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Clifford W Fong. Coenzyme Q 10 and Vitamin E synergy, electron transfer, antioxidation in cell membranes, and interaction with cholesterol. Eigenenergy Adelaide South Australia Australia. 2023. hal-03976270

HAL Id: hal-03976270

<https://hal.science/hal-03976270v1>

Submitted on 7 Feb 2023

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Coenzyme Q₁₀ and Vitamin E synergy, electron transfer, antioxidation in cell membranes, and interaction with cholesterol

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Keywords: Coenzyme Q₁₀; Vitamin E; Redox cycles; Antioxidation; Cell membranes; Statins; lipid peroxidation; Oxidative stress; Free radicals; Quantum mechanics;

Abbreviations

CoQ₁₀ Coenzyme Q₁₀ or Ubiquinone, CoQ₁₀H₂ Ubiquinol, CoQ₁₀H• Ubisemiquinone radical, Vit E vitamin E or α -tocopherol, LDL Low-density lipoprotein, HDL High-density lipoprotein, ϵ Dielectric constant, SolvEn Total solvation energy, Electrostatic solvation energy ESE, CDS Non-electrostatic cavity dispersion solvent structure of the first solvation shell, ΔG solvation free energy of truncated CoQ₁₀H•, ES First excited state excitation energy, HL HOMO-LUMO energy gap, HOMO highest occupied molecular orbital, LUMO lowest unoccupied molecular orbital, PUFA Polyunsaturated fatty acid.

Abstract

A zonal dielectric model of the cellular lipid membrane has been used to examine the location and properties of CoQ₁₀, CoQ₁₀H₂, α -tocopherol and cholesterol in the lipid bilayer. It is shown that the quinone/quinol heads of CoQ₁₀ and CoQ₁₀H₂, the chromanol head of α -tocopherol, and hydroxyl group of cholesterol interact with the ester carbonyl head group of the phospholipid bilayer where ϵ is 20.52. The excitation energies of the first excited state ES and HL energy gaps have been used to show that intermediate CoQ₁₀H• radical is particularly stable, and very similar to CoQ₁₀H₂ in kinetic stability, but more reactive as judged by the excitation energy. The results are strongly supportive of a likely additive or synergistic interaction when CoQ₁₀H₂ is combined with α -tocopherol. While α -tocopherol and CoQ₁₀H₂ have distinct lipoprotective antioxidant properties in biological membranes, their combined antioxidant activity should be markedly synergistic. Cholesterol can influence the interaction of CoQ₁₀, CoQ₁₀H₂, with α -tocopherol in the cellular phospholipid membrane in a complex fashion, made even more complex as all these entities are transported in the body by LDL. Statin users might benefit from dietary supplements of CoQ₁₀ and α -tocopherol to maintain antioxidant reserves in cell membranes.

Introduction

Coenzyme Q₁₀ (CoQ₁₀) exists within the body in the oxidised form (ubiquinone) and the reduced form CoQ₁₀H₂ (ubiquinol). These two redox forms are continually interchanged within cells as part of the normal function of CoQ₁₀. In the blood, CoQ₁₀ is transported in ubiquinol form (bound to low-density lipoprotein- and very low-density lipoprotein-cholesterol), irrespective of the initial dietary form (CoQ₁₀ or CoQ₁₀H₂, endogenous or exogenous as supplements).

CoQ₁₀ has a critical role supplying all cellular energy production in the electron transport chain of the mitochondrial respiratory process which produces ATP chemical energy. CoQ₁₀H₂ also plays an important role as an antioxidant that protects lipid-soluble cell membranes. CoQ₁₀H₂ is a major lipid-soluble antioxidant, protecting cell membranes and circulating lipoproteins from

oxidative damage by free radicals. CoQ₁₀H₂ is the only lipid-soluble antioxidant manufactured within the body. CoQ₁₀H₂ is also able to regenerate vitamins C and E back to their active fully reduced forms. The antioxidant function of CoQ₁₀ is attributed to its reduced ubiquinol CoQ₁₀H₂ form, which must be continuously regenerated from its ubiquinone oxidised form.

CoQ₁₀H₂ may also have anti-inflammatory action, particularly in elderly people. CoQ₁₀H₂ is the active form of CoQ₁₀ and represents between 93 – 99 per cent of the total CoQ₁₀ pool in human plasma and the lymph. CoQ₁₀ in the ubiquinol form is needed for antioxidant protection in the lymph and blood. When ubiquinol leaves the blood to enter the cells and tissues, it is converted to the ubiquinone form needed for the process of ATP energy production. [1] [Judy]

Turunen [2] has listed some pathological conditions which could involve lipids such as CoQ₁₀ and cholesterol from the mevalonate pathway. Dietary supplementation with CoQ₁₀H₂, appears positive in upregulating antioxidant capability and there is emerging research indicating beneficial effects of CoQ₁₀ supplementation in individuals on statin medications. [3] [Rodick] There is evidence that CoQ₁₀ supplementation positively affects mitochondrial deficiency syndrome and some of the symptoms of ageing. Cardiovascular disease and inflammation appear to be alleviated by the antioxidant effect of CoQ₁₀H₂. [4] [AAseth] Low CoQ₁₀ levels are related to inflammatory processes and oxidative stress, factors implicated in atherosclerosis, obesity, nonalcoholic fatty liver, as well as metabolic syndrome. Metabolic syndrome is a disease characterized by cardiovascular risk factors linked to obesity, dyslipidemia and hyperglycemia. CoQ₁₀H₂ deficiency interferes in a negative manner with inflammatory and oxidative parameters.

While the main function CoQ₁₀ is related to mitochondrial respiration, acting as an electron transporter, the cellular functions of coenzyme Q are multiple: it is present in all cell membranes, limiting the toxic effect of free radicals, it is a component of LDL, it is involved in the aging process, and its deficiency is linked to several diseases, possibly suppressing ferroptosis, a type of iron-dependent programmed cell death characterized by lipid peroxidation. [5] [Pallotti]

Most of the body's daily requirement for CoQ₁₀ is produced within the body. As people age the ability of the body to synthesize its own CoQ₁₀ decreases. CoQ₁₀ levels have also been shown to be depleted in a number of disorders, particularly heart disease. The synthesis of CoQ₁₀ is a complex multistage process (governed by at least 13 genes), requiring a number of amino acids, vitamins, and trace element precursors and cofactors, deficiency of any of which can adversely affect normal CoQ₁₀ production. Nutritional supplementation with CoQ₁₀ can provide a mechanism to maintain adequate levels within the body. [6] [Mantle]

Low-density lipoprotein (LDL) is a lipoprotein that carries cholesterol in the blood. The test for LDL cholesterol measures the amount of cholesterol bound to LDL in the blood. LDL cholesterol is undesirable because it deposits excess cholesterol in the walls of blood vessels and contributes to 'narrowing of the arteries' (atherosclerosis) inducing angina, heart attack and strokes. Hence LDL cholesterol is often termed 'bad' cholesterol. The HDL (high density lipoprotein) cholesterol test measures the amount of cholesterol carried by HDL particles in the blood. HDL particles actually remove excess cholesterol from the body. Having a high level of cholesterol carried by HDL particles is considered good and HDL cholesterol is often termed 'good' cholesterol.

