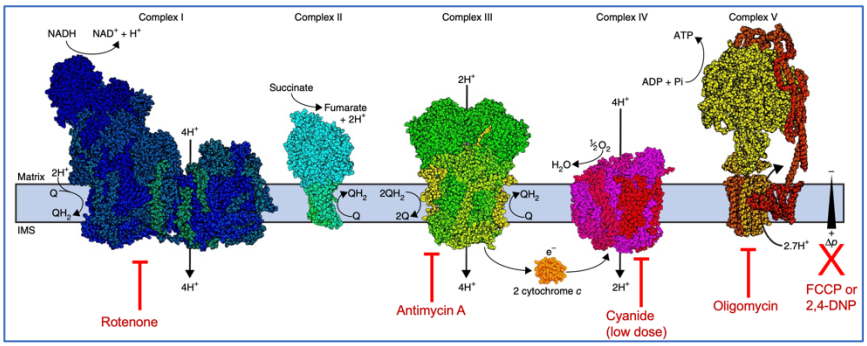
CH-313 (Autumn 2023: Instructor: Prof. Yimon Aye; PhD-student teaching fellow: Song Chen)
In-class Problem Set (Lecture 13-14) **There are 3 problems in total but we will only take the best-scoring two!**

Q1. Concepts and tools assessing mitochondrial function

(i) The following trace is obtained by measuring oxygen consumption rate (OCR) over time, in healthy cells in live culture (note: *not* in isolated mitochondria). Match X, Y, Z to the correct small-molecule(s) shown. Where none of the X, Y, Z is applicable, write 'n/a' in the blank. (**12 points**). **Note:** Figure shown may be of help to understand the function of the various small-molecules used.



Important note: after addition of X as shown, Y was added while X was still present in the system; however, both X and Y were washed out (i.e., removed from the system) before Z was added.

Glucose _____

Rotenone + Antimycin A _____

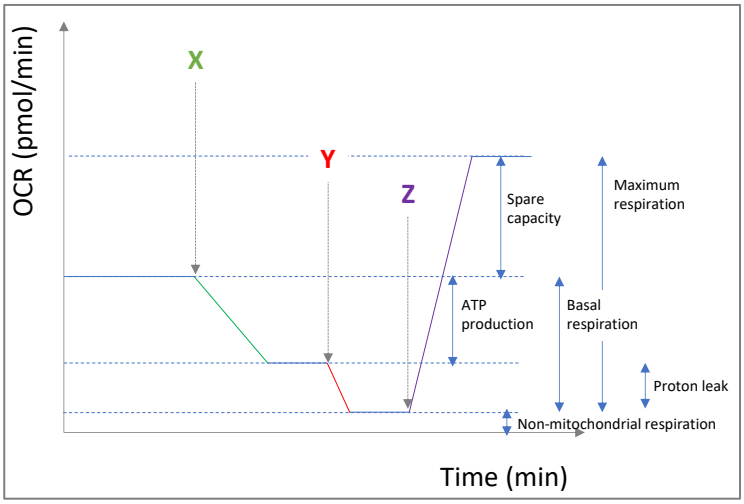
Oligomycin _____

FCCP or 2,4-DNP _____

2-Deoxyglucose _____

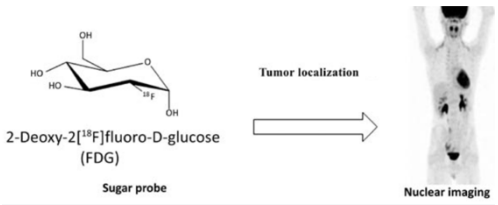
(note: 2-Deoxyglucose, a non-oxidizable glucose analog, inhibits glycolysis)

Cyanide (low dose) _____



(ii) If similar analysis as above were to be performed on cancer cells, what feature(s), *if any*, in this plot above would you expect to be different? Give a brief reason for your answer.

