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TOPICAL REVIEW

How We Came to Understand the "*Tumultuous Chemical Heterogeneity*" of the Lipid Bilayer Membrane

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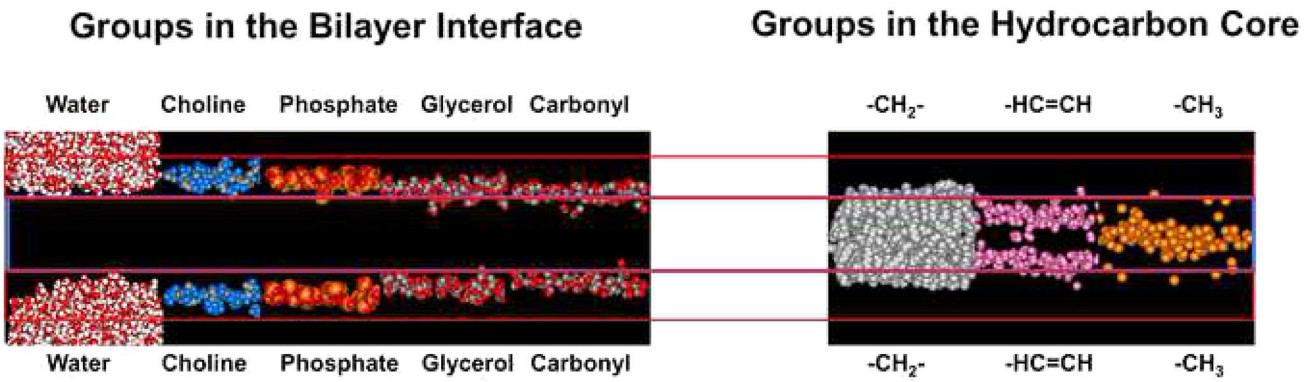
Abstract

The path to our modern understanding of the structure of the lipid bilayer membrane is a long one that can be traced from today perhaps as far back as Benjamin Franklin in the eighteenth century. Here, I provide a personal account of one of the important steps in that path, the description of the "Complete Structure" of a hydrated, fluid phase dioleoyl phosphatidylcholine bilayer by the joint refinement of neutron and X-ray diffraction data by Stephen White and his colleagues.

Graphic Abstract



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Introduction

Some authors contend that the path to our modern understanding of membrane structure began in 1774 when the American polymath Benjamin Franklin had an opportunity to become the first membrane biophysicist (Tanford 1989; Wang et al. 2013). However, Franklin and his contemporaries let the opportunity slip away—essentially unnoticed. Franklin poured oil on a small pond in England to test its ability to calm waves. He wrote "*The oil, though not more than a teaspoonful produced an instant calm over a space of several yards square, which spread amazingly…making all that quarter of the pond, perhaps half an acre, as smooth as a looking glass*". Franklin noted that the spreading oil pushed away objects that were floating on the surface of the water. He concluded "I *think it is a curious enquiry, and I wish to know whence it arises* (Brownrigg and Farish 1774)". The concept of the molecule was more than a century old in 1774, yet no one reasoned that Franklin had created a molecular monolayer on the surface of the water. Thus, no one at the time made what would have been *the first measurement of the thickness of a membrane-like film*—a teaspoonful (~ 5 ml) of oil spread over half an acre (~ 2000 m^2) has a thickness of 2.5 nm, a value that is remarkably close to half of the ~ 5 nm thickness of a lipid bilayer membrane. In fact, had this calculation been done at the time, it

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would have been the first measurement of the size of *any molecule*.

