Supporting Information

Allosteric modulation mechanism of the mGluR$_5$ transmembrane domain

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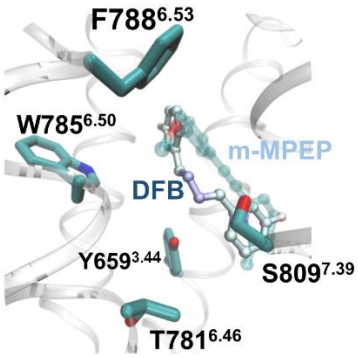

Figure S1. Initial binding mode of DFB obtained from docking. The position of m-MPEP is also shown (transparent) for comparison.

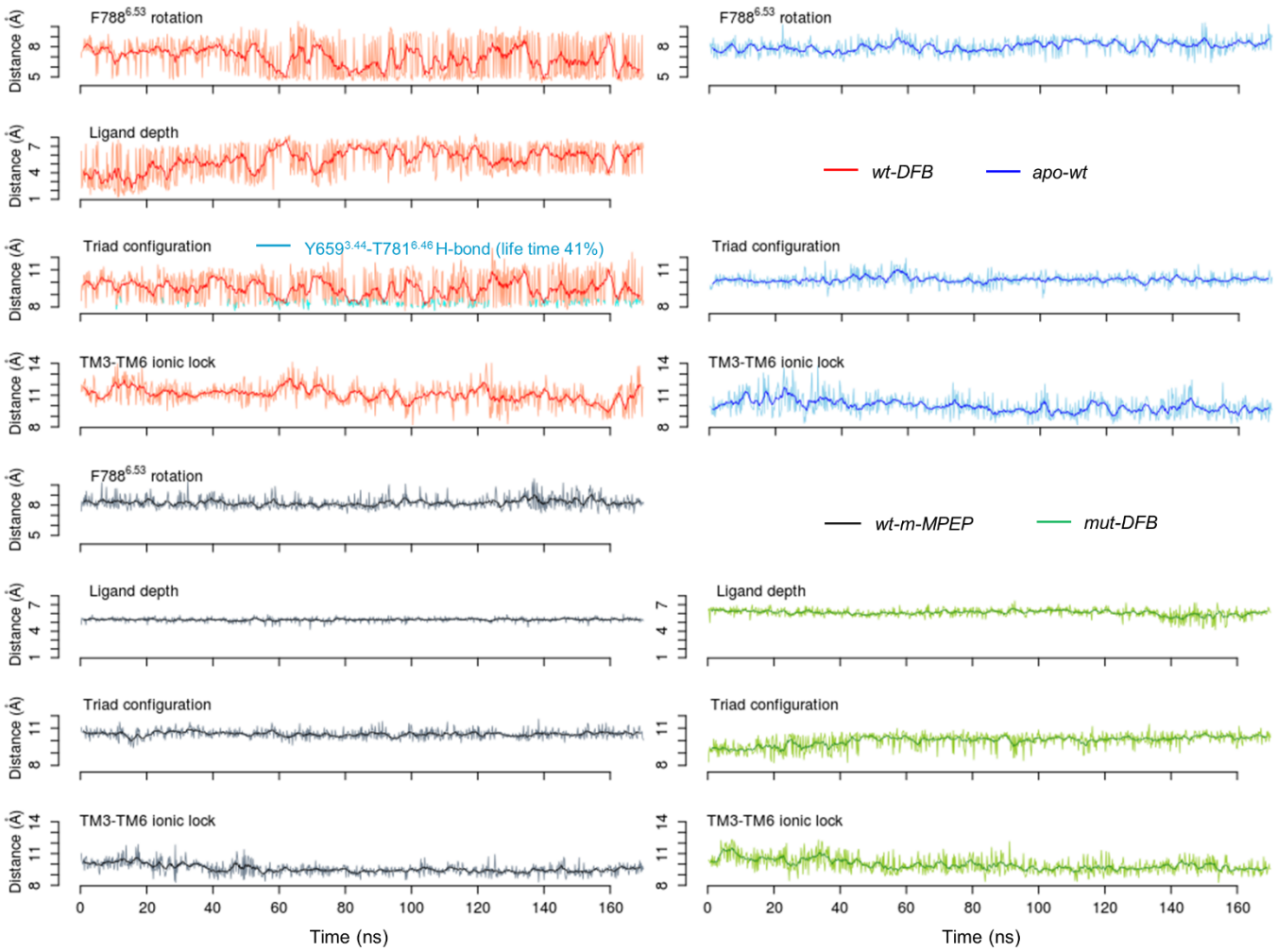

Figure S2. Time series plots of the rotation of F788$^{6.53}$ (as measured by the distance between F788$^{6.53}$ side chain and V806$^{7.36}$ Cα), the ligand binding modes (as measured by the center-of-mass distance between the ligand and the triad Cα atoms), the triad configuration (as measured by the T781$^{6.46}$-Y659$^{3.44}$ Cα distance), and the