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Distribution and Dynamics of Quinones in the Lipid Bilayer Mimicking Inner Membrane of Mitochondria

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Keywords
Electron transport chain, molecular dynamics simulations, free energy calculations, biological energy transduction

Abbreviations
MD, molecular dynamics; US, umbrella sampling; WHAM, weighted histogram analysis method; PMF, potential of mean force; Q, quinone or its analogue; PC, phosphatidylcholine; PE, phosphatidylethanolamine; CL, cardiolipin
Abstract

Quinone and its analogues (Q) constitute an important class of compounds that perform key electron transfer reactions in oxidative- and photo-phosphorylation. In the inner membrane of mitochondria, ubiquinone molecules undergo continuous redox transitions enabling electron transfer between the respiratory complexes. In such a dynamic system undergoing continuous turnover for ATP synthesis, an uninterrupted supply of substrate molecules is absolutely necessary. In the current work, we have performed atomistic molecular dynamics simulations and free energy calculations to assess the structure, dynamics, and localization of quinone and its analogues in a lipid bilayer, whose composition mimics the one in the inner mitochondrial membrane. The results show that there is a strong tendency of both quinone and quinol molecules to localize in the vicinity of the lipids’ acyl groups, right under the lipid head group region. Additionally, we observe a second location in the middle of the bilayer where quinone molecules tend to stabilize. Translocation of quinone through a lipid bilayer is very fast and occurs in 10-100 ns time scale, whereas the translocation of quinol is at least an order of magnitude slower. We suggest that this has important mechanistic implications given that the localization of Q ensures maximal occupancy of the Q-binding sites or Q-entry points in electron transport chain complexes, thereby maintaining an optimal turnover rate for ATP synthesis.
**Introduction**

ATP is used as a primary source of energy in all known forms of life. Its synthesis is driven by two key metabolic pathways: photosynthesis and respiration. In both processes, electron transfer reactions are used to establish a proton electrochemical gradient across bioenergetic membranes, and the proton gradient in turn is used to drive the synthesis of ATP \[^1\]. The transfer of electrons between different membrane-bound respiratory or photosynthetic complexes is accomplished by various mobile electron carriers such as cytochromes, plastocyanin, and quinone. Quinone or its analogues are particularly unique in the sense that they perform electron transfer reactions entirely in the hydrophobic domain of a membrane. They undergo one- or two-electron reduction reactions to form semiquinone or quinol \[^2\]; here we use the notation where Q stands for quinone as well as its analogues, such as quinols, and further classification to subclasses is made below when needed. The redox potentials of the pH dependent reactions associated with reduction are modulated by proteinaceous environments \[^2\], meaning that the temporal stability of any Q species strongly depends on its local environment.

In addition to its primary role as an electron/proton carrier, Q has been implicated in many other biological functions. For example, it has been proposed to play an important role in prevention of mitochondrial permeability transition pore \[^3\], in modulating proton transfer in mitochondrial membranes \[^4\], as well as in preventing proton leaks \[^5\]. Q has also been suggested to be associated with the organization of respiratory supercomplexes \[^6\] and in modulating mitofusin 2 in the outer mitochondrial membrane protein \[^7\].

Quinone or its analogues are found in all kingdoms of life. For instance, plastoquinone is used as an electron carrier in photophosphorylation, and menaquinone is employed in respiration by some bacteria \[^8\], \[^9\]. On the other hand, human mitochondria are primarily dependent on ubiquinone also known as coenzyme Q (Q\[^{10}\] that is quinone with 10 isoprene units, see Fig. 1). In humans, ubiquinone is synthesized biochemically in most tissues \[^10\], and its deficiency leads to many complex diseases \[^10\], \[^11\]. Further, due to its capability to undergo redox reactions, it possesses antioxidant properties \[^10\] and thereby effectively prevents the oxidation of mitochondrial lipids and proteins \[^12\].

