How proteins produce cellular membrane curvature

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Abstract | Biological membranes exhibit various function-related shapes, and the mechanism by which these shapes are created is largely unclear. Here, we classify possible curvature-generating mechanisms that are provided by lipids that constitute the membrane bilayer and by proteins that interact with, or are embedded in, the membrane. We describe membrane elastic properties in order to formulate the structural and energetic requirements of proteins and lipids that would enable them to work together to generate the membrane shapes seen during intracellular trafficking.

The extraordinarily beautiful and complex shapes of cells and cell organelles are fashioned by the physical forces that operate on their membranes (FIG. 1). A fundamental problem of molecular cell biology in conjunction with physics and mathematics is to understand the evolutionary, developmental and functional rationale for these shapes, as well as the mechanisms that are used by cells to produce them.

Each shape evolved for specific physiological reasons[1–3]. Cells without internal membranes, such as prokaryotic cells and erythrocytes, can take on an array of shapes for their purposes. The corkscrew shape of spirochetes, which are long and slender bacteria (FIG. 1), might favour their penetration and packing into host cells, and the biconcave disc-like shape of erythrocytes guarantees the optimal surface-to-volume ratio that is necessary for fast oxygen exchange between haemoglobin and the outside medium. Moreover, the ability of erythrocytes to change their shape easily in the hydrodynamic flow allows them to navigate within various blood vessels with varying flow rates. The plasma membranes of cells that have internal membranes undergo radical shape transformations when they develop intercellular contacts, or spread and move on a substrate[4]. Even more complex shapes can be achieved by endothelial cells, the plasma membranes of which can develop internal tubes that span the cell volume[5,6]. In mathematical terms, the formation of each of these tubes changes the membrane topology[7], progressively moving the surface to higher levels of shape complexity.

Intracellular membranes are even more dynamic and varied in their shapes. For example, the endoplasmic reticulum (ER) and the Golgi apparatus are complex systems of interconnected tubules, cylinders and discs (FIG. 1), and intracellular transport intermediates have a broad range of shapes from small (~50 nm) spheres and narrow tubes to tubular–saccular carriers[8–11].

There are many questions about this dazzling array of shapes for which we do not know the answers. For example, why do the ER and Golgi have distinctly different shapes (FIG. 1), with the Golgi being much more saccular and fenestrated and the ER being more tubular with small cisternae? Both shapes maximize the surface area while keeping the internal volume low, which allows efficient and fast protein trafficking in and out of these organelles. But why are they different? Is it related to the fact that the Golgi sorts specific proteins for export to specific organelles and sites on the plasma membrane? Are there domains of lipids and proteins that form in the Golgi, but not in the ER, and that stabilize the strange fenestrated and interconnected Golgi stacks?

To intelligently investigate such questions, which abound in cell biology, we must recognize that membrane curvature is generated as a result of a complex interplay between membrane proteins, lipids and physical forces that are applied to the membrane surface. Moreover, to control membrane curvature, cells must have sensors that feed back to the curvature-producing molecules. As the physical principles that underlie shape creation and sensing must be universal for all cells, the aim of this review is to describe these principles and consider their realization in one biological instance — intracellular membrane trafficking.

Physical principles underlying membrane shape

The surface of a cell, or cell organelle, is formed by a biological membrane. Therefore, the shape of a cell, or cell organelle, is determined by the membrane shape,

