People & Ideas

Michael Kozlov: A twist in membrane physics

Kozlov studies the biophysics of membrane shape and behavior.

At the most fundamental level, the formation and remodeling of intracellular vesicles, tubules, and cytoskeletal structures is dictated by the physical and mechanical properties of their components. Studying these properties and the forces needed to alter them can therefore bring a better understanding of the proteinaceous systems that provide such forces and that—quite literally—shape a cell's destiny. Michael Kozlov is working to do just that.

As a theoretical mechano-biophysicist, Kozlov employs concepts such as elasticity and thermodynamics to model the basic forces at work in cells. He has studied the forces that govern remodeling of the actin cytoskeleton at the leading edge of migrating cells (1) and investigated how membranes bend and remodel (2–5). His work on the physics of membranes has opened new windows on how membranous organelles form (3) and how their remodeling through membrane fission (4) and fusion (5) is achieved. We called him at his office at Israel's Tel Aviv University to hear how the forces that shape cells have also shaped his work.

THE BIG MOVE

You were trained as a physicist in Russia…

I was born in Moscow. My father was an engineer at an industrial research institute and my mom was a physician, so we were a middle-class family. But getting into university was a challenge for me because I was Jewish and my parents were not members of the Communist Party. I wanted to study physics, but I didn't expect to be accepted to one of the few places in Moscow where this could be done.

Eventually, after considerable trouble, I was accepted to the Engineering Physics Institute in Moscow to study theoretical physics. While I was there, I developed an interest in biology, and I did my master's thesis on a problem related to biology: calculating the shape of red blood cells. Then I did my PhD on membrane fusion at the Frumkin Institute of Electrochemistry in Moscow. I was there for six years after completing my PhD because I didn't belong to the section of Soviet society that could travel to Western countries.

Then the Soviet regime weakened, and the moment the possibility emerged I got an Alexander von Humboldt postdoctoral fellowship to work in West Berlin. I joined Wolfgang Helfrich's group initially for one year, but then I became a research associate in Helfrich's group. Six years later I joined Tel Aviv University. So, my first trip to the West was also my last one. I never returned to Russia.

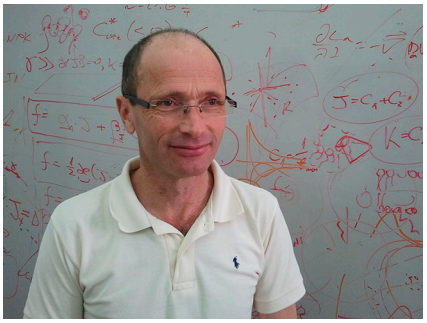
You first started studying membrane curvature while in Russia, though…

Yes. I have been interested in membrane curvature since first working on membrane fusion with Vladislav Markin, as a PhD student, inspired by the experimental work of my lifelong friend and colleague Leonid Chernomordik. That is why I joined Helfrich's lab, because he is a theoretical physicist who developed the major model for the bending elasticity of biological membranes. But while he was always interested in applying his theory to biological phenomena, Helfrich was a pure physicist. So, in his lab I worked on physico-chemical systems related to biology, but I didn't directly work on biological problems. It wasn't until I came to Tel Aviv that I started to talk to and collaborate with real biologists.

> "It seems intuitive to many people that a membrane… should not require force to bend."

Did any of your family remain in Russia?

No. Everyone is here in Israel. I don't have a large family, but my parents, my wife, and my kids are here. Actually, my daughter is now in Australia but hopefully on a temporary basis. [Laughs] Even my wife's mom and many of our friends are now here.



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Michael Kozlov

BENDING INTO SHAPE

One concept fundamental to your work is that membranes resist bending…

It seems intuitive to many people that a membrane—a very thin shell just four or five nanometers thick—should not require force to bend. But it turns out that even a thickness of a few nanometers is sufficient to make the membrane resistant to bending.

Depending on its lipid composition, a given membrane may prefer to remain flat, or it may spontaneously adopt a curved shape. It requires energy—that is, force application—to deviate from this preferred shape. It turns out that a membrane just four or five nanometers thick has sufficient rigidity to resist thermal undulations, for example, which is important for maintenance of specific shapes of the cell membranes. On the other hand, this rigidity is small enough to enable biologically feasible forces, on the order of piconewtons, to generate curvature.

How can membranes attain new shapes?

The basic mechanism is really simple: in order to generate curvature, one has to make the membrane's structure asymmetric. If the two lipid monolayers that constitute the membrane have a similar structure then the lipid bilayer will remain flat. But changing the properties or structure of one monolayer will inevitably cause the membrane to bend. This can be achieved, for example, by binding proteins to one membrane surface but not the other. Even low-affinity binding between a protein and a membrane monolayer can cause some curvature.

