Theoretical Investigation of Selective Ligand Binding Mode of Galanin Receptors

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Table S1. Galanin Receptors homologous templates, identified via BLAST.

S. No	Description	Max score	Total score	Query Cover	E value	Identity (%)	PDB ID
	GalR1						
1	Chain A, Kappa opioid receptor	147	147	79%	1e-41	33.69%	6VI4_A
2	Chain A, Orexin receptor type 1	133	133	77%	2e-36	33.57%	6TOD_A
3	Chain A, Soluble cytochrome b562, Delta-type opioid receptor chimeric protein	138	138	80%	2e-37	33.68%	4N6H_A
4	Chain R, N-formyl peptide receptor 2	104	104	81%	1e-25	29.32%	6OMM_R
	GalR2						
1	Chain A, Soluble cytochrome b562, Delta-type opioid receptor chimeric protein	135	135	78%	3e-36	31.73%	4N6H_A
2	Chain A, Fusion protein of Nociceptin receptor and cytochrome b562	150	150	71%	2e-41	35.00%	4EA3_A
3	Chain R, C-C chemokine receptor type 6,C-C chemokine receptor type 6	115	115	83%	2e-28	25.51%	6WWZ_R
4	Chain A, Substance-P receptor	92.8	92.8	66%	4e-21	28.09%	2KS9_A
	GalR3						
1	Chain A, Kappa opioid receptor	134	134	81%	8e-37	29.41%	6VI4_A
2	Chain A, Soluble cytochrome b562, Delta-type opioid receptor chimeric protein	127	127	81%	2e-33	31.51%	4N6H_A
3	Chain A, Soluble cytochrome b562,Delta-type opioid receptor	127	127	79%	3e-33	30.79%	4RWA_A
4	Chain R, Muscarinic acetylcholine receptor M1	95.9	95.9	79%	3e-22	27.33%	6OIJ_R

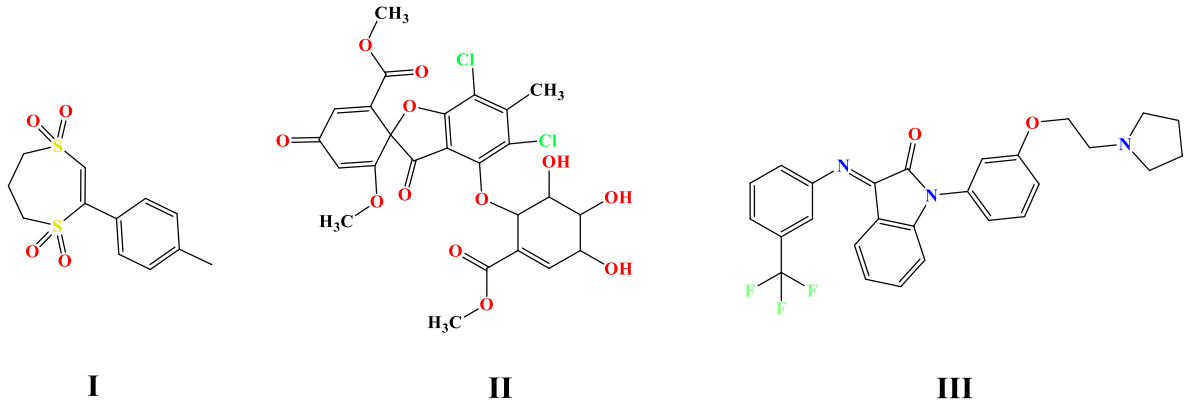

Figure S1. Structure of Galanin receptors ligands.

