Mechanisms of Photosensitized Lipid Oxidation and Membrane Permeabilization

Isabel O. L. Bacellar† and Mauricio S. Baptista*†

Departamento de Bioquímica, Instituto de Química, Universidade de São Paulo, Avenida Prof. Lineu Prestes 748, São Paulo, SP 05508-000, Brazil

ABSTRACT: Lipid oxidation encompasses chemical transformations affecting animals and plants in many ways, and light is one of the most common triggers of lipid oxidation in our habitat. Still, the molecular mechanisms and biological consequences of photoinduced lipid oxidation were only recently understood at the molecular level. In this review, we focus on the main mechanisms of photosensitized lipid oxidation and membrane permeabilization, dissecting the consequences of both singlet oxygen and contact-dependent pathways and discussing how these reactions contribute to chemical and biophysical changes in lipid membranes. We aim to enable scientists to develop novel and more efficient photosensitizers in photomedicine, as well as better strategies for sun protection.

INTRODUCTION

Photosensitized oxidations are involved in a multitude of phenomena triggered by the excitation of photosensitizer molecules by light, yielding excited states and other reactive species that ultimately elicit oxidative reactions. Because photosensitizers occur naturally in cells and biological structures, photosensitized oxidations are largely responsible for the detrimental effects of excessive sunlight in the skin and hair. In contrast, photosensitized oxidations are increasingly employed to treat diseases, such as cancer and bacterial infections, in the clinical modality named photodynamic therapy (PDT) (Figure 1). From the mechanistic perspective, extensive interaction between photosensitizers and lipid membranes often correlates with enhanced photodynamic efficiency, consistently with lipid bilayers surrounding the cell itself and many organelles. Since membranes are mostly composed of proteins and lipids, these biomolecules are the major targets of photoinduced cell inactivation.

In this mini-review, we focus exclusively on lipid damage and especially on membrane permeabilization, which compromises cell homeostasis and is cytotoxic. Indeed, many of the cell death routes operating in photosensitized oxidation conditions involve leakage of organelle content, resulting in regulated cell death pathways or—in the extreme case—disruption of the plasma membrane, leading to unregulated necrosis. Early studies on liposome permeabilization already attempted to assess membrane oxidation using colorimetric assays as general indicators of lipid oxidation. Even though the progress in the chemical analysis of oxidized lipids is clear, only recent studies described the molecular-level mechanisms of photoinduced lipid oxidation in conditions associated with membrane permeabilization.

Given the prominent role of membrane permeabilization in biological photosensitized oxidations, we aim to review the molecular mechanisms involved in this transformation and apply this knowledge to discuss ways of controlling the outcomes of PDT and skin photodamage. Even though the research on lipid oxidation extends beyond photosensitized oxidations, being broadly investigated in the contexts of food waste and disease (e.g., atherosclerosis), we limit our discussion to a variety of reactions elicited by photosensitizers. In addition to that, we constrain our discussion to monounsaturated lipids, which have been used in most of the mechanistic and biophysical studies on photosensitized oxidations. These lipids depend on external agents to be oxidized and generate fewer products than polyunsaturated lipids, which have bisallylic hydrogens and undergo auto-oxidation.

PHOTOSSENSITIZED OXIDATIONS IN THE CONTEXT OF LIPID OXIDATION AND MEMBRANE PERMEABILIZATION

Photosensitized oxidations start with light absorption by a photosensitizer, which is converted into an excited singlet state. This species may be converted to its lower excited triplet state by intersystem crossing, yielding a longer-lived species that has a higher probability of engaging in an electron or energy transfer process with molecules nearby. The latter mechanism is responsible for generating singlet oxygen \( \left[ \text{O}_2(\Delta) \right] \), an excited state of molecular oxygen that usually has a lifetime in the microsecond range and that is highly reactive against unsaturated lipids, proteins, and nucleic acids. In addition, both singlet and triplet excited states of photosensitizers are generally better reductants and oxidants than the ground-state photosensitizer, meaning that photosensitizers may engage in novel redox reactions after the absorption of light. The probability of excited states effectively

Received: October 1, 2019
Accepted: November 28, 2019
Published: December 12, 2019
engaging in biologically relevant redox reactions depends on their generation in the proximity of biological targets, given that their diffusion range is limited by their lifetimes. Moreover, these reactions face strong competition from singlet oxygen generation by excited triplet states: while photosensitizers usually have high singlet oxygen generation quantum yields and singlet oxygen is often considered a major player in PDT, the generation of superoxide radicals by direct electron transfer from excited triplet states to oxygen is believed to seldom occur. For this reason, superoxide radicals are unlikely to play a significant cytotoxic role if compared with singlet oxygen or photosensitizer excited state themselves.2,10

Several definitions were proposed to classify the mechanism of photosensitized oxidations, the most famous one being the classification in Type I and Type II originally proposed by C. S. Foote. This classification sorted processes depending on specific interactions of the excited state of the photosensitizer with a substrate or solvent (Type I) or with oxygen (Type II).11 This definition and others have been interpreted and employed in different ways in the literature (e.g., often the term “Type I” is used to describe electron-transfer reactions solely).10 In order to discuss the molecular details of photosensitized oxidation mechanisms with clarity, here we classify the first steps of photosensitized oxidations in the (i) contact-dependent pathway, in which the excited state of the photosensitizer directly reacts with the biological target, and (ii) contact-independent pathway, in which the excited state of the photosensitizer generates a mediator species (usually singlet oxygen), which diffuses and then reacts with the target (Figure 2). This definition shares similarities with the original classification by Foote, while emphasizing whether the oxidation of the biological target relies directly or not on an effective collision (i.e., direct reaction) with the excited state of the photosensitizer itself.

Contact-dependent pathways rely on the occurrence of an effective collision of the excited photosensitizer with the biological target (e.g., unsaturated lipids), triggering reactions such as initiation of lipid peroxidation by hydrogen abstraction. The effective collision needs to happen within the diffusion pathway of the excited state of the photosensitizer, which is limited by the excited state lifetime. Therefore, contact-dependent pathways clearly depend on the affinity and close proximity of the photosensitizer to its target (e.g., interaction

Figure 1. Photosensitized oxidations have both detrimental and therapeutic effects. Detrimental effects occur in damage to skin cells and hair exposed to excessive sunlight and due to the presence of natural photosensitizers. In contrast, artificial light sources and synthetic photosensitizers are used in photodynamic therapy (PDT) to selectively inactivate cancer cells, bacteria, and fungi. Both detrimental and therapeutic effects share the same basic mechanism, which results in biomolecule oxidation and cytotoxicity.

