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Comparative study on the binding of several per- and Polyfluoroalkyl Substances (PFAS) to the G Protein-coupled Estrogen Receptor (GPER) using molecular docking technology

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Abstract. Per- and Polyfluoroalkyl Substances (PFAS) constitute a class of persistent organic pollutants with estrogen-disrupting effects. The molecular mechanism underlying their mediation of rapid non-genomic signaling pathways through the G Protein-coupled Estrogen Receptor (GPER) remains incompletely elucidated. This study selected three representative PFAS—Perfluorooctane Sulfonate (PFOS), Perfluorooctanoic Acid (PFOA), and Perfluorobutyric Acid (PFBA)—based on the following criteria: PFOS and PFOA are the most ubiquitous traditional long-chain PFAS in the environment, characterized by potent bioaccumulative potential, while PFBA, as a representative short-chain PFAS, is widely used as a substitute for long-chain PFAS. Molecular docking techniques were employed to simulate and compare the interactions between these three PFAS and the GPER-specific agonist G1 with the receptor. The results indicated the following binding affinity order for ligands with GPER: G1 (-7.26 kcal/mol) > PFOS (-6.09 kcal/mol) > PFOA (-5.48 kcal/mol) > PFBA (-4.45 kcal/mol). Binding mode analysis revealed that PFOS exhibits the highest binding affinity due to its sulfonate group forming salt bridges and multiple halogen bonds. In contrast, PFOA and PFBA primarily engage in hydrophobic interactions and halogen bonds, with their binding affinity diminishing as the perfluorinated carbon chain length decreases.

Keywords: molecular docking, PFAS, GPER, PFOA, PFOS

1. Introduction

Per- and Polyfluoroalkyl Substances (PFAS) are a class of synthetic organic fluorine compounds defined by exceptional chemical stability and environmental persistence, widely used in industrial manufacturing and consumer goods. Owing to their resistance to degradation, PFAS have accumulated extensively in the global environment and living organisms, posing multiple health hazards, including endocrine-disrupting effects [1, 2]. Traditionally, the estrogenic effects of PFAS were thought to be primarily mediated through the Estrogen Receptor (ER). However, accumulating evidence demonstrates that PFAS possess weak binding affinity for

ER, implying the potential existence of alternative signaling cascades, such as GPER-mediated non-genomic signaling (GPER).

In recent years, investigations into the endocrine-disrupting effects of PFAS have expanded beyond traditional nuclear receptors to other potential targets [3]. Computational toxicology approaches, including molecular docking and molecular dynamics simulations, have been increasingly utilized to decipher the molecular interaction mechanisms between PFAS and diverse receptors [4, 5]. However, studies on the interaction mechanisms between PFAS with different structures—such as variations in carbon chain length and functional groups—and GPER remain relatively limited.

To fill this research gap, the present study selected three representative PFAS with distinct carbon chain lengths and terminal functional groups: Perfluorooctanesulfonic Acid (PFOS), Perfluorooctanoic acid (PFOA), and Perfluorobutyric Acid (PFBA)—three PFASs representative of varying carbon chain lengths and terminal functional groups. Employing molecular docking approaches, we investigated their binding modes, binding affinities, and interactions with key amino acid residues with GPER and its specific agonist G1. The study aimed to decipher the potential molecular mechanism underlying PFAS-mediated estrogenic activities via GPER, thereby providing a theoretical foundation for the environmental and human health risk assessment of PFAS.

2. Materials and methods

2.1. Preparation of ligand molecules

This study selected four ligand molecules for docking analysis, encompassing three representative PFAS congeners (Perfluorooctanoic Acid (PFOA), and Perfluorooctanesulfonic Acid (PFOS), Perfluorobutyric Acid (PFBA)) and the GPER-specific agonist G1 (as a positive control). PFOA and PFOS are the most ubiquitous traditional long-chain PFAS in the environment and have been globally classified as priority control pollutants; PFOA and PFOS are the most prevalent traditional long-chain PFAS in the environment and have been globally designated as priority pollutants for control; PFBA, a short-chain PFAS, is extensively employed as a substitute for long-chain PFAS; G1, a highly selective agonist of GPER, possesses a well-characterized binding mode with GPER and was employed as a positive control in the molecular docking assays.

