

1 ***Trans* palmitoleic acid, a dairy fat biomarker, stimulates insulin secretion**  
2 **and activates G protein-coupled receptors with a different mechanism than**  
3 ***cis* isomer**  
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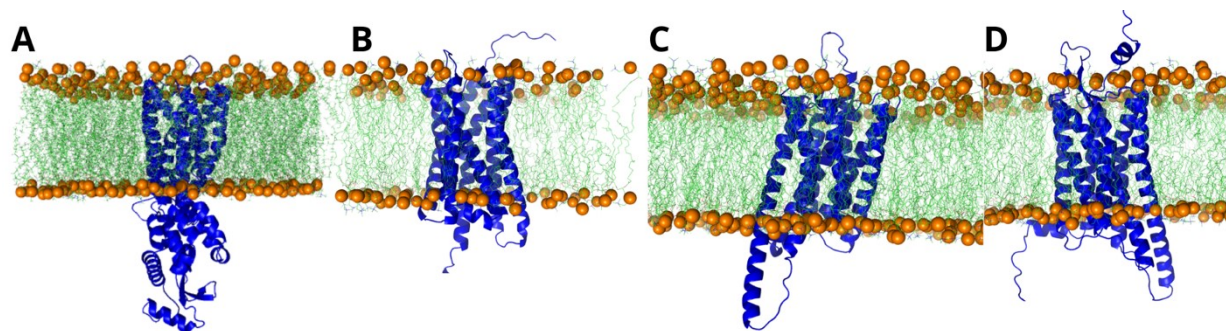
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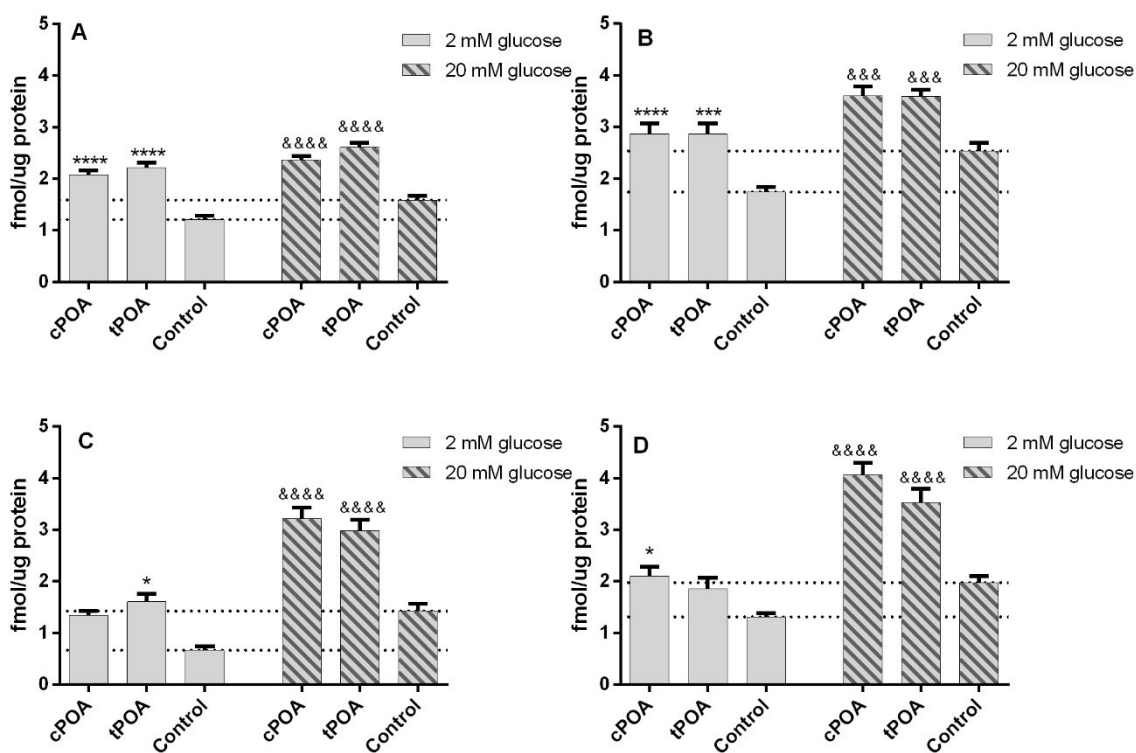
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26 Supplemental Figure 1. Structures of GPR40 (A), GPR55 (B), GPR119 (C) and GPR120 (D) in 1-  
 27 palmitoyl-2-oleoyl-*sn*-glycero-3-phosphatidylcholine/1-palmitoyl-2-oleoyl-*sn*-glycero-3-  
 28 phosphatidylglycerol/cholesterol membrane.

