Doctoral dissertation

Antimicrobial effect - decomposition of biological phenomena into physical approach - a theoretical model

Mateusz Rzycki

Abstract

The widespread problem of antibiotic resistance in bacteria requires the application of many interdisciplinary approaches. An example of that approach is to reduce it to the form of appropriate interactions. The principal aim of this dissertation became to develop physical and numerical models of biological membranes that allow the evaluation of the antimicrobial activity of Gemini-structured molecules. In this thesis, the approaches from physics, as well as chemistry and biology, have been combined in order to create probabilistic and numerical bacterial membrane models that enable the assessment of the surfactants' effects on physicochemical and thermodynamic bilayer properties. One of the proposed concepts assumes the description of the activity of the compound on the cell membrane through dipole-dipole interactions in a non-conservative field due to the viscosity of the medium. An employed strategy that reduces the problem to physical interactions aims toward providing a broader perspective and knowledge in the uneven fight against antibiotic-resistant bacteria.

The widespread antimicrobial resistance in bacteria was identified in 2015 as one of the top 10 global threats to human health by the World Health Organization. Unless new drugs are discovered by 2050, millions of people will become victims to drug-resistant bacteria every year. Especially overwhelming is the constant expansion of so-called superbugs, such as *Klebsiella pneumoniae*, which cause a wide spectrum of infections and exhibit ongoing acquisition of drug resistance even to antibiotics of last resort. There are many factors why microorganisms become resistant, including constant mutations, *i.e.* modification of genetic material, acquisition of resistance genes from other organisms (lateral transfer). The human factor also has a strong influence on bacterial resistance by frequent overuse of antibiotics, insufficient doses, or too short antibiotic therapy duration. Over the years, bacteria are escaping from the lethal activity of antibiotics by developing their own adaptive mechanisms. A promising approach to solve that issue is to focus on antiseptics that do not have a well-defined molecular target in bacterial cells, unlike current antibiotics, but target bacteria structures, in general. It has been reported that some of these agents selectively attack cell membranes inducing their destruction by emulsification. Several commonly used antimicrobial agents from the cationic surfactants (Gemini surfactants) family are commercially available. The most well-known are octenidine (OCT) and chlorhexidine (CHX), which are effective against both Gram-positive and Gram-negative bacteria. In addition, new compounds within the Gemini structures are synthesized each year and present potential antimicrobial activity. The comparison of the antibacterial efficacy of given compounds becomes problematic due to various protocols used by research groups. Therefore the selection of potential precursors that can substitute currently used antibiotics becomes challenging.

The first work in the thesis (Paper 1 - Section 6) was focused on delivering insights into the molecular interactions of OCT and CHX on negatively charged (*i.e* bacterial) and zwitterionic (*i.e.* eukaryotes) membranes. The use of numerical and experimental methods allowed to determine the preferential location of these compounds, their formation of aggregates, their destructive effect on

membranes, and to describe the changes in membrane mechanical parameters under the analyzed molecules' activity. The in-depth analysis allowed to propose a novel mechanism of the selective antibacterial compounds' action, especially for OCT - based on differences between emerging mechanical properties of bacterial and eukaryotic membranes, respectively.

In the further research stage (Paper 2 - Section 7), other Gemini-structured compounds with strong antibacterial activity were investigated. Since different protocols and bacterial strains are used in experimental studies, comparison of the antimicrobial efficiency remained unfeasible. Therefore we decided to provide a systematic theoretical approach, hence we collected a database of over 250 Gemini molecules with potential antibacterial activity. The performed quantum calculations allowed to optimize the force fields of all the collected molecules. Employing a standardized protocol based on molecular dynamics simulations, 25 molecules with estimated potential antibacterial activity were tested on three-component model of bacterial membrane. The analysis of membrane parameters allowed to define a pre-emulgation stage, and to select the 8 most promising precursors with possible strongest antimicrobial activity.

Computational studies often address questions regarding the accuracy and reliability of presented models to real biological structures. The models may differ significantly from real or experimental analogs, thus resulting in inaccurate conclusions. Therefore, in the following research (Paper 3 - Section 8), a comprehensive numerical model, reflecting the detailed (multicomponent) lipid structure of *Escherichia coli* bacteria was proposed. It was also determined whether the complexity may significantly affect the conclusions drawn from numerical analyses of simplified models. An in-depth analysis of structural, dynamic, and mechanical properties revealed that the complexity and composition of the bacterial membrane model are crucial. The potential lack of complexity in current numerical bacterial membrane models undoubtedly affects the fundamental origins of interactions, so that some biological phenomena may remain elusive or suppressed.

The awareness of the limitations in experimental and well-established numerical methods has inspired me to the development of a dedicated methodology and original simulation software for the rapid evaluation of antibacterial potential in Gemini surfactants (Paper 4 - Section 9). Molecular dynamics simulations allow to assess the compound behavior on membranes with the potential structure interactions. The free energy calculation methods enable to precisely determine the behavior of antibacterial molecules in contact with the membrane, indicating the location of energy barriers or preferential agent location. Construction of an accurate energy profile for Gemini-type molecule acting on the membrane is computationally demanding and time-consuming process. Simplification of the interaction scheme and limitation of redundant parameters allowed to develop a unique software called *Diptool*, a tool for rapid screening the membrane-agent interactions and for potential antimicrobial candidate selection. *Diptool* is primarily based on the dipole interactions of lipids and antimicrobial compounds, whereas a modified Verlet algorithm is responsible for the integrating equations of motion. Using an experimental set of Gemini surfactants we provided quantitative structure-activity relationship (QSAR) studies, which in the end allowed to select the most significant parameters that influence the agent antimicrobial activity. Implementation of those identified parameters within the *Diptool* allowed to assess whether the molecule penetrates the membrane and what the free energy distribution looks like. This, in the end, allows for a direct estimation of the agent's antibacterial potential. The tool was validated with the Adaptive Biasing Force approach, well-known from molecular dynamics. The greatest advantage of the *Diptool* software is its speed, since can provide results comparable to full-atom MD simulations but in a matter of minutes.

To summarize, in this dissertation a selective activity molecular mechanism of widely used antibacterial compounds OCT and CHX was proposed. Subsequently, a Gemini molecule database was developed and a unified numerical protocol to study antimicrobial compounds was proposed. Further, an improved and comprehensive numerical model of *E. coli* bacteria was delivered. Finally, an original methodology and software for the analysis of the antibacterial potential of Gemini-structured molecules were developed. The core of the dissertation consists of four scientific publications (Paper 1-4), in which

the above achievements were described, covering methodology, implementation stage, and verification of assumptions.