Institute of Biophysics,
Czech Academy of Sciences

Opponent's review on PhD thesis

Title: Development and characterization of light-producing deoxyribozymes

Thesis author: Kateřina Švehlová

Institution: Faculty of Science, Charles University, Prague; Institute of Organic Chemistry and Biochemistry, CAS, Prague

Supervisor: Edward Curtis, PhD

Opponent: Daniel Renčiuk, PhD

In the dissertation thesis, as already follows from the title, Kateřina Švehlová focused on the development and characterization of new deoxyribozyme, which is able to produce light. In principle, the final deoxyribozyme, called Supernova, catalyzes transfer of phosphate group from a commercially available substrate, CDP-Star, to the 5' end of the deoxyribozyme. The dephosphorylated substrate then decomposes over time, which is associated with the production of light. The thesis covers selection process and activity optimization of the deoxyribozyme, its structural analysis, subsequent optimization of reaction conditions and potential application of the deoxyribozyme as a sensor. In my opinion, the thesis completely fulfills the original aims stated on page 32.

Concerning the topicality, novelty and originality of the presented research, ribozymes and deoxyribozymes are currently one of the hot topics in science with significant application potential. The originality of the research might also follow from the mentioned patent application.

The thesis itself is based on two papers of the group recently published in renowned international impacted journals. Kateřina Švehlová is the first author of one of them, which was published in Angewandte Chemie Int. Ed. with current impact factor above 15. Besides that, Mgr. Švehlová is a co-author of four other publications in renowned journals (e.g. Nucleic Acids Research or J Biol Chem). The number of publications and the quality of journals indicates the quality of Mgr. Švehlová as a researcher.

The thesis is written in perfect English with no mistakes and complemented with a significant number of precise and clear schemes. The thesis starts with a 22 pages long introductory part briefly covering recent knowledge on catalytic nucleic acids and deoxyribozyme selection process, supported by more than a hundred of appropriate references. Despite the title of the thesis, major part of the introduction deals with RNA and ribozymes, which might be due to the historical reasons. This part is accompanied by a description of the in vitro selection process, subsequent analysis of the positive hits, mostly based on sequencing methods, and an overview of computational secondary and tertiary structure determination, including the comparative sequence analysis used later in the thesis.

The structure of the "Results" section is an unusual combination of original text, probably covering the results published in the Angewandte paper and the attached second publication in ChemBioChem together with its supplementary information. The Angewandte paper in the published form is not included in the thesis. As the presented results were already published and respective publications underwent standard reviewer process, I do not discuss the presented results. The presentation of the results within thesis is excellent.

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In terms of scientific quality, the thesis and associated publications represent truly an excellent piece of work. From the whole thesis it is obvious that the author has a deep knowledge of all aspects of the studied system, as well as of the methods used. She is also clearly aware of the limitations and potential drawbacks of the system. All these issues are well discussed, analyzed and potential solutions and future prospective are presented. In fact, any time during reading the thesis when I could ask something or had an idea for an additional experiment, the answer or experiment results were in the next few pages. Therefore, I have no major comments or questions about particular aspects of the thesis, but I would like to ask few more general points:

- Is there a reason why for substitution of variable loop regions four adenine sequence was used? Especially in context of the figure 8B in the ChemBioChem paper, where the second loop is suggested to coordinate one of the zinc cations?
- Could you very briefly sum up and explain some discrepancies between the ligation rate and rate enhancement or luminescence?
- Why is it necessary to purify the ligated products of selection on gel, when in the next PCR step only those with ligated adaptor for primer binding are amplified?

In conclusion, the presented doctoral thesis is far above average. Student proved creative capacity in research in Molecular biology and associated fields and the presented doctoral thesis fulfils all the requirements standardly demanded in the field.

I **recommend** the presented doctoral thesis for the defense and the **award of the title PhD** to Kateřina Švehlová.

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