

Model-Aided Design and Integration of Functionalized Hybrid Nanomaterials for Enhanced Bioremediation of Per- and Polyfluoroalkyl Substances (PFASs)

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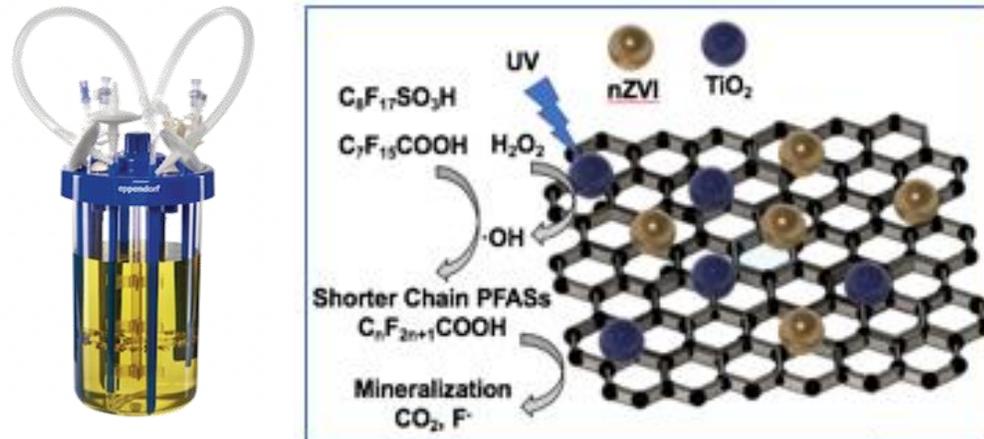
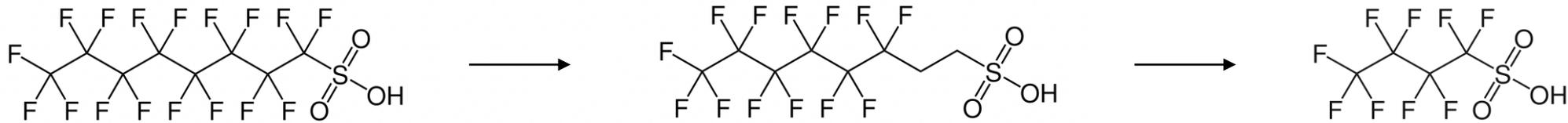
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Problem: Biodegradation of PFAS usually requires the existence of a C-H bond at the α -carbon of *poly*fluorinated compounds, producing shorter-chain and more persistent *per*fluorinated byproducts.

Solution: Enhance biodegradation of PFAS by pretreatment using catalytic nanomaterials to promote defluorination, then use enhanced microbial cultures (dehalogenators) to complete biodegradation.



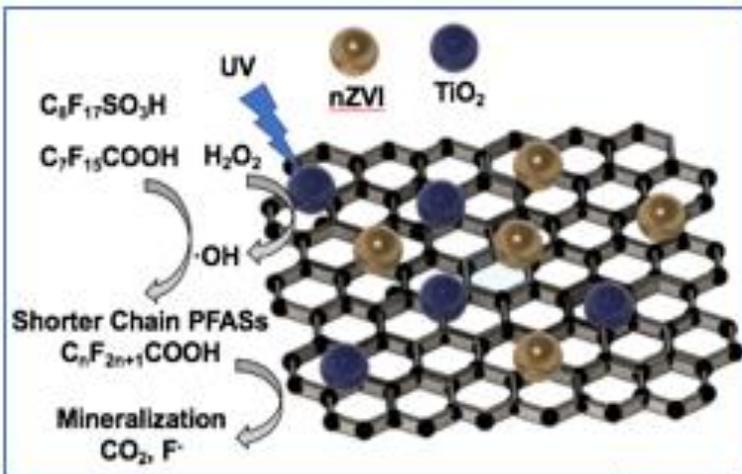
\rightarrow rGO-nZVI nanohybrids degrade long chain PFAS to shorter-chain PFAS with partial defluorination

Aims:

Aim 1: Synthesize multifunctional redox-active nano hybrids and evaluate their catalytic properties for PFAS degradation

Aim 2: Select microbial communities with high biodegradation potential for short-chain PFAS; identify community structure, functional genes, and enzymes for PFAS degradation

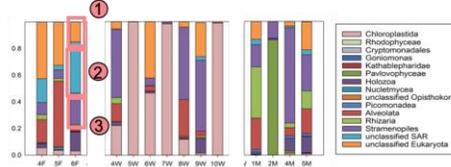
Aim 3: Perform molecular modeling to discover, detect, and refine enzymatic biodegradation for structurally diverse PFAS



Targeted and 'Omics
Characterization



1. Amplicon Sequencing



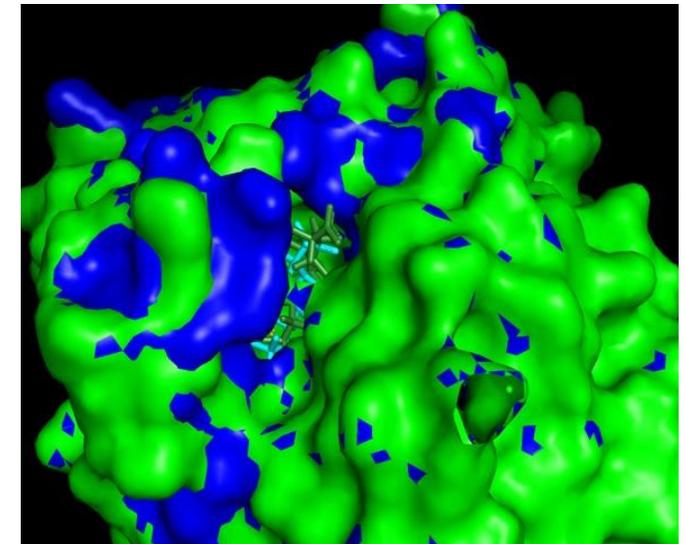
2. Metagenomics and transcriptomics



3. Target gene identification (qPCR)

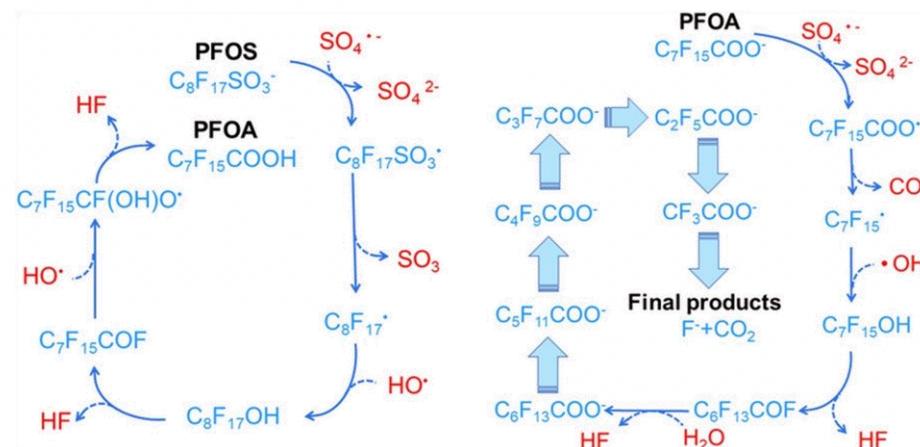
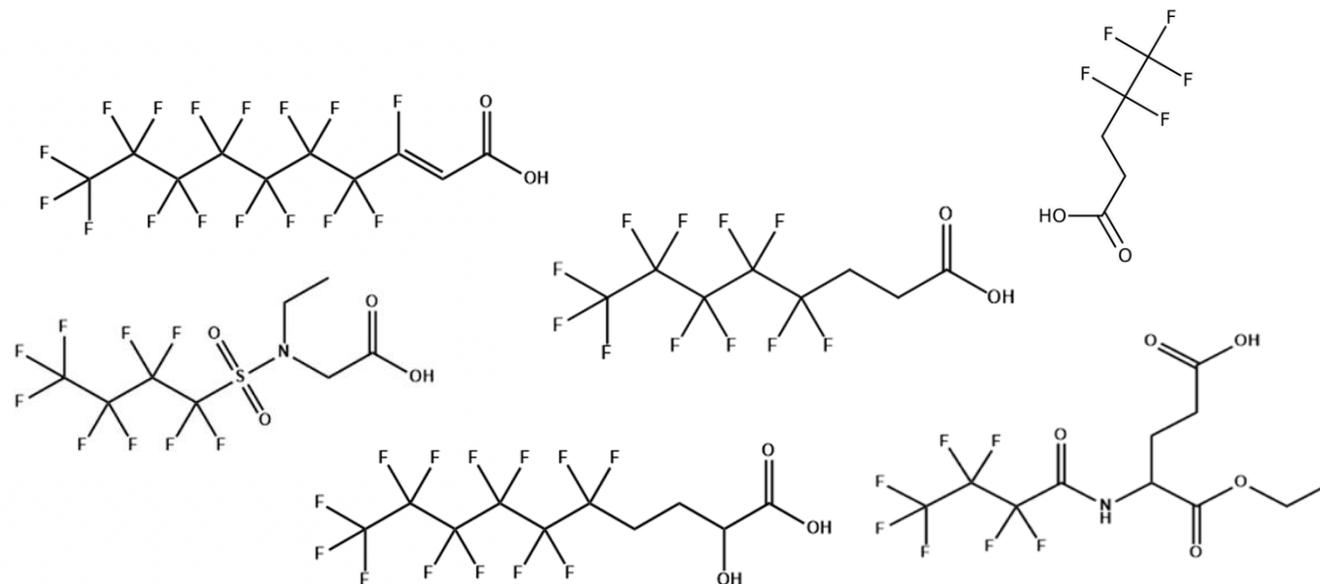


