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The Cubic Gyroid-based Membrane Structure of the Chloroplast in *Zygnema (Chlorophyceae zygnematles)*

Yuru Deng and Tomas Landh

Departments of Biophysical Sciences and Biomaterials, State University of New York at Buffalo, Buffalo, NY 14214, USA

Green algae do not form true grana. Instead the chloroplast membrane(s) tend to form more complex morphologies than the simple "lamellar-like" structures usually associated with cell organelles. One of us has recently shown that the chloroplasts of the green alga *Zygnema* form a 3-D structure described as a ***cubic membrane***, i.e. a triply self intersection-free membrane structure based on the family of periodic surfaces with cubic symmetries (Landh 1994). Accordingly, analysis of previously published electron micrographs reporting "lamellar lattices" in the *Zygnema revealed* them to be a primitive cubic membrane. This particular structure is, however, composed of several, approximately parallel, bilayers. Such a construction represent a multicontinuous cubic membrane described as foliated periodic cubic surfaces (PCS) each differing only in the magnitude and sign of their mean curvature. By definition such a multimembranous structure partitions space into n+1(where n equals the number of membranes) physically distinct, intertwined, but separate subspaces.

Due to the very complex geometries and symmetries of cubic membranes, their projected electron densities, as produced in transmission electron microscopy (TEM), are inherently difficult to decipher. However, the more complex the symmetries, the fewer number of projections are actually needed to recover the 3-D structure. Continuous membranes with cubic symmetries have in fact, recently been shown by one of us to be described by PCS's whose mathematical expressions are well known (Landh 1994). We have developed a template-correlative matching procedure in which mathematical simulations of projected electron density maps of PCS's are matched, through cross-correlation calculations, to the experimental TEM micrographs. As a part of this work a "library" of theoretical projections have been calculated as function of their potential, crystallographic direction, and section thickness.

We have observed cubic chloroplast membranes in TEM experiments using a sample preparation protocol following a modification of that previously published. Briefly, *Zygnema* (LB 923, UTEX) filaments from cultures grown under a 16-18 hr. light (3200 lux)-dark cycle were embedded in agar, fixed with 2% glutaraldehyde: formaldehyde (1:1) in 0.1 M cacodylate buffer containing 2.5% sucrose, washed, and postfixed in 2% osmium tetroxide.

A chloroplast membrane with cubic symmetry was observed in culture LB 923 after approximately 41 days of culture (Fig. 1a). In addition to the cubic membrane, there were a small, but significant, amount of lamellar-like morphologies "dispersed" throughout the cubic membrane morphology. As is seen in Figures 1 and 5 the membrane(s) of the lamellar configuration is continuous with those of the cubic, thus giving further evidence for the continuous membrane folding model put forward earlier. Utilizing the above mentioned technique we show that the chloroplasts of *Zygnema* terminal to their log phase of growth display a cubic membrane with gryoid-based morphology (Figs. 1b, 2, and 3). Furthermore, these chloroplast membranes seem to be continuous with the membranes inside the pyrenoid body through stalk lamellar-like morphologies. Inside the pyrenoid body the membranes refold into another gyroid-based cubic membrane (Fig. 4).

Intriguingly, we observe both single (Figs. 1, 2 and 3) and double gryoid-based membrane morphologies (Figs. 5 and 6), but no primitive-PCS based multicontinuous morphology as was identified in earlier publications. The basis of such a seemingly