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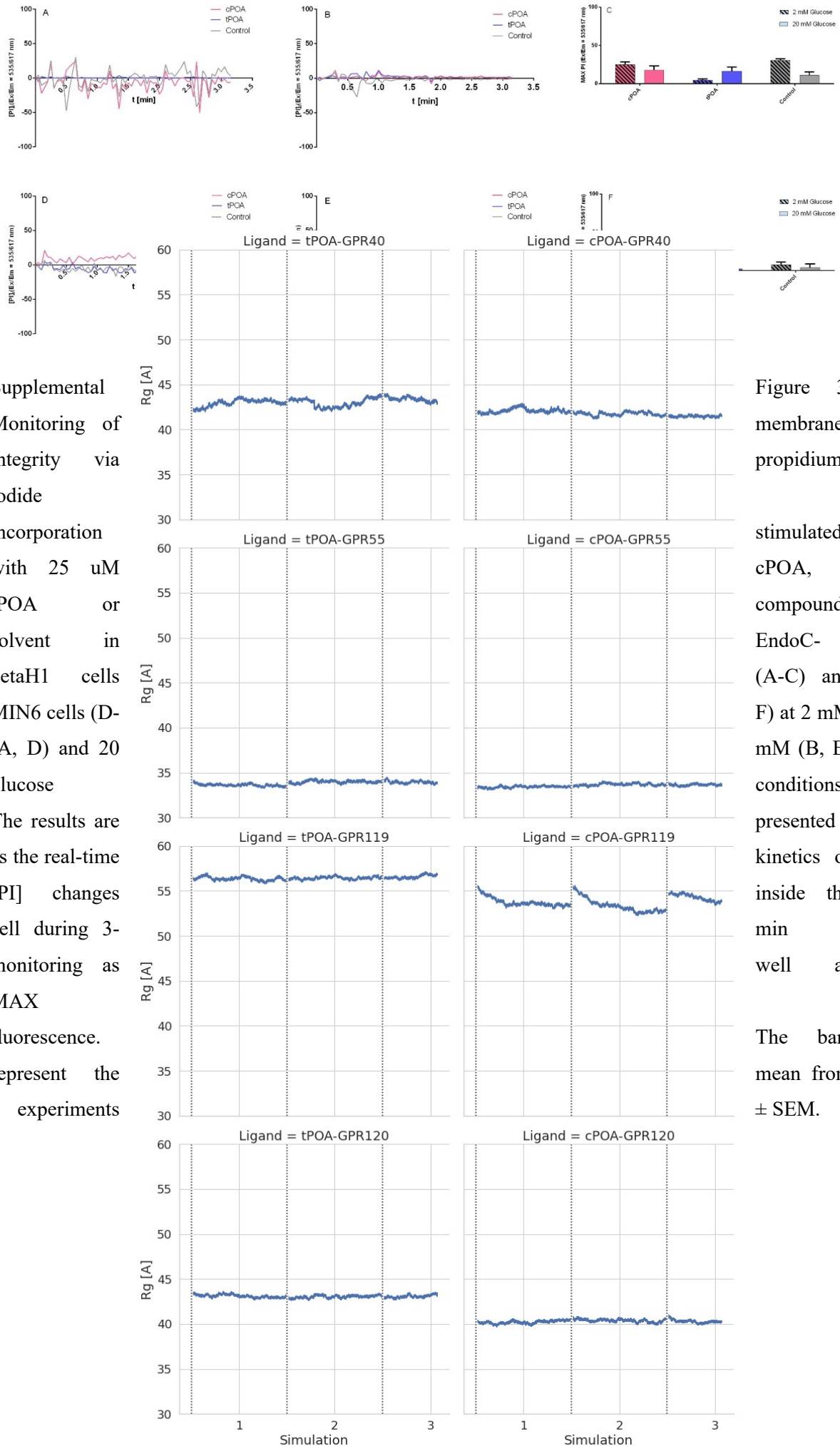
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31 Supplemental Figure 2. Acute and long-term effect of *cis* and *trans* isomers of palmitoleic on GSIS.  
