Harmful Singlet Oxygen Formation in Light Stressed Photosystem II

An Introductory Perspective

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Introduction

Photosynthetic organisms are exposed to oxidative stress when light energy absorbed exceeds light energy that can be used for photosynthesis or dissipated as heat. Photooxidative damage substantially reduces photosynthetic rates in plants, and is an active area of bioengineering research to improve crop yields. In excess irradiance, the light capturing apparatus photosystem II (PSII) produces a highly reactive oxygen species known as singlet oxygen. In the absence of adjoining antioxidants, singlet oxygen readily degrades photosynthetic structures and adjacent organic molecules, requiring costly repair and protection mechanisms. To approach the photooxidation subject, this paper begins with a brief introduction of relevant photochemistry, and then delves into the two singlet oxygen formation mechanisms in PSII. The first mechanism reviewed in this paper concerns long-lived excited chlorophyll A (Chl) in the PSII antenna complexes. The second involves charge recombination on the reaction center's P_{680} Chl.

Introduction to Photochemistry: Photon Absorption and Energy Dissipation

Photoexcitation is the absorption of photons by molecules that causes electron promotion from low energy molecular orbitals to higher energy molecular orbitals, more distant to the molecule's nuclei (Taiz, 2006). The excitation level of the molecule is denoted in subscript, with ground state being S_0. By thermodynamics, the excited state of a molecule is inclined to relax by dissipating energy (Taiz, 2006). Dissipation takes many forms, however these can be broadly categorized as either radiative; fluorescence and phosphorescence, and non-radiative; internal conversion, vibrational relaxation, intersystem crossing, and quenching ("Jablonski diagram",

2018). Jablonski Energy diagrams, presented in Figure A1, are commonly used to illustrate the principles of absorption and dissipation. The most relevant dissipation mechanisms in this photooxidation discussion are the non-radiative intersystem crossing and quenching.

Intermolecular Energy Transfers

When an excited molecule dissipates its excitation energy by reacting with another molecule, a quenching reaction is said to occur. Quenching reactions are fundamental to both the desirable and harmful reactions in photosynthesis. The excited molecule, denoted, M^* may react with a ground state quenching molecule Q in several ways (Taiz, 2006).

Physical quenching:	$M^* + Q \rightarrow M + Q + heat$	(i)
Energy transfer:	$M^* + Q \rightarrow M + Q^*$	(ii)
Charge separation:	$M^* + Q \rightarrow [M^+Q^-]$	(iii)

Physical quenching (i) is a common photoprotection mechanism in plants whereby Chl uses carotenoids to divert excess light energy into heat (Taiz, 2006). Energy transfer (i) is required to transfer light energy from peripheral pigments towards the PSII reaction center. Charge separation (iii) is the crucial reaction which forms the basis of the thylakoid electrochemical gradient in photosynthesis. Failed charged separation, so-called charge recombination, is the major contributor of PSII singlet oxygen. These reactions will be discussed further below.

Singlets and Triplets: Electron Configurations

The majority of ground state molecules exist in a singlet electronic configuration, with the notable exception of oxygen (Borden, 2017). This begs the question, what differentiates

singlet and triplet molecules? For an introductory level it suffices to understand that singlet molecules have all paired electrons, doublets have one unpaired electron, and triplets have two unpaired electrons (Warren, 2000). To ascertain the electronic pairing of diatomic molecules like oxygen, one can complete a molecular orbital diagram, as shown in Figure A2. The diagram shows that two electrons are unpaired in the pi-antibonding orbitals. However, a more simplified representation is appropriate for complex molecules like Chl. Thus, the simplified schematic representations of ground state singlet and triplet molecules are compared in Figure A3, and analogously in their excited state in Figure A4. The singlet and triplet character of molecules is crucial to a deeper understanding of aerobic reactions because singlet molecules are typically only reactive with other singlet molecules, and likewise, triplets with triplets. This explains the activation energy precluding thermodynamically favorable oxidation reactions such as the combustion between the building blocks of life and our ~20% oxygen atmosphere. Thankfully, while these reactions are thermodynamically favorable, kinetic limitations prevent our bodies from spontaneous combustion (Borden, 2017). However, these kinetic limitations can be overcome, as will be discussed in the context of photooxidative damage.

