



HAL
open science

Engineering lipid-based micro-RNA therapeutics for treatment of intervertebral disc degeneration

Hussein H Genedy, Paul Humbert, Bilel Laoulaou, Marion Fusellier, Catherine Le Visage, Catherine Passirani-Malleret, Jérôme Guicheux, Johann Clouet, Elise Lepeltier

► To cite this version:

Hussein H Genedy, Paul Humbert, Bilel Laoulaou, Marion Fusellier, Catherine Le Visage, et al.. Engineering lipid-based micro-RNA therapeutics for treatment of intervertebral disc degeneration. SF Nano 2023, Dec 2023, Montpellier, France. hal-04841115

HAL Id: hal-04841115

<https://hal.science/hal-04841115v1>

Submitted on 16 Dec 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Engineering lipid-based micro-RNA therapeutics for treatment of intervertebral disc degeneration

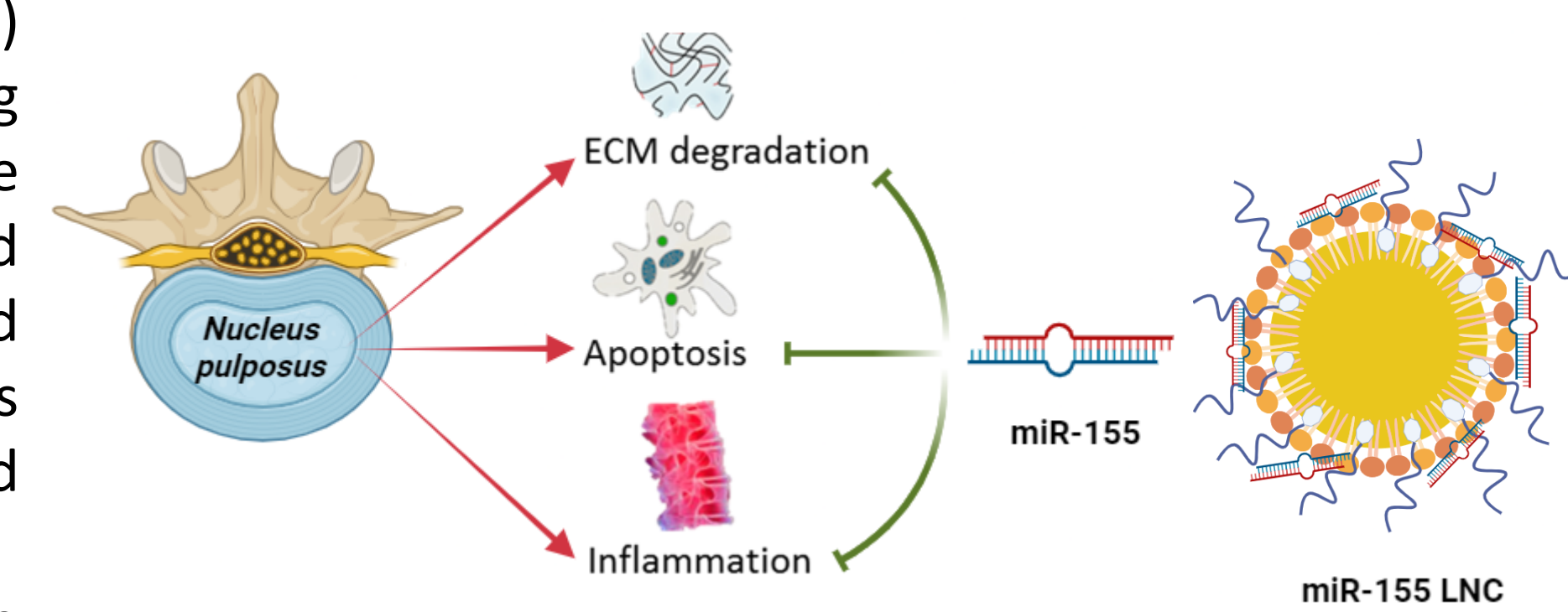
Hussein Genedy¹, Paul Humbert², Bilel Laoulaou², Marion Fusellier^{2,3}, Catherine Le Visage^{2,4}, Catherine Passirani¹, Jérôme Guicheux^{2,6,7}, Johann Clouet^{2,4,8*}, Élise Lepeltier^{1,9*}

¹ Univ Angers, Inserm, CNRS, MINT, SFR ICAT, F-49000 Angers, France; ² Nantes University, Oniris, CHU Nantes, INSERM, Regenerative Medicine and Skeleton, RMeS, UMR 1229, F-44000 Nantes, France; ³ Department of Diagnostic Imaging, CRIP, ONIRIS, College of Veterinary Medicine, Food Science and Engineering, Nantes F-44307, France; ⁴ Nantes Université, UFR Sciences Biologiques et Pharmaceutiques, Nantes, F-44035, France; ⁶ Nantes Université, UFR Odontologie, Nantes, F-44042, France; ⁷ CHU Nantes, PHU4 OTONN, Nantes, F-44093, France; ⁸ CHU Nantes, Pharmacie Centrale, PHU 11, Nantes, F-44093, France; ⁹ Institut Universitaire de France (IUF)

