Biomembranes and Lateral Nanoscale Inhomogeneities

Subjects: Cell Biology

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The nanoscale lateral inhomogeneities (nanodomains, NDs) in the lipid bilayer of cellular and model membranes can be divided into two large groups: (i) arising spontaneously and (ii) arising as a result of the influence of external factors (in relation to the components of the lipid bilayer) like other molecules (for example, peptides and proteins), changes in environmental parameters (temperature, degree of hydration, presence of ions, etc.), curvature of the membranes, etc. Undoubtedly, these processes are interrelated, since in order for a certain type of ND-based DMP to arise in the membrane under the influence of external factors, it is necessary that the undisturbed lipid bilayer itself (including water molecules and ions) is able in principle to spontaneously form such nanoscale structures.

Keywords: computer simulations ; dynamics of lipid membranes ; lipid–lipid H-bonding ; lateral heterogeneity of membrane ; mechanisms of nanodomain formation ; model biomembranes ; molecular dynamics ; mosaicity of membrane surface ; physico-chemical properties of lipid bilayers

1. Introduction

Apart from the barrier function separating contents of cells or cellular compartments from the exterior, lipid bilayer of biological membranes plays a critical role in numerous biochemical processes in the living organisms. Up to 80% of the mass in cell membranes is related to proteins, sterols, carbohydrates, and other non-lipidic components, which determine specificity and a broad spectrum of biological activities of the membranes, such as molecular and ion transport, cell communication and signaling, membrane fusion, and so on[1]. Intimate molecular details of this amazing machinery of cell membranes are far from being understood, although it has become clear that membrane lipids represent a very important “piece of the puzzle”. Instead of being a passive “sea” with polar surfaces and a hydrophobic core, where different proteins and other molecules can accomplish their functions, multicomponent lipid bilayers of cell membranes represent themselves as a highly active, dynamic, fine-tuning, and self-organizing medium [2].

Currently, it has become clear that all the roles of cell membranes mentioned above are largely due to the heterogeneity of properties of their lipid “skeleton” on different spatial and temporal scales. This is a fundamental property, which therefore requires in-depth analysis. It is obvious that the heterogeneous organization of lipid membranes is due to the physical and chemical nature of their constituent molecules, primarily amphiphilic lipids, as well as their wide diversity—apart from proteins, natural membranes contain hundreds of types of lipids and other compounds. Differences in head-groups and/or acyl chains in lipid molecules lead to a large variety of intermolecular interactions and thereby to non-ideal mixing of lipids in bilayers [3][4].

Often, when we talk about the heterogeneous nature of cell membranes, we mean their “layered” arrangement—zones with radically different physical and chemical properties are layers that alternate along the normal to the lipid bilayer plane. It is this organization of biomembranes that creates a reliable barrier that protects the contents of the cell from the external environment, ensures the correct insertion, assembling and functioning of numerous membrane proteins, membrane-active peptides, and other molecules. It is known that they are also heterogeneous in a number of key parameters—the density of membrane components (lipids, small molecules, water, ions, etc.). The properties of these surfaces largely determine the mechanisms of recognition of cell membranes and their model mimetics by external agents, such as proteins, peptides, and their supramolecular complexes, including viruses, etc.

It should also be kept in mind that such lateral inhomogeneities change over time, including equilibrium or quasi-equilibrium states (as far as they can be discussed in a living cell at all). As is shown below, the spatiotemporal scales of inhomogeneities (domains, clusters) vary in a wide range—from 1 to 10^3nm and from 0.1 ns to milliseconds. What are the properties of the membrane–water interface that exhibit inhomogeneities in their lateral distributions? The most important are: (1) their structural characteristics, expressed in terms of the density of molecules and individual atoms, as well as

describing the relief of the molecular surface of the lipid bilayer; (2) the surface distribution of their hydrophobic/hydrophilic and/or electrostatic properties; (3) dynamic parameters of the membrane components due to their lateral diffusion at different spatial scales—from integral macroscopic averages to the trajectories of individual molecules and their groups.

