Supplementary materials

Prediction of Accurate Binding Modes using Combination of classical and accelerated Molecular dynamics and Free Energy Perturbation Calculations: An Application to Toxicity Studies

Filip Fratev*[1, 2], Thomas Steinbrecher[3] and Svava Ósk Jónsdóttir[4]

[1] Department of Pharmaceutical Sciences, School of Pharmacy, The University of Texas at El Paso, 1101 N Campbell St, El Paso, TX 79902, USA

[2] Micar21 Ltd., Persenk 34B, 1407, Sofia, Bulgaria

[3] Schrödinger GmbH, Dynamostrasse 13, 68165 Mannheim, Baden-Württemberg, Germany

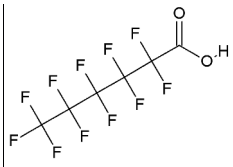
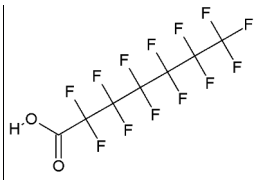
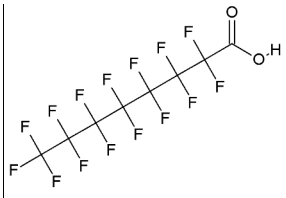
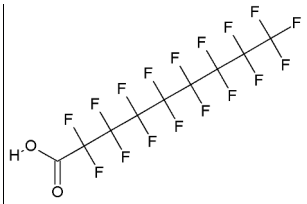
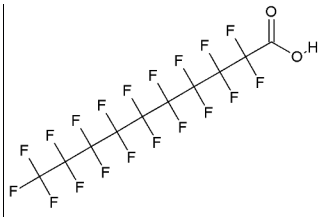
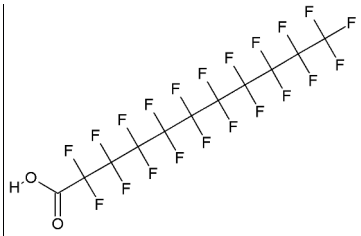
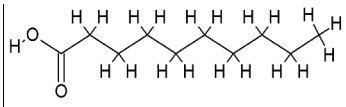
[4] insilTox ApS, Sverigesvej 20B, DK-2800 Kongens Lyngby, Denmark

**Corresponding author e-mail*: fratev@micar21.com, soj@insiltox.com

Supplemental materials:

Contain Supplementary table S1, figures S1-S8, Movie S1, Structural files (regular and induced fit docking outputs, first and last MD frame from cMD run 1 on PFuDA).

Table S1: Ligand names, 2D graphs and other basic information on the ligands studied

Ligand name (abbreviation)	Ligand name	Molecular weight [g/mol]	No. carbon atoms	2D graph
PFHxA	Perfluorohexanoic acid	314.05	6	
PFHpA	Perfluoroheptanoic acid	364.06	7	
PFOA	Perfluorooctanoic acid	414.07	8	
PFNA	Perfluorononaic acid	464.08	9	
PFDA	Perfluorodecanoic acid	514.09	10	
PFuDA	Perfluoroundecanoic acid	564.09	11	
DA	Decanoic acid	172.27	10	

