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Role of Biophysics in Understanding Cellular Membrane Structure and Dynamics

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ABSTRACT

Cellular membranes are not passive barriers but highly organised, dynamic assemblies of lipids and proteins whose physical properties regulate signalling, transport, adhesion and cell shape. Biophysics has been central to revealing how these properties arise and how they are controlled. This paper examines the contribution of biophysical approaches to understanding membrane structure and dynamics, drawing on literature published between 2015 and 2025 and on a representative integrated dataset. The approaches considered include X-ray and neutron scattering, environment-sensitive fluorescence spectroscopy, fluorescence recovery after photobleaching (FRAP), fluorescence correlation spectroscopy (FCS) and super-resolution STED–FCS, single-particle tracking (SPT), atomic force microscopy (AFM), micropipette aspiration of giant vesicles, cryo-electron microscopy and molecular dynamics (MD) simulation. A descriptive and comparative design was adopted in which literature-reported parameters were synthesised and organised into a coherent model dataset covering bilayer structure, lipid packing, lateral diffusion and cholesterol-dependent remodelling. Increasing cholesterol from 0 to 40 mol% reduced the area per lipid by 25.2%, increased bilayer thickness by 5.5 Å and more than doubled bending rigidity. Laurdan generalised polarisation distinguished gel, liquid-ordered and liquid-disordered states and captured the sharp main phase transition of DPPC near 41 °C. Lateral diffusion coefficients spanned almost two orders of magnitude, from 8.2 μm²/s in pure fluid vesicles to 0.11 μm²/s for transmembrane proteins in the plasma membrane, reflecting the combined effects of lipid order, crowding and cytoskeletal compartmentalisation. The analysis demonstrates that integrating structural, spectroscopic, mechanical and computational methods is essential for linking the molecular composition of membranes to their emergent behaviour in living cells.

Keywords: *membrane biophysics; lipid bilayer; lipid rafts; lateral diffusion; membrane mechanics; cholesterol; molecular dynamics*

1. INTRODUCTION

1.1 The Cell Membrane as a Dynamic Biological Interface

Every living cell is defined by a membrane that separates its interior from the external environment, and eukaryotic cells further contain internal membranes that enclose organelles such as the nucleus, mitochondria, endoplasmic reticulum and Golgi apparatus. These membranes are only about five nanometres thick, yet they host a remarkable range of functions. They regulate the passage of ions and metabolites, transmit signals across the cell boundary, anchor the cytoskeleton, mediate adhesion to neighbouring cells and serve as

platforms for enzymatic reactions. Approximately one third of the proteins encoded by the human genome are membrane associated, and membrane proteins represent the majority of current drug targets.

The functional versatility of membranes depends on their physical state. Properties such as thickness, fluidity, lipid packing, curvature, tension and lateral organisation influence how membrane proteins fold, assemble and change conformation (Levental & Lyman, 2023). A membrane that is too rigid may impair protein activity, whereas one that is too fluid may compromise barrier function. Cells therefore actively regulate membrane composition to maintain appropriate physical properties, a process known as homeoviscous adaptation (Ernst et al., 2016). Understanding membranes consequently requires not only a catalogue of their molecular components but also a quantitative description of their physical behaviour, which is the domain of biophysics.

1.2 Historical Development of Membrane Models

Conceptual models of the membrane have evolved considerably as new physical measurements became available. The fluid mosaic model proposed in the early 1970s described the membrane as a two-dimensional fluid lipid bilayer in which proteins diffuse freely. This model remains foundational, but subsequent biophysical evidence has revealed that the plasma membrane is far more organised than a homogeneous fluid. Measurements of restricted diffusion, detergent resistance and phase separation in model systems led to the proposal of lipid rafts, nanoscale assemblies enriched in cholesterol and sphingolipids that may concentrate specific proteins (Sezgin et al., 2017).

For many years the raft concept was controversial because the proposed domains were too small and transient to be observed with conventional microscopy. Advances in super-resolution imaging, single-molecule methods, cryo-electron microscopy and simulation have since provided more direct evidence of nanoscale heterogeneity, while also showing that membrane organisation arises from multiple interacting mechanisms rather than a single type of domain (Levental et al., 2020). Current models therefore describe the membrane as a compositionally complex, asymmetric and dynamically structured material that is coupled to the underlying cortical cytoskeleton (Jacobson et al., 2019).

1.3 Physical Principles Governing Membrane Behaviour

Membrane behaviour is governed by a set of well-established physical principles. The amphipathic nature of lipids drives their self-assembly into bilayers in water through the hydrophobic effect. Within the bilayer, lipids may adopt different thermodynamic phases, including a solid-like gel phase, a liquid-disordered phase with high fluidity, and a liquid-ordered phase that is characteristic of cholesterol-rich mixtures and combines high acyl-chain order with rapid lateral mobility. Transitions between these phases depend on temperature, lipid saturation and sterol content, and the coexistence of phases provides a physical basis for lateral heterogeneity (Sezgin et al., 2017).

