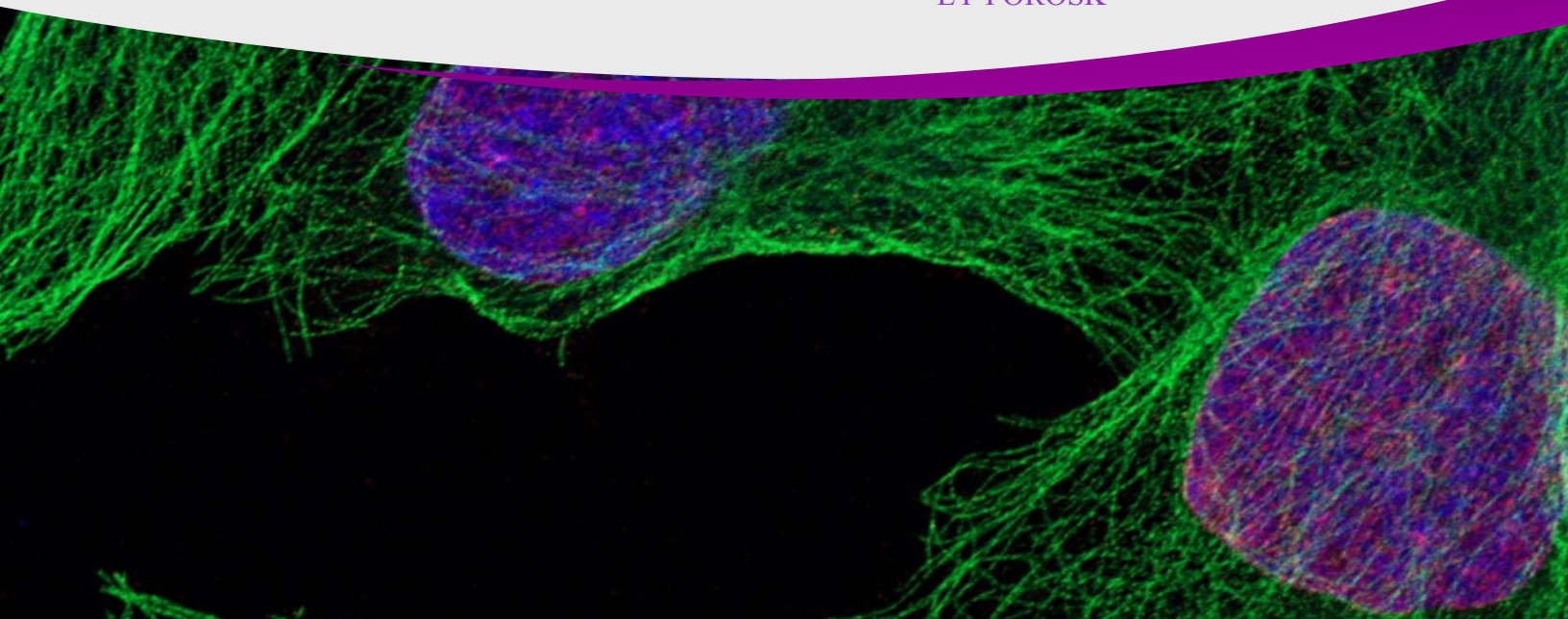
28th

Wilhelm Bernhard Workshop

on the Cell Nucleus

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LY POROSK



Organized by the University of Tartu, Institute of Technology

Local organizers: Prof. Reet Kurg, Margit Mutso, Kristiina Kurg, Merike Petuhov, Ly Porosk

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Functional screening of cell-penetrating peptides to enhance the delivery of RNAi-loadednucleic acid nanostructures

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RNA-based therapeutics, particularly RNA interfering (RNAi) small RNAs such as microRNAs(miRs) and small interfering RNAs (siRNAs), have emerged as promising therapies for multiple pathologies, including cardiac repair after ischemia. These small non-coding RNAs can modulate key regenerative pathways, offering potential for functional recovery post injury. However, their clinical application is hindered by the lack of safe and efficient delivery systems.

In this context, DNA nanotechnology offers a unique platform to address these limitations. Nucleic acid nanostructures (NANs) are highly programmable, biocompatible and exhibit remarkable structural versatility, allowing precise control over size, shape and functionalization. Their ability to protect and transport unstable nucleic acid cargos while minimizing toxicity and immunogenicity makes them ideal candidates for miR delivery in cardiac regenerative medicine.

To enhance the cellular uptake of these NANs, cell-penetrating peptides (CPPs) are being explored as functional moieties. CPPs are short peptides with positively charged residues that facilitate translocation across negatively charged cellular membranes. Their integration into NANs is driven by electrostatic interactions between the cationic peptide's residues and the anionic phosphate backbone of DNA. A critical parameter in this assembly is the nitrogen to phosphate (N/P) ratio, which marks the stability of the peptide-DNA complex and its physicochemical properties. By fine-tuning the N/P ratio, it is possible to optimize the formation of stable, compact hybrids that maximize cellular internalization while preserving the cargo integrity.

Peptide-functionalized NANs were developed as advanced delivery vehicles for cardio-therapeutic miRs. DNA origami NANs were assembled via a stepwise self-assembly process and characterized by gel electrophoresis and dynamic light scattering (DLS), confirming consistent dimensions (~50 ± 5 nm) and high structural stability. Hybrid nanocarriers were subsequently engineered by incorporating CPPs into the different NANs through controlled N/P ratios, resulting in significantly enhanced cellular uptake in U87-luc2 cells.

Ongoing efforts focus on optimizing this strategy by evaluating alternative CPPs and expanding their application to other DNA nanostructures, including tetrahedron and DNA nanohydrogels. These advancements aim to maximize the intracellular availability of therapeutic miRs, with the final goal of enabling efficient and non-viral gene therapies in human-induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) for cardiac regeneration.