Figures

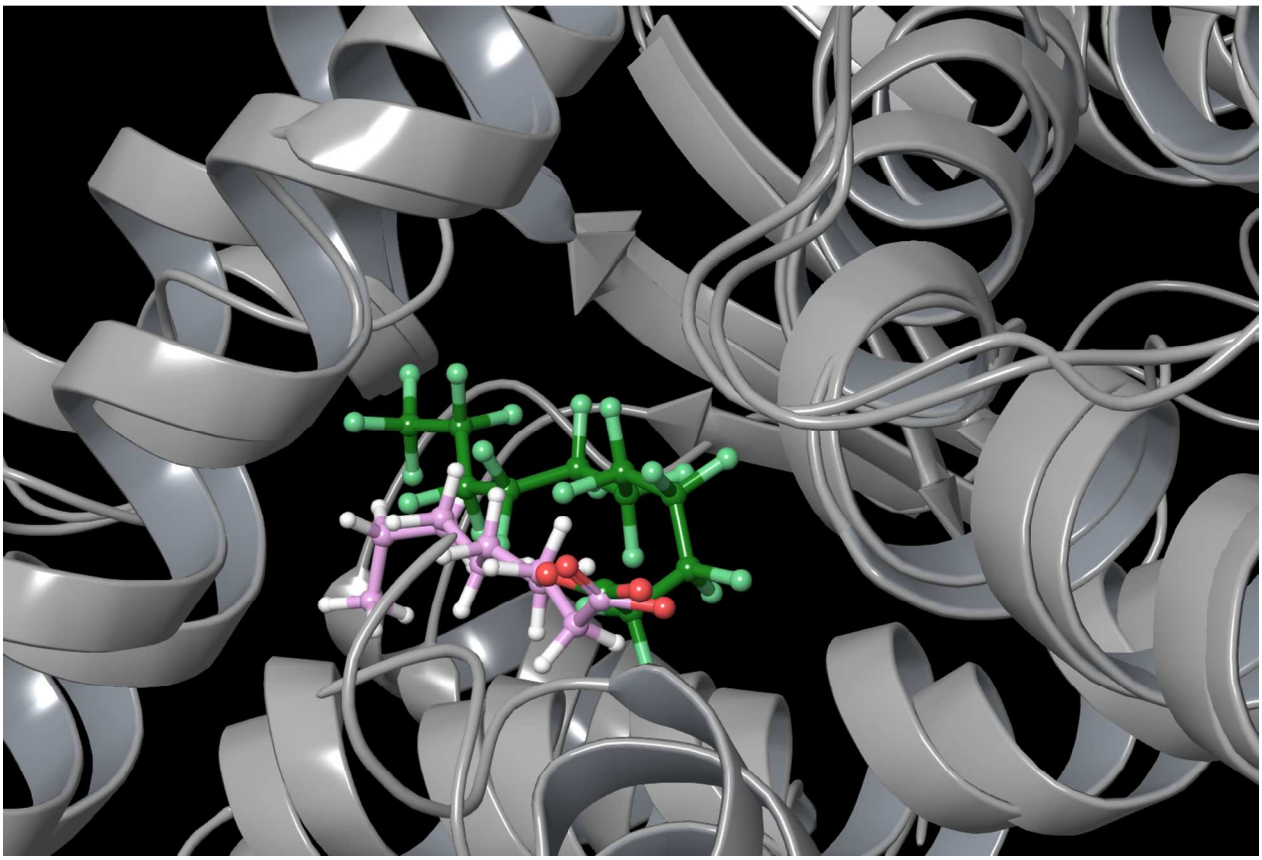


Figure S1. Comparison between the binding mode of DA (pink) obtained by X-ray crystallography and the most populated binding conformation (Binding mode 1) of PFuDA (green). The PFuDA conformation was obtained by averaging the cMD trajectories from 2x100 ns cMD simulations and subsequently the structure was minimized. Note that binding mode 1 is not the starting conformation.

