Supporting Information

(12 pages, 3 tables, and 5 figures)

Interactions of perfluorooctanesulfonate (PFOS) and 6:2 chlorinated polyfluorinated ether sulfonate (6:2 Cl-PFESA) with human serum albumin (HSA): A comparative study

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Supplementary methods

Cell viability analysis after treatment with various concentrations of HSA, PFOS, or 6:2 Cl-PFESA

We studied the potential influence of HSA, PFOS, and 6:2 Cl-PFESA in culture medium on JAR cell viability. In brief, JAR cells were first plated in 96-well plates (5 × 10^3 cells/ well) and cultured for 24 h. After washing three times with PBS, serum-free DMEM/F12 medium with different concentrations of HSA protein, PFOS, and 6:2 Cl-PFESA (Table S1) was added to the wells. Cells were then cultured for 48 h before cell viability measurement by MTT assay.

Fluorescence displacement assays

Fluorescence displacement experiments were performed using a Horiba fluorescence spectrometer (Fluoromax-4 spectrofluorometer). Briefly, purified peptides, 1,8-ANS, PFOS and 6:2 Cl-PFESA were dissolved and diluted with Tris-HCl buffer (pH 8.0) before experiment. Different concentration of 1,8-ANS was added into 1 μM peptide solution to make the final 1,8-ANS concentrations ranging from 0-30 μM (Table S1). After equilibrating at room temperature for 2 minutes, the fluorescence spectrum was scanned (scanning wavelength: 410-550 nm, excitation wavelength: 392 nm, slit width: 5 nm, 5 nm). Three repeated scans were performed, and the average value was used to calculate the Kd of 1,8-ANS to two peptides. To obtain the replacement curves of PFOS/6:2 Cl-PFESA to 1,8-ANS, different concentrations of PFOS or 6:2 Cl-PFESA was added into the 1,8-ANS-Peptide incubation system. Volume of the final system was 100 μL, and the final concentration of 1,8-ANS and PFOS/6:2 Cl-PFESA was

shown in Table S1. After equilibrating at room temperature for 5 minutes, fluorescence spectrum was scanned and repeated for three times, fluorescence intensity values at 470 nm were used to fit the replacement curve.

Table S1. Concentrations of HSA, purified peptides, 1,8-ANS, PFOS, and 6:2 Cl-PFESA used in different experiments.

Experiment	Chemical	Concentration (μM)	DMSO % (v/v)
Stock solution	PFOS	50 mM	100
	6:2 Cl-PFESA	50 mM	100
MTT	HSA	0, 0.15, 0.3, 0.6, 1.25, 2.5, 5, 7.5, 12.5, 25, 37.5, 50, 75, 100, 150	0
	PFOS	0, 6.25, 12.5, 25, 50, 100, 150, 200, 300, 400, 500, 600, 800, 1000, 1500	< 3%
	6:2 Cl-PFESA	0, 6.25, 12.5, 25, 50, 100, 150, 200, 300, 400, 500, 600, 800, 1000, 1500	< 3%
Ultrafiltration centrifugation	HSA	5	0
	PFOS	1, 1.5, 2, 2.5, 5, 10, 20, 30, 40, 45, 50	< 0.1%
	6:2 Cl-PFESA	1, 1.5, 2, 2.5, 5, 10, 20, 30, 40, 45, 50	< 0.1%
Fluorescence displacement	Peptide I/II	1	0
	1,8-ANS	0, 0.1, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, 10, 15, 20, 30	0
	PFOS	0, 0.1, 0.5, 1.5, 2.5, 5, 10, 20, 40, 80, 200, 500	< 1%
	6:2 Cl-PFESA	0, 0.1, 0.5, 1.5, 2.5, 5, 10, 20, 40, 80, 200, 500	< 1%

Table S2. Predicted and measured secondary structure contents of Peptide I and Peptide II.

	α-helix (%)	β-sheet (%)	Random coil (%)
Peptide I-predicted	65.1	0	34.9
Peptide I	74.3	6.8	18.9
Peptide II-predicted	69.9	0	30.1
Peptide II	71.5	5.7	22.8

Table S3. Top optimal sites and tightly binding sites obtained by molecular docking and trypsin proteolysis assay, respectively. Sites acquired by both methods were displayed with orange numbers.

