URSI AP-RASC, Sydney, Australia, 17 – 22 August 2025

RF Electromagnetic Fields on TRPM8 Receptors: A Molecular Dynamics Approach

Carmen Pisano[(1)], Laura Caramazza[(1,2)], Ludovica Ferri[(1)], Noemi Alvieri[(1)], Paolo Marracino[(3)], Federico Del Signore[(1)], Micaela Liberti[(1,2)] and Francesca Apollonio[(1,2)]
(1) BioEMLab Group, DIET@Sapienza, University of Rome, Italy
(2) Center for Life Nano-& Neuro-Science, Fondazione Istituto Italiano di Tecnologia (IIT), Rome, Italy
(3) Rise Technology S.r.l., L.re Paolo Toscanelli 170, Rome, Italy, 00121

Abstract

In recent years, increasing attention has been given to the potential biological effects of radiofrequency electromagnetic fields (RF-EMF), particularly regarding their interaction with molecular and cellular components. Previous studies using RF-EMF signals suggest a possible modulation of thermo-sensitive receptors, highlighting TRPM8 as a key candidate for assessing RF-EMF-related effects; among transient receptor potential (TRP) ion channels, TRPM8 plays a key role in thermosensation and cellular signaling. However, direct structural observations require sophisticated and costly methodologies. Molecular dynamics (MD) simulations represent a cutting-edge computational approach, providing deep insights into molecular conformational changes under external stimuli, thus complementing experimental studies and reducing the need for costly laboratory techniques.
This study employs MD simulations to investigate the interaction of a 26 GHz RF-EMF with the TRPM8 ion channel in a lipid bilayer. A rigorous multi-step approach ensured accurate molecular modeling and simulation. Comparative analyses under RF exposure and control conditions revealed that while TRPM8 remains structurally stable, RF-EMF induces specific modifications in the selectivity filter region, suggesting a potential modulatory effect on ion channel function. Although evidencing subtle effects, these preliminary results open the door for further investigation to understand the biological implications of RF exposure, especially in the context of 5G technology.

1. Introduction

The widespread use of radiofrequency electromagnetic fields (RF-EMFs) in telecommunications and medical applications has intensified research into their potential biological effects [1]. The 5G wireless communication technology operates across three frequency bands for 5G are: sub-GHz band (i.e., < 1 GHz), mid-band (i.e., between 1 GHz and 6 GHz), and mm-wave (i.e., with frequencies in the order of dozens of GHz and higher) [2]. For previous wireless communication technologies, the biological effects of RF-EMFs have been extensively studied, and exposure limits have been well defined by international guidelines [3]. To address the knowledge gap on possible 5G exposure effects, ongoing research projects [4] are specifically directed at conducting *in vitro*, *in vivo* and *in silico* experiments. Among the molecular targets of

