Effects of Ubiquinone-10 on the Stability and Mechanical Properties of Lipid Membranes

EMMA K. ERIKSSON
Abstract

Ubiquinones are a group of fat-soluble molecules present in many biological membranes. The most abundant version in humans, ubiquinone-10 (Q10), plays an important role in the mitochondrial respiration chain and also functions as a powerful antioxidant. Accumulating evidence suggests that Q10 also could have other functions in the membrane. The aim of this thesis has been to explore Q10’s possible role as a membrane stabilizer.

To investigate the potential effect of Q10 in membranes, liposomes with compositions of biological relevance were used as models systems. In lipid systems mimicking that of the inner membrane of the mitochondria, Q10 was found to lower the membrane’s permeability to hydrophilic solutes, render the membrane more resistant to rupturing and promote membrane lipid order. In models mimicking the plasma membrane of E.coli, Q10 was observed to decrease the water permeability and increase the elastic resistance against membrane deformation during osmotic shock. All in all, the results suggest a general membrane stabilizing effect of Q10. The results indicate, however, that the extent of, as well as the mechanisms behind, the membrane stabilizing effects of Q10 vary depending on the membrane lipid composition. Part of the reason for this can likely be traced back to differences in the intermembrane location of Q10.

Supplementary experiments, which facilitated the investigations of Q10 membrane effects, revealed that the choice of cuvette material was of importance for liposome leakage experiments with fluorescent hydrophilic dyes. The results of these experiments highlight the need to take liposome-cuvette interactions into account when planning and evaluating spectroscopic studies involving liposomes.

*Keywords*: ubiquinone-10, lipid bilayers, membrane stabilizer, liposome permeability, osmotic shock, cuvettes, solanesol

*Emma K. Eriksson, Department of Chemistry - BMC, Analytical Chemistry, Box 599, Uppsala University, SE-75124 Uppsala, Sweden.*

© Emma K. Eriksson 2018

ISSN 1651-6214
urn:nbn:se:uu:diva-361361 (http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-361361)
Till Alice
List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.

(*) First authorship is shared by these authors


Reprints were made with permission from the respective publishers.
Contribution report

The author wishes to clarify her contribution to the research presented in papers I-IV:

I  Took active part in performing experiments and analyzing data. Wrote the first draft of the manuscript.

II  Took active part in planning the study, performed the majority of the experiments and analyzed data. Wrote the first draft of the manuscript.

III  Took active part in planning the study, performing experiments, analyzing data and writing the manuscript.

IV  Planned, designed, performed and analyzed the majority of the experiments. Had the main responsibility for writing the manuscript.
Contents

1. Introduction ......................................................................................... 11
   1.1 Biological membranes ........................................................................ 11
   1.2 Ubiquinone-10 .................................................................................... 12
   1.3 Polar lipids.......................................................................................... 14
       1.3.1 Optimum aggregate structure ..................................................... 15
       1.3.2 Phospholipids .............................................................................. 17
       1.3.3 Lamellar phases and lipid order .................................................. 19
   1.4 Liposomes as model membranes ........................................................ 20
       1.4.1 Liposome formation .................................................................... 21
       1.4.2 Liposome stability ...................................................................... 21
   1.5 Thesis layout ...................................................................................... 22

2. Experimental techniques ...................................................................... 23
   2.1 Fluorescence assays ............................................................................ 23
       2.1.1 Leakage ....................................................................................... 23
       2.1.2 Fluorescence anisotropy ............................................................. 24
       2.1.3 Osmosis ...................................................................................... 25
   2.2 Cryo-TEM imaging ............................................................................ 28
   2.3 QCM-D ............................................................................................... 30
   2.4 Reference molecule ............................................................................ 32

3. Results and discussion .............................................................................. 33
   3.1 Paper I: Ubiquinone-10 in POPC membranes .................................... 33
       3.1.1 Design of model system .............................................................. 33
       3.1.2 Liposome permeability and spreading on silica surfaces ........... 34
       3.1.3 Lipid packing order and membrane density ............................... 37
   3.2 Paper II: Ubiquinone-10 in liposomes modelling the inner
      membrane of mitochondria ................................................................. 39
       3.2.1 Design of model system .............................................................. 39
       3.2.2 Liposome permeability and spreading on silica surfaces .......... 40
       3.2.3 Effect of solanesol and possible membrane locations of Q10 .... 41
       3.2.4 Roles of the major lipid components in the IMM model............ 43
3.3 Paper III: Osmo-protective effect of ubiquinone in liposomes modelling the plasma membrane of *E.coli* .................................................... 45
   3.3.1 Design of model system .............................................................. 45
   3.3.2 Verifying the osmo-protective effect .......................................... 46
   3.3.3 Quantifying the osmo-protective effect ...................................... 47
   3.3.4 Resistance against liposome deformation during osmotic shock .................................................................................................... 48
   3.3.5 Possible explanation for the osmo-protective effect of Q10 ...... 49
3.4 Paper IV: Influence of cuvette material on spectroscopic leakage experiments .............................................................................................. 51
   3.4.1 Leakage results affected by the choice of cuvette material ........ 51
   3.4.2 Effect of magnetic stirring .......................................................... 52
4. Concluding remarks ............................................................................. 55
Svensk sammanfattning ............................................................................. 56
Acknowledgements ....................................................................................... 59
References ..................................................................................................... 60
Abbreviations

### Chemical compounds and characterization techniques

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>CF</td>
<td>5(6)-carboxyfluorescein</td>
</tr>
<tr>
<td>Chol</td>
<td>Cholesterol</td>
</tr>
<tr>
<td>CL</td>
<td>Cardiolipin</td>
</tr>
<tr>
<td>cryo-TEM</td>
<td>Cryo-transmission electron microscopy</td>
</tr>
<tr>
<td>DPH</td>
<td>1,6-diphenyl-1,3,5-hexatriene</td>
</tr>
<tr>
<td>IR</td>
<td>Infrared</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear magnetic resonance</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate buffered saline</td>
</tr>
<tr>
<td>PC</td>
<td>Phosphatidylcholine</td>
</tr>
<tr>
<td>PE</td>
<td>Phosphatidylethanolamine</td>
</tr>
<tr>
<td>PG</td>
<td>L-α-phosphatidylglycerol</td>
</tr>
<tr>
<td>PI</td>
<td>Phosphatidylinositol</td>
</tr>
<tr>
<td>POPC</td>
<td>1-palmitoyl-2-oleoyl-sn-glycero-3-phosphatidylcholine</td>
</tr>
<tr>
<td>POPE</td>
<td>1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanolamine</td>
</tr>
<tr>
<td>POPG</td>
<td>1-palmitoyl-2-oleoyl-sn-glycero-3-phospho-(1′-rac-glycerol)</td>
</tr>
<tr>
<td>POPS</td>
<td>1-palmitoyl-2-oleoyl-sn-glycero-3-phospho-L-serine</td>
</tr>
<tr>
<td>PS</td>
<td>Phosphatidylserine</td>
</tr>
<tr>
<td>Q6</td>
<td>Ubiquinone-6</td>
</tr>
<tr>
<td>Q8</td>
<td>Ubiquinone-8</td>
</tr>
<tr>
<td>Q9</td>
<td>Ubiquinone-9</td>
</tr>
<tr>
<td>Q10</td>
<td>Ubiquinone-10, coenzyme Q10</td>
</tr>
<tr>
<td>QCM-D</td>
<td>Quartz crystal microbalance with dissipation monitoring</td>
</tr>
<tr>
<td>ROS</td>
<td>Reactive oxygen species</td>
</tr>
<tr>
<td>Soy-PI</td>
<td>L-α-phosphatidylinositol from soy</td>
</tr>
<tr>
<td>Soy-PS</td>
<td>L-α-phosphatidylserine from soy</td>
</tr>
<tr>
<td>Sol</td>
<td>Solanesol</td>
</tr>
<tr>
<td>Lipid mixtures</td>
<td>Description</td>
</tr>
<tr>
<td>---------------</td>
<td>-------------</td>
</tr>
<tr>
<td>BM</td>
<td>Bacterial membrane</td>
</tr>
<tr>
<td>BMM</td>
<td>Bacterial membrane model</td>
</tr>
</tbody>
</table>
1. Introduction

1.1 Biological membranes

Biological membranes have important roles in most physiological processes and without membranes, life, as we know it, would not exist. They can be described as thin semi-permeable films which separate two adjacent domains. The main function of some biological membranes is to compartmentalize living tissue, while the primary functions of others, in addition to forming compartments, is as anchors for proteins and to act as sites for cellular reactions.

The structural framework in a biological membrane is made up of the bilayer, which consists of a two-layer organization of amphiphilic molecules, mainly polar lipids, held together by non-covalent forces. Within the bilayer, there are also embedded proteins that ensure the essential biological functions (Figure 1). The general view of the biological membrane structure originates from the “Fluid Mosaic Model” proposed by Singer and Nicolson in 1972 (1). The central features of this model are based on the assumption that the bilayer consists of a homogeneous two-dimensional fluid lipid matrix into which proteins are inserted. In this model, the proteins are considered to diffuse freely in the lipid medium, limited only by the viscous resistance of the lipids. In updated versions of this model, the importance of the “mosaic” nature of the membrane structure has been emphasized. Interactions between membrane lipids, integral membrane proteins and other membrane-associated components apparently affect the lateral mobility and range of motion of particular membrane components. These non-random interactions have been considered to control the formation of functional platforms, i.e. lipid rafts, within the membrane. (2)

The lipid composition of biological membranes varies greatly, not only between different species but also between different tissues and depending on its location in the cell. In addition to possible lateral segregations within the membrane, there is also commonly an asymmetrical distribution of the lipids between the inner and outer leaflet of the bilayer. For example, almost all negatively charged lipids in eukaryotic cells are located in the inner leaflet facing the cytoplasm, while most lipids with large glycosylated head-groups are located in the outer leaflet facing the extracellular environment (3). In addition to their physiologically relevant effects on cells, the variety of lipids and their controlled organization has, effects on the mechanical
properties if the membrane (3, 4). In addition to the polar lipids and proteins, biological membranes also contain components such as sterols, which affect the membrane’s mechanical properties by condensing and stabilizing the lipid bilayer.

Figure 1. Schematic illustration of a biological membrane.

