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Review

Lipid diffusion in planar membranes investigated by fluorescence correlation spectroscopy

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ABSTRACT

Investigation of lipid lateral mobility in biological membranes and their artificial models provides information on membrane dynamics and structure; methods based on optical microscopy are very convenient for such investigations. We focus on fluorescence correlation spectroscopy (FCS), explain its principles and review its state of the art versions such as 2-focus, *Z*-scan or scanning FCS, which overcome most artefacts of standard FCS (especially those resulting from the need for an external calibration) making it a reliable and versatile method. FCS is also compared to single particle tracking and fluorescence photobleaching recovery and the applicability and the limitations of the methods are briefly reviewed. We discuss several key questions of lateral mobility investigation in planar lipid membranes, namely the influence which membrane and aqueous phase composition (ionic strength and sugar content), choice of a fluorescent tracer molecule, frictional coupling between the two membrane leaflets and between membrane and solid support (in the case of supported membranes) or presence of membrane inhomogeneities has on the lateral mobility of lipids. The recent FCS studies addressing those questions are reviewed and possible explanations of eventual discrepancies are mentioned.

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1. Introduction

Biological membranes are the site of a variety of vital biochemical processes in the cell; they act not only as a division between the interior and the exterior of a cell and cellular organelles, but also as an environment required for folding and activity of numerous proteins [1,2]. In spite of often a large mass fraction of proteins, a lipid bilayer is the key building block of each biological membrane forming its structural matrix and providing mechanical stability and low permeability to ions and large molecules. The fluid mosaic model of Singer and Nicolson [3] introduced the importance of lateral mobility of membrane components for kinetics and mechanisms of processes associated with membranes and, thus, inspired a vivid interest in investigation of lateral diffusion of lipids within membranes. Later studies have shown that biological membranes are not homogeneous, but contain domains, known as lipid rafts, differing in lipid and protein composition and in structural and dynamical parameters [2,4–9]. The membrane domains are considered highly dynamical structures, which are involved in signalling pathways and other cellular processes [1,10,11]; their investigation belongs, therefore, among the key topics of current membrane biology and biophysics.

Lateral diffusion coefficient of membrane lipids is one of the most important dynamical parameters of biological membranes and, as such, it is closely related to the membrane structure. Furthermore, it is accessible by a variety of experimental techniques, making it a very useful and convenient characteristic of membrane dynamics and organization. Since cellular membranes are substantially complex systems, where the lipid diffusion is influenced by rafts, proteins and interactions with cytoskeleton [12–15], various artificial model systems have been widely used to gain thorough understanding of how the presence and size of lipid domains, interaction with proteins and peptides and various other factors influence the lateral mobility of lipids. Such knowledge helps to understand better the findings of experiments in living cells and to develop theoretical models of lipid bilayers, ultimately leading to a more detailed understanding of structure and dynamics of biological membranes [16–19].

