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Improvement of amino ester hydrolase (AEH) via protein engineering, machine learning, and process engineering

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PURPOSE OF THE ABSTRACT
Amino ester hydrolase (AEH) catalyzes the synthesis of semisynthetic beta-lactam antibiotics and is a potential alternative to Pen G acylase (PGA). While AEH is more active and selective than PGA towards the synthesis of targets with (R)-phenylglycyl side chains than PGA, its substrate specificity is limited, its biophysical behavior is complex, and it is unstable at temperatures > 25oC.

We set out to improve the utility of AEH by improving its overall stability. While we had succeeded in stabilizing wild-type AEH from Xanthomonas campestris via site-directed mutagenesis [1], we now employ machine-learning based techniques, such as FireProt [2] and PROSS [3], to improve its thermal stability. In addition, we employed differential scanning fluorimetry (DSF) for enzyme unfolding, back reflection to study AEH aggregation, and analytical ultracentrifugation (AUC) to study AEH's oligomericity behavior [4] and improve its deactivation kinetics [5].

We found that mutating increasingly larger numbers of residues, up to 30% of the residues, led to dramatic stabilization, as measured by DSF via the melting temperature Tm (Figure 1). However, most variants were found to be inactive. Only the (re) discovery of a Ca2+-binding site in X. campestris AEH reliably recovered activity, ranging from small to sizable [6].

The development of AEH towards a biocatalyst useful in large-scale synthesis serves as an example of the need to explore various techniques and pathways and not just to rely on a single development path, whether via machine learning or experimental protein engineering.

FIGURES

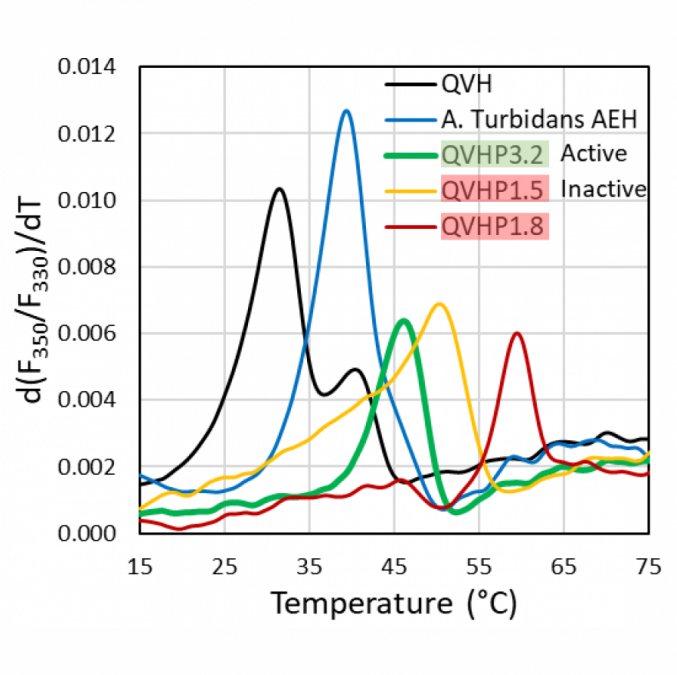

FIGURE 1

Melting temperature of AEH variants measured by Differential Scanning Fluorimetry (DSF.)

Melting temperatures indicated by peaks

FIGURE 2

KEYWORDS

amino ester hydrolase | stabilization | machine learning | protein engineering

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