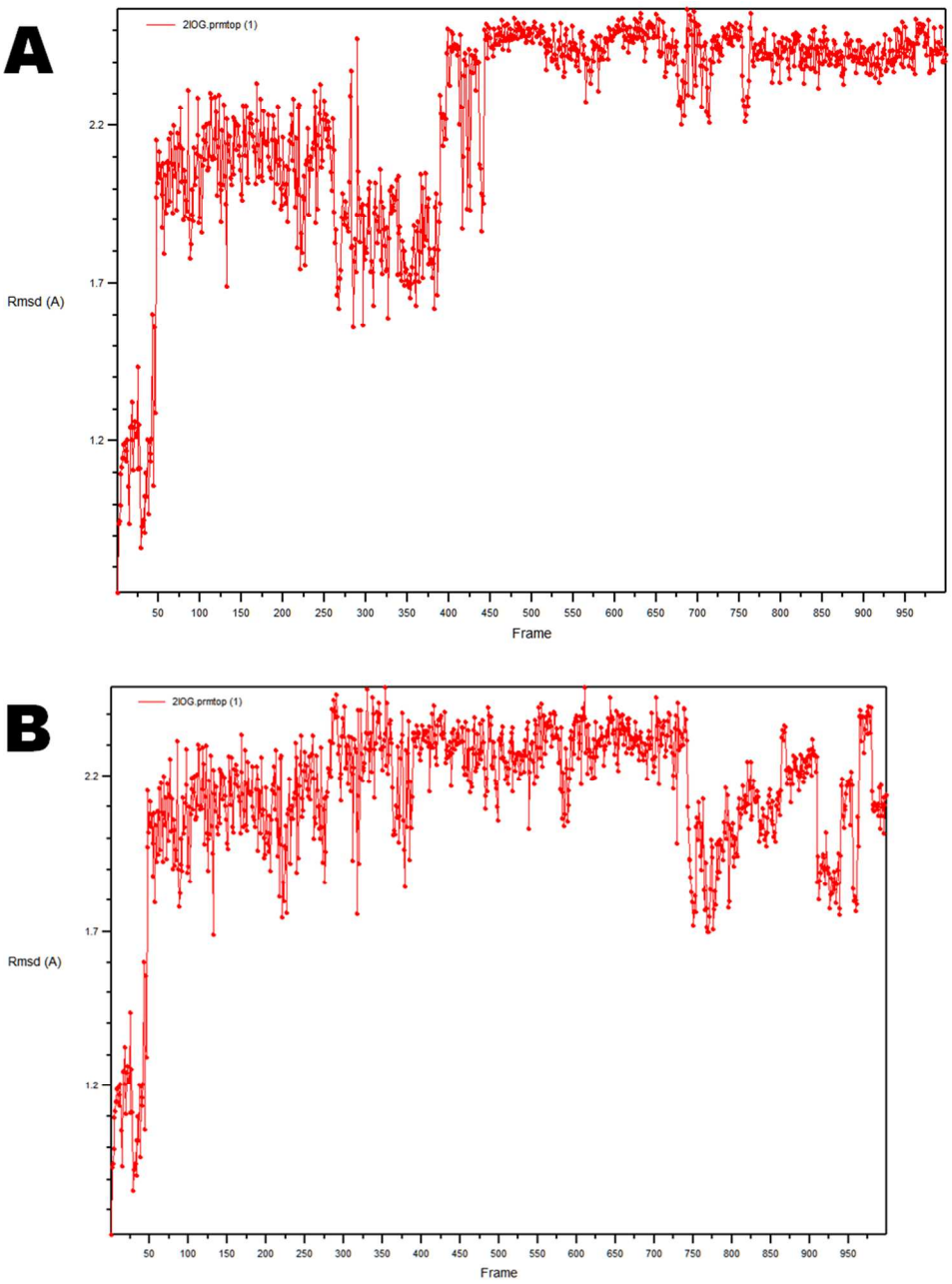


Figure S2. The RMSD of the PFuDA ligand during the cMD simulation ***(A)*** run1 and ***(B)*** run 2. 100 frames correspond to 10 ns, i.e. each simulation is 100 ns long. Note that the transitions at 5-50 ns (Binding mode 2) in run 1 and after 75 ns (Binding mode 3) are well visible.