TM3-TM6 ionic lock (as measured by the K665$^{3.50}$-E770$^{6.35}$ Cα distance). *wt-DFB* exhibited strong correlations between the F788$^{6.53}$ rotation, the alternation of the PAM binding mode and the reconfiguration of the triad, which were loosely coupled to the destabilization of the TM3-TM6 ionic lock. The reconfiguration of the triad also involved a new H-bond between T781$^{6.46}$ backbone and Y659$^{3.44}$ side chain (cyan).

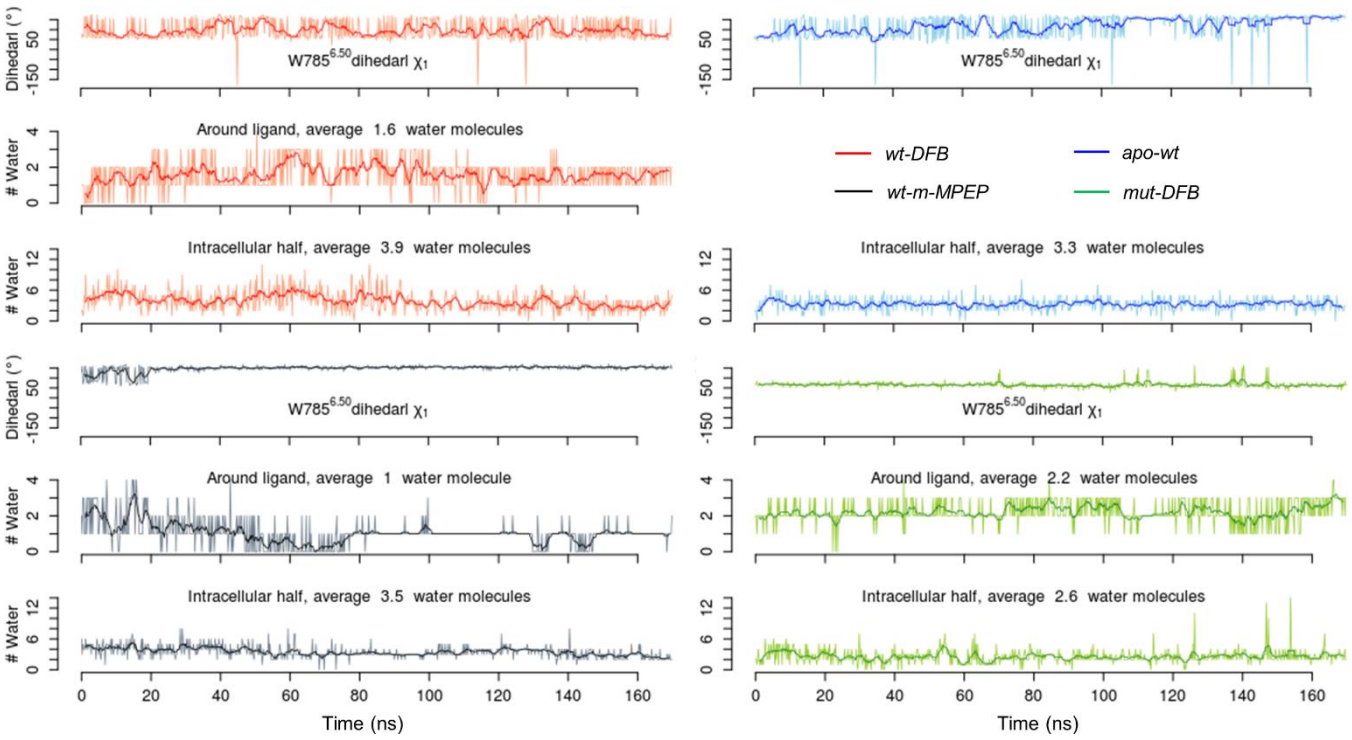

Figure S3. Time series plots showing correlations between the W785$^{6.50}$ dihedral angle χ_1 and the number of water molecules within the AM-pocket, which were loosely coupled to the hydration within the intracellular half of the 7TM.

