université PARIS-SACLAY
FACULTÉ DE PHARMACIE

Graduate School: Health and Drug Research
Master 2 Pharmaceutical Technology and Biopharmacy

Company/laboratory/public institution: Institut Galien Paris Saclay (UMR 8612)

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Supervision of trainee:

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Position: Chair Junior Professor & Associate professor

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Internship period: 20 January - 18 July 2025

Title of the project: Preparation and functionalization of liposomes with proteins for characterization via surface plasmon resonance imaging and capillary electrophoresis: a model for extracellular vesicle studies

Context: Extracellular vesicles (EVs), naturally secreted by cells and carry on their surface and in their cargo proteins and nucleic acids, targeting other cells, making them promising candidates for diagnosis and therapy. However they have very heterogeneous sizes and membrane proteins which make them hard to be analyzed. Liposomes are versatile synthetic lipid-based nanocarriers that can encapsulate a wide range of molecules and be functionalized with various biomolecules, making them an attractive tool for drug delivery and bioanalytical studies. Liposomes are similar to EVs in their aspect of having lipid membrane, however, as liposomes are "artificial", their properties and cargo could be finely tuned. This project will use liposomes as a model, mimicking EV characteristics, to develop and optimize separation and detection techniques, mainly capillary electrophoresis (CE) and phase-contrast surface plasmon resonance imaging (PC-SPRi). The aim is to prepare liposomes of different sizes and functionalize their surface with proteins of varying molecular weights. These functionalized liposomes will be characterized using conventional nanoparticles characterisation techniques as well as using CE and SPRi, to analyze their biophysical properties. This will serve as a model for understanding EV behaviour in biological systems

Objectives:

1. Establish a protocol for **liposome preparation**:
    - Prepare liposomes of different sizes using **microfluidics**, where the composition of aqueous and lipid phases, as well as other microfluidic parameters, will be optimised to obtain various sized liposomal preparations.
    - Attach proteins of both small and large molecular weight (albumin, lysozyme) to the surface of the liposomes, mimicking the membrane protein content of EVs. This will serve later for immuno-immobilization of liposomes
    - Evaluate the efficiency and stability of liposomes post-loading using techniques such as dynamic light scattering (DLS), nanoparticles tracking analysis (NTA), and zeta sizer.
2. Develop advanced analytical techniques for the characterization of EVs:
    - Investigate the separation and functionalization of liposomes using techniques involving diffusion and electromigration of liposomes in microcapillaries including Taylor Dispersion Analysis[1] (Obeid, 2023), and capillary electrophoresis[2-3] (Morani, 2020; Zohouri, 2024).
    - Develop the detection of functionalized liposomes using SPRi and PC- SPRi (Patent SYMMES-Livache[9]) for functionalized liposomes detection and comparison between the two methods.

- Perform initial tests for comparison of the synthesized "model System for EVs", liposomes, with the actual biological EVs

Expected outcomes: Develop optimized protocols for preparing liposomes of different sizes and attaching proteins with high efficiency and stability. Gain insights into the limit of detection in terms of resolution and concentration of SPRi and PC-SPRi techniques. Understand the possible behaviour of biological nanoparticles, when tested using same analytical techniques.

Technical Program:

- Week 1-4: familiarization with liposome synthesis, size optimization, and nanoparticle characterization techniques (DLS, NTA, Zetasizer).
- Week 5-8: Protein functionalization methods for liposomes, followed by stability assessments.
- Week 9-16: Optimization of analytical techniques (CE, TDA, SPRi) for detecting and characterizing functionalized liposomes.
- Week 17-20: Comparative analysis of liposome models with EVs, focusing on stability, surface properties, and detection sensitivity.
- Week 21-24: The final phase will involve compiling results, completing any remaining experiments, and proposing future research directions

Skills and competencies you will acquire by the end of the master's internship:

- Experience in preparing liposomes and functionalizing them with proteins.
- Proficiency in using advanced characterization techniques, including SPRi and CE
- Proficiency with various instrumental techniques like NTA, DLS, zeta-sizer.
- Engage in experimental design, critical thinking and problem-solving, driving innovative approaches in drug loading and diagnosis.
- Ability to work independently and collaboratively within a research team

Profile and required skills:

- Students in their second year of a master's program in chemistry, pharmaceutical formulation, biopharmaceutics, nanomedecines, biotechnology or relevant field are encouraged to apply.
- Motivation to engage in cutting-edge interdisciplinary science involving chemistry, microfluidics, physics, and biology.
- Strong scientific curiosity, attention to detail, and a collaborative mindset.