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a

Cocci

Rods

A spirochete

b

Endoplasmic reticulum

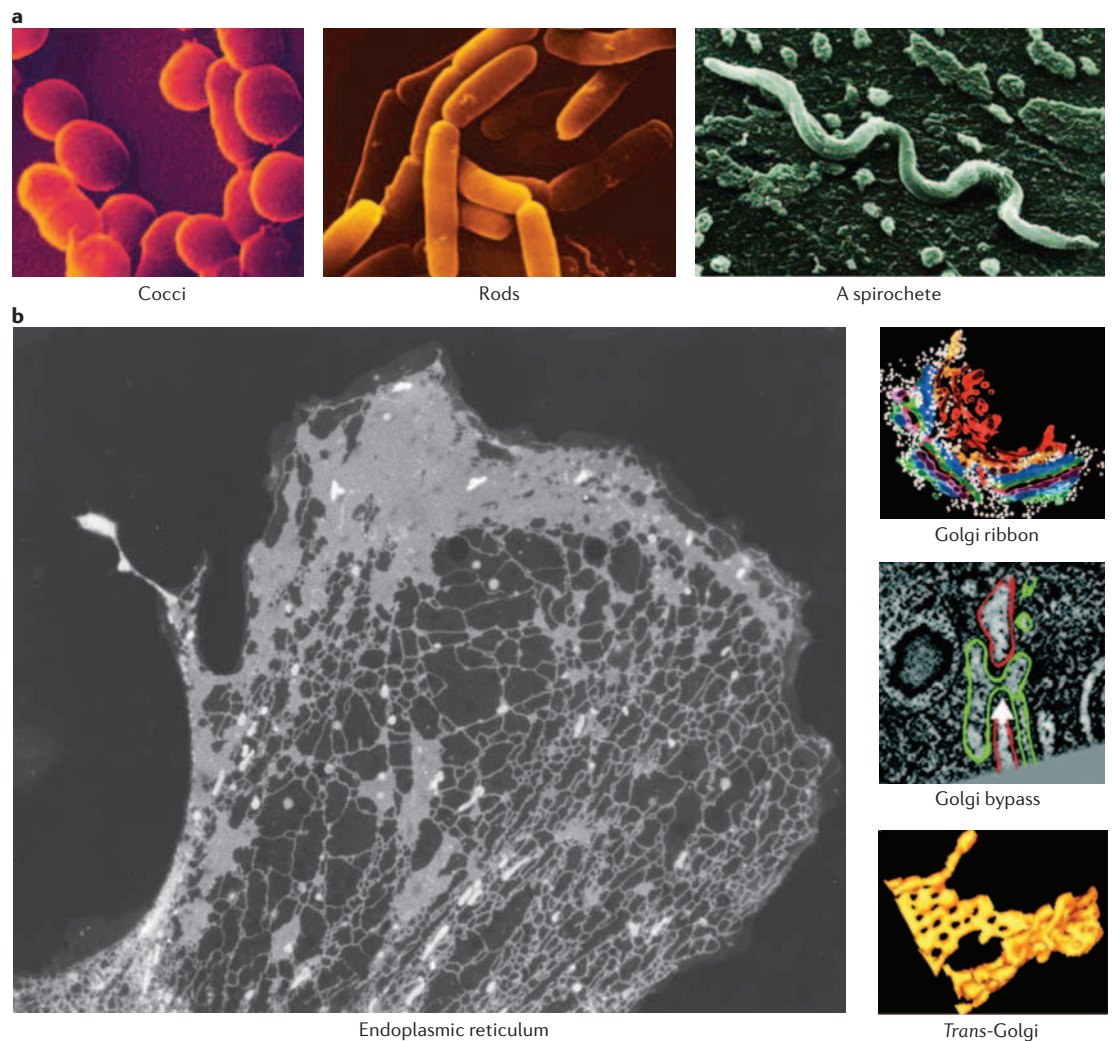Golgi ribbon

Golgi bypass

Trans-Golgi

Figure 1 | **The beautiful and complex shapes of cells and cell organelles. a** | These panels show the prokaryotic shapes of cocci, rods and a spirochete. These images are reproduced from REF. 88. **b** | The large image shows the stained endoplasmic reticulum of a 3T3 fibroblast cell as recorded by confocal microscopy. This image is reproduced with permission from REF. 89 © (1986) the Rockefeller University Press. From top to bottom, the three smaller images show a part of a tubular–saccular Golgi ribbon, an intracisternal connection of the Golgi (labelled Golgi bypass) and fenestrated Golgi forms (labelled *trans*-Golgi). These images were taken from an electron-micrograph tomography series. The Golgi ribbon image is reproduced with permission from REF. 90 © (2002) Macmillan Magazines Ltd, the Golgi bypass image is reproduced with permission from REF. 91© (2004) the National Academy of Sciences, and the *trans*-Golgi image is reproduced with permission from REF. 92 © (2004) Blackwell Publishing.

and understanding the mechanisms that control cellular shapes requires the geometrical and physics tools of membrane biophysics.

Membrane shapes involved in intracellular trafficking. Membrane shapes are described using the geometrical concepts of principal curvatures, c_1 and c_2, or radii of curvature, $R_1 = 1/c_1$ and $R_2 = 1/c_2$ (BOX 1). More commonly, two combinations of the principal curvatures are used: the total curvature, $J = c_1 + c_2$, and the Gaussian curvature, $K = c_1 \cdot c_2$ (REF. 12).

There are four basic shapes that are characterized by different combinations of values of the principal curvature: the flat, cylindrical, saddle-like and spherical shapes (BOX 2). The generation of intracellular membrane carriers involves all of these shapes. Uncoated plasma membranes as well as non-invaginating coated pits have an almost flat shape (BOX 2 part a). Coated endocytic vesicles and the coatomer protein (COP)-coated vesicles that operate in the ER–Golgi system have almost spherical shapes[8,10,13–20] (BOX 2 part d). Cylindrical shapes characterize the membrane carriers that operate between the ER and the Golgi apparatus and between the Golgi and the plasma membrane[8,9,11,21–23], as well as membrane tubules such as those formed by dynamin–GTP-γS[24] (BOX 2 part b). The membrane necks that connect budding transport vesicles and the membrane of origin at stages before complete vesicle

Box 1 | Membrane curvatures

A biological membrane is commonly described as a two-dimensional surface, which spans a three-dimensional space. So, to describe membrane shape, it is not sufficient to determine the membrane curling that is seen in a single cross-section of the object, because in general there are two curvatures that characterize the shape at each point in space. Mathematically, these two curvatures are called the principal curvatures, c_1 and c_2 (REF. 7), and their meaning can be understood by the following thought experiment. If you cross-section the membrane surface at a point under consideration using two planes that are perpendicular to the surface and orientated in two special directions called the principle directions (see figure), the principle curvatures are the curvatures of the two lines of intercepts between the planes and the surface, which have almost circular shapes in close proximity to the point under consideration. The radii of these two circular fragments, R_1 and R_2, are called the principle radii of curvature, and their inverse values, $c_1 = 1/R_1$ and $c_2 = 1/R_2$, are referred to as the two principle curvatures.
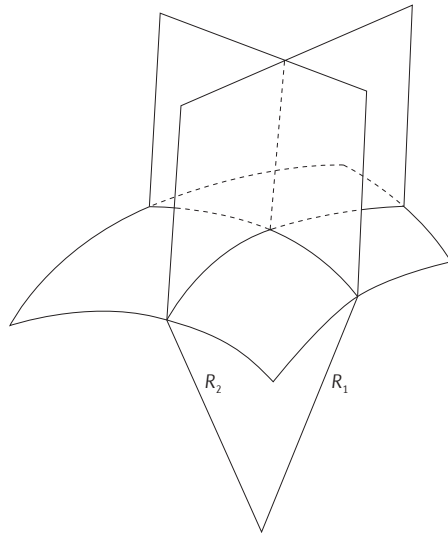
Importantly, the curvature can be positive or negative. To define the curvature sign, we need to distinguish between the two sides of the surface. For a lipid monolayer, one surface is covered by the phospholipid polar headgroups, whereas the other is hydrophobic. By convention, monolayer bending in the direction from the heads to the tails (which forms a shape with a bulging hydrophilic surface) produces positive curvature, whereas bending in the opposite direction results in negative curvature. For a cell membrane, the difference between the membrane sides is determined by their orientation with respect to the volume that is contained by the membrane. Positive curvature corresponds to bulging towards the outside medium. However, it should be noted that the convention regarding the curvature sign is arbitrary. For example, in mathematical books, an agreement opposite to the one mentioned here is usually adopted.

separation are characterized by saddle-like shapes (BOX 2 part c). In addition, saddle-shaped membrane fragments constitute the surface of the fenestrations of Golgi cisternae and of junctions between three or more cylindrical membrane portions of the ER.

The important challenge is to understand the mechanisms by which flat membranes are transformed into cylindrical and spherical membranes, which possess large curvatures. Furthermore, what are the forces that stabilize the saddle-like regions of membranes?

