



# Lipid peroxidation modifies the assembly of biological membranes “The Lipid Whisker Model”

Ángel Catalá\*

Facultad de Ciencias Exactas, Instituto de Investigaciones Fisicoquímicas Teóricas y Aplicadas-Centro Científico Tecnológico La Plata-Consejo Nacional de Investigaciones Científicas y Técnicas, Universidad Nacional de La Plata, Argentina

\*Correspondence: [catala@inifta.unlp.edu.ar](mailto:catala@inifta.unlp.edu.ar)

## Edited by:

Mario L. Diaz, Universidad de La Laguna, Spain

## Reviewed by:

Stefano Piotto, University of Salerno, Italy

Rodrigo F. M. De Almeida, Universidade de Lisboa, Portugal

**Keywords:** lipid peroxidation, Lipid Whisker Model, membranes, PUFAs, Fluid Mosaic Model

The aim of this opinion article is to point out the basic principles that modify the assembly of biological membranes during lipid peroxidation. With this objective in mind, I describe: the structural and functional properties of membranes, the transport and diffusion of oxygen regulated by cholesterol and fatty acids; the “Lipid Whisker Model” and finally analyzed the changes induced by lipid peroxidation in membrane structure and dynamics, both at the lipid and protein level.

Several reviews have appeared in recent years related to the kinetics and biology of lipid peroxidation products (Catalá, 2010; Yin et al., 2011; Pinchuk and Lichtenberg, 2014; Vigor et al., 2014; Davies and Guo, 2014). The analysis of lipid peroxidation products has been particularly important in the advancement of research in this field because of the complexity of product mixtures. I also discuss the effect of other membrane modifications, triggered by lipid peroxidation products and reducing sugars. Contrary to what is expected by the LWM, Garrec et al. (2014) have recently investigated the validity of the “floating peroxy radical” hypothesis by means of molecular modeling and predicted that the peroxy radical does not “float” at the surface of the membrane.

**Abbreviations:** 22:6 n-3, docosahexaenoic acid; ER, endoplasmic reticulum; CD36, fatty acid translocase; FMM, Fluid Mosaic Model; FRET, Förster Resonance Energy Transfer; LWM, Lipid Whisker Model; oxPL, oxidized phospholipid; 16:0, palmitic acid; PC, phosphatidylcholine; PUFAs, polyunsaturated fatty acids; 18:0, stearic acid.

## INTRODUCTION

When the Fluid Mosaic Model (FMM) of biological membrane structure was introduced 42 years ago, it was visualized as a basic model for cell membranes that could explain existing data on membrane proteins and lipid structures and their dynamics. According to the (FMM), a membrane was described as a biological fluid of proteins and lipids oriented in two dimensions. The basic structure of all cell and organelle membranes is the lipid bilayer. Protein molecules are distributed in different regions of the bilayer and perform diverse functions. Cell membranes are active, fluid structures, and most of their molecules are able to travel in the plane of the membrane (Singer and Nicolson, 1972). The viscosity of a lipid membrane largely depends on whether the acyl chains attached to glycerophospholipids are grouped into a rigid state or exist in a relatively disordered, fluid state. Long chain saturated fatty acids maximize Van der Waals forces, and increase the viscosity of the membrane (Chapman and Benga, 1984). Fluidity is defined as the ease of movement and represents the reciprocal of the viscosity of the membrane (Lee, 1991). The fluid properties of biological membranes are critical for various cell functions. Still slight changes in membrane fluidity may cause unusual function and pathological processes (García et al., 2005). After more than 40 years, the (FMM), described by Singer and Nicolson is still relevant to recognize the structure, function, and dynamics of biological membranes (Nicolson, 2014). However, several

biological processes cannot be explained on the basis of this typical phospholipid orientation and utilize other phospholipid conformations (Catalá, 2012) that are described in this article.

