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Direct method to study membrane rigidity of small vesicles based on atomic force microscope force spectroscopy

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Mechanical properties of lipidic membranes such as their bending rigidity are governing liposome morphology and play an important role in processes like membrane fusion and adhesion. Force versus deformation measurements are the most direct means to determine this, but so far experimental data is scarce and mainly stems from techniques that are limited to giant vesicles. We present atomic force microscope force spectroscopy as a method allowing force-deformation measurements of submicron vesicles. Bending rigidities of small unilamellar dipalmitoylphosphatidylcholine (DPPC) liposomes ($R<200$ nm) can be derived from the force-deformation data using analytical models based on shell theory and are in good agreement with independent measurements.

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Liposomes, which were discovered 40 years ago by Bangham [1], are spherical, bilayer vesicles that form spontaneously when certain phospholipids are dispersed in water. On the applied side, several products in fields as diverse as pharmaceutics, cosmetics, or food design take advantage of their chemical, microencapsulating, and surface properties. Especially homogeneous unilamellar vesicles in the size range 50–200 nm are widely used in this context since this size range offers a compromise between loading efficiency, stability, and ability to extravasate [2]. Mechanical properties of the lipidic membranes are key parameters for membrane shape and stability and play an important role in processes such as fusion and adhesion of vesicles [3]. So far, experimental techniques for measuring these mechanical properties are limited to giant unilamellar vesicles of at least 10 microns in diameter. For these, elastic constants can be derived from shape analysis if the membrane is soft enough to carry out pronounced thermal fluctuations using optical microscopy [4,5]. Alternatively, in a micropipette manipulation, the vesicle is aspired by the micropipette and shape changes are monitored with optical microscopy as a function of applied pressure difference [6]. However, the production and manipulation of giant unilamellar vesicles is experimentally rather demanding or even impossible. Therefore, methods that allow measurements on small unilamellar vesicles are of common interest. We explore in this paper the applicability of atomic force microscope (AFM) force spectroscopy (i.e., measurement of force vs distance curves) for such measurements.

In a previous work we have shown that AFM force spectroscopy can be used to measure directly the bending rigidity of micrometer-sized faceted vesicles [7]. Here we use this technique to probe the rigidity of small unilamellar liposomes ($R<200$ nm) and demonstrate that elastic constants of the membrane and the bending rigidity can be derived using an analytical model based on shell theory.

Small unilamellar liposomes were prepared by dissolving dipalmitoylphosphatidylcholine (DPPC) molecules in a mixture (1:1)v of $MeOH/CHCl_3$. A thin film of lipids is then formed on the wall of the flask by evaporation of the solvent under reduced pressure (48 h). A slow hydration at a temperature above the transition temperature of DPPC lipids ($T_m=42\,^\circ C$) by the addition of aliquots (150 μL) of PBS buffer (pH=7.2) leads to the formation, after shaking, of a solution of large multilamellar liposomes [8]. Small unilamellar liposomes are obtained by extruding (LiposoFast, basic, Avestin Inc., Ottawa, Canada) at $T>T_m$ the solution of large multilamellar liposomes through polycarbonate membranes with decreasing pores sized. The extrusion through a 400 nm diameter membrane and through a 200 nm diameter membrane leads to the formation of a solution of unilamellar liposomes with a Gaussian radius distribution centered around 120 nm with a full width at half maximum of 75 nm, as shown by the light scattering analysis of the radius distribution (Fig. 1).