	The top 3 optimal sites observed by molecular docking	**Tightly binding sites obtained by proteolysis and sequence alignment**
PFOS	site 6 > site 3 > site 2	Site 3, 4, 6 and 7
6:2 Cl-PFESA	site 2 > site 6 > site 3	site 1, 2, 7 and part of site 6

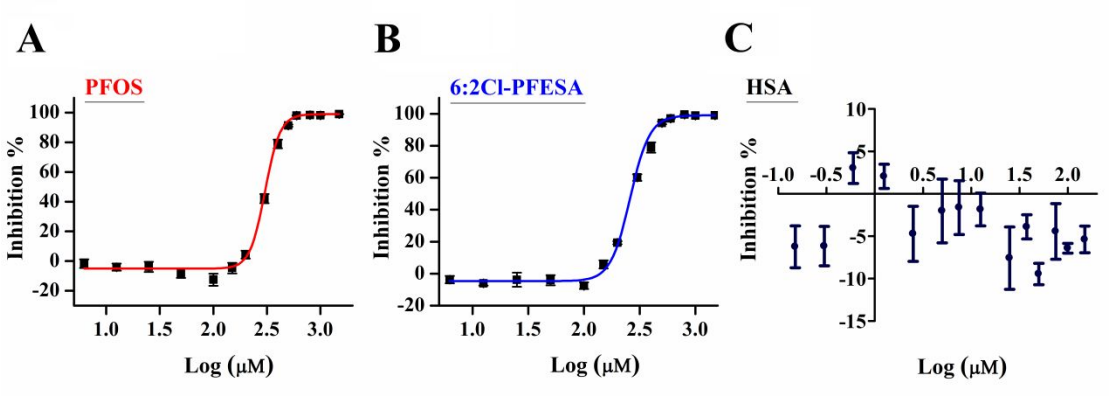
A
PFOS
Inhibition %
Log (μM)
B
6:2Cl-PFESA
Inhibition %
Log (μM)
C
HSA
Inhibition %
Log (μM)

Fig. S1

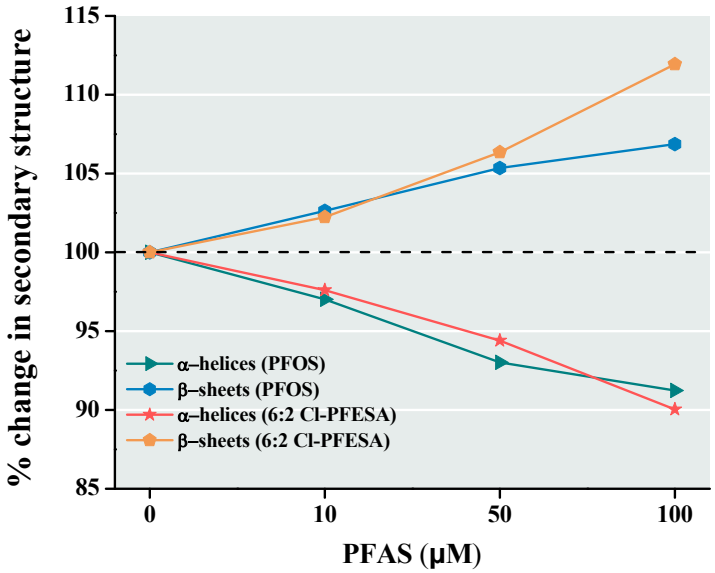
% change in secondary structure
115
110
105
100
95
90
85
0
10
50
100
PFAS (μM)
α–helices (PFOS)
β–sheets (PFOS)
α–helices (6:2 Cl-PFESA)
β–sheets (6:2 Cl-PFESA)

