Skoltech
Skolkovo Institute of Science and Technology

Jury Member Report – Doctor of Philosophy thesis.

Name of Candidate: Dmitry Sutormin

PhD Program: Life Sciences

Title of Thesis: Regulation of bacterial genome topology by topoisomerases

Supervisor: Professor Konstantin Severinov

Name of the Reviewer: Keir C. Neuman

I confirm the absence of any conflict of interest (Alternatively, Reviewer can formulate a possible conflict)	**Date: 21-08-2022**

The purpose of this report is to obtain an independent review from the members of PhD defense Jury before the thesis defense. The members of PhD defense Jury are asked to submit signed copy of the report at least 30 days prior the thesis defense. The Reviewers are asked to bring a copy of the completed report to the thesis defense and to discuss the contents of each report with each other before the thesis defense.

If the reviewers have any queries about the thesis which they wish to raise in advance, please contact the Chair of the Jury.

Reviewer's Report

Reviewers report should contain the following items:

- Brief evaluation of the thesis quality and overall structure of the dissertation.
- The relevance of the topic of dissertation work to its actual content
- The relevance of the methods used in the dissertation
- The scientific significance of the results obtained and their compliance with the international level and current state of the art
- The relevance of the obtained results to applications (if applicable)
- The quality of publications

The summary of issues to be addressed before/during the thesis defense

The thesis "REGULATION OF BACTERIAL GENOME TOPOLOGY BY TOPOISOMERASES" submitted by Dmitry Sutormin covers the development and application of in vivo sequencing methods to achieve base pair resolution of the cleavage sites of the three bacterial topoisomerases under a ride range of physiological conditions. The thesis includes a well-written and insightful overview of bacterial topoisomerase structure and function followed by detailed description of the development of the technique that was coined as "Topo-Seq" and concluding with the implementation of Topo-Seq to determine the locations and sequence context of the binding of DNA Gyrase, Topoisomerase IV, and Topoisomerase IA. The overall quality of the thesis and the results are exceptional. The thesis is well-organized and the writing is of high quality for the most part. The development of the Topo-Seq methodology is a major breakthrough that brings a new level of detail and precision in identifying the distribution and site-specific activity of the three major bacterial topoisomerases. The results, particularly, with Gyrase and TopoIA are exceptionally through and bring new important insights to the field of bacterial topology regulation and overall physiology. The level of scientific rigor is exceptionally high. The experimental design is well-conceived and include important controls. The conclusions are well-supported by the data, which is of very high quality. Given the importance of topoisomerases as targets for antibacterial therapies, it is likely that the techniques developed and the results obtained in this thesis will contribute to the further development and refinement of antibacterial agents. Overall this is an exceptional thesis that was a pleasure to read. This evaluation is reflected in the three first author publications that have arisen from the thesis that are all of very high quality, having been published in leading international journals and been widely appreciated within the topoisomerase community.

I have sent the candidate a marked copy of the thesis that includes edits and suggestions ranging from the correction of typos to discussion of alternative hypothesizes or the request to place aspects of the work in a broader context in the field. For the purpose of the formal thesis defense I would like to see the following issues addressed during the defense:

1. On page 13 of the thesis there is a discussion of the number of ATP hydrolyzed during one round of strand passage by type IIA topoisomerases. The conclusion that two ATP molecules are hydrolyzed is perhaps premature given the available data. It would be good to review this claim and discuss the available data.
2. Page 15. In a somewhat related point, which is important for some of the conclusions drawn later in the thesis, the relationship between DNA binding, DNA bending, cleavage, and topoisomerase gate opening and closing can be discussed and perhaps revised with respect to the role of DNA bending and also the order of gate closure, strand passage and religation over the catalytic cycle of type IIA topoisomerases.
3. Page 18. The discussion of poisoning of type II topoisomerase poisons is focused on their role in disrupting metal ion interactions. This should be expanded to include the notion of "interfacial inhibitors" that is an important aspect of inhibition.
4. Page 29. I would like to see a more detailed discussion of r-loop formation starting with a negatively supercoiled DNA molecule and including the impact of R-loop formation in capturing negative supercoiling (-Lk) on the overall process.
5. This will likely be part of the defense presentation but I would like to see a high level description of the development of the Topo-Seq approach and the small differences between the three implementations for the three main enzymes studied.
6. Page 51. In the defense I would like to see a brief discussion of the statistical approaches used to obtain the confidence intervals and to see a worked example of one of these calculations. They

are referenced in the thesis but I would like to see some indication of how they are applied in one instance.

7. Page 70. I would like to see more discussion on possible explanations as to why there is more cleavage by topo IV in the Gyrase S83l background. The notion of more Cpx molecules seems unlikely to me.
8. Page 73. The in vitro measurements of the sequence specificity of topo IV should be referenced and discussed in relation to the findings presented in the thesis.
9. Page 75. The use of Monte Carlo simulations to determine topoisomerase binding peaks is referenced but I would like to see a brief discussion of how this is achieved in the defense.
10. Once of the significant findings and broad conclusions from this thesis is the notion of constrained versus unconstrained supercoiling associated with transcription in different bacteria. I would like to see a broader discussion of this finding in the thesis presentation. Are there expected consequences of this difference on things such as global response to supercoiling? Also, are there other ways that this could be verified, or are there predictions that arise from this finding that can be tested?

Overall this is a terrific thesis. I am looking forward to the thesis defense.

Keir Neuman

Provisional Recommendation

☒ *I recommend that the candidate should defend the thesis by means of a formal thesis defense*

☐ *I recommend that the candidate should defend the thesis by means of a formal thesis defense only after appropriate changes would be introduced in candidate's thesis according to the recommendations of the present report*

☐ *The thesis is not acceptable and I recommend that the candidate be exempt from the formal thesis defense*