“Life” (or Molecular biology) exists at low Reynolds number, in salty water, and in a thermal bath!

What is the consequence of molecular biology happening in salty water?

Nerve signals! And Coulomb’s law is not the same!

THE NOBEL PRIZE IN PHYSIOLOGY OR MEDICINE 2023

Illustrations: Niklas Elmehed

Katalin Karikó Drew Weissman

"for their discoveries concerning nucleoside base modifications that enabled the development of effective mRNA vaccines against COVID-19"

THE NOBEL ASSEMBLY AT KAROLINSKA INSTITUTET

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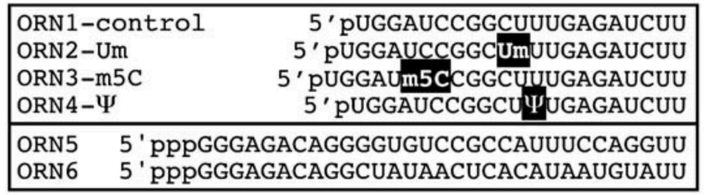

Suppression of RNA Recognition by Toll-like Receptors: The Impact of Nucleoside Modification and the Evolutionary Origin of RNA

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Summary

DNA and RNA stimulate the mammalian innate immune system through activation of Toll-like receptors (TLRs). DNA containing methylated CpG motifs, however, is not stimulatory. Selected nucleosides in naturally occurring RNA are also methylated or otherwise modified, but the immunomodulatory effects of these alterations remain untested. We show that RNA signals through human TLR3, TLR7, and TLR8, but incorporation of modified nucleosides m5C, m6A, m5U, s2U, or pseudouridine ablates activity. Dendritic cells (DCs) exposed to such modified RNA express significantly less cytokines and activation markers than those treated with unmodified RNA. DCs and TLR-expressing cells are potently activated by bacterial

thetic antiviral compound R-848 (Jurk et al., 2002), but a natural ligand has not been identified.

It has been known for decades that selected DNA and RNA molecules have the unique property to activate the immune system. It was discovered only recently that secretion of interferon in response to DNA is mediated by unmethylated CpG motifs acting upon TLR9 present on immune cells (Hemmi et al., 2000). For years, bacterial and mammalian DNA were portrayed as having the same chemical structure, which hampered the understanding of why only bacterial, but not mammalian, DNA is immunogenic. Recently, however, the sequence and structural microheterogeneity of DNA has come to be appreciated. For example, methylated cytidine in CpG motifs of DNA has proven to be the structural basis of recognition for the innate immune system. In light of this finding and given that multiple TLRs respond to RNA, a question emerges as to whether the immunogenicity of RNA is under the control of similar types of modification. This possibility is not unreasonable given that RNA undergoes nearly one hundred different nucleoside modifications (Rozenski et al., 1999). Importantly, the extent and quality of RNA modifications depend on the RNA subtype and corre-

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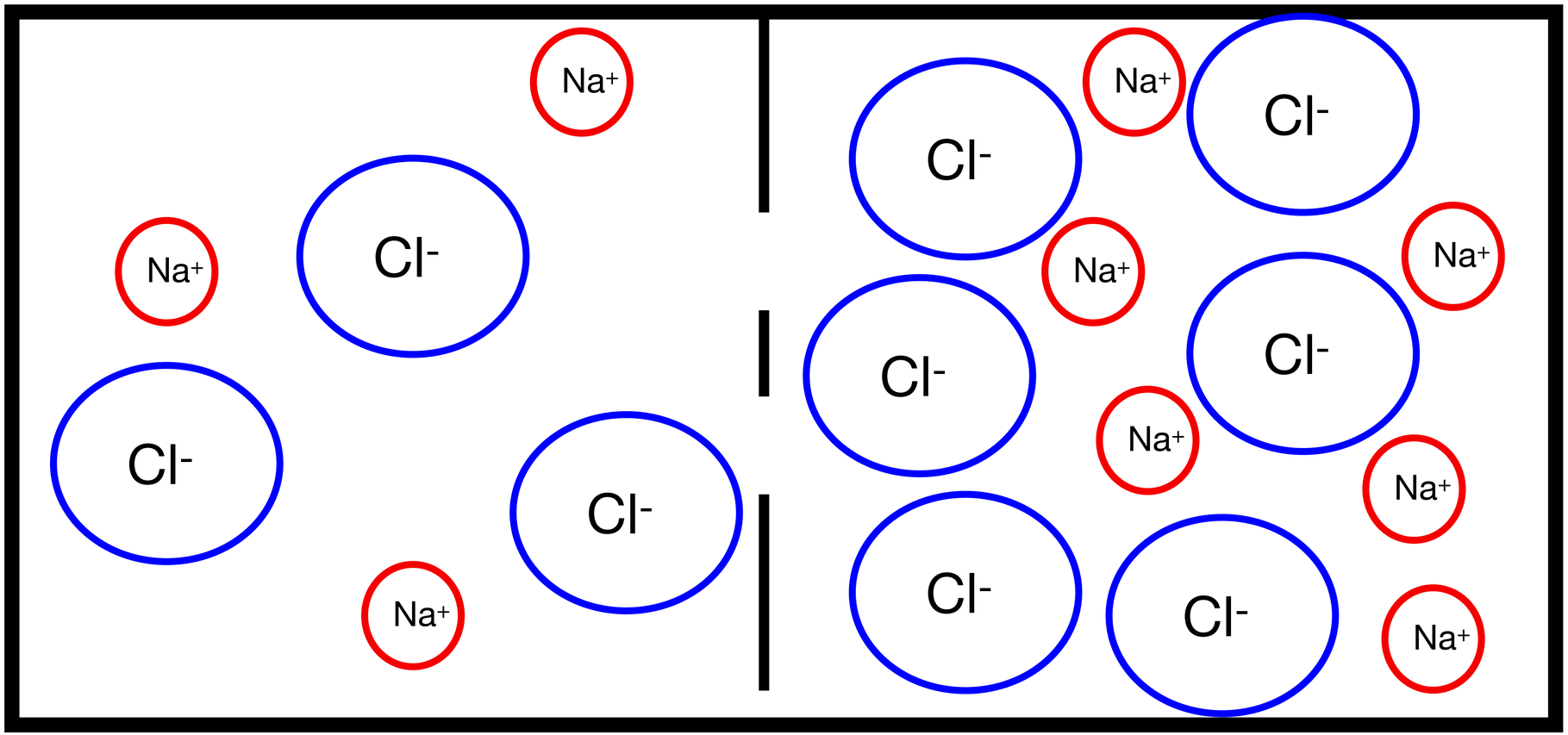
Consider a cell separated by a semi-permeable membrane