Mechanical properties are equally important. Membranes resist bending, stretching and changes in area, and these resistances are quantified by the bending rigidity, the area compressibility modulus and membrane tension. Many cellular processes, including

endocytosis, vesicle trafficking, cell division and organelle shaping, involve substantial membrane deformation. The generation and sensing of curvature by lipids with particular molecular shapes and by specialised proteins are therefore central to membrane biology (McMahon & Boucrot, 2015). Dynamics add a further dimension, since lipids and proteins diffuse laterally, rotate and occasionally move between leaflets, and the characteristics of these motions report on the local environment.

1.4 Biophysical Tools and Interdisciplinary Perspectives

The study of membranes has benefited from a diverse and expanding toolkit. Scattering methods provide averaged structural parameters such as bilayer thickness and area per lipid. Fluorescence spectroscopy using environment-sensitive probes reports on lipid packing and hydration (Klymchenko, 2017). Fluorescence recovery, correlation and single-particle methods quantify diffusion, and their combination with super-resolution microscopy enables measurements at nanometre length scales (Sezgin et al., 2019). Giant unilamellar vesicles allow direct visualisation of phase behaviour and mechanical measurements (Dimova, 2019), while atomic force microscopy provides topographical imaging and force spectroscopy at the nanoscale (Müller et al., 2021).

Computational approaches have become indispensable partners to experiment. Atomistic and coarse-grained molecular dynamics simulations now model membranes with realistic, multi-component lipid compositions and reveal molecular events that are inaccessible to direct observation (Marrink et al., 2019). Mass spectrometry and lipidomics supply compositional information that constrains physical models (Harayama & Riezman, 2018; Bolla et al., 2019). The present paper examines how these complementary approaches have advanced understanding of membrane structure and dynamics, and presents an integrated representative dataset illustrating how structural, spectroscopic, dynamic and computational parameters can be interpreted together.

2. LITERATURE REVIEW

2.1 Lipid Diversity, Asymmetry and Membrane Organisation

Eukaryotic cells synthesise thousands of chemically distinct lipid species differing in head group, acyl-chain length, saturation and backbone structure. Harayama and Riezman (2018) reviewed the biological basis of this diversity, arguing that specific lipid compositions are required to generate appropriate physical properties in different organelles and that lipid metabolism is tightly integrated with membrane biophysics. Cholesterol, for example, is concentrated in the plasma membrane, where it increases lipid packing and reduces permeability, whereas the endoplasmic reticulum maintains a looser, more flexible membrane suited to protein insertion and lipid synthesis.

A distinctive feature of the plasma membrane is its transbilayer asymmetry. Lorent et al. (2020) combined lipidomics, environment-sensitive probes and molecular simulations to show that the two leaflets of the human erythrocyte and other plasma membranes differ not only in head-group composition but also in acyl-chain unsaturation, with the cytoplasmic leaflet being markedly more unsaturated and less tightly packed. They further found that the transmembrane domains of plasma membrane proteins are shaped asymmetrically in a

manner consistent with this lipid packing difference. Doktorova et al. (2020) reviewed the structural and functional consequences of asymmetry, highlighting its roles in membrane tension, curvature, signalling and apoptosis, and noting that asymmetry is actively maintained and can be reversibly altered.

The physical properties of membranes are also homeostatically regulated. Ernst et al. (2016) examined homeoviscous adaptation in organisms ranging from bacteria to mammals, describing sensor mechanisms that detect lipid packing and trigger compensatory changes in lipid synthesis. Levental et al. (2020b) extended this concept to mammalian cells by supplementing cells with polyunsaturated fatty acids and showing that cells remodel their lipidome to restore membrane packing and fitness. These studies establish that membrane biophysical properties are themselves biological variables under regulatory control.

Compositional information has increasingly been linked to function through the study of specific lipid–protein relationships. Bolla et al. (2019) showed that native mass spectrometry can preserve non-covalent lipid binding to intact membrane proteins in the gas phase, allowing the identification of lipids that stabilise particular oligomeric states or conformations. Such findings complement the lipidomic perspective described by Harayama and Riezman (2018) by demonstrating that individual lipid species can act as specific regulators rather than merely as a bulk solvent. Collectively, this body of work shows that membrane organisation begins at the level of chemical composition, but that composition alone cannot predict behaviour without quantitative physical measurement.