Singlet Oxygen Production in PSII: Long-lived Excited States of Antenna Chl
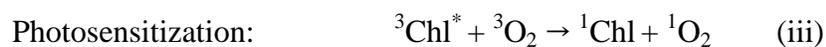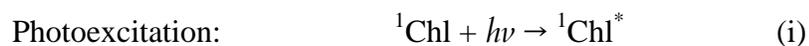
The first mechanism of singlet oxygen production in photosynthesis occurs in the PSII light-antenna receptor complex. The antenna contains five principal components which flank the PSII core complex (itself housing the reaction center). The antenna structure is illustrated in Figure A5. The largest antenna components are the LHCII S and LHCII M, and the smaller components are CP24, CP26 and CP29 (Xu, 2017). The antenna complexes are densely packed with chlorophyll A/ B, and very importantly also with carotenoids. As a whole, the role of the

PSII antenna is to absorb photosynthetically active radiation (PAR), convert it to electronic excitation energy, and efficiently funnel the excitations towards the reaction center. The cascading energy transfers from peripheral antenna pigments towards the reaction center are known as Förster resonance energy transfer (FRET) (Taiz, 2006). Antenna FRET is highly efficient, with 95 to 99% of absorbed PAR photons transferring energy to the RC's special trap Chl_{D1} (Nobel, 2009). However, FRET efficiency falls under light stress, in part because excitation energy is transferred to oxygen instead of the RC (Pospíšil, 2016).

During light stress, PSII antenna Chl molecules cannot transfer energy fast enough to the overexcited trap Chl_{D1}, and are said to remain in long-lived excited states (Pospíšil, 2016). As a result, enough time elapses for the Chl molecule to undergo the slowest dissipation mechanism, which is intersystem crossing. Intersystem crossing causes an electron in the Chl porphyrin ring to randomly invert its spin (Taiz, 2006). The one-electron spin inversion changes the electronic configuration of Chl; beginning with paired electrons, it ends up with two unpaired electrons. Thus, long-lived excited state singlet Chl has a probability of forming an excited triplet state configuration after enough time. Thereafter, the excited triplet Chl is no longer kinetically inhibited from reacting with triplet oxygen. In absence of antioxidant quenching, the triplet Chl reacts with triplet oxygen and forms singlet oxygen.

In photochemistry notation, the Chl ground state is singlet, denoted ^1Chl, while the oxygen ground state is triplet denoted $^3O_2$. When ^1Chl absorbs a photon (denoted hv), the excited molecule is written as $^1Chl^*$. In long-lived excited states, intersystem crossing may occur, forming the excited triplet Chl state, denoted $^3Chl^*$. The $^3Chl^*$ is now in an electronic configuration kinetically capable of reacting with ground state triplet $^3O_2$. This latter reaction, termed photosensitization, or spin-exchange, produces the harmful reactive oxygen species

singlet oxygen $^1O_2$. These reactions are schematically represented in the Jablonski diagram in Figure A6. The reactions described above can be summarized in equations as follows:

$$^1Chl + h\nu \rightarrow {}^1Chl^* \qquad \text{(i)}$$

Photoexcitation:

$$^1Chl^* \rightarrow {}^3Chl^* \qquad \text{(ii)}$$

Intersystem Crossing:

$$^3Chl^* + {}^3O_2 \rightarrow {}^1Chl + {}^1O_2 \qquad \text{(iii)}$$

Photosensitization:

From there on, $^1O_2$ will react almost immediately with nearby organic molecules, either damaging proteins, lipids, DNA, RNA, or harmlessly reacting with carotenoids, tocopherols, and other antioxidants (Taiz, 2006). Due to the dense concentration of carotenoids in the antenna, $^3Chl^*$ are usually harmlessly quenched. However, if the antenna is dissociated from the PSII core is can become a substantial source of $^1O_2$ (Pospíšil, 2016).

