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ABSTRACT

Cell membranes are dynamic and complex structures, and their composition and structure are major determinants of pathology. It is now commonly accepted that the membranes' physical properties, such as fluidity and thickness, are determining factors for permeability, partitioning of drug molecules, and protein aggregation. Membrane-interacting molecules can in some instances be expected to have a greater therapeutic potential than traditional therapies targeting receptors or enzymes. Alzheimer's disease is an example where traditional approaches thus far have been proven unsuccessful. With bacteria becoming resistant to more and more antibiotics, potential membrane based antibiotics provide an alternative route with great potential. Here, we provide a perspective on the basic mechanisms how physical membrane properties can affect diseases and the therapeutic potential of changing membrane lipid composition and properties to target those diseases. Neurodegenerative diseases, such as Alzheimer's disease, and infectious diseases, are prime examples among many others where the so-called Membrane-Lipid Therapy shows great potential for the development of new drugs and new therapies.

Biological membranes are the most important biological interface. Composed mainly of lipid molecules and proteins, they serve as a barrier to the external environment for the contents of the cell. 20%–30% of genes encode membrane-embedded proteins, and these proteins play important roles in cell signaling and cell adhesion. In early research of membranes and membrane proteins, lipid molecules were not considered as active participants in membrane processes. In 1972, shortly after it was determined that proteins may embed within the lipid membrane, Singer and Nicholson published their "Fluid Mosaic Model" of lipid membranes.[1] In this model, the membrane serves as a passive, unstructured, and two-dimensional liquid within which embedded proteins float, and lipid molecules act as solvents. The model quickly became popular as it allowed for the lateral diffusion of proteins, as well as the transverse diffusion of small molecules, such as oxygen or carbon dioxide. However, it is now widely accepted that such a model is too simplistic to explain complex biological processes.

In 1997, Simons and Ikonen[2] suggested the existence of transient structures in membranes, so-called "rafts," local fluctuations in density and composition. While evidence for these rafts is found in model membranes,[3–5] their existence in live cells remains controversial, in particular because of a lack of experimental proof, and the significance of membrane heterogeneity has been challenged. For instance, evidence was presented recently that proteins can stabilize ordered membrane domains, leading to their collective activation.[2] This mechanism is different from the original raft idea where proteins actively partition into preexisting ordered domains. More recently, there is increasing evidence that the lipid portion of the membrane takes an active role in numerous membrane processes.

The biophysical properties of membranes influence membrane functions, as well as the activity of essential proteins that regulate our cells. It is now widely accepted that the lipid-bilayer structure and dynamics are an essential contributor to membrane functionality.[6–8] Subtle modifications to the structure of membranes are of vital importance to maintain homeostasis.

For instance, many neurodegenerative diseases are associated with lipid alterations.[9–11] However, the majority of drugs targeting them is designed to interact with membrane receptors or enzymes. This limitation likely reduces the efficacy of our current therapies on the progression of these diseases. Including the cell membrane as a target has the potential for new treatments for numerous pathologies, as sketched in Fig. 1. Indeed, while the pharmaceutical world used to be

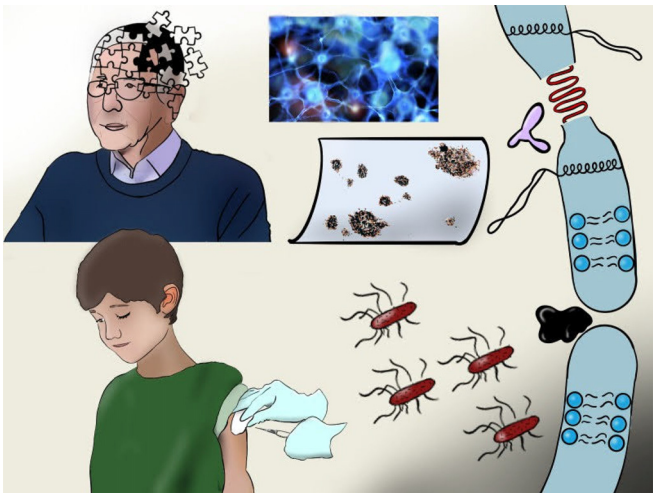

FIG. 1. Alzheimer's disease is the most common cause of dementia worldwide. It is marked clinically by gradual cognitive decline, and pathologically by the presence of senile plaques, which are formed through the aggregation of amyloid-β peptides in functional tissue of the brain. Membranes play a crucial role in the aggregation of these peptides by serving as a nucleation point at early stages of plaque formation. Antibiotic resistance is caused by bacteria that have become resistant to our existing antibiotics. By changing their membrane properties they prevent the antibiotic molecules from damaging the membrane, rendering the drug useless.

