3D-QSAR STUDY OF THE ENDOCRINE DISRUPTING EFFECT OF PERFLUOROOCTANE SULFONATES (PFOS) AND PERFLUOROOCTANOIC ACID (PFOA) ON HUMAN ESTROGEN, ANDROGEN AND THYROID RECEPTORS

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Perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) have become emerging persistent organic pollutants (POPs), but their health effects on humans remain controversial because of contradictory experimental and epidemiological studies. In this study, we used three-dimensional quantitative structure–activity relationship (3D-QSAR) method by applying Surflex-dock to study and compare the binding modes between PFOS, PFOA and eight other endocrine disrupting chemicals, and human estrogen receptor (hERα), human androgen receptor (hAR) and human thyroid receptor (hTRβ). Molecular docking and hydrogen bond studies indicated that PFOS and PFOA had high affinity potency toward hERα, hAR and hTRβ due to low free binding energies, while the highest value was obtained toward hTRβ. This means that PFOS and PFOA might have more disrupting effects on thyroid than on estrogen and androgen receptors. Hydrogen bonding interactions revealed that Met313 in hTRβ might act as the critical amino acid residue in the binding of ligand–receptor complex, which would provide an explanation for the interaction mechanisms. Our results provide an important reference and direction for the interaction mode and mechanism study between PFOS/PFOA and human endocrine systems.

Keywords: Endocrine disruption; 3D-Quantitative structure–activity relationship; 3D-QSAR; PFOS; PFOA; Molecular docking; Human receptors; Fluorinated pollutants; Mechanism of action.

A variety of structurally diverse natural and synthetic chemicals, classified as endocrine disrupting chemicals (EDCs)[1–3], including bisphenol A, benzo-(α)pyrene, phthalates, polychlorinated biphenyls (PCBs), etc., have been reported to interfere with endocrine system and ultimately disturb the normal function of tissues and organs. Given their physicochemical differences and distinct biological effects, it is not surprising that EDCs influence the

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endocrine system through many mechanisms, including the traditional estrogen/androgen/thyroid receptor-mediated pathways and targets for endocrine disruption.

Polyfluorinated chemicals (PFCs) have become emerging persistent organic pollutants (POPs) and been widely present in the environment, wildlife and humans[4–6]. The industrial production of perfluorooctane sulfonic acid (PFOS), perfluorooctanoic acid (PFOA) and their derivatives stopped in 2000, and the European Union has banned most of their uses from June 27th, 2008. On the Stockholm Convention on Persistent Organic Pollutants held in Geneva, Switzerland from May 4th to 8th, 2009, PFOS, its salts and perfluorooctane sulfonyl fluoride were proposed for inclusion in the Stockholm Convention due to their highly toxic and persistent properties.

Although a number of experimental and epidemiological studies focused on possible endocrine disruption, the health effects of perfluoroalkyl compounds on humans remain controversial. For example, PFOS and PFOA were shown to result in lower testosterone levels and higher oestradiol levels in cell cultures[7], altered estrogenic activities in primary cultured hepatocytes of freshwater male tilapia[8] and perturbation of thyroid hormone metabolism genes in male Sprague–Dawley rats[9]. PFOS significantly regulated the gene expression related to androgens, estrogens and thyroid development in Zebrafish embryos[10,11] and disturbed thyroid function[12–15] and neuroendocrine system[16] in rats. PFOA altered activities in estrogenic signaling in trout[17], affected genes responsible for thyroid hormone biosynthesis and estrogen-responsive genes in rare minnows[18] and disturbed estrogen-responsive genes in rare minnows[19]. Whereas some reports with contrary results are thought-provoking, such as that PFOS or PFOA showed no estrogenic effects on the medaka estrogen receptor alpha or human estrogen receptor in an *in vitro* yeast two-hybrid assay[20,21]. PFOS did not induce hypothyroid state or alter hypothalamic-pituitary-thyroid (HPT) activities[22,23], and had minimal effects on the expression of thyroid-related gene transcripts[24] in rats. Also, there were neither associations between non-occupational PFOS exposures from anglers in New York state and thyroid function[25], nor significant correlations between PFOS concentration in maternal and cord blood samples from pregnant women subjects and levels of thyroid-stimulating hormone or free thyroxine[26]. PFOA was not significantly associated with estradiol or testosterone in the serum measurement studies of workers[27], and no significant positive relationships with thyroid-stimulating hormone have been found in the study of a community with longstanding environmental exposure to PFOA[28]. Therefore, for the disrupting effects of PFOS and PFOA, owing to those above-mentioned con-

trary positive results with experiment animals and negative results with humans, there is a need for further study to explore the mode of action and mechanism of PFOS and PFOA on humans. Their potential endocrine disrupting effects can be then assessed. In the meantime, due to the difficulties in performing experiment and collecting samples for human studies, *in vitro* method would be the proper protocol.

Understanding protein–ligand interactions is essential for investigating the mode of action and mechanism, but those interactions are difficult to describe. In this study, focusing on the regions where steric and electrostatic effects play a dominant role in ligand–receptor interactions, we performed molecular modeling studies to dock PFOS and PFOA into the ligand binding domain (LBD) of the human estrogen receptor (hERα), human androgen receptor (hAR) and human thyroid receptor (hTRβ). Applying the Surflex-dock program, using three-dimensional quantitative structure–activity relationship (3D-QSAR) method, we have investigated the endocrine disrupting effects and the mode of action and mechanism of PFOS and PFOA on human receptors. The binding conformation, the hydrogen binding and the free binding energy were compared between three human receptors and PFOS/PFOA, as well as some known typical environmental EDCs with different chemical structures and suitable for 3D-QSAR module study, including bisphenol A, benzo(α)pyrene, phthalates, PCBs, etc.

METHODS

Modeling Dataset

PFOS, PFOA and eight different potential EDCs were selected, including bisphenol A, diethyl phthalate, benzyl butyl phthalate, dimethyl phthalate, benzo(α)pyrene, 2-chlorobiphenyl, 2,2′,4,4′-tetrachlorobiphenyl, and 2,3,3′,4,4′-pentachlorobiphenyl.

