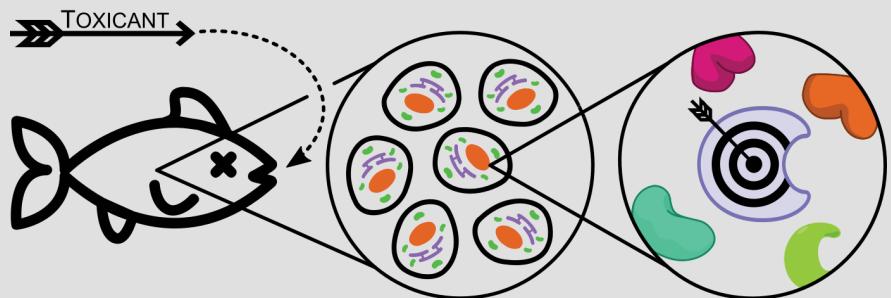


Chemical Proteomics Methods for Elucidating the Protein Targets of Environmental Contaminants

David Hall

PNNL Seminar,
Biological Sciences
Division

May 1st, 2022



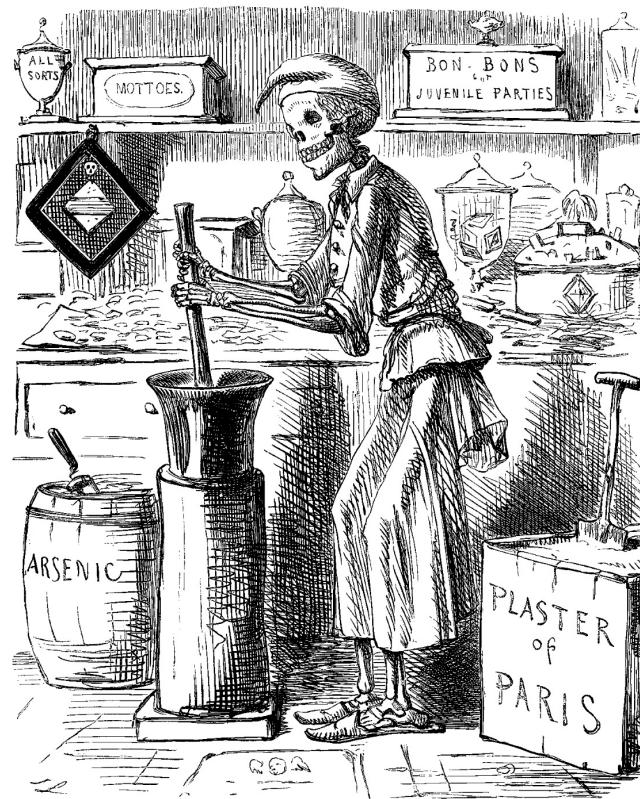
Slides available here: davidrosshall.github.io/pnnl-talk

1



G. McCaa
USBM

2



THE GREAT LOZENGE-MAKER.

A Hint to Paterfamilias.

J. Leech
Punch, 1858

3



PBS Wisconsin
(2020)

4



BBC UK
(1999)

5

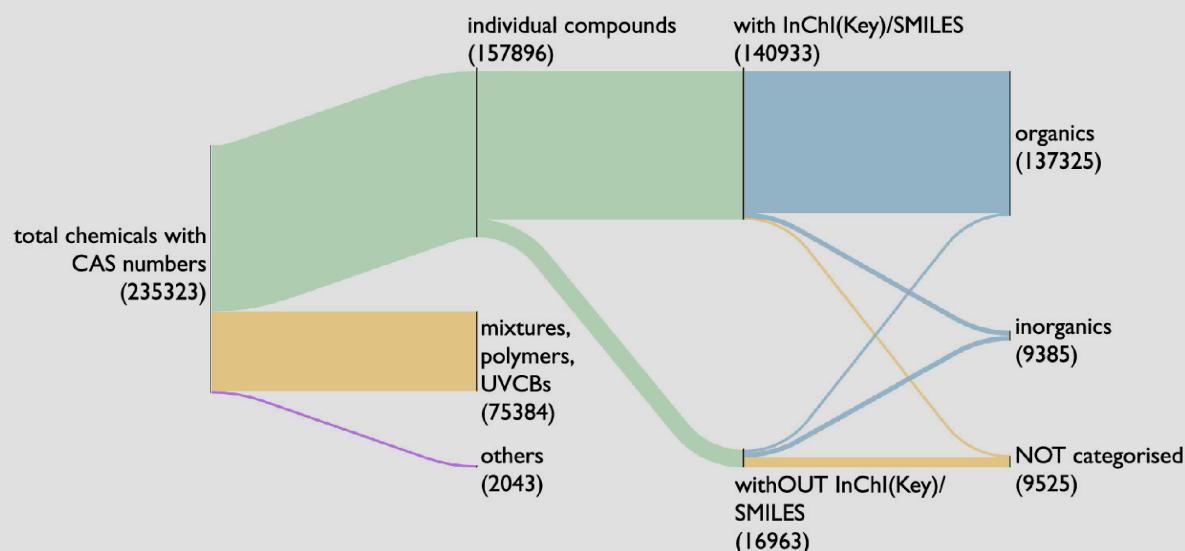


Caven
Images
(2021)

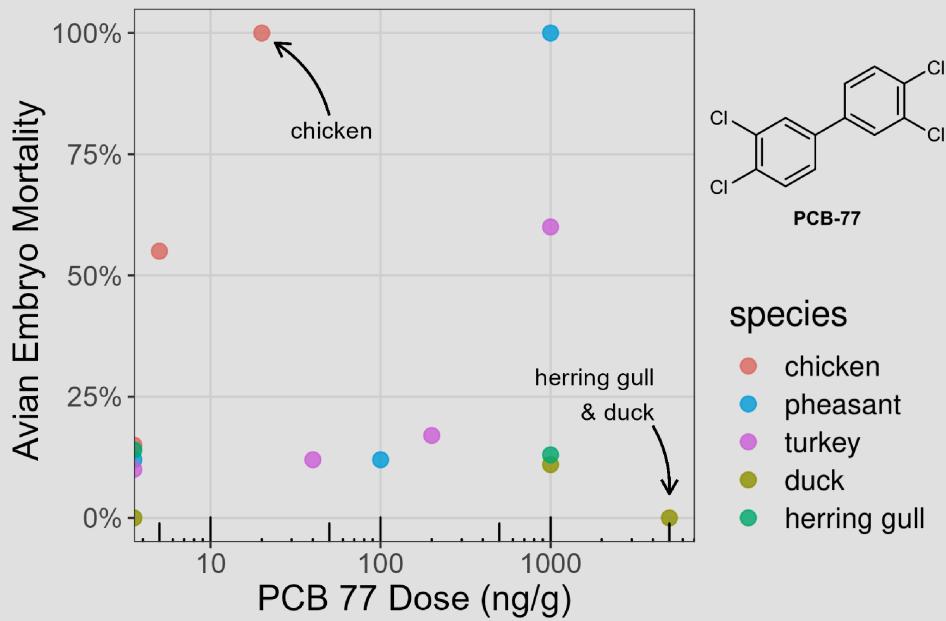
6

Traditional animal-based toxicity/risk assessments faces two major obstacles

Problem 1: The throughput of animal-based testing cannot cope with the number of chemicals in use



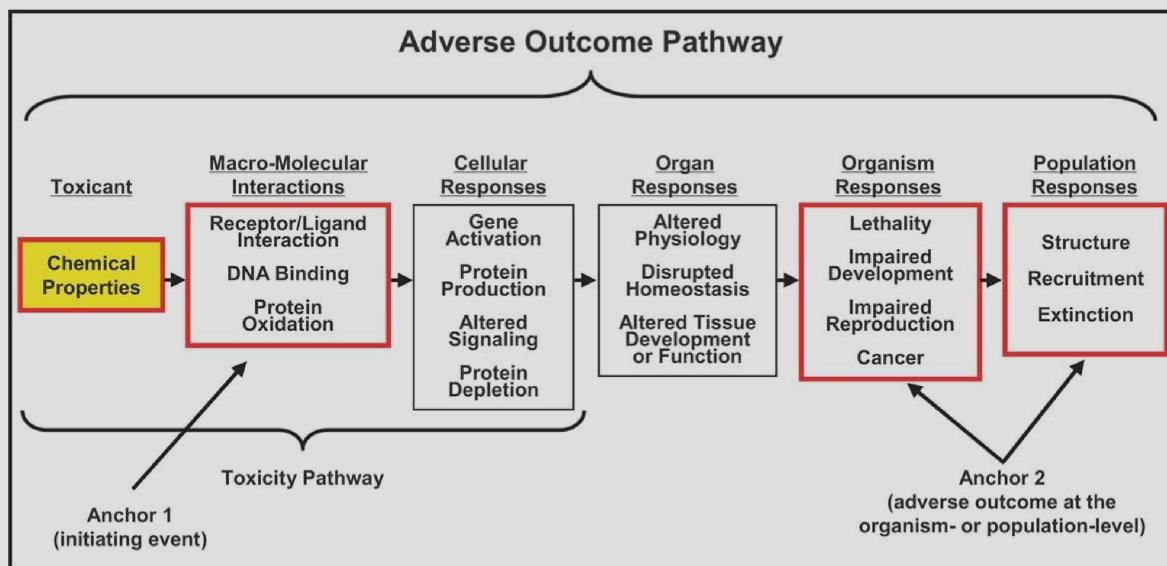
Problem 2: Single species testing does not reflect interspecies variation in toxic response(s)



Data from
Kennedy
et al., 1996

9

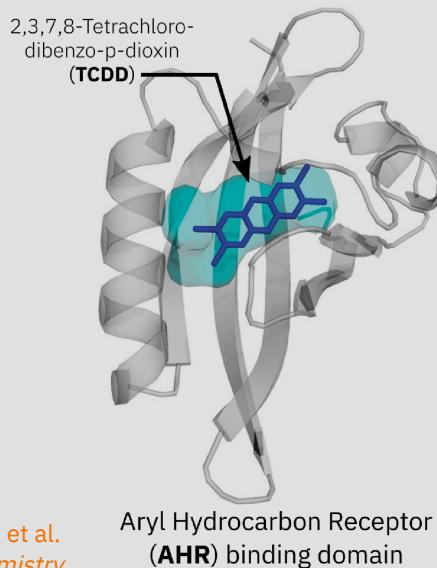
AOPs can address these issues, but animal testing fails to elucidate *molecular* mechanisms of toxicity



Ankley et al.
Env. Tox. &
Chem. 2010

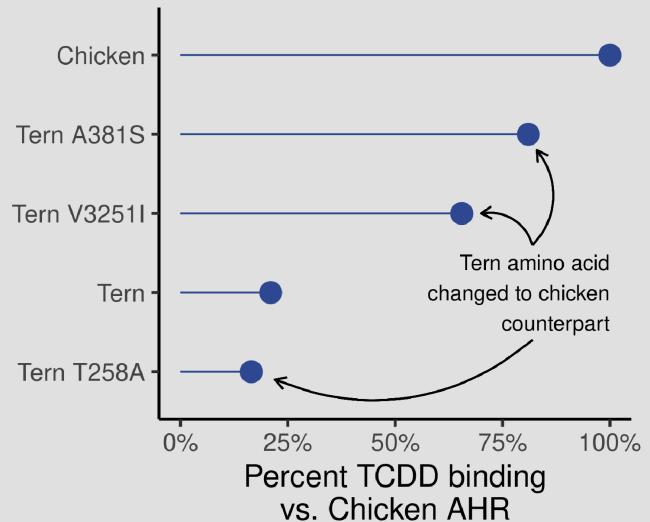
10

Small differences in protein structure can account for large disparities in interspecies toxicity



