Photosynthesis (SC/BIOL 4061) First Term Test (17 Oct 2011)

Answer four of the following five questions in the exam booklet provided.
When finished, please insert the question sheet and your crib sheet(s) in the exam booklet (all will be returned to you after grading). You should be able to answer each question on one to two pages. Excessive length is not encouraged.

Question One

There is both direct and indirect evidence for 'primordial' photosynthesis. Isotopic carbon discrimination is one example of evidence. Explain why it is used (and its interpretation). Knowing what you know about the effect of nuclear weapons testing and power plant accidents on carbon isotopic distributions, could it still be used today? Why?

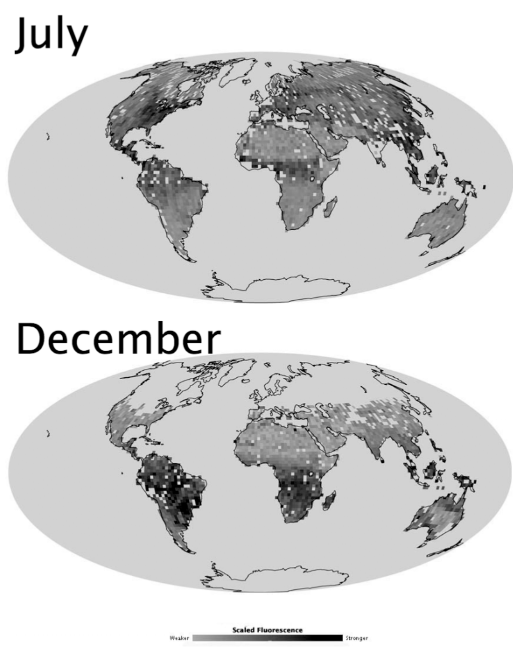
Question Two

Three thousand million years after the advent of oxygenic photosynthesis, earth is clearly out of equilibrium: dangerously high levels of oxygen (O_2) in the atmosphere and barely sufficient levels of carbon dioxide (CO_2). Would similar dis-equilibria have occurred if only anoxygenic photosynthesis had been occurring for the past three thousand million years? What would the dis-equilibria be? Explain.

Question Three

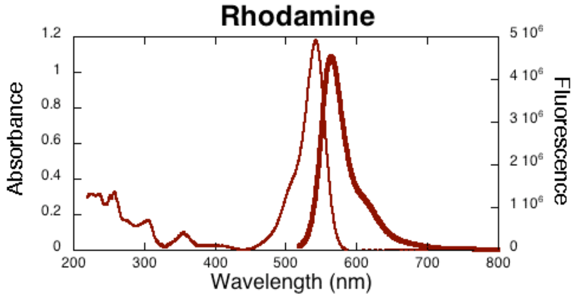
When a photon is 'slowed' as it passes through a medium like H_2O or flint glass, does its energy change? Explain.

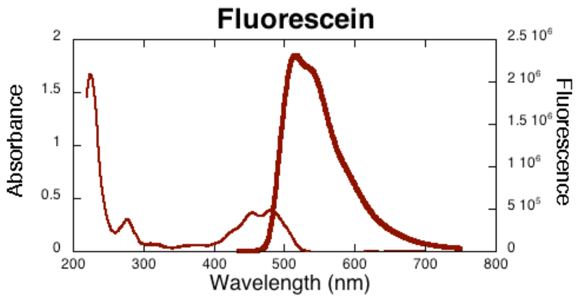
Question Four

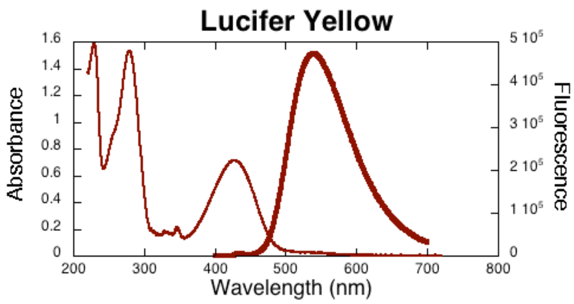


Shown is global fluorescence due to photosynthesis during July and December. Explain why global fluorescence could be used to evaluate photosynthetic activity and how it relates to photosynthetic productivity.