Introduction & objectives

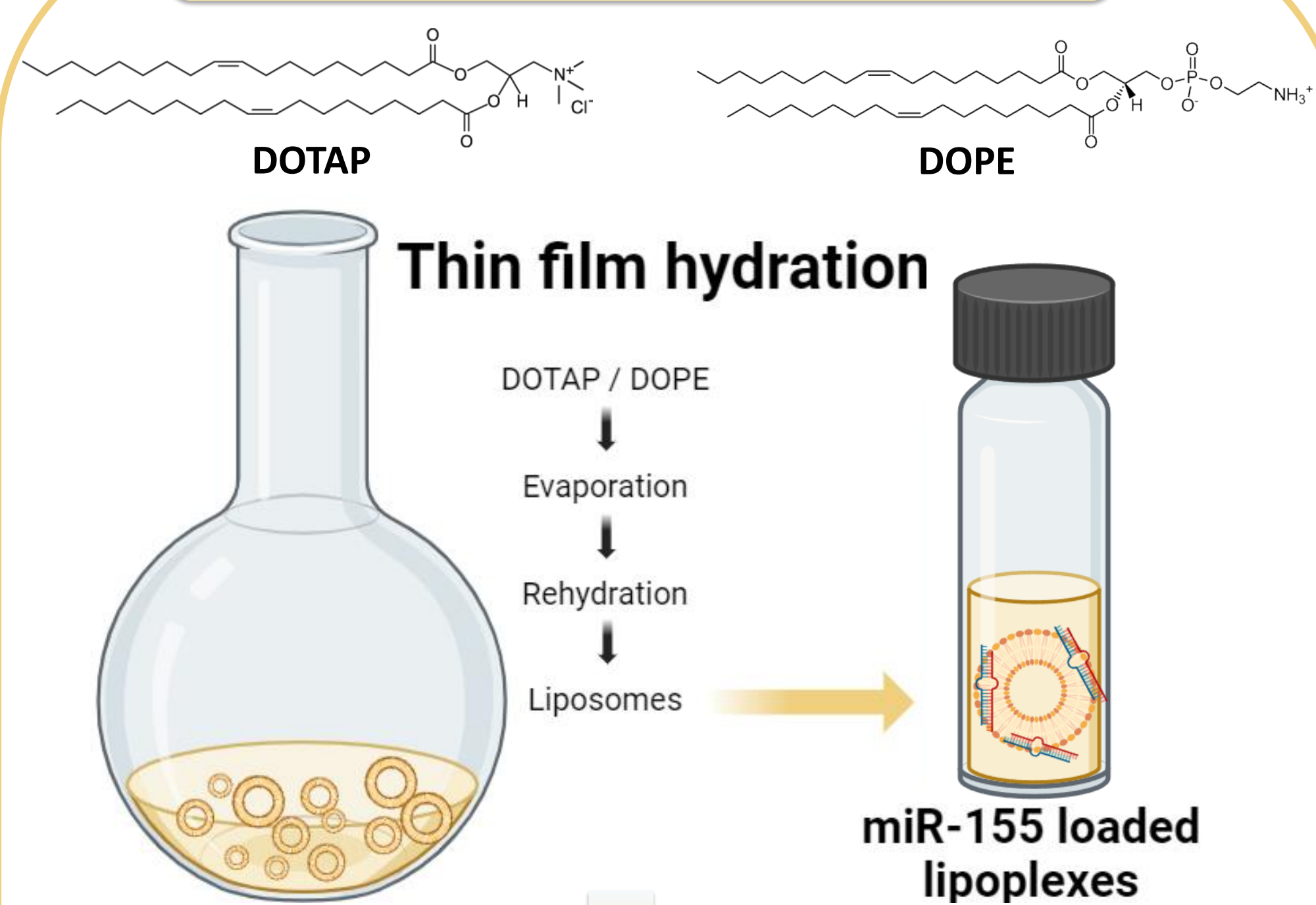
The past decade has witnessed an increased interest in harnessing RNA interference (RNAi) mechanisms for therapeutic applications, going all the way to the clinic and meeting the regulatory agencies' criteria. Given the accumulating evidence that multiple micro-RNAs (miRs) are dysregulated during disc degeneration, they could have a huge potential as biomarkers, targets, or active principles against this debilitating condition. Our miR of choice, miR-155, is downregulated in intervertebral disc degeneration (IVDD) and is known to be involved in protecting the IVD from apoptosis, decreasing degradation and controlling inflammation [1]. However, miRNA delivery encounters extracellular and intracellular barriers. Extracellularly, they would be rapidly degraded by endogenous nucleases and intracellularly, their uptake will be hindered by their relatively high molecular weight and negative charge. A promising technique to address this challenge is the vectorization of miRNAs within lipid nanocapsules (LNCs), providing both protection from degradation and improving their uptake within the scarce target cells, called *Nucleus pulposus* (NP), of the degenerated IVD [1,2].

In this study, our primary objectives are to formulate and comprehensively characterize miRNA-loaded LNCs. Additionally, we aim to compare two distinct methods of peptide adsorption for the surface decoration of LNCs. Through these objectives, we seek to optimize the delivery efficiency and therapeutic potential of miRNA-loaded LNCs, laying the groundwork for enhanced treatments in intervertebral disc degeneration.