There is a well known connection between CoQ₁₀ availability and the widespread use of statins to reduce cholesterol in the body. Adverse effects of statins include muscle problems and cognitive problems and peripheral neuropathy, or pain or numbness in the extremities like fingers and toes, are also widely reported. Statins lower CoQ₁₀ levels because they work by blocking the mevalonate pathway involved in cholesterol production but this is the same pathway by which CoQ₁₀ is produced. Statins also reduce the LDL lipoproteins that transport cholesterol, CoQ₁₀ and other fat-soluble antioxidants.

The loss of CoQ₁₀ leads to loss of cell energy and increased free radicals (by reduction of the antioxidant CoQ₁₀H₂) which, in turn, can further damage mitochondrial DNA. Loss of CoQ₁₀ may lead to a greater likelihood of symptoms arising from statins in patients with existing mitochondrial damage, since these people rely on ample CoQ₁₀ to help overcome this deficiency. Because statins may cause more mitochondrial problems over time, and as these mitochondrial processes tend to weaken with age, new adverse effects can also develop the longer a patient takes statin drugs. The risk of adverse effects goes up as age goes up so the benefit of statins may not exceed their risks in those over 70 or 75 years old, even those with heart disease. The connection between statins' antioxidant properties and mitochondrial risk helps explain a complicated finding that statins can protect against the very same problems, in some people, to which they may predispose others – problems such as muscle and kidney function or heart arrhythmia. [7] [Golomb 2008] Randomized clinical trials showed evidence of unfavorable statin (simvastatin and pravastatin) effects on energy and exertional fatigue, particularly for women. [8] [Golomb 2012] Okuyama [9] has summarized evidence that statin medications cause heart failure by depleting the heart muscle cells of Coenzyme Q10, heme A, and selenoproteins, thereby impairing mitochondrial ATP production.

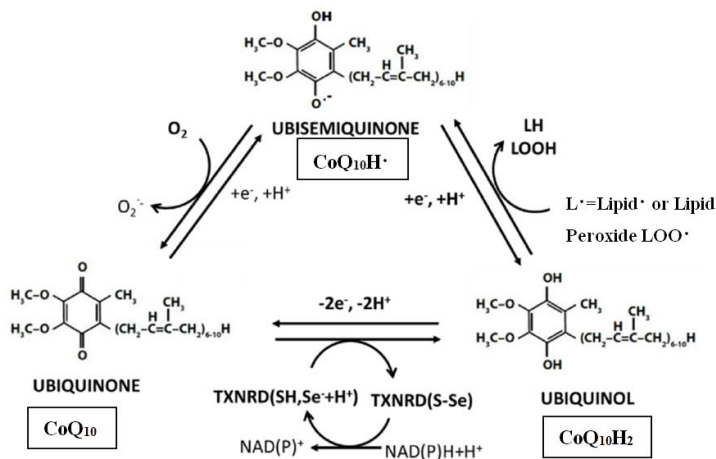


Figure 1. Redox cycle showing the interconversion amongst CoQ₁₀, CoQ₁₀H₂ and the radical intermediate CoQ₁₀H• in the mitochondrial membrane electron transport chain. CoQ₁₀H₂ may stop the lipoperoxidation chain reaction by trapping Lipid• / LOO• [adapted from [4] Aaseth]

Figure 1 shows the mitochondrial redox cycle of CoQ₁₀ (Ubiquinone), the fully oxidized form, the reduced form CoQ₁₀H₂ (Ubiquinol) and the half-reduced form anion radical CoQ₁₀H[•] (Ubisemiquinone anion radical) including redox cycling. In the extramitochondrial space thioredoxin reductase (TXNRD) is a major reducing enzyme with its selenol group and vicinal thiol, which catalyzes a two-electron reduction. CoQ₁₀H₂ may stop the lipoperoxidation chain reaction by trapping carbon centered lipid- and lipoperoxide radicals. CoQ₁₀H[•] formed in the respiratory chain in mitochondria may give rise to superoxides in the presence of molecular oxygen. Superoxides are in turn partitioned into molecular oxygen and hydrogen peroxide by superoxide dismutase (SOD). Critical to the functionality of CoQ₁₀ is that protons can concomitantly be taken up from one side of the membrane during the reduction of CoQ₁₀ and they can be released on the other side of the membrane when CoQ₁₀H₂ is oxidized in the reverse reaction.

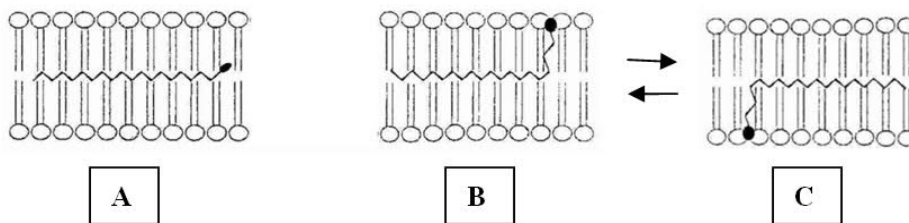


Figure 2. Schematic representation of CoQ₁₀ in lipid membrane bilayer in two configurations: (Right, B and C) dynamic flip flop between B and C with polar head of CoQ₁₀ located close to the polar phospholipid head groups of the upper and lower membrane surfaces, and (Left, A) oriented parallel to the interfacial plane, lying in the bilayer midplane space.

