

Visualizing biological membrane organization and dynamics

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Abstract. Biological membranes are fascinating. Santiago Ramón y Cajal, who received the Nobel prize in 1906 together with Camillo Golgi for their work on the nervous system, wrote “[...]in the study of this membrane[...] I felt more profoundly than in any other subject of study the shuddering sensation of the unfathomable mystery of life”¹. The visualization and conceptualization of these biological objects have profoundly shaped many aspects of modern biology, drawing inspiration from experiments, computer simulations, as well as from the imagination of scientists and artists. The aim of this review is to provide a fresh look on current ideas of biological membrane organization and dynamics by discussing selected examples across fields.

Keywords: Molecular visualization, Lipid bilayer.

1 Introduction

The field of biological membranes. Membranes are and have always been essential for biology, starting from their catalyzing role in the origins of life, be it by harnessing energy as ion gradients across them or by forming compartments for chemical reactions to take place, later on shaping organelles and transportation vessels. In their role as barriers they maintain concentrations, form (electrochemical) gradients used for energy conversion, ward off access of unwanted substances, enable a fine control over in- and efflux into a given compartment, and thereby complexify the delivery of drugs. Furthermore they are essential as matrices to support other (macro)molecules, in particular membrane proteins. They are able to store energy and they are intrinsically linked to signaling processes. Biological membranes have shaped important parts of biophysics, structural and molecular biology as well as molecular modeling.

¹ The full quotation is “*I must not conceal the fact that in the study of this membrane I for the first time felt my faith in Darwinism (hypothesis of natural selection) weakened, being amazed and confounded by the supreme constructive ingenuity revealed not only in the retina and in the dioptric apparatus of the vertebrates but even in the meanest insect eye. There, in fine, I felt more profoundly than in any other subject of study the shuddering sensation of the unfathomable mystery of life.*” from the autobiography *Recollections of My Life*.

A compelling image of several biological membrane structures in the neuron is depicted in Figure 1. The visualization of these objects is at the core of this review. By visualization, a term that may depend very much on the question being addressed, is meant the understanding that is gained about these objects and their properties by making them visible to our eyes and minds through abstractions, images, pictures and conceptualization. This interpretation closely follows the definition given by Ben Shneiderman: “The purpose of visualization is insight, not pictures” [1].

Early days of biological membrane investigation. A brief (and by no means complete) account on how membrane-related questions have accompanied the scientific progress over the last 50 years may start with the early visualization of bilayers and proteins in bilayers by freeze-fracture electron microscopy [2] as well as the observation of lateral diffusion of proteins in live cells [3, 4]. X-ray diffraction measurements of Engelman [5] and calorimetric studies by Reinert and Steim [6] were critical to the acceptance of the lipid bilayer as the fundamental structural element of the membrane. The fluid mosaic model of the structure of cell membranes introduced by Singer and Nicolson [7] provided a molecular vision that has deeply influenced many scientists. At about the same time, it had become clear that the association of protein complexes with membranes had functional consequences. Peter Mitchell's work [8] has significantly contributed to this realization, for instance by recognizing the importance of the electrochemical proton gradient for energy transformation. In these early days, Henderson and Unwin's pioneering investigation paved the way for profound and consequential effects on the field of membrane structure through the determination of the low-resolution electron microscopy (EM) structure of bacteriorhodopsin [9]. Early computational attempts look at the order-disorder transition in model bilayers [10].

Between 1980 and 1995, the first molecular simulations of membrane systems appeared, investigating first mono- [11], then bi-layers [12, 13] on a 10 to 100 ps time-scale, and in particular their phase behavior and the diffusion of solutes [14]. This progress made in molecular modeling enabled a straightforward atomic-level visualization of membrane systems filling an important gap with other existing techniques. During this period, in 1985, the Nobel prize in Physiology or Medicine was awarded jointly to Brown and Goldstein for their discoveries concerning the regulation of cholesterol metabolism [15, 16]. Another Nobel prize rewarded Deisenhofer, Huber and Michel for determining the three-dimensional structure of the photosynthetic reaction center [17]. This seminal work proved that the structure of membrane proteins could actually be determined, thereby providing detailed structural views of such objects which represents an important turning point in membrane biophysics. Pioneering structural studies use two- and three-dimensional electron and X-ray crystallography. At this time, the 2-stage model of membrane protein folding and oligomerization was introduced [18].

From 1995 to 1999, molecular simulations gained an order of magnitude in time-scale, from 100 ps to 1 ns, examining peptides and small membrane proteins inserted in lipid bilayers [19]. Nevertheless it became obvious that such calculations are ex-

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tremely expensive in terms of computational resources and specific methods as well as simplified models were developed to go further. For instance, in coarse grained models [20] the number of particles is typically reduced by about an order of magnitude. It became obvious that the structure determination of membrane proteins is lengthy and complicated; hence many avenues were pursued to improve this situation. In this context, lipidic cubic phases have been studied extensively [21, 22].

From 2000 to 2007, the 1 to 100 ns timescale becomes accessible and more complex phenomena are investigated, such as for instance electroporation [23] and the behavior of double bilayers. The scope of systems accessible through computational approaches has been strongly extended by the exponential development of coarse-grained simulation methods and force fields, leading to depictions of large membrane machinery and complex membrane mixtures. Membrane protein folding and insertion receive continued attention [24, 25]. High-resolution microscopy techniques and cryo-electron tomography enable to go further in understanding membrane systems at the cell level [26, 27]. More Nobel prizes concern protein structure determination by NMR, a technique later on applied to membrane proteins in a micellar environment [28, 29], and the structural elucidation of important classes of membrane proteins such as aquaporins [30] and ion channels [31, 32]. Around this time, the vision of membrane architecture based on the classical fluid mosaic model was refined to take into account the patchiness of the membrane [33], crowded by proteins, leading to segregated domains and variability in properties such as thickness and lipid composition.

For 10 years now, simulation times are within the 100 ns to 10 microsecond regime, and the complexity of systems under consideration has gone up steeply to reach multiple molecular assemblies such as membrane proteins in a trafficking organelle [34]. The attention received by these biomembrane investigations is due to several aspects. First, concerning membrane proteins, but also the ability to overcome membrane barriers, the pharmaceutical industry has major interests in these objects [35]. Membrane proteins, and ion channels in particular, are linked to many conditions such as Alzheimer, Parkinson, general anesthesia, tobacco addiction, drug resistance, bioenergetics and vision. Antimicrobial peptides [36, 37] bear importance as an alternative to antibiotics, to complement immune defenses and to fight bugs. Fundamental processes related to membranes and membrane proteins such as electroporation and the world of lipid phases are equally of interest. Some concepts continue to spawn animated discussions among scientists, such as lipid rafts [38, 39]. **Note that in this manuscript, the term raft will be used in a broad sense to denominate nanodomains in biological membranes, the existence of which seems beyond dispute.** Lipids themselves are intrinsically linked to our health, be it through cholesterol levels or conditions such as atherosclerosis, angina and infarct. Over recent years, the steep rise of the emerging field of lipidomics has led to renewed interest in biological membranes and an increasingly finer view on the role of individual lipid compositions [40-42].

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Scope of this review. In this review I will discuss how visualization contributes to shaping our mental image of membranes, membrane proteins and related processes. It is by no means an exhaustive review, but rather a personal view based on my own experience as well as exchanges with colleagues active in the broad field of biological membranes. **The aspects of visualization - in the broad sense that was introduced above - and conceptualization are central for the viewpoint adopted in this review.** The discussion will address three complementary aspects: depictions obtained from experiments, visualizations from computational methods and illustrations representing educated guesses to fill in missing data. The goal is to capture how the visualization (**again, in a rather broad sense**) of biological membranes supports and in some cases drives our understanding in the molecular biology of these objects.

A first focus concerns the **organization of biological membranes** in terms of a molecular view of these objects, hence spatial arrangements, geometry and deformations will represent important aspects. The focus may be on the lipids themselves, on protein within the bilayer or on reporter molecules. **Adding dynamics** (and hence to some extent noise) to initially static views is essential, as lipids are extremely dynamic objects with many different characteristic timescales, forming the second focus of the review. Dynamic views do not always make things clearer or easier to understand. On the other hand, many membrane properties can be described collectively, without considering the detailed dynamics of each individual lipid, and hence such an approximative **averaged picture** may be sufficient and convenient to gain insight into certain questions. A general difficulty is that much of the information obtained provides an indirect view only, for instance by following a given species in the membrane environment to better understand a biological process or mechanism. Hence the required **interpretation and extrapolation** to all pieces of the membrane puzzle is in itself a major challenge. The review attempts to highlight important ideas and advances on how visual cues are shaping scientists' understanding of biomembrane landscapes and molecular interactions. It reflects my personal view of what may be a critical overview of the biomembrane field. The **perspectives** focus on current challenges and the bottlenecks to future major advances. A few thoughts on areas that may deserve more attention from the scientific community are provided as well.

2 Experiments

2.1 Visualizing membrane structures

Experiment is a primary source of information for membrane objects and over the years the great variety of experimental approaches and their improvements extended the accessible window of observation to many levels of size, resolution and time-scales. The lipid bilayer represents a fundamental object of interest. Different views contribute to our understanding, such as a physico-chemical picture of lipids based on model membranes, phase diagrams, diffraction of bilayer stacks, etc., complemented by structural biology of isolated systems through X-ray diffraction and cryo-electron microscopy of membrane proteins. Then, AFM provides another level of detail, fol-

lowed by structural biology approaches on more or less intact systems through cryotomography and superresolution optical microscopy. At the entire cell level, the repertoire includes depictions through fluorescence and optical microscopy. In all these approaches, the resolution or underlying number of observables is a critical parameter. Static images are complemented by dynamics (or disorder) by a variety of approaches, for instance NMR. Let us start our journey with detailed information on bilayer structure, down to an atom level description, which can be gained in particular from crystallographic and atomic force microscopic (AFM) studies as illustrated in Figure 2.

Gaining detailed information on bilayer structure from experiment. The size scale spanned is about 4 orders of magnitude, in the nanometer range, between 0,2 and 800 nm. Crystallographic and neutron diffraction provided important details about lipid bilayers, going back to groundlaying neutron diffraction studies by Büldt et al. [43] and subsequent joint X-ray and neutron diffraction studies of fluid bilayer structure by Wiener and White [44, 45]. The archetypical visualization derived from such studies are scattering density profiles (Fig. 2A) that allow to measure the bilayer thickness in a direct way [44]. These early X-ray and neutron diffraction studies of bilayers were crucial in the development and testing of MD simulations, as they provide an experimental metric to compare to. Actually, such lipid bilayer profiles played a critical role in the acceptance of the bilayer hypothesis by showing the transbilayer distribution and dynamics of the lipid component groups such as phosphates and carbonyls along the bilayer. A different visualization using an electron density map can nicely depict the bigger picture of bilayer orientation in specific phases (Fig. 2B) [46]. This picture can be further refined through AFM at submolecular resolution depicting the membrane surface as shown in Fig. 2C [47]. More complex processes can be observed, such as membrane remodeling by peptides (Fig. 2D, [48]) or the formation of raft-like structures (Fig. 2E, [49]). The question how these rafts are built in terms of their thermodynamic equilibrium, their induction by raft fabric-binding proteins such as bacterial toxins and other lectins, etc. is precisely at the heart of ongoing investigations. Some influential work was performed by Kai Simons trying to understand the dynamic segregation of proteins and lipids into subdomains in the plasma membrane [50, 51]. Important contributions to the raft topic are reviewed elsewhere [52, 53]. AFM is a tool of choice for imaging the surface topography of membrane domains [54]. At the other end of the length scale, membrane objects in a cellular context have been known and visualized for a long time with stunning details.

Towards larger-scale direct observations of membrane structure. We typically jump x1000 from the nanometer to the micrometer scale, in the 1/100 to 10 micron range, to observe cellular objects constituted by membranes. Much information on the conformation of membranes can be obtained by optical light microscopy on a 10 micron scale [55]. The phenomena that have been observed include flexibility and undulations of membranes and lipid vesicle shape transformations. On a 10x smaller scale, Fig. 3A illustrates how electron-opaque stained lipids provide detailed transmission electron microscopy (TEM) pictures of various tissues, here highlighting the Golgi

apparatus and the distribution of very low density lipoprotein particles. Such staining techniques enabled early estimates of the width of lipid bilayers [56]. A particular interest has been devoted to the membrane curvature [57] and Fig. 3B to D depict chosen examples from the review by McMahon and Gallop emphasizing its key role in growth, division and movement [58]. Earlier images obtained by freeze etching provided some of the first views on such objects, albeit with artifacts due to the sample preparation (Fig. 3E, [59]). Among others, visualizations of liposomes, lipid phases and lipid rods could be obtained by this technique, at scales down to a tenth of a micron [60]. In combination with thin section electron microscopy [61], the rapid-freeze deep-etch images provide stunning details as shown in Fig. 3F for a plasma membrane with a high density of caveolae, revealing their specific coat texture. Complementary depictions of objects such as mitochondria can be obtained, for instance globally highlighting their membranes (Fig. 3G, [62]) or focusing on small particles attached to the surfaces of isolated mitochondrial membranes (Fig. 3H, [63]). With techniques such as cryo-electron microscopy of vitreous sections (CEMOVIS), one can zoom in to the very details of membrane structure in cell envelopes, for instance revealing the fact that bacteria have an outer membrane (Fig. 3I, [64]). Another helpful approach is to focus on the membrane plane using supported phospholipid bilayers and epifluorescence microscopy to depict liposomes, tubular structures and membrane defects [65].

An obvious question arises about how we can fill the space between those two extreme scales at the atomic level and the organelle one, as well as enrich the scope of objects and processes that can be addressed. Several solutions exist. Several classes of proteins may have intrinsic links with membranes and may hence act as reporters for the underlying membrane structures as illustrated in Figure 4.

Using reporter molecules to bridge scales of observation. The red blood cell has been studied for a long time, representing an emblematic membrane-based biological object. Its membrane is supported by a spectrin-actin lattice termed the membrane skeleton, which can be visualized for instance by negative staining EM (Fig. 4A, [66]). Hemagglutinin membrane distributions in relation to its possible ability to cluster in lipid rafts have been investigated by combining different approaches, for instance fluorescence photoactivation localization microscopy (FPALM), reaching subdiffraction resolution [67] with EM (Fig. 4B,C). The question of recruitment to lipid rafts has been investigated using single-molecule near-field optical microscopy (NSOM), localizing reporter molecules to approximately 3 nm accuracy in 250 nm small domains [68]. The sarcolemma is the cell membrane of a striated muscle fiber cell and represents another source of insight into biological membranes. Figure 4D shows sarcolemmal features revealed by staining the dystrophin network attached to it [69]. The eukaryotic cell membrane as well features characteristic protein connections to dense actin networks. Fluorescence correlation spectroscopy (FCS) and stimulated emission depletion (STED) experiments imaged by confocal microscopy have been used to illustrate the influence of this network on lipid phase separation [70].

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7 **A zoom on EM, X-ray and AFM approaches.** As mentioned above, EM and AFM
8 **techniques** have from their beginning been key approaches, sometimes in close link
9 to crystallography, shaping our mental images of membrane objects. Through subse-
10 quent refinement, and also by choosing specifically suitable objects, very in depth-
11 studies and visualizations could be achieved as depicted in Figure 5.

12 The purple membrane is one such particularly suited object studied extensively in
13 the literature. Early insights from electron microscopy revealed some of its features
14 with great detail, down to about 0.7 nm [9] as shown in Figure 5A and B. Crystallo-
15 graphic studies provided additional insight, also resolving the lipids surrounding the
16 membrane protein and essential water molecules for proton translocation (Fig. 5C,
17 [71]). AFM images refined the view by exposing the overall arrangement in the
18 membrane to a high precision [72] as in Figure 5D and recording conformational
19 changes, for example of surface-exposed loops [73], as in Figure 5E. These loops
20 could be further refined by a combined view from several techniques [74]. To what
21 extent X-ray crystallography provides an accurate picture of membrane lipids [75, 76]
22 has led to discussions and a critique in the scientific literature [77]. An alternative for
23 structural characterization of membrane proteins themselves is provided by NMR
24 spectroscopy [29] and can be extended to lipid-protein interactions [78]. The purple
25 membrane is not the only object suitable for in-depth characterization. Vesicle-bilayer
26 complexes have been visualized [79, 80] among the many important features on the
27 membrane surface. Figures 5F to I provide one other example on the stepwise visuali-
28 zation of membrane pore formation by a bacterial cytolysin that forms either ring- or
29 arc-shaped oligomers [81]. The time evolution of such processes can be followed.

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32 **Selected examples highlighting recent evolutions.** Our insight into such membrane
33 structures has recently significantly been heightened by the most advanced EM ap-
34 proaches. In particular single particle cryo-EM reconstructions and tomographic stud-
35 ies have significantly enriched the portfolio of available techniques to probe complex
36 and large-scale membrane structures as depicted in Figure 6.

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38 Membrane structures are ubiquitous and have diverse shapes, as for instance illustrat-
39 ed by the SEM image of vesicles at a nerve ending shown in Figure 6A or the various
40 membrane structures evidenced in the colored micrograph of Figure 1. Using more
41 recent cryo-EM approaches, through the so-called resolution revolution [82], even
42 more detailed depictions can be obtained. Specific interactions may be highlighted,
43 such as between liposomes and amyloid fibrils [83] in Figure 6B, the end binding of
44 actin filaments to the plasma membrane [26] in Figure 6C, or between SNARE pro-
45 teins and lipid bilayer nanodiscs [84]. Intermediate stages of important biological
46 processes such as mitochondrial fusion can also be witnessed as shown in Figure 6D
47 where two mitochondrial outer membranes are connected by a mitofusin docking
48 ring, leading to the formation of a pore [85]. Tomography now enables the 3 dimen-
49 sional reconstructions of whole cells, as illustrated in Figure 6E, that can be co-
50 refined by a combination with computational approaches [27]. Many membrane struc-
51 tures are thereby revealed. In specific cases such as viruses, this technique even per-
52 mits to resolve secondary structures of (membrane) proteins within their membrane
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7 environment [86]. An interesting extension to these approaches is to combine electron
8 microscopy with other techniques. For example, *in vivo* fluorescence video micros-
9 copy has been demonstrated as a suitable technique together with EM for following the
10 morpho-functional organization of the intracellular membrane trafficking pathways
11 and monitoring structures such as transport carriers [87].
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13 2.2 Adding dynamics to the picture

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15 Membranes are intrinsically fluid and dynamic objects as already mentioned in pass-
16 ing in the previous section. Specific experimental techniques have been developed to
17 be able to visualize the temporal dynamics of membrane phenomena. A very wide-
18 spread approach is to track the movement of domains or reporter molecules in mem-
19 branes.
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21 **Tracking the movement in the membrane.** Using single dye tracing, Schuetz et al.
22 imaged the lateral motion of single fluorescence labeled lipid molecules in native cell
23 membranes on a millisecond time scale, achieving a positional accuracy of ca. 50 nm
24 (Figure 7EA, [88]). Thereby they followed lipid-specific membrane microdomains.
25 Such a spatial resolution is necessary to distinguish different cases of lipid diffusion
26 as induced by barriers such as rafts, which is illustrated in Figure 7B,C [89]. Such
27 tracking experiments can also be combined with high resolution imaging, for instance
28 enabling insight into how the actin meshwork may condition the diffusion of mem-
29 brane proteins [90] as depicted in figures 7D and E. This combination provides a view
30 on the compartmentalization and barriers inducing anomalous diffusion in a mem-
31 brane environment. High-speed single-particle tracking techniques and their influence
32 on our understanding of the plasma membrane dynamics have been reviewed in light
33 of different underlying concepts [91] and extend the accessible timescales down to
34 about 1 microseconds temporal resolution, by achieving 2 nm spatial precision [92].
35 Such techniques have enabled to observe single lipids moving about rafts [93]. How-
36 ever, maybe more important than the precise “image” produced by such approaches,
37 their statistical treatment and analysis provide further insight [94]. The tracking of
38 single molecules is not the only approach to gain temporal insights. With improve-
39 ments in the AFM technique, it has been possible to track the surface of membranes
40 in incredible detail and with continuously improving time-resolution as depicted in
41 Figure 8.
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45 **AFM-based membrane surface tracking.** Early studies investigated phenomena
46 such as drug-membrane interactions, here the interaction of the antibiotic azithromy-
47 cin with lipid domains in model biomembranes (Figure 8A, [95]). By using time-lapse
48 images over 60 minutes a progressive erosion and disappearance of the bilayer gel
49 domains were observed with a certain type of bilayer, providing evidence that the
50 perturbation of lipid domains strongly depends on the lipid nature. Figure 8B revisits
51 the sullysin example discussed previously, now explicitly adding the temporal dimen-
52 sion completing the process on a ~20 min. scale. Much higher time resolution can
53 now be achieved using high-speed approaches [96]. Figure 8C features the sequence
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of separation of two OmpF proteins implying molecular shear and rotational movements, visiting a range of interaction states fully compatible with a coarse-grained simulation model. As previously discussed, our information is not always direct in terms of observing the membrane or membrane proteins themselves, but can also be guided by reporter molecules. Such is the case in the study of membrane fission related dynamin helix changes depicted in figures 8D and E [97]. The dynamin helix acts upon a membrane tubule. Another interesting feature of AFM is to probe force-induced conformational changes as has been done by monitoring the unfolding pathways of individual bacteriorhodopsins from the purple membrane [98]. Other sources of information on dynamics exist, often applied to model membranes from supported lipid bilayers, giant unilamellar vesicles and giant plasma membrane vesicles [99, 100]. By combining several approaches, additional insight may be gained and complementary pictures emerge as in the study of the formation of liquid ordered domains combining confocal microscopy with fluorescence microscopy and AFM [101]. In some cases, the dynamics of objects can be tracked in 3 dimensions [102, 103].

The dynamic information that is obtained by these various experimental approaches, either a series of fixed images at certain time intervals, or more recently videos, depict the sequence of events, but cannot properly account for the stochastic and random aspects of processes that are typically ensemble phenomena. Therefore one must keep in mind that our (partial) observations are driven by a given kinetics and only represent certain aspects of the true biological process.

Characterizing membrane dynamics naturally leads to imaging approaches, some of which have already been touched upon.

2.3 Imaging, just imagine..

Much progress has been made in imaging and many techniques and variants thereof are available. Lyman et al. point out the general progress made in visualizing dynamics and membrane organization through new experimental approaches [104], some of which are directly related to imaging. At the core, we find fluorescence-based approaches, able to reach submicron molecular dynamics measurements [105]. This may require specific developments, such as for instance nano-antennas capable of probing single-molecule dynamics in the plasma membrane of living cells [106, 107]. Improved spatial (super)resolution of the imaging has been achieved for electron and near-field microscopy, as well as fluorescence-based approaches [108-110], which had a clear impact on the study of membrane rafts [111, 112] and caveolae [113]. The most recent advances deliver volumetric 3D image series of subcellular processes, including endocytosis and membrane dynamics [114].

One of the many fluorescence-based techniques that provide useful insight into membrane systems is fluorescence lifetime imaging microscopy (flimscopy), which can for instance be applied to liposomes [115] and living cells [116]. Time-resolved fluorescence images may provide detailed insight into phenomena such as cell membrane permeabilization and DNA/membrane interaction by electric pulses at sub-

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7 second resolution [117] or in dynamin-catalyzed membrane fission and vesicle re-
8 lease [118].
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10 **Limiting the perturbation induced by labels and further progress.** A general
11 concern with many approaches is that tags might interfere with *in vivo* function. In
12 comparison to single-particle tracking of membrane components [119], where rela-
13 tively large gold particles or antibodies are used for tracking, smaller molecular labels
14 can be used for imaging membrane regions, for instance to monitor plasma-membrane
15 proteins [120]. The smaller labels reduce the impact such particles may have on alter-
16 ing the very dynamics under study. Label-free approaches such as interferometric
17 scattering (iSCAT) microscopy [121] and coherent brightfield (COBRI) microscopy
18 [122] can completely elude the issue of probe perturbation [123]. Single dyes can be
19 detected as well in fluorescence images, enabling the imaging of single molecule
20 diffusion [124]. The combination of photoactivated localization microscopy (PALM)
21 with live-cell single-particle tracking leads to spatially resolved maps of single-
22 molecule membrane protein motions [125].
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24 Some existing techniques that lacked imaging capabilities have been extended as is
25 the case for Fluorescence correlation spectroscopy (FCS). By imaging total internal
26 reflection fluorescence cross-correlation spectroscopy (ITIR-FCCS) data, diffusion
27 phenomena in lipid membranes could be probed with good temporal (millisecond)
28 and spatial (microns) resolution [126].
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30 Another area of progress concerns the increasing repertoire of (membrane) probes,
31 some of which are capable of sensing properties of their environment such as mem-
32 brane polarity [127], orientation [128] or tension [129]. Ratiometric biosensors have
33 been developed as well [130, 131]. Alternatively, very detailed insight into physico-
34 chemical membrane properties may be obtained by combining techniques. For exam-
35 ple, by coupling laser confocal scanning microscopy to microelectrochemistry, a pH
36 profile for membrane permeation processes can be imaged [132]. Similarly, electrical
37 measurements co-recorded with second harmonic generation (SHG) micrographs lead
38 to images describing structural and dynamic variations within a single bilayer [133].
39 SHG is a powerful technique, able to probe even fine details such as water chirality
40 and environment in a membrane environment [134].
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42 Many of the experimental techniques described so far rely on reporter molecules,
43 which can typically be among the lipids or among the membrane proteins. Hence the
44 acquired data is indirect and an important aspect is what is actually "seen" by a given
45 technique compared to what remains hidden. The probes may induce a preference for
46 specific lipid phases, hence introducing some bias in the measurements.
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48 **2.4 From average properties to simulations**

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50 Many ideas and concepts, less so comprehensive pictures, can be derived from the
51 measurement of average properties [135]. The bilayer density shown in Figure 2A is a
52 typical example, lipid order parameters as measured by NMR are another. Infor-
53 mation on the lipid-protein interface was obtained early on by ESR and NMR [136-
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138]. NMR spectra thereby enable insight into bilayer properties, polar lipid headgroup features, their angle with respect to the bilayer, etc., and are sensitive to the environment, for instance the ionic force. To provide a single emblematic example of the unique role such information may play to shape our conceptual images, lipid bicelles should be mentioned, for which indirect techniques such as NMR and neutron diffraction provide measurements [139, 140], that are, however, compatible with a range of models. They develop their full scope when combined with computational approaches [141-144] that will be discussed in the next section.