Having two concentration of ions. Only [Na] can diffuse across the membrane

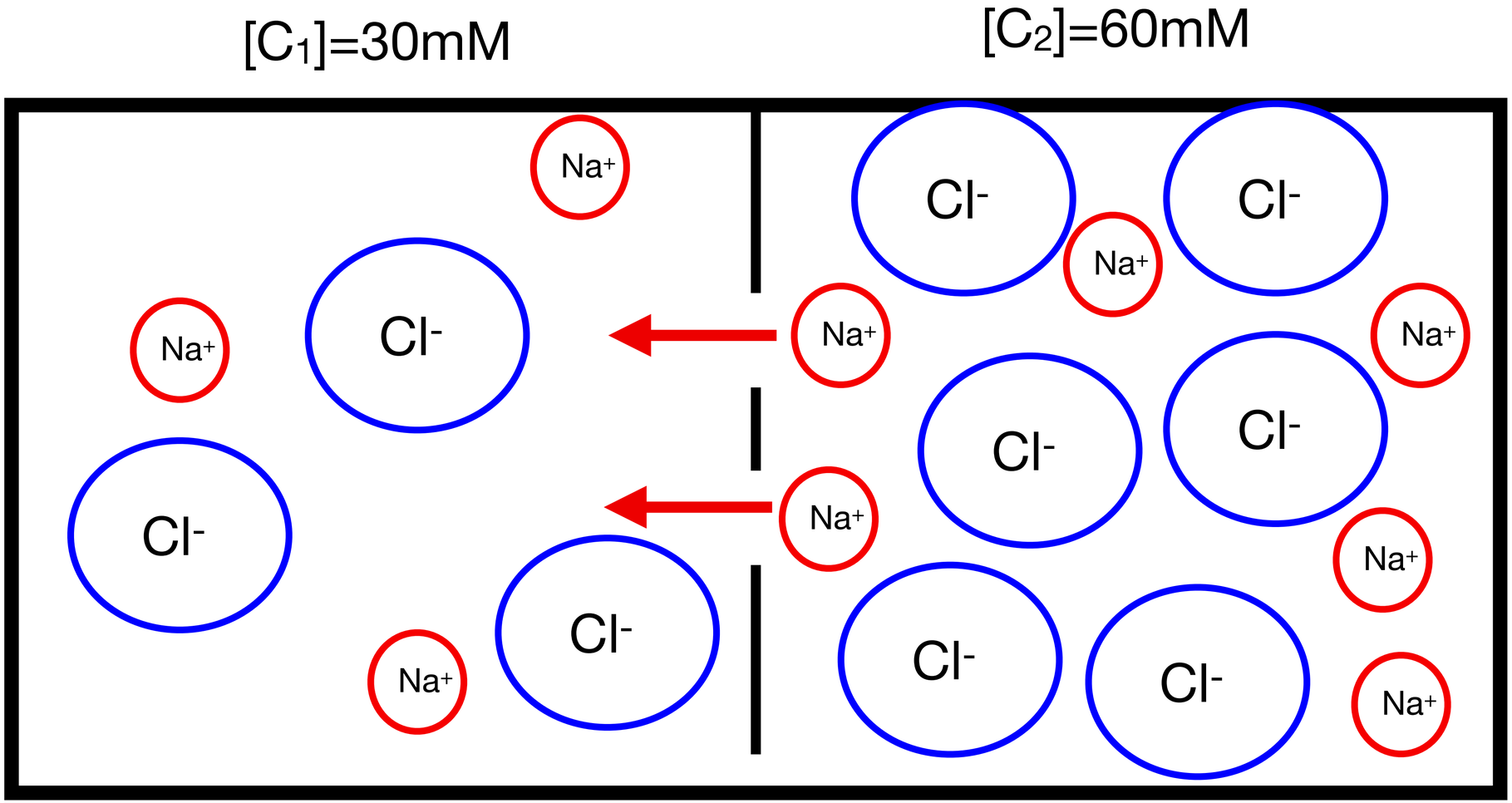
$[C_1]$=30mM

$[C_2]$=60mM



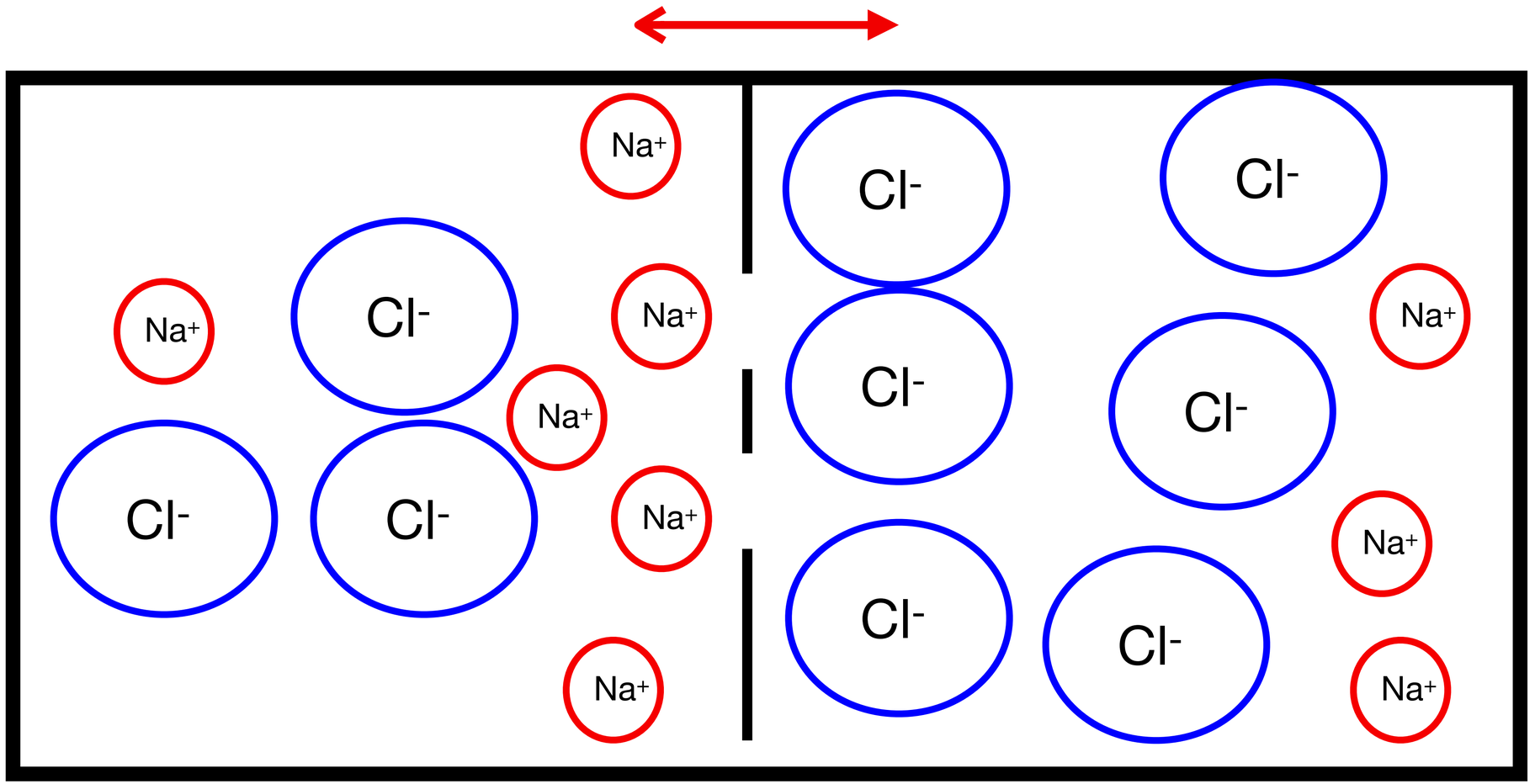
Cl- cannot diffuse through the pore

Na will diffuse from higher concentration to lower concentration

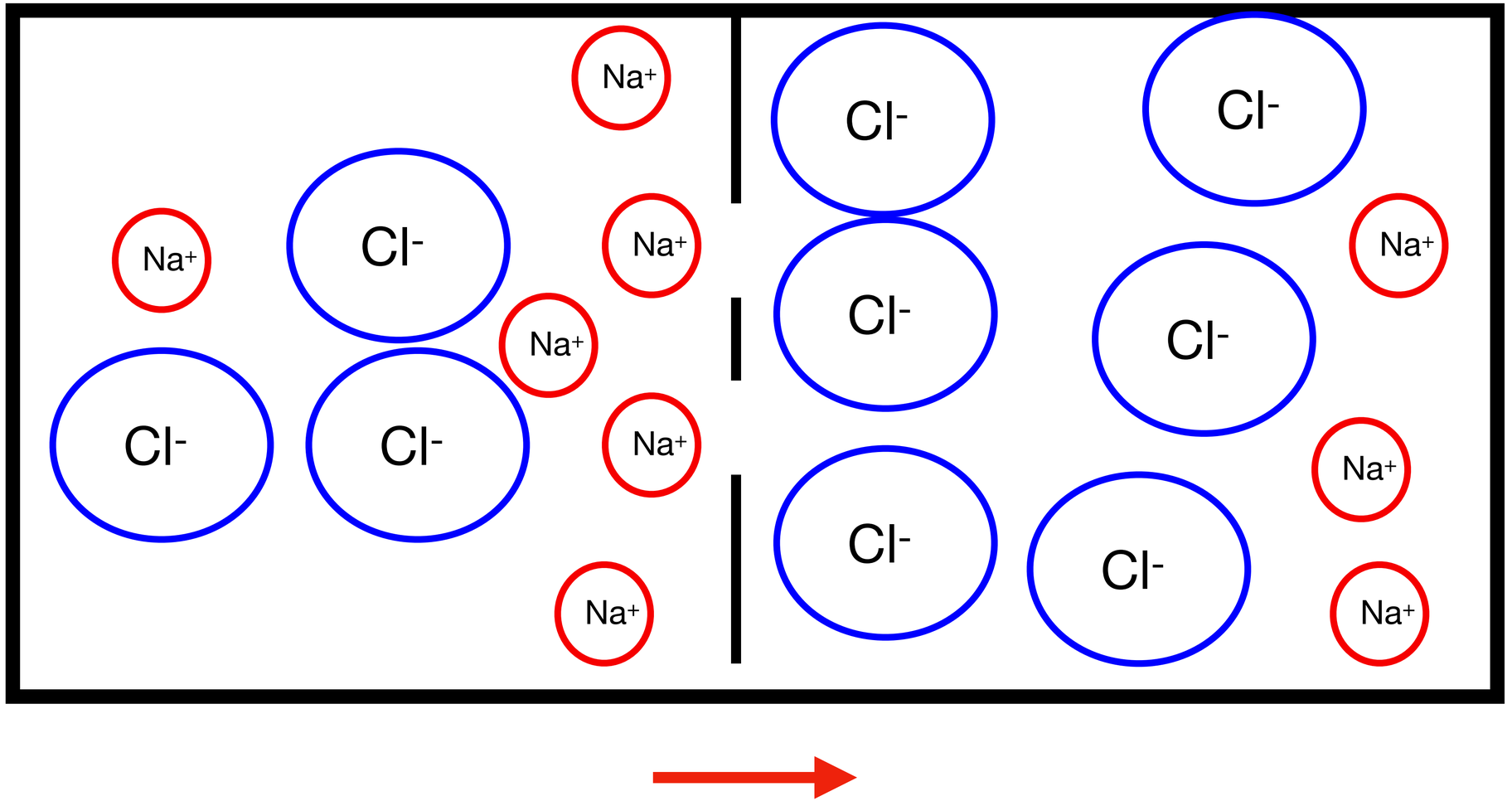


Cl- cannot diffuse through the pore

Opposite charges build up across the membrane

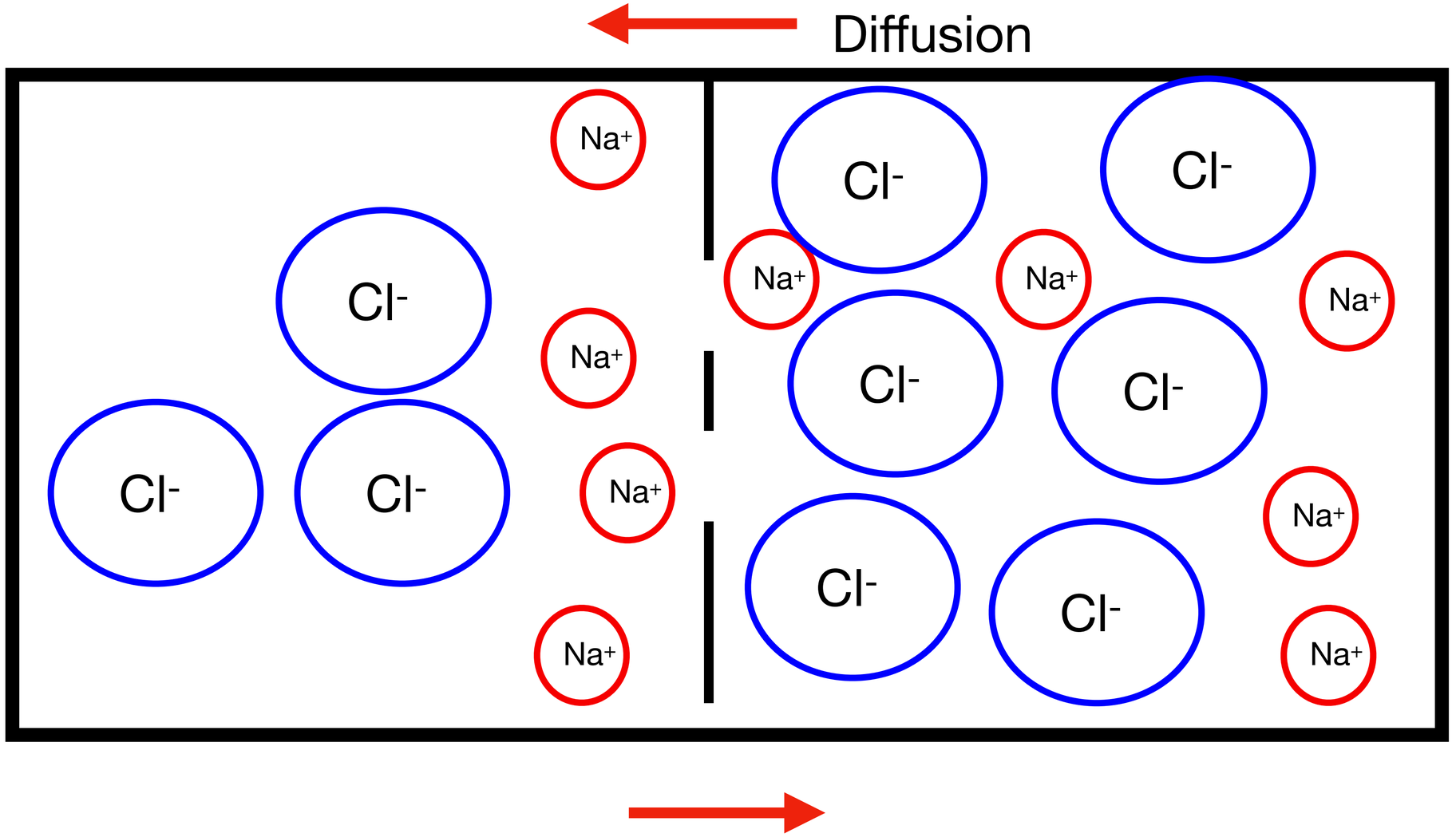


Opposite charges build up across the membrane



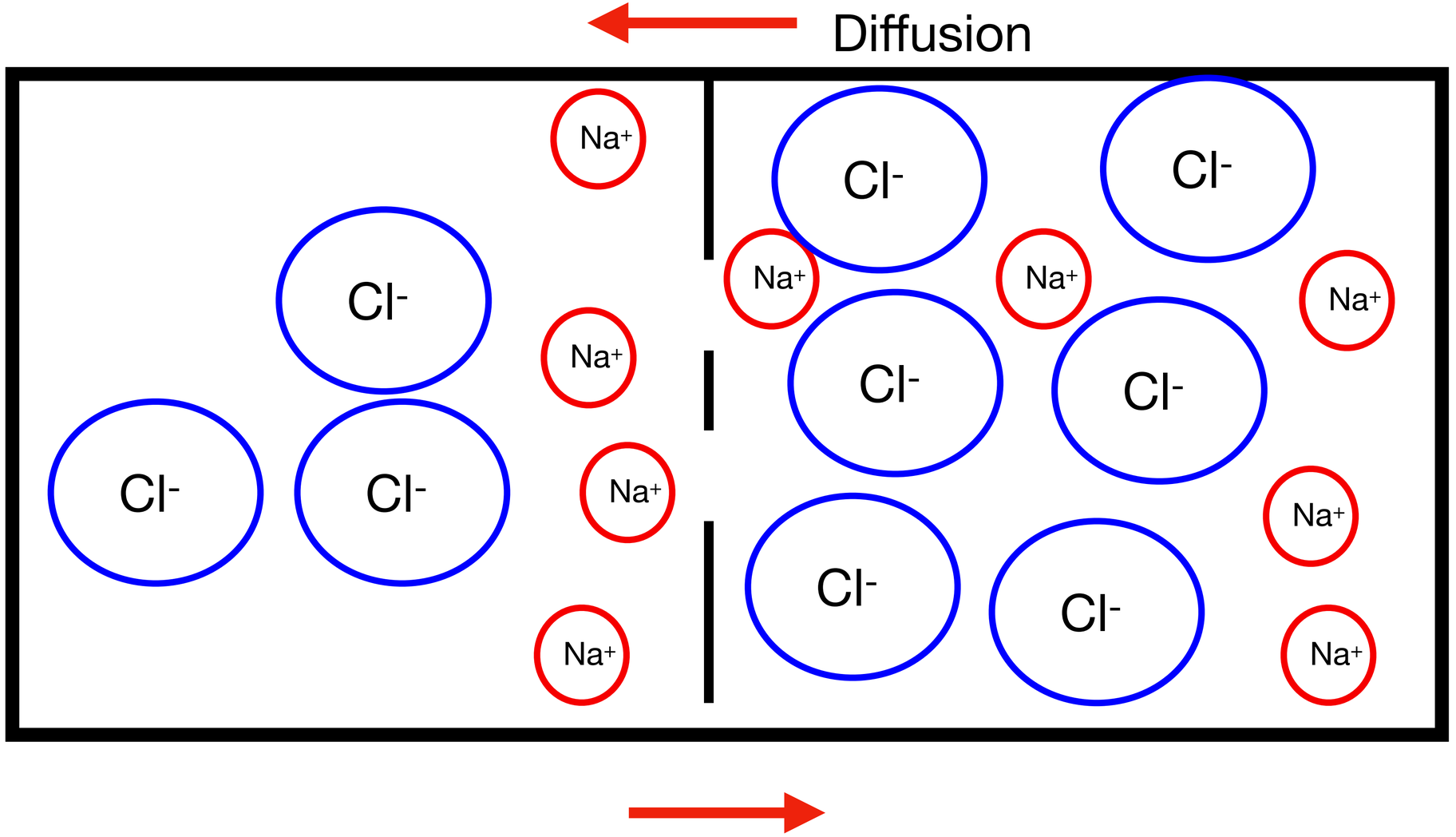
Some Na ions will be pulled back due to electrostatic potential difference

Two flows in the opposite direction



