Photosynthesis in silico
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Chapter V
Photosystem II in silico

**Exchange Pathways of Plastoquinone and Plastoquinol in the PSII Complex**

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**Abstract**

Plastoquinone (PLQ) serves as an electron carrier between photosystem II (PSII) and the cytochrome b$_{6}$f complex (Cyt b$_{6}$f). To understand how PLQs enter and leave PSII we performed coarse grained Martini simulations of PSII embedded in the thylakoid membrane, covering a total simulation time of more than 0.6 milliseconds. In addition to the two known channels, we observed a third channel for PLQ diffusion between the thylakoid membrane and the PLQ exchange cavity. Our simulations point to a diffusion mechanism in which all three channels serve as entry and exit channels, with the flux through channels I and III being significantly higher than through channel II. The exchange cavity serves as a PLQ reservoir. We predict the subunits cyt b559, PsbJ, ycf12, PsbK and possibly PsbX to play an important role in directing the fluxes through the different channels.

**Introduction**

Photosynthetic organisms convert light into chemical energy. This is a complex process involving four major protein complexes: photosystem II (PSII), cytochrome b$_{6}$f complex (Cyt b$_{6}$f), photosystem I and ATP synthase. The process starts at PSII, which extracts electrons from water. The electrons travel subsequently to Cyt b$_{6}$f and PSI, after which they reduce NADP$^{+}$. The electrons are transported between these protein complexes by charge carriers. Plastoquinone (PLQ) is the charge carrier responsible for the electron transport from PSII to Cyt b$_{6}$f. PLQ can be reduced to plastoquinol (PLQol) in a two step process, involving a semiplastoquinone intermediate (Figure 5.1).

PSII coordinates two PLQs, named $Q_{A}$ and $Q_{B}$, that are involved in the electron transfer process. The two plastoquinones $Q_{A}$ and $Q_{B}$ are symmetrically positioned around a non-heme iron. $Q_{A}$ is stationary and does not leave the protein. $Q_{B}$, however, leaves the protein after it has been double reduced and taken up two protons. The exact reduction mechanism is not completely clear yet, but involves both $Q_{A}$ and $Q_{B}$. First, $Q_{A}$ is reduced to $Q_{A}^{\cdot}$ by pheophytin (PHO) D1. $Q_{A}^{\cdot}$ is subsequently oxidized by $Q_{B}^{\cdot}$, resulting in $Q_{A}$ and $Q_{B}^{\cdot}$. $Q_{B}^{\cdot}$ is likely to take up a proton at this point, forming $Q_{B}H$ (Haumann & Junge, 1994; Müh, Glöckner, Hellmich, & Zouni, 2012; Okamura, Paddock, Graige, & Feher, 2000). A second electron is necessary to further reduce $Q_{B}H$.
to $Q_B^-\mathrm{H}^+$, after which it can take up a second proton forming $Q_B^-\mathrm{H}_2^+$ and leave the binding site. This electron also comes from PHO D1, via $Q_A^-$ (Müh et al., 2012). $Q_B^-\mathrm{H}_2^+$ leaves the binding site, thereafter a new $Q_B^-$ enters the binding site and the process can start again. In the X-ray structure of Guskov et al. a third PLQ is present, this PLQ is coined $Q_C^-$ (Guskov et al., 2009). $Q_C^-$ is however not present in the later Umena structure (Umena et al., 2011). The $Q_C^-$ site is located close to the $Q_B^-$ site, but the role of $Q_C^-$ is still highly debated (see below).

Whereas the $Q_A^-$ site is well buried inside the protein, the $Q_B^-$ and $Q_C^-$ sites connect to a small cavity located within the PSII complex, and are therefore indirectly accessible from the thylakoid membrane (see Figure 5.2, panel A and Figure 5.3). This so called PLQ/PLQol exchange cavity is filled with lipids: on the stromal side with the negative charged phosphatidylglycerol (PG) and sulfoquinovosyldiacylglycerol (SQDG) lipids and lumenally with digalactosyldiacylglycerol (DGDG) and monogalactosyldiacylglycerol (MGDG) lipids. The cavity has two channels that come out in the thylakoid membrane (Guskov et al., 2009). Channel I, containing the $Q_C^-$ tail, is flanked by cyt b 559α and cyt b 559β on one side and by PsbJ on the other side. It opens up to the centre of the thylakoid membrane. The $Q_C^-$ head group protrudes into the PLQ exchange cavity, where it interacts with lipid and cofactor tails, but not with any amino acids. Channel II, containing the $Q_B^-$ tail, is located between the D2 subunit and the α and β subunits of cyt b 559, opening up more on the stromal side compared to channel I. The $Q_B^-$ headgroup is located close to the non-heme iron.

