

Name: _____

Student ID: _____

Answer three of 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

Biologists (and microscopists) tend to consider refractive index to be the ratio of the speed of light in a vacuum to the speed of light in whatever substance the light passes through (water, glass or oil are most interesting to them): $n=c/v$. But there is a more complex definition of the refractive index (that is, it is a complex number having *real* (n) and *imaginary* (k) components): $\tilde{n} = n + i \cdot k$ (where i is the square root of -1 , $i = \sqrt{-1}$). Both n and k are shown and explained in the figure below from Johnsen's book entitled *Optics of Life*. Explain the relevance of the *complex* refractive index of water in the context of photosynthesis.

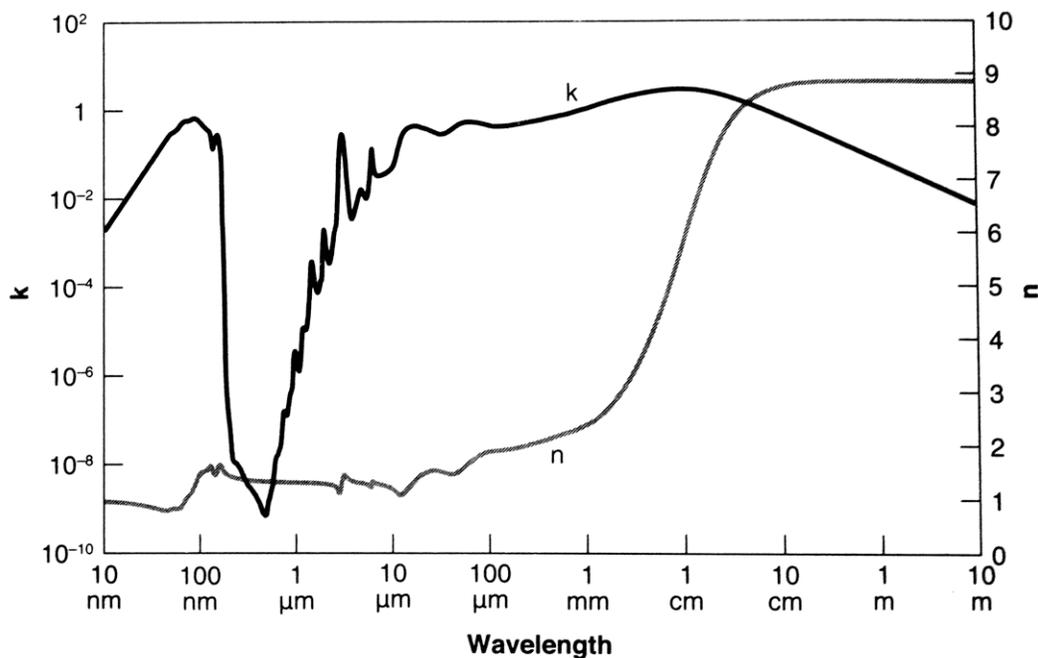
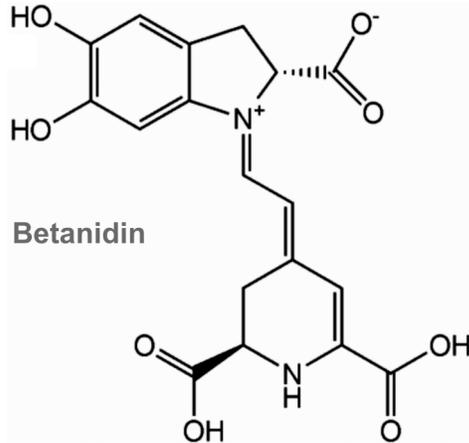


Figure 5.11: Real (n) and imaginary (k) components of the refractive index of water. The real component is what we usually think of as the refractive index; the imaginary component is closely related to the absorption coefficient. Note the gigantic drop in k in the visible range. Also notice that the real refractive index varies by nearly a factor of ten over the range shown. Data from Segelstein, 1981.

Question Two

The chemical structure is betanidin, closely related to the glucoside betanin (common in red beets and other plants). Predict its absorption spectrum (give the reason(s) for your prediction).



Would changes in pH affect its maximum absorbance wavelength? How? Explain clearly. Predict its fluorescence spectrum (a diagram of the absorbance and fluorescence spectra would be very helpful). Could this pigment be used in photosynthesis — either natural or artificial? Why?

