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Understanding, mimicking, and exploiting lipid membrane fusion in biomedicine and synthetic biology

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ABSTRACT

Lipid membrane fusion is a fundamental process underlying numerous biological functions in both physiological and pathological conditions. Understanding the physico-chemical mechanisms governing fusion, from the minimal molecular and structural requirements to the factors regulating its progression, is essential for elucidating complex biological phenomena and developing new biomedical and synthetic biology strategies. In this review, we adopt an interdisciplinary perspective to analyze how lipid composition, environmental conditions, and fusogenic agents influence the different stages of the fusion process, from membrane docking to fusion pore formation. We critically examine the main *in vitro* membrane models, discussing their advantages and limitations, and integrate experimental results with contributions from molecular simulations, which have allowed us to resolve the fusion intermediates and the underlying energy landscape at the nanoscale level. Particular emphasis is placed on synthetic fusogenic agents, including peptides, nucleic acids, nanoparticles, and polymers, highlighting their mechanisms of action and possibilities for rational design. Finally, we discuss emerging applications of membrane fusion in synthetic biology and biomedicine, with a focus on biomimetic systems, controlled drug delivery, and fusogenic lipid particles that promote endosomal escape. Overall, this review aims to provide a unifying conceptual framework linking fundamental principles of membrane fusion with advanced technological applications.



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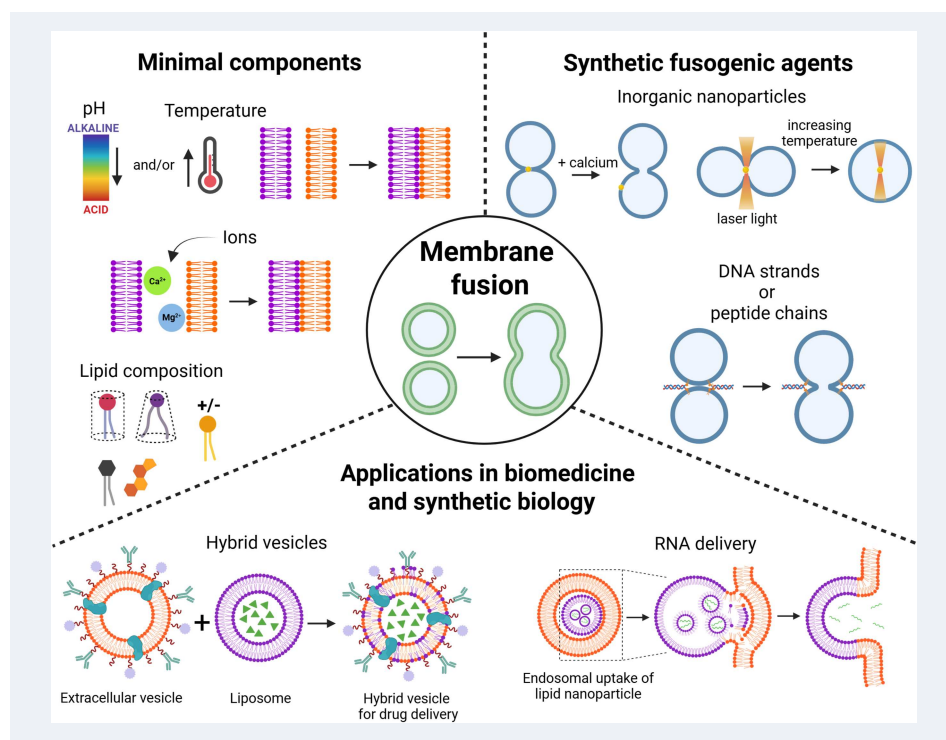
Biomembrane fusion; biomimetic systems; fusogenic agents; lipid membranes; nanomedicine

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1. Introduction

Membrane fusion is a fundamental biological process that enables a variety of essential cellular functions, including extracellular trafficking, synaptic transmission, fertilisation, and viral entry. Understanding how fusion occurs—which minimal components it requires, what triggers it, and how it progresses—remains a central challenge in biophysics and cell biology. This knowledge is valuable from a fundamental perspective, but it also holds biomedical relevance. On the one hand, a deeper understanding of fusion *in vivo* can help identify molecular dysfunctions associated with disease. Indeed, defects or dysregulation in fusion mechanisms are implicated in several pathological conditions, such as neurological and neurodegenerative diseases [1], muscle and development disorders [2], and cancer [3]. On the other hand, controlled fusion events are increasingly exploited to develop innovative therapeutic approaches, for example in the field of antivirals (see, for example, Xing et al. [4]) or in the design of targeted delivery systems, engineered to exploit pH-dependent fusogenicity to escape endosomes [4,5]. In parallel, the ability to induce and control membrane fusion *in vitro* has become a major objective in synthetic biology, where fusion can be used to manipulate compartmentalised systems and construct minimal mimetic models of biological function [6].

These scientific and technological drivers have motivated the design and development of synthetic fusogenic agents—such as peptides, nanoparticles, polymers, and nucleic acids—that can trigger fusion under controlled conditions. These tools offer not only mechanistic insight into the fusion process but also practical applications in drug delivery, gene transfer, and artificial cell engineering.

In this review, we adopt a physico-chemical perspective to explore the molecular mechanisms and synthetic strategies underlying membrane fusion.

In [Section 2](#), we dissect the fundamental stages of membrane fusion and identify the minimal molecular and structural requirements that govern this process. We look over both experimental and computational approaches to study fusion between model lipid membranes.

In [Section 3](#) we examine how fusion can be triggered and modulated by synthetic agents *in vitro*, such as peptides, nanoparticles, nucleic acids, and polymers.

In [Section 4](#) we review recent applications of fusion-based strategies in biomedicine and synthetic biology, ranging from controlled cargo loading and intracellular delivery to engineered fusion systems that replace or mimic natural fusion machineries.

2. Minimal components required for fusion

Membrane fusion plays a crucial role in the functioning of all living cells, but its underlying mechanisms are far from universal. Indeed, fusion processes vastly differ in complexity and molecular intermediaries across different biological contexts. In this section, we examine how both experimental model systems and computational simulations provide complementary insights into fusion mechanisms and outline the minimal molecular and structural requirements that drive these processes.

2.1. Experimental membrane models *in vitro*: the physico-chemical parameters influencing fusion processes

Membrane fusion is a multistep process involving docking, hemifusion, and full fusion with the formation of a fusion pore (Figure 1). Under physiological conditions, membrane fusion is regulated by a wide range of highly specialised proteins, which play an essential role in bringing membranes into close proximity and in lowering the energetic barriers associated with fusion. *In vivo*, these protein-mediated mechanisms are indispensable for ensuring both the efficiency and specificity of the fusion process. Nevertheless, each stage of the fusion process is also strongly modulated by the surrounding physico-chemical environment—particularly the lipid composition, ionic conditions, pH, and temperature—which collectively shape the energy landscape that must be navigated for membranes to merge. While acknowledging the central role of proteins in biological membrane fusion, the present work focuses specifically on how these physico-chemical factors contribute to fusion dynamics, as they can be systematically isolated and studied using simplified model systems and computational approaches.

Membrane models are valuable systems to understand the basic physical and chemical determinants of membrane fusion, regardless of narrow biological specificities. In Table 1, we identify the fundamental model membrane systems used in experimental fusion assays, highlighting their main advantages and limitations. Below, we outline how the progression of membrane fusion is shaped by the surrounding physico-chemical environment, in particular lipid composition, ionic conditions, pH, and temperature.