3 Simulations and computational approaches

A distinctive feature of molecular simulations is that all ingredients of a simulation system are seen with high fidelity, no reporter molecules or probes are necessary to observe biological membranes in atomistic detail. Hence, molecular simulations, and in particular the molecular dynamics (MD) technique, were crucially important as the primary tool for direct membrane visualization. Their accuracy comes with the limitation of comparatively small spatial scales, typically within the tenths of a nanometer to 1000 nanometer range. This scale is appropriate to compare to above-mentioned diffraction experiments, which were crucial for validating the transbilayer distribution and dynamics in the bilayer in the early days of MD simulations, see for instance the studies described in Refs. [12, 145, 146]. Timescales are short as well, from picoseconds to tens of microseconds, although this is somewhat depending on the underlying model of representation. Both time and spatial resolutions are high, routinely on the order of tenths of nanometers and picoseconds, respectively. An intrinsic limitation is that one can only observe the molecular species that were built into the model to be simulated. Hence, building accurate starting models for molecular membrane simulations including all relevant compounds is essential.

3.1 Building biological membrane models

A famous quote by Richard Feynman states, “What I cannot create, I do not understand”. Many tools for putting together membrane-related molecular constructions exist and a few of them are discussed here as they represent an important cornerstone for our molecular understanding of these systems. Unless the computational power behind simulations is sufficient for a model to fully equilibrate, it is essential to build the best possible initial systems for the computations to produce reliable results [147, 148].

Some of the available tools for building membrane systems [149] are directly integrated into the molecular modeling workflow, for instance as extensions to the popular Visual Molecular Dynamics software [150] or as part of widely used web services such as CHARMM-GUI [151-153]. Figure 9 illustrates a few examples of membrane building tools. A central issue is the packing of the lipid bilayer [154], which has been addressed for example through shape-based approaches [155], 2D bin-packing [156],

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7 or by using coarse-grain lipid templates [157]. Another trend is to take into account
8 the full complexity of lipid compositions [158-162]. Modern tools attempt to integrate
9 both aspects of packing and lipid composition as illustrated in Figure 9A-C. For set-
10 ting up membrane proteins in their environment, automated pipelines such as the
11 MemProtMD (<http://memprotmd.bioch.ox.ac.uk>) resource exist [163, 164]. The cur-
12 vature of membrane objects offers another challenge for building models, for instance
13 in the case of vesicles. If the strain induced by such curvature is not compensated for,
14 a model may be in a metastable tense state by construction, leading to biases that
15 could for instance substantially facilitate vesicle fusion. A link between curvature and
16 packing defects exists [165, 166]. Nowadays, the ambition is to build and visualize
17 cell-scale models with astonishing accuracy [167-172], see Figure 9 D and E for ex-
18 amples, an undertaking which can be greatly helped by efficiently exploiting GPUs
19 [173]. An emblematic example where such advanced model building yielded extreme-
20 ly valuable and scientifically sound insights is the study of a trafficking synaptic vesi-
21 cle [34] (Figure 10). The model provides a detailed picture in terms of protein and
22 lipid composition, vesicle size and copy number of its major constituents by integrat-
23 ing quantitative data and structural models.
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25 Quite naturally, such models require a rich repertoire of complementary simulation
26 approaches to bridge the different time- and length-scales.
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29 3.2 Simulating biological membrane dynamics at multiple scales 30

31 The vast majority of simulation and modeling approaches described hereafter inte-
32 grate motion and provide a dynamic view of lipid bilayer-related structure and func-
33 tion. An average image can be extracted as well, for instance in relation to aspects
34 such as ionic conductance, osmotic permeability, electrostatic potential, pressure pro-
35 file, etc. [174, 175]. Most simulations discussed here rely on a force field to represent
36 the lipid bilayer [176-180]. An overwhelming number of exciting studies exist in this
37 field, yet only a tiny fraction will be alluded to in the present review. For a more
38 complete account, the reader is referred to comprehensive reviews such as [181-188].
39 **The fundamental role that MD simulations played and still play in our understanding
40 of membrane structure has to be stressed, as it cannot be discussed in detail within the
41 scope of the present review.**

42 **Challenges and limitations.** Concerning the description of the various modeling
43 scales used to represent membrane systems, we will follow the nomenclature from
44 [189], mostly discussing representations at the bead- and elastic scales. Every detail
45 of a chosen model system can be followed using molecular dynamics, hence the
46 choice of the extent of the model and of the aspects that are to be analyzed is crucial.
47 Therefore, dealing with the complexity of membrane systems in terms of their repre-
48 sented size and the multiplicity of lipid components is essential. Examples include
49 attempts to capture plasma [161], thylakoid [190] and bacterial [159, 191] mem-
50 branes. An accurate representation of the diverse glycolipids poses some modeling
51 challenges; the asymmetry in membrane leaflet composition adds to the complexity.
52 Similar issues exist for the (membrane) protein representations. For example, the
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latter often neglect post-translational modifications that play crucial biological roles as has been shown for the epidermal growth factor receptor [192]. In the spirit of the famous quote “all science is either physics or stamp collecting” by Lord Rutherford, one may point out that observing a single (or even a handful) of molecular trajectories is more akin to stamp collecting than to capturing the true molecular dynamics (“physics”) of a biological system, which is often overlooked when MD results are interpreted. **Much more extended sampling – in our experience typically tens of replicas of relevant duration (which depends on system size, but as an order of magnitude say on the order of several microseconds at least) – is needed to obtain statistically reliable and significant data.**

Atomistic molecular dynamics simulations were among the first approaches to generate insight into membrane dynamics [10, 12-14, 193-195], for instance with respect to long-range undulatory and peristaltic modes [146, 196-198]. At the same time, the case of such membrane undulations illustrates the intrinsic time- and lengthscale limits such simulations have to come up against. **Figure 11 attempts to retrace a small selection of historic landmarks in the field. Figures 11A to C depict a progression in the simulations in terms of membrane complexity. Figure 11A is among the first depictions of a simulated pure lipid bilayer.** Inclusion of peptides and proteins into the membrane simulations paved the way to study large biological machinery, starting from model systems such as the gramicidin A channel [19, 199] shown in Figure 11B. The next step is to encompass a more complete membrane environment, for instance including the cell wall structure as depicted in Figure 11C [200]. Many membrane processes have been investigated **and in addition to complexifying the membrane itself, more intricate membrane phenomena have been simulated and visualized even for pure membranes. Among these, electroporation is particularly spectacular [23, 201] (Figure 11D). More recently cavitation bubble collapse has been investigated for drug delivery [202, 203]. Fig. 11E shows another level moving towards the simulation of large functional membrane patches: a landmark study investigates a multicomplex photosynthetic membrane as it most likely appears in the cell [204] (Figure 11E). It consolidates several experimental results in an MD simulation of a large multi-protein-membrane system.**

Coarse graining of membrane systems provided a significant step forward in terms of improving sampling, spatial and temporal scales [205-209] **involving many simulation groups such as those of Essex [210], Klein [20, 211], Marrink [212], Sansom [213, 214] and Voth [215].** Observing the lipid phase behavior as illustrated in figures 12A and B is a typical application [216] and bears for example some relevance in relation to the previously mentioned hemagglutinin distribution of Figure 4C [217]. Membrane fusion is another fascinating topic of foremost importance where our ideas have been shaped **at least** in part by molecular simulations [218-221]. Membrane remodeling in general can be investigated, for instance zooming in on tethers [222] (Figure 12C). Complex membrane protein environments can be treated with fewer limitations than at the all-atom level. For instance, the *Escherichia coli* cell envelope incorporating two membranes and various native membrane proteins has

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been simulated, revealing that both membranes curve in a manner dependent on the size of the embedded proteins [223] (Figure 12D). Similar approaches enabled the simulation of enveloped viruses whose shells encompass complex compositions [224-226]. A current challenge to further optimize coarse-grained membrane models is to remove the explicit solvent [227-230], which calls for some treatment of hydrodynamics [231]. We have recently set up an implicit water membrane model capable of describing for instance hydrodynamics within a lipid vesicle nanoreactor by extending previous work with the MUPHY software in line with the concepts described in [232]. An example of a vesicle nano-reactor in its flowfield is illustrated in Figure 12F. This effort is part of a general trend to bridge coarse-grained scales with the mesoscopic scale. Further examples involve understanding the formation of large membrane protein clusters, which has been investigated by training a mesoscale model incorporating thousands of outer membrane proteins [233] on coarse-grained molecular dynamics simulations of large protein assemblies [234], as previously performed on GPCRs [235, 236] (Figure 12E). A typical target of such mesoscale simulations is to recapitulate the **protein** diffusion characteristics. Other groups have investigated lateral diffusion in membranes [237, 238].

Such **mesoscale**, as well as **ad hoc modeling approaches** enable another step towards bigger biological objects. The previously described coarse-grained bead models can be further approximated through coarser beads. A series of very coarse coarse-grained models, some qualified as shape-based, others as ultra-coarse-grained [239], have been derived in several cases, for instance for transmembrane proteins [240] and as illustrated in Figure 13B for membrane sculpting BAR domains [241, 242]. Using dissipative particle dynamics (DPD), very large membrane sections and their remodeling can be investigated, providing a step forward on topics such as the previously mentioned fusion processes [243, 244] (Figure 13A). An alternative approach, already alluded to above, is to use implicit representations, which has been described for the membrane [245].

For specific questions, ad-hoc tuned coarse-grained representations can be designed, as for instance in relation to the dynamics in the photosynthetic thylakoid membranes [206]. The phase behavior of proteins in such photosynthetic membranes is recapitulated by such a model [246]. Phase separation in mixed lipid bilayers has also been investigated by bridging CG MD simulations to a mesoscopic model based on the phenomenological Landau-Ginzberg free-energy functional [247]. The red blood cell membrane was mentioned and depicted previously (Figure 4A). It can be simulated using elastic modeling [248, 249] as depicted in Figure 13D. Such elastic representations can be used to study protein mobility [250] and can be compared to coarse-grained models [251], showing that discrepancies arise for large distances. Another approach applied to the red blood cell, in particular to reproduce its static and dynamic light scattering features, has been to model it as triangulated closed surfaces [252] (Figure 13C), an approach that can also be applied to fluctuating membranes [253]. Similar **strategies** have been used to understand lipid membrane-mediated attraction between curvature inducing objects [102, 103], in close link with experiment. Such a link is needed, and adapted modeling **techniques** may fill an important gap in

complementing the most recent experimental data on membranes, as convincingly argued in [104].

3.3 Making sense: visualization and analysis

Visualization is key to understand and analyze the complex processes in molecular membrane simulations [254]. For instance, taking the preponderant molecular dynamics technique as an example, all information, visualization and analysis are derived from the time series of positions and velocities of the atoms (or particles for coarse-grained simulations) of the simulation system. The **repertoire of visual representations** is mostly the typical one for atomistic systems, applied to the membrane constituting molecular species, with a few additional ones that will be discussed hereafter. To highlight the lipid bilayer in a non-intrusive and simplified way, one may fit a deformable sheet through selected reference atoms or positions [255] (Figure 14A), or connect reference atoms such as the phosphorus from lipid headgroups as illustrated in Figure 14B. This representation is particularly useful with large systems, eventually containing several lipid bilayers [256]. To recapitulate the dynamics of lipid flow within the membrane, a combination of path line, vector field and streamline techniques can be used [257] as illustrated in Figure 14C. Many membrane simulations are carried out at coarse-grained resolution, hence an effortless way of displaying such systems facilitates the visualization task. Molecular visualization software such as UnityMol [258] start to incorporate native import filters for coarse grained topologies. This visualization capacity was used to visualize carbon nanotube perturbations of a lipid bilayer [259] (Figure 14D). Zooming into the fine details of glycolipids, specific – possibly abstracted – representations for the sugar moieties [260] (Figure 14E) are yet to be generalized, which applies to e.g. glycans as well. It seems likely that volume rendering approaches, as already used for some experimental membrane data representations in 3D [114], should bear promise for visualizing simulations of biomembranes. Concerning the representation of membrane proteins and their properties, the particular example of the depiction of hydrophobicity is compelling. Typically it is implemented through color variations. A confusingly large number of different color scales can be found in the scientific literature, without a clear consensus. An original method combines a black-to-white gradient with animated field lines [261] and has been applied to membrane proteins [262] (Figure 14F). Another aspect concerns our ability to display large membrane systems, in line with building cell-scale models as discussed above. Hence, performance and visualization efficiency are important aspects to be considered. The Wang tiling concept has been extended to membranes for precisely this purpose [173] as shown in Figure 14G.

Visualization for analysis. Such visualizations are typically closely connected to the analysis of simulation trajectories, a vast domain. Often the 2-dimensional nature of the bilayer is exploited in these analyses, which requires defining membrane leaflets in case of deformed membranes. Voronoi diagrams and Delaunay triangulation can be used for such analysis [263] (Figure 15A), as well as grid-based approaches [264]. Some solutions are extensions to visualization software such as VMD [265],

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7 others are intended for command-line use, such as FATSLiM [266]. The latter is particularly apt to handle curved membranes, as it relies on a local calculation of membrane normals illustrated in Figure 15B. MDAnalysis is another feature-rich command-line analysis tool that has been applied to many large membrane systems featuring a LeafletFinder algorithm [267]. A particular tool was developed to focus on local rather than averaged membrane properties, including area per lipid, order parameters, curvature and bilayer thickness [255]. Lipid bilayer packing defects are shown in Figure 15C, representing intrinsically local properties that can be analyzed with a geometrical approach [166, 268]. Image-processing techniques (Figure 15D) are another source of specific membrane analysis tools, as applied to detection of lipid phases during phase separation [269]. Concerning membrane proteins, the analysis of helix geometry and its visual abstraction (Figure 15E) were proposed [270] as well as the characterization of their particular topologies (Figure 15F), notably in relation to water networks prefiguring solute transport, based on Laguerre tessellation [271].

22 23 **4 Conceptual illustrations, animations and artistic depictions of** 24 **membrane systems**

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27 **In order to conceptualize ideas about bilayer structure and fundamental principles of**
28 **biological membranes, researchers have from the early days on recognized the crucial**
29 **importance of visual representations thereof.** Historically, illustrations as in figures 16
30 A and B were abundant in early papers on membranes in order to fill the gaps between the "raw" experimental and simulation data and their interpretation, **often pre-**
31 **dating the realization of the first experimental approaches providing actual visualiza-**
32 **tions. Such illustrations** typically represent schematic views of lipid bilayers [7] or pseudo-three-dimensional representations of their phases [272]. These images were triggered by research results requiring conceptualization to convey the underlying new ideas to the scientific community, be it about membrane protein insertion [18, 273] (Figure 16C to E), hydrophobic mismatch [274] (Figure 16F), the fluid mosaic model [7, 275] (Figure 16G), diffusion within the bilayer [276] (Figure 16H) or many other ideas. **Hence this figure is not only of historical importance, but also illustrates the way in which the scientific community crystallizes ideas and concepts in this field, which is intrinsically visual.** A broad variety of membrane depictions can be found in the literature, yet many among them are based on more (or sometimes less) educated guesses. The sheer scales at which membranes operate require some mental analogies for us to fully apprehend the characteristic size and length spectrum. To aid our imagination of these dimensions, a 'perceptive scale' [277] has been proposed to facilitate the interpretation of cellular scales.

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49 **Towards communication approaches.** Subsequently the motivation for illustrations expanded as recognition of the beauty and complexity of nanoscopic molecular assemblies grew. A move from data analysis, interpretation and (research) hypothesis generation to broader education, communication and learning operated [278-280]. This evolution opened the available toolset towards software from animation, illustra-

tion and cinema [281, 282], with specific extensions for biomolecules, and applications to membranes [283] as depicted in Figure 18A. Some new tools were designed to tackle the challenges of illustrating biological complexity [284]. Such illustrations have been marked by the touch of their creators with a certain artistic license [285], with a range of famous contributors such as David Goodsell [286-290] (Figure 17B), Janet Iwasa [280] (Figure 17A), Graham Johnson [291], Gaël McGill and many more. Some general guidelines have been suggested for illustrating biomolecular structural data [292]. The same content may be depicted differently according to the targeted audience as shown in figures 18B and C. Illustrations may also serve to raise awareness and stimulate scientific discussion. Such is the case for the pneumococcal life cycle, visually reviewed through watercolor paintings (Figure 17D) with a consistent scale recapitulating currently available experimental data [293]. Hence, quite naturally, such depictions evolve over time, when new knowledge becomes available. A good example, discussed in more detail in [294], may be the animation "The Inner Life of the Cell" from 2006 by Harvard BioVisions and Xvivo (<https://www.youtube.com/watch?v=wJyUtbn0O5Y>). Since then, our awareness of the crowdedness of cellular environments has risen significantly, and is not conveyed in the initial movie. Motions as well are unlike the very agitated perpetual molecular jiggling that we are now aware of. An updated version of the animation, "Inner Life of a Cell | Protein Packing" has since been released (<https://www.youtube.com/watch?v=uHeTQLNFTgU>).

The efficiency of illustrations and animations for learning concepts such as structure and composition of the cell membrane, chemical properties of the relevant molecules, membrane barrier function and transport mechanisms has been investigated [295]. The visual language used needs to be adapted, and has to remain sufficiently accurate and complex to convey for instance the dynamic nature of binding events in a membrane [296] as illustrated in Figure 17C. Tangible models can be used, for instance to convey the solubility properties of membrane proteins [297]. Serious games [298] may offer new opportunities for learning and some already address membrane systems, such as DocMolecules (Figure 18D, <https://youtu.be/gZyneEqaWcQ>), targeting to dock a drug to the right membrane receptor or Eukaryo (Figure 18E) [299] simulating a eukaryotic cell.

5 Perspectives and conclusion

The perspectives on visualizing biological membrane organization and dynamics are both extremely encouraging and full of challenges. The first challenge, already mentioned, concerns the complexities in composition, in scales and in diversity that are starting to be addressed by experiments, simulations and illustrations. On the experimental side, many key players remain largely hidden from scrutiny, such as for instance the ubiquitous cholesterol. Finding an efficient yet non-perturbing fluorescent cholesterol analog would allow to attend to our "blindness" and work is under way in that direction. On the simulation and visualization front, our tools are not yet as mature as for proteins, DNA or even sugars, where many representations with vari-

ous degrees of abstraction exist, tuned to capture essential parts of the biology of these molecules. As has been discussed above, our repertoire for pictorial lipid representations is still rather limited. Another tendency, that is to be largely welcomed, is the convergence of the various methods, be it experimental, computational or illustrative ones, to combine and confront related information. This is fully in line with what Lyman et al. described in their "modeling manifesto" [104] for the pair of experiments and simulations.

It should, however, be noted that we are still at the level where we attempt to accurately describe structure and dynamics of membranes, and only to some limited extent their (mostly) equilibrium energetics. In the future, such a molecular view may need to embrace thermodynamics more fully, to characterize where and in what form energy is distributed in a given configuration. In addition to the molecular dynamics, it is essential to capture the dynamics of energy flow. This requires to move beyond the equilibrium picture, as life in its essence is a non-equilibrium state. Our conceptual images need to evolve from a currently rather vague picture of enthalpy associated with biological membranes to a comprehensive mental representation of energy fluctuations and flow. This major challenge may become tractable by using modern tools to render the multidimensional massive data that can nowadays be acquired intuitively explorable [300]. Virtual reality approaches, visual analytics [301] and in general advanced data display and mining technologies [302] will assist this transformation [303, 304].

To conclude, our picture of biological membranes is already very rich and detailed, with meso- to nanoscale information from a variety of complementary sources, enabling us to relate molecular structures to the biological phenomena, as well as to characterize the pertaining biophysical properties. Many of the underlying techniques have seen significant progress enabling us to probe these membrane systems in depth. These improvements enable a convergence between real (experiments), virtual (simulations) and imaginary (illustrations) views. Yet we may still miss new comprehensive representations for these systems, sometimes necessary to develop new ideas. Therefore we may still be overlooking some fundamental concepts. For other biological objects of studies, such breakthroughs were made in close relation to our pictorial or mental conceptualization. Three emblematic examples include unraveling the mystery of the alpha helix, to which Pauling contributed decisively, depicting the secondary structure of proteins by cartoon representations pioneered by Richardson, or understanding the DNA double helix based on the works by Watson, Crick and Franklin. Entire branches of biology rely on these discoveries.

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Conflict of Interest

The author declares that he has no conflict of interest.

References

- [1] Card SK, Mackinlay JD, Shneiderman B. Readings in information visualization: using vision to think: Academic Press; 1999.
- [2] Tillack TW, Marchesi VT. Demonstration of the outer surface of freeze-etched red blood cell membranes. *J Cell Biol.* 1970;45:649-53.
- [3] Frye LD, Edidin M. The rapid intermixing of cell surface antigens after formation of mouse-human heterokaryons. *J Cell Sci.* 1970;7:319-35.
- [4] Poo M-M, Cone RA. Lateral diffusion of rhodopsin in the photoreceptor membrane. *Nature.* 1974;247:438.
- [5] Engelman DM. X-ray diffraction studies of phase transitions in the membrane of *Mycoplasma laidlawii*. *J Mol Biol.* 1970;47:115-7.
- [6] Reinert JC, Steim JM. Calorimetric detection of a membrane-lipid phase transition in living cells. *Science.* 1970;168:1580-2.
- [7] Singer SJ, Nicolson GL. The fluid mosaic model of the structure of cell membranes. *Science.* 1972;175:720-31.
- [8] Mitchell P. A general theory of membrane transport from studies of bacteria. *Nature.* 1957;180:134.
- [9] Henderson R, Unwin PN. Three-dimensional model of purple membrane obtained by electron microscopy. *Nature.* 1975;257:28-32.
- [10] Cotterill R. Computer simulation of model lipid membrane dynamics. *Biochimica et Biophysica Acta (BBA)-Biomembranes.* 1976;433:264-70.
- [11] Kox A, Michels J, Wiegel F. Simulation of a lipid monolayer using molecular dynamics. *Nature.* 1980;287:317.
- [12] Heller H, Schaefer M, Schulten K. Molecular dynamics simulation of a bilayer of 200 lipids in the gel and in the liquid crystal phase. *The Journal of Physical Chemistry.* 1993;97:8343-60.
- [13] Van der Ploeg P, Berendsen H. Molecular dynamics simulation of a bilayer membrane. *The Journal of Chemical Physics.* 1982;76:3271-6.
- [14] Bassolino-Klimas D, Alper HE, Stouch TR. Solute diffusion in lipid bilayer membranes: an atomic level study by molecular dynamics simulation. *Biochemistry.* 1993;32:12624-37.
- [15] Goldstein JL, Brown MS. Binding and degradation of low density lipoproteins by cultured human fibroblasts. Comparison of cells from a normal subject and from a patient with homozygous familial hypercholesterolemia. *J Biol Chem.* 1974;249:5153-62.
- [16] Nair P. Brown and Goldstein: the cholesterol chronicles. *Proc Natl Acad Sci U S A.* 2013;110:14829-32.

- [17] Deisenhofer J, Epp O, Miki K, Huber R, Michel H. Structure of the protein subunits in the photosynthetic reaction centre of *Rhodospseudomonas viridis* at 3Å resolution. *Nature*. 1985;318:618-24.
- [18] Popot JL, Engelman DM. Membrane protein folding and oligomerization: the two-stage model. *Biochemistry*. 1990;29:4031-7.
- [19] Woolf TB, Roux B. Structure, energetics, and dynamics of lipid-protein interactions: A molecular dynamics study of the gramicidin A channel in a DMPC bilayer. *Proteins*. 1996;24:92-114.
- [20] Shelley JC, Shelley MY, Reeder RC, Bandyopadhyay S, Klein ML. A Coarse Grain Model for Phospholipid Simulations. *The Journal of Physical Chemistry B*. 2001;105:4464-70.
- [21] de Kruijff B. Biomembranes. Lipids beyond the bilayer. *Nature*. 1997;386:129-30.
- [22] Landau EM, Rosenbusch JP. Lipidic cubic phases: a novel concept for the crystallization of membrane proteins. *Proc Natl Acad Sci U S A*. 1996;93:14532-5.
- [23] Tieleman DP. The molecular basis of electroporation. *BMC Biochem*. 2004;5:10.
- [24] Bowie JU. Solving the membrane protein folding problem. *Nature*. 2005;438:581-9.
- [25] White SH, Wimley WC. Membrane protein folding and stability: physical principles. *Annu Rev Biophys Biomol Struct*. 1999;28:319-65.
- [26] Medalia O, Weber I, Frangakis AS, Nicastro D, Gerisch G, Baumeister W. Macromolecular architecture in eukaryotic cells visualized by cryoelectron tomography. *Science*. 2002;298:1209-13.
- [27] Earnest TM, Watanabe R, Stone JE, Mahamid J, Baumeister W, Villa E, et al. Challenges of Integrating Stochastic Dynamics and Cryo-Electron Tomograms in Whole-Cell Simulations. *J Phys Chem B*. 2017;121:3871-81.
- [28] Hiller S, Garces RG, Malia TJ, Orekhov VY, Colombini M, Wagner G. Solution structure of the integral human membrane protein VDAC-1 in detergent micelles. *Science*. 2008;321:1206-10.
- [29] Fernandez C, Adeishvili K, Wuthrich K. Transverse relaxation-optimized NMR spectroscopy with the outer membrane protein OmpX in dihexanoyl phosphatidylcholine micelles. *Proc Natl Acad Sci U S A*. 2001;98:2358-63.
- [30] Murata K, Mitsuoka K, Hirai T, Walz T, Agre P, Heymann JB, et al. Structural determinants of water permeation through aquaporin-1. *Nature*. 2000;407:599-605.
- [31] Jiang Y, Lee A, Chen J, Cadene M, Chait BT, MacKinnon R. The open pore conformation of potassium channels. *Nature*. 2002;417:523-6.
- [32] Jiang Y, Lee A, Chen J, Cadene M, Chait BT, MacKinnon R. Crystal structure and mechanism of a calcium-gated potassium channel. *Nature*. 2002;417:515-22.
- [33] Engelman DM. Membranes are more mosaic than fluid. *Nature*. 2005;438:578-80.
- [34] Takamori S, Holt M, Stenius K, Lemke EA, Grønborg M, Riedel D, et al. Molecular anatomy of a trafficking organelle. *Cell*. 2006;127:831-46.
- [35] Terstappen GC, Reggiani A. In silico research in drug discovery. *Trends Pharmacol Sci*. 2001;22:23-6.

