First Year Report (2022/2023)
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Supervisors: Prof. Giulia Rossi, Dr. Davide Bochicchio

Research

My work is based on studying properties of condensed and soft matter systems at submolecular resolution using computational tools such as Molecular Dynamics and advanced sampling techniques. My PhD project has been drafted in the framework of the Multimodal Innovative THeranostic nanoSystem (MITHoS) project funded by MIUR-PRIN 2020. The MITHoS project aims at the development of a therapeutically active Zinc Oxide (ZnO) NPs used as an ultrasound-responsive agent against multiple myeloma cancer. MITHoS project involves 3 experimental units (PoliTO, UniTO, UniMI) and two computational units (CNR, UniGe).

Our group (UniGe) is working on the development of molecular models with coarse-grained resolution for the characterization of the structural and thermodynamical properties of the MITHOS nanoconstruct.

As a side project, I have been also contributed to the investigation of anion transporters, with a gold-core scaffold, and of their interactions with lipid bilayers of Large Unilamellar Vesicles (LUVs). This side-project is in collaboration with Michał Chmielewski, head of the Supramolecular Chemistry Laboratory at the University of Warsaw, Poland.

Computational investigations for the design of a Multimodal Innovative Nanosystem (MITHoS)

Drug delivery systems based on stimuli-responsive metal oxide nanoparticles (NPs) are being used for cancer treatment[1]. These smart nanoagents act as diagnostic and therapeutic devices (theranostic)[2], and they are promising alternatives to traditional chemotherapeutic approaches, as they have better targeting ability, reduced toxic effects, and they allow for better tuning of the dose/response behavior. Biomedical metal oxide nanoparticles rely on the NP surface functionalization. The nanocarriers can enter unhealthy cell through the targeting ligands (e.g., antibodies) that bind to the cell receptors. With the ultrasound stimulus, the drug is released in the intracellular environment damaging the target cell[3].

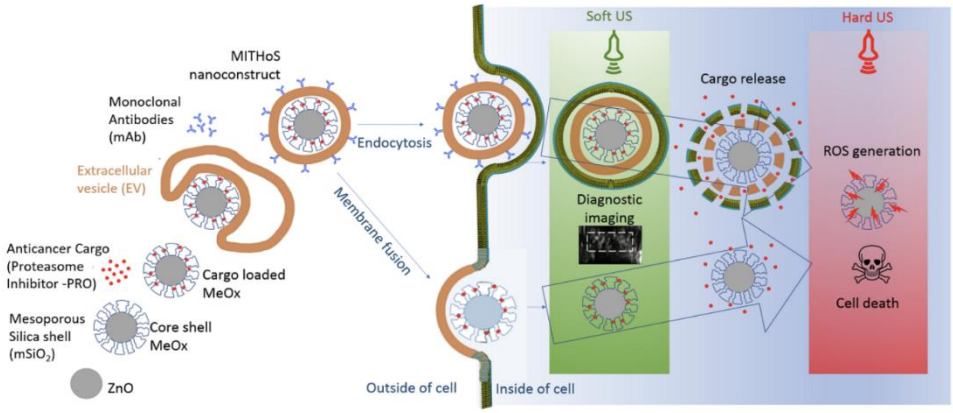

Figure 1 – The MITHOS project concept

Here, our target NP is a ZnO nanoparticle functionalized with a mixture of hydrophobic and hydrophilic ligands, namely oleic acid (OLA) and 3 (aminopropyl)trimethoxysilane (APTMES). The functionalized NP is then covered by a lipid membrane and used as an

ultrasound-responsive carrier of the anti-cancer drug Carfilzomib against multiple myeloma. One of the challenges that need to be faced during the synthesis of the nanoconstruct, is the optimization of the drug loading. The experiments performed in PoliTo have revealed a non-optimum loading of Carfilzomib in the NP-ligands-bilayer construct, and *our aim has been to use molecular simulations to elucidate the molecular mechanisms of drug-construct interactions, and suggest modifications to the NP ligand composition in order to enhance the efficiency of the drug loading step*.

