UNIVERSITÄT LEIPZIG

b-ACT matter
Forschungs- und Transferzentrum für bioaktive Materie

Lecture 5

Soft Matter Physics

Molecular association and cooperativity

- Recap: Gibbs distribution & chemical potential
- Free energy change of a reaction
- Equilibrium constant from mass action law
- Equilibrium constant from grand partition function
- Cooperativity in chemical reactions

Recap: Grand canonical ensemble

Open system with thermal + particle reservoir!

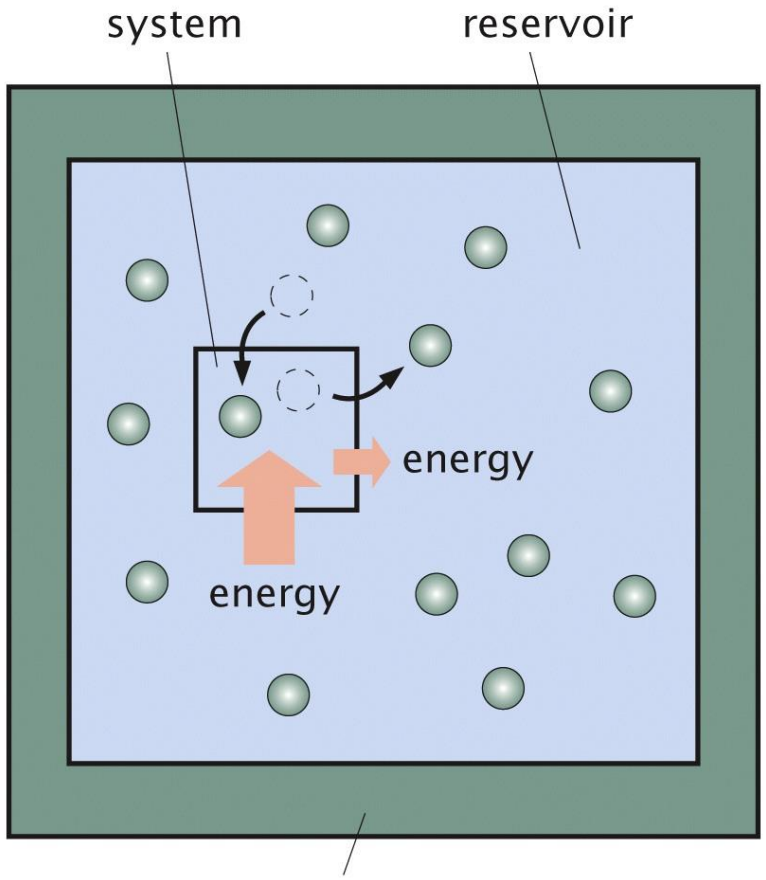


Figure 7.9 Physical Biology of the Cell, 2ed. (© Garland Science 2013)

Gibbs distribution for system with energy & particle exchange:

Energy to have N_s particles in system

Energy to take out N_s particles from reservoir

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

"Boltzmann-like" term comprising the total free energy change as sum of the free energy of the system with N_s particles and the free energy change from extracting N_s particles from the reservoir (concentration dependent)

Grand partition function $\mathcal{Z} = \sum_i e^{-\beta(E_s^{(i)} - N_s^{(i)}\mu)}$

Recap: Gibbs distribution and chemical potential

Gibbs distribution: $\rho\left(E_s^{(i)}, N_s^{(i)}\right) = \frac{e^{-\beta\left(E_s^{(i)} - \mu N_s^{(i)}\right)}}{\mathcal{Z}}$

(with $\beta = 1/k_{\mathrm{B}}\,T$)

Grand partition function:

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

Grand potential: $\Omega(T, V, \mu) = -k_B T\, ln\mathcal{Z} = F - \mu N = U - TS - \mu N = H - pV - TS - \mu N$

Average particle number in system: $\langle N \rangle = \sum_i N_i \rho_i = \frac{1}{\mathcal{Z}} \sum_i N_i e^{-\beta(E_i - \mu N_i)} \longrightarrow \langle N \rangle = \frac{1}{\beta} \frac{\partial}{\partial \mu} \ln \mathcal{Z}$

Recap: Gibbs distribution and chemical potential

Gibbs distribution: $\rho\left(E_s^{(i)}, N_s^{(i)}\right) = \frac{e^{-\beta\left(E_s^{(i)} - \mu N_s^{(i)}\right)}}{\mathcal{Z}}$

(with $\beta = 1/k_B\,T$)

Grand partition function:

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

Grand potential: $\Omega(T, V, \mu) = -k_B T\, ln\mathcal{Z} = F - \mu N = U - TS - \mu N = H - pV - TS - \mu N$

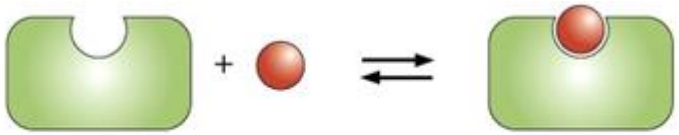
Average particle number in system: $\langle N \rangle = \sum_i N_i \rho_i = \frac{1}{\mathcal{Z}} \sum_i N_i e^{-\beta(E_i - \mu N_i)} \longrightarrow \langle N \rangle = \frac{1}{\beta} \frac{\partial}{\partial \mu} \ln \mathcal{Z}$

Chemical potential: $\mu(T, p, N) = \left(\frac{\partial G}{\partial N}\right)_{T,p}$

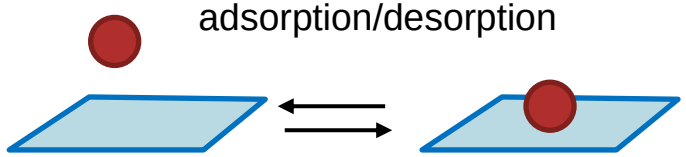
$\mu_i = \mu_{i,0} + k_B T \ln\left(\frac{c_i}{c_{i,0}}\right) \longrightarrow$ $c_{i,0}$= 1M

Examples of “chemical” reactions

$A + B \rightarrow C$

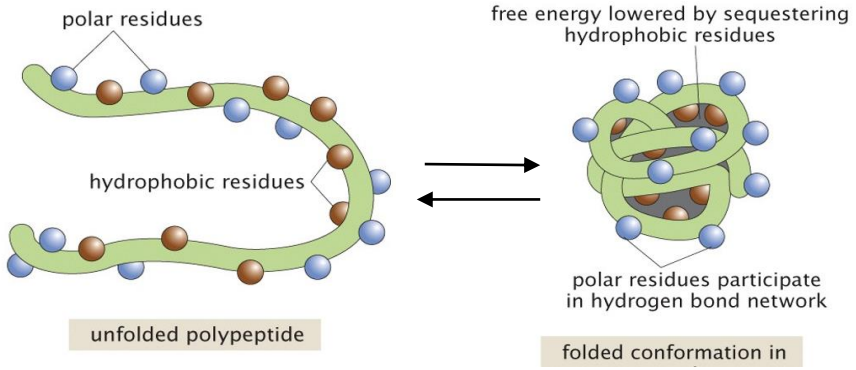


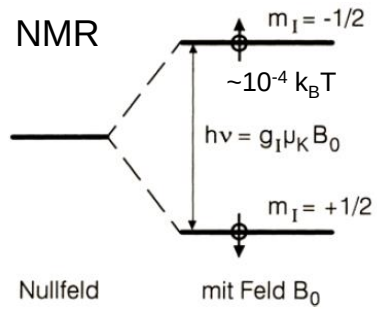
ligand binding and unbinding to receptor



$A \rightarrow B$

Globule formation (polymers, proteins)





What can we learn about relevant energy/entropy changes by looking at the involved species?

