Review

EXPLORING THE BINDING DYNAMICS OF BAR PROTEINS

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Abstract: We used a continuum model based on the Helfrich free energy to
investigate the binding dynamics of a lipid bilayer to a BAR domain surface of
a crescent-like shape of positive (e.g. I-BAR shape) or negative (e.g. F-BAR
shape) intrinsic curvature. According to structural data, it has been suggested
that negatively charged membrane lipids are bound to positively charged amino
acids at the binding interface of BAR proteins, contributing a negative binding
energy to the system free energy. In addition, the cone-like shape of negatively
charged lipids on the inner side of a cell membrane might contribute a positive
intrinsic curvature, facilitating the initial bending towards the crescent-like shape
of the BAR domain. In the present study, we hypothesize that in the limit of
a rigid BAR domain shape, the negative binding energy and the coupling
between the intrinsic curvature of negatively charged lipids and the membrane
curvature drive the bending of the membrane. To estimate the binding energy,
the electric potential at the charged surface of a BAR domain was calculated
using the Langevin-Bikerman equation. Results of numerical simulations reveal
that the binding energy is important for the initial instability (i.e. bending of
a membrane), while the coupling between the intrinsic shapes of lipids and
membrane curvature could be crucial for the curvature-dependent aggregation of
negatively charged lipids near the surface of the BAR domain. In the discussion,

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we suggest novel experiments using patch clamp techniques to analyze the binding dynamics of BAR proteins, as well as the possible role of BAR proteins in the fusion pore stability of exovesicles.

Key words: BAR proteins, Binding dynamics, Patch clamp, Charged lipids, Intrinsic shape

INTRODUCTION

The membrane curvature induced by Bin/Amphiphysin/Rvs (BAR) proteins has a major role in various biological processes such as cell shaping, cell growth, cell motility, receptor-ligand interactions, adhesion to the extracellular matrix, and intracellular signaling [1-6]. The BAR domains are homodimers, forming a typical crescent-like shape of positive (e.g. I-BAR) or negative (e.g. F-BAR) intrinsic curvature, depending on the side of the BAR domain rich with basic amino acids [7-10]. It was suggested that the basic amino acids at the concave or convex side interact with negatively charged lipids along the inner leaflet of the membrane, which may lead to demixing of lipids [7, 11]. In addition, two hydrophobic insertion loops, one of each monomer, were suggested to penetrate the inner membrane leaflet, thereby increasing its surface area and curvature [12]. Yet, the underlying dynamics of membrane bending concomitant to the aggregation of negatively charged lipids is poorly understood.

