DISCOVERY AND ENGINEERING OF NYLON HYDROLASES FOR PA66 RECYCLING

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Plastic waste is one of the biggest obstacles to developing a circular, sustainable economy. As of 2017, there have been ~8.3 billion tons of plastic produced, of which 6.3 billion tons are waste and only 9% has been recycled. This linear model is environmentally harmful, economically wasteful, and not sustainable. Mechanical recycling of polymers requires strict sorting of plastic waste that substantially increases costs, particularly for complex composites. Enzymes provide an alternative route to break down mixed polymer waste, with the potential to tailor specific enzymes to degrade different polymers in a mixed waste stream and produce targeted products.

Nylons encompass a family of polyamide (PA) thermoplastic polymers, produced at a scale of roughly 9 million tons in 2020. Since nylons and proteins share a common polyamide linkage, enzymes for nylon hydrolysis have evolved from the N-terminal nucleotide (Ntn) hydrolase superfamily, members of which more commonly cleave amide bonds in proteins. However, the limited set of currently characterized nylon hydrolases have low activity for intact polymeric nylon and instead preferentially hydrolyze smaller nylon oligomers to dimeric products. For enzymatic recycling, particularly of nylon composites, partial depolymerization to medium chain length oligomers would facilitate both separation of the composite elements and repolymerization.

We have identified, synthesized, expressed, and characterized a panel of 95 Ntn hydrolase enzymes with 25-40% pairwise amino acid identity. Unexpectedly, approximately one third of the tested enzymes showed nylon hydrolysis activity using pellets of pure nylons. The measured activity showed no correlation to the protein phylogeny. The most common hydrolysis products were linear tetramers, and different enzymes showed selectivity for PA6 or PA66. We developed a machine learning (ML) model to predict nylon hydrolysis activity and validated this model by synthesizing a second enzyme panel enriched in active sequences.

Starting from the most active enzyme identified in the diversity screen, we used directed evolution to further improve its activity. Site-saturation mutagenesis targets were identified through a variety of computational approaches and screened for increased hydrolytic activity. Results from these screens were used to refine the ML model and drive further rounds of engineering. Additional assays are in progress to characterize the enzyme structure, its interactions with the solid polymer, and alterations to properties of the residual polymer.
We expect this approach and our diverse panel of nylon hydrolases to be useful for recycling of a variety of polyamide polymers. More broadly, engineering enzymes to degrade solid polymers brings additional engineering challenges but also opens new applications for precision recycling of mixed substrates.