- 1
2
3
4
5
6
7 [36] Bechinger B. Structure and functions of channel-forming peptides: Magainins,
8 cecropins, melittin and alamethicin. *J Membr Biol.* 1997;156:197-211.
9 [37] Bechinger B. The structure, dynamics and orientation of antimicrobial peptides
10 in membranes by multidimensional solid-state NMR spectroscopy. *Biochim Biophys*
11 *Acta-Biomembr.* 1999;1462:157-83.
12 [38] Brown DA, London E. Functions of lipid rafts in biological membranes. *Annu*
13 *Rev Cell Dev Biol.* 1998;14:111-36.
14 [39] Simons K, Toomre D. Lipid rafts and signal transduction. *Nat Rev Mol Cell*
15 *Biol.* 2000;1:31-9.
16 [40] Wenk MR. The emerging field of lipidomics. *Nat Rev Drug Discov.* 2005;4:594-
17 610.
18 [41] Shevchenko A, Simons K. Lipidomics: coming to grips with lipid diversity. *Nat*
19 *Rev Mol Cell Biol.* 2010;11:593-8.
20 [42] Quehenberger O, Armando AM, Brown AH, Milne SB, Myers DS, Merrill AH,
21 et al. Lipidomics reveals a remarkable diversity of lipids in human plasma. *J Lipid*
22 *Res.* 2010;51:3299-305.
23 [43] Buldt G, Gally HU, Seelig A, Seelig J, Zaccai G. Neutron diffraction studies on
24 selectively deuterated phospholipid bilayers. *Nature.* 1978;271:182-4.
25 [44] Wiener MC, White SH. Structure of a fluid dioleoylphosphatidylcholine bilayer
26 determined by joint refinement of X-ray and neutron diffraction data. III. Complete
27 structure. *Biophys J.* 1992;61:434-47.
28 [45] Wiener MC, White SH. Structure of a fluid dioleoylphosphatidylcholine bilayer
29 determined by joint refinement of X-ray and neutron diffraction data. II. Distribution
30 and packing of terminal methyl groups. *Biophys J.* 1992;61:428-33.
31 [46] Sun WJ, Tristram-Nagle S, Suter RM, Nagle JF. Structure of the ripple phase in
32 lecithin bilayers. *Proc Natl Acad Sci U S A.* 1996;93:7008-12.
33 [47] Egger M, Ohnesorge F, Weisenhorn AL, Heyn S, Drake B, Prater C, et al. Wet
34 lipid-protein membranes imaged at submolecular resolution by atomic force
35 microscopy. *Journal of Structural Biology.* 1990;103:89-94.
36 [48] Shaw JE, Epan RF, Hsu JC, Mo GC, Epan RM, Yip CM. Cationic peptide-
37 induced remodelling of model membranes: direct visualization by in situ atomic force
38 microscopy. *J Struct Biol.* 2008;162:121-38.
39 [49] Saslowsky DE, Lawrence J, Ren X, Brown DA, Henderson RM, Edwardson JM.
40 Placental alkaline phosphatase is efficiently targeted to rafts in supported lipid
41 bilayers. *J Biol Chem.* 2002;277:26966-70.
42 [50] van Meer G, Stelzer EH, Wijnaendts-van-Resandt RW, Simons K. Sorting of
43 sphingolipids in epithelial (Madin-Darby canine kidney) cells. *J Cell Biol.*
44 1987;105:1623-35.
45 [51] Simons K, Garoff H, Helenius A. How an animal virus gets into and out of its
46 host cell. *Sci Am.* 1982;246:58-66.
47 [52] Simons K, Sampaio JL. Membrane organization and lipid rafts. *Cold Spring*
48 *Harb Perspect Biol.* 2011;3:a004697.
49 [53] Lingwood D, Simons K. Lipid rafts as a membrane-organizing principle.
50 *Science.* 2010;327:46-50.
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

[54] Giocondi MC, Yamamoto D, Lesniewska E, Milhiet PE, Ando T, Le Grimellec C. Surface topography of membrane domains. *Biochim Biophys Acta*. 2010;1798:703-18.

[55] Lipowsky R. The conformation of membranes. *Nature*. 1991;349:475-81.

[56] Bangham AD, Horne RW. Negative Staining of Phospholipids and Their Structural Modification by Surface-Active Agents as Observed in the Electron Microscope. *J Mol Biol*. 1964;8:660-8.

[57] Bassereau P, Jin R, Baumgart T, Deserno M, Dimova R, Frolov VA, et al. The 2018 biomembrane curvature and remodeling roadmap. *Journal of Physics D: Applied Physics*. 2018;51:343001.

[58] McMahon HT, Gallop JL. Membrane curvature and mechanisms of dynamic cell membrane remodelling. *Nature*. 2005;438:590-6.

[59] Branton D. Fracture faces of frozen membranes. *Proc Natl Acad Sci U S A*. 1966;55:1048-56.

[60] Deamer DW, Leonard R, Tardieu A, Branton D. Lamellar and hexagonal lipid phases visualized by freeze-etching. *Biochim Biophys Acta*. 1970;219:47-60.

[61] Rothberg KG, Heuser JE, Donzell WC, Ying YS, Glenney JR, Anderson RG. Caveolin, a protein component of caveolae membrane coats. *Cell*. 1992;68:673-82.

[62] Angermuller S, Fahimi HD. Imidazole-buffered osmium tetroxide: an excellent stain for visualization of lipids in transmission electron microscopy. *Histochem J*. 1982;14:823-35.

[63] Deutsch K, Krause W, Rosenthal S. An electron microscopical study of isolated mitochondrial membranes treated with osmium tetroxide, potassium permanganate, and formaldehyde. *Journal of Cell Science*. 1964;3:319-23.

[64] Zuber B, Chami M, Houssin C, Dubochet J, Griffiths G, Daffe M. Direct visualization of the outer membrane of mycobacteria and corynebacteria in their native state. *J Bacteriol*. 2008;190:5672-80.

[65] Tamm LK, McConnell HM. Supported phospholipid bilayers. *Biophys J*. 1985;47:105-13.

[66] Byers TJ, Branton D. Visualization of the protein associations in the erythrocyte membrane skeleton. *Proc Natl Acad Sci U S A*. 1985;82:6153-7.

[67] Hess ST, Gould TJ, Gudheti MV, Maas SA, Mills KD, Zimmerberg J. Dynamic clustered distribution of hemagglutinin resolved at 40 nm in living cell membranes discriminates between raft theories. *Proc Natl Acad Sci U S A*. 2007;104:17370-5.

[68] van Zanten TS, Cambi A, Koopman M, Joosten B, Figdor CG, Garcia-Parajo MF. Hotspots of GPI-anchored proteins and integrin nanoclusters function as nucleation sites for cell adhesion. *Proc Natl Acad Sci U S A*. 2009;106:18557-62.

[69] Straub V, Bittner RE, Leger JJ, Voit T. Direct visualization of the dystrophin network on skeletal muscle fiber membrane. *J Cell Biol*. 1992;119:1183-91.

[70] Honigsmann A, Sadeghi S, Keller J, Hell SW, Eggeling C, Vink R. A lipid bound actin meshwork organizes liquid phase separation in model membranes. *Elife*. 2014;3:e01671.

[71] Belrhali H, Nollert P, Royant A, Menzel C, Rosenbusch JP, Landau EM, et al. Protein, lipid and water organization in bacteriorhodopsin crystals: a molecular view of the purple membrane at 1.9 Å resolution. *Structure*. 1999;7:909-17.

- 1
2
3
4
5
6
7 [72] Muller DJ, Buldt G, Engel A. Force-induced conformational change of
8 bacteriorhodopsin. *J Mol Biol.* 1995;249:239-43.
9 [73] Miles M. Scanning probe microscopy. Probing the future. *Science.*
10 1997;277:1845-7.
11 [74] Heymann JB, Muller DJ, Landau EM, Rosenbusch JP, Pebay-Peyroula E, Buldt
12 G, et al. Charting the surfaces of the purple membrane. *J Struct Biol.* 1999;128:243-9.
13 [75] Fyfe PK, McAuley KE, Roszak AW, Isaacs NW, Cogdell RJ, Jones MR. Probing
14 the interface between membrane proteins and membrane lipids by X-ray
15 crystallography. *Trends Biochem Sci.* 2001;26:106-12.
16 [76] Essen L, Siegert R, Lehmann WD, Oesterhelt D. Lipid patches in membrane
17 protein oligomers: crystal structure of the bacteriorhodopsin-lipid complex. *Proc Natl*
18 *Acad Sci U S A.* 1998;95:11673-8.
19 [77] Pebay-Peyroula E, Rosenbusch JP. High-resolution structures and dynamics of
20 membrane protein–lipid complexes: a critique. *Curr Opin Struct Biol.* 2001;11:427-
21 32.
22 [78] Watts A. Nuclear magnetic resonance methods to characterize lipid-protein
23 interactions at membrane surfaces. *J Bioenerg Biomembr.* 1987;19:625-53.
24 [79] Kumar S, Hoh JH. Direct visualization of vesicle– bilayer complexes by atomic
25 force microscopy. *Langmuir.* 2000;16:9936-40.
26 [80] Schonherr H, Johnson JM, Lenz P, Frank CW, Boxer SG. Vesicle adsorption and
27 lipid bilayer formation on glass studied by atomic force microscopy. *Langmuir.*
28 2004;20:11600-6.
29 [81] Leung C, Dudkina NV, Lukoyanova N, Hodel AW, Farabella I, Pandurangan
30 AP, et al. Stepwise visualization of membrane pore formation by sulfolysin, a bacterial
31 cholesterol-dependent cytolysin. *Elife.* 2014;3:e04247.
32 [82] Kuhlbrandt W. Biochemistry. The resolution revolution. *Science.*
33 2014;343:1443-4.
34 [83] Milanesi L, Sheynis T, Xue WF, Orlova EV, Hellewell AL, Jelinek R, et al.
35 Direct three-dimensional visualization of membrane disruption by amyloid fibrils.
36 *Proc Natl Acad Sci U S A.* 2012;109:20455-60.
37 [84] Shi L, Shen Q-T, Kiel A, Wang J, Wang H-W, Melia TJ, et al. SNARE proteins:
38 one to fuse and three to keep the nascent fusion pore open. *Science.* 2012;335:1355-9.
39 [85] Brandt T, Cavellini L, Kuhlbrandt W, Cohen MM. A mitofusin-dependent
40 docking ring complex triggers mitochondrial fusion in vitro. *Elife.* 2016;5.
41 [86] Zhang W, Chipman PR, Corver J, Johnson PR, Zhang Y, Mukhopadhyay S, et al.
42 Visualization of membrane protein domains by cryo-electron microscopy of dengue
43 virus. *Nat Struct Biol.* 2003;10:907-12.
44 [87] Mironov AA, Polishchuk RS, Luini A. Visualizing membrane traffic in vivo by
45 combined video fluorescence and 3D electron microscopy. *Trends Cell Biol.*
46 2000;10:349-53.
47 [88] Schutz GJ, Kada G, Pastushenko VP, Schindler H. Properties of lipid
48 microdomains in a muscle cell membrane visualized by single molecule microscopy.
49 *EMBO J.* 2000;19:892-901.
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

[89] Eggeling C, Ringemann C, Medda R, Schwarzmann G, Sandhoff K, Polyakova S, et al. Direct observation of the nanoscale dynamics of membrane lipids in a living cell. *Nature*. 2009;457:1159-62.

[90] Sadegh S, Higgins JL, Mannion PC, Tamkun MM, Krapf D. Plasma Membrane is Compartmentalized by a Self-Similar Cortical Actin Meshwork. *Phys Rev X*. 2017;7.

[91] Kusumi A, Nakada C, Ritchie K, Murase K, Suzuki K, Murakoshi H, et al. Paradigm shift of the plasma membrane concept from the two-dimensional continuum fluid to the partitioned fluid: high-speed single-molecule tracking of membrane molecules. *Annu Rev Biophys Biomol Struct*. 2005;34:351-78.

[92] Hsieh CL, Spindler S, Ehrig J, Sandoghdar V. Tracking single particles on supported lipid membranes: multimobility diffusion and nanoscopic confinement. *J Phys Chem B*. 2014;118:1545-54.

[93] Wu HM, Lin YH, Yen TC, Hsieh CL. Nanoscopic substructures of raft-mimetic liquid-ordered membrane domains revealed by high-speed single-particle tracking. *Sci Rep*. 2016;6:20542.

[94] Schutz GJ, Schindler H, Schmidt T. Single-molecule microscopy on model membranes reveals anomalous diffusion. *Biophys J*. 1997;73:1073-80.

[95] Berquand A, Mingeot-Leclercq MP, Dufrene YF. Real-time imaging of drug-membrane interactions by atomic force microscopy. *Biochim Biophys Acta*. 2004;1664:198-205.

[96] Casuso I, Khao J, Chami M, Paul-Gilloteaux P, Husain M, Duneau JP, et al. Characterization of the motion of membrane proteins using high-speed atomic force microscopy. *Nat Nanotechnol*. 2012;7:525-9.

[97] Colom A, Redondo-Morata L, Chiaruttini N, Roux A, Scheuring S. Dynamic remodeling of the dynamin helix during membrane constriction. *Proc Natl Acad Sci U S A*. 2017;114:5449-54.

[98] Oesterhelt F, Oesterhelt D, Pfeiffer M, Engel A, Gaub HE, Muller DJ. Unfolding pathways of individual bacteriorhodopsins. *Science*. 2000;288:143-6.

[99] Sezgin E, Schwille P. Model membrane platforms to study protein-membrane interactions. *Mol Membr Biol*. 2012;29:144-54.

[100] Veatch SL, Cicuta P, Sengupta P, Honerkamp-Smith A, Holowka D, Baird B. Critical fluctuations in plasma membrane vesicles. *ACS Chem Biol*. 2008;3:287-93.

[101] Garcia-Saez AJ, Chiantia S, Schwille P. Effect of line tension on the lateral organization of lipid membranes. *J Biol Chem*. 2007;282:33537-44.

[102] van der Wel C, Vahid A, Saric A, Idema T, Heinrich D, Kraft DJ. Lipid membrane-mediated attraction between curvature inducing objects. *Sci Rep*. 2016;6:32825.

[103] van der Wel C, Vahid A, Saric A, Idema T, Heinrich D, Kraft DJ. Erratum: Lipid membrane-mediated attraction between curvature inducing objects. *Sci Rep*. 2016;6:37382.

[104] Lyman E, Hsieh CL, Eggeling C. From Dynamics to Membrane Organization: Experimental Breakthroughs Occasion a "Modeling Manifesto". *Biophys J*. 2018;115:595-604.

- 1
2
3
4
5
6
7 [105] Wawrezynieck L, Rigneault H, Marguet D, Lenne PF. Fluorescence correlation
8 spectroscopy diffusion laws to probe the submicron cell membrane organization.
9 *Biophys J.* 2005;89:4029-42.
- 10 [106] Winkler PM, Regmi R, Flauraud V, Brugger J, Rigneault H, Wenger J, et al.
11 Optical Antenna-Based Fluorescence Correlation Spectroscopy to Probe the
12 Nanoscale Dynamics of Biological Membranes. *J Phys Chem Lett.* 2018;9:110-9.
- 13 [107] Regmi R, Winkler PM, Flauraud V, Borgman KJE, Manzo C, Brugger J, et al.
14 Planar Optical Nanoantennas Resolve Cholesterol-Dependent Nanoscale
15 Heterogeneities in the Plasma Membrane of Living Cells. *Nano Lett.* 2017;17:6295-
16 302.
- 17 [108] Mateos-Gil P, Letschert S, Doose S, Sauer M. Super-Resolution Imaging of
18 Plasma Membrane Proteins with Click Chemistry. *Front Cell Dev Biol.* 2016;4:98.
- 19 [109] Izeddin I, Specht CG, Lelek M, Darzacq X, Triller A, Zimmer C, et al. Super-
20 resolution dynamic imaging of dendritic spines using a low-affinity photoconvertible
21 actin probe. *PLoS One.* 2011;6:e15611.
- 22 [110] Nair D, Hosy E, Petersen JD, Constals A, Giannone G, Choquet D, et al. Super-
23 resolution imaging reveals that AMPA receptors inside synapses are dynamically
24 organized in nanodomains regulated by PSD95. *J Neurosci.* 2013;33:13204-24.
- 25 [111] Simons K, Gerl MJ. Revitalizing membrane rafts: new tools and insights. *Nat*
26 *Rev Mol Cell Biol.* 2010;11:688-99.
- 27 [112] Sezgin E, Levental I, Mayor S, Eggeling C. The mystery of membrane
28 organization: composition, regulation and roles of lipid rafts. *Nat Rev Mol Cell Biol.*
29 2017;18:361-74.
- 30 [113] Parton RG. Caveolae--from ultrastructure to molecular mechanisms. *Nat Rev*
31 *Mol Cell Biol.* 2003;4:162-7.
- 32 [114] Liu T-l, Upadhyayula S, Milkie DE, Singh V, Wang K, Swinburne IA, et al.
33 Observing the cell in its native state: Imaging subcellular dynamics in multicellular
34 organisms. *Science.* 2018;360:eaq1392.
- 35 [115] Oida T, Sako Y, Kusumi A. Fluorescence lifetime imaging microscopy
36 (flimscopy). Methodology development and application to studies of endosome fusion
37 in single cells. *Biophys J.* 1993;64:676-85.
- 38 [116] Margineanu A, Hotta J, Vallee RA, Van der Auweraer M, Ameloot M, Stefan
39 A, et al. Visualization of membrane rafts using a perylene monoimide derivative and
40 fluorescence lifetime imaging. *Biophys J.* 2007;93:2877-91.
- 41 [117] Phez E, Faurie C, Golzio M, Teissie J, Rols MP. New insights in the
42 visualization of membrane permeabilization and DNA/membrane interaction of cells
43 submitted to electric pulses. *Biochim Biophys Acta.* 2005;1724:248-54.
- 44 [118] Pucadyil TJ, Schmid SL. Real-time visualization of dynamin-catalyzed
45 membrane fission and vesicle release. *Cell.* 2008;135:1263-75.
- 46 [119] Saxton MJ, Jacobson K. Single-particle tracking: applications to membrane
47 dynamics. *Annu Rev Biophys Biomol Struct.* 1997;26:373-99.
- 48 [120] Di Rienzo C, Gratton E, Beltram F, Cardarelli F. Fast spatiotemporal
49 correlation spectroscopy to determine protein lateral diffusion laws in live cell
50 membranes. *Proc Natl Acad Sci U S A.* 2013;110:12307-12.
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

- [121] Hsieh C-L. Label-free, ultrasensitive, ultrahigh-speed scattering-based interferometric imaging. *Optics Communications*. 2018;422:69-74.
- [122] Huang YF, Zhuo GY, Chou CY, Lin CH, Chang W, Hsieh CL. Coherent Brightfield Microscopy Provides the Spatiotemporal Resolution To Study Early Stage Viral Infection in Live Cells. *ACS Nano*. 2017;11:2575-85.
- [123] de Wit G, Danial JS, Kukura P, Wallace MI. Dynamic label-free imaging of lipid nanodomains. *Proc Natl Acad Sci U S A*. 2015;112:12299-303.
- [124] Schmidt T, Schutz GJ, Baumgartner W, Gruber HJ, Schindler H. Imaging of single molecule diffusion. *Proc Natl Acad Sci U S A*. 1996;93:2926-9.
- [125] Manley S, Gillette JM, Patterson GH, Shroff H, Hess HF, Betzig E, et al. High-density mapping of single-molecule trajectories with photoactivated localization microscopy. *Nat Methods*. 2008;5:155-7.
- [126] Sankaran J, Manna M, Guo L, Kraut R, Wohland T. Diffusion, transport, and cell membrane organization investigated by imaging fluorescence cross-correlation spectroscopy. *Biophys J*. 2009;97:2630-9.
- [127] Parasassi T, Krasnowska EK, Bagatolli L, Gratton E. Laurdan and Prodan as polarity-sensitive fluorescent membrane probes. *journal of fluorescence*. 1998;8:365-73.
- [128] Sund SE, Swanson JA, Axelrod D. Cell membrane orientation visualized by polarized total internal reflection fluorescence. *Biophys J*. 1999;77:2266-83.
- [129] Colom A, Derivery E, Soleimanpour S, Tomba C, Molin MD, Sakai N, et al. A fluorescent membrane tension probe. *Nat Chem*. 2018.
- [130] Cicchetti G, Biernacki M, Farquharson J, Allen PG. A ratiometric expressible FRET sensor for phosphoinositides displays a signal change in highly dynamic membrane structures in fibroblasts. *Biochemistry*. 2004;43:1939-49.
- [131] Tanimura A, Nezu A, Morita T, Turner RJ, Tojyo Y. Fluorescent biosensor for quantitative real-time measurements of inositol 1,4,5-trisphosphate in single living cells. *J Biol Chem*. 2004;279:38095-8.
- [132] Grime JM, Edwards MA, Rudd NC, Unwin PR. Quantitative visualization of passive transport across bilayer lipid membranes. *Proc Natl Acad Sci U S A*. 2008;105:14277-82.
- [133] Ries RS, Choi H, Blunck R, Bezanilla F, Heath JR. Black lipid membranes: visualizing the structure, dynamics, and substrate dependence of membranes. *The Journal of Physical Chemistry B*. 2004;108:16040-9.
- [134] Kocsis I, Sorci M, Vanselous H, Murail S, Sanders SE, Licsandru E, et al. Oriented chiral water wires in artificial transmembrane channels. *Sci Adv*. 2018;4:eaao5603.
- [135] Bloom M, Evans E, Mouritsen OG. Physical properties of the fluid lipid-bilayer component of cell membranes: a perspective. *Q Rev Biophys*. 1991;24:293-397.
- [136] Jost PC, Griffith OH. The lipid-protein interface in biological membranes. *Ann N Y Acad Sci*. 1980;348:391-407.
- [137] Seelig A, Seelig J. Effect of a single cis double bond on the structures of a phospholipid bilayer. *Biochemistry*. 1977;16:45-50.
- [138] Seelig J, Waespesarcevic N. MOLECULAR ORDER IN CIS AND TRANS UNSATURATED PHOSPHOLIPID BILAYERS. *Biochemistry*. 1978;17:3310-5.

- 1
2
3
4
5
6
7 [139] Triba MN, Devaux PF, Warschawski DE. Effects of lipid chain length and
8 unsaturation on bicelles stability. A phosphorus NMR study. *Biophys J.*
9 2006;91:1357-67.
- 10 [140] Triba MN, Warschawski DE, Devaux PF. Reinvestigation by phosphorus NMR
11 of lipid distribution in bicelles. *Biophys J.* 2005;88:1887-901.
- 12 [141] Pastor RW, Venable RM, Feller SE. Lipid bilayers, NMR relaxation, and
13 computer simulations. *Acc Chem Res.* 2002;35:438-46.
- 14 [142] König S, Sackmann E. Molecular and collective dynamics of lipid bilayers.
15 *Current Opinion in Colloid & Interface Science.* 1996;1:78-82.
- 16 [143] Berkowitz M. A Molecular Look at Membranes. *Current topics in membranes:*
17 Elsevier; 2016. p. 1-25.
- 18 [144] Beaugrand M, Arnold AA, Henin J, Warschawski DE, Williamson PT,
19 Marcotte I. Lipid concentration and molar ratio boundaries for the use of isotropic
20 bicelles. *Langmuir.* 2014;30:6162-70.
- 21 [145] Benz RW, Castro-Roman F, Tobias DJ, White SH. Experimental validation of
22 molecular dynamics simulations of lipid bilayers: a new approach. *Biophys J.*
23 2005;88:805-17.
- 24 [146] Tieleman DP, Marrink S-J, Berendsen HJ. A computer perspective of
25 membranes: molecular dynamics studies of lipid bilayer systems. *Biochimica et*
26 *Biophysica Acta (BBA)-Reviews on Biomembranes.* 1997;1331:235-70.
- 27 [147] Faraldo-Gómez JD, Smith GR, Sansom MS. Setting up and optimization of
28 membrane protein simulations. *European Biophysics Journal.* 2002;31:217-27.
- 29 [148] Yesylevskyy SO. ProtSqueeze: simple and effective automated tool for setting
30 up membrane protein simulations. *Journal of chemical information and modeling.*
31 2007;47:1986-94.
- 32 [149] Javanainen M, Martinez-Seara H. Efficient preparation and analysis of
33 membrane and membrane protein systems. *Biochim Biophys Acta.* 2016;1858:2468-
34 82.
- 35 [150] Humphrey W, Dalke A, Schulten K. VMD: visual molecular dynamics. *J Mol*
36 *Graph.* 1996;14:33-8, 27-8.
- 37 [151] Jo S, Lim JB, Klauda JB, Im W. CHARMM-GUI Membrane Builder for mixed
38 bilayers and its application to yeast membranes. *Biophysical journal.* 2009;97:50-8.
- 39 [152] Jo S, Kim T, Im W. Automated builder and database of protein/membrane
40 complexes for molecular dynamics simulations. *PloS one.* 2007;2:e880.
- 41 [153] Wu EL, Cheng X, Jo S, Rui H, Song KC, Dávila- Contreras EM, et al.
42 CHARMM- GUI membrane builder toward realistic biological membrane
43 simulations. *Journal of computational chemistry.* 2014;35:1997-2004.
- 44 [154] Sommer B. Membrane packing problems: a short review on computational
45 membrane modeling methods and tools. *Computational and structural biotechnology*
46 *journal.* 2013;5:e201302014.
- 47 [155] Sommer Br, Dingersen T, Gamroth C, Schneider SE, Rubert S, Krüger J, et al.
48 CELLmicrocosmos 2.2 MembraneEditor: a modular interactive shape-based software
49 approach to solve heterogeneous membrane packing problems. *Journal of chemical*
50 *information and modeling.* 2011;51:1165-82.
- 51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

[156] Bovigny C, Tamò G, Lemmin T, Maïno N, Dal Peraro M. LipidBuilder: a framework to build realistic models for biological membranes. ACS Publications; 2015.