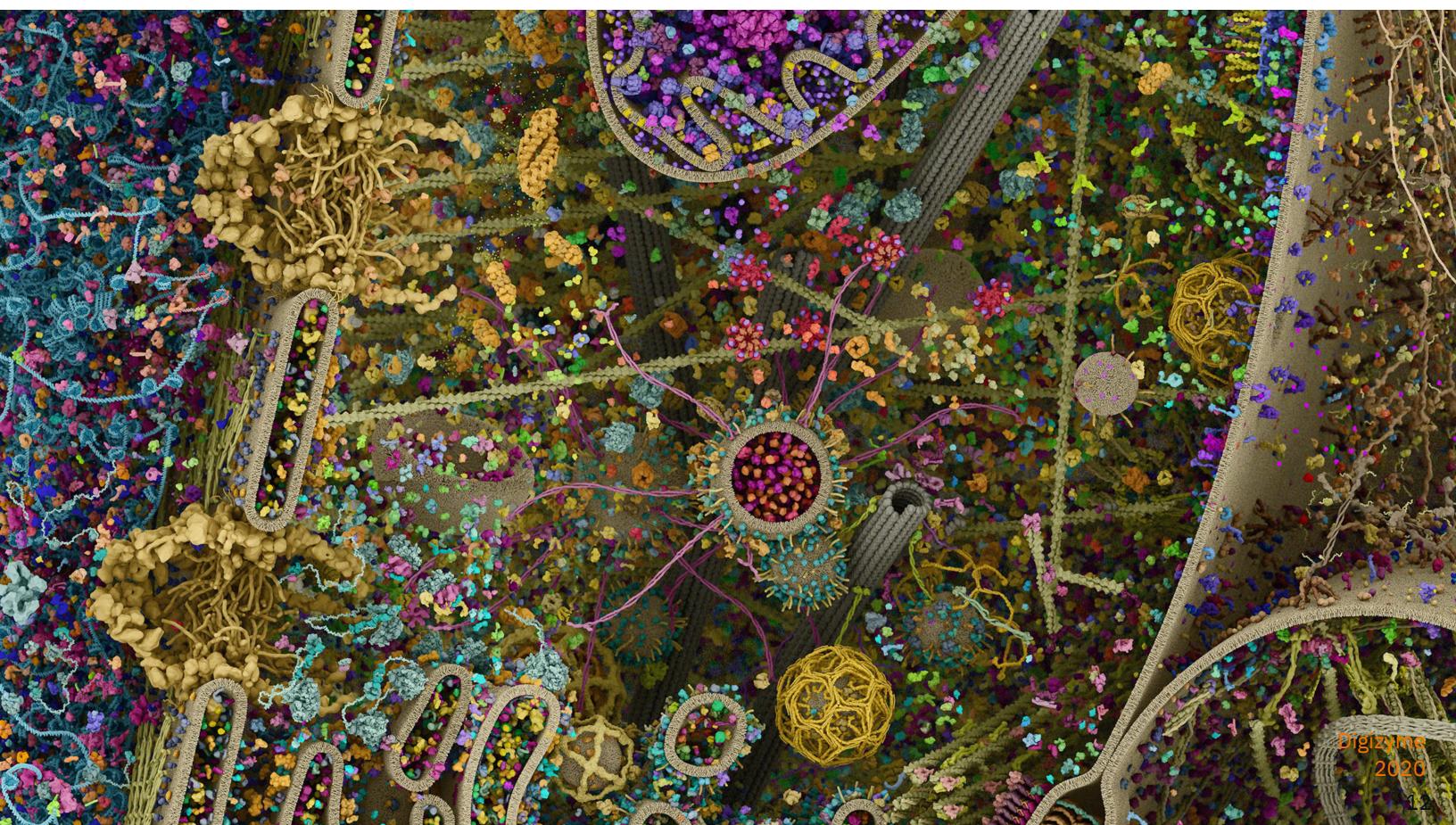
Aryl Hydrocarbon Receptor (AHR) binding domain

Pandini et al.
Biochemistry
2009



Karchner et al.
PNAS 2006

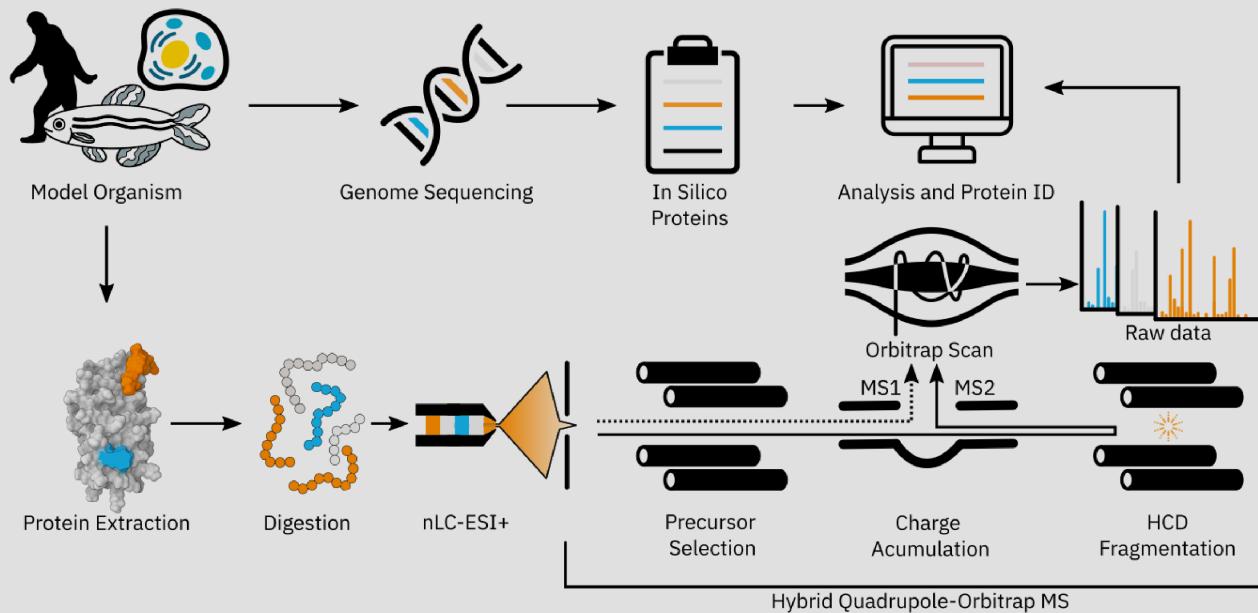
11



Digizyme
2020

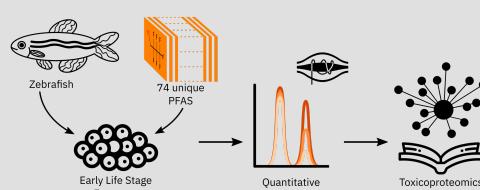
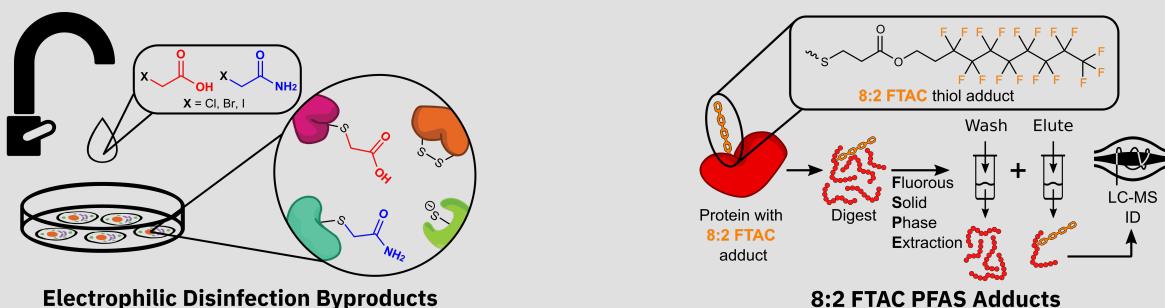
12

The identification and quantification of thousands of proteins is possible via LC-MS proteomics



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During my PhD, I've developed proteomic methods to explore environmental chemical/protein interactions



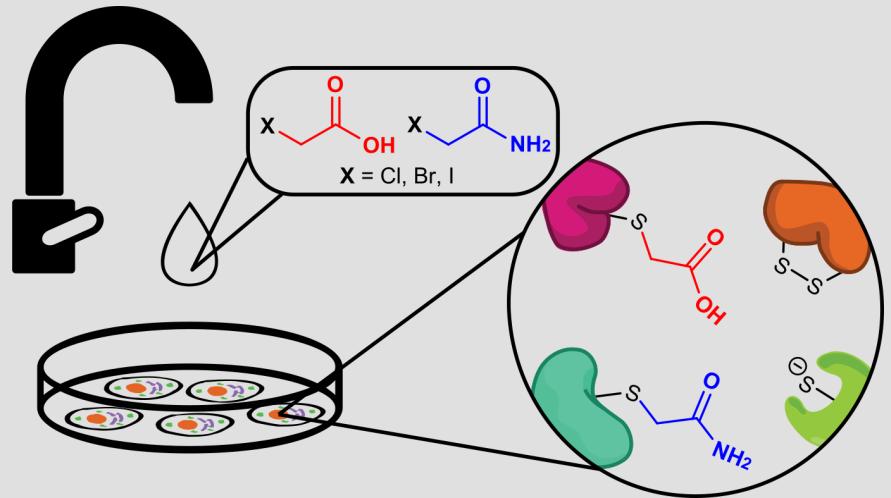
PFAS impacts on ELS zebrafish

14

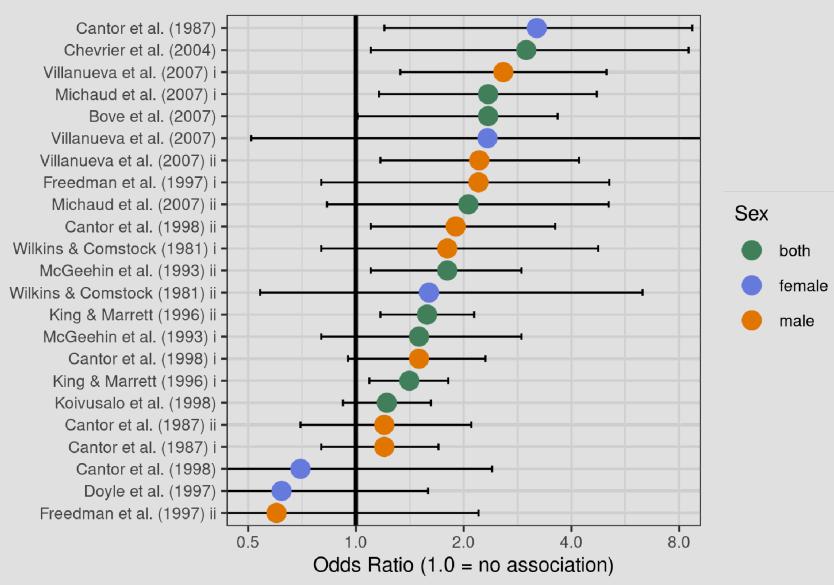
Project 1: Profiling Proteome Thiol Reactivity of Monohaloacetic Acids and Monohaloacetamides