## THE STRUCTURAL AND FUNCTIONAL PROPERTIES OF MEMBRANES ARE DETERMINED BY POLYUNSATURATED FATTY ACIDS

The fluid properties in biological membranes are recognized principally by the presence of polyunsaturated fatty acids (PUFAs) in phospholipids molecules located in both sites of the lipid bilayer. The nature and saturation of the attached fatty acid of the phospholipids generate spectacular effects on membrane packing and fluidity (Janmey and Kinnunen, 2006). The unsaturated fatty acids give a high degree of conformational flexibility to the unsaturated hydrocarbon chains in the membranes because they occupy a small wedge-shaped space. This generally results in looser packing and a more fluid membrane. In contrast, saturated fatty acids confer rigidity that results in a less fluid or more arranged membrane. The rigidity permits saturated fatty acids to group together tightly and forms a solid at inferior temperatures. When a new double bond is open in the fatty acid produces a “kink” in the molecule. For this reason unsaturated fatty acids, such as docosahexaenoic acid (22:6 n-3), assume numerous patterns since this fatty acid can turn around in the area of C–C bonds but not in the region of the rigid C=C bonds (Feller et al., 2002). Adjustments in the lipid composition modify the fluidity of

membranes. Lipid composition is modified during regulation of de novo synthesis at selected cellular places, distribution or transfer to new sites, and by specific modifying reactions. Stearyl CoA desaturase is in charge for the formation of monoethylenic fatty acids from saturated fatty acids by catalyzing the addition of a double bond into the ninth carbon of saturated C16:0 and C18:0 substrates (Enoch et al., 1976).

### THE TRANSPORT AND DIFFUSION OF OXYGEN IN MEMBRANES IS REGULATED BY CHOLESTEROL AND FATTY ACIDS

Lipid peroxidation and formation of reactive oxygen species are important chemical reactions that use oxygen and occur in cell membranes. The diffusion of oxygen into synthetic membranes prepared with phosphatidylcholine and cholesterol have been investigated by Subczynski et al. (1991). Oxygen permeability through the membrane in all the membranes studied in this work was limited by the presence of cholesterol. Thus, they showed an increase in oxygen transport in the central part of the synthetic membranes because cholesterol decreases oxygen transport in and around the head group regions, where the main obstacles to the oxygen permeability exist. Based on this explanation, it can be assumed that non-raft areas rich in PUFA-phospholipids and vitamin E will be more available by oxygen than lipid rafts areas containing sphingolipids and cholesterol. This condition will make several micro areas more vulnerable to lipid peroxidation than others.

### THE “LIPID WHISKER MODEL” IS SUITABLE TO EXPLAIN THE STRUCTURE OF OXIDIZED CELL MEMBRANES

The Lipid Whisker Model (LWM) (Greenberg et al., 2008) is an extension of the Fluid Mosaic Model suggested by Singer and Nicolson (1972). Recent studies into the conformation of oxidized phospholipid (oxPL) species recognized by CD36 within model membranes (Li et al., 2007) have led to the development of the LWM. A primary attribute of the (FMM) is that amphipathic phospholipids are oriented in an organization

lamellar mesophase with hydrophobic fatty acyl chains embedded within the interior of the membrane and the hydrophilic polar groups facing the aqueous environment. This lipid organization accepts fast lateral circulation of lipids and membrane proteins similarly within the planar membrane surface. It also causes the water-resistant character of cell membranes to hydrophilic molecules. But, recent information proposes that in peroxidized cell membranes, numerous of the oxPL classes adopt a particular conformation. Lipid peroxidation is achieved by addition of numerous polar molecules on fatty acid chains (Catalá, 2009). Consequently, when cell membranes undergo oxidation, if not adapted by the action of phospholipases, they may “produce whiskers” including a variety of oxidized sn-2 fatty acids of diverse structures. In the (LWM), the assembly of many oxPL within cell membranes is different compared with one observed in non-oxPL described in the (FMM). Biophysical studies have shown that the addition of an oxygen atom to the acyl chain produces a significant change that prevent its immersion in the interior of the membrane, however, the modified acyl chain is projected on the aqueous medium (**Figure 1**) (Subczynski et al., 1991; Greenberg et al., 2008).