Free energy change of a reaction

chemical reaction $x_A\,A + x_B\,B \rightarrow x_C\,C\;x_D\,D$

free energy change of single reaction

$$dG = \underbrace{\left(\frac{\partial G}{\partial N_A}\right)_{T,p}}_{\mu_A} dN + \underbrace{\left(\frac{\partial G}{\partial N_B}\right)_{T,p}}_{\mu_B} dN + \underbrace{\left(\frac{\partial G}{\partial N_C}\right)_{T,p}}_{\mu_C} dN + \underbrace{\left(\frac{\partial G}{\partial N_D}\right)_{T,p}}_{\mu_D} dN$$

$$dG = \underbrace{-x_A}_{\nu_A} dN\mu_A \underbrace{-x_B}_{\nu_B} dN\,\mu_B \underbrace{+x_C}_{\nu_C} dN\mu_C\;\underbrace{+x_D}_{\nu_D} dN\mu_D \longrightarrow \Delta_r G = \frac{dG}{dN} = \sum_i \nu_i \mu_i$$

ν_i are the stoichiometry factors

Free energy change of a reaction

chemical reaction

$$-\nu_A A + (-\nu_B)B \rightarrow \nu_C C + \nu_D D$$

(ν_A and ν_B negative)

free energy change of single reaction

$$\Delta_r G = \sum_i \nu_i \mu_i$$

$$= \sum_i \nu_i \mu_{i0} + k_B T \sum_i \ln\left(\frac{c_i}{c_{i0}}\right)^{\nu_i} = \Delta_r G^0 + k_B T \sum_i \ln\left(\frac{c_i}{c_{i0}}\right)^{\nu_i}$$

$$\Delta_r G = \Delta_r G^0 + k_B T \ln\left[\prod_i \left(\frac{c_i}{c_{i0}}\right)^{\nu_i}\right]$$

Entropy term due to different abundance of species *i*

make sure to insert c_i in units of [M]!

Standard free energy of reaction contains enthalpic & entropic part (molecular property)

(at standard conditions: p = 101.3 kPa, T = 298.15 K, c_{i0} = 1M)

Mass action law

chemical reaction $-\nu_A A + (-\nu_B)B \rightarrow \nu_C C + \nu_D D$

(ν_A and ν_B negative)

in equilibrium $\Delta_r G = 0 = \Delta_r G^0 + k_B T \ln\left[\prod_i \left(\frac{c_i}{c_{i0}}\right)^{\nu_i}\right]$

$$K_{eq}(T) = \left(\prod_i c_{i0}^{\nu_i}\right) e^{-\Delta_r G^0 / k_B T} = \prod_i c_i^{\nu_i}$$

equilibrium constant

ratio of reaction species provides equilibrium constant which is related to the standard free energy (change) of the reaction by:

$$\Delta_r G^0 = -k_B T \ln\left(K_{eq} \prod_i c_{i0}^{-\nu_i}\right)$$

Bimolecular association & dissociation constants

Association constant: is the equilibrium constant of a simple association reaction

$$L + R \leftrightarrow LR \longrightarrow K_a = \frac{1}{K_d} = \frac{[LR]}{[L][R]} \qquad [K_a] = M^{-1}$$

Dissociation constant: is the equilibrium constant of a simple dissociation reaction

$$LR \leftrightarrow L + R \longrightarrow K_d = \frac{1}{K_a} = \frac{[L][R]}{[LR]} \qquad [K_d] = M$$

Langmuir adsorption isotherm for bimol. association

$L + R \leftrightarrow LR$

as $[L] = [L]_0 - [LR]$ and $[R] = [R]_0 - [LR]$

with $[L]_0 \gg [R]_0$ (excess of ligand) → $[L] \approx [L]_0$

$$K_d = \frac{([R]_0 - [LR])[L]_0}{[LR]}$$

Langmuir isotherm

$$\rho_{bound} = \frac{[LR]}{[R]_0} = \frac{[L]_0}{[L]_0 + K_D} = \frac{[L]_0/K_D}{1 + [L]_0/K_D}$$

K_D is the ligand concentration at which the receptor is half occupied

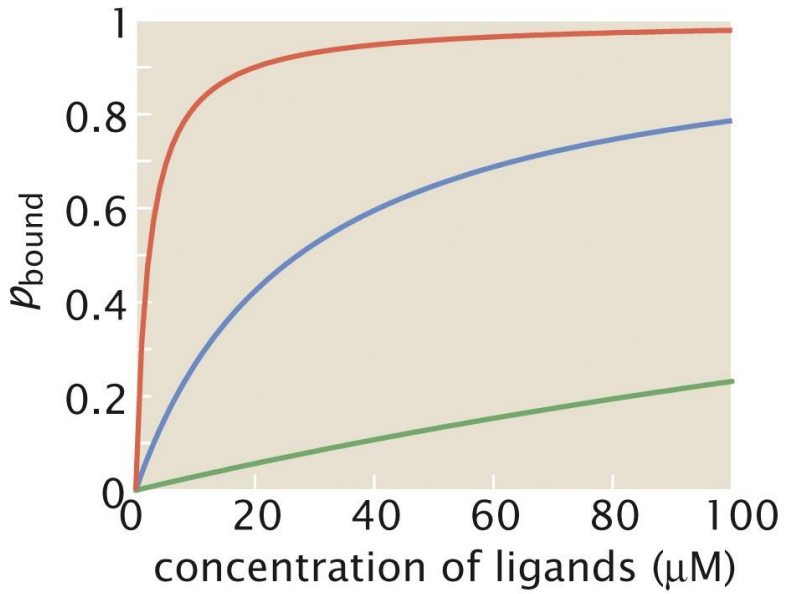
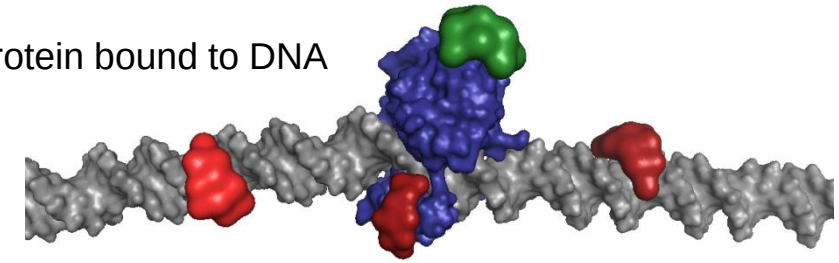


Figure 6.6 Physical Biology of the Cell, 2ed. (© Garland Science 2013)

How to determine an equilibrium constant?