Membrane elasticity. To create shapes, membranes must undergo deformations. The work the cell needs to do to deform membranes is determined by the structure and elasticity of the membrane. The membrane structure controls the non-deformed unstressed shape of the membrane, which is referred to as the 'spontaneous shape'. This shape is characterized by the spontaneous curvature of the membrane bilayer, J_s^B, and depends on the spontaneous curvatures of the inner and outer membrane monolayers, J_s^{in} and J_s^{out} (see Supplementary information S1 (box) and references therein; for a qualitative discussion of this issue, see REFS 25,26). The

elasticity of a membrane determines the forces that have to be applied to produce deviations from the spontaneous shape, as well as the energies that accumulate in the membrane as a result of such deviations. The membrane elasticity is characterized by the elastic moduli, such as the bending moduli of the membrane monolayer, κ_m, and bilayer, κ_B, the moduli of the Gaussian curvature of the monolayer and bilayer, $\bar{\kappa}_m$ and $\bar{\kappa}_B$, respectively, the membrane stretching modulus, Γ (see Supplementary information S1 (box) and references therein), and the modulus of the tilt of the hydrocarbon chains with respect to the membrane plane[27–30].

The common understanding of the energetics of membrane shaping is largely based on the seminal Helfrich theory of membrane bending elasticity (see REFS 12,31–35 and references therein; see also Supplementary information S1 (box)). A rigorous application of this theory requires a criterion that determines whether the membrane curvature is small or large. The scale that is used to assess the values of membrane curvature is based on membrane thickness, d. The curvature, c, is considered to be small if the corresponding radius of curvature, R, is much larger than the membrane thickness, $R \gg d$ (that is, the absolute value of the curvature, $|c|$, should satisfy the equation $|c| \cdot d \ll 1$) (FIG. 2a). In cases in which the curvature radius is close to the membrane thickness, $R \approx d$, (that is, $|c| \cdot d \approx 1$), the membrane curvature is regarded as being large (FIG. 2b). The use of Helfrich theory assumes small membrane curvatures. A characteristic radius of curvature, R, for intracellular membrane carriers is a few tens of nanometres (for example, see REFS 36–38), and is therefore only several times larger than the bilayer thickness, $d \approx 4$ nm. This corresponds to the limits of the applicability of the elastic theory, the predictions of which should be trusted in this case only on a semi-quantitative level.

Armed with an understanding of the definitions, conventions and laws that govern the geometry and elasticity of membranes (BOX 1; see also Supplementary information S1 (box)), we can discuss what is known about how organelles acquire their shapes. Although it is the interplay between lipids and proteins that determine membrane shaping, we discuss their contributions separately for clarity. Furthermore, we distinguish between proteins that function extrinsically to the membrane and those that function as membrane components.

Generation of membrane shapes by lipids

Cylindrical shape. The energy, F_{Cyl}, that is necessary to generate a cylindrical membrane of radius R and length L out of a flat membrane is given by $F_{Cyl} = \pi L \cdot \kappa_B \cdot \left(\frac{1}{R} - 2J_s^B \right)$. Lipids can produce a cylindrical shape on their own if the required energy vanishes, $F_{Cyl} = 0$, meaning that the spontaneous curvature of the lipid bilayer has a value of $J_s^B = 1/(2R)$.

The radii of membrane cylinders that form in intracellular membrane-transport pathways are typically ~25–30 nm (for example, see REFS 9,39). So, the spontaneous curvature necessary to generate such cylinders equals ~(1/50) nm^{-1}. As J_s^B results from a difference in the spontaneous curvatures of the monolayers,

Box 2 | Basic membrane shapes

Two combinations of the principle curvatures are commonly used to describe membranes: total curvature, $J = c_1 + c_2$, and Gaussian curvature, $K = c_1 \cdot c_2$. These values have a fundamental geometrical meaning[7], and they characterize membranes that have the properties of two-dimensional isotropic fluids (for example, lipid bilayers in liquid-crystalline state)[12].

There are several basic membrane shapes. There is a flat shape for which all curvatures equal zero ($c_1 = 0$ and $c_2 = 0$, so $J = 0$ and $K = 0$) (see figure, part **a**). There is a cylindrical shape, which has one positive principle curvature, $c_1 > 0$, whereas the second principle curvature equals zero, $c_2 = 0$. This produces a non-zero total curvature, $J = c_1 \neq 0$, and a zero Gaussian curvature, $K = 0$ (part **b**). There is a saddle-like shape (part **c**), for which the principle curvatures are equal in absolute value but have a different sign, $c_1 = -c_2$. This means that the total curvature equals zero, $J = 0$, whereas the Gaussian curvature is negative $K = -(c_1^2) < 0$. Finally, there is a spherical surface (part **d**), which has two equal principle curvatures, $c_1 = c_2$. This results in the relationship between the total and the Gaussian curvatures being defined by the equation $K = J^2/4$.

The images in the figure show biological examples of these basic membrane shapes. **a** | A membrane fragment under a flat clathrin lattice. **b** | A membrane tube under a dynamin coat. **c** | The neck of a membrane bud. **d** | Pure clathrin cages assembled *in vitro*. The images in parts **a** and **d** are courtesy of John Heuser, Washington University, USA (see Heuser Lab in the Online links box for more fascinating images). The images in parts **b** and **c** are reproduced with permission from REF. 87 © (2003) the National Academy of Sciences.