Because of the presence of two channels, there have been three different models for PLQ/PLQol diffusion proposed involving the $Q_B^-$ and $Q_C^-$ sites (Guskov et al., 2009), see Figure 5.2, panel B. In the ‘alternating’ mechanism, in which channel I and II are used both as an entry and as an exit, a single PLQ/PLQol always enters and leaves through the same channel. In the ‘wriggling’ mechanism PLQ enters via channel I and

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Figure 5.1: Structure of plastoquinone, plastosemiquinone and plastoquinol. The uptake of one electron by plastoquinone results in the radical semiplastoquinone, an additional electron and two protons result in PLQol.
PLQol leaves via channel II. This would be in line with the fact that PLQol is more polar than PLQ, preferring to leave through channel II which opens up closer to the membrane surface (Müh et al., 2012; Nowicka & Kruk, 2010). In the ‘single channel’ mechanism only channel II is used and channel I is occupied by a stationary PLQ molecule (Q_c) that might be involved in redox reactions with cyt b559 (Bondarava et al., 2003; Kaminskaya, Shuvalov, & Renger, 2007).

In Chapter IV we described a third channel leading from the thylakoid membrane towards the PLQ/PLQol exchange cavity. This channel allows lipids to exchange between the cavity and the bulk membrane. Channel III is a result of the movement of
PsbJ. The channel emerges when PsbJ moves towards cyt b559α which creates a tunnel between PsbJ on one side and PsbK and ycf12 on the other side. Channel III seems to be larger in size compared to channel I, especially at the luminal side.

In this Chapter we present the results of a coarse grained (CG) molecular dynamics (MD) study of the diffusion of PLQ and PLQol in and out of the PSII complex. We focus our analysis on the exchange pathways of PLQ and PLQol by quantifying the role of the different channels, including channel III. Previous computational work has focused on the energetics of the Qₐ and Qₐᵣ binding sites (Ishikita, Hasegawa, & Noguchi, 2011), and on simulation of PLQ either in solution (Ishikita et al., 2011) or in the thylakoid membrane (van Eerden et al., 2015). To our knowledge no simulations have been performed so far on the diffusion of PLQ in and out of PSII.

**Figure 5.3: Stromal and side view of the plastoquinone binding sites in PSII.** Subunits that line the PLQ/PLQol exchange channels are colored in red (cyt b 559 α +β), blue (D2), cyan (PsbJ) or green (PsbK and ycf12). Channel I is formed by cyt b 559α and cyt b 559β on one side and PsbJ on the other side. Channel II is formed by cyt b 559α and cyt b 559β on one side and D2 on the other side. Channel III is lined by PsbJ on one side and ycf12 and PsbK on the other side. Qₐ (cyan) is trapped inside D2. Qₐᵣ (orange) is partially located in channel II and QC (green) is partially located in channel I. Picture rendered from the structure of Guskov et al. (Guskov et al., 2009), note that PsbY is not present in the Umena structure (Umena et al., 2011).

**Methods**

**Simulation setup**

To study the dynamics of PLQ and PLQol exchange in the PSII complex, five independent simulations were performed. In these simulations the PLQ in the Qₐᵣ pocket was replaced with a PLQol and extra PLQs were added to the thylakoid membrane.
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The simulations are based on the crystal structure from Umena et al. with PDB ID: 3ARC (Umena et al., 2011). The protein was coarse grained together with all of its cofactors and embedded in a thylakoid membrane composed out of 2686 lipids and 1425 lipids for the dimer and monomer respectively using insane (Wassenaar, Ingólfssson, Böckmann, Tieleman, & Marrink, 2015a). The membrane was described previously (van Eerden et al., 2015) and is a realistic representation of the thylakoid membrane. It is composed out of the negative charged PG and SQDG, and the neutral MGDG and DGDG lipids with oleoyl and palmitoyl tails. The dimer/monomer systems were solvated with 73 144/40 648 CG water beads (representing four times as many water molecules). On top of that, 455/253 Na+ and 455/253 Cl− ions were added, which corresponds to approximately 100 mM NaCl, plus 1032/541 Na+ counter ions to neutralize the overall charge. The systems were minimized and in eight different equilibration runs the systems were heated to 298 K and the time step was increased to 10 fs. The obtained structures served as the starting structure for the other simulations. For a more in depth description of the simulation setup we like to refer to Chapter III.

