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Recent developments in the field of bending rigidity measurements on membranes

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ABSTRACT

This review gives a brief overview of experimental approaches used to assess the bending rigidity of membranes. Emphasis is placed on techniques based on the use of giant unilamellar vesicles. We summarize the effect on the bending rigidity of membranes as a function of membrane composition, presence of various inclusions in the bilayer and molecules and ions in the bathing solutions. Examples for the impact of temperature, cholesterol, some peptides and proteins, sugars and salts are provided and the literature data are discussed critically. Future directions, open questions and possible developments in this research field are also included.

Contents

1. Introduction

During their course of life, cells and some (parts of) cellular organelles undergo orchestrated and sometimes dramatic morphological changes involving the crucial participation of the cell membrane. Examples of such processes are clearly provided by cell division, neuron growing, autophagy, endocytosis, morphological transitions in the Golgi body and the endoplasmic reticulum. The increased interest towards understanding membrane shapes and morphological transitions occurring during these processes requires detailed knowledge of the membrane elastic properties.

Biological membranes possess a peculiar combination of elastic properties, namely incompressibility and very low bending rigidity. Since the pioneering work of Helfrich [1,2], this combination of characteristics has been the reason for initiating a significant number of studies and has kept the interest of membrane biophysicists focused on finding ways to precisely evaluate the bilayer elastic properties. These efforts have yielded a considerable amount of data collected on lipid bilayer systems, see e.g. Refs. [3–6] and the references therein. In the current review, we will attempt to provide a thorough overview of the available methods and will summarize some general trends in the dependence of the bending rigidity on various factors. However, because

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of the increasingly wide expansion of the work in the field observed in recent years, it is probably unavoidable that there will be unintentionally omitted studies.

In the following sections we will shortly introduce some of the experimental approaches developed for the assessment of the membrane bending rigidity. We will then discuss a few examples of how certain compounds present in the membrane or in the bathing medium can affect the elasticity of the bilayer.

2. Methods for measuring the bending rigidity

This section gives a brief description of several methods developed for the assessment of the membrane bending rigidity. Not surprisingly, Helfrich's contribution has been essential for the development of some of them, starting with his introduction of the role of bending elasticity in membrane systems [1].

The experimental model systems on which these methods have been developed comprise essentially giant unilamellar vesicles and bilayer stacks, the former being significantly more popular. In general, bilayer stacks exhibit slightly different properties from those of freely suspended single bilayers. In giant unilamellar vesicles, the membrane is fully hydrated and the bilayer fluctuations are not constrained by neighboring membranes, contrary to the case of bilayer stacks where steric interactions have been recognized and evaluated already in the early studies of Helfrich [7].

The approaches for deducing the bending rigidity can be classified in the following categories: (i) methods based on the analysis of thermal fluctuations of the membrane of giant vesicles; (ii) techniques relying on measuring the force to actively bend their membrane typically employing micropipettes, optical tweezers, electric or magnetic fields, and light; (iii) approaches based on scattering techniques; and (iv) molecular dynamic simulations. Recently, Nagle published a critical discussion about the values obtained with different methods [5]. Extensive reviews summarizing experimental values obtained on membranes with different compositions and using different techniques can be found in Refs. [3,4,6]. In the following subsections, we will predominantly focus on experimental techniques applied to giant vesicles and will briefly describe the rest of the approaches. To illustrate the extent to which the data not only depends on the measuring technique, but also on the environmental conditions which we will discuss in more detail later (such as immersing medium and presence of various molecules and salts), in Table 1 we have included a summary of measurements performed on one widely used phospholipid, palmitoyloleoylphosphatidylcholine (POPC).

2.1. Fluctuation spectroscopy

The most popular method for measuring the membrane bending rigidity seems to be fluctuation analysis, which was established almost 40 years ago by Brochard and Lennon on erythrocytes [19] and by Servuss et al. on tubular vesicles [20]. Later it was extended to giant vesicles [21] and the theory refined by Helfrich [22] and Milner and Safran [23]. The analysis of shape fluctuations of membranes and vesicles is based on collecting time sequences of snapshots as obtained by optical microscopy. The thermally induced fluctuations around equilibrium form are monitored and the mean square values of shape deviations are determined. The method has been applied to tubular vesicles [20,24,25], fractions of a vesicle [26,27], prolate [28] and quasispherical vesicles [10,21,29–35].

From an experimental point of view, the fluctuation spectroscopy method is probably the least demanding because it is based on direct video microscopy observation of giant vesicles, see Fig. 1. Fluctuation

Table 1
A summary of experimental data for the bending rigidity of POPC membranes and the effect of different inclusions or environmental factors. In the column describing the medium, for measurements on giant vesicles, we have indicated cases where the solutions inside and outside are asymmetric.

