Lecture of November 26: Chapter 14, *in vivo* Biology, and the Biophysics of Crowding

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The living *(in vivo)* cell is a crowded place

- Assuming E. Coli has a volume of $V_{Ecoli} \sim 1 \mu m^3$: about 20% of the volume is occupied by proteins; about 6% by RNA …
- Overall macromolecules occupied about 40 to 60% of the volume of a living cell.
- The dilute gas approximation used in many calculations is not valid.
- See data on next page for calculation

Data

Table 2.1: Observed macromolecular census of an *E. coli* cell. (Data from F. C. Neidhardt et al., Physiology of the Bacterial Cell, Sinauer Associates, 1990 and M. Schaechter et al., Microbe, ASM Press, 2006.)

Substance	% of total dry weight	Number of molecules
Macromolecules		
Protein	55.0	2.4×10^6
RNA	20.4	
23S RNA	10.6	19,000
16S RNA	5.5	19,000
5S RNA	0.4	19,000
Transfer RNA (4S)	2.9	200,000
Messenger RNA	0.8	1,400
Phospholipid	9.1	22×10^6
Lipopolysaccharide (outer membrane)	3.4	1.2×10^6
DNA	3.1	2
Murein (cell wall)	2.5	1
Glycogen (sugar storage)	2.5	4,360
Total macromolecules	**96.1**	
Small molecules		
Metabolites, building blocks, etc.	2.9	
Inorganic ions	1.0	
Total small molecules	**3.9**	

Data

Sizes

SLIDE 3

hydrogen atom radius 0.05 nm
water molecule radius 0.135 nm
covalent bond length ≈ 0.1 nm
H-bond, distance between atoms flanking H 0.27 nm
sugar, amino acid, nucleotide = 0.5 – 1 nm
electron microscope resolution 0.7 nm
Debye screening length (of physiological Ringer's solution) $\lambda_D \approx 0.7$ nm
Bjerrum length of water at room temp $\ell_B \equiv e^2/4\pi\varepsilon k_B T_r = 0.71$ nm; thus $4\pi\ell_B =$
diameter of DNA 2 nm
globular protein diameter 2–10 nm (lysozyme, 4 nm; RNA polymerase, 10 nm)
bilayer membrane thickness ≈ 3 nm
diameter of F-actin 5 nm

diameter of nucleosome 10 nm
E. coli flagellum radius 10 nm
synaptic cleft in chemical synapse 20–40 nm (myoneural junction, 50–100 nm)
poliovirus diameter 25 nm (smallest virus, 20 nm)
microtubule diameter 25 nm
ribosome diameter 30 nm (25 nm for *E. coli*)
casein micelle diameter 100 nm
thinnest wire in Pentium processor chip, about 100 nm
eukaryotic flagellum diameter 100–500 nm
width of transistor in consumer electronics ≈ 180 nm
optical microscope resolution 200 nm
vertebrate axon diameter 0.2–20μm
wavelength of visible light, 400–650 nm
size of bacterium 1μm (smallest, 0.5μm)
myofibril diameter 1–2μm
capillary diameter, as small as 3 μm

DATA

Specialized values

SLIDE 4

Water

Energy to break an intramolecular hydrogen bond in water, 1–$2k_{\mathrm{B}}T_{\mathrm{r}}$; hydrogen bond when two water molecules condense in vacuum, $8\,k_{\mathrm{B}}T_{\mathrm{r}}$.

Attraction energy of two 0.3nm ions in water, about $k_{\mathrm{B}}T_{\mathrm{r}}$.

Heat of vaporization of water $Q_{\mathrm{vap}} = 2.3 \cdot 10^6\,\mathrm{J\,kg^{-1}}$.

Oil–water surface tension, $\Sigma = 0.04\,\mathrm{J\,m^{-2}}$; air–water surface tension, $0.072\,\mathrm{J\,m^{-2}}$.

Number density of water molecules in pure water, 55 M; mass density of water at 20°C, $998\,\mathrm{kg\,m^{-3}}$.

Diffusion constant for generic small molecules in water, $D \approx 1\,\mu\mathrm{m}^2/\mathrm{ms}$. Specifically, for O_2 it's $2\,\mu\mathrm{m}^2/\mathrm{ms}$; for water molecules themselves, $2.2\,\mu\mathrm{m}^2/\mathrm{ms}$; for glucose, $0.67\,\mu\mathrm{m}^2/\mathrm{ms}$.