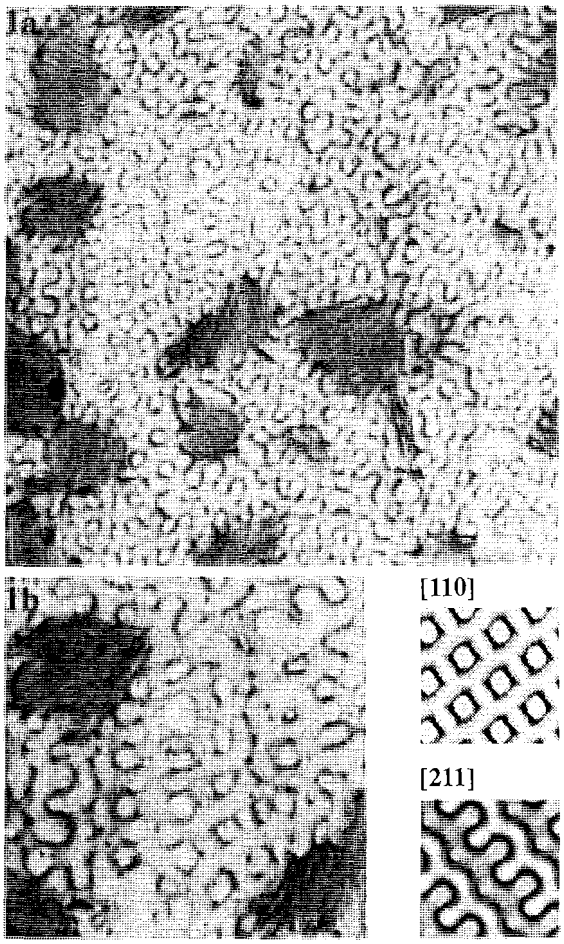

Fig. 1. TEM micrographs showing the gryoid cubic membrane morphology of a chloroplast in *Zygnema* (LB 923) (a) Overview. Note the continuous folding of the membrane between the lamellar-like and gryoid-based morphologies. Thus, while both structures, define, at least locally, two subspaces in the chloroplast, this can only be directly proven (by projections) through the study of the cubic membrane (b)Detail of the cubic membrane of which several regions can be matched to the theoretical projections a single membrane gryoid structure. The gryoid membrane has very close to zero potential i.e., an average mean curvature of zero, and the section is approximately a unit cell thick. Note the very well preserved details of the pro-jected electron density as compared to the theoretically determined expectation. Regions of the projection along the [211] and the [110] directions are indicated.

structural invariant choice is currently unknown. However, in the case of the invariant choice of the double diamond cubic membrane morphology of the prolamellar body of etiolated chloroplasts in higher plants, the underlaying structure-functional reason seems to be fulfilling a particular need-

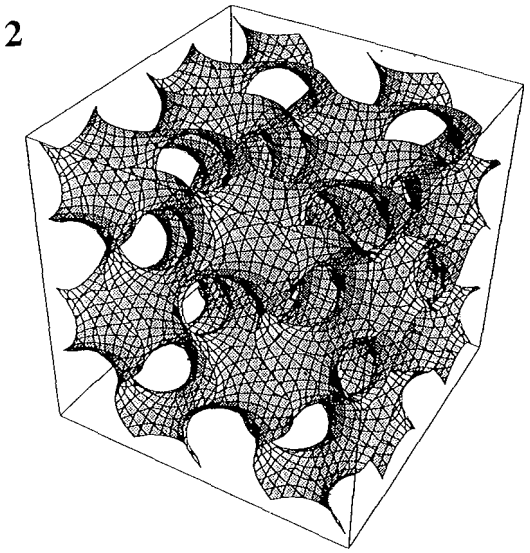

Fig. 2. The G-PCS (eight unit cells) shown as based on its nodal surface representation[1]. The group/subgroup relation is $Ia3d/I4_132$. Naturally, the space group of the cubic membranes must be that of the sugroup of type 2, i.e., the black-and-white subgroup, since the membrane is compositionally asymmetrical and thus lacks the mirror symmetry creating the invariance of the Ia3d spacegroup of the gyroid.

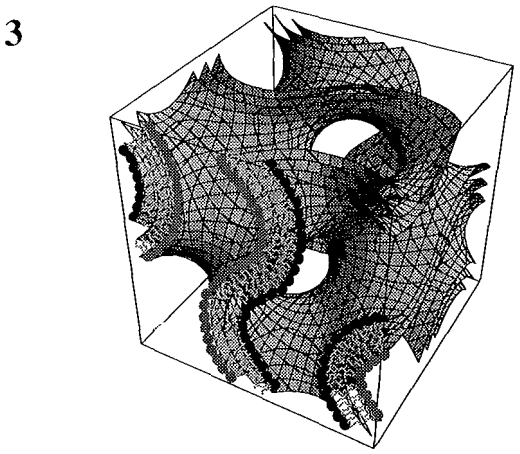

Fig.3. Three parallel G-based surfaces (one unit cell)of which the centered is the nodal (zero-potential), and the other two parallel surfaces are the constant surfaces (equi-potential). The potential differs only with respect to the sign. These parallel surfaces can be used to describe either: a single membrane, in which case the centered surface is the "imaginary" midbilayer surface and the two parallel surfaces are the two apolar/polar surfaces; or they can describe multimembranous (three membranes in this case) arrangements in whcih each surface describes the midbilayer surface of each membrane (see Figure 5 for examples depicting two membranes).

