through an intact cell membrane and react with the intracellular enzyme esterase, which converts it into an intensely green fluorescent calcein dye (excitation/emission 495nm/515nm). The polyanionic calcein dye remains confined inside intact cell membranes so the living cells are easily visible though a microscope. EthD-III can permeate only through damaged membranes and reacts with intracellular nucleic acid to emit intense red fluorescence inside dead cells (excitation/emission 530nm/635nm). So, a green color represents live intact cell membranes

whereas the red color represents ruptured cell membranes or dead cells. This assay is very useful for detecting both live and dead cells at the same time without any pre-treatment of the samples. The green and red stained cell photos were then processed by Image J software (available from the NIH website) to count the number of live and dead cells.

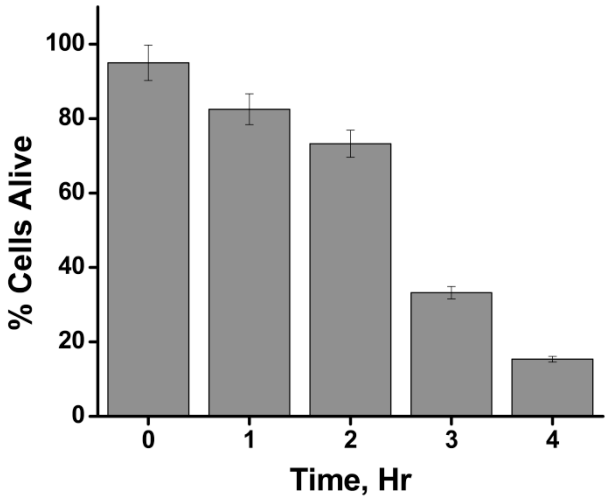

Figure S1: Cellular viability w.r.t time (hr) in presence of 500 μM menadione in buffer solution.

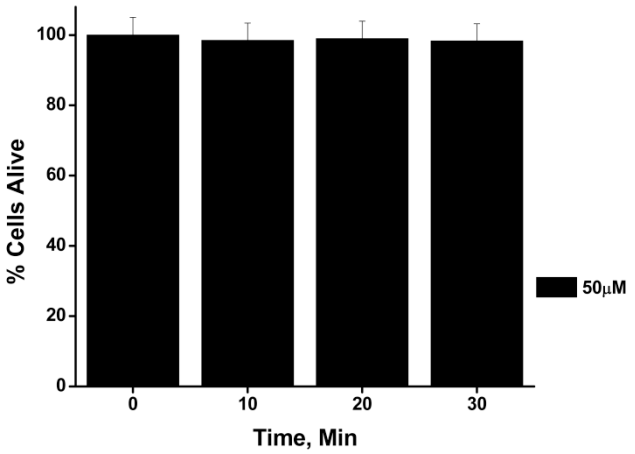

Figure S2: Cellular viability w.r.t time (min) on exposure of 500 μM menadione and 50 μM MK571. The cells were incubated in 50 μM MK571 solution for 60min before adding menadione and MK571 solution.

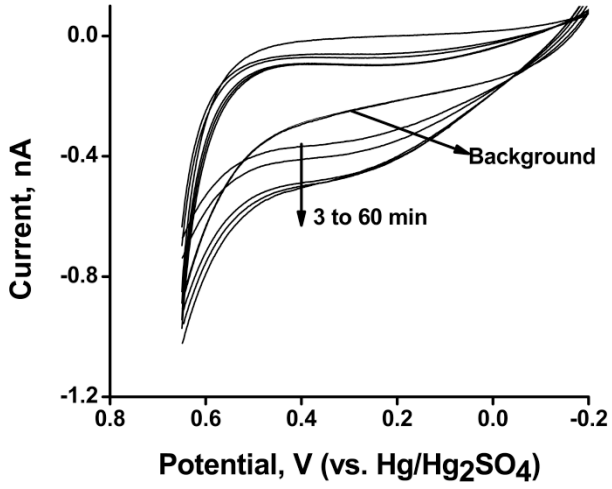

Figure S3: Cyclic voltammetry of thiodione efflux coming out from the monolayer of HeLa cells on exposure to 500 μM menadione. Tip: 10 μm Pt; Counter: 0.5 mm Pt; Reference: Hg/Hg_2SO_4.