Haloalkane dehalogenase (3U1T) in green. Mutated version (in blue) allows PFDA to dock further into the enzyme.

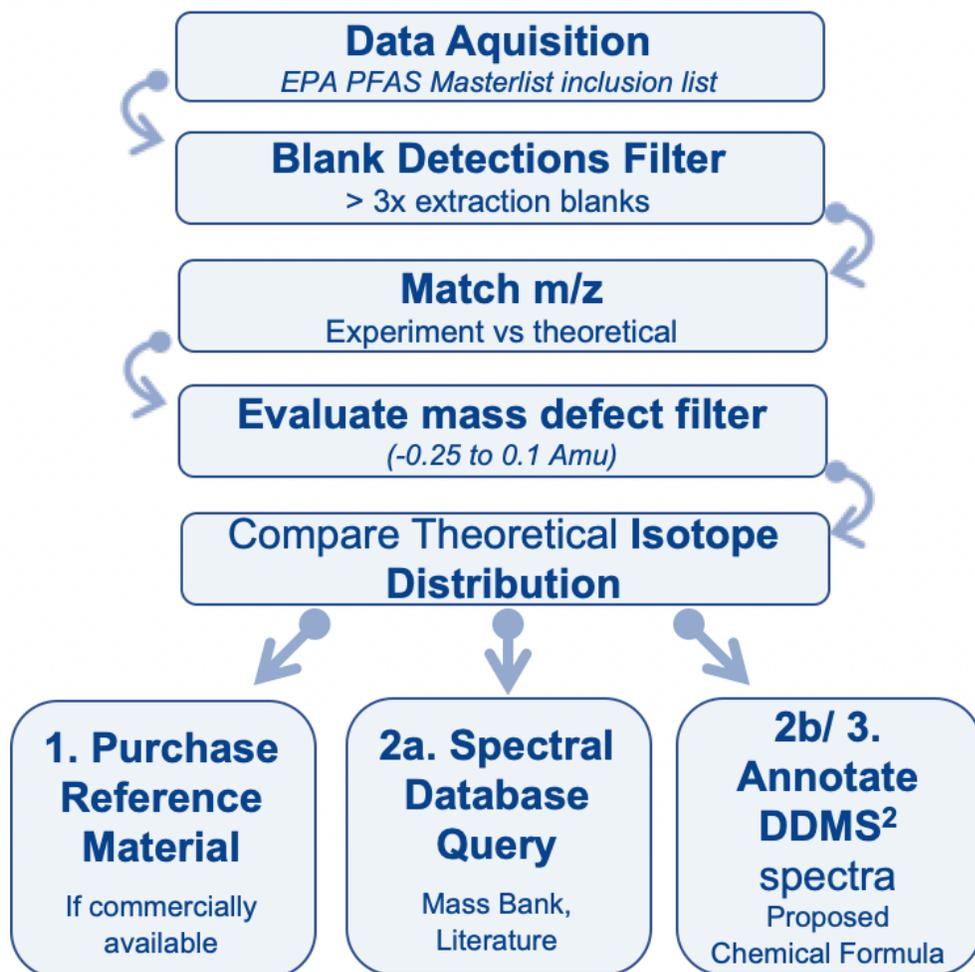


Analytical Challenges

- Identification & Quantification of PFAS degradation products is challenging *without reference standards*
- Short chain and poly fluorinated PFAS are known transformation products of redox PFAS degradation



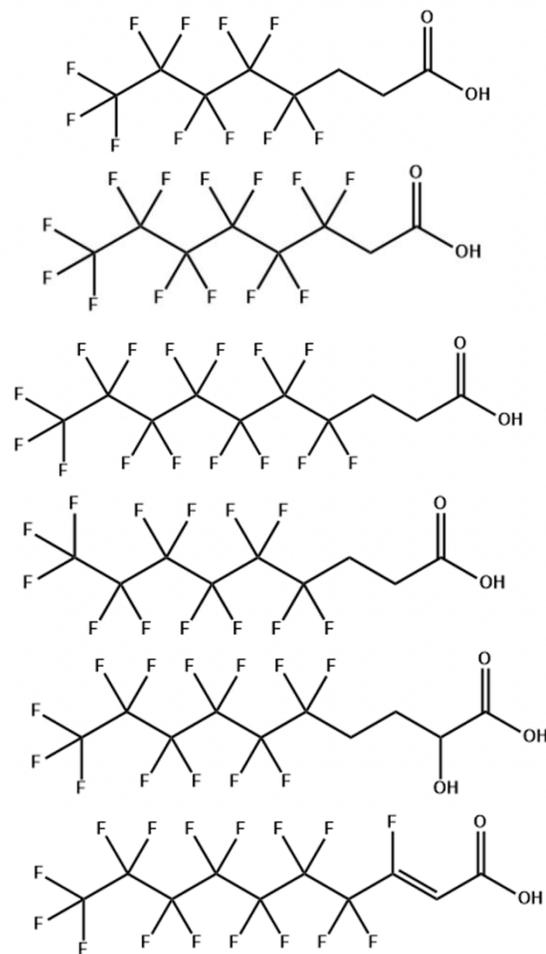
Suspect Screening Workflow:



