Final report

Award Number: DE-FG02-08ER15989

Recipient Institution: Arizona State University

Project Title: A Combined Genetic, Biochemical, and Biophysical Analysis of the A₁ Phylloquinone Binding Site of Photosystem I from Green Algae

Principal Investigator: Kevin E. Redding

Distribution limitations: none

Note: The original grant supporting this project (DE-FG02-00ER15097) was made to the University of Alabama. After the PI moved to Arizona State University in 2008, the DOE issued a new grant to ASU (DE-FG02-08ER15989) to allow completion of this work. At that time a 'final report' was submitted for the prior grant. This document represents the complete report summarizing all activities on the project funded by both grants.

Executive summary of project accomplishments

This project has resulted in the increase in our understanding of how proteins interact with and influence the properties of bound cofactors. This information is important for several reasons, including providing essential information for the re-engineering of biological molecules, such as proteins, for either improved function or entirely new ones. In particular, we have found that a molecule, such as the phylloquinone used in Photosystem I (PS1), can be made a stronger electron donor by placing it in a hydrophobic environment surrounded by negative charges. In addition, the protein is constrained in its interactions with the phylloquinone, in that it must bind the cofactor tightly, but not in such a way that would stabilize the reduced (negatively-charged) version of the molecule. We have used a combination of molecular genetics, in order to make specific mutations in the region of the phylloquinone, and an advanced form of spectroscopy capable of monitoring the transfer of electrons within PS1 using living cells as the material. This approach turned out to produce a significant savings in time and supplies, as it allowed us to focus quickly on the mutants that produced interesting effects, without having to go through laborious purification of the affected proteins. We followed up selected mutants using other spectroscopic techniques in order to gain more specialized information. In addition to the main project funded by this work, this grant supported several related side-projects that also increased our understanding about related issues (see details below).

Figure 1. Arrangement of the core cofactors of Photosystem I. The pseudo-C₂ symmetry axis is shown as a dotted gray line dividing the two branches. For sake of clarity, the phytol chains of the cofactors have been truncated and the terminal acceptors F_A and F_B are not shown. Reduction of the ec3 Chl(s) occurs in <100 ps, with subsequent electron transfer to the PhQ on a similar timescale. In the nanosecond timescale, the electron is transferred to F_X and then to F_A/F_B. Our main working model is that ET can proceed down either the A-branch or B-branch, leading subsequently to ET from either PhQ_A or PhQ_B with slower or faster kinetics, respectively.

Summary of proposed project

This project focuses on the phylloquinone (PhQ) cofactor of PS1. The primary electron donor (P₇₀₀, a pair of chlorophylls) is connected to the tertiary electron acceptor (F_X, an Fe₄S₄ cluster) by two branches of cofactors. Each branch contains a pair of chlorophylls (ec2 and ec3) and a PhQ (see Fig. 1). These cofactors are bound by two related membrane polypeptides, PsaA and PsaB, in a pseudo-C₂-symmetrical structure. We proposed to use a combination of site-directed mutagenesis and spectroscopic analysis in order to understand better how the PsaA/PsaB

polypeptides of PS1 modulate the properties of the PhQ cofactor. The following hypotheses were listed in the grant proposal as the primary ones that we would test:

- 1) Both branches of cofactors are active, and each PhQ plays a role in reducing F_X .
- 2) The redox properties of the PhQs are due to interaction with residues in PsaA/B.
 - a. These provide them with a hydrophobic environment.
 - b. Nearby (negative) charges lower PhQ's reduction potential.
 - c. Some residues are involved as H-bond donors to the quinone oxygen(s).
- 3) A corollary to the above hypothesis is that differences in the properties of the two PhQs must be due to differences in their environments.

The specific aims of this project were to

- 1) Modify the hydrophobic character of the PhQ niche
- 2) Reduce nearby negative charges
- 3) Evict PhQs from specific sites
- 4) Identify “chlamyquinone” (the quinone used in PS1 by *Chlamydomonas reinhardtii*)
- 5) Perform site-specific reconstitution of tail-channel mutants with tail-less quinones
- 6) Make combinations of mutations to test specific hypotheses

Figure 2. The PhQ_A site, based on the crystal structure of PS1 from *Thermosynechococcus elongatus* (Jordan et al, 2001). Important amino acid residues mentioned in the text are shown, along with a summary of the effects of various mutations made in the course of this project. Note that the numbering used here and throughout the text follows that of *T. elongatus*.

