Homework 3

Parth Bhargava · A0310667E

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Problem 1

Estimate the equilibration constant for ATP hydrolysis.

Solution

The ATP hydrolysis reaction is

$$\mathrm{ATP} + \mathrm{H_2\ O} \to \mathrm{ADP} + \mathrm{P}_i$$

At the biochemical standard state (pH 7, 25 °C, 1 M concentrations), the standard free energy of hydrolysis is $\Delta G^\circ \approx -30.5$ kJ/mol. The equilibrium constant relates to ΔG° via

$$K_{\mathrm{eq}} = e^{-\Delta G^\circ/(RT)}$$

At $T = 298$ K with $RT = 8.314 \times 298 = 2478$ J/mol:

$$K_{\mathrm{eq}} = e^{30500/2478} = e^{12.3} \approx 2.2 \times 10^5$$

Since $K_{\mathrm{eq}} = [\mathrm{ADP}][\mathrm{P}_i]/[\mathrm{ATP}]$, the products are overwhelmingly favoured at equilibrium.

Problem 2

Please estimate the amount of free energy released from ATP hydrolysis under the concentrations $[\mathrm{ATP}] \sim 1$ mM, $[\mathrm{ADP}] \sim 1$ mM, $[\mathrm{P}_i] \sim 0.4$ mM.

Solution

The actual free energy change at arbitrary concentrations is

$$\Delta G = \Delta G^\circ + RT \ln \frac{[\mathrm{ADP}][\mathrm{P}_i]}{[\mathrm{ATP}]}$$

The reaction quotient with $[\mathrm{ATP}] = 10^{-3}$ M, $[\mathrm{ADP}] = 10^{-3}$ M, $[\mathrm{P}_i] = 4 \times 10^{-4}$ M:

$$Q = \frac{10^{-3} \times 4 \times 10^{-4}}{10^{-3}} = 4 \times 10^{-4}$$

Using $\Delta G^\circ = -30.5$ kJ/mol and $RT = 2.478$ kJ/mol (at 298 K):

$$\Delta G = -30.5 + 2.478 \ln(4 \times 10^{-4}) = -30.5 + 2.478 \times (-7.82) = -30.5 - 19.4 = -49.9 \quad \mathrm{kJ/mol}$$

Converting to thermal units via $k_B T = RT/N_A$, or equivalently $\Delta G/(RT) = -49.9/2.478 \approx -20.1$. So about $20 k_B T$ of free energy is released per ATP hydrolysed under these physiological-like conditions — significantly more than the standard-state value of $12.3 k_B T$.

Problem 3

Please estimate the mixing entropy for ATP in water at concentrations 0.01, 0.1 and 1 millimolar.

Solution

The chemical potential of a solute at concentration c is $\mu = \mu_0 + k_BT\ln(c/c_0)$, where $c_0 = 1$ M is the standard reference. The mixing entropy per solute molecule is the entropic part of this free energy:

$$\Delta S_{\text{mix}} = -k_B \ln\frac{c}{c_0}$$

A lower concentration means more volume per molecule and hence more translational microstates.

$c = 0.01$ mM $= 10^{-5}$ M: $\Delta S = 5\ln 10 \cdot k_B \approx 11.5k_B$ per molecule $(T\Delta S \approx 11.5k_BT)$
$c = 0.1$ mM $= 10^{-4}$ M: $\Delta S = 4\ln 10 \cdot k_B \approx 9.2k_B$ per molecule $(T\Delta S \approx 9.2k_BT)$
$c = 1$ mM $= 10^{-3}$ M: $\Delta S = 3\ln 10 \cdot k_B \approx 6.9k_B$ per molecule $(T\Delta S \approx 6.9k_BT)$

Converting to molar units via $N_Ak_B = R$: for instance at $c = 1$ mM, $T\Delta S = 6.9RT \approx 4.1$ kcal/mol.