(iii) ^{18}F-FDG (see structure shown below) has revolutionized the Positron Emission Tomography (PET) imaging in cancer treatment in humans. Following intravenous injection of a small amount of ^{18}F-FDG, the PET scanner rotates around the relevant regions of the human body to capture locales of malignant tumor cells.



-Why do these cells show up brighter in the PET images than normal healthy cells? A brief phrase/text in your answer is sufficient.

-What is this phenomenon generally known as?

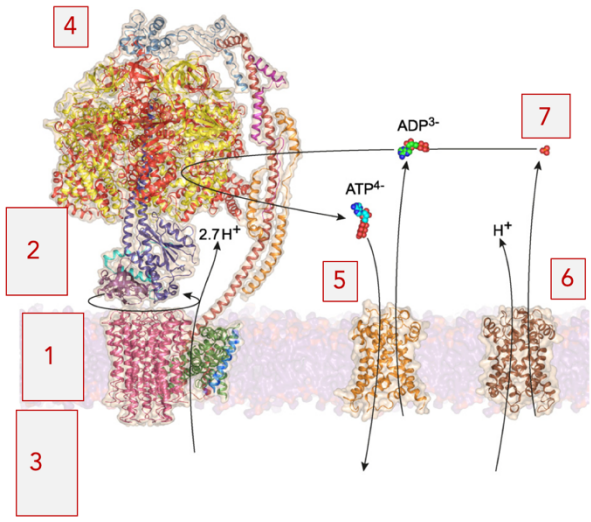
-What is the generally-accepted underlying molecular reason behind this phenomenon? A short phrase/text in your answer is sufficient.

Q2. This problem concerns chemical biology of ATP synthesis

(i) Provide the missing labels in the shown figure.

Note: The first answer is given for you.

1. Mitochondrial inner membrane
2. _____
3. _____
4. _____
5. _____
6. _____
7. _____

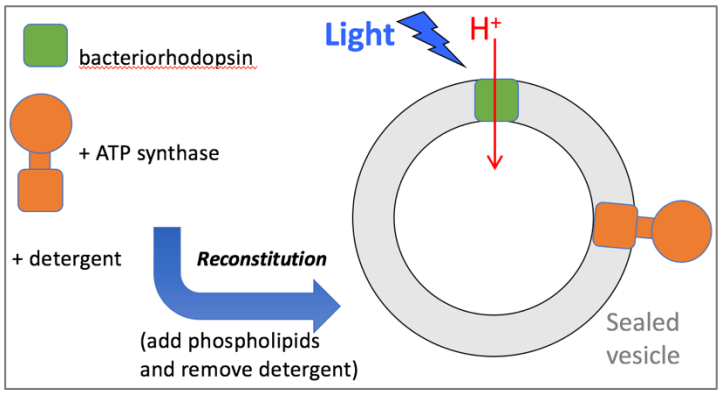


Early work that laid the grounds for ultimately revealing how ATP synthase works…

The 1997 Nobel prize in Chemistry recognizes the contributions made toward the biochemical understanding of ATP-synthesis machine, so-called 'ATP synthase'. In 1960, Efraim Racker and co-workers, albeit not the winners of this Nobel prize, isolated for the first time an enzyme from mitochondria which we now call ATP synthase. This problem takes you through some of the foundational studies from the Racker laboratory in their attempt to better understand how ATP synthesis ultimately occurs in mammalian mitochondria.

The Racker lab reconstituted into the membranes of the same vesicles, purified bacteriorhodopsin (which is a light-driven proton pump from a photosynthetic bacterium) and purified ATP synthase (from ox heart mitochondria). Assume that all molecules of bacteriorhodopsin and ATP synthase are oriented as shown in Figure, so that protons are pumped into the vesicle and ATP synthesis occurs on the outer surface.

(i) Draw out, using the existing figure, to show the anticipated outcome following addition of ADP and phosphate to the *external* medium and shining light into the suspension of vesicles.



