not necessarily need to be processed as two separate lines of information. Indeed, the first attempts have been made to directly combine observations with the constraints imposed by physical laws as represented by models[10]. Until now, studies using such data-assimilation techniques have not included interactive representations of the carbon cycle, but there is no formal obstacle to prevent that. Inclusion of such representations would produce estimates of the state of the coupled climate–carbon system that use all available information, and thus potentially provide stronger constraints on all of the processes involved. ■

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BIOPHYSICS

Joint effort bends membrane

Michael M. Kozlov

The curvature of cellular membranes is generated by proteins and lipids. A synthetic experimental system allows the interplay between protein- and lipid-generated bending mechanisms to be studied directly.

A living cell can be thought of as a building of extremely peculiar architecture, in which the walls are formed by films just a few nanometres thick — the cell membranes. The numerous rooms, halls and corridors of the building are the intracellular organelles, such as the endoplasmic reticulum, Golgi complex and mitochondria. These have irregular shapes, consisting of tubules and sheets that are a few tens of nanometres in cross-section[1]. The shapes are highly dynamic, undergoing constant changes and interconversions. What's more, separate nanospheres and nanotubules bud off from the cell's external and internal membranes, forming transport vessels that ferry biomolecules between destinations within cells.

The diversity and dynamics of membrane shapes are vital for the cell's physiology. One of the biggest challenges in cell biology and biophysics is therefore to understand the molecular mechanisms that enable cell membranes to bend easily and rapidly into highly curved, dynamic shapes. Reporting in the *Journal of the American Chemical Society*, Yu *et al.*[2] describe a promising synthetic model of membranes that can be used to assess experimentally the combined influence of lipids and proteins on membrane curvature.

At first glance, it is not obvious that a cell needs to make any special effort to bend its membranes. The structural basis of any cell membrane is a lipid bilayer only four nanometres thick, made up of two monolayers of molecules (a mixture of phospholipids and cholesterol). Surely, such a thin film of soft biological matter must flex easily, perhaps even in response to thermal fluctuations in the local environment?

In fact, the characteristic scale of the energy required for membrane bending is about 20 times higher than the energy provided by thermal fluctuations, which makes membranes sufficiently rigid to pose a challenge to the cell. To shape them into curved nanospheres or tubules, cells must thus employ special mechanisms. The simplest approach is to use the membrane's constituents — either the lipids themselves and/or proteins associated with the lipid bilayers — to somehow bring about bending. But how?

There are two main approaches. If a lipid bilayer consists of two identical monolayers, it tends to remain flat; because its structure is symmetrical with respect to the mid-plane, there are no physical reasons for it to bend in any direction. So, a straightforward way to cause membrane bending is to create bilayer asymmetry. The second approach is to impose physical constraints on the membrane, such as frames or scaffolds that enforce bilayer curvature. Both lipids and proteins can be used to implement each of these two strategies.

Bilayer asymmetry can be achieved by generating a difference in the lipid compositions of the two monolayers. Lipid molecules of different kinds can be seen as the elements of a mosaic, with various shapes similar to

FLUID DYNAMICS

Supersonic splash

Throwing a coin into a wishing well might not bring you your heart's desire, but it could cause an astonishing physical effect — a supersonic jet of air. That's if you follow the instructions of Stephan Gekle and colleagues at the University of Twente in the Netherlands and the University of Seville in Spain. They report in *Physical Review Letters* that when a cavity is transiently created by dropping an object into a fluid, the airflow inside it has much in common with that in a jet engine (S. Gekle *et al. Phys. Rev. Lett.* **104,** 024501; 2010).

The authors used an experimental set-up in which a circular disc was pulled into a tank of water by a motor, breaking the liquid's surface. To visualize the airflow in the wake of the disc, they passed smoke across the surface of the water and illuminated the resulting smoke-filled cavity with laser light. A high-speed camera was used to record the smoke's movement, taking pictures at up to 15,000 frames per second. From these pictures, Gekle *et al.* calculated the velocity of the smoke (and so also that of the airflow) at speeds of up to 10 metres per second.

