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Skolkovo Institute of Science and Technology

Thesis Changes Log

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

The thesis document includes the following changes in answer to the external review process.

Reviewer Comment 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".
Author: Corrected (Last line, p. 40).

Reviewer Comment 2) Line 5 on page 42. "prepareAR9" should be corrected as "prepare AR9".
Author: Corrected (Line 5, p. 42).

Reviewer Comment 3) Line 9 on page 42. "5000g" should be corrected as "5000 g".
Author: Corrected (Line 8, p. 42).

Reviewer Comment 4) Last line on page 44. Does "contain costs" mean "constrain costs"?
Author: Yes, corrected (Last line, p. 44).

Reviewer Comment 5) Line 18 on page 45. "MgCl2" should be corrected as "$MgCl_2$".
Author: Corrected (Line 18, p. 45).

Reviewer Comment 6) Last line on page 76. A table to show the quality of the dataset should be provided when the resolution limit of the crystal is mentioned
Author: The table with crystallographic statistics of the dataset was placed in Appendix C-1 (p.104)

Reviewer Comment 7) First line on page 77. The reason why the candidate expected there are "two RNAP molecules per asymmetric unit" should be provided especially when model building is not completed. Usually the number of molecules in an asymmetric unit is estimated by calculating Matthews coefficient.
Author: The table with Matthews coefficients (Appendix D-1 (p. 107)) and the reference to it (lines 3-4, p. 78) were added.

Reviewer Comment 8) Dataset tables and Matthews coefficient for other crystals should be also provided.
Author: The tables with crystallographic statistics and Matthews coefficients were placed in Appendix C (p.104-106) and Appendix D (p. 107-109).

Reviewer Comment 9) Figures 24, 27, 28. What map (e.g. 2Fo-Fc) at what contour level is shown? This

should be mentioned in the figure legends.
Author: Contouring levels for all electron density maps were added to the figure legends. The figure legends were also expanded to explain the phasing process and were supplemented with references to programs used.

Reviewer Comment 10) Some specific structures in AR9 RNA polymerase (e. g. lid, zipper, etc.) are explained in discussion. The electron density of these parts should be shown to prove those features are clear even in low resolution, uncompleted structure.
Author: Figures with fragments of the AR9 nvRNAP electron density map showing some features common to multisubunit RNAPs were placed in Appendix E (p. 110).

Reviewer Comment 11) The active site of RNAP contains two Me^{2+} ions, not just one (p. 15).
Author: The text on the p.15 was corrected accordingly.

Reviewer Comment 12) According to recent reports, transcriptional pausing is not accompanied by clamp opening but depends on other conformational changes in the elongation complex (Guo et al., Kang et al., Mol Cell 2018) (p. 21).
Author: The sentence about dependence of the transcriptional pausing on the clamp opening was deleted from the text (p.21).

Reviewer Comment 13) The description of sigma dissociation and the role of sigma finger in this process does not include all necessary references (pp. 33-34).
Author: Additional references were added (p.33, references 82, 83, 84).

Reviewer Comment 14) Some minor details are missing from Materials and Methods (such as the step of DNA precipitation after piperidine treatment in $KMnO_4$ footprinting).
Author: Corrected (Lines 8-10, p.48).