But if one wants to understand membrane bending inside cells—the curvatures

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of intracellular tubes, vesicles, and so on—then one has to understand how large curvatures are generated. By "large curvatures" I mean curvatures with radii around 20–30 nanometers. Based on experiments by my collaborator Harvey McMahon, we have suggested a specific mechanism for how this could take place, which we call the hydrophobic insertion mechanism.

According to this theory, amphipathic protein helices become embedded into the outer lipid monolayer of a membrane, entering the space between the lipid polar head groups and penetrating into the monolayer up to 30–40% of its thickness without spanning the whole membrane. This protein insertion splays the heads but not the hydrophobic tails of the lipid molecules, creating a strong asymmetry of the outer monolayer structure and hence of the whole membrane. We're still working to refine our models, but we think that only a modest concentration of hydrophobic insertions is needed to change the symmetry such that the membrane acquires large curvature.

What are some examples of proteins that do this?

There are quite a few proteins that can do this. A typical example is epsin. This protein is involved in endocytosis, and it has an amphipathic helical domain that inserts into the membrane. We have used epsin as a paradigm for proteins generating curvature by the hydrophobic insertion mechanism.

There is also another mechanism that proteins can use to generate curvature, which is called scaffolding. Scaffolding is employed by rigid, hydrophilic proteins that lack membrane-penetrating domains. These proteins simply interact with the membrane and, by sticking to the membrane, force it to adopt the same shape as themselves. Because of membrane bending rigidity, this shape is propagated to some extent within the membrane region surrounding the protein. F-BAR domains typically have this kind of scaffolding ability, but there are also proteins that combine both the scaffolding and the insertion mechanisms. For example, N-BAR domains have amphipathic helices that can insert into the membrane, but they may also use the scaffolding mechanism to generate membrane curvature.

The different properties of scaffolding and insertion proteins are biologically important. For example, we have a model that says that BAR-like scaffolds tend to generate curvature, but they don't contribute anything to the tendency of the membrane to undergo membrane fission. Therefore, they favor the formation of cylindrical membrane tubes. On the other hand, membrane insertions can drive membrane curvature and also a propensity to undergo fission, so they favor the formation of spheres. If there is a relative abundance of insertions over scaffolds, then membranes will undergo fission. We have a recent paper where our collaborators in McMahon's lab verified this model experimentally and showed how hydrophobic insertions could orchestrate fission of endocytic vesicles.

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Collaborators Kozlov and Tom Rapoport run down Masada mountain in Israel.

A SURPRISE TWIST

How about membrane topographies more complicated than tubules and vesicles?

I have been collaborating for a while with Tom Rapoport from Harvard. And Tom collaborates with Mark Terasaki, who has been working on electron microscopy of the endoplasmic reticulum. At a meeting, Tom showed me images of the endoplasmic reticulum in neuronal cells. He and Mark were very interested in understanding the three-dimensional structures that could give rise to a motif they saw in serial cross sections of the ER. Tom asked me about it because I am a theoretician and he and I have collaborated on the structure of individual ER sheets and tubes in the past. So, with my former student and current collaborator Tom Shemesh, I started thinking about this problem and also about the forces that could drive formation of such a motif in cross section. Through alternating cycles of theoretical modeling with experimental verification, the four of us came up with the theory that ER sheets within a stack are connected to each other via helicoidal ramps, similar to those found in a parking garage. As long as a helicoidal ramp that twists in one direction is surrounded by ramps twisting in the opposite direction, the stresses caused to the structure almost cancel each other out and the energy cost of the structure is very low. Therefore, ER sheets form a single stacked structure with helicoidal membrane connections. We're now trying to extend our understanding to other ER morphologies.

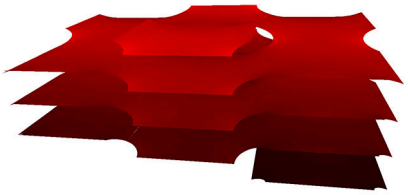

IMAGE COURTESY OF MICHAEL KOZLOV

A parking garage–like model of the ER.

> "We're now trying to extend our understanding to other ER morphologies."

What else are you bending your efforts toward?

[Laughs] One of the directions in my group that is not membrane related concerns the formation of actin structures within cells. We have a long-standing collaboration with Alexander Bershadsky (now mainly at MBI, Singapore) looking at cell adhesions and actin structures at the cell's leading edge. We're not doing all this work at the same time, of course. But these are the things we are thinking about right now.

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