As cell membranes are peroxidized “grow whiskers” because phospholipids are peroxidized, and several of its oxidized fatty acids are projected onto the surface (**Figure 1**). This change produced by the oxidation of a fatty acid chain in the membrane may be a trigger event to numerous biological activities.

### THE EFFECT OF OTHER MEMBRANE MODIFICATIONS, TRIGGERED BY LIPID PEROXIDATION PRODUCTS AND REDUCING SUGARS INTRODUCES CHANGES IN CELL MEMBRANE PHYSICO-CHEMICAL AND BIOLOGICAL PROPERTIES

During lipid peroxidation, biomolecules such as proteins or amino lipids can be covalently modified by lipid decomposition products (Catalá, 2009). For the case of aliphatic aldehydes (alkenals) such as 1-hexanal or 1-nonanal, the  $\epsilon$ -amino groups of the lysine residues in proteins can be modified through

the formation of a Schiff base.  $\alpha,\beta$ -Unsaturated aldehydes (alkenals) such as acrolein or 4-hydroxy-2-nonenal react with lysine, cysteine, and histidine through a Michael-type addition. Conversely, lipid hydroperoxide might covalently react with protein without serious decomposition of its structure. However, the mechanism of lipid hydroperoxide-derived protein modification is not so clear. Evidence for in situ ethanolamine phospholipid adducts with hydroxy-alkenals has been recently described.

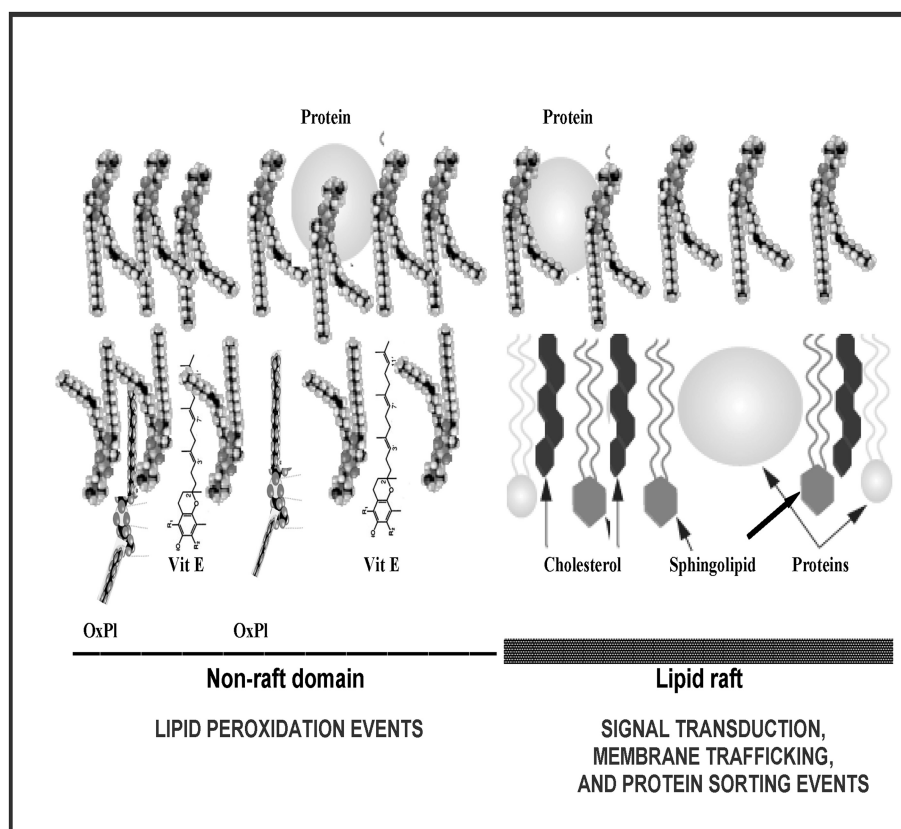
Non-enzymatic modification of aminophospholipids by lipid peroxidation-derived aldehydes and reducing sugars through carbonyl-amine reactions are thought to contribute to the age-related deterioration of cellular membranes (Naudí et al., 2013). Much evidence demonstrates the modification of aminophospholipids by glycation, glycoxidation and lipoxidation reactions.