Preparation of the Receptor Structures

Three traditional regulation receptor targets for human endocrine disruption effects were used for molecular docking and were acquired from protein data bank (PDB). They are human estrogen receptor alpha ligand binding domain in complex with the natural ligand 17beta-estradiol (EST) (hERα, with the PDB entry ID of 1ERE), human androgen receptor with metribolone (R18) (hAR, with PDB ID of 1E3G) and human thyroid hormone receptor beta with KB131084 (OEF) (hTRβ, with PDB ID of 2J4A).

Molecular Modeling

The three-dimensional structure building of small molecular ligands and all modeling were performed using molecular modeling software package Sybyl7.3 (Tripos Inc., St. Louis, Missouri, USA) running on a Linux workstation. Tripos standard molecular field, Gasteiger–Hückel charge and Powell energy optimation strategy were utilized for the energy minimizations and optimization of ligand structures. Energy convergence criterion was fixed at 0.05 kcal mol^{-1} (1 cal = 4.184 J), maximum iterations times were set at 1000, nonbond cutoff of 8 Å was adopted to consider the intramolecular interaction, and other parameters were used as default values. After the active center of receptors was analyzed by SOLV technique in SiteID program, molecular docking research was performed at the Linux workstation.

Molecular Docking

The binding interactions between small molecule ligands and hERα, hAR and hTRβ were analyzed by using Surflex-dock module in SYBYL7.3.

Before ligand docking, it is critical to search for the binding pocket of the protein. In this study, Ligand Mode was adopted to generate the protomol in the Surflex-dock program. Surflex-dock uses an empirical scoring function and a patented search engine to dock ligands into a protein's binding site. Docking is guided by the protomol, an idealized representation of a ligand that makes every potential interaction with the binding site. The protomol can be generated automatically or defined based on a cognate ligand or known active site. The ligand mode finds the cavity in the receptor protein based on the known ligand position. In addition, two parameters that can significantly affect the size and extent of the protomol generated are the threshold and the bloat value, which were set as default values. Threshold value (between 0.01 and 1) indicates how much the protomol can be buried in the protein, and increasing this number will decrease the volume. Bloat value provides a way to inflate the protomol in the number of Å (0–10) in 3D.

Surflex-dock's scoring function, which contains hydrophobic, polar, repulsive, entropic and solvation terms, was trained to estimate the dissociation constant (K_d) expressed in $-\log K_d$ unit. The free binding energies (kcal mol^{-1}) of protein–ligand complexes would be obtained according to the calculation of free energy of binding ($RT \ln K_d$, where RT = 0.59 kcal mol^{-1}).

RESULTS AND DISCUSSION

The Active Site of Ligand Binding Domain (LBD) in Receptors

The active sites of LBD in hERα, hAR and hTRβ calculated from SiteID program were shown in Fig. 1.

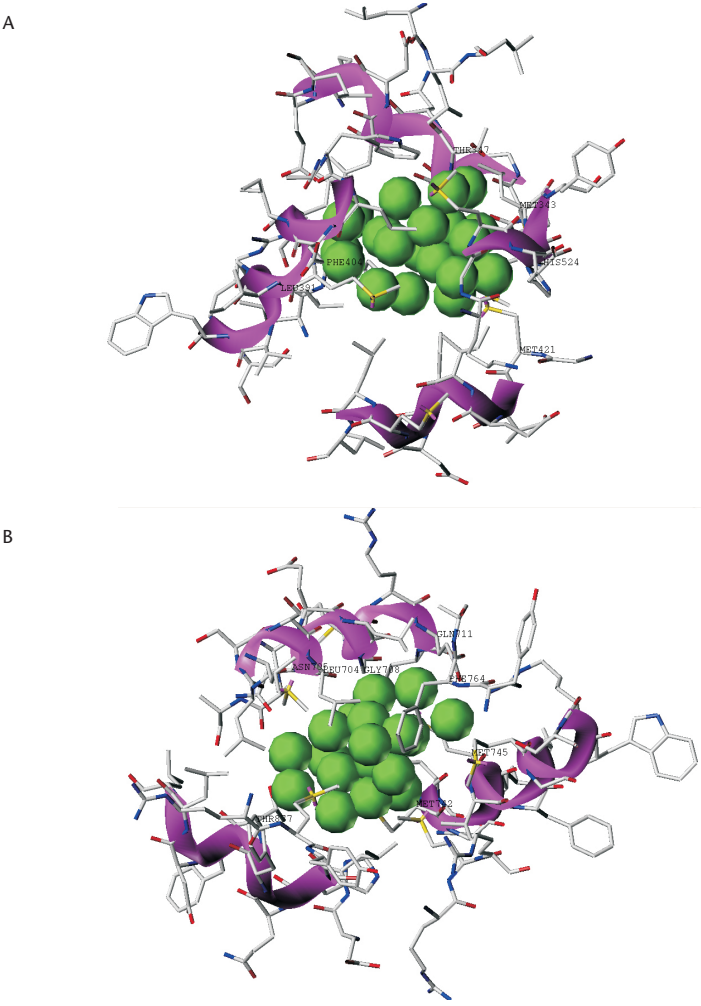


FIG. 1
The active solvent pockets of hERα (A), hAR (B) and hTRβ (C) receptors. The helix structure in the receptor is indicated in magenta, sheet structure in yellow, and other structure in cyan. Solvent pockets searched by SiteID program are shown with green sphere cluster

C

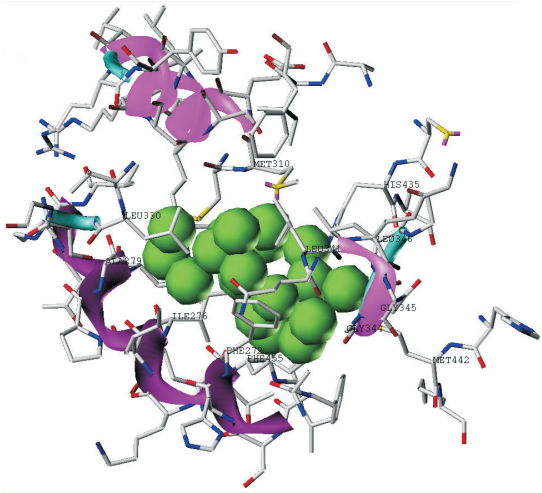


FIG. 1
(*Continued*)

hERα formed a hydrophobic cavity with pocket volume of about 315 Å^3 surrounded with most of hydrophobic critical amino acid residues, such as Leu384, Leu387, Leu391, Phe404, Ile424, Phe425, Leu346, Leu428, Gly521, Leu525, Leu540, Leu349, Ala350, etc. in 6 Å distance. The pocket volume of hAR was about 285 Å^3 surrounded with Leu701, Leu704, Leu707, Gly708, Val746, Phe764, Leu873, Phe876, Leu880, etc., and the pocket volume of hTRβ was about 90 Å^3 surrounded with Ala317, Leu330, Leu341, Leu346, Ile353, Phe272, Ile275, Ile276, Ala279, etc. Therefore, the pocket volume of the three receptors was in the order of hERα > hAR >> hTRβ, which could affect the differentiation of ligands that bind or incorporate into the receptor pockets.