Many experimental studies have been performed to decipher the localization of ubiquinone in lipid bilayers \[^13\], \[^14\], and references therein\]. It has been suggested that it resides somewhere in the lipid hydrocarbon chain region but not aligned along the hydrocarbon chains. Instead, this molecule is possibly lying along the membrane plane \[^13\]. On the contrary, X-ray diffraction studies show the residence of Q head group away from the center of the membrane \[^14\]. Given that the experimental results are inconclusive, computer simulation studies have also been carried out to understand the
detailed structural and dynamical features of quinone or its analogues [15, 16, 17, 18]. The available simulation data suggest ubiquinone to reside in the acyl chain region with considerably faster lateral diffusion compared to the lipids around it, or alternatively to stand upright under the lipid head groups in a manner where the ubiquinone head would lie next to the glycerol group of phosphatidylcholine (PC) lipids [15]. However, given that the simulation times of these early considerations [15-17] were short (usually less than 10 ns), and the studies were done in single-component PC bilayers, [15,18], one has to conclude that also the simulation data is inconclusive.

Figure 1. Chemical structures of the molecules considered in this study. Left-hand side: (top) oxidized Q (Q_{ox}) corresponding to ubiquinone, and (bottom) reduced and protonated Q (QH\(_2\)) referred to as ubiquinol. The isoprene (monomer) unit is shown explicitly in both molecular structures. Right-hand side: (top) cardiolipin, (middle) DLPC, and (bottom) DLPE.

Here, we use atomistic classical MD simulations to explore membranes with oxidized and reduced (as well as protonated) Q, including also its variants with regard to chain length. The membrane hosting Q in our work is a three-component lipid bilayer containing PC, PE (phosphatidylethanolamine), and cardiolipin – the most abundant lipids in the inner mitochondrial membrane. Our results provide a detailed and novel picture into the dynamics and energetics of Q in a membrane and highlight its relevance to bioenergetic systems. We find that Q head group
shows preference to localize in the vicinity of the lipid head group region, and its translocation across the hydrophobic milieu of the lipid bilayer is fast, which may have mechanistic consequences in biological energy transduction.

**Computational Methods**

We constructed model systems comprising oxidized quinone (Q\textsuperscript{ox}) or reduced quinol (QH\textsubscript{2}) in a lipid bilayer, whose composition mimics the contents of the inner mitochondrial membrane \[19, 20\]. Each model system included either 16 Q\textsuperscript{ox} or QH\textsubscript{2} molecules that were embedded in a symmetric lipid membrane comprised of 240 di-18:2-PC (DLPC), 208 di-18:2-PE (DLPE), and 64 cardiolipin (CL) molecules (see Figure 1). All four chains in CL had the structure of a diunsaturated 18:2 linoleic chain, since it is the predominant one found in human mitochondria \[21\]. CL carried a charge of -2e, therefore 128 Na\textsuperscript{+} counterions were added to neutralize the system. In addition, ~20,000 water molecules were used to hydrate the lipid bilayer.

We also explored how the Q chain length affected the partitioning to the lipid membrane. To this end, we studied both Q\textsuperscript{ox} and QH\textsubscript{2} with 1, 6, 8, 9, and 10 isoprene units. Starting structures for all these lipid bilayer systems were constructed from the equilibrated membrane systems obtained in our earlier studies \[22, 23\].

To parameterize lipid molecules and ions, we used the (all-atom) OPLS-AA force field \[24\] together with the recently developed parameters for lipid simulations \[25, 26, 27, 28\]. For water, we used the TIP3P model, which is compatible with the OPLS-AA force field \[29\]. The Nose-Hoover method was used with a time constant of 0.4 ps to couple the temperature (300 K) with separate heat baths for the membrane and the rest of the system \[30, 31\]. The reference pressure (1 bar) was maintained by the semi-isotropic Parrinello–Rahman barostat \[32\]. For long-range electrostatic interactions, we used the particle-mesh Ewald (PME) method \[33\]. The linear constraint solver (LINCS) algorithm was used to preserve the covalent bond lengths \[34\].

All simulations were carried out using the GROMACS 4.5 software package \[35\]. Prior to any MD simulation, the steepest-descent algorithm was used to minimize the energy of the initial configuration. The time step was then set to 2 fs, and for each model system we performed MD simulations over a period of ~200 ns.

