

Km Value Determination

The Michaeli-Menten constant, K_m , is often used as a measure of an enzyme's ability to bind a substrate.

First the average initial reaction velocity was calculated. The difference in absorbance was divided by the path length and molar extinction coefficient of P-Nitrophenol (17500 M cm^{-1}) [1].

$$\Delta A = A_f - A_i = \epsilon l c$$
$$\Delta A / l c = \Delta c$$

Where A is absorbance

ϵ is the molar extinction coefficient

C is the concentration (M)

Reaction velocity was determined by dividing the change in concentration by the time interval

$$V_o = \Delta c / \Delta t$$

Where V_o is the initial reaction velocity

This calculation was carried out for the concentration of substrate tested. A Lineweaver-Burk transform was applied to these data of the form

$$1/V_o = K_m/V_{\max} (1/[S]) + 1/V_{\max}$$

Where K_m is the Michaelis-Menten constant

V_{\max} is the maximum velocity of the reaction

[S] is the concentration of substrate

Using the slope and Y intercept of the graph, the K_m values were obtained for the mutant PETase enzymes. These data (Figure 1) are of the same magnitude as the reported wild type PETase (4.6 mmol) [2]

Figure 1.

Mutant	Km (mmol)
Wild Type	1.56
S238F W159H	3.34
Tianyu	2.66
Dimi 1	2.66
Dimi 2	1.58
Dimi 3	2.34
Dimi 4	1.54

Acknowledgements

- [1] Austin HP, Allen MD, Donohoe BS, Rorrer NA, Kearns FL, Silveira RL, Pollard BC, Dominick G, Duman R, Omari KE, et al. 2018. Characterization and engineering of a plastic-degrading aromatic polyestherase. *Proceedings of the National Academy of Sciences* 115.
- [2] Ma Y, Yao M, Li B, Ding M, He B, Chen S, Zhou X, Yuan Y. 2018. Enhanced Poly(ethylene terephthalate) Hydrolase Activity by Protein Engineering. *Engineering* 4:888–893.