(*nota bene* Question Five overleaf)







Question FIve

Excitation (narrow lines) and emission (thick lines) spectra are shown for three common fluorochromes. Construct a model of how they would be utilized in a light-harvesting scheme with a clear explanation of your reasons for the scheme you propose.

Finis!

KEY — Term Test One (17 October 2011)[1]

Question One: There are three common isotopes of Carbon: ^{12}C (99%), ^{13}C (1%) and ^{14}C (negligible). Because of its smaller size, enzymes discriminate in favor of ^{12}C (known as the kinetic isotopic effect) (25%). Therefore $^{12}C/^{13}C$ discrimination in 'ancient' carbon material can be used as indirect evidence for CO_2 fixation in the dark reactions of photosynthesis (25%). It does not provide evidence for whether the photosynthesis was anoxygenic or oxygenic (both will fix CO_2) (25%). Nuclear weapons (and power plant accidents) have elevated ^{14}C. While this impacts on ^{14}C carbon dating, it won't affect $^{12}C/^{13}C$ discrimination because: 1) ^{12}C and ^{13}C levels are unaffected; 2) ^{12}C and ^{13}C levels are much higher than ^{14}C; 3) old inorganic material is used for standards for mass spectrometric measurements of $^{12}C/^{13}C$ discrimination and won't be affected by ^{14}C elevation in the atmosphere. (25%)	
Question Two: The oxygenic disequilibria arise from the light reactions (H_2O + light —> O_2) and the dark reactions ($CO_2 + H_2O$ + ATP + NADPH —> $(CH_2O)_n$ + ADP + NADP) (25%). The second reaction (CO_2 depletion) will certainly occur in an 'anoxygenic' world (25%). For the first, instead of H_2O —> O_2, anoxygenic photosynthesizers tend to use H_2S —> S_2 (25%). H_2S is nowhere near as prevalent as H_2O, resulting in significant H_2S depletion early on. Thus, low CO_2 likely, H_2S starvation very likely (25%).	
Question Three: When velocity is slowed, the wavelength also changes so that the frequency is unchanged. $E = h\nu$, where ν is the frequency ($\nu=\upsilon/\lambda$, velocity/wavelength). Thus photon energy will be unchanged (100%).	
Question Four: Fluorescence arises from two major causes. One is prompt fluorescence (the exciton never gets used in photochemistry) (25%), the other is delayed (photochemistry is reversed and releases a photon) (25%). Fluorescence increases as photosynthetic activity increases. So, on the one hand, it is a direct measure of photosynthetic activity (25%). On the other hand, anything that adversely affects the reactions centers will cause higher fluorescence, so it is also a measure of photosynthetic inefficiency (25%).	
Question Five: Exciton transfer would follow the sequence of longer absorption wavelengths (Pigment$_{absorbance}^{emission}$): Lucifer Yellow$_{200–300}^{450–550}$ —> Fluorescein$_{400–500}^{500–600}$ —> Rhodamine$_{500–575}^{550–600}$ (75%) This sequence would enhance the ability of the light-harvesting complex to utilize UV light due to Lucifer Yellow, but the Fluorescein$_{400–500}^{500–600}$ —> Rhodamine$_{500–575}^{550–600}$ would 'trap a much larger number of photons (25%).	

[1] 25 points per question (4/5 questions).

Photosynthesis (SC/BIOL 4061) Second Term Test (14 Nov 2011)

Answer the following four questions in the exam booklet provided. When finished, please insert the question sheet and your crib sheet(s) in the exam booklet (all will be returned to you after grading). You should be able to answer each question on one to two pages. Excessive length is not encouraged.

Question One

Describe the mechanism(s) used in higher plant photosynthesis that balance photonic energy between 1) NADP reduction to NADPH and 2) ATP synthesis.

Question Two

In the context of electron transport within reaction centers of photosynthesis, describe 3 examples of chemically distinct compounds/mechanisms that mediate charge separation.

Question Three

What are the 'red drop' and red light enhancement? Explain why they provide evidence for two reaction centers in higher eukaryotic photosynthesis.