In contrast to three-dimensional (3D) objects, it is much more convenient and efficient to work with 2D distributions in the form of the so-called “Dynamic Molecular Portrait” (DMP) since they can be efficiently processed using numerical methods: their average characteristics and the corresponding standard deviations can be calculated; they can be subjected to digital filtering; maps of multiple states of one system or different systems can be compared, visualized, etc. Note that similar technologies for working with DMPs are also used for the analysis of non-planar biological molecular objects; in particular, globular proteins and/or their individual structural elements, for example, alpha-helices, etc. In such cases, the DMP is created by projecting the properties of the molecular surface onto the surface of a sphere (for ball-shape objects, such as globular proteins) [5] or cylinder (for rod-like objects, such as α-helix) [6] , respectively. In the context of this article, this is just a way of presenting data that is convenient to use when describing the time-dependent set of different physicochemical properties of the membrane-water interface (see above).

Given the fact that cell membranes are literally “stuffed” with proteins and other molecules [7], the study of the effects of lateral heterogeneity is very difficult due to the small areas of the “free” lipid bilayer. Therefore, such work is usually carried out on model systems that mimic the cell membrane—lipid bilayers consisting of one or more types of lipids. At the same time, for obvious reasons, smaller inhomogeneities—the so-called “nanodomains” (ND), or nanoclusters—are much less studied because of technical limitations of modern experimental methods. The characteristic size of NDs is less than 10 nm, which corresponds just to several tightly packed lipids.

There may be a feeling that due to the averaging, such ND-related phenomena are not able to significantly affect the macroscopic properties of the lipid bilayer, but this is not so at all! In particular, the role of microscopic heterogeneities in the membranes follows from the fact that the self-organization and functioning of the most important classes of membrane protein receptors, ion channels, enzymes, etc. may critically depend on the properties of the annular lipids that form one-two nearest molecular layers. Often, characteristics of the latter are very different from the “free” lipid bilayer membrane. Another example is that local (of the order of 10 nm) curvature defects on the membrane surface practically determine the binding of a number of important membrane-active peptides in these regions, affect the processes of membrane fusion, etc. Thus, it is necessary to understand the atomistic aspects of the formation and evolution of DMPs of the cell membranes. This approach is based on a detailed analysis of NDs: their identification, characterization, and delineation of the corresponding atomistic mechanisms. Some aspects of the problem are discussed in comprehensive recent reviews [8][9][10][11][12], but here, the author would like to express his thoughts on this problem based on the results of his own long-term research in this area.

2. Characteristics of Lateral Heterogeneities in Lipid Bilayers

The nanoscale lateral inhomogeneities (nanodomains, NDs) in the lipid bilayer of cellular and model membranes can be divided into two large groups: (i) arising spontaneously and (ii) arising as a result of the influence of external factors (in relation to the components of the lipid bilayer) like other molecules (for example, peptides and proteins), changes in environmental parameters (temperature, degree of hydration, presence of ions, etc.), curvature of the membranes, etc. Undoubtedly, these processes are interrelated, since in order for a certain type of ND-based DMP to arise in the membrane under the influence of external factors, it is necessary that the undisturbed lipid bilayer itself (including water molecules and ions) is able in principle to spontaneously form such nanoscale structures. Here, we review only the spontaneously formed lateral NDs and their characteristics, while the externally induced inhomogeneities, which are much more involved in the biological action of the membranes, require separate consideration.

The sections below are organized as follows: First, we discuss the available data on the detection and characterization of NDs in experiments with model lipid bilayers, and also demonstrate the possibilities of observing nanosized objects in the membranes of living cells. Then, we focus on critically examining the lessons of studying atomic-scale lateral inhomogeneities in membranes with the full arsenal of available modern tools. Finally, we turn to the results of the NDs analysis using computational approaches.

The simplest systems mimicking cellular membrane are lipid bilayers composed of different types of lipids. Studies of mixed lipid bilayers have therefore attracted considerable interest for a long time [13] . The most appropriate system allowing characterization of “microdomains” in the fluid state is a bilayer composed of two phospholipids—under such conditions, many of them demonstrate non-ideal mixing. At the same time, many more experiments have been conducted for more complex systems—lipid bilayers from a mixture of phosphatidylcholines (PC), sphingomyelin (SM), and cholesterol (Chol).

