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# Budding of giant unilamellar vesicles induced by an amphitropic protein $\beta_2$-glycoprotein I

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ABSTRACT

$\beta_2$-glycoprotein I ($\beta_2$GPI) is a plasma protein capable of binding reversibly to membranes, and is classified among the amphitropic proteins. Part of the protein intercalates into the outer membrane leaflet, altering the difference between the preferred areas of the membrane leaflets, which results in membrane shape transformations. Budding, as a specific example of such shape transformations, was studied using giant unilamellar vesicles. Our aim was to identify the vesicle parameters that influence the degree of membrane budding by studying this process qualitatively and quantitatively. A simple theoretical model has been developed and assessed against the experimental observations. The results show that $\beta_2$GPI binds in a concentration dependent manner, causing transitions between vesicle shapes with increasing numbers of buds. Higher numbers of buds are characteristic of larger and/or more flaccid vesicles. When the vesicle membrane is strained, a higher $\beta_2$GPI concentration is needed to produce the same effects as on the unstrained vesicle. Vesicles were found to be highly individual in their behaviour, so each was treated individually. Specific vesicle behaviour was found to be the consequence of the neck between the main vesicle body and the buds, which could be either open, closed for the exchange of solution, or closed for the exchange of both solution and membrane.



## 1. Introduction

The class of proteins defined as amphitropic [1] can translocate reversibly from the cytosol/plasma into the membrane of cells. Three membrane binding motifs have been proposed and described in detail [2]: (i) a lipid clamp that regulates the association of a protein with a specific lipid headgroup, (ii) a covalently bound lipid anchor that embeds in the membrane and (iii) an amphipathic helix that is embedded in the membrane parallel to the membrane surface, in such a way that the hydrophobic face of the helix orients towards lipids and the polar face to the aqueous phase. $\beta_2$-glycoprotein I ($\beta_2$GPI) is an amphitropic protein of type (iii) but a structurally distinct binding motif with a patch of positive charges and a hydrophobic loop inside this patch.

$\beta_2$GPI is a 50 kDa plasma protein. Its crystal structure reveals five domains connected in an elongated fish-hook shape (Fig. 1a). While the first four domains are regular short consensus repeats, the fifth domain differs structurally and functionally [3]. It contains a 20 $nm^2$ large patch containing 14 positively charged amino acid residues and a hydrophobic loop that consists of seven amino acid residues. The loop is approximately 1.5 nm long, 1.2 nm wide and 0.9 nm deep (Fig. 1a), and the area of the cross-section of the loop of about 1 $nm^2$. These characteristics indicate that the fifth domain is implicated in the membrane binding which is mediated by strong electrostatic interactions between its positive charges and the negative charges of the lipid polar headgroups [4]. Binding of $\beta_2$GPI does not take place in the absence of the latter. The affinity increases with increasing molar fraction of negatively charged lipids in the membrane and decreases with increasing ionic strength of the medium [5–9]. The association is further strengthened by hydrophobic interactions in which the hydrophobic loop of the fifth domain is anchored into the outer phospholipid leaflet of the membrane (Fig. 1b and c).

The biological role of $\beta_2$GPI is not certain, but it is believed that when inserted in the membrane it is recognized by antiphospholipid antibodies (aPL) [10] which are associated with antiphospholipid syndrome, an autoimmune disease [11]. One possible example of $\beta_2$GPI involvement in cellular processes is the formation of apoptotic blebs on cells losing transmembrane lipid asymmetry at the beginning of apoptosis. Abundant quantities of $\beta_2$GPI were detected in the blebs [12], suggesting that $\beta_2$GPI plays a role *in vivo* in budding and vesiculation of membranes. It could thus act as an immunological marker for macrophages when removing liposomes or apoptotic cells [13]. In an earlier study of interactions of $\beta_2$GPI with giant unilamellar vesicles (GUVs) [14], buds were observed to form on the surface of GUVs. Shape transformations of GUVs could thus constitute a promising tool with which to probe the effects of this type of protein on

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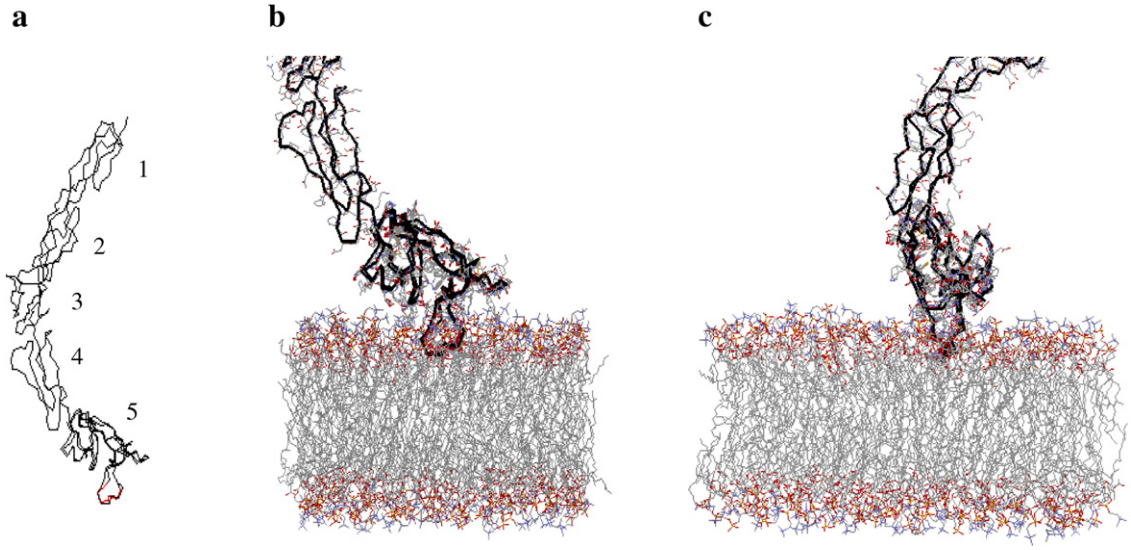


