Maintaining an osmotic balance

- **Different strategies**
  - Animal: pumping ions out
  - Plants/some bacteria: using a rigid shell
  - Amoeba: extruding water bubble periodically

Alberts, *et al.*, Molecular biology of the cell
Membranes as insulators

the hydrophobic nature of pure membranes prevents charges from crossing it

simple theoretical predictions estimate 100 kT to dehydrate an ion!!!
Nernst potential

charge separation means membrane can act as a **capacitor**

\[ c(x) \propto e^{-qV(x)/kT} \]

\[ \frac{c_1}{c_2} = \frac{e^{-zeV_1/kT}}{e^{-zeV_2/kT}} \]

\[ \Delta V = V_2 - V_1 = \frac{kT}{ze} \ln \frac{c_1}{c_2} \]

\( kT/e \sim 25 \text{ mV}, \text{ same order of magnitude as membrane potential (-100 mV)!} \)

**Nernst equation** relates chemical potential to electric potential.

+ , can cross  - , blocked

**PBoC 17.2.2**
Ion channels

Ion channels are used by cells to maintain concentrations out of equilibrium. The Nerst potential is the membrane potential at which there is no net flow of that ion, even if all ions can cross freely. The potential will be non-zero due to negatively charged proteins and DNA inside the cell.

Resting membrane potential: -90 mV

Cells use (gated) ion channels and pumps to maintain concentrations out of equilibrium.
Potassium (K+) channels

ubiquitous in living organisms
tetramer (four subunits)
over 80 types in mammals

highly selective for K+, even over smaller Na+
the pore is dynamic! Selectivity arises due to unique electrostatic environment created by backbone carbonyls


Voltage gating

for some channels, voltage induces conformational changes, opening or closing it

\( f \) is the fraction of the distance the charges move

\[
p_{\text{open}} = \frac{e^{-\beta \Delta \epsilon}}{1 + e^{-\beta \Delta \epsilon}}
\]

\[
\Delta \epsilon = \Delta \epsilon_{\text{conf}} - Q_f V_{\text{mem}}
\]

“gating charge” (\( Q_f \))

Voltage shifts the energy difference between two open and closed states

simulations measured gating charge to be 10.25e, close to exp. value of 13e

opening has sharp dependence on voltage (sigmoidal curve) - as expected for two-state system

Calculation of the gating charge for the Kv1.2 voltage-activated potassium channel.
Voltage gating models for K+ channel from MacKinnon!

helical screw now favored, arginines never exposed directly to membrane

paddle model largely ignored now


ion pumps

expend energy to drive ions against their electrochemical potentials

necessary to establish large concentration gradients, maintaining them uses 20-40% of the cell’s energy! (up to 70% in neurons)

crystal structure of Na+/K+ ATPase

Crystal structure of the sodium-potassium pump at 2.4 Å resolution
Shinoda, Ogawa, Cornelius & Toyoshima
Nature 459, 446-450 (2009)
numerous static structures permit rough visualization of catalytic cycle

Ca\textsuperscript{2+} ATPase
leak channels

K+ leak channel establishes the electric potential gradient, makes $V_{\text{mem}} = V_{\text{Nerst}}(\text{K}^+)$

crystal structure of two-pore domain K+ leak channel

gradients have multiple uses

ion gradients aren’t just for transporting other ions; they can be used to power transport of a number of other substrates (secondary active transporters)

Alberts, et al., Molecular biology of the cell
circuit model of ion channels

biological circuits are analogous to electronic ones

\[ C = \frac{Q}{V} \text{ (charge build-up near membrane)} \]

each channel is like a resistor with conductance \( g \)

\[ I = g(V_{\text{mem}} - V_{\text{Nernst}}) \text{ Ohm’s Law} \]
voltage gating in the circuit

For voltage-gated channels, the conductance $g$ becomes a function of $V$.

Result is a non-linear $I$-$V$ curve.
bistable switching

\[ \Delta Q = -(I_K + I_{Na}) \Delta t = C \Delta V_{mem} \]

\[ C \frac{dV_{mem}}{dt} = g_K(V_{Nernst}^K - V_{mem}) + g_{Na}(V_{Nernst}^{Na} - V_{mem}) \]

\[ V_{mem} = \frac{g_K V_{Nernst}^K + g_{Na} V_{Nernst}^{Na}}{g_K + g_{Na}} \]  
(steady state)

\[ g_{Na} = g_{Na}^{open} \frac{1}{1 + e^{\beta q(V^* - V_{mem})}} \]

two cases: \( g_{Na} \gg g_K \) or vice versa

\[ \frac{dV_{mem}}{dt} \]
\[ V_{mem} \]

\[ V_{Nernst}^K \quad V^* \quad V_{Nernst}^{Na} \quad V_{mem} \]

fixed point  
fixed point

\[ \Delta Q = -(I_K + I_{Na}) \Delta t = C \Delta V_{mem} \]

\[ V^* \] (threshold) determines switch between two dominant states
Action potentials

voltage pulse that travels down the cell membrane
Action potentials

1) signal raises voltage above threshold
2) voltage-gated Na+ channels open
3) membrane depolarizes
4) neighboring Na+ channels open (positive feedback loop)
5) Na+ channels close, K+ channels open, reversing the potential back to resting state
source of the signal

