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Citation	Khaykovich, Boris et al. "Thickness-radius relationship and spring constants of cholesterol helical ribbons." Proceedings of the National Academy of Sciences 106.37 (2009): 15663-15666. © 2009 National Academy of Sciences
As Published	http://dx.doi.org/10.1073/pnas.0907795106
Publisher	United States National Academy of Sciences
Version	Final published version
Citable link	http://hdl.handle.net/1721.1/55291
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Thickness–radius relationship and spring constants of cholesterol helical ribbons

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Contributed by George B. Benedek, July 15, 2009 (sent for review June 1, 2009)

Using quantitative phase microscopy, we have discovered a quadratic relationship between the radius R and the thickness t of helical ribbons that form spontaneously in multicomponent cholesterol–surfactant mixtures. These helical ribbons may serve as mesoscopic springs to measure or to exert forces on nanoscale biological objects. The spring constants of these helices depend on their submicroscopic thickness. The quadratic relationship ($R \propto t^2$) between radius and thickness is a consequence of the crystal structure of the ribbons and enables a determination of the spring constant of any of our helices solely in terms of its observable geometrical dimensions.

biological force spectroscopy | elasticity of thin films | phase-contrast microscopy in biophysics

The elastic properties of meso- and nanoscale thin elastic strips forming helical ribbons or tubules, have been the focus of active recent research in both biophysics and nanoscience communities (1–7). We have discovered that in a number of complex aqueous solutions containing a sterol (cholesterol in particular) and a mixture of surfactants, the sterol molecules may self-assemble into ribbons of helical shape (8). The geometry of the helical ribbons is characterized by the radius, width, thickness, contour length, and pitch angle, see figure 1*a* in ref. 9. Remarkably, the pitch angle is always either 11° or 54°, whereas axial length, width, and radius vary by two orders of magnitude in the range from 1 to ≈100 μm. These helical ribbons are fascinating objects for fundamental studies (2, 8–10). Furthermore, because low-pitch helical ribbons have spring constants in the range of 0.5 to 500 pN/μm (2), and the elongation of these springs from 1 μm up to 100 μm can easily be observed microscopically, it follows that they can be used as mesoscopic spring scales to measure forces between nanoscale biological objects in the range from 0.5 pN to 50 nN. For this and other applications, the ability to readily determine the spring constants of individual helices is of crucial importance. In this article, we establish the relationship between the spring constant of the low-pitch cholesterol helical ribbons and its readily observable dimensions: width, radius, and length.

Originally, it had been thought that cholesterol helical ribbons formed in surfactant mixtures had liquid crystalline structure and that their shape was governed by elastic properties of liquid crystalline layer (9, 11, 12). Recently, we have shown by X-ray diffraction that these helical ribbons are, in fact, single crystals with structure closely resembling that of cholesterol monohydrate (10). Having in mind the single-crystal nature of our ribbons, we have proposed that their helical shape is determined by a balance between two terms in the free energy of deformation of the cholesterol crystalline strip (2). The first term, the spontaneous bending energy, favors curling toward one of the two faces of the ribbon and is linear in curvature, $-K_s/R$. The second term is the elastic energy of bending a strip. This energy increases equally upon curling toward either face of the ribbon, and it is proportional to the square of curvature, K_{el}/R^2 . Mini-

mization of the sum of the two terms determines the radius of the helix, $R = 2K_{el}/K_s$. The quantitative theory for the observed geometrical and elastic properties of these helical ribbons is elaborated in ref. 2. If the ribbon is anisotropic, $K_{el}(\psi)$ depends on the angle ψ between the axis of bending and the ribbon edge [note that in thin ribbons, only cylindrical deformations are possible (13)]. The equilibrium pitch angle ψ_0 is the preferential bending direction, for which $K_{el}(\psi)$ is minimal. When the crystalline helical ribbon with contour length s is stretched or compressed, its axial length $l = s \sin \psi$, and therefore its pitch angle changes. The ribbon is forced to bend in a less preferable direction than in equilibrium, and its elastic energy increases. That leads to axial rigidity and a corresponding spring constant. We have shown (2) that the spring constant K_{spring} of such a crystalline helical ribbon is determined by its width (w), its contour length (s), and its radius (R) according to:

$$K_{spring} = \frac{8w}{R^2s} K_{\alpha}, \quad [1]$$

where the effective bending modulus

$$K_{\alpha} = \partial^2 K_{el}(\psi) / \partial (\sin \psi)^2 |_{\psi=\psi_0}$$

According to beam-bending theory for solid ribbons (13), we expect K_{α} to be proportional to the cube of the thickness t of the strip, $K_{\alpha} \propto t^3$.