The two-dimensional chemical structures of all ligand molecules were initially constructed using ChemDraw software, then imported into Chem3D for structural optimization and energy minimization to obtain stable three-dimensional conformations. The refined molecular structures were saved in PDB format, subsequently processed with AutoDock Tools (ADT) to add Gasteiger charges and define rotatable bonds, and finally converted to PDBQT format for molecular docking.

2.2. Receptor molecule preparation

The three-dimensional structure of the GPER protein is derived from the Protein Data Bank (PDB) with the code 8XOF. This structure corresponds to the cryo-EM structure of the Lys05-GPR30-Gq complex, resolved at 2.6 Å resolution. It comprises the intact seven Transmembrane Domains (7TM) of GPER, along with key functional regions including Intracellular Loops (ICLs) and Extracellular Loops (ECLs). The 8XOF structure harbors GPER's orthosteric binding pocket, formed by TM2, TM3, TM6, and TM7. This pocket contains critical amino acid residues involved in ligand binding that constitute a typical GPCR ligand-binding environment. Selecting this structure for molecular docking ensures the conformational accuracy of the binding pocket and the integrity of functional domains. Prior to docking, the native protein structure was subjected to the following preprocessing steps: water molecules, the native ligand, and other extraneous atoms

were removed using PyMOL software. Subsequently, polar hydrogen atoms were appended and Gasteiger charges were calculated using AutoDock Tools. Finally, the processed protein structure was saved in PDBQT format and designated as the rigid receptor for subsequent molecular docking simulations.

Based on the known positions of reference ligands within the 8XOF structure, the active binding pocket of GPER was identified. This pocket resides in the transmembrane region of GPER and forms a hydrophobic cavity delineated by several transmembrane helices. This cavity functions as the pivotal domain for ligand binding to GPER and the initiation of subsequent signal transduction.

2.3. Molecular docking process

Molecular docking simulations were performed using AutoDock Vina software, which employs gradient optimization algorithms and empirical scoring functions to efficiently and accurately predict ligand-receptor binding modes and binding affinities. Docking parameters were configured based on the crystal conformation of the Lys05 ligand in the PDB ID 8XOF structure, defining the active site pocket center coordinates (X = -14.5, Y = 9.0, Z = -23.5). A three-dimensional grid box of 25 Å × 25 Å × 25 Å was centered on the GPER active site, ensuring complete coverage of the entire active site and surrounding potential extension regions. The grid center precisely aligns with the geometric center of the GPR30 ligand-binding pocket, ensuring inclusion of all known ligand-interacting residues and coverage of hydrophobic interaction regions. Other parameters retained default software settings, including energy range=4 and exhaustiveness=8, to guarantee reliability and reproducibility of docking results.

For each ligand molecule, the Vina software outputs multiple binding conformations, ranked from highest to lowest binding affinity (binding energy). We select the conformation with the lowest binding energy (highest absolute value) as the optimal binding mode for that ligand, to be used in subsequent analyses.

2.4. Analysis method for docking results

For each ligand-receptor complex, Three-Dimensional (3D) visualization was conducted using PyMOL software to examine the spatial orientation of the ligand within the active pocket and the global binding topology. Furthermore, the Protein-Ligand Interaction Profiler (PLIP) online tool was utilized to conduct a comprehensive analysis of the intermolecular interactions between the ligand and GPER, including but not limited to hydrogen bonds, hydrophobic interactions, halogen bonds, and salt bridges.

Special emphasis was placed on the types, distances, and geometries of these interactions, as well as the specific amino acid residues involved. By systematically comparing the binding characteristics of different PFAS with G1, the binding affinity of PFAS to GPER and potential activation mechanisms were evaluated.

3. Results

3.1. Analysis of molecular docking results

Molecular docking simulations effectively identified the optimal binding conformations of four ligands (PFBA, PFOA, PFOS, and G1) with the GPER protein. Table 1 summarizes the binding energies of each ligand with GPER and the primary interaction types. A more negative binding energy denotes stronger ligand-receptor binding affinity and enhanced complex stability.

