Supplemental figures for

Detergent Induction of HEK 293A Cell Membrane Permeability Measured Under Quiescent and Superfusion Conditions using Whole Cell Patch Clamp

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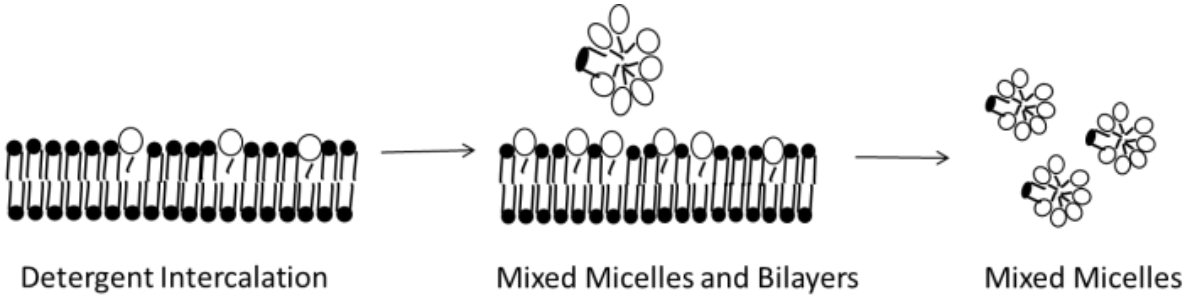

Figure S1. The solubilization of lipid membrane by detergents has been described to proceed in three phases: 1) The intercalation of detergents in the bilayer 2) Above a critical concentration, mixed bilayers and mixed micelles are formed 3) On complete solubilizaition, mixed micelles containing detergent and lipids are formed.

A

B

Phase	Main Channel	Traps	Traps and Compounds	Compounds
Preprime	1 psi (0-92 s) 0.3 psi (92-120 s)	5 psi (0-90 s) 1.5 psi (90-115 s)	6 psi (115-120s)	
Prime	1 psi (0-30 s) 0.4 psi (30-55 s)	6 Hg (50-55 s)	5 psi (0-20 s) 2 psi (20-50 s)	Not used
Trap	Pulse: 0 psi for 4.2s 0.25 psi for 0.8 s 15 such pulses	6 Hg (0-85 s)	Not used	Not used
Break	0.2 psi (0-20 s)	6 Hg (0-5 s) 10 Hg (5-15 s) 6 Hg (15-20 s)	Not used	Not used
Data Acquisition	0.16 psi (Duration of experiment)	6 Hg (Duration of experiment)	Not used	6 psi

C

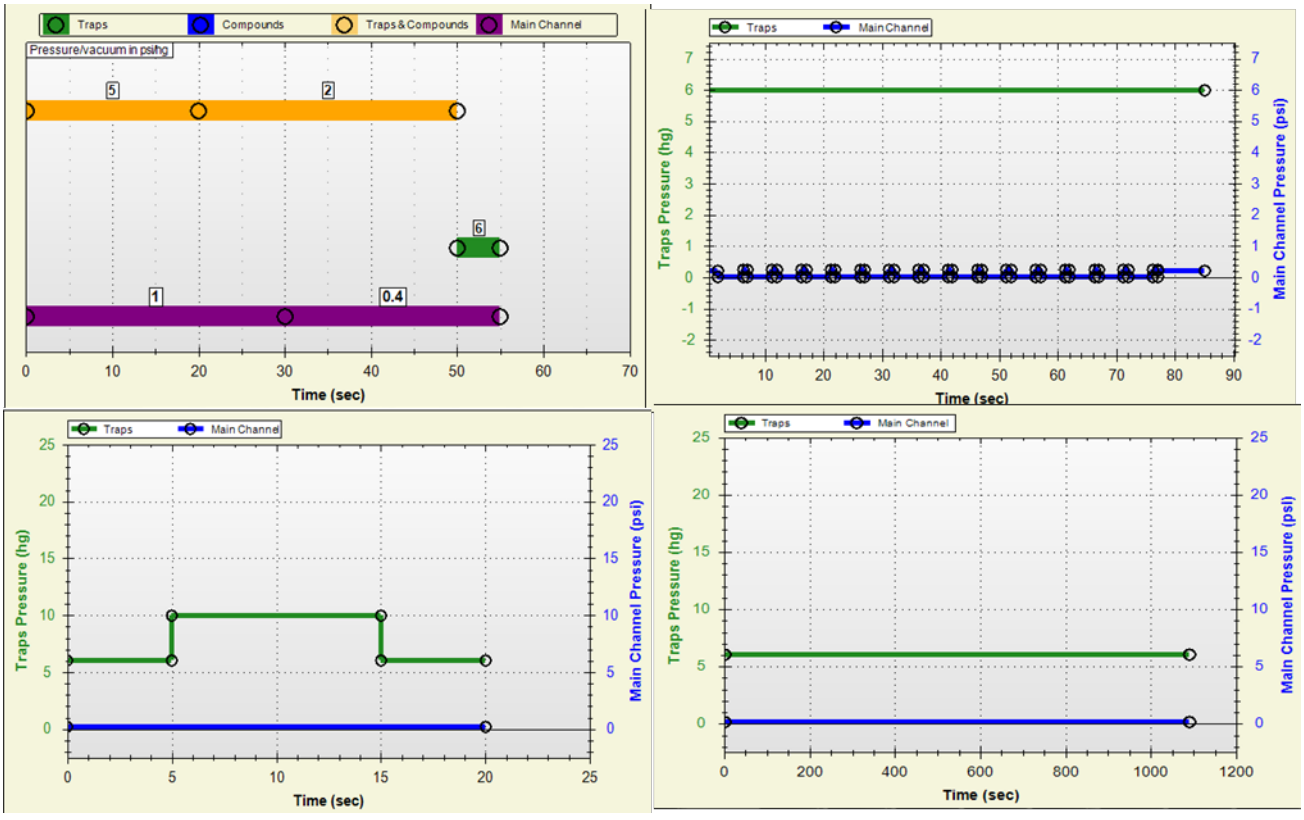
Traps
Compounds
Traps & Compounds
Main Channel
Pressure/vacuum in psihg
5
2
6
1
0.4
Time (sec)
Traps
Main Channel
Traps Pressure (hg)
Main Channel Pressure (psi)
Time (sec)
Traps
Main Channel
Traps Pressure (hg)
Main Channel Pressure (psi)
Time (sec)
Traps
Main Channel
Traps Pressure (hg)
Main Channel Pressure (psi)
Time (sec)

