Interaction of hydrophobic nanoparticles with a biological membrane

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Abstrakt

Energy barrier was explained as being due to bending rather than thickness change of the bilazer in the vicinity of nanoparticle.

Keywords: cell; biomembrane; Helfrich enetgy; nanoparticle;

1. Introduction

When interacting with cells, the first barrier than nanonparticles need to encounter is the plasma membrane. Interaction of nanoparticles with biological membranes have been studied over the past years, particularly in the context of their unique electornic, optical, catalytic and magnatic properties that make them extremely interesting for a variety of biomedical applications [1].

Embedding nanoparticles within liposomal layer have been used to construct stimuli-responsive liposomes [2]. Studies of vesicle-nanoparticle hybrids could also provide fundamental insight into distribution of nanomaterials within living cells and organisms [3]. Small nanoparticles (<5nm [4]) could be embedded within lipid bilayer [5] while the hydrophobic nanoparticles larger than about 6 nm in diameter tend to induce lipid adsorption around the nanoparticles to form micelle-like structures instead of vesicles [6]. Membrane properties such as the intrinsic membrane curvature also influence the nanoparticle-membrane interactions [7].

Rasch et al., 2010 showed that the state of 2 nm-sized gold nanoparticles embedded within the biological membrane depends also on the method of preparation of lipid vesicles. If the hybrids composed of gold nanoparticles and phosphatidylcholine (PC) lipid vesicles are prepared by extrusion, the nanoparticles form a dense monolayer in the hydrophobic core of the lipid bilayer without disrupting the structure of lipid molecules. Nanoparticle-vesicle hybrids prepared by the dialysis process induce aggreagation of nanoparticles thereby forming Janus-like vesicles. Rasch et al. hypothetised that there exists an energy penalization when the spherical nanoparticle is located in the midplane of the bilayer. Each nanoparticle induces void space that draws other nanoparticles together in the membrane.

The hypothesis of Rasch et al., 2010 explains clustering of nanoparticles and predicts an energy barrier between aggregated and separated state of nanoparticles. The primary aim of this study is to confirm the energy barrier hypothesis by using mathematical modeling. The secondary aim of this study is to evaluate the effect of membrane properties and particle size on membrane-nanoparticle energetics.

2. Methods

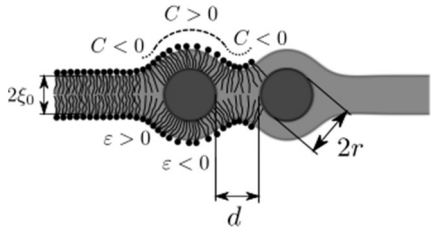

Fig. 1. *Geometry of the lipid bilayer with embedded nanoparticle of radius r at distance d. The thickness of the phospholipid hydroxycarbon tail layer in unperturbed planar lipid bilayer is denoted by* ξ_0. *Nanoparticles induce changes in the curvature of the bilayer leaflets C and changes in the membrane thickness due to deformation of hydroxycarbon tails* $\varepsilon=\xi/\xi_0$. *The presented geometry corresponds to peak energy for nanoparticle radius of 2 nm.*

The nanoparticle embedded within the lipid bilayer induces perturbation in bilayer thickness and bends the bilayer leaflets (Fig. 1). The shape of the lipid bilayer wrapping around the nanoparticle is governed by the interplay between the strain energy G_c and the bending energy G_b. The optimal shape is determined by the minimum of the total energy G .

$$G = G_b + G_c \tag{1}$$

where the energy of both, the outer and the inner membrane leaflets should be considered. The bending energy of the membrane monolayer with intrinsic curvature C_0 could be expressed as [8]

$$G_b = \frac{1}{2} k_b \int_A (2H - C_0)^2 \mathrm{d}A \tag{2}$$

where A is the membrane area, k_b is the bending modulus of phospholipid monolayer and H is the mean curvature.

A length mismatch between the thickness of the hydrophobic core of an unpreturbed monoloayer ξ_0 and the

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thickness of the deformed monolayer in the vicinity of the nanoparticle ξ describes the deformation of the hydroxycarbon tails. It is considered that the deformation is linearly elastic and that the hydrophobic binding is strong enough to prevent the exposure of hydrophobic residues to the water [9]. The deformation energy of hydroxycarbon tails is therefore expressed as [10]

$$G_c = \frac{1}{2} k_c \int_A \; k_c \left(\frac{\xi - \xi_0}{\xi_0}\right)^2 \mathrm{d}A \qquad (3)$$

where κ_c is the compression-expansion modulus of the lipid bilayer and $\frac{\Delta\xi}{\xi_0} = \varepsilon$ is strain of the hydroxycarbon core.