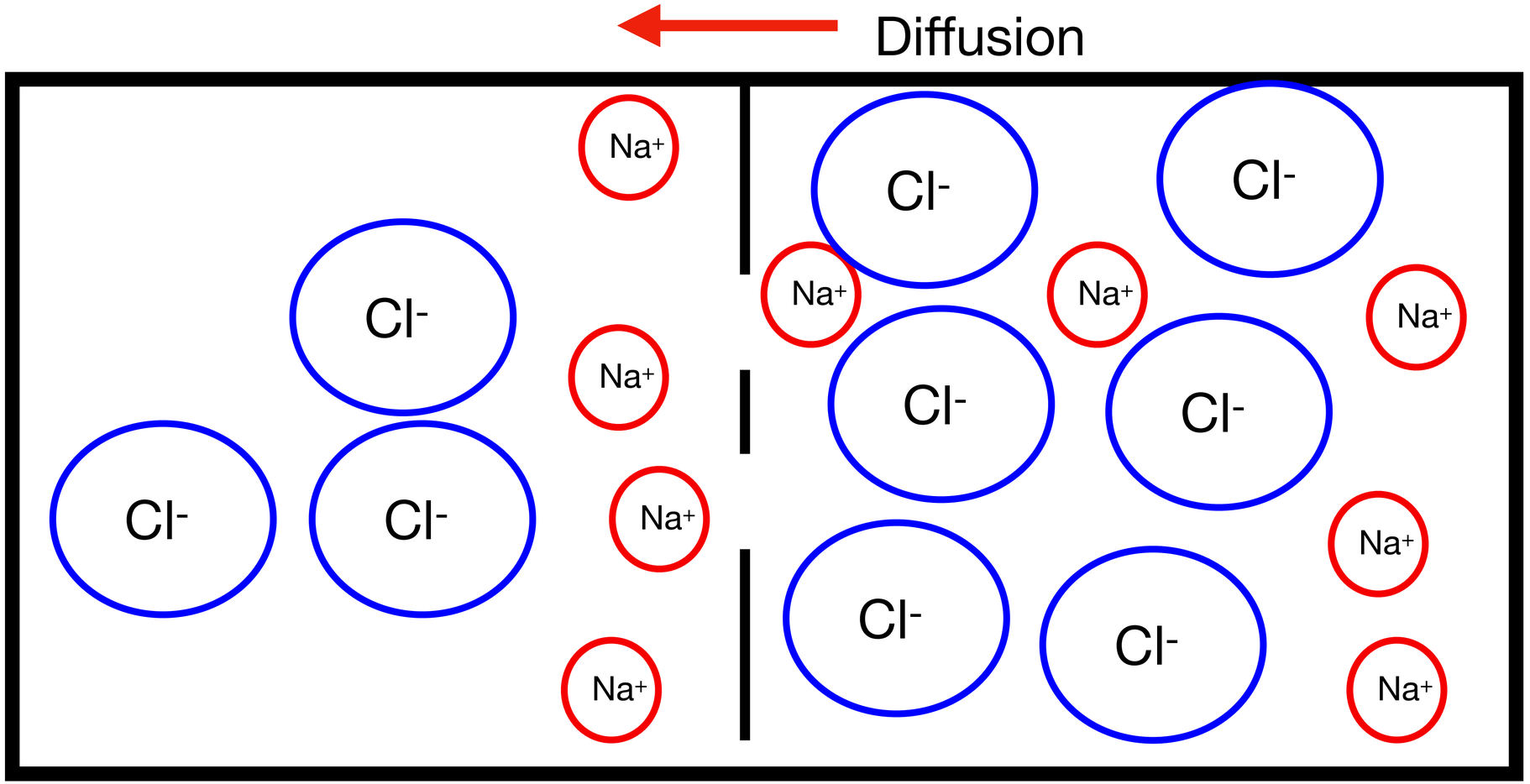
Pull back due to electrostatic forces

These two opposite flows balance => equilibrium



Pull back due to electrostatic forces

$$\text{Diffsion flow } \vec{J}_D = -D\frac{\partial C}{\partial x}\hat{x}$$



$$\text{Flow due to electrostaticpotential } \vec{J}_E = c\vec{v} = c\frac{\vec{f}}{6\pi\eta a}$$

These two opposite flows balance => equilibrium

$$D\frac{\partial C}{\partial x} = c\frac{f}{6\pi\eta a}$$

These two opposite flows balance => equilibrium

$$D\frac{\partial C}{\partial x} = c\frac{f}{6\pi\eta a}$$

$$D\frac{\partial C}{\partial x} = c\frac{q\frac{\partial V}{\partial x}}{6\pi\eta a}$$

