

Multilayer gyroid cubic membrane organization in green alga *Zygnema*

Ting Zhan^{1,2} · Wenhua Lv² · Yuru Deng^{1,2}

Received: 30 November 2016 / Accepted: 25 January 2017 / Published online: 7 February 2017
© Springer-Verlag Wien 2017

Abstract Biological cubic membranes (CM), which are fluid membranes draped onto the 3D periodic parallel surface geometries with cubic symmetry, have been observed within subcellular organelles, including mitochondria, endoplasmic reticulum, and thylakoids. CM transition tends to occur under various stress conditions; however, multilayer CM organizations often appear associated with light stress conditions. This report is about the characterization of a projected gyroid CM in a transmission electron microscopy study of the chloroplast membranes within green alga *Zygnema* (LB923) whose lamellar form of thylakoid membrane started to fold into multilayer gyroid CM in the culture at the end of log phase of cell growth. Using the techniques of computer simulation of transmission electron microscopy (TEM) and a direct template matching method, we show that these CM are based on the gyroid parallel surfaces. The single, double, and multilayer gyroid CM morphologies are observed in which space is continuously divided into two, three, and more subvolumes by either one, two, or several parallel membranes. The gyroid CM are continuous with varying amount of pseudo-grana with lamellar-like morphology. The relative amount and order of these two membrane morphologies seem to vary with the age of cell culture and are insensitive to ambient light condition. In addition, thylakoid gyroid CM continuously interpenetrates

the pyrenoid body through stalk, bundle-like, morphologies. Inside the pyrenoid body, the membranes re-folded into gyroid CM. The appearance of these CM rearrangements due to the consequence of *Zygnema* cell response to various types of environmental stresses will be discussed. These stresses include nutrient limitation, temperature fluctuation, and ultraviolet (UV) exposure.

Keywords Transmission electron microscopy · Multilayer · Gyroid · Cubic membrane · Thylakoid · *Zygnema*

Introduction

In certain species of *Zygnema*, the chloroplast thylakoid membranes tend to form more complex morphologies than the simple “lamellar-like” stack structures. We report here that the chloroplasts of green alga *Zygnema* sp. (LB923) form a 3D membrane structure described as a cubic membrane (CM), a highly ordered crystalline membrane organization based on a triply periodic curved surface with cubic symmetries (Landh 1995; Almsheerqi et al. 2006). The previous analysis reported that electron micrographs by McLean and Pessoney (1970) that contained “lamellar lattices” in *Zygnema* sp. revealed them to be a primitive CM (Landh 1996). This astonishing structure is, however, composed of several, parallel, phospholipid bilayers (Fig. 1). By definition, such a multilayer (or multi-membranous) structure partitions space into $(n + 1)$ (where n equals the number of parallel membranes) physically distinct, intertwined, but separate sub-spaces.

Due to the extremely complex geometries and symmetries of CM, their projected electron densities, as produced in transmission electron microscopy (TEM), are inherently difficult to decipher. However, the more complex the symmetries, the fewer numbers of projections are actually required to recover

Handling Editor: Andreas Holzinger

✉ Yuru Deng
dengyr@wibe.ac.cn

¹ Institute of Biomaterials and Engineering, Wenzhou Medical University, Zhejiang 325035, People’s Republic of China

² Wenzhou Institute of Biomaterials and Engineering, CNITECH, Chinese Academy of Sciences, Zhejiang 325001, People’s Republic of China

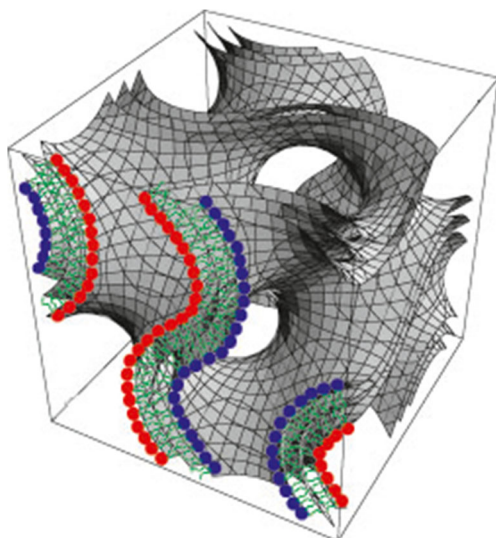


Fig. 1 The bilayer constellation of a 3D mathematical model gyroid (G) cubic membrane represents three parallel G-based cubic surfaces (one unit cell) in which the center is the nodal surface and the other two parallel surfaces are constant level surfaces. These parallel surfaces can be used to describe a multilayer gyroid CM organization

the 3D membrane shape (De Rosier and Klug 1968). The continuous membranes with cubic symmetries have, in fact, been shown to be described by cubic surfaces whose mathematical expressions are mathematically well characterized (Landh 1996; Hyde et al. 1997).