David Hall,
Kirsten Yeung,
and Hui Peng

Published in *ES&T*,
2020, 54, 23,
15191-15201

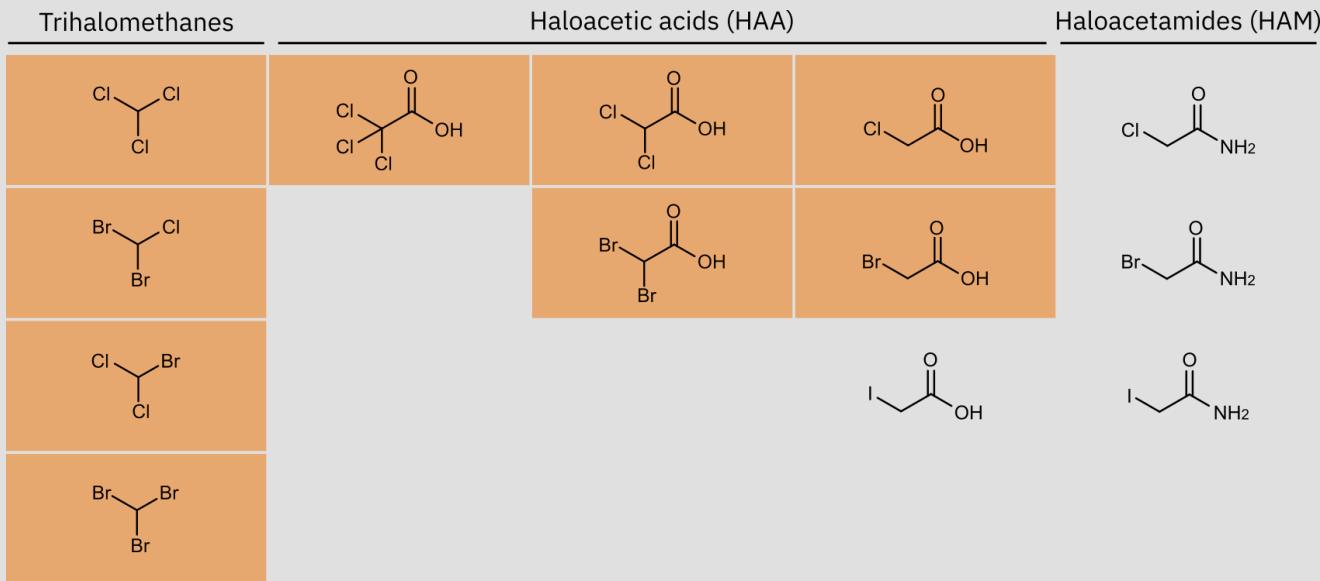


Long-term consumption of chlorinated water is epidemiologically linked to bladder cancer



Data from
Hruday
2009

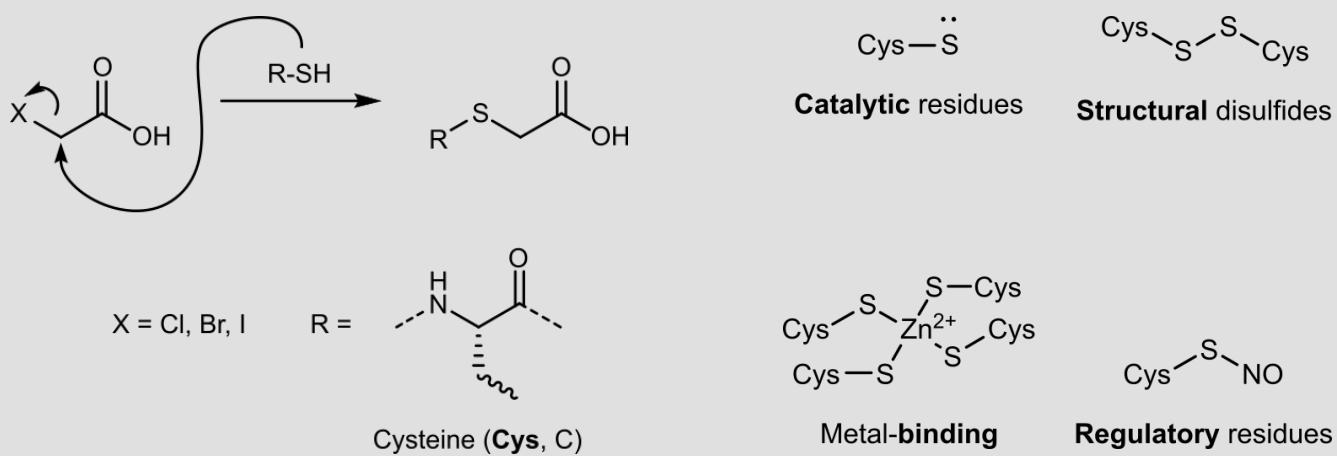
Adverse health outcomes of chlorinated water are caused by halogenated disinfection byproducts



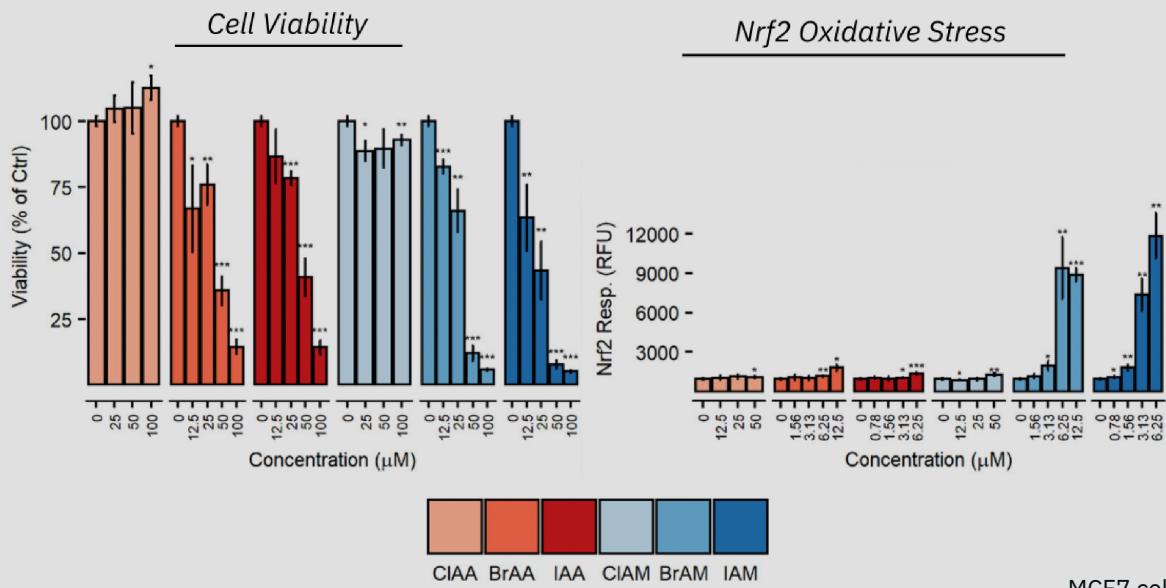
THMs and HAA5 are regulated in Canada

17

HAM and HAA geno- and cyto-toxicity is purportedly due to their reactivity with, potentially critical, cysteine residues



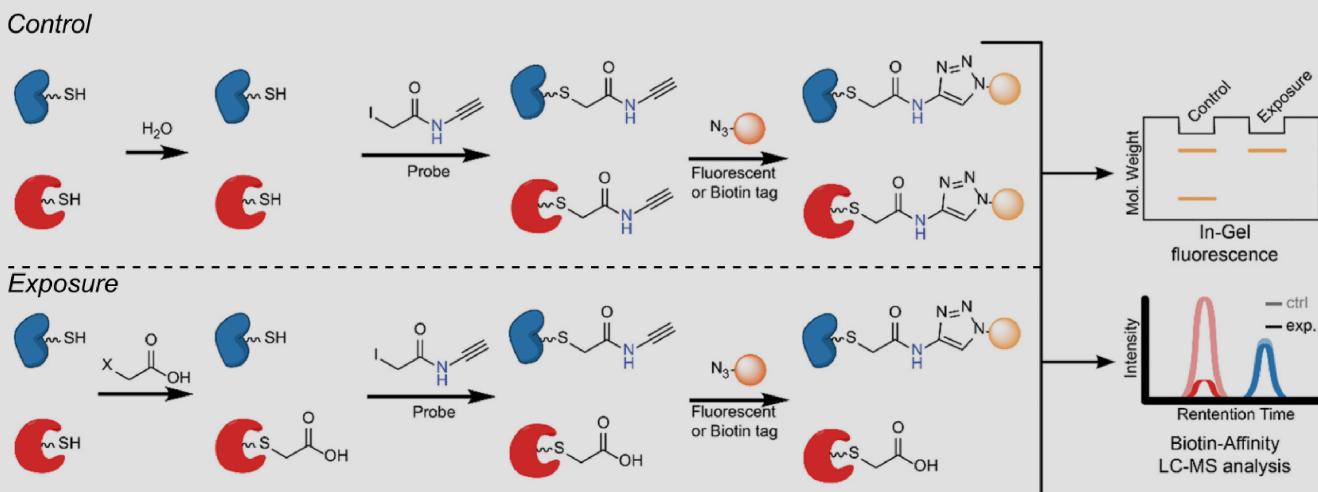
Order of DBP cytotoxicity is I > Br >> Cl,
but only HAMS induce Nrf2 oxidative stress



AA = acetic acid
AM = acetamide

19

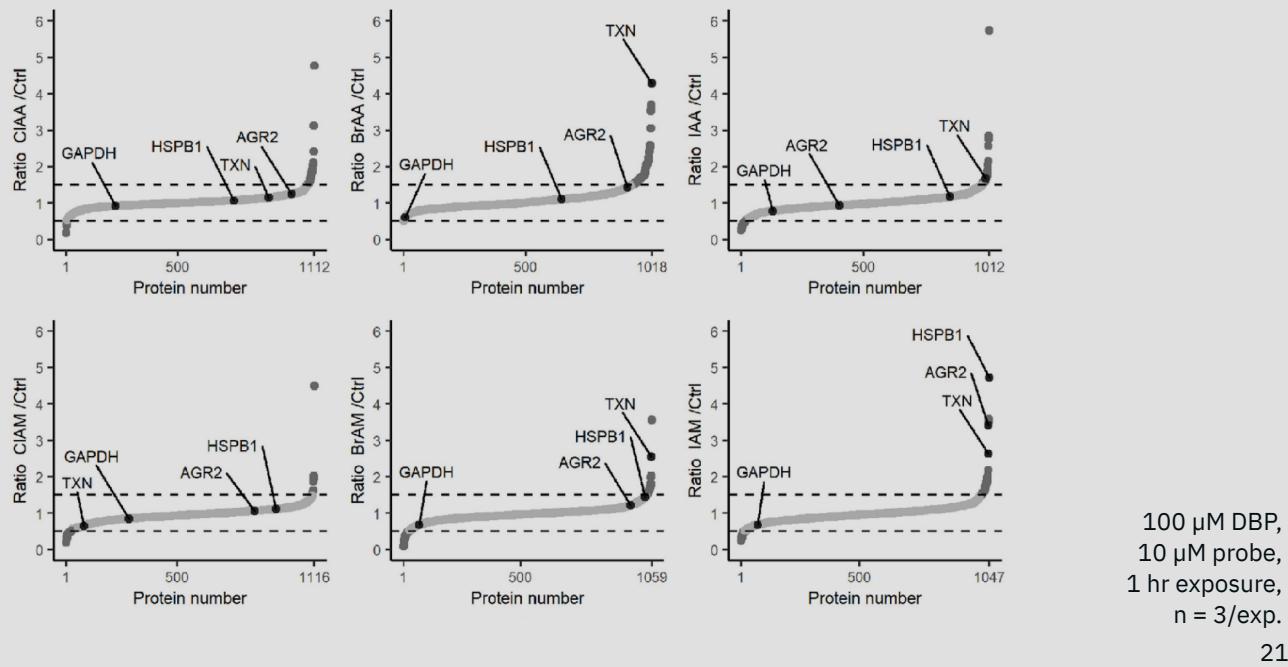
Activity-based protein profiling lends itself to identifying the protein targets of mHAAs and mHAMS