In addition to the above (non-biased) simulations, we also performed (biased) free-energy calculations to obtain the free energy profile of Q in the direction perpendicular to the membrane surface (Z-direction). Due to the high computational cost of these calculations, we considered only Q (both Q\textsuperscript{ox} and QH\textsubscript{2}) molecules with 1 (Q\textsubscript{1}) and 10 (Q\textsubscript{10}) isoprene units. The potential of mean
force (PMF) profiles were calculated using the umbrella sampling (US) method implemented in GROMACS through the g_wham tool \cite{36, 37}. The PMF was obtained using 30 sampling windows with a distance of 0.1 nm between each window in the direction of membrane normal (Z-direction). The distances shown in the PMF profiles below are given for the center of mass (COM) of the headgroup of Q with respect to the COM of the lipid bilayer. For each sampling window, we used an equilibration time of 30 ns followed by a production run of 70 ns. Lipid bilayers used in the free energy simulations were 4 times smaller than the ones used in unbiased simulations, and consisted of 60 DLPC, 58 DLPE, and 16 CL molecules. The total time of all simulations was 14 µs.

The interactions of Q molecules with their immediate surroundings were analyzed quantitatively (Table 1). For this, we mainly considered hydrogen bonds (H-bonds) and charge pairs (CPs). Hydrogen bonds between the Q head group and lipid/water molecules were evaluated based on the following geometrical criteria: the acceptor (A) - donor (D) distance shorter than 0.35 nm, and the angle between the D-A vector and the D-hydrogen vector < 35º \cite{38}. Meanwhile, charge pairs are interactions between the positively charged choline group of DLPC and negatively charged oxygen atoms on Q molecules, when the two are not more than 0.4 nm apart \cite{39}.

**Results**

*The Q head group resides under the lipid head group region but the occupancy depends on its oxidation state*

MD simulations shed light on the dominant positions occupied by the Q head group in the lipid bilayer. It is observed that the ubiquinol (QH\textsubscript{2}) head group position is centered at about 1.5 to 1.7 nm from the center of the lipid bilayer, which is about 0.5-0.7 nm below the position of the phosphate groups (Fig. 2a). The observed localization of the QH\textsubscript{2} head group is likely due to its polar interactions with the lipid head groups. This view is supported by the observation that the head groups of QH\textsubscript{2} with different isoprenoid tails align themselves largely to the same region in the membrane, with only a minor preference for being positioned closer to membrane center when the isoprenoid tail length increases (Fig. 2a).

The distribution of ubiquinone (Q\textsuperscript{ox}) also peaks at a distance of 1.5-1.7 nm from membrane center (Fig. 2b). However, in this case there is an additional strong peak in the middle of the membrane, highlighting how the oxidation (and protonation) state of the Q moiety has a profound effect on its positioning in the membrane. To clarify the underlying mechanism that differentiates the positioning of Q\textsuperscript{ox} from QH\textsubscript{2}, we analyzed the interactions between lipids and Q molecules. We found that due to the H-bond acceptor and donor groups in the QH\textsubscript{2} head group, QH\textsubscript{2} stabilizes in
the vicinity of the carbonyl moieties of the lipid molecules. In contrast, the observed lower density of Q$^{\text{ox}}$ molecules in the same region is apparently due to the missing H-bond donor group (see below).

**Figure 2.** Density profiles of (a) QH$_2$ and (b) Q$^{\text{ox}}$ in the lipid membrane, the membrane depth of zero corresponding to the membrane center. The density profiles have been computed over all the atoms in the head groups of Q molecules. Data are given for Q with different isoprenoid chain lengths that range from one (light blue) to 10 (green) monomers. For comparison, the density of phosphorous (P) atoms in the phosphate group of DLPC is also depicted (grey). The data have not been averaged over the two leaflets, thus the differences in the two leaflets characterize statistical fluctuations.

