Single-cell Transfection by Electroporation Using an Electrolyte/Plasmid-Filled Capillary

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SUPPORTING INFORMATION

Table S1: Program for 2-µm opening capillary pulling using a Sutter P-2000 CO_2 laser puller

Line No.	Heat	Filament	Velocity	Delay	Pull
1	250	0	30	200	0
2	250	0	30	200	0
3	250	0	30	200	0
4	270	0	30	200	0

Lipofectamine-mediated bulk transfection

A549 cells were cultured in antibiotic-free BME one day before transfection to reach 90% confluence at the time of transfection. Before transfection, the cells in the culture dish were washed 3 times with extracellular buffer following which 200 µL of serum-free Opti-MEM medium (Invitrogen) were added to the dish. In the meantime, 0.78 µg of plasmid and 1.5 µL of the Lipofectamine 2000 solution were each individually dissolved/diluted in 50 µL Opti-MEM. These solutions were combined and incubated at room temperature for 30 min to obtain the working plasmid/Lipofectamine solution. The working solution was added to the A549 cell dish dropwise and mixed with the medium gently by rocking the dish back and forth. After 4 hours' incubation at 37°C in a CO_2 incubator, 1.5 mL of BME were added to each dish before continuing overnight culture. We arrived at the quantities of plasmid and Lipofectamine 2000 by exploring a range of ratios of DNA (µg) and Lipofectamine 2000 solution (µL) (2 µg DNA: 1 µL Lipofectamine to 0.75 µg DNA: 3.75 µL Lipofectamine). We found that maximum transfection yield occurred at about 1:2 (0.78 µg DNA and 1.5 µL Lipofectamine).

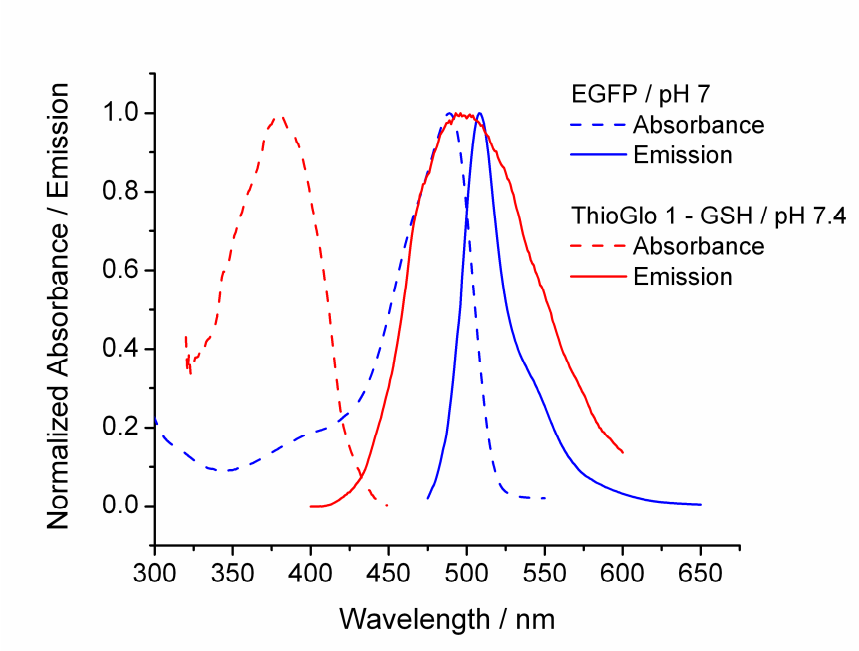

Figure S1: Absorbance and emission spectra of EGFP and Thioglo® 1-GSH adduct. The EGFP spectra are from Invitrogen/Molecular Probes product information.

Numerical simulation of electric field distribution around a 2-µm opening capillary

The simulation was done in Comsol Multiphysics 3.4.

Capillary drawing in Comsol

A typical capillary with a 2 µm tip opening and a taper length of 2 mm was chosen for the model. Because the length of the capillary (15 cm) was much larger than the tip opening, we only modeled part of the capillary, including the tapered 2 mm and 0.5 mm of untapered capillary. The real dimensions (outer diameter /inner diameter ~ distance from the tip end) were measured after taking images under objectives 20×, 40×, and 60×. These data were imported into OriginLab and fitted into 3 Sigmoidal Boltzman functions

(0-100 μm/100-600 μm/600-1600 μm) followed by smoothing. Finally, the outer and inner walls were each defined by 62 points (radius and axial distance).

