**Vitamin E Has Reduced Affinity for a Polyunsaturated Phospholipid: An Umbrella
Sampling MD Simulations Study**

Xiaoling Leng[†], Fangqiang Zhu and Stephen R. Wassall^{*}

Department of Physics, IUPUI, Indianapolis, IN 46202-3273

^{*}Email for correspondence swassall@iupui.edu

[†]Current address Institute of Molecular Biophysics, Florida State University, Tallahassee,
FL 32306–4380

This is the author's manuscript of the article published in final edited form as:

Leng, X., Zhu, F., & Wassall, S. R. (2018). Vitamin E Has Reduced Affinity for a Polyunsaturated Phospholipid: An Umbrella Sampling MD Simulations Study. The Journal of Physical Chemistry B. <https://doi.org/10.1021/acs.jpcb.8b05016>

ABSTRACT

Vitamin E is an essential micronutrient. The primary function of this lipid soluble antioxidant is to protect membrane phospholipids from oxidation. Whether vitamin E preferentially interacts with polyunsaturated phospholipids to optimize protection of the lipid species most vulnerable to oxidative attack has been an unanswered question for a long time. In this work we compared the binding of α -tocopherol (α toc), the form of vitamin E retained by the human body, in bilayers composed of polyunsaturated 1-stearoyl-2-docosahexaenoylphosphatidylcholine (SDPC, 18:0-22:6PC) and, as a control, monounsaturated 1-stearoyl-2-oleoylphosphatidylcholine (SOPC, 18:0-18:1PC) by umbrella sampling molecular dynamics (USMD) simulations. From the potential of mean force (PMF) as a function depth within the bilayer, we find that the binding energy of α toc is less in SDPC ($\Delta G_{bind} = 16.7 \pm 0.3$ kcal/mol) than in SOPC ($\Delta G_{bind} = 18.3 \pm 0.4$ kcal/mol). The lower value in SDPC is ascribed to the high disorder of polyunsaturated fatty acids (PUFA) that produces a less tightly packed arrangement. Deformation of the bilayer is observed during desorption, indicating that phosphatidylcholine (PC)-PC, as well as α toc-PC, interactions contribute to the binding energy. Our results do not support the proposal that vitamin E interacts more favorably with polyunsaturated phospholipids.

INTRODUCTION

Vitamin E stands for the name of a group of naturally occurring, biologically active tocopherol (tocopherol and tocotrienol) compounds.^{1,2} α -Tocopherol (α toc) is the form that is retained in the human body. This lipid-soluble antioxidant is an essential micronutrient. Neurological problems are among the symptoms of its deficiency in the diet.³ There is general agreement that the primary function for α toc is to protect polyunsaturated lipids that are extremely susceptible to attack from free radicals in membranes.^{2,4-6} The growing consumption of fish oil supplements rich in omega-3 polyunsaturated fatty acids (n-3 PUFA) that are taken up into membrane phospholipids underscores the importance of this role.⁷⁻¹⁰ Although the chemistry by which α toc inhibits the peroxidation of lipid chains is known,¹¹⁻¹² an open question is whether preferential interaction with polyunsaturated lipids exists.¹³⁻¹⁵

The α toc molecule is composed of a chromanol head group with a hydroxyl group at one end and a phytyl chain at the other end (Figure 1). It usually resides in a membrane with the hydroxyl group near the aqueous interface while the phytyl chain extends towards the center.¹⁶⁻¹⁸ In this arrangement the sacrificial hydroxyl group traps lipid peroxyl radicals when they rise up to the surface of the membrane and subsequently is recycled by a water-soluble reducing agent such as ascorbate.¹⁹ The general features of the location and orientation of α toc in phospholipid bilayers are reproduced in the few atomistic molecular dynamics (MD) simulations that have been published to date.²⁰⁻²² Our simulations demonstrated how efficiently α toc is able to fulfill its antioxidant role in a polyunsaturated phospholipid bilayer.²² They revealed that high disorder enhances the probability that a polyunsaturated chain comes into close proximity with the hydroxyl group on a chromanol group that resides at the surface of the bilayer. Additionally, we observed flip-flop for α toc is fast, which suggests the hydroxyl group can also easily penetrate

into the interior of a polyunsaturated membrane to intercept lipid peroxy radicals.

Here we follow up on our earlier simulations with an umbrella sampling molecular dynamics (USMD) simulation study that further compares the molecular organization of α toc in 1-stearoyl-2-docosahexaenoylphosphatidylcholine (SDPC, 18:0-22:6PC) and 1-stearoyl-2-oleoylphosphatidylcholine (SOPC, 18:0-18:1PC) bilayers. The molecular structure of SDPC with saturated stearic acid (SA, 18:0) for the *sn*-1 chain and docosahexaenoic acid (DHA, 22:6) containing 6 double bonds for the *sn*-2 chain is representative of a polyunsaturated phospholipid.²³ SOPC where the *sn*-2 chain is replaced by oleic acid (OA, 18:1) that has a single double bond serves as a monounsaturated control (Figure 1). From the results, we obtain estimates of the binding energy and rate of flip-flop for α toc and gain insight into the two processes. Does α toc prefer polyunsaturated phospholipids, as has been hypothesized,^{13,15} is a question that we address.