Figure S2. A shows the order and relative durations of the 4 phases in the experiment. B summarizes the pressure settings at the different phases. C is a graphic representation of the pressure settings.

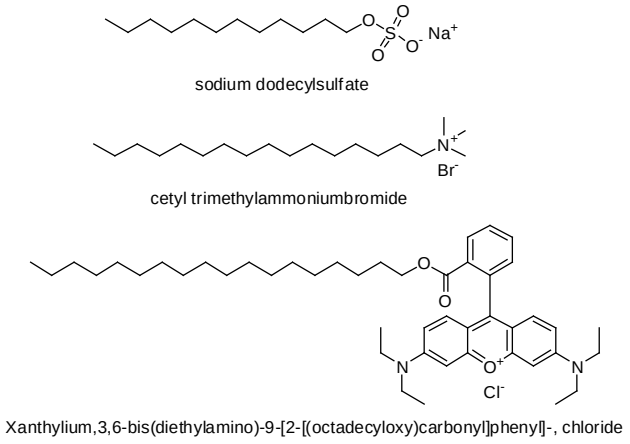

Figure S3. The detergents used in this study are anionic sodium dodecylsulfate and cationic cetyl trimethylammoniumbromide. The fluorescent agent xanthylium, 3,6-bis(diethylamino)-9-[2-(octadecyloxy)carbonyl]phenyl]-,chloride (Octadecyl Rhodamine B, ORB) was used because it is amphiphilic and is known to intercalate in membranes.

