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CHAPTER FOUR

THE CUBIC “FACES” OF BIOMEMBRANES

Zakaria A. Almsherqi, Felix Margadant, and Yuru Deng*

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Abstract

Biomembranes are traditionally viewed as flat phospholipid-bilayer sheets delineating the cell boundaries and dividing the cell into multiple subcellular organelles with specialized functions. However, biological membranes may also fold up into three-dimensional nanoperiodic arrangements, termed cubic membranes. This type of geometry is mathematically well described and extensively studied in lipidic cubic phase systems. This chapter will (1) summarize similarities and dissimilarities between cubic membranes and cubic phases; (2) provide an update on the experimental data describing the role of lipids,

* Corresponding author: Tel.: +65 6516 1935; Fax: +65 6778 8161; E-mail address: phsdy@nus.edu.sg

Cubic Membrane Laboratory, Department of Physiology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore

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proteins and electrostatic charges on the biogenesis of cubic membranes; and (3) discuss their potential function in intracellular macromolecular transport and as optical filters, as well as potential practical applications such as gene delivery vehicles.

1. Introduction

Cell membranes consist largely of lamellar lipid bilayers. However, a substantial number of membrane lipids can adopt bilayered nonlamellar phases such as hexagonal and cubic phases in water–lipid system. With the advent of electron microscopy (EM), membrane morphologies resembling cubic phases have been reported for a great assortment of organelles [1,2]. Until recently, the interpretation of three-dimensional (3D) structures observed in the two-dimensional (2D) TEM micrographs was challenging. Propitiously, Landh [3] managed to develop the method of direct template correlative (DTC) matching, a technique that can assess the presence of highly organized membrane organizations (specifically, cubic membranes) in biological specimens. DTC has revealed the abundance of cellular cubic membranes in TEM micrographs ranging from protozoa to human cells [3,4], in the mitochondria of amoeba [5], and more recently, in subcellular membrane compartments associated with cellular stress and viral infection [6]. The development of EM tomography has abetted the analysis of cubic membranes by allowing the confirmation of cubic structures in subcellular organelles of living organisms [7].

Cubic membranes are highly curved nanoperiodic structures with lipid–protein multibilayers that can be induced under different conditions such as stress, disease, and viral infection. Studies have also shown that cubic membranes are inducible by lipid or proteins alterations [1]. Although they can exist in living cells, the generation of such membrane architecture appears to be tightly regulated, as can be deduced from the dominance of lamellar bilayers in biology. Information on the specific mechanism of formation, potential functions, and significance for such cubic membrane transformation in biological systems is scarce, and studies on cubic membrane thus far remain at descriptive level [2].

Because of their unique patterns observed in TEM micrographs and great similarity to the bicontinuous lipidic cubic phases [8–11], cubic membranes (Fig. 1) have often been compared to self-assembled cubic lipidic phases in aqueous dispersions that are well characterized in vitro. Indeed, the efforts toward understanding the formation and functional roles of cubic membranes in biological systems have been enhanced by the efforts in investigating cubic phase formation and their behavior in lipid–water system.
The Cubic “Faces” of Biomembranes

2. Cubic Phase Versus Cubic Membrane

Nonlamellar arrangements of lipids in aqueous dispersions are well characterized in vitro and are of significant technological interest as biomimetics, matrices for membrane protein crystallization, and drug delivery systems [12]. Among these nonlamellar lipid mesophases, cubic phases attract great attention because of their unique feature of 3D periodicity. The type of surface symmetry of bicontinuous cubic phases based on triply periodic minimal surfaces may change, for instance, depending on the water content, pH, electrolyte concentration in the media, and the temperature, giving rise to various subtypes of cubic structures. At least seven cubic phases have been identified in water–lipid system [11]. In living systems, so far only three surface families have been identified to exist, and these three types of cubic membranes are schematically shown in Fig. 1 (D–F). They are designated according to their corresponding triply periodic minimal (or level) surfaces as gyroid (G), double diamond (D), or primitive (P) types of symmetry [4,13–15]. However, other cubic membrane symmetries might exist in living systems. These highly curved 3D periodic (n)-parallel
membrane bilayers representing “infinite periodic minimal surfaces” [16] divide space into \((n + 1)\) independent aqueous subcompartments.