### LIPID PEROXIDATION IN MEMBRANES: THE PEROXYL RADICAL DOES NOT “FLOAT”

Some key microscopic aspects of the lipid peroxidation reaction in cell membranes are still poorly studied. In particular, it is usually accepted that the propagation of the radical reaction in lipid bilayers is hampered by the rapid diffusion of peroxy intermediates toward the water interface, that is, out of the reaction region. Contrary to what is expected by the LWM, Garrec et al. (2014) have recently investigated the validity of this “floating peroxy radical” hypothesis by means of molecular modeling. Combining quantum calculations of model systems and atomistic simulations of lipid bilayers containing lipid oxidation products, these authors predict that the peroxy radical does not “float” at the surface of the membrane. Instead, it remains located quite deep inside the bilayer.

### THE CHANGES INDUCED BY LIPID PEROXIDATION IN MEMBRANE STRUCTURE AND DYNAMICS, BOTH AT THE LIPID AND PROTEIN LEVEL

The mechanisms and principles establishing the thousands of proteins and lipids that make up membrane bilayers in cells are still unclear. Mueller et al. reviewed the basic properties of biological membranes



**FIGURE 1 | Hypothetical model of the plasma membrane.** In terms of lipids, the heterogeneous membrane is believed to consist of a dispersed “lipid raft” phase, enriched in cholesterol, raft-associated proteins, and saturated lipids (such as sphingolipids), and the “non-raft” matrix phase enriched with phospholipids containing PUFAs and vitamin E. Vitamin E,

partition into domains that are enriched in polyunsaturated phospholipids increasing the concentration of the vitamin in the place where take place the lipid peroxidation process and oxidized phospholipids (oxPI) are formed. Reproduced from Catalá (2012). Copyright ©2012 Elsevier Masson SAS. All rights reserved.

and the most common theories for lateral segregation of membrane components before discussing an emerging model of a self-organized, multi-domain membrane or “patchwork membrane” (Mueller et al., 2012).

The oxidation of phospholipids has become a recent topic of interest within the field of membrane biophysics. Still, the exact mechanism of membrane injury by oxidized lipids is uncertain (Wong-Ekkabut et al., 2007).

Membrane rafts remain one of the most controversial issues in biophysics, because the methods for their detection are still far from perfect. Optical techniques rely heavily on fluorescent markers. In biological membranes, these probes are connected with lipids and thus define only the lipid part of the membrane. Therefore, they are “blind” to the presence of membrane proteins, unless FRET techniques between proteins and membrane probes are used.

Therefore, the imperative future challenge for the membrane probes will be to design molecules capable of monitoring lipid surrounding specifically around a given protein of interest (Klymchenko and Kreder, 2014).

Proteins and lipids in membrane processes are reciprocally dependent on each other. While the lipid domains as organizers of proteins has attracted wide-spread consideration, the question whether proteins control lipids or lipids control proteins in cell membranes is not a simple problem to solve. The fact that proteins exploit the biophysical properties of lipids including lipid charge and phase separation for membrane function is one of the reasons why membrane biology is such an attractive area of research (Rossy et al., 2014).

