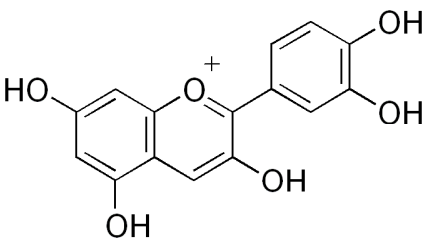
Photosynthesis (SC/BIOL 4061) First Term Test (8 Oct 2008)

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 are three types of evidence for 'primordial' photosynthesis. Some are consistent with the presence of photosynthesis (either anoxygenic or oxygenic). Some are consistent with oxygenic photosynthesis. Describe the three lines of evidence and why they support the 'primordial' presence of either anoxygenic and/or oxygenic photosynthesis.

Question Two
Describe two distinct examples of proteobacterial photosynthesizers that exhibit unusual pathways of carbon dioxide fixation, emphasizing their unique photosynthetic properties compared to oxygenic photosynthesizers, such as cyanobacteria, algae and higher plants. A typical e^- donor in proteobacterial groups is H_2S. Predict the S–H bond energy, compared to the O–H bond energy of H_2O (460 kJoules mol^{-1}).

Question Three


The chemical structure is cyanidin, an example of an anthocyanin. Predict its absorption spectrum (give the reason(s) for your prediction). Although it has anti-oxidative properties, it is not used in photosynthesis, and certainly not in photochemistry. Why isn't it used in photochemistry?

Question Four
Oxidative damage, and protection from oxidative damage, is now recognized as extraordinarily important, not just in chloroplasts but in mitochondria as well. Describe the causes of oxidative damage and two protective mechanisms involving carotenoids.

Question Five
Explain why light-harvesting complexes would play a central role in controlling thylakoid architecture and shunting of excitons to either non-cyclic and/or cyclic photosynthesis.

Question Six (extra credit)
What organism did Emerson and Arnold use in their oft-cited experiments establishing the concept of a light-harvesting antenna (genus and species please)?

KEY — Term Test One (8 October 2008)[1]

Question One: The layering of present-day stromatolites (uppermost layer: oxygenic cyanobacteria [$H_2O + CO_2 <–> (CH_2O)_n + O_2$] and facultative aerobes [$O_2 + (CH_2O)_n <–> CO_2 + H_2O$]; lowermost layer: anoxygenic proteobacteria [$H_2S + CO_2 <–> (CH_2O)_n + S$] and anaerobic heterotrophs [$(CH_2O)_n <–> CO_2 + EtOH$]) (25%) offers only indirect evidence at best for the appearance of oxygenic photosynthesis based solely on a correlate with expected evolution of O_2 producers from anoxygenic progenitors (8%). $^{12}C/^{13}C$ enrichment offers evidence of CO_2 fixation (25%), but are irrelevant to oxygenic/anoxygenic photosynthesis (8%). Deposits of oxidized iron ($Fe(II)_{water\ soluble} + O_2 –> Fe(III)_{water\ insoluble}$) (25%) offer the most direct evidence of oxygenic photosynthesis (8%).	
Question Two: Probably the best distinction is between green sulfur bacteria and green non-sulfur bacteria because of the use of the reverse Kreb Cycle ferredoxin as the reductant) in the former (which use H_2S as an e^- donor) (35%) and the hydroxypropionate pathway in the latter (which uses either H_2S or H_2 as an e^- donor) (35%). Other differences in preferred e– donor, variations in ATP and/or NADH production, and type of bacteriochlorophyll also exist. Bacteriochlorophyll absorbs at very long wavelengths (750–800 nm) that correspond to a low energy (about 150 kJoule mol^{-1}) compared to chlorophyll (680 nm / about 176 kJoule mol^{-1}), from which we could infer that the S–H bond of H_2S would have a lower bond energy than the O–H bond of H_2O (463 kJoule mol^{-1}) (I recollect published values of about 370 kJoule mol^{-1}) (30%).	
Question Three: With eight conjugated bonds, the A_{max} would be about 420 nm (50%). Photochemistry requires charge separation ($A + hv <–> A^* <–> A^+ + e^-$). With an existing positive charge on the molecule, the energetics of removing yet another electron would be prohibitive (50%).	
Question Four: Transfer of the chlorophyll triplet state to oxygen causes the formation of singlet state oxygen, capable of causing oxidative damage, most notably to components of the reaction center (30%). *Nota bene* Be careful to differentiate between singlet oxygen ($O_2 + Chl^T <–> {}^1O_2^{\bullet} + Chl$) and superoxide / perhydroxyl radical / hydrogen peroxide / hydroxyl radical / water ($e^- + O_2 <–> O_2^{\bullet -} + H^+ <–> HO_2^{\bullet -} + e^- + H^+ <–> H_2O_2 + e^- + H^+ <–> OH^{\bullet} + e^- + H^+ <–> H_2O$). Singlet oxygen is damaging, but does not cascade through the superoxide sequence of oxygen radicals. Carotenoids may quench the chlorophyll triplet state directly (30%), or, quench the singlet oxygen (30%). In either case, the resulting triplet state carotenoid dissipates the excited state as heat, returning to its ground state (10%).	
Question Five: Light harvesting complexes are the most efficient and direct way to allocate excitons to either PS I (cyclic and non-cyclic) or PS II (non-cyclic) (20%). Thus they have a key role in regulating the changes in granal architecture that allow either LHC to migrate (to PS I in the case of less granal stacking), or for LHC-PS II complexes to migrate (to PS I in the case of less granal stacking) (40%). The use of PQ/PQH_2 as the sensor of exciton allocation is sensible, since it effectively reports on the supply of reducing equivalents from PS II. At high PQH_2, more excitons must be allocated to PS I (40%) .	
Chlorella (0.5) pyrenoidosa (0.5)	