Figure 2. Excited states of photosensitizers oxidize substrates by contact-dependent or contact-independent pathways. (A) The photoexcitation of a photosensitizer (PS) in the ground singlet state \( S_0 \) results in an excited singlet state \( S_1 \), which may undergo intersystem crossing (ISC) and originate an excited triplet state \( T_1 \). In the contact-dependent pathway (B), the excited photosensitizer directly reacts with the target substrate. In contrast, in the contact-independent pathway (C), a mediator species is first formed by interaction or reaction with the excited photosensitizer. This mediator species, commonly singlet oxygen, may diffuse over hundreds of nanometers before reacting with the target substrate.
with lipid bilayers). In contrast, contact-independent pathways rely on the formation of a mediator species (e.g., singlet oxygen) via interactions or reactions involving the excited photosensitizer. In this case, it is not the excited photosensitizer, but this mediating, diffusive species that undergoes an effective collision with the biological target. Note that the mediator species may also be an excited state and have a limited diffusion length; however, the average diffusion length of singlet oxygen, which is the main agent in contact-independent pathways, is at least 1 order of magnitude larger than the thickness of lipid membranes. As a result, the effective collision between singlet oxygen and unsaturated lipids may happen considerably far from the original location where the photosensitizer excited state was generated, meaning that contact-independent pathways have a potentially larger action range if compared with contact-dependent ones and may still be biologically relevant even for photosensitizers that remain in aqueous solution instead of tightly interacting with biomolecules.

The overall contribution of each mechanism depends on specific properties of the photosensitizer (e.g., oxidation and reduction potentials) and on the available substrates for electron or energy transfer. From the kinetics point of view, the outcome of the photosensitization event is governed by the relative rate constant for each process and by the relative concentration of molecular oxygen and substrates for direct reactions. As mentioned above, since excited states have a limited lifetime, substrates must be available within the diffusion range of the excited photosensitizer. As a result, membrane binding is a prerequisite for contact-dependent pathways. This factor is especially relevant when contact-dependent processes elicited by singlet and triplet excited states are compared. Given that for organic molecules the former may live more than 1 order of magnitude less than the latter, excited singlet states may need to be generated already in molecular contact with the biological target. Instead, longer-lived excited species may have time to reach the target through diffusion. In addition to that, other factors such as photosensitizer aggregation and interaction with proteins also modulate the photosensitization mechanism, while the membrane itself additionally affects excited triplet state lifetimes.

The distinction between contact-dependent and contact-independent pathways applies solely to the initial steps of photooxidation. Once primary reaction products are formed (e.g., lipid radicals or reduced photosensitizer species), subsequent chemical reactions ensue according to the available species and their reactivity. These steps often do not directly involve excited photosensitizers or do not happen directly after photosensitizer excitation, meaning that at this stage the classification in contact-dependent and contact-independent mechanisms is no longer appropriate or obvious. Yet, these reactions may be of biological relevance and generate highly oxidant species such as hydroxyl radicals or even excited states. One example of such processes is the combination and ensuing decomposition of two peroxyl radicals, yielding singlet oxygen or excited carbonyls. Likewise, semireduced photosensitizer radicals may generate superoxide radicals by one-electron reduction of oxygen, demonstrating that the initial photosensitization step may trigger a variety of reactions that proceed without direct photon absorption.

Below, we first review studies describing the mechanisms and consequences of the contact-independent pathway. Next, we provide a detailed discussion of the primary processes involved in the contact-dependent pathway, followed by a review of the critical role of truncated lipid aldehydes in membrane permeabilization.

**Contact-Independent Pathway: Singlet Oxygen as a Mediator.** Singlet oxygen reacts via the ene reaction with unsaturated lipids, being those sterols or lipids bearing fatty acyl chains. Allylic hydrogens are required for the ene reaction to occur, meaning that saturated lipids have low reactivity toward singlet oxygen. The ene reaction yields allylic lipid hydroperoxides solely in the E (trans) configuration, with the number of positional isomers increasing for polyunsaturated lipids. For example, the oxidation of oleic acid (18:1 $\Delta^9$), which has a single unsaturation in the Z configuration, yields two positional isomers with a single unsaturation in the E configuration: 18:1 $\Delta^{10}$ and 18:1 $\Delta^8$, bearing –OOH groups in positions 9 and 10, respectively.

When singlet oxygen is the only oxidizing agent, oxidation of monounsaturated lipids exclusively yields lipid hydroperoxides. Lipid hydroperoxides accumulate in the membranes, being stable in the absence of high temperatures, acids, or transition metal ions. In addition to that, there is no evidence of the occurrence of a second ene reaction event involving the newly formed double bond in monounsaturated lipid hydroperoxides. In contrast, tandem singlet oxygen additions have been reported for polyunsaturated substrates (including lipids) in both the case of the ene reaction and other types of reactions carried out by singlet oxygen.

To assess the role of singlet-oxygen-mediated reactions compared with other processes elicited by photosensitizers, it is essential to consider the magnitude of the rate constant for the ene reaction between singlet oxygen and lipids. This parameter is usually determined by monitoring (i) the characteristic near-infrared (NIR) luminescence of singlet oxygen or (ii) changes in the concentration of products and reagents. The former method allows for direct measurements of the singlet oxygen lifetime, which is the reciprocal of its first-order decay constant. By varying the concentration of quenchers (e.g., lipids) and employing the Stern–Volmer relationship, bimolecular rate constants for singlet oxygen quenching ($k_q$) may be obtained. Nevertheless, $k_q$ encompasses both chemical and physical quenching, which cannot be separately determined by NIR luminescence. This fact is a significant limitation of this methodology, given that many compounds suppress singlet oxygen via both mechanisms. Instead, chemical quenching constants may be independently measured by monitoring changes in the chemical composition of samples, such as the formation of specific hydroperoxide isomers. The most significant drawback of this methodology is the interference of competing reactions (e.g., contact-dependent pathways), yielding common or similar products to ene reaction products, perhaps super estimating the singlet oxygen chemical quenching constant.

While saturated lipids quench singlet oxygen only physically, both chemical and physical deactivation channels are relevant for unsaturated lipids. For instance, a singlet oxygen luminescence study employing fatty acids in carbon tetrachloride solution showed that $k_q$ ranged from $10^3$ to $10^6 \text{M}^{-1} \text{s}^{-1}$ for saturated fatty acids, depending on the number of hydrogen atoms. For unsaturated fatty acids, the magnitude of $k_q$ additionally depended on the number of allylic and especially bisallylic hydrogens, as expected for chemical quenching. For oleic acid (18:1), the presence of one double bond led to a $k_q$ value of...
1.7 × 10^4 M⁻¹ s⁻¹, which is twice as high as for stearic acid (18:0). For the polyunsaturated linoleic (18:2) and linolenic (18:3) acids, the measured quenching constants were even higher than for oleic acid. Indeed, whereas for oleic acid the chemical quenching deactivation channel was estimated to have a 60% contribution to the overall quenching, this figure increased to 95% for arachidonate.

The determination of singlet oxygen quenching constants directly in lipid membranes is challenging. For NIR luminescence studies, data analysis in microheterogeneous systems requires models that are much more complex than the models usually employed for isotropic solutions since diffusion and partition of singlet oxygen must be considered. Moreover, the best available models for membrane systems are fairly insensitive to variations of singlet oxygen lifetime in membranes: because membranes are only a few nanometers thick, singlet oxygen diffuses to the aqueous medium within nanoseconds. As a result, shortly after the excitation pulse, the luminescence signal has a greater contribution originating from the membrane, while the luminescence resulting from the decay in aqueous medium dominates most of the ensuing luminescence kinetics. Nevertheless, even the brief contribution coming from the membrane translates poorly the suppression of singlet oxygen by lipids, given that the diffusion of singlet oxygen out of the membrane contributes to the signal as the main deactivation channel. Therefore, the literature lacks precise determinations of the singlet oxygen lifetime in membranes by NIR luminescence and, consequently, of the effect of lipid composition on this parameter. As an approximation for membrane-based environments, singlet oxygen lifetimes have been determined in phosphatidylcholine films. The measured values are in the range of ca. 5–20 μs, falling in the lower end for hydrated films and on the higher end for dry films.