Force deformation curves were obtained with a NANOWIZARD AFM (JPK instrument, Berlin) operating in a PBS buffer (pH=7.2) at room temperature on small unilamellar DPPC liposomes immobilized onto silicon substrates that were first cleaned in an air-plasma cleaner for 5 min ($P=0.2$ mbar) followed by a dipping in a Piranha solution (30% H_2O_2, 70% H_2SO_4) for 10 min (CAUTION: Piranha is

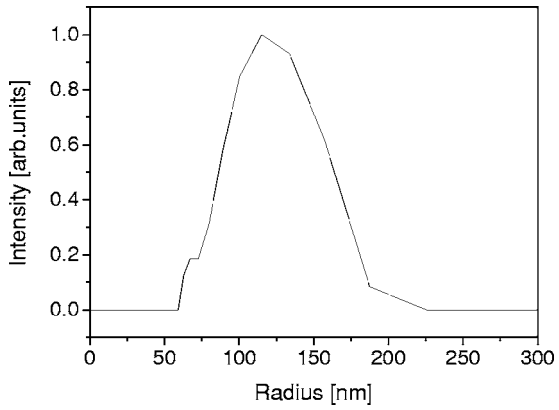

FIG. 1. DPPC-liposomes radius distribution determined by light scattering.

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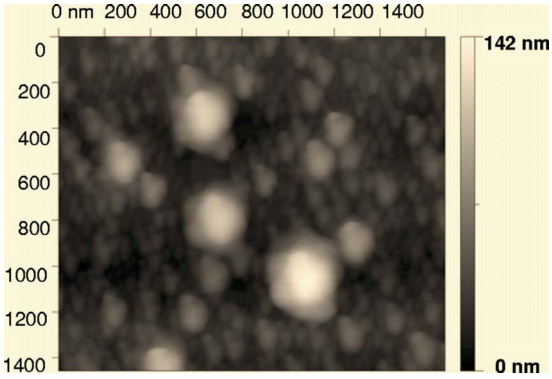

FIG. 2. (Color online) AFM image of DPPC-liposome immobilized on silicon substrate (PBS buffer, contact mode).

a vigorous oxidant and should be used with extreme caution) [9].

The spring constant of the rectangular cantilevers (MLCT-AUNM, Veeco) used in this study had a nominal spring constant of 20 pN/nm. However, the spring constants were calibrated by using both thermal oscillation [10] and Sader's methods [11]. For the force spectroscopy experiment, ~50 approach-retraction cycles were performed on each liposome. The maximum height observed by AFM imaging (Fig. 2) for the immobilized liposomes was around 200 nm indicating a partial spreading of the liposomes. AFM imaging was also used to check that the structure of the liposomes remains unchanged after the force vs distance measurements. For deformations on the order of the membrane thickness (~5 nm) we obtained an approximately linear and reversible thickness (approaching and retracting curves superimposed) typical for an elastic behavior in this regime (Fig. 3). For larger deformation the linear dependency of the force on the deformation is lost and a hysteresis is observed between the approaching and the retracting curves indicating the vanishing of the elastic behavior (results not shown). This behavior is typical for shelllike structures, which, other than massive particles, show a discontinuity in their deformation behavior: the buckling transition [12]. The prebuckling regime can be used to obtain quantitative information on elastic constants [13–15].

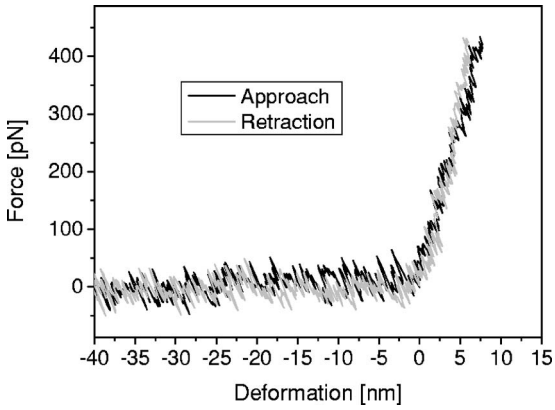

FIG. 3. Force vs deformation curves obtained on DPPC liposomes in the small deformation regime.