The five different simulations all started form the same initial structure, but with different seeds for the initial randomized velocities. In order to investigate the pathways of PLQ diffusion to the Q_b binding site, 69 PLQ molecules were inserted into each membrane leaflet at the start of the simulation, totalling to 138 free PLQs, which gives a concentration of about 5 mol % in the membrane. The PLQs were added to a pre-equilibrated bilayer by increasing the lateral dimensions of the box by 1.27 nm and adding 69 PLQs to each leaflet using the insane script (Wassenaar, Ingólfssson, Böckmann, Tieleman, & Marrink, 2015a).

The Martini force field version 2.2 (de Jong et al., 2013b) in conjunction with the Elnedyn elastic network (Periole et al., 2009) were used to model the interactions. The PLQ and PLQol parameters originate from (de Jong et al., 2015). The lipid parameters were taken from (López et al., 2013) with the modification as described in (van Eerden et al., 2015). GROMACS version 4.5.5 (Hess et al., 2008) was used to integrate the equations of motion with the standard Martini settings for the Martini force field (Marrink et al., 2007). The simulations were run in the isothermal-isobaric (NpT) ensemble. The temperature was set to 328 K using the V-rescale thermostat with a coupling constant of \( \tau_T = 2.0 \) ps (Bussi et al., 2007). The pressure was semi-isotropically coupled to an external bath of \( p=1 \) bar with a coupling constant of \( \tau_p = 1.0 \) ps and a compressibility of \( \chi = 3.0 \times 10^{-4} \) bar\(^{-1}\) using the Berendsen barostat (Berendsen et al., 1984). A shifted potential with a cutoff of 1.2 nm in conjunction with a dielectric constant of 15 was used to model the electrostatic interactions. The Van der Waals interactions were also calculated using a shifted potential, with a cut off of 1.2 nm and a switch at 0.9 nm.

The production simulations had a length of 88.7 µs and 62.8 µs for the dimer and the monomer, respectively. The five replicate simulations of the dimer including the PLQ in the bulk membrane had a length of 84.2 µs, 92.1 µs, 97.4 µs, 94.3 µs, and 106.5 µs,
summing up to almost 475 µs. In total, 626 µs was analysed. Trajectories were saved and analysed every 1 ns for the dimer and monomer simulation and every 500 ps for the five replicate simulations.

Analysis

The root mean square fluctuation (RMSF) of PLQ and PLQol was calculated using the Gromacs tool g_rmsf. The RMSF was calculated for the cofactors in each monomer separately to prevent artifacts caused by a non-perfect fit. After removing the jumps across the periodic boundaries and the translational and rotational motions from the dimer, the translational and rotational motions were removed separately for each monomer. Subsequently the RMSF was calculated of the cofactors.

PLQ diffusion pathways were visualized by calculating the time-average density of the cofactor throughout the simulation box. For this purpose all five simulations with PLQ in the thylakoid membrane were concatenated and fitted on the protein backbone using the Gromacs tool trjconv. The calculation of the density was done using the Volmap tool in VMD with the resolution set to 0.2 nm after fitting the simulation on the protein backbone (BB) bead (Humphrey et al., 1996). The threshold for showing occupancies was set to give a clear, qualitative view of the channels, while still being able to distinguish from the bulk. The PLQ densities reveal clear pathways in the simulation box with a threshold value of 8% (implying that at least in 8% of the frames a PLQ bead is present, which corresponds to roughly 2.4 times the occupancy of PLQ in the bulk).

