Magnetic field effects on the Adenosine A2A Receptor: a rigorous insight via molecular dynamics simulations

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Abstract. In this paper, we present the results of the application of an external static magnetic field with the Velocity Verlet algorithm for performing Molecular Dynamics simulations of the A2A receptor protein embedded in a phospholipidic bilayer. Molecular Dynamics simulations allow to understand at molecular level the interaction mechanisms between atoms under specific conditions. Here MD simulations of a receptor protein have been performed in presence of an external magnetic field in order to try to elucidate specific endpoints of interaction with the field. In particular, an insight on the binding site environment has been carried out.

Introduction.

Several studies have shown how the use of low frequency magnetic fields may have biological effects on different cells functions. It has been reported that the use of pulsed electromagnetic fields (PEMFs) increases the anti-inflammatory effect of different types of cells such as neutrophils, synoviocytes, chondrocytes and osteoblasts, with significant reduction in some of the most important inflammatory cytokines [1]-[2].

Increasing evidence suggests that the beneficial effects of PEMFs are mediated by the modulation of adenosine receptors (ARs), specifically increasing the expression of A2A. In particular, an accurate analysis of the kinetic parameters showed that these enzymes possess affinity that is not modified by the presence of PEMFs, while receptor density it is modified by PEMFs in function of the time and applied intensity [1]. Although the pathway of interaction underlying such effects, pointed out an involvement of adenosine receptors (ARs), it still remains to be elucidated which is the final mechanism responsible for the observed increase in density of receptors.

To this regard, simulations based on Molecular Dynamics (MD) may become a strategic tool to study molecules behavior. Recently authors have implemented a procedure to introduce a static homogeneous magnetic field in the Gromacs software, one of the most used environments for MD [3]. They already studied the action of a static magnetic field on the A2A receptor model, in simulations where the molecule was considered in a buffer aqueous environment [4].

In this work the receptor is considered in its physiological environment, therefore embedded in a phospholipid bilayer modeling a patch of membrane, separating two aqueous water reservoirs including Na and Cl ions. The simulations carried out are extremely long if compared with usual molecular dynamics runs and the investigation is focalized on the magnetic field action on the binding site of the receptor: in particular to the response of specific aminoacid, the cysteine, which are known in literature to have a recognized role in ligand binding [5]. Therefore the hypothesis we want to test is the following: magnetic field acting on cysteine residues which are at the entrance gateway of the ligand to the A2A receptor, modify their physiological orientation with a possible consequence on facilitating the entrance of the ligand.

Methods. MD simulations have been performed in NPT (number of particles, pressure and temperature constants) ensemble at a temperature of 300 K, in a box of 10x10x15 nm^3 of dimension (Fig. 1a). 272 lipids forming the membrane bilayer have been considered, moreover 60 and 70 ions of Na+ and Cl- respectively to neutralize the positive charge of the protein and 37.558 water molecules for a total number of atoms equal to 154.154. For the adenosine receptor A2A molecular model, the 3PWH PDB structure has been chosen, but in the unbound configuration in order to firstly analyze the protein behavior with no ligand (see Fig. 1a).