In previous theoretical studies, we used a continuum model to describe how membrane complexes (either proteins or lipids) that have spontaneous curvature could initiate the formation of membrane protrusions [12-15]. The coupling of the spontaneous curvature of membrane complexes (e.g. IRSp53 that has an I-BAR domain) to the membrane curvature [16, 17] is essential for the curvature-dependent flux of membrane complexes towards the tip of a growing membrane protrusion. Additionally, the interaction between negatively charged lipids and basic amino acids at the surface of a BAR domain would contribute a negative binding energy term to the system free energy, further facilitating the bending of the membrane. In the present study, we used a computational model in order to show how the negative binding energy initially bends the membrane towards the crescent-like shape of the BAR domain, and how the coupling between the intrinsic curvature of negatively charged lipids and the membrane curvature leads to the aggregation of negatively charged lipids near the surface of the BAR domain (Fig. 1). The membrane force due to the intrinsic curvature of negatively charged lipids on the membrane could have an important role in the attachment of the lipid bilayer to the BAR domain surface. The resultant bending of the lipid bilayer may lead to membrane invagination upon the attachment to F-BAR domain shapes (Fig. 1A) or to membrane exvagination in the case of I-BAR domain shapes (Fig. 1B).
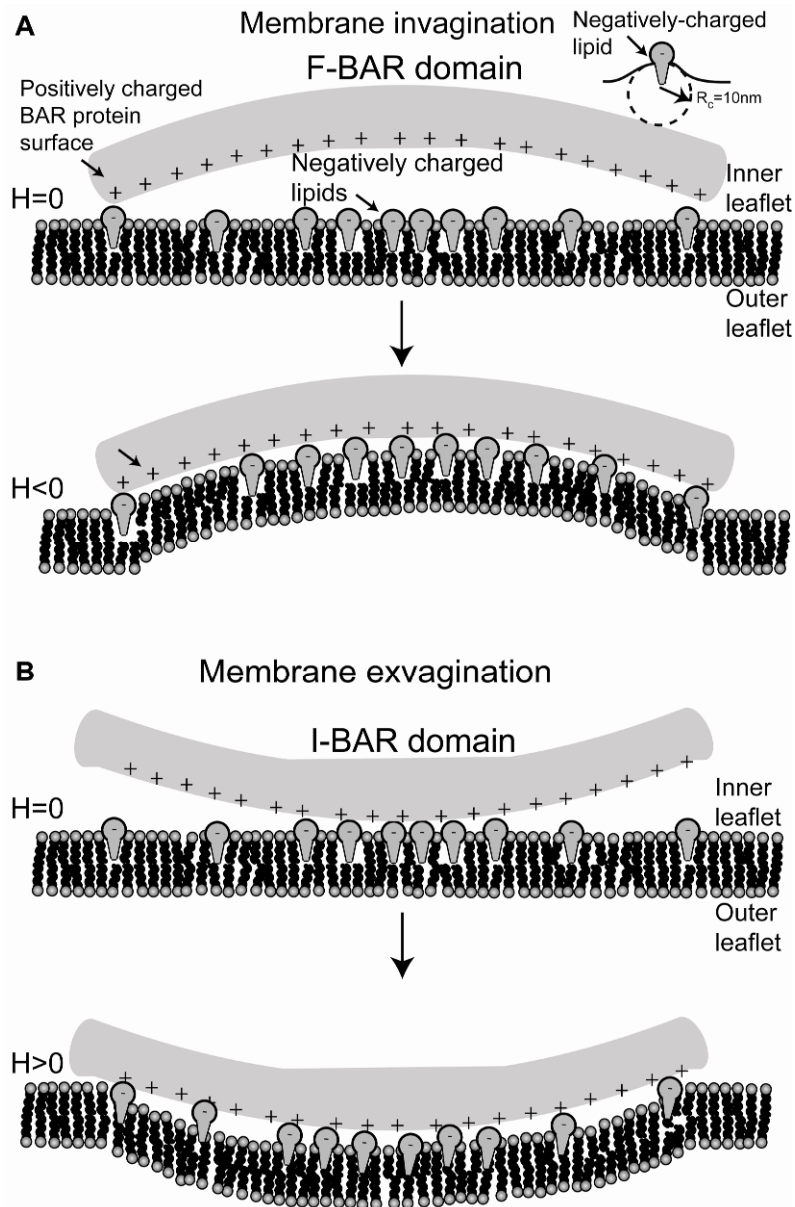
Fig. 1. Schematic diagram of the binding dynamics of a BAR domain to a lipid bilayer. The binding of a BAR domain could be driven by the attraction between negatively charged lipids and positively charged amino acids at the concave or convex regions of F-BAR (A) and I-BAR (B) domain shapes, respectively. The positive intrinsic curvature of negatively charged lipids may also facilitate the inward bending of the membrane. Note that the adhesion of the lipid bilayer to the rigid F-BAR (I-BAR) surface changes the local membrane curvature to negative (positive), initiating the formation of a membrane invagination (exvagination).

THE MODEL

We set out to investigate the membrane dynamics due to the binding of a lipid bilayer to a BAR domain of positive (concave, I-BAR shape model) or negative (convex, F-BAR shape model) intrinsic curvature. It is assumed that the BAR domain curved surface attracts negatively charged lipids to form a local Gaussian distribution. The model is a variation of previous theoretical studies [4, 13]. Our model is a coarse-grained model, in which the details of the molecular scale level are not described. The minimal length scale along the membrane that is relevant to this model is in the order of ~0.1 nm. The model is written as a set of equations of motion for the continuum fields that describe the membrane shape and lateral density of negatively charged lipids, including the actual forces acting on the membrane, and the details of the membrane elasticity. For the sake of simplicity, the modeled membrane contour of a cell surface is considered initially to be nearly flat.