Each tenfold dilution adds $k_B\ln 10 \approx 2.3k_B$ of mixing entropy per molecule. The large magnitude (several k_BT) shows that translational entropy is a significant contribution to the free energy of biomolecules in dilute solution.

Problem 4

A motor protein binds the microtubule with different affinity when the head is bound with ATP or ADP (hydrolysis product). The dissociation constant for the motor–microtubule binding in the presence of ATP and ADP is 6 mM and 25 mM, respectively. Calculate the binding energies for the ATP and ADP states.

Solution

For a single binding site, the probability of occupancy in the grand canonical ensemble is

$$P_{\text{bound}} = \frac{c/K_d}{1 + c/K_d}$$

where K_d is the dissociation constant. At $c = K_d$ the site is half-occupied. The underlying statistical weight for the bound state is $(c/c_0)e^{-\varepsilon/(k_BT)}$, where $\varepsilon < 0$ is the binding energy (the energy of the bound state relative to an unbound reference). Setting this weight equal to unity at $c = K_d$ gives

$$\frac{K_d}{c_0}e^{-\varepsilon/(k_BT)} = 1 \quad\Longrightarrow\quad K_d = c_0 e^{\varepsilon/(k_BT)}$$

Inverting:

$$\varepsilon = k_BT\ln\frac{K_d}{c_0}$$

ATP state ($K_d = 6$ mM $= 6\times 10^{-3}$ M):

$$\varepsilon_{\text{ATP}} = k_BT\ln(6\times 10^{-3}) = k_BT \times (-5.12) \approx -5.1k_BT$$

ADP state ($K_d = 25$ mM $= 2.5\times 10^{-2}$ M):

$$\varepsilon_{\text{ADP}} = k_BT\ln(2.5\times 10^{-2}) = k_BT \times (-3.69) \approx -3.7k_BT$$

The ATP-bound motor binds the microtubule about $1.4k_BT$ more strongly than the ADP-bound motor. This difference in affinity drives the mechanochemical cycle: ATP binding promotes tight attachment; hydrolysis to ADP triggers release.

Problem 5

In the toy model for cooperative binding, a mix of ligands and substrates contain a certain population in which only one of the two binding sites on a substrate is occupied. Please estimate the relative occurring probability for this single-binding population for ligand concentration $c/c_0 =$ 0.001, 0.01, 0.1 and 1. You may use $\Delta\varepsilon = -4k_BT$, and $J = -2k_BT$.

Solution

For a substrate with two binding sites, the four microstates and their grand canonical weights are:

(0, 0) — both empty: weight $= 1$
(1, 0) or (0, 1) — one occupied: weight $= (c/c_0)e^{-\Delta\varepsilon/(k_BT)}$ each
(1, 1) — both occupied: weight $= (c/c_0)^2 e^{-2\Delta\varepsilon/(k_BT)} e^{-J/(k_BT)}$

With $\Delta\varepsilon = -4k_BT$ and $J = -2k_BT$:

$$e^{-\Delta\varepsilon/(k_BT)} = e^4 \approx 54.60, \quad e^{-2\Delta\varepsilon/(k_BT)-J/(k_BT)} = e^{8+2} = e^{10} \approx 22027$$

Let $x = c/c_0$. The grand partition function is

$$\Xi = 1 + 2xe^4 + x^2e^{10}$$

and the probability of exactly one site being occupied is

$$P_1 = \frac{2xe^4}{\Xi}$$

Evaluating numerically:

c/c_0	$2xe^4$	x^2e^{10}	Ξ	P_1
0.001	0.1092	0.02203	1.131	**9.7 %**
0.01	1.092	2.203	4.295	**25.4 %**
0.1	10.92	220.3	232.2	**4.7 %**
1	109.2	22027	22137	**0.49 %**

The single-binding probability peaks at intermediate concentrations. At low c, both sites are mostly empty; at high c, the cooperative interaction ($J < 0$) strongly favours filling both sites simultaneously. This non-monotonic behaviour is a hallmark of cooperative binding.

