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Dr hab. Bartosz Różycki
Associate Professor
Institute of Physics
Polish Academy of Sciences
Aleja Lotników 32/46
02-668 Warszawa
Email: rozycki@ifpan.edu.pl
Phone: +48 22 116 3224

Report on the PhD thesis of Ms. Jeel Linesh Raval entitled "Shapes and shape transformations of vesicles induced by their adhesion to rigid surfaces"

The PhD thesis of Ms. Jeel Linesh Raval contributes to the research on adhesion-induced shape transformations of single- and multi-component vesicles. The system under study consists of a lipid vesicle in contact with a planar, rigid surface. The interactions between the lipid vesicle and the rigid surface are assumed to be attractive and modeled by a contact potential. The energy of elastic deformations of the vesicle is given by the spontaneous curvature model introduced originally by Wolfgang Helfrich in 1973 (Ref. [62]). This model describes the vesicle membrane as a smooth two-dimensional surface and involves to parameters of membrane elasticity, namely, the bending rigidity modulus and the spontaneous curvature.

The goal of the PhD thesis is to explore the vesicle equilibrium configurations, which are determined by energy minimization with constrains appropriate for the problem under study. It is assumed in this PhD thesis that the vesicle shapes are axisymmetric, which significantly simplifies the problem of energy minimization. As a matter of fact, the mathematical problem studied in this work is brought down to numerical minimization of a multivariable function with constrains implemented using the method of Lagrange multipliers.

Chapters 1, 2 and 3 are introductory whereas chapters 4, 5 and 6 contain original results of the PhD thesis. In chapters 4 and 5, vesicles with a single-component membrane are considered. Here, the dimensionless parameters of the model are: (i) the reduced volume v of the vesicle, (ii) the spontaneous curvature c_0 of the vesicle membrane, and (iii) either the reduced radius r of the adhesion zone (in chapter 4) or the reduced strength w of adhesion (in chapter 5). In chapter 6, vesicles with a two-component membrane are considered. The membrane components, denoted as A and B, are characterized by spontaneous curvatures c_0^A and c_0^B, respectively. An additional parameter here is the average fraction ϕ_{avg} of component A, which is assumed to take the value of 0.5.

Chapter 1 starts from an overview of elementary information on lipid bilayers and vesicles. There are several issues in section 1.1 that need to be explained:

- What is meant by "the critical length of the tail" and "the representative surface area" mentioned on page 2?
- The statement on page 5 about coexistence of the liquid-ordered and liquid-disordered phases is not precise and may be confusing. It should be made clear that the liquid-ordered and liquid-disordered phases can coexist in certain ternary mixtures of low-T_m lipids, high-T_m lipids and cholesterol. I am not aware of any studies reporting on the coexistence of the liquid-ordered and liquid-disordered phases in binary mixtures of lipids, as the paragraph on page 5 seems to imply.

- On page 1, the statement that "… they [amphiphilic molecules] could also aggregate to form bilayer structures which involve a large edge free energy, etc." is unclear to me.
- What is exactly the difference between "H-bonding" and "H-bonds" on page 1?
- There are no references to figures 1.1 and 1.2 in the text. Thus, these figures seem to be redundant.

Section 1.2 provides a brief review of literature on adhesion of lipid vesicles. There are several statements in this section that are vague or imprecise. In particular, the following questions arise when reading section 1.2:

- What are the "structural forces" mentioned on page 8? What is the physical origin of these forces?
- What precisely are the (V, A) and (P, A) ensembles mentioned on page 9? What are the differences between these ensembles?
- What are the "sticker molecules" and "repeller molecules" mentioned on page 9? Can one give examples of such molecules?
- What is actually meant by "… if there is a complex formation of membrane component with a macromolecule" on page 10?