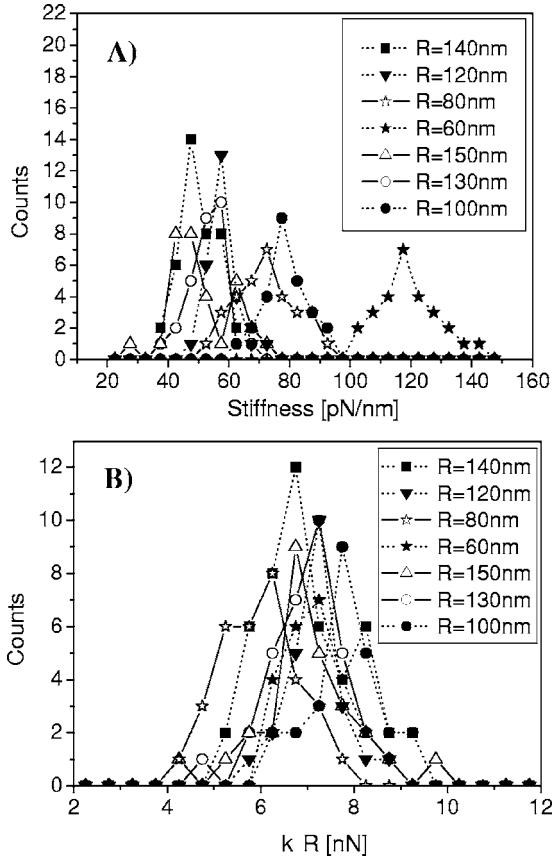

FIG. 4. (A) Distribution of the stiffness measured on DPPC liposomes with different sizes. (B) Normalized stiffness calculated using the analytical model based on shell theory.

Figure 4(a) shows the liposome stiffness (e.g., slope of the force-deformation curve) measured on several liposomes with different radii of curvature obtained from the topography of the structure. It is clearly demonstrated that the stiffness of the DPPC membrane increases when the liposome radius decreases, indicating a dependency of the liposome deformability on the radius.

This result shows that the deformation behavior cannot be modeled in this case by the frequently used Hertz model [16], which could neither explain the vanishing of the elastic behavior in the high deformation regime, nor the dependency of liposome stiffness on the radius. In contrast, both observations are predicted by shell deformation theory and for deformations on the order of the membrane thickness, we have previously found good agreement with finite element modeling and experimental results [17]. Based on this model, the liposome stiffness (k) scales as [7]:

$$k = \frac{4Eh^2}{R\sqrt{3(1-\nu^2)}},$$

where E denotes the effective Young's modulus, ν the Poisson ratio, R the radius of curvature of the spherical cap, and h the membrane thickness. To test the applicability of this model, we have rescaled the liposome stiffnesses by the liposomes' radius of curvature (as obtained from the AFM

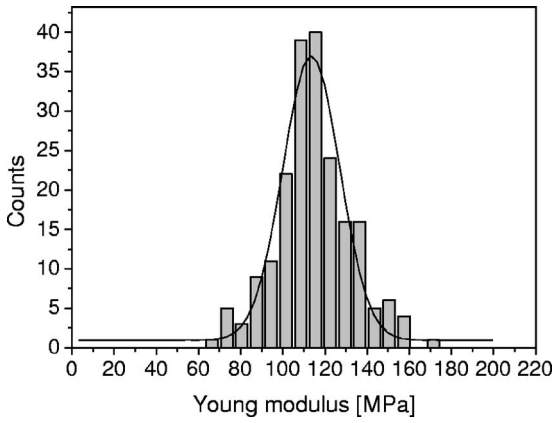

FIG. 5. Young modulus distribution of DPPC liposomes obtained by summation of the results independently of liposomes sizes.

topography measurements) and found that after this rescaling, the data collapses onto a master distribution [Fig. 4(b)].

Based on a Poisson ratio of 0.5 [18] and the radii of curvature, the effective Young's modulus of lipidic membrane can be determined. The summation, independent of the liposome sizes, of all the Young modulus measurements leads to a narrow Gaussian distribution with $E = 110 \pm 15$ MPa at room temperature (Fig. 5).