The researchers found that air is initially pulled into the expanding cavity at velocities similar to the plummeting disc's impact speed. But the walls of the cavity rapidly collapse because of water pressure, shrinking the cavity. This shrinkage reverses the direction of the airflow, ultimately causing a thin, fast air stream to surge up through the neck of the now-hourglass-shaped cavity (pictured; the diameter of the disc at the bottom of the cavity is 4 centimetres).

To calculate the air velocities that would be seen at higher speeds, Gekle *et al.* simulated

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the system mathematically. Their model incorporated equations used to describe airflow in supersonic jet engines, but with modifications that took into account the constantly changing shape of the cavities formed in their study. By combining experimental data with numerical simulations, the authors found that air can be pushed out of the cavities at supersonic speeds — even though the pressure inside the cavity is only 2% greater than atmospheric pressure.

Andrew Mitchinson

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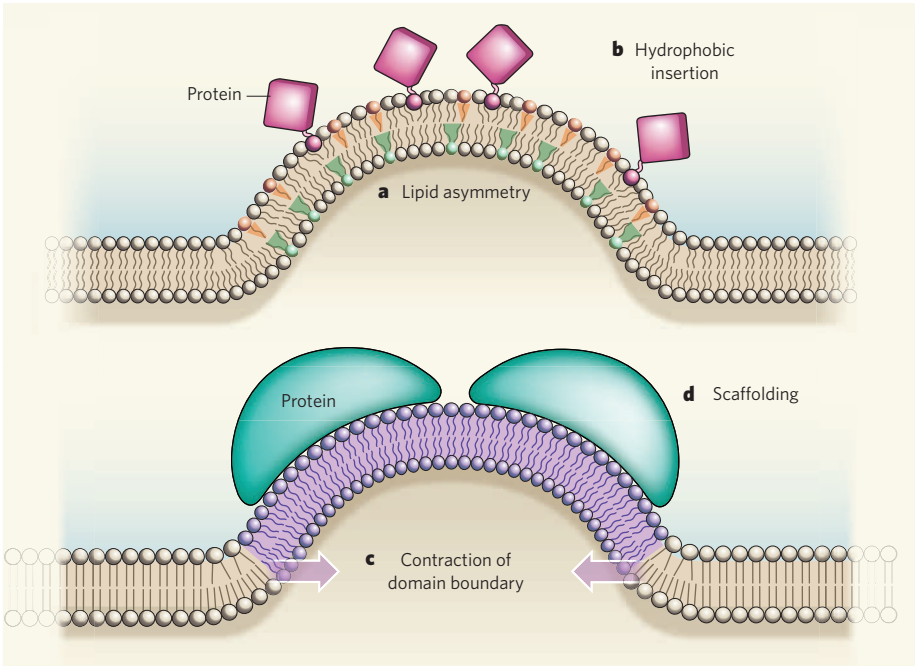

Figure 1 | Mechanisms of bending in lipid bilayers. **a**, Lipid asymmetry. This occurs when each monolayer is enriched with lipid molecules of different shapes (such as the orange and green molecules shown) and/or when one monolayer contains more lipid molecules than the other. **b**, Proteins cause membrane asymmetry by inserting their hydrophobic domains into one side of the bilayer. **c**, When bilayer matrices contain domains consisting of different lipid phases (such as the ordered (brown) and disordered (purple) regions shown), the boundaries between the domains tend to contract, causing the intervening region to bend. **d**, Finally, proteins bound to the bilayer can act as scaffolds that force curvature on the membrane. Yu *et al.*[2] report a synthetic model of membranes in which both hydrophobic insertion and domain-boundary contraction bring about bending.

cylinders, cones or inverted cones[3]. If, for example, there are more inverted cone-like molecules in the outer monolayer than in the inner monolayer, the bilayer will tend to adopt a concave shape (Fig. 1a). Alternatively, asymmetry can be created by introducing more lipid molecules into one monolayer than in the other[4]. The membrane will then bulge in the direction of the monolayer that has the larger number of molecules.