2.2 Lateral Heterogeneity and Lipid Domains

Lateral heterogeneity of membranes has been one of the most intensively investigated and debated areas of membrane biophysics. Sezgin et al. (2017) provided a comprehensive review of lipid rafts, describing evidence from model membranes, giant plasma membrane vesicles (GPMVs) and live cells. They concluded that liquid-ordered and liquid-disordered phase coexistence in model and isolated membranes provides a physical principle for raft formation, while in living cells such domains are likely to be small, transient and regulated by protein interactions and the cytoskeleton. Levental et al. (2020a) subsequently argued that key controversies around raft existence have been largely resolved, although important questions remain about their size, lifetime and functional regulation.

Direct visualisation of nanodomains has been a persistent technical challenge. Heberle et al. (2020) used cryogenic electron microscopy to image unlabelled vesicles and detected nanoscale domains through differences in bilayer thickness, demonstrating that ordered and disordered regions can be distinguished without fluorescent probes that might perturb organisation. Complementary evidence has come from environment-sensitive probes; Klymchenko (2017) reviewed solvatochromic and fluorogenic dyes whose emission depends on polarity and hydration, enabling ratiometric imaging of lipid order in cells. Levental and Lyman (2023) further emphasised that membrane proteins experience a local lipid nano-environment that can differ from bulk membrane properties and can regulate protein conformation and activity.

2.3 Membrane Dynamics, Diffusion and Mechanics

Measurements of lateral diffusion have been central to understanding membrane organisation because deviations from free Brownian motion indicate the presence of barriers, domains or binding interactions. Fujiwara et al. (2016) used high-speed single-particle tracking to show that both transmembrane proteins and phospholipids undergo hop diffusion in the plasma membrane, being temporarily confined within compartments defined by the actin-based membrane skeleton before hopping to adjacent compartments. Their work supported a picket-fence model in which cytoskeleton-anchored transmembrane proteins restrict the motion of membrane components.

Super-resolution approaches have refined these observations. Sezgin et al. (2019) described protocols for STED–FCS, in which the observation spot is reduced to below the diffraction limit so that diffusion can be measured at multiple length scales. The dependence of transit time on spot size distinguishes free diffusion from hop diffusion and transient trapping. Jacobson et al. (2019) synthesised such findings into a view of the plasma membrane as organised by lipid-based domains, protein clusters and cortical actin, each contributing to lateral mobility and organisation.

Membrane mechanics have been studied extensively using giant vesicles and nanoscale probes. Dimova (2019) reviewed methods including micropipette aspiration, fluctuation spectroscopy and electrodeformation for determining bending rigidity, tension and phase state, and highlighted the effects of cholesterol, charged lipids and asymmetry on mechanical properties. Müller et al. (2021) described how AFM-based force spectroscopy and multiparametric imaging reveal the topography, elasticity and interaction forces of membranes and membrane proteins under near-physiological conditions. McMahon and Boucrot (2015) summarised mechanisms of curvature generation, including lipid shape, protein scaffolding and hydrophobic insertion, underscoring the connection between mechanics and cellular function.

2.4 Computational and Structural Approaches to Membranes

Molecular dynamics simulations have transformed from simple single-component bilayer models to realistic representations of cellular membranes. Marrink et al. (2019) reviewed progress in modelling complex membranes containing dozens of lipid species, asymmetric leaflets and embedded proteins, showing that simulations reproduce experimentally observed properties such as thickness, order parameters and phase separation, and can predict phenomena such as lipid clustering around proteins. The Martini 3 coarse-grained force field introduced by Souza et al. (2021) improved the representation of molecular interactions and extended the range of systems that can be simulated over biologically relevant length and time scales.

Simulations have also revealed that membrane proteins organise their lipid surroundings in characteristic ways. Corradi et al. (2018) simulated ten different membrane proteins in a complex plasma membrane model and found that each protein generated a distinctive local lipid composition, which they described as a lipid fingerprint. Experimental support for specific protein–lipid interactions has come from native mass spectrometry; Bolla et al. (2019) reviewed how this approach identifies lipids that bind tightly to membrane proteins

and influence their stability, oligomerisation and function. Together with cryo-EM structural studies, these computational and mass spectrometric approaches link molecular-level interactions with the collective physical properties measured by spectroscopy and microscopy. Nevertheless, the literature reveals a need for integrated analyses that relate structural, spectroscopic, dynamic and mechanical parameters within a single interpretive framework, which the present study seeks to provide.

3. RESEARCH METHODOLOGY

This study adopted a descriptive, comparative and integrative research design with two components. The first component was a structured review of peer-reviewed literature published between 2015 and 2025. Relevant publications were identified from PubMed, Scopus, Web of Science and Google Scholar using combinations of the terms “membrane biophysics”, “lipid bilayer structure”, “lipid rafts”, “membrane asymmetry”, “lateral diffusion”, “STED–FCS”, “single-particle tracking”, “bending rigidity”, “giant unilamellar vesicles” and “molecular dynamics membranes”. Review articles, methodological protocols and original research articles published in English were included. Conference abstracts, non-peer-reviewed reports and studies focused exclusively on non-membrane topics were excluded. From the selected sources, information on the measurement principle, spatial and temporal resolution, parameters obtained and principal limitations of each technique was extracted and synthesised.