Table 1. Summary of molecular docking results between ligands and GPER

Ligand	Bond energy (kcal/mol)	Number of hydrogen bonds	Number of Halogen Bonds	Number of Hydrophobic Interactions	Other interactions
G1	-7.26	2	0	4	-
PFOS	-6.09	1	3	2	Salt Bridge (1)
PFOA	-5.48	0	2	3	-
PFBA	-4.45	0	1	1	-

The binding energy data demonstrate that the binding affinity order of the four ligands with GPER is G1 > PFOS > PFOA > PFBA. This rank order reflects discrepancies in binding stability between the ligands and GPER, which is further correlated with their potential biological activity (Table 1).

3.2. G1-GPER docking results

As shown in Figure 1, G1, a specific agonist of GPER, displayed the highest binding affinity (-7.26 kcal/mol), consistent with its biological function. Analysis of its binding mode revealed that G1 is deeply embedded within the transmembrane binding pocket of GPER, establishing stable interactions with surrounding amino acid residues primarily via hydrophobic interactions and hydrogen bonds.

Specifically, G1 forms four strong hydrophobic interactions with hydrophobic residues such as VAL214, ALA313, and PHE314. Particularly, its two extremely close contacts with PHE314 (at 3.22 Å and 3.12 Å, respectively) contribute substantially to the total binding energy via tightly packed van der Waals interactions. Additionally, G1 connects to amino acid residues within the binding pocket via two hydrogen bonds. Despite their limited quantity, these hydrogen bonds confer precise spatial orientation and binding specificity, effectively anchoring the ligand in the optimal spatial configuration to maximize favorable intermolecular interactions. This synergistic mechanism—dominated by hydrophobic interactions and complemented by hydrogen bonds for precise positioning—enables G1 to efficiently activate the GPER receptor.

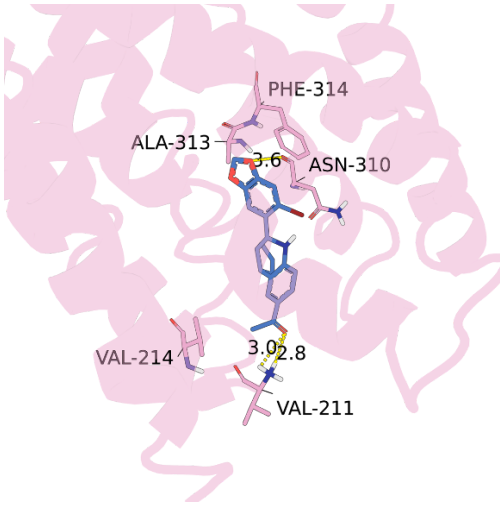

Figure 1. G1-GPER docking results

3.3. PFOS-GPER docking results

As shown in Figure 2, the binding energy between PFOS and GPER is -6.09 kcal/mol, second only to G1 but the strongest among the three PFAS congeners. PFOS displays a multifaceted interaction profile: in addition to one hydrogen bond, it forms three halogen bonds and one salt bridge, a phenomenon attributed to its distinctive molecular architecture.

The perfluorocarbon chain of PFOS establishes extensive hydrophobic contacts with hydrophobic residues in the GPER binding pocket, while its terminal sulfonate group interacts with polar residues via hydrogen bonds and salt bridges. Notably, PFOS forms a stable salt bridge with the HIS307 residue, and this charged interaction markedly augments binding stability. Multiple fluorine atoms in PFOS also form halogen bonds with surrounding amino acid residues. Collectively, these interactions contribute to PFOS's high-affinity binding to GPER. Accumulating evidence from prior studies has corroborated that PFOS exhibits higher binding affinity for GPER compared to other PFAS congeners, which is presumably attributable to its longer perfluorinated carbon chain and the highly electronegative sulfonate moiety.

