Trans palmitoleic acid, a dairy fat biomarker, stimulates insulin secretion and activates G protein-coupled receptors with a different mechanism than *cis* isomer

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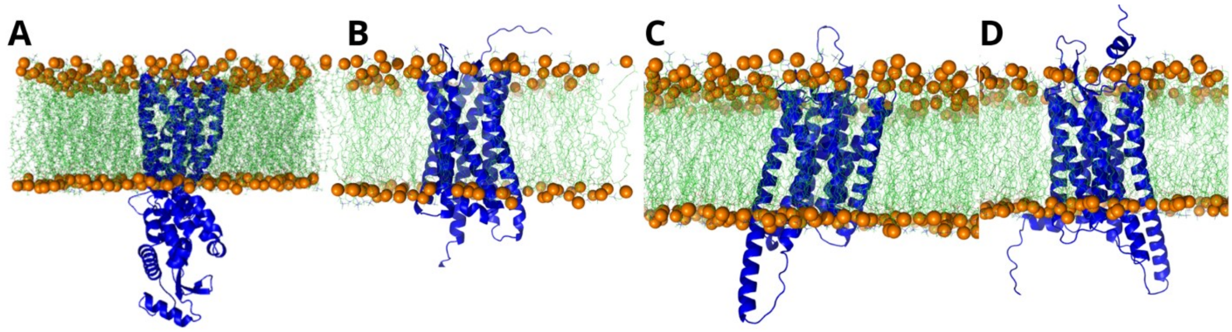
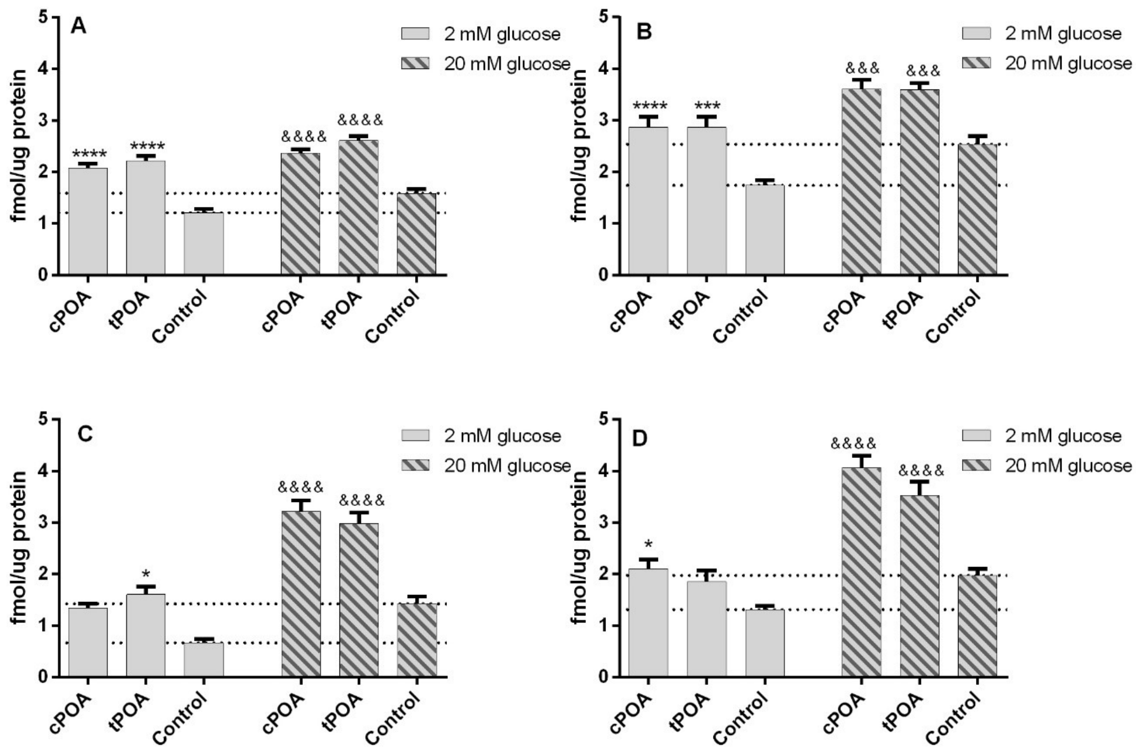
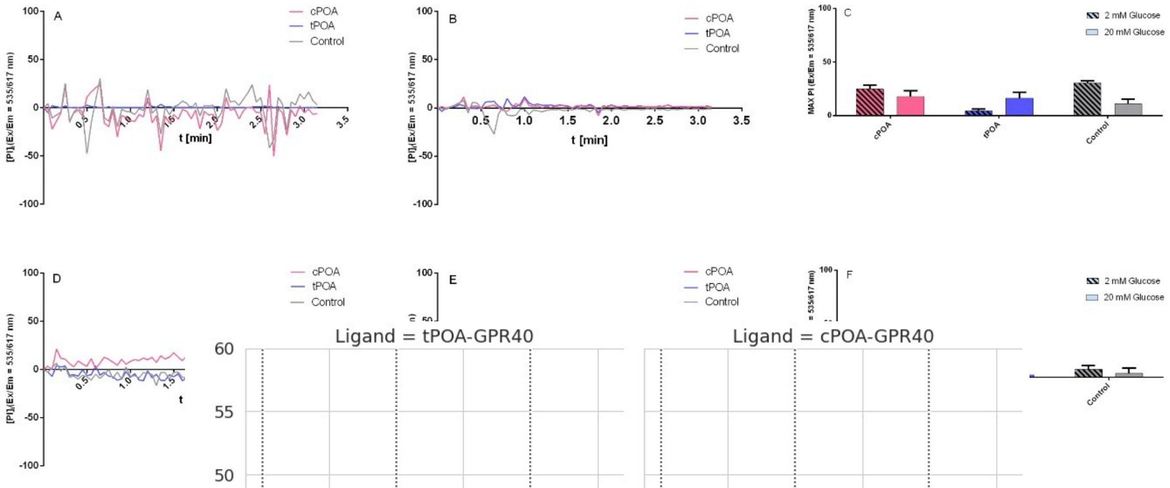
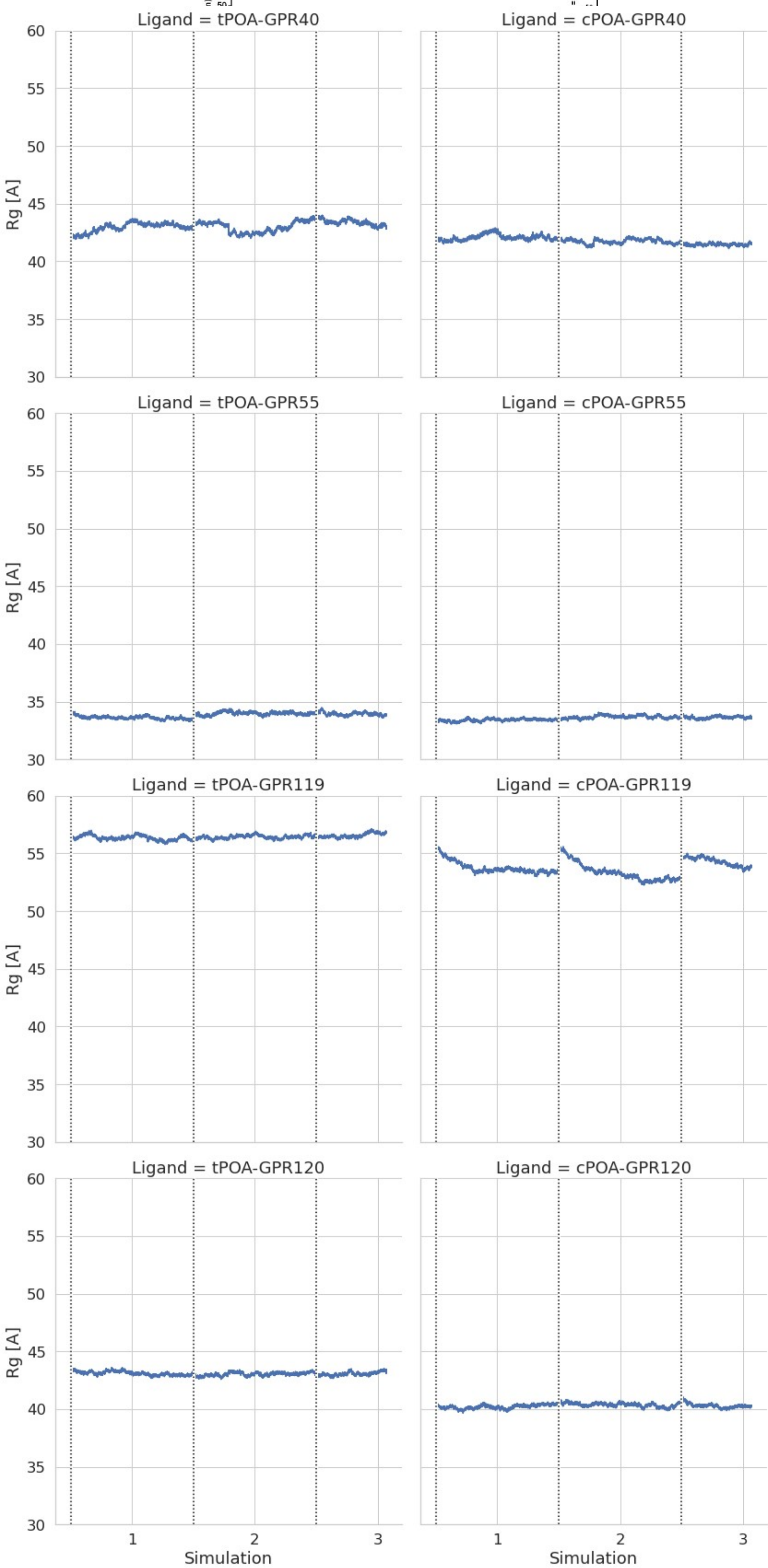


