# NMR Studies of Ubiquinone Location in Oriented Model Membranes: Evidence for a Single Motionally-Averaged Population

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Ubiquinones are key components in the electron transport chains of mitochondrial and bacterial membranes.[1] In their major physiological form ($UQ_{10}$), these molecules consist of an aromatic quinone head group and a hydrophobic tail of 10 repeating isoprene units (Figure 1). Determining the location and orientation of ubiquinones in membranes is essential for understanding their role as diffusible redox carriers. Conflicting models have been reported for the location of ubiquinones within native and artificial membranes.[2,3] Current proposals range from the formation of ubiquinone micelles spanning the entire bilayer to different coexisting pools, either in the bilayer midplane or closer to the lipid head groups. The lack of consensus between these studies stems in part from differences in the relative amount of ubiquinone incorporated into the membrane systems. An experimental approach is desired that combines high resolution and sensitivity with the ability to work at physiological ubiquinone concentrations (1−3 mol %).

By using specifically $^{13}C$-labeled ubiquinones[4] and high-resolution NMR of oriented membranes, we are able to detect resolved ubiquinone resonances at <1 mol % $UQ_{10}$ concentration. We reconstituted [4-$^{13}C$]$UQ_{10}$ into model bilayers consisting of a mixture of dimyristoyl phosphatidylcholine (DMPC) and dihexanoyl phosphatidylcholine (DHPC).[5] These lipids have been shown to form discoidal membranes orienting spontaneously in an external magnetic field in a temperature-dependent manner.[6] One advantage of these oriented systems is that angular information, which can be related to molecular orientation, is retained in the observed chemical shifts and dipolar splittings.[7] It has been shown in studies on glycolipids incorporated into oriented bilayers that rapid rotational diffusion around the membrane normal yields sharp resolved resonances which report on the orientation of the glycolipid head group relative to the motional director.[8] In a similar fashion, the carbonyl chemical shift of the quinone head group in oriented membranes can be measured and used to constrain the orientation of the ubiquinone ring. Different orientations would result in distinct chemical shifts of the labeled carbonyl.

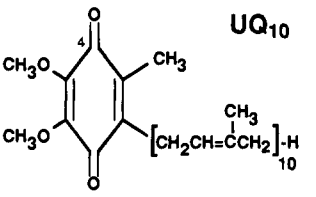


**Figure 1.** Chemical structure of ubiquinone. $UQ_{10}$ was labeled at the [4-$^{13}C$] carbonyl position.

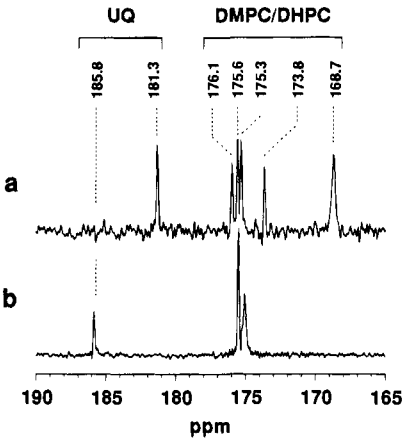


**Figure 2.** $^{13}C$ NMR spectra of DMPC/DHPC membranes containing 2 mol % [4-$^{13}C$]$UQ_{10}$ obtained at 308 K. (a) In the oriented samples (25% w/v), the [4-$^{13}C$]$UQ_{10}$ resonance is observed at 181.3 ppm. The lipid carbonyl signals are assigned according to the literature. The DMPC and DHPC *sn*-1 carbonyl resonances are at 168.7 and 173.8 ppm, respectively. The DHPC *sn*-2 carbonyl is found at 176.1 ppm, while the DMPC *sn*-2 carbonyl is split into a doublet at 175.3 and 175.6 ppm. (b) In the unoriented sample (15% w/v), the [4-$^{13}C$]$UQ_{10}$ resonance is found at 185.8 ppm and the lipid carbonyls are observed at 175.5 ppm (*sn*-2) and at 174.9 ppm (*sn*-1).

Figure 2 presents spectra of [4-$^{13}C$]-labeled $UQ_{10}$ in unoriented and oriented bilayers at 308 K. In the unoriented spectrum (Figure 2b), the isotropic ubiquinone resonance is observed at 185.8 ppm. Two isotropic resonances at 174.9 and 175.5 ppm are assigned to the *sn*-1 and *sn*-2 chain carbonyls of the phospholipids, respectively.[6] The difference in chemical shift may reflect a difference in hydrogen bonding.[9] Orientation of the sample produces a more complex spectrum (Figure 2a). Five of the lines, a doublet due to $^{31}P$ dipolar splitting and three singlets, belong to the DMPC and DHPC carbonyls.[6] The positions of these resonances relative to their isotropic values

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(5) [4-$^{13}C$]$UQ_{10}$ was dissolved in chloroform, added to a 5 mm NMR tube, and dried under nitrogen. DMPC and DHPC were codissolved in chloroform in a 3:1 molar ratio, dried, and added to the $UQ_{10}$-containing NMR tube by weighing in the appropriate amount of lipid to obtain a 1:50 molar ratio ($UQ_{10}$:lipids). Deuterated water was added to a final 25% (w/v) concentration for the oriented sample. In order to obtain an unoriented sample, DHPC was added to a final 2:1 DMPC/DHPC ratio and the sample was diluted to 15% (w/v). The samples were treated by a combination of heating, cooling, sonication, and centrifugation to obtain a homogeneous mixture. The samples were stored at −20 °C and allowed to equilibrate at room temperature for 1 day prior to NMR data collection.