Methods based on quantification of photooxidation products have also been employed to determine the rate constant for the reaction of singlet oxygen with lipids in membranes. By monitoring spectral changes caused by the formation of hydroperoxide conjugated dienes, a value of 7.5 × 10^5 M⁻¹ s⁻¹ was measured in egg yolk phosphatidylcholine liposomes. Paying attention to biophysical changes to the membrane, Weber et al. exploited the increase in membrane surface area caused by lipid hydroperoxides to derive the rate of lipid hydroperoxide formation in 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) giant unilamellar vesicles (GUVs) containing a membrane-anchored photosensitizer. The authors calculated a rate constant value of 3 × 10^6 M⁻¹ s⁻¹ and
estimated that ca. one in every five singlet oxygen molecules undergo chemical quenching under these conditions.14

Since the singlet oxygen generation quantum yield of photosensitizers is usually high, lipid hydroperoxides tend to be the major lipid oxidation product and accumulate in the membranes.5 Lipid hydroperoxides have different properties compared with their nonoxidized lipid counterparts and adopt a distinct conformation when inserted in the bilayer. Due to its capability of forming hydrogen bonds with water and lipid polar heads, the hydroperoxide group migrates to the polar head region of the bilayer, introducing a bend in the oxidized lipid chain. Computational and experimental studies show that the bending of the fatty acyl chain results in a 15−20% increase in membrane surface area upon full conversion of POPC or 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) to hydroperoxides (Figure 3A), which is accompanied by a 15−20% decrease in membrane thickness.5,22 Even though several other transformations were reported to occur due to the formation of lipid hydroperoxides (e.g., changes in lipid lateral phase separation behavior33 and a ca. 4-fold decrease in the membrane stretching modulus14), full conversion of monounsaturated lipids to hydroperoxides does not permeabilize the membrane toward sugars or cause pore opening, as observed in experiments with GUVs and also in computational simulations of membranes (Figure 3B).3,14,24,25 Furthermore, the formation of hydroperoxides was shown by molecular dynamics simulations to be insufficient to explain the dramatic increase in permeability of electroporated membranes.26

Contact-Dependent Pathway: Radical-Mediated Lipid Oxidation. Lipid peroxidation initiated by contact-dependent reactions starts with a direct reaction between a lipid and the excited state of a photosensitizer, being followed by radical-mediated reactions. Differently from singlet-oxygen-mediated lipid oxidation, which proceeds via a single type of reaction, the contact-dependent pathway involves many classes of reactions and yields different kinds of products depending on the photosensitizer and subtracts. For this reason, a significant part of the topics covered in this section is derived from general studies on lipid peroxidation, whenever possible relating to specific features of photosensitized oxidation. The discussion mainly focuses on monounsaturated lipids since we are mainly interested in the initial oxidation processes triggered by light.

Classically, lipid peroxidation is divided into three phases, which are initiation, propagation, and termination. Initiation refers to the creation of lipid carbon-centered radicals. These radicals quickly react with oxygen, forming peroxyl radicals. In the propagation step, peroxyl radicals abstract hydrogen atoms from nonoxidized lipids, forming lipid hydroperoxides and new carbon-centered radicals, which may engage in further propagation reactions and extend lipid oxidation. Nevertheless, the continuation of the propagation sequence may be interrupted if two peroxyl radicals react and form a nonradical species, which is considered a termination step.

Under photosensitized oxidation conditions, initiation may happen via a direct reaction between a nonoxidized lipid and the excited state of the photosensitizer. Excited states may abstract hydrogen atoms from lipids, forming lipid hydroperoxides and new carbon-centered radicals, which may engage in further propagation reactions and extend lipid oxidation. Nevertheless, the continuation of the propagation sequence may be interrupted if two peroxyl radicals react and form a nonradical species, which is considered a termination step.
allylic hydrogens was calculated as being 79 kcal mol$^{-1}$ for methyl oleate acid. For bisallylic hydrogens, the value is lower (e.g., 70 kcal mol$^{-1}$ for methyl linoleate),\textsuperscript{26} while for allyl hydrogens the value is estimated to be ca. 10 kcal mol$^{-1}$ higher.\textsuperscript{29} For this reason, saturated lipids are resistant to oxidation.

The excited triplet state of riboflavin was shown to abstract hydrogens from polyunsaturated methyl esters with rate constants larger than $10^5$ M$^{-1}$s$^{-1}$ but with values of the magnitude of $10^4$ M$^{-1}$s$^{-1}$ or smaller for the monounsaturated methyl oleate.\textsuperscript{28} The latter value is orders of magnitude smaller than the quenching rate of excited triplet states by oxygen,\textsuperscript{9} suggesting that singlet oxygen generation may be the major triplet deactivation route unless in oxygen-deprived samples or if the photosensitizer is in close contact with substracts for direct reactions. Excited triplet states of other classes of photosensitizers have also been shown to abstract hydrogen atoms of lipids in homogeneous solutions and in micelles, as is the case of urocanic acid, vitamin K, and benzophenone.\textsuperscript{30,31}

Specifically in the latter case, the rate of hydrogen abstraction was shown to depend on the number of available allylic and bisallylic hydrogens.\textsuperscript{31} A recent publication by us additionally showed that the bleaching rate of the amphiphilic photosensitizer DO15 increases in the presence of unsaturated lipids and depends on the concentration of double bonds, suggesting the occurrence of direct reactions between photosensitizers and lipids.\textsuperscript{5}

Lipid-carbon-centered radicals react with oxygen with rates of at least $10^7$ M$^{-1}$s$^{-1}$, yielding lipid peroxy radicals.\textsuperscript{7,8} This reaction is reversible and, together with radical stabilization by resonance structures, accounts for radical isomerization and the formation of a higher number of isomers than formed through the ene reaction.\textsuperscript{26} Indeed, radical-mediated oxidation of oleic acid yields isomers in both the E and Z configurations, with the oxygenated group attached to carbon numbers 8, 9, 10, or 11.\textsuperscript{5} The differences in number and type of positional isomers of hydroperoxides formed via radical- and singlet-oxygen-mediated oxidations are commonly employed to distinguish between these mechanisms. For example, by analyzing positional isomers of oxidized phenyl esters of oleic and linoleic acid, Chacon et al. concluded that riboflavin had the most significant contribution of radical chemistry among a group of photosensitizers including MB, erythrosine, and hematoporphyrin.\textsuperscript{35} Oxidation products of cholesterol are also often employed for this purpose, as its oxidation product 3β-hydroxy-5α-cholest-6-ene-5-hydroperoxide is considered a biomarker of singlet-oxygen-mediated oxidation.\textsuperscript{12}