Identification Match Factors:
m/z, retention time, fragmentation, mass defect

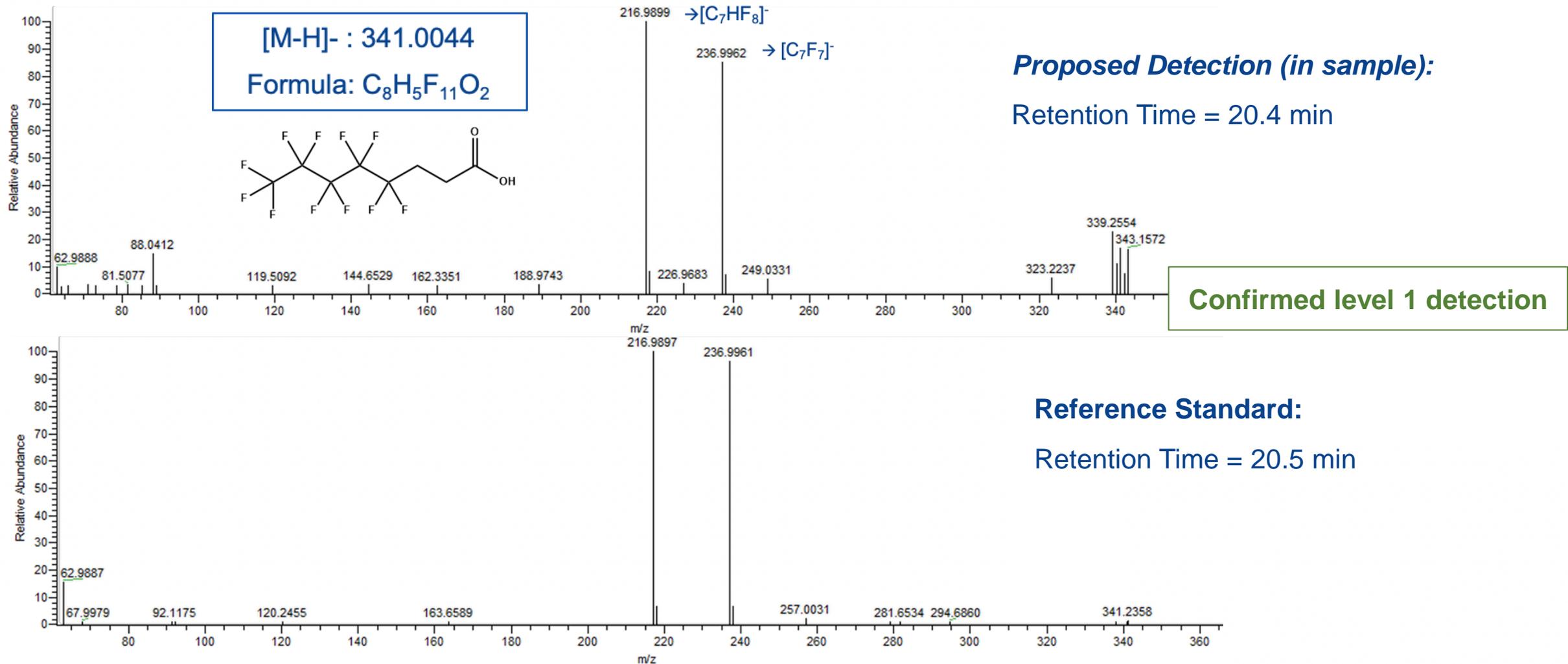
- Level 1** – reference standard
- Level 2a** – spectral database
- Level 2b** – diagnostic structural evidence from fragmentation
- Level 3** – Common PFAS fragment observed

Suspect Screening Results



Name	M/Z	Retention time	Chemical Formula	Confirmation Level
5:3 Fluorotelomer carboxylic acid†	341.0042	20.40	C ₈ H ₅ F ₁₁ O ₂	Level 1
6:2 Fluorotelomer carboxylic acid	376.9855	18.11	C ₈ H ₃ F ₁₃ O ₂	Level 2a
7:3 Fluorotelomer carboxylic acid†	440.9979	24.06	C ₁₀ H ₅ F ₁₅ O ₂	Level 2a
6:3 Fluorotelomer carboxylic acid†	391.0012	22.43	C ₉ H ₅ F ₁₃ O ₂	Level 2b
5,5,6,6,7,7,8,8,9,9,10,10,10-Tridecafluoro-2-hydroxydecanoic acid	421.0118	20.32	C ₁₀ H ₇ F ₁₃ O ₃	Level 3
(Z)-3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-hexadecafluorodec-2-enoic acid	456.9728	19.60	C ₁₀ H ₂ F ₁₆ O ₂	Level 3

Addressing Challenges – 2H,2H,3H,3H-Perfluorooctanoic acid



AIM 2: Assess the potential and efficacy of biodegradation and mineralization of short-chain PFAS

Comparison of potential biodegradation between selected untreated PFAS and nano treated PFAS by mix community culture

Analysis: PFAS, F-, DNA

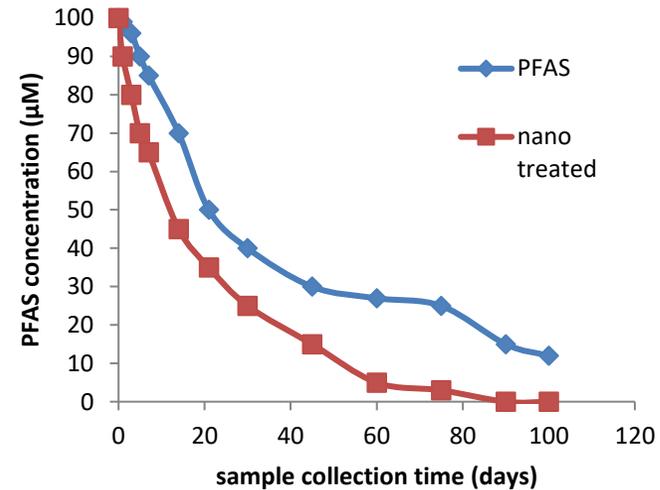
PFAS treated: PFOA, PFOS, PFBS, 6:2 FTCA, Nafion BP2, GENX



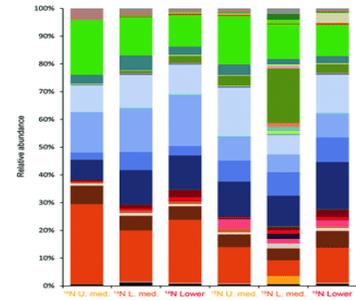
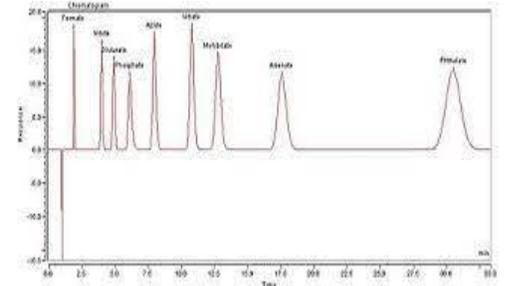
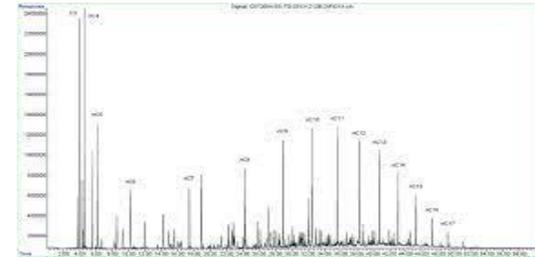
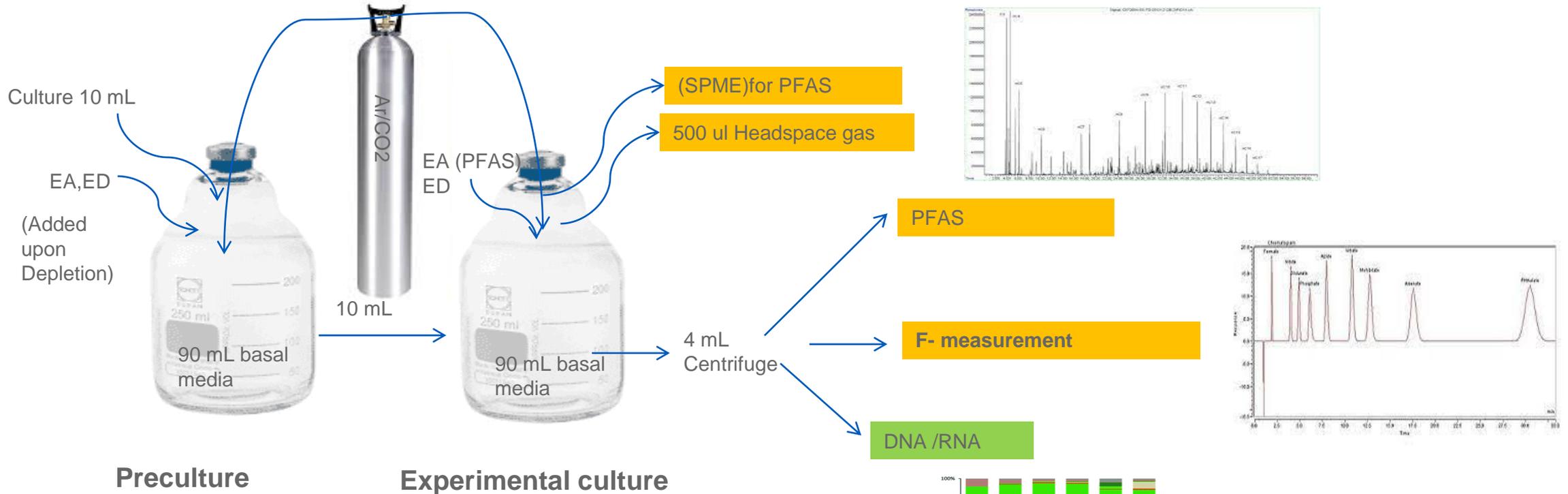
Nano Pretreatment



