

## 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:

I confirm the absence of any conflict of interest  (Alternatively, Reviewer can formulate a possible conflict)	<b>Yes</b>  <b>Date: 15-08-2022</b>
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*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

This PhD thesis concerns the action of DNA topoisomerases *in vivo*. In general the thesis is of high quality, containing a substantial amount of data that has already been accepted for publication. The thesis topic is the regulation of bacterial genome topology by topoisomerases, and the work directly addresses this by analysing the sites of gyrase, topo IV and topo I in the genomes of *E. coli* and *C. crescentus*. The candidate discusses in detail the biological relevance of the distribution of these topoisomerases. The work is carried out using innovative methods, some of which the candidate has developed himself.

The work addresses a fundamental issue in topoisomerase biology, namely the role of these enzymes in transcription, particularly with reference to the Liu and Wang twin supercoiled domain model, published in 1987. In the intervening years it has been challenging to address the hypotheses raised by this model; the candidate has succeeded in doing this. The work has, so far, led to the publication of two high-quality papers.

Chapters 1 & 2, Introduction and Objectives, are clear and authoritative; the Introduction is based on a review that was written by the candidate. It is very focussed on bacterial topoisomerases and it would be useful to discuss broader issues with the candidate. There are few errors, and most of these are minor errors of English that can easily be corrected. There are a few discussion points raised by the Introduction.

Chapter 3 comprises Materials and Methods. This appears to be complete and accurate, although there are a few errors of English and minor formatting errors.

Chapter 4 comprises the Results and mainly consists of work published by the candidate. The initial section deals with mapping gyrase and topo IV sites *in vivo* in *E. coli* and *C. crescentus* under various conditions. Much of this work is already published and is consequently of high quality, and mainly well-written, except for minor errors. A few comments to be discussed with the candidate:

- The Chapter could do with more preamble to give context.
- Fig. 9 could do with a key to symbols.
- It would be interesting to discuss with use of inhibitors will identify gyrase binding sites *in vivo*.
- Discuss the finding that strong gyrase sites are poor for supercoiling in the context of previous work.
- P. 52: explain the effects of increased drug concentration.
- P. 56: Fig 15 – is the increase in GCSs for high versus low transcribed genes really significant? (oxo result).
- P. 57: is there much difference +/- rif? (Fig 16)
- P. 60: what is the significance of the BIME results?
- P. 65: role of Azamat?
- P. 69: why HCl and NaOH?
- It would be interesting to discuss the possibility of carrying out the gyrase/topo I analysis in *Mycobacterium tuberculosis*.
- P. 84, Fig. 33D – grey rectangles are not clear.
- P. 84, Fig. 33E – why use EMSA?
- P. 87 – discuss the annihilation of +ve/-ve supercoils by RNA polymerase.
- P. 88 – explain the difference between the open and closed models.

Chapter 5 is a short conclusions chapter; points from this can be discussed in the viva. As far as I can see the reference section is appropriate.

A few general comments:

- There is frequent mis-use of the work 'the'.
- The paragraphing is not consistent.
- There is some text in red that needs to be altered.

#### 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*