The conversion of lipid peroxyl radicals into lipid hydroperoxides requires the abstraction of a hydrogen atom, usually from a nonoxidized lipid. This process yields a new carbon-centered radical, which may react with molecular oxygen and propagate lipid oxidation. The hydrogen abstraction step is usually the rate-limiting step of lipid oxidation, and for this reason, peroxyl radicals tend to accumulate and are considered the prevailing chain carriers during lipid oxidation.\textsuperscript{27,34} In solution, propagation rate constants vary from $10^{-1}$ to $10^4$ M$^{-1}$s$^{-1}$ with a 5-fold decrease being reported for 1,2-dilinoleoyl-sn-glycero-3-phosphocholine (DLPC) in lipid bilayers if compared with tert-butyl alcohol solutions.\textsuperscript{35} Since the bond dissociation energy (BDE) of OO–H bonds is relatively independent of the peroxyl radical structure, the rate constant of the propagation step depends mostly on the BDE of the available C–H bonds.\textsuperscript{27} Note that for methyl oleate there is not a substantial difference between the C–H BDE (79 kcal mol$^{-1}$) and the value reported for the OO–H BDE for a small organic substrate (85 kcal mol$^{-1}$).\textsuperscript{28,29} Standard one-electron reduction potential values ($E^0$, pH = 7) are also commonly used to predict the spontaneity of lipid oxidation reactions. This analysis confirms that lipids may be possible substrates for hydrogen abstraction by peroxyl radicals ($E^0$ = −0.77–1.44 V for allylperoxyl radical ROO*, H*/ROOH) at allylic sites ($E^0$ = 0.96 V for allyl*, H*/allyl–H in propene) and specially at bisallylic sites ($E^0$ = 0.60 V for PUFA*, H*/PUFA–H, for bisallylic hydrogens in polyunsaturated fatty acids, PUFA), even though it should be noted that peroxyl radicals have a wide range of reduction potentials\textsuperscript{34} and that biological conditions may be far off from the standard condition.

At later stages of lipid oxidation, lipid hydroperoxides formed through radical-mediated pathways or via the ene reaction (Figure 4, reaction 1) may start to accumulate and also undergo direct reactions with excited states of photosensitizers (Figure 4, reaction 3). Two different possibilities may occur: breakage of the O–OH bond (BDE = 47 kcal mol$^{-1}$) or the OO–H bond (BDE = 85 kcal mol$^{-1}$), leading to alkoxyl and peroxy radicals, respectively.\textsuperscript{29} While most of the studies on quenching of photosensitizer excited states by hydroperoxides report on ultraviolet-absorbing molecules that are likely nonrepresentative of common drugs employed in PDT, there is evidence that excited states of metalloporphyrin\textsuperscript{36} and phenothiazines react with hydroperoxides.\textsuperscript{37} Tanielian and Mechin showed that the excited triplet state of methylene blue (MB) is quenched by tert-butyl hydroperoxide with a bimolecular rate constant of $10^6$ M$^{-1}$s$^{-1}$ in deoxygenated chloroform. The authors concluded that a hydrogen atom transfer occurs from the hydroperoxide to the excited triplet state of MB via electron transfer, forming a peroxy radical and the protonated semireduced MB radical.\textsuperscript{37} The phenothiazine photosensitizer DO15 was additionally shown to be photobleached by lipid hydroperoxides in lipid bilayers, in conditions in which oxygenated lipid radicals were also detected.\textsuperscript{5}

The possible production of alkoxyl radicals by excited states of photosensitizers opens additional reaction channels for photoinduced membrane damage. Even though it is challenging to distinguish peroxyl and alkoxyl radicals experimentally, alkoxyl radicals usually have higher reaction rates and are less selective than peroxy radicals.\textsuperscript{9} Indeed, alkoxyl radicals may abstract hydrogens both from nonoxidized lipids and from the –OOH group of hydroperoxides,\textsuperscript{32} forming lipid alcohols that may also accumulate in membranes. Alkoxyl radicals may additionally be obtained by one-electron reduction of lipid hydroperoxides by reductants or metal ions.\textsuperscript{12} For instance, phosphatidylethanolamine alcohols and hydroperoxides were detected by mass spectrometry in bacteria irradiated with positively charged porphyrins.\textsuperscript{38}

Lipid hydroperoxides, peroxy radicals, and alkoxyl radicals may all yield nonradical products through reactions specific to each class of products. Hydroperoxides, for example, may undergo two-electron reduction to their corresponding radicals.\textsuperscript{12} Lipid radicals may undergo termination reactions forming lipid dimers, which may be stable and detectable in cell membranes.\textsuperscript{39} Nevertheless, the termination reaction of two peroxy radicals forms an unstable linear tetroxide dimer, which decomposes into a lipid ketone, a lipid alcohol, and molecular oxygen.\textsuperscript{9} This mechanism is known as the Russell–Zeldovich mechanism.
mechanism (Figure 4, reaction 4) and has been suggested to occur under photosensitized oxidation conditions, as indicated by the detection of lipid alcohol and ketones in equimolar concentrations.

Interestingly, either the ketone or oxygen is produced as an excited state, making the Russell mechanism a clear example of how later steps of lipid oxidation may yield reactive species that further contribute to the complexity of lipid oxidation processes.

Phospholipid aldehydes with truncated carbon chains were detected in DOPC lipid films irradiated with rhodamine-DPPE, in DLPC liposomes irradiated with a pterin derivative and recently detected and related to membrane permeabilization in POPC liposomes irradiated with the phenothiazine photosensitizers MB and DO15. The literature often proposes Hock cleavage as the mechanism of formation of truncated lipid aldehydes directly from lipid hydroperoxides. Nevertheless, this mechanism was never demonstrated for monounsaturated lipids and is supposed to involve acid catalysis. We recently showed that GUVs made from POPC hydroperoxides are impermeable to sugars and are as stable in low pH (pH > 3.5) as pristine POPC vesicles. The observation that not even membranes made of polyunsaturated lipid hydroperoxides are unstable at low pH refutes the hypothesis that the acid-catalyzed Hock cleavage plays a role in photosensitized membrane permeabilization.

In contrast, there is significant evidence that truncated lipid aldehydes may instead be formed through alkoxyl radical \( \beta \)-scission (Figure 4, reaction 5), a reaction in which the C–C bond adjacent to the carbon bearing the –O* group undergoes homolytic cleavage. This reaction yields a lipid aldehyde and a short-chain carbon-centered radical, which may determine the major products being formed (e.g., the formation of alkyl radicals is more favored than that of vinyl radicals). Tanielian et al. showed that irradiation of MB in a benzene/methanol mixture introduced hydroperoxide groups in the polymer cis-1,4-polybutadiene, a process that was followed by oxygen-independent polymer chain scission and photobleaching of MB. The authors attributed this result to MB converting hydroperoxides to alkoxyl radicals, which subsequently fragment via alkoxyl radical \( \beta \)-scission. More recently, we showed that the structures of truncated lipid aldehydes detected during membrane permeabilization were entirely consistent with their exclusive formation through alkoxyl radical \( \beta \)-scission, in conditions in which the formation of oxygenated lipid radicals was signaled by the fluorogenic probe \( \text{H}_{2}\text{B-PMHC} \).

