

BAR domains and membrane curvature: bringing your curves to the BAR

Jennifer L. Gallop¹ and Harvey T. McMahon¹

MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, U.K.

Abstract

BAR (bin, amphiphysin and Rvs161/167) domains are a unique class of dimerization domains, whose dimerization interface is edged by a membrane-binding surface. In its dimeric form, the membrane-binding interface is concave, and this gives the ability to bind better to curved membranes, i.e. to sense membrane curvature. When present at higher concentrations, the domain can stabilize membrane curvature, generating lipid tubules. This domain is found in many contexts in a wide variety of proteins, where the dimerization and membrane-binding function of this domain is likely to have a profound effect on protein activity. If these proteins function as predicted, then there will be membrane subdomains based on curvature, and thus there is an additional layer of compartmentalization on membranes. These and other possible functions of the BAR domain are discussed.

Introduction

Most cells and organelles are not simply round and therefore membranes do not generally adopt a minimum energy state, but have infoldings, protrusions, compressions and tubulations. These observed larger membrane shapes are effectively differences in local membrane curvature. It is interesting to consider the possibility that membrane curvature itself has a primary role in specifying the location of effector proteins, for example actin filaments, enzymic activities or scaffold proteins. It is already well recognized that distinct membrane morphologies with defined subregions of curvature are generated and maintained in an active fashion by the interplay of cytoskeletal elements and membrane trafficking pathways. Increasingly, membrane-curva-

¹Correspondence can be addressed to either author (email jennyg@mrc-lmb.cam.ac.uk or hmm@mrc-lmb.cam.ac.uk).

ture sensing, generating and stabilizing molecules are also being recognized to have important functions in membrane morphology [1–3]. These all work together to allow, among other functions, the active movement of cells in response to external cues, the budding and fusion of membrane compartments and the dividing of membrane areas into different subdomains.

There are many reviews on the role of the cytoskeleton in cell dynamics [4–6], but, in the present review, we concentrate on the role of BAR (bin, amphiphysin and Rvs161/167) domains [7]. These are membrane-binding domains that may prove to play a central role in binding and stabilizing areas of high membrane curvature. This protein module is found in many trafficking proteins (Table 1), and also proteins of unknown function.

The BAR domain — a curvature sensor

The BAR domain got its name from the conserved N-terminal region of bin, amphiphysin and Rvs161/167 proteins (all amphiphysin family members). This domain in amphiphysin is known to be responsible for membrane binding and dimerization of the protein [8,9]. In addition, both *in vitro* and *in vivo*, the domain leads to tubulation of membranes [3,9]. This tubulation is due, at least in part, to the banana shape of the domain (Figure 1A), which binds to membranes via its concave surface [7]. Each monomer of the BAR domain has three kinked α -helices (Figure 1A, arrows), which gives rise to a six-helix bundle on dimerization. The membrane interaction is purely electrostatic and thus amphiphysin can bind well to negatively charged membranes, given the high intensity of charge at the ends of the domain (Figure 1B). Moreover, if it is provided with membranes of higher curvature, it binds better, as it makes use of the positive charges on the concave surface [7]. Thus this domain is a dimerization and membrane-curvature-sensing module. Some of the other domains found in proteins with BAR modules are listed in Table 1. This leads to the discussion below on how the presence of BAR domains will affect protein function.

BAR domains as dimerization domains on membrane binding

In the BAR structure, the dimerization interface buries a large hydrophobic surface, and the edge of this dimerization surface forms part of the membrane-binding face. The dissociation constant for the dimer in solution is relatively weak. The K_d for the amphiphysin dimer is 6 μ M [7], and, for comparison, the Jun/Fos leucine zipper heterodimer has a K_d of 110 nM [10]. From the structure, we see that for curvature sensing to occur via the concave face of the banana-shaped domain, dimerization is necessary. Given the positive-charge distribution on this surface, we predict that membrane binding will promote dimerization. Inhibiting or promoting dimer formation could therefore be a regulatory mechanism for fine-tuning membrane binding.