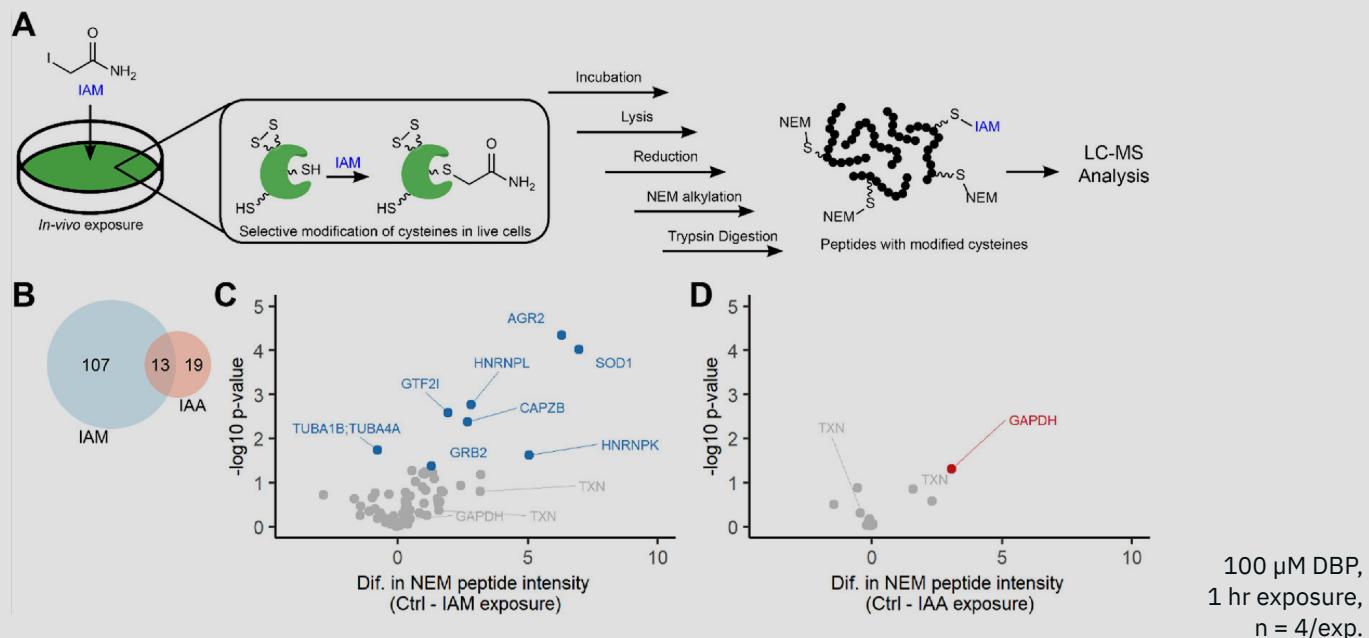
mHAA = monohaloacetic acid
mHAM = monohaloacetamide

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Biotin-affinity LC-MS analysis of 1 hr MCF7 cell exposures reveals compound and class specific protein targets



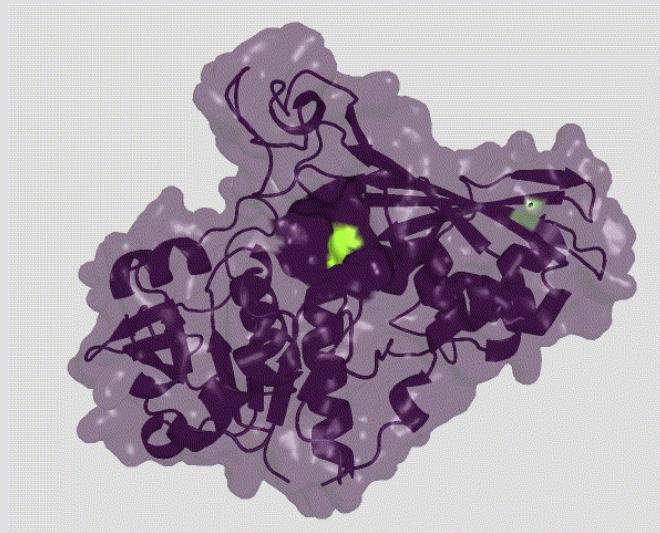
Direct adduct monitoring validated many of the suspected targets of IAM and IAA



NEM = N-ethylmaleimide

22

Adduct monitoring provides binding-site information such as modification of Cys152 on GAPDH by IAM and IAA



IAM = iodoacetamide

IAA = iodoacetic acid

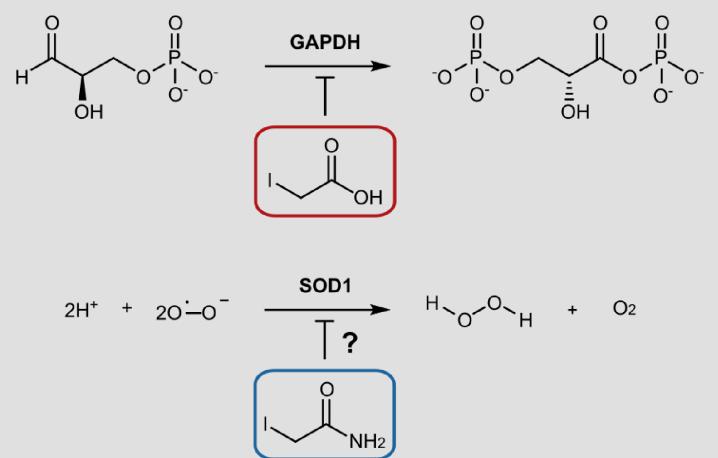
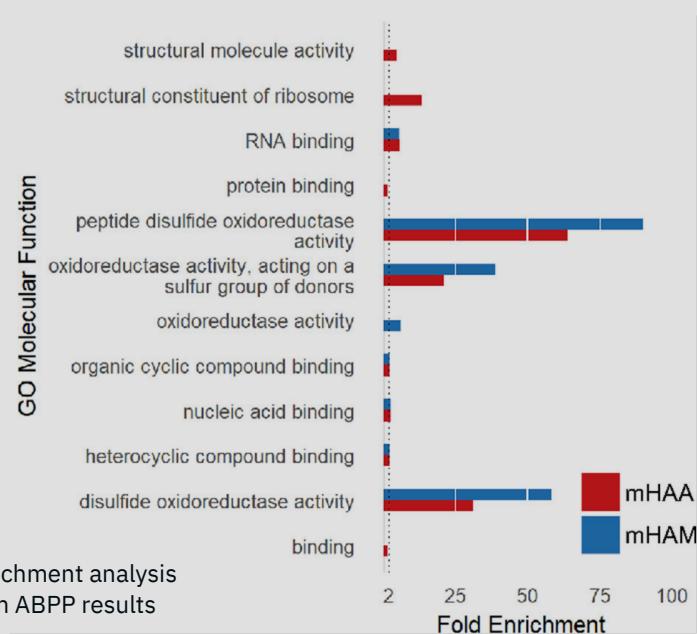
GAPDH = Glyceraldehyde 3-phosphate dehydrogenase

P04406

AlphaFold

23

mHAs preferentially react with redox proteins accounting for their stronger Nrf2-mediated oxidative stress responses



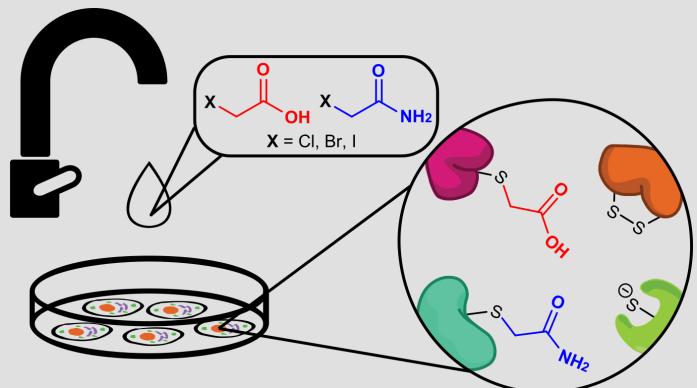
GAPDH = Glyceraldehyde-3-phosphate dehydrogenase
SOD1 = Superoxide dismutase [Cu-Zn]

Pals et al. ES&T 2011

24

Project 1: Conclusions

1. Class and compound specific differences in DBP cytotoxicity
2. ABPP and adduct monitoring can readily identify DBP protein targets
3. Differences in toxicity responses can be accounted for by variation in protein targets

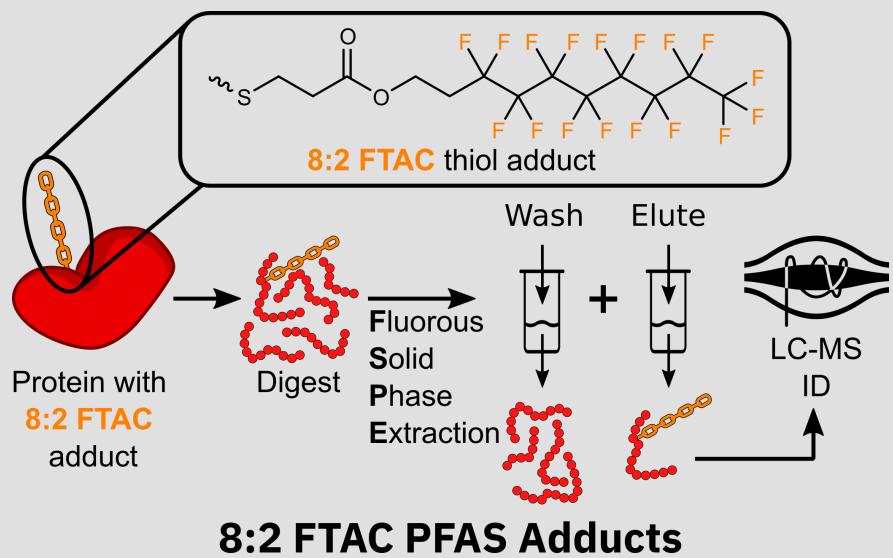


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Project 2: Querying the *In Vitro* Proteome Cysteine Reactivity of 8:2 Fluorotelomer Acrylate

