Acetylcholine receptor - 5 subunits \((2x, 1y, 1y, 1d)\): 4 homologous genes
- when 2 acetylcholine bind, opens lets in Na+
- 5 bent \(\alpha\)-helices inside, as shown by crystal structure
- when ligands bind, \(\alpha\)-helices swivel so channel opens; also forms polar environment to let ions through
  - 3rd amino acid charged lysine or arginine: S5/S6 make channel,
  - S4 moves through membrane
  - (on outside of membrane in excited cell, inside in resting cell)
  - \(\alpha\)-helices no polarines, hydrophobic amino acids
  - \(\gamma\) when positive inside; opens channel

Gating of ion channels selective: \(\text{Na}^+ \quad \text{K}^+\)
- size filtration w/ naked ions lets \(\text{Na}^+\) through, \(\text{K}^+\) out
- but ions don't exist naked in aqueous solution; have waters of hydration
  - the smaller the naked ion, the bigger the shell of hydration, so smaller ions actually bigger
  - to conduct naked ions through channel, must line up polar amino acids to replace/mimic shell of hydration
  - let through naked \(\text{K}^+\) (w/ polar proteins) but only sterically mimics shell of hydration of \(\text{K}^+\), \(\text{Na}^+\) too small to be fooled (can't touch enough polar groups)

Cloning membrane proteins:
1. purify, sequence, read genetic code backwards to make oligonucleotides, probe cDNA library
   - but hard to purify bic lipid proteins: need 2 tricks:
     1. pick animals full of channel proteins, eg: Torpedo torpido and Electrophorus electricus (electric eels and rays): electric organs full of channel proteins

2. cobra toxin binds to \(\text{ACh}\) channel and blocks, you can't breathe, etc
   - krait (seasnake, Bungarus multicinctus) makes bungarotoxin, principle component is
α-bungarotoxin: like cobra toxin but tighter binding (use for ACh receptor binding)

- get toxin, label w/ radioactive iodine, bind to electric animal homogenate, etc.
  use Edman degradation to get oligonucleotide to probe libraries

- inject cDNA into Xenopus oocytes (lots of ribosomes etc. waiting to be fertilized so can start translating protein)
  - if put in foreign DNA, can be made & trafficked right away
  - clone all 4 genes for 5 subunits; put in oocyte, express channel protein (test by
    patch clamp w/ ACh in micropipette, look for currents of right size)

- this system also worked for voltage-gated Na⁺ channel, but need different toxin
  - pufferfish fugu makes tetrodotoxin, causes action potential to disappear by blocking Na⁺ channels
  - liver has a lot of tetrodotoxin

2. once you get some channels, other often homologous (eg Na⁺ homolog to Ca²⁺ channels)

3. if no high-affinity toxin, take mutants (eg shaker), map mutation, clone gene positionally,
   look for membrane-spanning regions, test in oocytes

- Na⁺ Cl⁻ (in seawater): did fine until it wanted to eat
  ate proteins (negative charge)
  Na⁺ went in to balance charge,
  more ions inside → osmotic pressure,
  1st living thing blows up

- need to take control of own ionic destiny: fill self up w/ different type of ion so can
  balance ion coat concentrations inside, outside; eg w/ Na⁺/K⁺ pump (K⁺ uncommon
  in seawater)

- blood similar to seawater; more NaCl in it than in cells; more K⁺ in cells

- Ca²⁺ very low in cell, bc used as messenger (chide it eg in mitochondria, rise only when
  you want to have signal, eg synaptic vesicle fusion)

Na⁺ K⁺ ATPase pumps K⁺ in, Na⁺ out: makes 2 gradients

weaken, digitalis toxins that block this pump
Gradients + channels give you energy:

- will not flow until equal: when equilibrium reached, energy gained by going down concentration gradient will balance energy lost by going up voltage gradient: **Nernst equilibrium**
- can find voltage w/ **Nernst equation**

At Nernst equilibrium, for an ion crossing barrier: $\Delta E_{\text{chem}} = \Delta E_{\text{electrical}}$

- $G$ (free energy) = $G^{\circ} + RT \ln [K^+]$
  - $G^{\circ}$ energy in stuff itself
  - $\ln [K^+]$ energy to squash $K^+$ in concentrated form
    b/c hydrated, act like ideal gas

$$\Delta E = RT \ln \left[ \frac{[K^+]_{\text{initial}}}{[K^+]_{\text{final}}} \right]$$

Subtractive logs divide:

$$\Delta E = RT \ln \left[ \frac{[K^+]_{\text{initial}}}{[K^+]_{\text{final}}} \right]$$

$voltage = work\ done\ transporting\ charge\ up\ potential\ gradient\ divided\ by\ charge$

$$\Delta E_{\text{elec}} = qV$$

- for mole of electrons, $q = zF$
  - $z$ charge (e.g., +2 for $Ca^{2+}$, +1 for $K^+$)

$$RT \ln \left[ \frac{[K^+]_{\text{initial}}}{[K^+]_{\text{final}}} \right] = zF$$

Solve for voltage: $V = \frac{RT}{zF} \ln \left( \frac{[K^+]_{\text{initial}}}{[K^+]_{\text{final}}} \right)$
inside $K^+$-rich cell membrane only permeable to $K^+$ (not quite true, but approximate).

- net positive charge $\rightarrow$ outside, so inside negative

- practically, at $25^\circ C$, $V = 58 mV \log \frac{I_{in}}{I_{out}}$

\[
\frac{[28]}{58 mV \log \frac{I_{in}}{I_{out}}}
\]

\[
= \frac{58}{\log 20} = 58 (-\log 20)
\]

\[
= 58 (-1.301) = -69 mV
\]