Optional Winter Break Problem Set

BMES Cell Team

Fall 2020

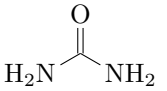
Name: KEY

Instructions:

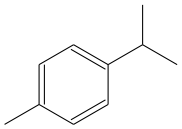
1. We will be holding a review session for this problem set on Tuesday, January 12th.
2. Although this problem set is optional, it is recommended that you attempt it on your own before the review session so that you can get extra practice.
3. Problems 6 and 8 contain "enrichment questions" that are intended to help you think critically and apply your knowledge beyond the field of Cell Research. Don't worry if you can't figure them out.
4. If you have any questions, please direct-message us on Slack, or send us an email at
   - `justinxchen22[at]gmail[dot]com`
   - `abekhtel[at]gmail[dot]com`

Problem 1.

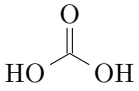
You are running a SDS gel electrophoresis experiment on each of the four molecules listed below. A constant current I_α flows from the cathode at the top of the plate to the anode at the bottom, where $I_\alpha < 0$. Additionally, you are using Tris-acetate buffer, which has a pH of 8.3, so some of these molecules may look different than the way they are drawn below.



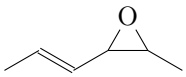
a



b



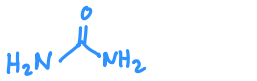
c



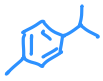
d

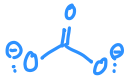
(a) Under these conditions, redraw molecules **a** to **d** in their protonated, deprotonated, or neutral state.

Since we are under basic conditions, we want these molecules to be deprotonated as much as possible, while maintaining stability. Thus,



(Unstable if deprotonated)





(Carbonate)

(b) Which molecule (**a** to **d**) would travel the *furthest* down the gel? Justify your answer by providing two different reasons.

The carbonate molecule (c) would travel the furthest because:

1. It is small compared to the other molecules on the list
2. It is charged, making it very polar and more attracted to the anode.

(c) Which molecule (**a** to **d**) would travel the *least* down the gel? Justify your answer by providing two different reasons.

Molecule (b) would travel the least because:

1. It is large compared to the other molecules on the list
2. It is fully composed of hydrocarbons, which are not polar and contain no partial charges

Problem 2.

Follow the directions below to perform a homology analysis of 6ZCT, one of the SARS-CoV-2 subproteins.

- Go to the Protein Data Bank (`https://www.rcsb.org/`), and search for `6ZCT`.
- Answer the following questions about the 6ZCT subunit:

(a) What nonstructural protein (nsp) is the 6ZCT subunit found in?

Nonstructural protein 10 (nsp10)

(b) How many amino acids is the 6ZCT subunit? What is the 1-letter abbreviation and full name of the fifth amino acid? (*Hint*: Click on the FASTA sequence file to find the full sequence.)

125 amino acids.
S = Serine

- Copy the FASTA sequence on the Protein Data Bank (PDB).
- Open up a new tab, and go to the website for SWISS MODEL (`https://swissmodel.expasy.org/`).
- Click on Start Modelling, and paste the FASTA sequence onto the "Target Sequence(s)" section.
- Name this project `p1_6ZCT` and begin building the model. This process will take around 10 to 15 minutes.

(c) Once your model for 6ZCT is built, click on the camera icon on the bottom right of the image. Under resolution, click extreme, and a png file should be downloaded to your computer. Upload this image at `https://forms.gle/mGPWYz1sgRnMY8JaA`.

Let's investigate another protein subunit called 7K3N. Using your knowledge about homology modeling from the 6ZCT subunit, answer the following questions about 7K3N.

(d) What nonstructural protein (nsp) is the 7K3N subunit found in?

Nonstructural protein 1 (nsp1)

(e) How many amino acids is the 7K3N subunit? What is the 1-letter abbreviation and full name of the fifth amino acid?

180 amino acids.
V = Valine

(f) Use SWISS Model to build a model for the 7K3N subunit. Name this project `p2_7K3N`, and upload your picture to the same link (`https://forms.gle/mGPWYz1sgRnMY8JaA`).

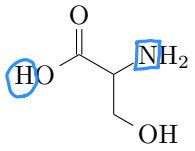
(g) What is the function of the nonstructural protein that corresponds to 7K3N?

