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We will begin our presentation in a few minutes…

Leadership and Excellence in Environmental Engineering and Science

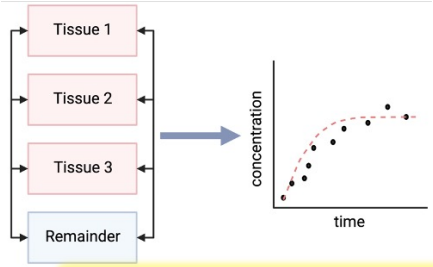
When persistence doesn't pay:
Integrative approaches to understand and predict the hazards of poorly studied per- and polyfluoroalkyl substances (PFAS).

Carla Ng
Department of Civil & Environmental Engineering

AAEES Webinar
Wednesday, January 15, 2025

University of Pittsburgh

The Ng Lab at Pitt: Legacy and Emerging Chemicals in Human-Environment Systems



Organisms as Reactors

- Organism models used to predict tissue distribution of chemicals.
- Guide *who and how* to *sample* to protect ecosystems.

Molecular Models

- Understanding toxicity and remediation for degradation of "forever chemicals" (PFAS).

Human Exposure via Food

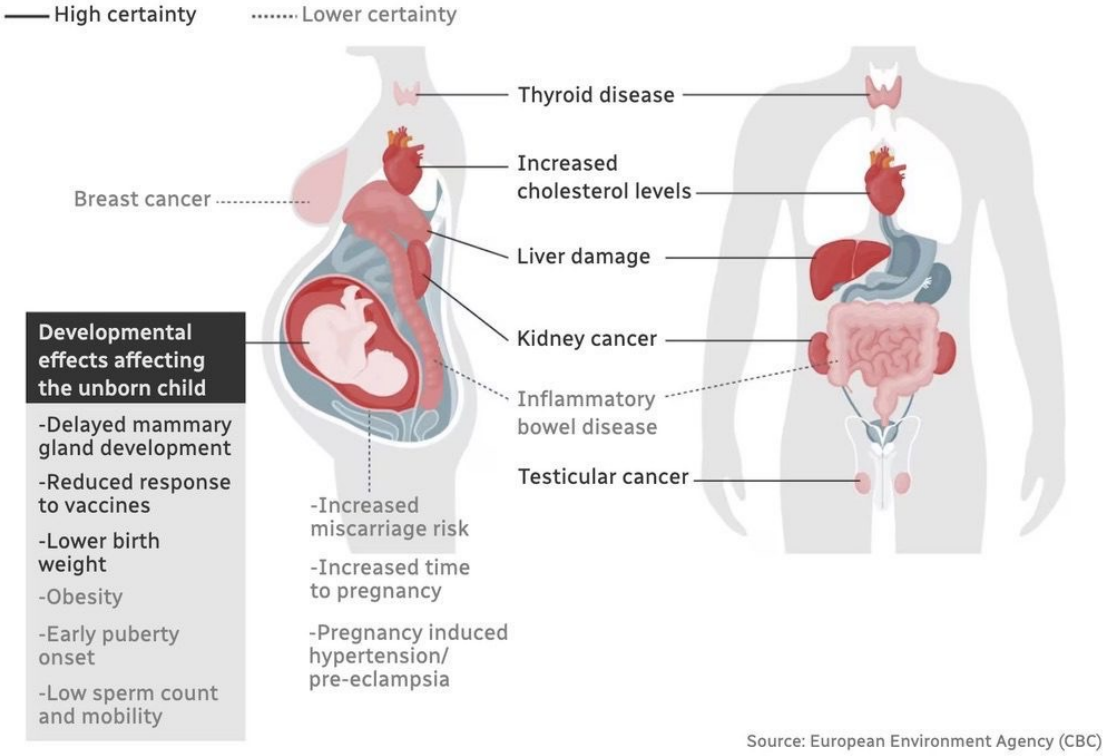
- PFAS in seafood and packaged foods.
- Pesticides, POPs, veterinary drugs in wild and farmed seafood.

Regional Contamination, Near and Far

- Sudden and chronic chemical releases in McKeesport, East Palestine, and Beaver County.
- Collaborative sampling in Ghana and Suriname.

PFAS: Globally distributed… and global toxicants?

Existing studies on PFAS show effects on a multitude of organ systems.



Source: European Environment Agency (CBC)

PFAS are known to disrupt fetal development, cause liver damage and increase circulating cholesterol.

The IARC recently classified PFOA as carcinogenic to humans, and PFOS as possibly carcinogenic to humans.

Other effects with lower certainty show differing results across studies or between humans and animals.

The compilation of this information has taken decades.

Tissue distribution observations drive theories:

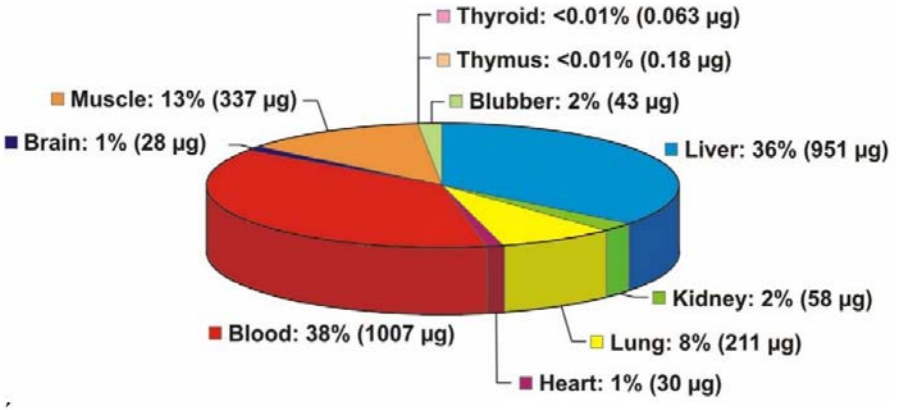
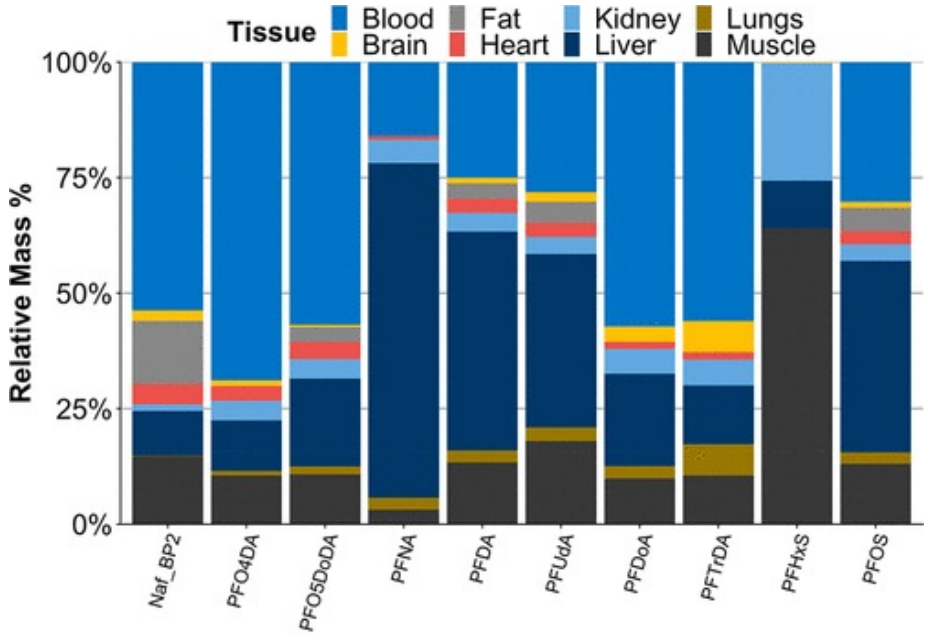


Fig. 1. PFC whole body burden distribution in percent and µg per tissue in brackets for harbor seals (*Phoca vitulina*) from the German Bight.

Ahrens et al. 2009: seals



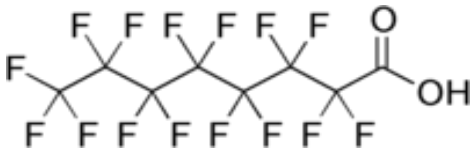
Robuck et al. 2021: Cape Fear River Estuary birds.

Patterns of PFAS distribution in different species show:

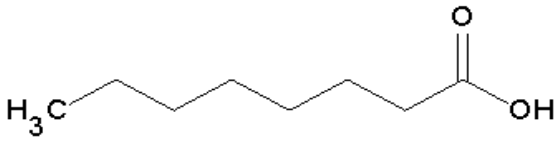
- Specificity (preference for specific tissues/components)
- Selectivity (different patterns for different PFAS).

What can PFAS molecular interactions tell us?

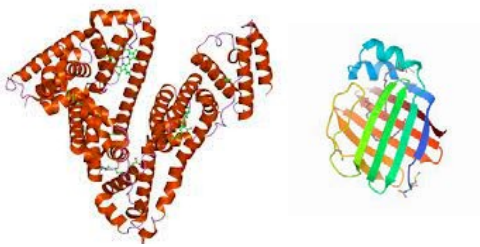
perfluorooctanoic acid (PFOA)

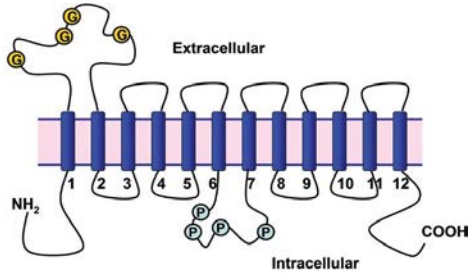


octanoic acid



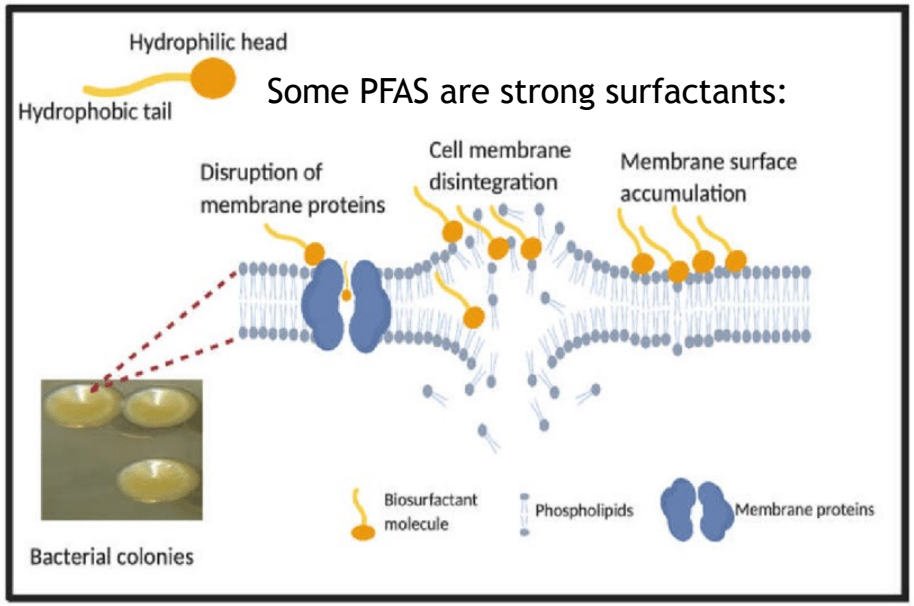
Fatty acid carriers in the body, e.g. serum albumin, liver fatty acid binding protein, bind PFAS.