Within this study, two rigid spherical nanoparticles of diameter *r* separated by the distance *d* (Fig. 1) are considered. The problem is reduced to two-dimensions, as shown in Fig. 1. Within two-dimensional formulation, the membrane is considered as an elastic beam with mean curvature (*H*=*C*/2 in Eq. (2)). The energy per lipid molecule *g* is

$$g = \frac{1}{2}\frac{a_0}{l}\sum_{i=b,c} \kappa_i \int_l \; X_i^2 \mathrm{d}l \qquad (4)$$

where *l* is the total length of the bilayer sheets at the hydrophic/water interface, *X* equals ($C - C_0$) and ($\Delta\xi/\xi_0$) for *i*=*b* (bending) and *i*=*c* (strain), respectively.

The minimum energy is obtained using a custom written optimization employing basin hopping method [11]. The membrane contour is discretized and each step of the random perturbation is followed by the local minimization using the sequential quadratic programming [12]. The simulated annealing acceptance test based on standard Monte Carlo was used [13]. To reduce computational demands, one quarter of geometry is solved considering symmetry about bilayer midplane and midplane between nanoparticles (Fig. 1). The model was verified by comparison of the results with the results of an analytical model [14]. The element size was chosen on the basis of the mesh convergence test. The parameters of the simulation are listed in Tab. 1. The range of the nanoparticle size is determined by the method of Gopalakrishnan et al, 2006 who observed no nanoparticles larger than 4 nm embedded in the lipid bilayer. To study the effect of membrane properties on the energy associated with the nanoparticle inclusion, the variation in intrinsic curvature C_0, bending k_b, and compression-expansion modulus κ_c are considered.

Table 1. *Parameters of the phospholipid membrane monolayer. Bold numbers denote the default values.*

Parameter		Value
nanoparticle size	*r* [nm]	1, **2**, 3, 4 [14]
intrinsic curvatur	C_0 [nm^{-1}]	-0.1, **-0.2**, -0.3 [16]
bending moduls	κ_b [k_bT]	5, **10**, 15 [17]
compression modulus	κ_b [k_bTnm^{-2}]	30, **45**, 60 [18]
monolyer thickness	ξ_0 [nm]	**1.47** [19]
lipid area	a_0 [^{2}]	**72.4** [20]

3. Results and Discussion

Our simmulations presented in Fig. 2 confirms hypothesis of Rasch et al, 2010, that there exists an energy barrier between disperse and aggregate state of nanoparticles embedded in the biological membrane. The condensed state of nanoparticle is energetically more favourable than dispersed state. Our results could explain aggregation of nanoparticle in the lipid bilayer prepared by dialysis which was observed by Bonnaud et al, 2014 and Rasch et al, 2010. Insertion of the first nanoparticle disturbs the membrane and renders it favourable for clustering of other particles. The energy barrier further stabilizes such cluster of nanoparticles. The overall energy and the area of the membrane affected by the nanoparticle is increased with the particle size. Large nanoparticles are unlikely to be embedded within the lipid bilayer [21] and their cellular internalization happens through the wrapping of the cell membrane around the nanoparticles.

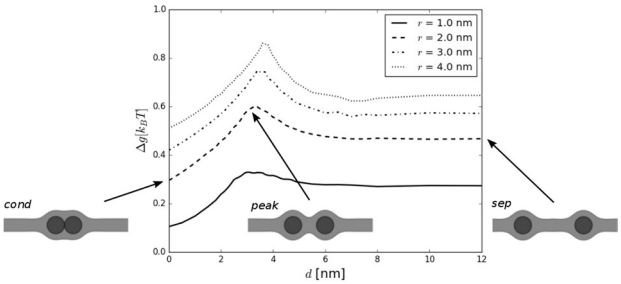

Fig. 2. *Difference between total energy per lipid molecule in the presence of nanoparticles and total energy of the planar lipid bilayer Δg, as a function of the distance between the nanoparticles d for varous sizes of nanoparticle.*

It could be expected, that a phospholipid monolayer with intrinsic curvature matching the nanoparticle would facilitate incorporation of other nanoparticles. This effect was observed for nanoparticle embedded into the micelle [22] and could be enhanced to achieve higher yield of bilayer lipid sorting [23]. However, our simmulations show that the intrinsic curvature of the nanoparticle-biomembrane assembly considerably affects the configuration of the membrane (Fig. 3A). The inclusion of the nanoparticle into the planar mebrane induces regions of positive curvature and regions of negative curvature (Fig. 1). The decrease of the bending energy pertaining to the region with positive curvature (Eq. (2)) is canceled by the increase of the bending energy pertaining to the region with negative curvature. The intrinsic curvature of phospholipids has also a limited effect on the height of the energy barrier (Fig. 4).