METHODS

MD simulations

We prepared two lipid membrane systems: SDPC with α toc and SOPC with α toc. In each case there are in total 80 phosphatidylcholine (PC) lipid molecules and 2 α toc molecules, which are divided equally between the two leaflets. The initial structures of these lipid bilayers were taken from our earlier published simulations containing 20 mol% α toc, which have been equilibrated and run for over 200 ns.²² We deleted the extra α toc molecules, leaving two of them - one in each leaflet - to get the bilayer content we needed. In order to ensure enough room to allow an α toc molecule to be fully pulled out of the bilayer into the aqueous phase, the bilayers were hydrated with 8562 water molecules that made a water layer of approximately 45 Å on each

side. A harmonic constraint was applied to the two α toc molecules to keep them 35 Å apart in the xy plane, which is approximately $\frac{1}{2}$ the diagonal length of the bilayer, to minimize interactions between them during the umbrella sampling process. We used the CHARMM C36p force field for all simulations.²⁴ Non-bonded (van der Waals and short-range electrostatic) interactions were cut off at 12 Å by a switching function starting at 10 Å and the long-range electrostatic interactions were calculated using the particle-mesh Ewald method with grid spacing 1.0 Å.²⁵ Periodic boundary conditions were applied to a rectangular box in which the x and y dimensions (in the plane of the membrane) were held equal while the z dimension (in the direction of the membrane normal) was varied independently. The temperature and pressure were kept at 37 °C and 1 atm, respectively, by the Langevin dynamics (damping coefficient 1/ps) and piston method.²⁶

Umbrella sampling

With USMD simulations we obtained the free energy, known as the potential of mean force (PMF), as a function of α toc's distance from the bilayer center. Then the energy cost of pulling an α toc molecule out from the bilayer, which we call the binding energy, was determined from the corresponding PMF profile.

A brief description of our procedure follows. The distance along the direction (z) of the membrane normal between the center of the bilayer and the center of mass of the rigid chromanol head group on each α toc molecule was chosen to represent the location of α toc - the reaction coordinate. The α toc molecules in both leaflets were restrained symmetrically with respect to the center of bilayer, which maintains the compositional balance of the bilayer and doubles the sampling efficiency. 45 windows were simulated with the value of z_i varying from 0

to 44 Å in increments of 1 Å. In each window i , the α toc molecules were restrained by a harmonic potential

$$U_i = \frac{1}{2}k(z - z_i)^2 \quad (1)$$

with a force constant $k = 3.68 \text{ kcal}/(\text{mol} \cdot \text{\AA}^2)$ that was chosen to optimize the separation between Gaussian distributions in adjacent windows according to an established criterion²⁷ at the corresponding z_i value. The initial structures in the windows were generated by a series of simulations that pulled the α toc molecules from the equilibrium position to $z = 0 \text{ \AA}$ or $z = 44 \text{ \AA}$ with a speed of 1 Å/ns. The Hamiltonian replica exchange method was applied during umbrella sampling to optimize the process.²⁸ Production runs were performed with NAMD2²⁹ on the Big Red II super computer at Indiana University. Their duration was 200 ns in each window, with the last 100 ns of the trajectories employed to perform the PMF calculation using the weighted histogram analysis method (WHAM).^{30,31} In the WHAM calculation, bin size is set to 0.01 Å, the lower and upper boundaries in SDPC are -1.95 Å and 55.92 Å and in SOPC are -1.77 Å and 55.74 Å. The numerical optimization was done by the BFGS algorithm, with the convergence tolerance set to 2^{-52} , the minimum relative change that can be represented by a double-precision floating-point number. Errors were estimated following a previously described protocol³¹ in which the statistical errors of the average positions are calculated by the block averaging method.³²

RESULTS

Potential of mean force

Figure 2 shows the PMF ($G(z)$) vs. position relative to the center of the bilayer calculated from our simulations for α toc in SDPC and SOPC. As expected, the shape of the plots resembles

that reported for cholesterol^{33,34} where, like α toc, the sterol consists of a rigid moiety (tetracyclic ring) with a hydroxyl group at one end and a branched chain at the opposite end.¹⁵ The variation of $G(z)$ is flat where α toc becomes completely disengaged from the membrane ($z \geq 35$ Å) in the water, and the baseline ($G = 0$) is set there. When α toc is brought into contact with phospholipid, $G(z)$ starts to drop as α toc penetrates more deeply within the membrane until the equilibrium location is reached (where $G(z) = G(z_{eq})$ exhibits a minimum). Then there is small increase in $G(z)$ towards the center of the bilayer ($z = 0$) that represents a barrier to movement from one side of the bilayer to the other. As in analogous studies on cholesterol,³⁴ we define the binding energy of α toc in the membrane to be $\Delta G_{bind} = -G(z_{eq})$ and the free energy barrier for the flip-flop of α toc between leaflets to be $\Delta G_{flip} = G(0) - G(z_{eq})$. Table 1 lists the magnitude of these quantities for SOPC and SDPC. Also included in the table are values for the equilibrium depth z_{eq} of α toc in each bilayer (10.1 Å in SDPC and 11.4 Å in SOPC), which qualitatively agree with estimates made from our previous standard all-atom MD simulations (11.3 Å in SDPC and 11.8 Å in SOPC).²² Exact agreement is not be expected in view of the increase in bilayer order and thickness that accompanies the higher concentration of the vitamin in the earlier work (20 vs. 2.5 mol%).

Binding energy

The binding energy ΔG_{bind} evaluated here for α toc by UBSMD simulations in SDPC (16.7 kcal/mol) is less favorable than in SOPC (18.3 kcal/mol) (Table 1). These values are comparable to the few other reports to date of a binding energy from USMD simulations for α toc in PC bilayers,^{35,36} although direct comparison is inappropriate given that the force fields are not the same. From the plots of PMF (Fig. 2), we can see that the differential in ΔG_{bind} between

SOPC and SDPC originates in the interfacial region ($z \approx 35 \text{ \AA}$) where αtoc starts to interact with the membrane. An abrupt change in the slope of the PMF curve occurs there in SOPC, from flat when αtoc is entirely in the aqueous phase to an approximately linear decline as the phytol sidechain and then the chromanol group penetrates more deeply into the bilayer. In SDPC, the change in slope of the PMF curve is initially more gradual ($31 \leq z \leq 35 \text{ \AA}$) and only subsequently at greater depth within the bilayer does the curve become approximately linear in slope and falls approximately parallel to the curve in SOPC. We attribute this behavior to the more loosely packed nature of the polyunsaturated bilayer that reduces the thickness of the bilayer. The PMF curve in SDPC consequently lies a bit above that in SOPC, resulting in a correspondingly smaller binding energy.