K_d for simple protein DNA complex from EMSA (electromobility shift analysis)

BcnI protein bound to DNA



gel electrophoresis apparatus

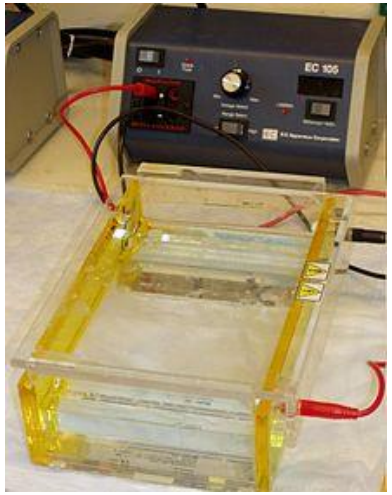
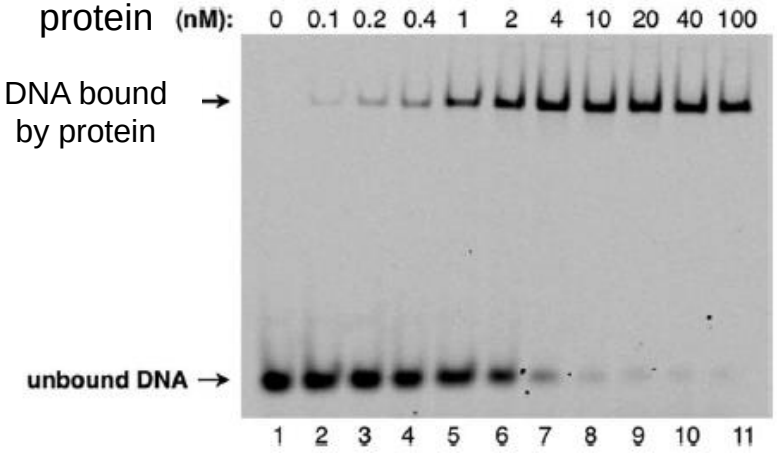
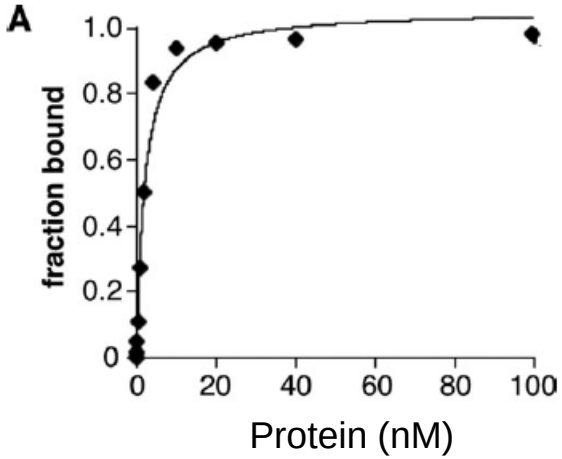
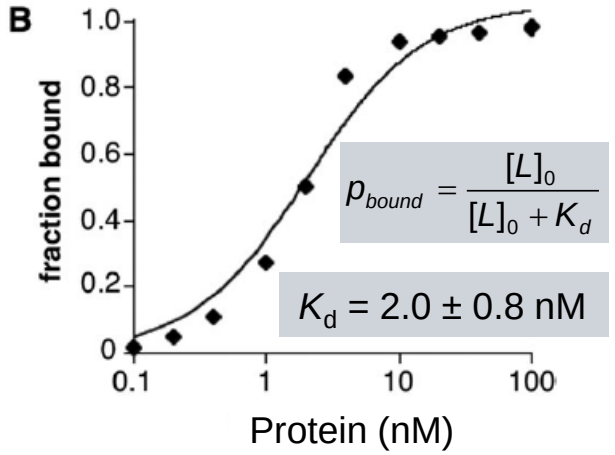


Image of the gel after electrophoresis



radiactively or fluorescently labeled DNA



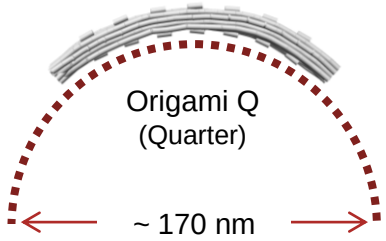


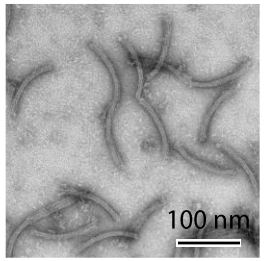
from BAMBED, Vol. 40, pp. 383–387, 2012

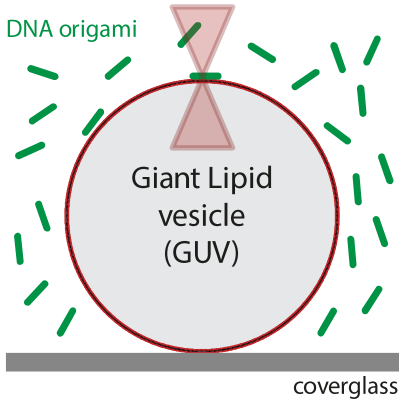
How to determine an equilibrium constant?

K_d for simple biomolecule (DNA origami)-lipid interactions from fluorescence microscopy

Biomimetic curved DNA origami







Stronger binding

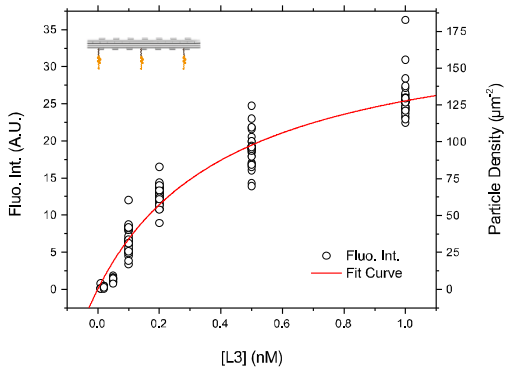
$K_d = 0.39 \pm 0.07$ nM

$K_d = 0.68 \pm 0.18$ nM

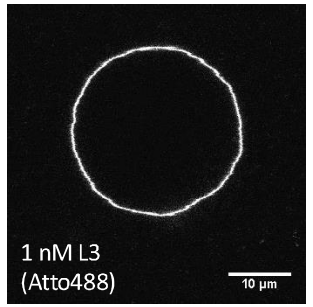
Weaker binding

$K_d = 2.0 \pm 0.6$ nM

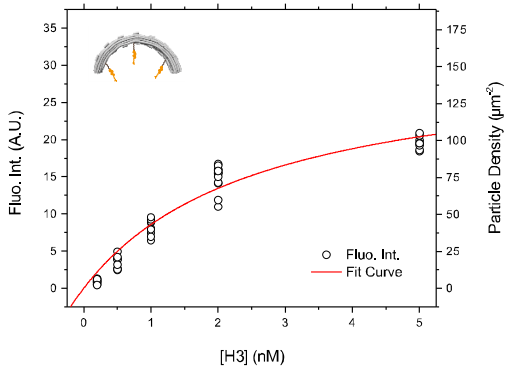
a



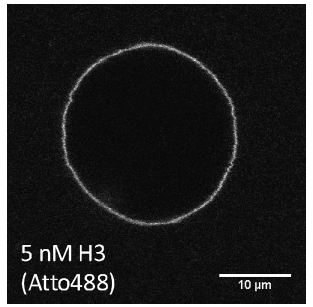
b



c



d



Franquelim HG, et al. (2018) ***Nat Commun***. 9(1): 811

Bimolecular association from Gibbs distribution

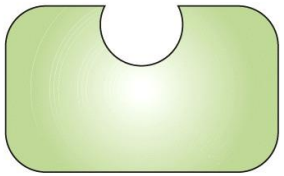
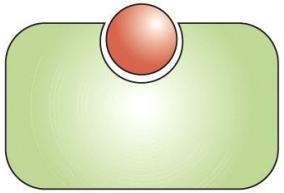
STATE	WEIGHT
 $\sigma = 0$	1
 $\sigma = 1$	$e^{-\beta(\varepsilon_b-\mu)}$