exclusively governed by drugs targeting proteins and nucleic acids, there has been a shift in recent years to molecules specifically targeting membrane lipids.[8] The field of this so-called Membrane-Lipid Therapy uses membrane-active pharmacology to develop treatments based on the regulation of the membrane lipid composition and the membranes' structure. While low concentrations of high-affinity drugs are sufficient to disrupt receptors, larger drug concentrations are typically required to change membrane properties sufficiently to affect membranes and membrane protein function.[12–14] This new approach has been shown promise as an alternative for conventional drugs both in terms of efficacy, specificity, and safety.[15] The aim of this article is to give a membrane perspective on disease intervention, where the involvement of membranes in disease is in some cases more evident than in others.

Degenerative diseases of the human brain have long been viewed among the most puzzling and difficult problems in biomedical sciences. Alzheimer's disease is the most common cause of dementia worldwide. In 2001, an estimated 24×10^6 people around the world were living with dementia, a number that is expected to double every 20 years, reaching 81.1×10^6 by 2040.[16] This disease is marked clinically by progressive cognitive decline and pathologically by the presence of senile plaques, which are formed through the aggregation of amyloid-β peptides in functional tissue of the brain.[17] As researchers have begun to uncover the mechanistic underpinnings of neurodegenerative diseases, it has become increasingly apparent that these diseases have both biochemical and biophysical roots.[18–22]

Amyloid peptides strongly interact with anionic, negatively charged membranes, which are commonly found in the human brain. The peptides spontaneously insert into those membranes and the nonpolar side chains partition in the hydrophobic membrane core,[23,24] leaving the peptide backbone lying in the interface and promoting the peptide to adopt a β-prone conformation while inducing a partial dehydration of the backbone. As a result, the formation of intra- and intermolecular peptide–peptide hydrogen bonds is favored in the two-dimensional axis of the membrane, i.e., aside from each other, rather than with three dimensions of motional freedom. The peptides transition from an helical α-state into a stretched, pathological β form. Those small, nanometer sized β-sheets form the nuclei for the growth of extracellular neurotoxic amyloid fibers and plaques.[25] The membrane provides a unique environment to facilitate this conformation of peptides into β-sheets, which are much more susceptible to the protein's environment than α-helices, which rely more heavily on the amino acid sequence.

At the same time, peptide insertion causes the membrane to distort to ensure that the entire hydrophobic region of the peptide is contained within the hydrophobic core. This hydrophobic mismatch occurs when the hydrophobic region of the peptides is larger or smaller than the bilayers' hydrophobic thickness. Local distortions surrounding the peptides cause a change in the membrane interface and can lead to long-ranged attractive forces between the membrane embedded peptides, affecting the energy barriers associated with peptide aggregation and favoring such.[8,26–30]

To visualize this concept, imagine a bucket half-filled with water and place a number of floating disks on the surface. The disks create small distortions of the water surface around the edge and induce negative curvature. The surface tension is then creating long-ranged interactions between the noninteracting disks, and the disks will begin to come together to minimize the total surface tension. Importantly, this interaction is independent of the properties of the disks but purely a property of the medium. In a similar manner, inhibiting negative membrane curvature becomes a potential target in anti-Alzheimer's treatments to prevent peptide aggregation. An increase in membrane fluidity for instance can reduce the hydrophobic mismatch and curvature.

As a consequence, the interface between membrane and peptide cluster becomes less favorable in thick and stiff membranes, which favors the formation of larger aggregates, while the corresponding energy mismatch is reduced in soft and thin membranes. Some common compounds and drugs have been speculated to have an effect in Alzheimer's disease, such as melatonin (the hormone that regulates sleep), acetylsalicyclic acid (Aspirin, a common pain killer), and curcumin (a traditional Indian spice). Experiments and simulations showed that melatonin does not change structural parameters of membranes and also has no effect on the size or extent of peptide clusters. Acetylsalicyclic acid led to membrane thickening and stiffening and to the formation of larger peptide aggregates by increasing hydrophobic mismatch and curvature. Curcumin on the other hand was found to make membranes softer and thinner, reducing the local curvature. As a result, curcumin can reduce the volume fraction of β-sheets and peptide aggregates by 70%.[31] The formation of large amyloid aggregates can potentially be inhibited at early stages of the disease by dissolving small nanometer sized nuclei by changing membrane properties.