Question Three

The appearance of high levels of oxygen in geological time is now known as the “Great Oxygenation Event” (Wikipedia), the belief that it was biologically induced — due to oxygenic photosynthesis— is common. One line of evidence in support of a “Great Oxygenation Event” is the appearance of red banded-iron sediments. There is an alternative — also biogenic— explanation¹:

Anoxic iron-rich sediment samples that had been stored in the light showed development of brown, rusty patches (in defined media containing ferrous iron, the product was a FeCO_3 precipitate). Two different types of purple bacteria (absorption spectra revealed peaks characteristic of bacteriochlorophyll a) oxidized colorless ferrous iron under anoxic conditions in the light to brown ferric iron. Neither strain utilized free sulfide; however, both strains grew on black ferrous sulfide (FeS), which was converted to ferric iron and sulfate. The existence of ferrous iron-oxidizing anoxygenic phototrophs may offer an explanation for the deposition of early banded-iron formations in an assumed anoxic biosphere in Archean times.

Could this be true? Is there any way to distinguish between the two possibilities (free oxygen *versus* electron donor to anoxygenic photosynthesizers)? Explain.

Question Four

Explain how light-harvesting complex II plays a central role in controlling thylakoid architecture and shunting of excitons to either non-cyclic and/or cyclic photosynthesis. Would the unique anoxygenic carbon dioxide fixation pathways favour non-cyclic or cyclic photosynthesis? Why?

¹ Ehrenreich A and Widdel F (1994) Anaerobic oxidation of ferrous iron by purple bacteria, a new type of phototrophic metabolism. *Applied and Environmental Microbiology* 60:4517–4526

KEY — Term Test One (16 October 2013)²

<p>Question One: In the context of photosynthesis, ‘k’ is the most important. Water absorbs the <i>least</i> at wavelengths between <i>ca</i> 200 nm and <i>ca</i> 800 nm. This is the ‘sweet spot’ for photosynthetically active radiation (PAR) (<u>8.5 points</u>). It also matches the maximal photon flux density of natural sunlight (<u>8.5 points</u>), and provides a photonic energy that is reasonably close to the energies associated with chemical bond conversion, but not UV energies that would cause molecules to break apart (<u>8.5 points</u>). Archaic photosynthesizers absorb maximally between <i>ca</i> 800 and 900 nm (bacteriochlorophyll λ_{\max}), where light availability would be less. Apparently, photosynthetic organisms evolved to match the maximal water transmittance (<u>8.5 points</u>).</p>	
<p>Question Two: The molecule has 7 conjugate bonds, so the predicted maximal absorbance (λ_{\max}) would be <i>ca</i> 400 nm (<u>5 points</u>). However, the two cyclic structures would cause a longer wavelength λ_{\max} (<u>5 points</u>). In fact, the reported λ_{\max} for betanidin is <i>ca</i> 490 nm³. pH will affect the λ_{\max}, probably strongly because of the presence of three carboxyl groups (<u>5 points</u>). It’s hard to predict the change in λ_{\max}; the removal of the hydrogen ions would provide ‘more’ π electrons, so a longer wavelength is likely (and reported in the literature) (<u>5 points</u>). The fluorescence spectra would be red-shifted by <i>ca</i> 20 nm or so. Thus a $\lambda_{\text{absorption}}$ of about <i>ca</i> 490 nm, a $\lambda_{\text{emission}}$ of <i>ca</i> 510 nm (<u>5 points</u>). There is no doubt it could be used as a light-harvesting pigment —collecting excitons to transfer to the reaction center (<u>4 points</u>). Its use in photochemistry is also possible. It depends upon how effectively it can donate an electron: betanidin + light \rightarrow betanidin* \rightarrow betanidin⁺ + e⁻ (<u>5 points</u>). It is being investigated for use in artificial photosynthesis⁴.</p>	
<p>Question Three: Yes, red banded-iron sediments could be formed by anoxygenic photosynthesis. Especially so given the experimental evidence that extant purple bacteria use ferrous iron to supply electrons (<u>8.5 points</u>). In terms of long-term impact (that is, geological time), it is <u>not</u> likely. The reason is that ferrous iron would eventually become depleted (<u>8.5 points</u>), while H₂O (as an e⁻ donor) would always be in excess (H₂O is an astonishing 55 M, and covers <i>ca</i> 75% of the globe) (<u>8.5 points</u>). The best possibilities for determining <u>which</u> biogenic process had occurred would be the presence of FeCO₃ and sulfate in the red banded iron sediments, since <u>neither</u> would arise from direct (O₂-mediated) ferrous to ferric conversion (<u>8.5 points</u>).</p>	
<p>Question Four: Without going into details, the major players in stacked <i>versus</i> unstacked thylakoids are the two photosystems (I and II) and plastoquinone (PQ <i>versus</i> PQH₂) (<u>12 points</u>). PQH₂ will build up if there is insufficient NADP, causing a shift to more cyclic photosynthesis involving less granal stacking and association of LHCII with PS I (<u>12 points</u>). The two major CO₂ fixation pathways in anoxygenic photosynthesis (reverse Krebs and hydroxy propionate) both require an excess of NADPH over ATP, and so would favour non-cyclic photosynthesis (<u>10 points</u>).</p>	
TOTAL	