Free energy profiles illustrate Q head group positioning to depend on oxidation state and tail length

The above results are supported by the free energy calculations. The PMF profiles obtained for ubiquinol (QH$_2$) indicate that the number of isoprene units in the chain makes a difference. For a short-tailed QH$_2$, we observe a deep minimum at a distance of 1.5 nm from the middle of the bilayer and a shallow minimum at the center, where free energy is about 9 kJ/mol higher than in the minimum closer to the membrane-water interface (Fig. 3). The free energy barrier from the lower to
the higher minimum is about ~11 kJ/mol, and ~2 kJ/mol in the opposite direction. When the chain length is increased to 10 isoprene units, both the location and the depth of the deeper minimum are shifted (Fig. 3). Now the position of the free energy minimum is around 0.8-1.2 nm and the free energy is about 3 kJ/mol lower than in the membrane center. The barrier from the lower to the higher free energy minimum is ~3 kJ/mol, and ~1 kJ/mol in the other direction. The difference between the two cases (1 vs. 10 isoprene) can be rationalized by the strongly hydrophobic nature of the isoprenoid tail, which also causes the sharp increase in the PMF as QH$_2$ is dragged from the membrane to the water phase. For a short-tail QH$_2$, the free energy barrier is then ~20 kJ/mol, while for the long-tail QH$_2$, the barrier is considerably larger.

The behavior with ubiquinone (Q$_{ox}$) is distinctly different (Fig. 3). The results show that inside the membrane, the Q$_{ox}$ molecules can diffuse freely from the membrane center along the membrane normal direction up to a distance of ~1.5 nm, where the free energy is still comparable to thermal energy. Here, the presence of a long tail (Q$_{10}$) only marginally affects the depth of the free energy minimum in comparison to the short-tailed Q$_1$ (Fig. 3). The length of the isoprenoid tail has a major effect only on the free energy barrier that one has to be overcome to access the water phase.

The free energy profile of Q$_1$ converges to bulk-water behavior within ~2.5 nm from membrane center, however the tail of Q$_{10}$ is so long that finding similar convergence for Q$_{10}$ would have required simulations with a substantially larger system size (as to the amount of water) and was therefore considered unnecessary for this work, where the main interest focuses on the behavior inside a lipid membrane. The fact that long-tail Q molecules are known to be non-soluble (or weakly soluble) in water supports this choice.

Overall, the agreement between the free energy results (Fig. 3) and the density distributions (Fig. 2) is very good, showing that both Q species (predominantly quinols) stabilize close to the lipid head groups. This may have mechanistic consequences as we discuss below.

**Translocation of Q across the membrane center is a rapid process**

The above density and PMF profiles indicate that Q may translocate through the hydrophobic milieu of a lipid bilayer. When this is analyzed, we indeed find a series of events in which the quinone (Q$_{ox}$) head group flips from one side of the bilayer to the other (Fig. 4, and Supplementary Video). Irrespective of the tail length, quinone molecules move freely across the lipid bilayer, in full agreement with the free energy profile in Fig. 4. The time scale of quinone (Q$_{ox}$) translocation is observed to be of the order of 10-100 ns, which is fast compared to the translocation time scale of quinols (QH$_2$), which are also found to flip-flop but with a time scale at least ten times longer – of
the order of 1000 ns (Fig. 4). The slow time scale of QH\(_2\) flip-flop coupled to observing those processes infrequently (with all tail lengths considered in the present study) is typical for rare event phenomena, which in this case is apparently due to the large free energy barrier (Fig. 3). In the same spirit, the continuous flow of Q\(^{\text{ox}}\) between the two bilayer leaflets reflects the low free energy barrier across the membrane center (Fig. 3).

Figure 3. PMF profiles of (a) QH\(_2\) and (b) Q\(^{\text{ox}}\) molecules along the direction perpendicular to the membrane surface (Z-direction). The position corresponds to the COM of the Q head group. In both cases, results are shown for two tail lengths: quinone with 1 (Q\(_1\)) or 10 (Q\(_{10}\)) isoprene monomers. The membrane depth of zero corresponds to the middle of the lipid bilayer.

For QH\(_2\), the number of translocation events we observe is low, about 8 events in all five simulations that altogether cover a time scale of ~1000 ns, which suggests a rate of about 0.5 events per microsecond per QH\(_2\). The error margins are high here due to low sampling, but the given number can be expected to be correct as the order of magnitude. Meanwhile, for Q\(^{\text{ox}}\) the translocation rate is faster by about an order of magnitude as Fig. 3b suggests, however the quantification of the translocation rate is here difficult due to continuous back-and-forth motion of Q\(^{\text{ox}}\) molecules across the membrane center.

It is interesting to point out that the flipping of QH\(_2\) takes place in the neighborhood of
cardiolipin molecules (Fig. 5), mechanistic implications of which are discussed below.

