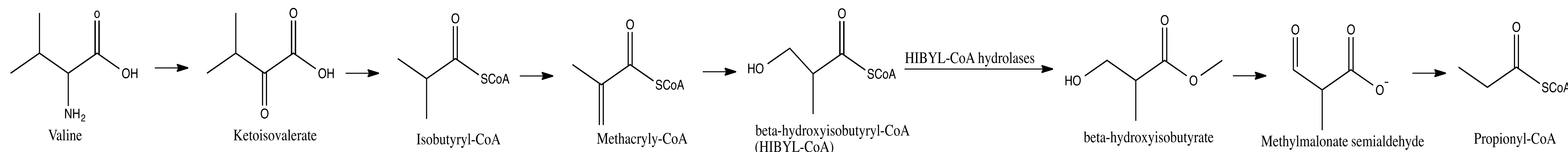


# Kinetic Characterization of CHY4 and CHY5 in the Catabolism of Valine in *Arabidopsis thaliana*



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## Introduction

Valine is an essential branched chain amino acid that is used as an energy source during times of plant growth and extended periods of darkness when normal energy sources are not available.<sup>1</sup> The degradation of valine yields propionyl-CoA, which may be further metabolized through respiration. Unlike other organisms, in which valine degradation takes place exclusively in the mitochondria, plants possess enzymes involved in valine degradation in both the mitochondria and peroxisomes.<sup>2</sup> This study specifically investigates the enzymes that catalyze the conversion of  $\beta$ -hydroxyisobutyryl-CoA to  $\beta$ -hydroxyisobutyrate in an irreversible process. In the plant species *A. thaliana*, there are eight isoforms of the hydrolase enzymes that are believed to catalyze this reaction. Deemed CHY1-8, CHY1-3 have peroxisomal targeting sequences while CHY4-6 have mitochondrial leader sequences, and all are part of a larger enzyme family that encompass many functions.

To date, only one peroxisomal hydrolase, CHY1, has been classified as a HIBYL-CoA hydrolase.<sup>3</sup> In order to confirm that valine degradation does in fact take place in the mitochondria, it is essential to classify each necessary enzyme with mitochondrial targeting sequences for the entire pathway. While previously none of the mitochondrial CHY enzymes have been classified, the present study investigates CHY4 and CHY5, both mitochondrial enzymes. Additionally, CHY4 was chosen for study as it is essential for plant germination. It is thought that in the absence of CHY4, a build up of the toxic intermediate methacrylyl-CoA proves fatal to the cell.

With previous work done by Scott Waters ('14) and Haley Dugan ('14) focused on expressing and purifying the CHY4 and CHY5 hydrolases, the present study seeks to classify CHY4 and CHY5 to determine if they are specific for this step in the valine degradation pathway, as HIBYL-CoA hydrolases, or if they also function in other pathways in the plant, such as the conversion of propionyl-CoA to acetyl-CoA.