In order to count the flux of PLQs and PLQols through the three channels, first the residues lining the cavity were determined. For each channel we defined two ‘walls’ that line the channel, one on each side, see Table 5.1. The diffusion of PLQ in or out of a channel can easily take several microseconds and due to its elongated nature it is possible for a PLQ to be present in two channels concurrently. Therefore the diffusion of PLQs was tracked on the level of the cofactor as a whole, as well as for each individual cofactor bead. For each frame in the simulation, we determined for each individual PLQ/PLOol bead if it resided within 0.6 nm of one of the walls. If a cofactor bead was in contact with two walls that form together a channel, the bead was assigned to that channel. In case it was in contact with three or more walls, it was assigned to the two walls with which it has the most contacts and in case these two walls line the same channel, the bead was assigned to this channel. Once a bead had been assigned to a channel and in a subsequent frame loses contact with one or both of the walls, it was checked whether the bead resides inside or outside the PLQ exchange cavity. For this purpose a group of residues in the back of the PLQ exchange cavity was defined (cavity residues), see Table 5.1. The centres of geometry (cog) of the cavity residues and of the channel wall residues that were in contact with PLQ/PLOol bead were calculated. Subsequently the distance in the XY plane between the cavity cog and the channel walls cog and the distance in the XY plane between the PLQ/PLOol bead and the cavity cog were determined. The PLQ bead was considered to be in the cavity when
the distance between the cavity residues and the bead was smaller than the distance between the cavity residues and channel walls. If the distance between the PLQ bead and the cavity residues was bigger than the distance between the cavity residues and the channel walls, the bead was considered to be outside the protein. Once it had been determined for all the beads of a cofactor if they reside in or out of the PLQ exchange cavity, it was determined if the cofactor as a whole resides in or outside the PLQ cavity. In order to only count an entry or exit when the cofactors beads diffused through the same channel, the status of a cofactor, as being in or outside the PLQ cavity, was updated only when more than 70% of the PLQ beads had diffused in or out of the PLQ cavity through the same channel. Each time a PLQ entered or left the cavity this was recorded, as well as which channel it used. This method, however, is not full proof, due to the movement of the channel walls. Therefore the simulations were visually inspected to verify each crossing and to check for missed crossings. Pictures were rendered in VMD and in Pymol (Humphrey et al., 1996; Schrödinger, LLC, 2010).

The distance between the PLQol headgroups and the non-heme iron were calculated using the Gromacs tool `g_dist`. For the PLQol headgroup the centre of mass of the three headgroup beads was used.

<table>
<thead>
<tr>
<th>Channel</th>
<th>Wall 1</th>
<th>Wall 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>PsbJ-TRP11 – PsbJ-LEU36</td>
<td>Cyt b 559α-SER16 – Cyt b 559α-TRP35 and Cyt b 559β-TYR13 – Cyt b 559β-PHE32</td>
</tr>
<tr>
<td>II</td>
<td>Cyt b 559α-SER16 – Cyt b 559α-TRP35 and Cyt b 559β-TYR13 – Cyt b 559β-PHE32</td>
<td>D2-PHE27 – D2-THR53</td>
</tr>
<tr>
<td>III</td>
<td>Ycf12-VAL18 – Ycf12-PHE37</td>
<td>PsbJ-TRP11 – PsbJ-LEU36</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cavity residues</th>
</tr>
</thead>
<tbody>
<tr>
<td>D1-SER268 – D1-ALA294</td>
</tr>
</tbody>
</table>

**Table 5.1: Residues lining the different channels and the center of the cavity.** Of some residues not all beads were included: D1-SER268 only SC1, D2-PHE27 only SC2 and SC3, Cyt b 559α-TRP35 only the backbone bead (BB), Cyt b 559β-TYR13 only beads SC1, SC2 and SC3, PsbJ-TRP11 only the SC2, SC3 and SC4 bead, PsbJ-LEU36 only the BB and ycf12-PHE37 only the BB.

**Results**

**PLQols can leave the Q<sub>b</sub> site on a submicrosecond time scale**

To test if PLQol has a higher tendency to leave the Q<sub>b</sub> site than PLQ (Chapter III), five replicate simulations of the PSII dimer were run in which the Q<sub>b</sub> PLQs were replaced by PLQols. These simulations also contained additional PLQs in the membrane (about 5 mol%), to see if PLQs can enter the PLQ exchange cavity and replace a PLQol in the Q<sub>b</sub> site, and/or occupy the Q<sub>c</sub> site. Note, the Q<sub>c</sub> site was left unoccupied at the start of these simulations.
Indeed we observe the spontaneous unbinding of PLQols from the Q_b site. From a total of ten PLQols (2 per dimer), five of the PLQols leave the Q_b site completely during the simulation. Out of these five, one PLQol escapes the protein through channel III ending up in the bulk thylakoid membrane. Two PLQols end up in the PLQ exchange cavity, and two other PLQols reach halfway through channel II. Additionally there are two PLQols that leave the binding site transiently, and later return to it. One of these PLQols completely enters the PLQ cavity and rebinds to the Q_b site after 22 µs. The other PLQol stays largely in place, only its headgroup turns away from the Q_b site. This happens however two times, in both cases the headgroup rebinds to the Q_b site after about 1 µs. Of the eight times a PLQols leaves the binding site (7 PLQols, of which one two times), there are three instances in which the PLQol leaves the binding site quickly (within 100 ns after the start of the simulation), two in which this happens within the first 1 µs, one after 6 µs and two after around 64 µs of simulation time. Here leaving the binding site is defined as the moment the distance between the PLQol headgroup and the non-heme iron is larger than 1.5 nm.