Table 1. Example BAR domain proteins, indicating common themes in their domain structure. PTB, phosphotyrosine-binding domain; Snx1, sorting nexin 1.

Protein	BAR membrane binding shown?	Other lipid-binding domains	GEF/GAP activity or small GTPase binding?	SH3 domain?	Other domains
Amphiphysins	Yes	N-terminal amphipathic helix	No	Yes	Endocytic motif domain
Endophilins	Yes	N-terminal amphipathic helix	No	Yes	
Nadrin/RICH	Yes	N-terminal amphipathic helix	RhoGAP activity	No	Proline repeats
BRAP/Bin2	No	N-terminal amphipathic helix	No	No	
APPLs	No	PH and PTB domains	Binds Rab5 GTP	No	
Arrfaptins	Yes	N-terminal region	Binds Arf, Rac, Arl	No	Sorting nexin domain
Snx1	Yes	PX	No	No	
Oligophrenins	Yes	PH	RhoGAP activity	Yes	
Centaurin β s	Yes	PH	ArfGAP activity	No	Ankyrin repeats
Tuba	No	None	RhoGEF activity	Yes (several)	

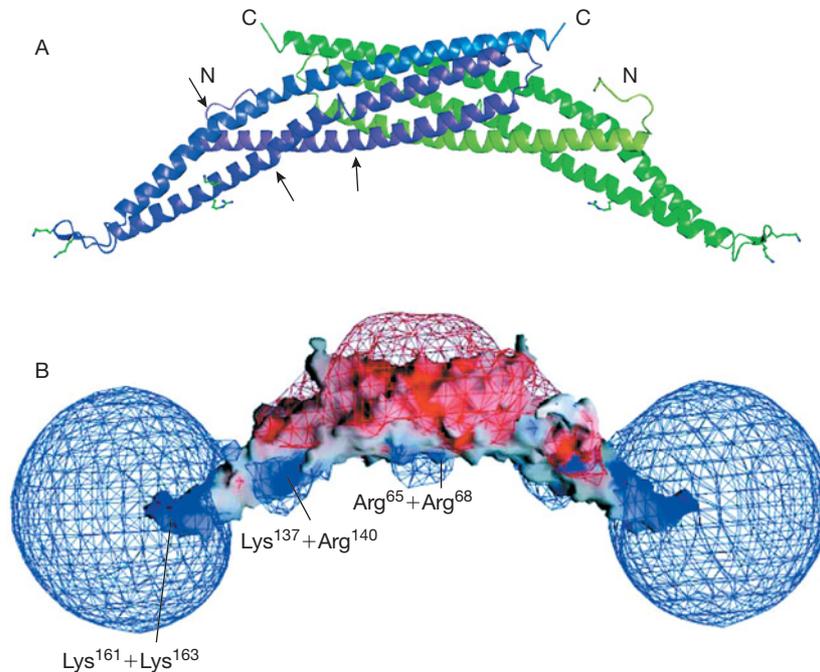


Figure 1 Structure of amphiphysin BAR domain. (A) Ribbon representation of the *Drosophila* amphiphysin BAR domain structure, which spans amino acids 25–240 (Protein Data Bank code 1URU). The banana shape is generated by dimerization and kinks in the α -helices. The overall structure is the same as an arfaptin C-terminal fragment (Protein Data Bank code 1I4D), although these proteins are only weakly homologous at the sequence level. (B) Surface-charge representation of the BAR domain (red, -10 kTe^{-1} , blue $+ \text{kTe}^{-1}$) showing electrostatic equipotential surfaces in mesh at 2 kTe^{-1} (+ blue, - red). The positively charged residues on the concave face are labelled. Kinks in α -helices are indicated by arrows.