Singlet Oxygen Formation in PSII: Reaction Center Mechanisms

The second mechanism of $^1O_2$ formation in PSII occurs in the reaction center. The RC is composed of a heterodimer of two protein complexes, the D1 and D2 branches (Taiz, 2006). The D1 branch is photosynthetically active and provides scaffolding for cofactors needed in charge separation (Pospíšil, 2016). Other important components of the RC are, the Oxygen Evolving Complex (OEC), the tyrosine residue (TyrZ), the Chl dimer P_{680} (also known as P_{D1}/P_{D2}), the primary electron acceptor Pheophytin (Pheo), and subsequent quinone electron acceptors Q_A (fixed) and Q_B (loose). The RC structure and electron flow described below are illustrated in Figure A7 with red arrows representing electron transport from the OEC to Q_B.

 Based on extensive work by Pospíšil (2016) and many other, it is possible to summarize the steps of charge separation as follows. After excitation of the trap Chl_{D1}^*, the excited state is delocalized over the primary acceptor Pheo, such that the resulting excitation state is annotated

$^1[Chl_{D1}Pheo]^*$. This system is destabilized by the high redox potential of Pheo relative to Chl_{D1} (Taiz, 2006). Thus, a charge separation occurs, where Chl_{D1} reduces Pheo with one electron, which forms the primary radical cation-anion pair $^1[Chl_{D1}{}^{+\bullet}Pheo^{-\bullet}]$ (Pospíšil, 2016). The dot superscript notation here indicates that both molecules host a molecular orbital with an unpaired electron, i.e, that they are radicals, yet the system as a whole remains singlet. The radical pair is stabilized by rapidly oxidizing the P_{680} Chl dimer, so that charge separation resides over $^1[P_{680}{}^{+\bullet}Pheo^{-\bullet}]$ (Pospíšil, 2016). The radical pair $^1[P_{680}{}^{+\bullet}Pheo^{-\bullet}]$ is further stabilized by two synchronous charge migrations: negative charge from $Pheo^{-\bullet}$ to QA which forms the radical $QA^{-\bullet}$ and neutralizes Pheo, and positive charge from $P_{680}{}^{+\bullet}$ to TyrZ which forms $TyrZ^{+\bullet}$, and neutralizes P_{680} (Pospíšil, 2016). The negative charge continues to migrate from Q_A to Q_B, and when two electrons reach the loosely bound Q_B, it is protonated by two stromal H^+ to form PQH_2 (Taiz, 2006). PQH_2 is liberated from PSII, and reduces Cytochrome b6f while supplying its protons to the thylakoid lumen (Taiz, 2006). Meanwhile, electron supply to $TyrZ^{+\bullet}$ is accomplished by the OEC Mn_4O_5Ca cluster, which splits water every time it accumulates four oxidizing equivalents (Taiz, 2006).

When forward electron flow is hindered, electrons flow backwards. Forward electron flow is hindered either due to overly reduced PQH_2 pool, thylakoid acidification, or insufficient electron input from the OEC (Pospíšil, 2016). When electrons flow backwards and meet electron holes, several charge recombinations can occur. One recombination where $^1[P_{680}{}^{+\bullet}Q_A^{-\bullet}]$ always leads to ground state P_{680}, is harmless (Pospíšil, 2016). Another recombination, where the singlet charge-pair $^1[P_{680}{}^{+\bullet}Pheo^{-\bullet}]$ can invert spin orientation to triplet $^3[P_{680}{}^{+\bullet}Pheo^{-\bullet}]$, is harmful and leads to $^3P_{680}{}^*$ (Pospíšil, 2016). Because the OEC splits water to produce oxygen, the RC is an oxygen rich environment. Thus, the $^3P_{680}{}^*$ derived from $^1[P_{680}{}^{+\bullet}Pheo^{-\bullet}]$ charge recombination has

a high probability of releasing its energetic state by a spin exchange reaction with ground state $^3O_2$ to form $^1O_2$ (Taiz, 2006). This latter charge recombination is the most substantial source of highly reactive singlet oxygen in light stress, and the primary cause of photoinhibtion.