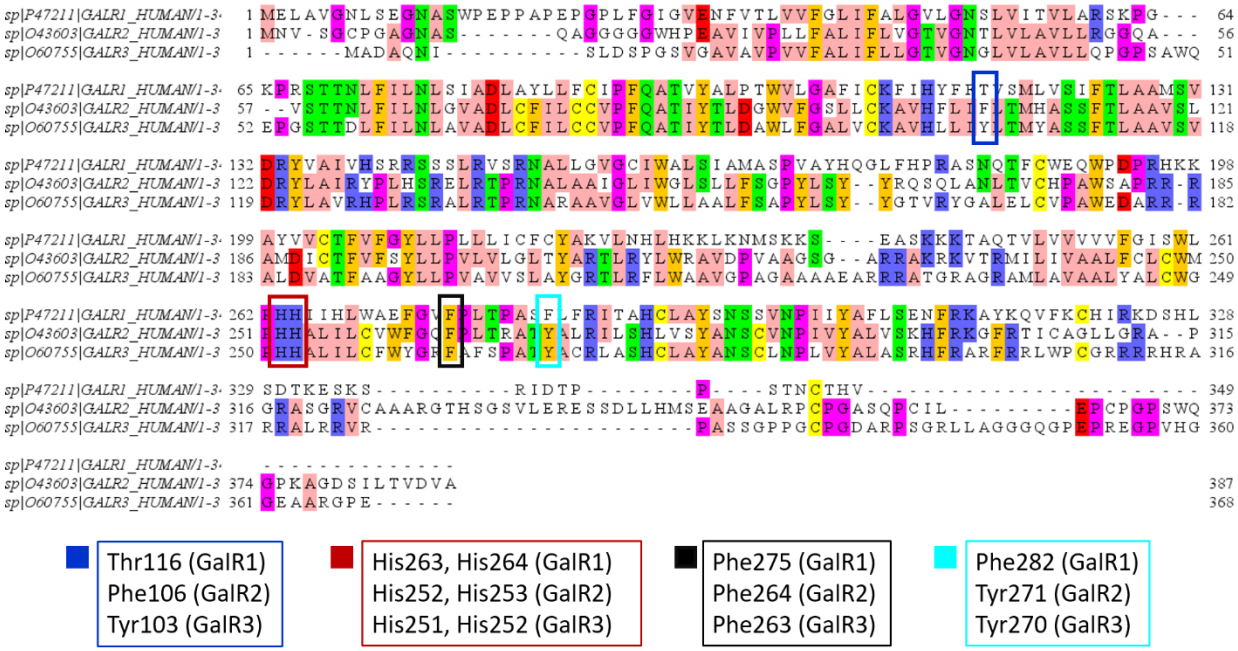

Figure S2. Sequence alignment of Galanin receptors. Similarity between the binding site of three receptors are annotated with the box of different colors.

The alignment of receptors sequence was achieved using ClustalW, and as expected, low sequence similarity was observed at the N and C terminus. However, transmembrane helices were perfectly align. A significant similarity between the binding site residues were observed, however inconsistency was also observed. For instance, Phe115 and Phe186 of GalR1 aligned to Ile105 and Val174 of GalR2 and Ile102 and Leu171 of GalR3, respectively. Similarly, Glu271 and His267 of

GalR1, were replaced by Tryptophan and Isoleucine residues in GalR2 and GalR3. These inconsistencies may account for the selectivity between the receptors.

