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Molecular Modelling of Nanoparticle Delivery through Normal and Cancer Cell Membranes

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Abstract **-** Nanoparticles find diverse applications in biomedical engineering, with targeted drug delivery (TDD) being a major focus. The experimental trials for the optimisation of TDD require a lot of time and effort. In this work, we perform molecular dynamics simulations to analyse the effect of the interaction of gold and silver nanoparticles with normal and cancer cell membranes to exploit their migration potential to cross the lipid bilayer membrane. The permeation of nanoparticles has been studied through both unconstrained and constrained simulations. Our observations hint at a pronounced affinity of nanoparticles to the hydrophobic tail region of lipid molecules, resulting in the pulling of lipid molecules along with the particles. We believe this observation holds promise for enhancing the functionalisation of nanoparticles in drug delivery applications. Furthermore, gold nanoparticles exhibit better penetration potential through the bilayer compared to silver nanoparticles. The insights gained from this study can be utilised in the development of effective in-silico drug screening models for cancer.

Keywords**:** In-silico modelling, MD simulation, Cancer, Targeted drug delivery, Membrane permeability

1. Introduction

Cancer represents a significant global health challenge. Also, it is the primary cause of mortality after cardiovascular diseases. The ongoing progress in cancer therapeutics research has contributed significantly to advancements in combating this formidable disease. Nanomaterials have emerged as transformative tools in disease diagnosis, treatment, and prevention methodologies. Notable breakthroughs include advancements in early detection and targeted drug delivery techniques (TDD). Targeted drug delivery involves administering medication in a manner that enhances concentration in specific target areas relative to other regions. This strategic approach has gained prominence, considering the limitations and side effects associated with conventional treatments. These side effects include off-target toxicities and the potential for secondary tumour generation due to intense radiation. TDD addresses these challenges by delivering anti-tumour agents directly to the intended site of action through encapsulation in nanocarriers, minimising the impact on normal cells. Given the crucial role of TDD in overcoming traditional treatment limitations, it is essential to analyse the interaction and affiliation of nanocarriers with the lipid bilayer membrane of the cancer cells to identify the potential for migration and internalisation. Additionally, understanding the difference in the behaviour of normal and cancer cell membranes along with the nanocarrier is essential in designing the delivery of drug-encapsulated nanocarriers into cancer cells. Studies on metallic nanoparticles, particularly gold and silver, have inevitable significance in this context due to the easiness of performing surface modifications.

In this work, we employed molecular dynamics simulations to examine the permeation of gold and silver nanoparticles through normal and cancer cell membranes. Typically, cancer cell membranes exhibit two distinctive features over normal cell membranes: an increased occurrence of phosphatidylserine (POPS) molecules in the upper[1] and a significant decline of cholesterol molecule concentrations throughout the membrane leaflets[2]. We have generated molecular models of normal and cancer cell membranes incorporated with nanoparticles accordingly. Our objective is to compare various cell membrane-nanoparticle combinations, elucidating differences in permeation mechanisms between normal and cancerous membranes.

2. Methodology

2.1 Generation of the lipid bilayer system

The atomistic models of lipid bilayers and the nanoparticles were generated using CHARMM-GUI[3]. We considered five types of molecules to construct the lipid bilayer: Cholesterol (CHL), Phosphatidylcholine (POPC), Phosphatidylethanolamine (POPE), POPS, and Sphingomyelin (PSM). Each membrane model consisted of 100 lipid molecules and ~4000 water molecules in each leaflet. The lipid proportions varied between the normal and cancer cell membranes, with their distribution across bilayer leaflets comprehensively outlined in Table 1, and the corresponding density profiles with respect to distance from the membrane centre of mass (COM) are shown in Fig. 1 and Fig. 2.

Table 1: Distribution of phospholipids and cholesterol molecules in the modelled membranes.

