ELECTRON EXTRACTION FROM THE $A_{1A}$ AND $A_{1B}$ SITES OF PHOTOSYSTEM I

A Dissertation in
Chemistry
by
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ABSTRACT

This dissertation describes an analysis of the kinetic properties of cyanobacterial Photosystem I (PS I) and how the principles learned can be applied for the general production of biofuels. First, I describe development of a robust and unique organic/inorganic photobiochemical nanoconstruct formed by the coupling of PS I and an inorganic thiolate stabilized Pt nanoparticle via a molecular wire. PS I serves to harvest and store light energy, while the Pt nanoparticle catalyzes the reduction of two protons to dihydrogen utilizing the electrons donated from PS I. While the idea to use a molecular wire to facilitate electron extraction from PS I is not new, this is the first successful work in which these two modules are tethered from the A_{1A} and A_{1B} (quinone) sites instead of from the terminal F_{B} iron-sulfur cluster. Using the menB mutant from PS I that contains a displaceable plastoquinone in the A_{1A} and A_{1B} binding sites, I reconstituted the site with a phylloquinone-like molecule that contains a terminated thiol bound to a Pt nanoparticle. I investigate how this system functions, as electron transfer to the Pt nanoparticle is unfavorable relative to both forward electron transfer and charge recombination. I proposed that dihydrogen production is made possible by suppressing the charge recombination channel via rapid reduction of P_{700}^{+} from an external donor. This proposal was tested by starting with intact PS I and sequentially removing the iron-sulfur clusters, measuring the rate of P_{700}^{+} reduction, and correlating dihydrogen production to a specific concentration of cytochrome c_{6}. Second, I investigate the electron transfer properties of PS I embedded in a room temperature glass provided by trehalose. I show that not only does immobilization have a significant impact on the electron transfer rates, but also that the
induced changes are nearly identical to those seen in low temperature glasses provided by glycerol. This method provides a unique opportunity to mimic low temperatures effects on PS I at room temperature as well as providing a means for long-term stabilization of protein function at room temperature.
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<table>
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<th>Description</th>
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<td>(NQ(CH₂)₁₅S)₂</td>
<td>1-[15-(3-Methyl-1,4-naphthoquinone-2-yl)]pentadecyl Disulfide</td>
</tr>
<tr>
<td>A₀</td>
<td>primary electron acceptor in Photosystem I</td>
</tr>
<tr>
<td>A₁A</td>
<td>A-side quinone intermediate electron acceptor in Photosystem I</td>
</tr>
<tr>
<td>A₁B</td>
<td>B-side quinone intermediate electron acceptor in Photosystem I</td>
</tr>
<tr>
<td>Asc</td>
<td>sodium ascorbate</td>
</tr>
<tr>
<td>β-DM</td>
<td>β-dodecylmaltoside</td>
</tr>
<tr>
<td>Chl</td>
<td>chlorophyll</td>
</tr>
<tr>
<td>Cyt c₆</td>
<td>cytochrome c₆</td>
</tr>
<tr>
<td>CW EPR</td>
<td>constant-wave electron paramagnetic resonance</td>
</tr>
<tr>
<td>DCPIP</td>
<td>2,6-dichlorophenolindophenol</td>
</tr>
<tr>
<td>EPR</td>
<td>electron paramagnetic spectroscopy</td>
</tr>
<tr>
<td>Fₐ</td>
<td>terminal iron-sulfur cluster acceptor in Photosystem I</td>
</tr>
<tr>
<td>Fₐ</td>
<td>terminal iron-sulfur cluster acceptor in Photosystem I</td>
</tr>
<tr>
<td>FeS</td>
<td>Iron-sulfur cluster</td>
</tr>
<tr>
<td>Fₓ</td>
<td>intermediate iron-sulfur cluster acceptor in Photosystem I</td>
</tr>
<tr>
<td>menB</td>
<td>Photosystem I where plastoquinone-9 occupies the A₁ site</td>
</tr>
<tr>
<td>menB/rubA</td>
<td>Photosystem I containing plastoquinone-9 and devoid of FeS clusters</td>
</tr>
<tr>
<td>menB/PsaC</td>
<td>Photosystem I containing plastoquinone-9 and devoid of the PsaC, PsaD, and PsaE subunits</td>
</tr>
<tr>
<td>Nd:YAG</td>
<td>neodymium-doped yttrium aluminum garnet</td>
</tr>
<tr>
<td>NP</td>
<td>nanoparticle</td>
</tr>
<tr>
<td>PQ-9</td>
<td>plastoquinone-9</td>
</tr>
<tr>
<td>Pt</td>
<td>platinum</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>P&lt;sub&gt;700&lt;/sub&gt;</td>
<td>primary electron donor of Photosystem I</td>
</tr>
<tr>
<td>P&lt;sub&gt;700&lt;/sub&gt;-A&lt;sub&gt;1&lt;/sub&gt; core</td>
<td>Photosystem I lacking the F&lt;sub&gt;X&lt;/sub&gt;, F&lt;sub&gt;A&lt;/sub&gt;, and F&lt;sub&gt;B&lt;/sub&gt; clusters</td>
</tr>
<tr>
<td>P&lt;sub&gt;700&lt;/sub&gt;-F&lt;sub&gt;X&lt;/sub&gt; core</td>
<td>Photosystem I lacking PsaC, PsaD, and PsaE subunits</td>
</tr>
<tr>
<td>P&lt;sub&gt;700&lt;/sub&gt;-F&lt;sub&gt;A&lt;/sub&gt; core</td>
<td>Photosystem I lacking the terminal F&lt;sub&gt;B&lt;/sub&gt; cluster</td>
</tr>
<tr>
<td>PMS</td>
<td>phenazine methosulfate</td>
</tr>
<tr>
<td>PS I</td>
<td>Photosystem I</td>
</tr>
<tr>
<td>PS II</td>
<td>Photosystem II</td>
</tr>
<tr>
<td>TEM</td>
<td>transmission electron microscopy</td>
</tr>
<tr>
<td>WT</td>
<td>wild type</td>
</tr>
<tr>
<td>TrEPR</td>
<td>transient electron paramagnetic resonance</td>
</tr>
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</table>
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DISSERTATION OVERVIEW

The chapters found herein discuss the creation and analysis of a novel tethering mechanism of a biological/inorganic nanoconstruct between Photosystem I (PS I) and a Pt nanoparticle (Pt-NP) that can produce dihydrogen upon illumination in the presence of electron donors. This system is also characterized mechanistically in terms of electron transfer to the NP.

Chapter 1 details the fundamental properties of PS I and why it is a promising choice for solar energy conversion. Theory is then put into practice by showing the many instances of in which PS I has been successfully used to generate dihydrogen.

Chapter 2 introduces my first successful attempt at wiring PS I to a Pt-NP through the A_{1A} and A_{1B} quinone-binding sites. First, it is demonstrated by time-resolved optical spectroscopy and transient EPR (TrEPR) spectroscopy that the quinone, \((\text{NQ(CH}_2)_5\text{S})_2\), is able to displace plastoquinone in the \textit{menB} mutant nearly quantitatively. Further, even with the Pt-NP attached, the synthesized quinone is able to occupy the A_{1A} (and likely A_{1B}) sites. This construct is then shown to produce dihydrogen upon illumination. It is demonstrated that dihydrogen is only produced using concentrations of cytochrome \(c_6\) that are sufficient to suppress charge recombination with \(P_{700}^+\).

Chapter 3 expands on the model described in Chapter 2. The terminal FeS clusters are sequentially removed to generate \(P_{700}-F_A\) cores, \(P_{700}-F_X\) cores, \(P_{700}-A_1\) cores. Each of these PS I cores is tested by time-resolved optical spectroscopy and TrEPR to ensure quinone incorporation. \(P_{700}^+\) reduction and dihydrogen production are measured as a function of cytochrome \(c_6\) concentration for each sample, again showing that dihydrogen is only produced when charge recombination with \(P_{700}^+\) is suppressed. It is demonstrated that even though the electron is transferred to the Pt-NP through the A_{1A} and A_{1B} sites, the electron has the redox potential of the terminal FeS cluster.
Chapter 4 describes the first attempts at measuring PS I kinetics in trehalose, a room temperature glass. Purified PS I is dissolved into a concentrated solution of trehalose and allowed to dry, forming a glass at room temperature. These PS I embedded glasses are analyzed using time-resolved optical spectroscopy at 830 nm and 480 nm at nanosecond resolution to measure the rate of charge recombination with P$_{700}^+$ and forward electron transfer from A$_1A$ and A$_1B$ to F$_X$, respectively. It is shown that the kinetics of electron transfer are significantly impacted by the immobilization of PS I and that the resulting kinetics are similar to those found in low temperature glasses.

Chapter 5 summarizes the important aspects of the previous chapters in terms of their applicability to practical devices. Additionally, the impact of this work is discussed in terms future experiments and how the design principles learned in this work can be applied to other light-harvesting systems.
Chapter 1

General Introduction
1.1 PROMISE AND CHALLENGES OF SOLAR ENERGY CONVERSION

As energy demands continually increase, so too must our ability to meet those demands. By 2040, the U.S. Energy Information Administration (EIA) estimates that worldwide energy consumption will increase 56% from current levels. Presently, the vast majority of our energy demands are met through the burning of fossil fuels. As our finite supply of these resources continues to dwindle, it is imperative that a cheap and readily available source of energy is found. One particularly attractive option is the free and virtually inexhaustible energy provided by the sun.

The problem of converting solar energy into stored chemical energy has an interesting history. In artificial systems, semiconductors such as TiO$_2$ are used as a means to harvest light energy. Absorption of a photon leads to an electron/hole pair, whereafter the electron and hole migrate toward the conduction and valence bands, respectively. If coupled to an appropriate catalyst, electrons in the conduction band can be transferred to do useful work. This idea has been successfully applied to semi-conductors such as TiO$_2$ and Al$_2$O$_3$, where dihydrogen is produced upon illumination when coupled to a catalyst$^{2-4}$. More recently, promising work involving water-splitting dye sensitized photoelectrochemical cells demonstrates the potential for light-induced water splitting when coupling a TiO$_2$ semiconductor to IrO$_2$$^{5,6}$.

While this work is encouraging, attempts at light harvesting and solar energy conversion from purely artificial means suffer from several practical limitations. (i) Most photo-reactive semiconductors to date require low abundance metals such as ruthenium and iridium, making them prohibitively expensive for large scale implementation. (ii) In order for electrons to enter the conduction band and perform useful work, light with energy greater than or equal to the bandgap must be absorbed. In systems based on TiO$_2$, this band gap is 3.2 eV, requiring light of wavelengths shorter than 350 nm. To combat this fundamental limitation, organic and inorganic dyes have been used to increase the spectral absorptive range of these photoelectrochemical cells, which
successfully expand the range of useable light from the near-UV to the visible region. However, the dyes themselves are often unstable upon long-term exposure to light and oxygen (in the case of organic dyes) or they require expensive materials such as bis(2,2’-bipyridine)(4,4’-diphosphonato-2,2’bipyridine)ruthenium(II). (iii) Artificial systems can suffer from low quantum yields (<1%) due to rapid charge recombination events. Overcoming these limitations has proven challenging, but one needs only look for inspiration from natural photosynthetic systems.

1.2 NATURAL SOURCES OF SOLAR ENERGY CONVERSION

1.2.1 Description of Photosystem I

Photosystem I (PS I), depicted in Figure 1.1, is a multi-subunit pigment-protein complex, whose cellular function is to convert light into reducing equivalents for the reduction of NADP$^+$ in the following manner:

$$\text{NADP}^+ + \text{H}^+ + 2\text{e}^- \rightarrow \text{NADPH}$$

The crystal structure of trimeric PS I from *Thermosynechococcus elongatus* has been resolved to 2.5 Å, providing detailed structural information on the organization of the pigments and cofactors. Each monomer contains 13 subunits, labeled PsaA, PsaB, PsaC, PsaD, PsaE, PsaI, PsaJ, PsaK, PsaL, PsaE, and PsaX. These subunits bind 96 chlorophylls (Chls), 22 carotenoids, four lipids, three [4Fe-4S] clusters, two phylloquinones, and many water molecules. The primary charge-separation, and hence the electron transfer cofactors, reside in the heterodimer formed from PsaA, PsaB, and the stromal subunit, PsaC. The cofactors, shown in Figure 1.2, are arranged in pseudo-C$_2$ symmetry that form two branches labeled ‘A’ and ‘B’. Note that both the spectroscopic and structural names are provided and the ‘A’ or ‘B’ associated with the name represents the branch in which the cofactor is active. For example, the $A_{1A}$ phylloquinone is
involved in electron transfer through the A-branch and the A₁B phylloquinone is involved in electron transfer through the B-branch. While there has been considerable debate on which branch(es) are active, the current consensus is that both branches function in transmembrane electron transfer. However, the A-branch is favored in cyanobacteria, as ~65% of electron transfer occurs through the A-branch, and ~35% through the B-branch.

Antenna pigment Chl molecules serve to absorb light at wavelengths less than 700 nm. This energy is transferred via a Förster mechanism to the primary donor, a special pair of Chl a/Chl a’ molecules denoted P₇₀₀ (Chl a’ is the 3’-epimer of Chl a) within 20-30 ps. The excited state P₇₀₀* has a redox potential of -1.2V, perhaps the most reducing cofactor in biology. Within ~4 ps, an electron is transferred to a Chl monomer on either the A-branch or the B-branch, known as A₀A and A₀B. Because of the relatively large distance between P₇₀₀ and A₀, a second accessory Chl (A₁A and A₁B) is likely involved as an electron transfer intermediate. This model, however, is not universally accepted. In recent years, ultrafast transient absorbance experiments suggest that the initial charge separation event occurs between A₁A/A₁B and A₀A/A₀B. P₇₀₀ would then donate an electron to the oxidized A₁A (or A₁B) Chl a molecule. Regardless of the mechanism, the first readily observable charge-separated state is that of P₇₀₀⁺ A₀⁻ or P₇₀₀⁺ A₀B⁻. The lifetime of this state would last for only 20 ns were it not for the presence of the phylloquinones located in the A₁A and A₁B sites. Electron transfer to the phylloquinone extends the lifetime from 20 ns to ~100 µs. From here, the A- and B-branches converge at the 4Fe-4S cluster denoted Fₓ, an interpolypeptide cluster ligated by both PsaA and PsaB. Finally, the electron is serially transferred to the 4Fe-4S clusters Fₐ and Fₐ, located in the PsaC subunit, resulting in the final charge separated state, P₇₀₀⁺ [Fₐ/Fₐ]⁻, which is stable for ~65 ms. In vivo, the electron is then transferred to a water soluble [2Fe-2S] ferredoxin to reduce NADP⁺, but lacking any terminal electron acceptors, the electron will recombine with P₇₀₀⁺. It is important to note that lifetime of the initial charge separated state P₇₀₀⁺ A₀⁻ is extended by several orders of magnitude, from a relatively short 20 ns to a physiologically
relevant 65 ms by donation through this series of acceptors. This gain in time, however, comes at the cost of free energy, as roughly 40% of the energy is lost from the initial state. **Figure 1.3** provides a summary of the redox potentials of the electron acceptors as a function of their edge-to-edge distance, while also providing rates of forward electron transfer and charge recombination at each step.

### 1.2.2 Application of PS I to solar energy production

PS I has several properties that make it an ideal candidate for light harvesting and energy conversion. (i) The embedded carotenoid and Chl $\alpha$ molecules provide a large spectral range in which the protein can absorb light, where wavelengths shorter than 700 nm are used$^{10}$. (ii) The quantum yield of PS I is nearly 1, where each absorbed photon results in the final charge-separated $P_{700}^{+}F_{B}^{-}$ state$^{20,22}$. This yield is observed regardless of the wavelength of light used for excitation. (iii) An impressive $\sim$38% of the energy from a blue photon and $\sim$59% of the energy of 700 nm photon are conserved in the final charge separated state. (iv) The $P_{700}^{+}F_{B}^{-}$ state is stable for $\sim$65 ms, which is enough time for the electron to be transferred to another module to perform reductive chemistry. (v) The terminal electron acceptor, $F_{B}$, has a standard biochemical midpoint potential of -580 mV$^{20}$, which is sufficiently negative to provide driving force for many electrochemical reactions, including the reaction (at pH 7.0):

$$2H^+ + 2e^- \rightarrow H_2$$

Finally, (vi) PS I can be manipulated both chemically and genetically. The stromal subunits (PsaC, PsaD, and PsaE) can be removed and reconstituted with modified variants$^{23-29}$. The terminal iron-sulfur (FeS) cluster can be selectively removed by treatment with HgCl$_2$$^{30,32}$. Specific pigments, including the Chls, carotenoids, and phylloquinones, can be removed by treatment with diethyl ether at varying levels of water saturation, leaving the rest of the cofactors intact and
functional. Lastly, genetic interruption of phylloquinone biosynthesis results in a plastoquinone–9 (PQ–9) molecule in the A1 binding site that is loosely bound, and hence readily displaced with a variety of non-native quinones. These factors highlight the potential of PS I as a promising source for light harvesting and subsequent generation of reducing power.

The first pioneering experiments done using PS I for solar energy conversion were performed by Greenbaum and colleagues, who employed the Hill reaction, which reduces ferric ions to ferrous ions. Using this method, platinum, ruthenium, and osmium were separately deposited onto chloroplasts by light-induced reduction of hexachloroplhatinate ([PtCl6]2-), hexachloroosmiulate ([OsCl6]2-) and hexachlororuthenate ([RuCl6]2-), respectively. On further exposure to light, these systems were shown to generate dihydrogen, with a maximum rate of 0.113 µmol H2 mg Chl–1 hr–1 provided by the osmium complex. This work was extended to isolated PS I RCs, from spinach, where in the presence of sodium ascorbate and plastocyanin, a maximum rate of 0.025 µmol H2 mg Chl–1 hr–1 was observed. This rate increased to 0.080 µmol H2 mg Chl–1 hr–1 when plastocyanin was crosslinked to the acceptor side of PS I.

To overcome the limitations of expensive materials and the probable inefficient structuring of the Pt catalyst around the PS I complexes, the hydrogenase (H2ase) enzyme was used. H2ases are a class of proteins that employ cheap and abundant metals such as nickel and/or iron to catalyze the reversible reaction:

\[ 2\text{H}^+ + 2e^- \leftrightarrow \text{H}_2 \]

In an attempt to eliminate diffusion-limited chemistry, researchers attempted to create a more controlled structure, whereby the H2ase is maintained at a close proximity to the terminal Fb cluster of PS I. To that end, several fusion complexes were developed. The first genetic fusion was between the PsaE subunit of PS I from *T. elongatus* and the small HoxK subunit of a [NiFe]-H2ase from *Ralstonia eutropha*. When the fusion protein was assembled with the larger HoxG subunit, the construct was able to produce dihydrogen in the presence of reduced methyl viologen. While
H₂ase activity was only ~16% that of the wild type (WT) enzyme, it provided evidence that this system could be functional. Indeed, when bound to trimeric PS I complexes devoid of the native PsaE subunit, a maximum rate of dihydrogen production of 0.58 µmol H₂ mg Chl⁻¹ hr⁻¹ was observed. An optimized system was developed by deposition onto a gold electrode, which produced 2,812 µmol H₂ mg Chl⁻¹ hr⁻¹ when illuminated. However, the fusion construct was only stable for several minutes before becoming inactive. A second fusion construct was created between the PsaE subunit of PS I from *Synechocystis* sp. PCC 6803 and a [NiFe]–H₂ase from *Desulfovibrio vulgaris*. Similar to the previous fusion construct, dihydrogen was generated at a modest rate, reaching a maximum of 0.3 µmol H₂ mg Chl⁻¹ hr⁻¹. These results showed that not only is H₂ase a viable catalyst for light-induced hydrogen production, but that careful assembly of the construct and subsequent removal of diffusion-limited chemistry is essential for increasing electron transfer rates.

To further extend the notion of increasing the proximity of the catalyst to the light-harvesting module, our laboratory pioneered systems based on covalent linkages between PS I and the catalyst. Grimme and coworkers first tested this concept by covalently linking a Pt nanoparticle to the F₅ cluster of PS I using a 1,6-hexanedithiol wire. This was accomplished by removal of the native PsaC subunit and subsequent replacement with a modified variant containing an open coordination site at the F₅ cluster. The tether binds the F₅ cluster by ‘chemical rescue’ to the Pt nanoparticle. Under fully optimized conditions, this system was shown to generate dihydrogen at a maximum rate of 312 µmol H₂ mg Chl⁻¹ hr⁻¹. The success of this system lead to combining the tethering concept that initially employed inorganic constructs with the naturally high rates of H₂ production provided by H₂ases. Lubner and colleagues tethered the F₅ site to an [FeFe]-hydrogenase from *Clostridium acetobutylicum* with a variety of dithiolated wires. When donor-side limitations are overcome by cross-linking cytochrome c₉, a remarkable rate of 2,200 ± 460 µmol H₂ mg Chl⁻¹ hr⁻¹ was observed. This
corresponds to 105 ± 22 e⁻PS I⁻¹s⁻¹, more than double the rate of natural photosynthesis (47 e⁻PS I⁻¹s⁻¹)⁶⁰.

The technologies discussed thus far all focus on obtaining electrons from the terminal F_B cluster. The question arises as to whether or not it is possible to tether PS I to a catalyst at sites earlier than the FeS clusters. Doing so would provide more reducing potential (E°' = −844 mV for the A₁Β site and E°' = −580 mV the F_B site), though at the cost of needing to extract the electron rapidly. As shown in Figure 1.3 the charge recombination time decreases sharply from 65 ms when the electron resides on F_A/F_B to 1 ms when the electron resides on F_X and 10/100 µs when the electron resides on A₁. Obtaining an electron from the phylloquinone in the A₁ site through a forward electron transfer step must therefore occur at on a time scale faster than ~1 µs to retain a high quantum yield.

