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 & Professor

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Internship period: 20 January - 18 July 2025

Title of the project: Development and comparison of methodologies for drug loading into biological nanoparticles: focus on Extracellular vesicles for therapeutic applications

Context: Extracellular Vesicles (EVs) are nanoparticles secreted by various cell types. They carry cellular content through the bloodstream from the parental cell to target cells. The cellular membrane of EVs protects their content from degradation and provides membrane receptors (e.g., tetraspanins) that bind to complementary receptors on target cells, triggering endocytosis and delivering the EV cargo. Because of their natural origin, biocompatibility, and ability to transfer a wide range of therapeutic agents, EVs are of significant interest as nanocarrier and drug delivery systems. They can serve as nanovectors to deliver drugs to target cells, protect therapeutic agents from the extracellular medium, and help cross biological barriers.

However, a significant challenge remains: finding an effective method to load large amounts of therapeutic agents into EVs without compromising their stability. Developing efficient loading methods and understanding the underlying mechanisms are crucial to enhancing the clinical and therapeutic potential of EVs.

Objectives:

1. Develop and optimize loading methods using bulk and microfluidic methodologies:
   - Develop techniques such as electroporation, sonication, Freeze-thaw cycles, passive loading, etc (Fig. 1) to load drugs (vitamine C, paracetamol, etc) into EVs derived from human plasma obtained commercially
   - Understand and correlate the loading mechanism with the physico-chemical properties of the loaded agent (hydrophilic: Ascorbic acid (Vitamine C) and Dopamine; amphiphilic: acetaminophen (paracetamol); larger biomolecules (miRNA)
   - Evaluate the efficiency and stability of EVs post-loading.
2. Establish a protocol to characterize loaded EVs:
   - Investigate various techniques to characterize EVs and their loading, including fluorescence, UV-visible spectroscopy, nanoparticle tracking analysis (NTA), western blot, zeta potential, dynamic light scattering (DLS), electrochemical methods, Taylor Dispersion Analysis[1] (Obeid, 2023), and capillary electrophoresis[2-3] (Morani, 2020; Zohouri, 2024).

Expected outcomes: Gain insights into the mechanisms of biomolecule loading into EVs, identifying key factors influencing the efficiency and stability of the loading process. Develop protocols for efficient and stable loading of therapeutic agents into EVs, and establish standardized procedures for the characterization and analysis of loaded EVs.

Technical Program:

- Week 1-4: The intern will begin by familiarizing themselves with the core concepts and practical techniques related to EV isolation (ultracentrifugation, filtration) and characterization (NTA, DLS, Zetasizer, TDA, CZE).
- Week 5-8: The intern will focus on learning drug loading methods, particularly the use of electrochemical techniques (electroporation), sonication, and freeze-thaw cycling, while evaluating their effects on EV stability.
- Week 9-16: The intern will optimize drug loading methods, testing the loading efficiency of various drugs using the previously learned techniques and assessing the stability of EVs. they should perform experiments in bulk and using microfluidic devices.
- Week 17-20: During this period, the intern will conduct detailed data analysis to evaluate how different methods affect drug loading efficiency and the stability of EVs. They will also begin preparing their Master's thesis and presentation.
- 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:

- Contribute and innovate in developing novel methods and protocols.
- Hands-on experience working with biological nanoparticles and complex media.
- Proficiency with various instrumental techniques like NTA, DLS, zeta-sizer, electrochemistry, UV-Vis spectroscopy, fluorescence, western blot.
- Engage in experimental design, critical thinking and problem-solving, driving innovative approaches in EV research.
- 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 technology,biotechnology or relevant field are encouraged to apply.
- Motivation to engage in cutting-edge interdisciplinary science involving chemistry, microfluidics, physics, and biology.
- Scientific curiosity, critical thinking, and a willingness to collaborate with different team members.

Perspectives: This internship could pave the way for PhD recruitment after the master's program.

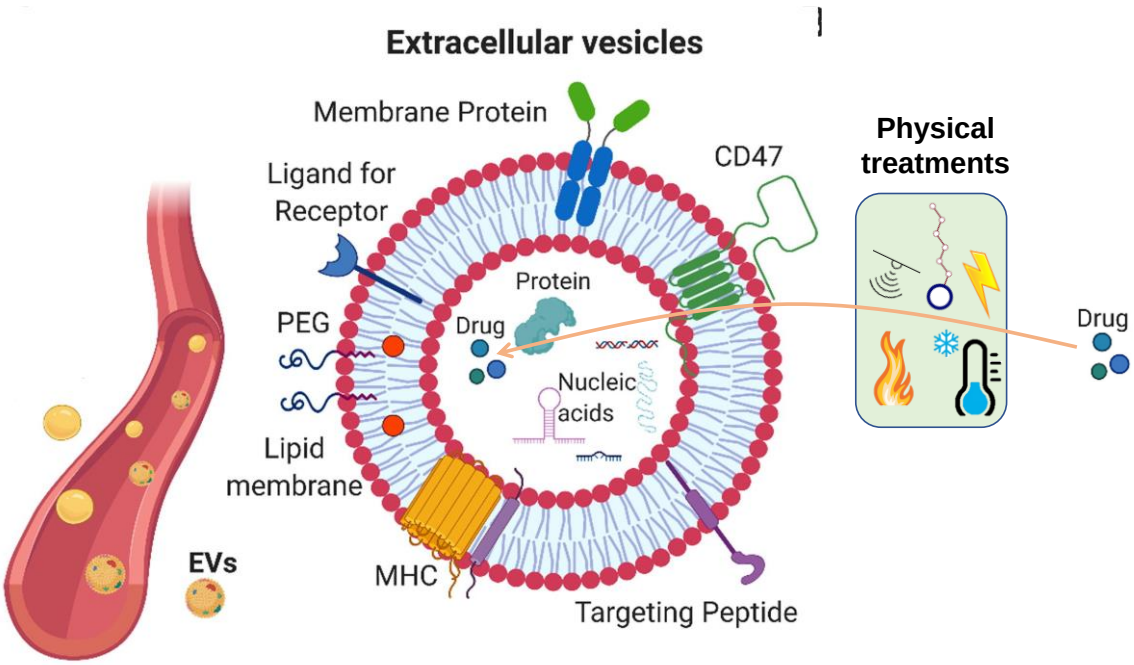

Fig. 1: EVs as a drug delivery system (DDS): proteins, hydrophilic drugs and nucleic acids (miRNA, siRNAs, mRNAs, etc.) can be loaded in the lumen of vesicles whereas targeting ligands, membrane proteins and lipophilic drugs can be incorporated in the membrane. Adapted from ref 6.

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
2. Zohouri, D. et al. (2024), Investigation of on-line electrokinetic enrichment strategies for capillary electrophoresis of extracellular vesicles. Journal of Chromatography A, 465116
3. Morani, M. et al. (2020). Electrokinetic characterization of extracellular vesicles with capillary electrophoresis: A new tool for their identification and quantification. Analytica Chimica Acta, 1128, 42-51.
4. Herrmann, I. K. et al. (2021). Extracellular vesicles as a next-generation drug delivery platform. Nature nanotechnology, 16(7), 748-759.
5. Du, S. et al. (2023). Extracellular vesicles: A rising star for therapeutics and drug delivery. Journal of Nanobiotechnology, 21(1), 231.
6. De Castilla, P. E. M. et al.(2021). Extracellular vesicles as a drug delivery system: A systematic review of preclinical studies. Advanced drug delivery reviews, 175, 113801.