Membrane composition	Temperature	Medium	Measuring technique	Bending rigidity (10^{-20} J)	Ref.
Pure POPC	Room	Doubly distilled water, 50–200 μM NaN_3, glue contaminants	Electro-deformation	2.46 ± 0.49	[8]
	24 °C	100 μM NaN_3	Electro-deformation	5.8 ± 1.2	[9]
	24 °C	100 μM NaN_3	Fluctuation analysis	3.9 ± 0.9	[9]
	25 °C	75 mM sucrose inside, 75 mM glucose outside	Fluctuation analysis	21.1 ± 0.4	[10]
	30 °C	Water	X-ray scattering	8.5	[11]
	25 °C	250 mOsm sucrose inside, 250 mOsm glucose outside	Micropipette aspiration	21.1 ± 0.4	[12]
	30 °C	100 mM NaCl, 10 mM Tris, pH 7.4, 2 mM EDTA	Fluctuation analysis	10	[13]
	22 °C	Tris/EDTA buffer	Fluctuation analysis	12.9 ± 0.4	[14]
	24 °C	Water	Fluctuation analysis	14.6	[15]
	20 °C	Water	Fluctuation analysis	15.99 ± 0.31	[16]
	20 °C	10 mM TRIS (for effects of other buffers see [16]	Fluctuation analysis	14.48 ± 0.19	[16]
	20 °C	100 mM NaCl	Fluctuation analysis	12.55 ± 0.33	[16]
	22 °C	Deuterated water	Neutron spin echo and dynamic light scattering	7.7 ± 0.8	[17]
	22 °C	Various salt solutions	Fluctuation analysis	Decrease with salt concentration	[4]
POPC with cholesterol or sterols	25 °C	250 mOsm sucrose inside, 250 mOsm glucose outside	Micropipette aspiration	Strong stiffening	[12]
POPC with cholesterol	22 °C	Deuterated water	Neutron spin echo and dynamic light scattering	Strong stiffening	[17]
POPC with lysolipids	25 °C	75 mM sucrose inside, 75 mM glucose outside	Fluctuation analysis	Decrease with lysolipid concentration	[18]
POPC with magainin	30 °C	100 mM of NaCl, 10 mM of Tris, pH 7.4, 2 mM of EDTA	Fluctuation analysis	Softening	[13]
POPC with fluorescent membrane probes	22 °C	Tris/EDTA buffer	Fluctuation analysis	Weak effect	[14]
POPC with 5 mol% triolein	24 °C	Water	Fluctuation analysis	6.6	[15]

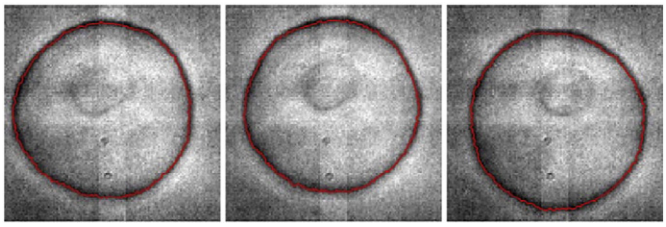

Fig. 1. Phase-contrast images of a vesicle exhibiting shape fluctuations. The detected vesicle contour is marked in red. The time lapse between the snapshots is several seconds. The vesicle diameter is approximately 25 μm. The solutions across the membrane are identical. Courtesy of Nico Fricke.

spectroscopy of giant vesicles and particularly, phase contrast observations are advantageous as an approach, because no guest molecules in the membrane (such as fluorescent dyes or deuterated molecules) are required. One of the few disadvantages of this method lies in the requirement that the vesicles should exhibit visible fluctuations implying low membrane tension. This method cannot be applied to vesicles in the gel phase.

The method has been continuously improved in the last decades [28–32,34,36–38]. Exhaustive overviews on the method development chronologically are provided in references [4,6,39]. A more recently reported version of the fluctuation spectroscopy method is based on detecting the membrane fluctuations using the so-called light sheet fluorescence microscopy [40], but this approach requires the presence of fluorescent dye (at relatively high concentration) in the membrane.

2.2. Methods based on mechanical deformation

This category of methods is probably the most diverse in terms of tools employed to apply a force to the membrane and bend it. One popular approach is the micropipette aspiration technique introduced by Evans and Needham [41–43] applied to membranes of different composition [44–51] and ubiquitously used nowadays as a manipulation technique providing a readout of the membrane tension, see e.g. [52–55]. Here, the mechanical tension generated via a suction pressure in the aspirating glass capillary, see Fig. 2, is used to measure the area stored in membrane undulations. In the low tension regime, the slope of the area dilation versus the logarithm of the tension yields the bending rigidity. One of the measured parameters is the length of the vesicle portion aspirated in the pipette. Compared to phase contrast, here the use of fluorescence is advantageous for higher precision of the measurement, see Fig. 2. Possible difficulties that one has to overcome while using this approach relate to potential adhesion of the membrane to the pipette, which sometimes can be overcome with appropriate coating.

Another method, less employed in the literature, is based on measuring the vesicle deformation induced by electric fields as introduced by Helfrich and co-workers [8,9,56]. During electrodeformation, a selected vesicle is subjected to an AC electric field of increasing strength and the induced shape deformation is recorded [8,9,35], see Fig. 3. Analogously to the micropipette aspiration method, the vesicle deformation is associated with a change in the apparent area resulting from flattening of the membrane undulations [43,57]. The tension of the deformed vesicle is obtained from the electric stresses, for detail see [35,58,59]. Typically, the conductivity conditions in these experiments are selected so that the vesicles attain prolate deformation [60–64] with elongation along the field direction, see Fig. 3. This method has not yet been applied to membranes containing charged species as the latter can exhibit inhomogeneous distribution over the vesicle surface upon application of electric field.

Measurements based on micropipette aspiration and electrodeformation yield, in general, lower values for the bending rigidity of membranes compared to those obtained with fluctuation spectroscopy, see e.g. [8,35,48,51]. The origin of this discrepancy lies in the fact that the relative area change measured via the methods with mechanical deformation may not be uniquely caused by pulling out membrane undulations, but also partially arise from stretching. The error in accessing the real surface tension in these micromechanical experiments and the associated artificial reduction in the measured bending stiffness with such methods has been theoretically treated by Henriksen and Ipsen [48] who introduced a correction factor to account for the observed discrepancy, see also Table 1. In comparing the results from different methods, one should also take into account potential effects of the solutions in which the measurements were performed. In particular, the presence of sugars in the medium (typically used to osmotically stabilize vesicles used in methods based on mechanical deformation), can lower the bending rigidity, see also Section 4.4.