During my first PhD year, we have been able to (i) develop a coarse-grained model of the functionalized NP (ii) characterize the drug adsorption on a functionalized ZnO surface, using two different ligand compositions (iii) investigate the NP-lipid bilayer interaction as well as drug distribution in the nanoconstruct.

Drug loading on a functionalized Zinc Oxide surface. Following the indications from our experimental partners, we considered two alternatives, positively charged ligands for the functionalization of the NP surface, namely (APTMES) or N-(2-Aminoethyl)-3-aminopropyltrimethoxysilane (l-APTMES). These ligands are both amine-terminated, but l-APTMES is more hydrophobic and should thus be able to better retain the drug on the NP surface. In order to derive the free energy of adsorption of the drug on a functionalized surface, we performed **metadynamics** simulations, in water and in ethanol (to mimick the experimental conditions). We found out that the free energy of binding of Carfilzomib to the functionalized surface changes considerably as a function of the ligand (see Figure 2), suggesting that l-Aptmes is the best alternative for drug loading. The experimental group in PoliTo is now testing this computational prediction. As a second result, we found that when oleic acid was added to both systems, it favored adsorption in both cases.

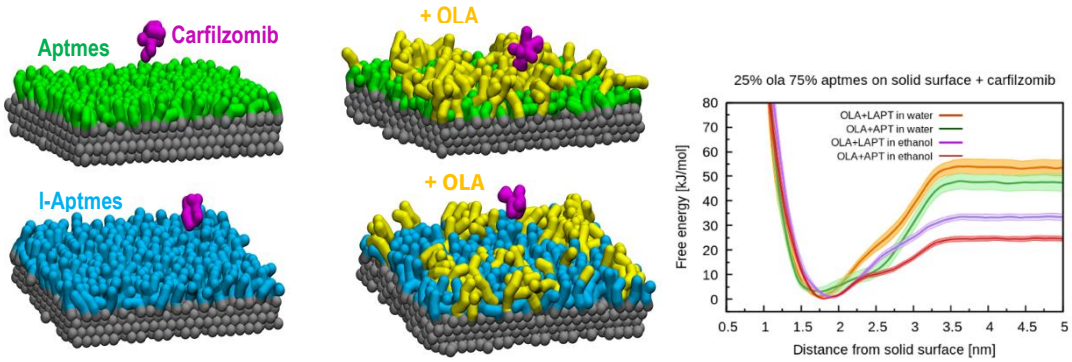

Figure 2. Left: Zinc Oxide surface functionalized with Aptmes, l-Aptmes, OLA+Aptmes, OLA+l-Aptmes. Right: Free energy of adsorption of Carfilzomib on functionalized ZnO surface

Drug loading on a functionalized surface in contact with a lipid bilayer. In the experiments, the final construct is constituted by the functionalized NP embedded by a lipid bilayer. It is thus necessary to include the bilayer in our modeling and use the simulations to understand i) what is the most favorable location for the drug within the whole construct, and ii) what is the free energy of binding of the drug within the whole construct. To this aim, we simulated the self-assembly of a pegylated lipid bilayer on top of a surface functionalized with l-Aptmes and oleic acid. The bilayer composition and the self-assembly process were tuned to mimic the experimental process. As a result of the bilayer self-assembly, we found that the equilibrated membrane had asymmetric composition. This is an interesting prediction coming from the simulations. Indeed, on the one hand, it is experimentally challenging to probe this asymmetry, and, on the other hand, it is likely that it can affect the drug distribution and binding energy.

As a result of the self-assembly of lipids and of the formation of the adsorbed bilayer, we found using unbiased MD simulations that Carfilzomib is preferentially located at the interface between the ligand layer and the lower lipid bilayer leaflet, as shown in Figure 3.

