Problem 1

a) To calculate RELATIVE quantum yield, you just need to determine the values of the emission (counts) on the graph for each of the three curves at their maxima, and then find the emission ratio of calmodulin/free Y & calmodulin+Ca^{2+}/free Y.

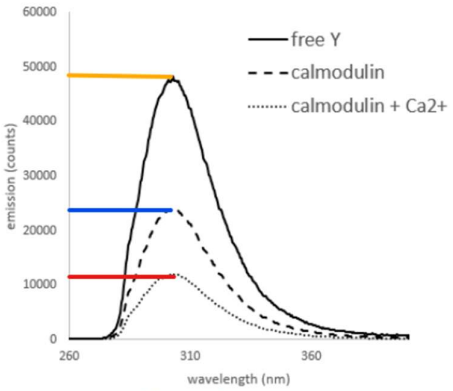


Figure 1

free Y ≈ 48000

calmodulin ≈ 23000

calmodulin+Ca^{2+} ≈ 12000

$\Phi_{relative}$(calmodulin/Y) ≈ 48 %

$\Phi_{relative}$(calmodulin+Ca^{2+}/Y) = 25 %

b) This behavior is explained by the effect of the solvent (water) on the fluorescence of the tyrosine residues and conformational changes in calmodulin structure upon binding calcium ions.

In the absence of the calcium ions calmodulin exists in closed (compact) conformation. Tyrosine will be buried inside the folded protein structure → higher fluorescence (relative quantum yield).

When bound to calcium ions, calmodulin gets open (extended) conformation. And thus tyrosine residues become more exposed to the solvent → water "quenches" fluorescence → lower relative quantum yield.

Problem 2

By definition, protein stability can be determined as the free Gibbs energy of the protein folding process. And we know that:

$\Delta G°_{fold} = -RT \ln(K_{fold})$ (1) and

$\Delta G°_{Urea} = \Delta G°_{H20} + m*[Urea]$ (2)

The problem asks to find $\Delta G°_{H20}$ (protein stability) and also the m-value.

Protein folding can be represented with this equilibrium:

U ⇌ N and thus

$$K_{fold} = \frac{[N]}{[D]} = \frac{\text{fraction\_native}}{\text{fraction\_denatured}} \quad (3)$$

K_{fold} at different urea concentrations can be determined from the given graph at several points (at least two), as the fluorescence intensity directly correlates with the protein's (un)folded fractions:

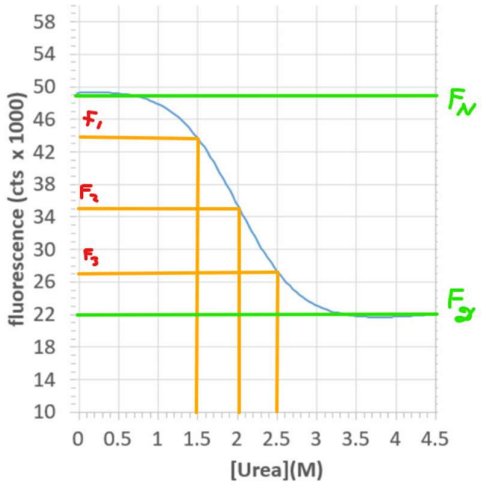


Figure 2

$$K_{fold} = \frac{F - F_D}{F_N - F} \qquad (4)$$

where F_D – fluorescence intensity of completely denatured protein; F_N — fluorescence intensity of the native (folded) protein. F — protein fluoresce at a given urea concentration.

(This eq. can be derived from the following reasonings:

$F = F_D$*fraction_denatured + F_N*fraction_native, and

fraction_denatured + fraction_native = 1)

From the plot:

$F_N = 49$; $F_D = 22$.

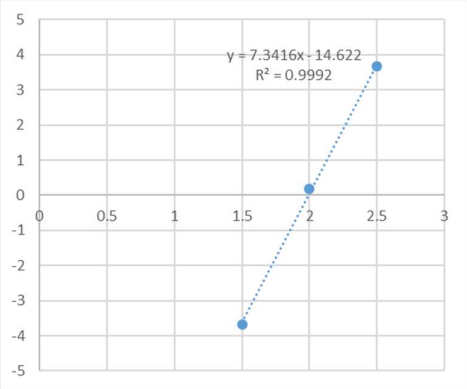
Let's take F values at urea concentration 1.5, 2, and 2.5 M:

$F_1 = 44$; $F_2 = 35$; $F_3 = 27$.