Fig. S2

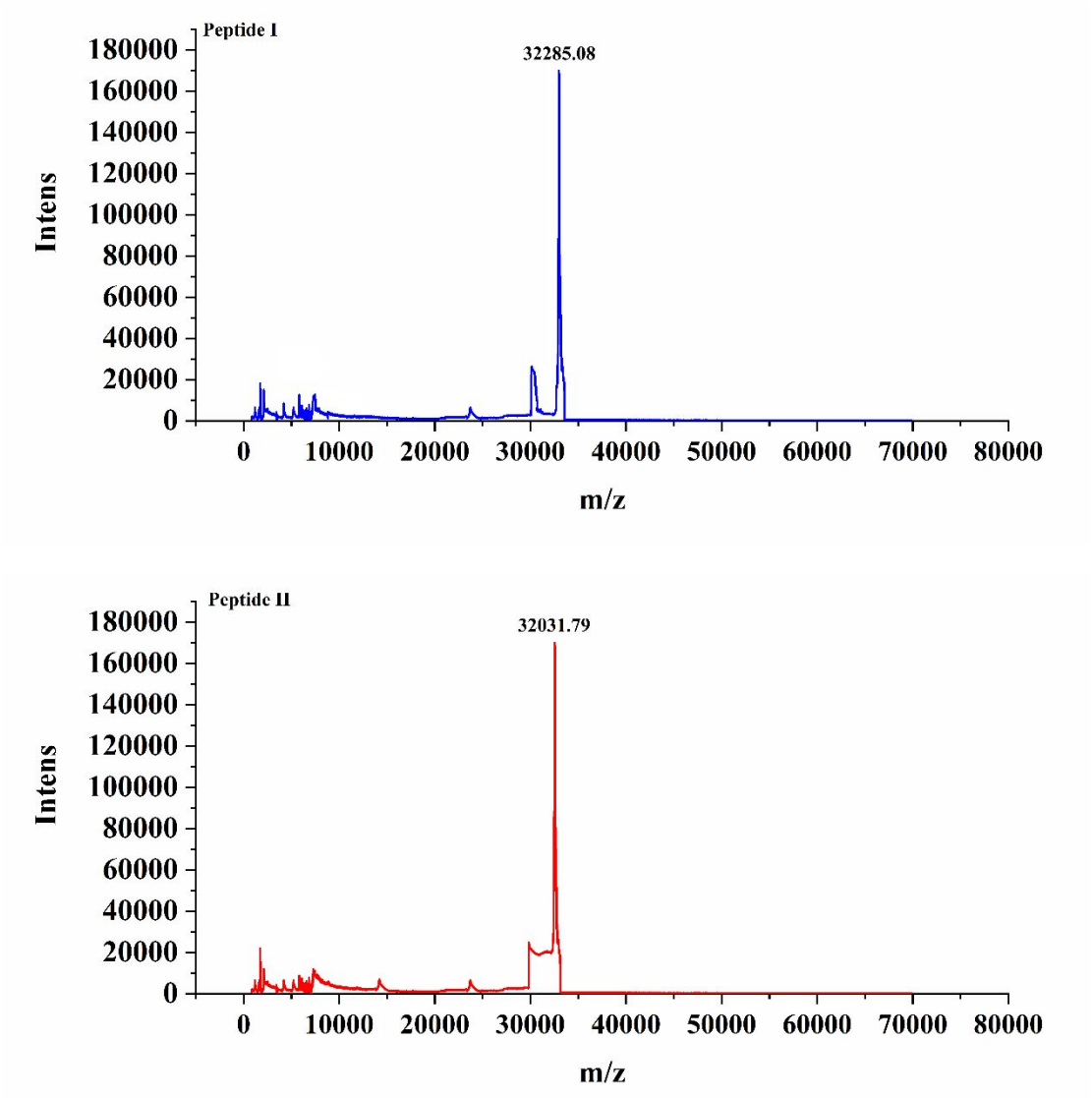
Peptide I
32285.08
Intens
180000
160000
140000
120000
100000
80000
60000
40000
20000
0
0
10000
20000
30000
40000
50000
60000
70000
80000
m/z
Peptide II
32031.79
Intens
180000
160000
140000
120000
100000
80000
60000
40000
20000
0
0
10000
20000
30000
40000
50000
60000
70000
80000
m/z