Problem 6

A protein can bind a piece of DNA double-helix at either site A or B as shown in the figure. The binding energy for either site is $\varepsilon = -3.5k_BT$ (k_B is the Boltzmann constant and T the temperature of the solution). The chemical potential for the protein molecule is $\mu = \mu_0 + k_BT\ln(c/c_0)$ with $\mu_0 = -2.2k_BT$ and c as the protein concentration ($c_0 = 1$ M).

(a) When the separation between sites A and B is $L = 20$ base pairs, the protein binding at the two sites is uncorrelated. What is the probability for only one site to be occupied for $c = 100$ μ M and 2 M, respectively? What is the probability for both sites to be simultaneously occupied by protein molecules for the two c values?

(b) When L is 10 base pairs, the protein binding at the two sites causes an overall free-energy drop of $J = -1.7k_BT$. What is the probability for both sites to be simultaneously occupied for $c = 100$ μM and 2 M, respectively?

(c) When L is 5 base pairs, the protein binding at the two sites causes an overall free-energy increase of $J = +1.7k_BT$. What is the probability for both sites to be simultaneously occupied for $c = 2$ M?

Solution

In the grand canonical formalism, each binding site is either empty (weight 1) or occupied (weight z). The Boltzmann weight for occupation includes the binding energy ε and the chemical potential $\mu = \mu_0 + k_BT\ln(c/c_0)$ of the protein in solution:

$$z = e^{-(\varepsilon-\mu)/(k_BT)} = \frac{c}{c_0}e^{-(\varepsilon-\mu_0)/(k_BT)}$$

The dissociation constant K_d is the concentration at which $z = 1$ (half-occupancy). Setting $c = K_d$:

$$K_d = c_0 e^{(\varepsilon-\mu_0)/k_BT}$$

so equivalently $z = c/K_d$.

With $\varepsilon - \mu_0 = -3.5 - (-2.2) = -1.3k_BT$:

$$K_d = e^{-1.3} \approx 0.272 \ \text{M}, \quad z = \frac{c}{c_0}e^{1.3} \approx 3.669\frac{c}{c_0}$$

Part (a): Uncorrelated binding ($L = 20$ bp)

The two sites are independent, so the partition function factorises:

$$\Xi = (1+z)^2 = 1 + 2z + z^2$$

$$P(\text{one occupied}) = \tfrac{2z}{(1+z)^2}, \qquad P(\text{both occupied}) = \tfrac{z^2}{(1+z)^2}$$

$c = 100$ μM $= 10^{-4}$ M: $\quad z = 10^{-4} \times 3.669 = 3.67 \times 10^{-4}$

$$P_1 \approx 2z \approx 7.3 \times 10^{-4}, \quad P_2 \approx z^2 \approx 1.3 \times 10^{-7}$$

At this dilute concentration the binding is very weak ($c \ll K_d$).

$c = 2$ M: $\quad z = 2 \times 3.669 = 7.338$

$$P_1 = \frac{14.68}{69.52} \approx 0.211, \quad P_2 = \frac{53.85}{69.52} \approx 0.775$$

Above K_d, both sites are predominantly occupied.

Part (b): Cooperative binding ($L = 10$ bp, $J = -1.7k_BT$)

The simultaneous occupancy now carries an extra Boltzmann factor $e^{-J/k_BT} = e^{1.7} \approx 5.47$:

$$\Xi = 1 + 2z + z^2 e^{-J/k_BT}, \quad P_{\text{both}} = \frac{z^2 e^{1.7}}{\Xi}$$

$c = 100$ μM: $z^2 e^{1.7} \approx 7.4 \times 10^{-7}$, $\Xi \approx 1.00$

$$P_{\text{both}} \approx 7.4 \times 10^{-7}$$

Compared to the uncorrelated case (1.3×10^{-7}), the cooperative interaction boosts the doubly-bound probability by a factor $e^{1.7} \approx 5.5$.