Question Four

Describe light-induced pH changes. At alkaline pH, some redox reactions will be affected, and the standard Gibbs free energy for ATP synthesis is significantly higher. Explain why. How does this affect ATP synthesis in the chloroplast?

KEY — Term Test Two (14 November 2011)[2]

Question One: Within the Z-scheme, we must differentiate between cyclic (ATP production) (PS I, cyt b_6f and PQ) and non-cyclic (NADP reduction and ATP production) (PSII, cyt b_6f, PS I, and PQ). PQ functions in both and regulates the balance based on its redox poise. If PQH_2 is high, that means that NADP is unavailable for reduction so there is a shift to cyclic photosynthesis (ATP production) as a consequence of LHC phosphorylation and unstacking of the grana.	
Question Two: Within the reaction center itself, the major sites of charge separation begin with the shift in midpoint potential of the exciton (P_{680} or P_{700}). Phaeophytin transiently accepts the e^- from $P_{680\text{ or }700}$. Quinone then transiently accepts the e^- from phaeophytin. In its positively charged state, the reaction center P_{680} separates e^- from the Mn complex in the water splitting complex via transfers from tyrosines and hisitidines.	
Question Three: A diagrammatic representation of the difference in absorbance and O_2 production shows that quantum efficiency declines mysteriously at about 700 nm. This is the red drop. Red light enhancement is the synergistic increase in photosynthetic activity by 680 and 700 nm light. The best explanation is that two photosystems work together, the PSII and PS I. This was confirmed by monitoring the effect of different wavelengths on cytochrome oxidation.	
Question Four: Redox reactions that involve both e^- and H^+ give rise to the delta pH across the thylakoid membrane. The stromal low [H^+] makes the process more difficult, since $2H^+ + 2e^- + NADP \longleftrightarrow NADPH + H^+$ will be more difficult if H^+ substrates are lower, also true for $2H^+ + 2e^- + PQ \longleftrightarrow PQH_2$. The pH dependence of the standard Gibbs free energy will increase the energetic barrier to ATP synthesis, working against the vectorial delta μ^{H+}.	

[2] The four questions were weighted equally. Partial credit was given, as deemed appropriate.

Photosynthesis (SC/BIOL 4061) Final Exam (16 Dec 2011)

Answer FIVE of the following SEVEN questions in the exam booklet provided.

When finished, please insert the question sheet and your crib sheet(s) in the exam booklet. You should be able to answer each question on one to two pages. Excessive length is not encouraged.

Please note that the answers are abbreviated and without diagrams, and thus represents the 'general' answer, but not the detailed one.

Question One

Explain the mechanism of fluorescence and describe the fates of an exciton. Mg-complexed porphyrins (chlorophylls) have two absorption bands (in the blue and near-red). Explain why selective pressure has maintained chlorophyll as the dominant photosynthetic pigment for 4000 million years? Would there be any disadvantage to a 'black' pigment (that absorbed all wavelengths in the visible part of the spectrum)? Why?

Absorption of photons with an energy matching an electron orbital transition results in a 'excited' electron in a higher energy orbital. When the exciton relaxes to the ground state, a photon is emitted, with an energy lower than the absorbed photon. This is fluorescence, and a fast process (pico to nanoseconds). In photosynthesis, fluorescence is wasted energy (not used for 'work'). Besides fluorescence, the fates of an exciton include 1) transfer to another pigment, 2) radiation-less relaxation to the ground state, 3) triplet formation and phosphorescence, and 4) photochemistry. Chlorophylls have two absorption bands, both can be used in photochemistry. Two bands are advantageous in that both blue and red portions of the solar spectrum can be used at lower cost (1 biochemically expensive pigment performs two functions). A black absorbing pigment would be even better than chlorophyll in this sense —capturing all useful wavelengths of solar radiation. The biochemical building blocks for such a black pigment have never evolved. A potential drawback is heat production with such a high efficiency photon-energy-trapper'.

Question Two

As a newly-minted BioEngineer, your goal is to improve on the 'dated' Z-scheme of photosynthesis. Propose a novel electron (and proton) transport chain mechanism that would increase the efficiency of ATP and NADPH production.

