Investigating the molecular basis of insect odorant receptor AgOR28 activation mechanism

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Introduction

Arthropod-borne viruses (arboviruses) are a rising concern with elevated temperatures and different weather patterns following man-made climate change. Mosquito species are key vectors of arboviral diseases, like chikungunya, dengue, malaria, and zika, posing significant public health challenges worldwide[1]. Control strategies to limit the prevalence of mosquitoes is thus highly alluring. One such strategy is to directly influence the behavior of mosquitoes through their olfaction. Odor targeted intervention can both be used to deter mosquitoes or attract to further disable them as a vector. Insects detect odors through odorant-activated ion channel complexes that are composed of an odorant receptor (OR) subunit and an odorant receptor co-receptor (Orco) which are thought to exist in a 1:3 OR/Orco ratio *in vivo*[2,3]. A recently published cryo-EM structure with a bound known agonist, *Ag*OR28 with 2,4,5-Trimethylthiazole (TMT)[2,4,5], precented an excellent opportunity to compare molecular dynamics (MD) simulations with experiments. Comparison of the apo cryo-EM structure with the agonist bound cryo-EM structure revealed and increase in the distance between transmembrane helix 7 (TM7) of the OR subunit to the Orco subunit diagonal to the OR (see **Figure 1.**, green arrow), indicating that the active state of the receptor open the ion channel via this movement. In this work we simulated the system in the apo state, TMT bound and acetophenone bound state, another known agonist[4,5].

The System

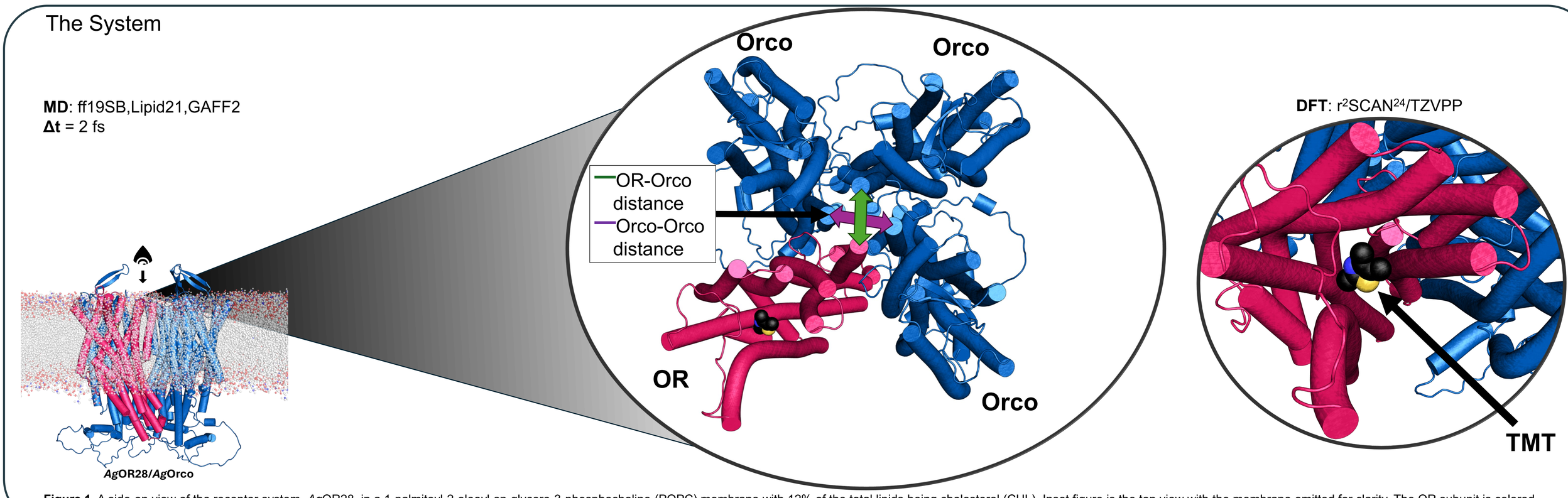

Figure 1. A side-on view of the receptor system, *Ag*OR28, in a 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) membrane with 12% of the total lipids being cholesterol (CHL). Inset figure is the top view with the membrane omitted for clarity. The OR subunit is colored magenta while Orco is blue, 2,4,5-Trimethylthiazole (TMT) is black with a blue nitrogen and a yellow sulfur. The OR-Orco distance is indicated with a green arrow while the Orco-Orco distance is purple (see **Figure 3.** for distance dynamics during the MD)