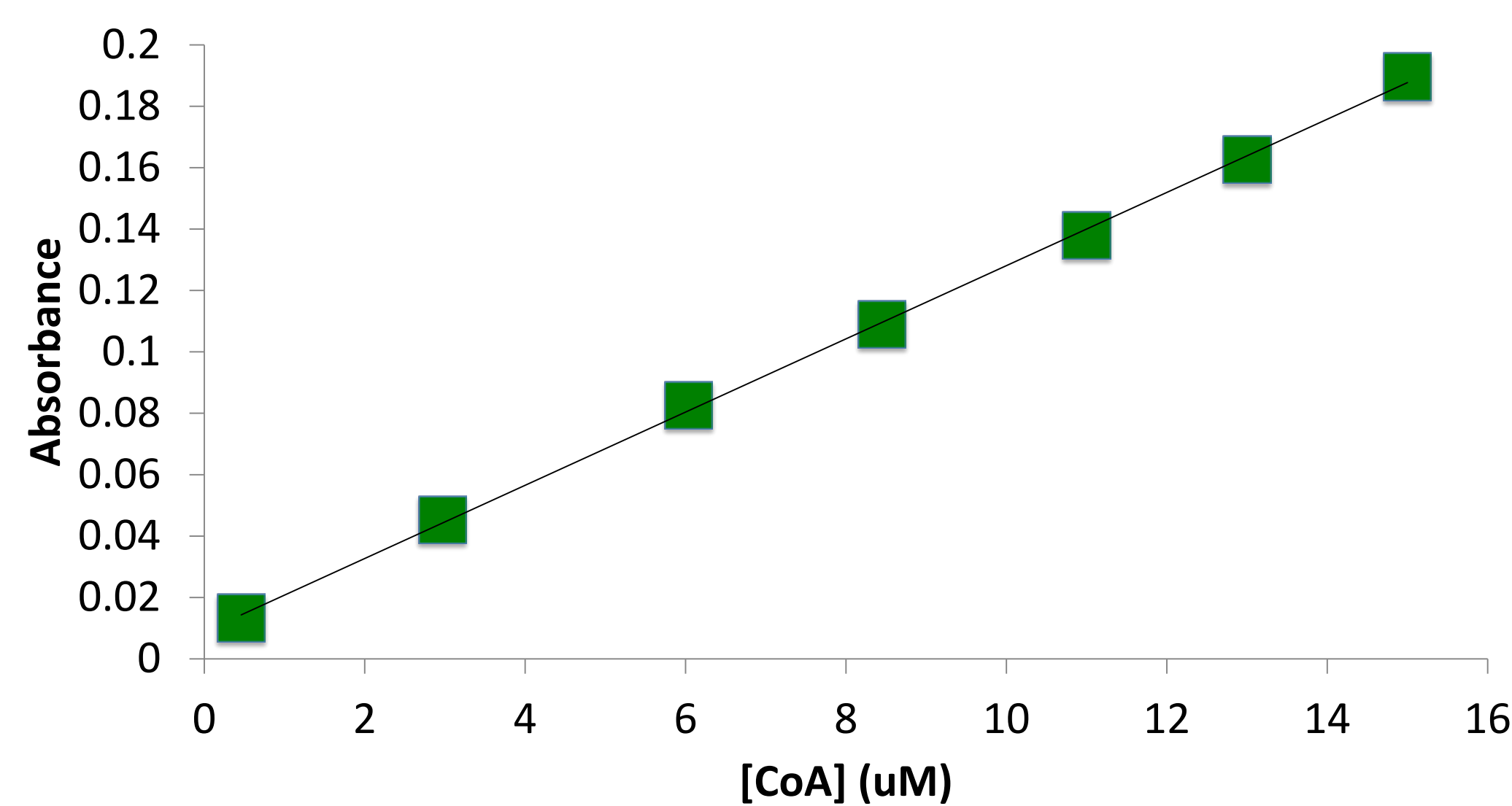


Figure 1. Standard curve of Absorbance vs. concentration of CoA ranging from 0.46-15 uM at 37°C in 0.2 M Tris-HCl pH 8, 1 mM DTNB ( $y = -11.9x + 8.8$ ).

