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Computational and Theoretical Approach to Deciphering Potential PFAS-Induced Toxicity on the Human Estrogen and Sperm Receptors, with Implications on Female Fertility

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Abstract: Despite warnings from researchers, the use of per- and polyfluoroalkyl substances (PFAS) in consumer products continues to rise, significantly increasing their presence in the environment and human bodies. The cautionary advice has not yet led to meaningful action and the problem persists. This study utilized computer-based simulations to investigate the potential harmful effects of PFAS on human fertility, specifically their impact on female fertility by binding to the human estrogen and sperm receptors, highlighting a possible toxic mechanism. Molecular docking simulations revealed that perfluorotetradecanoic acid (PFTeDA), perfluorotridecanoic acid (PFTriA), perfluorodecanesulfonic acid (PFDS), and perfluorododecanoic acid (PFDoA) exhibited high binding affinity on both protein targets, with binding affinities comparable to or exceeding those of the native ligand. PFTeDA demonstrated the highest binding affinity among the studied PFAS on both proteins. Molecular dynamics simulations confirmed the stability of PFTeDA binding at both targets, suggesting persistence at these biological sites. Density functional theory analysis revealed that PFDS and PFDoA possess high reactivity, indicating a propensity for interaction with fertility proteins. These findings suggest that these PFAS may pose significant toxicity to female fertility proteins, potentially leading to reproductive issues. Further research is imperative to elucidate the underlying mechanisms and to develop effective countermeasures against the potentially deleterious effects of these PFAS on human reproductive health, thereby informing evidence-based strategies for mitigating this critical threat to human fertility and reproductive well-being.

Keywords: per- and polyfluoroalkyl substances, human estrogen receptor, human sperm receptor, perfluorotetradecanoic acid

1. Introduction

Rapid industrialization has unleashed a torrent of toxic pollutants into the environment, imperiling human health and the ecosystem. Among the most insidious of these pollutants in recent times are per- and polyfluoroalkyl substances (PFAS), a group of synthetic chemicals characterized by their robust carbon-fluorine bonds [1]. PFAS have become ubiquitous in modern industries, finding applications in electronics, automotive, aerospace, construction, and consumer products such as clothing, adhesives, firefighting foam, furniture, non-stick cookware, and food packaging [2]. However, the large-scale production and use of PFAS have led to their widespread release into the environment through air emissions, industrial waste, and product degradation, contaminating soil, water, and air [3]. These persistent, bioaccumulative, and toxic compounds threaten human health and environmental sustainability, emphasizing the need for effective mitigation strategies to curb their impact and restore ecological balance.

PFAS have been recognized as endocrine disruptors capable of altering the body's delicate hormonal balance [4]. By mimicking hormone function, PFAS exposure can lead to a range of adverse health effects, including reduced fertility, pregnancy complications, congenital disabilities, developmental delays, cancer, metabolic disorders, and immune system dysfunction. PFAS can be ingested or absorbed through multiple exposure pathways, including eating contaminated food and water, inhaling contaminated air, and dermal exposure to contaminated consumer products. Exposure to PFAS has been linked to toxicity of sex hormones, particularly for vulnerable populations such as pregnant women, children, and adolescents, leading to disruptions in reproductive health. These compounds can bind to sex hormone receptors, altering sex hormone production and regulation and interfering with hormone signaling and balance. Recent studies have revealed significant associations between PFAS exposure and sex hormone levels. Specifically, perfluorodecanoic acid (PFDA), perfluorooctanesulfonic acid (PFOS), and perfluorohexanesulfonic acid (PFHxS) exposure have been linked to increased testosterone concentrations in males. In contrast, PFDA, perfluorooctanoic acid (PFOA), and PFOS exposure have positively correlated with free testosterone levels in women aged 20–49 [5]. The study also observed that n-PFOS exposure was positively associated with sex hormone-binding globulin levels in men over 20 and all females. Additionally, research has established a correlation between PFOA exposures

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and disrupted endocrine function, as evidenced by decreased serum testosterone levels in Leydig cell adenomas, as well as elevated estradiol levels in rodent models and altered steroid hormone profiles in polar bears [6]. Furthermore, research has established a correlation between exposure to PFDA and PFOS and serum estradiol concentrations in adolescent males, suggesting a potential endocrine-disrupting effect of these compounds on reproductive hormone homeostasis during pubertal development [7]. These findings highlight the potential endocrine-disrupting effects of PFAS on human health.

The genital system of the female gender is regulated by an intricate network of hormones, including follicle-stimulating hormone, luteinizing hormone, estrogen, and progesterone. The human estrogen receptor (ER), in particular, plays a pivotal role in regulating various physiological processes, with its two main receptor subtypes, ERα and ERβ, encoded by the ESR1 and ESR2 genes, respectively. ERα is primarily involved in reproductive processes, while ERβ is implicated in non-reproductive processes, including bone and cardiovascular health. Estrogens are essential for fertility in mammals, and their actions are critical at key points in the reproductive process, including the development of ovulatory follicles and the triggering of the midcycle preovulatory surge of gonadotropins [8]. The binding of endocrine-disrupting chemicals (EDCs) to the ERs can have a range of effects, including a reduced affinity for natural estrogens, leading to impaired estrogen signaling, alteration of ER structure and function, potentially leading to aberrant signaling and potential health impacts such as infertility and congenital disabilities [9]. The zona pellucida, a critical extracellular matrix surrounding the mammalian egg cell, plays a vital role in fertilization. Zona pellucida sperm-binding protein 3 (ZP3), also referred to as the sperm receptor, facilitates sperm penetration and fusion with the oocyte membrane [10]. The zona pellucida's functional integrity is essential for successful fertilization, and its disruption by xenobiotics can have deleterious effects on sperm binding and recognition, acrosome reaction, and sperm penetration. Consequently, this can lead to reduced fertility, decreased pregnancy rates, and an increased risk of miscarriage [11].