	c_1	c_2	J	K
a Plane	0	0	0	0
b Cylinder	+	0	+	0
c Saddle	+	−	0	−
d Sphere	+	+	+	+
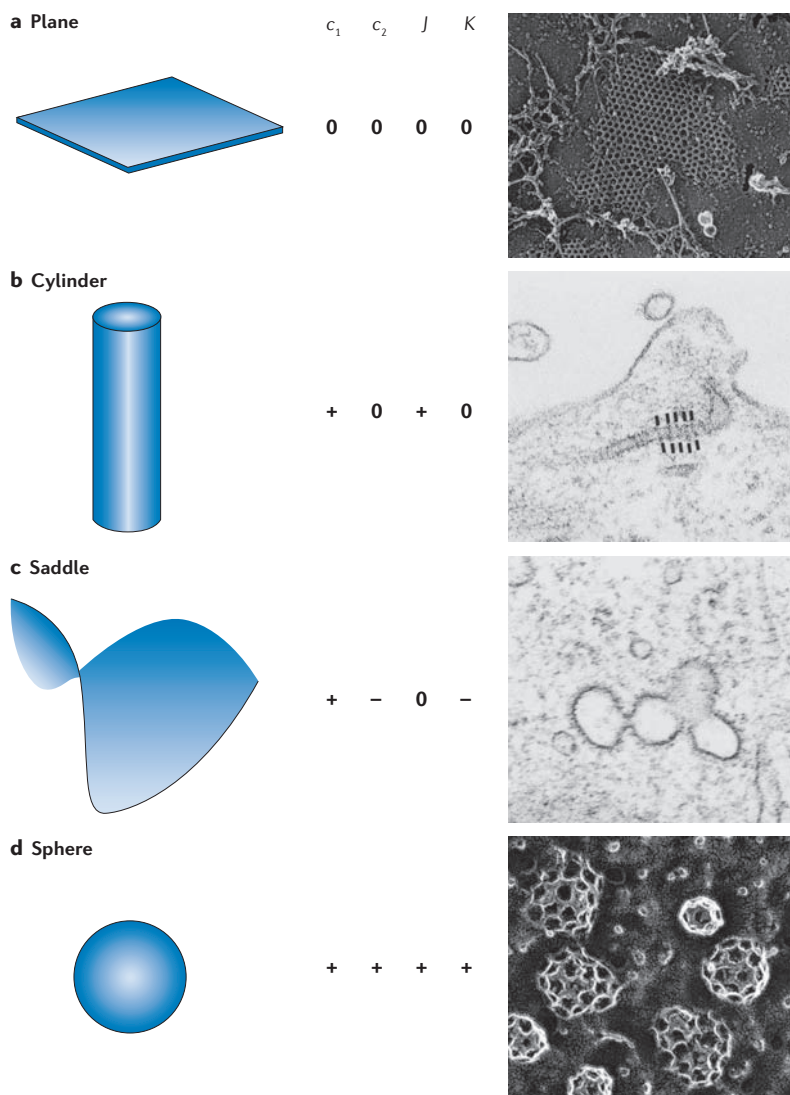
$J_s^B = (J_s^{out} - J_s^{in})/2$ (see Supplementary information S1 (box)), an unusual membrane lipid composition would be required to produce such curvature. The lipids cholesterol, dioleoylphosphatidylethanolamine (DOPE) and diacylglycerol are characterized by strongly negative spontaneous curvatures (TABLE 1) and therefore have the potential to generate a large membrane curvature. However, even for these lipids, the required J_s^B can be reached only if they are extensively concentrated in the internal monolayer. On the basis of the specific values of spontaneous curvatures for individual lipids, a bilayer spontaneous curvature of ~(1/50) nm^{-1} requires a difference of ~15% in the mole fraction of molecules such as cholesterol or DOPE between the internal and outer monolayers, provided that the contribution of the rest of the lipids to the spontaneous curvature of the monolayer is negligible. This degree of compositional asymmetry might be created in biological membranes, but requires special mechanisms and conditions. An example might be the presence of large membrane concentrations of an unconventional phospholipid, lysobisphosphatidic acid (LBPA), which is present in late endosomes and has been shown to generate, in conjunction with transmembrane pH gradients, multivesicular liposomes[40]. The liposome formations might result from an asymmetric distribution of LBPA between the two monolayers that is driven by a transmembrane difference in pH. Lipid asymmetry can also be produced by special protein machinery and metabolism[41]. Phospholipase A2 catalyses hydrolysis at the sn-2 position of phospholipids, which results in the release of fatty acids and lysophospholipids (the latter are characterized by positive monolayer spontaneous curvature; TABLE 1). As long as it is confined to a local area, increasing the lysophospholipid density in a membrane monolayer that is exposed to the function of phospholipase A2 can generate a local positive spontaneous curvature of the monolayer and, thereby, of the whole bilayer.

Another way to create a cylinder out of a flat membrane — known as the bilayer-couple mechanism[42] — is to generate an area difference between the membrane monolayers, $A^{out} - A^{in} \neq 0$ (see Supplementary information S1 (box)). Transformation of a flat membrane into a cylinder with a curvature radius of 30 nm requires an area difference (ΔA) between the monolayers of the cylindrical membrane that is equal to ~10% of the total area of the membrane mid-surface (A_{mid}), $\Delta A / A_{mid} \approx 0.1$. A similar extent of area asymmetry has been estimated as being needed for the formation of small (~100–200 nm) spherical vesicles out of large unilamellar liposomes[43]. This is a significant asymmetry, the creation and support of which would require a protein machinery that consumes energy continuously to transport lipids actively to counteract the background phospholipid flip–flop rate in the opposite direction. Vesicle formation from giant unilamellar liposomes, which have dimensions that are similar to those of cells, requires a lipid asymmetry of only 0.1%, but the resulting vesicles are much larger (~1 µm) than transport vesicles[43]. Creation of 100-nm vesicles from giant unilamellar liposomes would require

the involvement of protein scaffolds or the generation of considerable membrane tension[43].

When discussing the mechanisms of membrane shaping by lipids, a difference has to be emphasized between the effects of the area asymmetry, $A^{out} - A^{in}$, and of the monolayers spontaneous curvature asymmetry, $J_s^{out} - J_s^{in}$. The former has a 'global' character because it is based on the difference in the total areas of the monolayers, whereas the latter produces a local effect and does not average over the whole membrane area. Therefore, in many cases the $J_s^{out} - J_s^{in}$ factor is more effective than $A^{out} - A^{in}$ in shaping membranes (for more details, see Supplementary information S1 (box)).

In summary, the generation of cylindrical membrane-trafficking intermediates solely on the basis of lipid properties is likely to require an unusually large trans-membrane asymmetry of lipid composition, which probably requires a persistent energy input from proteins.

Spherical shape. The generation of a spherical membrane of radius R out of a flat membrane requires the energy $F_{Sph} = 8\pi\left(\kappa_B + \frac{1}{2}\bar{\kappa}_B\right) - 8\pi\kappa_B \cdot R \cdot J_s^B$. Similar to the case for a cylindrical membrane, a positive bilayer spontaneous curvature ($J_s^B > 0$) facilitates this process. The bilayer spontaneous curvature that is required to form a spherical membrane of radius R is determined by the equation $J_s^B = \frac{1}{R} \cdot \left(1 + \frac{1}{2} \cdot \frac{\bar{\kappa}_B}{\kappa_B}\right)$. The radii of spherical membrane carriers (transport vesicles) that are involved in intracellular membrane transport are ~30 nm. To estimate the values of J_s^B that would be required to form such carriers, we need to know the bending modulus of the membrane bilayer (κ_B) and the elastic modulus of the Gaussian curvature ($\bar{\kappa}_B$), which can vary over a wide range[44–47]. To provide an estimate of the minimal spontaneous curvature required, on the basis of recent results[47], we take $\bar{\kappa}_B$ as having the value $\bar{\kappa}_B = 2 \cdot \bar{\kappa}_m \approx -2 \cdot 0.8 \cdot \kappa_m \approx -6.4 \cdot 10^{-20} J$. Using this value and the common value for κ_B of $8 \cdot 10^{-20}$ J (for example, see REF. 48), for $R = 30$ nm, we obtain a bilayer spontaneous curvature of ~(1/50) nm^{-1}. If the modulus of the Gaussian curvature equals zero ($\bar{\kappa}_B = 0$), the required spontaneous curvature is even larger, ~(1/30) nm^{-1}. Similar to the case for cylindrical shapes, the generation of such large values of spontaneous curvature requires unusual conditions.

