Physical Principles in Biology
Biology 3550
Spring 2024

Lecture 32

Membrane Permeability and Introduction to Protein Folding

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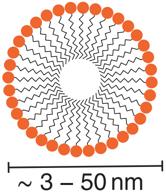
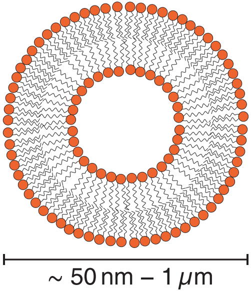
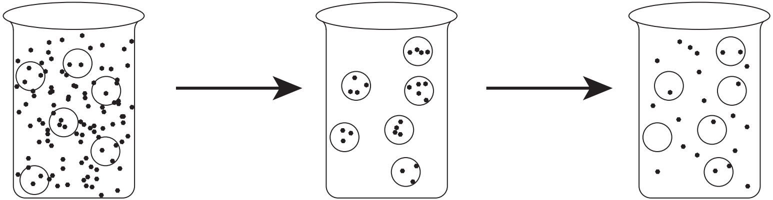
Micelles and Vesicles





- Micelles are formed by detergents and soaps; vesicles are formed by phospholipids.
- Micelles are made up of a single shell of detergent or soap molecules; vesicles are made up of lipid bilayers.
- Micelles are generally much smaller than vesicles.
- Different shapes and sizes of the micelles and vesicles reflect the different shapes of detergents and soaps (∼conical) and phospholipids (∼cylindrical).

Using Vesicles to Measure Permeability of Bilayers



- Form vesicles in presence of molecules of interest.
- Separate vesicles from external molecules.
- Allow molecules to diffuse across bilayers for a period of time.
- Separate vesicles from external molecules and measure concentrations.
- What determines rate of escape?
  Fick's first law!

Diffusion Across Vesicle Bilayer

- Fick's first law: $$J = -D\frac{dC}{dx}$$
- Concentration gradient: $$\frac{dC}{dx} \approx \frac{C_{\text{in}} - C_{\text{out}}}{\text{Bilayer thickness}}$$
- A parameter commonly used in this and other contexts: permeability coefficient, P (not to be confused with pressure!).

  Represents combination of diffusion coefficient and membrane thickness:

  $$P = \frac{D}{\Delta x}$$

  Units:

  $$\text{m}^2/\text{s} \div \text{m} = \text{m}/\text{s}$$

Fick's First Law Expressed Using the Permeation Coefficient

$$J = -D\frac{dC}{dx} \approx -D\frac{\Delta C}{\Delta x} = -P\Delta C$$

- In practice: Measure flux and calculate P

$$P = -\frac{J}{\Delta C}$$

- What do we need to know?
  - Concentration of molecules inside vesicle and out.
  - Rate of molecules diffusing (moles/time).
  - Surface area of vesicles (J has units of $\text{mol} \cdot \text{m}^{-2}\text{s}^{-1}$).
  - Don't need to know membrane thickness!
- P reflects both molecule and bilayer (or other kind of membrane)

Clicker Question #1

What kind of ions or molecules would you expect to have the **smallest** permeability coefficients for phospholipid bilayers?

A) Sugars

B) Amino acids

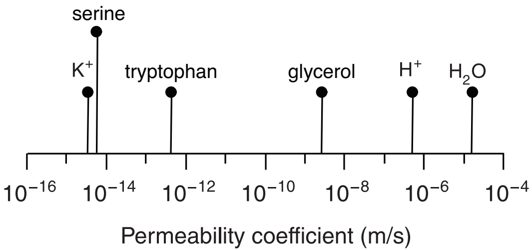
C) Water

D) Nucleotides

E) Small ions like Na^+, K^+ or Cl^-

All answers count for now!

Measured Permeability Coefficients



- Range of permeabilities is extremely wide: 9 orders of magnitude.
- Charged ions have very low permeability.
- Polar small molecules have low to medium permeabilities.
- Permeabilities of water and H^+ are actually quite high!

Data from: Chakrabarti, A. C. & Deamer, D. W. (1992). *Biochim. Biophys. Acta - Biomembranes*, 111, 171–177.
https://doi.org/10.1016/0005-2736(92)90308-9
Paula, S., Volkov, A. G., Van Hoek, A. N., Haines, T. H. & Deamer, D. W. (1996). *Biophys. J.*, 70, 339–348.
https://doi.org/10.1016/S0006-3495(96)79575-9

Comparing Permeability Across Bilayers with Diffusion Coefficients in Water

- $P = D/\Delta x$
- Can calculate an "effective diffusion coefficient" by assuming a value for Δx.