Through the method developed based on pattern and symmetry recognition between the original TEM micrographs and theoretical computer-generated 2D projection maps, three types of cubic membranes (gyroid, double diamond, primitive) have been identified (Landh 1996; Almsherqi et al. 2006, 2009). While the local shape in these CM is defined by periodic cubic surface, the global topology depends upon the continuity of the membranes. As a part of this work, a “library” of theoretical projections has been calculated as function of their potential, crystallographic viewed direction, number of membranes, and section thickness (Landh 1995, 1996; Almsherqi et al. 2006; Chong and Deng 2012).

Here, we report a structural analysis of TEM micrographs of thylakoid membrane morphologies of *Zygnema* based on the gyroid (G) surface compared to those published micrographs of primitive (P) surface by McLean and Pessoney (1970). The thylakoid CM were observed in a TEM study by using a sample preparation protocol following a modification of a previously published procedure (McLean and Pessoney 1970). Chloroplast thylakoid membranes form the CM morphology in a continuous culture of the *Zygnema* (LB923) approximately 41 days post-sub-culturing. Utilizing the image analyzing method established earlier (Landh 1995; Deng and Mieczkowski 1998; Almsherqi et al. 2006; Chong and Deng 2012), here we show that CM is based on gyroid

surface. All single, double, and multilayer gyroid CM are observed in which space is continuously partitioned into two, three, and more sub-spaces by one, two, or several parallel membranes. All these gyroid membranes are continuous with varying amount of pseudo-grana having lamellar-like thylakoid membrane morphology. The relative amount and order of these two morphologies seem to vary with the age of cell culture and are insensitive to ambient light condition. Moreover, gyroid CM continuously interpenetrate the pyrenoid body through stalk, bundle-like, morphologies. Inside the pyrenoid body, these membranes refold into gyroid CM morphologies. Our findings on the occurrence of gyroid CM transition in thylakoids at the end of log phase of *Zygnema* cell growth have led to a hypothesis for the role of CM transition as a survival strategy of cellular response to various environmental stress, including nutrient limitation in this study.

Materials and methods

Strain origin and cell culture condition

Green alga *Zygnema* sp. (LB 923) was obtained from the Department of Botany, University at Austin (UTEX) (Starr and Zeikus 1993). The culture was grown in soil water medium at 22 °C under 16–8-h light-dark cycle at light intensity of 3200 lx. *Zygnema* sp. is characterized by a pair of stellate chloroplasts connected by a bridge of cytoplasm in which the nucleus is found, in each cell. Each filament is divided into many single cells by the septa and is about 20 μm in width and several centimeters long. The pyrenoid, located in the center of each chloroplast, is traversed by numerous lamellae and is surrounded by the starch grains.

Transmission electron microscopy (TEM)

The 41-day-old *Zygnema* filaments were embedded in 1.5% agar and then fixed for 1 h at room temperature in 2% glutaraldehyde–2% formaldehyde (1:1) (primary fixation) with 0.1 M cacodylate buffer adding 2.5% sucrose, pH 7.2. After washing 4 times during 1 h with the same buffer, the cells were treated with 4% osmium tetroxide (secondary fixation) and buffer (1:1) for 1 h. The specimen was then washed four times with the same buffer and let sit overnight at room temperature. After being dehydrated in an acetone series, the cells were gradually infiltrated and embedded in Epon-Araldite in 60 °C oven for 48 h. Ultrathin sections of 50–70 nm were cut with a diamond knife on an ultra-microtome and post-stained in uranyl acetate (2%) and lead citrate (0.2%). Micrographs were taken on transmission electron microscope (JEOL 100 CX II).

TEM image analysis

A pattern recognition based on a direct template matching method is computer-generated projections which are used to match subdomains chosen from the area of interest (AOI). The latter are selected based on the apparent 2D order domain of a cubic membrane morphology displayed in a TEM micrograph. The projection direction and 2D order of the subdomain are initially assessed by its power spectrum, calculated using a fast Fourier transform (FFT) algorithm, as compared to the computer-generated template, and after cautious refinements; the resolution of the match is evaluated by a cross-correlation function (Unwin and Henderson 1975) between the subdomain and the refined template. All image calculations were performed using the image processing program package NIH Image (Landh 1996).

