Reviewer's report on a doctoral thesis: Development and use of deoxyribozymes that generate color and fluorescence

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The presented work concerns the development and improvement of the properties of DNA molecules, deoxyribozymes, that transfer the phosphate group from various substrates to the 5' hydroxyl group of the DNA molecules themselves. Such a reaction converts the substrate into the fluorescent or highly absorbing product. One of the long-term goals is the development of new sensor DNA molecules.

The work itself is written in nice and understandable English. The opening passages are quite accurate, but at the same time downright funny, which was clearly the intention. The placement of the researched topic in the historical context is therefore very successful, by no means boring reading (just look at the names of the individual sub-chapters). The introductory part could be recommended as a brief textbook or script for students interested in research of DNA. It contains a description of the most critical discoveries in the research of DNA structure and function, whether in nature or in the laboratory. In a lighter form, it also deals with the chirality of biological macromolecules or the origin of life on Earth. In the last part of the introduction, we find important information about in vitro evolution, including an explanation of the advantages and disadvantages of the individual approaches used. The introductory part excellently prepares the reader for the next chapters.

The work also contains the objectives of the work and a description of the author's most important results. There are really a lot of them. I appreciate the detailed workflow of the individual experiments, the quality of the presented images and the illustrative diagrams of the secondary structures of the investigated DNA molecules.

I greatly appreciate the thorough analysis of the conditions necessary for the activity of the discovered DNAzymes, for example the influence of monovalent and divalent metal cations, PEG, DMSO, pH, temperature.

Discussion and conclusions follow. The author critically comments on his own results and compares them with the available literature on comparable systems.

A description of the methods used is included at the end of the work. The dissertation includes all other parts that are required.

Although the thesis is written very nicely, I have some small complaints.

I don't like the wording "light producing DNAzyme", "fluorogenic and chromogenic signals" or "fluorogenic molecule". The reason is that the enzymes discussed do not actually produce light by themselves. I would consider fluorescence and absorbance to be signals. Fluorogenic and chromogenic are then ongoing reactions rather than signals and molecules.

The thesis contains a table of used oligonucleotides. This table (7.4 List of oligonucleotides used) is probably not properly labeled, although it is referenced many times (Table 1?).

It is not clear how the characteristic of the original library of sequences (Fig. R_6a, R_7a, the maximum of the graph at 1E+7) is connected with the information that 1.74E+14 different sequences were used (p. 39).

The author of the thesis is a co-author of four high-quality publications, of which two papers (first authorships) are directly related to the subject of the dissertation. I believe that the author has clearly demonstrated his ability for independent scientific work and an engaging presentation of its results.

I unequivocally recommend the work for defense and propose to grade it as excellent.

Questions:

Q1 The used protocol of in vitro evolution contains several critical steps. As a result one obtains the list of enriched DNA sequences with supposed enzymatic activity. However, it is not clear how the enrichment correlates with real DNAzyme activity. For example, the molecule "Hit 10" (Figure R_6) has higher activity than nine more abundant molecules. What is the reason for this inconsistency? How probable is the possibility that e.g. Hit 20 or Hit 50 molecules would be better than Hit 10 in terms of the activity?

Q2 Aurora and Apollon molecules have different folds than Supernova although they come from the Supernova library. Do you expect that the usage of completely different inicial libraries could end up with similar active

molecules (concerning the average mutational distance of variants in the libraries and the sequence lengths)?

Q3 Aptamers called SOMAmers contain unnatural and modified nucleotides as mentioned in the theses. Did you also consider such modifications to increase chemical variability?

Q4 In chapter 1.9.3, the fluorogen-activating RNA/DNA aptamers are described. Another approach is the use of enzymatically active RNA/DNA molecules that could produce much more fluorescent products. For that purpose the repeated action of the enzyme might be beneficial. Could you comment on the possibilities of regenerating DNAzymes by their dephosphorylation?

Q5 When characterizing the Aurora 2 molecule you expected 14 peaks in the proton NMR spectrum but only 12 were observed (Fig. R_10g). I understand that some peaks may overlap. But, is it possible to determine in which positions the expectation and the result differ? Which specific nucleotide interactions were not observed?

Q6 The cooperativity of Zn2+ ions binding is discussed (p. 60). However, how is it with the cooperativity of 4-MUP binding to Aurora 1 and 2? (Fig. R_14c).

31/8/2024
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