Enzyme Related Synthesis of Lipids and Fabrication of Planar Lipid Membranes

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Abstract

Biological membranes are structurally and functionally complex supramolecular assemblies. The intricate complexity itself poses a significant barrier in investigating the constituents, components, and their structure-activity relationships. Scientists employ two synthetic methods to reduce complexity: the top-down approach and the bottom-up approach. The top-down synthetic approach involves genome reduction of the living cell, leading to a collateral reduction in membrane complexity. In contrast, the bottom-up synthetic approach like chemical and enzymatic synthesis engages assembly of non-living components to construct biomimetic membrane structures with desired complexity. The synthetic routes catalyzed by enzymes are referred to as enzymatic synthesis, while routes utilizing a combination of catalytic systems and enzymes are known as chemo-enzymatic synthesis. The lipids prepared by enzymatic or chemo-enzymatic method are essential to integrate into a membrane in a manner that makes them amenable to various analytical techniques. Planar lipid bilayer method is one such versatile technique which can be used to prepare membranes from individual lipid molecules to utilize in various analytical procedures. Two planar lipid membranes types, black lipid membrane and supported lipid membrane, are more common than other types due to their less complexity in preparation. This review discusses the enzyme related synthesis of lipids, the method of preparation and challenges involved in fabricating black lipid membrane and supported lipid membrane models.

Introduction

The emergence of life relies heavily on the development of an interface that separates and isolates certain organic compounds from their surroundings. This crucial step is achieved through the presence of "membrane-forming materials" in prebiotic conditions. Among these materials, simple prebiotic amphiphilic molecules such as fatty acids and lipids demonstrate great potential as they can spontaneously self-assemble in an aqueous medium. This self-assembly leads to the formation of fluid-filled, membrane-bound compartments. These compartments effectively encapsulate and trap polar molecules from the surrounding environment, providing an enclosed space for unique chemical reactions to occur. The study of these self-assembled lipid structures offers valuable insights into the early stages of life's formation (Podolsky & Devraj, 2021; Garret & Grisham, 2016; Tymoczko et al., 2015). The compartments formed by these membranes serve as boundaries that selectively permit the transportation of certain molecules based on their hydrophobic properties. Within these compartments, organic compounds engage in biochemical reactions, giving rise to macromolecules that exhibit distinct chemical characteristics compared to the surrounding species. The stability of these membranous structures becomes influenced by various factors, including ionic strength, temperature, pH, minerals, geochemistry, membrane composition, membrane-molecule interactions, chain length and degree of unsaturation of the fatty acids (Podolsky & Devraj, 2021). These factors exert selection pressure on the membrane, dictating the types of structures that are best suited for the prevailing conditions. As a consequence of the dynamic interplay between these factors and the properties of the membrane, the compartments evolved into protocells, which are abiotic "cell-like" chemical systems.

The membranes of protocells are thought to be composed of a specific group of lipids called acylglycerols, which are amphiphilic molecules formed by the combination of glycerols and fatty acids under prebiotic conditions. In the evolutionary ladder, acylglycerols are slowly replaced by phospholipids. However, this adoption of phospholipids involved a trade-off between membrane permeability and stability. The composition of membrane phospholipids and acylglycerols became crucial in safeguarding the integrity of protocells and facilitating essential biochemical reactions within them (Podolsky & Devraj, 2021). As a result, protocells gradually evolved from simple structures to more complex entities, both in terms of their membrane composition and the internal chemical reactions. The continuous refinement of protocell membranes over the time has played a crucial role in the emergence of modern-day cells.

Biological membranes of modern cells are significantly more complex than their ancestral counterparts. They now consist of clusters of membrane-bound proteins such as cell-adhesion proteins, ion channels, enzymes, cell-surface receptors, cell markers, transporters, surface antigens, etc. Furthermore, the composition of the membrane varies across its plane, resulting in lateral heterogeneity and transverse asymmetry (Raven et al., 2017). These modifications help the cells to get involved in signal transduction (Belfiore et al., 2017), cell recognition (van Meer et al., 2008), migration, growth, reproduction, identification, anchoring, bulk transport, immune response (D'Arrigo & Servi, 2010), and maintaining cell morphology. Any changes in foundational lipid composition, membrane

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protein structure, and compositional dynamics might lead to pathological conditions (Dias & Nylandsted, 2021). To develop effective therapeutics and to understand the biological relevance of these structures, the physicochemical properties of the membrane constituents and components need to be studied while they reside in the membrane. However, due to the membrane's complexity and sensitivity, this task is not readily achievable.

