Receptor-mediated cellular uptake of gene-encoding DNA origami

*Viktorija Kozina, Samuel Beerkens, Annabelle Solder, Magdalena Sperl, and Hendrik Dietz**
Am Coulombwall 4a and Boltzmannstraße 11
Laboratory for Biomolecular Nanotechnology, School of Natural Sciences and Munich Institute of Biomedical Engineering, Technical University of Munich
Garching near Munich, Bavaria, Germany
viktorija.kozina@tum.de and dietz@tum.de

Effective delivery and expression of specific genes in target cells is crucial for many scientific and medical applications[1]. However, it is still challenging to package, deliver, and express nucleic acids of interest in a cell- or tissue-specific manner, and this issue typically needs to be addressed case-by-case[1]. It was previously shown that mammalian cells are capable of expressing the genes encoded into the DNA origami nanostructures[2], and that such structures can be actively imported into the cell nucleus[3], but the cellular uptake of nanostructures remains a major impediment. Internalization of DNA origami by mammalian cells has been an area of interest for many researchers due to the technique's high potential in the biomedical field. Passive[4] and receptor-mediated[5] uptake of DNA nanostructures of different sizes and shapes have been screened. While it was shown in both cases that mammalian cells are capable of internalizing the structures, they remained trapped in the endosomes and later degraded by the lysosomes[4,5].

The well-described structure[6] and internalization process[7] of the transferrin receptor led us to hypothesize that DNA origami structures functionalized with transferrin would get taken up by the cells more efficiently than non-transferrin functionalized structures, thus opening a path to exploit the transferrin system for targeted delivery of DNA origami structures into cells. Furthermore, we speculated that transferrin-functionalized structures may also show enhanced escape from endosomal entrapment since the transferrin receptor-mediated uptake does not exclusively lead to the degrading pathway[7]. We have validated the internalization theory both qualitatively and quantitatively using confocal microscopy and flow cytometry. We expanded our screening process to better understand the effects of ligand multivalency and patterning on the internalization process of DNA origami, as well as structure shapes and sizes. Finally, expression of mCherry and eGFP fluorescent proteins by mammalian cells was observed after they have been treated with mCherry- or eGFP-encoding DNA origami functionalized with transferrin, leading us to believe that some of the internalized structures were able to escape the endosome and be shuttled to the cell nucleus for further processing.

References:

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