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Classification of LexA mobility states during the SOS response in Escherichia coli using single-molecule tracking PALM

Kurzbeschreibung:

Antibiotic-resistant bacteria are amongst leading health threats in current society, affecting individuals of any age or region. Though naturally occurring phenomenon, antibiotic resistance in bacteria has rapidly increased in the past 20 years due to misuse of antibiotic treatments in healthcare, agriculture and animal husbandry. This ultimately leads to all known antibiotics losing their potency. Previously manageable bacterial infections such as pneumonia, tuberculosis, foodborne diseases and sepsis are becoming harder to treat which results in over 700 000 deaths each year due to antibiotic-resistance. Underlying this worldwide health threat, is the remarkable ability of bacteria to rapidly mutate and change their genetic information molecule – DNA, upon encountering the drug. A universal pathway to acquiring resistance in all bacteria is termed – SOS response, and the main gene driving this process is lexA. LexA regulates the entire network of genes, solely dedicated to increasing mutations which can lead to resistance to virtually any antibiotic. Tackling antibiotic resistance would require inhibiting and preventing the SOS response, thereby disabling bacteria from changing and adapting to drug-treatments. In order to advance the newest drug-development and inhibition strategies and prevent the current rise in antibiotic-resistant bacteria, I, together with my dear colleagues, set to learn more about this system and the way it is activated in the presence of the drug.

Escherichia coli, a bacterium universally present in human digestive system and known to cause multiple health threats when in its pathogenic form (urinary tract infections, respiratory infections, diarrhea and even meningitis) served as a model system for this antibiotic resistance study. We developed a novel genetic engineering strategy and used advanced microscopy techniques to study the system on the smallest scale. “Single-molecule tracking photoactivated localization microscopy” or smtPALM for short, is a state-of-the-art microscopy technique with an ability to “see” and track individual molecules with the precision of several billionths of a meter and a thousandth of a second. This incredible precision and speed allowed us to observe the very beginning of the bacterial reaction to antibiotics by monitoring the key protein in this process – a beforementioned, LexA. We observed this molecule adopting multiple dynamic states, which change rapidly upon antibiotic treatment in a predictable and dosage-dependent manner. I further investigated this by applying higher antibiotic concentrations, higher treatment times and engineered new bacterial mutants with high susceptibility to observe the dynamics of the initial steps in acquiring the resistance. A combination of genetic engineering, microbiology, advanced microscopy and biophysical modeling in my MSc project, had resulted in a first-time model of the dynamic states that LexA, a key protein in SOS regulatory network. Even though this had been an area of research for decades, fundamental knowledge on the very beginning of this process was obscure, hence the use of novel technologies could help us unravel the missing information and post and important base for further research. Future studies can expand on this knowledge and ultimately lead to novel drug development, targeting antibiotic resistance in the

very initial steps of this process, thereby drastically reducing the chances of mutations and hence treatment costs and hospitalization time . I was fortunate enough, and forever grateful, to be involved in such project which not only spurs further development and understanding in the field of bacterial antibiotic resistance, but also lays a foundation for future research in the at BCUBE - Center for Molecular Bioengineering in Dresden, on this topic of such importance in contemporary society.

Botschaft und Begründung der Bewerbung:

Having moved to Dresden two years ago as a foreigner, I was met with an incredible amount of support. Dresden's scientific excellence in the field of Biophysics stood out to me the most, and provided the perfect environment for me to do research at a level that I have never been able to do previously. Originally, I come from Serbia, where I have pursued my undergraduate in Molecular Biology at the University of Belgrade. I was lucky enough to be presented with the incredible potential that research and science can offer to modern society early in my studies, but never had the opportunity to practically participate in research in Serbia. Nevertheless, I was motivated understand the underlying causes of many of the major health issues that the society is facing – viral infections and vaccines, antibiotic resistance, cancer, diabetes etc. My diligence was recognized by being awarded multiple scholarships during my undergraduate education both from national ("State Scholarship", "Scholarship for exceptionally talented scholars and students", Scholarship "Dositeja" - Top 800 students in the country) and private sector ("Promedia doo" – specialists at lab supplies and microbiology reagents). Being honored to receive this support during my education, I was introduced the benefits and opportunities it opens up for further development and future career in research of any individual. It was in my undergraduate studies when I was introduced to the remarkable scientific community in Dresden, at the Biotechnology Center – BIOTEC, by an alumni student in his final year of PhD. I understood the large international community present there and the rapid development in interdisciplinary research in Dresden, bringing foreign individuals from various disciplines to collaborate and produce incredible research.