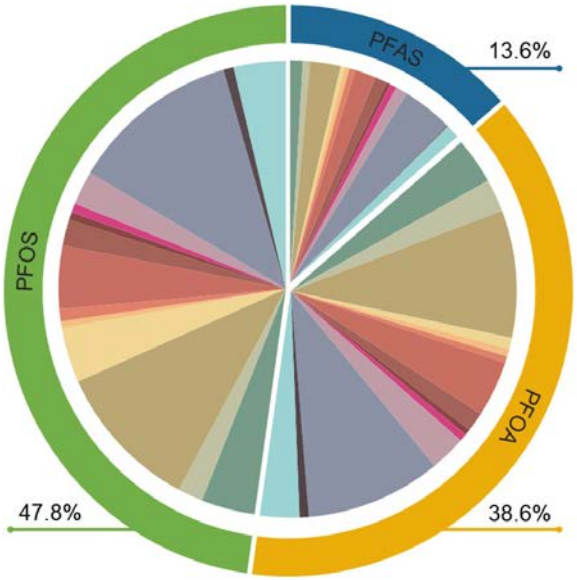
Nigam et al. 2015 Physiol Rev

Organic anion transport proteins and polypeptides in the liver, kidneys, … ?, mediate elimination rates.



But: still many known and unknown unknowns

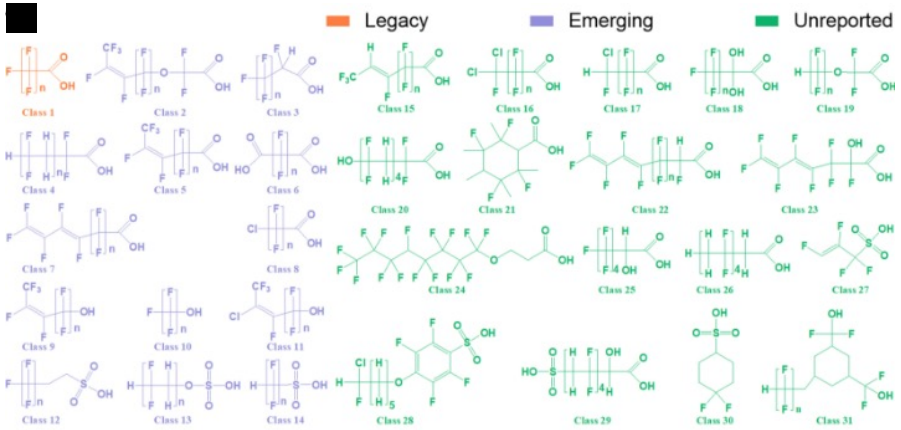
Most (>86%) toxicity studies have focused on only two PFAS: PFOA and PFOS. <15% addressed the thousands of other PFAS. Cell viability, endocrine, reproductive and metabolism effects were the most frequently studied endpoints.



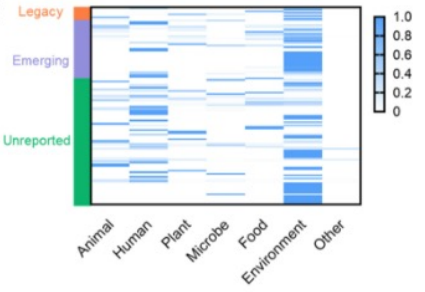
Number of publications

	PFAS	PFOA	PFOS
Body weight	44	159	184
Cancer	25	112	86
Cell	107	438	506
Heart	14	38	188
Bone	17	27	23
Nervous	22	23	41
Endocrine	73	191	216
Immune	43	67	89
Respiratory	13	20	27
Skin	24	26	31
Kidney	41	123	115
Metabolism	179	467	581
Genotoxicity	3	32	35
Reproductive	48	135	178

Wee and Aris, npj Clean Water 2023

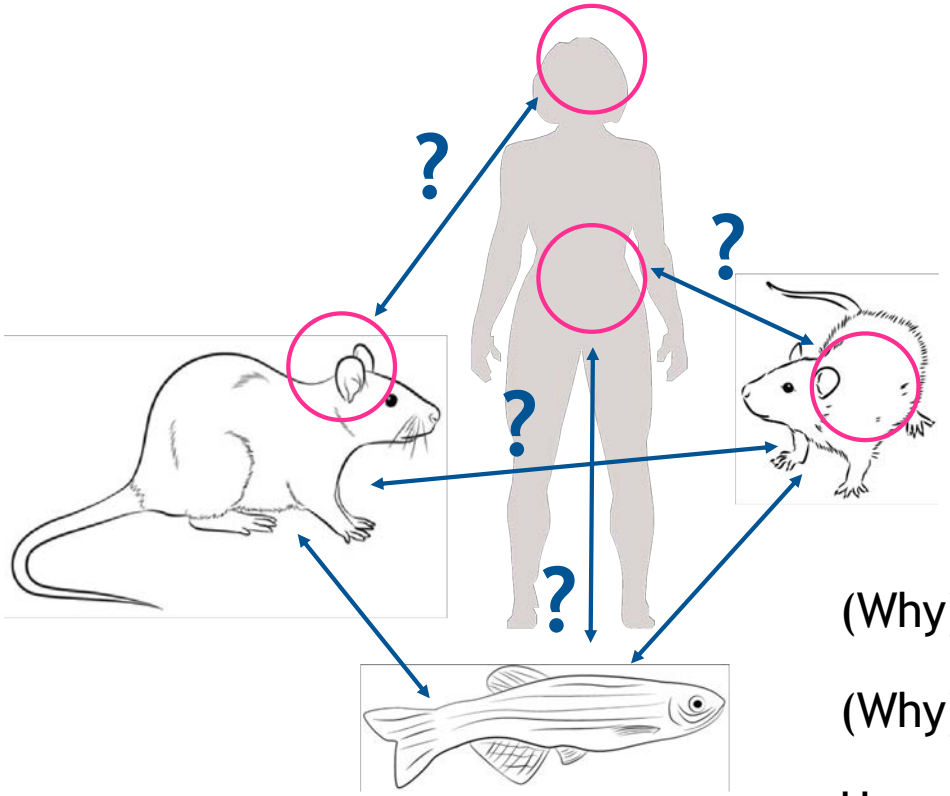


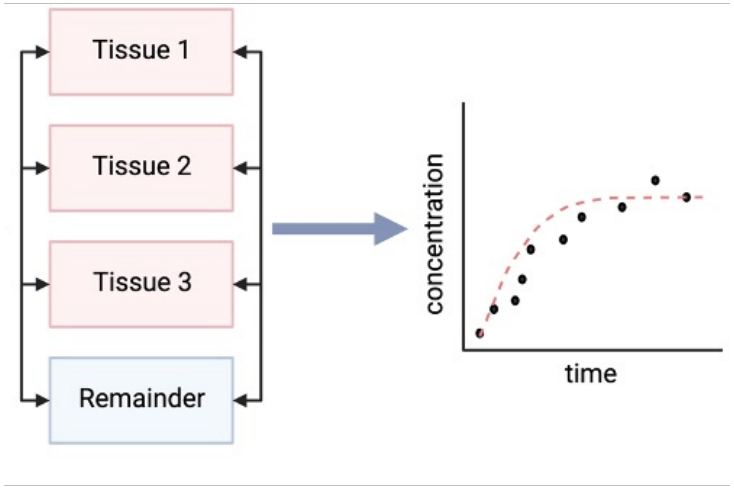
Retrospective analysis illustrates that humans, wildlife and the environment are exposed to many previously unreported PFAS, with no toxicity data and no standards available to enable laboratory testing.



Wang et al., Science Advances 2024

Key questions drive our integrative approaches:





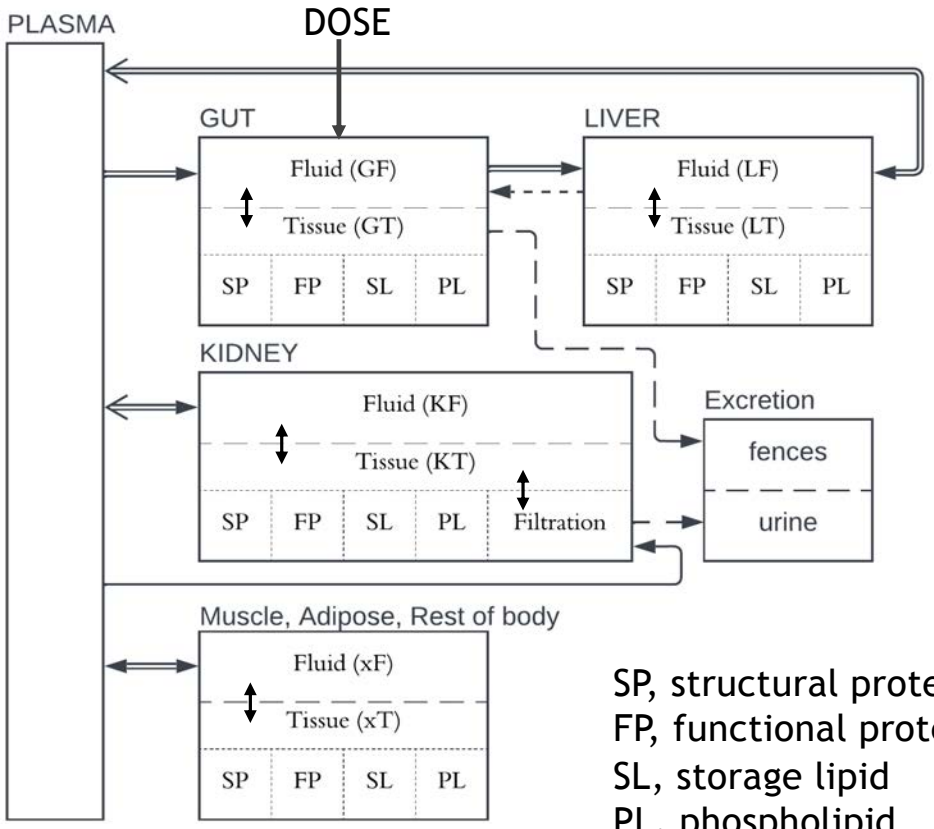
(Why) do PFAS bioaccumulate?