1.2 Ubiquinone-10

Ubiquinones are a group of fat-soluble molecules present in the membranes of most eukaryotic species and gram-negative bacteria. The compounds are named after their prevalence in cells, i.e. “ubiquitous quinones” (5). Structurally, ubiquinones consist of a substituted quinone moiety headgroup attached to an isoprenoid chain of different lengths, which varies among organisms. The most common human version of ubiquinone has 10 isoprenoid units and is called ubiquinone-10 (Q10) or coenzyme Q10 (Figure 2).

Ubiquinones can exist in three different redox states: fully oxidized (ubiquinone), partially reduced (semiubiquinone, a free radical intermediate) and fully reduced (ubiquinol). The ability of ubiquinone to undergo reversible redox cycling is responsible for many of its known functions, one of them being a cofactor of the mitochondrial respiration chain, where it accepts electrons from the respiratory complexes I (NADH dehydrogenase) and II (succinate dehydrogenase) and transfers them to complex III (cytochrome c oxidoreductase). Q10 can also transfer protons across the membrane, which provides energy for the ATP synthase (6). Consequently, Q10 is often described as having a central role in the energy production of cells and particularly high concentrations can be found in tissues with high energy requirements, such as heart, kidney, liver and muscles tissues (7). The reduced form also has a strong antioxidant effect, which protects membrane lipids from peroxidation (8). Q10 can also recycle and regenerate other antioxidants, such as vitamin E and vitamin C (9). Some less studied biological
aspects of Q10 include its potential anti-inflammatory effect (10, 11) and its potentially important role in cell growth (12) and certain forms of apoptosis (13).

Q10 is synthesized in all tissues. It is generally presumed that the levels are not dependent on the dietary contributions under normal conditions. Given its poor water solubility, the bioavailability of exogenous Q10 is low which limits its uptake and distribution (14, 15). Human Q10 levels range from about 8µg/g tissue in the lung to about 114 µg/g tissue in the heart (7). Within the cells about 40-50 % of the total amount is located in the inner mitochondrial membrane and smaller amounts are found in other organelles (e.g. the Golgi apparatus, endoplasmic reticulum, lysosomes) (15). Variations in Q10 levels in cells and tissues have been found to be related to certain diseases such as Alzheimer’s, cardiomyopathies and diabetes (6, 16). Also, a decline in Q10 production can be associated with aging (6, 15). Q10’s potential involvement in several diseases seems to be related, among other things, to its ability to keep the reactive oxygen species (ROS) under control (13). ROS are produced as by-products of the mitochondrial respiration chain(17). At low concentrations, they have beneficial effects. Under overproduction conditions however, these molecules seem to inhibit the normal function of cell components such as lipids, proteins and DNA (18), where the oxidative stress is considered to be related to chronic-degenerative diseases as well as the aging process (15).

The various confirmed and suggested functions of Q10 have motivated further and more detailed studies relating to the physico-chemical properties of Q10 in membranes. For example, the exact location and orientation of Q10 in lipid membranes has been a subject of debate (19-21). Typically, two possible orientations are considered: one where Q10 is totally embedded in the mid-plane of the apolar region and one where part of the molecule, the quinone moiety, resides closer to the polar headgroups. It is also possible that Q10 alternates between the two orientations (20). An overall central location of Q10 has been suggested to have a general membrane destabilizing effect, increasing both fluidity and permeability (19). Other studies, showing that the lysis of red blood cells could be prevented by the inclusion of ubiquinone-6 (Q6) in the membrane (22), suggest the contrary. Early studies with for example vitamin E, which is structurally similar to Q10, in lipid membranes indicated that it could have a membrane stabilizing role (23). The inclusion of cardanol in liposomes, also structurally similar to Q10, showed a similar leakage reducing effect as the one produced with cholesterol in liposomes (24). Together with more recent studies, showing that certain bacteria increase their production of endogenous ubiquinone-8 (Q8) when subjected to hyperosmotic salt stress (25), speculation has emerged concerning a potential membrane stabilizing effect of Q10 (26).

Interestingly, the amount of ubiquinone is high in membranes, such as the inner mitochondrial membrane and plasma membrane of gram negative bac-
teria, with low amounts of well-known membrane stabilizers, e.g. sterols (27, 28). Previous observations in our lab also showed that the incorporation of ubiquinone-10 in lipid membranes prolonged the stability of liposomes. The aim of the work in this thesis has been to further investigate the possible membrane stabilizing effects of Q10.

Figure 2. The molecular structure of ubiquinone-10 (Q10).

1.3 Polar lipids

Polar lipids are a group of membrane lipids that have amphiphilic properties. Common to all amphiphilic molecules is that they contain a hydrophilic, water loving, and a hydrophobic, water fearing, part (Figure 3). Polar lipids have a hydrophobic part typically consisting of two aliphatic chains and a hydrophilic headgroup which can be charged, zwitterionic or uncharged. When added to aqueous solutions at certain concentrations, polar lipids will start to aggregate spontaneously (self-assemble). The critical concentration needed for this assembly is quite low \( \leq 10^{-8} \) M for phospholipid species (29). Therefore, the majority of the lipids will be in aggregates rather than diffusing freely in the surrounding media.

Figure 3. A schematic illustration of a conventional polar lipid.
1.3.1 Optimum aggregate structure

Numerous structures can form during self-assembly, depending on several factors, such as the fundamental properties of the amphiphiles and the inter-molecular forces between them. Furthermore, the structural arrangement of amphiphiles can be affected by environmental conditions such as ionic strength, pH and temperature, or by amphiphile concentration.

The concept of a packing parameter can be employed to predict the preferred aggregate structure for a certain lipid. The idea is based on every molecule having an optimal headgroup area, \(a_0\), and a critical chain length, \(l_c\), which can be related to the hydrophobic chain volume, \(v\), by the packing parameter (30):

\[
N_s = \frac{v}{a_0 \cdot l_c}
\]  

(1)

A schematic illustration of how the aggregate structure can vary with packing parameter is shown in Figure 4. Low \(N_s\)-values suggest highly curved aggregates, such as globular micelles. Amphiphiles with \(N_s \approx 1\) typically form flat bilayer (lamellar) structures. Most lipids have packing parameters around 1, which is consistent with their general tendency to form lamellar structures. It is important to note that the packing parameter gives only a rough estimation of preferred aggregate structure and is only useful for dilute aggregate solutions.

Another way to analyze aggregate structure is to use the curvature concept. In this approach, the preferred mean curvature of the structure is of importance. The mean curvature \((H)\) is defined by (31):

\[
H = \frac{1}{2} \left( \frac{1}{R_1} + \frac{1}{R_2} \right)
\]  

(2)

where \(R_1\) and \(R_2\) are the radii of curvature in two perpendicular directions at a certain point at the surface. For a sphere, \(H = 1/R (R_1 = R_2)\) and for a planar bilayer, \(H = 0 (R_1, R_2 = \infty)\). A saddle shaped surface will also have \(H = 0 (R_1 = -R_2)\). The sign of the mean curvature by convention is positive if the surface curves towards the apolar region, e.g. normal micelles are defined as having positive curvature. The lipid spontaneous curvature \((H_0)\) is also related to the aggregate mean curvature. This is the curvature that an unconstrained film of lipids would adopt. The sign of the spontaneous curvature is defined as positive if the lipids curve towards oil and negative if the amphiphiles curve towards water.
Figure 4. A schematic overview showing the relationship between preferred aggregate structure, packing parameter and curvature.
1.3.2 Phospholipids

Phospholipids are a group of polar lipids that are very abundant in biological membranes. The molecular structure of a general phospholipid consists of a glycerol backbone connected to a phosphorus containing hydrophilic head-group and two hydrophobic fatty acid chains. There is a large range of possible fatty acid chain lengths and degrees of saturation. Also, there is a variety of possible headgroups. In eukaryotic cells, the phosphatidylcholine (PC) and phosphatidylethanolamine (PE) headgroups are very common (32). The numerous combinations of headgroups, chain lengths and degrees of saturation give great diversity in the phospholipid species present in biological membranes. The reasons for this generally large variety of phospholipids are however not yet completely understood.

The lipids used in this thesis were synthetic phospholipids (Figure 5) and phospholipids from extracts of soy, *E.coli* and bovine heart (Figure 6). The extracts include the special phospholipid, cardiolipin (CL), which is a di-phosphatidylglycerol, i.e. a lipid with two phosphatidic acid moieties connected by a glycerol backbone. This lipid is mainly found in the mitochondria of eukaryotic cells and in plasma membranes of bacteria (33, 34).

![Figure 5. Synthetic phospholipids used in this thesis.](attachment:figure5.png)
Figure 6. Predominant lipid structures present in lipid extracts used in this thesis.
1.3.3 Lamellar phases and lipid order

Phospholipid lamellar phases can display several thermotropic phase transitions in the presence of water. At low temperatures, the bilayers can be found in lamellar crystalline phase, \( L_c \), in which the lipids are tightly packed, and the membranes can be seen as crystal-like. At a specific temperature, the bilayer will transform into a lamellar gel phase, \( L_\beta \), in which the lipids still have a high conformational order, but the chains have a higher rotational disorder than in the \( L_c \) phase. By increasing the temperature further, the gel phase, with primarily all-trans configurations of the lipids, will transform into a liquid-crystalline phase, \( L_\alpha \). In the \( L_\alpha \) phase, the lipids are disordered, i.e. the hydrocarbons can have several gauche conformations, which give the membrane interior liquid-like properties. The specific temperature at which the lipid chains collectively melt is called the main transition temperature, \( T_m \). This temperature is greatly affected by both the length and degree of saturation of the fatty acid chains. For example, shorter fatty acid chains or higher quantities of unsaturations will thus favor the \( L_\alpha \) phase, giving a lower \( T_m \). Furthermore, phospholipids with saturated chains, can sometimes display an additional phase, the rippled phase, \( P_\beta' \), which can be observed between the \( L_\beta \) and the \( L_\alpha \) phase. This phase has a special out-of-plane, “sawtooth” structure containing alternating gel and liquid-like segments (35).