While there is, to this day, no cure or effective prevention for Alzheimer's disease, there is evidence that a healthy diet can slow down first occurrence and progression. A diverse range of bioactive nutrients and compounds found in natural products has been shown to play a potential role in the prevention of neurodegenerative diseases.

Resveratrol, found in grapes, caffeine, the main active component in coffee, β-carotene, found in orange fruits and vegetables, and Epigallocatechin gallate (EGCG), a component of green tea, are the most promising candidates. Thickening and stiffening of the membranes result in the formation of large peptide aggregates, as observed for resveratrol and caffeine. β-carotene shows some potential as it forms transmembrane aggregates, which result in breaking up the peptide aggregates into smaller β-sheets. The best result was obtained for EGCG, where thinning and fluidification of the membranes was found to completely dissolve the β-sheet peptide clusters and transition the peptides into their nonpathological α-form.[32]

At this point, the full mechanism for membrane-mediated amyloid aggregation remains elusive. However, from our current understanding, membranes provide a crucial framework for the processes involved in aggregation. At the early stages of peptide aggregation, the bilayer offers a site of high stability for the monomeric peptides, which allows neighboring peptides to coordinate hydrogen-bonding and folding and uncoiling of the peptide to form more stable β- and cross-β sheets. These processes make the membrane a key component in the nucleation of peptide aggregates and a prime target for the development of potential anti-Alzheimer's drugs.

With the worldwide rise of antibiotic resistance, the design and modification of current antibiotics are of critical importance.[33,34] The World Health Organization (WHO) considers the issue of antibacterial resistance as a top global threat to public health, threatening our ability to treat common infectious diseases, resulting in prolonged illness, disability, and death. It is estimated that 700 000 people worldwide die every year due to drug-resistant strains of bacterial infections, HIV/AIDS, tuberculosis, and malaria. Experts have warned that by 2050, the annual death toll will soar to 10×10^6 worldwide.[35]

The majority of antibiotics target intracellular biochemical pathways in bacteria and translocate across the bacterial membranes through active transport. It has been suggested that certain antibiotics can diffuse through the membranes passively. These drug molecules need to spontaneously partition in the bilayers and enter the hydrophobic membrane core to eventually enable transmembrane diffusion. Experiments and simulations have indeed found that some of these antibiotics can induce membrane disorder and alter membrane fluidity, and that their efficacy is correlated with their membrane affinities.[36] An important question with clinical relevance, therefore, is whether partitioning of the antibiotics leads to changes in membrane properties and to membrane damage or rupture, potentially increasing side-effects and toxicity.[37]

On the other hand, the membrane is a key target of many antimicrobial peptides, both biochemically via the inhibition of essential membrane regulating proteins and biophysically through the perturbation of lipid bilayers by inducing water permeation and eventually membrane rupture.[38] Polymyxins consist of cyclic lipopeptides that show a strong antimicrobial activity toward gram-negative bacteria. Although this class of antibiotics was first discovered in the mid-1900s, intravenous clinical use was discouraged due to the incidence of adverse side effects, such as renal failure. For this reason, they were deemed "last-line" antibiotics.[39] However, the emergence of "superbugs" and widespread antibiotic resistance has rendered many antibiotics ineffective in combatting infections, which has led to the increasing use of these last-line polymyxins.[34]

The nonspecific nature of polymyxin interactions with gram-negative bacterial membranes can, in particular, lead to the damage of renal epithelial cells, giving rise to nephrotoxicity. This ability of the drug to cause kidney damage hinders the clinical efficacy of intravenously administered polymyxins. It has been reported that nephrotoxicity occurs in 60% of patients.[40,41]

Renal membranes exist in a heterogeneity of cholesterol-depleted and cholesterol-rich regions.[42] Cholesterol is a common ingredient in eukaryotic cell membranes while bacterial cell membranes do not contain cholesterol.

