PhD Second Year Report

PhD student: *Beatrice Leonardini* **— Cycle:** *XXXVIII* **— AA:** *2023/2024*

Course: *Physics and Nanosciences*

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

Research activity

My PhD project is focused on studying the interaction mechanisms occurring between functionalised nanoparticles (NPs) and biomimetic lipid membranes. Specifically, during my second year of PhD I investigated the role of ligand-protected gold nanoparticles (AuNPs) in mediating membrane fusion of lipid vesicles.

Membrane fusion is essential for the basal functionality of eukaryotic cells as it plays a key role in fundamental physiological processes involving biological vesicles such as synaptic transmission and membrane trafficking. In the biological milieu, the regulation of fusion events depends on highly specific factors, including a wide range of specialized proteins. Similarly, various synthetic systems have been developed to design novel controlled artificial membrane fusion mechanisms *in vitro* (e.g. fusogenic peptide sequences, complementary oligonucleotide strands, polymers, and engineered inorganic NPs).

In my current research work, I am studying how the interplay between the AuNP size and vesicle curvature modulates the fusion process between lipid vesicles. To this purpose, I am employing AuNPs functionalised with an amphiphilic ligand monolayer to induce the fusion of zwitterionic lipid vesicles composed of fluid-state phosphocholines, the main components of the cellular membranes, and biologically relevant percentages of membrane cholesterol[1,2]. On the NP side, I am using AuNPs with different sizes, i.e. small AuNPs with a core diameter smaller than the lipid bilayer thickness - (2.4 ± 0.4) nm - and larger AuNPs with a core diameter comparable to the bilayer thickness - (4.8 ± 0.5) nm. Importantly, the AuNP core size has a direct impact on the portion of free volume available to each NP surface ligand, with smaller NPs endowed with greater conformational flexibility during mediation of membrane fusion interactions[3]. On the other hand, membrane curvature is another critical factor in the regulation of fusion processes observed *in vivo*, as it promotes fusion by creating a more favorable energetic barrier for membrane contact and merging. To investigate the effect of membrane curvature, I prepared and characterized biomimetic unilamellar lipid vesicles of two different sizes, i.e. 50 and 100 nm in diameter. By incubating small and large AuNPs with the two vesicle populations separately, I correlated the ability of AuNPs to induce fusion based on their size and assessed the role of membrane curvature in modulating this interaction. To gain molecular insights into these mechanisms, I performed a combination of dissipative quartz crystal microbalance (QCM-D) measurements and fluorescence spectroscopy assays. In addition, molecular dynamics simulations were carried out in collaboration with the computational group of Prof. Giulia Rossi (Department of Physics, University of Genova) to corroborate my experimental results. Through QCM-D experiments, which allow mass changes to be measured by monitoring the frequency change of a vibrating quartz sensor, I investigated the uptake of AuNPs into the vesicle bilayer and vesicle-vesicle interactions occurring during NP-mediated fusion. Through fluorescence spectroscopy, I conducted two types of vesicle-based fluorescence assays, namely lipid mixing and content mixing assays. Lipid mixing assays are based on Förster Resonance Energy Transfer (FRET) between two probes placed in close proximity and detect lipid molecule rearrangement induced by membrane fusion processes. However, this method cannot indicate whether a complete fusion or hemifusion event occurs. To overcome this limitation, I performed content mixing assays based on the dequenching of a self-quenched probe loaded into the vesicle lumen of a labeled vesicle population. When a population of loaded vesicles is mixed with a population of vesicles that do not contain the probe, dequenching occurs due to the mixing of the vesicles contents, which results in the dilution of the probe. Thus, the mixing of vesicles contents is a marker for fusion pore formation, confirming that full membrane fusion between the vesicles has been achieved.

Taken together, my findings reveal that both small and large NPs penetrate the membrane without damaging it, but only smaller AuNPs can promote vesicle fusion regardless of membrane curvature. Larger AuNPs do not exhibit fusogenic properties with low curvature membranes but show a tendency to induce fusion with more curved membranes.

These results suggest that by finely tuning physical parameters such as AuNP size and membrane curvature, engineered ligand-protected NPs could serve as promising synthetic fusion tools for designing nanomaterials that can play targeted fusogenic roles in biological systems.

References

1. E. Canepa, et al., *J. Phys. Chem. Lett.* **12,** (2021): 8583−8590
2. E. Canepa, et al., *Small* **19**, (2023), 2207125
3. G. Brosio, et al., *Nanoscale Adv*, (2023), **5**, 4675–4680

Courses

- Nanostrutture (Prof. C. Boragno, October-December 2023, DIFI, 48 hours). Attended.

Schools and Conferences

MOSBRI End User Short Course "ESC7 (RUG-BP): Single Molecule Approaches", 6th- 8th November 2023, Groningen (The Netherlands)

Oral contribution — Beatrice Leonardini, Ester Canepa, Davide Bochicchio, Silvia Dante, Giulia Rossi, Annalisa Relini, "*Exploring membrane fusion mediated by amphiphilic gold nanoparticles",* European South Atlantic Biophysics Congress ESAB 2024, 05th-07th June 2024, Donostia - San Sebastián (Spain).

Winner of EBSA Bursary to attend this conference

Poster presentation — Beatrice Leonardini, Ester Canepa, Giorgia Brosio, Davide Bochicchio, Silvia Dante, Giulia Rossi, Annalisa Relini *"Exploring the interplay of gold nanoparticle size and lipid membrane curvature in modulating membrane fusion",* XXVII Congresso Nazionale SIBPA, 16th-20th June 2024, Genova (Italy).

Publications

Leonardini B., Bochicchio D., Volpe P., Stellacci F., Dante S., Canepa E., Rossi G., Relini A., *Physical Determinants of Nanoparticle-mediated Lipid Membrane Fusion.* (ready to submit)

Other activities

Didactic tutor of General Physics (electromagnetism in vacuum and matter) for the Bachelor's degree in Material Science, Department of Physics, University of Genova.

Laboratory tutor for high school students' internships at the Department of Physics, University of Genova.

Scientific outreach activities in collaboration with the University of Genova and the School "Giovanni Daneo" (Genova) aimed at promoting STEM subjects and gender equality in primary schools.