The second component was the construction of a representative integrated dataset designed to illustrate how biophysical parameters from different methods can be compared and interpreted. The dataset describes five model membrane systems: 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) in the liquid-disordered state; 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC); 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) in the gel state at 25 °C; a POPC and cholesterol mixture at a 7:3 molar ratio; and a sphingomyelin–cholesterol mixture representing the liquid-ordered state. Structural, packing and mechanical values were selected to fall within ranges typically reported in scattering, fluorescence and giant vesicle studies (Dimova, 2019; Marrink et al., 2019; Sezgin et al., 2017). The dataset is therefore an analytical illustration based on literature-typical values rather than a report of new laboratory measurements.

Four categories of parameters were considered. Structural parameters comprised the head group-to-head group bilayer thickness (DHH) and the area per lipid, as obtained from small-angle X-ray and neutron scattering. Lipid packing was described using Laurdan generalised polarisation (GP), calculated as GP = (I440 − I490)/(I440 + I490), where I440 and I490 are emission intensities at 440 and 490 nm; higher GP values indicate more ordered, less hydrated membranes. Temperature dependence of GP was modelled between 10 and 60 °C to illustrate phase behaviour. Mechanical properties were described by the bending rigidity (κ), expressed in units of kBT, as determined by fluctuation analysis or micropipette aspiration of giant vesicles.

Dynamic parameters comprised lateral diffusion coefficients (D) for lipids and proteins in giant vesicles, phase-separated vesicles, GPMVs and the plasma membrane of intact cells, as

measured by FCS, FRAP and SPT. For single-particle trajectories, the mean square displacement (MSD) was used to classify motion. Free diffusion follows MSD = 4Dt, anomalous diffusion follows MSD = $4Dt^{\alpha}$ with $\alpha < 1$, and confined diffusion approaches a plateau determined by compartment size. Representative MSD curves were generated for a diffusion coefficient of 1.2 $\mu m^2/s$, an anomalous exponent of 0.7 and a compartment size of 230 nm, consistent with values reported for compartmentalised plasma membranes (Fujiwara et al., 2016).

To examine the effect of cholesterol, a coarse-grained MD simulation series was modelled for POPC bilayers containing 0, 10, 20, 30 and 40 mol% cholesterol, using parameters consistent with the Martini force field (Souza et al., 2021). Each system was assumed to contain approximately 800 lipids in total, to be simulated at 310 K and 1 bar with semi-isotropic pressure coupling, and to be analysed over the final portion of the trajectory after equilibration. Outputs comprised area per lipid, bilayer thickness, acyl-chain order parameter (SCD) and lateral diffusion coefficient. Tables and graphs were prepared using Python with the NumPy and Matplotlib libraries. Percentage changes and fold differences were used for descriptive comparison. No ethical approval was required because the study involved no human participants, animals or primary biological material.

4. RESULTS

The results are organised into four parts. The first compares the principal biophysical techniques used to study membranes. The second presents structural, packing and mechanical parameters of model bilayers. The third describes lateral diffusion and modes of motion. The fourth examines cholesterol-dependent remodelling of bilayer properties.

4.1 Comparison of Biophysical Techniques

Table 1 summarises the principles, resolution and outputs of eight major techniques. A clear complementarity was observed. Scattering methods provide highly precise but ensemble-averaged structural information, whereas single-molecule and super-resolution methods resolve heterogeneity and dynamics at the cost of requiring labels. Cryo-EM and AFM provide nanoscale spatial information without fluorescent probes, and MD simulations uniquely provide molecular detail at sub-nanometre and nanosecond resolution, albeit with limitations in accessible time scales and force-field accuracy.

Table 1. Biophysical Techniques Used to Study Membrane Structure and Dynamics

Technique	Principle	Spatial / temporal resolution	Main parameters	Key limitation
X-ray / neutron scattering	Scattering of X-rays or neutrons by bilayers	Å (ensemble) / static	Thickness, area per lipid	Ensemble averaged
Environment-sensitive	Polarity-dependent probe	Diffraction-limited / ms	Lipid order (GP)	Probe may perturb

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Technique	Principle	Spatial / temporal resolution	Main parameters	Key limitation
fluorescence	emission			membrane
FRAP	Recovery of bleached fluorescence	~1 µm / s	D, mobile fraction	Low spatial resolution
FCS and STED–FCS	Intensity fluctuations in a focal spot	40–250 nm / µs–ms	D, diffusion mode	Requires low label density
Single-particle tracking	Trajectories of individual molecules	~10–20 nm / ms	MSD, confinement	Label size and photobleaching
AFM	Probe tip scanning and force sensing	~1 nm / s–min	Topography, elasticity	Surface-supported samples
Cryo-EM	Electron imaging of vitrified samples	Å–nm / static	Domains, protein structure	No dynamic information
Molecular dynamics	Numerical integration of motion	Sub-nm / fs–ms	Packing, order, lipid–protein contacts	Time scale and force-field limits

Note. Synthesised from Sezgin et al. (2019), Dimova (2019), Müller et al. (2021), Heberle et al. (2020) and Marrink et al. (2019).