Lipid composition plays a central role in defining membrane curvature stress and stability, thereby impacting fusion intermediates. Lipids with a tendency for negative spontaneous curvature, such as phosphatidylethanolamine (PE), promote stalk formation and hemifusion by stabilising highly curved intermediates [18]. The role of cholesterol is more context dependent. In many protein-mediated systems

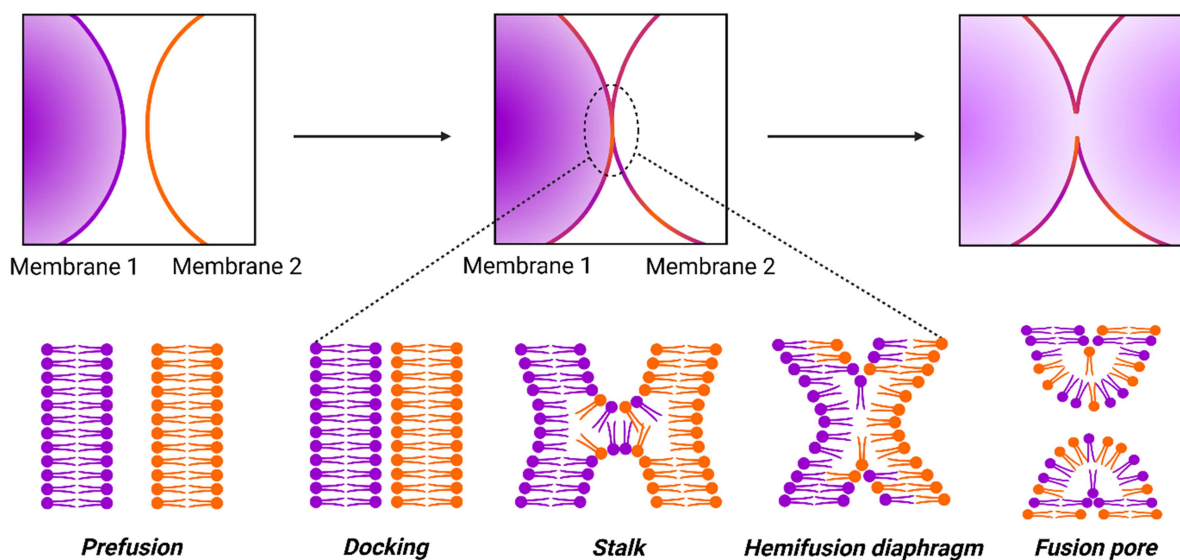

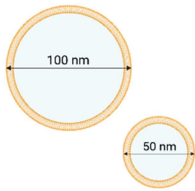
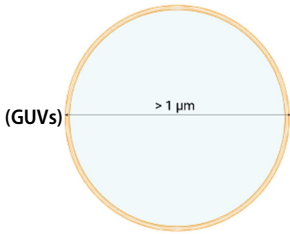


Figure 1. The metastable states along the fusion pathways. The transition from *prefusion* to *docking* requires overcoming a dehydration barrier. The transition from *docking* to *stalk* corresponds to the formation of the stalk state, in which the two hydrophobic parts of the outer leaflets come into contact. The *fusion pore* most often forms after the stalk elongates into a *hemifusion diaphragm*. More details on the mechanics of fusion will be provided in Section 2.2.

Table 1. Reconstituted membrane systems have been instrumental in dissecting the molecular mechanisms of membrane fusion under well-controlled conditions. Among them, supported lipid bilayers (SLBs), giant unilamellar vesicles (GUVs), and submicron liposomes such as large (LUVs) and small unilamellar vesicles (SUVs) are the most commonly used. Each system has distinct properties that make it more or less suitable for specific experimental questions, with characteristic advantages and limitations [7].

Model membrane	Characteristics	Advantages	Limitations
Supported lipid bilayers (SLBs) 	Planar bilayers adsorbed on solid substrates (e.g. glass or mica) Flat, stable membrane patches	Ideal for surface-sensitive techniques such as total internal reflection fluorescence microscopy Stable platform for single-vesicle studies Controlled observation of vesicle docking and fusion events	Strong interactions with the solid support may restrict lipid and protein mobility, confine membrane morphology to planar, introduce friction, and can denature large transmembrane proteins The thin hydration layer between the bilayer and substrate hampers the incorporation of bulky proteins and affects membrane mechanics and curvature Variants such as polymer-cushioned SLBs [8] or pore-spanning membranes [9] can partially alleviate these drawbacks by decoupling the bilayer from the support
Small and large unilamellar vesicles (SUVs and LUVs) 	Closed bilayer vesicles of approximately 50 nm (SUVs) and 100 nm (LUVs) in diameter Highly curved membranes with intrinsic tension, especially in SUVs, which are less stable than LUVs	High reproducibility, suitability for high-throughput assays, and compatibility with biochemical reconstitution of fusion proteins. Experiments have typically been conducted in bulk using ensemble fluorescence or scattering techniques [10,11]	They are not optically resolvable with conventional microscopy Their ensemble nature averages over many events, making it difficult to resolve transient intermediates and rare events Leakage and dye self-quenching can also complicate data interpretation, and curvature stress may influence fusion pathways
Giant unilamellar vesicles (GUVs) 	Larger vesicles (5–100 μm) Flat membranes at the molecular scale and resemble the plasma membrane	Observable under the microscope, namely allowing the visualisation of the fusion process Their size allows encapsulation of soluble markers and proteins GUVs can be manipulated with micropipettes, optical tweezers, electric fields, or microfluidic flows, enabling precise control over their mechanical state and environment [12–14]	GUV preparation can be more laborious, and yields may be low (depending on membrane composition), limiting throughput Reconstitution of fusion proteins into GUVs remains challenging, and fusion efficiencies are often low without strong fusogenic triggers [16,17]
		They are particularly valuable for visualising morphological changes (e.g. budding, tubulation) during fusion and for quantifying mechanical parameters such as membrane tension, see e.g. Cavalcanti et al. [15]	
		They require far less lipid material than LUV/SUV experiments	

and in bilayer stacks, cholesterol enhances fusion, for example, by increasing line tension at domain interfaces or modulating viral fusion protein activity [19–21]. However, in protein-free model membranes, cholesterol can increase membrane order, bending rigidity, and resistance to pore formation, thereby stabilising hemifusion-like states and suppressing the transition to full fusion [22–24]. For example, cholesterol has been shown to reduce fusion efficiency and increase mechanical resilience against pore opening in giant unilamellar vesicle (GUV)-based systems [23,24]. The presence of charged lipids can enhance electrostatic attraction between opposing membranes, increasing the probability of docking and lowering the hydration repulsion barrier [25,26]. Figure 2A illustrates how the membrane charge and fluidity of large unilamellar vesicles (LUVs) and GUVs modulate their fusion pathways, leading to efficient lipid and content mixing in fluid-phase vesicles and restricted fusion in rigid, saturated membranes.

Ions are classical fusogens. Divalent cations such as Ca^{2+} or Mg^{2+} reduce the repulsive hydration forces between bilayers, cross-bridge negatively charged lipid headgroups, and thereby promote membrane proximity [29–31]; similar behaviour is expected for multivalent cations [32]. In addition to favoring docking, Ca^{2+} can increase membrane tension, further accelerating hemifusion and pore formation [33–35]. Furthermore, Ca^{2+} ions have been shown to induce negative curvature in anionic lipid membranes, promoting fusion [36]. However, excessive cation concentrations can lead to uncontrolled aggregation and leakage, highlighting the importance of fine-tuning ion levels.

pH can modulate the charge state of lipids and alter their molecular shape, thereby affecting fusion competence. For example, oleic acid-containing liposomes remain stable at high pH but become fusogenic at acidic pH, as protonation neutralises headgroup charges, destabilising the bilayer and promoting stalk formation [37]. In GUV-based studies, lowering the pH can drive membrane adhesion and hemifusion by reducing electrostatic repulsion [38]. Acidic pH also triggers conformational changes in viral fusion proteins, as classically shown for influenza virus with supported lipid bilayers [39,40]. More recently, structural and biophysical analyses have further shown that acidic pH triggers highly specific conformational changes in these proteins, which act as molecular pH sensors to initiate fusion; these transformations, revealed by cryo-EM and experimental fusion assays, uncover how sharply tuned protein rearrangements at low pH directly promote membrane merging in both viral and cell-based systems, with similar pH- and protein-dependent mechanisms observed for extracellular vesicles (EVs) (Figure 2B) [27,41–43].

Temperature can play an important role in membrane fusion by modulating lipid phase transitions, membrane fluidity, and the lateral mobility of both lipids and proteins involved in the fusion process. As the temperature increases toward or above the lipid phase transition temperature, membranes become more fluid and permeable, and lipids expand, facilitating the merging of bilayers and promoting fusion [44,45]. Higher temperatures correlate with increased rates and extents of membrane fusion, while lower temperatures restrict the diffusion and lateral mobilisation of both lipids and fusion proteins and inhibit the overall fusion process. Fusion protein activity is also dependent on temperature, as exemplified by evidence that higher temperatures directly increase the fusogenicity of the SARS-CoV-2 spike protein [46].

In summary, the fusion pathway *in vivo* is not solely determined by the protein machinery but is strongly shaped by the physical environment: lipids set the curvature, ions and pH modulate electrostatic and hydration barriers, and temperature controls molecular mobility and kinetics. Tuning these parameters is therefore crucial for both mechanistic studies and the design of synthetic fusion systems.