Braasch-Turi [10] has summarized the three schools of thought regarding the location of CoQ₁₀ in lipid membranes, ie the quinone headgroup is located: 1) at or near the lipid headgroups, 2) within the acyl chains, or 3) within the bilayer midplane. The consensus is that at least part of the isoprenyl side chain is embedded within the bilayer midplane, and the headgroup is thought to extend into one of the membrane leaflets. This requires a bend (~90° turn) in the isoprenyl side chain to allow CoQ₁₀ to be accommodated within the bilayer. It is known that CoQ₁₀ is about the same length as a typical phospholipid bilayer if CoQ₁₀ was in a fully-extended conformation. Lenaz [11] showed that CoQ₃ is located throughout almost the whole bilayer thickness. Di Bernado [12] found for a series of CoQ homologues, that the cut-off for the folded conformation was four isoprenoid units. CoQ₁₀ in the straight chain trans configuration has a length of 56 Å, which is greater than the thickness of a phospholipid bilayer, reinforcing the structural requirement for some folding when inside the lipid membrane. [13] [Garg] So it appears that CoQ₁₀ has a folded conformation as in Figure 2 with three or four isoprenoid units at about 90° to the remaining six or seven isoprenoid units laying in the bilayer midplane region. CoQ₁₀H₂ is thought to have the same folded conformation as CoQ₁₀.

However most studies of the localization of CoQ₁₀ in lipid bilayers have been static studies, but it is known that CoQ₁₀ exerts a fast flip-flop between the bilayer leaflets. Certainly lipid bilayers

are dynamic flexible fluid entities. Lateral diffusion coefficients in two dimensions have been calculated to span values of 10^{-7} – 10^{-6} cm^2s^{-1} . It therefore been suggested that the most probable location of CoQ₁₀ is in the middle of the bilayer with the quinone ring oscillating between the bilayer surfaces by bending its long tail (Figure 2). [12] [Di Bernado] The (flip-flop) diffusion of the Q head group from one side of the membrane to the other occurs rapidly at room temperature. In the inner mitochondrial membrane, CoQ₁₀H₂ molecules are continuously oxidized close to one side of the membrane, whereas CoQ₁₀ is reduced on opposite side, in conjunction complemented with the release and uptake of protons, respectively. The rapid flip flop mechanism is critical for the optimal turnover of the entire electron transport chain. Some investigators propose a two site model for CoQ₁₀ in membranes, one site in the interior of the membrane and the other near its hydrocarbon-water interface. Garg [13] has shown CoQ₁₀ presence in lipid DMPC monolayers is dynamically biphasic, with one miscible phase occupying the interstitial space between the DMPC lipids, and the other immiscible phase is present as pure CoQ₁₀ domains. Cholesterol increased the immiscible fraction in the monolayers. Voltage-dependent pores in a lipid bilayer membrane model have been studied. [14,15,16] [Alobeedallah, Alobeedallah, Zhang] Voltage-dependent mitochondrial permeability transition pores have also been investigated. [2] [Turunen]

Antioxidant activities of CoQ₁₀ in cell membranes: CoQ₁₀ and particularly CoQ₁₀H₂ also act as a lipophilic free-radical-scavenging antioxidant in the plasma membrane. [17] [Bentinger] Present in nearly all cellular membranes, CoQ₁₀ / CoQ₁₀H₂ provide antioxidant protection, in part, by maintaining the reduced state of α -tocopherol (α -TOC) and ascorbic acid (ASC). Furthermore, CoQ₁₀ also regulates apoptosis by preventing lipid peroxidation. Other functions of CoQ₁₀ in the cell membrane include metabolic regulation, cell signaling, and cell growth through local regulation of cytosolic redox intermediates such as NAD(P)H. [18,19] [Navas] [López-Lluch] Figures 3 and 1 illustrate the antioxidant processes carried out by CoQ₁₀ / CoQ₁₀H₂ and α -tocopherol redox reactions in cell membranes, particularly lipid peroxidation.

Lipid peroxidation of cell membranes is involved in neurodegenerative diseases such as multiple sclerosis, Alzheimer's disease, Huntington's disease, and Creutzfeldt-Jakob disease, as well as in psychiatric diseases such as schizophrenia, bipolar, and other major depressive disorders. [Ramana] [20]

FSP1 is the oxidoreductase that reduces CoQ₁₀ in the plasma membrane. This FSP1/CoQ₁₀/NADH system alone is then thought to be sufficient to suppress lipid peroxidation and ferroptosis in the cell membrane. Ferroptosis is an iron-dependent form of cell death and is characterized by the formation of reactive lipid species. Since ferroptosis-mediated lipid damage can be prevented by two independent parallel mechanisms, the GPX4/GSH and FSP/CoQ₁₀ mechanisms, which are both derived from the mevalonate pathway, it is speculated that statins could be involved in ferroptosis mediated diseases. [21] [Santoro]

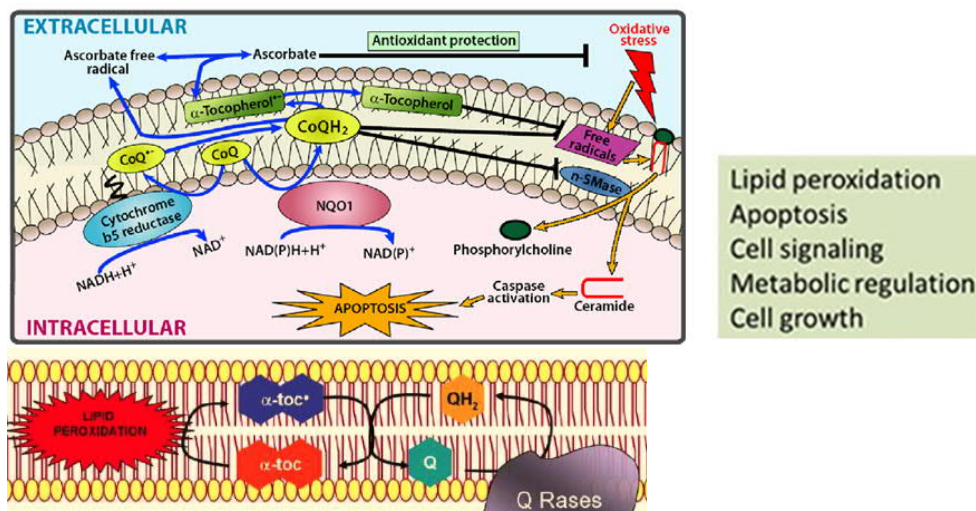


Figure 3. Schematic of CoQ₁₀, CoQ₁₀H₂ and α -tocopherol antioxidant redox cycles in cell membranes. Several membrane and cytosolic CoQ reductases (Q Rases) including the members of mitochondrial ETC maintain the redox cycle of CoQ₁₀ in membranes. CoQ₁₀H₂ can ameliorate lipid peroxidation by itself, but its major role in vivo is recycling α -tocopherol (α -toco) to its reduced state. (n-SMase, neutral-sphingomyelinase; NQO1, NAD(P)H:quinine) [adapted from [18] Navas, [19] Lopez Lluch]

The form of vitamin E used by humans is α -tocopherol, but it is well known that many people do not meet the recommended dietary allowance of 15 mg/day. Only α -tocopherol supplementation can reverse vitamin E deficiency symptoms. The main function of α -tocopherol in humans is that of a *fat-soluble antioxidant*. α -tocopherol can intercept peroxy radicals and thus prevent a chain reaction of lipid oxidation, and so maintain the integrity of cell membranes throughout the body. Other antioxidants, such as vitamin C and CoQ₁₀H₂, are capable of regenerating the antioxidant capacity of α -tocopherol.

