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Closing the gap: The approach of optical and computational microscopy to uncover biomembrane organization☆

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ABSTRACT

Biological membranes are complex composites of lipids, proteins and sugars, which catalyze a myriad of vital cellular reactions in a spatiotemporal tightly controlled manner. Our understanding of the organization principles of biomembranes is limited mainly by the challenge to measure distributions and interactions of lipids and proteins within the complex environment of living cells. With the recent advent of super-resolution optical microscopy (or nanoscopy) one now has approached the molecular scale regime with non-invasive live cell fluorescence observation techniques. Since in silico molecular dynamics (MD) simulation techniques are also improving to study larger and more complex systems we can now start to integrate live-cell and in silico experiments to develop a deeper understanding of biomembranes. In this review we summarize recent progress to measure lipid-protein interactions in living cells and give examples how MD simulations can complement and upgrade the experimental data. This article is part of a Special Issue entitled: Biosimulations edited by Ilpo Vattulainen and Tomasz Róg.

1. Introduction

1.1. Membrane organization

The cellular plasma membrane is the interface between the outside environment and the internal cytoplasm of the cell (Fig. 1). In this way, it can be seen as a protective barrier of the cell. However, it is more than that, since it is critically involved in many important cellular activities and a variety of cellular functions are localized at this interface zone for example signaling and sensing, selective transport of matter, cell adhesion or electrical action potential propagation. These functions result from the spatiotemporal organization of proteins and lipids into distinct complexes. The current challenge is to unravel the molecular interactions and rearrangements in the plasma membrane, and extract the underlying organization principles to understand how cellular function emerges (for example [1–4]). High-resolution mass spectroscopy has enabled to catalog the composition of cellular membranes in great detail (lipidomics, proteomics and glycomics). Additionally, the list of (membrane)-protein structures solved by Xray-diffraction, Nuclear Magnet Resonance (NMR) or Cryo-Electron-Microscopy (EM) continues to grow. Despite this progress, we still have problems establishing the connection between composition, structure and biological function. For example, it is still largely unclear why cells require thousands of different lipid species. How are biological functions robustly controlled and organized in such complex composite structures as cellular membranes? To answer these questions new approaches are required that allow dissecting molecular interactions in live cell membranes and integrate the results into a simulation framework that ideally reconstitutes biological structures and functions in silico based on physical interactions (bottom up). This review is structured in three parts. In the first part we will introduce some of the experimental and simulation methods that have shaped our current view of membrane organization. In the second part we will explain the recent advancements in the field of optical nanoscopy, a method that allows resolving fluorescently labeled structures with near molecular resolution in an almost non-invasive manner. In the last part we will discuss some of the current frontiers of membrane research, which require a combination of experimental and simulation approaches.

1.2. Experimental methods

Many different methods have been applied to investigate or model the organization of molecules in the plasma membrane of (living) cells. We can broadly group the approaches into three categories:

1) The 'omics' approach strives to identify and quantify the molecular components of membranes, e.g. protein-, lipid-, and sugar species. For example, the vast complexity of the eukaryotic lipidome, i.e. of the lipid composition of the cellular plasma membrane as well as the differential composition of organelle membranes, has been successfully established by high-resolution mass spectrometry [5,6].

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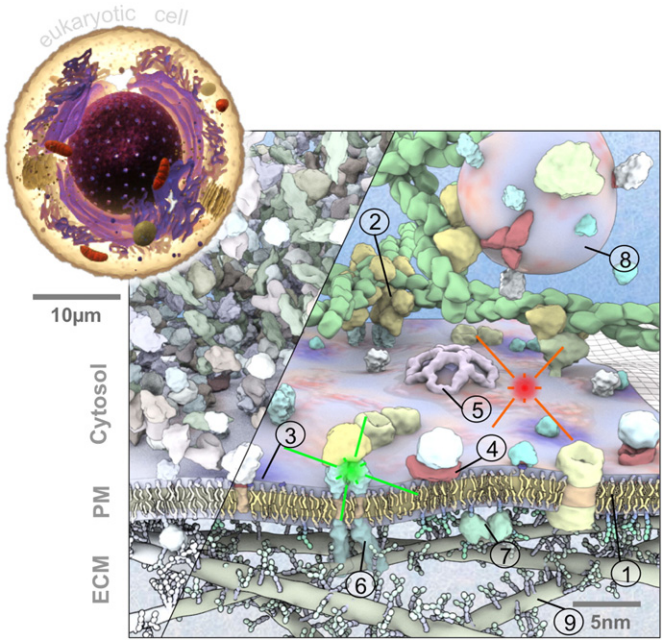

Fig. 1. Model of plasma membrane organization. Upper left: Model of eukaryotic cell with organelle membranes. Main picture: zoom in on the plasma membrane (PM) the extracellular matrix (ECM) and the cytosolic layer (Cytosol). Left section illustrates the crowding of proteins (~200 g/l) in the cytoplasm and the PM. Right side emphasizes important structures for membrane organization (1) Cholesterol and glyco-lipid enriched rafts. (2) Cell cortex membrane interactions (picket fence). (3) Clusters of poly-anionic lipids on the inner PM-leaflet. (4) Palmitoylated proteins on the inner PM-leaflet. (5) Site of endocytosis. (6) Cell adhesion receptors. (7) GPI-anchored proteins on the outer PM-leaflet. (8) Membrane turnover by vesicular transport. (9) Extracellular matrix. Figure is modified from [135].

