CPPs TAILORED DNA NANOCARRIERS AS A PLATFORM TOWARDS RNAi CARDIOTHERAPEUTICS

Marina Ripalda-Paredes[1,2], Marcos Sánchez-Barat[2,3], Ly Porosk[4], Laura Ordovás[2,3,5,6], Margus Pooga[4], Silvia Hernández Ainsa[1]

[1]Instituto de Nanociencia y Materiales de Aragón (INMA), CSIC-Universidad de Zaragoza, Zaragoza, Spain
[2]Instituto de Investigación Sanitaria de Aragón (IISA), Zaragoza, Spain
[3]Instituto de Investigación en Ingeniería de Aragón (I3A), Universidad de Zaragoza, Zaragoza, Spain
[4]Faculty of Science and Technology, Institute of Technology, University of Tartu, Tartu, Estonia
[5]Fundación ARAID, Zaragoza, Spain
[6]CIBER-BBN, Madrid, Spain

mripalda@unizar.es

Small interfering RNAs (siRNAs) and microRNAs (miRs) are powerful therapeutic tools that can address complex medical challenges such as, for example, modulating pathways implicated in the regeneration of ischemic cardiac tissue [1]. However, the clinical potential of RNA interfering (RNAi) therapies is limited due to issues like low nucleic acid stability in its unmodified form, poor membrane permeability and reduced endosomal escape [2].

To overcome these challenges, we utilize DNA nanotechnology, which uses Watson-Crick-Franklin base-pair recognition [3], as an innovative platform for delivering RNAi molecules. These hybrid DNA/RNA nanostructures (NANs) minimize immune responses associated with siRNA therapies. To further enhance cellular delivery, we functionalize these NANs with Cell Penetrating Peptides (CPPs). CPPs are cationic peptide sequences composed of 4 to 40 amino acids, and are known for their capability to transport a wide range of molecules across cellular membranes while maintaining cargo stability.[4].

By exploiting the electrostatic and hydrophobic interactions between the positively charged CPPs and the negatively charged DNA backbone, and by adjusting the nitrogen to phosphate (N/P) ratio to 2:1, we were able to fine-tune the physicochemical properties of the resulting CPP-NAN complexes, yielding efficient hybrid NANs.

In this proof-of-concept study, we tested only the DNA NANs without delivering therapeutic siRNAs. Two main types of NANs were investigated: DNA origami-based NANs (ORI) and DNA tetrahedron-based NANs (TD). The CPPs enhanced the internalization of both ORI (52 ± 4 nm) and TD (11 ± 1 nm) in U87 MG luc2 glioblastoma-like cells, without compromising cell viability (*Figure 1*).

This approach lays the groundwork for developing fully programmable, non-viral RNAi delivery systems. Current studies are focused on expanding this method to alternative NANs, such as DNA nanohydrogels (NHG) that carry therapeutic miRs. At the same time, we are optimizing CPP functionalization in human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) to identify the most effective combinations of ORI-CPP and TD-CPP for cardiac cell delivery.

Ultimately, this strategy contributes to establish fully programmable and non-viral RNAi delivery systems with application in processes such as cardiac regeneration.

References

[1] Gabisonia K, Prosdocimo G, Aquaro GD, Carlucci L, Zentilin L, Secco I, et al. MicroRNA therapy stimulates uncontrolled cardiac repair after myocardial infarction in pigs. Nature. 2019 May 16;569(7756):418–22.

[2] Moazzam, M., Zhang, M., Hussain, A., Yu, X., Huang, J., & Huang, Y. (2024). The landscape of nanoparticle-based siRNA delivery and therapeutic development. In *Molecular Therapy* (Vol. 32, Issue 2, pp. 284–312). Cell Press. https://doi.org/10.1016/j.ymthe.2024.01.005

[3] Kosara, S., Singh, R., & Bhatia, D. (2023). Structural DNA nanotechnology at the nexus of next-generation bio-applications: challenges and perspectives. In *Nanoscale Advances* (Vol. 6, Issue 2, pp. 386–401). Royal Society of Chemistry. https://doi.org/10.1039/d3na00692a

[4] Moreno-Vargas, L. M., & Prada-Gracia, D. (2025). Exploring the Chemical Features and Biomedical Relevance of Cell-Penetrating Peptides. In *International Journal of Molecular Sciences* (Vol. 26, Issue 1). Multidisciplinary Digital Publishing Institute (MDPI). https://doi.org/10.3390/ijms26010059

Figures

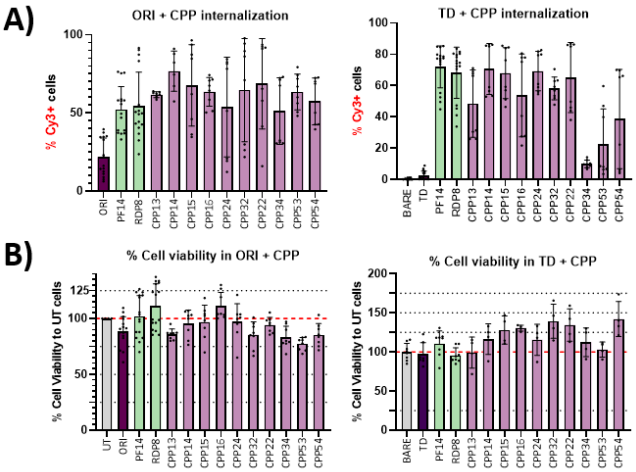

Figure 1. Internalization (A) and cell viability (B) on U87 MG luc2 cells of ORI – CPPs and TD - CPPs complexes.