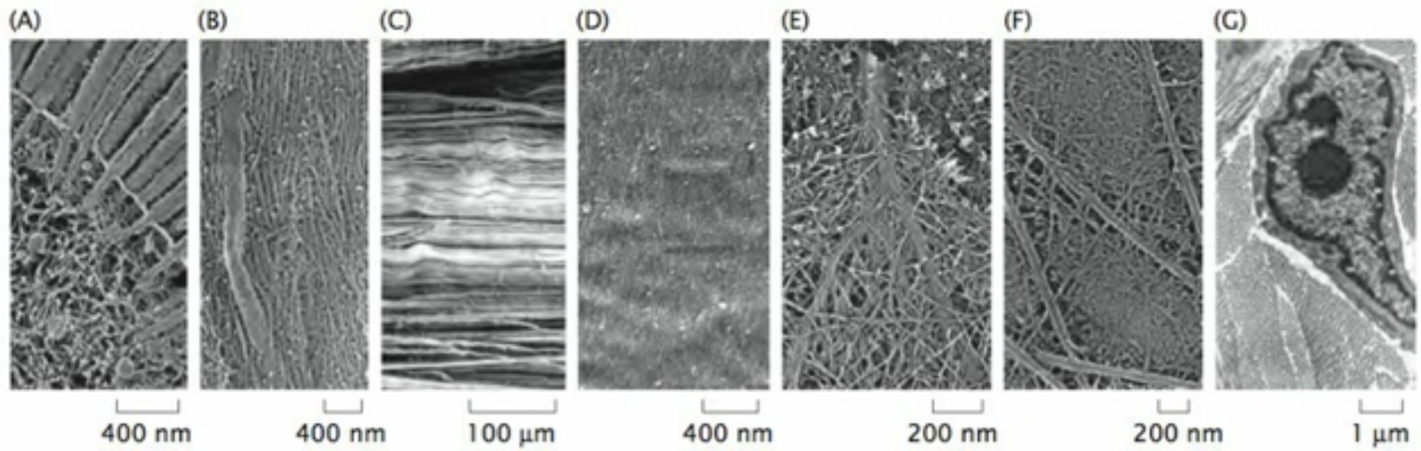
Diffusion constant for typical globular protein in water, $D \approx 10^{-2}\,\mu\mathrm{m}^2/\mathrm{ms}$.

Heat capacity of water at room temperature, $4180\,\mathrm{J\,kg^{-1}K^{-1}}$. This corresponds to $0.996\,\mathrm{cal\,cm^{-3}K^{-1}}$.

Thermal conductivity of water at 0°C, $0.56\,\mathrm{J\,s^{-1}\,m^{-1}\,K^{-1}}$; at 100°C it's $6.8\,\mathrm{J\,s^{-1}\,m^{-1}\,K^{-1}}$.

Viscosity of water at 20°C, $1.00 \cdot 10^{-3}\,\mathrm{Pa \cdot s}$; of air, $1.72 \cdot 10^{-5}\,\mathrm{Pa \cdot s}$; of honey, $0.1\,\mathrm{Pa \cdot s}$; of glycerol, $1.4\,\mathrm{Pa \cdot s}$. The effective viscosity of cell cytoplasm depends on the size of the object considered: For molecules smaller than 1 nm it's similar to that of water; for particles of diameter 6 nm (such as a $10^5\,\mathrm{g\,mole^{-1}}$ protein) it's about 3 times greater. For 50–500 nm particles it's 30–300 times as great as water, while the entire cell behaves as though its viscosity were a million times as great as water.

Filament Networks Architecture inside and outside the cell (Figure 14.1)



Crowding by Macromolecules alter Chemical Equilibria

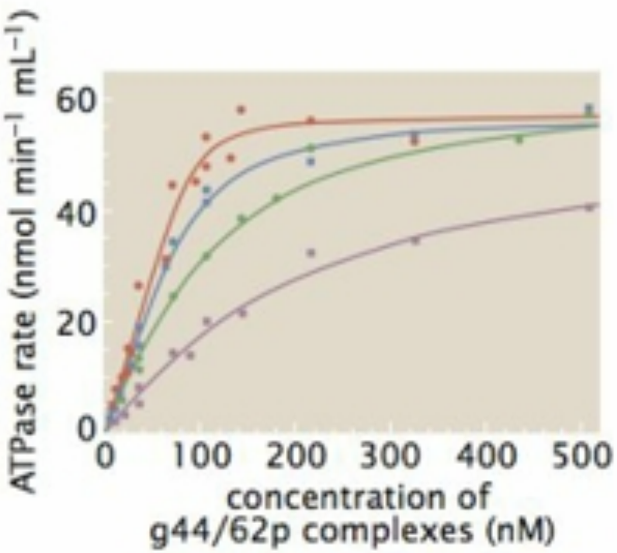


Figure 14.3: ATPase rate associated with T4 DNA replication. The different curves measure the ATPase rate as a function of the g44/62p (clamp loader) protein concentration as measured using different concentrations of polyethylene glycol as a crowding agent. The concentrations of polyethylene glycol going from the bottom to the top curve are 0, 2.5, 5, and 7.5 weight percent. (Adapted from T. C. Jarvis et al., *J. Biol. Chem.* 265:15160, 1990.)

