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De Novo Enzymes Development for PFAS Compounds Degradation

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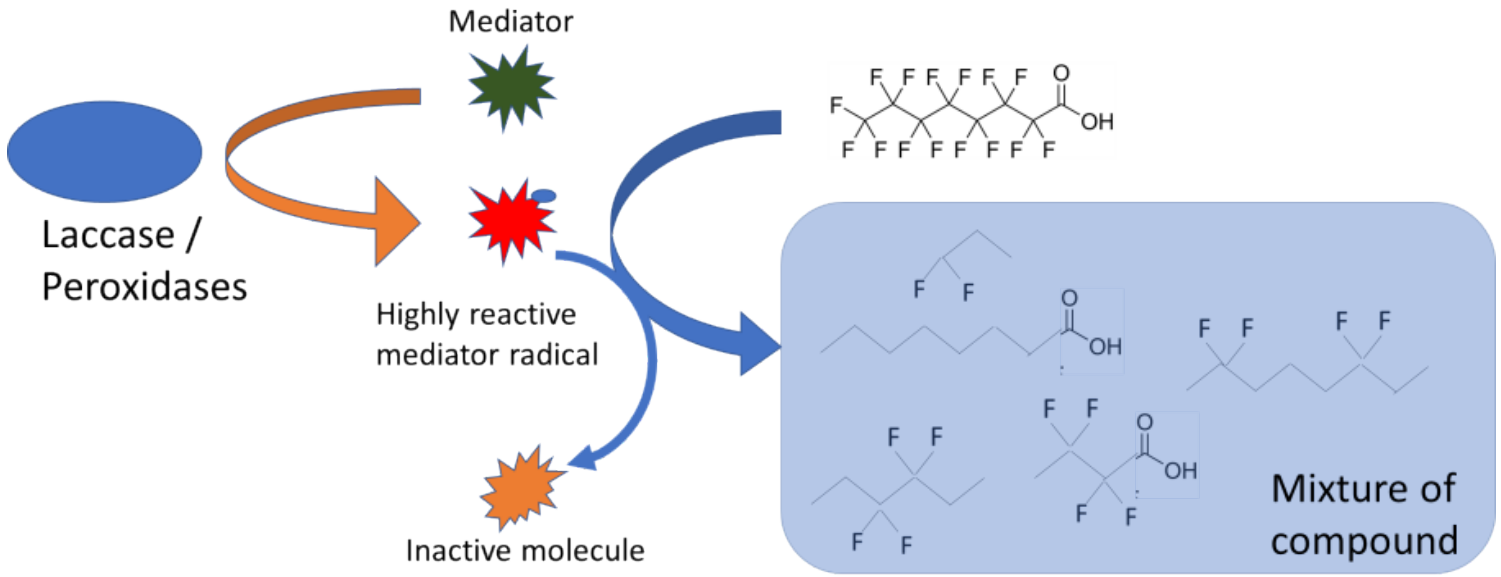
Can PFAS be Biologically Degraded?

- Polyfluorinated compounds (incl. precursors)
- Perfluorinated compounds
- Multiple pathways being investigated
  - Whole Cell (fungal or bacterial) – *In situ* applications
  - **Biocatalysts (enzyme mediators) – *Ex situ applications***
  - **Novel enzymes - *Ex situ applications***