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reflect a well-ordered sample. The coincidence of the lipid chemical shifts with those from corresponding samples without ubiquinones suggests that the addition of $UQ_{10}$ does not alter the orientational behavior of the DMPC/DHPC membranes.[10] The [4-$^{13}$C] ring carbonyl in $UQ_{10}$ leads to a *single* narrow resonance at 181.3 ppm, 4.5 ppm upfield from its isotropic position. The shift is much larger than that expected from a change in local environment and most likely results from nonisotropic averaging of the *static* nonaxially symmetric carbonyl tensor,[7,11] as for the lipid resonances. The direction and size of the observed shift constrain the orientational distribution of the chemical shift tensor with respect to the motional director, i.e. the bilayer normal. Because there are many ways to average orientational-dependent chemical shifts to arrive at a particular value, the observation of a single chemical shift does not allow us to uniquely define the orientation. However, since $\sigma_{33}$, the largest shielding element, is generally perpendicular to the carbonyl plane,[12] the direction of the shift clearly argues against large populations of orientations which would place $\sigma_{33}$ parallel with the bilayer normal, i.e. perpendicular to the static field. This rules out models with the ring plane parallel with the membrane midplane. The upfield shift is consistent with another class of models in which the ring plane is close to parallel to the lipid chains. Further experiments are planned using deuterium labels, multiple $^{13}$C labels to measure dipolar couplings, and additional chemical shifts to refine the head group orientation. Ubiquinones in a slowly exchanging separate phase undergoing isotropic motion either outside or within the membrane can also be ruled out by the absence of an isotropic ubiquinone signal in the oriented sample.

Having limited the number of models for the average orientation of $UQ_{10}$, we were able to address the depth of the ubiquinone head group with respect to the membrane surface by measuring $^{13}$C longitudinal relaxation times with and without the addition of $Gd^{3+}$.[13] The presence of a paramagnetic ion facilitates nuclear relaxation at a molecular site in a distance-dependent manner.[14] The hydrophobic region in the membrane

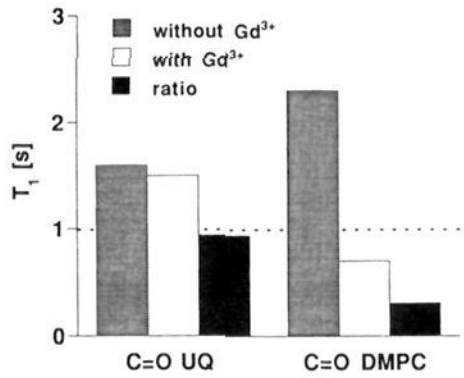


**Figure 3.** Comparison of $T_1$ relaxation times in oriented samples at 308 K with and without the addition of 0.6 mM $Gd^{3+}$. The ratios of the measured $T_1$ values are represented by the solid bars. The dashed line at a ratio of 1 serves as a reference line for an unaltered relaxation. The $T_1$'s for the DMPC *sn*-1 carbonyl (168.7 ppm) are shown.

interior, not accessible to the ion, is less influenced than the lipid head group. The observed carbonyl $T_1$ relaxation times obtained with the oriented sample are summarized in Figure 3. The lipid carbonyls are strongly effected by $Gd^{3+}$ as expected from their proximity to the membrane surface. In contrast, the [4-$^{13}$C]$UQ_{10}$ relaxation time is not significantly changed by the paramagnetic reagent. These results support a location of the ubiquinone head group buried in the lipid bilayer[2] and provide evidence against proposed models where the dominant location is one with the ring close to the lipid head group.[3]

In summary, NMR measurements of $^{13}$C-labeled ubiquinones in oriented membranes combine high resolution with sensitivity in a single experiment and open up a promising approach for determining the location of quinones in membranes. Our data show that the position and orientation of the $UQ_{10}$ head group at 308 K and physiological concentration are most consistent with models that place $UQ_{10}$ close to or in the bilayer midplane with the ring plane preferentially parallel to the lipid chains.

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(13) $T_1$ relaxation times were measured using an $\pi-\tau-\pi/2$ inversion recovery sequence. Typically 10 $\tau$ values were collected with 512 scans each. The $\pi$ pulse was 12 $\mu$s and the recycle delay 15 s. Intensity changes were fit to a single exponential.

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