ION GATING DRIVEN BY LIGANDS

- Ligand-gated channels.

ION-CHANNEL-COUPLED RECEPTORS

Figure 15-16a Molecular Biology of the Cell 5/e (© Garland Science 2008)
ION GATED CHANNELS: ACETYLCHOLINE

(A) acetylcholine

O
H₃C — C — O — CH₂ — CH₂ — N' — CH₃

CH₃

(B) heart muscle cell

receptor protein

acetylcholine

DECREASED RATE AND FORCE OF CONTRACTION

(C) skeletal muscle cell

CONTRACTION

(D) salivary gland cell

SECRETION

Figure 15-9 Molecular Biology of the Cell 5/e (© Garland Science 2008)
Data for the gating of nicotinic acetylcholine receptor

As in previous work (20), we have rendered these relatively insensitive receptors more responsive by introducing a single Leu-9/Ser mutation in the M2 region of the subunit (residue 262) (25, 26). This mutation lowers EC50 by a consistent factor of 40, but by itself does not activate receptors. The M2 9 residue is thought to lie some 50 Å from the binding site, and for the muscle receptor, the increased sensitivity probably arises because the channel is open longer and opens more frequently (C. Labarca, G. N. Filatov, H. Zhang, J. Li, and H. A. L., unpublished work), rather than because a desensitized state becomes conducting, as occurs for the analogous mutation in the homomultimeric receptor (27). It remains formally possible that there are subtle changes in the structure of the binding site because of incorporation of the 9 mutation (H. Zhang, P. Deshpande, C. Labarca, H. A. L., and J. Li, unpublished work) (28). The validity of extending the measurable range of ACh sensitivities by using the Leu-9/Ser mutation is supported by three entries in Table 2 (Trp, 5-Br-Trp, and 5-F-Trp), for which the ratios of EC50 values with and without the 9 mutation are the same.

While the results with 5-CN-Trp were suggestive, the most intriguing data come from studies of the fluorinated Trp derivatives. Beginning with 5-F-Trp and moving on to di-, tri-, and tetrafluoro derivatives produces a series of closely related compounds with substantial electronic but minimal steric changes. Within such a series, the ever-present concerns that F…3. (A) Representative voltage-clamp current traces from oocytes expressing Trp (wild type, Left) and 5,7-F2-Trp (Right) at 149. Bars represent application of ACh (M). (B) Dose–response relations and fits to the Hill equation for (left to right): Trp; 5-F-Trp; 5,7-F2-Trp; 5,6,7-F3-Trp; and 4,5,6,7-F4-Trp. EC50 values are given in Table 2. (C) Plot of log[EC50/EC50 (wild type)] vs. quantitative cation–binding ability at 149 for the same residues as in B. Data are from Table 2. The data fit the line y = 3.2 + 0.096x, with a correlation coefficient r = 0.99. Error bars are approximately the size of the markers.
States and Weights for Binding Problems

- We work out the probability of the binding probability by making a model of the solution as a lattice.

<table>
<thead>
<tr>
<th>STATE</th>
<th>ENERGY</th>
<th>MULTIPLICITY</th>
<th>WEIGHT</th>
</tr>
</thead>
<tbody>
<tr>
<td>(A)</td>
<td>$L e_{sol}$</td>
<td>$\frac{\Omega!}{L!(\Omega-L)!} \approx \frac{\Omega^L}{L!}$</td>
<td>$\frac{\Omega^L}{L!} e^{-\beta L e_{sol}}$</td>
</tr>
<tr>
<td>(B)</td>
<td>$(L-1)e_{sol} + \varepsilon_b$</td>
<td>$\frac{\Omega!}{(L-1)!(\Omega-L+1)!} \approx \frac{\Omega^{L-1}}{(L-1)!}$</td>
<td>$\frac{\Omega^{L-1}}{(L-1)!} e^{-\beta((L-1)e_{sol} + \varepsilon_b)}$</td>
</tr>
</tbody>
</table>

Figure 6.1 Physical Biology of the Cell (© Garland Science 2009)

Figure 6.4 Physical Biology of the Cell (© Garland Science 2009)
These simple binding curves illustrate the way in which the binding probability depends upon the $K_d$ or the binding energy.
EXPLORING PROMOTER ARCHITECTURE: CAN WE COMPUTE HOW CELLS DECIDE?

Figure 6.9 Physical Biology of the Cell (© Garland Science 2009)
EXPLORING PROMOTER ARCHITECTURE: CAN WE COMPUTE HOW CELLS DECIDE?