In addition, I do not agree with the statement on page 9 that "… at the microscopic level it is an inevitable prerequisite that the non-specific interactions are made very small such that they do not interfere with the binding of the ligand-receptor pairs". Weikl et al. (Eur. Phys. J. E 8, 59-66, 2002), for example, studied the adhesion of membranes with competing specific and generic interactions. Also Reister et al. (New J. Phys. 13, 025003, 2011) studied membranes adhering via both specific receptor-ligand bonds and non-specific interactions.

Section 1.3 starts with a brief discussion on the local spontaneous curvature of multi-component membranes with nano-domains. Then a mechanism of molecular segregation based on differences in the spontaneous curvature between the different membrane components is introduced. If section 1.3 was written clearly, it would be a useful introduction to the studies presented in chapter 6. It is not clear to me, for example, what kind of coupling is meant in the following sentence "In various biological works, they have hypothesized the segregation of components based on the coupling between the local membrane curvature and the concentration of the components [66–69]" found on page 12.

Section 1.4 defines the goal of the thesis. In particular, it is stated that "This thesis is an attempt to further the understanding of the adhesion-induced shape transformations of single and multicomponent vesicles".

The sentence "Separation of components inside the bio-membrane can help in the activation-deactivation of certain proteins, which may help in the regulation of some cell functions" on page 13 is vague. No references to biological studies that could support such a statement are given. What proteins and which cell functions are meant here? Can one give any examples?

There are many grammar errors in Chapter 1. Examples: "This chapter presents a brief introduction to the membrane structure and it's properties" on page 1; "Although the biological membranes are extremely soft structures but they are resistant against disruption …" on page 1; "… for e.g. …" at the bottom of page 2; "… like for e.g. …" on page 3; "… can undergo rotation about it's axis …" on page 3; "… the attractive forces between the adjacent molecules can be strong enough that they can be held tightly together …" on page 5; "… for vesicles with the vesicle membrane composed of single and multi-components" on page 8; "Shape change like budding was found to encourage phase segregation …" on page 10; "Such aggregation of components are possible …" on page 11; "We have seen some interesting shapes and shape transitions that occur in nature, and were also experimentally observed …" on page 12; "… the transition between the free vesicle state and it's adhered state has been identified …" on page 14.

Chapter 2 introduces the concept of bending energy of fluid membranes. In particular, the spontaneous curvature model and the area difference elasticity model are discussed. Comments and questions:

- On the top of page 18 one reads that "The total free energy of the bilayer membrane within the Helfrich-Evans model … can be described as [80-82, 85]". Does this total free energy describe the bilayer membrane at constant temperature and volume or at constant temperature and pressure? And can one determine the entropy of the bilayer membrane from this total free energy?
- The statement on page 16 that "It was found that the scale of the bending rigidity is larger compared to the scale of thermal energy, thus thermal fluctuations can be neglected …" seems to contradict the following sentence on page 3: "Membranes are extremely soft structures, and this is revealed by the thermally excited shape fluctuations which can be visible under a microscope [16]". If the thermally excited shape fluctuations of lipid membranes can be observed under an optical microscope, why can they be neglected in theoretical models?
- I do not understand the logic behind this sentence on page 17: "If the bilayer is unconnected and there are no edge restrictions, then such a bilayer will not be resistant to bending because the two monolayers will just slide past each other on being bent". First of all, what kind of connection (or lack of connection) is meant here? (The bilayer is unconnected to what?) Secondly, each of the two monolayers has some bending rigidity, so why would the bilayer be not resistant to bending?

There are language problems in chapter 2 as well. Examples: "… the height of the bilayer is very negligible compared to its lateral length dimension" on page 16; and "In fact, the pears vesicles are minimized at a lower energy minima when …" on page 19.

Chapter 3 contains a description of mathematical methods used in this work. In fact, the original results reported in chapters 4, 5 and 6 have been obtained within the mathematical framework introduced in chapter 3.