In line with our expectations, we thus find that PLQol has a higher propensity to leave the Q_b binding site than PLQ. In the simulations 5/10 PLQols per 500 µs leave the Q_b site versus 0/3 PLQs per 150 µs. Individual unbinding times vary from < 100 ns to up to > 88 µs.

Conformation of the existence of a third plastoquinone channel

To investigate how PLQs diffuse in and out of the PLQ exchange cavity, we calculated a PLQ density map, combining the data from the five replicate simulations.

The density map is visualized in Figure 5.4, showing the areas around the PSII dimer where it is more likely to encounter a PLQ or PLQol during the simulation.

The PLQ exchange cavity and the location of Q_a are clearly visible in the density. This implies that PLQ can spontaneously enter the exchange cavity from the bulk thylakoid membrane. Interestingly the density is not homogenously distributed within the cavity, but located more towards the cyt b559 and away from CP43. There are in fact three clear distinct pathways from the membrane towards the PLQ exchange cavity, indicated in Figure 5.4. These pathways correspond to channels I & II reported in the literature before (Ohnishi et al., 2007), and channel III that has been described in Chapter IV as a channel that allows lipids to exchange between the cavity and the bulk membrane. Our data thus confirms that channel III is also used by PLQ. Note that, in the density map channel III is only visible in the left monomer; the channel is however used in both monomers and becomes visible in the other monomer when the density threshold is reduced (data not shown). Despite the presence of PLQ in the exchange cavity, we did not observe in any of the simulations PLQ docking into the Q_b site, which is in principle possible in those cases in which PLQol has left. This is reflected by the lack of density at the Q_b site. Visual inspection of the trajectories show
Figure 5.4: Diffusion of PLQ and PLQol in and out of PSII. A) Stromal view on PSII with combined occupancy densities of PLQ and PLQol from the five replicate simulations. Indicated are the locations of the headgroups of QA, QB, and QC, as well as channel I, channel II, and channel III. The red circle approximately indicates the location of the PLQ exchange cavity. Note that in the right monomer channel III is not visible at the chosen threshold level. The protein is colored as in Figure 3.2A.

B) Time series of snapshots of diffusion of PLQ into PSII (left) and PLQol out of PSII (right), the PLQ and PLQol headgroup beads are shown as spheres for clarity. In the left panel the PLQ enters the complex via channel I. After entry the PLQ diffuses around in the PLQ exchange cavity, hereby it tail shortly sticks out of channel III. By the end of the simulation the PLQ headgroup is very close to the QB binding site. In the right panel the PLQol moves from its initial position at the QB site with its tail inside channel II, towards the PLQ exchange cavity. Subsequently its headgroups moves inside channel III, with the PLQ tail still in the exchange cavity. Hereby the headgroup remains in the channel opening.
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that subtle side chain reorientations that occur after unbinding of PLQol prevent easy access to this binding site. The Q_c site, on the other hand, is frequently visited by PLQs that use channel I, as is visible from the density.

Another interesting finding is the non-homogeneous distribution of PLQ around the protein (Figure 5.4). PLQ clearly has a preferred region of interacting with PSII, ranging approximately from subunits PsbZ to PsbH, with a few spots around CP43 and D1 where the PLQ tries to penetrate PSII. Remarkably, there is no density in and around the dimer cleft. It appears that PLQs accumulate at the side of the protein where they can enter the exchange cavity.

In summary, we find that PLQs accumulate around the PSII complex at sides close to the exchange cavity, and are able to enter and leave this cavity using three different channels. Of these, two channels correspond to the known channels I and II. The third channel is a novel channel that has not been reported before.

Channel I and III most intensively used by PLQ

To get an estimate about the plastoquinone fluxes through the three channels we counted how many PLQs and PLQols pass through each channel in the five different simulations. The data is shown in Table 5.3, split out over the in total ten different monomers. In total, we observed nineteen full entries and eleven full exits, over a total simulation time of more than 474 µs. In addition to the full flux events, many additional PLQs or PLQols are found partly trapped inside the channels at the end of the simulation.

The data shows that all channels are used, both as an entrance and as an exit to the PLQ cavity. We observed that a PLQ can leave the PLQ exchange cavity from a different channel than the channel it used to enter the cavity. It is however also common that they leave through the same channel as through which they entered.

