First Year Report (2024/2025)

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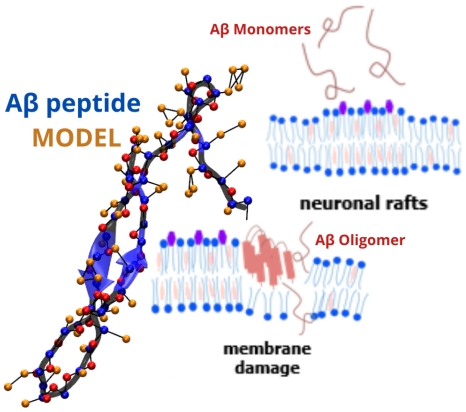
Research

Proteins are the key focus of my Ph.D. work, investigated through multiscale molecular simulations to understand their conformational dynamics and their role in shaping membrane properties.

During my first year I concentrated on two main research lines: (i) *NeuRaftAmyl (PRIN 2022)*, focused on the atomistic conformational behavior of Aβ peptides and on the development of a foldable coarse-grained (CG) model to study aggregation pathways; and (ii) *Tetraspanins in extracellular vesicles*, membrane proteins characteristic of EVs, with particular emphasis on the structural and mechanical impact of the tetraspanin CD9 on EV-like bilayers.

1. NeuRaftAmyl (PRIN 2022): Atomistic Aβ Peptides and Foldable CG Modeling

Amyloid plaques, primarily composed of Aβ peptides, are a hallmark of Alzheimer's disease [1]. It is widely recognized that small oligomers, rather than mature fibrils, are the most toxic species, damaging neuronal membranes and initiating the amyloid cascade [2, 3]. The aggregation process is highly heterogeneous, involving multiple nucleation pathways and metastable intermediates [4, 5, 6], and remains challenging to resolve due to the limited resolution of current experimental techniques. Molecular simulations can provide valuable insights, with atomistic models offering detailed resolution at the expense of computational cost.



Atomistic groundwork and manuscript in preparation. I performed extensive atomistic metadynamics simulations of Aβ42, while complementary simulations of Aβ40 were carried out in collaboration with the University of Messina group led by Prof. Valeria Conti Nibali. These datasets form the basis of a manuscript currently in preparation. My analysis emphasized not only the reconstruction of free-energy landscapes and structural ensembles, but also the development of *innovative procedures* for data reduction and interpretation, including a customized clustering strategy specifically designed for intrinsically disordered peptides. This approach has provided new insight into the conformational heterogeneity of Aβ and highlighted distinctive intermediates potentially relevant to the early steps of aggregation.

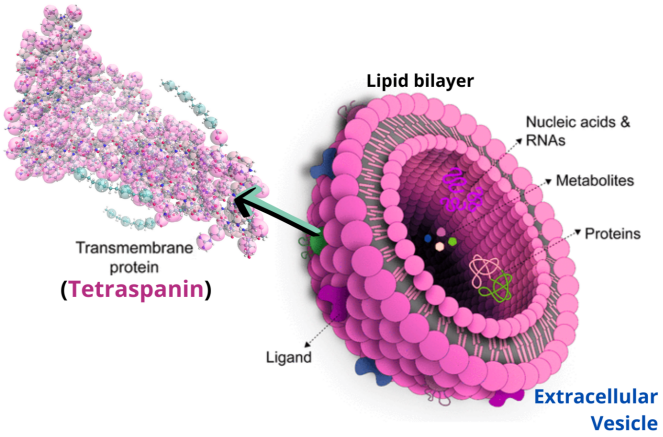
Foldable CG strategy. Building on the atomistic ensembles, I worked on an *innovative parameterization protocol* for a *foldable* CG model. Unlike conventional CG protein models tuned to native folds, the target model is required to *adaptively reorganize* its secondary-structure content across broad basins.

Expected outcomes. The foldable CG model will enable efficient exploration of monomer and early-oligomer landscapes, clarify routes toward aggregation-prone intermediates, and provide a platform for testing strategies to hinder initial aggregation steps. This work is conducted within the *NeuRaftAmyl* project funded by MUR (PRIN 2022).

Status: atomistic analysis completed and being written up; CG model under active parameterization and benchmarking against atomistic and experimental observables.

2. Tetraspanins in Extracellular Vesicles (CD9)

Extracellular vesicles themselves represent a heterogeneous population of nanosized lipid bilayer particles released by most cell types. They play crucial roles in intercellular communication by transferring proteins, lipids, and nucleic acids, thereby modulating recipient cell physiology. EVs have attracted great interest not only as mediators of normal and pathological processes, but also as promising platforms for biomarker discovery and therapeutic delivery.



Tetraspanins are a family of membrane proteins enriched in extracellular vesicles, where they act as molecular scaffolds that organize the so-called "tetraspanin web" and regulate intercellular communication [7]. Structural studies have highlighted the versatility of these proteins: CD9 and CD81, for instance, display conformational flexibility linked to oligomerization and functional regulation [8, 9].

Moreover, high-resolution structures have revealed cholesterol-binding pockets that could couple tetraspanin organization to membrane composition [10].

Beyond their physiological role, tetraspanin assemblies have been implicated in processes such as virus entry and immune modulation [11]. For these reasons, CD9 was selected as a reference system to investigate how tetraspanins influence the conformational and mechanical properties of EV-like bilayers.

Model development and current status. I developed Martini 3 coarse-grained models of CD9 inserted in EV-like lipid compositions. In collaboration with the group of Prof. Giacomello (Rome), which provided atomistic trajectories, I iteratively tuned the CG representation to *maintain a stable tertiary fold while preserving realistic conformational flexibility*. The work is still *ongoing*, but preliminary results have already revealed *distinct conformational substates* of CD9, which may be functionally relevant for its organization in membranes.

Towards membrane mechanics. Building on this model, I am setting up simulations to investigate how CD9 affects the *elastic and bending response* of EV membranes with prostasome-like composition. Planned analyses include tilt/splay fields, membrane thickness, curvature coupling, and protein–lipid interactions, with the aim of connecting CD9 structural dynamics to mesoscale properties linked to endosomal escape and fusion.

Courses and Exams

- **Atomistic Simulation with Machine Learning Interatomic Potentials** (Ph.D. course, DIFI, January–February 2025, 1.5 CFU). *Exam successfully passed.*
- **Machine Learning Methods for Physicists** (Master's course, DIFI, February–May 2025, 6 CFU). *Exam scheduled for October 2, 2025.*
- **Theoretical Foundations of Machine Learning** (MALGA organized, DIBRIS Genova, June 23–27, 2025, 3 CFU). *Project submitted on September 15, 2025 – evaluation pending.*
- **Scientific & Technical Writing** (short course, November 13 and 15, 2024, organized by Dockto in collaboration with ADI Genova, 1 CFU).

Schools

- **BIO-MEMOs School** – Biomimetic Models for Exploring Membrane Biophysical Properties in Health and Disease. Venice, January 27–31, 2025 (3 CFU). *Poster presentation.*

Conferences

- **European Biophysics Congress (EBSA 2025)**. Rome, June 30 – July 4, 2025. *Poster presentation.*
- **Conference on Frontiers in Atomistic Simulations: From Physics to Chemistry and Biology**. ICTP, Trieste, September 8–12, 2025. *Poster presentation.*
- **Workshop on Extracellular Vesicles and Particles (EVPs): Bridging the Gap Between Computational and Experimental Research**. Organized by my research group (*Nanobiocomp*), Genoa, September 17–19, 2025. *Poster presentation.*

References

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