Figure 6.13 Physical Biology of the Cell (© Garland Science 2009)
WHERE WE ARE HEADED: CAN WE COMPUTE HOW CELLS DECIDE?

Knobs to tune architecture

Simple activation

Fold-change \( = \frac{1 + ae^{\frac{v_{ap}}{kT}}}{1 + a} \)

Activator and helper

Fold-change \( = \frac{1 + a + he^{\frac{v_{ha}}{kT}}}{1 + h} \)

Unregulated promoter

Simple repression

Fold-change \( = (1+r)^{-1} \)

DNA looping

Fold-change \( = \left(1 + \frac{r_m}{1 + r_a} e^{\frac{\tau_{act} + \tau_{loop}}{kT}}\right)^{-1} \)

Bintu et al. (2005)
Some other examples

• Data and fits using our binding formula.

Figure 6.26  Physical Biology of the Cell (© Garland Science 2009)
SOME OTHER EXAMPLES

Table 2-1  Sample receptor/ligand binding parameters

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Uptake</th>
<th>Kd (mM)</th>
<th>Rmax (pmol kg⁻¹)</th>
<th>Km (mM)</th>
<th>L (μL ml⁻¹)</th>
<th>Emax (μmol kg⁻¹)</th>
<th>IC50 (μM)</th>
<th>IC50 (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transferrin</td>
<td>Transferrin</td>
<td>LPA</td>
<td>1.1 x 10⁻⁶</td>
<td>3 x 10⁻⁶</td>
<td>6.1</td>
<td>2 x 10⁻⁴</td>
<td>13</td>
<td>2 x 10⁻⁴</td>
</tr>
<tr>
<td>Cholinergic peptide</td>
<td>PHLP-II</td>
<td>Sepiolite</td>
<td>5.0 x 10⁻⁶</td>
<td>3 x 10⁻⁶</td>
<td>0.02</td>
<td>1 x 10⁻⁵</td>
<td>60</td>
<td>1 x 10⁻⁵</td>
</tr>
<tr>
<td>Muscarinic receptors</td>
<td>Muscarinic receptors</td>
<td>nAchR</td>
<td>1.1 x 10⁻⁵</td>
<td>3 x 10⁻⁵</td>
<td>0.001</td>
<td>3 x 10⁻⁶</td>
<td>20</td>
<td>3 x 10⁻⁶</td>
</tr>
<tr>
<td>TGF</td>
<td>TGF</td>
<td>AAM</td>
<td>1.8 x 10⁻⁶</td>
<td>3 x 10⁻⁶</td>
<td>0.14</td>
<td>1 x 10⁻⁵</td>
<td>11</td>
<td>1 x 10⁻⁵</td>
</tr>
<tr>
<td>Proteoglycan</td>
<td>Proteoglycan</td>
<td>PEC</td>
<td>1.4 x 10⁻⁶</td>
<td>3 x 10⁻⁶</td>
<td>0.15</td>
<td>1 x 10⁻⁵</td>
<td>65</td>
<td>1 x 10⁻⁵</td>
</tr>
<tr>
<td>Promoter</td>
<td>Promoter</td>
<td>TFI</td>
<td>1.1 x 10⁻⁶</td>
<td>3 x 10⁻⁶</td>
<td>0.1</td>
<td>2 x 10⁻⁶</td>
<td>2.5</td>
<td>2 x 10⁻⁶</td>
</tr>
<tr>
<td>EGF</td>
<td>EGF</td>
<td>1.5 x 10⁻⁷</td>
<td>1.5 x 10⁻⁷</td>
<td>0.15</td>
<td>5 x 10⁻⁸</td>
<td>1.6</td>
<td>5 x 10⁻⁸</td>
<td></td>
</tr>
<tr>
<td>Peroxide</td>
<td>Peroxide</td>
<td>2 x 10⁻⁷</td>
<td>7 x 10⁻⁷</td>
<td>0.1</td>
<td>5 x 10⁻⁸</td>
<td>5.2</td>
<td>5 x 10⁻⁸</td>
<td></td>
</tr>
<tr>
<td>mRNA bound to</td>
<td>mRNA bound to</td>
<td>lL3</td>
<td>2 x 10⁻⁷</td>
<td>1.3 x 10⁻⁷</td>
<td>0.15</td>
<td>5 x 10⁻⁸</td>
<td>10</td>
<td>5 x 10⁻⁸</td>
</tr>
<tr>
<td>IL-6 (mouse strain)</td>
<td>IL-6 (mouse strain)</td>
<td>IL-4</td>
<td>2 x 10⁻⁷</td>
<td>1.3 x 10⁻⁷</td>
<td>0.15</td>
<td>5 x 10⁻⁸</td>
<td>1.0</td>
<td>5 x 10⁻⁸</td>
</tr>
<tr>
<td>IL-6 (mouse strain)</td>
<td>IL-6 (mouse strain)</td>
<td>IL-4</td>
<td>2 x 10⁻⁷</td>
<td>1.3 x 10⁻⁷</td>
<td>0.15</td>
<td>5 x 10⁻⁸</td>
<td>1.0</td>
<td>5 x 10⁻⁸</td>
</tr>
</tbody>
</table>