To my knowledge, no calculation like this was done until more than 100 years later when researchers including Lord Rayleigh, Agnes Pockels, Irving Langmuir, and many others began to study lipid films in earnest (Rayleigh 1890; Pockels 1891; Langmuir 1917; Tanford 1989; Maget-Dana 1999). Rayleigh performed his famous oil film experiment, a carefully controlled laboratory version of Franklin's experiment, in 1890. He specifically designed the experiment to enable measurement of the thickness of an oil film, and reported a thickness of 1.6 nm (Rayleigh 1890). Ultimately, soap film work combined with the work of Meyer and Overton on general anesthetics (Overton 1901; Meyer 1901) around the turn of the century helped lead to the realization that living cells were bounded by a lipid film. Here, I recount how, two centuries after Franklin stilled the waves on Clapham Common, and one century after Lord Rayleigh stilled the movement of camphor chips on the surface of water in his laboratory, waves of another sort were used to describe the "Complete Structure" of a lipid bilayer membrane (Wiener and White 1992a). I discuss how this new image of the *Complete Structure* of the bilayer was acquired, how it transformed our understanding of bilayer structure by delineating how matter and thermal motions are distributed across the thickness of the bilayer, and how it catalyzed new insights in areas such as molecular dynamics simulations and membrane protein folding.

In this short account, I can mention or cite only a few of the scientists who contributed across several centuries, to our understanding of the structure of the lipid bilayer. But clearly, this long evolution of ideas was the collective effort of many exceptional scientists. The long combined efforts of some led to the consensus, in the mid-twentieth century, that the cell membrane was a fluid phase, lipid bilayer membrane. The combined efforts of others, including the researchers discussed here, led to our modern understanding of lipid bilayer structure and dynamics. Some of that rich history is described in two publications (Tanford 1989; Singer and Nicolson 1972) which, in my opinion, should be read by any student or researcher who is serious about studying membranes.

How was the "*Complete Structure*" Obtained?

In the biologically relevant fluid phase, lipid bilayers are two-dimensional fluids, with a structure that cannot be determined with atomic resolution. The story of the *Complete Structure* of the lipid bilayer is about the development of "liquid crystallography" by Stephen White and postdocs Glen King and Michael Wiener which was used to extract maximum structural insight from available lamellar X-ray and neutron diffraction. These authors published a series of papers culminating in the 1992 paper "*Structure of a Fluid Phase DOPC Bilayer by Joint Refinement of X-ray and Neutron Diffraction Data. III The Complete Structure*" by Wiener and White (Wiener and White 1992a). The first paper in the series, by King and White (King and White 1986), established a framework for analyzing neutron (and X-ray) diffraction data by using strip function models (Worthington 1969) of quasimolecular groups; bonded groups of atoms expected to behave coherently. Examples of natural quasimolecular groups in a phosphatidylcholine molecule include the choline headgroup, the phosphate, the glycerol backbone, the carbonyls, and the terminal methyl groups. The second paper in the series, by Wiener and White (Wiener and White 1991a), built upon previous work (Mitsui 1978; Wiener et al. 1989) to demonstrate how Gaussian functions can be used to describe the distributions of the quasimolecular groups. In this paper, Wiener and White also established a way to think about membrane diffraction. A stack of fluid phase bilayers is a near perfect one-dimensional crystal along the bilayer normal (Franks and Lieb 1979; Smith et al. 1987; Wiener and White 1991a). The small number of diffraction orders observed, usually 4–8, is not due to stacking/lattice disorder, but to the inherent length-scale that best describes the system. The third paper in the series (Wiener and White 1991b) established a "joint refinement" method in which the significant differences in X-ray and neutron atomic scattering cross-sections can be exploited to form a detailed image of the transbilayer distribution of quasimolecular groups by global fitting of data sets acquired using both techniques. The fourth, fifth and sixth papers (Wiener and White 1991c, 1992b; Wiener et al. 1991) described the distribution of the fatty acid double bonds, terminal methyl groups, and water. These papers also addressed the critical issue of scaling of neutron and X-ray diffraction data for the joint refinement. This body of work led to the *Complete Structure* paper in which the distribution of all lipid quasimolecular groups and water were determined by a global fit of X-ray and neutron membrane diffraction scattering factors. The global fit was unconstrained except for the positions and widths of the water and double bonds which had been measured in the previous papers. The center of mass and 1/e Gaussian half-widths of each quasimolecular group were individually allowed to vary. The result, Fig. 1a, was the time-averaged distribution (i.e. position and width) of each quasimolecular group along the bilayer normal.