Figure 7.10 Physical Biology of the Cell, 2ed. (© Garland Science 2013)

free energy of receptor $E = \varepsilon_b \cdot \sigma$

σ → state variable i.e., occupancy by ligand

ε_b → energy change upon ligand binding

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

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

Bimolecular association from Gibbs distribution

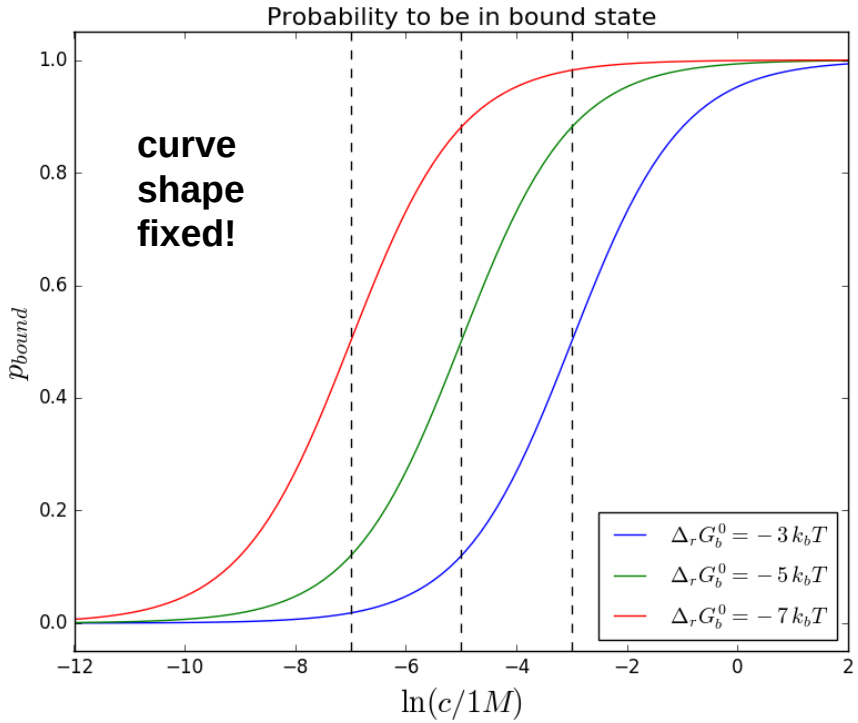
Inserting $\mu = \mu_0 + k_B T \ln(c/c_0)$:

$$\rho_{bound} = \frac{e^{-\beta(\varepsilon_b - \mu_0 - k_B T\, ln(c/c_0)}}{1 + e^{-\beta(\varepsilon_b - \mu_0 - k_B T\, ln(c/c_0)}}$$

Inserting $\Delta_r G^0_{bind} = \varepsilon_b - \mu_0$ and $K_d = c_0\, e^{\beta \Delta_r G^0_{bind}}$:

$$\rho_{bound} = \frac{(c/c_0) e^{-\beta \Delta_r G^0_{bind}}}{1 + (c/c_0) e^{-\beta G^0_{bind}}}$$

$$\rho_{bound} = \frac{c/K_d}{1 + c/K_d}$$ **Langmuir isotherm**



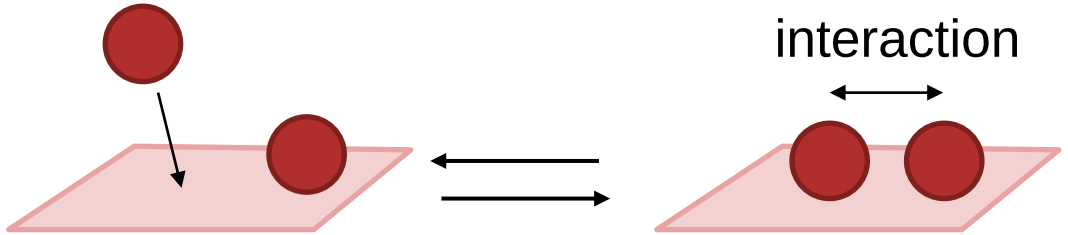
$$p_{bound} = \frac{1}{e^{\beta(\varepsilon_b - \mu)} + 1} = \frac{1}{e^{\beta(\Delta_r G^0_{bind} - k_B T \ln c)} + 1}$$

Changing equilibrium state population by sweeping an external potential

Cooperativity

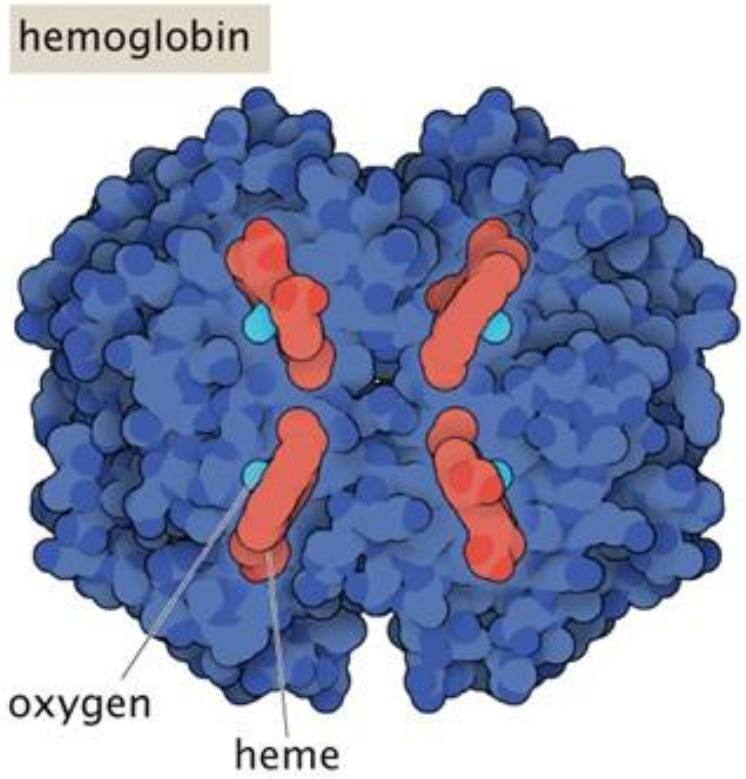
Identical elements (e.g. binding sites) of a system that act dependently of each other,
i.e. the occupation of an element state depends on the state of a neighboring element

Cooperative adsorption



gives commonly rise to nucleation phenomena

Hemoglobin – the model object of cooperativity



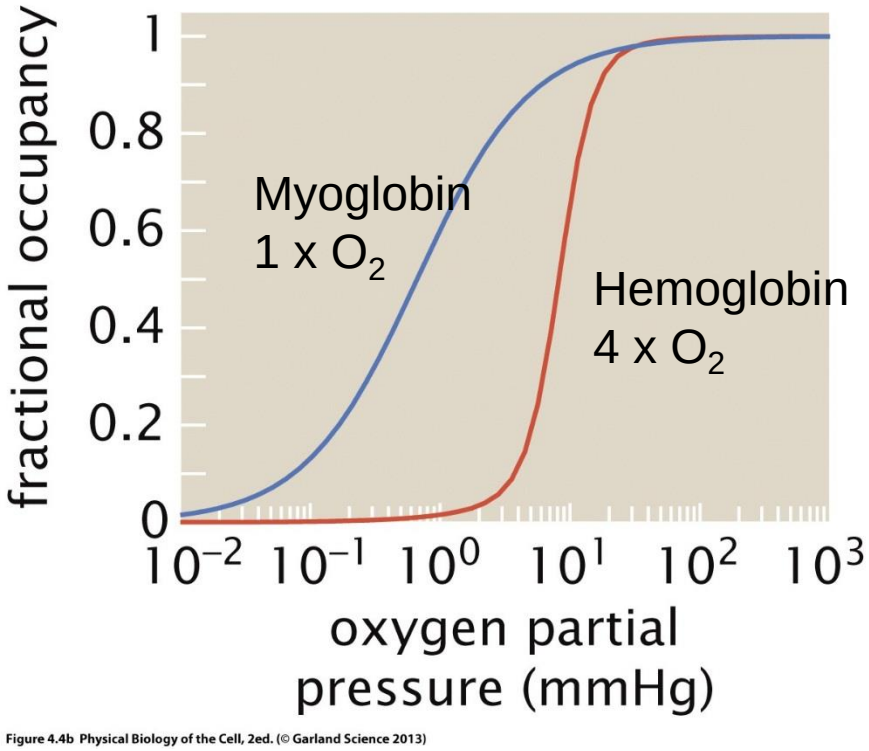


Figure 4.4b Physical Biology of the Cell, 2ed. (© Garland Science 2013)