To analyze the membrane related structural and functional phenomena, the complexity of the membrane needs to be simplified, or construction of a minimal model membrane becomes a necessity. Synthetic biologists use complementary approaches of top-down and bottom-up methods to address this challenge (Bhattacharya et al., 2019). The top-down approach involves reverse engineering the natural cells to their more primitive form by sequentially deleting "superfluous" genes and simplifying the overall cell complexity. This result is concomitant simplification of the membrane complexity. The matrix of these "primitive" cell membranes is less complex, possessing only the basic constituents and components to sustain life. The components can be isolated, purified, and cataloged. The types of membrane lipids and protein components for the model membrane can be chosen from the available genetic makeup of the cell.

In contrast, the bottom-up approach does not rely on real cells but instead focuses on synthesizing membrane-forming lipids and systematically assembling them to form membrane like structures. These synthetic structures are analogous to the actual membranes (Blanken et al., 2020). This approach is often preferred as it allows for the incorporation of both familiar and unorthodox lipid molecules into the membrane matrix. The behavior of these synthetic membranes can be programmed by controlling the chemical nature of their constituents and researchers can decide the type of the proteins the system should possess. This type of granular control over lipid composition and membrane protein incorporation cannot be duplicated by the top-down approach.

To effectively choose between the top-down and bottom-up approaches, researchers must possess a comprehensive understanding of lipid biosynthesis and the ability to manipulate these pathways. This knowledge enables them to construct membranes with greater control to mimic real membranes. Thorough exploration and investigation of the various lipid synthetic and fabrication methods are crucial, as they provide valuable insights to design functional membrane models. In this concise review, we discuss the enzyme related lipid synthesis pathways and different planar membrane fabrication methods which are being used to develop functionally advanced membrane models to perform membrane related studies.

1. Synthesis of lipid molecules

Biological membrane lipids consist of their own enzymatic machinery for maintenance and modification (Moessinger et al., 2014). Careful structuring of these lipids within the membranes enables the manifestation of inherent traits such as membrane fluidity (Matosevic & Paegel, 2013), lamellarity (Raven et al., 2017), selective permeability (Garret & Grisham, 2016), and spatiotemporal remodeling capabilities (Simons & Gerl, 2010). Contriving artificial membranes with aforementioned features may require lipids with special functionality (Devaraj, 2017). However, chemical routes for formulating such synthetic lipids involve multi-step processes and the utilization of two or more precursors (Fasoli et al., 2006). The synthetic routes exclusively catalyzed by enzymes are referred to as enzymatic synthesis, while routes utilizing a combination of catalytic systems and

enzymes are known as chemo-enzymatic synthesis (Bhattacharya et al., 2019). The subsequent sections will delve into these methods for synthesizing lipids.

1.1 Enzymatic methods

Membrane lipid biosynthesis of cells primarily involves the acylation reaction between long-chain acyl derivatives and polar groups (Gibellini & Smith, 2010). The enzyme responsible for catalyzing this reaction is acyltransferase, which is inherently integrated into the cell membrane. This integration is essential for the proper enzymatic activity (van Meer et al., 2008). Attempts to isolate or solubilize membrane enzymes into a reaction medium for desired lipid synthesis may result enzyme denaturation, as it relies on the membrane environment to maintain its native conformation and functionality.

In order to utilize enzymes that require a membrane template, it is necessary to create specific membrane-bound structures. One strategy is to synthesize liposomes using available lipids and incorporate the membrane related enzymes into these vesicles (Matosevic & Paegel, 2013). Membrane precursors can be supplied to the reaction medium, where they can reach the liposomes through diffusion. Depending on the nature of the precursors, they can be adsorbed or fused with the liposome membrane. Once incorporated, the enzymes initiate the membrane formation reaction, and the liposomes increase in size. When they reach a critical size, the liposomes bud off giving rise to "daughter" liposomes (Mercier et al., 2013). However, these liposome-embedded enzymes often exhibit limited phospholipid production, which may be attributed to the intrinsic requirement for specific accessory proteins by the chosen enzyme systems (Vance & Devaraj, 2021).

Repurposing certain enzyme systems by giving alternative chemical routes is another strategy in enzymatic synthesis. For example, the water-soluble mycobacterial ligase FadD10 (an enzyme involved in the fatty acid metabolism of *Mycobacterium tuberculosis*) reacts with fatty acids in the presence of ATP and Mg^{2+} to form fatty acid adenylates (FAAs) (Liu et al., 2013). FAAs are very common metabolites, and they can react non-enzymatically and chemoselectively with amine-functionalized lysolipids to form phospholipids.

The choice of enzymatic lipid synthesis is motivated by several factors, despite the challenges associated with finding suitable enzymes and related synthetic routes. These methods offer several advantages, including the requirement for mild reaction conditions, lower consumption of reagents compared to non-enzymatic approaches, reduced production of by-products (see Section 1.2), and the ability to yield enantiomerically pure final products. The consideration of stereochemistry becomes particularly relevant when investigating enantiospecific lipid interactions (Taniguchi et al., 2017). These reasons highlight the significance of enzymatic approaches in lipid synthesis and their potential for addressing specific research objectives in the field.