It was reported that the presence of cholesterol in the renal membranes leads to a significant reduction of membrane damage.[43] Surprisingly, cholesterol did not protect the membranes from drug insertion; however, it was found to even increase the membrane inserted peptide fraction. However, by stabilizing the bilayers' structure and suppressing lipid and peptide mobility, cholesterol reduces membrane indentation and thinning, peptide clustering, and water permeation, which minimizes membrane damage. Renal cells with less cholesterol appeared to be less well protected leading to polymyxin's nephrotoxicity. Cholesterol's molecular mechanism for protecting eukaryotic cells from the toxic effects of bacteria targeting ploymyxins lies therefore in the stabilization of the bilayer geometry. This mechanism is conceptually different from the effect described above: Membrane thickening and stiffening here lead to a stabilization of the membrane while in the case of Alzheimer's disease, membrane thickening increased hydrophobic mismatch and induced amyloid aggregation and potential damage. Body cells with a low cholesterol content are prone to being attacked by antibiotics, resulting in a high toxicity. Such findings are important for a more effective drug design as the global incidence of antibiotic resistance increases, in addition to understanding toxicology.

While polymyxin antibiotics have provided a critical option for clinicians in treating complex multidrug-resistant infections, the first polymyxin resistance has recently been reported from the emergence of *mcr*-1 in bacteria, a transferrable gene encoding for a phosphatidylethanolamine transferase enzyme, which mediates the addition of an ethanolamine to lipid A on the bacterial membrane. This alteration reduces the membrane charge gradient and increases lipid packing. Together, these changes have been shown to prevent the bactericidal activity by lipopeptide antibiotics.[44–47]

The two balancing forces that determine whether polymyxins can insert into membranes and create damage are (1) electrostatic attraction and (2) the "elastic" resistance of the membrane against penetration. The equilibrium between these two forces determines the location of the polymyxin B molecules in the membrane. The membrane surface density depends on the anionic lipid fraction. If the charge difference is larger than the repulsive forces, the polymyxin antibiotic will eventually be able to penetrate and create membrane damage. The loss of a negatively charged group in the bacterial membrane now reduces the affinity of the cationic polymyxin. Moreover, the addition of ethanolamine to lipids across the bacterial surface increases the volume of the membrane core and intermolecular attraction between adjacent lipids, ultimately increasing membrane stability and resistance to mechanical compression and membrane collapse.

In this model, the insertion depth of the polymyxin molecules is a linear function of the anionic fraction, and the corresponding data from experiments and simulations indeed show a linear behavior. The phenomenological spring constant k of resistance for anionic membranes was determined to be 2500 N/m, and a significantly higher resistance of 18 000 N/m was determined for resistant membranes

with the increased core density. The spring constant for graphite for comparison is 27 000 N/m for the in-plane interaction, and 3.5 N/m for the much weaker out-of-plane interactions between graphite layers.[48] The force constants measured for the polymyxin insertion are, thus, about one order of magnitude weaker than a covalent bond, however, about 1000 times stronger than a hydrogen bond. This biophysical model applies well to several gram-negative bacterial strains and can be used to predict resistance strength.[39]

Altogether, *mcr*-1 expression was found to affect the global physical properties of bacterial membranes, making resistant bacteria less attracted to the polymyxin and less susceptible to polymyxin insertion. Identifying the two key factors for bacterial resistance of this type of antibiotic presents an opportunity to improve existing antibiotics and inform the development of new antibiotics.

In summary, biological membranes that were initially thought of as simple physical barriers are now viewed as important, active components of the cell. Similarly, lipid composition, organization, and interaction between the different components are essential for membrane function. Membrane dysregulation is a main contributor to the etiology of many diseases, and little importance has been given to the biophysical properties of cell membranes when it comes to biomedical applications and disease therapies. In the past, this field has been entirely dominated by proteins and biochemical interactions. However, as our understanding of the complex processes involved increases, it becomes clearer that biophysical interactions cannot be neglected.

Combining biochemical and biophysical approaches has great potential for the treatment of diseases and the development of future drugs. While research in the biomedical field typically relies on low-resolution, high-throughput techniques using standard lab supplies and statistical analyses, high-resolution biophysical techniques in comparison are slow and low-throughput, requiring expensive and sophisticated research infrastructure, as well as mathematical modeling. As such, they only slowly find their way into biochemical and medical laboratories, often through fruitful collaborations between researchers from medicine and biomedical physics. Progress in the field is further hindered by the existing funding infrastructure, which strictly distinguishes funding for the natural and health sciences. One solution lies in the training of a new generation of researchers with profound knowledge in both areas. It is certainly a mistake to reduce physics and chemistry classes for health science students and not have physics and chemistry students take courses in the life and health sciences.