In summary, contact-dependent mechanisms may generate a large variety of products, which are expected to increase for polyunsaturated lipids. Yet, the role of contact-dependent mechanisms and the kinetics of membrane permeabilization were shown to follow similar trends for both monounsaturated and polyunsaturated lipids, suggesting the existence of common fundamental mechanisms and product classes. Lipid hydroperoxides and, additionally, lipid alcohols and lipid ketones do not seem to increase membrane permeability on their own. On the contrary, truncated lipid aldehydes were shown to have profound effects on bilayer permeability, as will be discussed in the following section.

The fact that the formation of aldehydes by alkoxyl radical \( \beta \)-scission invariably requires contact-dependent mechanisms reinforces the idea that singlet-oxygen-mediated oxidation is not enough for membrane permeabilization, even though photosensitizers typically have high singlet oxygen generation quantities and despite singlet oxygen being able to react up to hundreds of nanometers away from its original generation site. This is the case of the aforementioned photosensitizers MB and DO15, which lead to very different degrees of membrane oxidation in conditions in which both molecules deliver a similar flux of singlet oxygen molecules to the membrane. When added to a liposome solution, MB stays mostly in the aqueous phase (i.e., mostly in the form of freestanding molecules), while a higher fraction of DO15 molecules partitions in the membrane. In the conditions tested, MB takes much longer irradiation times to permeabilize membranes than DO15, and this results from the smaller number of effective collisions between the lipid double bonds and the excited triplet states of MB, which mostly need to diffuse from the aqueous solution. In contrast, many DO15 triplet excited states are generated within molecular contact with the biological target, allowing for higher efficiency of initiation of lipid oxidation and showcasing that extensive partitioning of photosensitizers in membrane systems is a key factor governing photodynamic activity. Because contact-dependent pathways require physical proximity of the photosensitizer and the right conformation and geometry for reactions with the target to occur, contact-dependent pathways may be very inefficient if compared with singlet oxygen generation in solution. This idea is reinforced by photosensitizer photobleaching quantum yields in the order of 10\(^{-4}\) to 10\(^{-5}\) in the presence of membranes, however, these contact-dependent events are the ones that cause actual damage in membranes.

**LIPID TRUNCATED ALDEHYDES AND MEMBRANE PERMEABILIZATION**

Most of the progress toward unraveling the permeabilization effects of photosensitizers in membranes results from three different approaches: microscopy observation of GUVs, permeability assays with liposomes, and computational studies. Around a decade ago, Caetano et al. reported the first systematic study of photoinduced membrane permeabilization in GUVs. Using DOPC vesicles, the authors showed that irradiation of membranes under high concentrations of MB caused vesicle explosion. Several studies then followed, providing a closer look at the transformations suffered by GUVs at milder oxidation conditions. At the onset of the experiments, GUVs are usually spherical and tense. The formation of hydroperoxides increases membrane surface area, which is often accompanied by an intensification of thermal fluctuations and fast changes of GUV shape. The GUV then recovers its spherical shape while accommodating the excess area in buds and strings (Figure 3A). Depending on the photosensitizer used, this stage is followed by membrane permeabilization.

Membrane permeabilization is typically detected through observation of GUVs by phase-contrast microscopy. For this application, GUVs are produced in sucrose solutions and diluted in glucose solutions, resulting in increases in membrane permeability that reduce the refraction index difference between the inner and the outer solutions (Figure 3A). GUVs containing 100% POPC or DOPC hydroperoxides can maintain sugar asymmetry to the same levels as nonoxidized lipids while GUVs treated with light and photosensitizers such as MB gradually lose contrast. In photooxidized GUVs, transient micrometric-sized pores are commonly observed during irradiation. Their lifetime increases from less than a second to 3–4 s when the viscosity of the medium is raised by
addition of glycerol. Yet, it is likely that nanometric pores also formed under these conditions and that they would be sufficient for sugar transport, although their size renders imaging and lifetime determination challenging. Even though pores cannot be observed in submicrometric-sized liposomes, these membrane systems provide insight into the chemical properties of pores and on their formation pathways. Indeed, the extent of the photoinduced permeabilization effect was shown to depend on the nature of the transported solute, being more significant for species with smaller charge density (e.g., 5(6)-carboxyfluorescein), as opposed to monatomic ions. Membrane permeabilization was also reported to be accompanied by an increase in lipid flip-flop rates, suggesting that the permeation of amphiphilic solutes would initially depend on water defects, which later evolve into open and less selective pores.

In contrast to lipid hydroperoxides, alcohols and ketones, lipid aldehydes with truncated chains were shown to increase membrane permeability. The permeabilization effect of aldehydes and its concentration dependence was thoroughly characterized in liposomes, employing membranes already formed in the presence of these oxidized lipids. Ytzhak and Ehrenberg studied the permeabilization effect of 1-palmitoyl-2-glutaryl-sn-glycero-3-phosphocholine (PGPC) and 1-palmitoyl-2-((9′-oxo-nanoyl)-sn-glycero-3-phosphocholine (ALDOPC) in liposomes of phosphatidylcholine from egg yolk. Using a potentiometric dye, they showed that 2 mol % of any of these oxidized lipids was enough to promote dissipation of a K+ electric diffusion potential in liposomes. Leakage was accelerated by increasing aldehyde concentrations up to 16 mol %, at which point membranes became unstable. When the oxidized lipids were substituted by 1-α-lysophosphatidylcholine from egg yolk, no dissipation effects were observed up to 20 mol %, with membrane destabilization occurring above 25 mol %. Runas and co-workers also investigated the effect of low levels of aldehydes on membrane permeability, employing GUVs with fixed percentages of 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) and cholesterol and with variable levels of 1-palmitoyl-2-linoleoyl-sn-glycero-3-phosphocholine (PLPC) and its oxidized product ALDOPC. Using a microfluidic approach, they observed that increasing the amount of ALDOPC from 0 to 2.5 mol % enhanced in 1 order of magnitude the membrane permeability to the hydrophilic and uncharged molecule PEG12-NBD. Only above 12.5 mol % of ALDOPC, membranes became permeable (yet still stable) to fluorescein-dextran of 40 or 2000 kDa, suggesting the opening of pores larger than 55 nm.

Molecular dynamics simulations provide fundamental mechanistic insight into the permeabilization effects of lipid aldehydes. These studies show that pore opening develops from randomly distributed lipids, which aggregate and form water defects. Similarly to lipid hydroperoxides, the carbonyl group of the aldehyde chain was shown to migrate to the polar head region of the lipid bilayer, where it establishes hydrogen bonds with the polar heads themselves and also with water molecules. In average, the oxidized carbon chains lay flat and parallel to the bilayer, in the so-called “extended conformation.” Nonetheless, the aldehyde chains experience an angular distribution that is wider than that of hydroperoxide chains, due to a lower average number of hydrogen bonds and due to their shorter length, enabling them to access more free volume.

