Kuroda Kenichi (Orcid ID: 0000-0002-2935-8351)

WIREs Nanomedicine & Nanobiotechnology
Review article

Title: Biomimetic Antimicrobial Polymers – Design, Characterization, Antimicrobial and Novel Applications

Authors:
Haruko Takahashi[1], Iva Sovadinova[2], Kazuma Yasuhara[3, 4], Satyavani Vemparala[5,6], Gregory A. Caputo[7]*, Kenichi Kuroda[8]*

Affiliations:
[1] Graduate School of Integrated Sciences for Life, Hiroshima University, 1-3-1 Kagamiyama, Higashi-Hiroshima, Hiroshima 739-8526, Japan
[2] RECETOX, Faculty of Science, Masaryk University, Kotlarska 2, Brno, Czech Republic
[3] Division of Materials Science, Graduate School of Science and Technology, Nara Institute of Science and Technology, 8916-5 Takayama, Ikoma, Nara 6300192, Japan.
[4] Center for Digital Green-innovation, Nara Institute of Science and Technology, 8916-5 Takayama, Ikoma, Nara 6300192, Japan.
[5] The Institute of Mathematical Sciences, CIT Campus, Taramani, Chennai, India 600113
[6] Homi Bhabha National Institute, Training School Complex, Anushakti Nagar, Mumbai, 400094, India
[7] Department of Chemistry & Biochemistry, Rowan University, Glassboro NJ 08028, USA
[8] Department of Biologic and Materials Sciences & Prosthodontics, School of Dentistry, University of Michigan, Ann Arbor MI 48176, USA

*Corresponding authors

Abstract

Biomimetic antimicrobial polymers have been an area of great interest as the need for novel antimicrobial compounds grows due to the development of resistance. These polymers were designed and developed to mimic naturally occurring antimicrobial peptides in both physicochemical composition and mechanism of action. These antimicrobial peptide mimetic polymers have been extensively investigated using chemical, biophysical, microbiological, and computational approaches to gain a deeper understanding of the molecular interactions that drive function. These studies have helped inform SARs, mechanism of action, and general physicochemical factors that influence the activity and properties of antimicrobial polymers. However, there are still lingering questions in this field regarding 3D structural patterning, bioavailability, and applicability to alternative targets. In this review, we present a perspective on the development and characterization of several antimicrobial polymers and discuss novel applications of these molecules emerging in the field.

1. Introduction

Antimicrobial polymers have been of great interest in the research community for the past decades. Many studies have been dedicated to the development of synthetic polymers which act by disrupting the bacterial cell membranes and causing bacterial cell death. We published a review article in this journal in 2013 and described the background and design principle of antimicrobial polymers which mimic host-defense antimicrobial peptides (AMPs).(Kuroda & Caputo, 2013) Since then, the antimicrobial polymer research has significantly evolved and expanded. The development of novel antimicrobial polymers has been largely driven by advances in polymer chemistry, which provided a diversity of functional groups and molecular shapes to facilitate the investigation of the structure-activity relationships in these molecules. Broader molecular diversity has increased the ability to fine-tune molecular properties, and thus the potential to translate the research outcomes to clinical applications. On the other hand, while the bacterial infections have been the primary target, the use of antimicrobial polymers has been expanded to address other challenges including drug-resistant cancer cells, environmental bacteria, and more. In addition to biological studies on efficacy and cytotoxicity, the design and development of antimicrobial polymers are also driven by knowledge obtained from fundamental research using biophysical methods and computational simulations. Specifically, the membrane-disrupting mode of action of the polymers has been studied by using model membrane systems, spectroscopic experiments, microscopic analysis, and molecular dynamics computational simulations. The combined use of these novel methods and novel applications has expanded the utility, but also the understanding, of these biomimetic polymers.

Here we provide an update on the state of the research field on cationic, biomimetic antimicrobial polymers. The central theme of this article is “new targets and new methods” in the studies on antimicrobial polymers. We begin with a review of the evolution of antimicrobial polymer research including early development and characterization. This includes the most recent trends in polymer chemistry approaches, although very briefly, as many excellent review articles are available on this topic.(Alfei & Schito, 2020; Ding, Wang, Tong, & Xu, 2019; Etayash & Hancock, 2021; Ghosh, Sarkar, Issa, & Haldar, 2019; H. Takahashi, Caputo, & Kuroda, 2021; J. Tan, Tay, Hedrick, & Yang, 2020) An analysis of novel targets incorporates highlights the more recent investigations of antimicrobial polymer usage in the studies of anticancer, anti-cyanobacterial, and antialgal

applications. The discussion will be extended to the new methods to study antimicrobial polymers using giant liposomes and molecular dynamic simulations to probe into the polymer-membrane interactions.

2. Rise of AMP-memetic polymers

The primary driving force behind antimicrobial polymer research has been the need for new antibiotics due to drug resistance in bacteria. The rise of antibiotic resistance in bacteria and other pathogens has been highlighted as an emerging threat to world health by a number of organizations.(Antimicrobial Resistance, 2022; CDC, 2019; Organization, 2018) This resistance phenomenon has arisen due to a variety of factors including natural evolution under selective pressure, over-prescription of antibiotics, and improper use of antibiotics, among others. These topics have been comprehensively reviewed elsewhere, and we direct interested readers to the referenced reports.(Alfei & Schito, 2020; J. Chen, Wang, Liu, & Du, 2014; Ding et al., 2019; Engler et al., 2012; Cansu Ergene, Yasuhara, & Palermo, 2018; Etayash & Hancock, 2021; Ghosh et al., 2019; Krumm & Tiller, 2017; W. Ren, Cheng, Wang, & Liu, 2017; Santos et al., 2016; Scott & Tew, 2017; H. Takahashi et al., 2021; Haruko Takahashi, Caputo, Vemparala, & Kuroda, 2017; J. Tan et al., 2020; X. Y. Zhou & Zhou, 2018)

Importantly in this context, membrane-active antimicrobial polymers have not demonstrated selective pressure on bacteria to develop resistant phenotypes, which provides promise for these molecules as new antibiotic agents.(I. P. Sovadinova, E.F.; Urban, M.; Mpiga, P.; Caputo, G.A.; Kuroda, K., 2011; Thoma, Boles, & Kuroda, 2014) However, as human cells also contain cell membranes, the lipid membrane-disrupting action by polymers is not inherently specific to bacteria, and thus potential toxicity of the polymers to host cells is a concern. In contrast to these polymers, many conventional antibiotic drugs are enzyme inhibitors which target enzymes involved in bacterial metabolic processes or the biosynthesis of bacterial cell structures. The specific targets of small-molecule antibiotic action can be subject to mutational or expression-level changes, resulting in the development of resistance.(Brauner, Fridman, Gefen, & Balaban, 2016; Crofts, Gasparrini, & Dantas, 2017; Fisher, Gollan, & Helaine, 2017; Hall & Mah, 2017; Kester & Fortune, 2014; Lazar et al., 2014; Peterson & Kaur, 2018) For small molecules, since the target

enzymes/structures exist only in bacteria and not in human cells, there is inherent selectivity for bacteria.

To address the challenge of selectivity, a new design concept based on host-defense antimicrobial peptides (AMPs) has emerged, which has contributed to a new perspective on the development of antimicrobial polymers. Naturally occurring AMPs have cationic and hydrophobic amino acid residues in their sequences. The cationic groups are attracted to the negatively charged bacterial cell surfaces. Upon binding to the bacterial membrane, AMPs often adopt an α-helical conformation with the cationic and hydrophobic domains segregated to opposite sides of the helix, allowing the hydrophobic domain to insert into the hydrophobic core of the lipid bilayer, causing membrane disruption or permeabilization.(Magana et al., 2020) While there are AMPs which adopt β-strand or disordered structures, the α-helical AMPs are both the most widely represented in nature and the most widely studied in the lab.(Huan, Kong, Mou, & Yi, 2020; Kang, Kim, Seo, & Park, 2017; Kohn et al., 2018; Mahlapuu, Bjorn, & Ekblom, 2020; Patrulea, Borchard, & Jordan, 2020; Saint Jean et al., 2018; Wildman, Lee, & Ramamoorthy, 2003) Because the bacterial cell surfaces are significantly negatively charged compared to mammalian cell membranes, the favorable electrostatic interactions lead to preferential binding of AMPs to bacteria over human cells, imparting selective activity. The intriguing part of this mechanism is that the antimicrobial activity of AMPs is driven by the physicochemical interactions between peptides and bacterial cell membranes, not specific biological interactions such as ligand-receptor binding, which require specific peptide sequences and targets. There have been numerous reports in the literature in which AMP sequences were modified using natural and non-natural amino acids which show equal or enhanced efficacy compared to the parent sequence. Early work from Oren and Shai demonstrated that synthetic AMPs composed of D-enantiomers of naturally occurring amino acids retained antimicrobial activity despite dramatic differences in peptide structure.(Oren & Shai, 1997; Shai & Oren, 1996) More recent work continues to demonstrate that AMPs partially or wholly composed of D-amino acids retain antimicrobial activity, resist protease digestion, and may have beneficial impacts on host cytotoxicity.(Hicks, Abercrombie, Wong, & Leung, 2013; Lu et al., 2020; Y. Zhao et al., 2016) Similarly, non-canonical amino acids have been incorporated into a variety of AMP sequences with mixed results. Depending on the sequence and the structure of the amino acid, these amino acids have been shown to improve activity, not impact activity, or in some

cases reduce or abolish AMP activity.(Hicks et al., 2013; Hitchner, Necelis, Shirley, & Caputo, 2021; Stone et al., 2019)

The physicochemical mechanism of AMPs has been also supported by peptide-mimetic molecules. "AMP mimetics" were traditionally referred to peptide-like molecules with defined sequences, including beta-peptides and peptoids, which can mimic the bioactive secondary conformations (helices and beta-sheet) of natural peptides.(Andreev et al., 2018; DeGrado, Schneider, & Hamuro, 1999; Godballe, Nilsson, Petersen, & Jenssen, 2011; Porter, Wang, Lee, Weisblum, & Gellman, 2000; Yoo & Kirshenbaum, 2008) These peptide-mimics are designed to form amphiphilic helical structures and showed antimicrobial activity, suggesting that the synthetic backbones could serve as a molecular platform, but not necessarily natural peptides (α-amino acids), although the helical structures are membrane-active conformations.

The knowledge that antimicrobial activity was driven, at least partly, by the amphiphilic, cationic nature of AMPs motivated researchers toward the development of AMP-mimetic polymers; specifically, if the physicochemical interactions drive selective binding to bacteria over human cells and membrane disruption to kill bacteria, defined sequences, secondary conformations, and peptide chains may not be the requisite for antimicrobial activity anymore. *Why not use synthetic random copolymers*? This possibility of polymer antibiotics attracted polymer chemists who were interested in the use of advanced polymer chemistry methods to design bioactive polymers and macromolecules. The approach of using random copolymers offers the inherent benefit of a simpler, often "one-pot" synthetic methodology.(Foster, Mizutani, Oda, Palermo, & Kuroda, 2017) Random copolymers with cationic and hydrophobic side chains, based on methacrylates, nylons, norbornenes, and other backbones were designed and indeed these copolymers exhibited both antimicrobial activity and selectivity.(Al-Badri et al., 2008; Cansu Ergene et al., 2018; Kuroda & DeGrado, 2005; M. W. Lee et al., 2014; Lienkamp et al., 2008; L. Liu et al., 2021; K. E. Locock et al., 2013; Palermo & Kuroda, 2010; E. F. Palermo, S. Vemparala, & K. Kuroda, 2012) From the polymer chemist's perspective, biological components or building blocks (antibiotics, peptides, etc.) are not required in this model, because the chemical composition affecting the properties (hydrophobicity, block sequences, molecular weight, molecular shapes, etc.) of polymers directly impacts their antimicrobial activity. This allows the full power of

polymer chemistry to utilize a much broader scope of chemical space to design and develop antimicrobial polymers. Moving beyond the functionalities accessible in naturally occurring amino acids allows for much more fine-grained analysis of structure-activity relationships (SARs), as well as opening the possibility to novel monomer compositions (block and random copolymers) which are uncommon in naturally occurring peptides.(W. Chin et al., 2018; Y. Oda, Kanaoka, Sato, Aoshima, & Kuroda, 2011; Yukari Oda et al., 2018) As such, innovations in synthetic approaches by polymer chemists were another big driver in the evolution and growth of this field.

Another important factor in the growth and development of the field of antimicrobial polymers is the straightforward activity assays which are an attractive and accessible model system for synthetic polymer chemists. Antimicrobial assays are not inherently complex experiments using commercially available and low-cost components. The antimicrobial activity of polymers is evaluated using a turbidity-based microdilution assay to determine the Minimal Inhibitory Concentration (MIC).(Wiegand, Hilpert, & Hancock, 2008) This assay is typically performed in 96-well plates in which serially diluted stocks of polymer solution are mixed with a bacterial suspension, and bacterial growth is determined by the turbidity of the culture after 18h of incubation. This assay is quick, allows for easy side-by-side replicates, and requires small amounts of the polymer under investigation. As compared to mammalian cell culture, the initial evaluation of antimicrobial activity is much more accessible for many researchers, although a detailed evaluation of the mechanism of action requires more in-depth microbiological studies. Additionally, the bacteria commonly used in MIC assays are *Escherichia coli* and *Staphylococcus aureus* which represent both Gram-negative and Gram-positive classes of bacteria, can be cultured overnight, and grow under simple aerobic conditions. These factors, combined with the increasing awareness and subsequent funding initiatives around developing new antimicrobials and combating antimicrobial resistance, have contributed to the growth and diversification of the antimicrobial polymers field.

3. AMP mimetic polymers and polymer chemistry

In naturally occurring AMPs, the cationic moieties drive binding to bacterial membranes and selectivity, while the hydrophobic moieties insert into the lipid bilayer core and cause membrane disruption, resulting in bacterial cell death.(Hitchner et al., 2019; Jiang et al., 2008; J. Li, Hu, Jian,

Xie, & Yang, 2021; Lopez Cascales et al., 2018; Rosenfeld, Lev, & Shai, 2010; Senetra, Necelis, & Caputo, 2020) Inspired by the mechanism, antimicrobial polymers are designed to have both cationic and hydrophobic groups as essential and minimal functional groups for antimicrobial activity.(Colak & Tew, 2012; Kuroda, Caputo, & DeGrado, 2009; Mukherjee, Ghosh, Bhadury, & De, 2017; Palermo, Lee, Ramamoorthy, & Kuroda, 2011; E. F. Palermo et al., 2012) In the early stages of this field, random copolymers with binary compositions of monomers with cationic or hydrophobic side chains were synthesized and evaluated for their antimicrobial activity.(Colak & Tew, 2012; Kuroda et al., 2009; Mukherjee et al., 2017) Based on AMPs and the polymers mimicking them, the well-accepted design principle is to create an optimal balance between the cationic and hydrophobic content of polymers which results in potent activity and selectivity. While hydrophobic groups are needed to disrupt bacterial membranes, polymers with too much hydrophobic character cause non-specific binding to human cell membranes, leading to undesired cytotoxicity.(Grace et al., 2016; Kuroda et al., 2009; Kuroki et al., 2017; K. E. S. Locock et al., 2013; Tyagi & Mishra, 2021) Palermo and coworkers thoroughly discussed these factors in their excellent review articles on the amphiphilic balance of antimicrobial polymers (C. Ergene & Palermo, 2018; Cansu Ergene et al., 2018; Palermo & Kuroda, 2010). The readers who are interested in how to achieve the optimal balance of polymers should refer to their articles.

This initial polymer design was based on α-helical AMPs such as magainins, which generally consist of 20–30 amino acid residues which translate to 2,000–3,000 g/mol molecular weight (MW). Therefore, the polymers were designed to be low MW, linear polymers in order to mimic the short peptide chains of α-helical AMPs. Indeed, higher MW polymers tend to show higher toxicity than those with low MWs, which also further supports the use of low MW polymers.(Fischer, Li, Ahlemeyer, Krieglstein, & Kissel, 2003; Gibney et al., 2012; Mellati et al., 2016) This is in contrast with earlier, more traditional antimicrobial polymers with high MWs which were prepared by free-radical polymerization.(Kenawy, Worley, & Broughton, 2007; Tashiro, 2001) These more facile methods were subsequently employed to create block copolymers with a variety of cationic and hydrophobic segments.(Judzewitsch, Nguyen, Shanmugam, Wong, & Boyer, 2018) Importantly, the block copolymers allow for mix-and-match combinations of block sizes and ordering/sequencing of blocks in the polymer, adding versatility and flexibility beyond the basic control of monomer composition. These sequences also self-

assemble to form multi-chain large structures such as aggregates and micellar nanoparticles in solution. The amphiphilic and macromolecular structures have provided more protein-like molecules which mimic the diverse classes of AMPs. In addition to linear polymers, the polymer platforms include branched polymers, dendrimers, graft polymers, micelles, and nanoparticles.(Alfei & Schito, 2020; J. Chen et al., 2014; Ding et al., 2019; Engler et al., 2012; Cansu Ergene et al., 2018; Etayash & Hancock, 2021; Ghosh et al., 2019; Krumm & Tiller, 2017; W. Ren et al., 2017; Santos et al., 2016; Scott & Tew, 2017; H. Takahashi et al., 2021; Haruko Takahashi et al., 2017; J. Tan et al., 2020; X. Y. Zhou & Zhou, 2018)

4. New targets

In this section, we present novel applications and future directions for antimicrobial polymers. Based on the fundamental principles and designs of antimicrobial polymers, new applications have emerged as additional areas of interest for this class of molecules.

4-1. Anti-cancer polymers

Cancer is a disease in which host cells exhibit altered characteristics due to genetic mutations to proto-oncogenes and/or tumor suppressor genes as a result of biological events or external stimuli, resulting in abnormal and uncontrollable cell growth.(Bray et al., 2018; Diederichs et al., 2016; Fouad & Aanei, 2017) The uncontrolled division of cancer cells eventually invades the surrounding tissues and causes malfunctions in organs or can spread through the blood and lymph to more distal sites in the body. According to the statement issued by the WHO, cancer is the second leading cause of death globally, and is responsible for an estimated 9.6 million deaths in 2018 that number was analyzed using GLOBOCAN.(Bray et al., 2018) Chemotherapy is a first-line therapy to treat cancer, however, cancer cells often develop resistance against chemotherapeutic agents, which presents a critical challenge in cancer therapy.(Housman et al., 2014) Although the majority of cancer cells are sensitive to the anti-cancer drugs, the small population which is resistant is then responsible for persistence in the tumor and recurrence and leads to failure of chemotherapy and refractory cancer diagnoses.(Cree & Charlton, 2017; Housman et al., 2014; Schmidt & Efferth, 2016) Furthermore, with continued treatment, multi-drug resistance (MDR) can emerge and is one of the major causes of failure of this mode of treatment.(Gillet & Gottesman, 2010) Many studies have been devoted to develop new anti-cancer

drugs which do not result in resistance development.(T. Hu, Li, Gao, & Cho, 2016; Kuwano, Sonoda, Murakami, Watari, & Ono, 2016; Leary, Heerboth, Lapinska, & Sarkar, 2018; Maik-Rachline & Seger, 2016) However, it has been a significant challenge to identify molecular targets in cancer cells which are immune to resistance development because of the high mutation rate and rapid cell division which are hallmarks of cancer cells.

The standard cancer chemotherapy approach uses therapeutics which target the rapidly dividing cells, i.e., the majority of cancer cells, primarily acting to disrupt DNA synthesis or other vital cell replication processes. For example, Paclitaxel, a cytoskeletal drug targeting tubulin, binds to the mitotic spindle assembly and subsequently inhibits chromosome segregation during cell division.(Yusuf, Duan, Lamendola, Penson, & Seiden, 2003) Furthermore, there are several drugs which are approved for use as both anti-cancer drugs and antibiotics called as antitumor antibiotics, such as Adriamycin(Skovsgaard & Nissen, 1975), bleomycin(Hecht, 2000) and mitomysin(Galm et al., 2005). Mainly these drugs act as DNA intercalators, blocking DNA replication and/or transcription.(Galm et al., 2005; Hecht, 2000) These molecules highlight the shared fundamental bioactivities and potential treatment targets in cancer cells and bacteria. Thus, anti-cancer drugs share common strategies with antibiotics that interfere with the cellular mechanisms such as macromolecular synthesis required for cell division and proliferation to treat diseases caused by rapidly dividing cells: cancer and bacterial infections.

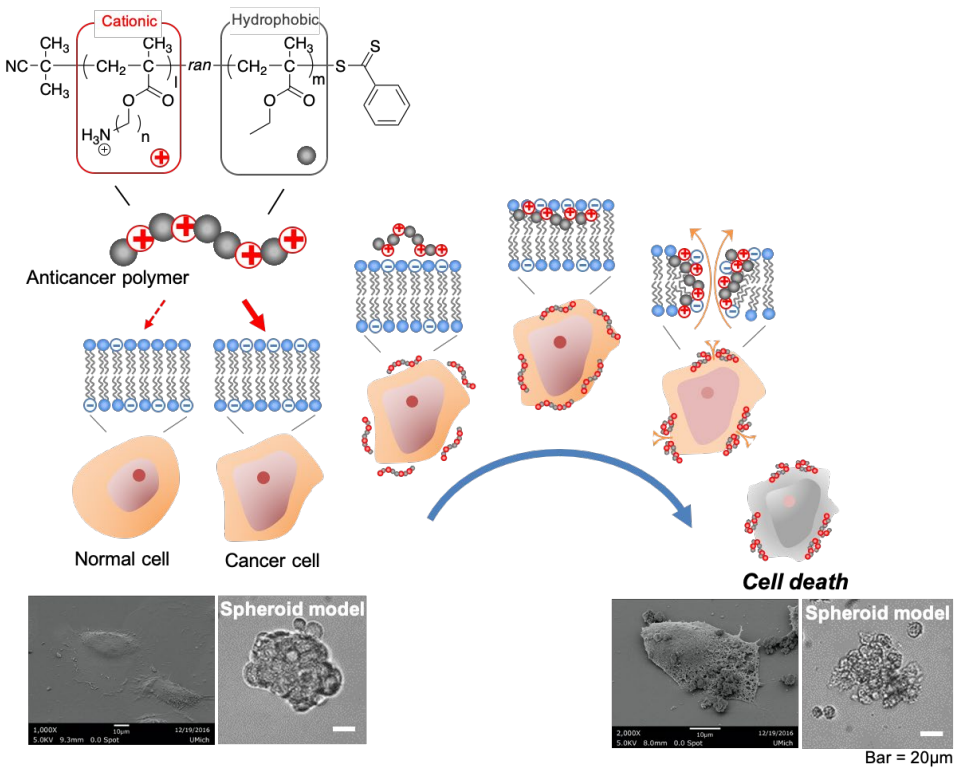


Figure 1. Cationic amphiphilic methacrylate copolymers with anticancer activity. Polymers selectively bind to the cancer cell membrane and disrupt it, resulting in cell death. Adapted from (H. Takahashi et al., 2019) with permission.

As might be expected given the commonality of drug mechanisms of action, there are also commonalities in the mechanisms by which cancer cells and bacteria acquire drug resistance. In both cancer and bacterial cells, a common mechanism underlying the resistance phenotype is efflux pump systems, which enhance the removal of antibiotics or anti-cancer drugs from the cytosol.(Lowrence, Subramaniapillai, Ulaganathan, & Nagarajan, 2019) Another important parallel in the resistance mechanisms is the local environment around the cells. Cancer cells often form clusters of cells, i.e. tumors, which results in limited diffusion of chemotherapeutic agents to the cells at the interior of the tumor.(Belli et al., 2018; M. N. Wang et al., 2017) This shielding through aggregation is also observed in bacterial biofilms.(Jamal et al., 2018; S. L. Percival, McCarty, & Lipsky, 2015) Similarly, cells in the interior of tumors and biofilms both have limited access to nutrients and oxygen due to the physical restrictions, and often enter a dormant or senescent state, limiting the effectiveness of drugs that target actively dividing cells.(Chakrabarty, Chakraborty, Bhattacharya, & Chowdhury, 2021; Klapper, Gilbert, Ayati, Dockery, & Stewart,

2007; Steiner, 2021) As the majority of anti-cancer drugs and antibiotics act on cellular machinery involved in cell growth and division, cancer cells and bacteria display resistance to treatments when in a quiescent or dormant state since the cell division processes are inactive.