Cubic phases are often interspersed in the phase diagram between the other main phases and they have often been given the generic term “intermediate phase.” Similarly, the coexistence of different subtypes of cubic membranes or their existence together with the other membrane organizations (annulated lamellae, Karmellae, hexagonal, etc.) within the same cell organelle is frequently seen, pointing to the ease with which lamellar and bicontinuous cubic membranes or other organized membrane structures are interconverted. In vitro studies using monoolein membranes containing negatively charged dioleoylphosphatidic acid have shown that the electrostatic interactions caused by surface charge of the membranes [17], charged short peptides such as poly-L-lysine [18], Ca\(^{2+}\) concentration [19], and low pH [20] play important role in the phase transition between lamellar and cubic phases as well as on the stability of cubic phases. Likewise, the weak molecular interactions, for example, by membrane proteins, may lead to transformation of Endoplasmic reticulum (ER) membrane from lamellar into cubic in mammalian cells suggesting that electrostatic interactions may play an important role in that process [18,21]. In a recent experimental study on the effects of divalent cations in the isolation buffers for mitochondria, we were able to demonstrate that the presence of high concentration of Ethylenediaminetetraacetic acid (EDTA) up to 10 mM preserved mitochondrial cubic membranes in vitro (manuscript in preparation). As the pH, temperature, and the nature and the concentration of the electrolytes in the cellular milieu are relatively constant, it is likely that the composition and the weak molecular interactions between the membrane components (mainly lipids and proteins) are the essential factors affecting cubic membrane morphology and integrity in vivo. It is important to note that the asymmetry of biological membranes with respect to the two leaflets is also likely to affect cubic membrane formation, in particular as a consequence of lipid and protein composition, and their interaction with different internal and external ion milieu of the membrane-bound organelles.

With the reference to the morphology of lipid–water mixture, there is a “mirror plane” through the lamellar phase, phases on the water-rich side (type I), and those in water-poor side (type II). It has been argued that the latter must control the morphology of the cubic membrane [8] and hence that the cubic membrane structures must be of the inverted type (type II) rather than of “normal” type (type I). All known lipid–water and lipid–protein–water systems that exhibit phases in equilibrium with excess water are of the inverted type (type II). Thus, water activity is an important factor to determine the structure of cubic membranes. Inverted cubic phases have been observed with very high water activity (70–90%), in the mixtures of lipids, in lipid–protein systems, in lipid–polymer systems [22], and in lipid and lipopolysaccharide mixtures [23,24].
The other major difference between cubic phase and cubic membrane is the lattice size. Most cubic phases in lipid–water systems exhibit unit cell parameters not larger than 20 nm, while in cellular cubic membranes the lattice size is usually larger than 50 nm. However, in lipid–protein–water, lipid–poloxamer–water, and lipid–cationic surfactant–water systems, cubic phases with lattice size of 50 nm have also been observed [3]. On the other hand, the lattice size of cubic membranes is rarely less than 50 nm (e.g., in prolamellar bodies) and usually ranges from 50 to 500 nm. Cubic membranes with large lattice size (500 nm) were observed in chloroplast membranes of green algae *Zygnema* [25].

Investigations on physical properties of cubic phase samples show that cubic phase are completely transparent and optically isotropic; thus, they have often been overlooked for solutions. Furthermore, the cubic phases have been at times mistaken for nonequilibrium isogel and claimed to belong to the group of “microemulsions.” This might be due to the fact that cubic phase when formed may be pseudostable for a long time at nonequilibrium temperatures. It may also take considerable time before the cubic structure is formed. In some cases, the cubic phases occur transiently in the biochemical process. Similarly, the infrequent observation of cubic membrane in the cells under normal conditions could be due to the transient transformation of biomembranes for short time into cubic morphology. However, the “unbalanced” or stress conditions of the cell may favor cubic membranes arrangements. This also may explain the discrepancy in reporting cubic membrane formation from different research groups examining the same cell type under similar experimental conditions.