Example: SAFF concentrate

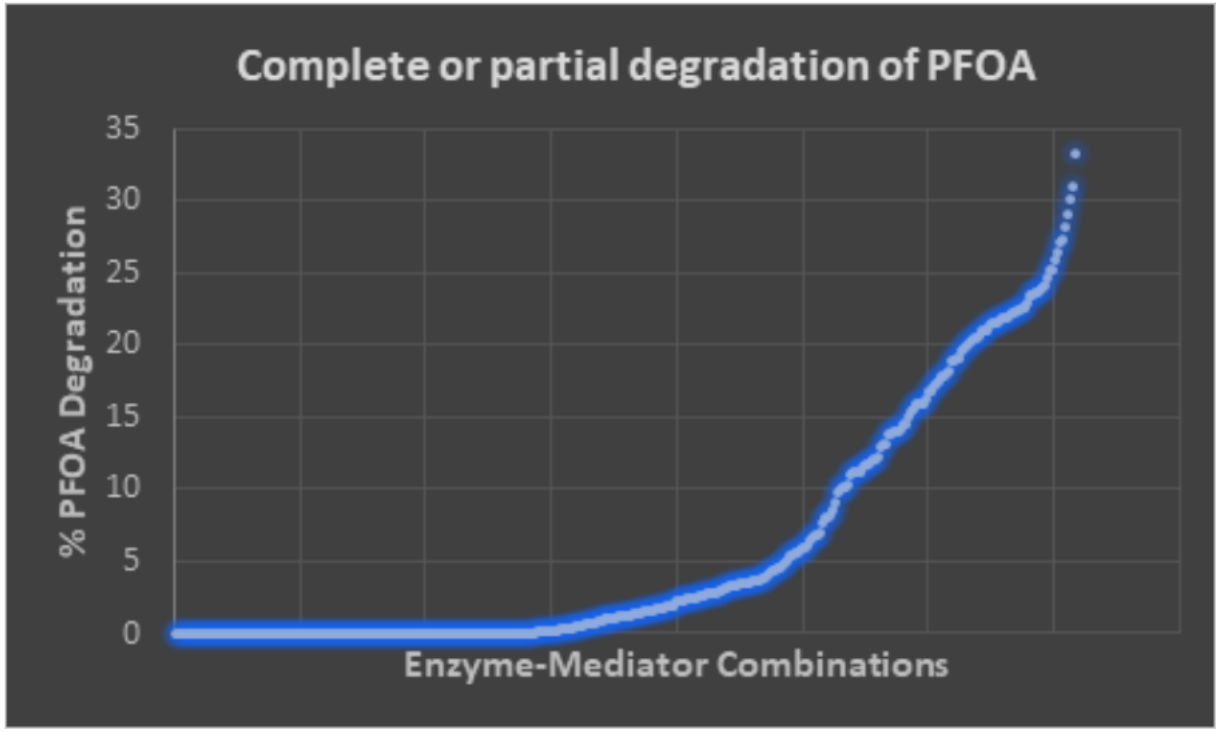
Biocatalysis (Enzyme mediator)



- Ligninolytic enzymes degrade emerging contaminants (not just PFAS)
- They oxidize “mediators” (phenolic compounds and aromatic amines), which creates radicals
- Radical is very highly reactive and oxidizes any molecule it comes in contact with

Enzyme-Mediator screen Results

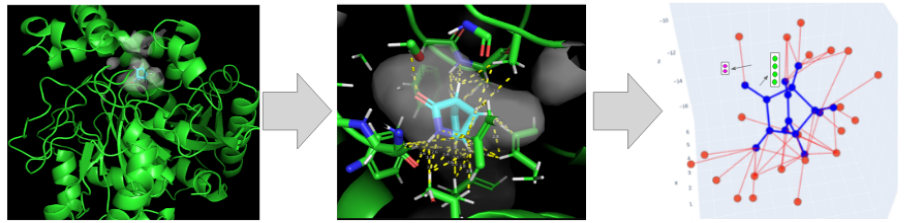
- High throughput screening of enzyme-mediators pairs showed ~30% PFOA degradation
- Around 500 enzyme constructs were tested against 70+ mediators to identify mediator with highest redox potential



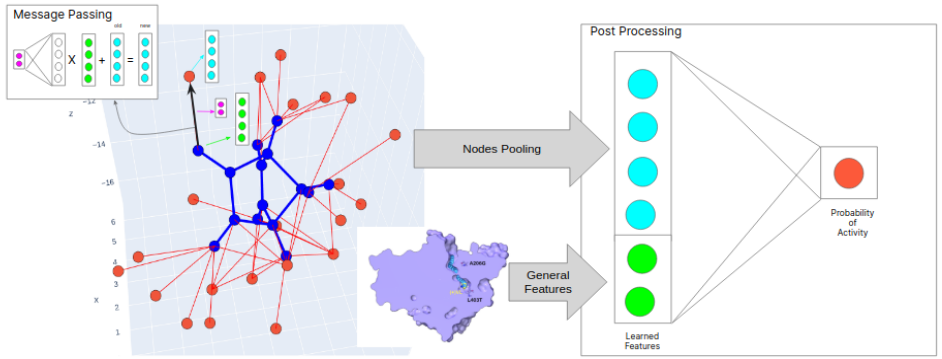
Novel Enzymes

- Working with Aether Biomachines to develop novel enzymes to degrade PFAS

- Ultra-high throughput experimental platform enables building thousands of variants, running miniaturized enzymatic experiments on high density plates

