

Localization of coenzyme Q₁₀ in the center of a deuterated lipid membrane by neutron diffraction

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Abstract

Quinones (e.g., coenzyme Q, CoQ₁₀) are best known as carriers of electrons and protons during oxidative phosphorylation and photosynthesis. A myriad of mostly more indirect physical methods, including fluorescence spectroscopy, electron-spin resonance, and nuclear magnetic resonance, has been used to localize CoQ₁₀ within lipid membranes. They have yielded equivocal and sometimes contradictory results. Seeking unambiguous evidence for the localization of ubiquinone within lipid bilayers, we have employed neutron diffraction. CoQ₁₀ was incorporated into stacked bilayers of perdeuterated dimyristoyl phosphatidyl choline doped with dimyristoyl phosphatidyl serine containing perdeuterated chains in the natural fluid-crystalline state. Our data show CoQ₁₀ at the center of the hydrophobic core parallel to the membrane plane and not, as might be expected, parallel to the lipid chains. This localization is of importance for its function as a redox shuttle between the respiratory complexes and, taken together with our recent result that squalane is in the bilayer center, may be interpreted to show that all natural polyisoprene chains lie in the bilayer center. Thus ubiquinone, in addition to its free radical scavenging and its well-known role in oxidative phosphorylation as a carrier of electrons and protons, might also act as an inhibitor of transmembrane proton leaks.

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1. Introduction

Redox-active quinones with long polyisoprenoid chains like coenzyme Q are ubiquitous in eukaryotes. They fulfill two established functions as electron/proton carriers in bioenergetic membranes and as antioxidants [1]. Since all of the organelle membranes that contain them are exposed to H⁺ gradients, they may also serve as inhibitors of H⁺ leakage [2].

Ubiquinone (CoQ₁₀) is a 50-carbon polyisoprene with a terminal quinone domain. It is synthesized in all of the mammalian organelle membranes investigated [3]. It is essential for ATP synthesis in mitochondria as a carrier of electrons and protons in oxidative phosphorylation and in other proton-pumping membranes, presumably by the Q-cycle [4].

The structurally and functionally very similar plastoquinone is employed in photosynthetic membrane systems. The function of the polyisoprenoid domain of CoQ₁₀ is generally assumed to anchor the quinone to the hydrophobic domain of the bilayer. Nonetheless, a short aliphatic chain has been shown to suffice for this purpose in vitro [5].

The localization of CoQ₁₀ within membranes has been the subject of many studies [6,7]. The studies, using NMR spectroscopy [5,8,9], fluorescence [10], differential infrared spectroscopy [11], linear dichroism [12], and differential scanning calorimetry [13] have concluded with differing and often conflicting interpretations.

It has recently been established that squalane, an analogue of squalene, which appears to inhibit proton leakage across alkaliphile membranes [14], lies in the bilayer center of phospholipid bilayers [15]. The “water cluster” model [2] for proton leakage across lipid bilayers suggests that hydrocarbons in the center of straight chain lipid bilayers may serve as an inhibitor of proton leakage in addition to any other functions it

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may have in the cell. Thus, quinone polyisoprenoids may inhibit proton leakage in addition to their other biological roles.

Neutron diffraction is ideal and the most direct tool for localizing molecular building blocks in lipid bilayers. Due to the large difference in the coherent scattering length b of a proton ($b_H = -3.74$ fm) and a deuteron ($b_D = +6.67$ fm) an ideally isomorphous replacement can be achieved. In this study, a method is applied in which the membrane constituent CoQ₁₀ is protonated and the bilayer lipids are deuterated. Using this approach we report that CoQ₁₀ is resident in the center of perdeuterated dimyristoyl phosphatidyl choline (DMPC) bilayers doped with 5 mol% dimyristoyl phosphatidyl serine (DMPS) containing perdeuterated chains.

2. Materials and methods

Perdeuterated dimyristoyl phosphatidyl choline (*d*DMPC, 67 deuterons, 98% purity) and the net negatively charged lipid dimyristoyl phosphatidyl serine containing perdeuterated chains (*d*DMPS, 54 deuterons, 98% purity) were purchased from Avanti Polar Lipids, Alabaster, AL. Non-deuterated ubiquinone (89% minimum) was obtained from Sigma Chemicals.

