Radical damage in lipids investigated with the fragment molecular orbital method

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Abstract

To quantify the thermodynamics for hydrogen abstraction lipids, the fragment molecular orbital method (FMO) is used to calculate structures and energies of the reactants and products. The analytic second derivative is developed for the open-shell Hartree-Fock formulation of FMO and used to calculate zero point energy corrections. The accuracy of FMO is evaluated for a lipid model and the errors in reaction energies are found not to exceed 0.5 kcal/mol. The reaction energies determined for multiple sites in two lipids are used to discuss likely sites and pathways of radical initiation in membranes.

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1. Introduction

Oxygen is necessary for human life, but it is also slowly killing us [1,2]. Mitochondria couple cellular respiration with an oxidative phosphorylation reaction to generate adenosine triphosphate (ATP), which is used as a source of chemical energy by the cell. Side products of mitochondria activity are reactive oxygen species (ROS), which account for 1-5% of products [3,4]. Thus, mitochondria are an endogenous source of ROS, however there are additional exogenous sources such as metal-ion-catalyzed reactions and ionizing radiation [5,6]. The persistent presence of ROS results in a state of oxidative stress that ultimately leads to apoptosis or cell death [7,8]. Oxidative stress is a contributor to cancer and multiple amyloid diseases such as diabetes, rheumatoid arthritis, Alzheimer's and Parkinson's diseases [5,9]. Aging is thought to be an accumulation of damaged biological structures due to oxidative stress [10]. Although there are normal physiological processes that utilize ROS for cell signaling, ROS reactions are typically viewed as deleterious chemical processes [4,11].

Oxidative stress is a chronic physiological state where oxidation reactions occur with greater frequency and overwhelm the biological repair mechanisms [12]. Oxidative stress causes chemical reactions where highly reactive species oxidize biomolecules [10,13,14,15], ROS damage protein, DNA, carbohydrates, and lipids [2,11,15,16,17]. Oxidation reactions require electron migration, and often produce open-shell radical intermediates. Oxidative chemistry is a component in many physiological conditions, yet the molecular mechanisms remain poorly

1 understood. Investigation of mechanisms of radical reactions is challenging because the radical
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4 species are typically short-lived and thus difficult for experimental observation.
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7 Life is possible in part because biological functions are compartmentalized by membranes
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10 surrounding cells, organelles, and vesicles. Lipids are an integral component in membranes.
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13 The physical properties of the membranes are controlled by lipid composition, and lipid
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16 chemistries affect function. Lipids are amphipathic molecules with both hydrophobic (“fears
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19 water”) and hydrophilic (“loves water”) groups. Lipids form bilayers with the hydrophobic
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22 portion of the molecules on the interior, and the hydrophilic portion of the molecules on the
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25 exterior. The hydrophilic lipid heads contain a negatively charged phosphate group and a
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28 positively charged choline group, whereas the hydrophobic tails contain long fatty acid chains.
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32 Lipid oxidation is thought to occur on the carbon between the double bonds in polyunsaturated
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35 fatty acids (PUFA) tails [2,18]. The carbon between two double bonds is preferred because the
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38 resulting radical intermediate is stabilized through resonance of the unpaired electron with two
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41 double bonds. Radical damaged PUFAs are repaired by a cellular process involving tocopherol
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44 and ascorbate [18].
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48 Hydrogen abstraction (HA) is the first step in the oxidative pathway initiated by reactive
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51 oxygen or nitrogen species, associated with oxidative stress. Further oxidation reactions lead to the
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54 formation of other lipid oxidation products ($R\cdot$, $RO\cdot$, $ROO\cdot$, $ROOH$) [11]. Aerobic oxidation
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57 chemistry in lipids also leads to cleavage reactions. The lipid oxidation products decompose and
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1 cleave the fatty acid chain, with the major products being aldehydes such as malondialdehyde and
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4 4-hydroxynonenal.
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7 A mechanism for oxidation under aerobic conditions consists of an initiation, a propagation
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10 and a termination.
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13 Initiation:

23 Propagation:

33 Termination:

47 Oxygen initiates the pathway in HA reactions, by producing two hydroxyl radicals ($\cdot\text{OH}$) and
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49 two lipid radicals ($\text{R}\cdot$). Hydroxyl radical can initiate another HA reaction to make additional lipid
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51 radicals. The addition of oxygen to the lipid radical produces the lipid peroxy radical ($\text{ROO}\cdot$).
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54 Both the lipid peroxy radical and the lipid radical propagate and create additional radical species.
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58 The radicals are terminated when any two combine and create a bond between them.
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1 This work seeks to understand the effects of oxidative stress on a typical lipid. The lipid
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4 palmitoyl oleoyl phosphatidyl choline (POPC) is selected because it is one of the most prevailing
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7 phospholipids. POPC has a 16:0 chain for the first tail, a 18:1 chain on the second tail, and a
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10 phosphatidyl choline head. In membranes, POPC forms monolayers in the liquid-crystal phase,
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13 which is the biologically most relevant phase. The peroxidation of linoleic acid catalyzed by the
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16 enzyme 15-lipoxygenase was studied theoretically [19]. Also, hydrogen abstraction for peptides
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19 was investigated theoretically [20]. Typically, lipids and membranes are studied with molecular
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22 mechanics (MM) [21,22,23,24]. The cost of quantum-mechanical (QM) methods results in a
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25 necessity to constrain a molecular system size and to consider only the pertinent functional groups,
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28 either the head and backbone [25,26,27] or tail [28].
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32 Recent developments in fragment-based QM methods significantly extend a possible system
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35 size[29], and some methods are developed specifically for open-shell (radical) systems [30,31,32].
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38 The fragment molecular orbital method (FMO) performs fragment calculations in the electrostatic
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41 field of the whole system [33,34,35,36]. FMO is interfaced with the restricted open-shell
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44 Hartree-Fock (HF), ROHF, and second order Møller-Plesset perturbation theory (ROMP2)
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47 methods suitable for calculating open-shell systems [37,38,39,40].
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51 Previously, FMO has been used to examine three (proteins, carbohydrates, and nucleic acids)
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54 of the four main types of biological molecules. In this Letter, validation studies are conducted for
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57 the fourth type, lipids. The effect of various fragmentation schemes is examined on a lipid model,
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and FMO is applied to predict most likely hydrogen abstraction sites as a result of a chemical reaction of POPC with a hydroxyl radical. The reaction energies for various sites are computed with zero point energy corrections (ZPE), to obtain which the analytic Hessian is developed for FMO-ROHF. Dispersion interactions important in hydrophobic species are accounted for by performing ROMP2 calculations.

2. Methodology and computational details

The FMO energy for a system divided into N fragments is evaluated as [37]

$$E = \sum_I^N E'_I + \sum_{I>J}^N (E'_{IJ} - E'_I - E'_J + \text{Tr}(\Delta \mathbf{D}^{IJ} \mathbf{V}^{IJ})), \quad (1)$$

where E'_I and E'_{IJ} are the internal energies of fragments and their pairs, respectively, obtained by subtracting the contribution of the embedding electrostatic potential $\mathbf{V}$ from the respective HF energies. The density transfer matrix is defined as the difference between the electron densities of pairs and individual fragments, $\Delta \mathbf{D}^{IJ} = \mathbf{D}^{IJ} - (\mathbf{D}^I \oplus \mathbf{D}^J)$.

In this Letter, the analytic Hessian for FMO-ROHF was developed by reformulating the Hessian for unrestricted Hartree-Fock [41]; an arbitrary number of open-shell fragments may be used in the FMO-ROHF Hessian. Differentiating the energy in Eq. 1 with respect to nuclear coordinates a and b , one obtains

$$\frac{\partial E}{\partial a \partial b} = \sum_I^N \frac{\partial E'_I}{\partial a \partial b} + \sum_{I>J}^N \left(\frac{\partial E'_{IJ}}{\partial a \partial b} - \frac{\partial E'_I}{\partial a \partial b} - \frac{\partial E'_J}{\partial a \partial b} + \frac{\partial \text{Tr}(\Delta \mathbf{D}^{IJ} \mathbf{V}^{IJ})}{\partial a \partial b} \right) \quad (2)$$

Explicit equations are given in the supplemental materials. Although FMO-ROHF can be thought as a particular case of FMO-UHF with the restriction that α and β spin orbitals are identical, and FMO-UHF Hessian was developed earlier [41], the equations for the Hessian need to be derived specifically for ROHF due to the presence of a single Fock matrix for both α and β spins. The derivative of $Tr(\Delta\mathbf{D}^{IJ}\mathbf{V}^{IJ})$ for ROHF is rather similar to UHF, but the derivatives of the internal energies E'_I and E'_{II} related to the Fock matrix require modifications, as described in the supplemental materials. Additionally, there are differences between ROHF and UHF in coupled perturbed equations, and ROHF has an extra two-electron interaction term because of the restriction on spin orbitals [40]. The Raman activities for FMO-ROHF were implemented using the finite field approach [42] and IR intensities were also developed.