Flip-flop

Between the equilibrium position (z_{eq}) and the center of membrane ($z = 0$), there is an increase in the PMF which denotes a barrier to the transverse movement of αtoc (Fig. 2). The barrier reflects that insertion of the hydrophilic OH group on the chromanol head group into the hydrophobic interior of the bilayer is energetically unfavorable. A plot (Figure S3, Supporting Information) of the number of water molecules within $5 \text{ \AA}$ from the hydroxyl group in different simulation windows reveals desolvation accompanies the rise in free energy from z_{eq} ($z \approx 10 \text{ \AA}$) to near the center of the bilayer ($z \approx 3 \text{ \AA}$), implying that the dehydration energy contributes to the flip-flop barrier. That the barrier in SDPC ($\Delta G_{flip} = 1.9 \text{ kcal/mol}$) is lower than in SOPC ($\Delta G_{flip} = 2.5 \text{ kcal/mol}$) indicates that flip-flop of αtoc from one side of the bilayer to the other is easier in the polyunsaturated than monounsaturated case (Table 1). A calculation of the rate of flip-flop now follows.

To estimate the rate of flip-flop for α toc, we adopt an approach applied to cholesterol in the past.³⁴ A complete flip-flop is defined as α toc moving from z_{eq} (equilibrium position) in one leaflet to z_{eq} in the other leaflet. In this process the molecule moves from z_{eq} to the membrane center ($z = 0$) and then to z_{eq} on the other side of the bilayer. The respective rate constants k_f (from z_{eq} on one side of the bilayer to $z = 0$) and k_d (from $z = 0$ to z_{eq} on the other side of the bilayer) that characterize these motions are related via

$$k_f = k_d e^{-\frac{\Delta G_{flip}}{RT}}, \quad (2)$$

and equate to the rate of flip-flop (from z_{eq} on one side to z_{eq} on the other side of the bilayer)

$$k_{flip} = \frac{1}{(k_f^{-1} + k_d^{-1})} \times \frac{1}{2}. \quad (3)$$

The factor of $\frac{1}{2}$ is included in eq. 3 to take into account the possibility that an α toc molecule that has reached the center of the membrane can return to its original leaflet as well as cross over to the other leaflet. To obtain an estimate of the value of k_d , 10 standard MD simulations were performed with α toc initially located at the center of membrane and the time (t_d) it takes to move back to z_{eq} without harmonic restraint was measured.

Table 2 lists the results of our calculations. They reveal that the average rate of flip-flop $\bar{k}_{flip}$ is approximately 3 times greater in SDPC ($\bar{k}_{flip} = 7.5 \times 10^5 \text{ s}^{-1}$) than SOPC ($\bar{k}_{flip} = 2.5 \times 10^5 \text{ s}^{-1}$). The difference is qualitatively consistent with the greater number of flip-flop events observed for α toc in the two membranes in our previous standard simulations.²² Over 180 ns of simulation there were 22 flip-flops ($k_{flip} \sim 3 \times 10^6 \text{ s}^{-1}$) in SDPC as opposed to just 2 ($k_{flip} \sim 3 \times 10^5 \text{ s}^{-1}$) in SOPC. The level of agreement with the values calculated for $\bar{k}_{flip}$ in the current study is reasonable given the somewhat rudimentary nature of the model used here and the poor statistics of an estimate based upon counting only a limited number of instances of flip-flop in

the short duration of the earlier simulations.

Membrane deformation

Insight into the effect of desorption of α toc on membrane structure may be gleaned from inspection of the simulations obtained during umbrella sampling. Deformation of the SDPC and SOPC membrane is observed in the windows where α toc has been partly pulled out from the membrane. Figure 3A shows this deformation as a plot of $z(r)$, which is the average z coordinate (relative to the center of the bilayer) of the central C2 atom on the glycerol backbone, against their lateral distance r from α toc in different windows – i.e. α toc held at different depths in the membrane. The curves are mostly flat from window 0, where the α toc molecule is at the middle of the bilayer, to window 10, which is its equilibrium position. For windows 20 and 30, when α toc is in the interfacial region, nearby ($r \leq 15$ Å) there is an increase in $z(r)$. This indicates the phospholipid molecules located near α toc extend into water more than ones farther away - in other words, the pulling of α toc out of the bilayer deforms the membrane. The snapshot presented in Figure 3B gives an illustration of the deformation. Once α toc is completely in water, the membrane returns to normal as shown in window 35 where no deformation is apparent. We take the difference in $z(r)$ between the window ($r = 5$ Å) where it is greatest in magnitude and where α toc is at its equilibrium depth as a measure of how much the membrane is deformed when α toc is removed. The difference is 4.7 Å (between windows 30 and 10) in SDPC, which is less than the difference of 5.6 Å in SOPC (between windows 31 and 11). Membrane deformation, we note, was also observed in other USMD simulations³⁷ and has been applied to estimate the physical property of membranes.³⁸

Behavior at aqueous interface

In the replica exchange method, it is possible that neighboring windows swap their replicas and cause them to take a random walk between the windows.³⁹ In our simulations, we found examples of replicas that migrate through the interface between a window with α toc entirely in water and a window with α toc in contact with the membrane. The snapshots in Figure 4 give an illustration of the two states. With the trajectories of such replicas, we can take a close look at how α toc leaves the membrane into the water and, vice versa, enters the membrane from water. When α toc leaves the membrane, it begins from a state that has the chromanol head in the water and the phytyl tail is still inside the membrane and extends down into the interior (Fig. 4A). When α toc becomes fully pulled into the water (by replica exchange), the orientation of the chromanol and the whole molecule including the phytyl tail quickly becomes random and surrounded by water (Fig. 4B). On the other hand, when α toc enters the membrane from water, the phytyl chain encounters the membrane first. We did not find that water molecules penetrate into the membrane with the phytyl chain in this process.