2.1. Preparation of stacked bilayers

Chloroform solutions containing *d*DMPC/*d*DMPS (9:1 mol/mol) with or without an additional 5 mol% perprotonated ubiquinone were prepared. The total lipid weight for each preparation was 10 mg. Oriented samples were prepared as described [15]. Dry samples were then rehydrated for 24 h at room temperature in an atmosphere of 98% relative humidity, maintained with a saturated K₂SO₄ solution.

2.2. Neutron diffraction

Neutron diffraction measurements were carried out on the membrane diffractometer V1 at the Berlin Neutron Scattering Center of the Hahn-Meitner-Institute, Berlin (Germany) with a neutron wavelength of $\lambda = 0.452$ nm in thermostated aluminum cans at 98% relative humidity. The procedure is described in detail elsewhere [15]. Briefly, the diffraction intensities were measured at four different D₂O/H₂O contrasts (100:0, 80:20, 50:50, and 0:100) at $T = (25 \pm 0.1)$ °C with rocking scans around the expected Bragg position θ by $\theta \pm 2^\circ$. Up to 6 orders were detected for each sample. Diffraction patterns of *d*DMPC/*d*DMPS bilayers were examined with or without 5 mol% protonated ubiquinone. The measured intensities were scaled with the integrated scattering length of the water layer using the known differences in scattering length of subsequent D₂O/H₂O ratios. The D₂O/H₂O contrast variation also facilitates the phase assignment [16]. The square roots of the scaled intensities, corrected with absorption and Lorenz factors, produced the structure factor amplitudes. The scattering length density profile $\rho(z)$ is given by:

$$\rho(z) = \rho_0 + \frac{2}{d} \sum_{h=1}^n \pm F(h) \cos\left(\frac{2\pi h z}{d}\right)$$

where ρ_0 is the total scattering length density of the bilayer, $F(h)$ are the scaled structure factors and d the unit cell length. For more details to evaluate the localization of labels in biological membranes, see, e.g., [17,18].

3. Results

The rocking scans around the first order peaks display a mosaicity inferior to 0.5 degrees demonstrating the excellent quality of the samples. As already reported [19], few small yellow droplets of ubiquinone are expelled from the lipid bilayers, but do not disturb the alignment of the membranes.

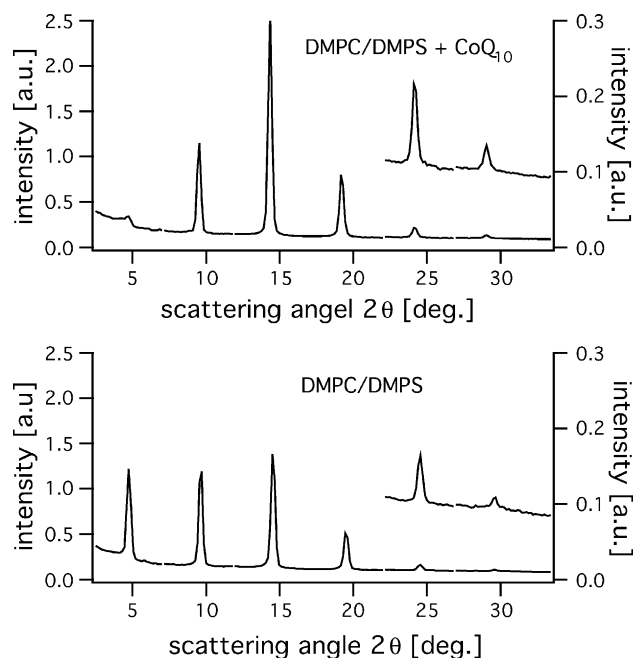


Fig. 1. Diffraction patterns of the stacked bilayers of *d*DMPC/*d*DMPS (9:1 mol/mol) sample at 25 °C with and without protonated ubiquinone at D₂O/H₂O=100:0 contrast. Up to six orders are visible. In particular, at 100% D₂O content, the diffraction pattern of the sample containing deuterated lipids and protonated ubiquinone is dominated by the third order peak.