From the above, it is clear that lipid chain order is important for lamellar phase states. Membranes with a certain lipid composition will have a certain lipid chain order and will be in a certain phase state. However, by introducing further membrane components, such as cholesterol (Figure 7), the lipid order can be affected. Cholesterol is a kind of lipid that is frequently found in eukaryotic membranes. In phospholipid bilayers, cholesterol will sit with the hydroxyl group near the lipid headgroups, while the stiff ring system will be embedded in the hydrophobic part of the membrane. In liquid crystalline phase membranes, cholesterol generally introduces order to the lipid chains. In gel phase membranes however, it induces disorder. By including more than 20-30 mol % cholesterol in phospholipid bilayers, the phase transition between gel and liquid-crystalline phase is smeared out. This results in a new liquid-ordered phase, \( L_\omega \), in a large temperature span (36). The \( L_\omega \)-phase has mixed properties with the gel and liquid crystalline phase, where the lipids have a high lipid chain order while still allowing a rapid lateral diffusion.
1.4 Liposomes as model membranes

Liposomes can be used as model membrane systems to investigate the properties of biological membranes (Figure 8). This type of model makes it possible to study the properties and effects of individual membrane components by customizing the liposomal composition and its surroundings.

Liposomes are closed lipid bilayers, usually spherically shaped, with an aqueous core. They can consist of one, or several, bilayers and the size can range from ~20 nm to several micrometers in diameter. Generally, when using liposomes as model systems, unilamellar liposomes, i.e. liposomes having only one bilayer, are desirable. However, liposome production can sometimes also generate bi- or multilamellar structures, depending on the lipid composition and the preparation technique.

The liposome structure makes it possible to encapsulate hydrophilic compounds in the inner aqueous center and hydrophobic substances within the lipid bilayer. These unique properties have been used in the food, pharmaceutical and cosmetic industry (37-39).

Figure 7. Molecular structure of cholesterol.

Figure 8. A schematic illustration of a liposome. (Figure by permission from Göran Karlsson.)
1.4.1 Liposome formation
The formation of spherical bilayer shells from flat bilayer sheets requires an input of energy. The cost in energy comes from the fact that it is necessary to create smaller bilayer segments, where unfavorable hydrophobic edges are exposed to the aqueous solution, and to bend the bilayer. The input in energy can come from mechanical energy by extrusion or from acoustic energy by tip sonication, for example. The mechanism of liposome formation can then be understood by considering the interplay between the edge tension (the energy required to keep the hydrophobic edge exposed to the surrounding aqueous solution) and curvature energy (related to the energy needed to bend the flat bilayer). When the lipid bilayer closes, the edge tension vanishes. (40-42)

1.4.2 Liposome stability
There are many aspects of liposome stability. From a thermodynamic point of view, liposomes are seldom equilibrium structures. The fact that they usually have a limited stability in suspension and can produce different size distributions with the same lipid components supports this. The stability of liposomes in suspension can be defined by their colloidal stability, which is related to their propensity to flocculate and fuse. Their partial stability in suspension can sometimes be described as liposomes being in ‘kinetic traps’, referring to their kinetic stability. There are several methods for increasing the stability of liposomes in suspension. For example, it is possible to incorporate an amount of PEGylated lipids (43), lipids connected to hydrophilic polymers, which prolongs the colloidal stability of liposomes by introducing steric forces. Also, it is possible to stabilize liposomes electrostatically. This can be obtained when the surface charge density on the liposomes is high enough and the electrostatic repulsion is larger than the attractive van der Waals forces.

Another aspect of liposome stability is their chemical stability, which is the resistance to, for example, oxidation and hydrolysis processes. Well-packed bilayers will reduce the access of oxidizing or hydrolyzing agents, connecting thus the chemical stability to the mechanical stability of lipid membranes. The mechanical stability, which is the stability defined by the intramembrane cohesivity, can be related to liposome properties such as permeability, the ability to withstand attacks from surfactants, and the ability of liposomes to sustain osmotic shock. By including cholesterol in the lipid membrane for example, the mechanical stability of the liposome can be increased. When discussing membrane stability or liposome stability throughout this thesis, it is mechanical stability that is implied. (44, 45)
1.5 Thesis layout

The overall aim of this thesis is to describe the membrane stabilizing function of Q10 and investigate the mechanisms behind it.

In *Paper I* our objective was to investigate the effect of ubiquinone-10 in simple POPC membranes. In *Paper II* we progressed to a more complex lipid membrane model mimicking that of the inner mitochondrial membrane, thus simulating one of the most relevant lipid environments for ubiquinone-10. In *Paper III* our goal was to investigate the osmo-protective effect of ubiquinones, in this case using a membrane model mimicking the lipid composition in the plasma membrane of *E. coli* bacteria. The investigations in *Paper IV* were done in parallel with those in *Papers I and II*, and the aim was to investigate and clarify the origin of some curious effects observed after changing the cuvette material used during spectroscopic measurements.
2. Experimental techniques

2.1 Fluorescence assays

2.1.1 Leakage

Liposome permeability experiments to estimate membrane stability were performed by studying the release of the fluorescent hydrophilic compound 5(6)-carboxyfluorescein (CF). The method uses the ability of CF to self-quench at high concentrations, where about 95% of the CF signal will be quenched at 100 mM (46). The standard procedure is to prepare liposomes in a highly quenching concentration of CF, normally 100 mM CF in 10 mM phosphate buffer. The free CF solution is then replaced by PBS buffer (isotonic to the CF-solution) by gel filtration. Upon CF leakage from the liposomes, the fluorescence signal increases due to CF dilution. Prior to the experiments, the samples are diluted to a suitable concentration to ensure a leaked CF signal within the linear fluorescence to concentration range (~3-30 µM total lipid concentration) (47). The degree of leakage over time is calculated by:

\[ \chi_{CFrel} = \frac{(I(t) - I_0)}{(I_{tot} - I_0)} \]  

(3)

where \( I(t) \) is the time-dependent fluorescence intensity, \( I_0 \) is the initial fluorescence intensity and \( I_{tot} \) is obtained after complete liposome leakage induced by the addition of a surfactant (often Triton X-100 (polyethylene glycol tert-octylphenyl ether)). The underlying concept of the leakage experiment is visualized in Figure 9.

In addition to spontaneous leakage, the surfactant promoted leakage from liposomes was also monitored. Via stopped-flow measurements using a rapid mixing device, the fluorescence could be monitored after mixing CF-containing liposomes with equal volumes of surfactant solutions. The degree of leakage was calculated by Equation 3.

In Papers I and II, both spontaneous and surfactant-induced CF leakage were recorded to examine the effect of membrane-incorporated Q10 in simple systems (POPC) and more complex lipid systems (mimicking the inner mitochondrial membrane). In Paper III, measurements of osmotic stress-induced leakage were performed to increase the understanding of how the plasma membrane of E.coli counteracts osmotic stress. The effect of the
choice of cuvette material on leakage measurements was investigated in *Paper IV*.

![Diagram of leakage](image)

*Figure 9. Unquenching of carboxyfluorescein (CF) by liposomal leakage.*

### 2.1.2 Fluorescence anisotropy

Steady-state fluorescence anisotropy was measured to estimate the lipid packing order in lipid membranes. Experiments were performed by incorporating a hydrophobic fluorescent probe, 1,6-diphenyl-1,3,5-hexatriene (DPH), in the membrane with a probe to lipid ratio of 1:1000. DPH is a rod-like probe which generally sits parallel to the acyl chains in the lipid membrane and resides on average about midway between the headgroups and the bilayer center (48). Therefore, the measured anisotropy reflects the order in the hydrophobic part close to the headgroup region rather than the order in the center of the membrane.

The possibility of photo-selective excitation of the fluorophore by polarized light allows determining of fluorescence anisotropy. Recordings can thus be performed by equipping the spectrometer with two polarizing filters, polarizing both the excitation and the emission light (*Figure 10*). The magnitude of the obtained fluorescence anisotropy is affected by the motional freedom/rotational diffusion of the probe. Fluorophores dissolved in aqueous non-viscous solutions typically display anisotropies close to 0, since the surrounding media allows the probe to have unrestricted motional freedom. On the other hand, membrane-bound probes, where the fluorescence emission is faster than the molecular rotations of the probe, will give rise to a measurable fluorescence anisotropy (49, 50). A high anisotropy value indicates a high lipid packing order (50, 51) and a low number of packing defects.
The steady-state anisotropy ($<r>$) was calculated by:

$$<r> = \frac{(I_{VV} - GI_{VH})}{(I_{VV} + 2GI_{VH})}$$  \hspace{1cm} (4)

where $G=I_{HV}/I_{HH}$ is an instrumental correction (grating) factor and $I_{XY}$ are the fluorescence intensities measured with the different combinations of the polarizers ($X=$excitation, $Y=$emission, $H=$horizontal, $V=$vertical). Anisotropy measurements were performed in Papers I-III.

Figure 10. Schematic figure showing the setup during fluorescence anisotropy measurements. The figure depicts one of the four combinations of polarizers, e.g. where both polarizers are set to a vertical mode, enabling measurement of the signal denoted $I_{VV}$. (Figure adapted from (49).)

2.1.3 Osmosis

The liposomes’ resistance to osmotic stress was characterized by determining the osmotic water permeability coefficient ($P_f$) and the final relative volume of liposomes after osmotic shock ($V_{final}/V_0 = X(\infty)$) in Paper III. Experiments were performed by monitoring the self-quenching of encapsulated CF in liposomes after a sudden increase in outer osmolarity. A customized approach was developed, originating from previously described experiments (52, 53) related to the permeability equation:
\[
\frac{dX(t)}{dt} = -\frac{P_t V_w(SAV)}{RT} (\Delta P_{os})
\]  

where \(X(t)\) is the relative volume of liposomes (with respect to the initial volume) as a function of time, \(V_w\) is the molar volume of water and \(SAV\) is the initial surface to area volume ratio. \(\Delta P_{os} = RT(\text{OsC}_{\text{out}} - \text{OsC}_{\text{in}(0)})/X(t)\) is the osmotic pressure difference across the membrane where \(\text{OsC}_{\text{out}}\) and \(\text{OsC}_{\text{in}}\) are the initial outer and inner osmolarities. Since several significant mechanisms are not considered in this model, a refined version was developed as described below (see supporting information in Paper III for more details).