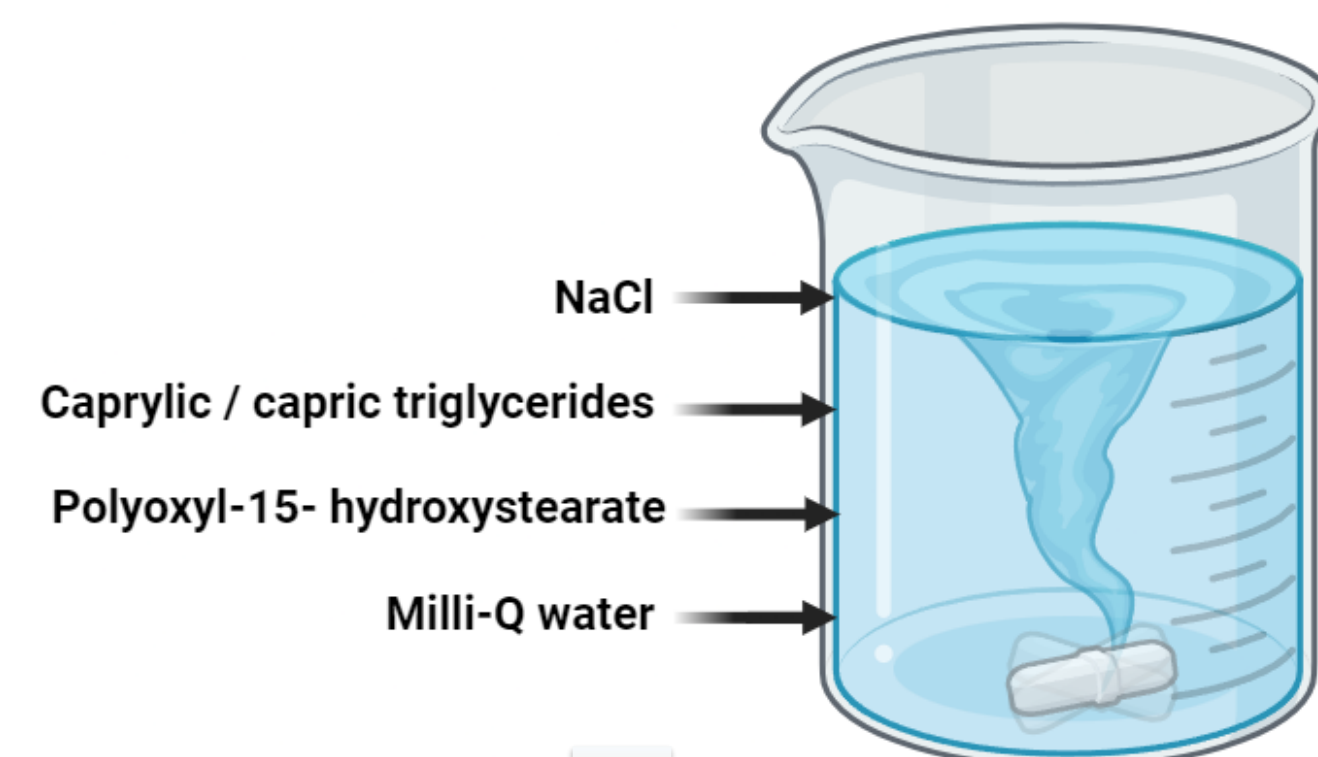


Material & Method

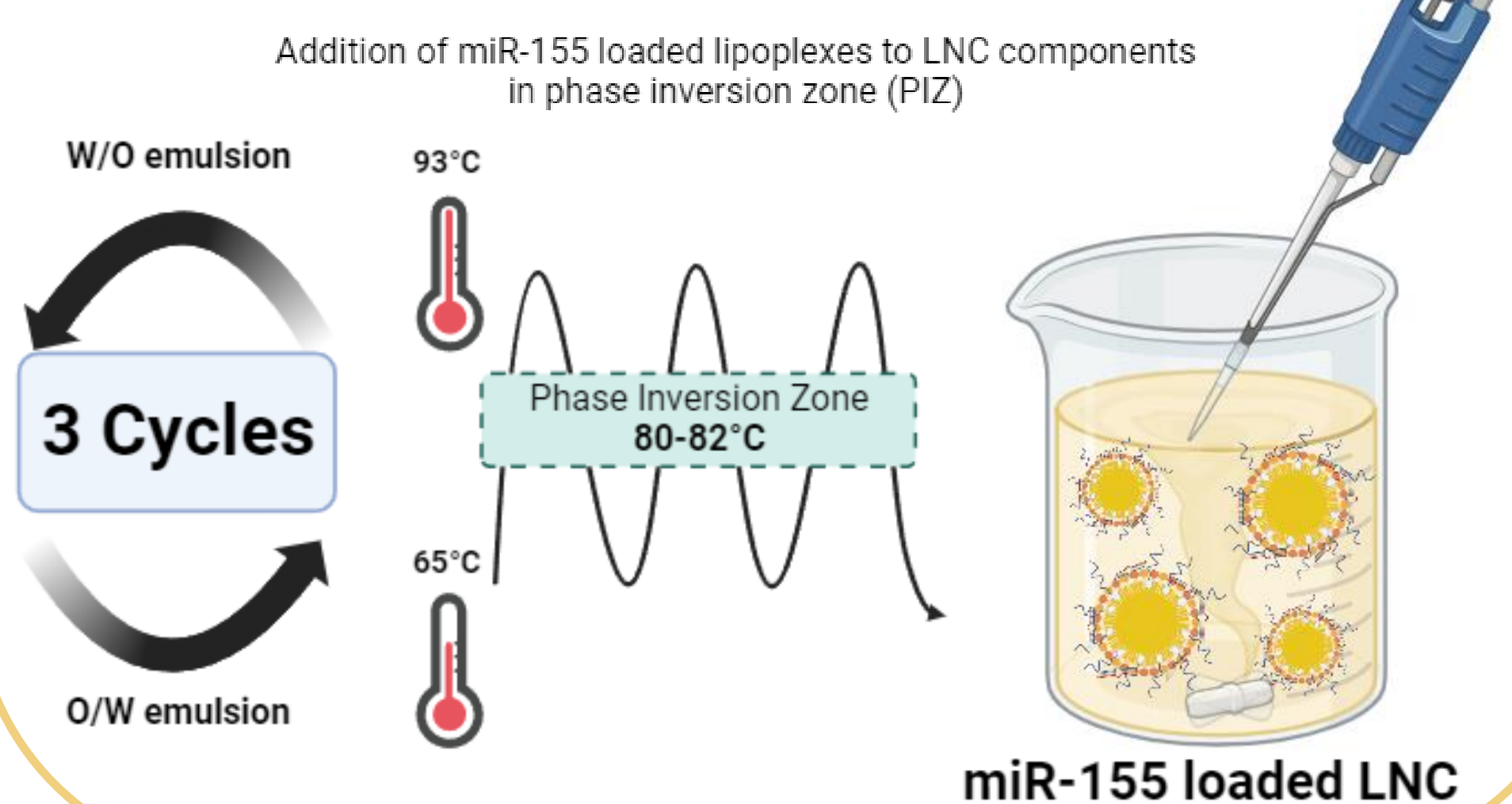
miR-155 LNC formulation