Fig. 1 shows the diffraction patterns obtained from the two samples containing, or not containing, protonated ubiquinone at 100:0 D₂O/H₂O contrast. Up to six diffraction orders were recorded for each sample with pronounced relative intensity changes between the samples. The measured *d*-spacing for the samples were equal to (5.28 ± 0.04) nm for the pure lipid sample and (5.38 ± 0.04) nm for the lipid sample with the ubiquinone, respectively, suggesting no change in the membrane thickness. Profiles of the scattering length densities (i.e., the membrane profiles) are calculated as the Fourier sum of the structure factors. The scattering length density profiles for the deuterated lipid sample with protonated ubiquinone are illustrated for the various D₂O/H₂O atmospheres in Fig. 2, demonstrating the effect of the contrast variation. The unit cell is chosen so that the midplane of the lipid membrane is in the center and the water layers are at its outer edges. At 0% D₂O, the contrast between the water layer and the lipid membrane is largest, reflecting the negative scattering length density (sld) of H₂O (-0.56×10^{10} cm⁻²) and the positive average scattering length density of the deuterated lipids at about $+5.9 \times 10^{10}$ cm⁻². At 100% D₂O contrast the scattering length densities of lipids and water nearly match (sld(D₂O) = 6.33×10^{10} cm⁻²) and the scattering density profile reflects smaller local changes in this entity. The scattering length density profiles for the two samples with and without ubiquinone in an atmosphere of 100% D₂O are shown in Fig. 3 together with the corresponding difference density profile. The protonated ubiquinone shows up as a negative density difference due to the negative scattering length of the protons. The difference densities between the samples with and without the ubiquinone for all four measured contrast ratios are displayed in Fig. 4 and reflect its location in

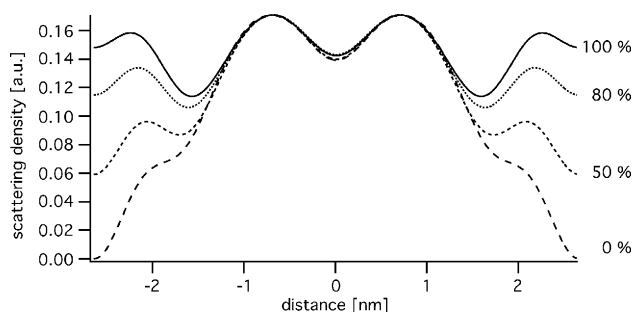


Fig. 2. The effect of the contrast variation on the calculated density profiles are shown in the case of the deuterated lipid membrane containing the protonated ubiquinone. The decreasing D_2O content from 100% to 0% in the water composition gives rise to a decrease in the scattering length density in the aqueous layers of the membrane stack. The scattering length density in the center of the bilayer is not effected in the experimental error limit.

the bilayer center. In Fig. 4, this difference is clearly seen in the middle of the lipid bilayer. The experimental noise level due to counting statistics and Fourier truncation errors are reflected in the wavy features at the edges of the profiles. An artist view reflecting our neutron diffraction result is shown in Fig. 5, ubiquinone (dark gray) with its quinone ring is located in the center of the lipid bilayer near the terminal methyl groups, with minimal penetration into the acyl chain region.

4. Discussion

The aim of the present study is the unequivocal determination of the location of coenzyme Q_{10} in lipid bilayers, or more precisely, the orientation and the depth in the membrane profile. For this, we used the most direct method: neutron diffraction in combination with H/D contrast, here deuterated lipids and protonated CoQ_{10} . CoQ_{10} contains 10 isoprene units (50 carbons and 80 hydrogens) with a series of ten hinged planes (double bonds) and a quinone (9 carbons and 9 hydrogens). It needs not remain in an extended conformation, but may be curled in the membrane structure. Our approach is different from the “typical” neutron diffraction analysis on

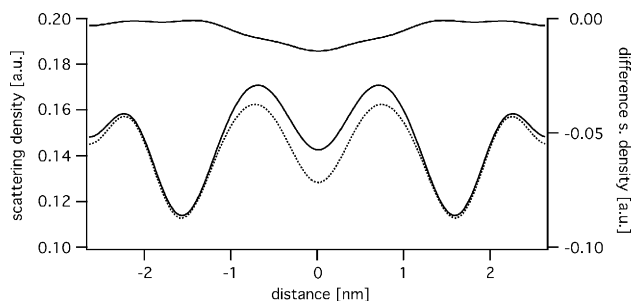


Fig. 3. Scattering density profile of the $dDMPC/dDMPS$ (90:10 mol%) stacked bilayers without (lower solid line) or with (dotted line) protonated ubiquinone. The two minima represent the headgroups in the bilayers cross section. The trough in the middle is the bilayers center. The results are the Fourier transform of the structure factors calculated from the diffractogram in Fig. 1. Upper solid line: ubiquinone distribution calculated as the difference between the two scattering density profiles shown in the lower part of the figure. The central minimum reflects the position of the protonated ubiquinone, in the middle of the unit cell, i.e., in the center of the membrane.