Another popular technique consists of pulling a membrane tube out of a giant vesicle [65–67]. The tube is typically formed via subjecting the vesicle to fluid drag [66,68] or by exerting a force via a membrane-attached bead which sediments under gravity [65,69,70], or is manipulated with electromagnetic field [67] or optical tweezers [71]; for illustration of the last approach see Fig. 4. The tube radius can be obtained from geometrical parameters using the conservation of membrane area and total vesicle volume, see e.g. [72]. Measuring the tube radius as a function of the axial force on the tube yields the bending rigidity. The tube pulling technique receives increasing popularity in measurements on membranes decorated with proteins, see e.g. [73–77], however the analysis should be performed with caution and the tension associated with spontaneous curvature should be carefully considered [78].

A couple of other more unconventional techniques have been applied to evaluate the bending rigidity when the membrane is in the gel phase. They all demand particular experimental requirements in terms of laboratory equipment. One of them, optical dynamometry, is based on bending the membrane of a giant vesicle by displacing two membrane-attached beads away from each other by means of optical

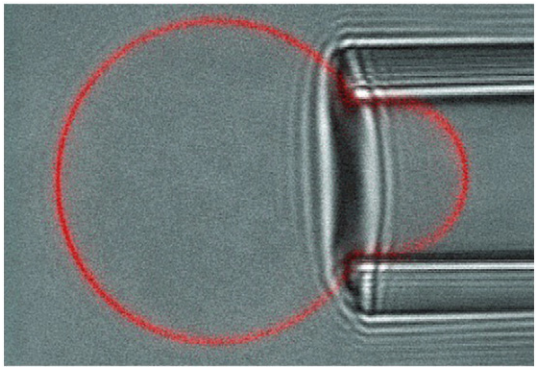

Fig. 2. Vesicle aspirated in a micropipette: an overlay of phase-contrast and confocal cross-section images. The membrane was fluorescently labeled (false red color). The inner diameter of the glass capillary is approximately 10 μm. Courtesy of Nico Fricke.

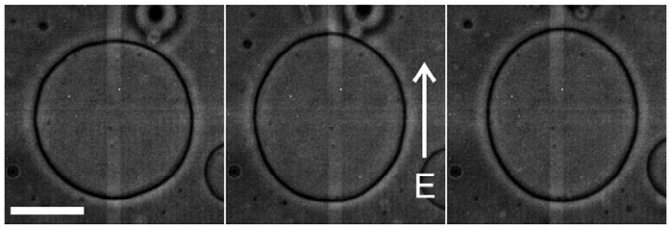

Fig. 3. Deformation of a quasispherical vesicle in electric field: phase contrast images acquired at field amplitudes 2, 10 and 20 kV/m and frequency of 300 kHz. The scale bar corresponds to 25 μm. Adapted from reference [35] (http://pubs.rsc.org/en/content/articlelanding/2010/sm/b920629a) with permission from The Royal Society of Chemistry.

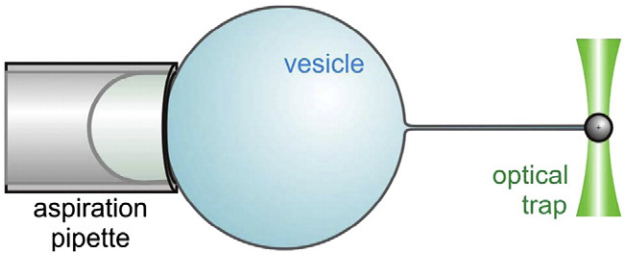

Fig. 4. Tube pulling as a method for measuring the bending rigidity of membranes. In this example, a bead manipulated via an optical tweezers is used to pull a tube out of a giant vesicle. The vesicle is held by an aspiration pipette used to measure the membrane tension. The optical trap can be employed to measure the equilibrium pulling force. The bending rigidity can be evaluated from the slope of the increase of the equilibrium tube force with the square root of the tension, see e.g. [67].

tweezers [79]. The beads have an almost point contact with the bilayer and their displacement leads to out-of-plane bending of the membrane, see Fig. 5A. Because of the point-like contact of the beads to the membrane, the applied force is not in the plane of the bilayer and thus the contribution from stretching is rather negligible. Another approach, an all-optical method, relies on pressing on a giant vesicle with an optical force and measuring the vesicle deformation under a confocal microscope using two laser beams [80,81], for schematic illustration see Fig. 5B. This technique was termed differential confocal microscopy. Yet another applied technique, called optical stretching, is based on manipulating the vesicles with laser beams from two opposing optical fibers [82,83].

2.3. Scattering techniques

The scattering techniques mentioned in the introduction of Section 2 comprise diffuse X-ray scattering (see e.g. [84–89]) on highly oriented lipid bilayer stacks, neutron and X-ray reflectivity on floating bilayers [90–93], and neutron spin echo combined with dynamic light scattering [17,94] on extruded large unilamellar vesicles. (Note that the effect of deuterated water used in some of these studies on the membrane bending rigidity has not been investigated so far, even though it is well known that the hydrogen bonding in these systems and water structure is changed [95].) An approach developed in the group of Sackmann based on the combination of flickering and scattering (dynamic reflection interference contrast microscopy) applied to cells and giant vesicles [96,97] can be also grouped in this category.

