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Review of the PhD thesis of Manu Kumar

I have received for a review a PhD thesis with the title Design and production of highly programmable DNA-protein cage hybrid nanostructures, by Manu Kumar, performed under the supervision of Prof. dr. Jonathan Heddle.

The PhD thesis is clearly written, with an introduction and overview of the field and its relevance, stated aims, two sections on the results in two main areas, a description of used methods and materials for the experimental work, a discussion of results concerning the current knowledge and its potential impact.

This PhD thesis describes in the first section the design of ferritin cages based on mutants that affect the Mg-ion requirement for cage oligomerization and their detailed characterization and the second section of results on the design of DNA cages into which the ferritin cages can fit.

The topic of PhD, design of programmable biomolecules, that includes both polypeptide- and DNA-based polymers is of high interest as it enables the control of the matter at the nanoscale with several potential applications. It can also contribute to the understanding of the structure-function of ferritins and protein cages in general.

In the first part, the candidate prepared and analyzed several mutants of TmFtn with mutations at position 65, replacing the wild-type Glu residue. Replacement by cationic residues Lys and Arg generates cage that was stable already in the absence of Mg ions as it stabilized the ionic interactions between the subunits in the cage. The variants were stable and the crystal structures confirmed that there were no large modifications in the 3D structure and adopted octahedral cage geometry. Iron mineralization remained unperturbed for all tested ferritin mutants. Those results demonstrate strong effect of a single residue on the cage assembly.

The second part of results described the design of a DNA-based cage designed to accommodate ferritin cages. Those cages were conjugated with ssDNA segments via surface-exposed Lys residues. DNA cage was designed to present complementary ssDNA that can bind ssDNA conjugated to the ferritin. Results demonstrate successful conjugation, formation of DNA cages, and their occupation with ferritin cages, depending on the ratio of ferritin cages and DNA gloves. AFM and negative stain EM images confirm the desired assembly. Iron could be loaded to ferritin cages in the presence of DNA gloves. This part of the results demonstrates that DNA can be combined with protein cages, where DNA has certain specific advantages regarding programmability.

The result from the first part of PhD thesis was published in the Nanoscale journal with the candidate as the first author and has therefore also been scientifically reviewed.

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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.

Prof. dr. Roman Jerala