As described, many cancer cells are resistant or tolerant to drugs through many different mechanisms. The traditional drug design may not be able to mitigate this problem. Recently, a non-traditional anti-cancer drug designed via a biomimetic approach was tested by mimicking AMPs which act by targeting cell membranes. The lipid bilayer structure of cell membranes of cancer cells is not directly associated with the majority of resistance mechanisms and hence the cell membranes are a promising candidate as a target site for cancer cells as well as bacteria. Indeed, some AMPs are also active against cancer cells through targeting cancer cell membranes.(Felicio, Silva, Goncalves, Santos, & Franco, 2017) One of the characteristics of cancer cells is enrichment of anionic phosphatidylserine (PS) lipids in the outer leaflet of the cell membrane compared to normal cells. The presence of PS imparts an anionic net charge to the lipid membrane surface, similar to that of bacteria (although smaller in magnitude). The higher net negative charge of the cancer cell surface facilitates the binding of cationic AMPs to cancer cells and imparts a degree of selectivity over normal cells. Parallel to the activity against bacteria, AMPs disrupt cancer cell membranes or target the intracellular components, resulting in cell death.(Gaspar, Veiga, & Castanho, 2013; Jafari, Babajani, Sarrami Forooshani, Yazdani, & Rezaei-Tavirani, 2022; Tornesello, Borrelli, Buonaguro, Buonaguro, & Tornesello, 2020)

This common mechanism by which AMPs can kill bacterial and cancer cells promoted researchers to extend the AMP-mimetic design of antimicrobial polymers to develop anticancer polymers toward the treatment of drug-resistant cancer. Recently, Yang and Hedrick tested their design approaches by preparing membrane-active polymers and macromolecules which were effective in killing drug-resistant cancer cells. In this work, macromolecular self-assemblies of cationic diblock polycarbonates selectively bound to cancer cells over normal cells and caused necrotic cell death.(Park et al., 2018) The polymers showed potent activity against multiple cancer cell lines including doxorubicin-resistant breast cancer cells and cancer stem cells. In addition, the same group also demonstrated that biodegradable triblock copolymers showed in vitro potent activity against multi-drug resistant cancer cells, and multiple treatments with the polymers at sub-

lethal doses do not induce resistance.(Zhong et al., 2019) These polymers also suppressed tumor growth and inhibited metastasis in the mouse tumor model. As another example, cationic amphipathic peptoids were active against a broad range of cancer cell lines including multidrug-resistant cells *in vitro* and inhibited tumor growth *in vivo*.(Huang et al., 2014) Furthermore, our laboratory has also demonstrated that methacrylate copolymers with random sequences of cationic and hydrophobic sidechains selectively targeted and disrupted cancer cell membranes, causing necrosis (**Fig. 1**).(H. Takahashi et al., 2019) As this mechanism is not dependent on cell proliferation, the polymers killed dormant prostate cancer cells that poorly responded to the conventional anti-cancer drug docetaxel. Additionally, these polymers also lysed aggregated cells and dispersed spheroids, indicating that these polymers may be effective in treating tumors. In other cases, some naturally-derived biopolymers including chitosan and their derivatives(Adhikari & Yadav, 2018) and polysaccharides from *Rhynchosia minima* root(Jia et al., 2015) have shown inherent anticancer activity, but the activity is not driven by design and their mechanism is not fully understood.

In general, human cell membranes are inherently more resistant to membrane-active AMPs compared to bacterial cell membranes. Antimicrobial polymers have been designed to maintain this biocompatibility; however, anticancer polymers need to target human cell membranes in order to be effective. In principle, the selective activity of polymers against cancer cells over normal cells relies on the difference in the net negative charges between the normal and cancer cell types.(L. T. H. Tan et al., 2017) Bacterial cell membranes have a significantly higher net negative charge than normal healthy human cell membranes, which favors the selective activity of AMPs and polymers against bacteria. However, the difference between normal and cancer cells is subtle; in the outer leaflet of the cell membrane, there is only ~10% increase of anionic PS lipids in cancer cells over normal cells.(Utsugi, Schroit, Connor, Bucana, & Fidler, 1991) Therefore, it is likely to be difficult for the polymers to distinguish them completely. However, it is interesting that phosphatidylethanolamine (PE) lipid which also exists in the outer leaflet of cancer cell membranes as well as being enriched in bacterial cell membranes, can play an important role in the membrane disrupting mechanism of small molecules and peptides.(L. T. H. Tan et al., 2017) Taken together, there are clearly differences in lipid composition between healthy and cancer cells that can be targeted.

It has been demonstrated that membrane-active polymers do not induce resistance in cancer cells.(Park et al., 2018) Similar to bacteria, we also speculate that the membrane-active mechanism would not contribute to resistance development due to the large fitness cost of dramatic alterations in lipid compositions. However, the number of studies on anti-cancer polymers in the field has been limited, and further investigations will be needed to determine the likelihood of resistance development against these polymers. As a more advanced application, anticancer polymers and/or peptides may have synergy with anti-cancer drugs. For example, combination therapy using membrane-active peptides and doxorubicin/epirubicin has already been investigated.(J. Zhao, Huang, Liu, & Chen, 2015) As compared to using drugs and polymers/peptides as individual, distinct therapeutic agents, combination therapy may be more beneficial and clinically useful because it could reduce the doses of these agents, thus reducing toxicity and side effects to normal cell membranes. As already reported in the antimicrobial polymers(D. D. Yang et al., 2021), this approach can be extended to drug delivery carriers enabling delivery through cancer cell membranes. Synthetic polymers are a versatile platform to design and construct drug conjugates, micelles/self-assemblies, and macromolecules for drug delivery systems. We will be able to equip them with the ability to target cell membranes.

4-2. Antialgal and anticyanobacterial polymers

Although the promising antimicrobial activity and selectivity of peptide-mimetic antimicrobial polymers against human bacterial pathogens for biomedical applications are well documented, their applicability to prevent, control and mitigate unwanted excessive growth or biofouling of environmentally-relevant harmful microorganisms is neglected. There is a substantial gap in knowledge about the interaction of biomimetic synthetic polymers with microorganisms in the aquatic environment, such as cyanobacteria, green algae, or heterotrophic bacteria, which is surprising because transferring a successful polymer-based antimicrobial platform from biomedical applications into a water management sector could lead to a promising platform for developing a new type of bioinspired polymers agents or coatings applicable in various natural or engineered water systems.

Algae and cyanobacteria are photosynthetic aquatic microorganisms essential in providing oxygen, fixing nitrogen and cycling nutrients for other organisms and sustaining life on the Earth. They occur in all types of water, including salt, fresh, and brackish water, and in places with sufficient sunlight, moisture, and nutrients. Their growth is generally considered positive for aquatic ecosystems. However, their intensive and uncontrolled growth, attachment and settlement are a growing ecological, public health, economic, and technical concern in water management in natural or engineered environments. Specifically, a massive and exceptional overgrowth of harmful algae or cyanobacteria in water bodies forms blooms and produces toxins (cyanotoxins), both substantially reducing water quality and its potential use, safety, and sustainability for drinking water production, recreational purposes, fisheries, and agriculture or aquacultures.(Han, Clarke, & Pratt, 2014; Organization, 2003) Harmful algal (HAB) or cyanobacterial (HCB) blooms are driven by combining anthropogenic-induced eutrophication processes and rising environmental temperatures and are expected to increase in frequency and intensity worldwide.(Briand, Bormans, Gugger, Dorrestein, & Gerwick, 2016; Paerl & Paul, 2012) Additionally, cooling water systems provide a favorable environment for the uncontrolled growth of cyanobacteria and algae, which can adversely affect their efficient operations, promote metal corrosion, and accelerate wood deterioration in many industries, including water treatment or power plants and chemical, petrochemical and food industries.(Di Pippo, Di Gregorio, Congestri, Tandoi, & Rossetti, 2018; Hauer, Capek, & Bohmova, 2016) Finally, undesired attachment and settlement of aquatic microorganisms, including algae or cyanobacteria, on synthetic surfaces (biofouling) causes economic, environmental, or safety-related negative effects on the aquatic industries.(Selim et al., 2017) Therefore, treatment strategies allowing preventing, controlling or mitigating unwanted and explosive cyanobacterial and algal propagation, attachment and settlement have attracted scientific and regulatory attention. Multiple approaches have been employed to control undesired organisms including various chemicals, e.g., metal salts and organometallic compounds, biological agents, and mechanical or physical forces. However, these approaches are often limited by non-specificity, low efficiency, or non-target toxicity.(Matthijs, Jancula, Visser, & Marsalek, 2016) For example, the treatment with commercially available cyanocides based on general herbicidal chemicals such as Diuron or Endothall in open aquatic environments generally induces resistant aquatic bacteria and algae and causes unselective elimination of non-target aquatic organisms, disrupting water ecology or the secondary

pollution.(Matthijs et al., 2016; Tixier, Sancelme, Bonnemoy, Cuer, & Veschambre, 2001) Bioactive and biomimetic synthetic polymers in a solution or as surface coatings can overcome some disadvantages.

A limited number of synthetic bioinspired and biomimetic polymers studied in solution for their potential applicability in the aquatic environment and water management are summarized in Table 1. These represent various structures, from polyethyleneimines through polymethacrylates to polymeric nanoparticles or dendrimers. The most used aquatic photosynthetic organisms were freshwater species - algae *Desmodesmus quadricauda* and *Chlamydomonas reinhardtii* and cyanobacteria *Anabaena* sp., *Microcystis aeruginosa* and *Synechococcus elongatus*. *D. quadricauda* and *C. reinhardtii* are ubiquitous freshwater green algae in many natural and industrial water systems and model organisms for antialgal tests and biofouling.(Arora & Sahoo, 2015; Janssen, Vangheluwe, & Sprang, 2000) However, both can become a common part of extensive water blooms or colonize submerged surfaces (biofouling).(Arora & Sahoo, 2015) *Anabaena* sp., *M. aeruginosa* and *S. elongates* are freshwater cyanobacteria commonly utilized as model cyanobacterial species in experimental studies. *A. flos-aquae* and *M. aeruginosa* produce cyanotoxins (microcystins) that can be a dominant species of harmful cyanobacterial blooms (HABs), producing cyanotoxins and causing human health and ecological problems.(Lyon-Colbert, Su, & Cude, 2018; Preece, Hardy, Moore, & Bryan, 2017)

Even though algal and cyanobacterial cells differ in their cell type (algae which are eukaryotic *vs.* prokaryotic cyanobacteria) and structure (size, surface, volume, cell wall, membrane composition), the cell surfaces of these organisms are negatively charged due to the de-protonation of carboxyl and sulfate groups in the algal cell envelope(J. Liu et al., 2013; Wyatt et al., 2012) or the anionic components of peptidoglycan including murein in the cyanobacterial cell walls(Hoiczyk & Hansel, 2000) and extracellular polysaccharide glycocalyx layer surrounding cell wall(Bryant, 2006). Thus, amphiphilic and cationic biomimetic polymers can interact with an algal or cyanobacterial cell through an electrostatic attraction, similar to their interactions with human bacterial pathogens. Therefore, it is not surprising that some polymers, whose applicability to fighting undesired environmentally relevant photosynthetic aquatic microorganisms in solution has been explored so far, previously displayed promising antimicrobial activity against human pathogens by interacting

with their negatively charged membranes (for example, polymethacrylates or polyethyleneimines) (**Table 1**). Additionally, cyanobacterial and algal plasma, thylakoid, and/or chloroplast membranes have an amphiphilic (amphipathic) character derived from the phospholipids which have a polar head group and two hydrophobic fatty acid tails.(Alberts et al., 2015) Polymers can interact with these membranes through electrostatic and hydrophobic interactions, which can lead to their disruption and leakage of intercellular content.

Most polymers interacting with algal or cyanobacterial cells in solution (**Table 1**, illustration in **Fig. 2**) inhibited growth (algostatic and/or cyanostatic effect), and some of them caused cell death (algicidal and/or cyanocidal effect). The proposed molecular mechanisms of algicidal or cyanocidal activity of biomimetic polymethacrylates, branched PEIs, PPI-DEN, or cationic PAMAM dendrimers cover 1) binding to the cell surface, 2) crossing the cell wall, and 3a) destabilizing and permeabilizing plasma membrane, leading to membrane disruption and cell lysis or 3b) polymer internalization and disrupting structure and function of chloroplasts or thylakoids, leading to chlorophyll and photosynthesis deteriorations.(Gonzalo et al., 2015; Přemysl Mikula et al., 2021; Premysl Mikula et al., 2018; Petit, Debenest, Eullaffroy, & Gagné, 2012; Petit, Eullaffroy, Debenest, & Gagné, 2010) PAMAM dendrimers and core-shell polystyrene-copper oxide nanoparticles also elevated ROS (reactive oxygen species) production in algal and/or cyanobacterial cells.(Gonzalo et al., 2015; Petit et al., 2012; Petit et al., 2010; Saison et al., 2010) Only N-halamine derivatized PAM nanoparticles exhibited selectivity to cyanobacteria over algae, likely by a preferential attachment to cyanobacterial cell surfaces.(Sadhasivam, Gelber, Zakin, Margel, & Shapiro, 2019) Finally, some polymers induced cyanobacterial (PEIs(Arrington, Zeleznik, Ott, & Ju, 2003; Premysl Mikula et al., 2018; Zeleznik, Segatta, & Ju, 2002), PPI-DEN(Premysl Mikula et al., 2018), PDADMAC-PSFA(Lv, Zhang, & Qiao, 2018)) or algicidal (PAMAM dendrimers(Perreault, Bogdan, Morin, Claverie, & Popovic, 2012), core-shell polystyrene-copper oxide nanoparticles(Saison et al., 2010)) cell aggregation (flocculation, illustration in **Fig. 2**). Branched PEIs with MW 12 or 1.1 kDa aggregated selectively cyanobacterial over algal cells.(Premysl Mikula et al., 2018) Specifically, they effectively flocculated *S. elongatus* cells and accelerated their settling down. In contrast, the flocs of *M. aeruginosa* cells did not settle, but they floated in a medium under the surface (**Fig. 2**). However, PEIs did not cause aggregation of algal cells of *D. quadricauda* and *C. reinhardtii*.

In addition to polymers in solution, numerous studies investigated polymers-based coatings for their potential anti-biofouling or fouling-release properties on various surfaces, mostly for marine applications. Amphiphilic polymer platforms designed by sequence-controlled chemistries to combine the benefits of hydrophilic and hydrophobic surfaces are currently conceived as one more promising strategy to implement special interface functions in various industries and technologies. The experiments utilized common, widespread marine micro-and macro-fouling organisms (the most used microorganisms marine algae *Ulva* sp. and their zoospores and *Navicula* sp.) and measured cell attachment, settlement, and viability on the studied surface.(Leonardi & Ober, 2019) For example, amphiphilic surface-active PDMAEMA brushes or SPE/BMA copolymers displayed anti-biofouling properties against algae and zoospore settlement as well as growth-inhibitory activity.(Schardt et al., 2021; Yandi et al., 2017) The recent developments in this field focusing on both advantages and drawbacks of these coatings have been reviewed intensively elsewhere, and we encourage the interested reader to explore these references.(Galli & Martinelli, 2017; Leonardi & Ober, 2019; Qiu et al., 2022)

Studies reporting the interaction of bioinspired and biomimetic polymers with algae and cyanobacteria indicate that they are a simple, cost-effective and versatile polymer platform for designing potent antimicrobials effective in controlling and mitigating the undesired growth of environmentally relevant algae or cyanobacteria. This platform can be also utilized for effective and selective polymer-induced flocculation and removal of cyanobacteria or algae in water management and biotechnological processes. We envision that the applicability of a combination of various anti-algal and anti-cyanobacterial biomimetic polymers may allow their selective interactions with algal or cyanobacterial cells and their selective removal, which has a great potential for treating health problems and environmental and technical issues caused by phototrophic microorganisms. However, practical aspects of the real-world applications of these polymers, especially in open systems such as water reservoirs, need to be deeply explored. First, the anti-algal and anti-cyanobacterial activities of polymers should be confirmed under conditions more closely related to their potential applications and external environmental factors, such as the pH, dose, ionic strength, and temperature must be taken into account. For example, natural or industrial waters contain a broad range of Ca^{2+} or Mg^{2+} concentrations, which interfere with the

antimicrobial activity of cationic polymers in high concentrations due to high ionic strength reducing the electrostatic to microbial cells.(I. Sovadinova et al., 2011) Secondly, polymer safety to the non-target beneficial biota must be assessed, especially in open systems. Finally, polymers and each polymer component should be biodegradable and their bioproducts non-toxic (environmentally friendly polymers). Overall, we are just at the beginning of the study on the potential of bioinspired and biomimetic polymers in the aquatic environment and water management, further research is needed to discover their practical applicability in this field.

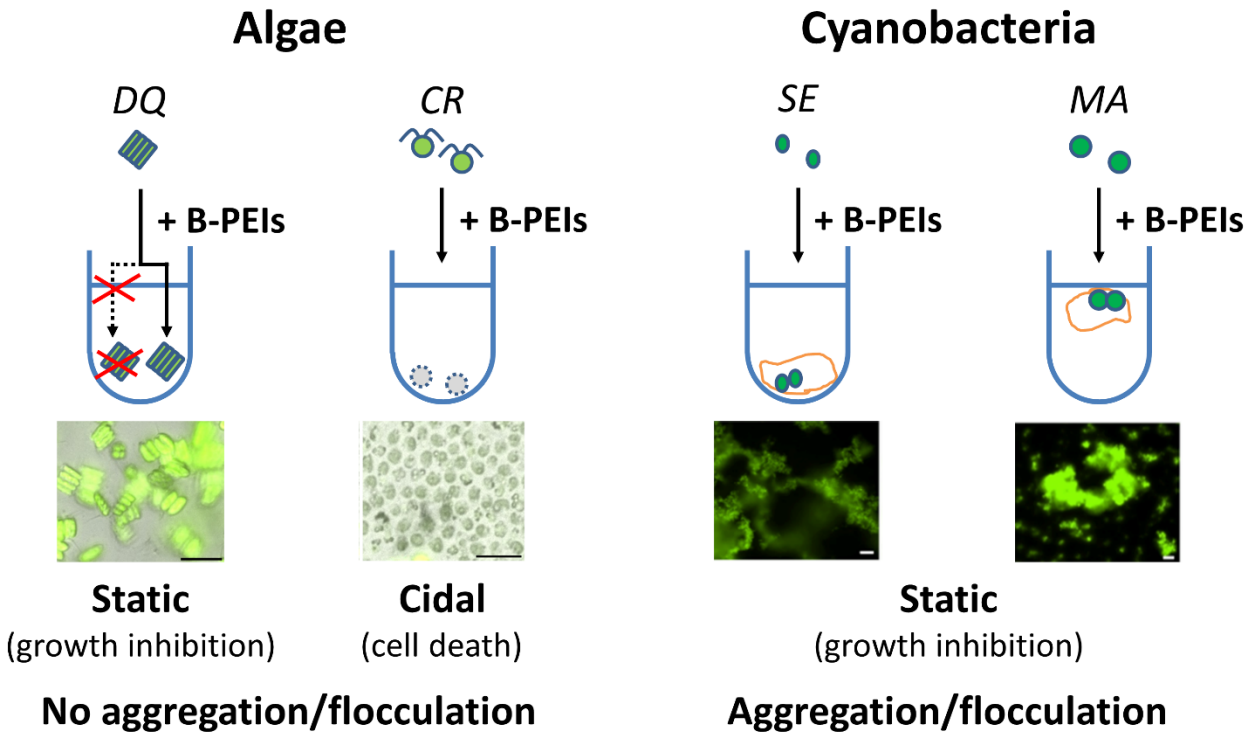


Figure 2. Selective interactions of B-PEIs with algal and cyanobacterial cells. B-PEI, branched poly(ethylene imine), CR, *Chlamydomonas reinhardtii*; DQ, *Desmodesmus quadricauda*; MA, *Microcystis aeruginosa*; SE, *Synechococcus elongatus*. Reprinted from (Premysl Mikula et al., 2018) with permission.

Table 1. Examples of synthetic bioinspired and biomimetic polymers with potential applicability in the aquatic environment and water management due to their interactions with cyanobacteria or algae in solution.

Polymer	Studied target species	Studied non-target aquatic species	Activity & Mechanisms	REFs
Amylose-fatty ammonium salt inclusion complexes	▪ Pathogenic alga *Prototheca wickerhamii*	▪ Not studied	▪ Growth inhibitory activity – algistatic effect	(Hay et al., 2020)
Biomimetic PMAs	▪ Freshwater cyanobacteria *Microcystis aeruginosa* and *Synechococcus elongatus* ▪ Freshwater algae *Desmodesmus quadricauda* and *Chlamydomonas reinhardtii*	▪ Not studied	▪ Growth inhibitory activity – algistatic and cyanostatic effects ▪ Algicidal activity against *C. reinhardtii* and cyanocidal against picocyanobacterium *S. elongatus* and microcystin-producing cyanobacterium *M. aeruginosa* ▪ Structure-depending effects	(Přemysl Mikula et al., 2021)
Core-shell polystyrene-copper oxide nanoparticles	▪ Not studied	▪ Freshwater alga *Chlamydomonas reinhardtii* as a model of ubiquitous algae	▪ Induction of algal cell aggregation, cellular granulosity, ROS production and chlorophyll deterioration and photosynthesis	(Saison et al., 2010)
Indole derivatives and acrylate resins	▪ Marine or brackishwater algae *Phaeodactylum tricornutu*m, *Nitzschia Closterium* and *Skeletonema costatum*	▪ Not studied	▪ Growth inhibitory activity – algistatic effect	(Feng, Ni, Yu, Zhou, & Li, 2019)
N-halamine derivatized PAM NPs	▪ Freshwater cyanobacterium *Microcystis aeruginosa* ▪ Freshwater cyanobacteria *Pseudanabaena sp.* and *Limnothrix* sp. isolated in the field ▪ Natural HCB samples	▪ Freshwater algae *Desmodesmus* sp.*, Monoraphidium* sp. and diatom *Nitzschia* sp. (diatom) isolated in the field	▪ Broad-spectrum cyanocidal activity, including toxic and potential bloom-forming species ▪ Selectivity to cyanobacteria over algae ▪ Toxic to diatom *Nitzschia* sp. ▪ Likely a preferential attachment of NPs to cyanobacterial cells	(Sadhasivam et al., 2019)
PAMAM dendrimers cationic (G2-G5)	▪ Not studied	▪ Freshwater alga *Chlamydomonas reinhardtii* as a representative of ubiquitous algae	▪ Algicidal activity with larger size dendrimers more toxic than the smaller size ones ▪ Internalization in both algal and cyanobacterial cells ▪ G4 dendrimers stimulated photosynthesis in *C. reinhardtii* resulting in elevated ROS	(Petit et al., 2012; Petit et al., 2010)

			production and a modification in the expression of genes involved in antioxidant and photosynthetic processes	
PAMAM ethylenediamine core dendrimers amine- and hydroxyl-terminated (G2-G4)	▪ Freshwater cyanobacterium *Anabaena* sp.	▪ Freshwater alga *Chlamydomonas reinhardtii* as a model of ubiquitous algae	▪ Growth-inhibitory activity – algistatic and cyanostatic effects, increasing with generation number ▪ Cationic dendrimers more toxic than their hydroxyl-terminated counterparts ▪ Elevated ROS production in both algal and cyanobacterial cells	(Gonzalo et al., 2015)
PAMAM nanoglycodendrimers coated with mannose G0	▪ Not studied	▪ Freshwater alga *Chlamydomonas reinhardtii* as a model of ubiquitous algae	▪ Aggregation of algal cells caused by interactions with the cell wall, inducing inhibition of cellular division and some decrease in photosynthetic activity	(Perreault et al., 2012)
PDADMAC-PSFA	▪ Freshwater cyanobacterium *Microcystis aeruginosa*	▪ Not studied	▪ Flocculation and removal of cyanobacterial cells without harming them	(Lv et al., 2018)
PEIs branched MW 0.5-12 kDa	▪ Freshwater cyanobacteria *Microcystis aeruginosa* and *Synechococcus elongatus* ▪ Freshwater algae *Desmodesmus quadricauda* and *Chlamydomonas reinhardtii*	▪ Not studied	▪ Growth inhibitory activity – algistatic and cyanostatic effects ▪ Selective cytocidal activity to *C. reinhardtii* over other phototrophic microorganisms ▪ Selective flocculation of cyanobacterial cells over algal cells ▪ Structure-dependent effects	(Premysl Mikula et al., 2018)
PEIs MW 2-750 kDa	▪ Freshwater cyanobacteria *Anabaena flos-aquae*	▪ Not studied	▪ Induction of flocculation and flotation (or sedimentation) of buoyant cyanobacterial cells ▪ PEIs with high MW more effective in flocculation ▪ No significant morphological changes in the PEI-exposed cells	(Arrington et al., 2003; Zeleznik et al., 2002)
PPI tetramine dendrimer G1 with a diaminobutane core	▪ Freshwater cyanobacteria *Microcystis aeruginosa* and *Synechococcus elongatus* ▪ Freshwater algae *Desmodesmus quadricauda* and *Chlamydomonas reinhardtii*	▪ Not studied	▪ Growth inhibitory activity – algistatic and cyanostatic effects ▪ Cytocidal activity to algae *C. reinhardtii*, *D. quadricauda* and cyanobacterium *M. aeruginosa*	(Premysl Mikula et al., 2018)

TiO2-embedded expanded polystyrene balls	▪ Freshwater cyanobacterium *Microcystis aeruginosa*	▪ Not studied	▪ Growth-inhibitory activity –cyanostatic effect	(Joo et al., 2020)

G, generation; HCB, harmful cyanobacterial bloom; MW, molecular weight; NPs, nanoparticles; PAM, polyacrylamide; PAMAM, poly(amidoamine); PDADMAC, polydiallyldimethylammonium chloride; PEI, Poly(ethylene imine); PMA, poly(methyl acrylate); PPI, polypropylenimine; PSFA, polysilicate ferrite aluminum; ROS, reactive oxygen species.