The free energy of the model is based on the Helfrich energy form, including the binding energy of negatively charged lipids to positively charged regions along the BAR domain, and configurational entropy of negatively charged lipids [4, 13-14]:

$$F = \int (\frac{1}{2}\kappa(2H - \bar{H}n)^2 + \sigma_0 - \alpha n + kTn_s n(\ln(n)-1))ds, \qquad (1)$$

where κ is the membrane bending rigidity, n is the area fraction density of negatively charged lipids, $\bar{H}$ is the effective intrinsic curvature of negatively charged lipids, H is the local mean membrane curvature, n_s is the saturation density of negatively charged lipids, σ_0 is the membrane surface tension, α is the binding energy, k is the Boltzmann constant, T is temperature and $ds = d_m \cdot dl$ is an element of membrane area, where d_m is the dimension of the membrane perpendicular to the contour and dl is a line element along the contour. The first term describes the bending energy due to the mismatch between the membrane curvature and the spontaneous curvature of negatively charged lipids. The second term describes the surface tension energy. The third term describes the negative binding energy of negatively charged lipids to positively charged regions along the BAR domain, which is estimated from the electrostatic attraction (described below). The fourth term describes the entropic contribution due to the lateral thermal motion of the negatively charged lipids in the membrane in the limit of small n. The derivation leading to Eq. 1 is a one-dimensional version of the more general expressions derived in previous studies [18, 19]. These expressions recover the familiar form for small undulations of a flat membrane in the Monge gauge form.

We derive the equations of motion of the membrane contour using the derivation of the free energy (Eq. 1) with respect to the membrane coordinate and concentration of negatively charged lipids [13]. To take into account the drag due

to viscous forces, we assume for simplicity only local friction forces [4, 13], with coefficient ξ. The equation of motion of the membrane is

$$\xi \frac{\partial \mathbf{r}}{\partial t} \cdot \mathbf{n} = -\frac{\delta F(s)}{\delta \mathbf{n}} \qquad (2)$$

where ξ is the coefficient of the local friction force due to viscous drag of the fluids surrounding the membrane, $\mathbf{r}$ is the radial vector in the (x,y) coordinate system, t is time, $\mathbf{n}$ is the normal direction, and $\dfrac{\delta F(s)}{\delta \mathbf{n}}$ is the derivation of Eq. 1 with respect to the x and y directions. Here we consider only the changes along the y direction. The derivation of the free energy is projected to give the forces normal to the membrane contour [13]. See the Supplementary material at http://dx.doi.org/10.2478/s11658-011-0013-0 for the membrane contour forces and fluxes derived from the derivation of the free energy. The following is the list of parameter values incorporated in our model: $\xi = 1\,\text{s}^{-1}\text{g }\mu\text{m}^{-2}$, $D = 0.002\,\mu\text{m}^2\text{s}^{-1}$, $\alpha = 5\,kT$, $n_s = 1500\,\mu\text{m}^{-2}$, $\kappa = 100\,kT$, $\bar{H} = 100\,\mu\text{m}^{-1}$, $\sigma = 0.1\,kT$, where kT is the thermal energy (for more details see Supplementary information).

In order to estimate the binding energy between the negatively charged head groups of a single lipid molecule and the positively charged surface of a BAR domain, we estimated the electric potential at the charged surface of the BAR domain. To calculate the surface potential, the corresponding Poisson's equation for the charged surface of a BAR domain in contact with an electrolyte solution was solved using the following Langevin-Bikerman differential equation [20-22]:

$$\nabla \cdot (\varepsilon_0 \varepsilon_r(\mathbf{r}) \nabla \phi) = -\rho_{free}(\mathbf{r}), \qquad (3)$$

where $\rho_{free}(\mathbf{r})$ is macroscopic (net) volume charge density of counterions and coions in the electrolyte solution in contact with the charged surface of the BAR domain:

$$\rho_{free}(\mathbf{r}) = -2e_0 n_s n_0 \frac{\sinh(e_0 \phi / kT)}{H(\phi, E)} \qquad (4)$$

and $\varepsilon_r(\mathbf{r})$ is the relative permittivity:

$$\varepsilon_r(\mathbf{r}) = 1 + n_s n_{0w} \frac{p_0}{\varepsilon_0} \frac{F(p_0 E / kT)}{H(\phi, E)\, E}, \qquad (5)$$

where

$$F(u) = L(u)\frac{\sinh u}{u}, \quad L(u) = (\coth(u) - 1/u), \qquad (6)$$

$$H(\phi, E) = 2n_0 \cosh(e_0 \phi / kT) + n_{0w}\frac{\sinh(p_0 E / kT)}{(p_0 E / kT)}. \qquad (7)$$

For the sake of simplicity the relative permittivity (Eq. 5) was approximated by a step function with the value epsilon $\varepsilon_r(\mathbf{r} = \mathbf{r}_{surf})$ (determined by Eq. 5) in the region ($\mathbf{r}_{surf} \leq \mathbf{r} \leq \mathbf{r}_{surf} + \mathbf{a}$), where a=0.32 nm and $\varepsilon_r(\mathbf{r})$=78.5 in the region ($\mathbf{r} > \mathbf{r}_{surf} + \mathbf{a}$). Here ϕ is the electric potential, $E = |\nabla\phi|$ is the magnitude of the electric field strength, ε_0 is the permittivity of the free space (vacuum), e_0 is the elementary charge, kT is the thermal energy, n_0 is the bulk number density of positively and negatively charged ions in electrolyte solution, n_{0w} is the bulk number density of water, $n_s = n_{0w} + 2n_0$ is the number density of lattice sites [20-22], p_0 is the magnitude of the water dipole moment. Eq. 3 takes into account the finite size of ions and the spatial variation of relative permittivity due to orientational ordering of water molecules near the charged surface of the BAR domain. The following boundary conditions were taken into account [20-22]:

$$\nabla\phi(\mathbf{r} = \mathbf{r}_{surf}) = -\frac{\sigma\mathbf{n}}{\varepsilon_0\varepsilon_r(\mathbf{r} = \mathbf{r}_{surf})} , \nabla\phi(\mathbf{r} \to \infty) = 0 , \qquad (8)$$

where $\mathbf{n}$ is the normal unit vector in the direction of $\nabla\phi$ and σ the effective surface charge density of the BAR domain. In this work Eq. 3 was solved numerically [20] using the Finite Element Method (FEM) within the program package Comsol Multiphysics 3.5a (COMSOL AB, Stockholm). The boundary conditions (Eq. 8) are taken into account.
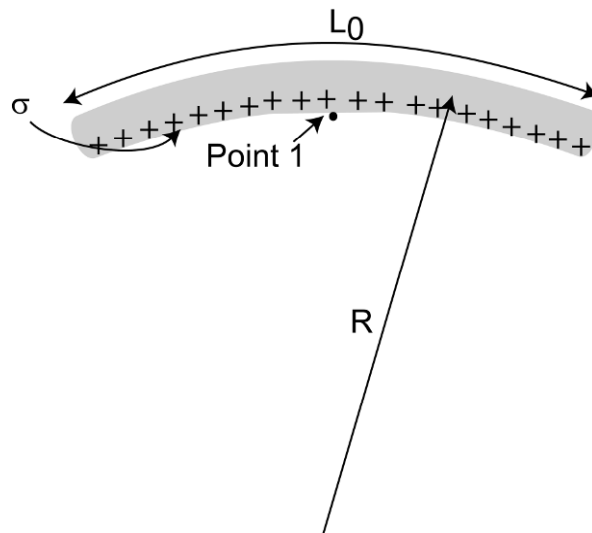

Fig. 2. Schematic diagram for the calculation of the electric field of a BAR protein. The electric field is calculated as a function of the BAR protein radius of curvature at point 1. The surface charge density of positively charged amino acids is σ, and the total length of the BAR domain is L_0.