It is important to note that the PSII reaction center contains two β-carotene molecules (Car_{D1} and Car_{D2}), yet, these are not in sufficient proximity to $^3P_{680}^*$ to enable the spin exchange reaction required to transfer the triplet state from the Chl dimer to carotenoids, since such a reaction requires orbital overlap and a distance smaller than ~3.6Å (Telfer, 2002; Krieger-Liszkay, 2004; Pospíšil, 2016). However, this is not an evolutionary flaw in design. Interestingly, Krieger-Liszkay (2004) notes that if these two β-carotenes were close enough to react, then they would be rapidly degraded by oxidation of strongly electrophilic radical $P_{680}^{+•}$, and furthermore, their oxidation would interfere with the successful oxidation of water. Thus the function of the RC β-carotenes is not to quench $^3P_{680}^*$, but instead to quench the resulting $^1O_2$ (Krieger-Liszkay, 2004). However, these two β-carotenes do not suffice to quench singlet oxygen produced in the RC. Singlet oxygen also attacks the D1 protein in a controlled damage reaction (Krieger-Liszkay, 2004). In fact, the D1 protein is rapidly repaired and turned over even under low light conditions, and its controlled degradation seems to act as a safety valve to absorb oxidative stress (Krieger-Liszkay, 2004). Nevertheless, under extreme light, oxidative damage emerging from the RC will spread to other PSII proteins, the thylakoid lipid bilayer, and beyond, resulting in photoinhibition, and upregulation of photoprotection genes, such as carotenogenesis (Pospíšil, 2016).

Conclusion

This paper introduced basic photochemistry knowledge required to understand how light stress produces the reactive oxygen species singlet oxygen in PSII. Two main mechanisms of singlet

oxygen formation were reviewed. The first mechanism reviewed involved long-lived excited state Chl in the antenna forming triplet Chl. The second mechanism involved charge recombination induced formation of triplet P_{680} in the RC. It was argued that the PSII antenna is not a substantial contributor to photooxidatve damage because it is densely packed with carotenoid antioxidants that quench triplet Chl photosensitizers. Contrarily, the RC is a substantial contributor to photooxidative damage because the distance between the photosensitizer $^{3}P_{680}^{*}$ and nearby antioxidants exceeds the orbital overlap requirement for harmless quenching. In conclusion, future developments in precision bioengineering may target the PSII reaction center for much needed improvements in photosynthetic rates and crop yields.

