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Skolkovo Institute of Science and Technology

Jury Member Report – Doctor of Philosophy thesis.

Name of Candidate: Maria Sokolova

PhD Program: Life Sciences

Title of Thesis: Functional and Structural Analysis of a Non-Canonical RNA Polymerase Encoded by Giant Bacteriophage AR9

Supervisor: Prof. Konstantin Severinov

Chair of PhD defense Jury: Prof. Yuri Kotelevtsev ***Email**: y.kotelevtsev@skoltech.ru*

Date of Thesis Defense: 15 June 2018

Name of the Reviewer: Shunsuke Tagami

I confirm the absence of any conflict of interest (Alternatively, Reviewer can formulate a possible conflict)	**Signature:**  **Date: 14-05-2018**

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 dissertation submitted by Maria Sokolova describes her research on functional and structural analysis of an RNA polymerase from a giant bacteriophage.

The dissertation contains careful and well-designed biochemical analysis of the non-canonical RNA polymerase and explains its unusual features. The candidate first performed purification of AR9 RNA polymerase from the native sample and identified its subunit composition. Then, the mechanism of promoter recognition was analyzed by biochemical experiments including in vitro transcription, primer extension, footprinting analyses. The results have shown that AR9 RNA polymerase can bind the promoters only when they contain uracils in specific positions. Furthermore, it turned out AR9 RNA polymerase can displace nascent RNA from ssDNA, which is a property not observed for other RNA polymerases.

The dissertation also describes the laborious efforts for structural analysis of the enzyme although it has not reached its completion. However, considering that structural analysis of an RNA polymerase, a large enzyme that attains multiple conformations is usually highly challenging, the trials performed here should not be undervalued. The applied methods and strategies in this project including X-ray crystallography and art-of-state CryoEM techniques have been perfectly reasonable and completion of the structural analysis is mostly promised. The partially modeled structure of AR9 RNA polymerase has already shown some differences from canonical RNA polymerases so that we can expect unique structural features of this enzyme will be revealed when the structural analysis is completed.

In summary, the quality and overall structure of the thesis is sufficient. The topic, actual contents and methods used in the dissertation are appropriate. The thesis described unique mechanical and structural features of AR9 RNA polymerase that will offer important implications on how RNA polymerases evolved and diverged. Therefore, the results shown in the dissertation will be highly appreciated in the field. Some parts of these works were already published as high-quality articles on three international scientific journals. Therefore, the dissertation meets the international level and will be ready for the formal defense once minor points mentioned below are corrected.

• Issues to be addressed before/during the thesis defense

1. Line 2 on page 41. "RNAP was purified from infected with PBS2 *B. subtilis* cells" should be corrected as "RNAP was purified from *B. subtilis* cells infected with PBS2".

2. Line 5 on page 42. "prepareAR9" should be corrected as "prepare AR9".

3. Line 9 on page 42. "5000g" should be corrected as "5000 g"

4. Last line on page 44. Does "contain costs" mean "constrain cost"?

5. Line 18 on page 45. "MgCl2" should be corrected as "$MgCl_2$".

6. Fig 13. Some positions outside the conserved sites (-12, -8, -1) still have significant effects. Is there any explanation for this? It is mentioned that "the AR9 DNA is very AU-rich and may be present in partially single stranded form" in discussion of chapter 3. Is it possible that AT-richness around a promoter contributes to transcription initiation efficiency? The candidate may discuss relationship between promoter efficiency and sequence outside the conserved sites.