2.2. The contribution of molecular simulations to the understanding of the multi-step fusion process

2.2.1. Structure of fusion intermediates and transition mechanisms

In the early 2000s, the first molecular simulations of membrane fusion were pivotal in transforming fusion from a purely phenomenological description into a molecularly resolved process. The structure of the first hydrophobic connection between the fusing bilayers and the mechanistic pathway leading to the opening of the fusion pore have been debated for a long time, with simulations being an important complement to experimental investigation [18]. Based on previous theoretical suggestions (see, for example, Kozlovsky et al. [47], Chernomordik et al. [48], Jahn et al. [49], and references therein), early work by Noguchi et al. [50], using a solvent-free coarse-grained model, captured spontaneous fusion between small vesicles and

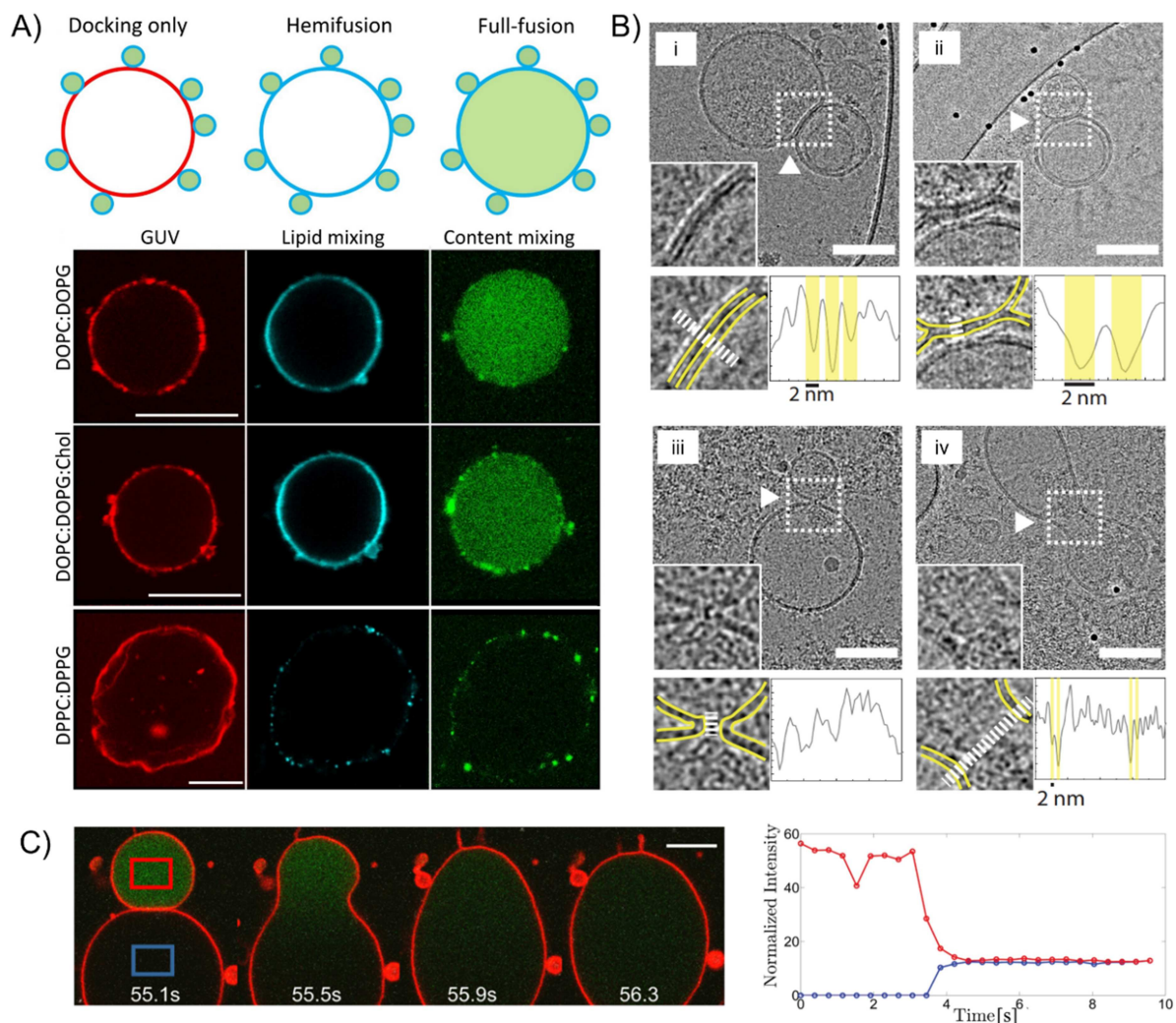


Figure 2. Metastable states along the membrane fusion pathway are visualised by confocal and cryo-electron microscopy. (A) Confocal images illustrating the membrane fusion pathway of LUVs with GUVs, from docking to hemifusion and fusion pore formation (scale bar: 10 μ m). Negatively charged GUVs with different membrane phases were labelled with DPPE–rhodamine (red) and incubated with positively charged LUVs (DOTAP:DOPE, 1:1 molar ratio) labelled with Atto Cyan as a lipid-mixing marker and fluorescein–dextran as a content marker. LUVs efficiently fuse with fluid-phase GUVs composed of unsaturated lipids (DOPC/DOPG, with or without cholesterol), as evidenced by robust lipid mixing (cyan) and content mixing (green). In contrast, for more rigid GUVs enriched in saturated lipids (DPPC/DPPG), LUVs dock, appearing as diffraction-limited spots in both lipid and content mixing channels, but do not undergo extensive lipid mixing or content transfer. Adapted from Lira et al. [23], published by Elsevier, Inc., on behalf of the American Society for Biochemistry and Molecular Biology and distributed under the Creative Commons CC BY license. (B) Cryo-electron images revealing extracellular vesicle (EV)–LUV fusion intermediates (scale bar: 100 nm). (i) Contact between vesicles (docking); (ii) hemifusion (white arrow indicates the location where the two bilayers merge into one); (iii) initial fusion pore formation (white arrow indicates the fusion pore); (iv) fusion pore enlargement and lumen mixing between fused vesicles (white arrow indicates the extended neck where the vesicles fused). Bottom panel: line profile showing bilayer leaflets. The yellow and white lines indicate the membrane leaflets and regions where the line profile was acquired, respectively. Adapted from Morandi et al. [27], published by Oxford University Press on behalf of the National Academy of Sciences and distributed under the Creative Commons CC BY license. (C) Confocal images showing lumen mixing during the fusion of two fluid-phase GUVs (scale bar: 10 μ m). The lipid membranes are labelled in red with Texas Red™ DHPE (TR). One GUV contains only a sucrose solution, while the other contains sucrose mixed with calcein (green). Calcein fluorescence intensity is measured in the two boxed regions within the GUVs during the fusion process. Adapted with permission from Rorvig-Lund et al. [28], copyright 2015 American Chemical Society.

confirmed the formation of a narrow lipidic bridge between adjacent membranes, the so-called stalk, as a key intermediate connecting apposed bilayers. In the same years, Müller [51,52], using Monte Carlo simulations and a simple model of membrane-forming amphiphiles, hypothesised that the stalk would have been responsible for the membrane destabilization leading to pore opening. Ohta-lino et al. [53], in one of the first all-atom molecular dynamics (MD) simulations of two dimyristoylphosphatidylcholine (DMPC) bilayers in close apposition, tested the formation of an inverse micelle intermediate, as proposed by Siegel [54], as well as the stalk hypothesis. All together, these simulation works agreed on an important fact that dehydration of lipid headgroups can induce significant rearrangements of lipid conformations. Simulations have directly shown that alkyl chains lying nearly parallel to the membrane surface can act as a trigger for the stabilisation of hydrophobic connections between bilayers [55]. This idea will turn out to be crucial, as most of the subsequent and even current investigations will focus on how the physico-chemical membrane properties (composition, curvature, stress, asymmetry, etc), as well as the introduction of biological or synthetic fusogens, may favour such lipid conformational changes and, in turn, fusion. Risselada et al [56]. investigated the transformation of the metastable stalk state into an expanded hemifusion diaphragm, proposing that tension in the cis leaflets, as such, due to ion-induced condensation effects, may promote stalk elongation.