The pervasive presence of EDCs in our environment through various sources, including plastics, pesticides, and personal care products, coupled with the alarming rise in human infertility, underscores the urgent need for comprehensive research into the binding affinity of these chemicals to reproductive proteins and their subsequent toxic effects. This is essential for understanding the underlying mechanisms of EDC-induced reproductive toxicity and also developing effective strategies to mitigate their harmful effects. This knowledge will inform evidence-based policies and guidelines for minimizing human exposure to EDCs and protecting reproductive health. The present study employed computational approaches to investigate the binding affinity and potential toxicity of some common PFAS at two critical protein targets: the human ER (ESR1) and the mammalian sperm receptor, ZP3. It aimed to elucidate the molecular interactions between PFAS and these receptors, shedding light on the potential mechanisms of endocrine disruption and reproductive toxicity associated with PFAS exposure. By leveraging computational methods, this study provides valuable insights into the possible risks of specific PFAS to these female fertility proteins.

2. Methodology

The flow chart for the bioinformatics analyses in this study is shown in Figure 1.

2.1. Ligand identification and preparation

A selection of prominent PFAS commonly utilized in commercial product manufacturing was chosen for this study (Supplementary Table 1). Their three-dimensional structural data files were obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). To ensure optimal molecular geometries, the compounds' energies were minimized using the PyRx virtual screening tool and the Universal Force Field methodology, aligning them with their authentic equilibrium conformations. The optimized structures were then converted into AutoDock-compatible ligand files (pdbqt) for subsequent molecular docking simulations, enabling the investigation of their binding interactions.

2.2. Preparation of protein targets

The three-dimensional structures of the human ER (ESR1; PDB ID: 3OS8) and the mammalian sperm receptor (ZP3; PDB ID: 3D4C) (Figure 2) were retrieved from the Protein Data Bank (PDB) database (https://www.rcsb.org/). The crystallographic water molecules and extraneous residues were removed from the protein structures, and the amino acids comprising the active sites were identified using UCSF Chimera 1.14 [12]. Subsequently, the protein structures were energy-minimized. Gasteiger charges were assigned during the Dock Prep protocol to generate optimized structural conformations, ensuring the proteins were in a suitable state for further analysis.

2.3. Molecular docking analysis

Molecular docking simulations of PFAS ligands with protein targets were performed using AutoDock Vina in PyRx software [13]. The binding sites on the protein receptors were defined using a grid box with specified dimensions: $x = 28.714$, $y = 17.288$, $z = 20.599$ for the human ER (ESR1), and $x = 21.429$, $y = 24.221$, $z = 21.227$ for the mammalian sperm receptor (ZP3). Docking analysis yielded protein-ligand complexes, whose binding affinity scores were exported to a CSV file. The protein-ligand interactions, including hydrogen bonding and hydrophobic contacts, were visualized using Biovia Discovery Studio 4.5, providing a detailed understanding of the binding modes and molecular interaction patterns.

2.4. Molecular dynamic (MD) simulation studies

To complement molecular docking studies, which predict ligand binding in a static context, MD simulations were employed to investigate the structural stability of the highest-affinity PFAS-protein complexes in a physiological environment. The starting coordinates for the 100 ns all-atom MD simulations were performed using Desmond [14]. The protein-ligand complexes underwent preprocessing via the Protein Preparation Wizard, incorporating optimization and minimization protocols to refine their structural configurations. The System Builder module was utilized to generate the simulation periodic box, and solvation was achieved using the OPLS all-atom force field in conjunction with the SPC water model. 0.15 M NaCl was added to mimic physiological conditions, and the NPT ensemble at 300 K and 1 atm pressure was selected. To facilitate analysis, trajectories were saved at 10 ps intervals. Simulation stability was evaluated by monitoring the protein and ligand's root-mean-square deviation (RMSD) over time, offering valuable insights into the protein-ligand complexes' dynamic behavior, binding stability, and conformational fluctuations.