Without Pretreatment



AIM 2: Assess the potential and efficacy of biodegradation and mineralization of short-chain PFAS



Experimental Workflow

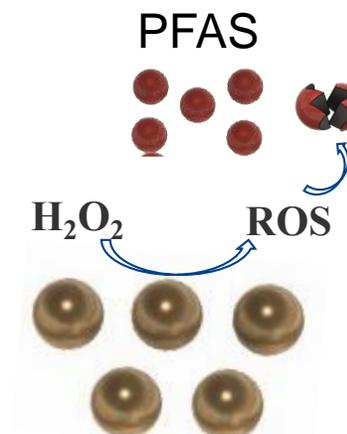
Aim 1: Synthesize multifunctional redox-active nanohybrids and evaluate their catalytic properties for PFAS degradation

PFAS Degradation by Redox-Active Nanomaterials

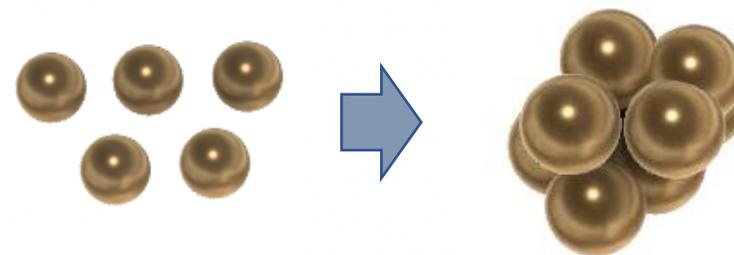
Promise: Redox active metal nanoparticles such as *nZVI* or Fe^0 can degrade PFAS through heterogeneous Fenton reactions, however, with *limited efficacy*.



Challenge: Metal or metal oxide nanoparticle catalysts aggregate in aqueous systems and loose surface area and reactivity

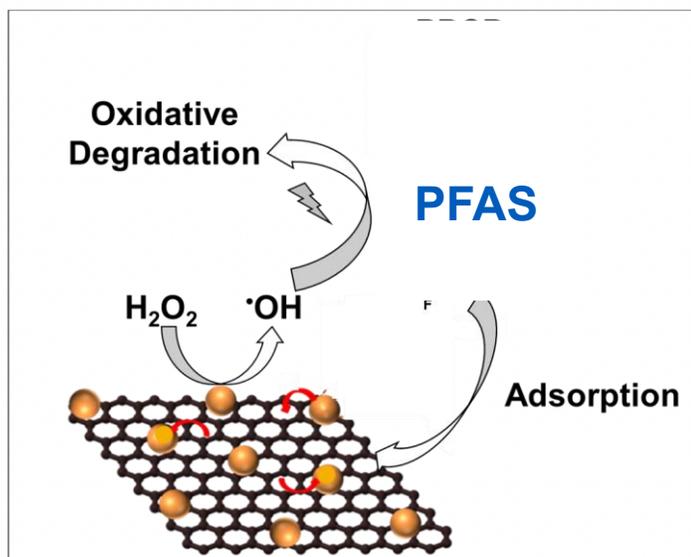


Nanoscale Zero Valent Iron (nZVI)

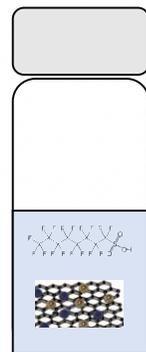
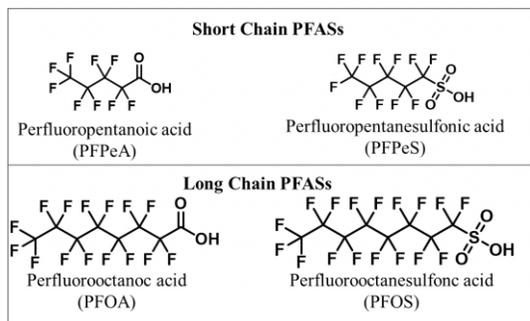


Particle Aggregation

Addressing Challenge – Hybridization of Nanomaterials

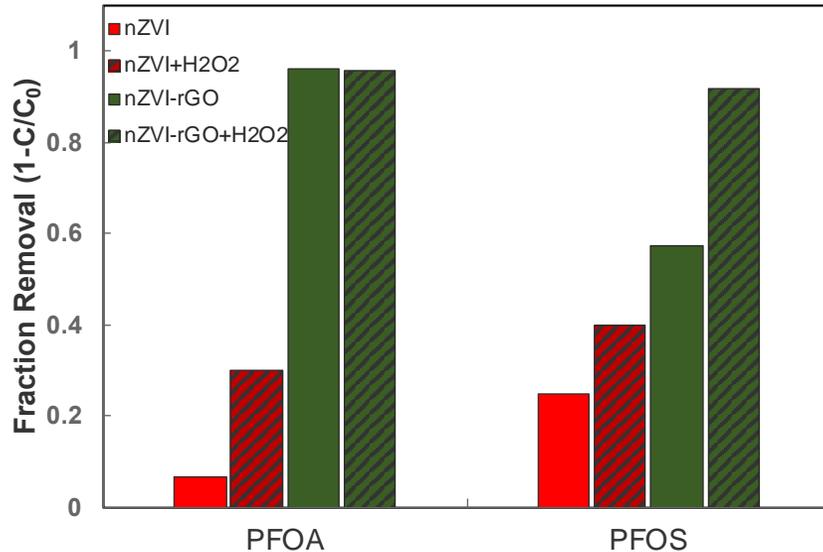


- Place iron nanoparticles on 2D graphene
- Graphene resists nZVI aggregation
- Graphene is highly conductive because of its delocalized electrons and can enhance the catalysis process
- Graphene is all-carbon surface and hence can adsorb PFASs on the nano hybrid surface

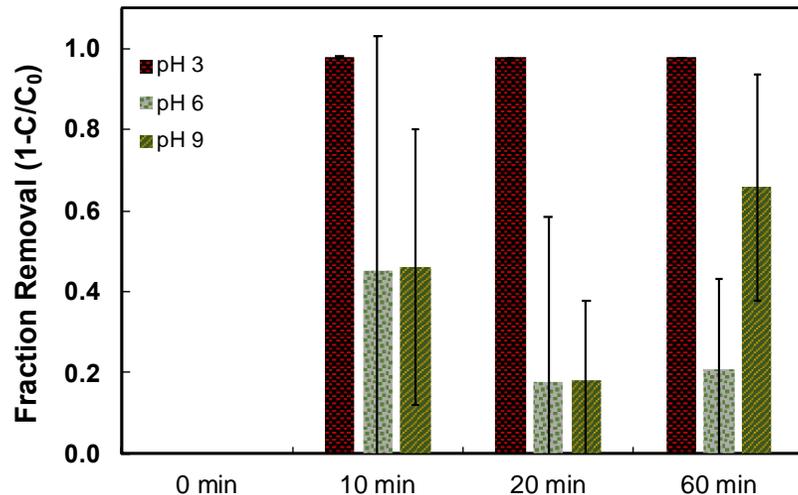


- PFAS solution dosed with 5 mg of nanohybrid
- Mixture were placed on the shaker for 60 min, and aliquots were collected at 0, 10, and 20, and 60 min
- Aliquots were centrifuged and supernatant was collected
- Sample were analyzed on LC MS/MS to quantify the change in PFAS

PFAS Removal Efficiency by Nanohybrids: Effect of H₂O₂ & pH



- Low removal were observed for nZVI compared to rGO-nZVI in presence and absence of H₂O₂ for both the compounds
- Removal of both PFOA and PFOS increased for nanohybrid materials by >30%
- >92% removal was achieved for nZVI-rGO in presence of H₂O₂ within 20 min for both PFOA and PFOS
- >95% removal was achieved with nanohybrid within 10 min for the pH of 3
- No any fixed pattern were observed for the removal of PFOS for pH of 6 and 9