Now, we can find, K_{fold} and then $\Delta G°_{Urea}$ values.

Urea [M]	F	Kfold	deltaG, kJ/mol
1.5	44	4.4	-3.670781926
2	35	0.928571	0.183607837
2.5	27	0.227273	3.670781926

Plottting deltaG vs urea concentration and doing linear regression, we get:



From the obtained regression equation:

$\Delta G°_{H20}$ = -14.6 kJ/mol

m-value = 7.3

NOTE:

It's not necessary to take 3 or more data points and do linear regression to solve this problem.

Taking two points and solving a simple system of 2 equations is sufficient to get a reasonable estimation of the numbers asked for.

Problem 3

a) The Förster radius (R_o) values depend on

- Orientation factor κ2
- Spectral Overlap Integral:
- Acceptor extinction coefficient
- Refractive Index n of the medium
- Quantum Yield of the Donor

b) To find the distance between the dyes one needs to use two equations:

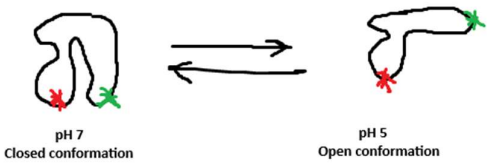
$E = 1 - \frac{\tau_{DA}}{\tau_D}$ (1) and

$r = R_0(\frac{1-E}{E})^{\frac{1}{6}}$ (2)

At pH 7 we get r = 3.74 nm

At pH 5 we get r = 5.2 nm

c) Something like this:



Problem 4

1)

a) At physiological conditions 2 different main phases coexist:

- Liquid-disordered phase L_d. Consists primarily of phospholipids with unsaturated hydrocarbon chains, allowing significant rotational and lateral freedom. Has loose packing and high fluidity.

- Liquid-ordered phase L_o. Has tight packing and higher order in the acyl chains. This phase is enriched in saturated lipids and cholesterol.

b) Cholesterol promotes formation of the L_o phase. It increases the packing density and rigidity of fluid membranes while preventing the crystallization of the membrane into a solid gel phase.

The rigid structure of cholesterol intercalates between lipid tails, which restrict the movement of neighboring methylene groups.

c) Protein A (Palmitoylated): Localizes to the Liquid-ordered L_o phase as palmitate is a long, linear fatty acid. Its straight chain packs efficiently with the ordered lipids and cholesterol.

• Protein B (Farnesylated): Localizes to the Liquid-disordered L_d phase. Farnesyl is a bulky,

branched, unsaturated isoprenoid. Its structure disrupts the tight packing, which favors the loosely

packed disordered phase.

2)

We calculate the bending energy using $G = \frac{1}{2}\kappa \int (c_1 + c_2)^2 dA$. We know: Bending rigidity $\kappa = 20\, k_B T$. Sheet area $A = 50\,\text{nm} \times 50\,\text{nm} = 2500\,\text{nm}^2$.

Conversion Factor (ATP to $k_B T$):
We assume physiological temperature ($T \approx 310$ K):

$$RT \approx 2.58\,\text{kJ/mol}$$

$$\text{Energy of 1 ATP} = \frac{30.5\,\text{kJ/mol}}{2.58\,\text{kJ/mol}} \approx 11.8\, k_B T$$

a)

The sheet rolls into a tube / cylinder of length $L = 50$ nm and circumference $2\pi R = 50$ nm. Since the curvature is the inverse of the radius of the circle that fits the surface, we get curvatures: $c_1 = 1/R$, $c_2 = 1/\infty = 0$ (∞ because one of the sides of the tube is flat and thus has infinite radius). Hence:

$$E_{\text{tube}} = \frac{1}{2}\kappa \int \left(\frac{1}{R}\right)^2 dA = \frac{1}{2}\kappa \frac{A}{R^2}$$