- Signals are converted from electrical to chemical at the end of one neuron and then converted back to electrical at the next neuron (chemical synapse)

- Neurotransmitters are either **excitatory** (open Na+ channels, initiating depolarization)
  - acetylcholine
  - serotonin
  - glutamate

- or **inhibitory** (open Cl- channels)
  - γ-Aminobutyric Acid (GABA)
  - glycine

- Cells can also join connexons (specialized channels) directly at so-called gap junctions for faster response (electrical synapse)
Cable equation

Discretize membrane into small patches

Kirchhoff’s rules

$$i(x - dx) - i(x) = i_r(x) = \Delta g_{\text{patch}} [V(x) - V_N] + \Delta C_{\text{patch}} \frac{\partial V(x, t)}{\partial t}$$

Ohm’s Law

$$V(x + dx) - V(x) = - i(x) R_{\text{int}}$$

$$\frac{dV}{dx} = - \frac{R_{\text{int}}}{\Delta x} i(x)$$

Cable equation!

$$\lambda^2 \frac{\partial^2 V(x, t)}{\partial x^2} - \tau \frac{\partial V(x, t)}{\partial t} = g_{\text{patch}} [V(x) - V_N]$$
properties of the cable equation

if \( g \) is constant (i.e., does NOT depend on \( V \)), then **linear** cable equation results in:

\[
\lambda^2 \frac{\partial^2 V(x, t)}{\partial x^2} - \tau \frac{\partial V(x, t)}{\partial t} = \alpha V(x, t)
\]

result is a passive spread that decays quickly over distance (no traveling wave)

response is **all or nothing**

resulting potential is independent of signal
depolarization waves

numerical solutions to the (non-linear) cable equation

signal below threshold voltage potential decays over time

signal above threshold voltage traveling depolarization wave, but no spikes! (still not identical to action potential)

PBoC 17.4.3
Hodgkin-Huxley model

squid giant axon - up to 10 cm in length, 1 mm in diameter!

large size makes experiments easy, permitted Hodgkin/Huxley to develop mathematical model of nerve cell behavior

J. Physiol. (1952) 117, 500-544

A QUANTITATIVE DESCRIPTION OF MEMBRANE CURRENT AND ITS APPLICATION TO CONDUCTION AND EXCITATION IN NERVE

By A. L. HODGKIN and A. F. HUXLEY
From the Physiological Laboratory, University of Cambridge

Alan Hodgkin  Andrew Huxley
1 paper = 1 Nobel Prize (1963)

*actually five papers, but this was the important one
Hodgkin-Huxley model

**REMEMBER**: conductivities \( G \) are functions of \( V \) themselves!

States of a given channel (or gate within a channel):

Master equation:

\[
\frac{dp_i}{dt} = \alpha_i(V) (1 - p_i) - \beta_i(V) p_i
\]

Steady state at resting, clamping voltages

\[
\frac{dp_i}{dt} = 0
\]

\[
\begin{align*}
p_i^0(V_r) &= \frac{\alpha_i(V_r)}{\alpha_i(V_r) + \beta_i(V_r)} \\
p_i^\infty(V_c) &= \frac{\alpha_i(V_c)}{\alpha_i(V_c) + \beta_i(V_c)}
\end{align*}
\]

Probability for a single gate:

\[
p_i(t) = p_i^\infty(V_c) - [p_i^\infty(V_c) - p_i^0(V_r)] e^{-t/\tau_i(V_c-V_r)}
\]
Hodgkin-Huxley model

\[ p_i(t) = p_i^\infty (V_c) - [p_i^\infty (V_c) - p_i^0 (V_r)]e^{-t/\tau_i (V_c-V_r)} \]

conductivity may depend on more than one “gate” that must all be open at once

\[ G_i = \bar{g}_i \prod_i p_i \]

HH model fit to experimental data

K+: best fit produced by 4 gates, identical response is sustained (all gates activating)

\[ G_K = \bar{g}_K n^4 \]

conductivity for K+  

Hodgkin-Huxley model

\[ p_i(t) = p_i^\infty(V_c) - [p_i^\infty(V_c) - p_i^0(V_r)]e^{-t/\tau_i(V_c-V_r)} \]

colorbox{conductivity with different driving voltages}

\[ G_i = \bar{g}_i \prod p_i \]

response for Na+ is transient, (at least) one gate inactivates channel

Na+: best fit produced by 3 activating gates and a single inactivating gate

\[ G_{Na} = \bar{g}_{Na} m^3 h \]

Important note: everything at this stage is just parameter fitting!

putting it all together

\[
\lambda^2 \frac{\partial^2 V(x, t)}{\partial x^2} - \tau \frac{\partial V(x, t)}{\partial t} = \\
\bar{g}_N m^3 h [V(x, t) - V_{Na}] + \bar{g}_K n^4 [V(x, t) - V_K] + \bar{g}_L [V(x, t) - V_L]
\]