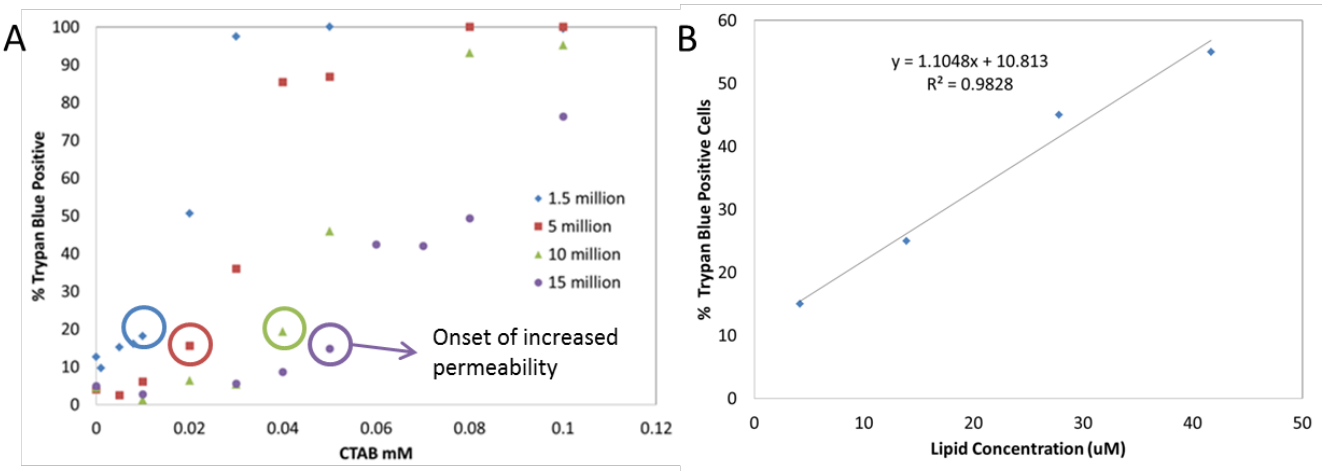

Figure S4: 1.5 – 15 million cells were treated with different concentrations of CTAB for 15 minutes. The cells were centrifuged and resuspended in ECS. 10 µL of cell suspension was added to 10 µL of trypan blue and 80 µL of PBS. The number of trypan blue positive cells was then counted using a hemocytometer. (A). Percent of trypan blue positive cells after treatment with CTAB. Circles indicate the point of onset of increased permeability to trypan blue as an example of point of onset of lipid perturbation. (B). CTAB concentration at the onset of increase in percent of trypan blue positive cells with respect to lipid concentration. The partition constant was thus evaluated similar to experiments reported in literature. The ratio of detergent to lipid in the bilayer (R_b) was 1.1 ± (0.1). The intercept was 11± (3) mM. The partition constant was calculated to be 48,000 M^{-1}.

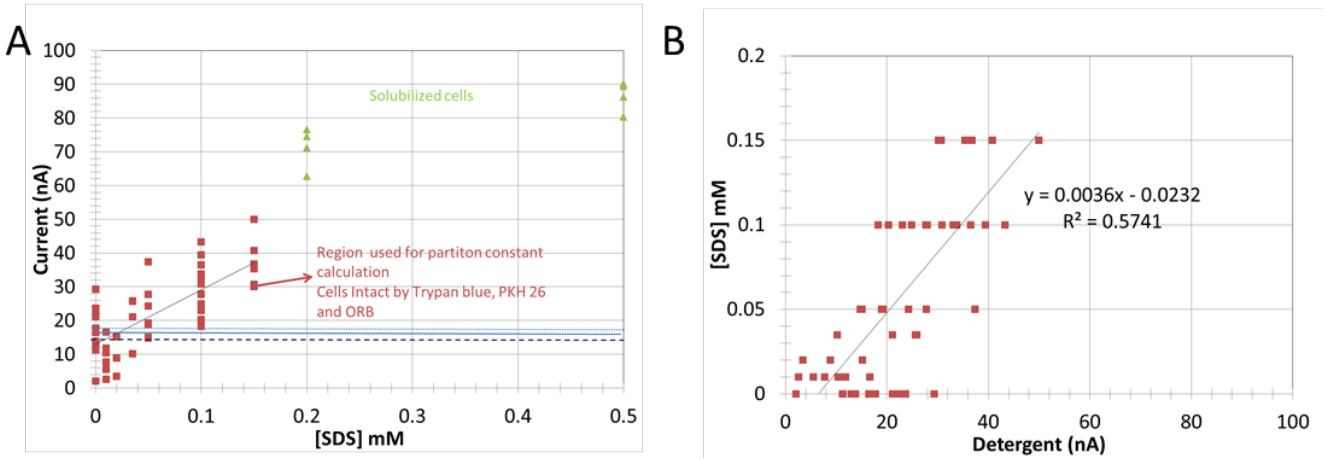

Figure S5. Due to variability in current data, the partition constant for patch clamp study was evaluated in a manner different from studies in literature. **A** shows currents when 1.5 million cells were treated with increasing concentrations of SDS. Data from region I where cells exhibit currents of about 50 nA was used for further analysis. **B** shows the inverse of plot A. The slope in **B** gives the detergent necessary to increase the current by 1 nA. The detergent necessary to increase the current by 15 nA was calculated using the slope from the linear regression in **B**. This was plotted with respect to the lipid concentration (figure 9 B) in order to obtain estimates for R_b and K.

