

Bioaccumulation & Impacts of Novel & Legacy PFAS in Wildlife of Coastal North Carolina

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Acknowledgements:

- **Belcher Lab:**
Theresa Guillette, PhD
Matt Guillette
Madi Polera
Thomas Jackson



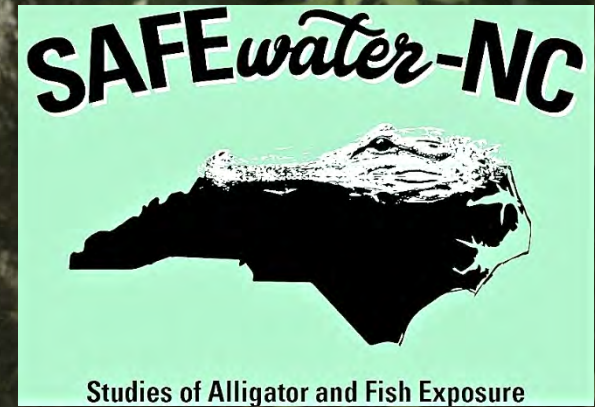
- Helen Nguyen
Chris Scheibly
Gabe Bendfeldt
Aubrey Sasser



- Mark Strynar, PhD
- James McCord, PhD



- Alicia Davis
- Kyle Rachels
- Clint Morgenson



The BIG Questions:

- Are PFASs present and accumulating in NC wildlife?
 - Which ones and for how long?
 - Do “replacement” PFAS bioaccumulate?
- Are the levels found associated with indicators of adverse wildlife/ecosystems health?
- What can this tell us about effects on humans?

The Concept of One Health:

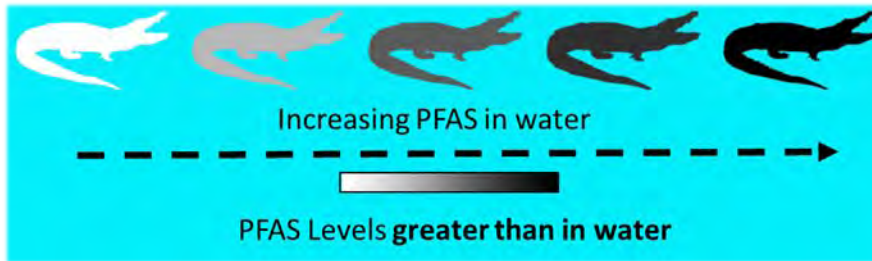
Studies in sentinel species can act as an early warning of environmental problems that are impacting humans



