

AFFINITY CALCULATIONS FOR LIPOPHILIC MODULATORS BINDING TO ISOLATED SITES ON GABA(A) RECEPTORS



SRUTHI MURLIDARAN ^A, REZA SALARI ^{A,B}, JÉRÔME HÉNIN ^C AND GRACE BRANNIGAN ^{A,B}

^ACENTER FOR COMPUTATIONAL AND INTEGRATIVE BIOLOGY, ^BDEPARTMENT OF PHYSICS, RUTGERS UNIVERSITY, CAMDEN, NJ AND ^CINSTITUT DE BIOLOGIE PHYSICO-CHIMIQUE, CNRS, PARIS.

MOTIVATION

- 1. Most anesthetics, some hormones and neurosteroids are known to modulate GABA(A) receptors.
- 2. The molecular mechanisms of these ligands are still unclear. The exact binding sites of these modulators in GABA(A) receptor, the sites they occupy at clinical concentrations and how they affect gating and ion conduction in receptor are the questions yet to be answered.
- 3. Docking studies followed by Molecular Dynamics(MD) simulations employing free energy perturbation (FEP) calculations, are being used to compare the binding affinities of the exogenous modulators at the inter-, intra-subunit and pore sites.

INTRODUCTION

- GABA is the primary inhibitory neurotransmitter in Central Nervous System; inhibition is partially transduced by extracellular binding to the type A GABA receptor, an anionic pLGIC.

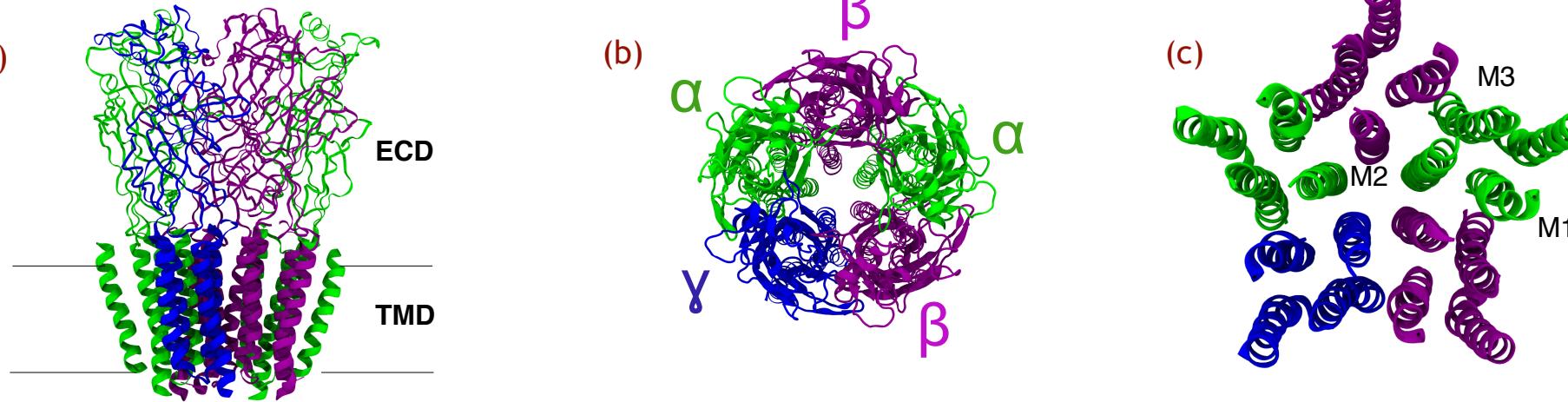


FIG1: GABA_A Receptor, front view (a). GABA_A has three domains ECD, TMD and IMD with 5 subunits. GABA_A Receptor, top (b) and Cross-section view (c). The TMD has four membrane spanning α helices(M1-M4), with M2 lining the pore.

- General Anesthetics are small molecules that induce immobilization, unconsciousness and amnesia by depressing neuronal signaling.
- Depending on the route of administration, the general anesthetics are further classified as intravenous(Propofol) and inhalational (Sevoflurane, Isoflurane) anesthetics.