The creation of spherical membranes with a 30-nm radius using bilayer-couple mechanisms requires a monolayer area difference of 20% (see Supplementary information S1 (box)), which is even more than the area difference that is necessary to generate cylindrical intermediates.

In conclusion, on the basis of the properties of lipids, the generation of spherical membrane carriers also requires a substantial and persistent energy input that is mediated by proteins.

Fenestrated shapes. The key membrane elastic property that could drive the formation of fenestrated structures is the modulus of the Gaussian curvature of the bilayer, $\bar{\kappa}_B$. According to a fundamental geometrical theorem (Gauss-Bonnett theorem), the
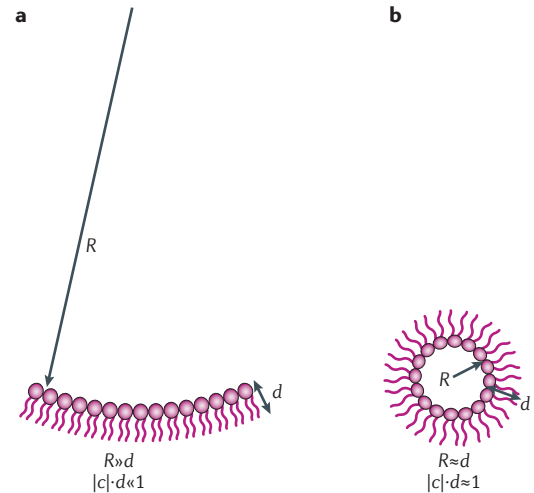
Isotropic
The same in all directions.

Lysophospholipids
Phospholipids (or derivatives of phosphatidic acid) that lack one of their fatty acyl chains.

Figure 2 | Determining membrane curvature. The common understanding of the energetics of membrane shaping is largely based on the seminal Helfrich theory of membrane bending elasticity (see REFS 12,31-35 and references therein; see also Supplementary information S1 (box)). A rigorous application of this theory requires a criterion that determines whether the membrane curvature is small or large. The scale that is used to assess the values of membrane curvature is based on membrane thickness, d. **a** | The curvature, c, is considered to be small if the corresponding radius of curvature, R, is much larger than the membrane thickness, $R>>d$ (that is, the absolute value of the curvature, $|c|$, should satisfy the equation $|c| \cdot d <<1$). **b** | In cases in which the curvature radius is close to the membrane thickness, $R \approx d$, (that is, $|c| \cdot d \approx 1$), the membrane curvature is regarded as being large.

formation of a membrane self-connection that leads to fenestration results in a change in the integral of the Gaussian curvature of -4π. The corresponding change in the energy of the Gaussian curvature of the bilayer, $F_K = \bar{\kappa}_B \int dA \cdot K$, equals $-4\pi \cdot \bar{\kappa}_B$. Therefore, the formation of fenestrations requires changes in the modulus of the Gaussian curvature of the bilayer towards positive values and, conversely, the elimination of fenestrations must involve the variation of $\bar{\kappa}_B$ in the negative direction. The value of $\bar{\kappa}_B$ can be controlled by the spontaneous curvature of membrane monolayers[44,47,49], so that, in principle, the lipid composition can determine the tendency of a membrane to become more or less fenestrated. However, estimations show that the feasible variations in lipid composition are not sufficient to change the sign of the modulus of the Gaussian curvature, and therefore cannot drive fenestration formation. So, protein function also seems to be required to change membrane topology.

Generation of membrane shapes by proteins
Proteins can change membrane curvature by applying forces to membrane surfaces. In the following subsections, we consider two types of force — pulling forces and bending forces.

Pulling membranes using molecular motors. One realistic way to generate a cylindrical (tubular) membrane

Table 1 | **Effective spontaneous curvature, J_s, for different lipids**

Lipid	J_s (nm^{-1})	References
Lysophospholipids		
L-lyso PC*	1/5.8	93
O-lyso PC*	1/3.8	93
P-lyso PC*	1/6.8	93
LPA*	1/2	94,95
L-lyso PE*	< 1/40	93
O-lyso PE*	< 1/40	93
S-lyso PE*	< 1/40	93
Other lipids		
DOPS‡	1/14.4	96
DOPC*	−(1/20) to −(1/8.7)	97,98
PA*	−(1/4.6)	94,95
DOPE	−(1/3)	99
Cholesterol*	−(1/2.9) to −(1/2.3)	97
DCG*	−(1/1.3)	98
DAG*	−(1/1.01)	98,99

*These lipids were studies in dioleoylphosphatidylethanolamine (DOPE). ‡Dioleoylphosphatidylserine (DOPS) was studied at physiological pH. DAG, diacylglycerol; DCG, dicaprylglycerol; DOPC, dioleoylphosphatidylcholine; L-lyso, lysolauryl; LPA, lysophosphatidic acid; O-lyso, lysooleoyl; PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; P-lyso, lysopalmitoyl; S-lyso, lysostearoyl.

is to apply a localized pulling force, Φ_{pull}, to an initially flat membrane. The force provides the energy needed for membrane tube formation and generates lateral tension, γ_c, in the emerging cylindrical membrane. The relationship between the pulling force and the radius, R, of membrane curvature can be calculated based on considerations of the membrane mechanical equilibrium (for example, see REFS 50–52) and is given by $\Phi_{pull} = 2\pi \cdot \kappa_B \cdot \left(\frac{1}{R} - J_s^B\right)$. The corresponding lateral tension in the membrane tube is $\gamma_c = \kappa_B \cdot \frac{1}{R} \cdot \left(\frac{1}{R} - J_s^B\right)$.

