PhD First Year Report

Phd student: *Beatrice Leonardini* — **Cycle:** *XVIII* — **AA:** *2022/2023*

Course: *Physics and Nanosciences*

Supervisors: Prof. *Annalisa Relini*, Dr. *Ester Canepa*

Research activity

My PhD project aims at studying the interaction mechanisms occurring between functionalized nanoparticles (NPs) and biomimetic cell membranes. Over the last decades, NPs have been extensively studied to develop novel biomedical applications, which lead to the inevitable interaction between NPs and the cell membrane. Since exogenous agents as NPs could alter the membrane integrity from both a structural and a functional point of view, understanding the NP-membrane interaction mechanisms is a priority in the field. In this context, during my first year of PhD, I have worked on the preparation and characterization of model membranes composed by different lipid mixtures, and I investigated their interaction with amphiphilic gold nanoparticles (AuNPs) of different core sizes (in the range 2-5 nm). The experiments have been performed within a collaboration with the computational group of Prof. Giulia Rossi.
Below is a summary of these research activities.

Effect of NP size on lipid membrane fusion. Lipid membrane fusion is a critical event underlying several vital processes for the cell, including cargo transport, reproduction and communication. Membrane-embedded AuNPs with a gold core smaller than the lipid bilayer thickness (about 5 nm) can be employed as synthetic fusogens to modulate vesicle fusion under physiological conditions [1]. I further investigated the fusogenity of larger AuNPs with a core size comparable to the bilayer thickness. To this purpose, I used fluorescence spectroscopy assays based on fluorescence resonance energy transfer (FRET) and fluorescence dequenching to measure the mixing of vesicle membrane lipids and aqueous contents, respectively. AuNPs exhibited a different behaviour depending on the gold core size. Large AuNPs showed a greater tendency to induce lipid mixing between vesicles, evidence compatible with the occurrence of vesicle hemifusion states. On the other hand, small AuNPs seemed to be more efficient in promoting mixing of vesicle aqueous contents. Moreover, at the Materials Characterization Facility of IIT (Dr. Silvia Dante, Dr. Mirko Prato) I performed Dissipative Quartz Crystal Microbalance (QCM-D) measurements to quantitatively detect AuNPs uptake into the membrane. QCM-D is a highly sensitive technique for real-time detection of mass and structural changes occurring in thin samples, such as lipid membrane-AuNPs system, deposited on the surface of a quartz crystal sensor. The results show that both large and small AuNPs enter the bilayer, but at the same concentration the uptake kinetics of large AuNPs is slower than that of small AuNPs.

Effect of NP size on lipid membrane permeability. To understand the size effects on the interaction of AuNPs with lipid membranes, I am also investigating the osmotic stress that is induced on vesicles with and without AuNPs by a sugar concentration gradient between the inner contents of the vesicles and the outer solution. In the presence of a sugar concentration gradient, water diffusion would induce changes in the vesicles volume. This study aims to reveal the formation of membrane defects (such as nanopores) that form upon passive penetration of AuNPs and alter the permeability of the lipid bilayer. To this purpose, I am carrying out UV-Vis spectroscopy experiments, measuring the absorbance of the vesicle solution at different concentrations of sugar in the outer medium. In addition, testing membrane permeability with AuNPs of different sizes is

interesting to have an indication whether AuNPs aggregate in the membrane. In fact, computational simulations have observed that in the presence of NP aggregates in the membrane, nanoscale channels are formed that allow water molecules to pass through.

Membrane bilayer asymmetry. In parallel to the above main research topics, I am starting to optimize a protocol to obtain model lipid membranes with improved biomimicry. Membrane asymmetry is one of the main features of biological membranes, which actively maintains a difference in the lipid composition of the inner and outer leaflets of the bilayer. However, biomimetic systems commonly used in model studies are limited to symmetric bilayers. Recently, several protocols based on lipid exchange mediated by cyclodextrin (CD) have been introduced to obtain asymmetry in model membranes [2]. Using this approach, I am obtaining asymmetric vesicles composed of neutral and charged lipids with a higher percentage of charged lipids in the inner leaflet. Dynamic Light Scattering (DLS) and zeta potential measurements are employed to provide information on the hydrodynamic diameter and surface charge of the vesicles, respectively. In particular, zeta potential measurements correlate with the amount of charged lipids in the outer leaflet. These data could give a first indication of the successful exchange of charged lipids with neutral lipids in the vesicle outer leaflets. To this purpose, further measurements with complementary techniques such as NMR (Nuclear Magnetic Resonance) spectroscopy are planned.

References

[1] Canepa, E. *et al.* Cholesterol-Containing Liposomes Decorated With Au Nanoparticles as Minimal Tunable Fusion Machinery. *Small* **2023**, 19, 2207125.

[2] Doktorova, M. *et al*. Preparation Of Asymmetric Phospholipid Vesicles For Use As Cell Membrane Models. *Nat. Protoc.* **2018**, 13, 2086–2101.

Attended courses

- Spectroscopies and Materials for photonics *(Prof. M. Canepa, Prof. F. Bisio, March-June 2023, DIFI, 48 hours)*
- Biosensing *(Prof. O. Cavalleri, Prof. E. Angeli, Prof. P. Canepa, May-June 2023, DIFI, 20 hours)*
- Atomic Force Spectroscopy *(Prof. A. Relini, June-July 2023, DIFI, 20 hours)*

Conferences

Oral contribution — B. Leonardini, D. Odino, S. Errico, R. Ferrando, F. Chiti, C. Canale and A. Relini, *Probing the nanomechanical properties of lipid membranes: the effect of trodusquemine,* 108th National Congress of Italian Society of Physics (SIF), Milan, 12 – 16 September 2022**.**

Oral contribution — B. Leonardini, D. Odino, S. Errico, R. Ferrando, F. Chiti, C. Canale and A. Relini, *Effect of trodusquemine on the nanomechanical properties of biomimetic neuronal membranes on solid support,* XXII GEM Congress "Membranes: structure, dynamics and function", Autrans, France, 14 – 17 March 2023.

Publications

B. Leonardini, *"Probing the nanomechanical properties of lipid membranes: the effect of trodusquemine"*, Il Nuovo Cimento - Colloquia and Communications in Physics.