Harnessing Mirror-Image Peptides: Nanoparticle Vectors for Nucleic Acid Delivery in Cancer Treatment

Andrea Banfi[1], Lucia Salvioni[1], Miriam Colombo[1], Davide Prosperi[1]

E-mail: a.banfi17@campus.unimib.it
[1] *NanoBioLab*, Department of Biotechnology and Biosciences, University of Milano-Bicocca, Milan, Italy

Keywords: peptide-based nanoparticles, targeted therapy, nucleic acids nanodelivery, immunotherapy, cancer

Abstract:

The development of efficient delivery systems for nucleic acid-based cancer therapies is an ongoing challenge. Peptide-based systems are emerging as promising alternatives due to their tunable physiological properties. Nevertheless, their clinical translation is hindered by limited stability and susceptibility to proteolytic degradation.
Here, we investigate D-peptides, protease-resistant synthetic peptides composed exclusively of D-amino acids (aa), as nanovectors for nucleic acid (NA) delivery. Due to their inverted chirality, Mirror-Image Peptides (MIPs) exhibit outstanding resistance to protease activity while retaining biological functionality. Among several candidates, we selected a 30-aa charged peptide sequence capable of self-assembling into nanostructures by complexing with negatively charged NAs.
Using both manual and automated microfluidic-based synthesis, we generated nanoparticles complexed with either RNA or double-stranded DNA. Microfluidic synthesis provided superior size control compared to manual methods and, importantly, offers a scalable and reproducible platform suitable for future clinical translation. Stability and protease-resistance were assessed *via* gel electrophoresis, revealing significantly enhanced NA retention in MIP-based nanoparticles relative to their natural L-form counterparts. D-form nanoparticles were further evaluated in MDA-MB-231 luciferase-expressing cells, were they achieved siRNA-mediated gene silencing efficiencies comparable to L-form nanoparticles.
The goal of this platform is to activate the cGAS-STING innate immune pathway by delivering dsDNA to the tumor microenvironment. This pathway is a central cytosolic DNA-sensing mechanism that triggers type I interferon production and a broad antitumor immune response. Preliminarily studies using a macrophage reporter cell lines (THP1-Dual™) confirmed robust pathway activation, supporting the underlying rationale. In parallel, epifluorescence imaging demonstrated efficient NP uptake in both cancer cells and immune cells, key players in orchestrating effective antitumor responses.