



Molecular Dynamics Studies on the Effects of RF EM fields on Biological Membranes

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Abstract

Radiofrequency (RF) electromagnetic (EM) fields, with frequencies above 6 GHz and into the ‘millimetre wave’ range are recently being employed for emerging telecommunication technologies, opening again the issue of investigating possible biological effects.

In particular, a study on the comprehension of the basic interaction mechanisms at the cellular and molecular levels is needed. Here, molecular dynamics (MD) simulations can give the chance to evaluate the interactions between RF signals and biological matter, occurring in a nanosecond timescale at the atomistic level. Authors investigate the physical mechanisms induced on a patch of biological membrane by applying Continuous Wave (CW) signals, at 1 and 26 GHz, comparing their effects with static field ones.

1 Introduction

Over the last decades, telecommunication technologies based on RF EM fields have been developed for a variety of wireless communications applications, with frequencies ranging from hundreds of kHz to hundreds of GHz, such as wireless power transfer (WPT), internet of things (IoT), and 5G mm-wave technologies [1]. The increasing use of such EM fields has been sustaining the research on the possible biological effects induced at different levels of the biological scale. In this regard, the updated guidelines on the acceptable levels of RF EM fields (100 kHz-300 GHz) have been recently published by the International Commission on Non-Ionizing Radiation Protection (ICNIRP) [2].

A consensus on the interaction between EM fields and the biological system is that the possible effects start from the molecular level and lead to macroscopic changes at the organism level [3]. Due to the high frequency content of the EM fields employed above 6 GHz, the interaction with the human body is superficial, remaining at the level of the skin tissue. Recent microdosimetric studies investigated this exposure using different realistic models of skin cells, giving an estimation of induced electrical quantities such as absorbed power densities [4], [5].

Cells and intracellular structures are protected by thin membranes composed of a double lipid layer with embedded proteins, which separate their chemical content from the extracellular environment and act as a barrier for ion and compound passage. This allows for the creation of

electrochemical gradients across the membrane and induces a transmembrane potential (TMP). For example, the TMP of the membrane is currently used in medicine to allow the transfer of therapeutics, typically by applying pulsed electric fields [6], but more recently also CW EM fields [7]. For these reasons, the cell membrane, with its aqueous environment, represents a suitable target to evaluate the effects of the EM fields application through MD simulations. Atomistic simulations aim to compute macroscopic quantities by analysing physico-chemical phenomena occurring at the microscopic level, thus, allowing the investigation of the conformational and energetical changes of systems due to the interaction with their environment, as well as with an applied external EM field. MD simulations have the advantage of considering all or most atoms in a studied system, though at a significant computational cost. In MD studies, biological membranes are mainly modelled as patches of lipid bilayers in an aqueous environment. Usually, literature studies the electrical stimulation of biological membranes by applying static electric fields to investigate permeabilization mechanisms for medical applications [8], [9]. Despite its relevance for the physical understanding of the interaction with membranes, the response of a lipid bilayer to RF EM fields has currently been less examined, except for the THz frequencies [10], [11].

In this work, MD investigations on a lipid bilayer in its aqueous environment have been performed to evaluate its response when external EM stimuli at 1 and 26 GHz are applied. Here, the focus has been centred on analysing the ions redistribution and the behaviour of water molecules at the interface with the lipid bilayer due to CW EM fields, comparing outcomes with the reference situation of a static field application.

2 Material and Methods

2.1 Membrane Model Systems

The biological membrane system was built with 512 phospholipid molecules of POPC and it is hydrated using more than 53000 SPC water molecules as in [12]. The aqueous environment is filled with 88 K⁺ and 88 Cl⁻ ions to reproduce the ionic solution of intra- and extracellular media typically surrounding cells, for a 0.09 M final concentration [8], with a zero net charge. Figure 1-A shows the starting configuration model, with the lipid bilayer, composed of hydrophilic heads and hydrophobic chains,

placed at the center of the cubic box (with $L=13$ nm), and water molecules are distributed on the upper and lower regions of the box, outside the hydrophobic layers. A second 3D system is prepared considering a water solution without ions, as a comparison.

