Estimating the Bioaccumulation Potential of Per- and Polyfluoroalkyl Substances (PFAS) Across Species by Integrative In Silico Approaches

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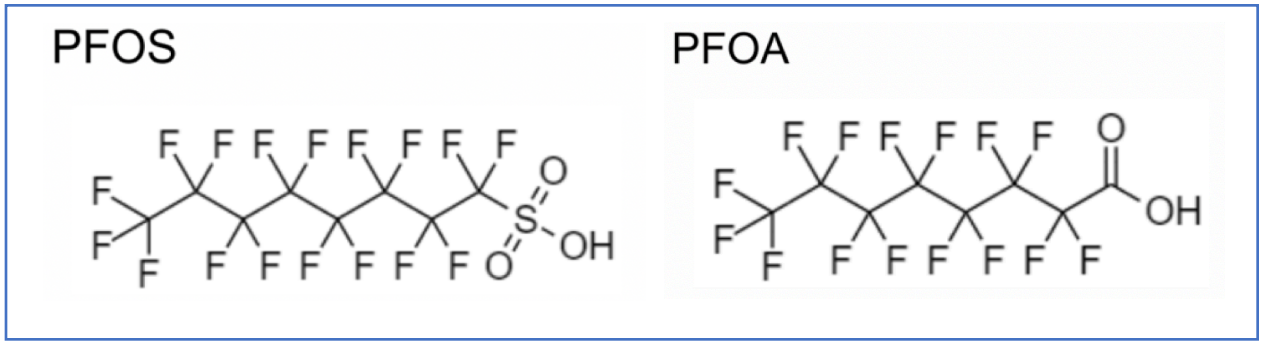
INTRODUCTION

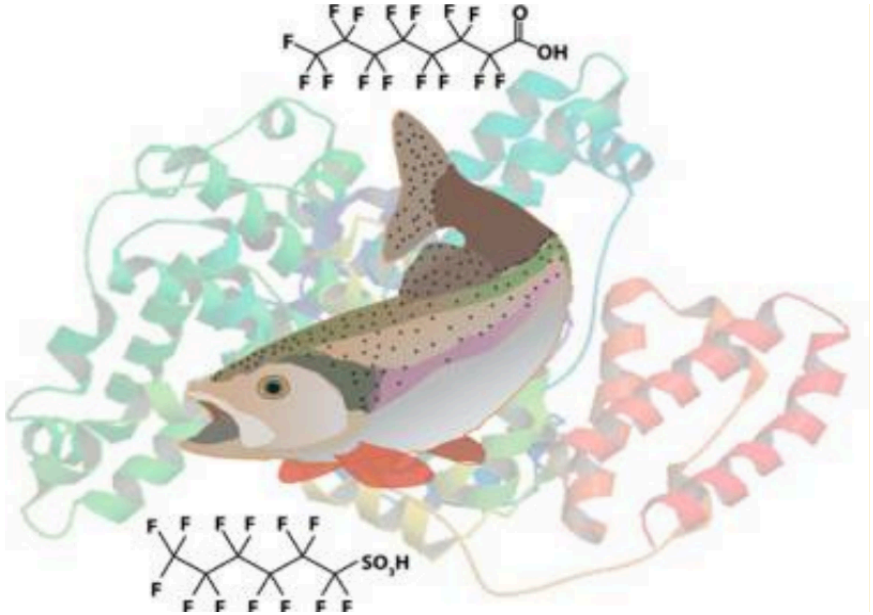
- **Per- and polyfluoroalkyl substances (PFAS)**