2.4. Molecular dynamics simulations

The calculation of the bending rigidity from molecular dynamics simulations, both from atomistic and coarse-grained models, has been realized by means of different approaches. The most commonly used method, introduced by Goetz et al. [98], is based on sampling the height fluctuation spectrum of a tensionless bilayer from molecular dynamics trajectories, see also [99–101]. Another approach is based on measuring the bending rigidity from a response to a force deforming the membrane [102] or from considering the energy required to deform the membrane [103]. Yet another method mimics an experimental approach relying on measuring the force necessary to hold a membrane tube and is based on simulating a tubular membrane spanning the simulation box [104].

3. Effect of the membrane phase state

It is well known that many molecular species can influence the phase state of the membrane. Temperature is however, the most direct means to modulate the lipid phase state and as a consequence affecting the bending rigidity of the membrane. The majority of the techniques described in the previous section apply to fluid membranes. For example, even though fluctuation spectroscopy is considered by many groups as the most precise technique for measuring the bending rigidity, it cannot be applied to membranes in the gel phase, and probably even to stiffer fluid membranes such as membranes in the liquid ordered phase with bending rigidity which is an order of magnitude higher than that of "typical" fluid membranes exhibiting bending rigidity around 10^{-19} J.

Fluctuation spectroscopy was however used to assess the temperature dependence of the bending rigidity of membranes approaching the main phase transition temperature, T_m, of the lipid [105]. This approach was applied to vesicles made of dimyristoylphosphatidylcholine and a decrease in the bending rigidity at temperatures close and above T_m was observed [106], see Fig. 6. This trend was later confirmed from diffuse X-ray scattering from bilayer stacks [107]. In Fig. 6, the data are presented as a function of the reduced temperature, $T - T_m$ (this way of plotting allows for comparison of data for different lipids). Later, the approach of bending gel-phase membranes by means of the method of optical dynamometry as illustrated in Fig. 5A was used to measure the bending rigidity below and close to the main phase transition temperature [79,108], see Fig. 6. The bending modulus was found to exhibit an anomalous decrease in the vicinity of T_m. This temperature

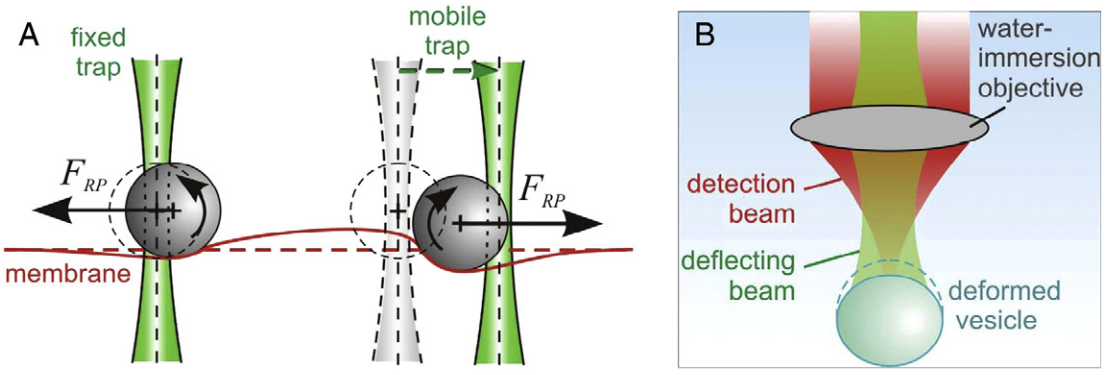

Fig. 5. Schematic presentation of two unconventional methods for measuring the bending rigidity of gel-phase membranes. (A) In the method of optical dynamometry [79], the vesicle membrane is bent by means of manipulating two beads with optical tweezers. The plane of the membrane at rest is indicated with the red dashed line and the initial position of the beads is shown with their dashed contours. The beads adhere to the membrane with a point-like contact. One trap (left) is kept fixed, while the second one (right) is displaced shifting the center of the trapped bead to the right and creating out-of-plane bending of the membrane. The bent membrane is shown with the solid red curve. The radiation pressure force exerted by the optical tweezers is indicated as F_{RP}. (B) In the all-optical method of differential confocal microscopy [80,81], an optical force (green beam) is applied to deform the vesicle, while the deformation is detected via a second more tightly focused beam (red). The bending rigidity is calculated from the deformation and the work done by the optical force.

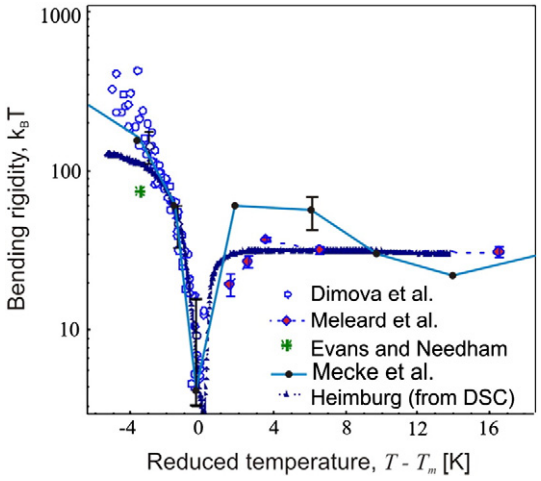

Fig. 6. Temperature dependence of the bending rigidity close to the main phase transition temperature T_m. The open circles represent data adapted from Ref. [79] using the approach of optical dynamometry as shown in Fig. 5A. The red diamonds show data adapted from Ref. [106] and obtained with fluctuation spectroscopy. The green asterisk is a single-point data form Ref. [41] collected from micropipette aspiration. The black dots and solid line represent data adapted from Ref. [109] from neutron reflectivity on floating bilayers in the vicinity of a solid support. The solid blue triangles are data calculated from the heat capacity curve of dipalmitoylphosphatidylcholine liposome suspension in Refs. [110,112].