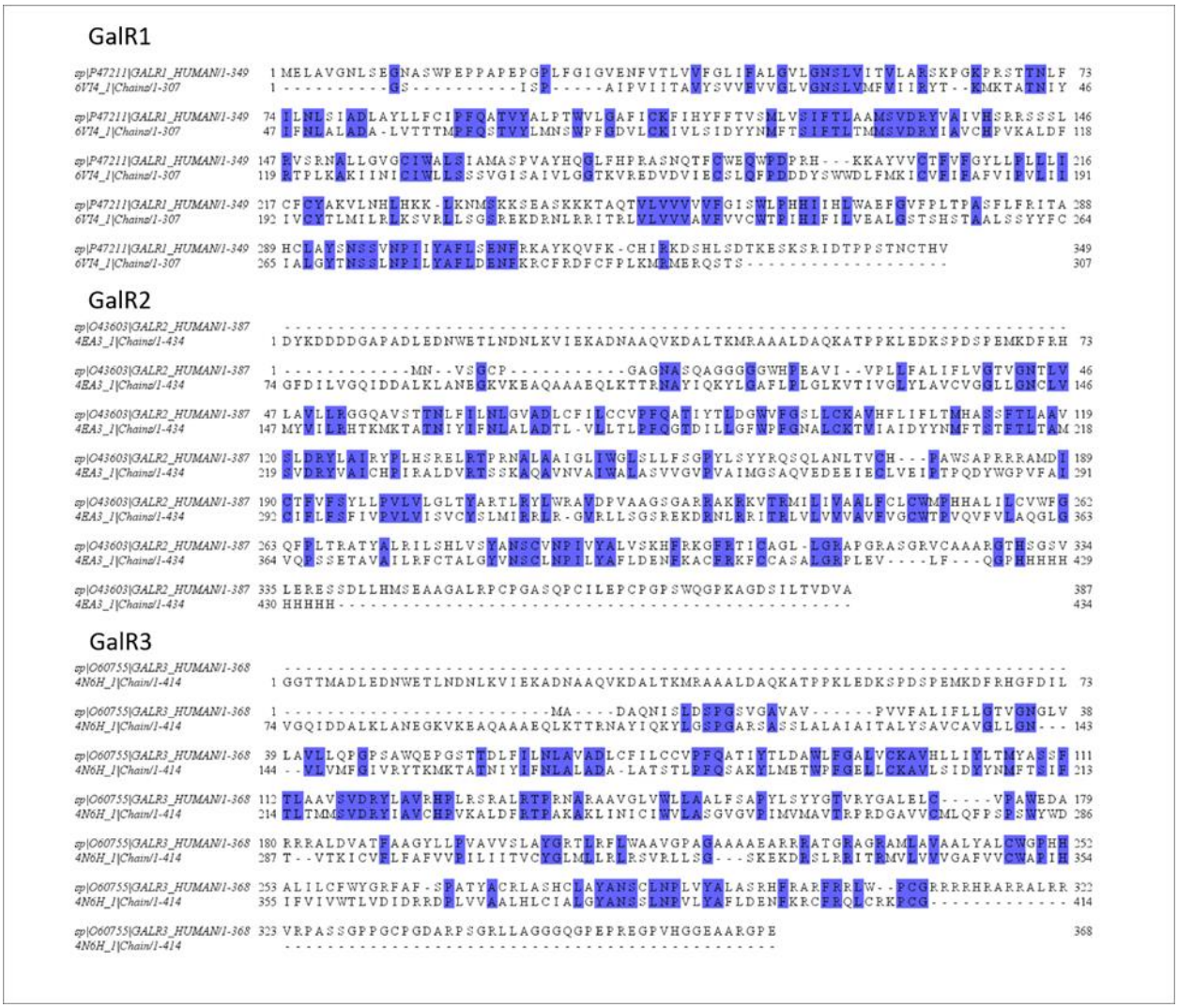

Figure S3. Sequence alignment of three receptors with the templates used for the construction of homology models. Low sequence identities were observed for the three receptors with their templates (33.6% for GalR1, 35.0% for GalR2 and 31.5% for GalR3).