1.3 ROLE OF QUINONES IN ELECTRON TRANSFER

Quinones are a versatile class of molecules that play an important role in electron transfer⁶¹. Formally defined as having a fully conjugated cyclic dione structure, they encompass a wide array of compounds⁶². The two types of quinones found in oxygenic phototrophic RCs are derivatives of 1,4-benzoquinone and 1,4-naphthoquinones (Figure 1.4). PQ-9⁶³ is a class of benzoquinone containing methyl groups in the 5 and 6 positions and a polyprenyl group substituted in the 3 position. Two PQ-9 molecules are found in the PS II reaction center, denoted Q_A and Q_B, and each quinone serves a different function (See 64 and 65 for a review). Q_A is a bound electron transfer intermediate, serving to transfer one electron to from the pheophytin primary electron acceptor to Q_B. Q_B, however, functions as a mobile electron/proton carrier. When doubly reduced, Q_B becomes protonated by the surrounding environment, taking on the plastoquinol form, and serves as a mobile two-electron/two-proton carrier between PS II and the cytochrome b₆f complex. Alternatively, PS
I employs phylloquinone, a naphthoquinone derivative with a methyl group in the 2-position, and a polyprenyl group substituted at the 3-position\textsuperscript{66,67}. Unlike the PQ-9 of PS II, phylloquinone is a bound electron transfer intermediate between the A\textsubscript{0} electron acceptors and the terminal FeS clusters. It is thought that the redox potential of the quinone is modulated by almost –300 mV by its interaction with the protein environment.

1.3.1 Manipulation of the A\textsubscript{1} sites of PS I

In addition to its versatility, another attractive property of the quinone in PS I is how readily it can be manipulated. Changing the identity of the quinone in the binding site has allowed researchers to determine much information about the functional role of the phylloquinone. Currently, there exist two methods of quinone manipulation: (i) chemical removal via treatment with organic solvents and (ii) genetic interruption of the phylloquinone biosynthetic pathway.

Ikegami and colleagues were the first to find that the quinone (and various other pigments) can be removed from PS I by treatment with diethyl ether\textsuperscript{33}. Importantly, these reaction centers retained functionality, even when only 9 Chl molecules were present per P\textsubscript{700}\textsuperscript{37}. It was soon discovered that other solvent systems, such as hexane/methanol, work equally well at removing the maximum number of Chls while maintaining functional charge separation\textsuperscript{68,69}. Diethyl ether, however, remains an optimal choice for pigment extraction because of its potential selectivity. By changing the water content of the solvent, it is possible to control the amount of Chls removed. Water saturated ether will remove >90\% of the Chls, whereas dry ether removes relatively few Chls\textsuperscript{37-39}. Regardless of the solvent choice, all of the carotenoids and both of the phylloquinones are removed.

In pigment extracted PS I particles, it was discovered that functional electron transfer can be restored by reconstitution of the A\textsubscript{1A} and A\textsubscript{1B} sites with various quinones. This was first observed
through the incorporation of 2-methyl-1,4-napthoquinone, a derivative of phylloquinone lacking the phytol tail\textsuperscript{15}, into ether treated PS I. Here it was shown that that photooxidation of P\textsubscript{700} was accelerated in the presence of a naphthoquinone. Since then, researchers have successfully incorporated different quinones containing benzo--\textsuperscript{70-72}, naphtho--\textsuperscript{37,39,68,69,73}, and anthraquinone\textsuperscript{36,74} headgroups, finding that in nearly all cases, quinone uptake results in functional electron transfer to the FeS clusters.

Alternatively, a second method of quinone displacement is possible through genetic manipulation of phylloquinone biosynthesis. Johnson and coworkers discovered that if biosynthesis of the phylloquinone was precluded by inhibiting the menA, menB, menD, or menE genes, the A\textsubscript{1A} and A\textsubscript{1B} sites become occupied by a PQ-9 molecule commonly associated with PS II\textsuperscript{42,43,45}. The more positive redox potential of PQ-9 relative to phylloquinone significantly impacts electron transfer through the protein. Most notably, there was a significantly decreased charge recombination time, from 65 ms in WT PS I, to 3 ms in PQ-9 containing PS I\textsuperscript{45}. While forward electron transfer from A\textsubscript{1} to F\textsubscript{X} was found to be thermodynamically uphill, the presence of the FeS clusters ensured that electron transfer from A\textsubscript{1} to F\textsubscript{B} was favorable overall. This was demonstrated by CW electron paramagnetic resonance (EPR) spectroscopy that measured the photoaccumulation of the reduced [F\textsubscript{A}/F\textsubscript{B}]\textsuperscript{--} clusters, and by flavodoxin reduction experiments that showed electrons were still able to be transferred to flavodoxin, albeit at lower rates than in phylloquinone containing PS I\textsuperscript{47}.

A particularly useful property of PQ-9 containing PS I is that PQ-9 is loosely bound and easily displaced by a variety of naphthoquinones. Two methods are available for PQ-9 replacement: (i) supplementing cells with the target quinone, and (ii) incubating purified PS I particles with the target quinone\textsuperscript{42,43,45,75}. When naphthoquinone-like molecules such as 2-methyl-1,4-naphthoquinone and phylloquinone are used, WT kinetics are restored in both time-resolved optical and EPR experiments. This discovery has allowed researchers to study fundamental properties of
the quinone binding site, such as the presence of hydrogen bonding from the protein to the phylloquinone, without the damaging effects of ether treatment\(^7\). Incorporation of various quinones with PS I using the menB mutant is still an active area of study and an important tool for measuring the effects thermodynamics on electron transfer.

### 1.3.2 Tethering of PS I through the A\(_1\) site

Terasaki and coworkers were the first to realize the potential of PS I tethering through the A\(_{1A}\) and A\(_{1B}\) sites\(^{40,41,77}\). In their pioneering work, they successfully coupled PS I to a field-effect transistor (FET) for the development of a biophotosensor. By taking advantage of the empty binding site afforded by ether treated PS I, a phylloquinone-like molecule,\(1\)-(15-(3-methyl-1,4-naphthoquinone-2-yl)]pentadecyl disulfide, was able to reconstitute the A\(_{1A}\) and A\(_{1B}\) sites. As the headgroup was identical to that of the native phylloquinone, it was easily recognized by the protein. Moreover, this quinone contained a thiol-functionalized tail that was subsequently attached to a gold nanoparticle, which served as an intermediate between PS I and the electrode. Quinone uptake was monitored by measuring \(P\)\(_{700}\) oxidation at 701 nm following excitation with blue light. While PS I activity was nearly diminished in ether treated PS I, it was restored to near WT-like levels by quinone reconstitution.

The biophotosensor was made by depositing a self-assembled monolayer (SAM) of 1,2-benzenedimethanethiol on the surface of a gold electrode. PS I tethered through the A\(_{1A}\) and A\(_{1B}\) sites to the gold nanoparticle was then connected to the electrode by interaction of the free thiols with the SAM. Upon illumination, it was found that a current was generated, presumably by electron transfer from the PS I particles (a model is depicted in Figure 1.5). An action spectrum showed that the maximum photocurrent was obtained at 680 nm, and that the pattern mimicked the absorption spectrum of Chl \(a\). Moreover, it was shown that the voltage difference between the gate
and the source changed from -3.3 V to -5.4 V upon exposure to light, and that this difference depended on the intensity of the incident light\(^40\). These data demonstrated the first instance of electron removal from the quinone site of PS I.

To ensure that the quinone was indeed responsible for the signals, they repeated their experiments on a system in which the thiol moiety of the quinone was replaced with a viologen derivative\(^41\). Spectroscopic measurements of viologen-based naphthoquinone at 600 nm showed that the viologen was not reduced when intact PS I was used, indicating that the quinone must be in the A\(_{1A}\) and/or A\(_{1B}\) binding sites for electron transfer to occur. This construct was then fixed onto a gold electrode containing a SAM of 3-mercapto-1-propanesulfonic acid, and photocurrent action spectra were measured. Similar to their previous work, the highest photocurrent was observed at 680 nm, and the profile resembled that of the PS I absorption spectrum. Controls were measured in which (i) the quinone was not reconstituted into the binding site and (ii) the reconstituted PS I particles were not immobilized onto the surface. In the former, no photocurrent responses were observed, and in the latter, only a weak response was measured that did not depend on the wavelength of light\(^41\).

While these experiments provide preliminary insights into electron removal from the A\(_1\) sites, there are several areas that need addressed. First, a more robust series of spectroscopic measurements must be made that would elucidate the mechanism of electron transfer through this system. Time-resolved optical measurements should be employed to directly monitor electron transfer before any statements can be made regarding electron transfer through the wire\(^78\). TrEPR can also be used to determine the properties of the quinone when it resides in the A\(_{1A}\) and A\(_{1B}\) binding sites\(^66,79,80\). Secondly, if a practical device is to be made using this tethering technology, it must be robust. Using ether treated PS I severely reduces the optical cross-section, leading to decoupled Chls and inefficient energy transfer to the trap. Moreover, longevity of the PS I complex is severely compromised, as it is now susceptible to oxidative damage due to the lack of
carotenoids. Carotenoids serve an essential function by quenching the highly reactive singlet oxygen species\(^{81-83}\), and any practical device based on carotenoid-less PS I would have a limited lifetime. It is the primary aim of this thesis to address these issues by creating and analyzing a robust light harvesting system based on electron transfer from the \(A_{1A}\) and/or \(A_{1B}\) sites of PS I.

1.3.3 Practical considerations for longevity

While the use of biological based materials for light harvesting and solar energy conversion is extensive, longevity is a practical consideration that must be addressed before a functional device can be developed. Proteins and other biological materials can lose function by denaturation, a process that is often accelerated by heat, solvents, certain inorganic compounds, and irradiation. Cells have the ability to simply remake a protein, however this luxury is lost in most devices. Therefore, inhibiting this process would be an ideal strategy to maintain long-term functionality.

Although fatty acids\(^{84}\) and anti-inflammatory drugs\(^{85}\) have been shown to inhibit denaturation, perhaps the most promising candidate is a disaccharide known as trehalose\(^{86}\). Long known as a means of stabilizing biomolecules, it is found in many organisms, including: eubacteria, archaea, fungi, invertebrates, and lower plants\(^{86}\). In these natural systems, trehalose is used to protect against osmotic stress\(^{87}\), desiccation\(^{88}\), high temperatures, and is used as a cryoprotectant\(^{89}\). In laboratory settings, it has been shown to inhibit protein denaturation\(^{90}\), promote drought resistance in plants\(^{91}\), and even suppress body odor\(^{92}\).

Recently the effects of protein immobilization by trehalose have been studied in Type II reaction centers from spinach\(^{93}\) and the purple bacterium \textit{Rhodobacter sphaeroides}\(^{94,95}\). In spinach, steady-state \(O_2\) evolution was significantly enhanced and the functionality of the protein was increased when assayed over a series of several months\(^{93}\). Two studies in \textit{R. sphaeroides} examined the effects of electron transfer and charge recombination of the reaction center embedded in a
trehalose matrix. It was discovered that the charge recombination rate was increased, which was attributed to an inability of the protein to change between light- and dark-adapted states\textsuperscript{94,95}. While type I reaction centers have not been studied, it seems likely that both electron transfer and protein longevity will be impacted. An additional aim of this thesis will be to make the first known measurements of cyanobacterial PS I in a trehalose-glass matrix.
1.4 REFERENCES


1.5 FIGURE LEGENDS

**Figure 1.1** Crystal structure of a PS I monomer from *T. elongatus* at 2.5 Å resolution (PDB entry 1JB0). PsaA is depicted in red, PsaB in blue, PsaC in orange.

**Figure 1.2** Arrangement of electron transfer cofactors in PS I from *T. elongatus*. The spectroscopic labels are shown beside each cofactor, with the crystallographic names in parenthesis. The A- and B-branches are labeled.

**Figure 1.3** Depiction of the accepted values of the redox potential (y-axis) as a function of their edge-to-edge distance (x-axis), along with measured rates of electron transfer. Forward electron transfer is denoted in solid lines and charge recombination is denoted as dashed lines.

**Figure 1.4** Chemical structures of the naphtho- and benzoquinone derivatives, phylloquinone and PQ-9, respectively.

**Figure 1.5** Model of PS I attached to a FET via the A₁ site
Figure 1.1
Figure 1.2
Figure 1.3
Figure 1.4
Chapter 2

Light-Mediated Hydrogen Generation in Photosystem I: Attachment of a Napthoquinone-Molecular Wire-Pt Nanoparticle to the A$_{1A}$ and A$_{1B}$ Sites

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2.1 ABSTRACT

The molecular wire-appended naphthoquinone 1-[(15-(3-methyl-1,4-naphthoquinone-2-yl)]pentadecyl disulfide [(NQ(CH$_2$)$_{15}$S)$_2$] has been incorporated into the A$_{1A}$ and A$_{1B}$ sites of Photosystem I (PS I) in the menB variant of Synechocystis sp. PCC 6803. Transient electron paramagnetic resonance studies show that the naphthoquinone headgroup displaces plastoquinone-9 from the A$_{1A}$ (and likely A$_{1B}$) sites to a large extent. When a Pt nanoparticle is attached to the molecular wire by reductive cleavage of the disulfide and reaction with the resulting thiol, the PS I–NQ(CH$_2$)$_{15}$S–Pt nanoconstruct evolves dihydrogen at a rate of 67.3 μmol of H$_2$ (mg of Chl)$^{-1}$ h$^{-1}$ [3.4 e$^-$ (PS I)$^{-1}$ s$^{-1}$] after illumination for 1 h at pH 6.4. No dihydrogen is detected if wild type PS I, which does not incorporate the quinone, is used or if either (NQ(CH$_2$)$_{15}$S)$_2$ or the Pt nanoparticle is absent. Time-resolved optical studies of the PS I–NQ(CH$_2$)$_{15}$S–Pt nanoconstruct show that the lifetimes of forward electron transfer to and charge recombination from the iron–sulfur clusters are the same as in native PS I. Thus, electrons are not shuttled directly from the quinone to the Pt nanoparticle during either forward electron transfer or charge recombination. It is found that the rate of dihydrogen evolution in the PS I–NQ(CH$_2$)$_{15}$S–Pt nanoconstruct depends strongly on the concentration the sacrificial electron donor cytochrome $c_6$. These observations can be explained if the iron–sulfur clusters are involved in stabilizing the electron; the ~50 ms residence time of the electron on F$_A$ or F$_B$ is sufficiently long to allow cytochrome $c_6$ to reduce P$_{700}^+$, thereby eliminating the recombination channel. In the absence of P$_{700}^+$, slow electron transfer through the molecular wire to the Pt catalyst can occur, and hence, H$_2$ evolution is observed.
2.2 INTRODUCTION

The conversion of solar radiation into stored chemical energy through entirely artificial means has proven to be challenging, often requiring low-abundance metals such as ruthenium or iridium and frequently suffering from low quantum yields. In contrast, photosynthetic reaction centers in plants and bacteria employ abundant metals such as iron, manganese, and calcium and offer impressively high quantum yields. Photosystem I (PS I), in particular, is relevant to biofuel research because it generates a low-potential reductant capable of reducing protons to dihydrogen. The cofactors and the kinetics of electron transfer in PS I are depicted in Figure 2.1. Although the inherent lifetime of the initial charge-separated state between the donor P$_{700}^+$ and the acceptor A$_0^-$ is ~30 ns, the lifetime is lengthened by electron transfer through a series of acceptors that include two phylloquinones, A$_{1A}$ and A$_{1B}$, and three [4Fe-4S] clusters, F$_X$, F$_A$, and F$_B$. When the electron is present on any given cofactor, there is competition between forward electron transfer and backward electron transfer, the latter leading ultimately to charge recombination with P$_{700}^+$. Because the rates of forward electron transfer (depicted as solid lines) are typically several orders of magnitude greater than the rates of charge recombination (depicted as dashed lines), the quantum efficiency of electron transfer to the terminal iron-sulfur (FeS) clusters borders on 100%$^{3,4}$. This high quantum efficiency occurs at the expense of Gibbs free energy; nevertheless, as much as 59% of the energy of a 700 nm photon is conserved in the final P$_{700}^+$ F$_B^-$ charge-separated state$^4$.

A number of methods have been devised for attaching catalysts to PS I for light-driven dihydrogen production. In a series of pioneering applications, Greenbaum and colleagues deposited Pt, Os, or Ru onto both thylakoid membranes and PS I complexes by using the reducing power of the acceptors to generate the free metal from the salt$^{5,11}$. These modified reaction centers evolved dihydrogen in light when supplied with sacrificial donors to P$_{700}^+$. The use of deposited Pt has been greatly extended by a number of other groups$^{12-15}$ in an attempt to produce a practical solar
conversion device. Another method attempts to position the catalyst close to but not in intimate contact with PS I. One example is a [NiFe]-hydrogenase enzyme that has been genetically fused with PsaE and introduced into a PsaE-less variant of PS I\textsuperscript{16-18}. The result is that the [NiFe]-hydrogenase is bound close to the terminal FeS clusters of PS I, thereby allowing it to act as an acceptor for electrons that have been transferred from P\textsubscript{700} to F\textsubscript{A} or F\textsubscript{B}. Another example is the use of a molecular wire to attach a Pt catalyst\textsuperscript{19,20} or a [FeFe]-hydrogenase\textsuperscript{21-23} to the F\textsubscript{B} cluster of PS I. The molecular wire similarly restricts the range of distances between the electron transfer cofactors, making it suitable for rapid electron transfer. Both methods allow the electron to tunnel from the terminal FeS cluster to the catalyst in a short period of time\textsuperscript{24}, and both report high rates of generation of dihydrogen under illumination.

The question of whether it is possible to extract the electron from an earlier acceptor in PS I arises. Steric constraints imposed by the PsaA–PsaB heterodimer make it difficult to devise a method for attaching a molecular wire to the F\textsubscript{X} cluster. However, a variety of techniques exist to introduce alternative quinones into the A\textsubscript{1A} and A\textsubscript{1B} sites. Solvent extraction with diethyl ether\textsuperscript{25-30} or a hexane/methanol mixture\textsuperscript{31,32} removes the majority of the chlorophylls (Chls) and most, if not all, of the quinones and carotenoids, allowing a wide range of naphthoquinones\textsuperscript{26,28,30-33}, anthraquinones\textsuperscript{34,35}, and some benzoquinones\textsuperscript{36-38} to be incorporated into the A\textsubscript{1A} and A\textsubscript{1B} sites. This method was recently exploited by Terasaki et al.\textsuperscript{39}, who introduced the substituted naphthoquinone, NQ(CH\textsubscript{2})\textsubscript{15}S-Au, into the A\textsubscript{1A} and A\textsubscript{1B} sites to fabricate a biophotosensor. A genetic method for introducing alternative quinones is also available, which involves interruption of the menA, menB, menD, or menE gene\textsuperscript{40-44}. With any of these genes inactivated, the biosynthesis of phylloquinone is blocked, and plastoquinone-9 (PQ-9) acts as a surrogate in the A\textsubscript{1A} and A\textsubscript{1B} sites. PQ-9 is loosely bound and can be displaced with a variety of substituted naphthoquinones\textsuperscript{43,45,46} or anthraquinones\textsuperscript{47}. This method of quinone exchange has an advantage over solvent extraction in that all of the Chls and carotenoids are retained in the PS I complexes.
For electrons to be transferred from the terminal FeS clusters through an alternative pathway, one of two conditions must be met. Either the rate of forward electron transfer through the alternative pathway must exceed the rate of charge recombination to $P_{700}^+$, or the rate of reduction of $P_{700}^+$ by an external donor must exceed the rate of charge recombination so that recombination is prevented. Because of the long lifetime (65 ms) of the $P_{700}^+ [F_A/F_B]^- $ charge-separated state, either of these conditions is readily met when a molecular wire incorporating a Pt nanoparticle or a [FeFe]-hydrogenase enzyme is attached to the terminal $F_B$ cluster. However, a more complicated situation is encountered if the electron is to be removed from $A_{1A}$ and $A_{1B}$. Because the quinone is not the terminal acceptor, the electrons must be transferred to the catalyst during either a forward electron transfer step or charge recombination. For forward electron transfer, the rate of electron transfer along the alternative pathway must exceed that of electron transfer from the quinone to $F_X$. However, if the electron is transferred during charge recombination, then external reduction of $P_{700}^+$ must outcompete the recombination event.

In this study, we extend the work of Terasaki et al.\textsuperscript{39} to determine whether a construct based on Pt instead of Au can generate dihydrogen under illumination and whether the transfer of the electrons from the $A_{1A}$ and $A_{1B}$ sites occurs during the forward electron transfer step or charge recombination. We expect that the Pt nanoparticle will not be able to penetrate into the hydrophobic region of the protein; thus, the alkyl chain must be long enough to span the distance between the $A_1$ site(s) and the surface of the protein. On the other hand, the rate of electron transfer between the quinone and the nanoparticle is expected to decrease as the length of the tether increases. Thus, there should be an optimal length for the tether corresponding to the minimal distance between the $A_1$ site and the protein surface. On the basis of the structure, we estimate this distance to be on the order of half the transmembrane distance of 30 Å. The length of the alkyl tether depends on its conformation, but the maximal length of a 15-carbon alkyl chain is roughly 22.5 Å. Hence, a construct with a 15-carbon chain should be sufficient to span the distance. This substituted
naphthoquinone can be incorporated into the A\textsubscript{1A} and A\textsubscript{1B} sites of menB PS I complexes by displacing the exchangeable PQ-9. We show that when the naphthoquinone is tethered to a Pt nanoparticle, it can still be exchanged into the A\textsubscript{1A} and A\textsubscript{1B} binding sites and that this construct generates dihydrogen upon illumination. However, we also show that the rate of dihydrogen production depends on the concentration of the external donor, cytochrome c\textsubscript{6} (Cyt c\textsubscript{6}), present in solution. Thus, we propose that the trapping occurs during charge recombination and that the FeS clusters are involved in stabilizing the electron, thereby allowing a longer residence time that permits Cyt c\textsubscript{6} to reduce P\textsubscript{700}\textsuperscript{+} and eliminate the recombination channel.