What Did We Learn from the "*Complete Structure*"?

The "Complete Structure" of a fluid phase DOPC bilayer was built upon the work of many researchers who had previously studied bilayer structure and dynamics using techniques such as electron microscopy and NMR in addition to

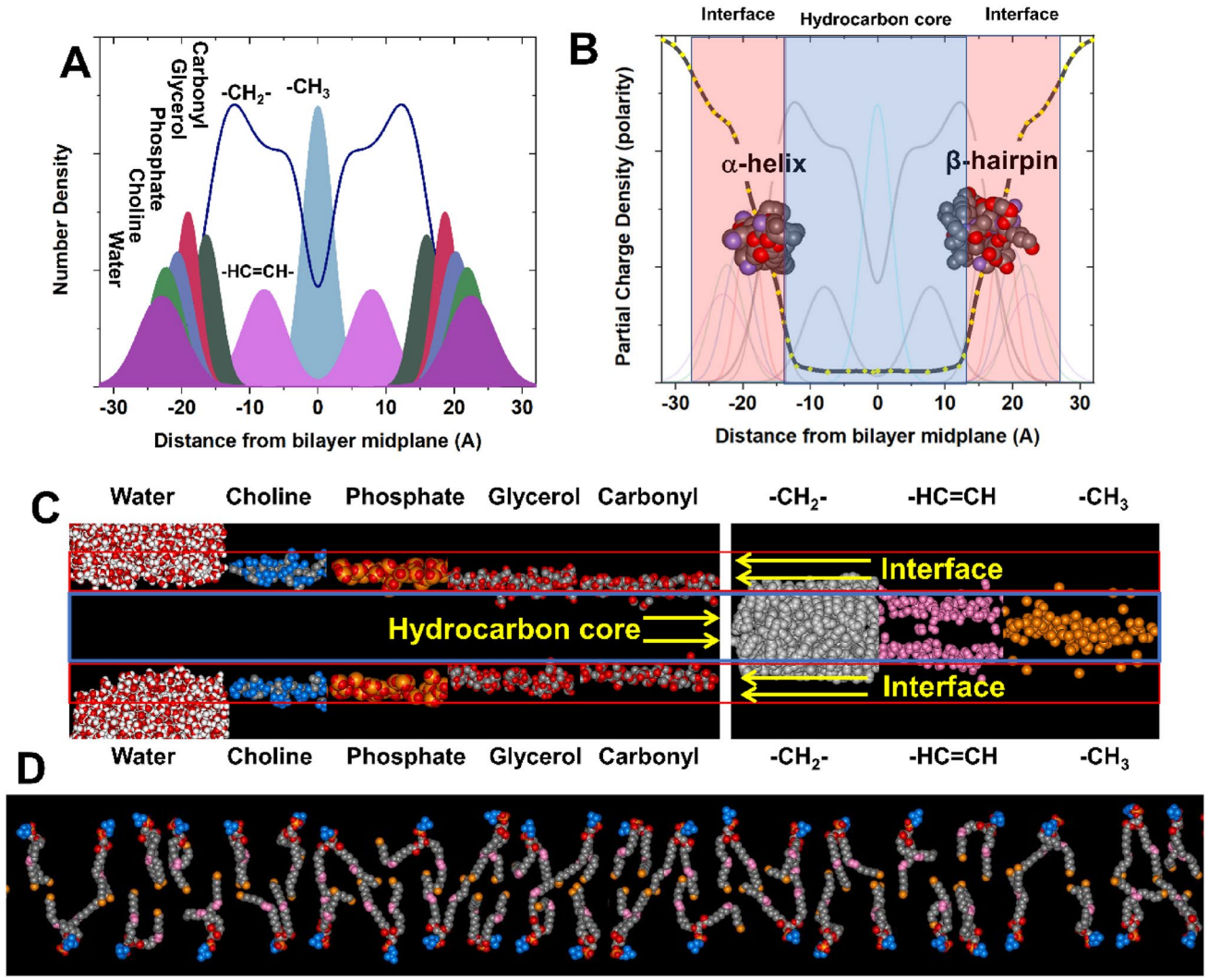

Fig. 1 The "tumultuous chemical heterogeneity" of the fluid phase lipid bilayer membrane. **a** Number density distributions of quasimolecular groups determined in the *Complete Structure* of a dioleoyl phosphatidylcholine (DOPC) bilayer determined using joint refinement of X-ray and neutron lamellar diffraction (Wiener and White 1992a). The 5.4 waters and 28 $-CH_2-$ groups have been scaled down for display. **b** Number densities enable calculation of partial charge density, a reasonable surrogate measure of polarity across the bilayer. The *Complete Structure* showed that the interfacial zone occupies fully half the thickness of the bilayer, bridging the bulk water and hydrocarbon core with a continuous gradient of polarity that is wide enough to fully accommodate folded α-helices and β-hairpin structures which may sample the edge of the hydrocarbon core. **c** DOPC bilayer deconstructed into individual quasimolecular parts. Validated molecular dynamics simulations show the distributions of these quasimolecular groups in a hydrated, fluid phase DOPC bilayer. **d** Snapshots of individual lipid conformers separated out from a realistic simulation of a DOPC bilayer (Benz et al. 2005). The thermal motion of individual groups and whole lipid molecules is revealed. Coloring of atoms is the same as in (**c**)