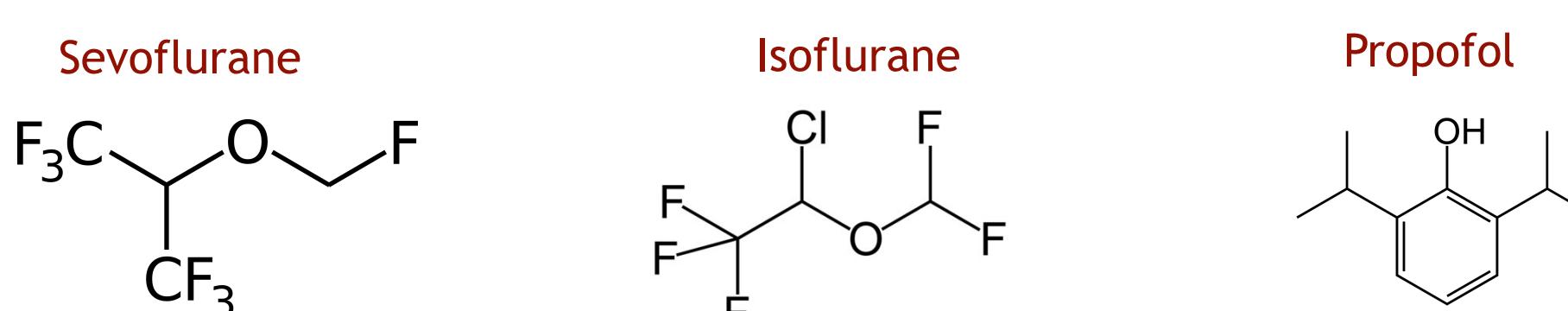
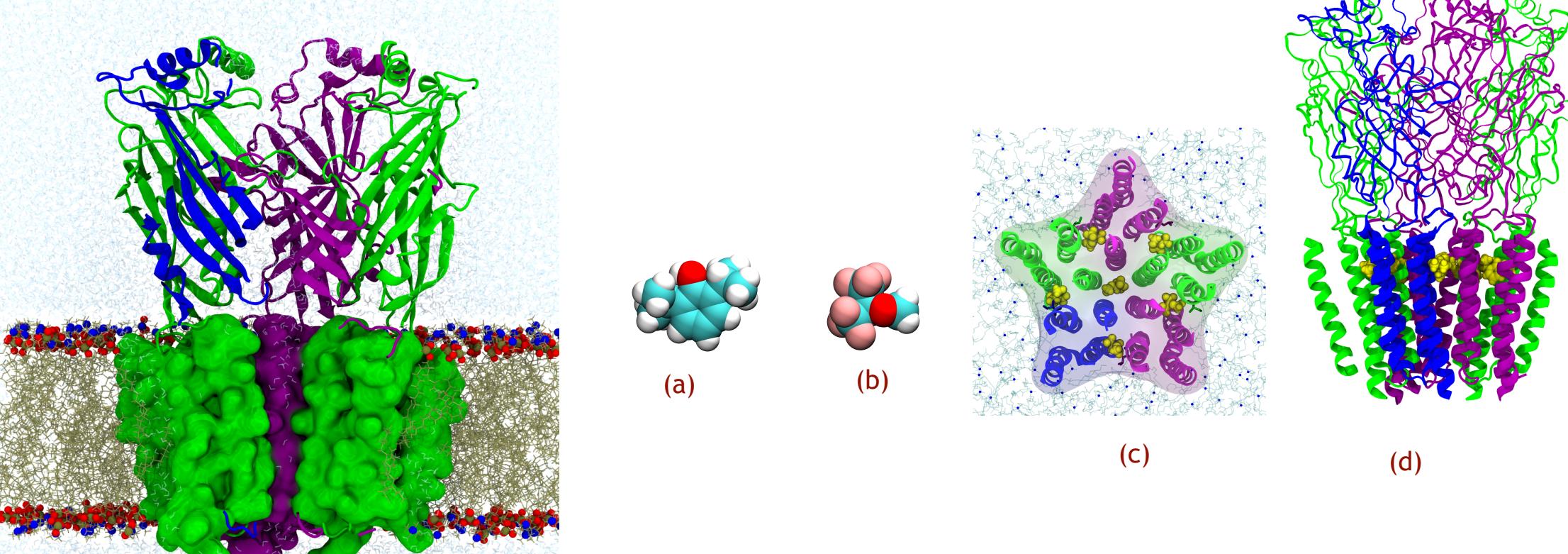