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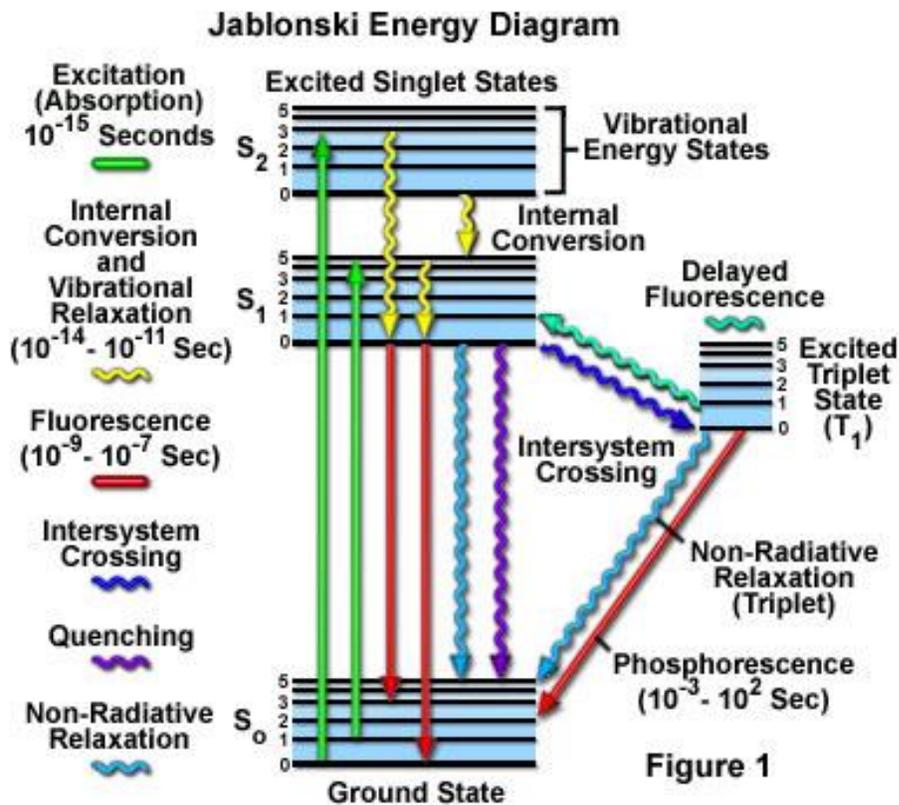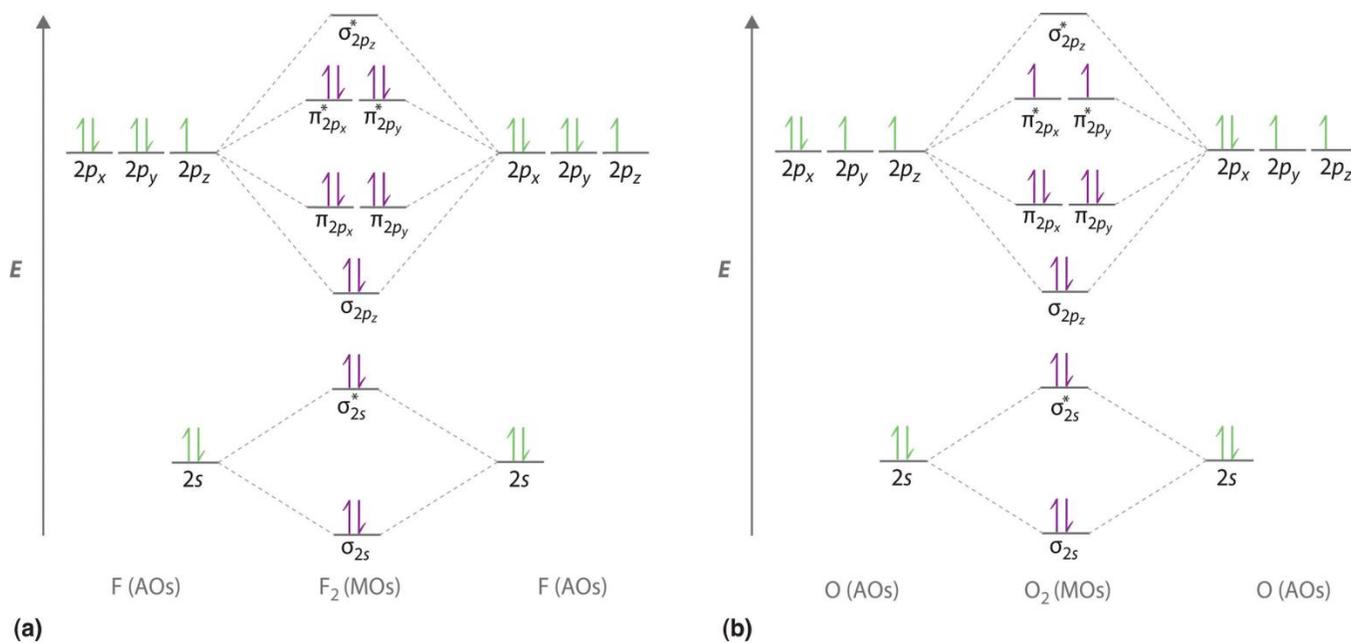Xu, P. (2017, August 02). Functional organization of photosystem II antenna complexes: CP29 under the spotlight. Retrieved from http://www.sciencedirect.com/science/article/pii/S0005272817301135

Appendix

Figure A1: Jablonski Diagram

Excitation from ground state S_0 to higher electronic states S_1 or S_2 shown in light green. Notable

dissipation mechanisms illustrated are internal conversion and vibrational relaxation in yellow,

which release heat, and quenching in purple, which transfers excitation energy to another

molecule. Also critical to photooxidation is intersystem crossing in dark blue, which changes the

electron configuration of the molecule. From, Fluorescence Resonance Energy Transfer (FRET)