Membrane deformation is also apparent when α toc enters the membrane from bulk water, which is illustrated in Figure S4 (Supporting Information) by snapshots of α toc re-inserting into the bilayer of SOPC from bulk water during the replica-exchange process (Fig. S4A). An accompanying plot of average z coordinate for the central C2 atom of the glycerol backbone in SOPC as a function of lateral distance from the α toc molecule, furthermore, indicates that the bilayer is flat before α toc contacts the membrane and then is deformed after the phytyl side chain re-inserts and interacts with the lipid (Fig. S4B). The amount of deformation is comparable to while α toc is at a similar depth when pulled out of the membrane. The implication is that the deformation is energetically favorable for the whole system when α toc is partially inserted into

the membrane, presumably due to the α toc-PC interactions.

Chromanol orientation

How the orientation of the chromanol group on α toc changes with depth in the membrane was mapped with a two-dimensional probability histogram $\rho(z, r_z)$. The orientation is represented by the tilt of the chromanol ring r_z , as defined by $r_z \equiv \cos(\theta)$ where θ is the angle that the C3-C6 vector on the chromanol group makes with the bilayer normal (Fig. 5A). It can be shown that if the orientation is completely random and uniform in 3-dimensional space, r_z will obey a uniform distribution between -1 and +1. z and r_z are divided into bins that are 0.5 Å and 0.02 in respective size. The values of $\rho(z, r_z)$ at each z are normalized so that they sum to one.

The two-dimensional histograms of orientation for the chromanol group obtained in SDPC and SOPC are plotted in Figure 5B. They are similar in the two membranes. As would be expected, the chromanol group has no preferred direction when the entire α toc molecule is in water ($z > 35$ Å). When the phytyl chain penetrates the bilayer ($z < 35$ Å), it packs parallel to the surrounding phospholipid chains and the orientation of the chromanol group becomes constrained in a distribution that tends to line up with the bilayer normal ($r_z = 1$, $\theta = 0^\circ$). Near the disordered center of the bilayer ($z \sim 0$ Å), however, the situation changes and the orientation becomes almost uniformly distributed again. It indicates that α toc would undergo a lot of reorientation as it crosses the membrane, and that our simulations have a good sampling of the orientation of the chromanol.

DISCUSSION

Vitamin E binds less strongly to a polyunsaturated phospholipid

The role of α toc in the plasma membrane is to protect polyunsaturated phospholipids from oxidation, an activity that requires close contact with the lipid peroxy radicals formed during the chain reaction by which oxidation proceeds. Because the global concentration of α toc is small (< 1 mol% of all lipids),¹⁶ how it can effectively protect the comparatively larger amount of polyunsaturated phospholipid remains a question.¹⁵ The answer may lie in the unique membrane properties of PUFA chains that contain multiple -C-C=C-C- units. The C-C bonds next to each C=C bond have a shallow rotational energy barrier, so that PUFA chains are tremendously disordered.⁴⁰ This disorder provides a membrane environment necessary for the function of specific proteins.⁴¹ It also produces an aversion for cholesterol that drives the formation of PUFA-rich domains depleted in the sterol,⁴² leading to the hypothesis that α toc preferentially co-localizes with PUFA in such domains to increase the local concentration of α toc around the lipid species most in need of protection from oxidation.¹⁵

Our simulations appear to contradict the proposal that α toc and polyunsaturated phospholipids preferentially interact with each other. They reveal that there is a higher binding energy for α toc associated with monounsaturated SPC (18.3 kcal/mol) than with polyunsaturated SDPC (16.7 kcal/mol) (Table 1). A partition coefficient $K_{SDPC}^{SOPC} = 10.9$ (SPC with respect to SDPC), correspondingly, was evaluated from the profile of PMF (Fig. 2) by integrating the probabilities associated with $G(z)$ via

$$K_{SDPC}^{SOPC} = \frac{\int e^{-\frac{G_{SPC}(z)}{RT}} dz}{\int e^{-\frac{G_{SDPC}(z)}{RT}} dz}. \quad (5)$$

The limits of the integrals are between $z = 0$ (α toc at the center of the membrane) and the value of z where the profile levels off (α toc no longer in membrane), while R and T are respectively the gas constant and temperature. These results imply that were SDPC and SPC domains (hypothetically) to exist in a membrane, α toc would prefer SPC over SDPC - i.e. the

polyunsaturated domain would not be favored. A somewhat analogous preference for saturated over unsaturated PC was found in coarse grain (CG) MD simulations run on vitamin E added to a binary mixture of 1,2-dipalmitoylphosphatidylcholine (DPPC, 16:0-16:0PC) and 1,2-dilinoleoylphosphatidylcholine (18:2-18:2PC).⁴³ However, the situation is far more complex in a biological membrane. Plasma membranes contain cholesterol, sometimes >50 mol% of total lipid,⁴⁴ with an affinity for predominantly saturated sphingolipids that drives segregation into sphingolipid-rich/cholesterol-rich domains (lipid rafts).⁴⁵ We postulate that the high concentration of cholesterol in these domains will exclude α toc into PUFA-rich/sterol-poor (non-raft) regions. To test this possibility, we plan to perform UBSMD simulations comparing the binding energy of α toc for bilayers in the presence of cholesterol. CG simulations that can reproduce the heterogeneous distribution of lipids in a membrane⁴⁶ offer another approach that we plan to try to directly observe the partitioning of α toc between domains.