The cubic phases are viscous, but there is a wide range in stiffness; in some cubic phases, the samples may be hard and like Plexiglas, in others they almost flow. In some experimental work, it has been found that at shear strain amplitudes a cubic phase shows linear elastic properties [26]. Such a strain hardening is probably due to the occurrence of dislocation and is evidence for a periodic 3D network. On the other hand, the physical properties of cubic membrane have not been investigated yet. However, our recent experimental data may shed light on some physical properties of cubic membranes such as their ability to interaction with macromolecules and the optical interference filtering of light of certain wave lengths (see section 6.2.)

3. **Order in Chaos**

Intracellular membranes are generally considered to have no specific pattern or organization and very frequently adopt spherical geometry, delineated by one phospholipid-bilayer membrane that separates the interior from the exterior. Thus, the existence of nonlamellar highly organized
structures of biological membranes, such as inverted hexagonal ($H_{II}$) or cubic ($Q_{II}$) phases, has long been a matter of dispute. As a matter of fact, the major experimental limitations of identifying 2D periodic hexagonal or 3D periodic cubic membrane morphologies in (living) cells are caused by the dimensions of these structures and by limited analysis tools at this size range in vivo. In vitro, complex lyotropic liquid crystalline phases, such as, lamellar ($L_a$), $H_{II}$, or $Q_{II}$ structures, are readily amenable to a range of experimental techniques, such as small-angle X-ray scattering, nuclear magnetic resonance, and differential scanning calorimetry. These techniques, however, are not feasible in whole cells because of limited resolution power and the strong background noise. Thus, the experimental approach to uncover nonlamellar membrane structures in (fixed) biological samples is restricted to TEM, leaving a wide range of interpretations for 3D structures between fact and artifact.

Possibly the best-characterized cubic membrane transition was observed in the mitochondrial inner membranes of the free-living giant amoeba (*Chaos carolinense*). In this organism, mitochondrial inner membranes undergo dramatic changes in 3D organization upon food depletion, providing a valuable model system to study induced membrane reorganization. Within one day of starvation, about 70% of mitochondria undergo this morphological transition, which after 7 days of starvation is observed in virtually all mitochondria [27]. Intriguingly, this process is fully reversible to wild-type morphology upon refeeding [5]. Using electron tomography, we have unequivocally demonstrated that inner mitochondrial membranes in *C. carolinense* cells adopt cubic morphology [7]. This starvation-triggered transition is accompanied by alterations in cellular oxidative stress response, suggesting that cubic membrane formation may be related to oxidative stress [28].

Highly organized cubic membrane structure is not restricted to be found only in amoeba *Chaos* cells; rather, careful analysis of published TEM micrographs has revealed the existence of cubic membrane morphologies in numerous cell types from all kingdoms of life and in virtually any membrane-bound cell organelles, especially smooth ER, plasma membrane, inner nuclear membrane, mitochondrial inner membrane, and chloroplast thylakoid membranes [2,3]. Not surprisingly, the ER was found to be the most prominent target of morphological alterations because of its highly convoluted structure and central functions in membrane lipid synthesis, assembly, secretion of membrane and secretory proteins, ion homeostasis, and membrane quality control. Thus, these morphologies (because of their complex morphology in TEM micrographs) appear under numerous nicknames in the literature, such as “undulating membranes” [29], “cotte de mailles” [30], “membrane lattice” [31], “crystalloid membranes” [32], “paracrystalline ER” [33], “tubuloreticular structures” [3,4,34], and “organized smooth ER” (OSER) that is formed in response to elevated
levels of the membrane-resident protein cytochrome b(5) [21]. Quite remarkably, the most prominent examples of ER expansion are related ER stress condition and may occur, for example, as a response to hypoxia stress [35], drug treatment [36], or tumors [29], or specifically, upon stimulation of lymphocytes with lipopolysaccharide [37] or interferon [38]. Tubuloreticular structures are also formed in SARS coronavirus infected cells [39] and, indeed, have been subsequently identified to resemble cubic membrane architecture [6]. Cubic membrane structures, therefore, have significant potential as ultrastructural markers in pathology.

