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Review of PhD thesis of Gerrit Wilkens

I received for a review a PhD thesis with the title Control of nanostructures biological macromolecular assemblies using DNA nanotechnology, by Gerrit Wilkens, performed under the supervision of dr. Jonathan Heddle.

The PhD thesis is well written, with an introduction and overview of the field and its relevance, with new important results, clear description of used methods in the experimental work, discussion of results and its place with respect to the current knowledge and its potential impact and attribution of work of other researchers.

This PhD thesis describes the design of two platforms based on DNA nanotechnology, first, design of catenated single stranded DNA circles for the formation of catenated DNA origamis and second, the design of DNA origami circle templated-formation of liposomes and their manipulation and purification using magnetic beads.

In the first part, DNA catenanes were formed by a two-step procedure based on plasmid DNA. In the first step dsDNA catenanes are formed from a large plasmid using Tn3 resolvase using two attachment sites within the same plasmid, and in the second step, dsDNA catenanes were converted into the ssDNA catenanes by application of a nickase and exonucleases. The resulting ssDNA catenanes were subsequently converted into two DNA origamis using appropriate staples and visualized using AFM. The strategy is original and efficient and the results convincingly documented. This works has also undergone a peer review and has been published in ACS Nanotechnology Au.

The second part describes formation of liposomes anchored to the DNA ring via cholesterol modified DNA oligos, similar as reported before. The novelty of this work is coupling DNA rings to magnetic beads, which facilitated removal of excess lipids, purification and could in principle be used for more complex manipulation, such as e.g. insertion of proteins into the generated liposomes. Release of DNA circle-liposome assemblies or liposomes has been achieved using DNA displacement and the release products have been characterized by gel electrophoresis and AFM. Combination of DNA origami and lipids with magnetic separation clearly represents a favorable improvement that might lead to more efficient production and purification of designed assemblies based on DNA.

In conclusion, the PhD thesis presents an original scientific contribution to science and therefore in my opinion fulfils the requirements for the achievement of the PhD title.

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