

① The energy to form a pore is the lower of the hydrophilic and hydrophobic energies. Thus:

- $r \approx 45 kT$ (at $V_m = 0$)
- E^* represents a local maxima for pore formation energy. This occurs at r^*
 $L \approx 0.5 \text{ nm} = \text{min. radius of hydrophilic pores.}$

• h is the membrane thickness $\sim 5 \text{ nm}$

• ϵ_w, ϵ_m are permittivities of water and membrane
 $L 2\epsilon_0 - 5\epsilon_0$
 $L 80\epsilon_0$

$\epsilon_0 = 8.85 \times 10^{-12} \text{ F/m} = \text{permittivity of vacuum}$

★ Notice that in UFR the membrane potential decreases the energy by affecting the capacitive energy stored in the membrane:

- The surface charge density, $g = (\epsilon_m E_m - \epsilon_w E_w)$
 $L \text{ electric field in the cytoplasm}$
 $L \text{ in the membrane}$

In general:

$E_m \gg E_w$ so $g = \epsilon_m E_m$

Example: $E_a^{\text{applied}} = 100 \text{ V/m}$ cell radius = $20 \mu\text{m}$ how do we get E_m ?

$E_m = \frac{V_m}{h} \Rightarrow g = \epsilon_m \frac{V_m}{h}$ how do we get V_m ?

\Rightarrow Spherical cell model!

at $\theta = 0 \Rightarrow V_m = \left(\frac{3}{2}\right) \left(\frac{R_m}{R_m + R_a}\right) E_0 r \cos \theta$ at $\theta = 0$
 $\text{if } R_m \gg R_a \text{ we can ignore and}$

$V_m = 3 \text{ mV} \Rightarrow E_m = 6 \times 10^5 \text{ V/m} \Rightarrow g = 2.66 \times 10^{-4} \text{ C/m}^2$ ($\epsilon_m = 5\epsilon_0$)

If the charge exists in the form of monovalent ions ($1.6 \times 10^{-19} \text{ C}$)

$\Rightarrow \sim 1700 \text{ ions/nm}^2$

$U = \frac{1}{2} \frac{Q^2}{C} = \boxed{\frac{1}{2} QV}$

So the energy stored by this charge separation reduces the energy required to form a pore.

2) Now, if we apply ~~an~~ an electric field to a spherical cell in a bath what will happen?

- Suppose there is a threshold value for E_m that causes pore formation
→ What is the response of the spherical cell?
- What is its baseline state?

$$V_{m,rest} \approx -80 \text{ mV} \Rightarrow E_m \approx \frac{80 \text{ mV}}{5 \text{ nm}} = \boxed{160 \frac{\text{KV}}{\text{cm}} !}$$

- Now suppose we apply a field. Will the membrane respond uniformly?

No: The region of hyperpolarization (near the anode) is preferentially electroporated why?

This goes away ^{partially} when working with biphasic pulsed and completely with AC pulses where:

$$V_m = \left(\frac{3}{2}\right) \left(\frac{R_m}{R_m + R_a}\right) E_0 r \cos \theta \underbrace{\left(1 + (2\pi f r)^2\right)^{-1/2}}_{\text{AC steady state term}}$$

Back to pore energy: Why does $E(r)$ look different?

For hydrophilic pores:

$B = 1.4 \times 10^{-19} \text{ J}$ - "steric repulsion energy"

$\gamma = 1.8 \times 10^{-11} \text{ J/m}$ - "Edge energy"

σ = Surface tension of ~~pore~~ ^{bilayer} = "membrane tension"

F = electric force

$$E(r) = (\text{1st term}) + (\text{second term}) - (\text{third term}) - (\text{fourth term})$$

1st term = steric repulsion of polar heads as $r \rightarrow 0$ ($E(r)$ increases)

2nd term = Energy to "bend" the bilayer to form the hydrophilic pore lining.

3rd term = The pore decreases the membrane tension lowering energy.

★ 4th term = Effect of V_m . Different from hydrophobic pores because now it can conduct current flow. → not a dielectric.

$r_h = 0.97 \text{ nm}$ = characteristic length for electric force

$r_t = 0.31 \text{ nm}$ = correction for toroidal shape.

→ "Electric force to expand the pore.

When $r < r_t \Rightarrow$ hydrophobic

3) Creation and rescaling of pores:

A population of pores existing at time, t has a "pore density ~~function~~ distribution," $n(r, t)$ such that the number of pores per unit area with radii between r and $r+dr$ is:

$n(r, t) dr \rightarrow$ this term has V in it, but we won't look at it specifically.

Under certain conditions New and Krassowski showed that

$$N(t) = \int_0^{\infty} n(r, t) dr \quad \text{is the density of hydrophilic pores.}$$

The equilibrium pore density for a given V_m is:

$$N_{eq}(V_m) = N_0 e^{g(V_m/V_{ep})^2} \quad \text{where}$$

$N_0 = 1.5 \times 10^9 \text{ m}^{-2}$ = equilibrium pore density at $V_m = 0$

$g = \left(\frac{r_m}{r_*}\right)^2$ $r_m = 0.8 \text{ nm}$ = min. energy radius at $V_m = 0$

$V_{ep} = 0.258V =$ characteristic voltage of electroporation

We can now define the temporal relationship for pores:

$$\frac{dN}{dt} = \alpha e^{(V_m/V_{ep})^2} \left(1 - \frac{N}{N_{eq}(V_m)}\right) \quad (\star)$$

So:

- Hydrophilic pores appear at a rate exponentially dependent on the square of V_m
- Note: There is not a distinct threshold for pore formation, but a large increase does occur at $V \sim V_{ep} \Rightarrow V_m < 1V \sim$ "subthreshold"
- This can also describe rescaling after a shock (when N is larger than N_0). The rescaling time constant is $\sim 3s$.
- The only pores that rescale according to (\star) are ones with r near r_m . If $r \gg r_m$ it is either more complicated or irreversible.