Crowding alters Kinetics (Hydrodynamics) in cells

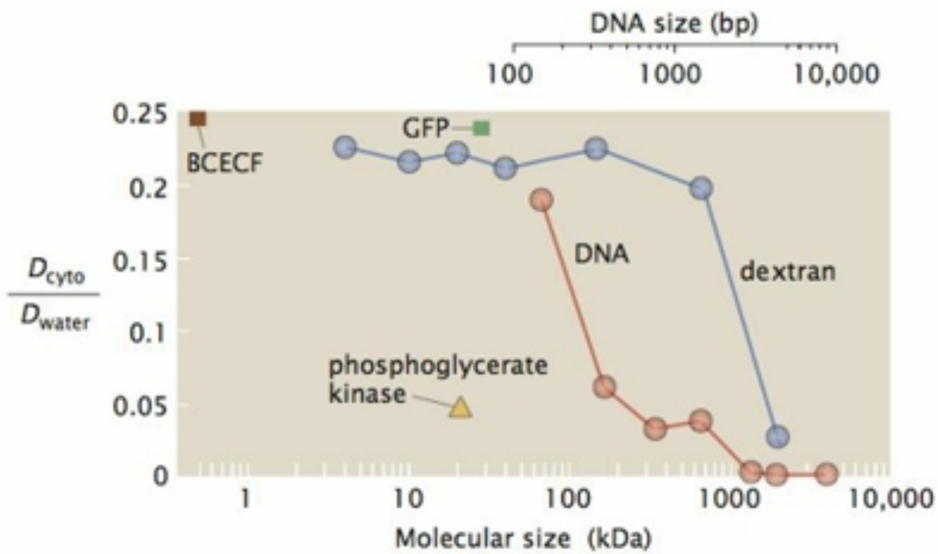
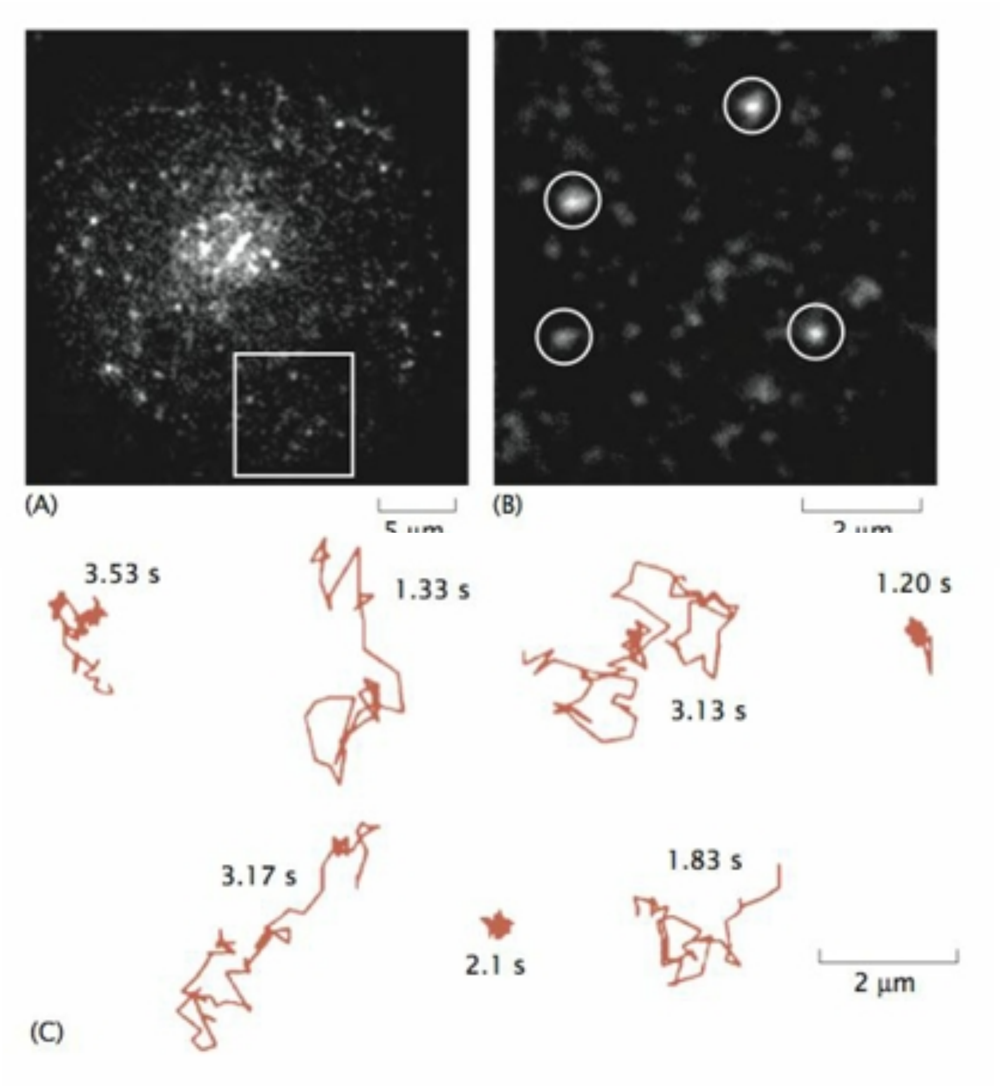