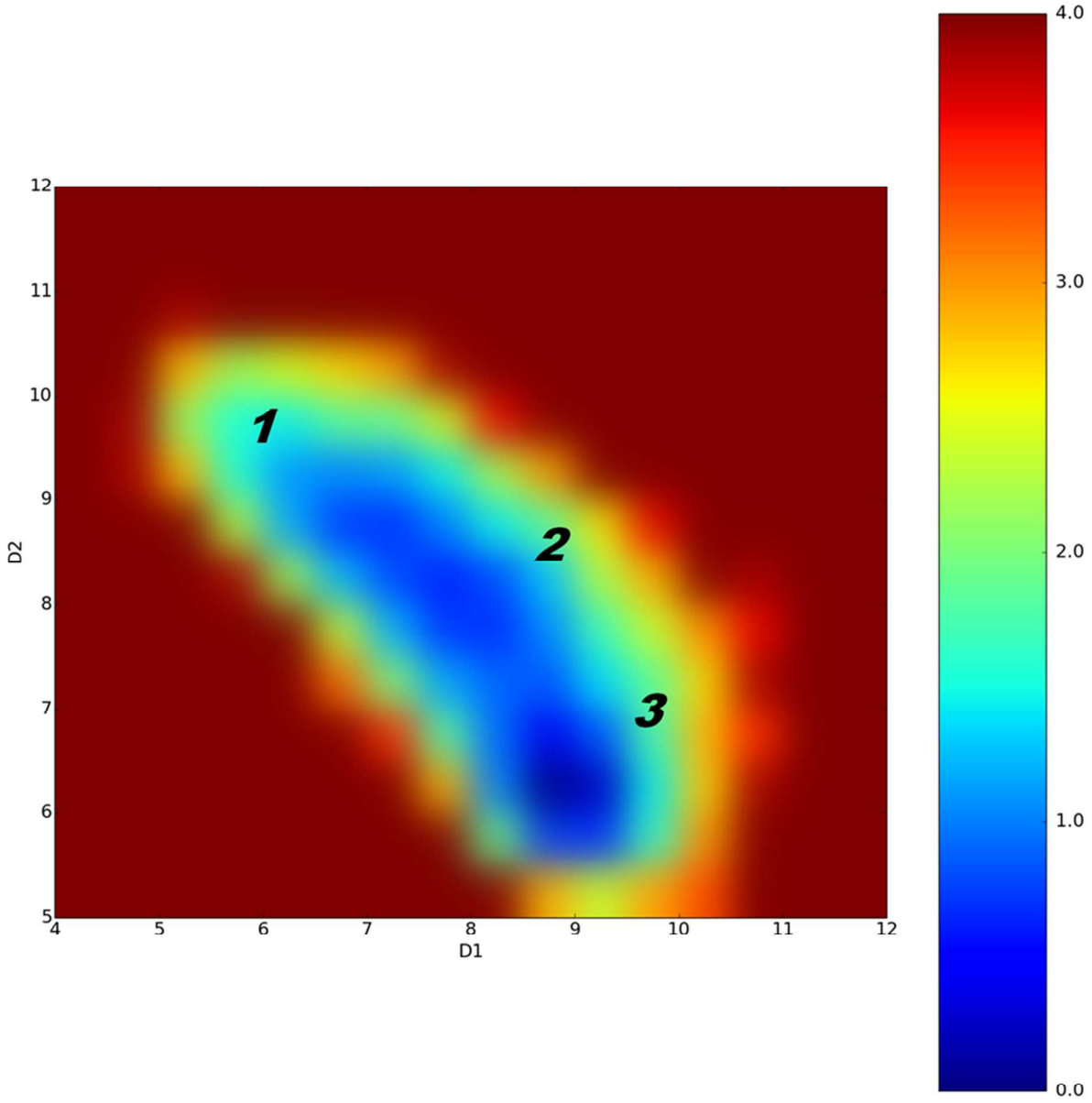


Figure S3. The free energy map of the PFOA conformations obtained by the combined 2x100 ns cMD simulations (in kcal/mol). D1 and D2 coordinates are the distances in Å between the carbon atom of the CF_3 group (the end of ligand) and the Cα atoms of Phe282 (helix 3) and Tyr473 (helix 5), respectively.