These two opposite flows balance => equilibrium

$$D\frac{\partial C}{\partial x} = c\frac{q\frac{\partial V}{\partial x}}{6\pi\eta a}$$

$$\frac{dC}{C} = \frac{-q}{D6\pi\eta a}\frac{dV}{dx}dx$$

Integrate both sides

(Note: Converted the partial derivatives to ordinary derivatives because, at equilibrium, the system is independent time, and only position (x) matters)

These two opposite flows balance => equilibrium

$$D\frac{\partial C}{\partial x} = c\frac{q\frac{\partial V}{\partial x}}{6\pi\eta a}$$

$$\int_{x_1}^{x_2}\frac{dC}{C} = \int_{x_1}^{x_2}\frac{q}{D6\pi\eta a}\frac{dV}{dx}dx$$

Integrate both sides

These two opposite flows balance => equilibrium

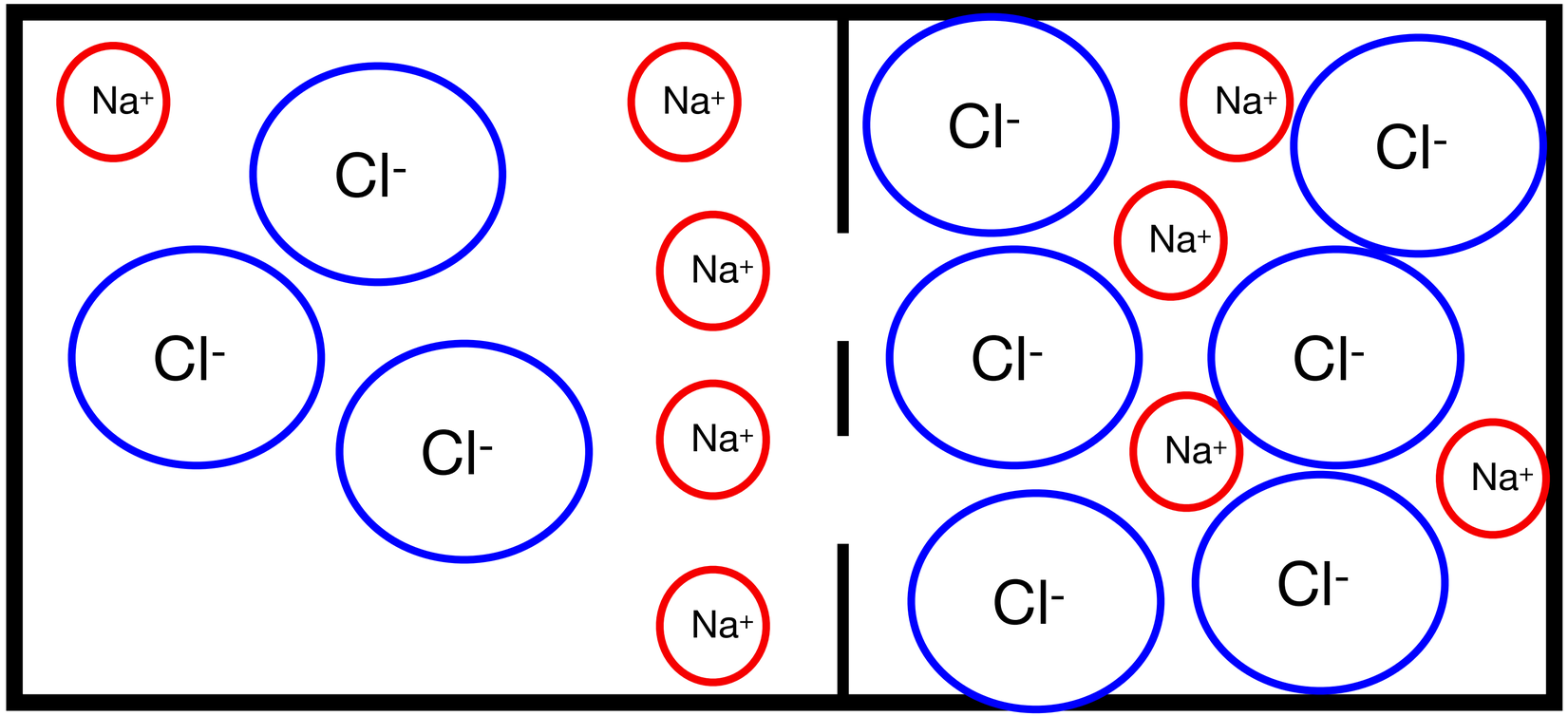
$$\int_{x_1}^{x_2} \frac{dC}{C} = \int_{x_1}^{x_2} \frac{q}{D6\pi\eta a} \frac{dV}{dx} dx$$