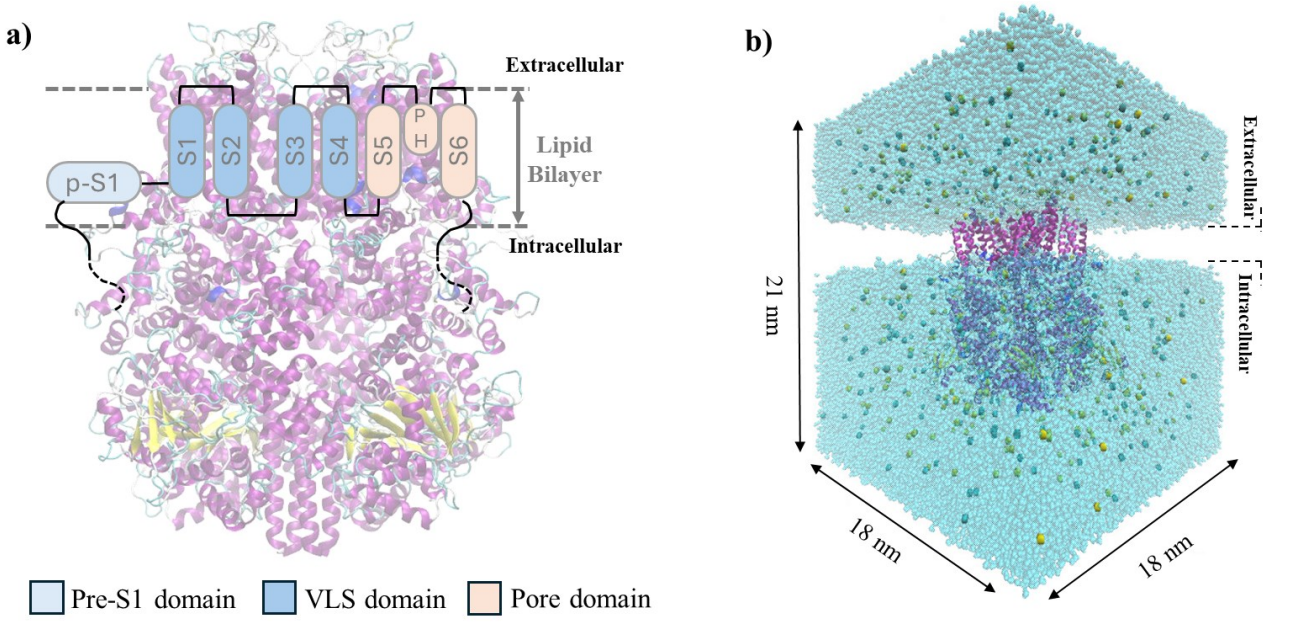

Figure 1 hTRMP8 model. hTRPM8 model and schematic representation of Pre-S1, VLD and Pore domain position respect to the lipid bilayer (a) and model h-TRPM8 embedded in the hydrated membrane environment (b).

interest, TRP channels, including TRPM8, play a crucial role in sensory perception and cellular homeostasis [5]. TRPM8, a non-selective calcium-permeable ion channel, is primarily involved in cold sensation and is activated by temperature variations and chemical ligands [6]. Previous *in vitro* and *in vivo* results indicate involvement in thermal adaptation and thermogenesis when exposed to low-level RF signals (900 and 1800 MHz) and suggest potential alterations in the functioning of thermo-sensitive receptors [7], [8], [9]. In addition, the study of [9] suggests that the activity and/or conformation of cold-sensitive ion channels, such as TRPM8, may be influenced by RF exposure. Understanding these interactions is challenging due to the complexity of experimental setups required to observe structural changes in TRPM8 directly. Molecular dynamics (MD) simulations provide a powerful and indispensable alternative, offering an unparalleled ability to probe biomolecular systems at atomic resolution, allowing for the detailed study of conformational changes under controlled conditions [10]. By leveraging *in silico* approaches, this study aims to explore potential non-thermal effects of 26 GHz RF-EMF exposure on TRPM8. Structural variations in the receptor will be analyzed, with a particular focus on the selectivity filter and gate region, hypothesizing a possible gating mechanism induced by RF-EMF exposure.

2. Methods

To investigate the effects of RF-EMFs on TRPM8, the molecular model of human (h-) TRPM8 was implemented using MODELLER [11] starting from models in the open-source Protein Data Bank (PDB). Two templates representing different bound states belonging to the Parus Major) [12] were chosen to cover the mutually missing residue parts as much as possible. The homology procedure verifies the alignment of the target and template sequences and generates several models (i.e. 50 structures), which are finally evaluated with a qualitative assessment test to identify the optimized 3D atomic structure that better resembles the target sequence [11]. The final model (~140000 atoms) shown in Fig. 1 (a), was incorporated into a phospholipid membrane system surrounded by an ionic solution, ensuring a realistic physiological environment, as shown in Fig. 1(b). Fig. 1(a) emphasizes with a schematic representation the position of the TRPM8 Voltage Sensor Like (VLS) domain, the Pre-S1 and the Pore domain with respect to the lipid bilayer and the intracellular/extracellular environments. The membrane environment was built in CHARMM-GUI [13] starting from a patch of lipid bilayer (~900 POPC molecules) and ionic solutions (~180000 water molecules and NaCl ions). The simulative box dimensions are 18x18x21 nm^3. The protein was embedded within the equilibrated membrane using GROMACS (v. 2021.5) commands and energy minimization, equilibration, and relaxation steps were conducted to ensure system stability before applying the RF-EMF. Each MD simulation was performed in the NPT ensemble, with temperature and pressure kept at 310 K and 1 bar, respectively. The production runs included 200 ns of simulation without (control) and an additional 200 ns with applied RF-EMF at 26 GHz. The field intensity was chosen in line with literature as 5×10^7 V/m. Root-mean-square deviation (RMSD) analysis was conducted to assess the potential conformational effects on the receptor. Additionally, a targeted analysis of the ion channel pore was conducted to assess a possible gating mechanism induced by RF-EMF exposure.