[157] Wassenaar TA, Ingólfsson HI, Böckmann RA, Tieleman DP, Marrink SJ. Computational lipidomics with insane: a versatile tool for generating custom membranes for molecular simulations. *Journal of chemical theory and computation*. 2015;11:2144-55.

[158] Wen PC, Mahinthichaichan P, Trebesch N, Jiang T, Zhao Z, Shinn E, et al. Microscopic view of lipids and their diverse biological functions. *Curr Opin Struct Biol*. 2018;51:177-86.

[159] Parkin J, Chavent M, Khalid S. Molecular Simulations of Gram-Negative Bacterial Membranes: A Vignette of Some Recent Successes. *Biophys J*. 2015;109:461-8.

[160] Ingólfsson HI, Carpenter TS, Bhatia H, Bremer PT, Marrink SJ, Lightstone FC. Computational Lipidomics of the Neuronal Plasma Membrane. *Biophys J*. 2017;113:2271-80.

[161] Ingólfsson HI, Melo MN, van Eerden FJ, Arnarez C, Lopez CA, Wassenaar TA, et al. Lipid organization of the plasma membrane. *J Am Chem Soc*. 2014;136:14554-9.

[162] Sharma S, Kim BN, Stansfeld PJ, Sansom MS, Lindau M. A Coarse Grained Model for a Lipid Membrane with Physiological Composition and Leaflet Asymmetry. *PLoS One*. 2015;10:e0144814.

[163] Stansfeld PJ. Computational studies of membrane proteins: from sequence to structure to simulation. *Current opinion in structural biology*. 2017;45:133-41.

[164] Stansfeld PJ, Goose JE, Caffrey M, Carpenter EP, Parker JL, Newstead S, et al. MemProtMD: Automated Insertion of Membrane Protein Structures into Explicit Lipid Membranes. *Structure*. 2015;23:1350-61.

[165] Vanni S, Hirose H, Barelli H, Antonny B, Gautier R. A sub-nanometre view of how membrane curvature and composition modulate lipid packing and protein recruitment. *Nature communications*. 2014;5:4916.

[166] Vamparys L, Gautier R, Vanni S, Bennett WD, Tieleman DP, Antonny B, et al. Conical lipids in flat bilayers induce packing defects similar to that induced by positive curvature. *Biophysical journal*. 2013;104:585-93.

[167] Im W, Liang J, Olson A, Zhou H-X, Vajda S, Vakser IA. Challenges in structural approaches to cell modeling. *Journal of molecular biology*. 2016;428:2943-64.

[168] Johnson GT, Autin L, Al-Alusi M, Goodsell DS, Sanner MF, Olson AJ. cellPACK: a virtual mesoscope to model and visualize structural systems biology. *Nature methods*. 2014;12:85.

[169] Johnson GT, Goodsell DS, Autin L, Forli S, Sanner MF, Olson AJ. 3D molecular models of whole HIV-1 virions generated with cellPACK. *Faraday discussions*. 2014;169:23-44.

[170] Ingólfsson HI, Arnarez C, Periole X, Marrink SJ. Computational 'microscopy' of cellular membranes. *J Cell Sci*. 2016;jcs. 176040.

- [171] Sommer B, Bender C, Hoppe T, Gamroth C, Jelonek L. Stereoscopic cell visualization: from mesoscopic to molecular scale. *Journal of Electronic Imaging*. 2014;23:011007.
- [172] Biere N, Ghaffar M, Doebbe A, Jäger D, Rothe N, Friedrich BM, et al. Heuristic modeling and 3D stereoscopic visualization of a *Chlamydomonas reinhardtii* cell. *Journal of integrative bioinformatics*. 2018;15.
- [173] Klein T, Autin L, Kozlíková B, Goodsell DS, Olson A, Gröller ME, et al. Instant Construction and Visualization of Crowded Biological Environments. *IEEE transactions on visualization and computer graphics*. 2018;24:862-72.
- [174] Aksimentiev A, Schulten K. Imaging alpha-hemolysin with molecular dynamics: Ionic conductance, osmotic permeability, and the electrostatic potential map. *Biophysical Journal*. 2005;88:3745-61.
- [175] Gullingsrud J, Schulten K. Lipid bilayer pressure profiles and mechanosensitive channel gating. *Biophysical Journal*. 2004;86:3496-509.
- [176] Klauda JB, Venable RM, Freites JA, O'Connor JW, Tobias DJ, Mondragon-Ramirez C, et al. Update of the CHARMM all-atom additive force field for lipids: validation on six lipid types. *J Phys Chem B*. 2010;114:7830-43.
- [177] Poger D, Van Gunsteren WF, Mark AE. A new force field for simulating phosphatidylcholine bilayers. *J Comput Chem*. 2010;31:1117-25.
- [178] Ulmschneider JP, Ulmschneider MB. United Atom Lipid Parameters for Combination with the Optimized Potentials for Liquid Simulations All-Atom Force Field. *J Chem Theory Comput*. 2009;5:1803-13.
- [179] Dickson CJ, Madej BD, Skjevik AA, Betz RM, Teigen K, Gould IR, et al. Lipid14: The Amber Lipid Force Field. *J Chem Theory Comput*. 2014;10:865-79.
- [180] Piggot TJ, Pineiro A, Khalid S. Molecular Dynamics Simulations of Phosphatidylcholine Membranes: A Comparative Force Field Study. *J Chem Theory Comput*. 2012;8:4593-609.
- [181] Ingolfsson HI, Lopez CA, Uusitalo JJ, de Jong DH, Gopal SM, Periole X, et al. The power of coarse graining in biomolecular simulations. *Wiley Interdiscip Rev Comput Mol Sci*. 2014;4:225-48.
- [182] Marrink SJ, de Vries AH, Tieleman DP. Lipids on the move: simulations of membrane pores, domains, stalks and curves. *Biochim Biophys Acta*. 2009;1788:149-68.
- [183] Lindahl E, Sansom MS. Membrane proteins: molecular dynamics simulations. *Curr Opin Struct Biol*. 2008;18:425-31.
- [184] Khalili-Araghi F, Gumbart J, Wen PC, Sotomayor M, Tajkhorshid E, Schulten K. Molecular dynamics simulations of membrane channels and transporters. *Curr Opin Struct Biol*. 2009;19:128-37.
- [185] Stansfeld PJ, Sansom MS. Molecular simulation approaches to membrane proteins. *Structure*. 2011;19:1562-72.
- [186] Bennett WF, Tieleman DP. Computer simulations of lipid membrane domains. *Biochim Biophys Acta*. 2013;1828:1765-76.
- [187] Perilla JR, Goh BC, Cassidy CK, Liu B, Bernardi RC, Rudack T, et al. Molecular dynamics simulations of large macromolecular complexes. *Current opinion in structural biology*. 2015;31:64-74.

[188] Chavent M, Duncan AL, Sansom MS. Molecular dynamics simulations of membrane proteins and their interactions: from nanoscale to mesoscale. *Current opinion in structural biology*. 2016;40:8-16.

[189] Baaden M, Lavery R. There's plenty of room in the middle: multi-scale modelling of biological systems. *Recent Adv In Structural Bioinformatics*, Research Signpost India, AG de Brevern, ed. 2007.

[190] van Eerden FJ, de Jong DH, de Vries AH, Wassenaar TA, Marrink SJ. Characterization of thylakoid lipid membranes from cyanobacteria and higher plants by molecular dynamics simulations. *Biochimica et Biophysica Acta (BBA)-Biomembranes*. 2015;1848:1319-30.

[191] Wu EL, Fleming PJ, Yeom MS, Widmalm G, Klauda JB, Fleming KG, et al. E. coli outer membrane and interactions with OmpLA. *Biophys J*. 2014;106:2493-502.

[192] Azimzadeh Irani M. Correlation between experimentally indicated and atomistically simulated roles of EGFR N-glycosylation. *Molecular Simulation*. 2018;44:743-8.

[193] Wendoloski J, Kimatian S, Schutt C, Salemme F. Molecular dynamics simulation of a phospholipid micelle. *Science*. 1989;243:636-8.

[194] Venable RM, Zhang Y, Hardy BJ, Pastor RW. Molecular dynamics simulations of a lipid bilayer and of hexadecane: an investigation of membrane fluidity. *Science*. 1993;262:223-6.

[195] Smit B, Hilbers P, Esselink K, Rupert L, Van Os N, Schlijper A. Computer simulations of a water/oil interface in the presence of micelles. *Nature*. 1990;348:624.

[196] Lindahl E, Edholm O. Mesoscopic undulations and thickness fluctuations in lipid bilayers from molecular dynamics simulations. *Biophys J*. 2000;79:426-33.

[197] Marrink S, Mark A. Effect of undulations on surface tension in simulated bilayers. *The Journal of Physical Chemistry B*. 2001;105:6122-7.

[198] Sharma P. Entropic force between membranes reexamined. *Proceedings of the National Academy of Sciences*. 2013;110:1976-7.

[199] Woolf TB, Roux B. Molecular dynamics simulation of the gramicidin channel in a phospholipid bilayer. *Proc Natl Acad Sci U S A*. 1994;91:11631-5.

[200] Samsudin F, Boags A, Piggot TJ, Khalid S. Braun's Lipoprotein Facilitates OmpA Interaction with the Escherichia coli Cell Wall. *Biophysical journal*. 2017;113:1496-504.

[201] Tarek M. Membrane electroporation: a molecular dynamics simulation. *Biophys J*. 2005;88:4045-53.

[202] Man VH, Truong PM, Li MS, Wang J, Van-Oanh NT, Derreumaux P, et al. Molecular Mechanism of the Cell Membrane Pore Formation Induced by Bubble Stable Cavitation. *J Phys Chem B*. 2018.

[203] Fu H, Comer J, Cai W, Chipot C. Sonoporation at Small and Large Length Scales: Effect of Cavitation Bubble Collapse on Membranes. *J Phys Chem Lett*. 2015;6:413-8.

[204] Chandler DE, Strumpfer J, Sener M, Scheuring S, Schulten K. Light harvesting by lamellar chromatophores in *Rhodospirillum rubrum*. *Biophys J*. 2014;106:2503-10.

- 1
2
3
4
5
6
7 [205] Saiz L, Klein ML. Computer simulation studies of model biological
8 membranes. *Acc Chem Res.* 2002;35:482-9.
9 [206] Schneider AR, Geissler PL. Coarse-grained computer simulation of dynamics in
10 thylakoid membranes: methods and opportunities. *Front Plant Sci.* 2013;4:555.
11 [207] Marrink SJ, Tieleman DP. Perspective on the Martini model. *Chemical Society*
12 *Reviews.* 2013;42:6801-22.
13 [208] Ayton GS, Noid WG, Voth GA. Multiscale modeling of biomolecular systems:
14 in serial and in parallel. *Curr Opin Struct Biol.* 2007;17:192-8.
15 [209] Shinoda W, DeVane R, Klein ML. Computer simulation studies of self-
16 assembling macromolecules. *Curr Opin Struct Biol.* 2012;22:175-86.
17 [210] Orsi M, Essex JW. The ELBA force field for coarse-grain modeling of lipid
18 membranes. *PLoS One.* 2011;6:e28637.
19 [211] Shinoda W, DeVane R, Klein ML. Zwitterionic lipid assemblies: molecular
20 dynamics studies of monolayers, bilayers, and vesicles using a new coarse grain force
21 field. *J Phys Chem B.* 2010;114:6836-49.
22 [212] Marrink SJ, De Vries AH, Mark AE. Coarse grained model for semiquantitative
23 lipid simulations. *The Journal of Physical Chemistry B.* 2004;108:750-60.
24 [213] Bond PJ, Sansom MS. Insertion and assembly of membrane proteins via
25 simulation. *J Am Chem Soc.* 2006;128:2697-704.
26 [214] Bond PJ, Holyoake J, Ivetac A, Khalid S, Sansom MS. Coarse-grained
27 molecular dynamics simulations of membrane proteins and peptides. *Journal of*
28 *structural biology.* 2007;157:593-605.
29 [215] Izvekov S, Voth GA. A multiscale coarse-graining method for biomolecular
30 systems. *J Phys Chem B.* 2005;109:2469-73.
31 [216] Ackerman DG, Feigenson GW. Multiscale modeling of four-component lipid
32 mixtures: domain composition, size, alignment, and properties of the phase interface.
33 *The Journal of Physical Chemistry B.* 2015;119:4240-50.
34 [217] Parton DL, Tek A, Baaden M, Sansom MS. Formation of raft-like assemblies
35 within clusters of influenza hemagglutinin observed by MD simulations. *PLoS*
36 *Comput Biol.* 2013;9:e1003034.
37 [218] Risselada HJ, Kutzner C, Grubmuller H. Caught in the act: visualization of
38 SNARE-mediated fusion events in molecular detail. *Chembiochem.* 2011;12:1049-55.
39 [219] Marrink SJ, Mark AE. The mechanism of vesicle fusion as revealed by
40 molecular dynamics simulations. *Journal of the American Chemical Society.*
41 2003;125:11144-5.
42 [220] Durrieu MP, Bond PJ, Sansom MS, Lavery R, Baaden M. Coarse-grain
43 simulations of the R-SNARE fusion protein in its membrane environment detect long-
44 lived conformational sub-states. *Chemphyschem.* 2009;10:1548-52.
45 [221] Kasson PM, Kelley NW, Singhal N, Vrljic M, Brunger AT, Pande VS.
46 Ensemble molecular dynamics yields submillisecond kinetics and intermediates of
47 membrane fusion. *Proc Natl Acad Sci U S A.* 2006;103:11916-21.
48 [222] Baoukina S, Marrink SJ, Tieleman DP. Molecular structure of membrane
49 tethers. *Biophys J.* 2012;102:1866-71.
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

- [223] Hsu P-C, Samsudin F, Shearer J, Khalid S. It Is Complicated: Curvature, Diffusion, and Lipid Sorting within the Two Membranes of *Escherichia coli*. *The journal of physical chemistry letters*. 2017;8:5513-8.
- [224] Reddy T, Sansom MS. The Role of the Membrane in the Structure and Biophysical Robustness of the Dengue Virion Envelope. *Structure*. 2016;24:375-82.
- [225] Reddy T, Sansom MS. Computational virology: From the inside out. *Biochim Biophys Acta*. 2016;1858:1610-8.
- [226] Reddy T, Shorthouse D, Parton DL, Jefferys E, Fowler PW, Chavent M, et al. Nothing to sneeze at: a dynamic and integrative computational model of an influenza A virion. *Structure*. 2015;23:584-97.
- [227] Izvekov S, Voth GA. Solvent-free lipid bilayer model using multiscale coarse-graining. *The Journal of Physical Chemistry B*. 2009;113:4443-55.
- [228] Arnarez C, Uusitalo JJ, Masman MF, Ingolfsson HI, de Jong DH, Melo MN, et al. Dry Martini, a coarse-grained force field for lipid membrane simulations with implicit solvent. *J Chem Theory Comput*. 2015;11:260-75.
- [229] Wang Z-J, Deserno M. A systematically coarse-grained solvent-free model for quantitative phospholipid bilayer simulations. *The Journal of Physical Chemistry B*. 2010;114:11207-20.
- [230] Brannigan G, Lin LCL, Brown FLH. Implicit solvent simulation models for biomembranes. *Eur Biophys J Biophys Lett*. 2006;35:104-24.
- [231] Zgorzki A, Lyman E. Toward Hydrodynamics with Solvent Free Lipid Models: STRD Martini. *Biophysical journal*. 2016;111:2689-97.
- [232] Sterpone F, Melchionna S, Tuffery P, Pasquali S, Mousseau N, Cragnolini T, et al. The OPEP protein model: from single molecules, amyloid formation, crowding and hydrodynamics to DNA/RNA systems. *Chem Soc Rev*. 2014;43:4871-93.
- [233] Chavent M, Duncan AL, Rassam P, Birkholz O, Hélie J, Reddy T, et al. How nanoscale protein interactions determine the mesoscale dynamic organisation of bacterial outer membrane proteins. *Nature communications*. 2018;9.
- [234] Fowler PW, Hélie J, Duncan A, Chavent M, Koldsø H, Sansom MS. Membrane stiffness is modified by integral membrane proteins. *Soft Matter*. 2016;12:7792-803.
- [235] Koldsø H, Sansom MS. Organization and dynamics of receptor proteins in a plasma membrane. *Journal of the American Chemical Society*. 2015;137:14694-704.
- [236] Periole X, Knepp AM, Sakmar TP, Marrink SJ, Huber T. Structural determinants of the supramolecular organization of G protein-coupled receptors in bilayers. *J Am Chem Soc*. 2012;134:10959-65.
- [237] Lu L, Voth GA. Systematic coarse-graining of a multicomponent lipid bilayer. *J Phys Chem B*. 2009;113:1501-10.
- [238] Voegelé M, Hummer G. Divergent Diffusion Coefficients in Simulations of Fluids and Lipid Membranes. *J Phys Chem B*. 2016;120:8722-32.
- [239] Grime JM, Voth GA. Highly scalable and memory efficient ultra-coarse-grained molecular dynamics simulations. *Journal of chemical theory and computation*. 2013;10:423-31.
- [240] Madsen JJ, Sinitskiy AV, Li J, Voth GA. Highly Coarse-Grained Representations of Transmembrane Proteins. *J Chem Theory Comput*. 2017;13:935-44.

- 1
2
3
4
5
6
7 [241] Arkhipov A, Yin Y, Schulten K. Four-scale description of membrane sculpting
8 by BAR domains. *Biophys J*. 2008;95:2806-21.
- 9 [242] Yu H, Schulten K. Membrane sculpting by F-BAR domains studied by
10 molecular dynamics simulations. *PLoS Comput Biol*. 2013;9:e1002892.
- 11 [243] Shillcock JC, Lipowsky R. The computational route from bilayer membranes to
12 vesicle fusion. *Journal of Physics: Condensed Matter*. 2006;18:S1191.
- 13 [244] Shillcock JC, Lipowsky R. Tension-induced fusion of bilayer membranes and
14 vesicles. *Nature materials*. 2005;4:225.
- 15 [245] Tanizaki S, Feig M. A generalized Born formalism for heterogeneous dielectric
16 environments: application to the implicit modeling of biological membranes. *The*
17 *Journal of chemical physics*. 2005;122:124706.
- 18 [246] Schneider AR, Geissler PL. Coexistence of fluid and crystalline phases of
19 proteins in photosynthetic membranes. *Biophysical journal*. 2013;105:1161-70.
- 20 [247] Shi Q, Voth GA. Multi-scale modeling of phase separation in mixed lipid
21 bilayers. *Biophys J*. 2005;89:2385-94.
- 22 [248] Brown FL. Elastic modeling of biomembranes and lipid bilayers. *Annu Rev*
23 *Phys Chem*. 2008;59:685-712.
- 24 [249] Lin LC-L, Brown FL. Brownian dynamics in Fourier space: membrane
25 simulations over long length and time scales. *Physical review letters*.
26 2004;93:256001.
- 27 [250] Brown FL. Regulation of protein mobility via thermal membrane undulations.
28 *Biophysical journal*. 2003;84:842-53.
- 29 [251] West B, Brown FL, Schmid F. Membrane-protein interactions in a generic
30 coarse-grained model for lipid bilayers. *Biophys J*. 2009;96:101-15.
- 31 [252] Mauer J, Peltomaki M, Pobleto S, Gompper G, Fedosov DA. Static and
32 dynamic light scattering by red blood cells: A numerical study. *PLoS One*.
33 2017;12:e0176799.
- 34 [253] Gompper G, Kroll D. Triangulated-surface models of fluctuating membranes.
35 *Statistical mechanics of membranes and surfaces*: World Scientific; 2004. p. 359-426.
- 36 [254] Telenius J, Vattulainen I, Monticelli L. Visualization of Complex Processes in
37 Lipid Systems Using Computer Simulations and Molecular Graphics. *Lipidomics*:
38 Springer; 2009. p. 317-38.
- 39 [255] Gapsys V, de Groot BL, Briones R. Computational analysis of local membrane
40 properties. *J Comput Aided Mol Des*. 2013;27:845-58.
- 41 [256] Darré L, Tek A, Baaden M, Pantano S. Mixing atomistic and coarse grain
42 solvation models for MD simulations: let WT4 handle the bulk. *Journal of chemical*
43 *theory and computation*. 2012;8:3880-94.
- 44 [257] Chavent M, Reddy T, Goose J, Dahl AC, Stone JE, Jobard B, et al.
45 Methodologies for the analysis of instantaneous lipid diffusion in MD simulations of
46 large membrane systems. *Faraday Discuss*. 2014;169:455-75.
- 47 [258] Lv Z, Tek A, Da Silva F, Empereur-mot C, Chavent M, Baaden M. Game on,
48 science - how video game technology may help biologists tackle visualization
49 challenges. *PLoS One*. 2013;8:e57990.
- 50 [259] Lelimosin M, Sansom MS. Membrane perturbation by carbon nanotube
51 insertion: pathways to internalization. *Small*. 2013;9:3639-46.
- 52
53
54
55
56
57
58
59
60
61
62
63
64
65

[260] Perez S, Tubiana T, Imberty A, Baaden M. Three-dimensional representations of complex carbohydrates and polysaccharides--SweetUnityMol: a video game-based computer graphic software. *Glycobiology*. 2015;25:483-91.

[261] Andrei RM, Callieri M, Zini MF, Loni T, Maraziti G, Pan MC, et al. Intuitive representation of surface properties of biomolecules using BioBlender. *BMC bioinformatics*. 2012;13:S16.

[262] Catoire LJ, Damian M, Baaden M, Guittet E, Baneres JL. Electrostatically-driven fast association and perdeuteration allow detection of transferred cross-relaxation for G protein-coupled receptor ligands with equilibrium dissociation constants in the high-to-low nanomolar range. *J Biomol NMR*. 2011;50:191-5.

[263] Lukat G, Krüger J, Sommer Br. APL@ Voro: A voronoi-based membrane analysis tool for GROMACS trajectories. *Journal of chemical information and modeling*. 2013;53:2908-25.

[264] Allen WJ, Lemkul JA, Bevan DR. GridMAT- MD: a grid- based membrane analysis tool for use with molecular dynamics. *Journal of computational chemistry*. 2009;30:1952-8.

[265] Guixa-Gonzalez R, Rodriguez-Espigares I, Ramirez-Angueta JM, Carrio-Gaspar P, Martinez-Seara H, Giorgino T, et al. MEMBPLUGIN: studying membrane complexity in VMD. *Bioinformatics*. 2014;30:1478-80.

[266] Buchoux S. FATSLiM: a fast and robust software to analyze MD simulations of membranes. *Bioinformatics*. 2016;33:133-4.

[267] Michaud- Agrawal N, Denning EJ, Woolf TB, Beckstein O. MDAnalysis: a toolkit for the analysis of molecular dynamics simulations. *Journal of computational chemistry*. 2011;32:2319-27.

[268] Vanni S, Vamparys L, Gautier R, Drin G, Etchebest C, Fuchs PF, et al. Amphipathic lipid packing sensor motifs: probing bilayer defects with hydrophobic residues. *Biophysical journal*. 2013;104:575-84.

[269] Jefferys E, Sansom MS, Fowler PW. NRas slows the rate at which a model lipid bilayer phase separates. *Faraday Discuss*. 2014;169:209-23.

[270] Dahl AC, Chavent M, Sansom MS. Bendix: intuitive helix geometry analysis and abstraction. *Bioinformatics*. 2012;28:2193-4.

[271] Esque J, Sansom MSP, Baaden M, Oguey C. Analyzing protein topology based on Laguerre tessellation of a pore-traversing water network. *Sci Rep*. 2018;8:13540.

[272] Rosevear F. Liquid crystals: the mesomorphic phases of surfactant compositions. *J Soc Cosmet Chem*. 1968;19:581-94.

[273] Steck TL. The organization of proteins in the human red blood cell membrane. A review. *J Cell Biol*. 1974;62:1-19.