It is a "host translation inhibitor," meaning that it will prevent transcription from taking place in the host cell. This will ultimately prevent the host cell from producing proteins that are necessary for it to do its job.

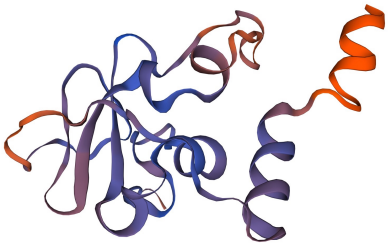
(h) Suppose you are running a Western blot experiment to compare the structures of 6ZCT and 7K3N. Which of these two subunits would travel further down the gel? Explain.

The 6ZCT subunit would travel further because it contains less amino acids and is therefore smaller.

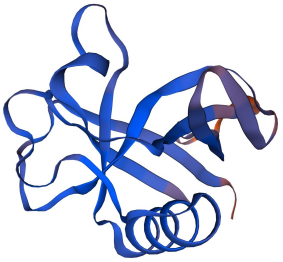
(i) The following figure shows the chemical structure of *serine*, an amino acid found in many proteins. Circle the most acidic hydrogen, and box the most basic atom in the molecule.



(c)



(f)



Problem 3.

Tsukemen is a popular Japanese dish comprised of ramen noodles and an excess of heavily concentrated broth. These components are kept in separate bowls, as the broth is used for dipping (almost like a sauce). After the noodles are consumed, there should still be a significant amount of broth left over. The server would dilute the leftover broth with a lightly concentrated solution to turn it into soup. This last step can be repeated multiple times. To make this problem simple, however, let's assume that the lightly concentrated solution is infinitely dilute. In other words, it's just water.

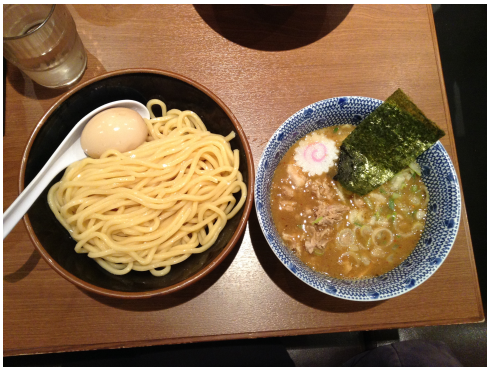


Figure 1. Getting hungry yet?

(a) Suppose you take your friends to a popular Tsukemen restaurant in Sawtelle. The server starts you off with γ_0 mL of broth with concentration C_0. After using up γ_1 mL of the broth, you decide to ask the waiter to fill your bowl back up with the "lightly concentrated" soup base. *What is the concentration of your soup after the first dilution?*

Let's start off with the dilution equation, defined by equation (1).

$$M_1 V_1 \equiv M_2 V_2 \tag{1}$$

We know the original concentration is C_0. Thus,

$$M_1 = C_0 \tag{2}$$

Since the dilution doesn't happen after you consume γ_1 mL of the original γ_0 mL of broth, the "original" volume is given by:

$$V_1 = \gamma_0 - \gamma_1 \tag{3}$$

Since the waiter fills your bowl back up with the soup base, the final concentration is given by:

$$V_2 = \gamma_0 \tag{4}$$

Continued from previous page...

Combining equations (1) thru (4) and solving for M_2:

$$C_0(\gamma_0 - \gamma_1) = M_2\gamma_0$$

$$M_2 = \frac{C_0(\gamma_0 - \gamma_1)}{\gamma_0} \quad \text{or} \quad C_0 - \frac{C_0\gamma_1}{\gamma_0} \qquad (5)$$

(b) Let's say that you consumed another γ_1 mL of the soup and ask for another refill. *What is the concentration of your soup after the second dilution?*

Let's redefine M_1 and M_2. Let our new M_1 be M_2 from part (a), as given by equation (5), and let M_2 be the concentration we are solving for. V_1 and V_2 should remain the same. Thus,

$$M_1 = \frac{C_0(\gamma_0 - \gamma_1)}{\gamma_0} \qquad (6)$$

Combining equations (1), (3), (4), and (6):

$$\frac{C_0(\gamma_0 - \gamma_1)}{\gamma_0}(\gamma_0 - \gamma_1) = M_2(\gamma_0)$$

$$M_2 = \frac{C_0(\gamma_0 - \gamma_1)^2}{\gamma_0^2} \qquad (7)$$

(c) Assuming you repeated this process many more times, *what would the concentration of your soup be after the nth dilution?*

$$M_n = \frac{C_0(\gamma_0 - \gamma_1)^n}{\gamma_0^n} \qquad (8)$$

Problem 4.

