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The lipid network

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ABSTRACT

Natural cell membranes are composed of a remarkable variety of lipids, conferring specific biophysical properties for proteins and organisms to function. Knowledge of the lipid composition of membranes may allow the design of new membrane active drugs, which will further our understanding of why lipids are so diverse. Crafting a relevant model of a cell membrane with controlled composition is becoming an art, with the ability to reveal the molecular mechanisms of biological processes and lead to better treatment of pathologies. By matching physiological observations from *in vivo* experiments to high-resolution information, more easily obtained from *in vitro* studies, complex interactions at the lipid interface are determined. The role of the lipid network in biological membranes is, therefore, the subject of increasing attention.

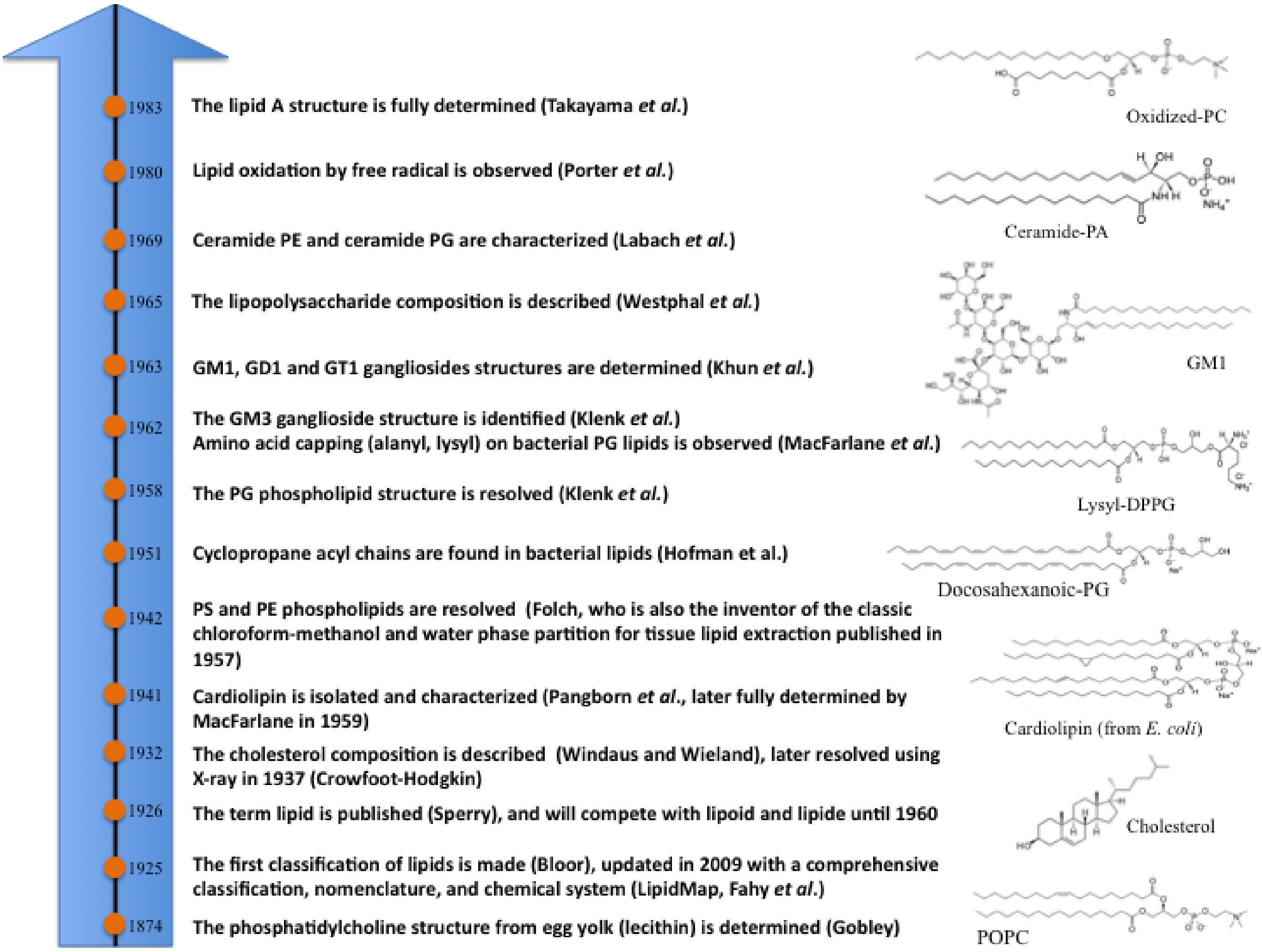
Keywords: model membrane, lipid composition, lipid transformation, lipid targeting, membrane active peptides.