Membrane	POPC		POPS		POPE		CHL		PSM	
	Upper leaflet	Lower leaflet	Upper leaflet	Lower leaflet	Upper leaflet	Lower leaflet	Upper leaflet	Lower leaflet	Upper leaflet	Lower leaflet
Normal	25	10	0	22	8	26	38	38	29	4
Cancer	25	12	18	25	8	42	17	17	32	4

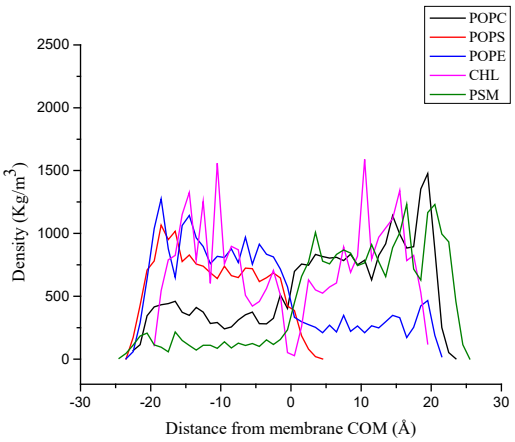

Fig. 1. The density profile of membrane components in normal cell membrane model.

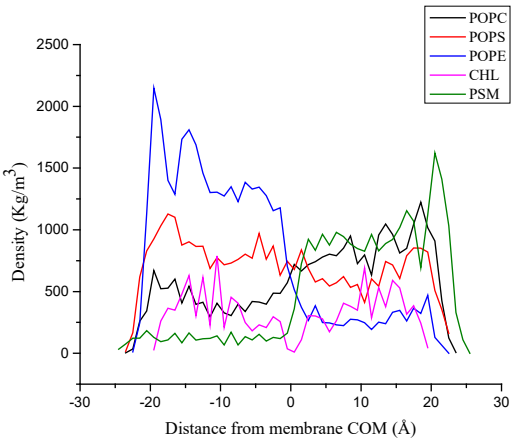

Fig. 2. The density profile of membrane components in cancer cell membrane model.

2.2 Equilibration of lipid bilayer system

The generated lipid models were minimised and equilibrated by classical molecular dynamics simulation techniques using NAMD program. During the equilibration, the position restraints were applied to nitrogen atoms with a force constant 3 kcal/mol/Å^2. A constant temperature of 310 K was obtained using Langevin thermostat, and the constant pressure of 1 bar was maintained using Nose-Hoover Langevin piston pressure control. To ensure the rigidity of water molecules, the SETTLE algorithm was implemented, while all the covalent bonds were constrained using the RATTLE algorithm. The interactions, including both bonded and non-bonded, were characterised using the CHARMM36 force field parameters. System coordinates were saved at every 2 fs interval. Visualisation, analysis, and post-processing of trajectories were carried out using Visual Molecular Dynamics (VMD) software.

2.3 Steered molecular dynamics (SMD)

To investigate the nanoparticle diffusivities, it is essential to sample configurations and energies along the z-axis of the bilayer membrane. A classical molecular dynamics simulation spanning 2.5 μs was conducted; however, the particles did not traverse the membrane even after this extended duration. This lack of permeation could possibly be due to the entrapment of nanoparticles in the local free energy minima[4]. To elucidate the permeation mechanism, it is imperative to generate free energy profiles of nanoparticles across the bilayer membrane, necessitating enhanced sampling techniques such as Adaptive Biasing Force (ABF), Umbrella Sampling, etc.

To generate the configurations for adapting the sampling process, the SMD technique, spanning 240 ns was employed. The nanoparticle was initially positioned 3 nm above the COM of the bilayer and systematically pulled along the Z-direction towards the membrane centre, with a pulling velocity of 5.3×10^{-7} nm/ps and an applied force constant of 3 kcal/mol/Å^2.