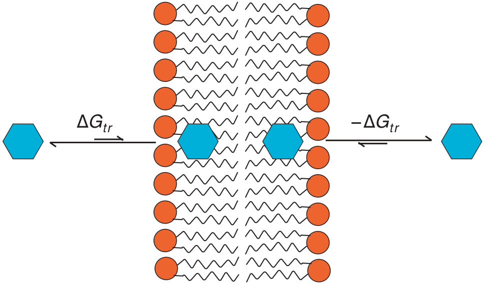
  $$D = P\Delta x$$

  Assume $\Delta x = 4\,\text{nm}$
- For ions (other than H^+):

  $$\begin{aligned} D &= P\Delta x = 10^{-14}\,\text{m/s} \times 4 \times 10^{-9}\,\text{m} \\ &= 4 \times 10^{-23}\,\text{m}^2/\text{s} \end{aligned}$$
- For small polar molecules:

  $$\begin{aligned} D &= P\Delta x = 10^{-10}\,\text{m/s} \times 4 \times 10^{-9}\,\text{m} \\ &= 4 \times 10^{-19}\,\text{m}^2/\text{s} \end{aligned}$$
- Compare to $D = 10^{-10}\,\text{m}^2/\text{s}$ for small molecules in water.

Quantitative Model for Permeability



- Molecule rapidly equilibrates between aqueous and lipid phases.
- Molecule diffuses across lipid phase.
- Diffusion is rate limiting, but overall rate depends on fraction of molecules in the lipid phase:

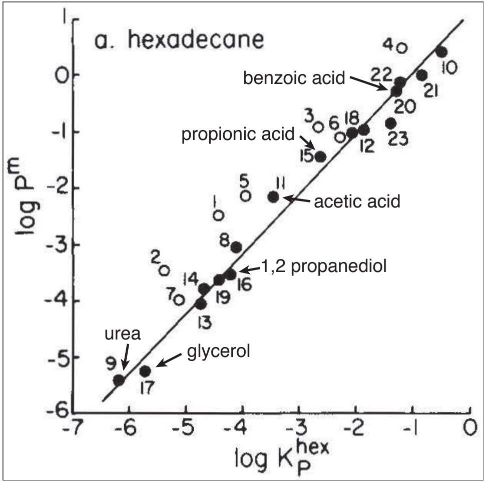
$$P = K_{\mathrm{tr}} D_{\mathrm{l}} / \Delta x$$

K_{tr} is equilibrium constant between phases.

D_{l} is diffusion coefficient in lipid.

- Model predicts a correlation between measured ΔG_{tr} and P for molecules of similar size.

Correlation Between Permeability and Solubility in Non-polar Liquids



- Values are for small polar molecules.
- P^m: Bilayer permeability coefficient (cm/s).
- K_p^{hex}: Partition coefficient from water to hexadecane.
- Correlation supports "solubility diffusion" model.

Walter, A. & Gutknecht, J. (1986). Permeability of small nonelectrolytes through lipid bilayer membranes. *J. Membrane Biol.*, 90, 207–217.
http://dx.doi.org/10.1007/BF01870127

An Alternative Model

- Bilayers randomly form holes that quickly reseal.
- What would this model predict?
- Some sort of transient pore model likely explains high permeabilities of water and H ·

Some Questions about the Origins of Life on Earth

What are some of the fundamental things that living organisms have to do?

- Collect nutrients from environment.
- Convert nutrients into other compounds and useful forms of energy.
- Build complicated macromolecules, including enzymes and genetic material.
- Create compartments bounded by membranes.
- Reproduce themselves.

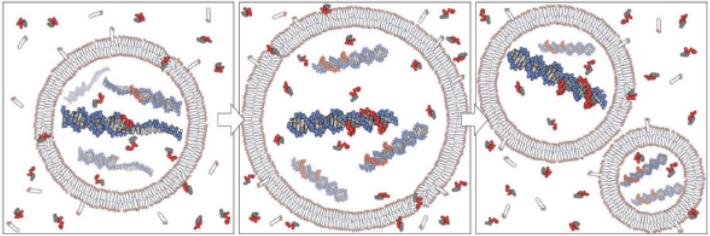
Which came first, proteins or nucleic acids?