In this article, we report measurements of the thickness of cholesterol helical ribbons using quantitative phase microscopy (14, 15). We found a universal relationship, independent of solution conditions, between the thickness of the helix and its radius, consistent with $R \propto t^2$, as shown on Fig. 1. Because the bending modulus K_{α} is determined only by internal elastic properties of the crystalline ribbon and its thickness, the radius of the helix can be used as a measure of the effective elastic modulus of the ribbon, according to relationship $K_{\alpha} \propto t^3 \propto R^{3/2}$ with a universal proportionality coefficient. This relationship is of great importance for application purposes because it enables selection of cholesterol ribbons with desired spring constants based solely on microscopically observed geometrical parameters of the ribbon. Furthermore, we argue that our approach has broader applicability to a variety of nanoscale tubules and helical ribbons constituted of inorganic thin films (4, 5, 16) or thin single crystals such as ZnO and InP (17, 18).

Author contributions: B.K., W.C., A.L., R.R.D., M.S.F., and G.B.B. designed research; B.K., N.K., W.C., C.H., and Y.S. performed research; B.K., N.K., and W.C. analyzed data; and B.K., N.K., W.C., A.L., M.S.F., and G.B.B. wrote the paper.

The authors declare no conflict of interest.

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that utilizes acoustooptical modulators to shift the frequency of the reference beam by 1.25 kHz relative to that of the sample beam. Four interference images are taken by a camera with the frame rate of 5 kHz, four times larger than the frequency shift of reference beam. As a result, the relative phase between the sample and reference beams differs by $\pi/2$ between consecutive interference images. The phase image of a sample is then calculated from these four interference images by using so-called phase-shifting interferometry algorithm (14). We measure the phase difference between the sample and reference beams with milliradian precision, which translates into a few nanometers resolution in the optical thickness of our samples.

Our instrument uses a coherent light source, and the phase images display a fixed pattern of diffraction from particles in the beam path, such as dust or micelles, as well as from the specimen itself. To remove the background diffraction pattern and thus enhance the signal-to-noise ratio of phase detection, we synthesize phase images taken at different angles of illumination, so that only images from the specimen in the focal plane add up constructively (15).

To use Eq. 2, we assume that our helices have the same index of refraction as cholesterol monohydrate crystals, which have essentially the same structure

as our helical strips (10). Because, to the best of our knowledge, this index of refraction has not been reported, we grew flat single crystals of cholesterol monohydrate, sufficiently thick to allow direct determination of their thickness microscopically by focusing on polystyrene beads adsorbed on the upper and lower surfaces and thin enough to permit the use of phase microscopy to measure phase retardation (in water solutions of potassium thiocyanate, having refractive indices of either $n = 1.4152$ or 1.4406). Using Eq. 2, we found that the refractive index of cholesterol monohydrate along the c axis, which is perpendicular to both the plate shaped crystal and the ribbon surface, is $n = 1.522 \pm 0.003$. Interestingly, this value falls into the range 1.49 ± 0.06 , which was measured by ellipsometry for a cholesterol monolayer on air–water interface (24).

ACKNOWLEDGMENTS. We gratefully acknowledge the support provided by the Department of Energy, Division of Materials Sciences and Engineering, Office of Basic Energy Sciences under Award DE-FG02-04ER46149. This work was also supported by National Center for Research Resources of the National Institutes of Health Grant P41-RR02594-18, National Science Foundation Grant DBI-0754339, and the Hamamatsu Corporation.

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