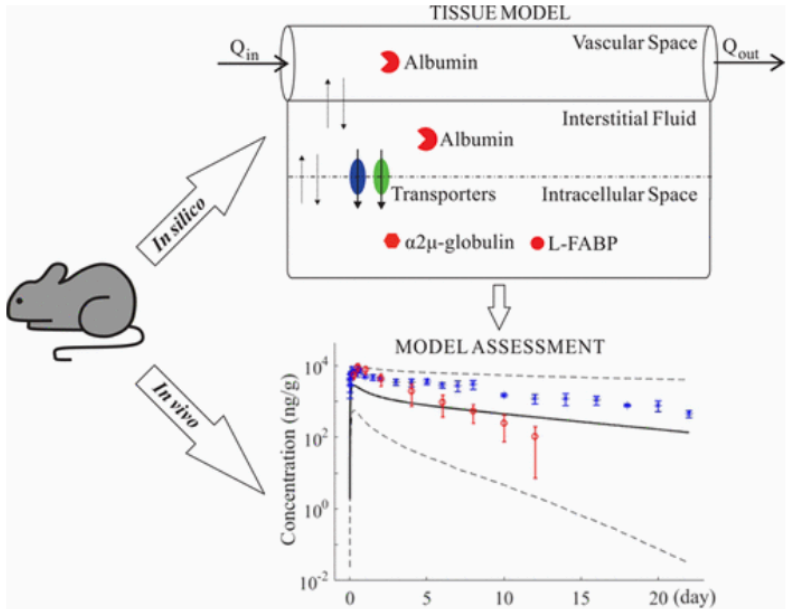
  - Many PFASs are extremely persistent in the environment
  - They have been detected in tissues from species as diverse as whales, birds, fish, and even invertebrates, covering the range of trophic levels

INTRODUCTION

- **Bioaccumulation**
  - ❖ Accumulated in blood, liver, and kidney tissue
  - ❖ Bind to proteins including serum albumin and liver-type fatty acid binding protein (LFABP)



Ng and Hungerbühler *Env Sci & Tech,* **2013**



Cheng and Ng, *Env Sci & Tech,* **2017**

- ❖ **PFAS-protein interactions** play an essential role in determining PFAS bioaccumulation potential in animals