4. Biogenesis of Cubic Membranes

Cell membrane morphology probably controlled by the principles of self-assembly and/or self-organization is likely to adopt an optimally organized structure under the influence of selective conditions. This is a dynamic process, perhaps restricted to submembrane domains, and short-lived, and is dependent on the lipid as well as protein components of the membrane.

It is now well established that proteins may induce phase transition in lipid membranes resulting in new structures not observed in pure lipid–water systems [8]. Depending on the structure and nature of the proteins, their interactions with phospholipid bilayers can be manifested in very different ways. On the other hand, evidence from in vitro studies clearly differentiates membrane-forming lipids for their propensity to form non-lamellar structures, according to the molecular shape concept. The structural role of the membrane lipids in promoting cubic membrane formation in vivo is undisputed; however, only very recent evidence obtained from amoeba Chaos cells has correlated specific alterations of membrane lipids to cubic membrane formation in vivo [40]. Although lipids extracted from these cells may assemble to cubic phases in vitro, marked differences in lattice size clearly indicate that additional factors—presumably proteins—exist in vivo and determine the overall 3D appearance of these structures.

4.1. Role of Membrane-Resident Proteins in Cubic Membrane Formation

Cubic membrane formation is frequently associated with the overexpression of certain ER-resident proteins and, to a lesser extent, with overexpression of some inner mitochondrial membrane proteins. Several membrane proteins have been shown to induce cubic membrane formation upon experimental dys-regulation, for example, HMG-CoA reductase [41–44], cytochrome b(5) [21], msALDH [32] InsP3 receptor [35],
Detailed study on the overexpression of specific ER-resident proteins such as cytochrome b(5) in COS-7 cells revealed that the biogenesis of OSER structures may involve weak homotypic interactions between cytoplasmic domains of proteins and may underlie the formation of other stacked membrane structures within the cells as well [21]. Time-lapse imaging of OSER biogenesis revealed that these structures formed rather quickly once a threshold level of OSER-inducing proteins was exceeded; OSER formation also involved gross remodeling of surrounding tubular ER. In this system, the attachment to the cytoplasmic domain of different ER-resident membrane proteins of green fluorescent protein (GFP) that is capable of low affinity, head-to-tail dimerization was sufficient to induce OSER formation upon overexpression of cytochrome b(5) in living cells [21]. Recent evidences shows that the formation of cubic membranes, in the ER could also be triggered by controlled dimerization of “artificial” membrane proteins in mammalian tissue culture cells [49]. Homotypic low affinity interactions between cytoplasmic domains of proteins thus can transform tubular ER into stacked lamellae or crystalloid structures; such a mechanism may underlie the reorganization of other organelles into stacked membrane structures as well [21] and provides an intriguing model system to investigate the cellular and molecular requirements for cubic membrane formation.

4.2. Role of Membrane Lipids in Cubic Membrane Formation

Up to date, cubic membrane formation is mainly associated with overexpression of certain membrane-bound proteins. Although cubic phases can be formed by “nonlamellar” lipids in vitro, only very few data directly correlate cubic membrane formation with cellular lipid profiles in vivo [50]. In one study, it was shown that the molar ratio of monogalactosyl diacylglycerol to digalactosyl diacylglycerol was higher in the prolamellar body (PLB) fraction that exhibits cubic membrane morphology than in the prothylakoid fraction [50]. Also, the content of glycolipids and protochlorophyllides was increased in the PLB fraction [50]. Detailed lipid analysis of amoeba Chaos cells with cubic membrane organization in their mitochondria revealed an unusually high concentration of highly polyunsaturated fatty acids (C22:5; docosapentaenoic acid) [40]. Three predominant lipid species, namely plasmalogen PE (C16:0/C22:5), plasmalogen PC (C16:0/C22:5), and diacyl-PI (C22:5/C22:5), were identified in amoeba Chaos lipid extracts and their relative amounts doubled under starvation stress conditions [40]. A rich body of data suggests that plasmalogens—which are also present in mammalian cell membranes—may serve as mediators of membrane dynamics because of their high propensity to form inverted hexagonal structures [51]. This property has also suggested a potential role...
for plasmalogens in facilitating membrane fusion processes (for review see Ref. [52]). Biophysical studies have also shown that the presence of plasmalogen PE lowers the lamellar (Lα) to hexagonal-phase (HII) transition temperature [53]. Interestingly, Chinese hamster ovary (CHO) cells, which display massive membrane rearrangements upon HMG-CoA reductase overexpression, are also rich in plasmalogen lipids (up to 11% of their total phospholipids), especially plasmalogen PE [54].

