Engineering the thermal stability of a PET degrading enzyme [ID-333425]

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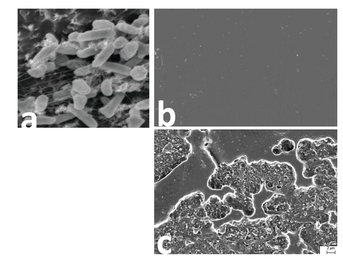
Introduction

Polyethylene terephthalate (PET) is a semi-crystalline polyester made from terephthalic acid and ethylene glycol. Its outstanding properties such as chemical resistance, lightweight and stability promote its widespread use with the downside of accumulation in the environment in the form of microplastic causing problems on all levels. For PET derived microplastic a new perspective arose with the finding of the enzyme PETase in *Ideonella sakaiensis*.[1] This enzyme is the key for the organism's ability to fully break down PET and use it as a nutrition source. It therefore features as the first known PET degrading enzyme with substantial activity at ambient temperatures, but this limits its utilization for microplastic related applications like recycling and decontamination. One way to develop PETase into an enzyme with the desired properties is rational protein design. Here we use the PROSS[2] algorithm to optimize IsPETase. We further compare and combine our results with those from Cui et al[3] who applied their GRAPE algorithm, thereby revealing synergistic combinations of mutations from both approaches.



Ideonella sakaiensis[3](**a**), solid PET surface before (**b**) and after incubation with isPETase (**c**).

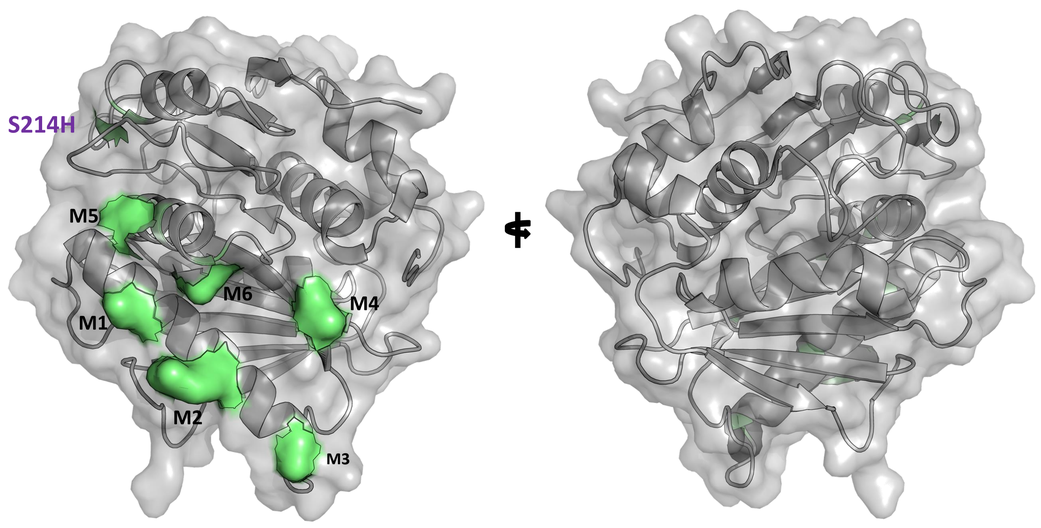
PROSS – Algorithm

- **Fully automated and unsupervised**
- **Phylogenetic analysis**: Depends on sequence alignments to identify potential stabilizing mutations for higher thermostability.
- **Rosetta Scoring:** Individual and pairwise scoring of the suggested mutation.
- **Output:** Seven variants from conservative to progressive number of mutations. Here we used an intermediate design bearing 7 mutations: **S214H** and six further mutations, here labelled M1-M6.