Section 3.1 introduces the arc-length parametrization of shapes of axisymmetric vesicles. In this parametrization any vesicle shape is given by the tangent angle θ as a function of the contour arc-length s. The function $\theta(s)$ is then approximated by a finite number N of Fourier modes, which enables to reduce the problem of functional minimization to a simpler problem of minimization of a multivariable function. Section 3.2 contains an exact derivation of mathematical expressions for the principal curvatures of any axisymmetric vesicle surface. Section 3.3 introduces the energy functional for an axisymmetric vesicle adhering to a planar and rigid surface.

There are two comments about equation (3.3.2) on page 25. Firstly, the integrand function $H(s)$ is not defined here. Secondly, the upper limit of integration, $L_s + r(L_s)$, is explained only on page 30, following equation (3.5.3). This explanation would be in place immediately after equation (3.3.2). In addition, there are also several important questions pertaining to the discussion following equation (3.3.2):

- What software has been used to perform the minimization?
- What is the algorithm used? Is it a steepest descent method? Or a Monte Carlo simulated annealing, perhaps?
- What is the numerical accuracy of the method used?
- And, last but not least, what is the error resulting from the truncation of the Fourier series to the first N modes?

Sections 3.4 and 3.5 introduce the energy functionals for vesicles with single- and multi-component membranes, respectively. There are several questions pertaining to section 3.5:

- The theoretical description of the two-component vesicles, as introduced in section 3.5, does not take into account the entropy of mixing of the components A and B. This seems to be in

contradiction with the discussion on page 12: "Thus, the gain in bending energy due to the lateral segregation of components competes with the loss of entropy of mixing". Why is the mixing entropy neglected in this study? How would the results presented in chapter 6 change if the mixing entropy was taken into account in the theoretical model and numerical calculations?

- On page 29 one reads that "The individual rigidities of component A and component B are assumed to be equal in our calculations". Why is this assumption made here? Can it be somehow justified? What is a molecular picture behind this assumption?
- It is assumed on page 30 that "the spontaneous curvature is a linear function of the concentration". Can one somehow substantiate this assumption? And what are the limits of applicability of equation (3.5.4)?
- The local concentration ϕ of component A is introduced on page 29. Is it the molar concentration or the mass concentration? Or perhaps, it is the molar fraction of component A?
- I do not understand the last sentence on page 28, i.e. "... one kind of lipid forms a complex with a large molecule like that of a macromolecule". What type of a macromolecule is meant here?

There are some language errors also in chapter 3, for example: "… the vesicle is characterized by it's spontaneous curvature value …" on page 26; "This kind of multi-component system is more closer to …" on page 28; "… a vesicle membrane composed of two kinds of membrane components …" on page 28.

Chapter 4 contains original results of energy minimization with constraints on the reduced radius r of the adhesion zone, which is one of the relevant parameters in this chapter. The shapes of the adhered vesicles have been studied for several values of the reduced volume v and of the reduced spontaneous curvature c_0. Namely, the diagrams of vesicle shapes have been obtained for $v = 0.545$ and $c_0 = 0$ (figure 4.4), for $v = 0.8$ and $c_0 = 2.4$ (figure 4.6), for $v = 0.7277$ and $c_0 = 2.4$ (figure 4.8), for $v = 0.545$ and $c_0 = 3$ (figure 4.10), for $v = 0.705$ and $c_0 = 3$ (figure 4.12), and for $v = 0.89$ and $c_0 = 3$ (figure 4.14). It is not clear to me why these particular values of v and c_0 have been chosen in this study. What is special about these values of v and c_0?

The diagrams shown in figures 4.4, 4.6, 4.8, 4.10, 4.12, and 4.14 indicate the range of stability for different shapes (oblate, prolate, and stomatocyte) of the adhered vesicles. These diagrams constitute the main results presented in chapter 4. Interestingly, adhesion-induced vesicle budding has been observed in certain cases with $c_0 > 0$. Importantly, most of the results presented in chapter 4 have been published by Jeel Raval and Wojciech Góźdź in an article entitled "Shape Transformations of Vesicles Induced by Their Adhesion to Flat Surfaces", ACS Omega 5, 16099-16105 (2020).