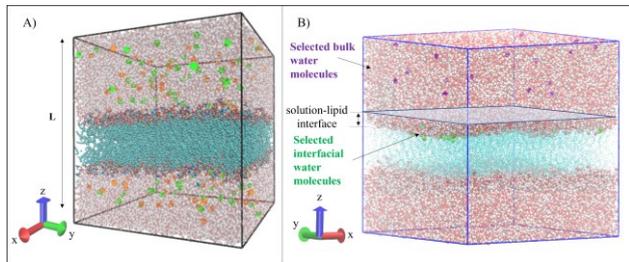


Figure 1. A) 3D model of the biological system composed of POPC (hydrophobic chains with cyan atoms and polar heads with atoms in blue and red), water molecules (in transparent view), and ions (K^+ in orange and Cl^- in light green, shown not in scale). B) Example of the groups of water molecules selected for the analysis, respectively in green and light violet for interfacial and bulk water.

2.2 MD simulation settings and analysis

All MD simulations presented in this work were carried out with GROMACS software, version 4.6.5 [13]. The temperature and the pressure are kept constant at 310 K and 1 bar by coupling the system with temperature and pressure baths (relaxation times of 0.1 ps and 1 ps, respectively), to perform simulations in the ensemble characterized by a number of molecules (N), pressure (P) and temperature (T) as constant values, also defined NPT. Periodic boundary conditions are applied to the three dimensions. Further details of the simulation settings could be found in [8]. The starting configurations, as the one shown in Figure 1 for the ionic solution, are obtained after a production time of 100 ns to equilibrate each of the two systems.

In this work, a total of five simulations have been performed to evaluate the effect of exogenous EM fields on biological membranes, each of them with a production time of 10 ns. Two simulations were performed as controls on the two models with and without ions when any external stimuli are imposed on the system. While three simulations were carried out by applying: (i) a static electric field; (ii) a CW EM field at 1 GHz; and (iii) a CW EM field at 26 GHz. Such external fields are added along the positive z -direction of the Cartesian coordinate system, thus normal to the lipid bilayer, and with an intensity of 0.2 V/nm, a suitable value (within the time-range here considered) for the study of intact double-layer structures prior to the appearance of electropores, well discussed in the literature [9]. The perturbation effect of the EM fields on the biological system elements (molecules and ions) has been evaluated considering the potential profile along the z -axis ($\Psi(z)$), using the Poisson equation as follows:

$$\Psi(z) = -\frac{1}{\epsilon_0} \int_0^z dz' \int_0^{z'} \rho(z'') dz'' \quad (1)$$

In equation (1), ϵ_0 is the vacuum permittivity, ρ is the charge density, and the potential is computed from $z=0$ to 13 nm, dividing the total length of the system into 0.025 nm slabs [14]. For static field simulations, the potential has been averaged over the whole simulation duration. Conversely, for CW EM the atoms and molecules behaved as time-dispersive objects, thus responding to the field in an oscillating fashion with constants time delay. Data taken around each density's positive peak have been then considered for the computation. Moreover, going into detail about the physical mechanisms occurring during the EM perturbations, we analyzed the behavior of two actors of the molecular system: ions and water molecules. For the ions, we evaluated their density distribution and their role in building-up the TMP. For the water, we focused on their intrinsic behavior both near the lipid membrane and far from it, following the procedure previously fine-tuned in [8]. For this purpose, a selection of two groups of 15 water molecules (i) on the interface with the lipid bilayer and (ii) on the bulk of the ionic solution, is performed when the EM perturbation is at regime (see Figure 1-B). The defined water-lipid interface is about 0.5 nm spaced. The behavior of these two groups of water molecules is investigated at the 10th peak of the EM field at 26 GHz, evaluating the hydrogen bond (H bond) of each water molecule. Data are provided in comparison to the static field perturbation and the control (no field) simulation.