The larger the spontaneous curvature, the smaller the force required to form a membrane tube of radius R. For symmetrical bilayers with a zero spontaneous curvature, $J_s^B = 0$, the required pulling force is inversely proportional to the cylinder radius, $\Phi_{pull} = 2\pi \cdot \kappa_B \cdot 1/R$. This relationship has been verified in experiments[51,53,54] and has been used to measure the bilayer bending modulus, κ_B. Using the value $\kappa_B \approx 8 \cdot 10^{-20}$ J, it can be readily estimated that for a tube with a radius of ~25 nm the pulling force must equal ~20 pN, which is close to the force developed by several molecular motors[55]. This means that cylindrical intracellular membrane carriers can be formed by molecular motors[56–58].

The tension that is created by the pulling force within the generated cylindrical tube of $R \approx 25$ nm and $J_s^B = 0$ equals $\gamma_c = \kappa_B \cdot 1/R^2 \approx 0.1$ mNm^{-1}. Note that this tension is, in general, different from the tension in the flat membrane, γ_f, which serves as a reservoir of material for the tube. Moreover, there is a minimal value of the tension, γ_c^{min}, that has to be generated in a cylindrical membrane of radius R in order to pull it out of the flat reservoir. A thermodynamic analysis shows that

this minimal tension equals $\gamma_c^{min} = \gamma_f + \frac{1}{2} \cdot \kappa_B \cdot \frac{1}{R} \cdot \left(\frac{1}{R} - 2J_s^B\right)$. For a vanishing spontaneous curvature, $J_s^B = 0$, and $R \approx 25$ nm, the minimal tension in the tube, γ_c^{min}, exceeds the tension in the flat membrane, γ_f, by ~0.06 mNm^{-1}. Moreover, there is a minimal value of the pulling force, Φ_{pull}^{min}, that has to be applied to the flat membrane surface in order to generate a tube. This value is given by $\Phi_{pull}^{min} = 2\pi \left(\sqrt{2 \cdot \kappa_B \cdot \gamma_f} - \kappa_B \cdot J_s^B\right)$, and the radius of the resulting cylindrical membrane equals

$$R^\star = \sqrt{\frac{\kappa_B}{2 \cdot \gamma_f}} .$$

These relationships predict that the larger the tension in the initial flat membrane, γ_f, the larger the minimal force, Φ_{pull}^{min}, needed to pull out a cylindrical tube and the smaller the radius of the resulting tube. This explains the qualitative results[58] on the relationship between the initial tube-formation force and membrane tension.

Bending membranes using proteins. Membrane curvature can be produced by proteins that bind to the membrane surface. There are three key mechanisms of membrane bending by proteins, which are not mutually exclusive.

The first mechanism is the scaffold mechanism (FIG. 3a,b). It assumes that proteins can function as scaffolds that present the intrinsic curvature of the protein to the lipid[59]. Such proteins must satisfy several criteria in order to bend lipid bilayers locally. Most importantly, the intrinsic shape of a protein, or protein network, must allow it to expose a curved interaction surface to the lipid bilayer. Such a shape can result either from the tertiary protein structure or from a surface that is formed by protein–protein interactions. Furthermore, the protein or proteins must have sufficient intrinsic rigidity to counteract the tendency of the lipid bilayer to relax to its state of spontaneous curvature. Protein rigidity is poorly understood. Finally, the protein must have a sufficient affinity for the lipid polar headgroups to make the lipid bilayer fit to the shape of the protein. The energy of protein–membrane binding has to exceed the membrane-bending energy.

The second mechanism is called the local spontaneous curvature mechanism (FIG. 3c). It is based on the local deformation of membranes that occurs when the amphipathic moieties of proteins become embedded in the lipid matrix. A shallow insertion of an amphipathic protein helix into only the upper part of a membrane monolayer has the role of a wedge which perturbs the packing of the lipid polar headgroups and results in a local monolayer deformation.

Last, proteins can change membrane shapes according to the bilayer-couple mechanism. If the amphipathic protein domains penetrate only one lipid monolayer, they can produce an area difference between the membrane leaflets and the membrane will develop curvature to compensate for this area asymmetry. In practical terms, the bilayer-couple mechanism of protein function seems to be the least biologically relevant. This is related to the global nature of the bilayer-couple effect based on the difference between

the total areas of the two lipid monolayers. In a key biologically relevant situation, such as membrane-carrier formation, protein insertion is expected to occur only locally in membrane spots that have areas that are negligible compared to the total membrane area. Even if the local membrane concentration of the inserted protein domains was considerable, the effect would be averaged over the total membrane area, so it would provide a negligible contribution to the total area difference between the membrane leaflets.

Proteins bend membranes: biological examples
Scaffold mechanism. The scaffold mechanism is straightforward and might underlie the function of many well-studied membrane-curving proteins[59]. It proposes that all of the protein coats that are known to cover the surface of membrane invaginations and buds function as scaffolds for membrane curvature (FIG. 3b). Within this idea, the COPI and COPII complexes and the clathrin–adaptor-protein complexes provide scaffolds for spherical curvature, whereas dynamin and BAR (Bin, amphiphysin, Rvs)-domain-containing proteins (including endophilin) wrap around membranes and provide scaffolds for cylindrical curvature[26].

The clearest way to visualize the scaffold mechanism is to consider the formation of cylindrical membranes by dynamin and BAR-domain-containing proteins. The dynamin helix self-assembles in the absence of lipid into rings and helices[60], which means that it is characterized by the intrinsically bent shape of a 'split lock washer' (recent structural data confirm this; J. Hinshaw, personal communication). Furthermore, dynamin binds to lipid membranes and forms cylindrical coats that have the same helical structure and cross-section radius as the pure dynamin helix[36,61–68]. This means that the rigidity of the dynamin coat is greater than that of the lipid bilayer and that dynamin binding to lipids is sufficiently strong to allow the scaffold mechanism to work in membrane shaping and, probably, membrane fission[67,69–72].