Knowledge of cellular mechanisms has been obtained by relating *in vitro* and *in vivo* observations. *In situ* studies of highly entangled cell networks provide us with challenging hypotheses that high-resolution experiments using cell models try to validate or *vice-versa*. In general, membrane-active molecules respond differently to specific lipid composition, which can be encountered either within a single cell membrane – such as a lipid domain – or by trafficking through different membrane layers. As the variety of lipids is linked progressively to cellular regulation, it is becoming important to craft relevant membrane models and to analyze information obtained from different lipid compositions. This review begins with a brief overview of the history of lipids and model membranes before expanding on the differences obtained using natural membranes, membrane models and between models of various lipid compositions. Finally, how membrane complexity due to the diversity of the lipid components can be used to advantage for specific targeting will be described.

A complex network builds over a century

The immense variety of lipids was not immediately obvious. The term lipid itself has evolved, first used by Sperry in 1926 (Sperry 1926) but definitively adopted in 1960, competing with the terms lipoid or lipide until then. Meanwhile, numerous lipid structures were determined, amongst the first that of the phosphatidylcholine structure from egg yolk in 1874 by the French scientist Gobley. The sense of diversity already existed in this period due to the exponential rate of discovery of different fatty acids that constitute the acyl chain of lipids.

In between the two world wars, the renowned structure elucidation of cholesterol led to two Nobel prizes, in 1927 (Windaus and Wieland with the chemical structure) and 1937 (Crowfoot-Hodgkin with the first X-ray crystal structure). The main phospholipids were discovered between 1941 with cardiolipin (CL) (Pangborn 1941) then phosphatidylethanolamine (PE), phosphatidylserine (PS) in 1942 (Folch 1942), phosphatidylglycerol (PG) in 1958 (Benson and Maruo 1958) and finally phosphatidylinositol (PI) in 1959 (Pizer and Ballou 1959). The ceramide and ganglioside lipid structures were resolved in the sixties (Klenk and Padberg 1962; Kuhn and Egge 1963; Kuhn and Wiegandt 1963; LaBach and White 1969) as post-synthesis modifications of bacterial lipids headgroups by amino-acid addition (Macfarlane 1962). Lipopolysaccharide and lipid A structure determinations were achieved in the mid eighties (Takayama et al. 1983); and acyl chain modifications were discovered in 1980 (Porter et al. 1980), illustrating the diversity of lipid species composing the vital - and functional - cell membranes of all living organisms.



1983 The lipid A structure is fully determined (Takayama *et al.*)

1980 Lipid oxidation by free radical is observed (Porter *et al.*)

1969 Ceramide PE and ceramide PG are characterized (Labach *et al.*)

1965 The lipopolysaccharide composition is described (Westphal *et al.*)

1963 GM1, GD1 and GT1 gangliosides structures are determined (Khun *et al.*)

1962 The GM3 ganglioside structure is identified (Klenk *et al.*)
Amino acid capping (alanyl, lysyl) on bacterial PG lipids is observed (MacFarlane *et al.*)

1958 The PG phospholipid structure is resolved (Klenk *et al.*)

1951 Cyclopropane acyl chains are found in bacterial lipids (Hofman et al.)

1942 PS and PE phospholipids are resolved (Folch, who is also the inventor of the classic chloroform-methanol and water phase partition for tissue lipid extraction published in 1957)

1941 Cardiolipin is isolated and characterized (Pangborn *et al.*, later fully determined by MacFarlane in 1959)

1932 The cholesterol composition is described (Windaus and Wieland), later resolved using X-ray in 1937 (Crowfoot-Hodgkin)

1926 The term lipid is published (Sperry), and will compete with lipoid and lipide until 1960

1925 The first classification of lipids is made (Bloor), updated in 2009 with a comprehensive classification, nomenclature, and chemical system (LipidMap, Fahy *et al.*)