(Why) do they have preferential tissue distribution?

How does this impact toxicity?

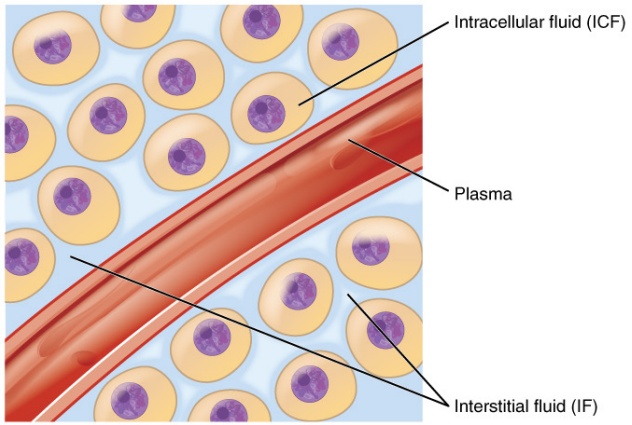
Traditional (K_{OW}-based) metrics fall short.

Ruiwen Chen



SP, structural protein
FP, functional protein
SL, storage lipid
PL, phospholipid

Goal: Move from 2-phase partitioning to multi-phase distribution.



https://commons.wikimedia.org/wiki/File:2702_Fluid_Compartments_ICF_ECF.jpg

$$K_{Tissue-Fluid} = \mathrm{K}_{PL} f_{\mathrm{PL}} + \mathrm{K}_{SL} f_{SL} + \mathrm{K}_{SP} f_{SP} + \mathrm{K}_{FP} f_{FP}$$

In vitro assays to explore PFAS "partitioning"

Ruiwen Chen

Environmental Science & Technology

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pubs.acs.org/est

Article

Deriving Membrane–Water and Protein–Water Partition Coefficients from In Vitro Experiments for Per- and Polyfluoroalkyl Substances (PFAS)

Ruiwen Chen, Derek Muensterman, Jennifer Field, and Carla Ng*

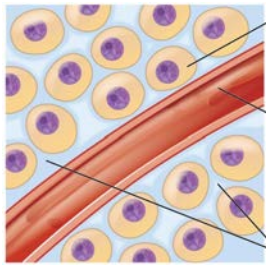
Cite This: https://doi.org/10.1021/acs.est.4c06734

In this work, we investigated the distribution of PFAS to phospholipid membranes and HSA from the aqueous phase via laboratory measurements with 60 PFAS and subsequent modeling. Phospholipid membrane–water partition coefficients (K_{MW}) were measured with SSLM and specific binding to HSA was estimated by equilibrium dialysis. Then, the specific-binding curves from equilibrium dialysis were extrapolated to simulate the PFAS HSA/Water distribution ($D_{HSA/W}$).

Chen et al. Environ. Sci. Technol. 2025, 59, 1, 82–91

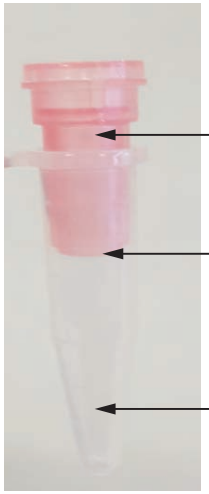
Parsing out drivers of tissue distribution

$$K_{tissue-fluid} = K_{PL} f_{PL} + K_{SL} f_{SL} + K_{SP} f_{SP} + K_{FP} f_{FP}$$



SP, structural protein
FP, functional protein
SL, storage lipid
PL, phospholipid

Equilibrium dialysis for protein-PFAS interactions (HSA) and SSLM assay (Transil assay) for PFAS-phospholipid interactions.



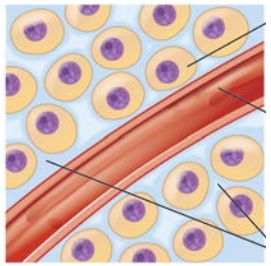
Protein and PFAS in buffer; protein restricted to 100µL volume within dialysis cup.

Dialysis membrane with molecular weight cutoff (MWCO) smaller than protein.

PFAS in buffer can diffuse across dialysis membrane to reach equilibrium between bound and unbound fraction.

Parsing out drivers of tissue distribution

$$K_{tissue-fluid} = K_{PL} f_{PL} + K_{SL} f_{SL} + K_{SP} f_{SP} + K_{FP} f_{FP}$$



SP, structural protein
FP, functional protein
SL, storage lipid
PL, phospholipid

Measuring the SSLM (PL=phospohlipid) part *in vitro*:

$$K_{MW} = \frac{C_{PFAS,M}}{C_{PFAS,W}} = \frac{\left(C_{spike} - C_{PFAS,W}\right) \cdot V_W}{C_{PFAS,W} \cdot M_{PL}}$$

Note: for very hydrophobic PFAS, needed to account for sorption to assay surfaces!

Measuring the HSA (FP=functional protein) part *in vitro*:

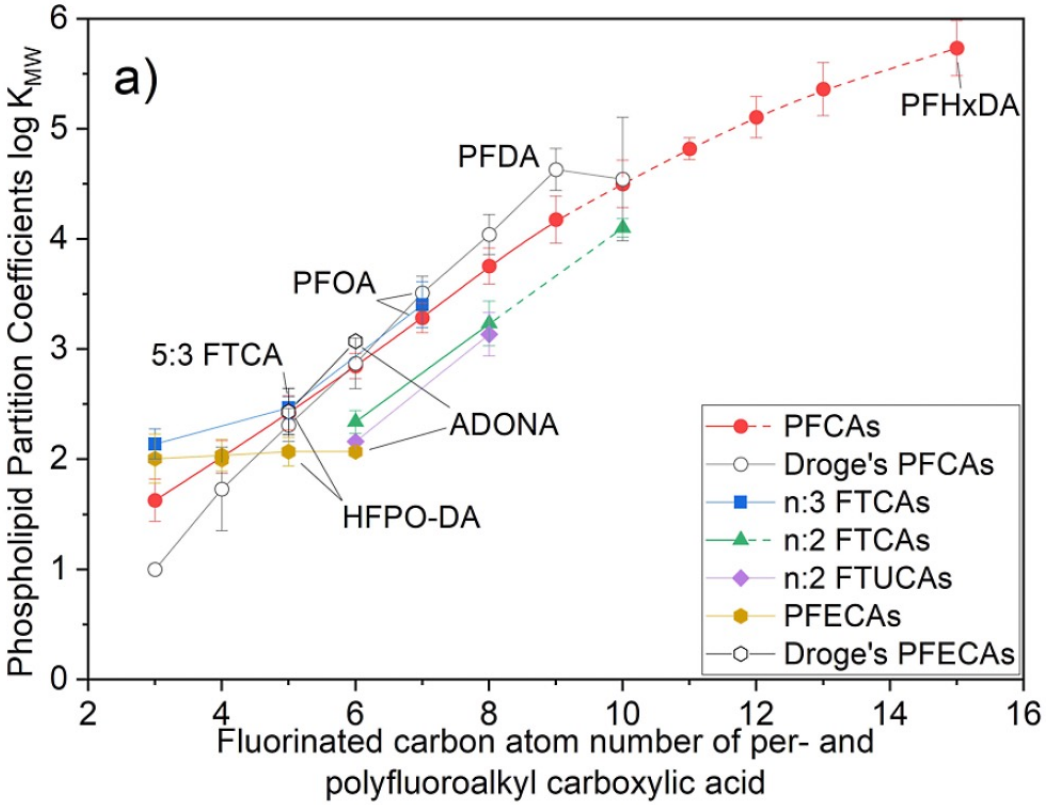
$$K_A = \frac{[\text{HSA}\cdot\text{PFAS}]}{[\text{HSA}]\cdot[\text{PFAS}]} = \frac{1}{K_D} = \frac{k_{on}}{k_{off}}$$

$$\text{total specific binding} = \frac{[\text{HSA}\cdot\text{PFAS}]}{[\text{total HSA}]} = \frac{[\text{PFAS}^*]\cdot B_{max}}{[\text{PFAS}^*] + K_D}$$

$$\rightarrow \quad D_{HSA/W} = \frac{B_{max}\cdot\rho_{HSA}}{\left(K_D + \frac{C_{aq}}{MW_{PFAS}}\right)\bullet MW_{HSA}}$$

More complicated!

Results: Some PFAS prefer membranes



For per- and polyfluoroalkyl carboxylic acids, our results generally agree with and extend the foundational datasets generated by Stephen Droge (https://pubs.acs.org/doi/10.1021/acs.est.8b05052, https://pubs.rsc.org/en/content/articlelanding/2021/em/d1em00327e).

