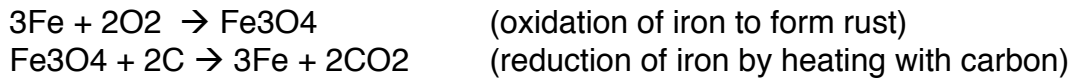


Photosynthetic Light Reactions

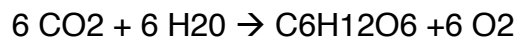
A. Oxidation-Reduction Reactions:



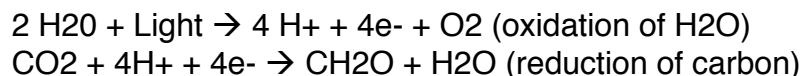
- **Reduction** is the removal of oxygen, the addition of electrons, or the addition of hydrogen atoms; conversely, **oxidation** is the addition of oxygen, the removal of electrons, or the removal of hydrogen atoms.
- In phytoplankton, these oxidation-reduction reactions are catalyzed by the presence of **photosynthetic pigments**

Chlorophyll does **NOT** by itself lead to a chemical reaction---cooked spinach is still green, but it is no longer capable of photosynthesizing

- In plants, we often refer to photosynthesis as:



- Which can be broken down into two separate reactions:



- These are the **Light Reactions** and **Dark Reactions**
- All photosynthetic organisms (except for some bacteria) use **oxygenic** photosynthesis, meaning they produce oxygen. All have chlorophyll a as the primary reaction pigment, in the form of PS I and PSII (but not all algae use Chl a as the primary antenna pigment)

B. History of Photosynthetic Unit

- Early 20th Century, realized that Chlorophyll was a catalyst (wasn't destroyed during the reaction), and thought that it somehow absorbs light, produces O₂, and fixes CO₂
- 1932, Emerson and Arnold set up an experiment with a bunch of equipment to give very short, very bright flashes:
 - Allowed the dark time between flashes to vary

- Long time between flashes (long dark period), O₂ evolution is independent of temperature
- Short time interval, strongly temperature dependent (enzymes involved)
- Took about 2500 chl molecules to produce O₂ (turns out it's between 1500-2500 on average)
- Determined 3 things (VERY IMPORTANT):
 - Light and Dark reactions
 - High light, dark reactions (enzymes) are limited
 - Many chlorophyll molecules are required
- Von Warburg (1920s)
 - Determined that the O₂ produced requires CO₂ to be consumed
 - According to his measurements, it takes 4 electrons to carry out this reaction (so each O₂ molecule requires 4 electrons, or photons)
 - Based on this calculation, Von Warburg determined that the **quantum yield** for O₂ production is 0.25 (1 oxygen molecule requires 4 photons, so the **yield** based on **quanta** is 1/4)
- So, we know chlorophyll is involved in photosynthesis, and it takes at least 4 electrons to produce O₂. This reaction has light and dark reactions, and involves a lot of chl molecules
- 1943, Emerson and Lewis asked what the other pigments (besides Chl a) were doing...
 - Shined other color lights on algae, discovered it's not as efficient
 - When they shined far-red light, very inefficient (red drop)
 - Shine blue AND red light, more efficient than either one by itself
 - Means there must be TWO independent light reactions (PSII, PSI)
 - PSI does NOT produce oxygen!
 - So, Von Warburg was technically correct in that it takes 4 electrons to produce O₂, but it takes 8 electrons to complete photosynthesis
- In Summary, we now know that algae (and plants, and bacteria) achieve photosynthesis by using a group of pigment molecules working together...the entire functional unit (the minimum collection of pigments, etc. needed to photosynthesize) is made up of a collection of pigments and biochemical reactants:

PSU – Photosynthetic Unit, the functional unit of photosynthesis

PS II – Photosystem II (also called the Reaction Center), where Oxygen is produced

PSI – Photosystem I, necessary to complete photosynthesis

Antenna Pigments – all the rest of the pigments associated with the PSU
Light Harvesting Complex – the physical structure of the antenna pigments

C. Quantum Yields of Photosynthesis

- **Quantum Yield** is simply the efficiency with which a reaction proceeds...how fast does the reaction go as a function of the number of absorbed photons?
 - *Moles of photons absorbed per moles product*
 - NOT going to be equivalent for different products (for example, quantum yield of oxygen doesn't have to equal the quantum yield of CO₂ fixation)
 - Why did the Von Warburg Experiment come up with the wrong answer?
 - He was a feared Nobel Laureate at that time, so nobody wanted to question his results—he could ruin your career!
 - Von Warburg used CO₂ starved Chlorella (a green alga), and white light...so he was actually shining blue and red light on the algal culture
 - Came up with quantum yield for O₂ of 0.25, meaning you need to absorb 4 electrons for each O₂ produced
 - Emerson in 1950s, said it was 0.10 to 0.125 (or about 8 electrons)