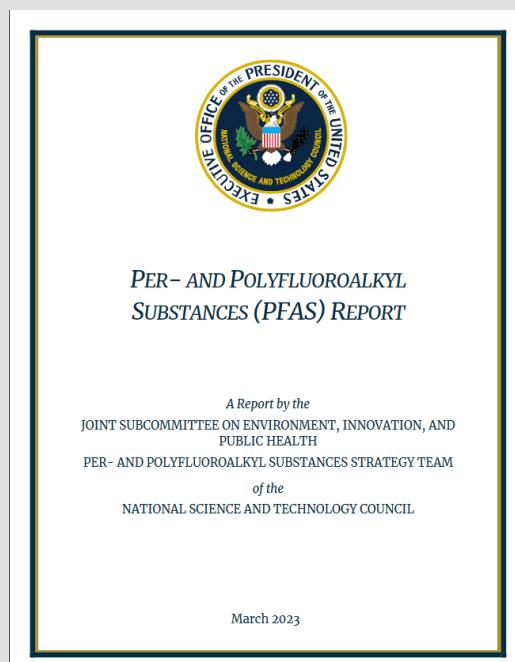
Submitted for publication

David Hall,
Jeremy
Gauthier, and
Hui Peng



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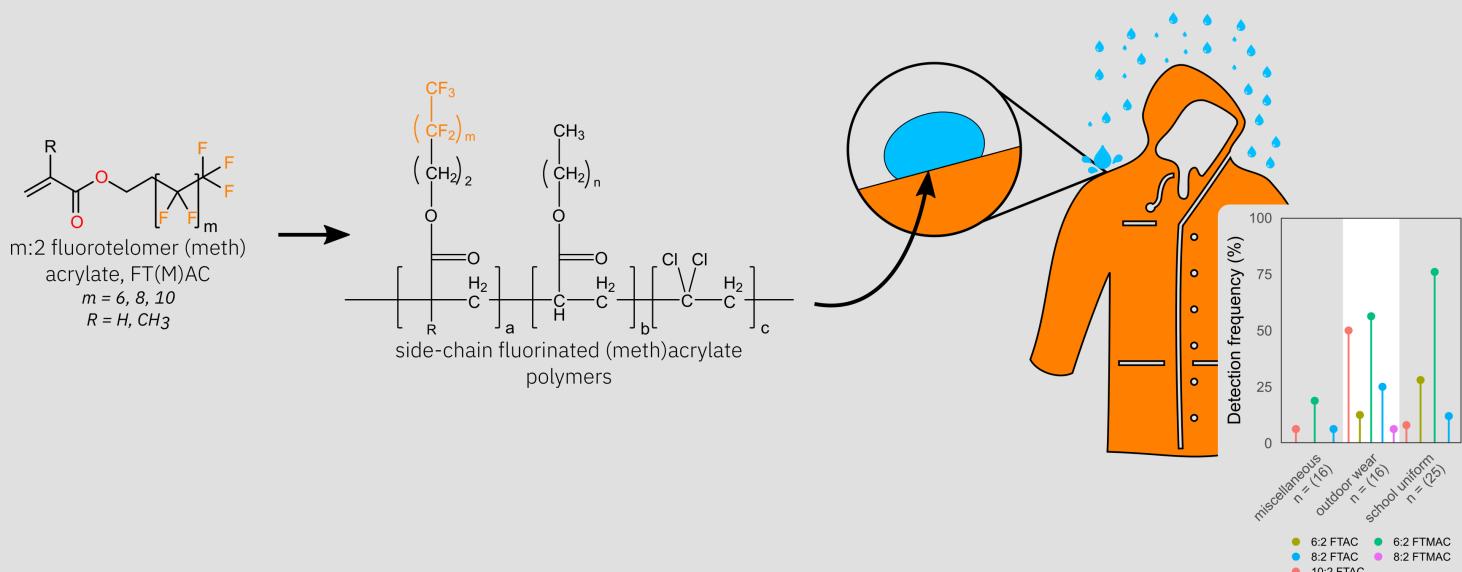
Decades of widespread use, along with mobility and **persistence**, have resulted in **ubiquitous PFAS contamination worldwide**. The persistence of PFAS is concerning as some chemicals can **accumulate in tissues...**



Per- and Polyfluoroalkyl Substances (PFAS) Report (2023)

27

FT(M)AC monomers are detected in fluoropolymer treated textiles, among other consumer products

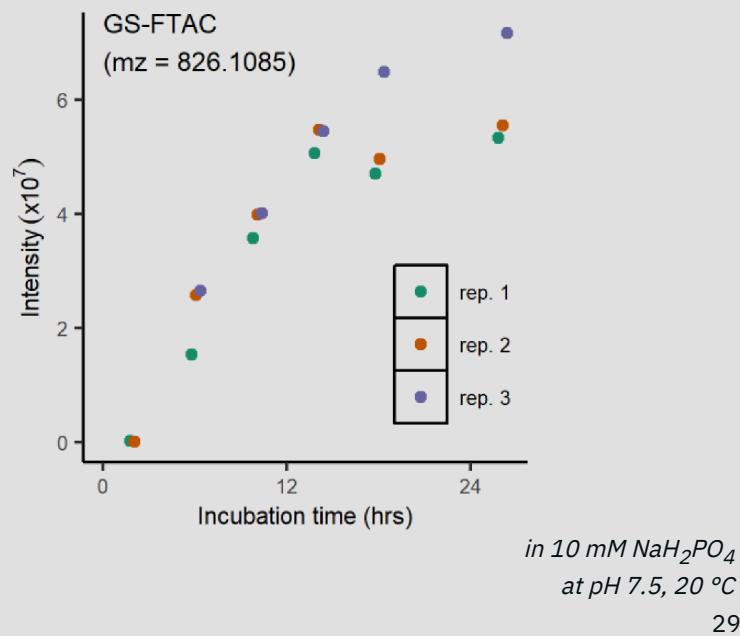
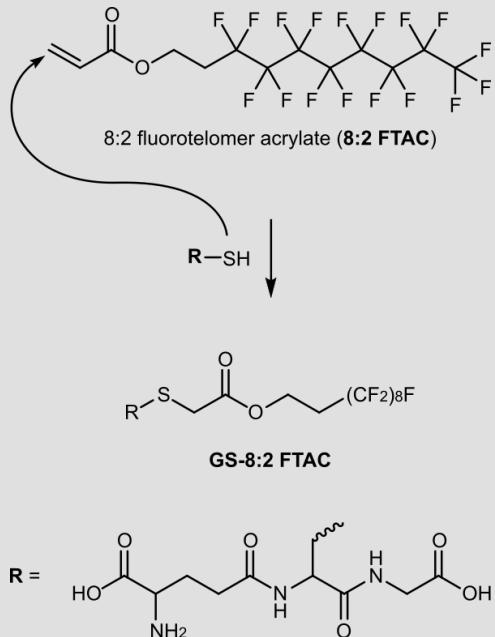


See Evich et al. *Science* (2022)
for review on PFAS

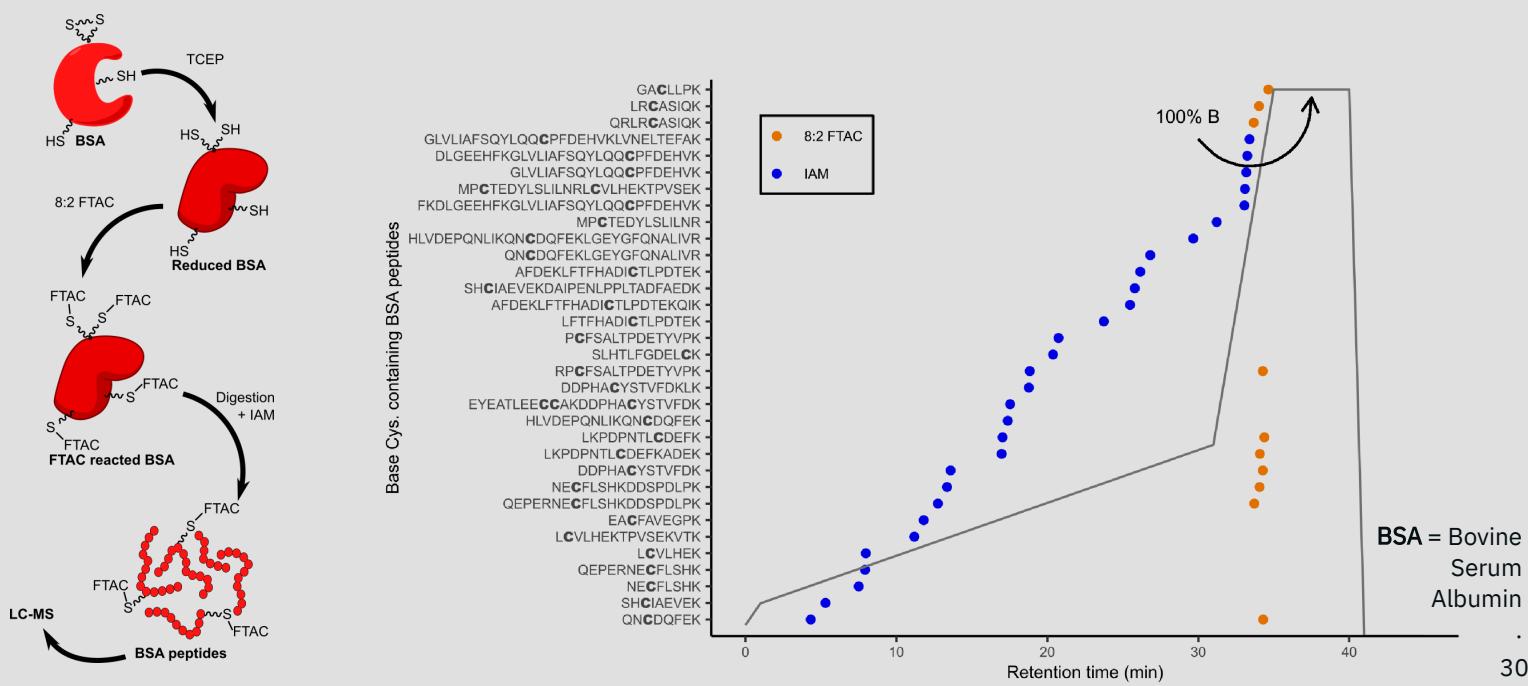
Data from Xia et al. *ES&T* (2022)

28

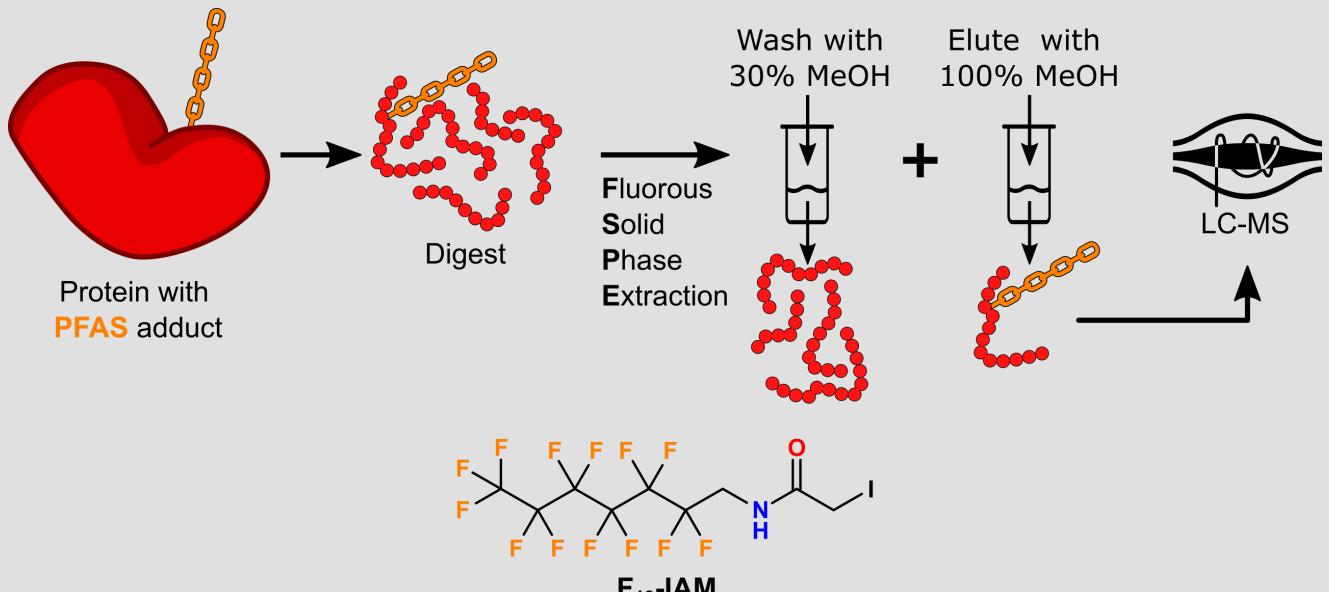
LC-MS monitoring confirms that 8:2 FTAC reacts with glutathione (GSH) *in vitro*



Detection of 8:2 FTAC modified peptides hampered by low-intensity and coelution of modified peptides



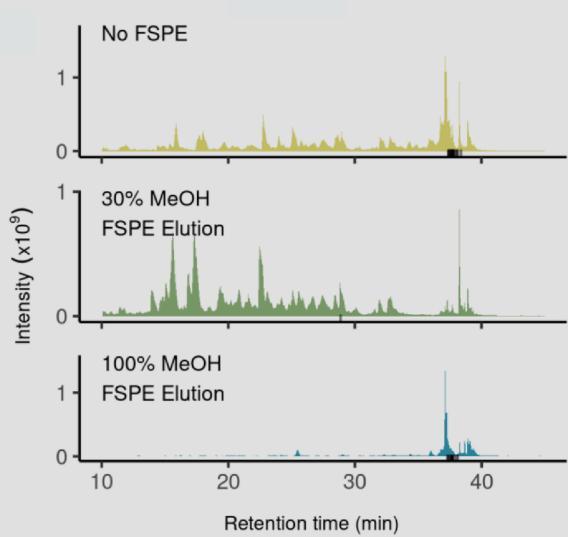
Fluorous Solid Phase Extraction enables the selective enrichment of long-chain PFAS adducted peptides