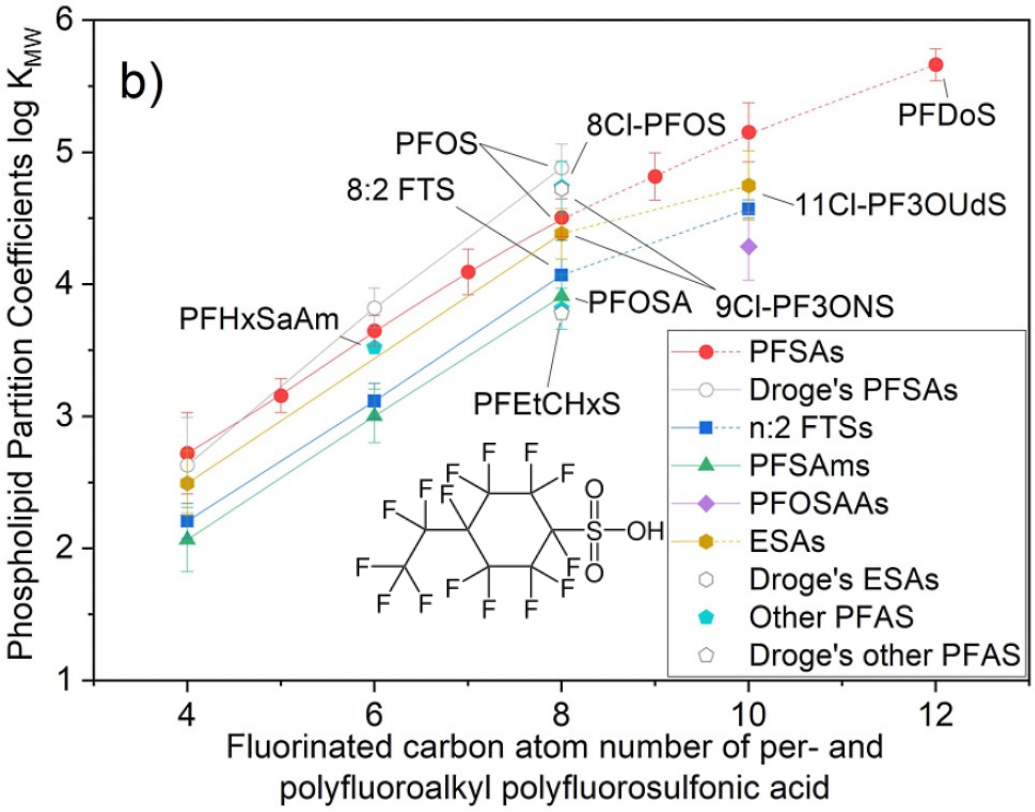
We found an increase of 0.36 ± 0.01 in K_{MW} per FC.

PFCAs and FTCAs generally increase with chain length, though n:2 FTCAs and FTUCAs are on a different intercept.

No such relationship observed for ether carboxylic acids (PFECAs).

Chen et al. Environ. Sci. Technol. 2025, 59, 1, 82–91

Results: Some PFAS prefer membranes



Greater diversity of groups represented within the sulfonates, but slopes are consistent:
We see a 0.37± 0.02 log-unit increase per FC.

Sulfonamides and fluorotelomers fall at lower end of intercepts, suggesting effect of lower surface activity on membrane interactions.

Among C8 sulfonates, PFOS has the highest K_{MW} whereas the cyclic PFEtCHxS has the lowest.

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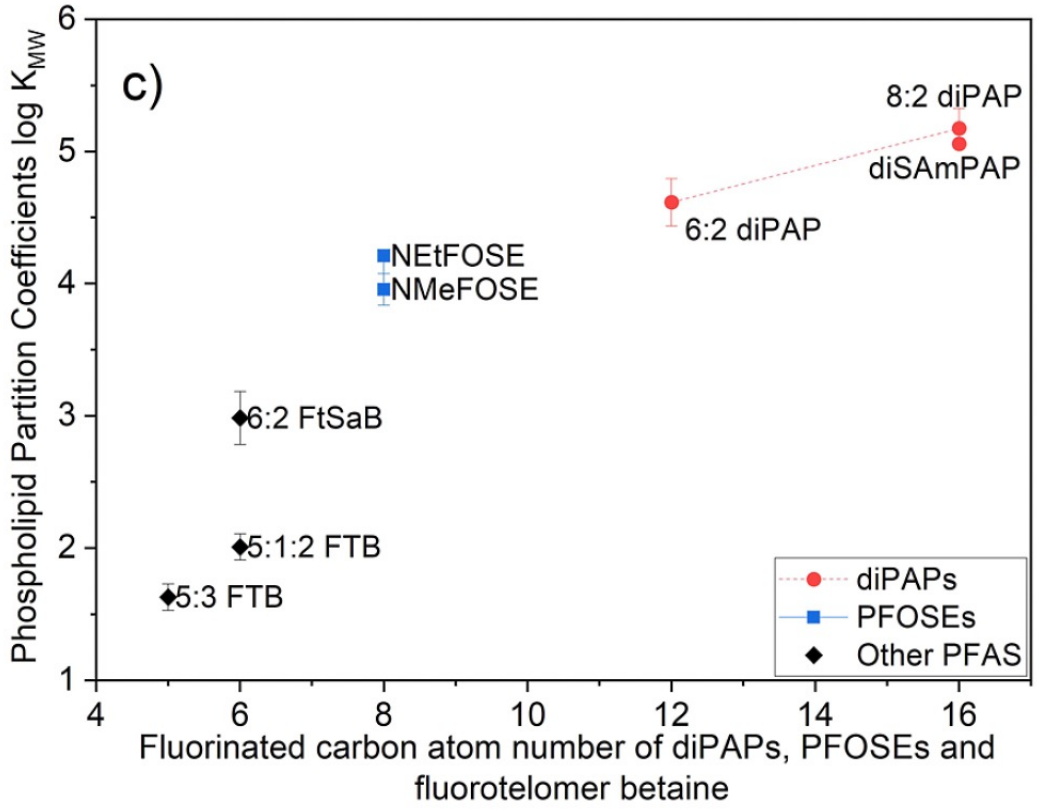
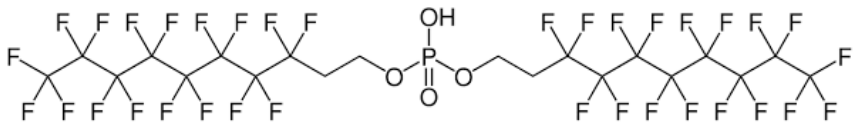
Results: Some PFAS prefer membranes



For other groups, too few data points across FCs for clear relationships.

DiPAPs show high K_{MW} due to large number of FC groups.



8:2 diPAP

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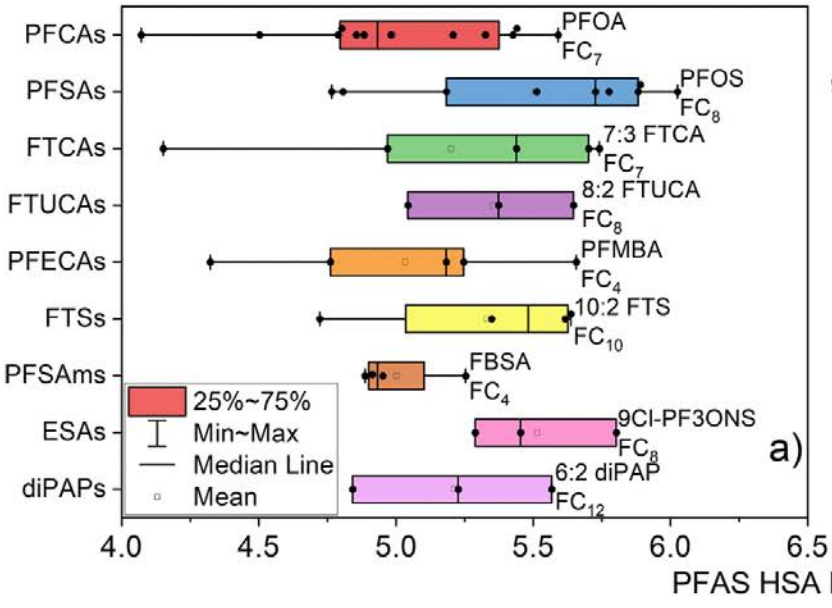
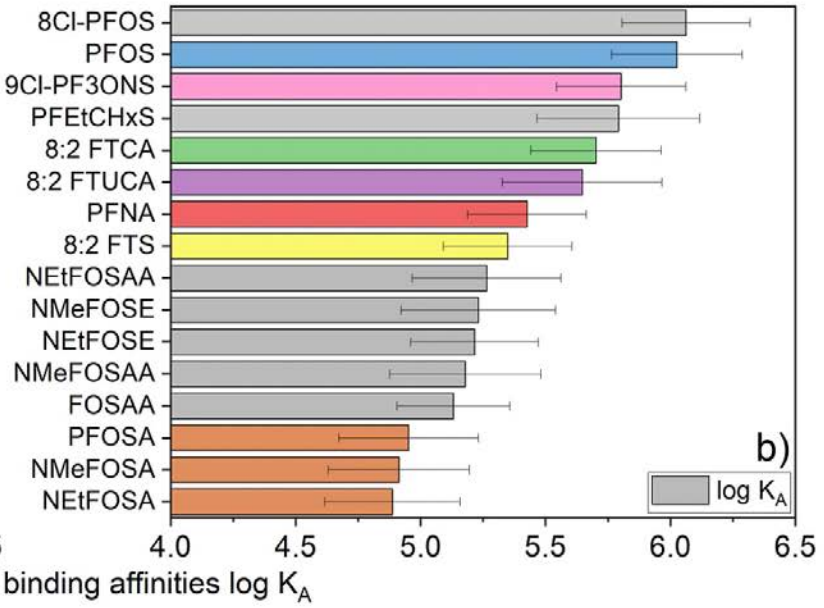
Proteins: HSA interactions are more variable

Across all chain lengths:



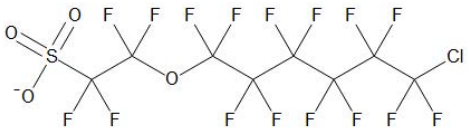
Focus on “C8” baseline:



PFAS HSA binding affinities log K_A

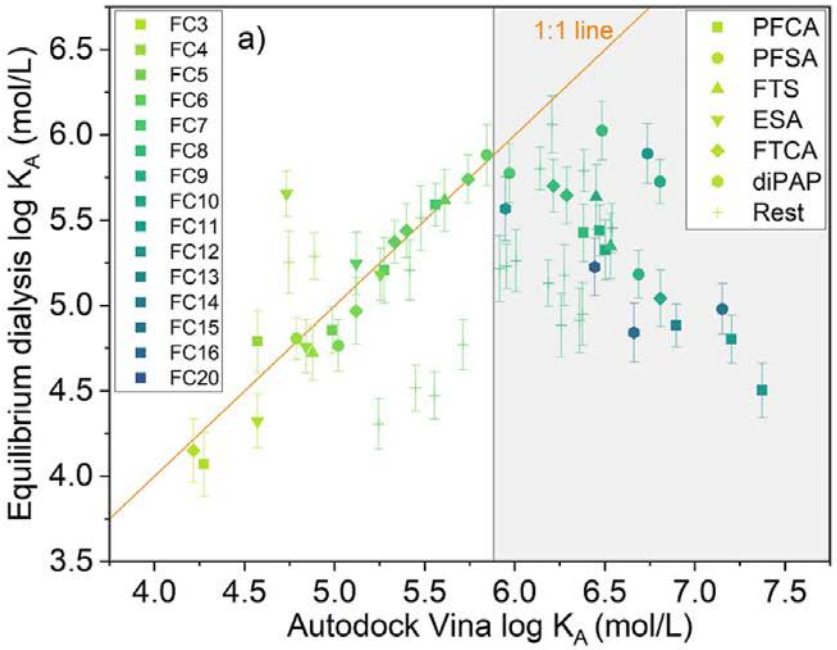
PFOS has the highest affinity for HSA across all groups and chain lengths (including longer-chain sulfonates) for the well-represented groups.