3 Results and discussion

3.1 Calculation of membrane properties

The quantification of lipid bilayer deformation is a pivotal factor in understanding the interaction between nanoparticles and lipid molecules. Calculating the membrane properties holds significance for analysing the molecular dynamics simulations of lipid bilayer models. The area per lipid (APL) values for 240 ns simulation are shown in Fig. 3. Throughout the simulation, the APL values in the two membranes were nearly identical. The average cross-sectional area of lipid molecules in the membrane leaflets, that is, the area per lipid, is an important criterion for ensuring that the system is equilibrated to a steady state. Here, the APL value calculated for both membranes lie in the 45-55 Å^2 range, which is very close to the experimental findings[5], [6].

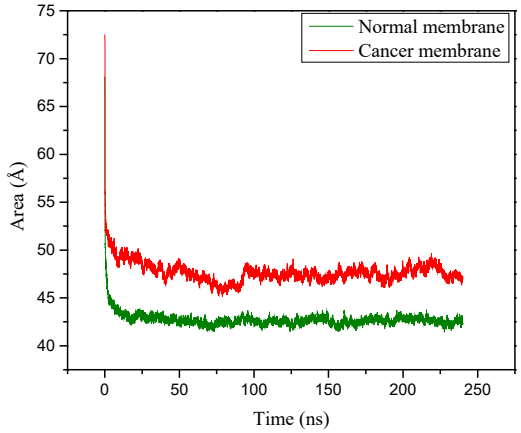

Fig. 3. The area per lipid values for the membrane models without the nanoparticles.

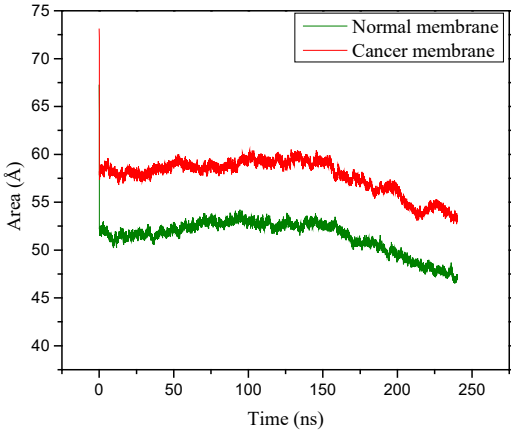

Fig. 4. The area per lipid values for the membrane models with the nanoparticles.

3.2 Nanoparticle migration through the cell membrane models

A prominent variation in the APL value was observed when the nanoparticles were introduced into the membrane system (as shown in Fig. 4). This indicates the presence of nanoparticles in the system. A sudden decrease in the area can be observed in both cancer and normal membranes. This is due to the encapsulation of the nanoparticle by the lipid molecules (as shown in Fig. 5). The silver nanoparticles were also found to follow the same pattern. This encapsulation can be attributed to the hydrophobic nature of the nanoparticles [7], which gives an insight into the properties of potential capping agents. This property can be utilised for the better functionalisation of these nanoparticles.

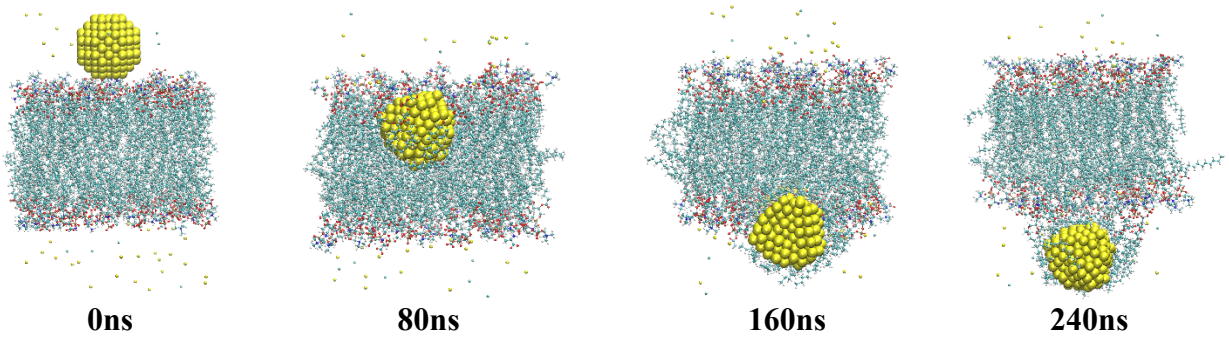

Fig. 5. Interaction of gold nanoparticles with cancer membrane model. Water molecules have not been displayed for the purpose of clarity.

