FINAL SCIENTIFIC REPORT

Nanovehicle fine-tuning for improved anticancer drug delivery (NanoCanTune)

SUMMARY

The project ***Nanovehicle fine-tuning for improved anticancer drug delivery (NanoCanTune)*** was aimed at developing new liposomal vehicles as controlled release systems for the antitumor agent 5-fluorouracil (5-FU) by investigating key aspects of the properties and behavior of nanovehicles in a biological context. This goal was achieved through a multidisciplinary experimental approach, incorporating **synthesis and physico-chemical characterization techniques**, **bioinformatics**, and **proteomics**, targeted towards: (1) investigating the stability of liposomal formulations, and (2) identifying the optimal liposomal formulation for which the drug release properties of the antitumor agent 5-FU are prolonged. The hypothesis underlying this project is that by adjusting the composition of the protein coating (protein corona) formed on the liposomal surface when nanovesicles are in a biological environment might be a promising strategy to improve therapeutic specificity and efficacy. Meanwhile, current findings present contradictory results regarding the membrane affinity and permeability of 5-FU in liposomal structures, highlighting a critical need for clarification and deep understanding of the precise mechanisms involved in the delivery and distribution of 5-FU in liposomes. Therefore, identifying a liposomal formulation that presents an optimal profile of 5-FU release, combined with stability in a biological context, is crucial for the development of new anticancer therapies with improved release kinetics.

Specifically, this project employed a **multidisciplinary approach**, which included both **synthesis and physico-chemical characterization techniques**, as well as the use of **molecular modeling** and **proteomic methods** to successfully achieve the proposed objectives. Initially, liposomal nanovehicles with different lipid compositions, with or without the antitumor drug 5-FU, were prepared using the thin-film hydration technique. The nanovehicles were thoroughly characterized by various physico-chemical methods, including dynamic light scattering (DLS) and transmission electron microscopy (TEM), and for those containing 5-FU, the encapsulation efficiency and controlled release properties *in vitro* were also determined. This allowed for the identification of formulations with 5-FU that had the best release kinetics, as well as highlighted the compositional parameters influencing encapsulation efficiency. Additionally, the behavior of nanovehicles in a biological context was studied to describe the protein corona through electrophoretic profile characterization for each composition. In terms of molecular modeling, the influence of lipid composition on the

distribution of 5-FU in membrane systems was detailed (Figure 1), with partial overlap being identified between computational and experimental results.

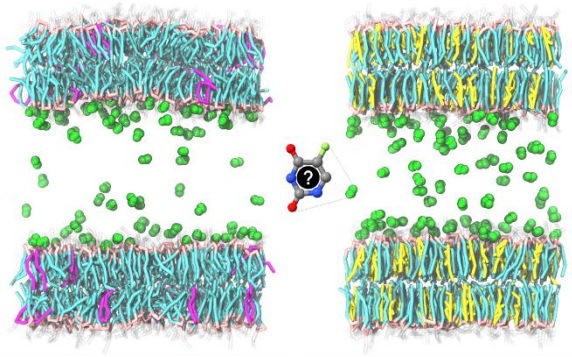

Figura 1. Schematic representation of the influence of lipid composition on the preference of the 5-FU molecule for various regions in the simulated molecular systems; The carbon chains of the phospholipid DPPC are colored in turquoise, cholesterol lipid in yellow, and the positively charged lipid DOTAP in magenta. 5-FU is represented as green spheres.

Thus, we could highlight the challenges in the direct translation of molecular models into experimental realities and, implicitly, the complex balance between lipid composition, encapsulation efficiency, and release kinetics from liposomal systems with 5-FU. Specifically, the results generated in this project emphasized the importance of an integrated approach, combining computational and experimental methodologies, to unravel the complexity of drug-lipid membrane interactions towards developing more efficient liposomal nanovehicles. The experimental results of this project are expected to have a significant scientific impact, being of particular fundamental relevance. Furthermore, the obtained liposomal systems, especially the one containing 10 mol% DOTAP, which presented an optimal release profile for the antitumor drug 5-FU, and does not undergo significant physico-chemical alterations in a biological context, have a high application potential due to the improved release profile and, implicitly, the minimization of repeated drug dosing to achieve the expected therapeutic effect. Moreover, these systems could lay the groundwork for developing other new formulations with improved properties. The most significant result of the project are its publications, one article already having been accepted for publication in the scientific journal *ACS Omega*, along with a Supplementary Cover Art, which will facilitate its visibility among the journal's readers, and two additional articles being under evaluation in scientific journals within the top 25% most relevant to their field (*Scientific Reports* and *Molecular Pharmaceutics*). The cumulative impact factor of journals that have accepted or are evaluating manuscripts with results from this project is >10, suggesting an excellent impact on the scientific community. The project director participated in 4 international conferences and carried out a mobility in Cluj-Napoca, for participation in the workshop "A week-long

exploration of Quantum Chemistry through Jupyter Notebooks", thus **all indicators regarding result dissemination have been met**. Additionally, the project director has successfully laid the foundations for the research direction in the field of liposomes at the host institution, which will improve and strengthen the institution's research direction in nanomedicine, an objective proposed through the H2020-ERA-Chairs project (No. 952390), which is ongoing at the same host institution.

Project director,
Dr. Pomohaci Roxana-Maria