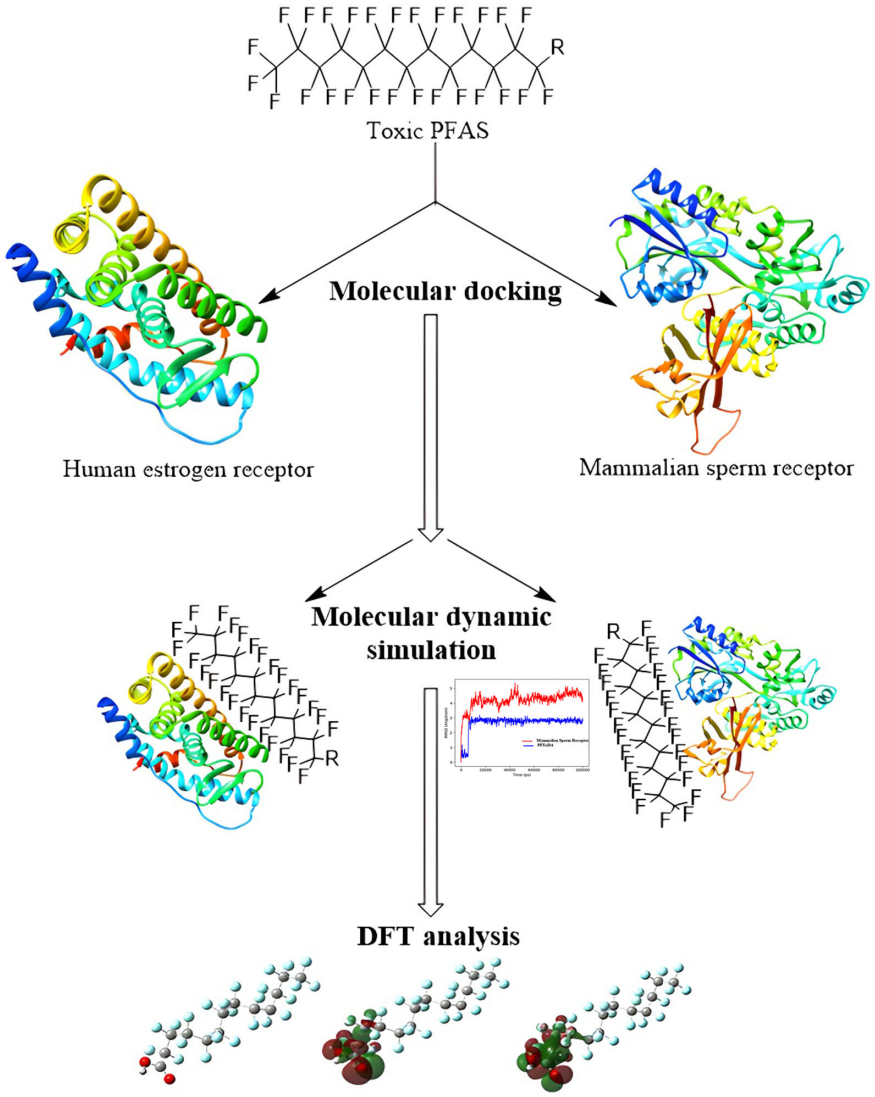

Figure 1. Schematic representation of the bioinformatics workflow

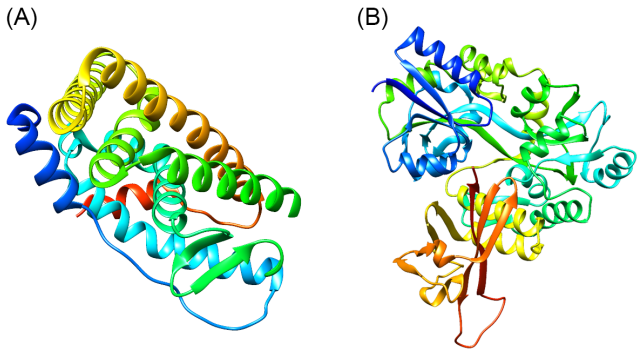

Figure 2. Crystal structure of prepared molecular targets: (A) the human estrogen receptor and (B) the mammalian sperm receptor

2.5. Density functional theory (DFT) calculation

Quantum mechanical calculations, utilizing DFT, were performed to elucidate the molecular geometry and electronic structure of the investigated chemical systems, providing a detailed understanding of their atomic-level properties. Unconstrained geometry optimization was followed by ground-state calculations using Gaussian 09, employing the B3LYP/6-311G(d,p) method to elucidate the molecular electronic structure [15, 16]. The Gauss View 6 graphical interface was utilized to visualize output and checkpoint files. The optimized molecular systems yielded essential parameters, including the energy of the highest occupied molecular orbital (EHOMO) and the energy of the lowest unoccupied molecular orbital (ELUMO), which were used to calculate the energy gap (E_{gap}) (Equation (1)) for each molecule, providing valuable insights into their electronic structure and reactivity.

$$E_{gap} = E_{LUMO} - E_{HOMO} \quad (1)$$

3. Results and Discussion

3.1. Molecular docking studies

Molecular docking is a computational technique that simulates protein-ligand binding, predicting optimal orientation and affinity. It is essential in structure-based drug design, as it helps identify

potential drug candidates and their interactions with biomolecules as they bind to target sites. Molecular docking can also be used to predict toxicity at protein targets. By analyzing the binding of a ligand to a protein, molecular docking can help identify potential toxic effects by revealing binding to unintended protein targets, where strong binding to a protein can indicate potential toxicity, which can lead to adverse effects. This technique has been used to study the toxicity of nanoplastics [17] and PFAS [13] to the human placental enzymes. The findings from these studies demonstrated that these compounds could hamper the normal development of the human fetus by inhibiting key enzymes in the placenta. Molecular docking simulations, however, are limited by their reliance on static protein structures, simplified scoring functions, and neglect of explicit solvent effects, which can lead to inaccurate predictions of binding modes and affinities. Furthermore, docking algorithms may struggle to capture the flexibility and dynamics of protein-ligand interactions, potentially overlooking alternative binding conformations or underestimating the importance of induced fit effects. As a result, potential hits or binding modes identified through docking may require experimental validation to confirm their accuracy. This highlights the need for integrative approaches combining computational and experimental methods to obtain reliable insights into molecular interactions. The binding affinities of the studied PFAS at the human ER were compared with the native ligand 4-[1-benzyl-7-(trifluoromethyl)-1H-indazol-3-yl]benzene-1,3-diol (KNO) in this study, and the results are shown in Table 1.

Research has consistently shown a significant correlation between maternal exposure to PFAS and adverse birth outcomes. Specifically, studies have found that increased levels of PFOS, PFOA, and PFHxS in mothers are associated with lower birth weights, particularly in female infants, with estimated reductions ranging from 50 to 100 grams per unit increase in maternal PFAS levels [18]. Furthermore, prenatal exposure to certain PFAS compounds, including PFOS and PFDA, has been linked to an elevated risk of preterm birth and miscarriage, with evidence suggesting a direct linear relationship between exposure levels and these adverse outcomes [19, 20]. The relationship between PFAS exposure and hormone regulation remains unclear due to inconsistent findings in existing research. In vitro studies have demonstrated that PFOA and PFOS can exhibit both estrogenic and antiestrogenic properties, thereby disrupting steroid hormone production and potentially impacting reproductive and endocrine systems. Notably, the literature suggests that PFOS may have a dual effect on estrogenic activity, with some studies indicating that it can mimic estrogenic properties. In contrast, others have observed an inverse association between PFOS exposure and estradiol levels [21]. Furthermore, cell-based assays have revealed that high concentrations of PFOA and PFOS can increase estrone and progesterone production [22], potentially leading to hormone imbalances. However, the complexity of this relationship is underscored by studies that have found no association between PFOA exposure and sex hormone levels. These inconsistent findings highlight the need for further investigation to elucidate the mechanisms underlying PFAS-induced hormone disruption and to determine the potential risks to human health. In this study, the binding affinity of PFTeDA (–10.9 kcal/mol) was significantly higher than the value obtained for the control (–10.5 kcal/mol) at the human ER. PFTriA, PFDS, and PFDoA also gave very considerable binding affinities at this target. These findings indicate that the compounds could bind efficiently to this target, preventing estrogen from making contact with its receptor, which could impact toxicity and disrupt reproductive health. The binding affinity of the PFAS was found to increase directly with the length of their carbon chain, indicating a positive correlation between the number of carbon atoms in their molecular structure and their ability to bind to this target. This is consistent with experimental reports that long-chain PFAS have created the most significant problems due to their persistence, bioaccumulation, and toxicity [23].