Addition of chlorine increases PFOS affinity for HSA (as seen also for the chloro-fluoro ether).

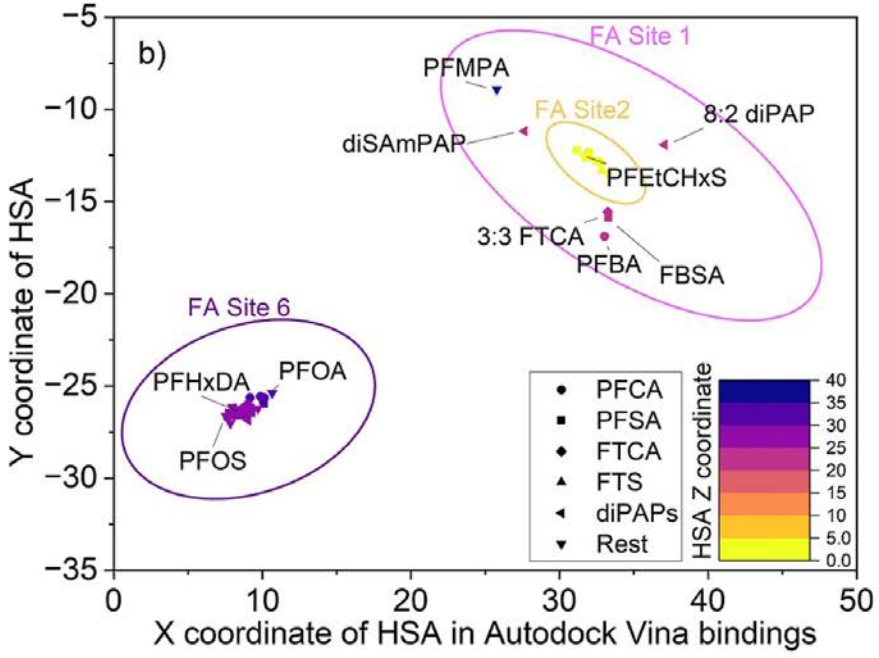


Chen et al. Environ. Sci. Technol. 2025, 59, 1, 82–91

Docking has limitations for long chain lengths:



Good agreement for log K_A < 6



No clear relationship with binding site.

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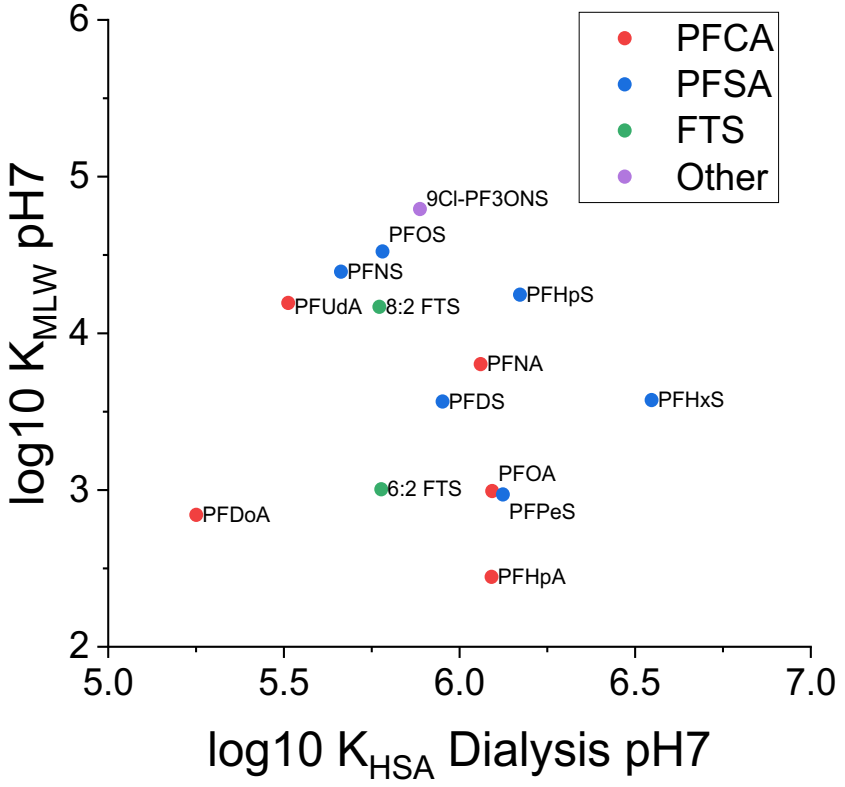
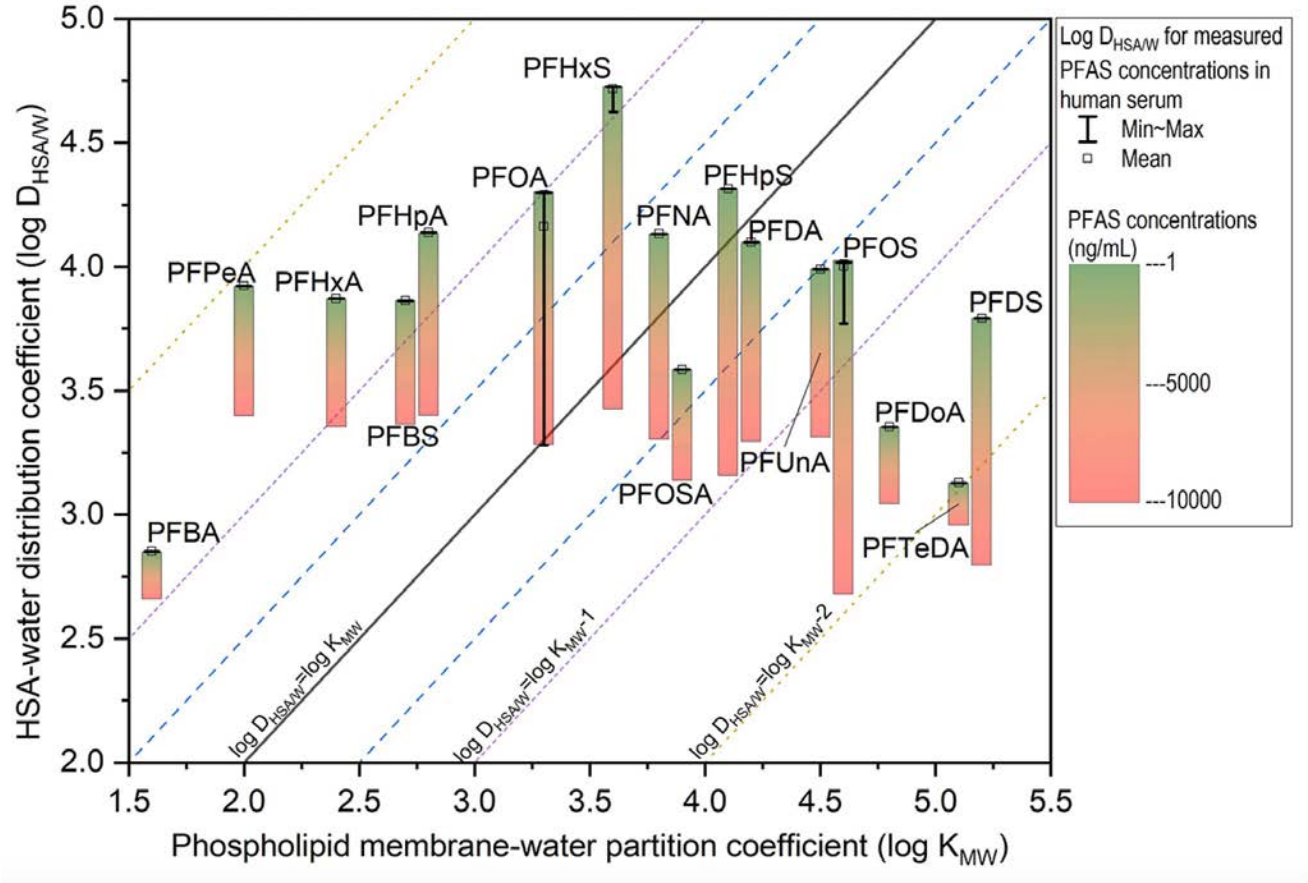
Proteins and phospholipids: complementary data.



- No strong correlation between membrane (K_{MLW}) and protein(K_A for HSA) binding.
- Suggests different mechanisms and influence of chain length/structural features at play.
- **This is good news! These are complementary, not redundant data.**

Non-saturable partitioning meets saturable binding:



- **Distribution** of PFAS between free and bound phases: larger effect of "exposure concentration" on HSA than membrane binding.
- **What are the implications for occupational exposure and typical toxicity experiments?**

Chen et al. Environ. Sci. Technol. 2025, 59, 1, 82–91

Take-homes from membrane and HSA assays:

- Consistent relationship between increase in KMW and fluorinated carbons (~0.36 log units per additional FC). [slope of curve]
- Intercepts make the difference across functional groups and types (e.g. why sulfonates have higher KMW than carboxylates for the same # of FC).