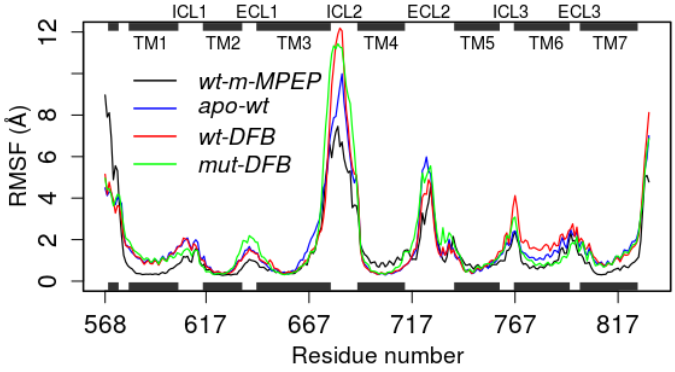

Figure S4. Cα RMSF of the four systems during the 170 ns of simulations.

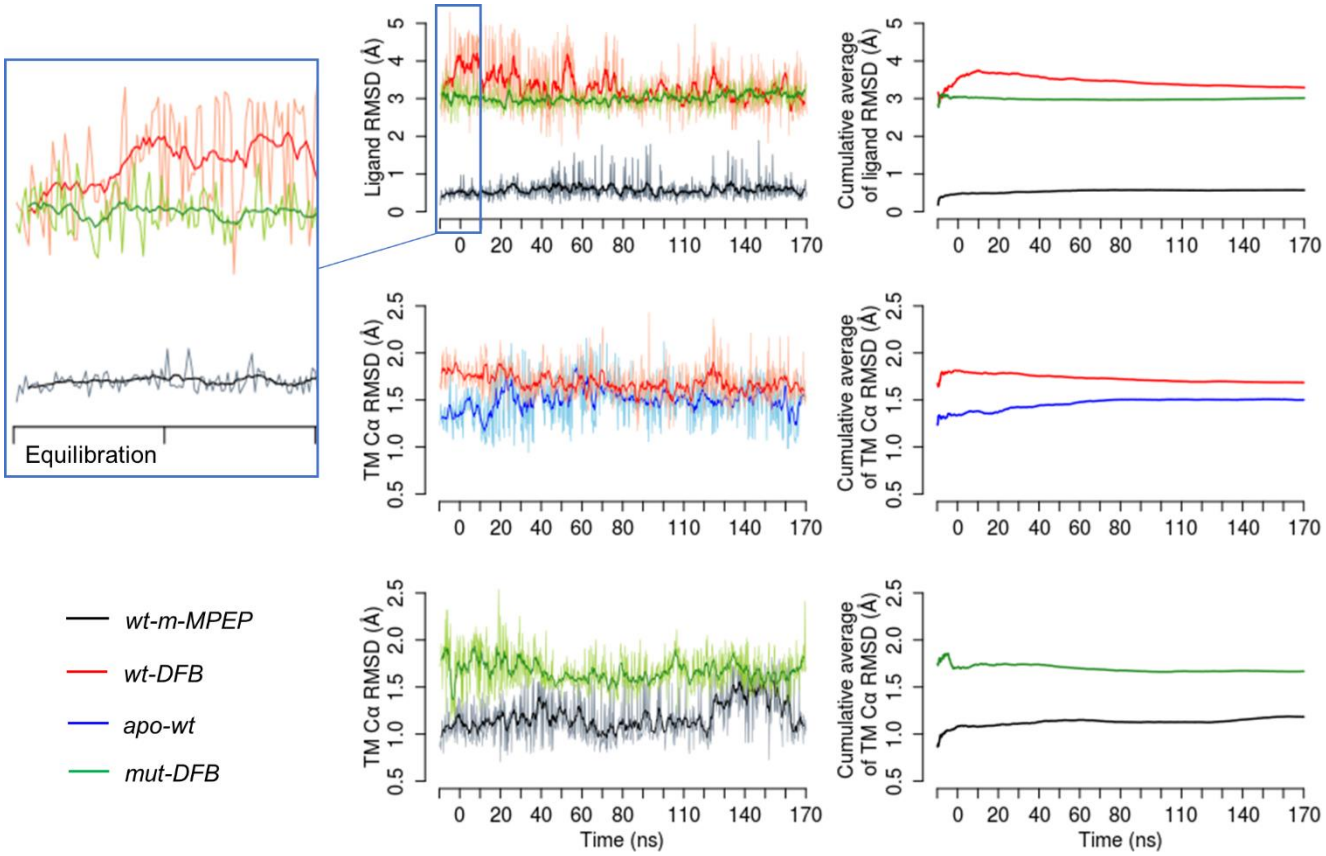

Figure S5. (Left) RMSD of the ligand heavy atoms and the TM domain Cα atoms during the equilibration and the production phases with respect to the initial structures. **(Right)** Cumulative average of the RMSDs showing convergence of the simulations.

Table S1. Number of contacts[a] between the intracellular loops, averaged over each simulation trajectory

	ICL1-ICL2	ICL1-ICL3	ICL2-ICL3	ECL1-ECL2	ECL1-ECL3	ECL2-ECL3
apo-wt	1±3	28±8	4±8	73±21	0	1±2
wt-m-MPEP	0	32±6	5±10	59±8	0	3±5
wt-DFB	1±5	18±14	7±19	67±17	0	1±2
mut-DFB	2±8	25±13	22±23	31±7	0	1±2

[a] Within 6 Å of distance. Multiple contacts with the same atom within one simulation frame were counted only once.

Supplementary Methods

VMD [1] was used to embed the 7TM models in a bilayer of POPC lipids. The system was solvated in a periodic 94 × 96 × 110 $Å^3$ periodic box of explicit water and neutralized with Cl^- ions. The final simulation systems consisted of ~63,000 atoms each, including ~12,800 water molecules, 154 POPC and 15 Cl^- ions.

Two cycles of energy minimization (5,000 steps of steepest descent and 5,000 steps of conjugate gradient) were performed. During the first cycle, 200 kcal·mol^{-1}·Å^{-2} positional restraints were applied on the protein (and the ligand when present). The system