Table 1. Binding affinity of the PFAS on the human estrogen receptor

PFAS	Binding affinity (kcal/mol)
PFTeDA	–10.9
PFTriA	–10.5
KNO (control)	**–10.5**
PFDS	–10.4
PFDoA	–10.2
PFNS	–10.0
PFOS	–9.8
PFDA	–9.7
PFOSA	–9.5
PFNA	–9.3
PFHps	–8.8
PFOA	–8.4
PFHpA	–8.2
PFHxS	–8.0
PFPeS	–7.8
GenX	–7.5
PFHxA	–7.3
PFBS	–7.2
PFPeA	–7.0
PFMOBA	–6.9
PFBA	–6.4

The protein-ligand complex of the ER and the hit compounds are shown in Figure 3. The protein-ligand interactions showed a marked diversity and specificity in the binding modes. In addition to hydrogen and halogen bonding, the importance of non-covalent interactions, such as carbon-hydrogen bonds, halogen bonds, pi-alkyl interactions, and alkyl interactions, was also highlighted. These interactions are often specific to particular ligands and amino acid residues, and they play a crucial role in modulating the binding affinity and specificity of ligands to the protein. The binding modes and interactions of PFAS with the protein also appear to be influenced by the presence of specific amino acid residues. The presence of MET421 and ARG394 seems critical for the binding of PFTeDA, while ARG394 plays a key role in the binding of PFTriA. These observations underscore the significance of considering the unique amino acid residues present at the binding site when predicting the binding modes and interactions of PFAS with proteins. The orientation and interaction characteristics of different PFAS and control compounds at the binding sites also exhibit notable diversity and specificity. These differences in binding orientation and interaction characteristics are likely influenced by the unique chemical properties of each ligand and the amino acid residues present at the binding site. The fluorine atoms in PFAS play a crucial role in their strong interactions with protein receptors. Fluorine is highly electronegative and has a strong ability to attract electrons. Their small size allows them to occupy a smaller space than other halogens, enabling PFAS to fit snugly into the binding site of protein receptors. These properties allow fluorine

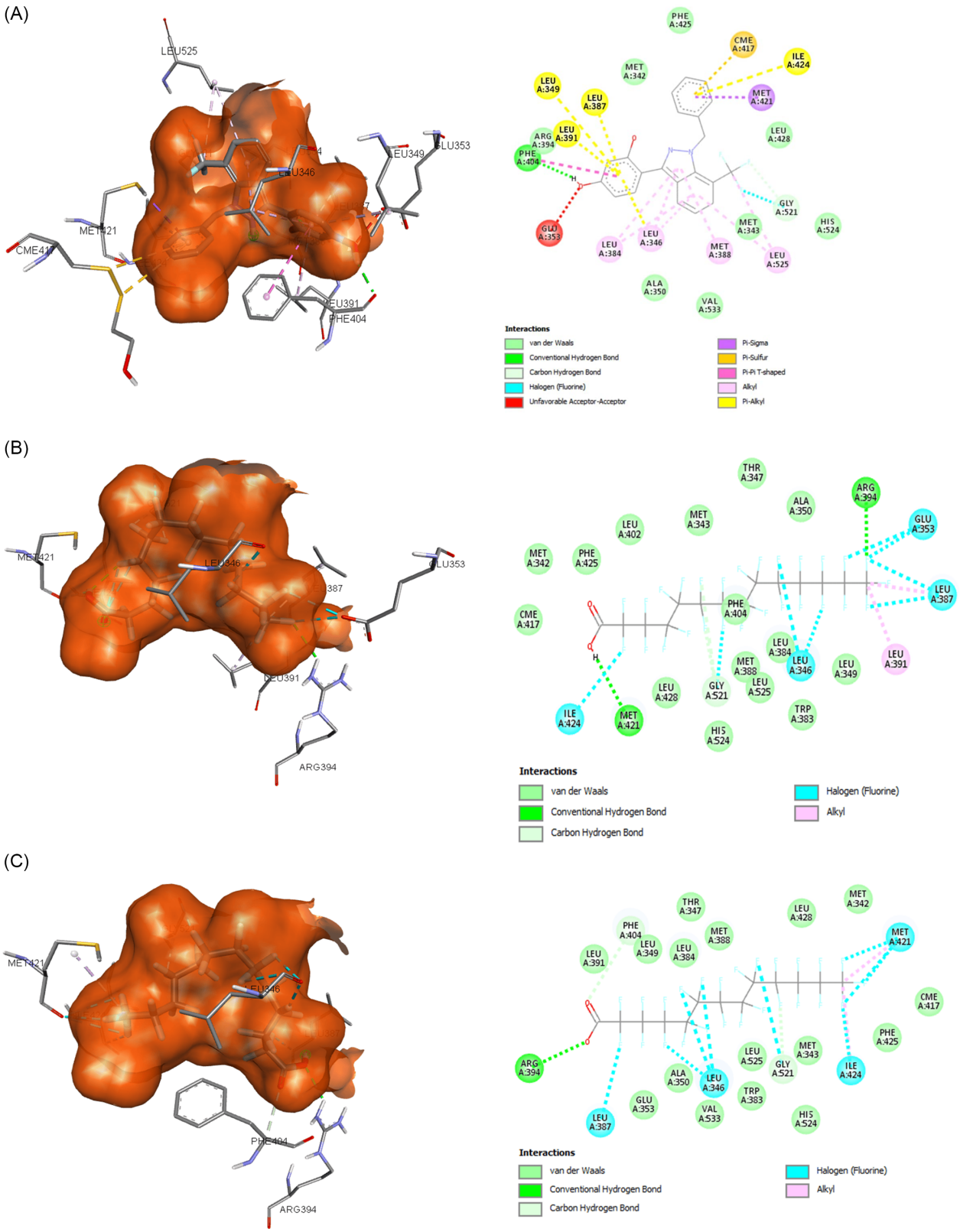

Figure 3. 3D (left) and 2D (right) interaction models of (A) KNO (Control), (B) PFTeDA, and (C) PFTriA on the estrogen receptor

Table 2. Binding affinity of the PFAS on the mammalian sperm receptor

PFAS	Binding affinity (kcal/mol)
PFTeDA	–10.2
PFDS	–10.2
PFTriA	–10.1
PFDoA	–9.9
PFDA	–9.3
PFNA	–9.3
PFOS	–9.3
PFNS	–9.2
PFOA	–9.1
PFOSA	–9.1
PFHps	–8.9
PFHxS	–8.6
PFHpA	–8.2
PFHxA	–7.9
PFPeS	–7.9
GenX	–7.8
Alpha maltose (control)	**–7.7**
PFMOBA	–7.3
PFBS	–7.2
PFPeA	–7.0
PFBA	–6.4

to form strong electrostatic interactions with the protein receptors, enhancing binding affinity. Also, their ability to participate in hydrogen bonding with the protein receptor further stabilizes the interaction. The strong interactions between PFAS and protein receptors, mediated by the fluorine atoms, can lead to various biological effects, including the disruption of normal cellular function and toxicity. These points highlight the need to understand fluorine's role in PFAS-protein interactions better.