3.3 Comparison of the interaction of gold and silver NPs with the cell membrane models

A distinct energy difference is evident upon analysing the SMD simulation results for nanoparticle interaction across cell membranes. The gold nanoparticle is found to exhibit a better penetration tendency to traverse cancerous membranes compared to normal membranes (Fig. 6). In contrast, silver nanoparticle displays only a marginal difference in penetration between the two membrane types (Fig. 7). Notably, gold nanoparticles appear to be more efficient in reaching the core of the defined cell membrane model. We have observed fluctuations in the required energy for nanoparticles to cross the membranes. Simulations with reduced pulling rates and longer timesteps may be needed to optimise this. These additional simulations will provide a comprehensive understanding of the interaction dynamics, facilitating improved strategies for nanoparticle transport across cell membranes.

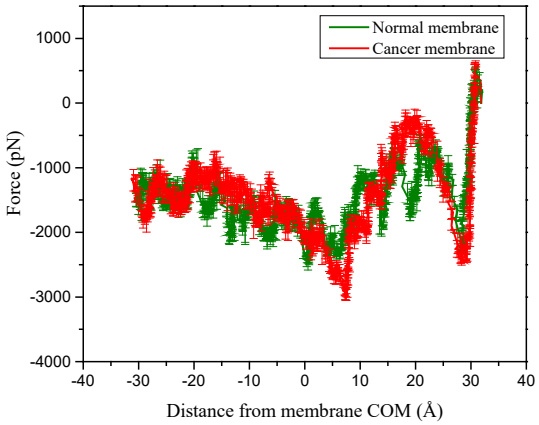

Fig. 6. SMD analysis for gold nanoparticle.

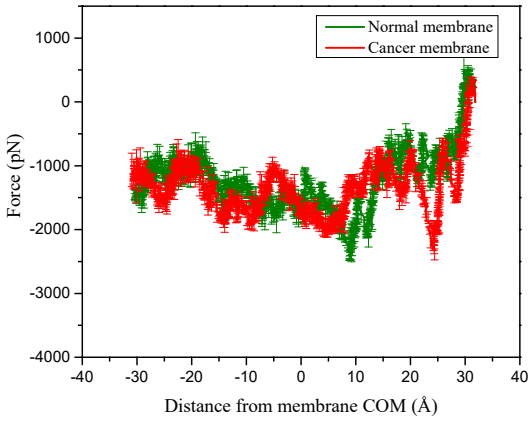

Fig. 7. SMD analysis for silver nanoparticle.

4. Conclusion

Analysis of the interaction of nanocarriers with the cell membrane models is crucial in designing targeted drug delivery. We have modelled multiple combinations of nanoparticle-cell membrane systems to examine the migration potential of the nanoparticles. Classical molecular dynamics simulations reveal that the complete membrane permeation of gold and silver nanoparticles requires several microseconds, possibly due to local energy barriers. Enhanced sampling methods must be adopted to generate the free energy landscape of the cell membrane-nanoparticle interactions. The sampling of the total system can be performed using the output of the SMD simulation at different intervals. Also, preliminary investigations from the SMD trajectory reveal that, compared to silver, gold nanoparticles show a better tendency to traverse the same membrane.

Furthermore, nanoparticle affinity for lipid hydrophobic groups can guide the selection of effective functionalising agents. These insights are essential for advancing strategies for successful nanoparticle transport across cell membranes in the context of targeted drug delivery.

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