FIG2: Inhalation (Sevoflurane, Isoflurane) and Intravenous (propofol) anesthetics.

- MD-FEP simulations of these ligands could shed light onto their molecular interactions with the receptor and help us design novel site specific drugs with more specific actions.

SYSTEM SETUP

- The MD simulation of GABA_A receptor, with NAMD2.10[3], CHARMM36 [4] were generated using an $\alpha 1\beta 3\gamma 2$ GABA_A model derived from an $\alpha 1\beta 1\gamma 2$ GABA_A model used previously. [1]
- Docking calculations were performed using AUTODOCK(Vina), for identifying the possible binding sites for propofol, sevoflurane and T3 in GABA_A Receptor.



- At the conclusion of the traditional MD simulation, standard binding affinities for propofol and sevoflurane in each distinct site were calculated using separate 24 ns Alchemical Free Energy Perturbation (AFEP) simulations.

RESULTS AND DISCUSSION

PROPOFOL (PFL)

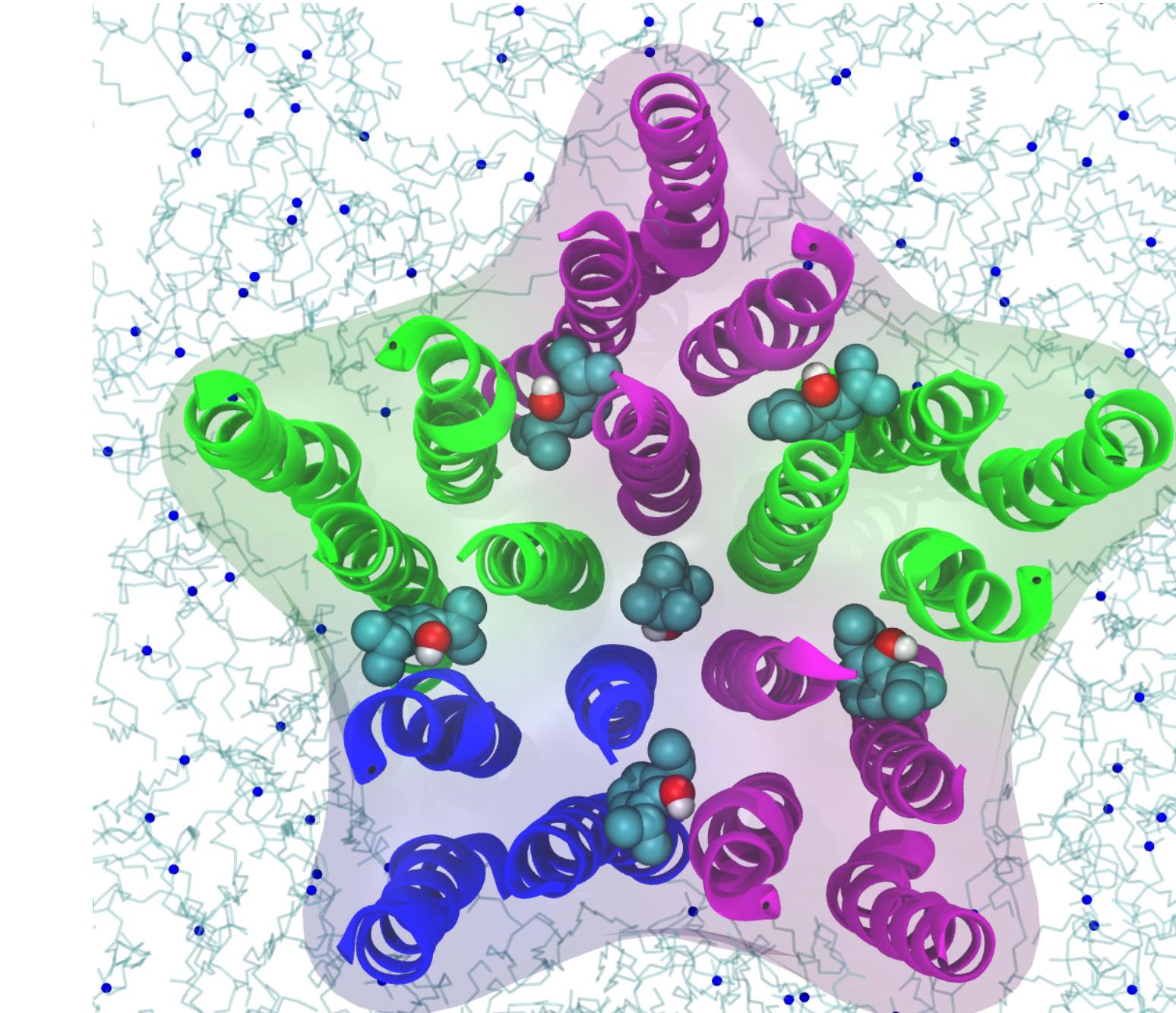


FIG 5: PFL - Standard MD simulation - Snap-shot of a view from EC domain, after 500ns of simulation.