$$\frac{k_B T}{q} \ln \frac{C_1}{C_2} = V_1 - V_2$$

Einstein, $D = \frac{k_B T}{6\pi\eta a}$

At equilibrium, we get a potential difference across the membrane

$$V_2 - V_1 = \Delta V = \frac{k_B T}{q} \ln \frac{C_1^{eq}}{C_2^{eq}}$$

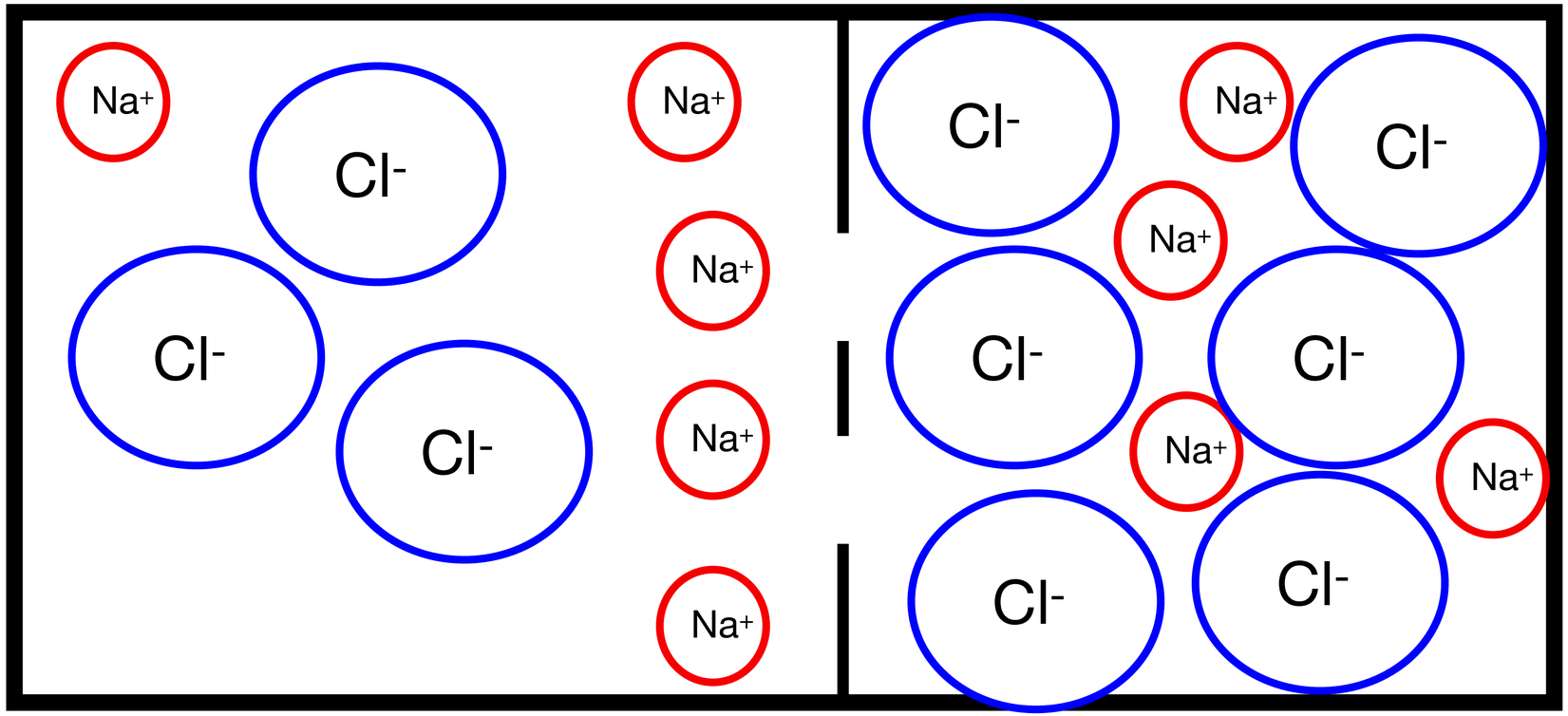


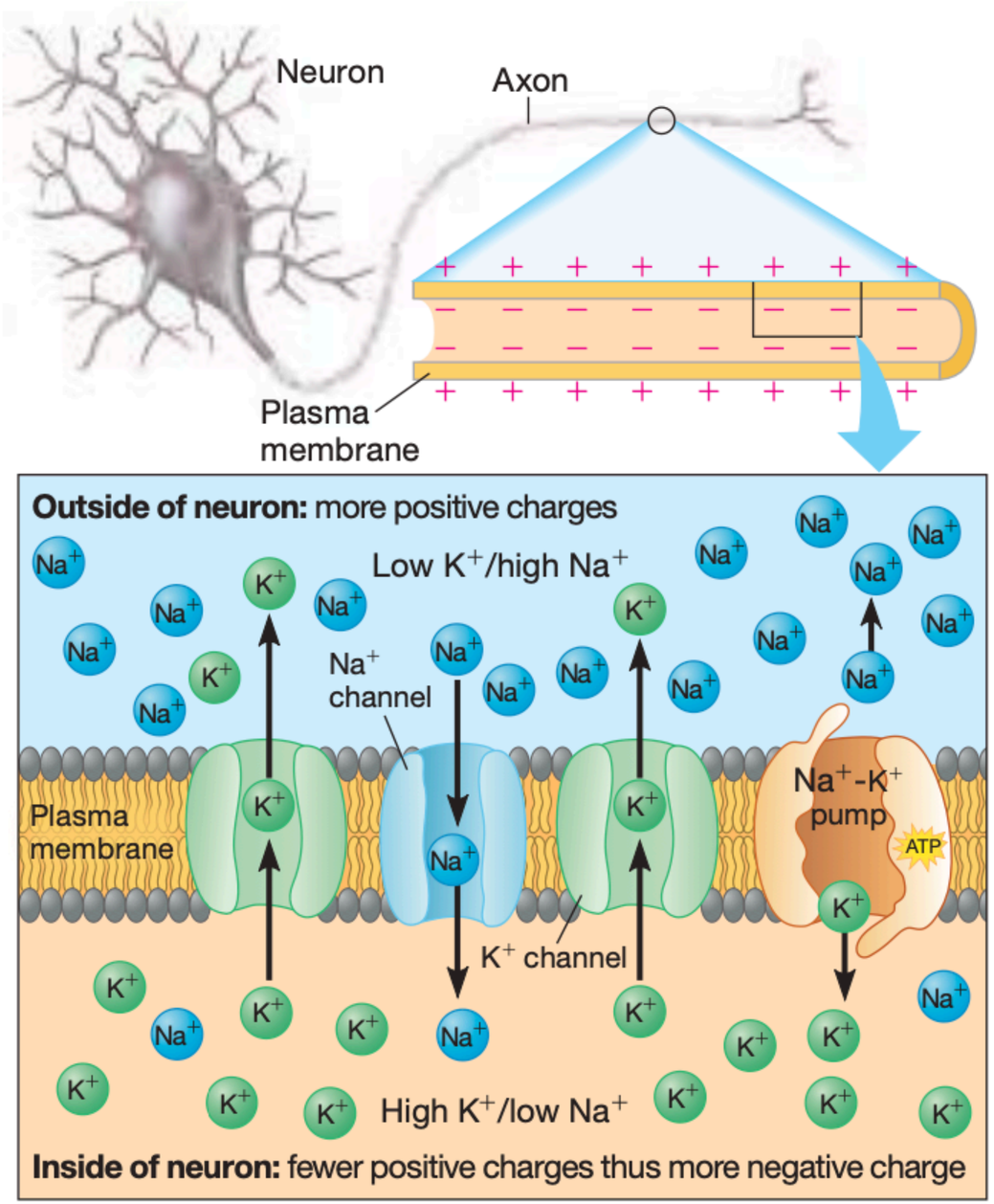
Nernst equation

“Resting” potential

Nernst equation gives the potential difference across a semi-permeable cell membrane, at equilibrium ("Resting" potential)

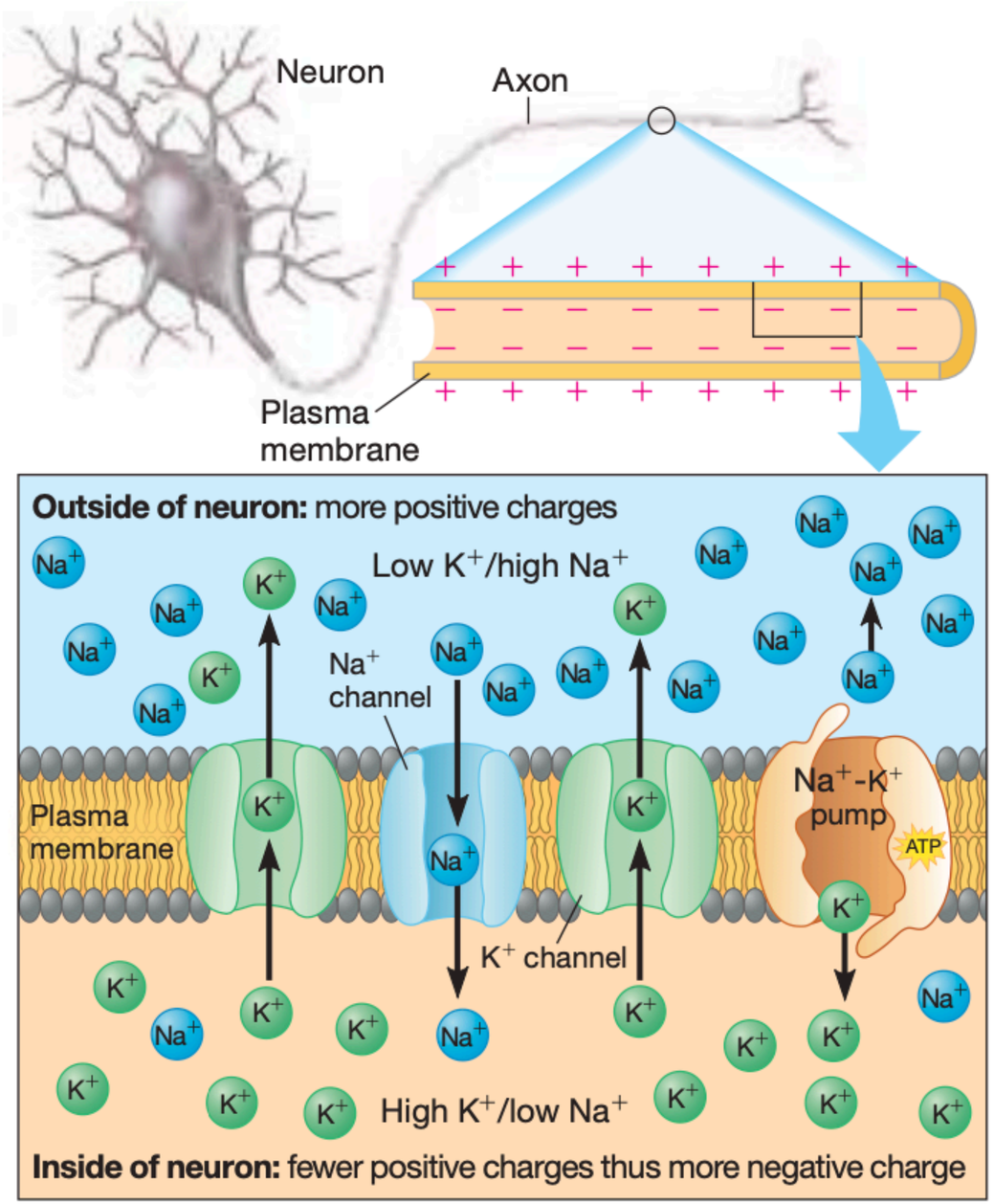
$$V_1 - V_2 = \Delta V = \frac{k_B T}{q} \ln \frac{C_1^{eq}}{C_2^{eq}}$$





▲ **Figure 28.3** How the resting potential is generated

Electrostatic potential difference across neuronal cell membrane



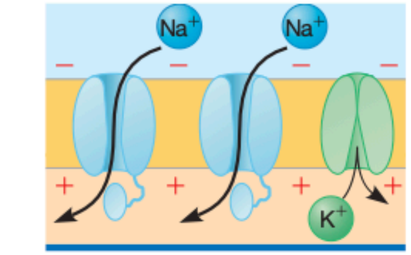
▲ **Figure 28.3** How the resting potential is generated

Change in this potential is the “nerve signal”

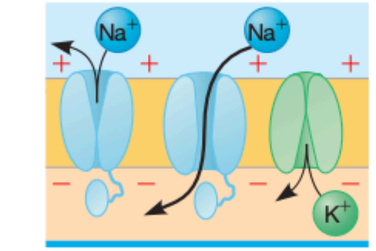
Any stimulus — a sound, tap on the knee—can act as a stimulus.

They can open the ion gates and change the potential!

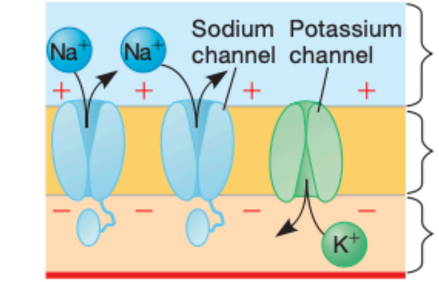
“Action potential” in neurons



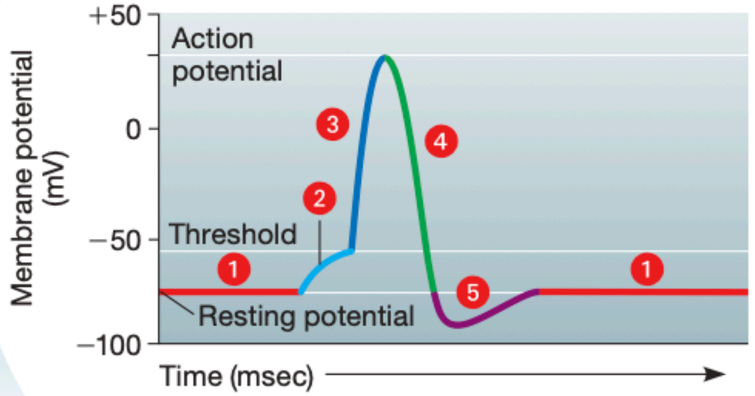
3 More Na^+ channels open; K^+ channels remain closed; interior of cell becomes more positive. Membrane polarity becomes the reverse of resting state.

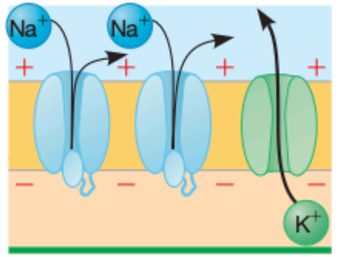


2 **Depolarization:** A stimulus opens some Na^+ channels; if threshold is reached, an action potential is triggered.



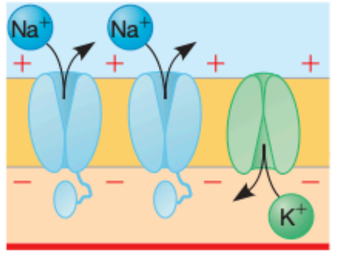
1 **Resting state:** Voltage-gated Na^+ and K^+ channels are closed; resting potential is maintained by ungated channels (not shown).





4 **Repolarization:** Na^+ channels close and inactivate; K^+ channels open, and K^+ rushes out; interior of cell becomes more negative than outside.

5 The K^+ channels close relatively slowly, causing a brief undershoot.



1 Return to resting state.

See: Campbell, Chapter 28

The salty water consequence: Coulomb’s law is nor more the same!

Positively charged protein

Negatively charged protein

Positively charged protein

What is the interaction energy?

