Title: Optimizing enzymes for plastic upcycling using machine learning design and high throughput experiments

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Project Goals: We aim to create new and optimized PET-depolymerizing enzymes (PETases) useful for industrial application. [**Aim 1]** Design novel PETases that are significantly different (25-65+ mutations) from known PET-depolymerizing enzymes and contain unique properties useful for performant enzymatic PET recycling and upcycling. Introducing many simultaneous mutations, while maintaining function, will enable us to more efficiently search for altered properties that depend on primary amino acid sequence. **[Aim 2]** Optimize previously described PETases by testing millions of mutagenized variants using directed evolution. Starting with existing functional PETases and exploring small changes in many distinct sequences using a novel ultra-HTP functional assay, we will optimize enzymes with improved properties by varying experimental conditions. **[Aim 3]** Characterize performance metrics of new and optimized PETases in detail including solvent tolerance, stability, catalytic rate, and substrate promiscuity.

Abstract Text:

Plastic use is ubiquitous in the modern world, and polyethylene terephthalate (PET) is one of the most abundantly produced plastics (and the most highly produced polyester), with ~65 million metric tons manufactured annually. To the consumer, PET is likely most recognizable as the plastic used to make beverage bottles. Like many plastics, traditional mechanical or chemical means of PET deconstruction and upcycling are costly and inefficient.

Recently, biological enzymes capable of breaking down PET into its basic building blocks (terephthalic acid and ethylene glycol) have garnered significant attention as an attractive means of dealing with the plastic problem. These enzymes are currently undergoing pilot studies for implementation in enzyme-based recycling. However, there are significant limitations to current enzymes, including the need to perform costly pre-processing of the plastic waste before the enzymes are able to work. Further optimization of these enzymes is necessary to make the

process profitable and thereby incentivize commercialization of this biology-based green recycling technology.

We aim to apply recent advances in artificial intelligence and machine learning to design new versions of enzymes capable of breaking down PET. Based on preliminary experiments using this evolution-informed computational design strategy, we believe we can create a highly diverse set of enzymes that have exceptional properties useful for industrial recycling. Testing these enzymes is typically labor intensive, but using a new robotically-enabled platform we will be able to experimentally characterize key enzymatic properties of thousands of these designed enzymes.

In addition to applying machine learning approaches to design new enzymes, we have developed a novel method that, by experimentally testing millions of small changes to enzyme structure, enables us to optimize existing enzymes that are known to break down plastic. The key to this approach is that we encapsulate individual variations of each enzyme in single droplets together with plastic nanoparticles creating a "mini reaction", and then we select those droplets which successfully break down PET to isolate the 'winning' enzyme variants.

Ultimately, we believe that the result of these studies will be the discovery of highly-optimized enzymes capable of breaking down PET plastics in an industrial recycling setting, enabling a powerful and green solution to the plastic problem.

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