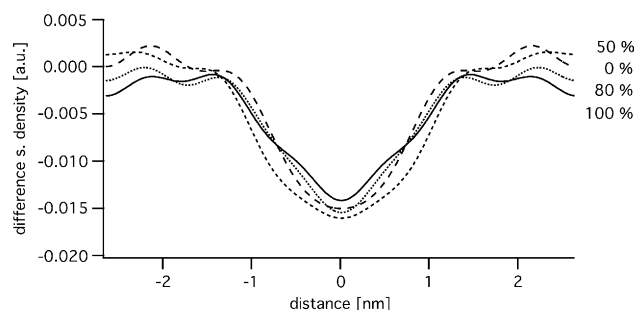


Fig. 4. Density profiles for all measured D_2O/H_2O contrasts. The central minimum in the scattering length density is caused by the protonated ubiquinone. This position corresponds to the lipid bilayer center. The wavy features at the outer edges of the density profiles are due to counting statistics and Fourier truncation errors and reflect the overall error in the membrane profiles calculated.

stacked lipid membranes wherein the localization of a small deuterated molecule (or domain) is determined in per-protonated bilayer lipids. In this study, we localized the per-protonated ubiquinone in a perdeuterated bilayer; an approach now possible due to the availability of per-deuterated lipids. Our neutron diffraction experiments yielded a set of scattering length density profiles from samples with deuterated lipids and deuterated lipids plus protonated ubiquinone at a series of D_2O/H_2O ratios of the solvent. From the respective difference density profiles (Fig. 4), the location of ubiquinone is demonstrated unambiguously in the bilayer center of lipid membranes in the physiologically relevant fluid-crystalline state.

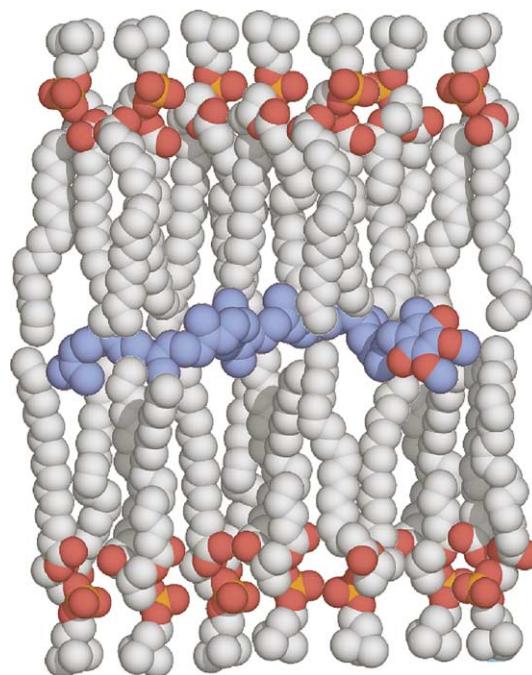


Fig. 5. An artist view of the lipid membrane with embedded ubiquinone derived from the neutron scattering density profiles (Fig. 4). The ubiquinone molecule (dark gray) is sandwiched between the two lipid layers of the membrane. Its conformation is not necessarily straight but its location is confined parallel to the membrane plane with only little intrusions into the lipid acyl chains.

Our previous data on the localization of squalene in phospholipid bilayers [15], together with the present data for CoQ₁₀ show that all polyisoprenes and polyisoprene domains lie flat in the bilayer center—between the bilayer leaflets. A careful review of the localization of polyisoprenes, including especially ubiquinone, lays out the contradictory data that leaves the question open to theoretical statements at best [7]. It is important therefore to have our definitive data on the subject.

The incorporation of CoQ₁₀ into the lipid bilayer does not change the repeat distance of the membrane stack in the experimental error limit (the increase is only 0.1 nm). This finding is in accordance with previous X-ray experiments on DMPC and DPPC membranes in the presence of ubiquinone [19]. Inspection of the membrane profiles in Fig. 3 suggests that the membrane thickness does not change, the density minimum at ± 1.6 nm does not change its position on the membrane profile.

