Modeling Ion Channels in the Gigaseal

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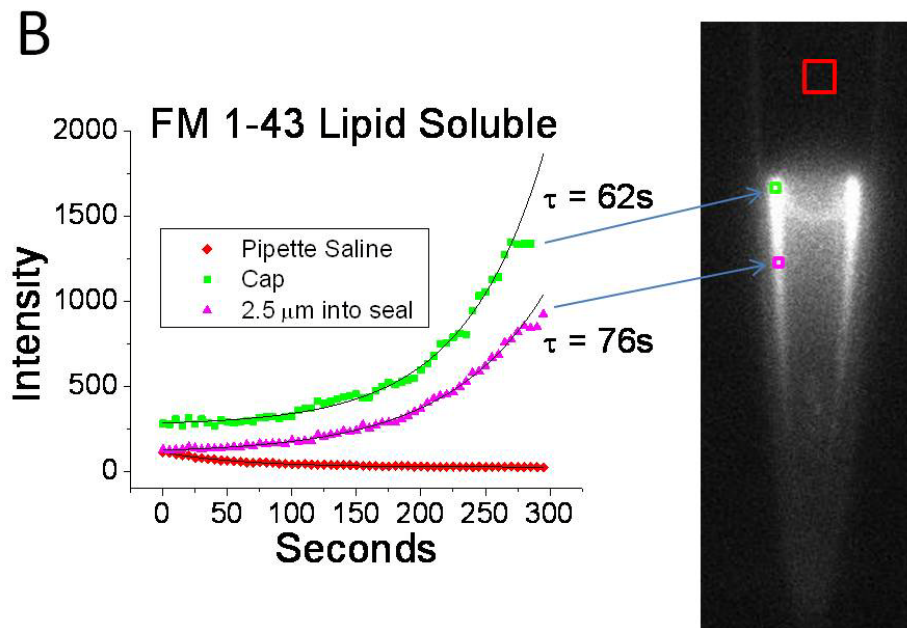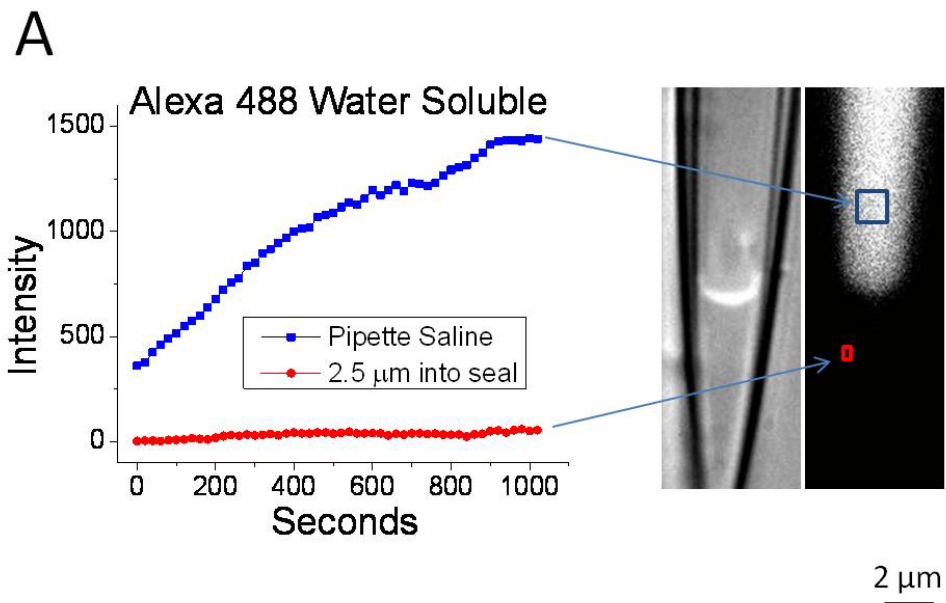
Supporting Material

FIGURE S1. Water and lipid soluble dye movement in the patch seal. A patch pipette was frontfilled 30 seconds with saline containing 140 mM KCl, 10 mM HEPES pH 7.2, 2 mM MgCl$_2$, and 1 mM EGTA. The pipette was then backfilled with the same saline containing dye. A patch was rapidly formed on an HEK cell and the dye was allowed to diffuse down the seal. Bright field and fluorescence (510 nm) images are shown side by side to indicate the patch cap position, and the ROIs (regions of interest) in the pipette saline and the seal region are indicated by colored boxes. Fluorescence was monitored as described in (3).

(A) Alexa 488 intensity in the saline above the seal continues to increase over ~10 min (blue trace) as the backfill diffuses up to the patch, but can observe no fluorescence from the seal (red trace).

(B) With the lipid soluble dye FM 1-43, fluorescence in the seal increases over time (green trace – ROIs shown in the fluorescence image to the left). Over the same time the background fluorescence from unbound FM 1-43 in the pipette saline shows no significant change (red trace). FM 1-43 (611 Da) is slightly larger than the water soluble Alexa 488 (570 Da). The data suggests that the seal bilayer properties are quite uniform since the gradient of fluorescence is smooth.