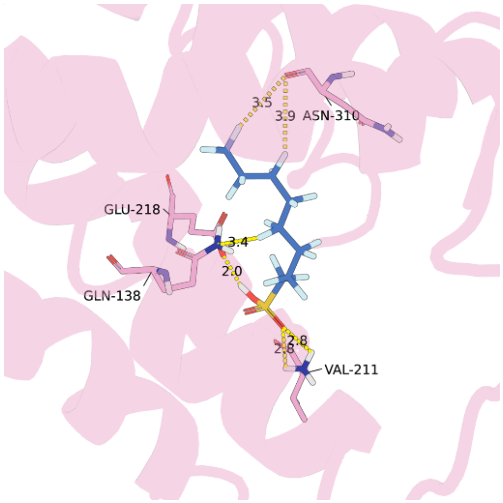

Figure 2. PFOS-GPER docking results

3.4. PFOA-GPER docking results

As depicted in Figure 3, the binding energy between PFOA and GPER is -5.48 kcal/mol, corresponding to a moderate binding affinity. In contrast to PFOS, PFOA primarily relies on its fully fluorinated carbon chain to form hydrophobic interactions with hydrophobic residues (such as LEU137, MET133, and VAL214) within the GPER binding pocket, with multiple halogen bonds further reinforcing the ligand-receptor interaction.

Notably, although the carboxyl group at the PFOA terminal possesses potential for hydrogen bonding, no stable hydrogen bonds were detected in the docking model. This observation may be associated with the spatial orientation of the carboxyl group and the local hydrophobic microenvironment within the binding pocket. This finding is consistent with results from prior investigations, indicating that PFOA primarily interacts with GPER through hydrophobic forces and halogen bonds rather than polar interactions. Molecular dynamics simulations have further validated that PFOA binding to GPER induces conformational changes in the receptor, resulting in expansion of the binding cavity and disruption of the ion-lock motif—key hallmarks of GPER activation.

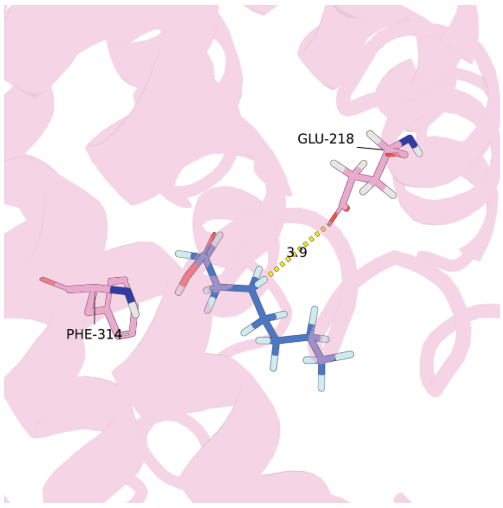

Figure 3. PFOA-GPER docking results

3.5. PFBA-GPER docking results

As shown in Figure 4, PFBA, representing short-chain PFAS, exhibits the weakest binding energy with GPER (-4.45 kcal/mol). This is primarily ascribed to its abbreviated perfluorinated carbon chain, which restricts effective hydrophobic contact with the GPER binding pocket. PFBA forms only a limited number of intermolecular interactions with GPER, including one halogen bond and one hydrophobic interaction. While direct literature on PFBA-GPER binding interactions remains limited, environmental fate studies have demonstrated substantial discrepancies in physicochemical properties and environmental behavior between short-chain and long-chain PFAS [6]. This provides indirect support for the inference that their binding capacities to biological targets may differ at the molecular level.

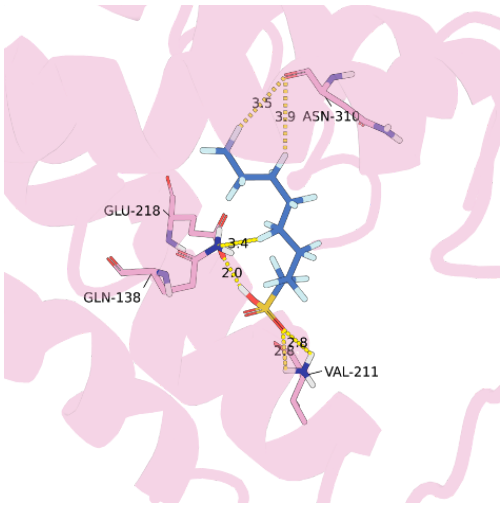

Figure 4. PFBA-GPER docking results

Due to PFBA's relatively small molecular size, it cannot form sufficient effective contacts within GPER's comparatively large binding pocket, resulting in poor binding stability. This observation is consistent with experimental findings: short-chain PFAS congeners generally exhibit attenuated biological activity and endocrine-disrupting potentials. Despite PFBA's limited direct binding affinity to GPER, its high

environmental concentrations and high mobility may still pose potential risks, particularly in scenarios involving long-term low-dose exposure.