Simulation Model

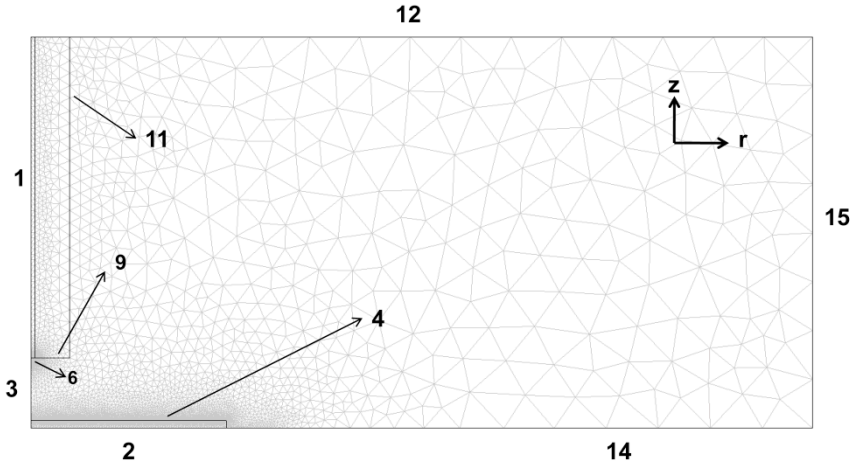

Figure S4: The mesh distribution of Comsol Multiphysics model in 2-D axial symmetry.

We assumed that the thiodione present in the solution undergoes simple two-electron transfer as shown below, and the tip was held at diffusion-controlled potential to avoid any kinetics complications.

$$\text{T (soln)} \longrightarrow \text{Oxidized T (soln)} + 2e$$

Where, T represents thiodione species.

Since the redox species T and oxidized T 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 T was given as $c_T(r,z,t)$ and the diffusion equation in cylindrical coordinates was described as

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

Where, r and z are the coordinates as shown in Figure S3; t represents time; c and D represent the concentration and diffusion coefficient of species R. The other species used in the simulation were as follows: M = menadione outside cell; M1 = menadione inside cell; T = thiodione outside cell; T1= thiodione inside cell; G = glutathione inside cell. Separate variables were allocated for thiodione molecules both inside (T1) and outside (T) the cell to represent the different diffusion coefficient inside and outside the cell. Also, thiodione concentration inside the cell depends on the complicated balance of glutathione and menadione concentration as well as reaction rate as described in Table 2.

The simulation model described above was solved by a finite element method where the mesh was increased in exponential grid fashion to generate a two-dimensional grid. Finer mesh distribution was used at the regions where sharp changes in the concentration gradients were noticed. The details about the boundary conditions are described in table below:

Boundary in COMSOL model	**Description**	**Boundary Conditions**
1, 3	Axial symmetry	Symmetry
6	Electrode Surface	T = 0
2, 9,11 & 14	Insulation sheath	Insulation
4	Cellular membrane	Flux of M = $-k_{in}*(R-R1)$ Flux of M1= $k_{in}*(R1-R)$ Flux of T= P*(T1-T)
12 & 15	Bulk Solution	T = 0 M = 0.5

Table 1: The boundaries in comsol multiphysics model showed in figure S3 and their corresponding boundary conditions.

Initially the menadione concentration inside the cell was zero and the concentration in the bulk solution was 0.5 mM or 0.5 mol/m^3. Appropriate menadione concentration was used for other thiodione concentration curve fitting. The diffusion coefficient of menadione outside the cell was considered as 8×10^{-10} m^2/s and the diffusion coefficient of all species inside cell was assumed as 1×10^{-10} m^2/s. The tip (RG=10) was located at 100 μm away from the substrate or 90 μm from cell surface. The current at the electrode was determined by

$$I_{tip} = \int_{r=0}^{r=a} 2\pi n F D_T r \frac{\partial c_1}{\partial z} dr$$

Where, n = 1; F= 96485 C/mol; and $D_T = 4\times10^{-10}$ m^2/s; a = tip radius, m

Though the real size of the substrate or cellular monolayer is in the order of several cm, in the simulation model we choose 250 μm in radial length or 500 μm as a substrate size to save computation time without affecting any scientific outcome. In addition, for a 10 μm tip size; substrate size ≥ 200 μm will not have any significant affect on the tip concentration measurement.