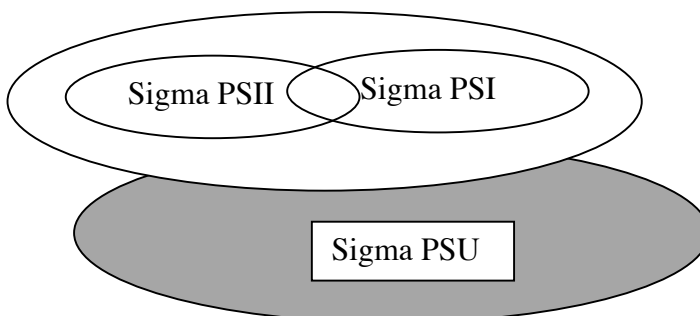
D. Effective Absorption Cross-Sections

- If you flash an algal culture with bright light, the oxygen produced increases in a Poisson Function, which can be described as:

$$Y/Y_{\max} = 1 - e^{-\sigma E}$$

Where Y is the yield (oxygen production), sigma is the slope, and E is the flash energy

- The “effective” cross section means it doesn't have anything to do with the physical size...it's a theoretical measurement, that makes it easier to measure
- Quantum Yield of Photosynthesis is the relative ratio of the cross-section of PSII to the entire PSU
- So, if the ONLY reaction is absorption by PSII and PSI, maximum yield is 0.125



E. Physical Structure of Photosynthesis

- To make photosynthesis work, we must have the PSU embedded in a membrane, which provides physical support, access to chemicals (CO₂, nutrients, etc) and sets up a gradient for the products (to keep the energy sources and sinks separated)
 - The cellular organelle responsible for photosynthesis is the **chloroplast**
 - The **thylakoid** is the structural unit of photosynthesis.
 - Both photosynthetic prokaryotes and eukaryotes have these flattened sacs/vesicles containing photosynthetic chemicals.
 - Only eukaryotes have chloroplasts with a surrounding membrane.
 - Thylakoids are stacked like pancakes in stacks known collectively as **grana**.
 - The areas between grana are referred to as **stroma**
- Although this is all very complicated, it's not really well known why the structure is so convoluted...for our purposes, it's enough to know that the PSUs are embedded in a cellular organelle
- A chemical gradient is set up across the thylakoid membranes once the photochemistry starts, because Hydrogen ions are pumped across...the outer side has a pH of about 8, while the inner side has a pH of about 6, setting up a **proton gradient**

F. Photophosphorilation and the Z-Scheme

- **Photophosphorylation** is the process of converting energy from a light-excited electron into the pyrophosphate bond of an ADP molecule
- Once a light quanta hits the PSU, it is funneled down to the Reaction Center
- From there, it forms an **exciton** by "pulling" an electron off of a Mn molecule
- The exciton can do one of three things:
 - Release the energy as heat
 - Release the energy as fluorescence
 - Achieve charge separation, and pass the electron on to another molecule
- If it achieves charge separation, the electron then "cascades" down an electrochemical gradient from one electron acceptor to another, via the **electron transport chain** and the **cytochrome-b6f complex**
- Along the way, the energy released allows an ATP-pump to phosphorylate ADP to ATP: $ADP + P \rightarrow ATP$

- The electron is now passed to P700, the specialized chlorophyll molecule at the center of PSI
- Another photon (so, the second one) is absorbed by PSI, allowing that electron to be “pumped up” to a high excitation level (forming a second exciton)
- This electron cascades down through Ferredoxin/Flavodoxin compounds, forming NADPH
- This entire process is called the “Z-scheme”, and describes the light-reactions of photosynthesis
- There are two special cases which can short-circuit this general scheme:
 - 1) PSI can operate by itself (***cyclic photophosphorilation***) to produce ATP, without the production of NADPH (which means no O₂ production, and no ability to fix CO₂)
 - 2) The ***Mehler Reaction*** can use PSII to PSI, but ends up using all of the oxygen consumed...this can do two things. First, it can reduce the oxygen concentration around the cell, and second, it “burn off” excess light-energy

