Skoltech
Skolkovo Institute of Science and Technology

Jury Member Report – Doctor of Philosophy thesis.

Name of Candidate: Afanasii Stepanov

PhD Program: Life Sciences

Title of Thesis: Fluorescent sensors of epigenomic changes during stem cells differentiation

Supervisor: Professor Dmitriy Chudakov

Co-supervisor: Dr. Nadezhda Gurskaya

Name of the Reviewer: Prof. Dr. Igor Adameyko

I confirm the absence of any conflict of interest	**Date: 09-10-2024**

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

1. Brief Evaluation of the Thesis Quality and Overall Structure of the Dissertation

The thesis titled "Fluorescent Sensors of Epigenomic Changes During Stem Cells Differentiation" by Afanasii Stepanov is of high quality both in terms of content and presentation. The research is well-grounded, the development of novel genetically encoded epigenetic probes (GEEPs) is an innovative advancement, and the application of these sensors to real-time analysis of histone modifications during stem cell differentiation addresses a critical gap in epigenetic studies. The overall structure of the dissertation is coherent, with a logical flow that guides the reader through the introduction of the problem, development of the tools, experimental results, and conclusion. Each chapter is well-organized, beginning with clear objectives and leading to conclusions based on the presented data. The inclusion of multiple methodologies, from genetic engineering to live-cell imaging and bioinformatics, demonstrates the thesis's multidisciplinary approach. Mainly, the quality of this Thesis rests in the narrated research results, which are significant and are absolutely sufficient for advancing to a PhD defense stage. Indeed, the development of genetically encoded sensors for live-cell imaging of histone modifications is a major innovation, and the application of these tools to study stem cell differentiation provides valuable new insights into the dynamics of epigenetic regulation. The experimental scope of the thesis is broad and well-executed, and the research has far-reaching implications for a variety of biological fields.

2. The Relevance of the Topic of Dissertation Work to Its Actual Content

The topic of the dissertation is highly relevant to the content. The title accurately reflects the primary focus of the research, which is the development and application of fluorescent sensors to monitor epigenomic changes during stem cell differentiation. The thesis successfully adheres to this theme by:

- Developing genetically encoded sensors for specific histone modifications (H3K9me3 and H3K9ac),

- Demonstrating their use in tracking epigenetic changes in real-time during the differentiation of induced pluripotent stem cells (iPSCs),

- Correlating these modifications with gene expression changes.

The project addresses important biological questions about the role of epigenetic modifications in cell differentiation, making the topic well-aligned with the actual content.

3. The Relevance of the Methods Used in the Dissertation

The methods employed in the dissertation are highly relevant and appropriate for the research questions being addressed. The student has used a wide range of advanced techniques, each carefully chosen to meet the study’s goals:

- Genetic engineering methods, such as "Golden Gate" cloning, were used to construct the genetically encoded epigenetic probes.

- Fluorescence microscopy and live-cell imaging were essential for visualizing histone modifications in real time, a central aim of the thesis.

- Stem cell culture and differentiation protocols were used to investigate dynamic epigenetic changes in a biologically relevant system.

- Machine learning and bioinformatics were employed to analyze complex imaging data and to correlate histone modifications with transcriptional changes using RNA sequencing.

These methods are at the forefront of current molecular biology and epigenetics research, demonstrating the student's familiarity with cutting-edge techniques. The combination of these techniques enabled the research to produce meaningful insights into epigenetic regulation.

4. The Scientific Significance of the Results Obtained and Their Compliance with the International Level and Current State of the Art

The results presented in the thesis are scientifically significant and contribute to the international level of research in the fields of epigenetics, stem cell biology, and live-cell imaging. The development of genetically encoded epigenetic probes (GEEPs) for tracking histone modifications in live cells is an innovative achievement. This approach addresses limitations of existing methods such as chromatin immunoprecipitation (ChIP) and fixed-cell immunofluorescence by enabling real-time monitoring of epigenetic changes. Furthermore, the application of these sensors in stem cell differentiation provides novel insights into the temporal dynamics of histone modifications, such as H3K9me3 and H3K9ac, which are crucial for understanding gene regulation during differentiation. The thesis findings are highly relevant to current research, and the use of advanced microscopy, bioinformatics, and computational approaches places this work at the cutting edge of the field. The research meets international standards for high-impact work in molecular biology and epigenetics, as demonstrated by the quality of experimental design, data analysis, and interpretation of results.

5. The Relevance of the Obtained Results to Applications (if applicable)

The results obtained in this thesis have potential applications in several fields. The ability to monitor epigenetic changes in real-time during stem cell differentiation could improve the efficiency and precision of protocols used to guide stem cells into specific lineages. This is particularly important for developing cell-based therapies. Given that many cancers involve dysregulation of histone modifications, these sensors could be adapted to study epigenetic changes in live cancer cells, aiding in the development of new therapeutic strategies. The live-cell imaging tools developed in this thesis could be used in high-throughput screening to identify compounds that modulate histone modifications. This could lead to the discovery of new drugs targeting epigenetic pathways. While the thesis is primarily focused on the development of research tools, the potential translational impact of these tools in therapeutic settings is promising.

6. The Quality of Publications

The publications resulting from this thesis are of high quality. The research findings align with international standards for publications in molecular biology, particularly in the areas of epigenetics, stem cell research, and live-cell imaging technologies. The methods and results described in the thesis are novel and impactful, and the data is rigorously analyzed, making the work highly suitable for publication in reputable scientific journals.

7. The Summary of Issues to be Addressed Before/During the Thesis Defense

Overall, the thesis is strong, and there are no issues or points that should be addressed before or during the defense.

Provisional Recommendation
X Yes, *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*