 32 MIN6 cells were treated with cPOA and tPOA (25  $\mu$ M) for 1,5 h (A) and 24 h (B). EndoC- $\beta$ H1 cells  
 33 were treated with cPOA and tPOA (10  $\mu$ M) for 1,5 h (C) and 24 h (D). The bars represent the means  $\pm$   
 34 SEM from at least 3 independent experiments. \*\*\*\* $p$  < 0.0001, \*\*\* $p$  < 0.001, \* $p$  < 0.05 vs 2 mM glucose  
 35 control; &&&& $p$  < 0.0001, &&& $p$  < 0.001, && $p$  < 0.01 vs 20 mM glucose control.

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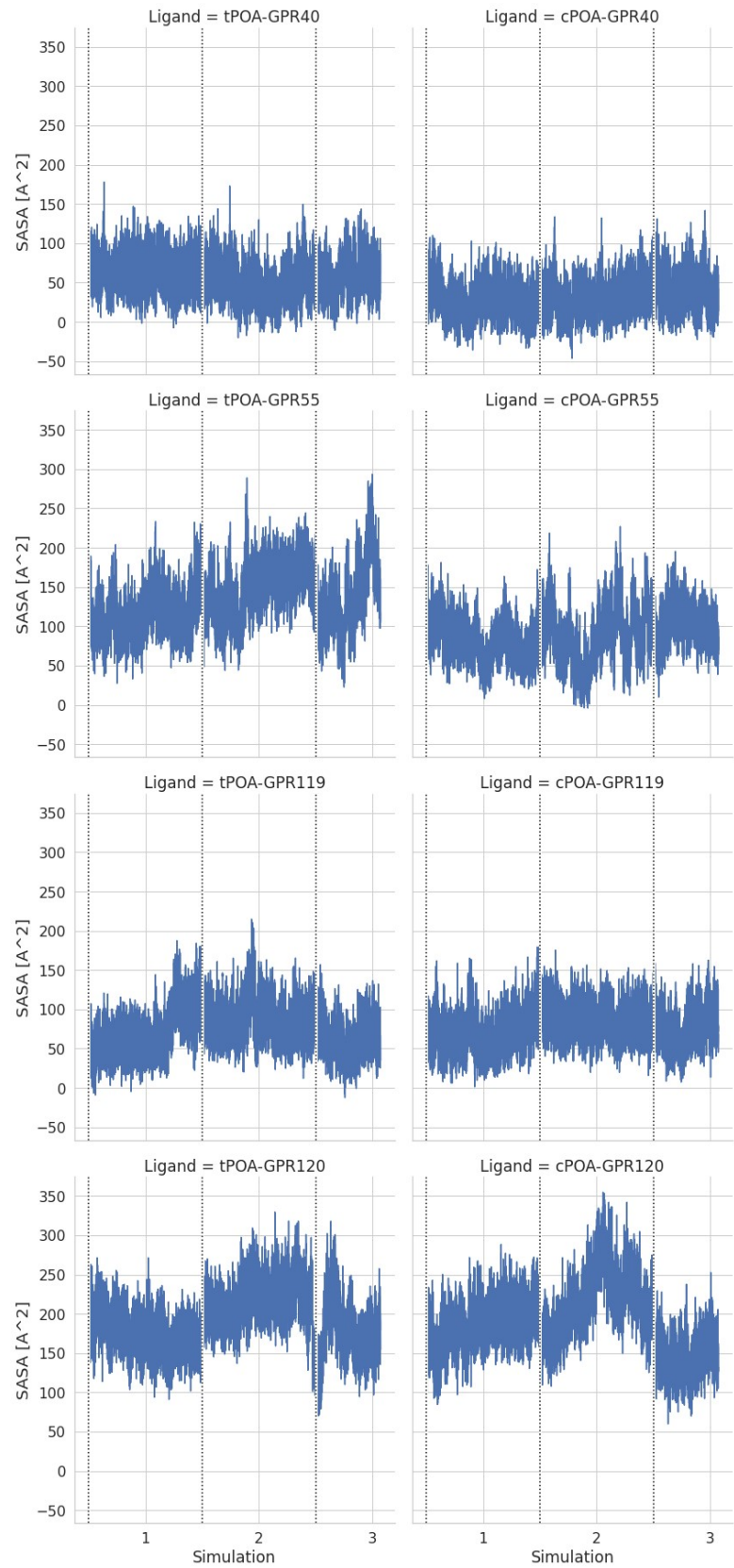
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 40 Supplemental  
 42 Monitoring of  