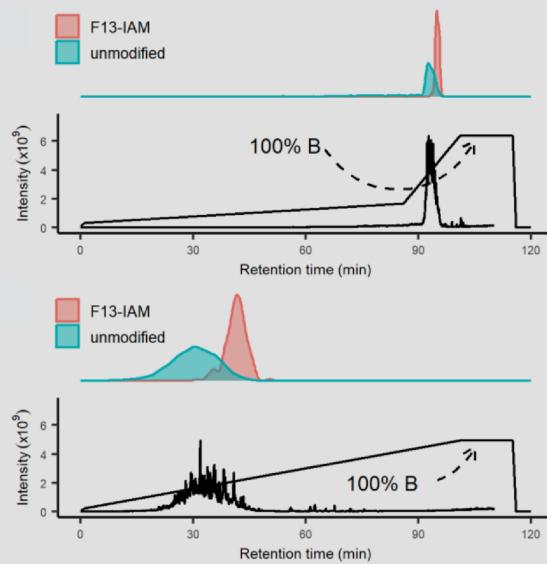
Based on
Brittain
et al. 2005

31

FSPE enriches very hydrophobic and PFAS modified peptides which can be further resolved using C18 nLC



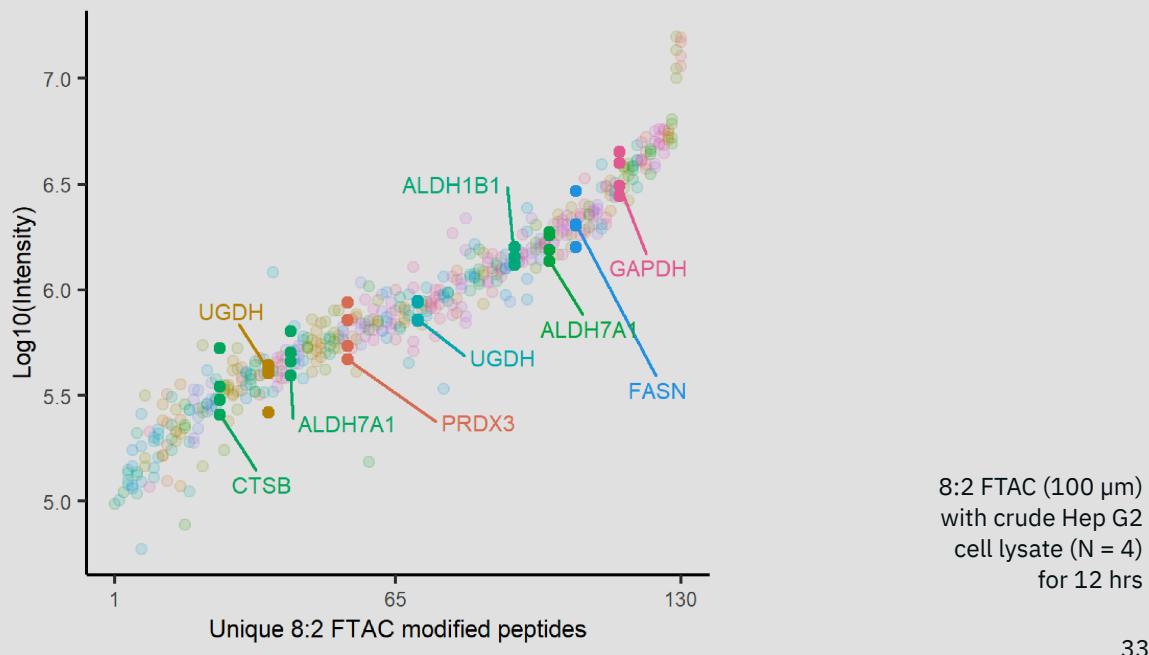
$F_{13}\text{-IAM}$ reacted with BSA



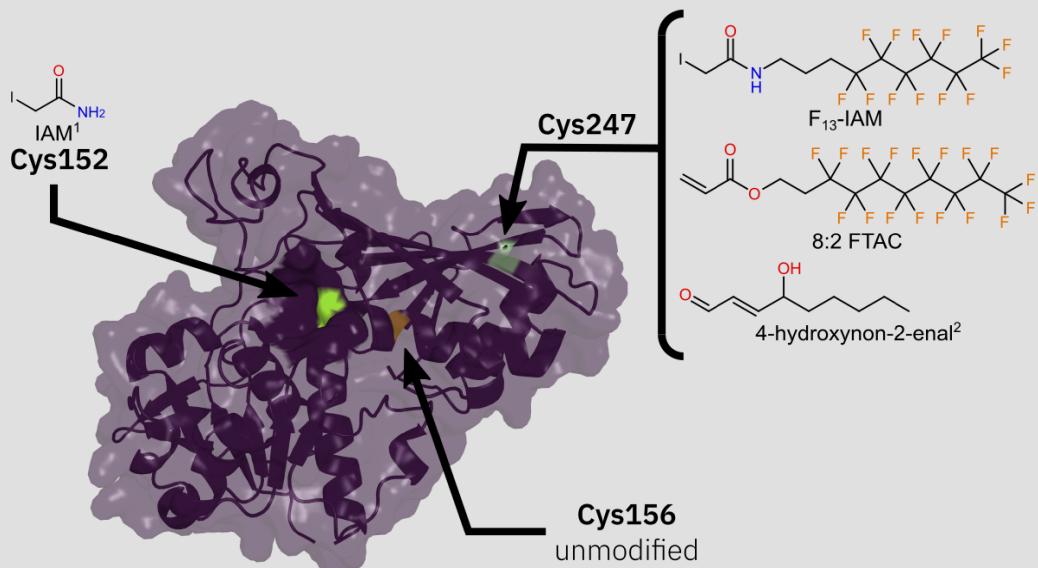
$F_{13}\text{-IAM}$ reacted with crude lysate

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FSPE enrichment helped to identify over 107 proteins modified by 8:2 FTAC in crude cell lysates

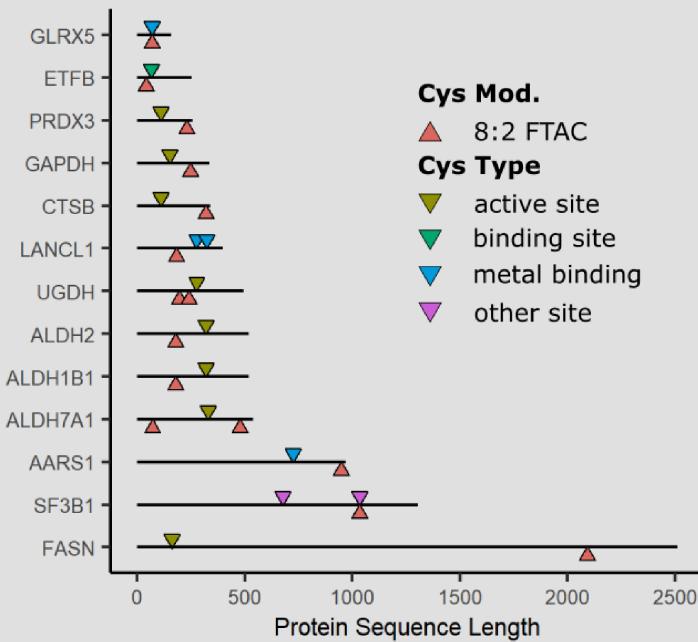


8:2 FTAC and F₁₃-IAM only modify GAPDH Cys247
and not the catalytic Cys152 residue



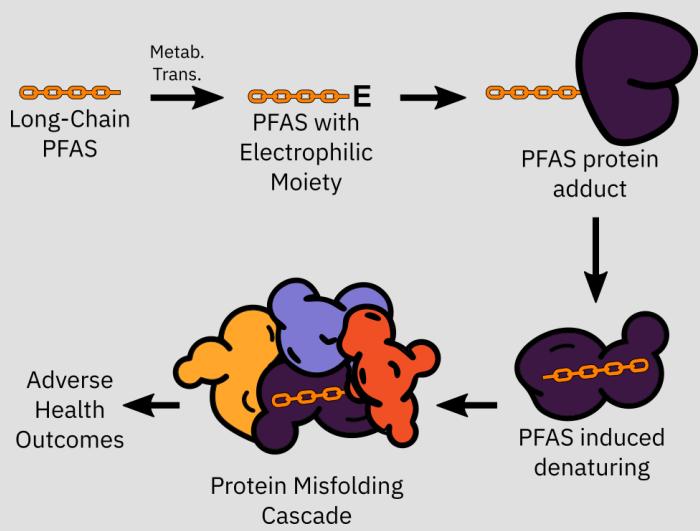
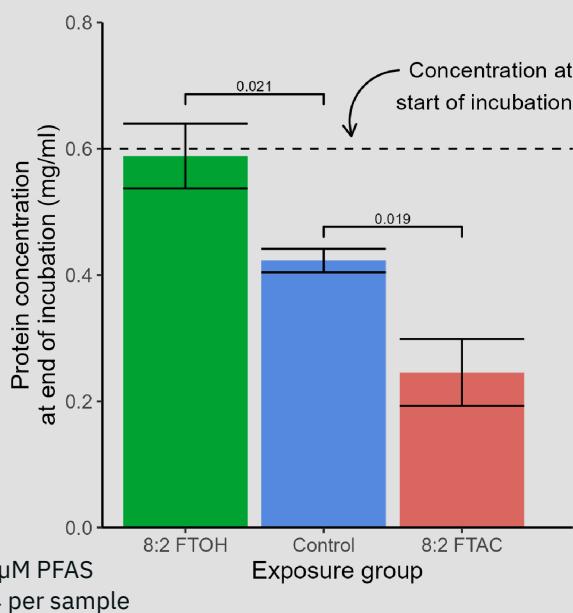
¹Hall et al., 2020
²Yang et al., 2005

Only 9 of 107 8:2 FTAC modified proteins have annotated nucleophilic residues, none of which are modified



35

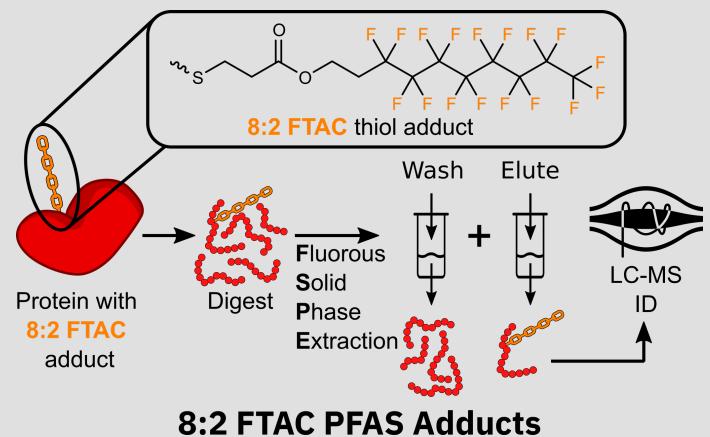
8:2 FTAC adducts promote protein aggregation in crude cell lysates; suggesting new possible mechanism of toxicity



36

Project 2: Conclusions

1. 8:2 FTAC does react with protein thiols
2. FSPE selectively enriches peptides with long-chain PFAS modifications
3. PFAS modifications occur outside of predicted regions
4. PFAS modification decreases protein stability