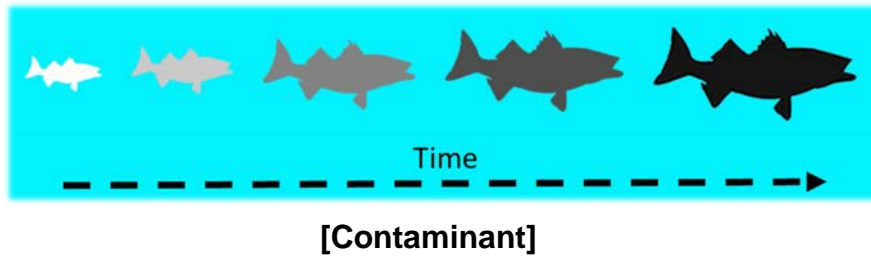
Sentinel Species

Bioconcentration, Bioaccumulation, Biomagnification

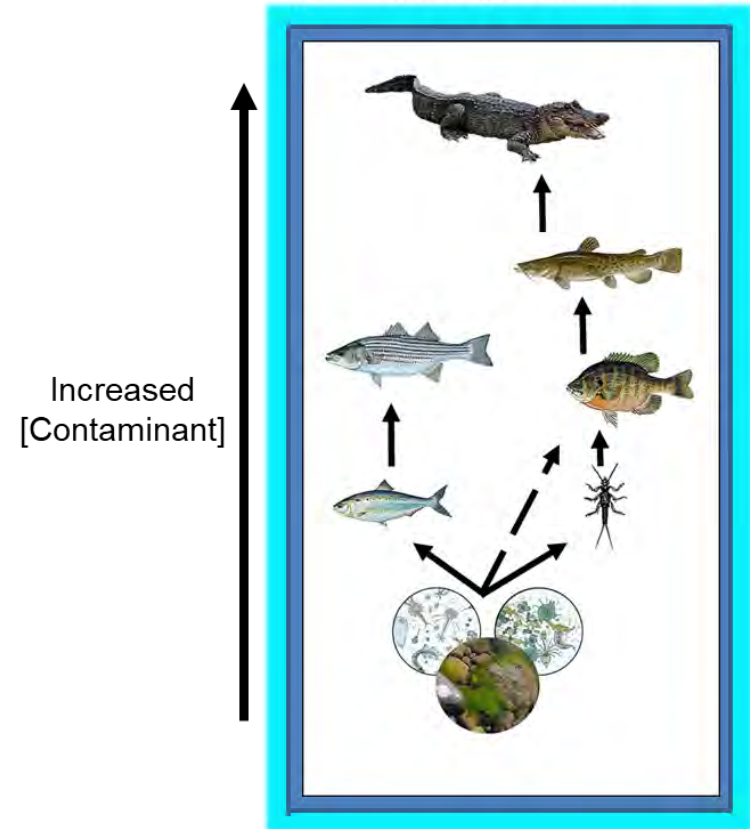
Bioconcentration/Bioaccumulation



Persistent Organic Pollutants (POPS) or Toxic Metals

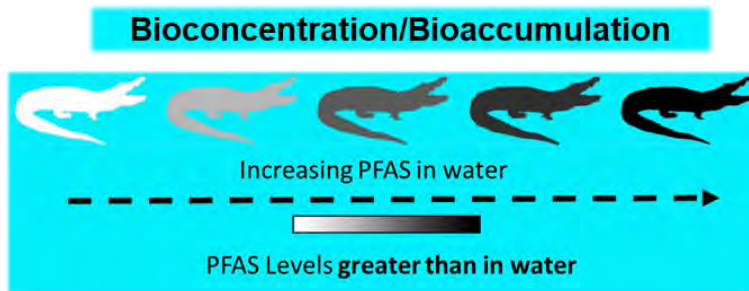


Biomagnification



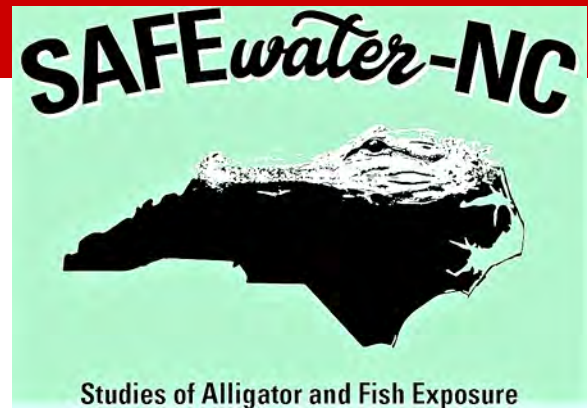
Contaminant is eliminated much slower than it is absorbed

PFAS are bioaccumulating in aquatic vertebrates living in contaminated waters

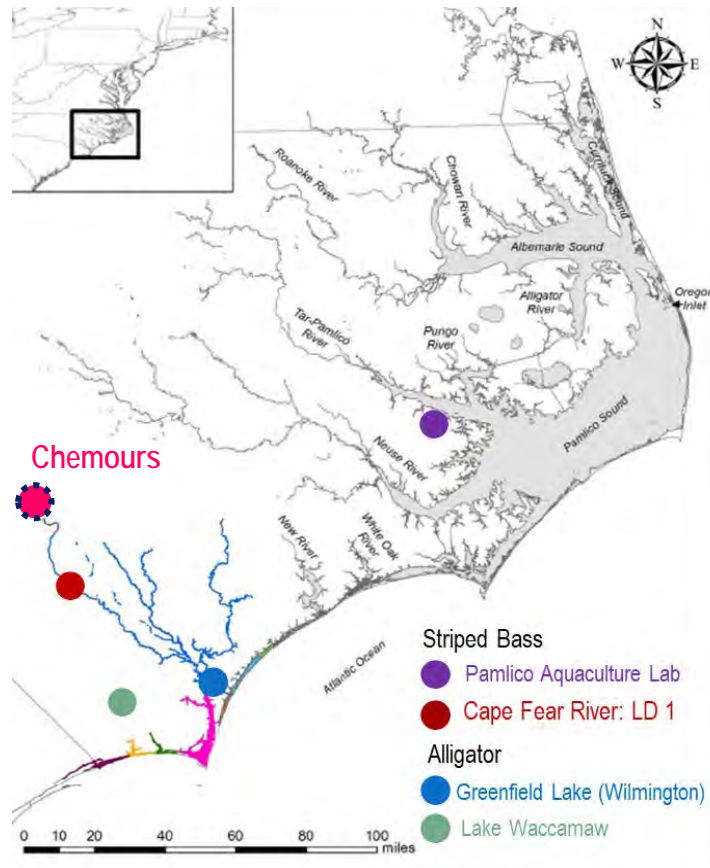


- PFAS in Cape Fear River Water = ppt
- Blood of Fish and Alligator = **ppb-ppm**