[274] Lee AG. How lipids affect the activities of integral membrane proteins. *Biochim Biophys Acta*. 2004;1666:62-87.

[275] Jain MK, White HB, 3rd. Long-range order in biomembranes. *Adv Lipid Res*. 1977;15:1-60.

[276] Galla HJ, Hartmann W, Theilen U, Sackmann E. On two-dimensional passive random walk in lipid bilayers and fluid pathways in biomembranes. *J Membr Biol*. 1979;48:215-36.

- 1
2
3
4
5
6
7 [277] Zoppe M. Towards a perceptive understanding of size in cellular biology. *Nat*
8 *Methods*. 2017;14:662-5.
9 [278] Iwasa JH. Bringing macromolecular machinery to life using 3D animation.
10 *Current opinion in structural biology*. 2015;31:84-8.
11 [279] McGill G. Molecular movies... coming to a lecture near you. *Cell*.
12 2008;133:1127-32.
13 [280] Iwasa JH. Animating the model figure. *Trends in cell biology*. 2010;20:699-
14 704.
15 [281] Johnson GT, Autin L, Goodsell DS, Sanner MF, Olson AJ. ePMV embeds
16 molecular modeling into professional animation software environments. *Structure*.
17 2011;19:293-303.
18 [282] Andrei R, Pan C, Zoppè M. BioBlender: Blender for Biologists. *BlenderArt*
19 *Magazine*. 2010;31:27-32.
20 [283] Ghaffar M, Biere N, Jäger D, Klein K, Schreiber F, Kruse O, et al. 3D
21 modelling and visualisation of heterogeneous cell membranes in Blender.
22 *Proceedings of the 11th International Symposium on Visual Information*
23 *Communication and Interaction: ACM*; 2018. p. 64-71.
24 [284] Le Muzic M, Autin L, Parulek J, Viola I. cellVIEW: a tool for illustrative and
25 multi-scale rendering of large biomolecular datasets. *Eurographics Workshop on*
26 *Visual Computing for Biomedicine: NIH Public Access*; 2015. p. 61.
27 [285] Goodsell DS, Johnson GT. Filling in the gaps: artistic license in education and
28 outreach. *PLoS Biology*. 2007;5:e308.
29 [286] Goodsell DS. Visual methods from atoms to cells. *Structure*. 2005;13:347-54.
30 [287] Goodsell DS. Illustrating the machinery of life: Viruses. *Biochemistry and*
31 *Molecular Biology Education*. 2012;40:291-6.
32 [288] Goodsell D. Cellular Landscapes in Watercolor. *Journal of Biocommunication*.
33 2016;40.
34 [289] Goodsell DS. Eukaryotic cell panorama. *Biochemistry and Molecular Biology*
35 *Education*. 2011;39:91-101.
36 [290] Goodsell DS. *Escherichia coli*. *Biochemistry and Molecular Biology Education*.
37 2009;37:325-32.
38 [291] Gramling C. Illustration. *Science*. 2005;309:1990-.
39 [292] Johnson GT, Hertig S. A guide to the visual analysis and communication of
40 biomolecular structural data. *Nature Reviews Molecular Cell Biology*. 2014;15:690.
41 [293] Engholm DH, Kilian M, Goodsell DS, Andersen ES, Kjærgaard RS. A visual
42 review of the human pathogen *Streptococcus pneumoniae*. *FEMS microbiology*
43 *reviews*. 2017;41:854-79.
44 [294] Zimmer C. Watch Proteins Do the Jitterbug. *New York Times*. 2014;10.
45 [295] Rundgren CJ, Tibell LAE. CRITICAL FEATURES OF VISUALIZATIONS
46 OF TRANSPORT THROUGH THE CELL MEMBRANE-AN EMPIRICAL STUDY
47 OF UPPER SECONDARY AND TERTIARY STUDENTS' MEANING-MAKING
48 OF A STILL IMAGE AND AN ANIMATION. *Int J Sci Math Educ*. 2010;8:223-46.
49 [296] Jenkinson J, McGill G. Visualizing Protein Interactions and Dynamics:
50 Evolving a Visual Language for Molecular Animation. *CBE-Life Sci Educ*.
51 2012;11:103-10.
52
53
54
55
56
57
58
59
60
61
62
63
64
65

[297] Cooper AK, Oliver-Hoyo MT. Creating 3D Physical Models to Probe Student Understanding of Macromolecular Structure. *Biochemistry and Molecular Biology Education*. 2017;45:491-500.

[298] Baaden M, Delalande O, Ferey N, Pasquali S, Waldispuhl J, Taly A. Ten simple rules to create a serious game, illustrated with examples from structural biology. *PLoS Comput Biol*. 2018;14:e1005955.

[299] Yuen D, Cartwright S, Jacob C, Acm. *Eukaryo: Virtual Reality Simulation of a Cell*. New York: Assoc Computing Machinery; 2016.

[300] Sommer B, Baaden M, Krone M, Woods A. From Virtual Reality to Immersive Analytics in Bioinformatics. *Journal of integrative bioinformatics*. 2018;15.

[301] Trellet M, Férey N, Flotyński J, Baaden M, Bourdot P. Semantics for an integrative and immersive pipeline combining visualization and analysis of molecular data. *Journal of integrative bioinformatics*. 2018;15.

[302] Maes A, Martinez X, Druart K, Laurent B, Guégan S, Marchand CH, et al. MinOmics, an Integrative and Immersive Tool for Multi-Omics Analysis. *Journal of integrative bioinformatics*. 2018.

[303] Hirst JD, Glowacki DR, Baaden M. Molecular simulations and visualization: introduction and overview. *Faraday discussions*. 2014;169:9-22.

[304] Doutreligne S, Cragolini T, Pasquali S, Derreumaux P, Baaden M. UnityMol: Interactive scientific visualization for integrative biology. *Large Data Analysis and Visualization (LDAV), 2014 IEEE 4th Symposium on: IEEE; 2014*. p. 109-10.

[305] Bunnell TM, Jaeger MA, Fitzsimons DP, Prins KW, Ervasti JM. Destabilization of the dystrophin-glycoprotein complex without functional deficits in alpha-dystrobrevin null muscle. *PLoS One*. 2008;3:e2604.

[306] Edidin M. Lipids on the frontier: a century of cell-membrane bilayers. *Nat Rev Mol Cell Biol*. 2003;4:414-8.

Figure Captions

Figure 1. Colorized Scanning Electron Micrograph showing the internal structure of a single neuron. The membranes of the endoplasmic reticulum are bright green. The ER and other parts of the cell are covered in membrane bound cytoplasmic proteins and ribosomes, which are blue. The Golgi complexes are a dull green, their products, lysosomes and vesicles, are yellow. The mitochondria are red. The outer membrane of the cell is turquoise. Microscopy and image processing: James Tyrwhitt-Drake, UVic AMF. Sample preparation: Dr. Patrick Nahirney, UVic Medical Sciences. See also: <http://gigapan.com/gigapans/147187>.

Figure 2. Visualization of lipid bilayer structural properties. **A:** Neutron scattering density profile of a fluid dioleoylphosphatidylcholine bilayer determined by joint refinement of X-ray and neutron diffraction data [44]. **B:** electron density map of the ripple phase in lecithin bilayers [46]. **Image copyright 1996 National Academy of Sciences.** **C:** AFM image of the surface of a DMPG membrane; the image is 2.4 x 3.0 nm², its height about 0.05 nm [47]. **D:** Membrane remodeling by the melittin peptide

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7 showing a gross disruption at high peptide concentration [48]. **E:** sphingomyelin
8 rafts protruding from a DOPC background with an almost exclusively raft-associated
9 glycosylphosphatidylinositol-anchored protein shown as yellow peaks [49].

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11 **Figure 3 : Cell-integral biological objects formed by membranes. A:** Transmis-
12 sion electron microscopy (TEM) of stained lipid bilayers depicting the Golgi appa-
13 ratus [62]. **B:** fenestrations in the Golgi (from C. Hopkins and J. Burden, Imperial
14 College London) [58]. **C:** Tubule on endosomes (from P. Luzio and N. Bright, Uni-
15 versity of Utah) [58]. **D:** HIV-1 viral budding (from W. Sundquist and U. von
16 Schwedler, University of Utah) [58]. **E:** An onion root tip shown with etching and
17 featuring the surfaces of the cytoplasmic matrix (CM), vacuolar contents (VC), vac-
18 uolar membrane faces (VF), spherosomes (S), nuclear membrane (N) and endoplas-
19 matic reticulum (ER) [59]. **F:** Caveolae in the plasma membrane of a fibroblast from
20 ref. [61], **Fig. 1. G:** TEM image highlighting mitochondrial membranes [62]. **H:** Sec-
21 tion of an isolated mitochondrial membrane with a three-layered structure and small
22 particles attached [63]. **I:** Cryo-electron microscopy of vitreous sections (CEMOVIS)
23 of the cell envelope of *mycobacteria smegmatis* featuring plasma membrane (PM),
24 inner wall zone (IWZ), medial wall zone (MWZ), granular layer (GL) and outer
25 membrane (OM) [64].
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28 **Figure 4 : Insight on biological membranes through characteristic protein**
29 **structures. A:** Protein associations in the erythrocyte membrane skeleton, in particu-
30 lar junctional complexes involving spectrin [66]. **B:** Hemagglutinin protein clusters in
31 live cells obtained by fluorescence photoactivation localization microscopy (FPALM)
32 [67]. **Copyright 2007 National Academy of Sciences. C:** TEM image of
33 hemagglutinin clusters in fibroblast membrane sheets [67]. **Copyright 2007 National**
34 **Academy of Sciences. D:** Dystrophin organization on the sarcolemma [305]. **E:** Actin
35 network influence on lipid phases in model membranes [70].
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38 **Figure 5 : Near atomic scale insights on membrane objects. A:** Three-
39 dimensional potential map spanning the purple membrane obtained by electron mi-
40 croscopy [9]. **B:** A model of a single bacteriorhodopsin molecule constituting the
41 purple membrane [9]. **C:** Crystal structure highlighting protein, lipid and water organ-
42 ization in the purple membrane [71]. **D:** Cytoplasmic surface of the purple membrane
43 showing individual bacteriorhodopsin molecules at subnanometer resolution [72]. **E:**
44 Force-induced conformational change of bacteriorhodopsin surface loops [73]. **F:**
45 Negatively stained EM of arc- and ring-shaped sullysin oligomer assemblies that per-
46 forate a liposome membrane [81]. **G:** AFM topography of sullysin on a supported
47 lipid bilayer [81]. **H:** Detailed AFM topography of a complete sullysin ring [81]. **I:** A
48 sullysin arc only partially enclosing the dark hole in the membrane [81].
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51 **Figure 6 : Towards membrane machinery at a larger scale and in 3 dimen-**
52 **sions. A:** Scanning electron microscope image by Tina Carvalho, University of Ha-
53 waii at Manoa depicting a nerve ending. It reveals vesicles shown in orange and blue
54 color. Image under a CC-BY-NC-SA license. **B:** A rendered 3D model of a distorted
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liposome interacting with fibrils and surrounded by small vesicles [83]. **C**: The actin cytoskeleton of a eukaryotic cell visualized by cryoelectron tomography, here zooming in on the actin-membrane interaction [26]. **D**: Two docked mitochondria (red) at an intermediate stage of outer membrane fusion with an apparent toroidal pore of 40 nm diameter [85]. The blue volumes at the interface are attributed to a docking ring, possibly formed by mitofusin protein assemblies. **E**: Tomographic reconstruction of a *S. cerevisiae* cell with a portion of the cell wall in magenta, mitochondrion in green, ER in orange. Reprinted with permission from [27]. Copyright 2017 American Chemical Society.

Figure 7 : Reporting on membrane dynamics by tracking individual molecules or domains. **A**: Single molecule microscopic visualization of lipid microdomains in a muscle cell membrane [88]. **B**: Following molecules in a confocal microscope is limited in detail due to the large detection area [89]. **C**: STED is able to discriminate between freely diffusing and hindered lipids due to its small subdiffraction spot size [89]. **D**: The image depicts the trajectories of individual potassium channels shown in cyan overlaid on an actin PALM image shown in red [90], scale bar is 2 micrometer. **E**: Enlargement of two areas of panel D illustrating confinement in a large compartment (left) and hopping between two compartments (right) [90], scale is x4 compared to panel D.

Figure 8 : (High-speed) AFM tracking of membrane processes. **A**: Real-time imaging of drug-membrane interactions [95]. **B**: Real-time imaging of the prepore-to-pore transition by sulysin with prepore intermediates appearing as diffuse streaks in the top panel, becoming more clearly defined in the center panel, leading to the pore state shown on the bottom [81]. **C**: High-speed AFM of the outer membrane protein F extends timescale resolution to hundreds of microseconds [96]. **D**: Membrane tubule constriction through the dynamin helix related to membrane fission [97]. **E**: Kymograph illustrating morphological changes of the dynamin helix along the tubule axis depending on GTP treatment or not [97].

Figure 9 : Membrane building tools. **A**: Molecular system representing a typical gram-negative bacterial outer membrane with lipopolysaccharide [167]. **B**: Building an outer mitochondrial membrane model with the CELLmicrocosmos MembraneEditor. Reprinted with permission from [155]. Copyright 2011 American Chemical Society. **C**: Setting up a cytochrome bc1 complex in a hexagonal prism within a 5-component bilayer. Reprinted with permission from [157]. Copyright 2015 American Chemical Society. Views are from the side, from the bottom and overviewing the lattice. **D**: Result of applying a cellPACK recipe for stochastic packing of a whole HIV-1 virion model in its envelope [169] - published by The Royal Society of Chemistry. **E**: Modeling the Golgi apparatus in 3D from freeze-fracture SEM data [172]. On the left a cut model highlighting cleaved surfaces as in an SEM image. In the middle the 3D model, on the left rendering with final material.

Figure 10 : Molecular model of an average synaptic vesicle. An outside view of the vesicle based on space-filling models of all macromolecules at near atomic resolution [34].

Figure 11. Atomistic molecular simulation examples. **A:** Simulation of the gel phase of the POPC bilayer. Reprinted with permission from [12]. Copyright 1993 American Chemical Society. **B:** Gramicidin A channel in a 1:8 ratio simulation in DMPC [19]. **C:** Membrane protein simulation snapshot from the end of a 200 ns atomistic simulation with a cell wall model [200]. OmpA dimer is red and blue for each protomer, Braun's lipoprotein is pink, the membrane is cyan (LPS) and grey (other phospholipids), and cell wall is green. **D:** Top view of the lipid bilayer of initially ca. 25×29 nm at six different times during an electroporation simulation spanning ca. 4 ns [23]. © Tieleman; licensee BioMed Central Ltd. 2004. **E:** Modeling of a photosynthetic membrane patch, showing a corresponding AFM topograph, the model of the chromatophore patch containing 39 LH2 and 7 LH1-RC complexes, with a close-up on an open and closed LH1 monomer [204].

Figure 12 : Examples of coarse-grained simulations. **A:** Determination of Lo and Ld lipid phase patches demarcated by a thick black line in a coarse-grained simulation of a 4-component DPPC (blue), PUPC (green) DUPC (red) and cholesterol (yellow) lipid mixture. DUPC ratio increases from left to right. Reprinted with permission from [216]. Copyright 2015 American Chemical Society. **B:** CG Model of a hemagglutinin A cluster in domain-forming membranes of DPPC (headgroups in light blue), DLiPC (headgroups in dark blue) and cholesterol in green [217]. The protein is shown in orange, phospholipid tails in gray and white. **C:** A membrane tether pulled by external force, shown in side and cross-section views [222]. **D:** Coarse-grained model encompassing the two membranes of *E. coli* with several membrane proteins [223]. The AcrABZ-TolC efflux pump is lime green, inner membrane protein AqpZ and outer membrane protein OmpA are pink, the lipids are cyan (LPS), grey (POPE), blue (POPG) and red (cardiolipin). This is a snapshot from the end of a 10 microsecond CG simulation. **E:** Mesoscale fluctuations of a plasma membrane model with increasing protein content [235] revealing organization and dynamics of receptor proteins. On the left, without any proteins present, in the middle with single TM helices (in red) from the gp130 cytokine receptor, on the right with GPCRs of the S1P1 receptor (in pink). **F:** Illustration of a vesicle nano-reactor simulation using a dry Martini-like lipid representation coupled with Lattice-Boltzmann hydrodynamics. On the left, the exterior water flow around the vesicle is depicted, on the right a cross-sectional cut reveals the overall fluid dynamics in the system. Unpublished results courtesy of A. Brandner, S. Timr and F. Sterpone.

Figure 13 : Examples of mesoscopic membrane simulations. **A:** Tense vesicle fusion with a tense square membrane patch observed by DPD [244]. **B:** Membrane tubules induced by an F-BAR domain lattice, starting from an edge-to-edge fusion forming a T-junction [242]. On the left, the membrane tubule is shown with F-BAR domains from the side. In the middle and on the right, the tubule lipids are shown

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7 from top at 0 and 30 microseconds, respectively. **C:** Triangulated model of the red
8 blood cell used for studying its light scattering features [252]. **D:** An elastic representation
9 of the red blood cell membrane with anchoring to the spectrin network highlighting
10 specific pinning sites [230].

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12 **Figure 14 : Specific visualization methods applicable to membranes and**
13 **membrane proteins.** **A:** Sheet representing the membrane center colored by local
14 order parameter with cholesterol molecules represented on top [255]. **B:** SNARE
15 complex in a double membrane system from [256] with each headgroup represented
16 by a green network connecting phosphor atoms. **C:** Correlated dynamics for upper
17 and lower leaflet of a planar membrane depicted by streamlines [257]. **D:** Native
18 coarse-grained representation of a membrane-inserted carbon nanotube filled with
19 water [259]. **E:** Representation of an LPS lipid combining SugarRibbons and
20 HyperBalls-licorice representations in SweetUnityMol [260]. **F:** Hydrophobicity representation
21 of a GPCR following [261, 262] highlighting electric field lines through
22 animation and hydrophilicity through darkening. See
23 <http://www.baaden.ibpc.fr/pub/blt2/jbn11.html> for an animated version on-line. **G:**
24 Cross-sectional view of a lipid-bilayer membrane populated by lipid tiles [173].
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27 **Figure 15 : Analysis examples producing visual representations.** **A:**
28 APL@Voro user interface and typical color-coded analysis result [263]. **B:** Difference
29 between area per lipid analysis for a curved membrane patch. On the left, analysis in a
30 plane, on the right FATSliM approach using local Voronoi cells [266]. **C:** Packing
31 defects determined by a geometrical method shown in blue, acyl chains in yellow,
32 polar heads in gray. A peptide inserting into the membrane is colocated [166, 268]. **D:**
33 Image processing to determine lipid domain boundaries from [269] - **Published by**
34 **The Royal Society of Chemistry.** **E:** Bendix membrane protein helix representations
35 colored by an angle-indicative heatmap [270]. **F:** Laguerre tessellation analysis of the
36 water network through the FepA membrane protein showing a water path through the
37 protein bridging the media on both sides of the membrane (left) and isolated water
38 inclusions (centre) with an inset zooming in on a selected inclusion [271].
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41 **Figure 16: Selected historic illustrations from the literature.** **A:** Schematic
42 cross-sectional view of a phospholipid bilayer with filled circles representing
43 headgroups and wavy lines depicting the fatty acid chain [7]. **B:** Alternating layer
44 structure of a neat surfactant phase (left) and middle phase structure of surfactant/water
45 systems (right) [272]. **C:** Hypothetical membrane protein insertion for the
46 major erythrocyte membrane [273, 306]. Carbohydrates exposed to the extracellular
47 space are drawn attached to the proteins. **D:** Cross-sectional view illustrating the lipid-
48 globular protein mosaic model of membrane structure [7]. **E:** Two-stage model of
49 membrane protein insertion illustrated for an alpha-helical integral membrane protein.
50 **Reprinted with permission from [18]. Copyright 1990 American Chemical Society.**
51 First trans-bilayer alpha-helices form, then they assemble into their final tertiary
52 architecture. **F:** Hydrophobic thickness illustrated. On the left a perfect match between
53 protein and bilayer, on the right a hydrophobic mismatch leading to a lipid bilayer
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7 distortion around a membrane protein [274]. **G:** A typical biomembrane in various
8 degrees of schematization. The top panel illustrates organized and disorganized lipids
9 forming discrete regions. The centre panel schematizes ordered and disordered re-
10 gions as plates. The bottom panel depicts a molecular view on the long range organi-
11 zation and compound distribution [275]. **H:** A possible pathway for a molecule diffus-
12 ing randomly in a biological membrane [276].

13
14 **Figure 17: Contributions from medical illustrators and artists.** **A:** Three still
15 frames from an animation depicting clathrin-mediated endocytosis by Janet Iwasa in
16 collaboration with Tomas Kirchhausen [280]. **B:** Transport from the Golgi featuring a
17 clathrin cage illustrated by David Goodsell [289]. **C:** Opening frames from four ani-
18 mations representing the same receptor-ligand binding event with varying degree of
19 detail and crowding by Jodie Jenkinson and Gaél McGill [296]. **D:** Watercolor of
20 pneumococcal cell wall synthesis with apparent membrane, membrane proteins, cell
21 wall structure and peptidoglycan layers drawn by Høyer Engholm [293].
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24 **Figure 18: From 3D modeling to communication and serious games.** **A:** Lipid
25 bilayer perspective on a 3D thylakoid membrane model rendered with Blender [172,
26 283]. **B:** Choice of molecular representations according to a given audience. Here, a
27 GPCR represented in a detailed lipid bilayer to make an engaging web banner image
28 [292]. **C:** The same GPCR as in **B** rendered for a game prototype to engage teenagers
29 [292]. **D:** Screenshot of the DocMolecules serious game prototype showing a drug
30 molecule floating over a membrane in search for its receptor. **E:** Eukaryo simulation
31 screenshot depicting the eukaryotic cellular space with control elements to adjust the
32 level of detail and a minimap for location reference [299].
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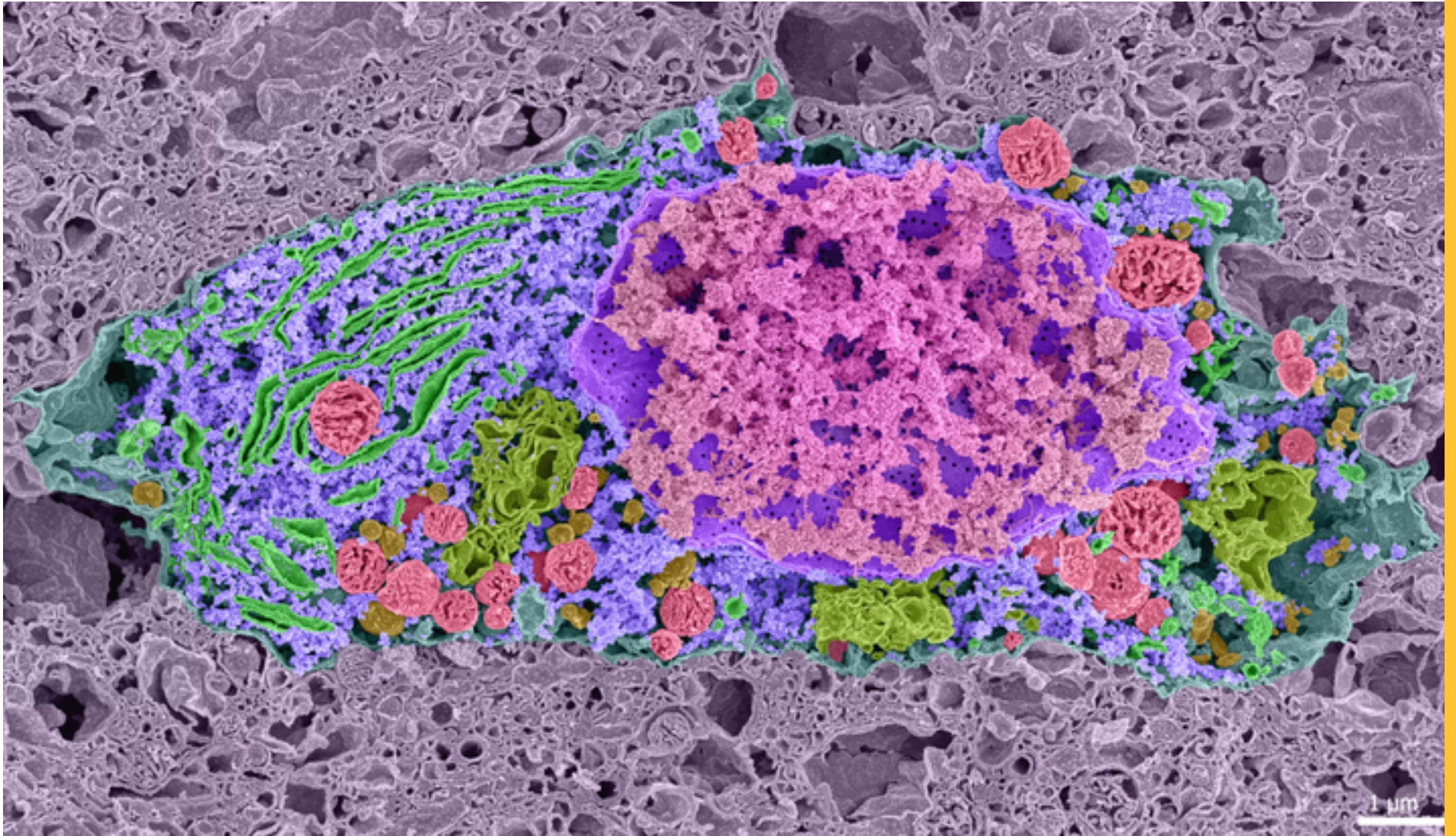