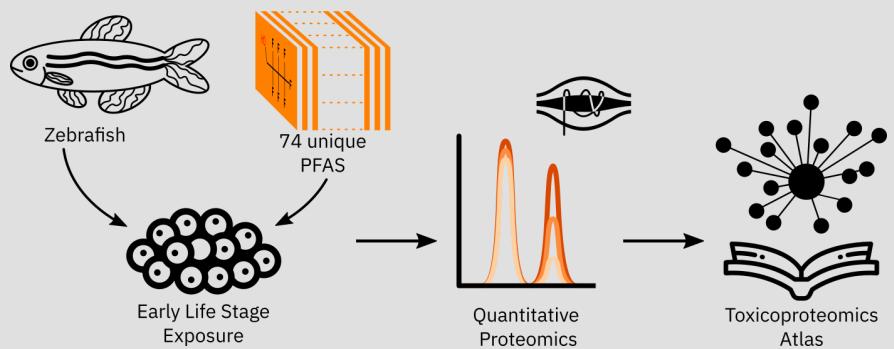


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Project 3: Toxicoproteomics Atlas of Per- and Polyfluoroalkyl Substances in Early-Life Stages Zebrafish (*Danio rerio*)

David Hall,
Jiajun Han, Wen
Gu, Diwen Yang,
and Hui Peng

Manuscript in
preparation



38

1 hpf



IMP
Vienna
Biocenter

39

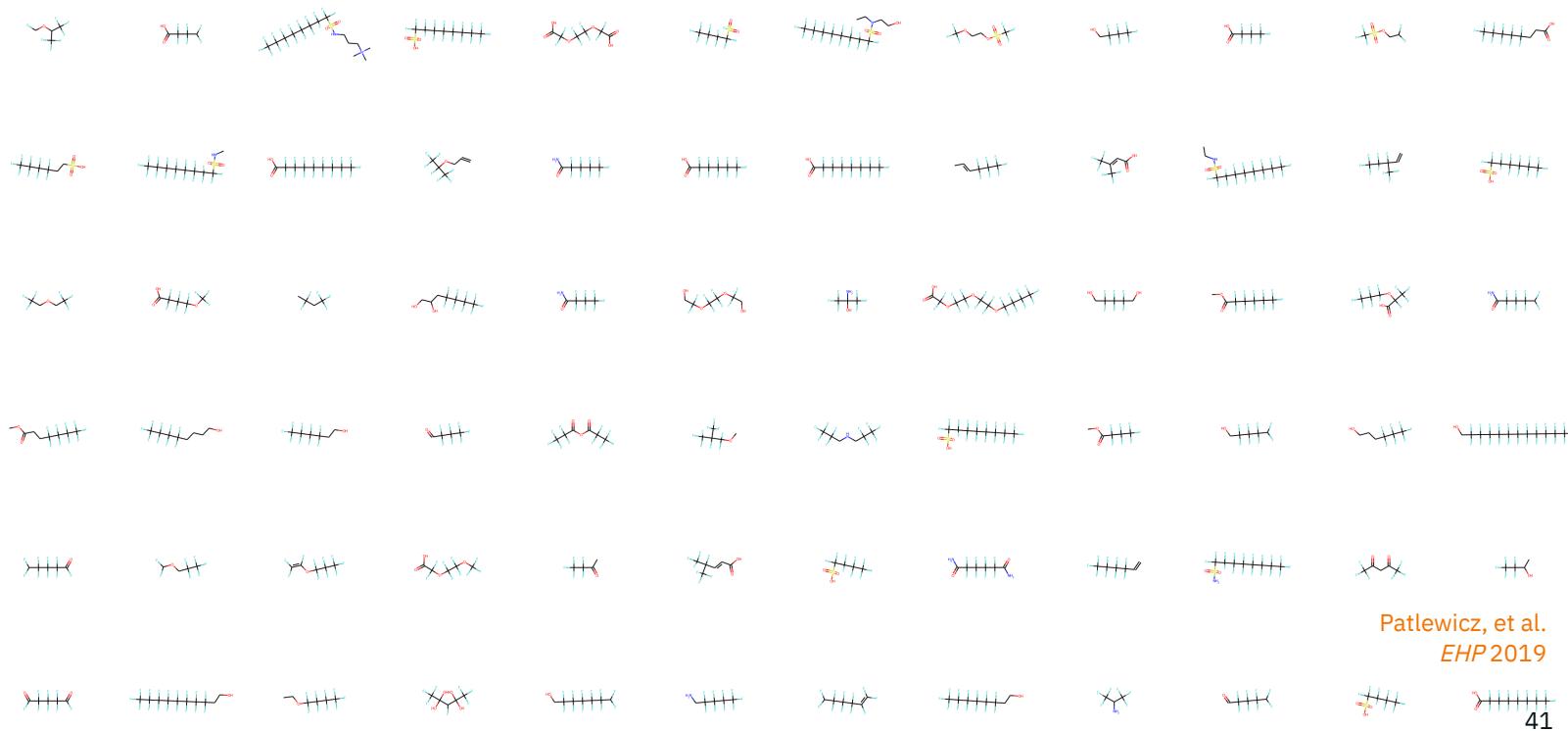
120 hpf



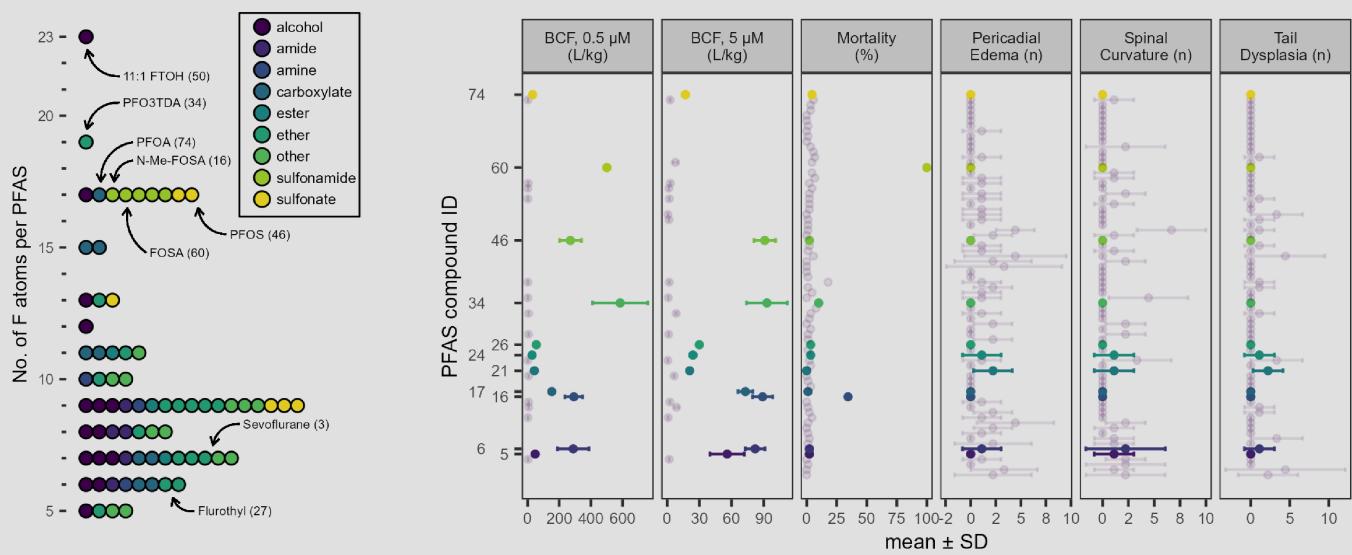
Cavanagh &
McCarthy, 2014

40

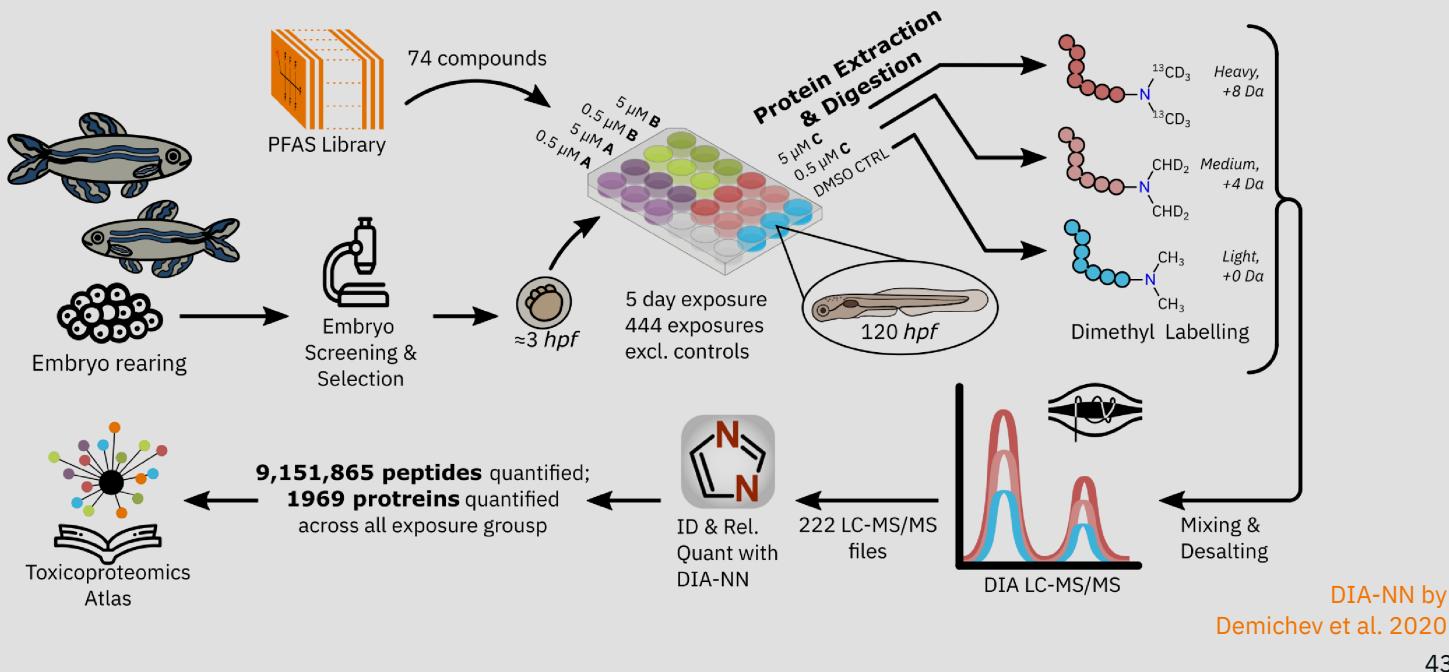
PFAS are a structurally diverse class of compounds



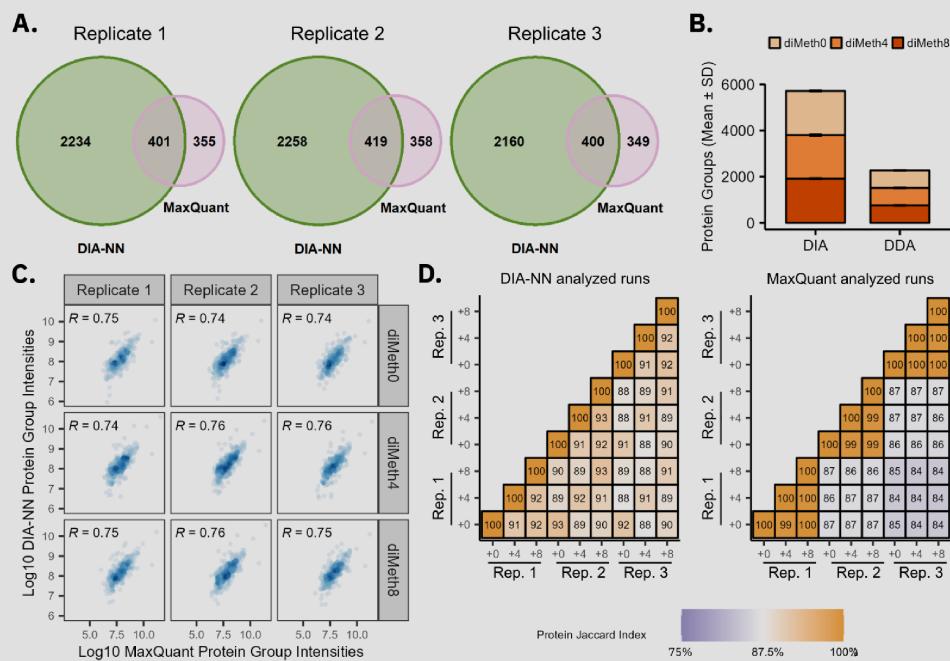
Traditional fish embryo toxicity endpoints are poor predictors of weak acute and sublethal toxicities