Langmuir adsorption isotherm describes myoglobin but does not describe hemoglobin binding!!!
→ binding of O_2 at one sites increases K_{eq} for binding a second O_2
→ **cooperativity** seen as a more sudden/steeper transition

Hypothetical “Dimoglobin”

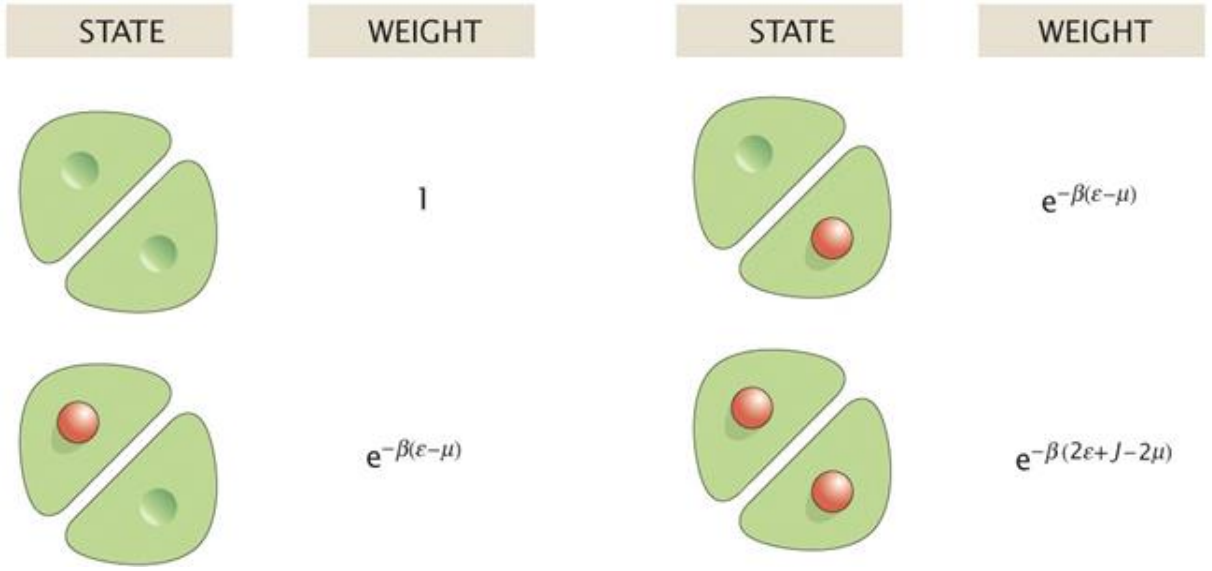


Figure 7.17 Physical Biology of the Cell, 2ed. (© Garland Science 2013)

free energy of system

$$E = \varepsilon(\sigma_1 + \sigma_2) + J\sigma_1\sigma_2$$

J is measure of cooperativity

↓

mutual interaction energy between the two bound ligands

two state variables for each binding site:

$\sigma_1 = 0 \; or \; 1$ → binding site 1 unbound/bound by O_2

$\sigma_2 = 0 \; or \; 1$ → binding site 2 unbound/bound by O_2

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

Hypothetical “Dimoglobin” – probability of states

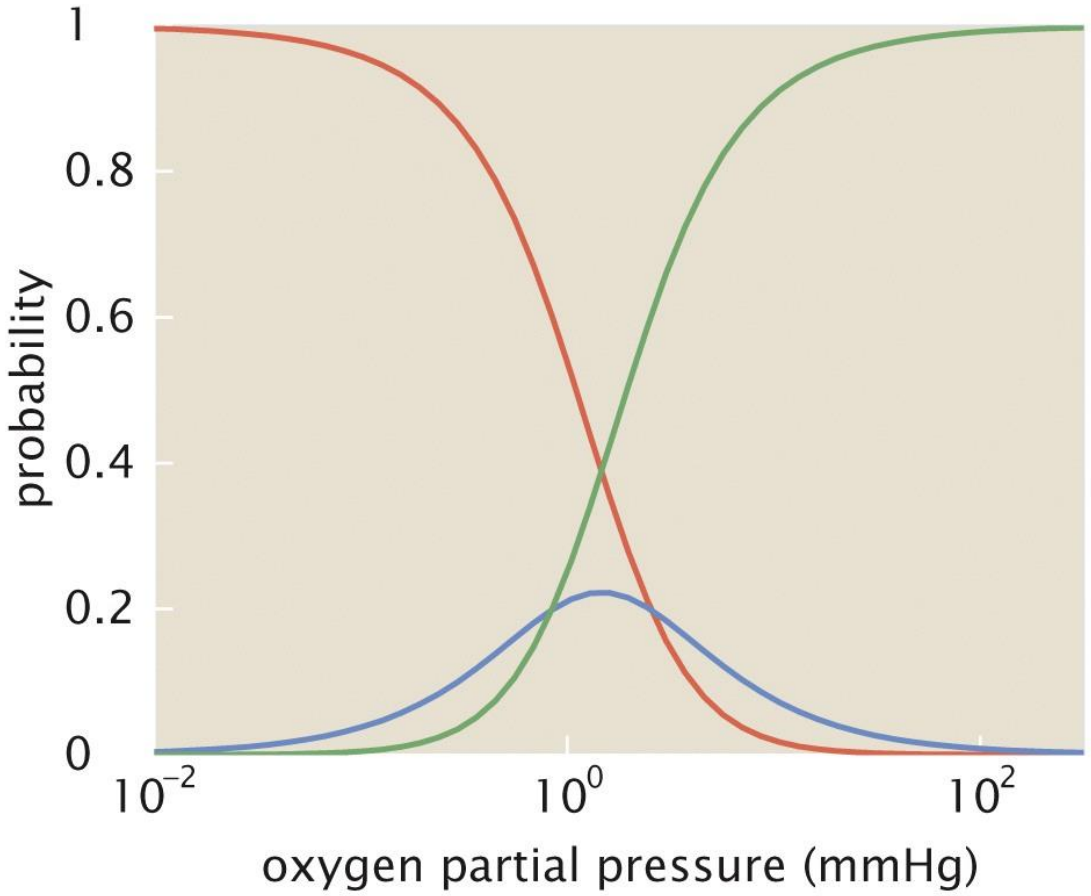
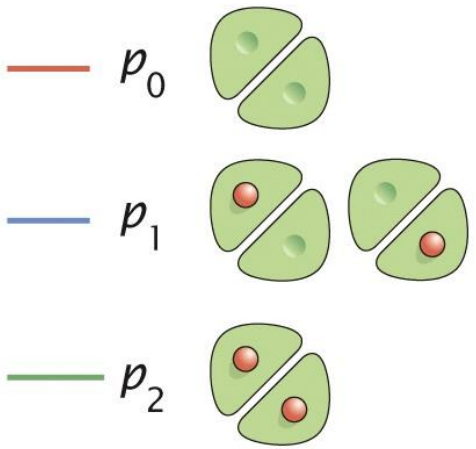