Taking advantage of our data we have considered the following molecular description of ubiquinone in phospholipid bilayers. Using the volumetric lipid data of Nagle and Tristan-Nagle [20] and the neutron scattering length of elements [21], the neutron scattering length of deuterated myristoyl (CD₂)₁₂–CD₃ – the acyl chain of the lipid used in these experiments – is calculated to $b_{\text{myr}} = 266.5$ fm, with a volume of 390×10^{-24} cm³ we calculate the neutron scattering length density to 6.8×10^{10} cm⁻². This is to be compared to the scattering length density of the total protonated CoQ₁₀, which is estimated to 0.4×10^{10} cm⁻², using the scattering length of 78.81 fm and an estimated volume of 2000×10^{-24} cm³. Incorporation of the protonated ubiquinone in the perdeuterated lipid matrix would therefore reduce the scattering length density where it resides. Our data (Figs. 3 and 4) prove that the polyisoprene domain of ubiquinone is at the center of the bilayer, i.e., sandwiched

between the two lipid monolayers. From the width of the difference density feature, one can conclude that the polyisoprene chain runs predominantly parallel to the membrane plane. To illustrate the molecular distribution along the bilayer profile, we construct a model where molecular groups are represented by Gaussian distributions (Fig. 6). A comparison of these data with that of squalene [15] suggests that both polyisoprenes and polyisoprene domains in general have the same conformation in straight-chain lipid bilayers: they lie parallel to the membrane plane in the bilayer center. Thus, this statement applies to the myriad of polyisoprenes found in natural membranes. Only the membranes of the archaea lack straight-chain lipids, which are replaced by diphytanyl chains and which also contain polyisoprenes such as squalene [22]. The localization of polyisoprenes in these bilayers is yet to be established.

4.1. Previous attempts to localize ubiquinone in bilayers

There have been many attempts using indirect measurements to localize ubiquinone in bilayers. Our neutron diffraction experiments have unequivocally localized its polyisoprene domain at the bilayer center. Using differential scanning calorimetry (DSC) to measure the phase transition temperature T_m , Katsikakis and Quinn [10,23,24] concluded, that coenzyme Q₁₀ at up to 20 mol% codispersions in dipalmitoyl phosphatidyl choline (DPPC) lies in the center of the bilayers. Fluorescence quenching measurements [25] are also consistent with the conclusion that ubiquinone resides in the bilayer center. In contrast, Lenaz et al. [26] have concluded from fluorescence studies, having the fluorophore at different heights in the bilayer, “that CoQ analogues oscillate between the two bilayer surfaces not extending beyond the glycerol region.”

Katsikas and Quinn have shown [23], using X-ray diffraction, that tocopherol, another polyisoprene, thickens the bilayer, in contrast to the present study for CoQ₁₀. They suggested on this basis, that tocopherol is in or near the bilayer center. In contrast, Stidham et al. [19] reported in their ¹³C NMR study on egg phosphatidylcholine liposomes containing ubiquinone, a marked effect on the ¹³C spin lattice relaxation times of the hydrocarbon chain atoms near the lipid head groups, but nearly no effect on the atoms nearest to the bilayer center, suggesting ubiquinone's location in the head group region. They concluded that the ubiquinone is not located preferentially between the monolayers. A more extensive study was conducted by Cornell et al. [5]. Using deuterated ubiquinone and examining its ²H NMR quadrupole coupling they found that the Q₁₀ isoprenoid chain is in a mobile environment, physically separated from the cooperative motions and orientational constraints of the bilayer lipid chains. They thus suggested that the Q₁₀ isoprenoid chain *might* lie in the center of the bilayer. These investigators also synthesized a ubiquinone analogue in which the ten isoprene groups have been replaced by a perdeuterated saturated tridecyl chain. The ²H NMR quadrupole coupling showed

