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The following exam references Sedlak, E. and Robinson N.C. (2015) Destabilization of the Quaternary Structure of Bovine Heart Cytochrome c Oxidase upon Removal of Tightly Bound Cardiolipin. **Biochemistry** *(in press)*.

1. The authors use a 100 mM phosphate buffer (pH 7.4) as part of the protein purification process. The relevant pKa of phosphate is 7.21. If you wanted to prepare 1 L of this buffer:

a) How many total moles of phosphate (acid and basic forms) will be in the final solution?

b) How many total moles of the "acidic" form of phosphate (NaH_2PO_4) will be in the final solution?

c) How many total moles of the "basic" form of phosphate (Na_2HPO_4) will be in the final solution?

d) How many grams of the "acidic" form of phosphate (NaH_2PO_4) will be added to make the final solution?

e) How many grams of the "basic" form of phosphate (Na_2HPO_4) will be added to make the final solution?

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2. The authors use horse heart cyctochrome c, the DNA sequence of which may be found below:

ATGCTTAACC ACATTCCTCC TTGTTTAGGC ATCACCTGGA AAGAGGAAAC
ACTGATGGAA TATTTGGAGA ATCCCAAGAA GTACATCCCT GGAACAAAAA
TGATCTTTGC TGGCATTAAG AAGAAGACAG AAAGAGAAGA CTTAATAGCT
TATCTCAAAA AAGCTACTAA TGAGTAA

a) If you want to amply the cytochrome c gene from a horse chromosome using PCR and primers that are 20 bases in length, what would be the sequence of the primers?

5'-_ _-3'

5'-_ _-3'

3.

a) Which amino acids contain a carboxylic acid or a carboxylate in their sidechain?

b) Which amino acids contain an amine in their sidechain?

c) Which amino acids contain an amide in their sidechain?

d) Which amino acids contain sulfur in their sidechain?

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3. Cytochrome c oxidase is composed of 13 subunits that interact at the quaternary structural level. The authors study (*i*) the strength of the interaction between subunit III and the whole cytochrome c oxidase complex, (*ii*) the strength of the interaction between subunit VIIa and the whole cytochrome c oxidase complex, and (*iii*) whether the phospholipid Cardiolipin affects the strength of the quaternary interactions. The authors reported the fraction of complexes with bound Subunits III and VIIa under various perturbing concentrations of GuHCl:

Subunit III with Cardiolipin		Subunit III minus Cardiolipin		Subunit VIIa with Cardiolipin		Subunit VIIa minus Cardiolipin	
[GuHCl]	Fraction Bound	[GuHCl]	Fraction Bound	[GuHCl]	Fraction Bound	[GuHCl]	Fraction Bound
0	1	0	1	0	1	0	1
0.2	1.05	0.15	0.9	0.2	0.96	0.15	0.96
0.4	1.05	0.3	0.88	0.4	1.01	0.3	0.85
0.6	0.97	0.45	0.82	0.6	1	0.45	0.825
0.75	0.85	0.55	0.8	0.8	0.89	0.55	0.78
0.9	0.8	0.7	0.62	1	0.8	0.7	0.55
1.2	0.5	0.8	0.41	1.16	0.45	0.8	0.26
1.4	0.35	1.1	0.125	1.32	0.39	1.1	0.06
1.5	0.18	1.3	0	1.5	0.24	1.3	0
1.6	0.12	1.5	0	1.66	0.18	1.5	0
1.8	0.07	1.7	0	1.82	0.1	1.7	0
2.2	0.1	1.9	0	2.1	0.1	1.9	0

*for your convenience these data tables may be found on the course Moodle page by the exam paper.

Modeling the data as we did in the Mb stability lab to determine the equilibrium constant in the absence of GuHCl (K°_{eq}) is a way to assess the stability of the quaternary structure with respect to each subunit, where:

$$K^{\circ}_{eq} = \frac{[\text{Dissociated complex}][\text{Subunit III}]_{free}}{[\text{Whole complex}]} \text{ or } K^{\circ}_{eq} = \frac{[\text{Dissociated complex}][\text{Subunit VIIa}]_{free}}{[\text{Whole complex}]}.$$

The smaller the value of K°_{eq}, the more tightly the complex interacts. The larger the value of K°_{eq}, the weaker the complex interacts. Use Excel and the SolverStat macro to model the authors' data from the tables above to the following equation (modified from the Mb lab):

$$\text{Fraction Bound} = \frac{1}{K^{\circ}_{eq}\left(e^{m[GuHCl]}\right)+1}$$

a) Fill in the following table to report your results:

	Subunit III		Subunit VIIa	
	(+) Cardiolipin	(-) Cardiolipin	(+) Cardiolipin	(-) Cardiolipin
Keq°	(±)	(±)	(±)	(±)
m	(±)	(±)	(±)	(±)

b) The authors conclude that Cardiolipin strengthens the quaternary interactions for Subunit III and Subunit VIIa. Do your modeling results support this conclusion? Explain.

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4. Open the “High-Resolution Three Dimensional Structure of Horse Cytochrome c” in PyMol.

a) Determine the length between the terminal amine of arginine 38 and the nearest carboxylate oxygen of the heme cofactor. This is a charge—charge interaction.

b) Determine the length between the amide nitrogen of side chain of asparagine 52 and the nearest carboxylate oxygen of the heme cofactor. This is a hydrogen bond interaction.