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Poznań, 20.09.2018

Review of Doctoral Thesis

Title: Structurally defined RNA nanoparticles for gene expression regulation

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Introduction

The subject of the reviewed PhD thesis is rooted in the rational design of functional nanoparticles using RNA motifs. The thesis is written in English and occupies a total of 128 pages. It comprises (in order of occurrence): acknowledgements, the list of author's achievements (papers and conference presentations), an English and a Polish abstract, list of abbreviations, table of contents, 14 chapters organized in 4 sections, 3 appendices, and a bibliography. The main text is illustrated with 53 colored figures and 5 tables which are not listed separately. The list of references contains 113 publications.

The layout of the thesis corresponds to the structure of a scientific article defined for journals in the field of life sciences. From my perspective, it is quite an unusual choice. However, the presentation of the results is adequate. The description of a problem and its solution is precise and clear. Figures and tables are informative and perfectly complement the text.

Assessment

Section I: Introduction. In the first section of the thesis, the author provides a well-written introduction to the subject of RNA nanoparticles. The section is divided into six chapters. The first one includes a presentation of known RNA classes and functions. Next two chapters outline the architecture of RNA molecules and briefly discuss their susceptibility to the 3D modeling by selected automated methods. The fourth chapter describes in detail the process of RNA architectonics design, also known as tectoRNA

rational design. The author considers the importance of selected motifs of RNA structure in designing RNA nanoparticles and their functionalization. This chapter is particularly valuable and significantly facilitates an understanding of the research conducted by the doctoral student. The fifth chapter explains the process of RNA processing in the cell, starting from its genesis and leading through molecule maturation. The section concludes with chapter six, including a brief reflection on the current and possible applications of RNA nanoparticles with a particular focus on biomedicine.

The outline of the section, its contents, including figures, and cited references are relevant. The section quite comprehensively introduces the subject which is studied in the doctoral dissertation. The only shortcoming noticed in this part of the thesis concerns the relatively superficial treatment of *in silico* methods. In Chapter 3, D.M. Jędrzejczak lists four structure databases, but no additional comment about them is given (Why these databases are mentioned while others are not? What is the difference between them?). In Chapter 4, the author lists several modelling tools but does not distinguish between the secondary and the tertiary structure prediction. In my opinion, these two problems should be treated separately, if only because the history of creating algorithms for the secondary structure prediction of RNA is already 50 years old, while the automated 3D structure prediction methods started to be developed intensively only in this century.

Section II: Results and discussion. The second section is the essential part of the reviewed doctoral thesis because it is here that the author presents the results of her scientific work. The section occupies 58 pages and is divided into five chapters (Chapter 7 – Chapter 11). Each of them presents one of the research stages conducted by the author throughout the doctorate. Every chapter presents the achievements at a given stage of the research. The first reported success is finding a new RNA motif, the three-way junction (referred to as 3wj-nRA) with structural features that ensure its enzymatic processing. The author presents a protocol used to predict the secondary, and next, the tertiary structure of the motif using selected *in silico* methods. The protocol is pictured in Figure 9. In this scheme, names of three resources are miswritten. Correct names are RNAfold, RNA FRABASE, RNAComposer instead of RNA fold, FRABASE, RNA Composer. I also do not understand how the pdf file can be used in RNA 3D structure prediction (see page 21). Probably, rather a strange formulation of the sentence is misleading here.

Next chapter (Chapter 8) focuses on the *in silico* design of RNA structures. First, it discusses the prototyping of triangular RNA nanoparticle. This process follows the RNA tectonics and rational design approach. The author presents a way of constructing a single 3wj-nRA-based monomer and then

combining three such monomers into a triangular object. Additionally, the procedure of designing siRNA for further functionalization of the nanoparticle is presented. The synthesis of computationally designed RNAs in the laboratory is explained in Chapter 9. Next, in Chapter 10, D.J. Jędrzejczyk describes in details the experiments performed to confirm the structure of synthesized RNA molecules and the processing of the constructed monomers. This part of the section is perfectly illustrated by high-quality colored figures and tables including RNA sequences and secondary structures in parenthesis notation.