Negatively charged protein

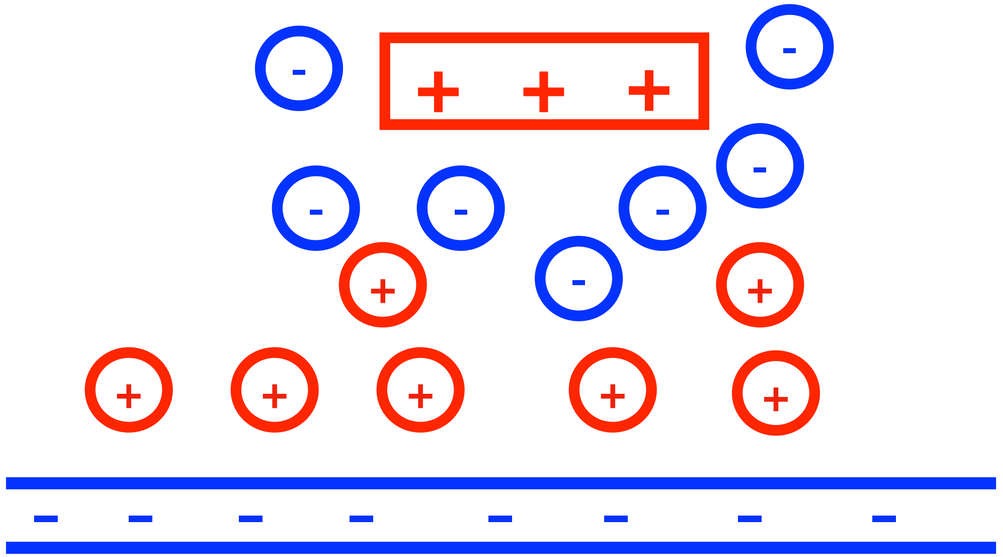
Positively charged protein

What is the interaction energy?

Negatively charged protein

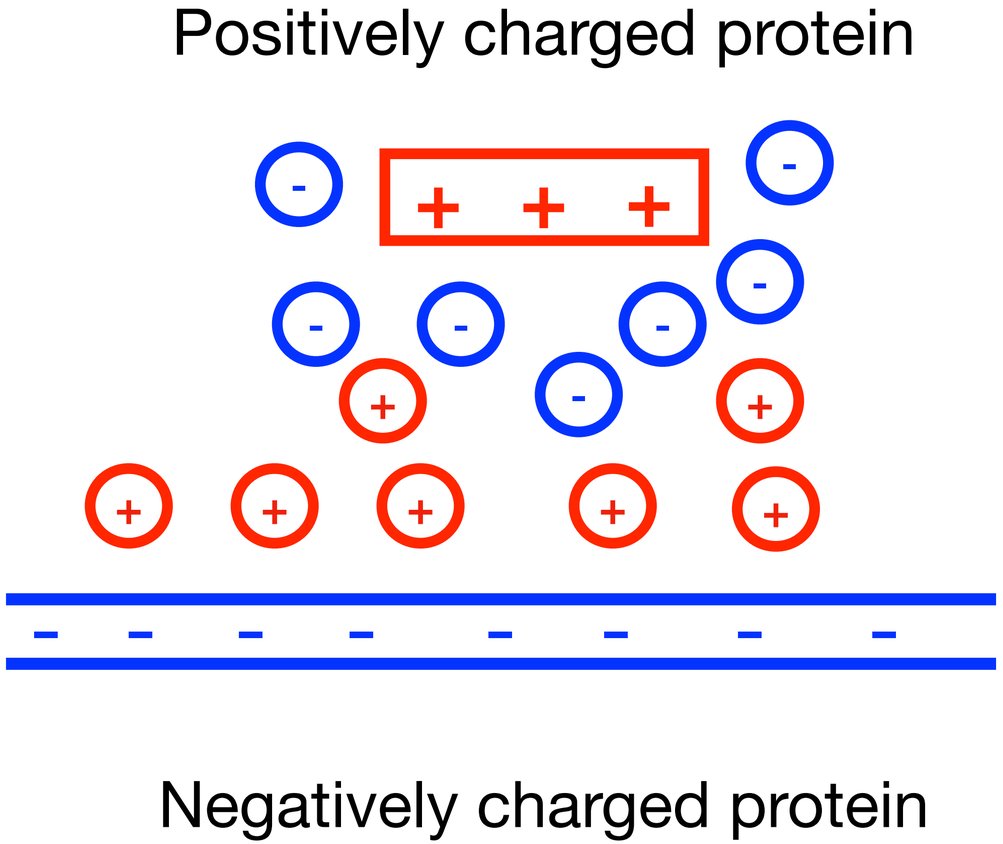
$$E = \frac{kQ_DQ_P}{r}$$

Positively charged protein



Negatively charged protein

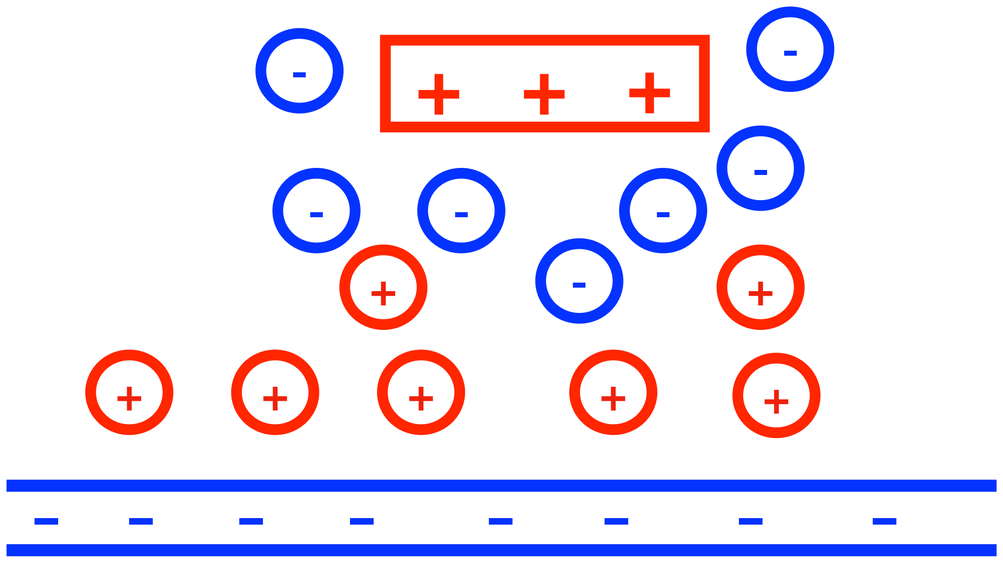
BUT, molecular biology is in salty water!