Results

A good preservation of the thylakoid membranes in the TEM study has been obtained following a modification of fixation and staining protocols of McLean and Pessoney (1970), and the ultrastructural features of *Zygnema* (LB 923) shown in Fig. 2 are generally in agreement with their report. However, utilizing computer simulation of TEM and direct template matching method, we show that the thylakoid membranes of *Zygnema* terminal to the log phase of cell growth display a CM with gyroid (G) morphology, rather than the primitive (P) morphologies that were identified in the analysis of Landh (1996) on the micrographs reported by McLean and

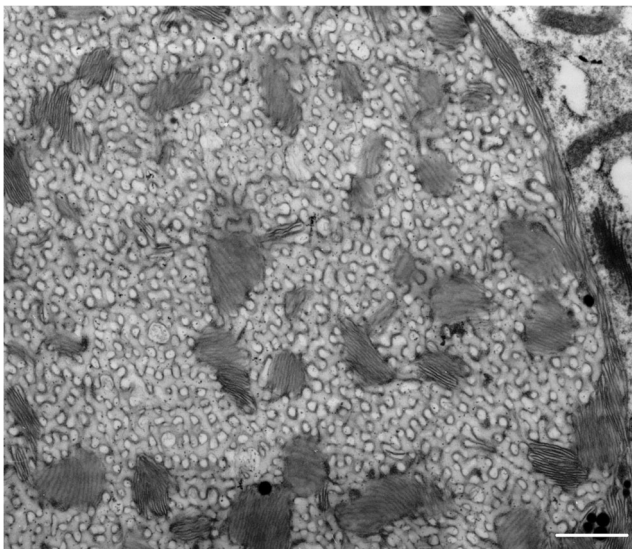


Fig. 2 An overview of TEM micrograph shows part of the chloroplast of *Zygnema* (LB 923). Lamellar-like thylakoid membrane morphology to gyroid CM transition occurred at the end of log phase of cell growth. Scale bar = 1 μm

Pessoney (1970). Most intriguingly, we observe not only single membrane constellations (Fig. 3), but also double (Fig. 4a) and multilayer gyroid CM morphologies (Fig. 4b).

Gyroid CM transition in the chloroplasts of *Zygnema* (LB 923)

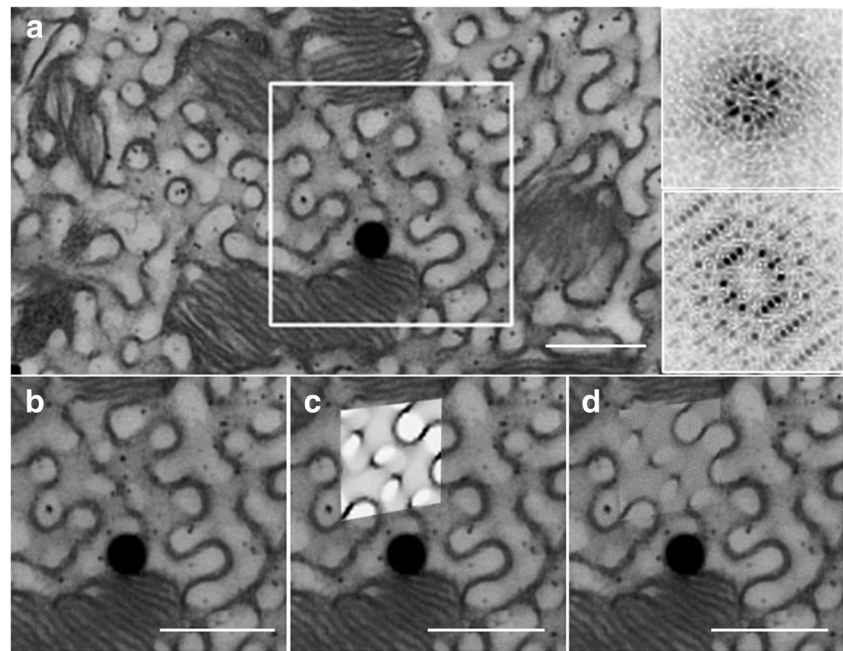
The membrane in this constellation of gyroid CM is conveniently described by reference to three parallel gyroid cubic surfaces, as schematically shown in Fig. 1. These parallel surfaces can be used to describe either a single bilayer membrane, in which case the centered surface is the “imaginary” mid-bilayer surface and two parallel surfaces are the two apolar/polar interfaces, or a set of multiple (≥ 2) bilayer membranes, in which each gyroid surface describes the mid-bilayer surface.

At the late log phase and stationary phase of cell cultures, we observed morphologies of thylakoid membranes of *Zygnema* (LB 923) cultures that displayed lamellar to gyroid CM transition (Figs. 2, 3, 4, 5 and 6). As seen in the Figs. 2 and 3, the gyroid membrane morphology coexists with the conventional lamellar-like membrane architecture. Analysis of the variation of projected electron density maps of the single membrane gyroid CM revealed that the sample thickness is in the order of half a unit cell of the lattice. Figure 3 exemplifies the effect of specimen thickness along some projection directions. A direct analysis of this CM was hampered by the apparently large unit cell size. Consequently repeating crystallographic motifs of a plane group was generally only seen in small areas relative to the total area of CM domain. Thus an AOI representing the same projection direction in an image was small which limits the use of the template matching method. Though it is less direct to analyze, these projection maps contain, however, more information than those displaying a single projection direction. An example of CM image analysis is shown in Fig. 3, in which subdomain correlates nicely with the theoretical projected electron density of a single membrane gyroid CM. The gyroid CM morphology domain shown in the TEM micrograph of Fig. 3 exhibits cubic symmetry in several subdomains. A well-ordered subdomain (boxed) which is shown to correspond to a projection of a single gyroid CM along [211] direction generated from a specimen with a thickness of approximately half a unit cell.