Hydrogen Bonding and Interactions of Aromatic Rings Between Receptors and Ligands

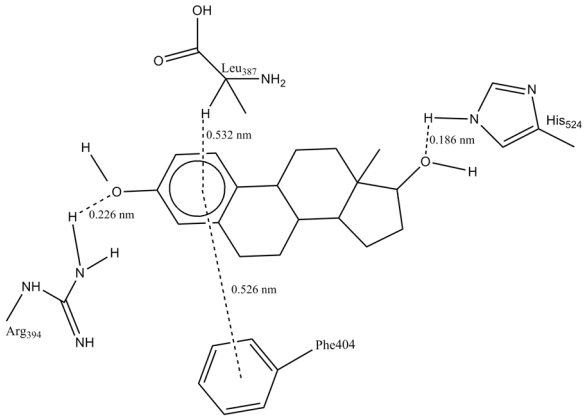
Hydrogen bonds between hydrogen and oxygen, nitrogen and halogen atoms, stacking interactions of aromatic rings, interactions between functional H atoms and aromatic rings between receptors and ligands were also identified (Table I) to help to understand the interactions of the protein–ligand complexes, which allowed us to determine the amino acid residues involved in the recognition of endocrine disrupting ligands. Dashed lines indicate the interactions, and the distances are shown, too. The other EDCs not shown in Table I formed no hydrogen bonds with the three receptors.

TABLE I
Hydrogen bonds and interactions of aromatic rings between receptors and small molecular ligands[a]

Name	hERα (1ERE)	hAR (1E3G)	hTRβ (2J4A)
Natural ligand	EST (I)	R18 (II)	OEF (III)
PFOA	IV	V	VI
PFOS	VII	VIII	IX
Bisphenol A	X	XI	XII
Diethyl phthalate	XIII	XIV	XV

[a] Structures I–XV see below.

I



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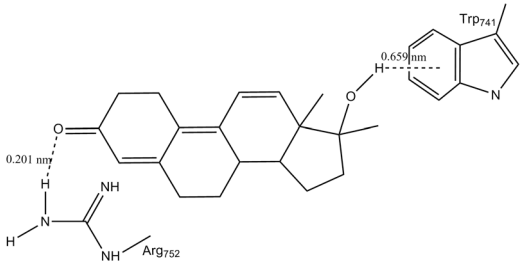

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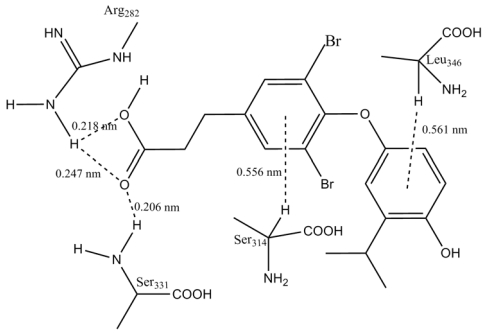
Arg282
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NH
H
N
O
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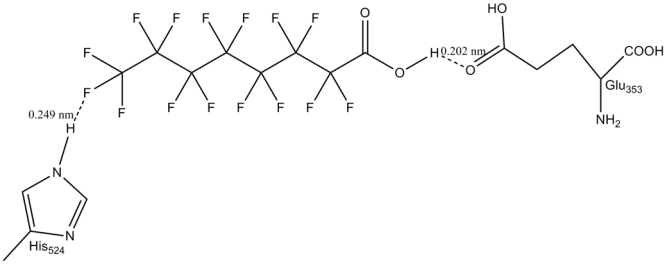
H
0.247 nm
O
0.206 nm
H
H
N
Ser331
COOH
Br
COOH
Leu346
NH2
H
0.561 nm
O
0.556 nm
Br
H
Ser314
COOH
NH2
OH

IV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F
F
F
F
F
F
F
F
F
F
F
F
F
F
F
O
O
H
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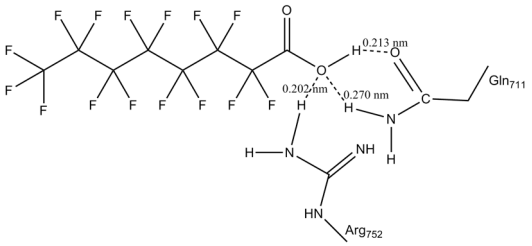
HO
O
COOH
Glu353
NH2
0.249 nm
H
N
N
His524

V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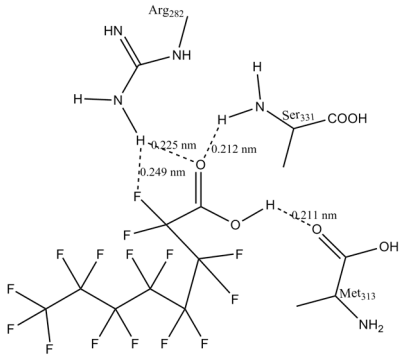
0.270 nm
H
H
N
C
Gln711
H
N
NH
H
HN
Arg752