dependence was later confirmed by another technique based on neutron reflectivity [90,109] as displayed in Fig. 6, and by differential confocal microscopy [80] (illustrated in Fig. 5B). This anomaly in the behavior of the bending rigidity was predicted on the bases of analysis of heat capacity data from suspension of small vesicles [110]. Using micropipette aspiration, the bending rigidity in the gel phase can be also deduced from measurements on the compressibility modulus [41], single data point is illustrated with an asterisk in Fig. 6.

The results from different techniques all lead to a view that the bending rigidity decreases considerably near T_m on both sides of the transition. Basically, the membrane becomes more flexible because each monolayer becomes more compressible. It is then easy to locally bend the membrane by decreasing the density of the outside leaflet and increasing that of the inside one. In the general theory by Hansen et al. [111], each monolayer is modeled as a 2D fluid close to a critical point, which is the source of the diverging compressibility.

To close this section, let us mention that an increasing amount of data is now also being reported on fluid multicomponent membranes. These bilayers can exhibit the coexistence of two fluid phases: liquid disordered and liquid ordered, see e.g. [113–117]. While the former is close to fluid single component lipid membranes in terms of bending rigidity, the latter is stiffer as shown with measurements based on fluctuation analysis and vesicle electrodeformation [35] or tube pulling [75,76].

4. Effects of additives and inclusions on the membrane bending rigidity

In this section, we will consider the effect of "inclusions" in the membrane, such as cholesterol, charged lipids, anchored polymers, peptides which insert in the membrane, proteins and species adsorbing at the membrane. Some of them can strongly modulate the bending rigidity of the membrane. Indeed, because fluid lipid membranes are so soft and flexible, in certain cases the membrane bending rigidity can be used as a direct indicator for the presence or adsorption of certain molecular species in the membrane. Data are available even on how the membrane bending rigidity can be affected by the presence of different fluorescent labels commonly used in visualizing membrane domains [14].

4.1. Cholesterol

It is a popular perception that cholesterol is unhealthy. However, this molecule is a vital component of mammalian cell membranes constituting up to about 50 mol% of the total lipid in the animal cell plasma membrane [118,119]. Then, why does the cell need so much of it? Indeed, cholesterol is a key player in stabilizing membrane domains or rafts which are believed to be responsible for the correct functioning of membrane proteins. Cholesterol also modulates the physical properties of membranes.

A widely accepted view in the 90's and the beginning of this century has been that cholesterol increases the membrane bending rigidity [12,32,43,70,106]. However, recently it has become clear that this effect is not universal but rather depends on the specific architecture of the lipid building the membrane [35,120,121]. The bending rigidity of membranes made of dioleoylphosphatidylcholine (DOPC) and cholesterol does not show any significant correlation with the cholesterol content, see Fig. 7. This observation was first reported by the group of Nagle [120–122] based on measurements of the diffuse X-ray scattering from DOPC-cholesterol bilayer stacks, and later confirmed from fluctuation analysis and electrodeformation of giant vesicles [35] (see also [53] and the supporting material of Ref. [73] where data for the bending rigidity obtained from tube pulling is reported). Furthermore, cholesterol mixtures with other lipids, such as sphingomyelin (SM) exhibit a reduction in the bending rigidity with increasing cholesterol amounts in the membrane [8,9], see Fig. 7. At room temperature, the examined SM-cholesterol mixtures are in the liquid-ordered phase, which is why they exhibit relatively higher bending rigidity. In addition, the trend with increasing cholesterol fractions is neither that of DOPC nor that of other previously studied lipids exhibiting stiffening (see also Table 1 for data on POPC).

To summarize, the effect of cholesterol is not universal, but rather specific to the type of lipid. The bending rigidity of saturated or many mono-unsaturated lipids increases with cholesterol content (see data summarized in [4]), while that of double-unsaturated lipids such as DOPC is independent of the cholesterol fraction. In sphingomyelin membranes the bending rigidity is found to decrease with increasing cholesterol content.

We cannot argue that these trends in the behavior of the different membrane types directly couple to and are universal for the specific degree of unsaturation. A more plausible view would be that the effect of cholesterol depends on the individual molecular architecture of the lipid combining the effect of unsaturation and acyl chain length, and probably the lipid interfacial region. Recent simulations indicate that tilt and splay deformations have important contribution to the overall

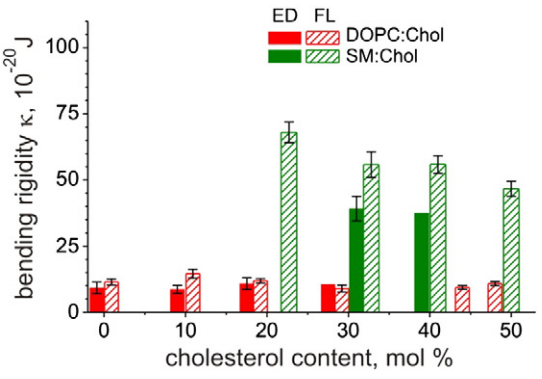

Fig. 7. Bending rigidity of cholesterol-doped DOPC and SM membranes as measured by electrodeformation (ED) of giant vesicles (solid bars) and by fluctuation spectroscopy, (FL) (hatched bars) at 23 °C. The error bars show the standard error from the mean value of a population of vesicles. Adapted from reference [35] (http://pubs.rsc.org/en/content/articlelanding/2010/sm/b920629a) with permission from The Royal Society of Chemistry.