For comparison with alternate techniques, the bending rigidity (K_C) can be calculated from [12]: $K_C = Eh^3/[12(1-\nu^2)]$ and is found to be $330 \pm 50\ k_B T$ for the DPPC membrane at room temperature. This value is in good agreement with previous measurement on similar phospholipid membranes in a gel state obtained either on giant liposomes [5,19] or on a supported lipid bilayer [20] indicating the relevance of our method. This value is much higher that the value found by Liang *et al.* [21] for egg-PC small unilamellar liposomes. However, we believe that the use of the Hertz model to analyze AFM force-distance measurements on vesicles leads to an underestimation of the bending rigidity.

In conclusion, we have demonstrated that AFM force spectroscopy, when it is associated with a model based on shell theory, is a versatile method to directly measure the rigidity of the membrane of small vesicles ($\varnothing < 200$ nm). Since this AFM force spectroscopy can be carried out on small vesicles, it complements existing techniques that rely on giant vesicles.

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[1] A. D. Bangham, M. M. Standish, and J. C. Watldm, J. Mol. Biol. **13**, 238 (1965).
[2] D. D. Lasic, TIBS **16**, 307 (1998).
[3] R. Lipowsky and E. Sackmann, *Structure and Dynamics of Membranes* (Elsevier Science, Amsterdam, 1995).
[4] F. Brochard, P. G. de Gennes, and P. Pfeuty, J. Phys. (Paris) **37**, 1099 (1976).
[5] C.-H. Lee, W.-C. Lin, and J. Wang, Phys. Rev. E **64**, 020901 (2001).
[6] J. R. Henriksen and J. H. Ipsen, Eur. Phys. J. E **14**, 149 (2004).
[7] N. Delorme, M. Dubois, S. Garnier, A. Laschewsky, R. Weinkamer, T. Zemb, and A. Fery, J. Phys. Chem. B **110**, 1752 (2006).
[8] C. Delajon, T. Gutberlet, R. Steitz, H. Mohwald, and R. Kratsev, Langmuir **21**, 8509 (2005).
[9] N. Delorme, J.-F. Bardeau, A. Bulou, and F. Poncin-Epaillard, Thin Solid Films **496**, 612 (2006).
[10] J. L. Hutter and J. Bechhoefer, Rev. Sci. Instrum. **64**, 1868 (1993).
[11] J. E. Sader, I. Larson, P. Mulvaney, and L. R. White, Rev. Sci. Instrum. **66**, 3789 (1995).
[12] L. D. Landau and E. M. Lifschitz, *Theory of Elasticity* (Pergamon Press, New York, 1986).
[13] E. Helfer, S. Harlepp, L. Bourdieu, J. Robert, F. C. MacKintosh, and D. Chatenay, Phys. Rev. E **63**, 021904 (2001).
[14] F. Dubreuil, N. Elsner, and A. Fery, Eur. Phys. J. E **12**, 215 (2003).
[15] J. P. Michel, I. L. Ivanovska, M. M. Gibbons, W. S. Klug, C. M. Knobler, G. J. L. Wuite, and C. F. Schmidt, Proc. Natl. Acad. Sci. U.S.A. **103**, 6184 (2006).
[16] J. Domke and M. Radmacher, Langmuir **14**, 3320 (1998).
[17] E. Reissner, J. Math. Phys. (Cambridge, Mass.) **31**, 109 (1952).
[18] D. E. Laney, R. A. Garcia, S. M. Parsons, and H. G. Hansma, Biophys. J. **72**, 806 (1997).
[19] R. Dimova, B. Pouligny, and C. Dietrich, Biophys. J. **79**, 340 (2000).
[20] J. Daillant, E. Bellet-Amalric, A. Braslau, T. Charitat, G. Fragneto, F. Graner, S. Mora, F. Rieutord, and B. Stidder, Proc. Natl. Acad. Sci. U.S.A. **102**, 11639 (2005).
[21] X. Liang, G. Mao, and K. Y. S. Ng, Colloids Surf., B **34**, 41 (2004).