Dimethyl labelling combined with plexDIA balances instrument time, sample depth, and quantification

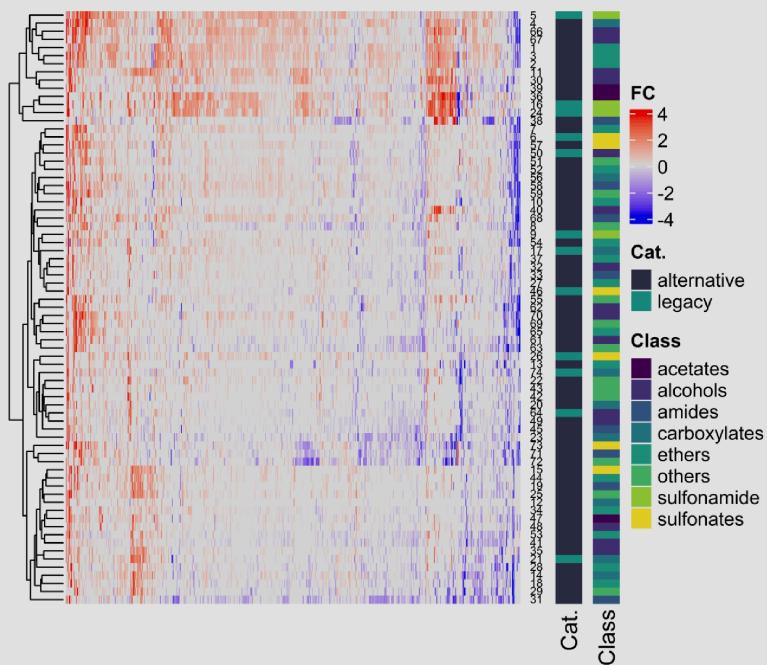


Reanalysis of identical samples shows plexDIA outperforms DDA in coverage and data completeness



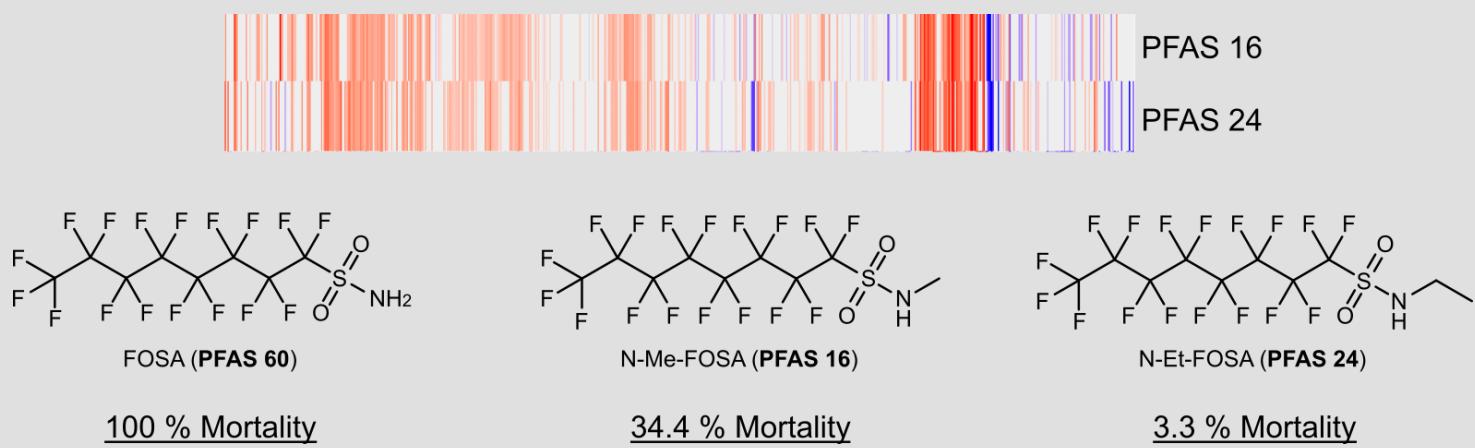
Proteome response to PFAS exposure belies chemical classification, suggesting more complex mechanisms

5 μ M PFAS exposure,
PFAS 60 omitted due to
100% mortality



45

Despite similarities in chemical structure and proteome responses, sulfonamides have divergent rates of mortality

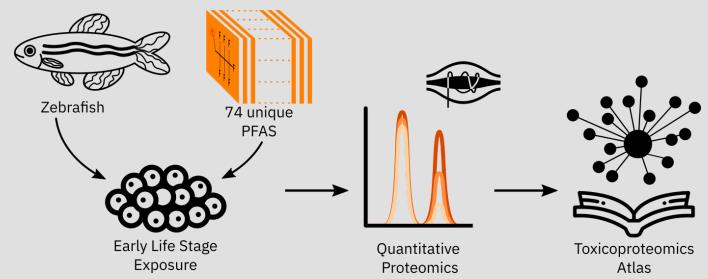


5 μ M PFAS results
PFAS 60 omitted due to
100% mortality

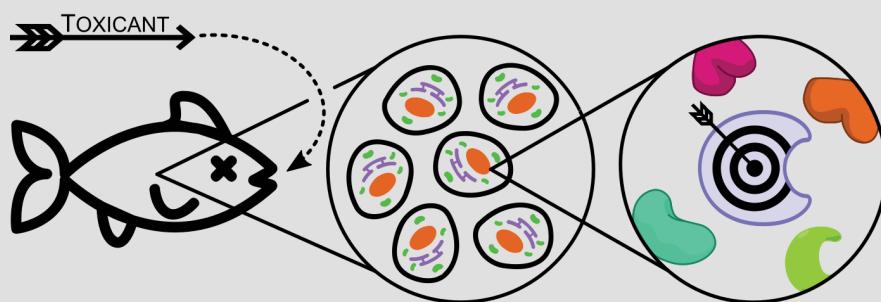
46

Project 3: Conclusions

1. DIA proteomics + ELS zebrafish exposure is adaptable to high-throughput screening
2. Toxicoproteomic response of PFAS exposure typically belies classification
3. Proteomics reveals insights into mechanisms not observable until many days/weeks post hatching

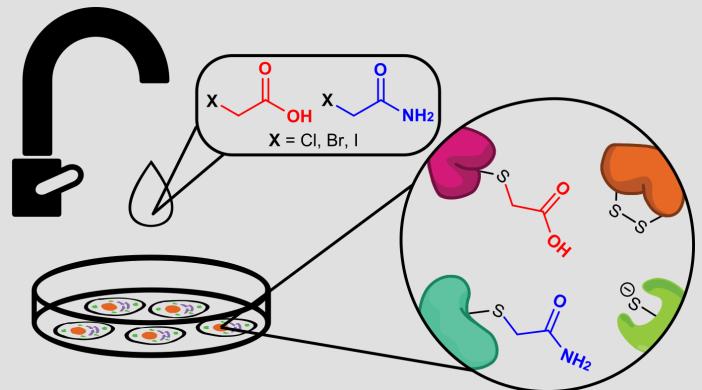


Concluding remarks for my PhD work



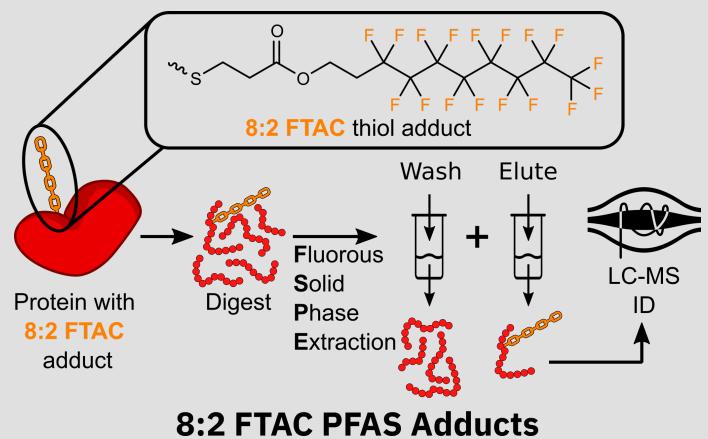
Concluding remarks for my PhD work

- **Project 1** demonstrated how chemical proteomics can elucidate mechanisms of toxicity



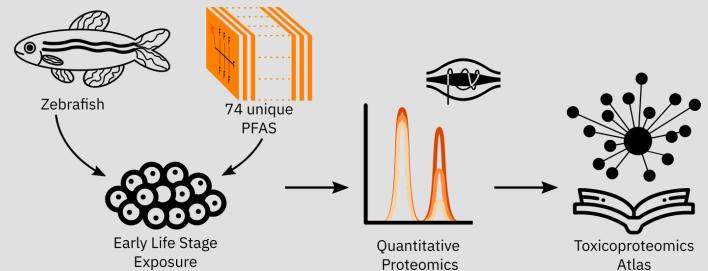
Concluding remarks for my PhD work

- **Project 1** demonstrated how chemical proteomics can elucidate mechanisms of toxicity
- **Project 2** led to the development of an a chemical proteomics approach to study long-chain PFAS protein adducts

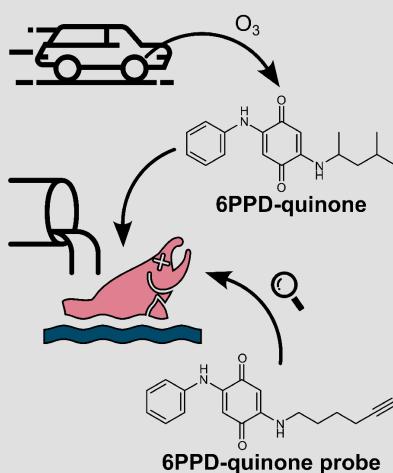


Concluding remarks for my PhD work

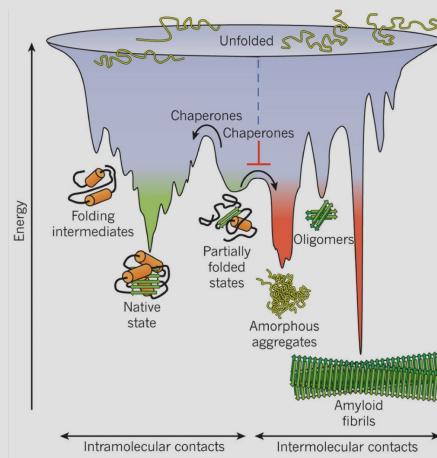
- **Project 1** demonstrated how chemical proteomics can elucidate mechanisms of toxicity
- **Project 2** led to the development of an a chemical proteomics approach to study long-chain PFAS protein adducts
- **Project 3** analyzed the proteome responses of 74 PFAS to create the largest toxicoproteomics dataset to date



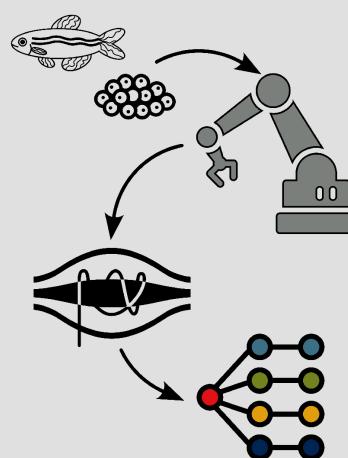
Looking to the future, work should expand proteomics methods, and explore downstream insights



Adapting ABPP



Protein Aggregation



HTS Proteomics

Chemical Proteomics Methods for Elucidating the Protein Targets of Environmental Contaminants

Questions?