α -Tocopherol is known to be the most effective *lipid-soluble*, chain-breaking antioxidant, protecting cellular membranes from being attacked by lipid peroxy radicals. Vitamin E prevents the propagation of lipid peroxy radicals in cellular membranes. Lipid peroxy radicals react 1,000 times faster with vitamin E than with polyunsaturated fatty acids [22] [Buettner] Vitamin E is transported in the blood by the plasma lipoproteins and erythrocytes. α -tocopherol is carried from the gut to the liver in chylomicrons where they are incorporated as chylomicron remnants. Catabolism of chylomicrons takes place in the systemic circulation through the action of cellular lipoprotein lipase. Vitamin E can be transferred to high density lipoprotein (HDL) and then to low density lipoprotein (LDL) and very low density lipoprotein (VLDL). Most α -tocopherol enters peripheral tissues within the intact lipoprotein through the LDL receptor pathway. α -tocopherol protects the fats in LDLs from oxidation. [23, 24] [Wang, Kagan]

While α -tocopherol and CoQ₁₀H₂ have distinct lipoprotective antioxidant properties in biological membranes, their combined antioxidant activity is markedly synergistic. While it is likely that α -tocopherol is the initial chain-breaking antioxidant during lipid peroxidation, both CoQH₂ and CoQ₁₀H• appear to efficiently recycle the resultant vitamin E phenoxyl radical back to its

biologically active reduced form. It is thought that physical interactions of α -tocopherol and coenzyme Q within the environment of the membrane lipid bilayer facilitate the recycling of α -tocopherol by ubiquinone and ubiquinol. [24] [Kagan] Buettner's [22] standard one electron reduction potentials indicate that α -tocopherol can easily remove an electron or H from $\text{CoQ}_{10}\text{H}_2$, indicating that vitamin E is a superior antioxidant than $\text{CoQ}_{10}\text{H}_2$.

Supplementation of CoQ_{10} with vitamin E has been shown to markedly decrease atherosclerosis and inflammation, both of which are precursors to heart disease, in baboons and mice. [25,26] [Wang, Thomas] There is some supporting small trial results in humans where antioxidant supplementation significantly increased large and small artery elasticity in patients with multiple cardiovascular risk factors. This beneficial vascular effect was associated with an improvement in glucose and lipid metabolism as well as decrease in blood pressure. Also significant increase in HDL-cholesterol and marginal declines in triglycerides as well as total cholesterol levels were observed in antioxidant treated patients. [27] [Shargorodsky]

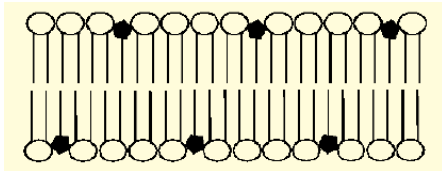


Figure 4. Schematic showing α -tocopherol (black arrowheads) in a phospholipid bilayer membrane, with the chromanol head of α -tocopherol is oriented towards the phospholipid head of the bilayer. The chromanol head is hydrogen bonded to the ester carbonyl of the phospholipid. [24] [Wang]

The likely structure of α -tocopherol in a lipid bilayer membrane is shown in Figure 4. It was concluded that α -tocopherol rotates about an axis perpendicular to the plane of the phospholipid bilayer, with the motion of the rigid chromanol ring is more constrained (via hydrogen bonding to the phospholipid headgroup) than the aliphatic phytol side chain with the frequency of rotamers increasing with distance towards the hydrophobic centre of the bilayer. [24] [Wang]

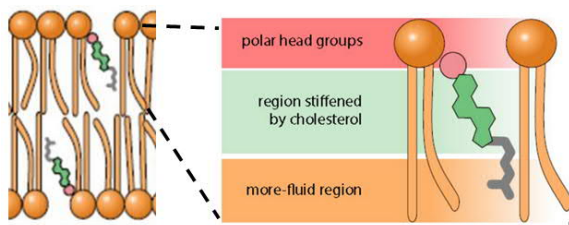


Figure 5. Schematic of the effect of cholesterol on the dynamic fluidity of the cell membrane phospholipid bilayer where cholesterol lowers membrane fluidity at high temperatures and increases membrane fluidity at low temperatures. The hydroxyl group of

cholesterol forms a hydrogen bond with the ester group in the head of the phospholipid bilayer.

Cholesterol reduces permeability of lipid membranes. Cholesterol plays a critical role in the function of the cell membrane with about 25-30% of lipid in the cell membrane being cholesterol. Cholesterol plays a role in membrane fluidity but its most important function is in reducing the permeability of the cell membrane. Cholesterol helps to restrict the passage of molecules by increasing the packing of phospholipids. Cholesterol can fit into spaces between phospholipids and prevent water-soluble molecules from diffusing across the membrane. Figure 5 [28,29] [Andrade, Krause]

Using electrical impedance spectroscopy (EIS) it was found to examine the effects of cholesterol on the electrical/dielectric properties of lipid membranes tethered to a solid modified gold substrate have shown: (a) when cholesterol was present in the tethered membranes at a concentration of 10% (mol/mol); the thickness of the membranes increased and the membrane conductance decreased, (b) when cholesterol was present in the tethered membrane at more than 30% (mol/mol) the membranes then became thinner and possessed a much larger electrical conductance. The EIS allowed a distinction to be made between a hydrophobic region in the center of the bilayer and another hydrophobic region further out towards the polar head region, in addition to the polar head region itself. Cholesterol was found to have the largest effect on the inner hydrophobic region, although the outer hydrophobic region was also affected. [14] [Alobeedallah]