In the final, most extensive chapter of this section (Chapter 11), the author presents results of her study on the regulatory potential of trimeric nanoparticles and their constituent monomers, built upon 3wj-nRA motifs. This part is particularly important as the aim of the author's doctoral research was to design RNA nanoparticles for gene expression regulation. The regulatory potential of triangular RNA and its monomers was estimated using the green fluorescent protein gene expression assay. Two cellular lines, HeLa and MDA-MB-231/GFP-RFP, were used in the tests. Thus, the chapter contains a description of both cell lines' cultivation, optimization of gene expression systems, cytotoxicity and function analysis. Next, gene expression regulation is discussed for both systems. It includes an explanation of experiments performed to measure fluorescence intensity applying a plate reader and a flow cytometer. The author observes regulatory effect and concludes that RNA nanoparticle constructed using 3wj-nRA motif applies to different regulatory functions released by enzymatic processing of the motif. A detailed inspection of the reported results is possible thanks to numerous photos and charts supplementing the description and extensive comment. The experimental part of the work is presented in a comprehensive and transparent way with great attention to detail. I consider the whole section II of the reviewed thesis to be of high scientific value.

Section III: Conclusions. This section occupies only one page and contains a concise summary of the research results obtained by the author of the reviewed dissertation. The content is relevant and well written. Conclusions are drawn in an appropriate way and show that the author has achieved the objective of her research project. The content of this section does not raise any objections.

Section IV: Materials and methods. In this section, D.J. Jędrzejczyk gives detailed technical information about laboratory experiments that were conducted as part of the research work. The results of the experiments have been described in the preceding chapters. Experimental procedures described here are not an original result of the author. However, their precise knowledge is necessary in the case of willingness to repeat the conducted experiments and to verify the obtained research results. In the three chapters into which this section is divided, the following wet-lab experiments are described: RNA

synthesis (Chapter 12), RNA structure determination (Chapter 13), and the green fluorescent protein gene expression assay (Chapter 14). First, the author focuses on methods applied to synthesize RNA molecules, in particular, long RNA fragments that – unlike the short ones – were not synthesized by an automated method. She provides the details of DNA amplification protocol, the reaction of *in vitro* run-off transcription, RNA purification process, and monomer association into the single nanoparticle. Next chapter is devoted to an explanation of structure determination study, encompassing gel electrophoresis, radioactive labelling reaction, and Dicer processing. The tests aimed at verifying whether the synthesized molecules adopted a structure similar to the *in silico* prediction. Finally, D.J. Jędrzejczyk elaborates on the GFP gene expression regulation study that was conducted with two independent cell lines, HeLa and MDA-MB-231/GFP/RFP. She clearly describes the process of cell cultivation, maintenance, and further treatment.

The section contains a very detailed description of all laboratory experiments, resulting in repetitive experiments and verifiable results obtained in the study. It would be appropriate to include in this section also the parameters of computational methods used in the research. Such information is usually provided in scientific publications describing studies that apply information technology methods, even when they are used with default settings. It allows the computational experiment to be unambiguously defined and makes it easily reproducible.

Appendices. Appendices A, B, and C contain tables and figures that supplement the presentation of the results obtained by the author. They are well-formatted, clear and informative.

Summary

Based on the reviewed doctoral thesis, I conclude that Dominika Jędrzejczak demonstrates the ability to present the results of research correctly and convincingly. She can independently conduct scientific work and conclude accurately. She proved to be familiar with the current state of knowledge about the problem addressed in the thesis. She possesses the general theoretical knowledge in the field of structural biology and bioengineering.

The reviewed doctoral dissertation contains an original contribution to the area of bioengineering. Main research results include: identification of the new three-way junction motif, designing trimeric RNA nanoparticle comprising the new motif, experimental confirmation of the nano-object 3D structure, functionalization of the created trimer with siRNA molecule. The results obtained by the author were published in good journals and presented many times at scientific conferences in Poland and abroad, gaining recognition and two awards for the best oral presentation. The thesis is

excellently written and well documented. There are no typing errors. The text is written in a clear and concise manner. The figures and tables are shown properly. The conclusions confirm that the objective of the work was successfully accomplished.

Recommendation

The submitted dissertation entitled "Structurally defined RNA nanoparticles for gene expression regulation" constitutes the original solution of the scientific problem and it meets all the requirements for doctoral theses specified in the Act on Academic Degrees and Academic Title (Art. 13), currently in force in Poland. Thus, I recommend the admission of Dominika Joanna Jędrzejczyk to further stages of the doctoral dissertation process. Moreover, taking into account the publication record, conference activity, multidirectional approach to conducting research in the doctoral project, as well as the importance and potential applicability of the results achieved by the student, I apply for the distinction of the doctoral thesis referred to in this review.

Marta Szachniuk