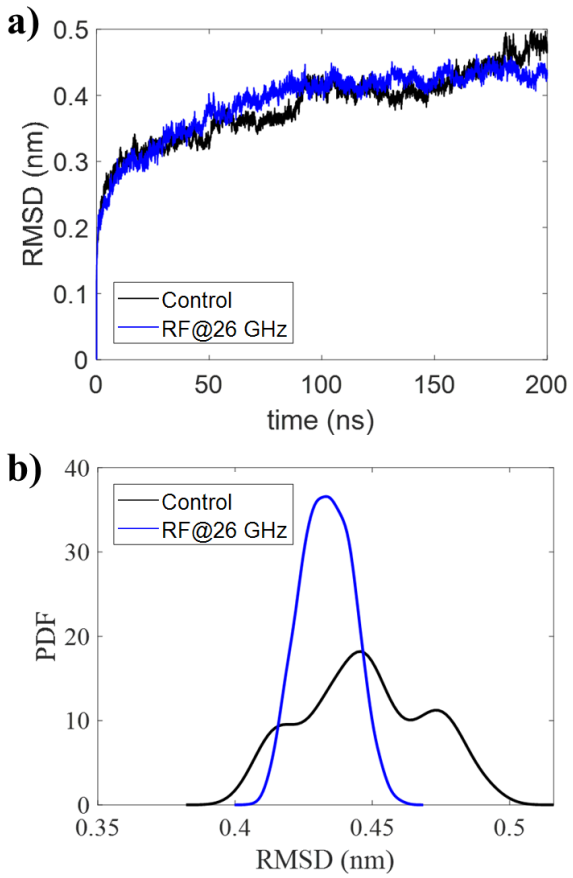

Figure 2 RMSD in time and PDF, evaluated over 200 ns and the last 50 ns respectively, for control and exposed (26 GHZ) simulations (a)-(b).

3. Results

To investigate the potential role of RF-EMF exposure on the 3D conformation of the ion channel the root mean square deviation (RMSD) over 200 ns of simulation has been evaluated. The RMSD analysis, illustrated in Fig. 2 (a)-(b) respectively in time and with the relative probability density function (PDF) focusing on the last 50 ns, was performed for both control and RF-exposed conditions. The results in time confirm that TRPM8 remains structurally stable under RF-EMF exposure, with no significant unfolding observed. The RMSD values for the exposed condition closely follow those of the control, indicating that the global conformational stability of the protein is preserved. Focusing on the PDF analysis, the RMSD distribution for the RF-exposed condition appears more narrowly centered around a slightly higher RMSD

