LARGE SCALE MOLECULAR DYNAMICS SIMULATIONS OF BIOMOLECULAR SYSTEMS IN MEMBRANE ENVIRONMENT

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Consiglio Nazionale delle Ricerche

Istituto di Chimica Biomolecolare

MEMBRANE PROTEINS

Membrane proteins are large biomolecular systems formed by one or more monomers including:

✓ G-protein coupled receptors (GPCRs)

✓ Receptor-Channels belonging to the family of Transient Potential Receptor (TRP) channels (non-selective cations-permeable channels)

✓ Ion Channels (Ca^{2+}, K^+, Cl^-, Na^+)
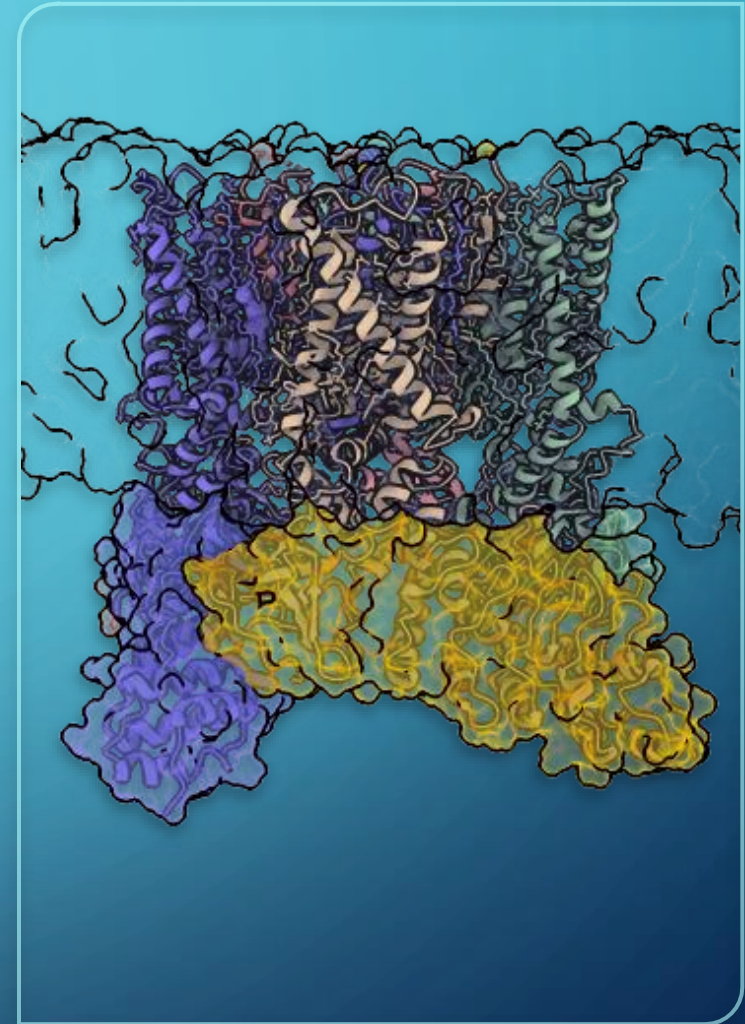
MEMBRANE PROTEINS IN DRUG DISCOVERY

- ✓ Membrane proteins are relevant therapeutic targets for a wide range of diseases.

- ✓ Molecular dynamics (MD) simulations of these biological systems represent a valuable tool to characterize ligand-protein interactions and large protein conformational changes occurring upon ligand binding at atomic level.

MD SIMULATIONS IN MEMBRANE ENVIRONMENT

The simulation of these biomolecular systems in their «native» conditions requires the embedding of proteins in a lipid bilayer, water and ions, which dramatically increases the total number of atoms and interactions to describe. This, in turn, results in huge computational costs and dimensions of trajectory files to store.