The amphipathic helix of N-BAR domains may lead to stabilization or driving of membrane curvature

A frequent modification of BAR domains is an N-terminal sequence that is predicted to form an amphipathic helix (Figure 2), and, in all cases where this helix is found, these are called N-BAR domains [7]. In the case of amphiphysin, the N-terminal sequence was shown to form a helix on membrane binding [7]. This N-terminal amphipathic helix is important for the membrane interaction of amphiphysin, endophilin and RICH/nadrin [7,11,12]. The N-terminal amphipathic helix is also present in BRAP (breast-cancer-associated protein)/Bin2, one of the first BAR domains to be identified [13,14]. Based on our modelling of these sequences as α -helices, we see that the polar face of the helix is largely positive (Figure 2A), which suggests an interaction with either a

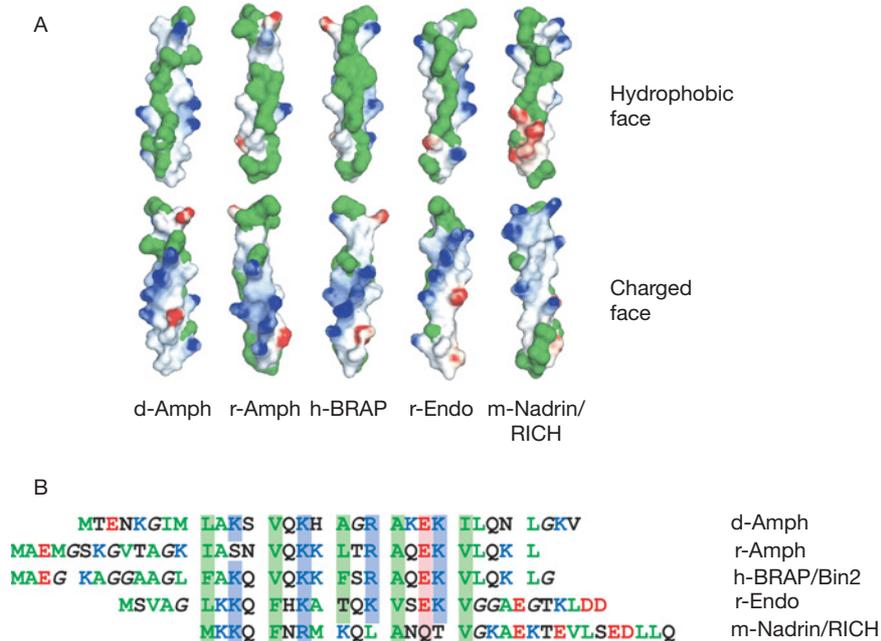


Figure 2 Amphipathic helix predictions for N-BAR domains. (A) α -Helical models of a range of N-terminal sequences from BAR domain proteins show the polar (red negatively charged, blue positively charged) and non-polar (green) faces. **(B)** Alignments of the N-terminal sequences based on the regions of hydrophobicity and charge in the modelled helices. The glycine residues indicated in italics will give rise to flexibility in the helix, and it can be seen that where glycine residues occur, there is a misalignment of polar and non-polar residues when modelled on a straight α -helix. This is most striking in RICH/nadrin, where there is a negatively charged patch on the hydrophobic face towards the C-terminal end of the sequence. Amph, amphiphysin; Endo, endophilin. d, *Drosophila*; r, rat; h, human; m, mouse.

negative surface of another protein (potentially the convex face of the BAR domain) or with negatively charged lipid head-groups.

One explanation for the observed superior binding of N-BAR domains is the membrane insertion of the hydrophobic face of this helix in the hydrophobic phase of the membrane. Membrane insertion of the amphipathic helix could have a number of consequences. If the helix does insert into the bilayer, then it will increase the residency time of the domain on the membrane (as seen with epsin [15]), and this increased residency will allow formation of a more effective framework to drive or stabilize membrane-curvature changes. Insertion of an amphipathic helix also has the consequence of displacing lipids leading to the generation of local curvature (Figure 3A). With a high concentration of BAR proteins, helix insertion may contribute significantly to membrane curvature. If the helix inserts into the bilayer perpendicular to the BAR domain, then