- Genetic information is encoded by nucleic acids.
- Proteins are needed to synthesize nucleic acids.
- Now know that some RNA molecules have catalytic activities, suggesting that something like RNA may have come before proteins and DNA.

When Did Membranes Enter the Picture?

- Compartmentalization would favor local reactions and prevent competing reactions from stealing reagents.
- Without pores, bilayers prevent escape of molecules, but also their entry!
- Some fatty acids can form bilayers that small polar and charged molecules can cross, but that larger molecules can't.

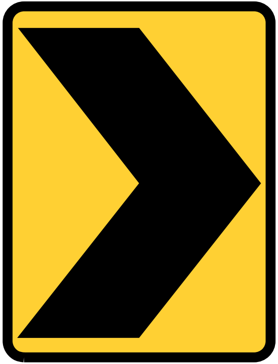
A Model for Primitive Proto-cells



- Primitive reactions lead to formation of polymers, possibly RNA-like.
- Polymers become trapped in semi-permeable vesicles.
- Precursors to polymers diffuse into vesicles and add to the polymers, effectively trapping them.
- Polymers grow, forcing vesicles to grow, and eventually divide.

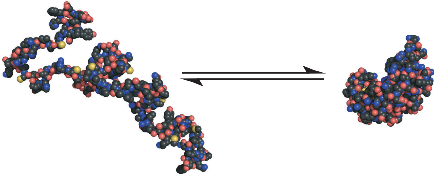
Mansy, S. S., Schrum, J. P., Krishnaurthy, M., Tobé, S., Treco, D. A. & Szostak, J. W. (2008). *Nature*, 454, 122–125. http://dx.doi.org/10.1038/nature07018

Warning!



Direction Change

Another Thermodynamic Process in Biology: Protein Folding and Unfolding



- Unfolded proteins:
  - Broad ensembles of rapidly interconverting conformations.
- Folded (native) proteins:
  - Compact, well-defined conformations.
  - *Usually* the functional state.
  - Motions are restricted, but can be essential for function.
- Three-dimensional structure forms after (or during) synthesis.
- Many proteins can unfold reversibly, and the process has been extensively studied *in vitro*.

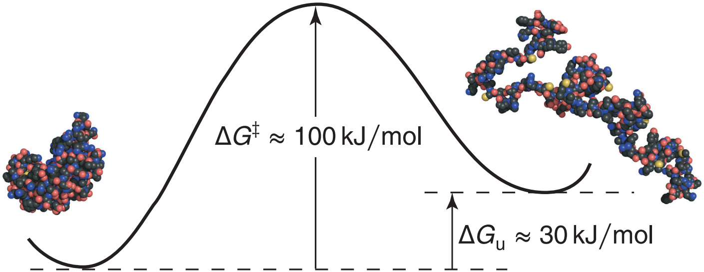
Protein Unfolding: A Simplified Summary

- For small, single-domain proteins ($\approx$ 100 amino acids), unfolding is well described as a “two-state” process:

$$\mathrm{N} \rightleftharpoons \mathrm{U}$$

  Partially folded molecules are rarely detected at equilibrium.

- Free energy profile for unfolding and refolding:



Equilibrium Constant for Unfolding

- Calculate K_u from $\Delta G_\mathrm{u}^\circ$ at 298 K

$$\Delta G_\mathrm{u}^\circ = -RT \ln K_\mathrm{u}$$

$$K_\mathrm{u} = e^{-\Delta G_\mathrm{u}^\circ/(RT)} = e^{-(30\times 10^3\ \mathrm{J/mol})/(8.314\ \mathrm{J/(mol\cdot K)}\times 298\ \mathrm{K})}$$

$$K_\mathrm{u} = \frac{[\mathrm{U}]_\mathrm{eq}}{[\mathrm{N}]_\mathrm{eq}} \approx 6 \times 10^{-6}$$

- Only a very small fraction of molecules are unfolded at any instant.
- But, unfolding can be relatively fast, and individual molecules will sample the unfolded state at some point.
- What determines the overall equilibrium between native and unfolded states?
- What determines which three-dimensional structure a particular sequence will form?