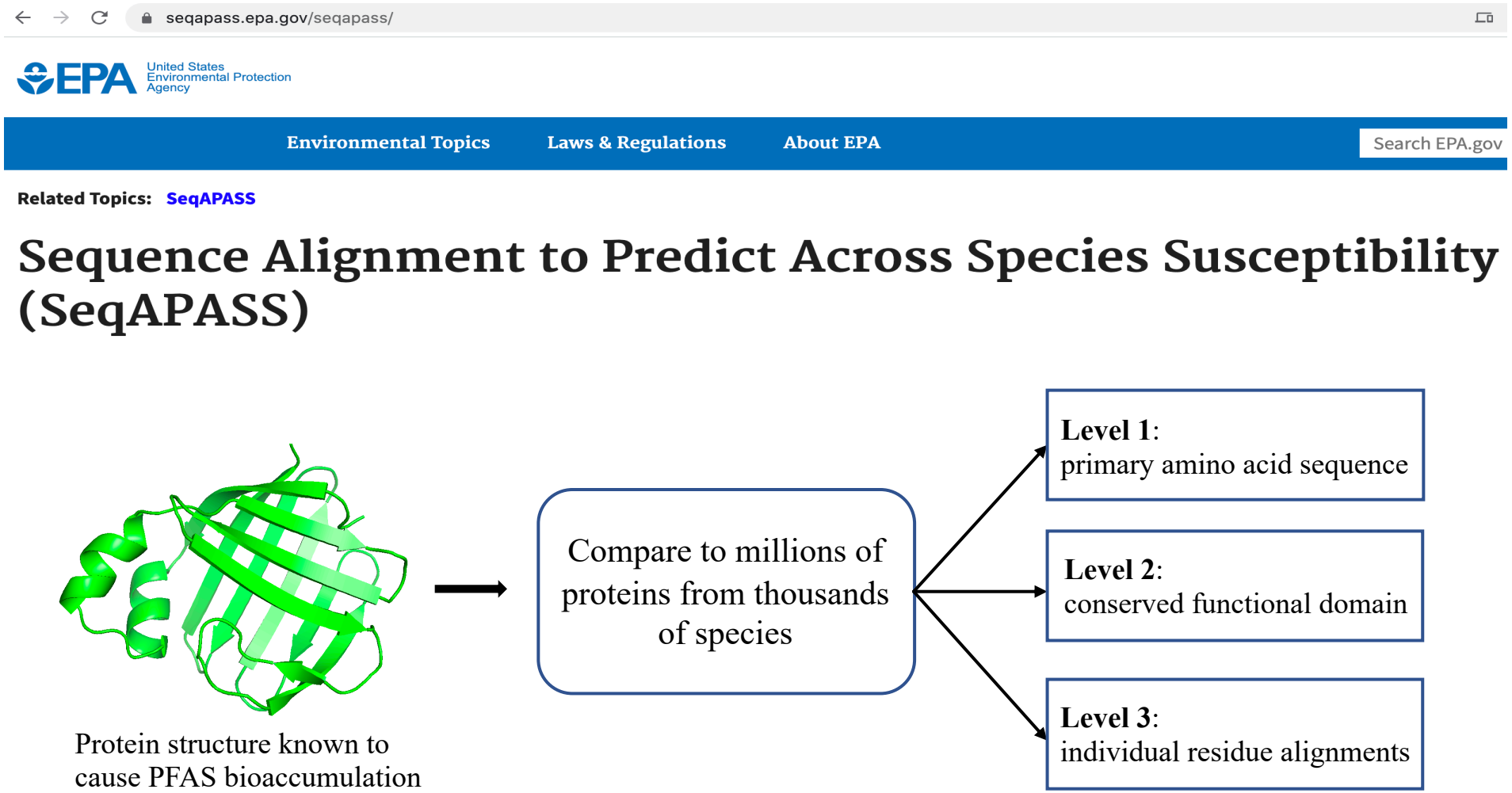
INTRODUCTION

- **Large number of PFAS**

  - ❖ More than 4000 PFASs on global market, from OECD (Organization for Economic Cooperation & Development)
  - ❖ In silico methods hold great promise for evaluating bioaccumulation potentials
  - ❖ Integrative in silico approach including two complementary tools:
    - ➢ SeqAPASS
      - Sequence and structure of proteins;
      - No PFAS-protein interactions included, but can rapidly extrapolate for large number of species
    - ➢ Molecular dynamics
      - Function of protein (i.e., protein binding affinity)