One of the first references to nanoscale molecular clusters can be found in [14]. The term has been used to refer to anomalies observed in a number of physical properties of organic liquids near the freezing point. It has been proposed that the mean molecular density within the cluster is higher than for freely dispersed molecules, and internal rotational freedom is inhibited for molecules within the cluster. The fraction of clusters present in alkanes just above the melting point was estimated as c.a. 10%, with the average cluster size being 3–4 molecules.

The first experimental indications that atomic-scale inhomogeneities are present in model lipid membranes began to be obtained in the 1970s based on the study of model lipid bilayers using X-ray [15] , ESR spin-probes [16], and small-angle neutron diffraction [17]. In these works, inhomogeneities in the acyl chain region, including areas bordering the polar heads of lipids were discussed. At the same time, it was formulated that such clusters represent "… short-lived, more densely packed arrangement of molecules within an environment of freely dispersed molecules" [16].

Important information about transiently stable but mostly dynamic NDs was obtained by a variety of biophysical methods, like Förster resonance energy transfer (FRET) analysis [18][19], interferometric scattering microscopy [20], stimulated emission depletion (STED) microscopy [21] differential scanning calorimetry (DSC) [22] examined the details of their formation by stearoyl-SMs (SSMs) using FRET measurements in lipid bilayers containing SSM and its enantiomer (ent-SSM), dioleoyl-phosphatidylcholine (DOPC), and Chol. [20] succeeded to achieve dynamic imaging of nanoscopic lipid domains without any labels. Using phase-separated droplet interface bilayers they resolved the diffusion of domains as small as 50 nm in radius and observed formation of NDs, destruction, and dynamic coalescence with a domain lifetime of 220 ± 60 ms.

For instance, consistent employment of FRET and small-angle neutron scattering (SANS) permitted to significantly narrow the uncertainty in domain size estimates for DOPC and palmitoyloleoylphosphatidylcholine (POPC) mixtures with SM/Chol [18]. The authors applied fluorescence correlation spectroscopy on planar plasmonic antenna arrays with different nanogap sizes to assess the dynamic nanoscale organization of model biological membranes. As a result, the measured diffusion data were consistent with the coexistence of transient NDs in both liquid-ordered (Lo) and liquid-disordered (Ld) microscopic phases of multicomponent lipid bilayers. Coexisting of microscale phase separation with nanoscopic domains led to suggestion that such transient assemblies in model bilayers might be similar to those occurring in living cells, which in the absence of raft-stabilizing proteins are poised to be short-lived.

In contrast to the situation with NDs existing inside larger L_o domains, routine experimental methods (e.g., optical spectroscopy) often do not reveal large domains in the water–lipid mixtures. A simple and accessible method to measure domain sizes below optical resolution (<200 nm) in distearoylphosphatidylcholine was proposed in [23]. The existence of NDs within larger L_o phase domains has been revealed by fluorescence lifetime and 2H NMR experiments [24]. Based on the results of nitroxide quenching methods and FRET experiments it was shown that NDs exist in this mixture and their radius gradually decreased from ≥15 to <4 nm as temperature increased from 10 to 45 °C. As reported in [25], single molecules partitioning into and escaping from the raft-mimetic Lo domains were directly visualized in a continuous manner with unprecedented clarity. As a result, it was possible to measure the temperature dependence of domain and ultra-nanodomain formation for vesicles composed of various mixtures containing a high-Tm lipid (SM) or dipalmitoyl phosphatidylcholine (DPPC)), low-Tm lipid (DOPC or POPC) at various concentrations of Chol (T_m is the melting temperature.). The observation that domain size is more sensitive to membrane composition than domain formation has implications for how membrane domain properties may be regulated in vivo.