Figure 7.18 Physical Biology of the Cell, 2ed. (© Garland Science 2013)

Probabilities of each of the distinct state:



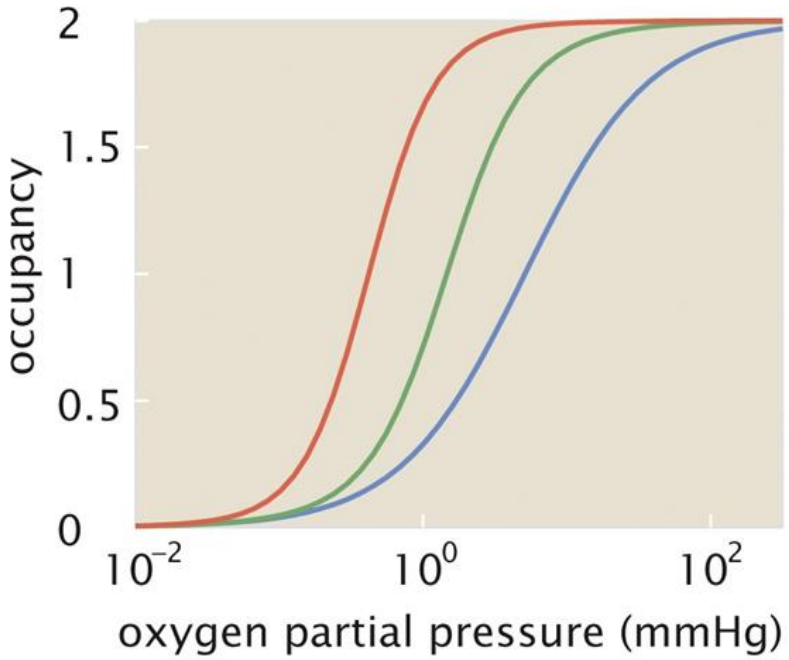
$$\rho_0 = \frac{1}{Z}$$

$$\rho_1 = \frac{2e^{-\beta(\varepsilon-\mu)}}{Z}$$

$$\rho_2 = \frac{e^{-\beta(2\varepsilon+J-2\mu)}}{Z}$$

single-ligand state (ρ_1) is only transiently occupied to a low degree!

“Dimoglobin” – Mean number of oxygens bound



$\Delta\varepsilon = \varepsilon_b - \mu_0 = -5\ k_BT$

Cooperativity factor J

- 0 k_BT
- −2.5 k_BT
- −5 k_BT

Mean number of bound O_2:

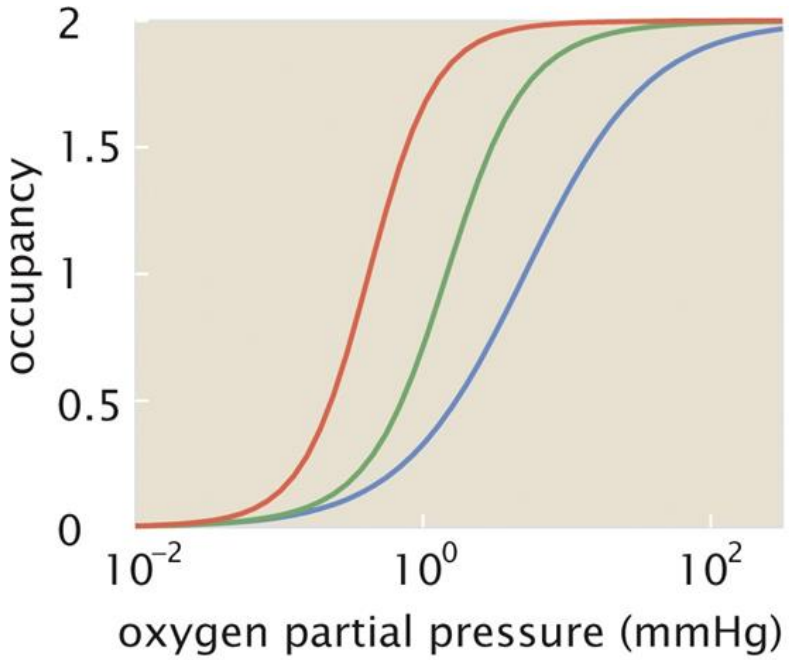
$$\langle N\rangle = \frac{1}{\beta}\frac{\partial}{\partial\mu} ln\, Z = \frac{1}{\beta}\frac{1}{Z}\frac{\partial}{\partial\mu} Z$$

$$\langle N\rangle = \frac{2e^{-\beta(\varepsilon-\mu)} + 2e^{-\beta(2\varepsilon+J-2\mu)}}{1 + e^{-\beta(\varepsilon-\mu)} + e^{-\beta(\varepsilon-\mu)} + e^{-\beta(2\varepsilon+J-2\mu)}}$$

Inserting $\mu = \mu_0 + k_BT \ln(c/c_0)$:

$$\langle N\rangle = \frac{2(c/c_0)e^{-\beta\Delta\varepsilon} + 2(c/c_0)^2 e^{-\beta(2\Delta\varepsilon+J)}}{1 + 2(c/c_0)e^{-\beta\Delta\varepsilon} + (c/c_0)^2 e^{-\beta(2\Delta\varepsilon+J)}}$$

“Dimoglobin” – Mean number of oxygens bound



$\Delta\varepsilon = \varepsilon_b - \mu_0 = -5\ k_BT$

Cooperativity factor J

- 0 k_BT
- −2.5 k_BT
- −5 k_BT

If $J = 0$ → no cooperativity

$$\langle N \rangle = 2\frac{(c/c_0)e^{-\beta\Delta\varepsilon}}{1+(c/c_0)e^{-\beta\Delta\varepsilon}} = \mathbf{2}\frac{\boldsymbol{c/K_d}}{\mathbf{1}+\boldsymbol{c/K_d}}$$

2 independent Langmuir isotherms

Mean number of bound O_2:

$$\langle N \rangle = \frac{2(c/c_0)\mathrm{e}^{-\beta\Delta\varepsilon} + 2(c/c_0)^2\mathrm{e}^{-\beta(2\Delta\varepsilon+J)}}{1+2(c/c_0)\mathrm{e}^{-\beta\Delta\varepsilon} + (c/c_0)^2\mathrm{e}^{-\beta(2\Delta\varepsilon+J)}}$$

- $\boldsymbol{J < 0}$; **Cooperative binding**
(i.e. increased affinity for the second ligand)

- $\boldsymbol{J > 0}$; **Anti-cooperative binding**
(i.e. affinity for the second site is reduced)

