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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]

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