4. Discussion

4.1. Relationship between PFAS structure and GPER binding affinity

Carbon chain length is one of the key factors influencing binding affinity. Longer perfluorinated carbon chains offer increased hydrophobic contact interfaces, facilitating extensive hydrophobic interactions with hydrophobic amino acid residues in the GPER binding pocket. PFBA (C4) displays the weakest binding capacity due to its shorter carbon chain; PFOA (C8) possesses a medium-length carbon chain and exhibits moderate binding affinity; whereas PFOS (C8), despite having the same carbon chain length as PFOA, features a terminal sulfonate group. Relative to PFOA's carboxyl group, this sulfonate group mediates more diverse intermolecular interactions, including salt bridges and stronger hydrogen bonds. Consequently, PFOS demonstrates higher binding affinity than PFOA.

This observation is consistent with the findings of Liang et al., whose spectral analysis and computational simulations similarly confirmed that Perfluorosulfonates (PFSAs) generally exhibit stronger binding affinity to GPER than Perfluorocarboxylates (PFCAs) of corresponding chain lengths [7]. Additionally, the degree of fluorination in PFAS emerges as a critical determinant of binding affinity. Fully fluorinated carbon chains can form halogen bonds with protein residues via highly electronegative fluorine atoms. Although these bonds exhibit weak interaction energy, their cumulative effect is significant, effectively enhancing binding stability.

4.2. Interaction mechanism between PFAS and GPER and potential estrogenic effects

Molecular docking results reveal a potential interaction mechanism between PFAS and GPER. Analogous to G1, PFAS primarily binds to the transmembrane domain of GPER, a tightly packed binding pocket rich in hydrophobic residues. However, in contrast to G1, PFAS relies more heavily on hydrophobic interactions and halogen bonds rather than an extensive hydrogen bond network.

Notably, both PFOS and PFOA can interact with key residues such as HIS307 and PHE314, which are located within the activation-critical region of GPER. Prior studies have established that effective ligand engagement with these residues is indispensable for GPER activation. Upon PFAS binding to this domain, they may induce a conformational change in GPER similar to that of natural ligands, leading to the outward movement of transmembrane helices and the release of ion locks, consequently triggering downstream signaling cascades.

This proposed interaction mechanism provides a molecular-level rationale for the findings of numerous experimental investigations. For example, a research team from Lanzhou University discovered via molecular dynamics and multi-omics analysis that PFOA binding to GPER can activate GPER-mediated downstream signaling cascades, including the Endocrine Resistance Pathway (ERP) and the Estrogen Signaling Pathway (ESP) [8].

4.3. Environmental health risk alert

The findings of this study offer novel and substantial insights for assessing the environmental health risks of PFAS. Although short-chain PFAS (such as PFBA) are often widely used as perceived safer alternatives to long-chain PFAS due to their comparatively low bioaccumulative potential in the environment, this study confirms their ability to bind to GPER. This suggests that in regions with high local contamination or specific

exposure scenarios, short-chain PFAS may still elicit appreciable biological responses via this signaling pathway. More alarmingly, long-chain PFAS (particularly PFOS) exhibit high-affinity binding to GPER. This indicates that traditional PFAS may potentially disrupt endocrine homeostasis by activating GPER-mediated rapid non-genomic signaling cascades.

GPER is ubiquitously expressed in multiple tissues and organs including mammary glands, reproductive systems, and cardiovascular systems, where it precisely regulates core physiological processes such as cell proliferation, differentiation, and metabolism [9]. Through the GPER signaling pathway, PFAS theoretically may promote breast cancer cell migration, disrupt normal reproductive system function, and disturb energy metabolism balance, thereby constituting a suite of adverse health outcomes. Recent large-scale environmental studies have reinforced these concerns. Data reveal diverse PFAS contamination profiles, including PFOA-dominant patterns closely associated with industrial emissions and domestic sewage, as well as PFOA/PFOS co-dominant patterns [10]. The persistent presence and bioaccumulation of these PFAS in environmental media undoubtedly prolong exposure windows for human populations.