At the start of the simulation each monomer contains only one PLQ in the QA site and one PLQol in the QB site. In most of the monomers, the entry flux is higher than the exit flux (Table 5.3), resulting in a net increase in the amount of PLQs present in the PLQ exchange cavity. The average gain per monomer amounts to 0.8 PLQs. At the end of the simulation the combined PLQ/PLQol concentration inside the PLQ exchange cavity is on average 16 ± 3 mol %, to be compared with the bulk membrane concentration of 5 mol %. When only considering PLQ the concentration is 9 ± 2 mol %. It is likely that this is still an underestimation, as equilibration of the PLQ population in the exchange cavity is a slow process. On average, every 50 µs a PLQ enters the PLQ cavity.
Comparing the fluxes between the channels, it appears that channel I and III are more or less equally used by PLQ, but significantly less PLQs pass through channel II (by a factor of approximately seven compared to either channel I or III, taking into account all full entries and exits). On top of that the ratio between complete passes and incomplete passes is especially low in channel II. In this channel PLQs often get stuck and do not completely pass through the channel. These two observations are very likely a result of the smaller size of channel II. Channel I has dimensions of 1.0 × 2.0 nm², while the dimensions of channel II are only 1.0 × 1.2 nm² (Kern, Zouni, Guskov, & Krauss, 2009). Channel III is very similarly sized as channel I, at the start of the simulations its dimensions are approximately 1.1 × 2.0 nm². During the simulation the shape and size of channels I and III however fluctuates significantly, with sizes temporarily increasing by 50-100%, see below.

Movement of Cyt b559, PsbJ, PsbK and ycf12 affect channel opening

Visual inspection of our simulation trajectories reveals that the subunits lining the PLQ channels are dynamic, and can move with respect to each other. In particular, there is movement of the helices cyt b559α+β, PsbJ, PsbK and ycf12. These relative movements can either result in constriction or even complete closure of a channel, or result in an increased capacity of a channel due to a larger channel opening. This is especially noticeable for channels I and III. In Figure 5.5 representative snapshots of the subunits lining the channels in one of the monomers are shown to illustrate the different channel states (open/closed). Visual inspection further shows that PLQs can enter the channels in two orientations, headgroup first or tail first.

Table 5.3: Number of PLQs and PLQols diffusing in and out of the PLQ exchange cavity through the three channels. In bold are the cofactors that completely diffuse through a channel, while the numbers after the ‘+’ represent the number of cofactors that still remain somehow attached or inside the PLQ channel and do not fully enter or leave the PLQ exchange cavity. The numbers are split out over the five independent simulations, but sums are given as well.
In Figure 5.5A, PsbJ is close to cyt b 559. This results in the closure of channel I and the opening of channel III. In channel III there is indeed a PLQ, while the tail of the Q\textsubscript{e} is seen sticking outside of channel II. In Figure 5.5B, PsbJ moved towards ycf12 while cyt b559\textalpha moved towards cyt b559\textbeta. This results in an extra wide opening of channel I, which makes it possible that several PLQs are inside this channel simultaneously. It can be seen that PLQs can enter in two orientations. One of the PLQs enters channel I with its tail first, that almost reaches the QC site, the PLQ in channel II enters with its headgroup first. Although PsbJ and ycf12 almost closed off channel III, a PLQ head group managed to still enter channel III at the stromal side. In Figure 5.5C, a PLQ is located in channel II, while the head group of the Q\textsubscript{e} peaks out of channel III, which is wide open due to the approach of PsbJ towards cyt b559\textalpha. Again, this causes closure of channel I. In panel D of Figure 5.5, both channels I and III are closed, due to the approaching of all three subunits cyt b559\textalpha, PsbJ, and ycf12. There is a PLQ visible in the PLQ exchange cavity, which has reoriented after entering the cavity, its headgroup occupies the Q\textsubscript{e} site. Next to PsbK there are two PLQs that penetrate from the membrane into the PLQ cavity. None of these two completely enters into the PLQ cavity though, and they diffuse back into the membrane. In all the simulations we did not observe any of the PLQs reaching into the PLQ cavity by using this pathway next to PsbK. This can also be inferred from the density map in Figure 5.4, where in both monomers there are small patches of density visible between PsbK and PsbZ. The density is however not contiguous with the density in the PLQ cavity. Figure 5.5E shows another snapshot, where all three channels are open and simultaneously occupied. Note that the position of PsbX varies significantly in the different panels, which could influence the flux through channel II.