During osmotic shock it is often assumed that the final relative volume \(X(\infty)\) of the liposomes is known and equal to \(\text{OsC}_{\text{in}}/\text{OsC}_{\text{out}}\), e.g. that liposomes will shrink until equal osmolarities are achieved, without any resistance against deformation. By introducing \(\Delta P_{el}\), being the elastic pressure exerted by the lipid membrane upon deformation, the model was refined to:

\[
\frac{dX(t)}{dt} = -\frac{P_t V_w(SAV)}{RT} (\Delta P_{os} - \Delta P_{el})
\]  

The value of \(X(\infty)\), when assumed to be known, is also commonly used to establish a relationship (often assumed to be linear) between the relative volume \(X(t)\) and the fluorescence intensity. In Paper III, a new way of calculating the relative liposome volumes from the relative fluorescence intensities was established without the need to assume final conditions and by considering the following:

- Both encapsulated and leaked CF contributes to the total fluorescence intensity.
- The concentration of CF inside the liposomes is affected not only by water transport but also by CF leakage.

The processes occurring during osmotic shock are summarized in Figure 11. From these considerations, the following relationship between relative fluorescence intensities and relative liposome volume could be established:

\[
X(t) = \frac{(f(t)-1)[\text{[CF]}_{\text{lip}(0)}(1-Z)]}{\tau_1 \ln \left[\frac{1}{A_1}(x_{\text{CF}}(t) - y_0 - f(t) + (f(t)-1)Z)\right]}
\]  

where \(f(t)\) is the time-dependent fraction of leaked CF (determined from separate experiments), \([\text{CF}]_{\text{lip}(0)}\) is the concentration of the CF solution used for liposome preparation, \(x_{\text{CF}}(t)\) is the relative fluorescence intensity after subjecting liposomes to osmotic stress and \(y_0\), \(A_1\) and \(\tau_1\) are parameters de-
scribing the exponential relationship between the relative fluorescence and the encapsulated CF (calculated for every sample). $Z$ is a parameter accounting for changes in initial encapsulated CF concentration due to elapsed time between separation and the start of the osmosis experiment and is given by:

$$Z = \frac{r_1(x_1-x_{\text{lip}(\text{CF}_{\text{lip}}(0))})}{r_1+[\text{CF}]_{\text{lip}}(0)(x_{\text{lip}}(\text{CF}_{\text{lip}}(0))-y_0)} \tag{8}$$

where $x_{\text{lip}}(\text{CF}_{\text{lip}}(0))$ is the relative fluorescence directly after separation and $x_1$ is the relative fluorescence right before the osmosis experiment. To summarize, by determining the following parameters:

- $x_1$ = relative fluorescence of non-stressed liposomes
- $x_{\text{CF}}$ = relative fluorescence after subjecting the liposomes to stress
- $f$ = fraction of leaked CF in a fully quenched system (100 mM CF)
- $x_{\text{lip}}(\text{CF}_{\text{lip}}(0))$ = relative fluorescence of non-leaked liposomes
- the empirical values of $y_0$, $r_1$, and $A_1$ (determined from a calibration curve of $x_{\text{lip}}(\text{CF}_{\text{lip}}(0))$ at different $[\text{CF}]_{\text{lip}}(0)$)

the ratio $X(t) = V_2/V_1$ can be obtained.

To determine the relationship between $X(t)$ and $\Delta P_{el}$, experiments with different outer osmolarities were performed. At equilibrium conditions, $\Delta P_{el} = \Delta P_{os} = RT(Os_{\text{out}} - Os_{\text{in}(0)}/X(\infty))$. However, no universal correlation was found. Therefore, the relationship between each experimental point for each sample was modeled as linear, giving a final permeability equation:

$$\frac{dX(t)}{dt} = X'(t) = -P_fV_w(SAV)\left(\text{Os}_{\text{out}} - \frac{\text{Os}_{\text{in}(0)}}{X(t)} - (m_XX(t) + b_X)\right) \tag{9}$$

where $m_X$ and $b_X$ are the $X$-dependent slope and $y$-intercept values.

To calculate the osmotic water permeability coefficient $P_f$, curves with $X(t)$ vs. $t$ were fitted with an exponential equation $X(t) = a_0 + Ae^{-kt}$, where $a_0 = X(\infty)$. Thereafter $X'(t)/P_f$ was calculated for every experimental point using Equation 9 and the obtained curve was fitted with $X'(t)/P_f = -A_2e^{-kt}$. By combining the two fittings, $P_f$ could be obtained:
2.2 Cryo-TEM imaging

Cryogenic transmission electron microscopy (cryo-TEM) is a very useful method for characterizing colloidal particles or aggregates in aqueous media, for example self-assembled lipid structures such as liposomes (54). The cryogenic containment of the sample during preparation and imaging enables direct visualization without sample perturbation, where no drying, stain, labeling or replica is needed. The TEM-imaging technique uses differences in electron densities in the sample to form an image. By irradiating the specimen with an electron beam, it is possible to obtain a contrast between the water matrix and the lipid structures, giving a two-dimensional image (Figure 12). The technique is especially valuable as a supplement to other qualitative characterization methods e.g. to confirm the production of certain aggregates or to increase sample understanding, as used in Papers I-III.

The specimen is prepared by depositing a small drop of sample onto a copper grid which is covered by a holey polymer film (Figure 13a). The excess liquid is removed by a filter paper, giving thin sample films which span the holes in the polymer support. The grid is then immersed in liquid ethane kept at 100 K, causing instant vitrification of the sample film and thus avoiding rearrangement of water and the lipids. The entire preparation step is performed inside a climate chamber (Figure 13b), with high humidity and controllable temperature. To maintain a vitrified state of the sample, and to avoid water condensation forming ice crystals on top of the sample surface, the specimen is carefully handled in a cooled nitrogen atmosphere during

$P_f = \frac{kA}{A_2}$  (10)

Figure 11. Processes occurring during osmotic shock.
mounting and transfer to the electron microscope. Analysis is then performed by irradiating the sample with electrons under high vacuum conditions in transmission mode. (55)

Aggregates from about 4-5 nm up to ~500 nm size are suitable for observation with cryo-TEM. The relatively small differences in electron density between water and amphiphiles limit the contrast and therefore also the dimensions that can be resolved. The upper limit is controlled by the thickness of the sample film produced during specimen preparation, where water in films thicker than 500 nm will give rise to high background electron scattering. (55)

During image interpretation, it is important to be aware of possible structural artefacts. In addition to overlaying ice crystals and sample burning arising from long-time exposure of electrons, one frequently occurring artefact is the presence of invaginated liposomes (Figure 12). The sample films have very high surface-to-volume ratios making them very sensitive to evaporation, and a modified salt concentration in the sample film can force liposomes to release water and possibly to collapse (56). Liposomes with soft membranes will have less resistance to membrane deformation and will thus more frequently form invaginated structures. Keeping a high humidity in the preparation chamber counteracts this effect. Another important artefact is the occurrence of size sorting caused by the biconcave form of the sample film. Large objects thus tend to reside in the thickest part of the sample film while small or flat objects are more likely to be found in the thinnest part. Very large objects, with dimensions bigger than the sample film, are not possible to observe. By investigating many sample areas, and keeping the possible artefacts in mind, cryo-TEM can be used for estimating particle structure and size.

Figure 12. Interpretation of cryo-TEM images. From left to right: 1) spherical liposome, 2) flattened liposome viewed sideways, 3) flattened liposome facing forward, 4) invaginated liposome, 5) invaginated liposomes with opening facing the electron beam, 6) bilamellar liposome. (Figure by permission of Göran Karlsson.)
2.3 QCM-D

Quartz crystal microbalance with dissipation monitoring (QCM-D) is a technique used to monitor mass depositing on surfaces. It works by recording changes in frequency and energy dissipation factor using an oscillating sensor (Figure 14). With this equipment, it is possible to study the interactions between liposomes and different surfaces and also, in cases where liposomes attach and spread on the surface, to estimate lipid membrane density. The method is very sensitive and can record mass density changes in the ng/cm$^2$ regime. (57-59)

A QCM sensor consists of a quartz crystal wedged in between two electrodes. The sensing surface of the crystal is normally coated with a thin layer of a desired material, e.g. silica, polystyrene, gold, etc. By applying an AC electric field, mechanical stress is induced in the quartz crystal due to the inverse piezoelectric effect. The QCM instrumentation then records the shift in resonance frequency ($\Delta f$) due to mass changes in the deposited film. If rigid and evenly distributed films are formed on the surface, then the recorded $\Delta f$ can be used to estimate the adsorbed mass density ($m$) by Sauerbrey equation (60):

\[
\Delta f = \frac{2 fm}{d^2} \times \sum m_i
\]

where $f$ is the resonance frequency, $m$ is the mass of the deposited film, $d$ is the thickness of the crystal, and $m_i$ is the mass per unit area of each individual film layer.
\[ m = -C \frac{\Delta f_n}{n} \]  \hspace{1cm} (11)

where \( C \) is the mass sensitivity constant (17.7 ng·cm\(^{-2}\)·Hz\(^{-1}\) for a crystal with a fundamental frequency of 5 MHz) and \( n \) is the overtone number.

Using the QCM-D technique, it is also possible to monitor the energy dissipation factor, which is a measure of the damping or dissipation (\( \Delta D \)) of the oscillation when the voltage is turned off. The dissipation factor is related to the rigidity of the adsorbed material, thus allowing differentiation between, for example, (stiff) bilayers and surface attached (soft) liposomes (57).

Experiments with QCM-D instrumentation are in general performed with a continuous sample flow until frequency and energy dissipation signals have stabilized. By replacing the sample solution with a buffer, further sample information can be obtained about the attachment of the adsorbed structures.

The QCM-D technique was used in Papers I, II and IV. In Paper I, the focus was on distinguishing between interactions of liposomes and silica for different samples, where fingerprint curves (\( \Delta D \) vs. \( \Delta f \)) (59, 61) were used to identify different processes in certain time periods. Membrane densities were also determined. In Paper II the aim was to differentiate between the interactions of liposomes with different lipid compositions, and silica. In Paper IV the aim of the QCM-D measurements was to discern possible interactions between liposomes and different cuvette materials.

![Figure 14. A typical QCM-D recording for liposome adhesion and bilayer formation on the sensor surface.](image)
2.4 Reference molecule

Solanesol (Sol), a molecule similar to Q10, but with a hydroxyl group instead of the quinone moiety in Q10, was used in several experiments as a reference substance (*Figure 15*). The purpose of having this molecule as a reference was to probe the significance of the quinone moiety on the observed membrane effects. Also, as there was no unified model of the location and orientation of Q10 in lipid membranes, the hydrophobic solanesol molecule, which is thought to reside mostly in the center of the lipid membrane, facilitated these investigations.