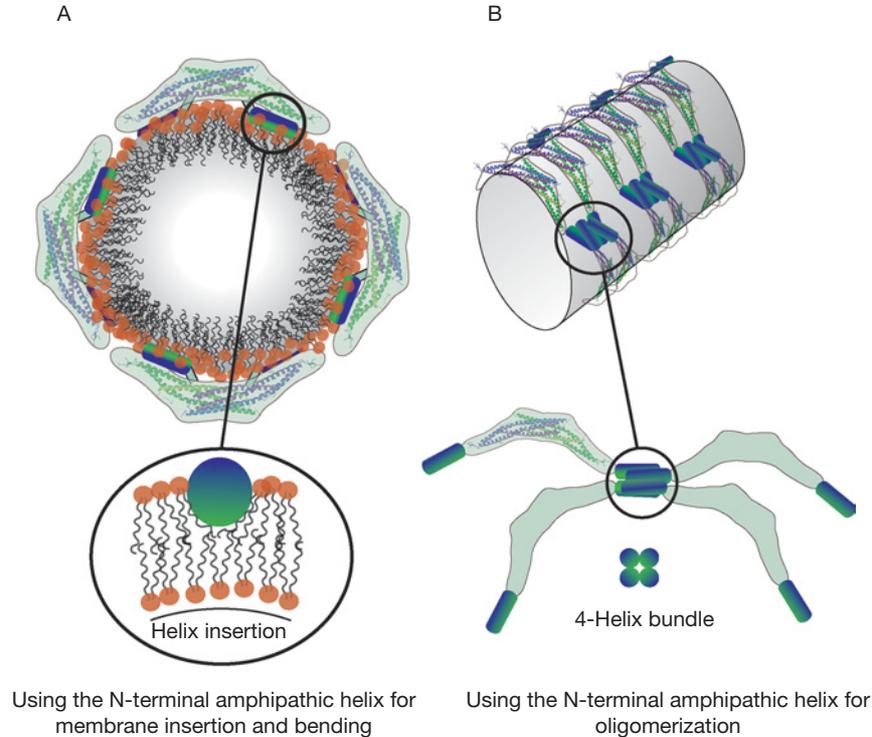


Figure 3 Role of amphipathic helix in membrane binding. (A) Helix insertion into the outer leaflet could cause increased membrane binding of N-BAR domains. (B) Alternatively, the amphipathic helices could form helical bundles and mediate oligomerization on the surface of the tubule.

this will act to drive one-dimensional curvature, i.e. lipid tubules. If it inserts in parallel to the BAR domain, then the N-BAR domain will generate two-dimensional curvature, i.e. lipid vesicles. In liposome tubulation experiments, there is often a fine transition between complete tubulation via an N-BAR domain and the formation of vesicles, and thus the generation of two-dimensional curvature has been observed.

An alternative model for the action of the N-terminal helix (which needs to be tested) may be to promote higher-order oligomerization on membranes by formation of a three or four-helix bundle which packs the hydrophobic residues in the centre, thus providing a better framework for driving tubulation (Figure 3B). Indeed, striations are sometimes observed on N-BAR domain tubules and cross-linking on liposomes gives rise to higher-order species [11,12]. Of course, these striations may simply be due to side-by-side packing of this banana-shaped molecule, and this conformation will readily allow cross-linking.

For amphiphysin and endophilin, the amphipathic helix is not essential for *in vitro* liposome tubulation ([7], and J. L. Gallop, unpublished work). Even if it is tempting to assume that insertion of the amphipathic helix is solely respon-

sible for driving tubulation, mutation of a key hydrophobic residue, (Phe¹⁰ in r-endophilin 1, Phe⁹ in r-amphiphysin 1) and indeed deletion of the amphipathic helix still results in tubule formation at high protein concentrations, and, at lower concentrations, the proteins bind better to smaller liposomes. This suggests an amphipathic helix is an important alteration to the BAR domain that helps promote membrane curvature.

Coincidence detection of membrane curvature and composition

BAR domains are frequently found in combination with other lipid binding domains (see Table 1). These domains include PH (pleckstrin homology) domains (which bind phosphoinositides) and PX (phox) domains (which bind 3-phosphorylated phosphoinositides). This conjugation of the BAR domain to other domains with defined lipid specificity means that the protein not only selects a particular membrane compartment, but also selects areas of membrane curvature on that compartment. If both domains are required for membrane binding then the protein acts as a logical AND gate, or coincidence detector. This mechanism is used frequently in signalling pathways, where, for instance, localization and a binding partner are required for activation [16]. This gives rise to precise localization of binding partners and enzymic activities. Centaurin β 2 requires both functional BAR and PH domains in order to tubulate intracellular membranes in COS cells [7]. In the case of sorting nexin1, a BAR and a PX domain combination leads to the localization of the protein to tubular extensions on endosomes [17].