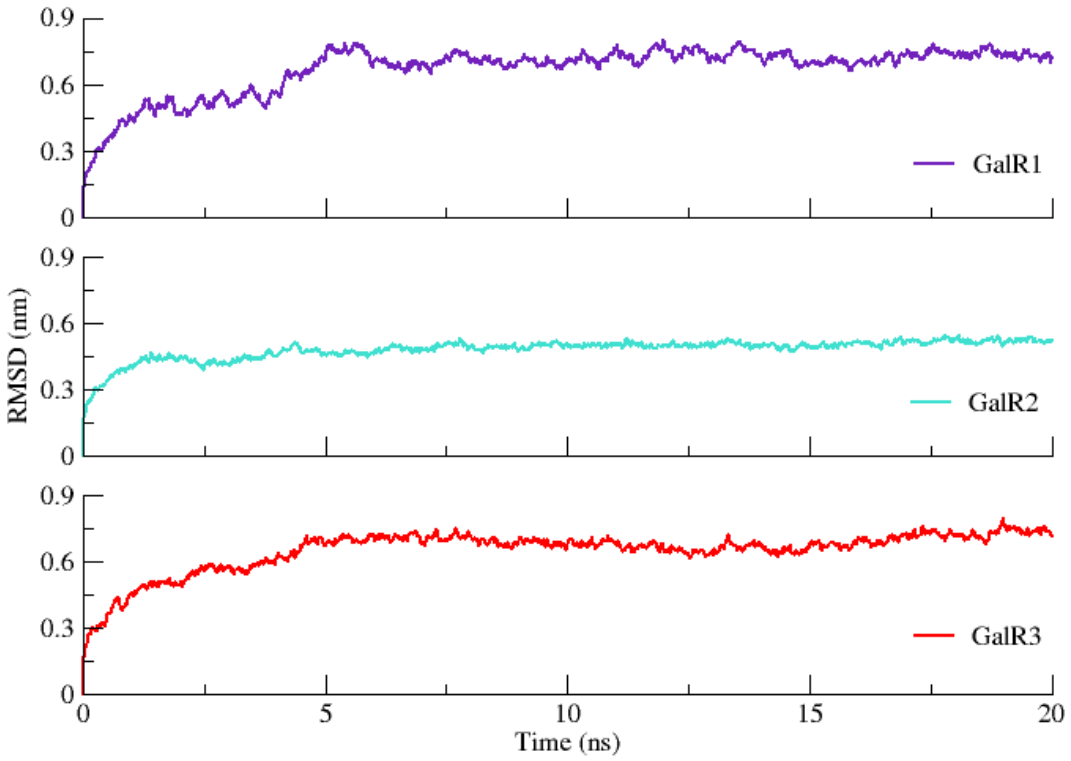

Figure S4. Root Mean Square Deviation of the generated homology models of the three receptors.