MD SIMULATIONS (AMBER 20)

✓ G-protein coupled receptors (GPCRs):

CB_2R/ligand complex ~ **100.000** atoms total - **2GPUs: ~14-15h and 64Gb/100ns**

✓ Receptor-Channels belonging to the family of Transient Potential Receptor (TRP) channels (non-selective cations-permeable channels):

TRPV3 (tetramer)/ligand complex ~ **300.000** atoms total **- 2GPUs: ~21-22h and > 100Gb/100ns**

✓ Ion Channels (Ca^{2+}, K^+, Cl^-, Na^+):

VDAC (dimer) /ligand complex ~ **140.000** atoms total - **2GPUs: ~18h and 79Gb/100ns**

MD SIMULATIONS (AMBER 20)

- ✓ G-protein coupled receptors (GPCRs):

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MD SIMULATIONS USING IBISCO CLUSTER

MD of CB$_2$R-ligand complexes on microsecond timescale to:

✓ assess the effect of a fluorescent probe ligand pendant on the overall complex stability;

✓ evaluate the ability of the reactive group to reach lysine residues to identify the most promising ones;

✓ characterize the receptor conformational changes upon binding;

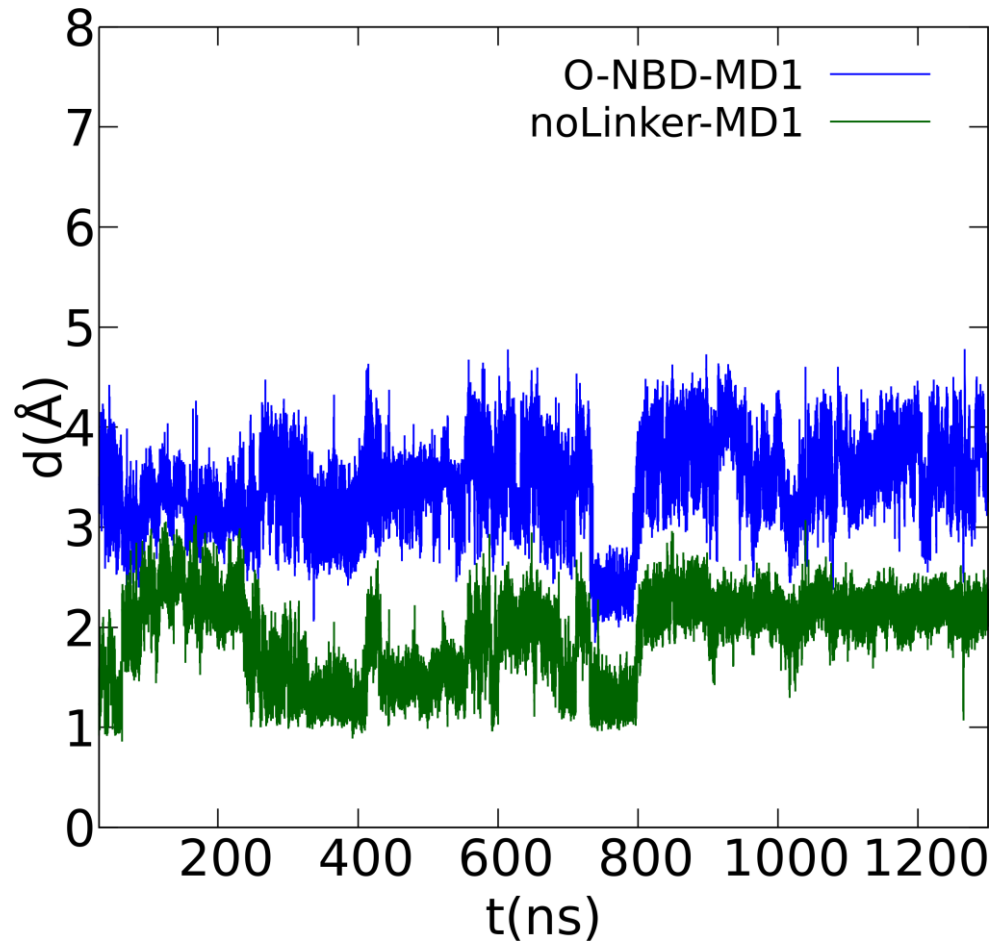✓ identify the higher affinity ligand between two epimers.