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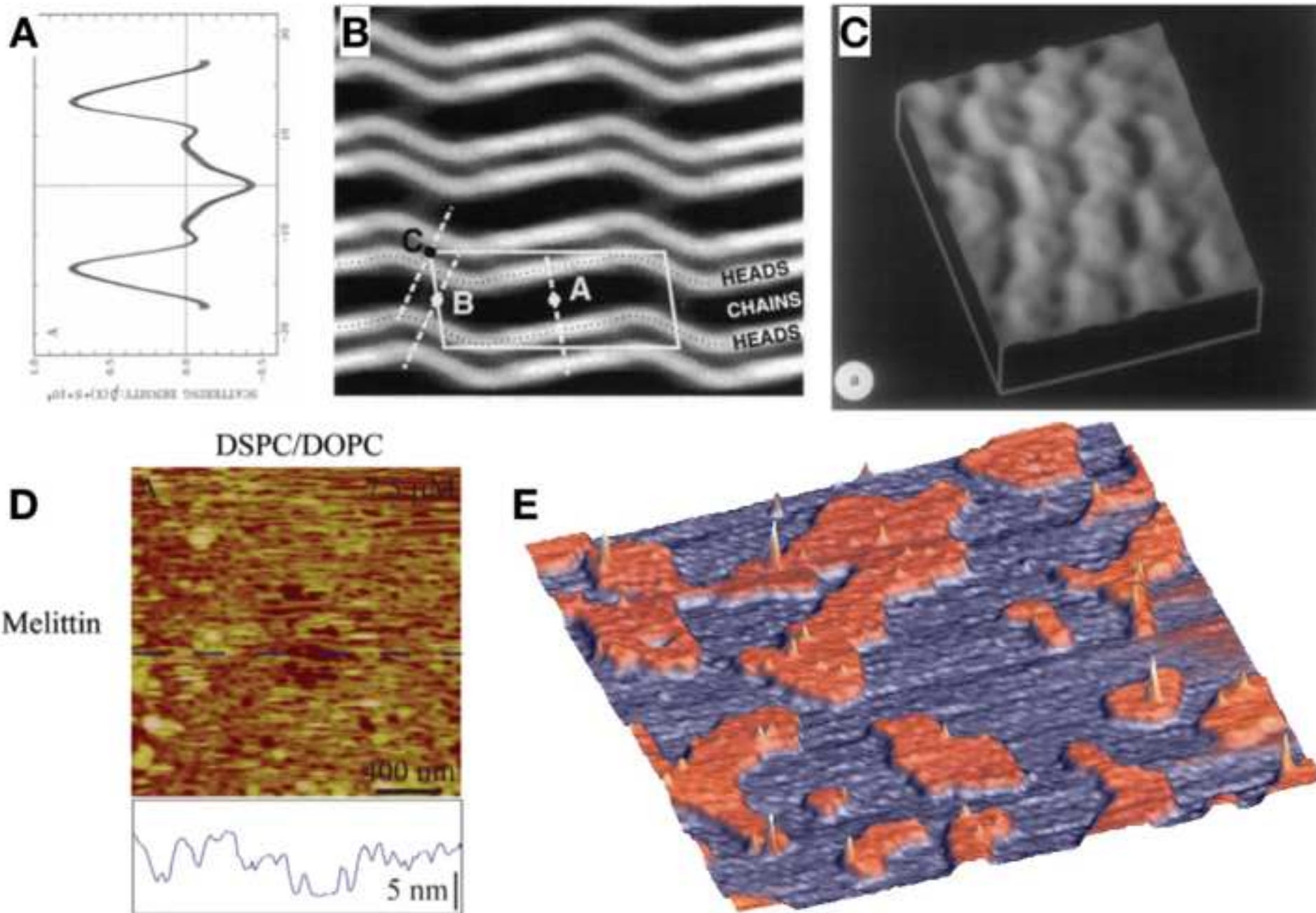


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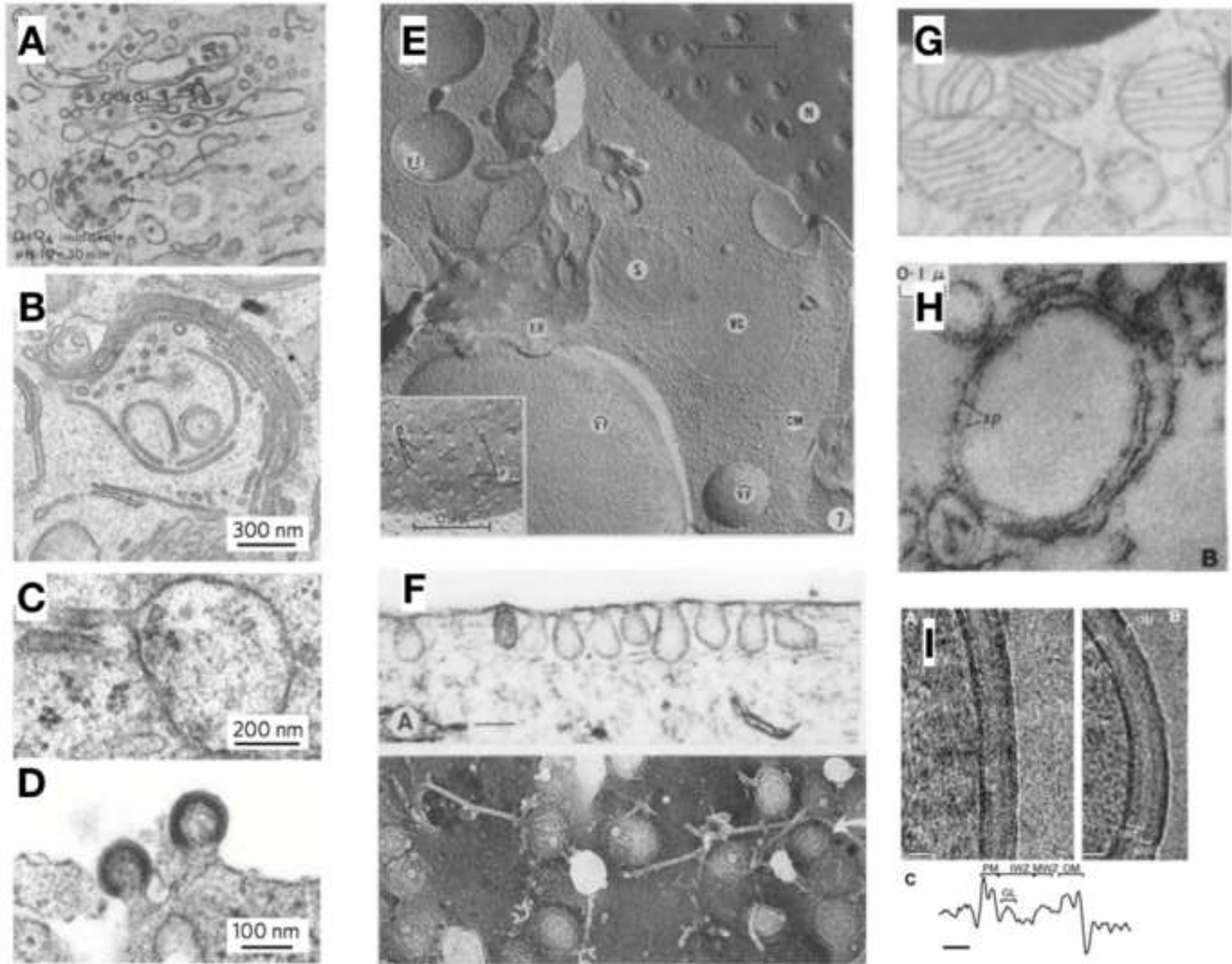


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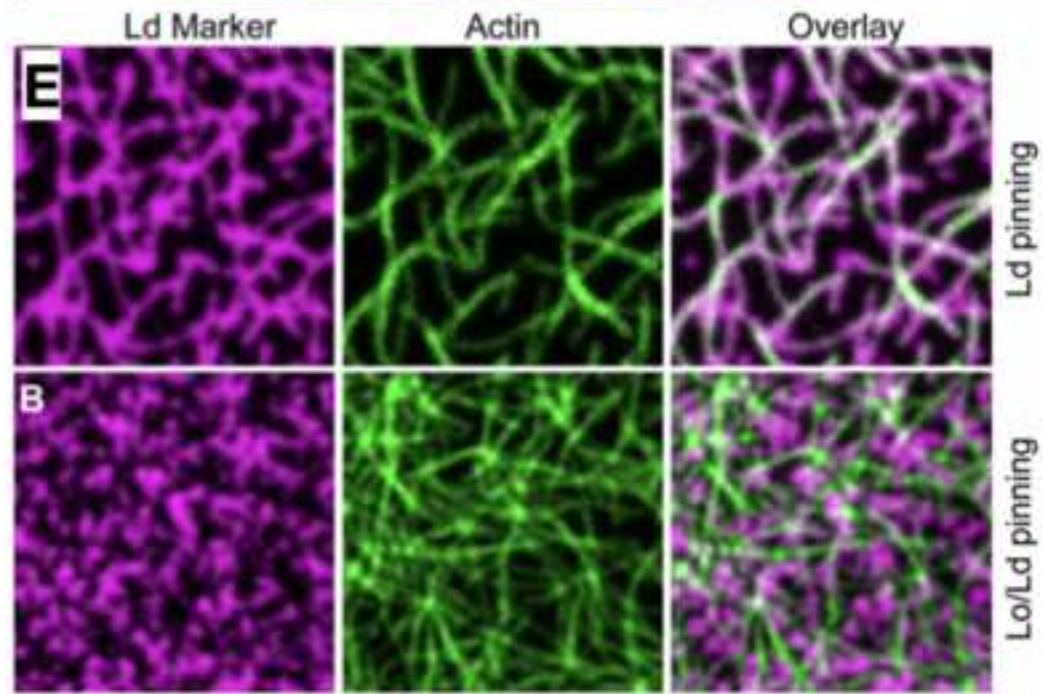
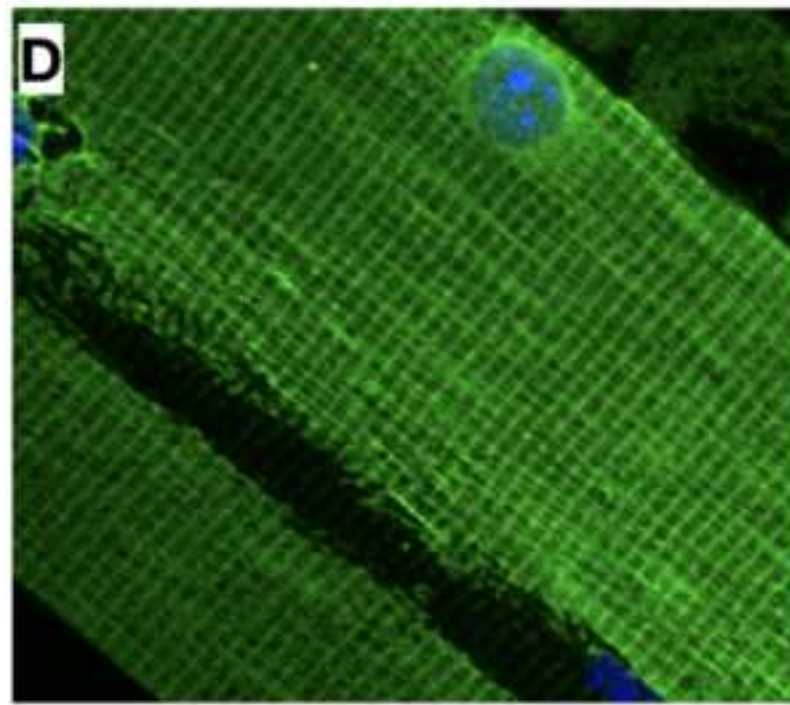
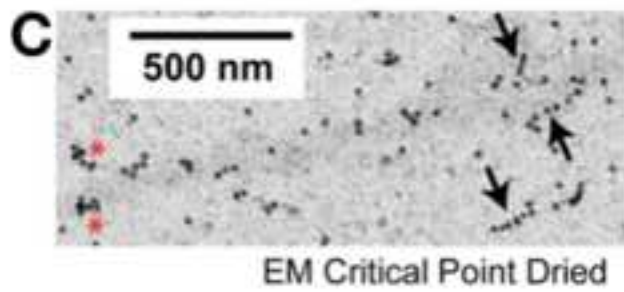
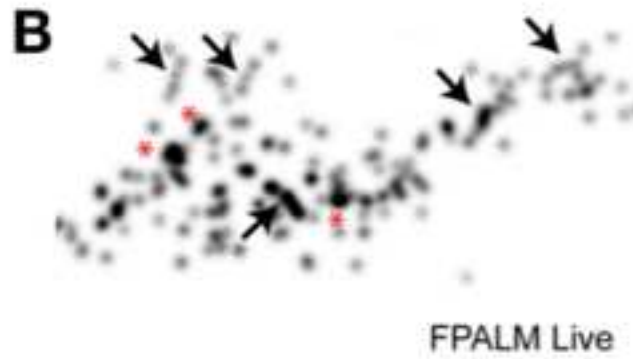
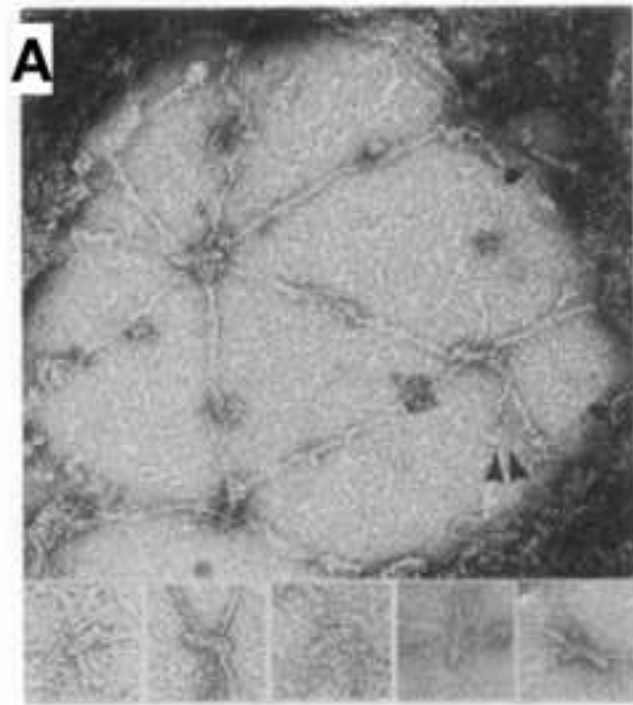


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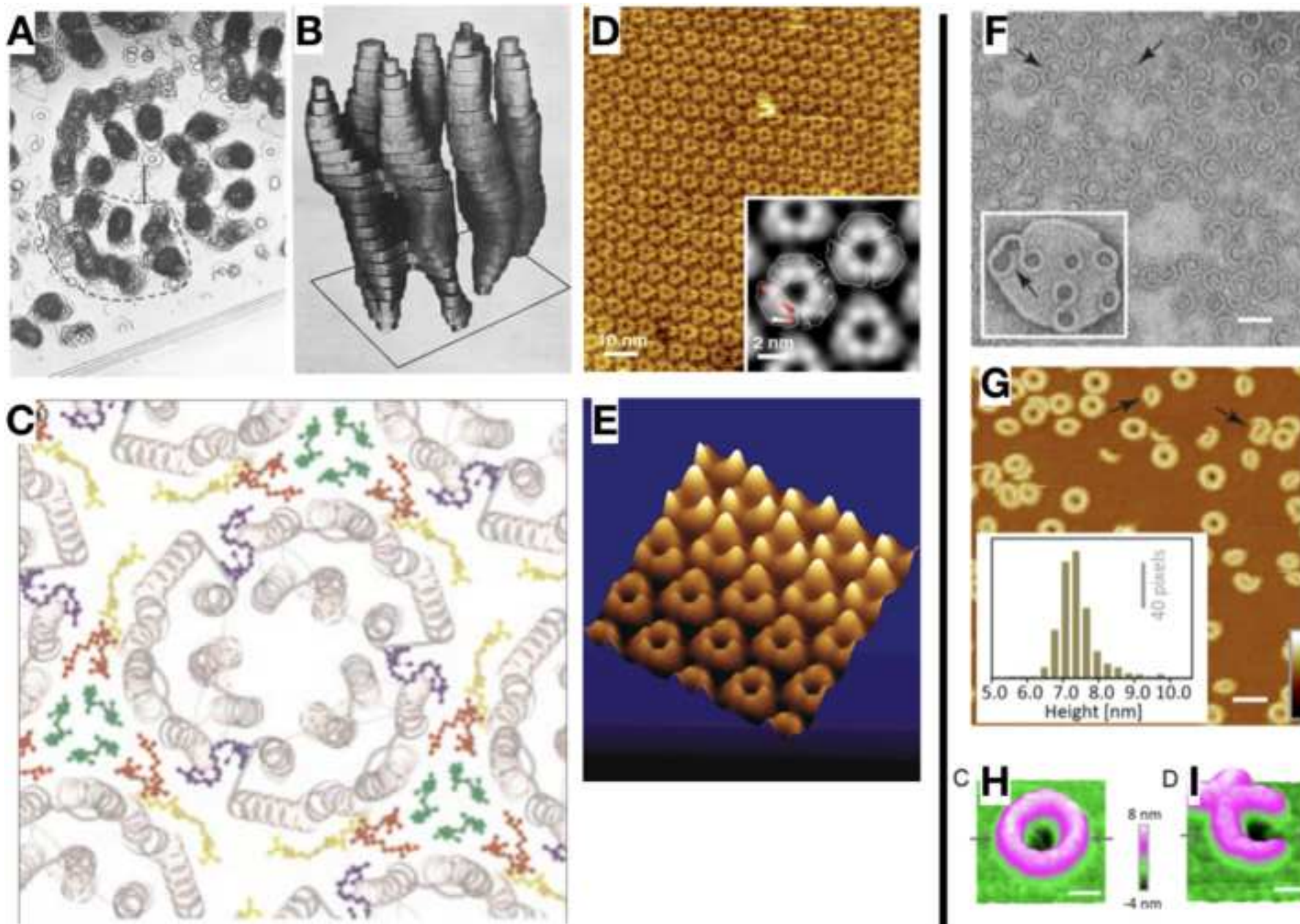


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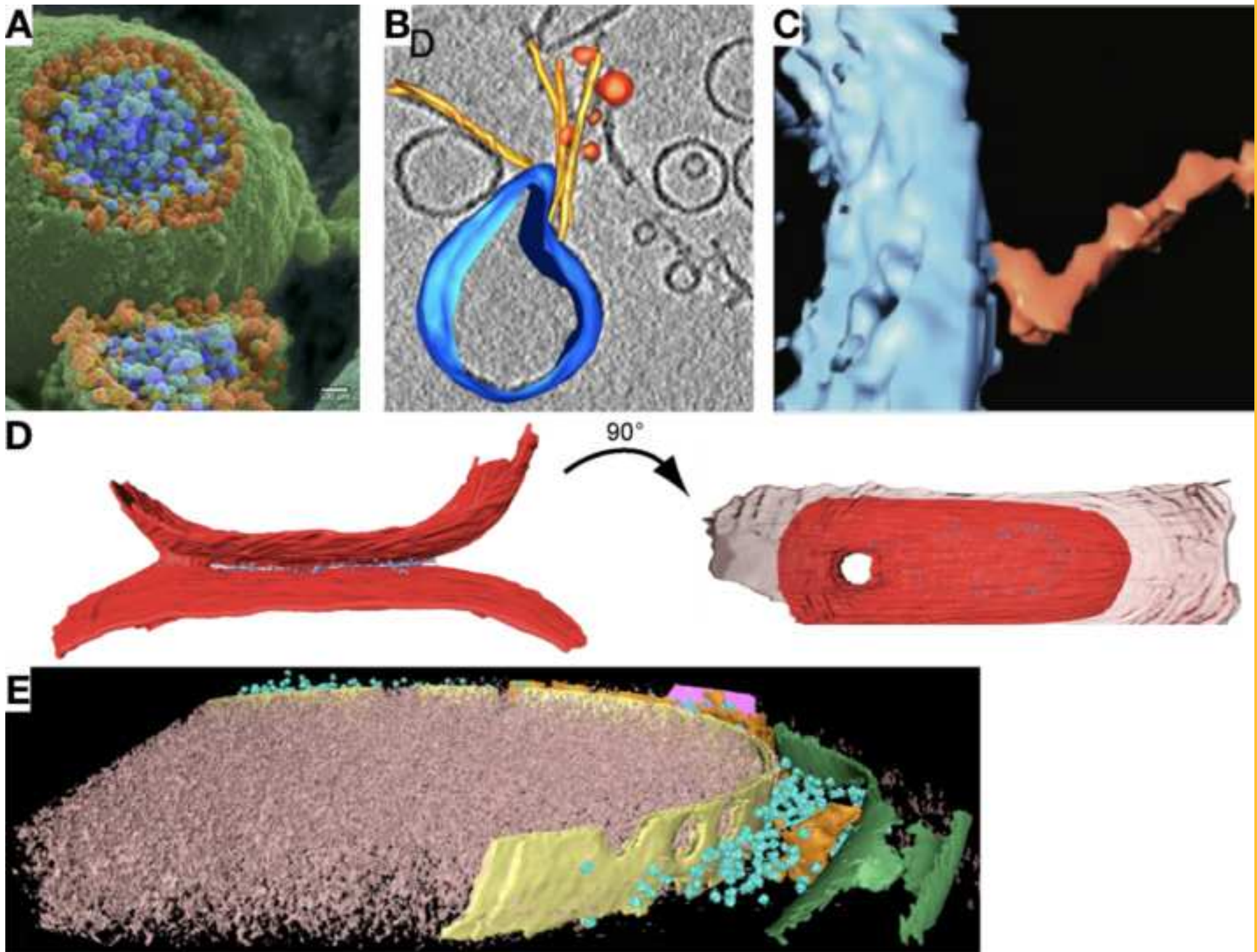


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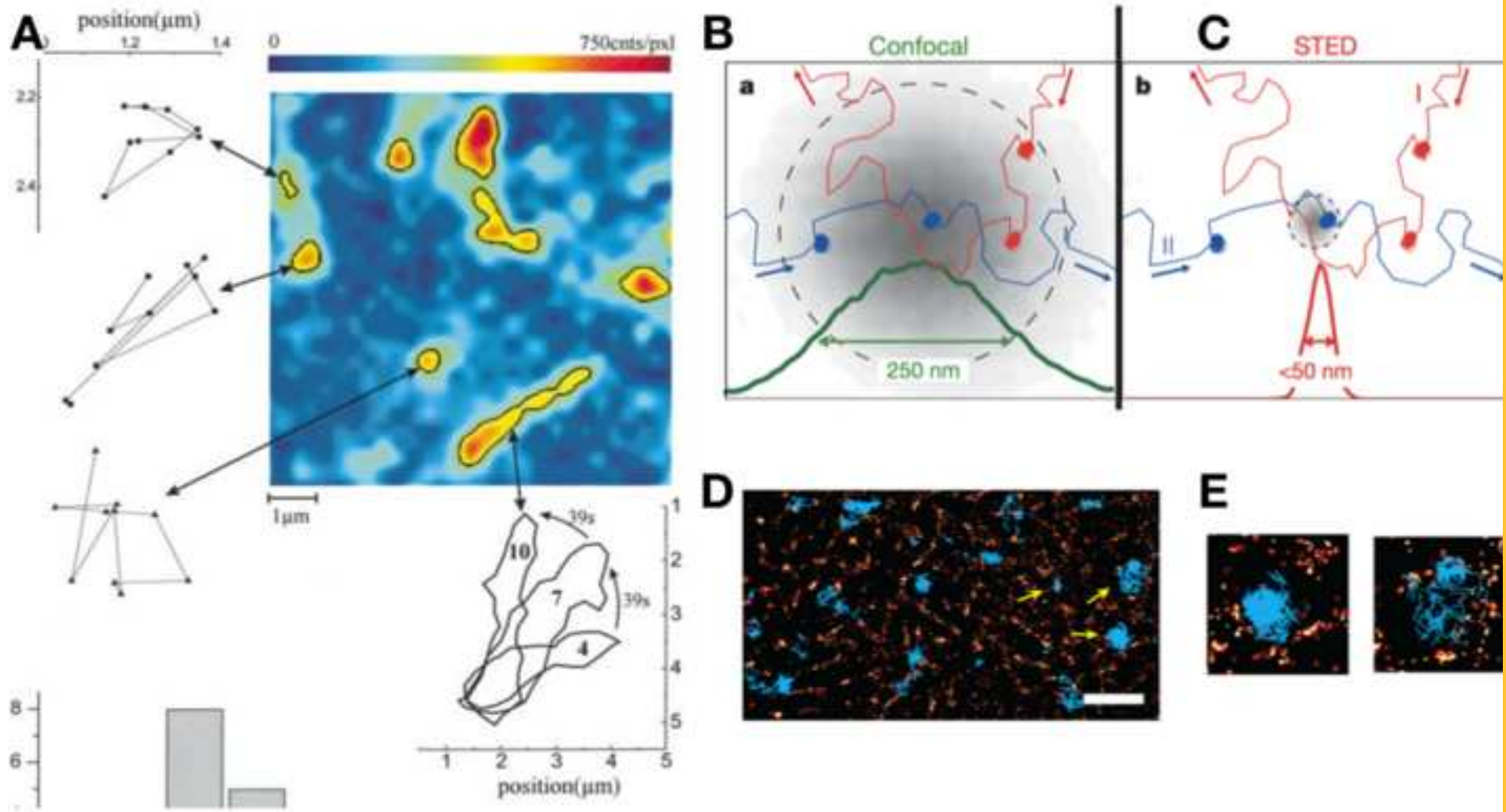