Structure and projections of the double membrane gyroid CM

The double membrane gyroid CM morphology is made up by two parallel membranes based on two parallel gyroid surfaces. Representative analysis of the double membrane gyroid CM morphology of *Zygnema* (LB 923) through a direct comparison between the double membrane gyroid CM morphology and its theoretical projection along the [221] direction is

Fig. 3 A TEM image analysis of the chloroplast thylakoid CM morphology. The box area of interested AOI (**a**) is shown in **b** and matched to an undistorted (**c**) and an adjusted (**d**) projection along the [211] generated for a thickness of 0.5 unit cell. Note the very characteristic pattern of gyroid CM whose electron density is matched with high accuracy. The FFT's which are the calculated (not scaled) on the experimental AOI (*upper*) and on the theoretical (*lower*) generally support a cubic symmetry and they display similar spectrums but with different relative peak intensities. Scale bars = 500 nm



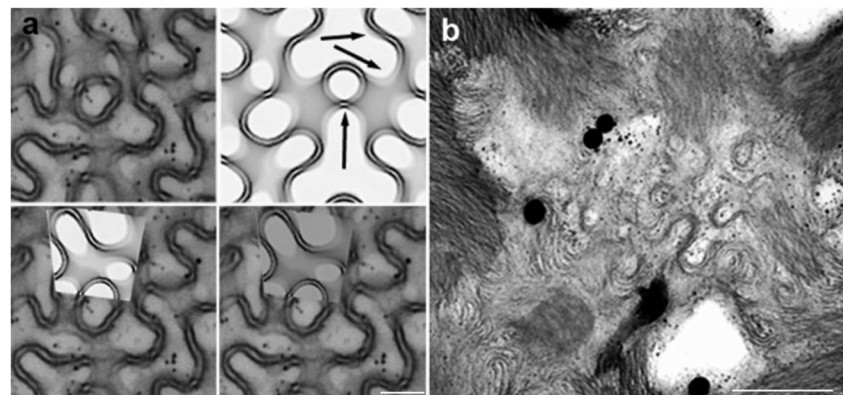
shown in Fig. 4a. As with the single gyroid membrane morphology, a minute preservation of the fine details of the projected electron density is observed (Fig. 4a, arrows). Not only can this be taken as very good indication for a gyroid-based 3D CM structure but, importantly, it is also a very good evidence of the double membrane nature of the structure (compare the projected electron density maps in Fig. 3).

The complexity of the motif of the unit cell somewhat restricts the use of direct template matching method since the symmetry of the maps is now even more sensitive to the projected direction. Figure 4a shows an attempt to identify part of the lattice motif. However, since direct template matching method only allows for restricted distortions, the degree of matching is somewhat smaller than in the case of single membrane gyroid structure. Still, the agreement of the fine variation of electron density map is fair, and the resolution of the correlation is only slightly less than that of the single membrane gyroid CM morphology.

Structure of the multilayer gyroid CM morphology

Occasionally, we have observed a multilayer (≥ 3) convoluted configuration of the thylakoid membranes at the late log phase of cell growth. The size of domains exhibiting these morphologies was, however, too small to allow an unambiguous structural analysis. An example is shown in Fig. 4b. Still, since the fine details of the electron density maps correlate to a high degree with that of computer-simulated projections of multilayer gyroid-based CM structures, we can assume that the symmetry is maintained. Due to the small size of the domains (about 1 μm), the number of parallel membranes could not be determined. The domain shown in Fig. 4b seems to display 4–8 membranes arranged in a pairwise fashion, and, as is apparent, the number of membranes revealed depends on the projection direction, making it appear as though the number varies.