2.3 MATERIALS AND METHODS

2.3.1 Synthesis and Characterization of 2-(15-bromopentadecyl)-3-methyl-1,4-naphthoquinone

A 500 mL round-bottom flask is charged with 2-methyl-1,4-napthoquinone (1.48 g, 8.62 mmol), silver nitrate (2.96 g, 17.30 mmol), 15-bromohexadecanoic acid (4.29 g, 12.80 mmol), ammonium persulfate (3.97 g, 17.40 mmol), ddH\textsubscript{2}O water (70 mL) and acetonitrile (70 mL). The mixture was heated to reflux and vigorous stirred for 2 h. The organic layers were removed and the water phase was extracted with ethyl acetate three times. After combining the organic phases, they were washed with brine and dried over sodium sulfate. The solvent was removed by distillation and the residue was purified using a silica gel column using hexane:ethyl acetate at a ratio of 100:1. A yellow solid was obtained in a yield of 65% and used in the next step of the procedure. \textsuperscript{1}H NMR (CDCl\textsubscript{3}, 400 MHz) \textsuperscript{\delta} 8.1 (dd, 2H), 7.8 (dd, 2H), 3.45 (t, 2H), 2.6 (t, 2H), 2.2 (s, 3H), 1.9 (quin, 2H), 1.50-1.20 (m, 24H). FTIR in cm\textsuperscript{-1}(solid): 2916, 2849, 1653, 1588, 1290, 715 LC-MS (EI, ToF) \textit{m/z} 460 (M\textsuperscript{+}).
2.3.2 Synthesis and Characterization of 1-[15-(3-Methyl-1,4-naphthoquinone-2-yl)]pentadecyl Disulfide

A 250 mL round-bottom flask was charged with thiourea (1.46 g, 18.0 mmol), 2-(15-bromopentadecyl)-3-methyl-1,4-naphthoquinone (1.77 g, 3.84 mmol), and ethanol (75 mL). The reaction was refluxed under N\textsubscript{2} gas for 24 hours. After 24 hours, tetraethylenepentamine (1.53 mL, 7.66 mmol) was added and the solution was refluxed for an additional 24 hours. The reaction was quenched with water and extracted 3 times with ethyl acetate. The combined organic layers were combined, washed with brine and dried using sodium sulfate. Using a rotating evaporator the ethyl acetate was removed and the residue was purified with a silica gel column with a hexane/ethyl acetate gradient (100:1, 50:1, 25:1 und 15:1). If necessary the purification was repeated and a yellow solid was obtained in a yield of 35% \textsuperscript{1}H NMR (CDCl\textsubscript{3}, 400 MHz) δ 8.1 (dd, 4H), 7.7 (dd, 2H), 2.7 (t, 4H), 2.6 (t, 4H), 2.2 (s, 6H), 1.7 (quin, 4H), 1.6-1.2 (m, 48H) FTIR in cm\textsuperscript{-1}(solid): 2917, 2850, 2566, 1653, 1619, 1588, 1289, 717 LC-MS (EI, ToF) m/z 827 (M\textsuperscript{+})

2.3.3 Preparation of Pt Nanoparticles

Pt nanoparticles were prepared according to literature procedures\textsuperscript{48}. One milliliter of mercaptosuccinic acid (23.7 mM) and 10 mL of hexachloroplatinic acid (3.38 mM) were mixed and stirred vigorously in 18.2 MΩ H\textsubscript{2}O. Five milliliters of sodium borohydride (67.6 mM) was added to the reaction solution. The mixture immediately turned from yellow to brown. The reaction
was completed when the solution turned dark brown. The resulting particles were characterized via transmission electron microscopy and determined to be 3–5 nm in diameter.

2.3.4 PS I Growth and Purification

Wild type (WT) PS I complexes were isolated from *Synechocystis* sp. PCC 6803 and purified in accordance with the literature\(^4^9\). The purified PS I trimers were resuspended in buffer containing 0.05% (v/v) Triton X-100 and 20% (v/v) glycerol in 50 mM Tris-HCl (pH 8.3). The *menB* variant was grown and purified according to published methods\(^4^0\). Cells were grown in β-HEPES medium supplemented with 5 mM glucose and 25 μg/mL spectinomycin. PS I trimers from the variant were isolated using the same protocol that was used for the WT with the exception that β-dodecyl maltoside (β-DM) was used instead of Triton X-100.

2.3.5 Incorporation of Quinone into the *menB* Variant of PS I

Quinone incorporation for transient electron paramagnetic resonance (EPR) and time-resolved optical studies was achieved via a 16–24 h incubation of *menB* PS I with a 100-fold excess of either phylloquinone or (NQ(CH\(_2\))\(_{15}\)S)\(_2\). The sample was washed twice with Tris-HCl (pH 8.3) containing 0.05% β-DM over a 50 kDa Centricon membrane to remove both the residual naphthoquinone and the displaced PQ-9.
2.3.6 Nanoconstruct Assembly and Dihydrogen Analysis

The NQ(CH\(_2\)\(_{15}\)S–Pt adduct was made by reductive cleavage of (NQ(CH\(_2\)\(_{15}\)S)\(_2\) by the Pt nanoparticles \emph{in situ}. The concentration of Pt in the nanoparticle reaction mixture was 2.11 mM. On the basis of the atomic radius of Pt and the nanoparticle radius of \(\sim 4\) nm and assuming close packing of the atoms, there should be \(\sim 10^4\) atoms per nanoparticle. Hence, the concentration of Pt nanoparticles in the reaction mixture was \(\sim 0.2\) μM. A total of 2 mL of the Pt nanoparticles was incubated with 100 μL of a 20 mM solution of the target naphthoquinone in the dark while the mixture was vigorously stirred for \(>7\) days. The nanoconstruct was assembled via a 16–24 h incubation of 75 μL of the NQ(CH\(_2\)\(_{15}\)S–Pt adduct, \emph{menB} or WT PS I at a final concentration of 5.8 μg/mL Chl (6.5 μM P\(_{700}\)), and either Tris or MES buffer at pH 8.3 or 6.4, respectively, to a final volume of 250 μL. Following incubation, soluble electron donors were added to final concentrations of 30 μM 2,6-dichlorophenolindophenol (DCPIP), 100 mM sodium ascorbate, and 100 μM Cyt \(_c\). For experiments monitoring the effect of Cyt \(_c\) concentration, the PS I concentration was increased to 50 μg/mL, and the donor concentrations were changed to 10 μM DCPIP, 5 mM sodium ascorbate, and 0–100 μM Cyt \(_c\). The headspaces of the assembled nanoconstruct solutions were purged in the dark for 10 min using ultrapure argon and samples were exposed to continuous illumination using a 100 W xenon arc lamp (996 μmol of photons m\(^{-2}\) s\(^{-1}\)). The temperature was maintained using a clear polycarbonate flask filled with ddH\(_2\)O to remove infrared radiation. Periodically, 200 μL of headspace was removed with an airtight locking syringe and analyzed with a Shimadzu GC-8A gas chromatograph equipped with a ShinCarbon 80/100 column (2 m \(\times\) 2 mm) and thermal conductivity detector (detector current of 100 mA) with ultrapure N\(_2\) as the carrier gas (flow rate of 0.75 mL/min).
2.3.7 Transient EPR Spectroscopy

Time/field transient EPR data sets were measured using a modified ER 200D-SRC spectrometer with either a Bruker ER 041 X-MR X-band or a Bruker ER 051 QR Q-band microwave bridge. For the X-band measurements, a Flexline ER 4118 X-MD-5W1 dielectric resonator was used, and for the Q-band experiments, an ER 5106 QT-W cylindrical resonator was used. A Surelight-Continuum Nd:YAG laser operating at 10 Hz and a wavelength of 532 nm and 4.0 mJ/pulse provided single-turnover flashes. The temperature was controlled using an Oxford Instruments CF935 gas flow cryostat. The transient EPR signal was collected in direct detection mode with a home-built broadband amplifier (bandwidth of >500 MHz), digitized using a LeCroy LT322 500 MHz digital oscilloscope, and saved on a personal computer for analysis. Samples were pretreated with 1 mM sodium ascorbate and 50 μM phenazine methosulfate prior to analysis. The samples were adapted to the dark for 20 min on ice to ensure complete reduction of P700+ and frozen in the dark before being illuminated.

2.3.8 Time-Resolved Optical Studies at 820 and 480 nm

Forward electron transfer and charge recombination kinetics in the PS I samples was monitored by transient absorbance changes at 480 and 820 nm following a single-turnover laser flash at 532 nm using a laboratory-built, dual-beam spectrometer as described previously. Samples were placed in a 10 mm × 2 mm quartz cuvette. The cuvette was placed such that the optical path length was 10 mm. The measuring and reference beam intensities were balanced using a variable density optical filter wheel. The difference signal was amplified with a model 11A33 differential comparator and processed using a DSA610 digital signal analyzer (Tektronix,
Beaverton, OR). Measurements at 480 nm included a 480 laser diode (OptoEngine MBL-H-480-30mW) as the measuring beam and a mechanical shutter that opened the measuring beam 2 ms prior to the flash. The spectrometers were controlled using software written in LabView (National Instruments), and the data were analyzed using the graphics capability of IGOR (Wavemetrics). The samples contained 50 μg/mL Chl, Tris-HCl (pH 8.3), 10 μM DCPIP, 5 mM sodium ascorbate, and 0.05% (w/v) β-DM.

2.4 RESULTS

2.4.1 Transient EPR Spectroscopy

Our first objective was to confirm that PQ-9 could be displaced by (NQ(CH2)15S)2 in menB PS I. We used (NQ(CH2)15S)2 rather than NQ(CH2)15SH in the incorporation studies with the menB variant to prevent reactions with the reactive sulfhydryl group. At low temperature, reversible electron transfer in PS I occurs repeatedly between P700 and A1A-, resulting in the spin-polarized P700+ A1A- radical pair that can be observed by transient EPR (TrEPR)51 (see ref 51 for a short overview). The TrEPR spectrum is sensitive to the structure and orientation of the quinone as well as its interaction with its binding site. Low-temperature TrEPR spectra of WT PS I and menB PS I before and after incubation with (NQ(CH2)15S)2 are shown at X-band (Figure 2.2A) and Q-band (Figure 2.2B). The spectra of the WT and menB samples are similar to those reported previously42, and at both microwave frequencies, changes after incubation with (NQ(CH2)15S)2 are seen primarily in the low-field region of the spectrum, which is dominated by contributions from A1A−−, while the high field region, where the contributions from P700++ appear, is largely unchanged. At Q-band, the components of the quinone g tensor are partially resolved and the spectra are sensitive to g
anisotropy. The delocalization of the electron over two rings in the naphthoquinone headgroups of (NQ(CH₂)₁₅S)₂ and phylloquinone leads to a g anisotropy smaller than that of PQ-9, which contains a benzoquinone headgroup with only one ring. The difference between the structure of the two quinones results in an upfield shift of the features in the quinone portion of the Q-band spectrum after incubation (Figure 2.2B).

The Q-band spectrum of the PS I–(NQ(CH₂)₁₅S)₂ sample (Figure 2.2B, solid spectrum) is almost identical to that of WT PS I (Figure 2.2B, dotted spectrum), which is expected, as phylloquinone and (NQ(CH₂)₁₅S)₂ have the same headgroup. At X-band, the g anisotropy is not resolved and the spectrum is more sensitive to hyperfine couplings than at Q-band. In PQ-9, the two methyl groups and the methylene group of the side chain have significant hyperfine couplings, which overlap to give a broad inhomogeneous line shape. In the WT and (NQ(CH₂)₁₅S)₂ samples, the presence of only a single methyl group leads to a decrease in the inhomogeneous line width, and as a result, the height of the central absorption and low-field emission increases. In WT PS I, the fact that the C₄ carbonyl group is H-bonded to the protein while the C₁ carbonyl group is not results in high electron density on C₂ of the naphthoquinone ring and leads to prominent, albeit only partially resolved, hyperfine splitting from the 2-methyl group. This splitting is most evident as a shoulder on the low-field side of the central absorptive peak (Figure 2.2A). Although the X-band spectrum of the PS I–(NQ(CH₂)₁₅S)₂ sample (Figure 2.2A, solid spectrum) is almost identical to that of WT PS I (Figure 2.2A, dotted spectrum), the shoulder due to the 2-methyl hyperfine splitting is slightly less pronounced.

There are several possible reasons for this difference. One is that the long side chains in phylloquinone and (NQ(CH₂)₁₅S)₂ may have different orientations of the first methylene group relative to the quinone ring. The hyperfine couplings of the two-methylene protons depend on this orientation, and an increase in their values would increase the inhomogeneous line width and lower the resolution of the 2-methyl hyperfine splitting. Another is that the spin density distribution on
the naphthoquinone headgroup may differ slightly in the two quinones such that the density on C₂ is lower in the (NQ(CH₂)₁₅S)₂ sample. It is also possible that the sample incubated with (NQ(CH₂)₁₅S)₂ still contains a small amount of residual PQ-9 in the A₁₆ site, which would also tend to lower the resolution of the spectrum. Even though TrEPR does not directly provide information about the A₁₆ site, it can be assumed on the basis of the structural similarity with the A₁₆ site that (NQ(CH₂)₁₅S)₂ has similarly displaced PQ-9.

The TrEPR data show that (NQ(CH₂)₁₅S)₂ has a high affinity for the A₁₆ (and presumably A₁₆) binding site without the Pt nanoparticle attached. Corresponding experiments with the Pt nanoparticle bound to the tether are not feasible because the nanoparticles aggregate and precipitate at the concentrations needed for EPR experiments. However, time-resolved optical spectroscopy, which can be performed at lower concentrations, can be used to characterize the incorporation.

2.4.2 Time-Resolved Optical Spectroscopy at 820 nm

Flash excitation of PS I leads to an absorbance change at 820 nm due to the formation P₇₀₀⁺, and the kinetics of this absorbance change can be used to monitor charge recombination from the acceptor side of the complex. The following experiments were conducted in the absence of Cyt c₆ so that the charge recombination kinetics would not be obscured by rapid forward donation to P₇₀₀⁺. In WT PS I, two major kinetic components are observed after a laser flash (Figure 2.3A). The event with a lifetime of 65 ms represents P₇₀₀⁺[F₅/F₆]⁻ charge recombination, and the component with a lifetime of 1.1 s represents donation of an electron from DCPIP to P₇₀₀⁺ in those reaction centers in which the electron has been lost to dioxygen. In menB PS I, which contains PQ-9 in the A₁₆ and A₁₆ binding sites, the amplitude of the absorbance change [3.7 milli-optical density units (mOD)] is slightly lower than that of the WT (4 mOD) and P₇₀₀⁺[F₅/F₆]⁻ charge recombination is
accelerated, showing a lifetime of 3.1 ms (Figure 2.3B). A minor component with a (long) lifetime unresolved on this time scale is also present, which represents donation of an electron from DCPIP to P700+. Faster charge recombination between P700+ and [F6/F8]− in menB PS I occurs because the midpoint potential of PQ-9 is more oxidizing than that of phylloquinone. This results in a smaller Gibbs free energy difference between A1A or A1B and Fx, thereby increasing the population of electrons on the quinone relative to the FeS clusters. Because charge recombination proceeds by backward transfer of an electron from [F6/F8]− through Fx and A1A/A1B, the change in relative populations leads to faster depletion of the electrons from the FeS clusters and, hence, faster reduction of P700+. The smaller amplitude of the slow kinetic phase is due to the residence time of the electron on F8 being shorter in menB PS I. The probability of an electron being transferred from F8− to dioxygen is correspondingly lower.

When (NQ(CH2)15S)2− is added to menB PS I (Figure 2.3C), the amplitude of the absorbance change at the onset of the flash (3.6 mOD) is similar to that of the menB variant (3.7 mOD), but the 3.1 ms kinetic phase is largely replaced by two kinetic phases with lifetimes of 45 ms (0.9 mOD) and 2.0 s (2.3 mOD). The 2.2 ms kinetic phase (0.4 mOD) is similar to that of the dominant kinetic phase in the menB variant and is assigned to a minor fraction of PS I complexes that still contain PQ-9. The 45 ms and 2.0 s events are similar to those seen in WT PS I (Figure 2.3A) and are due to P700+[F6/F8]− charge recombination and donation of an electron from DCPIP to P700+, respectively. The resumption of a long-lived charge-separated state is consistent with replacement of PQ-9 with a naphthoquinone, and with forward electron donation to FA and F8.

When the NQ(CH2)15S–Pt adduct was incubated with menB PS I (Figure 2.3D), the total amplitude of the absorbance change at the flash was similar to that of the menB variant. However, the 45 ms and 1.3 s kinetic events are present as in the WT, indicating that the majority of the electrons are similarly transferred to the FeS clusters. This result shows that the NQ(CH2)15S–Pt adduct is able to displace PQ-9 in the menB variant but also implies that the transfer of an electron to the Pt
nanoparticle does not compete with forward transfer to the FeS clusters. The origin of the minor, 370 \( \mu s \) event is unknown; however, it does represent a return of an electron to \( P_{700}^+ \). Because of the distance involved, we consider it unlikely that this event represents charge recombination between an electron on the Pt nanoparticle and \( P_{700}^+ \).

2.4.3 *Time-Resolved Optical Spectroscopy at 480 nm*

Electron transfer beyond A\(_{1A}\) and A\(_{1B}\) can be studied more directly using time-resolved optical absorption spectroscopy at 480 nm, a wavelength that detects the electrochromic band shift of carotenoids in the vicinity of the quinones\(^{55,56}\). The measurement at 480 nm is a proxy for the transient redox changes of the quinones and is more reliable than the measurement at 380 nm because the amplitude of the absorbance change does not depend on the identity of the quinone occupying the A\(_{1A}\) and A\(_{1B}\) sites. In WT PS I, two kinetic components are observed after a laser flash (*Figure 2.4A*). The event with a lifetime of 17.1 ns represents the forward transfer of an electron from A\(_{1B}^-\) to F\(_X\), and the event with a lifetime of 207 ns represents the transfer of an electron from A\(_{1A}^-\) to F\(_X\)\(^{57}\). The fast and slow components are present in a ratio of 30:70, which is typical for cyanobacterial PS I. In *menB* PS I, two kinetic events are resolved, a minor component with a lifetime of 34.3 ns (*Figure 2.4B*) and a major component with a lifetime of 23.2 \( \mu s \) (*Figure 2.4C*; note the different time scale). The fast and slow components are present in a ratio of 25:75. The slow phase is consistent with a previous study in which a major kinetic phase with a lifetime of \(~15 \mu s\) was assigned to the transfer of an electron from PQ-9 to F\(_X\) in *menB* PS I\(^{41}\). The origin of the 34 ns phase is uncertain. At least two kinetic phases of forward electron transfer to F\(_X\) are expected when PQ-9 occupies the A\(_{1B}\) and A\(_{1A}\) sites, and as discussed in ref 41, it is possible that a broad distribution of kinetic phases exists. (Further characterization of these kinetic components is
beyond the scope of this work.) When native phylloquinone is incubated with \textit{menB} PS I, two components, with lifetimes of 23.5 and 358 ns, are resolved that are similar to the lifetimes of the transfer of an electron from $A_{1B}^-$ and $A_{1A}^-$ to $F_X$ in WT PS I (Figure 2.4D). This result shows that phylloquinone largely displaces PQ-9 in \textit{menB} PS I\textsuperscript{43}. Similarly, when (NQ(CH\textsubscript{2})\textsubscript{15}S\textsubscript{2}) is incubated with \textit{menB} PS I, two components, with lifetimes of 22.0 and 454 ns, are resolved (Figure 2.4E) that resemble the fast and slow kinetic phases, respectively, in phylloquinone-reconstituted \textit{menB} PS I. When the NQ(CH\textsubscript{2})\textsubscript{15}S–Pt nanoparticle adduct is incubated with \textit{menB} PS I (Figure 2.4F), two components, with lifetimes of 16.5 and 386 ns, are similarly resolved that resemble the fast and slow kinetic phases, respectively, in phylloquinone-reconstituted \textit{menB} PS I. (The lower signal-to-noise ratio in this sample is due to a greater amount of scattering of the measuring beam in the sample containing a Pt nanoparticle.) This result is consistent with the study conducted at 820 nm, and together, the data indicate that the majority of electrons are transferred to the $F_X$ cluster and further to $F_A$ and $F_B$. It also confirms that (i) the presence of the Pt nanoparticle does not interfere with the ability of the naphthoquinone group to occupy the $A_{1A}$ and $A_{1B}$ sites and (ii) the similar amplitudes illustrate a nearly quantitative displacement of PQ-9.

