

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:

I confirm the absence of any conflict of interest.	Signature:  Date: 18th August 2020
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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 thesis entitled “*Characterization and application of CRISPR-Cas enzymes*” by Iana FEDOROVA, under the supervision of Professor Konstantin SEVERINOV provides insights into the key effectors that drive the mechanism of action of bacterial adaptive immune systems. In my expert opinion, the overall thesis content, quality, conclusions, narrative and structure all meet or exceed the requirements for superior graduate work at the PhD level at a world class institution like the Skolkovo Institute of Science and Technology and peer institutions with Doctoral programs in life sciences.

Briefly, the candidate provides an introduction to CRISPR-Cas immune systems and the repurposing of their effector Cas nucleases for genome editing, notably Cas9 and Cas12. Chapters 1 and 2 present the characterization of two CRISPR-Cas9 type II-C systems (leading to two lead-authorships in *NAR* for Iana FEDOROVA). Chapters 3,4 and 5 present collaborative work documenting the structure of Cas12 (a *Cell* paper), genome editing using Cas12 (a *Nature Biotechnology* paper) and characterize the Cas12e cleavage (a *RNA Biology* paper). Chapter 6 delves more into CRISPR biology with focus on the interesting adaptation step (a *Nature Communications* paper).

Importantly, this comprehensive dissertation focuses on the study of CRISPR-Cas immune systems, which happen to be both scientifically fascinating and industrially broadly useful and applicable. Indeed, this topic is arguably the most compelling, timely and competitive field across all life sciences, notably as the driver of the genome editing revolution currently underway. Accordingly, this topic in general and this work in particular are of high interest to the scientific community and a broad and rapidly expanding readership. This makes the work presented here most relevant and the detailed content therein valuable and insightful. The all-encompassing focus on CRISPR-Cas systems, and biochemical characterization of Cas effectors, and their use for genome editing render this dissertation complete from basic science (Cas12a, LbCas12a and DpbCas12e biochemistry), to technology development (novel PpCas9, CcCas9, DfCas9) and its application (for genome editing).

With regards to materials, techniques, protocols and technical details encompassed in the work discussed and used to generate the data presented throughout the dissertation, current, timely and relevant methods were used across the board. Noteworthy, this is consistent with world-class work previously done under the supervision of Professor Konstantin SEVERINOV, and it is noteworthy and noted in the acknowledgements that Iana FEDOROVA had the opportunity to be trained in and exploit techniques developed by the esteemed Professor Feng ZHANG. Noteworthy, co-authorships with other world-class CRISPR experts (e.g. Eugene KOONIN, John VAN DER OOST) is documented, as well as the role as communicating author for one of the papers presented in the thesis (Selkova et al., *RNA Biology*; together with Pr. Konstantin SEVERINOV), showcasing the senior role also played by the student in training.

The significance and overall impact of the results obtained throughout the presented chapters not only comply with international level and the current state of the art, but in many ways extend beyond them, as illustrated by the journals in which this work was published, notably lead-authorships in *Nucleic Acids Research*, as well as co-authorships for papers published in the very best journals in the world, notably *Cell* and *Nature Biotechnology*. The number and quality of these publications, which also encompass co-authorships in *RNA Biology* and *Nature Communications* render the overall quality of the publication record competitive at the international level.

With regards to relevance of the obtained results, besides the editorial interest of the aforementioned journals, the condoning of the published work by referees during the peer-review process, and the reception by the readership, I personally see value in the potential of the technologies for actual genome editing. This is perhaps best documented by the filing of 5 patents, including some already granted.

Overall, I enthusiastically recommend that the candidate should defend the thesis by means of a formal thesis defense and hereby state that the submitted dissertation is comprehensive and articulate, meeting international standards of distinguished academic work, which is unusually compelling and timely given interest in CRISPR-Cas systems and their tremendous potential for genome editing. This work complements the published literature and has opened new avenues of research in this important field of study with tangible applications for these specific effectors. Under the supervision of Pr. Konstantin SEVERINOV, the student has benefited from superior mentorship and guidance, complemented by collaborative training with other world leaders (e.g. KOONIN, ZHANG, VAN DER OOST), and gained insights through the pursuit of intellectual property and participation in international scientific meetings and conferences, providing comprehensive and formative training .

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