# LIGAND-DIRECT CB$_2$R COVALENT PROBES
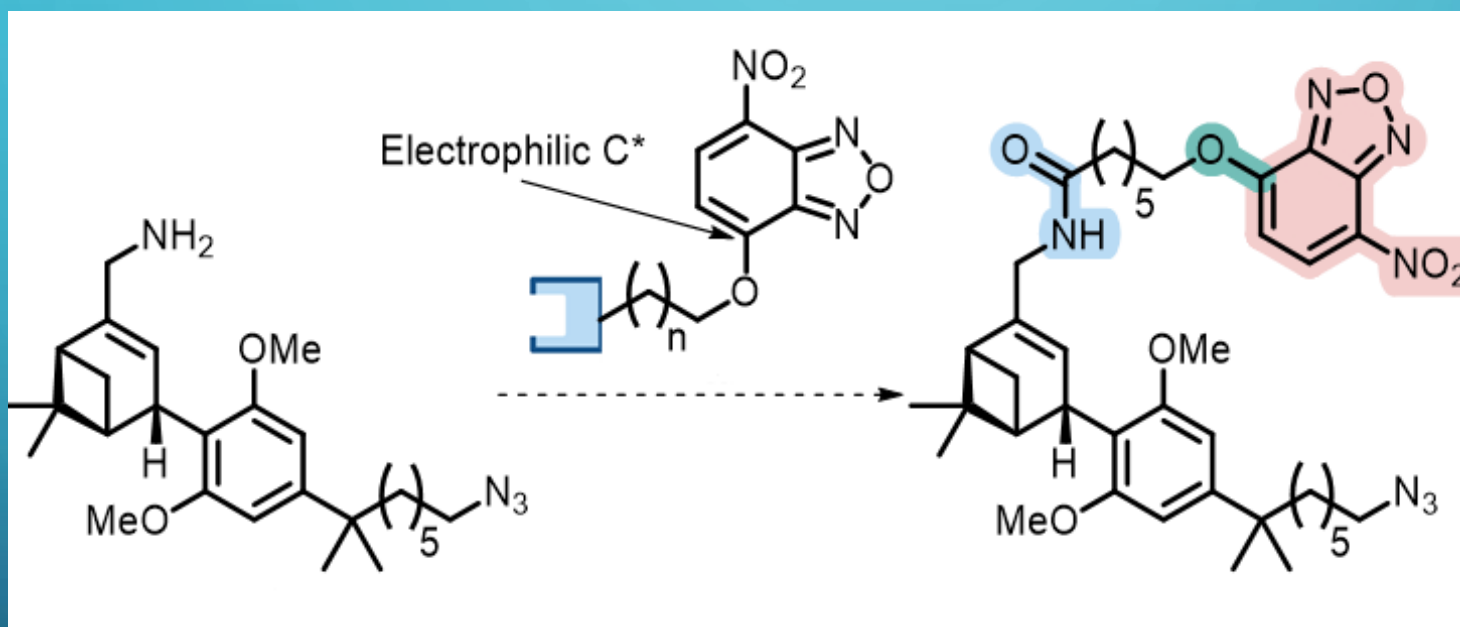
O-linked NitroBenzoxaDiazoles (NBD) are non-fluorescent
N-linked NBD are highly fluorescent
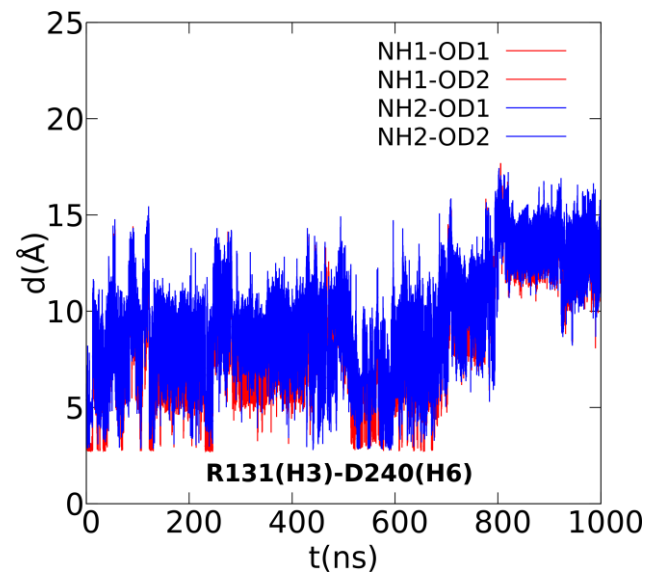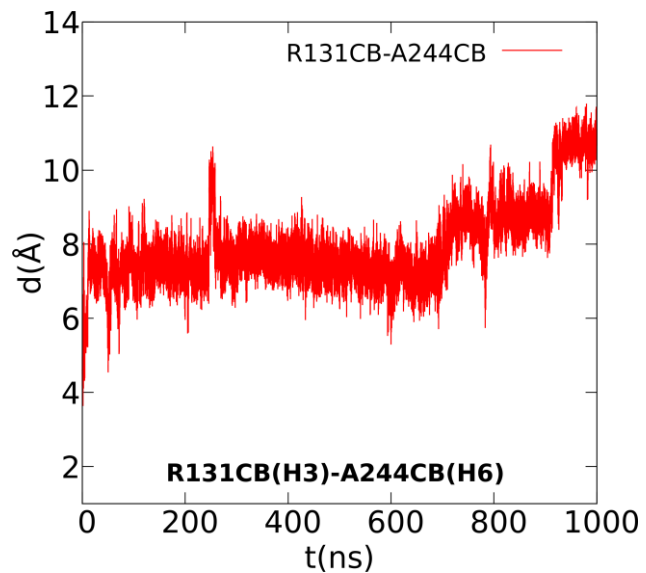
J. Am. Chem. Soc. 2023;145(28):15094-15108