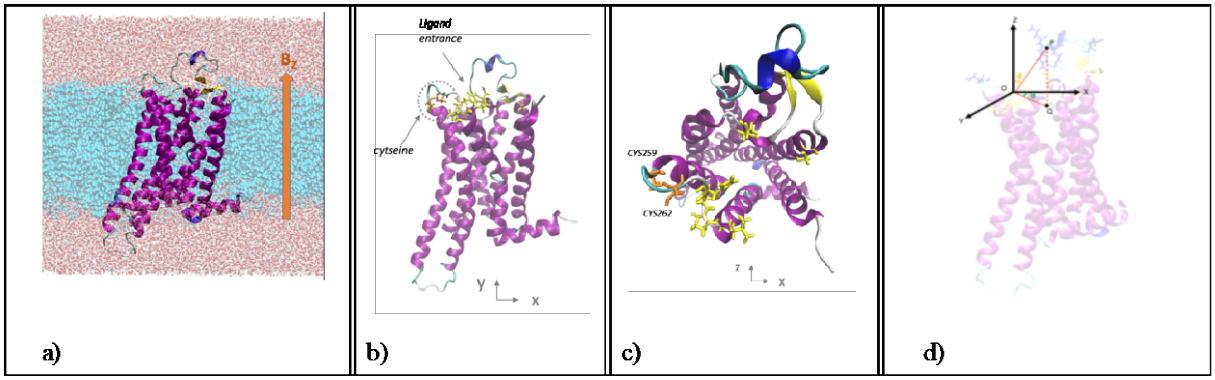

Fig. 1 Molecular model of the A2A receptor. a) model of the receptor in membrane; b) lateral view with detail of cysteines; c) top view; d) φ and ψ angles.

To implement the magnetic field, we employed the Velocity Verlet (VV) algorithm, in which the Lorentz force acts on the charged particles, which perform Larmor oscillations at the Larmor frequency when an external magnetic field is applied [3]. The magnetic field has been considered as reported in Fig. 1a, therefore normal to the patch of the membrane. After a 100 ns of equilibration, molecular simulations have been performed both with no field applied and with an intensity of the B field equal to 1T, for a total duration of 270 ns.
The simulations have been analyzed in terms of local outcomes related to the orientation of single residues with the applied field. In particular the dipole moment of two cysteine residues (CYS262 and CYS259) at the entrance of the binding site environment (Fig. 1 b and c) has been studied either looking at the three components of the dipole vector or evaluating its projection on the direction of the field (z) and perpendicular to it (plane xy) as reported in Fig. 1d.

Results. No statistically significant variations have been observed for the secondary structure of the receptor meaning that the protein structures itself is not affected by the field. However, a finer analysis has been conducted selecting the two cysteine residues. Figure 2 reports the three components of the two residues comparing the unexposed situation with the exposed one.