Fig. 4 Multilayer gyroid CM organizations. **a** Analysis of a double-membrane gyroid CM morphology. Direct comparison of the fine details of the 2D projection map. Arrows indicate signature patterns. Undistorted (*lower left*) and adjusted (*lower right*) matches to the simulated [221] projection map (*upper right*). Scale bar = 250 nm. **b** The constellation of multilayer gyroid CM morphology. Scale bar = 1 μm



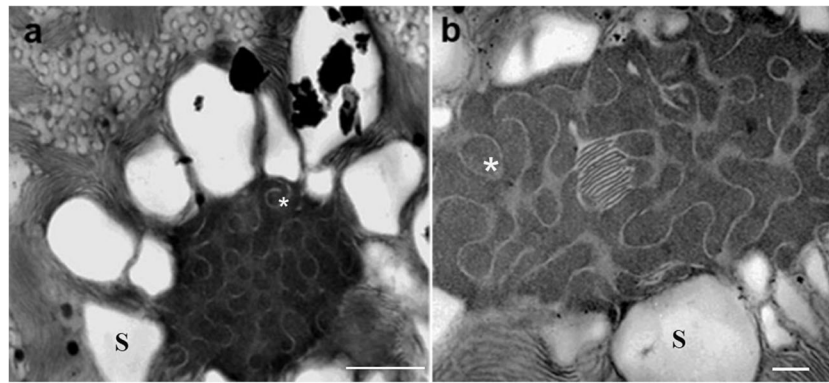


Fig. 5 The gyroid CM appears in the pyrenoid body of *Zygnema* (LB 923). **a** Through a continuous membrane folding process gyroid CM in the chloroplast thylakoids to lamellar-like morphology which forms connective stalk-like or sheet-like bundles of membranes leading into

the pyrenoid body (*) surrounded by the starch granules (S) and at which point they fold back to gyroid cubic morphology. **b** In certain cut section, lamellar-like thylakoid membrane directly fold into the gyroid pyrenoid body (*) (**a** scale bar = 1 μm ; **b** scale bar = 250 nm)

The gyroid CM morphology in the pyrenoid body

In addition to gyroid CM formed in classical thylakoids, we have also observed a gyroid CM morphology formed within the pyrenoid body as shown in Fig. 5. It seems to form through a continuous membrane folding process of the gyroid morphology in the chloroplast thylakoids to lamellar-like morphology which forms connective stalk- or sheet-like bundles of membranes leading into the pyrenoid body at which point they fold back to gyroid cubic morphology. Even though this CM morphology is yet to be rigorously analyzed, there is little doubt about its gyroid-based configuration based on the pattern recognition of our library of 2D projection maps.

Continuous membrane folding

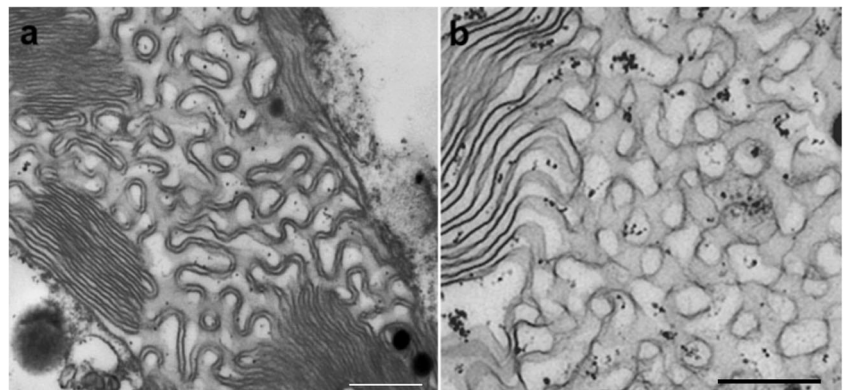
So far, we have made extensive use of the continuous nature of CM as basis in the underlying triply periodic, and hence intersection-free and physically independent, surface(s). In some fortunate sections, it is also possible to see the intersection-free folding between lamellar and cubic membrane transition. In particular, the lamellar-like morphologies are shown to be continuous with those of gyroid CM in Fig. 6, thus supporting a continuous membrane folding process

(Landh 1995). Most importantly, whereas CM allows for unambiguous distinguishing of the dividing of the 3D chloroplast inner space, the lamellar morphology alone does not. Since lamellar and cubic, two morphologies are continuous, the various spaces identified in the CM morphology as a reference structure can be traced back throughout the lamellar, and its relative unrelated location mapped out in what otherwise appears to be the identical spaces.

Discussion

Zygnema, unlike most other green algae, do not form true grana. Instead, the chloroplast thylakoid membranes tend to form, in addition to “lamellar-like” pseudo-grana, more complex morphologies than the relatively simple structures usually formed by photosynthetically active chloroplast membranes. The thylakoid membranes of *Zygnema* transform from lamellar lattice-like structure to CM shape, a striking similar membrane structure with a 3D nano-periodicity appearing in other cell types and subcellular organelles (mitochondria and ER) under various stress conditions as well (Almsharqi et al. 2009).