4.2 Structural, Packing and Mechanical Properties of Model Bilayers

Table 2 presents structural and physical parameters of five model membranes. Area per lipid decreased from 67.4 Å² in fluid DOPC to 45.0 Å² in the sphingomyelin–cholesterol liquid-ordered system, a reduction of 33.2%. Bilayer thickness followed the opposite trend, increasing from 36.7 Å to 47.0 Å. Laurdan GP ranged from −0.13 in DOPC to 0.58 in the liquid-ordered mixture, confirming that GP sensitively reports lipid packing. Bending rigidity increased approximately fivefold across the series, from 19 kBT in DOPC to 95 kBT in the sphingomyelin–cholesterol mixture, showing that ordered membranes are substantially stiffer.

Table 2. Structural and Physical Parameters of Model Membrane Systems at 25 °C

Membrane system	Phase state	Area per lipid (Å²)	Thickness DHH (Å)	Laurdan GP	Bending rigidity κ (kBT)

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Membrane system	Phase state	Area per lipid (Å²)	Thickness DHH (Å)	Laurdan GP	Bending rigidity κ (kBT)
DOPC	Liquid-disordered	67.4	36.7	−0.13	19
POPC	Liquid-disordered	64.3	38.5	−0.05	23
POPC:Chol (7:3)	Liquid-ordered-like	50.6	43.2	0.27	48
DPPC	Gel	47.9	44.2	0.55	n.d.
SM:Chol (1:1)	Liquid-ordered	45.0	47.0	0.58	95

Note. SM = sphingomyelin; Chol = cholesterol; n.d. = not determined because gel-phase vesicles do not show fluid fluctuations. Values are representative of literature ranges.

Figure 1 displays the temperature dependence of Laurdan GP for three representative membranes. DPPC showed a sharp sigmoidal decrease in GP from approximately 0.55 to −0.22 centred near 41 °C, corresponding to the main gel-to-fluid transition. In contrast, DOPC remained in the fluid state throughout the temperature range and showed a gradual linear decline in GP. The POPC:cholesterol mixture maintained intermediate, positive GP values with a shallow temperature dependence, consistent with the ability of cholesterol to buffer membrane order against temperature change.

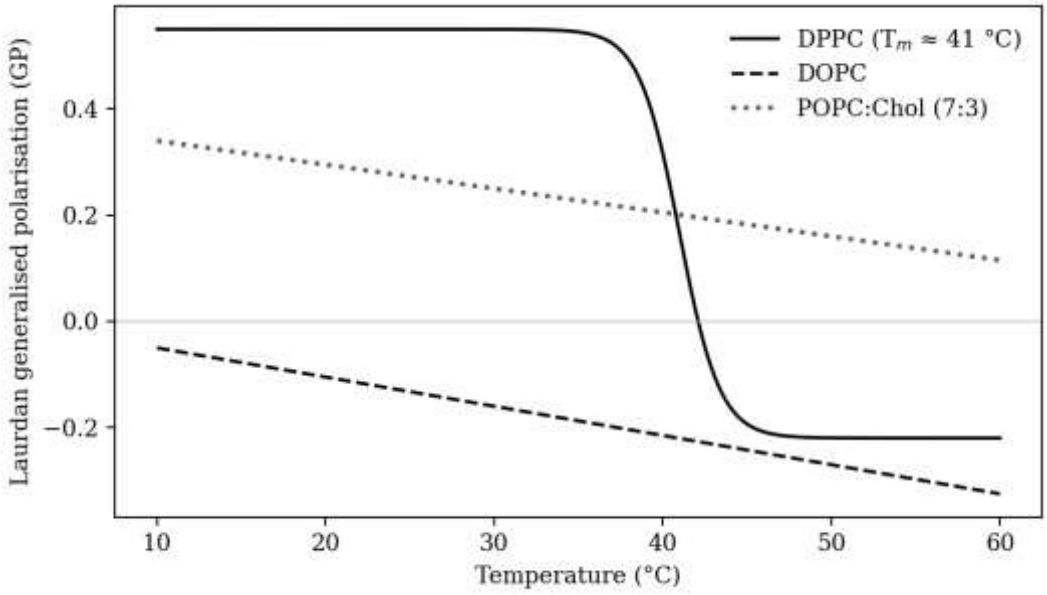


Figure 1. Temperature Dependence of Laurdan Generalised Polarisation in Model Membranes

