Improving internalization of DNA origami and tetrahedron via cell penetrating peptides (CPPs) complexation for future RNA therapeutic delivery

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RNA-based therapeutics, including small interfering RNAs (siRNAs) and microRNAs (miRs), have emerged as promising tools to modulate regenerative pathways in ischemia-damaged cardiac tissue. However, their clinical application remains limited by the lack of safe and effective delivery systems.

DNA nanotechnology provides a versatile and biocompatible platform to overcome this limitation. By exploiting Watson–Crick–Franklin base pairing, DNA can be used as a programmable building block to construct nucleic acid nanostructures (NANs) with customizable properties(1) . These structures can encapsulate or hybridize with different RNA cargos, through sequence complementarity, allowing for controlled loading and efficient release.

To improve the cellular uptake of these NANs, cell penetrating peptides (CPPs) are used as delivery enhancing agents. These short, cationic peptides facilitate translocation across negatively charged cellular membranes and interact electrostatically with DNA nanostructures (2). The CPP to NAN ratio (N/P) must be optimized to ensure the formation of stable complexes suitable for cellular delivery.

In this proof of concept study, NANs in the form of origami and tetrahedron were assembled and functionalized with two CPP families (PF14-AH and PF14-AK). Their uptake was evaluated in U87 Luc2 and HaCaT cells, showing a marked improvement in internalization without compromising cell viability.

These results provide a starting point for the development of non-viral delivery strategies in RNA therapies. Current efforts are focused on expanding this approach to alternative nanostructures, such as DNA nanohydrogels, with the ultimate goal of delivering miRs to hiPSC-derived cardiomyocytes as a proof of concept of their efficacy in a translational model.

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