Fig. S3

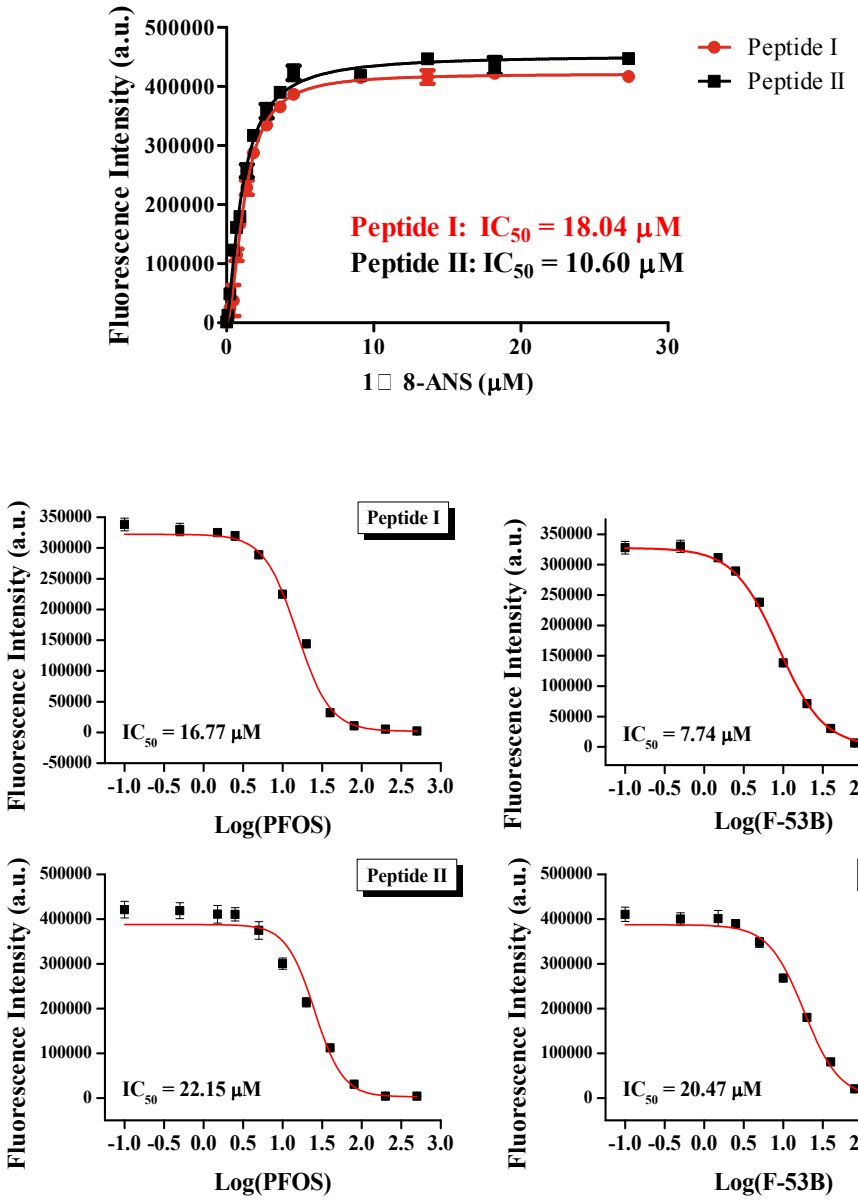
Fluorescence Intensity (a.u.)
500000
400000
300000
200000
100000
0
0
10
20
30
1□ 8-ANS (μM)
Peptide I
Peptide II
Peptide I: IC50 = 18.04 μM
Peptide II: IC50 = 10.60 μM
Peptide I
IC50 = 16.77 μM
Log(PFOS)
Peptide I
IC50 = 7.74 μM
Log(F-53B)
Peptide II
IC50 = 22.15 μM
Log(PFOS)
Peptide II
IC50 = 20.47 μM
Log(F-53B)

Fig. S4

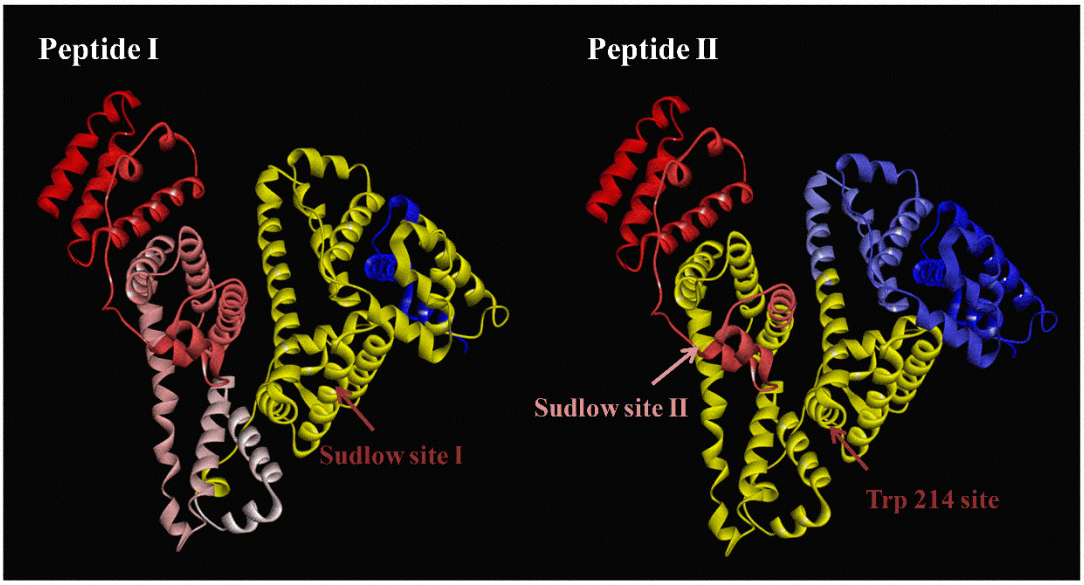
Peptide I
Peptide II
Sudlow site I
Sudlow site II
Trp 214 site

Fig. S5