LNC Components

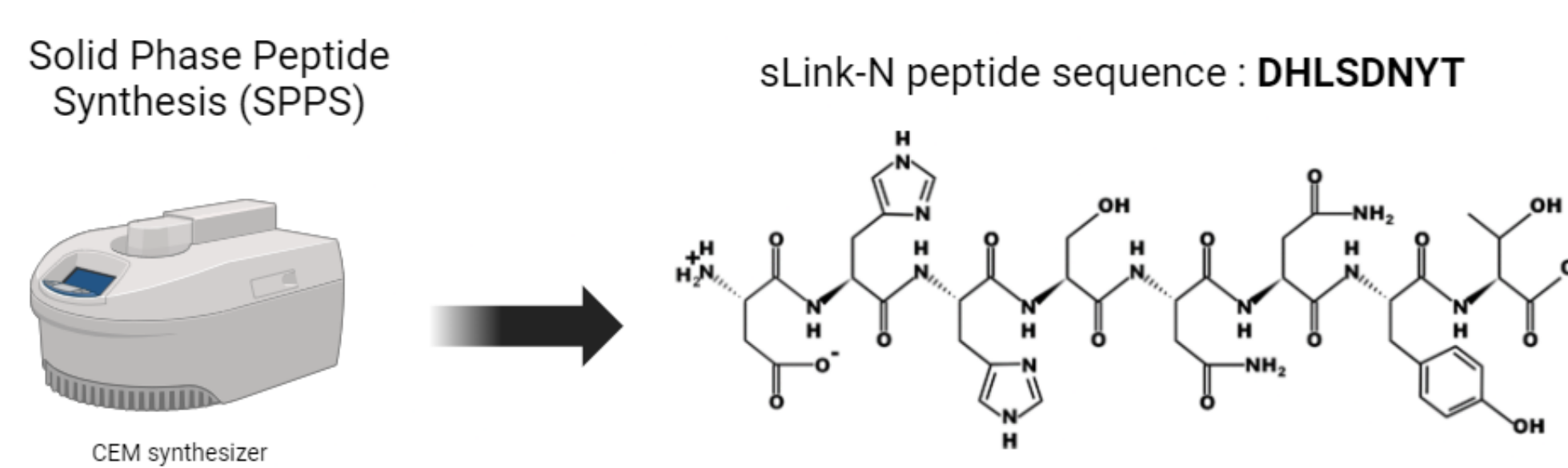


Phase inversion process



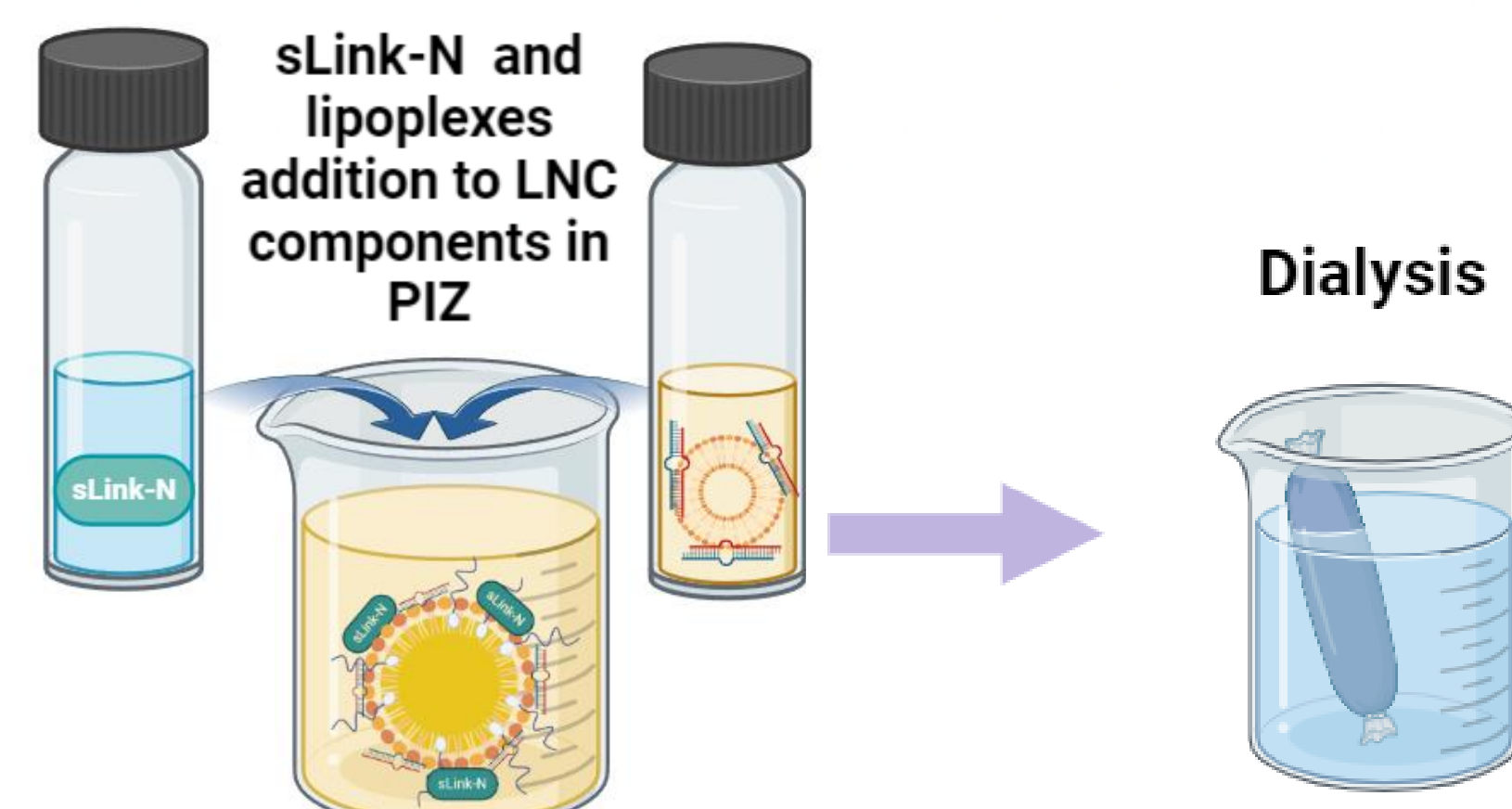