As mentioned earlier, the electrostatic charge of the ions in the medium plays an important role in cubic phase formation and stability as well as cubic membrane transformation. Sequestering divalent cations such as Ca^{2+} and Mg^{2+} seems to be essential to isolate intact cubic membranes from biological samples. Furthermore, pathological conditions result in disturbance of intracellular Ca^{2+} homeostasis that are frequently associated with cubic membrane formation (e.g., muscular dystrophy) [2]. Thus, the electrostatic interaction between the membrane components that leads to cubic membrane formation might be affected by the concentration of the intracellular electrolytes and pH, which subsequently may affect cubic membrane transformation and integrity.

5. Induction of Cubic Membranes Through Integrated Alteration of Membrane Lipids and Proteins

Frequent observation of ER membranes transformation into cubic structure provides a valuable experimental system to analyze the underlying molecular mechanisms of cubic membrane formation. ER is the most prominent membranous network in the cell responsible for the synthesis and trafficking of a wide range of lipids and proteins. It is also a critical organelle for Ca^{2+} homeostasis. As a central regulator of protein synthesis, trafficking, and targeting, the ability of ER to adapt its capacity to manage synthetic, metabolic, and other adverse conditions is of paramount importance for the cell. Under conditions that challenge ER function and induce cubic membrane formation, particularly an overexpression of recombinant proteins as well as disturbance in calcium storage and redox status, the overloaded ER with unfolded or misfolded proteins would overwhelm ER function [55]. To maintain homeostasis, the ER has evolved functions to sense the stress in its lumen and transduce signals to the cytoplasm and the nucleus in an attempt to adapt for cell survival. These responses including transcriptional activation and translational attenuation are collectively termed the unfolded protein response (UPR) [56]. The three major pathways in which ER responds to stress conditions (see Fig. 2) have also been implicated in the regulation of cellular lipogenesis and likely in cubic
membrane formation. In response to low sterol levels, sterol regulatory element binding protein (SREBP) proteins translocate from ER to the Golgi and are processed into active transcription factors [57]. Activation of SREBP proteins is critical for the regulation of target genes involved in

**Figure 2** Proposed model illustrating the effects of ER stress on the regulatory machinery controlling ER protein and lipid homeostasis and cubic membrane formation. Dashed lines indicate known factors which induce cubic membrane formation (directly or indirectly) in living systems. SREBP, sterol regulatory element binding protein; UPR, unfolded protein response. See text for details.
cholesterol metabolism (SREBP2) or lipid (SREBP1) synthesis [58]. SREBP transcription factors induce cholesterol and unsaturated fatty acid syntheses for new membrane formation and ER expansion, probably, to accommodate the newly formed proteins. Interestingly, both overexpression of transmembrane protein and increased levels of unsaturated fatty acids (and probably the cholesterol) have been linked to cubic membrane formation [40].

It will be fascinating to propose this integrated network of responses to cellular stress and how it relates to the membrane transformation of the ER. ER stress could induce transformation of its morphology from lamellar into cubic organization in response to and through integrated alteration of membrane lipid and protein content in response to ER stress conditions. Cubic membranes thereafter may play a role in ameliorating ER stress through facilitating macromolecule transportation between the ER and the cytoplasm or transporting unfolded or misfolded proteins from ER lumen to the phagosomes (see section 6.1.).

