PhD Third Year Report

PhD student: *Beatrice Leonardini* — **Cycle:** *XXXVIII* — **AA:** *2024/2025*

Course: *Physics and Nanosciences*

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

Research activity

During my PhD, I investigated different biophysical mechanisms underlying the interaction between amphiphilic gold nanoparticles (AuNPs) and biomimetic membranes. In the third year, I finalized the study on membrane fusion initiated in the first two years (see previous reports and [1]), then I extended my research to exploring the interaction of NPs with more biomimetic cell models, specifically giant unilamellar vesicles (GUVs). Finally, I focused on experiments aimed to elucidate changes in membrane permeability in the presence of NPs.

NP-GUV interaction. GUVs are a widely used platform for investigating the interactions between exogenous agents and cell-sized model membranes. Their micrometric size and the tunable lipid composition allow them to mimic the essential properties of cellular membranes, making them an important tool for studying key aspects of membrane-NPs interactions (e.g., membrane morphological deformation and membrane poration) [2]. Moreover, a major advantage of GUVs is their compatibility with optical microscopy techniques, which enable single-vesicle resolution and real-time observation of membrane changes.
I conducted this part of my PhD project during a research stay abroad (October 2024 – February 2025) in collaboration with the group of Dr. Rumiana Dimova at the Max Planck Institute of Colloids and Interfaces (Potsdam, Germany). I acquired expertise in preparing fluorescently-labeled GUVs using both the electroformation method and the PVA (polyvynl alcohol)-assisted method. These two approaches are complementary, as they allow vesicle preparation under different salt concentrations, an important parameter to reproduce experiments under physiologically ionic conditions. The aim of the study was to investigate the influence of AuNPs on the structural integrity and stability of zwitterionic DOPC (1,2-dioleoyl-*sn*-glycero-3-phosphocholine) GUVs. I employed AuNPs with a gold core of 3.2 nm diameter, functionalized with negatively charged ligands. AuNPs were incubated with the vesicles under iso-osmotic conditions at a high lipid to NP ratio (approximately 50:1), in order to elucidate the role of NP aggregates in inducing membrane remodeling processes such as spontaneous tubulation, pore formation, or vesicle rupture. I investigated these interactions using confocal fluorescence and phase-contrast microscopy. Using labeled GUVs, I performed experiments to quantify the number of tubular structures formed in the presence or absence of NPs, while simultaneously monitoring phase-contrast images to detect membrane pore formation. To support these experiments, I also conducted a fluorescence leakage assay by adding GUVs to a fluorescent dye solution and monitoring dye permeation into the vesicle lumen.
Analysis of the acquired images revealed that, despite the high NP to vesicle ratio, no significant enhancement in the formation of tubular structures occurred upon NP incubation and no detectable increase in membrane permeability was observed. These findings suggest that the amphiphilic coating of the AuNPs plays a crucial role in stabilizing NP–membrane interactions and hindering disruptive effects on bilayer integrity. These findings are consistent with our previous results [3].

Membrane permeability. The experiments consistently indicate that our AuNPs do not destabilize membranes under osmotic equilibrium conditions. However, molecular dynamics simulations, performed in collaboration with the group of Prof. Giulia Rossi, revealed that NP aggregation within membranes can form narrow channels, enabling water flux and thus increasing membrane permeability without inducing pore formation or structural damage [4]. To observe this effect, vesicles have to be studied under osmotic stress conditions. In the final part of my PhD, I performed experiments to detect this phenomenon, including small-angle X-ray scattering (SAXS). SAXS provides quantitative information on electron density differences at the nanoscale, including vesicle shape, size, and bilayer properties (e.g., thickness and fluidity). Data analysis is ongoing and additional experiments are planned.

References

[1] B**.** Leonardini, et al., *Nanoscale*, **2025**, 17, 8923-8932.

[2] M. A. S. Karal, et al., *Chemistry and Physics of Lipids*, **2020**, 230, 104916.

[3] E. Canepa, et al., *Scientific Reports***, 2021**, 11, 1256.

[4] E. Lavagna, et al., *Nanoscale*, **2020**, 12, 9452.

Schools and Conferences

XXIX SIBPA School: "Biomimetic models for exploring membrane biophysical properties in health and disease", 27th - 31st January 2025, Venice (Italy). **Poster presentation**.

15th EBSA Congress 2025, 30th June - 4th July 2025, Rome (Italy). **Poster presentation**.

To be attended:

EVPs Workshop: "Extracellular vesicles and particles (EVPs): bridging the gap between computational and experimental research", 17th - 19th September 2025, Genoa (Italy). **Poster presentation**.

110th National Congress of Italian Society of Physics (SIF), 22nd - 26th September 2025, Palermo (Italy). **Oral contribution**.

Publications

B. Leonardini, D. Bochicchio, P. Volpe, F. Stellacci, S. Dante, E. Canepa, G. Rossi, A. Relini, *Physical determinants of nanoparticle-mediated lipid membrane fusion, **Nanoscale**, 2025, **17**, 8923-8932.*

D. Odino, **B. Leonardini**, S. Errico, D. Barbut, M. Zasloff, R. Ferrando, F. Chiti, C. Canale, A. Relini, *Bottom effect in phase-separated supported lipid bilayers: evaluation of intrinsic Young's modulus and effect of trodusquemine.* (ready to be submitted)

Exams

Seminar, XXIX School of Pure and Applied Biophysics: "Biomimetic models for exploring membrane biophysical properties in health and disease", 3 CFU. *Passed.*

Nanostrutture (Prof. C. Boragno, October-December 2023, DIFI, 48 hours), 6 CFU. *Passed.*

Atomic Force Spectroscopy (Prof. A. Relini, June-July 2023, DIFI, 20 hours), 3 CFU. *Passed*.

Planned for September 2025:

Spettroscopie e Materiali per la Fotonica (Prof. M. Canepa, March-June 2023, DIFI, 48 hours), 6 CFU.

Other activities

Teaching activity: didactic tutor for the course *"Fisica e laboratorio di misure fisiche"*, 1st year of the bachelor's degree in Biology (42 hours).

Scientific outreach activity: collaboration with the University of Genoa and the Istituto Comprensivo Maddalena-Bertani (Genoa) within a PNRR project, promoting STEM subjects and gender equality in primary schools (10 hours).