      - Slow, but provide additional insight into PFAS-protein interactions

METHODS

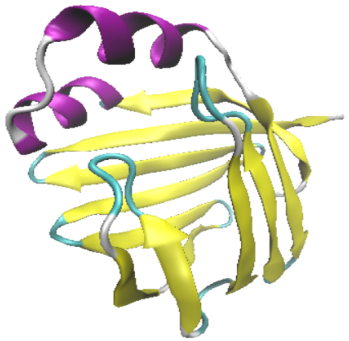
SeqAPASS Workflow



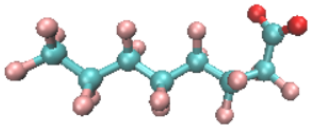
LaLone et al., *Toxicol Sci.*, **2016**

METHODS

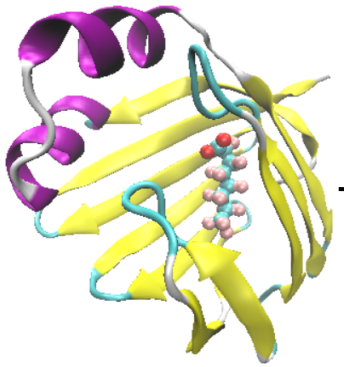
❑ Molecular dynamics (MD) Workflow



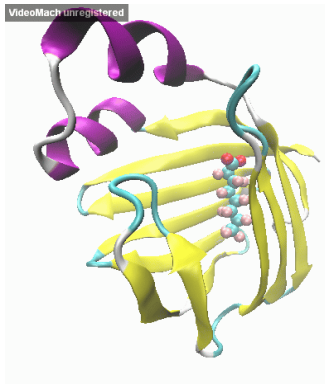
Homology modeling (Phyre2) or PDB



Avogadro or PDB



Molecular docking (Autodock vina)



Molecular dynamics (AMBER 14)

$$\Delta G_{bind} = \frac{1}{N}\sum_{i=1}^{N} G_i^{Complex} - G_i^{Protein} - G_i^{PFAS}$$

$$G = E_{bond} + E_{electrostatic} + E_{van\ der\ Waals} + G_{polar} + G_{nonpolar} - TS$$