\textbf{2.4.4 Light-Induced Dihydrogen Generation}

We next tested whether the PS I–NQ(CH\textsubscript{2})\textsubscript{15}S–Pt construct could evolve dihydrogen in the light. These experiments were conducted at a low Chl concentration, 5.8 μg/mL, to minimize self-shielding. A detailed time course for dihydrogen production under constant illumination at pH 6.4 in the presence of 100 μM Cyt \textit{c}_6 is shown in Figure 2.5. After a rapid rise, the rate of dihydrogen evolution reached a maximum of 42.5 μmol of H\textsubscript{2} (mg of Chl)\textsuperscript{-1} h\textsuperscript{-1} [2.2 e\textsuperscript{-} (PS I)\textsuperscript{-1} s\textsuperscript{-1}] after illumination for 1 h; thereafter, the rate declined with a half-time of \textasciitilde9 h. In a series of triplicate
runs, the maximal rate of dihydrogen production was 67.6 ± 8.2 μmol of H₂ (mg of Chl)⁻¹ h⁻¹ [3.4 e⁻ (PS I)⁻¹ s⁻¹] at pH 6.4 and 44.3 μmol of H₂ (mg of Chl)⁻¹ h⁻¹ [2.3 e⁻ (PS I)⁻¹ s⁻¹] at pH 8.3, both measured after illumination for 1 h (Table 1).

The controls for these experiments are listed in Table 1. No dihydrogen was detected (i) in the presence of WT PS I, the NQ(CH₂)₁₅S–Pt construct, Cyt c₆, and light; (ii) in the presence of menB PS I, Pt nanoparticles, Cyt c₆, and light but in the absence of (NQ(CH₂)₁₅S)₂; (iii) in the presence of menB PS I, Cyt c₆, and light but in the absence of the NQ(CH₂)₁₅S–Pt construct; (iv) in the presence of menB PS I, (NQ(CH₂)₁₅S)₂, Cyt c₆, and light but in the absence of Pt nanoparticles; (v) in the presence of menB PS I, the NQ(CH₂)₁₅S–Pt construct, and light but in the absence of Cyt c₆; or (vi) in the presence of menB PS I, the NQ(CH₂)₁₅S–Pt construct, and Cyt c₆ but in the absence of light. The detection limit for dihydrogen after illumination for 1 h was 0.034 μmol of H₂ (mg of Chl)⁻¹ h⁻¹.

2.4.5 Effect of Cyt c₆ on Rates of Dihydrogen Production and P₇₀₀⁺ Reduction

The question is how dihydrogen can be evolved if there is no evidence of altered electron transfer through the acceptor chain. If, as the transient absorbance data indicate, the electrons are transferred to the FeS clusters rather than through the molecular wire to the Pt nanoparticle, we would expect that dihydrogen would be evolved only at electron donor concentrations that suppress charge recombination between P₇₀₀⁺ and [Fₐ/Fₐ⁻]. Under these conditions, the electron would equilibrate among the acceptors, but the slow kinetic leak through the span of the molecular wire would ultimately lead to trapping of the electron on the Pt nanoparticle. We therefore studied the effect of Cyt c₆ concentration on the rate of dihydrogen produced in the PS I–NQ(CH₂)₁₅S–Pt construct. To correlate the results with the 820 and 480 nm optical studies shown in Figures 2.3
and 2.4, the experiment was conducted at a Chl concentration 10-fold higher than those of the experiments reported in Table 1. This results in significant self-shading, which leads to a lower rate of evolution of dihydrogen on a per Chl basis. As shown in Figure 2.6, no dihydrogen was detected if only the sacrificial donor, sodium ascorbate, or if only sodium ascorbate and 30 μM DCPIP are used as donors. Similarly, no dihydrogen was detected in the additional presence of either 0.01 or 0.1 μM Cyt c₆. When the concentration of Cyt c₆ was increased to 2 μM, however, dihydrogen was detected [0.05 μmol of H₂ (mg of Chl)⁻¹ h⁻¹], and doubling the concentration of Cyt c₆ to 4 μM resulted in a 10-fold increase in the rate of dihydrogen evolved [1.05 μmol of H₂ (mg of Chl)⁻¹ h⁻¹]. Increasing the concentration of Cyt c₆ to 100 μM resulted in a doubling of the rate of dihydrogen evolved [2.65 μmol of H₂ (mg of Chl)⁻¹ h⁻¹], but higher concentrations of Cyt c₆ led to only marginally higher rates. The absence of a linear relationship between Cyt c₆ concentration and dihydrogen production and the abrupt onset of light-induced dihydrogen production at 2 μM Cyt c₆ indicate that more than simple kinetics is governing the reaction.

Figure 2.7 plots the rate of reduction of P₇₀₀⁺ as a function of Cyt c₆ concentration. Note that the abscissa is plotted on a logarithmic scale. In the presence of only sodium ascorbate, or in the additional presence of 30 μM DCPIP, the e⁻ lifetime of P₇₀₀⁺ is ∼65 ms (compare to Figure 2.3A); hence, P₇₀₀⁺ reduction is dominated by charge recombination from [F₅₇₅/F₄₈₀]⁻. Under these conditions, the forward transfer of an electron to P₇₀₀⁺ is so slow that it cannot outcompete charge recombination. When Cyt c₆ is added to the reaction mixture, the forward transfer of an electron to P₇₀₀⁺ is similarly outcompeted by charge recombination at concentrations of 100 nM and 0.1 μM. However, at Cyt c₆ concentrations of >1 μM, the forward transfer of an electron to P₇₀₀⁺ is faster than charge recombination according to the following sequence (values of e⁻ in parentheses): 2 μM (45 ms), 4 μM (28 ms), 16 μM (12 ms), 50 μM (3 ms), and 100 μM (1.2 ms). From Figure 2.6, it is clear that dihydrogen is evolved only at Cyt c₆ concentrations of >2 μM. Hence, dihydrogen evolution occurs only under conditions where the charge recombination channel from [F₅₇₅/F₄₈₀]⁻ to
We suggest that the FeS clusters are involved in stabilizing the electron in the PS I–NQ(CH₂)₁₅S–Pt construct because the longer residence time they afford allows the charge recombination channel to be eliminated by the forward donation of an electron to P₇₀₀⁺.

2.5 DISCUSSION

A useful way to describe electron transfer in PS I is by the spreading of equilibrium model⁵⁴, ⁵⁸, which takes as its foundation the experimental finding that the closer the reaction is to the primary charge separation step, the larger the rate constant (Figure 2.1). The spreading of equilibrium model takes into account the observation that at each step, the forward rate constant is considerably larger than the charge recombination rate constant, usually by several orders of magnitude. Accordingly, the process of light-driven electron transfer can be considered a series of steps in which all of the previous steps are in quasi-equilibrium. The equilibrium process spreads sequentially over time from the picosecond domain to the millisecond domain, terminating in a final quasi-equilibrium state. When this state is achieved, the electron has a finite probability of residing on each of the electron acceptors, the amount depending on the equilibrium constant separating any given acceptor–donor pair. Hence, there is a finite probability that any given A₁A or A₁B site will be in the reduced state. Given midpoint potentials for the electron acceptors of −844 mV for A₁B, −671 mV for A₁A, −700 mV for Fₓ, −520 mV for Fₐ, and −580 mV for Fₜ (see ref 33), the application of the partition function states that the probability is 2.8 × 10⁻³ that the electron will reside on A₁A and 3.7 × 10⁻⁶ that the electron will reside on A₁B during this final state. In the absence of external donors and acceptors, the electron would be permanently distributed in this manner were it not for the existence of the charge recombination channel, which occurs to P₇₀₀⁺ via a
tunneling mechanism from $A_{1A}^-$ or $A_{1B}^{-}$\textsuperscript{58}. The electron will ultimately be bled from the acceptor chain and the PS I cofactors returned to the ground state.

The attachment of the NQ(CH\textsubscript{2})\textsubscript{15}S–Pt adduct provides a third option for the electron on $A_{1A}^-$ and $A_{1B}^-$ (Figure 2.8). The ability of the electron to be transferred through the molecular wire to the Pt nanoparticle will be a function of the forward rate constant, $k_2$, relative to the forward transfer of an electron to the FeS clusters (simplified here as a single rate constant, $k_1$) and the charge recombination channel, $k_r$. The rate of transfer of an electron from the quinone to the Pt nanoparticle, $k_2$, is governed by the driving force of the reaction ($\Delta G$), the reorganization energy ($\lambda$), and the electronic coupling ($|V|^2$). A rough estimate of the driving force can be obtained by taking the difference between the midpoint potential of the quinone–semiquinone couple and midpoint potential of the proton–dihydrogen couple. There are no experimentally determined values for the midpoint potentials of $A_{1A}$ or $A_{1B}$; instead, the calculated midpoint potentials of $-671$ mV for $A_{1A}$ and $-844$ mV for $A_{1B}$ will be used because the $173$ mV difference in midpoint potentials agrees reasonably well with the experimentally determined rates of fast to slow electron transfer steps attributed to $A_{1A}^-$ to $F_X$ and $A_{1B}^-$ to $F_X$, respectively (see ref 33 for a review). The proton–dihydrogen couple has a standard biochemical midpoint potential ($E^\circ$) of $-430$ mV. Thus, the change in Gibbs free energy ($\Delta G$) from $A_{1A}^-$ is $-241$ mV and $A_{1B}^-$ is $-414$ mV, and reduction of protons to dihydrogen will be highly favorable on both branches.

The reorganization energy ($\lambda$) associated with reduction of the Pt nanoparticle is likely to be very small because of the large surface area over which the electron is distributed. The effect on $\lambda$ of changing the size of an electron acceptor has been studied by comparing fullerene (C\textsubscript{60}) to smaller acceptors such as naphthalenediimide in donor–acceptor complexes\textsuperscript{59}. As one might expect, C\textsubscript{60} has a small reorganization energy because of the delocalization of the charge, and the same should apply for a Pt nanoparticle. Thus, the activation energy is expected to be relatively large. The electronic coupling is determined to a large extent by the distance between the naphthoquinone
headgroup and the Pt nanoparticle. It is difficult to estimate this distance because of the number of variables involved, including the uncertain conformations of the methylene groups in the hydrocarbon chain. Ideally, the average distance between the center of the first and last carbon atoms of a freely rotating hydrocarbon would be 1.50\(l\), where \(l\) is the carbon–carbon distance of 1.54 Å\(^6\). The distance between the naphthoquinone headgroup and the Pt nanoparticle could therefore be as much as 22.5 Å were the hydrocarbon chain to be fully extended. In contrast, the edge-to-edge distance between the quinones in the A\(_{1A}\) and A\(_{1B}\) sites is 9 Å. Given that the electronic coupling decreases exponentially with distance and a significant activation energy is expected, it is clear that under nearly all reasonable configurations of the molecular wire–Pt nanoparticle adduct, \(k_2\) will be much smaller than \(k_1\); hence, the electron will have only a small probability of being transferred to the Pt nanoparticle during forward electron transfer. This assessment is supported by the time-resolved optical data shown in Figure 2.4.

However, we also need to consider \(k_2\), \(k_{-2}\), and \(k_c\). The relative magnitude of \(k_2\) and \(k_c\) can be estimated from the kinetics of electron transfer in PS I in the absence of the FeS clusters. In PS I complexes from the \(rubA\) variant, which lacks F\(_X\), F\(_A\), and F\(_B\) but contains phylloquinone in the A\(_{1A}\) and A\(_{1B}\) sites, two kinetic phases are present with lifetimes of 15 and 95 µs\(^6\)\(^1\),\(^6\)\(^2\). The lifetimes of the P\(_{700}^+\) A\(_{1A}^-\) and P\(_{700}^+\) A\(_{1B}^-\) charge separated states in the absence of F\(_X\), F\(_A\), and F\(_B\) are therefore 3 orders of magnitude shorter than the lifetime of the P\(_{700}^+\) [F\(_A^+\)/F\(_B^+\)]\(^-\) charge-separated state in WT PS I. Although bidirectional electron transfer through the two branches of cofactors was not appreciated at the time of the \(rubA\) study, it seems reasonable that one kinetic phase is derived from P\(_{700}^+\) A\(_{1A}^-\) charge recombination and the other from P\(_{700}^+\) A\(_{1B}^-\) charge recombination. Given that the Gibbs free energy change between P\(_{700}^+\) and A\(_{1B}^-\) is larger than that between P\(_{700}^+\) and A\(_{1A}^-\), and provided the reaction is in the normal region of the Marcus curve, we tentatively assign the 15 µs kinetic phase to the former while the 95 µs kinetic phase can be assigned to the latter. In the absence of F\(_X\), F\(_A\), and F\(_B\), the electron on either A\(_{1A}\) or A\(_{1B}\) has only two available routes. It can either
recombine with $P_{700}^+$ with rate constant $k_t$ or be transferred to the Pt nanoparticle with rate constant $k_2$. The fate of the electron will be determined by the relative values of $k_2$ and $k_t$. While the value of $k_2$ is not known, the large distance between the naphthoquinone headgroup and the Pt nanoparticle as well as the presence of $\sigma$-bonds throughout the hydrocarbon tether will most certainly render $k_2$ much lower than $k_t$. This assessment is also supported by the time-resolved optical data shown in Figure 2.4.

We also need to consider the relative values of $k_2$ and $k_{-2}$. The $k_2/k_{-2}$ ratio is given by the equation $k_2/k_{-2} = \exp(-\Delta G/kT)$; using our estimate for $\Delta G$ of $-241$ mV for the A branch and $-414$ mV for the B branch, we obtain a $k_2/k_{-2}$ ratio of $10^4$ (A branch) or $10^7$ (B branch). In either case, it is clear that this ratio is very large, and hence, the reverse transfer of an electron from the Pt nanoparticle to $A_{1A}$ and/or $A_{1B}$ is expected to be extremely slow and can be neglected. Because $k_2$ is much smaller than $k_t$ and $k_1$, the transfer of an electron to the Pt nanoparticle can occur only if charge recombination is inhibited. The reduction of $P_{700}^+$ by Cyt $c_6$, which occurs on the millisecond time scale, is faster than charge recombination from $[F_A/F_B]^-$, which occurs with a lifetime of 65 ms. Thus, the recombination channel, $k_r$, can be eliminated when the FeS clusters are present. Under these conditions, the lifetime of the electron on the acceptor chain becomes very long, and we propose that a small leak through the span of the molecular wire allows electrons to be transferred to the Pt nanoparticle. Because $k_{-2}$ is negligible, the electrons become trapped. The rate of trapping is given by $k_{-4}(k_2/k_1)$. These rate constants are not known accurately, but $k_1$ is on the order of $10^7$ s$^{-1}$. In the absence of the FeS clusters, the charge recombination from $A_{1A}^-$ occurs with a lifetime of 95 $\mu$s, so $k_t \approx 10^4$ s$^{-1}$. Using these two values, the observed lifetime of 65 ms for charge recombination from $F_B$ in native PS I yields a $k_{-1}$ of $\approx 1.6 \times 10^4$ s$^{-1}$. If we arbitrarily assume that $k_2$ is one order of magnitude smaller than $k_t$, we obtain a rate of $\sim 1$ s$^{-1}$ for the trapping of electrons on the Pt nanoparticle. When two electrons are serially transferred to the Pt nanoparticle, the release
of dihydrogen makes electron transfer irreversible, thereby depleting the acceptor side of electron(s) until the next photon strikes the reaction center.

This work adds to the number of practical methods for attaching a Pt nanoparticle to the acceptor side of PS I for the purpose of achieving light-driven hydrogen evolution. These include self-organized platinization, the electrostatic attachment of a Pt nanoparticle, the attachment of a Pt nanoparticle via a molecular wire to the $F_B$ cluster, and, now, the attachment of a Pt nanoparticle via a molecular wire to the $A_{1A}$ and $A_{1B}$ binding sites. Although the selection of which method to choose for a particular device will depend on the details of the application, the wide variety of attachment points demonstrates the versatility of PS I as a photocatalyst for solar hydrogen production.

2.6 ACKNOWLEDGEMENTS

We thank Dr. Junlei Sun for assistance in conducting the time-resolved optical measurements at 480 nm and Dr. Carolyn Lubner and Prof. Ron Koder for valuable discussions.
2.7 REFERENCES


II. Rubredoxin is required for the in vivo assembly of F(X) in Synechococcus sp. PCC 7002 as shown by optical and EPR spectroscopy, *J Biol Chem* 277, 20355-20366.

2.8 SCHEMES

Scheme 2.1 Synthesis of 1-[15-(3-Methyl-1,4-naphthoquinone-2-yl)]pentadecyl Disulfide.

1. 

2. 

yield 60%

yield 33%
2.9 TABLES

**Table 2.1** Maximum rate of dihydrogen production under various sample conditions

<table>
<thead>
<tr>
<th>Sample</th>
<th>Rate of H₂ production [μmol of H₂ (mg of Chl)⁻¹ h⁻¹]</th>
</tr>
</thead>
<tbody>
<tr>
<td>menB PS I, NQ(CH₂)₁₅S–Pt, Cyt c₆, pH 6.5, hv</td>
<td>67.3 ± 8.2a</td>
</tr>
<tr>
<td>menB PS I, NQ(CH₂)₁₅S–Pt, Cyt c₆, pH 8.3, hv</td>
<td>44.3 ± 5.3a</td>
</tr>
<tr>
<td>WT PS I, NQ(CH₂)₁₅S–Pt, Cyt c₆, pH 6.5, hv</td>
<td>0.0ᵃᵇ</td>
</tr>
<tr>
<td>menB PS I, Pt, Cyt c₆, pH 6.5, hv</td>
<td>0.0ᵃᵇ</td>
</tr>
<tr>
<td>menB PS I, Cyt c₆, pH 6.5, hv</td>
<td>0.0ᵃᵇ</td>
</tr>
<tr>
<td>menB PS I, (NQ(CH₂)₁₅S₂), Cyt c₆, pH 6.5, hv</td>
<td>0.0ᵃᵇ</td>
</tr>
<tr>
<td>menB PS I, NQ(CH₂)₁₅S–Pt, pH 6.5, hv</td>
<td>0.0ᵃᵇ</td>
</tr>
<tr>
<td>menB PS I, NQ(CH₂)₁₅S–Pt, Cyt c₆, pH 6.5</td>
<td>0.0ᵃᵇ</td>
</tr>
</tbody>
</table>

ᵃAfter illumination for 1 h.ᵇDetection limit of 0.034 μmol of H₂ (mg of Chl)⁻¹ h⁻¹.
2.10 FIGURE LEGENDS

**Figure 2.1.** Depiction of the midpoint potential in millivolts (ordinate) and edge-to-edge distance (abscissa) of the PS I cofactors. The solid lines represent forward electron transfer lifetimes and dashed lines charge recombination lifetimes. Although the latter are depicted as direct to $P_{700}^+$, charge recombination proceeds by way of backward electron transfer to (at least) $A_{1A}$ and $A_{1B}$. The A-and B-branches are depicted as $A_{0A}/A_{1A}$ and $A_{0B}/A_{1B}$, respectively.

**Figure 2.3.** TrEPR spectra of PS I at 90 K. Comparison of WT PS I with menB PS I before and after incubation with (NQ(CH$_2$)$_{15}$S)$_2$ at (A) X-band and (B) Q-band. A equals absorption, and E equals emission.

**Figure 2.3.** Time-resolved optical spectra measured at 820 nm after a saturating laser flash: (A) WT PS I, (B) menB PS I, (C) menB PS I incubated with (NQ(CH$_2$)$_{15}$S)$_2$, and (D) menB PS I incubated with the NQ(CH$_2$)$_{15}$S–Pt adduct. The data are shown as dots, and the fitted spectra are shown as solid lines. The residual of the fit is depicted above each of the plots. The long-lived phase in the spectra represents the transfer of an electron from the external donor in reaction centers that have lost the electron on the acceptor side to dioxygen. The reaction mixture contained PS I at 50 μg/mL Chl, 5 mM sodium ascorbate, 10 μM DCPIP, and 0.05% β-DM in 50 mM Tris buffer (pH 8.3).
Figure 2.4. Time-resolved optical spectra measured at 480 nm after a saturating laser flash: (A) WT PS I, (B) menB PS I, (C) menB PS I on a microsecond time scale, (D) menB PS I incubated with phylloquinone, (E) menB PS I incubated with (NQ(CH$_2$)$_{15}$S)$_2$, and (F) menB PS I incubated with the NQ(CH$_2$)$_{15}$S–Pt adduct. The data are shown as dots, and the fitted spectra are shown as solid lines. The residual of the fit is depicted above each of the plots. Because P$_{700}$ also absorbs at this wavelength, the long-lived phase in the spectra represents long-lived P$_{700}^+$ that does not decay on this time scale. The reaction mixture contained PS I at 50 μg/mL Chl, 5 mM sodium ascorbate, 10 μM DCPIP, and 0.05% β-DM in 50 mM Tris (pH 8.3).

Figure 2.5. Time course of the rate of dihydrogen evolution during illumination of the PS I–NQ(CH$_2$)$_{15}$S–Pt construct at pH 6.4. The reaction mixture contained WT PS I at 5.8 μg/mL Chl, 100 μM Cyt c$_6$, 5 mM sodium ascorbate and 30 μM DCPIP in 20 mM MES buffer (pH 6.5).

Figure 2.6. Bar graph depicting the rate of dihydrogen evolution as a function of external electron donors. The reaction mixture contained WT PS I at 50 μg/mL Chl in 50 mM Tris buffer (pH 8.3). Sodium ascorbate and DCPIP were present at concentrations of 5 mM and 10 μM, respectively, in all but the ascorbate-only experiment.

Figure 2.7. Time course of P$_{700}^+$ reduction as a function of Cyt c$_6$ concentration. The reaction mixture contained WT PS I at 50 μg/mL Chl, 5 mM sodium ascorbate, and 10 μM DCPIP in 50 mM Tris buffer (pH 8.3).