The mechanism of poration has been recently investigated by Bubnis and Grubmüller in planar, zero-tension membranes [57] by means of atomistic simulations. According to these authors, the membrane initially deforms by local thinning, which is energetically more favourable than creating a bare hydrophobic opening. As thinning progresses and approaches the transition region, water begins to intrude through the narrow slab. This initial water penetration is necessary but not sufficient to complete pore formation. What actually marks passage through the transition state is the moment when the lipid headgroups from the two leaflets meet along the pore axis. This headgroup contact occurs just before the pore becomes fully established and provides the most reliable indicator of successful nucleation. More recently, by a combination of MD simulations and field-based lipid calculations, Spencer et al. [58]. identified a pool of possible mechanisms leading to final pore formation just at the rim of the hemifusion diaphragm: from a purely physical, continuous perspective, membrane tension and line tensions control the possibility to make rim pores metastable and even drive them to overcoming the critical rim pore radius, leading to complete fusion. At the molecular level, line tension at the edge of the rim pore and of the hemifusion diaphragm could be regulated by lipid lateral separation, by lipid diffusion or by lipid passive (for sterols) or active flip flops. A tight regulation of line tensions by a specialised molecular machinery is most likely responsible for such regulation *in vivo*, especially in situations such as during synaptic neurotransmitter release from a vesicle, in which pore opening and closing is a transient, reversible process.

2.2.2. The energy required for fusion

To fully understand which steps in the fusion pathway act as kinetic bottlenecks and be able to predict the relative thermodynamic stability of the fusion intermediates, it is crucial to map out the free energy landscape connecting the fully hydrated membranes to the pore state. These transitions involve rare, concerted rearrangements of many lipids, solvent molecules and ions and happen on experimental time scales that range from hundreds of nanoseconds to hours [7,59], particularly in *in vitro* systems, where docking can be the rate limiting step [60,61]. As standard MD with atomistic or sub-molecular resolution cannot approach these time scales, enhanced sampling techniques are required. At the methodological level, enhanced-sampling methods fall into several broad classes (for a complete review, see Henin et al. [62]. and references therein): methods that bias the dynamics along specific collective variables (umbrella sampling, adaptive biasing force, metadynamics and its most recent developments); generalised-ensemble or tempering approaches, that modulates the overall energy function, thereby affecting all degrees of freedom simultaneously (replica exchange, parallel tempering); and finally path-based or path-finding methods (string method, transition path sampling, forward flux sampling). More recently, machine-learning approaches to enhanced sampling have become increasingly popular [63].

In the context of membrane fusion, enhanced sampling has been applied to compute the potential of mean force (PMF) profiles for specific transitions (e.g. from two apposing bilayers to stalk formation, from a hemifused diaphragm to fusion pore opening). Coarse-grained MD simulations combined with the string method [64,65], as well as umbrella sampling [55], enabled Müller et al. to estimate the free energy barrier

connecting the lamellar phase to the stalk phase, yielding a value of approximately $15 k_B T$. Using the string method once again, Smirnova et al. [66] demonstrated that the relative stability of the lamellar and stalk states can invert depending on the degree of hydration, with the stalk becoming stabilised at extremely low hydration levels. In the same study, reduced hydration was shown to lower the free energy barrier for stalk formation by several $k_B T$ s. The role of lipid composition during stalk formation has recently been addressed systematically, again using coarse-grained simulations by Hub and co-workers [22]. These authors quantified the free energy profiles for stalk formation across more than 100 lipid compositions, reporting barrier heights ranging from roughly 15 to 60 kJ/mol ($6\text{--}24 k_B T$) depending on headgroup and tail saturation. In their survey, PE- and unsaturated lipids markedly stabilised the stalk, whereas PC- and saturated species increased the barrier and favoured the lamellar phase. Cholesterol was found to favour stalk formation, though no accumulation of cholesterol at the stalk was observed. Finally, fusion between identical, symmetric membranes mimicking the composition of either the inner or the outer mammalian plasma membranes revealed that the inner leaflet is significantly more prone to stabilise the stalk, with free energy differences between the lamellar and stalk state differing by about 50 kJ/mol ($20 k_B T$). The use of a coarse-grained model has made possible the quantification of stalk formation barriers and stalk stability over a large variety of membrane lipid compositions. Recent attempts at calculating the same quantities via atomistic force fields seem to corroborate the finding of the coarse-grained model in terms of the energy required for stalk formation [67].

More caution, in terms of the reliability of coarse-grained models, and in particular of the Martini model [68], should be used when looking at the energetics of the final step of the fusion process, namely, pore formation. Hu et al. [69], for instance, used the Martini force field to provide an estimate of pore-formation energy barriers for 18 different lipid types, finding values ranging between 75 and almost 300 kJ/mol. These energies are way larger than those reported in atomistic simulations, for example by Bennet et al. [70] (17 and 78 kJ/mol for DLPC and DPPC, respectively); by Hub et al. [71,72] (about 50 kJ/mol for DMPC, 75 kJ/mol for POPC); by Bubnis et al. [57] (about 45 kJ/mol for DMPC). The reason for this discrepancy is likely to be found in Martini's underestimation of polar interactions in non-polar environments, and in its intrinsic limitation at capturing the formation of H-bonds between water and lipid headgroups. Both factors disfavour the formation of water defects spanning the membrane thickness at the CG level [73,74].

3. Synthetic fusogenic agents

Synthetic fusogens provide a powerful and versatile mean to trigger membrane fusion in a controlled and tunable manner, enabling both mechanistic studies and technological applications. Synthetic fusogens can be rationally designed to exploit individual physical ingredients of the fusion process, favoring, e.g. membrane dehydration, membrane disorder and curvature generation. Over the past two decades, a wide range of synthetic strategies—spanning peptides, nucleic acids, polymers, and inorganic nanomaterials—have been developed to promote fusion. In this section, we review the main classes of synthetic fusogens, highlighting their underlying mechanisms of action and discussing how their physical and chemical features can be tuned to modulate distinct stages of the fusion pathway.

3.1. Peptides

Peptide fusogens span a broad design space, but a useful organising principle is biological inspiration. The first class of bio-inspired fusogenic peptides includes peptides designed to mimic fusion mediated by SNARE (soluble N-ethylmaleimide sensitive factor attachment protein receptor) proteins, whose mechanism of action is shown in Figure 3A. These peptides [75–77] can assemble in the form of membrane-anchored coiled-coils. Each peptide is conjugated to a lipophilic molecule, such as cholesterol or a phospholipid, through a flexible poly-ethylene glycol (PEG) spacer, as shown in Figure 3B. When complementary peptide sequences are linked to opposing membranes, coiled-coil formation helps overcoming the energy barrier to initiate membrane contact and fusion. The PEG spacer, in addition to providing flexibility to the construct, also contributes to membrane dehydration. However, it also has a steric effect that is detrimental to fusion: its length was shown to be inversely