You are hired as an "imagineer" for Disney Parks and Resorts®, and your first job is to create a standard curve to optimize the taste and appearance of their blue milk, a popular drink sold in Star Wars Galaxy's Edge. After running some spectrophotometric experiments, you have received the following data about the relationship between concentration and absorbance.

Concentration	Absorbance
0.125 M	0.22
0.248 M	0.49
0.510 M	0.73

(a) Using a linear regression calculator, find the best-fit curve of the data shown above, and write the equation below. Round the slope to four decimal places, and round the y-intercept to the nearest whole number.

$A = 1.2605C$

(b) Assuming the molar absorptivity constant for blue milk is ε_{blue}, what was the length of the cuvette you used to obtain this data?

$A \equiv \varepsilon_{blue} \ell C$ (from Beer's Law)

$\therefore \quad \varepsilon_{blue} \ell = 1.2605$

$\therefore \ell = \frac{1}{\varepsilon_{blue}} (1.2605)$

(c) Given the following relationship between absorbance (A) and transmittance (T), derive an equation for transmittance in terms of the concentration C. Let $0 < T < 1$.

$$A \equiv -\log(T)$$

$A = 1.2605C$

$A = -\log(T)$

$\log(T) = -1.2605C$

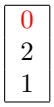
$\therefore \quad T = 10^{-1.2605C}$

Problem 5.

(a) You are using a P20 μ-pipette to draw up a PBS solution for cell culture. The volume-display window appears as follows. How many μL of the solution would be pipetted at this setting?

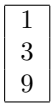
34 µL

(b) You are using a P1000 μ-pipette to draw up DMEM for cell culture. The volume-display window appears as follows. How many mL of the solution would be pipetted at this setting?



210 µL = 0.21 mL

(c) You are using a P200 μ-pipette to draw up ethyl acetate, a common organic solvent. The volume-display window appears as follows. How many μL of the solution would be pipetted at this setting?



139 µL

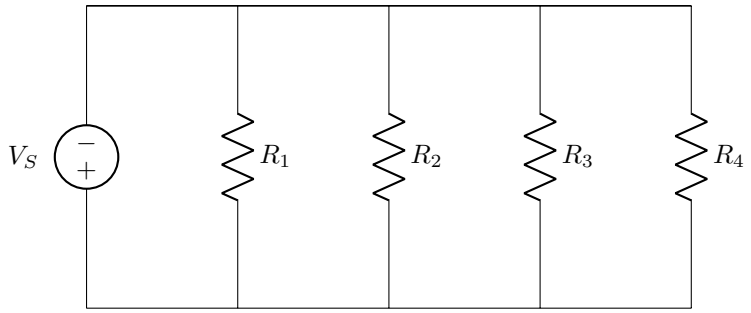
(d) Polyethylene glycol (PEG) is a polymer known for its unusually high viscosity. Because of this, you had a hard time pipetting it with a standard μ-pipette. What could you do to make it easier to pipette, while maintaining the same precision?

(Note: You cannot use a larger pipette because that would alter the precision.)

The pipette tip may be too small. You can use scissors to cut off the tip of the pipette tip. The precision won't be altered since you are still using the same pipette.

Problem 6.

The process of gel electrophoresis can be thought of as a circuit comprised of resistors in parallel, as shown by the schematic below. V_S represents the value for a DC voltage source, while R_x represents the different resistances of each well.