Figure 2.8. Energy diagram of the PS I–NQ(CH$_2$)$_{15}$S–Pt construct. The forward transfer of an electron to the FeS clusters is simplified as a single rate constant, $k_1$. 
Figure 2.1
Figure 2.2

A

X-Band

--- menB
--- menB + (NQ(CH₂)₄S)₂
--- wild type

B

Q-Band

--- menB
--- menB + (NQ(CH₂)₄S)₂
--- wild type

346 347 348 349 350 351
B₀ / mT

1204 1206 1208 1210 1212
B₀ / mT
Figure 2.3
Figure 2.4

A. Time, µs

B. Time, µs

C. Time, µs

D. Time, µs

E. Time, µs

F. Time, µs
Figure 2.5

![Graph showing H₂ Rate (umol H₂/mg chl • Hr⁻¹) over Time (Hours).]
Figure 2.6

H$_2$ Production Rate

Rate of Hydrogen Production
(µmolH$_2$·mg$_{chl}$·hr$^{-1}$)

Ascorbate
DPIP/Asc
0.01 µM Cyt. C$_6$
0.1 µM Cyt. C$_6$
2 µM Cyt. C$_6$
4 µM Cyt. C$_6$
100 µM Cyt. C$_6$
Figure 2.7
Figure 2.8
Chapter 3

Electron Transfer from the A_{1A} and A_{1B} sites to a Tethered Pt Nanoparticle Requires the FeS Clusters for Suppression of the Charge Recombination Channel

[Published as a paper titled "Electron transfer from the A_{1A} and A_{1B} sites to a tethered Pt nanoparticle requires the FeS clusters for suppression of the recombination channel" by Michael Gorka, Adam Perez, Carol S. Baker, Bryan Ferlez, Art van der Est, Donald A. Bryant, and John H. Golbeck (2015) *Journal of Photochemistry and Photobiology B: Biology*]
3.1 ABSTRACT

In this work, a previously described model of electron withdrawal from the $A_{1A}/A_{1B}$ sites of Photosystem I (PS I) was tested using a dihydrogen-producing PS I–NQ(CH$_2$)$_3$S–Pt nanoconstruct. According to this model, the rate of electron transfer from $A_{1A}/A_{1B}$ to a tethered Pt nanoparticle is kinetically unfavorable relative to the rate of forward electron transfer to the iron-sulfur (FeS) clusters. Dihydrogen is produced only when an external donor rapidly reduces P$_{700}^+$, thereby suppressing the charge recombination channel and allowing the electron in the FeS clusters to proceed via uphill electron transfer through the $A_{1A}/A_{1B}$ quinones to the Pt nanoparticle. We tested this model by sequentially removing the FeS clusters, F$_B$, F$_A$, and F$_X$, and determining the concentration of cytochrome $c_6$ (Cyt $c_6$) at which the backreaction was outcompeted and dihydrogen production was observed. P$_{700}$-$F_A$ cores were generated in a $menB$ insertionally inactivated strain by removing $F_B$ with HgCl$_2$; P$_{700}$-$F_X$ cores were generated in a $menB$ $psaC$ insertionally inactivated strain that lacks $F_A$ and $F_B$, and P$_{700}$-$A_1$ cores were generated in a $menB$ $rubA$ insertionally inactivated strain that lacks $F_X$, $F_A$ and $F_B$. Quinone incorporation was measured using transient electron paramagnetic resonance spectroscopy and time-resolved optical spectroscopy. Cyt $c_6$ was titrated into each of these PS I preparations and the kinetics of P$_{700}^+$ reduction were measured. A similar experiment was carried out on PS I–NQ(CH$_2$)$_3$S–Pt nanoconstructs assembled from these PS I preparations. This study showed that the concentration of Cyt $c_6$ needed to produce dihydrogen was comparable to that needed to suppress the backreaction. We conclude that the FeS clusters serve to ‘park’ the electron and thereby extend the duration of the charge-separated state; however, in doing so, the redox advantage of removing the electron at $A_{1A}/A_{1B}$ is lost.
3.2 INTRODUCTION

The Photosystem I (PS I) reaction center is a multi-subunit, pigment-protein complex found in plants and cyanobacteria that, upon illumination, generates and maintains a long-lived charge-separated state\(^1\)\(^,\)\(^2\). Its core consists of a heterodimer of the PsaA and PsaB proteins in which the electron transfer cofactors are arranged in two pseudo-C2 symmetric functionally active branches denoted A and B\(^3\)\(^-\)\(^5\), spanning the width of the thylakoid membrane\(^6\). The \(\sim100\) chlorophylls (Chls) that form the antenna system in cyanobacterial PS I serve to absorb light and transfer energy to the primary donor (P\(_{700}\)). The kinetics and pathway of the initial charge separation are not firmly established and several different models have been proposed recently\(^7\)\(^-\)\(^9\) However, in all cases, charge separation between P\(_{700}\), a Chl \(\alpha'/\alpha\) special pair (Chl \(\alpha'\) is the 13\(^2\) epimer of Chl \(\alpha\)) and either of the Chl acceptors A\(_{0}\) or A\(_{0b}\) is complete within a few ps or less. To increase the lifetime of the charge separation, the electron is transferred along a chain of cofactors that includes two phylloquinone (A\(_{1A}/A_{1B}\)) and three [4Fe-4S] clusters (F\(_{X}\), F\(_{A}\), and F\(_{B}\)). The latter two clusters are bound to the PsaC subunit that is located on the stromal side of the membrane. The final charge-separated state, P\(_{700}\)^{*}\(F_{B}^-\), is stable for \(\sim65\) ms, which is sufficient time for the electron to be transferred to a soluble [2Fe-2S] ferredoxin\(^6\)\(^,\)\(^10\)\(^,\)\(^11\). Figure 3.1 provides a comprehensive view of the electron transfer kinetics in PS I.

PS I has a number of properties that make it a promising candidate for use as a photosensitizer in the production of solar biofuels. Not only does it have a high quantum yield of charge separation, estimated to be \(>0.98\)\(^1\), but it is exceptionally stable to high light and high temperatures. Additionally, the midpoint potential of the final acceptor, F\(_{B}\), is sufficiently negative to reduce protons to dihydrogen in the presence of a suitable catalyst\(^1\).

Greenbaum and colleagues were the first to exploit this capability by depositing Ru, Os, and Pt catalysts on thylakoid membranes and on PS I particles and observing dihydrogen
production on exposure to light\textsuperscript{13-19}. More recently, a number of groups have successfully coupled both enzymatic and inorganic catalysts to PS I for dihydrogen production\textsuperscript{20-32}. All of these attempts focused on obtaining the electron from the reduced F\textsubscript{B} cluster at the terminus of the transfer chain. However, Gibbs free energy is lost as the electron progresses from A\textsubscript{0} to F\textsubscript{B} (Figure 3.1); hence, if the electron could be extracted earlier in the electron transfer chain, it could be obtained at a more reducing potential. For example, direct electron transfer from the A\textsubscript{1B} quinone would allow the electron to be available at a midpoint potential of −845 mV\textsuperscript{5}.

Terasaki and coworkers were the first to pioneer a system of electron withdrawal from the A\textsubscript{1} sites for use in a biophotosensor\textsuperscript{33}. In their work, PS I was treated with diethyl ether to remove phylloquinone from the A\textsubscript{1A} and A\textsubscript{1B} sites, which were then reconstituted with a synthesized naphthoquinone derivative. However, chemical extraction removes all of the carotenoids and most of the Chls\textsuperscript{34-40}, which leads to a decrease in the optical cross-section for photon capture. Additionally, the lack of carotenoids renders the reaction center susceptible to oxidative damage. Thus, we attempted to reconstitute the A\textsubscript{1A} and A\textsubscript{1B} sites in a larger and more robust system containing all of the carotenoid and Chl molecules by using the menB strain of PS I.

The menB gene encodes 1,4-dihydroxy-2-naphthoyl-CoA synthetase, a necessary enzyme in the phylloquinone biosynthesis pathway\textsuperscript{41,42}. When the menB gene is inactivated, phylloquinone is not synthesized, and the A\textsubscript{1A} and A\textsubscript{1B} sites are occupied by plastoquinone-9 (PQ-9)\textsuperscript{43-47}, a mobile electron carrier that shuttles electrons from Photosystem II to the cytochrome b\textsubscript{6}f complex and transports protons from the stroma to the lumen. This substituted benzoquinone binds loosely to the A\textsubscript{1A} and A\textsubscript{1B} sites of PS I and can be readily displaced with a variety of substituted naphthoquinones\textsuperscript{46,48,49}. We took advantage of this ability to displace PQ-9, to introduce a menaquinone with a head group identical to that of the native phylloquinone, but with a carbon chain long enough to reach the surface of the protein. When a Pt nanoparticle is bound to the
thiolated end of the substituted menaquinone and the resulting PS I–NQ(CH$_2$)$_{15}$S–Pt nanoconstruct is illuminated, it evolves dihydrogen in the presence of a sacrificial electron donor.$^{32}$

In a previous publication$^{32}$ we proposed a hypothesis for how this system might function. In the model proposed, the rate of electron transfer to the attached nanoparticle is kinetically unfavorable relative to the rate of forward electron transfer from $A_{1A}^-$ and $A_{1B}^-$ to the iron-sulfur (FeS) clusters (200 ns and 20 ns, respectively) and to the rate of charge recombination from the reduced FeS clusters to $P_{700}^+$ (~65 ms). Under these conditions, the electron in the FeS clusters ultimately recombines with $P_{700}^+$, and no dihydrogen is produced. However, if charge recombination is suppressed by reducing $P_{700}^+$ rapidly with an external donor, the electron is transferred instead to the Pt nanoparticle. In essence, the FeS clusters serve to ‘park’ the electron, thereby extending the lifetime of the charge separated state and making it possible for an external donor to reduce $P_{700}^+$. The key to dihydrogen production, then, is the suppression of the charge recombination channel.

If this model is correct, then faster charge recombination times from earlier acceptors should require higher concentrations of Cyt $c_6$ to produce dihydrogen. In this work, we test the proposed model by sequentially removing the FeS clusters $F_B$ ($P_{700}$-$F_A$ cores), $F_A/F_B$ ($P_{700}$-$F_X$ cores), and $F_X$, $F_A/F_B$ ($P_{700}$-$A_1$ cores) from PS I and measuring the charge recombination kinetics and dihydrogen production as a function of Cyt $c_6$ concentration for each sample.
3.3 MATERIALS AND METHODS

3.3.1 Cell growth and PS I purification

Strains used for the purification of PS I include the wild type (WT) strain, the menB insertionally inactivated strain of *Synechocystis sp.* PCC 6803 (hereafter menB strain), and a menB rubA insertionally inactivated strain of *Synechococcus sp.* PCC 7002 (hereafter menB rubA strain). The *Synechocystis sp.* PCC 6803 menB gene was disrupted with the *aadA* gene conferring spectinomycin resistance. The *Synechococcus sp.* PCC 7002 menB rubA strain was created by the insertional inactivation of menB and rubA by antibiotic resistance genes for gentamicin resistance (*aacC1*) and for kanamycin resistance (*aphII*), respectively. These strains were grown and PS I was purified as described previously.\cite{43,50,51} Cells were harvested by centrifugation, broken by three passes through an M-110EH-30 microfluidizer processor (Microfluidics). Cell debris was removed via low speed centrifugation (2000 × g) and the resulting supernatant was centrifuged at 158,000 × g for 1 h to pellet the thylakoid membranes. Membranes were solubilized with 1% (w/v) β-dodecylmaltoside (β-DM) for 1 h at 4°C in the dark. The PS I trimers were purified on a 5 to 20% sucrose density gradient in 50 mM Tris-HCl buffer (pH 8.3), 0.05% (w/v) β-DM, subsequently pelleted, and suspended in 50 mM Tris-HCl buffer (pH 8.3) containing 20% (w/v) glycerol and 0.05% (w/v) β-DM. The menB rubA strain did not produce PS I trimers; hence, PS I monomers were collected from the first gradient, dialyzed, and the contaminating PS II was removed using a DEAE-sepharose anion exchange column (Sigma) with 50 mM Tris-HCl buffer (pH 8.0), 0.05% β-DM. PS I monomers were eluted at 75-100 mM NaCl and PS II was eluted at 200 mM NaCl. The purity of the fractions was confirmed by fluorescence emission spectroscopy at 77 K (data not shown).
The menB psaC deletion strain of Synechococcus sp. PCC 7002 (hereafter menB psaC strain) was constructed by knock-out with antibiotic resistance genes for gentamicin (aacC1) and kanamycin (aphII) into the psaC and menB genes, respectively, using a PS I-less background strain as described previously. Cells were grown in A(+ media, supplemented with gentamicin, kanamycin, and 0.1% (w/v) glycerol, under low light conditions (12 μmol m\(^{-2}\) s\(^{-1}\)). Cells were harvested and broken with three passes through an M-110EH-30 microfluidizer processor (Microfluidics). Cell debris was removed via low speed centrifugation (2000 × g) and the resulting supernatant was spun at 158,000 x g for 1 h to pellet the thylakoid membranes. The membranes were solubilized with 1% (w/v) β-DM for 1 h at 4°C in the dark, and the resulting solution was partly purified over a 5-20% sucrose density gradient in 50 mM Tris-HCl buffer (pH 8.3), 0.05% (w/v) β-DM. Because no PS I trimers were present, PS I monomers were collected, dialyzed, and the contaminating PS II was removed using a DEAE-sepharose anion exchange column (Sigma) with 50 mM Tris-HCl buffer (pH 8.0), 0.05% β-DM. PS I monomers were eluted at 75-100 mM NaCl and PS II was eluted at 200 mM NaCl. The purity of the fractions was confirmed by fluorescence emission spectroscopy at 77 K (data not shown).

### 3.3.2 Removal of the F\(\text{B}\) Cluster

F\(\text{B}\) was removed by incubation of PS I with HgCl\(_2\) under conditions similar to those of a previously described method. A 0.8 mg/mL Chl solution of menB PS I in 50 mM Tris-HCl buffer (pH 8.3) containing 0.05% (w/v) β-DM was incubated with 1 mM HgCl\(_2\) for 24 h at 4°C. The residual HgCl\(_2\) was removed by ultrafiltration using a 50 kDa cutoff membrane (Amicon) in the dark at 4°C. The buffer, 50 mM Tris-HCl (pH 8.3), was exchanged after two, three, and 14 h.
The final concentrate was suspended in 50 mM Tris-HCl buffer (pH 8.3) 0.05% (w/v) β-DM, 20% (w/v) glycerol, and stored at −80°C until used.

### 3.3.3 Two-Step Synthesis and Characterization of 1-[15-(3-methyl-1,4-naphthoquinone-2-yl)]pentadecyl disulfide

Scheme 3.1 shows the two-step synthesis of 1-[15-(3-methyl-1,4-naphthoquinone-2-yl)]pentadecyl disulfide. A typical process is as follows:

#### 3.3.3.1 2-(15-bromopentadecyl)-3-methyl-1,4-naphthoquinone

A 500 mL round-bottom flask is charged with 2-methyl-1,4-naphtoquinone (1.48 g, 8.62 mmol), silver nitrate (2.96 g, 17.30 mmol), 15-bromohexadecanoic acid (4.29 g, 12.80 mmol), ammonium persulfate (3.97 g, 17.40 mmol), ddH₂O water (70 mL) and acetonitrile (70 mL). The mixture was heated to reflux and vigorously stirred for two hours. The organic layers were removed and the water phase was extracted with ethyl acetate three times. After combining the organic phases, they were washed with brine and dried over sodium sulfate. The solvent was removed by distillation and the residue was purified using a silica gel column using hexane:ethyl acetate at a ratio of 100:1. A yellow solid was obtained in a yield of 65%. 1H NMR (CDCl₃, 400 MHz) δ 8.1 (dd, 2H), 7.8 (dd, 2H), 3.45 (t, 2H), 2.6 (t, 2H), 2.2 (s, 3H), 1.9 (quin, 2H), 1.50-1.20 (m, 24H). FTIR in cm⁻¹ (solid): 2916, 2849, 1653, 1588, 1290, 715 LC-MS (EI, ToF) m/z 460 (M⁺).
3.3.3.2 1-[15-(3-methyl-1,4-naphtoquinone-2-yl)]pentadecyl disulfide

A 250 mL round-bottom flask was charged with thiourea (1.46 g, 18.0 mmol), 2-(15-bromopentadecyl)-3-methyl-1,4-naphthoquinone (1.77 g, 3.84 mmol), and ethanol (75 mL). The reaction was refluxed under N\textsubscript{2} gas for 24 hours. After 24 hours, tetraethylenepentamine (1.53 mL, 7.66 mmol) was added and the solution was refluxed for an additional 24 hours. The reaction was quenched with water and extracted 3 times with ethyl acetate. The combined organic layers were washed with brine and dried using sodium sulfate. Using a rotating evaporator, the ethyl acetate was removed and the residue was purified with a silica gel column with a hexane/ethyl acetate gradient (100:1, 50:1, 25:1 und 15:1). If necessary, the purification was repeated and a yellow solid was obtained in a yield of 35% \textsuperscript{1}H NMR (CDCl\textsubscript{3}, 400 MHz) \(\delta\) 8.1 (dd, 4H), 7.7 (dd, 2H), 2.7 (t, 4H), 2.6 (t, 4H), 2.2 (s, 6H), 1.7 (quin, 4H), 1.6-1.2 (m, 48H) FTIR in cm\textsuperscript{-1}(solid): 2917, 2850, 2566, 1653, 1588, 1289, 717 LC-MS (EI, ToF) \(m/z\) 827 (M\textsuperscript{+})

3.3.4 Synthesis of Pt Nanoparticles

Pt nanoparticles were synthesized as described previously\textsuperscript{61}. One milliliter of 23.7 mM mercaptosuccinic acid was added to 10 mL of 3.38 mM hexachloroplatinic acid and stirred vigorously. The reaction was initiated by adding 5 mL of 67.6 mM sodium borohydride to the solution. The reaction was considered complete when the solution turned dark brown. The resulting particles were characterized via transmission electron microscopy and found to be ~5 nm in diameter.
3.3.5 Quinone Incorporation

Incorporation of various quinones into PS I complexes from the various menB strains was carried out as described previously. A 100-fold excess of the target quinone was added to a solution of PS I for 16-24 h. Excess quinone was removed by washing twice with 50 mM Tris-HCl (pH 8.3), containing 0.05% (w/v) ß-DM using a 50 kDa ultrafiltration membrane (Amicon).

3.3.6 Time-Resolved Optical Studies at 830 nm

Electron transfer kinetics in the PS I complexes were monitored by transient absorbance changes at 830 nm following a single-turnover laser flash at 532 nm using a laboratory-built, dual-beam spectrometer as described previously. The measuring beam was provided by an 830 nm laser diode (Crystalaser, model number DL830-100-O). Samples were placed in a 10 mm × 4 mm quartz cuvette which was oriented such that the optical path length was 10 mm. The measuring and reference beam intensities were balanced using a variable density optical filter wheel. The difference signal was amplified with a differential amplifier (Thor Labs PDB460A) and processed using a 12-bit, 200 MS/s, NI-5124 PCI card (National Instruments). The samples contained 50 μg/mL Chl, Tris-HCl (pH 8.3), 10 μM 2,6-dichlorophenolphindophenol (DCPIP), 5 mM sodium ascorbate, and 0.05% (w/v) ß-DM.

3.3.7 CW X-Band EPR of HgCl₂ Treated Samples

CW X-band measurements were carried out using an ELEXSYS EPR spectrometer equipped with a Super X CW-EPR microwave bridge and a high Q resonator (Bruker BioSpin)
Corp, Billerica, MA). The sample was cooled using an ESR 900 cryostat (Oxford Instruments, Austin, TX). A Coherent Verdi-V5 laser (500 mW at 532 nm) provided the excitation and was passed through a 3 X beam expander (Oriel Instruments, Irvine, CA). Samples used for measurements were prepared at a concentration of 0.8 mg/mL Chl PS I and treated with 10 µM DCPIP and 5 mM ascorbate, dark adapted for 45 minutes, and frozen in the dark before analysis.

### 3.3.8 Transient EPR Spectroscopy

Time/field transient EPR data sets were measured using a modified ESP 300E spectrometer equipped with an ER 042 MRH X-band microwave bridge and a Flexline ER 4118 X-MD-5W1 dielectric resonator (Bruker BioSpin Corp., Billerica, MS). A Vibrant 355II laser (Opotek, Inc., Carlsbad, CA) running at 10 Hz and a wavelength of 532 nm provided single-turnover flashes at 20 mJ per pulse. The temperature was controlled using a CF935 continuous flow cryostat (Oxford Instruments, Austin, TX). The transient EPR signal was collected in direct detection mode with a home-built broadband amplifier (bandwidth >500 MHz), and digitized using a 500 MHz bandwidth, 8-bit, 2GS/s, Model CS85G PCI card (GaGe Instruments). The software for data acquisition was written in-house using LabView (National Instruments). Samples were measured at a concentration of 2 mg/mL Chl and pretreated with 1 mM sodium ascorbate and adapted to the dark for 45 min following a freezing in the darkness with liquid nitrogen. The data was analyzed using IGOR (Wavemetrics, Portland, OR).