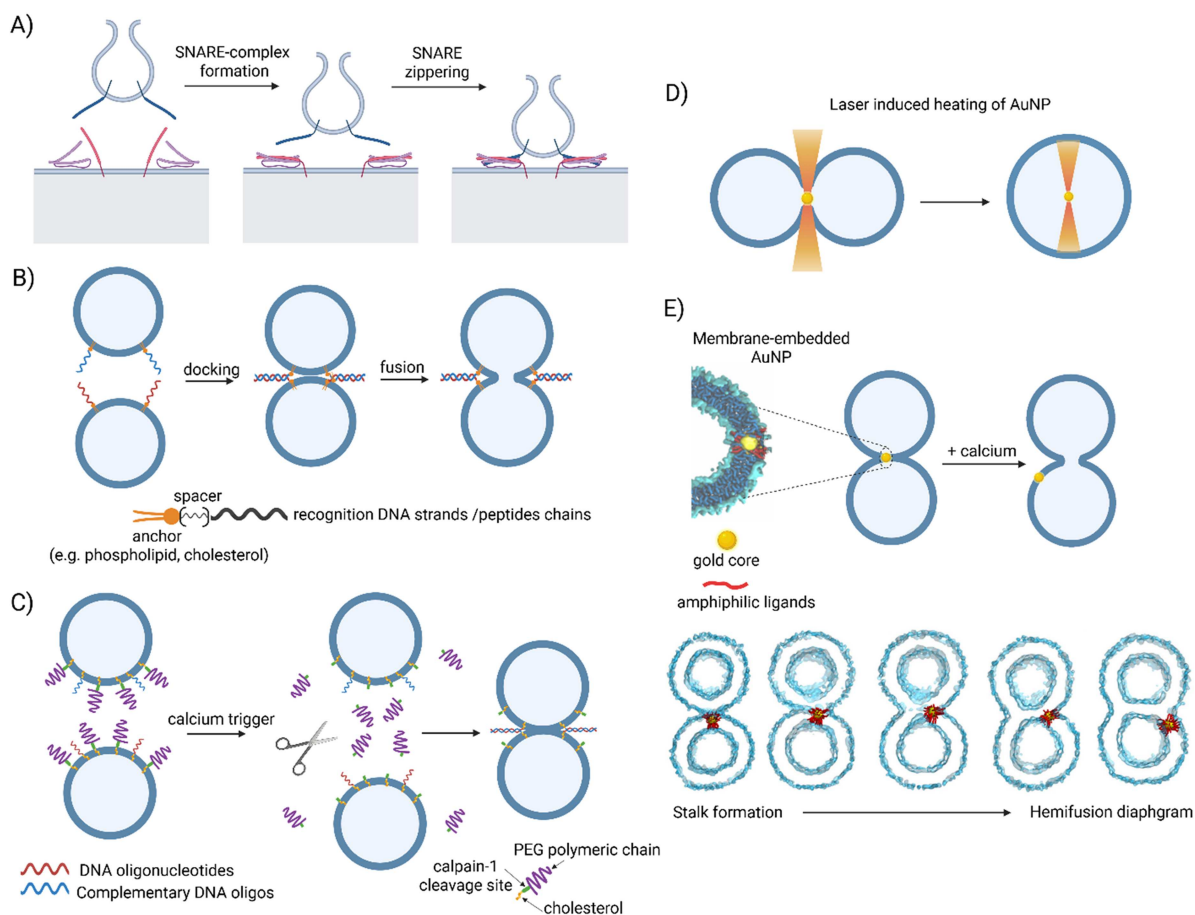


Figure 3. Synthetic membrane fusion mechanisms. (A) Schematic steps of SNARE-mediated membrane fusion: the initial membrane approach, SNARE complex formation and membrane apposition, and progressive zippering will lead to membrane hemifusion and fusion pore formation. (B) Sketch of vesicle fusion mediated by complementary DNA strands or coiled-coil peptides with each strand linked to a lipophilic moiety (e.g. cholesterol or phospholipid) through a flexible spacer. Inspired by Löffler et al. [90]. (C) Schematic illustration of calcium-triggered DNA-mediated membrane fusion. Complementary DNA oligonucleotides form double helices to drive fusion. PEG chains attached via a calpain-cleavable linker inhibit DNA interactions in the absence of calcium; upon calcium addition, calpain-1 removes the PEG chains, allowing DNA bundle formation between membranes and promoting fusion. Inspired by Hsu et al. [89]. (D) Adjacent vesicles fused through laser heating of an AuNP located between them, resulting in complete mixing of lipids and vesicle lumens. Inspired by Rorvig-Lund et al. [28]. (E) Amphiphilic gold NPs penetrate the bilayer, lowering the critical energy barriers for the initial membrane approach and stalk formation through sulphonate–lipid interactions, while calcium ions promote stalk expansion and fusion pore formation (top panel). Bottom panel: computational simulations showing NP–membrane interactions from stalk formation to hemifusion diaphragm (only the phospholipid headgroups are represented) [101].

proportional to the fusion rate [78]. Coiled-coil formation is based on the interaction of helical peptides with opposite net electric charge. In particular, peptides are denoted ‘E peptides’ or ‘K peptides’, depending on the prevalence of negatively charged glutamic acid (E) or positively charged lysine (K) residues, and their interaction is based on the formation of E/K complexes. It has been shown that while E peptides promote interactions with only their complementary K peptide counterparts, K peptides play a dual role in membrane docking, as they also directly interact with the membrane, favoring the formation of lipid protrusions [79,80]. As discussed in the previous sections, the protrusion of lipid tails is one of the rare events triggering stalk formation. Recently, it has been shown that membrane anchoring of the E peptide on both opposing membranes with the addition of a free K peptide dimer formed by two parallel helices stabilised by a disulphide bridge provides an improved architecture to exploit the dual role of the K peptide, resulting in high content mixing efficiency (70%) [81].

Beyond coiled-coil architectures, a wide range of membrane-active synthetic peptides can induce hemifusion or full fusion by directly perturbing the bilayer structure. In membrane-enveloped viruses such as, for example, the influenza virus, the fusion peptide is a highly conserved region of the genome across many different virus subtypes and, as such, an important target for antiviral therapies [82]. The mechanism by which these peptides and their synthetic counterparts cause viral entry may involve fusion but also, more generally, membrane destabilization or disruption (e.g. poration). For a review, see, for example, Lozada et al. [83] and Ahmad and Khan [84]. The possible coexistence of a proper fusion mechanism and of membrane damages leading to content leakage has also been proposed and supported by molecular simulations [67]. A decrease of pH is often the trigger of virus-inspired peptide fusogenic activity [84].

A similar overlap between fusogenic and disruptive mechanisms has been reported for antimicrobial peptides, which—while primarily evolved to permeabilize membranes—can promote lipid mixing, hemifusion, or full fusion under specific conditions, reflecting a shared reliance on amphipathic insertion and defect stabilisation [85–88].

3.2. Nucleic acids

Another SNARE-inspired strategy to induce membrane fusion involves the use of complementary DNA oligonucleotide strands to promote liposome docking [89–91]. DNA hybridisation between the liposomes in contact favours the formation of an adhesion patch, which evolves into a stalk upon lipid mixing between the outer leaflets of the adhered bilayers and finally gives rise to the opening of the fusion pore. A variant of this approach is based on peptide nucleic acids (PNA) [92,93].

In this approach, the oligonucleotide strand is anchored to the membrane using a lipophilic molecule (e.g. a couple of linear aliphatic chains with appropriate functionalization), with or without a PEG spacer between the lipophilic anchor and the nucleotide strand. Different anchor designs have been explored, including the use of a macrocyclic polyaza crown ether scaffold to increase the distance between the aliphatic chains from 0.3 nm when they are linked to the same N-atom, to 0.7 nm when they are linked to the crown ether. In this way, the two chains are decoupled and interact separately with the lipid membrane, improving the anchoring of the oligonucleotide [90].

Lipid-DNA conjugates have been employed to induce the fusion of three liposome populations in cascade, exploiting orthogonally hybridising sets of membrane-anchored oligonucleotides and resulting into a fusion yield of 50%–80% per stage [91].

To control the onset of the fusion process, PEG chains anchored to the membrane by cholesterol were introduced to shield the DNA strands, as sketched in Figure 3C. A cleavage site for calpain, a protease activated by calcium, was introduced between the cholesterol anchor and PEG. In this way, calcium addition triggers PEG cleavage from the membrane anchor, thus exposing the DNA oligonucleotides and allowing their hybridisation [89].

Lipidated oligonucleotides were also used to set up lipid nanoreactors for the synthesis of carbohydrate mimetics, exploiting the DNA-programmed fusion of aqueous sub-attoliter compartments [94].

3.3. Inorganic nanoparticles

The major advantage of inorganic nanoparticles (NPs) as fusogenic agents lies in their tunable physico-chemical properties, which enable controlled regulation of membrane fusion efficacy. By tailoring NP properties, the different stages of the fusion process can be modulated both *in vitro* and *in vivo*. The key structural parameters of NPs include the material, size, and shape of the NP core, as well as the NP surface functionalization.

Metal and metal-oxide nanoparticles, such as gold and silica, are common candidates for nanomedicine applications because of their biocompatibility and versatile surface chemistry. In particular, gold nanoparticles (AuNPs) are attractive because of their unique optical properties, namely, localised surface plasmon resonance, which enables efficient light-to-heat conversion. This feature has been exploited to achieve laser-induced membrane fusion with high spatial and temporal control (see Figure 3D for a sketch of the fusion process). Optically controlled fusion, in which plasmonic AuNPs are irradiated by a laser

while being optically trapped between two membranes, has proven effective for inducing fusion in both synthetic vesicles and living cells [28,95–97]. NP surface functionalization and size play a key role in mediating interactions with membranes, as they modulate electrostatic and amphiphilic interactions with lipids, as well as the surrounding ionic environment. Nanoparticles with a purely hydrophilic surface, such as SiO₂ NPs, primarily interact with lipid headgroups and are not expected to penetrate the membrane hydrophobic core. Nevertheless, Arribas Perez et al. [98], showed that SiO₂ NPs with diameters of approximately 30 nm can induce hemifusion and full fusion. This effect arises from the membrane curvature and tension generated upon wrapping by the lipid bilayer. In contrast, several studies have demonstrated that amphiphilic surface coatings are essential to promote partial NP penetration into the bilayer hydrophobic core, thereby activating fusion pathways that more closely resemble protein-inspired fusogenic mechanisms. By combining computational studies and experimental techniques such as fluorescence assays, Tahir et al. [99], showed that amphiphilic, negatively charged AuNPs can mediate the fusion of zwitterionic DOPC vesicles (Figure 3E). Further insight into these interactions was provided by Canepa et al. [100], who again combined experimental assays and molecular simulations and demonstrated that these NPs lower the critical energy barriers associated with the initial membrane approach and stalk formation via sulphonate–lipid interactions, while calcium ions trigger stalk expansion into fusion pore formation. Canepa et al. [100] also showed that increasing the cholesterol content in membranes significantly enhances fusion efficiency. There is broad consensus that cholesterol, by reducing acyl-chain shielding, enhances hydrophobic contacts between apposing membranes, thereby promoting fusion events. Simulations further suggested that cholesterol accumulation at the stalk may contribute to its stabilisation.