Alligator: Study Approach



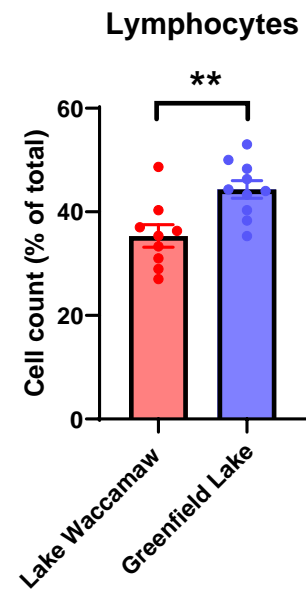
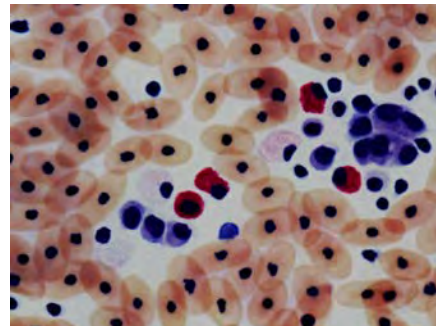
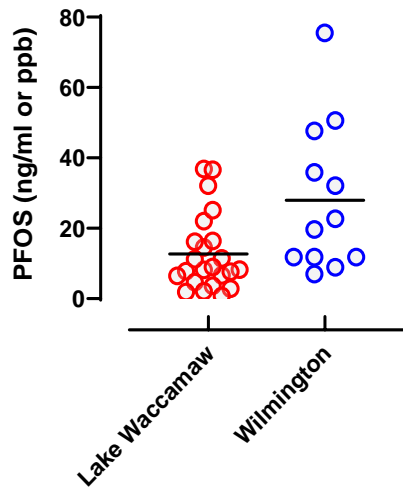
Approach:

- Active capture adult alligators (6 foot +) and Juvenile
- Sample: Blood/Serum
 - Morphometric measures
 - Determine sex
- Quantitate serum PFAS levels (LC/MS/MS)
- Blood chemistry/hormones/liver enzymes
- Immune function: lysozyme and immune cell counts

- 1) Identify a reference population
 - Lake Waccamaw (Lumber River watershed)
- 2) Compare Cape Fear & Wilmington Area
 - Greenfield Lake

Preliminary Findings:

- Increased serum PFAS are found in Cape Fear River alligators (and fish)
- Higher contamination levels - increases in unhealed “lesions” and poor body condition
- Site-specific differences in immune function, liver enzymes, and blood chemistry



Analysis is ongoing.....

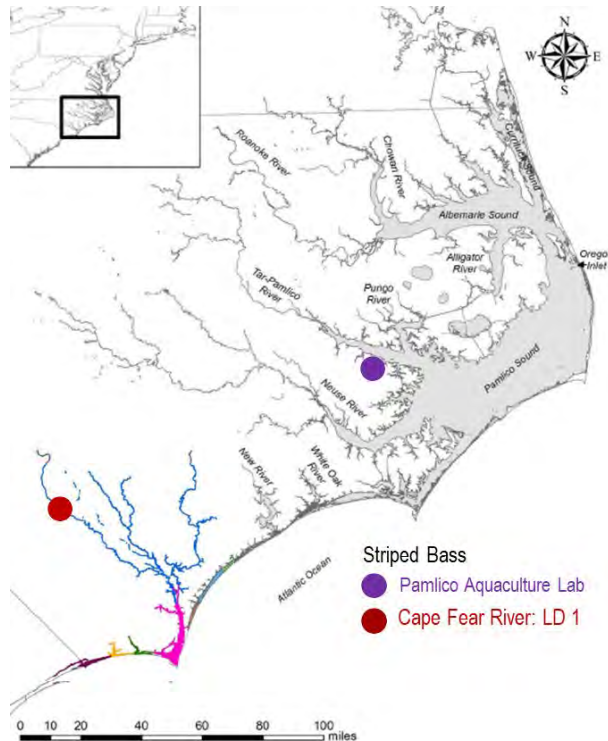
Striped Bass: *Morone saxatilis*



Commercial and recreational fishery in North Carolina valued at more than **\$94 million annually**

