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## Enzymes for a sustainable plastic waste management

During the last 80 years, about 10 billion tons of plastics have been synthesized, and their ongoing crude oil-derived production still accounts for the vast majority of products (~90%), compared to bio-based (~1%) and recycled (~9%) alternatives. Current thermo(mechanical) and chemical recycling technologies typically involve high energy costs and lead to a material downgrade with each cycle. A large fraction of plastic waste is disposed of in landfills or even released uncontrolled into the environment. In addition, the fragmentation of synthetic polymers has led to the omnipresence of micro- and nanoparticles across the globe, with severe detrimental effects on the environment and likely on human health.

In recent years, enzymes and microbes have emerged as viable options for the sustainable biocatalytic re- and upcycling of plastics, paving the way toward circular economies. However, due to the diversity of polymer types and their inherent recalcitrance to biodegradation, these enzymes still require significant improvement and adaptation before they can be applied at industrial scale. In 2016, the discovery of a bacterium named *I. sakaiensis* that degrades and metabolizes polyethylene terephthalate (PET) with the help of two naturally evolved key enzymes, PETase and MHETase, triggered global interest and research initiatives in this field. We determined the first crystal structure of *I. sakaiensis* MHETase bound to its substrate and enhanced the enzyme based on these structural insights. This landmark study enabled us to secure funding through the Helmholtz Sustainability Challenge (FINEST project), where a five-member sub-consortium (FINEST | MICROPLASTICS) is dedicated to developing enzymes for the degradation of organic fine particulates (microplastics). Together with FINEST project partners, numerous PET hydrolases have already been structurally characterized in their substrate-bound state and improved. Currently, a hydrolase active against the highly abundant and teratogenic plasticizer diethyl phthalate (DEP) is under investigation. Within FINEST, a collaboration with HZDR will also explore the development of a protein/peptide-based micro- and nanoplastics detection assay to better monitor these potentially hazardous, omnipresent particles. Complementary to this, the recently launched PUreValue project (a FINEST satellite) focuses on the highly complex polymer polyurethane, with the goals of enabling its biocatalytic valorization for upcycling as well as developing approaches for environmental monitoring. Here, we obtained the first structure of urethanase SP2, an amidase that can hydrolyze the carbamate bond of polyurethanes. Structural data enabled us to improve the enzyme toward simpler substrate snippets, and we are now pursuing a redesign of the enzyme using AI-based protocols to increase its activity on larger substrates. Finally, HZB participates in the Horizon Europe project UPCYCLE, which aims to improve the life cycle costs of enzymatic polyester hydrolysis at industrial scale. In summary, HZB is deploying its expertise in structural biology, biochemistry, biotechnology, and AI-based protein design to drastically advance the recycling and valorization of hydrolysable polymers, thereby contributing to a significant reduction in greenhouse gas emissions and global environmental pollution.

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