X-ray and neutron diffraction. For example, see (Luzzati and Husson 1962; Stoeckenius 1962; Stoeckenius 1962; Luzzati 1968; Fettiplace et al. 1971; Wilkins et al. 1971; Levine and Wilkins 1971; Hitchcock et al. 1974; Worcester and Franks 1976; Büldt et al. 1978; Zaccai et al. 1979). Many aspects of the structure of fluid phase lipid bilayer membranes had previously been glimpsed by these influential prior studies. However, the *Complete Structure* of a hydrated, fluid phase DOPC bilayer provided a holistic image of bilayer structure and dynamics. The fluid bilayer shows substantial thermal disorder in the transbilayer distributions of the lipid groups. Wiener and White showed that Gaussian distributions accurately describe the time-averaged positions of quasimolecular groups along the bilayer normal. Comparison of the hard sphere widths with the experimentally determined widths showed that there are motional gradients. The glycerol backbone moiety has the lowest thermal motion, but thermal motion increases in both directions; toward the headgroup moieties and toward the acyl chains. The greatest thermal disorder occurs in the terminal methyl groups (Mihailescu

et al. 2011). A few years after the *Complete Structure*, Postdoc Kalina Hristova and White (Hristova and White 1998) made critical measurements of how bilayer thermal motions change as a function of hydration level, enabling the description of a more biologically relevant fluid phase bilayer in excess water.

Paradoxically, although the transbilayer Gaussian distributions of quasimolecular groups are broad, their centers and widths are each determined with high precision in the *Complete Structure*. Thus, these experimentally determined transbilayer distributions of atomic groups can (and should!) be used to validate molecular dynamics simulations of lipid bilayers as White and colleagues have done (Benz et al. 2005). Experimentally validated (i.e. realistic) thermal motions (Fig. 1c, d) and subsequent lateral pressure profiles across the bilayer remain critical parameters for correctly modelling peptide/protein insertion, folding and structure in membrane simulations.