Accomplishments

Combining mutations that alter directionality of ET within PS1 with those that slow reoxidation of PhQ_A (hypothesis 1 & aim 6): We combined two of the mutants that slow ET from PhQ_A (PsaA-W697F and PsaA-S692A) with one that redirects ET from the A-branch to the

B-branch (PsaA-Y696F; Li et al, 2006). The primary reason for doing this was to test our interpretation of the ET kinetics even further. We had a very specific prediction for this combination of mutations: the effects of the mutations would be additive. That is, we would see both the change in amplitudes seen in the PsaA-Y696F mutant, and the slowing of the kinetic component attributed to PhQ_A^- reoxidation seen in the PhQ_A site mutants. As can be seen in Figure 3, this prediction is met in both the PsaA-S692A/Y696F and the PsaA-Y696F/W697F double mutants. This work was published in *Photochemistry and Photobiology* (publication #8).

These results have the exciting implication that it is possible to control independently the yield of reduction of each PhQ (*i.e.* directionality) and their rate of reoxidation. Although this was implied from the previous work on the single mutants, the results from the double mutants demonstrates that this can be done *simultaneously*. In principle, we had no right to expect this with these specific combinations of mutations, given how close they are to each other in the primary and tertiary structure of PS1. The close convergence of the changes in rates and amplitudes seen in the single mutants and double mutants, however, demonstrates just how independent their effects are. Thus, the structure of PS1 may be robust enough to accommodate multiple mutations without experiencing extensive interference from synergistic effects. Although it may be risky to over-generalize this phenomenon, these results at least open the possibility of re-engineering the internal working of PS1 in a controlled manner.

Figure 3. Pump-probe spectroscopic characterization of single and double mutant PS1 *in vivo*. Semi-log plot in ns- μ s timescale of absorption decay at 380 nm, which corresponds to reoxidation of PhQ_B^- (usually ~ 20 ns) and PhQ_A^- (usually ~ 200 ns) (Guergova-Kuras et al, 2001). Panel A: comparison of PsaA-Y696F (diamonds), PsaA-W697F (squares), and PsaA-Y696F/W697F (circles). Panel B: comparison of PsaA-Y696F (diamonds), PsaA-S692A (squares), PsaA-S692A/Y696F (circles). Taken from Publication #8 (Santabarbara et al, 2008).

Eviction of PhQs (aims 3 and 5): We have evidence that the PsaA-L722Y mutation causes eviction of the quinone from the PhQ_A site, due to steric clash between the Tyr and the phytyl chain of the quinone. These include: (1) lower amplitude of the ~ 200 -ns component (Fig. 4); (2) altered DAS for the faster decay component (Fig. 4); and (3) lowered amplitude of the $4\text{-}\mu\text{s}$ decay component corresponding to re-reduction of P_{700}^+ (normalized to the initial bleaching). We have shown that the faster DAS is likely a superposition of the ~ 20 -ns decay component plus a new component corresponding to back-reaction from the $\text{P}_{700}^+ \text{ec}_3^-$ state, which should take place in the 10-30 ns timescale. We purified PS1 from this mutant and the corresponding B-side mutation (PsaB-L706Y) and we have repeated these measurements with the particles. Dr. Santabarbara also quantified the amount of $^3\text{P}_{700}$ triplet formed by charge recombination in PS1 RCs in which forward ET from ec_3 is blocked, and found that the PsaA-L722Y mutant had a significantly increased amount of $^3\text{P}_{700}$ triplet. We estimate, based all these measurements, that

approximately 20-40% of the PhQ_A sites in the Psa-L722Y mutant are empty. The incomplete nature of the eviction precluded doing some of the experiments we had planned on doing (*e.g.* specific aim 5), and we thus decided to drop further work on this mutant. (In addition, the fact that it was unstable and poorly accumulated in the cell made it very difficult to work with.)

Figure 4. Decay-associated spectra of fitted kinetic components with $\tau_1=10-30$ ns (A) or $\tau_2=100-260$ ns (B) in WT strain (thick solid lines), PsaA-L722Y (open diamonds, dashed lines), PsaA-L722T (open stars, dash-dotted lines), and PsaB-L706Y (solid circles, dotted lines). Spectra were internally normalised on bleaching (430 nm) of the $t=0$ spectrum. Taken from Publication #6 (Santabarbara et al, 2010).

