

Jury Member Report – Doctor of Philosophy thesis.

Name of Candidate: Andrey Krivoy

PhD Program: Life Sciences

Title of Thesis: Primed CRISPR-Cas adaptation in type I-E system of Escherichia coli: use of single-molecule and biochemical assays to verify models of the phenomenon at molecular level


Supervisor: Prof. Konstantin Severinov

Chair of PhD defense Jury: Prof. Konstantin Lukyanov

Email: k.lukyanov@skoltech.ru

Date of Thesis Defense: November 30, 2018

Name of the Reviewer:

I confirm the absence of any conflict of interest	Signature:  Date: 15-11-2018
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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

The present dissertation studies molecular mechanisms of phenomenon of primed CRISPR-Cas adaptation. A series of elaborated experiments were performed to distinguish between two alternative models of the primed adaptation. The first model suggests conformational changes of the Cascade effector complex as the main cause of priming, whereas the second model postulates that kinetics of interactions determines whether interference or priming occurs. The obtained results unambiguously demonstrated that the first model is wrong. In turn, this indirectly supports the second (kinetic) model. In general, this work represents a significant step forward in mechanistic understanding and methodology development in the field of CRISPR-Cas functioning. This work is a basic study and does not have immediate practical applications; however, some technical advances (such as single-molecule magnetic tweezers assay) can potentially be applicable for a variety of other investigations.

The topic of dissertation is fully relevant to its actual content. The author used a cutting edge method of magnetic tweezers together with classical techniques. The techniques used are adequate to the processes under investigation; all experiments are thoroughly designed and controlled.

The publications are impressive. One paper, with the first authorship of Andrey Krivoy, was published in Nucleic Acids Research – a top journal (impact factor 11.6) in the field of DNA and RNA studies. Second paper was published in Methods in Enzymology (impact factor 2.0 according to Web of Science, and 3.8 according to SCImago Journal Rank) – a recognized series of protocols published since 1955.

Some minor criticism and questions:

Fig. 10C and corresponding text (p. 51-52): “Overall, the effect of increased temperature was even more significant and together with Mg²⁺ resulted in approximately 8-fold increase of R-loop formation rate”. The effect of temperature itself (without Mg²⁺) remains unclear.

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