Striped Bass: can live in **both salt and fresh water**

- Cape Fear River Population – Do not migrate
 - riverine/estuarine
 - good model of Cape Fear contaminants
- No natural reproduction in the Cape Fear River
- Tar/Pamlico, Neuse and Cape Fear Rivers:
 - Essentially 100% are hatchery progeny
 - Analyzed fish were between ~2-7 year old
 - Residents of the Cape Fear River from 1-6 years



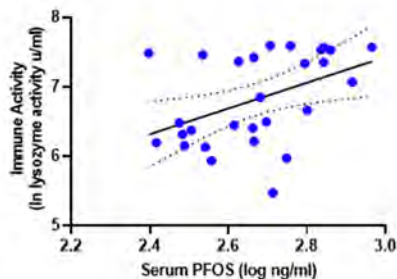


• Approach:

- Targeted and untargeted high-resolution mass spectrometry of blood
 - Measure known (≥ 23)
 - Detect unknown PFAS

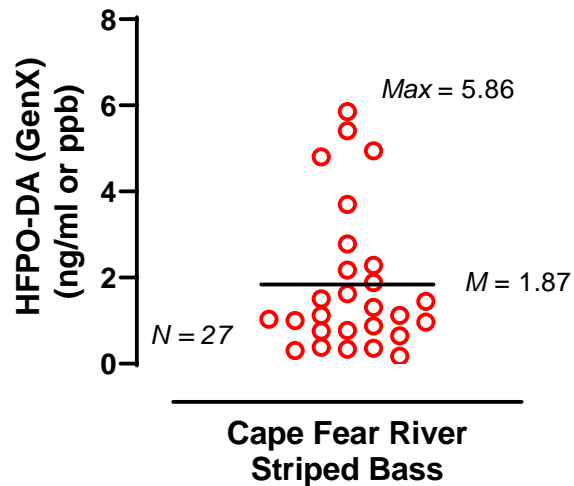
- Blood chemistry and health-related biomarkers
 - Liver
 - Kidney
 - Immune system
 - Hormones

- Regression analysis to detect associations between total and individual PFAS concentrations and health endpoints

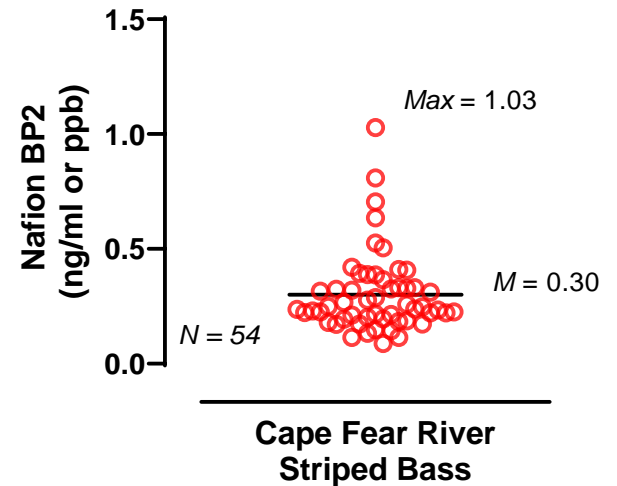


PFAS in Cape Fear Striped Bass Serum

- GenX was detected in half the samples
- Nafion BP 2 was detectable in 78% of samples



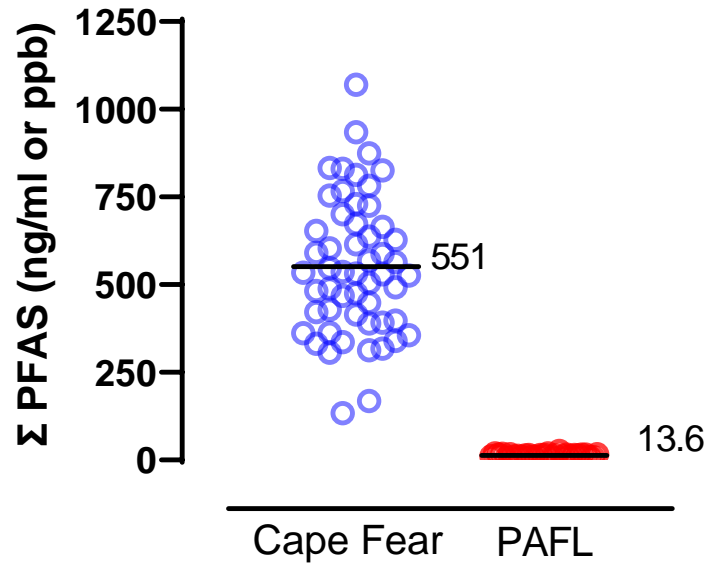
52.6% (30/57 <LOQ 0.24 ng/ml)