### 3.3.9 Nanoconstruct Assembly and Dihydrogen Analysis

The NQ(CH$_2$)$_{15}$S–Pt adduct was constructed as described previously$^{32}$. Briefly, 2 mL of ~0.2
μM Pt nanoparticles were incubated with 100 μL of a 20 mM solution of (NQ(CH₂)₁₅S)₂ in the dark vigorously stirring the mixture for ~7 days. The nanoconstruct was assembled by 16–24 h incubation of 75 μL of the NQ(CH₂)₁₅S–Pt adduct with PS I at a final concentration of 50 μg/mL Chl (0.58 μM P₇₀₀), in 50 mM Tris-HCl buffer (pH 8.3) containing 0.05% (w/v) β-DM to a final volume of 250 μL. After assembly, soluble electron donors were added in the dark to final concentrations of 10 μM DCPIP, 5 mM ascorbate, and up to 200 μM Cyt c₆. Prior to illumination, the headspace of the assembled nanoconstruct was purged in the dark for 10 min using ultrapure argon. The samples were exposed to continuous illumination using a 100 W xenon arc lamp (996 μmol of photons m⁻² s⁻¹). The temperature was maintained using a clear polycarbonate flask filled with doubly distilled H₂O to remove infrared radiation. A total of 200 μL of headspace was removed at regular intervals with an airtight locking syringe and analyzed with a Shimadzu GC-8A gas chromatograph equipped with a ShinCarbon 80/100 column (2 m × 2 mm) and thermal conductivity detector (detector current of 100 mA) with ultrapure N₂ as the carrier gas (flow rate of 0.75 mL/min). Samples that produced no dihydrogen were measured over the course of 48 h of illumination.
3.4 RESULTS

3.4.1 CW EPR Studies of Hg-Treated PS I

The terminal FeS cluster, F\textsubscript{B}, has been shown to be quantitatively and selectively removed from PS I by treatment with HgCl\textsubscript{2}\textsuperscript{55,56,58-60}. While the mechanism is not fully understood, it is thought that Hg interacts with the sulfur bonds, causing the [4Fe-4S] cluster to disintegrate. The efficiency of cluster removal can be assessed using low-temperature CW EPR spectroscopy, wherein illumination of a frozen PS I sample promotes a single electron from P\textsubscript{700} to either the F\textsubscript{A} or F\textsubscript{B} cluster. In WT PS I, F\textsubscript{A} and F\textsubscript{B} are reduced according to their relative redox potentials, and resonances associated with F\textsubscript{A} and F\textsubscript{B} occur in a \textasciitilde4:1 ratio. In the case in which F\textsubscript{B} is missing or inactive, only the rhombic resonances associated with F\textsubscript{A} would be expected.

The CW EPR data on Hg-treated men\textit{B} PS I complexes are shown in Figure 3.2. In the men\textit{B} strain (top), resonances associated with F\textsubscript{A} (2.05, 1.95, and 1.86) and F\textsubscript{B} (2.07, 1.93, 1.88) are visible\textsuperscript{63}. However, upon treatment with HgCl\textsubscript{2} (bottom), the F\textsubscript{B} resonances disappear and only resonances from F\textsubscript{A} are observed. Additionally, one sees a shift in the \textit{g} value for the high field trough of F\textsubscript{A} from 1.86 in the control PS I complexes to 1.84 in the Hg-treated preparation consistent with published results\textsuperscript{55}. Both of these observations indicate that F\textsubscript{B} is missing in the Hg-treated PS I complexes.

3.4.2 Transient EPR Spectroscopy

Transient EPR (TrEPR) spectroscopy at low temperatures has been used extensively to study the light-induced radical pair P\textsubscript{700}••+A\textsubscript{1A}•– in PS I (references\textsuperscript{64,65} provide reviews). The TrEPR
spectrum of $P_{700}^{++}$ $A_{1A}^-$ is spin-polarized and is sensitive to the orientation of the quinone in the binding site, the chemical structure of the quinone and the nature of its binding to the protein. Figure 3.3 shows the X-band TrEPR spectra of PS I complexes from the WT, the $menB psaC$ strain ($P_{700}$-FX cores), and from the $menB rubA$ strain ($P_{700}$-A$_1$ cores). Figure 3.3A shows a comparison of the WT and the $P_{700}$-FX cores before and after addition of (NQ(CH$_2$)$_{15}$S)$_2$. The corresponding comparison for the $P_{700}$-A$_1$ cores is shown in Figure 3.3B. There are clear differences in the spectral features before and after addition of the substituted naphthoquinone (dotted and dashed spectra, respectively). The differences in the spectra arise primarily from the different hyperfine couplings in PQ-9 and menaquinone. The two methyl groups associated with the PQ-9 head group have substantial hyperfine interactions, and these overlapping splittings give rise to a broad/featureless spectrum$^{44,66}$. This characteristic spectrum is seen in both the $P_{700}$-FX cores and the $P_{700}$-A$_1$ cores prior to (NQ(CH$_2$)$_{15}$S)$_2$ incorporation. However, when a molecule containing a naphthoquinone head group occupies the binding site, the single methyl group leads to partially resolved hyperfine couplings, most visible as the shoulder on the low-field absorptive feature. The partial delocalization of the electron over two rings of the naphthoquinone head group instead of one ring of the PQ-9 head group leads to a smaller $g$-anisotropy$^{67}$, which also contributes to the spectral differences. However, at X-band this effect is relatively minor. The spectra taken following addition of the quinone are very similar to that of the WT (solid spectrum) indicating that in both cases the quinone has been successfully incorporated into the $A_{1A}$ (and likely $A_{1B}$) site in both the $P_{700}$-FX cores and $P_{700}$-A$_1$ cores.

In addition to the spectrum of $P_{700}^{++}$ $A_{1A}^-$ a contribution from $^3P_{700}$ is also observed for the $P_{700}$-FX cores and $P_{700}$-A$_1$ cores when the spectrum is collected over a wider field range (data not shown). The triplet background has been removed from the spectra in Figure 3.3 by making a linear baseline correction. The spin polarization pattern of the $^3P_{700}$ spectrum indicates that it is formed by charge recombination and it remains present following incubation of menaquinone into
the P_{700}-F_X and P_{700}-A_1 cores. Thus, we conclude that a fraction of the quinone binding sites in these cores are unable to bind either PQ-9 or (NQ(CH_2)_15S)_2. However, it is not possible to reliably quantify the size of this fraction from TrEPR data.

### 3.4.3 Time-Resolved Optical Spectroscopy

Time-resolved optical spectroscopy at 830 nm can be used to monitor the rate of P_{700}^+ reduction either from the electron acceptors or from an external electron donor. At low concentrations of external electron donors, the experiment assesses the charge recombination kinetics from the electron acceptor chain. At sufficiently high concentrations of an external electron donor, the experiment assesses forward electron transfer kinetics from the external donor. We discuss each of the PS I preparations in turn below.

#### 3.4.3.1 P_{700}-F_A cores from Hg-Treated PS I

WT PS I shows canonical ~65 ms kinetics after a saturating flash, characteristic of charge recombination from the terminal F_A/F_B cluster(s) (Figure 3.4A). PS I from the menB strain, which contains PQ-9 in the A_{1A} and A_{1B} sites, shows characteristic 3-4 ms kinetics after a saturating flash^{46} (Figure 3.4B). After incubation with (NQ(CH_2)_15S)_2, the 3-4 ms phase is nearly quantitatively replaced with a ~50 ms WT-like phase^{52}. PS I complexes from the menB strain that had been treated with HgCl_2 show a ~2 ms phase after a saturating flash (Figure 3.4C) reflecting the absence of the terminal F_B cluster, and addition of (NQ(CH_2)_15S)_2 results in a longer kinetic phase with a lifetime of ~25 ms (Figure 3.4D), which shows that PQ-9 can be displaced by the substituted menaquinone. Some fast phase remains, however, which could be due to non-displaced PQ-9 or to PS I cores in
which F_A is additionally damaged, the latter resulting in P_{700}-F_X cores that show characteristic ~1-2 ms charge recombination kinetics. In this sample, we estimate ~42% naphthoquinone incorporation into the A_{1A} and A_{1B} sites.

The attempted suppression of P_{700}^{+} F_{A}^{−} charge recombination in the Hg-treated PS I sample with Cyt c_{6} is shown in Figure 3.5A. After addition of (NQ(CH_{2})_{15}S)_{2}, suppression of the backreaction occurs only at concentrations of Cyt c_{6} that exceed 10 µM (17:1, Cyt c_{6}:P_{700}). This is a higher concentration than the ~1 µM of Cyt c_{6} needed to suppress P_{700}^{+} [F_{A}/F_{B}]^{−} charge recombination in WT PS I (1.7:1, Cyt c_{6}:P_{700}).

3.4.3.2 P_{700}-F_X cores from the menB psaC strain

The charge recombination kinetics in P_{700}^{+} F_X^{−} cores from the menB psaC strain are depicted in Figure 3.4E. Two kinetic phases are present with lifetimes of ~25 µs and 409 µs. A more thorough interpretation of these phases will be forthcoming in a separate article, but for the purpose of this study, we assume that both are due to charge recombination from A_{1A}^{−} and A_{1B}^{−}. After addition of (NQ(CH_{2})_{15}S)_{2}, two new phases appear, with lifetimes of 1.4 ms, and ~2 s (Figure 3.4F). Because a naphthoquinone occupies the A_{1A} and A_{1B} sites, the 1 ms phase is most likely charge recombination from F_X^{−}, while the 2 s phase is forward electron transfer to P_{700}^{+} due to electron loss to dioxygen from the surface-exposed F_X cluster. A small amount of the fast kinetic phase is still present, indicating that either some of the PQ-9 has not been displaced or the sites are unable to accept the substituted menaquinone. Either way, we estimate ~25% naphthoquinone incorporation into the A_{1A} and A_{1B} sites.

The attempted suppression of P_{700}^{+} F_X^{−} backreaction in PS I from the menB psaC strain with Cyt c_{6} is shown in Figure 3.5B. After addition of (NQ(CH_{2})_{15}S)_{2}, suppression of the
backreaction occurs only at concentrations of Cyt c₆ exceeding 200 µM (345:1, Cyt c₆:P₇₀₀). This is a much higher concentration than the ~10 µM of Cyt c₆ (17:1, Cyt c₆:P₇₀₀) needed to suppress the P₇₀₀⁺Fₐ⁻ charge recombination in the Hg-treated PS I sample lacking Fₐ.

### 3.4.3.3 P₇₀₀⁺A₁ cores from the menB rubA strain

The charge recombination kinetics in P₇₀₀⁺A₁⁻ cores from the menB rubA strain are shown in Figure 3.4G. In this preparation, charge recombination is biphasic, with lifetimes of 60 µs and 750 µs. Again, we assume that these kinetic phases are due to charge recombination from A₁A and A₁B. It is interesting that these lifetimes are both ~2-fold greater than in the presence of the Fₓ iron-sulfur cluster. One possibility for this difference is that the electrostatic environment around the quinone is different in the presence and absence of the Fₓ cluster, which would have an influence on the midpoint potential or the reorganization energy (or both). On addition of (NQ(CH₂)₁₅S)₂, the charge recombination kinetics become significantly faster, with lifetimes of 15 µs and 200 µs. Titration of the P₇₀₀⁺A₁⁻ cores with Cyt c₆ is shown in Figure 3.5C. Even the highest concentrations of Cyt c₆ that we were able to attain, 300 µM (517:1, Cyt c₆:P₇₀₀), were unable to outcompete the 15 µs and 200 µs charge recombination from A₁A⁻/A₁B⁻.

### 3.4.4 Dihydrogen Production in the Various PS I Core Preparations

Table 3.1 shows the rate of dihydrogen production attained in PS I–NQ(CH₂)₁₅S–Pt nanocostructs prepared from the various PS I cores. In the P₇₀₀⁻Fₐ cores, the onset of dihydrogen production occurs at 10 µM Cyt c₆, and the rate increases until a maximum of 0.92 ± 0.15 µmol H₂·mg chl⁻¹·h⁻¹ is reached at 200 µM Cyt c₆. The P₇₀₀⁻Fₓ cores require a significantly higher
concentration of Cyt \( c_6 \) before any dihydrogen is observed. Only at 200 µM Cyt \( c_6 \) was dihydrogen observed, and the rate was 0.11 ± 0.04 µmol H\(_2\)·mg chl\(^{-1}\)·h\(^{-1}\). No dihydrogen was observed in P\(_{700}\)-A\(_1\) cores at any concentration of Cyt \( c_6 \). Additionally, no dihydrogen was observed in control samples that were (i) not illuminated; (ii) lacking soluble electron donors; (iii) lacking the quinone wire; (iv) lacking the Pt nanoparticle; and (vi) when phylloquinone occupies the A\(_{1A}\) and A\(_{1B}\) sites.
3.5 DISCUSSION

Electron transfer among the electron acceptors of PS I is best described by a quasi-equilibrium model. The salient feature of this model is that at each step, the rate of forward electron transfer is considerably larger (by several orders of magnitude) than the rate of charge recombination. When quasi-equilibrium is achieved (at about 1 µs), the electron has a finite probability of residing on each electron transfer cofactor according to its equilibrium constant, which is determined by the change in Gibbs free energy relative to its donor and acceptor pairs. Charge recombination with \( P_{700}^+ \) occurs via thermal repopulation from (most likely) the \( A_{1A}/A_{1B} \) sites rather than by direct electron transfer to \( P_{700}^+ \) from the reduced FeS clusters.

A general diagram for the proposed electron transfer routes in the PS I–NQ(CH\(_2\)\(_{15}\)S–Pt nanoconstruct is depicted in Scheme 3.2, where \( k_{1/-1} \) is electron transfer to/from the FeS clusters, \( k_{2/-2} \) is electron transfer to/from the Pt nanoparticle, \( k_r \) is the charge recombination channel, and \( k_f \) is the reduction of \( P_{700}^+ \) by an external donor. In a previous publication, we proposed that \( k_2 \) is slower than both \( k_r \) and \( k_1 \), and only by eliminating \( k_r \) can the electron proceed to the Pt nanoparticle. This was demonstrated by a correlation between the concentration of Cyt \( c_6 \), the rate of \( P_{700}^+ \) reduction, and the onset of dihydrogen production. It was shown that dihydrogen was only generated at Cyt \( c_6 \) concentrations that reduced \( P_{700}^+ \) faster than charge recombination from the \([F_A/F_B]^-\) clusters, i.e., when charge recombination is suppressed by making \( k_f > k_r \). It follows that if the terminal acceptors were sequentially removed, this would result in a condition in which \( k_r \) is increased, and \( k_f \) would also necessarily need to increase for any electron transfer to occur to the Pt nanoparticle.

In the \( P_{700}^-F_A \) core produced by Hg-treatment of the \textit{menB} strain, the absence of the \( F_B \) cluster causes the charge recombination time to decrease from \(~65 \text{ ms}\) in the control to \(~20 \text{ ms}\) in the treated sample containing \((\text{NQ(CH}_2\text{)_{15}S})_2\). The increase in \( k_r \) is not difficult to overcome using
reasonable concentrations of Cyt c₆ (10 µM), which satisfies the condition in which \( k_f > k_r \), resulting in suppression of charge recombination. It is only when these conditions are satisfied that the production of dihydrogen occurs. However, suppression of \( k_r \) becomes significantly more difficult as additional electron acceptors are removed. The P₇₀₀-Fₓ core protein provides an insight into the limits of the conditions in which charge recombination can be out competed with an external donor.

In this sample, the rate of charge recombination is increased ~60-fold relative to the WT and only by increasing the concentration of Cyt c₆ by a factor of 20 is \( k_f > k_r \). The effect of increasing \( k_r \) becomes most apparent in the P₇₀₀-A₁ core. Here, \( k_r \) has become sufficiently large that the relatively slow diffusion-based chemistry of Cyt c₆ donation cannot result in a condition in which \( k_f > k_r \). We therefore conclude that electron extraction from the A₁ sites is contingent on suppression of the charge recombination channel. For forward donation from Cyt c₆ to be able to reduce P₇₀₀⁺, the FeS clusters are required to lengthen the charge-separated state to the ms time range.

The question arises as to the reduction potential of the electron when withdrawn from the A₁A₁/A₁B sites in these various PS I core preparations. According to the Nernst Equation:

\[
E = E^o + \left(\frac{RT}{nF}\right) \ln\left([Q_r]/[Q_o]\right)
\]

(1)

where \( E \) is the potential of the electron, \([Q_r]\) is the concentration of the reduced species and \([Q_o]\) is the concentration of the oxidized species.

If we assume quasi-equilibrium between cofactors, the relative concentration of reduced components is a function of the equilibrium constant between each donor/acceptor pair:\(^{58,69}\):

\[
[P^*] \approx \frac{1}{Z}, [A_0^-] \approx \frac{K_{PA_0}}{Z}, [A_-] \approx \frac{K_{PA_1}}{Z}, [F_X^-] \approx \frac{K_{PF_X}}{Z}, [F_A^-] \approx \frac{K_{PF_A}}{Z}, [F_B^-] \approx \frac{K_{PF_B}}{Z}
\]

\[Z \approx 1 + K_{PA_0} + K_{PA_1} + K_{PF_X} + K_{PF_A} + K_{PF_B}\]

(2)

where \( K_{PA_0} \) is the equilibrium constant between \( P^* \) and \( A_0 \), \( K_{PA_1} \) is the equilibrium constant between \( P^* \) and \( A_1 \), etc. Expanding these terms results in the following:

\[K_{PA_1} \approx K_{PA_0} K_{A_0 A_1}\]
If \( K_{XY} \) is expressed in terms of the free energy difference, \( \Delta G_{XY} = -RT\ln(K_{XY}) \), and substituted into equation (2):

\[
[A_0^-] \approx e^{\frac{-\Delta G_{PA0}}{RT}} Z
\]

\[
[A_1^-] \approx e^{\frac{-\Delta G_{PA0} - \Delta G_{A0A1}}{RT}} Z...
\]

\[
[F_{A}^-] \approx e^{\frac{-\Delta G_{PA0} - \Delta G_{A0A1} - \Delta G_{A1FX} - \Delta G_{FXFA} - \Delta G_{FAB}}{RT}} Z
\]

(4)

According to different estimations, \( \Delta G_{PA0} \) varies from -40 to -250 mV. This leads to values of \( \Delta G_{A0A1} \) which may vary from -250 to -550 mV (though these values do not significantly impact the outcome of this analysis). Given that \( \Delta G_{A1FX} = -100 \) mV, and \( \Delta G_{FXFA} = -105 \) mV, it follows that at quasi-equilibrium, the concentration of reduced species heavily favors the terminal acceptors. This results in a situation such that for the P\(_{700}\)-F\(_A\) cores and P\(_{700}\)-F\(_X\) cores, respectively:

\[
[F_{A}^-] \gg [F_{X}^-] > [A_1^-] > [A_0^-]
\]

\[
[F_{X}^-] > [A_1^-] > [A_0^-]
\]

By evaluating the Nernst equation for each cofactor, we are left with a generalized form:

\[
E_{tot} = \sum_{Q=P, A} E_Q^0 + \frac{(RT)}{nF} \ln(K_{PQ}/Z),
\]

(5)

Where \( E_{tot} \) is the total redox potential of the electron, \( E_Q^0 \) is the redox potential of a given cofactor \( Q \), and \( K_{PQ} \) is the equilibrium constant between P\(_{700}\) and a cofactor \( Q \). Substitution of equation (3) yields

\[
E_{tot} = \sum_{Q=P, A} E_Q^0 + \frac{(RT)}{nF} \ln(Q^-)
\]

(6)
In the case of the $P_{700}$-$F_A$ core sample, the contribution from the previous acceptors is negligible, and the resulting equation simplifies to:

$$E_{tot} \approx E^0_{F_A} + \left(\frac{RT}{nF}\right) \ln[F_A^-]$$

(7)

The same holds true for the $P_{700}$-$F_X$ core sample wherein:

$$E_{tot} \approx E^0_{F_X} + \left(\frac{RT}{nF}\right) \ln[F_X^-]$$

(8)

Thus, in the case of the $P_{700}$-$F_A$ core protein, the electron has the redox potential of the $F_A$ cluster ($\sim -520$ mV), and in the $P_{700}$-$F_X$ core protein, the electron has the redox potential of the $F_X$ cluster ($\sim -700$ mV). Therefore, although the FeS clusters serve to extend the lifetime of the charge-separated state, in doing so, the redox advantage of removing the electron at $A_{1A}/A_{1B}$ is lost. The key to obtaining the electron at a lower reduction potential than the FeS clusters lies in finding a fast electron donor to $P_{700}^+$ that can suppress the faster charge recombination reaction in the $P_{700}$-$A_1$ core preparations.
3.6 CONCLUSION

In this study we have provided evidence that suppression of the charge recombination channel is required for electron transfer from $A_{1A}/A_{1B}$ to a tethered Pt nanoparticle that would otherwise be unfavorable due to kinetic constraints. The key is retention of one or more FeS clusters to increase the time of charge separation such that an external electron donor can outcompete the charge recombination reaction. However, the electron is extracted from $A_{1A}$ and $A_{1B}$ at the potential of the terminal FeS cluster. The description used here is not specific to PS I and applies to any system in which charge recombination takes place via the extended equilibrium mechanism. The results show that although extracting the electron from a low potential acceptor close to the donor can become feasible by extending the lifetime of the charge-separated state using acceptors further from the donor, this comes at the expense of reduction potential. Thus, if a system is to be designed to generate an electron at the low potential of the quinone in the $A_{1B}$ site ($\sim$ –845 mV) the electron must be extracted directly from this cofactor by blocking the fast charge recombination with $P_{700}^+$ ($\sim$10 µsec). Only then will the electron be forced to proceed via the thermodynamically favorable but kinetically slower route to the catalytic center tethered to the quinone.


