BE/APH161 – PHYSICAL BIOLOGY OF THE CELL

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ION GATING DRIVEN BY LIGANDS

- Ligand-gated channels.

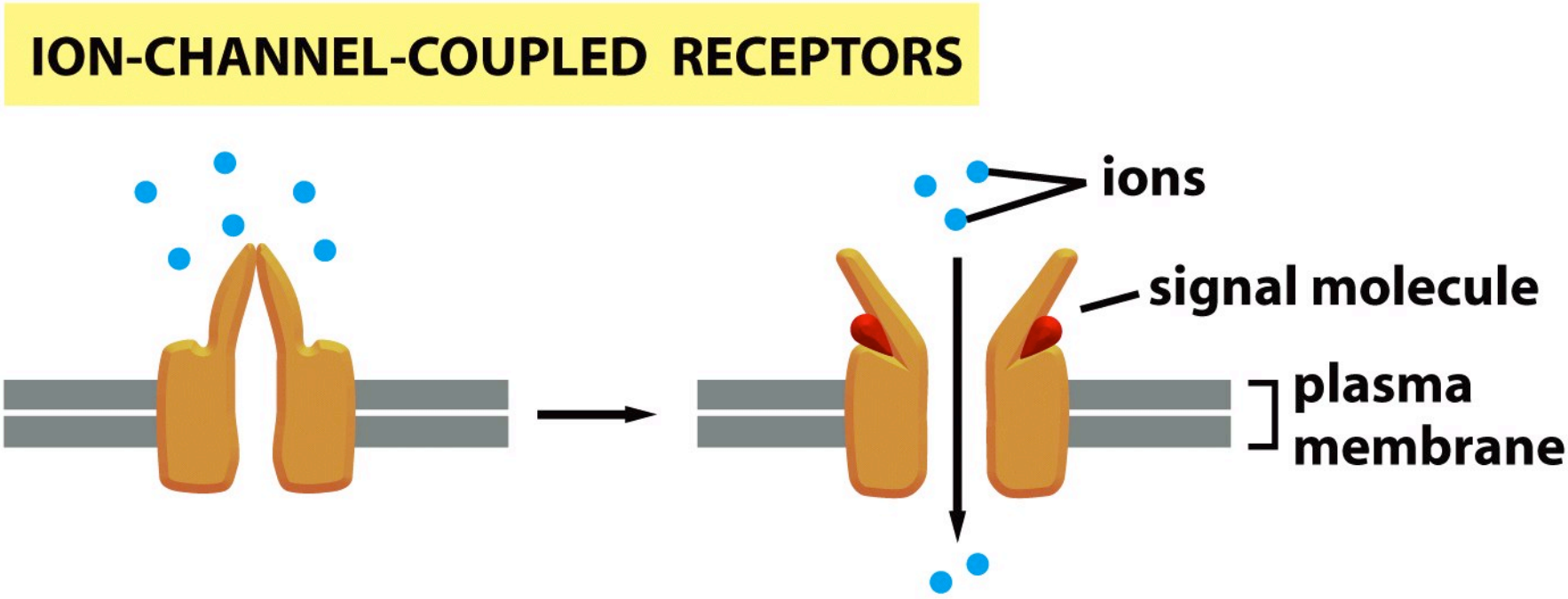


Figure 15-16a Molecular Biology of the Cell 5/e (© Garland Science 2008)

ION GATED CHANNELS: ACETYLCHOLINE

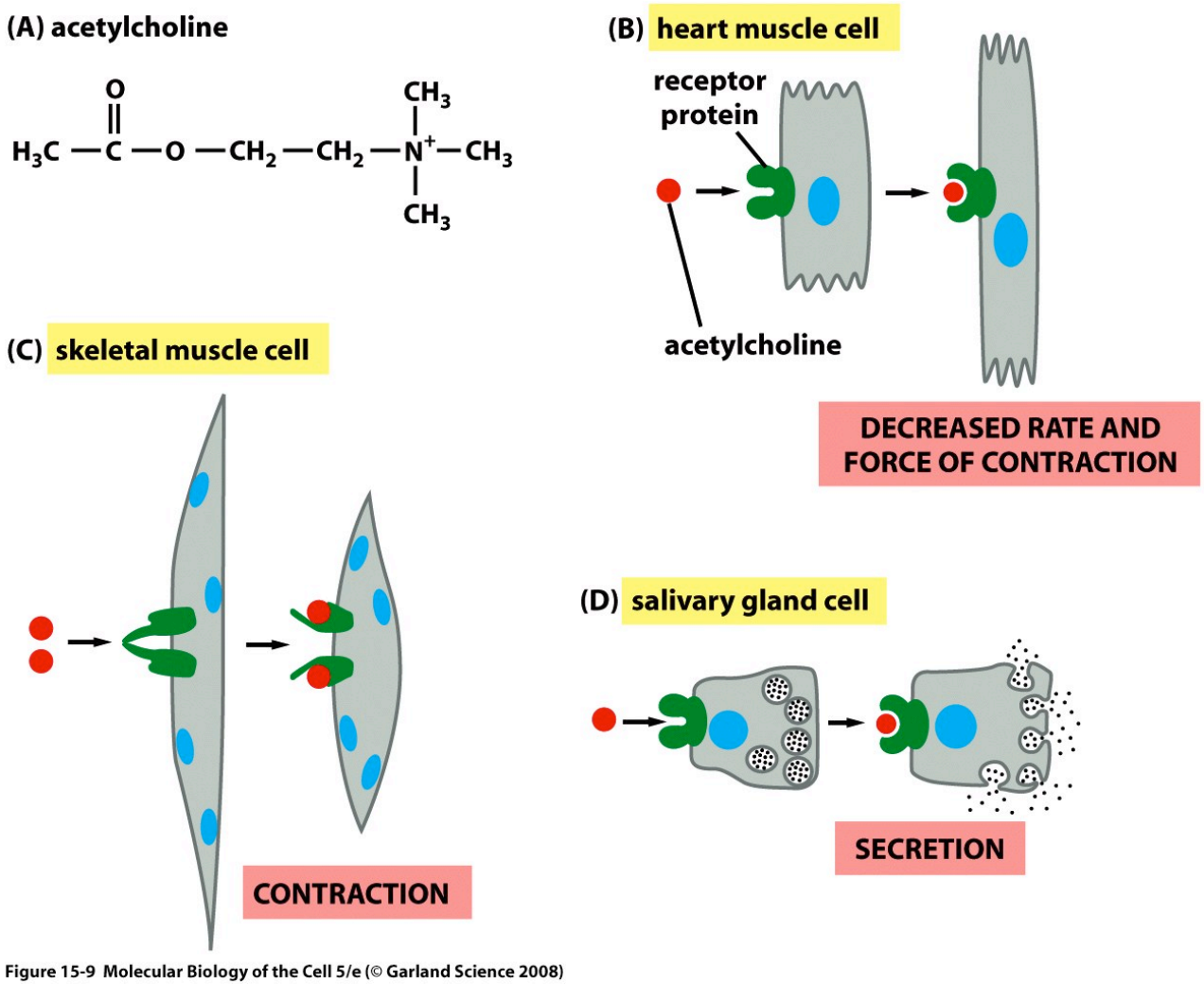
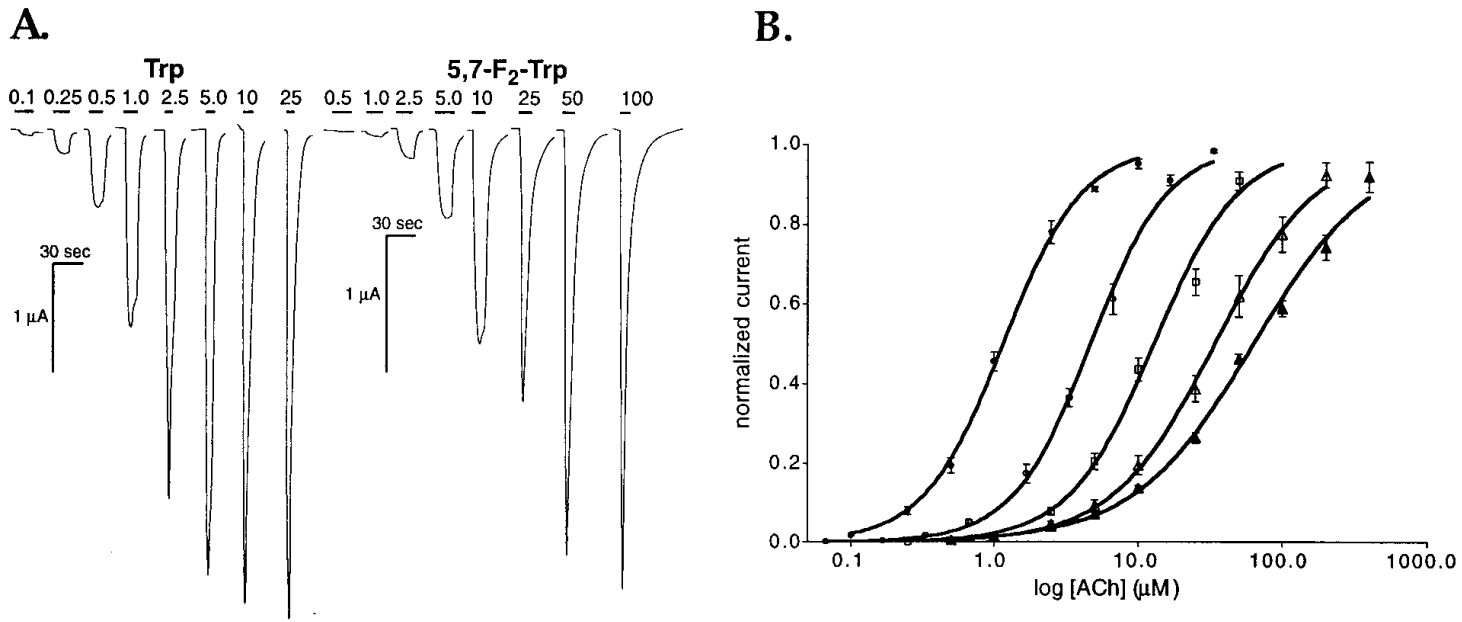


Figure 15-9 Molecular Biology of the Cell 5/e (© Garland Science 2008)

DATA FOR THE GATING OF NICOTINIC ACETYLCHOLINE RECEPTOR



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.

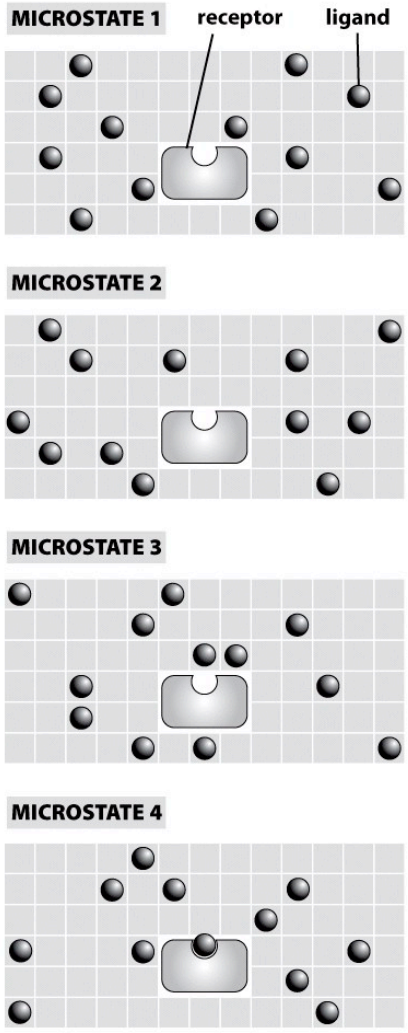


Figure 6.1 Physical Biology of the Cell (© Garland Science 2009)

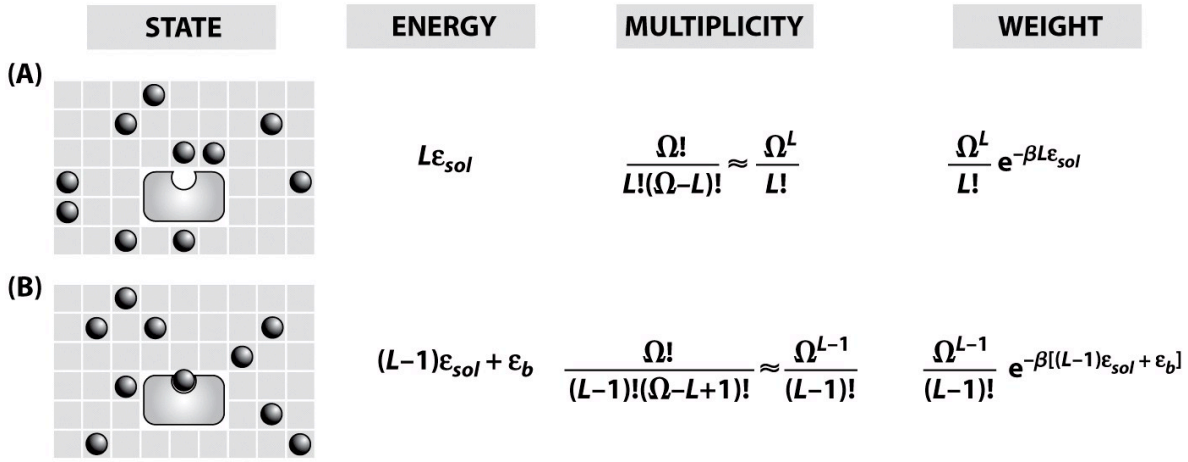


Figure 6.4 Physical Biology of the Cell (© Garland Science 2009)

BINDING CURVES AND BINDING FREE ENERGY

- These simple binding curves illustrate the way in which the binding probability depends upon the Kd or the binding energy.