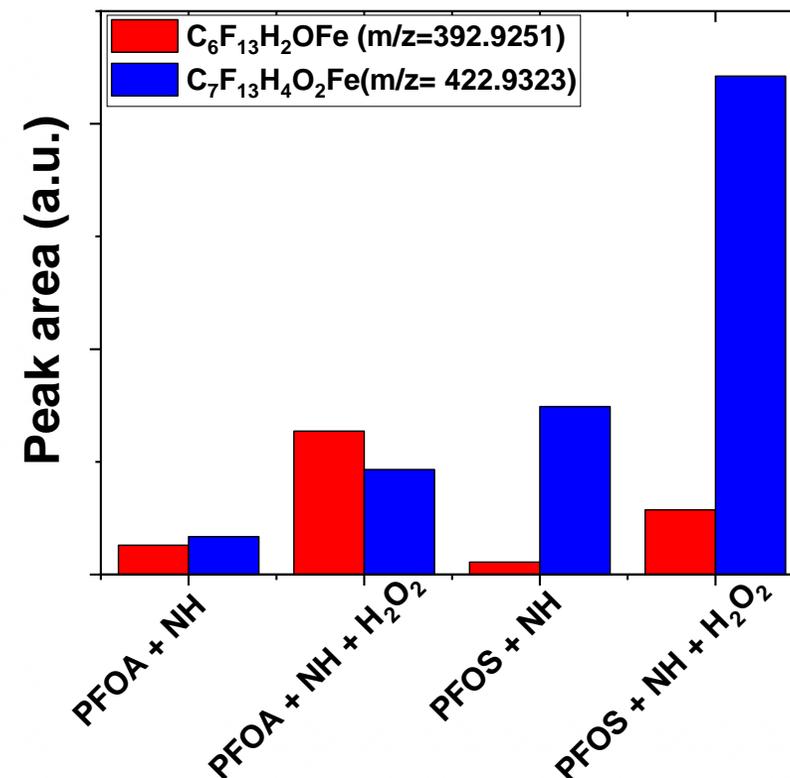
Identifying PFAS Degradation Products

- Liquid chromatography high resolution mass spectroscopy (LC-HRMS) was performed
- Non-target analyses identified two unique **PFAS-Fe complexes** of shorter chain than the tested PFASs
- $C_7H_4O_2F_{13}Fe$ and $C_6H_2OF_{13}Fe$

Possible Mechanism

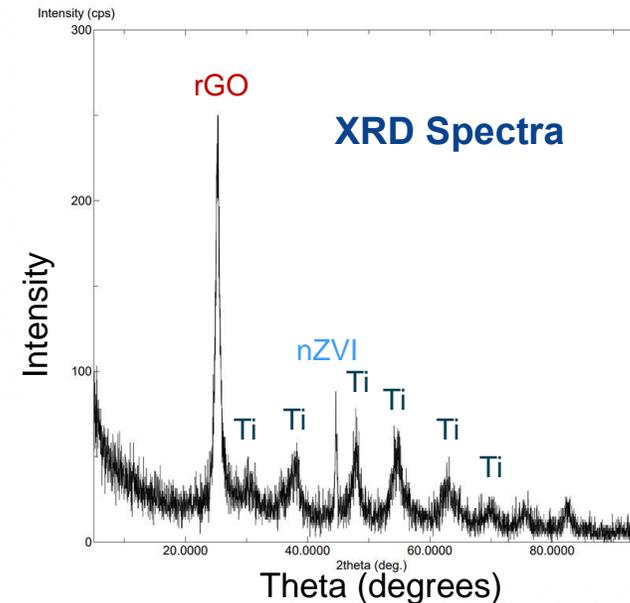
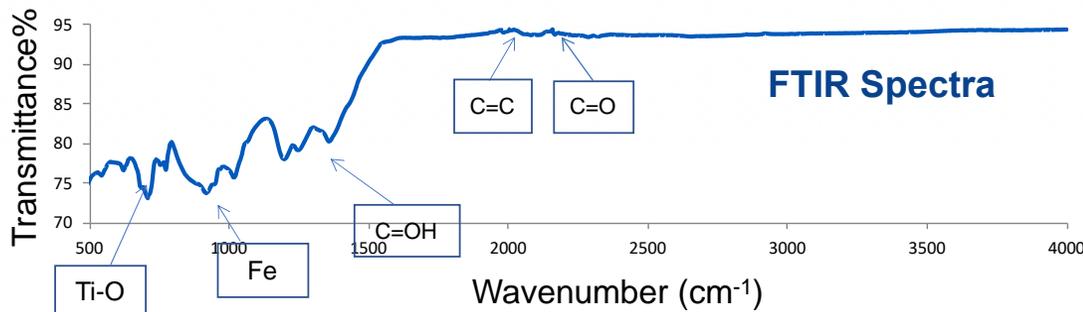
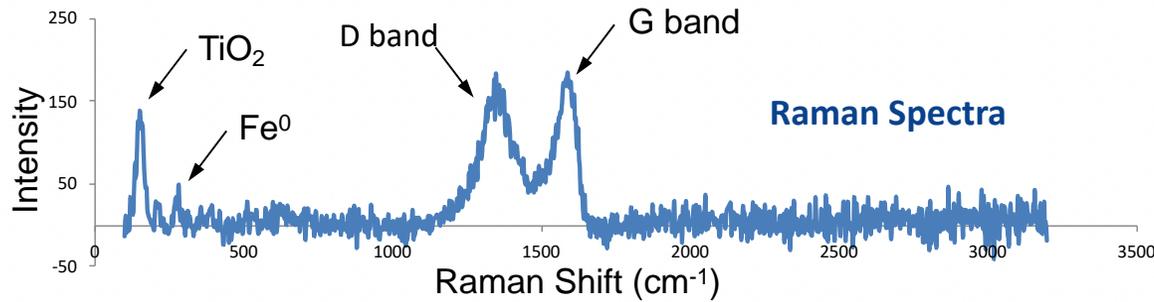
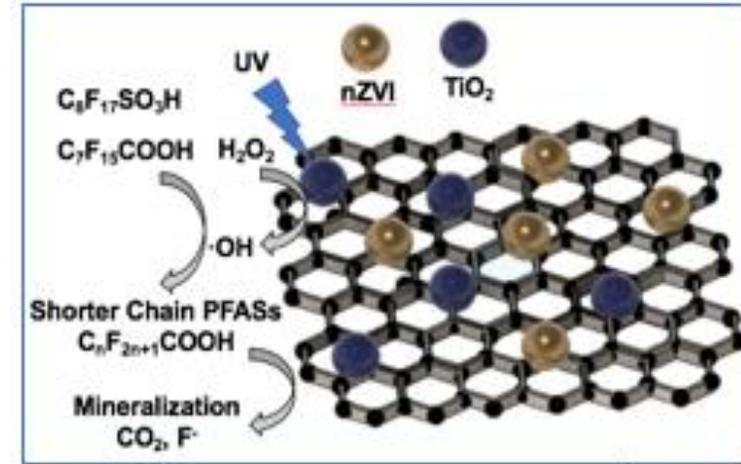


Decarboxylation and chain-shortening follow, and iron complexation occurs



Improving Nanohybrid Performance: Hierarchical Assembly

- Combine photocatalytic TiO_2 with the graphene-iron catalyst to improve performance
- TiO_2 photocatalyst can degrade contaminants via simultaneous generation of holes and electrons
- TiO_2 also creates a phenomenon called strong metal-support interaction (SMSI) and enhances the catalyst performance