The binding affinities of the PFAS at the mammalian sperm receptor are given in Table 2.

Most PFAS studied had binding affinities much higher than alpha maltose, the protein's native ligand. PFTeDA and PFDS showed the highest binding affinity, followed by PFTriA and PFDoA. These four PFAS also had the highest binding affinity and toxicity at the ER. Perfluorotetradecanoic acid (PFTeDA) is a long-chain PFAS with 14 carbon chain length. It is used to produce industrial and commercial materials, including insecticides, detergents, photographic films, and firefighting foams. It has been detected at varying concentrations in humans, shellfish, and wastewater sludge. PFTeDA has been reported to induce mitochondrial damage and oxidative stress in zebrafish embryos and larvae and has also been linked to decreased testosterone production in male zebrafish [24]. Perfluorodecanesulfonic acid (PFDS) is a perfluoroalkyl substance found in lake trout and used in many products, including clothing, food packaging, and non-stick cookware. Its presence in human systems has been linked to various health issues, including immune system, reproductive problems, and cancer [25]. Perfluorotridecanoic acid (PFTriA) is a perfluorinated compound used as a laboratory chemical and in research to study the effects of perfluorinated compounds on the environment and human health. Exposure to PFTriA has led to a decrease in the production of testosterone in male zebrafish [26]. Perfluorododecanoic acid (PFDoA) does not have a direct application or use. Still, it is instead a breakdown product of other PFAS like perfluorooctanoic acid (PFOA), perfluorohexanesulfonic acid (PFHxS), and perfluorobutanesulfonic acid (PFBS) [27, 28].

The finding that the mammalian sperm receptor is highly susceptible to binding with multiple PFAS, as revealed in this study, raises significant concerns. PFAS binding to the sperm receptor could alter sperm motility, viability, and fertility, potentially leading to reduced reproductive success. Chronic PFAS exposure may lead to long-term reproductive consequences, including reproductive damage, affecting future generations.

The interactions of the hit PFAS and the mammalian sperm receptor are shown in Figure 4. Closely examining these interactions reveals a striking diversity and specificity in the binding modes, highlighting the complex nature of PFAS-protein interactions. One of the most notable features of the binding modes is the prevalence of conventional hydrogen bonding interactions. These interactions are critical for stabilizing the binding of PFAS to the protein, and the unique chemical properties of each PFAS compound likely influence their specificity. In addition to hydrogen bonding, the importance of non-covalent interactions, such as pi-lone pair, halogen, pi-sigma, and pi-alkyl interactions, are also highlighted. These interactions are often specific to particular PFAS compounds and amino acid residues, and they play a crucial role in modulating the binding affinity and specificity of PFAS to the protein.

The orientation and interaction characteristics of different PFAS and the control compounds at the binding sites also exhibit notable diversity and specificity. PFTeDA adopted a distinct binding orientation, interacting with GLU45, ASN13, ASN151, LYS16, ARG67, TRP63, and TRP341 through hydrogen bonding, while PFDS binds in a different orientation, engaging with GLU154, ARG67, ASN13, LTS16, TRP231, and TRP63. The control compound, in contrast, exhibited a unique binding orientation distinct from both PFTeDA and PFDS. These differences in binding orientation and interaction characteristics are likely influenced by the unique chemical properties of each PFAS compound and the specific amino acid residues present at the binding site. The binding modes and interactions of PFAS and the control compound with the protein also appear to be influenced by the presence of specific amino acid residues. These observations highlight the importance of considering the specific amino acid residues present at the binding site when predicting the binding modes and interactions of PFAS with proteins.

3.2. MD simulation

MD simulation is a computational approach that analyzes atomic and molecular motions over time. It facilitates ab initio protein structure prediction and identification of receptor-compatible compounds with minimal disruption to the active site [29]. This approach, however, is limited by its reliance on classical mechanics, simplified force fields, and relatively short simulation timescales, which can restrict their ability to capture slow conformational changes or complex interactions involving multiple timescales. Additionally, MD simulations may be sensitive to initial conditions, force field parameters, and simulation protocols, which can introduce variability and uncertainty into the results. Consequently, MD simulations may not always accurately predict long-term stability, binding free energies, or kinetic rates, and as such, complementary experimental or computational approaches may be required to validate and refine the findings. The dynamic stability of protein-ligand complexes in this study was investigated by analyzing their backbone RMSD relative to