[1] 25 points per question (4/5 questions); 8 points for question 6 (extra credit).

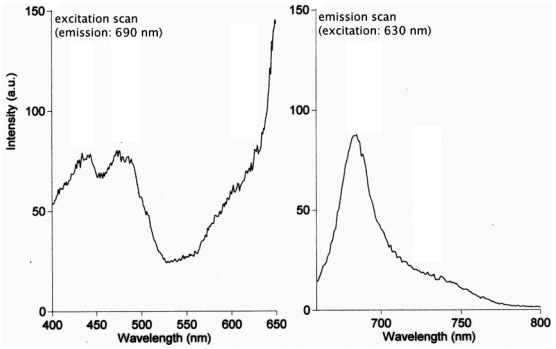
Photosynthesis (SC/BIOL 4061) Final Exam (1 Mar 2009)

Answer FIVE of the following SIX 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.

Question One

The excitation and emission scans (left) are from a suspension of the photosynthetic alga *Eremosphaera viridis*. Contrast these scans with those you would expect for chlorophyll in a solvent (such as acetone). Explain why the algal excitation (absorption) scan has very broad peaks in the 400–550 nm range compared to the narrow absorption peak of chlorophyll.



Question Two

In algae, mosses and higher plants, chloroplasts are mobile. In *Eremosphaera viridis*, when the cell is irradiated with very high light intensities, the chloroplasts move from peripheral orientation (a uniform distribution at the surface of the spherical cell) to a dense aggregate in the center of the cell. Explain why. Predict what wavelengths of light would cause chloroplast movement, giving your reason(s).

Question Three

For redox reactions that occur in the light reactions of photosynthesis, give 2 examples of chemically distinct compounds that transport electrons *and* protons (H^+). Explain their role(s) in NADPH and ATP production.

Question Four

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^+)?

Question Five

If the photorespiratory pathway is knocked out (by mutagenesis), photosynthetic cells are more sensitive to photo-oxidation. Explain why, giving your reason(s).

Question Six

As a newly-minted bioengineer of photosynthesis, you decide to develop a single-cell photosynthetic algae that uses the C4 pathway instead of a carbon concentrating mechanism. Diagram in detail an appropriate cellular/biochemical architecture that would allow the C4 pathway to operate in a single cell during illumination.