The Hessian was implemented in the FMO code [43] in GAMESS-US [44] and parallelized with the generalized distributed data interface [45]. This program was used for all calculations; Facio [46] was used for making FMO input files. Calculations were performed on the community cluster “Carter” within Information Technologies at Purdue Research Computing center.

The accuracy of FMO-ROHF Hessian was evaluated in comparison with full ROHF Hessian. For this purpose, vibrational enthalpy and entropy, as well as IR and Raman spectra were calculated for a mixture of 10 oxygen molecules (triplet spin state) and 31 nitrogen molecules (singlet spin state) in the high spin coupling for dimers [40], with the total multiplicity of 21. The geometry was optimized with full and FMO-based ROHF, and the obtained minimum for each

method was used for Hessian calculations.

The reactions studied in this Letter can be described as

The lipid and lipid radical were computed with restricted closed shell and open-shell methods, respectively. FMO is used for lipids and hydroxyl and water are computed with full QM. The 6-31G* basis set is used in all calculations. The default values of thresholds were used and the fragmentation in FMO was done using the hybrid orbital projection operator [47]. For radical species, doublet spin states were computed.

First, the FMO method is validated in lipids by comparing the energetics of HA reactions to a full QM reference (throughout this work, “full” with respect to QM methods implies a calculation without fragmentation) for 1,2-dibutyl glycerol model system. This diglyceride, containing 32 atoms, is the lipid without the head and long tails.

Then, the FMO method is applied to HA in POPC, in the gas phase. The structure of POPC was extracted from a single snapshot of classical MD trajectory of a solvated lipid bilayer [48] and optimized with FMO-MP2/6-31G*. The structure of diglyceride model system was generated from the POPC structure by removing the head and long tails and optimized with MP2/6-31G*. In this way, POPC and diglyceride systems have similar conformations, which is important for the analysis of relative HA enthalpies.

The calculation setup was as follows. The reaction enthalpies ΔH at 0 K were obtained by

adding the electronic energies ΔE and ZPE corrections ΔE^{ZPE} as

$$\Delta H = \Delta E + \Delta E^{ZPE} \quad (4)$$

where the reaction energy ΔE is obtained by subtracting the energies of reactants and products following Eq. 3 ΔE^{ZPE} were obtained with HF (open or closed shell, as appropriate) Hessians for HF optimized structures. It was found that for two POPC sites, 28 and 211, ROHF failed to converge, so for those reactions unrestricted HF and MP2 were used [41,49].

3. Results and discussion

3.1 Accuracy of FMO-ROHF Hessian

The accuracy of FMO-ROHF is evaluated in comparison with full ROHF on a test system composed of 10 oxygen and 31 nitrogen molecules (divided into 1 molecule per fragment). The Raman spectra are shown in Figure 1 (the corresponding IR spectra had no peaks so they are not plotted). FMO-ROHF reproduces full ROHF results without fragmentation reasonably well. The low frequency region has somewhat larger errors because these normal modes are delocalized over several oxygen and nitrogen molecules.

The maximum and root mean square deviation for frequencies is 2.56 and 0.74 cm^{-1} , respectively. The vibrational enthalpy and entropy difference between FMO-ROHF and full ROHF calculations is 0.6 kcal/mol and -0.1 cal/(mol • K), respectively.

3.2. Validation of FMO for HA reactions in lipids

The diglyceride model shown in Figure 2 has a three-carbon glycol backbone; the four-carbon tails are attached at carbons 1 and 2 in the glycol backbone, and the OH head is attached at carbon 3. Different fragmentation patterns are necessary for describing different HA radical products in order to avoid fragmenting near radical sites. Potential sites for HA are along the backbone (carbons 1, 2 and 3) and on tails (carbons 12-14 and 21-24). For convenience, numbering in the diglyceride is consistent with numbering in the POPC lipid.

The full QM and FMO reaction energies for various HA reaction in the diglyceride are displayed in Table 1. The FMO scheme provides excellent agreement with full QM calculations; the error in most cases is less than 0.1 kcal/mol, two larger deviations (0.52 and 0.11 kcal/mol) are caused by the delocalization to the allylic group adjacent to the reaction site. For two sites (12 and 3), two different fragmentation schemes were applied to check the effect of the fragmentation pattern on the accuracy. It was found that the difference is negligible.