6. Biophysical Properties of Cubic Membranes

6.1. Interaction with Macromolecules

Intracellular and interorganelle trafficking of macromolecules and communication are active areas of research, yet no unified model exists. To what extent intracellular trafficking depends on or can be optimized through specific types of membrane organization, for example, as continuous 3D space partitioners or networks, is unknown. In our recent experimental study, we have shown that isolated mitochondria with cubic membrane organization from starved amoeba cells incorporate and retain short oligonucleotides (ODN) with high efficiency [59]. Although the molecular basis for the high affinity of ODN to cubic membranes has not been uncovered yet, it is reasonable to assume that it is strongly promoted by electrostatic interactions with the highly curved and extended membrane surface. The ability of cubic membranes to uptake and retain biomacromolecules within their channels might explain the vital role of cubic membrane organization in viral replication, assembly, and excretion from the infected cell [60]. The model for cubic membranes as a virus assembly site “virus factory” [2] is strongly supported by TEM images of virus particles captured in the process of budding off from cubic membranes [6]. The time-course of host cubic membrane formation that occurs immediately before RNA virus maturation further supports a functional role for cubic membranes as a specialized site for viral replication and assembly [61]. The interconnected convoluted channel structure of cubic membranes could provide an ideal architecture for local concentration and storage of viral precursor molecules, with adequate interaction with the host cytoplasm. The 3D continuous membrane network with surface pores that are
50–80 nm in diameter might offer a preferable environment as a transport system or a passageway for selective entry of viral proteins, nucleic acids, and other precursors for de novo assembly of virion particles.

In the three dimensions of a cell, the partitioning of the intracellular space into subvolumes—leading to an interpenetrated pipeline system—could in principle be regulated by membrane organization, which ultimately controls spatiotemporal activities, including intracellular trafficking and transportation, and fast response to physiological alterations such as changing temperature, osmolarity, or pH during pathological or cell stress condition. Cubic membranes may facilitate enrichment—or exclusion—of certain lipids and proteins, as a prerequisite for vesicle-mediated subcellular trafficking. They may also function as partitioners within the membrane-bound organelles, to create subdomains of specific molecular composition and function. It has been repeatedly noted that the stage of cell division and differentiation has a strong influence on the formation of cubic membranes [2]. Cells that undergo rapid cell division and differentiation, such as the differentiating sieve elements, cells during spermatogenesis, or tumor cells, are well overrepresented in the collection of cubic-membrane-forming cells [2]. Obviously, during cell division and differentiation events, there is a greater need for regulated spatiotemporal transport, as well as cellular communication, which is particularly well illustrated during spermatogenesis. In the case of differentiating sieve elements, cubic membranes might have a particular role in the assimilated transport process.

6.2. Optical Properties

The photonic properties of bicontinuous or multicontinuous cubic phases based on triply periodic level surfaces (G, D, and P) have been studied intensively [62–64]. In general, the lattice size of the photonic crystal has to be of the same magnitude as the wavelengths to be controlled. Photonic crystals based on triply periodic level surfaces with the largest photonic band gap (PBG) are of type D and G cubic structures [62]. It has been shown that D and G architectures present very wide and complete PBG [62]. The same G-surfaces are used to describe cubic membrane organization of “lens mitochondria” observed in *Tupaia* species [2,65,66]. As both lens mitochondria and photonic crystals with PBG properties share the same geometry (G type), it is interesting to speculate that cubic membrane architecture in the lens mitochondria of *Tupaia* species may function as a photonic crystal with a lattice size close to UV wavelength and their PBG property might be able to block or direct UV light from reaching the outer segment of photoreceptors.

Our preliminary results from a comprehensive 3D simulation show that the light transport through the cubic membrane arrangement yielded the preliminary result that photonic crystal properties might not be required for the lens mitochondria to operate as a wide-angle, broad-band UV filters in
front of the photoreceptors. Our simulations inscribe in a simplified assembly of such membranes the power to reduce UV transmission as much as 20% beyond a certain frequency, with the interference filter also showing sufficiently steep transitions not to block the visible light from the eye (Fig. 3).