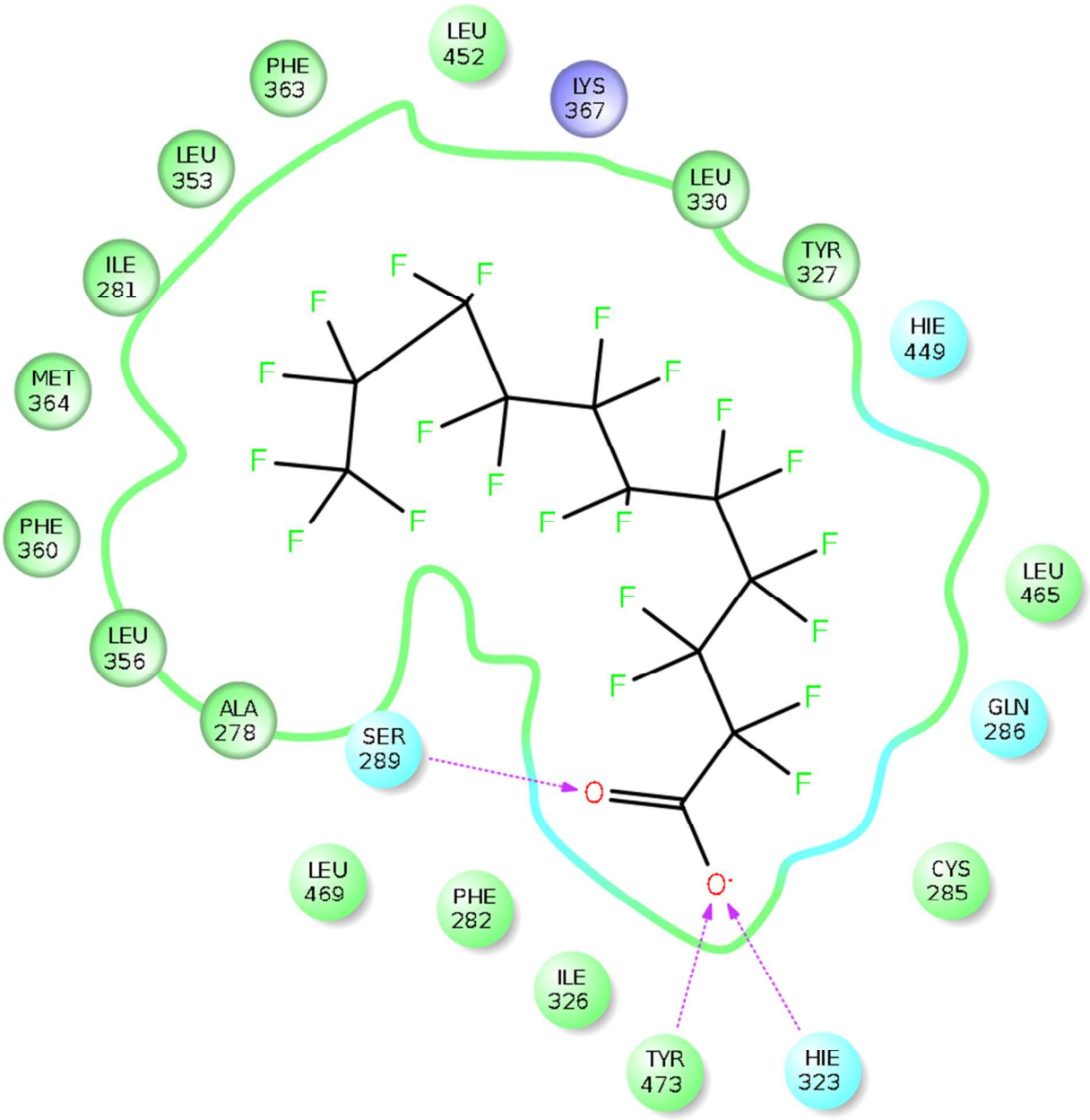


Figure S4. Detected PFuDA ligand-receptor interactions for cMD run 1 (130 ns simulation). The H-bonds are marked with dotted lines and the blue and green spheres show the areas of electrostatic and hydrophobic interactions, respectively.