Fig. 6 Continuous membrane folding process in the chloroplast thylakoid of *Zygnema* (LB 923). **a** Lamellar-like thylakoid membranes fold into either double-membrane gyroid CM or **b** single-membrane gyroid CM morphologies (**a** scale bar = 500 nm; **b** scale bar = 500 nm)



The CM morphology of the chloroplast thylakoid membranes in *Zygnema* (LB 923) started to appear in the cultures at the late log phase and stationary phase of cell growth. At this aging stage, the photosynthetic apparatus is fully developed, and since the appearance of CM is insensitive to light conditions, being opposite to the case of CM in the etioplast (also called prolamellar body (PLB)) of higher plants, it is rather the stage of cell differentiation and/or cell culturing age which seems to determine such CM conversion in green alga *Zygnema*.

Unlike the lamellar to CM transformation in this report, a recent 3D reconstruction study (Kowalewska et al. 2016) of using EM tomography technique to demonstrate a continuous membrane folding process between CM to lamellar-like, without dispersion into vesicles, during the Runner bean (*Phaseolus coccineus*) chloroplast biogenesis process. The 3D reconstruction data nicely support our 2D TEM data (Figs. 2 and 6) of having reversed lamellar-CM transition in continuous membrane folding model proposed by Landh (1995).

The most complex family of the various symmetrically governed architectures of chloroplast thylakoid membrane morphologies are CM, of which the gyroid morphology (Fig. 1) is the only one identified in *Zygnema* (LB 923) (Figs. 2, 3, 4, 5 and 6) in this report. However, other type of CM in the same strain of *Zygnema* analyzed earlier by Landh (1996) on previously published electron micrographs (McLean and Pessoney 1970) reporting “lamellar lattices” in the same strain of *Zygnema* revealed them to be a primitive (P)-based CM morphology. Curiously, McLean and Pessoney (1970) suggested it to have a structure identical to that of the prolamellar body described by the model of Gunning (1965). PLB has been shown to be invariantly and unambiguously described as a double diamond (D) CM (Landh 1996; Gunning 2001; Williams et al. 1998; Selstam et al. 2007). This only exemplifies the perplexing complexity of deciphering the multifaceted projections of CM. Furthermore, the particular P-based CM in *Zygnema* reported by McLean and Pessoney (1970) is, unlike the gyroid CM in this report, always composed of several, approximately parallel, bilayers. Recall that such a construction represents a multilayer cubic membrane described as foliated parallel cubic surfaces.

Is there any particular reason to explain why all the cultures of *Zygnema* (LB 923) investigated show only gyroid CM in this study, while the *Zygnema* reported earlier (McLean and Pessoney 1970) only seemed to form multilayer P-based CM morphologies? Does it suggest that the cell organelles optimize their spatial interior organization not only based on surface area, but also the functional aspect of a particular symmetry? Would gyroid-based CM fold into P-based CM overtime during nutrient limitation by some minor manipulation of the membrane structure to serve different function for the

purpose of cell survival? Clearly, the identification of crystallographically defined 3D membrane shape would lay the fair basis for the further exploration on the structure-function relationship of biological membranes.

Another example of CM in the prolamellar body of higher plants is always described by a D-based cubic surface with a rather constant potential and lattice size independent of species, and light or culture conditions. Landh (1995) has suggested that this particular structure might be selected to act as wave blockers through the existence of biological band-gap materials. Thus, it was suggested that the D-based CM structure of the PLB might be selected due to its efficiency in “capturing” certain photons with the wavelength necessary for the conversion of protochlorophyll to chlorophyll. Even though this hypothesis remains to be experimentally proven, it has introduced the concept of crystallographically determined structure-function aspects of cell membranes, which is a novel functional attribute of cell membranes based on their 3D organizations. These, and similar concepts, are currently being intensely explored theoretically in other light-associated biological system: the multilayer (8–12 layers) gyroid CM arrangements in the “lens mitochondria” of the small mammals treeshrew’s retinal photoreceptors (Almsherqi et al. 2012) in addition to the case of photosynthetically active *Zygnema* chloroplast; we might then conjecture that there exists some optical property for the multilayer CM organizations.

The fact that there are more than two spaces inside the chloroplast thylakoids challenges the conventional view of the topology of chloroplast interior. CM could thus act as an optimized organizational architecture principle. The multiple (≥ 3) space organization, which has been identified in conjunction with all classical membrane-bound organelles throughout almost all kinds of cell types of all kingdoms, can only be realized through the scheme of CM design (Landh 1995, 1996; Almsherqi et al. 2006, 2009). Due to the continuous membrane folding process, these physically distinct spaces might be also present in other morphologies; nevertheless, they are not morphologically distinguishable. Lamellar to CM folding represents one of the most clear-cut evidences for the multiple compartmentation within the organelle of a single cell such as green alga *Zygnema* chloroplasts and/or ameba *Chaos* mitochondria (Deng and Mieczkowski 1998) in addition to treeshrew’s (*Tupaia glis* and *Tupaia belangeri*) “lens mitochondria” in the retinal cone photoreceptor cells (Almsherqi et al. 2012).