Occasionally, the truncated chains populate the hydrophobic region of the bilayer and even interact with the oxidized groups from the opposing leaflet, increasing the dielectric constant inside the membrane and decreasing membrane thickness. Not only that, hydrogen-bonded water molecules are also carried inside the bilayer by the mobile aldehyde chains, originating transient water bridges that may progress into pores. Depending on the degree of membrane oxidation, the resulting membrane pores may be stable for hundreds of nanoseconds and often remain open for the remainder of the simulation. The extended conformation of lipid aldehydes additionally increases the lipid–lipid distance and favors water penetration, as supported by experimental measurements, showing increased membrane hydration in oxidized membranes. Simulations revealed that increasing fractions of 1-palmitoyl-2-(5′-oxo-valeroyl)-sn-glycero-3-phosphocholine (POVPC) (15–66 mol %) in DOPC membranes altered water permeation across the membrane from the passage of single-water molecules to the passage of small clusters, with transient water defects occurring solely in the headgroup region. In the range of 75–100 mol % of POVPC, larger water defects appeared, some of which evolved into transmembrane water-filled pores. The opening of pores, which occurred at shorter times at higher aldehyde concentrations, increased the number of transported water molecules by 2 orders of magnitude if compared with transport across intact membranes. Moreover, the overall conical shape of lipid aldehydes (packing parameter ≈0.5) is also believed to contribute to the stabilization of pore edges and micelle-like structures, in contrast to the cylindrical shape of hydroperoxides and nonoxidized lipids (packing parameter ≈1).

Therefore, it is clear that the permeabilization effects of lipid aldehydes result from a precise combination of properties, including molecular conformation, chain mobility, and hydrogen bonding capabilities. Interestingly, the truncated lipid carboxylic acid 1-palmitoyl-2-azelaoyl-sn-glycero-3-phosphocholine (PAzePC) was shown to adopt the extended conformation when in the protonated form, similarly to its aldehyde counterpart ALDOPC. Nonetheless, experiments in POPC multilamellar vesicles in a broad pH range revealed solely an increase in the disorder of the hydrophobic region of the bilayer, yet without the opening of pores. Even in the case of aldehydes, membrane permeability was shown to be influenced by additional molecules present in the membrane. For instance, both aldehyde fragments produced as a result of lipid chain break and cholesterol were shown to reduce the susceptibility of membranes to pore opening. Therefore, it should be expected that other oxidized species formed in the membrane may modulate the permeabilization effect of aldehydes, evidencing the need for studying the permeabilization of membranes oxidized in situ.

Only recently, truncated lipid aldehydes were quantified during lipid membrane permeabilization, by derivatization with the probe 1-pyrenebutyric hydrazide (PBH) and analysis by mass spectrometry. Phospholipids with aldehyde chains containing 8-, 9-, and 10-carbon atoms were detected in very low concentrations (ca. 1 mol %) exclusively in POPC membranes undergoing permeabilization. Remarkably, the photosensitizer MB was unable to permeabilize membranes or form aldehydes in conditions in which the more hydrophobic photosensitizer DO15 promoted both effects. Nonetheless, 40-fold more prolonged irradiation of samples with MB reproduced the results obtained with DO15, indicating a correlation between membrane permeabilization...
and aldehyde formation. In addition to that, molecular dynamics simulations showed that only lipid aldehydes promoted pore opening and significantly decreased (ca. 2-fold) the permeation free energy barrier of water if compared with pristine POPC membranes (Figure 3C). Lipid hydroperoxides, alcohols, and ketones did not enhance membrane permeability in any manner, suggesting that the membrane permeabilization effect is caused exclusively by very low percentages of truncated lipid aldehydes.

Simulations typically require higher concentrations of oxidized lipids for pores to be observed in comparison to experimental studies. One possible cause of this discrepancy is the fact that membranes may exhibit lipid phase separation, having nano- or microsized domains that may concentrate oxidized lipids to levels more compatible with simulations. Discrepancies could also arise from the membranes oxidized in situ being under nonequilibrium conditions and susceptible to forces that may contribute to pore opening. For instance, Yusupov et al. showed that applying a constant electric field perpendicularly to the membrane plane favored pore opening in simulated oxidized membranes. Higher aldehyde concentrations decreased the time needed for pore formation and the threshold electric field required for pore opening, while hydroperoxides did not exhibit a clear concentration dependence or enhanced pore formation significantly if compared with a nonoxidized bilayer. In photooxidized GUVs, the opening of pores was also associated with the tension created by reducing membrane area at constant volume, given that the membrane surface area decreases at later oxidation stages due to the formation of truncated lipid species.

CONTROLLING LIPID OXIDATION IN THE CONTEXTS OF SKIN CARE AND PDT

Recent mechanistic studies on photosensitized oxidations have demonstrated the importance of contact-dependent pathways in order to irreversibly damage biological structures and provided a molecular-level explanation of why photosensitizer efficiency often correlates with membrane binding. Contact-dependent processes are expected to have enhanced chemical and spatial specificity compared with contact-independent ones, given that they do not rely on the diffusion of a mediator species (i.e., singlet oxygen) and that they depend on specific reactions between the photosensitizer and its targets. Direct reactions involving photosensitizers have also been shown to be fundamental for generating vascular damage in a new modality of treatment of prostate cancer, which has already received approval in several countries. In this case, the photosensitizer is incorporated into human serum albumin, where it undergoes a fast electron transfer reaction yielding radical species in the protein hydrophobic pocket.

The formation of excited states and their effective collision with a target are the crucial steps in determining photosensitizer efficiency. Even though singlet oxygen oxidizes lipids through the one reaction and the resulting lipid hydroperoxides make the membrane thinner, this transformation is insufficient for membrane permeabilization. The decisive step toward pore opening occurs when excited states of photosensitizers directly react with unsaturated lipids, starting a multistep process that ultimately generates truncated lipid aldehydes and permeabilizes membranes. The fact that the most relevant reaction requires direct involvement of the photosensitizer indicates that the initial damage may be confined to the nanometer scale. Therefore, strategies to control the transformations happening in the skin during sunlight exposition should likely consist of a combination of excited state and lipid radical quenching. For example, polyunsaturated lipids with bisallylic hydrogens replaced by deuterium atoms have been shown to inhibit lipid oxidation reactions depending on hydrogen abstraction; hence, one could envision the incorporation of these modified lipid species in sunscreen formulations aiming at preventing contact-dependent processes. The development of such strategies would likely also benefit from accompanying lipidomic studies, in order to unravel the main reactions and lipid oxidation products that must be prevented.

Since contact-dependent reactions require direct involvement of photosensitizers, it remained an open question whether photobleaching of photosensitizers was correlated with membrane permeabilization. Tasso and co-workers answered this question by testing membrane permeabilization by a series of porphyrazines that suffer photobleaching by an electron abstraction reaction in the excited singlet state. Even though all the studied photosensitizers had similar singlet oxygen generation quantum yields and membrane binding efficiencies, the ones with higher photobleaching rates permeabilized membranes to a greater extent. This result proves that one-electron reactions are essential to lipid oxidation processes and that photobleaching is not necessarily as detrimental as usually portrayed in the literature but a consequence of efficient membrane damage. Therefore, developing strategies for photosensitizer regeneration should become a priority when designing new drugs and protocols for PDT, in addition to aiming for photosensitizers whose excited states selectively engage in direct reactions with unsaturated lipids and perhaps lipid hydroperoxides. Moreover, targeting photosensitizers to specific insertion depths and domains of lipid membranes may also significantly improve the efficiency of contact-dependent reactions.