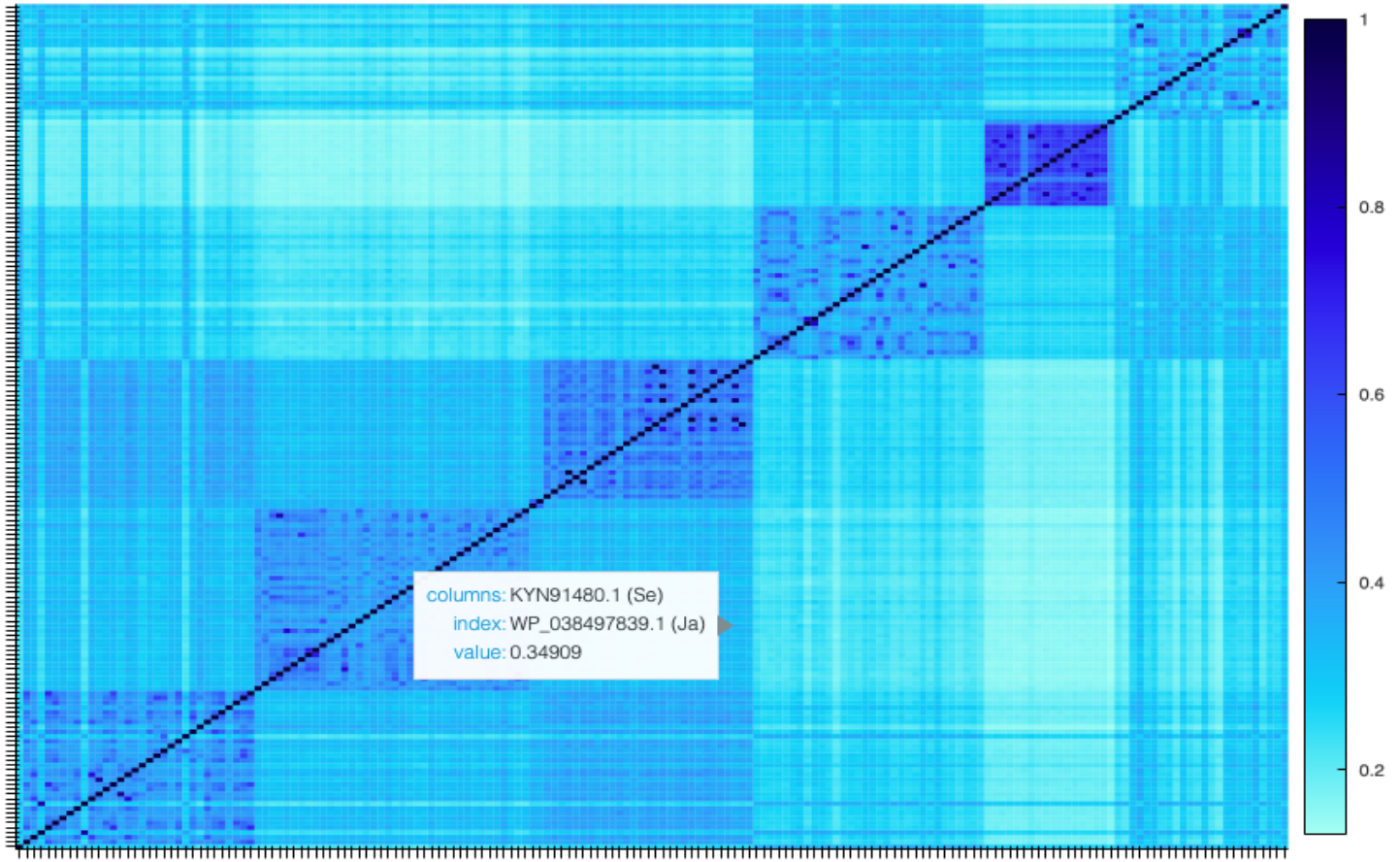


- AI-Physics hybrid models consist of a Physics simulator that predicts binding pose/s of the substrate within the enzyme, followed by pose parameterization and a message passing graph neural network