Properties of the two PhQ-binding sites (hypotheses 2 and 3): One of our central hypotheses is that the hydrophobic environment of the PhQ niche is key to making it so reducing. We have made mutations of the key hydrophobic residues that surround the 2-methylnaphthoquinone headgroup of PhQ_A:

- 1) PsaA-Trp693: to Phe (our original mutation), Leu, Ala
- 2) PsaA-Phe685: to Trp, His, Asn
- 3) PsaA-Leu718: to Thr, Tyr, Trp

Some of these (PsaA-W697A, PsaA-L722W) did not accumulate PS1, which is a common occurrence and likely related to problems with assembly. We have two very exciting results.

One was the first mutation that actually *accelerates* this ET step: PsaA-L722T. Description of this mutant was combined with that of PsaA-L722Y in publication #6 (Santabarbara et al, 2010). Although the acceleration was rather modest (*i.e.* ~ 2 -fold; see Fig. 5), it uncovered a phenomenon that we had not previously observed: inter-quinone electron transfer. Since the reduction potential of PhQ_A is more positive than that of PhQ_B or F_X, ET from PhQ_B to F_X can be followed by ET from F_X to either F_A/F_B or PhQ_A. The latter event results in an apparent increase in the amount of ET from PhQ_A. Thus, in the past we have overestimated the amount of ET from PhQ_A and underestimated the amount of ET from PhQ_B. This hypothesis explained very nicely the result that the proportion of ET from PhQ_B seemed to increase in the PsaA-L722T mutant (Figures 4 and 5).

Figure 5. Schematic representation of the energetics of PhQ^+ oxidation, and resulting simulation of population evolution in three cases: WT, PsaA-L722T (isoenergetic scenario), and PsaA-L722T (exergonic scenario). The dashed grey lines in latter panels are the simulation of total PhQ^+ population evolution for the WT, in order to allow direct comparison. **Energetic Schemes:** black solid lines: WT levels; dashed-grey lines show the suggested mutation-induced shift of standard midpoint potentials. The black arrows show the ET reactions included in the simulations, also shown are the *inverse* values of the calculated rate constants (units of ns). **Kinetic simulations:** Population evolution of total PhQ^+ (solid black lines), PhQ_A^+ (dashed red line), PhQ_B^+ (dashed blue line), and F_x (dash-dotted golden lines). The dashed grey line is total PhQ^+ in the WT. Also shown are simulated observable lifetimes (τ_i) and relative amplitudes (A_i) for the total PhQ^+ population. Taken from Publication #6 (Santabarbara et al, 2010).

Dr. Santabarbara was able to simulate the data from several other mutants very satisfactorily. As it turns out, lowering the reduction potential of PhQ_A (even by a relatively small amount) has a large effect on the amount of inter-quinone ET, but raising the reduction potential has a much smaller effect, since this potential is already more positive than that of F_x . This explains why we missed it in our earlier analyses of mutants that slowed ET from PhQ_A . The effect is impossible to ignore, however, in the PsaA-F689A mutant, where the reduction potential of PhQ_A becomes so high that it visibly affects the relative proportion of ET from PhQ_A and PhQ_B (see below). Interestingly, in the PsaB-L706Y mutant, we saw a similar effect as in the PsaA-L722T mutant. In this case, however, ET became so fast that we could hardly see it, as much of it was occurring before the first time point we could measure, resulting in an apparent decrease in the amplitude of the component with decay time below 20 ns (Fig. 4).

We have one more paper to publish on further analysis of the PsaA-L722T mutant (Publication #2; Mula et al, 2012). In collaboration with the groups of Art van der Est (Brock Univ.) and Gary Hastings (Georgia State Univ.), we have performed time-resolved EPR experiments and theoretical modeling. The EPR experiments indicate that the hyperfine coupling to the 2-methyl protons becomes *larger* in the PsaA-L722T mutant, as the low-field should become more distinct (Fig. 6A). This was exactly contrary to our expectations – we thought that the faster rate of forward ET from PhQ_A indicated a weaker H-bond from the PsaA-Leu722 amide, which would be accompanied by a *smaller* hyperfine coupling to the 2-methyl group. The modeling studies might explain this discrepancy – they indicate that the introduced Thr residue may, on occasion, serve as an alternate H-bond donor to PhQ_A (Fig. 6B).

