

Jury Member Report – Doctor of Philosophy thesis.

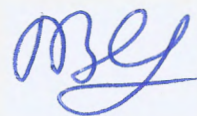
Name of Candidate: Iana Fedorova

PhD Program: Life Sciences

Title of Thesis: Characterization and application of CRISPR-Cas enzymes

Supervisor: Professor Konstantin Severinov

Name of the Reviewer: Petr Sergiev

<p>I confirm the absence of any conflict of interest</p> <p>(Alternatively, Reviewer can formulate a possible conflict)</p>	<p>Signature:</p>  <p>Date: 18-08-2020</p>
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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

PhD thesis “CHARACTERIZATION AND APPLICATION OF CRISPR-Cas ENZYMES” by Iana Fedorova comprises several interconnected studies united by the common theme of CRISPR-Cas system function and biotechnology application. The presented work was published in 6 manuscripts in the top level scientific journals, including absolutely leading ones, such as Cell and Nature biotechnology. Iana is the first author in two of them, published in Nucleic acids research making her personal contribution and scientific level unquestionable. Moreover, in the manuscript most recently published in the RNA biology journal, she is the last author, indicating her independent and leading contribution to the study.

The field of CRISPR-Cas system is highly dynamic, at the cutting edge of molecular biology and its biotechnology applications, such as genome editing, including genome therapeutics. This field, on one hand, allows to publish in on average higher impact journals, but on the other hand, it is also extremely competitive. One of the challenges of the field at the moment is to find smaller Cas nucleases to deliver their genes alongside with sgRNAs in a single safe and efficient viral vector, such as AAV.

In her work Iana first studied several new Cas nucleases which aimed to replace bulky Sp Cas9 in genomic editing applications. To begin, Iana studied *C. cellulolyticum* CRISPR/Cas system. Its PAM site consensus was determined and an attempt, unfortunately unsuccessful, to construct efficient sgRNA was performed. Next, the study was continued by an analysis of two more Cas9 enzymes, of *DeFluviimonas sp. 20V17* and *P. pneumotropica*. For both systems, PAM sites and cleavage preferences were determined and confirmed by the mutational analysis. Temperature dependencies of cleavage efficiencies were determined, which is important for genome engineering applications in mammalian hosts. For both enzymes sgRNA design was successfully achieved. Both enzymes were tested for their ability to drive DNA cleavage in mammalian system. While PpCas9 was found to be efficient in this assay, DfCas9 was inactive. It should be said, that PpCas9 is among the smallest enzymes of the kind, and as such, is potentially a good substitution for SpCas9 in biomedical applications.

Two chapters describe the work done while Iana was at her internship in the world leading laboratory of Feng Zhang. While her personal contribution into these couple of papers was not the main, still, it is a great achievement. These works were published in the top level scientific journals Cell and Nature biotechnology. Both chapters were devoted to a study of another blend of Cas nucleases, Cas12a/Cpf1. In the first paper the structure of the functional complex of above mentioned enzyme was described, while the second paper described an application of this system for multiplex genome cleavage, facilitated by the ability of Cas12a to process CRISPR array primary transcript.

In the paper published in RNA biology, Iana demonstrated the ability of Cas12e enzyme to produce 5'-overhangs at cleaved targets and, most interestingly, the dependence of overhang size on crRNA length.

Finally, in the paper int Nature communications, Iana described a new high throughput method for determination of short DNA intermediates of new spacer adaptation. Strand specificity of the developed technique allowed to suggest a particular structure of the DNA intermediates, being blunt at the PAM-distal and protruding at the PAM proximal end.

Presented thesis is a top level work perfectly done in the cutting edge research area. Only few minor questions raised while reading this text.

1. How reproducible were depletion/cleavage assays, say, those described on the page 39? How many biological replicates were taken for NGS analysis and what was the correlation between them?
2. CcCas9 was tested in a heterologous system. Are there any indications that all results should ideally apply for the natural host?
3. Author noted that the terminal nine nucleotides of *C. cellulolyticum* H10DRs have a sequence similar to bacterial extended -10 promoter consensus element (page 43). It is an interesting observation. Where, in your opinion, terminators of transcription are located?
4. Why, in your opinion, sgRNA was not functional for CcCas9?
5. How could you explain that some targets containing ideal PAM sites, such as target 3 (page 69) were not recognized by PpCas9? Would it limit possible biotechnology application? Are there any structural explanations for the difference in target recognition?
6. A difference in the specificity observed for in vitro and in vivo data (page 73) question the validity of in vitro based specificity tests, employed in other experiments. How this difference could be explained? Kinetics, thermodynamics of binding? Competition with DNA binding proteins, such as histones?
7. Figure 7c and 8. How indel frequency might be negative?

These questions are of course just a minor issue, while an overall impression on the thesis is superb. The work presented is a top level research which makes the reader enthusiastic about the achievements of the author. There are absolutely no doubts, that PhD thesis "CHARACTERIZATION AND APPLICATION OF CRISPR-Cas ENZYMES" meets all possible criteria for successful PhD thesis. Iana Fedorova is clearly a clever, successful, thoughtful, hardworking independent scientist, who certainly deserves PhD degree.

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