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1.2 Chemo-enzymatic method

These methods take a distinct approach by employing "acylation" reactions with the help of enzymes to synthesize lipid molecules (Podolsky & Devaraj, 2021). Unlike enzymatic synthesis, the precursors and the final lipid molecules may not necessarily share any structural or chiral resemblance to the canonical membrane lipids (Martin et al., 2021).This innovative strategy allows for the exploration of diverse chemical landscapes and the synthesis of lipid molecules with unique properties, providing a departure from traditional enzymatic approaches. Here, the primary emphasis lies in the design of a lysolipid-like precursor. This precursor is strategically engineered to chemically prime it for reactions with fatty acid analogues, resulting in the formation of molecules that can be unconventional or chemically tweaked canonical lipids. The significance of this method (D'Arrigo et al., 2007) is beyond its technical intricacies, as it plays a pivotal role in redefining life in chemical terms. The initial step in this method starts with the synthesis of lysophosphatidylcholines – the precursor.

1.2.1 Synthesis of Lysophospatidylcholines (LPCs)

The reaction pathway begins with the synthesis of 2-lysophosphatidylcholine (2-LPC), a naturally occurring most abundant phospholipid in membranes (Figure 1.1). The reason to begin with 2-LPC is that current unorthodox reaction trends heavily rely on the ability for its functionalization. To synthesize 2-LPC, both natural and unnatural precursors can be used as starting materials with desired chiral centers. The canonical procedure includes three steps; the preparation of regioselective acyl-substituted glycerol backbone, subsequent phosphorylation, and amination (D'Arrigo & Servi, 2010). The major challenge in this procedure is the regioselective incorporation of the acyl chain into glycerol/glycerol derivative. The incorporated acyl group can undergo intramolecular migration and can be catalyzed by both acids and bases (D'Arrigo & Servi, 2010). Therefore, a mixture of 1-acyl and 2-acyl substituted glycerol derivatives will be obtained. Careful control over pH and certain reagents can suppress the side reaction but cannot be eliminated (D'Arrigo & Servi, 2010). This equilibration becomes a problem in product purification procedures.

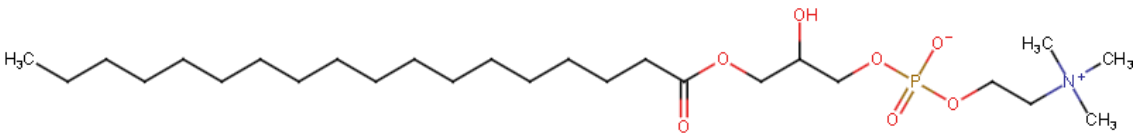

Figure 1.1: A typical structure of 2-lysophosphatidylcholine

Deacylation of natural phosphatidylcholines (PCs) is another alternative approach to preparing 2-LPCs (Anaokar et al., 2019). Acid hydrolysis of PCs gives

glycerophosphorylcholines (GPCs) (Figure 1.2), and esterification with the desired fatty acids gives 1,2-diacyl-phosphatidylcholines. Exploiting certain classes of phospholipases to hydrolyze the fatty acid chain at position *sn*-2 can give 2-LPCs. Tin-mediated monoacylation of GPCs is another promising method to synthesize 2-LPCs and involves no enzymes. Once the synthesis of 2-LPC is accomplished, the functional group at *sn*-2 can be chemically primed to undergo reactions with fatty acid analogues.

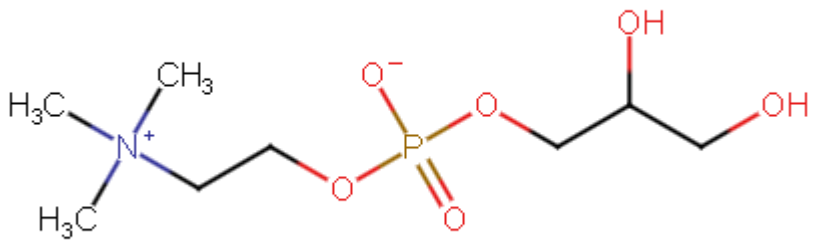

Figure 1.2: Structure of glycerophosphorylcholine

1.2.2 Functionalization of 2-LPCs

The acylation of the *sn*-2 positions of 2-LPCs initiates the functionalization process. By introducing a chemical "linker" at the *sn*-2 position, specific fatty acid derivatives can be selectively incorporated into the lipid structure. Acting as a reactive intermediate, the "linker" facilitates the formation of the desired lipid molecule through chemoselective reactions with the targeted fatty acid derivatives. Careful consideration of the linker's structure, reactivity, and sensitivity (Flores et al., 2020) enables fine-tuning of the reaction conditions to optimize the yield of the desired lipids (Podolsky & Devraj, 2021). This chemo-enzymatic approach offers a promising avenue for the efficient synthesis of tailored lipid molecules with desired functional properties.