Taken together, our simulations point to a plastic behaviour of the plastoquinone exchange channels. Subunits cyt b559\textalpha+\textbeta, PsbJ, PsbK and ycf12 are causing changes in channel openings by moving closer together or further apart, causing the channel sizes to vary greatly over time.

**Discussion**

In this chapter we have investigated the behaviour of PLQ and PLQol in PSII, based on coarse-grained molecular dynamics simulations. The CG approach allowed us to simulate the full PSII dimer system, including all cofactors and embedded in a realistic thylakoid membrane environment, on an aggregate time scale of more than 0.6 ms. Here we discuss our results in light of the current literature view on plastoquinone binding and exchange pathways. In our simulations with PLQ occupying the Q\textsubscript{e} site, both Q\textsubscript{A} and Q\textsubscript{e} remain stationary. This is in line with their function in photosynthesis. Experiments suggest that is relatively difficult to remove Q\textsubscript{A} from its binding site (Araga et al., 1993; Diner, de Vitry, & Popot, 1988; Ermakova-Gerdes & Vermaas, 1998). On top of that we find a relation between the movement of PsbT and the mobility of Q\textsubscript{A}, in line with an experimental established relation between Q\textsubscript{A} and PsbT (Ohnishi et al., 2007). The Q\textsubscript{e} PLQ is only expected to leave the site after being
Photosystem II in silico
Figure 5.5: Stromal and side views of the left PSII monomer with different states of channels I & III and PLQs in the three different channels. Only PLQs in the vicinity of the three channels are shown. Subunits that line the PLQ/PLQol exchange channels are colored and labelled. The PLQs are colored light green and the two PLQols (Q_b) orange. The positions of the channels are indicated by their number (I, III and III). Channel I is formed by cyt b 559α and cyt b 559β on one side and PsbJ on the other side. Channel II is formed by cyt b 559α and cyt b 559β on one side and D2 on the other side. Channel III is lined by PsbJ on one side and ycf12 and PsbK on the other side. Dashed circles indicate the approximate location of the position of the headgroup in the Q_b (red) and Q_c (light blue) binding sites.

converted to PLQol. This is confirmed by our simulations: we observe that while all PLQs remain in the Q_b pocket, PLQols diffuse out of this binding site. The affinity of PLQ and PLQols for the Q_c site appears weak. Although the site is frequently visited by PLQ/PLQols diffusing through channel I, actual binding is not observed. We hypothesize that the Q_c site in the crystal structure of Guskov et al. (Guskov et al., 2009) either originates from a PLQ trapped inside the channel under the crystallization conditions, or represents a number of weaker binding spots around the Q_c site.

The exchange cavity serves as a PLQ reservoir

The simulations with 5 mol % PLQ in the membrane show that there are preferential regions on PSII where PLQ interacts, and from which PLQ can enter the exchange cavity. Importantly, our data suggest that the PLQ cavity could function as a PLQ reservoir. We observed a gain of 0.8 PLQ per monomer (leading to a local concentration about twice the bulk membrane concentration of PLQ), and considering our simulations did not reach equilibrium yet, the equilibrium population could be even higher. Note that the PLQ cavity reservoir should not be confused with the total PLQ pool, which is likely located for a large part outside the PSII complex in the thylakoid membrane, and estimated to contain from 9-10 PLQs to up to 30 (Govindjee, 2004; Kolber & Falkowski, 1993). The entry kinetics of PLQ, on average one PLQ per 50 µs per monomer, is fast compared to both the first reduction step of Q_b towards Q_b\(^{-}\) that has a time constant of a few hundreds of microseconds (Kern & Renger, 2007) and the minimum time required for reoxidation of Q_A, which is usually around 600 µs (Kolber & Falkowski, 1993). This implies that a PLQ is always available close to the Q_b site to replace PLQ upon its reduction to PLQol.