HA is the most exothermic at sites 3, 12 and 22 in the diglyceride. Radicals on carbon sites adjacent to oxygen or carbonyl are more energetically favorable than on the sites adjacent to saturated carbons. The magnitude of the HA energies decreases as the distance from the carbonyl increases, as seen in the series 12, 13 and 14 and concurrently in 22, 23, and 24. Reaction energies along the glycol backbone (sites 1 and 2, at 10 kcal/mol and 11 kcal/mol, respectively) are similar to the energies at the sites that are two carbons away from the carbonyls (sites 13 and 23,

which are both 11 kcal/mol).

3.3. Application of FMO to study HA in POPC

POPC molecule is shown in Fig. 3 and atomic coordinates are given in Table S1 in SI. Sites 1, 2, and 3 are adjacent carbons in the glycol backbone in POPC. Tail length is an important differentiator; lipid tails are numbered from the start of the tail at the carbon in the carbonyl functional group in the ester linkage to the end of the tail. The second tail in POPC is unsaturated, with a cis double bond between the 9th and 10th carbons. Carbons 28 and 211 are allylic sites that are expected to be the most prone for oxidation.

Different fragmentation schemes (see Fig. 3; more information is given in the supplemental materials) are necessary to avoid fragmentation next to radical sites. Carbon-carbon single bonds are selected for fragmentation in all patterns. HA at sites 1, 17, 18, 24, 25, 26, 29, and 214 use pattern I; HA at carbon 12 uses pattern II; HA at site 211 uses pattern III; and HA at site 28 uses pattern IV. Patterns I, II and VI have six fragments, and pattern III has five fragments per lipid. For each HA reaction, the neutral POPC is calculated with the same fragmentation scheme as the radical product.

HA reaction enthalpies were determined for selected sites in POPC, using FMO (Figure 4). The terminal allylic site 211 is the most favorable for HA, with the energy of -22.3 kcal/mol. This is not surprising as the radical is stabilized by π interactions with a nearby double bond. Interestingly the other allylic site 28 is not as favored as site 211. This might be due to a more

restricted conformation of the site 28 that is on the glycol backbone side of the double bond compared the 211 site that is on the tail terminus side of the double bond.

Sites 12 and 25 both have slightly higher reaction enthalpy at -18.1 and -18.3 than the allylic site 28 at -16.7 kcal/mol. In site 12 the radical site is adjacent to carbonyls and stabilized by interactions with them. The sites that are not adjacent to either allyl or carbonyl groups, for example, carbons 17, 18, 24, 25, and 214, have an average HA energy value of -15.3 ± 1.9 kcal/mol, with a range from -13.8 to -18.3 kcal/mol. The least favored HA energy of -5.7 kcal/mol is observed for carbon 29 that participates in a double bond.

Saturated carbons in the diglyceride tails show a decrease in the magnitude of the HA energy as the distance from the ester group and the site number increases (see Table 1 for the diglyceride system). In POPC, the saturated carbons in the POPC tails (sites 17, 18, 24 and 25) do not show the same trend, apart from a noticeable decrease in the HA energy magnitude between the sites adjacent to the allyl or carbonyl groups and the other saturated sites. We hypothesize that the sites near the end of the tail experience additional destabilization of the radical products, which can explain the observed decrease in the magnitude of the HA energy in the diglyceride model. Additionally, local non-covalent interactions might play a role in stabilizing or destabilizing the HA energy at each given site.

HA energies in diglyceride and POPC are compared in Figure 5 and Table 2. The magnitudes of the HA energies at sites 1, 12, and 22 are 1.5-2 kcal/mol lower in the diglyceride than in POPC.

1 Site 24, which is the terminal site in the diglyceride, shows the greatest deviation of 5.3 kcal/mol.

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4 These results suggest that the diglyceride is an appropriate model for lipids if oxidation processes
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7 are of interest, with a possible exception for the terminal sites, where the tails, truncated in the
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10 diglyceride make some contribution.

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13 The importance of dispersion can be seen in Figures 4 and 5, where both ROHF and ROMP2
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16 results are shown. ROHF considerably underestimates reaction energies in a consistent fashion, the
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19 contribution due to the correlation energy is on the order of 10 kcal/mol in POPC and somewhat
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22 less in diglyceride. The most outstanding difference is observed for site 1 in POPC: ROHF predicts
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25 a negligible reaction enthalpy, while adding ROMP2 correlation changes it to a very substantial
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28 value. Site 214 shows a similar trend.