To guarantee coordination of cellular activities, cells use membrane contact sites (MCSs) between the membranes of

diverse organelles. MCSs are domains where two membranes come to close proximity, typically less than 30 nm, and create microdomains that favor exchange between two organelles. Since the endoplasmic reticulum (ER) is the most widespread cellular membrane network, it is thus not surprising to find the ER involved in most MCSs within the cell. The ER contacts diverse compartments such as mitochondria, lysosomes, lipid droplets, the Golgi apparatus, endosomes, and the plasma membrane. Taken into account these observations, several levels of complexity have to be considered when analyzing the changes induced by lipid peroxidation in membrane structure and dynamics, both at the lipid and protein level.

In my opinion and in the light of new investigations, several critical aspects of lipid peroxidation in biological membranes, such as their assembly

and structural organization need to be revisited.

## AUTHOR NOTE

The author is a member of Carrera del Investigador Científico, Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina.

## ACKNOWLEDGMENT

Studies in the author laboratory were supported by PIP-0157 National Research Council (CONICET).

## REFERENCES

- Catalá, A. (2009). Lipid peroxidation of membrane phospholipids generates hydroxyl-alkenals and oxidized phospholipids active in physiological and/or pathological conditions. *Chem. Phys. Lipids* 157, 1–11. doi: 10.1016/j.chemphyslip.2008.09.004
- Catalá, A. (2010). A synopsis of the process of lipid peroxidation since the discovery of the essential fatty acids. *Biochem. Biophys. Res. Commun.* 399, 318–323. doi: 10.1016/j.bbrc.2010.07.087
- Catalá, A. (2012). Lipid peroxidation modifies the picture of membranes from the “Fluid Mosaic Model” to the “Lipid Whisker Model.” *Biochimie* 94, 101–109. (This article belongs to a special issue: Lipids in all their states Edited by Erick J. Dufourc.). doi: 10.1016/j.bbrc.2010.07.087
- Chapman, D., and Benga, G. (1984). “Biomembrane fluidity – studies of model and natural membranes,” in *Biological Membranes*, Vol. 5, ed D. Chapman (London, UK: Academic Press), 1–56.
- Davies, S. S., and Guo, L. (2014). Lipid peroxidation generates biologically active phospholipids including oxidatively N-modified phospholipids. *Chem. Phys. Lipids* 181, 1–33. doi: 10.1016/j.chemphyslip.2014.03.002
- Enoch, H. G., Catalá, A., and Strittmatter, P. (1976). Mechanism of rat liver microsomal stearyl-CoA desaturase. Studies of the substrate specificity, enzyme-substrate interactions, and the function of lipid. *J. Biol. Chem.* 251, 5095–5103.
- Feller, S. E., Gawrisch, K., and MacKerell, A. D. Jr. (2002). Polyunsaturated fatty acids in lipid bilayers: intrinsic and environmental contributions to their unique physical properties. *J. Am. Chem. Soc.* 124, 318–326. doi: 10.1021/ja0118340
- García, J. J., Martínez-Ballarín, E., Millán-Plano, S., Allué, J. L., Albendea, C., Fuentes, L., et al. (2005). Effects of trace elements on membrane fluidity. *J. Trace Elem. Med. Biol.* 19, 19–22. doi: 10.1016/j.jtemb.2005.07.007
- Garrec, J., Monari, A., Assfeld, X., Mir, L. M., and Tarek, M. (2014). Lipid peroxidation in membranes: the peroxy radical does not “float.” *J. Phys. Chem. Lett.* 5, 1653–1658. doi: 10.1021/jz500502q
- Greenberg, M. E., Li, X. M., Gugiu, B. G., Gu, X., Qin, J., Salomon, R. G., et al. (2008). The lipid whisker model of the structure of oxidized cell membranes. *J. Biol. Chem.* 283, 2385–2396. doi: 10.1074/jbc.M707348200