elasticity of cholesterol-doped membranes [123]. Potential contributions from changes in the membrane thickness in the presence of cholesterol could also play a role in modulating the membrane rigidity. The experimental data confirm not only that the old widely accepted view about the rigidifying and stabilizing effect of cholesterol on membranes is ungrounded, but also that as a rule, generalization about the effect of cholesterol on the material properties of membranes is not realistic.

4.2. Charged lipids

In general, if a membrane molecular species is not electroneutral, it will contribute to the surface charge of the membrane. Increasing surface charge is associated with increasing bending rigidity. A substantial bulk of theoretical studies has addressed the effect of the surface charge on the membrane bending rigidity (including again valuable contributions from Helfrich), see e.g. [124–128]. The rise in the bending rigidity with increasing fraction of charged species is expected considering the stronger repulsion in the bilayer plane (for example between the polar heads of charged lipids), which effectively suppresses the membrane undulations and thus increases the membrane rigidity [125]. The presence of the electric double layer surrounding a charged membrane is also predicted to give rise to the bending rigidity [125,126,129].

While the theoretical modeling for the effect of membrane charge on the bending rigidity has been well documented, systematic experimental studies on giant vesicle membranes containing charged lipids are still rather limited (note that studies on the electrostatic effects on the bending rigidity in stacks of membranes are complicated because of the multitude of interactions between layers). Almost no effect has been detected in phosphatidylcholine (PC) membranes doped with phosphatidylserine (PS) when employing tube pulling [69]. A more significant change in the bending rigidity was detected on PC bilayers where the surface charge was modulated via changes in the pH [130]. A significant increase in the bending rigidity of PC/PS membranes at pH 5 as a function of the fraction of the charged lipid has been reported in Refs. [6,131,132]. Probably the strongest effect was recently observed on PC vesicles containing a fraction of phosphatidylglycerol [133], where the bending rigidity was observed to increase around threefold compared to that of the pure PC membranes. Additional data on the effect of various charged species in the membrane can be found. For example, some experimental studies have investigated the effect of charged surfactants [134] and phosphatidic acids [135] in the membrane.

4.3. Other inclusions, anchored and adsorbed species

A number of molecular species which insert or adsorb to the membrane are approximately electroneutral or charged but in the presence of salt impart only weakly to electrostatic-based changes in the bending rigidity. In this case, the bending rigidity is effectively modulated by the impact of the molecule on the membrane thickness. Example for such molecules is provided by some peptides, whose effect on the membrane rigidity strongly depends on their conformation and orientation in the bilayer and is pronounced already at small concentrations (around 1 mol%) of the peptides in the membrane. For instance, alamethicin and valinomycin are known to induce membrane thinning and thus a strong decrease in the bending rigidity [87,136–138]. Magainin was also found to dramatically soften the bilayer, which was interpreted with a continuum model taking into account the local membrane curvature induced by the peptide and the intermolecular peptide–peptide interactions [13]. Another peptide such as the HIV fusion peptide FP23, for which thinning of the membrane (on the order of 3 Å) was observed was also found to significantly soften the membrane [51,139,140], see Fig. 8. This peptide was also suggested to induce decoupling of the composing membrane leaflets as deduced from measurements on the bending and stretching elasticity moduli [51]. The decrease in the bending rigidity caused by the presence of this peptide in the bilayer

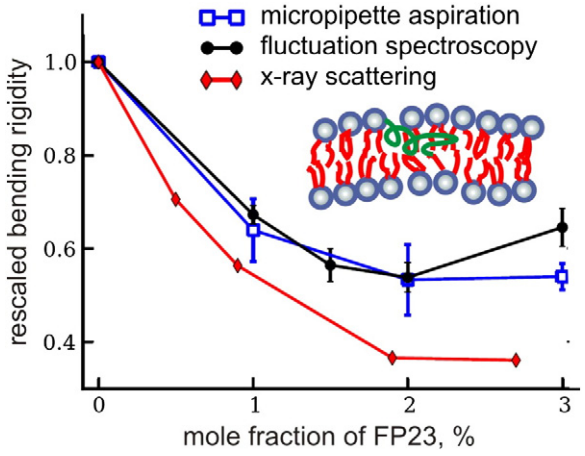

Fig. 8. Relative changes of the bending rigidity of DOPC bilayers as a function of the concentration of the transmembrane peptide FP23. The data were rescaled by the bending rigidity of the pure DOPC bilayer. Two of the datasets correspond to the results obtained with fluctuation analysis (solid circles) and micropipette aspiration (open squares) [51]. The error bars represent standard errors. The third dataset (solid diamonds) is collected from diffuse X-ray scattering from Ref. [139]. The cartoon in the inset illustrates the decoupling effect the peptide has on the bilayer. Data adapted from reference [51] with the permission of IOP Publishing Ltd.

is similar to that observed with co-surfactants [141] and theoretically expected for transmembrane inclusions, see e.g. references [142–145]. The mechanism behind the membrane softening in this case is related to coupling of the intercalated molecules to the local membrane curvature and depends strongly on the molecular geometry. Bulky transmembrane proteins such as bacteriorhodopsin do not influence the membrane rigidity [146], even though their activity can magnify the fluctuations in the membrane. Other proteins, however, can induce some mechanical effect, see e.g. [147]. More examples of molecules affecting the bending rigidity of POPC membranes are given in Table 1.