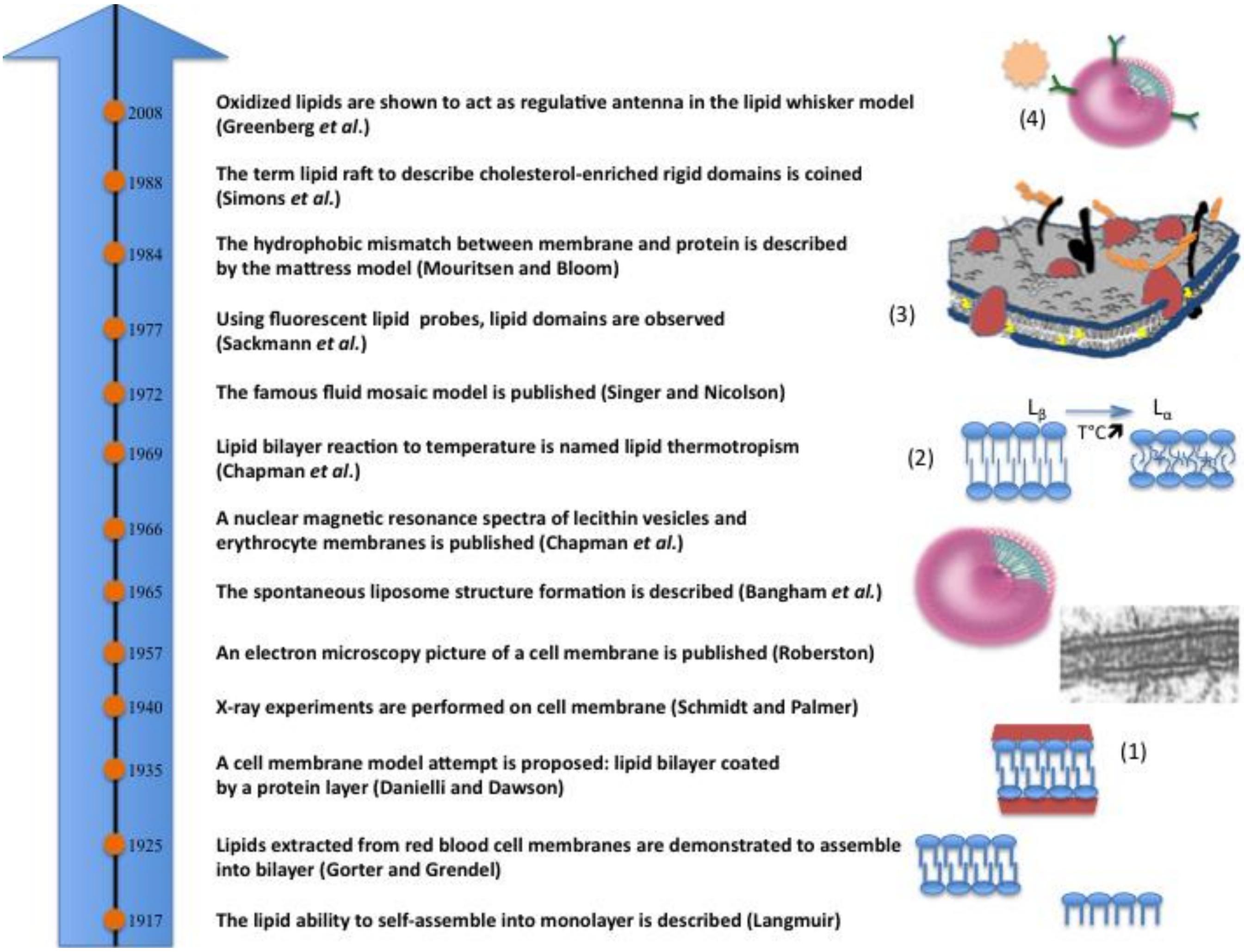
1874 The phosphatidylcholine structure from egg yolk (lecithin) is determined (Gobley)

Fig 1. Timeline of various lipid discoveries. POPC: 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine. PG: glycero-3-phosphoglycerol. DPPG: 1,2 -dipalmitoyl-sn-glycero-3-phosphoglycerol. GM1: monosialotetrahexosylganglioside. PA: glycero-3-phosphatidic acid. PE: glycero-3-phosphoethanolamine. PS: glycero-3-phosphoserine. PI: glycero-3-phosphoinositol.

Evolution of understanding of lipid membranes

The original ideas of cell membrane composed of lipids came from the work of Quincke in 1888, later confirmed by Overton in 1895, when cell permeability to ionic and hydrophobic molecules was studied. The concept of lipid self-organization into monolayer at the water-air interface was demonstrated by Langmuir in 1917. He used the initial work of Pockels, and indeed even her sink, to make the now famous Langmuir trough. Eight years later, Gorter and Grendel extracted lipids from red blood cells and came to the conclusion that lipids were assembled into bilayers if the area per lipid and the cell surface were to match (Gorter and Grendel 1925). The first attempt to describe a cell membrane model was made by Danielli and Dawson in 1935 (Danielli and Davson 1935). They described that lipids form a bilayer sandwiched by a layer of proteins, erroneously confirmed by Schmidt and Palmer using X-ray techniques (1941) (Palmer and Schmitt 1941; Palmer et al. 1941). This marked the explosion of structural work using high-resolution techniques. Robertson observed the first organelle membranes using electron microscopy in 1957 and came up with the "unit membrane" or railroad track model (Robertson 1957).