④ I-V relationships for pores:

The total current, I_p , through K pores is:

$$I_p(t) = \sum_{j=1}^K i_p(r_j, V_m) \quad i_p = \text{I-V relation for one pore.}$$

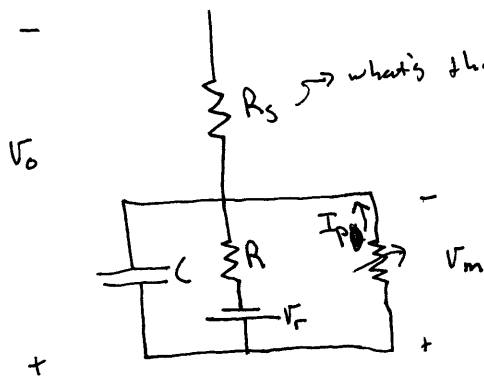
With a cylindrical pore:

$$R_p = \frac{h}{S \pi r^2} \quad S = \text{conductivity of pore solution} \approx 2 \text{ S/m}$$

To account for large pores where the trans-membrane potential is lower:

$$i_p(r, V_m) = \frac{V_m}{R_p + R_i} \quad R_i = \frac{1}{2S r}$$

Since pores contribute a membrane current they impact V_m :



$$C = C_m A$$

$$R = R_m / A$$

what's this? From which we get:

$$R_s C \frac{dV_m}{dt} = V_0 - V_m - \frac{R_s}{R} (V_m - V_r) - R_s I_p$$

This assumes:

- 1) C is constant (though it actually decreases by $\sim 2\%$)
- 2) No active membrane currents. These were found to be negligible compared to Electroporation by De Bruin and Krassowska.

Several phases:

- 1) Membrane charging
- 2) Pore creation
- 3) Pore evolution
- 4) Postshock pore shrinkage
- 5) Resealing.

1) Membrane Charging:

Assuming a $50 \mu\text{m}$ spherical cell exposed to $V_0 \gg V_r$, ms shock let's look at the phases. Initially the membrane is intact and at rest.

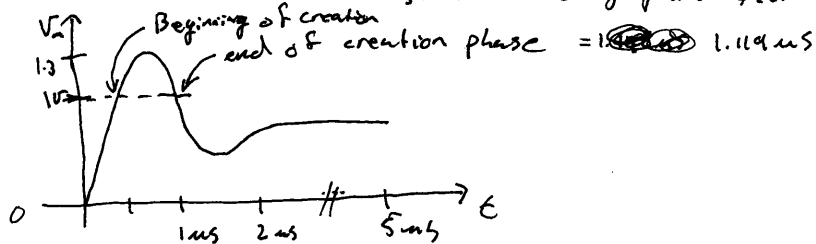
• Since there are no pores initially $I_p = 0 \Rightarrow$

$$V_m(t) = V_{\text{rest}} + \frac{V_0}{1 + R_s/R} (1 - e^{-t/\tau}) \quad \tau = \frac{R_s C}{1 + R_s/R}$$

5) If V_0 is below threshold then this process charges as we've seen before.
For stronger V_0 this model breaks down since pores will begin to form.
The first pore is formed when the density (N) times the membrane area (A) is equal to 1. This happens at $\sim 0.652 \mu s$ for a $3V$ shock.

2) Pore Creation:

When V_m exceeds threshold ($\sim 1V$ here) the pore formation rate markedly increases (as before). This shunts current across the membrane through a relatively low resistance. This slows the charging and then reverses it.



3) Pore evolution:

A much slower process than 1,2.

Hydrophilic pores are $r_* = 0.5 nm$ at first. They then grow but to no more than $4 nm$ by the end of the creation phase.

They expand or shrink due to two factors

1) Drift - $\frac{dr}{dt}$ to decrease bilayer energy
 $\hookrightarrow =$ ~~a function of E~~ a function of E

2) Diffusion - random increases/decreases in r due to thermal fluctuations

\rightarrow Most pore evolution happens after creation has stopped and V_m has reached a steady state

\rightarrow Small $\sim 1 nm$ pores and much larger ones form. Not a smooth transition in between.

4) Postshock Shrinkage:

Only $2 \mu s$ after the shock the membrane potential dropped to $-0.024 V$ from $0.4 V$. ($V_r = -0.08$ normally, but the persistent pores lead to some residual depolarization)

When all "large" pores become "small" $\sim 1 nm$ pores this phase ends.

This takes $\sim 22 \mu s$.

5) Pore resealing: Pores disappear and membrane integrity (and conductivity) is restored
 $t = 35 \mu s$ and $V_m \rightarrow V_r$