MD SIMULATIONS USING IBISCO CLUSTER

The rmsd MD trajectory of the ligand with inclusion/exclusion of flexible linker showed that it does not affect the overall stability of the ligand within the orthosteric site.
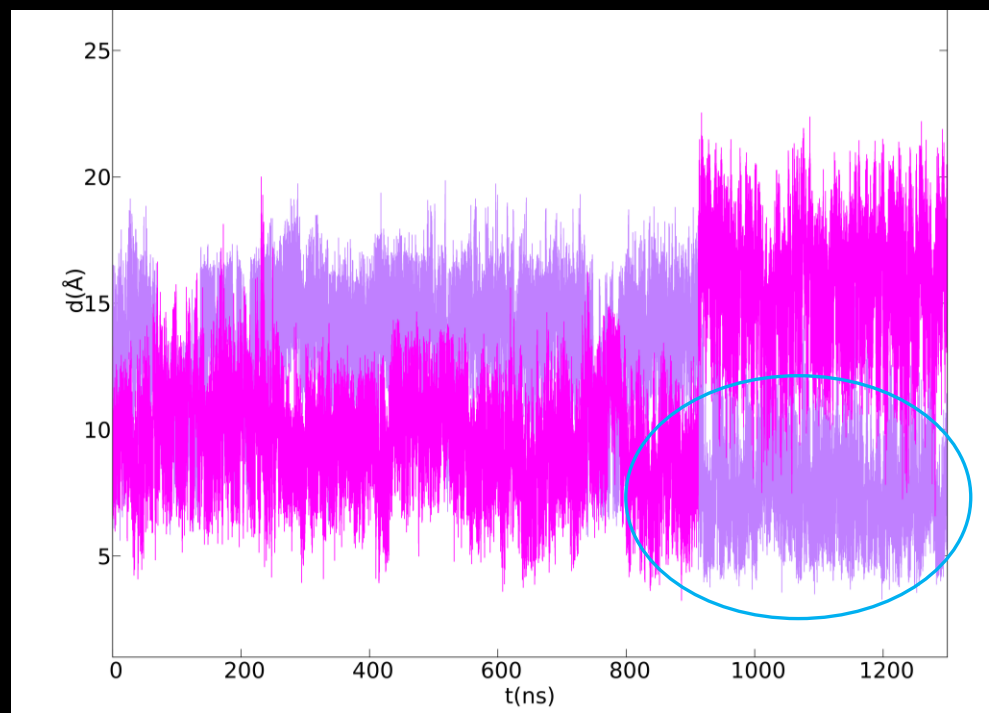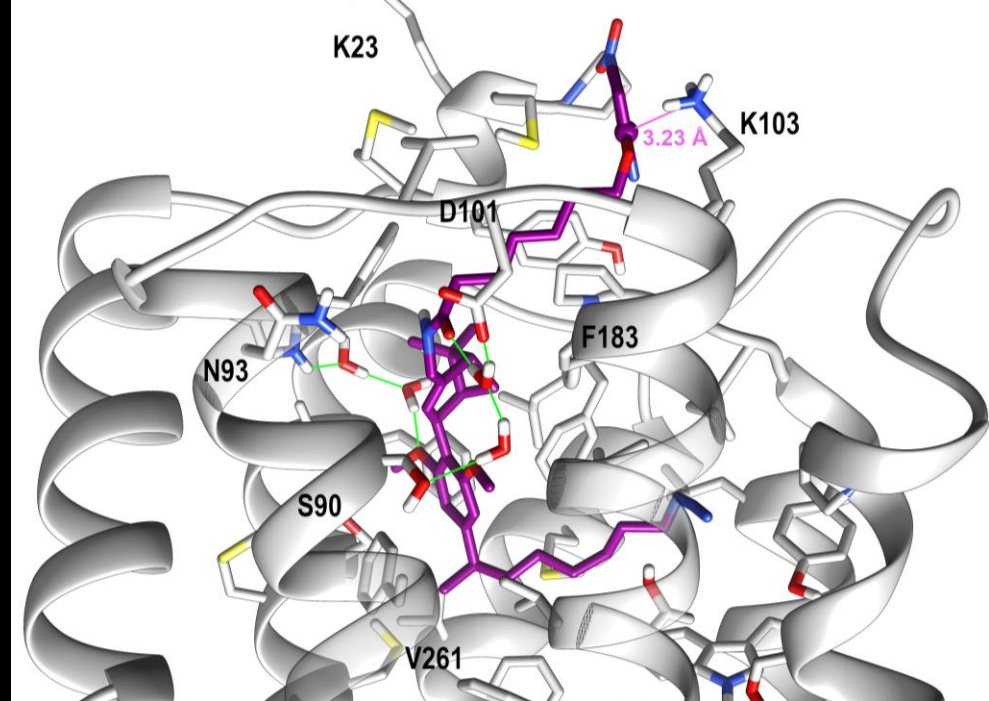