Analytical model of seal diffusion

A fluorescent probe with concentration $c(x,t)$ diffuses along the membrane with diffusion coefficient D and in may also dissolve in the water of the seal with rate constant k. Its concentration at the dome facing the pipette is A exp(mt) + B (reflecting the backfill diffusion) and in water (under the dome) it is assumed to be negligible. Then the equation is

$$\frac{dc(x,t)}{dt} = D\frac{d^2c(x,t)}{dx^2} - kc(x,t)$$

Eq. S (1)

with initial condition

$$c(x,0) = 0$$

Eq. S (2)

and boundary condition

$$c(0,t) = \phi(t) = A\exp(mt) + B$$

Eq. S (3)

One can solve this equation to obtain the following function

$$c(x,t) = \frac{1}{2}Ae^{mt}\left\{\begin{array}{l}\exp\left(-x\sqrt{\frac{(k+m)}{D}}\right)erfc\left[\frac{x}{2\sqrt{Dt}} - \sqrt{(k+m)t}\right] + \\ \exp\left(x\sqrt{\frac{(k+m)}{D}}\right)erfc\left[\frac{x}{2\sqrt{Dt}} + \sqrt{(k+m)t}\right]\end{array}\right\} +$$

$$\frac{1}{2}B\left\{\begin{array}{l}\exp\left(-x\sqrt{\frac{k}{D}}\right)erfc\left[\frac{x}{2\sqrt{Dt}} - \sqrt{kt}\right] + \\ \exp\left(x\sqrt{\frac{k}{D}}\right)erfc\left[\frac{x}{2\sqrt{Dt}} + \sqrt{kt}\right]\end{array}\right\}$$

Eq. S (4)

By fitting this equation to the observed data (Fig. S1B), we estimate the concentration at the dome ($x_0 = 0$) and at a point, say $x_1 = 2.5$ µm from the dome. While Eq. S4 is rather

cumbersome, it can be simplified for large times. If time t is large enough, Eq. S4 can be reduced to

$$c(x,t) = A\exp\left(mt - x\sqrt{\frac{(k+m)}{D}}\right) + B\exp\left(-x\sqrt{\frac{k}{D}}\right)$$

Eq. S (5)

To find the parameters k, m, and D, one merely has to measure time dependence of concentration in two points, say x_0 and x_1 and solve the corresponding equations. Notice that rate of change of concentration does not depend on the coordinates: it is always given by the same exponential dependence $\exp(mt)$. We fit the raw data of Fig. S1B in both positions simultaneously with the same exponential (Fig. S2) and solved for the diffusion constant.
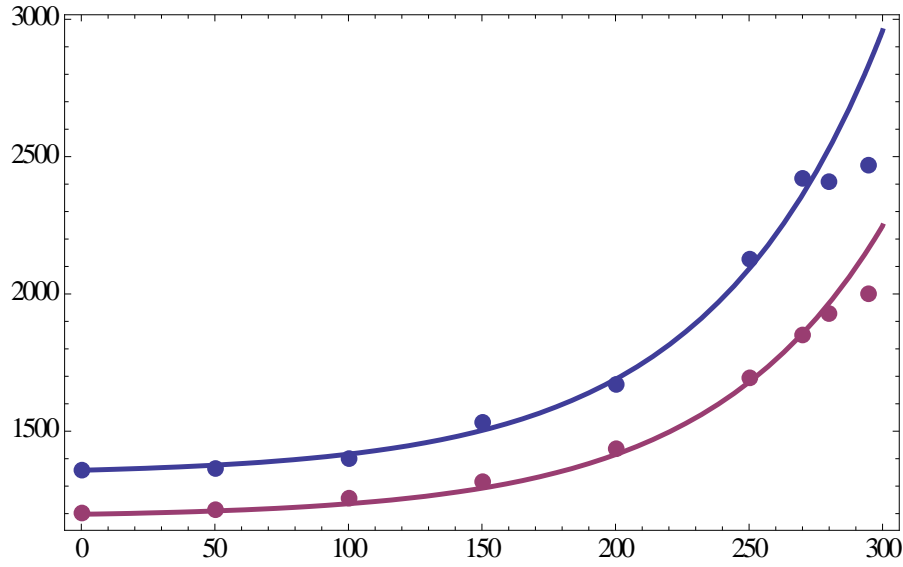

FIGURE S2. A fit of the time dependence of FM1-43 intensity in the seal at positions x=0 and x=2.5 μm (From the data of Fig. S1B).

The fitting curves are for x = 0

$$c(x_0,t) = 16\exp(t/65) + 1342 ;$$

Eq. S (6)

and for x_1 = 2.5 μm

$$c(x_1,t) = 10.5\exp(t/65) + 1187 .$$

Eq. S (7)

Therefore

$$A = 16 \quad \text{and} \quad m = \frac{1}{65} s^{-1}$$

Eq. S (8)

Comparing Eq. S5 and S7 one can find equations for parameters k and D:

$$A\exp\left(-x_1\sqrt{\frac{(k+m)}{D}}\right) = 10.5; \quad B\exp\left(-x_1\sqrt{\frac{k}{D}}\right) = 1187$$

Eq. S (9)

Solution of these equations provides the following parameters:

$$k = 1.427*10^{-3} s^{-1} \quad \text{and} \quad D = 0.592*10^{-8} cm^2 s^{-1}$$

Eq. S (10)

The desorption rate k suggests exchange times on the order of ms making the assumption of an equilibrium distribution of dye in the seal quite reasonable on the time resolution of the experiment.