In order to explore the full potential of therapies that regulate membrane properties, extensive contributions are still required. These include better model systems representing the complexity of lipid composition in different cells, as well as animal models of disease with known lipid alterations. Additionally, further knowledge of the membrane structure and its repercussions on pathophysiology is necessary to move the field ahead. Finally, clarification of the role of lipids in intracellular activities will contribute to a better and deeper understanding of therapeutic targets, leading to better and more efficient treatments for various lipid related diseases.

DATA AVAILABILITY

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

REFERENCES

[1]S. Singer and G. L. Nicolson, "The fluid mosaic model of the structure of cell membranes," Day Good Membr. Viruses Immunopathol. **175**, 7–47 (1972).
[2]K. Simons and E. Ikonen, "Functional rafts in cell membranes," Nature **387**, 569–572 (1997).
[3]L. Toppozini, S. Meinhardt, C. L. Armstrong, Z. Yamani, N. Kučerka, F. Schmid, and M. C. Rheinstädter, "Structure of cholesterol in lipid rafts," Phys. Rev. Lett. **113**, 228101 (2014).
[4]M. C. Rheinstädter and O. G. Mouritsen, "Small-scale structure in fluid cholesterol–lipid bilayers," Curr. Opin. Colloid Interface Sci. **18**, 440–447 (2013).
[5]J. D. Nickels, M. D. Smith, R. J. Alsop, S. Himbert, A. Yahya, D. Cordner, P. Zolnierczuk, C. B. Stanley, J. Katsaras, X. Cheng *et al.*, "Lipid rafts: Buffers of cell membrane physical properties," J. Phys. Chem. B **123**, 2050–2056 (2019).
[6]W. K. Subczynski and A. Wisniewska, "Physical properties of lipid bilayer membranes: Relevance to membrane biological functions," Acta Biochim. Pol. **47**, 613–626 (2000).
[7]P. V. Escribá, J. M. González-Ros, F. M. Goñi, P. K. Kinnunen, L. Vigh, L. Sánchez-Magraner, A. M. Fernández, X. Busquets, I. Horváth, and G. Barceló-Coblijn, "Membranes: A meeting point for lipids, proteins and therapies," J. Cell. Mol. Med. **12**, 829–875 (2008).
[8]R. Phillips, T. Ursell, P. Wiggins, and P. Sens, "Emerging roles for lipids in shaping membrane-protein function," Nature **459**, 379–385 (2009).
[9]V. Martín, N. Fabelo, G. Santpere, B. Puig, R. Marín, I. Ferrer, and M. Díaz, "Lipid alterations in lipid rafts from Alzheimer's disease human brain cortex," J. Alzheimer's Dis. **19**, 489–502 (2010).
[10]S. Sameni, L. Malacrida, Z. Tan, and M. A. Digman, "Alteration in fluidity of cell plasma membrane in Huntington disease revealed by spectral phasor analysis," Sci. Rep. **8**, 734 (2018).
[11]S. S. Antollini and C. Fabiani, "Alzheimer's disease as a membrane disorder: Spatial cross-talk among beta-amyloid peptides, nicotinic acetylcholine receptors and lipid rafts," Front. Cell. Neurosci. **13**, 309 (2019).
[12]K. F. Herold, O. S. Andersen, and H. C. Hemmings, "Divergent effects of anesthetics on lipid bilayer properties and sodium channel function," Eur. Biophys. J. **46**, 617–626 (2017).
[13]M. Zhang, T. Peyear, I. Patmanidis, D. V. Greathouse, S. J. Marrink, O. S. Andersen, and H. I. Ingólfsson, "Fluorinated alcohols' effects on lipid bilayer properties," Biophys. J. **115**, 679–689 (2018).
[14]R. Kapoor, T. A. Peyear, R. E. Koeppe, and O. S. Andersen, "Antidepressants are modifiers of lipid bilayer properties," J. Gen. Physiol. **151**, 342–356 (2019).
[15]P. V. Escribá, "Membrane-lipid therapy: A historical perspective of membrane-targeted therapies–from lipid bilayer structure to the pathophysiological regulation of cells," Biochim Biophys Acta Biomembr. **1859**(9 Pt. B), 1493–1506 (2017).
[16]C. P. Ferri, M. Prince, C. Brayne, H. Brodaty, L. Fratiglioni, M. Ganguli, K. Hall, K. Hasegawa, H. Hendrie, Y. Huang *et al.*, "Global prevalence of dementia: A Delphi consensus study," The Lancet **366**, 2112–2117 (2005).
[17]C. L. Masters, G. Multhaup, G. Simms, J. Pottgiesser, R. Martins, and K. Beyreuther, "Neuronal origin of a cerebral amyloid: Neurofibrillary tangles of Alzheimer's disease contain the same protein as the amyloid of plaque cores and blood vessels," EMBO J. **4**, 2757–2763 (1985).
[18]J. Hardy and D. J. Selkoe, "The amyloid hypothesis of Alzheimer's disease: Progress and problems on the road to therapeutics," Science **297**, 353–356 (2002).
[19]D. Eisenberg and M. Jucker, "The amyloid state of proteins in human diseases," Cell **148**, 1188–1203 (2012).
[20]M. Goedert, "Alzheimer's and Parkinson's diseases: The prion concept in relation to assembled Aβ, tau, and α-synuclein," Science **349**, 1255555 (2015).
[21]J. Nasica-Labouze, P. H. Nguyen, F. Sterpone, O. Berthoumieu, N.-V. Buchete, S. Coté, A. D. Simone, A. J. Doig, P. Faller, A. Garcia, A. Laio, M. S. Li, S. Melchionna, N. Mousseau, Y. Mu, A. Paravastu, S. Pasquali, D. J. Rosenman, B. Strodel, B. Tarus, J. H. Viles, T. Zhang, C. Wang, and P. Derreumaux, "Amyloid β protein and Alzheimer's disease: When computer simulations complement experimental studies," Chem. Rev. **115**, 3518–3563 (2015).
[22]R. Ahmed, M. Akcan, A. Khondker, M. C. Rheinstädter, J. C. Bozelli, R. M. Epand, V. Huynh, R. G. Wylie, S. Boulton, J. Huang *et al.*, "Atomic resolution