1.3 Application of lipids / lipid structures

Lipid molecules synthesized through enzymatic and chemo-enzymatic approaches have the remarkable ability to self-assemble into supramolecular structures, such as vesicles. These vesicles hold significant potential for further functionalization as artificial cells or as versatile platforms for drug delivery (Hindley et al., 2020). Researchers can leverage current synthetic capabilities to incorporate diverse components, including drugs, proteins, and minigenomes (transcriptional-translational/TXTL machinery), into these vesicles to study natural cellular behavior with minimal components. Unorthodox lipid vesicles offer distinct advantages as drug delivery systems compared to enzymatically synthesized vesicles. Unlike enzymatically synthesized vesicles, which may possess evolutionary biochemical relationships that can be exploited by host systems for elimination, unorthodox vesicles can

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be deliberately designed to be non-immunogenic, non-toxic, and resistant to enzymatic degradation. To assess their potential applications, it is crucial to fabricate the lipid molecules or their assemblies, such as vesicles, into model membranes, enabling comprehensive investigation using available techniques.

2. Model membrane fabrication

Lipid molecules can exist as individual molecules or as vesicles in the reaction medium. By changing the reaction medium, the vesicles can be disassembled into individual lipid molecules or vice versa. This is a very important requirement for fabrication procedures because they require starting materials to be in a particular form or state (Castellana & Cremer, 2006; Tamm & McConnell, 1985). For most scientific investigations, the membranes assembled as planar bilayer structures are preferred (Tsemperouli et al., 2019; Ries et al., 2004; Frey et al., 2018).

2.1 Applicability of commercially available lipid molecules

Commercially available lipid molecules are commonly used for fabricating model membranes, with their suitability depending on the specific nature of the investigation. When studying components that are bio-orthogonal with membrane lipids, readily available lipids serve as an inert matrix or "docking centers" without posing any issues. However, for investigations focusing on lipid composition, dynamics of lipid-anchored proteins, membrane heterogeneity, membrane modification, transmembrane protein folding, membrane curvature, or compositional dynamics, the standard lipid solutions may not be suitable. In such cases, researchers must resort to more intricate and laborious manual synthetic approaches to create custom lipid compositions that precisely meet the experimental requirements.

The components that need to be integrated into the model membrane matrix also influence the type of fabrication. For example, to probe the structure-related functionality of membrane proteins, the membrane should have the ability to incorporate proteins, conserve their native conformation, provide freedom to move within the fabricated region, and should have solution access to both interfaces. The following sections provide an overview of some commonly employed fabrication methods in the field.

2.2 Black lipid membrane (BLM)

The typical setup has two chambers separated by a micrometer thick Teflon septum (Kapoor et al., 2008). The septum contains an aperture (~0.001 mm diameter) and sometimes the septum can be adjusted in a certain direction (Montal & Mueller, 1972; Lidgard & Jones, 1975). Initially, the chambers are filled with analyte solution, and the lipid solution is dissolved in a suitable organic solvent. This prepared solution can be painted as a film over the aperture or added to both analyte-filled chambers as a condensed monolayer (Rajapaksha et al., 2013). Dipping the septum into the trough or changing the liquid height of the monolayer-loaded chambers can lead to bilayer formation on the aperture. Bright-field microscopy, scanning nonlinear optical microscopy and electrochemical impedance spectroscopy (Tsemperouli et al., 2019) can be used to confirm both the bilayer formation and the membrane thickness based on the type of the BLM method used.

This synthetic membrane closely mimics the microenvironment of a real membrane. With aqueous solutions covering both sides of the membrane, diffusion of constituents and components within the membrane is unhindered, allowing for fusion of membrane-bound structures. Transmembrane proteins of interest can be purified from real cells either as detergent-extracted or vesicle-incorporated forms and can be successfully integrated into the bilayer. Through probing the analyte chambers, their activities such as ion channel currents, transport kinetics, and receptor affinities for ligands/inhibitors can be studied (Montal & Darszon, 1981). The obtained results can be extrapolated to real scenarios and are reproducible with proper care (Urban et al., 2016). This approach enables the investigation of various phenomena, including the effects of ionophore antibiotics, pore-forming toxins, protein-lipid interactions, and drug interactions on membrane structure, integrity, surface reactions, and transverse diffusion of lipid molecules (Drachev et al., 1974). Overall, this method is well-suited for studying the functionality of unknown proteins. In the following section, the challenges in fabricating the lipids as BLM are discussed.

