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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: Petr Sergiev

I confirm the absence of any conflict of interest 	**Date: 09-09-2021**

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

Next generation sequencing methods revolutionaraized our understanding of the chromatin folding in living cells at a scale from nucleosomes to chromosomes. Thanks to the Hi-C family of techniques scientific community become aware of topologically associated domains (TAD) as structural unit of interphase chromatin. While TADs describe chromatin compaction at a "local" scale, global chromatin folding was described as falling into (at least) two compartments. While the "wet science" methodology of Hi-C advanced significantly in recent years, there was an urge to upgrade the bioinformatic analysis of these data accordingly.

Aleksandra Galitsyna contributed the major bioinformatics calculations to several key studies on Hi-C applications to the chromatin structure of the bulk and individual cells of several organisms. She advanced the methods of bioinformatic support of Hi-C experimentation to a new level. In the first paper presented, "Nuclear lamina integrity is required for proper spatial organization of chromatin in Drosophila" Aleksandra demonstrated that chromatin association with nuclear lamina is not absolutely required for the formation of TADs, but rather contribute to the degree of chromatin compaction as well as transcriptional silencing of lamina-associated domains. While some LADs became decompacted upon LAM-KD, overall compaction of chromatin is increased. Devoid of contacts with lamina, chromatin segregation into A and B compartments is blurred. The value of this part for the scientific community is huge, as it dismissed the idea that lamina association is a primary driver for chromatin compaction and TAD formation. Now it is clear that lamina association is a secondary contributor that "enforce" compactization of certain genomic regions.

In the following manuscript entitled "Cumulative contact frequency of a chromatin region is an intrinsic property linked to its function" Aleksandra demonstrated that CCF depends on the chromatin state, namely it is increasing for the active chromatin. As a remarkable observation, syntenic genome regions of mice and human demonstrated (somewhat) similar CCF, indicating a cross-species conservation of this parameter.

In a paper "A machine learning framework for the prediction of chromatin folding in *Drosophila* using epigenetic features" Aleksandra applied an arsenal of computational methods to find predictors of TAD boundaries based on ChipSeq experimental data. It appeared that Chriz protein binding and active chromatin modifications, such as H3H4me2 and 3 are the best sort of such predictors. The study was continued by a remarkable work on a single cell Hi-C "Order and stochasticity in the folding of individual Drosophila genomes". For the success of this study Aleksandra contributed a new set of quality control algorithms allowed to filter out a number of artifacts. It allowed authors of the manuscript to visualize TADs formation at a level of individual cells corroborating the data obtained with bulk Hi-C. Finally, Aleksandra presented a nice summary of computation methods developed to analyze data generated by Hi-C family of methods, which is published in Briefings in Bioinformatics.

As a summary, I have to state that the work of Aleksandra Galitsyna which was included into her thesis is a significant advancement of bioinformatics support for the chromatin structure analysis. The top quality of the data analysis matches an outstanding set of journals, where her results were published. I have not a single doubt that Aleksandra Galitsyna should defend the thesis by means of a formal thesis defense.

As a future path to the development of better understanding of chromatin folding via HiC based methods I would suggest to address the possibility to map interactions of (i) homologous chromosomes and sister chromatids, (ii) non-unique DNA regions, such as centromeres, telomeres, nucleolar rDNA etc. as chromatin folding of these regions might and even definitely IS different from that of unique DNA regions.

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 candidote be exempt from the formal thesis defense*