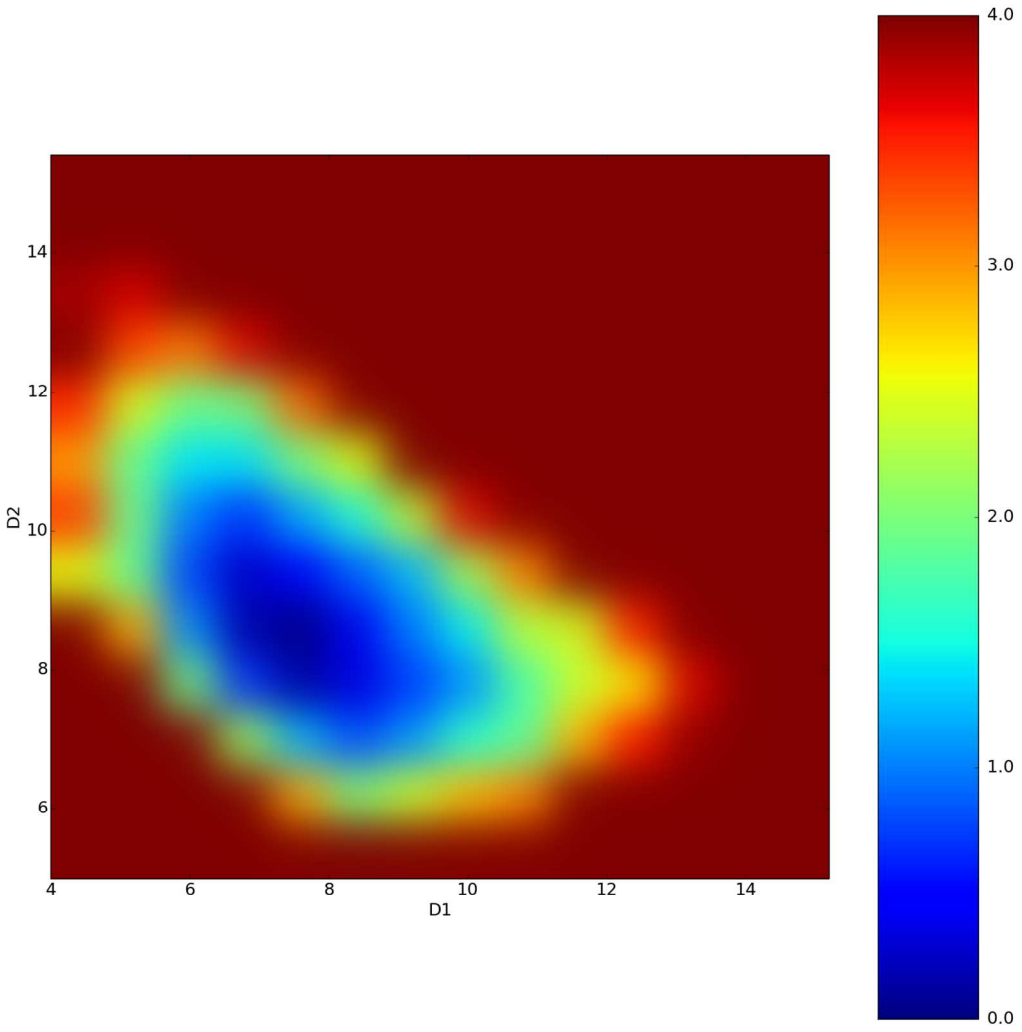


Figure S5. Reweighted free energy map of the PFOA conformations obtained by the combined aMD simulations (in kcal/mol). D1 and D2 coordinates are the distances in Å between the carbon atom of the CF_3 group (the end of ligand) and the Cα atoms of Phe282 (helix 3) and Tyr473 (helix 5), respectively.

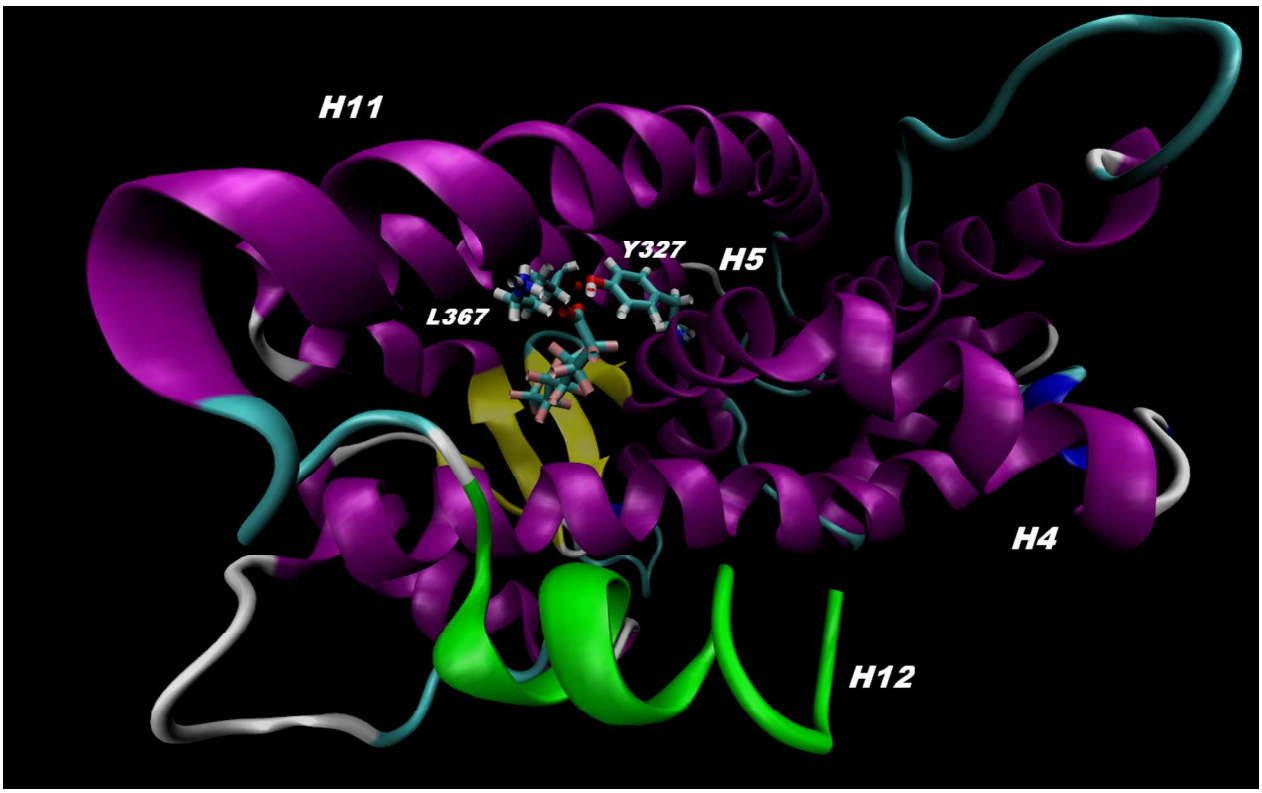


Figure S6. Antagonist H12 conformation detected during the movement of the PFOA ligand in the aMD simulation.