2.2.1 Challenges in BLM

The major disadvantage of this method is that the membrane's surface tension is insufficient to maintain it for a longer period, hence imposing a time limitation (Shi & Baugmart, 2014). So only the chemical events within the lifetime of a BLM can be recorded. Another important parameter to maintain is the osmolarity of the chambers. If there's a significant difference in osmolarities, the membrane can be deformed, blocked, ruptured, and possibly convert back to vesicles (Shi & Baugmart, 2014). This encouraged researchers to develop other fabrication methods which are free from these drawbacks. In the following section, we will explore one such alternative approach that offers improved stability and functionality, thereby eliminating the aforementioned limitations.

2.3 Supported lipid bilayer (SLB)

The fabrication of supported lipid bilayers (SLBs) involves the systematic assembly of lipid molecules onto a solid support, which significantly enhances their stability. The stability of SLBs arises from the strong interaction between the lipid molecules and the solid substrate, which provides mechanical support and prevents the bilayer from rupturing or collapsing. This attachment helps overcome the surface tension limitations of BLMs and allows SLBs to be maintained for longer periods of time. Commonly Langmuir-Blodgett (LB) technique (Roberts, 1990) is used for fabrication. Lipid molecules are mixed in water-immiscible solvent and a few drops are added to the water surface of the LB trough, the solution spreads rapidly and covers the entire surface area available. Later the volatile solvent evaporates and leaves the lipids at the air-water interface. The distance between the lipid molecules will be large at this stage and the molecules will be randomly distributed. Reducing the surface area by increasing the surface pressure induces phase transition on the monolayer, and ultimately a compact solid phase of monolayer (Roberts, 1990) called Langmuir film will result. At this stage, the monolayer will have intermolecular distances and orientations identical to the leaflets of the actual membrane. This monolayer should be

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transferred to the substrate twice to create the inner and upper leaflets of the membrane.

The properties of SLBs, such as lipid composition, packing density, and orientation, can be precisely tailored and controlled during the fabrication process. This control enables the creation of well-defined and reproducible experimental conditions, allowing for accurate measurements and detailed investigation. However, despite these advantages, their fabrication is not without challenges. In the next section, the challenges encountered during the fabrication and component integration on SLBs will be discussed.

2.3.1 Challenges in SLB

The major problem associated with these structures is that they cannot be exposed to air, and the introduction of air onto the membrane can compromise membrane integrity. However, coating the bilayer with certain materials can improve membrane stability (Holden, 2004 & Daniel, 2006). The method of incorporating transmembrane proteins in this method is also problematic. Because co-dissolving synthesized (Robelek, 2007) or extracted membrane proteins with the lipid solutions and adding them to the LB trough will expose them to the air-water interface. This can denature the whole enzyme or some parts of it that might be important for functioning. To overcome this, the desired membrane proteins are incorporated into vesicles, allowing them to be adsorbed onto the solid support. The vesicles slowly rupture and expand as a bilayer on the substrate.

Problem arises when the proteins tend to have favorable interactions with the solid support due to their proximity to the membrane. If the protein is involved in surface reactions, the interaction with the solid support increases the activation barrier for lateral diffusion, and the dynamic reactions associated with the proteins will be slower or even possibly absent. Therefore, the kinetic parameters obtained regarding the proteins in this fabrication method will be different from the actual value (Castellana & Cremer, 2006). Additionally, proteins having a domain organization that spans transversely might also be affected by this method. Because the domains that need to be protruded outside the membrane for proper functioning can interact with the substrate, resulting in denaturation or inactivation (Castellana & Cremer, 2006).

The nature of the substrate can also affect the imitative behavior of synthetic membranes because all surfaces are not microscopically smooth and will possess different adsorption sites/defects with different affinity and valency for the same adsorbent (Castellana & Cremer, 2006). Therefore, preferential adsorption and distribution of certain components can occur during fabrication. This in turn affects membrane fluidity, reaction kinetics, and membrane integrity.

Elevating the fabricated membrane from the substrate to a certain distance might relieve the system from mentioned drawbacks (Wong et al., 1999). This can be done by “cushioning” the substrate with a polymeric substance or self-assembling a functionalized monolayer onto the substrate before following the above fabrication procedure. The cushioning effect is analogous to paint sealants, cutting off any imperfections, adding thickness, and decoupling unwanted interactions between the substrate and the membrane (Wong et al., 1999).

SLBs are often preferred over BLMs because they can be probed by several surface analytical tools such as atomic force microscopy (Sarkis & Vié, 2020), vibrational sum-

frequency generation spectroscopy, fluorescence correlation spectroscopy, fluorescence recovery, and surface plasmon resonance. This allows for the characterization of membrane topology, the movement of lipid molecules, phase transition (Prenner, 1997), spatiotemporal information, and domain organizations (Melby et al., 2016).