VI


Arg282
HN
NH
H
N
H
N
Ser331
COOH
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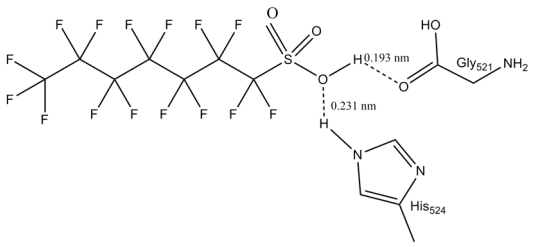
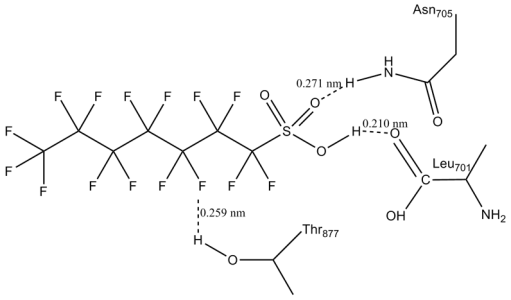
0.249 nm
O
F
F
O
H
0.211 nm
O
OH
F
F
F
F
F
F
F
F
F
F
F
F
F
F
F
Met313
NH2

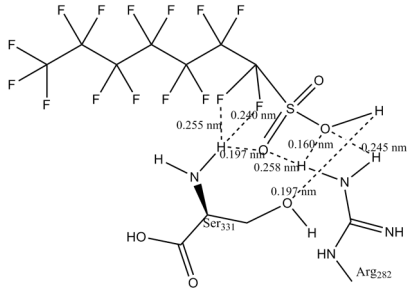
VII


0.193 nm
Gly521
NH2
0.231 nm
His524

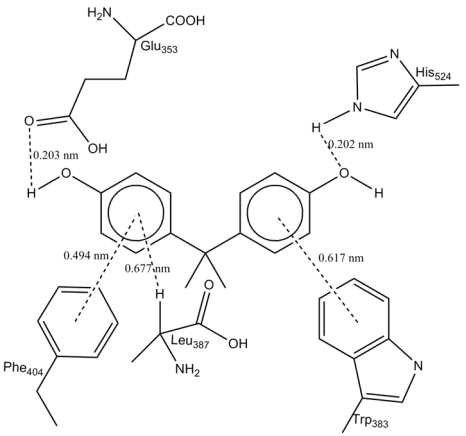
VIII


Asn705
0.271 nm
0.210 nm
Leu701
0.259 nm
Thr877

IX


0.240 nm
0.255 nm
0.160 nm
0.245 nm
0.197 nm
0.258 nm
0.197 nm
Ser331
Arg282

X


Glu353
His524
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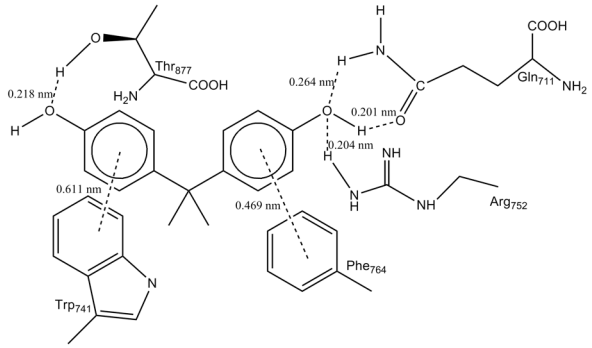
0.202 nm
0.494 nm
0.677 nm
0.617 nm
Leu387
Phe404
Trp383

XI



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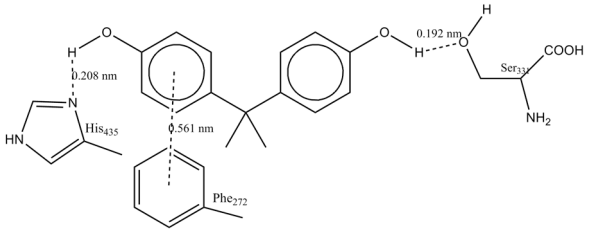

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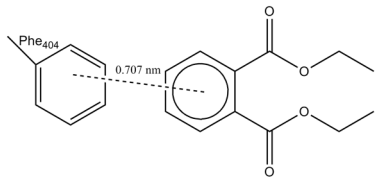
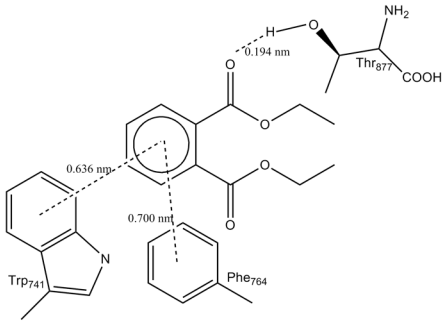
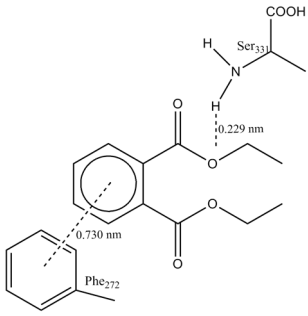


XIV



XV



For the natural ligands of the three receptors studied, EST forms two hydrogen bonds with His524 and Arg394 of hERα using the two hydroxy groups on its ring structure, one stacking interaction between the aromatic rings of EST and Phe404, and one interaction between the aromatic ring of EST and the functional alpha hydrogen of Leu387. R18 forms one hydrogen bond with Arg752 of hAR using its carbonyl group, and one interaction between the functional hydroxy hydrogen of R18 and the aromatic ring of Trp741. OEF froms three hydrogen bonds with Arg282 and Ser331 of hTRβ using its carboxy group, two interactions between the two aromatic rings of OEF and the functional alpha hydrogens in Ser314 and Leu346, respectively. Among the eight different potential EDCs, bisphenol A and diethyl phthalate could form hydrogen bonds with hERα, hAR or hTRβ receptors attributed to their hydroxy group or carboxy group, and also some stacking interactions of aromatic rings, interactions between functional H groups and aromatic rings for receptors and small molecular ligands.

However, for PFOA, there is no interactions of aromatic rings but there are some hydrogen bonds between hydrogen and oxygen, nitrogen and halogen atoms due to the molecular structure. In hERα, the hydroxy group of the carboxy group of PFOA formed one hydrogen bond with Glu353 and the fluorine atom in the other end of PFOA formed another hydrogen bond with the hydrogen atom on the imidazole ring of His524. In hAR, the hydroxy group of the carboxy group of PFOA formed three hydrogen bonds with Gln711and Arg752. And in hTRβ, the carboxy group of PFOA formed three hydrogen bonds with Ser331, Arg282 and Met313 and the fluorine atom on the carbon atom next to the carboxy group of PFOA formed the fourth hydrogen bond with the hydrogen atom on the side chain of Arg282.