$c = 2$ M: $z^2 e^{1.7} = 53.85 \times 5.47 = 294.8$, $\Xi = 310.5$

$$P_{\text{both}} = \frac{294.8}{310.5} \approx 0.950$$

Part (c): Anti-cooperative binding ($L = 5$ bp, $J = +1.7k_BT$)

Now $e^{-J/k_BT} = e^{-1.7} \approx 0.183$, suppressing simultaneous binding:

$$\Xi = 1 + 2(7.338) + (7.338)^2 \times 0.183 = 1 + 14.68 + 9.84 = 25.51$$

$$P_{\text{both}} = \frac{9.84}{25.51} \approx 0.386$$

The steric clash ($J > 0$) at short separation halves the doubly-bound probability compared to the uncorrelated case (0.775).

Problem 7

A follow-up question from question 6, but for two different proteins binding the same DNA duplex.

Two different proteins A and B bind to a double-stranded DNA at site 1 and site 2, respectively. The binding energy for the proteins are $\varepsilon_A = -3k_BT$ and $\varepsilon_B = -5k_BT$ (k_B is the Boltzmann constant and T the temperature of the solution). The chemical potential is $\mu_A = \mu_{A0} + k_BT \ln(c_A/c_0)$ for protein A and $\mu_B = \mu_{B0} + k_BT \ln(c_B/c_0)$ for protein B with $\mu_{A0} = +1.2k_BT$, $\mu_{B0} = -1.2k_BT$. c_A and c_B are the protein concentration, and $c_0 = 1$ M.

(1) What is the dissociation constant for protein A and protein B, respectively?

(2) The DNA may have four states: (a) no site is occupied, (b) site 1 is occupied only, (c) site 2 is occupied only, (d) both sites are simultaneously occupied. If the protein binding at the two sites is uncorrelated, what is the free-energy difference between state (a) and state (d) at $c_A = c_B = 2.4$ μM?

(3) In the case of uncorrelated binding, the average number of protein A bound on the DNA depends on the protein's concentration c_A as

$$\langle N_A \rangle = \frac{c_A/c_0}{K_{d1} + c_A/c_0}$$

Derive K_{d1} in terms of ε_A and μ_{A0}.

(4) In the case of correlated binding, the average number of protein A bound on the DNA, $\langle N_A \rangle$, follows the same formula as in (3). But K_{d1} now depends also on the concentration of protein B (c_B) and on the free-energy change J due to the simultaneous binding of the two proteins. Derive the new K_{d1} in terms of c_B and J.

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Solution

Part (1): Dissociation constants

As derived in Q4 and Q6, the fugacity for binding a protein with energy ε and solution chemical potential $\mu = \mu_0 + k_BT\ln(c/c_0)$ is $z = (c/c_0)e^{-(\varepsilon-\mu_0)/k_BT}$. The dissociation constant satisfies $z = 1$ at $c = K_d$, giving $K_d = c_0 e^{(\varepsilon-\mu_0)/k_BT}$:

$$K_{dA} = c_0 e^{(\varepsilon_A-\mu_{A0})/k_BT} = 1\ \ \mathrm{M} \times e^{(-3-1.2)} = e^{-4.2} \approx 0.015\ \ \mathrm{M} = 15\ \ \mathrm{mM}$$

$$K_{dB} = c_0 e^{(\varepsilon_B-\mu_{B0})/k_BT} = 1\ \ \mathrm{M} \times e^{(-5+1.2)} = e^{-3.8} \approx 0.022\ \ \mathrm{M} = 22\ \ \mathrm{mM}$$

Part (2): Free-energy difference between state (a) and state (d)