5. New methods

Mechanistic studies on the membrane-disrupting mechanisms of AMP-mimetic polymers have been advanced by biophysical and computational approaches. In particular, model lipid vesicles or liposomes can provide a new insight into the polymer-membrane interactions. Liposomes have been extensively used for the study of AMPs and the mechanism of action, these peptides exert. Specifically, the bilayer binding, penetration, and disruption caused by AMPs have been investigated using a variety of spectroscopic and computational approaches using model membranes.(A. Chakraborty et al., 2021; Ladokhin, Selsted, & White, 1997; Schifano & Caputo, 2021; Sepehri, PeBenito, Pino-Angeles, & Lazaridis, 2020; Y. Tamba, Ariyama, Levadny, & Yamazaki, 2010; Tang, Signarvic, DeGrado, & Gai, 2007) These same approaches are being extended to AMP-mimetic polymers to gain a greater understanding of the mechanism of action.

5-1. Cell-sized giant vesicles

In recent years, several researchers extensively conducted a microscopic observation of the AMP-induced perturbation of a lipid bilayer using cell-sized giant unilamellar vesicles (GUVs). GUVs with a diameter of several tens of micrometers allow *in situ* observation of membrane dynamics such as membrane permeability, morphological changes, and domain formation in a single vesicle level by optical microscopy.(Dimova & Marques, 2020) Yamazaki *et al.* have reported the kinetic model of the AMP-induced pore formation in a lipid bilayer based on the single GUV technique.(Hasan, Karal, Levadnyy, & Yamazaki, 2018; Karal, Alam, Takahashi, Levadny, & Yamazaki, 2015; Y Tamba & Yamazaki, 2005, 2009) They have found that Magainin-2 permeabilizes the lipid bilayer by the transition from the binding state to the pore-forming state(Y Tamba & Yamazaki, 2005), and the pores are activated by the lateral tension of the lipid bilayer (Karal et al., 2015). Huang *et al.* have also utilized GUVs to quantitatively evaluate the expansion of membrane area as well as the change in vesicle volume due to peptide binding in combination with the micropipette aspiration technique.(M. Lee, Sun, Hung, & Huang, 2013; M. T. Lee, Hung, Chen, & Huang, 2008) This chapter introduces the mechanistic study of the interaction of AMP-mimetic polymethacrylate derivatives with lipid bilayers revealed by the microscopic observation of GUVs.

The permeabilization of lipid bilayers induced by polymethacrylate random copolymers with hydrophilic (cationic) and hydrophobic side chains was evaluated using GUVs.(Tsukamoto et al., 2021) GUVs employed for the microscopic observation were prepared by the gentle hydration method.(Reeves & Dowben, 1969) An 8:2 mixture of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine (POPE) and 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phospho-(1'-*rac*-glycerol), sodium salt (POPG) was employed to mimic the *E. coli* membrane whereas an erythrocyte model membrane was prepared with 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) alone. To monitor the leakage from GUVs, sucrose was entrapped in the inner aqueous lumen at a high concentration (200 mM), and the GUVs were then diluted with an aqueous solution of glucose with a matched osmolarity. Due to the difference in the refractive indexes between sucrose and glucose solutions, the inside of the GUVs shows darker contrast compared to the outside in the phase-contrast microscopic image. Thus, the polymer-induced release of the sucrose can be monitored as a decrease in the darkness (gray value) inside the GUVs of interest. Here, we have synthesized the random copolymers of aminoethyl methacrylate as a hydrophilic unit and methyl (MMA) or *n*-butyl methacrylate (BMA) as a hydrophobic unit for the GUV study (**Fig. 3A**). Antimicrobial activity against *E. coli* and hemolytic toxicity assays revealed that PM_{34}, which contains 34% MMA, exhibited selective antimicrobial activity over hemolytic toxicity, whereas PB_{36}, containing 36% BMA, displayed both high antimicrobial activity and hemolytic toxicity.

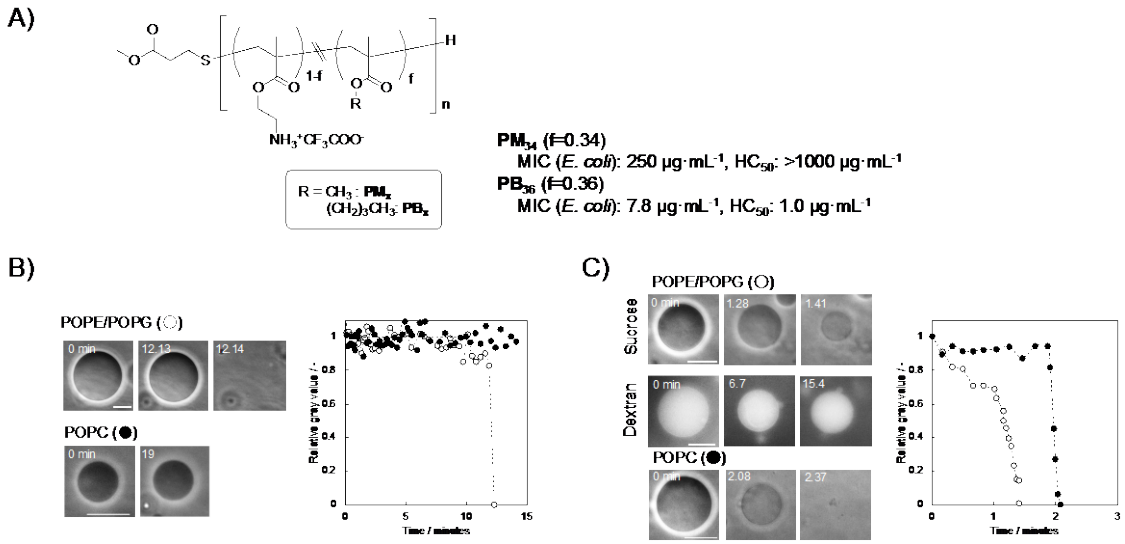


Figure 3. Permeation of GUV membranes induced by the polymethacrylate random copolymers. Chemical structures of the polymers (A). Permeation of the GUVs induced by PM_{34} (B) and PB_{36} (C) polymers. Figures are reprinted with permission from (Tsukamoto et al., 2021). Copyright 2021 American Chemical Society.

The release of the sucrose as a result of membrane disruption by the polymer was well-correlated with the corresponding biological activities. Specifically, the PM_{34} polymer that shows antimicrobial activity with diminished toxicity, induced the selective disruption of the *E. coli* model POPE / POPG GUVs and the release of the entrapped sucrose whereas the POPC GUVs as erythrocyte model remained intact (**Fig. 3B**). In contrast, biocidal PB_{36} polymer with high antimicrobial activity and hemolytic toxicity induced the leakage of the sucrose from both GUVs (**Fig. 3C**). This result indicates that the polymethacrylate derivatives disrupt membranes by the selective recognition of the specific lipid properties and such selectivity is affected by the chemical structure of the hydrophobic groups in the polymer. Interestingly, we confirmed that both PM_{34} and PB_{36} polymers induced leakage of entrapped sucrose from POPE / POPG GUVs, however, the mechanism of the membrane disruption was different: the PM_{34} polymer caused the sudden rupture of GUVs after a certain induction period, while the PB_{36} polymer induced gradual leakage immediately after addition of the polymer but the spherical morphology of the GUV was retained. These membrane disruption mechanisms by the polymers are somewhat similar to what is observed with AMPs. Specifically, membrane disruption caused by the PM_{34} and PB_{36} polymers corresponds to the carpet model and the pore formation model proposed in the AMPs study, respectively.(Shai, 2002) The pores formed by the PB_{36} polymer appear to have a defined size because the large molecular weight marker (RITC-dextran, 70 kDa) did not leak from GUVs under the same conditions where sucrose exhibited complete leakage. Furthermore, the kinetic analysis of the population of disrupted GUVs suggests the autocatalytic reaction of the polymer-induced membrane disruption in which the rate of GUV burst was increased with time. This unique kinetic behavior is likely to originate in the cooperative mechanism of the action in which a polymer chain that is bound to the membrane recruits a free (unbound) polymer chain from the solution. These nucleation events require time for the polymers to interact before permeabilization can take place. Taken together, it was demonstrated that antimicrobial polymethacrylate derivatives enhance the

permeability of a lipid bilayer in mechanisms similar to that of AMPs, and the chemical structure of the hydrophobic side chains differentiates the permeation mechanism of lipid bilayers.

More recently, we have investigated the formation of the phase-separated domains on GUVs as another mode of perturbation of lipid bilayer induced by the polymethacrylate derivatives.(Yasuhara, Tsukamoto, Kikuchi, & Kuroda, 2022) Lipid domains in which specific lipids and membrane proteins are segregated in the cell membrane are known to be responsible for the several pathways of signal transduction.(Simons & Toomre, 2000) Lipid domains have recently also been linked to the structure and composition of lipid bilayers such as lipid asymmetry across the bilayer.(Kakuda, Suresh, Li, & London, 2022) Heterogeneity in the lipid bilayer originated in the lateral phase separation or domain formation is also implicated in the action mechanism of the AMPs. Based on the model membrane study, Epand *et al.* have found that several AMPs with multiple cationic groups induce membrane destabilization by clustering negatively charged lipids in the bacterial membrane.(Epand & Epand, 2011) To examine the polymer-induced formation of lipid domains, AMP-mimetic polymethacrylate random copolymers without and with rhodamine moiety (PE_{44} and RPE_{42}, respectively) were synthesized by the atom transfer radical polymerization (ATRP) of cationic aminobutyl methacrylate and hydrophobic ethyl methacrylate (**Fig. 4A**). For the visualization of the domains on the GUV, POPE / POPG (8:2) GUVs were stained with Texas Red® 1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine, triethylammonium salt (TR-DHPE). In the fluorescence image of the GUVs, uniform fluorescence was observed on the GUV surface before the addition of the polymer, confirming the excellent miscibility of both lipids (**Fig. 4B**). The addition of the PE_{44} to the GUVs resulted in the coexistence of the dark and bright regions in the same GUV membrane, indicating the formation of lipid domains. The thermal analysis by the differential scanning calorimetry (DSC) has also confirmed that the addition of the PE_{44} polymer induced the formation of the POPE-rich phase in the POPE / POPG (8:2) multilamellar vesicles. Taken together, it was suggested that similar to some AMPs, PE_{44} polymer forms phase-separated lipid domains by the clustering of POPG, an anionic phospholipid in the membrane.

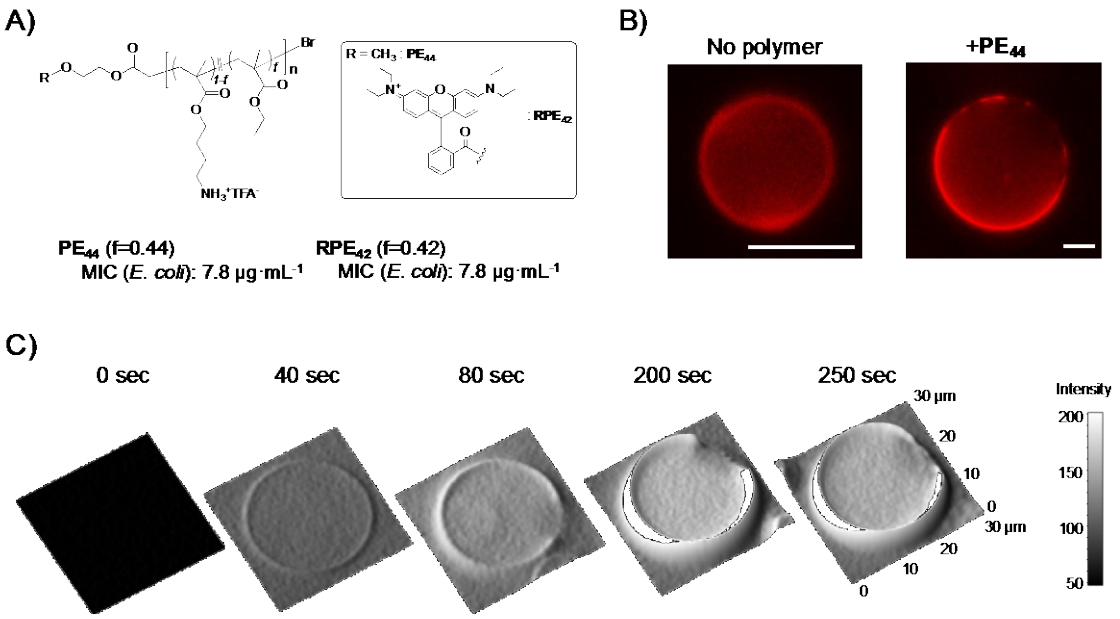


Figure 4. Formation of lipid domains in the POPE / POPG (8:2) GUV membrane induced by the polymethacrylate random copolymers. Chemical structures of the polymers (A). Clustering of lipids in the GUV membrane induced by PE_{44} as revealed by the heterogeneous distribution of TR-DHPE fluorescence (B). Time-lapse profile of the binding and localization of RPE_{42} on the GUV (C). Figures are modified from (Yasuhara et al., 2022) with permission.

We also evaluated the binding of the polymer to the membrane and its localization using a Rhodamine-labeled polymer (RPE_{42}, **Fig. 4C**). Upon the addition of RPE_{42}, the fluorescence intensity gradually increased on the surface of the POPE/POPG GUVs, indicating the binding of the polymer to the lipid bilayer. The fluorescence was uniformly distributed on the entire surface of the GUV immediately after the addition of the polymer, indicating a near homogenous distribution of the polymer around the vesicle. However, we observed the development of dark and bright domains in the membrane after a certain period, indicating the heterogeneous localization or clustering of the polymer on the lipid bilayer. Since the time-course of the polymer binding estimated by the fluorescence intensity displayed a sigmoidal time dependency, the binding of the polymers to the lipid bilayer proceeds in a cooperative manner. Significant enhancement of the polymer binding was observed simultaneously at the time when the heterogeneity in the GUV membrane was produced. This observation suggests that the formation of lipid domains creates vacant binding sites that can accept additional free polymers from an aqueous phase. A series of GUV studies introduced here suggested that polymethacrylate random

copolymer mimics not only the biological activities of the AMPs but also their action mechanism on the lipid bilayer.

5-2. Computational simulations

Large-scale computer simulations, both at atomic and coarse grain resolution, can play a significant role in probing the structural and functional aspects of antimicrobial agents (AM agents) and their interactions with model bacterial membranes.(G. Bocchinfuso, Bobone, Mazzuca, Palleschi, & Stella, 2011; Gianfranco Bocchinfuso et al., 2009; Horn, Cravens, & Grossfield, 2013; Khandelia & Kaznessis, 2007; Leontiadou, Mark, & Marrink, 2006; Jianguo Li et al., 2012; J. Li et al., 2013; D. Liu et al., 2004; Matyus, Kandt, & Tieleman, 2007; Mihajlovic & Lazaridis, 2012; Orioni et al., 2009; Polyansky et al., 2010; Romo, Bradney, Greathouse, & Grossfield, 2011; Rzepiela, Sengupta, Goga, & Marrink, 2010; Santo, Irudayam, & Berkowitz, 2013; Sengupta, Leontiadou, Mark, & Marrink, 2008; Soliman, Bhattacharjee, & Kaur, 2009; Stavrakoudis, Tsoulos, Shenkarev, & Ovchinnikova, 2009; Tew et al., 2002; Thøgersen, Schiøtt, Vosegaard, Nielsen, & Tajkhorshid, 2008; von Deuster & Knecht, 2011; Y. Wang, Schlamadinger, Kim, & McCammon, 2012; X. Zhao, Yu, Yang, Li, & Huang, 2015) Some of the aspects that simulations can give insight into include: the structural landscape of the AM agents in solution and at the water-lipid interface, the role of various functional moieties in specific interactions with membranes, composition-dependent membrane reorganization as a response function to AM agent interaction. Herein, we particularly focus on our atomistic simulations of AM agent-model membrane systems using methacrylate-based polymers as a platform.

Recognizing hydrophobic and cationic functional groups as the fundamental components in biomimetic polymer design, the earlier set of simulations focused on these two groups as the only ingredients of AMP-mimetic polymer simulations. The presence of these two types of groups is expected to induce facial amphiphilicity, along the randomly arranged sequence of copolymer backbone, which is implicated in the disruption of the bacterial membrane. Very early sets of simulations of such copolymers in the solution phase revealed that the overall 3D conformations of these biomimetic polymers are very sensitive to varying polymer lengths and sequences.(Ivanov et al., 2006) The conformations that are adopted by the polymers in solution are important precursors to understanding aggregation in solution, detection, interactions with model bacterial

membranes, and subsequent partitioning. The solution simulations also strongly suggested that a pre-existing, rigid structure is not an essential design element as the polymers adopted well-defined stable structures in solution phase, as seen in **Figure 5** (Ivanov et al., 2006) dictated by the sequence. These early sets of simulations also indicate that the composition, control of overall hydrophobicity, and charge are important ingredients in fine-tuning the selectivity and subsequent activity of such designed biomimetic polymers.

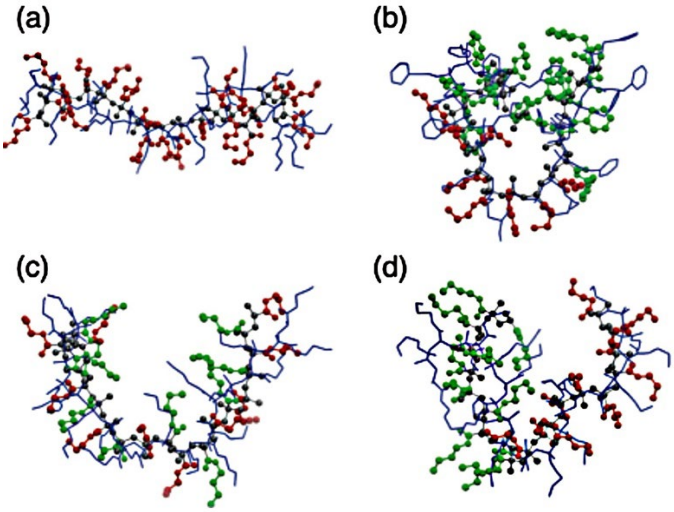


Figure 5. Sequence dependent conformations of a polymer of length 20 in solution phase. Hydrophobic and charged groups are coloured in green and red respectively. Adapted with permission from (Ivanov et al., 2006). Copyright 2006 American Chemical Society.

The next set of simulations (E. F. Palermo et al., 2012) was focused on understanding the role of cationic side-chain spacer arms for effective antimicrobial activity as monitored from conformational analysis of the several polymers near the water-membrane interface. These simulation studies showed that the copolymers adopt facially amphiphilic conformations with segregation of cationic and ethyl groups along the polymer backbone when bound to a bacterial-mimetic model lipid bilayer as seen in **Figure 6**. The cationic space arms have also been shown to be required to be of a certain length, consistent with experiments, for both facial amphiphilicity and the depth of polymer insertion into the lipid bilayer. This underscores the optimal composition of the polymers in terms of effective overall hydrophobic content and the functionally relevant

structures. These results were the first to demonstrate the acquisition of functional structures by random copolymers depending on the environment.

The simulations of interactions of a single binary polymer (with hydrophobic and cationic functional groups) with bacterial model lipid bilayers are not sufficient to probe into the cooperative mechanism by which multiple polymers interact with the lipid bilayers. Large scare atomistic simulations of multiple binary polymers both in solution and with model bacterial membranes were set up to address these issues.(Baul, Kuroda, & Vemparala, 2014) The solution simulations of multiple binary polymers reveal that weak aggregates are formed in solution and no particular evidence of complete phase separation is observed over the simulation timescale. The role of weak polymer aggregates is crucial in the partitioning of polymers into the lipid bilayers environment. The simulations of an aggregate of binary copolymers with the lipid bilayers reveal that the aggregate bind to the lipid bilayers surface first. As the polymer-lipid bilayers interaction is more favorable than that polymer-polymer interactions in the aggregate, a single polymer chain is seen to leave the polymer aggregate and partition into the bacterial lipid bilayers, acquiring facial amphiphilicity after complete partitioning. This timely release of the polymers from the aggregate continues throughout the simulation timescale, as seen in **Figure 7**. The presence of multiple aggregated polymers near the surface has a profound effect on the membrane properties, which were not observed when only a single polymer was present, underscoring the cooperative effects of multiple polymers. Clustering of anionic POPG lipid molecules was induced due to the presence of multiple polymers, resulting in lateral heterogeneity of the membrane thickness strongly suggesting thickening of the membrane. The partitioned polymers were seen in the vicinity of the thick-thin phase boundaries giving strong indications of the detection of bilayer defects by the membrane-active agents like the antimicrobial polymers studied here.

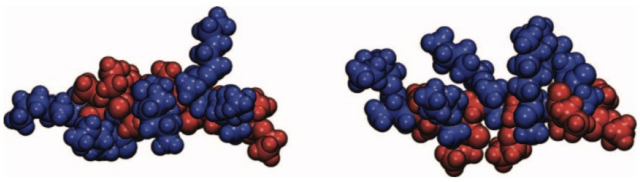


Figure 6. Acquired Facial amphiphilic structures in solution and water-membrane interface. Adapted from (Baul et al., 2014) with permission.

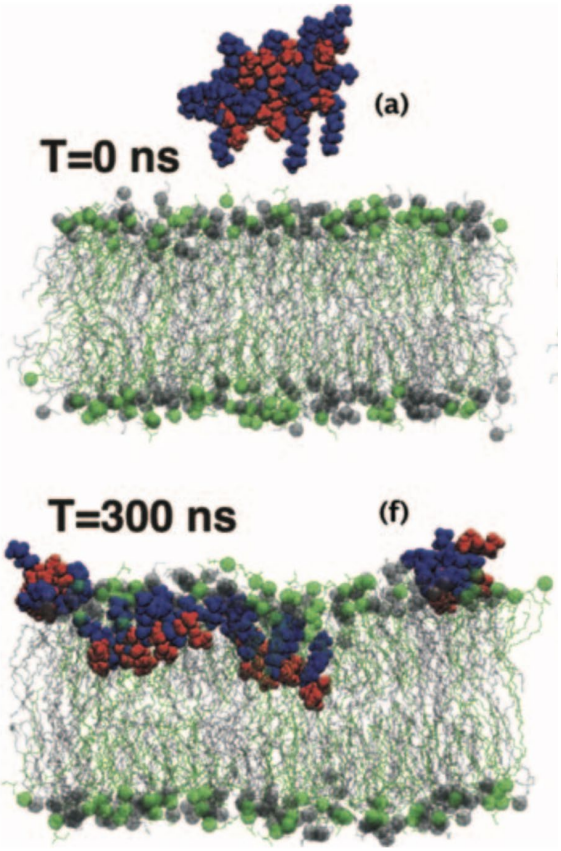


Figure 7. Partitioning of methacrylate-based polymers into the bilayer. Water and ions are not shown for clarity. The cationic and hydrophobic groups of E4 polymers are shown in blue and red, respectively and the acquisition of facial amphiphilicity after partitioning can be clearly seen. Adapted from (Baul et al., 2014) with permission.

An exploration of naturally occurring antimicrobial peptides suggests that the antimicrobial agents have functional groups such as polar and negatively charged amino acids in addition to the functionally relevant binary composition of hydrophobic and positively charged groups. Thus, the next set of investigations focused on this biologically inspired composition of ternary polymers with hydrophobic, polar and positively charged groups. Specifically, the interest is in understanding (1) the role of polar groups in the conformational landscape of ternary polymers (2) the smearing or delocalization of overall hydrophobicity of the polymers via the introduction of

polar groups, which may play a crucial role in promoting weak aggregates in solution which aids partitioning into membranes. Detailed simulations(Garima Rani, Kuroda, & Vemparala, 2020) of aggregation dynamics of ternary polymers in solution were conducted and compared to the binary polymer aggregation to delineate the role of inclusion of polar functional groups in overall polymer-polymer interactions. The simulations clearly demonstrate that introduction of polar groups leads to increased conformational fluctuations and the formation of more loosely-packed, open aggregates in contrast to the robust aggregates formed in the case of binary polymers, as seen in **Figure 8**. The role of replacing some of the hydrophobic groups with overall charge neutral polar groups is (1) increased solubility of the polymers due to reduced ability of hydrophobic groups to interact and cluster, and (2) maintaining polymer-polymer attractive interactions via weak attractive electrostatic interactions with the charged groups and contributing to aggregate formation. This study demonstrates the functionally tunable role that polar groups play in polymer-polymer interaction in the solution phase.