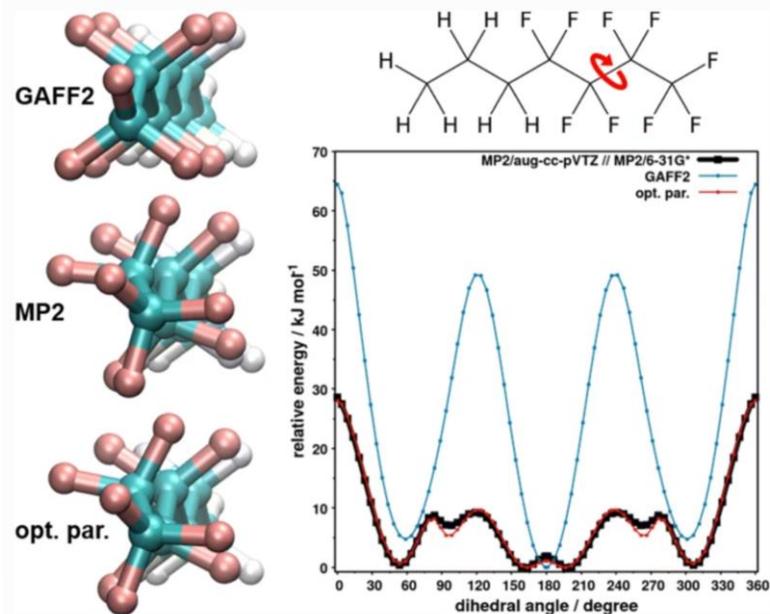


Aim 3: Perform molecular modeling to discover, detect, and refine enzymatic biodegradation for structurally diverse PFAS

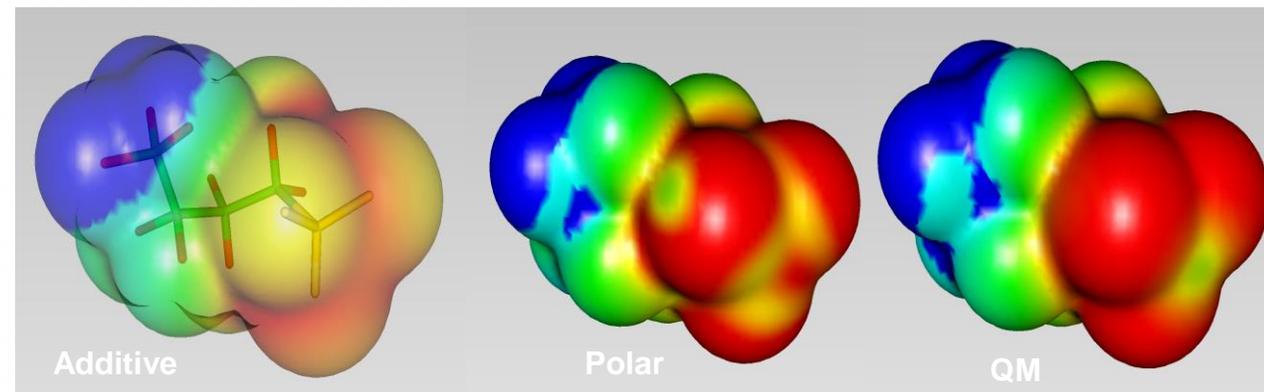
Preliminary Problem: Fluorinated molecules are not well parameterized in Molecular Dynamic (MD) forcefields. The lack of parametrization can lead to errors within the MD simulations. There is also concern of how the anion is treated in polar vs additive forcefields. The impact that this will have on results is unknown.

Solution: Evaluate additive and popular forcefields, used in popular MD software, for PFAS and related molecules. Create optimized parameterization set for the Amoeba forcefield that accurately simulates PFAS.

Fig. 1



Left:
 $\text{CH}_3(\text{CH}_2)_2(\text{CF}_2)_3\text{CF}_3$
 from MD with parameters autogenerated by GAFF2 forcefield, quantum mechanical results at the MP2/6-31G* level, and MD with an optimized torsion angle parameter.
 Right: The difference in torsion angle energy barriers for each structure. (Träg and Zahn 2019)

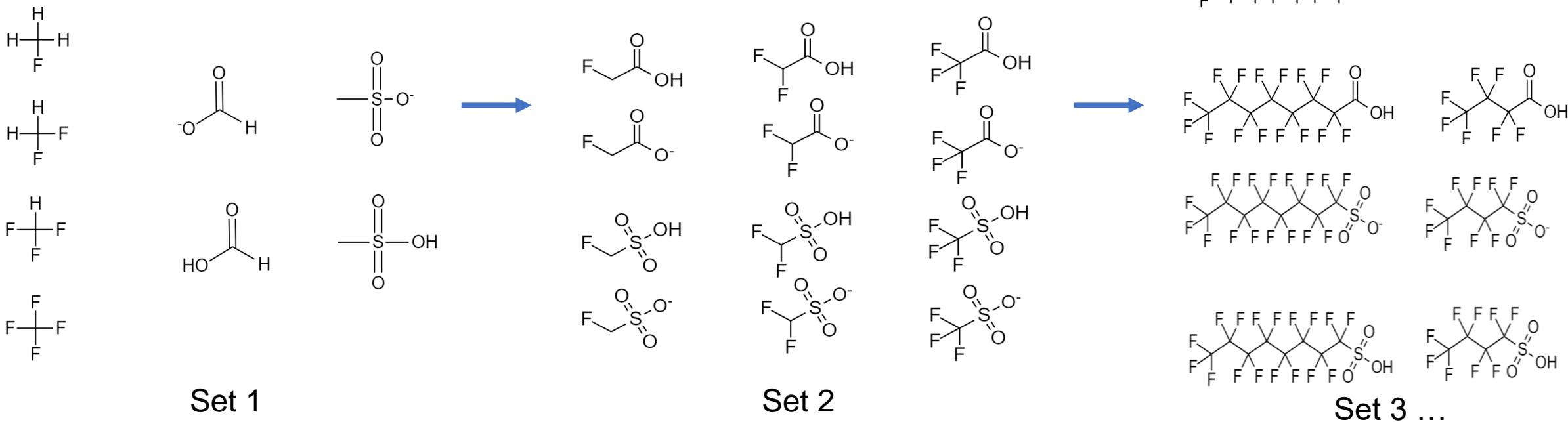


Representation of $\text{C}_4\text{F}_9\text{SO}_3^-$ electrostatic potential mapping by an additive forcefield (left) and polar forcefield (middle) compared to quantum mechanical on the B3LYP/6-311+G** level

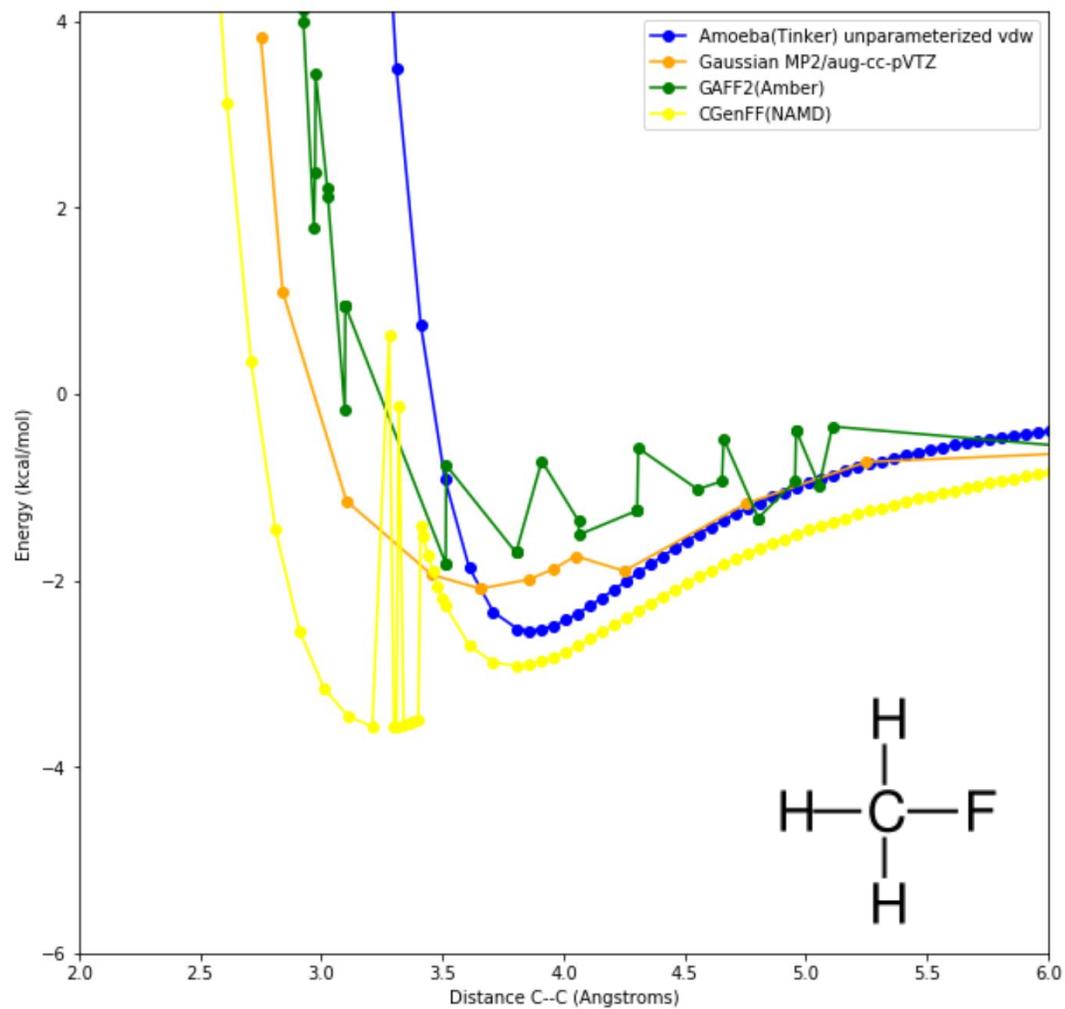
Challenge: PFAS largely lack experimental data needed to properly evaluate MD simulations.

