



# Peroxisome-Proliferator Activated Receptors as a Macromolecular Target for Chemical Toxicity: Models of the Interactions of PPARs with Perfluorinated Organic Compounds

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research & development

IIC-2

## Science Question

How can perfluorinated compounds interact with key biomolecular receptors and how do they compare to other anthropogenic materials in terms of biological affinity?

## Research Goals

The goal of this research is to develop molecular models of the interaction between the peroxisome-proliferator activated receptor and persistent environmental pollutants, perfluorinated organic compounds (PFCs).

## Background Information

The *Peroxisome Proliferator Activated Receptors* (PPARs) are a class of nuclear receptors that modulate both transcription and multiple metabolic processes, and are implicated in a variety of metabolic disorders linked to lipogenesis, adipose tissue accumulation, fatty-acid oxidation pathways, type-II diabetes and metabolic syndrome to name a few (Figure 1).

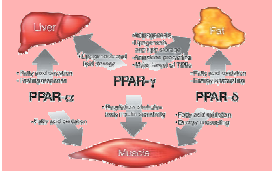


Figure 1. PPAR Receptor, function and distribution (From: Ronald M Evans, Grant D Baruch & Ying Xu Wang. PPARs and the complex journey to obesity. Nature Medicine 10, 353-361 (2004))

It is known that the persistent environmental pollutants PFOA (perfluorooctanoic acid) and PFOS (perfluorooctane sulfonate) are specific PPAR $\alpha$  agonists in wide variety of animal species (See Figure 2). The dose of these chemicals to the biomolecular site of action is increased due to persistent bioaccumulation (half-life of 4.4 years in humans) and unequal tissue distribution.

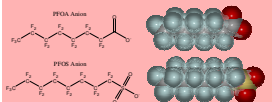


Figure 2: Structural formula of PFOA & PFOS esters, with respective CPK models (right)

Despite *in vivo* studies in animal species, the specific binding affinities and molecular modes of binding of this class of compounds for PPARs have yet to be identified and evaluated. Molecular modeling methods are used to gain insightful information in the mode of action and estimate the relative binding affinities of PFOA, PFOS and other related chemicals in comparison to known agonist, antagonists and putative natural ligands of the PPARs will greatly facilitate the Agency's task of evaluating the risk due to chemicals in this environmentally relevant class.

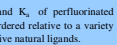
## Methods/Approach: I.,II.,III.

Many toxicological processes may be studied using the paradigms of this study. As a result, methods investigated may have a far reaching effect for evaluating the risks of this and other classes of chemicals and other macromolecular targets. Three main approaches of computational molecular interaction modeling methods are exploited:

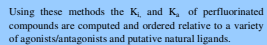
### I. Multi-Fragment Search (Solvent Mapping)



### II. Homology Modeling / Ligand Docking



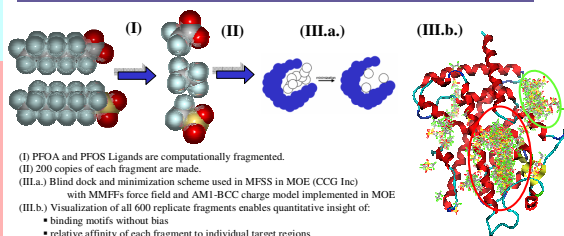
### III. Virtual High-Throughput Screening



Using these methods the  $K_i$  and  $K_a$  of perfluorinated compounds are computed and ordered relative to a variety of agonist/antagonists and putative natural ligands. The above methods are used specifically to (1) elucidate potential sites of binding to a given receptor and rank site selectivity using classical molecular mechanics force-field approaches (2) compute the binding affinity of the test PFCs compared to other known strong binding and natural agents and (3) determine the relative binding affinities of a diverse subset of compounds including PFCs.

## Methods and Approach Used

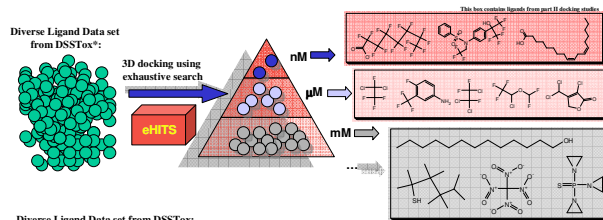
### I. Multi-Fragment Search (Solvent Mapping) Binding Site Analysis



- (I) PFOA and PFOS ligands are computationally fragmented.
- (II) 200 copies of each fragment are made.
- (III.a) Blind dock and minimization scheme using in MFSS in MOE (CCG Inc) with MMFFs force field and AM1-BCC charge model implemented in MOE
- (III.b) Visualization of all 400 replicate fragments enables quantitative insight of:
  - binding motifs without bias
  - relative affinity of each fragment to individual target regions
  - constitutive fragment overlap as potential "full dock" analyses (i.e. Blind determination of potential binding sites by overlapping chemical regions)
  - AGONIST binding site > RXR binding motif > Co-factor binding motif

This data suggests that perfluorosulfonate, perfluorocarboxylate, and fluorocarbon moieties appear highly clustered in the PPAR LBD pocket (red circle in III.b.) associated with agonist activity. Similarly this analysis suggests that the PPAR/RXR heterodimerization site (green circle in III.b.) as an additional potential site whereby it may have antagonist properties. We perform the next two phases (Ligand docking and V-HTS) of these studies in the major binding pocket (shown in red circle).