² 34 points per question (3/4 questions).

³ Journal of Photochemistry and Photobiology A: Chemistry. 240:5–13. 2012.

⁴ Solar Energy. 86(5):1563–1575. 2012.

Name: _____

Student ID: _____

Answer three of 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

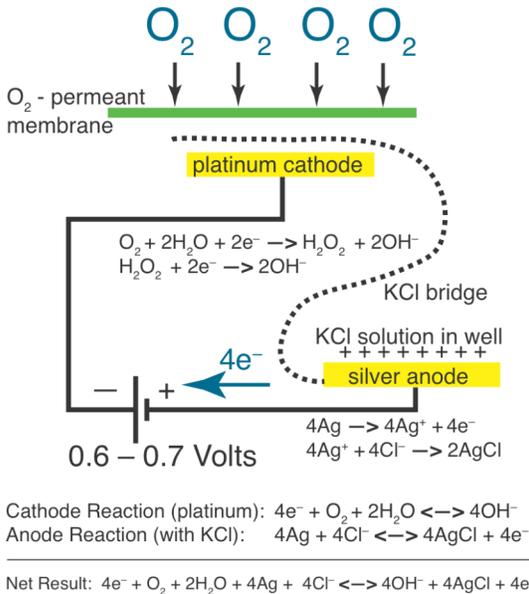
Explain the experimental approach used to identify the P680 of Photosystem II and P700 of Photosystem I. Diagrams of spectra will be helpful.

Question Two

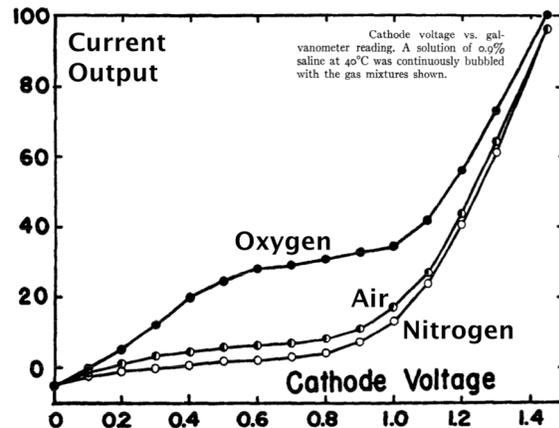
How would changes in pH affect the redox reactions of the Clark-type oxygen electrode? Could a Clark electrode be used as part of an artificial leaf system? Explain.

REDOX REACTIONS:

THE OXYGEN ELECTRODE (CLARK-TYPE) AS A CASE STUDY.



Below is a graph of current *versus* voltage from the Clark et al original paper¹.



Question Three

For redox reactions that occur in the light reactions of photosynthesis, give 2 examples of chemically distinct compounds that only transport electrons (e^-) *not* protons (H^+). Explain their role(s) in NADPH and ATP production.

Question Four

Explain the structure, bioenergetics and kinetics of water-splitting in Photosystem II.

¹ Clark LC Jr, Wolf R, Granger D and Taylor Z (1953) Continuous recording of blood oxygen tensions by polarography. Journal of Applied Physiology 6:189-193.