KEY

Question One

Chlorophyll in acetone (or some other solvent in which the chlorophyll molecules would be completely solvated in a homogeneous dielectric) exhibits very sharp peaks ±25 nm): absorption in two bands (420 nm and 660 nm) and fluorescence in a doublet (ca 675 nm and 710 nm). In constrast, chlorophyll in the algae has a very broad absorption peak (400–500 nm) and emission peak (670–700 nm with a shoulder at 725 nm). There are two possible explanations for the broadness of the absorption peak in the algae. One is the heterogeneous dielectric for molecules embedded in a diversity of ways within lipid regions and hydrophobic regions of the light-harvesting protein complexes. The other is the broadening effect of molecule to molecule transfers, which would tend to 'red-shift' the absorption to varying degrees. Finally auxillary pigments (carotenoids, for example, might contribute to the absorption spectrum of the algae.

Question Two

The major effect of aggregating the chloroplasts in the center of the cell would be to limit the absorptive cross-sectional area. At high light intensities, this would minimize photo-oxidative damage to the light harvesting systems (both chlorophyll molecules and the reaction centers) of the chloroplasts. The maximal photo-oxidative effect is likely to occur at shorter wavelengths, so its likely the wavelengths in the range 400–500 nm would mediate chloroplast aggregation at the center of the cell. This may (or may not) be chlorophyll. Carotenoids could be a sensor of high light intensity, but how would signaling be mediated? Chlorophyll, even the reaction centers, would be more likely; but in fact, flavins have been implicated in chloroplast movements.

Question Three

Plastoquinone is one example of a redox mediator that transfers both electrons and protons ($PQ + 2e^- + 2H^+ <----> PQH_2$). Flavins undergo the same redox reaction ($FAD + 2e^- + 2H^+ <----> FADH_2$). NADP might be considered an example, but it is a terminal molecule, not involved in transport of e^- and H^+ in the light reactions of photosynthesis. By transporting H^+ across the membrane, or removing H^+ from one side of the membrane, PQ and FAD create a delta pH gradient across the membrane that is used for the synthesis of ATP by the ATP synthetase.

Question Four

The enzyme is made up of two structural components, the CF_o embedded in the membrane, comprised primarily of c subunits (plus a smaller number of a and b subunits). The CF_i is comprised of three units each of subunits alpha and beta, plus single units of gamma, delta, and epsilon. Rotation is believed to cause the conformational changes required for ATP synthesis on the alpha/beta subunits. The hydronium ion has a structure similar to sodium ions, implying a bona fide channel must exist in the CF_o. In contrast, protons could only traverse the membrane via step-wise protonations and deprotonations of appropriate H^+-accepting groups (for example, carboxyls and amines).

Question Five

The photorespiratory pathway provides 'relief' from photo-oxidation in three ways. First, it requires additional ATP and reducing equivalents, so it serves as a sink for excess reductive equivalents produced in photosynthesis, thereby protecting the chloroplast from photo-oxidative damage. The reducing equivalents are the reduced ferredoxin required to regenerate glutamate from alpha-ketoglutarate; the ATP is required in the regeneration of phosphoglycerate from glycerate. Second, it creates [CO_2], providing an additional source for continued carbon dioxide fixation, competing with the oxygenase reaction of RuBisCO at the same time. Third, in the absence of PGA regeneration, there is a very significant *net* loss of PGA for continued operation of the Calvin Cycle. The consumption of O_2 in the oxygenase reaction will occur whether or not photorespiratory pathway is functional, and can't be considered part of the protective envelope conferred by photorespiration. However, the accumulation of the phosphoglycollate product of the oxygenase reaction would be detrimental to the photosynthetic process.

Question Six

The open-ended nature of this question is constrained by two parameters: both C4 and C3 pathways must operate simultaneously (that is, during illumination), and they must occur in the same cell. Should they be spatially separated? It would be more efficient if they were. In fact, if the PEPCase reaction occurred near the plasma membrane, to capture HCO_3^- from outside of the cell, it would mimic the normal Carbon Concentrating Mechanism(s) of aquatic autotrophs. Then, the product of PEPCase (oxaloacetate) could be transported to the chloroplast, where it could be reduced to malate (using NADPH), decarboxylated to pyruvate (using NADP), phosphorylated to phosphoenolpyruvate (using ATP), and exported to the cytoplasm where it would be available for PEPCase. Another alternative would be to use PEP carboxykinase to release the CO_2 from oxaloacetete (transported into the chloroplast) and regenerate PEP. The major imbalance in either scenario is the net movement of phosphate out of the chloroplast, which must be freely imported into the chloroplast as a substrate for ATP synthesis.