Figure 14.4: Diffusion constants in cells. The plot shows the ratio of the measured cellular diffusion constant to that for the same molecule in water for several different molecules, including a series of DNA molecules of different sizes and BCECF, a small fluorescent molecule. (Adapted from A. S. Verkman, *Trends Biochem. Sci.* 27:27, 2002.)

Crowding alters Kinetics (Hydrodynamics) in cells



This is figure 14.4
See class notes for calculation to verify this behavior.

Crowding Effect on Binding

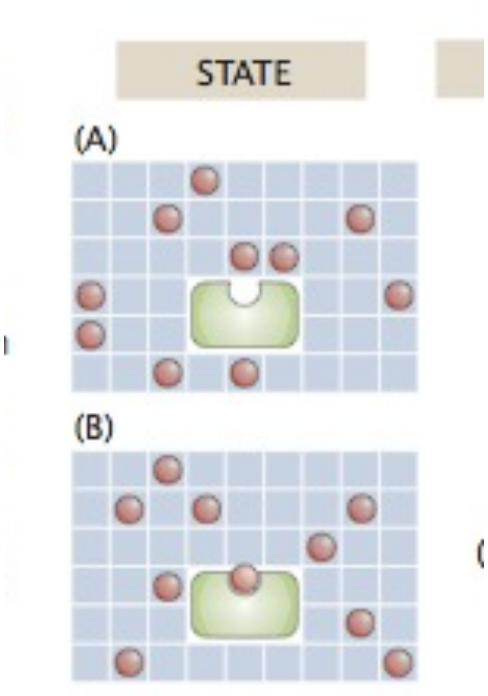


Figure 6.4 Binding with
no crowding

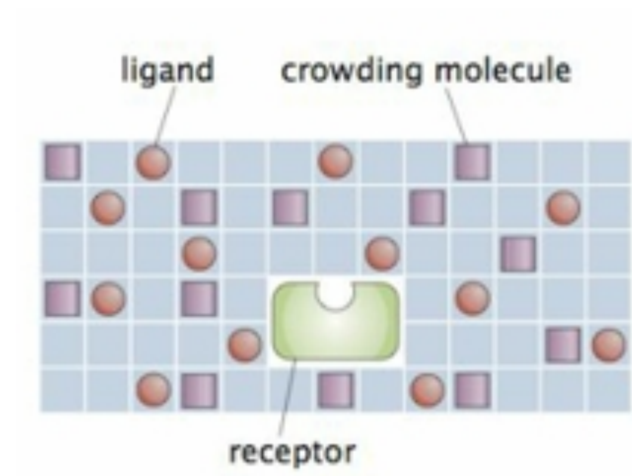


Figure 14.9 Binding with
crowding Molecules

Binding without Crowding

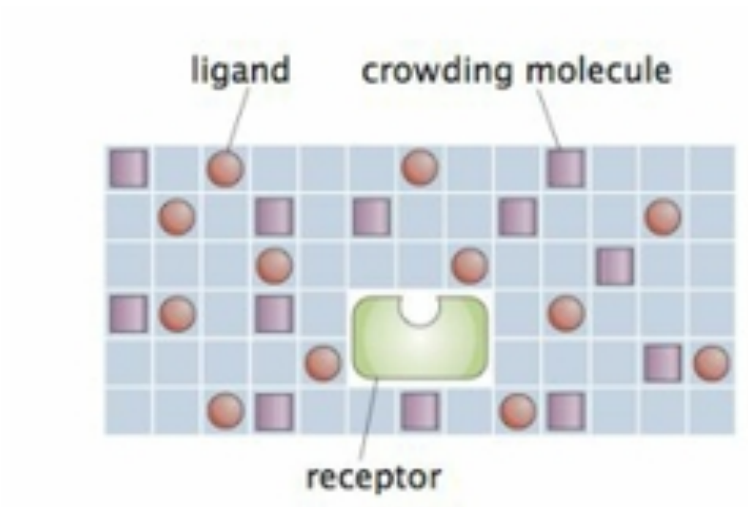
$$Z(L,\Omega) = e^{-\beta L\varepsilon_{sol}} \frac{\Omega!}{L!(\Omega - L)!} + e^{-\beta\varepsilon_b} e^{-\beta(L-1)\varepsilon_{sol}} \frac{\Omega!}{(L-1)![\Omega - (L-1)]!}. \tag{6.14}$$