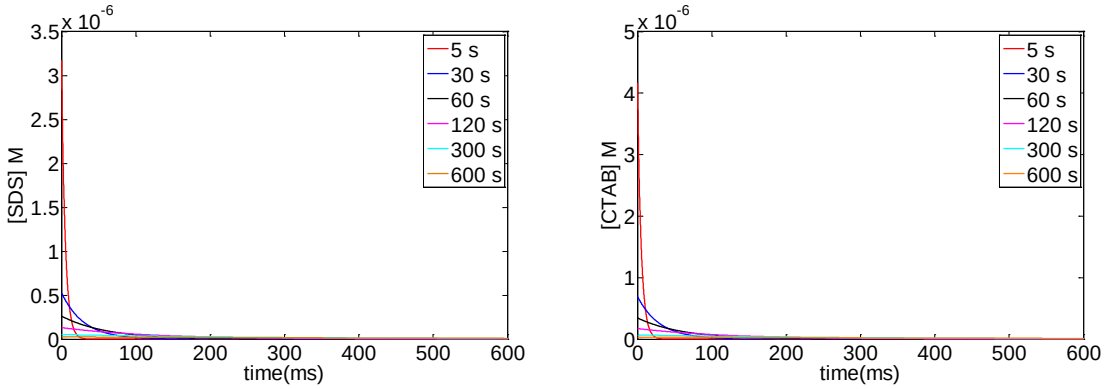

Figure S6: We assumed that a monolayer of detergent molecules was adsorbed on the surface of the microfluidic channel. SDS and CTAB were modeled to have surface areas of 0.3 and 0.4 nm^2 respectively[1,2]. We modeled the concentration of detergent that cells would be exposed to assuming exponential release of the detergent from the surface with time constants ranging from 5 s to 600 s. The highest concentrations of detergent for release profiles with different time constants for SDS and CTAB range from 0.02 - 3 µM and 0.04 – 4 µM CTAB respectively. Thus, cells are exposed respectively to SDS and CTAB at concentrations 70-10,000 fold and 3 - 250 fold less than the concentrations necessary to induce membrane permeability in the kinetic experiments.

Analysis of errors for partition constant:

The partition constant K is calculated using our estimates for R and D_w. Thus, uncertainties in the estimation of R and D_w both contribute to the estimation of K. The first step in estimating the error in the estimation of K is to estimate the uncertainty associated with the values of R and D_w. This is followed by an error propagation formula to estimate the error in K.[3]

Step 1: The standard errors for the slope (R) and intercept (D_w) presented in figure 9B are calculated

In Figure 9B, detergent concentration at 15 nA increase in current (Y) is plotted versus lipid concentration (X) and the data is fit to a line (equation a)

$$Y_{fit} = b_0 + b_1 * X \quad \text{(a)}$$

The variability of the observed Y values around the regression line is given by equation b.

$$s = \sqrt{\frac{(\sum Y_{observed} - Y_{fit})}{n-2}} \quad \text{(b)}$$

Standard error for the estimates for slope (b_1) and intercept (b_0) were calculated using equations c and d.

$$SE_{b_0} = s * \sqrt{\frac{1}{n} + \frac{\bar{X}^2}{(X-\bar{X})^2}} \quad \text{(c)}$$

$$SE_{b_1} = \frac{s}{\sqrt{(X-\bar{X})^2}} \quad \text{(d)}$$

Step 2: Calculation of error in K due to errors in the measurement of R and D_w

Partition constant is calculated as in equation e

$$K = \frac{R + \delta R}{(R + \delta R + 1) * (D_w + \delta D_w)} = \frac{1}{\frac{D_w \pm \delta D_w}{R \pm \delta R} + (D_w \pm \partial D_w)}$$

According to reference 3,

$$\frac{A \pm \delta A}{B \pm \delta B} = \frac{A}{B} \pm \frac{B}{A} \left(\frac{\partial A}{A} + \frac{\delta B}{B}\right)$$

Thus the estimate for K simplifies to

$$K = \frac{1}{\left(\frac{D_w}{R} + D_w\right) \pm \left[\frac{D_w}{R}\left(\frac{\delta D_w}{D_w} + \frac{\delta R}{R}\right) + \delta D_w\right]} \quad \text{(e)}$$

References:

1. de Aguiar, H. B.; Strader, M. L.; de Beer, A. G.; Roke, S. Surface Structure of Sodium Dodecyl Sulfate Surfactant and Oil at the Oil-in-Water Droplet liquid/liquid Interface: A Manifestation of a Nonequilibrium Surface State. *J Phys Chem B* **2011**, *115*, 2970-2978.
2. Ravera, F.; Ferrari, M.; Liggieri, L.; Loglio, G.; Santini, E.; Zanobini, A. Liquid–liquid Interfacial Properties of Mixed nanoparticle–surfactant Systems. *Colloids Surf. Physicochem. Eng. Aspects* **2008**,*323*, 99.
3. Bevington, P. R., *In Data Reduction and Uncertainty Analysis for the Physical Sciences* ; McGraw-Hill: New York, 1969.