The BAR domain is presented as a two-dimensional arch of curvature radius 20 nm and an approximate length of 20 nm. The electric field is calculated at point 1 at the domain surface (Fig. 2). The surface charge density $\sigma = 0.2$ As/m^2 of the concave side of the BAR domain is considered uniform. Note that the total protein length $L_0 = 20$ nm. The surface potential $\phi_{surf} = 130$ mV was calculated for a dipole moment of water, which is $p_0 = 4.794$ D, the bulk concentration of salt is $n_0 / N_A = 0.15$ mol/l and the bulk concentration of water $n_{0w} / N_A = 55$ mol/l. From these results the binding energy $\alpha \approx e_0 \phi_{surf} = 5$ kT was estimated.

RESULTS

The contributions of the intrinsic curvature of negatively charged lipids and the negative binding energy to the membrane dynamics during the attachment of a lipid bilayer to the surface of a BAR domain were determined using numerical simulations in MATLAB (for more details see Supplementary information). By assuming that a BAR domain attracts negatively charged lipids, the initial condition for the density of negatively charged lipids is a Gaussian distribution, assuming that the BAR domain is being attracted to hot spots of negatively charged lipids (Fig. 1). The BAR domain is of a crescent-like shape of positive or negative intrinsic curvature, modeling the membrane-bending dynamics due to I-BAR and F-BAR domain shapes, respectively. The BAR domain is positioned at close contact to the membrane surface, such that the crescent-like shape makes contact with the membrane surface at the middle of the I-BAR shape model, or at the edges of the F-BAR model (Fig. 1). It is assumed that the BAR domain is rigid, having a constant shape throughout the simulation. Due to short-range van der Waals attraction forces, membrane regions that exceed a threshold distance of 0.1 nm from the BAR domain are trapped to the end of the simulation. The negative binding energy term (i.e. $-\alpha n$ <0) could be added selectively to membrane regions at a close distance to the BAR domain surface. Numerical simulations (using MATLAB) were employed in order to reveal the separate and combined effects of the above-mentioned coupling and negative binding energy, which increase the instability of the system. In our numerical continuum model, the negatively charged lipids have a positive intrinsic (cone-like) curvature of 0.1 nm^{-1}, which means that their preferred radius of curvature is 10 nm. The saturation density of negatively charged lipids is one lipid per nm^2. The attraction to the BAR domain is captured in the negative binding energy term ($\alpha = 5$ kT). Results of numerical simulations reveal that an initial Gaussian distribution of negatively charged lipids of positive intrinsic curvature would drive the membrane inward bending (Fig. 3). The membrane is being adhered to the rigid BAR protein, functioning as a mold changing the local membrane curvature to the intrinsic curvature of the BAR domain. Consequently, the local membrane curvature at the vicinity of the I-BAR domain shape is positive, while

the local membrane curvature at the vicinity of the F-BAR domain shape is negative (Fig. 3). To have a better understanding, the membrane force derived from the free energy was separated into a force due to the negatively charged lipids and to the usual restoring force due to the spontaneous membrane curvature. It is observed that the force due to the negatively charged lipids is larger, enabling the adhesion of the membrane to the BAR domain surface (Fig. 3).
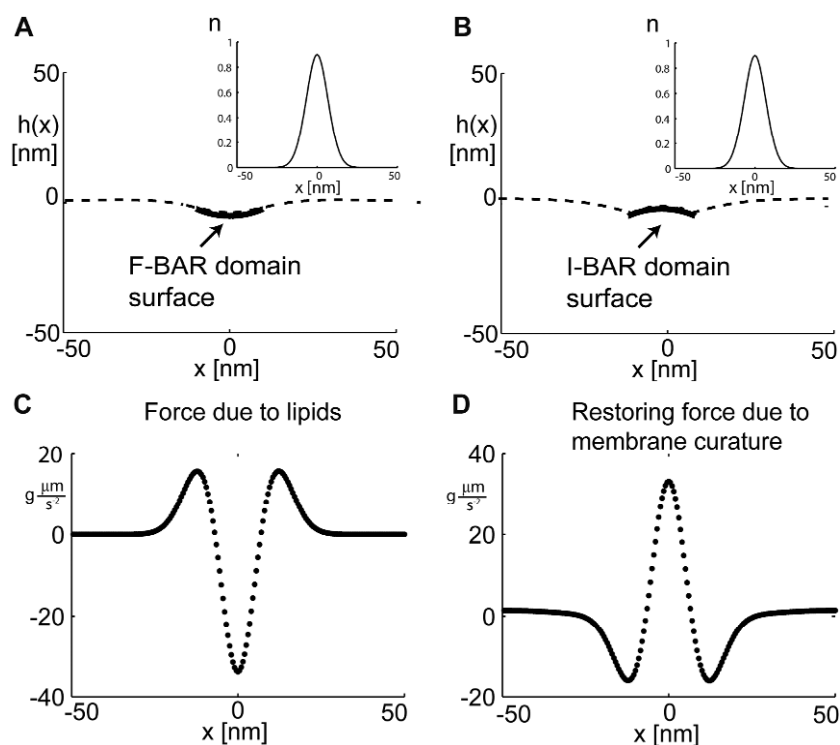