**Figure 4.** Trajectories of the center of mass of the head groups of selected (a) quinol (QH$_2$) and (b) quinone (Q$^\circ$) molecules with tail length of 10 units. The coordinate $Z = 0$ nm corresponds to the center of the membrane. Trajectory of each molecule is shown with a different color.

**Figure 5.** Snapshots illustrating the translocation of quinol (Q$_{10}$) through a lipid bilayer. Notably, the translocation takes place in the vicinity of CL. Quinol is shown in yellow, CL in white, DLPC in light blue, and DLPE in dark blue.
Interactions at the membrane-water interface indicate strong interactions between Q and PC, and a weak interplay between Q and CL

In order to further characterize the molecular basis of the observed localization of Q, we examine the polar interactions between the Q head group, and the lipid and water molecules. The data presented in Table 1 show that the number of interactions is much larger for quinol. This is expected, since quinol is more hydrophilic due to the presence of hydroxyl groups in this molecule in comparison to the carbonyl groups in quinone (see also Fig. 1). Second, it is observed that quinol forms the largest number of interactions with DLPC molecules, a smaller but still significant number with DLPE, while the number of interactions with CL is extremely small (Table 1). In contrast, in the case of quinone, we observe less hydrogen bonds with water and charge pairs with DLPC molecules, and practically no interactions with DLPE and CL. Additionally, the data in Table 1 display no clear effects of the tail length on the polar interactions analyzed. One exception is Q\textsuperscript{ox} (with 10 isoprene units), which displays much larger number of polar interactions with the surroundings, than Q\textsuperscript{ox} with shorter tails.

Table 1. Polar interactions of different Q species with lipid and water molecules.

<table>
<thead>
<tr>
<th></th>
<th>Quinol</th>
<th>Quinone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tail length</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>CP\textsuperscript{1}</td>
<td>0.43</td>
<td>0.36</td>
</tr>
<tr>
<td>DLPC\textsuperscript{2}</td>
<td>0.24</td>
<td>0.25</td>
</tr>
<tr>
<td>DLPE\textsuperscript{3}</td>
<td>0.32</td>
<td>0.28</td>
</tr>
<tr>
<td>CL\textsuperscript{2}</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>Water\textsuperscript{3}</td>
<td>1.24</td>
<td>1.24</td>
</tr>
</tbody>
</table>

\textsuperscript{1} Charge pairs (CP) between Q and DLPC lipids.

\textsuperscript{2} Hydrogen bonds of Q with water and lipid molecules.

\textsuperscript{3} Charge pairs (CP) between Q and DLPC lipids.

Discussion

In the current study, we studied the localization of Q in a lipid bilayer, whose composition largely matches the lipid content in the inner mitochondrial membrane. We observed that the Q molecules predominantly interact with PC molecules rather than PE, and very rarely with CL. This preference most likely originates from differences in lipid head group size relative to the size of non-polar tails; this ratio is the largest in PC and the smallest in CL. This observation is analogous to the “umbrella effect” introduced to explain the ordering effect of cholesterol \cite{40}. In the umbrella model, one assumes that the large PC head group protects the hydrophobic part of cholesterol (which has a very small head group), thus preventing it from unfavorable interactions with water \cite{41}. Although this mechanism was initially proposed for cholesterol, it has been found useful also in explaining the
behavior of other lipids characterized by small head groups, such as CL, diacylglycerol, phosphatidylic acid, and ceramide \[^{42, 43, 44, 45}\]. A similar scenario can be envisaged here, where the large head group of PC shields a quite hydrophobic Q from unfavorable interactions with the aqueous phase.