MD SIMULATION AND FREE ENERGIES

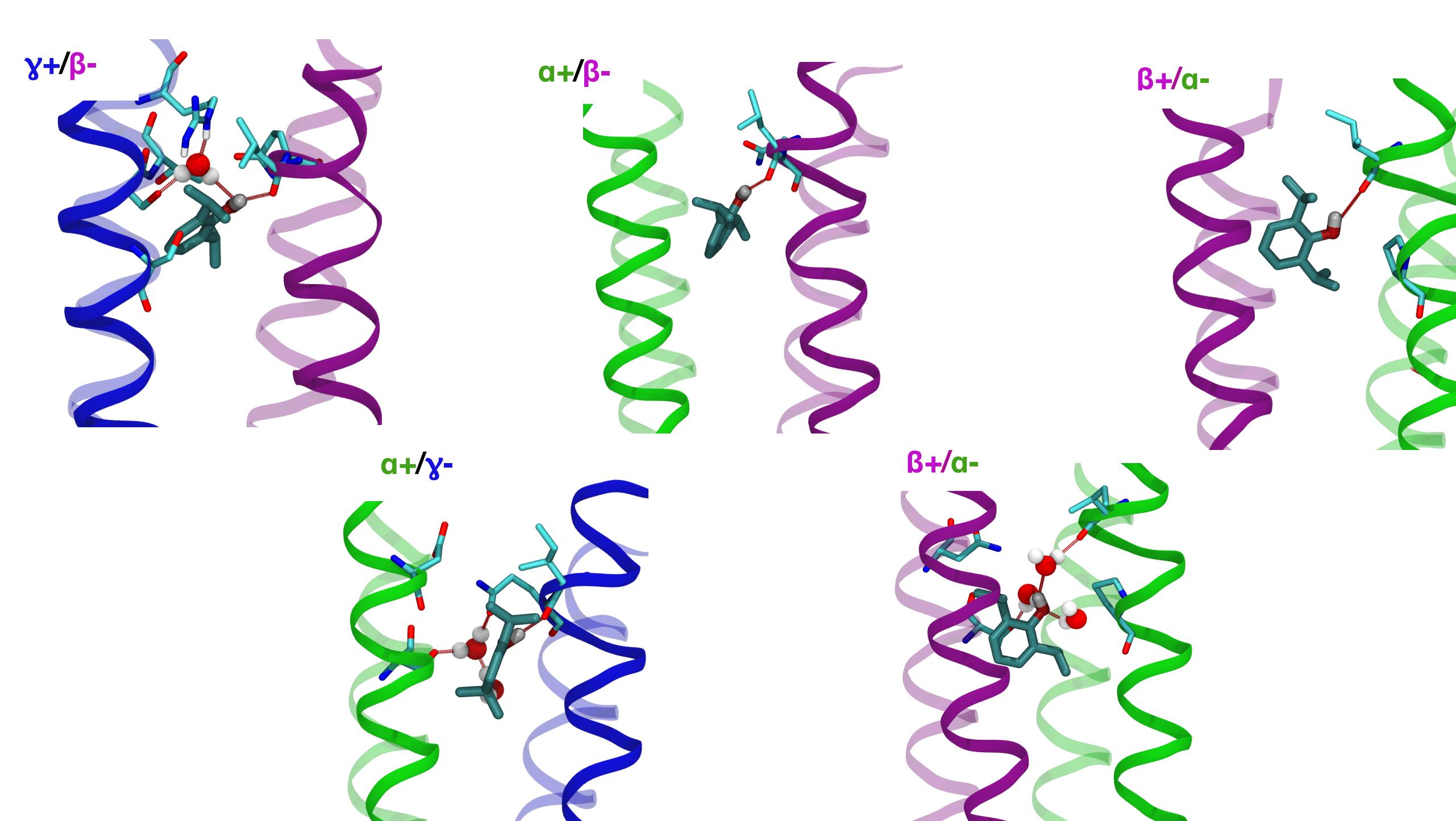


FIG 6: PFL interactions at different intersubunit sites

Binding site	α/β^-	β/α^-	γ/β^-	α/γ^-
ΔG_{bind} (kcal/mol)	-7	-4.8/-6.1	-11.3	-6.7
Kd (μM)	6	600/35	4×10^{-3}	12
H-bond Occupancy	0.9	0.06/0.2	0.8	0.7

PFL EC₅₀ = 0.5-5 μM [9]

SEVOFLURANE (SEV)