The size and the shape of NPs are also critical determinants of the fusogenic capacity. For amphiphilic NPs, even small variations in NP size can significantly alter stalk formation and hemifusion [101–103]. Molecular simulations have shown that NP core curvature strongly influences both the kinetics and thermodynamics of stalk formation, with smaller NPs exhibiting lower free-energy barriers and more stable stalks [104]. Similarly, systematic computational analyses of different NP morphologies—from spherical to prolate shapes—have demonstrated that the NP shape and membrane affinity affect the surface contact area, membrane wrapping, and overall fusogenic behaviour [103]. Finally, the NPs actively contribute to regulate the interplay with membrane curvature [101] and membrane tension [98].

Taken together, these findings highlight the potential of inorganic NPs as versatile platforms for the rational design of fusogenic nanomaterials.

3.4. Polymers

Polymer-assisted synthetic membrane fusion can result from a variety of mechanisms, depending on the polymer structure and its interplay with membrane lipid components. In some cases, a direct interaction between the polymer and the membrane is supposed to take place, as for polycations with different degrees of hydrophobicity interacting with membranes containing negatively charged lipids [85,105] or hydrophobic polyelectrolyte interacting with neutral PC vesicles [106]. In both cases a strong membrane destabilization is present, giving rise to leaky fusion, while for anionic amphiphilic polymers interacting with positively charged membranes, hemifusion with subsequent vesicle precipitation has been reported [107].

Computational studies at different resolutions have shown that polymers, including polyelectrolytes, dendrimers and amphiphilic block copolymers, can induce significant membrane deformation (see, for example, Rossi and Monticelli [108,109] and references therein). Although direct molecular simulations of polymer-induced membrane fusion remain scarce, the available literature indicates that polymer-driven membrane remodelling, often involving changes in curvature, lipid packing and local ordering, can lower the energetic cost of highly deformed intermediates. Depending on the polymer architecture and interaction mode, such effects may lead to membrane disruption via poration or, alternatively, to the stabilisation of stalk-like intermediates along the fusion pathway.

A different scenario is involved in membrane fusion induced by PEG. The mechanism of PEG-mediated vesicle fusion is based on depletion interactions [110,111]. The polymer is excluded from a region adjacent to each of the opposing membranes, producing an attractive osmotic force that promotes membrane dehydration and membrane–membrane contact. It was suggested that membrane dehydration causes an

asymmetry in the lipid packing pressure in the two bilayer leaflets, favoring lipid rearrangement and stalk formation [111]. It has been proposed that the optimal range of PEG molecular weights for fusion is 8000–10,000; lower-molecular-weight PEG would not be able to induce a significant depletion force, while higher molecular weight PEG would be adsorbed to the membrane surface to a significant extent, such as to eliminate the depletion attraction and give rise to a repulsive steric barrier to aggregation [110].

4. Recent applications

Combining experimental studies and molecular simulations on model and synthetic fusogenic systems provides critical insights into the mechanisms of membrane fusion. Beyond deepening our fundamental understanding, these findings pave the way for the development of platforms with increasing biological relevance. In this section, we highlight recent studies in which membrane fusion is employed to design engineered synthetic biologic and drug delivery systems.

4.1. Fusion for synthetic biology and mimetic systems

While vesicles are convenient models to study the lipid membrane and its dynamics, they can fall short when investigating more biologically relevant systems. Indeed, mimicking the complex lipid makeup of cell membranes is particularly complicated when one considers the lipid diversity across living domains and cell types [112,113]; for example, phosphatidylglycerol (PG) belongs more to prokaryota [114,115] and phosphatidylcholine (PC) and phosphatidylserine (PS) to eukaryota [116]. Even across bacteria, there is wide variety across gram+ and gram- bacteria [114,117]. Beyond lipid composition, cell membranes typically hold a large amount of protein; membrane proteins make up 50% of the cell membrane mass [118].

The production of biomimicking membranes *in vitro* is a challenge because of the sheer number and diversity of proteins and lipids in cell membranes. Therefore, only the main structural features and functions of the original system are implemented in the model system, resulting in a simplified view of the aspects that are considered relevant for each specific study. There are now multiple methods for protein reconstitution, both in GUVs [119,120] and in smaller vesicles [121–124]. In addition, the compositional complexity of model membranes can be enhanced by exploiting membrane fusion as an assembly strategy, enabling the controlled transfer of lipids and proteins from biologically derived vesicles to synthetic membranes. The fusion of model membranes either with EVs, which are directly produced by cells [125,126], or with artificial cell-derived vesicles, such as those reconstituted from red blood cell membrane components [127], has been used to progressively approximate the molecular composition of living cell membranes. EVs were fused to GUVs using detergents [128] or calcium ions [129]; charge-mediated fusion was exploited to fuse oppositely charged proteoliposomes and GUVs to reconstitute proteins into GUVs [130], also preserving protein orientation [131].

Beyond the use of fusion to mimic the lipid and protein makeup of cell membranes, higher levels of complexity can be reached by employing vesicles as microcompartments and triggering small-scale reactions to reproduce simple cell functions. Indeed, Ishmukhametov et al. [132] exploited charge-mediated fusion to trigger a sequence of fusion events that ultimately resulted in the minimal electron transfer chains necessary for ATP synthesis [132]. As in biological processes, it is crucial that the steps take place in order, generalised and uncontrolled fusion of the microcompartments is not advisable; one way to restrict and specialise fusion events was to produce liposomes bearing mutually recognisable complementary chemical or DNA groups, exploiting the coil-coiled mechanism described in Section 3 [133,134].

Furthermore, complex compartment-based reactions have been successfully carried out by controlling the opening and closing of pore proteins across the leaflets shared by hemifused vesicles [135–137] without the need to resort to full fusion and more carefully controlling the sequence of content mixing across microcompartments.

Finally, artificial pancreatic beta cells analogues were built by encapsulating small vesicles loaded with insulin to mimic the storage granules inside a giant vesicle whose membrane is equipped with a mechanism to sense changes in glucose levels. An increase in glucose levels is converted into a decrease in pH inside the artificial cell, triggering fusion of the small vesicles with the artificial cell membrane, with

subsequent insulin release. Additionally, in this case, fusion takes place through a coiled-coil E/K peptide mechanism, which is made possible by pH-induced deshielding of K peptides located in small vesicle membranes. Restoring normoglycemic conditions reshields the ISVs, and this behaviour could be repeated for several cycles [138].

In addition to biomimetic fusogenic systems built from typical cellular membrane components, synthetic polymers-based vesicles have long been explored as alternative closed-shell compartments, commonly referred to as polymersomes [139]; for a broader comparison between liposomes and polymersomes, see Rideau et al. [140]. Hybrid vesicles composed of both lipids and polymers have also been employed in fusion studies, offering tunable mechanical and compositional properties that bridge lipid bilayers and polymer membranes [141–143]. Polymersomes and hybrid vesicles are able to undergo fusion via electrostatic interactions; charge-assisted fusion between anionic polymersomes and viruses has been shown to result in antiviral activity due to the increased size of the fused particle and the loss of viral fusogenic activity [144]. The use of amphiphilic comb polymers in which a hydrophilic zwitterionic backbone is electrostatically linked to hydrophobic tails allows obtaining membranes with thickness, flexibility, and lateral mobility typical of liposomes [145].

To summarise, fusion may be used to produce vesicles with a more biologically relevant lipid makeup, as well as to reconstitute proteins in vesicles without overly affecting their native environment and structure. Furthermore, it is a powerful tool in synthetic cell research [146], as controlled fusion of vesicles acting as microcompartments can pave the path to reproduce cellular activities *in vitro*, employing lock-and-key specialised fusion switches acting as logical gates.