Fig. 3. Mechanisms driving the adhesion of a lipid bilayer to different BAR domain shapes. The inward bending of the membrane towards an F-BAR (A) or an I-BAR (B) domain shape is driven by the positive intrinsic curvature of negatively charged lipids and the negative binding energy due to the attraction between negatively charged lipids and positively charged amino acids (Fig. 1). The initial distribution of negatively charged lipids is of a Gaussian shape (inset). The two main forces acting on the membrane are the force due to the positive intrinsic curvature of lipids (C), and the restoring membrane force towards its initial zero spontaneous curvature (D).

DISCUSSION

A coarse-grained continuum mechanical model of the membrane is employed to investigate the membrane bending dynamics upon the binding of an individual I-BAR or F-BAR domain membrane protein (Fig. 1). The negative binding energy due to the attraction between negatively charged lipids and positively charged amino acids was estimated from calculations of the electrostatic

potential using the Langevin-Bikerman equation (Fig. 2). Additionally, it was assumed that the negatively charged lipids at the inner leaflet of the lipid bilayer had a positive intrinsic curvature (i.e. cone-like shape), enabling the adhesion of the lipid bilayer to the BAR domain surface (Fig. 3). It was demonstrated that in the limit of a rigid BAR domain, the BAR domain surface functioned as a mold changing the local curvature of the membrane according to its intrinsic curvature. For example, the adhesion of the lipid bilayer to an I-BAR domain shape may result in a positive membrane curvature (outward bending), which may initiate the formation of a membrane exvagination (e.g. membrane protrusions or exovesicles). On the other hand, the adhesion of a lipid bilayer to an F-BAR domain shape could result in a negative membrane curvature (inward bending), leading to the formation of a membrane invagination (e.g. membrane tubules or endovesicles). The dual function of negatively charged lipids, facilitating both exvaginations and invaginations, could be important, giving the cell membrane the flexibility needed to respond to different cellular functions.

Possible patch clamp experiments to explore the binding dynamics of BAR domains

Patch clamp techniques are mostly employed for the study of ion channels in cells, where the activity of individual ion channels can be recorded [23]. At a negative membrane resting potential of a cell, the membrane is depolarized by the application of a positive potential difference that brings the membrane potential closer to zero. On the other hand, the cell could be hyperpolarized by the injection of a negative potential difference that reduces the membrane potential below the resting potential [24]. In neuronal cells, a negative resting potential is maintained by the action of passive ion channels and active transport mechanisms. However, in a pure liposome system without ion channels that maintain a negative resting potential, the resting membrane potential is close to zero. Nevertheless, the effects of a negative and a positive potential difference on the membrane potential should be similar to a normal cell. We propose that by inducing stable and uniform membrane charge densities it could be possible to study the binding dynamics of BAR proteins using patch clamp techniques (Fig. 4). In the whole-cell mode one can introduce molecules into the cytosol that can affect various signaling pathways targeting the cytosol and plasma membrane [25-26]. Moreover, in the cell attached configuration elementary properties of single vesicle fusion process can be studied [27]. In particular, the use of an inside-out patch would allow the experimenter to manipulate the membrane charge density near the BAR domain protein, which may lead to the binding and bending of the patched membrane. The main advantage of patch clamp techniques is the capacity to investigate a single BAR domain protein under a controlled environment or *in vivo* conditions, testing the hypothesis that lipid demixing and changes in the membrane charge densities are crucial for the binding dynamics of BAR proteins. Finally, the role of electrostatic attraction, using patch clamp techniques, could also consider the effects of variations in the