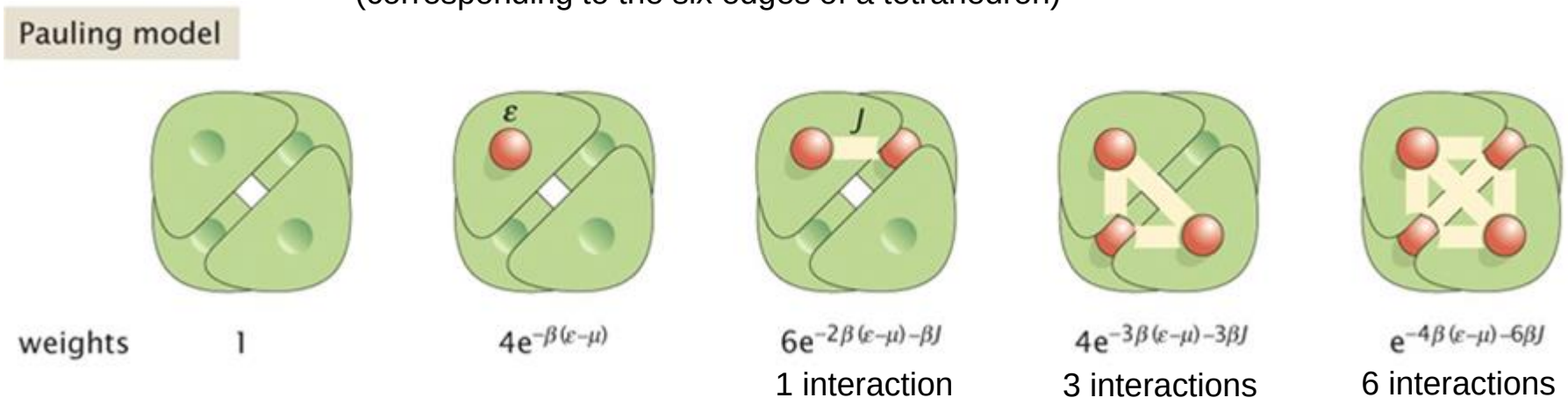
Hemoglobin – with all four binding sites

Real hemoglobin

(max. 4 O_2)

6 pairwise ligand-ligand interactions
(corresponding to the six edges of a tetrahedron)

Pauling model



$$\mathcal{Z} = \underbrace{1}_{0\text{ bound}} + \underbrace{4e^{-\beta(\varepsilon-\mu)}}_{1\text{ bound}} + \underbrace{6e^{-2\beta(\varepsilon-\mu)-\beta J}}_{2\text{ bound}} + \underbrace{4e^{-3\beta(\varepsilon-\mu)-3\beta J}}_{3\text{ bound}} + \underbrace{e^{-4\beta(\varepsilon-\mu)-6\beta J}}_{4\text{ bound}}$$

Mean number of bound O_2:

$$\langle N \rangle = \frac{4e^{-\beta(\varepsilon-\mu)} + 12e^{-\beta(\varepsilon-\mu)-\beta J} + 12e^{-3\beta(\varepsilon-\mu)-3\beta J} + 4e^{-4\beta(\varepsilon-\mu)-6\beta J}}{1 + 4e^{-\beta(\varepsilon-\mu)} + 6e^{-2\beta(\varepsilon-\mu)-\beta J} + 4e^{-3\beta(\varepsilon-\mu)-3\beta J} + e^{-4\beta(\varepsilon-\mu)-6\beta J}}$$

Hemoglobin – compare model with data

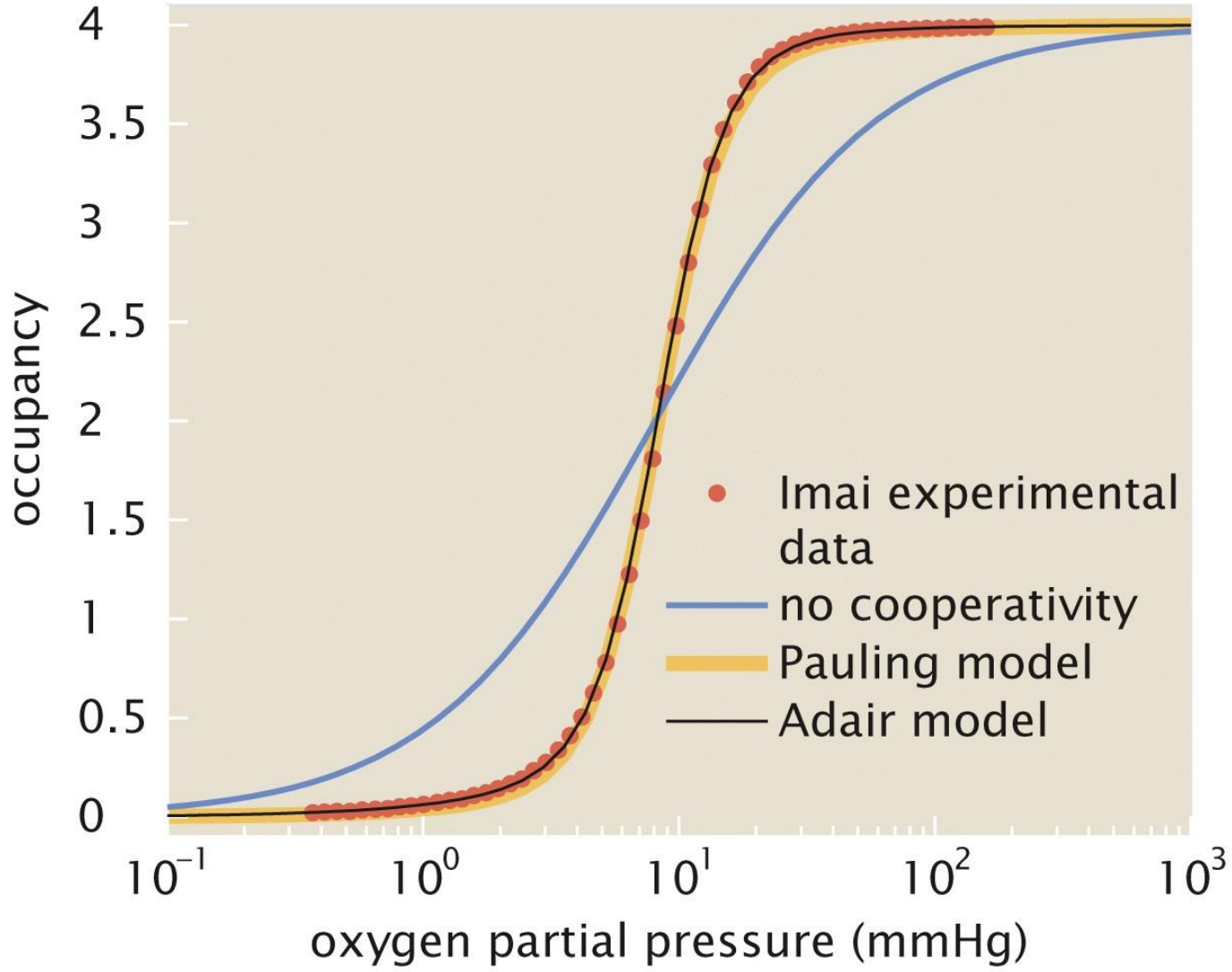
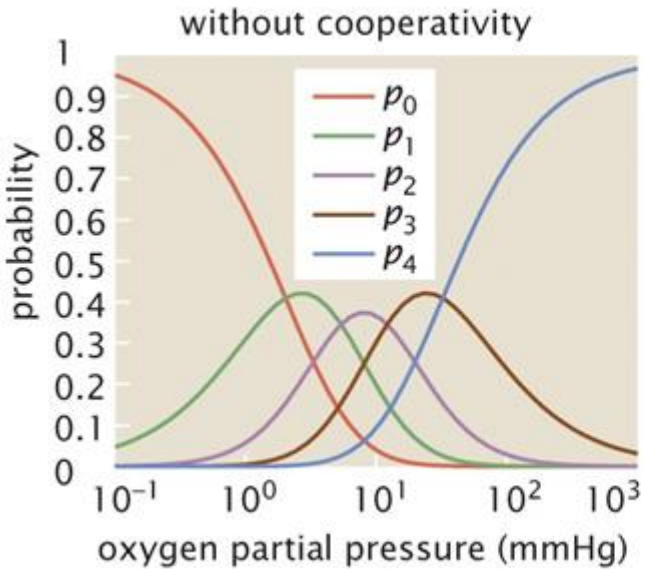