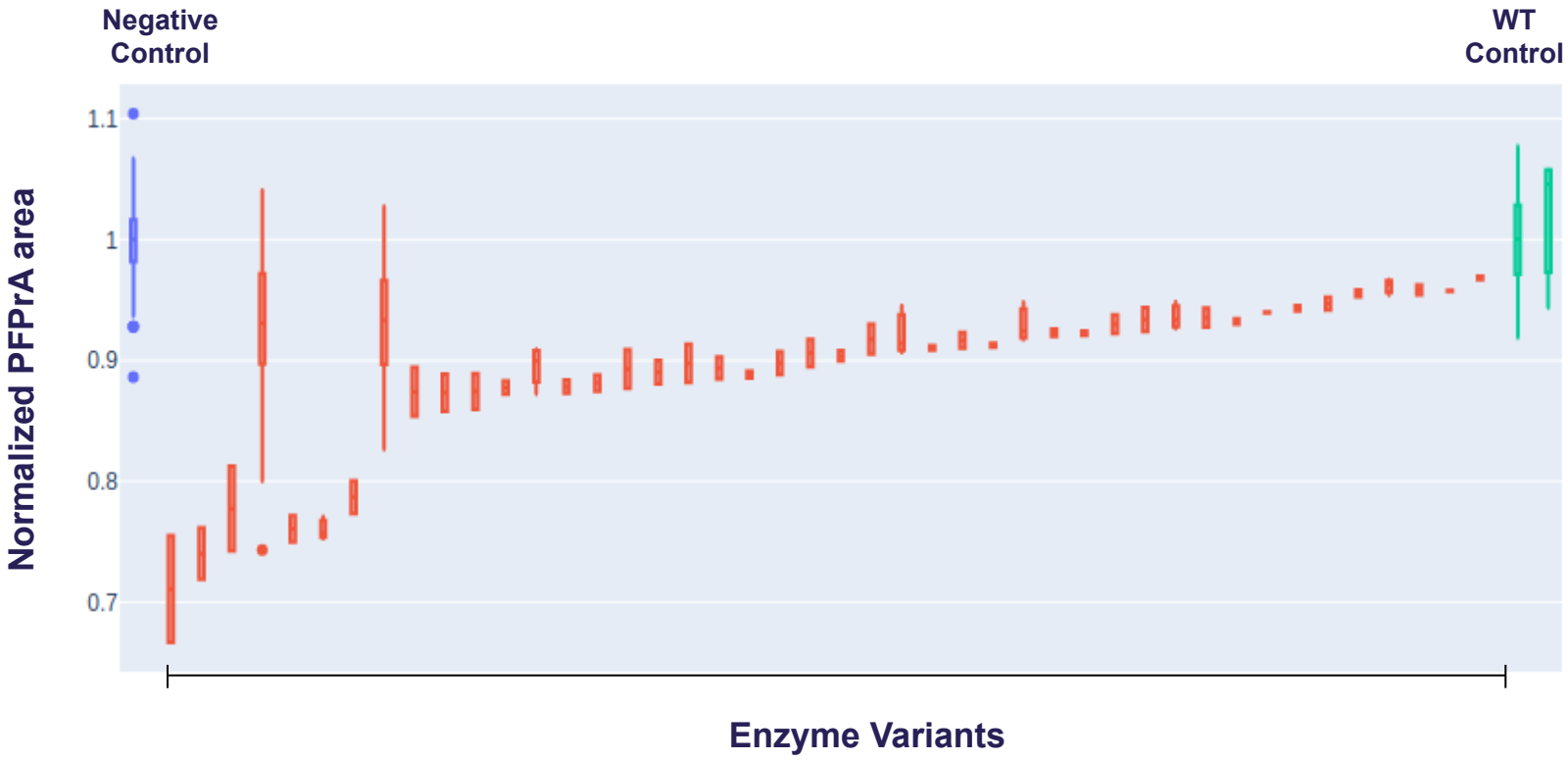


Where to Start?