3.7 REFERENCES


phyloquinone biosynthetic pathway mutants of Synechocystis sp. PCC 6803, J. Biol. Chem. 276, 39512-39521.


3.7 SCHEMES

Scheme 3.1 Synthesis of 1-[15-(3-Methyl-1,4-naphthoquinone-2-yl)]pentadecyl Disulfide.

1.

\[
\text{Scheme 3.1 Synthesis of 1-[15-(3-Methyl-1,4-naphthoquinone-2-yl)]pentadecyl Disulfide.}
\]

\[
\begin{align*}
\text{Scheme 3.1 Synthesis of 1-[15-(3-Methyl-1,4-naphthoquinone-2-yl)]pentadecyl Disulfide.}
\end{align*}
\]

2.

\[
\text{yield 68%}
\]

\[
\text{yield 33%}
\]
Scheme 3.2. Energetic diagram of the PS I nanoconstruct, where $k_{1/1}$ represents the rate constant to/from all available FeS clusters, $k_{2/2}$ represents the rate constant to/from Pt nanoparticle, $k_r$ is the recombination channel, and $k_f$ represents the rate of donation from Cyt $c_6$. 
### 3.8 TABLES

**Table 3.1** Rates of dihydrogen production for various PS I core samples tethered through the A₁ sites.

<table>
<thead>
<tr>
<th>[Cyt $c_o$]</th>
<th>$menB$ PS I$^{10}$</th>
<th>$P_{700}$-F$_A$ cores</th>
<th>$P_{700}$-F$_X$ cores</th>
<th>$P_{700}$-A$_1$ cores</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 2 µM</td>
<td>0.05 ± 0.01</td>
<td>0.00$^{1,2}$</td>
<td>0.00$^{1,2}$</td>
<td>0.00$^{1,2}$</td>
</tr>
<tr>
<td>10 µM</td>
<td>1.30 ± 0.27</td>
<td>0.16 ± 0.07</td>
<td>0.00$^{1,2}$</td>
<td>0.00$^{1,2}$</td>
</tr>
<tr>
<td>50 µM</td>
<td>-</td>
<td>0.37 ± 0.13</td>
<td>0.00$^{1,2}$</td>
<td>0.00$^{1,2}$</td>
</tr>
<tr>
<td>100 µM</td>
<td>2.65 ± 0.35</td>
<td>0.67 ± 0.08</td>
<td>0.00$^{1,1}$</td>
<td>0.00$^{1,2}$</td>
</tr>
<tr>
<td>200 µM</td>
<td>-</td>
<td>0.92 ± 0.15</td>
<td>0.11 ± 0.04</td>
<td>0.00$^{1,2}$</td>
</tr>
</tbody>
</table>

$^{1}$After 48 hours of illumination; $^{2}$Limit of detection is 0.034 (µmol H₂ mg₉chl⁻¹ hr⁻¹)
3.9 FIGURE LEGENDS

**Figure 3.1** Edge-to-edge distance and midpoint potential of PS I cofactors. Solid lines represent forward electron transfer lifetimes and dashed lines represent charge recombination lifetimes. The A– and B–branches are depicted as $A_{0A}/A_{1A}$ and $A_{0B}/A_{1B}$, respectively. It is not known whether $A_{0A}$ and $A_{0B}$ have different reduction potentials; they are separated here for clarity purposes only.

**Figure 3.2** CW-EPR light-minus-dark difference spectra of PS I from the *menB* strain (top) and Hg-treated PS I from the *menB* strain (bottom) at X-band. Spectrometer conditions: microwave power, 20mW; microwave frequency, 100 kHz; modulation amplitude, 20 G; temperature, 15 K; 8 averages.

**Figure 3.3** X-band TrEPR spectra of PS I core complexes at 90K. Comparison of WT PS I with PS I complexes prepared from (A) the *menB psaC* strain, and (B) the *menB rubA* strain before and after incubation with (NQ(CH$_2$)$_{15}$S)$_2$. A=absorption, and E=emission.

**Figure 3.4** Time-resolved optical kinetics at 830 nm after a saturating laser flash in (A) WT PS I, (B) PS I complexes prepared from the *menB* strain; (C) Hg-treated PS I complexes from the *menB* strain; (D) Hg-treated PS I complexes from the *menB* strain with (NQ(CH$_2$)$_{15}$S)$_2$; (E) PS I complexes from the the *menB psaC* strain; (F) PS I complexes from the *menB psaC* strain incubated with (NQ(CH$_2$)$_{15}$S)$_2$; (G) PS I complexes from the *menB rubA* strain; and (H) the PS I complexes from the *menB rubA* strain incubated with (NQ(CH$_2$)$_{15}$S)$_2$. The data are shown as dots and the fitted spectra are shown as solid lines. The residual of the fit is depicted above each of the
plots. The reaction mixture contained PS I at 50 μg/mL Chl, 5 mM sodium ascorbate, 10 μM DCPIP, and 0.05% (w/v) β-DM in 50 mM Tris-HCl buffer, pH 8.3.

**Figure 3.5** $P_{700}^+$ dark reduction as a function of Cyt $c_6$ concentration measured at 830 nm. Samples are PS I complexes from (A) the Hg-treated menB strain plus (NQ(CH$_2$)$_{15}$S)$_2$; (B) the menB psaC strain plus (NQ(CH$_2$)$_{15}$S)$_2$; and (C) the menB rubA strain plus (NQ(CH$_2$)$_{15}$S)$_2$. Samples contained WT PS I at 50 μg/mL Chl, 5 mM sodium ascorbate, and 10 μM DCPIP in 50 mM Tris-HCl buffer, pH 8.3
Figure 3.1
Figure 3.2

[Diagram showing EPR spectra for 'As Isolated' and '+ HgCl₂' with labeled g-values and magnetic field (mT) range.]
Figure 3.3
Figure 3.4
Figure 3.5

A

B

C
Chapter 4

Studies of Cyanobacterial Photosystem I Embedded into a Room

Temperature Trehalose Glass Matrix

[Contributing authors include Michael Gorka, Nastia Petrova, Dr. Vasily Kurashov, Dr, Alexy Semenov, and Dr. John Golbeck]
4.1 ABSTRACT

Studying proteins at their glass transition temperature ($t_g$) has yielded important information on how protein dynamics affect function. However, these experiments suffer from practical limitations, as cryogenic temperatures and solutions contain high concentrations of glycerol (>65% w/v) are required. In recent years, research has focused on implementing trehalose as a means of overcoming these limitations. Trehalose is a naturally occurring disaccharide that has myriad uses in a variety of organisms, including protection against desiccation. Although the mechanism and the nature of its interaction with protein is not fully understood, the unusually high $t_g$ of trehalose makes it a prime candidate for studying the effects of protein immobilization at room temperature. Although other proteins have been studied, photosynthetic reaction centers (RCs) are poorly represented in the current body of literature. This work represents the first study of Photosystem I (PS I) from cyanobacteria embedded in a room temperature trehalose glass. We use time-resolved optical spectroscopy to measure both forward electron transfer and charge recombination in wild type PS I, P$_{700}$-F$_X$ cores, and P$_{700}$-A$_1$ cores. The results demonstrate that protein immobilization significantly impacts the rates of electron transfer that can be attributed to functional inhomogeneity in which PS I RCs populate various sub-states. Moreover, as this system is comparable to low temperature glasses, the effects can be mimicked and studied at room temperature.
4.2 INTRODUCTION

Examining the function of proteins at their glass transition temperature is an area of study that has received much attention in recent years. Yet, analysis of these systems can be difficult because of practical limitations that require cryogenic temperatures and the presence of high concentrations of glycerol\textsuperscript{1,2}. Recently, however, researchers have had success in using saccharides to form glasses at or near-room temperature. Perhaps the most useful of those studied is the disaccharide, trehalose.

It has long been known that nature uses trehalose as a means of stabilizing biomolecules. Trehalose is produced in many species of eubacteria, archaea, as well as certain fungi, invertebrates, and lower plants, being important for many environmental adaptations\textsuperscript{3}. For instance, trehalose is synthesized in \textit{Escherichia coli} during times of osmotic stress\textsuperscript{4}, in roundworms as a protection against desiccation\textsuperscript{5}, and it was found to play an important role in cryoprotection in \textit{Lactobacillus acidophilus}\textsuperscript{6}.

The reason for the myriad uses of trehalose in nature is attributed to its unique chemical and physical properties. Identified as a kosmotrope, the trehalose/water interaction is stronger than the interaction between water molecules. As such, it organizes water around itself\textsuperscript{7}. Moreover, its surprisingly stable glycosidic oxygen renders it unusually rigid and much less susceptible to hydrolysis\textsuperscript{8}. Perhaps its most interesting property is the high glass transition temperature ($t_g$); at 117°C, trehalose has the highest glass transition temperature of all the mono and disaccharides. The result of this property is that even when moderately hydrated, it is still able to stay below its $t_g$ at room temperature, and this is especially advantageous for studying immobilized proteins at non-cryogenic temperatures\textsuperscript{7}.

The mechanism of protein stabilization by trehalose is not well understood, and different models have been put forth:
1. The **high-viscosity model** proposes that it is the vitrification of the sugar matrix that is responsible for its stabilizing properties. Here, fluctuations of the bulk solvent are disallowed by the inherent viscosity of the glassy matrix, which significantly reduces internal protein dynamics\textsuperscript{7,9}.

2. The **water-replacement model** suggests that as water molecules (and thus hydrogen bonds) are lost in the drying process, they are subsequently replaced by trehalose molecules. The internal motions of the protein are restricted by the size and rigidity of the trehalose molecules that now function to provide hydrogen bonds to the protein\textsuperscript{7,10,11}.

3. The **water-entrapment model** proposes that there is almost no interaction between the protein and the trehalose matrix. Instead, it is the kosmotropic (water-ordering) property that drives the inhibition of protein dynamics. The trehalose molecules order waters around themselves instead of the protein, forming a shell which locks in a limited range of protein conformations\textsuperscript{7,12}.

It should be noted that the models above are not necessarily mutually exclusive, though each proposes a unique mechanism of protein interaction with the trehalose matrix. In reality, the effects are most likely a combination of different models. Of course, this issue is complicated by the fact that membrane-bound proteins are only soluble in aqueous media in the presence of surfactants, and accounting for this additional interaction can be difficult.

The effects of immobilization by trehalose have been recently studied in the Type II reaction center (RC) of the purple bacterium *Rhodobacter sphaeroides*\textsuperscript{13,14}, and in Photosystem II (PS II) from spinach\textsuperscript{15}. In each of these systems, glass formation had a significant impact on the function of the proteins. In the purple bacterial RC, dehydrated glasses had a much faster charge recombination time due to the rigidity of the protein, resulting in an inability to change between light- and dark-adapted states. Interestingly, in spinach PS II, trehalose was shown to not only
enhance steady-state O$_2$ evolution, but to also significantly inhibit inactivation during extended storage$^{15}$. We are, however, unaware of any published studies that have examined electron transfer in the Type I reaction center, Photosystem I (PS I), embedded in a trehalose matrix.

PS I from cyanobacteria is an ideal system in which to study the effects of protein dynamics on electron transfer. The crystal structure has been resolved down to 2.5 Å$^{16}$, and the distances between cofactors and their locations within the protein are known. Moreover, both chemical and genetic manipulations on PS I have been employed and their effects on protein structure and function are known$^{17-19}$. Perhaps most importantly, the thermodynamics and kinetics of electron transfer have been studied in depth and their models are well understood$^{1, 2, 20-23}$.

PS I is a pigment protein complex composed of 13 subunits whose function is to create and maintain a long-lived charge separated state upon exposure to light. The 96 antennae chlorophyll (Chl) serve to harvest light and, via a Förster mechanism, transfer the energy to the trap, P$_{700}$, a Chl dimer$^{23}$. Initial charge separation occurs within 20 ps to a Chl monomer denoted A$_0$$^{24}$. In order to prolong the lifetime of charge separation, the electron is transferred through a series of cofactors including one of two phylloquinone molecules (A$_{1A}$ and A$_{1B}$), and three [4Fe-4S] clusters, denoted F$_X$, F$_A$, and F$_B$. The resulting state, P$_{700}^+ F_B^-$, is stable for ~65 ms, which is long enough to be physiologically relevant$^{16, 25, 26}$. A summary of electron transfer in PS I is described in Figure 4.1.

This work provides the first spectroscopic measurements for both forward electron transfer and charge recombination in PS I in a room temperature glass. Using time-resolved optical spectroscopy at 830 nm and 480 nm, we examine charge recombination and forward electron transfer rates in different PS I preparations. The effects of the FeS clusters are examined by focusing on intact PS I, P$_{700}$-F$_X$ cores, and P$_{700}$-A$_1$ cores (the latter provided by the rubA mutant). The results show that glass formation significantly impacts the rates of electron transfer in terms of P$_{700}^+$ reduction, and phylloquinone re-oxidation, and that the observed rates are comparable to those seen in low temperature glasses.
4.3 MATERIALS AND METHODS

4.3.1 Growth and Purification of PS I

Wild type (WT) PS I from *Synechocystis sp.* PCC 6803 and PS I from the *rubA* variant of *Synechococcus sp.* PCC 7002 were isolated and purified in accordance with published methods.\(^\text{27, 28}\) Cells were harvested by centrifugation and broken by three passes through an M-110EH-30 microfluidizer processor (Microfluidics). Low speed centrifugation (2000 \(\times\) g) was used to remove cell debris. The thylakoid membranes in the supernatant were pelleted by centrifugation at 158,000 x g for 1h. Membranes were solubilized with 1\% (w/v) \(\beta\)-dodecylmaltoside (\(\beta\)-DM) for 1 h at 4°C in the dark. The resulting WT PS I trimers were initially purified on a 5 to 20\% sucrose density gradient in 50 mM Tris-HCl buffer (pH 8.3), 0.05\% (w/v) \(\beta\)-DM. Purified PS I trimers were pelleted with a second 5-20\% sucrose density gradient lacking \(\beta\)-DM, and suspended in buffer containing 0.05\% \(\beta\)-DM and 20\% (v/v) glycerol in 50 mM Tris-HCl (pH 8.3). PS I from the *rubA* mutant did not produce trimers and thus the monomers were collected from the first gradient; the contaminating PS II was removed with a DEAE-sepharose anion exchange column (Sigma) with 50 mM Tris-HCl buffer (pH 8.0), 0.05\% \(\beta\)-DM. PS I monomers were eluted at 75-100 mM NaCl and PS II was eluted at 200 mM NaCl. The purity of the fractions was confirmed by fluorescence emission spectroscopy at 77 K.
4.3.2 Generation of $P_{700}$-$F_X$ cores

$P_{700}$-$F_X$ cores were generated as previously described$^{17}$. In short, PS I particles were incubated with 6.8 M urea for ~30 minutes until all detectable PsaC was removed. The resulting PS I was diluted 10x with ddH$_2$O to quench the reaction and the urea and dissociated proteins were removed by dialysis against 50 mM Tris-HCl buffer (pH 8.3) using a 50 kDa membrane.

4.3.3 Preparation of Trehalose Glass

Trehalose glass samples were prepared as follows. A 40 µL solution of 0.8 M trehalose containing 1 mg/mL PS I (providing a ratio of 80,000:1 trehalose:PS I), 10 µM 2,6-dichlorophenolindophenol and 5 mM sodium ascorbate was made in 50 mM Tris-HCl buffer (pH 8.3) with 0.05% β-DM. Once properly mixed, the solution was pipetted onto an optical glass slide, forming a thin layer. The slide was initially dried in a desiccator containing a saturated solution of lithium chloride under continual flow of ultra-pure argon for ~5 hours. The sample was then placed into a separate desiccator containing a saturated solution of lithium chloride, under an atmosphere of ultra-pure argon for a minimum of 5 days prior to spectroscopic measurements. Samples were prepared and stored at room temperature in the dark.

4.3.4 Time-Resolved Optical Spectroscopy at 830 nm

Charge recombination kinetics in the PS I-trehalose complexes were monitored by transient absorbance changes at 830 nm following a single-turnover laser flash at 532 nm using a laboratory-built, dual-beam spectrometer as described previously$^{29}$. The actinic beam was from a ND:YAG
laser (DCR-11, Spectra-Physics) with a 3 ns pulse width flashed every 4 seconds and the intensity was lowered to <5% with neutral density filters. The measuring beam was provided by an 830 nm laser diode (Crystalaser, model number DL830-100-O). Samples were placed in sealable cuvette that was purged for 5 minutes with argon prior to measuring, to maintain the exclusion of water. During measurements, the glass slide was oriented such that it was at a 45-degree angle relative to both the actinic and probe beams. The intensity of the probe beam on the sample and reference arm were balanced using a variable density optical filter wheel. In front of each detector were two 830 nm interference filter (FL830-10, Thor Labs) and a 532 nm notch filter (NF533-17, Thor Labs) to remove stray actinic light. The difference signal was amplified with a differential amplifier (Thor Labs PDB460A), and the data were processed using a 12-bit, 200 MS/s, NI-5124 PCI card (National Instruments).

**4.3.5 Time-Resolved Optical Spectroscopy at 480 nm**

Phylloquinone re-oxidation rates were obtained by measuring at 480 nm following a single-turnover laser flash at 532 nm using a laboratory-built, dual-beam spectrometer as described previously. The measuring beam was provided by a 480 laser diode (OptoEngine MBL-H-480-30mW). In order to minimize the actinic effect of 480 nm light, the beam was put through a mechanical shutter that opened 2 ms prior to the laser flash, expanded using a beam expander, and subsequently condensed prior to the detectors with a beam condenser. The glass slide was placed such that it was perpendicular to the probe beam and at a 45-degree angle to the actinic light. Measuring and reference beam intensities were balanced using a variable density optical filter wheel. The difference signal was amplified with a differential amplifier (Thor Labs PDB460A) and processed using a PCI card (National Instruments). Data were analyzed using the graphics
capability of IGOR (Wavemetrics) and the traces were fit using a stretched multieponential fitting program.

4.4 RESULTS

4.4.1 Time-Resolved Optical Spectroscopy of WT PS I

Time-resolved optical spectroscopy at 830 nm is a commonly used method for measuring the charge recombination kinetics of PS I following a single turnover laser flash. The rate of reduction of $P_{700}^{+}$ can provide information about the kinetics and thermodynamics of the cofactors in the electron transfer chain and from which cofactor the electron is returning.

Measurements at 480 nm are able to monitor re-oxidation of the $A_{1A}$ and $A_{1B}$ quinones by taking advantage of the electrochromic bandshift of a $\beta$-carotene near the quinones$^{31}$. Most frequently, it is used to measure the rate of forward electron transfer from $A_{1A}$ and $A_{1B}$ to $F_X$. There are several aspects of this experiment that must be addressed. First, as we are not directly observing the quinone, the measurements are not specific to the identity of the quinone, i.e. whether it is $A_{1A}$ or $A_{1B}$. Second, 480 nm light has an actinic effect that initiates photochemistry and therefore we must reduce the actinic effect of the probe beam by limiting the measurement window to 400 µs. Finally, $P_{700}^{+}$ absorbs at 480 nm and the spectra always contain a relatively small contribution from $P_{700}^{+}$.

The spectra at 830 nm and 480 nm for WT, $P_{700}$-$F_X$ core, and $P_{700}$-$A_1$ core (from the rubA mutant) are presented below:
4.4.1.1 Wild Type PS I

Figure 4.2A,C shows the spectra taken at 830 nm for intact WT PS I in solution (Figure 4.2A) and in fully dried trehalose (Figure 4.2C). WT PS I in solution has well characterized charge recombination kinetics, with a major component having a lifetime of 65 ms ascribed to charge recombination from \([F_A/F_B]^-\), and a longer component having a lifetime of \(~2\) s assigned to reduction of \(P_{700}^+\) by DCPIP due to electron loss on the acceptor side to dioxygen. In a dried trehalose glass, the charge recombination kinetics are altered and they are characterized by several overlapping decays. The most significant decay of \(P_{700}^+\) occurs with a half-life of \(~100\) µs, with other contributions of roughly equal amplitude, having half-lives of \(~1\) ms and \(~20\) ms. Charge recombination kinetics were also measured after 9 months of storage at room temperature in a desiccator at 11% relative humidity and the results were identical (data not shown).

Figure 4.2B,D shows spectra taken at 480 nm at for WT PS I in solution (Figure 4.2B) and in fully dried trehalose (Figure 4.2D). In solution we observe two major kinetic phases with half-lives around 20 ns and 200 ns in a ratio of 1:2 (fast:slow). These phases are assigned to electron transfer from \(A_{1A}\) to \(F_X\) (200 ns) and \(A_{1B}\) to \(F_X\) (20 ns) and the remaining portion of the spectra that has not decayed (\(~1/3\) of the total signal) is attributed to the decay of \(P_{700}^+\). However, we observe much different kinetics in the dried trehalose samples. Both the 20 ns and 200 ns phases are missing and new kinetic phases with lifetimes of \(~74\) ns and \(~12\) µs (note the different time scale) are observed in a ratio of 1:2 (fast:slow) in addition to a slow phase at \(~150\) µs. We tentatively assign the \(~70\) ns phase to electron transfer from the B-side quinone to \(F_X\), the \(~200\) ns phase to electron transfer between the A-side quinone and \(F_X\), and the \(~150\) µs phase to charge recombination (see discussion for details).
4.4.1.2 \(P_{700}F_X\) cores

Figure 4.3A shows spectra taken at 830 nm at for \(P_{700}F_X\) cores in fully dried trehalose glass. In solution, it is well understood that charge recombination from the \(F_X\) cluster occurs with a lifetime of 1 ms. In the glass samples, however, multiple kinetic phases are present. As in the WT trehalose glass, we measure kinetic phases with half-lives of \(~100\) µs and \(~1\) ms, albeit with different relative ratios. However, in contrast to the WT sample, we do not observe any phases longer than \(1\) ms.