AUTHOR INFORMATION

Corresponding Author
E-mail: baptista@iq.usp.br.

ORCID ©
Mauricio S. Baptista: 0000-0001-7079-7666

Present Address
Département de Biochimie, Université de Montréal, C.P. 6128, Succursale Centre-ville, Montréal, QC, H3C 3J7, Canada.

Notes
The authors declare no competing financial interest.

Biographies
Isabel O. L. Bacellar studied Chemistry and Biochemistry at the University of São Paulo (Brazil). She carried out both her undergraduate and graduate research under the supervision of Prof. Mauricio S. Baptista, studying the effects of photosensitizers on lipid membranes, with emphasis on membrane permeabilization. Since concluding her doctoral studies in 2017, Isabel is a postdoctoral fellow at the University of Montreal (Canada).

Mauricio S. Baptista is a professor of Biochemistry at the University of São Paulo (Brazil). He earned Bachelor (1990) and Master (1992) degrees in Biochemistry from USP and holds a doctoral degree (1996) in Chemistry from Marquette University (USA). He did his postdoctoral training at UW-Madison (1997) and was visiting professor (2006) at the Université Joseph Fourier (Grenoble-France). His main interests are photochemistry/photobiology, membranes/interfaces, and mechanisms of cell death, where he published over 180 papers. He serves as Associate Editor of the Photochemical & Photobiological Sciences (RSC) and is part of the editorial board of Scientific Reports (Nature).

ACKNOWLEDGMENTS

Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP, grants 2013/11640-0 and CEPID Redoxoma 2013/07937-8), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES – Finance Code 001) are acknowledged for financial support.

REFERENCES

(1) Chiarelli-Neto, O.; Pavan, C.; Ferreira, A.; Uchoa, A. F.; Severino, D.; Baptista, M. S. Generation and Suppression of Singlet Oxygen in Hair by Photosensitization of Melanin. Free Radical Biol. Med. 2011, 51 (6), 1195–1202.

(2) Bacellar, I. O. L.; Tsubone, T. M.; Pavan, C.; Baptista, M. S. Photodynamic Efficiency: From Molecular Photochemistry to Cell Death. Int. J. Mol. Sci. 2015, 16 (9), 20523–20559.

(3) Alves, E.; Moreirinha, C.; Faustino, M. A.; Cunha, Á.; Delgadillo, I.; Neves, M. G.; Almeida, A. Overall Biochemical Changes in Bacteria Photosensitized with Cationic Phosphoryns Monitored by Infrared Spectroscopy. Future Med. Chem. 2016, 8 (6), 613–628.

(4) Anderson, S. M.; Krinsky, N. I. Protective Action of Carotenoid Pigments against Photodynamic Damage to Liposomes. Photochem. Photobiol. 1973, 18 (5), 403–408.

(5) Bacellar, I. O. L.; Oliveira, M. C.; Dantas, L. S.; Costa, E. B.; Junqueira, H. C.; Martins, W. K.; Durantini, A. M.; Cosa, G.; Di Mascio, P.; Wainwright, M.; et al. Photosensitized Membrane Permeabilization Requires Contact-Dependent Reactions between Photosensitizer and Lipids. J. Am. Chem. Soc. 2018, 140 (30), 9606–9615.

(6) Tasso, T. T.; Schlothauer, J. C.; Junqueira, H. C.; Matias, T. A.; Araki, K.; Liandra-Salvador, E.; Antonio, F. C. T.; Homem-de-Mello, P.; Baptista, M. S. Photobleaching Efficiency Parallels the Enhancement of Membrane Damage for Porphyrine Photosensitizers. J. Am. Chem. Soc. 2019, 141 (39), 15547–15556.

(7) Halliwell, B.; Gutteridge, J. M. C. Free Radicals in Biology and Medicine, 5th ed.; Oxford University Press: Oxford, 2015.

(8) Yin, H.; Xu, L.; Porter, N. A. Free Radical Lipid Peroxidation: Mechanisms and Analysis. Chem. Rev. 2011, 111 (10), 5944–5972.

(9) Di Mascio, P.; Martinez, G. R.; Miyamoto, S.; Ronsein, G. E.; Medeiros, M. H. G.; Cadet, J. Singlet Molecular Oxygen Reactions with Nucleic Acids, Lipids, and Proteins. Chem. Rev. 2019, 119 (3), 2043–2086.

(10) Baptista, M. S.; Cadet, J.; Di Mascio, P.; Ghogare, A. A.; Greer, A.; Hamblin, M. R.; Lorente, C.; Nunez, S. C.; Ribeiro, M. S.; Thomas, A. H.; et al. Type I and Type II Photosensitized Oxidation Reactions: Guidelines and Mechanistic Pathways. Photochem. Photobiol. 2017, 93 (4), 912–919.

(11) Foote, C. S. Definition of Type I and Type II Photosensitized Oxidation. Photochem. Photobiol. 1991, 54 (5), 659–659.

(12) Girotti, A. W. Photosensitized Oxidation of Membrane Lipids: Reaction Pathways, Cytotoxic Effects, and Cytoprotective Mechanisms. J. Photochem. Photobiol. B 2001, 63 (1–3), 103–113.

(13) Bacellar, I. O. L.; Cordeiro, R. M.; Mahling, P.; Baptista, M. S.; Röder, B.; Hackbarth, S. Oxygen Distribution in the Fluid/Gel Phases of Lipid. Biochim. Biophys. Acta, Membr. 2019, 1861 (4), 879–886.

(14) Weber, G.; Charitat, T.; Baptista, M. S.; Uchoa, A. F.; Pavan, C.; Junqueira, H. C.; Guo, Y.; Baulin, V. A.; Itti, R.; Marques, C. M.; et al. Lipid Oxidation Induces Structural Changes in Biomimetic Membranes. Soft Matter 2014, 10 (24), 4241–4247.

(15) Neff, W. E.; Frankel, E. N.; Weisleder, D. Photosensitized Oxidation of Methyl Linolate. Secondary Products. Lipids 1982, 17 (11), 780–790.

(16) Ghogare, A. A.; Greer, A. Using Singlet Oxygen to Synthesize Natural Products and Drugs. Chem. Rev. 2016, 116 (17), 9994–10034.

(17) Nonell, S.; Braslavska, S. E. Time-Resolved Singlet Oxygen Detection. Methods Enzymol. 2000, 319, 37–49.

(18) Krasnovsky, A. A.; Kagan, V. E.; Minin, A. A. Quenching of Singlet Oxygen Luminescence by Fatty Acids and Lipids. FEBS Lett. 1983, 155 (2), 233–236.

(19) Hackbarth, S.; Röder, B. Singlet Oxygen Luminescence Kinetics in a Heterogeneous Environment – Identification of the Photosensitizer Localization in Small Unilamellar Vesicles. Photochem. Photobiol. Sci. 2015, 14 (2), 329–334.