The trend we saw here for the binding of α toc is the same as seen for cholesterol. Greater affinity for saturated than unsaturated lipids has been measured for cholesterol by experiment^{47,48} and USMD simulation.^{33,34} Upon reflection, this observation is not too surprising given the similarity in molecular structure of cholesterol and α toc.¹⁵ Both molecules possess a rigid moiety with a hydroxyl group, which usually anchors at the aqueous interface, at one end and a hydrophobic chain, which extends to extend towards the middle of the bilayer, at the opposite end. Like in the case of cholesterol, the more disordered lipid environment in a polyunsaturated bilayer is responsible for the differences in binding energy for α toc. SDPC is thinner and more loosely packed (larger area per lipid) than SOPC.²² Thus, in the plot of PMF the change of $G(z)$ is more gradual at the aqueous interface (z between 30-35 Å), as well as near the center of membrane (from z_{eq} to $z = 0$), for α toc in SDPC compared to SOPC (Fig. 2).

Our USMD simulations furnish further details of the desorption process. In the windows where α toc is sticking out from the membrane, we noticed deformation of the membrane (Fig. 3). The implication is that not only α toc-phosphatidylcholine (PC) interactions, but also PC-PC interactions, contribute to the binding energy ΔG_{bind} . Snapshots from the replica exchange method also reveal the strongly hydrophobic nature of the behavior of α toc near the aqueous interface (Fig. 4). The water layer that surrounds an α toc molecule when it is in water does not follow it into the bilayer hydrophobic interior. This avoids the possibility that a water pore forms during the simulation, which might affect the accuracy of the PMF by perturbing membrane order.⁴⁹

Vitamin E flip-flops more often in a polyunsaturated bilayer

Flip-flop of phospholipids across protein-free membranes is slow because the hydrophobic interior of a lipid bilayer is an energetically unfavorable environment for hydrophilic head groups.⁵⁰ The timescale of the process is hours or longer, depending upon head group and chain composition.⁵¹ Reflecting the faster flip-flop that accompanies increased unsaturation, the rate measured is 40% greater for SDPC vs. SOPC.⁵² Few estimates of the rate of flip-flop exist for α toc.¹⁶ A more rapid timescale of seconds or less, akin to cholesterol,⁵³ is the growing consensus in recent work.²⁰⁻²²

The smaller energy barrier at the center of the bilayer identified in the profile of PMF for SDPC than SOPC (Fig. 2) shows that it is easier for α toc to translocate across a thinner, highly disordered polyunsaturated membrane. This assessment is confirmed by the faster rate of flip-flop calculated for SDPC than SOPC (Table 2), and agrees with the higher incidence of flip-flop observed for α toc in SDPC vs. SOPC in our previous standard simulations.²² The same trend of

1
2
3 faster flip-flop in more unsaturated, disordered membranes was seen for cholesterol in MD
4 simulations.^{34,54,55} Fast flip-flop for α toc in SDPC implies the vitamin could easily penetrate the
5
6 interior of a polyunsaturated bilayer to intercept lipid peroxy radicals, which would help in the
7
8 protection against oxidation.²² Our simulations also provide insight on the path that the flip-flop
9
10 of α toc takes. An upright orientation that remains lined up with the bilayer normal applies to the
11
12 chromanol group throughout most of the bilayer in both SDPC and SOPC (Fig. 5B). In the
13
14 vicinity middle of the bilayer, in contrast, the orientation becomes random so that the chromanol
15
16 group can invert and place the OH group towards the aqueous interface on the opposite side of
17
18 the membrane. This scenario is similar to that envisaged for the flip-flop of cholesterol in lipid
19
20 membranes studied by the USMD method.^{34,54}
21
22
23
24
25
26
27

28 **Concluding remarks**

29
30
31 In summary, our USMD simulations demonstrate that the dynamics and organization of
32
33 vitamin E in many respects resemble that of structurally similar cholesterol. Paralleling the
34
35 behavior of cholesterol in phospholipid bilayers, the binding energy of α toc is lower and its flip-
36
37 flop is faster with increased acyl chain unsaturation. Our findings do not support the proposal
38
39 that vitamin E binds preferentially to polyunsaturated phospholipids. We postulate that the
40
41 overwhelming higher concentration of cholesterol found in an animal plasma membrane,
42
43 however, may cause α toc to co-localize with polyunsaturated phospholipids. According to this
44
45 idea, cholesterol displaces α toc from regions enriched in saturated lipids for which the sterol has
46
47 high affinity into regions enriched in polyunsaturated lipids for which the sterol has poor affinity.
48
49
50
51
52
53

54 **ASSOCIATED CONTENT**

1
2
3 **Supporting Information**
4

5 Additional results derived from simulations.
6
7

8
9
10 **ACKNOWLEDGEMENTS**
11

12 This work was supported by the School of Science, IUPUI. The simulations were performed on
13
14 the Big Red II supercomputer at Indiana University.
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