Substituting $A = L \cdot 2\pi R$:

$$E_{\text{tube}} = \frac{1}{2}\kappa \frac{L \cdot 2\pi R}{R^2} = \pi\kappa \frac{L}{R} = \pi\kappa \frac{50}{(50/2\pi)} = 2\pi^2 \kappa$$

$$E_{\text{tube}} = 2\pi^2 (20\, k_B T) \approx 395\, k_B T$$

$$\text{ATP required} = \frac{395}{11.8} \approx 33\text{–}34 \text{ molecules of ATP}$$

b)

Curvatures: $c_1 = c_2 = 1/R$. The total bending energy of a closed sphere is topological and independent of radius (Helfrich energy).

$$E_{\text{sphere}} = \frac{1}{2}\kappa \int \left(\frac{2}{R}\right)^2 dA = \frac{1}{2}\kappa \left(\frac{4}{R^2}\right)(4\pi R^2) = 8\pi\kappa$$

$$E_{\text{sphere}} = 8\pi (20\, k_B T) = 160\pi\, k_B T \approx 503\, k_B T$$

$$\text{ATP required} = \frac{503}{11.8} \approx 42\text{–}43 \text{ molecules of ATP}$$

(Credit goes to Axel Brunetta for nicely solving the problem and typing down all the bulkyish equations.)

3)

Answers to this question can be easily found in the course presentation slides / any subject-related textbook.

Problem 5

1)

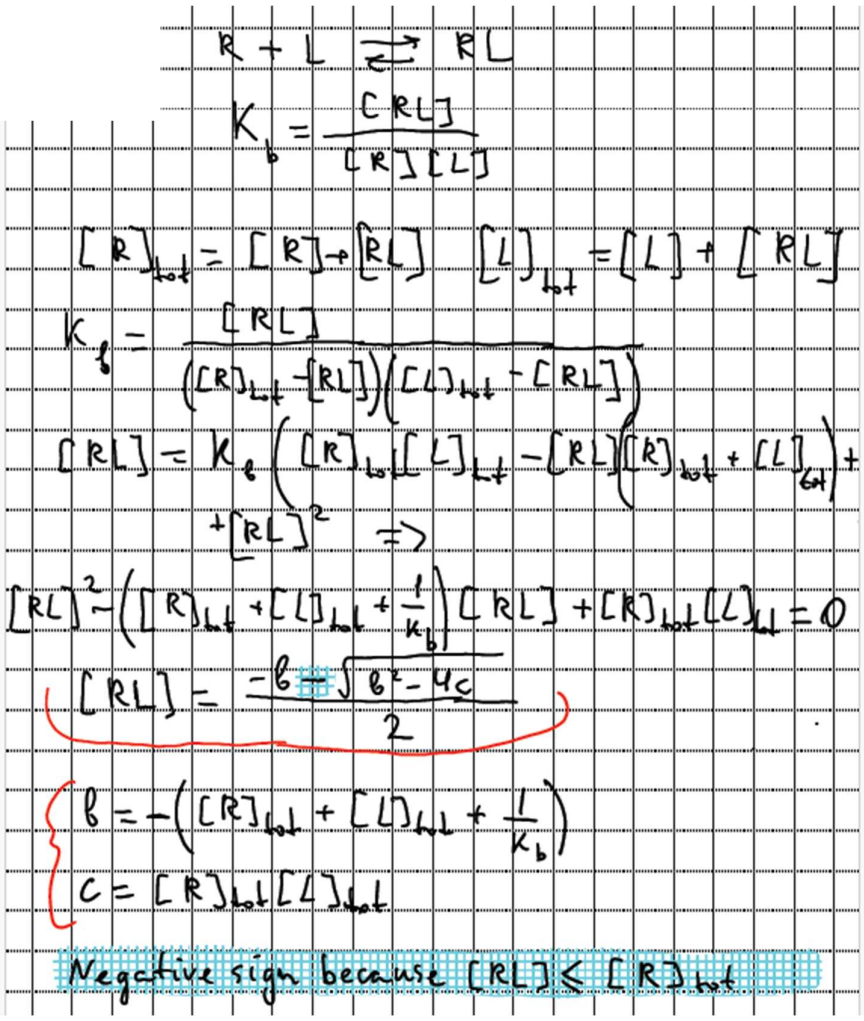
a) For the first inhibitor (orange curve), the stoichiometry is 1:1. For the second (blue), it's 1:2 (enzyme:inhibitor).