In a recent MD simulation study of Q\(^{\text{ox}}\) and QH\(_2\) in a lipid bilayer, Galassi and Arantes obtained results \[^{18}\], which are in qualitative agreement with ours. They showed that the Q head group prefers to localize at around 1.6 nm away from the center of the lipid bilayer \[^{18}\], however they did not see its distribution in the center. In contrast to our results, they did not observe Q “flip-flops” in simulations whose lengths were comparable to ours \[^{18}\]. Moreover, their PMF profiles showed high barriers (40-60 kJ/mol), which would slow down the diffusion of Q through the hydrophobic region, extending the time scale to micro- or milliseconds \[^{18}\]. The most likely reason for these variations compared to our work is the considerable difference in membrane composition; in their study, Galassi and Arantes used a single-component POPC bilayer, whereas in our case it is a many-component lipid bilayer including CL that is abundant only in mitochondrial membranes. Our earlier studies on translocation of porphyrine through lipid bilayers showed similar effects: in single-component lipid bilayers there was a large barrier (ca. 20 kJ/mol) in the middle of a bilayer, while in a two-component lipid bilayer this barrier reduced to ca. 2 kJ/mol \[^{41}\]. This suggests that the composition of a lipid membrane plays an important role in inter-leaflet diffusion of small molecules along the membrane normal direction. Moreover, our preliminary data suggest that CL molecules may play an important role in the translocation of Q molecules; by maintaining minimal interactions with the Q molecules (Table 1), they may facilitate the initiation of the translocation process. A second relevant difference between our study and the work by Galassi and Arantes \[^{18}\] is the degree of unsaturation in lipids. In our investigation, all lipid tails are modeled as diunsaturated linoleic (18:2) tails, whereas in ref. \[^{18}\] the tails have a different level of unsaturation; one tail with saturated palmitoyl, and another with monounsaturated oleoyl.

Structural analysis of various proteins involved in electron transfer reveals that the Q-binding sites located on the protein surface, or the regions through which Q molecules diffuse into and out from the protein, are all located close to the membrane-water interface and right under the lipid head group region (see Fig. 6). In our work, we observed that Q molecules (Q\(^{\text{ox}}\) or QH\(_2\), and their tail variants) reside in a similar region inside a lipid membrane (Figs. 2-4), right under the lipid head groups, showing that in the inner mitochondrial lipid membrane the Q molecules are positioned in a manner that is quite ideal to enter proteins associated with the electron transfer chain complexes. This finding is especially important in mitochondria, where the inner membrane has a high protein-to-lipid ratio, and for an optimal turnover rate of ATP synthesis, a continuous supply of
such substrates is required.

Figure 6. Q-binding sites in various electron transfer systems. The Q-binding sites are displayed in yellow, and the surrounding protein system in blue surface representations. Shown here are I – Nqo8 subunit of NADH:quinone oxidoreductase (PDB id 4HEA [46]), III – cytochrome b domain of quinol:cytochrome c oxidoreductase (PDB ids 1BCC and 2BCC [47]), IV – subunit I of quinol oxidase (PDB id 1FFT [48]), PSII – Q<sub>A</sub>/Q<sub>B</sub> binding domains of photosystem II (PDB id 3WU2 [49]), and qNOR – quinol dependent nitric oxide reductase (PDB id: 3AYG [50]). Crystallographically observed Q-binding site in complex I is buried deep inside the protein, and here the entry point for Q on the protein surface is displayed in yellow. In PSII, only Q<sub>B</sub> site is displayed.

X-ray crystallography has successfully identified Q-binding sites in many proteins [46, 47, 48, 49, 50]. However, in some enzyme complexes, such as complex I, a “second” Q-binding site has not yet been observed by X-ray studies, even though there is experimental data suggesting its presence (see references in ref. 51). Based on our results that Q molecules localize close to the membrane-water interface, we speculate that the “second” Q-binding site at the membrane-protein interface is likely to be located close to the N-side of the membrane.

Our PMF calculations (Fig. 3) and unbiased simulations (Fig. 4) showed that the (flip-flop) diffusion of the Q head group from one side of the membrane to the other occurs rapidly at room
temperature. We surmise that the low activation barriers of the flipping process may have important functional consequences. For instance, in the inner mitochondrial membrane, quinol molecules are continuously oxidized close to the P-side of the membrane, whereas quinone is reduced on the N-side, complemented with the release and uptake of protons, respectively [51]. A quinone molecule formed upon oxidation on the P-side, such as by the activity of complex III (or also quinol oxidase in bacteria), would undergo a flip to reach the N-side and be reduced by complex I or III. Such a scenario requires Q molecules to flip rapidly in the inner mitochondrial membrane, which is critical for the optimal turnover of the entire electron transport chain.

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