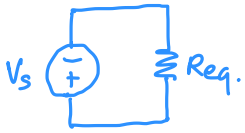


(a) Using an online search engine, look up the formula for equivalent resistance for resistors in parallel. Write the formula in the box below, and use that formula to derive the equivalent resistance of R_1 to R_4 in parallel. Also, draw a simplified schematic using the equivalent resistor.

$$\frac{1}{R_{eq}} = \sum_{n=1}^{N} \frac{1}{R_n} = \frac{1}{R_1} + \frac{1}{R_2} + \cdots + \frac{1}{R_N}$$

$$\frac{1}{R_{eq}} = \frac{1}{R_1} + \frac{1}{R_2} + \frac{1}{R_3} + \frac{1}{R_4}$$

$$R_{eq} = \frac{1}{\frac{1}{R_1} + \frac{1}{R_2} + \frac{1}{R_3} + \frac{1}{R_4}}$$



(b) Ohm's Law is arguably the most fundamental equation of circuitry and electronics. It is defined by the following equation, and it relates the voltage drop across a resistor to the current flowing through it.

$$V_{ab} \equiv IR$$

On the original circuit, what is the current I_3 running through the resistor R_3? On the simplified circuit you drew in part (a), what is the current I_{eq} running through the equivalent resistor R_{eq}? Please express your answers in terms of V_S and R_1 through R_4.

By definition of parallel resistors, the voltage difference of all resistors is the same as that of the voltage source. Thus,

$$V_S = I_3 R_3$$

$$I_3 = \frac{V_S}{R_3}$$

On the simplified circuit,

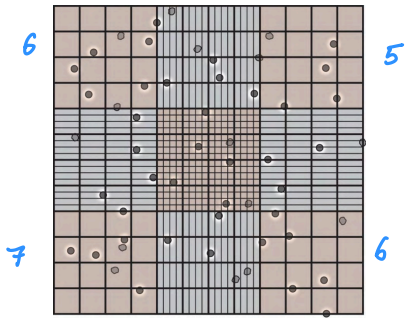
$$V_S = I_{eq} R_{eq}$$

$$I_{eq} = \frac{V_S}{R_{eq}}$$

$$I_{eq} = V_S \left(\frac{1}{R_1} + \frac{1}{R_2} + \frac{1}{R_3} + \frac{1}{R_4} \right)$$

Problem 7.

Suppose you are running an experiment with Human Aortic Endothelial Cells (HAECs), and you want to find the cell concentration. Upon your first attempt in using the hemocytometer, you discovered that the cells were too concentrated, so you took a small sample of the cell solution and diluted it by a factor of 30. After re-examining the hemocytometer under a microscope, you obtain the following data.



(a) Estimate the cell concentration of the *original* cell solution.

$\bar{x} = \frac{6+6+5+7}{4} = 6$

Concentration $= \bar{x} \cdot 10^4 \cdot$ Dilution factor $= 180 \cdot 10^4$ cells/mL

(b) Suppose your friend from UC Berkeley works for a hemocytometer manufacturing company, and sent a prototype to your lab for testing. Unfortunately, he mistyped the dimensions of the hemocytometer when he was designing it. Instead of the quadrants measuring 1mm×1mm×0.1mm, they are now 4mm× 2mm × 0.3mm. Derive a formula to calculate the cell concentration using this new hemocytometer. If the above image (not drawn to scale) had these dimensions, what would the cell concentration of the *original* cell solution be?

Volume of 1 quadrant:

$V_{quad} = 4\text{mm} \cdot 2\text{mm} \cdot 0.3\text{mm} = 2.4\ \text{mm}^3$
$= 2.4 \times 10^{-3}$ mL

Thus,

$$\text{Concentration} = \left(\frac{\bar{x}}{2.4 \times 10^{-3}}\right) \cdot \text{Dilution Factor}$$

$$= \frac{6}{(2.4 \times 10^{-3})} \cdot 30 = 7.5 \cdot 10^4 \text{ cells/mL}$$

Problem 8.

Note: *Although this is a differential equations problem, I have designed it in such a way that prior experience isn't necessary. I have included hints and guidelines throughout all parts of this problem to help you out.*

The SIR model is a system of differential equations used to model viral spread. S stands for the number of individuals who are *susceptible* to a virus, I stands for the number of people who are *infected*, and R stands for the number of people who have *recovered*. Assuming there are no deaths or births throughout a given time-frame, we can define the total population N as:

$$N \equiv S + I + R \tag{1}$$

(a) Recall that rate of change is given by the derivative. In the box below, write a differential equation to model the rate of change for the number of people who are *susceptible* to the virus. Denote any constant multiplicative factors as β, and think carefully about whether the rate of change is positive or negative. Your equation should be in terms of S, I, R, t and β, but not all of these values need to be used. Write all derivatives in Leibniz's notation.