Similarily, in hERα, the hydroxy group of the sulfonic group of PFOS formed two hydrogen bonds with His524 and Gly521. In hAR, the hydro-

gen atom on the hydroxy group of the sulfonic group of PFOS formed one hydrogen bond with Leu701, the oxygen atom of the sulfonic group formed the second hydrogen bond with Asn705, and the fluorine atom on the third carbon from the sulfonic group of PFOS formed the third hydrogen bond with Thr877. And in hTRβ, the hydroxy group of the sulfonic group of PFOS formed three hydrogen bonds with Ser331 and Arg282, the oxygen atom of the sulfonic group formed two hydrogen bonds with Ser331 and Arg282, and the fluorine atom on the carbon atom next to the sulfonic group of PFOS formed two hydrogen bonds with Ser331 and Arg282.

Meanwhile, the multiple hydrogen bonds between PFOS or PFOA and the receptors formed binding network, resulting in several six- or five-membered ring structures, which would help strengthen the interactions between PFOS or PFOA and human receptors.

Surflex-Docking for Ligands and Receptors

The mechanism of the selective binding of ligands to hERα, hAR and hTRβ was further explored with Surflex-docking. The scoring for each docked protein–ligand complexes was performed to evaluate the docking results, and the free binding energies (kcal mol^{-1}) of docked complexes were obtained and shown in Table II. It can be seen that the free binding energies of receptor–PFOS/PFOA complexes were equal or less than free binding energies for the most of the receptor–EDC complexes, such as PCBs, benzo(α)pyrene, phthalates, and bisphenol A.

Figures 2, 3 and 4 show the Surflex-docking results for some ligands fitted into the putative binding pockets in hERα, hAR and hTRβ. The ligands shown in Figs 2, 3 and 4 are the natural ligands for each receptor, the EDCs with the lowest free binding energy for each receptor, PFOS and PFOA, respectively.

For the 3D-structures of the docked hERα complexes illustrated in Fig. 2, bisphenol A showed the lowest free binding energy (–11.65 kcal mol^{-1}) with hERα. For PFOS and PFOA, the carbon backbone and the lipophilic moieties were incorporated into the hydrophobic pocket formed by hydrophobic amino acid residues of hERα. And the hydrophilic hydroxy group of the carboxy group or the sulfonic group at the other end of the structure of PFOS or PFOA formed hydrogen bonds with the amino acid residues of hERα to strengthen the intermolecular interaction between hERα and PFOS/ PFOA.

For the docked hAR-ligand complexes shown in Fig. 3, the lowest free binding energy with hAR was found for benzo(α)pyrene (–12.87 kcal mol^{-1}).

Similarily, for PFOS and PFOA, the carbon backbone was nearly submerged in the hydrophobic pocket formed by hydrophobic amino acid residues of hAR with no excess of steric blocking. The hydrophilic hydroxy group of the carboxy group or the sulfonic group at the other end of the structure of PFOS or PFOA formed hydrogen bonds with the amino acid residues of hAR to strengthen the intermolecular interaction between hAR and PFOS/PFOA.

For the docked hTRβ-ligand complexes in Fig. 4, attention should be paid to the small volume of the putative pocket. This means that the steric conformation of the ligands would affect the interaction between ligands and hTRβ to a large extent, and the cavity could distinguish slightly different ligands with distinct binding properties. The lowest free binding energy with hTRβ was shown for benzyl butyl phthalate (–11.72 kcal mol^{-1}). For PFOS and PFOA with no ring structure, most of the carbon backbone was surrounded by the active pocket with no excess steric blocking, and the hydrophilic hydroxy group of the carboxy group or the sulfonic group at the other end of the structure of PFOS or PFOA formed hydrogen bonds with the amino acids of hTRβ, to strengthen the intermolecular interaction between hTRβ and PFOS/PFOA.

TABLE II
Free binding energies (in kcal mol^{-1}) for ligands and receptors

Ligands	Free binding energy for receptors		
	hERα	hAR	hTRβ
PFOS	–7.64	–8.13	–8.22
PFOA	–7.39	–6.98	–10.32
Bisphenol A	–11.65[a]	–9.46	–11.47
Benzo(α)pyrene	–5.99	–12.87[a]	–4.17
Diethyl phthalate	–7.69	–6.38	–10.18
Benzyl butyl phthalate	–10.59	–6.22	–11.72[a]
Dimethyl phthalate	–6.49	–7.37	–7.11
2-Chlorobiphenyl	–8.06	–6.68	–9.83
2,2′,4,4′-Tetrachlorobiphenyl	–4.88	–4.63	–6.98
2,3,3′,4,4′-Pentachlorobiphenyl	–6.26	–2.67	–5.64

[a] The ligand with the lowest free binding energy for each receptor.