Peptide decoration

sLink-N peptide synthesis



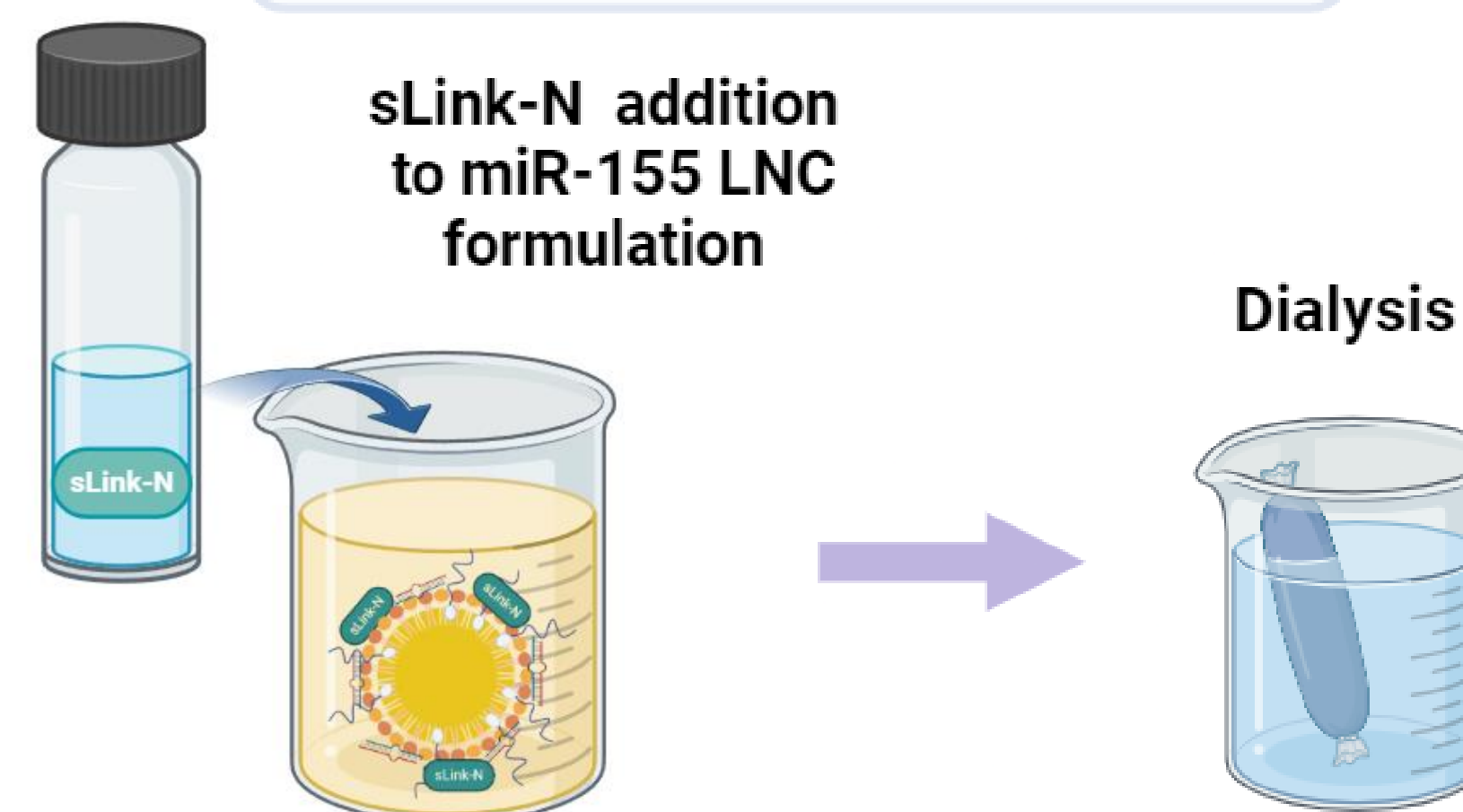
Two decoration methods:

Per-formulation adsorption



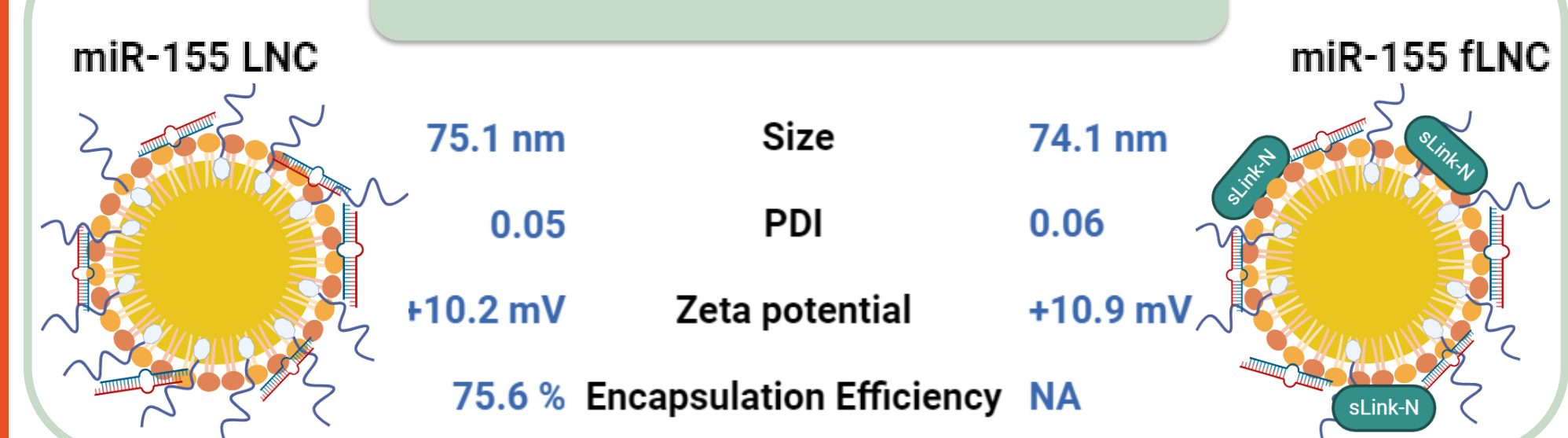
VS

Post-formulation adsorption

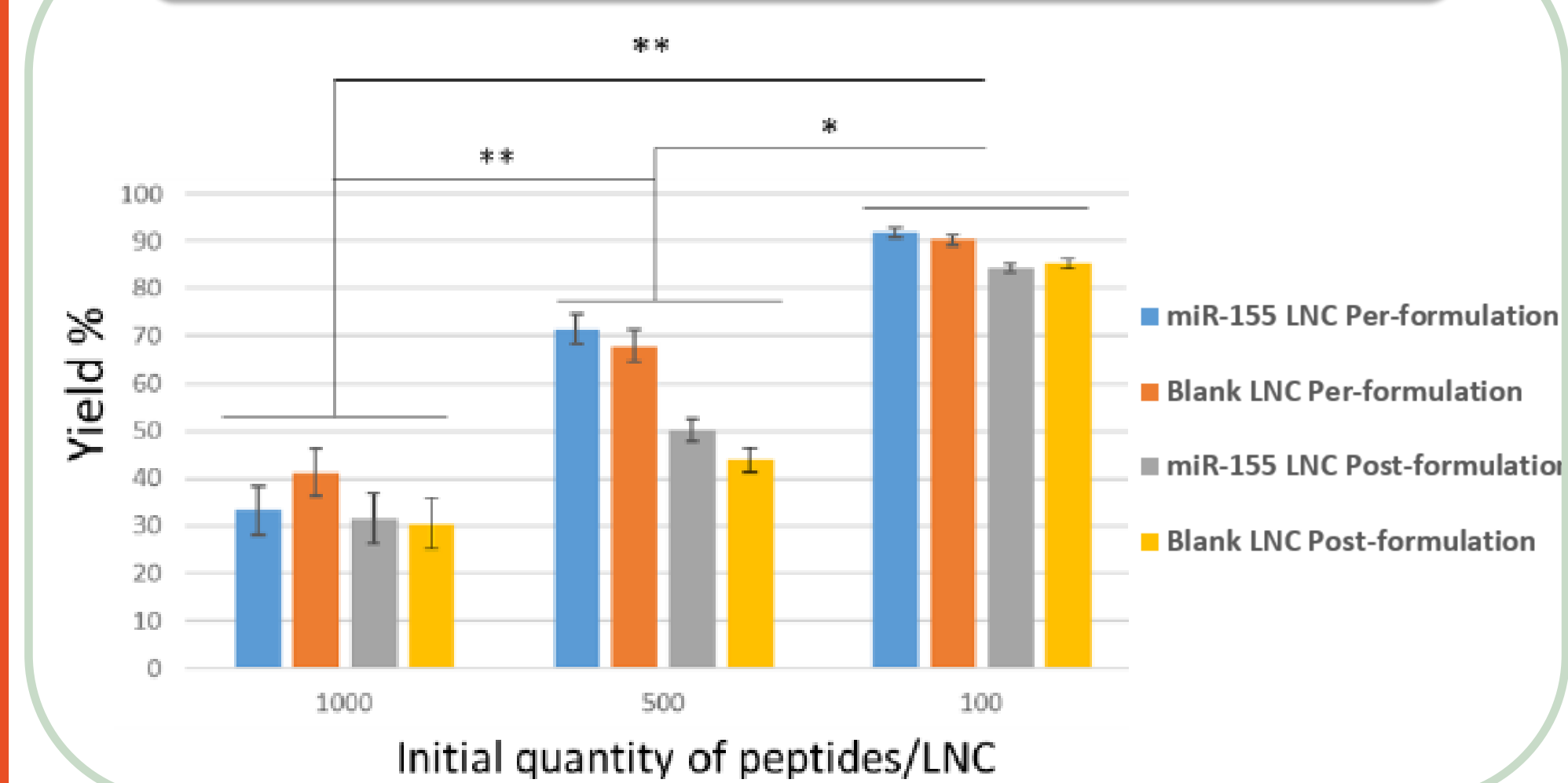


Results

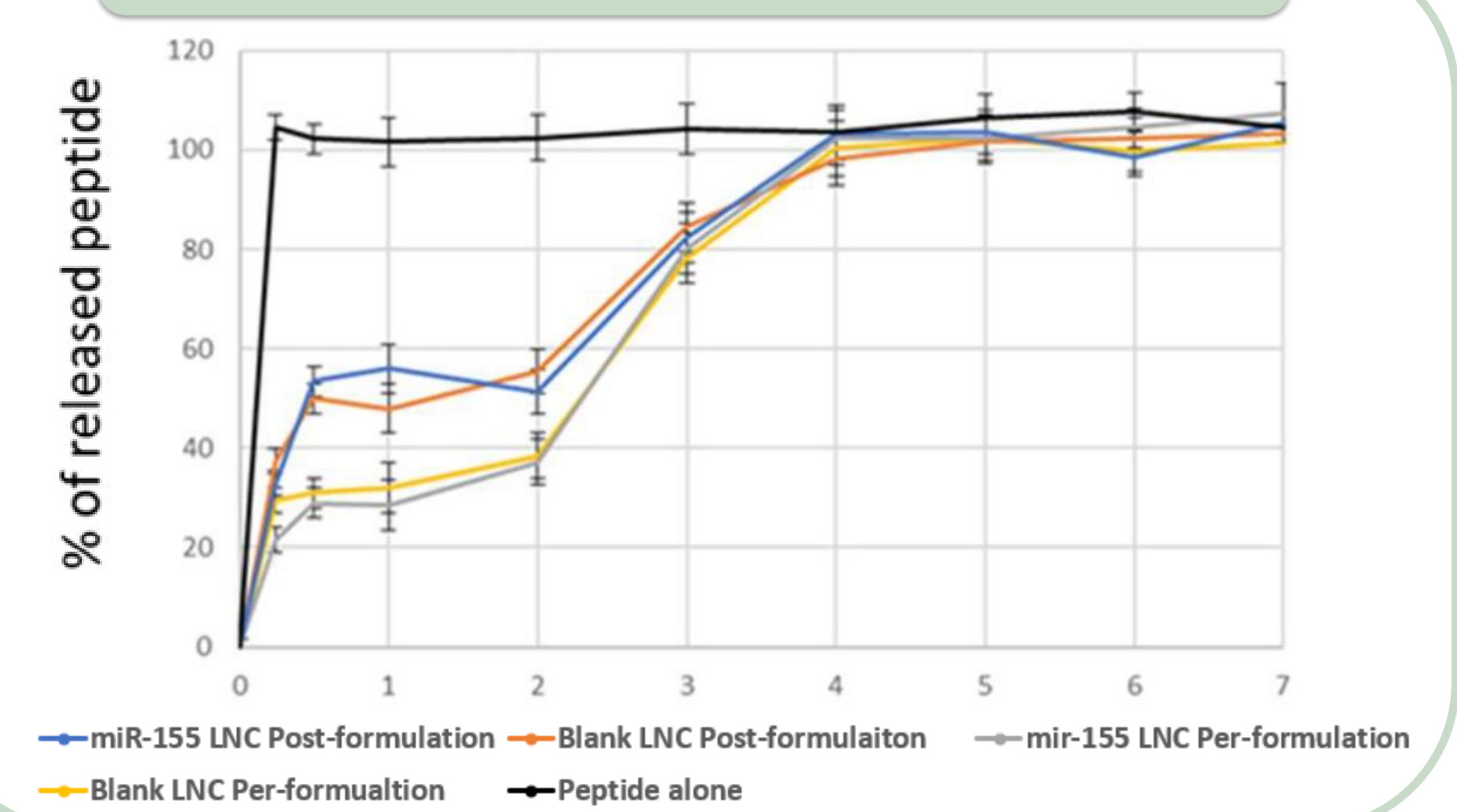
Characterization



Per/Post-formulation yield

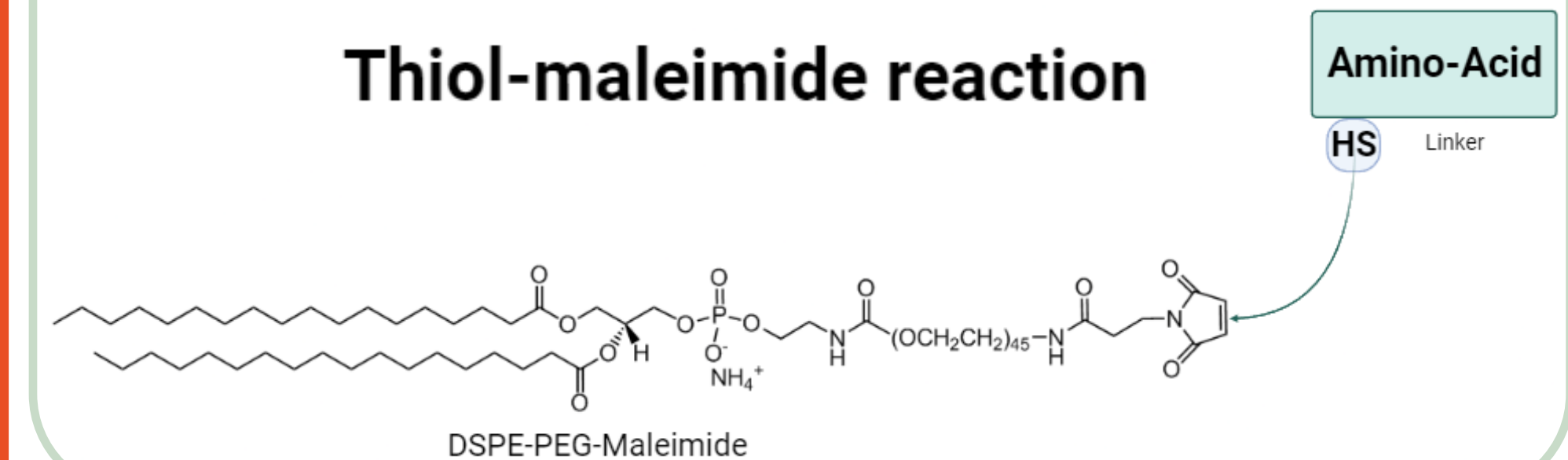


Peptide release kinetics



Perspective

Thiol-maleimide reaction



Discussion & Conclusion

The surface decoration of LNCs exhibits negligible influence on particle or surface charge. While encapsulation efficiency and drug loading for functionalized LNCs await determination, our exploration of peptide adsorption methods highlights the superiority of the per-formulation approach, particularly with an initial peptide quantity of 500 peptides/LNC.