Extraordinarily, recognizing the scarce financial conditions that can affect individuals from less developed countries when moving to Germany, Haniel Stiftung's and Klaus Tschira's foundation offers the Go-West scholarship to the best student coming from Middle or Eastern Europe to Dresden. I am grateful to have been selected as the top student for this award amongst many international students enrolled in my course, as it allowed me to focus on my studies and research. Keeping my academic record amongst the very best of the class, I have been awarded the same scholarship for my second year of studies too. During the second year I have joined the biophysics lab of Michael Schlierf at the Center for Molecular Bioengineering – BCUBE, where I could use my microbiology background in combination with the state-of-the-art microscopy techniques to tackle one of the most threatening health concerns in modern society, according to the World Health Organization – antibiotic resistance in bacteria. I was lucky to be part of a great team to produce novel research and advancements in the field of antibiotic resistance.

My work has ultimately resulted in two scientific publications regarding a novel method of genetic engineering and application of advanced microscopy techniques to

investigate the initial stages in the bacterial reaction to antibiotic treatment. My contribution to this work includes a first authorship paper published in the peer-reviewed journal of the American Chemical Society – Synthetic biology, and a second authorship paper under review in Science Advances journal of the American Association for the Advancement of Science – AAAS. Being financially supported before I am aware of the recognition given for the hard work on the noble cause and I feel appreciative to be in the position to apply for the Dresden Excellence Award this year. If recognized by this award, I would use it as a platform to represent Dresden and impact other foreign students, in my home country and where I am currently located, to explore Dresden's scientific community which can offer tremendous opportunities for future scientists in various fields of research.

Nächste Ziele und Vorhaben:

Following my MSc in Dresden, I became inspired by the interdisciplinary approaches in science, combining multiple fields such as biology, physics and computational science in tackling real-world problems and issues. For this reason, I continued my research and education as a PhD in a biophysics lab at the Technical University of Delft, Department of Bionanoscience in the lab of a Dist. Prof. Cees Dekker. My project, similarly, deals with the basic principles of bacterial function, how is the DNA organized and how it reacts to different conditions such as antibiotic presence. Benefiting from the experience and knowledge I obtained in Dresden, I use new, advanced microscopy techniques to study bacteria on the smallest scales - down to nanometers resolution (a billionth of a meter). In addition, I use polymer physics, which I learned the basics of during my Master's, to describe the interactions of proteins with the DNA in a system isolated from live cells.

I am highly motivated and fascinated with scientific research, and I hope to continue working in academia throughout my career until ultimately, I can teach and supervise my own students as a professor, transferring the enthusiasm and novel views onto them as some of the great professors have done for me. I keep strong connection to the city of Dresden both personally, since many of my friends are located there continuing their scientific careers, as well as scientifically. Even after the completion of my MSc thesis, I have engaged in collaborative work as a visiting scientist in the city of Dresden during last Autumn, working on a novel genetic engineering method that I have started during my Master's education, useful for biophysical studies on virtually all proteins. Additionally, I try to encourage younger students in Serbia to apply and explore the science in Dresden, by holding invited talks about my MSc work and experience in Dresden back at the University of Belgrade. I am thrilled to see that the city of Dresden has been awarded with a national grant for the Physics of Life research, maintains the "Cluster of Excellence" status, and overall how fast the science community is expanding. A strong collaborative network between Center for Regenerative Therapies (CRTD), Centers for Molecular Bioengineering and Biotechnology (BCUBE and BIOTEC) as well as outstanding Max Planck Society, Institute of Polymer Research Dresden (IPF), Center for Neurodegenerative Diseases (DZNE), Medical center and many others, give a unique research experience with high support and opportunities for researchers in all stages in their careers. I am humbled to have had the opportunity to be a part of it and most

sincerely hope I will return there one day to continue contributing to the research of Dresden on my own.