Figure 6 Analysis of the PsaA-L722T mutant. **A:** X-band TREPR spectra taken at 80K. The spectra are the average signal intensity in a 400-ns wide time window centered at 850 ns after the laser flash. **B:** Histograms of possible H-bond distances from molecular dynamics simulations of PS1.

In our studies of the PsaA-Leu722 substitution mutants, we joined forces with the group of John Golbeck (Penn. State Univ.), who had made a similar mutant in the cyanobacterium, *Synechocystis* sp. PCC6803 (S.6803): PsaA-L722W. Although this mutation precluded assembly of PS1 in the green alga *C. reinhardtii*, in the cyanobacterium it was stable enough to work with. We found a similar story for the kinetics in PsaA-L722W as we had seen in the PsaA-L722T algal mutant. Reoxidation of PhQ_A was accelerated by a factor of 2-3. We also saw a similar redistribution of the amplitudes to favor the fast component, going from 1:4 (fast:slow) in WT to almost 1:1 in the PsaA-L722W mutant at 480 nm (Publication #5; Srinivasan et al, 2011)

Our second class of mutants comprise substitutions at PsaA-Phe689. This brings us to our second major discovery: we have a new record for the effect of a mutation upon the rate of ET from PhQ_A to F_x – the PsaA-F689N mutation results in a 100-fold retardation. In this mutant, the $\text{PhQ}_A \rightarrow F_x$ step is the slowest one in PS1, which is a unique situation. The PsaA-F689W mutant slows ET down 10-fold, which is currently the second largest effect. We are currently writing a manuscript describing the PsaA-Phe689 mutants (Publication #1; Santabarbara et al, 2012).

Figure 7. Decay of absorption at 480 nm in ns- μ s timescale as a semi-log plot. This wavelength corresponds to bandshifts of pigments near the PhQs, as well as P_{700}^+ ; thus, it monitors reoxidation of PhQ_B^- (usually ~ 20 ns) and PhQ_A^- (usually ~ 200 ns) and re-reduction of P_{700}^+ (usually ~ 5 μ s) (Guergova-Kuras et al, 2001). Decay kinetics are presented for WT (black squares), and the mutants PsaA-L722T (green triangles), PsaA-F689W (red circles), and PsaA-F689N (blue diamonds).

Temperature dependence of ET reactions in PS1 *in vivo*

We performed an initial temperature-dependence study to understand the role of temperature in the initial ET processes in living cells (Publication #9; Santabarbara et al, 2009). Although the focus of this paper was on the reduction of P_{700}^+ by plastocyanin *in vivo*, we were able to estimate the activation energy of forward ET from PhQ_A and PhQ_B to be 107-130 meV and 8-47 meV, respectively. These agree reasonably well with the estimates of 65-110 meV and 7-15 meV, obtained in work with purified cyanobacterial PS1 *in vitro* using a more extensive temperature range (Agalarov and Brettel, 2003).

Work on *Acaryochloris marina*

Dr. Santabarbara did some pump-probe work on the unusual cyanobacterium, *Acaryochloris marina*, which is unique in that it uses the pigment chlorophyll *d* as its major pigment, which absorbs lower energy light than Chl *d*. It also has a naturally occurring ‘mutation’ in the axial ligand to the ec3_B cofactor from Met to Leu. This is a mutation that we had analyzed previously and showed that it resulted in inhibition of charge separation on the affected branch (Publication #9; Byrdin et al, 2006). Because of this fact, it had earlier been suggested that ET within *A. marina* PS1 would be unidirectional, using the A-branch exclusively. We have disproved this hypothesis. Surprisingly, Stefano found that not only does it maintain use of the B-branch, but it might use the B-branch more than in other organisms. Alternatively, it might be simply easier to see B-side ET in *A. marina*, due to the fact that forward ET from the two PhQs is substantially slower, with decay times of about 85-90 and 350 ns (Fig. 8), instead of the time more typical of cyanobacteria (*i.e.* ~ 15 -20 and ~ 250 -300 ns). At this time, we have no good explanation for the

slower ET from PhQ_B. This is a subject that Dr. Santabarbara will pursue in his independent career. These results were just published in *BBA* (Publication #3; Santabarbara et al, 2012).