The ions “screen” the effective interaction between DNA and protein

Computing screened electrostatic potential

Positively charged protein



Negatively charged protein

$$\vec{\nabla} \cdot \vec{E} = \frac{-\rho}{\epsilon_0 \epsilon_r}$$

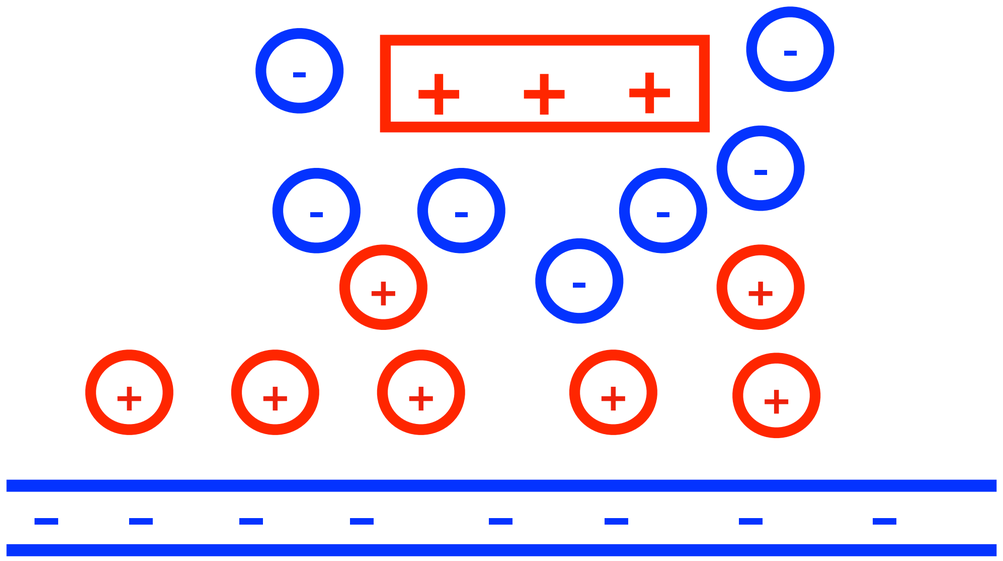
$$\vec{E} = -\vec{\nabla} V$$

$$\nabla^2 V = \frac{\rho}{\epsilon_0 \epsilon_r}$$

ρ = density of charged particles = probability of finding charged particles

Computing screened electrostatic potential

Positively charged protein



Negatively charged protein

$$\vec{\nabla} \cdot \vec{E} = \frac{\rho}{\epsilon_0 \epsilon_r}$$

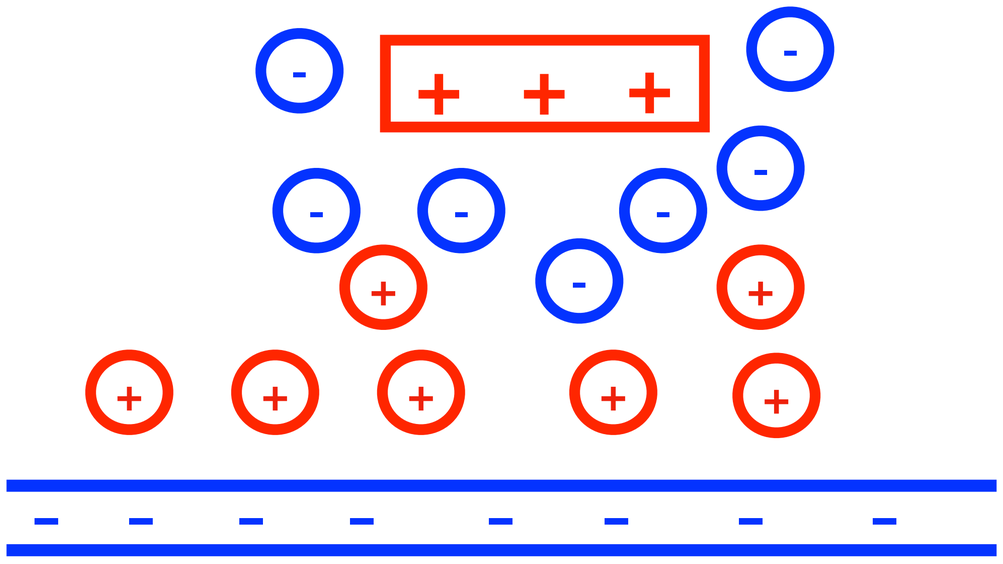
$$\vec{E} = -\vec{\nabla} V$$

$$\nabla^2 V = \frac{-\rho}{\epsilon_0 \epsilon_r}$$

$$\rho = \text{density or concentration of charged particles} = \sum_i q_i P_i$$

Computing screened electrostatic potential

Positively charged protein



Negatively charged protein

$$\nabla^2 V = \frac{-\rho}{\epsilon_0 \epsilon_r}$$

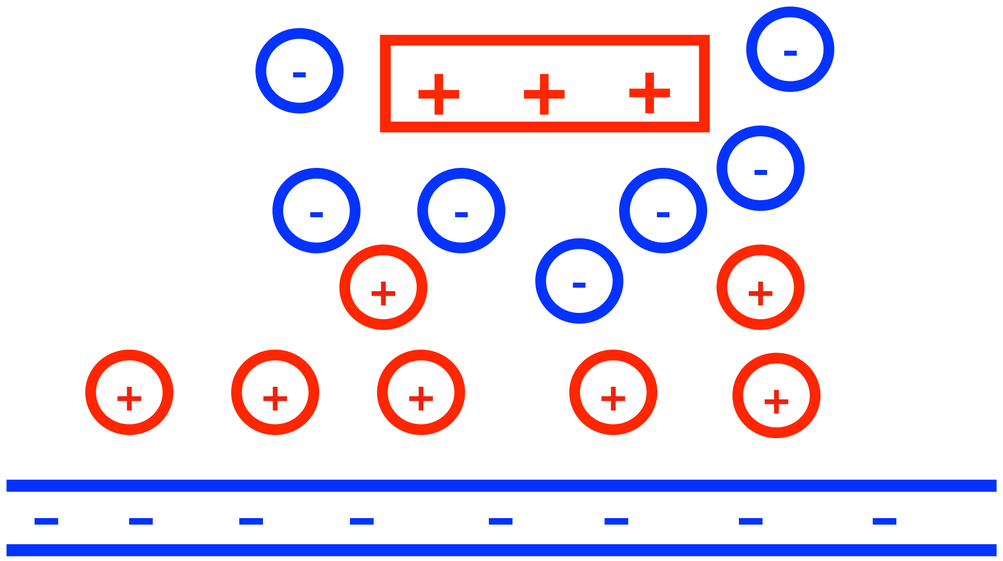
$$\rho = \sum_i q_i P_i$$

$$P_i = A \exp\left(\frac{-q_i V}{k_B T}\right)$$

$$\exp\left(-\frac{q_i V}{k_B T}\right) \approx 1 - \frac{q_i V}{k_B T}$$

Computing screened electrostatic potential

Positively charged protein



Negatively charged protein

$$\nabla^2 V = \frac{-\rho}{\epsilon_0 \epsilon_r}$$

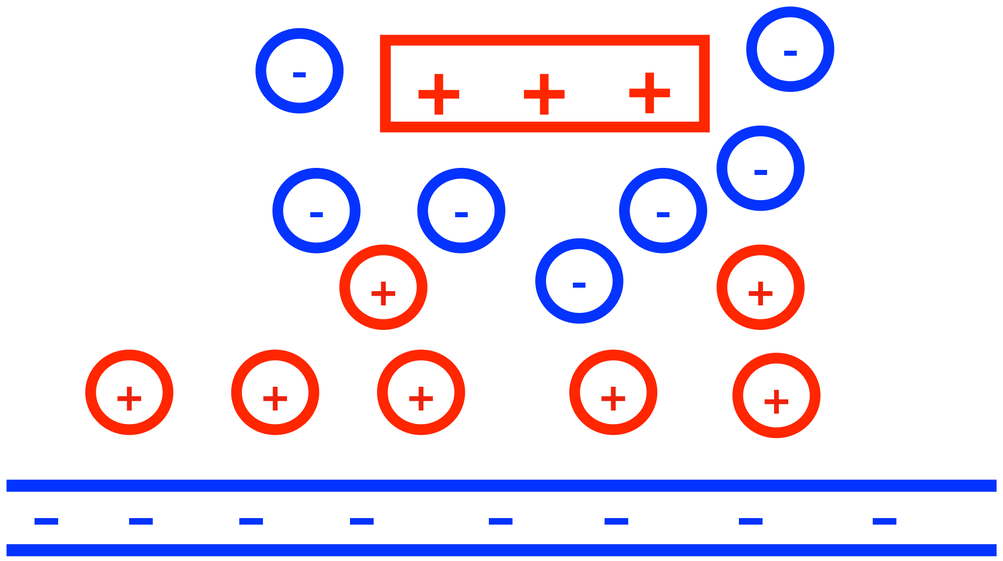
$$\rho = \sum_i q_i P_i$$

$$\nabla^2 V = \frac{-A}{\epsilon_0 \epsilon_r} \sum_i q_i \left(1 - \frac{q_i V}{k_B T}\right)$$

Overall system is charge neutral $\Rightarrow \sum_i q_i = 0$

Computing screened electrostatic potential

Positively charged protein



Negatively charged protein

$$\nabla^2 V = \frac{-A}{\epsilon_0 \epsilon_r} \sum_i q_i \left(1 - \frac{q_i V}{k_B T}\right)$$

Overall system is charge neutral $\Rightarrow \sum_i q_i = 0$

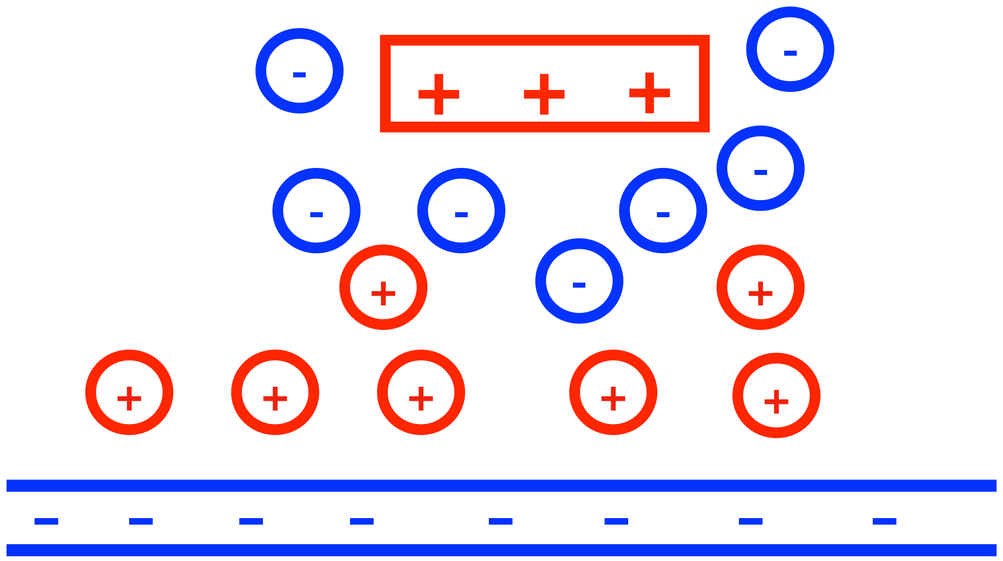
$$\nabla^2 V = \frac{A}{\epsilon_0 \epsilon_r} \sum_i \left(\frac{q_i^2 V}{k_B T}\right)$$

$$\lambda_D = \sqrt{\sum_i \frac{\epsilon_0 \epsilon_r}{A} \frac{k_B T}{q_i^2}}$$

$$\nabla^2 V = \left(\frac{1}{{\lambda_D}^2}\right) V$$

Screened electrostatic potential

Positively charged protein



Negatively charged protein

$$\nabla^2 V = \left(\frac{1}{{\lambda_D}^2}\right) V$$

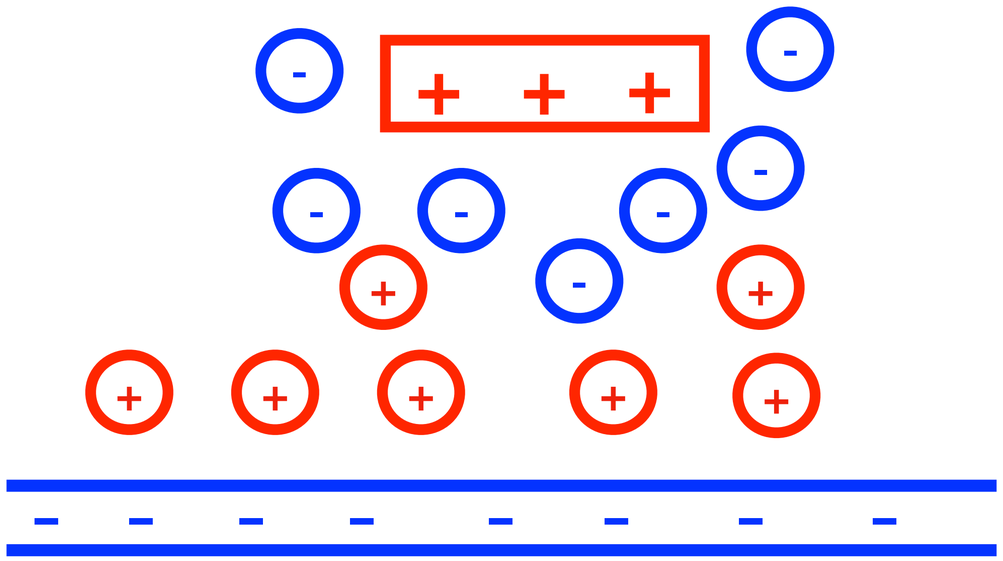
$$V = V_0 \exp\left(\frac{-r}{\lambda_D}\right)$$

$$\lambda_D = \sqrt{\sum_i \frac{\epsilon_0 \epsilon_r}{A} \frac{k_B T}{q_i^2}}$$