### III. Virtual High-Throughput Screening for PPAR Affinity



- Diverse Ligand Data set from DSSTox:**
- ~3600 ligands selected. In addition to \* data set used in part II Docking studies
  - AChTol, MW<600, parent only, organic only, single form
  - Exhaustive search algorithm used to sample ligand conformational space of receptor pocket using eHTS
  - Empirically parameterized scoring based on known ligand/receptor complexes.
  - Dock at same site as determined by I.Multi-Fragment Search (above)
  - Bin the results and sort by order of tightest binding (top of pyramid) to weakest binding (bottom of pyramid)
  - Representative hits are shown for each affinity class, selected as the top of each respective binding strength bin.

### II. Homology Modeling / Ligand Docking / Population Analysis

LIGAND	TRIVAL NAME	CLASS
OLA	conjugated linoleic 18:2 (9,11)	DIETARY FA
ALA	alpha-linolenic 18:3 (9,12,15)	DIETARY FA
LA	linoleic acid 18:2 (9,12)	DIETARY FA
OLA	oleic acid	DIETARY FA
PFOA_S	perfluorooctanoic_sulfonated	FLUOROCARBON
PFOA_L	perfluorooctanoic_linear	FLUOROCARBON
PFOS	perfluorooctane_sulfonated	FLUOROCARBON
OCT	octanoic acid	DIETARY FA
OK	oleic acid	DIETARY FA
SPH	sphingosine	LIPID/PEPTIDE
RDS	retinol	VITAMIN
TRP	tryptophan	HYDROPHOBIC
METP	methamphetamine	DRUG
TTA	trans-2-octadecenoic acid	DIETARY FATTY ACID

PPAR Docking studies comprised of (a) Docking a series of dietary fatty acids, fluorocarbons, known PPAR  $\alpha$ ,  $\beta$ ,  $\gamma$  and LXR agonists into "apo" forms (\*) modeled from PPAR  $\gamma$  IPRG in the case of PPAR  $\alpha$ ,  $\beta$  only) agonist bound conformation (117G, 2BAW and 2PRG for PPAR  $\alpha$ ,  $\beta$ ,  $\gamma$  respectively). Docking performed in largest binding site determined by MOE-Dock, based on crystal packing, connecting with the agonist binding site in the LBD as found in other PDB structures. Charges of ligands assigned based on AM1-BCC charge model. MMFFx force-field used for optimization, empirical scoring function used for Ki.

(b) Example of the binding pocket (117G) with the lowest energy pose for each of the circled hits are shown overlaid as space-filling models (CPK).

(c) Shown below: Population-based pose docking scores for "apo" and "active" forms arranged on a grid, ordered by frequency into  $K_i$  bins. This analysis suggests: for PPAR- $\alpha$  ~10-100 X increased affinity of the fluorocarbon class relative to the agonist class for the apo structure, similar order of magnitude as CIP/PPAR agonist for the active-bound conformation. In the case of PPAR  $\beta/\gamma$  similar orders of magnitude were found between putative natural ligands and isoform specific agonists. Population maps colored by frequency into affinity bins based on clustering pose energies.

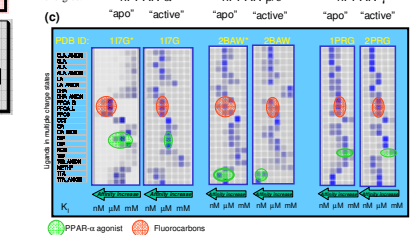


Figure 5: Respective ligands are shown in close-up of active site as CPK models in the 117G PPAR  $\alpha$  crystal structure. Note how the PFOA (linear and branched) and caproic acid (a known agonist) appear to be overlaid whereas PFOA occupies a slight offset in the same pocket.

## Results/Conclusions

- Alternative sites for PFOA/PFOS interaction with PPARs (i.e. also RXR heterodimer site):
  - \*agonist binding motif in LBD is "most probable" docking site, however RXR heterodimer domain is also probable!

\*Significantly higher affinity for PFOA/PFOS to apo form of protein in the PPAR  $\alpha$  isoform (~10-100x CIP agonist)

\*Potentially many more PFCs that may interact with PPAR or other NR studied in this preliminary HTS investigation.

## Impact and Outcomes

- This research supports the Agency's goals in the multi-year plans for Human Health and Endocrine Disruption. It addresses the significant Agency need for predictive models for hazard identification in the sub-area of (1) QSAR and other computational approaches, and (2) high throughput screening.
- This project supports two of the objectives of the NCCT as stated in the Computational Toxicology Framework:
  - \*2) develop predictive models for categorizing/prioritizing chemicals in the environment, and
  - \*3) improve quantitative risk assessments.

\*Molecular modeling and other tools derived from computational chemistry can be used in conjunction with available experimental data to develop methods for screening environmental chemicals for bio-affinity enabling a more efficient risk assessment process.

## Future Directions

- Select a minimal subset of PFCs and other PPAR specific ligands in order to experimentally evaluate the  $K_i$  (or conversely  $K_a$ ) and validate the accuracy of these models.
- Evaluate more ligand binding assays of PPAR, in addition to PFOA and PFOS itself in a variety of conditions that are representative of the cellular domains and functional forms in which they are found.
- Develop interaction maps of known agonists/antagonists for receptor
- Evaluate more nuclear receptors at multiple binding domains to generate *in silico* chemical genomics data, not solely develop new docking models.
- Model the PFOA/PFOS interactions with Human Serum Albumin and OAT-2 (organic anion transporter II) required for ligand sequestration and clearance respectively.

## References

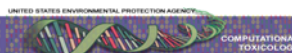
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Long Term Goal II

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