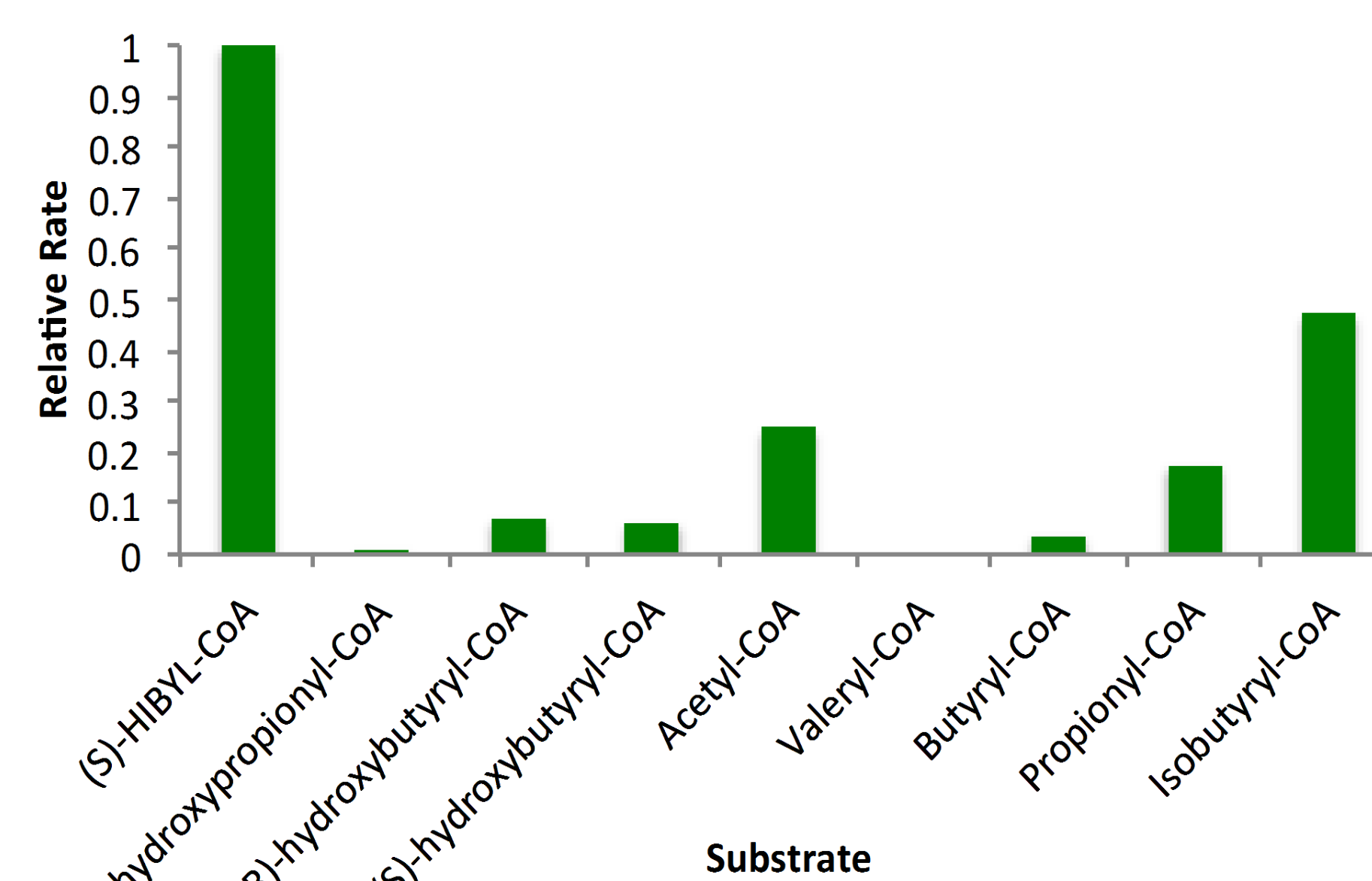


Figure 2. Substrate specificity of CHY5 (1.57 ug) at 37°C in 0.2 M Tris-HCl pH 8, 1 mM DTNB buffer. CHY5 is most active with the HIBYL-CoA substrate.