REFERENCES

- (1) Zingg, J. M. Vitamin E: an overview of major research directions. *Mol. Aspects Med.* **2007**, *28*, 400-422.
- (2) Niki, E.; Traber, M. G. A history of vitamin E. *Ann. Nutr. Metab.* **2012**, *61*, 207-212.
- (3) Ulatowski, I.; Manor, D. Vitamin E trafficking in neurologic health and disease. *Ann. Rev. Nutr.* **2013**, *33*, 87-103.
- (4) Packer, L. Vitamin E is nature's master antioxidant. *Sci. Am. Sci. Med.* **1994**, *1*, 54-63.
- (5) Wang, X.; Quinn, P. J. Vitamin E and its function in membranes. *Prog. Lipid Res.* **1999**, *38*, 309-336.
- (6) Traber, M. G.; Atkinson, J. Vitamin E, antioxidant and nothing more. *Free Rad. Biol. Med.* **2007**, *43*, 4-15.
- (7) Calder, P. C. Marine omega-3 fatty acids and inflammatory processes: Effects, mechanisms and clinical relevance. *Biochim. Biophys. Acta* **2015**, *1851*, 469-484.
- (8) Hou, T. Y.; McMurray, D. N.; Chapkin, R. S. Omega-3 fatty acids, lipid rafts, and T cell signaling. *Eur. J. Pharmacol.* **2016**, *785*, 2-9.
- (9) Shaikh, S. R. Biophysical and biochemical mechanisms by which dietary N-3 polyunsaturated fatty acids from fish oil disrupt membrane lipid rafts. *J. Nutr. Biochem.* **2012**, *23*, 101-105.
- (10) Raederstorff, D.; Wyss, A.; Calder, P. C.; Weber, P.; Eggersdorfer, M. Vitamin E function and requirements in relation to PUFA. *Brit. J. Nutr.* **2015**, *114*, 1113-1122.
- (11) Burton, G. W.; Ingold, K. U. Vitamin E: application of the principles of physical organic chemistry to the exploration of its structure and function. *Acc. Chem. Res.* **1986**, *19*, 194-201.
- (12) Alessi, M.; Paul, T.; Scaiano, J. C.; Ingold, K. U. The contrasting kinetics of peroxidation

of vitamin E-containing phospholipid unilamellar vesicles and human low-density lipoprotein. *J. Am. Chem. Soc.* **2012**, *124*, 6957-6965.

(13) Diplock, A. T.; Lucy, J. A. The biochemical modes of action of vitamin E: a hypothesis. *FEBS Lett.* **1973**, *29*, 205-210.

(14) Kagan, V. E. Tocopherol stabilizes membranes against phospholipase A, free fatty acids, and lysophospholipids. *Annals. NY Acad. Sci.* **1989**, *570*, 121-135.

(15) Atkinson, J.; Harroun, T.; Wassall, S. R.; Stillwell, W.; Katsaras, J. The location and behavior of α -tocopherol in membranes. *Mol. Nutr. Food Res.* **2010**, *54*, 641-651.

(16) Atkinson, J.; Epand, R. F.; Epand, R. M. Tocopherols and tocotrienols in membranes: a critical review. *Free Rad. Biol. Med.* **2008**, *44*, 739-764.

(17) Marquardt, D.; Kučerka, N.; Katsaras, J.; Harroun, T. A. α -Tocopherol's location in membranes is not affected by their composition. *Langmuir* **2015**, *31*, 4464-4472.

(18) Ausili, A.; de Godos, A. M.; Torrecillas, A.; Aranda, F. J.; Corbalán-García, S.; Gómez-Fernández, J. C. The vertical location of α -tocopherol in phosphatidylcholine membranes is not altered as a function of the degree of unsaturation of the fatty acyl chains. *Phys. Chem. Chem. Phys.* **2017**, *19*, 6731-6742.

(19) Marquardt, D.; Williams, J. A.; Kučerka, N.; Atkinson, J.; Wassall, S. R.; Katsaras, J.; Harroun, T. A. Tocopherol activity correlates with its location in a membrane: a new perspective on the anti-oxidant vitamin E. *J. Am. Chem. Soc.* **2013**, *135*, 7523-7533.

(20) Qin, S-S.; Yu, Z-W.; Yu, Y-X. Structural and kinetic properties of α -tocopherol in phospholipid bilayers, a molecular dynamics simulation study. *J. Phys. Chem. B* **2009**, *113*, 16537-16546.

(21) Qin, S-S.; Yu, Z-W. Molecular dynamics simulations of α -tocopherol in model

membranes. *Acta Phys. - Chim. Sin.* **2011**, *27*, 213-227.

(22) Leng, X.; Kinnun, J. J.; Marquardt, D.; Ghefli, M.; Kucerka, N.; Katsaras, J.; Atkinson, J.; Harroun, T. A.; Feller, S. E.; Wassall, S. R. α -Tocopherol is well designed to protect polyunsaturated phospholipids: MD simulations. *Biophys. J.* **2015**, *109*, 1608-1618.

(23) Leng, X.; Kinnun, J. J.; Cavazos, A. T.; Canner, S. W.; Shaikh, S. R.; Feller, S. E.; Wassall, S. R. All n-3 PUFA are not the same: MD simulations reveal differences in membrane organization for EPA, DHA and DPA. *Biochim. Biophys. Acta* **2018**, *1860*, 1125-1134.

(24) Klauda, J.B.; Monje, V.; Kim, T.; Im, W. Improving the CHARMM force field for polyunsaturated fatty acid chains. *J. Phys Chem. B* **2012**, *116*, 9424-9431.

(25) Darden, T.; York, D.; Pedersen, L. Particle mesh Ewald: an $n \cdot \log(n)$ method for Ewald sums in large systems. *J. Chem. Phys.* **1993**, *98*, 10089-10092.

(26) Feller, S. E.; Zhang, Y.; Pastor, R. W.; Brooks, R. B. Constant pressure molecular dynamics simulation: the Langevin piston method. *J. Chem. Phys.* **1995**, *103*, 4613-4621.

(27) Bereau, T.; Swendsen, R. H. Optimized convergence for multiple histogram analysis. *J. Comp. Phys.* **2009**, *228*, 6199-6129.

(28) Fukunishi, H.; Watanabe, O.; Takada, S. On the Hamiltonian replica exchange method for efficient sampling of biomolecular systems: application to protein structure prediction. *J. Chem. Phys.* **2002**, *116*, 9058-9067.