3 Results

The potential profile across the solution-lipid-solution system is reported in Figure 2-A for the control simulations, in which no exogenous stimulus has been applied to the system. In particular, the two configurations in the presence and absence of ions in the water solution present similar results, as expected. In fact, in the highly conductive ionic solution, the net charge is zero and the ions' distribution is nearly uniformly distributed within the solution. Moreover, the potential profiles show that the voltage drops of about 0.7 V are mainly focused across the interface of the solution with the low-dielectric bilayer. Lower drops of about 0.15 V are detected inside the lipid bilayer and localized between the polar head and the hydrophobic chains. Data are in line with the literature on hydrated membranes [14]. When applying an external EM field, the potential profile changes across the lipid bilayer, with a decrease in all the curves (see Figure 2-B). According to the literature, with these data, it is possible to evaluate the TMP induced on the biological membrane due to the application of the exogenous EM field, as the voltage drop across the membrane. From Figure 2-B it is evident that the static field determines the highest TMP value of about 2.7 V, with respect to the two CW EM signals which induce 2.1 and 1.8 V for 1 and 26 GHz, respectively. When CW EM fields are applied, the time in which the electrical intensity is at its maximum value reduces with frequency.

Thus, TMP evaluation indicates that the CW EM stimuli affect the *local* electric properties of the biological membrane, in a way that the TMP is lowered when the CW signal is applied with respect to a static field at the same intensity. To further investigate *local* electrostatic environment, the density of the ions along the z-axis has been reported in Figure 2-C and D, respectively for K^+ and Cl^- ions. Specifically, Figure 2-C shows that CW EM fields at 1 and 26 GHz determine a potassium distribution that is quite in line with the one obtained with a static field. In particular, potassium ions are mostly placed on the solution at the bottom of the bilayer. A reduction of the density of about 34 and 28 % (for CW EM at 1 and 26 GHz, respectively) at the solution-lipid interface with respect to the static field case is observed. Ion density increase of about 14 % is detected for both the CW signals on the upper part of the box. Regarding chloride ions (Figure 2-D), while for the static case, the peak in ion distribution is shifted further from the membrane, in both the CW fields this behavior is less evident.

All these data indicate that, although a direct coupling of the RF field with water and lipids is clearly observable as reported in [15] when CW fields are applied in our MD simulations the redistribution of ions is not able to build-up a *local* electric field at the interface with the membrane (as in the case of the static field), thus causing a lower TMP value with respect to the static field application (Fig. 2-B). Finally, from the time-course of the H bonds value of the two water clusters (i.e. at the membrane interface and in the bulk medium), we extracted the histograms presented in

Figure 3. As expected, the bulk water behavior is similar for each simulation condition, with mean values around 3 (see Table I). On the contrary, interfacial water behavior strictly depends on the external stimuli applied. In particular, the average H bond value for the interfacial water molecules is about 1.71 for the CW EM field at 26 GHz (like the 1.76 for the control), reaching values up to 3.02 for the static field exposure. From these results, it is apparent how only uniform fields (i.e. static, pulsed, etc) are able to induce electrostatic rearrangement of the water molecules in the system. On the contrary, due to the high frequency of the CW EM signal, the water molecules collective motion (within the cluster) is restricted, with no appreciable differences between the bulk to the interface regions. Consequently, the interfacial water remains bound to the lipid membrane during the whole simulation time. The most fascinating finding is that the behavior of the interfacial water is significantly altered by the exogenous field.

Table I. Average H bond and standard deviation values.

	Avg H bond \pm st.dev.		
	Control	Static Field	CW-Field @26 GHz
Bulk water	3.33 \pm 0.86	3.23 \pm 0.27	3.42 \pm 0.21
Interfacial water	1.76 \pm 0.90	3.02 \pm 0.43	1.71 \pm 0.15