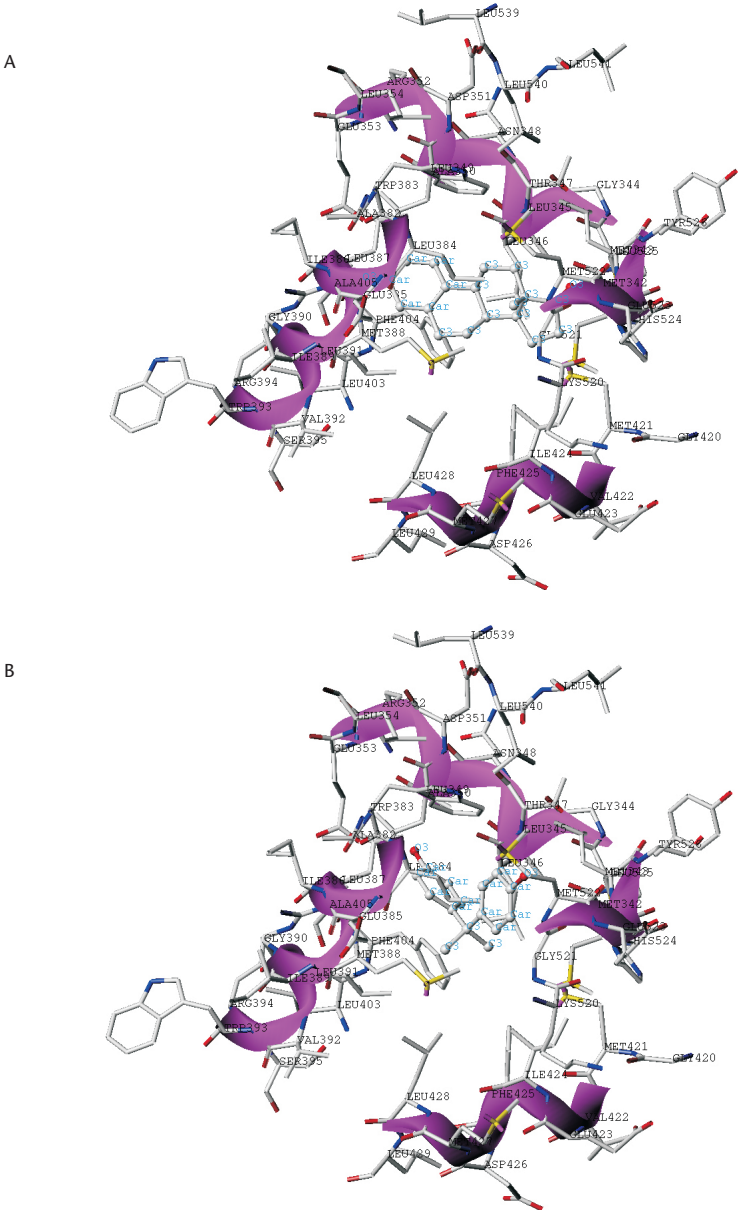
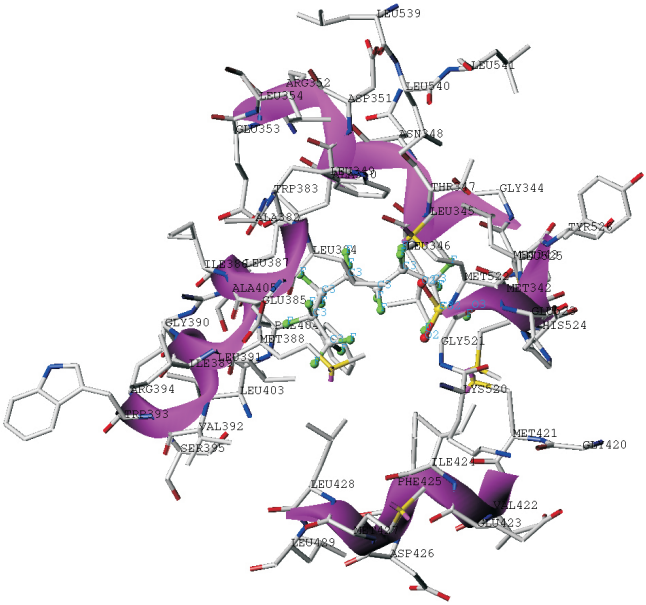


FIG. 2

The interaction between ligands (ball and stick style) and hERα (line style). The ligands are EST (A), Bisphenol A (B), PFOS (C) and PFOA (D). The helix structure in the receptor is indicated in magenta, sheet structure in yellow, and other structure in cyan

C



D

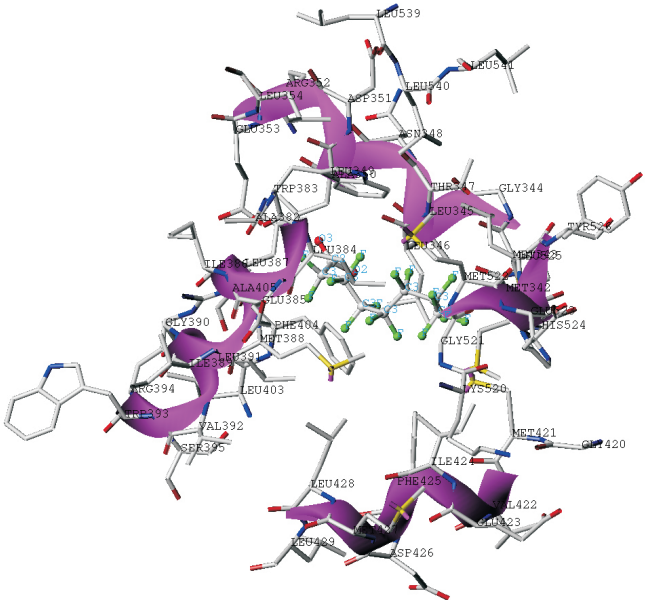


FIG. 2
(*Continued*)