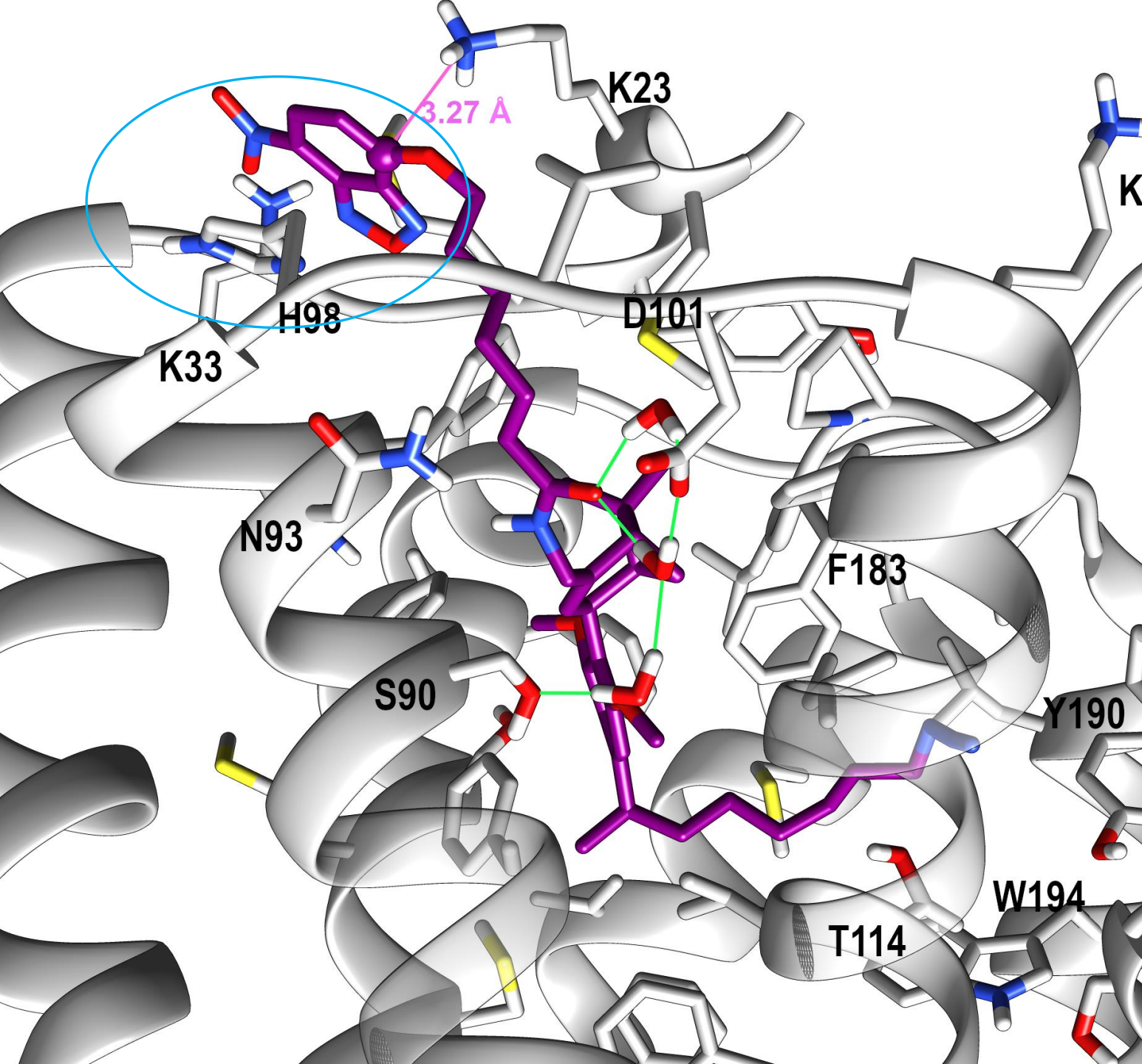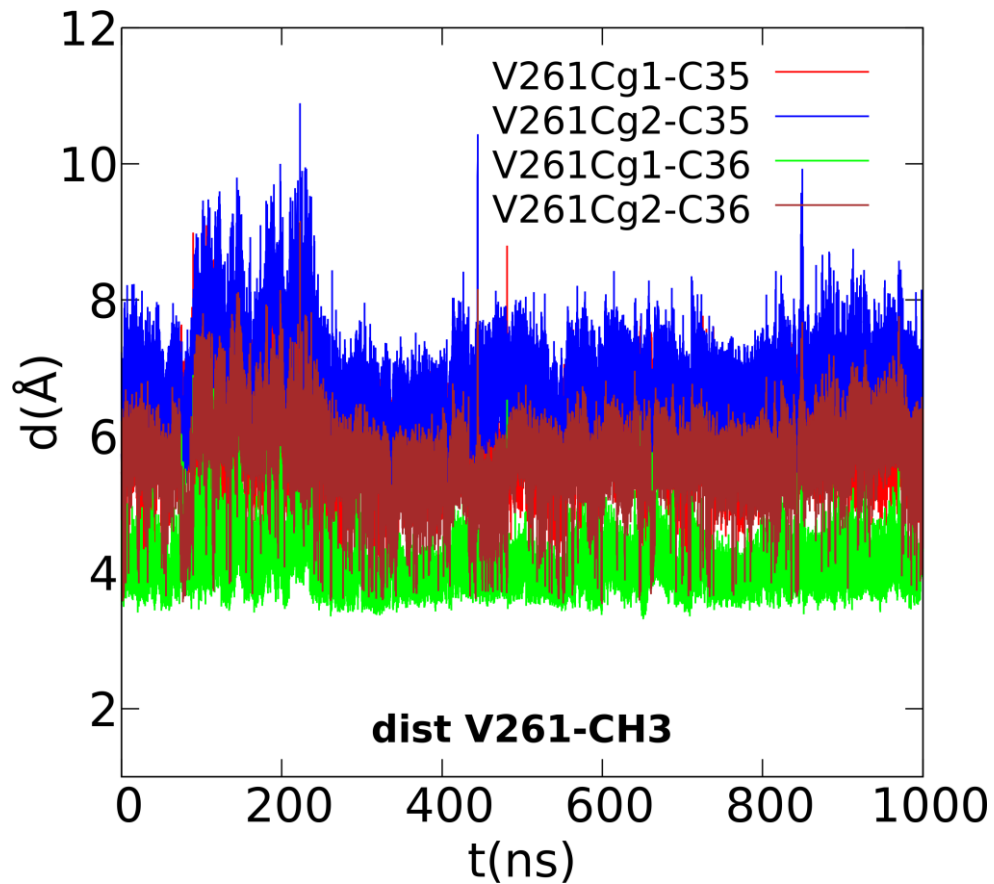
MD SIMULATIONS USING IBISCO CLUSTER