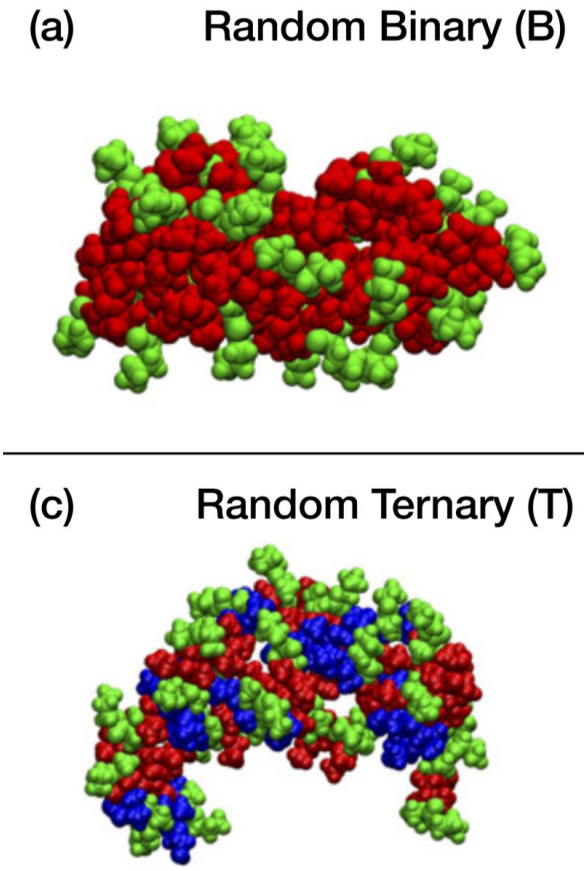


Figure 8. Aggregation morphology of random copolymers of binary and ternary functional group composition in solution phase. Adapted from (Garima Rani et al., 2020) with permission.

Subsequently, the interaction of ternary methacrylate polymers, composed of charged cationic, hydrophobic, and neutral polar groups, with model bacterial membranes was studied using extensive atomistic simulation.(G. Rani, Kuroda, & Vemparala, 2021) Conformational analysis of ternary polymers partitioned into bacterial membranes reveals the fundamental way in which they differ from binary polymers: the absence of facially amphiphilic structures, as seen in **Figure 9**. The ternary polymers exhibit a strong preference for acquiring globular and folded conformations in the bacterial lipid bilayer, along the lipid bilayer normal, and partition deeper into the nonpolar bilayer core. This clearly suggests that the introduction of polar groups frustrates the propensity of the binary polymers to adopt facially segregated structures and shows a novel membrane partitioning mechanism without amphiphilic conformations. The conformation of partitioned ternary polymer also suggests that the inclusion of more functional groups allows us to mimic a class of globular antimicrobial peptides like the defensins.(Kouno et al., 2008; D. Takahashi, Shukla, Prakash, & Zhang, 2010) This paves the way to systematically study the inclusion of more functional groups into the biomimetic design of polymers.

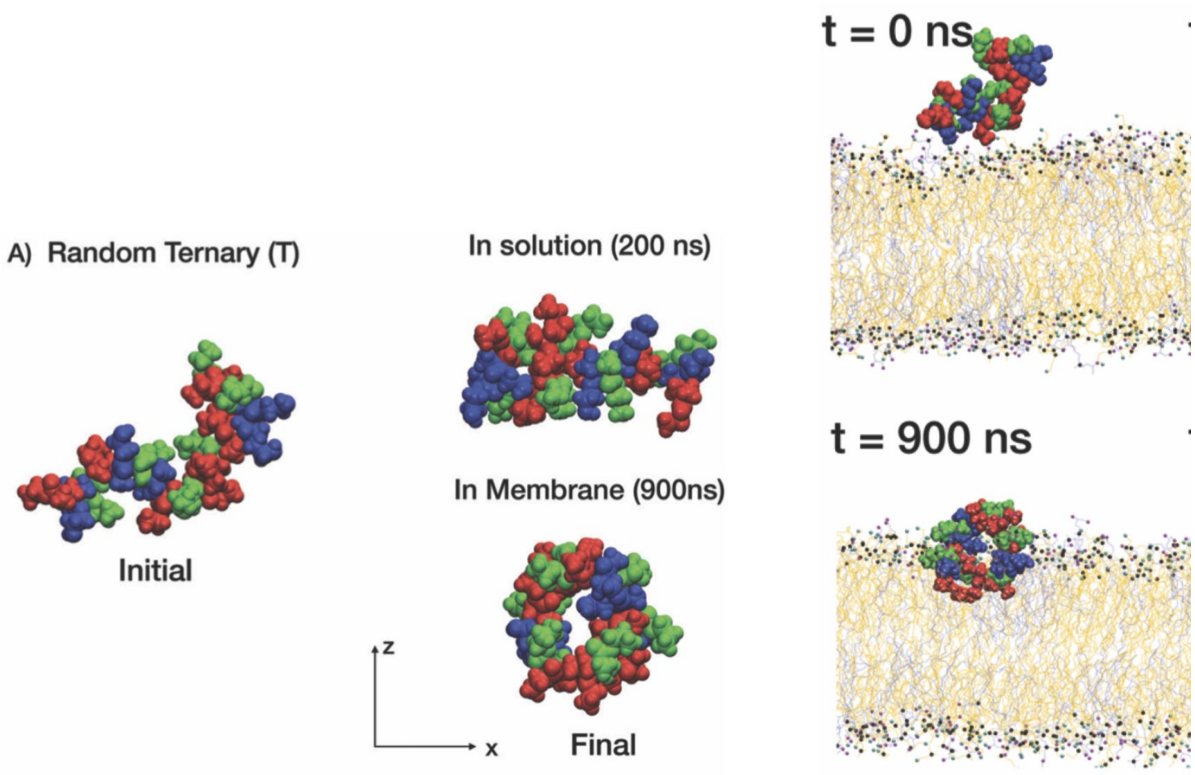


Figure 9. The evolution of ternary polymer structure in solution and in lipid bilayer phase. Snapshots of the structures at the beginning and at the end of 900 ns is also shown. Adapted from (Garima Rani et al., 2020) with permission.

6. Challenges and future perspective.

What challenges remain to be addressed? As antimicrobial polymers have been developed to address the emerging issue of antibiotic-resistant bacterial infections. Therefore, clinical implementation would be the ultimate goal of antimicrobial polymers. To achieve this goal, the polymers should be potent and selective to bacteria over humans. Ideally, high antimicrobial activity and no toxicity to humans are desired. Here we discuss the challenges to be addressed in our future studies and potential approaches.

6-1. Monomer sequences in polymers.

Many studies have reported the optimization strategy of cationic/hydrophobic balance. However, synthetic polymers are distinctively different from antimicrobial peptides in terms of sequences and conformation; polymers are heterogeneous. Many studies have used random or statistical

polymers with binary cationic and hydrophobic monomer compositions. For the clarification of terminology, "random" indicates that the reactivities of two different monomers are the same, and thus the average composition of the monomers is always the same as the feed composition during polymerization. On the other hand, "statistical" refers to polymerization of monomers with different reactivities, and the monomer composition in polymer chain shifts during polymerization, giving gradient or block-like sequences.

Here we use the following example to explain the heterogeneity in monomer sequences. The composition of cationic and hydrophobic monomers is often determined by analysis of ^{1}HNMR spectra. Because the monomer composition is the average value from all polymer chains contained in the sample, individual polymer chains have different monomer compositions. Some polymer chains have more cationic monomers, and others have more hydrophobic monomers than the average. In addition, the monomer distribution of these polymer chains may be random or gradient due to compositional drift. Polymers also contain polymer chains with different length or molecular weight, while recent advanced techniques of living/controlled polymerization provide very narrow molecular weight distribution of polymers. Therefore, a polymer sample is a very heterogeneous mixture of many polymer chains with different monomer compositions and sequences, molecular weights, and different populations (the number of polymer chains). It would be reasonable to assume that these polymer chains have different activity profiles. Consequently, the antimicrobial activity and toxicity of polymer products are collective results from different activities of the polymer chains in the product.

The challenge is that we cannot identify the active polymer species or relative contributions of polymer chains to the collective biological activities. Recently, antimicrobial activity of block copolymers with defined block sequences were studied.(Judzewitsch et al., 2018; Kuroki et al., 2017) These studies will provide a new approach to create more peptide-like defined polymers as well as provide a new insight into the "true" structure-activity relationship of antimicrobial polymers. However, the heterogeneous structure of polymers may be advantageous to prevent the resistance selection in bacteria because the polymer chains with different structures may have different cellular targets. This will be discussed later.

6-2. Cationic properties and binary monomer compositions.

Cationic functionalities are one of the critical components found in antimicrobial polymers and the template antimicrobial peptides. Wong and coworkers demonstrated that antimicrobial polymers typically have more cationic groups as compared to natural AMPs.(K. Hu et al., 2013) Indeed, the number of net cationic charges of magainin II (23 amino acids) and LL-37 (37 amino acids) are +4 and +6, respectively.(Zasloff, 2002) However, for example, antimicrobial methacrylate copolymers have > 10 cationic groups on average.(Edmund F. Palermo, Satyavani Vemparala, & Kenichi Kuroda, 2012) Our lab previously demonstrated that the 3-4 cationic groups of methacrylate copolymers are sufficient to exert effective in antimicrobial activity,(Mortazavian, Foster, Bhat, Patel, & Kuroda, 2018) which is consistent with the patterns in AMPs. In addition, from the optimization principle of cationic/ hydrophobic balance, one may think that polymers with more cationic groups are less cytotoxic to human cells because more cationic polymers bind more effectively to bacteria due to the enhanced electrostatic attractions. However, the human cell surfaces are also slightly negatively charged, and therefore as the polymer chains are more cationic the polymers bind to human cells more effectively, which may lead to increased toxicity to human cells. The confusion around the role of cationic functionalities stems from the mainstream approach in the field which use polymers composed of binary compositions of cationic and hydrophobic monomers. In many of these studies, the monomer ratio has been traditionally used as one of parameters to characterize the polymer rather than the number of monomers in a polymer chain. It might be more appropriate that the cationic charges and hydrophobicity of polymers should be decided as the numbers of these groups required for antimicrobial functions, but not relative ratios in binary compositions. Put simply, the ratio of monomers in a polymer chain may not be the sole driver of activity, but also the objective number of these groups in the chains. Therefore, binary copolymers inherently have this limitation in terms of the peptide-mimetic design, and a new approach may require more monomer components to be incorporated to more accurately mimic natural AMPs.

Gellman and Wong synthesized ternary nylon copolymers with cationic, hydrophobic, and hydroxyl groups and demonstrated that the hemolytic activity was reduced as compared to binary polymer counterparts while the antimicrobial activity was retained.(S. Chakraborty et al., 2014) Our lab also synthesized ternary methacrylate copolymers with cationic, hydrophobic, and

hydroxyl groups.(Mortazavian et al., 2018) The hydroxyl group was selected because it is neither ionic or hydrophobic so that it will not contribute to the electrostatic and hydrophobic interactions of the polymer chains with the bacterial membrane. Similar to the results of nylon copolymers, the methacrylate ternary copolymers showed reduced hemolytic activity than those with the same numbers of cationic and hydrophobic groups. This result may suggest that the 3rd component is needed to give a "space" between the functional groups in the monomer sequences for optimal activities. These studies may also indicate that there are the definite numbers of cationic and hydrophobic groups and the optimal size of polymers for potent activities and low toxicity. Therefore, it may be important to design antimicrobial polymers as a whole polymer chain, like peptides. We also argue that the conformation of the polymer chains is also important to consider because the polymer chains form membrane-active amphiphilic structures on the bacterial membranes.(Haruko Takahashi et al., 2017) Importantly, the incorporation of these hydroxyl containing groups is consistent with the AMP-mimetic design, with over 77% of AMPs in the APD3 antimicrobial peptide database containing at least one Ser residue.(G. Wang, Li, & Wang, 2016)

In addition, antimicrobial polymers have been very simple as compared to AMPs because they contain only cationic and hydrophobic groups, which are the essential and minimal components for the antimicrobial action. However, the amino acids in AMP sequences have more functions (Deplazes, Chin, King, & Mancera, 2020; Koehbach & Craik, 2019; Lehrer & Lu, 2012; Pazgier, Hoover, Yang, Lu, & Lubkowski, 2006; Persson, Killian, & Lindblom, 1998; H. Sun, Greathouse, Andersen, & Koeppe, 2008; Yau, Wimley, Gawrisch, & White, 1998); the conventional binary monomer design may have been missing out on the benefits of evolutionary functionalized functionalities of AMPs. Some studies have investigated this by incorporating amino acid functional groups. For example, Locock reported that tryptophan-rich polymers, 45 showed antimicrobial activity against *S. aureus*.(Locock et al., 2014) Our lab also incorporated carboxylic acids to cationic amphiphilic methacrylate copolymers to investigate hydrogen bonding.(Bhat, Foster, Rani, Vemparala, & Kuroda, 2021) The AMP-mimetic design can be extended to peptide-like chemical functionalities to improve the antimicrobial activity and selectivity of antimicrobial polymers.

6-3. Polymer stability, degradation, and biodistribution

One of the drawbacks of AMPs is proteolytic degradation, resulting in a short lifetime under the physiological conditions, which is a significant disadvantage for the therapeutic use of AMPs. This is one of motivations to use synthetic polymers which are not prone to quick degradation. However, stable antimicrobial polymers stay in the infection sites and continue to kill commensal bacteria. The polymers may also interfere the tissue healing process. These polymers may also accumulate in some organs, which may lead to systemic side effects. One potential approach is biodegradable polymers. Yang and Hedrick synthesized biodegradable antimicrobial polycarbonates(Cheng et al., 2015; Willy Chin et al., 2013; Nederberg et al., 2011; Ong et al., 2016; C. Yang et al., 2019) and we also developed antimicrobial self-degrading polyesters.(Mizutani et al., 2012) Recently, Palermo and coworkers developed antimicrobial self-immolative polymers which can be de-polymerized to monomers by external stimuli.(Cansu Ergene & Palermo, 2017) While these are promising beginnings, the appropriate balance of stability vs. degradation will likely be a parameter that will vary based on the application, infectious agent, and location of the infection in the host.

The efficacy of polymers is also largely affected by the environmental factors. For example, the blood and serum contain many proteins, DNA, lipids, polysaccharides, and cells, which bind to the polymers by electrostatic and hydrophobic interactions. This non-specific binding of serum components compromises polymer's activities and reduce the active polymer chains, requiring higher polymer concentrations for treatment. The MIC values of antimicrobial polymers (and AMPs) are in the order of micromolar, which is orders of magnitudes higher than antibiotics (MIC ~ nanomolar). Therefore, the polymer concentrations required at the infection sites are inherently high and are further increased due to non-specific binding of serum proteins. In the body, AMPs are locally secreted and degrade quickly. This may imply that local secretion can provide high concentrations of AMPs to kill bacteria, but AMPs degrade before causing any side effects; they are not meant to be circulate and patrol the body for invasion of pathogens. To increase local polymer concentrations and avoid systemic toxicity, it would be reasonable and practical if antimicrobial polymers are used for topical applications. If anyone wants to use the polymers for the inside of the body, it will be significant challenge to meet these conditions for the polymers to be effective. To mimic the peptide mechanism, one potential approach may be use of drug delivery

system, which can target the infection sites and increase the local concentration of the polymers. One potential approach is to target the acidic environment of infection sites in which biofilms are acidic.(X. Chen, Daliri, Tyagi, & Oh, 2021; Hitchner et al., 2019; Kyzioł, Khan, Sebastian, & Kyzioł, 2020; Moriarty, Elborn, & Tunney, 2007; Steven L Percival, McCarty, Hunt, & Woods, 2014; Riga, Vöhringer, Widyaya, & Lienkamp, 2017; Xiong et al., 2017)

6-4. Mode of action and cellular targets

The major focus in the field has been the membrane-disrupting mechanism of polymers. However, some polymers kill bacteria in different ways. For example, Locock and coworkers have demonstrated that cationic methacrylate polymers penetrate the cell membrane of *S. mutans* and bind to DNA in the cytosol.(Michl et al., 2020) Some AMPs were reported to target the intercellular components in bacteria including DNA.(D'Souza, Necelis, Kulesha, Caputo, & Makhlynets, 2021; Le, Fang, & Sekaran, 2017; Necelis, Santiago-Ortiz, & Caputo, 2021) While these mechanisms are reported for specific AMPs, it is likely that any AMPs can simultaneously bind to different sites in bacteria including the cell wall, membrane, and intracellular proteins and DNA/RNA, and this promiscuous targeting may individually or collectively contribute to killing of bacteria.(Mahlapuu et al., 2020) These multiple target sites for AMPs may also contribute to the low likelihood of resistance development in bacteria against AMPs(Mahlapuu et al., 2020) because bacteria need to respond to multiple antimicrobial mechanisms, overloading the bacterial resistance capacity. Because traditional small-molecule antibiotics act by specifically fitting into the active sites of proteins and DNA structures, slight changes in the shapes of these active sites lead to large reduction in antibiotic affinity. In contrast to antibiotics, the binding affinity of AMPs to theses target sites is low (~μM), but the targets are broad and non-specific. The biological implication of this may be a multi-target strategy to act against bacterial resistance, but instead, AMPs may sacrifice high affinity to be able to bind to a broader set target sites in the target-rich environment of the cell. In the same context, AMP-mimetic polymers are inherently not susceptible to resistance development in bacteria because the polymer chains may also bind to many components in bacteria. In addition, the inherent heterogeneity of polymer samples may enhance this strategy because the polymer chains with different compositions may bind to different binding sites with higher affinities. However, non-specific binding of polymers does not favor the selectivity to bacteria over human cells. This also represents the paradox in our polymer design

towards potent activity and selectivity. If we want the polymers selective to bacteria over human cells and show potent activity, we will make efforts to design the polymers to target bacterial components and structures which do not exist in human cells, with high affinity. However, as the polymer target becomes more specific, the likelihood of resistance development would also increase, which resemble to antibiotics. The question would be how much the polymers should or can be specific to bacteria.

6-5. Model membrane studies

Model membrane studies have been a major contributing factor to understanding the biophysical and physicochemical interactions that drive the mechanism of action of AMPs and antimicrobial polymers. These model membrane systems have the advantage of being more experimentally tractable especially with spectroscopic approaches as well as being able to explicitly control lipid bilayer composition. Our studies have shown clear lipid-dependence on binding, partitioning, and bilayer destabilization by both AMPs and antimicrobial polymers.(Kohn et al., 2018; Kuroda et al., 2009; Kuroda & DeGrado, 2005; Tsukamoto et al., 2021) Similarly, in silico models and MD simulations can be constructed to directly complement and parallel the experimental systems.(Ivanov et al., 2006; G. Rani et al., 2021) While these systems have been deeply informative, the model systems do not always faithfully recapitulate the complexity of the bacterial membrane. Specifically, bacterial membranes are more complex including a wider variety of lipid types, polysaccharides, proteins, and the inherent lipid asymmetry in the two leaflets of the bilayer. Recently, the London group has developed a robust system to create asymmetric model lipid vesicles with explicit control over the inner and outer membrane compositions, but these systems have yet to be studied with AMPs or antimicrobial polymers.(Doktorova et al., 2018; M. H. Li, Raleigh, & London, 2021) Our group and others have begun to extensively use live bacterial systems to monitor membrane permeabilization using a variety of approaches including reporter enzymes and DNA binding probes, however newer approaches are needed to be more widely applicable across species and organism type.(A. Chen et al., 2020; Semeraro et al., 2022; D. D. Yang et al., 2021; Z. Zhou et al., 2019) While these methods are providing new tools for the study of antimicrobial polymers, there is a continued need for new approaches to accurately recreate the complexity of the bacterial membrane while remaining compatible with the spectroscopic and analytical approaches to interrogate the molecular behavior.

6-6. Computational approaches

The time scales of antimicrobial action, observing complete membrane disruption, are presently not amenable to all-atom simulations. In addition, exploring the phase space of combinations of functional groups and their sequences for optimal design of an effective biomimetic antimicrobial polymer is also beyond the scope of all-atom simulations due to the computational cost involved. Coarse grain simulations, with their reduced representation of interaction sites enabling larger system size and longer simulation time scales can be promising in this direction. However, this also involves effective optimization and evolution of realistic coarse grain models for both bilayers of interest and class of polymers one wants to study. Coarse grain models like MARTINI(Rzepiela et al., 2010; Su, Marrink, & Melo, 2020) have been successful in exploring the bilayer poration and are strong candidates to explore membrane-lysis due to antimicrobial peptides as well as polymers. Another aspect is the exploration of phase diagram of different functional groups to explore the optimal combination for effective antimicrobial action. Understanding the role of sequence of the functional groups is another aspect for effective design of biomimetic polymers. Techniques such as machine learning are being explored currently to address these issues.

6-7. Industrial and clinical applications

Because of the research history of AMP-mimetic polymers, we consider that their first and primary applications would be antibiotic alternatives. While many studies have been conducted in academia and industries, there are no industrial or clinical applications of AMP-mimetic polymers yet; the hurdles for commercialization as therapeutics appear to be still significantly high due to safety issues and regulations as well as challenges in the material development as described above. However, some natural AMPs and derivatives have been clinical used, including daptomycin and colistin, and some synthetic AMPs are also under clinical tests.(C. H. Chen & Lu, 2020) The commercialization of AMPs will provide a pathway to clinical use of other membrane-active antibiotic agents including AMP-mimetic antimicrobial polymers. As described in this article, these polymers are also active against tumors as well as algal and cyanobacterial cells. While the primary target has been bacterial infections, we will be able to expand the scope of potential applications of antimicrobial polymers in clinical and commercial products.

While the commercialization pathway and market analysis are beyond the scope of this article, there is certainly a demand and market interest for antimicrobial materials. Antimicrobial and antifouling surfaces have consistently generated significant industrial attention for applications ranging from submerged marine machinery to paints to food service/preparation surfaces to hospital and medical/medical-device applications. Currently, the majority of antimicrobial components applied to these surfaces are either thin-film coatings containing an antimicrobial or plastics which have a small-molecule antimicrobial agent imbedded in the material itself.(Ahmed et al., 2022; Goderecci et al., 2017; Luo, Wu, Li, & Loh, 2019; X. Ren & Liang, 2016; D. Sun, Babar Shahzad, Li, Wang, & Xu, 2015; Tyagi & Mishra, 2022) However, these approaches have limitations. Coatings are inherently susceptible to wear, abrasion, or other modes of degradation that can impact performance. Imbedded antimicrobials have the potential to alter materials properties of the base plastic polymer.(M. C. Chen, Koh, Ponnusamy, & Lee, 2022; Kottmann, Mejía, Hémery, Klein, & Kragl, 2017; Marturano, Cerruti, & Ambrogi, 2017) Nonetheless, there is significant industrial interest in these areas, with the antimicrobial plastics market share currently standing at ~$40B annually with projections of increasing to ~$66B annually by 2026.(MarketsandMarkets) Thus, there is a ripe opportunity to expand the application of antimicrobial polymers into these types of applications. This has the potential to drive additional research on how the antimicrobial polymers can be incorporated into a variety of applications, as well as development of polymers with inherent antimicrobial properties that also have desired materials properties for certain applications.

7. Conclusion

This review article provides updates on AMP-mimetic polymers since our previous review. While many studies in the field have been focused on bacterial infections, the AMP-mimetic design may lead to the development of new therapeutics which can address other clinical problems including cancer. In the same context, this polymer approach can also be useful to control outbreaks of algae and cyanobacteria. These may exemplify the practicality and potential of this approach for industrial and clinical applications. There is also significant interest in antimicrobial materials, specifically antimicrobial plastics, which may be amenable to the use of antimicrobial polymers. In addition, while the antimicrobial assays are generally accessible, there is a concrete need for

additional efforts in studying these systems with both computational and biophysical systems. These studies will yield greater depth of understanding of the fundamental physico-chemical processes that underlie the mechanism of action of the polymers. This includes assay and method development to approach complex systems of heteropolymers in complex biosystems. However, this will require a significant amount of effort and systematic studies designed to interrogate the broad variability present in antimicrobial heteropolymers.