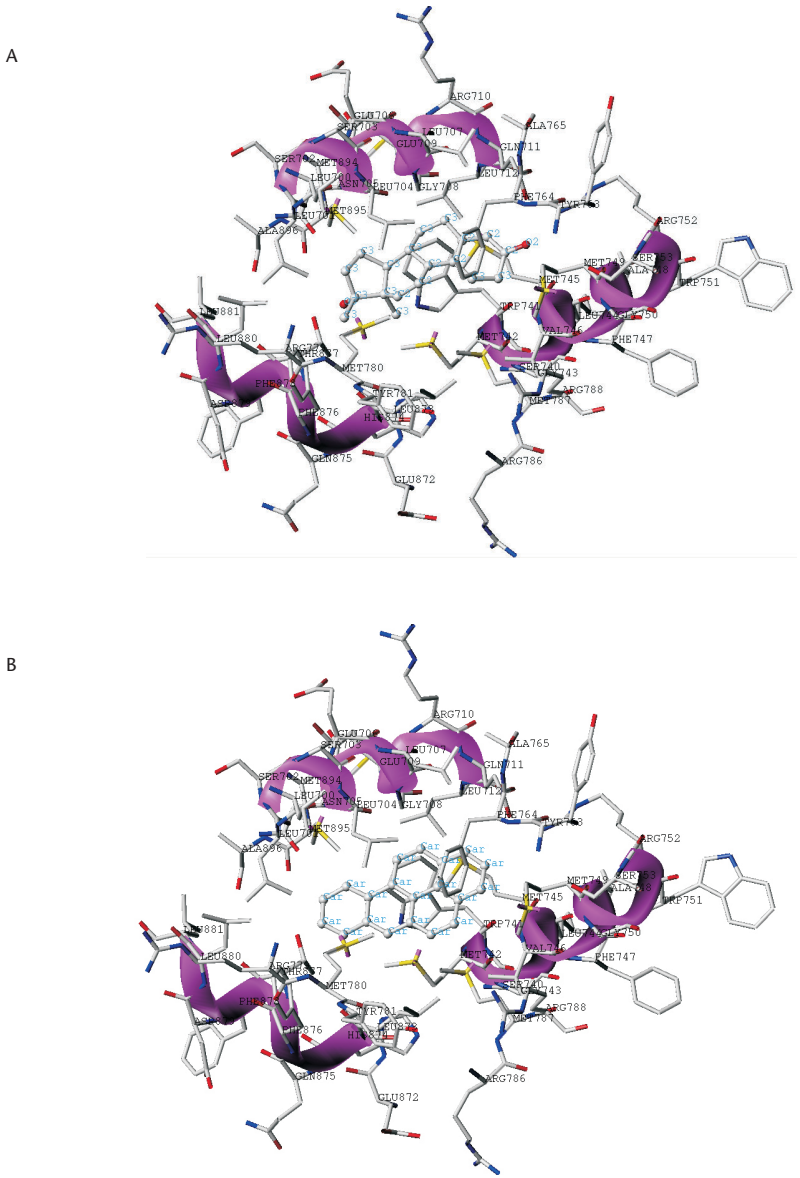
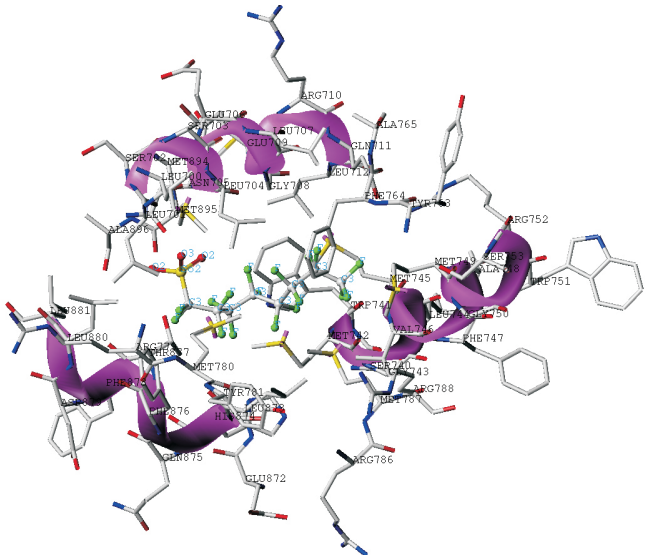


FIG. 3
The interaction between ligands (ball and stick style) and hAR (line style). The ligands are R18 (A), benzo(α)pyrene (B), PFOS (C) and PFOA (D). The helix structure in the receptor is indicated in magenta, sheet structure in yellow, and other structure in cyan

C



D

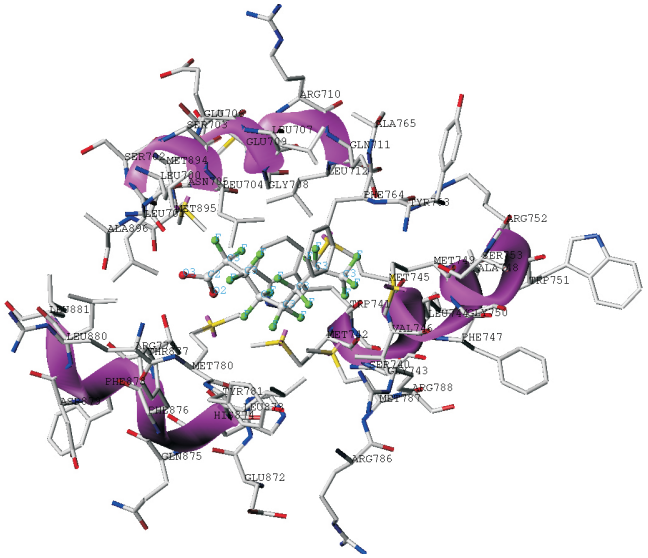


FIG. 3
(*Continued*)

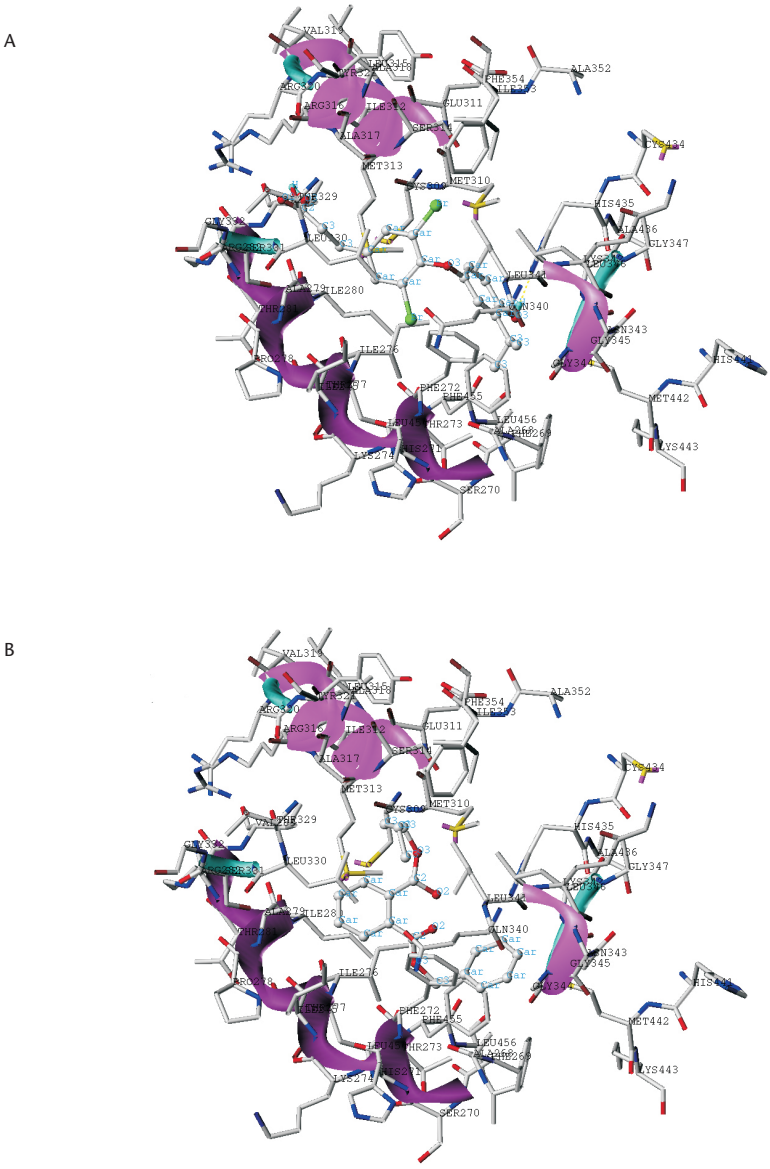
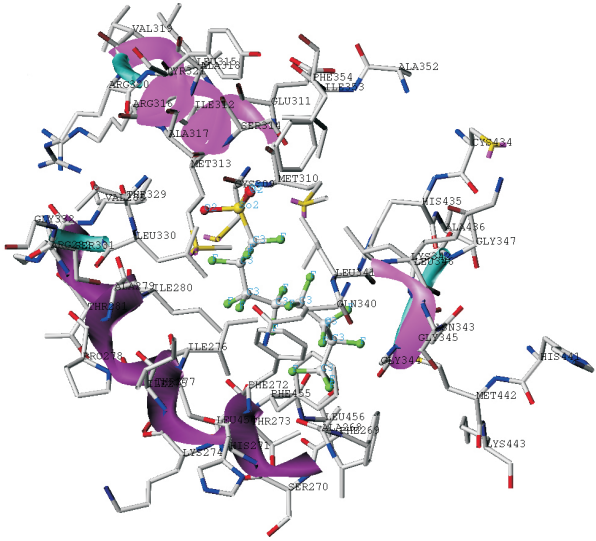


