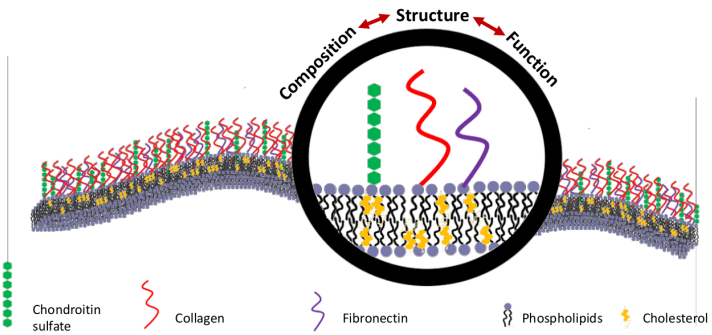
Investigation of the interface between the plasma membrane and the extracellular matrix for biotechnological applications

Project description.

Several biological processes, such as signal transduction and infection by pathogens e.g parasites or viruses, **take place at the cell surface and specifically at the interface between the plasma membrane (PM) and the extracellular matrix (ECM)**. The high compositional complexity of both PM and ECM limits their direct extraction from cells and investigation with biophysical methods. Simpler model systems, i.e. lipid bilayers, are typically used to mimic and study the cell surface. **Currently-used lipid bilayers** exhibit a too simple lipid composition (only 1-2 lipid species) and do not include the interface with the ECM components [1]. Consequently, they **are far from being reliable models of the cell surface.** On the other hand, having **access to more realistic models of the mammalian cell surface is crucial for** biotechnological applications such as the development platforms for **testing drug efficacy against viral infections.** This project is aimed at **developing functionalised lipid bilayers as advanced models of the mammalian cell surface including the PM-ECM interface**.(Figure 1). PM-bilayers will be produced as supported lipid bilayers (SLBs) and as vesicles in solution with the most representative lipid species of the mammalian PM, i.e. zwitterionic phosphatidylcholine (PC), the negatively charged phosphatidylserine (PS) and cholesterol (CHOL)[1]. A recently reported protocol will be optimised to functionalise the PM-bilayers with the most abundant ECM components, i.e. chondroitin sulphate, fibronectin and collagen [3,4]. We will systematically vary both the lipid composition of the PM-bilayers and ECM-layer surface density. Building up the complexity of the systems in a stepwise manner in terms of PM lipid composition and protein/carbohydrates in the ECM will provide a detailed understanding of the structure and function of each one of the main components of the mammalian cell envelope, opening up new possibilities for structural and biochemical investigations with sub-nanometer resolution. **Neutron reflectometry (NR) and small angle neutron scattering (SANS) will be used to provide the missing structural characterisation of the PM-ECM interface**.



State of the art

The lipids, are the most abundant PM components and provide its structural scaffold [2]. The outer PM surface is in contact with the ECM, i.e. a complex network of carbohydrates (e.g. chondroitin sulphate (CS)) and proteins (e.g. collagen and fibronectin)[5]. **A deeper characterisation of the PM-ECM interface is needed to better understand its biological functions as well as its role during cell infection by pathogens.** Recently, an experimental protocols was reported to decorate lipid bilayers with either ECM proteins, such as fibronectin and collagen, or carbohydrates [3,4,8]. Briefly, two modified phospholipids that bear an ammino (-NH2) and carboxy (-COOH) group exposed to the bulk solvent, are added to the lipid bilayer to form an amide bond with the carbohydrates (-NH2), and with collagen and fibronectin (-COOH). However, **in these studies the composition of the lipid bilayer included only a single lipid species, which is not sufficient to mimic the PM, and a detailed structural characterisation is missing.** Among all, neutron scattering techniques such as **NR and SANS are particularly suited for studying lipid bilayer structure** as they i) can provide information with a few Å resolution, ii) can distinguish biomolecules

such as lipids and proteins because of their different cross section, and iii) can provide information on a specific parts of the sample by selective deuteration[7]. **The Institut Laue Lagevin (ILL) provides high flux NR and SANS instrumentation, which is crucial for the proposed study.**