Supplemental Figure 1. Structures of GPR40 (A), GPR55 (B), GPR119 (C) and GPR120 (D) in 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphatidylcholine/1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphatidylglycerol/cholesterol membrane.

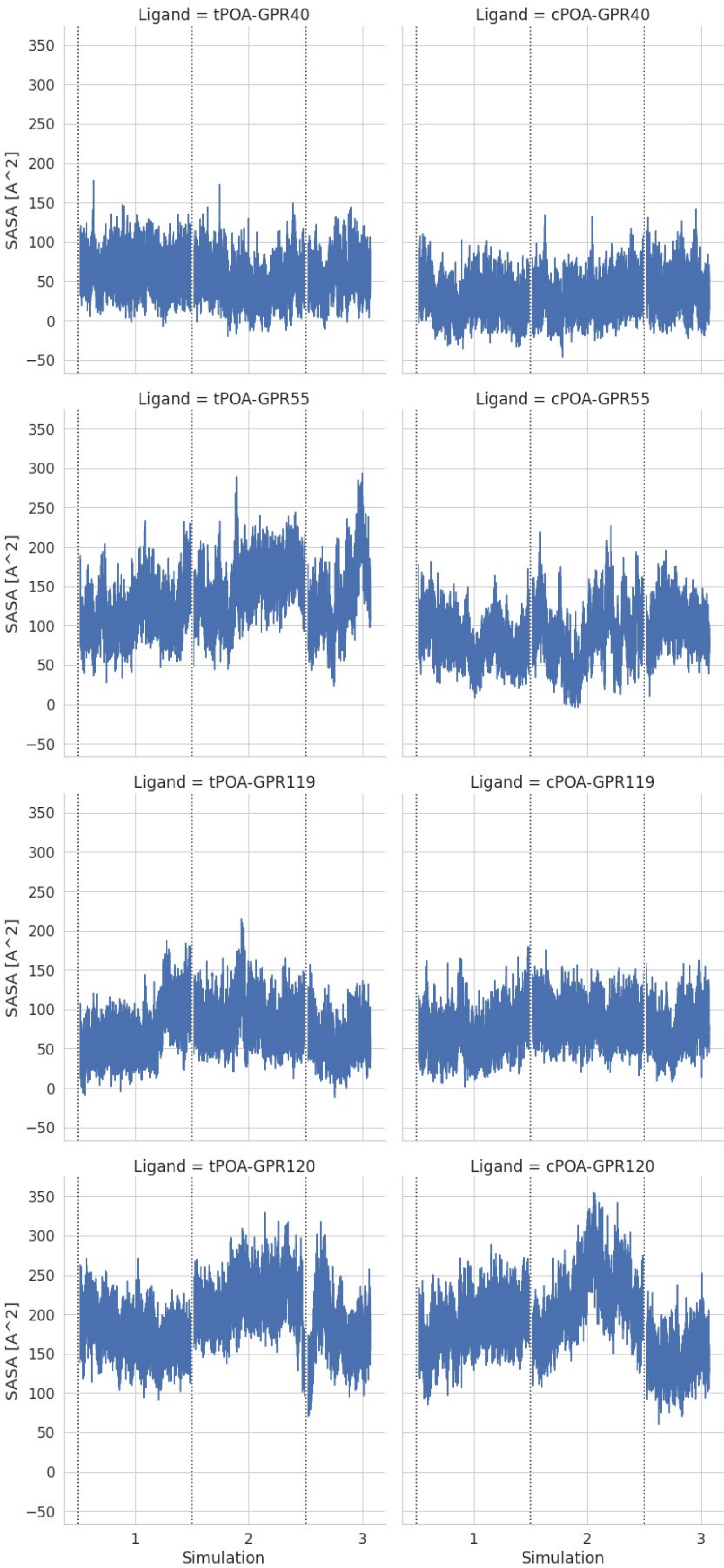


Supplemental Figure 2. Acute and long-term effect of c*is* and *trans* isomers of palmitoleic on GSIS. MIN6 cells were treated with cPOA and tPOA (25 µM) for 1,5 h (A) and 24 h (B). EndoC-βH1 cells were treated with cPOA and tPOA (10 µM) for 1,5 h (C) and 24 h (D). The bars represent the means ± SEM from at least 3 independent experiments. ****p < 0.0001, ***p < 0.00, 1*p<0.05 vs 2 mM glucose control; &&&&p < 0.0001, &&&p < 0.001, &&p<0.01 vs 20 mM glucose control.