Since we only observed gyroid CM in *Zygnema* cultures older than 41 days, the age of cell culture seems to potentiate the induction of CM transformation. Tentatively, it seems as if it forms during the encystment cycle. This is supported by a study of Berkaloff (1967) on the encystment in *Protosiphon botroides* during which a multilayer gyroid CM organization appears, similar to our study on *Zygnema* sp. (LB923). This

suggests a function of CM as a center of organization of molecules in 3D space. In addition, the formation of a gyroid CM inside the pyrenoid body (Fig. 5) seems to be through a continuous membrane folding process. Does it suggest a necessity of space segregation of components involved in starch synthesis (main function of pyrenoid body)? It thus seems plausible that the CM formation in *Zygnema* might be involved in the encystment process, since the need for food reserve is highly demanded at this stationary phase of the growth. This also can be taken as a good evidence of the continuous membrane folding model (Landh 1995) in which the membranes and their bounded spaces are continuously flowing, suggesting that the intracellular volume relations are regulated through various feedback mechanisms.

Interestingly, few reports on the ultrastructure of *Zygnema* and its relation to the excellent cell cope with diurnal temperature fluctuation and UV intensities, to support that *Zygnema* is well prepared themselves to the unfavorable environmental conditions such as high or low temperature stress (Stamenković et al. 2014), desiccation tolerance, and insensitive to UV exposure (Holzinger et al. 2009), suggesting that some kind of cell protective mechanisms exist in this amazing microalga.

CM structure in the chloroplasts previously observed in *Zygnema* sp. by McLean and Pessoney (1970) was not always observed in other *Zygnema*-related studies. Nevertheless, the same gyroid CM have been induced in *Zygnema* by stress temperatures, both heat and chilling treatment (Stamenković et al. 2014). Such appearance has been suggested to serve the first line in cell protection against temperature stress. This is along the line of our hypothesis of CM as an integrated antioxidant defense system (Deng and Almsherqi 2015). Furthermore, the theoretical simulation data on a multilayer gyroid CM arrangements in the “lens mitochondria” of retinal cone photoreceptors of tree shrew (*Tupaia belangeri*) is in support of multilayer CM as optical UV filters (Almsherqi et al. 2012). The simulation result has led us to speculate that CM function in green alga *Zygnema* might be associated with UV protection. It has been reported that *Zygnema* cells are extremely well-adapted to ambient solar radiation enabling their excellent cope with experimental UV exposure (Holzinger et al. 2009). The result is in support of our hypothesis that CM may work as UV filters (Almsherqi et al. 2012).

The analogous topologies of lamellar-to-cubic membrane transition at the much smaller nanometer scales have been observed in vitro studies (Angelova et al. 2015; Angelov et al. 2009; Angelov et al. 2015). The co-existence of 3 phase nanostructures appeared in lipid membrane-type particles after BDNF proteins uploaded (Angelova et al. 2015) which is similar to those observed in the numerous TEM micrographs in biological system (Almsherqi et al. 2009). However, there is no multilayer gyroid structure found in the self-assembled lipid systems as it is in the biological system. Moreover, the

lattice size of cubic phases in lipid systems is usually 10-fold less compared to the cellular cubic membrane (Almsherqi et al. 2009). The reasons for the differences in both systems are not clear for now, and it requires more further investigations to unfold the puzzles.

Conclusions

TEM studies and image analysis show that the ultrastructure of thylakoid membranes in green alga *Zygnema* sp. (LB 923) is based on an intersection-free membrane folding between lamellar-like and CM-based morphologies. Analysis of subdomains of TEM micrographs using pattern recognition and direct template matching method shows that CM morphology is based on gyroid surfaces. Since one, two, or multiple separate membrane constellations can be identified in which each membrane is draped on parallel gyroid cubic surfaces with particular potentials, defining two, three, or more physically distinct spaces, respectively.

The invariant choice of gyroid CM morphology in *Zygnema* (LB923) allows for the study of specific structure-function relationship based on the symmetry and its activities alone. Curiously, ambient light does not seem to be a primary factor in the choice of symmetry. The cell age of the culture seems to be the key for such CM transformation in this study. The identification of various space constellations unambiguously defined in CM morphology allows for the study of spatial relations in other morphologies, as well as through the concept of continuous membrane folding.

