PhD proposal 3: Mechanisms of Extracellular Vesicles (EVs) internalization by cells

1. Consortium

- University awarding the degree: University of Trieste
  Proposed supervisors: Dr. Loredana Casalis; Dr. Pietro Parisse
  Proposed academic supervisor: Dr. Loredana Casalis

- Proposing CERIC partner facility: NanoInnovation Laboratory, Elettra Sincrotrone Trieste, Basovizza, Italy
  Lead proponent: Dr. Loredana Casalis

- Contributing CERIC Partner facilities:
  Graz University of Technology, Scattering Facilities, Graz, Austria
  Lead collaborator: Prof. Dr. Heinz Amenitsch
  SISSI-Bio, Elettra – Sincrotrone Trieste
  Lead collaborator: Dr. Lisa Vaccari
  Small Angle Neutron Scattering, Budapest Neutron Center, Budapest, Hungary
  Lead collaborator: Dr. László Almásy
  Cryo-EM Facility Solaris, Krakow, Poland
  Lead collaborator: Dr. Sebastian Glatt

2. Scientific background (ca. 0.5 page)

Small extracellular Vesicles (EVs) are nanoscale natural vesicles 30-200 nm in size, ensuring the transport of molecules between cells throughout the body, strongly impacting on the fate of the recipient cells and affecting pathophysiological conditions as cancer, neurodegenerative and immuno diseases. For that, EVs are considered optimal candidates for disease diagnostics as well as for drug delivery, and are expected to influence the worldwide biomedical landscape in the next few years. However, the complexity of EVs origin and composition and their small size, make them a challenge to many available bioanalytical methods. EVs are a heterogenous collection of membrane-bound carriers with complex cargoes including proteins, lipids and nucleic acids. Cell type specificity of EVs biogenesis, realease and uptake is still debated, so are EVs uptake and the involved targeting cell receptors or internalization patways (cell membrane fusion; endocytosis). Therefore, a multi-technique, nano/microscopy approach is absolutely mandatory to find the exact correlation between EVs physico-chemical properties, cargo content and the actual cells-EVs interaction mechanisms.

We aim here to contribute to the understanding of the mechanisms of cell-cell communication mediated by EVs. We propose a multi-technique approach to study the interaction of EVs obtained from (breast) cancer cell lines and sorted according to protocols developed in our lab, with cell membranes as well as with artificial membrane systems as surface-supported lipid bilayers (SLB) and liposomes, engineered in order to pick up the most relevant parameters in such complex molecular landscape. We will work with systems of increasing complexity with different lipid composition (mixing of glycolipids; sphingolipids; cholesterol) to mimic cell plasma membranes with the presence of lipid rafts; we will add then functional molecules as ganglioside and/or fusion proteins as SNARE or caveolin, to clarify their role in EVs uptake.

3. Outline of the experimental protocol

Thus, the aim of this work is:

- The isolation and characterization of breast cancer cells derived small EVs.
- The definition of the specific membrane domains involved in EVs uptake in normal target cell lines.
- The study of the EVs internalization by simple and complex model membranes in order to identify the specific molecules facilitating or contrasting their uptake mechanisms.
- The definition of physical changes in recipient cell lines upon interactions with specific, sorted and characterized EVs subpopulations.

The project requires a panel of structural/spectroscopical techniques as:
a. AFM to characterize the size, shape and stiffness of the vesicles and the morphology changes in interaction with SLB/cells
b. Fluorescence Microscopy (including TIRF) to follow EVs interaction with SBL and cell membranes/artificial systems
c. Nano-IR analysis with photothermal expansion modality to get information on molecular cargo from different EVs sub-populations
d. Small Angle Scattering (X-rays and neutrons) using liposomes and Neutron + X-ray Reflectometry on supported lipid membranes to follow EVs-membranes interaction.
The thorough analysis as a function of lipid/lipid+protein composition and type of EVs will allow to disentangle the basis of uptake/fusion mechanisms of EVs.

4. The expected impact of the proposed research on the overall quality and capability of CERIC

This study is expected to answer many unresolved current questions on the role of extracellular vesicles in cell-cell communication in healthy and diseased conditions. Providing significant data on the physical properties and conformational changes induced in the modeled and target cell membranes should advance our understanding of EVs internalization at the level of membrane uptake pathways and signaling regulation. Identifying the specific molecular membrane facilitators of their cellular uptake could thus be targeted for their on/off switching and beneficial manipulation. This in future would encourage a more rational design of small EVs as feasible biocompatible drug delivery vehicles for the treatment of cancer and other pathological diseases.

5. **Estimated cost, if relevant** (tuition fee, salary/stipend, travel)

Year	Tuition fees	Salary	10% *	Stay abroad	Mobility	Tot per year
1st	€ 496.00	€ 18,850.00				€ 19,346.00
2nd	€ 496.00	€ 18,850.00	€ 1,534.00	€ 4,712.00¥	€ 600.00	€ 26,192.00
3rd	€ 496.00	€ 18,850.00	€ 1,534.00		€ 600.00	€ 21,480.00
Adm Costs						€ 1,800.00
Total						€ 68,818.00
* Funds available for the research activity of the PhD, per regulation						
¥ Funds available for 6-months abroad						

Annex 1: CVs of (co)supervisors

Prof. Dr. Loredana Casalis is a physicist with a PhD in Condensed Matter Physics. Her expertise is in surface bio-functionalization and in the exploitation of scanning probe microscopies to investigate the biophysics of protein interactions, enzymatic reactions on surfaces, and to develop nanoscale devices for quantitative diagnostics and disease monitoring. Her group has optimized procedures for the nanografting of unstructured proteins relevant to neurodegenerative diseases, to study fibrillation in-situ, on bare surfaces and in model membrane layers and to detect disease biomarkers circulating in body fluids, as proteins and extracellular vesicles. She has also established experience with synchrotron radiation based structural and spectroscopic techniques. She published more than 80 papers in international peer reviewed journals. She is teaching Biophysics at the University of Trieste, and is a member of the Teachers' Board of the PhD course in Nanotechnology of the University of Trieste and of the PhD course in Neurobiology of SISSA (International School for Advanced Studies) in Trieste. She has been unit coordinator of several research European and National Projects in the field of nanotechnology for biomedicine.

Dr. Pietro Parisse is a physicist with a PhD in Condensed Matter Physics. After joining the Nano Lab in late 2008 he moved his interests towards biophysics and nanotechology applied to biomedicine. The main focus of his research is the study of structure and functionality of biomolecules (DNA, RNA, proteins) and nanovesicles by means of scanning probe techniques and biophysical approaches, with the dual aim of: elucidating their role in cellular processes connected to cancer disease; developing new strategies for the detection and discovery of novel cancer related biomarkers. He published more than 50 papers in international peer reviewed journals and recently has been principal investigator of an Interreg Italia-Austria project on Extracellular Vesicles.