Approach: Parameterize and compare starting with “PFAS building blocks” with more available data, then continue to longer and branched PFAS molecules.

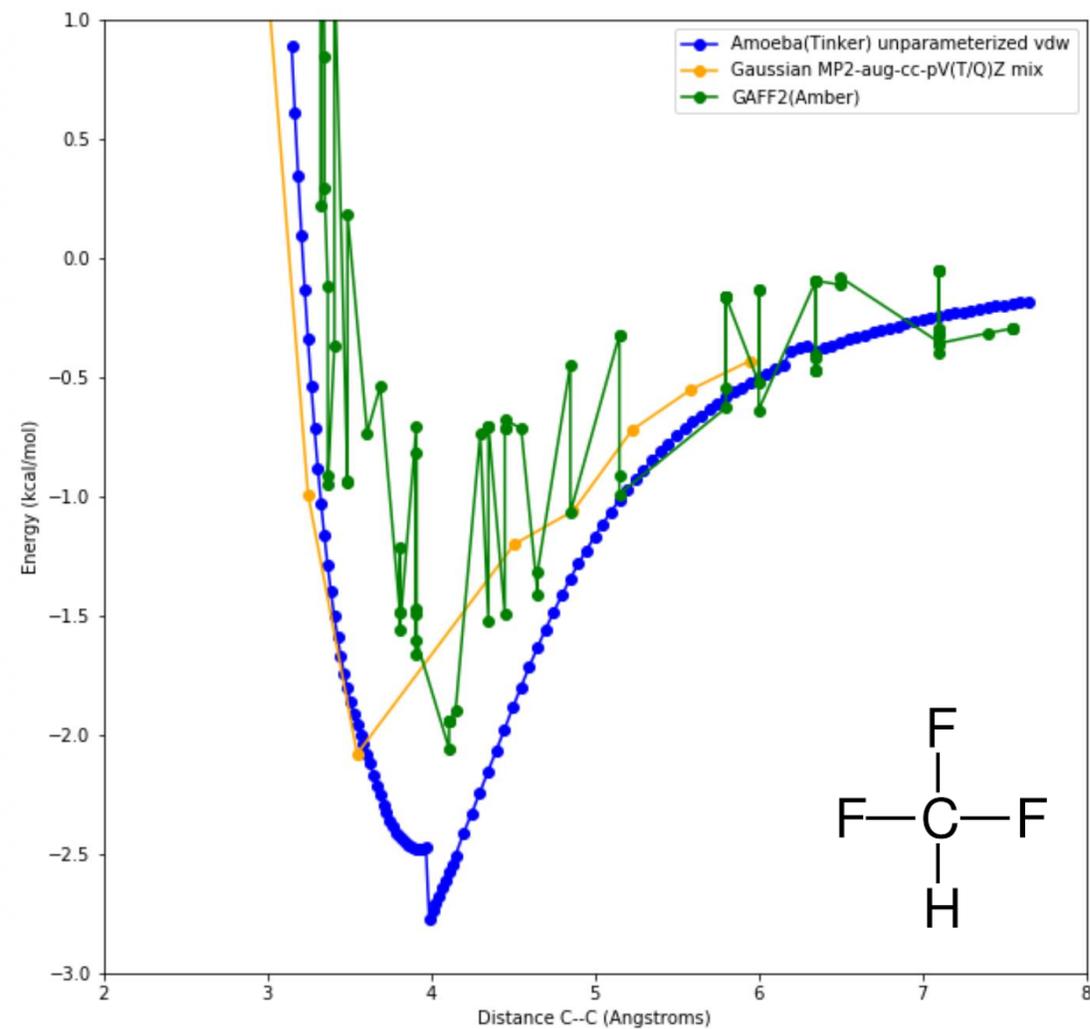
Current focus: Dimer Energies, Enthalpy of Vaporization, and Density.