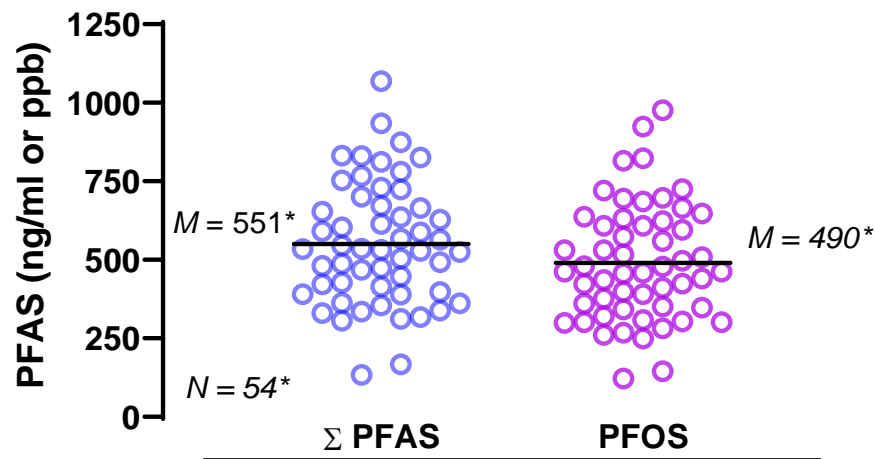
Detected in 78% of samples

PFAS in Striped Bass Serum

- Total PFAS is >40 higher in Striped Bass from the Cape Fear River



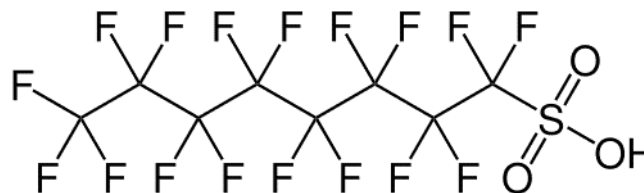
PFOS accounted for 89% of PFAS present in serum of Striped Bass from the Cape Fear River



**Cape Fear River
Striped Bass**

PFOS = 89% of Total PFAS

* concentration of 4 samples >LOQ

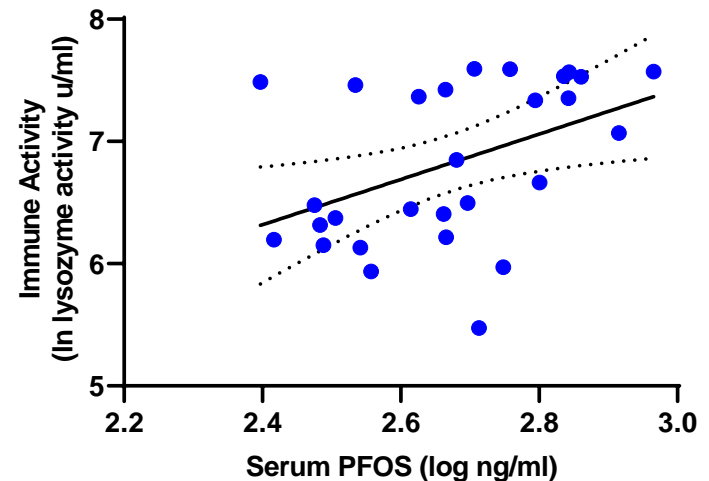
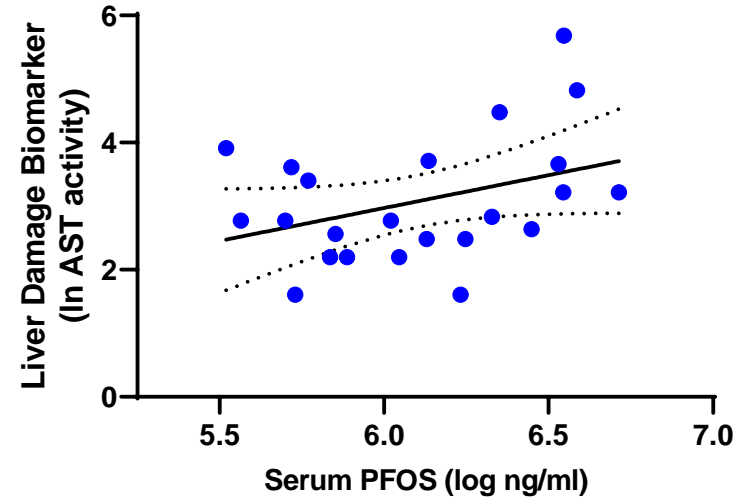


