

## Abstract

### Title

Shape Transformations of Free and Confined Lipid Vesicles:  
Three-Dimensional Analysis of Confocal Microscopy and Dynamically-  
Triangulated Membrane Simulation

(拘束条件の違いによるベシクル変形機構の解明 :

共焦点顕微鏡による三次元形状解析と動的三角格子模型による数値計算)

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In this thesis, we will discuss shape deformations of lipid vesicles to understand cells and cell organelles using a simple model. In order to quantify their shapes, we have not only performed experiments but also employed analytical, theoretical, and simulation methods.

Living body consists of several kinds of cells and cell organelles. We focus on two important factors of their morphologies: 1. Their shapes depend on their function 2. They are confined by other membranes. For example red blood cells (RBCs) have a biconcave disk shape so-called discocyte; this allows large deformations with a fixed area and volume so that RBCs can flow in microvessels narrower than themselves. These discocyte shapes can be observed in lipid liposomes. They are reproduced by minimizing the membrane bending energy with area and volume constraints.

One of the most successful models is the area-difference-elasticity (ADE) model constructed by S. Svetina *et al.* in 1989 [1]. They reproduced vesicle shapes by minimizing the membrane bending energy and the area-difference-elasticity energy with area and volume constraints. They quantify all the shapes by using two geometrical parameters; reduced volume  $v$  and reduced area difference  $\Delta a_0$ . It succeeded to reproduce starfish shape and other shapes, which are hard to make by the previous models.

On the other hand, problems still remain. To compare theoretical shapes with experimental results, we have to calculate  $v$  and  $\Delta a_0$  from microscopic images. It provides cross section lies in the focal plane, and then 2D images can only be used to quantify axisymmetric shapes. In the latest studies, researchers focus on more complex and biological systems. Then it is important to construct analytical method to compare experimental results with theoretical prediction in quantitatively.

In this thesis we focus on following two topics.

## 1. Three-dimensional analysis of lipid vesicle transformations [2]

Here we study a unilamellar vesicle to construct analytical method in order to compare experimental results with the predictions of theoretical models quantitatively. First we observed several three-dimensional images of vesicle shapes by using fast confocal microscopy. Shape deformations were induced by osmotic pressure difference. Then we constructed an original analytical method to calculate geometrical parameters  $v$  and  $\Delta a$  directly from experimental results. Finally we succeeded in quantifying non-axisymmetric shapes that were previously difficult to compare. Additionally, we observed remarkable aspect of deformation process,  $v$  stays almost constant but  $\Delta a$  decreases or increases step-like with time. This result supported the ADE model very well. It shows a possibility that there are lipid molecules reservoirs attached onto the membrane surface to induce the shape deformation.

## 2. Morphological variation of vesicle confined in spherical vesicle [3]

In order to quantify the effects of outer space constraint, we study multilamellar vesicles especially which have double-bilayer and outer vesicle is sphere.

We observed 3D images of double-bilayer vesicles. Then we construct simulation method based on dynamically-triangulated membrane model [4] including the ADE energy. The observed shapes include not only double stomatocyte which reported by O. Kahraman [5] but also following new shapes: discoidal invagination similar as mitochondria crista structure, doublet which means two hemisphere connected by small neck close to embryo structure of fertilized eggs. These shapes were well reproduced by our simulations and ADE model.

Through these two studies, we succeeded to construct following two basic vesicle models. First we succeeded to construct three-dimensional analysis method. It can become a powerful tool to understand more complicated shapes link to cell organelles. Actually it has already used for self-reproducing vesicle by Imai group and helps to figure out reasonable experimental condition [6]. Secondly, the confined system can be the simplest model of crista structure and embryo system. For crista, we observed discoidal invagination like cristae. On the other hand for embryo, we can also consider it as a simple model.

## Reference

- [1] S. Svetina and B. Žekš, *Eur. Biophys. J* **17**, 101 (1989).
- [2] A. Sakashita, N. Urakami, P. Ziherl, and M. Imai, *Soft Matter* **8**, 8569 (2012).
- [3] A. Sakashita, M. Imai, and H. Noguchi, arXiv:1309.3426v1.

- [4] H. Noguchi and G. Gompper, *Phys. Rev. E* **72**, 011901 (2005).
- [5] O. Kahraman, N. Stoop, M. M. Müller, *Nes J. Phys.* **14** 095021 (2012).
- [6] T. Jimbo, N. Urakami, P. Zihler, Y. Sakuma, and M. Imai, in preparation.