 44 integrity via  
 46 iodide  
 48 incorporation  
 50 with 25  $\mu$ M  
 52 tPOA or  
 54 solvent in  
 56 betaH1 cells  
 58 MIN6 cells (D-  
 60 (A, D) and 20  
 62 glucose  
 64 The results are  
 66 as the real-time  
 68 [PI] changes  
 70 cell during 3-  
 72 monitoring as  
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 78 3 experiments  
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Figure 3. membrane propidium stimulated cPOA, compound EndoC- (A-C) and F) at 2 mM mM (B, E) conditions. presented kinetics of inside the min well as The bars mean from  $\pm$  SEM.

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Supplemental Figure  
gyration of receptors  
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simulation with  
complexed ligand

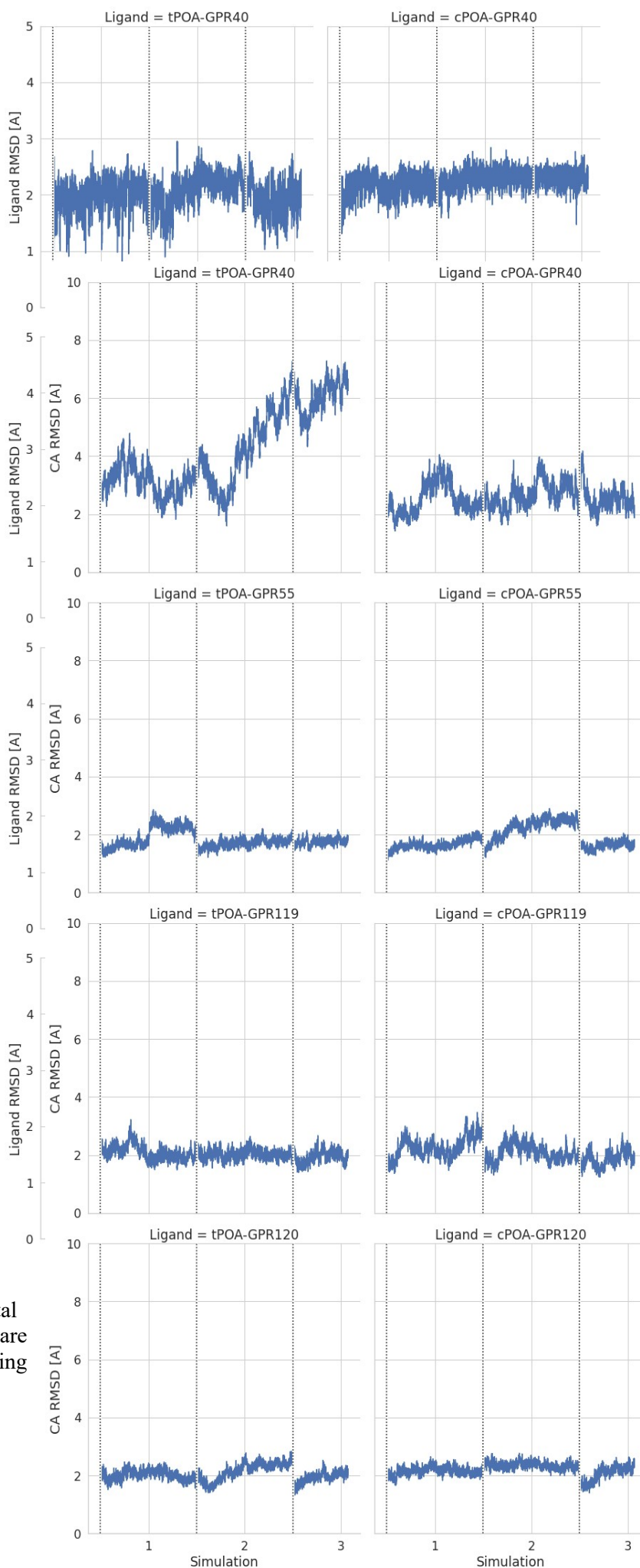
4. Radius of  
throughout  
dynamics  
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186 Supplemental Figure 5. Solvent-accessible surface area of each complexed ligand during molecular  
187 dynamics simulations

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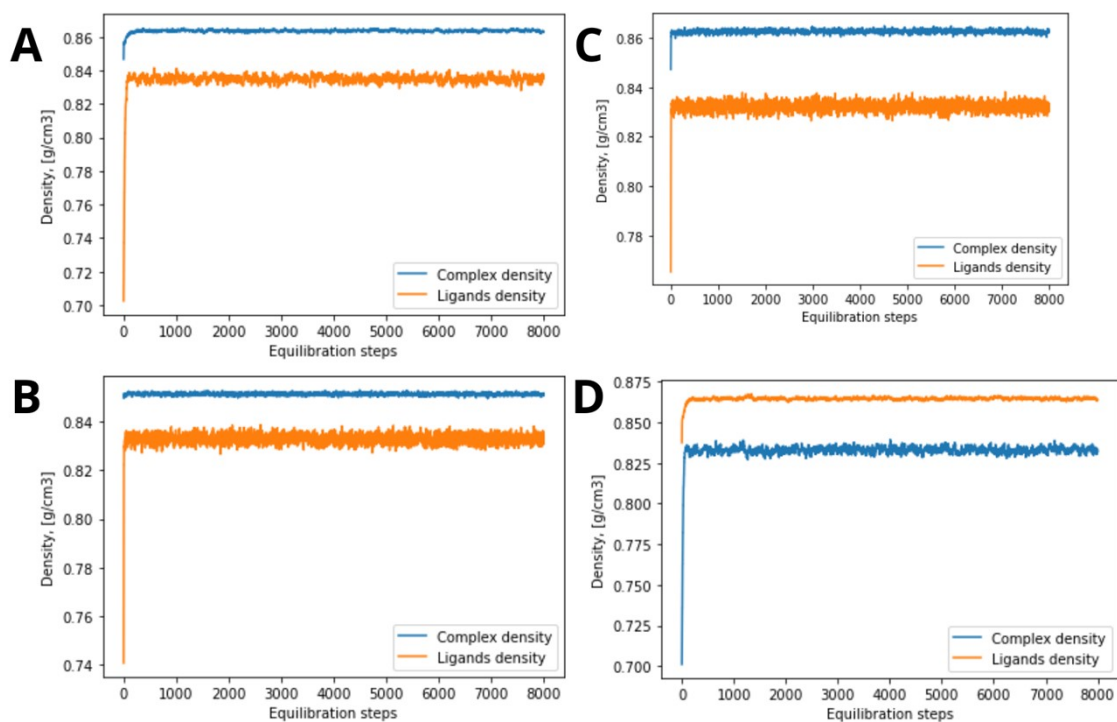


Supplemental  
 mean square  
 ligands during  
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Figure 6. Root  
 deviation of  
 each molecular