Proteins can generate membrane asymmetry by inserting their hydrophobic domains into the lipid bilayer matrix on one side of a membrane[5] (Fig. 1b), causing the membrane to bulge towards the affected monolayer. Most membrane-bound proteins have the potential to do this, because they already have hydrophobic domains inserted into membranes to anchor themselves. A theoretical analysis[6] of this hydrophobic insertion mechanism has revealed that the largest membrane curvatures are generated by shallow insertions that penetrate the external membrane monolayer only to about a third of its thickness. Common protein domains, such as amphipathic α-helices (which contain both hydrophobic and hydrophilic parts) and short hydrophobic loops, induce membrane curvature in this way, and are predicted to be much more effective than lipids in doing so[6].

Physical constraints that cause curvature in purely lipid membranes emerge if the lipid molecules are organized into patches of different phase state, such as ordered and disordered regions. Generally, the boundaries of such patches are in higher energy states than the rest of the membrane. This gives rise to a property known as line tension (which has dimensions of energy per unit length of the boundary), analogous to the surface tension associated with interfaces of immiscible media. Just as surface tension constricts the surface area of interfaces, line tension constricts patch boundaries. This causes patches to bulge, generating membrane curvature[7] (Fig. 1c). Protein molecules, on the other hand, physically constrain membranes if they have intrinsically curved shapes and attach to the bilayer surface along their bent faces — they simply impress their curvatures on the membranes[3,5,8] (Fig. 1d).

Although all of the above-mentioned mechanisms of membrane bending have been suggested and verified experimentally[3,5,8], the importance and effectiveness of the interplay between lipid- and protein-based modes remains largely unexplored[9]. Yu and colleagues' experimental model of a membrane[2] offers a unique possibility to resolve this issue. Their system consists of giant unilamellar vesicles (GUVs). At the size scales involved in intracellular membrane bending, the GUV membranes can be thought of as being essentially flat, because the vesicles' radii are much greater than the membrane thickness.

The membranes are composed of soft, liquid domains of a lipid known as DOPC (1,2-dioleoyl-*sn*-glycero-3-phosphocholine) enclosed within a stiff, gel-like lipid called DPPC (1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine), thus forming 'lakes' of DOPC within the 'land' of DPPC. In some experiments, the authors added cholesterol to the membranes to modify the physical properties of the lake and land regions, such as the line tension of the lake boundaries. The authors' system reproduces at least one of the lipid-based mechanisms of membrane bending: bulging of the lakes driven by boundary contraction.

But Yu *et al.* also added a protein to the membrane — melittin, a relatively short antimicrobial peptide containing 26 amino-acid residues. Some of the melittin molecules inserted themselves at shallow depths into the outer monolayer of the lipid lakes, and laid parallel to the membrane surface. These molecules bent the lipid lakes using the hydrophobic insertion mechanism. Other melittin molecules inserted perpendicularly to the membrane surface. These molecules formed transmembrane complexes that served as aqueous pores, facilitating curvature generation by removing geometrical constraints that otherwise maintain the volume of the GUVs. Yu and colleagues' system thus combines for the first time these lipid- and protein-based mechanisms of membrane bending.

Although the lake-like regions of the authors' GUVs bend, bud and even break away to form new, smaller vesicles, the curvatures generated in this system are much larger than those of intracellular vesicles and tubes. The physical forces controlling membrane bending *in vivo* cannot therefore be completely explained by the mechanisms built into Yu and colleagues' model. Moreover, the exact interplay between lipid-generated line tension and protein-generated hydrophobic insertion, and its role in determining curvature in the model system[2], remain to be clarified. Nevertheless, the authors' GUVs provide a general, promising platform for investigating how interactions between diverse proteins and lipids in membranes affect the shaping of those membranes. ■

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