Designing Novel Enzymes for Complete Degradation of Recalcitrant Polyamides

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Project Goals:

The goal of this project is to design enzymes capable of complete depolymerization of nylon 6 and nylon 66 and engineer bacterial strains able to metabolize the degradation products to higher-value sustainable materials.

As of 2015, a total of 6.3 billion tons of plastic waste had been generated globally. It is estimated only 9% of this total had been recycled, while 12% had been incinerated to recover energy values, and the remainder entered landfills or the natural environment. New technologies are needed to address this ever-growing problem. While closed-loop recycling methods offer new potential routes for dealing with plastic waste, competition with cheap, fossil fuel-derived precursors is likely to inhibit progress on this front for the foreseeable future. An alternative approach, harnessing the power of biology to not just depolymerize plastics back to their monomer precursors, but convert them into higher-value products offers stronger economic incentives and in turn would be expected to drive more rapid and widespread adoption. Toward that end, our work focuses on combining cutting-edge computational protein design and synthetic biology to address the challenge of complete biodegradation and upcycling of the recalcitrant polymers nylon 6 and nylon 66. Although natural enzymes have been shown to be able to degrade amorphous portions of polyamides such as nylon 6 and nylon 66, complete enzymatic degradation has not been demonstrated. We hypothesize this to be due in large part to a lack of natural enzymes able to efficiently catalyze degradation of the crystalline portion of the polymer. We are computationally designing enzymes to alleviate this limitation by introducing and optimizing polyamide hydrolysis activity in scaffolds with open active sites. In conjunction, we are screening and engineering bacterial strains able to metabolize nylon 6 and nylon 66 degradation byproducts directly into central metabolism. Once achieved, such platform strains can be used to produce a wide variety of fermentation products from central metabolites. Integration of our designer nylon 6 and nylon 66 depolymerizing enzymes into these engineered hosts will provide a novel, elegant, and cost-effective consolidated fermentation process for nylon upcycling to higher-value sustainable materials.

References

1. Geyer, R., Jambeck, J. R. & Law, K. L. Production, use and fate of all plastics ever made. *Sci. Adv.* 3, e1700782 (2017).
2. Kakudo, S., Negoro, S., Urabe, I. & Okada, H. Nylon oligomer degradation gene, nylC, on plasmid pOAD2 from a Flavobacterium strain encodes endo-type 6-aminohexanoate oligomer hydrolase: purification and characterization of the nylC gene product. *Appl. Environ. Microbiol.* **59**, 3978-3980 (1993).

3. Kinoshita, S. *et al.* 6-Amniohexanoic acid cyclic dimer hydrolase. A new cyclic amide hydrolase produced by Acromobacter guttatus KI72. *Eur. J. Biochem.* **80**, 489-495 (1977).
4. Kato, K. *et al.* Amino acid alterations essential for increasing the catalytic activity of the nylon-oligomer-degradation enzyme of Flavobacterium sp. *Eur. J. Biochem.* **200**, 165-169 (1991).
5. Yasuhira, K. *et al.* 6-Aminohexanoate oligomer hydrolases from the alkalophilic bacteria Agromyces sp. Starin KY5R and Kocuria sp. Strain KY2. *Appl. Environ. Microbiol.* **73**, 7099-7102 (2007).

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