map of the soluble amyloid beta assembly toxic surfaces," Chem. Sci. **10**, 6072–6082 (2019).
[23]H. Dies, L. Toppozini, and M. C. Rheinstädter, "The interaction between amyloid-β peptides and anionic lipid membranes containing cholesterol and melatonin," PLoS One **9**, e99124 (2014).
[24]M. A. Barrett, R. J. Alsop, T. Hauß, and M. C. Rheinstädter, "The position of Aβ22-40 and Aβ1-42 in anionic lipid membranes containing cholesterol," Membranes **5**, 824–843 (2015).
[25]A. Khondker, R. J. Alsop, and M. C. Rheinstädter, "Membrane-accelerated amyloid-β aggregation and formation of cross-β sheets," Membranes **7**, 49 (2017).
[26]N. Dan, P. Pincus, and S. Safran, "Membrane-induced interactions between inclusions," Langmuir **9**, 2768–2771 (1993).
[27]M. M. Müller, M. Deserno, and J. Guven, "Interface-mediated interactions between particles: A geometrical approach," Phys. Rev. E **72**, 061407 (2005).
[28]C. L. Armstrong, E. Sandqvist, and M. C. Rheinstädter, "Protein-protein interactions in membranes," Protein Pept. Lett. **18**, 344–353 (2011).
[29]J. Tang, R. J. Alsop, M. Backholm, H. Dies, A.-C. Shi, and M. C. Rheinstädter, "Amyloid-β 25–35 peptides aggregate into cross-β sheets in unsaturated anionic lipid membranes at high peptide concentrations," Soft Matter **12**, 3165–3176 (2016).
[30]O. N. Yogurtcu and M. E. Johnson, "Cytosolic proteins can exploit membrane localization to trigger functional assembly," PLoS Comput. Biol. **14**, e1006031 (2018).
[31]A. Khondker, R. J. Alsop, S. Himbert, J. Tang, A.-C. Shi, A. P. Hitchcock, and M. C. Rheinstädter, "Membrane-modulating drugs can affect the size of amyloid-β 25–35 aggregates in anionic membranes," Sci. Rep. **8**, 1–16 (2018).
[32]I. P. Gastaldo, S. Himbert, U. Ram, and M. C. Rheinstädter, "The effects of resveratrol, caffeine, β-carotene, and epigallocatechin Gallate (EGCG) on amyloid-β25-35 aggregation in synthetic brain membranes," Mol. Nutr. Food Res. (in press) (2020).
[33]R. Laxminarayan, A. Duse, C. Wattal, A. K. Zaidi, H. F. Wertheim, N. Sumpradit, E. Vlieghe, G. L. Hara, I. M. Gould, H. Goossens *et al.*, "Antibiotic resistance—the need for global solutions," Lancet Infect. Dis. **13**, 1057–1098 (2013).
[34]A. Khondker and M. C. Rheinstädter, "How do bacterial membranes resist polymyxin antibiotics?," Commun. Biol. **3**, 77 (2020).
[35]G. Humphreys and F. Fleck, "United Nations meeting on antimicrobial resistance," World Health Org. Bull. World Health Org. **94**, 638 (2016).