Figure 8. Decay associated spectra (DAS) derived from global analysis of laser flash-photolysis kinetics in whole cells of *A. marina* (A) and *Synechocystis* sp. PCC 6803 (B). Panel A (*A. marina*) and B (*Synechocystis*) show the DAS of the sub-microsecond decay phases. Empty squares: “fast” ns component; Filled circles: “slow” ns component; dash-dotted line is the sum of the two phases. Taken from Publication #3 (Santabarbara et al, 2012)

Double reduction of plastoquinone in PS1 (related to specific aims 3, 5, and 6)

We purified PS1 from the *menD1* mutant, which is unable to synthesize phylloquinone, so plastoquinone (PQ) occupies the quinone-binding site instead (Lefebvre-Legendre et al, 2007). Dr. Michael McConnell discovered that repetitive flashes of these particles would result in the double reduction of the incorporated PQ to plastoquinol (PQH₂). The process was accelerated by lowering pH, indicating that a protonation step is likely limiting double reduction (Fig. 9). Addition of the PsaA-L722T mutation also sped up PQH₂ production, lending further support to the idea that the H-bond from the PsaA-Leu722 amide N, although it slows forward ET from the quinone, also helps to blocks its protonation, thereby protecting it from double reduction. This was published in *Biochemistry* last month (Publication #4; McConnell et al, 2011).

Identification of ‘chlamyquinone’ (specific aim 4)

We had produced preliminary data indicating that the major naphthoquinone in *C. reinhardtii* was not actually phylloquinone, but a hydroxylated version of phylloquinone, most likely the 5-monohydroxyphylloquinone seen previously in *Euglena* (Ziegler et al, 1989). Our work was interrupted during the move to ASU, and then 2 years ago I found that a similar project examining the naphthoquinones of multiple algal species had been taken up by my colleague, Dr. Yuichiro Takahashi (Okayama Univ.), and that they were much further along than we were at the time. (He did not know that we had been working on it.) Accordingly, we dropped that project. Their paper was just published (Ozawa et al., 2012) and agrees perfectly with our data.

Figure 9. Photo-inactivation of PS1 particles purified from *C. reinhardtii* cells (WT and PsaA-L722T backgrounds) harboring either PhQ (A) or PQ (B) in the A_1 -binding pocket assayed at pH 8 (open symbols) or 5.5 (closed symbols). PS1 particles in assay buffer at pH 8 were flashed at 15 Hz with saturating laser flashes (532 nm, 6 ns duration) for the indicated time, and then periodically assayed with a single flash after a 1-min dark recovery period. A 20-min dark recovery followed each illumination period (indicated by vertical dotted line). Average of three independent experiments $\pm$ the standard error. Taken from Publication #4 (McConnell et al, 2011).

Human Resources:

One cannot help but notice the ubiquity of Stefano Santabarbara's contribution to this project. He was an extremely talented and ambitious postdoc, the best I have ever had. Fortunately, in the summer of 2010 he was able to obtain a permanent position as a researcher and group leader at the Institute of Biophysics in Milano. (Stefano is Italian, but as is widely known, obtaining a research position in Italy is extremely difficult, especially for someone who has spent as many years abroad as Stefano did.) I am extremely gratified that this project resulted in boosting the career of such a promising young scientist.

In the lab currently is a new postdoctoral fellow, Dr. Michael McConnell, who is the first author on a paper published last month. He is also very ambitious and hard-working and will be seeking a job soon.