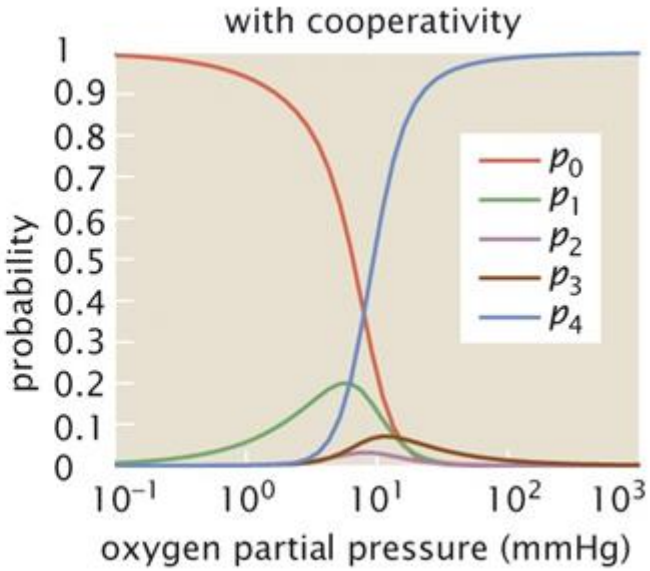
Figure 7.23 Physical Biology of the Cell, 2ed. (© Garland Science 2013)

Hemoglobin – with all four binding sites

Adair model: includes tri- and tetravalent interactions to have even more fun ☺ (not just pairwise)

$$E = \varepsilon \sum_{\alpha=1}^{4} \sigma_\alpha + \frac{J}{2} \sum_{\alpha,\gamma}{}' \sigma_\alpha \sigma_\gamma + \frac{K}{3!} \sum_{\alpha,\beta,\gamma}{}' \sigma_\alpha \sigma_\beta \sigma_\gamma + \frac{L}{4!} \sum_{\alpha,\beta,\gamma,\delta}{}' \sigma_\alpha \sigma_\beta \sigma_\gamma \sigma_\delta$$

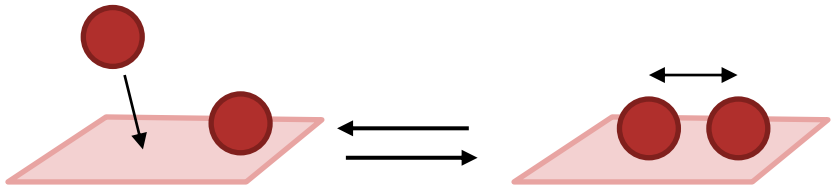




cooperativity effectively eliminates intermediate states

limits binding to narrow conc. range
(sudden treshold, all-or-none binding)

Cooperative ligand binding – formula using mass action law



$$R + \boldsymbol{n}\, L \rightleftharpoons L_{\boldsymbol{n}}R$$

$$[L_n R] = [R]\frac{[L]^n}{{K_\mathrm{d}}^n}$$

n^{th} power of dissociation constant

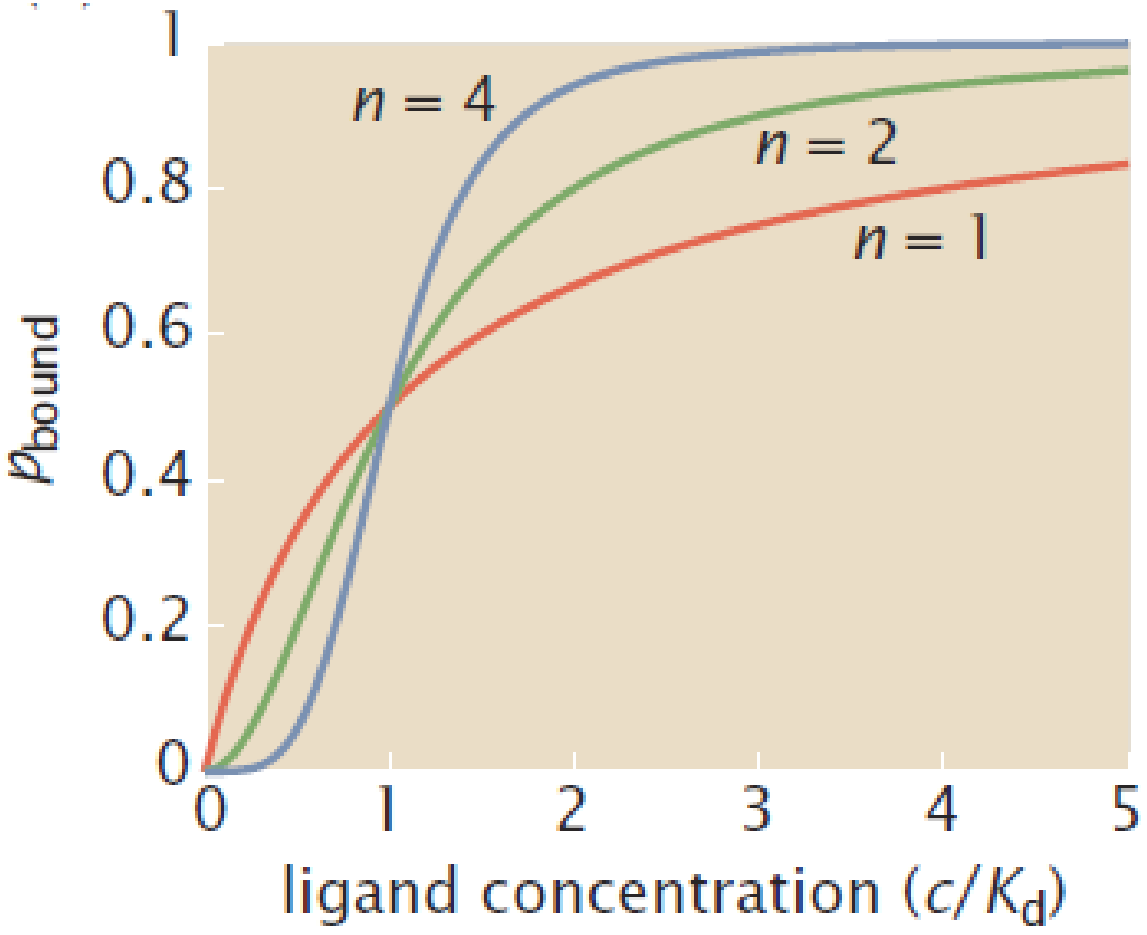
$$(K_\mathrm{d})^{\boldsymbol{n}} = \frac{[L]^{\boldsymbol{n}}[R]}{[L_{\boldsymbol{n}}R]} = c_0^{\boldsymbol{n}} e^{\beta(n\Delta\varepsilon)}$$

Probability that receptor carries n ligands:

$$\rho_{bound} = \frac{[L_n R]}{[R] + [L_n R]} = \frac{([L]/K_\mathrm{d})^{\boldsymbol{n}}}{1 + ([L]/K_d)^{\boldsymbol{n}}}$$

Hill function with **Hill coefficient $\boldsymbol{n}$**

Hill function for different Hill coefficients



With increasing Hill coefficient
(cooperativity)

↓

Curve becomes sigmoidal (S-shaped)

Hill function is empirical way to cooperativity

Fitting hemoglobin data provides $n < 4$