b) The interactions are exothermic and ΔH for both of them are roughly -5 units (kcal/mol or something, as units were not indicated on the graph).

c) A “simple” answer (but not really correct) is the following: which inhibitor binds stronger can be estimated from the steepness of the curves near the mid-point. Blue curve is steeper than orange → “blue” inhibitor binds better.

BUT these two inhibitors bind with different stoichiometry (1:1 vs 1:2), therefore their binding is described by different mathematical models, and therefore we cannot directly compare their binding affinity based on the steepness on the curve. So, we cannot confidently say which binding is stronger.

2)



3)

$$dQ = d[RL] \cdot \Delta H_{app} \cdot V_C \implies \frac{1}{V_C}\frac{dQ}{d[L]_{tot}} = \Delta H_{app}\frac{d[RL]}{d[L]_{tot}}$$

Thus, the final step to get eq. 1 is to find the differential of $d[RL]/d[L]_{tot}$. Then substitute back X_r and r and make some final rearrangements.

4)

a-c) I’m attaching an Excel file with the solution.

The obtained numbers:

ΔH = -15.1 kcal/mol $K_b = 4.87*10^5\ M^{-1}$ ΔG = -7.75 kcal/mol ΔS = -24.5 cal/(mol*K)

Problem 6

How to calculate the ΔH and ΔS of primers' annealing using nearest-neighbor method is well explained in the video attached with the problem. After enthalpy and entropy are calculated, T_m for the primers can be found.

<u>For forward primer:</u>

ΔH = -74.85 kJ/mol

ΔS = -2004.6 J/(mol*K)

Tm = 76 °C

<u>For reverse primer:</u>

ΔH = -71.34 kJ/mol

ΔS = -1926.7 J/(mol*K)

Tm = 72 °C

Thus, annealing for the PCR with these primers should be done at ~67–69 °C.

Here is a screenshot of a simple Python script to help calculate the enthalpies and entropies:

```
enthalpies_dict = {'AA':-8.4, 'TT':-8.4, 'AT':-6.5, 'TA':-6.3,'CA':-7.4,'TG':-7.4,'GT':-8.6,'AC':-8.6,
                   'CT':-6.1,'AG':-6.1,'GA':-7.7, 'TC':-7.7,'CG':-10.1,'GC':-11.1,'GG':-6.7,'CC':-6.7}
entropies_dict = {'AA':-23.6, 'TT':-23.6, 'AT':-18.8, 'TA':-18.5,'CA':-19.3,'TG':-19.3,'GT':-23,'AC':-23,
                   'CT':-16.1,'AG':-16.1,'GA':-20.3, 'TC':-20.3,'CG':-25.5,'GC':-28.4,'GG':-15.6,'CC':-15.6}
import re
def occurrences(text, sub):
    return len(re.findall('(?={0})'.format(re.escape(sub)), text))

oligo = 'AGCGGATAACAATTTCACACAGG'   <- insert your primer sequence
counter_occurances = {k: 0 for k in enthalpies_dict}
enthalpy = []
entropy = []
for key in enthalpies_dict:
    n_times = occurrences(oligo, key)
    counter_occurances[key] = n_times
    enthalpy.append(round(n_times*enthalpies_dict[key], 3))
    entropy.append(round(n_times*entropies_dict[key], 3))
enthalpy = round(sum(enthalpy)*4184,3)
entropy = round((sum(entropy)-5.9)*4.184,3)

print(f'number of all neighbours:\n {counter_occurances}')
print(f'Enthalpy = {enthalpy} J/mol')
print(f'Entropy = {entropy} J/mol*K')
```

returns the output with ΔH and ΔS values

The calculated above temperatures will differ from those found with the NEB Tm Calculator, as NEB calculator also makes a correction for buffer composition of the PCR solution, in particular salt concentration.