Voltage-dependent pores were examined in a lipid bilayer membrane model. The inclusion of 10 M% cholesterol within the membrane lipid caused a decrease in the membrane conductance, however, the inclusion of higher levels of cholesterol increased the membrane conductance. [14,15] [Alobeedallah] Cholesterol usually comprises about 30% of the cell membrane. [16] [Zhang]

The analysis of electron transfer and redox reactions taking place in membranes is often based on assuming the solvent is a continuum and whose dielectric constant is analogous to that of apolar methylenic solvents. Martins [30] has measured the equivalent dielectric constants and their thermal behavior, for POPC and mixed POPC/cholesterol bilayers, using the pyrene Ham Effect. Pyrene locates in the ordered phospholipid headgroup section of bilayers, in the hydrophobic acyl chain region close to the glycerol moiety and first methylenic groups in phospholipid bilayers. The dielectric constants averaged transversally in space and laterally in time (due to pyrene lateral diffusion) were measured. At 20 °C, POPC bilayers display a dielectric constant ϵ analogous to 1-propanol which is much higher than that of an apolar solvent. Also, POPC bilayers with high cholesterol content exhibit higher dielectric constants than those of lower cholesterol level. The cholesterol molecules are arranged perpendicular to the bilayer surface, with the hydroxyl group positioned near the middle of the phospholipid headgroup region of the POPC molecule. [31] [Arrais] Cholesterol, in the biologically relevant liquid crystalline state, exhibits an ordering effect of phospholipids and decreases the membrane permeability of polar molecules. The relative dielectric constant of the aliphatic core of the DOPC bilayer is $\epsilon \approx 2.8$. [32] [Valincius]

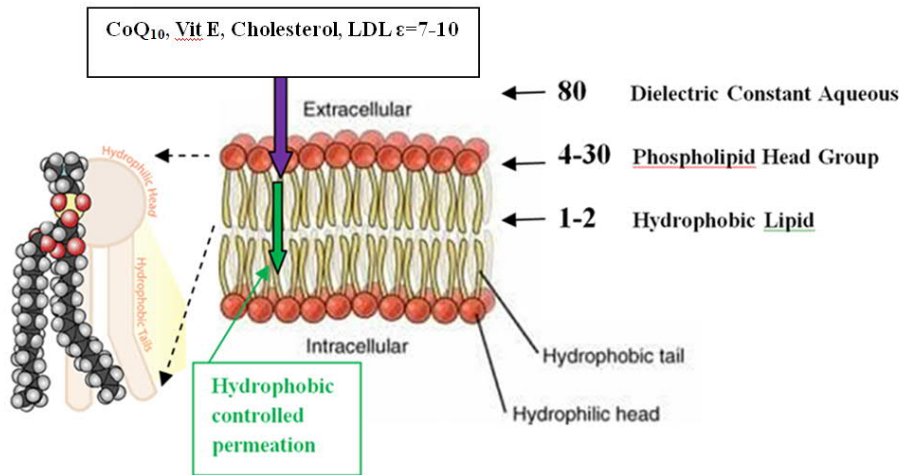


Figure 6. Schematic representation of CoQ₁₀, Vitamin E, Cholesterol in LDL ($\epsilon=7-10$) penetrating into the phospholipid bilayer of a cell membrane showing dielectric ϵ gradient of bilayer

Shinkarev [33] has studied the flash photolysis binding sites of CoQ₁₀, CoQ₁₀H₂, and the radical intermediate CoQ₁₀H• and concluded that CoQ₁₀H₂ escapes the binding site very much faster than CoQ₁₀H•, suggesting a large energetic barrier for the CoQ₁₀H•. However the high apparent dielectric constant in the vicinity of the quinone ring ($\epsilon \approx 25$) near the bilayer headgroup should result in a relatively small electrostatic stabilization energy of CoQ₁₀H•. Shinkarev proposed that that much of the kinetic stabilization of the CoQ₁₀H• was achieved by the topology of the binding site in which CoQ₁₀H• can exit the binding site only by moving its quinone head towards the center of the membrane. The large energetic penalty of transferring the charged headgroup to the membrane dielectric was proposed to explain the observed kinetic stability of the CoQ₁₀H•.

CoQ₁₀ also plays a role in the regulation of *mitochondrial permeability transition pores*. The PTP behaves like a voltage-dependent channel, favoring a closed conformation at high membrane potentials and an open conformation at low membrane potentials. Upon Ca²⁺ accumulation, many factors can modulate the PTP to open or close. Under physiological conditions, it is thought that the PTP serves as a fast Ca²⁺ release channel in the mitochondria. [2] [Turunen]

Study Objectives

To examine the dynamic quantum mechanical properties of CoQ₁₀, CoQ₁₀H₂, the radical CoQ₁₀H•, α -tocopherol and cholesterol to determine how electron transfer and antioxidation processes are governed in mitochondrial and cell membranes

Results

Central to any study of redox electron transfer processes in mitochondrial and cell membranes is based on the dynamic and fluid properties of the membrane. Transitory pores in the membrane and membrane voltage potentials and electrical fields mean that the dielectric properties of membranes are important determinants of electron transfer properties of embedded molecules. In this study, we use a basic dielectric ϵ representation of how CoQ₁₀, α -tocopherol and cholesterol carried from the blood plasma via LDL ($\epsilon=7-10$) can enter the cell membrane penetrating into the phospholipid bilayer of a cell membrane. The dielectric ϵ gradient of the bilayer is shown in Figure 5 [34,35,36] [Fong, Tian, Gramse]. The LDL environment is lipophilic, so CoQ₁₀, α -tocopherol and cholesterol leave a lipophilic or hydrophobic environment ($\epsilon=7-10$) to enter a membrane environment where $\epsilon=4-30$ near the phospholipid headgroup, to $\epsilon=1-2$ in the interior of the membrane. CoQ₁₀ /CoQ₁₀H₂ is structurally similar to α -tocopherol with polar heads and long aliphatic side chains, and cholesterol is mainly hydrophobic with an end hydroxyl group. (Figure 6) It is noted that the desolvation energy for CoQ₁₀, α -tocopherol and cholesterol leaving a hydrophobic LDL environment would be lower than the desolvation energy for typical drugs leaving a hydrophilic mostly aqueous blood plasma environment to enter the cell membrane.