The rate constant for homogeneous reaction between menadione and GSH was assumed to be fast, on the order of 4 to 6 × 10^{-3} s^{-1}, to maintain a balance between menadione diffusing inside the cell and undergoing conjugation reaction and thus avoiding any negative concentration both inside and outside cell. The adjusted parameters for three different concentrations of menadione are given in the table below:

	Menadione Concentration		
	500 µM	**250 µM**	**125 µM**
Rate of menadione uptake, m/s	1.6×10^{-8}	1.6×10^{-8}	1.6×10^{-8}
Rate of thiodione transport, m/s	1.6×10^{-7}	1.6×10^{-7}	1.6×10^{-7}
Rate of homogeneous reaction, s^{-1}	4	5	6
Glutathione concentration, mol/m^3	20	10	5

Table 2: The fitted simulation parameters for respective menadione concentration.

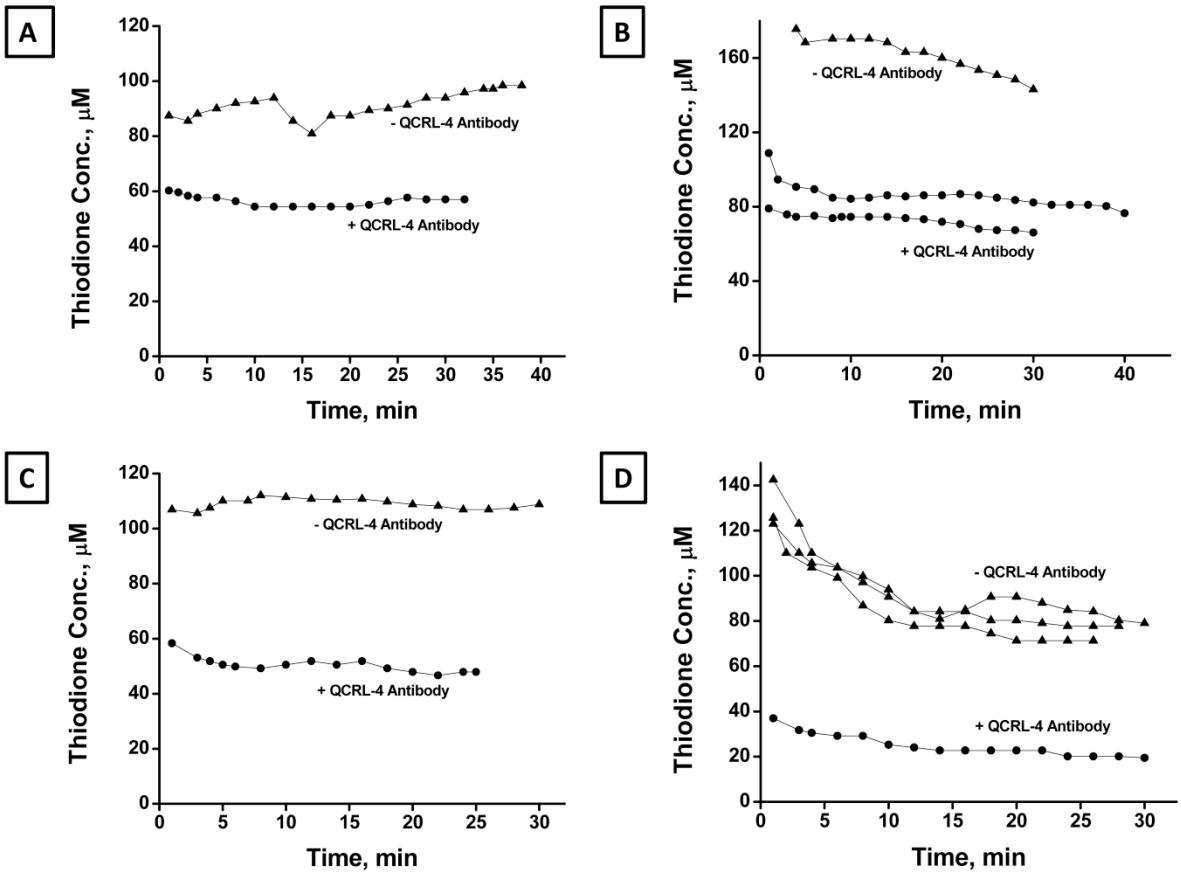

Figure S5: Thiodione concentration (µM) w.r.t time (min) when exposed to 500 µM menadione to transfected (solid circle) and non-transfected (solid triangle) HeLa cells with QCRL-4 antibody. (A-D) represents four independent set of experimental data. Tip-substrate distance in each experiment was maintained at 80 µm.