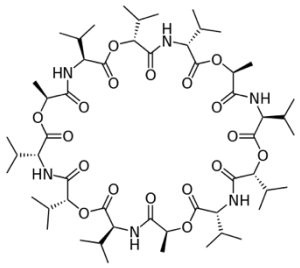
(ii) What would happen to this outcome if the vesicles were prepared without being careful to remove all the detergent (which makes the bilayer leaky to protons)?

(iii) If the ATP synthase molecules were randomly oriented so that about 50% faced the outside of the vesicle and 50% faced the inside, what would happen to this outcome?

If the bacteriorhodopsin molecules were randomly oriented, what outcome would you expect?

(iv) In fewer than 2 sentences, comment (praise or criticize) on the validity of this experiment that utilizes components from widely-divergent unrelated organisms.

Q3. This problem concerns mitochondrial electrochemical gradient Treatment of valinomycin renders the inner membrane (IM) of the mitochondria permeable to K^+ ions (which are abundant in the cytosol). Given the chemical structure of valinomycin below and considering in-class discussions on mitochondria function as well as the Figure in Part A above, explain, ***in 3 sentences***, how might valinomycin serve to make IM permeable to K^+?



For full credit, your answers must integrate the three following terms:
<<*hydrophobicity/hydrophobic*, *intermembrane space*, *matrix*>>

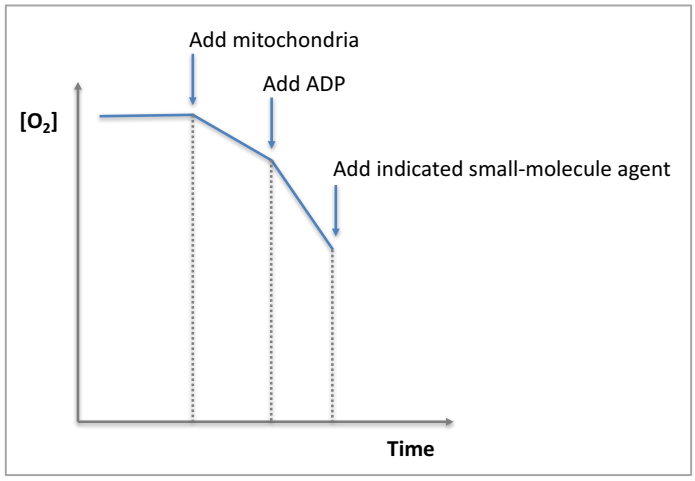
(ii) Based on Figure in Part A and in-class discussions, the two components of "proton-motive force" are:

_____ and _____

(iii) What would happen to ATP synthesis as a result of valinomycin treatment? Circle the correct answer:

Blocked Slowed No Change

(iv) The plot to the right shows time-dependent oxygen consumption by isolated mitochondria in a potassium phosphate-buffered solution containing succinate as the sole source of electrons for the ETC (electron transport chain). After a short interval, ADP was added followed by a small-molecule agent and the rates of oxygen consumption were measured by the respective slope.



In the plot below, draw out the expected slope if the small-molecule agent is:

a) Valinomycin. Label your line clearly as V.
b) W such that W renders IM permeable to H^+. Label your line clearly as W.
c) X such that X inhibits the ADP/ATP transporter. Label your line clearly as X.
d) Y such that Y inhibits ATP synthase. Label your line clearly as Y.
e) Z such that Z inhibits the enzyme that converts succinate to fumarate (see Figure in Problem 1 above). Label your line clearly as Z.

(v) Proton-motive force (also known as electrochemical gradient) is also used to transport metabolites across the IM. Based on Figure in Problem 1 (previous page) and in-class discussions, circle the letter(s) corresponding to cases where metabolite transport occurs along with the proton-motive force. ***There may be more than one correct letter.***

A. Antiport of aspartate and glutamate (where aspartate enters matrix while Glutamate exits matrix)
B. Symport of hydrogen phosphate (HPO_4^{2-}) and $2H^+$, into matrix
C. Symport of pyruvate anion and H^+, into matrix
D. Antiport of citrulline and ornithine [where citrulline (neutural metabolite) exits matrix while ornithine (positively-charged metabolite) enters matrix]