Receptor conformational change upon agonist binding:

- breakage of the ionic lock Arg (H3)-Asp(H6);
- increased distance between helices H3-H6.

LIGAND HIGHER AFFINITY TOWARD HUMAN OVER MURINE CB$_2$R

due to Val261$^{6.51}$Ala, Ser90$^{2.60}$Asn and Asn93$^{2.60}$Ile replacements:
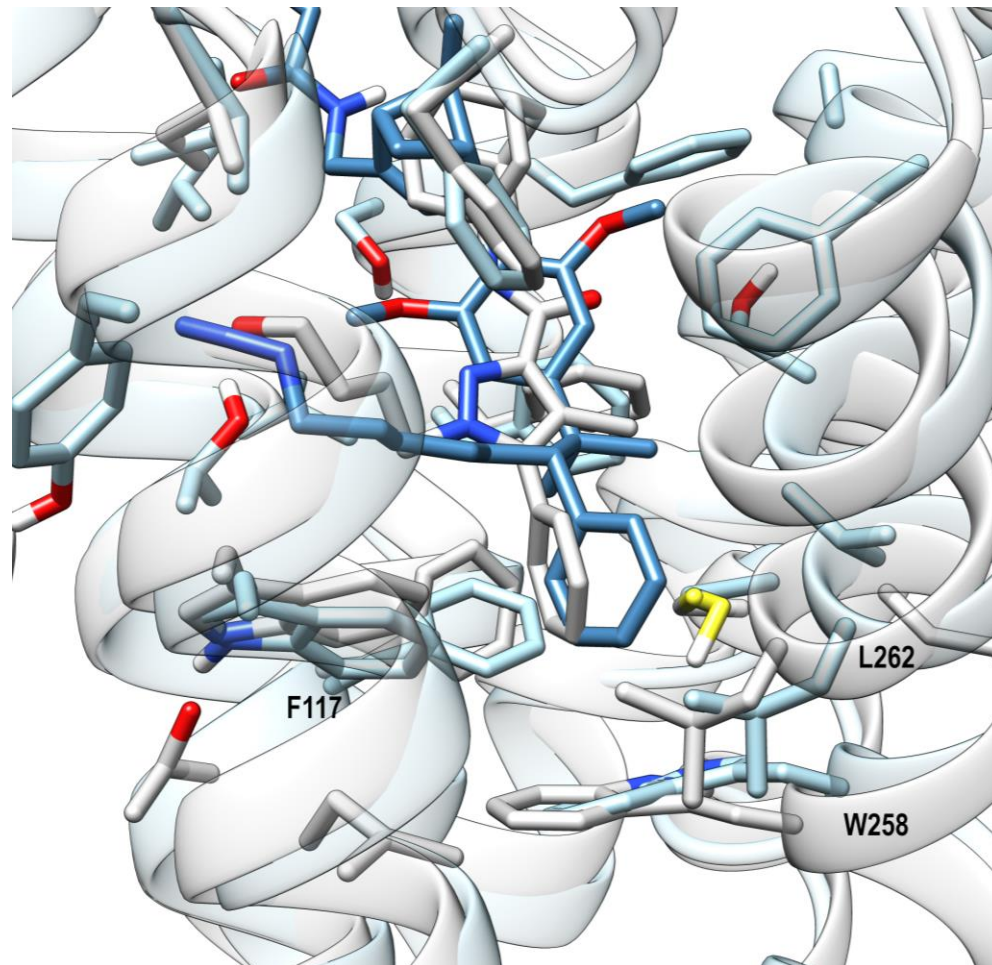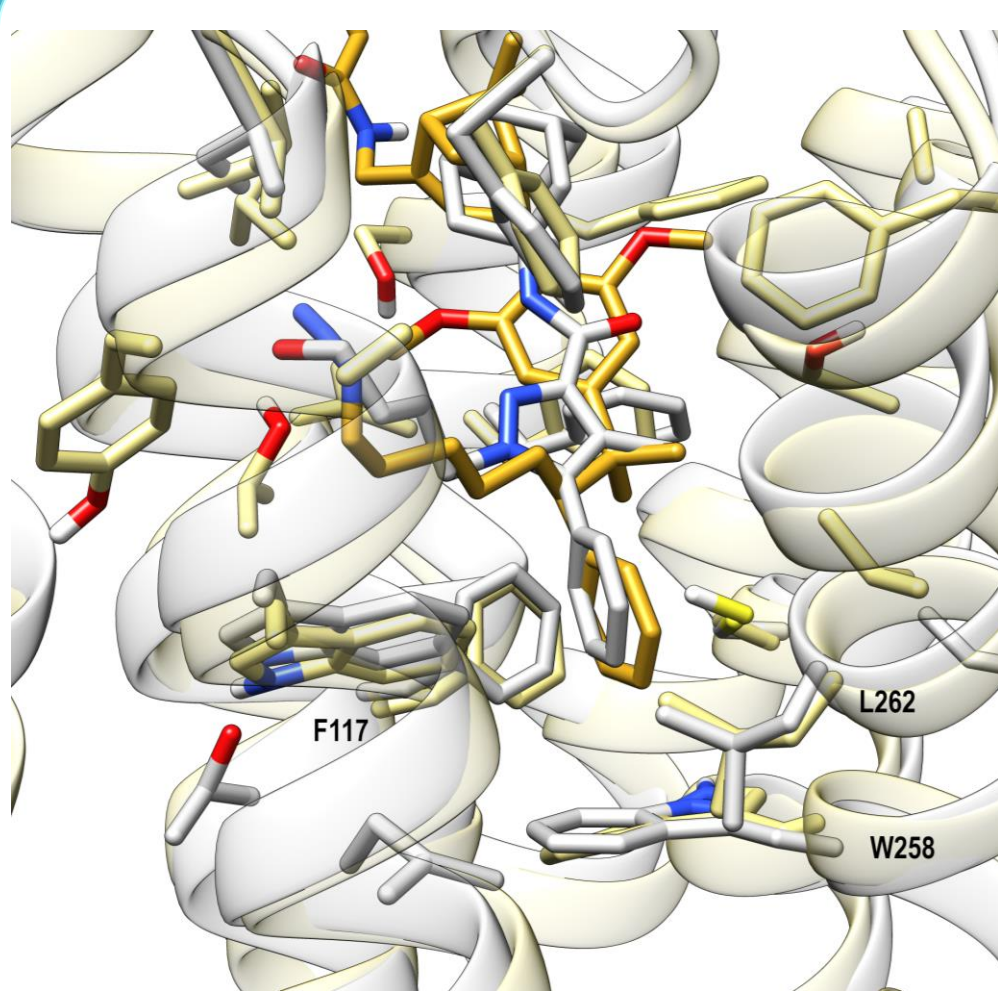
➢ Val261$^{6.51}$ sidechain forms stabilizing hydrophobic interactions with the ligand dimethyl group.