Rasch et al, 2010 hypothetised that the energy barrier consists mainly of the contribution of the phospholipid tail stretching. The increase of the compression-expansion modulus causes the increase of the energy required for inclusion of the nanoparticle (Fig. 3B), but not as much as the increase of the bending modulus (Fig. 3C). Our simulations show that bending of the monolayer is the decisive factor influencing the membrane energy. Increase of the bending stiffness of the phosholipid layers increases the bilayer energy associated with nanoparticle inclusion (Fig. 3C) and the height of the energy barrier separating

the condensed (Fig. 4A) and the dispersed (Fig. 4B) state of nanoparticles. The impact of bending modulus on membrane energy could be explained by larger area of lipid leaflets influenced by curvature pertrubation than areas requiring extensive change in membrane thickness (Fig. 1).

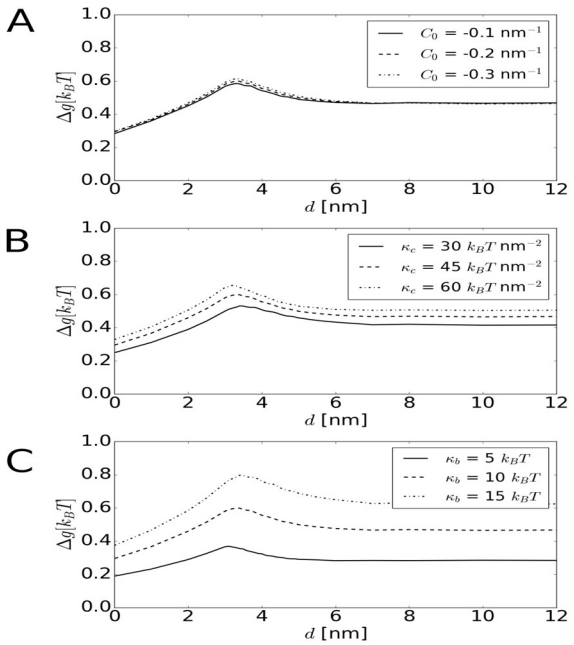

Fig. 3. *Effect of variation in (A) intrinsic curvature C_0, (B) compression-expansion modulus k_c, and (C) bending modulus on energy per lipid molecule in the presence of nanoparticles, relative to planar lipid bilayer Δu. d denotes the distance between the nanoparticles.*

Within this study a biomembrane composed of single lipid is considered. The lipid mixture with various intrinsic curvatures [16] could influence nanoparticle-biomembrane interaction. We may expect lipid sorting at the highly curved nanoparticle surface [23]. In this case, the Gaussian curvature should be included in equation (2) and entropic contribution due to change in lipid distribution should be considered.

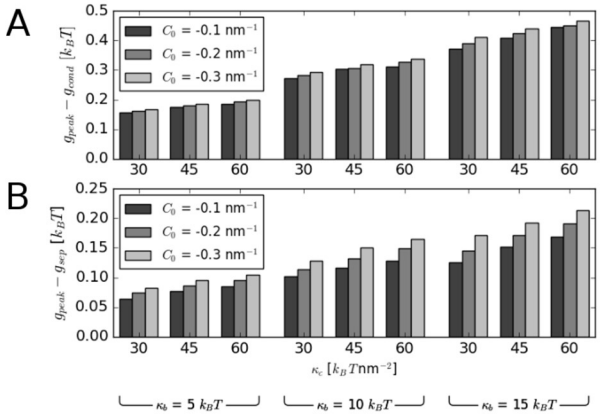

Fig. 4. *The height of the energy barrier with respect to (A) condensed and (B) separated phase of nanoparticles.*

The presented study is based on two-dimensional analysis by taking the second principal curvature in the membrane to be zero. In three-dimensions, the non-zero second curvature in Eq. (2) and integration in Eq. (4) should be performed over a large membrane patch. It would probably change the absolute values of energy, but would probably not influence the main mechanism of the energy barrier explained within the proposed study.

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