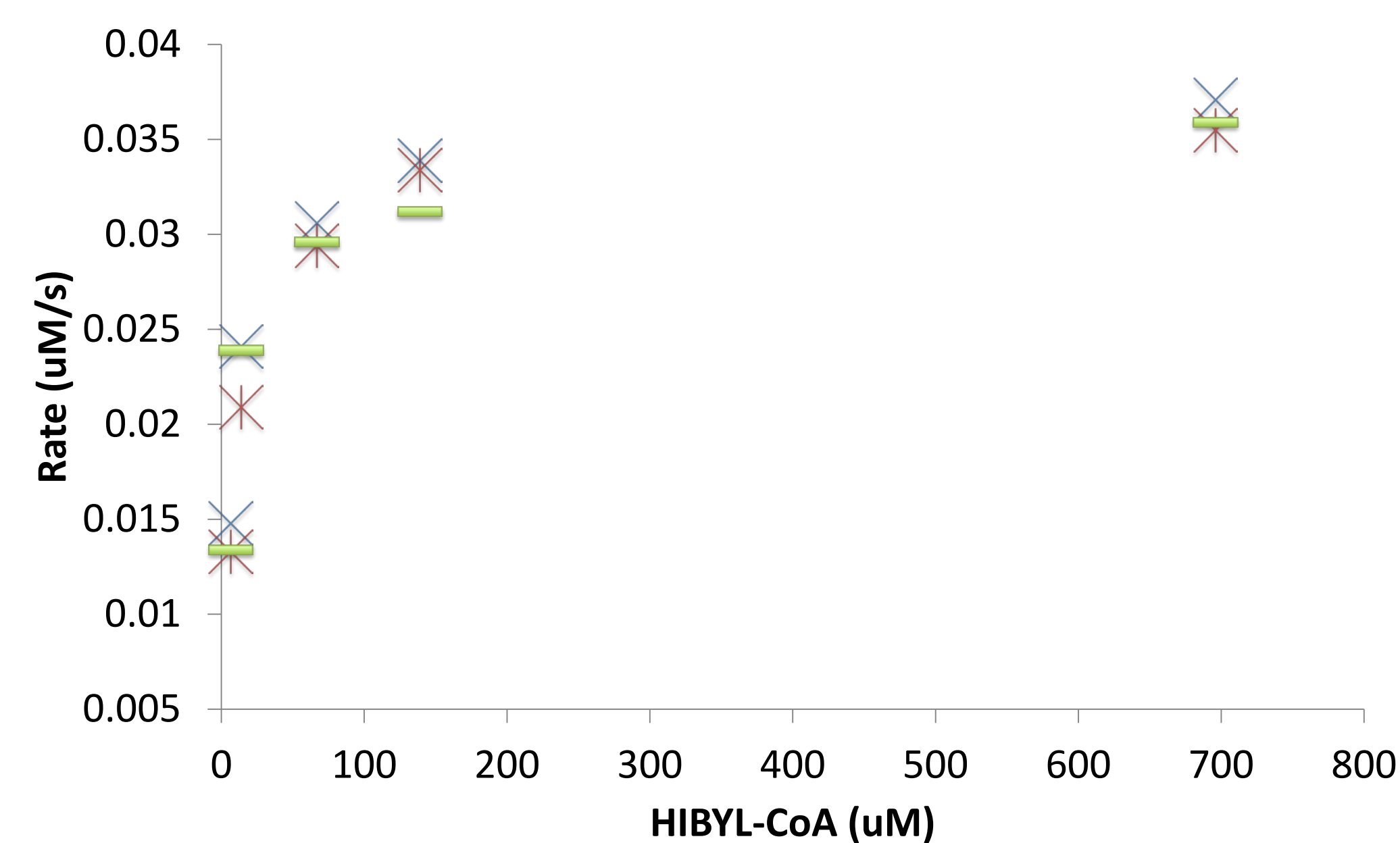


Figure 4. Michaelis-Menten plot of rate vs. concentration of substrate ranging from 0.67-140 uM at 37°C in 0.2 M Tris-HCl pH 8, 1 mM DTNB buffer with 1.57 ug CHY5 ( $K_m: 9.5 \pm 0.8$  uM,  $V_{max}: 3.60 \times 10^{-2} \pm 7 \times 10^{-3}$  uM/s).