(29) Phillips, J. C.; Braun, R.; Wang, W.; Gumbart, J.; Tajkhorshid, E.; Villa, E.; Chipot, C.; Skeel, R. D.; Kalé, L.; Schulten, K. Scalable molecular dynamics with NAMD. *J. Comp. Chem.* **2005**, *26*, 1781-1802.

(30) Kumar, S.; Bouzida, D.; Swendsen, R. H.; Kollman, P. A.; Rosenberg, J. M. The weighted histogram analysis method for free-Energy calculations on biomolecules 1. The

method. *J. Comp. Chem.* **1992**, *13*, 1011-1021.

(31) Zhu, F.; Hummer, G. Convergence and error estimation in free energy calculations using the weighted histogram analysis method. *J. Comp. Chem.* **2012**, *33*, 453-465.

(32) Flyvbjerg, H.; Petersen, H. G. Error-estimates on averages of correlated data. *J. Chem. Phys.* **1989**, *91*, 461-466.

(33) Zhang, Z.; Lu, L.; Berkowitz, M. L. Energetics of cholesterol transfer between lipid bilayers. *J. Phys. Chem. B* **2008**, *112*, 3807-3811.

(34) Bennett, W. D.; MacCallum, J. L.; Hinner, M. J.; Marrink, S. J.; Tieleman, D. P. Molecular view of cholesterol flip-flop and chemical potential in different membrane environments. *J. Am. Chem. Soc.* **2009**, *131*, 12714-12720.

(35) Meng, F. Molecular simulation of α -tocopherol passing across DPPC lipid using potential of mean force and accelerated molecular dynamics method. *J. Theor. Comput. Chem.* **2013**, *12*, 1341011-1-1341011-16.

(36) Boonnoy, P.; Karttunen, M.; Wong-ekkabut, J. Alpha-tocopherol inhibits pore formation in oxidized bilayers. *Phys. Chem. Chem. Phys.* **2017**, *19*, 5699-5704.

(37) Neale, C.; Pomes, R. Sampling errors in free energy simulations of small molecules in lipid bilayers. *Biochim. Biophys. Acta* **2016**, *1858*, 2539-2548.

(38) Pieffet, G.; Botero, A.; Peters, G. H.; Forero-Shelton, M.; Leidy, C. Exploring the local elastic properties of bilayer membranes using molecular dynamics simulations. *J. Phys. Chem. B* **2014**, *118*, 12883-12891.

(39) Sugita, Y.; Kitao, A.; Okamoto, Y. Multidimensional replica-exchange method for free-energy calculations. *J. Chem. Phys.* **2000**, *113*, 6042-6051.

(40) Feller, S. E. Acyl chain conformations in phospholipid bilayers: a comparative study of

docosahexaenoic acid and saturated fatty acids. *Chem. Phys. Lipids* **2008**, *153*, 76-80.

(41) Grossfield, A.; Feller, S. E.; Pitman, M. C. Contribution of omega-3 fatty acids to the thermodynamics of membrane protein solvation. *J. Phys. Chem. B* **2006**, *110*, 8907-8909.

(42) Wassall, S. R.; Stillwell, W. Polyunsaturated fatty acid–cholesterol interactions: domain formation in membranes. *Biochim. Biophys. Acta* **2009**, *1788*, 24-32.

(43) Muddana, H. S.; Chiang, H. H.; Butler, P. J. Tuning membrane phase separation using nonlipid amphiphiles. *Biophys. J.* **2012**, *102*, 489-497.

(44) Stillwell, W. *An Introduction to Biological Membranes (Second Edition)*. Elsevier Science, Oxford, UK, **2016**.

(45) Lingwood, D.; Simons, K. Lipid rafts as a membrane-organizing principle. *Science* **2010**, *327*, 46–50.

(46) Risselada, H. J.; Marrink, S. J. The molecular face of lipid rafts in model membranes. *Proc. Natl. Acad. Sci.* **2008**, *105*, 17367-17372.

(47) Nui, S. L.; Litman, B. J. Determination of membrane cholesterol partition coefficient using a lipid vesicle-cyclodextrin binary system: effect of phospholipid acyl chain unsaturation and headgroup composition. *Biophys. J.* **2002**, *83*, 3408-3415.

(48) Williams, J. A.; Wassall, C. D.; Kemple, M. D.; Wassall, S. R. An EPR method for measuring the affinity of a spin labeled analog of cholesterol for phospholipids. *J. Membrane Biol.* **2013**, *246*, 689-696.

(49) Neale, C.; Madill, C.; Rauscher, S.; Pomès, R. Accelerating convergence in molecular dynamics simulations of solutes in lipid membranes by conducting a random walk along the bilayer normal. *J. Chem. Theor. Comput.* **2013**, *9*, 3686-3703.

(50) Contreras, F.-X.; Sánchez-Magraner, L.; Alonso, A.; Goni, F. M. Transbilayer (*flip-flop*)

lipid motion and lipid scrambling in membranes. *FEBS Lett.* **2010**, *584*, 1779-1786.

(51) Homan, R; Pownall, H. J. Transbilayer diffusion of phospholipids: dependence on headgroup structure and acyl chain length. *Biochim. Biophys. Acta* **1998**, *938*, 155-166.

(52) Armstrong, V. T.; Brzustowicz, M. R.; Wassall, S. R.; Jenski, L. J.; Stillwell, W. Rapid flip-flop in polyunsaturated (docosahexaenoate) phospholipid membranes. *Arch. Biochem. Biophys.* **2003**, *414*, 74-82.

(53) Hamilton, J. A. Fast flip-flop of cholesterol and fatty acids in membranes: Implications for membrane transport proteins. *Curr. Opin. Lipidol.* **2003**, *14*, 263-271.

