MOLECULAR DYNAMICS SIMULATIONS ASSESSING MECHANISTIC FUNCTIONING OF NEW-GENERATION ANTIBACTERIALS AND HYDROPHOBIC GATING OF ION-CHANNELS

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NOVEMBER 2023

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by

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Submitted
in fulfillment of the requirement of the degree of doctor of philosophy

to the

INDIAN INSTITUTE OF TECHNOLOGY DELHI
NOVEMBER 2023

Dedicated to

My Family

Certificate

This is to certify that the thesis titled "**MOLECULAR DYNAMICS SIMULATIONS ASSESSING MECHANISTIC FUNCTIONING OF NEW-GENERATION ANTIBACTERIALS AND HYDROPHOBIC GATING OF ION-CHANNELS**" is being submitted by **Ms. Monika Kumari** to the Department of Chemistry, Indian Institute of Technology Delhi, for the award of the degree of **Doctor of Philosophy**. This thesis is a record of bonafide research work carried out by her under my supervision. In my opinion, the thesis has reached the standards fulfilling the requirements of the regulations relating to the degree.

The results contained in this thesis have not been submitted to any other University or Institute for the award of any degree or diploma.

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ACKNOWLEDGMENTS

"No one who achieves success does so without acknowledging the help of others. The wise and confident acknowledge this help with gratitude."

-Alfred North Whitehead

With God's grace and blessings, I have this opportunity to express my gratitude to those who have helped and supported me during this journey.

First, I begin by sincerely appreciating and acknowledging the invaluable contribution of my advisor, Prof. Hemant K. Kashyap, in fulfilling this endeavor. I am immensely grateful for his unwavering support and guidance through my Ph.D. His profound knowledge of the field, insightful feedback, and continuous encouragement has been instrumental in shaping the quality and direction of my research work.

I would also like to extend my sincere gratitude to my research committee members, Prof. Shashank Deep, Prof. Pramit K. Chowdhury, and Prof. Sujin B. Babu (Department of Physics), for their timely evaluations, insightful discussions, and constructive suggestions. I am also thankful to Prof. Siddharth Pandey (Head, Department of Chemistry) and Ex. Heads (Prof. Narayanan D. Kurur, Prof. Anil J. Elias, and Prof. Ashok K Ganguli) for providing necessary departmental facilities. I would also like to thank my collaborators, Dr. Shounak Roy and Prof. Amit Jaiswal from IIT Mandi and Prof. Tapan K. Nayak from KSBS, IIT Delhi, for the stimulating discussions and suggestions. Thanks to the entire team of the central supercomputing facility at IIT Delhi (HPC IITD) for providing the computational resources essential for carrying out the work reported in this thesis. I gratefully acknowledge the financial support from UGC-India through research fellowship and DST-SERB and IIT Delhi for providing travel grants to attend conferences.

I have been blessed with a very supportive and loving family who was there with me every step. My mother, Mrs. Amita Devi, and my sister, Dr. Pinki Yadav, have always been my inspiration and helped me make the right decisions in life. I devote this thesis to both of them. My father, Mr. Suresh K. Yadav, and my brother, Pushpender, are the two pillars of my strength and confidence. They are my true source of courage and positive energy in every situation. I also thank my siblings for always being by my side through the highs and lows and being my best

friends. I owe all my accomplishments to my family. I am tremendously grateful to my brother-in-law, Mr. Mandeep Poonia, and Mama, Mr. Manoj Yadav, for their unconditional support and words of encouragement. Words can't do justice to the selfless contribution of Mr. Shiv Naresh Yadav in this journey; still, I thank him for always having faith in me and motivating me in moments of weakness and self-doubt. I owe a tremendous sense of appreciation to all my labmates during my Ph.D. I extend my heartfelt appreciation and gratitude to my seniors, Dr. Pratibha Kumari, Dr. Shobha Sharma, Dr. Supreet Kaur, Dr. Aditya Gupta, Dr. Vikas Khatri, and Dr. Harender S. Dhattarwal for their generous help and timely advice. Their friendly attitude and constant guidance have been pivotal for my personal and professional growth. Thanks Akshay for always cheering up for my every small accomplishment and being a true friend along this journey. I would also like to thank Shobhna for the cooperation and helpful discussions and other lab members, Navneet Singh, Mrityunjaya K. Jha, Ayishwarya Dutta, and Puneet Kumar, for bringing energetic vibes and an amicable environment to the lab. I am also thankful to my friends from hostel, Ragini Jain, Dr. Nancy Varshney, Dr. Kirandeep, and Sumana for their cheerful presence. A special thanks to Dr. Shilpa Sharma for being my go-to person and patiently listening my prattles about random situations. I hope our bond lasts forever. I extend my earnest gratitude to Dr. Parul Jain and Dr. Shifali Kalra for being the best roommates and treating me like a younger sister with affection and care. Finally, I am thankful to all the people who have directly and indirectly helped me during this journey.