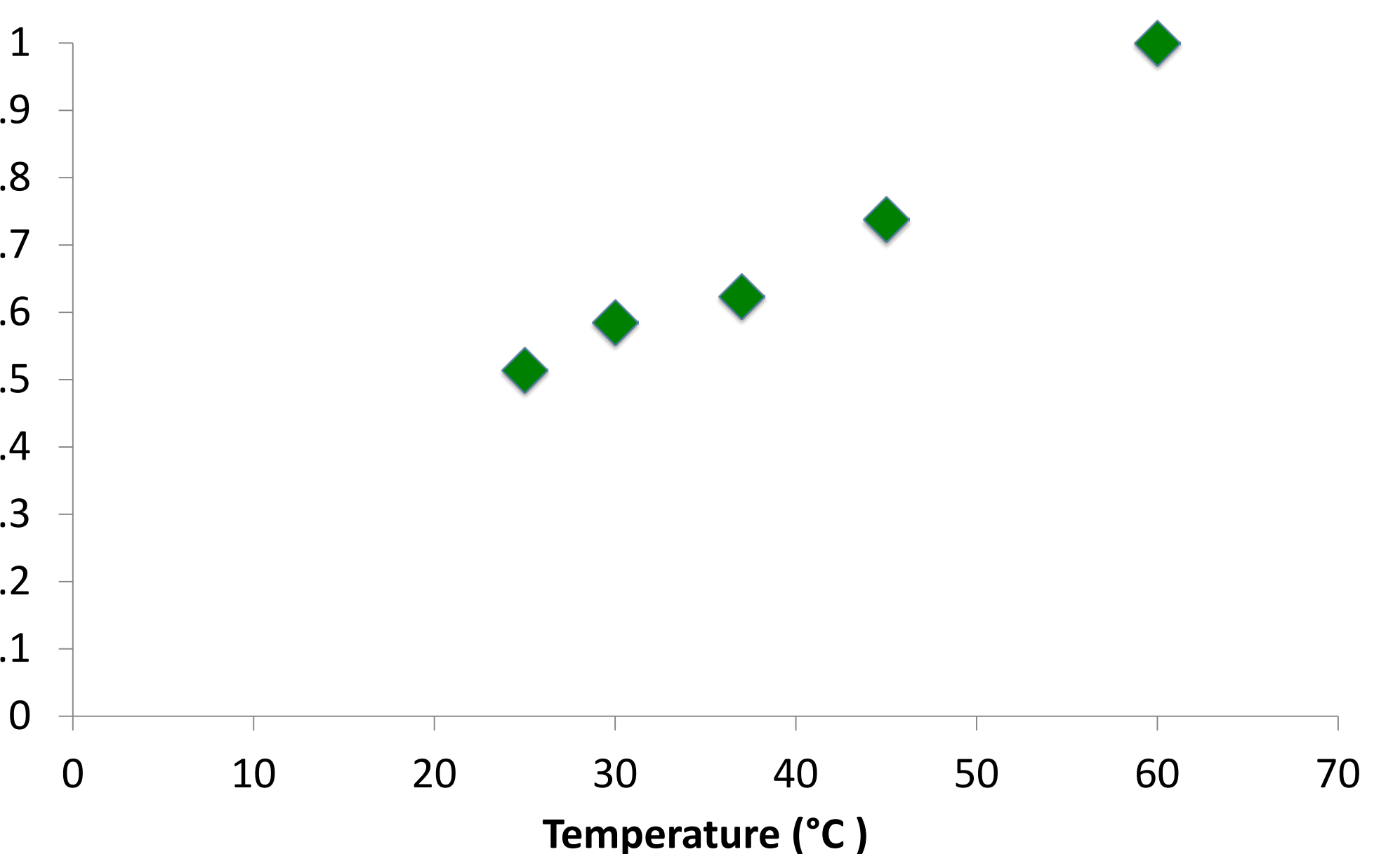


Figure 3. CHY5 temperature assay in 0.2 M Tris-HCl pH 8, 1 mM DTNB buffer (1.57 ug CHY5, 0.14 mM S-HIBYL-CoA). CHY5 is most active at higher temperatures.