Here is several comments and questions about the content of chapter 4:

- On page 33 one reads that "The phase diagram was reproduced with 160 Fourier amplitudes for $c_0 = 0$ as shown in Fig. 4.1 and with 80 Fourier amplitudes for non-zero reduced spontaneous curvatures as shown in Fig. 4.2 and Fig. 4.3". What is the reason for changing the number of Fourier modes in the numerical calculation? Why are 160 modes used in one case and only 80 in other cases?
- Figures 4.5, 4.7, 4.9, 4.11, 4.13, and 4.15 seem to be redundant because they are never mentioned in the main text of the thesis.
- Such statements as "Such a shape transformation may lead to budding, which may be important in biological processes" on page 54 or "The adhesion of a membrane surrounding biological cells is a phenomenon that may be important in many biological processes" on page 55 are vague. What biological processes are meant here exactly?
- On page 36 one reads that "We are convinced that within the assumed parameterization of the vesicle membrane, we have found all the solutions". Can one give any scientific argument to

support this conviction?

Chapter 5 contains original results of energy minimization without the constraints on the reduced radius r of the adhesion zone. Here, in contrast to the study presented in chapter 4, the relevant parameter is the reduced strength w of adhesion. The major objective of the research presented in chapter 5 is to examine how the shapes of the adhered vesicles are influenced by the reduced spontaneous curvature c_0, the reduced volume v, and the reduced adhesion strength w. In addition, the minimal strength of adhesion necessary to stabilize the vesicle shapes belonging to different classes, or branches, is investigated. The importance of the free vesicle shape for its susceptibility to adhesion is also discussed. Interestingly, it is shown that vesicle budding may be induced by changes in the adhesion strength. Importantly, most of the results presented in chapter 5 have been published by Jeel Raval, Aleš Iglič and Wojciech Góźdź in an article entitled "Investigation of Shape Transformations of Vesicles, Induced by Their Adhesion to Flat Substrates Characterized by Different Adhesion Strength", International Journal of Molecular Sciences 22, 13406 (2021).

Like other chapters of this thesis, chapter 5 does not read well, mostly because of numerous grammar errors and vague statements. Already the first sentence of chapter 5, "This chapter explores the study of the vesicle system under adhesion where the adhesion is introduced in the form of adhesion strength …", is quite awkward. The last sentence on page 69 reads "… when a sticker molecule is attached to a pole of a vesicle, it is possible to realize the scenario presented by our calculations". How could this be done in practice (in experiments)? If a molecule is attached or adsorb to a fluid lipid membrane, would it not diffuse within the membrane rather than stay fixed at a vesicle pole? Also the last sentence on page 72 "We can speculate that such segregation governed by adhesion can be used in biotechnological applications to collect nanoparticles like scavengers (leukocytes) do in the human body" is confusing. What kind of nanoparticles is meant here? And how can the adhesion-governed segregation be implemented in the human body?

Chapter 6 contains original results of energy minimization of a two-component vesicle. Here, not only the equilibrium shapes of the vesicle membrane but also the equilibrium distributions of membrane components are investigated. The dimensionless parameters of the model considered in chapter 6 are: the reduced radius r of the adhesion zone, the reduced volume v of the vesicle, the average fraction ϕ_{avg} of membrane component A, and the reduced spontaneous curvatures c_0^A and c_0^B of components A and B. However, the analysis presented in chapter 6 is restricted to the following set of the vesicle parameters: $v = 0.95$, $\phi_{avg} = 0.5$, $c_0^A = 8$ and $c_0^B = 0$. It would be interesting to know why this particular set of parameter values has been chosen here.

Seven branches of minimum-energy shapes are identified and discussed in chapter 6: two branches corresponding to oblate vesicles, one branch corresponding to prorate vesicles, two branches corresponding to pear-down vesicles, and three branches corresponding to pear-up vesicles. Interestingly, it has been observed that increasing the adhesion radius can either increase or decrease the degree of spatial segregation of components A and B, depending on the shape of the adhered vesicle. This observation is arguably the main result of the analysis presented in chapter 6.