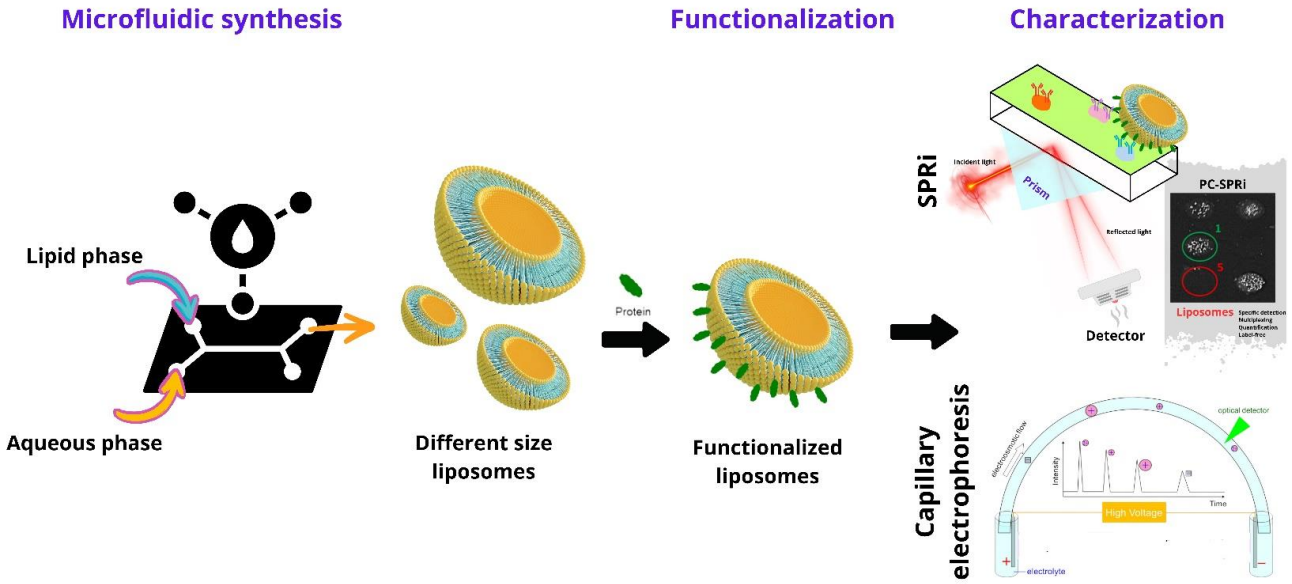

Fig. 1: Liposome preparation, functionalization and characterizationVarious sizes of liposomes will be synthesized using microfluidic platform. Proteins will be incorporated on the surface of liposomes then the separation of liposomes would be done using capillary electrophoresis and their immobilization using antibodies using SPRi

References:

1. Obeid, S. et al. (2023), Fast, simple and calibration-free size characterization and quality control of extracellular vesicles using capillary Taylor dispersion analysis. Journal of Chromatography A, 1705, 464189
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4. Lamaa, Diana, et al. "Overcoming Solubility Challenges: Liposomal isoCoQ-Carbazole as a Promising Anti-Tumor Agent for Inoperable and Radiation-Insensitive cancers." ChemBioChem (2024): e202400062.
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8. Ismail, A. et al. Contactless Bio-Electrofunctionalization of Planar Micropores. Advanced Materials Technologies 6, 2001154 (2021)
9. C-C. Andrei, C.HERRIER, S. SLIMANI, Y. Hou-Broutin, C. Moreau, T. Livache. (2022). Patent : FR2200324. A Procédé de détection d'objets biologiques par imagerie par résonance plasmonique de surface.