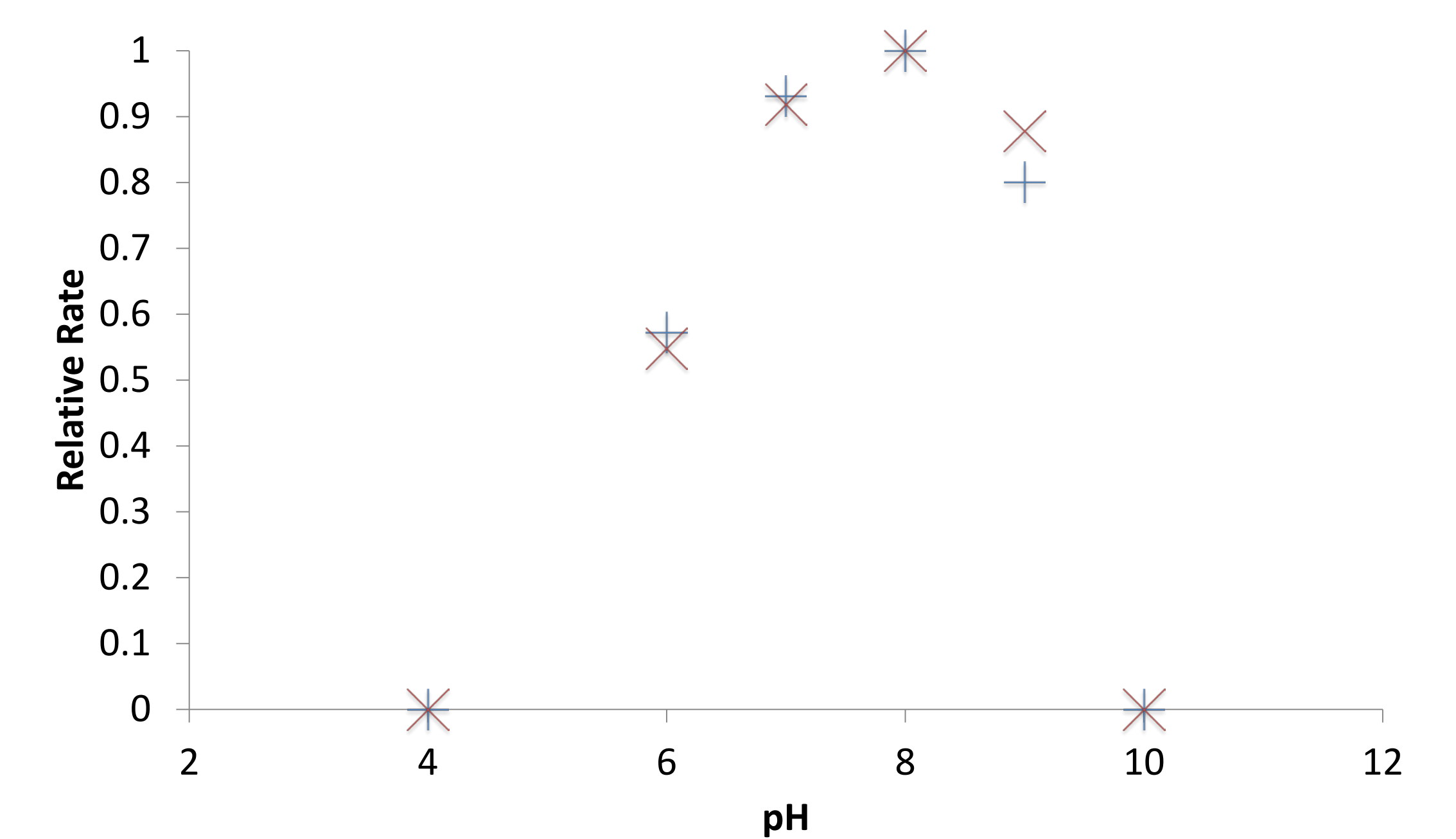


Figure 5. CHY5 pH assay in 0.2M Acetate-Mes-Tris-Glycine, 1 mM DTNB buffer at 37°C (1.57 ug CHY5, 0.14 mM S-HIBYL-CoA). CHY5 is most active at pH 8.