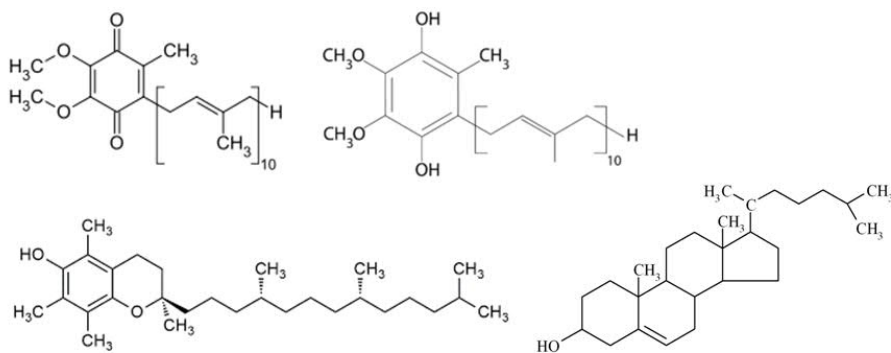


Figure 6. Ubiquinone (CoQ₁₀), ubiquinol (CoQ₁₀H₂) top left and right, α -tocopherol (vitamin E), cholesterol bottom left and right.

Lomize [37] has studied energy minimization of proteins in the hydrophobic region of membranes using an implicit solvation model ie minimizing transfer energy of the protein from water to the membrane hydrocarbon core approximated by the nonpolar solvent decadiene ($\epsilon=2.3$). The binding of a protein to the membrane is driven by hydrophobic interactions and opposed by desolvation of polar and charged groups. Implicit methods for modeling protein electrostatics require dielectric properties of the system to be known, and Li [38] has shown that the average dielectric constant inside the protein is relatively low, about ϵ 6–7, and reaches a value of about ϵ 20–30 at the protein's surface. The relative dielectric constant of the aliphatic core of DOPC bilayer is $\epsilon \approx 2.8$. [32] [Valincius]

Martins [30] has measured the equivalent dielectric constants and their thermal behavior, for POPC and mixed POPC/cholesterol bilayers, using the pyrene Ham Effect. Pyrene locates in the ordered phospholipid headgroup section of bilayers, in the hydrophobic acyl chain region close to the glycerol moiety and first methylenic groups in phospholipid bilayers. POPC bilayers

display a dielectric constant ϵ analogous to 1-propanol (ϵ 20.5) which is much higher than that of an apolar solvent.

The results of Lomize, Li, Valincius, Martins [37,38,32,30] are consistent with a dielectric gradient for a phospholipid bilayer membrane shown in Figure 1. In this study we now examine the properties of CoQ₁₀, α -tocopherol and cholesterol in a phospholipid bilayer by immersing these molecules in a vacuum ϵ 1, n-octane ϵ 1.94, n-octanol ϵ 9.86, 1-propanol ϵ 20.52, methanol ϵ 32.61. Octane is taken to be representative of the hydrophobic inner core of the bilayer, with propanol representative of the region near the phospholipid headgroup, (and octanol representative of the region between octane and propanol), and methanol representative of the bilayer properties close to the surface of the bilayer and the extracellular environment. The implicit SMD solvation model is used to determine the electrostatic and non-electrostatic solvation energies of CoQ₁₀, α -tocopherol and cholesterol in these solvents, thus indirectly describing how individual molecules of CoQ₁₀, α -tocopherol and cholesterol would behave in different parts of a bilayer membrane, from the hydrophobic inner region ϵ 1-2 to the regions close to the extracellular surface of the bilayer ϵ 4-30 (see Figure 5). The non-electrostatic (CDS) solvation energies are comprised of dispersion, cavitation, covalent components of hydrogen bonding, hydrophobic effects in bonding interactions which might occur between the polar and hydrogen bonding elements of CoQ₁₀, α -tocopherol and cholesterol and the carbonyl ester head group of the phospholipid bilayer.

In view of the wide spread evidence that fast dynamic processes are involved in cell membrane processes, particularly as discussed in the Introduction involving CoQ₁₀, α -tocopherol and cholesterol etc, either kinetic or kinetic stability methods must be used. As there is little kinetic data available, we have chosen to describe electron transfer reactions in dynamic cell membrane processes using quantum mechanical TD DFT methods. These methods are the HOMO-LUMO energy gap (HL) as a measure of kinetic stability, and the excitation energy of the first excited states (ES) of CoQ₁₀, α -tocopherol and cholesterol involved in electron transfer redox reactions governed by the cell membrane voltage potential of the bilayer itself or transient pores in the bilayer. The HL and ES values of CoQ₁₀, α -tocopherol and cholesterol and their intermediate species are calculated in various solvents to mimic the dielectric gradient of the bilayer from inner hydrophobic regions ϵ 1-2 to the regions close to the extracellular surface of the bilayer ϵ 4-30. (see Table 1)

Discussion

There is substantial literature evidence that CoQ₁₀, α -tocopherol and cholesterol have specific conformations in the cell membrane where the quinone/quinol heads of CoQ₁₀ and CoQ₁₀H₂, the chromanol head of α -tocopherol, and hydroxyl group of cholesterol are located near the ester carbonyl head group of the phospholipid bilayer, with the hydrocarbon tails extending deep into the hydrophobic interior. (Figures 2,3,4) Certainly, these conformations involve polar and hydrogen bonding interactions between the heads of CoQ₁₀, α -tocopherol and cholesterol and the

ester carbonyl head group of the phospholipid bilayer, whereas the hydrocarbon tails involve hydrophobic interactions deep in the membrane interior. It is also clear that all these conformations involve rapid dynamic interactions, as a result of flip flops, voltage-dependent transient pores and similar processes within the fluid membrane.