Acknowledgments

This work was partially supported by JSPS KAKENHI Grant Number 21K12684 (to HT). Iva Sovadinova thanks the RECETOX Research Infrastructure (No LM2018121) and project CETOCOEN EXCELLENCE (No CZ.02.1.01/0.0/0.0/17_043/0009632) financed by the Czech Ministry of Education, Youth and Sports for supportive background. This work was supported from the European Union's Horizon 2020 research and innovation programme under grant agreement No 857560. This publication reflects only the author's view and the European Commission is not responsible for any use that may be made of the information it contains. This material is based upon work supported by the National Science Foundation under Grant No. (DMR-2004305 BMAT). Any opinions, findings, and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the National Science Foundation. We would like to thank the Department of Biological and Materials Sciences, School of Dentistry, University of Michigan.

References

Adhikari, H. S., & Yadav, P. N. (2018). Anticancer Activity of Chitosan, Chitosan Derivatives, and Their Mechanism of Action. *International Journal of Biomaterials*, 29. doi:10.1155/2018/2952085

Ahmed, S., Sameen, D. E., Lu, R., Li, R., Dai, J., Qin, W., & Liu, Y. (2022). Research progress on antimicrobial materials for food packaging. *Critical Reviews in Food Science and Nutrition, 62*(11), 3088-3102. doi:10.1080/10408398.2020.1863327

Al-Badri, Z. M., Som, A., Lyon, S., Nelson, C. F., Nüsslein, K., & Tew, G. N. (2008). Investigating the Effect of Increasing Charge Density on the Hemolytic Activity of Synthetic Antimicrobial Polymers. *Biomacromolecules, 9*(10), 2805-2810. doi:10.1021/bm800569x

Alberts, B., Johnson, A., Lewis, J., Morgan, D., Raff, M., Roberts, K., & Walter, P. (2015). *Molecular Biology of the Cell* (J. Wilson & T. Hunt Eds. 6th Edition ed.). New York: W.W. Norton & Company.

Alfei, S., & Schito, A. M. (2020). Positively Charged Polymers as Promising Devices against Multidrug Resistant Gram-Negative Bacteria: A Review. *Polymers, 12*(5), 47. doi:10.3390/polym12051195

Andreev, K., Martynowycz, M. W., Huang, M. L., Kuzmenko, I., Bu, W., Kirshenbaum, K., & Gidalevitz, D. (2018). Hydrophobic interactions modulate antimicrobial peptoid selectivity towards anionic lipid membranes. *Biochim Biophys Acta Biomembr, 1860*(6), 1414-1423. doi:10.1016/j.bbamem.2018.03.021

Antimicrobial Resistance, C. (2022). Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. *Lancet*. doi:10.1016/S0140-6736(21)02724-0

Arora, M., & Sahoo, D. (2015). Green algae. In *The Algae World* (pp. 91-120): Springer.

Arrington, S. A., Zeleznik, M. J., Ott, D. W., & Ju, L.-K. (2003). Effects of polyethyleneimine on cyanobacterium Anabaena flos-aquae during cell flocculation and flotation. *Enzyme and microbial technology, 32*(2), 290-293.

Baul, U., Kuroda, K., & Vemparala, S. (2014). Interaction of multiple biomimetic antimicrobial polymers with model bacterial membranes. *J Chem Phys, 141*(8), 084902. doi:10.1063/1.4893440

Belli, C., Trapani, D., Viale, G., D'Amico, P., Duso, B. A., Della Vigna, P., . . . Curigliano, G. (2018). Targeting the microenvironment in solid tumors. *Cancer Treatment Reviews, 65*, 22-32. doi:10.1016/j.ctrv.2018.02.004

Bhat, R., Foster, L. L., Rani, G., Vemparala, S., & Kuroda, K. (2021). The function of peptide-mimetic anionic groups and salt bridges in the antimicrobial activity and conformation of cationic amphiphilic copolymers. *RSC Advances, 11*(36), 22044-22056. doi:10.1039/d1ra02730a

Bocchinfuso, G., Bobone, S., Mazzuca, C., Palleschi, A., & Stella, L. (2011). Fluorescence spectroscopy and molecular dynamics simulations in studies on the mechanism of membrane destabilization by antimicrobial peptides. *Cell Mol Life Sci, 68*(13), 2281-2301. doi:10.1007/s00018-011-0719-1

Bocchinfuso, G., Palleschi, A., Orioni, B., Grande, G., Formaggio, F., Toniolo, C., . . . Stella, L. (2009). Different mechanisms of action of antimicrobial peptides: insights from fluorescence spectroscopy experiments and molecular dynamics simulations. *Journal of peptide science: an official publication of the European Peptide Society, 15*(9), 550-558.

Brauner, A., Fridman, O., Gefen, O., & Balaban, N. Q. (2016). Distinguishing between resistance, tolerance and persistence to antibiotic treatment. *Nature Reviews Microbiology, 14*(5), 320-330. doi:10.1038/nrmicro.2016.34

Bray, F., Ferlay, J., Soerjomataram, I., Siegel, R. L., Torre, L. A., & Jemal, A. (2018). Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *Ca-a Cancer Journal for Clinicians, 68*(6), 394-424. doi:10.3322/caac.21492

Briand, E., Bormans, M., Gugger, M., Dorrestein, P. C., & Gerwick, W. H. (2016). Changes in secondary metabolic profiles of Microcystis aeruginosa strains in response to intraspecific interactions. *Environ Microbiol, 18*(2), 384-400. doi:10.1111/1462-2920.12904

Bryant, D. A. (2006). *The molecular biology of cyanobacteria* (Vol. 1): Springer Science & Business Media.

CDC. (2019). Antibiotic Resistance Threats in the United States, 2019. *U.S. Department of Health and Human Services, CDC*. Retrieved from https://www.cdc.gov/drugresistance/pdf/threats-report/2019-ar-threats-report-508.pdf

Chakrabarty, A., Chakraborty, S., Bhattacharya, R., & Chowdhury, G. (2021). Senescence-Induced Chemoresistance in Triple Negative Breast Cancer and Evolution-Based Treatment Strategies. *Front Oncol, 11*, 674354. doi:10.3389/fonc.2021.674354

Chakraborty, A., Kobzev, E., Chan, J., de Zoysa, G. H., Sarojini, V., Piggot, T. J., & Allison, J. R. (2021). Molecular Dynamics Simulation of the Interaction of Two Linear Battacin Analogs with Model Gram-Positive and Gram-Negative Bacterial Cell Membranes. *ACS Omega, 6*(1), 388-400. doi:10.1021/acsomega.0c04752

Chakraborty, S., Liu, R., Hayouka, Z., Chen, X., Ehrhardt, J., Lu, Q., . . . Gellman, S. H. (2014). Ternary Nylon-3 Copolymers as Host-Defense Peptide Mimics: Beyond Hydrophobic and Cationic Subunits. *Journal of the American Chemical Society, 136*(41), 14530-14535. doi:10.1021/ja507576a

Chen, A., Karanastasis, A., Casey, K. R., Necelis, M., Carone, B. R., Caputo, G. A., & Palermo, E. F. (2020). Cationic Molecular Umbrellas as Antibacterial Agents with Remarkable Cell-Type Selectivity. *ACS Appl Mater Interfaces, 12*(19), 21270-21282. doi:10.1021/acsami.9b19076

Chen, C. H., & Lu, T. K. (2020). Development and Challenges of Antimicrobial Peptides for Therapeutic Applications. *Antibiotics (Basel), 9*(1). doi:10.3390/antibiotics9010024

Chen, J., Wang, F. Y. K., Liu, Q. M., & Du, J. Z. (2014). Antibacterial polymeric nanostructures for biomedical applications. *Chemical Communications, 50*(93), 14482-14493. doi:10.1039/c4cc03001j

Chen, M. C., Koh, P. W., Ponnusamy, V. K., & Lee, S. L. (2022). Titanium dioxide and other nanomaterials based antimicrobial additives in functional paints and coatings: Review. *Progress in Organic Coatings, 163*, 106660. doi:https://doi.org/10.1016/j.porgcoat.2021.106660

Chen, X., Daliri, E. B.-M., Tyagi, A., & Oh, D.-H. (2021). Cariogenic biofilm: Pathology-related phenotypes and targeted therapy. *Microorganisms, 9*(6), 1311.

Cheng, J., Chin, W., Dong, H., Xu, L., Zhong, G., Huang, Y., . . . Fan, W. (2015). Biodegradable Antimicrobial Polycarbonates with In Vivo Efficacy against Multidrug-Resistant MRSA Systemic Infection. *Adv Healthc Mater, 4*(14), 2128-2136. doi:10.1002/adhm.201500471

Chin, W., Yang, C., Ng, V. W. L., Huang, Y., Cheng, J., Tong, Y. W., . . . Yang, Y. Y. (2013). Biodegradable Broad-Spectrum Antimicrobial Polycarbonates: Investigating the Role of

Chemical Structure on Activity and Selectivity. *Macromolecules, 46*(22), 8797-8807. doi:10.1021/ma4019685

Chin, W., Zhong, G. S., Pu, Q. Q., Yang, C., Lou, W. Y., De Sessions, P. F., . . . Yang, Y. Y. (2018). A macromolecular approach to eradicate multidrug resistant bacterial infections while mitigating drug resistance onset. *Nature Communications, 9*, 14. doi:10.1038/s41467-018-03325-6

Colak, S., & Tew, G. N. (2012). Amphiphilic polybetaines: the effect of side-chain hydrophobicity on protein adsorption. *Biomacromolecules, 13*(5), 1233-1239. doi:10.1021/bm201791p

Cree, I. A., & Charlton, P. (2017). Molecular chess? Hallmarks of anti-cancer drug resistance! *Bmc Cancer, 17*, 8. doi:10.1186/s12885-016-2999-1

Crofts, T. S., Gasparrini, A. J., & Dantas, G. (2017). Next-generation approaches to understand and combat the antibiotic resistome. *Nature Reviews Microbiology, 15*(7), 422-434. doi:10.1038/nrmicro.2017.28

D'Souza, A. R., Necelis, M. R., Kulesha, A., Caputo, G. A., & Makhlynets, O. V. (2021). Beneficial Impacts of Incorporating the Non-Natural Amino Acid Azulenyl-Alanine into the Trp-Rich Antimicrobial Peptide buCATHL4B. *Biomolecules, 11*(3), 421. Retrieved from https://www.mdpi.com/2218-273X/11/3/421

DeGrado, W. F., Schneider, J. P., & Hamuro, Y. (1999). The twists and turns of beta-peptides. *J Pept Res, 54*(3), 206-217. doi:10.1034/j.1399-3011.1999.00131.x

Deplazes, E., Chin, Y. K. Y., King, G. F., & Mancera, R. L. (2020). The unusual conformation of cross‐strand disulfide bonds is critical to the stability of β‐hairpin peptides. *Proteins: Structure, Function, and Bioinformatics, 88*(3), 485-502.

Di Pippo, F., Di Gregorio, L., Congestri, R., Tandoi, V., & Rossetti, S. (2018). Biofilm growth and control in cooling water industrial systems. *FEMS Microbiol Ecol, 94*(5). doi:10.1093/femsec/fiy044

Diederichs, S., Bartsch, L., Berkmann, J. C., Frose, K., Heitmann, J., Hoppe, C., . . . Wullenkord, R. (2016). The dark matter of the cancer genome: aberrations in regulatory elements, untranslated regions, splice sites, non-coding RNA and synonymous mutations. *Embo Molecular Medicine, 8*(5), 442-457. doi:10.15252/emmm.201506055

Dimova, R., & Marques, C. M. (2020). The giant vesicle book. Retrieved from https://public.ebookcentral.proquest.com/choice/publicfullrecord.aspx?p=5981840

Ding, X. K., Wang, A. Z., Tong, W., & Xu, F. J. (2019). Biodegradable Antibacterial Polymeric Nanosystems: A New Hope to Cope with Multidrug-Resistant Bacteria. *Small, 15*(20), 29. doi:10.1002/smll.201900999

Doktorova, M., Heberle, F. A., Eicher, B., Standaert, R. F., Katsaras, J., London, E., . . . Marquardt, D. (2018). Preparation of asymmetric phospholipid vesicles for use as cell membrane models. *Nat Protoc, 13*(9), 2086-2101. doi:10.1038/s41596-018-0033-6

Engler, A. C., Wiradharma, N., Ong, Z. Y., Coady, D. J., Hedrick, J. L., & Yang, Y. Y. (2012). Emerging trends in macromolecular antimicrobials to fight multi-drug-resistant infections. *Nano Today, 7*(3), 201-222. doi:10.1016/j.nantod.2012.04.003

Epand, R., & Epand, R. (2011). Bacterial membrane lipids in the action of antimicrobial agents. *J. Pept. Sci., 17*(5), 298-305. doi:10.1002/psc.1319

Ergene, C., & Palermo, E. F. (2017). Cationic Poly(benzyl ether)s as Self-Immolative Antimicrobial Polymers. *Biomacromolecules, 18*(10), 3400-3409. doi:10.1021/acs.biomac.7b01062

Ergene, C., & Palermo, E. F. (2018). Antimicrobial Synthetic Polymers: An Update on Structure-Activity Relationships. *Curr Pharm Des, 24*(8), 855-865. doi:10.2174/1381612824666180213140732

Ergene, C., Yasuhara, K., & Palermo, E. F. (2018). Biomimetic antimicrobial polymers: recent advances in molecular design. *Polymer Chemistry, 9*(18), 2407-2427. doi:10.1039/C8PY00012C

Etayash, H., & Hancock, R. E. W. (2021). Host Defense Peptide-Mimicking Polymers and Polymeric-Brush-Tethered Host Defense Peptides: Recent Developments, Limitations, and Potential Success. *Pharmaceutics, 13*(11), 1820. Retrieved from https://www.mdpi.com/1999-4923/13/11/1820

Felicio, M. R., Silva, O. N., Goncalves, S., Santos, N. C., & Franco, O. L. (2017). Peptides with Dual Antimicrobial and Anticancer Activities. *Frontiers in Chemistry, 5*, 9. doi:10.3389/fchem.2017.00005

Feng, K., Ni, C., Yu, L., Zhou, W., & Li, X. (2019). Synthesis and evaluation of acrylate resins suspending indole derivative structure in the side chain for marine antifouling. *Colloids and Surfaces B: Biointerfaces, 184*, 110518.

Fischer, D., Li, Y., Ahlemeyer, B., Krieglstein, J., & Kissel, T. (2003). In vitro cytotoxicity testing of polycations: influence of polymer structure on cell viability and hemolysis. *Biomaterials, 24*(7), 1121-1131. doi:https://doi.org/10.1016/S0142-9612(02)00445-3

Fisher, R. A., Gollan, B., & Helaine, S. (2017). Persistent bacterial infections and persister cells. *Nature Reviews Microbiology, 15*(8), 453-464. doi:10.1038/nrmicro.2017.42

Foster, L. L., Mizutani, M., Oda, Y., Palermo, E. F., & Kuroda, K. (2017). Design and Synthesis of Amphiphilic Vinyl Copolymers with Antimicrobial Activity. *Polymers for Biomedicine: Synthesis, Characterization, and Applications*, 245.

Fouad, Y. A., & Aanei, C. (2017). Revisiting the hallmarks of cancer. *American Journal of Cancer Research, 7*(5), 1016-1036. Retrieved from <Go to ISI>://WOS:000402257100001

Galli, G., & Martinelli, E. (2017). Amphiphilic polymer platforms: Surface engineering of films for marine antibiofouling. *Macromolecular Rapid Communications, 38*(8), 1600704.

Galm, U., Hager, M. H., Van Lanen, S. G., Ju, J. H., Thorson, J. S., & Shen, B. (2005). Antitumor antibiotics: Bleomycin, endiynes, and mitomycin. *Chemical Reviews, 105*(2), 739-758. doi:10.1021/cr030117g

Gaspar, D., Veiga, A. S., & Castanho, M. A. (2013). From antimicrobial to anticancer peptides. A review. *Front Microbiol, 4*, 294. doi:10.3389/fmicb.2013.00294

Ghosh, C., Sarkar, P., Issa, R., & Haldar, J. (2019). Alternatives to Conventional Antibiotics in the Era of Antimicrobial Resistance. *Trends in Microbiology, 27*(4), 323-338. doi:10.1016/j.tim.2018.12.010

Gibney, K. A., Sovadinova, I., Lopez, A. I., Urban, M., Ridgway, Z., Caputo, G. A., & Kuroda, K. (2012). Poly(ethylene imine)s as Antimicrobial Agents with Selective Activity. *Macromolecular Bioscience, 12*(9), 1279-1289. doi:https://doi.org/10.1002/mabi.201200052

Gillet, J. P., & Gottesman, M. M. (2010). Mechanisms of multidrug resistance in cancer. *Methods Mol Biol, 596*, 47-76. doi:10.1007/978-1-60761-416-6_4

Godballe, T., Nilsson, L. L., Petersen, P. D., & Jenssen, H. (2011). Antimicrobial beta-peptides and alpha-peptoids. *Chem Biol Drug Des, 77*(2), 107-116. doi:10.1111/j.1747-0285.2010.01067.x

Goderecci, S. S., Kaiser, E., Yanakas, M., Norris, Z., Scaturro, J., Oszust, R., . . . Hettinger, J. D. (2017). Silver Oxide Coatings with High Silver-Ion Elution Rates and Characterization of Bactericidal Activity. *Molecules, 22*(9). doi:10.3390/molecules22091487

Gonzalo, S., Rodea-Palomares, I., Leganés, F., García-Calvo, E., Rosal, R., & Fernández-Piñas, F. (2015). First evidences of PAMAM dendrimer internalization in microorganisms of environmental relevance: A linkage with toxicity and oxidative stress. *Nanotoxicology, 9*(6), 706-718.

Grace, J. L., Huang, J. X., Cheah, S.-E., Truong, N. P., Cooper, M. A., Li, J., . . . Whittaker, M. R. (2016). Antibacterial low molecular weight cationic polymers: dissecting the contribution of hydrophobicity, chain length and charge to activity. *RSC Advances, 6*(19), 15469-15477. doi:10.1039/C5RA24361K

Hall, C. W., & Mah, T. F. (2017). Molecular mechanisms of biofilm-based antibiotic resistance and tolerance in pathogenic bacteria. *Fems Microbiology Reviews, 41*(3), 276-301. doi:10.1093/femsre/fux010

Han, W., Clarke, W., & Pratt, S. (2014). Composting of waste algae: a review. *Waste Manag, 34*(7), 1148-1155. doi:10.1016/j.wasman.2014.01.019

Hasan, M., Karal, M., Levadnyy, V., & Yamazaki, M. (2018). Mechanism of Initial Stage of Pore Formation Induced by Antimicrobial Peptide Magainin 2. *Langmuir, 34*(10), 3349-3362. doi:10.1021/acs.langmuir.7b04219

Hauer, T., Capek, P., & Bohmova, P. (2016). Main photoautotrophic components of biofilms in natural draft cooling towers. *Folia Microbiol (Praha), 61*(3), 255-260. doi:10.1007/s12223-015-0429-4

Hay, W. T., Fanta, G. F., Rich, J., Evans, K. O., Skory, C. D., & Selling, G. W. (2020). Antimicrobial properties of amylose-fatty ammonium salt inclusion complexes. *Carbohydrate Polymers, 230*, 115666.

Hecht, S. M. (2000). Bleomycin: New perspectives on the mechanism of action. *Journal of Natural Products, 63*(1), 158-168. doi:10.1021/np990549f

Hicks, R. P., Abercrombie, J. J., Wong, R. K., & Leung, K. P. (2013). Antimicrobial peptides containing unnatural amino acid exhibit potent bactericidal activity against ESKAPE pathogens. *Bioorg Med Chem, 21*(1), 205-214. doi:10.1016/j.bmc.2012.10.039

Hitchner, M. A., Necelis, M. R., Shirley, D., & Caputo, G. A. (2021). Effect of Non-natural Hydrophobic Amino Acids on the Efficacy and Properties of the Antimicrobial Peptide C18G. *Probiotics Antimicrob Proteins, 13*(2), 527-541. doi:10.1007/s12602-020-09701-3

Hitchner, M. A., Santiago-Ortiz, L. E., Necelis, M. R., Shirley, D. J., Palmer, T. J., Tarnawsky, K. E., . . . Caputo, G. A. (2019). Activity and characterization of a pH-sensitive antimicrobial peptide. *Biochim Biophys Acta Biomembr, 1861*(10), 182984. doi:10.1016/j.bbamem.2019.05.006

Hoiczyk, E., & Hansel, A. (2000). Cyanobacterial cell walls: news from an unusual prokaryotic envelope. *Journal of bacteriology, 182*(5), 1191-1199.

Horn, J. N., Cravens, A., & Grossfield, A. (2013). Interactions between fengycin and model bilayers quantified by coarse-grained molecular dynamics. *Biophys J, 105*(7), 1612-1623. doi:10.1016/j.bpj.2013.08.034

Housman, G., Byler, S., Heerboth, S., Lapinska, K., Longacre, M., Snyder, N., & Sarkar, S. (2014). Drug Resistance in Cancer: An Overview. *Cancers, 6*(3), 1769-1792. doi:10.3390/cancers6031769

Hu, K., Schmidt, N. W., Zhu, R., Jiang, Y., Lai, G. H., Wei, G., . . . Yang, L. (2013). A critical evaluation of random copolymer mimesis of homogeneous antimicrobial peptides. *Macromolecules, 46*(5), 1908-1915.

Hu, T., Li, Z., Gao, C. Y., & Cho, C. H. (2016). Mechanisms of drug resistance in colon cancer and its therapeutic strategies. *World Journal of Gastroenterology, 22*(30), 6876-6889. doi:10.3748/wjg.v22.i30.6876

Huan, Y. C., Kong, Q., Mou, H. J., & Yi, H. X. (2020). Antimicrobial Peptides: Classification, Design, Application and Research Progress in Multiple Fields. *Frontiers in Microbiology, 11*, 21. doi:10.3389/fmicb.2020.582779

Huang, W., Seo, J., Willingham, S. B., Czyzewski, A. M., Gonzalgo, M. L., Weissman, I. L., & Barron, A. E. (2014). Learning from Host-Defense Peptides: Cationic, Amphipathic Peptoids with Potent Anticancer Activity. *Plos One, 9*(2), 10. doi:10.1371/journal.pone.0090397

Ivanov, I., Vemparala, S., Pophristic, V., Kuroda, K., DeGrado, W. F., McCammon, J. A., & Klein, M. L. (2006). Characterization of nonbiological antimicrobial polymers in aqueous solution and at water-lipid interfaces from all-atom molecular dynamics. *J Am Chem Soc, 128*(6), 1778-1779. doi:10.1021/ja0564665

Jafari, A., Babajani, A., Sarrami Forooshani, R., Yazdani, M., & Rezaei-Tavirani, M. (2022). Clinical Applications and Anticancer Effects of Antimicrobial Peptides: From Bench to Bedside. *Front Oncol, 12*, 819563. doi:10.3389/fonc.2022.819563

Jamal, M., Ahmad, W., Andleeb, S., Jalil, F., Imran, M., Nawaz, M. A., . . . Kamil, M. A. (2018). Bacterial biofilm and associated infections. *Journal of the Chinese Medical Association, 81*(1), 7-11. doi:10.1016/j.jcma.2017.07.012

Janssen, C., Vangheluwe, M., & Sprang, P. V. (2000). A brief review and critical evaluation of the status of microbiotests. *New microbiotests for routine toxicity screening and biomonitoring*, 27-37.

Jia, X. J., Zhang, C., Qiu, J. F., Wang, L. L., Bao, J. L., Wang, K., . . . He, C. W. (2015). Purification, structural characterization and anticancer activity of the novel polysaccharides from Rhynchosia minima root. *Carbohydrate Polymers, 132*, 67-71. doi:10.1016/j.carbpol.2015.05.059

Jiang, Z., Vasil, A. I., Hale, J. D., Hancock, R. E., Vasil, M. L., & Hodges, R. S. (2008). Effects of net charge and the number of positively charged residues on the biological activity of amphipathic alpha-helical cationic antimicrobial peptides. *Biopolymers, 90*(3), 369-383. doi:10.1002/bip.20911

Joo, J. C., Kim, G. Y., Lee, M. J., Ahn, C. H., Lee, S., Park, J. R., & Kim, J. K. (2020). Growth Inhibition of Microcystis aeruginosa Using TiO(2)-Embedded Expanded Polystyrene Balls. *J Nanosci Nanotechnol, 20*(9), 5775-5779. doi:10.1166/jnn.2020.17637

Judzewitsch, P. R., Nguyen, T. K., Shanmugam, S., Wong, E. H. H., & Boyer, C. (2018). Towards Sequence-Controlled Antimicrobial Polymers: Effect of Polymer Block Order on Antimicrobial Activity. *Angew Chem Int Ed Engl, 57*(17), 4559-4564. doi:10.1002/anie.201713036

Kakuda, S., Suresh, P., Li, G., & London, E. (2022). Loss of plasma membrane lipid asymmetry can induce ordered domain (raft) formation. *J Lipid Res, 63*(1), 100155. doi:10.1016/j.jlr.2021.100155

Kang, H. K., Kim, C., Seo, C. H., & Park, Y. (2017). The therapeutic applications of antimicrobial peptides (AMPs): a patent review. *Journal of Microbiology, 55*(1), 1-12. doi:10.1007/s12275-017-6452-1

Karal, M., Alam, J., Takahashi, T., Levadny, V., & Yamazaki, M. (2015). Stretch-Activated Pore of the Antimicrobial Peptide, Magainin 2. *Langmuir, 31*(11), 3391-3401. doi:10.1021/la503318z

Kenawy, E.-R., Worley, S., & Broughton, R. (2007). The chemistry and applications of antimicrobial polymers: a state-of-the-art review. *Biomacromolecules, 8*(5), 1359-1384.

