Second Year Report (2023/2024)
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Computational investigations for the design of a Multimodal Innovative Theranostic Nanosystem (MITHOS)

In our group, we are working on supporting the development of an ultrasound-responsive nanoagent (MITHOS) to treat multiple myeloma in clinical applications. We employ computational methods such as molecular dynamics and advanced sampling, working at submolecular resolution thanks to the development of coarse-grained molecular models, to characterize the structural and thermodynamical properties of the MITHOS nanoconstruct. The nanoconstruct comprises zinc oxide (ZnO) nanoparticles (NPs) functionalized with oleic acid (OLA) and 3(aminopropyl)-trimethoxysilane (LAPT) ligands, which are helpful for the stabilization of the NPs aggregate in the water environment. The NP aggregate is loaded with the drug carfilzomib (CFZ), and a lipid bilayer (LB) covers the overall assembly (Figure 1. (a)).

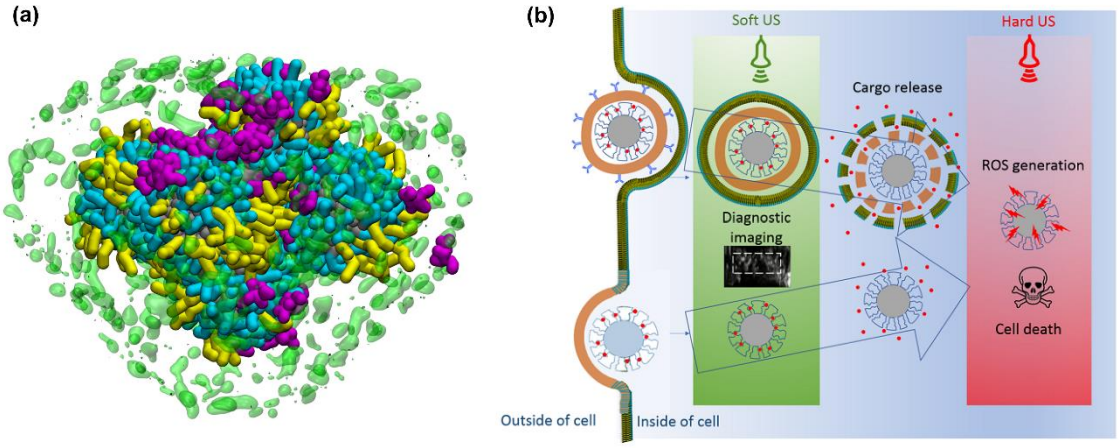

Figure 1. (a) MITHoS nanoconstruct – Silver: ZnO NPs, Yellow: OLA, Cyan: LAPT, Magenta: CFZ, Green: Lipids' heads. (b) Scheme of MITHoS exposition to the ultrasounds (US).

During my first PhD year, we characterized the drug-ligands interaction for optimal drug loading on a functionalized ZnO surface. We also investigated the CFZ preferential site within the functionalized ZnO surface+LB complex, concluding that CFZ is located in the region between the ligands layer and the lower lipid bilayer leaflet. Please see the previous report for more details on the project and prior results. Having assembled and optimized the nanoconstruct components, during my second PhD

year, we tried to understand the ultrasound-stimulated mechanism of action with MITHoS and how it could trigger the cargo release.