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292 Supplemental Figure 7. Root mean square deviation of protein C $\alpha$  atoms of each receptor throughout  
293 molecular dynamics simulations



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

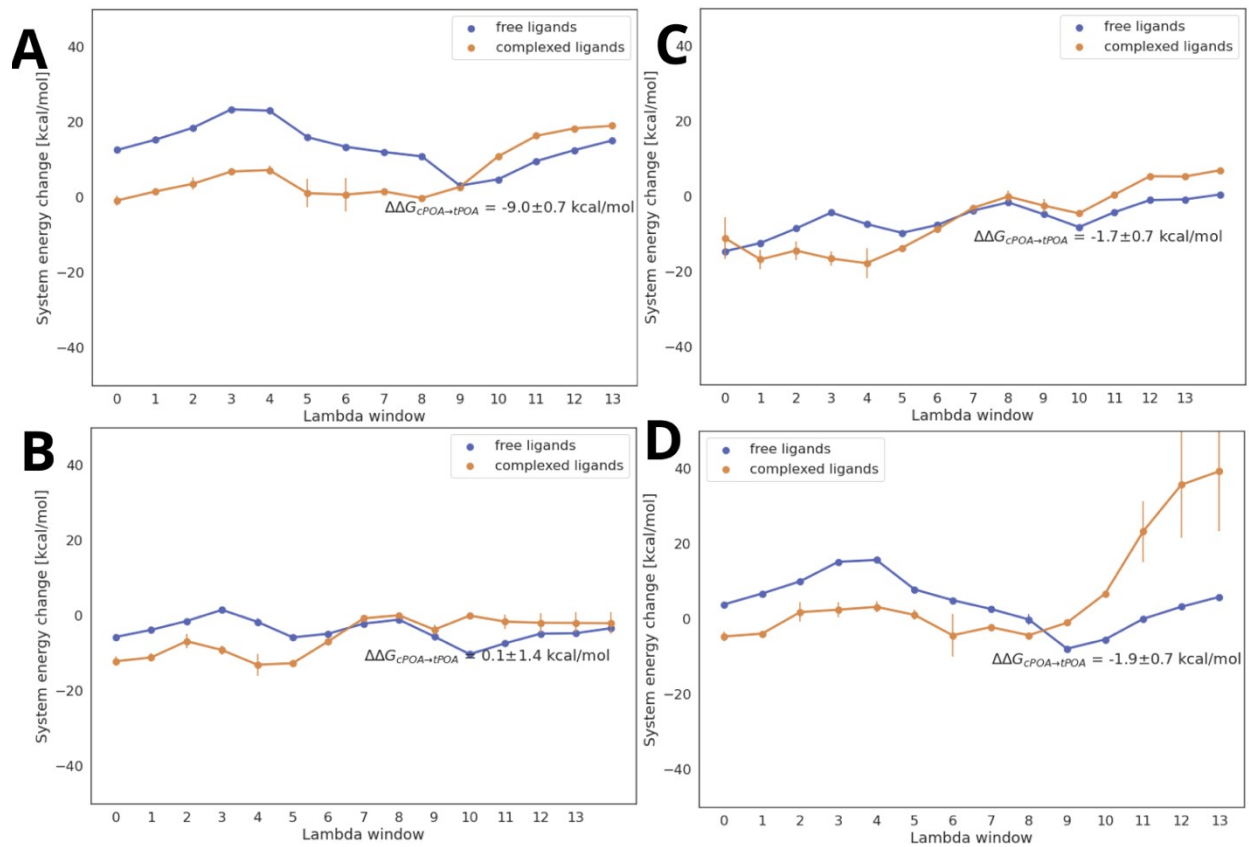
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 301 Supplemental Figure 9. System energy changes during cPOA-tPOA transformation. Total  $\Delta\Delta G$  is  
 302 obtained through trapezoid integration of the area under each curve, followed by calculating the  
 303 difference between the complex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