Main findings

- OR-Orco distance between the apo and TMT bound MDs did not show any indication of activation of the receptor, i.e. elongation of the OR-Orco distance, while acetophenone bound MDs did, see **Figure 3.**
- Acetophenone is known to induce less activation than TMT, in oocytes experiments[4,5] ; Flaw in our setup !
- 17 snapshots were selected from the TMT MDs for QM/MM SPE calculations. This revealed up to a 0.14 difference in Natural Population Analysis[29] (NPA) charge
- NPA (see **Figure 2. b)**) indicated TMT's S having a different charges compared to RESP charges, here dubbed Amber FF charges, see **Figure 2. a)**
- If the TMT's S charge was changed from –0.11 (Amber FF) to 0.5 (NPA), S would interact more strongly with GLU75, a negatively charged amino acid, stabilizing binding
- QM region only fluctuated 0.05 charge at most (min/max), with the noticeable exception of S of cysteine, which differed by 0.1
- QM region charges do not change compared to the Amber FF used
- Atomic Decomposition of London Dispersion energy (ADLD) showed that most of the stabilization via dispersion is due to the S of TMT, see **Figure 2. c)**

Charge distribution

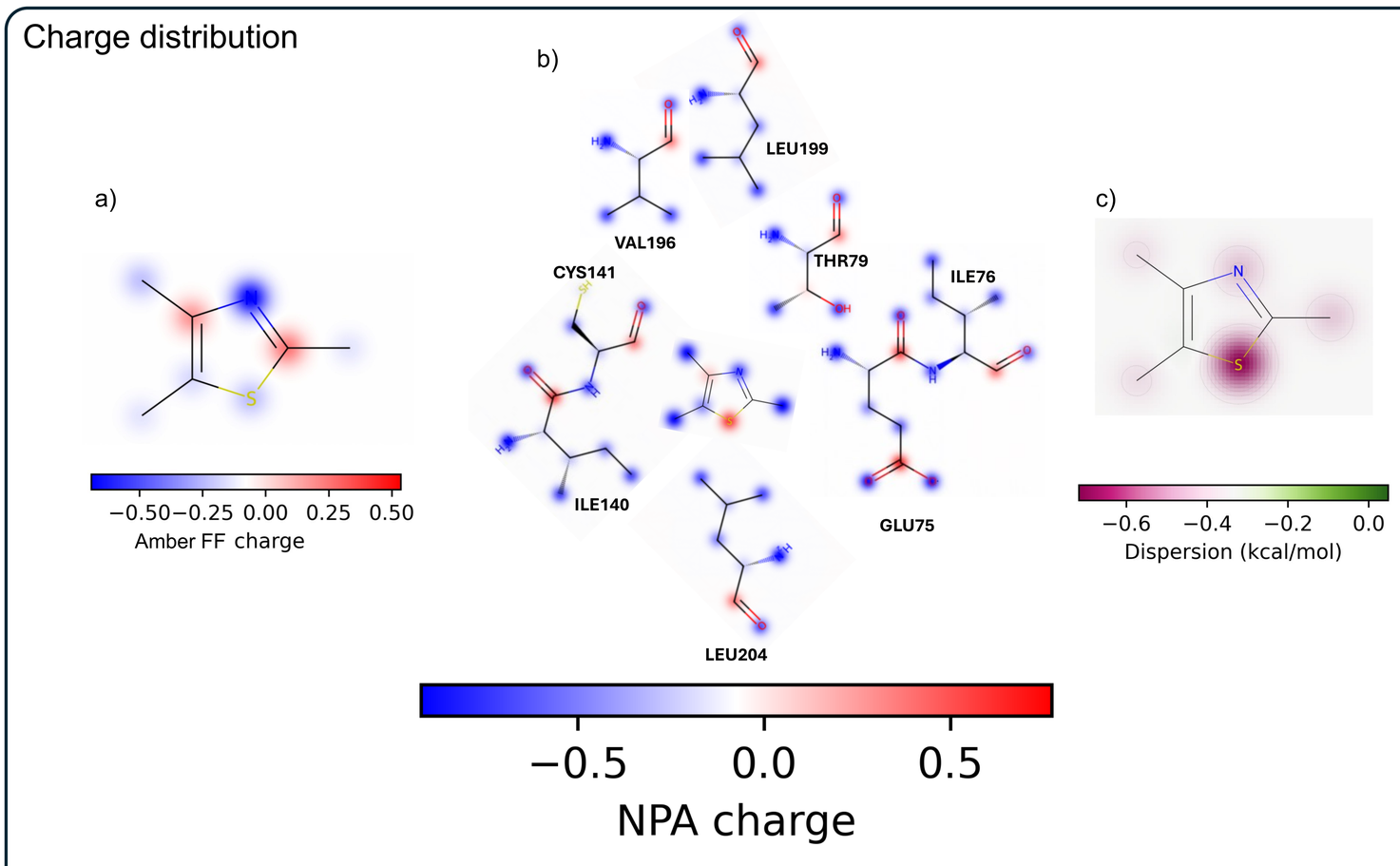

Figure 2. Molecular representations of the ligand and the protein environment. a) Heatmap of the charges as calculated via RESP and used for MD. Blue is negative charge and red positive. b) Natural Population Analysis[] (NPA) of the ligand and QM region of the lowest energy snapshot from MD. Blue is negative charge and red is positive charge. Note that the scale used is the same for a) and b). c) Dispersion stabilization contribution to the energy via the Atomic Decomposition of London Dispersion energy (ADLD) of the gas phase TMT subtracted from the bound TMT. Purple is high stabilization contribution while green is no contribution.