**Figure 3** Cubic membrane simulation in 2-dimension (2D). (A) A TEM micrograph representing a cubic membrane ‘slice’ (Left) was used to simulate the light transport across cubic membrane structure in 2D. This structure is assumed to extend infinitely in the Z-axis (Middle). The multi-membrane layers are locally stratified - they are arranged in parallel assemblies which are curved on a larger scale but whose interference properties locally resemble a stratified interference filter (Right). (B) A graph of the light reflection properties across a lens mitochondrion. The percentage of light reflection depends on the wavelength and the angle of incidence of the light beam. It shows the strong rejection of short wavelengths. (C) A typical change of refractive index as experienced by a beam of light traversing the cubic membrane structure. Preliminary results from the 3D simulation of two compartments with identical refractive index.
So a dual hypothesis can be tested with this work. And even if the photonic properties of the membrane assembly prove to be insufficient, there is strong evidence that their optical properties are significantly more far reaching than have been demonstrated before, with most of the presumptions being testable theoretically and computationally. The numerical framework, however, is too computationally demanding to be exhaustively implemented. We tested and deemed sufficient the use of lower orders of diffraction only in a Monte-Carlo scattering simulation which simulates the full phase and polarization evolution of the light transition. These simplifications are also applicable to large assemblies and frameworks and allow for quantitative predictions of much more complex cubic membrane assemblies. Our computer simulation of cubic membrane organization highlighted a potential novel role of cubic membranes in mammalian retinal tissues.

7. Potential Applications of Cubic Membranes

Over the past few years, bicontinuous cubic phase liquid crystals have been investigated for their applicability to controlled delivery of active ingredients and as matrices for membrane protein crystallization. Recent studies on the physical properties of the “biological analog” of cubic phase (cubic membranes) may suggest a range of potential applications.

7.1. Cubic Membrane as a Vehicle for Gene Transfection

Lipid polymorphism is an essential aspect to consider in designing liposomes for biologically active agent delivery [67]. For example, a lamellar phase comprising parallel strands of DNA sandwiched between fluid lipid bilayers is a prevailing system for DNA packaging [68], and the subsequent induction of a phase transition from the multilamellar “sandwich” structure to an inverted hexagonal or cubic phase is associated with the efficient release of DNA molecules [69]. The potential application of dispersed liquid crystalline phases such as bicontinuous cubic phase dispersions (known as cubosomes) for drug delivery has received increasing interest [70]. However, their potential as gene delivery systems is likely to be limited: the ability of cubic phase to interact with DNA is largely dependent on the charge interaction between negatively charged DNA molecules and cationic surfactants/lipids forming cubic phases [71]. However, the interaction of well-organized cubic membrane with ODNs may not require charge interaction with DNA molecules.

It is known that compaction and/or condensation of DNA is crucial for its delivery into the target cells. Moreover, membrane fusion is a process that requires intermediates with highly curved structure. It has been illustrated that during viral infection, viral fusion peptides promote the formation of inverted lipidic phases and nonlamellar-forming lipids, such as PE, to increase the rate of viral fusion in many cases [72,73]. As mentioned above,
cubic membranes, because of the presence of abundant nonlamellar-forming lipids, such as plasmalogens and polyunsaturated fatty acids (such as Docosapentaenoic acid (DPA)), may be able to undergo membrane fusion readily [74,75] once they approach the cellular plasma membranes and the nuclear membrane. This might be supported by the observation that lipoplexes containing plasmalogen are able to undergo membrane fusion readily [74,75] upon interaction with the cellular plasma membranes.

Studies evaluating transfection efficiency of gene carriers highlighted one of the most crucial barriers in the ability of DNA/vector complex to release DNA after cellular entry. These findings all stressed on the importance of nonlamellar phases, one of which being the micellar cubic phase [75] for the release of DNA from the transfection complexes. Lipoplex forming cubic phases, which assemble the structure found in cubic membrane, were found to have intermediate efficiency for DNA release, higher than that of lamellar phases.

Although the experimental data on using cubic membranes or cubic membrane-derived lipids in gene transfection are promising, more studies are required to assess their efficiency and toxicity.