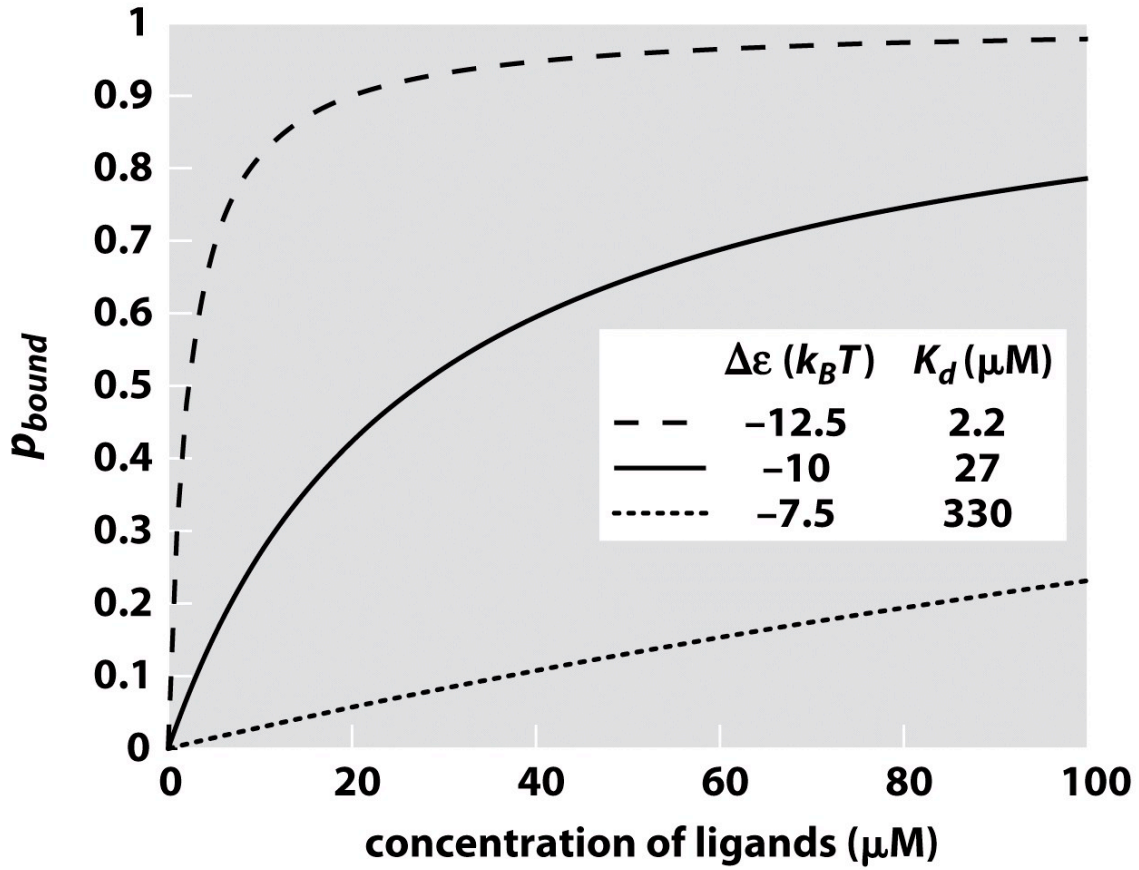


Figure 6.6 Physical Biology of the Cell (© Garland Science 2009)

EXPLORING PROMOTER ARCHITECTURE: CAN WE COMPUTE HOW CELLS DECIDE?

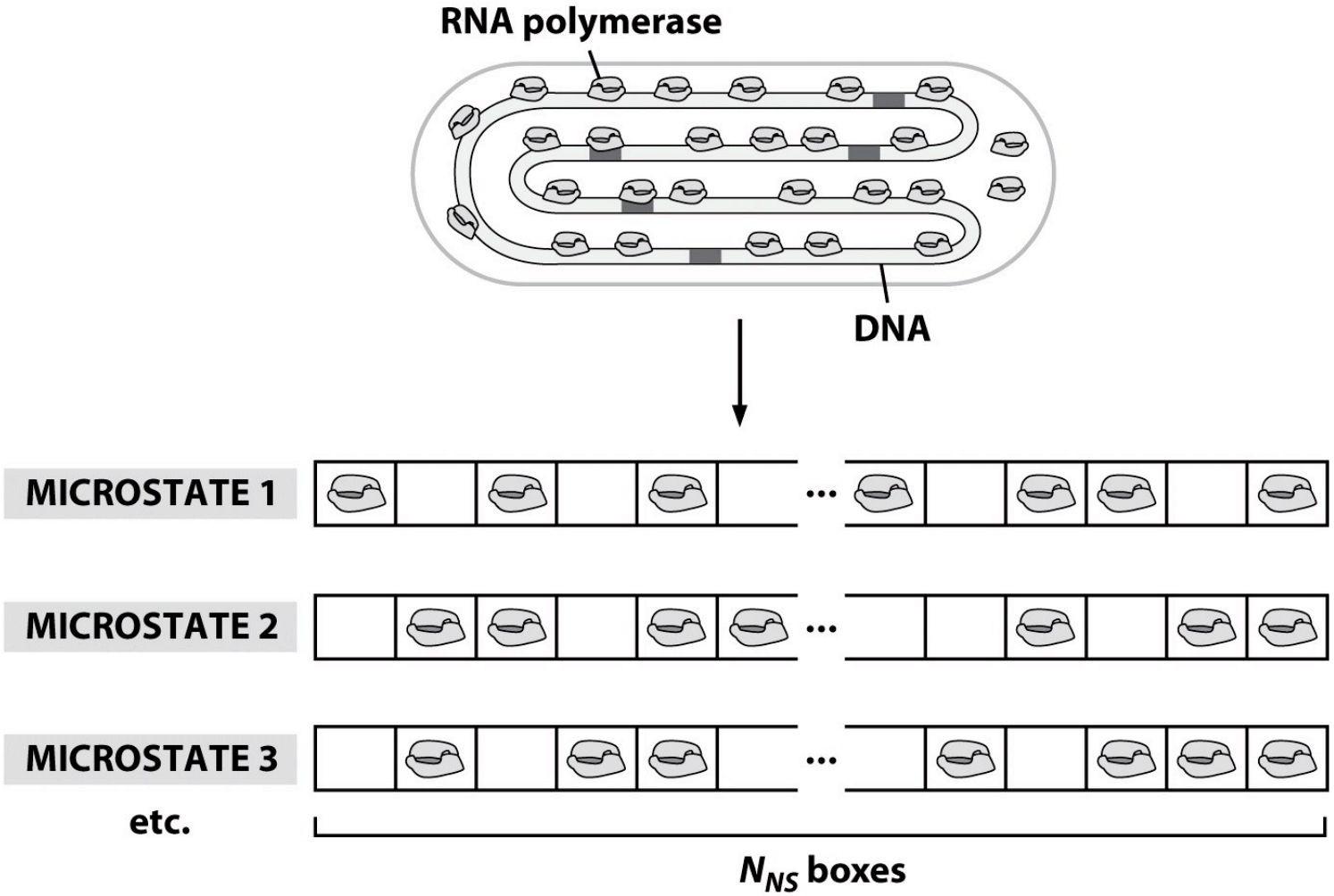


Figure 6.9 Physical Biology of the Cell (© Garland Science 2009)

EXPLORING PROMOTER ARCHITECTURE: CAN WE COMPUTE HOW CELLS DECIDE?

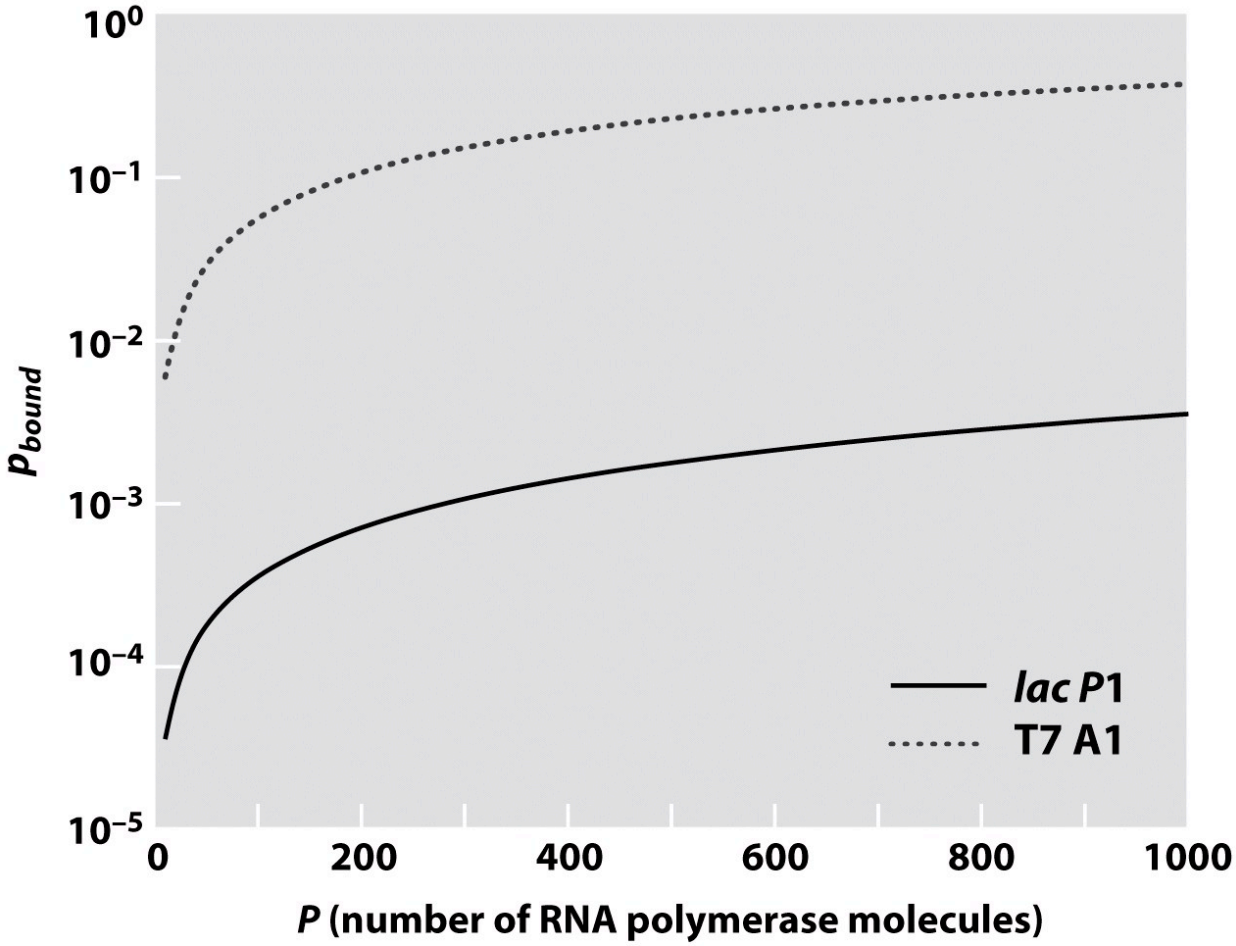
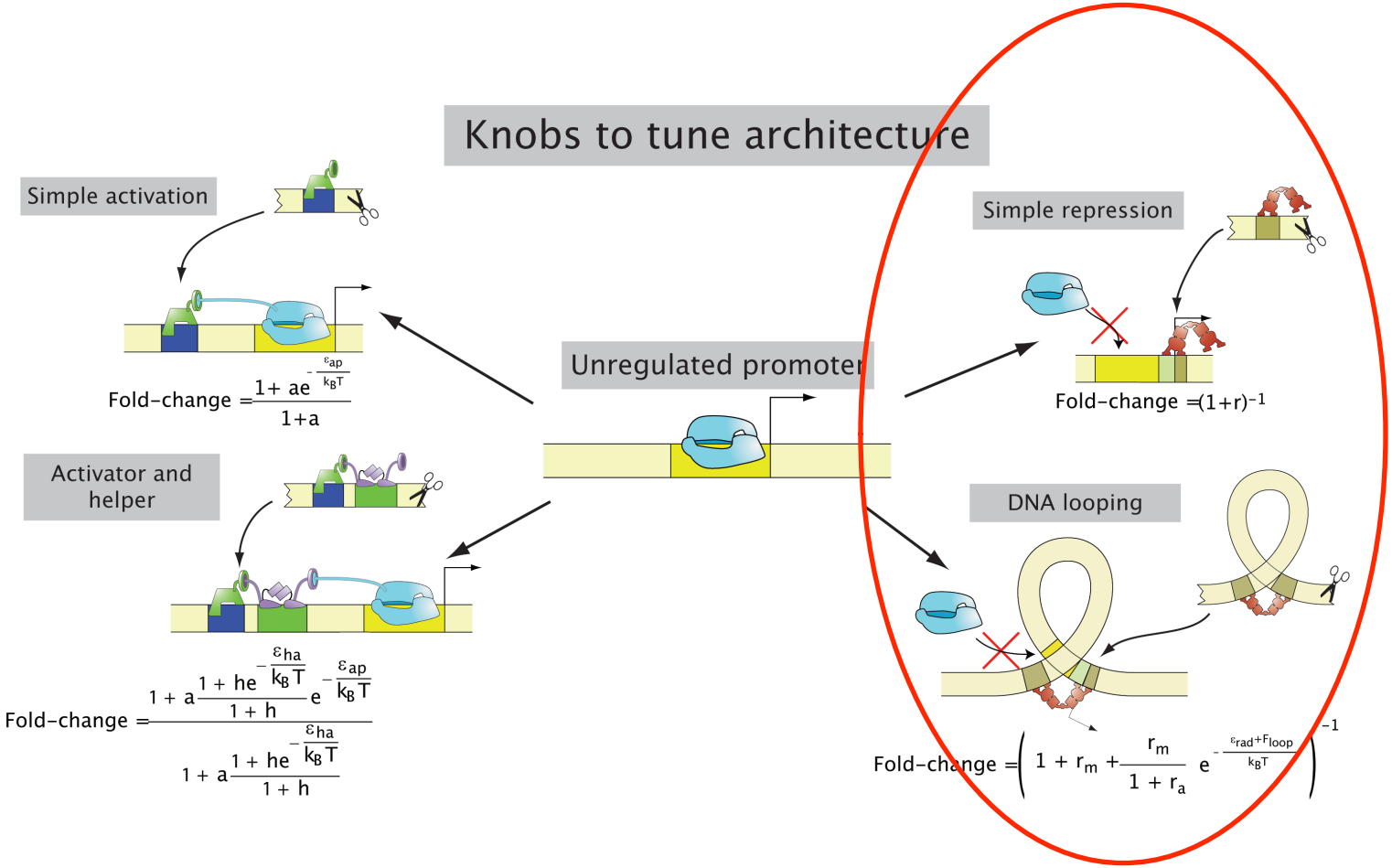


Figure 6.13 Physical Biology of the Cell (© Garland Science 2009)

WHERE WE ARE HEADED: CAN WE COMPUTE HOW CELLS DECIDE?