- 183 enzymes from 7 enzyme classes
- Minimal sequence overlap



Aether algorithms are able to predict enzyme variants that can result in significant **defluorination**



- Pentafluoropropionic acid (PFPrA) is a perfluorinated compound that was used to test whether Aether algorithms can generate novel activity i.e. defluorination.
- We observed 5 to 30 % degradation within 24 hours for this type of compound and chemistry demonstrating the predictability of the algorithms.

Enzymes tested in early screens can also degrade other **PFOS-like substrates** under similar conditions

11 additional enzymes that degrade PFOS-like substrates

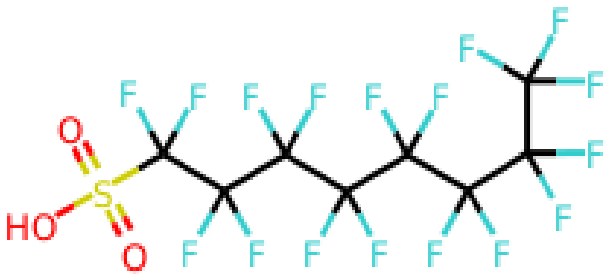
Aether Enzyme Families	% Depletion of other PFAS Substances		
	PFOSA	EtFOSAA	8:2 FTS
ENZ045	15 %	NA	NA
ENZ047	15 %	NA	15 %
ENZ048	16 %	NA	NA
ENZ209	20 %	NA	20 %
ENZ212	20 %	25 %	NA
ENZ224	16 %	NA	NA
ENZ227	14 %	NA	NA
ENZ243	15 %	40 %	NA
ENZ246	25 %	25 %	NA
ENZ260	NA	NA	NA
ENZ272	NA	NA	20 %
ENZ293	NA	NA	28 %

- Aether uses a strategic approach of combinatorial substrate screening to enable discovery of numerous alternative enzymes to degrade families of toxic compounds.
- Using this approach, we have demonstrated degradation of other halogenated compounds in the PFAS family in the first phases of a high throughput screening campaign performed in microtiter well plates using automated workflows.
- In addition to **Enz209** that has been engineered to degrade PFOS (as shown on the next slide), we identified ***11 additional enzymes*** that degrade PFAS compounds (PFOS-like substrates)

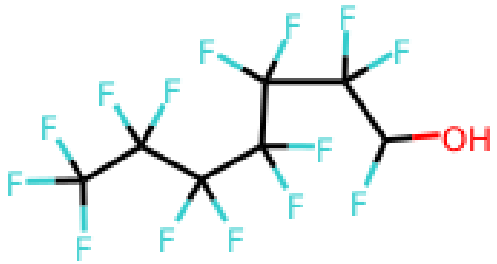
Preliminary data obtained via LCMS suggests **partial defluorination of PFOS**

PFOS contains **17 Fluorine atoms** and is potentially broken down into a C7 alcohol containing product with **14 Fluorine atoms**

PFOS (17 F)



C7 alcohol (14 F)



• Preliminary data from targeted mass detection using LCMS suggests partial defluorination of PFOS to a C7 alcohol. To our knowledge this is the ***first demonstration of enzymatic defluorination of a perfluorinated compound under ambient conditions***.

Summary

- Our research has shown that both fungal and bacterial enzymes can lead to a 25% to 30% degradation of PFAS compounds.
- It should be noted that the potential for engineering fungal enzymes such as laccases and peroxidases is limited, as they target mediators rather than PFAS compounds directly.
- On the other hand, novel bacterial enzyme variants have the advantage of being able to target PFAS compounds directly, and therefore can be further developed for improved activity.
- We confirmed the defluorination by monitoring the degradation products on LCMS

Acknowledgement

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  - DeNovo enzymes
- Ginkgo Bioworks
  - Fungal enzymes and mediators
- UCLA and Shaily's LAB
  - Fungal whole-cell and enzymes

Thank you

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