$$p_{bound} = \frac{e^{-\beta\varepsilon_b} \frac{\Omega^{L-1}}{(L-1)!} e^{-\beta(L-1)\varepsilon_{sol}}}{\frac{\Omega^L}{L!} e^{-\beta L\varepsilon_{sol}} + e^{-\beta\varepsilon_b} \frac{\Omega^{L-1}}{(L-1)!} e^{-\beta(L-1)\varepsilon_{sol}}}. \tag{6.17}$$

$$p_{bound} = \frac{(L/\Omega)e^{-\beta\Delta\varepsilon}}{1 + (L/\Omega)e^{-\beta\Delta\varepsilon}}, \tag{6.18}$$

Figure 6.4 Binding with **no crowding**

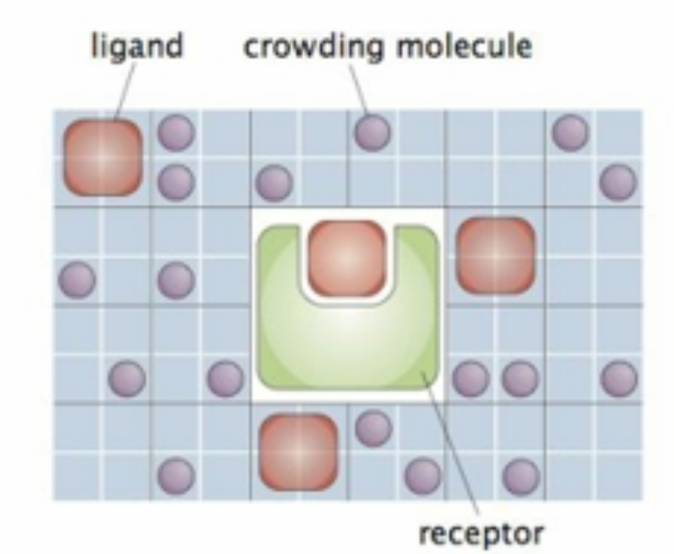
Crowding Effect on Binding: Ligand and Crowding Molecule are the same size

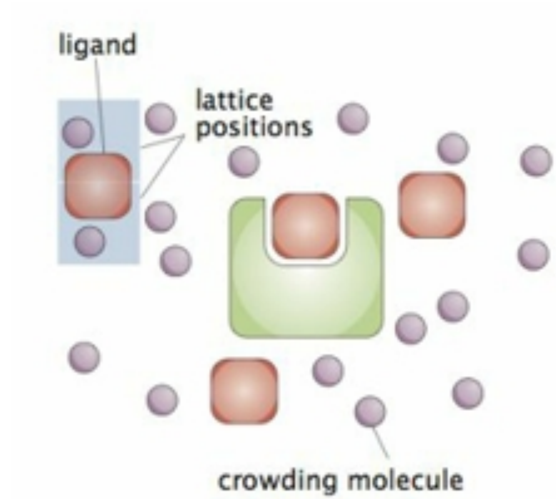


$$Z_{\mathrm{sol}}(L, C) = \frac{\Omega!}{L!C!(\Omega - L - C)!} \mathrm{e}^{-\beta L \varepsilon_{\mathrm{L}}^{\mathrm{sol}}} \mathrm{e}^{-\beta C \varepsilon_{\mathrm{C}}^{\mathrm{sol}}},$$

Class note will show that there's no effect!

Crowding Effect on Binding: Large Ligands and Small Crowding Molecules





- It is entropically favorable for large ligand to bind
- This “force” is called a depletion force