*Figure 15.* The molecular structure of solanesol.
3. Results and discussion

3.1 Paper I: Ubiquinone-10 in POPC membranes

3.1.1 Design of model system

In *Paper I*, the POPC membrane was chosen as a simple, yet relevant, model for ubiquinone’s natural environment in cellular membranes. The POPC lipid is often used in biomimetic experiments, since it is a common component in biological membranes. The saturated chain in the *sn*-1 position and the unsaturated chain in the *sn*-2 position give fluid membranes in a large temperature range and mimic the overall mammalian fatty acid composition. Also, POPC membranes have defined and well-known characteristics compared to what is the case with lipid extracts or complex lipid mixtures.

Apart from choosing a relevant lipid, it was also necessary to explore the maximum amount of Q10 possible to incorporate in POPC membranes. Typically, in the ubiquinone rich inner mitochondrial membrane the amount of ubiquinone is about 0.5-2 mol % (62, 63), which led us to use 2 mol % as a starting point. By determining the Q10 content (for Q10 total determination, see details in *Paper I*) in combination with phosphorus analysis and cryo-TEM investigations, it was revealed that there is a saturation limit in the range between 3.3 and 6 mol % of Q10 in the POPC membrane. As can be seen in the cryo-TEM images (*Figure 16*), dense oil-like structures appear when the concentration of Q10 is above this limit. By comparing the images from the sample with pure Q10 (treated in the same way as the lipid sample) showing Q10 crystals, it was clear that the aggregates formed must consist of a large amount of Q10 mixed with some lipid. To avoid complications arising from an expelled lipid-rich Q10 phase, the Q10 membrane concentration was limited to 3.3 mol % in further experiments.
3.1.2 Liposome permeability and spreading on silica surfaces

Permeability experiments were performed to explore the effect of Q10 on lipid membranes. Highly hydrophilic compounds, such as 5(6)-carboxyfluorescein (CF), are thought to leak primarily through the formation of defects and transient pores in the bilayer. It is expected that membrane additives which reduce the number or lifetime of these structures will thus reduce the rate of leakage. In Paper I, leakage experiments were performed over long periods of time and were best fitted with the two-term exponential equation as described by Agmo Hernández et al. (64):

\[ x_{CF_{rel}}(t) = 1 + C_1 e^{-t/\tau_1} + C_2 e^{-t/\tau_2} \]  \hspace{1cm} (12)

where \( x_{CF_{rel}}(t) \) is the fraction of released CF as a function of time. From the two time constants a parameter \( A \) can be determined, \( A = \tau_1^{-1} + \tau_2^{-1} \), where a larger \( A \)-value corresponds to a higher initial leakage rate.

The results (Table 1) showed that the incorporation of Q10 in POPC membranes decreases the \( A \)-values, compared to pure POPC. Moreover, a greater effect was achieved with a higher amount of Q10 in the membrane. Also, the initial leakage rate from liposomes containing 40 % cholesterol was comparable to the one with 3.3 mol % Q10, indicating that Q10 is more efficient at reducing the rate of leakage than cholesterol. By comparing the results obtained with solanesol and Q10 containing liposomes, it was revealed that the quinone moiety is important for decreasing the leakage rate.
Table 1. A-values, describing membrane leakage, for different lipid compositions. *Data from reference (64).

<table>
<thead>
<tr>
<th>Lipid composition</th>
<th>A/(10^-4s^-1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>POPC</td>
<td>4.65 ± 0.3</td>
</tr>
<tr>
<td>POPC:Q10 (2 mol % Q10)</td>
<td>3.15 ± 0.2</td>
</tr>
<tr>
<td>POPC:Q10 (3.3 mol % Q10)</td>
<td>2.83 ± 0.4</td>
</tr>
<tr>
<td>POPC:Sol (2 mol % solanesol)</td>
<td>5.04 ± 0.8</td>
</tr>
<tr>
<td>POPC:Chol (40 mol % cholesterol)</td>
<td>2.34 ± 0.7</td>
</tr>
</tbody>
</table>

Permeability experiments were also performed in the presence of surfactants to further verify and explore the effect of Q10 in membranes. By adding micelle-forming surfactants such as C_{12}E_{8} (octaethylene glycol monododecyl ether), the membrane permeability of hydrophilic compounds is generally increased. Surfactants are known to accumulate and stabilize transient pores and defects by their tendency to form structures with high spontaneous curvature (65, 66). This can occur at concentrations well below membrane saturation. The fast leakage achieved by the presence of surfactants allows a fit with a pseudo-first order leakage profile:

\[ x_{\text{CF} \text{rel}}(t) = 1 - e^{-kt} \] (13)

where \( x_{\text{CF} \text{rel}}(t) \) is the fraction of released CF as a function of time and \( k \) is the rate constant. This fit assumes a homogeneous distribution of surfactant in all liposomes, thus giving an equal leakage from all the liposomes. When comparing leakage results, it is also important to know if there is a difference in the partitioning of the surfactants depending on the membrane composition. Both characteristics were investigated in Paper I and the results indicated that the surfactants were distributed evenly between the liposomes and that there was no significant difference in partitioning between the compositions tested. Surfactant induced leakage results are presented in Figure 17. By comparing the leakage profiles with their associated rate constants, it is clear that Q10 has a leakage protective effect also in the presence of surfactants. The negligible effect of solanesol suggests that the quinone headgroup is essential for the membrane stabilizing effect.
Another way of examining membrane properties is to study the interaction between liposomes and surfaces, which can be done by using the QCM-D technique. Possible interaction pathways include vesicle adhesion and rupture (complete or incomplete) and irreversible or reversible adhesion of intact vesicles. In some cases, no vesicle-surface interaction is observed. The process of liposome-substrate attachment is promoted by the gain in energy obtained from vesicle-surface adhesion. The cost in elastic energy when the liposomes deform, however, counteracts this effect. Even more energy is needed for the liposome to rupture and form bilayers. How the liposomes interact with the surface will thus depend strongly on the mechanical stability and rigidity of the membrane, which can be connected to the cohesive forces between the lipids. (58, 67, 68)

In *Paper I*, liposome-silica surface interactions were studied as it has been observed that adhesion and subsequent liposome spreading is favorable on silica-based materials (69). *Figure 18* shows the resulting fingerprint curves ($\Delta D$ vs. $\Delta f$) for experiments performed with different lipid compositions. The initial slight increase in dissipation factor together with the large decrease in frequency, seen for all compositions, suggests fast liposome rupture and bilayer formation. The subsequent steeper slope (after the dotted vertical line) suggests the attachment of soft structures, most likely intact liposomes. The membrane properties of liposomes with different compositions were differentiated by studying the events occurring during rinsing of the sensor. Concerning the POPC and the POPC:Sol samples, intact liposomes were completely removed during rinsing suggesting that they were
attached on top of the already formed bilayer. The samples containing Q10, however, showed a significantly higher dissipation factor after rinsing, suggesting that some intact liposomes remain bound. This indicates that not all POPC:Q10 liposomes rupture after they attach to the surface, which suggests that the membrane presents a certain resistance to rupturing and spreading. Differences in whether liposomes attach and spread over the sensor surface are most likely linked to membrane stability and rigidity, as previous mentioned. From these results it was thus concluded that Q10 increases the mechanical stability of the membrane.

Figure 18. Fingerprint curves for different lipid compositions. The height of each cell corresponds to a dissipation shift of $2.5 \times 10^{-6}$.

3.1.3 Lipid packing order and membrane density

To understand more about the membrane properties affected by Q10 incorporation, lipid membrane order was investigated by measuring the fluorescence anisotropy of DPH ($\langle r \rangle$). The lipid order, e.g. acyl chain order, is a measure of how tightly packed the lipids are. When lipids have high order, there is a lower probability of the lipids forming membrane defects and transient pores. By extension, properties such as membrane permeability can be related to membrane order. The obtained anisotropy results (Figure 19)
showed that Q10 has a membrane ordering effect, where a higher concentration gives a higher order. Solanesol did not make any significant contribution to the membrane order, suggesting that the quinone moiety is also essential for the ordering effect. Although it could be debated that differences between Q10 and solanesol could arise from the extra isoprenoid unit in Q10, previous fluorescence anisotropy studies of Q10 and Q9 (ubiquinone with 9 isoprene units) prepared by ethanol injection, showed that both ubiquinones increase the lipid chain order in an almost identical fashion. (70) This indicates that the small difference in length would not be the reason for the potential differences observed with Q10 and solanesol.

By comparing the results from liposomes with Q10 and ones with cholesterol (a known membrane ordering compound), it was shown that fewer Q10 molecules than cholesterol were needed to achieve a certain membrane ordering effect. Since Q10 was found to have an upper concentration limit (between 3.3 and 6 mol % in POPC membranes), it was however not possible to reach the lipid order produced with 40 mol % cholesterol, which is a commonly used concentration to achieve stable membranes during drug delivery.

![Figure 19. Fluorescence anisotropy for different lipid compositions.](image)

To further investigate the reason for the leakage reducing and membrane stabilizing effect of Q10 in POPC membranes, the QCM-D data from section 3.1.2. was used to calculate membrane density. To account for frequency shifts caused by intact liposomes, the fingerprint curves were used (instead of raw QCM-D curves). The frequency shift at the point where the two pro-
cesses cross, i.e. when the process of bilayer formation changes to the process of adhesion of intact liposomes (vertical dotted line in Figure 18), was used to estimate the mass of the lipid bilayer. The extracted $\Delta f$ values were corrected to account for the bulk effect, i.e. the frequency shift caused by changes in solution density and viscosity when liposomes are present, and the results were verified with the ones expected for POPC with good agreement.

The results (Table 2) clearly show that Q10 increases the membrane density in the supported lipid bilayer. A higher lipid density is related to more tightly bound lipids which can be coupled to increased membrane rigidity. It is fair to assume that the lipid condensing effect would also apply for membranes free in suspension. The comparably higher density obtained for the 3.3 mol % Q10 sample than for 2 mol % Q10 samples and the negligible increase in membrane density by solanesol fit well with the previous observations concerning the impact on membrane order for each sample. Also, a more condensed and ordered membrane by the addition of Q10 explains well the observations of lowered liposome leakage and increased resistance to liposome rupture on silica surfaces. The fact that Q10 but not solanesol exhibits an effect fits best with the assumption that the quinone moiety at least partially resides close to the lipid headgroups (in contrast to a completely intermembrane location).