31 32 33 34 35 36 **5. Conclusions**

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39 The analytic FMO-ROHF Hessian has been developed and applied to study reaction enthalpies
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42 of radical reactions. The accuracy of FMO-ROHF Hessian is evaluated by taking a comparison
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45 with full *ab initio* Hessian, and a reasonable agreement has been obtained for both Raman
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48 spectrum and vibrational enthalpy and entropy. The error of FMO-ROHF method in evaluating
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51 reaction energies of the diglyceride does not exceed 0.5 kcal/mol for 11 patterns of the reaction
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54 sites and fragmentations.

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58 In the application of FMO to the hydrogen abstraction reaction in a typical lipid POPC, dozens
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1 of FMO-based MP2 geometry optimizations and HF Hessians have been conducted for the systems
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4 consisting of 134 atoms (133 for the radical). The most favorable and unfavorable sites have been
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7 identified with implications in analyzing radical damage in lipids. The importance of including the
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10 electron correlation for hydrophobic lipids has been clearly demonstrated.
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13 The effect of molecular environment on the reaction energies has been probed by comparing a
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16 minimal (diglyceride) and realistic (POPC) lipids, as well as different sites in each lipid. The
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19 radical stabilization due to the resonance with adjacent double bonds tends to increase the stability
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22 of the radical product whereas abstraction of hydrogen from a double bond decreases it. Sites with
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25 a similar molecular environment of the two lipids have similar reaction energies, whereas
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28 elongating the chain in POPC considerably changes the energy for the terminal site in the
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31 diglyceride. The two tails are not symmetric in lipids as is obvious from their molecular structures,
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34 and the reaction energies also differ.
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39 The validation of FMO for lipids opens a new field of future applications of FMO to lipids and
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42 membranes, including development of drug delivery systems [50]. The fragmentation pattern
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45 established in this work can be used for agglomerates of lipids, such as membranes. The validation
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48 studies can be used as a basis for future development of tools for automatic fragmentation of lipids,
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51 facilitating large-scale applications of FMO to lipids and membranes.
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53 54 55 56 57 58 **Acknowledgments** 59 60 61

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TABLES

Table 1. Hydrogen abstraction (HA) reaction energies ΔE (kcal/mol) for the diglyceride.

HA site	fragmentation pattern	Full QM ΔE	FMO ΔE	FMO error
1	A	-11.22	-10.71	0.52
12	A	-16.07	-16.08	-0.01
12	B	-16.07	-16.06	0.01
13	B	-11.92	-11.93	0.00
14	B	-9.47	-9.48	-0.01
2	B	-10.04	-9.93	0.11
22	A	-16.80	-16.84	-0.04
23	A	-11.41	-11.44	-0.03
24	A	-9.25	-9.30	-0.05
3	A	-16.94	-16.99	-0.05
3	B	-16.94	-16.98	-0.03

Table 2. Selected hydrogen abstraction reaction energies ΔE (kcal/mol).

lipid	site type	site	ΔE
diglyceride	backbone	3	-16.9
diglyceride	backbone	12	-16.1
diglyceride	backbone	22	-16.8
POPC	backbone	12	-17.9
POPC	backbone	22	-18.1
POPC	allyl	28	-15.7
POPC	allyl	211	-22.2

FIGURE CAPTIONS

Figure 1. Raman spectra for a molecular cluster composed of 10 oxygen and 31 nitrogen molecules. The red solid and blue dotted lines shows the spectra from FMO-ROHF and full ROHF Hessians, respectively. The spectra are divided into (a) low, (b) middle and (c) high frequency regions. The frequency is in cm^{-1} and the Raman activity in A^4/u .

Figure 2. Fragmentation patterns A (shown in blue) and B (shown in red) for the diglyceride model.

Figure 3. Fragmentation schemes used in FMO2 calculations for POPC (sn stands for stereospecifically numbered). The first tail is composed of carbon atoms 11-118, the second tail is composed of carbon atoms 21-218. Hydrogen atoms are not displayed.

Figure 4. Reaction enthalpies ΔH (kcal/mol) for various hydrogen abstraction (HA) sites in POPC.

Figure 5. Hydrogen abstraction (HA) reaction energies ΔE (kcal/mol) for selected sites in the diglyceride and POPC.

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Figure 1.

Figure 2.

Fragmentation Patterns: I, II, III, IV

Figure 3.

Figure 4.

Figure 5.

Radical damage in lipids

$$E = \sum_i^N E_i' + \sum_{i>j}^N (E_{ij}' - E_i' - E_j') + \sum_{i>j}^N \text{Tr}(\Delta D^{ij} \mathbf{V}^{ij})$$