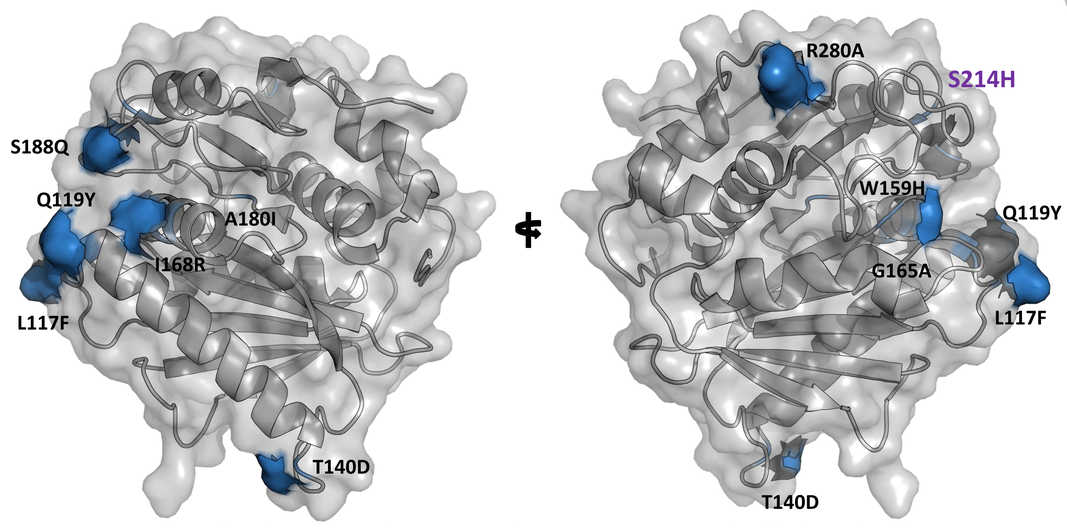
GRAPE – Algorithm

- **Semi-automated, iterative, supervised, experiment based**
- **Prediction of stabilizing mutants:** Based on a combination of statistical energy function, force-field based energy function (Rosetta) plus FoldX and a phylogenetic analysis. After filtering this yielded 21 mutations.
- **Biochemical Analysis and Clustering:** The 21 mutations were individually analysed and clustered upon Tm, activity and location using K-means algorithm.
- **Greedy algorithm:** Efficient combining of synergetic mutations to sample the energetic landscape overcoming epistatic effects. Iterative biochemical analysis, clustering and recombination.
- **Output:** Final variant with 10 mutations including S214H as suggested by PROSS.

Biochemical Characterization



Predicted structure of Pross-PETase1 in cartoon representation (dark grey) and protein surface in light grey. Mutations suggested by PROSS marked in green.



Predicted structure of DuraPETase in cartoon representation (dark grey) and protein surface in light grey. Mutations suggested by GRAPE marked in blue.

- **Analysis Pross-PETase1:**
  - Comparable activity to isPETase on PET at 30°C
  - **Tm:** 56°C, 9°C improvement

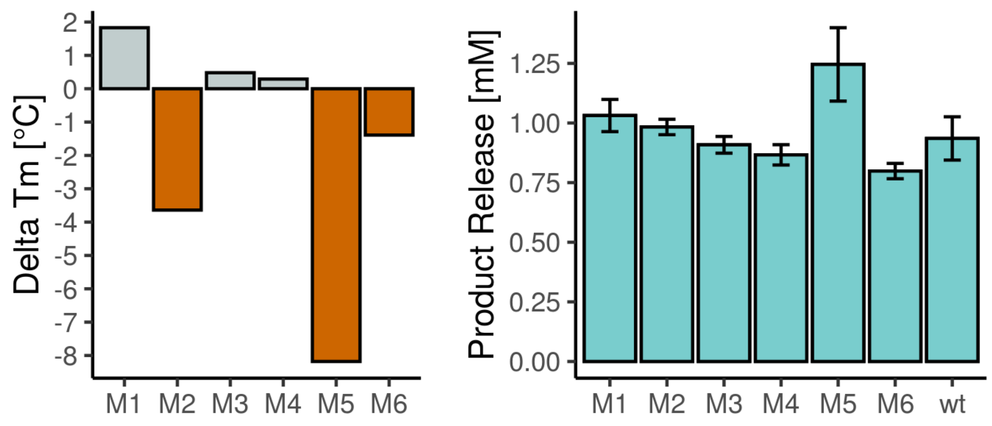
- **Analysis DuraPETase:**
  - Enhanced PET degradation of 23% at mild temperatures[3], high activity at elevated temperatures
  - **Tm:** 76°C, 29°C improvement

S214H is suggested by both approaches and shows very high stabilization potential according to Cui et al[3].

- What are the contributions of the remaining mutations M1-M6 in Pross-PETase1?
- Testing M1-M6 individually on top of DuraPETase

➢ The analysis suggests that only M1, M3 and M4 are beneficial for thermal stability while the other mutations are destabilizing. The activity of all variants is comparable to DuraPETase wt.

➢ Detailed analysis of activity profile in progress screening for synergistic combinations of mutations.



Difference in Tm of DuraPETase-M1-M6 compared to DuraPETase wt (left). Activity of DuraPETase variants M1-M6 on PET showing total product release (right).

References: [1] Yoshida et al. DOI: 10.1126/science.aad6359, [2] Goldenzweig et al. DOI: 10.1016/j.molcel.2016.06.012, [3] Cui et al. DOI: doi.org/10.1101/787069

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