Kester, J. C., & Fortune, S. M. (2014). Persisters and beyond: Mechanisms of phenotypic drug resistance and drug tolerance in bacteria. *Critical Reviews in Biochemistry and Molecular Biology, 49*(2), 91-101. doi:10.3109/10409238.2013.869543

Khandelia, H., & Kaznessis, Y. N. (2007). Structure of the antimicrobial beta-hairpin peptide protegrin-1 in a DLPC lipid bilayer investigated by molecular dynamics simulation. *Biochim Biophys Acta, 1768*(3), 509-520. doi:10.1016/j.bbamem.2006.11.015

Klapper, I., Gilbert, P., Ayati, B. P., Dockery, J., & Stewart, P. S. (2007). Senescence can explain microbial persistence. *Microbiology (Reading), 153*(Pt 11), 3623-3630. doi:10.1099/mic.0.2007/006734-0

Koehbach, J., & Craik, D. J. (2019). The Vast Structural Diversity of Antimicrobial Peptides. *Trends Pharmacol Sci, 40*(7), 517-528. doi:10.1016/j.tips.2019.04.012

Kohn, E. M., Shirley, D. J., Arotsky, L., Picciano, A. M., Ridgway, Z., Urban, M. W., . . . Caputo, G. A. (2018). Role of Cationic Side Chains in the Antimicrobial Activity of C18G. *Molecules, 23*(2), 17. doi:10.3390/molecules23020329

Kottmann, A., Mejía, E., Hémery, T., Klein, J., & Kragl, U. (2017). Recent Developments in the Preparation of Silicones with Antimicrobial Properties. *Chemistry – An Asian Journal, 12*(11), 1168-1179. doi:https://doi.org/10.1002/asia.201700244

Kouno, T., Fujitani, N., Mizuguchi, M., Osaki, T., Nishimura, S.-i., Kawabata, S.-i., . . . Kawano, K. (2008). A novel β-defensin structure: a potential strategy of big defensin for overcoming resistance by Gram-positive bacteria. *Biochemistry, 47*(40), 10611-10619.

Krumm, C., & Tiller, J. C. (2017). Chapter 15 Antimicrobial Polymers and Surfaces – Natural Mimics or Surpassing Nature? In *Bio-inspired Polymers* (pp. 490-522): The Royal Society of Chemistry.

Kuroda, K., & Caputo, G. A. (2013). Antimicrobial polymers as synthetic mimics of host-defense peptides. *Wiley Interdiscip Rev Nanomed Nanobiotechnol, 5*(1), 49-66. doi:10.1002/wnan.1199

Kuroda, K., Caputo, G. A., & DeGrado, W. F. (2009). The role of hydrophobicity in the antimicrobial and hemolytic activities of polymethacrylate derivatives. *Chemistry, 15*(5), 1123-1133. doi:10.1002/chem.200801523

Kuroda, K., & DeGrado, W. F. (2005). Amphiphilic polymethacrylate derivatives as antimicrobial agents. *J Am Chem Soc, 127*(12), 4128-4129. doi:10.1021/ja044205+

Kuroki, A., Sangwan, P., Qu, Y., Peltier, R., Sanchez-Cano, C., Moat, J., . . . Perrier, S. (2017). Sequence Control as a Powerful Tool for Improving the Selectivity of Antimicrobial Polymers. *ACS Applied Materials & Interfaces, 9*(46), 40117-40126. doi:10.1021/acsami.7b14996

Kuwano, M., Sonoda, K., Murakami, Y., Watari, K., & Ono, M. (2016). Overcoming drug resistance to receptor tyrosine kinase inhibitors: Learning from lung cancer. *Pharmacology & Therapeutics, 161*, 97-110. doi:10.1016/j.pharmthera.2016.03.002

Kyzioł, A., Khan, W., Sebastian, V., & Kyzioł, K. (2020). Tackling microbial infections and increasing resistance involving formulations based on antimicrobial polymers. *Chemical Engineering Journal, 385*, 123888.

Ladokhin, A. S., Selsted, M. E., & White, S. H. (1997). Sizing membrane pores in lipid vesicles by leakage of co-encapsulated markers: pore formation by melittin. *Biophys J, 72*(4), 1762-1766. doi:10.1016/S0006-3495(97)78822-2

Lazar, V., Nagy, I., Spohn, R., Csorgo, B., Gyorkei, A., Nyerges, A., . . . Pal, C. (2014). Genome-wide analysis captures the determinants of the antibiotic cross-resistance interaction network. *Nature Communications, 5*, 12. doi:10.1038/ncomms5352

Le, C. F., Fang, C. M., & Sekaran, S. D. (2017). Intracellular Targeting Mechanisms by Antimicrobial Peptides. *Antimicrobial Agents and Chemotherapy, 61*(4), 16. doi:10.1128/aac.02340-16

Leary, M., Heerboth, S., Lapinska, K., & Sarkar, S. (2018). Sensitization of Drug Resistant Cancer Cells: A Matter of Combination Therapy. *Cancers, 10*(12), 18. doi:10.3390/cancers10120483

Lee, M., Sun, T., Hung, W., & Huang, H. (2013). Process of inducing pores in membranes by melittin. *Proc. Natl. Acad. Sci. U. S. A., 110*(35), 14243-14248. doi:10.1073/pnas.1307010110

Lee, M. T., Hung, W. C., Chen, F. Y., & Huang, H. W. (2008). Mechanism and kinetics of pore formation in membranes by water-soluble amphipathic peptides. *Proc. Natl. Acad. Sci. U. S. A., 105*(13), 5087-5092. doi:10.1073/pnas.0710625105

Lee, M. W., Chakraborty, S., Schmidt, N. W., Murgai, R., Gellman, S. H., & Wong, G. C. L. (2014). Two interdependent mechanisms of antimicrobial activity allow for efficient killing in nylon-3-based polymeric mimics of innate immunity peptides. *Biochimica et Biophysica Acta (BBA) - Biomembranes, 1838*(9), 2269-2279. doi:https://doi.org/10.1016/j.bbamem.2014.04.007

Lehrer, R. I., & Lu, W. (2012). alpha-Defensins in human innate immunity. *Immunol Rev, 245*(1), 84-112. doi:10.1111/j.1600-065X.2011.01082.x

Leonardi, A. K., & Ober, C. K. (2019). Polymer-based marine antifouling and fouling release surfaces: Strategies for synthesis and modification. *Annual review of chemical and biomolecular engineering, 10*, 241-264.

Leontiadou, H., Mark, A. E., & Marrink, S. J. (2006). Antimicrobial peptides in action. *J Am Chem Soc, 128*(37), 12156-12161. doi:10.1021/ja062927q

Li, J., Hu, S., Jian, W., Xie, C., & Yang, X. (2021). Plant antimicrobial peptides: structures, functions, and applications. *Bot Stud, 62*(1), 5. doi:10.1186/s40529-021-00312-x

Li, J., Lakshminarayanan, R., Bai, Y., Liu, S., Zhou, L., Pervushin, K., . . . Beuerman, R. W. (2012). Molecular dynamics simulations of a new branched antimicrobial peptide: A comparison of force fields. *The Journal of Chemical Physics, 137*(21), 215101.

Li, J., Liu, S., Lakshminarayanan, R., Bai, Y., Pervushin, K., Verma, C., & Beuerman, R. W. (2013). Molecular simulations suggest how a branched antimicrobial peptide perturbs a bacterial membrane and enhances permeability. *Biochim Biophys Acta, 1828*(3), 1112-1121. doi:10.1016/j.bbamem.2012.12.015

Li, M. H., Raleigh, D. P., & London, E. (2021). Preparation of Asymmetric Vesicles with Trapped CsCl Avoids Osmotic Imbalance, Non-Physiological External Solutions, and Minimizes Leakage. *Langmuir, 37*(39), 11611-11617. doi:10.1021/acs.langmuir.1c01971

Lienkamp, K., Madkour, A. E., Musante, A., Nelson, C. F., Nüsslein, K., & Tew, G. N. (2008). Antimicrobial Polymers Prepared by ROMP with Unprecedented Selectivity: A Molecular Construction Kit Approach. *Journal of the American Chemical Society, 130*(30), 9836-9843. doi:10.1021/ja801662y

Liu, D., Choi, S., Chen, B., Doerksen, R. J., Clements, D. J., Winkler, J. D., . . . DeGrado, W. F. (2004). Nontoxic membrane - active antimicrobial arylamide oligomers. *Angewandte Chemie International Edition, 43*(9), 1158-1162.

Liu, J., Zhu, Y., Tao, Y., Zhang, Y., Li, A., Li, T., . . . Zhang, C. (2013). Freshwater microalgae harvested via flocculation induced by pH decrease. *Biotechnology for biofuels, 6*(1), 1-11.

Liu, L., Courtney, K. C., Huth, S. W., Rank, L. A., Weisblum, B., Chapman, E. R., & Gellman, S. H. (2021). Beyond Amphiphilic Balance: Changing Subunit Stereochemistry Alters the Pore-Forming Activity of Nylon-3 Polymers. *Journal of the American Chemical Society, 143*(8), 3219-3230. doi:10.1021/jacs.0c12731

Locock, K. E., Michl, T. D., Valentin, J. D., Vasilev, K., Hayball, J. D., Qu, Y., . . . Haeussler, M. (2013). Guanylated polymethacrylates: a class of potent antimicrobial polymers with low hemolytic activity. *Biomacromolecules, 14*(11), 4021-4031. doi:10.1021/bm401128r

Locock, K. E. S., Michl, T. D., Stevens, N., Hayball, J. D., Vasilev, K., Postma, A., . . . Haeussler, M. (2014). Antimicrobial Polymethacrylates Synthesized as Mimics of Tryptophan-Rich Cationic Peptides. *Acs Macro Letters, 3*(4), 319-323. doi:10.1021/mz5001527

Locock, K. E. S., Michl, T. D., Valentin, J. D. P., Vasilev, K., Hayball, J. D., Qu, Y., . . . Haeussler, M. (2013). Guanylated Polymethacrylates: A Class of Potent Antimicrobial Polymers with Low Hemolytic Activity. *Biomacromolecules, 14*(11), 4021-4031. doi:10.1021/bm401128r

Lopez Cascales, J. J., Zenak, S., Garcia de la Torre, J., Lezama, O. G., Garro, A., & Enriz, R. D. (2018). Small Cationic Peptides: Influence of Charge on Their Antimicrobial Activity. *ACS Omega, 3*(5), 5390-5398. doi:10.1021/acsomega.8b00293

Lowrence, R. C., Subramaniapillai, S. G., Ulaganathan, V., & Nagarajan, S. (2019). Tackling drug resistance with efflux pump inhibitors: from bacteria to cancerous cells. *Critical Reviews in Microbiology, 45*(3), 334-353. doi:10.1080/1040841x.2019.1607248

Lu, J., Xu, H., Xia, J., Ma, J., Xu, J., Li, Y., & Feng, J. (2020). D- and Unnatural Amino Acid Substituted Antimicrobial Peptides With Improved Proteolytic Resistance and Their Proteolytic Degradation Characteristics. *Frontiers in Microbiology, 11*. Retrieved from https://www.frontiersin.org/article/10.3389/fmicb.2020.563030

Luo, Z., Wu, Y.-L., Li, Z., & Loh, X. J. (2019). Recent Progress in Polyhydroxyalkanoates-Based Copolymers for Biomedical Applications. *Biotechnology Journal, 14*(12), 1900283. doi:https://doi.org/10.1002/biot.201900283

Lv, L., Zhang, X., & Qiao, J. (2018). Flocculation of low algae concentration water using polydiallyldimethylammonium chloride coupled with polysilicate aluminum ferrite. *Environmental technology, 39*(1), 83-90.

Lyon-Colbert, A., Su, S., & Cude, C. (2018). A systematic literature review for evidence of Aphanizomenon flos-aquae toxigenicity in recreational waters and toxicity of dietary supplements: 2000–2017. *Toxins, 10*(7), 254.

Magana, M., Pushpanathan, M., Santos, A. L., Leanse, L., Fernandez, M., Ioannidis, A., . . . Tegos, G. P. (2020). The value of antimicrobial peptides in the age of resistance. *Lancet Infect Dis, 20*(9), e216-e230. doi:10.1016/S1473-3099(20)30327-3

Mahlapuu, M., Bjorn, C., & Ekblom, J. (2020). Antimicrobial peptides as therapeutic agents: opportunities and challenges. *Critical Reviews in Biotechnology, 40*(7), 978-992. doi:10.1080/07388551.2020.1796576

Maik-Rachline, G., & Seger, R. (2016). The ERK cascade inhibitors: Towards overcoming resistance. *Drug Resistance Updates, 25*, 1-12. doi:10.1016/j.drup.2015.12.001

MarketsandMarkets. Antimicrobial Plastics Market by Additive (Inorganic, Organic), Type (Commodity Plastics, Engineering Plastics, High-Performance Plastics), Application and Region (APAC, North America, Europe, MEA, South America) - Global Forecast to 2026. Retrieved from https://www.marketsandmarkets.com/Market-Reports/antimicrobial-plastic-market-20591555.html

Marturano, V., Cerruti, P., & Ambrogi, V. (2017). Polymer additives. *2*(6). doi:doi:10.1515/psr-2016-0130

Matthijs, H. C. P., Jancula, D., Visser, P. M., & Marsalek, B. (2016). Existing and emerging cyanocidal compounds: new perspectives for cyanobacterial bloom mitigation. *Aquatic Ecology, 50*(3), 443-460. doi:10.1007/s10452-016-9577-0

Matyus, E., Kandt, C., & Tieleman, D. P. (2007). Computer simulation of antimicrobial peptides. *Current medicinal chemistry, 14*(26), 2789-2798.

Mellati, A., Valizadeh Kiamahalleh, M., Dai, S., Bi, J., Jin, B., & Zhang, H. (2016). Influence of polymer molecular weight on the in vitro cytotoxicity of poly (N-isopropylacrylamide). *Materials Science and Engineering: C, 59*, 509-513. doi:https://doi.org/10.1016/j.msec.2015.10.043

Michl, T. D., Hibbs, B., Hyde, L., Postma, A., Tran, D. T. T., Zhalgasbaikyzy, A., . . . Locock, K. E. S. (2020). Bacterial membrane permeability of antimicrobial polymethacrylates: Evidence for a complex mechanism from super-resolution fluorescence imaging. *Acta Biomaterialia, 108*, 168-177. doi:10.1016/j.actbio.2020.03.011

Mihajlovic, M., & Lazaridis, T. (2012). Charge distribution and imperfect amphipathicity affect pore formation by antimicrobial peptides. *Biochim Biophys Acta, 1818*(5), 1274-1283. doi:10.1016/j.bbamem.2012.01.016

Mikula, P., Mlnaříková, M., Nadres, E. T., Takahashi, H., Babica, P., Kuroda, K., . . . Sovadinová, I. (2021). Synthetic biomimetic polymethacrylates: promising platform for the design of anti-cyanobacterial and anti-algal agents. *Polymers, 13*(7), 1025.

Mikula, P., Mlnarikova, M., Takahashi, H., Babica, P., Kuroda, K., Blaha, L., & Sovadinova, I. (2018). Branched Poly (ethylene imine) s as Anti‐algal and Anti‐cyanobacterial Agents with Selective Flocculation Behavior to Cyanobacteria over Algae. *Macromolecular Bioscience, 18*(10), 1800187.

Mizutani, M., Palermo, E. F., Thoma, L. M., Satoh, K., Kamigaito, M., & Kuroda, K. (2012). Design and Synthesis of Self-Degradable Antibacterial Polymers by Simultaneous Chain- and Step-Growth Radical Copolymerization. *Biomacromolecules, 13*(5), 1554-1563. doi:10.1021/bm300254s

Moriarty, T., Elborn, J., & Tunney, M. (2007). Effect of pH on the antimicrobial susceptibility of planktonic and biofilm-grown clinical Pseudomonas aeruginosa isolates. *British journal of biomedical science, 64*(3), 101-104.

Mortazavian, H., Foster, L. L., Bhat, R., Patel, S., & Kuroda, K. (2018). Decoupling the Functional Roles of Cationic and Hydrophobic Groups in the Antimicrobial and Hemolytic Activities of Methacrylate Random Copolymers. *Biomacromolecules, 19*(11), 4370-4378. doi:10.1021/acs.biomac.8b01256

Mukherjee, I., Ghosh, A., Bhadury, P., & De, P. (2017). Side-Chain Amino Acid-Based Cationic Antibacterial Polymers: Investigating the Morphological Switching of a Polymer-Treated Bacterial Cell. *ACS Omega, 2*(4), 1633-1644. doi:10.1021/acsomega.7b00181

Necelis, M. R., Santiago-Ortiz, L. E., & Caputo, G. A. (2021). Investigation of the Role of Aromatic Residues in the Antimicrobial Peptide BuCATHL4B. *Protein Pept Lett, 28*(4), 388-402. doi:10.2174/0929866527666200813202918

Nederberg, F., Zhang, Y., Tan, J. P., Xu, K., Wang, H., Yang, C., . . . Yang, Y. Y. (2011). Biodegradable nanostructures with selective lysis of microbial membranes. *Nat Chem, 3*(5), 409-414. doi:10.1038/nchem.1012

Oda, Y., Kanaoka, S., Sato, T., Aoshima, S., & Kuroda, K. (2011). Block versus Random Amphiphilic Copolymers as Antibacterial Agents. *Biomacromolecules, 12*(10), 3581-3591. doi:10.1021/bm200780r

Oda, Y., Yasuhara, K., Kanaoka, S., Sato, T., Aoshima, S., & Kuroda, K. (2018). Aggregation of Cationic Amphiphilic Block and Random Copoly(vinyl ether)s with Antimicrobial Activity. *Polymers, 10*(1). doi:10.3390/polym10010093

Ong, Z. Y., Coady, D. J., Tan, J. P., Li, Y., Chan, J. M., Yang, Y. Y., & Hedrick, J. L. (2016). Design and synthesis of biodegradable grafted cationic polycarbonates as broad spectrum antimicrobial agents. *Journal of Polymer Science Part A: Polymer Chemistry, 54*(8), 1029-1035.

Oren, Z., & Shai, Y. (1997). Selective lysis of bacteria but not mammalian cells by diastereomers of melittin: structure-function study. *Biochemistry, 36*(7), 1826-1835. doi:10.1021/bi9625071

Organization, W. H. (2003). Algae and cyanobacteria in freshwater. In *Guidelines for safe recreational water environments* (pp. pp. 136–154). Geneva.

Organization, W. H. (2018). *Monitoring Global Progress On Addressing Antimicrobial Resistance (AMR)*. Retrieved from

Orioni, B., Bocchinfuso, G., Kim, J. Y., Palleschi, A., Grande, G., Bobone, S., . . . Stella, L. (2009). Membrane perturbation by the antimicrobial peptide PMAP-23: a fluorescence and molecular dynamics study. *Biochimica et Biophysica Acta (BBA)-Biomembranes, 1788*(7), 1523-1533.

Paerl, H. W., & Paul, V. J. (2012). Climate change: links to global expansion of harmful cyanobacteria. *Water Res, 46*(5), 1349-1363. doi:10.1016/j.watres.2011.08.002

Palermo, E. F., & Kuroda, K. (2010). Structural determinants of antimicrobial activity in polymers which mimic host defense peptides. *Appl Microbiol Biotechnol, 87*(5), 1605-1615. doi:10.1007/s00253-010-2687-z

Palermo, E. F., Lee, D. K., Ramamoorthy, A., & Kuroda, K. (2011). Role of cationic group structure in membrane binding and disruption by amphiphilic copolymers. *J Phys Chem B, 115*(2), 366-375. doi:10.1021/jp1083357

Palermo, E. F., Vemparala, S., & Kuroda, K. (2012). Cationic spacer arm design strategy for control of antimicrobial activity and conformation of amphiphilic methacrylate random copolymers. *Biomacromolecules, 13*(5), 1632-1641. doi:10.1021/bm300342u

Palermo, E. F., Vemparala, S., & Kuroda, K. (2012). Cationic Spacer Arm Design Strategy for Control of Antimicrobial Activity and Conformation of Amphiphilic Methacrylate Random Copolymers. *Biomacromolecules, 13*(5), 1632-1641. doi:10.1021/bm300342u

Park, N. H., Cheng, W., Lai, F., Yang, C., de Sessions, P. F., Periaswamy, B., . . . Hedrick, J. L. (2018). Addressing Drug Resistance in Cancer with Macromolecular Chemotherapeutic

Agents. *Journal of the American Chemical Society, 140*(12), 4244-4252. doi:10.1021/jacs.7b11468

Patrulea, V., Borchard, G., & Jordan, O. (2020). An Update on Antimicrobial Peptides (AMPs) and Their Delivery Strategies for Wound Infections. *Pharmaceutics, 12*(9), 39. doi:10.3390/pharmaceutics12090840

Pazgier, M., Hoover, D. M., Yang, D., Lu, W., & Lubkowski, J. (2006). Human beta-defensins. *Cell Mol Life Sci, 63*(11), 1294-1313. doi:10.1007/s00018-005-5540-2

Percival, S. L., McCarty, S., Hunt, J. A., & Woods, E. J. (2014). The effects of pH on wound healing, biofilms, and antimicrobial efficacy. *Wound Repair and Regeneration, 22*(2), 174-186.

Percival, S. L., McCarty, S. M., & Lipsky, B. (2015). Biofilms and Wounds: An Overview of the Evidence. *Advances in Wound Care, 4*(7), 373-381. doi:10.1089/wound.2014.0557

Perreault, F., Bogdan, N., Morin, M., Claverie, J., & Popovic, R. (2012). Interaction of gold nanoglycodendrimers with algal cells (Chlamydomonas reinhardtii) and their effect on physiological processes. *Nanotoxicology, 6*(2), 109-120.

Persson, S., Killian, J. A., & Lindblom, G. (1998). Molecular ordering of interfacially localized tryptophan analogs in ester- and ether-lipid bilayers studied by 2H-NMR. *Biophys J, 75*(3), 1365-1371. doi:10.1016/s0006-3495(98)74054-8

Peterson, E., & Kaur, P. (2018). Antibiotic Resistance Mechanisms in Bacteria: Relationships Between Resistance Determinants of Antibiotic Producers, Environmental Bacteria, and Clinical Pathogens. *Frontiers in Microbiology, 9*, 21. doi:10.3389/fmicb.2018.02928

Petit, A.-N., Debenest, T., Eullaffroy, P., & Gagné, F. (2012). Effects of a cationic PAMAM dendrimer on photosynthesis and ROS production of Chlamydomonas reinhardtii. *Nanotoxicology, 6*(3), 315-326.

Petit, A.-N., Eullaffroy, P., Debenest, T., & Gagné, F. (2010). Toxicity of PAMAM dendrimers to Chlamydomonas reinhardtii. *Aquatic toxicology, 100*(2), 187-193.

Polyansky, A. A., Ramaswamy, R., Volynsky, P. E., Sbalzarini, I. F., Marrink, S. J., & Efremov, R. G. (2010). Antimicrobial Peptides Induce Growth of Phosphatidylglycerol Domains in a Model Bacterial Membrane. *Journal of Physical Chemistry Letters, 1*(20), 3108-3111. doi:10.1021/jz101163e

Porter, E. A., Wang, X., Lee, H. S., Weisblum, B., & Gellman, S. H. (2000). Non-haemolytic beta-amino-acid oligomers. *Nature, 404*(6778), 565. doi:10.1038/35007145

Preece, E. P., Hardy, F. J., Moore, B. C., & Bryan, M. (2017). A review of microcystin detections in estuarine and marine waters: environmental implications and human health risk. *Harmful Algae, 61*, 31-45.

Qiu, H., Feng, K., Gapeeva, A., Meurisch, K., Kaps, S., Li, X., . . . Baum, M. (2022). Functional polymer materials for modern marine biofouling control. *Progress in Polymer Science*, 101516.

Rani, G., Kuroda, K., & Vemparala, S. (2020). Aggregation dynamics of methacrylate binary and ternary biomimetic polymers in solution. *arXiv: Soft Condensed Matter*.