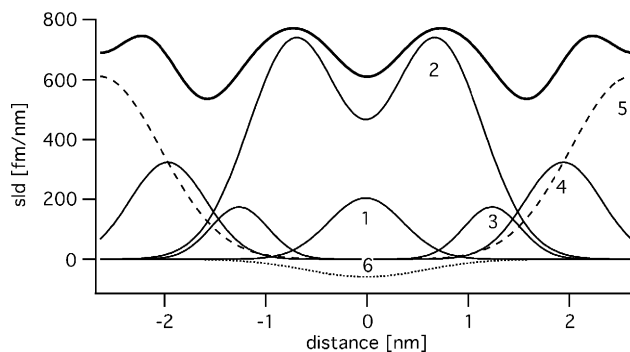


Fig. 6. Model of the deuterated DMPC bilayer containing the protonated ubiquinone with a 100% D₂O water layer. The different molecular groups of the lipids are represented by the Gaussian distributions 1,2,3,4, corresponding to the terminal methyl groups (1), the acyl chains (2), the glycerol backbone (3), and the phosphatidyl head group (4). The water distribution (5) is calculated by the difference of the density profiles at 100% and 0% D₂O, respectively. The model Gaussians 1 to 5 with the appropriate calculated scattering length densities were first fitted to the density profile of the membrane sample without ubiquinone, by varying the position, width, and amplitude, but keeping the integral to the calculated scattering length density. The parameters of the model Gaussian 6 (dotted curve), which describes the ubiquinone distribution, were then determined by fitting the parameters to the density profile of the membrane containing protonated ubiquinone, while keeping the parameters of Gaussians 1 to 5 constant.

that this analogue is oriented *parallel to the chains* of the phospholipids and that its quinone domain is in the region of the phospholipid headgroups. Thus two features of straight-chain lipid bilayers seem to provoke the isoprenes to reside in the bilayer center. First, the cooperative motions of the chains in each monolayer are disrupted by intrusion of branched hydrocarbons within that monolayer. Second, the two monolayers are uncoupled so that the *lateral* cooperative motions [27] of the phospholipid acyl chains within one monolayer do not affect the motions of the other. In a recent study using NMR chemical shift-polarity correlation Afri et al. [9] concluded that the ubiquinone ring lies in the lipid head group region but distant from the lipid–water interface and place a considerable portion of the isoprenoid side chain parallel to the lipid acyl chains, an interpretation which is not in line with our neutron diffraction result.

4.2. Implications of CoQ in the bilayer center

CoQ is best known as a carrier of electrons and protons in mitochondria during oxidative phosphorylation as is the ubiquinone analog plastoquinone in chloroplasts during photophosphorylation [28]. As part of the mitochondrial electron transport chain CoQ transfers electrons from Complex I and II to Complex III, all embedded in the inner mitochondrial membrane. To facilitate electron transport, CoQ should be freely diffusing in its membrane environment to encounter the binding pockets in the protein complexes involved [6,29]; this is best if CoQ₁₀ resides in the membrane midplane, as shown by our investigation.

Ubiquinone is also well known as an antioxidant in eukaryotes where it has wide distribution throughout many organelle membranes [30]. Interest has increased in the specific location of the quinone in the lipid bilayer cross-section with the emergence of high-resolution structures of the proteins that bind it [31].

Our data in Figs. 3 and 4 prove that the polyisoprene domain of ubiquinone, like that of squalane [15], lies in the bilayer center parallel to the membrane plane. Thus long polyisoprenes and polyisoprenes can be assumed to have such a residence. Long polyisoprenes include tocopherol, dolichol, plastoquinone, carotenes, and lycopene.

The presence of these polyisoprenes in the bilayer center raises a question as to the area occupied by them since they appear to be restricted to the cleavage plane. Each isoprene unit based on models and assuming a conformation parallel to the bilayer plane, occupies about 0.4–0.5 nm². Since the isoprene units are sandwiched between the two monolayers we need only consider the area of one monolayer with an area of 0.64 nm² per lipid. Each CoQ₁₀ contains 10 isoprenes and a quinone ring. Thus, it covers, if it resides as a monolayer, 4.4–5.5 nm², or an average of 5 nm². This occupies the area of about 8 lipid molecules. Thus a 6 mol% of CoQ₁₀ is sufficient to provide a full surface coverage of each monolayer for the total lipids of the bilayer. The concentration of ubiquinone in biological membranes is widespread and largest in mitochondria [1]. Using published

data with up to 7 nmol CoQ per mg protein [32], the surface coverage in mitochondria membranes lies between 10% and 30%. Such a calculation suggests that CoQ₁₀ can represent a serious barrier for, e.g., proton diffusion across the bilayer of, say, the inner membrane of the mitochondrion. Taken this together with our localization of both squalane and ubiquinone in the bilayer center, suggests, in accordance with the “water cluster” mechanism for proton leakage [2], that these polyisoprenes may inhibit proton leakage. Such inhibition would be another biological function for them, in addition to their role in redox systems as electron/proton carriers and as antioxidants.

Acknowledgements

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