You need an electron source, so PS II and the water-splitting enzyme have to be retained. One could envision multiple PS I – like photosystems that extend out to longer wavelengths (similar to the bacteriochlorophylls of the anoxygenic bacteria). These could be utilized to maximize the electrochemical proton gradient and thereby ATP synthesis. Bifurcation of the "PS II" to either NADP reduction or the "PS I – like" reaction centers (and thus ATP production) would control for the relative requirements for NADPH and ATP in photosynthesis and other biochemical requirements in the cell. The likely drawback is the biochemical 'cost' of producing multiple PS I – like photosystems. Note that this alone would not affect NADPH production, limited by the electron source, water. For this, an alternative electron donor system would have to be constructed. Possible electron donors include H2S and H2CO3.

Question Three

Diagram and explain the major structural components of the ATP synthetase. Why does the ATP synthetase use hydronium ions (H_3O^+) rather than protons (H^+)?

Alpha, beta, gamma, delta and epsilon make up the CF1 component, a, b, and c make up the CF0 proton channel. A mix of three sites that bind ADP and Pi or ATP either tightly or loosely are found in the alpha and beta structure, rotation of the complex causes conformational changes that result in net ATP production, mediated by the gamma subunit. Evidence for hydronium ions comes from known Na+ gradient-utilizing bacteria. The hydronium ion would have a size similar to Na+. Unlike H+, it could not participate in proton wires via protonation/de-protonation of carboxyls and amines.

Question Four

Describe the activation and enzymatic mechanism of RuBisCO.

The light-activated ‘activase’ binds RuBP and CO_2 to the active site to cause activation of RuBisCO. The carbamylated active site then adds a CO_2 to the 5-C RuBP to form a 6-C intermediate, followed by hydrolysis to two 3-C phosphoglycerates. Replacing the CO_2 with O_2 results in one phosphoglycerate and a glycolate (2-C) with no net carbon assimilation.

Question Five

If two plants —one C3, the other C4— are placed in a closed system (with ample light, water, etcetera), the C4 plant will eat the C3 plant. Explain why.

Two things are ‘in play’. First the C4 PEPCase has an effective compensation point (for CO_2) of zero because it uses HCO_3^- as the substrate. By comparison, the C3 RuBisCO has a compensation point of about 40 ppm CO_2 and thus C4 is a more efficient scavenger of CO_2. Second, elevated oxygen in the closed system will result in more oxygensase activity of the C3 RuBisCO, hence photorespiration and CO_2 release! In addition, to survive, the C3 will respire, releasing CO_2 that is then used by the C4: thus cannibal activity is occurring!

Question Six

Explain what is meant by DIC (dissolved inorganic carbon), including the relation between pH and the various inorganic carbon species.

Dissolved CO_2 is converted to bicarbonate (H_2O + CO_2 yields H^+ plus HCO_3^-, pK about 6.4), and to carbonate at a more alkaline pH (pK of about 10.4). The sum of CO_2, bicarbonate and carbonate is the DIC. At alkaline pH, the DIC can be very high. In oceans, much is precipitated out with Mg^{2+} and Ca^{2+}. In a single cell: if the cytoplasmic pH is more alkaline than the extracellular pH, CO_2 can diffuse in, be converted to bicarbonate, resulting in an accumulation of DIC within the cell, supporting CO_2 fixation in photosynthesis.

Question Seven

What is a ‘flux control coefficient’? Besides enzymes, there are other elements that can affect total photosynthetic activity. Nitrogen assimilation is one. Suggest another one, explain why.

The flux control coefficient is the slope of Ps activity versus amount of enzymatic activity. It is an effective way to determine how much control a single enzyme confers on a complicated pathway. Its major usage is in bioengineering in a systems context: what enzyme activities can be changed to increase total Ps throughput? Some major elements that affect photosynthesis are principally those that affect the extent of export from the photosynthetic tissue. Phosphate exerts such control. Alternatively, one can think of more global elements, such as sulfate, which use reducing equivalents from photosynthesis to be reduced to sulfur and sulfhydryls, and are required as a minor building block of many components of the electron transport chain and in proteins.