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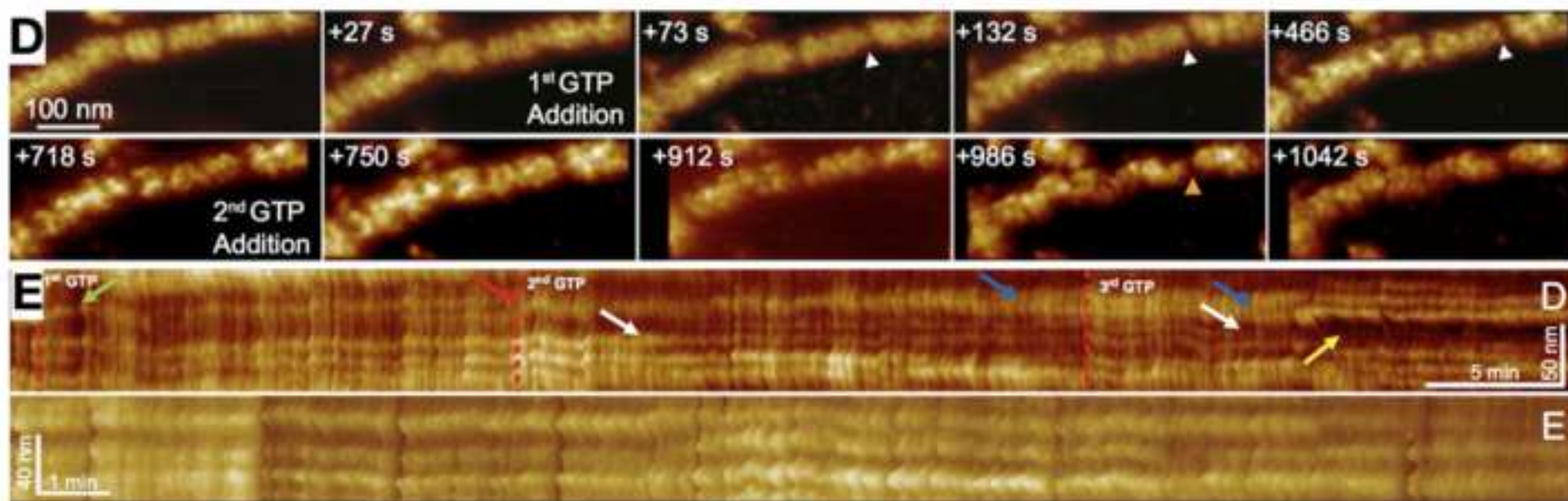
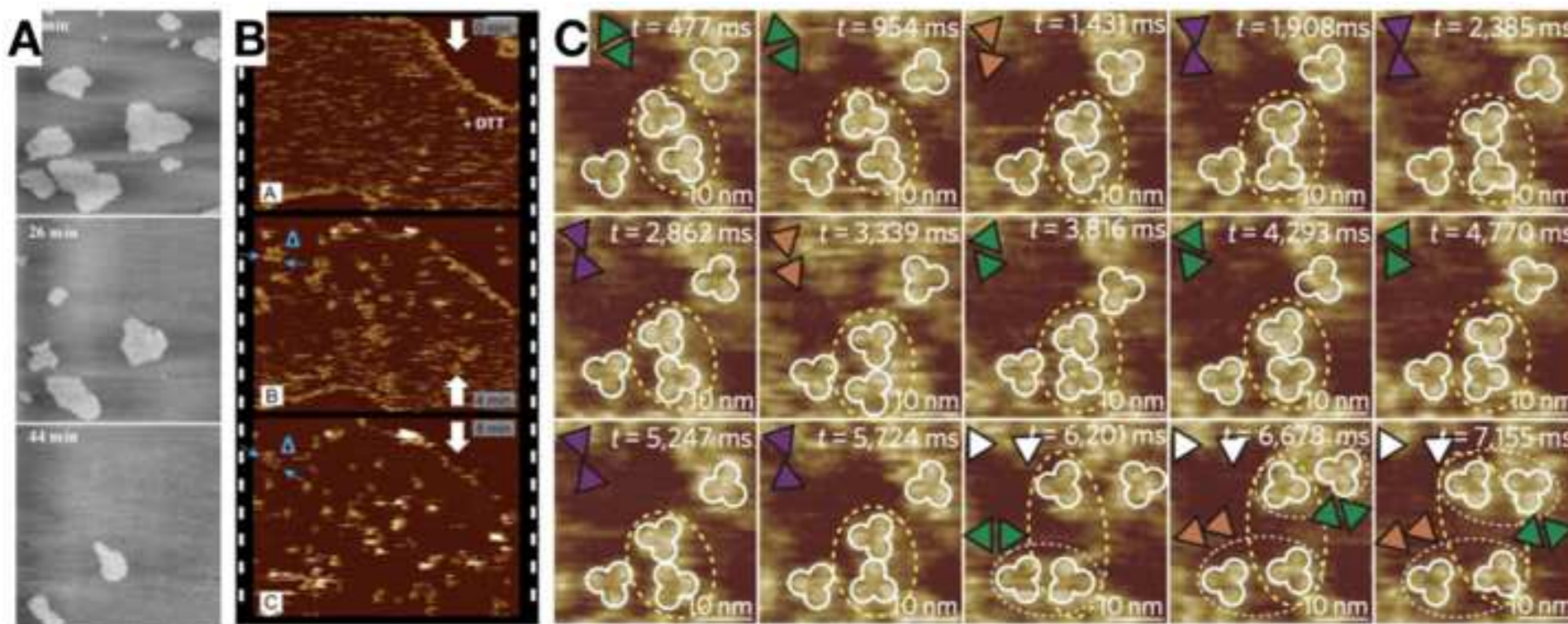


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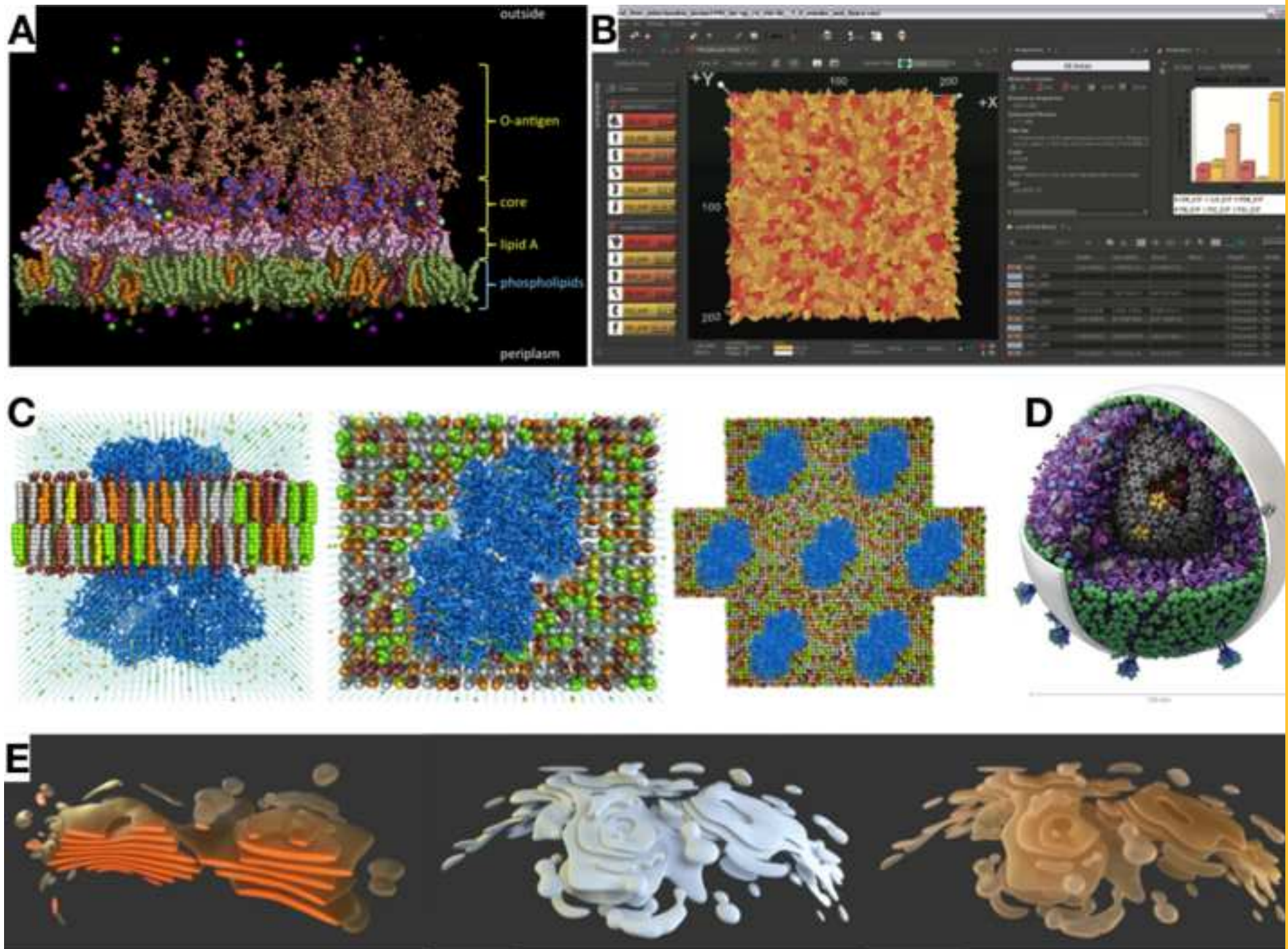


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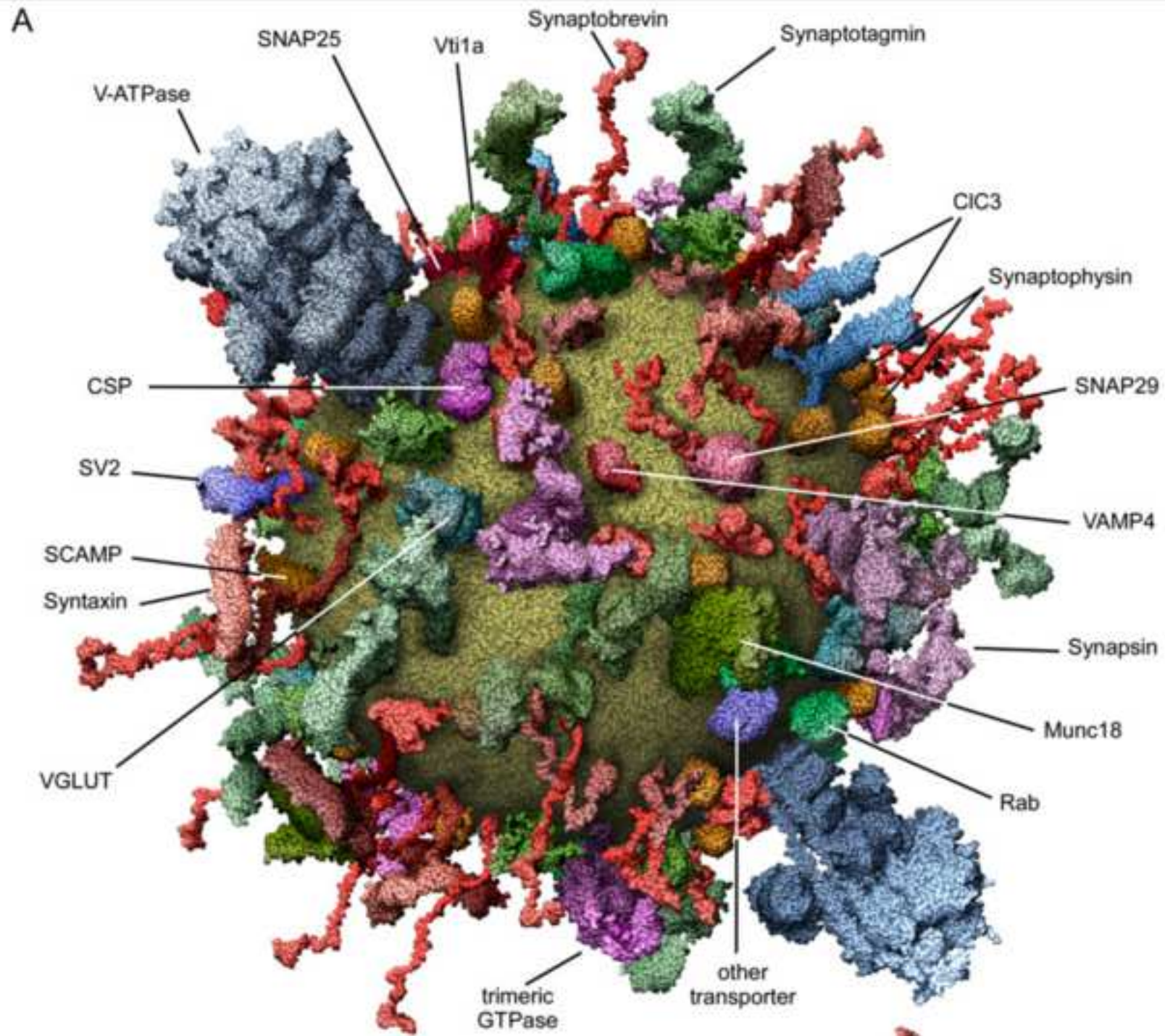


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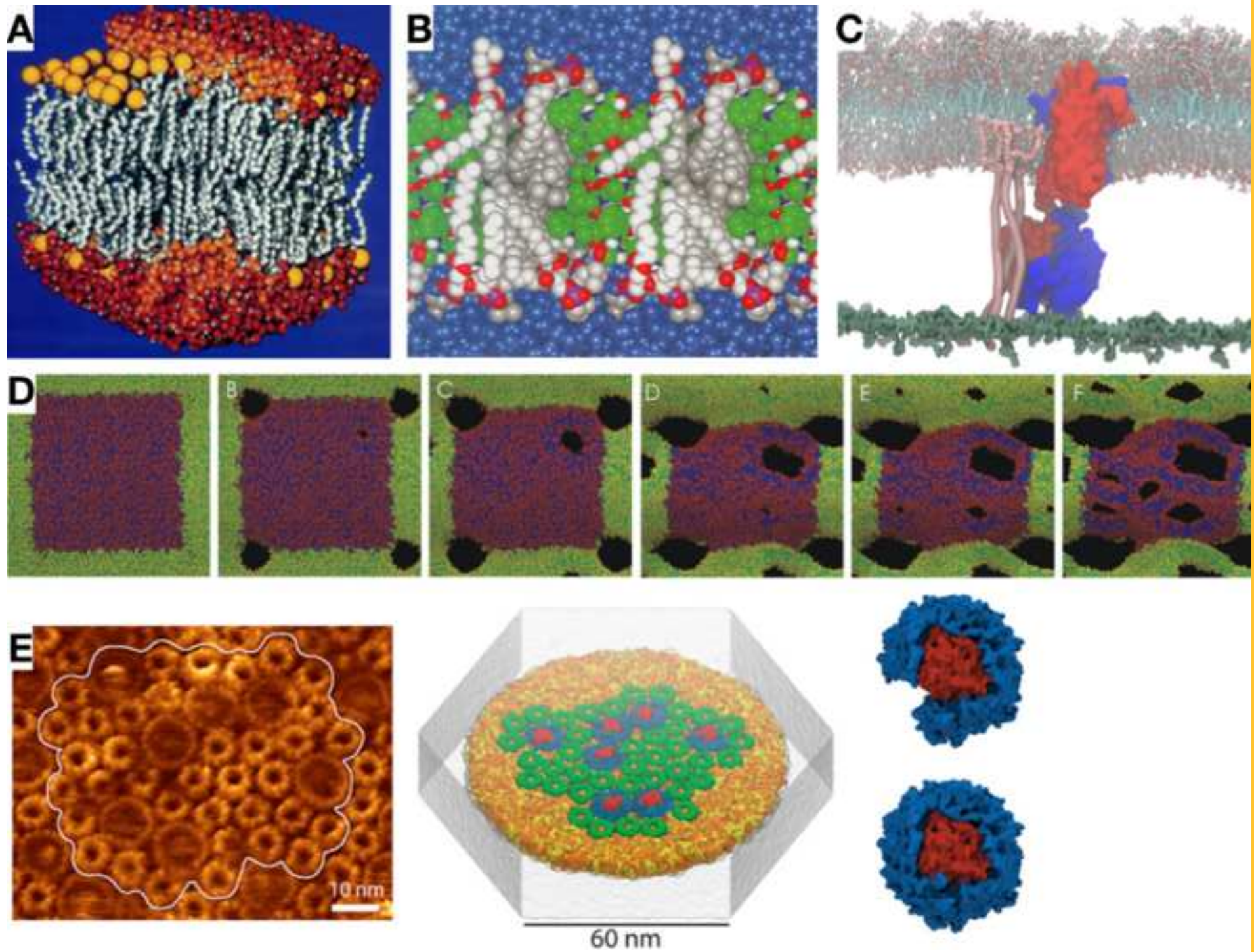


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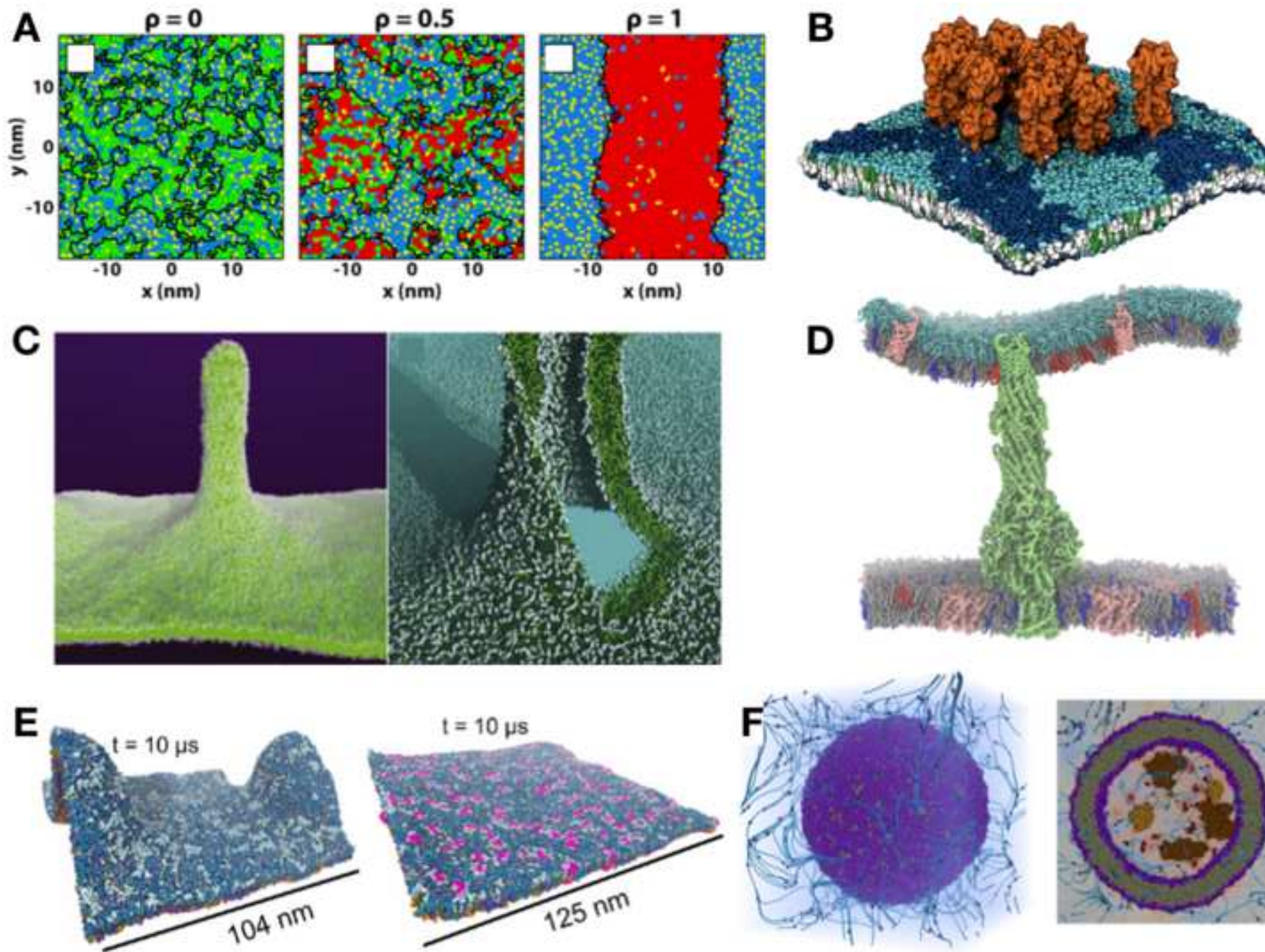


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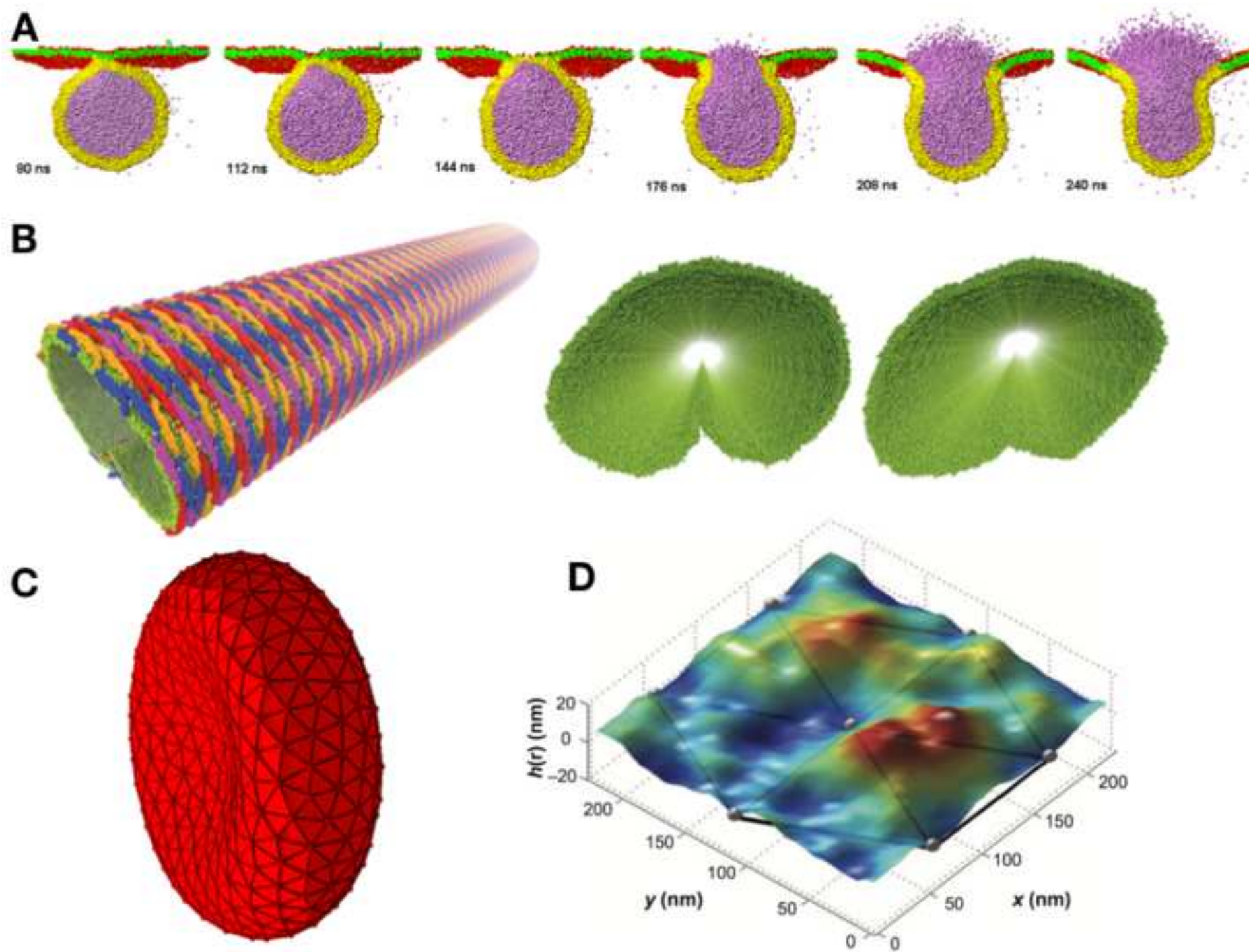