Moving a protein from solution to a binding site changes the free energy by $\varepsilon - \mu$. For both proteins bound (uncorrelated):

$$\Delta F = (\varepsilon_A - \mu_A) + (\varepsilon_B - \mu_B)$$

At $c_A = c_B = 2.4\ \mu\mathrm{M} = 2.4 \times 10^{-6}\ \mathrm{M}$ (expressing all energies in units of k_BT):

$$\mu_A/k_BT = \mu_{A0}/k_BT + \ln(c_A/c_0) = 1.2 + \ln(2.4 \times 10^{-6}) = 1.2 - 12.94 = -11.74$$

$$\mu_B/k_BT = \mu_{B0}/k_BT + \ln(c_B/c_0) = -1.2 + \ln(2.4 \times 10^{-6}) = -1.2 - 12.94 = -14.14$$

$$\Delta F = (-3 + 11.74) + (-5 + 14.14) = 8.74 + 9.14 = 17.9 k_BT$$

State (d) is $17.9 k_BT$ higher in free energy than state (a). The doubly-bound state is extremely unlikely at these dilute concentrations ($c \ll K_d$), with a Boltzmann weight ratio $e^{-17.9} \approx 1.7 \times 10^{-8}$.

Part (3): K_{d1} for uncorrelated binding

When binding at the two sites is independent, site 1 has two states:

Empty: weight 1
Occupied by A: weight $z_A = (c_A/c_0)e^{-(\varepsilon_A-\mu_{A0})/k_BT}$

The average occupancy is

$$\langle N_A \rangle = \frac{z_A}{1 + z_A} = \frac{c_A/c_0}{c_A/c_0 + e^{(\varepsilon_A-\mu_{A0})/k_BT}}$$

Comparing with the given formula $\langle N_A \rangle = (c_A/c_0)/(K_{d1} + c_A/c_0)$:

$$K_{d1} = e^{(\varepsilon_A-\mu_{A0})/k_BT}$$

Part (4): K_{d1} for correlated binding

With interaction energy J when both sites are simultaneously occupied, the DNA has four microstates:

$(0, 0)$: weight 1
$(A, 0)$: weight z_A
$(0, B)$: weight $z_B = (c_B/c_0)e^{-(\varepsilon_B-\mu_{B0})/k_BT}$
(A, B): weight $z_A z_B e^{-J/k_BT}$

The average number of A bound:

$$\langle N_A \rangle = \frac{z_A + z_A z_B e^{-J/k_BT}}{1 + z_A + z_B + z_A z_B e^{-J/k_BT}} = \frac{z_A\left(1 + z_B e^{-J/k_BT}\right)}{(1 + z_B) + z_A\left(1 + z_B e^{-J/k_BT}\right)}$$

Dividing numerator and denominator by $\left(1+z_B e^{-J/k_BT}\right)$:

$$\langle N_A\rangle = \frac{z_A}{\frac{1+z_B}{1+z_B e^{-J/k_BT}} + z_A} = \frac{c_A/c_0}{K_{d1}^{\text{eff}} + c_A/c_0}$$

where the effective dissociation constant is

$$K_{d1}^{\text{eff}} = e^{(\varepsilon_A-\mu_{A0})/k_BT} \cdot \frac{1+z_B}{1+z_B e^{-J/k_BT}}$$

Substituting $z_B = (c_B/c_0)e^{-(\varepsilon_B-\mu_{B0})/k_BT}$:

$$K_{d1}^{\text{eff}} = K_{d1}^{\text{uncorr}} \times \frac{1+(c_B/c_0)e^{-(\varepsilon_B-\mu_{B0})/k_BT}}{1+(c_B/c_0)e^{-(\varepsilon_B-\mu_{B0}+J)/k_BT}}$$

When $J<0$ (cooperative), $e^{-J/k_BT}>1$, so K_{d1}^{eff} decreases and A binds more readily in the presence of B. When $J>0$ (anti-cooperative), binding is suppressed. When $J=0$, the uncorrelated result is recovered.

Problem 8

In a toy model for biomolecules, a four-residue protein has three groups of possible conformations (namely unfolded state, intermediate state and folded state). Each group of conformations share the same energy (E) as shown. The number of conformations for each group is $\Omega = N_{\text{IR}} \times N_R$, in which N_{IR} is the number of conformational variations due to inter-residue arrangement, and N_R is the number of conformational variations due to atomic arrangement inside the residues. For the four-residue protein, $N_R = 16$ and N_{IR} is given below for each conformational group. Consider the protein molecule in a solution with temperature $T = 25$ °C.