➢ Ser90$^{2.60}$Asn and Asn93$^{2.60}$Ile substitutions alter the steric hindrance of the ligand binding site and disrupt the network of water-mediated H-bonds, respectively.
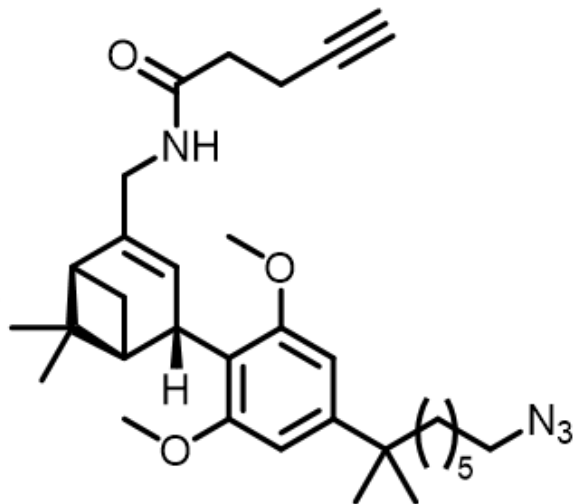
CB$_2$R agonist
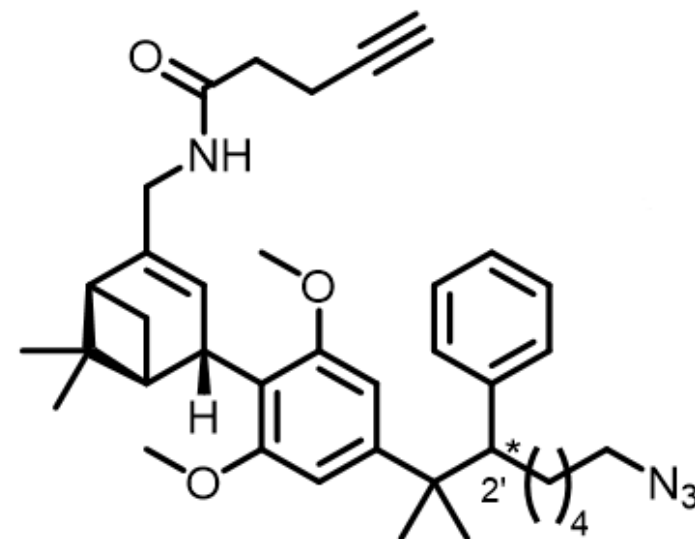
CB$_2$R inverse agonist epimers

Identification of R epimer as the higher affinity ligand from $\Delta\Delta G_{calc.}$

CONCLUSIONS

IBiSCo cluster allowed MD simulations of large biomolecular systems with affordable computing times, providing insights into:

- the binding modes of ligands within the receptor binding site;
- the conformational preferences of flexible ligand portions driven by receptor interactions;
- receptor conformational changes occurring upon ligand binding.

THANK YOU FOR YOUR ATTENTION !!