G. The Dark Reactions

- The light reactions provide not only the reducing power in NADPH but also the energy of ATP, both essential for producing sugars from CO₂.
- ATP is produced through an enzyme called ATP synthase, using ADP (adenosine diphosphate), inorganic phosphate (Pi) and energy from a proton motive force (pmf) across the thylakoid membrane. This pmf is composed of two components: an electrical potential across the thylakoid membrane and a proton gradient across the thylakoid membrane.
- The proton gradient comes from the storing up of protons (hydrogen ions) inside the lumen, giving a pH of 6 inside the lumen and pH of 8 outside, in the stroma. Then, basically, protons escaping out from the thylakoid lumen through a central core of the enzyme ATP synthase (embedded in the membrane) which catalyzes the phosphorylation of ADP and the release of ATP on the stromal side.
- The byproducts of the light reactions are an excess of ATP and NADPH—these are now used in the ***Calvin-Benson Cycle***, named after Melvin Calvin and Andy Benson, for his pioneering work with ¹⁴C-labeled compounds to trace the reactions.
- The key enzyme in the Calvin Cycle is the one that catalyzes the transformation of the 5-carbon sugar ribulose-5-phosphate and the single-carbon carbon dioxide to two 3-carbon 3-phosphoglycerates—this is the enzyme ribulose-1-5-biphosphate carboxylase or Rubisco
- The cycle runs 6 times, each time incorporating a CO₂ molecule, and ultimately produces glucose, which is then used for everything else in the cell

H. Measuring Photosynthesis

Several ways to measure photosynthesis:

1) Oxygen evolution – using either an electrode, or by using H_2O^{18} . Add heavy water, measure how much appears in the “head space” over time. Extremely precise, but requires very expensive equipment. This measures “gross photosynthesis”

2) ^{14}C Method—most commonly used. Measures the acid-stable organic carbon that is assimilated over time. Einar Steeman-Nielsen came up with this in 1952.

- This is a true “tracer” method because add small quantity of radioisotope, which doesn’t perturb the system.
- Have to account for the “discrimination” of ^{14}C vs. ^{12}C though, because plants don’t like to use heavy isotopes.
- Problems: first, it’s radioactive.
Second, it measures something between net and gross productivity, because oxygen evolution can occur without carbon fixation.
Third, it’s done in a bottle

3) Photo-acoustics --When light is absorbed, produces heat. By putting a sample in a sealed, air-tight vessel and shining light on it, produces heat, causes expansion, which produces a pressure wave. If there’s a microphone inside, can acoustically measure the heat production.

4) Fluorescence – This relies on the idea that once a photon is absorbed, it has to go to heat, fluorescence, or photochemistry.

- Big advantage, because it’s passive, non-destructive, and can be measured without using a bottle. Also instantaneous. Finally, the only things that fluoresce are things with chlorophyll, so it’s very specific to primary production.
- Big disadvantage—we have to assume a known relationship between the quantum yield of fluorescence and photosynthesis

I. Photosynthesis versus Irradiance Curves

Using any of these methods for measuring photosynthesis, we can determine how the plankton respond to light (irradiance). If we plot the photosynthesis as a function of the light, we produce a **Photosynthesis versus Irradiance curve** (P vs. E curve).

- Advantage: if we have a P vs. E curve for any water sample, we have an exact mathematical representation of how the phytoplankton respond to light (so we can solve for photosynthesis at any irradiance level)
- These curves can be divided into a number of parts:
 - alpha (α) is the initial slope of the curve, and indicates how light-sensitive it is (how quickly it reaches maximum photosynthesis)
 - beta (β) is the slope of the photo-inhibited region (if there is one), and describes how quickly high-light will damage the plankton
 - P_{\max} describes the maximum photosynthesis for a given set of conditions under no light limitation, and is representative of fully saturated (light and RUBISCO) photosynthesis
 - E_k describes the half-way point between 0 and P_{\max} , and is the point at which the algae switch from light-limited to RUBISCO-limited growth
- In general, the steeper the initial slope (alpha) the more likely that the plant (or algal cell) is “adapted” to low-light conditions...similarly, the more photo-inhibition, the more likely the plant is not well adapted to high light.
- P_{\max} is more difficult to interpret, but often is related to the ecological strategy of the plant...whether it is a fast growing, invasive species or a slower growing, but more competitive (in the long run) species.
- P_{\max} is difficult to compare from organism to organism unless there’s some sort of normalization to biomass...so you might compare P_{\max} normalized to cell number, or P_{\max} normalized to chlorophyll. If the latter, the notation is P_{\max}^B , which means it’s been normalized to biomass (or chlorophyll). This accounts for the fact that we rarely measure the photosynthesis of an individual cell. So, as an example, we would get very different P_{\max} values if there were 1,000 versus 1,000,000 cells per liter, but if we normalize to biomass (cell # or chlorophyll), that should “even things out”