(54) Jo, S.; Rui, H.; Lim, J. B.; Klauda, J. B.; Im, W. Cholesterol flip-flop: insights from free energy simulation studies. *J. Phys. Chem. B* **2010**, *114*, 13342-13348.

(55) Bennett, W. F. D; Tieleman, D. P Molecular simulation of rapid translocation of cholesterol, diacylglycerol, and ceramide in model raft and nonraft membranes. *J. Lipid Res.* **2012**, *53*, 421-429.

Table 1 Energetic terms derived from the PMF profile of *atoc*.

	Binding Energy (kcal/mol)	Flip-flop barrier (kcal/mol)	Equilibrium depth (Å)
SDPC	16.7±0.3	1.9±0.2	10.1
SOPC	18.3±0.4	2.5±0.1	11.4

Table 2 Kinetic properties of α toc flip-flop in SDPC and SOPC at 310 K. Data are taken from the average of 20 cases (2 α toc in 10 standard MD simulations) - 16/20 cases in SDPC and 15/20 cases in SOPC (α toc did not reach equilibrium within 60 ns in the rest of the cases). The range of t_d and derived rate constants are shown for reference, as well as the average rate of flip-flop.

	t_d (ns)	k_d (s ⁻¹)	k_f (s ⁻¹)	k_{flip} (s ⁻¹)	$\bar{k}_{flip}$ (s ⁻¹)
SDPC	8 to 55	1.3×10^8 to 1.8×10^7	5.7×10^6 to 8.3×10^5	2.7×10^6 to 4.0×10^5	7.5×10^5
SOPC	8 to 60	1.3×10^8 to 1.7×10^7	2.2×10^6 to 2.9×10^5	1.1×10^6 to 1.4×10^5	2.5×10^5

FIGURE LEGENDS

Figure 1 Molecular structure of α toc, SDPC and SOPC.

Figure 2 PMF for α toc partitioning from water to the center of the bilayer in SDPC (red) and SOPC (green). The PMF was set to zero in the aqueous phase (Fig. S1 in Supporting Information depicts an alternative alignment with the PMF set to zero at z_{eq}). Error bars are the standard error calculated using block averaging. Supporting Information, in addition, includes two-dimensional PMF (in z and $r_z \equiv \cos(\theta)$ to represent orientation) that were also calculated from our 1-dimensional sampling data (Fig. S2).

Figure 3 A. Average z coordinate (with maximum discrepancy of 0.4 Å) of the central C2 atom of the glycerol backbone in PC lipids as a function of lateral distance r from the α toc molecule at in different windows (different depths of the chromanol group).

B. Snapshot of the system when α toc has been half-pulled out from the membrane (SDPC). α Toc is shown as a space-filling model, the head groups of PC lipid are shown in an orange surface representation and the acyl chains of PC lipids are shown as blue lines.

Figure 4 Snapshots from the trajectory of one replica that swaps between the windows of α toc in and out of the membrane - α toc with the chromanol group restrained in the water and the phytol chain inside the membrane (A), and with the whole molecule completely in water (B). α Toc is shown as a space-filling model (chromanol group colored in light blue and phytol chain colored in orange), water molecules within a distance of 5 Å from α toc are shown as blue balls, and PC lipids with their head group within a lateral distance of 10 Å from α toc are shown in

licorice representation.

Figure 5 Probability distribution for the orientation of the chromanol group on α toc (A) as a function of depth in SDPC and SOPC (B). The orientation is denoted by $r_z \equiv \cos(\theta)$, where θ is the angle that the C3-C6 vector on the chromanol group makes with the bilayer normal.

α toc

SDPC

SOPC

Figure 1

Figure 2

A

B

Figure 3

Figure 4

A

B

Figure 5

Toc Graphic

Figure 1. Molecular structure of α toc, SDPC and SOPC.

46x26mm (600 x 600 DPI)

PMF for atoc partitioning from water to the center of the bilayer in SDPC (red) and SOPC (green). The PMF was set to zero in the aqueous phase (Fig. S1 in Supporting Information depicts an alternative alignment with the PMF set to zero at z_{eq}). Error bars are the standard error calculated using block averaging. Supporting Information, in addition, includes two-dimensional PMF (in z and $r_z \equiv \cos(\theta)$ to represent orientation) that were also calculated from our 1-dimensional sampling data (Fig. S2).

776x529mm (72 x 72 DPI)

Figure 3. (A). Average z coordinate (with maximum discrepancy of $0.4 \text{ \AA}$) of the central C2 atom of the glycerol backbone in PC lipids as a function of lateral distance r from the αtoc at in different windows (different depths of the chromanol group). (B). Snapshot of the system when αtoc has been half-pulled out from the membrane (SDPC). αToc is shown as a space-filling model, the head groups of PC lipid are shown in an orange surface representation and the acyl chains of PC lipids are shown as blue lines.

146x260mm (300 x 300 DPI)

Figure 4. Snapshots from the trajectory of one replica that swaps between the windows of α toc in and out of the membrane - α toc with the chromanol group restrained in the water and the phytyl chain inside the membrane (A), and with the whole molecule completely in water (B). α Toc is shown as a space-filling model (chromanol group colored in light blue and phytyl chain colored in orange), water molecules within a distance of 5 Å from α toc are shown as blue balls, and PC lipids with their head group within a lateral distance of 10 Å from α toc are shown in licorice representation.

60x44mm (300 x 300 DPI)

Figure 5. Probability distribution for the orientation of the chromanol group on atoc (A) as a function of depth in SDPC and SOPC (B). The orientation is denoted by $r_z \equiv \cos(\theta)$, where θ is the angle that the C3-C6 vector on the chromanol group makes with the bilayer normal.

102x127mm (300 x 300 DPI)