A Comparative Study of Neuroactive Ligands Docking to Muscarinic M1 Type GPCR

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Introduction and aim

Mosquitoes are the primary vectors of diseases which kill a million people each year. Malaria remains the major killer of children under five years old, taking the life of a child every two minutes. Unfortunately, commonly used repellents (i.e. DEET) were found to be neurotoxic in humans and their effectiveness is diminishing as mosquitoes become resistant. Therefore, there is a high need for a new generation of mosquito repellents. One of their modes of action goes through the **G-protein coupled receptors** (GPCRs).

GPCRs play a crucial role in signal transduction and are targets of 30-50% of all modern drugs. **Muscarinic acetylcholine receptors** (mAChRs), the subfamily of rhodopsin family α-type GPCRs, are the key element in both human and insect nervous systems. These receptors modulate a variety of physiological functions, such as airway, eye and intestinal smooth muscle contraction, heart rate and glandular secretions. While in humans 5 types are present (M1-M5), insect receptors form 3 groups A-C, from which A type is the most similar to the human M1.

By using **molecular dynamics (MD) and docking** tools we investigated the conformational changes in muscarinic acetylcholine receptors, in response to ligand binding in orthosteric and allosteric sites. Knowing the molecular basis of these changes is the crucial to finding compounds that would serve as **selective malaria vectors repellents having no side effects in humans**. Here we compare docking and MD results obtained for two distinct (X ray and homology based) initial structures of the human M1 GPCR.

Methods and protocols

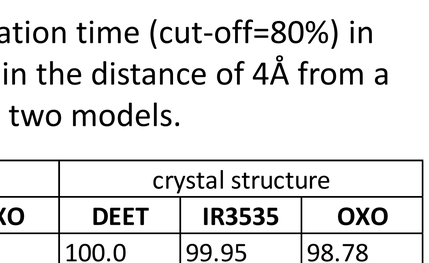
- ligand preparation, topology and parameters finding
- determination of the protein orientation in membrane
- molecular docking to M1 protein
- generation of CHARMM inputs for the protein-ligand-membrane complex for MD simulations
- equillibration and 100 ns long MD simulation
- trajectory analysis

CHARMM-GUI AutoDock Vina: Smina

Systems and results

- human M1 muscarinic acetylcholine receptor homology model (P11229 UniProtKB) and crystal structure (PDB: 5CXV)
- ligands: DEET, IR3535, oxotremorineM
- plasma membrane: 20-Å DOPC bilayer
- force field: CHARMM36
- temperature: 303.15 K
- pressure: 1 atm