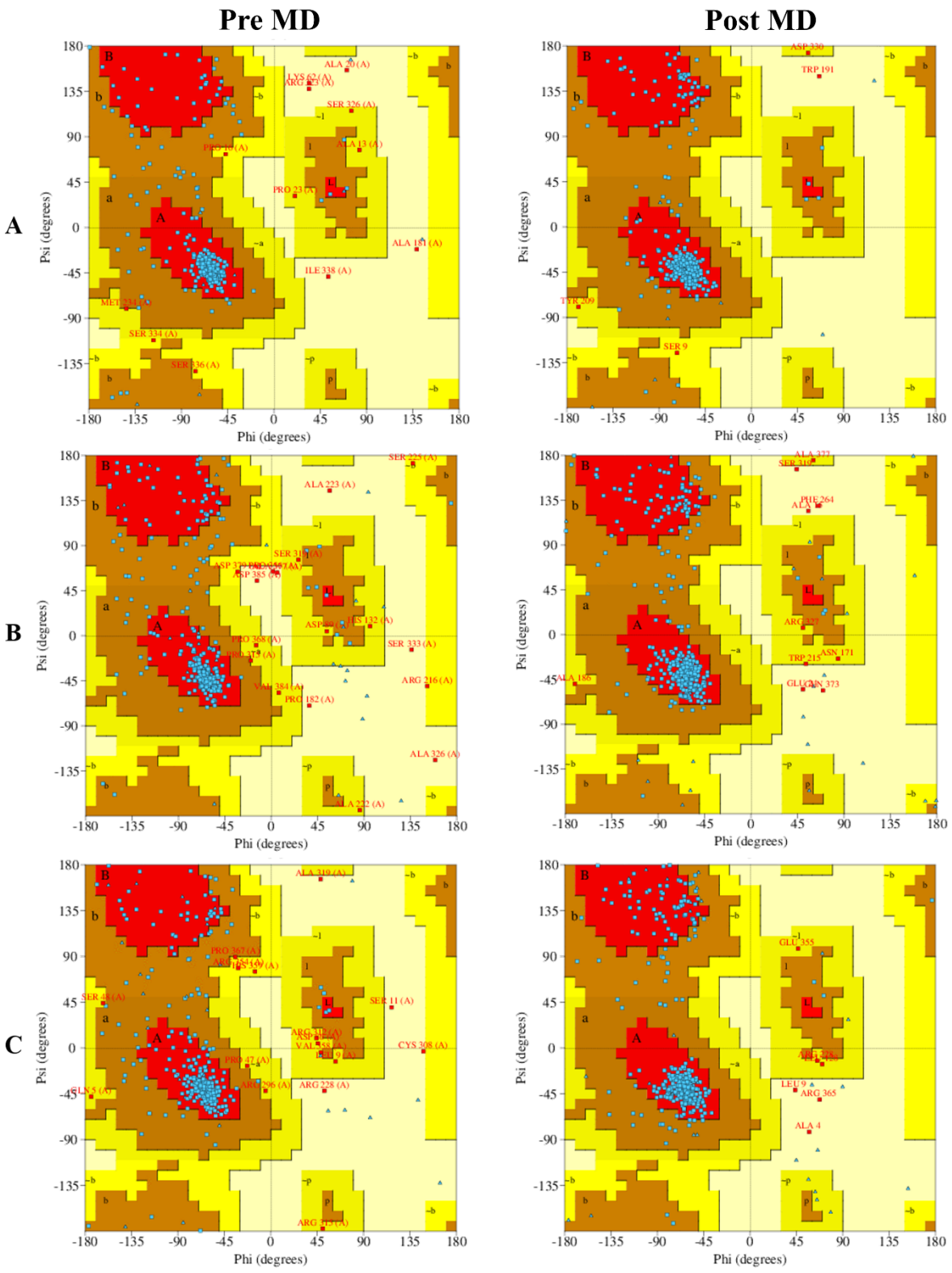

Figure S5. Ramachandran plots of Galanin receptors before and after minimization by MD. **A)** GalR1, **B)** GalR2 and **C)** GalR3.