Project implementation

The PhD student will attend the PhD School at University of Perugia (UniPG). **A 3-year PhD program** will be organised according to the following WPs: **WP1 (year 1): Establishing a protocol for the production of PM-ECM vesicles and SLBs** with tuneable composition and preliminary characterisation; **WP2 (year 2): characterisation of the produced samples with NR, SANS** and complementary lab techniques; **WP3 (year 3): Finalisation of data collection, data analysis, thesis and manuscript preparation.**

The PhD program will be based on the close collaboration between UniPG and the ILL. **The supervisor at UniPG, Alessandra Luchini (AL)**, is a young researcher with a well establish experience in the preparation of membrane models and their characterisation with neutron scattering techniques [1,9,10]. AL is a frequent user of the ILL instruments and has a very close collaboration with the ILL platform for natural deuterated lipid extraction (L-Lab) [9]. **AL will profit from the collaboration with Prof. Andrea Orecchini and Alessandro Paciaroni at UniPG in the supervision of the PhD student. The supervisor at ILL, Nicolò Paracini (NP),** will support the PhD student during neutron scattering data collection and interpretation. **During WP1, the PhD student will be based at UniPG, under the supervision of AL**, and will learn about sample preparation. Together, they will establish a reliable sample production pipeline for the entire project. The PhD student will be trained in the use of lab techniques such as light scattering, UV-, CD- and infrared spectroscopy. These will be fundamental for collecting preliminary data to be included in beamtime proposals. **During WP2 and WP3, the PhD student will spend between 12 and 18 months at the ILL under the supervision of NP**. Together with AL, NP will train the PhD student in NR and SANS, and support the student during data collection. **NP is the instrument responsible for the FIGARO reflectometer at ILL and his research focuses on the study of biological membranes with neutron scattering techniques. At ILL, the PhD student will also profit from training and experience in complementary techniques**, such as quartz crystal microbalance with dissipation monitoring, ellipsometry and Brewster angle microscopy. The last year of the PhD programm (WP3) will involve mostly data analysis and the preparation of the PhD thesis and manuscripts for publications. Given the complexity of the sample composition, SANS data could be the most challenging to interpret. Therefore, the design of SANS experiments and their data analysis will be done within an already existing collaboration with the biophysics group at UNIPVM in Ancona, namely under the supervision of Prof. Francesco Spinozzi, who has a longstanding experience in SANS data analysis. Frequent meetings with the supervisors (AL and NP), as well as with the other collaborators will be organised through the entire project duration to provide the PhD student the support needed to carry out the planned WPs.

Impact and dissemination

The PM-ECM interface is the outermost cell interface and it has to be crossed by drugs or pathogens to enter the cell. **Having a reliable and advanced model of such interface is crucial for several biotechnological applications**, such as drug and vaccine testing and discovery. The results produced by the proposed PhD program will be disseminated through high-impact journal publications and conference contributions. This project will also impact the career of both the supervisor at UniPG, a young researcher starting to build her profile as future group leader, and that of the hired **PhD student**, who **will benefit from the highly interdisciplinary and international collaboration with a world leading neutron facility as ILL.**

References

[1]Luchini, A. et al., Biomimetics, 6(1), 3, 2021. [2]Harayama, T. et al., Nat Rev Mol Cell Biol, 19, 281-296, 2018.[3]Altgärde, N. et al., JCIS, 390 (1), 258-266, 2013.[4]Ceridório, L.F. et al., Colloids and Surfaces B, 141, 595-601, 2016. [5]Bosman, F. T. et al., The Journal of Pathology, 200 (4), 423-428, 2003. [6] Lakey, et al., Biophysics Review, 3, 021307, 2022. [7]Huang, C. et al., Biomacromolecules, 11 (5), 1231-1240, 2010. [8]Luchini, A. et al., JCIS, 585, 376-385, 2021. [9] Luchini, A. et al., Scientific Reports, 11, 14867, 2021.