New Insights on the structural features of *Apis Mellifera* Nicotinic Acetylcholine Receptors

Rémi Gojard[1], Jean Yves Le Questel[1], Nicolas Galland[1], Emiliane Taillebois[2], Steeve Hervé Thany[2]

[1] *Nantes Université, CNRS, CEISAM, UMR 6230, F-44000 Nantes, France*
[2] *Université d'Orléans, USC INRAE 1328, Laboratoire Physiologie, Ecologie et Environnement (P2E), 1 rue de Chartres, 45067 Orléans, France*

Apis mellifera bees, recognized as the main pollinating insects, are crucial to agriculture, food, and food security. They are under threat from a number of factors, in particular the use of neonicotinoid insecticides.[1] These molecules belong to group 4 of the IRAC (Insecticide Resistance Action Committee) classification of insecticides and act as competitive modulators of nicotinic acetylcholine receptors (nAChRs). The latter are homo or hetero-pentameric transmembrane proteins responsible for signal transmission in the nervous system. Ligand binding occurs at the interface between two subunits, in an interaction pocket consisting of three loops from the main subunit (A, B, C) and three loops from the complementary one (D, E, F).[2] Numerous subunits of the nAChRs in honeybees have been sequenced.[3] However, structural information on the environment of neonicotinoids and derivatives in the binding site of bees nAChRs remains unknown.
Our project takes place in the context of a collaboration with electrophysiologists that have been able to measure ionic currents of various heteromeric bees nAChRs (αn)x-(βm)y (n=2,3,4; m=2; x,y=2,3 or x,y=3,2) in presence of agonists.
In the absence of experimental information on the structure of bees nAChRs, molecular modeling methodologies (docking, molecular dynamics (MD)) are essential. The starting point of the present study was to model the extracellular domains of *Apis mellifera* bee α2-β2 nAChRs in the two possible stoichiometries: (α2)2-(β2)3 and (α2)3-(β2)2 using the AlphaFold-Multimer program.
Our main goal is to determine the binding interactions of a set of neonicotinoids within, in a first attempt, the (α2)2-(β2)3 and (α2)3-(β2)2 surroundings. The importance of the loops in the agonist binding has led us to investigate in more details their flexibility, through the use of the MOE program, using as a reference a co-crystal structure of acetylcholine (ACh) complexed with an acetylcholine binding protein (AChBP, 3WIP PDB entry), a recognized surrogate for the ligand binding domain of nAChRs.
Molecular docking studies of various ligands (ACh, nicotine and insecticides) on the different conformations of nAChRs selected have then been carried out using the Autodock Vina program.
Our study allows to (i) highlight the importance of the (α2)x-(β2)y loops in the binding of various ligands; (ii) determine the interactions of different insecticides compared to one endogenous ligand (ACh) and one exogenous ligand (nicotine); (iii) rank the various insecticides with respect to ACh. We plan to investigate in a more comprehensive way through MD simulations relevant examples in order to refine the preliminary trends obtained through docking.
The present work provides informations on the binding of neonicotinoids and derivatives to bees nAChRs. We hope that with the MD simulations that we will carry out, our results will pave the way toward the understanding, at the atomic level, of the toxic effects of neonicotinoids.

Bibliography :

[1] M. C. Tackenberg *et al.*, « Neonicotinoids disrupt circadian rhythms and sleep in honey bees », *Sci. Rep.*, vol. 10, nº 1, p. 17929, oct. 2020, doi: 10.1038/s41598-020-72041-3.
[2] B. Selvam *et al.*, « Imidacloprid and thiacloprid neonicotinoids bind more favourably to cockroach than to honeybee α6 nicotinic acetylcholine receptor: Insights from computational studies », *J. Mol. Graph. Model.*, vol. 55, p. 1-12, févr. 2015, doi: 10.1016/j.jmgm.2014.10.018.
[3] A. Witwicka, F. López-Osorio, V. Patterson, et Y. Wurm, « Expression of subunits of an insecticide target receptor varies across tissues, life stages, castes, and species of social bees », *Mol. Ecol.*, vol. 32, nº 5, p. 1034-1044, 2023, doi: 10.1111/mec.16811.