Notes: The estimated number of receptors per cell, Kd, the association rate constant for the binding of the ligand, and the dissociation rate constant are indicated for the indicated receptors and ligands. (a) univalent ligand for the receptors. (b) AEG = AEG-755. (c) For the receptors; EGF = EGF, PHLP-II = PHLP-II, TFI = TFI, TGF = TGF, AAM = AAM, AAM = AAM, PEC = PEC, PEC = PEC, TFI = TFI, TFI = TFI, and TFI = TFI. (d) Rmax = Rmax, Rmax = Rmax, Rmax = Rmax, Rmax = Rmax, and Rmax = Rmax.

GIBBS' SECOND LAW

• One idea only: to find the privileged terminal state of a system, maximize the entropy.
• A corollary: minimize the free energy – this is for a system in contact with a heat bath.
• My point here is to get us all to think about the chemical potential.

Figure 5.27 Physical Biology of the Cell (© Garland Science 2009)
The Gibbs Distribution

System in contact with an energy reservoir

Probability for finding the system in microstate $i$:

$$p(E_i) = \frac{1}{Z} e^{-E_i/k_B T} \quad \text{Boltzmann distr.}$$

$$Z = \sum_{i=1}^{N} e^{-E_i/k_B T} \quad \text{- partition f.}$$

$$\langle E \rangle = \frac{1}{Z} \sum_{i=1}^{N} E_i p(E_i) = \frac{1}{Z} \sum_{i=1}^{N} E_i e^{-E_i/k_B T} = -\frac{\partial}{\partial\beta} \ln Z$$

$T_{\text{reservoir}}$ controls av. energy $\langle E \rangle$ of the system

System in contact with a particle and energy reservoir

Probability for finding the system in microstate $i$:

$$p(E_s^{(i)}, N_s^{(i)}) = \frac{e^{-\beta (E_s^{(i)} - \mu N_s^{(i)})}}{\mathcal{Z}}$$

Gibbs distr.

grand partition f.

$$\mathcal{Z} = \sum e^{-\beta (E_s^{(i)} - N_s^{(i)} \mu)}$$

$$\langle N \rangle = \frac{1}{\mathcal{Z}} \sum_i N_i e^{-\beta (E_i - N_i \mu)} = \frac{1}{\beta} \frac{\partial}{\partial\mu} \ln \mathcal{Z}$$

$\mu_{\text{res.}}$ controls av. # of particles $\langle N \rangle$ in the syst.
LIGAND-RECEPTOR BINDING: STATE VARIABLE DESCRIPTION

• Consider a single receptor in contact with the surrounding heat bath and particle reservoir.
• Two-state (b/u), $\sigma$ is an indicator of the state of binding
• The energy is

$$E = \varepsilon_b \sigma$$

$\varepsilon_b < 0$

• Evaluate aver. # of ligands bound, $\langle N \rangle$:

$$\langle N \rangle = \frac{1}{\mathcal{Z}} \sum_i N_i e^{-\beta(E_i - N_i \mu)}$$

$\mathcal{Z} = \sum_{\sigma=0}^1 e^{-\beta(\varepsilon_b \sigma - \mu \sigma)} = 1 + e^{-\beta(\varepsilon_b - \mu)}$

$$\langle N \rangle = \frac{e^{-\beta(\varepsilon_b - \mu)}}{1 + e^{-\beta(\varepsilon_b - \mu)}}$$

can also be computed as $\frac{1}{\beta} \frac{\partial}{\partial \mu} \ln \mathcal{Z}$

• Recall that the chem.potential of an ideal solution is

$$\mu = \mu_0 + k_BT \ln \left( \frac{c}{c_0} \right)$$

$\Rightarrow$

$$\langle N \rangle = \frac{\frac{c}{c_0} e^{-\beta \Delta \varepsilon}}{1 + \frac{c}{c_0} e^{-\beta \Delta \varepsilon}}$$

$\Delta \varepsilon = \varepsilon_b - \mu_0$ is the energy difference upon taking the ligand from solution and placing it on the receptor
Cooperativity and Binding

- Interestingly, many (if not most) of the real world binding problems we care about in biology do not satisfy the simple binding model (sometimes called the Langmuir adsorption isotherm) we have worked out so far.