PFAS exposures in Striped Bass are associated with liver and immune function changes

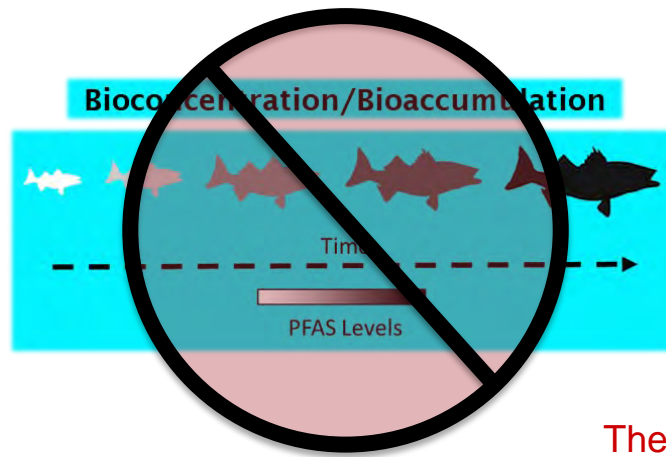
Comparison of liver enzyme activity and immune enzyme activity

With **regression analysis** found:

Enzyme concentrations are increased with increasing PFAS

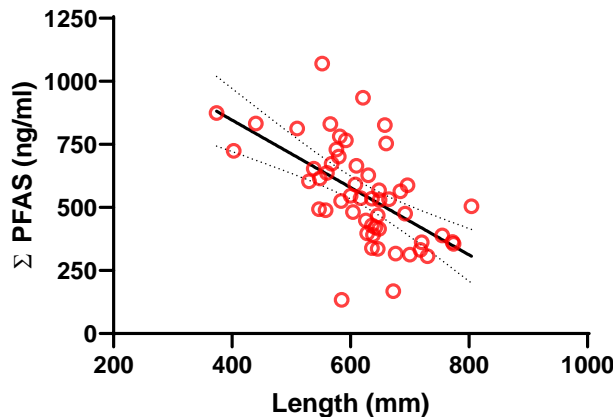


PFAS in Striped Bass Serum: Bioaccumulation is very complicated



In striped bass PFAS bioaccumulation is not a simple analogy to POPS or toxic metals