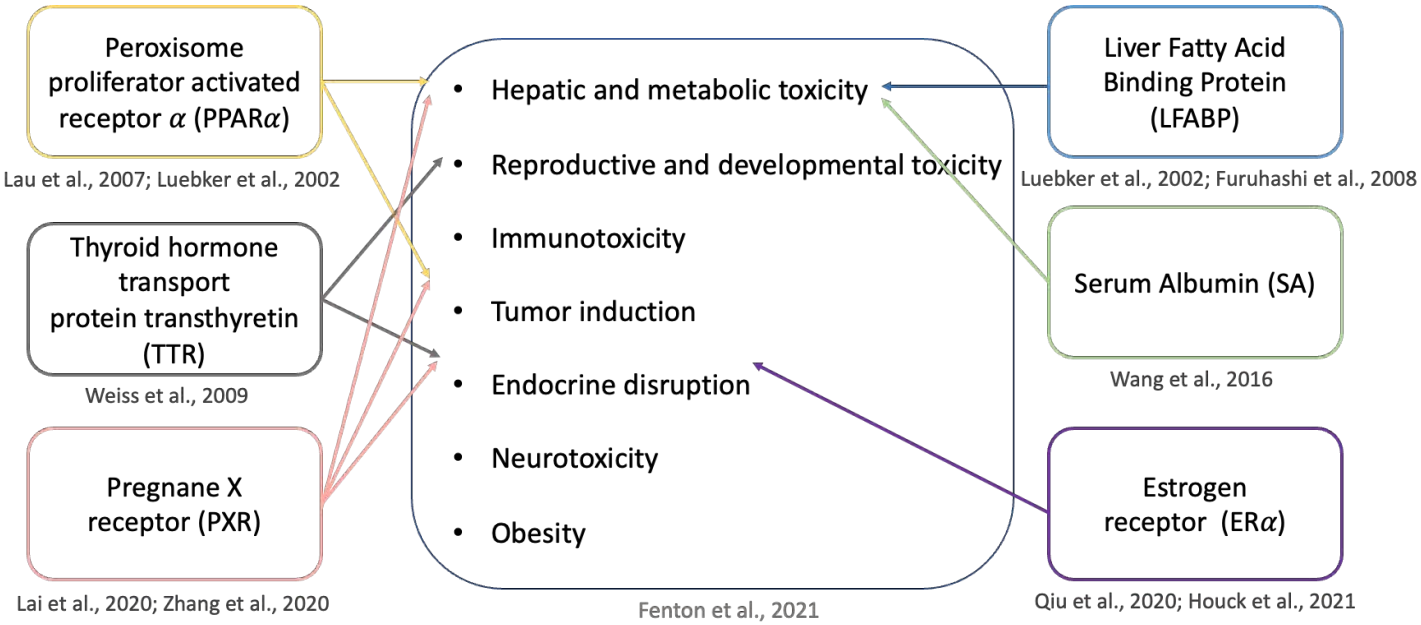
- HSA is different-- both in terms of chain length relationship and saturability.
- For chain length and functional group, there appears to be a "sweet spot" (i.e. PFOS) for maximum affinity.

- **Need to consider the interplay of membrane and protein interactions and the influence of exposure dose!**

- But what does this mean beyond distribution: what is the *toxic effect*?

Using interactions to categorize hazard: Molecular Screening

Molecular interactions (e.g. MIEs) can inform toxic mechanisms for chemicals.



Problem: We lack data on most PFAS, for many proteins!

Approach: Generate data *in silico* to rank many PFAS.

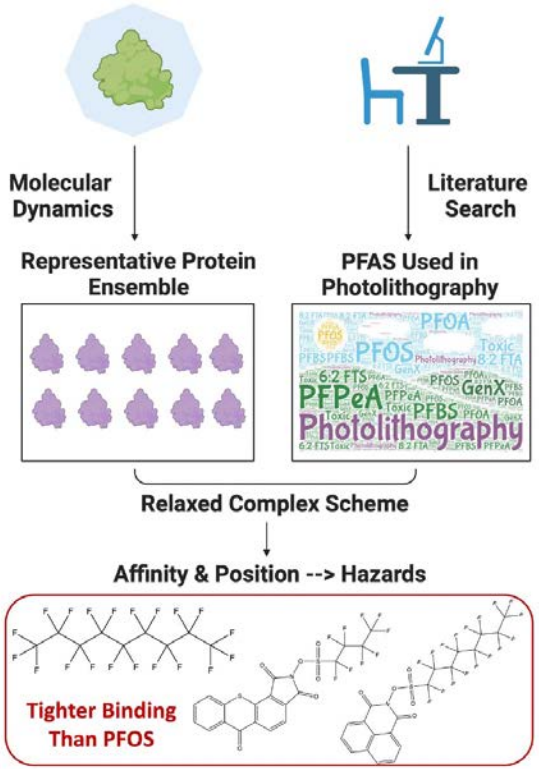
Case study: PFAS used in photolithography.

Yuexin Cao

High-throughput Screening of Protein Interactions with Per- and Polyfluoroalkyl Substances (PFAS) Used in Photolithography

Yuexin Cao[a] and Carla A. Ng[a,b*]

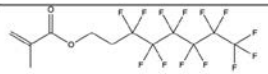
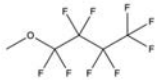
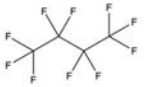
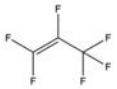
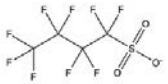
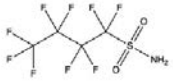
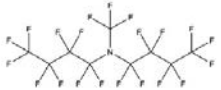
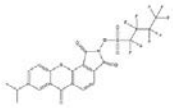
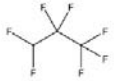
Diverse per- and polyfluoroalkyl substances (PFAS) are widely used in photolithography, and demand for semiconductor manufacturing is growing to support technological and energy transitions. PFAS are persistent in the environment and have been associated with significant health risks, yet remain inadequately studied. Our study, focusing on high-throughput screening of protein interactions with PFAS in photolithography, provides crucial insights into the molecular-level interactions of these substances with proteins. Understanding these interactions is essential for assessing the potential hazards of PFAS, guiding future regulations, and developing safer alternatives, thereby addressing a growing public health concern.



Cao & Ng, J HazMat, Accepted

Building the screening dataset

We identified 221 photolithography-relevant PFAS uses in 7 photolithography processes (including 16 specific applications) and 15 PFAS structural types (please see our SI for full list!) .
96 PFAS were selected for modeling.

PFAS Type	Definition	Counts	Example Modeled Structure
FTBS	Fluorotelomer-based substances	4	
HFE	Hydrofluoroethers	7	
PASFBS	Perfluoroalkyl carboxylic fluoride (PASF)-based substances	34	
PFAKA	Perfluoroalkanes	6	
PFAKE	Perfluoroalkenes	4	
PFSA	Perfluoroalkane sulfonic acids	4	
PFSAM	Perfluorosulfonamides	4	
PTA	Perfluoroalkyl-*tert*-amines	3	
SCFA	Side-chain fluorinated aromatics	11	
SFA	Semifluorinated alkanes	2	

Cao & Ng, J HazMat, Accepted

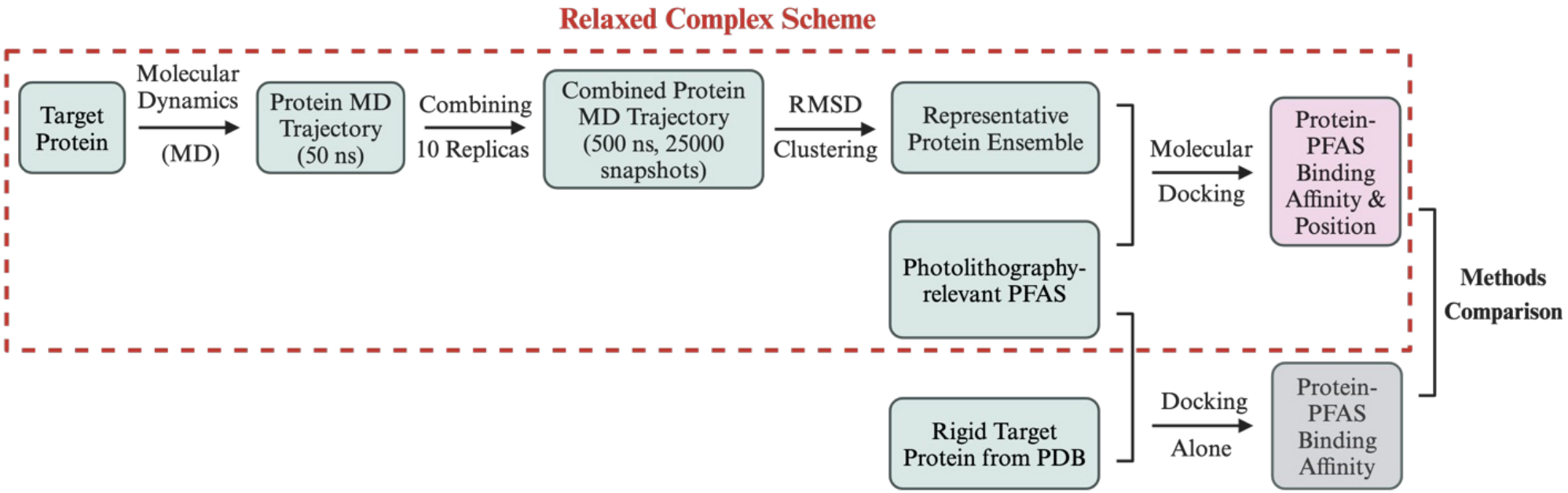
Building the screening dataset

We targeted 5 receptors relevant to bioaccumulation and toxicity of PFAS and modeled them across 4 species to compare model organisms to humans.

Proteins	ID in Protein Data Bank or entry identifier in UniProt			
	Human (Homo sapiens)	Rat (Rattus norvegicus)	Mouse (Mus musculus)	Zebrafish (Danio rerio)
Liver fatty acid binding protein (LFABP)	3STM	1LFO	P12710*	Q1AMT3*# & 2QO4#
Serum albumin (SA)	4L8U	P02770*	P07724*	-
Peroxisome proliferator activated receptor α (PPARα)	6LXA	P37230*	P23204*	A6XMH7*
Peroxisome proliferator activated receptor γ (PPARγ)	6MS7	O88275*	P37238*	A6XMH6*
Transthyretin (TTR)	4KY2^	1KGI	P07309*^	B8JLL8*^

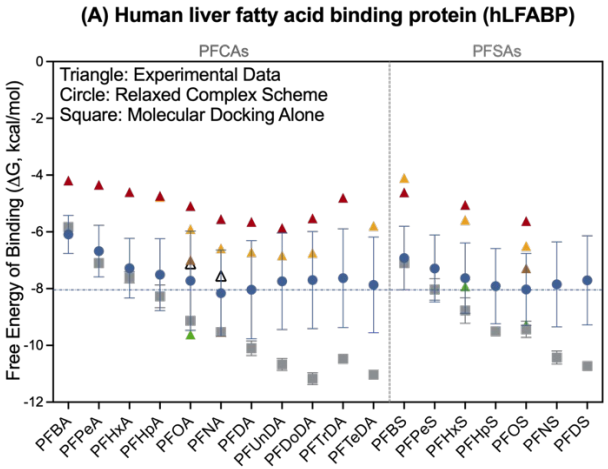
Cao & Ng, J HazMat, Accepted

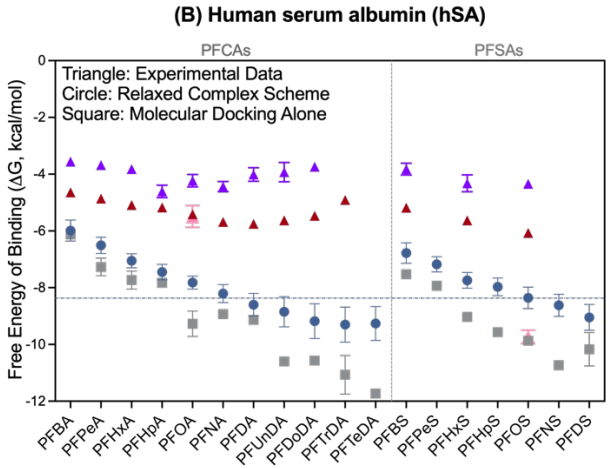
Balancing accuracy and efficiency: *Relaxed Complex Scheme*

