TFM offers 2025-2026

Project title: Bioprocess development for the production of cosmetic ingredients in Plant Stem Cells (VEG4COS).

Summary: Platform development for the biotechnological production of ingredients for cosmetic applications based on ***in vitro*** **culture of plant stem cells** as an alternative to extracting ingredients from plants' biomass, to produce anti-aging, anti-oxidants, and other ingredients with cosmetic activity.

The interest of companies focused on the production of cosmetic ingredients is moving fast from the classical ingredient extraction from plants to biotechnological-based bioprocesses that allow the no stationary production, improve reproducibility and product quality. The student will join a team of the project VEG4COS composed by 2 PhD candidates. The project has granted by Acció (Nuclis, Genralitat de Catalunya) in collaboration a well stablished company of the cosmetic ingredients sector.

The objective of this Project is to explore the use of plant stem cells as a sustainable source of bioactive compounds for cosmetic applications. Starting from differentiated cell lines derived from explants, the project involves a process of selection and screening to identify the most resilient, fast-growing, and productive cell cultures. These selected lines will then be transferred to suspension cultures, where different elicitation methods will be tested to enhance the production of flavonoids and polyphenols. In the most promising cases, biochemical assays will be carried out to evaluate the antioxidant activity of the produced molecules, which will also be tested in an *in vitro* platform using human cell models (hNDF). The overall aim is to contribute to the development of innovative and environmentally responsible cosmetic ingredients through plant cell biotechnology.

Additionally the project pursues to stablish the bases of the bioprocess based on Plant Stem Cells at bench scale bioreactors (from shake flasks up to 5-liter Bioreactor). This work will include the study of the main culturing and bioprocessing conditions, the development of bioprocess monitoring tools, bioprocess intensification to increase productivities, and if possible, production of different batches of the ingredient of interest and its characterization.

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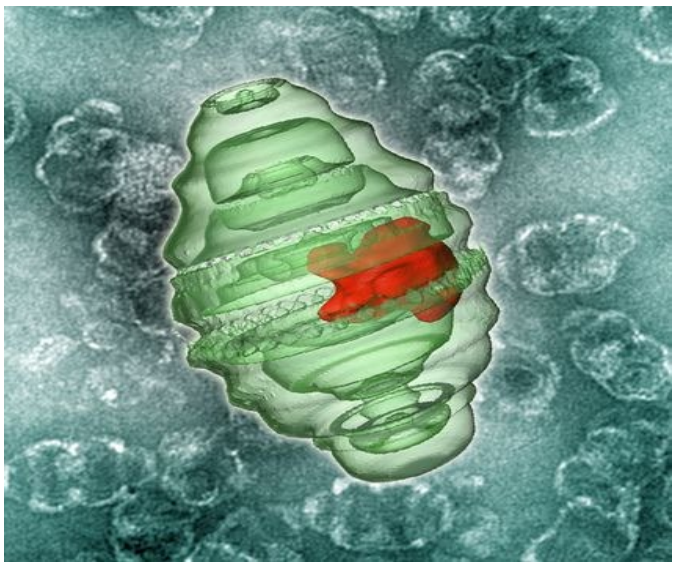
Project title: Production of Vaults: a virus-like-particle with a lock-open mechanism allowing the cargo of therapeutical as a novel drug delivery system.

Precision personalized medicine seek for next-generation biomaterials to serve as drug delivery systems with "smart" functional properties, including accurate recognition, self-organization and adaptability. Several strategies are currently inspired by the prospect of controlling the precise protein architectures as an alternative to the classic delivery systems. Among them, a particular virus like particles (VLP) named vaults represent a particularly attractive case, being about 40 nm -width-, 70 nm -length in size. Their natural function is not yet completely elucidated, although several functions related to nuclear transport, immune response, and drug multiresistance in cancer cells have been hypothesized. These nanocapsules are composed of different protein constituents and they can be produced in large quantities by expression of recombinant versions of the "major vault protein" (MVP) alone. Importantly, the vault particle represents an assembly of half-vaults. Under acidic conditions, the vaults can be reversibly opened and loaded with small molecules or biopharmaceuticals.



Microscopy image of isolated Vaults, and vault 3D reconstruction

The project consists in engineering HEK293 (human cell line) to produce engineered vaults with improved functionalities for cell tracking, quantification and specific purification. Initially, heterologous expression of MVP protein will be performed in HEK293 cells, and then purified by SEC and characterized by means of cytometry, western blot, NTA (nano-tracking analysis) and cryo-TEM. Once purified, vaults will be loaded with a small reporter molecule to assess functionality and loading capacity. Then, vaults uptake by recipient wild-type HEK293 cells will allow the assessment of vaults functionality and the potential of vaults to be used as delivery systems. Once purified, Vaults can be loaded with drugs/mRNA, by taking advantage of the dynamics of the nanoparticle (can be opened by lowering the pH).

The second part of the project consists in modifying MVP protein expression by fusing eGFP to N terminus of MVP to provide a fluorescent labelling to vaults, and StrepTag

to the C-terminus (located outside the cupula structure), what will ease vaults purification, tracking and quantification. Also targeting peptides will be fused to MVP to provide specific cell targeting to direct the drug delivery to specific recipient cells.

This piece of work will be conducted in collaboration with a PhD student and Dr Ana Belén Cuenca, head of Pharmaceutical Chemistry Department, whose team will further chemically modify the produced vaults to provide with improved features.

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Project title: Development of a platform to engineer small extracellular vesicles for peptide-based therapies targeting AAT deficiency.

Summary: The working hypothesis is that small extracellular vesicles (sEVs) can serve as an efficient delivery system for therapeutic proteins and peptides, which can be endogenously loaded by engineering the donor cell lines. Specifically, the present proposal focuses on developing an innovative therapeutic approach that employs small extracellular vesicles (sEVs) as a delivery system for functional alpha-1 antitrypsin (AAT) to protect alveoli from enzymatic degradation in patients sulfureting of AAT deficiency. This innovative treatment can be administrated via inhalation, directly to the lungs.

Building on this hypothesis, the primary goal of the project is to design endogenous loading strategies by expressing recombinant fusion proteins composed of hATT and sEVs specific markers. To achieve this goal, the proposal identifies four milestones that address key challenges in the development of EV-based therapeutics: **a)** achieving efficient protein (AAT) loading on/into EVs, **b)** overcoming limitations in scalable and efficient EV purification methods, **c)** ensuring effective delivery of EVs to pulmonary tissue, and **d)** establishing a robust, scalable biomanufacturing process for EV production. To achieve this goal, we have defined three specific objectives:

The **first objective** focuses on the engineering of HEK293 cells to produce esEVs efficiently loaded with AAT. This objective involves the expression of AAT fused to LAMP2B or TSG101. The strategy includes the expression of cleavable HisTag/StrepTag on the esEV surface to facilitate esEVs purification.

This proposal belongs to the project **TherPeEVs** kindly founded by the Ministerio de Ciencia e Innovación (Plan Estatal de Investigación Científica y Innovación). The student will work within a research group together with a Polish PostDoc researcher, so a good level of English is essential.

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