MM-PBSA calculation

✓ **Protein binding affinity** is quantified by equilibrium association constant (K_A) :

$$K_A = e^{-\Delta G_{bind}/RT}$$

=> ΔG_{bind} - Free energy of binding

Cheng and Ng, *Env Sci & Tech,* **2018**

METHODS

❑ Materials

LFABP structures across 7 different species

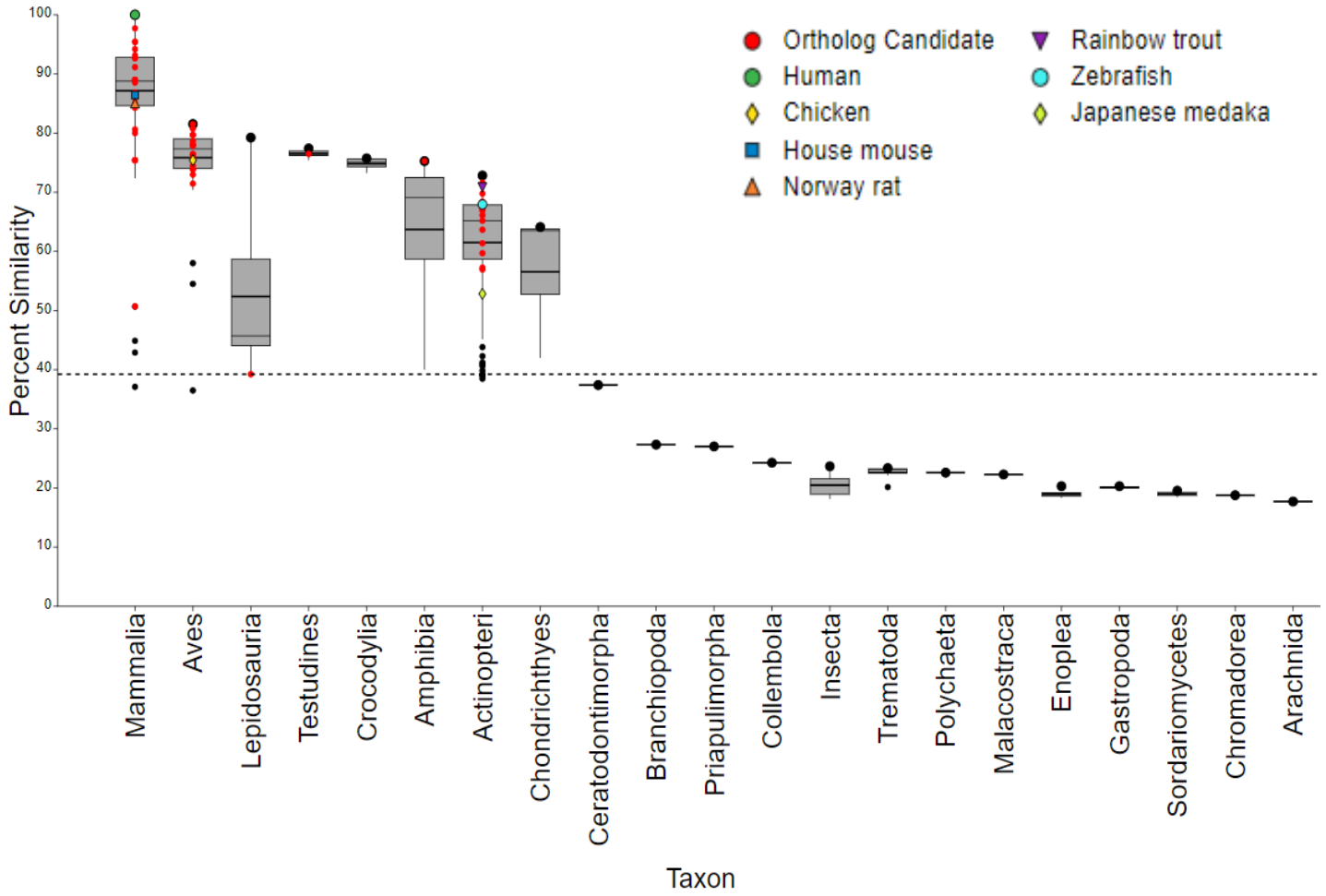
- Liver-type fatty acid binding protein (LFABP) is used as protein proxy for bioaccumulation assessment
- human and rat LFABP structures are available in Protein Data Bank (PDB)
- chicken, zebrafish, rainbow trout, Japanese medaka, and fathead minnow structures were generated using Phyre2