4.2. Fusion for drug delivery

Fusion based on complementary DNA strands was exploited for programmable RNA loading of extracellular vesicles, aimed to EV-mediated delivery of RNA therapeutics [147]. Fusion triggered by dimeric coiled-coil peptides was used for efficient delivery of doxorubicin [81]. However, in the build-up of therapeutic applications, mechanical methods such as sonication and co-extrusion are often used to fuse artificial vesicles with natural or artificial cell-derived vesicles, with the aim of making the membrane of the former more similar to that of the target cells. Recent applications of this approach include tumour-targeted drug [148] or short interfering RNA (siRNA) [149] delivery, cartilage-targeted drug delivery in the treatment of osteoarthritis [150], and dendritic cell-targeted systemic lupus erythematosus treatment [127].

4.3. Enhancing fusogenicity to favour endosomal escape of drug/RNA vectors

One of the major bottlenecks in bionanomedicine and drug delivery is the efficient intracellular release of therapeutic cargoes—such as drugs, peptides, or nucleic acids—into the cytosol of cells by functionalized vectors. Following cell uptake via endocytosis, most extracellular delivery vehicles become trapped within endosomes, which are subcellular vesicles enclosed by a lipid bilayer that retain therapeutic molecules and prevent them from reaching their intended subcellular targets. Endosomal entrapment is typically followed by trafficking to lysosomes—acidic, membrane-bound cellular compartments where the therapeutic payload is degraded—leading to a substantial reduction in therapeutic efficacy. Consequently, efficient endosomal escape into the cytosol represents a critical requirement for any synthetic delivery vector. This limitation is particularly pronounced in the context of RNA therapeutics, including messenger RNA (mRNA), siRNA and antisense oligonucleotides [151]. Despite their clinical success, state-of-the-art lipid nanoparticles (LNPs) remain strongly constrained by inefficient endosomal escape. Comparative studies have consistently shown that endosomal escape is a major bottleneck for LNP-based delivery strategies, with typically ~1% or less internalised cargo successfully reaching the cytosol [152].

Effective cytosolic delivery therefore requires strategies that enhance membrane fusion between drug nanocarriers, such as LNPs, and endosomal membranes to allow for cytosolic release of the therapeutic cargo. To achieve controlled and efficient endosomal escape, researchers are actively developing approaches to enhance fusogenicity through the design of specific compositional and structural ‘escape-promoting’ features within delivery vehicles. Here, we review the main aspects that have been most

extensively investigated in recent years to enhance lipid membrane fusogenicity and favour endosomal escape of LNPs—the current gold standard lipid-based vectors in clinical RNA delivery. Specifically, we will focus on lipid composition and lipid molecular structure as central determinants of LNP performance.

LNPs have demonstrated their clinical value in the delivery of nucleic acids for the treatment of both genetic and infectious diseases, as exemplified by Onpattro® siRNA delivery to the liver and the COVID-19 mRNA vaccines Comirnaty® (Pfizer-BioNTech) and Spikevax® (Moderna). LNPs are strategically engineered to efficiently encapsulate and protect nucleic acids, improve particle stability, facilitate cellular uptake, and promote endosomal escape of the therapeutic cargo via fusion with the endosomal membrane. A typical LNP design involves the encapsulation of the nucleic acid payload within a liquid-crystalline core containing inverse micellar structures, which sequester and protect nucleic acids from environmental degradation [153]. LNPs typically consist of four key components that work synergistically to enable efficient cytosolic delivery [151]: (1) *ionisable cationic lipids*, which become positively charged at acidic pH; (2) *glycerophospholipids* (or ‘helper lipids’), which are located primarily on the LNP surface and stabilise the particle structure and enhance shelf-life and circulation stability; (3) *sterol lipids* (e.g. cholesterol), which improve membrane integrity and help prevent premature leakage of the RNA cargo in biological fluids; and (4) *PEG-lipid conjugates*, which are mainly incorporated at the particle surface via lipid tails, where they form a steric barrier that helps prevent particle aggregation and opsonization by the immune system.

Although LNP formulations typically share this common set of four fundamental components, variations in lipid design—such as differences in chemical structure, number of tails, chain length, and degree of saturation—can be strategically tailored to enhance delivery properties [154,155]. Among these components, ionisable cationic lipids play a major role in enabling cytosolic delivery by leveraging their engineered pH-sensitive and charge-tunable fusogenic properties. In the last twenty years, a wide range of ionisable lipids have been developed for RNA delivery, with the ultimate goal of enhancing fusogenicity to favour endosomal escape [156,157]. These lipids are broadly categorised into five structural families: unsaturated (featuring double bonds in the hydrophobic tails), multi-tail (which increase lipid packing), polymeric (incorporating polymer or dendrimer elements) [151], biodegradable (containing cleavable ester or disulphide bonds for reduced toxicity and controlled release), and branched-tail (designed to destabilise endosomal membranes through enhanced packing defects and curvature stress) [158–161]. The chemical structures of the most representative ionisable lipids used in the clinical development of RNA therapeutics are shown in Figure 4A. In parallel with experimental research, artificial intelligence (AI) models trained on structural data have recently emerged as a strategic tool for the *in silico* identification of promising ionisable lipid candidates with enhanced nucleic acid delivery [162].

Despite their chemical diversity, all ionisable lipids contain tertiary amine groups that become protonated—and thus positively charged—under acidic conditions, i.e. when the pH is below the intrinsic pKa of the lipid's polar headgroup. Charge-tunable aminolipids with pKa values between 6.0 and 7.0 have been optimised for effective pH-sensitive performance [162]. At acidic pH, protonation of these lipids facilitates the formation of stable RNA-loaded LNPs through electrostatic interaction with the negatively charged phosphate backbone of the nucleic acid, promoting efficient RNA encapsulation into the LNP core. In the bloodstream (pH 7.35–7.45, above the lipid's pKa), ionisable lipids remain largely neutral. This minimises nonspecific interactions with negatively charged serum proteins, thereby reducing clearance by the immune system and lowering systemic toxicity. Upon cellular uptake, the LNPs enter the acidic endosomal environment (pH 5.0–6.0), where the ionisable lipids become re-protonated. Here, they come into contact with the endosomal membrane, which is enriched in negatively charged lipids such as phosphatidylserine, polyphosphoinositides and bis(monoacylglycero)phosphate (also known as lysobisphosphatidic acid) [166].

This pH-dependent protonation and electrostatic interaction with anionic endosomal lipids, triggered by the acidic endosomal environment, can induce lipid rearrangements and phase transitions that may be fusogenic through the transient formation of defects [167–169]. One example of lipid phase change induced by the endosomal pH decrease is shown in Figure 4B and C, namely, the transition from the lamellar (L) phase to the inverted hexagonal (H_{II}) phase upon interaction between protonated ionisable lipids and the negatively charged lipids of the endosomal membrane [163,170–172]. Synergistic experimental and *in silico* results show that the pH dependence of the lipid ionisation state is not only