## CHY4

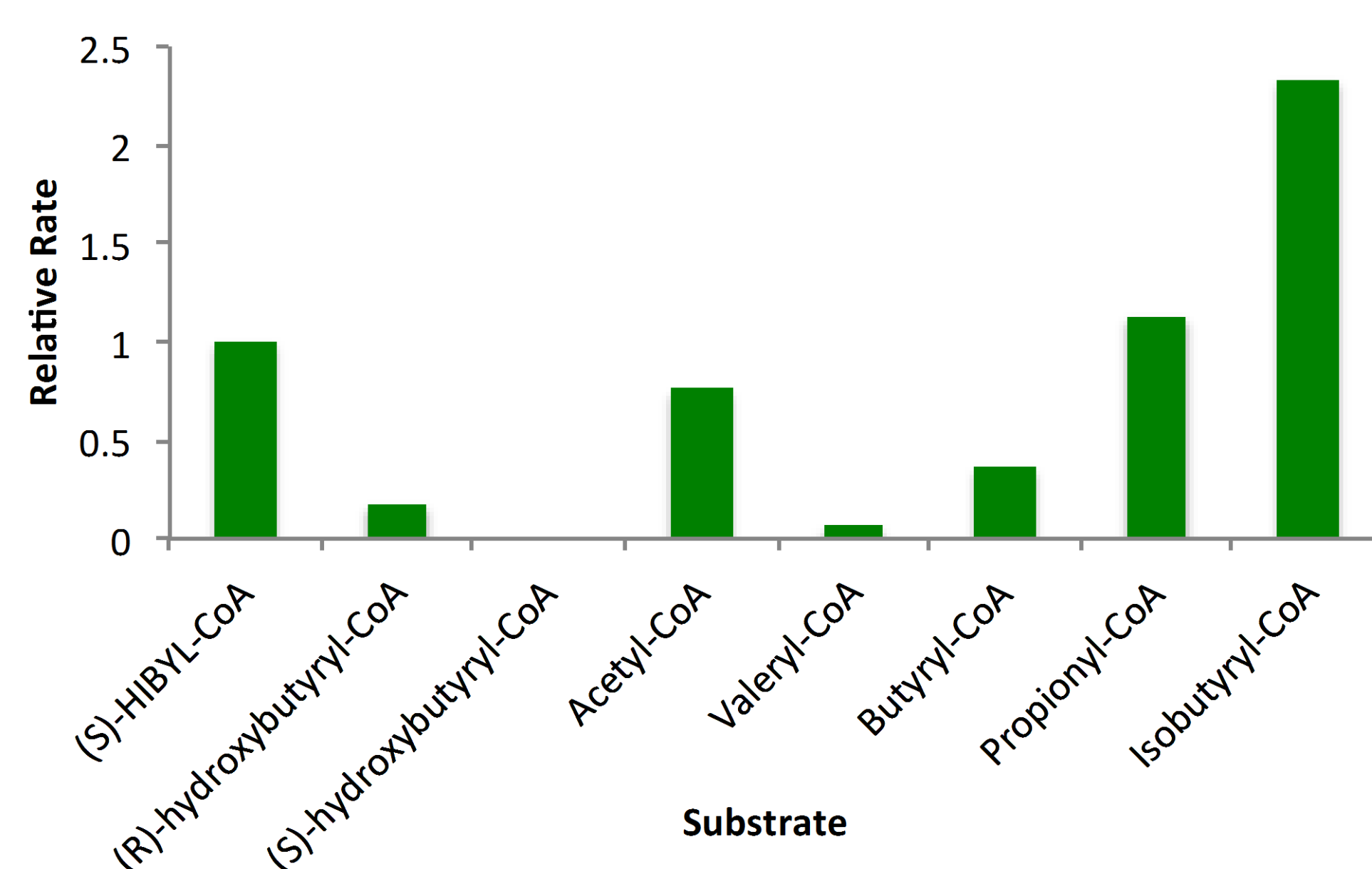


Figure 6. Substrate specificity of CHY4 (3.2 ug) at 37°C in 0.2 M Tris-HCl pH 8, 1 mM DTNB buffer. CHY4 is most active with isobutyryl-CoA.