The use of extracted phospholipids to reconstitute cell membranes was achieved by Bangham in 1965 (Bangham et al. 1965a) and the term liposome was coined. Summarizing several observations from *in vivo* to *in vitro* studies, Singer and Nicolson determined that cell membranes were highly fluid and homogeneous and established the fluid mosaic model in 1972 (Singer and Nicolson 1972), where proteins are rapidly diffusing in a "sea" of lipids. Following this period, several biophysical concepts flourished: lipid phases (Chapman 1975; Marsh 1991), lipid domains (Karnovsky et al. 1982; Stier and Sackmann 1973), membrane plasticity (Chapman 1988; Gitler and Rudy 1972), lipid polymorphism (Cullis and de Kruijff 1979), membrane asymmetry (Rothman and Lenard 1977), the lipid raft model (1988) (Simons and Ikonen 1997) and more recently the lipid whisker models of oxidised lipids (2008) (Greenberg et al. 2008) refined our understanding of lipid membrane functionalities. But despite the tremendous advances in high-resolution techniques, it is still difficult to control membrane organization, lipid structures and interplay with proteins to understand cellular processes. The common strategy to overcome these difficulties is to match *in vivo* functional information with *in vitro* structural data using relevant membrane models.



2008 Oxidized lipids are shown to act as regulative antenna in the lipid whisker model (Greenberg *et al.*)

1988 The term lipid raft to describe cholesterol-enriched rigid domains is coined (Simons *et al.*)

1984 The hydrophobic mismatch between membrane and protein is described by the mattress model (Mouritsen and Bloom)

1977 Using fluorescent lipid probes, lipid domains are observed (Sackmann *et al.*)

1972 The famous fluid mosaic model is published (Singer and Nicolson)

1969 Lipid bilayer reaction to temperature is named lipid thermotropism (Chapman *et al.*)

1966 A nuclear magnetic resonance spectra of lecithin vesicles and erythrocyte membranes is published (Chapman *et al.*)

1965 The spontaneous liposome structure formation is described (Bangham *et al.*)

1957 An electron microscopy picture of a cell membrane is published (Roberston)

1940 X-ray experiments are performed on cell membrane (Schmidt and Palmer)

1935 A cell membrane model attempt is proposed: lipid bilayer coated by a protein layer (Danielli and Dawson)

1925 Lipids extracted from red blood cell membranes are demonstrated to assemble into bilayer (Gorter and Grendel)

1917 The lipid ability to self-assemble into monolayer is described (Langmuir)

Fig 2. Timeline of membrane structure conception. (1) Danielli and Dawson's model of the protein layer (red) coating the lipid bilayer (Blue). (2) In the gel L_β phase , acyl chains are rigid; increasing the temperature promotes transition to the liquid-crystalline L_α phase where acyl chains are highly disordered. (3) Singer and Nicolson's fluid mosaic model: The membrane model is described to be **fluid** because of lipids (blue/grey) and proteins (red) diffuse laterally, meaning the membrane is not solid. The membrane is depicted as **mosaic** because it is composed of many macromolecules having different properties, such as glycolipids (black) or cholesterol (yellow). (4) The lipid whisker model represents the latest evolution in membrane structure and function understanding. When lipids acyl chains are oxidized (green), they become hydrophilic and are exposed towards the aqueous phase, serving as signal receptor for molecules such as enzymes (béchamel).

Influence of specific lipid composition on protein function

Crafting membrane models is becoming a trying task as more and more lipids are discovered, purified and characterized. In order to assess the role of specific lipid structures in modulating protein function, a simple strategy is to form models of minimal lipid composition and evaluate the relevance of the structural information obtained with physiological observations.

A simple and probably the most used lipid class is diacyl phosphatidylcholine (PC). This was considered to be the most important lipid in eukaryotic cells because PCs represent the largest part of organelle membranes and readily form liposomes, bilayers arranged in spherical structures, in aqueous phase (Bangham et al. 1965b; Huang 1969; Papahadjopoulos and Miller 1967). PC liposomes are well characterized and used as a template of many cell systems, but in many cases do not reflect a sufficient or adequate lipid environment for proteins to function properly. Indeed, the layer surrounding a membrane protein - the annular lipids – must have the right biophysical properties (rigidity, hydrophobic thickness, electrostatic potential, structure) to render a protein fully functional (Contreras et al. 2011; Epand 2007; Marsh 2008). For instance, HorA, a bacterial ATP-binding cassette multidrug transporter, was shown to lose ATP hydrolysis and substrate transport functions when the protein was reconstituted in PC vesicles instead of PE vesicles, the main lipid of bacterial membranes (Gustot et al. 2010). The structural modifications of the protein were insignificant while the important change was observed in the transmembrane segment (TM) orientation where the helices opened wider by 10° in PE *versus* PC-containing vesicles. In this case, comparing observations from two models composed of a single lipid seems straightforward, but as natural membranes are composed of a multitude of lipids, the relevance of these observations needs further investigation.