Acknowledgments We thank Tomas Landh for his help on the TEM image analysis and the insightful inputs for the discussion. We also thank Mark Mieczkowski for providing “Cubic Membrane Simulation Projection” program (QMSP). We also thank the Electron Microscopy Center at Wenzhou Medical University. This work is supported by grants from the National Natural Science Foundation, China (Grant No: 31670841) and Wenzhou Institute of Biomaterials and Engineering (Grant No: WIBE2015010-02) to Y.D.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

- Almsherqi ZA, Kohlwein SD, Deng Y (2006) Cubic membranes: a legend beyond the flatland of cell membrane organization. *J Cell Biol* 173:839–844
- Almsherqi ZA, Landh T, Kohlwein SD, Deng Y (2009) Chapter 6 cubic membranes: the missing dimension of cell membrane organization. *Int Rev Cell Mol Biol* 274:275–341
- Almsherqi Z, Margadant F, Deng Y (2012) A look through “lens” cubic mitochondria. *Interface Focus* 2:539–545

- Angelova A, Angelov B, Mutafchieva R, Lesieur S (2015) Biocompatible mesoporous and soft nanoarchitectures. *J Inorg Organomet Polym Mater* 25:214–232
- Angelov B, Angelova A, Vainio U, Garamus VM, Lesieur S, Willumeit R, Couvreur P (2009) Long-living intermediates during a lamellar to a diamond-cubic lipid phase transition: a small-angle X-ray scattering investigation. *Langmuir* 25:3734–3742
- Angelov B, Angelova A, Drechsler M, Garamus VM, Mutafchieva R, Lesieur S (2015) Identification of large channels in cationic PEGylated cubosome nanoparticles by synchrotron radiation SAXS and Cryo-TEM imaging. *Soft Matter* 11:3686–3692
- Berkaloff C (1967) Ultrastructural changes of the chloroplast thylakoids during the development of the encystment. *J Microsc (France)* 6: 839–852
- Chong K, Deng Y (2012) The three dimensionality of cell membranes: lamellar to cubic membrane transition as investigated by electron microscopy. *Methods Cell Biol* 108:317–343
- De Rosier DJ, Klug A (1968) Reconstruction of three dimensional structures from electron micrographs. *Nature* 217:130–134
- Deng Y, Almsharqi ZA (2015) Evolution of cubic membranes as antioxidant defence system. *Interface Focus* 5:20150012
- Deng Y, Mieczkowski M (1998) Three-dimensional periodic cubic membrane structure mitochondria of amoeba *Chaos carolinensis*. *Protoplasma* 203:16–25
- Gunning BES (1965) The greening process in *Plast. 1*. The structure of the prolamellar body. *Protoplasma* 60:111–130
- Gunning BES (2001) Membrane geometry of ‘open’ prolamellar bodies. *Protoplasma* 215:4–15
- Holzinger A, Roleda MY, Lütz C (2009) The vegetative arctic freshwater green alga *Zygnema* is insensitive to experimental UV exposure. *Micron* 40:831–838
- Hyde S, Andersson S, Larsson K, Blum Z, Landh T, Lidi S, Ninham BW (1997) Cytomembranes and cubic membrane system revisited. In: The language of shape: the role of curvature in condensed matter: physics, chemistry and biology. Elsevier, Amsterdam, pp 257–338
- Kowalewska Ł, Mazur R, Suski S, Garstka M, Mostowska A (2016) Three-dimensional visualization of the tubular-lamellar transformation of the internal plastid membrane network during runner bean chloroplast biogenesis. *Plant Cell* 28:875–891
- Landh T (1995) From entangled membranes to eclectic morphologies: cubic membranes as subcellular space organizers. *FEBS Lett* 369: 13–17
- Landh T (1996) Cubic cell membrane architectures. Taking another look at membrane-bound cell spaces. PhD Thesis. Lund University, Sweden
- McLean RJ, Pessoney GF (1970) A large quasi-crystalline lamellar lattice in chloroplasts of the green alga *Zygnema*. *J Cell Biol* 45:522–531
- Selstam E, Schelin J, Williams W, Brain A (2007) Structural organization of prolamellar bodies (PLB) isolated from *Zea mays*. Parallel TEM, SAXS and absorption spectra measurements on samples subjected to freeze-thaw, reduced pH and high-salt perturbation. *Biochim Biophys Acta* 1768:2235–2245
- Stamenković M, Woelken E, Hanelt D (2014) Ultrastructure of *Cosmarium* strains (Zygnematophyceae, Streptophyta) collected from various geographic locations shows specie-specific differences both at optimal and stress temperatures. *Protoplasma* 251:1491–1509
- Starr RC, Zeikus JA (1993) UTEX- the cell culture collection of algae at the University of Texas at Austin. *J Phycol* 14:47–100
- Unwin PNT, Henderson R (1975) Molecular structure determination by electron microscopy of unstained crystalline specimens. *J Mol Biol* 94:425–440
- Williams WP, Selstam E, Brain T (1998) X-ray diffraction studies of the structural organization of prolamellar bodies isolated from *Zea mays*. *FEBS Lett* 422:252–254