9 PFAS structures

- 6 PFCAs: PFBA(C4), PFPA(C5), PFHxA(C6), PFHpA(C7), PFOA(C8), PFNA(C9)
- 3 PFSAs: PFBS(C4), PFHxS(C6), PFOS(C8)

RESULTS

- **SeqAPASS – Level 1 & Level 2**



- 302 of the 347 aligned species are similar to human
- **Fathead minnow** did not have any common domains with the human query sequence
- No SeqAPASS Level 2 runs were submitted because no functional domains were identified as specific hits in NCBI's Conserved Domains database

RESULTS

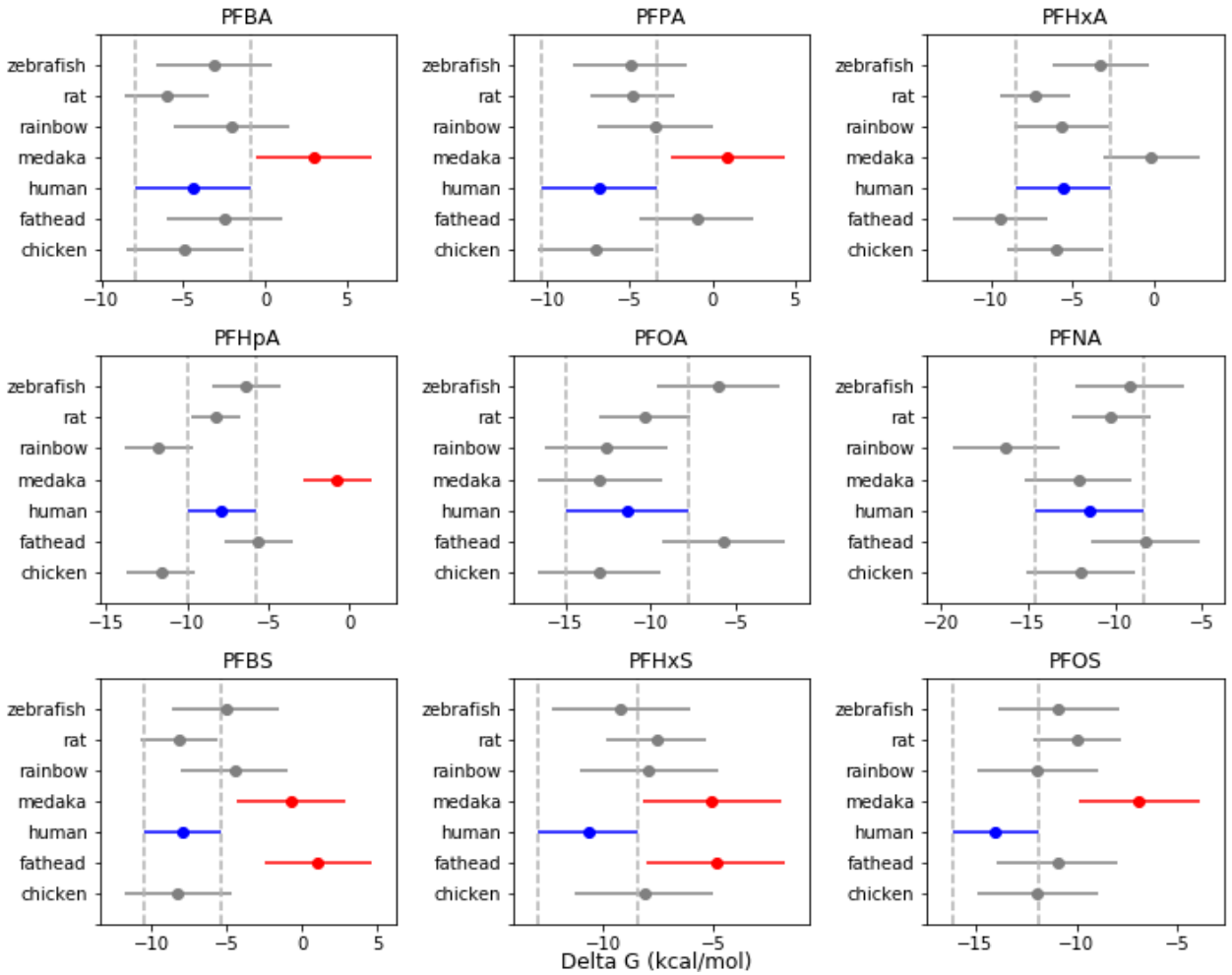
- **SeqAPASS – Level 3**

Table 1. Identification of Potential Critical Amino Acids Across Species

Human Amino Acid Position	Type 1 Primates, Ruminants, Whales/dolphins	Type 2 Rodents and other mammals, Fish, Amphibians, Testudines	Type 3 Aves, Lepidosauria Chondrichthyes	Type 4 Crocodylia	SeqAPASS Level 3 Prediction of Similar to Human LFABP Template	Mutation in DUET	Stability Change from DUET (ΔΔG, kcal/mol)
50	Phenylalanine (F)	Valine (V) Isoleucine (I) Leucine (L)	Valine (V) Isoleucine (I) Leucine (L)	Phenylalanine	Yes No No No	 F50V F50I F50L	 -1.196 (Destabilizing) -0.808 (Destabilizing) -0.893 (Destabilizing)
54	Alanine (A)	Threonine (T)	Threonine	Threonine	Yes No	 A54T	 -0.195 (Destabilizing)
81	Threonine (T)	Alanine (A) Glycine (G)	Alanine	Threonine	Yes No No	 T81A T81G	 -0.749 (Destabilizing) -0.023 (Destabilizing)
93	Threonine (T)	Threonine Valine	Alanine		Yes Yes No	 T93V T93A	 0.031 (Stabilizing) -1.004 (Destabilizing)
97	Asparagine (N)	Glycine	Glycine	Glycine	Yes No	 N97G	 0.521 (Stabilizing)