4.3 Lateral Diffusion and Modes of Motion

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Table 3 compares lateral diffusion across membrane systems of increasing complexity. The highest diffusion coefficient was observed in pure DOPC giant vesicles (8.2 μm²/s by FCS). In phase-separated vesicles, lipids in the liquid-disordered phase diffused at 6.4 μm²/s, whereas those in the liquid-ordered phase diffused 7.5-fold more slowly at 0.85 μm²/s. Lipid diffusion in GPMVs, which retain plasma membrane composition but lack the cytoskeleton, was 3.1 μm²/s, fivefold faster than lipid diffusion in the intact plasma membrane (0.62 μm²/s). Transmembrane proteins in the plasma membrane showed the slowest diffusion (0.11 μm²/s) and the lowest mobile fraction (68%). FRAP values were systematically slightly lower than FCS values, reflecting the larger length scales sampled.

Table 3. Lateral Diffusion of Membrane Components in Model and Cellular Membranes

System	D by FCS (μm²/s)	D by FRAP (μm²/s)	Mobile fraction (%)	Dominant diffusion mode
DOPC giant vesicle (lipid)	8.2	7.6	98	Free
Liquid-disordered phase (lipid)	6.4	5.9	97	Free
Liquid-ordered phase (lipid)	0.85	0.78	95	Free, slowed
GPMV (lipid)	3.1	2.8	94	Free
Plasma membrane (lipid)	0.62	0.51	88	Hop / transient trapping
Plasma membrane (TM protein)	0.11	0.08	68	Hop / confined

Note. TM = transmembrane; GPMV = giant plasma membrane vesicle. Values are representative of literature ranges.

Figure 2 presents the FCS diffusion coefficients on a logarithmic scale, illustrating a 75-fold range between the fastest and slowest systems. The stepwise reduction from pure vesicles to cells indicates that lipid order, compositional complexity and cytoskeletal interactions each contribute to reduced mobility.

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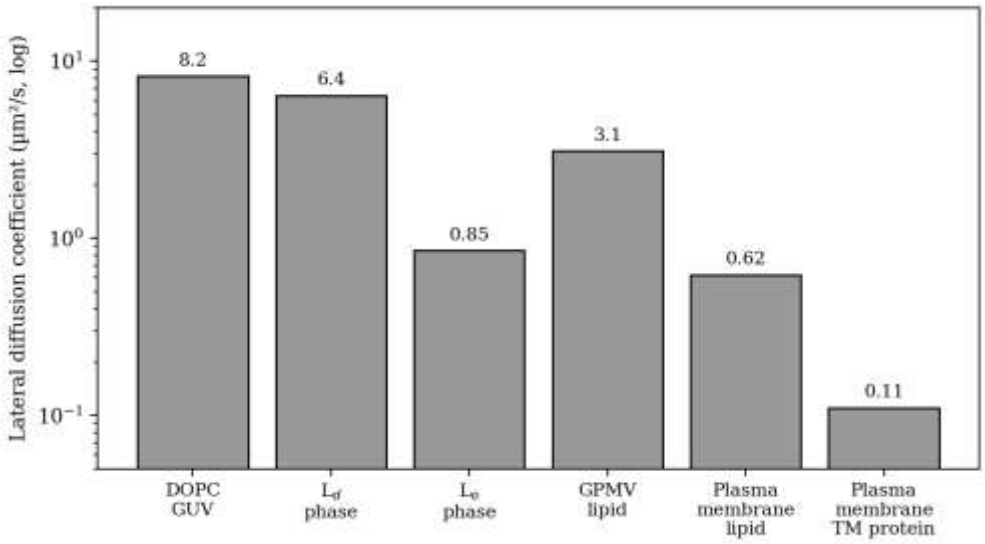


Figure 2. Lateral Diffusion Coefficients Across Membrane Systems (Logarithmic Scale)

Figure 3 shows representative mean square displacement curves for four diffusion modes. Free diffusion produced a linear increase in MSD with time. Anomalous subdiffusion produced a sub-linear curve, while confined diffusion rapidly reached a plateau of approximately 0.018 μm², corresponding to the 230 nm compartment size. Hop diffusion combined short-term confinement with slow long-range displacement, producing the characteristic shape observed for plasma membrane components by high-speed single-particle tracking.