$$\frac{\partial S}{\partial t} = -\beta SI$$

(b) Now write a differential equation to model the rate of change for the number of people *infected* with the virus. Denote any *new* constant multiplicative factors as γ, and think carefully about how this equation relates to the equation you wrote in part (a). Also, pay special attention to the signs $(+/-)$ of each term. Your equation should be in terms of S, I, R, t, β, and γ, but not all of these values need to be used. Write all derivatives in Leibniz's notation.

$$\frac{\partial I}{\partial t} = +\beta SI - \gamma I$$

(c) Lastly, write a differential equation to model the rate of change for the number of people who *recovered* from the virus. Think carefully about how this equation relates to the equation you wrote in part (b), and pay attention to whether the rate of change is positive or negative. Your equation should be in terms of S, I, R, t, β, and γ, but not all of these values need to be used. Write all derivatives in Leibniz's notation.

$$\frac{\partial R}{\partial t} = +\gamma I$$

(d) What is the sum of the equations you wrote in parts (a) through (c). What does this tell you about the necessity of all three equations to solve this problem?

$$\frac{\partial S}{\partial t} + \frac{\partial I}{\partial t} + \frac{\partial R}{\partial t} = -\beta SI + \beta SI - \gamma I + \gamma I$$

$$\boxed{\frac{\partial S}{\partial t} + \frac{\partial I}{\partial t} + \frac{\partial R}{\partial t} = 0}$$

This tells you that only two of the three equations are linearly independent, and thus, only two of them are required to solve the problem.

(e) Let's simplify the system of differential equations you wrote in parts (a) through (c). First, we can write the derivative of I in terms of S. (If you haven't taken multivariate calculus yet, you can ignore the ∂ sign and replace it with d).

$$\left(\frac{\partial I}{\partial S}\right)$$

Since your differential equations should be time-dependent, let's write an equivalent form of the above equation by introducing t.

$$\left(\frac{\partial I}{\partial S}\right) = \left(\frac{\frac{\partial I}{\partial t}}{\frac{\partial S}{\partial t}}\right) \tag{2}$$

Substitute the equations you wrote for parts (a) and (b) into the right side of equation (2). Write the new differential equation below.

$$\left(\frac{\partial I}{\partial S}\right) = \frac{\left(\frac{\partial I}{\partial t}\right)}{\left(\frac{\partial S}{\partial t}\right)} = \frac{+\beta SI - \gamma I}{-\beta SI} \Rightarrow \boxed{\left(\frac{\partial I}{\partial S}\right) = \frac{\gamma}{\beta S} - 1}$$

(f) Using the method of separable equations you learned in the Module 1, solve the differential equation you wrote in part (e). Assume that at $t = 0$, the number of people infected is I_0.

$$\int \partial I = \int \left[\frac{\gamma}{\beta S} - 1\right] \partial S$$

$$I = \frac{\gamma}{\beta}\int \frac{1}{S}\,\partial S - \int \partial S$$

$$I = \frac{\gamma}{\beta}\ln(S) - S + C$$

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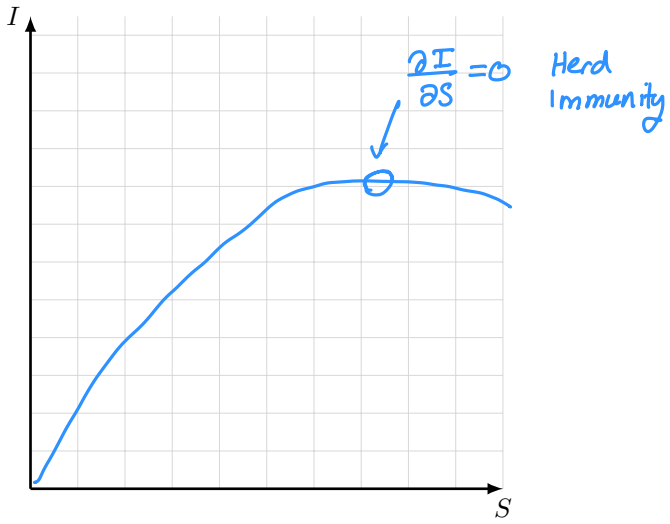
Applying the initial condition,

$$I = \frac{\gamma}{\beta} \ln(S) - S + I_0$$

(g) Graph your solution to part (f) below. Since this is a non-numerical problem, we are more concerned with the behaviour of the graph than the accuracy.