NAMD Scalable Molecular Dynamics
ProDy Protein Dynamics & Sequence Analysis



M1 homology model

Fig. 1

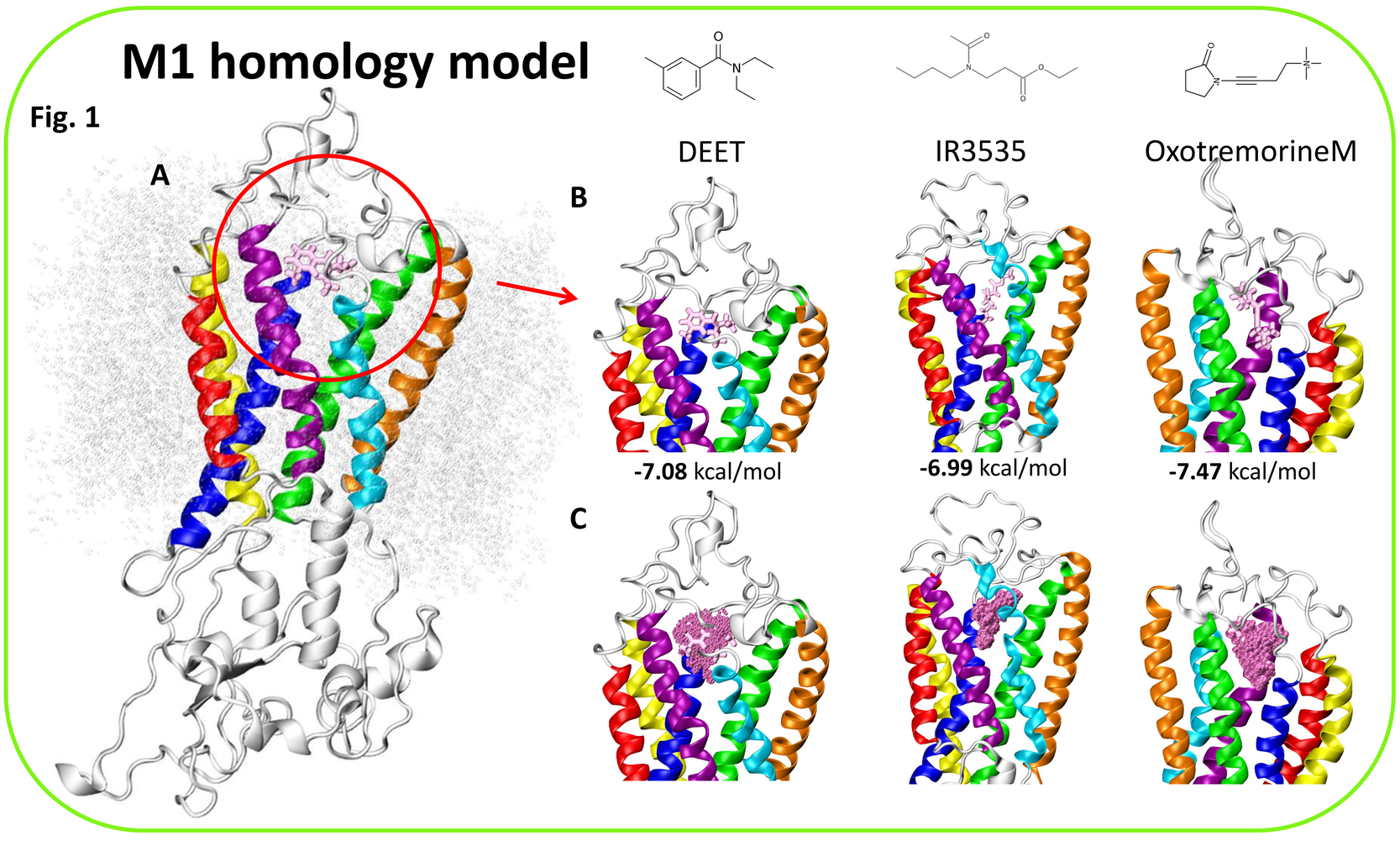


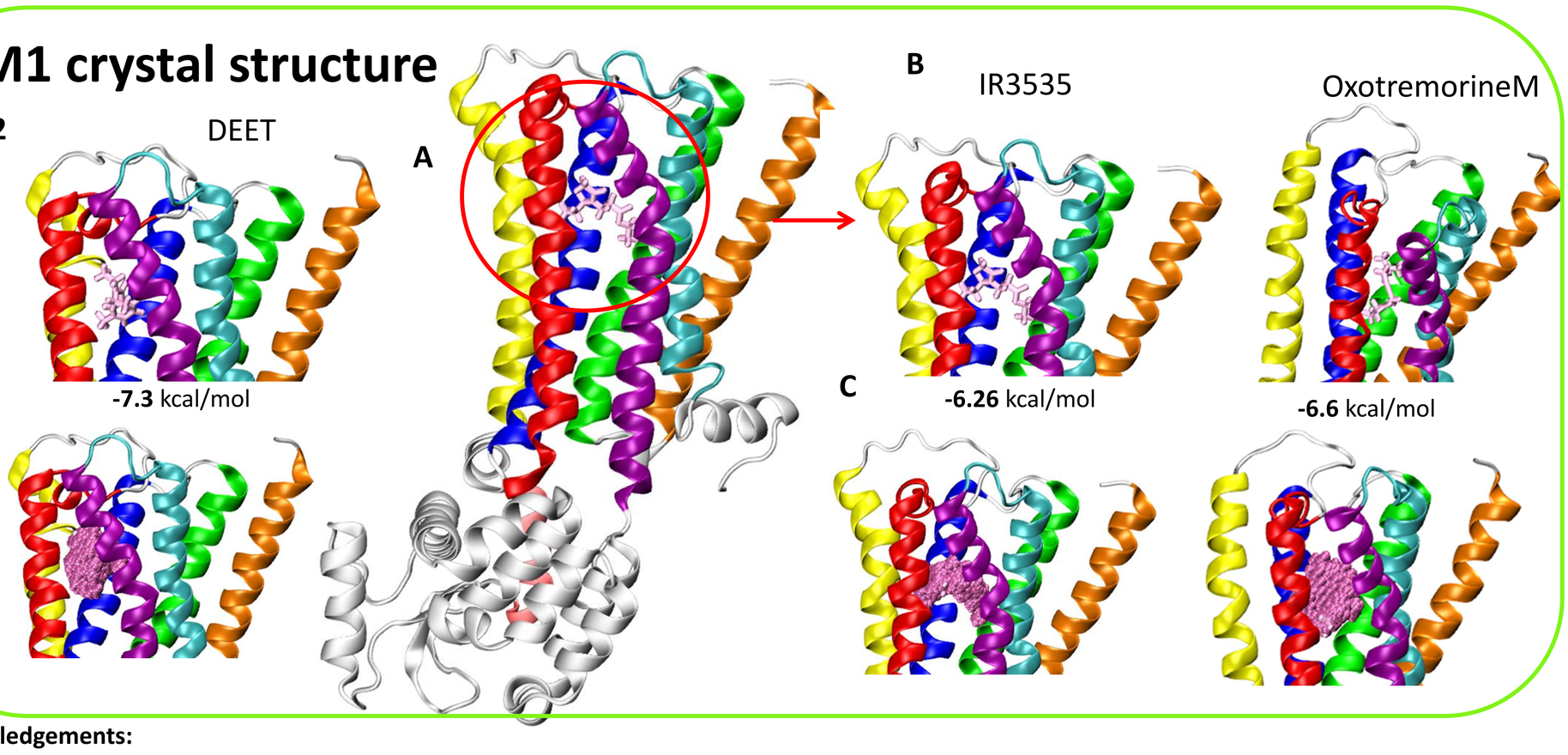
Fig. 1, 2 Molecular docking to M1 mAChR in DOPC plasma membrane
(A) whole receptor with DEET (1A) and IR3535 (2A)
(B) docking poses with the best affinity
(C) pockets occupied by ligand during 100 ns MD simulation

Tab. 1 Percentages of simulation time (cut-off=80%) in which a given residue is within the distance of 4Å from a ligand in both two models.

receptor	homology model			crystal structure		
resid\lig	**DEET**	**IR3535**	**OXO**	**DEET**	**IR3535**	**OXO**
TYR106		100		100.0	99.95	98.78
SER109		93.5		99.0	100.0	100.0
TRP378		98.1		99.98	100.0	100.0
TYR381		100	100	99.71	98.15	99.76
TYR404	97.3	96.1	83.7			99.96
CYS407		100			100.0	100.0
TYR408		99.4	100		100.0	99.0

M1 crystal structure

Fig. 2



Conclusions

- The lowest SMINA score docking poses are located deeper in the crystal structure than in the homology model ➔ DIFFERENT DOCKING POCKETS
- Ligands' docking sites in the crystal structure correspond well to the orthosteric site occupied by co-crystallized mAChRs antagonist tiotropium
- All ligands remained bound to M1 within 100 ns MD
- Positions of IR3535 form the most compact cluster in MD

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