Interaction of Functionalized C$_{60}$ Nanoparticles with Lipid Membranes

Kevin Gasperich
Advisor: Dmitry Kopelevich

ABSTRACT

The recent rapid development of applications for fullerenes has led to an increase in the manufacture of these nanoparticles and their derivatives. However, little is known about their effects on biological systems. Experimental studies suggest that these particles are significantly cytotoxic and that their cytotoxicity decreases with increasing solubility in water. This study focuses on characterizing the transport of functionalized C$_{60}$ nanoparticles through a phospholipid bilayer using coarse-grained molecular dynamics simulations. The energy barrier for bilayer penetration is shown to increase with functionalization and increased solubility in water.

INTRODUCTION

With all of the recent developments of novel applications for nanoparticles (NP), it is increasingly important to understand the effects of NP on biological systems. New applications of manufactured NP are being actively developed and the NP production is steadily increasing. With the rising abundance of these particles, it is important to determine if they pose any health hazards. Several studies have observed cytotoxic effects of fullerenes. For example, significant oxidative brain damage was observed in fish after exposure to fullerenes (Oberdörster, 2004). Damage to human cells was also observed after exposure to low concentrations of fullerene (Sayes et al., 2004). However, specific mechanism of this toxicity is still not well understood. Moreover, despite the apparent negative health effects of fullerenes, it has been observed that functionalization can reduce their cytotoxicity. The toxicity of fullerenes and their derivatives are observed to decrease with increasing solubility in water (Sayes et al., 2004).

The current study represents a preliminary look at the effects of functionalization of fullerenes on their interactions with a phospholipid bilayer. The goal of this work is to examine the differences between functionalized and non-functionalized fullerenes with respect to interactions with a dipalmitoylphosphatidylcholine (DPPC) phospholipid bilayer. Molecular dynamics simulations were used to model the behavior of the system.
To run the simulations efficiently, the size of the molecules in the system motivated us to use coarse grained models rather than atomistic ones. Because of the timescale anticipated for transportation of NP across the bilayer, an indirect method of calculating the characteristics of transport was used. Specifically, constrained simulations (Marrink & Berendsen, 1996) were used to model the process.

The constrained simulations were used to determine dependence of the potential of mean force (PMF) acting on the particle on the distance of the particle from the center of the membrane. The PMF data was calculated for functionalized C\textsubscript{60} and compared to data for the transport of non-functionalized C\textsubscript{60}.

**METHODS**

**Coarse-Grained Modeling**

The large size of the molecules involved and the accuracy of modern coarse-grained (CG) models motivated us to use one of these models in our simulations. DPPC lipids and water were modeled by the MARTINI model (Marrink et al., 2007), which uses four-to-one mapping of large (non-hydrogen) atoms to CG beads. The CG model of NP was made up of 20 beads, 10 hydrophilic and 10 hydrophobic, arranged in two separate hemispheres. Models of all species are shown in Figure 1, using different colored beads to represent different bead types.

![Figure 1: Atomistic and CG models of species considered in the current work.](image)

**Force Modeling**

Forces for non-bonded interactions in the molecular dynamics simulation were modeled using Lennard-Jones potential,

\[ U_{LJ}(r) = 4\varepsilon \left[ \left( \frac{\sigma}{r} \right)^{12} - \left( \frac{\sigma}{r} \right)^{6} \right]. \]  

(1)
Here, $\varepsilon$ is the strength of interaction, $\sigma$ is the effective bead diameter, and $r$ is the distance between the beads. The value of the interaction strength $\varepsilon$ depends on the types of beads (polar, nonpolar, etc.) involved in the interactions. In addition, interactions between charged CG beads were modeled using a screened Coulombic potential.

Bonds were modeled by harmonic length and angle potentials,

$$U_{\text{bond}}(r) = \frac{K_{\text{bond}}}{2}(r - r_0)^2$$

(2)

$$U_{\text{angle}}(\theta) = \frac{K_{\text{angle}}}{2} (\cos \theta - \cos \theta_0)^2$$

(3)

In Eq. (2), $K_{\text{bond}}$ is the force constant, $r$ is the instantaneous bond length, and $r_0$ is the equilibrium bond length. In equation (3), $K_{\text{angle}}$ is the force constant, $\theta$ is the instantaneous bond angle, and $\theta_0$ is the equilibrium bond angle.