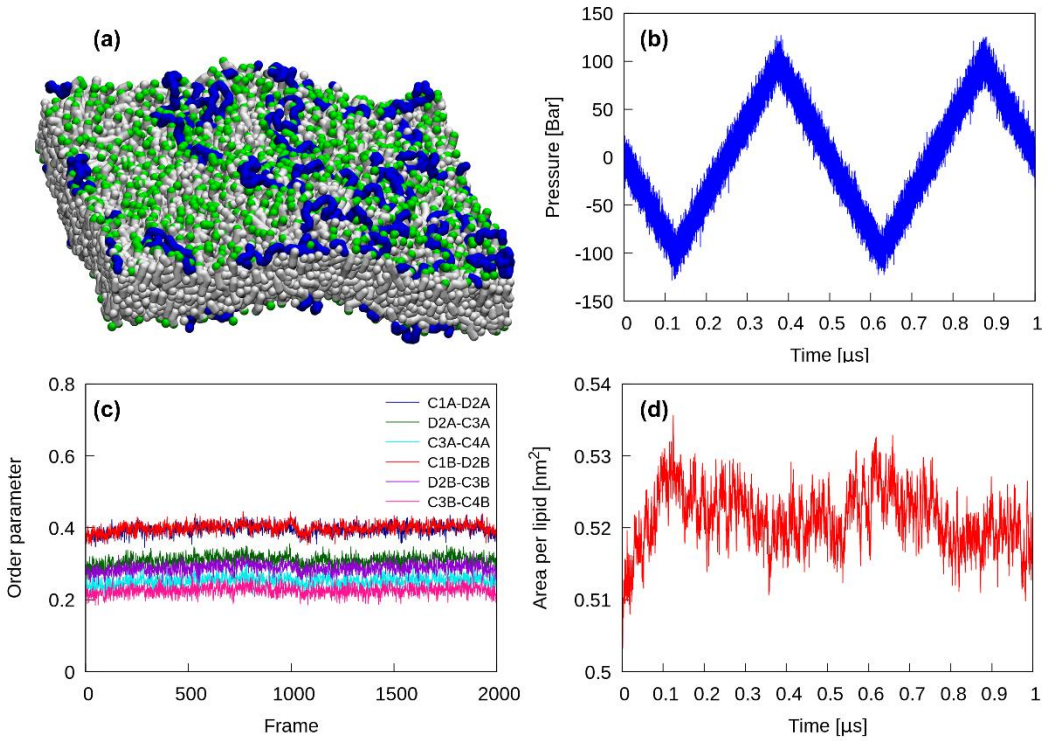

Figure 2. (a) Lipid bilayer contains 50 % DOPA, 10 % DOPC, 38.5 % Cholesterol, 1.5 % DSPE-PEG. (b) 2 periods of pressure wave against simulation time. (c) Lipid tail order parameter. (d) Area per lipid per leaflet. Theses esults were obtained using a frequency of 2 MHz and an amplitude of 100 Bar.

Ultrasounds for therapeutic drug delivery

It is well known that combining ultrasounds (US) with NPs can enhance drug delivery's efficacy and reduce drug side effects through improved translocation of drug-loaded particles through physiological barriers [1]. This approach generally works by sending ultrasound to the treatment target to induce acoustic cavitation. In turn, the interactions between cavitation bubbles and living cells would trigger the so-called "sonoporation", characterized by the temporary disruption of cell membrane integrity, to facilitate the uptake of drug molecules into cells [2]. Drug release from MITHoS should be achieved in the presence of both Soft-US (intensity: 0.1 – 2.0 W/cm2, pressure: 100 kPa – 1 MPa, frequency: 1 MHz) and Hard-US (focused, pressure: 1 MPa – 10 Mpa, frequency: 1-3 MHz).

Effect of ultrasounds on the lipid bilayer

Different mechanisms related to pressure variations can in principle alter drug release. At high US intensities (experimental values around 200-300 bar), cavitation is expected to take place in water samples containing nanoparticles [3], in turn causing mechanical damage to the membrane. At lower US intensities (up to about 100 bars), mechanisms of drug release are less clear. During this year, I have tried to simulate the simplest scenario, in which a lipid bilayer is subject to a cycle of increasing and decreasing pressure in the [-100:100] bar range. In order to impose controlled

pressure cycles in our Molecular Dynamics simulations, I wrote an in-house Python code. Before running the script, the user must give a few input ultrasound parameters, such as the number of cycles of interest for the ultrasound wave, the period of each cycle, the pressure amplitude, and the pressure increment of each simulation window. Then, different simulation windows are