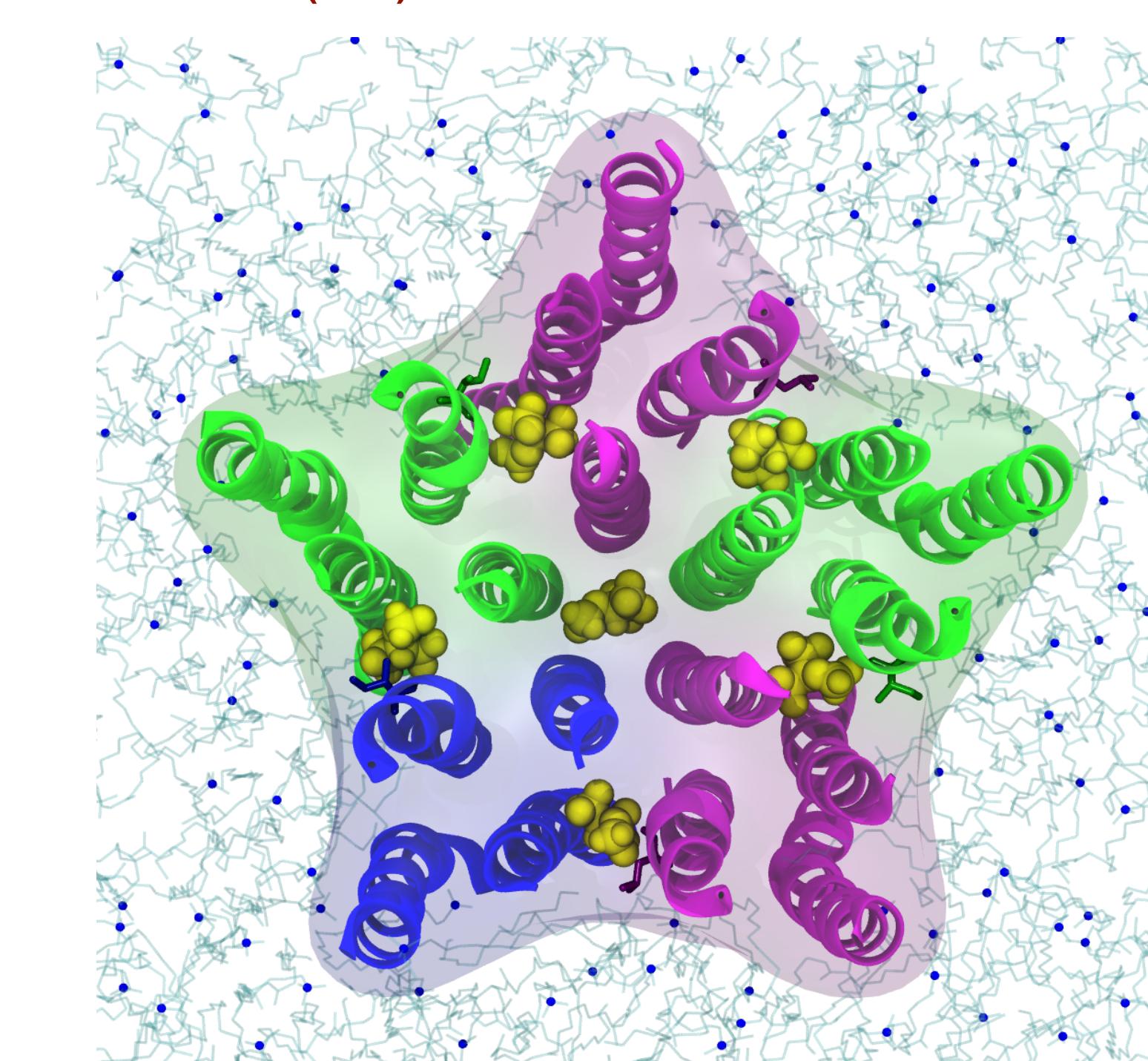


FIG 7: SEVO - Standard MD simulation - Snap-shot of a view from EC domain, after 150ns of simulation.

I索FLURANE (ISO)