Monika Kumari

Abstract

In this thesis, we report molecular dynamics (MD) investigations of two major contemporary biological concerns, (i) providing distinct functioning mechanisms of promising next-generation antibacterials and (ii) a detailed structural analysis along with the elucidation of Na^+ ion permeation pathway of acetylcholine receptor (AChR) transmembrane proteins.

In the first part, we outline different molecular mechanisms adopted by novel antibacterials to exert their bactericidal action against Gram-positive and Gram-negative bacterial membranes. The motivation behind this work arises from the rapid emergence of multi-drug resistant bacteria and the quest for new-generation antibacterials with minimal probability to induce bacterial resistance acquisition. Membrane-directed antibacterials such as polycations and two-dimensional (2D) nanomaterials that disrupt the bacterial membrane integrity as their mode of action can effectively address the concern. A comprehensive understanding of the molecular interactions responsible for the membrane destructing action is essential to efficiently modulate their antimicrobial potency. We first studied the antibacterial action of quaternary ammonium-substituted derivative of a natural polysaccharide, pullulan, against both Gram-negative (*Escherichia coli* (*E. coli*)) and Gram-positive (*Staphylococcus aureus* (*S. aureus*)) bacterial membranes. Next, we explored the molecular basis of broad-spectrum antibacterial activity of 2D molybdenum disulfide (MoS_2) against the cellular membranes of *E. coli* and *S. aureus* bacterial strains. The efficacy of these materials has been evaluated in terms of their deleterious impact on the structural features of the bacterial membranes. The primary interactions responsible for the destructive action of these prospective antibacterial candidates have been assessed through their pair interactions energies and H-bonds with the lipid components of the bacterial membranes. The emergence of inhomogeneity in lateral distribution of lipids has been deciphered using computed lateral number density profiles. Various other structural properties of bacterial membranes such as tail fluidity, surface roughness, lipid lateral diffusion, membrane thickness etc. have been computed to quantitatively estimate the disintegrating action of these antibacterial candidates.

In the second part of the thesis, we have elucidated the mechanism of hydrophobic

gating in the acetylcholine receptor (AChRs) channel pore. Neuromuscular AChRs are hetero-pentameric, ligand-gated ion channels, which play critical roles in muscle contraction and cognition. The binding of the neurotransmitter acetylcholine (ACh) to two target sites promotes a global conformational change of the receptor that opens the channel and allows ion conduction across the channel pore. The 'gate' is a complex, independent, macromolecular machine in AChR channel pore that regulates ion conduction. We have performed both equilibrium and non-equilibrium atomistic MD simulations for the structural analysis and deciphering the underlying free energy of Na^+ ion permeation in recently solved structures of ligand-gated AChRs. In addition, the impact of leucine (hydrophobic) to serine (hydrophilic) mutation at the lower end of the hydrophobic barrier was studied to elucidate the probable presence of hydrophobic residue interactome.

सार

इस थीसिस में, हम दो प्रमुख समकालीन जैविक चिंताओं की आणविक गतिशीलता (MD) जांच की रिपोर्ट करते हैं, (i) आशाजनक अगली पीढ़ी के जीवाणुरोधी के विशिष्ट कार्य तंत्र प्रदान करते हैं और (ii) Na^+ आयन पारगम्य मार्ग की व्याख्या के साथ एक विस्तृत संरचनात्मक विश्लेषण एसिटाइलकोलाइन रिसेप्टर (एसीएचआर) ट्रांसमेम्ब्रेन प्रोटीन। पहले भाग में, हम ग्राम-पॉजिटिव और ग्राम-नेगेटिव बैक्टीरियल झिल्ली के खिलाफ अपनी जीवाणुनाशक कार्रवाई करने के लिए नवीन जीवाणुरोधी द्वारा अपनाए गए विभिन्न आणविक तंत्रों की रूपरेखा तैयार करते हैं। इस कार्य के पीछे की प्रेरणा बहु-दवा प्रतिरोधी बैक्टीरिया के तेजी से उभरने और बैक्टीरिया प्रतिरोध अधिग्रहण को प्रेरित करने की न्यूनतम संभावना के साथ नई पीढ़ी के जीवाणुरोधी की खोज से उत्पन्न होती है। झिल्ली-निर्देशित जीवाणुरोधी जैसे कि पॉलीकेशन और द्वि-आयामी (2D) नैनोमटेरियल जो बैक्टीरिया झिल्ली की अखंडता को बाधित करते हैं क्योंकि उनकी क्रिया का तरीका प्रभावी ढंग से चिंता का समाधान कर सकता है। आणविक अंतःक्रियाओं की व्यापक समझ झिल्ली को नष्ट करने की क्रिया के लिए जिम्मेदार लोगों की रोगाणुरोधी क्षमता को कुशलतापूर्वक नियंत्रित करना आवश्यक है। हमने सबसे पहले ग्राम-नेगेटिव (एस्चेरिचिया कोली (ई. कोली)) और ग्राम-पॉजिटिव (स्टैफिलोकोकस ऑरियस (एस. ऑरियस)) बैक्टीरियल झिल्ली दोनों के खिलाफ प्राकृतिक पॉलीसेकेराइड, पुलुलन के चतुर्धातुक अमोनियम-प्रतिस्थापित व्युत्पन्न की जीवाणुरोधी कार्रवाई का अध्ययन किया। इसके बाद, हमने ई. कोली और एस. ऑरियस बैक्टीरियल उपभेदों के सेलुलर झिल्ली के खिलाफ 2D मोलिब्डेनम डाइसल्फ़ाइड (MoS_2) की व्यापक-स्पेक्ट्रम जीवाणुरोधी गतिविधि के आणविक आधार का पता लगाया। इन सामग्रियों की प्रभावकारिता का मूल्यांकन जीवाणु झिल्ली की संरचनात्मक विशेषताओं पर उनके हानिकारक प्रभाव के संदर्भ में किया गया है। इन संभावित जीवाणुरोधी उम्मीदवारों की विनाशकारी कार्रवाई के लिए जिम्मेदार प्राथमिक इंटरैक्शन का मूल्यांकन किया गया है। उनकी जोड़ी जीवाणु झिल्ली के लिपिड घटकों के साथ ऊर्जा और एच-बंधों की परस्पर क्रिया करती है। लिपिड के पार्श्व वितरण में असमानता का उद्भव हुआ है, गणना की गई पार्श्व संख्या घनत्व प्रोफाइल का उपयोग करके व्याख्या की गई। जीवाणु झिल्लियों के कई अन्य संरचनात्मक गुणों जैसे पूंछ की तरलता, सतह का खुरदरापन, लिपिड पार्श्व प्रसार, झिल्ली की मोटाई आदि की गणना मात्रात्मक रूप से अनुमान लगाने के लिए इन जीवाणुरोधी उम्मीदवारों की विघटित कार्रवाई की गई है।