Conclusion

This review explored the synthesis of lipid molecules through enzymatic and chemo-enzymatic methods, driven by discovery of lipid synthetic pathways and the availability of diverse chemical compounds. These advancements have opened up new possibilities for investigating the potential applications of synthesized lipids. However, in order to fully understand and harness their capabilities, it is crucial to fabricate these lipid molecules into functional structures – artificial membranes. Continued efforts in improving fabrication methods and achieving precise control over membrane composition, structure, and functionality have expanded the possibilities for targeted investigations and enabled a deeper understanding of biological processes. Out of many synthetic pathways for synthesizing lipid molecules, we believe the methods involved with enzymes give more reliability and control. Considering the membrane probing methods, planar lipid membrane methods are easier to handle, have broad experimental scope and cheaper to make. This step-by-step construction of increasingly complex artificial membranes paves the way for further exploration of membrane-related phenomena in a controlled environment, ultimately leading to valuable insights and advancements in scientific and medical applications.

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References

Anaokar, S., Kodali, R., Jonik, B., Renne, M. F., Brouwers, J. F. H. M., Lager, I., Patton-Vogt, J. (2019). The glycerophosphocholine acyltransferase Gpc1 is part of a phosphatidylcholine (PC)-remodeling pathway that alters PC species in yeast. *The Journal of Biological Chemistry*, *294*(4), 1189–1201. doi:10.1074/jbc.ra118.005232

Belfiore, A., Malaguarnera, R., Vella, V., Lawrence, M. C., Sciacca, L., Frasca, F., Morrione, A., & Vigneri, R. (2017). Insulin receptor isoforms in physiology and disease: An updated view. *Endocrine Reviews*, *38*(5), 379–431. https://doi.org/10.1210/er.2017-00073

Bhattacharya, A., Brea, R. J., Niederholtmeyer, H., & Devaraj, N. K. (2019). A minimal biochemical route towards de novo formation of synthetic phospholipid membranes. *Nature Communications*, *10*(1), 300. https://doi.org/10.1038/s41467-018-08174-x

Blanken, D., Foschepoth, D., Serrão, A. C., & Danelon, C. (2020). Genetically controlled membrane synthesis in liposomes. *Nature Communications*, *11*(1), 4317. https://doi.org/10.1038/s41467-020-17863-5

Castellana, E. T., & Cremer, P. S. (2006). Solid supported lipid bilayers: From biophysical studies to sensor design. *Surface Science Reports*, *61*(10), 429–444. https://doi.org/10.1016/j.surfrep.2006.06.001

Daniel, S., Albertorio, F., & Cremer, P. S. (2006). Making lipid membranes rough, tough, and ready to hit the road. *MRS Bulletin*, *31*(7), 536–540. https://doi.org/10.1557/mrs2006.139

D'Arrigo, P., Fasoli, E., Pedrocchi-Fantoni, G., Rossi, C., Saraceno, C., Tessaro, D., & Servi, S. (2007). A practical selective synthesis of mixed short/long chains glycerophosphocholines. *Chemistry and Physics of Lipids*, *147*(2), 113–118. https://doi.org/10.1016/j.chemphyslip.2007.03.008

D'Arrigo, P., & Servi, S. (2010). Synthesis of lysophospholipids. *Molecules (Basel, Switzerland)*, *15*(3), 1354–1377. https://doi.org/10.3390/molecules15031354

Devaraj, N. K., & Finn, M. G. (2021). Introduction: Click chemistry. *Chemical Reviews*, *121*(12), 6697–6698. doi:10.1021/acs.chemrev.1c00469

Devaraj, N. K. (2017). In situ synthesis of phospholipid membranes. *The Journal of Organic Chemistry*, *82*(12), 5997–6005. https://doi.org/10.1021/acs.joc.7b00604

Dias, C., & Nylandsted, J. (2021). Plasma membrane integrity in health and disease: significance and therapeutic potential. *Cell Discovery*, *7*(1), 4. https://doi.org/10.1038/s41421-020-00233-2

Drachev, L. A., Jasaitis, A. A., Kaulen, A. D., Kondrashin, A. A., Liberman, E. A., Nemecek, I. B., Ostroumov, S. A., Semenov, A. Y., & Skulachev, V. P. (1974). Direct measurement of electric current generation by cytochrome oxidase, H+-ATPase and bacteriorhodopsin. *Nature*, *249*(5455), 321–324. https://doi.org/10.1038/249321a0

Fasoli, E., Arnone, A., Caligiuri, A., D'Arrigo, P., de Ferra, L., & Servi, S. (2006). Tin-mediated synthesis of lyso-phospholipids. *Organic & Biomolecular Chemistry*, *4*(15), 2974–2978. https://doi.org/10.1039/b604636c