Rani, G., Kuroda, K., & Vemparala, S. (2021). Towards designing globular antimicrobial peptide mimics: role of polar functional groups in biomimetic ternary antimicrobial polymers. *Soft Matter, 17*(8), 2090-2103. doi:10.1039/d0sm01896a

Reeves, J. P., & Dowben, R. M. (1969). Formation and properties of thin-walled phospholipid vesicles. *J. Cell Physiol., 73*(1), 49-60. doi:10.1002/jcp.1040730108

Ren, W., Cheng, W. R., Wang, G., & Liu, Y. (2017). Developments in Antimicrobial Polymers. *Journal of Polymer Science Part a-Polymer Chemistry, 55*(4), 632-639. doi:10.1002/pola.28446

Ren, X., & Liang, J. (2016). 9 - Smart anti-microbial composite coatings for textiles and plastics. In M. F. Montemor (Ed.), *Smart Composite Coatings and Membranes* (pp. 235-259): Woodhead Publishing.

Riga, E. K., Vöhringer, M., Widyaya, V. T., & Lienkamp, K. (2017). Polymer‐Based Surfaces Designed to Reduce Biofilm Formation: From Antimicrobial Polymers to Strategies for Long‐Term Applications. *Macromolecular Rapid Communications, 38*(20), 1700216.

Romo, T. D., Bradney, L. A., Greathouse, D. V., & Grossfield, A. (2011). Membrane binding of an acyl-lactoferricin B antimicrobial peptide from solid-state NMR experiments and molecular dynamics simulations. *Biochimica et Biophysica Acta (BBA)-Biomembranes, 1808*(8), 2019-2030.

Rosenfeld, Y., Lev, N., & Shai, Y. (2010). Effect of the hydrophobicity to net positive charge ratio on antibacterial and anti-endotoxin activities of structurally similar antimicrobial peptides. *Biochemistry, 49*(5), 853-861. doi:10.1021/bi900724x

Rzepiela, A. J., Sengupta, D., Goga, N., & Marrink, S. J. (2010). Membrane poration by antimicrobial peptides combining atomistic and coarse-grained descriptions. *Faraday Discuss, 144*, 431-443; discussion 445-481. doi:10.1039/b901615e

Sadhasivam, G., Gelber, C., Zakin, V., Margel, S., & Shapiro, O. H. (2019). N-halamine derivatized nanoparticles with selective cyanocidal activity: Potential for targeted elimination of harmful cyanobacterial blooms. *Environmental Science & Technology, 53*(15), 9160-9170.

Saint Jean, K. D., Henderson, K. D., Chrom, C. L., Abiuso, L. E., Renn, L. M., & Caputo, G. A. (2018). Effects of Hydrophobic Amino Acid Substitutions on Antimicrobial Peptide Behavior. *Probiotics and Antimicrobial Proteins, 10*(3), 408-419. doi:10.1007/s12602-017-9345-z

Saison, C., Perreault, F., Daigle, J.-C., Fortin, C., Claverie, J., Morin, M., & Popovic, R. (2010). Effect of core–shell copper oxide nanoparticles on cell culture morphology and photosynthesis (photosystem II energy distribution) in the green alga, Chlamydomonas reinhardtii. *Aquatic toxicology, 96*(2), 109-114.

Santo, K. P., Irudayam, S. J., & Berkowitz, M. L. (2013). Melittin creates transient pores in a lipid bilayer: results from computer simulations. *J Phys Chem B, 117*(17), 5031-5042. doi:10.1021/jp312328n

Santos, M. R. E., Fonseca, A. C., Mendona, P. V., Branco, R., Serra, A. C., Morais, P. V., & Coelho, J. F. J. (2016). Recent Developments in Antimicrobial Polymers: A Review. *Materials, 9*(7), 33. doi:10.3390/ma9070599

Schardt, L., Martínez Guajardo, A., Koc, J., Clarke, J. L., Finlay, J. A., Clare, A. S., . . . Laschewsky, A. (2021). Low fouling polysulfobetaines with variable hydrophobic content. *Macromolecular Rapid Communications*, 2100589.

Schifano, N. P., & Caputo, G. A. (2021). Investigation of the Role of Hydrophobic Amino Acids on the Structure-Activity Relationship in the Antimicrobial Venom Peptide Ponericin L1. *J Membr Biol*. doi:10.1007/s00232-021-00204-y

Schmidt, F., & Efferth, T. (2016). Tumor Heterogeneity, Single-Cell Sequencing, and Drug Resistance. *Pharmaceuticals, 9*(2), 11. doi:10.3390/ph9020033

Scott, R. W., & Tew, G. N. (2017). Mimics of Host Defense Proteins; Strategies for Translation to Therapeutic Applications. *Current Topics in Medicinal Chemistry, 17*(5), 576-589. doi:10.2174/1568026616666160713130452

Selim, M. S., Shenashen, M. A., El-Safty, S. A., Higazy, S. A., Selim, M. M., Isago, H., & Elmarakbi, A. (2017). Recent progress in marine foul-release polymeric nanocomposite coatings. *Progress in Materials Science, 87*, 1-32. doi:10.1016/j.pmatsci.2017.02.001

Semeraro, E. F., Marx, L., Mandl, J., Letofsky-Papst, I., Mayrhofer, C., Frewein, M. P. K., . . . Pabst, G. (2022). Lactoferricins impair the cytosolic membrane of Escherichia coli within a few seconds and accumulate inside the cell. *Elife, 11*. doi:10.7554/eLife.72850

Senetra, A. S., Necelis, M. R., & Caputo, G. A. (2020). Investigation of the structure-activity relationship in ponericin L1 from Neoponera goeldii. *Pept Sci (Hoboken), 112*(3). doi:10.1002/pep2.24162

Sengupta, D., Leontiadou, H., Mark, A. E., & Marrink, S. J. (2008). Toroidal pores formed by antimicrobial peptides show significant disorder. *Biochim Biophys Acta, 1778*(10), 2308-2317. doi:10.1016/j.bbamem.2008.06.007

Sepehri, A., PeBenito, L., Pino-Angeles, A., & Lazaridis, T. (2020). What Makes a Good Pore Former: A Study of Synthetic Melittin Derivatives. *Biophys J, 118*(8), 1901-1913. doi:10.1016/j.bpj.2020.02.024

Shai, Y. (2002). Mode of action of membrane active antimicrobial peptides. *Biopolymers, 66*(4), 236-248. doi:10.1002/bip.10260

Shai, Y., & Oren, Z. (1996). Diastereoisomers of cytolysins, a novel class of potent antibacterial peptides. *J Biol Chem, 271*(13), 7305-7308. doi:10.1074/jbc.271.13.7305

Simons, K., & Toomre, D. (2000). Lipid rafts and signal transduction. *Nat. Rev. Mol. Cell Biol., 1*(1), 31-39. doi:10.1038/35036052

Skovsgaard, T., & Nissen, N. I. (1975). ADRIAMYCIN, AN ANTITUMOR ANTIBIOTIC - REVIEW WITH SPECIAL REFERENCE TO DAUNOMYCIN. *Danish Medical Bulletin, 22*(2), 62-73. Retrieved from <Go to ISI>://WOS:A1975V866000002

Soliman, W., Bhattacharjee, S., & Kaur, K. (2009). Interaction of an antimicrobial peptide with a model lipid bilayer using molecular dynamics simulation. *Langmuir, 25*(12), 6591-6595. doi:10.1021/la900365g

Sovadinova, I., Palermo, E. F., Urban, M., Mpiga, P., Caputo, G. A., & Kuroda, K. (2011). Activity and Mechanism of Antimicrobial Peptide-Mimetic Amphiphilic Polymethacrylate Derivatives. *Polymers, 3*(3), 1512-1532. doi:10.3390/polym3031512

Sovadinova, I. P., E.F.; Urban, M.; Mpiga, P.; Caputo, G.A.; Kuroda, K. (2011). Activity and Mechanism of Antimicrobial Peptide-Mimetic Amphiphilic Polymethacrylate Derivatives. *Polymers, 3*(3), 1512-1532.

Stavrakoudis, A., Tsoulos, I. G., Shenkarev, Z. O., & Ovchinnikova, T. V. (2009). Molecular dynamics simulation of antimicrobial peptide arenicin-2: beta-hairpin stabilization by noncovalent interactions. *Biopolymers, 92*(3), 143-155. doi:10.1002/bip.21149

Steiner, U. K. (2021). Senescence in Bacteria and Its Underlying Mechanisms. *Front Cell Dev Biol, 9*, 668915. doi:10.3389/fcell.2021.668915

Stone, T. A., Cole, G. B., Ravamehr-Lake, D., Nguyen, H. Q., Khan, F., Sharpe, S., & Deber, C. M. (2019). Positive Charge Patterning and Hydrophobicity of Membrane-Active Antimicrobial Peptides as Determinants of Activity, Toxicity, and Pharmacokinetic Stability. *J Med Chem, 62*(13), 6276-6286. doi:10.1021/acs.jmedchem.9b00657

Su, J., Marrink, S. J., & Melo, M. N. (2020). Localization Preference of Antimicrobial Peptides on Liquid-Disordered Membrane Domains. *Frontiers in Cell and Developmental Biology, 8*. doi:10.3389/fcell.2020.00350

Sun, D., Babar Shahzad, M., Li, M., Wang, G., & Xu, D. (2015). Antimicrobial materials with medical applications. *Materials Technology, 30*(sup6), B90-B95. doi:10.1179/1753555714Y.0000000239

Sun, H., Greathouse, D. V., Andersen, O. S., & Koeppe, R. E., 2nd. (2008). The preference of tryptophan for membrane interfaces: insights from N-methylation of tryptophans in gramicidin channels. *J Biol Chem, 283*(32), 22233-22243. doi:10.1074/jbc.M802074200

Takahashi, D., Shukla, S. K., Prakash, O., & Zhang, G. (2010). Structural determinants of host defense peptides for antimicrobial activity and target cell selectivity. *Biochimie, 92*(9), 1236-1241. doi:10.1016/j.biochi.2010.02.023

Takahashi, H., Caputo, G. A., & Kuroda, K. (2021). Amphiphilic polymer therapeutics: an alternative platform in the fight against antibiotic resistant bacteria. *Biomater Sci, 9*(8), 2758-2767. doi:10.1039/d0bm01865a

Takahashi, H., Caputo, G. A., Vemparala, S., & Kuroda, K. (2017). Synthetic Random Copolymers as a Molecular Platform To Mimic Host-Defense Antimicrobial Peptides. *Bioconjugate Chemistry, 28*(5), 1340-1350. doi:10.1021/acs.bioconjchem.7b00114

Takahashi, H., Yumoto, K., Yasuhara, K., Nadres, E. T., Kikuchi, Y., Buttitta, L., . . . Kuroda, K. (2019). Anticancer polymers designed for killing dormant prostate cancer cells. *Scientific Reports, 9*, 11. doi:10.1038/s41598-018-36608-5

Tamba, Y., Ariyama, H., Levadny, V., & Yamazaki, M. (2010). Kinetic pathway of antimicrobial peptide magainin 2-induced pore formation in lipid membranes. *J Phys Chem B, 114*(37), 12018-12026. doi:10.1021/jp104527y

Tamba, Y., & Yamazaki, M. (2005). Single giant unilamellar vesicle method reveals effect of antimicrobial peptide magainin 2 on membrane permeability. *Biochemistry, 44*(48), 15823-15833. doi:10.1021/bi051684w

Tamba, Y., & Yamazaki, M. (2009). Magainin 2-Induced Pore Formation in the Lipid Membranes Depends on Its Concentration in the Membrane Interface. *J.Phys. Chem. B, 113*(14), 4846-4852. doi:10.1021/jp8109622

Tan, J., Tay, J., Hedrick, J., & Yang, Y. Y. (2020). Synthetic macromolecules as therapeutics that overcome resistance in cancer and microbial infection. *Biomaterials, 252*, 40. doi:10.1016/j.biomaterials.2020.120078

Tan, L. T. H., Chan, K. G., Pusparajah, P., Lee, W. L., Chuah, L. H., Khan, T. M., . . . Goh, B. H. (2017). Targeting Membrane Lipid a Potential Cancer Cure? *Frontiers in Pharmacology, 8*, 6. doi:10.3389/fphar.2017.00012

Tang, J., Signarvic, R. S., DeGrado, W. F., & Gai, F. (2007). Role of helix nucleation in the kinetics of binding of mastoparan X to phospholipid bilayers. *Biochemistry, 46*(48), 13856-13863. doi:10.1021/bi7018404

Tashiro, T. (2001). Antibacterial and bacterium adsorbing macromolecules. *Macromolecular Materials and Engineering, 286*(2), 63-87.

Tew, G. N., Liu, D., Chen, B., Doerksen, R. J., Kaplan, J., Carroll, P. J., . . . DeGrado, W. F. (2002). De novo design of biomimetic antimicrobial polymers. *Proceedings of the National Academy of Sciences, 99*(8), 5110-5114.

Thøgersen, L., Schiøtt, B., Vosegaard, T., Nielsen, N. C., & Tajkhorshid, E. (2008). Peptide aggregation and pore formation in a lipid bilayer: a combined coarse-grained and all atom molecular dynamics study. *Biophysical journal, 95*(9), 4337-4347.

Thoma, L. M., Boles, B. R., & Kuroda, K. (2014). Cationic Methacrylate Polymers as Topical Antimicrobial Agents against Staphylococcus aureus Nasal Colonization. *Biomacromolecules, 15*(8), 2933-2943. doi:10.1021/bm500557d

Tixier, C., Sancelme, M., Bonnemoy, F., Cuer, A., & Veschambre, H. (2001). Degradation products of a phenylurea herbicide, diuron: synthesis, ecotoxicity, and biotransformation. *Environ Toxicol Chem, 20*(7), 1381-1389. doi:10.1897/1551-5028(2001)020<1381:dpoaph>2.0.co;2

Tornesello, A. L., Borrelli, A., Buonaguro, L., Buonaguro, F. M., & Tornesello, M. L. (2020). Antimicrobial Peptides as Anticancer Agents: Functional Properties and Biological Activities. *Molecules, 25*(12). doi:10.3390/molecules25122850

Tsukamoto, M., Zappala, E., Caputo, G., Kikuchi, J., Najarian, K., Kuroda, K., & Yasuhara, K. (2021). Mechanistic Study of Membrane Disruption by Antimicrobial Methacrylate Random Copolymers by the Single Giant Vesicle Method. *Langmuir, 37*(33), 9982-9995. doi:10.1021/acs.langmuir.1c01047

Tyagi, A., & Mishra, A. (2021). Optimal Balance of Hydrophobic Content and Degree of Polymerization Results in a Potent Membrane-Targeting Antibacterial Polymer. *ACS Omega, 6*(50), 34724-34735. doi:10.1021/acsomega.1c05148

Tyagi, A., & Mishra, A. (2022). Progress of Antimicrobial Plastics and Its Applications. In M. S. J. Hashmi (Ed.), *Encyclopedia of Materials: Plastics and Polymers* (pp. 1040-1046). Oxford: Elsevier.

Utsugi, T., Schroit, A. J., Connor, J., Bucana, C. D., & Fidler, I. J. (1991). ELEVATED EXPRESSION OF PHOSPHATIDYLSERINE IN THE OUTER-MEMBRANE LEAFLET OF HUMAN TUMOR-CELLS AND RECOGNITION BY ACTIVATED HUMAN BLOOD MONOCYTES. *Cancer Research, 51*(11), 3062-3066. Retrieved from <Go to ISI>://WOS:A1991FM97800052

von Deuster, C. I., & Knecht, V. (2011). Competing interactions for antimicrobial selectivity based on charge complementarity. *Biochim Biophys Acta, 1808*(12), 2867-2876. doi:10.1016/j.bbamem.2011.08.005

Wang, G., Li, X., & Wang, Z. (2016). APD3: the antimicrobial peptide database as a tool for research and education. *Nucleic acids research, 44*(D1), D1087-D1093.

Wang, M. N., Zhao, J. Z., Zhang, L. S., Wei, F., Lian, Y., Wu, Y. F., . . . Guo, C. (2017). Role of tumor microenvironment in tumorigenesis. *Journal of Cancer, 8*(5), 761-773. doi:10.7150/jca.17648

Wang, Y., Schlamadinger, D. E., Kim, J. E., & McCammon, J. A. (2012). Comparative molecular dynamics simulations of the antimicrobial peptide CM15 in model lipid bilayers. *Biochimica et Biophysica Acta (BBA)-Biomembranes, 1818*(5), 1402-1409.

Wiegand, I., Hilpert, K., & Hancock, R. E. (2008). Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. *Nat Protoc, 3*(2), 163-175. doi:10.1038/nprot.2007.521

Wildman, K. A. H., Lee, D. K., & Ramamoorthy, A. (2003). Mechanism of lipid bilayer disruption by the human antimicrobial peptide, LL-37. *Biochemistry, 42*(21), 6545-6558. doi:10.1021/bi0273563

Wyatt, N. B., Gloe, L. M., Brady, P. V., Hewson, J. C., Grillet, A. M., Hankins, M. G., & Pohl, P. I. (2012). Critical conditions for ferric chloride‐induced flocculation of freshwater algae. *Biotechnology and bioengineering, 109*(2), 493-501.

Xiong, M., Bao, Y., Xu, X., Wang, H., Han, Z., Wang, Z., . . . Chen, J. (2017). Selective killing of Helicobacter pylori with pH-responsive helix–coil conformation transitionable antimicrobial polypeptides. *Proceedings of the National Academy of Sciences, 114*(48), 12675-12680.

Yandi, W., Mieszkin, S., Callow, M. E., Callow, J. A., Finlay, J. A., Liedberg, B., & Ederth, T. (2017). Antialgal activity of poly (2-(dimethylamino) ethyl methacrylate)(PDMAEMA) brushes against the marine alga Ulva. *Biofouling, 33*(2), 169-183.

Yang, C., Lou, W. Y., Zhong, G. S., Lee, A., Leong, J. Y., Chin, W., . . . Yang, Y. Y. (2019). Degradable antimicrobial polycarbonates with unexpected activity and selectivity for treating multidrug-resistant Klebsiella pneumoniae lung infection in mice. *Acta Biomaterialia, 94*, 268-280. doi:10.1016/j.actbio.2019.05.057

Yang, D. D., Paterna, N. J., Senetra, A. S., Casey, K. R., Trieu, P. D., Caputo, G. A., . . . Carone, B. R. (2021). Synergistic interactions of ionic liquids and antimicrobials improve drug efficacy. *iScience, 24*(1), 101853. doi:10.1016/j.isci.2020.101853

Yasuhara, K., Tsukamoto, M., Kikuchi, J., & Kuroda, K. (2022). An Antimicrobial Peptide-Mimetic Methacrylate Random Copolymer Induces Domain Formation in a Model Bacterial Membrane. *Journal of Membrane Biology*. doi:10.1007/s00232-022-00220-6

Yau, W. M., Wimley, W. C., Gawrisch, K., & White, S. H. (1998). The preference of tryptophan for membrane interfaces. *Biochemistry, 37*(42), 14713-14718. doi:10.1021/bi980809c

Yoo, B., & Kirshenbaum, K. (2008). Peptoid architectures: elaboration, actuation, and application. *Curr Opin Chem Biol, 12*(6), 714-721. doi:10.1016/j.cbpa.2008.08.015

Yusuf, R. Z., Duan, Z., Lamendola, D. E., Penson, R. T., & Seiden, M. V. (2003). Paclitaxel Resistance: Molecular Mechanisms and Pharmacologic Manipulation. *Current Cancer Drug Targets, 3*(1), 1-19. doi:10.2174/1568009033333754

Zasloff, M. (2002). Antimicrobial peptides of multicellular organisms. *Nature, 415*(6870), 389-395. doi:10.1038/415389a

Zeleznik, M. J., Segatta, J. M., & Ju, L.-K. (2002). Polyethyleneimine-induced flocculation and flotation of cyanobacterium Anabaena flos-aquae for gas vesicle production. *Enzyme and microbial technology, 31*(7), 949-953.

Zhao, J., Huang, Y. B., Liu, D., & Chen, Y. X. (2015). Two hits are better than one: synergistic anticancer activity of a-helical peptides and doxorubicin/epirubicin. *Oncotarget, 6*(3), 1769-1778. doi:10.18632/oncotarget.2754

Zhao, X., Yu, H., Yang, L., Li, Q., & Huang, X. (2015). Simulating the antimicrobial mechanism of human beta-defensin-3 with coarse-grained molecular dynamics. *J Biomol Struct Dyn, 33*(11), 2522-2529. doi:10.1080/07391102.2014.1002424

Zhao, Y., Zhang, M., Qiu, S., Wang, J., Peng, J., Zhao, P., . . . Wang, R. (2016). Antimicrobial activity and stability of the D-amino acid substituted derivatives of antimicrobial peptide polybia-MPI. *AMB Express, 6*(1), 122. doi:10.1186/s13568-016-0295-8

Zhong, G. S., Yang, C., Liu, S. Q., Zheng, Y. R., Lou, W. Y., Teo, J. Y., . . . Yang, Y. Y. (2019). Polymers with distinctive anticancer mechanism that kills MDR cancer cells and inhibits tumor metastasis. *Biomaterials, 199*, 76-87. doi:10.1016/j.biomaterials.2019.01.036

Zhou, X. Y., & Zhou, C. C. (2018). Design, Synthesis and Applications of Antimicrobial Peptides and Antimicrobial Peptide-Mimetic Copolymers. *Progress in Chemistry, 30*(7), 913-920. doi:10.7536/pc171125

Zhou, Z., Ergene, C., Lee, J. Y., Shirley, D. J., Carone, B. R., Caputo, G. A., & Palermo, E. F. (2019). Sequence and Dispersity Are Determinants of Photodynamic Antibacterial Activity Exerted by Peptidomimetic Oligo(thiophene)s. *ACS Appl Mater Interfaces, 11*(2), 1896-1906. doi:10.1021/acsami.8b19098