Structural analysis

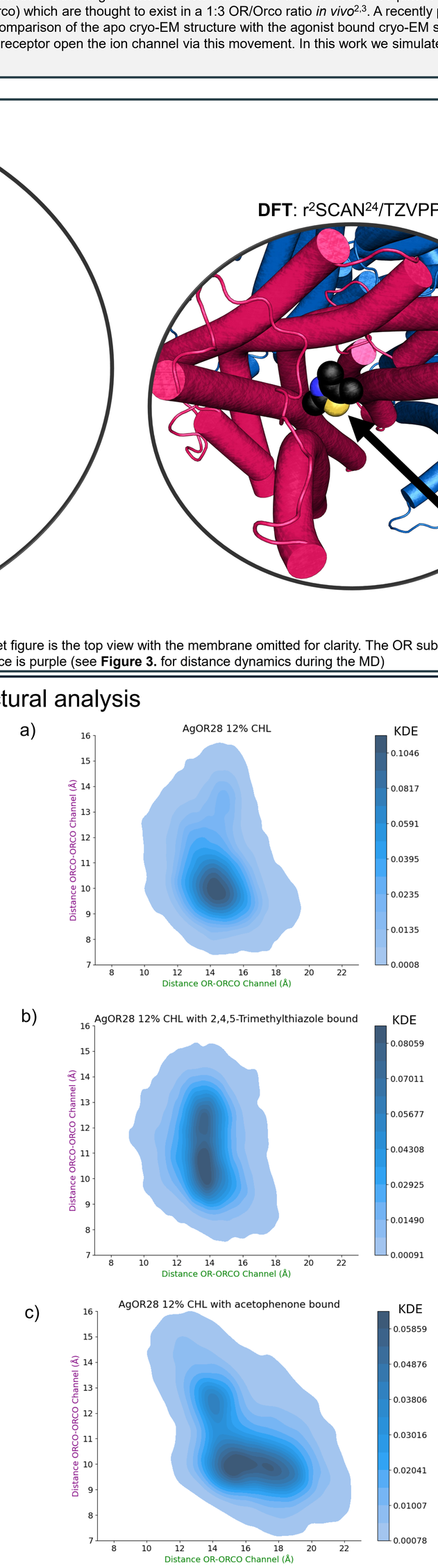

Figure 3. A kernel density estimate (KDE) plot of the evolution of OR-Orco vs. Orco-Orco distances over the trajectory of the MD runs, a) apo, b) TMT, and c) acetophenone 9x replicas of 100ns each. OR-Orco/Orco-Orco distance axis is colored green/purple, respectively, as the distance is in **Figure 1.**

Conclusions

- The well-established RESP protocol for parametrizing ligands fails for TMT, as it has a polarizable sulfur
- This results in incorrect binding dynamics as the sulfur does not interact correctly with the protein environment
- Additionally, London dispersion provides additional stabilization which is not accounted for in our modelling
- The dispersion stabilization contribution of the protein environment via the sulfur is about –0.7 kcal/mol for the lowest energy snapshot and could be important for accurately simulating the system, further study is needed

Outlook

- Re-parameterization of the ligand with better charges and subsequent MD to verify that TMT activates the channel
- Try polarizable force fields; as they are more costly, getting a clear idea if they are needed, is pertinent
- Geometry optimization of the lowest energy snapshots (ongoing)

Electronic Supplementary Information

Methodology

The cryo-EM structure[2], *Ag*OR28 PDB: 8V3C, was used to construct the system. PROPKA3[6] protonation states were used. PACKMOL-memgen[7] was used for a POPC/12% cholesterol membrane (see **Figure 1.**). TIP3P water model[8] was used and the system was neutralized with Na^+/Cl^- ions (0.15 M final concentration). All molecular dynamics (MD) simulations used the AMBER ff19SB[9] force field (FF) for proteins.

Ligands: Gaussian16[10] B3LYP[11-13]/6-31G*[14], electrostatics HF/6-31G*. Parameters and partial charges via RESP method[15] (Antechamber and General Amber Force Field 2 (GAFF2)). Lipid21 FF for lipid bilayers. AmberTools[16] for topology and parameter files.

Amber24[17] energy minimization was followed by equilibration with GROMACS[18]. Production MD were conducted in the NPT ensemble at 1 bar (C-rescale barostat[21]) and 300 K (V-rescale thermostat[20]) with periodic boundary conditions and Δt=2 fs. H-bonds constrained with LINCS[19]. MDAnalysis[22] was use for analysis.

Quantum Mechanics/Molecular Mechanics (QM/MM) single point energy (SPE): ORCA[23] r²SCAN[24]/TZVPP[25,26] with D4 dispersion correction[27,28]. The 2,4,5-Trimethylthiazole (TMT) agonist and surrounding environment were treated with QM (QM region) while the rest of the protein was treated as point charges.

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Full details and references in ESI

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