Microscopy. (n.d.). Retrieved from https://www.olympus-lifescience.com/en/microscope-

resource/primer/techniques/fluorescence/fret/fretintro/

Figure A2: F_2 and O_2 Molecular Orbitals

The molecular orbitals of F_2 (left) and O_2 (right) are contrasted here. F_2 is singlet since it has no unpaired electrons, while O_2 is triplet since it has two unpaired electrons in its pi-anti bonding orbitals. From, Libretexts. (2018, November 26). 11.5: Molecular Orbital Theory. Retrieved from

https://chem.libretexts.org/Bookshelves/General_Chemistry/Map:_General_Chemistry_(Petrucci
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Ground State

Singlet | Triplet

Energy

HOMO

Ground State
Singlet:
e.g Chl A

SOMO
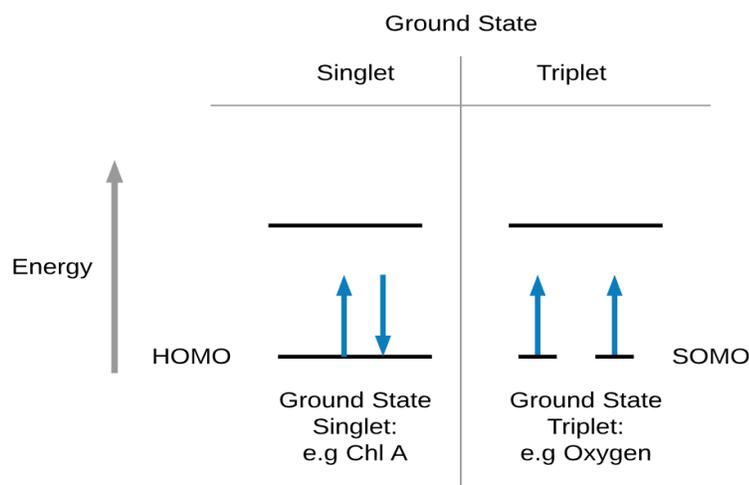
Ground State
Triplet:
e.g Oxygen

Figure A3: Ground state singlet and triplet molecules.

Electron spin indicated by blue arrows. Ground state singlet molecules have paired electrons in their highest occupied molecular orbital (HOMO). Triplet molecules have unpaired electrons in their singularly occupied molecular orbitals (SOMO), making them diradicals. From Leger, 2018.
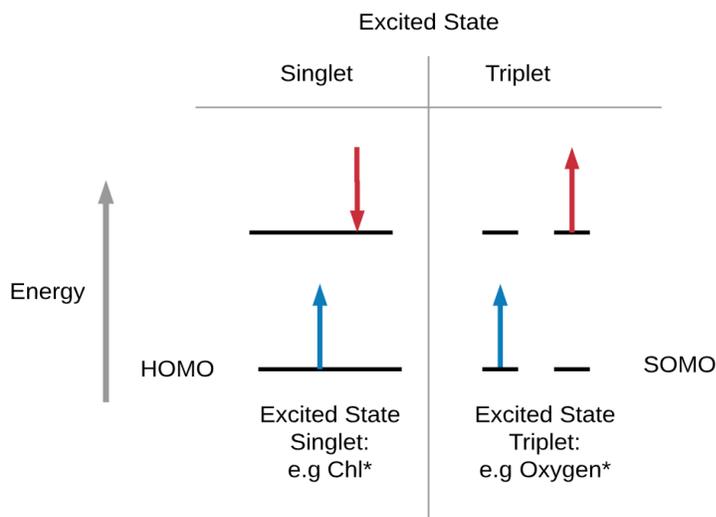
Excited State

Singlet | Triplet

Energy

HOMO

Excited State
Singlet:
e.g Chl*

SOMO

Excited State
Triplet:
e.g Oxygen*

Figure A4: Excited state Singlet and triplet molecules.

