Chapter 14
Crowding Effect of Cytoplasm
The Cell is Crowded

• When we modeled ligand-receptor binding reactions, polymer configurations or diffusion of particles, we assumed:
  a) Ideality: Molecules do not interact with each other
  b) Homogeneity: Environment is uniform.

• Most biochemical experiments in a test tube are performed at cellular pH and ionic strength, but we ignore the need for correction for extract dilution with molecular crowding.

• Ideality and homogeneity assumptions are not true inside cells.
• Mean spacing between proteins inside the cytoplasm (~10 nm) is on the order of their size (3-5 nm).
• The cytoplasm consists of various macromolecular networks that are densely crowded together.
Consequences of Crowding

1. Crowding Alters Biochemical Equilibria

- Previously, we showed how to write a partition function for occupancy of the receptor.
- These results will be modified by the presence of crowding agents.
- The presence of crowding agents effectively reduces the volume in which ligand molecules can distribute themselves.
- Entropic cost of stealing one ligand from solution decreases.
- Therefore, the presence of crowding molecules favor binding of ligand to receptor.
The Role of Crowding in Biochemical Equilibria

in the absence of crowding agents,

when there are L ligand molecules in solution, and none bound to the receptor

\[
Z(L) = \frac{\Omega!}{L! (\Omega - L)!} e^{-\beta L \varepsilon^\text{sol}_L} + \frac{\Omega!}{(L - 1)! (\Omega - L + 1)!} e^{-\beta [(L-1)\varepsilon^\text{sol}_L + \varepsilon_b]}
\]

\[
p_{\text{bound}} = \frac{\frac{\Omega!}{(L - 1)! (\Omega - L + 1)!} e^{-\beta [(L-1)\varepsilon^\text{sol}_L + \varepsilon_b]}}{Z} = \frac{1}{1 + \frac{\Omega}{L} e^{\beta \Delta \varepsilon}}
\]

where \( \Delta \varepsilon_L = \varepsilon_b - \varepsilon^\text{sol}_L \)

in the presence of C crowding agents

\[
Z(L, C) = \frac{\Omega!}{L! C! (\Omega - L - C)!} e^{-\beta [L \varepsilon^\text{sol}_L + C \varepsilon^\text{sol}_C]} + \frac{\Omega!}{(L - 1)! C! (\Omega - L - C + 1)!} e^{-\beta [(L-1)\varepsilon^\text{sol}_L + C \varepsilon^\text{sol}_C + \varepsilon_b]}
\]

\[
p_{\text{bound}} = \frac{\frac{\Omega!}{(L - 1)! C! (\Omega - L - C + 1)!} e^{-\beta [(L-1)\varepsilon^\text{sol}_L + C \varepsilon^\text{sol}_C + \varepsilon_b]}}{Z} = \frac{1}{1 + \left(\frac{\Omega - L - C}{L}\right) e^{\beta \Delta \varepsilon_L}}
\]
The Role of Crowding in Biochemical Equilibria

\[ p_{\text{bound}} = \frac{1}{1 + \left( \frac{\Omega - L - C}{L} \right) e^{\beta \Delta \varepsilon_L}} \]

Assuming that \( L \ll \Omega \),

crowding will be significant when \( C \) is comparable to \( \Omega \)

\[ \Delta \varepsilon = -5kT \]

\[ \Omega = 1000 \]
• If the size of the crowding agent is significantly smaller than the ligand, we need to modify our model.

• Assume that each ligand can fill up \( r \) small boxes, whereas each crowding molecule can fill a single small box.

• If we have \( \Omega \) large boxes,

\[
Z(L, C) = \frac{\Omega!}{L!(\Omega - L)! C! (r\Omega - rL)!} \frac{(r\Omega - rL)!}{(r\Omega - rL - C)!} e^{-\beta [L \varepsilon_L^{\text{sol}} + C \varepsilon_C^{\text{sol}}]} + \frac{\Omega!}{(L - 1)!(\Omega - L + 1)! C! (r\Omega - rL - C + r)!} \frac{(r\Omega - rL + r)!}{(r\Omega - rL - C + r)!} e^{-\beta [(L-1) \varepsilon_L^{\text{sol}} + C \varepsilon_C^{\text{sol}} + \varepsilon_b]}
\]

since \( L \ll \Omega \),

\[
p_{\text{bound}} = \frac{1}{1 + \frac{\Omega}{L} (1 - \phi_c)^r e^{\beta \Delta \varepsilon_L}}
\]

where \( \phi_c = \frac{C}{r\Omega} \) is the volume fraction of crowding molecules in solution
Depletion Forces: Order from Disorder

- In a solution containing small and large molecules, as the large particles approach one another, small particles are excluded from the volume between them.
- As they get even closer, volume available for small molecules increase.
- This has the effect of inducing entropic force of attraction between the large particles.
Crowded Dynamics

- We envisioned that enzymatic reactions in cells are dominated by diffusion and their overall concentration.
- However, most enzymes are not free to diffuse, and certain intermediary complexes are too low to account for observed reaction rates.
- Often, this problem is solved by forming a multiprotein complex or closely locating multiple enzymes within the pathway into a small compartment.
Crowded Dynamics: Protein Folding

• In vitro, many proteins can refold back after denaturation in buffer solution.
• Within cells, proteins tend to aggregate through interactions between their hydrophobic regions.
• To solve the folding problem within a cytoplasm, cells have molecular machines that compartmentalize peptides into hydrophobic chambers.
• Molecular Chaperons consume ATP energy to open the chamber, allow the peptide to enter the chamber and close the chamber and allow the peptide to fold properly by preventing the aggregation with others.

http://www.youtube.com/watch?v=USuO70m2kew&feature=related see 40th minute
Crowded Dynamics: Diffusion in Crowded Environments

- For a particle to hop to a new position, that new position cannot be occupied.
- Assume that the volume fraction occupied by the molecules is $\phi$.
- The probability that chosen site is occupied is again $\phi$.
- *Probability of the particle to jump right or left would be:*

\[
\begin{align*}
\text{Probability of the particle to jump right or left would be:} & \\
\begin{array}{c}
\text{TRAJECTORY} \\
\end{array} & \begin{array}{c}
\text{PROBABILITY} \\
\frac{1}{2} (1 - \phi) \\
\phi \\
\end{array} & \begin{array}{c}
\text{DISPLACEMENT x} \\
a \\
-a \\
0 \\
\end{array}
\end{align*}
\]

\[
p_{\text{right}} = p_{\text{left}} = \frac{1}{2} (1 - \phi)
\]

\[
p_{\text{stay}} = \phi
\]

*To compute the diffusion constant, we evaluate the rms displacement of a single step*

\[
< x^2 > = p_{\text{right}} a^2 + p_{\text{left}} a^2 + p_{\text{stay}} 0 = a^2 (1 - \phi) \quad \text{where } a \text{ is the step length.}
\]

\[
D = D_0 (1 - \phi)
\]