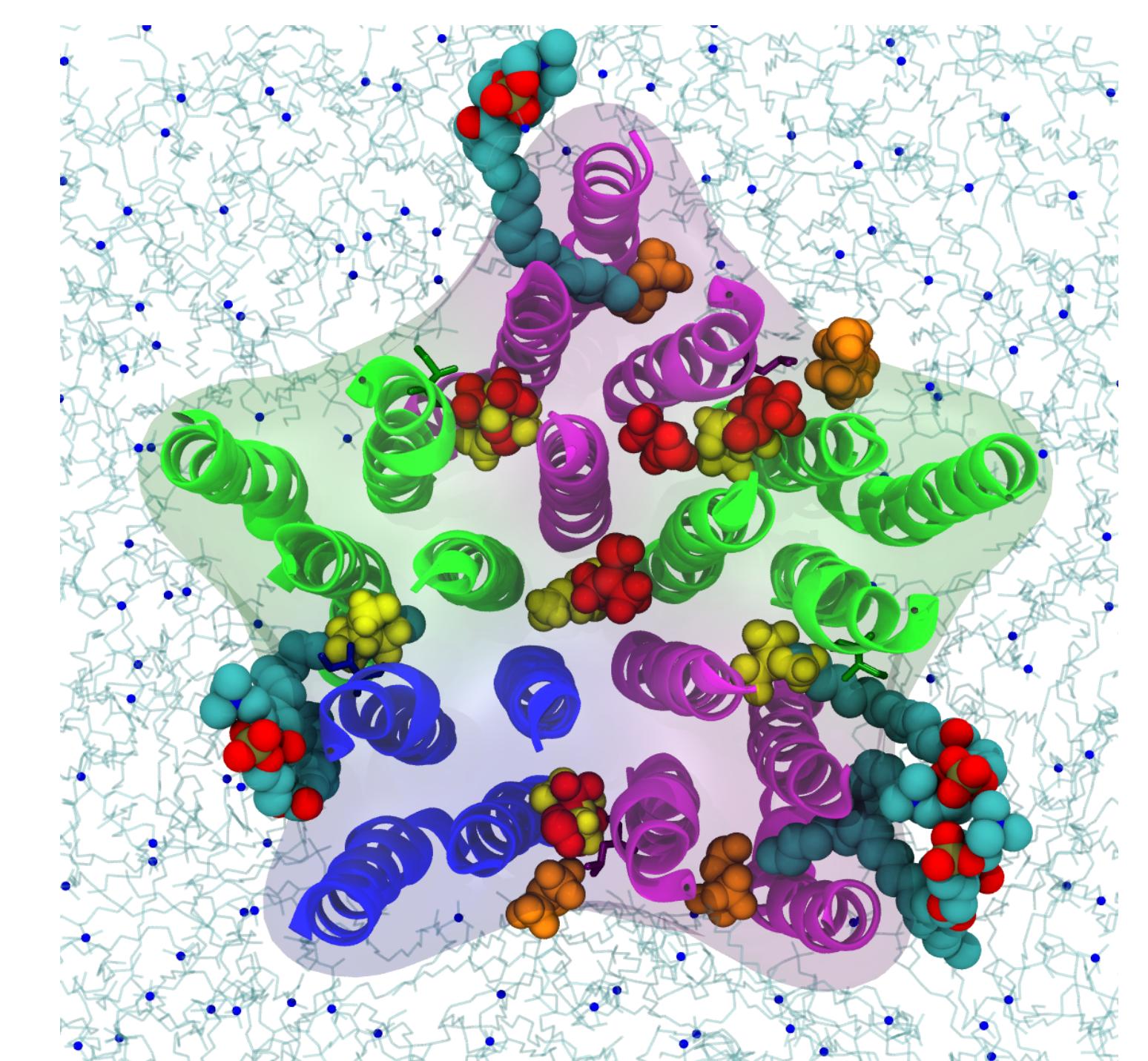


FIG 8: SEVO - Flooding simulation - Snap-shot of a view from EC domain, after 1.6/s of simulation. (Red)SEV are the molecules bound to intersubunit sites;(Yellow) SEV are final positions of molecules from std.MD simulations.