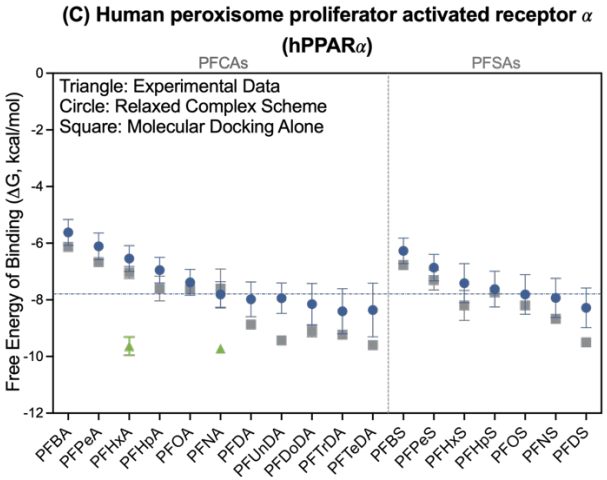


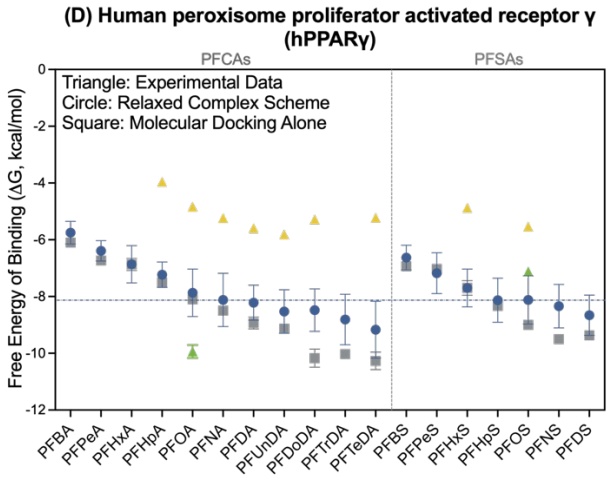
Cao & Ng, J HazMat, Accepted

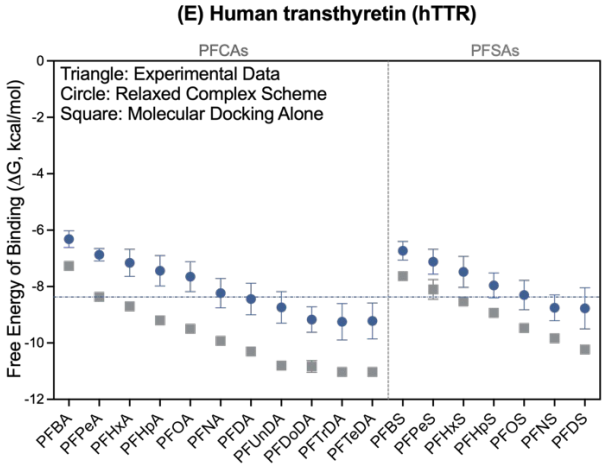
Validation of Relaxed Complex Scheme





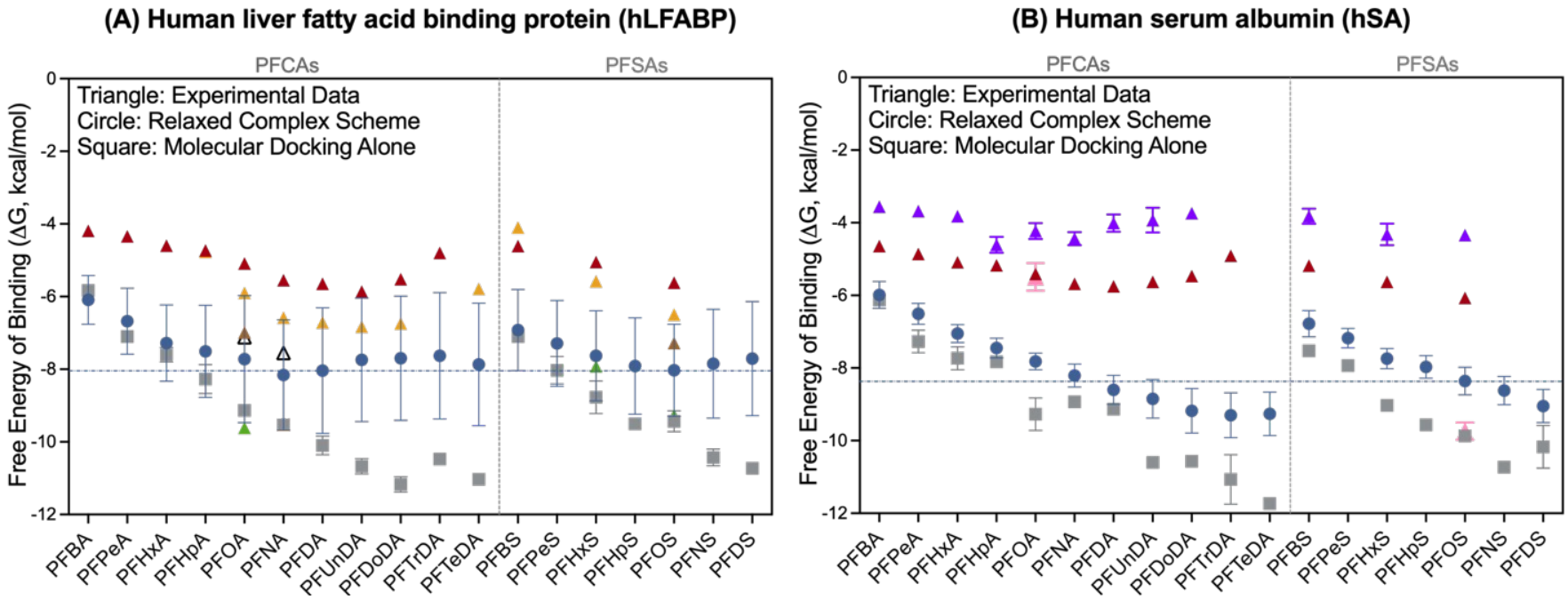






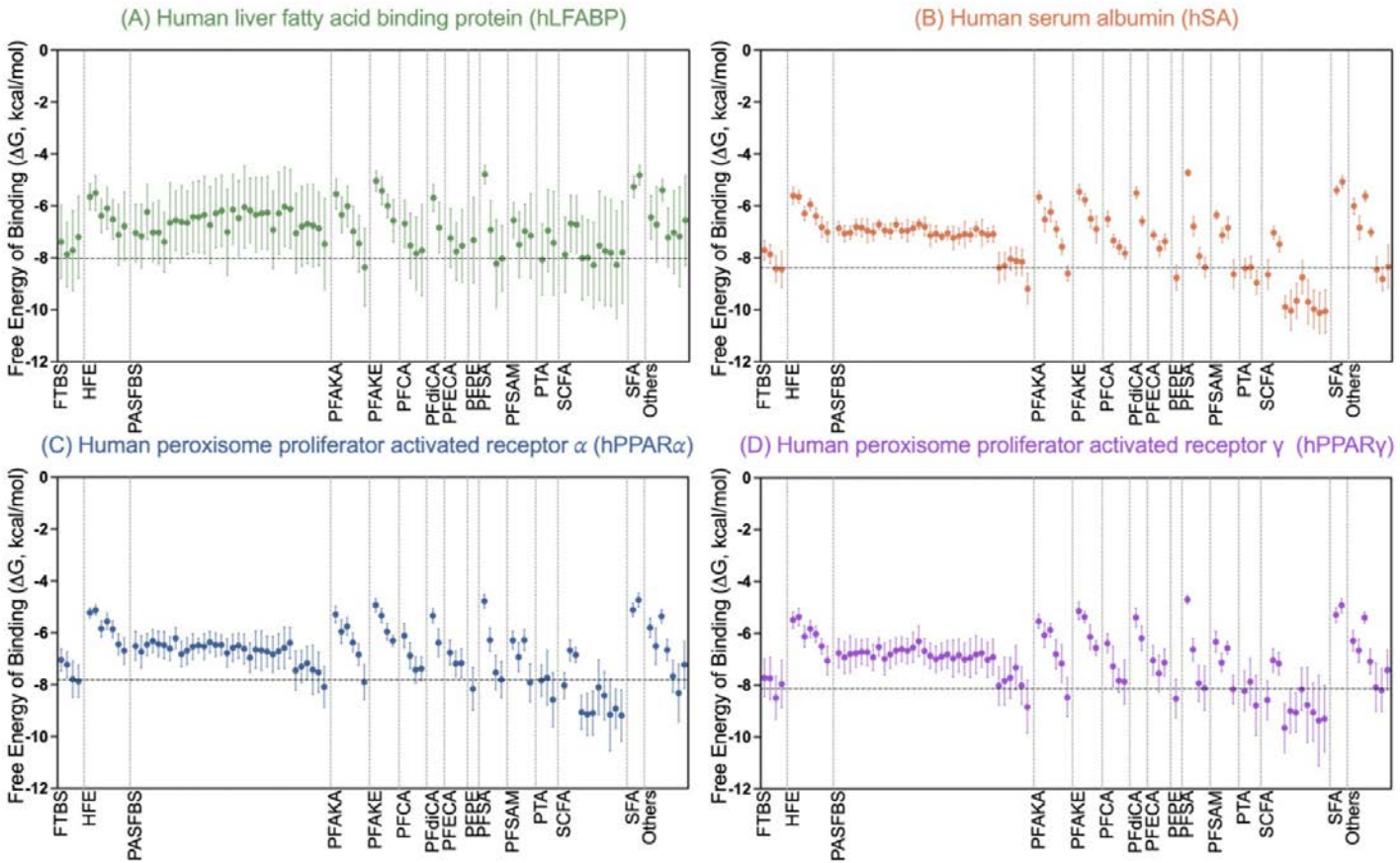
Validation focused on PFAS and receptors with most experimental data available, comparing the RCS with docking alone.