Three channels are independently used as entry and exit pathways

Our simulations provide direct evidence for the existence of designated PLQ/PLQol exchange pathways. Surprisingly, we find the exchange taking place via three distinct channels, rather than two channels that are usually assumed. Apart from channel I and channel II that have been described before (Guskov et al., 2009), we observed a third channel between subunits PsbJ and ycf12/PsbK. The simulations show that channels I & III are used more or less equally, but that the narrower channel II is used...
significantly less. The subunits lining the three channels can undergo conformational changes, modulating the relative and absolute opening or closure of channels I and III in particular. In the literature, three different PLQ exchange mechanisms have been proposed: the alternating, the wriggling, and the single channel mechanism (Guskov et al., 2009), cf. Figure 5.2. The simulations match each mechanism to a certain extent, but not any of them fully. Our data matches with the alternating mechanism in the sense that both channels are used as an entry and an exit. It differs to the point that a PLQ does not have to leave through the same channel as through which it entered. Our simulations agree with the wriggling mechanism to the point that both channels are used. In the wriggling model channel I is only used as an entry and channel II only as an exit. We observe that PLQs enter to the PLQ cavity using both channel I and II (and III) and that one PLQol leaves the binding site through channel III, which means that PLQol probably uses channel I as an exit as well. Our simulations do not agree with the single channel mechanism in which only channel II is actively used and channel I is occupied by a stationary PLQ. We indeed see that PLQs and PLQols use channel II, but the flux through channel I is significantly larger. In all three mechanisms the PLQs do directly diffuse to the Q₈ site after entering a channel and directly leave the complex after being reduced to PLQol. In our simulations we observe that the PLQ exchange cavity can function as a kind of local reservoir of PLQs, where PLQs can reorient. The latter is especially relevant because some of the PLQs enter tail first.

Limitations of our approach

It is imperative that the results that we found are verified experimentally. Molecular dynamics represent a strong tool in probing time and length scales that are experimentally difficult to access. They are however still based on models and are therefore prone to inaccuracies and errors. Specific limitations pertaining to the CG Martini model that was used in our study are discussed in Chapter I. Particular to this chapter is however the fact that Martini beads are too fat for flat molecules. Due to this the CG PLQ and PLQol are fatter than their atomistic equivalents. This could especially influence the diffusion of PLQ through Channel II, which is the smallest of all channels. Mutants studies have already shown that PsbJ is likely to be involved in the electron transport from Q₈ to the PLQ pool, pointed to the importance of cyt b559 for photosynthesis, and revealed that PsbX has an influence on plastoquinone turnover (Katoh & Ikeuchi, 2001; Lind, Shukla, Nyhus, & Pakrasi, 1993; Nowaczyk et al., 2012; Ohad et al., 2004; Pakrasi, Williams, & Arntzen, 1988; Regel et al., 2001; Shinopoulos & Brudvig, 2012). However, a way to really assess the existence of channel III in vitro could be by measuring the distance between the various helices over time. This could be achieved by the inclusion of fluorescent or electromagnetic probes in helices cyt b559α, PsbJ and ycf12 and subsequently reading out the distances between the subunits over time using FRET or EPR measurements. In this way it might be possible to verify if the movement of the helices is large enough to allow for the closure and opening of the different channels. Closing channels, by crosslinking the helices that form them, might be another approach to study the existence and the usage of the
various channels. One might be able to close, for example, channel III by crosslinking PsbJ, ycf12 and PsbK together and subsequently measure the effect on the redox potential of Qa and the PLQ pool. In this way one might be able to assess if channel III is really used.

Conclusion

Based on large scale simulations, we have been able to shed important light on the mechanism by which PLQs and PLQols can diffuse in and out of the PSII complex. Our simulations do not fully agree with any of the three diffusion mechanisms described in the literature (Guskov et al., 2009). Instead they point to a less organized, less determined model. Eight main observations can be made. 1) Three different channels exist that all can be used as an entry and an exit channel. 2) The entry and exit channel do not have to be the same for an individual PLQ. A number of PLQs enter and leave through the same channel, but others do this by a different channel. 3) PLQs can pass through the channels in at least two different orientations, with their headgroup first or with their tail first. 4) PLQs do not directly dock at the Qb site from the channel, instead they first enter in the PLQ exchange cavity where they can diffuse around and reorient themselves. 5) PLQs can accumulate in the PLQ exchange cavity forming a PLQ reservoir. 6) The PLQols from the Qb site can probably use any channel as an exit. Although there is no recording of a PLQol passing through channel II, one expects that the PLQols ending up in the PLQ exchange cavity could use any channel as an exit. 7) The flux through channel I and III is more or less equal and several times larger than through channel II. 8) The relative and absolute flux through channels I and III is influenced by the relative conformations of cyt b559, PsbJ, ycf12 and PsbK. Possibly PsbX might be able to influence the flux through channel II. Combining the eight observations, a model appears in which primarily channel I and III are used, each channel functioning as an uncorrelated entry and exit of PLQ/PLQol. The PLQ exchange cavity can function as a local PLQ supply in which the PLQs and PLQols can reorient and there is a regulatory function of subunits cyt b559, PsbJ, ycf12 and PsbK.