BASE CAMP

CHARACTERIZING ENZYME AND SUBSTRATE INTERACTIONS

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WHAT ARE ENZYMES, AND HOW DO BIOCHEMISTS STUDY THEM?

- Enzymes are proteins that help speed up chemical reactions
- They work by lowering the activation energy of the reaction, which increases the rate of the reaction.
- The substrate enters the active site of the enzyme, the enzyme then changes shape slightly, and the substrate binds to the active site (enzyme-substrate complex), then the products leave the active site of the enzyme
- **What if the substrate is a peptide? How does that work?**
  - Certain proteases favor one peptide over another
  - The application is based on IMF and characteristics of amino acids/side chains
  - **Michaelis-Menten Kinetics**: The equation describes the initial velocity as a function of substrate concentration
    - V-max: represents the maximum velocity achieved by the system, at maximum (saturating) substrate concentrations
    - Km: corresponds to the concentration of substrate needed to reach half of the maximum reaction rate.
    - Ex. Papain prefers Valine so it will have a lower Km as there is a higher affinity for the substrate and enzyme compared to Papain and Arginine.

STEPS WE TOOK TO STUDY ENZYMES

SYNTHESIZING

Making the peptide with **PS3**.

PURIFYING

Collecting the sample that *should* contain the nucleopeptide with the **HPLC**.

CONFIRMATION

Finding the molecular weight to confirm that the enzyme has been purified using the **MALDI**.

TESTING

Collect data on the fluorescence at different concentrations of our reaction to later identify what enzyme we were given using the **Microplate Reader**.

Instruments Used

Synthesizer

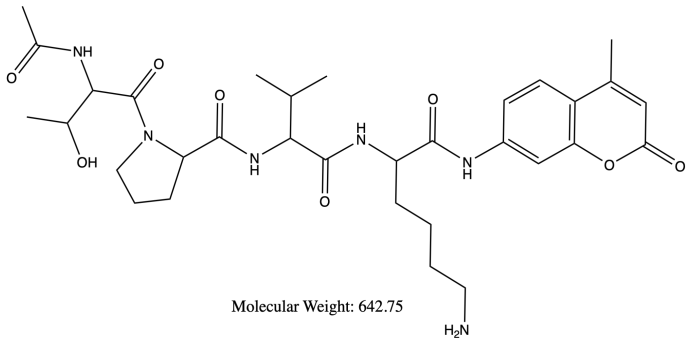
HPLC

MALDI

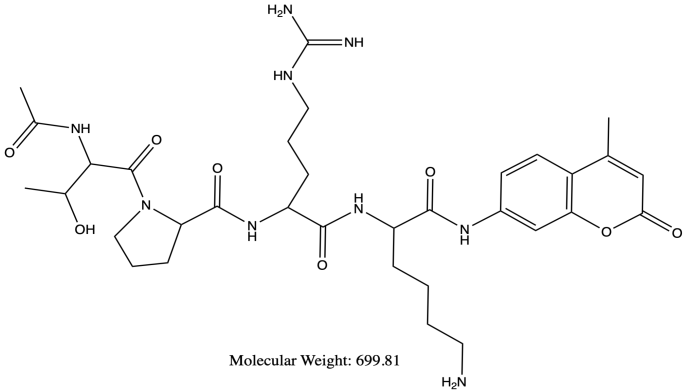
Microplate Reader

Picture of TPVK and TPRK

Our substrates

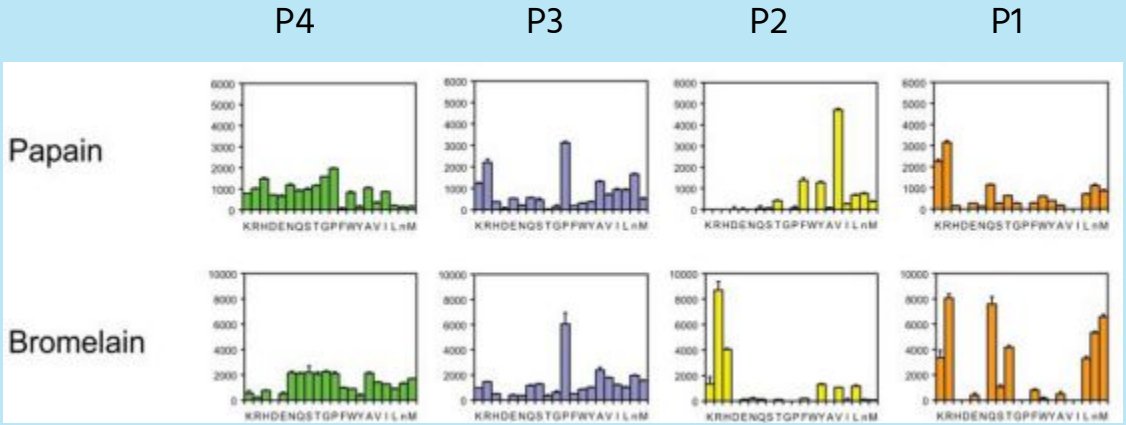


Ac-TPVK-amc



Ac-TPRK-amc

Charts of Bromelain and Papain P2 Sites



Substrate Profiling of Cysteine Proteases Using a Combinatorial Peptide Library Identifies Functionally Unique Specificities

Hypotheses

With the substrate TPRK, bromelain is expected to have a high Vmax and low Km because bromelain prefers the positively charged R over the hydrophobic V in the P2 substrate position.