The BAR domain has a banana-like shape[73,74], and its concave surface binds the lipid membrane (FIG. 3c). It therefore satisfies the criterion of having the correct intrinsic shape. In addition, the protein has 12 positively charged residues on its concave surface, which allows it to interact strongly with the negatively charged polar headgroups of the lipid molecules. The energy of this interaction is predicted to be 6–12 kcalmol^{-1} per domain for membranes that contain 15–30% negatively charged lipid headgroups[75]. As four BAR domains circle cylindrical membranes, this energy is more than the membrane-bending energy (which is about $20k_BT$), which means that the criterion of a high affinity of the protein for the membrane is also satisfied[75]. In general, the electrostatic interaction between lipids and proteins is one of the important factors that determines membrane shape. Although there are no data available regarding the intrinsic rigidity of BAR domains, presumably the bundling of the helices that constitute a BAR domain lends rigidity. The curvature of many membrane tubes that are covered by these
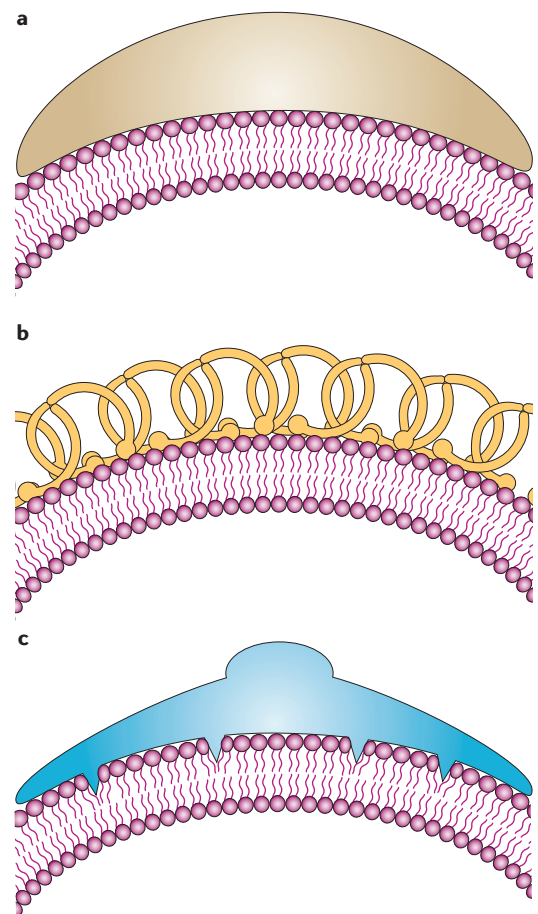

Figure 3 | **Mechanisms by which proteins can generate membrane curvature. a** | The scaffold mechanism. A rigid protein, or protein domain (for example, the BAR (Bin, amphiphysin, Rvs) domain), that has an intrinsic curvature binds to the membrane surface and bends the membrane beneath it. **b** | Polymerized coat proteins, which are sometimes linked to membranes through adaptor proteins (not shown), stabilize membrane curvature. **c** | The local spontaneous curvature mechanism is mediated by the insertion of amphipathic moieties of proteins between the polar headgroups of lipid molecules. A BAR domain is shown here inserting amphipathic helices into the monolayer it contacts[74].

domains is close to the curvature of the concave BAR-domain surface. This implies that the protein domains are more rigid than lipid bilayers, which would satisfy the last criterion of the scaffold mechanism.

For all of the other protein complexes that are assumed to function according to the scaffold mechanism, the data justifying this assumption are not available at present. Clathrin and its complex with adaptor proteins, which is required for membrane binding, self-assembles in the absence of lipid into cages, the polyhedral shapes of which can be approximated by spheres that have curvatures comparable to, or even smaller than, the curvatures of clathrin-coated vesicles[16,76,77]. This means that clathrin complexes are characterized by an intrinsically curved shape that is necessary for the scaffold mechanism. In fact, by

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Clathrin–adaptor-protein complexes
Adaptor proteins recruit clathrin to membranes and concentrate specific transmembrane proteins in clathrin-coated areas of the membrane. Clathrin is a protein that exists in a trimeric form called a triskelion, and clathrin triskelia polymerize to form cage-like structures.

BAR domain
('Bin, amphiphysin, Rvs' domain). A domain that is found in a large family of proteins. It forms a banana-like dimer, and binds to and tubulates lipid membranes.

Endophilins
A family of proteins that contain a BAR (Bin, amphiphysin, Rvs) domain with extra amphipathic helices. Endophilin-1 binds to and tubulates lipid membranes.

changing its adaptor proteins, clathrin can change the curvature of the clathrin-coated vesicle. On the other hand, clathrin complexes can also bind to membranes as flat arrays, and they do not fulfil the criterion of sufficient rigidity. Pure clathrin is estimated to be less rigid than lipid membranes[78]. The rigidity of clathrin–adaptor-protein complexes is of the same order of magnitude as that of lipid membranes, which is insufficient to bend membranes effectively.

The structure of one component (Sec23–Sec24) of the COPII coat complex has a concave surface that fits a circle with a 30-nm radius[79]. Moreover, under optimal conditions, Sec23–Sec24 and Sec13–Sec31 self-assemble into spheroids[37,79–81]. So, similar to clathrin, the final polymerized protein structure of Sec23–Sec24 plus Sec13–Sec31 has sufficient intrinsic curvature to operate using the scaffold model. What is known about the rigidity of the proteins that constitute the COPII complex? The Sec23–Sec24 dimer is held together by only the relatively weak forces of a short interacting β-strand, so it is unlikely that Sec23–Sec24 has sufficient rigidity to bend membranes. In addition, compared with the radii of COP-coated vesicles that can be formed *in vitro*, the large variation in the radii of lipid-free coats that were formed by the *in vitro* assembly of purified proteins indicates that they have insufficient rigidity to bend membranes[37]. Furthermore, Sec23–Sec24, Sec13–Sec31 or a complex of the two cannot bind to membranes, so they do not have the lipid-binding affinity required by the scaffold model. The COPII complex is therefore unlikely to work using the scaffold mechanism.

Local spontaneous curvature mechanism. A leading candidate for the generation of local spontaneous curvature through the insertion of an amphiphatic domain into the lipid matrix is epsin[82]. When added to liposomes, epsin creates small tubules. The structure of the ENTH (epsin N-terminal homology) domain of epsin has been solved and it does not seem to be a scaffold, as it is a small globular protein. However, it has a high binding affinity for membranes if phosphatidylinositol-4,5-bisphosphate is present in the membrane[83]. The ability of epsin to curve membranes is related to the insertion of its ENTH-domain amphipathic helix into the membrane bilayer. Mutations that prevent this insertion prevent epsin from inducing membrane curvature. However, more information is needed about the depth of the insertion and the orientation of the epsin amphiphatic helix within the membrane in order to estimate the local spontaneous curvature that can be induced by this protein. Presumably, the amphipathic N-terminal region of endophilin functions in a similar fashion, independently of its putative enzymatic activity[84].

Amphiphysin might also function according to the local spontaneous curvature mechanism. The amphipathic helix of amphiphysin might insert into lipid monolayers and generate local deformation. In fact, mutations that reduce the hydrophobicity of amino acids in this helix result in a much higher concentration of amphiphysin being needed to produce cylindrical membrane curvature[73,84]. As in the case of epsin,

there is not sufficient information at present to allow quantitative estimations of the effect of amphiphysin on the local spontaneous curvature to be obtained.