Another significant revelation in the *Complete Structure* was the true nature of the interface between the hydrocarbon core and the bulk water. Memorably described in the *Complete Structure* as a region of "tumultuous chemical heterogeneity", the interfaces of a fluid bilayer occupy fully half the total thickness of the bilayer. All lipid groups, including the terminal methyl groups (Mihailescu et al. 2011), spend some of their time in the interface due to their thermal motion. The interface also contains a significant amount of water. The time-averaged density of these groups creates a gradient of polarity, Fig. 1b, that forms a broad zone of transition between the very polar bulk water phase and the very non-polar hydrocarbon core, in the center of the bilayer. Importantly, the *Complete Structure* showed that the interfacial zones each occupy ~ 15 Å along the bilayer normal, more than wide enough to encompass whole elements of protein secondary structure (Fig. 1b). Hristova, with White and others, demonstrated experimentally that amphipathic α-helical peptides are readily accommodated within the bilayer interfacial zone (Hristova et al. 1999, 2001). The existence of these broad interfacial zones, with physical properties that are very different from the hydrocarbon core, means that a minimum of two hydrophobicity scales are needed to describe the thermodynamics of peptides and proteins partitioning into a lipid bilayer (White and Wimley 1999); at least one for the interface(Wimley and White 1996) and one for the hydrocarbon core (Wimley et al. 1996). Further, the broad interfacial zones mean that the thermodynamic cost of partitioning charged and polar molecules in the bilayer is much lower than previously thought (Hessa et al. 2005; Schow et al. 2010; Hristova and Wimley 2010).

Nonetheless, for peptides and proteins that partition into the bilayer interface, the reduced polarity, compared to bulk water, greatly increases the thermodynamic favorability of hydrogen bonded-secondary structure, giving rise to a very strong coupling between membrane binding and folding, a concept that has been useful in the understanding of membrane active peptides as well as membrane proteins (White and Wimley 1999).

Although the hydrocarbon core occupies only half the total thickness of the bilayer, the *Complete Structure* verified what many other researchers had previously concluded; the ~ 25–30 Å thick hydrocarbon core has a low abundance of water and lipid polar groups, making it one of the most hydrophobic micro-environments known in biology. Gunnar von Heijne, with White and others, later showed that the translocon, the protein machinery that folds and inserts membrane proteins in the endoplasmic reticulum, acts in accordance with the transmembrane distribution of polarity first revealed in the *Complete Structure* of DOPC (Ojemalm et al. 2011). The effective hydrophobicity sensed by the amino acids in a potential membrane spanning helix is highest in the center of the membrane, becoming lower as the amino acid in question is moved away from the hydrocarbon core towards either interfacial zone. While the mechanistic details of translocon-mediated insertion and folding are still being investigated, the *Complete Structure* of DOPC set the stage for understanding the role of the bilayer physical properties in the process.

Conclusion

Although Benjamin Franklin and his contemporaries missed their chance in 1774 to be the first membrane biophysicists, many other great scientists helped evolve our view of the structure of the lipid bilayer membrane. For a long time, the prevailing cartoon image of a lipid bilayer had been that of a hard-edged slab of hydrocarbon, created by lipids with a thin interface and little or no thermal motion along the bilayer normal. Such simplistic cartoons of bilayers not only imply the wrong structure and dynamics, but also ignore the immense *compositional* and spatial complexity of cellular membranes (Lorent et al. 2020). Here, I have highlighted how Stephen White and colleagues contributed to our modern view of the bilayer by giving us the *Complete Structure* of a fluid phase lipid bilayer membrane. They helped us to see the whole structural and dynamic complexity previously reported, in part, by many others. Unfortunately, old ideas, and simplistic cartoons, die only very slowly. Incorrect and unrealistic depictions of static, rigid, uniform membrane structures, with thin interfaces, have not yet been eradicated from textbooks, scientific papers, and the internet. Such images may adversely affect how scientists and students think about membrane biology, so we should do our best to present realistic images of bilayer structure and dynamics.