Unfolded state: $E = 2.00$ kcal/mol, $N_{\text{IR}} = 1$
Intermediate (partially folded) state: $E = 1.00$ kcal/mol, $N_{\text{IR}} = 2$
Folded state: $E = 0$, $N_{\text{IR}} = 1$

(a) Calculate the free energy for the unfolded state, the intermediate state and the folded state.

(b) Calculate the probability of finding the molecule in the folded state.

(c) Suppose the protein has a higher number of conformational variations due to atomic arrangement inside the residues, namely $N_R = 256$. Calculate the free energy for the folded state, and calculate the probability of finding the molecule in the folded state.

Solution

At $T = 25$ °C $= 298$ K, the thermal energy scale is $k_BT = 0.593$ kcal/mol.

The free energy of a macrostate with energy E and multiplicity Ω is

$$F = E - TS = E - k_BT\ln\Omega = E - k_BT\ln(N_{\text{IR}} \times N_R)$$

Part (a): Free energies

With $N_R = 16$:

Unfolded ($E = 2.00$ kcal/mol, $\Omega = 1\times16 = 16$):

$$F_U = 2.00 - 0.593\ln 16 = 2.00 - 0.593\times 2.773 = 2.00 - 1.64 = 0.36 \;\; \text{kcal/mol}$$

Intermediate ($E = 1.00$ kcal/mol, $\Omega = 2\times16 = 32$):

$$F_I = 1.00 - 0.593\ln 32 = 1.00 - 0.593\times 3.466 = 1.00 - 2.06 = -1.06 \;\; \text{kcal/mol}$$

Folded ($E = 0$, $\Omega = 1 \times 16 = 16$):

$$F_F = 0 - 0.593 \ln 16 = -1.64 \;\; \text{kcal/mol}$$

The folded state has the lowest free energy: it is the thermodynamically stable state.

Part (b): Probability of the folded state

The Boltzmann weight of each macrostate is $W_i = \Omega_i e^{-E_i/(k_B T)}$:

$$W_U = 16 \times e^{-2.00/0.593} = 16 \times e^{-3.37} = 16 \times 0.0344 = 0.55$$

$$W_I = 32 \times e^{-1.00/0.593} = 32 \times e^{-1.69} = 32 \times 0.185 = 5.93$$

$$W_F = 16 \times e^{0} = 16$$

$$Z = 0.55 + 5.93 + 16 = 22.48$$

$$P_F = \frac{W_F}{Z} = \frac{16}{22.48} \approx 71.2\%$$

Part (c): Higher intra-residue multiplicity ($N_R = 256$)

The free energy of the folded state becomes:

$$F_F = 0 - 0.593 \ln(1 \times 256) = -0.593 \times 5.545 = -3.29 \;\; \text{kcal/mol}$$

However, the probability is unchanged. Since N_R is a common factor across all three states, it multiplies every Boltzmann weight equally:

$$W_i = \big(N_{\text{IR},}i \times N_R\big) e^{-E_i/k_B T} = N_R \times \underbrace{\big(N_{\text{IR},}i \times e^{-E_i/k_B T}\big)}_{\text{same as before}}$$

Explicitly with $N_R = 256$: $W_U = 256 \times 0.0344 = 8.81$, $W_I = 512 \times 0.185 = 94.9$, $W_F = 256$. The ratio:

$$P_F = \frac{W_F}{Z} = \frac{256}{8.81 + 94.9 + 256} = \frac{256}{359.7} \approx 71.2\%$$

The factor N_R cancels identically in the probability ratio. Each free energy shifts by the same amount $-k_B T \ln(N_R/16) = -0.593 \ln 16 = -1.64$ kcal/mol, leaving the folding equilibrium unaffected. Only inter-residue conformational differences between states determine the folding probability.