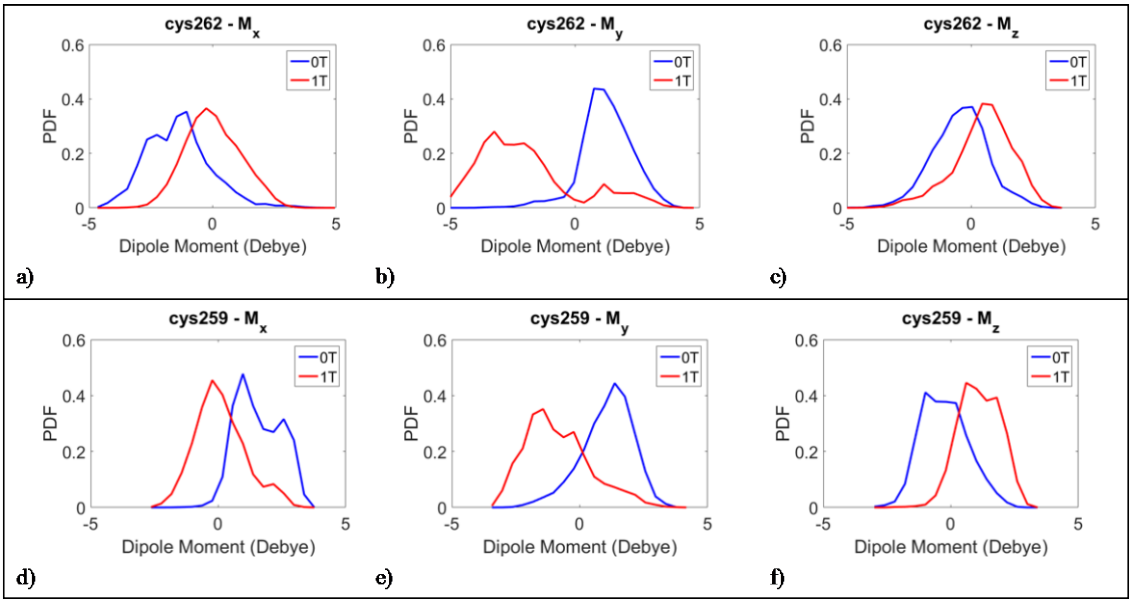

Fig. 2 Dipole moment of the two cysteines residues. X, Y and Z components for CYS262 upper row, X, Y and Z components for CYS259 lower row.

It is possible to observe that the influence of the field is evident in all the three components but especially for the y-one in both cysteine residues. Therefore a second analysis has been conducted evaluating the projection of the dipole moments of CYS262 and CYS259 on the B field axis (φ angle). Figure 3 reports how the B field modifies the orientation of the two cysteine residues; in fact φ angle becomes more oriented and aligned with the field in both cases, hence possibly facilitating the entrance of the ligand.

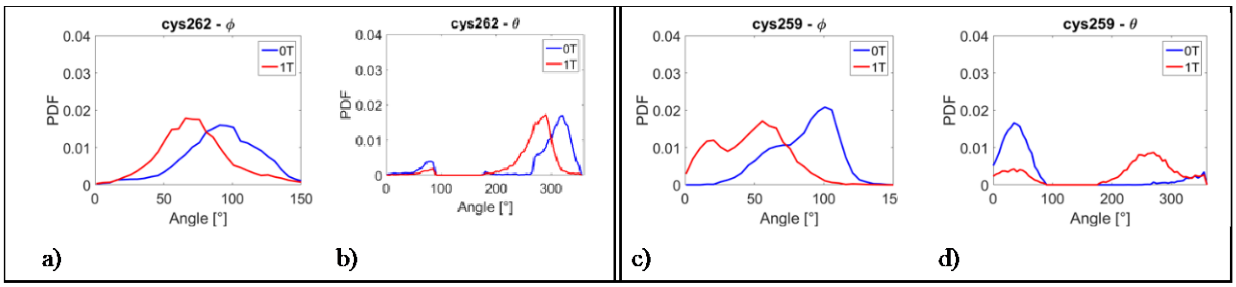

Fig. 3 Projection of the dipole moments of CYS262 and CYS259 on z axis (B field), angle ψ and on the plane orthogonal, angle φ.

Conclusions. In this paper we used the implementation of a static external magnetic field based on the introduction of the Lorentz force into the VV algorithm, inside the Gromacs software, in order to explore the effects of a magnetic field on the A2A receptor molecular model. In particular an insight on the binding site environment has been carried out. A clear effect of B field orientation for two cysteine residues, which are well known to have an important role in the binding of the ligand, is shown. This may open the way for an interpretation of the experimental data at the basis of the observed increase in density of A2A receptor.

REFERENCES

[1] K. Varani, et al.,, “Effect of low frequency electromagnetic fields on A2A adenosine receptors in human neutrophils”, British journal of pharmacology, 136 (1):57-66, 2002
[2] K. Varani et al., “Adenosine receptors as a biological pathway for the anti-inflammatory and beneficial effects of low frequency low energy pulsed electromagnetic fields”, Mediators of inflammation, 2017, 2017:2740963
[3] della Valle E. et al., “Magnetic molecular dynamics simulations with Velocity Verlet algorithm", 32nd URSI GASS Conference, Montreal,19 - 20 August 2017
[4] Della Valle, et al. Magnetic Molecular Dynamics Simulations of A2A Receptor in Solution (2018) 2018 2nd URSI Atlantic Radio Science Meeting, AT-RASC 2018.
[5] De Filippo et al., Role of extracellular cysteine residues in the adenosine A2A receptor, Purinergic Signalling (2016) 12:313–329.