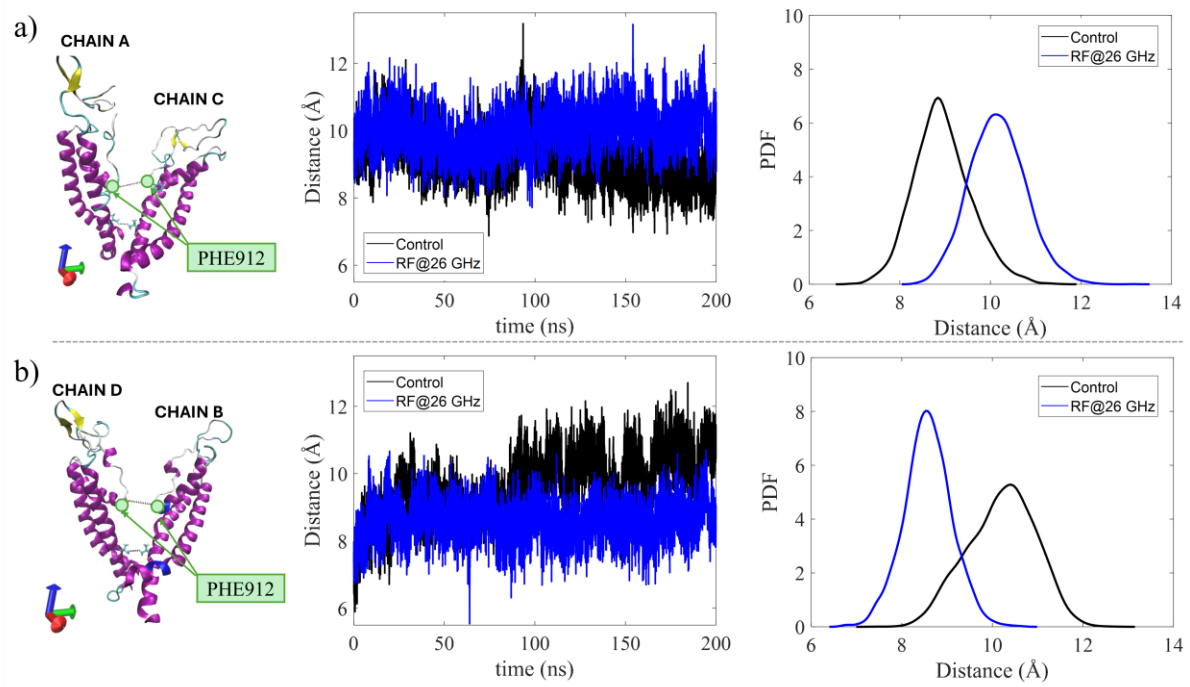

Figure 3 Distance between pairs of residues located on opposing chains: (a) monomer A-C, (b) monomer B-D. From left to right distance in time and PDF.

value compared to the control. This suggests that while the overall structural integrity of TRPM8 is maintained, the exposure may induce a more constrained conformational state, reducing the flexibility observed in the control condition. The broader RMSD distribution in the control indicates a higher degree of conformational variability, whereas RF exposure seems to stabilize the protein within a more restricted conformational ensemble.

To assess the potential impact of RF-EMF on channel gating, an analysis of specific residues located within the pore region of TRPM8 was performed. Fig. 3 illustrates the distance between pairs of residues positioned on opposing chains, which contributes to the 3D open pathway of the ion channel. Panel (a) and (b) of Fig. 3 present, from left to right: (i) a structural representation of the analyzed monomer pairs (A-C and B-D, respectively) with a focus on PHE912 positioning on each chain, (ii) the temporal evolution of the minimum distance between residues over 200 ns, and (iii) the corresponding PDF in the last 50 ns. Notably, the PDF plots reveal a shift in the exposed condition compared to the control, suggesting a potential alteration in the pore architecture under RF-EMF exposure. These changes may indicate a subtle but measurable effect on the structural dynamics of the gating region, which could influence ion channel function.

4. Conclusions

This work presents a computational study on the effects of RF-EMF on TRPM8 with E-field intensity of 5×10^7 V/m, providing a detailed molecular-scale analysis of receptor stability and conformational flexibility. Our results confirm that RF exposure does not induce large-scale unfolding or destabilization of the receptor but leads to subtle yet measurable modifications in its structural dynamics. Specifically, the narrower RMSD probability distribution suggests a reduction in conformational flexibility, potentially indicating a more constrained structural state under RF-EMF exposure. However, our analysis of the ion channel pore region reveals shifts in residue pair distances, particularly within the activation gate. These findings suggest that RF-EMF may subtly influence the gating mechanism, potentially affecting ion channel function, a role that should be further elucidated through future studies.