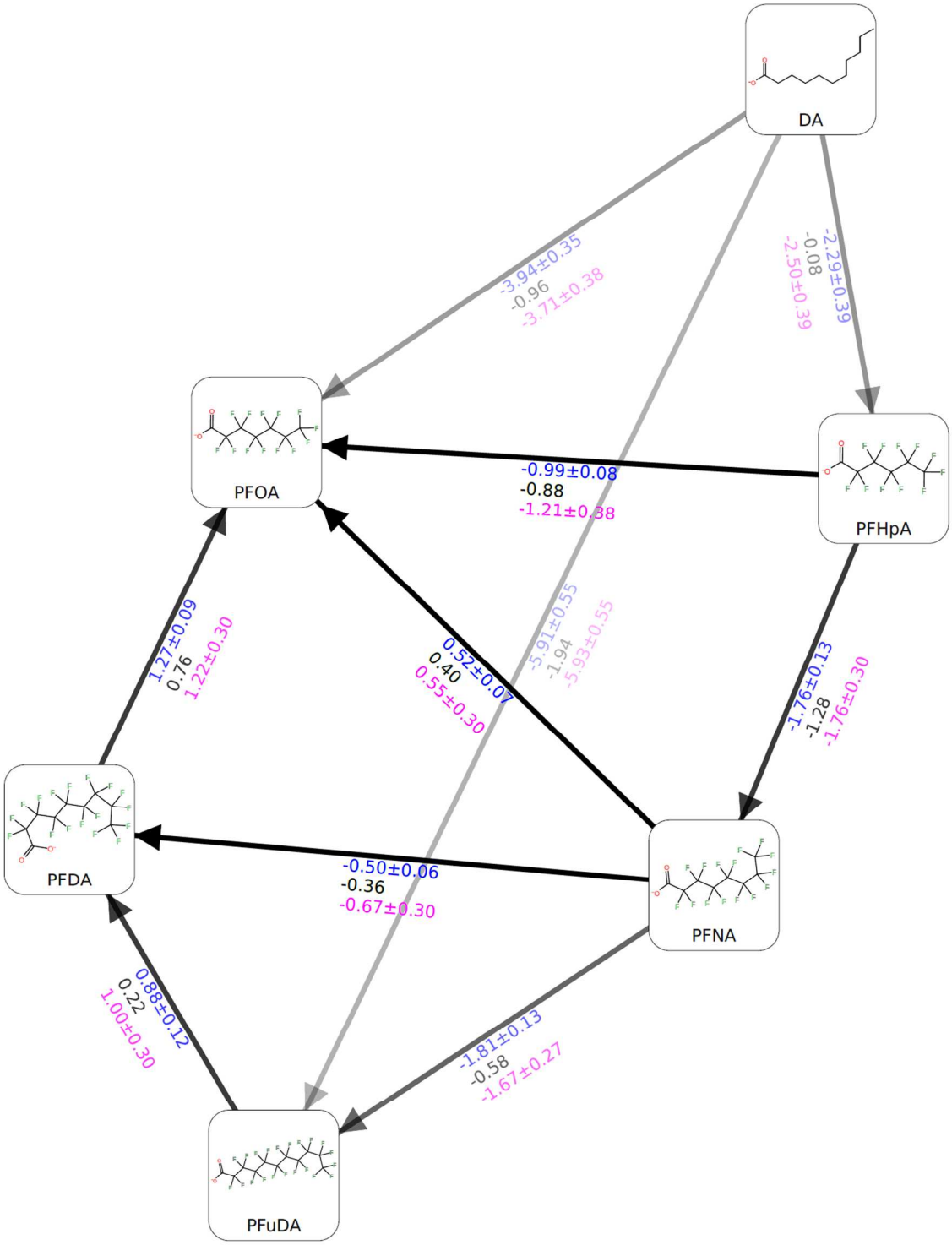


Figure S7. The FEP+ results based on the Rosiglitazone binding mode and 1FM6 pdb-structure. Predicted ΔΔG values for each individual permutation are shown. The ligands experimental relative binding affinities ΔΔG (kcal/mol), and the computed values by BAR (Bennett raw) and cycle closure estimation of ΔΔG are written in black, blue and magenta color, respectively.