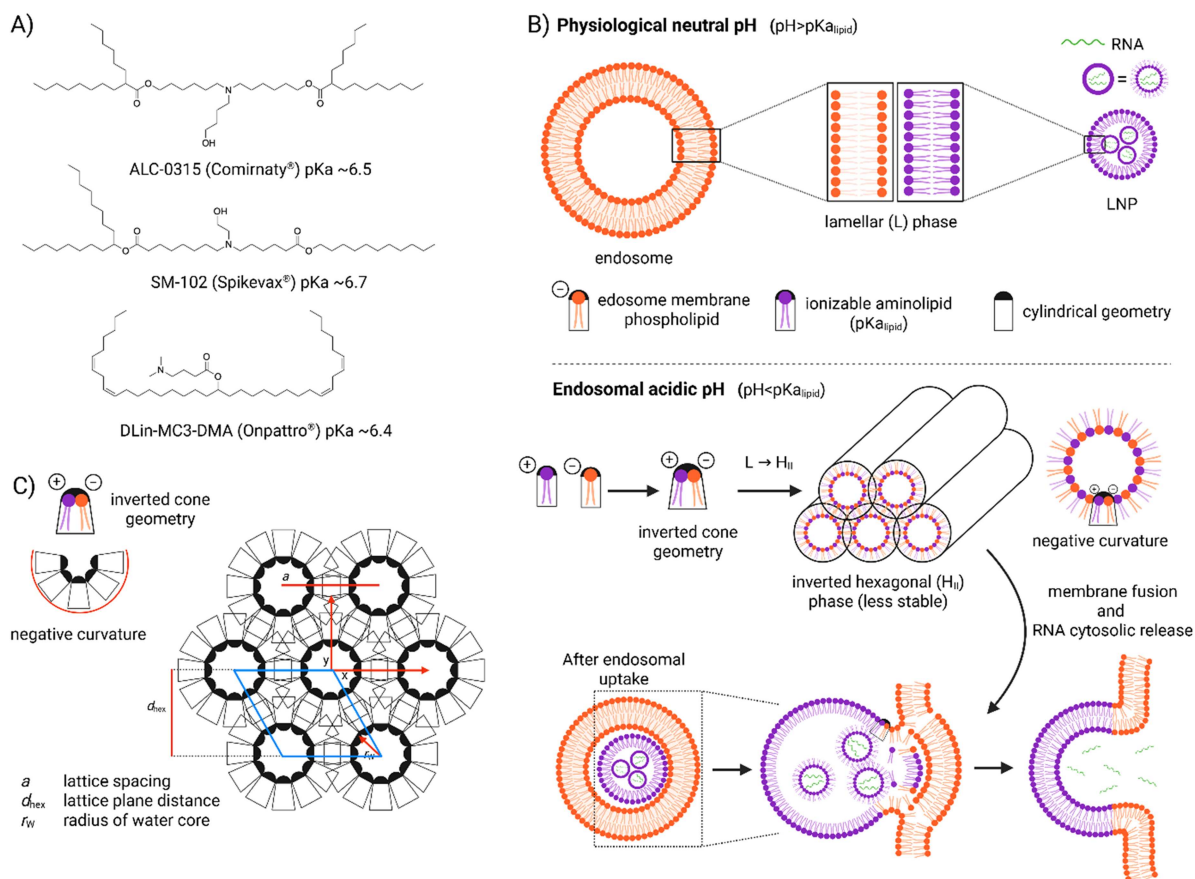


Figure 4. Applicative example of lipid membrane fusion in drug delivery: the case of lipid nanoparticles. (A) Representative chemical structures of cationic ionisable lipids used in LNP design to enhance membrane fusogenicity. Despite differences in molecular structure, these lipids share a key feature: the presence of charge-tunable amine groups whose protonation state varies with pH. These amines are protonated (positively charged) at acidic pH values below their pKa, facilitating electrostatic interactions and fusion with endosomal lipid membranes. (B) Schematic illustration of the lamellar-to-hexagonal phase transition ($L \rightarrow H_{II}$) occurring during LNP interaction with the endosomal membrane. Within the acidic environment of the endosomal compartment, protonated ionisable lipids interact with anionic lipids located on the inner leaflet of the endosomal membrane, forming cone-shaped cationic–anionic lipid pairs that are not compatible with a stable lamellar organisation. This interaction drives the transition toward the less stable H_{II} phase, resulting in the destabilization of both the LNP shell and the endosomal bilayer and ultimately enabling fusion-mediated release of the RNA cargo into the cytosol. Illustration shown in (B) inspired by Semple et al. [163]. and Chatterjee et al. [164]. (C) Schematic representation of lipid arrangement in the H_{II} phase. The H_{II} phase is a nonlamellar mesophase characterised by an array of cylindrical aqueous channels surrounded by inverted lipid monolayers arranged in a two-dimensional hexagonal geometry in the xy plane. Each lipid tubule is perpendicular to this plane and is filled with water and ions, which are not shown for clarity. Lipids are radially distributed along the cylinders surfaces, with the polar headgroups facing the aqueous cores. Illustration shown in (C) inspired by Ramezanpour et al. [165].

determined by the aminolipids' headgroup pKa values but also significantly influenced by the presence of RNA and the interplay between lipid shape, packing and headgroup chemistry [173]. The fusion between LNP and the endosomal membrane seems to be a necessary step toward RNA delivery to the cytosol, yet the latter should be considered a distinct process, as discussed by Phillip et al. [167]. Beyond lipid composition, the fusogenicity of LNPs can be enhanced by the specific packing of lipids and nucleic acids into LNP–RNA complexes. This structural organisation represents a powerful design feature that can lower the energetic cost of fusion and fusion-pore formation with target membranes [174].

Despite the recognised importance of lipid composition, packing, and fusogenic phase behaviour, a major challenge in the rational design of LNPs with high delivery efficiency remains the incomplete understanding of their internal structural features. Three primary categories of self-assembled lipid nanostructures in water have been reported in LNPs, largely determined by the specific lipid molecular

systems and the inclusion of structurally active lipids: lamellar vesicles (L), inverted hexagonal (H_{II}), and bicontinuous cubic phases (Q_{II}) [174,175]. In the lamellar phase, nucleic acids are sandwiched between lipid bilayers; in H_{II} , they are embedded within water-filled tubular lipid structures [176]; and in Q_{II} , they localise within interconnected aqueous nanochannel domains [177]. The hexagonal phase can be distorted, resulting in worm-like micelles [153]. Importantly, recent findings have shown that LNP-RNA complexes with bicontinuous cubic and inverse hexagonal internal structures exhibit significantly enhanced fusogenicity with endosomal membranes compared to conventional L phase systems [174,178]. These results underscore the critical role of the internal nanoparticle structure in facilitating the topological transitions required for efficient LNP endosomal escape, for example, by lowering the energetic cost of fusion and fusion-pore formation with the endosomal membrane [174]. Indeed, LNP structural organisation may depend sensitively on specific aspects of lipid composition, be highly heterogeneous even within a single particle, exist out of thermodynamic equilibrium, and undergo transitions between different metastable states along the journey to the endosome. Molecular simulations are providing valuable insights, although the field remains under active discussion, especially concerning the role of pH changes [179–181], and model development is still in its early stages [182]. Cholesterol is another critical component of LNP formulations, contributing to membrane fluidity and increasing bilayer rigidity. These effects are crucial for the structural stability of LNPs in biological fluids while also helping enhance encapsulation efficiency and reduce premature RNA leakage [151,183]. Recent findings have further unveiled cholesterol's implication in facilitating endosomal escape by promoting membrane fusion [184–186]. Although this effect is supported by experimental evidence, the underlying mechanisms remain poorly understood. Several hypotheses have been proposed to explain cholesterol's involvement in favoring the endosomal escape of LNPs. One suggests that the conical molecular geometry of cholesterol can lower the energy for stalk formation by providing intrinsic negative membrane curvature [187,188]. Additionally, the presence of a sterol lipid like cholesterol may alter the internal structure of LNPs, potentially inducing non-lamellar (e.g. inverted hexagonal or cubic) phases that are more disruptive to endosomal membranes, as illustrated in Figure 4B and C [189]. Structure–function studies further support the critical role of sterol lipids in tuning LNP fusogenicity. Fine-tuning cholesterol content or substituting it with structural analogues—like phytosterols—have been shown to lead to the formation of LNPs with polymorphic shapes, multilamellarity and internal defects (such as lipid partitioning) and these structural differences correlate with improved cytosolic delivery efficiency [184,190,191].

5. Conclusions

The ability to understand, control and mimic lipid membrane fusion is no longer limited to mechanistic questions in basic research but has become a strategic topic for the engineering of biologically relevant synthetic systems and the development of novel biomedical strategies. The physicochemical parameters influencing membrane fusion, from lipid composition and environmental conditions to the action of artificial fusogens, finely regulate the efficiency, selectivity, and reproducibility of processes that underlie many emerging applications.

In bottom-up synthetic biology, a quantitative understanding of fusion mechanisms is essential for the reliable design of biomimetic membrane systems. On the one hand, membrane fusion has been increasingly exploited to enable the selective and controlled transfer of lipids and proteins from biological membranes to synthetic models, such as lipid vesicles, thereby increasing compositional complexity in biomimetic studies and applications. On the other hand, the ability to programme specific and sequential fusion events has enabled the construction of vesicle-based artificial protocells and membrane-bound microcompartments capable of mimicking simple cellular functions without resorting to complex biological machinery. In these contexts, lipid membrane fusion emerges as a powerful functional nanoengineering tool whose performance critically depends on precise control of materials and experimental conditions [6,192].

In the biomedical field, lipid membrane fusion plays an equally strategic role in addressing some of the key challenges that still limit advances in nanomedicine, including the efficient loading of therapeutic cargoes into vesicular nanocarriers, their effective interaction with cellular membranes, and endosomal escape. The success of drug delivery, particularly for nucleic-acid-based therapeutics such as RNA, largely

depends on the ability to induce controlled fusion events. Without a detailed understanding of the energy barriers and intermediates involved in fusion, the optimisation of biomedical delivery systems that rely on membrane fusion mechanisms remains largely empirical.

Only by integrating well-controlled experimental model systems, molecular simulations, and rational design strategies will it be possible to transform membrane fusion from a stand-alone, difficult-to-predict phenomenon into a functionally engineerable process that can be integrated into biological and biomimetic approaches. This transition is now essential to enable the rational design of more complex biomimetic systems as well as more effective therapeutic applications.

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