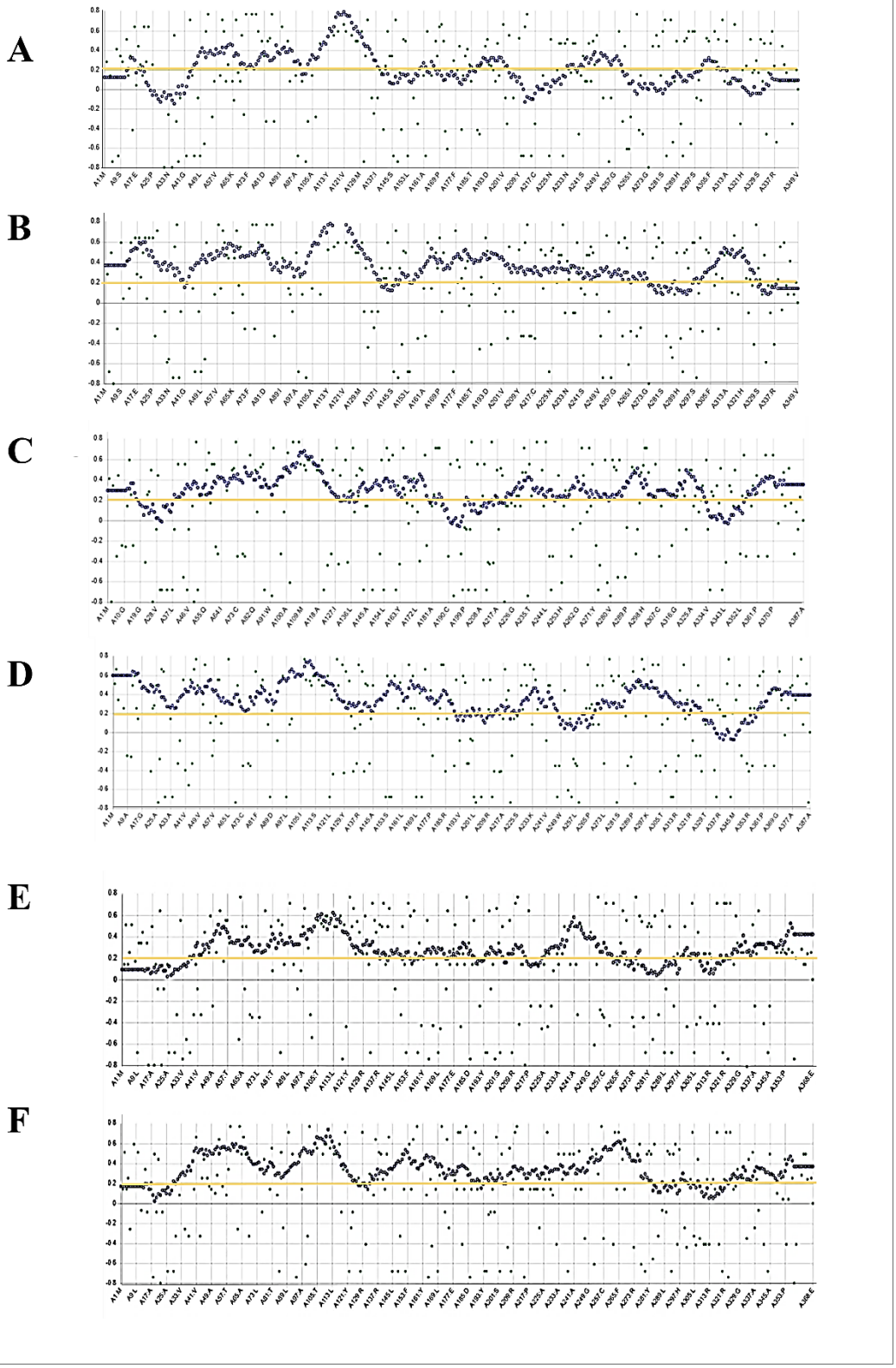

Figure S6 Verify 3D plot of Galanin receptors. **A)** GalR1 pre-MD, **B)** GalR1 post-MD, **C)** GalR2 pre-MD, **D)** GalR2 post-MD, **E)** GalR3 pre-MD and **F)** GalR3 post-MD.