<table>
<thead>
<tr>
<th>Lipid composition</th>
<th>m / (ng/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>POPC</td>
<td>452</td>
</tr>
<tr>
<td>POPC:Sol (2 mol % solanesol)</td>
<td>462</td>
</tr>
<tr>
<td>POPC:Q10 (2 mol % Q10)</td>
<td>483</td>
</tr>
<tr>
<td>POPC:Q10 (3.3 mol % Q10)</td>
<td>521</td>
</tr>
</tbody>
</table>

3.2 Paper II: Ubiquinone-10 in liposomes modelling the inner membrane of mitochondria

Our previous findings based on investigations with POPC membranes showed that the inclusion of Q10 has a general membrane stabilizing effect. Even though POPC lipids provide a biologically relevant model, biological membranes generally consist of several different lipids (and proteins, etc.) which could have effects on membrane properties, such as membrane bending rigidity and lipid spontaneous curvature. Also, previous molecular dynamics simulations indicate that the ubiquinone-lipid interaction varies depending on the lipid species (71, 72). This led us to explore the effect of Q10 in a more complex lipid system.
3.2.1 Design of model system

Since one of Q10s most important known functions is to participate in the energy production of the cell, the highest membrane concentrations can be found in the inner membrane of the mitochondria, especially in cells with high rates of metabolism, such as heart, kidney and liver cells (73). Therefore, the next step of investigating the effect of Q10 in membranes was to use a mixture of lipids resembling the lipid composition of the inner membrane of the mitochondria.

Since no extracts separating the inner and outer membrane of the mitochondria were available, we composed a lipid mixture with the composition POPE: POPC: CL: Soy-PI: Soy PS (49.2: 43: 6.1: 1: 0.7), denoted IMM. This composition mimics the lipid headgroup composition and the ratio of unsaturated and saturated fatty acid chains of the inner membrane of rat liver mitochondria (33, 74). Characterizing the IMM mixture by calorimetry confirmed a liquid crystalline phase at room temperature ($T_m \sim 16 \degree C$), and cryo-TEM investigations showed the possibility to produce liposomes. This led us to proceed with the IMM lipid mixture throughout Paper II.

3.2.2 Liposome permeability and spreading on silica surfaces

By monitoring how IMM liposomes (IMM, IMM:Q10 and IMM:Sol) attach and spread on silica surfaces, differences in membrane rigidity could be discerned. The results showed that both IMM liposomes and IMM:Sol liposomes attach and spread on silica surfaces, similar to the case with POPC liposomes. IMM:Q10 liposomes, on the other hand, attached and spread only partially, which resulted in intact liposomes remaining on the surface after rinsing.

Concerning the IMM and IMM:Sol results, mass densities could be calculated and were determined as 488 ng/cm$^2$ (IMM) and 528 ng/cm$^2$ (IMM:Sol). Interestingly, solanesol seems to have a membrane condensing effect in the IMM membrane. Also, the density of pure IMM is higher than that of the pure POPC membrane. In the Q10 case, the attachment and rupture processes were not sufficiently separated to enable mass density estimation for IMM:Q10. However, the incomplete liposome spreading indicates that the incorporation of Q10 increases the mechanical stability of IMM.

To further understand the effect of Q10 in the IMM model, spontaneous and surfactant-mediated leakage was monitored for the IMM liposomes. The spontaneous leakage results (Figure 20a) showed that not only Q10 but also solanesol have a leakage reducing effect. IMM liposomes were also shown to have a generally slower leakage than POPC liposomes. Studies monitoring surfactant-induced leakage (Figure 20b) revealed a similar trend to the spontaneous leakage experiments, although in this case solanesol had a somewhat stronger membrane protecting effect than Q10. The effects on
leakage due to the incorporation of Q10 or solanesol could not be attributed
to differences in surfactant partitioning between the membranes (for details
concerning partitioning see Paper II).

Given that both Q10 and solanesol have a similar membrane protecting
effect in IMM, the results suggested that the quinone moiety was not essen-
tial for reducing the leakage rate. It was thus reasonable to assume that it is
the presence of a long hydrophobic chain in the membrane that produces the
effect. To test this hypothesis, further surfactant-induced experiments were
performed with liposomes containing hexadecane. The results (Figure 20)
showed a leakage reducing effect with the inclusion of hexadecane, thus
confirming that a long hydrocarbon chain is sufficient to obtain a leakage
reducing effect in IMM liposomes.

3.2.3 Effect of solanesol and possible membrane locations of
Q10

The leakage protecting effect caused by the presence of solanesol in the hy-
drophobic membrane core is most likely related to the presence of an intrin-
sic curvature elastic stress in the IMM model membrane. This intrinsic stress
in the membrane is most likely linked to the presence of the two lipids POPE
and CL, which constitute a large part of the IMM model. These lipids have a
propensity to form structures with negative spontaneous curvature (75). By
forcing POPE and CL into membranes with zero or even positive curvature,
membrane strain would therefore arise. A further strain, promoting negative
curvature, is expected from the inclusion of large molecules, such as solane-
sol, in the hydrophobic core. Since CF leakage mainly occurs through transi-
ten pores (76, 77), in which structures with high curvature are formed, it can
be safely assumed that the leakage rate would be suppressed by factors pro-
moting negative curvature.

From the results, it is quite possible that a proportion of Q10 is located in
the hydrophobic core of the membrane, considering that the leakage protect-
ing effect is similar to the one obtained with solanesol. However, in the IMM
membrane, some Q10 molecules must also be located with the quinone moi-
ety closer to the lipid headgroups (as believed for POPC membranes), given
that an increase in lipid order is obtained. Previous experiments, both empir-
ical and molecular dynamics simulations, support the idea that Q10 could
have different membrane locations, where the location depends on the sur-
rounding lipid components and lateral pressures in the membrane (71, 72,
78, 79). The suggested mechanisms by which Q10 decreases the CF release
in IMM membranes are summarized in Figure 21.
Figure 20. Leakage recordings for different IMM samples: a) Spontaneous leakage and b) Surfactant induced leakage. The mixing ratios were 25:1 for IMM:Q10 and IMM:Sol, whereas for IMM:hexadecane it was 13:1.
3.2.4 Roles of the major lipid components in the IMM model

To explore the roles of the major lipid components in the IMM membrane, further experiments were performed. Anisotropy and cryo-TEM studies revealed that CL had a disordering effect in POPC membranes, and that pure CL membranes tended to be very soft and flexible. On the other hand, studies concerning POPE showed that POPE had an ordering effect on POPC membranes, and that the membranes produced tended to be very rigid (for detailed results, see Paper II). Together with the results from studies monitoring surfactant-induced leakage in simplified systems where CL or POPE were excluded (Figure 22), it was concluded that POPE is the component in IMM which is responsible for a changed leakage trend compared with that seen with POPC. In other words, POPE is required for solanesol to exert a leakage reducing effect similar to or stronger than that of Q10. The effect of POPE could be attributed to significant hydrogen bonding between PE headgroups, which would induce a high lateral membrane pressure and therefore propel Q10 or similar hydrophobic molecules to the center of the bilayer. Interestingly, it was also seen from the surfactant-induced leakage experiments that the inclusion of CL further increased the resistance against surfactant action. The explanation for this is likely coupled to CL’s propensity to promote structures with negative curvature, which would in turn reduce the formation of structures with positive curvature (e.g. transient pores).
Figure 22. a) Surfactant-induced leakage in a simplified IMM composition without CL and b) surfactant-induced leakage in simplified IMM composition without POPE. The lipid:Q10 and lipid:Sol ratios were 25:1 in all cases (see Paper II for more details).
3.3 Paper III: Osmo-protective effect of ubiquinone in liposomes modelling the plasma membrane of *E.coli*

Previous reports revealed that *E.coli* metabolically adapts when grown in or exposed to hyperosmotic conditions. This adaption includes, among other things, a substantial increase in ubiquinone-8 (Q8, the bacterial version of Q10) (25, 80). This led us to explore the osmo-protective effect of ubiquinones in lipid membranes mimicking the plasma membrane of *E.coli* bacteria.

3.3.1 Design of model system

Since no extract separating the plasma membrane from the outer membrane in *E.coli* was available, membranes with POPE, PG(*E.coli*) and CL(*E.coli*) (molar ratio of 75:19:6, denoted BM), were studied. The lipids in the BM mixture mimic the headgroup composition of the cytoplasmic membranes of *E.coli* bacteria (81) and also the naturally occurring presence of cyclopropane fatty acids is accounted for by using bacterial PG and CL.

For further quantitative investigations, another bacterial model membrane consisting of POPE, POPG and CL(heart, bovine) (75:19:6, denoted BMM), was used. The headgroup composition of the BMM mixture also reflects the one in the plasma membrane of *E.coli*, but the mixture has lipids with well-defined fatty acid substitutions. Pure POPC-based liposomes were included in the studies, since the properties of their membranes were well characterized in *Paper I*.

Cryo-TEM investigations confirmed the possibility of forming liposomes with both the BMM and the BM lipid composition (*Figure 23* and *Figure 24*) and calorimetric investigations showed that it was possible to perform all experiments at 25 °C and still remain in the liquid crystalline phase ($T_{m}(BMM) \sim 20.5$ °C and $T_{m}(BM) \sim 18$ °C).

*Figure 23*. Cryo-TEM image confirming BMM-liposomes. The scale bar is 100 nm.
3.3.2 Verifying the osmo-protective effect

As a first step, there was a need to verify the osmo-protective effect of ubiquinones in our BM system. Liposomes, before and after being subjected to osmotic stress, were studied by cryo-TEM analysis (Figure 24). The micrographs revealed that the unstressed samples were dominated by spherical unilamellar liposomes, but also contained some distorted/flattened unilamellar liposomes and a small population of multilamellar/multivesicular structures. After the addition of 50 mM salt, the proportions of the different structures changed and a general increase in the population of flat liposomes was seen. The micrographs thus revealed that the main way for BM liposomes to react to osmotic stress was by releasing water and forming flattened liposomes. This is in contrast to the most common osmotic stress response of liposomes, which is to form invaginations (see Figure 12). Images of samples containing Q8 and Q10 were studied (by classifying and counting structures) and compared to images obtained using pure BM lipids. The proportion of multilamellar/multivesicular liposomes was seemingly constant for all samples before and after osmotic shock. Differences could be seen in the populations of spherical and flat liposomes. There was a 40 \% drop in the proportion of spherical unilamellar liposomes in the Q10 sample and a corresponding drop of 56 \% in the Q8 sample, due to osmotic shock. This was in contrast to the 65 \% drop in the population of spherical liposomes observed in the pure BM sample. The results indicate that both Q8 and Q10 have an osmo-protective effect in the BM model. To get quantitative results, further investigations were needed.