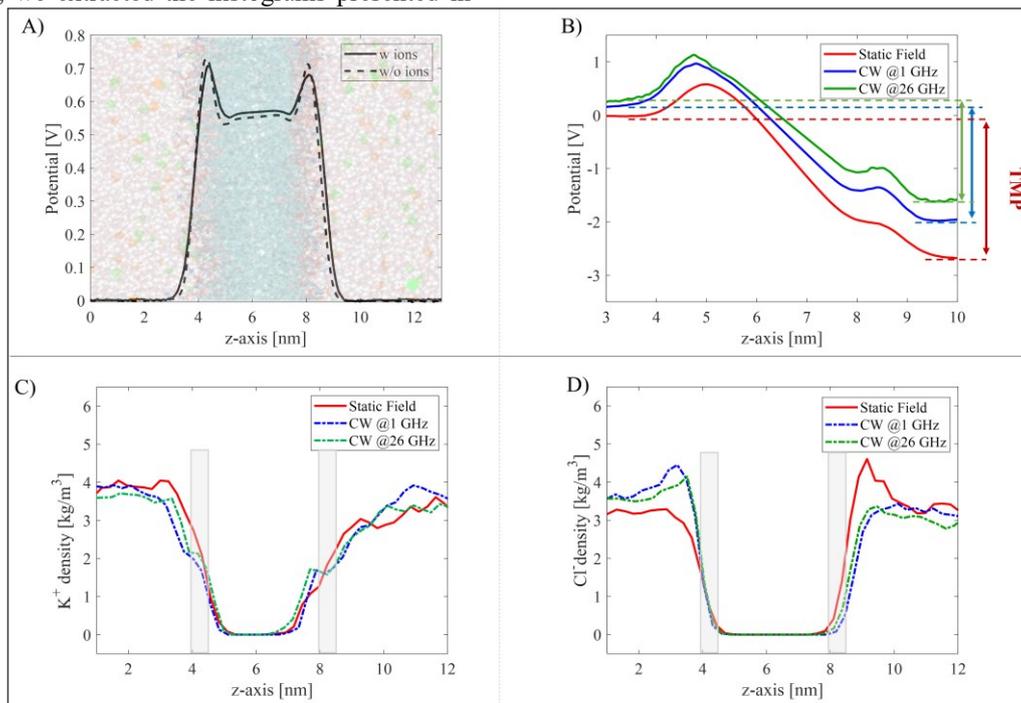


Figure 2. A) Potential along the z-axis for control simulations on lipid hydrated with ionic solution (solid line) and water (no ions included, dashed line). B) Potential profiles across the membrane in three conditions: Static Field, CW @1 GHz, and @26 GHz (respectively red, blue and green curve). K^+ (C) and Cl^- (D) density profiles for a static field (red solid curve), CW @1 GHz and @26 GHz (respectively blue and green dashed curves). Grey areas represent the solution-lipid transitions.

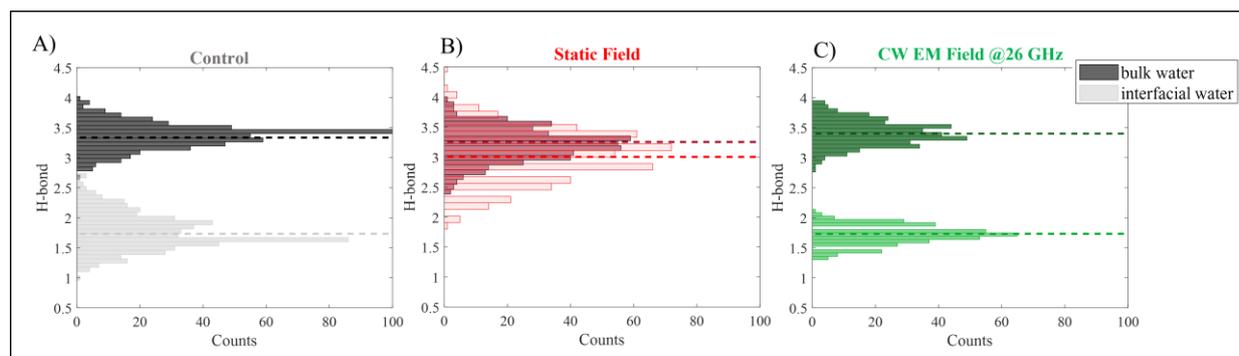


Figure 3. Histograms of the H bond of the selected bulk (dark) and interfacial (light) water molecules in the control simulation (A) and when exogenous fields are applied: the static field (B) and the CW EM field at 26 GHz (C). The trend of the 50th percentile of each distribution is reported in dashed lines for bulk (dark) and interfacial (light) water.

The application of a high-frequency CW signal on the hydrated membrane reduces the mobility of the interfacial water, resulting in the rigidification of the first few water layers that are in contact with the membrane.