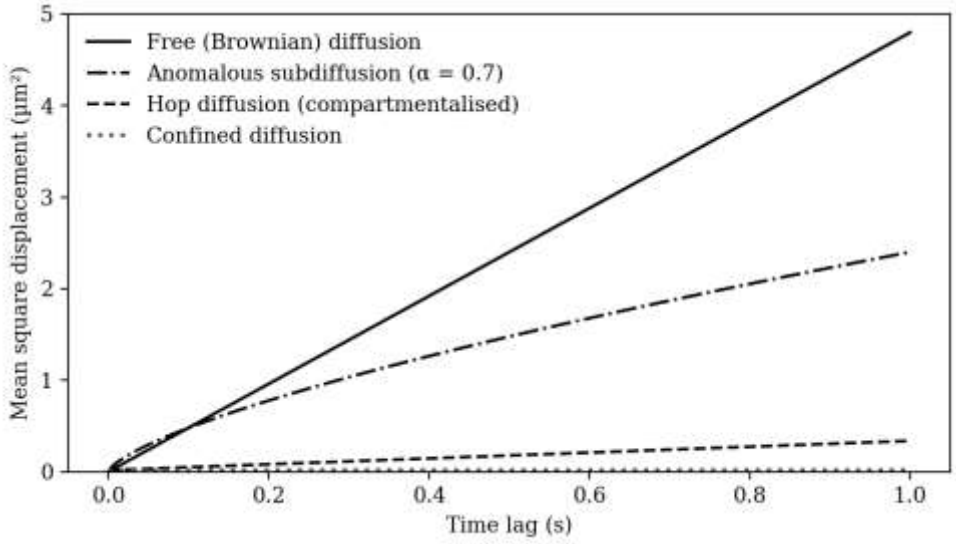


Figure 3. Representative Mean Square Displacement Curves for Different Diffusion Modes

4.4 Cholesterol-Dependent Remodelling of the Bilayer

Table 4 reports the modelled effects of cholesterol concentration on POPC bilayers. Increasing cholesterol from 0 to 40 mol% reduced the area per lipid from 64.3 Å² to 48.1 Å²

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(25.2% decrease), consistent with the condensing effect of cholesterol. Bilayer thickness increased from 38.5 Å to 44.0 Å, and the acyl-chain order parameter rose from 0.17 to 0.31. The lateral diffusion coefficient fell from 9.5 μm²/s to 3.7 μm²/s, a 61.1% reduction. Changes were largest between 0 and 30 mol% and began to plateau at higher concentrations, suggesting approach to saturation of cholesterol's ordering effect.

Table 4. Effect of Cholesterol Content on Simulated POPC Bilayer Properties at 310 K

Cholesterol (mol%)	Area per lipid (Å²)	Thickness DHH (Å)	Order parameter SCD	D (μm²/s)
0	64.3	38.5	0.17	9.5
10	58.9	40.1	0.21	7.8
20	54.2	41.8	0.25	6.1
30	50.6	43.2	0.29	4.6
40	48.1	44.0	0.31	3.7

Note. Coarse-grained simulation values; area per lipid calculated per phospholipid-equivalent. Values are representative of literature ranges.

Figure 4 illustrates the inverse relationship between area per lipid and bilayer thickness as cholesterol increases. The two parameters changed in a coordinated manner, reflecting the straightening and ordering of phospholipid acyl chains around the rigid sterol ring system.