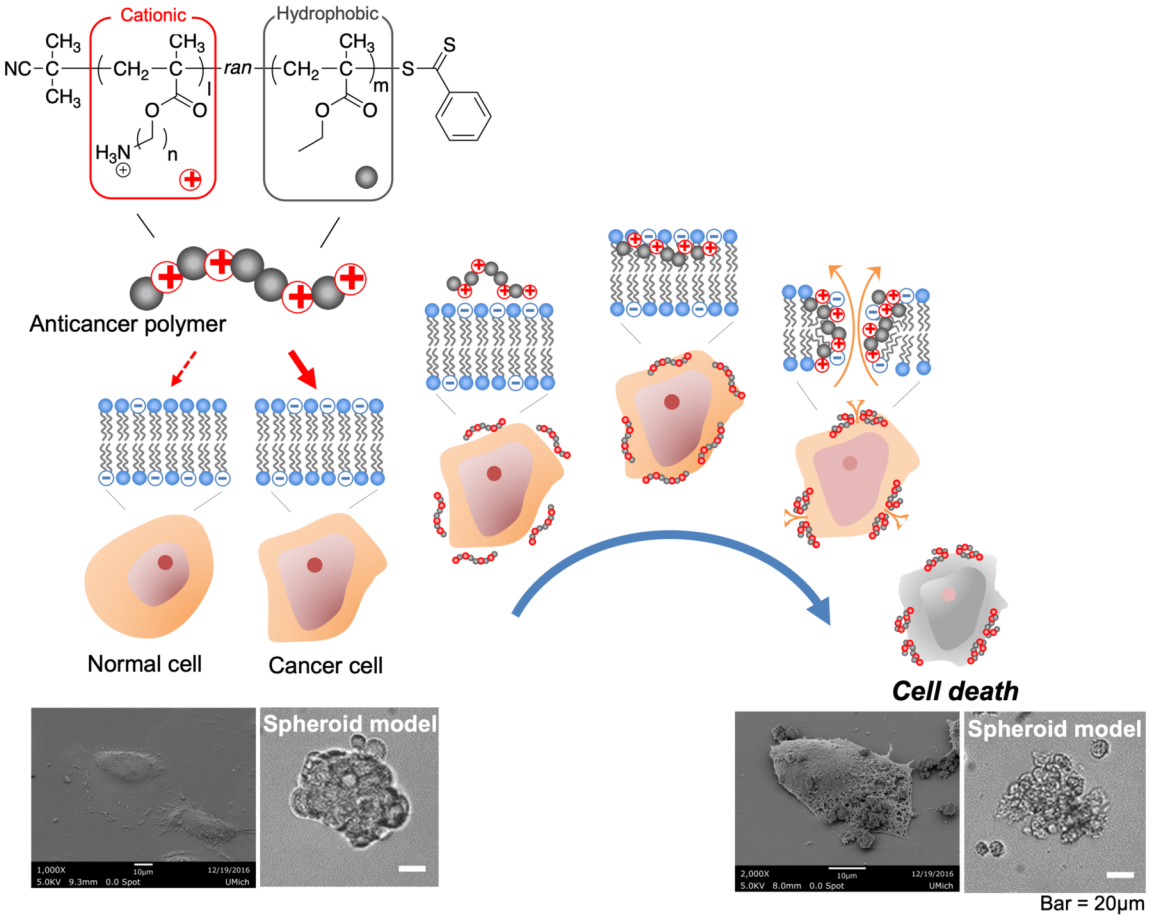
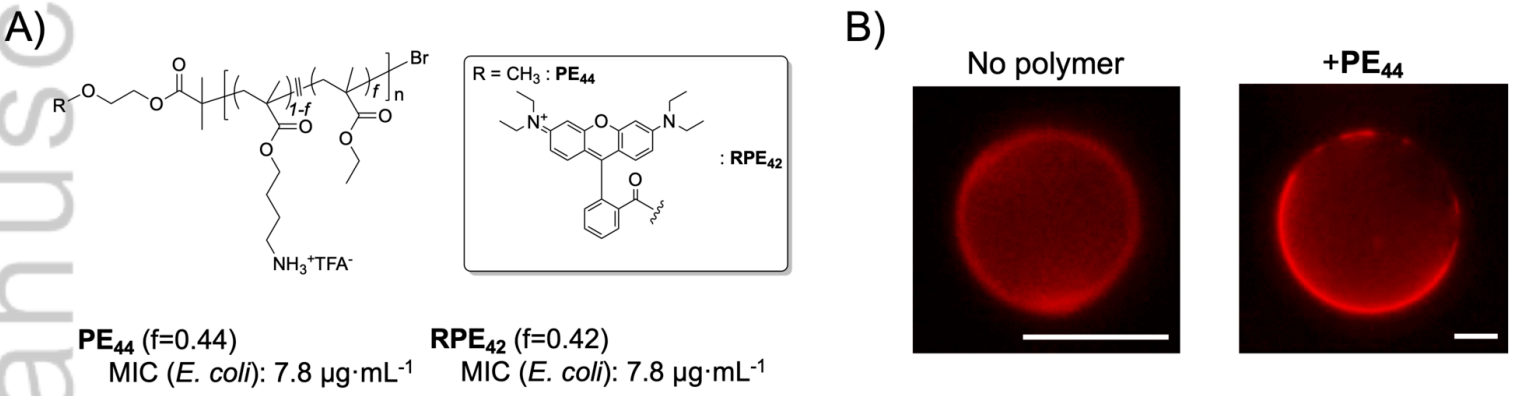


fig1.tiff

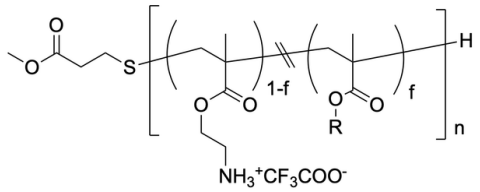


PE_{44} (f=0.44)
MIC (*E. coli*): 7.8 µg·mL^{-1}

RPE_{42} (f=0.42)
MIC (*E. coli*): 7.8 µg·mL^{-1}

fig4.tiff

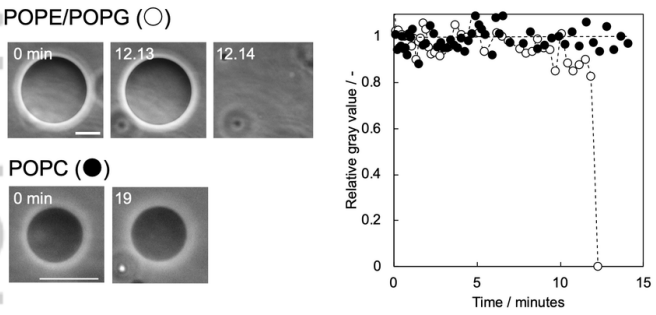
A)



R = CH_3 : **PM_x**
$(CH_2)_3CH_3$: **PB_x**

PM_{34} (f=0.34)
MIC (*E. coli*): 250 μg·mL^{-1}, HC_{50}: >1000 μg·mL^{-1}
PB_{36} (f=0.36)
MIC (*E. coli*): 7.8 μg·mL^{-1}, HC_{50}: 1.0 μg·mL^{-1}

B)



C)

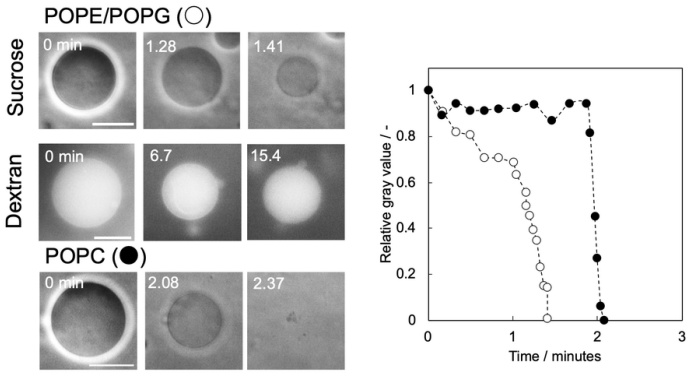


Fig3.tiff

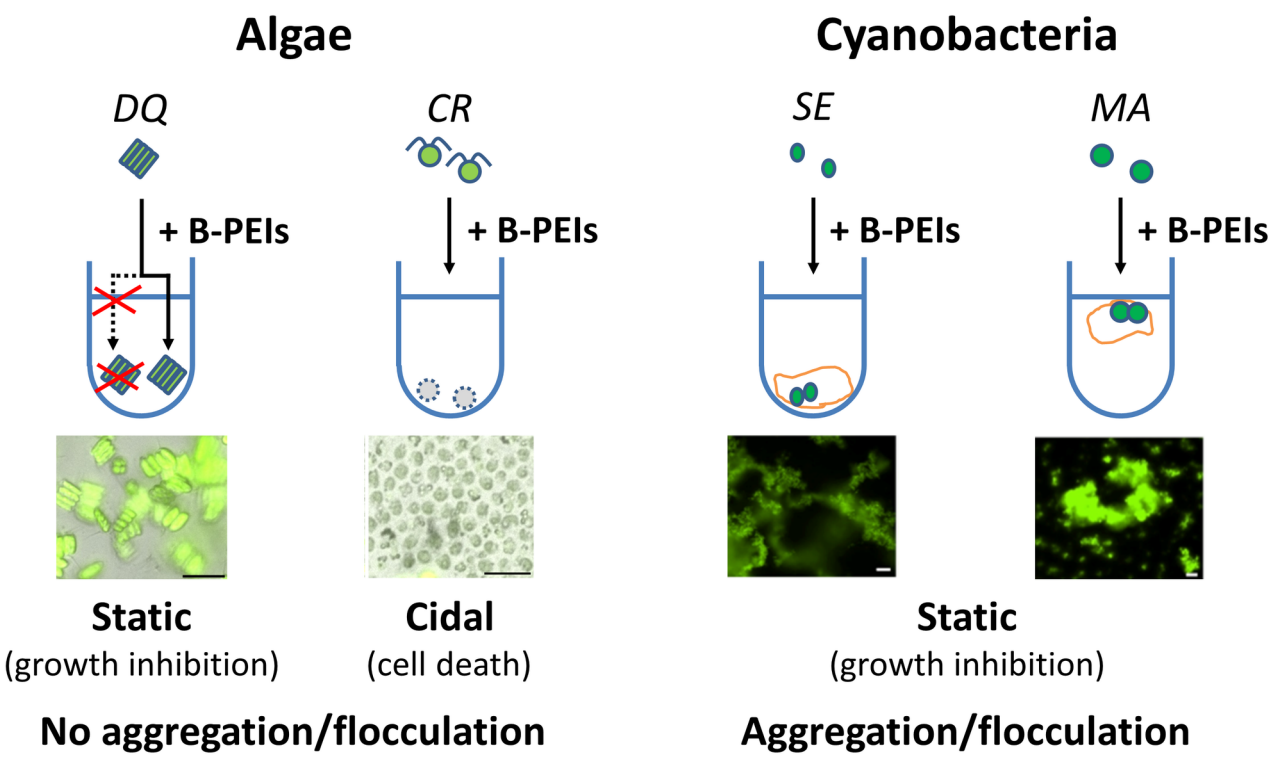
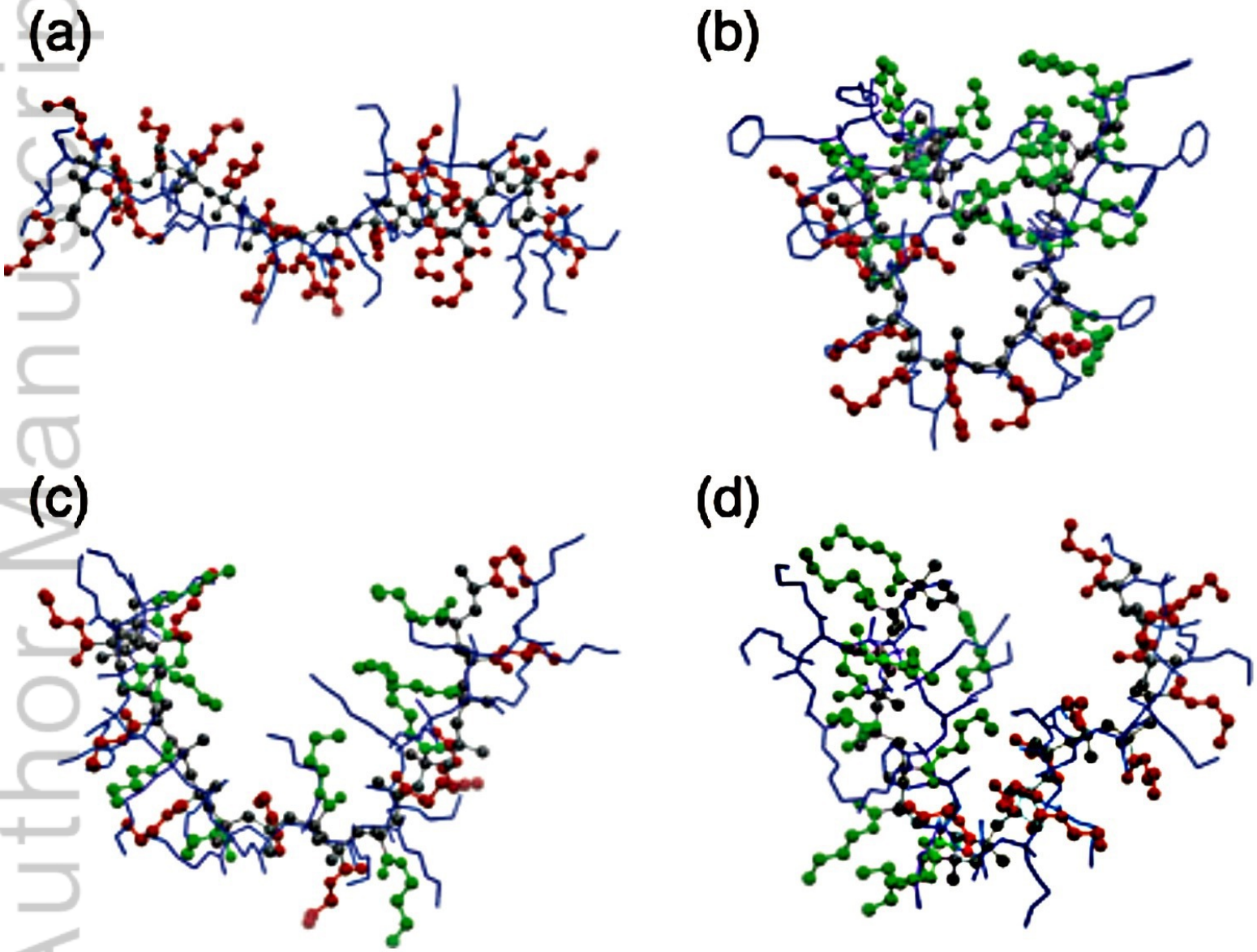
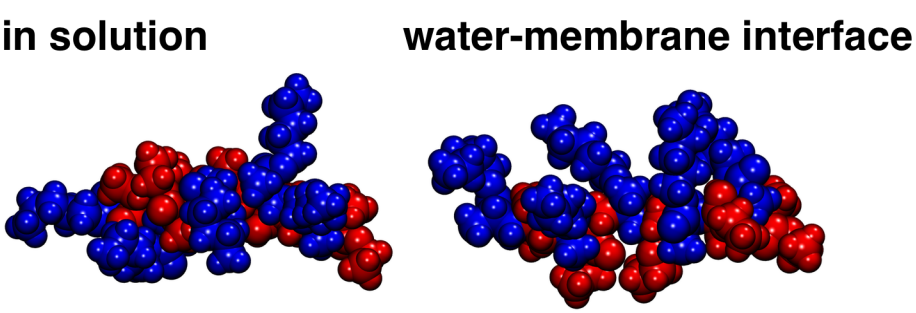


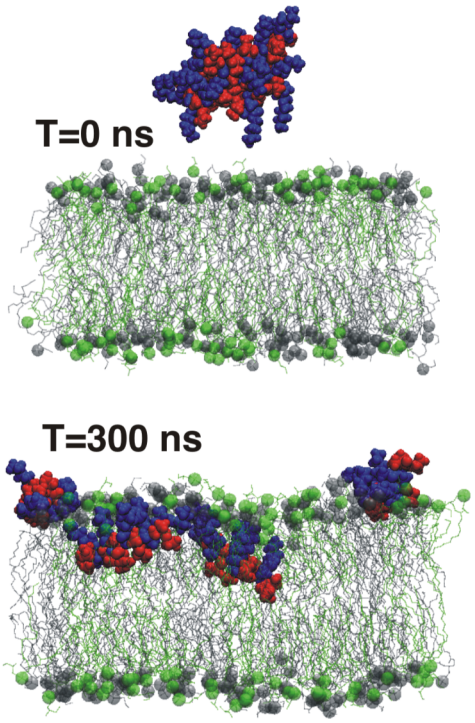
Figure 2.tif



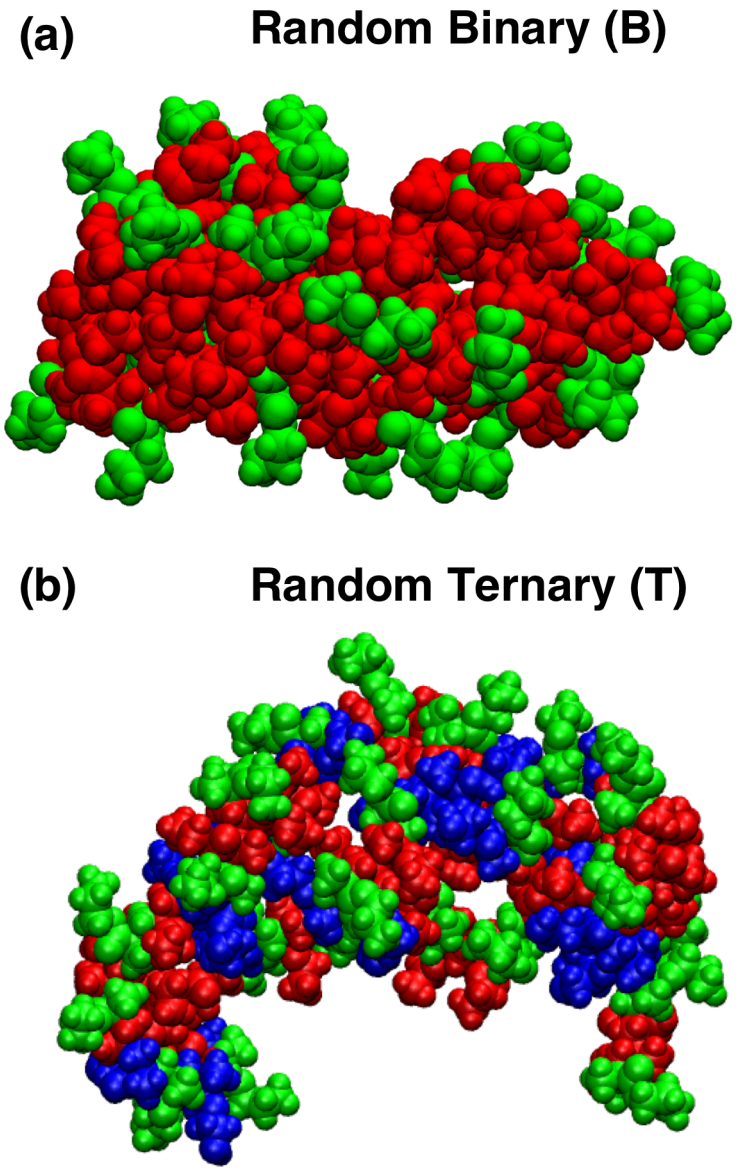
HR-Figure5.jpeg



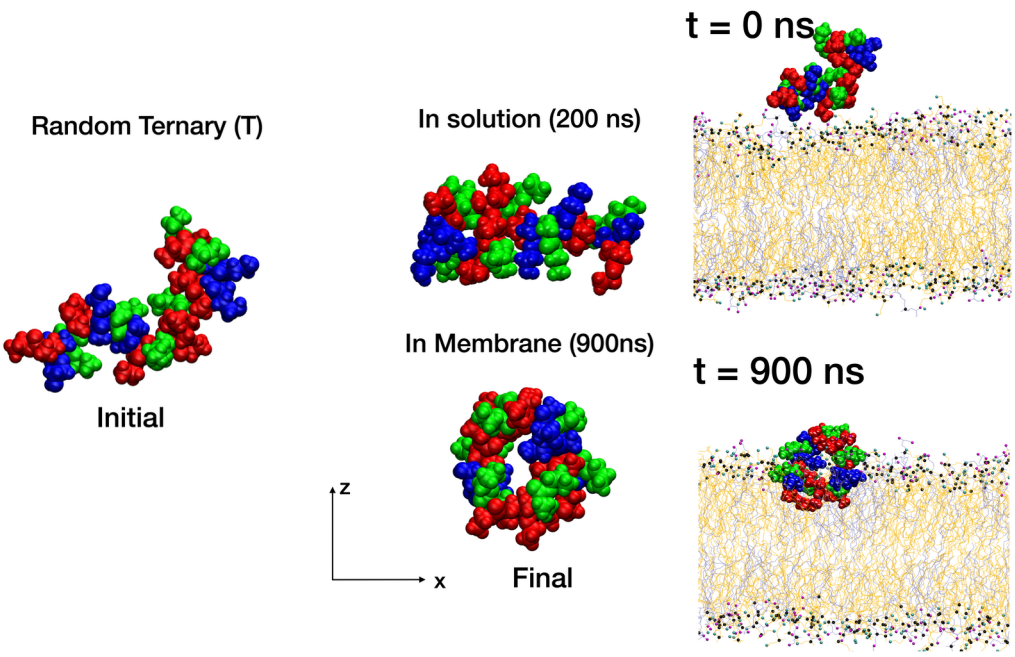
HR-Figure6.tiff



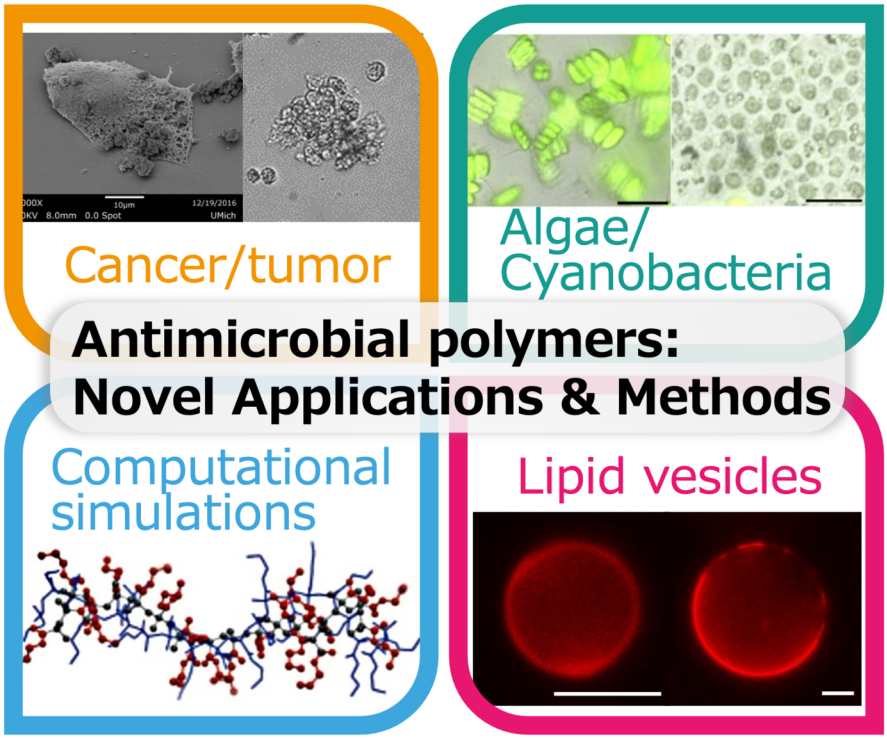
HR-Figure7.tiff



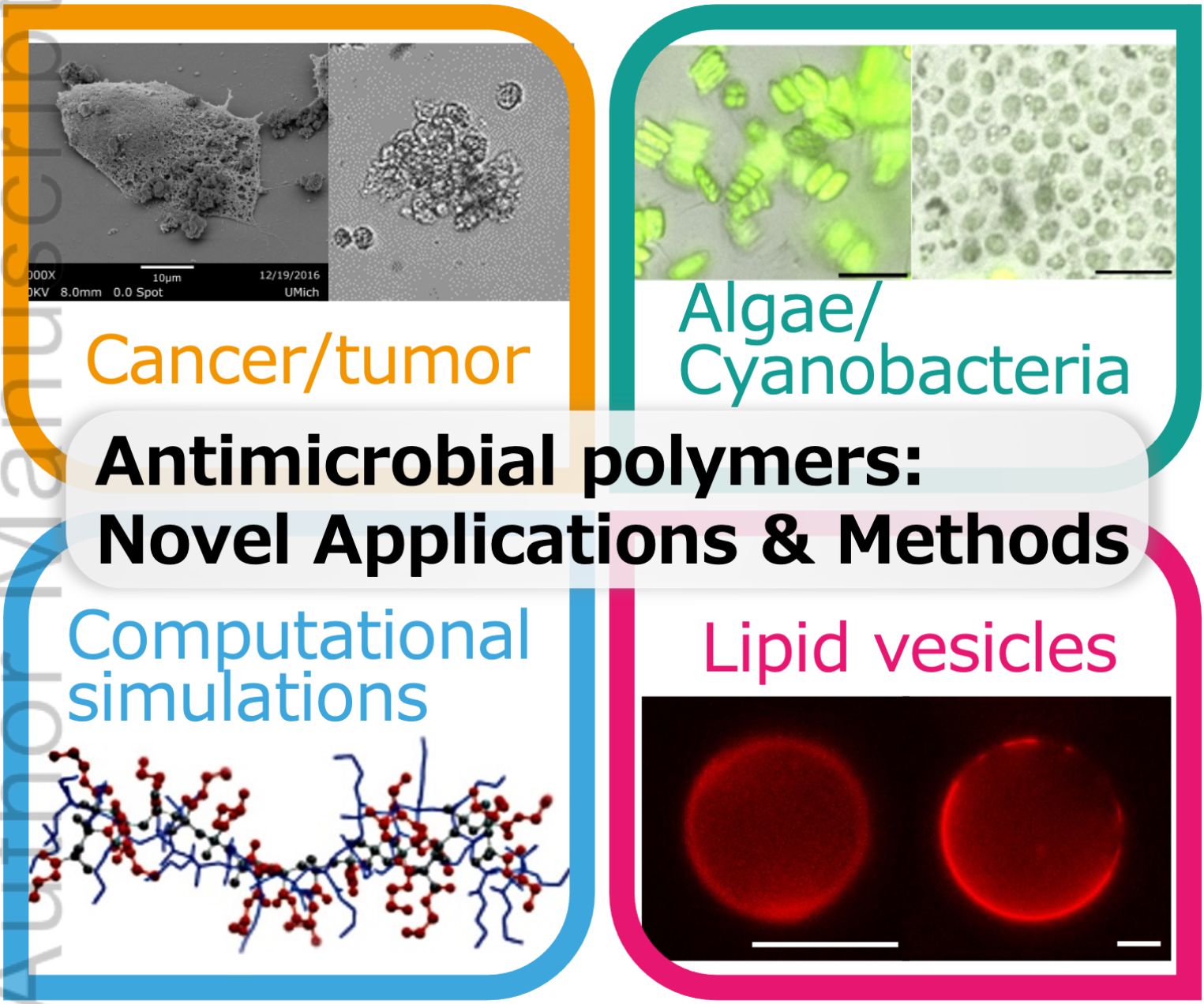
HR-Figure8.tiff



HR-Figure9.tiff



TOC.tiff



WNAN_1866_TOC.png