Alzheimer's disease offers a prime example of the difficulty in comparing observations from slightly more complex models. In this severe pathology affecting aging populations, the death of neurons may be linked to the aggregation of the amyloid-beta (Aβ) peptides on neuronal membrane, and many studies have tried to correlate the formation of amyloid fibrils with a specific lipid environment (Gehman and Separovic 2011; Di Paolo and Kim 2011; Sani et al. 2011a). Liposomes made of PC/PE, PC/GM1 or PC/PS were used to pinpoint the effect of a specific lipid (Sani et al. 2011a). Large differences in aggregation kinetics and fibril morphologies were observed: electrostatic interactions induced by anionic lipid (PS) delayed the fibril formation but produced long and thin fibrils while steric effects with the bulky GM1 lipids or interaction with the tight PE surface induced fast forming and coarse fibrils. Meanwhile, to determine toxicity, comparisons with observations on live neuronal cells are necessary. The aggregation of Aβ peptides on live neuroblastoma cells showed that cell-bound Aβ oligomer size distribution is shifted towards larger aggregates (Johnson et al. 2011). The lipid matrix either triggered a rapid increase in aggregate size or the oligomers clustered at specific sites on the membrane. Is it possible that the pathologic aggregation of Aβ is associated with a specific lipid environment? Cecchi *et al.* demonstrated that Aβ aggregation was less toxic against undifferentiated cognate cell models than with differentiated neuron cells containing higher amount of GM1 (Cecchi et al. 2011). Reducing the PE content by nearly 50% in differentiated human neuroblastoma cells dramatically decreased the neurotoxicity of Aβ peptides in another study (Cazzaniga et al. 2011). Although unable to conclude any specific role of a particular lipid - it could simply be that Aβ forms different fibril types at different rates in different lipid environments – lipids do play a role. However, even data from real cell systems and controlled membrane models, can lead to ambiguity, often delaying the cure of severe pathologies. The effect of different lipid compositions on membrane active molecules is discussed in the following examples.

Specific lipid affinity of antimicrobial peptides in bacterial membrane models

Eukaryotic and prokaryotic cells have little resemblance in their lipid composition - a good thing! Symbiosis is sometimes highly beneficial for both species but on other occasions a deadly war occurs at the membrane interface. Gram-negative bacteria can expose glycolipids – or lipopolysaccharides – on their external membrane surface, which act as endotoxins, stimulating immune response and inflammation in eukaryotic cells. To fight microbial infection, antibiotics were made but have become ineffective when facing evolved mutants, which are dangerously increasing in number (Andersson and Hughes 2011; Canton and Morosini 2011). To fight these new superbug generations, natural host defense peptides – or antimicrobial peptides (AMP) - are being studied intensively for their ability to disrupt membrane of specific lipid composition (Brown and Hancock 2006; Fernandez et al. 2011; Hadley and Hancock 2010; Lohner 2009; Epand and Epand 2011; Lundbaek et al. 2010; Sani et al. 2011b; Shai 2002). AMP affinity for bacterial membrane is generally triggered by electrostatic interactions: the positive cationic peptides interact preferentially with negatively charged anionic lipids, PG and CL (Lohner and Prossnigg 2009; Epand and Epand 2011). Contrastingly they have low affinity for eukaryotic membranes, which are usually neutral with high concentration of cholesterol that decreases the lipid membrane permeability. Thus, it seems straightforward to compare AMP interactions with membrane models of composition mimicking bacterial and eukaryotic cells. The impact of diverse acyl chains and headgroups lipid structures can be then individually assessed, providing valuable information in understanding how these peptides 'see' the bacterial membrane.

AMP activity can depend strongly on a specific interaction with a particular lipid structure. Cyclic synthetic arginine or tryptophan-rich AMP peptides have minimal effect against PE/PG membranes but interact strongly with PC, promoting permeabilisation of neutral membranes. However, these peptides are highly potent against *E. coli* and removal of the bacterial outer membrane drastically reduces peptide activity. LPS-doped PC membranes demonstrated the decisive role of O-antigen and outer core polysaccharides for peptide binding and partitioning (Bagheri et al. 2011; Junkes et al. 2011). The role of LPS – but not PG - as a critical partner is further shown for the NK2 peptide – another porcine AMP. Indeed, the weak interaction with PG membranes could not explain the bactericidal activities against *E. coli* bacteria (Hammer et al. 2010; Willumeit et al. 2005). Using LPS extracted from *E. coli* and *P. mirabilis*, the differences in activity could be identified in the LPS-binding step where the net charge and charge distribution difference of the two LPS was proposed to be responsible for the specific activities (Hammer et al. 2010). The porcine AMP PG1 inserted into anionic membrane composed of PG and lipid A (precursor of LPS) or into neutral PC membranes but not in PE lipids, which mostly constitute bacterial plasma membranes, confirming that not only the charge of the membrane surface but also the fine structure of the headgroup is an important parameter for AMP activity. On the Gram-positive side, the lantibiotic nisin combines targeting of lipid II (precursor of peptidoglycans) with membrane permeabilization (Wilmes et al. 2011). Nisin bound to the pyrophosphate moiety of lipid II and induced a stable membrane pore. Although interaction between the cationic peptide and the negatively charged model membranes is observed *in vitro*, nisin and various defensins have been shown to inhibit the cell wall biosynthesis by segregating the lipid II (Schneider et al. 2010; Wiedemann et al. 2001). Furthermore, lipid modification in bacteria changes the effect of membrane-active molecules. For instance, the aminoacylation of PG and teichoic acid lipids in the Gram-positive *S. aureus* is indicated as a potential cause of AMP resistance because it increases the positive charges on the bacterial membrane surface, to ultimately “hide” the electrostatic attraction with cationic peptides (Andra et al. 2011; Peschel 2002). Similar modification of LPS and lipid A were observed in Gram-negative bacteria as well, where the addition of the amino acyl group reduced the binding of cationic molecules (Koprivnjak and Peschel 2011).