- Janmey, P. A., and Kinnunen, P. K. J. (2006). Biophysical properties of lipids and dynamic membranes. *Trends Cell Biol.* 16, 538–546. doi: 10.1016/j.tcb.2006.08.009
- Klymchenko, A. S., and Kreder, R. (2014). Fluorescent probes for lipid rafts: from model membranes to living cells. *Chem. Biol.* 21, 97–113. doi: 10.1016/j.chembiol.2013.11.009
- Lee, A. G. (1991). Lipids and their effects on membrane proteins: evidence against a role for fluidity. *Prog. Lipid Res.* 30, 323–348. doi: 10.1016/0163-7827(91)90002-M
- Li, X. M., Salomon, R. G., Qin, J., and Hazen, S. L. (2007). Conformation of an endogenous ligand in a membrane bilayer for the macrophage scavenger receptor CD36. *Biochemistry* 46, 5009–5017. doi: 10.1021/bi700163y
- Mueller, N. S., Wedlich-Söldner, R., and Spira, F. (2012). From mosaic to patchwork: matching lipids and proteins in membrane organization. *Mol. Membr. Biol.* 29, 186–196. doi: 10.3109/09687688
- Naudí, A., Jové, M., Ayala, V., Cabré, R., Portero-Otín, M., and Pamplona, R. (2013). Non-enzymatic modification of aminophospholipids by carbonyl-amine reactions. *Int. J. Mol. Sci.* 14, 3285–3313. doi: 10.3390/ijms14023285
- Nicolson, G. L. (2014). The fluid-mosaic model of membrane structure: still relevant to understanding the structure, function and dynamics of biological membranes after more than 40 years. *Biochim. Biophys. Acta* 838, 1451–1466. doi: 10.1016/j.bbamem.2013.10.019
- Pinchuk, I., and Lichtenberg, D. (2014). Analysis of the kinetics of lipid peroxidation in terms of characteristic time-points. *Chem. Phys. Lipids* 178, 63–76. doi: 10.1016/j.chemphyslip.2013.12.001
- Rosy, J., Ma, Y., and Gaus, K. (2014). The organization of the cell membrane: do proteins rule lipids? *Curr. Opin. Chem. Biol.* 20, 54–59. doi: 10.1016/j.cbpa.2014.04.009
- Singer, S. J., and Nicolson, G. L. (1972). The Fluid Mosaic Model of the structure of cell membranes. *Science* 175, 720–731. doi: 10.1126/science.175.4023.720
- Subczynski, W. K., Hyde, J. S., and Kusumi, A. (1991). Effect of alkyl chain unsaturation and cholesterol intercalation on oxygen transport in membranes: a pulse ESR spin labeling study. *Biochemistry* 30, 8578–8590. doi: 10.1021/bi00099a013
- Vigor, C., Bertrand-Michel, J., Pinot, E., Oger, C., Vercauteren, J., Le Faouder, P., et al. (2014). Non-enzymatic lipid oxidation products in biological systems: assessment of the metabolites from polyunsaturated fatty acids. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* 964, 65–78. doi: 10.1016/j.jchromb.2014.04.042
- Wong-Ekkabut, J., Xu, Z., Triampo, W., Tang, I. M., Tieleman, D. P., and Monticelli, L. (2007). Effect of lipid peroxidation on the properties of lipid bilayers: a molecular dynamics study. *Biophys. J.* 93, 4225–4236. doi: 10.1529/biophysj.107.112565
- Yin, H., Xu, L., and Porter, N. A. (2011). Free radical lipid peroxidation: mechanisms and analysis. *Chem. Rev.* 111, 5944–5972. doi: 10.1021/cr200084z

**Conflict of Interest Statement:** The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 15 September 2014; accepted: 18 December 2014; published online: 12 January 2015.

Citation: Catalá Á (2015) Lipid peroxidation modifies the assembly of biological membranes “The Lipid Whisker Model”. *Front. Physiol.* 5:520. doi: 10.3389/fphys.2014.00520

This article was submitted to *Membrane Physiology and Membrane Biophysics*, a section of the journal *Frontiers in Physiology*.

Copyright © 2015 Catalá. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) or licensor are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.