Flores, J., White, B. M., Brea, R. J., Baskin, J. M., & Devaraj, N. K. (2020). Lipids: chemical tools for their synthesis, modification, and analysis. *Chemical Society Reviews*, *49*(14), 4602–4614. doi:10.1039/d0cs00154f

Frey, C. M., Barth, H., Kranz, C., & Mizaikoff, B. (2018). Horizontal black lipid bilayer membranes for studying pore-forming toxins. *Analytical Methods: Advancing Methods and Applications*, *10*(26), 3153–3161. https://doi.org/10.1039/c8ay01122b

Garrett, R. H., & Grisham, C. M. (2016). *Biochemistry* (6th ed.). Brooks Cole.

Gibellini, F., & Smith, T. K. (2010). The Kennedy pathway--De novo synthesis of phosphatidylethanolamine and phosphatidylcholine. *IUBMB Life*, *62*(6), 414–428. https://doi.org/10.1002/iub.337

Hindley, J. W., Law, R. V., & Ces, O. (2020). Membrane functionalization in artificial cell engineering. *SN Applied Sciences*, *2*(4). https://doi.org/10.1007/s42452-020-2357-4

Holden, M. A., Jung, S.-Y., Yang, T., Castellana, E. T., & Cremer, P. S. (2004). Creating fluid and air-stable solid supported lipid bilayers. *Journal of the American Chemical Society*, *126*(21), 6512–6513. doi:10.1021/ja048504a

Kapoor, R., Kim, J. H., Ingolfson, H., & Andersen, O. S. (2008). Preparation of artificial bilayers for electrophysiology experiments. *Journal of Visualized Experiments: JoVE*, *20*. https://doi.org/10.3791/1033

Lidgard, G. P., & Jones, M. N. (1975). d-Glucose permeability of black lipid membranes modified by human erythrocyte membrane fractions. *The Journal of Membrane Biology*, *21*(1), 1–10. https://doi.org/10.1007/bf01941058

Liu, Z., Ioerger, T. R., Wang, F., & Sacchettini, J. C. (2013). Structures of Mycobacterium tuberculosis FadD10 protein reveal a new type of adenylate-forming enzyme. *The Journal of Biological Chemistry*, *288*(25), 18473–18483. doi:10.1074/jbc.M113.466912

Martin, H. S., Podolsky, K. A., & Devaraj, N. K. (2021). Probing the role of chirality in phospholipid membranes. *Chembiochem: A European Journal of Chemical Biology*, *22*(22), 3148–3157. https://doi.org/10.1002/cbic.202100232

Matosevic, S., & Paegel, B. M. (2013). Layer-by-layer cell membrane assembly. *Nature Chemistry*, *5*(11), 958–963. https://doi.org/10.1038/nchem.1765

Melby, E. S., Mensch, A. C., Lohse, S. E., Hu, D., Orr, G., Murphy, C. J., … Pedersen, J. A. (2016). Formation of supported lipid bilayers containing phase-segregated domains and their interaction with gold nanoparticles. *Environmental Science. Nano*, *3*(1), 45–55. doi:10.1039/c5en00098j

Mercier, R., Kawai, Y., & Errington, J. (2013). Excess membrane synthesis drives a primitive mode of cell proliferation. *Cell*, *152*(5), 997–1007. https://doi.org/10.1016/j.cell.2013.01.043

Moessinger, C., Klizaite, K., Steinhagen, A., Philippou-Massier, J., Shevchenko, A., Hoch, M., Ejsing, C. S., & Thiele, C. (2014). Two different pathways of phosphatidylcholine synthesis, the Kennedy Pathway and the Lands Cycle, differentially regulate cellular triacylglycerol storage. *BMC Cell Biology*, *15*(1), 43. https://doi.org/10.1186/s12860-014-0043-3

Montal, M., Darszon, A., & Schindler, H. (1981). Functional reassembly of membrane proteins in planar lipid bilayers. *Quarterly Reviews of Biophysics*, *14*(1), 1–79. https://doi.org/10.1017/s0033583500002079

Montal, M., & Mueller, P. (1972). Formation of bimolecular membranes from lipid monolayers and a study of their electrical properties. *Proceedings of the National*

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Academy of Sciences of the United States of America, *69*(12), 3561–3566. https://doi.org/10.1073/pnas.69.12.3561

Podolsky, K. A., & Devaraj, N. K. (2021). Synthesis of lipid membranes for artificial cells. *Nature Reviews Chemistry*, *5*(10), 676–694. https://doi.org/10.1038/s41570-021-00303-3