These insights add to the understanding of RF-EMF interactions with biological systems, supporting future experimental validation and investigations into possible non-thermal effects on ion channels.

5. Acknowledgements

This work has been developed in the framework of and supported by the GOLIAT project funded by the European Union's Horizon 2020 HORIZON-HLTH-2021-ENVHLTH-02-01.

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," *J Expo Sci Environ*

Epidemiol, vol. 31, no. 4, pp. 585–605, Jul. 2021, doi: 10.1038/s41370-021-00297-6.

[2] L. Chiaraviglio, A. Elzanaty, and M. S. Alouini, "Health risks associated with 5G exposure: A view from the communications engineering perspective," *IEEE Open Journal of the Communications Society*, vol. 2, pp. 2131–2179, 2021, doi: 10.1109/OJCOMS.2021.3106052.

[3] "ICNIRP." Accessed: Feb. 15, 2025. [Online]. Available: https://www.icnirp.org/en/activities/news/news-article/rf-guidelines-2020-published.html

[4] "CLUE-H: EMF and Health Cluster." [Online]. Available: https://www.emf-health-cluster.eu/

[5] M. Zhang, Y. Ma, X. Ye, N. Zhang, L. Pan, and B. Wang, "TRP (transient receptor potential) ion channel family: structures, biological functions and therapeutic interventions for diseases," Dec. 01, 2023, *Springer Nature*. doi: 10.1038/s41392-023-01464-x.

[6] E. Olivares *et al.*, "TRPM8-dependent dynamic response in a mathematical model of cold thermoreceptor," *PLoS One*, vol. 10, no. 10, Oct. 2015, doi: 10.1371/journal.pone.0139314.

[7] A. Pelletier *et al.*, "Effects of chronic exposure to radiofrequency electromagnetic fields on energy balance in developing rats," *Environmental Science and Pollution Research*, vol. 20, no. 5, pp. 2735–2746, May 2013, doi: 10.1007/S11356-012-1266-5/FIGURES/6.

[8] H. J. Ruigrok *et al.*, "Activation of the TRPV1 Thermoreceptor Induced by Modulated or Unmodulated 1800 MHz Radiofrequency Field Exposure," *https://doi.org/10.1667/RR14877.1*, vol. 189, no. 1, pp. 95–103, Oct. 2017, doi: 10.1667/RR14877.1.

[9] T. C. Mai, S. Delanaud, V. Bach, A. Braun, A. Pelletier, and R. de Seze, "Effect of non-thermal radiofrequency on body temperature in mice," *Sci Rep*, vol. 10, no. 1, Dec. 2020, doi: 10.1038/s41598-020-62789-z.

[10] P. Marracino *et al.*, "Tubulin response to intense nanosecond-scale electric field in molecular dynamics simulation," *Scientific Reports 2019 9:1*, vol. 9, no. 1, pp. 1–14, Jul. 2019, doi: 10.1038/s41598-019-46636-4.

[11] B. Webb and A. Sali, "Comparative Protein Structure Modeling Using MODELLER," *Curr Protoc Bioinformatics*, vol. 54, no. 1, pp. 5.6.1-5.6.37, Jun. 2016, doi: 10.1002/CPBI.3.

[12] M. M. Diver, Y. Cheng, and D. Julius, "Structural insights into TRPM8 inhibition and desensitization," *Science (1979)*, vol. 365, no. 6460, pp. 1434–1440, Sep. 2019, doi: 10.1126/science.aax6672.

[13] J. Lee *et al.*, "CHARMM-GUI Input Generator for NAMD, GROMACS, AMBER, OpenMM, and CHARMM/OpenMM Simulations Using the CHARMM36 Additive Force Field," *J Chem Theory Comput*, vol. 12, no. 1, pp. 405–413, Jan. 2016, doi: 10.1021/acs.jctc.5b00935.