liposome lipid compositions as well as the effects of mutations in BAR proteins known to alter their affinity and binding to cell membranes.
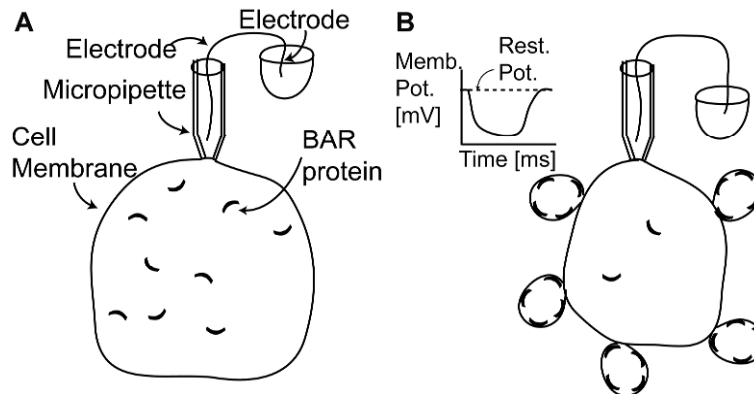

Fig. 4. Schematic diagram showing use of the patch clamp technique to study the binding dynamics of BAR proteins. A pure liposome, rich in BAR proteins of crescent-like shapes distributed mainly in the cytoplasm, is being patch clamped (A). The application of a potential difference (see inset; dotted line is the resting membrane potential) hyperpolarizes the membrane potential, enabling the binding of BAR proteins, which may initiate the formation of large-curvature (small-radius) vesicles (B).

On the role of BAR protein dynamics in the stability of a fusion pore

While the suggested experiment uses an artificial model of a pure liposome system, in a real cell, there are negatively charged lipids and lipid rafts (i.e. lipid areas enriched with cholesterol and sphingolipids) that dynamically demix due to changes in the membrane charge density [28, 29]. For example, during post-synaptic potentials (PSPs), both the binding of BAR proteins and the distribution of lipids may be altered. This possible interaction would also lead to demixing of membrane constituents that diffuse and aggregate at specific membrane regions [28, 29].

Aggregation of BAR proteins at specific membrane regions could also assist processes where bending of the membrane plays an important role, such as exocytosis. In exocytosis the merger of the vesicle and plasma membranes leads to formation of the fusion pore. The fusion pore is an aqueous channel connecting the vesicle lumen with the extracellular medium and is most likely a region of high curvature [30]. As such, it is an ideal membrane region where BAR proteins could potentially contribute to its formation, stability or even to the transition from the narrow diameter intermediate fusion pore state to either a) complete dilation (full-fusion exocytosis), or b) subsequent closure (transient exocytosis).

Direct methods to study the role of BAR proteins in exocytosis are whole-cell and cell-attached patch-clamp measurements of membrane capacitance (C_m). C_m is a parameter linearly related to plasma membrane area, which reflects the activity of exo- and endocytosis [31]. We propose to study the effects of BAR