Prenner, E. J., Lewis, R. N., Neuman, K. C., Gruner, S. M., Kondejewski, L. H., Hodges, R. S., & McElhaney, R. N. (1997). Nonlamellar phases induced by the interaction of gramicidin S with lipid bilayers. A possible relationship to membrane-disrupting activity. *Biochemistry*, *36*(25), 7906–7916. https://doi.org/10.1021/bi962785k

Rajapaksha, S. P., Wang, X., & Lu, H. P. (2013). Suspended lipid bilayer for optical and electrical measurements of single ion channel proteins. *Analytical Chemistry*, *85*(19), 8951–8955. https://doi.org/10.1021/ac401342u

Raven, P. H., Johnson, G. B., Mason, K. A., Losos, J., & Singer, S. (2016). *Raven, biology, 2017, 11E (AP edition) AP focus review guide* (11th ed.). McGraw-Hill Education.

Ries, R. S., Choi, H., Blunck, R., Bezanilla, F., & Heath, J. R. (2004). Black lipid membranes: Visualizing the structure, dynamics, and substrate dependence of membranes. *The Journal of Physical Chemistry. B*, *108*(41), 16040–16049. https://doi.org/10.1021/jp048098h

Robelek, R., Lemker, E. S., Wiltschi, B., Kirste, V., Naumann, R., Oesterhelt, D., & Sinner, E.-K. (2007). Incorporation of in vitro synthesized GPCR into a tethered artificial lipid membrane system. *Angewandte Chemie (International Ed. in English)*, *46*(4), 605–608. https://doi.org/10.1002/anie.200602231

Roberts, G. (Ed.). (1990). *Langmuir-Blodgett Films* (1990th ed.). Kluwer Academic/Plenum.

Sarkis, J., & Vié, V. (2020). Biomimetic models to investigate membrane biophysics affecting lipid-protein interaction. *Frontiers in Bioengineering and Biotechnology*, *8*, 270. https://doi.org/10.3389/fbioe.2020.00270

Shi, Z., & Baumgart, T. (2014). Dynamics and instabilities of lipid bilayer membrane shapes. *Advances in Colloid and Interface Science*, *208*, 76–88. doi:10.1016/j.cis.2014.01.004

Simons, K., & Gerl, M. J. (2010). Revitalizing membrane rafts: new tools and insights. *Nature Reviews. Molecular Cell Biology*, *11*(10), 688–699. https://doi.org/10.1038/nrm2977

Tamm, L. K., & McConnell, H. M. (1985). Supported phospholipid bilayers. *Biophysical Journal*, *47*(1), 105–113. https://doi.org/10.1016/s0006-3495(85)83882-0

Taniguchi, R., Inoue, A., Sayama, M., Uwamizu, A., Yamashita, K., Hirata, K., Yoshida, M., Tanaka, Y., Kato, H. E., Nakada-Nakura, Y., Otani, Y., Nishizawa, T., Doi, T., Ohwada, T., Ishitani, R., Aoki, J., & Nureki, O. (2017). Structural insights into ligand recognition by the lysophosphatidic acid receptor LPA6. *Nature*, *548*(7667), 356–360. https://doi.org/10.1038/nature23448

Tsemperouli, M., Amstad, E., Sakai, N., Matile, S., & Sugihara, K. (2019). Black lipid membranes: Challenges in simultaneous quantitative characterization by electrophysiology and fluorescence microscopy. *Langmuir: The ACS Journal of Surfaces and Colloids*, *35*(26), 8748–8757. https://doi.org/10.1021/acs.langmuir.9b00673

Tymoczko, J. L., & Berg, J. M. (2015). *Biochemistry: A Short Course: Third edition* (3rd ed.). W.H. Freeman.

Urban, P., Kirchner, S. R., Mühlbauer, C., Lohmüller, T., & Feldmann, J. (2016). Reversible control of current across lipid membranes by local heating. *Scientific Reports*, *6*(1), 22686. https://doi.org/10.1038/srep22686

van Meer, G., Voelker, D. R., & Feigenson, G. W. (2008). Membrane lipids: where they are and how they behave. *Nature Reviews. Molecular Cell Biology*, *9*(2), 112–124. https://doi.org/10.1038/nrm2330

Vance, J. A., & Devaraj, N. K. (2021). Membrane mimetic chemistry in artificial cells. *Journal of the American Chemical Society*, *143*(22), 8223–8231. https://doi.org/10.1021/jacs.1c03436

Wong, J. Y., Majewski, J., Seitz, M., Park, C. K., Israelachvili, J. N., & Smith, G. S. (1999). Polymer-cushioned bilayers. I. A structural study of various preparation methods using neutron reflectometry. *Biophysical Journal*, *77*(3), 1445–1457. doi:10.1016/S0006-3495(99)76992-4

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