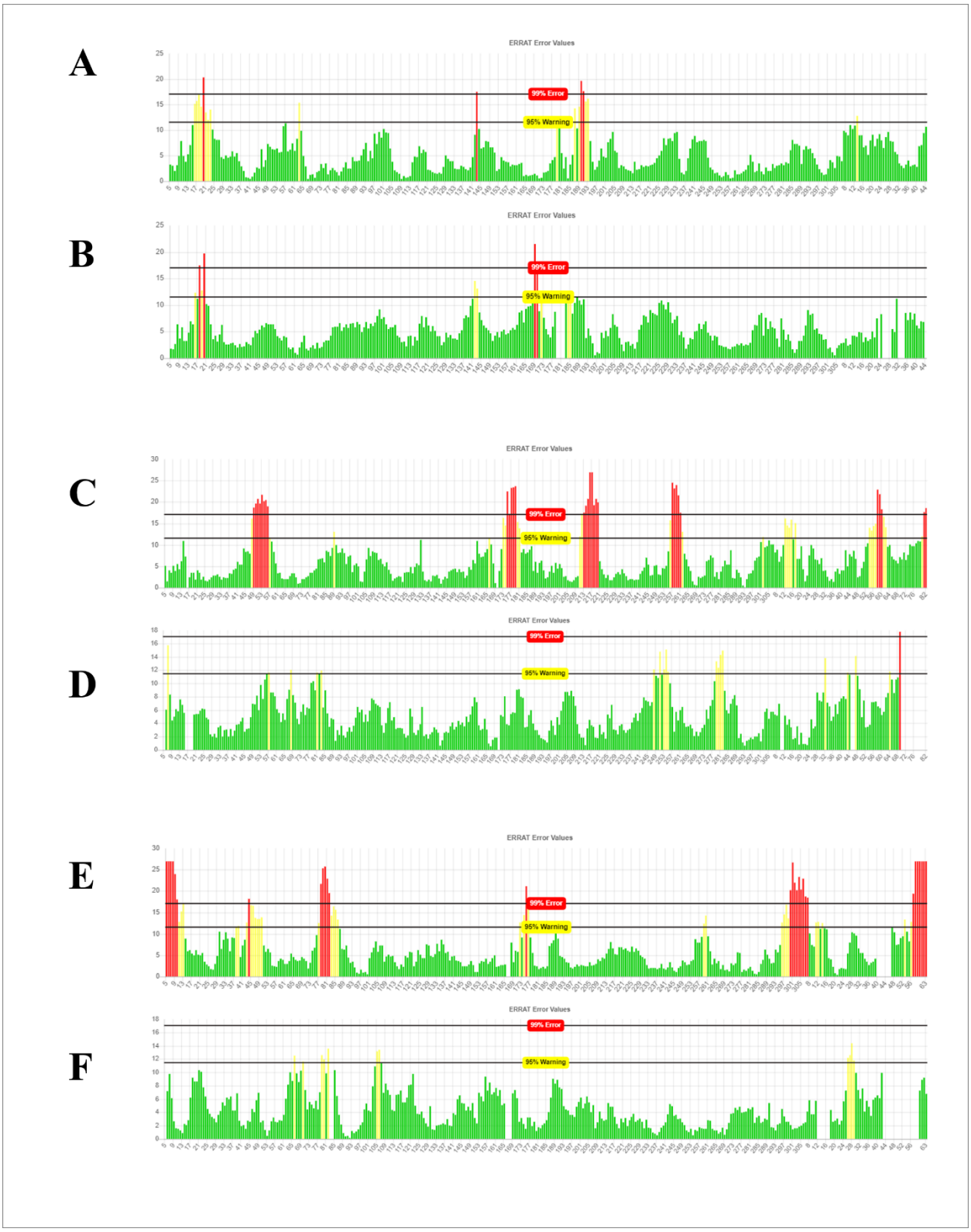

Figure S7. ERRAT plot of Galanin receptors. **A)** GalR1 pre-MD, **B)** GalR1 post-MD, **C)** GalR2 pre-MD, **D)** GalR2 post-MD, **E)** GalR3 pre-MD and **F)** GalR3 post-MD.

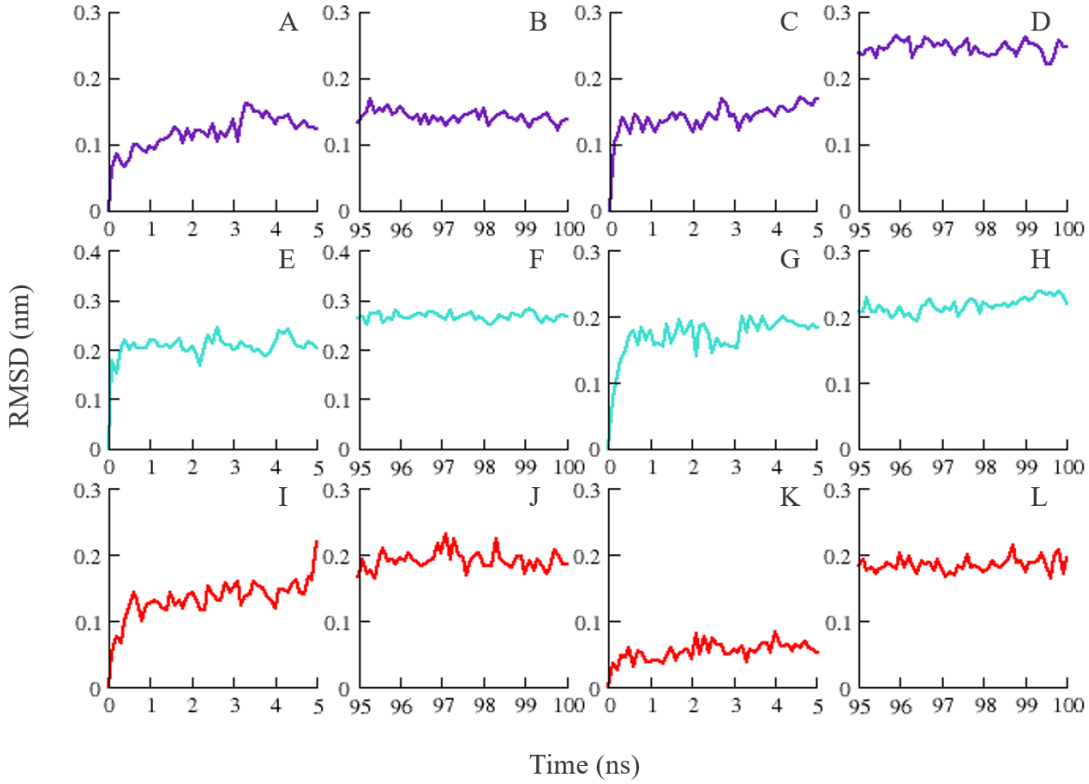

Figure S8. Root Mean Square Deviation of GalR1 (Violet), GalR2 (Cyan), and GalR3 (Red) in complex with compound I and III during initial 5ns (A, C, E, G, I, K) and final 5ns (B, D, F, H, J, L).