Not only phospholipid headgroup charge and structure can modulate interactions between AMP and membranes, acyl chains also appear to play very important part. LL-37 - a human AMP - induced a quasi-interdigitated phase in negatively charged PG model membranes, promoted disk-like micelles in neutral C16 PC (16 carbons long acyl chains) bilayers but induced interdigitation in longer chain C18 and C20 PC bilayer membranes (Sevcsik et al. 2007). PG1 - a porcine AMP – inserted into anionic membrane composed of PG and lipid A (precursor of LPS) or PC. But an increase in acyl chain saturation or incorporation of sterols, which both enhance the rigidity of the membrane hydrophobic core, reduced the ability of PG1 to insert deep into the membrane (Gidalevitz et al. 2003). Modifications occur in the life cycle of bacteria that change the fluidity of the bacterial membrane as well, and thereby the potential insertion of AMP into the lipid hydrophobic core. Bacteria can modulate their lipid structure and composition during their growth. For instance, on entering the stationary phase after their exponential growth, bacterial membranes displayed higher amounts of branched and cyclopropane acyl chains (Grogan and Cronan 1997). In *L. pneumophila*, modifications of the acyl chains produced a resistant strain against the AMP warnericin RK (Verdon et al. 2011). Also *B. infantis* was resistant to rifaximin due to an increase in the amount of cyclopropane and saturated acyl chains, which induced a reduction in membrane permeability for the antibiotic moiety (Vitali et al. 2008). Using solid-sate NMR, Dufourc *et al.* demonstrated that incorporation of a cyclopropane ring decreased the fluidity of the acyl chains core from the bilayer surface to the site of the cyclopropane ring with a much more disordered region thereafter to the center of the bilayer (Dufourc et al. 1983). A similar decrease in the temperature of the gel to liquid-crystal phase transition and a very significant broadening of the transition profile was observed in PC and PE membrane models. The cyclopropane-containing chains had a poor packing ability due to the bulky substituent effect (Perly et al. 1985) and may change the interaction of AMP with bacterial membrane during the growth cycle.

Overall, the balance between electrostatic interactions, specific recognition and penetration depth driven by hydrophobic interactions modulates very complex molecular mechanisms that cannot be simply studied with classic model membranes. Recently, the use of complex lipids, such as LPS, lipid A or lipid II, demonstrated their crucial roles in either enabling AMP to rupture the plasma membrane. It remains critical to improve the membrane models to reach relevant information that will improve the design of more potent and more specific membrane active molecules.

High-resolution structure in live cells

The best model of natural cell membrane is the living cell. Until recently, molecular structure was limited to simple membrane models, such as PC micelles. The in-cell NMR, developed by Dotsch, has overcome this limitation. They impressively resolved the structure of over-expressed proteins *in situ* (Serber et al. 2001; Serber et al. 2006). In 2001, they clearly demonstrated that the structure of mercury-detoxification MerA bacterial protein, over-expressed in *E. coli*, differed from *in vitro* models (Serber and Dotsch 2001). This technique is constantly improved and is a valuable way of providing the most relevant structural information (Maldonado et al. 2011; Selenko and Wagner 2007). Recently, the technique was applied to study membrane proteins, a very difficult task due to the resolution and stability limitations induced by the lipid environment. Fu and co-workers successfully managed to refine the TM segment of an apolipoprotein E receptor, LR11, involved in Alzheimer disease, using an in-cell solid-state NMR approach (Fu et al. 2011). They compared the secondary structure of the TM segment of LR11 in native membrane and PC micelles and found minor discrepancies between the two environments. Although bacterial membranes are not representative of eukaryotic cell membranes, this approach has many attractive advantages: little manipulation of the protein, such as purification and refolding steps, and investigation *in situ* where membrane heterogeneity and cellular activities are more realistic. Ultimately, the use of eukaryotic living cells will provide an even better environment for native proteins.

