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NANOSCIENCE CENTER
FACULTY OF SCIENCE
AARHUS UNIVERSITY

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Assessment and Recommendation

Dear committee,

I have reviewed the PhD thesis of Dominika Jedrzejczyk (DJ) entitled "Structurally defined RNA nanoparticles for gene expression regulation" describing her PhD studies done under the supervision of professor Arkadiusz Chworos.

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The thesis describes the main PhD project of DJ that has focused on rational design of RNA nanoparticles and at using these to deliver small interfering RNAs (siRNAs) to knockdown a target protein in human cells. The thesis consists of an introduction (18 pages) to RNA structure, RNA nanotechnology and RNA biology, a result and discussion section (60 pages) describing both bioinformatics, RNA biochemistry and cell work, a conclusion (1 page), materials and methods (22 pages), appendices, and a reference list.

DJ has published two articles as main author: A research article describing a bioinformatics study to identify a new RNA structural motif, and a review article summarizing the research fields relevant to her work. It should be noted that the main work presented in the thesis has still not been published but will represent her most important experimental contribution. DJ has contributed to two other research articles and to four post conference communications. DJ has further participated and contributed to several conferences and most impressively given a talk on the topic of the thesis at an EMBO Workshop in Stockholm, Sweden.

The PhD thesis is very well presented, clearly written and demonstrates expert knowledge of the relevant research fields. It should be mentioned that the research topic is interdisciplinary and requires knowledge in several areas such as RNA structure and function, RNA bioinformatics and 3D modeling, RNA biology and the unique RNA nanotechnology approach. The introduction shows that DJ has detailed insight in all of these topics.

The result section of the thesis contains both a bioinformatics study, a rational design part, and extensive experimental results. The identification of the novel motif 3wj-nRA is explained well and is based on the central databases and tools. The workflow for rational design of triangular nanoparticles and siRNA functionalization is elegantly explained and illustrated. Synthesis, assembly and Dicer cleavage of the RNA nanoparticles (section 9 and

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10) are carefully done with proper controls and nicely annotated. The experimental work on target gene expression and knockdown efficiency of the RNA nanoparticles is carefully done with proper statistics and controls and demonstrates a good scientific approach to a complex experimental problem. Experiments are very carefully done by use of several experimental techniques (plate reader, fluorescence microscopy, and flow cytometry) to verify the validity of results. The data shows consistent knockdown effects of siRNAs and multivalent RNA nanoparticles and important knowledge is obtained about differences between cell systems and delivery methods. The conclusion was reached that the regulatory siRNA fragments incorporated into the nanoparticle were effectively released and triggered targeted GFP gene silencing and that the regulatory effect was prolonged when induced with structuralized RNA compared to unstructured siRNAs.

Minor comments:

1) In general, the main text is clearly written, but there are a few places where I can't completely follow the argument. This might be due to fast writing or my lack of understanding. I should mention that this is very few places.

2) Abbreviations are nicely listed in the front matter but are not introduced and used systematically in the main text.

3) Concerning the identification of the 3wj-nRA it would have been nice to make it clearer in the text that it is a prediction that could be finally verified by structure determination by X-ray crystallography or Nuclear Magnetic Resonance study.

4) The heading "Structure determination" (section 10) is not fitting for the assembly and Dicer experiments. Structure determination is normally used for biophysical techniques aimed at determining the atomic structure of a given molecule.

5) Figure 18 shows that a fraction of the RNA structures form multimers. It would have been nice to provide an estimate of the yield of timer complexes versus multimers and also a discussion of how this would affect transformation yield and knockdown efficiency.

6) For the experimental part it would have been nice to add a set of RNA constructs that helps to demonstrate the positive effect of having a "structuralized RNA scaffold". This could have been a flexible 3-way junction that would not have performed well in trimer assembly and possibly also in knockdown experiments.

7) In section 11 it is a bit confusing that fluorescence-activated cell sorting (FACS) is used, when no sorting is necessary for the reported results. It would have been better just to describe that a flow cytometer was used to measure the fluorescence of individual cells.

The minor comments above are meant as helpful suggestions and does not affect my main conclusion that the PhD thesis of DJ is of high scientific quality and demonstrates that DJ has the necessary skills and knowledge

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in the research field. I can therefore recommend acceptance of the thesis for further stages of evaluation towards her final PhD degree. In fact, I find that the thesis is of very high quality because of the difficulty of the interdisciplinary topic and the experimental procedures that I can also recommend to have it awarded a prize for excellent work.

Yours sincerely,

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