was then gradually heated to 310 K during 1 ns, with 200 kcal·mol^{-1}·Å^{-1} restraints on the protein atoms (and the ligand when present). Bonds involving hydrogen atoms were constrained using the LINCS algorithm, allowing for a 2-fs time step. Van der Waals and short-range electrostatic interactions were cut off at 8 Å. Long-range electrostatic interactions were computed using Particle Mesh Ewald summation (PME). Two phases of 5-ns equilibration were performed in the *NPT*-ensemble (P = 1 bar, T= 310 K), with then without 15 kcal·mol^{-1}·Å^{-1} restraints on the protein and the ligand. Semi-isotropic pressure coupling was applied using the Andersen-Parrinello-Rahman barostat [2, 3], allowing the simulation box in the *z*-axis (perpendicular to the lipid bilayer) to vary independently of the *x*-*y* plane. Temperature coupling was realized using the Nose-Hoover thermostat [4].

Supporting references

1. Humphrey, W.; Dalke, A.; Schulten, K., Vmd: Visual Molecular Dynamics. *J Mol Graph* **1996**, 14, 33-8, 27-8.

2. Parrinello, M.; Rahman, A., Polymorphic Transitions in Single Crystals: A New Molecular Dynamics Method. *J Appl Phys* **1981**, 52, 7182-7190.

3. Andersen, H. C., Molecular Dynamics Simulations at Constant Pressure and/or Temperature. *J Chem Phys* **1980**, 72, 2384-2393.

4. Nosé, S.; Klein, M. L., Constant Pressure Molecular Dynamics for Molecular Systems. *Mol Phys* **1983**, 50, 1055-1076.