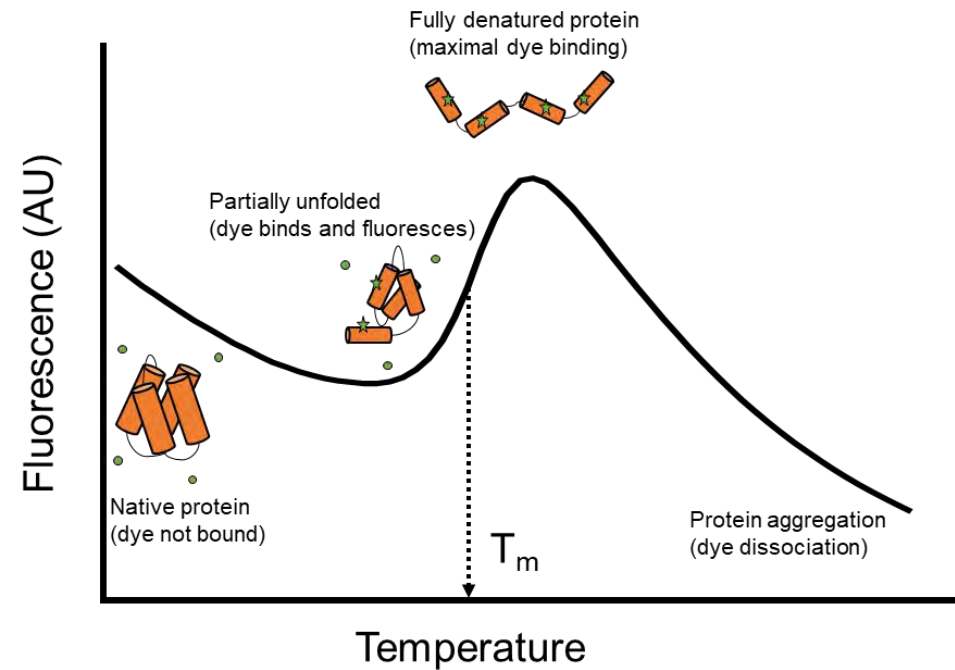
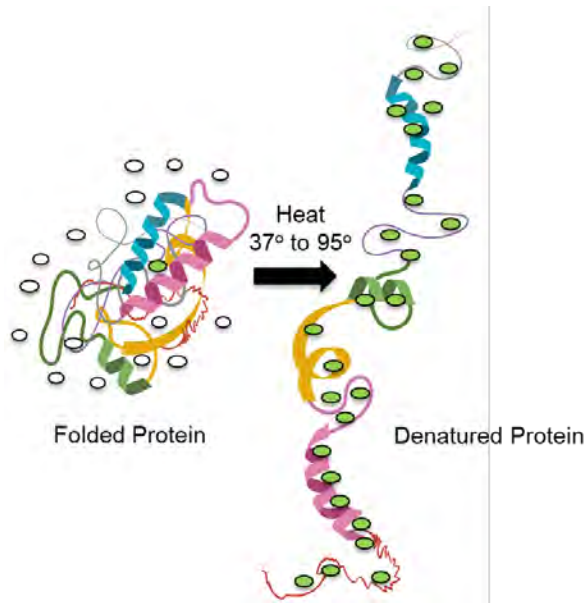
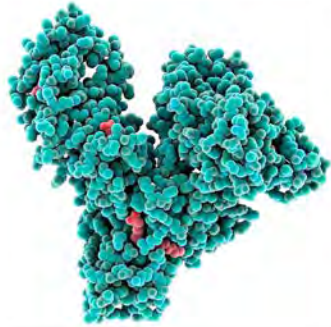
There is a major need to understand fundamental toxicokinetic properties of PFAS



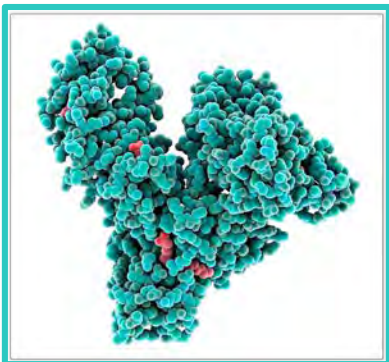
PFAS:

- Can be both polar and hydrophobic
 - Can bind proteins (albumin, FABP)
 - Accumulate in highly perfused tissues
 - Some may bind phospholipids
- ❖ Affinity of individual PFAS to serum proteins

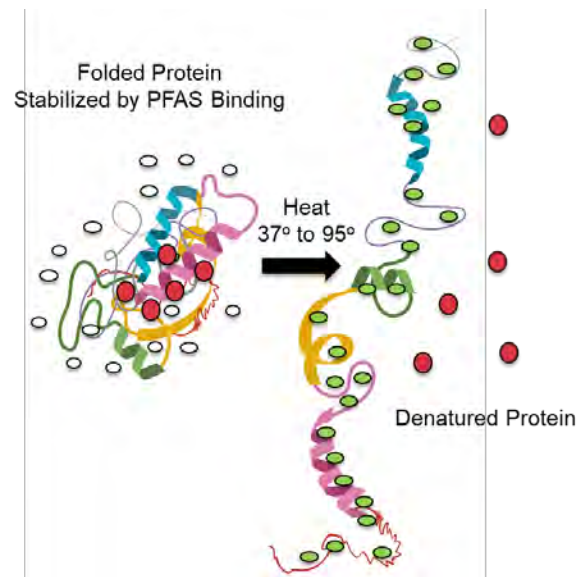
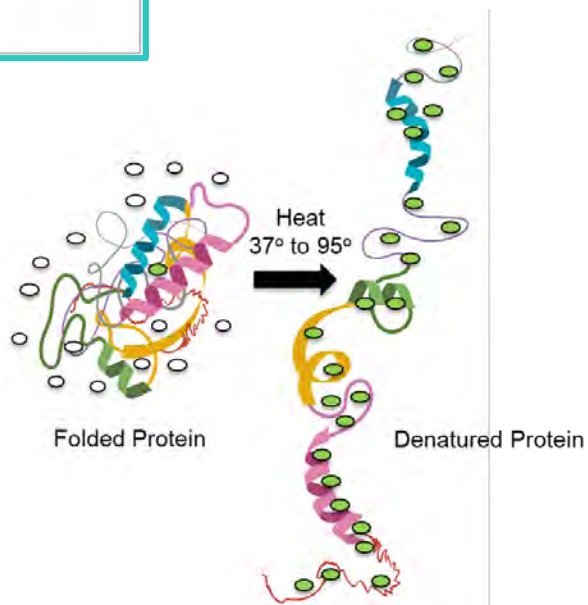
A Novel HT PFAS Albumin Binding Assay