RESULTS

- **MD Workflow**



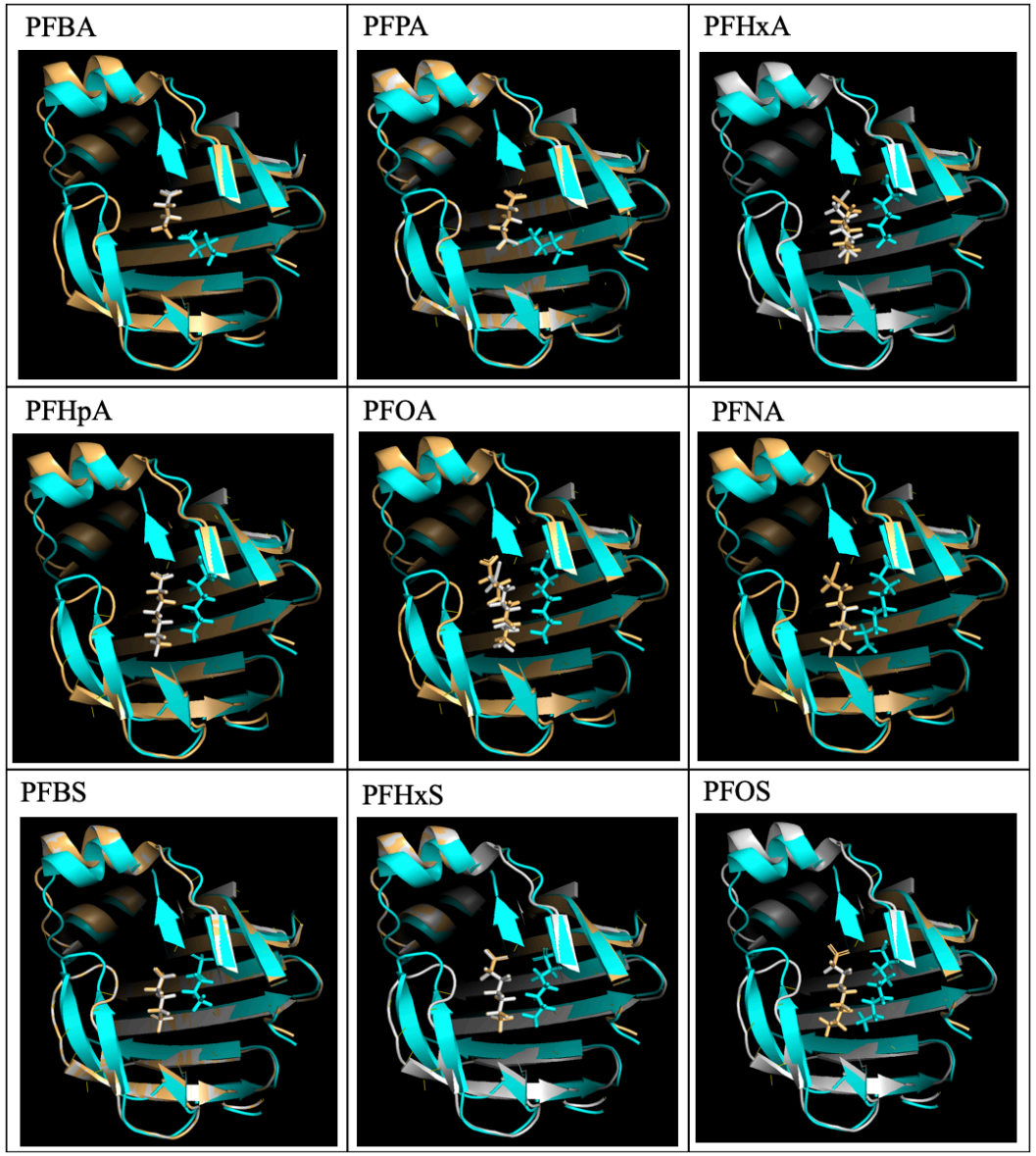
Multiple comparison (Tukey test) between human LFABP and other LFABPs for PFAS. Blue is human LFABP; red indicates significant difference ($p < 0.05$); gray means no difference from human LFABP ($p > 0.05$).

- **Japanese medaka** has significantly weaker LFABP binding affinity compared to human for all PFAS ligands ($P < 0.05$) except PFHxA, PFOA and PFNA
- **Fathead minnow** shows significantly weaker LFABP binding affinity than human for PFBS and PFHxS ($P < 0.05$)

Statistical summary over all 9 tested PFAS for different LFABPs

LFABPs	Max ΔG_{bind}	Min ΔG_{bind}	Mean ΔG_{bind}
chicken	-4.89333	-12.9956	-9.2
human	-4.39333	-13.9894	-8.89
rainbow trout	-2.01111	-16.2389	-8.45975
rat	-4.85333	-10.3439	-8.06698
zebrafish	-3.12778	-10.8956	-6.44444
fathead minnow	1.024444	-10.9344	-5.25457
Japanese medaka	2.956667	-12.9867	-3.86617