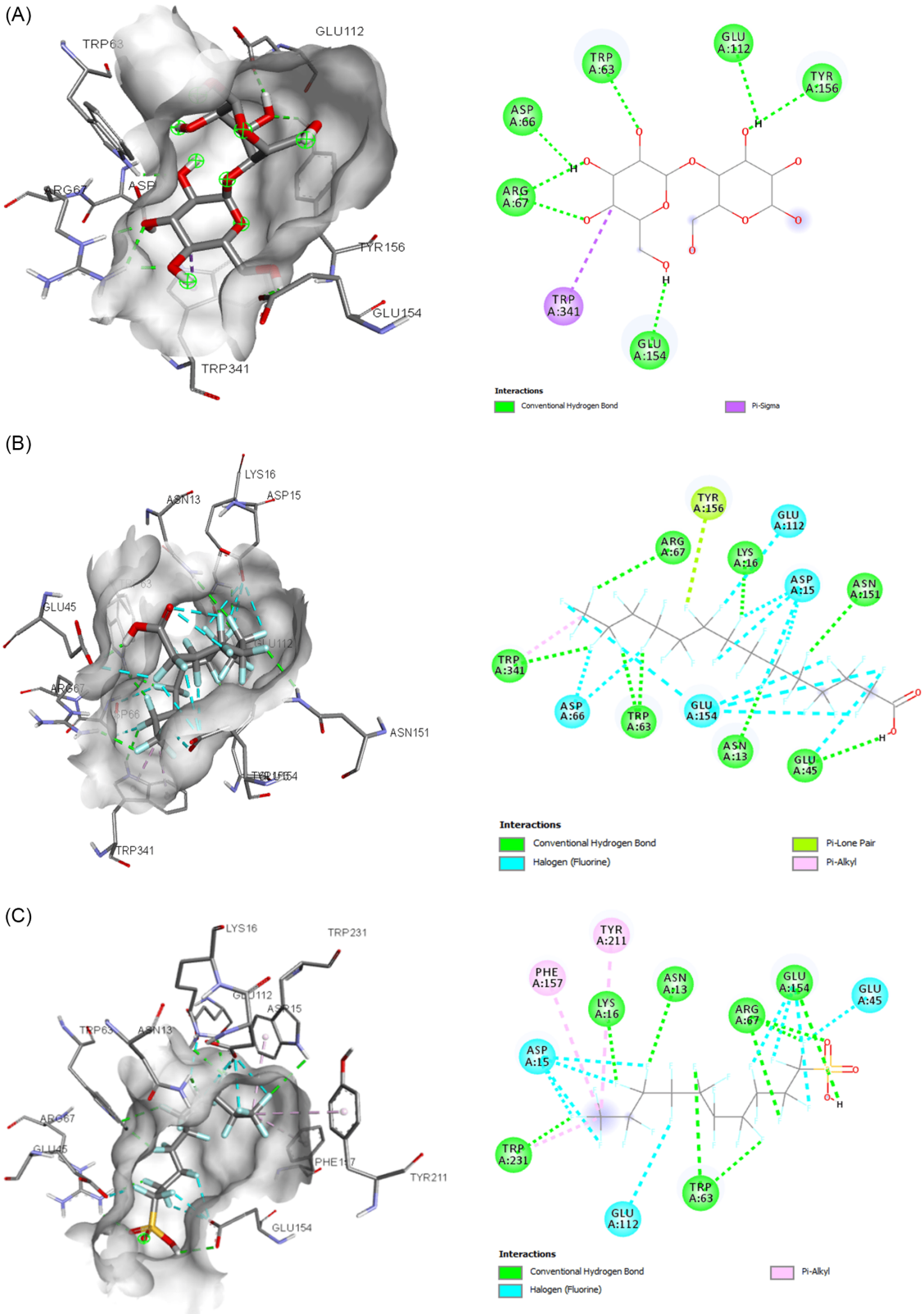

Figure 4. 3D (left) and 2D (right) interaction models of (A) alpha maltose (control), (B) PFTeDA, and (C) PFDS on the mammalian sperm receptor

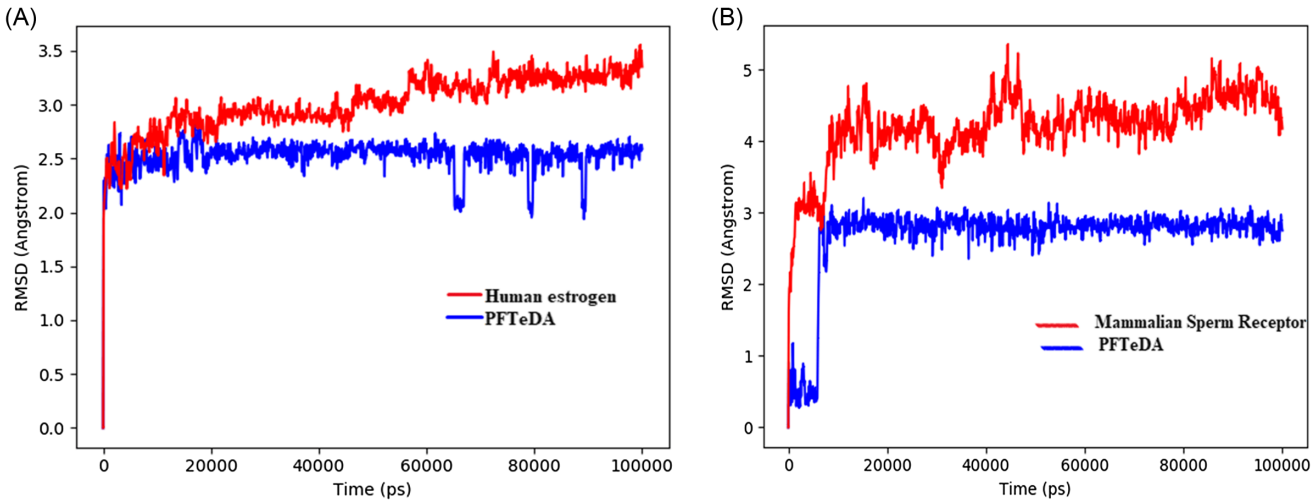

Figure 5. Ligand-free apo-protein RMSD (red) and protein-ligand complex RMSD (blue) for (A) human estrogen receptor-PFTeDA and (B) mammalian sperm receptor-PFTeDA