References

Benz RW, Castro-Roman F, Tobias DJ, White SH (2005) Experimental validation of molecular dynamics simulations of lipid bilayers: a new approach. Biophys J 88:805–817

Brownrigg W, Farish M (1774) Of the stilling of waves by means of oil. Extracted from Sundry letters between Benjamin Franklin, LL. D. F. R. S., William Brownrigg, M. D. F. R. S. and the Reverend Mr. Farish. Philos Trans 64:445–460

Büldt G, Gally HU, Seelig A, Seelig J, Zaccai G (1978) Neutron diffraction studies on selectively deuterated phospholipid bilayers. Nature (London) 271:182–184

Fettiplace R, Andrews DM, Haydon DA (1971) The thickness, composition and structure of some lipid bilayers and natural membranes. J Membr Biol 5:277–296

Franks NP, Lieb WR (1979) The structure of lipid bilayers and the effects of general anesthetics: an X-ray and neutron diffraction study. J Mol Biol 133:469–500

Hessa T, White SH, Von HG (2005) Membrane insertion of a potassium-channel voltage sensor. Science 307:1427

Hitchcock PB, Mason R, Thomas KM, Shipley GG (1974) Structural chemistry of 1,2 dilauroyl-DL-Phosphatidylethanolamine: Molecular conformation and intermolecular packing of phospholipids. Proc Natl Acad Sci USA 71:3036–3040

Hristova K, White SH (1998) Determination of the hydrocarbon core structure of fluid dioleoylphosphocholine (DOPC) bilayers by x-ray diffraction using specific bromination of the double-bonds: Effect of hydration. Biophys J 74:2419–2433

Hristova K, Wimley WC (2010) A look at arginine in membranes. J Membr Biol 239:49–56

Hristova K, Wimley WC, Mishra VK, Anantharamiah GM, Segrest JP, White SH (1999) An amphipathic α-helix at a membrane interface: a structural study using a novel X-ray diffraction method. J Mol Biol 290:99–117

Hristova K, Dempsey CE, White SH (2001) Structure, location, and lipid perturbations of melittin at the membrane interface. Biophys J 80:801–811

King GI, White SH (1986) Determining bilayer hydrocarbon thickness from neutron diffraction measurements using strip-function models. Biophys J 49:1047–1054

Langmuir I (1917) The constitution and fundamental properties of liquids and solids. II. Liquids. J Am Chem Soc 39:1848–1906

Levine YK, Wilkins MHF (1971) Structure of oriented lipid bilayers. Nat New Biol 230:69–72

Lorent JH, Levental KR, Ganesan L, Rivera-Longsworth G, Sezgin E, Doktorova MD, Lyman E, Levental I (2020) Plasma membranes are asymmetric in lipid unsaturation, packing and protein shape. Nat Chem Biol 16:710

Luzzati V (1968) Biological membranes. In: Chapman D (ed) Membrane. Academic Press, New York

Luzzati V, Husson F (1962) The structure of the liquid-crystalline phases of lipid-water systems. J Cell Biol 12:207–219

Maget-Dana R (1999) The monolayer technique: a potent tool for studying the interfacial properties of antimicrobial and membranelytic peptides and their interactions with lipid membranes. Biochim Biophys Acta 1462:109–140

Meyer H (1901) Zur theorie der alkolnarkose: Der einfluss wechselnder temperatur sur wirkungsstarke und theilungscoefficient dar narcotica. Naunyn Schiedebergs Arch Pharmacol 46:388–396

Mihailescu M, Vaswani RG, Jardon-Valadez E, Castro-Roman F, Freites JA, Worcester DL, Chamberlin AR, Tobias DJ, White SH (2011) Acyl-chain methyl distributions of liquid-ordered and -disordered membranes. Biophys J 100:1455–1462

Mitsui T (1978) X-ray diffraction studies of membranes. Adv Biophys 10:97–135

Ojemalm K, Higuchi T, Jiang Y, Langel U, Nilsson I, White SH, Suga H, von Heijne G (2011) A polar surface area determines the efficiency of translocon-mediated membrane-protein integration into the endoplasmic reticulum. Proc Natl Acad Sci USA 108:E359–364