BAR domains in creating or stabilizing membrane subdomains

One of the most important functions of the BAR domain may well be in the generation of membrane subcompartments. One such subdomain is the T-tubule network in muscle, which is an extension of the plasma membrane into the muscle cell. This network is dependent on the presence of amphiphysin, and, in its absence, there is a defect in excitation–contraction coupling [3]. The tubular extension on endosomes also require the presence of another BAR protein, sorting nexin1, and failure to form this subdomain on the endosomes leads to defects in intracellular membrane trafficking [17].

BAR domains as regulated recruiters of downstream effectors

We have already referred to the probable increased dimerization potential of BAR domains on membranes. SH3 (Src homology 3) domains are found in many BAR domain proteins, and a BAR protein on a membrane will present two SH3 domains for interactions. This will favour the binding of dimeric SH3-binding proteins or monomeric proteins with multiple SH3-binding sites. Amphiphysin

and endophilin both have C-terminal SH3 domains that recruit the mechanochemical GTPase dynamin and the lipid phosphatase synaptojanin to sites of endocytosis. Dynamin is an oligomer and so will bind better to oligomeric BAR proteins, while synaptojanin is a monomer, but has multiple SH3-interaction sequences. SH3-domain interactions can be very weak (in the order of 1 mM), and thus the initial dimerization of the SH3 protein via the BAR domain–membrane interaction may be important for achieving the downstream effect mediated by the SH3 interaction.

BAR domain proteins and small G-proteins

A striking proportion of the BAR domain proteins so far identified either activate the hydrolysis or exchange of nucleotides on small G-proteins [GAP (GTPase-activating protein) and GEF (guanine nucleotide-exchange factor) activities]. This highlights that membrane-curvature sensing is coupled with enzymic activities, both directly, as with GAPs and GEFs, and indirectly, as in the case of synaptojanin bound to endophilin. The regulation of Rho GTPase activity is particularly interesting because of its role in the control of actin polymerization and cytoskeletal remodelling [18], which is associated with changes in membrane morphology. Knockdown of oligophrenin 1 (a BAR protein; see Table 1) in neurons leads to defects in dendrite spine formation [19]. We can hypothesize that the role of the BAR domain, in conjunction with the PH domain, is localization of the GAP activity to the correct membrane position, for instance where spines protrude from dendrites. Tuba [20], another BAR protein, has Rho GEF activity and multiple SH3 domains that bind dynamin, N-WASP (neural Wiskott–Aldrich syndrome protein) and other actin-regulating proteins. Tuba may therefore be a link between the membrane-curvature events that accompany dynamin-mediated fission and cortical actin rearrangements.

There are also two examples of BAR domains that bind small G-proteins directly, and, based on homology, there are likely to be more. Arfaptin binds to a variety of G-proteins, including Rac1 and Arf1 [21]. Rac binds to the membrane-binding surface, while Arf competes with this interaction, but might not bind to the same site. It is known that Arf promotes the interaction of many proteins with membranes, and thus the exact function of this G-protein in arfaptin membrane interaction will be an important paradigm to solve. APPL is also proposed to bind to Rab5 GTP via its BAR+PH domain [22]. The exact interaction mode has not been solved, but by analogy with arfaptin, it is likely to modulate BAR domain membrane binding.

Conclusions

Sensing membrane morphology via protein domains and using this to influence cellular processes is a novel concept in biology. We have presented some ideas of how this can work at a molecular level, but further experiments are needed to verify or disprove these ideas. Given the large number of proteins

that have potential BAR domains, the importance of membrane morphology in cell structure and function, and the conservation of BAR modules from yeast to man, we can predict that these modules will have a broad and significant impact on diverse biological processes.

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