Using the dielectric zonal model of the lipid bilayer, Figure 5, it is shown for the species in Table 1 that several key features emerge:

- (a) The non-electrostatic CDS values for CoQ₁₀, CoQ₁₀H₂, α -tocopherol and cholesterol in an environment ranging from ϵ 1.94 to 32.61 are remarkably similar, indicating that bonding interaction from the quinone/quinol heads of CoQ₁₀ and CoQ₁₀H₂, the chromanol head of α -tocopherol, and hydroxyl group of cholesterol interact with the ester carbonyl head group of the phospholipid bilayer behave in a very similar mode. For example, the CDS values for CoQ₁₀ from the middle of the bilayer ϵ 1.94 is -24.05 kcal/mol. The CDS is comprised largely of cavitation, dispersion, and hydrophobic effects especially from the long aliphatic side chain). The CDS is -5.55 kcal/mol at ϵ 8.96, then CDS is -6.86 kcal/mol at ϵ 20.52, then a CDS -4.41 kcal/mol at ϵ 32.64 near to the extracellular surface of the bilayer. *This same trend is also true for CoQ₁₀H₂, α -tocopherol and cholesterol.* This result is consistent with the finding of Martins that a ϵ 20.52 (1-propanol) is the likely ϵ value for the lipid bilayer near the ester carbonyl head group of phospholipid bilayers. [30] [Martins] The change in CDS values from n-octanol ϵ 8.96 to 1-propanol ϵ 20.52 then to methanol ϵ 32.64 would be expected to reflect smooth changes in cavitation, H bonding and polarization in the solvent cavity, but these results indicate that 1-propanol is the optimal environment for CoQ₁₀, CoQ₁₀H₂, α -tocopherol and cholesterol.
- (b) The total solvation energies (SolEn) includes the electrostatic solvent energy (ESE) plus the CDS non-electrostatic energy. The ESE values increase smoothly as ϵ varies from 1.94 to 8.96 to 20.52 and 32.74 for CoQ₁₀, CoQ₁₀H₂, CoQ₁₀H[•], α -tocopherol and cholesterol.
- (c) It is noted that the same CDS and ESE trends in ϵ are seen for the radical intermediate CoQ₁₀H[•] showing that the dielectric environment surrounding CoQ₁₀, CoQ₁₀H₂, and CoQ₁₀H[•] are very similar, indicative of a very similar location and dynamic conformation in the fluid bilayer, with its flip flop configurations for these species. (Figure 3).
- (d) To demonstrate that it is the quinone/quinol heads of CoQ₁₀ and CoQ₁₀H₂, that interact with the ester carbonyl head group of the phospholipid bilayer that determine the properties of CoQ₁₀H[•], Table 1 shows the ΔG_{solv} of the truncated CoQ₁₀H[•] (removed the last nine isoprenyl units from the side chain). It is seen that ΔG_{solv} for CoQ₁₀H[•] trun has the same dependence on ϵ as shown for CoQ₁₀, CoQ₁₀H₂, (see (a) above). ΔG_{solv} of the truncated CoQ₁₀H[•] have values of -7.73, -9.85, -13.92 and -11.47kcal/mol for the ϵ values 1.94, 8.96, 20.52 and 32.64. The minimum value of -13.92kcal/mol at ϵ 20.52 (1-propanol) clearly shows the greatest stability occurs when the quinone/quinol heads of CoQ₁₀ and CoQ₁₀H₂, interact with the ester carbonyl head group of the phospholipid bilayer where the dielectric environment is around 20.52.
- (e) The excitation energies of the first excited state ES and HL energy gaps are also shown in Table 1. CoQ₁₀ is more reactive and has a lower ES 2.88eV and HL 5.38eV than CoQ₁₀H₂

ES 4.90eV and HL 7.43eV as expected. $\text{CoQ}_{10}\text{H}^\bullet$ is shown to be remarkably stable with a HL of 7.49eV (almost the same as the HL of $\text{CoQ}_{10}\text{H}_2$ 7.43eV) but with a lower excitation energy 1.82 ES. The stability of the $\text{CoQ}_{10}\text{H}^\bullet$ appears to be the result of extensive electron delocalization across the quinone/quinol moiety, which is confirmed by examining the vertical addition of an excess electron to CoQ_{10} as shown for $\text{CoQ}_{10}+\text{e}^-$ vert which has 2.41eV ES and 2.70eV HL with both values indicating high reactivity.

Shinkarev [33] has studied the flash photolysis binding sites of CoQ_{10} , $\text{CoQ}_{10}\text{H}_2$, and the radical intermediate $\text{CoQ}_{10}\text{H}^\bullet$. It was proposed that that much of the kinetic stabilization of the $\text{CoQ}_{10}\text{H}^\bullet$ is achieved by the topology of the binding site in which $\text{CoQ}_{10}\text{H}^\bullet$ can exit the binding site only by moving its quinone head towards the center of the membrane. However this study shows that $\text{CoQ}_{10}\text{H}^\bullet$ has an inherently high kinetic stability per se, very similar to $\text{CoQ}_{10}\text{H}_2$.

- (f) α -tocopherol has 4.80eV ES and 7.25eV HL, both values very similar to $\text{CoQ}_{10}\text{H}_2$ 4.90 ES and 7.43eV HL. The close similarities indicate very comparable reactivity, and given that both α -tocopherol and $\text{CoQ}_{10}\text{H}_2$ are similarly located in the bilayer (near the phospholipid head group) and that chromanol and quinone/quinol heads of α -tocopherol and $\text{CoQ}_{10}\text{H}_2$ are similar in molecular size, then it is likely that α -tocopherol and $\text{CoQ}_{10}\text{H}_2$ would show additive or synergistic behaviour in a lipid bilayer. This finding is consistent with some evidence that α -tocopherol and $\text{CoQ}_{10}\text{H}_2$ co-supplementation can have synergistic antioxidative effects. [23,25,26,27] [Kagan, Wang, Thomas, Shargorodsky]
- (g) The α -tocopherol radical has 1.09eV ES and 7.75eV HL, compared to 1.82eV ES and 7.49eV HL for $\text{CoQ}_{10}\text{H}^\bullet$, again showing complementary additive or synergistic properties if both radicals were present in a lipid bilayer.
- (h) It is also shown that the phenoxide $\text{CoQ}_{10}\text{H}^-$ intermediate 2.73eV ES and 3.03eV HL is far less stable than intermediate $\text{CoQ}_{10}\text{H}^\bullet$, 1.82eV ES and 7.49eV HL, ruling it out as a likely species during the conversion of CoQ_{10} to $\text{CoQ}_{10}\text{H}_2$.
- (i) The data for cholesterol is consistent with previous findings that the hydroxyl group is positioned near the middle of the phospholipid headgroup region of the POPC molecule, the same location where the quinone/quinol heads of CoQ_{10} and $\text{CoQ}_{10}\text{H}_2$, the chromanol head of α -tocopherol interact with the phospholipid headgroup. POPC bilayers with high cholesterol content usually exhibit higher dielectric constants than those of lower cholesterol level. [31] [Arrais] Cholesterol exhibits an ordering effect on phospholipids and decreases the membrane permeability of polar molecules. [32] [Valincius] Membrane concentrations of cholesterol might affect levels of CoQ_{10} or $\text{CoQ}_{10}\text{H}_2$ and/or α -tocopherol particularly by influencing ϵ or by competing for the ester carbonyl site of the head group of the phospholipid bilayer, where CoQ_{10} , $\text{CoQ}_{10}\text{H}_2$ and α -tocopherol also interact at this same site. Statins can lower LDL cholesterol levels, but also lower CoQ_{10} levels, because both originate from the mevalonate cycle, hence affecting antioxidation levels. However α -tocopherol and CoQ_{10} are both transported by LDL, so statin users might benefit from dietary supplements of CoQ_{10} and α -tocopherol to maintain antioxidant reserves.