Model and parameters

The simulation of SCEP utilized a conductive Media DC with 2D axial symmetry. This model solved a partial differential equation: $-\nabla(\sigma\nabla V) = Q$, where σ is the conductivity, V is the electrical potential, and Q is the current source (gradient of current density). Figure S2 shows the modeling geometry and boundary conditions. Parameters and constants used are listed in Table S2. Two model navigators, d_{cout} and d_{cins}, separate the inside cell and outside cell domains. They are related by membrane boundary condition set to be $J_n\ (d_{cout}) = (V_i\text{-}V_o)*\sigma_m/\Delta$ and $J_n\ (d_{cins}) = (V_o\text{-}V_i)*\sigma_m/\Delta$ where Vs are defined on the inside surface of the membrane, V_i, and the outside surface, V_o, σ_m is the membrane conductivity, Δ is the membrane thickness, and J_n is the current density normal to the membrane. The voltage at the simulated capillary untapered end is related to the applied voltage with an equation, $V_0 = V_{app} + E_{y\_dcout} \times (L_{tot} - L_{sim})$ where E_{y_dcout} is the y component of the electric field (in the symmetry axis direction) in the outside cell domain. Accurate calculation of V_0 requires turning on the "weak boundary condition". The cell-to-tip distance was set to 4 μm and the temperature T was set to 298.15 K for the simulation.

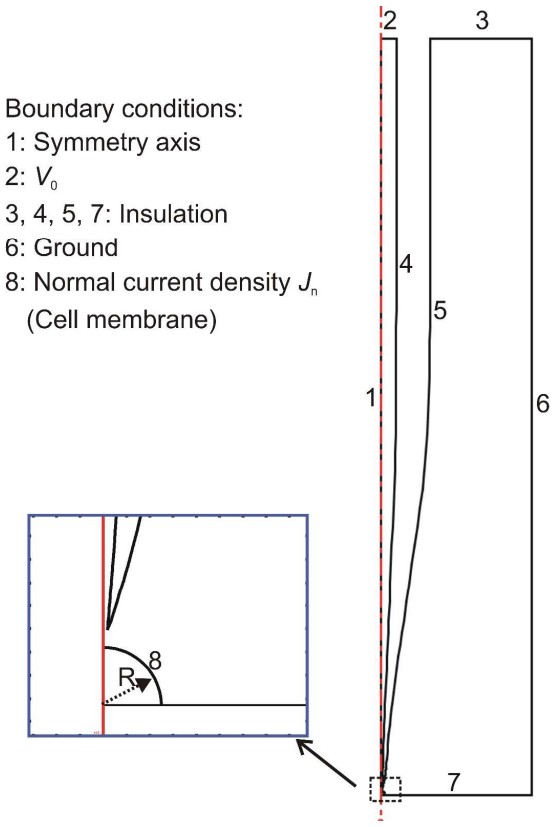

Figure S2: Modeling geometry. The actual shape of the capillary tip is used. Boundary conditions are described in the figure. The capillary is positioned perpendicular to the dish surface and is centered above the cell. Rotational symmetry is used to simplify the simulation.

Table S2: Parameters and constants for numerical simulation

Total capillary length L_{tot}	0.15 (m)
Simulated capillary length L_{sim}	0.0025 (m)
Applied voltage V_{app}	500 (V)
Conductivity of extracellular buffer σ_s *	$C_A + C_B (T - 273.15) + C_c (T - 273.15)^2$ (S/m)
Conductivity of cytoplasm σ_c	$\frac{13}{60} \times \sigma_s$ (S/m)
Conductivity of cell membrane σ_m	5.3×10^{-5} (S/m)
Cell membrane thickness Δ	7×10^{-9} (m)
Tip-Cell distance d	4 (μm)
Cell radius R	12.5 μm

* *The value of σ_s was measured by applying an electric potential across two electrodes (plates) immersed in a test solution under different temperature and fitted into a polynomial curve. C_A=1.11669, C_B=0.01838 and C_c = 1.65 × 10 $^{-4}$.*