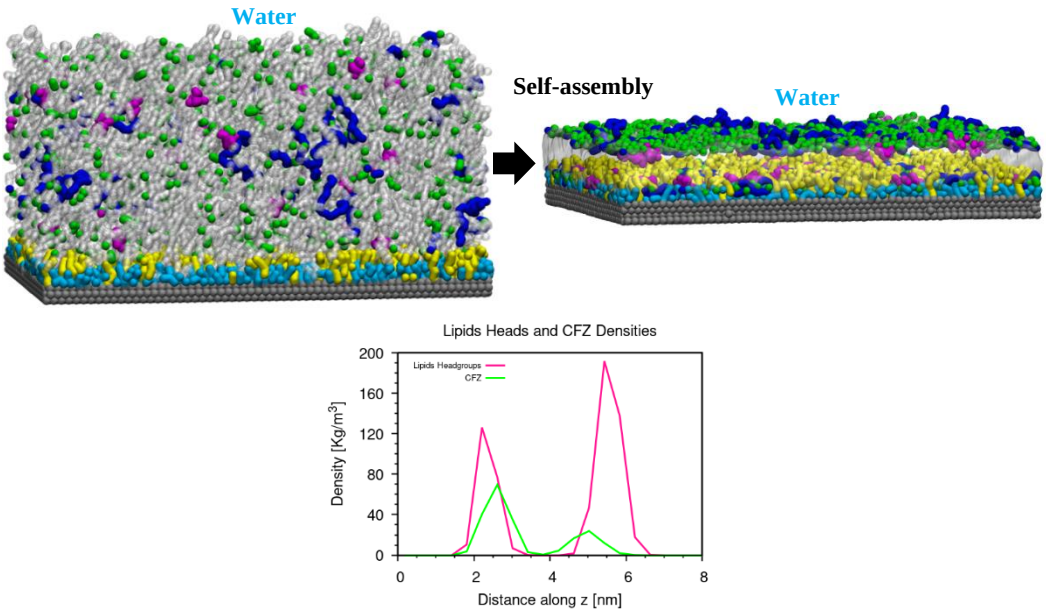

Figure 3. Up: Functionalized surface plus lipids and drugs molecules. Down: Distributions of lipids headgroups and Carfilzomib

We are currently performing Metadynamics simulations on this system, in order to derive a free energy profile for the adsorption of Carfilzomib as a function of the distance from the rigid substrate, in presence of both the ligands and the lipid bilayer. Preliminary results are shown in Figure 4.

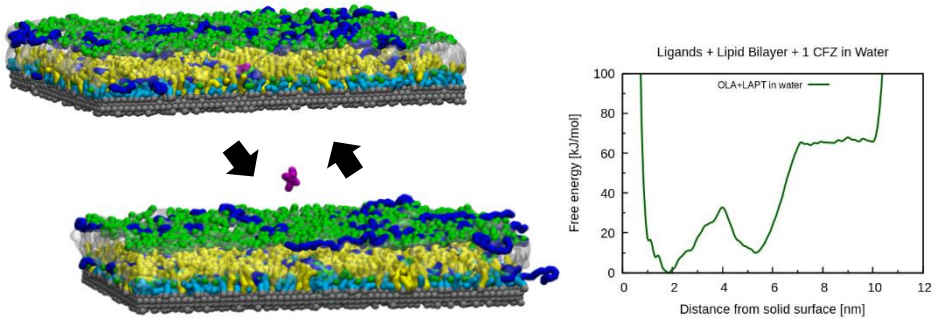

Figure 4. Left: Functionalized surface plus lipid bilayer and one Carfilzomib, Right: Carfilzomib free energy of adsorption

Attended courses

- Atomic Force Spectroscopy (Annalisa Relini, May-July 2023 (DIFI), 20 hours – 3 credits)
- Microscopic and Spectroscopic Techniques for the Analysis of Surfaces and Interfaces (Renato Buzio (CNR-SPIN), Andrea Gerbi (CNR-SPIN); Letizia Savio (CNR-IMEM), 20 hours – 3 credits)

Schools

- Flagship School "MolSim-2023", CECAM-NL - Amsterdam Science Park, University of Amsterdam, 09th - 20th January 2023 (3 credits)

Conferences

- Joint CMD 30 – FisMat 2023 conference, Politecnico di Milano, Italy, 4th – 8th September 2023
    - Poster: *Computational Investigations for the Design of a Multimodal Innovative Theranostic Nanosystem (MITHoS).*

References:

[1] Dhas, Namdev, et al. *J Control Rel* 333 (2021): 188
[2] Sharma, Harshita, et al. *Drug discovery today* 20.9 (2015): 1143
[3] Anjum, Sumaira, et al. *Cancers* 13.18 (2021): 4570.