Conclusion

A zonal dielectric model of the cellular lipid membrane has been used to examine the location and properties of CoQ₁₀, CoQ₁₀H₂, α -tocopherol and cholesterol in the lipid bilayer. It is shown that the quinone/quinol heads of CoQ₁₀ and CoQ₁₀H₂, the chromanol head of α -tocopherol, and hydroxyl group of cholesterol interact with the ester carbonyl head group of the phospholipid bilayer where ϵ is 20.52.

The excitation energies of the first excited state ES and HL energy gaps have been used to show that intermediate CoQ₁₀H[•] radical is particularly stable, and very similar to CoQ₁₀H₂ in kinetic stability, but more reactive as judged by the excitation energy.

It is particularly noteworthy that α -tocopherol has 4.80eV ES and 7.25eV HL, both values very similar to CoQ₁₀H₂ 4.90eV ES and 7.43eV HL. Similarly the α -tocopherol radical has 1.09eV ES and 7.75eV HL, compared to 1.82eV ES and 7.49eV HL for CoQ₁₀H[•]. These data are strongly supportive of a likely additive or synergistic interaction when CoQ₁₀H₂ is combined with α -tocopherol. While α -tocopherol and CoQ₁₀H₂ have distinct lipoprotective antioxidant properties in biological membranes, their combined antioxidant activity should be markedly synergistic.

Cholesterol can influence the interaction of CoQ₁₀, CoQ₁₀H₂, with α -tocopherol in the cellular phospholipid membrane in a complex fashion, made even more complex as all these entities are transported in the body by LDL. Statin users might benefit from dietary supplements of CoQ₁₀ and α -tocopherol to maintain antioxidant reserves in cell membranes.

Table 1.

	Vacuum ϵ 1 eV	n-Octane ϵ 1.94 kcal/mol	n-Octanol ϵ 9.86 kcal/mol	1-Propanol ϵ 20.52 kcal/mol	Methanol ϵ 32.61 kcal/mol
CoQ ₁₀	2.88 ES 5.38 HL	-24.05 CDS -32.31 SolvEn -8.26 ESE	-5.55 CDS -30.57 SolvEn -25.02 ESE	-6.86 CDS -34.51 SolvEn -27.65 ESE	-4.41 CDS -34.05 SolvEn -29.64 ESE
CoQ ₁₀ H ₂	4.90 ES 7.43 HL	-23.55 CDS -31.16 SolvEn -7.61 ESE	-6.39 CDS -30.56 SolvEn -24.17 ESE	-7.70 CDS -34.43 SolvEn -26.73 ESE	-5.56 CDS -34.18 SolvEn -28.62 ESE
CoQ ₁₀ H [•]	1.82 ES 7.49 HL	-23.52 CDS -30.78 SolvEn -7.26 ESE	-5.99 CDS -28.17 SolvEn -22.18 ESE	-7.29 CDS -31.83 SolvEn -24.54 ESE	-5.07 CDS -31.38 SolvEn -26.31 ESE
CoQ ₁₀ +e ⁻ vert	2.41 ES 2.70 HL				
CoQ ₁₀ H ⁻	2.73 ES 3.03 HL	-23.72 CDS -49.14 SolvEn -25.42 ESE	-6.20 CDS -71.67 SolvEn -65.47 ESE	-7.50 CDS -78.59 SolvEn -71.09 ESE	-5.28 CDS -81.04 SolvEn -75.76 ESE
α -Tocopherol	4.80 ES 7.25 HL	-12.13 CDS -15.20 SolvEn -3.07 ESE	-5.58 CDS -16.20 SolvEn -10.62 ESE	-6.20 CDS -17.97 SolvEn -11.77 ESE	-5.29 CDS -17.95 SolvEn -12.66 ESE
α -Toco [•] rad	1.09 ES 7.75 HL				
α -Toco+e ⁻ vert	0.06 ES 0.52 HL				
Cholesterol		-9.81 CDS -11.93 SolvEn -2.12 ESE	-5.26 CDS -13.07 SolvEn -7.81 ESE	-5.76 CDS -14.34 SolvEn -8.58 ESE	-5.08 CDS -14.42 SolvEn -9.34 ESE
CoQ ₁₀ H [•] trunc	1.82 ES 7.52 HL	-7.73 Δ G 1.84 ES -4.35 CDS	-9.85 Δ G 1.91 ES 2.69 CDS	-13.92 Δ G 1.90 ES 2.34 CDS	-11.47 Δ G 1.88 ES 3.35 CDS

Footnotes: ES First excited state excitation energy, HL HOMO-LUMO energy gap, CDS cavity-dispersion-solvent structure, SolvEn Total solvation energy, ESE electrostatic solvation energy, Δ G solvation free energy of truncated CoQ₁₀H[•] kcal/mol, vert vertical electron transfer

Experimental Methods

All calculations were carried out using the Gaussian 09 package. Energy optimizations were at the DFT/B3LYP/6-31G(d,p) (6d, 7f) GD3BJ level of theory for all atoms in vacuo, n-octane, n-octanol, 1-propanol and methanol using the IEFPCM/SMD solvent model. [47-49] Selected optimizations at the DFT/B3LYP/6-31⁺G(d,p) (6d, 7f) GD3BJ level of theory gave very similar results to those at the lower level. Energy and thermodynamic calculations were conducted at the DFT/B3LYP/6-31+G(d) (6d, 7f) with empirical dispersion GD3BJ level of theory with optimised geometries, using the IEFPCM/SMD solvent model. HOMO and LUMO calculations were for delocalized orbitals. TD DFT vertical calculations were conducted at the TD DFT/B3LYP/6-31G+(d,p) (6d, 7f) with the empirical dispersion GD3BJ correction for compounds with optimized geometries in vacuo, n-octane, n-octanol, 1-propanol and methanol using the IEFPCM/SMD solvent model.

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