Bintu et al. (2005)

Some other examples

- Data and fits using our binding formula.

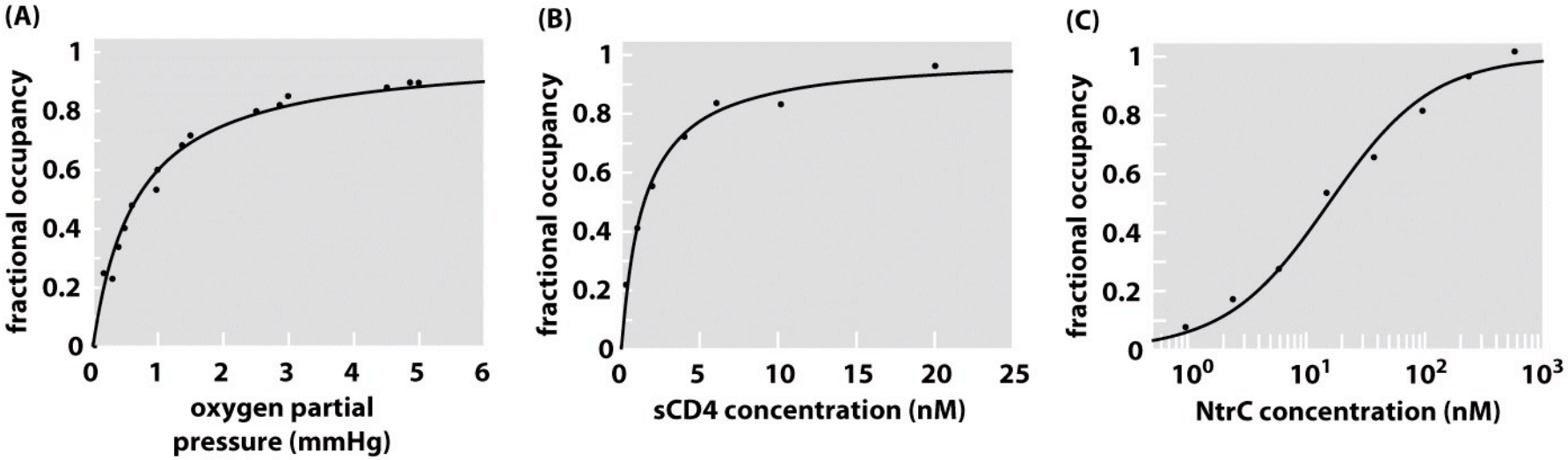


Figure 6.26 Physical Biology of the Cell (© Garland Science 2009)

SOME OTHER EXAMPLES

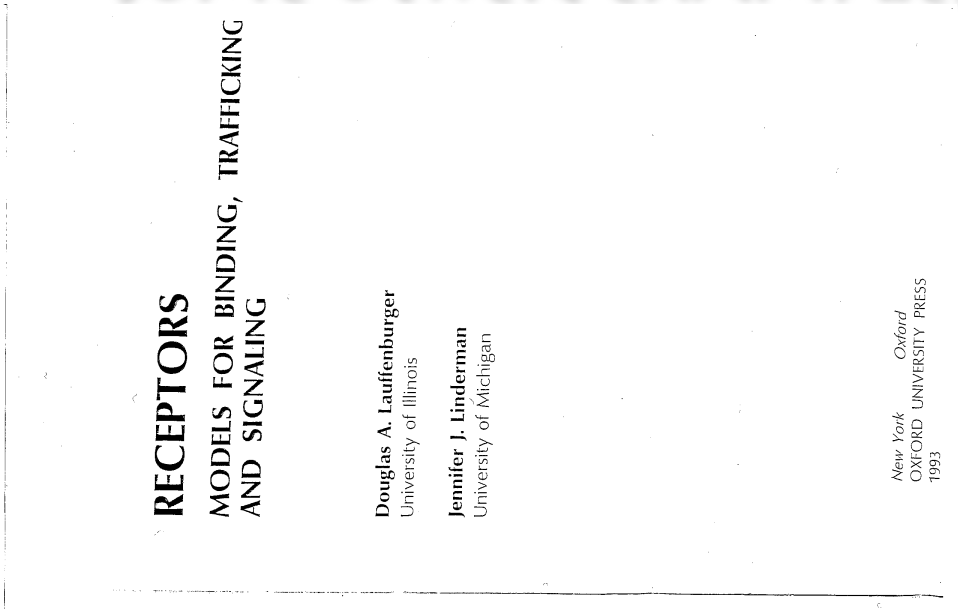


Table 2–1 Sample receptor/ligand binding parameters

Receptor	Ligand	Cell type	R_T (#/cell)	k_f ($M^{-1}\,min^{-1}$)	k_r (min^{-1})	K_D (M)	$t_{95\%}$ ($L_0 = K_D$) (min)	Reference
Transferrin	Transferrin	HepG2	5×10^4	3×10^6	0.1	3.3×10^{-8}	15	Ciechanover *et al.* (1983)
Fc_γ	2.4G2 Fab	Mouse macrophage	7.1×10^3	3×10^8	0.0023	7.7×10^{-12}	650	Mellman and Unkeless (1980)
Chemotactic peptide	FNLLP	Rabbit neutrophil	5×10^4	2×10^7	0.4	2×10^{-8}	3.7	Zigmond *et al.* (1982)
Interferon	Human interferon-α_2a	A549	900	2.2×10^8	0.072	3.3×10^{-10}	20	Baijer *et al.* (1989)
TNF	TNF	A549	6.6×10^3	9.6×10^8	0.14	1.5×10^{-10}	11	Baijer *et al.* (1989)
β-adrenergic	Hydroxybenzylpindolol	Turkey erythrocyte	—	8×10^8	0.08	1×10^{-10}	19	Rimon *et al.* (1980)
α_1-adrenergic	Prazosin	BC3H1	1.4×10^4	2.4×10^8	0.018	7.5×10^{-11}	83	Hughes *et al.* (1982)
Insulin	Insulin	Rat fat-cells	1×10^5	9.6×10^8	0.2	2.1×10^{-8}	7.5	Lipkin *et al.* (1986b)
EGF	EGF	Fetal rat lung	2.5×10^4	1.8×10^8	0.12	6.7×10^{-10}	12.5	Water *et al.* (1990)
Fibronectin	Fibronectin	Fibroblasts	5×10^3	7×10^8	0.6	8.6×10^{-9}	2.5	Akiyama and Yamada (1985)
Fc_ε	IgE	Human basophils	—	3.1×10^8	0.0015	4.8×10^{-12}	1000	Pruzansky and Patterson (1986)
IL-2 (heavy chain)	IL-2	T lymphocytes	2×10^3	2.3×10^7	0.015	6.5×10^{-10}	100	Smith (1988)
IL-2 (light chain)			1.1×10^4	8.4×10^8	24	2.9×10^{-8}	0.06	
IL-2 (heterodimer)			2×10^3	1.9×10^8	0.014	7.4×10^{-12}	110	

Shown are the measured number of receptors per cell R_T, the association rate constant k_f, the dissociation rate constant k_r, and the equilibrium dissociation constant $K_D = k_r/k_f$. The time required to reach 95% of equilibrium receptor binding when no bound receptors are initially present, $t_{95\%}$, is calculated from $t_{95\%} = -\ln(0.05)/(k_f(1 + L_0/K_D))$ for the case of $L_0 = K_D$. HepG2 = human hepatoma cell line; 2.4G2 Fab = Fab portion of 2.4G2 antibody against receptor; FNLLP = *N*-formylnorleucylleucylphenylalanine; A549 = human lung alveolar carcinoma; TNF = tumor necrosis factor; hydroxybenzylpindolol is an antagonist to the receptor; EGF = epidermal growth factor; IgE = immunoglobulin E; IL-2 = interleukin 2; prazosin is an antagonist to the receptor; BC3H1 = smooth muscle-like cell line; RBL = rat basophilic leukemia cell line.

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.

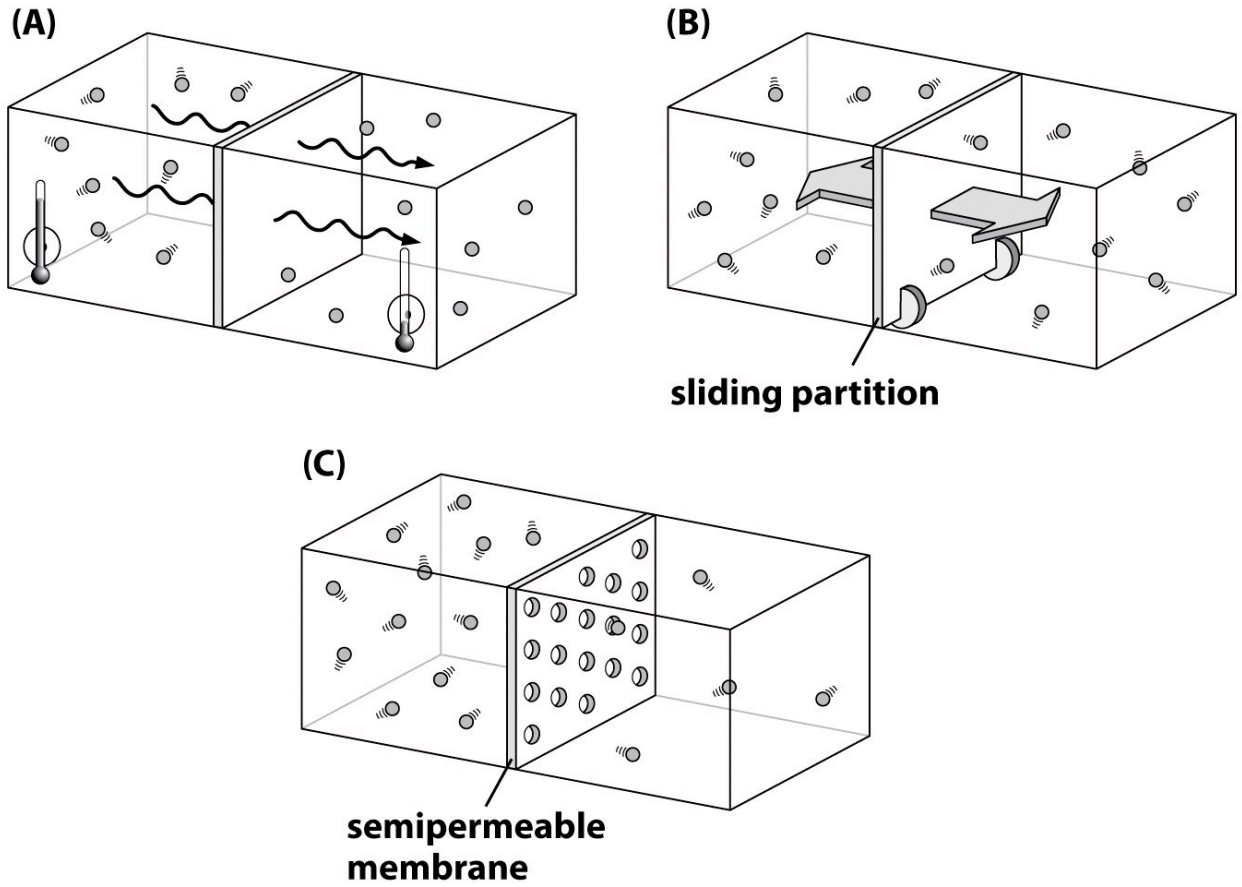
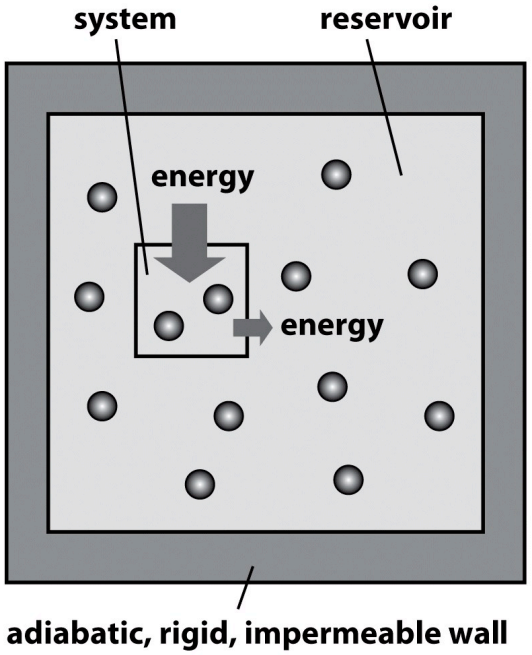


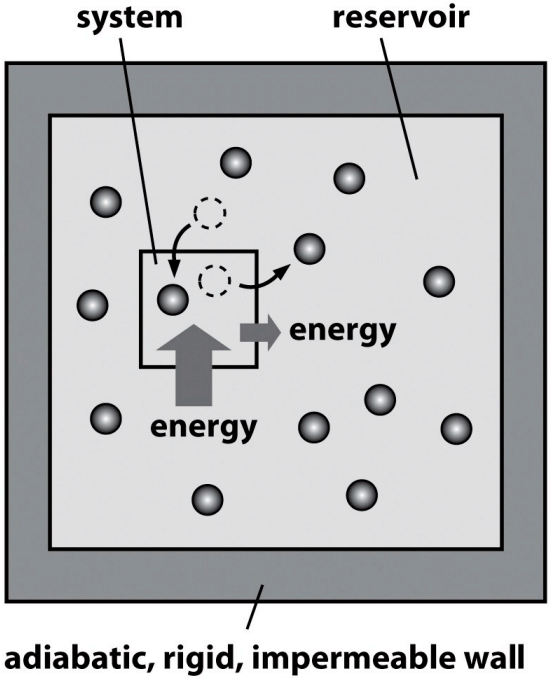
Figure 5.27 Physical Biology of the Cell (© Garland Science 2009)

THE GIBBS DISTRIBUTION

System in contact with an energy reservoir



System in contact with a particle and energy reservoir



Probability for finding the system in microstate *i*:

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

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

$$\beta = 1/k_B T$$

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

$T_{reservoir}$ controls av.energy <*E*> of the system

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

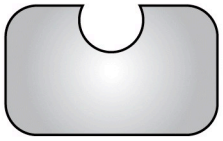
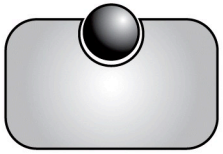
$$\langle N \rangle = \frac{1}{\mathcal{Z}} \sum_i N_i e^{-\beta(E_i - N_i \mu)} = \frac{1}{\beta} \frac{\partial}{\partial \mu} \ln \mathcal{Z}$$

$\mu_{res.}$ controls av. # of particles <*N*> 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), σ is an indicator of the state of binding
- The energy is

$$E = \varepsilon_b \sigma \qquad \underbrace{\varepsilon_b < 0}_{\text{favorable interaction btw L and R}}$$

STATE	WEIGHT
 $\sigma = 0$	1
 $\sigma = 1$	$e^{-\beta(\varepsilon_b - \mu)}$

- Evaluate aver. # of ligands bound, <N>:

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

Contact of the system with a thermal reservoir

Contact with a particle reservoir

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

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

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

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 $\mu = \mu_0 + k_B T \ln (c/c_0)$

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

$\Delta\varepsilon = \varepsilon_b - \mu_0$ *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.