The small GTPase Sar1, which is one of the core COPII proteins, has been shown to curve lipid bilayers by membrane insertion of its N-terminal amphiphatic α-helix[85]. Replacing hydrophobic residues in the α-helix with alanine impaired the ability of Sar1 to tubulate membranes. Sar1 is therefore a candidate for curving membranes using the local spontaneous curvature mechanism.

Interplay between different mechanisms. Does membrane shaping in intracellular carrier formation occur according to one of these mechanisms or all of them simultaneously? The budding machinery of endocytic vesicles includes: clathrin–adaptor-protein complexes, which might work only according to the scaffold mechanism, but seem to be too flexible to bend membranes effectively; and epsin, which is able to induce membrane curvature on its own using the local spontaneous curvature mechanism. The involvement of both epsin and clathrin–adaptor-protein complexes implies that their interplay guarantees the robustness of the membrane-deformation process. Epsin might first bind to a phosphatidylinositol-4,5-bisphosphate patch on the membrane. This binding bends the membrane according to the local spontaneous curvature mechanism and attracts clathrin–adaptor-protein complexes, which, in turn, bind more epsin[82]. Clathrin might then stabilize the curved shape of the emerging bud by polymerizing to form a coat. The interplay between clathrin and epsin might also have a role downstream of vesicle fission. As a result of phosphatidylinositol-4,5-bisphosphate hydrolysis after clathrin polymerization (without replenishment from the plasma membrane by lateral diffusion along the inner leaflet of the bud neck), epsin might dissociate from the vesicle membrane, causing the spontaneous curvature to return to its initial value. This would give rise to elastic stresses, which tend to unbend the membrane of the vesicle and promote coat disassembly.

The formation of membrane necks that connect clathrin-coated buds and the plasma membrane could be carried out by dynamin alone, as it is able to tubulate membranes using the scaffold mechanism. However, within the cell, dynamin operates together with amphiphysin and endophilin, which, given their amphipathic helices, might function, at least partially, according to the local spontaneous curvature mechanism[73,84] (FIG. 3c). Again, such protein interplays are probably necessary to ensure the reliability of membrane-neck formation and fission.

As discussed above, the COP complexes are unlikely to provide a sufficiently rigid scaffold to bend intracellular membranes into small buds and vesicles. So, in these systems, we also expect to see interplay between the different mechanisms of membrane bending. Membrane shaping by the COPII coat is most probably driven by the synergistic action of the Sec23–Sec24 plus Sec13–Sec31 scaffold and Sar1 generating the local spontaneous curvature[85]. Further elaboration on this

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Spheroid
A geometric shape that is similar to a sphere but that is not exactly spherical (for example, a mis-shaped ball).

Epsin
A protein that contains an ENTH (epsin N-terminal homology) domain and is involved in clathrin-mediated endocytosis.

ENTH domain
(epsin N-terminal homology domain). A domain of epsin and several other proteins that binds to and tubulates lipid membranes.

Amphiphysin
A BAR (Bin, amphiphysin, Rvs)-domain-containing protein that binds to clathrin-coated vesicles during budding. Amphiphysin binds to and tubulates lipid membranes.

ArfGAP1

(Arf GTPase-activating protein-1). A small GTPase-activating protein that is responsible for catalysing Arf1 GTP hydrolysis. ArfGAP1 is needed to build up the coatomer protein (COP)I coats of intracellular transport vesicles.

issue requires more information regarding the structures and membrane interactions of the proteins that are involved in COP-mediated membrane bending.

Sensing curvature

The idea that proteins can sense curvature is a twenty-first-century concept in the field of membrane curvature and trafficking. It emerged with the recent identification of proteins that have membrane-binding affinities that depend on the radius of membrane curvature. So, what are the mechanisms and protein properties of curvature sensing?

First, proteins that can induce curvature by scaffolding or by inserting their amphipathic domains into the lipid matrix can sense curvature. Such curvature sensing can be based on the membrane-curvature dependence of the effective energy of protein binding to the bilayer surface and protein insertion between the polar headgroups. Indeed, the total effective binding and/or insertion energy consists of the energy of the protein–membrane interaction and the energy of membrane deformation that results from protein attachment to its surface. The latter contribution vanishes and the effective binding energy is minimal if the membrane curvature matches the shape of the protein and no deformation is needed. Otherwise, the effective binding energy increases with an increasing mismatch between the membrane curvature and protein shape.

Second, proteins that have an intrinsic curvature but are too flexible to bend the membrane can still function as curvature sensors. The binding and/or insertion energy of such proteins depends on the membrane curvature. On binding to the membrane, the protein undergoes deformation, so that the elastic energy of the protein contributes to the effective binding energy. This contribution equals zero when the membrane and the protein shapes fit to each other, but grows with an increasing mismatch of shape.

Finally, there is evidence that the enzymatic activity of a protein can function as a membrane-curvature sensor. This has been shown for ArfGAP1 (Arf GTPase-activating protein-1), a protein that is involved in coated-vesicle formation[86]. However, the detailed mechanism of this phenomenon remains unclear. We feel that it is unlikely that changes in enzyme activity with curvature are a reflection of the spreading of headgroups (as was proposed in REF. 86). Rather, we propose that the binding of non-rigid proteins to a curved surface might bend the protein to some extent, which might alter the proximity of crucial amino acids in or near the active site and therefore the enzyme activity.

Conclusions

At this stage in our analysis of structural data, we suggest three mutually non-exclusive mechanisms of membrane-curvature generation by proteins — the scaffold mechanism, the local spontaneous curvature mechanism and the bilayer-couple mechanism. Which method a protein uses is determined by the depth of penetration of the hydrophobic domain of the protein into the bilayer matrix. Considering elastic energy, we conclude that neither the bilayer-couple mechanism nor the spontaneous curvature of lipids alone is likely to drive membrane bending for trafficking in living cells. We have therefore focused on the protein properties that are necessary to guarantee the success of the local spontaneous curvature and scaffold mechanisms. We show that only a few curvature-generating proteins (BAR-domain-containing proteins and dynamin) possibly function according to the scaffold mechanism alone. For all other proteins, it seems that the scaffold and local spontaneous curvature mechanisms might function together. Further experimental research that addresses the unexplored elastic properties of proteins and liquid ordered and crystalline membrane domains is needed to verify these conclusions.

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