The plasma membrane of living cells possesses a large number of asymmetrically distributed or anchored biomacromolecules. Presumably, the simplest mimetic of this macromolecule/membrane architecture is provided by flexible polymers for which one end contains the membrane anchor whereas all other polymer segments experience effectively repulsive interactions with the membrane [148]. The anchored polymers can form mushroom state at low surface concentrations and brush states at high surface concentrations [149,150]. Theoretical studies have predicted the increase of the overall bending stiffness of the membrane due to the anchored polymers [150], and later this was experimentally confirmed [148]. Peripheral or adsorbed proteins can also lead to the increase of membrane bending rigidity. For example, the binding of avidin to biotinylated bilayers was shown to stiffen the membrane [151] while the formation of two-dimensional streptavidin crystals on the surface of the vesicles rigidifies them [152].

What is probably most poorly studied and remains to be addressed is the effect of molecules with sugar moieties on the bending rigidity of membranes. Glycolipids are important components in the outer leaflet of biological membranes. On the one hand, they attract increasing interest in membrane studies due to the development of new synthesis schemes and a broad range of medical applications [153]. On the other hand, they constitute a large fraction of the lipids in the myelin sheaths of human nerve cells in the brain and some of them such as the ganglioside GM1 modulate the plasticity of neurons, see e.g. [154]. The author is not familiar with reported studies evidencing the direct effect of these molecules on the membrane bending rigidity. A reasonable presumption could be that they would decrease the bending rigidity since simple sugars have been already demonstrated to lead to this effect (see next section). Indeed, some recent studies provide evidence in this direction for the ganglioside GM1 [155]. However, contrary to this expectation,

the water-soluble polysaccharide chitosan, which adsorbs to the membrane, has been recently reported to increase the bending rigidity of neutral and charged membranes [133]. Obviously, the molecular architecture of the interacting molecules is important and further studies are needed for a clear understanding of the effect of sugar-like molecules to emerge.

4.4. Sugars and salts

Here, we will consider how the bending rigidity of membranes is influenced by an important factor – sugars and salts in the solution bathing the membrane. Regarding sugars, studies in this direction are relevant for understanding the stabilizing effect of carbohydrates on membranes. A number of reports provide evidence for the stabilization effect of small sugars in particular (see e.g. Ref. [156] and references therein), which is employed in protection against drought but also in cryo- and biopreservation. The origin of this stability can be sought in the behavior of physical parameters such as the bending rigidity of lipid bilayers. The only data known to the author is provided from systematic studies of the groups of Mitov and Vitkova [6,157–160] (and supported by a single measurement on DOPC vesicles reported in Ref. [51]). Note that some of the results on the effect of sugars appear contradictory in terms of the magnitude of the effect, probably because of differences in the data analysis and the used experimental techniques. Both micropipette aspiration and fluctuation spectroscopy on giant vesicles revealed a strong decrease in the bending rigidity of membranes when exposed to mono- and oligosaccharide solutions with concentration up to around 0.3 M. The impact of sucrose is illustrated in Fig. 9 with data from Ref. [158].

The observed decrease in the bending stiffness with increasing sugar concentrations is somewhat consistent with recent studies on the interaction of sugars with membranes demonstrating nonmonotonous concentration dependence, namely adsorption at low concentrations and depletion at high concentrations [161]. In concentration ranges as those explored in Fig. 9, membrane thinning as reported in Ref. [161] could explain the reduction in the bending rigidity. At higher concentrations however, sugars are expelled from the membrane [161] and one could expect that the trend in Fig. 9 will change. Unfortunately, data are still not available.

Obviously, further studies in this direction are required, particularly because of the broad use of sugars in the preparation of giant vesicles. In order to increase the optical contrast, to osmotically stabilize the vesicles and to have them settle at the bottom of the experimental chamber for easy observation, giant vesicles are typically grown in sucrose solution and subsequently diluted in isotonic glucose solutions. (Fluctuation analysis of vesicles with inside–outside asymmetric sugar solutions were performed in Ref. [162] but only the effect on the spontaneous curvature was reported.) The majority of data provided from micropipette aspiration measurements are collected on vesicles prepared in sugar solutions which are employed to ensure constant volume of the vesicles. Compared to fluctuation analysis in the absence of sugars, micropipette aspiration was found to yield lower values for the bending rigidity at higher sugar concentrations [51].

The softening of the membrane by sugars could be expected to have strong impact on measurements where the vesicles are exposed to osmotic swelling or deflation [163,164] and this issue has not been addressed so far. Studies on "third-party" molecules performed in the presence of sugars could also be influenced, but probably not so much when relative changes in the bending rigidity are discussed. However, it is not obvious whether the presence of sugars does not affect the interaction with the membrane of the molecules of interest when the studies are performed on vesicles grown in sugar solutions. Critical discussion of measurements performed in the presence and absence of sugars is included in the recent article of Nagle [5]. Clearly, the effect of sugars should not be undermined but considered carefully. During the revision stage of this review, the author became aware of systematic studies on the bending rigidity of bilayer stacks measured with diffuse X-ray scattering in the Nagle lab [165]. They report no significant effect of glucose on the membrane mechanics which further emphasizes the need to perform more careful studies and clarify differences in the data obtained with bilayer stacks and giant vesicles.