![Figure 24. Representative cryo-TEM images showing BM samples a) before and b) after osmotic shock. Images were obtained for a) BM:Q10 (1.6 mol % Q10) liposomes in an isotonic solution, and b) BM liposomes subjected to osmotic shock with 50 mM excess NaCl. The labels denote the different structures observed: S = spherical unilamellar liposomes; M = Bi- or multilamellar/multivesicular liposomes, and C = collapsed unilamellar liposomes. The latter can be observed at different orientations where top and sideways views are denoted by white and black labels, respectively. The scale bar is 100 nm.](image-url)
3.3.3 Quantifying the osmo-protective effect

To quantify the osmo-protective effect of ubiquinones, water permeability coefficients were determined \( (P_l) \) (Figure 25). For the BM model, variations between experiments were larger than the difference observed with Q8 \( (P_l = (4.13 \pm 0.23) \times 10^{-3} \text{ cm s}^{-1}) \), Q10 or solanesol. The observed variations were partly attributed to the heterogeneous fatty acid composition present in the bacterial extracts of PG and CL lipids. This variety of fatty acids can result in a heterogeneous liposome composition, causing variations in the overall permeability. Therefore, further experiments were performed with BMM membranes (and POPC membranes), with more well-defined lipids. Q10 was preferred over Q8 since its length fits better with the fatty acid chains of the lipids in the BMM model and it also allowed comparison with the results in Papers I and II.

A generally higher osmotic water permeability coefficient was found for pure BMM membranes than for POPC membranes. This increase can be attributed to the headgroups in BMM (PE, PG and CL) which have the possibility to form hydrogen bonds with water and can thus promote water adsorption onto, and transport through, the membrane. A decreased permeability coefficient was obtained when Q10 was included in both BMM and POPC membranes, showing that there is a quantifiable osmo-protective effect of Q10. When solanesol was included, the permeability coefficient was not significantly affected in any of the models tested, thus suggesting that the quinone moiety is important for the osmo-protective effect.

![Figure 25. Water permeability coefficients \( (P_l) \) for different lipid compositions. The Lipid:Q10 and Lipid:Sol ratios are 25:1 in all cases. * Indicates a significant difference according to an unpaired \( t \)-test at a 95 % confidence level.](image)
To investigate potential correlations between permeability and lipid order, fluorescence anisotropy measurements were performed. The results showed that Q10 has an ordering effect in BMM membranes (for more details, see Paper III). Compared to the previous investigations in Paper I, a higher general order in BMM membranes was observed than in POPC membranes. The magnitude of the ordering effect of Q10 was however a little lower in the BMM model compared with the POPC model. No clear correlation could be seen between the permeability and lipid order in the BMM system however, suggesting that differences in water permeability could not be attributed to membrane order only. In POPC membranes however, a correlation between permeability coefficients and membrane order was observed. This suggests that the osmo-protective effect of Q10 occurs via different mechanisms depending on the membrane composition.

3.3.4 Resistance against liposome deformation during osmotic shock

Usually when studying the water permeability of membranes, the fact that liposomes can present a resistance to deformation is normally not considered. Ideally, the final relative volumes of liposomes $X(\infty)$ (after osmotic shock) should be equal to the ratio between the inner and outer osmolarity ($OsC_{in(0)}/OsC_{out}$) at the starting point. By evaluating and comparing $X(\infty)$ with $OsC_{in(0)}/OsC_{out}$ for the BMM and POPC systems, further information concerning membrane resistance to osmotic shock were revealed (Figure 26).

As expected for soft membranes, no resistance to deformation was found for pure POPC liposomes with low quantities of added salt (50 mM excess). For high salt concentrations (250 mM excess) some resistance could be seen. This was attributed to the membrane’s resistance to keep the liposome from collapsing completely. This resistance arises after an already large liposome deformation.

The somewhat unexpected BMM results showed that there is a certain resistance to deformation only at low salt concentrations. At high salt concentrations the liposomes seem to shrink beyond what is necessary to achieve isoosmolar conditions, implying that the elastic tension in the membrane (surprisingly) promotes deformation. When Q10 and solanesol are included in the BMM membrane, the results showed that there was a resistance to membrane deformation at high salt concentrations. This suggests that both Q10 and solanesol have an osmo-protective effect in BMM which is separate from the effect on the permeability coefficient. However, and in contrast, no membrane opposing effect of Q10 or solanesol could be detected in POPC liposomes.
Taken together with the permeability results, the incorporation of Q10 seems to provide osmo-protection by affecting both the osmotic water permeability and the ability of the membrane to deform. The extent of each mechanism seems to depend on the lipid composition in the membrane.

Figure 26. Comparison between final relative volumes after osmotic shock \((X(\infty))\) and the initial inner and outer \((O_{\text{in}}/O_{\text{out}})\) osmolarity ratios for different lipid compositions, at two concentrations of excess salt.

3.3.5 Possible explanation for the osmo-protective effect of Q10

The promoted deformation of BMM liposomes, seen as a reaction to high osmotic stress, most likely relates to the presence of charged lipids (19% POPG and 6% of the double charged CL) and the propensity of POPE and CL to form structures with negative curvature (75). After subjecting BMM liposomes to osmotic shock, a screening of the headgroup repulsion between charged lipid headgroups leads to a more negative lipid spontaneous curvature. Liposomes treated this way may accommodate the excess area by flattening instead of forming invaginated structures when water flows out. The large population of flattened liposomes/oblate structures formed after osmotic shock, observed by cryo-TEM, supports this theory. During the membrane flattening process, it is also feasible to consider a possible lipid segregation in the membrane, where the curved parts would be enriched with POPG and CL (giving soft membranes), and the flat parts would contain primary POPE (giving rigid membranes). Enrichment with CL is supported by previous studies showing that CL accumulates in the curved poles of bacterial cells (34).
A possible explanation for the osmo-protective effect of Q10 in BMM can be extrapolated from the assumption that lipids segregate when salt is added. In Paper II it was concluded that the solubility of Q10 and solanesol in POPE was very low. Other experiments showed that it was possible to include high concentrations of Q10 (up to 15%) in CL membranes and that Q10 slightly promotes the formation of negative curvature structures in CL membranes (see supporting material in Paper III). It is therefore possible that Q10 (and solanesol) would accumulate in the CL-rich curved parts of the flattened liposome, thus preventing the liposomes from deforming further. Figure 27 illustrates the proposed lipid and Q10 segregation due to osmotic stress.

The osmo-protective effect of Q10 can therefore be described by two complementary mechanisms: 1) decreased water permeability as a result of an increased lipid packing order and 2) an increased elastic stress-induced pressure opposing liposome deformation and therefore also water flow. The latter mechanism is independent of the quinone moiety, as seen from the results with solanesol containing liposomes.

Figure 27. Visualization of the possible rearrangement of lipid and Q10 molecules arising from osmotic shock in the lipid model mimicking the E.coli plasma membrane.
3.4 Paper IV: Influence of cuvette material on spectroscopic leakage experiments

Throughout the membrane investigations in Papers I and II, liposome leakage experiments were performed by studying the leakage of the fluorescent hydrophilic dye carboxyfluorescein (CF). During the data collection process, unexpected discrepancies between experiments were seen when using different cuvette materials. Generally, when performing spectroscopic measurements in the visual range, it is assumed that the resulting data should be independent of the material used, since most materials show good transparency in that range. Quartz cuvettes are frequently preferred over plastic ones due to their high chemical stability and low surface roughness. However, the fact that liposomes can attach, and in some cases rupture, on hydrophilic surfaces is seldom considered when performing and interpreting spectroscopic measurements with liposomal samples.

3.4.1 Leakage results affected by the choice of cuvette material

As can be seen from the leakage recordings for POPC and IMM liposomes (membrane model mimicking the inner mitochondrial membrane, Paper II), performed with both quartz and polystyrene cuvettes, there were large discrepancies between the leakage profiles (Figure 28). For both samples, the overall leakage was faster when using a quartz cuvette. Also, concerning the results with quartz, an initial slow leakage process could be seen in the first minutes for POPC (see inset i in Figure 1a in Paper IV) and for the first ~2.5 h for IMM.

Since it was only the cuvette material which distinguished the experiments, further studies following liposomal interaction on silica and polystyrene surfaces were done with QCM-D (Figure 28c-f). From these results it was observed that the liposomes studied interact with silica surfaces by attaching and spreading onto the surfaces. For IMM liposomes, a larger critical concentration before rupture than for POPC liposomes was seen. On the other hand, no interactions between liposomes and the polystyrene surface were observed.

From the leakage and the QCM-D results, it was concluded that leakage curves obtained with polystyrene cuvettes ought to be the least disrupted and most correct since no significant liposome-surface interaction was seen. An explanation for the leakage results seen with quartz cuvettes was proposed as follows. The initial slow leakage was attributed to the accumulation of liposomes on the cuvette surface, since adsorbed intact liposomes have previously been shown to leak more slowly than liposomes free in solution (64). The increase in leakage thereafter was suggested to be a result of liposomes rupturing on the surface, thus releasing their whole CF content at once. After
complete surface coverage by ruptured liposomes, the remaining liposomes should leak in an undisrupted spontaneous way.

For the POPC sample, the mechanism well describes the results since after 2.5 h, the leakage profiles in quartz and polystyrene cuvettes are very similar. For the IMM sample, an additional mechanism must be involved, since the fast leakage rate obtained in quartz cuvettes persists for a longer period, probably related to the larger critical concentration of liposomes needed for liposome rupture. Results related to this additional mechanism are discussed in the next section.

Additional leakage and QCM-D experiments with a lipid composition of POPE:CL (94:6), showing no difference between the results with quartz and polystyrene and no liposome-surface interaction with any of the materials (see Paper IV), confirmed the correlation between the liposome-cuvette surface interactions and altered leakage curves.