- The classic example (i.e. the hydrogen atom of binding problems) is hemoglobin.

---

**Figure 4.4 Physical Biology of the Cell © Garland Science 2009**
Hemoglobin as a case study in cooperativity

- Hemoglobin - the classic example of ligand-receptor binding
- Cooperativity: the binding energy for a given ligand depends upon the # of ligands that are already bound to the receptor
- Intuitively: conformational change upon binding => the next ligand experiences a different binding energy

The protein hemoglobin: 4 polypeptide chains (2 α-chains, 2 β-chains), each carries a heme group => protein can bind up to 4 molecules of O₂

The heme group includes a porphyrin ring (gray line) + iron

Oxygen binds to heme on the hemoglobin molecules

several 100s hemoglobin molecules

Red blood cell

Hemoglobin molecule

apps.uwhealth.org
The nature of the Hill function

![Graph showing the Hill function with different values of n: n = 1, n = 2, n = 4. The graph plots \( p_{\text{bound}} \) against ligand concentration \( K_d \).](image)

Figure 6.27 Physical Biology of the Cell (© Garland Science 2009)
Hemoglobin as a case study in cooperativity

• Hemoglobin-oxygen binding: language of two-states occupation variables. State of system is described with the vector

\[
(\sigma_1, \sigma_2, \sigma_3, \sigma_4)
\]

where \(\sigma_i\): \(\sigma_i = 0\) (unbound), \(\sigma_i = 1\) (bound)

• Q.: what is the average # of bound \(O_2\) molecules as a function of the \(O_2\) concentration (or partial pressure)?

A toy model of a dimoglobin

• To illustrate the idea of cooperativity: imagine a fictitious dimoglobin (=dimeric hemoglobin) molecule which has 2 \(O_2\) binding sites (e.g., clams)

• \((\sigma_1, \sigma_2)\) \(\Rightarrow\) 4 distinct states

• The energy of the system:

\[
E = \varepsilon(\sigma_1 + \sigma_2) + J\sigma_1\sigma_2
\]

Energy associated with \(O_2\) being bound to one of the 2 sites

measure of the cooperativity
A TOY MODEL OF A DIMOGLOBIN

- The grand partition function (sum over the 4 states):

\[ \mathcal{Z} = 1 + \frac{1}{2} e^{-\beta (\epsilon - \mu)} + \frac{1}{2} e^{-\beta (\epsilon - \mu)} + e^{-\beta (2\epsilon + J - 2\mu)} \]

Single occupancy
Both sites occupied

- compute the probabilities for each classes of states: unoccupied, single occupancy, double occupancy

Parameters used: \( \Delta \epsilon = -5 \ k_B T, J = -2.5 \ k_B T, c_0 = 760 \ \text{mmHg} \)
• Membrane proteins are characterized in some cases by transmembrane alpha helices and cytosolic domain that passes along the signal.
Coupling receptors to enzyme action

• Receptor binding changes the probability of the “active” state.

Figure 15-16c Molecular Biology of the Cell 5/e (© Garland Science 2008)
• A wonderful and important topic for our consideration is that of posttranslational modifications.

• One of the tricks performed by the cytoplasmic side of a receptor (or its partners) is to do some posttranslational modification.
• In bio systems, changes in envir. conditions => the activity of an enzyme must be rapidly altered

• One of the most important regulatory modes in all of biology: regulation of protein activity by covalent attachment of phosphate groups

• The substrate for protein phosphorylation: target protein and ATP

• The enzyme: protein kinase (transfers the terminal phosphate group from ATP to a chemical group on a protein)

• A phosphate group carried 2 “-” charges => causes a dramatic change in the local charge distribution on the surface of the protein => drastic, large scale effect on protein structure and ability to bind

• This alteration is reversible: protein phosphatase
The diversity of kinases

• “The whole molecular control network, leading from the receptors at the cell surface to the genes in the nucleus, can be viewed as a computing device; and, like that other computing device, the brain, it presents one of the hardest problems in biology.”

• Catalytic domains shown in green. Roughly 250 aa long.

Figure 15-70 Molecular Biology of the Cell 5/e (© Garland Science 2008)
What is the fraction of activated proteins? How does it depend on the state of phosphorylation?