Other important solutes to discuss are salts and ions in general, having in mind the high ionic strength of physiologically relevant solutions. The effect of salts on the bending rigidity of membranes would of course depend on whether and how strongly these ions adsorb to the membrane. "Simple" salts such as NaCl are sometimes simplemindedly considered as inert to the lipid bilayer, but there is abundant evidence for adsorption of the constituting ions to the membrane, see e.g. Ref. [166] and references therein. For the moment, a unified vision about the effect of salts on the membrane rigidity is still missing. Studies on multilayer systems indicate swelling of PC bilayers in the presence of 0.5 M NaCl, which was interpreted as resulting from rigidifying of the membrane [167]. No significant changes in the bending rigidity of multiple PC bilayers in the presence of 1 M KCl and 0.1 M KBr were observed [168]. Somewhat contradictory to these observations, measurements based on fluctuation spectroscopy of giant vesicles provide direct evidence for the decrease in the bending rigidity of membranes in the presence of various types of salts [4,169] (once again underlying the difference in the behavior of single membranes as in giant vesicles and multiple layers as in multilayered systems). Fig. 10 exemplifies the effect of NaCl on the bending rigidity of POPC membranes in the concentration range 0–0.1 M NaCl. These measurements exhibit similar trends

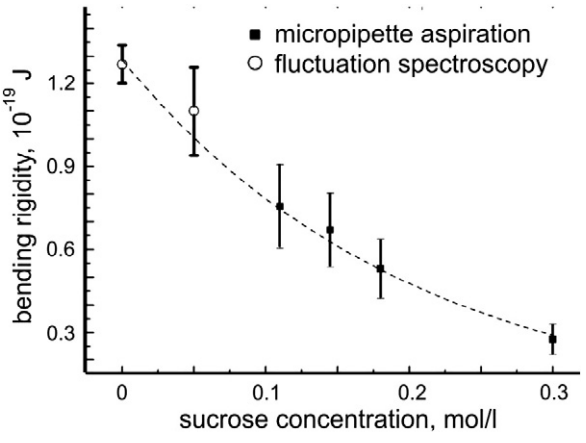

Fig. 9. Bending rigidity of stearoyloleoylphosphatidylcholine membranes as a function of sucrose concentration in the bulk: data collected via micropipette manipulation (solid squares) and fluctuation analysis (open circles) of giant vesicles. Data adapted from reference [158] with the permission of the publisher (Taylor & Francis Ltd.).

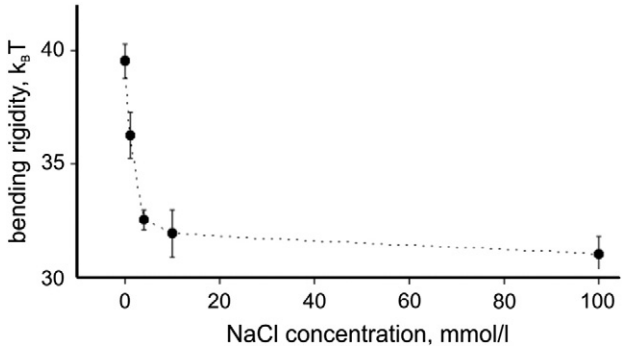

Fig. 10. Bending rigidity of POPC membrane as a function of sodium chloride concentration. Error bars represent standard deviations among a population of vesicles. The data is reproduced from Ref. [169].

as those observed in our lab [170]. Major decrease in the bending rigidity is observed already in the millimolar concentration range of various salts [169].

Probably, a word of caution should be added at the end of this section. The total lipid concentration in suspension of giant vesicles is typically in the micromolar range. Commercial salts and sugars have, in general, purity of between 98.0% and 99.8%. If one studies the effect of 0.1 M salt or 0.1 M sugar solution on the bending rigidity of giant vesicles, the concentration of the impurities might be already on the order of 0.2 mM to 2 mM, i.e., high compared to the total lipid concentration. Depending on their origin, impurities might actually exert an influence on their own. For example, impurities have been reported to change the phase state of PG membranes [171]. Effects resulting from the use of lipids from different producers also cannot be excluded. Differences in the material properties of membranes prepared from lipids purchased from two different producers have been already reported [172]. The use of plasticware in some labs may also turn to be a source of contamination [173]. Sealing agents in experimental chambers has also been recognized to influence the bending rigidity [8,9].

5. Concluding remarks

This review described very briefly the available experimental techniques for assessing the bending elasticity of lipid membranes. More emphasis was given to outlining the effect of various molecular species and ions on the membrane bending rigidity, as well as indicating some open questions demanding the attention of membrane biophysicists and requesting careful studies. On several occasions, it was demonstrated that the bending rigidity of membranes is a sensitive indicator for the presence of inclusions or adsorbing species.

What is probably still missing in the large bulk of research available in the literature is data resolving potential effects of buffering agents. A couple of studies have suggested that some buffers might indeed affect the mechanical properties of lipid membranes [174,175]. Swelling of multilayered stacks in different buffers was interpreted as resulting from potential membrane softening [174] or buffer-induced electrostatic repulsion [175]. (Indeed, during the revision stage of this review, Bouvraus et al. published a careful study demonstrating the influence of several buffering agents on the bending rigidity of membranes [16].) Studies in this direction are generally lacking in the literature and are called for, particularly nowadays when a large number of techniques for measuring the membrane rigidity have become commodity of many research groups.

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