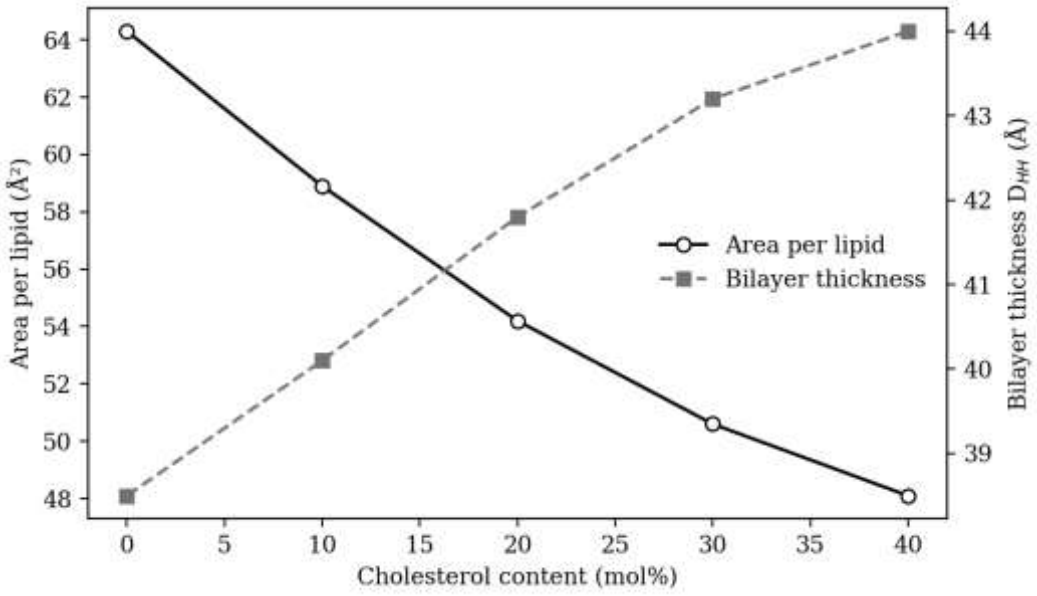


Figure 4. Effect of Cholesterol on Area per Lipid and Bilayer Thickness

Taken together, the four tables and four figures present a consistent picture in which composition determines packing, packing determines mechanical and dynamic properties, and the cellular environment imposes additional constraints through asymmetry, crowding and the cytoskeleton. Parameters obtained by independent techniques were mutually

consistent: systems with smaller area per lipid also displayed higher GP, greater bending rigidity and slower diffusion. This convergence illustrates the value of combining structural, spectroscopic, mechanical and computational approaches rather than relying on any single measurement.

The results also highlight the importance of length scale and time scale when interpreting biophysical data. FRAP, which samples motion over micrometre distances and seconds, consistently reported slightly lower diffusion coefficients than FCS, which samples sub-micrometre distances over milliseconds. In compartmentalised membranes this difference is expected, because long-range motion is limited by barriers that are not encountered during short observation periods. Similarly, scattering and simulation describe average bilayer properties, whereas fluorescence imaging and single-particle tracking reveal the local variation around that average. Recognising these differences prevents apparent inconsistencies between methods from being misinterpreted as experimental error and instead allows them to be used as sources of information about membrane organisation (Jacobson et al., 2019; Sezgin et al., 2019).

5. CONCLUSION

This paper has examined the role of biophysics in understanding the structure and dynamics of cellular membranes, drawing on literature published between 2015 and 2025 and on a representative integrated dataset. The review shows that biophysical research has transformed the conception of the membrane from a homogeneous two-dimensional fluid into a compositionally diverse, asymmetric and dynamically organised material. Studies of lipid diversity and asymmetry have shown that membrane composition is tuned to generate specific physical properties and is homeostatically regulated. Research on lateral heterogeneity has largely resolved long-standing debates about lipid rafts while revealing their small, transient and regulated nature.

The integrated results illustrate how complementary methods reveal different aspects of the same underlying physics. Scattering and simulation quantified the condensing and thickening effects of cholesterol, Laurdan spectroscopy captured changes in packing and phase behaviour, giant vesicle mechanics showed that ordered membranes are substantially stiffer, and diffusion measurements demonstrated that lipid order, compositional complexity and the cytoskeleton progressively restrict mobility. The convergence of these independent parameters supports a coherent structure–property framework linking molecular composition to emergent membrane behaviour.

Several limitations must be acknowledged. The quantitative dataset was constructed from literature-typical values rather than generated through new experiments, and real measurements vary with temperature, buffer, probe choice, instrumentation and analysis methods. Model membranes cannot fully reproduce the complexity of living cells, and fluorescent probes may perturb the systems they report on. Despite these limitations, the analysis confirms that no single biophysical technique can capture the full complexity of

membranes, and that integrated, multi-scale approaches are essential for understanding how membrane structure and dynamics support cellular function.

6. FUTURE WORK

Future research should prioritise the validation of the integrated framework presented here using experimental data acquired under matched conditions. Measuring structure, packing, mechanics and diffusion in the same membrane preparations would reduce variability arising from differences between laboratories and methods, and would allow stronger quantitative relationships among parameters to be established. Extending such measurements to asymmetric model membranes, which more faithfully reproduce the plasma membrane, is a particularly important goal given the growing evidence that leaflet asymmetry influences packing, tension and protein function.

Methodological developments are also expected to broaden the scope of membrane biophysics. Label-free imaging by cryo-electron tomography and advanced AFM may allow nanodomains to be observed directly in native cellular membranes, avoiding the perturbations introduced by probes. Faster and more photostable super-resolution methods could enable diffusion and organisation to be mapped simultaneously across whole cells. In parallel, whole-organelle and whole-cell-scale simulations using coarse-grained force fields, combined with machine learning analysis of trajectories, could connect molecular interactions with cellular-scale membrane remodelling.

Finally, greater attention should be directed towards the physiological and pathological relevance of membrane biophysics. Alterations in membrane composition and physical properties are associated with ageing, metabolic disease, neurodegeneration, infection and cancer, and many drugs act on or within membranes. Integrating lipidomics, native mass spectrometry and biophysical measurements in disease-relevant cells could reveal how membrane properties change in pathology and how they might be targeted therapeutically. Such work would translate fundamental biophysical insight into improved understanding of health and disease.

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