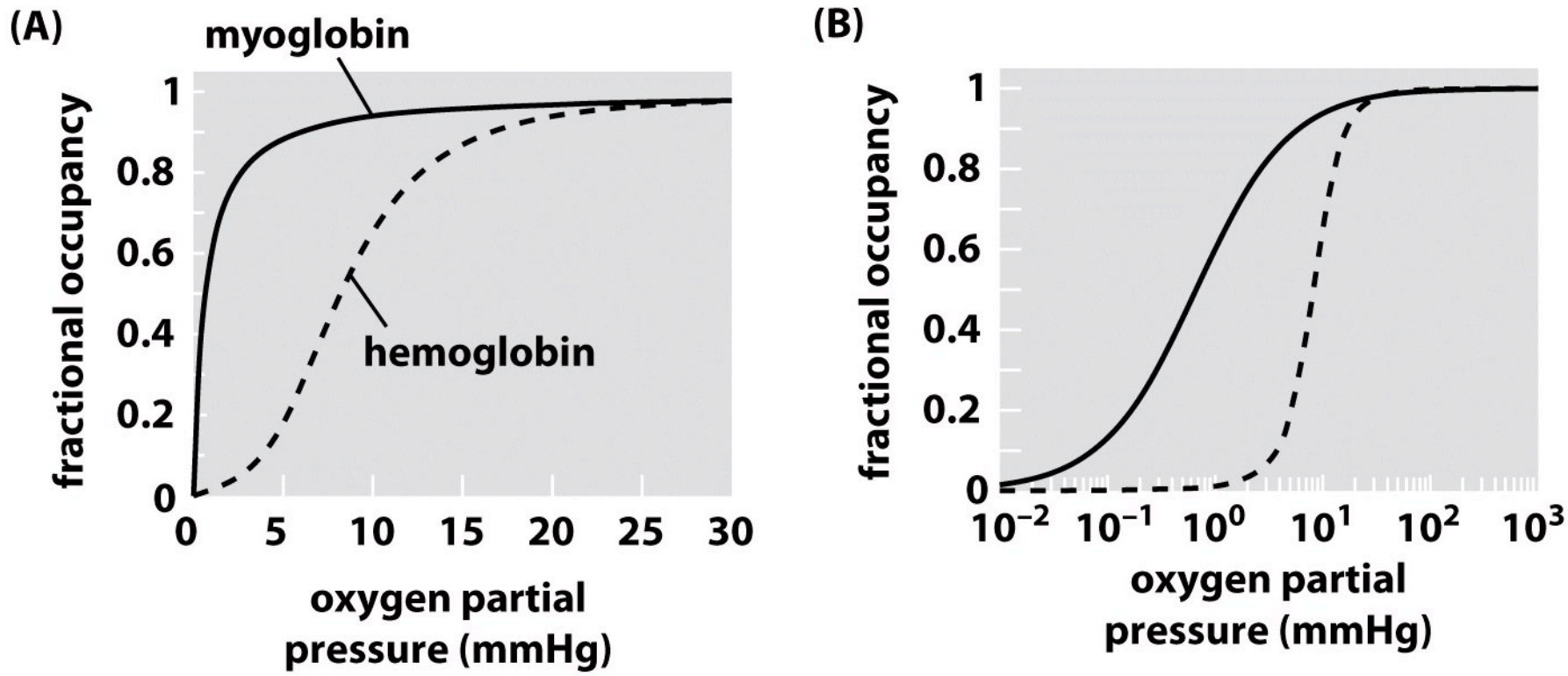
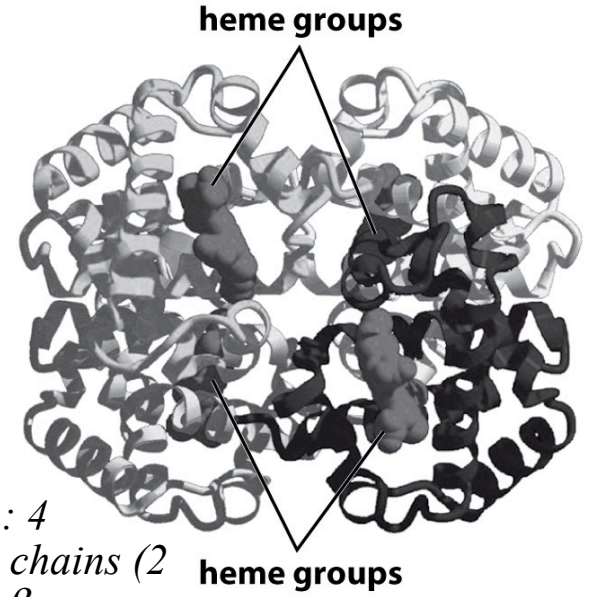


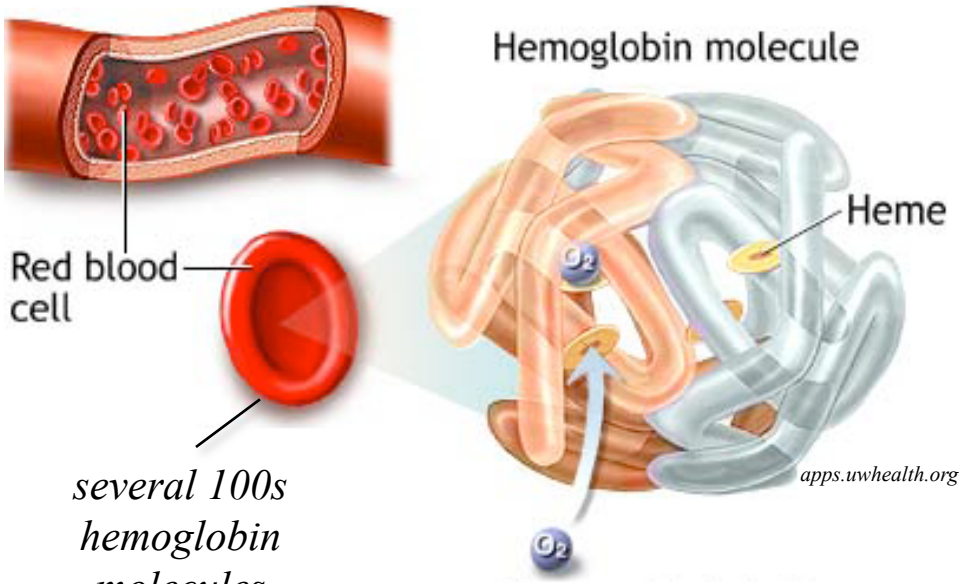
Figure 4.4 Physical Biology of the Cell (© Garland Science 2009)

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

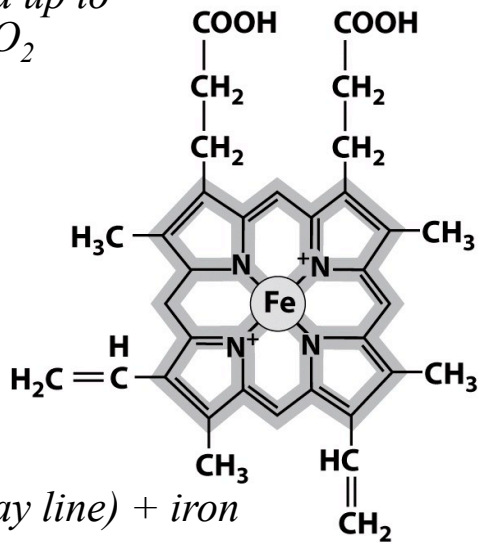


The protein hemoglobin: 4 polypeptide chains (2 α-chains, 2 β-chains), each carries a heme group => protein can bind up to 4 molecules of O_2



several 100s hemoglobin molecules

Oxygen binds to heme on the hemoglobin molecules



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

THE NATURE OF THE HILL FUNCTION

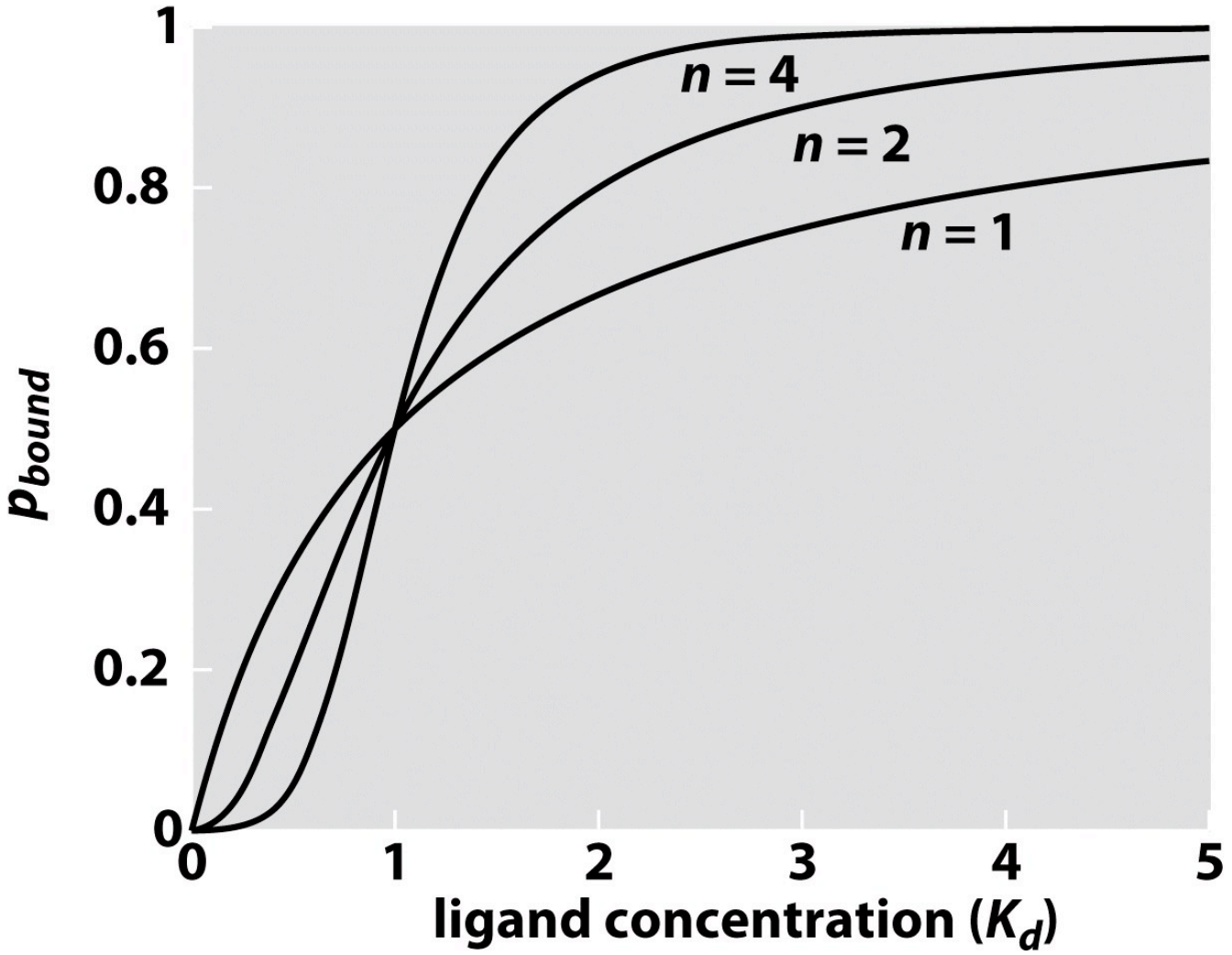


Figure 6.27 Physical Biology of the Cell (© Garland Science 2009)

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 σ_i: $\sigma_i = 0$ (unbound), $\sigma_i = 1$ (bound)