In a similar approach, high-resolution experiments were conducted on live mitochondria with a focus on membrane structure alterations. In 2009, Sani and co-workers were able to characterize the response of *ex vivo* mitochondrial membranes under calcium stress (Sani et al. 2009). The integrity of this organelle was preserved under magic angle spinning conditions in solid-state NMR experiments, and a clear change in membrane was observed upon calcium overload. Interestingly, the resolution was sufficient to identify the main phospholipids comprising the mitochondrial membrane (PE, PC and CL), which permitted tracking of individual lipid response. Combined with physiological experiments, only the outer membrane was shown to be altered by calcium stress. *In situ*, calcium did not interact with the inner membrane where most of CL lipids are located. This was an unexpected result as calcium has been shown to induce strong alteration in mitochondrial membrane models containing CL lipids by inducing hexagonal structures (Cullis et al. 1978; Macdonald and Seelig 1987). Correlation of *in vitro* data to *in vivo* results is rarely a straightforward task; live cells will remain extremely heterogeneous with unique lipid compositions while membrane models are simply unable to reflect the diversity of interactions modulating cellular mechanisms.

Natural cell membranes are obviously the best system to use, but their individual complexity, fragility and tedious low yield extraction (especially eukaryotic organelles) motivate the continued use of membrane models. High-resolution information are more easily obtained from *in vitro* studies, it is only the relevance with the biological context that remains the main issue.

Perspective

As we advance our knowledge on the lipid diversity and functionality of each molecular species existing in biological membranes, new perspectives in the regulation of cellular mechanisms appear. Lipids are now recognized as potent signaling molecules and, therefore, offer targets to design novel therapeutic treatments. Improvements in mass spectrometry are enabling fast and accurate screening of complex membrane compositions, and composing a membrane model better resembling a natural cell is almost achievable. However, many biophysical properties are still to be described as new membrane compositions, organization and structure continue to be discovered. Although models are often restricted by the requirements of the experimental technique, their constant improvement will provide better correlation between *in vitro* and *in vivo* studies. Techniques that allow monitoring of living cell membranes at atomic or molecular resolution are still to be improved, but experimental limitations are constantly pushed further away, building up our understanding of a lipid network composed of thousands of potential regulative lights.

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1983 The lipid A structure is fully determined (Takayama *et al.*)

1980 Lipid oxidation by free radical is observed (Porter *et al.*)

1969 Ceramide PE and ceramide PG are characterized (Labach *et al.*)

1965 The lipopolysaccharide composition is described (Westphal *et al.*)

1963 GM1, GD1 and GT1 gangliosides structures are determined (Khun *et al.*)

1962 The GM3 ganglioside structure is identified (Klenk *et al.*)
Amino acid capping (alanyl, lysyl) on bacterial PG lipids is observed (MacFarlane *et al.*)

1958 The PG phospholipid structure is resolved (Klenk *et al.*)

1951 Cyclopropane acyl chains are found in bacterial lipids (Hofman et al.)

1942 PS and PE phospholipids are resolved (Folch, who is also the inventor of the classic chloroform-methanol and water phase partition for tissue lipid extraction published in 1957)

1941 Cardiolipin is isolated and characterized (Pangborn *et al.*, later fully determined by MacFarlane in 1959)

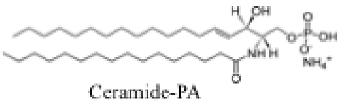
1932 The cholesterol composition is described (Windaus and Wieland), later resolved using X-ray in 1937 (Crowfoot-Hodgkin)

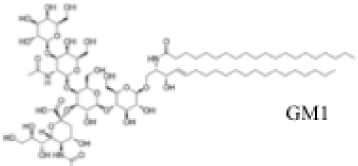
1926 The term lipid is published (Sperry), and will compete with lipoid and lipide until 1960

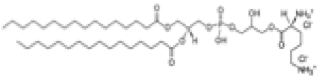
1925 The first classification of lipids is made (Bloor), updated in 2009 with a comprehensive classification, nomenclature, and chemical system (LipidMap, Fahy *et al.*)