Set 1: Fluoromethane and Trifluoromethane Dimer Energies

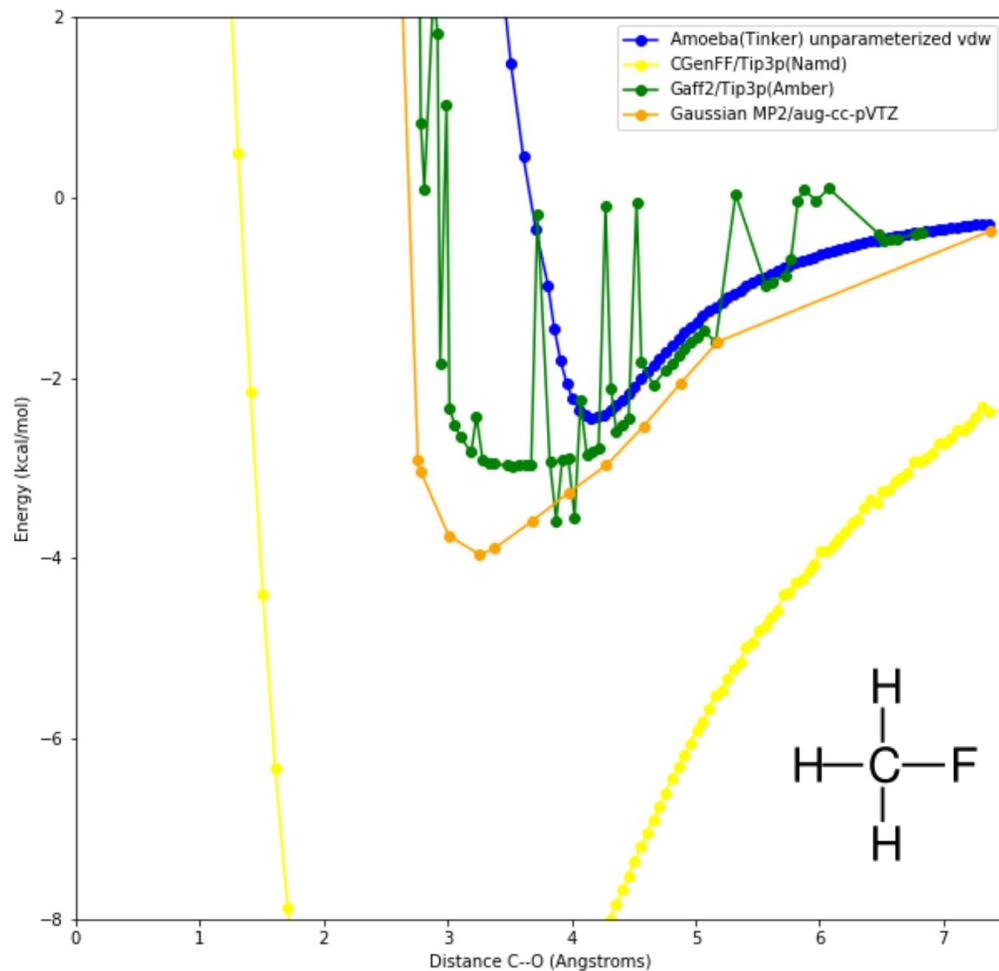


Fluoromethane

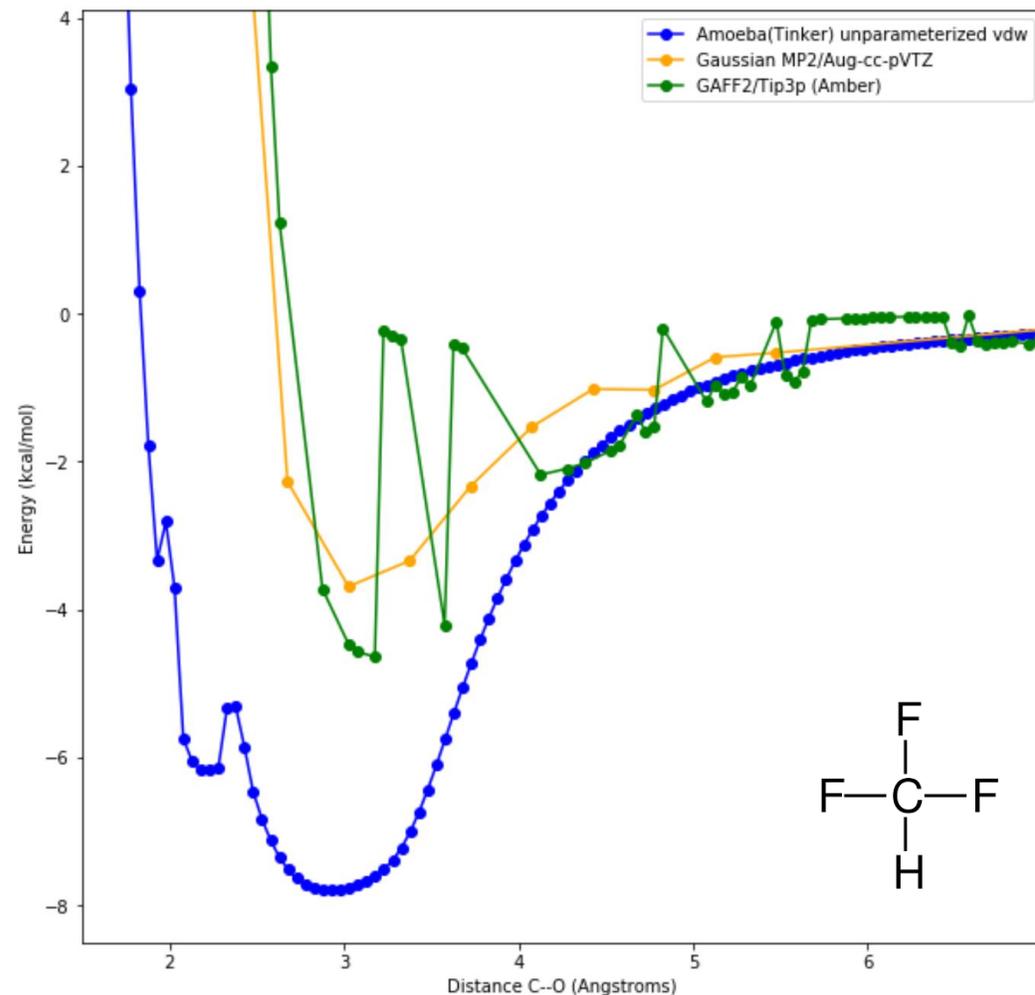


Trifluoromethane

Set 1: Fluoromethane and Trifluoromethane Water Dimer Energies



Fluoromethane

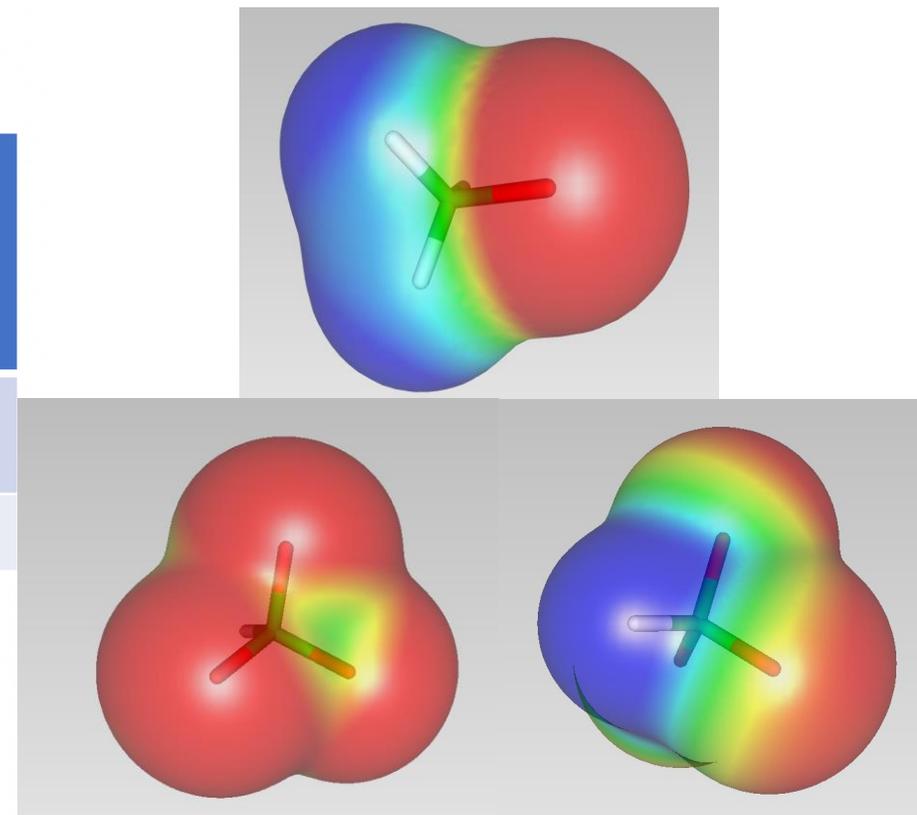


Trifluoromethane

Set 1: Fluoromethane and Tetrafluoromethane Enthalpy of Vaporization, Density, Electrostatic Potential Maps

Molecule	Temp. (K)	NIST Heat of Vaporization (kcal/mol)	Amber (GAFF2) Heat of Vaporization (kcal/mol)	Percent Error (%)	NAMD (CGenFF) Heat of Vaporization (kcal/mol)	Percent Error (%)
CFH ₃	194.75	3.989	3.479	12.79	4.959	19.56
CF ₃ H	190.97	3.99	6.089	52.61	N/A	N/A

Molecule	Temp. (K)	NIST Density (g/mL)	Amber (GAFF2) Density (g/mL)	Percent Error	NAMD (CGenFF) Density (g/mL)	Percent Error (%)
CFH ₃	194.75	0.881	0.8151	7.46	0.8818	0.091
CF ₃ H	190.97	1.4463	1.5396	6.45	N/A	N/A



Electrostatic Potential Mapping at the MP2/aug-cc-pVTZ level.

Top: CFH₃ Max:23.85 kcal/mol, Min:-26.98 kcal/mol, red < -12.55 kcal/mol- blue > 12.55 kcal/mol.

Bottom: CF₃H Max=35.85 kcal/mol, Min= -9.76 kcal/mol, red < -6.275 kcal/mol- blue > 28.237 kcal/mol



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Co-I: Nirupam Aich
Environmental Eng, UB



Co-I: Ian Bradley
Environmental Eng, UB



Co-I: Carla Ng
Environmental Eng, Pitt



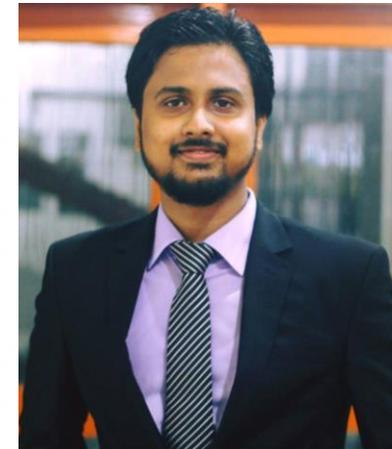
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