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

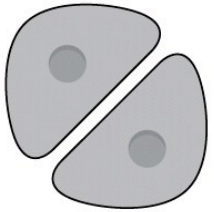
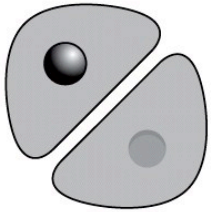
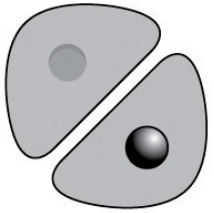
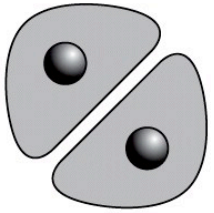
A TOY MODEL OF A DIMOGLOBIN

- To illustrate the idea of cooperativity: imagine a fictitious dimoglobin [=dimeric hemoglobin] molecule which has 2 O_2 binding sites (*e.g., clams*)
- (σ_1, σ_2) => 4 distinct states
- The energy of the system:

$$E = \underbrace{\varepsilon}(\sigma_1 + \sigma_2) + \underbrace{J}\sigma_1\sigma_2$$

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

measure of the cooperativity

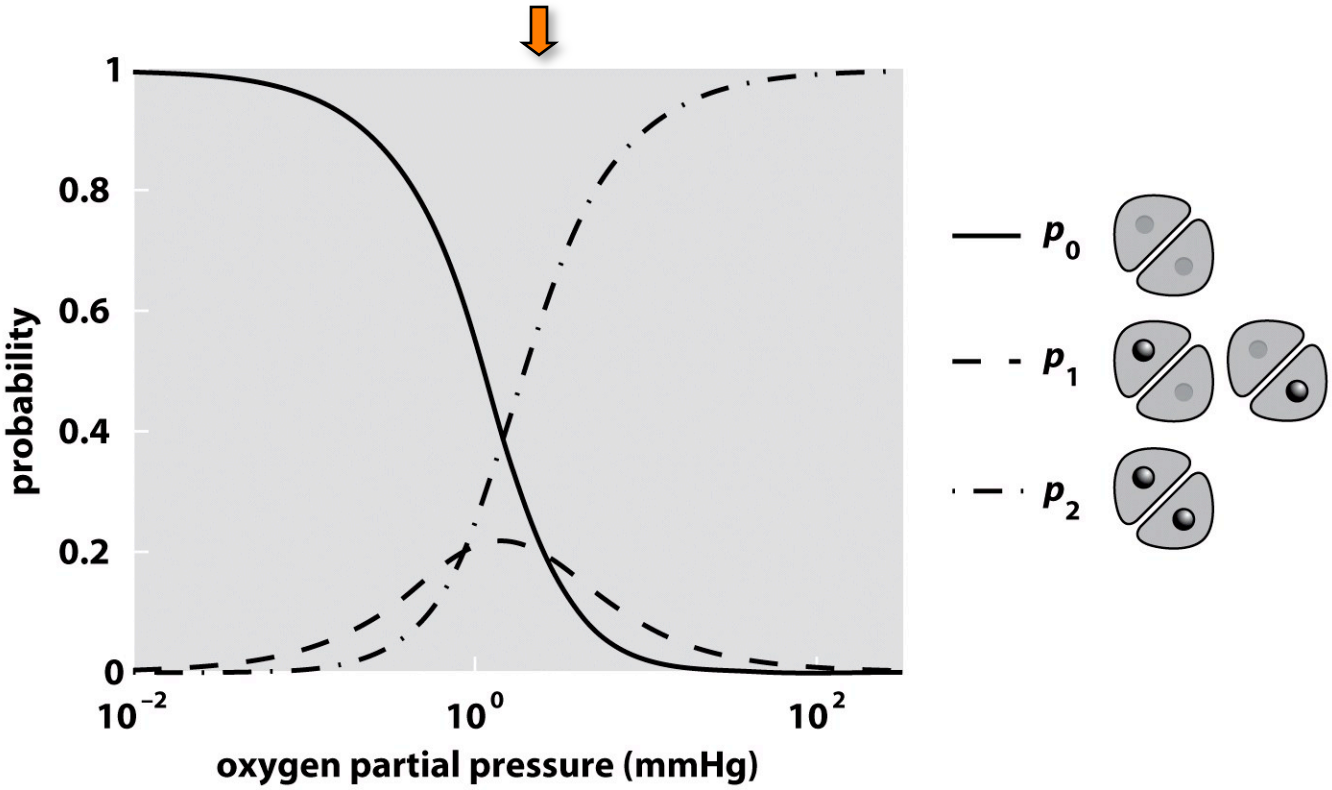
STATE	WEIGHT
	1
	$e^{-\beta(\varepsilon-\mu)}$
	$e^{-\beta(\varepsilon-\mu)}$
	$e^{-\beta(2\varepsilon+J-2\mu)}$

A TOY MODEL OF A DIMOGLOBIN

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

$$\mathcal{Z} = 1 + \underbrace{e^{-\beta(\varepsilon-\mu)} + e^{-\beta(\varepsilon-\mu)}}_{\text{Single occupancy}} + \underbrace{e^{-\beta(2\varepsilon+J-2\mu)}}_{\text{Both sites occupied}}$$

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



STATE	WEIGHT
	1
	$e^{-\beta(\varepsilon-\mu)}$
	$e^{-\beta(\varepsilon-\mu)}$
	$e^{-\beta(2\varepsilon+J-2\mu)}$

Parameters used: $\Delta\varepsilon = -5\ k_BT$, $J = -2.5\ k_BT$, $c_0 = 760$ mmHg

TALKING ACROSS THE MEMBRANE

- Membrane proteins are characterized in some cases by transmembrane alpha helices and cytosolic domain that passes along the signal.

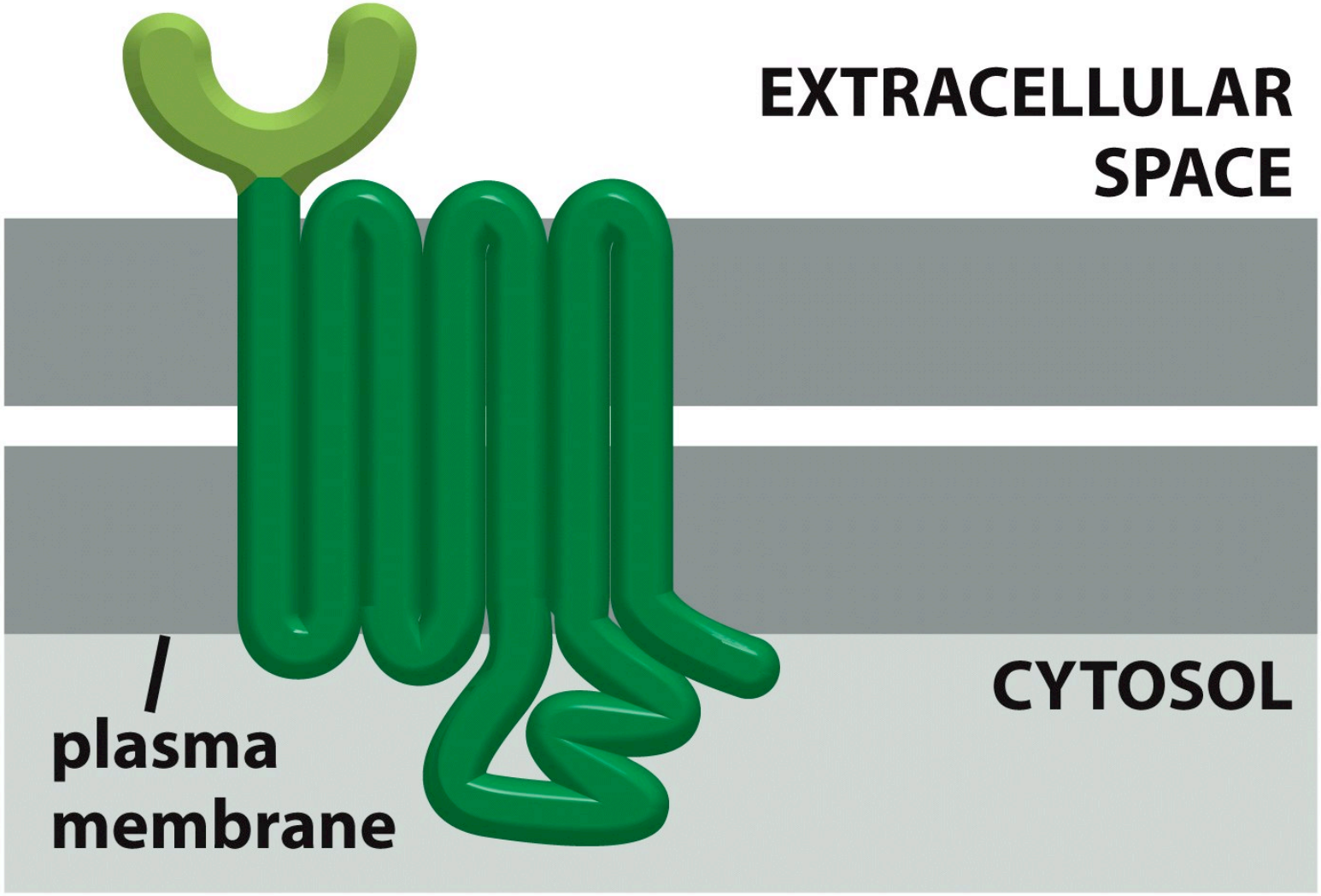


Figure 15-30 Molecular Biology of the Cell 5/e (© Garland Science 2008)

COUPLING RECEPTORS TO ENZYME ACTION

- Receptor binding changes the probability of the "active" state.

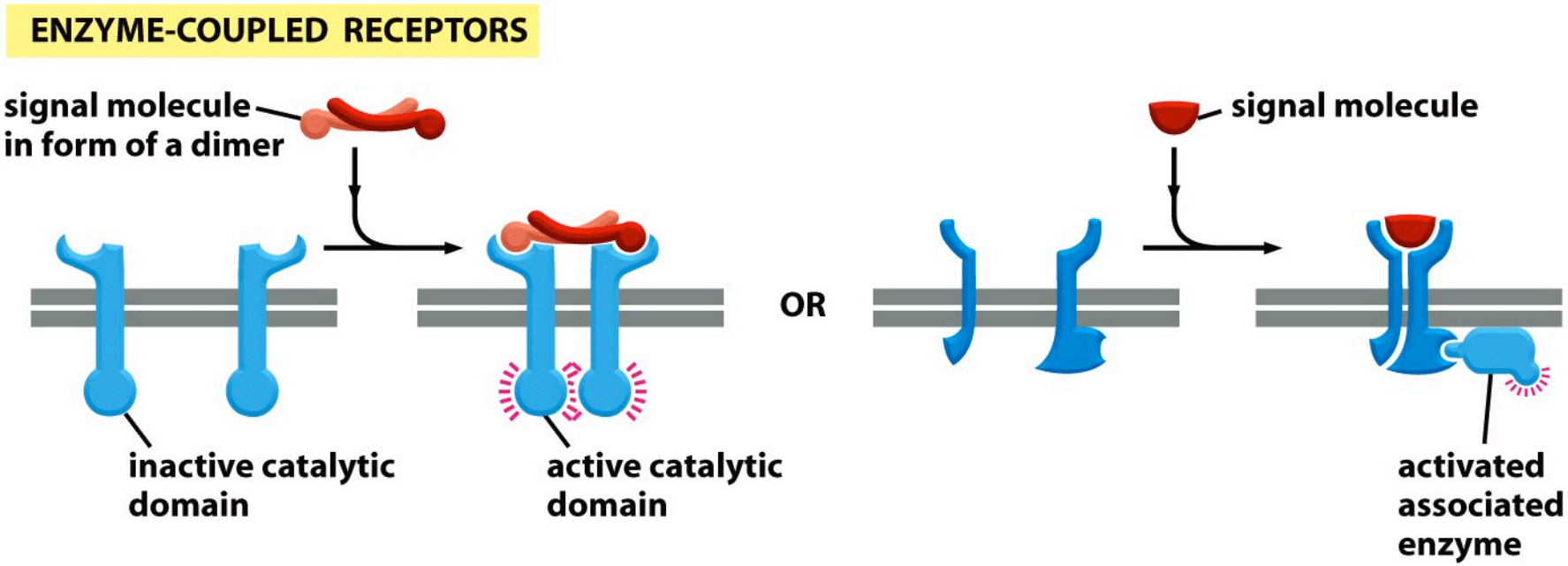


Figure 15-16c Molecular Biology of the Cell 5/e (© Garland Science 2008)

DOING WORK TO CHANGE THE PROTEIN STATE

- 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.