1874 The phosphatidylcholine structure from egg yolk (lecithin) is determined (Gobley)

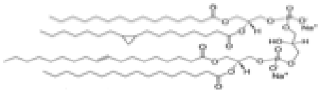

Oxidized-PC

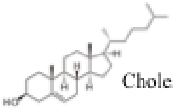

Ceramide-PA

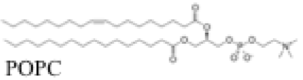

GM1

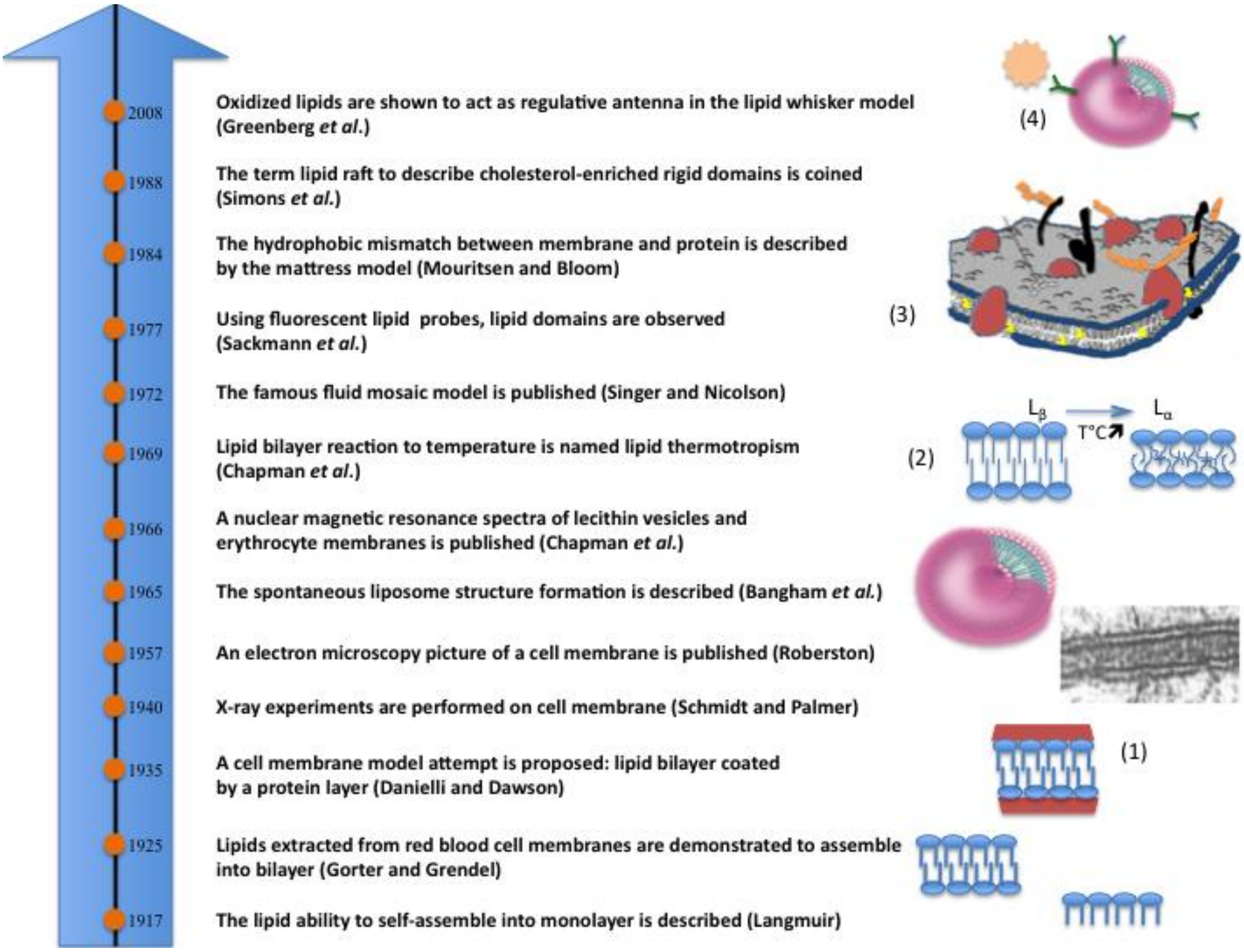

Lysyl-DPPG


Docosahexanoic-PG


Cardiolipin (from *E. coli*)


Cholesterol


POPC



2008 Oxidized lipids are shown to act as regulative antenna in the lipid whisker model (Greenberg *et al.*)

1988 The term lipid raft to describe cholesterol-enriched rigid domains is coined (Simons *et al.*)

1984 The hydrophobic mismatch between membrane and protein is described by the mattress model (Mouritsen and Bloom)

1977 Using fluorescent lipid probes, lipid domains are observed (Sackmann *et al.*)

1972 The famous fluid mosaic model is published (Singer and Nicolson)

1969 Lipid bilayer reaction to temperature is named lipid thermotropism (Chapman *et al.*)

1966 A nuclear magnetic resonance spectra of lecithin vesicles and erythrocyte membranes is published (Chapman *et al.*)

1965 The spontaneous liposome structure formation is described (Bangham *et al.*)

1957 An electron microscopy picture of a cell membrane is published (Roberston)

1940 X-ray experiments are performed on cell membrane (Schmidt and Palmer)

1935 A cell membrane model attempt is proposed: lipid bilayer coated by a protein layer (Danielli and Dawson)

1925 Lipids extracted from red blood cell membranes are demonstrated to assemble into bilayer (Gorter and Grendel)

1917 The lipid ability to self-assemble into monolayer is described (Langmuir)