In contrast to model lipid membranes, which usually consist of several types of lipids and often include Chol or its analogues, there is much less experimental work on the study of NDs in natural cell membranes. Second, natural membranes impose strict requirements for instrumental measurements—they must be non-invasive, so as not to affect the viability of cells. It was concluded that the non-invasive optical recording of molecular time traces and fluctuation data in tunable nanoscale domains is a powerful new approach to study the dynamics of biomolecules in living cells. Fluorescence burst analysis and fluorescence correlation spectroscopy performed for cell membranes on nanoantennas of different gap sizes gave similar results for SM trapped in Chol-enriched NDs [26] with characteristic size and lifetime—10 nm and 100 μs, respectively.

Although the experimentally-derived information about NDs in vivo is much less detailed than that obtained on model lipid bilayers, the proposed techniques are fully biocompatible and thus provide various new opportunities for biophysics and live cell research to reveal details that in principle cannot be probed in measurements on oversimplified lipid mixtures.

Summing up the experimental data on NDs in model and natural membranes, it should be noted that the most reliable methods for detecting nanoscale inhomogeneities are obtained either by the joint application of complementary methods, or by significant modifications of existing approaches with ultra-high resolution. As already noted, the very nature of NDs

in dynamic liquid water–lipid mixtures requires the work of experimenters on the verge of possibilities. However, in addition to the emerging technical difficulties, the current situation has a positive side, since it contributes to the rapid development of instrumental technologies, the introduction of new methods of sample preparation, and data analysis, etc.

It is important that, in addition to the already traditional systems containing SM, Chol, and a number of other components that significantly induce the spontaneous formation of NDs (and, therefore, are most convenient for conducting experiments), model membranes made of lipids that are not prone to raft formation are increasingly being studied (reviewed in [9]). Since the mosaicity parameters of the lipid/water interface can vary greatly with changing external conditions, the latter are often modified to achieve the clearest picture. Together with the above-mentioned modifications of the detection methods, this gives the desired result, improving the quality of experimental data on spontaneously formed NDs. The most important conclusion, which follows from the results of quite numerous independent experimental studies of lateral inhomogeneities in both model lipid bilayers and natural cell membranes, is that NDs actually exist. Starting from about 2004, atomistic simulations revealing NDs in different lipid bilayers have become rather common (e.g., [9][27][28]).

For a long period of time, such computational work on the properties of NDs aroused natural skepticism, given the number and nature of approximations originally inherent in the in silico methods. Therefore, right now, in connection with the experimental confirmation of the ND-related effects, there is a great opportunity not only to generalize the accumulated calculated data and correlate them with those observed in experiments, but also to start joint coordinated research on the topic under consideration. An excellent presentation of the computational work already carried out on the analysis of NDs in membranes is given in the reviews [11][29][30]. Finally, it is of great interest to analyze the changes in the DMP depending on the composition of the membranes and other factors.

Based on the results obtained using computational methods, the following conclusions can be formulated regarding the conditions of existence and spatiotemporal characteristics of NDs in model membranes:(1) Computer simulations of model membranes clearly indicate the existence of NDs on the lipid bilayer surface. Moreover, such objects were first discovered “at the tip of the pen” (i.e., in silico), and only later observed in the direct experiments described above and others. It is important that the computational data about the mechanisms of NDs formation and the influence of various factors on them (environmental conditions, etc.) are reproduced for the same systems using different calculation technologies: force fields, the level of approximations used (all-atom, united-atom, coarse-grained, etc.), computational protocols of sampling, and other modeling parameters. This indicates that the NDs are not an artifact caused by the choice of computational methods for obtaining data and processing them;(2) In computer models, it is possible to reproduce well the effect on the DMP of model lipid bilayers of such factors as temperature, the chemical nature of lipids in the membrane, a certain ionic composition, the role of the opposite monolayer, the presence of embedded “alien” objects with different parameters of mobility, etc.

3. Correspondence between Experimental and Computational Data

As indicated above, both experimental and computational methods contribute significantly to our understanding of the physical nature of lateral NDs, their dynamics, and biological impact. The joint use of these approaches makes it possible to obtain the most adequate information about NDs from independent sources. In addition, such self-consistent analysis contributes to the mutual enrichment of computer and “wet” experiments, thus ensuring rapid progress in this field. At the same time, bridging simulation and experiment is not always a simple task (see [51] for recent review).