Excitation energy causes electron promotion (red electron) to a higher energy orbital. From Leger, 2018.
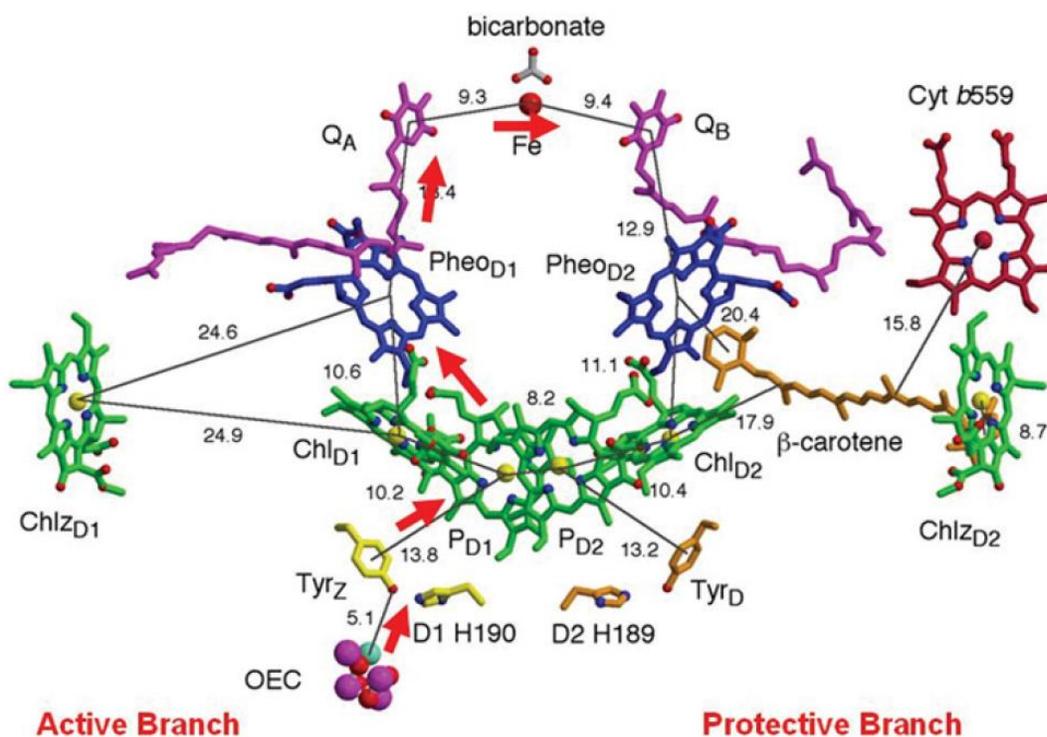
Figure A5: Photosystem II Reaction Center.

The red arrows show electron pathway from water split at the Oxygen Evolving complex (OEC), to tyrosine residue, to P_{680}, to trap Chl_{D1}, to $Pheo_{D1}$, to QA, to QB. After reduction QB detached and joins the PQH2 pool. From, Barber, J. (2006, November). Photosystem II: An enzyme of global significance. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/17052167
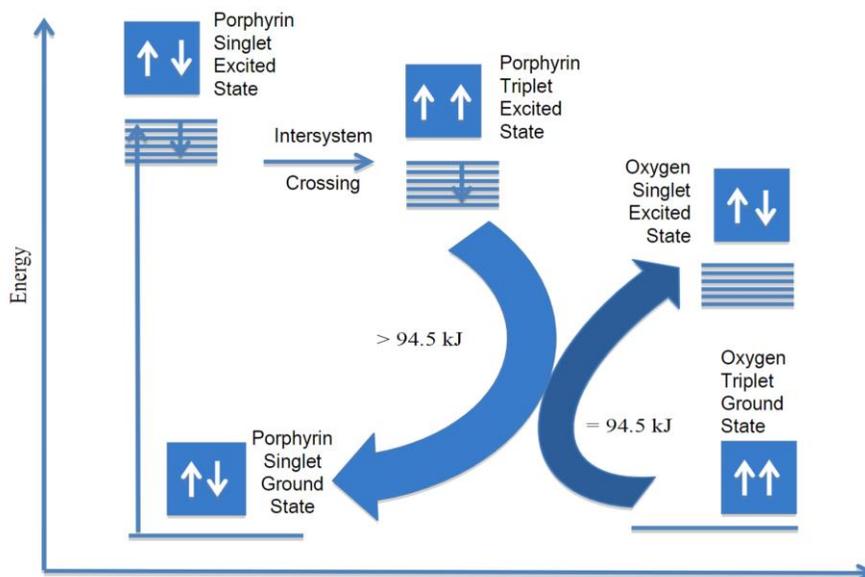
Figure A6: Formation of singlet oxygen by photosensitization.