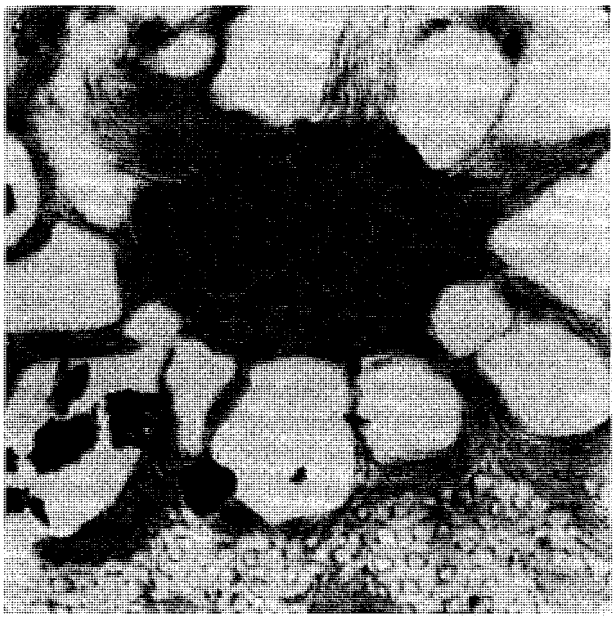

Fig. 4. G-PCS membrane in the pyrenoid body surrounded by starch granules (electron transparent). Note the folding of the cubic to lamellar-like morphology which forms a connective bundle or stalk of membrane into the pyrenoid body. (a≈500 nm)

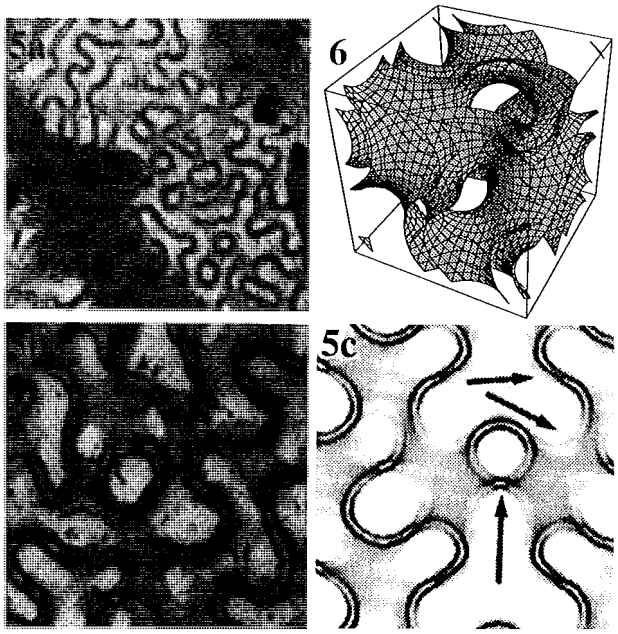

Fig. 5. Analysis of the double G-CPS based cubic morphology of *Zygnema* (LB 923). (a) Overview showing lamellar-like andcubic mor-phologies. Comparison between a enlarged view (b) of the doble G-PCS and its theoretical projection (c) along the [221] direction. Note the minute preser-vation of the fine details of the projected electron density (arrows) proving the double membrane structure.

namely, that of capturing photons.

The reason for the existence of multiple separate subspaces in organelles is currently unknown, but several theories have been put forward (Landh 1994, Landh and Deng).

Fig. 6. One unit cell of the double membrane gyroid. Note that even though this structure define three subspaces of divergent volume of the chloroplast, it has the same symmetry as the colored single membrane, i.e., $I4_132$.

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