(h) On the your graph above, circle the point that corresponds to herd immunity. This is the point where enough people have recovered from a particular virus to effectively end an epidemic.

(i) Throughout the graph, what is the sign of the second derivative?

$$\left(\frac{\partial^2 I}{\partial S^2}\right)$$

Negative = Concave down.

Problem 9.

You are working in a tissue engineering laboratory to develop a treatment for Achilles tendon injuries. Your goal is to deliver tendon stem/progenitor cells (TSPCs) to the site of injury and support proliferation of the seeded TSPCs.

Your collaborators have suggested that you experiment with Pluronic as the TSPC cell carrier. Pluronic is a thermosensitive hydrogel that is liquid at room temperature and gelates at body temperature. Your collaborators hypothesize that injecting liquid Pluronic with TSPCs and growth factors will generate a suitable environment for the elongation and maturation of TSPCs.

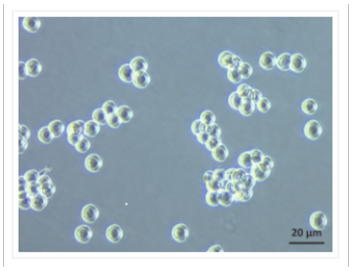
(a) How would you determine if the TSPCs are surviving within the hydrogel?

You can conduct a live-dead stain on the hydrogel and visualize it under a fluorescent microscope.

(b) How would you assess the elongation of TSPCs within the hydrogel?

ImageJ analysis of fluorescent images.

Suppose your friend from USC was performing a cell culture experiment with an epithelial cell line. When you checked her cells yesterday, they appeared to be fine. However, when she spilt the cells again today, they appeared as follows:



(c) What mistake did she make when splitting the cells?

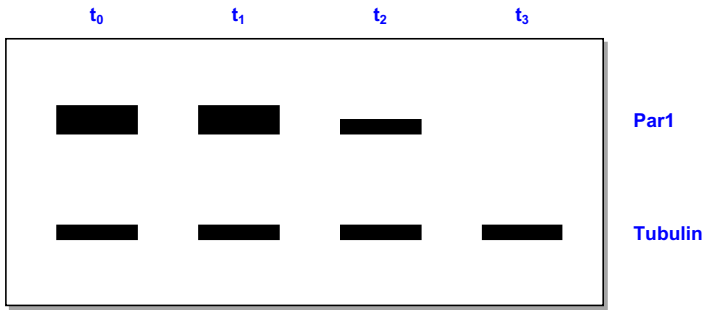
The cells were exposed to trypsin for a long time. Trypsin detaches cells from the surface by destroying membrane-bound proteins. In this image, the cells seem to be floating around in media.

Problem 10.

You are investigating the *Par1* gene, which you believe plays a major role in the embryonic development of mice. In your experiment, you isolate protein samples from the gene at four different time-points, as indicated below. Note that dpf stands for *days post fertilization*.

t_0	Early Embryonic Stage (7 dpf)
t_1	Late Embryonic Stage (14 dpf)
t_2	Newborn
t_3	Adults (3 months old)

Then, you perform a Western Blot to analyze your results. In addition to taking samples of the Par1 protein, you isolate samples of tubulin to run alongside your Par1 blot. Tubulin is a protein that plays a role in maintaining the structural integrity of cells; it is found at constant levels throughout the developmental process. The following diagram shows the results of your experiment.



(a) What was the purpose of running tubulin alongside Par1 in your Western Blot?

Tubulin serves as the control group to ensure the Blot is running correctly. It is a good control group because it is expressed across all developmental stages. This allows us to use it as a reference to compare with the expression of Par1 protein.

(b) Based on your experimental results, do you think the *Par1* gene is expressed during the embryonic stages?

Yes, it is prevalent at times t_0 and t_1. At the newborn stage (t_2), it begins to fade out. It is not present in adult mice (t_3).

(c) Based on your experimental results, do you think the *Par1* gene is necessary for adult mice?

It is <u>not</u> necessary for adult mice since it is not expressed.