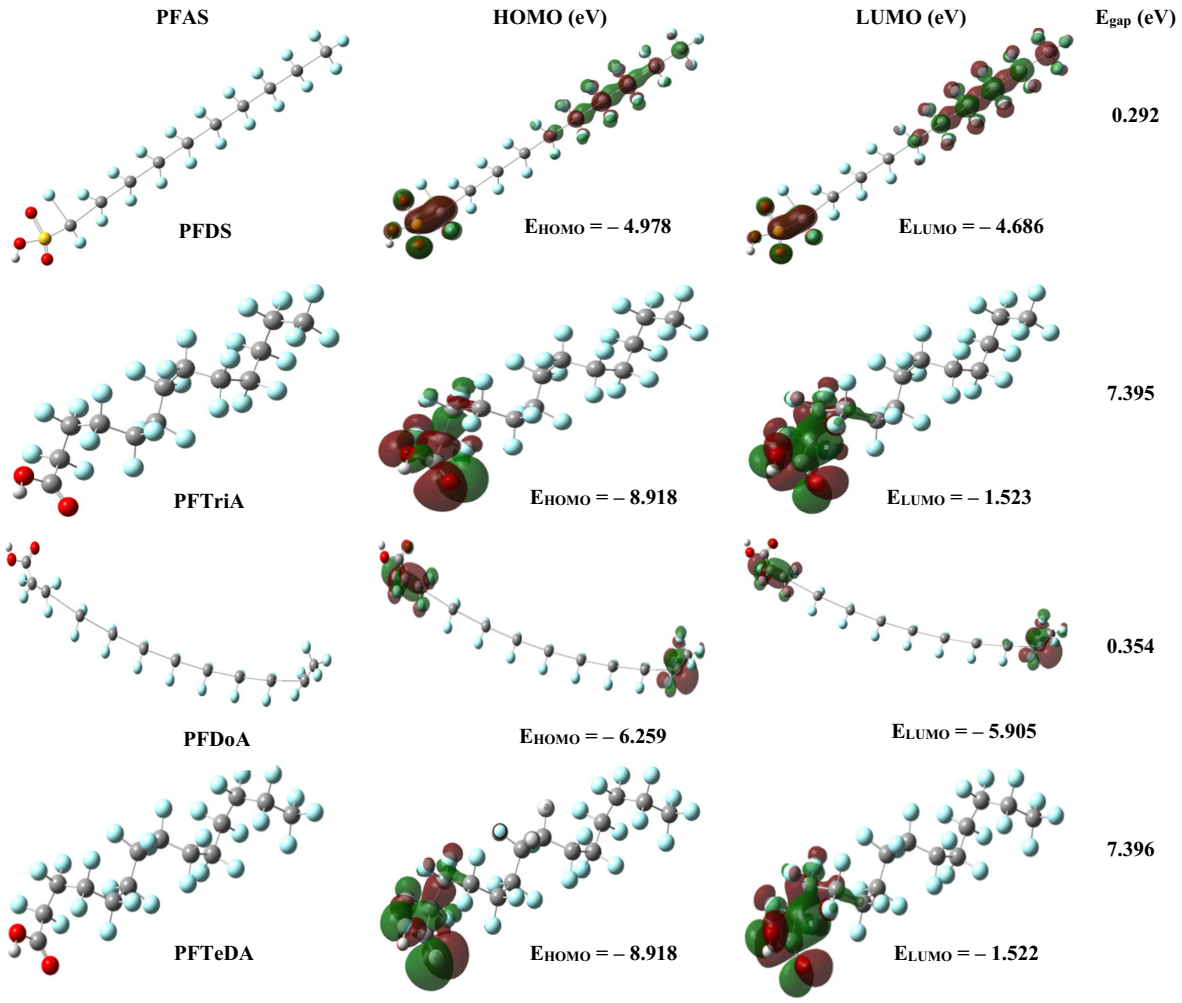

Figure 6. Contour plots of the HOMO-LUMO orbitals of the hit PFAS

the apo-protein over a 100 ns simulation period (Figure 5). The RMSD of the apo-protein of the human ER increased as the simulation progressed, indicating a gradual deviation from its initial structure. Notably, the RMSD remained relatively stable until 60 ns, after which it plateaus at approximately 3.2–3.3 Å. The RMSD of the apo-protein of the mammalian sperm receptor increased significantly within the first 20 ns, from approximately 3.1 Å to 4.2 Å, indicating a substantial conformational change. However, from 40 ns to 100 ns, the RMSD remained relatively stable at approximately 4.0 Å, suggesting that the protein has adopted a new stable conformation. These observations indicated that the apo-proteins underwent an initial conformational rearrangement, potentially involving the loosening of functional domains, followed by a stabilization of the altered structure. The changes in stability may impact the biological function of these receptors by altering the binding affinity or specificity for their ligands, potentially leading to changes in signal transduction or downstream effector responses. RMSD fluctuations within 1–4 Å were observed for the protein-ligand complexes. RMSD values >4 Å indicate significant protein conformational changes, implying ligand dissociation from its binding site [30]. The high stability of the studied complexes gives an indication of their unique ability to persist in this state and confer toxicity at these sites, hampering fertilization and inducing infertility in the organism. At the inception of the simulation, the protein targets experienced conformational changes that equilibrated at 60 ns and 10 ns at an average RMSD of about 3.3 Å and 4.0 Å till the end of the simulation for the human ER and mammalian sperm receptor, respectively.

The PFTeDA complexes gave very stable RMSD plots, achieving equilibrium with an average RMSD of 2.5 Å from the beginning of the simulation and 2.8 Å from 10 ns for the human ER and mammalian sperm receptor, respectively. The exceptional binding affinity between PFTeDA and the fertility proteins raises concerns about long-term reproductive toxicity, potentially culminating in fertility complications that may persist for an extended duration.

3.3. DFT analysis

DFT is a computational approach used to study the electronic structure and behavior of complex systems with multiple electrons. By applying DFT, the energies of frontier molecular orbitals (HOMO and LUMO) and the energy gap, which are essential indicators of molecular reactivity, can be determined. It offers a robust computational approach for assessing the reactivity of compounds, as a smaller energy gap is indicative of increased reactivity, which in the present context is associated with elevated toxicity. In contrast, a larger one suggests reduced toxicity, providing valuable insights into the potential biological activity of these compounds. The toxicity of the hit PFAS was assessed and compared using their energy gap values, and the results are shown in Figure 6. The values decreased in the other PFTeDA > PFTriA > PFDoA > PFDS.

The smaller the energy gap of a molecule, the more likely it would bind easily to protein targets. A small energy gap facilitates electron transfer and high reactivity, which is essential for forming strong interactions with protein targets. It can also indicate good bioavailability, as the molecule can easily cross cell membranes and reach its target protein. PFDS and PFDoA are crucial PFAS that warrant significant attention in the context of the studied fertility proteins, as they are utilized in the manufacture of non-stick and stain-resistant coatings for cookware and food packaging materials [31, 32]. Given their widespread daily use in numerous households, thoroughly investigating their potential impacts on human health and fertility is essential. The ubiquitous presence of these compounds in everyday consumer products underscores the importance of understanding their effects on fertility proteins and human reproductive health.

4. Conclusion

A comprehensive computational investigation evaluated the toxicity potential of selected PFAS at estrogen and mammalian sperm receptors. The results revealed that PFTeDA, PFTriA, PFDS, and PFDoA exhibited high binding affinity at fertility receptor proteins, with a direct correlation observed between PFAS carbon chain length and binding efficacy. MD simulations demonstrated remarkable stability of PFTeDA-protein complexes, suggesting persistence and potential toxicity at protein sites, which may lead to infertility. DFT calculations indicated that PFDS and PFDoA were the most reactive compounds, potentially targeting multiple fertility-related proteins in humans. These findings suggest that PFAS may cause endocrine disruption and infertility in humans, highlighting the need for caution in the use of consumer products containing these compounds.