[36]A. Khondker, D. J. Malenfant, A. K. Dhaliwal, and M. C. Rheinstädter, "Carbapenems and lipid bilayers: Localization, partitioning, and energetics," ACS Infect. Dis. **4**, 926–935 (2018).
[37]A. Khondker, I. Passos-Gastaldo, G. D. Wright, and M. C. Rheinstädter, "Membrane interactions of non-membrane targeting antibiotics: The case of aminoglycosides, macrolides and fluoroquinolones," BBA Biomembr. (in press) (2020).
[38]K. A. Brogden, "Antimicrobial peptides: Pore formers or metabolic inhibitors in bacteria?," Nat. Rev. Microbiol. **3**, 238–250 (2005).
[39]A. Khondker, A. K. Dhaliwal, S. Saem, A. Mahmood, C. Fradin, J. Moran-Mirabal, and M. C. Rheinstädter, "Membrane charge and lipid packing determine polymyxin-induced membrane damage," Commun. Biol. **2**, 67 (2019).
[40]C. J. Kubin, T. M. Ellman, V. Phadke, L. J. Haynes, D. P. Calfee, and M. T. Yin, "Incidence and predictors of acute kidney injury associated with intravenous polymyxin B therapy," J. Infect. **65**, 80–87 (2012).
[41]N. A. Berglund, T. J. Piggot, D. Jefferies, R. B. Sessions, P. J. Bond, and S. Khalid, "Interaction of the antimicrobial peptide polymyxin B1 with both membranes of E. coli: A molecular dynamics study," PLoS Comput. Biol. **11**, e1004180 (2015).
[42]M. G. Márquez, N. O. Favale, F. L. Nieto, L. G. Pescio, and N. Sterin-Speziale, "Changes in membrane lipid composition cause alterations in epithelial cell--cell adhesion structures in renal papillary collecting duct cells," Biochim. Biophys. Acta (BBA)-Biomembr. **1818**, 491–501 (2012).
[43]A. Khondker, R. J. Alsop, A. Dhaliwal, S. Saem, J. M. Moran-Mirabal, and M. C. Rheinstädter, "Membrane cholesterol reduces polymyxin B nephrotoxicity in renal membrane analogs," Biophys. J. **113**, 2016–2028 (2017).
[44]P. Khakbaz and J. B. Klauda, "Probing the importance of lipid diversity in cell membranes via molecular simulation," Chem. Phys. Lipids **192**, 12–22 (2015).
[45]J. McPhee and S. Lewenza, "From pigs to patients: Transmissible, single gene-mediated resistance to colistin," J. Med. Microbiol. Diagn. **5**, 221 (2016).
[46]L. Guo, K. B. Lim, C. M. Poduje, M. Daniel, J. S. Gunn, M. Hackett, and S. I. Miller, "Lipid a acylation and bacterial resistance against vertebrate antimicrobial peptides," Cell **95**, 189–198 (1998).
[47]A. Rice and J. Wereszczynski, "Atomistic scale effects of lipopolysaccharide modifications on bacterial outer membrane defenses," Biophys. J. **114**, 1389–1399 (2018).
[48]M. C. Rheinstädter, K. Schmalzl, K. Wood, and D. Strauch, "Protein-protein interaction in purple membrane," Phys. Rev. Lett. **103**, 128104 (2009).