Table S2. Docking score and values of different energy components after docking.

Receptor	GalR1		GalR2		GalR3	
Ligand ID	**I**	**III**	**I**	**III**	**I**	**III**
Score (kcal/mol)	-8.7	-6.4	-7.1	-10.4	-7.2	-9.0
Vander wal Energy (kcal/mol)	11.1	13.1	3.12	13.0	11.9	3.6
Electrostatic Energy (kcal/mol)	-0.9	29.7	-0.4	31.0	30.6	0.05
Total Energy (kcal/mol)	-178271.7	-144996.6	-78338.9	-149945.9	-78338.9	-116576.7
Torsion Energy (kcal/mol)	107.4	30.01	105.0	29.3	107.4	17.0

Parameter file for Compound I

;; Generated by CHARMM-GUI (http://www.charmm-gui.org)

;;

;; psf2itp.py

;;

;; Correspondance:

;; jul316@lehigh.edu or wonpil@lehigh.edu

;;

;; GROMACS topology file for HETA

;;

[moleculetype]

; name nrexcl

HETA 3

[atoms]

; nr type resnr residu atom cgnr charge mass

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3 CG2R61 0 LIG C3 3 -0.116 12.0110 ; qtot -0.384

4 CG2R61 0 LIG C4 4 -0.115 12.0110 ; qtot -0.499

5 CG2R61 0 LIG C5 5 0.001 12.0110 ; qtot -0.498

6 CG2R61 0 LIG C6 6 -0.115 12.0110 ; qtot -0.613

7 CG2R61 0 LIG C7 7 -0.116 12.0110 ; qtot -0.729

8 CG2D1O 0 LIG C8 8 0.193 12.0110 ; qtot -0.536

9 CG2D1O 0 LIG C9 9 0.040 12.0110 ; qtot -0.496

10 SG3O2 0 LIG S10 10 0.264 32.0600 ; qtot -0.232

11 OG2P1 0 LIG O11 11 -0.375 15.9994 ; qtot -0.607

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   16     SG3O2     0     LIG    S16     16      0.265    32.0600   ; qtot -0.685
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Parameter file for Compound III

;; Generated by CHARMM-GUI (http://www.charmm-gui.org)

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;; psf2itp.py

;;

;; Correspondance:

;; jul316@lehigh.edu or wonpil@lehigh.edu

;;

;; GROMACS topology file for HETA

;;

[moleculetype]

; name nrexcl

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; nr type resnr residu atom cgnr charge mass

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3 CG2R61 0 LIG C3 3 -0.116 12.0110 ; qtot -0.384

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5 CG2R61 0 LIG C5 5 0.001 12.0110 ; qtot -0.498

6 CG2R61 0 LIG C6 6 -0.115 12.0110 ; qtot -0.613

7 CG2R61 0 LIG C7 7 -0.116 12.0110 ; qtot -0.729

8 CG2D1O 0 LIG C8 8 0.193 12.0110 ; qtot -0.536

9 CG2D1O 0 LIG C9 9 0.040 12.0110 ; qtot -0.496

10 SG3O2 0 LIG S10 10 0.264 32.0600 ; qtot -0.232

11 OG2P1 0 LIG O11 11 -0.375 15.9994 ; qtot -0.607

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15 14 13 27 9

15 14 13 28 9

13 14 15 16 9

13 14 15 31 9

13 14 15 32 9

29 14 15 31 9

29 14 15 32 9

30 14 15 31 9

30 14 15 32 9

16 15 14 29 9

16 15 14 30 9

14 15 16 17 9

14 15 16 18 9

8 16 15 14 9

8 16 15 31 9

```
  8   16   15   32    9
 17   16   15   31    9
 17   16   15   32    9
 18   16   15   31    9
 18   16   15   32    9

[ dihedrals ]
; ai       aj        ak        al        funct    q0        cq
  8    9    5   16    2
  9    8   10   26    2

#ifdef REST_ON
#include "../restraints/HETA_rest.itp"
#endif
```