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Skolkovo Institute of Science and Technology

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: Ivana Ivančić-Baće

I confirm the absence of any conflict of interest (Alternatively, Reviewer can formulate a possible conflict)	**Signature:** I. Ivančić Bace **Date: 12-11-2018**

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 PhD thesis of the candidate Andrey Krivoy is original, it is of very high quality and the thesis dissertation is clearly written, well described and of excellent experimental results. The topic and methods of the thesis are relevant to its actual content and are of excellent scientific significance compliant with the international level and current state of the art. The thesis itself does not imply biotechnological application but the development of the single-molecule approach could be used in many other researches that might give applicable outcomes. The publications resulted from this thesis are of very high scientific quality and are published in the top scientific international journals. The aim of the research of this thesis was to better understand the mechanisms of primed adaptation at the single-molecule level using magnetic tweezers and changes in torque applied to single DNA molecules on type I-E CRISPR-Cas system of *Escherichia coli*. The research was accompanied by bulk biochemical assays and *in vivo* (quantitative and qualitative) analysis of primed adaptation. The thesis finishes with an attempt to reconstruct primed adaptation in the test tube *in vitro*. The hypothesis of the thesis was that either conformational changes in the Cascade effector complex decides if priming versus interference will occur, or kinetics of interaction between fully or partially matching targets. To differentiate between these two outcomes, the candidate developed the single-molecule magnetic tweezes experiment to measure the formation and properties of R-loops using purified Cascade complex (with g8 crRNA), plasmid containing g8 protospacer with consensus ATG PAM and targets variants with mutations in PAM (CCG) or seed region or PAM distal parts. In addition, variations in crRNA length were also made by making shorter or longer crRNAs (from 14 nt to 38 nt). Interestingly, full sized R-loops were formed for each PAM and seed mutant apart from CCG negative control and locking was much stronger than observed before for *S. thermophiles* Cascade. Stable R-loop formation was not observed for short -18 and -12 variants, while shorter variants (-6 and -3) supported locking and +6 variant was similar to *wt*. *In vitro* cleavage by Cas3 showed that the rate and efficiency of Cas3 cleavage strongly correlates to R-loop dissociation time, but that there was no correlation between mismatch position and priming *in vivo*. The same spacers were acquired for every seed mutant that was shown by high throughput sequencing. The most interesting mutant was T4G because it locked R-loops and recruited Cas3 as *wt* but adaptation was much higher than in *wt*. Since Cascade binding was slower in this mutant, this suggested that slower interference that supported R-loop formation is what supports more efficient primed adaptation. In other words, kinetic model better explains primed adaptation. Reconstitution of primed adaptation *in vitro* unfortunately failed, thus requiring further optimization but this was not the scope of this thesis.

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*