Model:

The “structural” state of the protein (active/inactive):

\[ \sigma_s: \quad \sigma_s = 0 \Rightarrow \text{inactive}, \]
\[ \sigma_s = 1 \Rightarrow \text{active} \]

The state of phosphorylation of the protein:

\[ \sigma_p: \quad \sigma_p = 0 \Rightarrow \text{unphosphorylated}, \]
\[ \sigma_p = 1 \Rightarrow \text{phosphorylated} \]

The state of phosphorylation can alter the relative energies of the active and inactive states

\[ \Rightarrow \text{at equilibrium, most of the phosphorylated molecules will be in active form} \]

\[ I_1 \text{ and } I_2 \text{ are the electrostatic interaction energies btw the two charges in the active and inactive states} \]

Figure 7.10 Physical Biology of the Cell (© Garland Science 2009)
• Using the $\sigma$ variables, the free energy of the protein is

$$G(\sigma_P, \sigma_S) = (1 - \sigma_P)[(1 - \sigma_S)\varepsilon + \sigma_S(\varepsilon - I_1)]$$

which simplifies to

$$G(\sigma_P, \sigma_S) = \varepsilon \sigma_S - I_2 \sigma_P + (I_2 - I_1)\sigma_S\sigma_P$$

=> states&weights:
PHOSPHORYLATION: TWO INTERNAL STATE VARIABLES

- From the states and weights:

\[
p_{\text{active}} = \frac{e^{-\beta G(\sigma_S=1, \sigma_P=0)}}{\sum_{\sigma_S=0,1} e^{-\beta G(\sigma_S, \sigma_P=0)}} = \frac{e^{-\beta \varepsilon}}{e^{-\beta \varepsilon} + 1}
\]

Probability of the protein being in the active state, if it is not phosphorylated

\[
p_{\text{active}}^* = \frac{e^{-\beta G(\sigma_S=1, \sigma_P=1)}}{\sum_{\sigma_S=0,1} e^{-\beta G(\sigma_S, \sigma_P=1)}} = \frac{e^{-\beta(\varepsilon-I_1)}}{e^{-\beta(\varepsilon-I_1)} + e^{\beta I_2}}
\]

Probability of the protein being in the active state, if it is phosphorylated

- The change in activity due to phosphorylation:

\[
\frac{p_{\text{active}}^*}{p_{\text{active}}} = \frac{1 + e^{\beta \varepsilon}}{1 + e^{\beta(\varepsilon+I_2-I_1)}}
\]

Figure 7.11 Physical Biology of the Cell (© Garland Science 2009)
PHOSPHORYLATION: TWO INTERNAL STATE VARIABLES

\[
\frac{p^*_{\text{active}}}{p_{\text{active}}} = \frac{1 + e^{\beta \varepsilon}}{1 + e^{\beta (\varepsilon + I_2 - I_1)}}
\]

- In the toy model in the figure,

\[\varepsilon \approx 5 \, k_B T \]
\[I_2 - I_1 \approx -10 \, k_B T \]

\[
\Rightarrow \quad \frac{p^*_{\text{active}}}{p_{\text{active}}} \approx 150
\]

-increase in activity upon phosphorylation

- In the cell, the increase in activity upon phosphorylation spans from factors of 2 to 1000.

Figure 7.10 Physical Biology of the Cell (© Garland Science 2009)
• A more precise realization of the implementation of signaling.
• We begin with an example that is simple both conceptually and mathematically, namely, prokaryotic two-component signal transduction.
Two-Component Signal Transduction

- Next few slides are courtesy of Michael Laub (MIT) and Mark Goulian (Upenn) – experts in the quantitative dissection of signaling networks.

- This figure shows the generic features of the two-component signal transduction systems.
COORDINATING MULTIPLE SIGNALING SYSTEMS IN A SINGLE CELL

animation by Mark Gouilan
**Phosphotransfer Profiling**

\[ HK + ATP^* \rightarrow HK\sim P^* + ADP \]

(use complete set of purified RRs)

incubate, separate by SDS-PAGE

\[ HK\sim P^* + RR \rightarrow HK + RR\sim P^* \]
Assessing Specificity: Phosphotransfer Profiling

C. crescentus PhoR profile – 60 min phosphotransfer reactions

C. crescentus PhoR profile – 5 min phosphotransfer reactions

- histidine kinases exhibit a strong kinetic preference in vitro for their in vivo cognate substrate
- specificity based on molecular recognition
Once we finish with our concrete example of chemotaxis, we will turn to the way in which cells decide where to put new actin filament and that will make us face this question of signal integration.
G-protein coupled receptors as an example

G-PROTEIN-COUPLED RECEPTORS

inactive receptor
inactive G protein
inactive enzyme
activated receptor and G protein
activated enzyme
activated G protein

signal molecule

Figure 15-16b Molecular Biology of the Cell 5/e (© Garland Science 2008)