Publications produced during the life of this project

Peer-reviewed articles

1. Santabarbara, S., F. Rappaport, and K.E. Redding. (2012). Mutations of PsaA-Phe689 have drastic effects upon forward electron transfer from PhQ_A to F_X in *Chlamydomonas reinhardtii*. *Manuscript in prep.*
2. Mula, S., M.D. McConnell, A. Ching, H. Gordon, G. Hastings, K.E. Redding and A. van der Est. (2012). Introduction of a Hydrogen Bond between Phylloquinone A_{1A} and a Threonine Side-chain OH Group in Photosystem I. *Manuscript in prep.*
3. Santabarbara, S., B. Bailleul, K.E. Redding, J. Barber, F. Rappaport, and A. Telfer. (2012). Kinetics of phyllosemiquinone oxidation in the Photosystem I reaction centre of *Acaryochloris marina*. *Biochim. Biophys. Acta*. **1817**:328-35.
4. McConnell, M.D., J.B. Cowgill, P.L. Baker, F. Rappaport and K.E. Redding*. Double Reduction of Plastoquinone to Plastoquinol in Photosystem I. *Biochemistry* **50**:11034-46.
5. Srinivasan, N., S. Santabarbara, F. Rappaport, D. Carbonera, K. Redding, A. van der Est, J.H. Golbeck. (2011). Alteration of the H-Bond to the A(1A) Phylloquinone in Photosystem I: Influence on the Kinetics and Energetics of Electron Transfer. *J. Phys. Chem. B* **115**:1751-9.
6. Santabarbara, S., K. Reifschneider, A. Jasaitis, F. Gu, G. Agostini, D. Carbonera, F. Rappaport, K.E. Redding*. (2010). Inter-quinone electron transfer in Photosystem I as evidenced by altering the hydrogen bond to the phylloquinone(s). *J. Phys. Chem. B* **114**:9300-12.
7. Santabarbara, S., K.E. Redding, F. Rappaport. (2009). Temperature dependence of the reduction of P₇₀₀⁺ by tightly bound plastocyanin *in vivo*. *Biochemistry*, **48**:10457-66.
8. Santabarbara, S., A. Jasaitis, F. Gu, F. Rappaport, K.E. Redding. (2008). Additive Effect of Mutations Affecting the Rate of Phylloquinone Reoxidation and Directionality of Electron Transfer within Photosystem I. *Photochem. Photobiol.*, **84**:1381-7.
9. Byrdin, M., Santabarbara, S., Gu, F., Fairclough, W., Heathcote, P., Redding, K. and Rappaport, F. (2006) Assignment of a kinetic component to electron transfer between iron-sulfur clusters F_X and F_{A/B} of Photosystem I. *Biochim. Biophys. Acta*, **1757**, 1529-38.
10. Petrenko, A., K. Redding, and L. Kispert (2005). The influence of the structure of the radical cation dimer pair of aromatic molecules on the principal values of a g-tensor: DFT predictions. *Chem. Phys. Lett.* **406**, 327-331.
11. Petrenko, A. and K. Redding* (2004). Intermolecular Electron Transfer and Exchange Integrals in Photosystem I. *Chem. Phys. Lett.* **400**, 98-103.
12. Li, Y., M.-G. Lucas, T. Konovalova, B. Abbott, F. MacMillan, A. Petrenko, V. Sivakumar, R. Wang, G. Hastings, F. Gu, J. van Tol, L.-C. Brunel, R. Timkovich, F. Rappaport, and K. Redding* (2004). Mutation of the putative hydrogen-bond donor to P₇₀₀ of Photosystem I. *Biochemistry* **43**, 12634-47.
13. Petrenko, O., A.L. Maniero, J. van Tol, F. MacMillan, Y. Li, L.-C. Brunel, and K. Redding* (2004). A high-field EPR study of P₇₀₀⁺⁺ in wild-type and mutant Photosystem I from *Chlamydomonas reinhardtii*. *Biochemistry* **43**, 1781-1786.
14. Konovalova, T.A., L.D. Kispert, and K. Redding* (2004). Photo- and chemically-produced phylloquinone biradicals: EPR and ENDOR study. *J. Photochem. Photobiol. A* **161**, 255-260.
15. Wang, R., V. Sivakumar, Y. Li, K. Redding and G. Hastings (2003). Mutation Induced Modulation of Hydrogen Bonding to P700 Studied Using FTIR Difference Spectroscopy. *Biochemistry* **42**, 9889-9897.

16. Guergova-Kuras, M., B. Boudreaux, A. Joliot, P. Joliot and K. Redding* (2001). Evidence for two active branches for electron transfer in photosystem I. *Proc. Natl. Acad. Sci.* **98**, 4437-4442.
17. Boudreaux, B., MacMillan, F., Teutloff, C., Agalarov, R., Gu, F., Grimaldi, S., Bittl, R., Brettel, K., and Redding, K.* (2001). Mutations in both sides of the Photosystem I reaction center identify the phylloquinone observed by electron paramagnetic resonance spectroscopy. *J. Biol. Chem.*, **276**, 37299-37306.

Book chapters

18. Redding, K. (2008). Photosystem I. In D. Stern (ed.), *The Chlamydomonas Sourcebook: Volume 2*. Academic Press.
19. Redding, K.* and A. van der Est. (2006). The Directionality of Electron Transfer in Photosystem I. In J. Golbeck (ed.), *Photosystem I: The Plastocyanin:Ferredoxin Oxidoreductase in Photosynthesis*. Kluwer Academic Publishers, Dordrecht. pp. 413-438.
20. F. Rappaport, B. Diner, and K. Redding. (2006). Optical measurements of secondary electron transfer in Photosystem I. In J. Golbeck (ed.), *Photosystem I: The Plastocyanin:Ferredoxin Oxidoreductase in Photosynthesis*. Kluwer Academic Publishers, Dordrecht. pp. 223-244.