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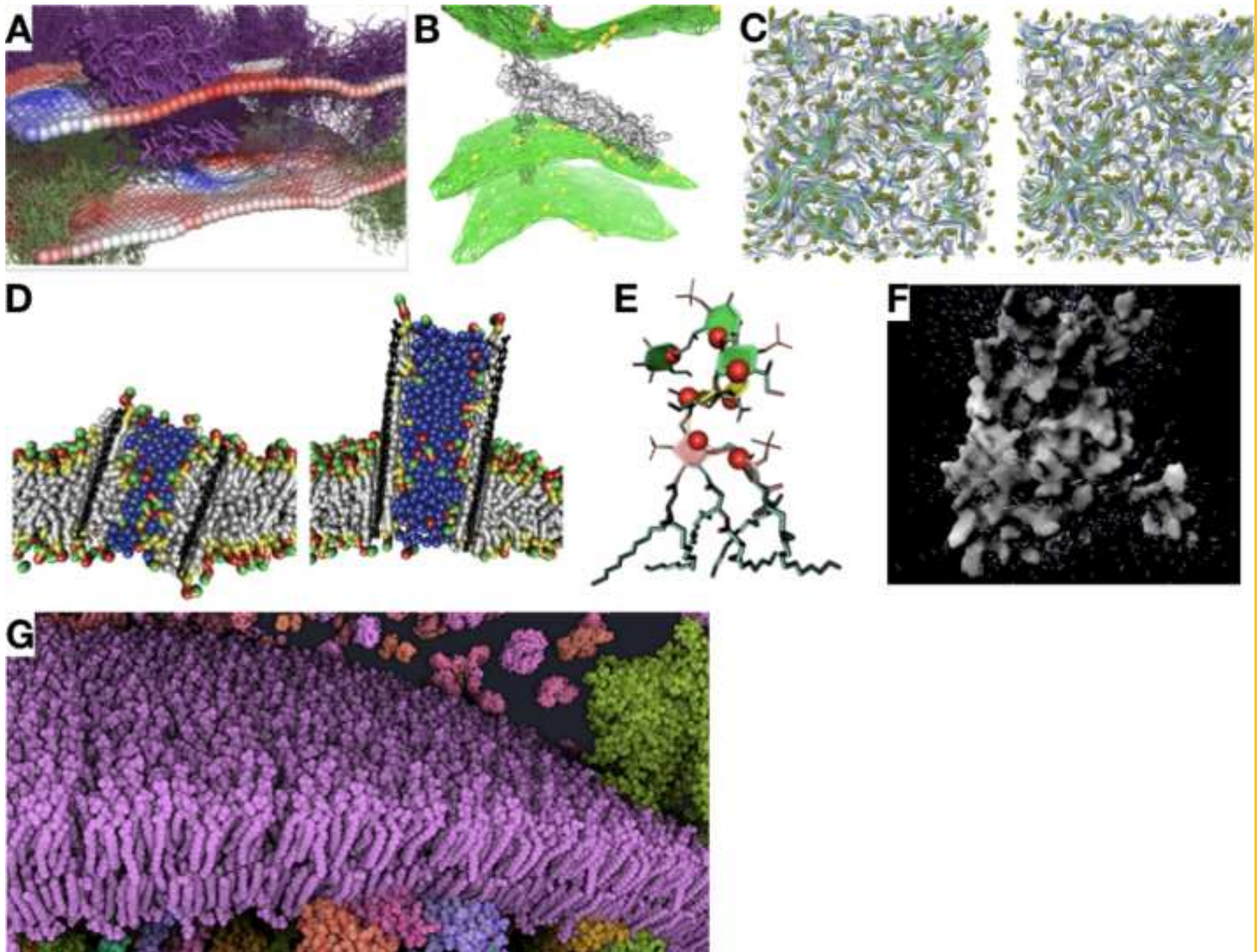


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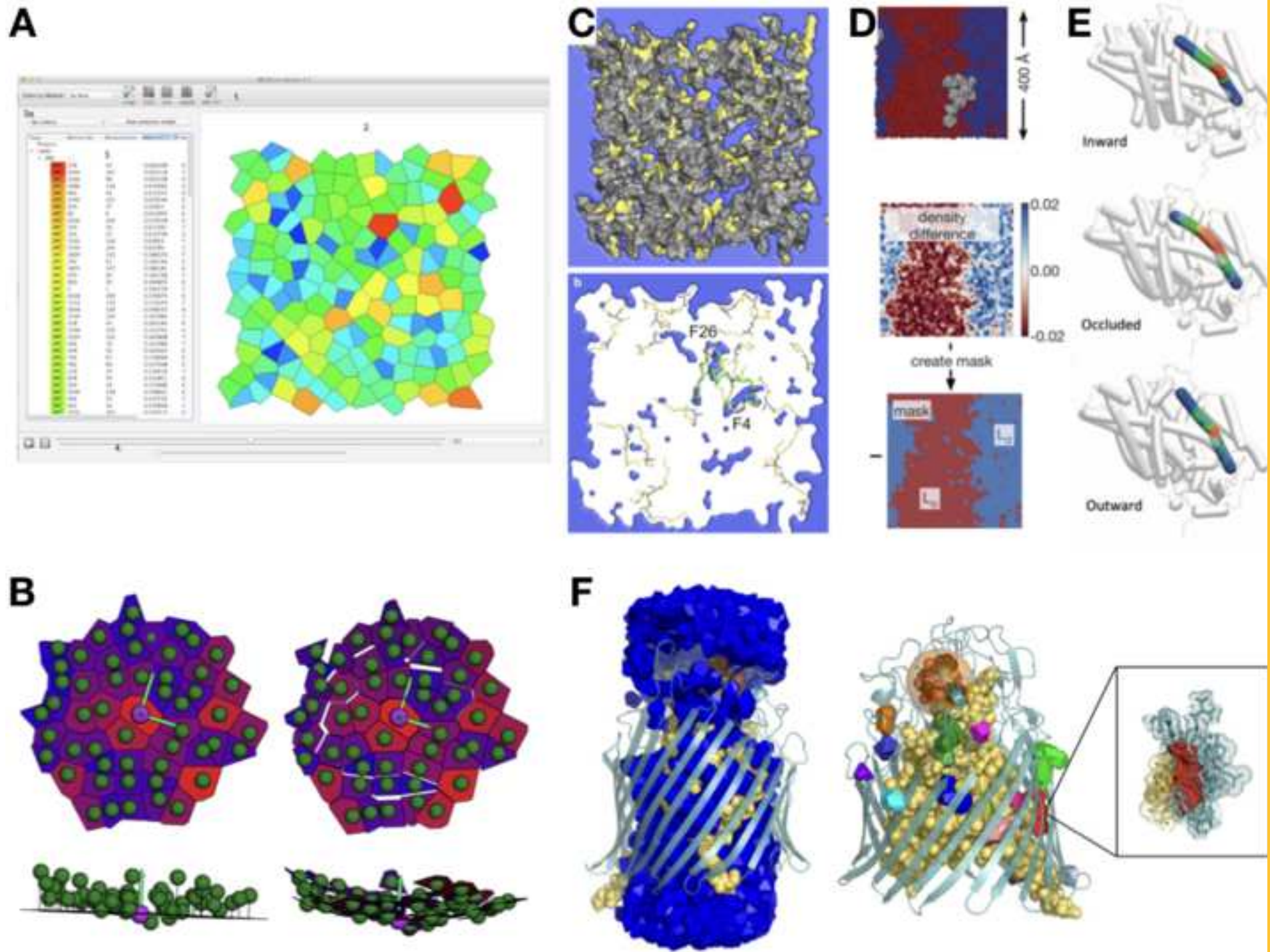


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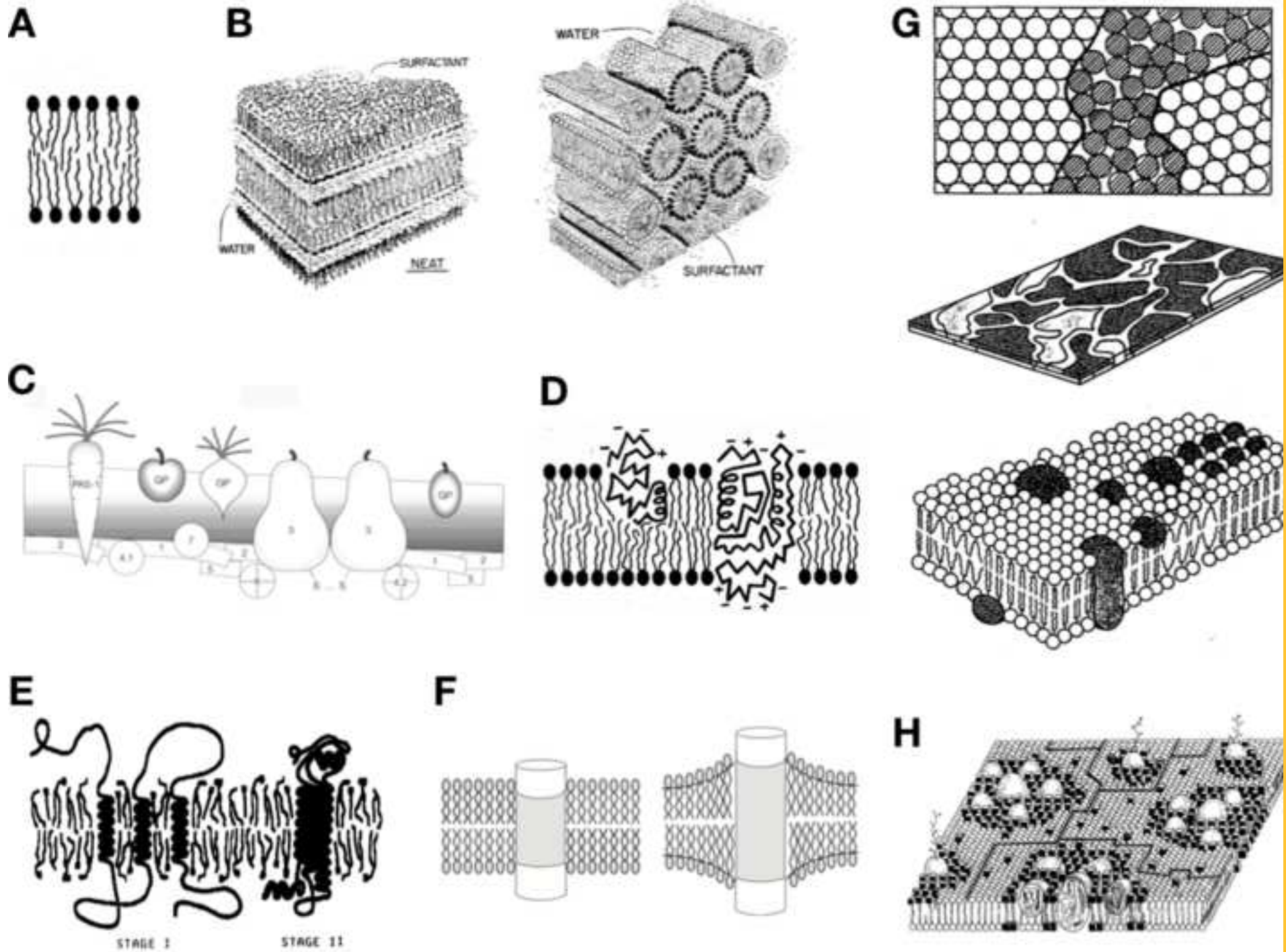


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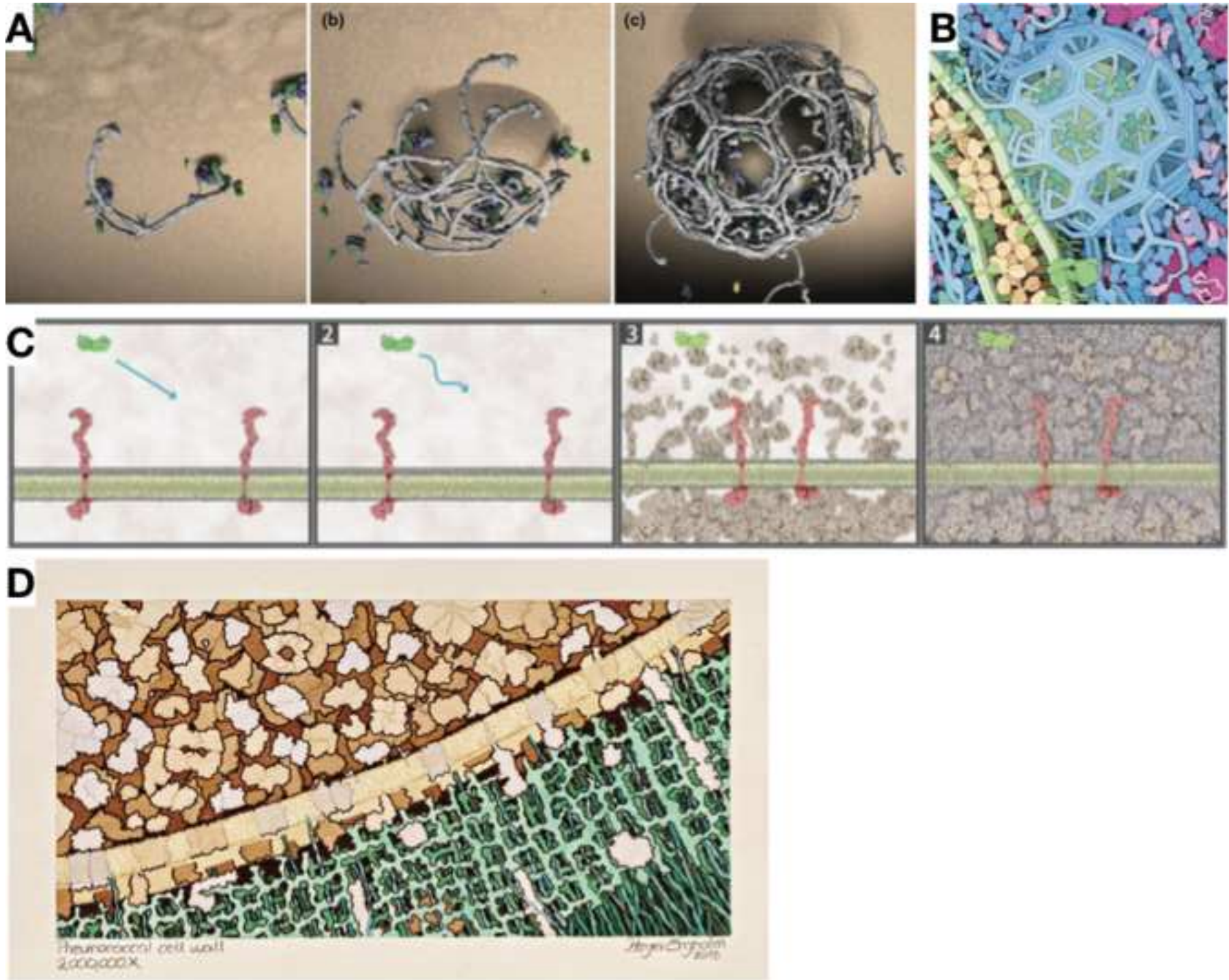
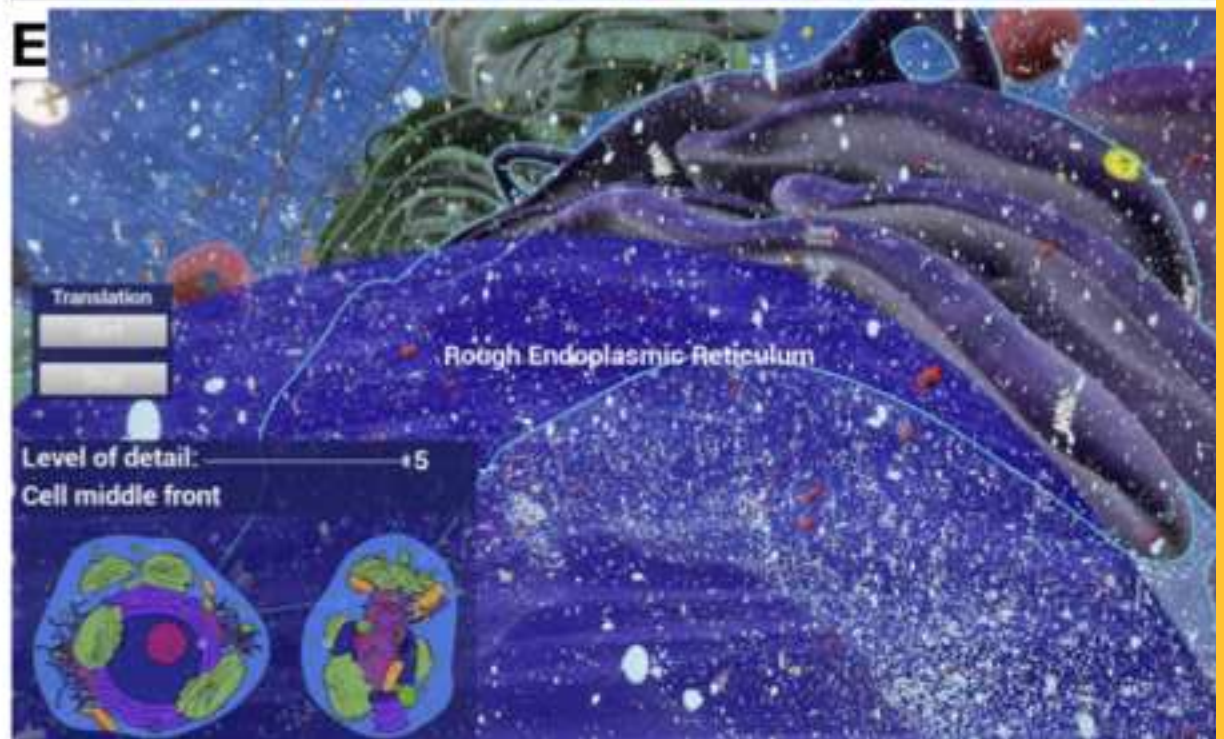
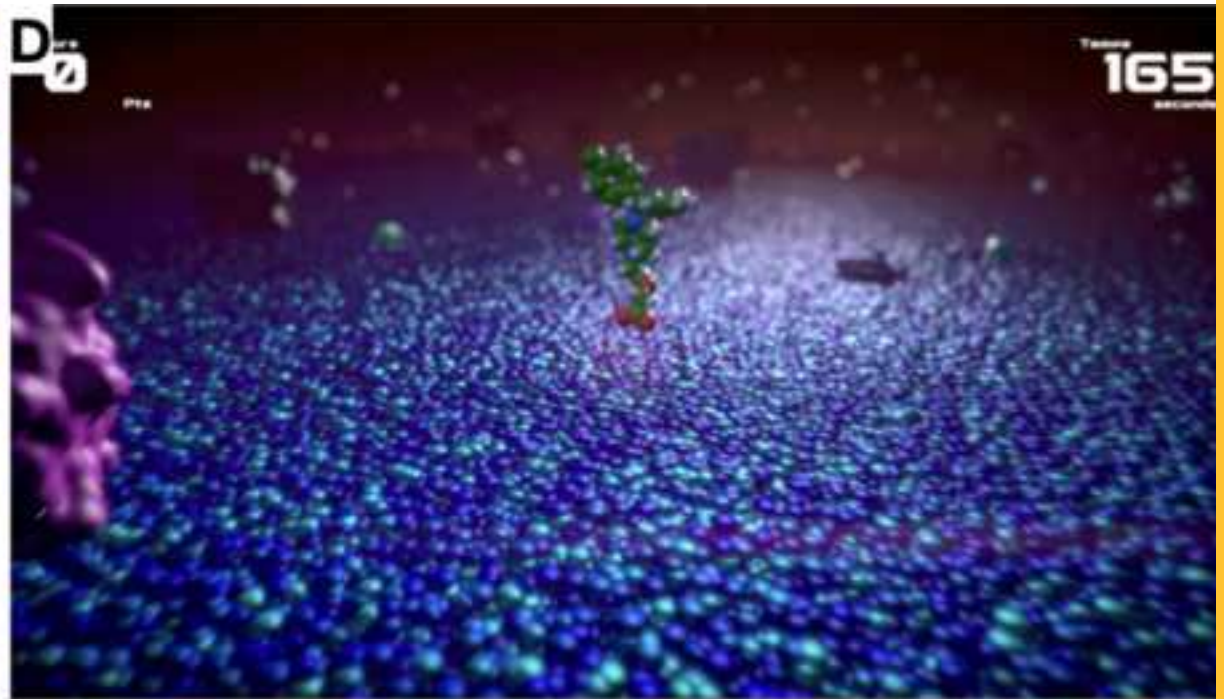
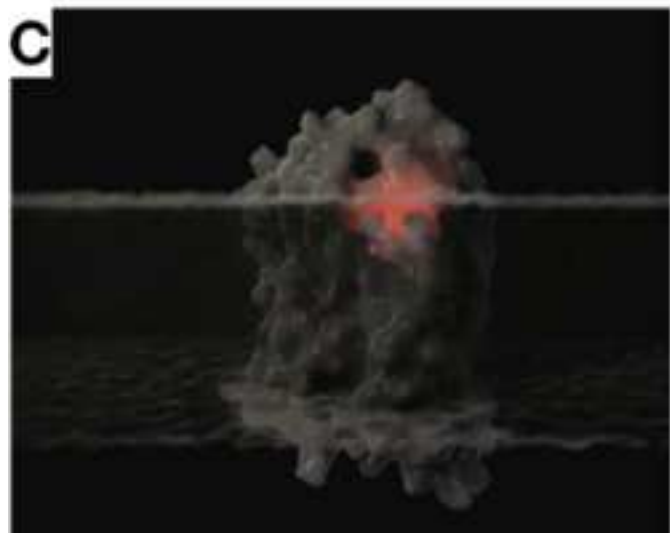
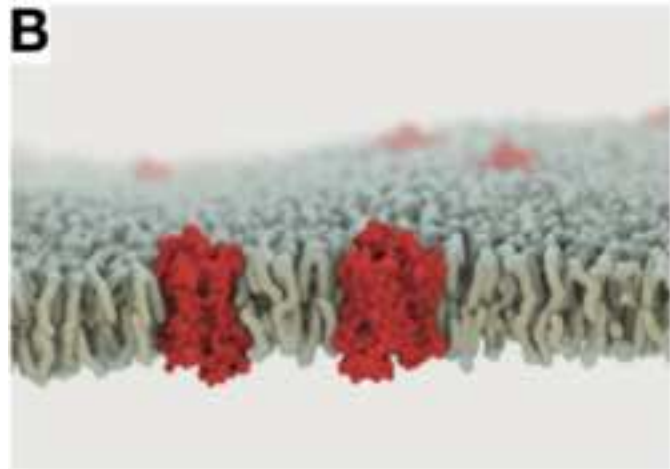
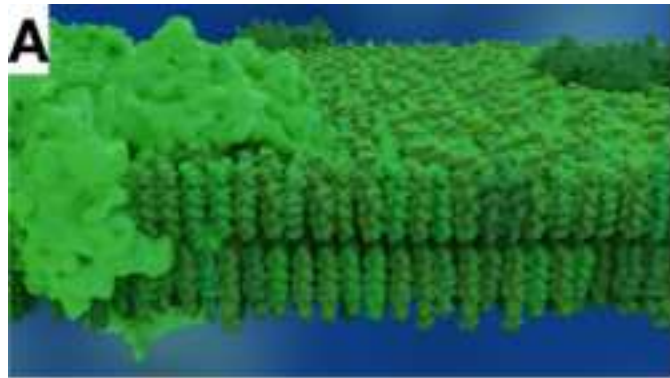
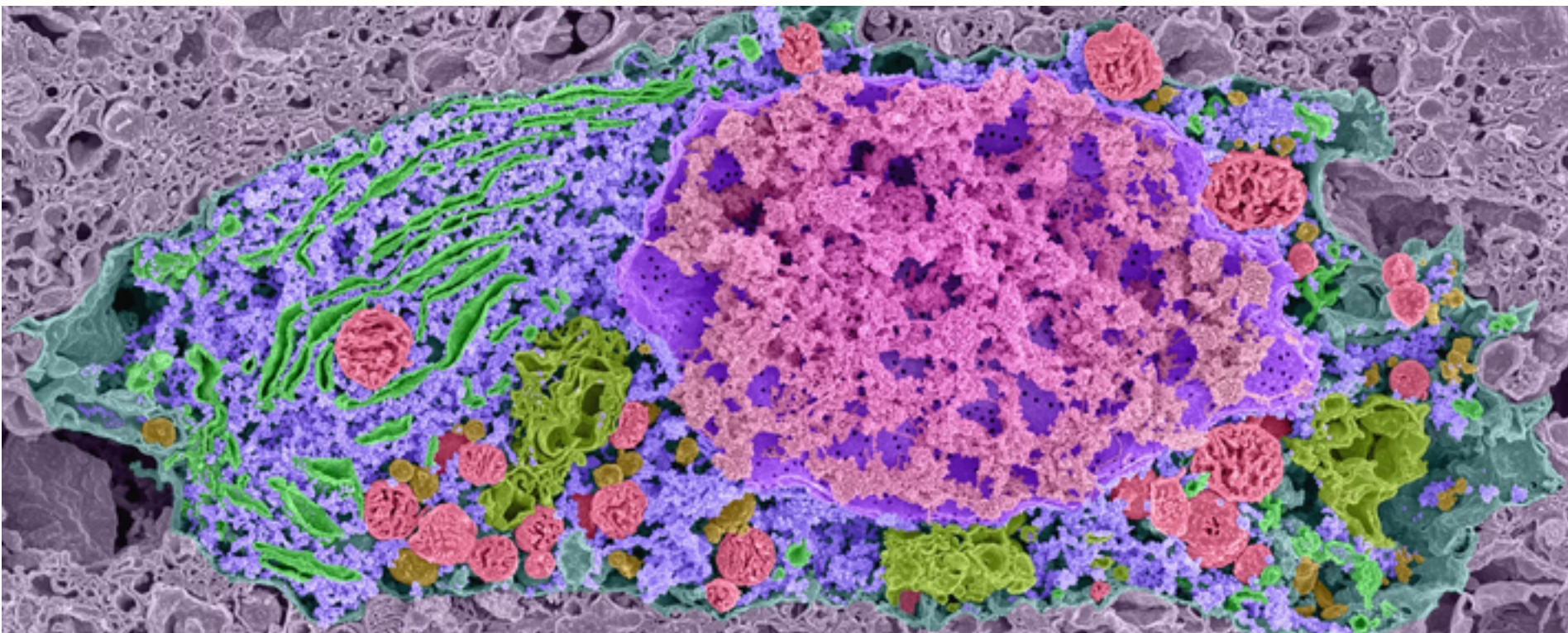


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- visualization of biological membranes has determined key aspects of modern biology
- experimental, computational and illustrative approaches shape our understanding
- membrane dynamics spans multiple time and length scales
- complexities in composition, in scales and in diversity are a major challenge
- describing membrane energetics and energy flow may be the next step