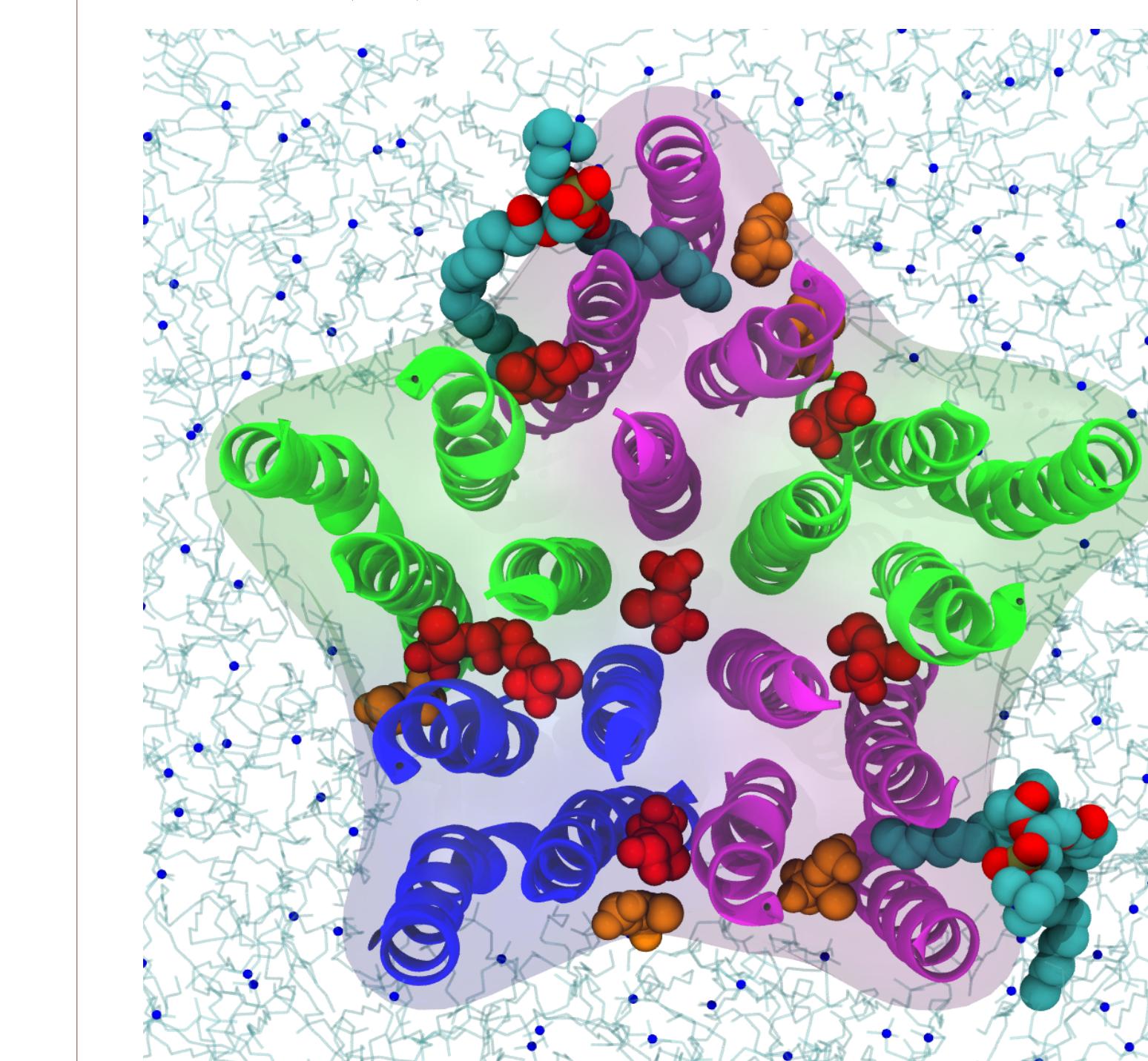


FIG 9: ISO - Flooding simulation - Snap-shot of a view from EC domain, after 400ns of simulation. (Red)ISO are the molecules bound to intersubunit sites



FIG 10: ISO - Flooding simulation - Snap-shot of a view from EC domain, after 400ns of simulation. (Red)ISO are the molecules bound to intersubunit sites

Binding site	α/β^-	β/α^-	γ/β^-	α/γ^-
ΔG_{bind} (kcal/mol)	-7.5	-7/-6	-5	-5
Kd (μM)	3	7/40	200.0	200.0

SEV EC₅₀ = 300-1000 μM [10]

SUMMARY

- Any ligand facing the β^- side of the subunit seems to H-bond with $\beta L223$ backbone oxygen very persistently in case of PFL and relatively stronger in case of SEV.
- H-bonding favorability of the site confers affinity, in PFL.
- AFEP predicts binding specificity to subunit interfaces, with (strongest to weakest) PFL - $\gamma/\beta^- > \alpha/\beta^- > \alpha/\gamma^- > \beta+/a^-$; SEV - $\alpha/\beta^- > \beta+/a^- > \gamma/\beta^- > \alpha/\gamma^-$.
- Flooding simulations of SEV validates the binding sites justified through FEP calculations, in addition to identifying a β -intrasubunit site and multiple occupancy of high affinity sites.
- Flooding simulation of ISO identifies binding at all the intersubunit sites and an EC sites at the α/γ^- interface.

FUTURE WORK

PROPOFOL:

- Identifying the key residues interactions at different intersubunit sites to explain the affinity values.

SEVOFLURANE:

- Identifying binding sites from the flooding simulations and performing FEP calculations;Understanding the binding pathway of sevoflurane from flooding simulations.

I索FLURANE:

- Performing FEP calculations for the binding sites identified through flooding.

REFERENCES

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