**Fig. 1.** (a) Schematic representation of $\beta_2$GPI (Protein Data Bank Identification Number: 1c1z). The structure shows an extended chain of five domains with an overall fish-hook appearance with the hydrophobic loop on the fifth domain marked in red. (b) The hydrophobic loop embedded in a membrane (the image is obtained with molecular dynamics). (c) View of the hydrophobic loop in the membrane from approximately 90° to that in b which reveals the keel-like part of the inserted portion of the protein at its distal end. It is evident that the majority of the hydrophobic loop of the protein is inserted in the region of polar headgroups of phospholipids and only the keel-like part reaches deeper into where we presume the neutral surface is. The area of the cross-section of the keel-like part is smaller than that of the cross-section of the whole inserted portion of the protein.

membranes, in particular by quantitative evaluation of the shape behaviour of flaccid GUVs in their presence. It is our purpose to identify the vesicle parameters crucial to influencing vesicle behaviour in the presence of $\beta_2$GPI. The approach introduced in this study may also be of use in studies of the effects of a variety of other proteins that have been identified to directly bind and deform membranes, e.g. proteins involved in membrane traffic processes [15].

The outline of the paper is as follows: we first present a theoretical background for mechanisms by which the binding of amphitropic proteins to the outer leaflet of membranes affects vesicle shapes. This provides a lead for designing the most informative experiments. The description and outcome of the necessary control experiments are presented in the Supplementary information. The results of the dependence of budding of GUVs on $\beta_2$GPI concentration are examined critically in the Discussion section by comparing them with the predictions of the proposed model.

## 2. Theoretical background

Vesicle budding is a specific example of vesicle shape transformations. The fact that amphitropic proteins affect vesicle shape indicates a coupling between their binding properties and the elastic energy of the membrane. This coupling is manifested by the mutual interdependence of the number of bound proteins and the vesicle shape. Here we shall treat this problem by minimizing free energy of the system ($G$), which is the sum of the elastic energy of the phospholipid membrane ($W$) and the free energy of the membrane bound proteins ($G_p$)

$$G = W + G_p. \tag{1}$$

In defining $W$ we shall take into account that we are concerned with the shape behaviour of flaccid vesicles, i.e. vesicles with excess membrane area with respect to the vesicle volume. Because the membrane is composed of two phospholipid leaflets, the relevant terms of the elastic energy of the vesicle membrane are the membrane-local [16] and non-local [17–20] bending energies, expressed as

$$W = \frac{1}{2}k_c\oint(C_1 + C_2 - C_0)^2 dA_0 + \frac{1}{2}\frac{k_r}{h^2 A_0}(\Delta A - \Delta A_0)^2, \tag{2}$$

where $k_c$ and $k_r$ are the local and nonlocal bending moduli of the bilayer, respectively, $C_1$ and $C_2$ are the principal curvatures of the membrane and $C_0$ is its spontaneous curvature. Integration is over the preferred membrane area $A_0$. $\Delta A$ is the difference between the areas of the outer and the inner leaflets and is given by $\Delta A = h\oint(C_1 + C_2)dA_0$, where $h$ is the distance between the neutral surfaces of the membrane leaflets, and $\Delta A_0$ the preferred (equilibrium) difference between the areas of the outer and the inner leaflets.

The preferred membrane area, $A_0$, and the preferred area difference, $\Delta A_0$, can be expressed in terms of the number of molecules of membrane components and their areas. Since biological membranes and thus experimental membrane systems are multicomponent, there is a possibility of multiple species of phospholipids in the membrane. We shall treat the case in which the proteins are inserted into the outer leaflet of the symmetrical and laterally homogeneous bilayer membrane. The element of the protein inserted into the outer leaflet increases the preferred area of that leaflet while the preferred area of the inner leaflet remains the same. $A_0$ and $\Delta A_0$ depend therefore on the number of bound proteins ($N_P$). The preferred areas of the outer and inner leaflets are then $A_{0,2} = \sum_{i=1}^{n_2} A_{L,i}N_{2,i} + A_pN_p$ and $A_{0,1} = \sum_{i=1}^{n_1} A_{L,i}N_{1,i}$, respectively, with $A_{L,i}$ the area of the $i$th component of $n_2$ species of phospholipid molecules in the outer leaflet and of $n_1$ species of phospholipid molecules in the inner leaflet, $N_{2,i}$ and $N_{1,i}$ the numbers of the $i$th component of $n_2$ and of $n_1$ species of phospholipid molecules in the outer and the inner leaflets, respectively, and $A_P$ the effective area of the protein insertion. The vesicle preferred area is then [19]

$$A_0 = \frac{1}{2}\left(A_{0,2} + A_{0,1}\right) \tag{3}$$

and the preferred area difference is

$$\Delta A_0 = A_{0,2} - A_{0,1}. \tag{4}$$

The protein insertion also affects the spontaneous curvature of the outer leaflet and, consequently, of the membrane ($C_0$). By taking into consideration the approximation that $C_0$ depends on the intrinsic curvatures of the protein ($C_P$) and phospholipid molecules ($C_{L,i}$) of the