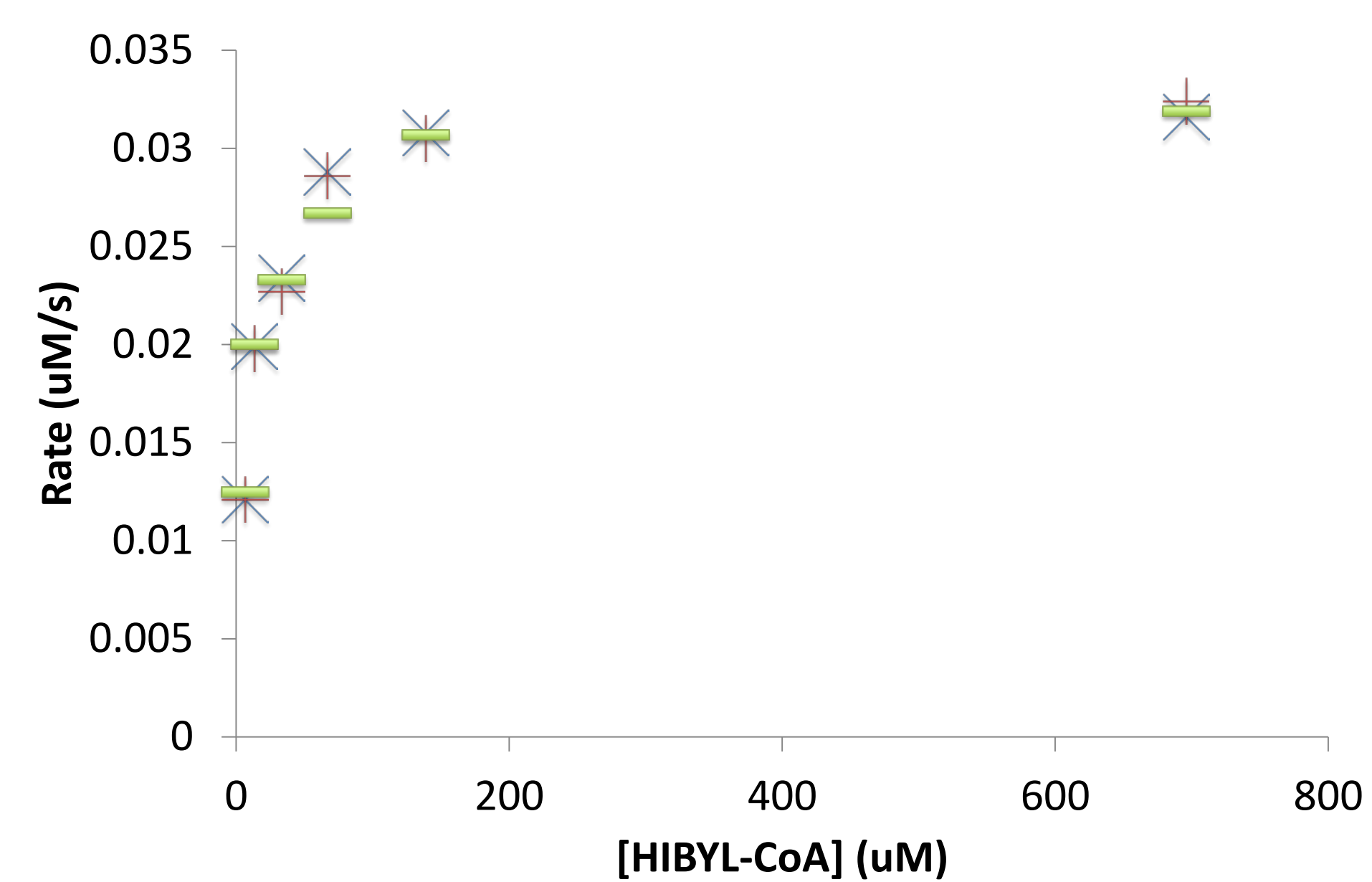


Figure 7. Michaelis-Menten plot of rate vs. concentration of substrate ranging from 13.4-696 uM at 37°C in 0.2 M Tris-HCl pH 8, 1 mM DTNB buffer with 1.57 ug CHY4 ( $K_m: 9.5 \pm 0.8$  uM,  $V_{max}: 3.21 \times 10^{-2} \pm 6 \times 10^{-4}$  uM/s).

## Conclusions

- CHY5 exhibited specificity for the HIBYL-CoA substrate and similar kinetic behavior to CHY1, strongly suggesting that it is in fact a HIBYL-CoA hydrolase that functions optimally at pH 8.
- CHY 4 displayed higher activity with isobutyryl-CoA, propionyl-CoA and acetyl-CoA, suggesting that this enzyme may have broader substrate specificity than other HIBYL-CoA hydrolases.
- Future work will re-investigate enzyme dependence on temperature with a modified assay procedure to overcome limitations of the current assay.

## References and Acknowledgements

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