Validation of Relaxed Complex Scheme



RCS improves both relative and absolute accuracy of binding affinity predictions, while maintaining reasonable computational efficiency.

Can we use interactions to categorize hazard?



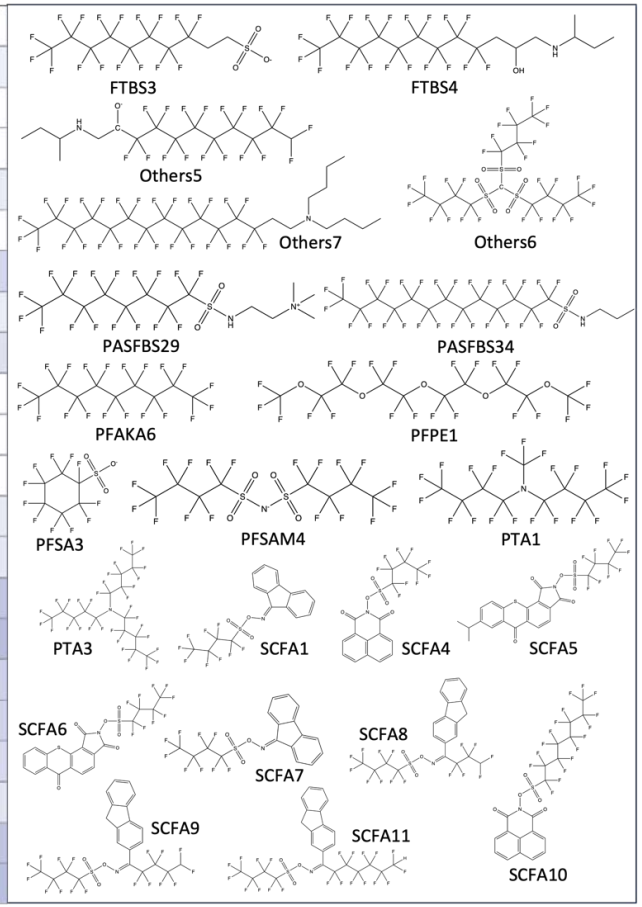
Different groups show different levels of affinity, often related to chain length, which varies across groups.

Note horizontal line is predicted affinity of our benchmark chemical PFOS.

A notable outlier: side-chain fluorinated aromatics (SCFAs).

Can we use interactions to categorize hazard?

	hLFABP	hSA	hPPARα	hPPARγ	hTTR
FTBS3		0.06		0.37	
FTBS4		0.08	0.06		0.03
Others5		0.08			
Others6		0.45	0.51	0.08	
Others7					0.16
PASFBS29		0.02			
PASFBS34		0.83	0.27	0.72	0.90
PFAKA6	0.33	0.24	0.09	0.35	0.11
PFPE1		0.41	0.35	0.40	0.55
PFSA3	0.19				
PFSAM4		0.27	0.11	0.04	
PTA1	0.04	0.04	0.03	0.11	
PTA3		0.59	0.77	0.67	0.23
SCFA1		0.28	0.22	0.46	0.32
SCFA10	0.24	1.76	1.11	1.25	1.54
SCFA11		1.70	1.38	1.18	1.87
SCFA4		1.53	1.25	1.53	1.17
SCFA5		1.68	1.33	0.88	1.03
SCFA6	0.25	1.30	1.28	0.94	0.81
SCFA7		0.39	0.29	0.04	0.42
SCFA8		1.34	0.61	0.65	1.59
SCFA9		1.62	1.35	0.93	1.83

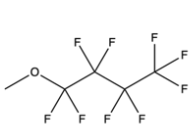
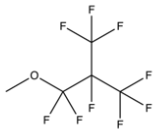
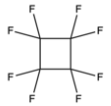
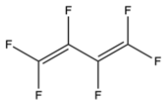
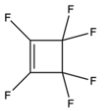
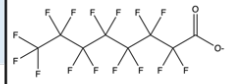
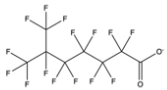
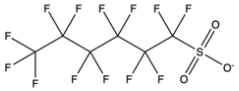


High affinity PFAS are dominated by molecules with many CF groups as well as aromatic PFAS and one cyclic PFAS.

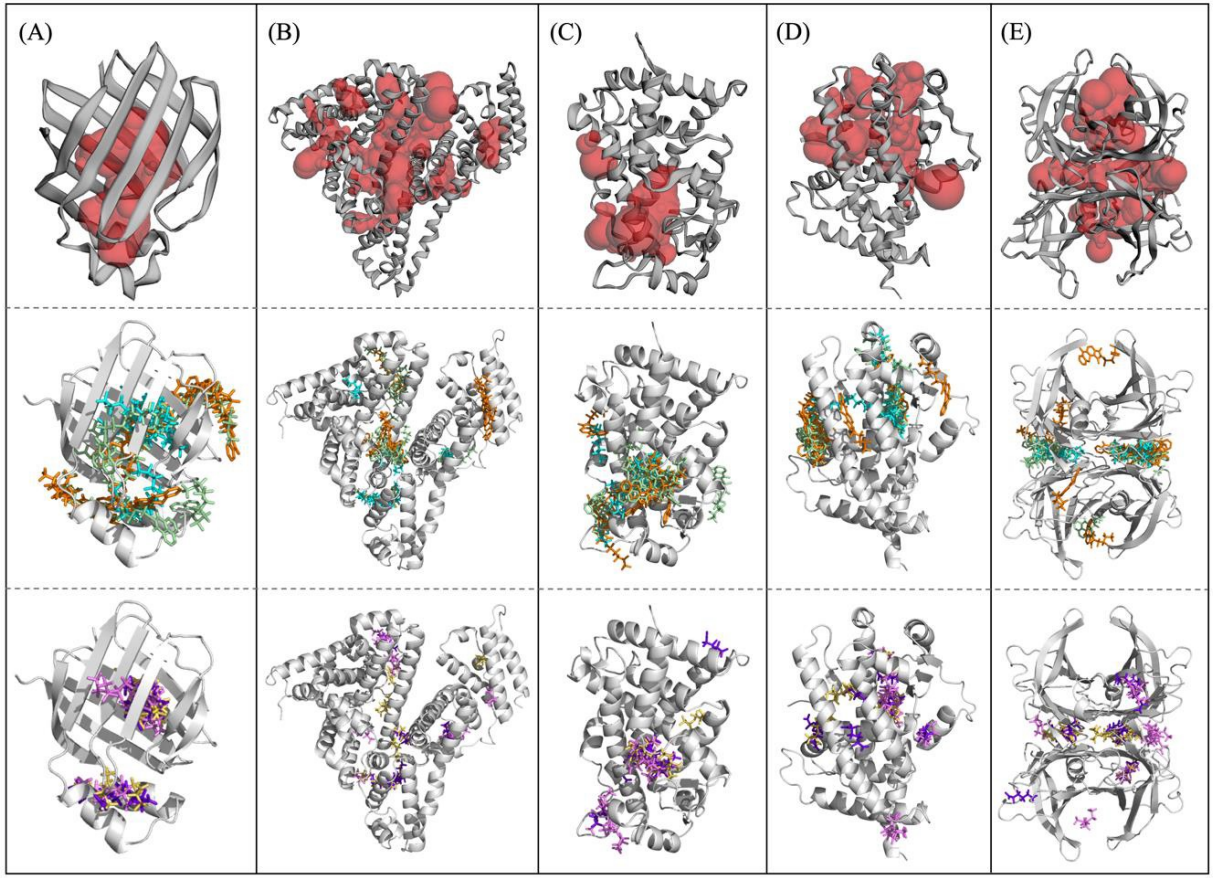
Higher affinity for these PFAS are observed across the modeled human receptors except for LFABP- potential limitation of binding pocket size?

These are largely untested PFAS, most without standards available to carry out experimental assays, but warrant prioritization.

Observations for linear vs. cyclic PFAS within groups

Chain Length	Structural Form	PFAS ID	hLFABP	hSA	hPPARα	hPPARγ	hTTR	
4 fluorinated carbons	Linear	HFE3	-6.38	-6.29	-5.84	-6.13	-6.29	 HFE3
	Branched	HFE4	-6.09	-5.95	-5.56	-5.83	-6.01	 HFE3
4 fluorinated carbons	Linear	PFAKA2	-6.34	-6.53	-5.95	-6.07	-6.38	 PFAKA2
	Cyclic	PFAKA3	-6.01	-6.23	-5.75	-5.87	-6.34	 PFAKA3
4 fluorinated carbons	Linear	PFAKE2	-5.42	-5.77	-5.33	-5.37	-5.69	 PFAKE2
	Cyclic	PFAKE3	-6	-6.5	-5.95	-6.14	-6.04	 PFAKE3
7 fluorinated carbons	Linear	PFCA4	-7.72	-7.82	-7.38	-7.87	-7.65	 PFCA4
	Branched	PFCA3	-7.83	-7.59	-7.43	-7.83	-7.52	 PFCA3
6 fluorinated carbons	Linear	PFHxS	-7.63	-7.74	-7.41	-7.7	-7.48	 PFHxS
	Cyclic	PFSA3	-8.22	-7.94	-7.53	-7.93	-8.27	 PFSA3

Can we use binding sites to categorize hazard?



Top row: active sites of (A) LFABP, (B) HSA, (C) PPAR a, (D) PPAR g, (E) TTR.

Middle row: Binding positions of strongly-binding PFAS.

Bottom row: Binding positions of weakly-binding PFAS.

What molecular simulations can and can't do:

Predicted protein binding affinities were influenced by PFAS structural features: fluorinated chain length, molecular size, and the presence of aromatic rings

Also affected by the dimensions of the protein binding pockets (e.g. limitations in LFABP).

Notably, 22 PFAS were predicted to bind more strongly than PFOS, suggesting their potential for bioaccumulation and adverse biological effects.

Needs to be further validated with outcomes of protein binding – only one component in a complex cascade!

Some take-home messages for PFAS

- There remain a large number of untested PFAS across many categories of use (and subsequent human exposure).
- Modeling strategies allow us to increase throughput on PFAS evaluation, but are not a panacea.
- Require data for training, validation, and evaluation.
- Combining insights from in vitro and in silico approaches can help to fill these gaps and prioritize chemicals for further study while avoiding animal use.
- In the meantime, treatment and destruction technologies are urgently needed, both inside and outside the box.

Acknowledgments

- Dr. Jennifer Field (OSU) – SERDP/Concawe collaboration on protein and membrane interactions.
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Questions?

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