3.4.2 Effect of magnetic stirring
The insight about possible further mechanisms affecting liposome leakage (IMM results) together with results from DPPC liposomes (see Paper IV), showing that the attachment of liposomes with no rupturing process also affects the leakage profile, led to investigations concerning the effect of magnetic stirring. By comparing the leakage from DPPC liposomes at certain points in time for quartz and polystyrene cuvettes, with and without stirring, it was clear that the stirring process increased the leakage rate in quartz cuvettes (Figure 29). The increase is likely explained by a mechanical, stirring-induced, rupturing of liposomes attached to, or close to, the cuvette bottom. This effect is likely the reason for the unexplained leakage increase for IMM liposomes described in the previous section, where liposomes were seen to accumulate to some extent before spreading over the surface. Consequently, two processes were identified as affecting the leakage in quartz cuvettes: liposome adhesion and spreading on the cuvette surface and mechanical rupturing of adhered liposomes by magnetic stirring.
Figure 28. Liposome CF leakage performed with polystyrene and quartz cuvettes for a) POPC liposomes and b) IMM-liposomes, and QCM-D experiments with c) POPC on silica, d) POPC on polystyrene, e) IMM on silica and f) IMM on polystyrene.
Figure 29. Effect of magnetic stirring on DPPC liposome leakage. Quartz cuvette with magnetic stirring – filled squares, quartz cuvette with no magnetic stirring – empty diamonds, polystyrene cuvettes with magnetic stirring – filled triangles and polystyrene cuvettes with no magnetic stirring – empty circles.
4. Concluding remarks

The results presented in this thesis show that ubiquinone-10 has a general stabilizing effect on lipid membranes. This effect includes promoting an increased lipid order and packing, a decreased permeability to hydrophilic solutes and water, and an increased resistance to liposome deformation and liposome rupturing on surfaces. This membrane stabilization can be compared to the membrane effects of cholesterol, although the mechanisms behind may differ. Also, results concerning liposome-cuvette interactions ensured the reproducibility of the experiments, which facilitated the investigations of Q10 membrane effects.

It was shown that the extent of the membrane stabilizing effect of Q10, and how it is achieved, depends on the membrane composition. For example, a decreased leakage rate was found to be related only to an increased lipid packing order for the POPC system, while in the lipid composition mimicking the inner mitochondrial membrane a decreased leakage was also related to other parameters. Further, the increased resistance against osmotic shock mediated by Q10 was shown as decreased osmotic water permeability and increased resistance against liposome deformation for membranes mimicking the plasma membrane of *E. coli*, while only osmotic water permeability was affected by the inclusion of Q10 in POPC membranes. These results agree with a composition-dependent location of Q10 in the membrane and may explain the non-unified view concerning Q10 membrane location. Additionally, liposome-surface interactions were also shown to be strongly dependent on the membrane composition. Taken together, this demonstrates that trends cannot always be generalized concerning lipids and lipid membranes.

The results presented in this thesis provide an overall picture of the effect and behavior of Q10 in lipid membranes. Future supplementary experiments, with NMR and/or IR spectroscopy for example, could be useful to help verify the results presented here. Also, studies of the effects of the reduced form of Q10, ubiquinol, would be valuable, since a large proportion of Q10 exists as ubiquinol in biological membranes. Furthermore, to increase the understanding of Q10’s effect on the inner mitochondrial membrane and the plasma membrane of gram negative bacteria, the models could be further developed. This could be done by considering proteins and other membrane components that could have effects on membrane properties, both in themselves and possibly in synergy with Q10.
Svensk sammanfattning

För att en cell ska fungera måste det finnas något som skiljer cellen från dess omgivning, d.v.s. det måste finnas ett cellmembran (Figur 1). Utöver det yttre hölje som skyddar cellen så finns det även membran inuti cellen som separerar organellerna, cellens organ, från omgivningen. Membranen har till uppgift att verka både som barriärer och som förankringsytor för många av cellens proteiner. Dessa proteiner deltar i livsviktiga processer i cellen, till exempel i energiproduktionen. Vissa små molekyler, som vatten, kan enkelt passera cellens membran. Större molekyler (exempelvis socker och aminosyror) och joner (salter) måste ta hjälp av membranbundna proteiner för att kunna transporteras genom membranet.

Grundstommen i cellens membran består av ett dubbelskikt av en speciell sorts molekyler som har amfifila egenskaper. Det betyder att de innehåller både en fettlöslig (hydrofob) och en vattenlöslig (hydrofil) del (Figur 1). Eftersom miljön i cellen till största del består av vatten så kommer dessa amfifila molekyler att arrangera sig på ett sätt så att de hydrofila delarna är riktade mot vattnet. Detta för att de hydrofoba delarna helst inte vill blanda sig med vatten, precis som att olja och vatten inte gärna blandar sig. Det finns väldigt många olika geometriska konstellationer som amfifila molekyler kan arrangera sig i när de befinner sig i vatten. Hur de arrangerar sig beror på molekylenas egenskaper. De vanligaste amfifila molekylenas i cellens membran kallas polära lipider och de aggererar sig helst i formen av ett dubbelskikt, även kallat bilager. I bilagret kan de flesta lipiderna förflytta sig inom det lager, eller skikt, som de befinner sig i, vilket gör att membranet ofta kan räknas som ”flytande” i två dimensioner.

Utöver proteiner och polära lipider finns även andra fettlösliga molekyler i cellmembran, till exempel kolesterol. I ett lipidmembran sätter sig kolesterolmolekylerna mellan lipidmolekylen och gör så att lipiderna blir mer utsträckta och packar sig tättare. Detta gör att membranet blir mer täthet/sträckstarkt och därför räknas kolesterol som en molekyl som kan stabilisera membran.

En annan fettlöslig molekyl som finns i de flesta cellmembran är ubikinon. Ubikinon finns i många varianter och den som är vanligast i människor är ubikinon-10 (Q10). I membranen så skyddar den bl.a. lipiderna mot oxidation. I det inre av mitokondriens (en av cellens organellerna) två membran förekommer Q10 i särskild hög halt. Anledningen till detta är att Q10 deltar i cellens energiproduktion och denna process sker i mitokondrien. Det finns
vanligen väldigt lite kolesterolmolekyler i de membran som innehåller mycket Q10. Även i vissa bakteriemembran finns det relativt höga halttrt av ubikinon. Därför har det övergripande målet med den här avhandlingen varit att studera om Q10, utöver de egenskaper man redan känner till, även har en membranstabiliserande effekt.

Figur 1. Schematisk bild av ett cellmembran, en polär lipid och en liposom.

För att kunna studera Q10s inverkan på membranens egenskaper måste man använda sig av ett lämpligt modellsystem. En vanlig metod för detta är att använda liposomer som modellmembran. Liposomens struktur kan liknas vid en ihålig fettpartikel där höljet består av ett membran av lipider (Figur 1). Med denna typ av struktur är det möjligt att skräddarsy vad membranet består av och därmed är det möjligt att undersöka effekten av exempelvis Q10.
I delarbete I så studerade vi hur Q10 fungerar i membran bestående av endast en lipidsort (POPC lipider). Denna lipid ger ett enkelt och väldefinierat system där membranegenskaperna kan jämföras med de som cellmembran har. Resultaten visade att en tillsats av Q10 i membranet ger en generell membranstabiliserande effekt.

I delarbete II så utvecklade vi membranmodellen till att likna lipidsammansättningen i det inre mitokondriemembranet. Resultaten visade att också i detta membran så hade Q10 en membranstabiliserande effekt. Resultaten visade också att upphovet till den membranstabiliserande effekten hos Q10 varierade med lipidsamman- sättningen i membranet.

I delarbete III så studerade vi membran som liknande ett av de membran som omger E.coli bakterier. Målet med detta arbete var att studera om Q10 har någon inverkan på ett membrans reaktion vid osmotisk chock, d.v.s. vid yttre påfrestningar av hög saltkonzentration. Resultaten visade att Q10 minskar genomläppligheten av vatten och ökar motståndet mot membraneformation vid osmotisk påfrestning.

Delarbete IV gjordes parallellt med delarbete I och II. Syftet med detta arbete var att undersöka några speciella experimentella resultat som uppstått i de andra delarbetena. Resultaten från denna studie visade att liposomerna i vissa fall kan interagera med provhållmaterialet (kyvettmaterialet) vid spektroskopisk analys på ett sätt som kan påverka de experimentella resultaten.
Acknowledgements


Tack till min biträdande handledare Victor för att du har lärt mig massor, för att du alltid ser det positiva i allt och för att du har orkat förklara saker om och om igen. Tack också för ett bra samarbete på labb och för alla skratt!

Tack till Karin, Sara A, Amelie och Malin, för att ni gav mig massor av livskunskap under många och långa fikastunder! Jag var mer än förberedd på livet som förälder när Alice kom =). Tack Anna för all hjälp i början av min doktorandtid.

Tack Jonny, för att du lärde mig att köra kryo-TEM och för all hjälp som jag har fått med kryo-bilder och annat fix och trix som behövts! Tack Philipp, Lars och Sara L för all hjälp under slutfasen av min doktorandtid.

Tack till alla mina vänner och släkt som har funnits där genom åren och hejat på! Tack Lisa, som har alltid har lyssnat på mig och stöttat mig i mitt arbete, även om det jag gjort kanske inte känns särskilt begripligt.

Ett stort tack till både mina föräldrar och mina svärföräldrar, som inte bara har stöttat mig genom åren, utan som även har gjort det möjligt för mig, genom barnpassning och annan hjälp, att slutföra min avhandling! Tack Mamma och Pappa för att ni också har lagt grunden för att jag ska kunna uppnå mina mål!

Tack Micke och Alice, för att ni har stått ut med mig under tiden jag skrivit min avhandling. Och tack för att ni finns och gör livet lite mer livat, ni är det bästa jag har!
References


A doctoral dissertation from the Faculty of Science and Technology, Uppsala University, is usually a summary of a number of papers. A few copies of the complete dissertation are kept at major Swedish research libraries, while the summary alone is distributed internationally through the series Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Science and Technology. (Prior to January, 2005, the series was published under the title “Comprehensive Summaries of Uppsala Dissertations from the Faculty of Science and Technology”.)