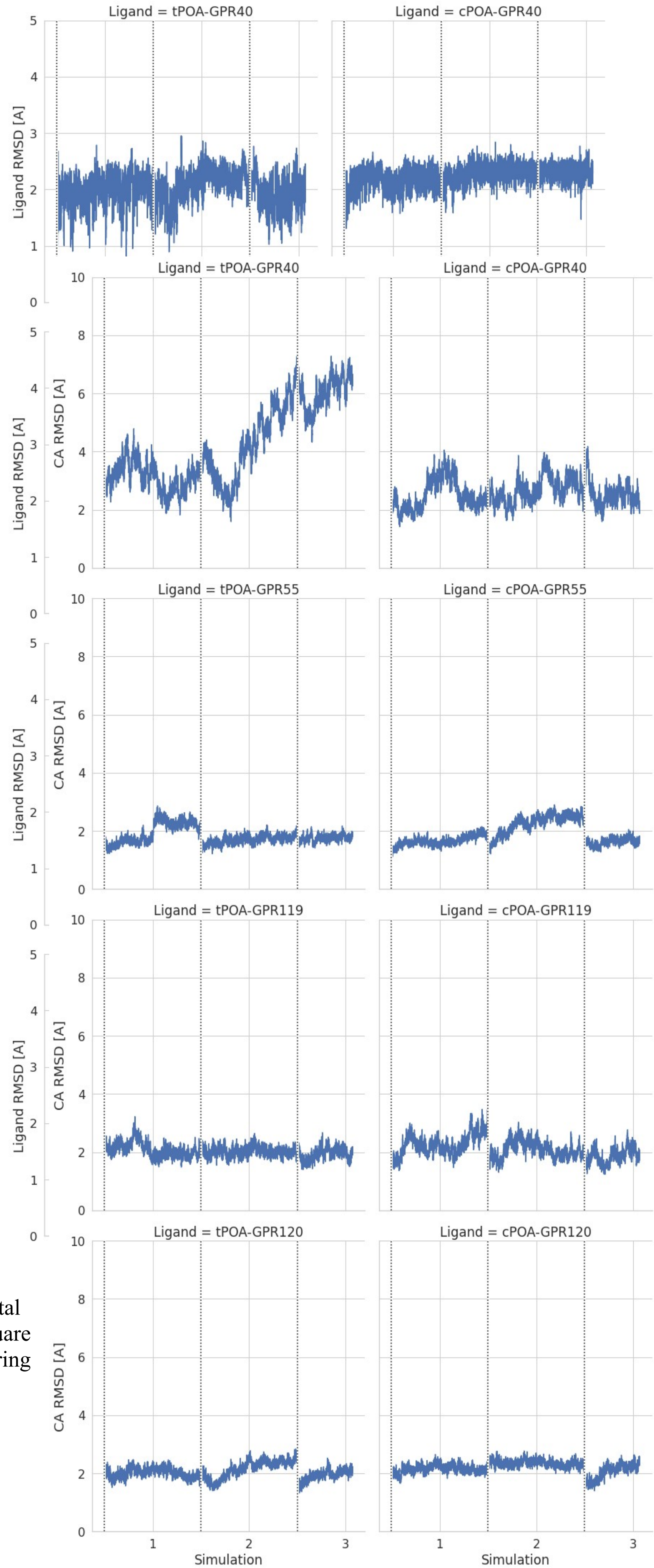


Supplemental Figure 3. Monitoring of membrane integrity via propidium iodide incorporation stimulated with 25 uM cPOA, tPOA or compound solvent in EndoC-betaH1 cells (A-C) and MIN6 cells (D-F) at 2 mM (A, D) and 20 mM (B, E) glucose conditions. The results are presented as the real-time kinetics of [PI] changes inside the cell during 3-min