Overton E (1901) Studien uber die Narkose. Gustav Fischer, Jena

Pockels A (1891) Surface tension. Nature 43:437–441

Rayleigh L (1890) Measurements of the amount of oil necessary in order to check the motions of camphor upon water. Proc R Soc Lond 47:364–367

Schow EV, Freites JA, Cheng P, Bernsel A, Von HG, White SH, Tobias DJ (2010) Arginine in membranes: the connection between molecular dynamics simulations and translocon-mediated insertion experiments. J Membr Biol 239:35–48

Singer SJ, Nicolson GL (1972) The fluid mosaic model of the structure of cell membranes. Science 175:720–731

Smith GS, Safinya CR, Roux D, Clark NA (1987) X-ray study of freely suspended films of a multilamellar lipid system. Mol Cryst Liq Cryst 144:235–255

Stoeckenius W (1962a) Some electron microscopical observations on liquid-crystalline phases in lipid-water systems. J Cell Biol 12:221–229

Stoeckenius W (1962b) In: Harris RJC (ed) The interpretation of ultrastructure. Academic Press, New York

Tanford C (1989) Ben Franklin stilled the waves. An informal history of pouring oil on water with reflections on the ups and downs of scientific life in general. Duke Univesity Press, Durham

Wang DN, Stieglitz H, Marden J, Tamm LK (2013) Benjamin Franklin, Philadelphia's favorite son, was a membrane biophysicist. Biophys J 104:287–291

White SH, Wimley WC (1999) Membrane protein folding and stability: physical principles. Annu Rev Biophys Biomol Struct 28:319–365

Wiener MC, White SH (1991a) Fluid bilayer structure determination by the combined use of X-ray and neutron diffraction. I. Fluid bilayer models and the limits of resolution. Biophys J 59:162–173

Wiener MC, White SH (1991b) Fluid bilayer structure determination by the combined use of X-ray and neutron diffraction. II. "Composition-space" refinement method. Biophys. J 59:174–185

Wiener MC, White SH (1991c) Transbilayer distribution of bromine in fluid bilayers containing a specifically brominated analog of dioleoylphosphatidylcholine. Biochemistry 30:6997–7008

Wiener MC, White SH (1992a) Structure of a fluid dioleoylphosphatidylcholine bilayer determined by joint refinement of X-ray and neutron diffraction data. III. Complete structure. Biophys J 61:434–447

Wiener MC, White SH (1992b) Structure of a fluid dioleoylphosphatidylcholine bilayer determined by joint refinement of X-ray and neutron diffraction data. II. Distribution and packing of terminal methyl groups. Biophys. J 61:428–433

Wiener MC, Suter RM, Nagle JF (1989) Structure of the fully hydrated gel phase of dipalmitoylphosphatidylcholine. Biophys J 55:315–325

Wiener MC, King GI, White SH (1991) Structure of a fluid dioleoylphosphatidylcholine bilayer determined by joint refinement of x-ray and neutron diffraction data. I. Scaling of neutron data and the distribution of double-bonds and water. Biophys J 60:568–576

Wilkins MHF, Blaurock AE, Engelman DM (1971) Bilayer structure in membranes. Nat New Biol 230:72–76

Wimley WC, White SH (1996) Experimentally determined hydrophobicity scale for proteins at membrane interfaces. Nature Struct Biol 3:842–848

Wimley WC, Creamer TP, White SH (1996) Solvation energies of amino acid sidechains and backbone in a family of host-guest pentapeptides. Biochemistry 35:5109–5124

Worcester DL, Franks NP (1976) Structural analysis of hydrated egg lecithin and cholesterol bilayers. II. Neutron diffraction. J Mol Biol 100:359–378

Worthington CR (1969) The interpretation of low-angle X-ray data from planar and concentric multilayered structures. Biophys J 9:222–234

Zaccai G, Büldt G, Seelig A, Seelig J (1979) Neutron diffraction studies on phosphatidylcholine model membranes. II. Chain conformation and segmental disorder. J Mol Biol 134:693–706