4 Conclusions

In this work, a numerical study at the atomistic level with the use of MD simulations has been presented to quantify the effect of two different EM signals on biological membranes: a static field, commonly used in literature, and a CW EM field, characterized by two frequencies of 1 and 26 GHz. Here, the macroscopic quantity of the induced TMP reveals that the static field can exert a stronger force on the system, with respect to the high frequencies of CW signals. This macroscopic quantity is related to the atomic rearrangement, such as ions and water redistributions.

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References

- [1] K. Karipidis, R. Mate, D. Urban, R. Tinker, and A. Wood, “5G mobile networks and health—a state-of-the-science review of the research into low-level RF fields above 6 GHz,” *JESSE*, vol. 31, no. 4. Springer Nature, pp. 585–605, Jul. 01, 2021.
- [2] G. Ziegelberger et al., “Guidelines for limiting exposure to electromagnetic fields (100 kHz to 300 GHz),” *Health Physics*, vol. 118, no. 5. 2020.
- [3] F. Apollonio et al., “Feasibility for microwaves energy to affect biological systems via nonthermal mechanisms: A systematic approach,” *IEEE Trans Microw Theory Tech*, vol. 61, no. 5, pp. 2031–2045, 2013.
- [4] Z. Haider et al., “Local Dosimetry at Cellular and Subcellular Level in HF and Millimeter-Wave Bands,” *IEEE Journal of Microwaves*, vol. 1, no. 4, 2021.
- [5] L. Caramazza et al., “A microdosimetric study at the cellular and intracellular level using a 3D realistic cell model,” in *2021 51st EuMC*, Apr. 2022, pp. 626–629.
- [6] T. Kotnik, L. Rems, M. Tarek, and D. Miklavčič, “Membrane Electroporation and Electroporation: Mechanisms and Models,” *Annu Rev Biophys*, vol. 48, no. 1, pp. 63–91, May 2019.
- [7] T. Garcia-Sanchez, C. Merla, J. Fontaine, A. Muscat, and L. M. Mir, “Sine wave electroporation reveals the frequency-dependent response of the biological membranes,” *Biochim Biophys Acta Biomembr*, vol. 1860, no. 5, 2018.
- [8] P. Marracino et al., “Electric-driven membrane poration: A rationale for water role in the kinetics of pore formation,” *Bioelectrochemistry*, vol. 143, 2022.
- [9] M. Tokman, J. H. J. Lee, Z. A. Levine, M. C. Ho, M. E. Colvin, and P. T. Vernier, “Electric Field-Driven Water Dipoles: Nanoscale Architecture of Electroporation,” *PLoS One*, vol. 8, no. 4, 2013.
- [10] J. Tang et al., “Terahertz Electric Field-Induced Membrane Electroporation by Molecular Dynamics Simulations,” *J Memb Biol*, vol. 251, no. 5–6, pp. 681–693, Dec. 2018.
- [11] P. T. Vernier, Z. A. Levine, M. C. Ho, S. Xiao, I. Semenov, and A. G. Pakhomov, “Picosecond and Terahertz Perturbation of Interfacial Water and Electroporation of Biological Membranes,” *J Memb Biol*, vol. 248, no. 5, 2015.
- [12] O. Berger, O. Edholm, and F. Jähnig, “Molecular dynamics simulations of a fluid bilayer of dipalmitoylphosphatidylcholine at full hydration, constant pressure, and constant temperature,” *Biophys J*, vol. 72, no. 5, pp. 2002–2013, 1997.
- [13] D. van der Spoel et al., “GROMACS User Manual version 4.5.6,” www.gromacs.org, 2010.
- [14] J. Melcr, D. Bonhenry, Š. Timr, and P. Jungwirth, “Transmembrane Potential Modeling: Comparison between Methods of Constant Electric Field and Ion Imbalance,” *J Chem Theory Comput*, vol. 12, no. 5, pp. 2418–2425, May 2016.
- [15] L. Caramazza et al., “Response of Hydrated Lipid Biayers to RF em Fields: Molecular Dynamics Investigations (2021) 2021 IEEE MTT-S International Microwave and RF Conference, IMARC 2021, DOI: 10.1109/IMARC49196.2021.9714568