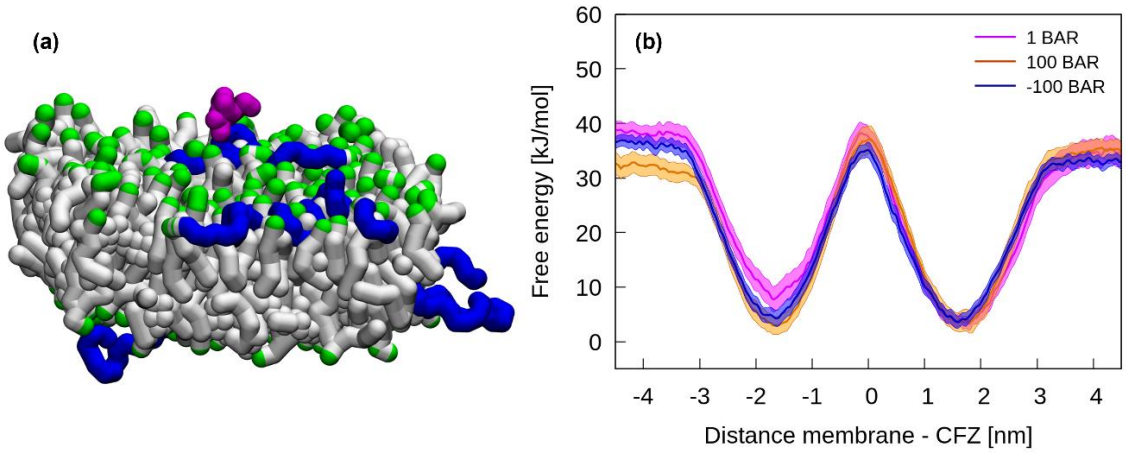

Figure 3. (a) Lipid bilayer contains 50 % DOPA, 10 % DOPC, 38,5 % Cholesterol, 1.5 % DSPE-PEG. (b) Free energy of adsorption of CFZ in the lipid bilayer

created, and the pressure increases or decreases by the desired increment modeling the pressure wave. Here, we assumed that the US wave imposes the same pressure along all three coordinate directions, and used the barostat with a semi-isotropic pressure coupling in which all the diagonal components of the pressure tensor are set to the same value, but the box dimension along the membrane normal is modified by the barostat independently on the in-plane box dimensions. A lipid bilayer (Figure 2. (a)) subject to the pressure wave was simulated, controlling the pressure changes (Figure 2. (b)) using our code. We used two frequencies, 2 MHz and 3.33 MHz, with a pressure amplitude of 100 bar (within the Hard-US regimes used in the experiments). To quantify the US effect on the bilayer in water, we calculated some structural quantities, namely the lipid tails' order parameter (Figure 2. (c)) and the area per lipid per leaflet (Figure 2. (d)). In all simulations, the pressure wave did not have a large impact on the lipid bilayer's structure.

Effect of the ultrasounds on the drug release

To assess the impact of the applied pressure on the free energy profile associated to the membrane translocation, metadynamics simulations (Figure 3. (a)) were performed with fixed pressure applied in a semi-isotropic way at 1 bar, 100 bar, and -100 bar. From the simulation, we calculated the CFZ free energy of adsorption (Figure 3. (b)). As can be seen in the image above, the difference between the three profiles falls within errorbars. Our results indicate that pressure per se has no influence on the free energy profile of drug release. The next steps will be 1) to understand whether the hypothesis of Pzz = Pyy = Pxx is correct, or if a different MD set-up could better reproduce the experimental conditions, 2) to evaluate the possibility to simulate the cavitation process, including the presence of the whole nanoconstruct (NPs + LB + CFZ + water) in the simulations.

Attended courses

- Advanced Computational Physics (Riccardo Ferrando (DIFI), Diana Nelli (DIFI), June-July 2024, 20 hours – 3 credits)
- Biosensing (Elena Angeli (DIFI), Ornella Cavalleri (DIFI), Paolo Canepa (DIFI), May-June 2024, 20 hours – 3 credits)

Conferences

- XXVII Congresso Nazionale SIBPA 2024, Sala delle Grida - Palazzo della Borsa -Piazza De Ferrari, Genova, Italy, 16th – 20th June 2024. Oral presentation: Computational Investigations for the Design of a Multimodal Innovative Theranostic Nanosystem (MITHoS).
- NANOMIB CONFERENCE - Recent Advances in Nanomedicine: Opportunities and Challenges, Università degli Studi di Milano Bicocca, Italy, 29th February – 1st March 2024. Poster presentation: Computational Investigations for the Design of a Multimodal Innovative Theranostic Nanosystem (MITHoS).

References

[1] Tharkar, P., Varanasi, R., Wong, W. S. F., Jin, C. T., & Chrzanowski, W. (2019). Nano-enhanced drug delivery and therapeutic ultrasound for cancer treatment and beyond. *Frontiers in Bioengineering and Biotechnology*, *7*, 324.
[2] YuAlfred, C. H. (2022). Ultrasound-mediated drug delivery: sonoporation mechanisms, biophysics, and critical factors. *BME frontiers*.
[3] Gu, Y., Li, B., & Chen, M. (2016). An experimental study on the cavitation of water with effects of SiO2 nanoparticles. Experimental Thermal and Fluid Science, 79, 195-201.