**Species Details**

The cellular membrane was modeled by a dipalmitoylphosphatidylcholine (DPPC) lipid bilayer. DPPC is a phospholipid with two hydrophobic tail groups. The water soluble head group of DPPC is zwitterionic and consists of phosphate and choline groups. The head group is modeled by two charged beads, the glycerol backbone is modeled by two non-polar beads, and the tails are each modeled by four apolar beads.

Water is also present in the system, and four water molecules are modeled by a single polar bead. The functionalized fullerene NP, $\text{C}_{60} \text{(OH)}_{24}$, was modeled by 10 hydrophilic and 10 hydrophobic beads, arranged on the opposite sides of a dodecahedron.

**Simulation Details**

All simulations were performed with the GROMACS 4.0 software package. All simulations used Nose-Hoover temperature coupling to keep the system at 323 K. The system was maintained at 1 bar with isotropic pressure coupling during self assembly, and using semiisotropic coupling (isotropic in the directions parallel to the bilayer) during the simulations of NP transport. The time step for all simulations was 0.02 ps.

Water (19896 beads) and DPPC (592 molecules) were placed randomly in a $15\times15\times15$ nm$^3$ cell and allowed to self-assemble into a bilayer. After equilibration of the self-assembled bilayer, the system of coordinates was defined with the $z$-axis normal to the
plane of the bilayer. The system was shifted in so that \( z=0 \) corresponds to the bilayer center of mass.

NP was inserted 8 nm away from the bilayer. Because of the large timescale involved in transport of large molecules across a phospholipid bilayer, an artificial force was imposed to pull the NP through the bilayer. The particle was pulled with a constant velocity in the \( z \)-direction (0.01 nm/ps). The slow velocity ensured that the artificial forces imposed on the system did not cause any large deformations of the bilayer.

After the NP was pulled through the bilayer, forty frames from the resulting trajectory were used in further calculations. These frames were taken at regular intervals along the \( z \)-axis, and made up the initial configurations for the constraint simulations.

Each initial configuration underwent energy minimization. Next, the distance along the \( z \)-axis between the center of mass of the bilayer and the center of mass of the NP was constrained. The force required to constrain the NP was used to calculate the potential of mean force (PMF) acting on the particle at each of the forty positions.

**CALCULATION**

Calculation of the potential of mean force (PMF) acting on the NP was based on the assumption that transport of the NP can be accurately described by the Langevin equation

\[
m \ddot{z}(t) + \gamma(z) \dot{z}(t) + \frac{dG(z)}{dz} = \Gamma(z,t) \quad (5)
\]

Here, \( m \) is the mass of the particle, \( \gamma \) is the friction coefficient, \( G(z) \) is the PMF, and \( \Gamma \) is the random Brownian force due to fluctuations of the lipids and water molecules surrounding the nanoparticle. Because the NP is constrained in the simulation, the acceleration and velocity of the particle are both zero, eliminating the first two terms of the equation. The random force is normally distributed with zero mean. Because the rest of the terms are zero during our simulation, the average constraint force is equal to the \( z \) derivative of the PMF.

**RESULTS**

The PMF acting on the functionalized fullerene is shown in Figure 2 along with the PMF acting on a non-functionalized \( \text{C}_{60} \) NP. A figure showing the approximate locations of the heads and tails of the DPPC molecules in the bilayer is also shown to help interpret the data.
As expected, the minimum potential for the functionalized NP occurs just inside the DPPC head groups. This represents the position where the hydrophilic half of the NP will reside within the head group region, and the hydrophobic half will reside within the tail group region. The negligible potential barrier for bilayer entry indicates that the particle will readily enter the bilayer. The high potential as the NP approaches the center of the bilayer shows that the functionalized particle will not easily penetrate the bilayer. It also will not readily enter the tail region of the DPPC. This suggests that the functionalized NP could be less toxic than the non-functionalized fullerene: It does not penetrate far into the bilayer, so it will be less likely to cause damaging mechanical stresses.

**REFERENCES**


Oberdörster, E., “Manufactured nanomaterials (fullerenes, C$_{60}$) induce oxidative stress in the brain of juvenile largemouth bass,” *Environ. Health Perspect.*, 2004, 112, 1058-1062