proteins in cultured cells. For example, melanotrophs, pituitary cells from the pars intermedia, are a convenient cell type to study effects of lipids or proteins on the cumulative exocytosis in a cell [32]. A 300 second cytosol dialysis by a pipette solution containing BAR proteins should be sufficient to elicit an increase/decrease in C_m, as is sufficient in the case where we use a pipette containing high-Ca^{2+} solution (Fig. 5A). This experiment would include C_m measurements in a whole-cell configuration. On the other hand, cell-attached C_m measurements [30] (Fig. 5B) would reveal elementary fusion pore properties (i.e. fusion pore dwell time and fusion pore conductance) in cells where BAR proteins were either overexpressed or microinjected in the cells prior to the C_m measurement. Since fusion pore properties can best be determined for transient fusion pores, one would have to decide on a convenient cell type, such as anterior pituitary lactotrophs, where transient fusion is the predominant type of fusion.
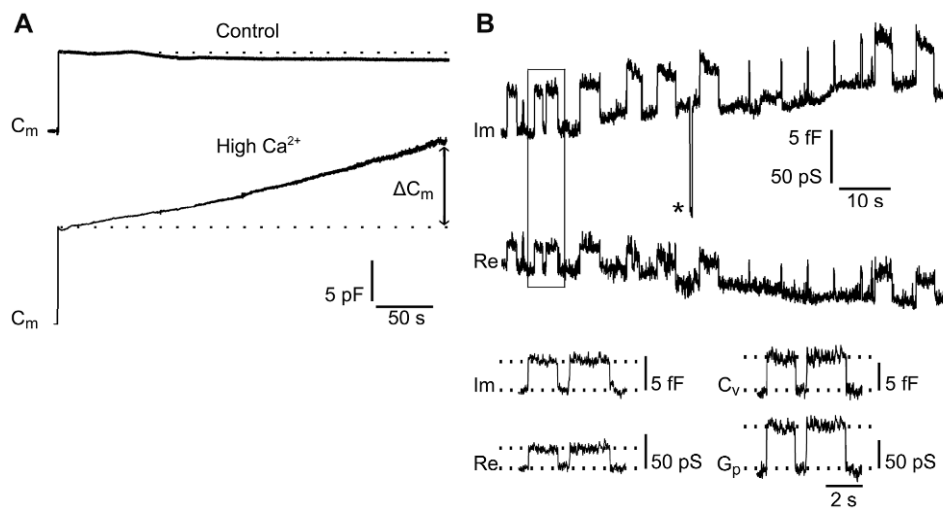

Fig. 5. Whole-cell and cell-attached membrane capacitance measurements are convenient techniques to study the binding dynamics of BAR proteins. (a) Whole-cell measurement of time-dependent changes in C_m of individual pituitary cell dialyzed with a patch pipette containing normal intracellular solution (control; top trace) and intracellular solution with added 1 μM free Ca^{2+} (high Ca^{2+}; bottom trace). Dashed lines denote resting C_m. Note the difference in C_m (ΔC_m) in a cell dialyzed with intracellular solution containing high Ca^{2+}, compared to the cell dialyzed with intracellular solution only. ΔC_m was measured 300 s after the establishment of the whole cell recording. (b) Representative part of a cell-attached measurement of individual pituitary cell, where reversible capacitance steps most likely mirror repetitive transient exocytotic events of a single vesicle. Note that transient I_m steps often exhibit crosstalk with the R_e trace even though the phase angle was set correctly (asterisks indicate calibration pulse in Im trace without projection to the R_e signal). Transient events with detectable projections can be used to calculate fusion pore conductance (G_p) and vesicle capacitance (C_v), as shown for the two transient events from the insets.

To conclude, in this paper we show that the coupling between the intrinsic curvature of negatively charged lipids and membrane curvature is crucial for the binding dynamics of a lipid bilayer to I-BAR and F-BAR domain surfaces. Unlike previous theoretical studies that investigated the equilibrium binding of a lipid bilayer surface and concomitant demixing [29, 33, 34], according to our knowledge the present study that takes into account the membrane bending energy is the first approach analyzing the membrane bending dynamics during the attachment of a BAR domain protein. It is proposed that demixing of lipids could be due to the coupling between the intrinsic curvature of negatively charged lipids and the membrane curvature. In future dynamical studies, the role of demixing and curvature-driven fluxes of negatively charged lipids like cardiolipin [35] should be investigated to have a better understanding of the mechanisms underlying the binding dynamics of BAR proteins.

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