Development of a highly optimized engineered PETase enzyme for plastic degradation

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The uncontrolled accumulation of plastic waste in the environment has long begun to impact on the natural ecosystems and to pose an existential threat to all forms of life on our planet. Advanced technical solutions to the plastic waste management problem are therefore in urgent demand. To this end, enzymatic approaches to plastic degradation hold great promises as novel and more efficient enzymes are constantly being developed. . Leaf-branch Compost Cutinase (LCC) is a naturally occurring PETase that has been reported to outperform all other PET-degrading enzymes known to date. This enzyme has been noticeably engineered in 2020[1], leading to the so-called ICCG variant (Tm = 94.0°C), the current gold standard. We have engineered a Leaf-branch Compost Cutinase (LCC), named DRK3, that features enhanced PETase activity and thermal stability relative to gold standard ICCG.[2] The DRK3 mutant shows a Tm > 98 °C and remarkable activity on amorphous PET films beyond 6 days at 68°C. The high resolution crystal structure of DRK3 shows no significant changes in its folding relative to both the wild type LCC (r.m.s.d. = 0.257 Å) and the ICCG variant (r.m.s.d. = 0.152 Å). The catalytic triad (D210, H242, and S265) overlaps perfectly with the catalytic triad in the parent enzymes. The mutations introduced in the design process are found mostly on the surface of the enzyme and away from the catalytic triad. We hypothesize that the increase in stability provided by the additional surface charges may help to keep the surface-exposed catalytic site in place even at high temperatures. This is confirmed by MD simulations, which show that at increasing temperatures DRK3 features a lower r.m.s.f., particularly near the key catalytic residue H242.
The enzymatic degradation of PET holds immense promises as a sustainable solution to the escalating crisis of plastic waste accumulation.

References

1. Tournier, V. et al. Nature 2020, 580 (7802), 216

2. Bhattacharya S. et al. Submitted