monitoring as well as MAX fluorescence. The bars represent the mean from 3 experiments ± SEM.



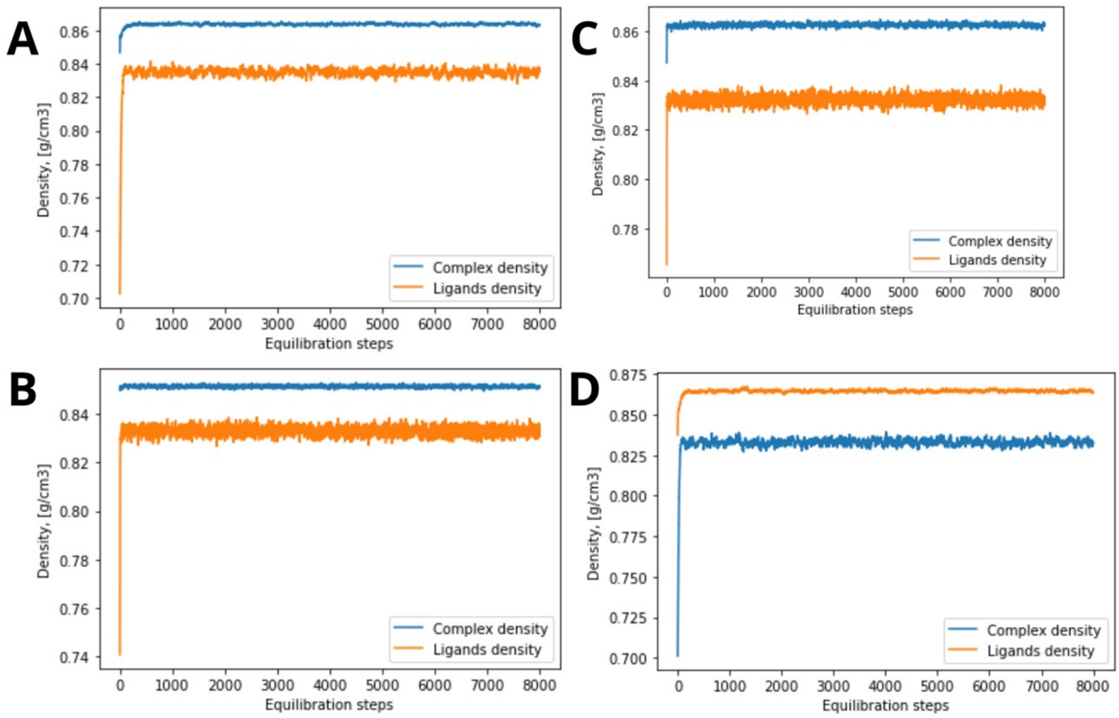
Supplemental Figure 4. Radius of gyration of receptors throughout each molecular dynamics simulation with respect to complexed ligand

Supplemental Figure 5. Solvent-accessible surface area of each complexed ligand during molecular dynamics simulations