Figure 4.3B shows spectra taken at 480 nm at for \(P_{700}F_X\) cores in fully dried trehalose glass. Changes similar to those observed in the WT are observed. The \(~20\) ns and \(200\) ns phases are replaced kinetic phases having lifetimes of \(~68\) ns and \(12\) µs phases (note the different time scale), in a ratio of 1:2 (fast:slow). There is also a slow kinetic phase with a lifetime of \(~100\) µs. We again assign these phases to electron transfer from the B-side quinone (65 ns phase), the A-side (12 µs phase) quinone to \(F_X\), and to charge recombination from the quinone site (\(~100\) µs phase).

4.4.1.3 \(P_{700}A_1\) cores

Figure 4.4A, C shows spectra taken at 830 nm at for \(P_{700}A_1\) cores in solution (Figure 4.4A) and in fully dried trehalose glass (Figure 4.4C). The kinetics are similar in both samples, with lifetimes of 10 µs and 100 µs in a ratio of 2:1 (fast:slow). Since the quinones are the terminal acceptors in these samples, the phases are attributed to charge recombination between \(A_{1A}^-\) and \(P_{700}^+\) and \(A_{1B}^-\) and \(P_{700}^+\), respectively\(^{28}\). The presence of a 20 ns phase is attributed to charge recombination between \(A_0^-\) and \(P_{700}^+\), a result of empty \(A_1\) binding sites. The fraction of this contribution is significant, and it is estimated that \(~40\)% of the binding sites are empty.
Figure 4.4B,D shows 480 nm spectra for rubA PS I in solution (Figure 4.4B) and in dried trehalose glass (Figure 4.4D). In both samples, significant kinetic phases with lifetimes of 10 µs and 100 µs appear, again, in a ratio of approximately 2:1. These results correlate with the 830 nm data that shows charge recombination of each quinone with P700+. There is also 20 ns phase that is again attributed to charge recombination between P700+ and A0−.

4.5 DISCUSSION

4.5.1 Analysis of Kinetic Phases

I will first evaluate the charge recombination kinetics determined at 830 nm, as previous studies at this wavelength are perhaps the best characterized and understood. The multiple phases observed in the WT trehalose sample appear to be in good agreement with what is understood about charge recombination kinetics of the various cofactors of PS I23. The 100 µs, and 1 ms, kinetic phases in the glass are assigned to charge recombination from A1− to P700+, and Fx− to P700+ respectively. However, the assignment of the 30 ms phase is uncertain. The accepted value for charge recombination from Fb− to P700+ is 65 ms. When this cofactor is removed with mercuric chloride and only Fa remains, the charge recombination time changes to ~20 ms32. The observed value of 30 ms lies closer to Fa than Fb, leaving several possible explanations. Electron transfer between Fa and Fb is thermodynamically uphill and could be inhibited in the majority of immobilized samples in which internal motions are restricted. In this case, charge recombination would occur from Fa−. Conversely, as is seen in bacterial RCs, where immobilization alters the charge recombination time due to an inability to change conformations13,14, immobilization of PS
I may shorten the lifetime of F_B from 65 ms to 30 ms. Further experiments are required to determine which of these explanations are more plausible.

A similar effect is seen in the P_{700}-F_X core sample, where charge recombination phases with lifetimes of 100 µs, and 1 ms can be assigned to A_1^- and F_X^-, respectively. The lack of any kinetic phases longer than 1 ms in the P_{700}-F_X core gives added reliability to the assignment of the slowest phase seen in the WT to charge recombination from [F_A/F_B]^-, and provides evidence that charge recombination is not merely slowed to different degrees from the F_X cluster.

In analyzing the electron transfer kinetics of A_1^- to F_X as measured at 480 nm, the values of 70 ns and 12 µs can be reasonably assigned to A_{1B}^- to F_X and A_{1A}^- to F_X respectively. First, the ratio between the fast and the slow phases (1:2 fast:slow) is in agreement with previously determined values for electron transfer from A_{1A}^- and A_{1B}^- to F_X. Moreover, the activation energy for the A_{1A}^- to F_X transition (110-220 meV) is much higher than the A_{1B}^- to F_X transition (17 meV), and thus the effect of temperature (or in this case, immobilization) on the rate would be predicted to be appreciably higher for A side re-oxidation. Additionally, the 12 µs phase seen at 480 nm for both samples does not have a compliment at 830 nm, indicating that it is not due to charge recombination. Finally, both the WT and P_{700}-F_X core are in good agreement with each other, as would be expected. In support of this assessment, Lüneberg and colleagues demonstrated that the rates of electron transfer from the quinones to the F_X cluster are identical in samples with and without the PsaC subunit (and thus, without F_A and F_B)^{13}.

While the charge recombination lifetimes in both the WT and P_{700}-F_X core samples appear to deviate from their traditional values, those in the P_{700}-A_1 cores do not. In the latter, the 480 nm and 830 nm spectra in solution are identical to those in the trehalose glasses. As there are no FeS clusters for available for forward electron transfer, we are confident in assigning 10 µs and 100 µs kinetic phases to charge recombination from A_{1A}^- and A_{1B}^-, respectively.
One possible explanation for the changes in charge recombination times in the trehalose glasses for the WT and P700-\(\text{F}_X\) core is a functional inhomogeneity that gives rise to different sub-states. These various sub-states within the glass would restrict electron transfer from different cofactors by changing the distance or redox potential of the cofactors. In this model, described in Figure 4.5, the 100 µs kinetic phase would be due to sub-states in which electron transfer from \(\text{A}_1^-\) to \(\text{F}_X\) is blocked, the 1 ms phase would arise from sub-states where electron transfer from \(\text{F}_X^-\) to \(\text{F}_A\) is blocked, and the 30 ms phase is due to sub-states where charge recombination occurs from \([\text{F}_A\text{F}_B^-]\). This would explain why the various phases are nearly identical to the accepted values for each cofactor.

4.5.2 Comparison with Low Temperature Glasses

In order to compare the function room temperature trehalose glasses with their low temperature glycerol complements, we can examine the experimentally determined values found in previous work. In pioneering studies carried out by Schlodder, et.al.\(^1\), and Agalarov, et.al.\(^2\), the forward rate of electron transfer from \(\text{A}_1^-\) to \(\text{F}_X\) in low temperature glasses was measured as a function of sample temperature. Using their data, they were able to approximate the activation energy of electron transfer from each quinone using the Arrhenius Equation:

\[
k = A e^{-E_a/RT}
\]

where \(k\) is the rate in units of \(s^{-1}\), \(E_a\) is the activation energy, and \(T\) is the absolute temperature in Kelvin. In their measurements, Schlodder and coworkers and Agalarov and coworkers determined the activation energies for the fast and slow phases to be 17 meV\(^2\) and 110-220 meV\(^{1/2}\), respectively. While there is considerable uncertainty in the slow phase, this can be
attributed to the noise level of their experiments and the differences in the biological samples (PS I was isolated from different species, in both monomer and trimeric forms).

Table 1 summarizes a comparison of the experimental parameters between room temperature trehalose glasses and low temperature glycerol glasses. Determination of the activation energy was done by inserting the rates measured in this experiment, along with the $t_g$ for a 65\% glycerol solution ($\approx 170$ K). With these parameters in mind, we calculated an activation energy of 18 meV for the fast phase, and 70 meV for the slow phase, both of which are in reasonable agreement with the previously determined values.

The determination of temperature represents the ‘effective’ temperature of the trehalose glasses using the $E_a$ and rates determined in this work. According to our calculations, the ‘effective’ temperatures for the fast and slow phases are $\approx 160$ K and $\approx 191$ K, respectively.

By examining the electron transfer rates, it is possible to predict the difference between what we observe and what we should expect from a room temperature glass. By using values for the $E_a$ and $T$ of 17 meV/110 meV and 170 K, respectively, we find that our values for the fast phase are in good agreement with the previously determined values. A significant difference occurs, however, when comparing the slow phase, which is an order of magnitude faster than predicted.

The difference in the slow measurement could arise from several factors: (1) The resolution of our spectroscopic measurements are significantly faster, 1 ns/point as opposed to 10 ns/point; (2) similarly, our time window is considerably larger, as our measurements extend out to 400 $\mu$s, as opposed to 6 $\mu$s; (3) small errors in calculating the $E_a$ will lead to large differences in expected values for electron transfer rate and ‘effective’ temperature; (4) the nature of the interaction of trehalose with proteins is not yet fully understood, particularly in proteins in which there is relatively little structural data available to examine.

In addition to forward electron transfer, Schlodder and coworkers measured charge recombination as a function of temperature in glycerol glasses\(^1\). It was found that as they
approached the glass transition temperature, the decay became multiphasic, and the ratio of fast-to-slow phases increased. At the $t_g$, it was found that only 60-70% of the PS I complexes participated in forward electron transfer to the FeS clusters, with the remaining 30-40% being due to charge recombination from $A_{1^-}$. In trehalose glass samples, there is a similar effect, with ~57% of charge recombination attributed to the FeS clusters and ~43% attributed to charge recombination from $A_{1^-}$.

4.6 CONCLUSION

In this work, we have performed the first study on electron transfer rates for PS I embedded in a room temperature glass. We have provided evidence that more than a simple consideration of the thermodynamics between the donor/acceptor pairs is required for electron transfer through this reaction center. To be specific, the rates of electron transfer are significantly slowed as the rigidity of the protein increases. Additionally, we have shown that the behavior of room temperature trehalose glasses is comparable to their behavior at low temperatures. This result opens up new and exciting opportunities for future research. A natural extension of this work would be to measure forward electron transfer rates and charge recombination rates as a function of water content. Water is a known plasticizer of trehalose glasses, and by increasing the water content, the inherent rigidity of the protein matrix decreases. This might allow one to mimic different temperatures by simply altering the water content. Additionally, the introduction of exogenous quinones into PS I using the menB mutant could vary the redox properties of the quinone and its effects on electron transfer in glasses. Electron paramagnetic resonance studies could be performed to both confirm the rates of forward electron transfer, as well as determine the properties of the reduced FeS clusters in WT samples (if indeed there are any). Moreover, the thermostability exhibited by these samples over
long periods of time would be beneficial for examining PS I complexes from *Chlamydomonas reinhardtii*, which are known to aggregate quickly, making spectroscopic measurements particularly difficult. Finally, the reproducibility of the measurements over a period of 9 months indicates trehalose glass is a promising matrix through which to maintain a PS I based solar cell.
4.7 REFERENCES


[21] Shinkarev, V. P., Vassiliev, I. R., and Golbeck, J. H. (2000) A kinetic assessment of the sequence of electron transfer from F(X) to F(A) and further to F(B) in photosystem I: the value of the equilibrium constant between F(X) and F(A), *Biophys. J.* 78, 363-372.


4.8 TABLES

**Table 4.1** Comparison of experimental parameters determined for low temperature glycerol glasses and room temperature trehalose glasses

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Glycerol Glass</th>
<th>Trehalose Glass</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fast Phase</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$E_a$*</td>
<td>17 meV</td>
<td>18 meV</td>
</tr>
<tr>
<td>$T^\dagger$</td>
<td>170 K</td>
<td>162 K</td>
</tr>
<tr>
<td>$k$</td>
<td>64 ns</td>
<td>70 ns</td>
</tr>
<tr>
<td><strong>Slow Phase</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$E_a$*</td>
<td>110 meV</td>
<td>70 meV</td>
</tr>
<tr>
<td>$T^\dagger$</td>
<td>170 K</td>
<td>191 K</td>
</tr>
<tr>
<td>$k$</td>
<td>330 µs</td>
<td>12 µs</td>
</tr>
</tbody>
</table>

*using a $T$ of 170K; †Using experimentally determined $E_a$
4.8 FIGURE LEGENDS

**Figure 4.1** Redox potential for electron transfer cofactors in PS I as a function of edge-to-edge distance. Solid lines: Forward electron transfer; dashed lines: Charge recombination.

**Figure 4.2** On top: Time-resolved optical kinetics at 830 nm for WT PS I in solution (A) and in trehalose glass (B). On bottom: Time-resolved optical kinetics at 480 nm for WT PS I in solution (C) and in trehalose glass (D). Data is represented as blue dots, and the fitted spectra as solid red lines.

**Figure 4.3** Time-resolved optical kinetics at 830 nm (A) and 480 nm (B) for P$_{700}$-F$_X$ cores in trehalose glass. Data is represented as blue dots, and the fitted spectra as solid red lines.

**Figure 4.4** On top: Time-resolved optical kinetics at 830 nm for P$_{700}$-A$_1$ cores in solution (A) and in trehalose glass (B). On bottom: Time-resolved optical kinetics at 480 nm for P$_{700}$-A$_1$ cores in solution (C) and in trehalose glass (D). Data is represented as blue dots, and the fitted spectra as solid red lines.

**Figure 4.5** Depiction of sub-states of PS I when embedded in trehalose glass for WT (top) and P$_{700}$-F$_X$ cores (bottom) as predicted by 830 nm kinetics. Red crosses represent functionally prohibited electron transfer from various cofactors. Below each sub-state is the phase to which it is attributed and its fraction of the total signal.
Figure 4.1
Figure 4.2
Figure 4.3

A

89.15 μs, 7.1 mOD
1.13 ms, 3.4 mOD

B

67.98 ns, 1.6 mOD
12.45 μs, 2.9 mOD
90.07 μs, 0.6 mOD
Figure 4.4
Figure 4.5

100 µs
44%

1 ms
30%

30 ms
26%

100 µs
67%

1 ms
33%
Chapter 5

Concluding Remarks and Future Directions
The work presented in this thesis details the first attempts to fully elucidate the mechanism and requirements for electron extraction from the A$_{1A}$ and A$_{1B}$ sites of photosystem I (PS I), and the first work to employ such a system for the production of solar fuels. It has been demonstrated that this mechanism is atypical when compared to the prevailing paradigm and provides two important ideas. (1) A reservoir of higher potential electron acceptors can serve to ‘park’ the electron and gain an increase in the lifetime of a charge-separated state. (2) Electron transfer can then occur through a kinetically unfavorable route as long as via the charge recombination channel is blocked. This, in particular, represents a way to think about facilitating electron transfer in both natural and artificial systems, making it be possible to tether multiple unique catalytic centers to each PS I complex or perhaps any light harvesting module.

5.1 EFFICACY OF A$_1$ TETHERING FOR PRACTICAL DEVICES

While this particular tethering strategy has been successful, it is important to evaluate it relative to the alternative method of wiring the F$_B$ site. This methodology has solved two fundamental problems that occur in other PS I tethering strategies. The first is that protein structure remains unchanged. Methods that tether through the terminal FeS cluster currently require the removal of the stromal subunits PsaC, PsaD, and PsaE and reconstitution with modified versions of the same proteins. As the binding of these subunits is particularly strong, 6.8 M urea is used to remove them entirely. As might be expected, this process irreparably damages a fraction of PS I complexes, often by destroying the F$_X$ cluster and in some cases, damaging the A$_1$ binding site such that quinones can no longer bind. However, by employing the menB mutant and simply displacing the plastoquinone, no protein damage is incurred and all PS I complexes remain functional. The only limitation in this scenario is the percentage of PS I complexes that allow complete
displacement of the plastoquinone; while nearly quantitative in menB PS I from *Synechocystis* sp PCC 6803, they are less than completely displaced in menB PS I from *Synechococcus* sp. PCC 7002. The second is that the molecular wire has ends specifically functionalized for each binding site. One problem currently limiting the efficiency of F<sub>B</sub>-based nanoconstructs is that the wires that are employed are non-specific. As each end is terminated in a thiol, it is not only probable, but likely, that non-specific tethering produces non-functional configurations, namely PS I-PS I and catalyst-catalyst constructs. These would significantly lower the efficiency, as only 1/3 of all PS I complexes would be active in generating dihydrogen. However, as the quinone presumably only binds to the A<sub>1A</sub> and A<sub>1B</sub> sites of PS I and as the thiol only binds to the catalyst, any homodimeric PS I-PS I and catalyst-catalyst constructs are avoided.

That said, several limitations are observed in this system that make it not an ideal choice for use as a practical device, particularly when compared to F<sub>B</sub> tethering. The most obvious shortcoming of this system is the relatively low rate of dihydrogen production. The maximum rate of dihydrogen produced for Pt nanoparticles tethered through the F<sub>B</sub> site is about 4.5 times higher than through the A<sub>1</sub> site. This effect becomes even more apparent when compared to systems using [Fe-Fe]-hydrogenase as a catalyst, in which the rates are over 20 times higher. Another limitation is the redox potential of the extracted electron. As the electron maintains the redox potential of the terminal cofactor during charge recombination, no gain in driving force is offered by removal from an internal cofactor. In order to gain the added redox potential, the charge recombination channel must be inhibited at rates perhaps only accessible by deposition onto an electrode surface. This prospect has proven challenging for many groups, and many have tried with little success. Perhaps the most promising system involves embedding into osmium gels, though these systems do still rely on ultimately on diffusion-based chemistry.

While there are clear limitations for *in vitro* systems, an *in vivo* system may show more promise. When suddenly exposed to light after being dark adapted, the cell simply cannot process
the number of electrons being produced, leading to photoaccumulation of reduced species in the electron transfer chain. Assembly of this nanoconstruct inside of a cell may provide a passive switch, allowing ‘backed up’ electrons to become useful by transferring through the wire when exposed saturating light intensities. This would increase the electron throughput of the protein, allowing more work to be done with the same amount of light. The difficulty, however, would be in engineering a membrane-based system where a functional enzyme would not only be embedded in the thylakoid membrane near to the PS I complexes, but also be covalently bonded via the quinone wire.

5.2 FUTURE WORK IN TREHALOSE MATRIX

The results when embedding PS I into a trehalose matrix are promising and provide a window into a new and exciting area of research. This work has shown that not only does a trehalose glass impact rates of electron transfer, but that the results are comparable to those at low temperatures. As we are now able to accurately mimic low temperatures while at room temperature, examining the kinetics and thermodynamics of the electron acceptor chain will be much easier and provide higher quality information.

While there are many possible research paths to take, two particularly attractive options are measuring electron transfer rates (i) at different water contents and (ii) with different cofactor identities. Water is a known plasticizer of trehalose glasses and as water content increases, so does the fluidity of the matrix. It should therefore be possible to compare the effects of trehalose glass with different water content to glycerol glasses at different temperatures. Moreover, we have a powerful tool in which to alter the thermodynamics of cofactors in PS I. By employing the menB mutant, we have an opportunity to change the nature of the interaction between the A1 and Fx.
cofactors. Introduction of strongly oxidizing or reducing quinones would be predicted to have a strong effect on electron transfer rates to the $F_X$ site (if it is allowed to occur at all). By selecting quinones of specific redox potentials, it may be possible to find a redox ‘tipping point’ where protein dynamics are required to allow for electron transfer through the PS I reaction center.

Thorough understanding of this room temperature glass and its effect on electron transfer in PS I could allow for developments in several areas. Perhaps the most obvious implementation of this system would be in PS I based solar cells. As longevity is one of the primary concerns of biological based systems, a trehalose-based matrix may be the solution. This work indicates that trehalose can help address two common problems with a PS I based system: (i) electron loss to molecular oxygen and (i) protein denaturation. Unless strictly anaerobic, molecular oxygen will act as an electron acceptor, resulting in not only ‘unusable’ electrons, but also in reduced oxygen species, which could damage the protein. However, after repeated flashes of the PS I embedded trehalose glass, we see no difference in the total absorbance of the sample. With no ability to reduce $P_{700}^+$ with external donors due to sample rigidity, it is likely that molecular oxygen is somehow inhibited from interacting with the protein. While the mechanism is unclear, it could be a structural issue, where molecular oxygen cannot reach the cofactors, a kinetic issue, where the rapid recombination rate out competes electron transfer to dioxygen, or both. Regardless, this would drastically increase the efficiency and longevity of a nanoconstruct, as more electrons will be available to be used, and the system will not be damaged by reduced oxygen species. Longevity will also be increased by inhibiting denaturation. After studying these glasses over a period of several months, no loss in absorbance or change in kinetic phases was observed, indicating that even at room temperature, the protein remains functional for extended periods of time.

Two obvious challenges to this prospect are: (i) rapid recombination and (ii) limited diffusion. If charge recombination is too fast and electron transfer to the terminal clusters is inefficient, the total efficiency of the construct will be severely diminished. According to this work,
only ~20% of the electrons make it to the $F_{A}/F_{B}$ clusters and presumably fewer would make it through a molecular wire. However, this effect may be manipulated if the sample is selectively hydrated and kept at a constant relative humidity above 11%, though this has not been validated. Additionally, being diffusion limited will inhibit sacrificial electron donors such as cytochrome and ascorbate from interacting with the protein. Placing PS I on an electrode surface, however, would alleviate this issue and allow $P_{700}^+$ to be reduced. Clearly, this work opens up many opportunities for research into manipulating the effects room temperature trehalose glasses and its applications to PS I based system.
5.3 REFERENCES


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- “Interaction of Cations with DNA at Interfaces as Studied by Sum Frequency Generation” Presentation offered at Physical Chemistry Seminar, The Pennsylvania State University. February 19^{th}, 2010
- “Microwave Assisted Synthesis of Tetrahydrofuran via Acid Catalyzed Cyclo-dehydration of 1,4-butanediol.” Work presented at the American Chemical Society National Meeting. New Orleans, Louisiana. April 2008, 6^{th}-10^{th}