(1)

\[
\frac{dn}{dt} = \alpha_n(V)(1 - n) - \beta_n(V)n
\]

(2)

\[
\frac{dm}{dt} = \alpha_m(V)(1 - m) - \beta_m(V)m
\]

(3)

\[
\frac{dh}{dt} = \alpha_h(V)(1 - h) - \beta_h(V)h
\]

(4)

One simplifying approximation: shape of the wave does not change, just moves with speed \(\theta\)

\[
\frac{\partial^2 V(x, t)}{\partial x^2} \approx \frac{1}{\theta^2} \frac{\partial^2 V(x, t)}{\partial t^2}
\]

no Matlab in 1952!!!
putting it all together

Complete model (solved numerically) now reproduces action potential spikes!

Original fit from 1952 is excellent!

“Only one calculation was complete; the other two calculations were not carried beyond the middle of the falling phase due to the labor involved…”

Hodgkin and Huxley, 1952, J. Physiol.
what is going on at the molecular level?

Hodgkin and Huxley did not even know that *channels* existed, let alone *gates* within them!

now we understand more about the basis of the action potential

both Na+, K+ channels start (and end) in closed state

http://faculty.washington.edu/chudler/ap.html
what is going on at the molecular level?

different channels (even in the same family) open at different voltages and with different speeds (this affects those $\alpha(V)$ and $\beta(V)$ terms)

![Diagram showing various potassium channel response curves](image)

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What are the “gates”?

In Na+ (and K+) activation gates are the voltage sensors, which are not necessarily independent but all must move to open.


Full channel is actually over 2000 residues long, comprised of homologous sub-domains

inactivation gate is at loop between two domains - moves to block channel
Applied electric field

In simulation, voltage is imposed through applied $E$ field

solution reacts to the field

field is canceled in the bulk solution; potential difference $V$ is focused to membrane (non-conducting) region

$V = L_z E_{app}$

Constant electric field simulations of the membrane potential illustrated with simple systems.
Applied electric field

If simulation box is **doubled** in length, how should $E$ change to keep $V$ constant?

$$V = L_z E_{\text{app}}$$

but what to use for $L_z$?

Applied electric field

but what to use for $L_z$?

potential drop is focused to membrane - maybe use $L_{\text{mem}}$?

in that case, $E_{\text{app}}$ should not change to keep $V$ the same

No!!!

Regardless of how system reacts, $V$ is a function of $L_z$

$L_z = L$, $E_{\text{app}} = E_0$

$L_z = 2L$, $E_{\text{app}} = 0.5 E_0$

trapezoidal cutout
and a pore

**Note:** non-equilibrium properties can depend on $L_z$!

“To obtain the applied voltage, $V$, from $E$, we assumed that the entire potential drop occurs across the SF (6, 59); this implies $V = E\Delta z$, where $\Delta z = 13.4 \pm 0.2$ Å is the distance between Thr374:Oy and Tyr377:O...”

claimed quantitative agreement of $IV$ curves with experiments

But $V$ was under-reported by a factor of 6!!!

a “correction” is given

At high voltages, the permeation rate was in accordance with our previously reported $K_{\text{v}1.2}$ pore-only simulations, after the simulated voltages from the previous study were recalculated using the correct method, new insight into which is provided here.

and in the Appendix

“We here present a different derivation of the relation $V = l_z \cdot E$ between the applied field and the potential drop that is intended to make more intuitively clear why this relation holds and the relation $V \approx l_M \cdot E$ ($l_M$ is the membrane thickness) that we used earlier (Jensen et al., 2010) is incorrect.”
comparing the $I/V$ curves
A problem! the two solutions are continuous due to periodic boundary conditions

one solution: have **two** membranes and thus **two** separate solvent regions

*computationally expensive!*

simulating concentration gradients

another solution: change the chemical potential of the ion species in the two compartments

apply a small force \( f \) at the box edge to create an energy difference

volume, not voltage

\[ p_1 = \frac{V_1 e^{-\epsilon}}{V_1 e^{-\epsilon} + V_2} \]

\[ p_2 = \frac{V_2}{V_1 e^{-\epsilon} + V_2} \]

\[ \epsilon = f d / kT \]

Assuming \( V_1 = V_2 \),

\[ r = \frac{C_1}{C_2} = e^{-f d / kT} \]

Molecular dynamics simulations of membrane proteins under asymmetric ionic concentrations.

simulating concentration gradients

OmpF, 1M KCl above, 0.1 KCl below

reversal potential cancels out field from the ions, given by Goldman-Hodgkin-Katz (GHK) equation:

\[
V_{\text{rev}} = \frac{kT}{e} \ln \left( \frac{p_K [K^+]_o + p_{Cl} [Cl^-]_i}{p_K [K^+]_i + p_{Cl} [Cl^-]_o} \right)
\]

\[V_{\text{rev}} = 26-27 \text{ mV (experiment)}\]

\[V_{\text{rev}} = 28.6 \text{ mV (simulation)}\]