Planar lipid membranes are frequently used in studies of lateral mobility as models of cellular membranes. Two main types of planar lipid membranes are supported lipid bilayers (SLBs) [20–23] and giant unilamellar vesicles (GUVs) [24–27]. Although the latter ones are, strictly speaking, not planar, their large diameters (in order of tens of μm) and, therefore, negligible local curvatures allow determination of lateral diffusion within their membranes by the same experimental approaches which are used in the case of SLBs [28–30]. An obvious advantage of a GUV is that it represents a free-standing bilayer which is in size similar to a cell. However, their preparation protocols are rather demanding and are usually limited to conditions of low ionic strengths [24,27,31], although protocols allowing GUV preparation under physiological conditions have been also described [32]. An alternative free-standing model membrane can be prepared by spreading a bilayer over an aperture (40–150 μm) in polytetrafluoroethylene septum [33]. SLBs are formed on hydrophilic surfaces such as glass, mica, fused silica [34,35] or self-assembled alkanethiol monolayers [36,37] via adsorption and fusion of lipid vesicles [35,38,39] or via Langmuir–Blodgett and Langmuir–Schaefer techniques [22,40,41]. They are easy to prepare, stable and retain their fluidity thanks to a thin aqueous layer (in the order of nm) separating the bilayer from the support [42–44], but the proximity of the solid surface, nevertheless, influences the properties of the bilayer including lateral mobility of its constituents [45–47]. Soft polymer layers ("polymer-cushioned" bilayers) [39,48–51] or linear polymer spacers covalently coupled to lipid head groups ("polymer-tethered" bilayers) [52–55] are sometimes used to increase the distance between the solid support and the lipid bilayer. The larger distance, then, allows reconstitution of membrane proteins into the bilayer [48,49,56], but the tethered lipids may obstacle lateral mobility of its constituents [53]. Lipid bilayers deposited on micro particles of diameters in the range from a few μm to tens of μm (to mimic the size of cells) represent another alternative to SLBs [56,57]. SLBs or GUVs formed from isolated plasma membranes form an important bridge between artificial planar membranes and living cells [32,58,59]. The differences among various model systems in terms of lipid lateral diffusion will be discussed in Section 5.1. Apart from being a model of biological membranes, SLBs are also interesting for their technological applications as biocompatible surfaces for sensors, medical implants or in separation devices [20,60,61]. Biotechnological applications of SLBs represent another motivation for investigations of their dynamical properties such as lateral diffusion of lipids.

Experimental techniques for characterisation of lateral diffusion in planar lipid bilayers are typically based on optical microscopy and the three main approaches contain fluorescence correlation spectroscopy (FCS) [62–65], fluorescence recovery after photobleaching (FRAP) also known as fluorescence photobleaching recovery (FPR) [66–70] and single particle tracking (SPT) or single dye tracing (SDT) [55,71–74]. The latter two techniques were developed primarily for characterisation of mobility in membranes or microtubules [67,72,75–78]; FCS was introduced to the field very soon after its introduction in 1970s [31,79,80]. All of the above mentioned techniques can be readily used to study lateral diffusion in the membranes of living cells allowing direct comparison with model systems [13,81–88]. We will, however, not discuss the specificities of measurements on living cells. In this review, we are focusing on FCS, but we will also briefly introduce the basic principles of the other two methods (in Section 4) in order to discuss the main differences among them and the impact of those on the comparability of their results. We will review the recent progress in FCS investigations of lateral diffusion in planar lipid membranes and discuss the influence of several factors including the presence of membrane inhomogeneities, peptide and protein insertion, ionic strength or frictional coupling between the two leaflets of the bilayer.

2. Basic theory of lateral diffusion in membranes

The free Brownian lateral diffusion in planar systems is described by the Einstein relation

$$\left\langle r^2(t)\right\rangle = \left\langle (r(t)-r(0))^2\right\rangle = 4Dt, \tag{1}$$

where $\left\langle r^2(t)\right\rangle$ is the mean square displacement (MSD), D is a constant called diffusion coefficient and t is time. More precisely, we should in this sense talk about lateral self-diffusion, to distinguish this process driven by thermal fluctuations around the equilibrium from diffusion driven by concentration gradients [89,90]. Several theories have been developed to relate the phenomenological parameter D to the microscopic properties of the diffusing molecule and the membrane. In the case of planar lipid membranes two distinct cases are distinguished according to the size of the diffusing molecules with respect to the size of lipids (which are the basic building blocks of the membrane). The diffusion of molecules similar in size to lipids or smaller is usually theoretically treated using the free area theory [91–93], while the diffusion of proteins much larger than lipids can be treated as diffusion in a viscous continuum [93–95]. According to the free area theory, lipid molecules perform a two-dimensional random walk and for each step a molecule requires sufficient free area to move into and certain minimal energy (activation energy E_a) to perform the step. E_a depends on frictional coupling of the lipid with other lipid molecules, surrounding aqueous phase and, in the case of SLBs, with the solid surface [93]. A model was derived, which relates D to E_a, area per lipid at given temperature $a(T)$ and to the minimal cross-sectional area per lipid molecule a_0. Knowledge of lipid areas $a(T)$ and a_0 (obtained by other experimental methods) allows determination of E_a from temperature dependences of D. Although the free area theory is rather