

Jury Member Report – Doctor of Philosophy thesis.

Name of Candidate: Aleksandra Galitsyna

PhD Program: Life Sciences

Title of Thesis: Chromatin folding in individual cells

Supervisor: Professor Mikhail Gelfand

Name of the Reviewer:

I confirm the absence of any conflict of interest

(Alternatively, Reviewer can formulate a possible conflict)



Date: 19-09-2021

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

In the presented PhD thesis “Chromatin folding in individual cells” Aleksandra Galitsyna investigated chromatin folding in individual cells of *Drosophila melanogaster* and related aspects.

The thesis bases on five publications of Aleksandra Galitsyna. The five corresponding chapters of the PhD thesis (Chapters 4-8) comprise a core of the thesis, where Aleksandra in each chapter presents the corresponding paper preceded by a short description. The other chapters contain Introduction (Chapter 1), Background (Chapter 2), Thesis Objectives (Chapter 3), and Conclusion (Chapter 9).

Chapter 4 is devoted to the question whether nuclear lamina is a driving force of chromatin formation in *Drosophila*. It was found that nuclear lamina helps inactive chromatin to be in compact conformation; however, it is not crucial of the chromatin formation.

Chapter 5 is devoted to the development of an interpretable machine-learning method to predict chromatin structure from the epigenetic features. It was found that the main factors defining the boundaries of topologically associating domains (TADs) are insulator protein Chriz and histone modification H3K4me3.

Chapter 6 is devoted to the establishing of the biological relevance of cumulative contact frequency (CCF). CCF is commonly used to normalize the Hi-C maps to make them comparable between different experiment and species. However, this procedure removes the biological signal contained in CCF. In this part of the thesis, it was found that CCF is positively correlated with active chromatin conformation.

Chapter 7 is the central part of the thesis devoted to the structure of chromatin in individual cells of *Drosophila*. This is the first publication revealing the chromatin formation in individual cells in insects. It was found that the TAD boundaries correlate well with that found previously in mammals. Another important finding is that inactive chromatin forms stable TADs while active chromatin arrangement is variable between individual cells.

Chapter 8 is devoted to the overview of data analysis methods of single-cell Hi-C data. The review is detailed and comprehensive giving the picture of this fast-growing area of research.

The results of the presented works are scientifically significant and comply with the international level and current state of the art. The work is perspective for fundamental research and future applications. The publications are of high quality, the number of publications suits the requirements for the publication-based PhD thesis.

The only question I have to Aleskandra concerns the analysis accomplished in Chapter 6: How do you think, would it be more convenient and effective to analyze correlation matrix (see Fig.3C of Lieberman-Eiden et al., 2009) rather than contact matrix? Maybe, in this scenario one could avoid iterative correction and/or have better characteristics for bulk/single-cell analysis?

The remarks that I have are minor:

1. The Glossary looks like a list of abbreviations. Are there any terms that are not abbreviated?
2. Page 12: “Striking conservation of mechanisms was suggested because TADs are present across a wide range of species, including mammals, insects, and nematodes.” May the formation of TADs is inevitable? It may turn out that the existence of long DNA molecules within nuclear is impossible without formation of TADs.
3. Page 15: “Proline ... is a very rigid aminoacid, typically not forming hydrogen bonds.” This should be reformulated because word “typically” implies that sometimes hydrogen bond still can be formed. However, as we know from physics, if a chemical group can form hydrogen bond then it

must always form it in water. What Aleksandra meant here is, probably, that N-terminal group of proline never forms a hydrogen bond, thus, making proline unfavorable in most regions of alpha-helices and beta-sheets.

The other remarks are just few typos, the list of them is given to Aleksandra.

The dissertation conforms to high international standards. It has a clear structure; the topic corresponds to the actual content.

To summarize, I rate the PhD thesis of Aleksandra Galitsyna as very important, of a high quality and scientifically significant.

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