(20) Baier, J.; Maier, M.; Engl, R.; Landthaler, M.; Bäumler, W. Time-Resolved Investigations of Singlet Oxygen Luminescence in Water, in Phosphatidylcholine, and in Aqueous Suspensions of Phosphatidylcholine or HT29 Cells. J. Phys. Chem. B 2005, 109, 3041–3046.

(21) Dearden, S. J. Kinetics of O2(1Δg) Photo-Oxidation Reactions in Egg-Yolk Lecithin Vesicles. J. Chem. Soc., Faraday Trans. 1 1986, 82 (5), 1627.

(22) Rosa, R. De; Spinozzi, F.; Itri, R. Hydroperoxide and Carboxyl Groups Preferential Location in Oxidized Biomembranes Experimentally Determined by Small Angle X-Ray Scattering: Implications in Membrane. Biochim. Biophys. Acta, Membr. 2018, 1860 (11), 2299–2307.

(23) Tsubone, T. M.; Baptista, M. S.; Itri, R. Understanding Membrane Remodelling Initiated by Photosensitized Lipid Oxidation. Biochim. Biophys. Acta 2019, 254, 106263.

(24) Boonnoy, P.; Jarerattanachat, V.; Karttunen, M.; Wong-ekkabut, J. Bilayer Deformation, Pores, and Micellation Induced by Oxidized Lipids. J. Phys. Chem. Lett. 2015, 6 (24), 4884–4888.

(25) Van der Paal, J.; Neys, E. C.; Verlackt, C. C. W.; Bogaerts, A. Effect of Lipid Peroxidation on Membrane Permeability of Cancer and Normal Cells Subjected to Oxidative Stress. Chem. Sci. 2016, 7 (1), 489–498.
(26) Rems, L.; Viano, M.; Kasimova, M. A.; Mlikavíc, D.; Tarek, M. The Contribution of Lipid Peroxidation to Membrane Permeability in Electropermeabilization: A Molecular Dynamics Study. Bioelectrochemistry 2019, 125, 46–57.

(27) Pratt, D. A.; Mills, J. H.; Porter, N. A. Theoretical Calculations of Carbon–Oxygen Bond Dissociation Enthalpies of Peroxyl Radicals Formed in the Autoxidation of Lipids. J. Am. Chem. Soc. 2003, 125 (19), 5801–5810.

(28) Huvaere, K.; Cardoso, D. R.; Homem-De-Mello, P.; Westermann, S.; Skibsted, L. H. Light-Induced Oxidation of Unsaturated Lipids as Sensitized by Flavins. J. Phys. Chem. B 2010, 114 (16), 5583–5593.

(29) Blanksby, S. J.; Ellison, G. B. Bond Dissociation Energies of Organic Molecules. Acc. Chem. Res. 2003, 36 (4), 255–263.

(30) Barclay, L. R.; Basque, M. C.; Stephenson, V. C.; Vinquist, M. R. Photooxidations Initiated or Sensitized by Biological Molecules: Singlet Oxygen versus Radical Peroxidation in Micelles and Human Blood Plasma. Photochem. Photobiol. 2003, 78 (3), 248–255.

(31) Marković, D. Z.; Patterson, L. K. Radical Processes in Lipids. Selectivity of Hydrogen Abstraction from Lipids by Benzoepheno Triplet. Photochem. Photobiol. 1989, 49 (5), 531–535.

(32) Frankel, E. N. Chemistry of Free Radical and Singlet Oxidation of Lipids. Prog. Lipid Res. 1984, 23 (4), 197–221.

(33) Chaon, J. N.; Jamieson, G. R.; Sinclair, R. S. Dye Sensitised Photo-Oxidation of the Methyl and Phenyl Esters of Oleic and Linoleic Acids. Chem. Phys. Lipids 1987, 43 (2), 81–99.

(34) Buettner, G. R. The Pecking Order of Free Radicals and Antioxidants: Liperoxidation, α-Tocopherol, Ascorbate. Arch. Biochem. Biophys. 1993, 300 (2), 535–543.

(35) Barclay, L. R. C. 1992 Synergy Award Lecture Model Biomembranes: Quantitative Studies of Peroxidation, Antioxidant Action, Partitioning, and Oxidative Stress. Can. J. Chem. 1993, 71 (1), 1–16.

(36) Gantchev, T. G.; Sharman, W. M.; van Lier, J. E. Metallophthalocyanines Photosensitize the Breakdown of (Hydro-) Peroxides in Solution to Yield Hydroxyl or Alkoxyl and Peroxyl Free Radicals via Different Interaction Pathways. Photochem. Photobiol. 2003, 77 (5), 469–479.

(37) Tanielian, C.; Mechlin, R. Alkyl Hydroperoxides as Electron Donors in Photochemical Reactions. J. Photochem. Photobiol., A 1997, 107 (1–3), 291–293.

(38) Alves, E.; Santos, N.; Melo, T.; Maciel, E.; Dória, M. L.; Faustino, M. A. F.; Tomé, J. P. C.; Neves, M. G. P. M. S.; Cavaleiro, J. A. S.; Cunha, A.; et al. Photodynamic Oxidation of Escherichia Coli Membrane Phospholipids: New Insights Based on Lipidomics. Rapid Commun. Mass Spectrom. 2012, 27 (3), 2717–2728.

(39) Frank, H.; Thiel, D.; MacLeod, J. Mass Spectrometric Detection of Cross-Linked Fatty Acids Formed During Radical-Induced Lesion of Lipid Membranes. Biochem. J. 1989, 260 (3), 873–878.

(40) Sankhagowit, S.; Wu, S. H.; Biswas, R.; Riche, C. T.; Povinelli, M. L.; Malmstadt, N. The Dynamics of Giant Unilamellar Vesicle Oxidation Probed by Morphological Transitions. Biochim. Biophys. Acta, Biophys. Membr. 2014, 1838 (10), 2615–2624.

(41) Vignoni, M.; Urrutia, M. N.; Junqueira, H. C.; Greer, A.; Reis, A.; Baptista, M. S.; Itri, R.; Thomas, A. H. Photo-Oxidation of Unilamellar Vesicles by a Lipophilic Pterin: Deciphering Membrane Photodamage. Langmuir 2018, 34 (50), 15578–15586.

(42) Brinkhorst, J.; Sara, S. J.; Pratt, D. A. Hock Cleavage of Cholesterol Sr-Hydroperoxide: An Ozone-Free Pathway to the Cholesterol Ozonolysis Products Identified in Arterial Plaque and Breath. J. Am. Chem. Soc. 2008, 130 (37), 12224–12225.

(43) Caetano, W.; Haddad, P. S.; Itri, R.; Severino, D.; Vieira, V. C.; Baptista, M. S.; Schröder, A. P.; Marques, C. M. Photo-Induced Destruction of Giant Vesicles in Methylen Blue Solutions. Langmuir 2007, 23 (3), 1307–1314.

(44) Gardner, H. W. Oxygen Radical Chemistry of Polyunsaturated Fatty Acids. Free Radical Biol. Med. 1989, 7 (1), 65–86.