In summary, a comprehensive environmental health risk assessment of PFAS is imperative, especially when accounting for their potential to activate the GPER signaling pathway. This involves not only re-evaluating traditional long-chain PFAS but also incorporating the potential impacts of short-chain alternatives into regulatory frameworks [11].

5. Conclusion

The results of this study indicate that the binding affinity of PFAS to GPER is closely related to their carbon chain length and functional group type. The order of binding strength is G1 > PFOS > PFOA > PFBA. PFAS primarily bind to GPER through hydrophobic interactions and halogen bonds. PFOS, bearing a terminal sulfonate moiety, further mediates salt bridges and hydrogen bonds, conferring it the highest binding affinity among the three PFAS congeners.

From the perspective of environmental health risk, this study provides molecular-level evidence for the endocrine-disrupting effects of PFAS, supporting the notion that PFAS have the potential to mediate rapid estrogenic responses by directly binding to GPER and activating its downstream signaling cascades.

A key limitation of this study is the exclusive reliance on static molecular docking methods, which cannot fully reflect the dynamic conformational changes occurring during protein-ligand binding. Furthermore, the limited range of PFAS compounds examined fails to comprehensively reflect the diversity of this extensive chemical family. Future research should integrate molecular dynamics simulations, in vitro cellular experiments, and in vivo studies to comprehensively validate the biological consequences of PFAS-GPER interactions. Expanding the research scope to incorporate a broader range of emerging PFAS alternatives will yield a more robust scientific foundation for the comprehensive environmental health risk assessment of PFAS.

References

[1] Ecke, F., Ytrehus, B., & Evander, M. (2025). Biomagnification and potential health effects of per-and polyfluoroalkyl substances (PFAS) in a terrestrial food web. *Scientific Reports, 15*(1), 31003.

[2] Kemp, M. J., Thoppe, K., & Jones, K. (2025). Evaluating health impacts of exposure to PFAS mixtures: A systematic review of epidemiological studies using mixture methods. *Critical Reviews in Toxicology, 54*(9), 587-605.

[3] Wang, J., Li, X., Zhang, Y., & Chen, L. (2016). Role of GPR30 in apoptosis induced by perfluorooctane sulfonyl compounds. *Journal of Toxicology, 30*(5), 349–352.

[4] Guan, R., Luan, F., & Li, N. (2022). Identification of molecular initiating events and key events leading to endocrine disrupting effects of PFOA: Integrated molecular dynamic, transcriptomic, and proteomic analyses. *Chemosphere, 307*, 135881.

[5] Cao, H., Zhou, Z., & Hu, Z. (2022). Effect of enterohepatic circulation on the accumulation of per-and polyfluoroalkyl substances: Evidence from experimental and computational studies. *Environmental Science & Technology, 56*(5), 3214–3224.

[6] Huang, K., Li, Y., & Bu, D. (2022). Trophic magnification of short-chain per-and polyfluoroalkyl substances in a terrestrial food chain from the Tibetan Plateau. *Environmental Science & Technology Letters, 9*(2), 147–152.

[7] Liang, W., Chen, Y., & Wei, Y. (2024). Analysis of binding modes between three perfluorosulfonates and GPER based on computational simulation and multiple spectral methods. *Toxics, 12*(5), 315.

[8] Qiu, Z. (2021). *Study on the effect of estrogen interference mediated by perfluorooctanoic acid PFOA through membrane receptor GPER* [Doctoral dissertation, Lanzhou University]. https://doi.org/10.27204/d.cnki.glzhu.2021.000868

[9] Liu, M., Zhao, W., & Ma, C. (2025). Perfluoroalkyl and polyfluoroalkyl substances interact with platelet glycoprotein Ibα and exacerbate thrombosis. *Journal of Hazardous Materials, 483*, 138506.

[10] Guo, Z. F., Ateia, M., & Borgomeo, E. (2025). Asymmetrical contamination of anionic PFAS across global freshwater reservoirs. *Water Research, 270*, 124940.

[11] Srivastava, R. K., & Srivastava, S. (2025). Mapping PFAS behavior via meta-analysis of soil dynamics, predictive modeling and policy integration. *Science of the Total Environment, 1000*, 180399.