KEY — Term Test One (18 November 2013)²

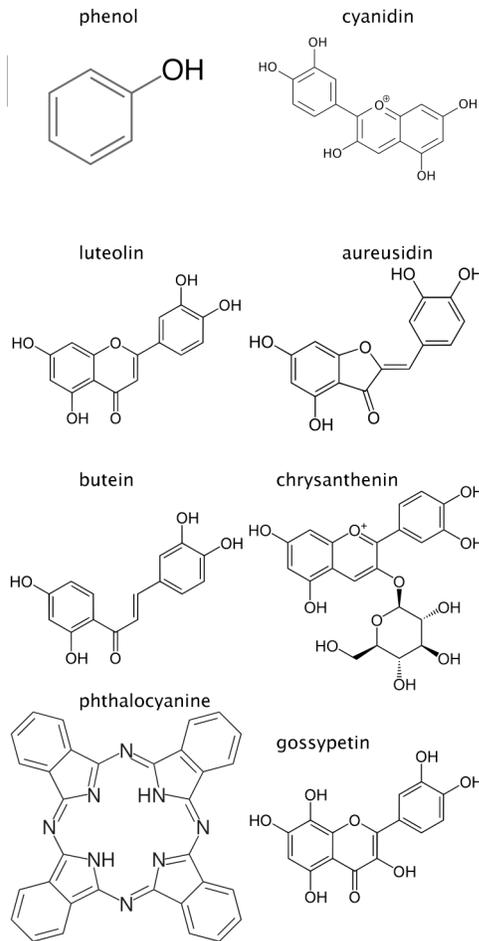
<p>Question One: The technique used was differential spectroscopy. When the reaction center pigment (P680 or P700) donates an electron to the quinone, it becomes positively charged and is unable to absorb light (8.5 points). To observe this very small change in overall absorbance, the chlorophyll molecules in the light-harvesting complexes need to be removed. Then, absorption spectra are recorded in the dark and with high levels of light sufficient to saturate the remaining chlorophylls in their excited state (8.5 points). The <i>difference spectra</i> reveal ‘negative’ changes at wavelengths of 700 nm and 680 nm (8.5 points). To distinguish between the two photosystems, it is probably necessary to inhibit one or the other photosystem selectively (8.5 points).</p>	/34
<p>Question Two: Differences in pH will certainly affect the currents caused by oxygen reduction to water in the Clark electrode, because hydroxides are a product in the overall redox reaction (10 points). Alkaline pH will cause the current to decline (9 points). In experimental work, the alkalization causes a drift in the current (decreasing) over relatively long measurement times. Flushing the chamber and stirring both allow the hydroxide anions to diffuse away, so the effect under normal conditions is relatively small (5 points). It’s conceivable that some aspects of the Clark electrode could be used in an artificial leaf system if a negative potential was applied (to oxidize water). The problem is that the reaction is non cyclic. That is, AgCl will disappear, and acidification will occur. So, additional components are required (10 points).</p>	/34
<p>Question Three: Two examples of redox reactions that do not involve protons are the Fe-S complexes, and cytochromes (hemes) (10 points). Both of these are found at multiple sites in the electron transport chain of photosynthesis. Cytochromes and Fe-S systems occur in the <i>cyt b₆f</i> complex. Fe-S systems are also found in Photosystem I just before NADP reduction (10 points). Neither one of these redox agents directly mediates ATP synthesis (dependent on the <i>delta</i>-pH gradient across the thylakoid membrane), but both do so indirectly – by accepting electrons from plastoquinone (which releases its protons into the thylakoid lumen) and donating electrons to NADP (causing proton consumption on the stromal side of the thylakoid membrane (10 points). Without them, net vectorial movement of protons could not occur (4 point)</p>	/34
<p>Question Four: With some brevity, water splitting occurs with step-wise electron loss that requires 4 photons in toto. This corresponds to removal of 4 H[•]: Bond energies for O—H are 463 kcal/mole (× 4=1852), for O=O it is 498 kcal/mole (× 2=996). So, a net energy input of 856 kcal/mole is required for the reaction (8 points). The structure involves two components directly responsible for e⁻ transfer: a complex of 4 manganese that undergo changes in oxidation state (5 points) as they remove e⁻ from the water molecules and a tyrosine that—in its radical form tyr[•]— transfers successive e⁻ to P₆₈₀⁺ (6 points) Structural support is provided by acidic residues that surround the manganese complex, and Ca²⁺ and Cl⁻ (5 points) The overall reaction is relatively slow. Each step requires about 100 microseconds, with the exception of e⁻ removal from water, which takes about 1 millisecond (10 points).</p>	/34
TOTAL	

² 34 points per question (3/4 questions).

Photosynthesis (SC/BIOL 4160) Final Exam (20 Dec 2013)

Answer FIVE of the 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

Using the compounds shown to the left, design a light harvesting system. Show your estimates of peak absorbance wavelengths and indicate the pathway of exciton transfer from one compound to the next, with an explanation why a particular sequence is necessary.