Let’s highlight the most significant problems: (1) It is very difficult to apply exactly the same conditions for observing NDs in both approaches. This is necessary in order to compare the results of calculations with experimental data and make mutual adjustments in case of discrepancies. We are talking, in particular, about the molecular/ionic composition of the medium surrounding the lipid bilayer; observation times (in all-atom MD, they are still limited to microseconds). In addition, the calculations have difficulties with the parametrization of force fields for molecules used in the experiment, but for which there is no reliable structural data to create, for example, the topologies necessary for calculating the energy. The correct accounting of the ionization states of the molecules, including the local pH values, is a particular difficulty. Under experimental conditions, only the integral values of these parameters can be controlled (including the data obtained using molecular pH-sensors), although the charge states of individual components of the membrane can change. (2) As mentioned above, special reporter groups—probes of various nature (fluorescent, spin, etc.)—are often used in experimental studies of NDs, which can distort the finely regulated interactions in the membrane. (3) The quality of the conformational sampling in the calculations of the most complex membrane systems containing up to 10^8 particles does not guarantee the accumulation of a representative ensemble of states—with all the ensuing consequences. This is also due to the limited size of the objects available for analysis, which in many cases does not allow the calculations to correctly reproduce a number of fundamental properties of the membranes, such as the shape of the entire surface and

the roughness of different scales that occur on it (the so-called curvature effects). (4) Both experimental and computational approaches have their own errors and limitations, some of which are extremely difficult (sometimes impossible!) to control, which therefore makes it questionable direct comparison of the results. (5) Although today we are struggling to understand at the molecular level the structure of simple model membranes, modern experimental technologies go much further, allowing *in vivo* studies of the biological aspects of the functioning of more and more complex membrane systems, where the most interesting phenomena occur in the cell. This creates a significant gap between the real needs of biomedicine and the capabilities of rigorous physical methods, which are so far effective only with simple models. These include all the computer approaches used today. Therefore, many of the results of experiments on the membranes of living cells cannot yet be reproduced in the simulation.

4. Conclusions

Concluding the story about NDs and related phenomena of spontaneous lateral clustering in lipid bilayers, it should be noted that the presented DMPs are specific for each membrane system of a given composition and considered under particular conditions (temperature, pressure, degree of hydration, etc.). Visual and informative tools of mapping and visualizing the surface properties of model lipid bilayers allow quick and reliable drawing of conclusions about both the integral characteristics of the membrane (for example, the blurred or, conversely, the contrast distribution of MHP or EP), and about the nanoscale parameters, expressed, e.g., in the distributions of NDs by size, lifetime, chemical composition, types of intra-and intermolecular interactions, etc. (e.g., [31]). The uniqueness of the DMPs allows not only to rationally explain the differences in the properties of lipid bilayers (which in itself is of fundamental importance!), but also to design new membrane materials with specified properties, in particular, by varying their composition and/or external conditions.

The above-mentioned feature of the model lipid bilayers to vary the parameters of the DMPs (expressed in terms of the lateral distribution of NDs), apparently, is one of the most important fundamental properties of the membranes, since it allows them to very smoothly adjust their surface properties, reacting to external conditions. If in the case of spontaneous NDs, we are considering, we are talking about such factors as temperature, lipid composition, ion profile, etc., then a number of additional factors appear in natural membranes that strongly affect the picture of DMPs. Among them, of course, the main role is played by integral proteins already present in the membrane, peptides, proteins and other molecules binding on the surface, etc. It is known that in such processes, local areas of the membrane (primarily those in contact with such external agents) experience significant disturbances, adapting to “alien” agents (e.g.,[32][33]). The mechanisms of these processes are directly related to the formation/rearrangement of the NDs pattern. However, this is the subject of a separate consideration. Some important aspects of the biological impact of ND-related phenomena are discussed in excellent recent reviews [8][9][11][30].

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