Exercise Sheet 2 – CH – 311 **6/11/2024**

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1. You need to synthesize a very stable, helical peptide as an inhibitor of a particular receptor. Computational modeling revealed that a peptide of the sequence RRE**K**TAFT**YKL**IE**E**HVLIRGE could be a good binder as a helix. The bold residues make critical interactions with the protein. However, upon synthesizing the peptide, it is not very helical and does not bind well.

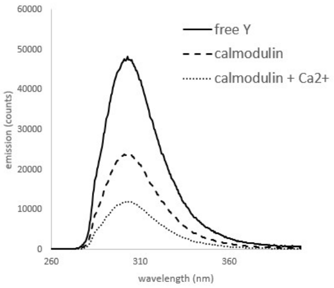
Give 3 strategies how the structure of this peptide can be stabilized, e.g. what amino acids should be changed, or what other modifications could be employed.

2. Describe the key assumption of the Zimm-Bragg model for helix formation. From your view, what is the key result from this model and why has it been important?

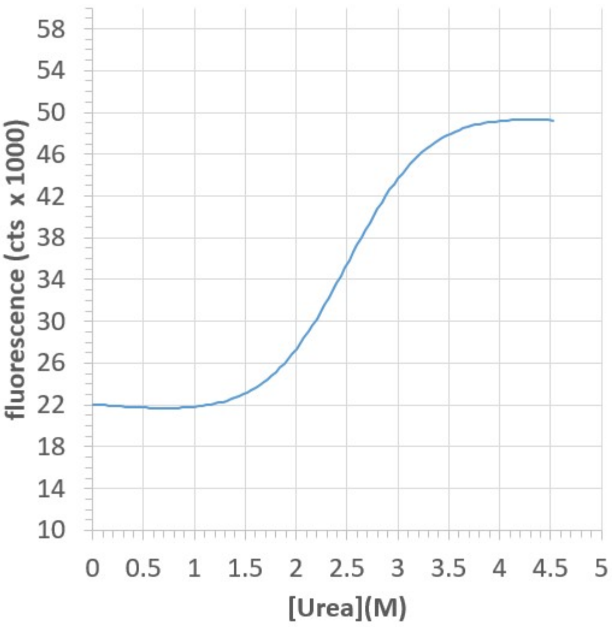
3. The figure displays the fluorescence spectrum of free tyrosine, the tyrosine in calmodulin with and without Ca2+.

a. Calculate the relative quantum yield of Y in calmodulin +/- Ca2+, relative to free tyrosine.

b. Explain this behavior.



4. You measure urea unfolding of a small protein using tryptophan fluorescence and obtain the following data (see below). Determine the **protein stability** in the absence of urea and calculate **the m-value**. Use a ruler and the picture below to determine the parameters.



5. Polymerase chain reaction (abbreviated PCR) is a laboratory technique for rapidly producing (amplifying) millions to billions of copies of a specific segment of DNA, which can then be studied in greater detail. PCR involves using short synthetic DNA fragments called primers to select a segment of the genome to be amplified, and then multiple rounds of DNA synthesis to amplify that segment.

a) Shortly, describe the principle of PCR and the components needed to perform it.

One important parameter to consider to successfully run PCR is the annealing temperature of the primers. The annealing temperature must be ~5 ºC lower than the lower T_m of the primers, where T_m is a primer's melting temperature.

In order to correctly choose the annealing temperature, one first has to calculate melting temperatures of the primers. This can be done by the Nearest-Neighbour Method—a thermodynamic model which can help calculate ΔH, ΔS & ΔG of DNA duplex formation and subsequently its T_m. In a nutshell, to calculate the T_m, one first needs to sum all enthalpy and entropy contributions of adjacent nearest-neighbour base pair stacks along the DNA sequence, which are empirical data and are shown in the table below, and then use the following formula:

$$T_m = \frac{\Delta H^o_{helix}}{\Delta S^o_{helix} + R ln(C_T/4)}$$

where C_T is the total strand (primer + template DNA (which can be neglected)) concentration.

Imagine you have to run PCR with the following primers:
Forward primer: 5’ CCCAGTCACGACGTTGTAAAACG 3’
Reverse primer: 5’ AGCGGATAACAATTTCACACAGG 3’
You are planning to use Taq polymerase. The primers concentration is 200 nm.

b) Calculate the T_m for the forward and reverse primers using the information from the table below and equation above. At what temperature would you do the annealing step? Compare your answer with the one obtained by the online T_m calculator (use NEB Tm Calculator). Why do you think your results might differ? *(To simplify your calculations, you can use Excel Sheets to solve this problem)*

Thermodynamic Parameters for DNA Helix Initiation and Propagation in 1 M NaCl[a]

propagation sequence	ΔH° (kcal/mol)	ΔS° (eu)	ΔG°_{37} (kcal/mol)
AA/TT	−8.4 ± 0.7	−23.6 ± 1.8	−1.02 ± 0.04
AT/TA	−6.5 ± 0.8	−18.8 ± 2.3	−0.73 ± 0.05
TA/AT	−6.3 ± 1.0	−18.5 ± 2.6	−0.60 ± 0.05
CA/GT	−7.4 ± 1.1	−19.3 ± 2.9	−1.38 ± 0.06
GT/CA	−8.6 ± 0.7	−23.0 ± 2.0	−1.43 ± 0.05
CT/GA	−6.1 ± 1.2	−16.1 ± 3.3	−1.16 ± 0.07
GA/CT	−7.7 ± 0.7	−20.3 ± 1.9	−1.46 ± 0.05
CG/GC	−10.1 ± 0.9	−25.5 ± 2.3	−2.09 ± 0.07
GC/CG	−11.1 ± 1.0	−28.4 ± 2.6	−2.28 ± 0.08
GG/CC	−6.7 ± 0.6	−15.6 ± 1.5	−1.77 ± 0.06
initiation at G·C[b]	(0)	(−5.9 ± 0.8)	+1.82 ± 0.24
initiation at A·T[c]	(0)	(−9.0 ± 3.2)	(+2.8 ± 1)
symmetry correction[d]	0	−1.4	+0.4
5′-terminal T·A bp[e]	+0.4	0	+0.4

[a] Errors are standard deviations. Extra significant figures are given for ΔH° and ΔS° to allow accurate calculation of the T_M. Values in parentheses involve assumptions about the initiation process (see text). [b] Initiation parameter for duplexes that contain at least one G·C base pair. [c] Initiation parameter for duplexes that contain only A·T base pairs. [d] Symmetry correction applies only to self-complementary sequences. [e] To account for end effects, duplexes are given the penalty listed for each terminal 5′-T·A-3′ base pair. Note this penalty is not applied to sequences with terminal 5′-A·T-3′ base pairs (see text).

SantaLucia et. al. Biochemistry 35.11 (1996): 3555-3562. https://doi.org/10.1021/bi951907q

For more information, check the article referred above, or this video with the detailed explanation how the Nearest-Neighbour Method works:

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