Given these findings, we recommend that regulatory authorities and industries take proactive measures to minimize human exposure to PFAS. Specifically, we suggest integrating these findings into food packaging and consumer product safety standards to ensure safer human-use products. Furthermore, these results provide science-based decision-making support for regulatory authorities to develop and enforce policies limiting the use of PFAS in consumer products. Finally, we encourage relevant industries to improve their production processes to reduce or eliminate the use of PFAS in products, thereby mitigating potential risks to human health and the environment.

Ethical Statement

This study does not contain any studies with human or animal subjects performed by any of the authors.

Conflicts of Interest

The authors declare that they have no conflicts of interest to this work.

Data Availability Statement

The data that support this work are available upon reasonable request to the corresponding author.

Author Contribution Statement

Maryjane Ada Nnabuchi: Validation, Formal analysis, Investigation, Resources, Data curation, Writing – original draft. **Chidi Edbert Duru:** Conceptualization, Methodology, Software, Resources, Writing – review & editing, Visualization, Supervision, Project administration.

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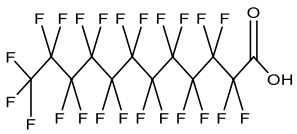
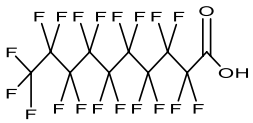
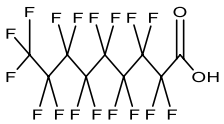
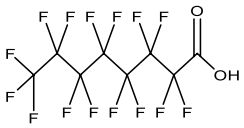
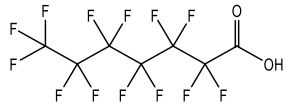
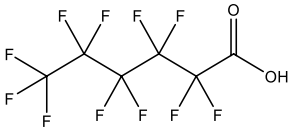
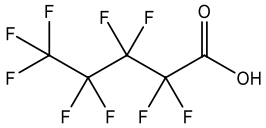
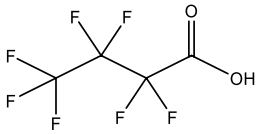
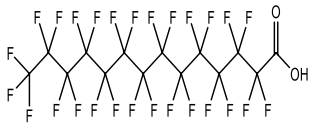
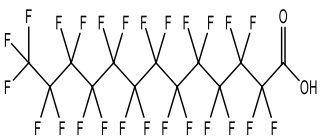
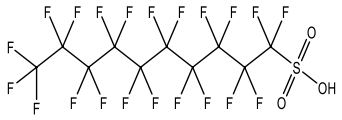
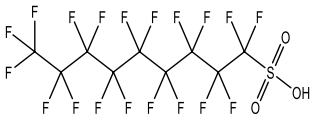
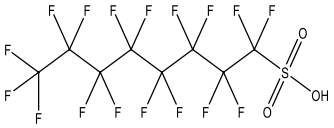
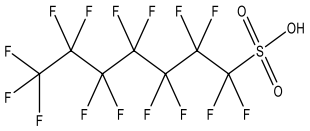
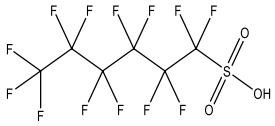
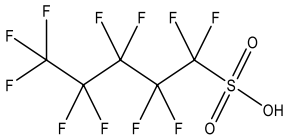
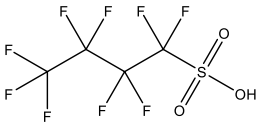
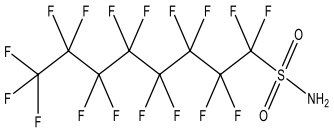
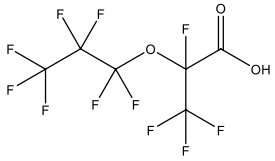
Supplementary Information

Supplementary Table 1. PFAS used in the study

PFAS	PubChem CID	Formula	Structure
Perfluorododecanoic acid (PFDoA)	67545	$C_{12}HF_{23}O_2$	
Perfluorodecanoic acid (PFDA)	9555	$C_{10}HF_{19}O_2$	
Perfluorononanoic acid (PFNA)	67821	$C_9HF_{17}O_2$	
Perfluorooctanoic acid (PFOA)	9554	$C_8HF_{15}O_2$	
Perfluoroheptanoic acid (PFHpA)	67818	$C_7HF_{13}O_2$	
Perfluorohexanoic acid (PFHxA)	67542	$C_6HF_{11}O_2$	
Perfluoropentanoic acid (PFPeA)	75921	$C_6HF_{11}O_2$	
Perfluorobutanoic acid (PFBA)	9777	$C_4HF_7O_2$	
Perfluorotetradecanoic acid (PFTeDA)	67822	$C_{14}HF_{27}O_2$	
Perfluorotridecanoic acid (PFTriA)	3018355	$C_{13}HF_{25}O_2$	
Perfluorodecanesulfonic acid (PFDS)	67636	$C_{10}HF_{21}O_3S$	
Perfluorononanesulfonic acid (PFNS)	86998	$C_9HF_{19}O_3S$	

(*Continued*)

Supplementary Table 1. (*Continued*)

PFAS	PubChem CID	Formula	Structure
Perfluorooctanesulfonic acid (PFOS)	74483	$C_8HF_{17}O_3S$	
Perfluoroheptanesulfonic acid (PFHps)	67820	$C_7HF_{15}O_3S$	
Perfluorohexanesulfonic acid (PFHxS)	67734	$C_6HF_{13}O_3S$	
Perfluoropentanesulfonic acid (PFPeS)	75922	$C_5HF_{11}O_3S$	
Perfluorobutanesulfonic acid (PFBS)	67815	$C_4HF_9O_3S$	
Perfluorooctanesulfonamide (PFOSA)	69785	$C_8H_2F_{17}NO_2S$	
Perfluoro-2-methyl-3-oxahexanoic acid (GenX)	114481	$C_6HF_{11}O_3$	
Perfluoro(4-methoxybutanoic) acid (PFMOBA)	12498036	$C_5HF_9O_3$