थीसिस के दूसरे भाग में, हमने एसिटाइलकोलाइन रिसेप्टर (एसीएचआर) चैनल छिद्र में हाइड्रोफोबिक गेटिंग के तंत्र को स्पष्ट किया है। न्यूरोमस्कुलर एसीएचआर हेटेरो-पेंटामेरिक, लिगैंड-गेटेड आयन चैनल हैं, जो मांसपेशियों के संकुचन और अनुभूति में महत्वपूर्ण भूमिका निभाते हैं। न्यूरोट्रांसमीटर एसिटाइलकोलाइन (एसीएच) का बंधन दो लक्ष्य स्थलों तक रिसेप्टर के वैश्विक गठनात्मक परिवर्तन को बढ़ावा देता है जो चैनल खोलता है और चैनल छिद्र में आयन संचालन की अनुमति देता है। 'गेट' AChR चैनल छिद्र में एक जटिल, स्वतंत्र, मैक्रोमोलेक्यूलर मशीन है जो आयन चालन को नियंत्रित करती है। हमने लिगैंड-गेटेड एसीएचआर की हाल ही में हल की गई संरचनाओं में संरचनात्मक विश्लेषण और Na^+ आयन पारगमन की अंतर्निहित मुक्त ऊर्जा को समझने के लिए संतुलन और गैर-संतुलन परमाणु एमडी सिमुलेशन दोनों का प्रदर्शन किया है। इसके अलावा, हाइड्रोफोबिक बाधा के निचले सिरे पर ल्यूसीन (हाइड्रोफोबिक) से सेरीन (हाइड्रोफिलिक) उत्परिवर्तन के प्रभाव का अध्ययन हाइड्रोफोबिक अवशेष इंटरैक्टोम की संभावित उपस्थिति को स्पष्ट करने के लिए किया गया था।

Permissions

Permissions have been taken from the respective journals to reprint the publications related to the work presented in this thesis.

List of Publications Related to Work Presented in this Thesis

1. **Monika Kumari**, Shounak Roy, Amit Jaiswal, and Hemant K. Kashyap, Anionic Lipid Clustering Mediated Bactericidal Activity and Selective Toxicity of Quaternary Ammonium Substituted Polycationic Pullulan Against *Staphylococcus aureus* Bacterial Membrane, *Langmuir* **2022**, 38, 8065–8076.

2. **Monika Kumari** and Hemant K. Kashyap, MoS_2 Nanosheets Induced Destructive Alterations in *Escherichia coli* Bacterial Membrane, *Soft Matter* **2022**, 18, 7159-7170.

3. **Monika Kumari** and Hemant K. Kashyap, Wrapping-trapping versus Extraction Mechanism of Bactericidal Activity of MoS_2 Nanosheets against *Staphylococcus aureus* Bacterial Membrane, *Langmuir* **2023**, 39, 5440–5453.

4. **Monika Kumari**, Nadira Khatoon, Rachita Sharma, Sushanth Adusumilli, Anthony Auerbach, Hemant K. Kashyap, Tapan K. Nayak, Hydrophobic Gating of the Acetylcholine Receptor Channel Pore, **2023**. [In revision]

Contents

List of Figures

List of Tables