Question Two

Magnesium-porphyrins are known to exhibit two absorption peaks (Q and Soret bands). Is there any evolutionary advantage to using pigments with two absorption peaks in photosynthesis? Explain.

Question Three

Regulation of the Calvin Cycle involves 'reductive' signal transduction. Explain why and how. Besides the role for reducing equivalents, propose one other signal transduction mechanism that almost certainly regulates the activity of the Calvin Cycle, indicating clearly why and how it would function.

Question Four

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

Question Five

Describe the metabolic cost of photorespiration in a C3 plant and explain the advantages of recently bio-engineered variants. C3 mutants that lack a functional photorespiratory pathway are more sensitive to photo-oxidation. Why?

Question Six

What is a 'flux control coefficient'? How is it measured and why is it important in bio-engineering?

KEY — Final Exam (20 December 2013)¹

<p>Question One: From lowest wavelength to highest: phenol (300 nm) → chrysanthenin (410 nm, due to +ve charge) = cyanidin (410 nm, due to +ve charge) → aureusidin (420 nm) = luteolin (420 nm) = butein (420 nm) = gossypetin (420 nm) → phthalocyanine (500 and 700 nm). Exciton transfer must occur from lowest wavelength (highest energy) to longest wavelength (lowest energy) (10 points). Most of the compounds will absorb at about 420 nm; in aggregate, they increase the total extinction coefficient at this wavelength. There may be some variations due to details of the chemical structures (notable the positive charge on chrysanthenin and cyanidin means fewer electrons for the pi orbital) (5 points). Pthalocyanin has a macro-cyclic structure, and –like chlorophyll porphyrins– will absorb at dual wavelengths (5 points).</p>	/20
<p>Question Two: The value of dual absorption bands is that a wider range of wavelengths can be absorbed (10 points). There is no reason to think that a judicious mix of multiple pigments would be worse, probably better at absorbing a wider range of wavelengths (5 points). Thus, no compelling reason to use chlorophyll (especially since many organisms rely on linear tetrapyrrolles with one absorbance band) (<u>5 points</u>).</p>	/20
<p>Question Three: Many of the enzymes in the Calvin cycle are regulated by reversible reduction of sulfhydryl bonds via the redox mediator ferredoxin. When light causes the reduction of NADPH, it creates a highly reduced environment, including a pool of reduced ferredoxin. Thus, reduction of sulfide bonds to sulfhydryls effectively ‘tells’ the Calvin cycle enzymes that photosynthesis is active (10 points). During photosynthesis, there is net vectorial movement of protons from the stroma into the thylakoid lumen, resulting in an alkaline stroma (where the enzymes of the Calvin cycle are located). It is highly likely that these enzymes have a alkaline pH optima for maximal activity (<u>10 point</u>)</p>	/20
<p>Question Four: The subunit makeup of the enzyme has two major parts: The membrane localized a, b and c CF_o component, and the cytosol-localized alpha, beta, gamma, delta and epsilon CF₁ component (<u>5 points</u>). Hydronium ions pass through the CF_o – causing rotation of the CF₁ and thereby ATP synthesis due to conformational changes (<u>5 points</u>). Protons would traverse the membrane via proton wires, a mechanism completely at variance with the movement of the large hydronium ion through channel structures in the CF_o (<u>10 points</u>)</p>	/20
<p>Question Five: The aggregate cost of a traditional photorespiratory pathway is NADH, ATP and the loss of an amino group (re-incorporating the amino group requires ferredoxin and ATP) (<u>10 point</u>). The bio-engineered photorespiratory pathway (glycolate to glyoxylate to tartronate semi-aldehyde to glycerate) involves <u>no net energy input</u> (NAD is reduced, then oxidized) (<u>5 point</u>). C3 photorespiratory mutants will accumulate glycolate and glyoxylate (the latter will result in production of H₂O₂ with a deleterious impact on protection from oxidative damage.</p>	/20
<p>Question Six: Basically, it measures the relative contribution of each enzyme to the total metabolic flux through a pathway, thereby identifying potential ‘choke-points’ that may be modified to increase overall metabolic flux. It is measured by modifying the amount of a specific enzyme in the pathway (either decreasing or increasing it) and assessing the impact on overall metabolic flux.</p>	/20
TOTAL	

¹ 20 points per question (5/6 questions).