- Hydrophobic dye binding sites sequestered in folded protein
- Denatured protein – dye binding sites exposed
- Measure fluorescence (497nm) increase to calculate T_m

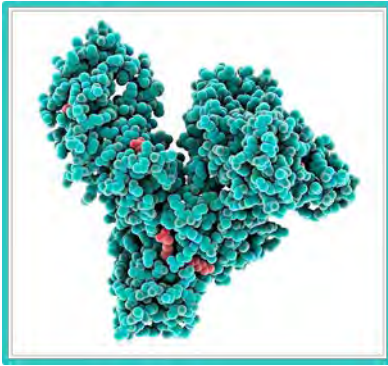


A Novel HT PFAS Albumin Binding Assay



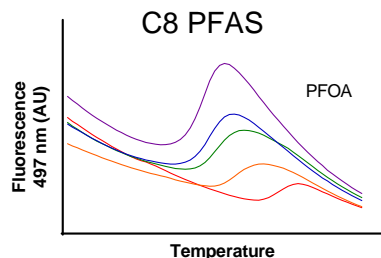
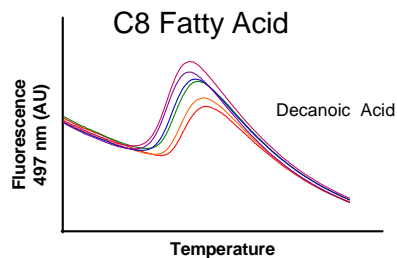
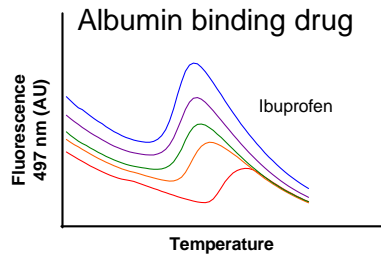
- Hydrophobic dye binding sites sequestered in folded protein
- Denatured protein – hydrophobic dye binding sites exposed
- Measure fluorescence increase to calculate T_m

- Bound ligand increases protein stability
- Compare impact of increasing concentrations of ligand/PFAS on T_m (ΔT_m)



A Novel HT PFAS Albumin Binding Assay - proof of concept -

Concentration Response

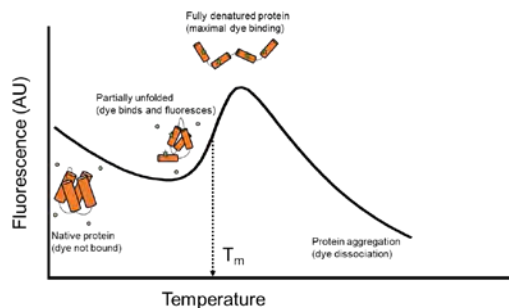


Compound Name	Abbreviation	Cas No.	Chain Length	ΔT_m ($^{\circ}C$)	EC10 [μM]
Perfluorobutanoic Acid	PFBA	375-22-4	4	5.5	344
Perfluoropentanoic Acid	PFPeA	2706-90-3	5	7.7	446
Perfluorohexanoic Acid	PFHxA	307-24-4	6	10.8	632
Perfluoroheptanoic Acid	PFHpA	375-85-9	7	16.4	910
Perfluorooctanoic Acid	PFOA	335-67-1	8	17.8	1183

Increasing chain length stabilizes structure



Short chain binds with higher affinity



- Able to calculate K_d and rapidly compare relative binding properties of a variety of PFAS at different proteins

QUESTIONS??



Question 1:

What physiochemical information is necessary to better characterize the fate and transport of individual or groups of PFAS?

Question 2:

What physiochemical information is necessary to better characterize the potential for individual or groups of PFAS to have adverse effects?