7.2. Cubic Membrane as “Structure” Antioxidants

It had been established that starved amoeba Chaos contains greater levels of free radicals than fed cells [28]. Meanwhile, starvation induces cubic membrane transformation of their inner mitochondrial membranes. As the appearance of cubic membranes coincides with the increase in ROS production, it is probable that the structural transition in the mitochondrial inner membrane may play an important part of the cell’s protective response to oxidative stress [28].

It is also possible that the alterations in lipid content during the starvation period play a protective role against Reactive oxygen species (ROS). As mentioned earlier, cubic transition of amoeba Chaos mitochondria when food is withdrawn is accompanied by major changes in fatty acid composition [40]. In particular, DPA content increased by 1.6-folds during the fasting process and is the most distinguished fatty acid species in Chaos lipids under starvation conditions. Recent investigations show that long chain polyunsaturated fatty acids (LC-PUFAs) such as DPA may have antioxidant abilities [40]. In one study [76], it was noted that fatty acid micelles containing LC-PUFAs were able to scavenge greater amounts of superoxide anions than that containing saturated fatty acids. In another investigation, it was observed that Escherichia coli cells with elevated levels of LC-PUFAs were resistant to hydrogen peroxide damage [77]. These cells exhibit lower intracellular concentrations of hydrogen peroxide than the control strain when treated with exogenous hydrogen peroxide [77]. In their study, Okuyama and colleagues [77] postulated that the membrane-shielding
effects observed in cells with elevated levels of LC-PUFAs may be attributed to the inherent structure of phospholipids that contain these fatty acids.

It is probable that phospholipids containing both LC-PUFAs and saturated fatty acids form more hydrophobic interfaces between the phospholipid bilayers. This hydrophobic interface could prevent the diffusion of hydrogen peroxide within cubic membrane compartment, thus protecting biomolecules contained from oxidative damage (Fig. 4). Furthermore, cubic membranes isolated from starved amoeba Chaos are found to be rich in plasmalogen PC [40]. This phospholipid can be preferentially oxidized to produce a relatively stable radical, thus inhibiting the chain reaction initiated by ROS [78]. Hence, the increased plasmalogen PC content in cubic membranes is possibly another factor that makes cubic membranes a good shield against ROS (Fig. 4).

7.3. Cubic Membranes as Optical Filters

Interference from low-grade optical components is a novel aspect of cubic membranes and differs substantially from synthetic interference filters. Previous studies have suggested that double membrane-bound “ellipsosomes”
and megamitochondria observed in the retina of *Tupaia* species [65,66] might play a role in altering the spectral sensitivity of the photoreceptors to act as filters which pass only longer wavelengths (Long pass filter) or as microlenses to enhance the photon capture in the outer segment of the photoreceptors. However, in most of these studies the 3D structure and physical characteristics of these specialized mitochondria have not been explored. The apparent 3D ultrastructural similarity of lens (cubic) mitochondria to photonic crystals and our computational simulation result strongly suggest a role of the cubic membranes as “optical filters.” However, a full 3D computer simulation of the light transport across the cubic membranes in lens mitochondria and experimental investigations are necessary to test this hypothesis beyond doubt. Should the full simulation confirm that the lens mitochondria feature orientation independent properties, this will call for extensive experimental work to explore the full use of complex layered optical filters in biological systems.

### 8. Conclusion Remarks

In conclusion, cubic membrane formation in biological systems, which is induced by lipid and protein alterations, drug intervention, or stress, is a widespread phenomenon that is only poorly understood. Current analyses of nonlamellar membrane arrangements are largely restricted to the descriptive level; the identification of inducible membrane systems, such as in virus-infected cells or the transition of mitochondrial inner membranes of amoeba *Chaos* to cubic morphology upon starvation, however, opens new avenues for understanding the molecular mechanisms and cellular requirements underlying these membrane arrangements.

*In vitro* studies on cubic phases and artificial membranes on the one hand, and *in vivo* lipidomic and proteomic analysis of cubic membranes on the other, hold great promise to uncover the molecular mechanism of cubic membranes formation in living cells, and their potential function(s).

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