Proceedings

21. Byrdin, M., Cohen, R., Fairclough, W., Gu, F., Golbeck, J., Heathcote, P., Redding, K. and Rappaport, F. (2005) Secondary Electron Transfer In Photosystem I: What Transient Absorption Can Tell. In van der Est, A. and Bruce, D. (eds.), *Photosynthesis: Fundamental Aspects to Global Perspectives*. Allen Press, Lawrence, Kansas. pp. 36-38.
22. Gu, F., Byrdin, M., Rappaport, F., van der Est, A., MacMillan, F. and Redding, K. (2005) Mutational analysis of the two phylloquinones in Photosystem I. In van der Est, A. and Bruce, D. (eds.), *Photosynthesis: Fundamental Aspects to Global Perspectives*. Allen Press, Lawrence, Kansas. pp. 101-103.
23. K. Redding*, R. Bittl, F. MacMillan, C. Teutloff, F. Gu, S. Grimaldi, and B. Boudreaux. Mutational analysis of the binding sites of the phylloquinones in PS I. in *PS2001 proceedings: 12th International Congress on Photosynthesis*, CSIRO Publishing, Collingwood
24. K Gibasiewicz, VM Ramesh, S Lin, K Redding, NW Woodbury, AN Webber. (2001). Mutations of ligands to connecting chlorophylls perturbs excitation dynamics in the core antenna of PSI from *Chlamydomonas reinhardtii*. in *PS2001 proceedings: 12th International Congress on Photosynthesis*, CSIRO Publishing, Collingwood
25. P Heathcote, IP Muhiuddin, SEJ Rigby, S Carter, S Purton, F Gu, K Redding and MCW Evans. Evidence for two functional phylloquinones in Photosystem I from *Chlamydomonas reinhardtii* in *PS2001 proceedings: 12th International Congress on Photosynthesis*, CSIRO Publishing, Collingwood

Other References used:

- Agalarov, R. and K. Brettel (2003). Temperature dependence of biphasic forward electron transfer from the phylloquinone(s) A(1) in photosystem I: only the slower phase is activated. *Biochim. Biophys. Acta* **1604**: 7-12.

Guergova-Kuras M, Boudreaux B, Joliot A, Joliot P, Redding K (2001) Evidence for two active branches for electron transfer in photosystem I. *Proc Natl Acad Sci U S A* **98**: 4437-4442.

Jordan P, Fromme P, Witt HT, Klukas O, Saenger W, Krauss N (2001) Three-dimensional structure of cyanobacterial photosystem I at 2.5 Å resolution. *Nature* **411**: 909-917.

Lefebvre-Legendre, L.; Rappaport, F.; Finazzi, G.; Ceol, M.; Grivet, C.; Hopfgartner, G.; Rochaix, J. D. (2007). Loss of phylloquinone in *Chlamydomonas* affects plastoquinone pool size and photosystem II synthesis. *J Biol Chem* **282**:13250-63.

Li Y, van der Est A, Lucas MG, Ramesh VM, Gu F, Petrenko A, Lin S, Webber AN, Rappaport F, Redding K (2006) Directing electron transfer within Photosystem I by breaking H-bonds in the cofactor branches. *Proc Natl Acad Sci U S A* **103**: 2144-2149

Ozawa, S.; Kosugi, M.; Kashino, Y.; Sugimura, T.; Takahashi, Y. (2012) 5'-Monohydroxyphylloquinone is the Dominant Naphthoquinone of PSI in the Green Alga *Chlamydomonas reinhardtii*. *Plant Cell Physiol* **53**: 237-43.

Ziegler, K.; Malderner, I.; Lockau, W. (1989) 5'-Monohydroxyphylloquinone as a component of Photosystem I. *Naturforsch. C* **44**:468-472.

Abbreviations used:

Chl, chlorophyll; DAS, decay-associated spectrum/spectra; ELDOR, electron- electron double resonance; ENDOR, electron-nuclear double resonance; EPR, electron paramagnetic resonance; ET, electron transfer; F_x, an Fe-S cluster serving as tertiary electron acceptor; PhQ, phylloquinone; P₇₀₀, pair of chlorophylls serving as primary electron donor; TR, time-resolved