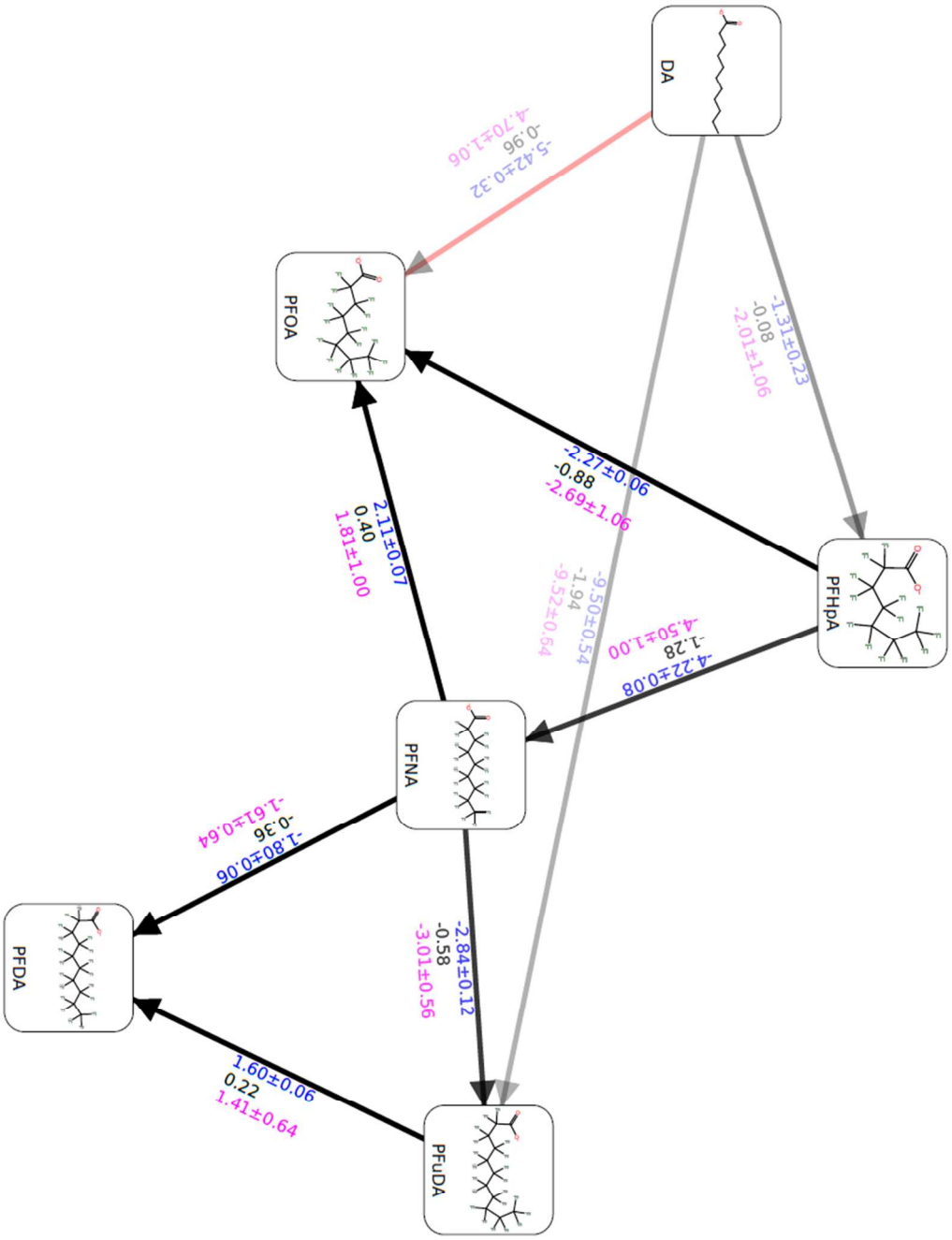


Figure S8. The FEP+ results based on the cMD derived structure for the PFuDA-PPARγ complex and original FEP+ protocol. Predicted ΔΔG values for each individual permutation are shown. The ligands experimental relative binding affinities ΔΔG (kcal/mol), and the computed values by BAR (Bennett raw) and cycle closure estimation of ΔΔG are written in black, blue and magenta color, respectively.