FIG. 4
The interaction between ligands (ball and stick style) and hTRβ (line style). The ligands are OEF (A), benzyl butyl phthalate (B), PFOS (C) and PFOA (D). The helix structure in the receptor is indicated in magenta, sheet structure in yellow, and other structure in cyan

C



D

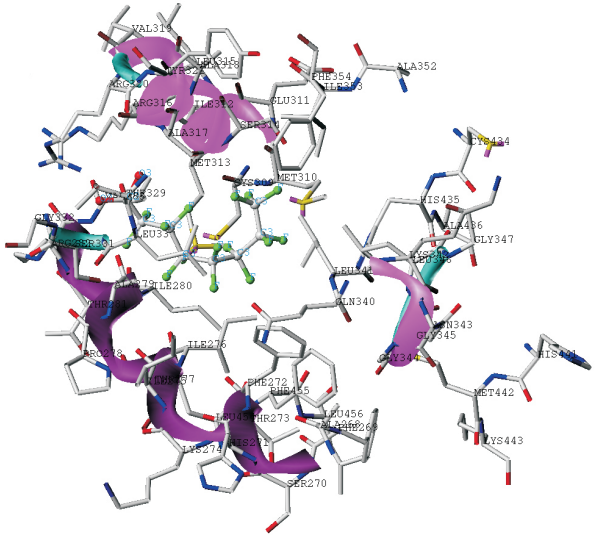


FIG. 4
(*Continued*)

The Interaction Mechanism Speculation for Receptors and PFOA/PFOS

According to the hydrogen bonding analysis and the docking results for ligands and receptors, it could be speculated that PFOS and PFOA have high interaction potency toward hERα, hAR and hTRβ and might be environmental endocrine disrupting pollutants for human beings. This was consistent with the positive endocrine disrupting results for PFOS and PFOA in experiments with animals[7–19].

The number of hydrogen bonds formed between PFOS/PFOA and the three receptors was in the order of hTRβ > hAR > hERα, and multiple hydrogen binding network structures were formed between PFOS/PFOA and receptors. Due to the different free binding energies, the affinity between PFOS/PFOA and receptors was in the order of hTRβ > hAR > hERα for PFOS, and hTRβ > hERα > hAR for PFOA. It could be presumed that PFOS/PFOA have higher affinity for hTRβ than for hERα and hAR. This might mean that PFOS/PFOA likely interfere the human endocrine system mainly through the thyroid receptor-mediated pathway, and to a less extent through the estrogen/androgen receptor-mediated pathway. In fact, the results obtained in this study were consistent with previously reported findings in animals[12–15,18].

Additionally, analyses of hydrogen bond interactions confirmed that Arg282, Ser331 and Met313 in hTRβ play relatively important roles in binding potency. The hydrogen atoms of the side chain of Arg282, the backbone hydrogen atom of Ser331 and the backbone oxygen atom of Met313 were inclined to form hydrogen bonds with the polar moieties at one end of the structure of PFOS or PFOA, such as the hydroxy group of the carboxy group or the sulfonic group. Especially, the hydrogen atoms of the side chain of Arg282 formed the major network of the hydrogen bonds with hTRβ. Interestingly, the carbonyl oxygen atom of the carboxy group of the backbone of Met313 in the hTRβ formed a hydrogen bond with the hydrogen atom of the carboxy group at the end of the structure of PFOA, which did not appear in the interaction between hTRβ and its natural ligand OEF. From these results, it appears that Met313 plays a critical role in the hydrogen bond interactions of the protein–ligand complex, which may provide a breakthrough point for the interaction mechanism study of the endocrine disrupting effect of PFOS and PFOA on humans.

CONCLUSIONS

We applied Surflex-docking to study and compare the interaction modes and mechanisms between a set of endocrine disrupting chemicals and human estrogen receptor, androgen receptor and thyroid receptor. Molecular docking results indicated that PFOS and PFOA have high affinity potency toward hERα, hAR and hTRβ due to the low free binding energies and might be environmental endocrine disrupting pollutants for human beings. Docking and hydrogen bond studies demonstrated that PFOS and PFOA have greater affinity potency for hTRβ than for hERα and hAR, which means that PFOS and PFOA might represent more disrupting effects toward thyroid than toward estrogen and androgen signaling pathways. Hydrogen bond interactions revealed that Met313 in hTRβ might act as the critical amino acid residue in the binding of ligand–receptor complex, which could provide an explanation for the interaction mechanism and need further experimental research to verify. Our results indicate the possible endocrine disrupting effects and pathways for PFOS/PFOA to affect humans, and could provide an important reference and direction for the study of the effects of PFOS and PFOA on human endocrine systems.

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