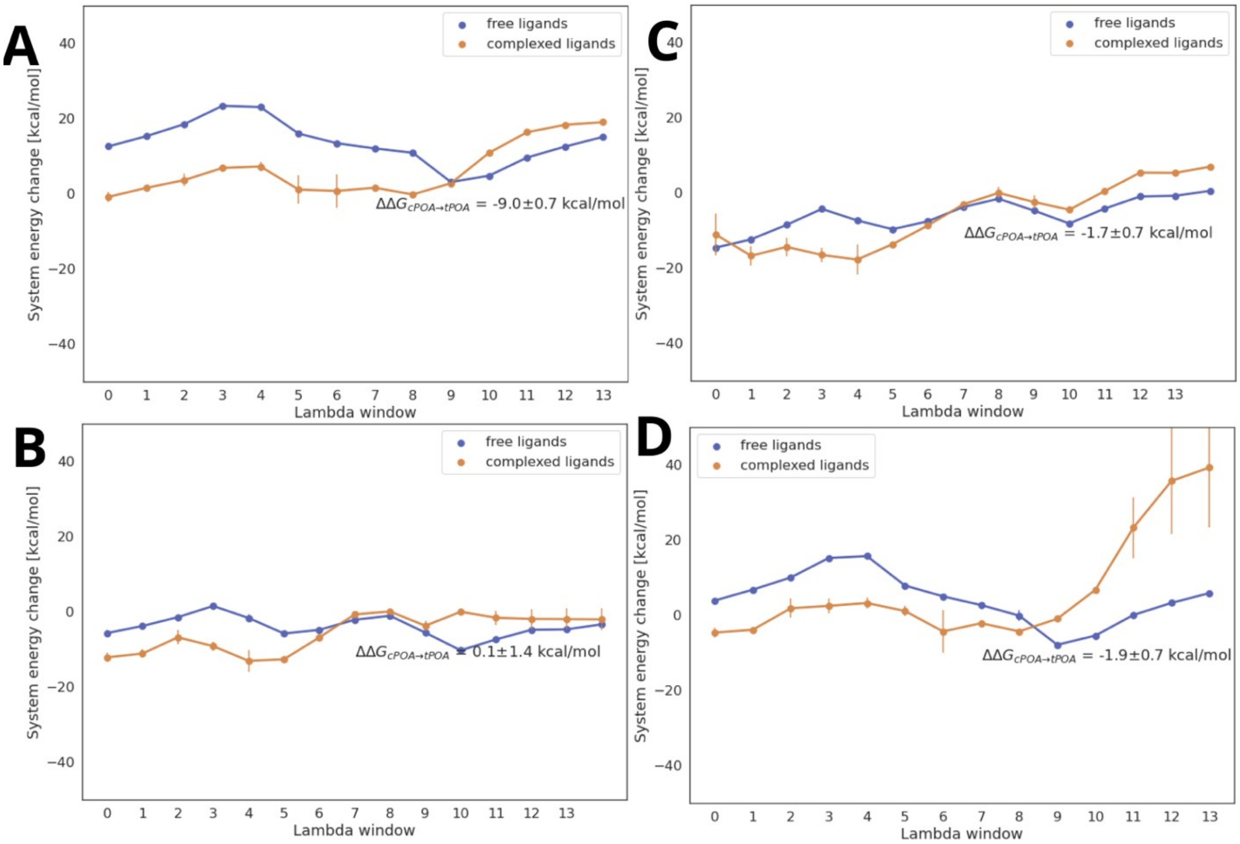


Supplemental Figure 6. Root mean square deviation of ligands during each molecular dynamics

Supplemental Figure 7. Root mean square deviation of protein Cα atoms of each receptor throughout molecular dynamics simulations



Supplemental Figure 8. Density of systems during equilibration procedure for thermodynamic integration



Supplemental Figure 9. System energy changes during cPOA-tPOA transformation. Total ΔΔG is obtained through trapezoid integration of the area under each curve, followed by calculating the difference between the complex state (complexed ligands, orange line) and protein-free state (free ligands, blue line). Each point represents the value from three repetitions and standard deviation. As one may notice, in each case, the energy of both systems was almost identical when the presence of tPOA was slightly higher than cPOA. (A) depicts GPR40, (B) GPR55, (C) GPR119 and (D) GPR120 systems.