Stability studies over 7 days reveal a slower release with the per-formulation method at 48 hours, suggesting enhanced grafting of peptides during LNC formulation. However, no substantial difference in peptide release is noted after 72 hours. The per-formulation method emerges as the most viable option, offering improved stability without compromising loaded LNC integrity.

Future investigations may compare this method with covalent peptide grafting, utilizing a modified sequence featuring a terminal cysteine. This could provide insights into potential synergies, advancing our understanding of surface functionalization techniques.

In summary, our results endorse the per-formulation method for the peptide adsorption, showcasing its stability and controlled release kinetics. This approach holds promise for optimizing micro-RNA therapeutics delivery, laying a foundation for enhanced treatments in IVDD.

References:

- [1] Clouet, J (2019). Intervertebral disc regeneration: From cell therapy to the development of novel bioinspired endogenous repair strategies. *Advanced Drug Delivery Reviews*
 [2] Le Moal, B (2022). Lipid nanocapsules for intracellular delivery of microRNA: A first step towards intervertebral disc degeneration therapy. *International Journal of Pharmaceutics*.

Acknowledgment:

This study was supported by grants from the Agence de la Biomédecine, the Institut National de la Santé et de la Recherche Médicale (INSERM), the Région des Pays de la Loire, ANR PRC 2022 (NEMESIS project). The authors gratefully acknowledge the technical assistance they received from personnel of MINT and RMeS laboratories.