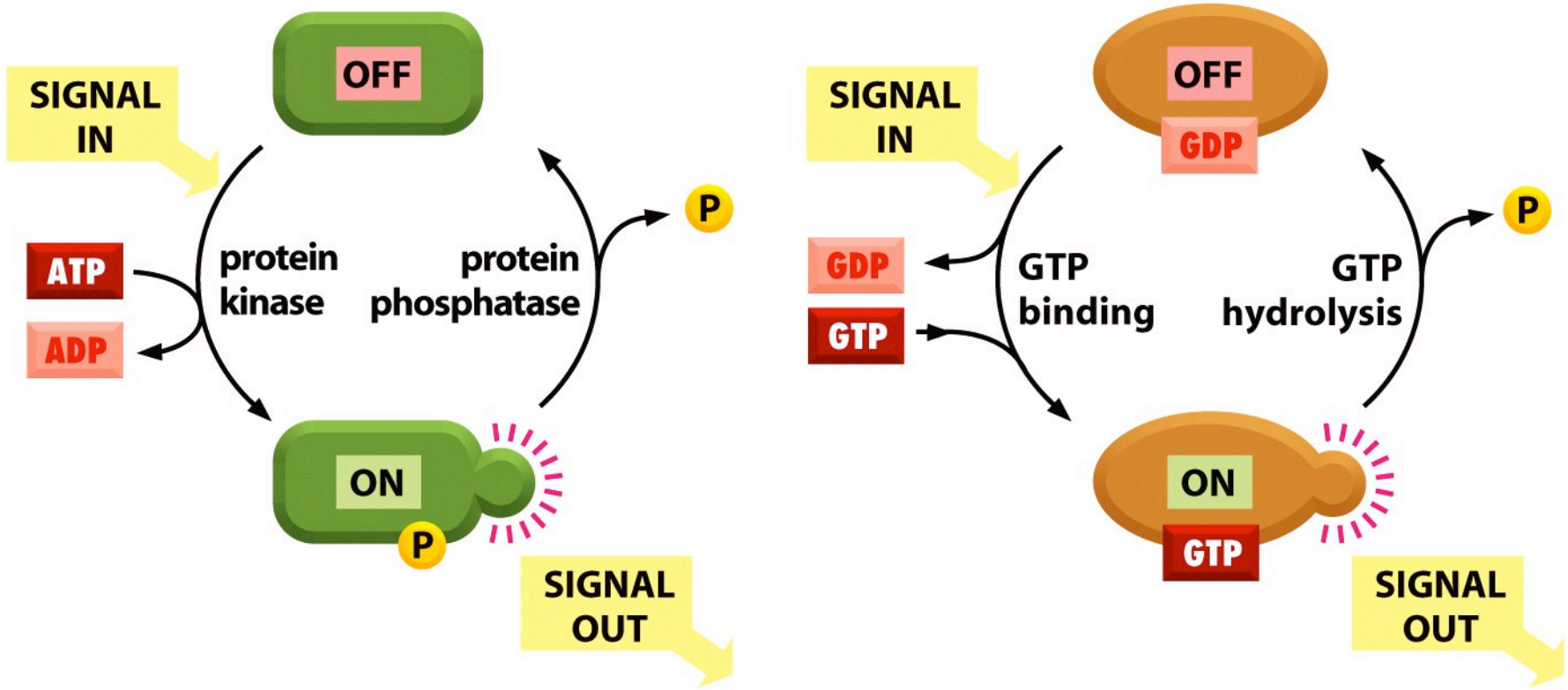
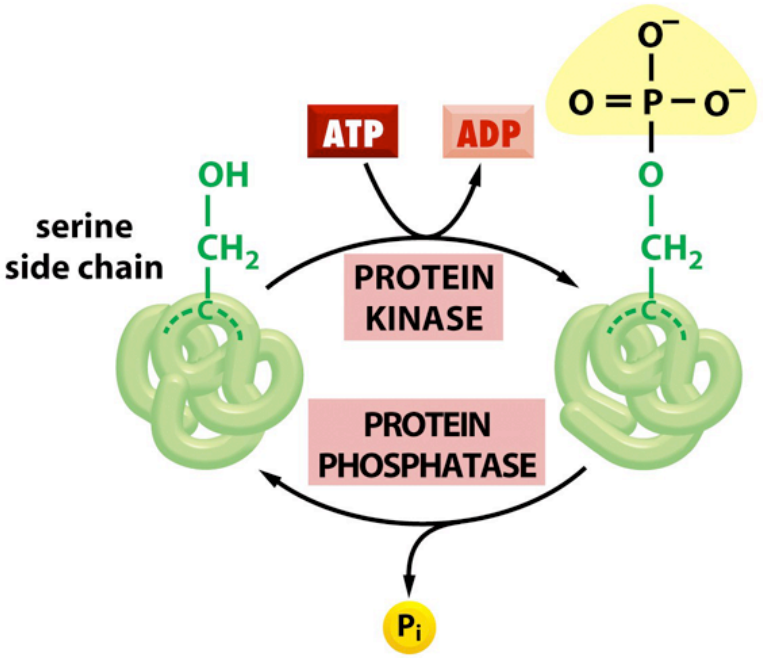


Figure 15-18 Molecular Biology of the Cell 5/e (© Garland Science 2008)

PHOSPHORYLATION

- 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



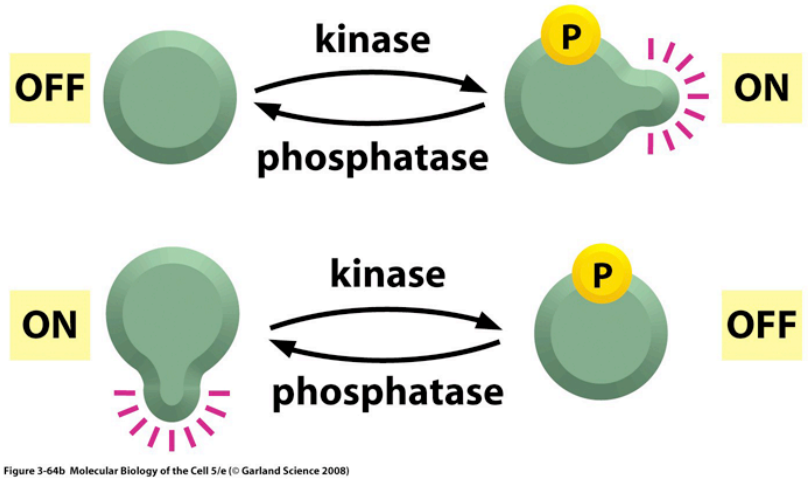


Figure 3-64b Molecular Biology of the Cell 5/e (© Garland Science 2008)

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.

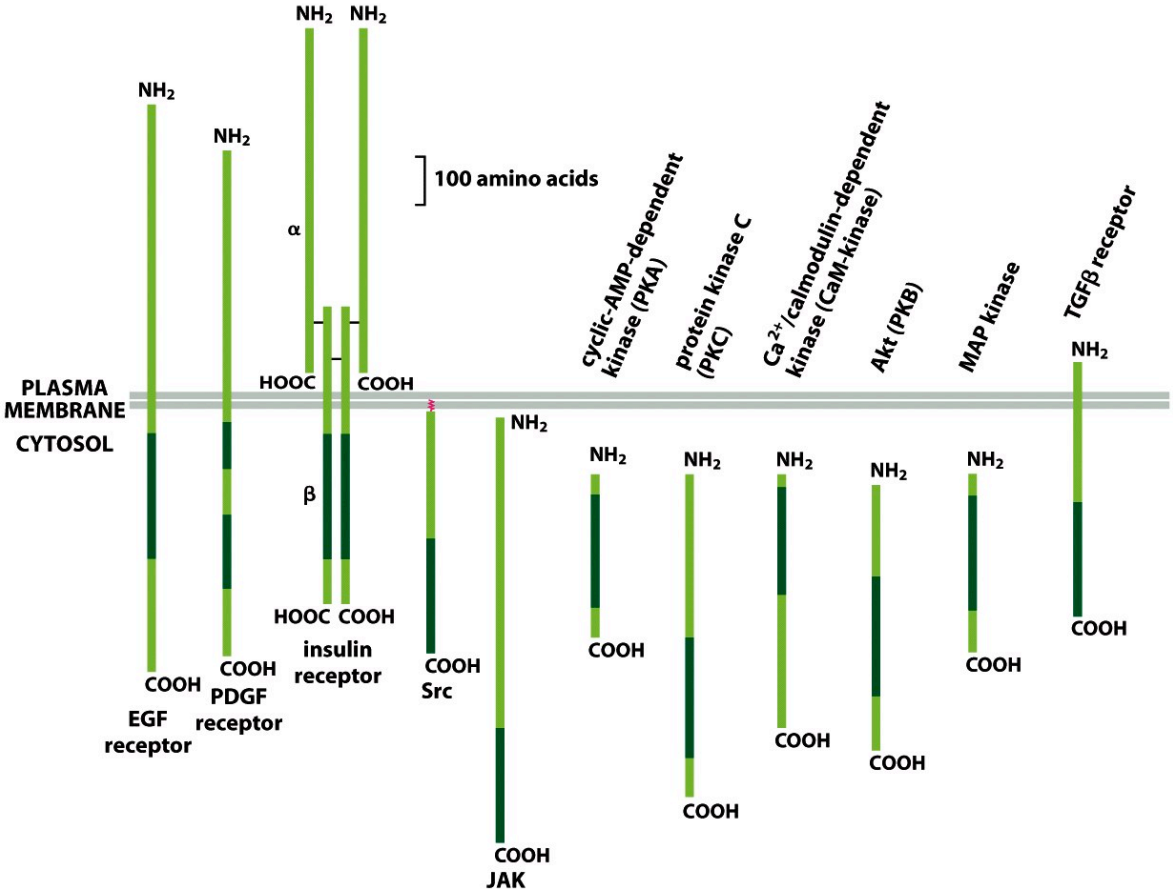


Figure 15-70 Molecular Biology of the Cell 5/e (© Garland Science 2008)

PHOSPHORYLATION: TWO INTERNAL STATE VARIABLES

- 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):

σ_s: $\sigma_s = 0$ => inactive,
$\sigma_s = 1$ => active

The state of phosphorylation of the protein:

σ_p: $\sigma_p = 0$ => unphosphorylated,
$\sigma_p = 1$ => phosphorylated

- The state of phosphorylation can alter the relative energies of the active and inactive states
=> at equilibrium, most of the phosphorylated molecules will be in active form

- I_1 and I_2 are the electrostatic interaction energies btw the two charges in the active and inactive states

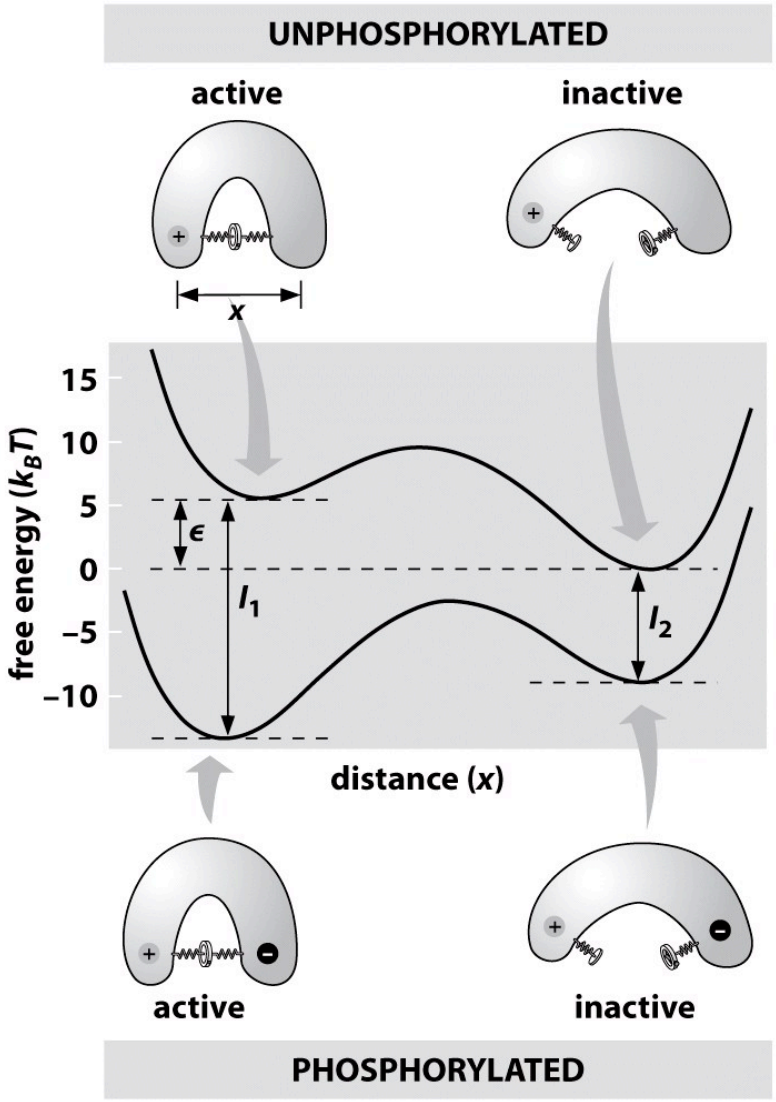


Figure 7.10 Physical Biology of the Cell (© Garland Science 2009)

PHOSPHORYLATION: TWO INTERNAL STATE VARIABLES

- Using the σ variables, the free energy of the protein is

$$G(\sigma_P, \sigma_S) = (1-\sigma_P)[(1-\sigma_S)0 + \sigma_S\varepsilon] + \sigma_P[(1-\sigma_S)(-I_2) + \sigma_S(\varepsilon - I_1)]$$

which simplifies to

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

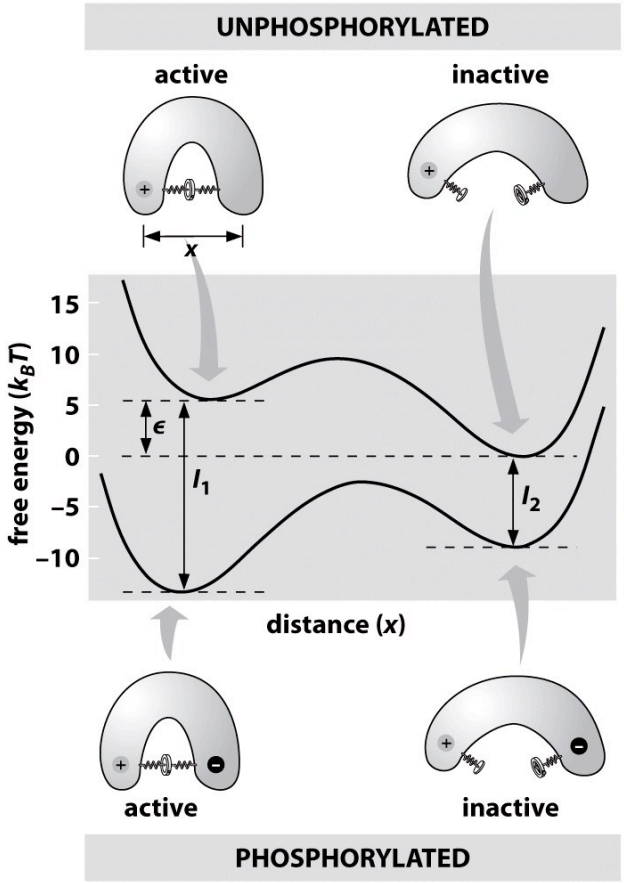


Figure 7.10 Physical Biology of the Cell (© Garland Science 2009)

=> states&weights:

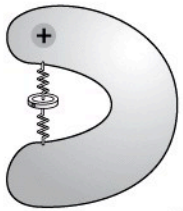
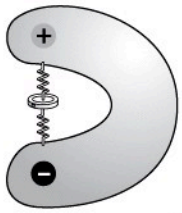
STATE	ENERGY	WEIGHT
 $\sigma_P = 0, \sigma_S = 0$	0	1
 $\sigma_P = 0, \sigma_S = 1$	ε	$e^{-\beta\varepsilon}$
 $\sigma_P = 1, \sigma_S = 0$	$-I_2$	$e^{\beta I_2}$
 $\sigma_P = 1, \sigma_S = 1$	$\varepsilon - I_1$	$e^{-\beta(\varepsilon - I_1)}$

Figure 7.11 Physical Biology of the Cell (© Garland Science 2009)

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:

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

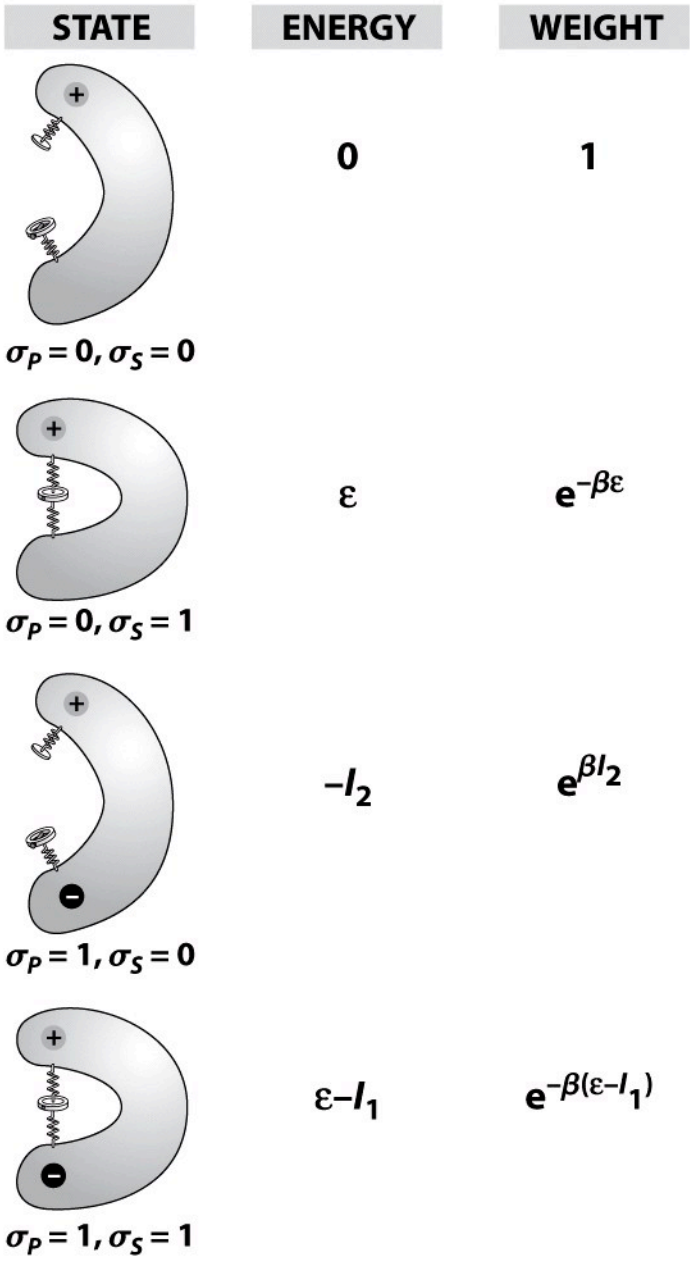


Figure 7.11 Physical Biology of the Cell (© Garland Science 2009)

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,

$$\varepsilon \approx 5\ k_B T$$

$$I_2 - I_1 \approx -10\ k_B T$$

$$\Rightarrow \quad p^*_{\text{active}}/p_{\text{active}} \approx 150$$

-increase in activity upon phosphorylation

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

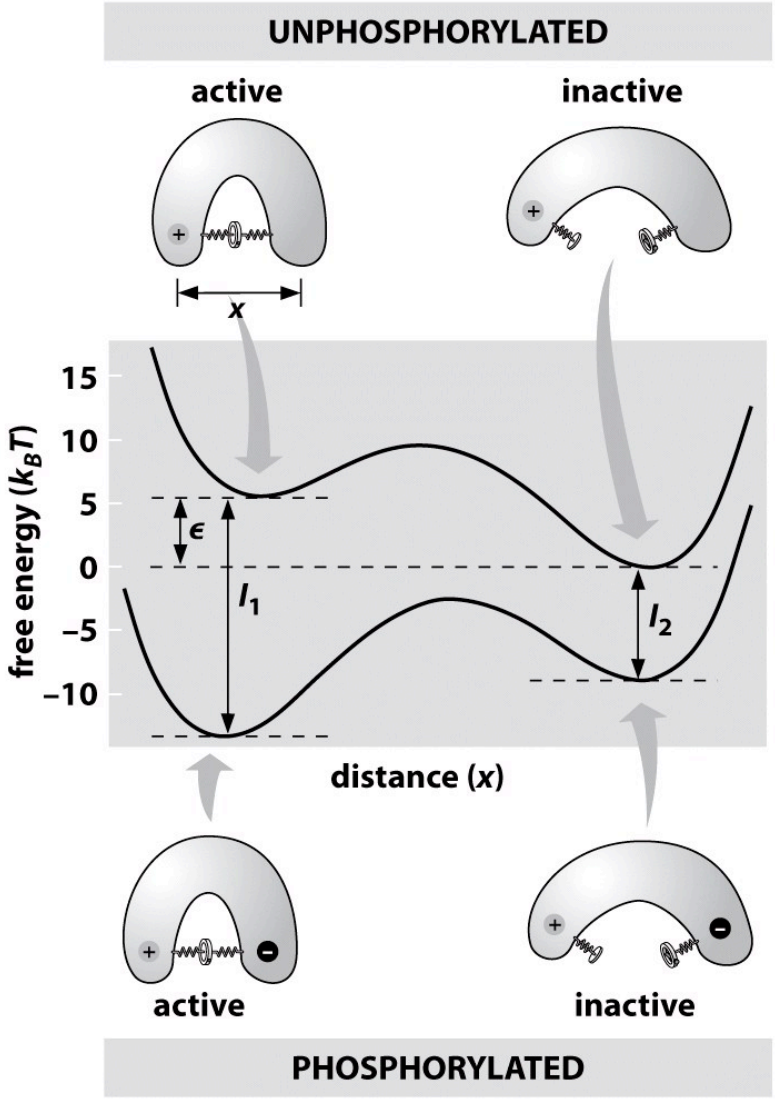


Figure 7.10 Physical Biology of the Cell (© Garland Science 2009)

EUKARYOTIC SIGNAL TRANSDUCTION

- 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..

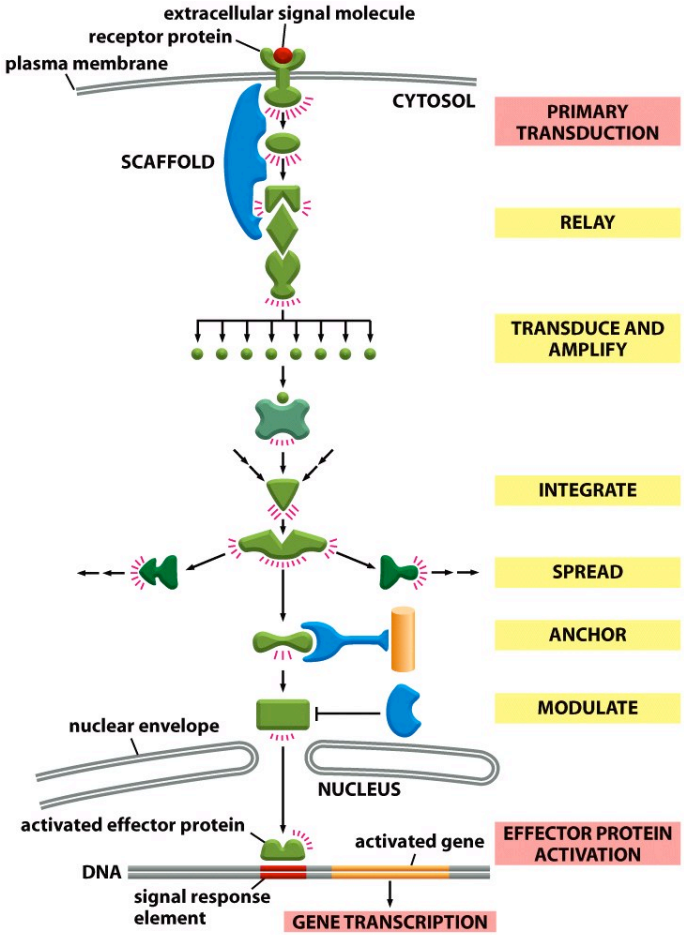
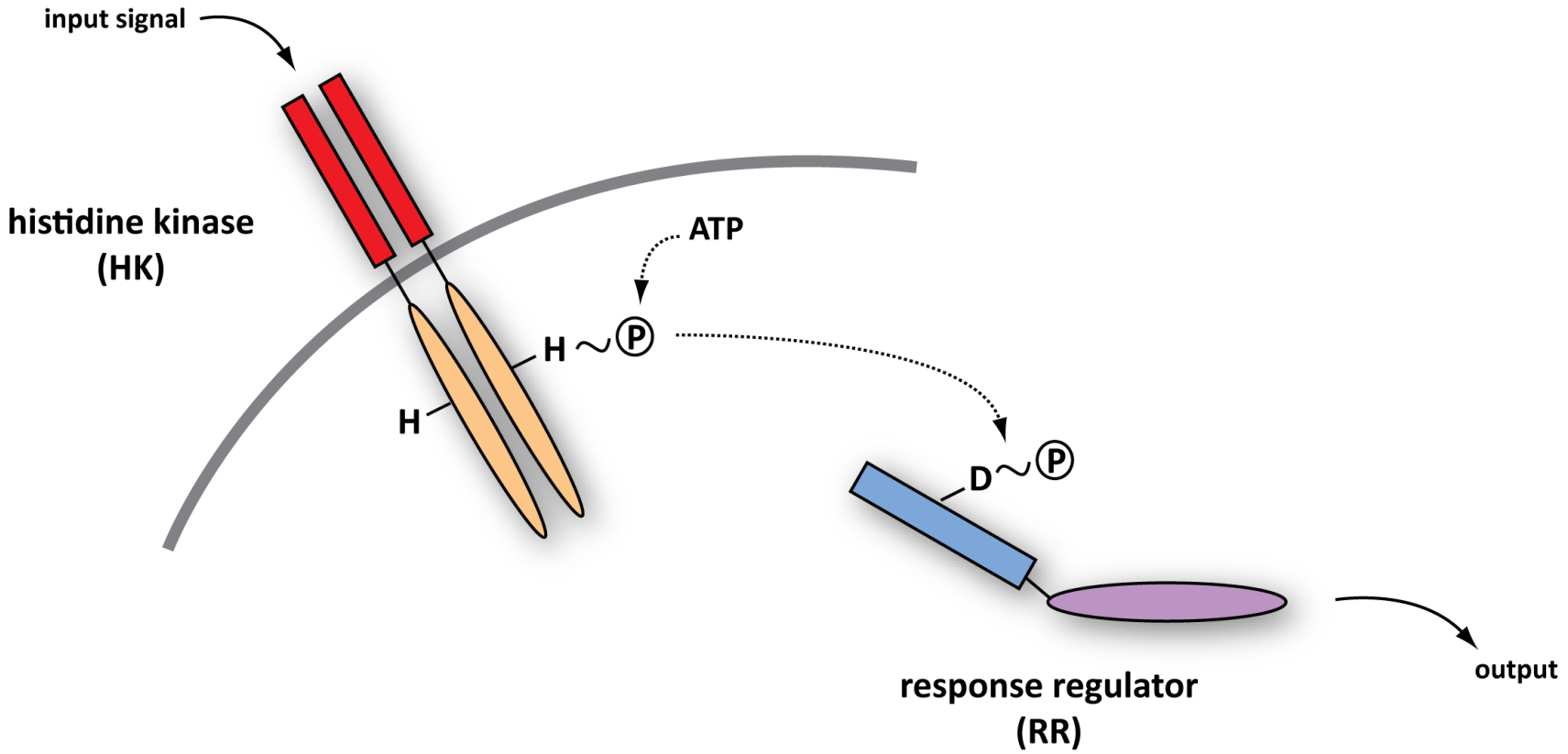