Additionally, mass spectrometry in combination with genetic and biological tools allows creating metabolic interaction networks as well as membrane protein interactomes [7,8]. Mapping the lipid-protein interactome is not yet fully possible. However, the development of photo-cross-linkable lipid analogs is a promising direction towards this goal [9]. Overall, these tools have greatly advanced our knowledge about membrane composition and have the potential to build up an unbiased comprehensive interaction network of membrane components.

2) The structural biology approach tries to work out the ultra-structure of membranes and its components usually with tools that are incompatible with live-cell observations. Methods such as X-ray diffraction, NMR and Cryo-EM provide us with detailed insights into protein folding and conformations as well as lipid bilayer packing. Unfortunately, these methods are restricted to purified proteins or simple membrane reconstitutions or in the case of cryo-EM which requires fixation of cells (freezing). Still, these methods are indispensible for understanding membranes as they build the foundation for a molecular understanding and are the input for molecular dynamics simulations.
3) To analyze membranes in the complex environment of living cells optical far-field microscopy is usually the method of choice, since it allows observation of structures and dynamics in an almost non-invasive manner. Specificity is achieved by fluorescence labeling of the molecule of interest. Unfortunately, the full potential of far-field fluorescence microscopy is limited by constraints in spatial and temporal resolution. The temporal resolution, i.e. the speed with which an image is recorded, determines whether the movements of membrane molecules or their spatial distribution into, for example, transient clusters may be accurately captured. Yet, the acquisition speed of the detectors, such as cameras, the scanning speed in case of raster-scanning microscopes such as the confocal device, as well as the microscopes' detection efficiency and fluorescent labels' light emission are all restricted and therefore limit the temporal resolution of any far-field fluorescence microscope to milliseconds to seconds. The spatial resolution of far-field fluorescence microscopy is on the other hand limited by diffraction to about 200 nm for visible light [10]. Consequently, many cellular features such as molecular assemblies in the plasma membrane appear blurred in the microscope image, since they are of smaller size. Additionally, molecular assemblies such as complexes or clusters can sometimes not be resolved in space and time, since the involved molecules might distribute evenly between an un-clustered and clustered state, giving no contrast between the two states in the final fluorescence image. Fortunately, over the years novel tools have been developed to tackle these limitations. Spectroscopic tools such as FCS (fluorescence correlation spectroscopy) [11,12] enable the observation of molecular diffusion and interaction dynamics with down to below millisecond time resolution, and even over space if combined with fast beam-scanning or camera recordings [13–17]. Complementarily, single-particle tracking (SPT) allows disclosing those dynamics by following single isolated fluorescently-tagged molecules over space and time, with a millisecond time resolution that is only limited by the acquisition speed of the detectors (e.g. camera) (see for example [18,19]). Enforcing its capabilities, the latter limitation has for example been tackled by interferometric scattering microscopy (iSCAT) [20]. Super-resolution fluorescence microscopy approaches such as STED (stimulated emission depletion [21]), RESOLFT (reversible switchable optical light fluorescence transition [22–24]), PALM (photoactivation light microscopy [25,26]), (d)STORM ((direct) stochastic reconstruction microscopy [27,28]) or GSDIM (ground state depletion microscopy followed by individual molecule return [29]) and resolution-improved approaches such as 4-Pi/I^5M [30,31] or SIM (structured illumination microscopy [32,33]) have enabled live-cell imaging with spatial resolution below 200 nm, partially even down to the macromolecular scale (for a recent review see for example [34]). Obviously, the combination of both, i.e. FCS- and SPT-based super-resolution approaches such as STED-FCS [35,36] or PALM-SPT [37] are tremendously valuable tools for investigating molecular plasma membrane organizations [38,39].

1.3. Why we need simulations

Even though great progress has been made to measure membrane structure and dynamics with higher spatiotemporal resolution, our current understanding of the self-organization principles in biological membranes is still fragmentary. The main reasons are: 1) The best light microscopy methods are able to observe only up to three or four molecular species simultaneously and often only at sparse labeling conditions. Consequently, over 99% of the molecules are invisible in the measurement, and interactions have to be inferred indirectly from the behavior of reporter molecules. In the context of membranes there is an additional bias towards protein–protein interactions because we have no functional method to label lipids and other small molecules. 2) Measuring dynamics in membranes is key to reveal organization mechanisms. However, in fluorescence microscopy high spatial and temporal resolution are often not achievable at the same time. 3) Like all biological systems, biomembranes are multi-scale systems where function emerges at different hierarchical levels across space and time. Consequently, building a bottom up (from molecules to cells) model of cell membranes requires information over the complete spectrum (fast to slow and small to large) and its integration into a multi-scale model.

Overcoming all these limitations on the experimental level will require a major breakthrough in the way we infer information