Boundary condition etc gives, $V_0 \propto \frac{1}{r}$

Screened electrostatic potential or screened-Coulomb potential

Positively charged protein



Negatively charged protein

$$V = \frac{B}{r}\exp\left(\frac{-r}{\lambda_D}\right)$$

$$\lambda_D = \sqrt{\sum_i \frac{\epsilon_0 \epsilon_r}{A}\frac{k_B T}{q_i^2}}$$

$$\lambda_D = \text{Debye length} \approx 1\text{nm}$$

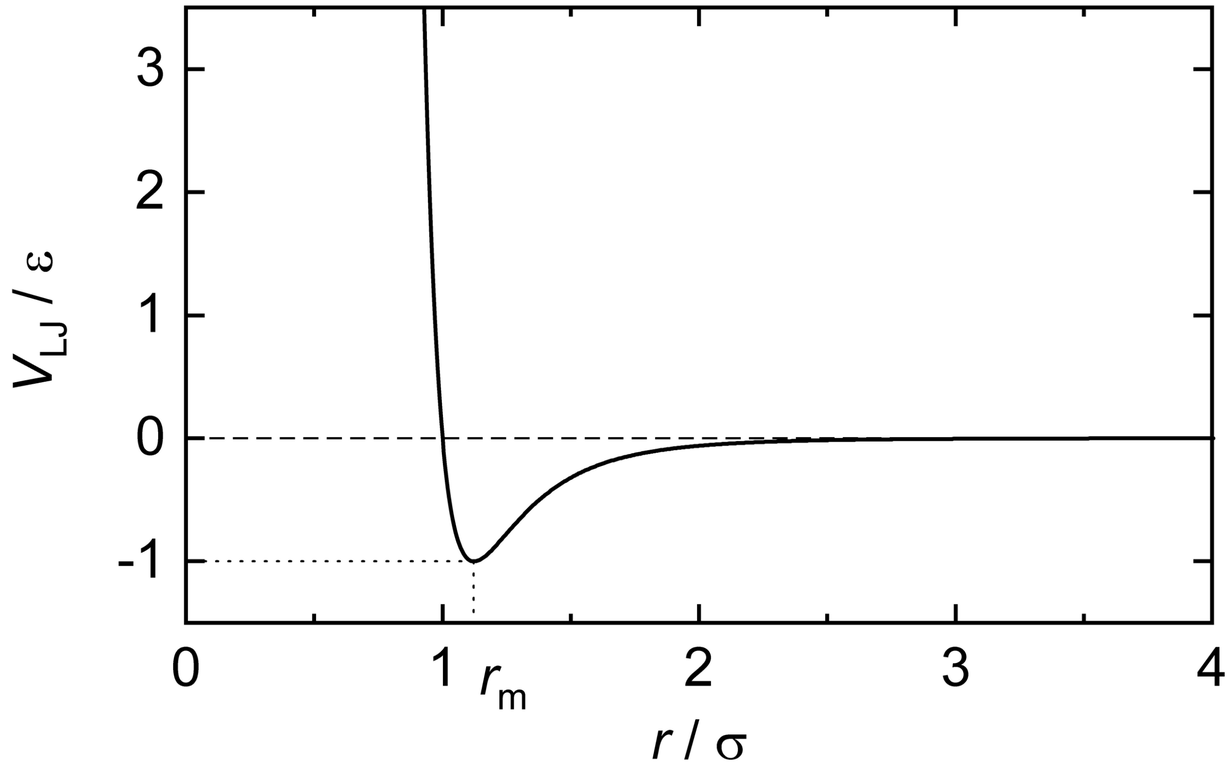
Negligible electrostatic interaction when distance >> 1nm

Other interaction energies

Inter-molecular effective potential

Lennard-Jones energy

$$V_{\mathrm{LJ}}(r) = 4\varepsilon \left[\left(\frac{\sigma}{r} \right)^{12} - \left(\frac{\sigma}{r} \right)^{6} \right],$$



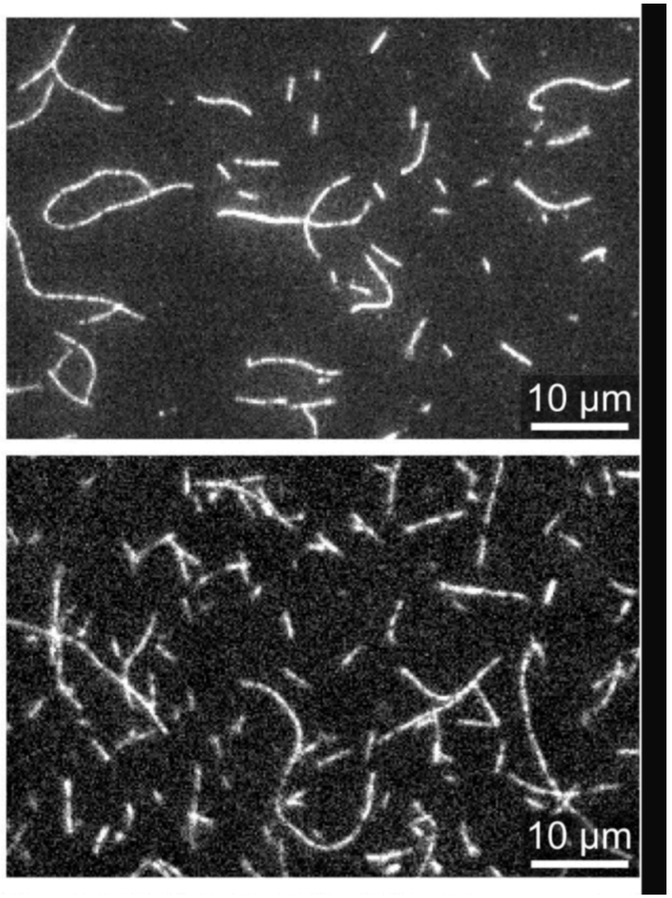
Short-range attraction; steric repulsion

3-body, 4-body potentials

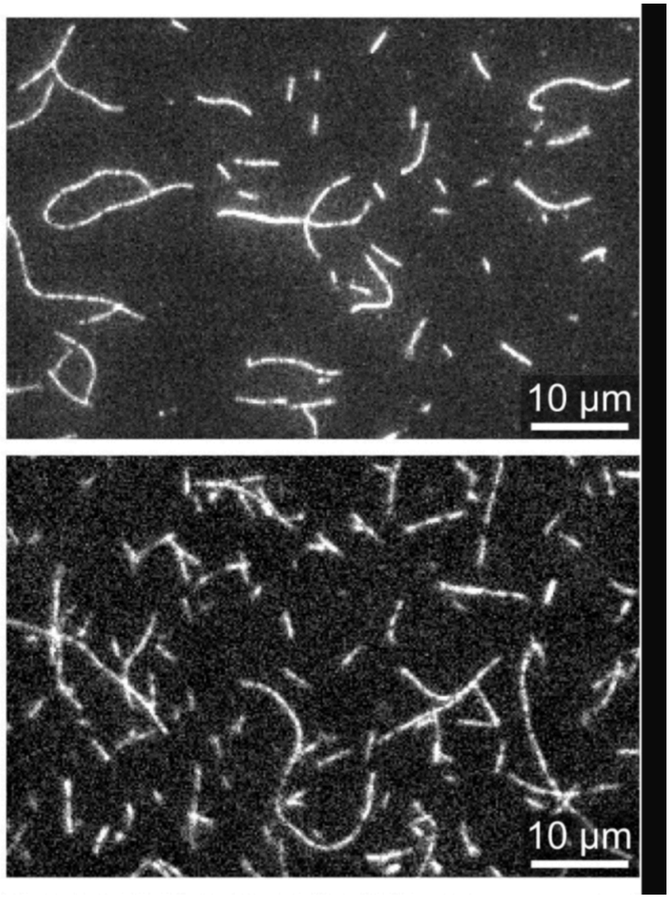
Curvature

Twist

By looking at microscopic images of bio-filaments (like actin or even DNA), can we say something about their properties?

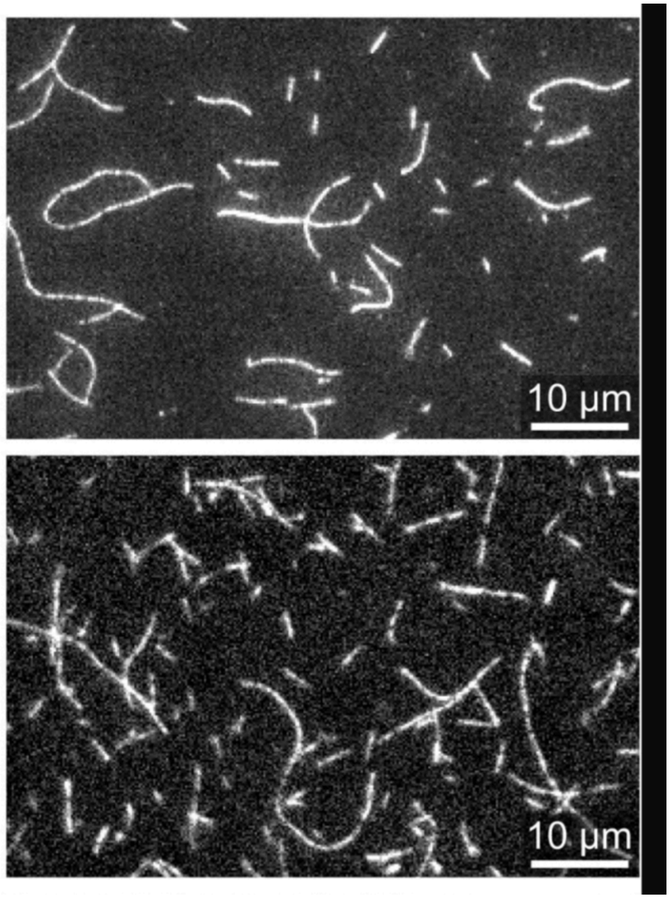


By looking at microscopic images of bio-filaments (like actin or even DNA), can we say something about their properties?



Can the thermal fluctuations make them bend?

By looking at microscopic images of bio-filaments (like actin or even DNA), can we say something about their properties?



Can the thermal fluctuations make them bend?

Elasticity Bendability, rigidity

Will affect force generation

Summary

- Ions channels across membranes lead to electrostatic potential difference
- Nernst equation
- Neurons: propagation of signal. Action potential
- Interaction between two charged macro-molecules like DNA and protein
- Screened due to the presence of ions
- Screened electrostatic potential falls exponential. Negligible beyond 1nm
- Other interaction energies in biology