

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:

<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: 12-09-2020 DD-MM-YYYY</p>
---	---

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

I am writing to comment on the doctoral thesis submitted by Ms. Iana Federova entitled “Characterization and application of CRISPR-Cas enzymes.” As some background, I lead the RNA Synthetic Biology group at the Helmholtz Institute for RNA-based Infection Research, and I have a joint appointment as a W2 professor in the medical faculty of the University of Würzburg. Prior to joining the HIRI in 2018, I was a professor in the department of Chemical and Biomolecular Engineering at North Carolina State University starting in 2011. For the past nine years, my independent research program has focused on the intersection between CRISPR biology and technologies. My group’s work has touched on the same topics presented in this thesis, including Cascade-Cas3, Cas9, Cas12a nucleases, and newly discovered Type V nucleases as well as their applications in bacteria and human cells. This prior work well positions me to comment on the overall quality, completeness, and impact of the work.

As a summary, Ms. Federova pursued the characterization and initial application of diverse CRISPR nucleases. The beginning of her thesis provides an overview of CRISPR-Cas systems as adaptive immune systems and the different types of nucleases associated with these systems. The first and second chapters report the characterization of Cas9 nucleases from Type II-C CRISPR-Cas systems, with Ms. Federova as a co-first author in both chapters. The third and fourth chapters report the first crystal structure of Cas12a and the use of Cas12a arrays for multiplexed editing, where Ms. Federova made various small contributions to each project as part of her internship at the Broad Institute. These contributions earned her middle authorship on the resulting publications. The fifth chapter reports the characterization of how the Cas12e nuclease cuts DNA. Here, Ms. Federova was a co-corresponding author with her thesis advisor. Finally, in the last chapter on gaining insights into the process of spacer acquisition by the Type I CRISPR-Cas system in *E. coli*, Ms. Federova made a small contribution that earned her middle authorship.

Overall, Ms. Federova assembled a diverse collection of work that has substantively advanced our understanding of Cas nucleases including Cas9, Cas12a, and Cas12e as well as spacer acquisition. This combined work resulted in five already-published research articles and one manuscript in submission—an impressive output for one PhD student. The published article from the fifth chapter lists Ms. Federova as a co-corresponding author, which is also atypical for a PhD student but speaks to her progression toward a scientist who can arrange and conduct independent research. Ms. Federova also employed a wide collection of experimental techniques ranging from *in vitro* cleavage assays to genome editing in mammalian cells.

The introduction of the thesis was extensive and covered a range of topics relating to CRISPR-Cas systems, including their origin and function in relation to other prokaryotic defense systems, the mechanistic steps comprising adaptive immunity, and their functional diversity. This section also provides good context for all of the experimental work of the thesis and briefly highlights how each chapter fits within the broader field. The next two chapters provide an excellent spread of techniques as part of characterizing the two Cas9 nucleases, including RNA-seq analysis of crRNA biogenesis, PAM determination using a high-throughput screen in *E. coli*, *in vitro* DNA cleavage assays, indel formation assays in human cells, and accompanying statistical analyses. The findings establish new Cas9 nucleases for CRISPR technologies and provide some insights that help differentiate these nucleases from the many others available for use. All experiments were well performed and resulted in quality work that successfully navigated the peer review process at highly respectable journals. I was admittedly surprised that Chapters 3, 4, and 6 appear as official thesis chapters given Ms. Federova’s smaller contributions to the work, although her specific contributions are clearly articulated at the beginning of each chapter. I also understand that this format is officially allowed by Skoltech’s thesis guidelines and therefore do not hold this formatting against Mr. Federova.

In summary, Ms. Federova has made a number of substantive contributions to the CRISPR field and fully demonstrated her ability to plan experiments, perform a wide range of experimental techniques, interpret results, and place them in the context of the larger field.

The work was well performed, and much of it already was vetted for scientific quality and completeness through peer review at well-respected journals. At most, I noticed some inaccurate comments made in the introduction, although I consider these more minor given the breadth of information Ms. Federova covered.

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