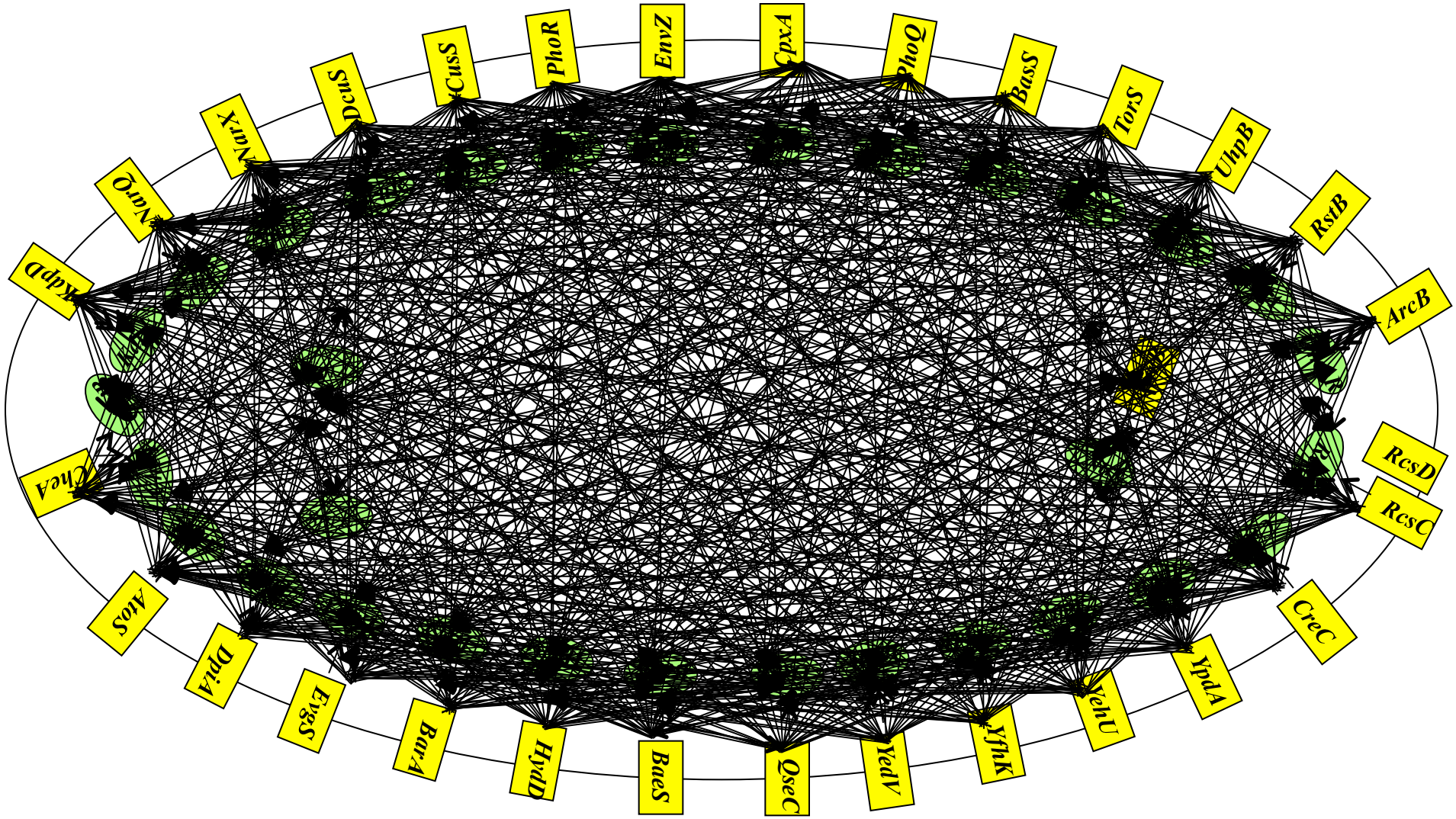
Figure 15-17 Molecular Biology of the Cell 5/e (© Garland Science 2008)

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.

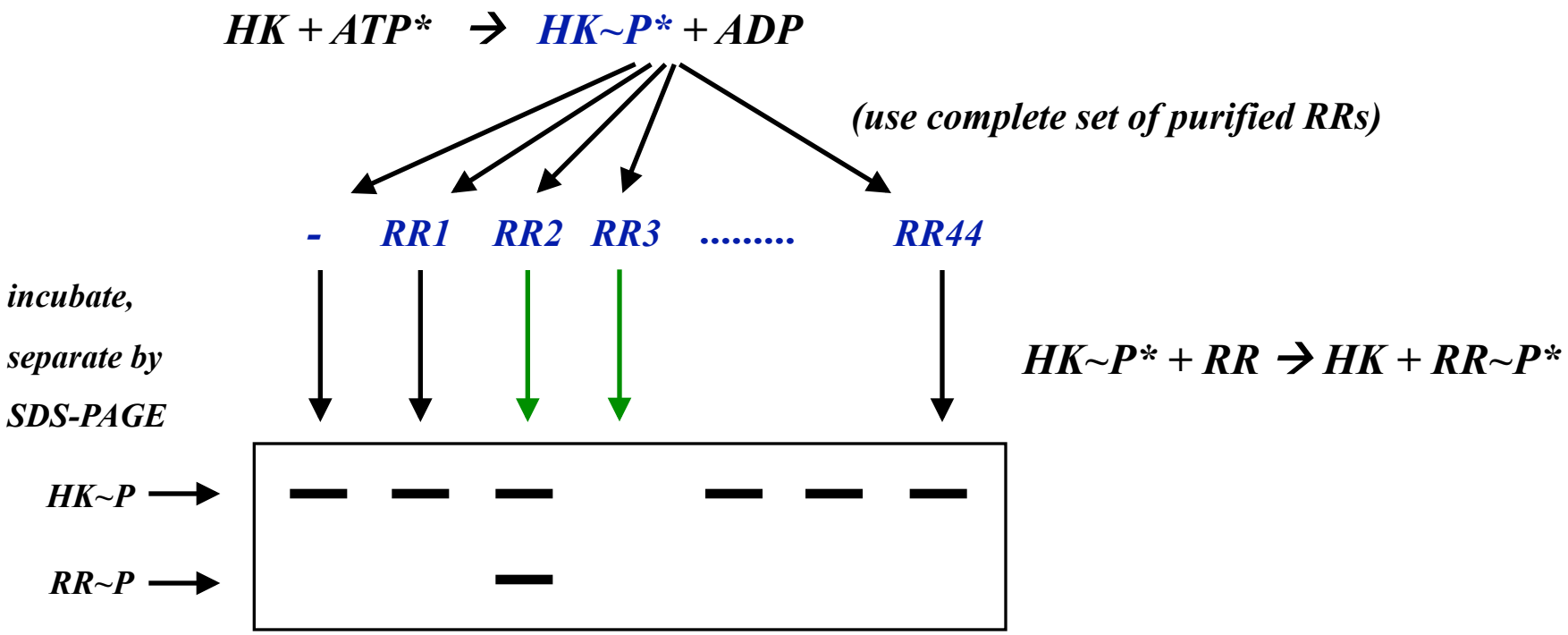


COORDINATING MULTIPLE SIGNALING SYSTEMS IN A SINGLE CELL



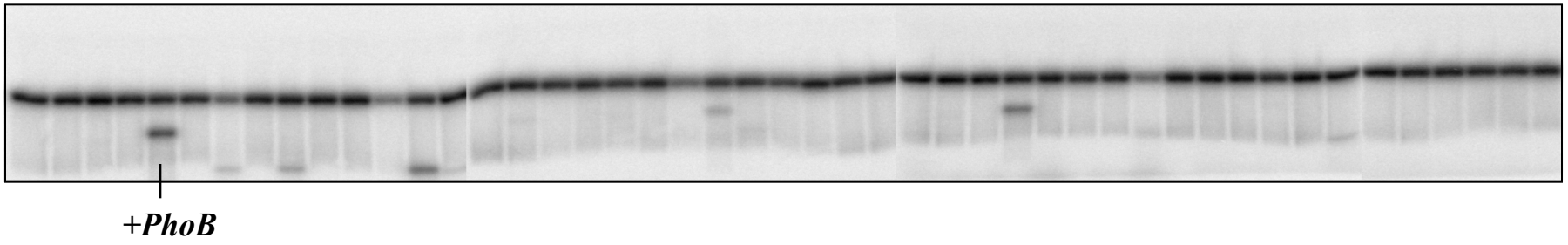
animation by Mark Gouilan

PHOSPHOTRANSFER PROFILING



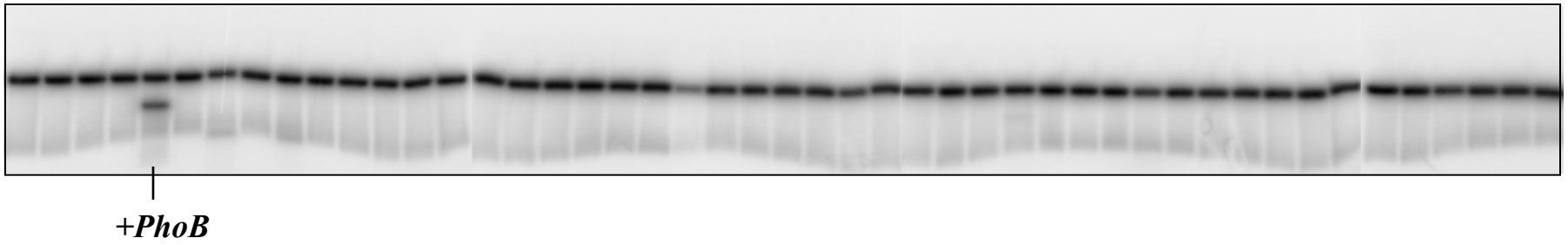
ASSESSING SPECIFICITY: PHOSPHOTRANSFER PROFILING

C. crescentus PhoR profile – 60 min phosphotransfer reactions



+PhoB

C. crescentus PhoR profile – 5 min phosphotransfer reactions



+PhoB

➔ ***histidine kinases exhibit a strong kinetic preference in vitro for their in vivo cognate substrate***

➔ ***specificity based on molecular recognition***

SIGNAL INTEGRATION

- 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.

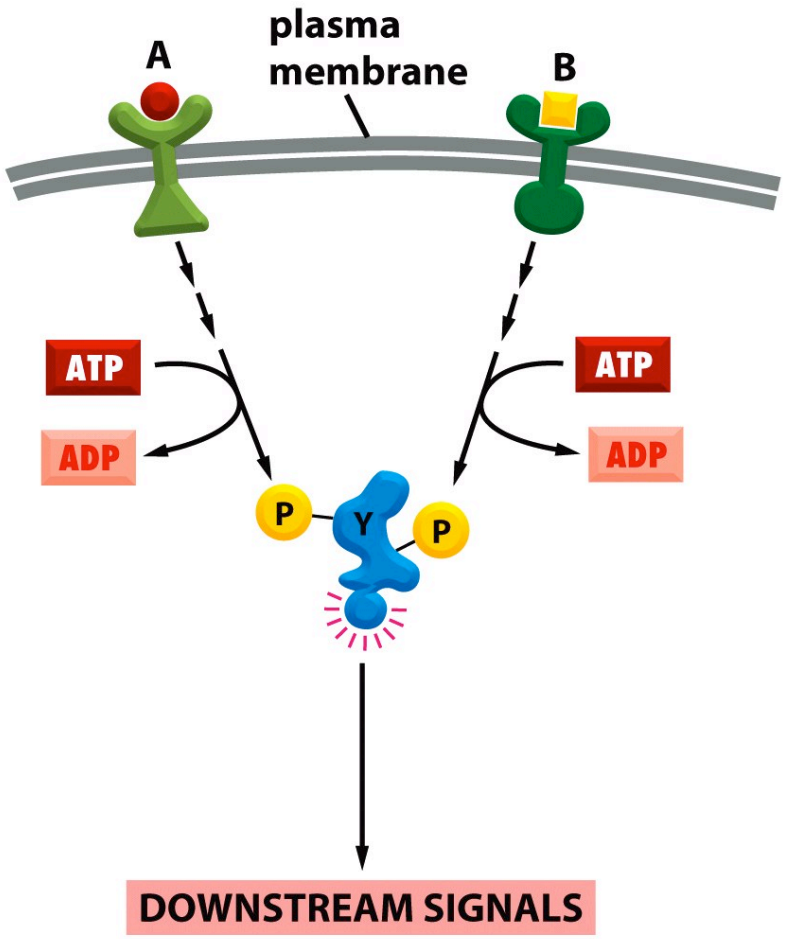


Figure 15-20 Molecular Biology of the Cell 5/e (© Garland Science 2008)

G-PROTEIN COUPLED RECEPTORS AS AN EXAMPLE

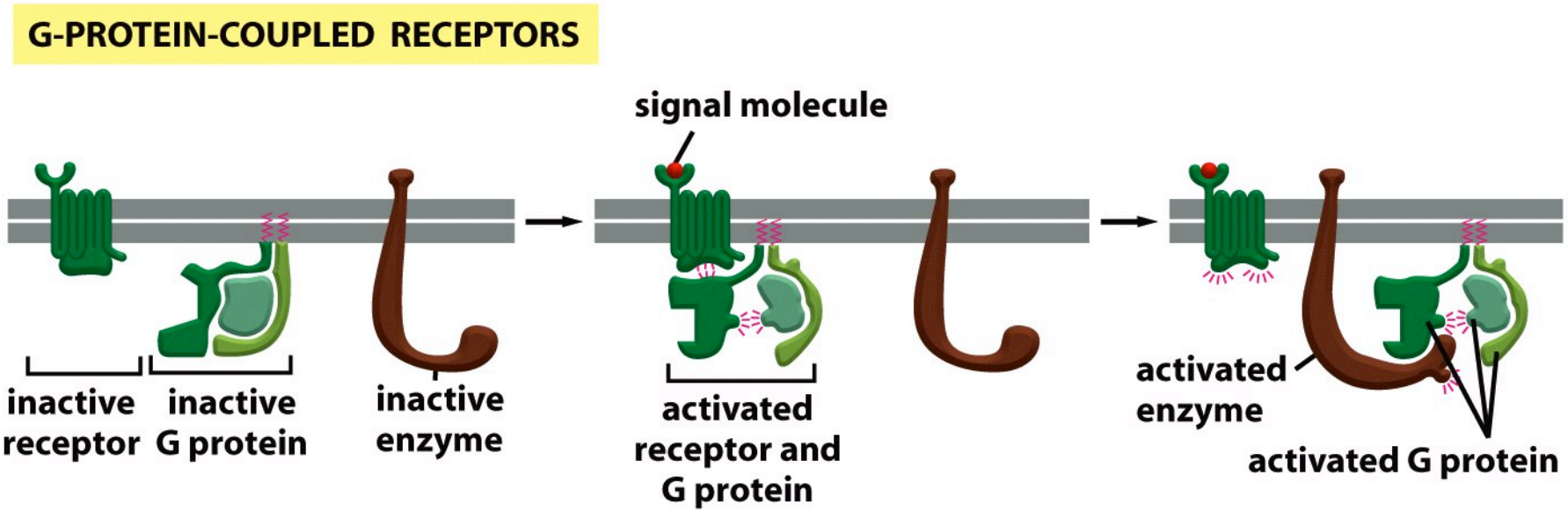


Figure 15-16b Molecular Biology of the Cell 5/e (© Garland Science 2008)