It is stated on page 76 that "the role of the entropy of mixing is neglected". This statement is not commented or discussed in any details. It would be interesting to know why the mixing entropy is neglected and how one can justify this neglect. Also, how are the results expected to change if the mixing entropy is included in the analysis?

Chapter 7 is a brief summary of the original results reported in the PhD thesis of Ms. Jeel Linesh Raval. It is somewhat awkward that this summary contains no literature references. One would expect the results of the numerical studies to be placed here in a broader context of research on vesicle adhesion. In addition, the PhD thesis contains no discussion on how the numerical results presented in chapters 4, 5 and 6 can be tested in experiments. Chapter 7 would be a suitable spot for such a discussion. Also, such statements as "… cells can alternate between strong and weak segregation in

order to regulate the activation and de-activation of cell activities" on page 97 are vague and should not appear in scientific publications.

In summary, in my opinion, the quality of the PhD thesis of Ms. Jeel Linesh Raval is rather low. The amount of language errors, flaws in logic, and unclear or imprecise statements in the PhD thesis – as indicated in this report – makes it somewhat questionable whether "the doctoral dissertation demonstrates the candidate's general theoretical knowledge in a discipline (or disciplines) and the ability to conduct research or artistic work independently" as required by Art. 187.1, Act of 20 July 2018, the Law on Higher Education and Science.

It should be noted that figures 4.4, 4.6, 4.8, 4.10, 4.12 and 4.14 in chapter 4 seem to be identical to figures 1-6 in the following publication: Jeel Raval and Wojciech Góźdź, "Shape Transformations of Vesicles Induced by Their Adhesion to Flat Surfaces", ACS Omega 5, 16099-16105 (2020). A possible problem is that no information about the reuse of these figures is given in the PhD thesis of Ms. Jeel Linesh Raval. I suspect that this may be considered as self-plagiarism.

It also should be noted that figures 5.1-5.8 in chapter 5 seem to be identical to figures 1-8 in Reference [111]: Jeel Raval, Aleš Iglič and Wojciech Góźdź, "Investigation of Shape Transformations of Vesicles, Induced by Their Adhesion to Flat Substrates Characterized by Different Adhesion Strength", International Journal of Molecular Sciences 22, 13406 (2021). No information about the reuse of the figures published in Reference [111] is given in the PhD thesis of Ms. Jeel Linesh Raval. Again, I suspect that this may be considered as self-plagiarism.

Among the major problems of this thesis is also the lack of information about the software and numerical algorithms used to obtain the results reported in chapters 4, 5 and 6. This raises questions about accuracy and reproducibility of the results.

In the view of the aforementioned issues, I request that Ms. Jeel Linesh Raval provides the following additional information and statements in her PhD thesis:

- Chapter 3 should contain detailed information about the software and numerical algorithms used to obtain the results reported in chapters 4, 5 and 6.
- Chapter 4 should contain a reference to the publication of Jeel Raval and Wojciech Góźdź entitled "Shape Transformations of Vesicles Induced by Their Adhesion to Flat Surfaces", ACS Omega 5, 16099-16105 (2020); and it should be clearly stated in chapter 4 that figures 4.4, 4.6, 4.8, 4.10, 4.12 and 4.14 are taken from this publication.
- It should be clearly stated in chapter 5 that figures 5.1-5.8 are taken from Reference [111].

Only when these additions are incorporated in the PhD thesis of Ms. Jeel Linesh Raval, I will be able to judge whether the thesis meets the requirements for doctoral dissertations as specified in Art. 187, Act of 20 July 2018, the Law on Higher Education and Science.

In addition to the amendments listed above, I encourage Ms. Jeel Linesh Raval to correct the language errors throughout the PhD thesis (see my comments on previous pages of this report).

Bartosz Różycki