RESULTS

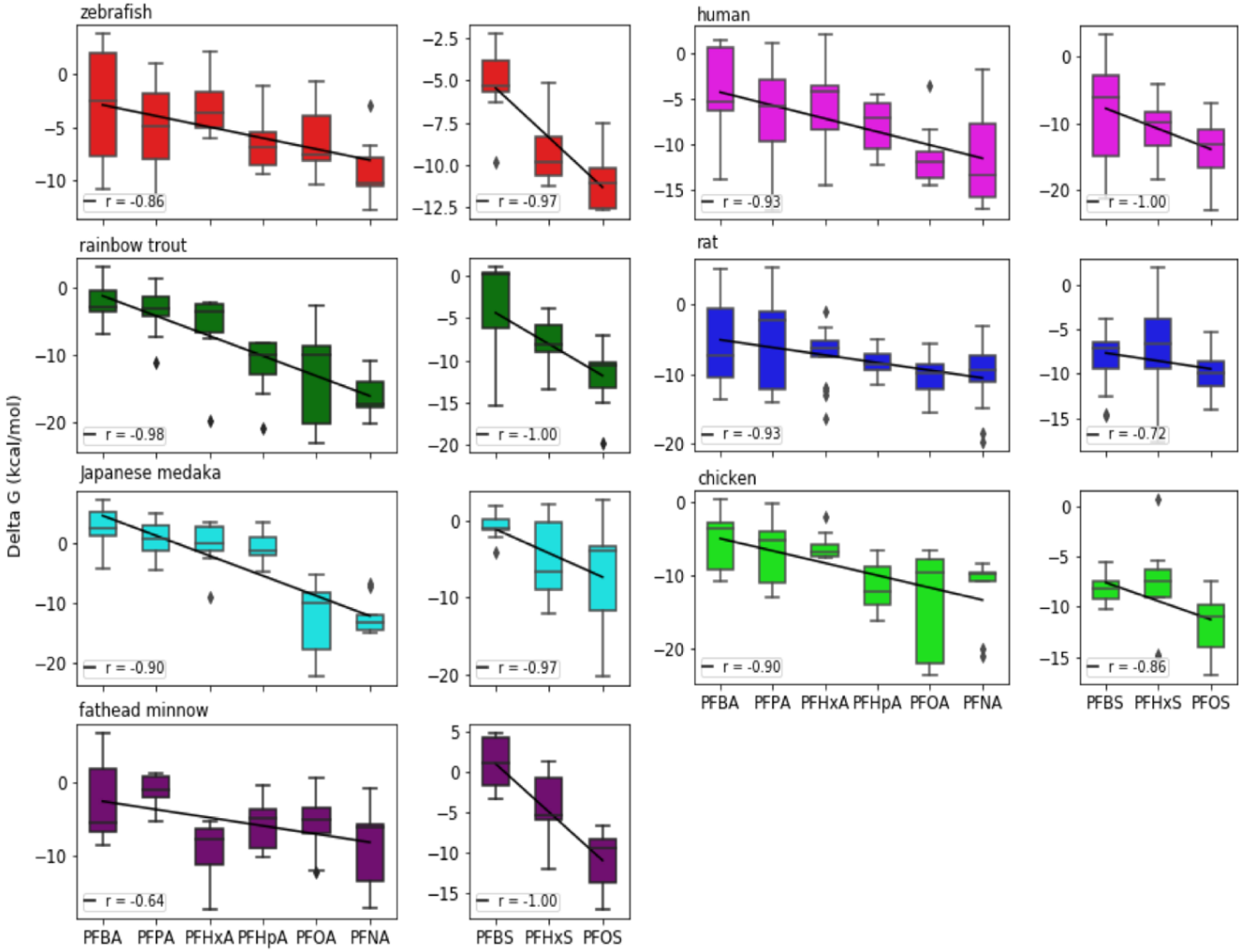


The PFAS binding poses for human (cyan color), Japanese medaka (orange color) and fathead minnow (grey color) LFABP after sequence alignment indicates:

- the positions of all PFAS ligands are quite different between human and the two fish species
- the position of ligands is closer to the bottom of the LFABP binding pocket, the binding affinity also tends to be stronger

RESULTS

- **MD Workflow**



Distribution of ΔG_{bind} for different PFAS-LFABP complexes across species

Insights into how the chemical structures of PFAS influence their protein binding behavior:

- In all LFABP systems, a quite strong negative relationship was observed for both LFABP versus PFCAs and LFABP versus PFSAs, with the correlation coefficient ranging from **-0.64** to **-1.0**.

CONCLUSION

By integrating SeqAPASS and the molecular dynamics workflow, our approach:

- Provides insights into the bioaccumulation potential across different species from the evaluation of both the structure and function of the critical protein LFABP
- Suggests that rat, chicken, zebrafish and rainbow trout are better representative species than Japanese medaka and fathead minnow for predicting bioaccumulation and toxicity in humans