A porphyrin ring acts a photosensitizer after undergoing intersystem crossing. From, Photodynamic therapy A new light for the developing world. (n.d.). Retrieved from https://www.researchgate.net/publication/308889253_Photodynamic_therapy_A_new_light_for_the_developing_world
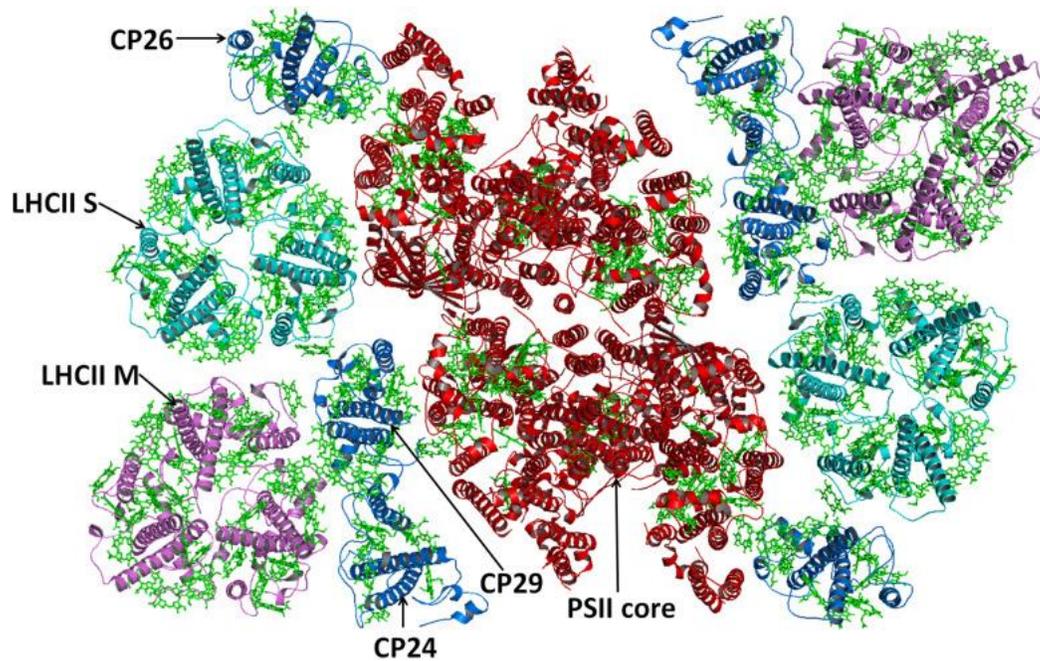
Figure A7: Photosystem II, top view.

The PSII core contains the reaction center where light driven charge separation and water

oxidation occurs. Major light harvesting antenna complexes LHII S and LHII M and minor

complexes CP 24, CP 26 and CP29 are visible. From,

https://www.sciencedirect.com/science/article/pii/S0005272817301135

Explaining terminology: singlet, doublet, and triplet.

Electron pairing refers to coupling of electrons with opposite spin, i.e opposite orientation of the angular momentum, which cancels (paired) or amplifies (unpaired) influence of electrons' magnetic moment on the molecule. Electrons can spin up (ms= +½) or spin down (ms= -½), where ms is the spin quantum number. For a singlet system all spins pair, resulting in total zero spin of the system S=0. In doublet, one unpaired electron results in S=1/2 . In triplets, two unpaired electrons results in total spin S=1. Then, by the spin-angular momentum vector equation L = 2s +1. Singlet, L =1. Doublet, L= 2. Triplet, L=3.