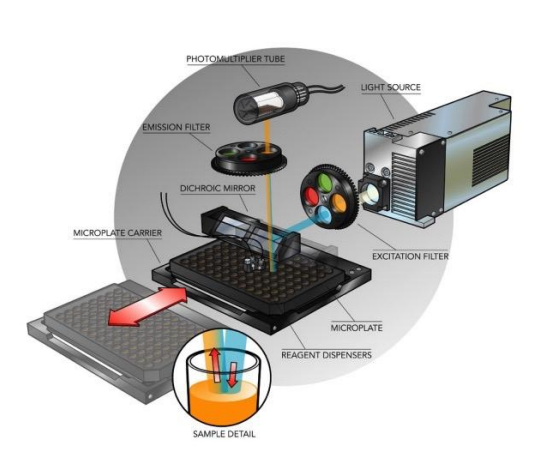
With the substrate TPVK, papain is expected to have a higher Vmax and lower Km compared to the TPRK because papain prefers the hydrophobic V over the positively charged arginine.

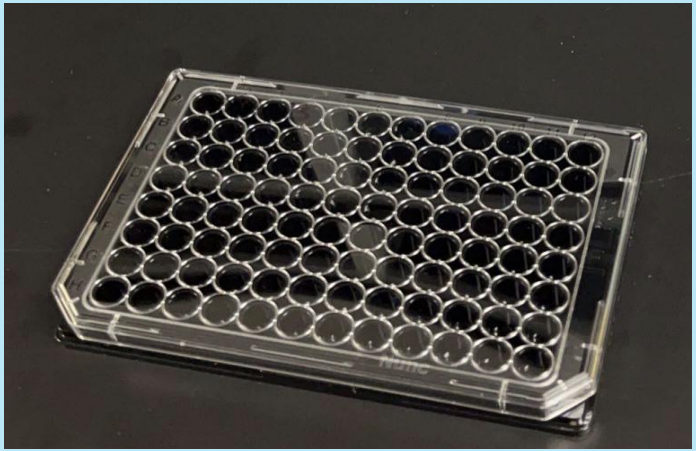
METHODS

- Creating a standard curve (serial dilutions)
- Used equation $C_1V_1=C_2V_2$ to create a range of enzyme concentrations (altering substrate concentration to make **Michaelis-Menten curve***).

METHODS (continued)

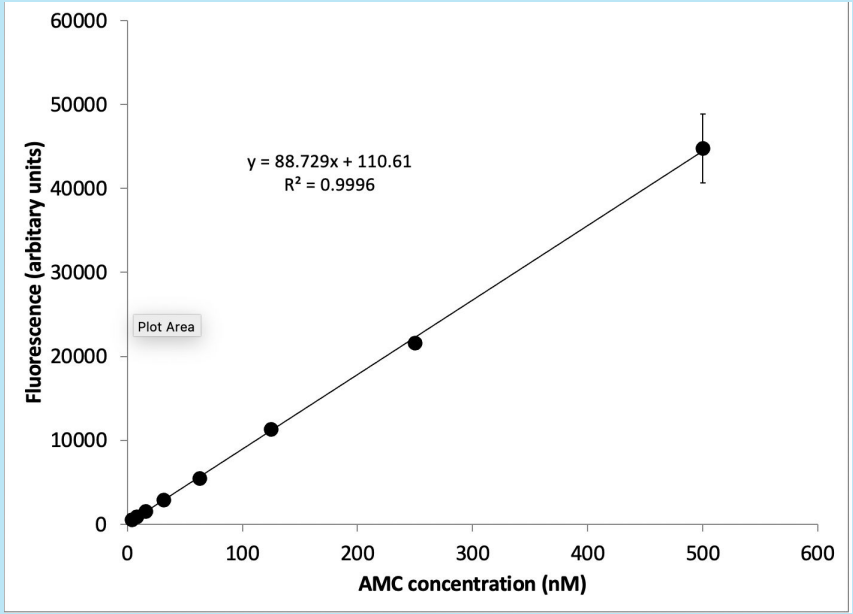
- Solutions A and B were unknown
    - They were either the enzyme Bromelain or Papain.
- Used black absorbance well plates for the Microplate reader.
    - Used 2 plates; One used for Solution A and one used for Solution B. Both had concentrations of TPVK and TPRK with buffer (in separate rows).





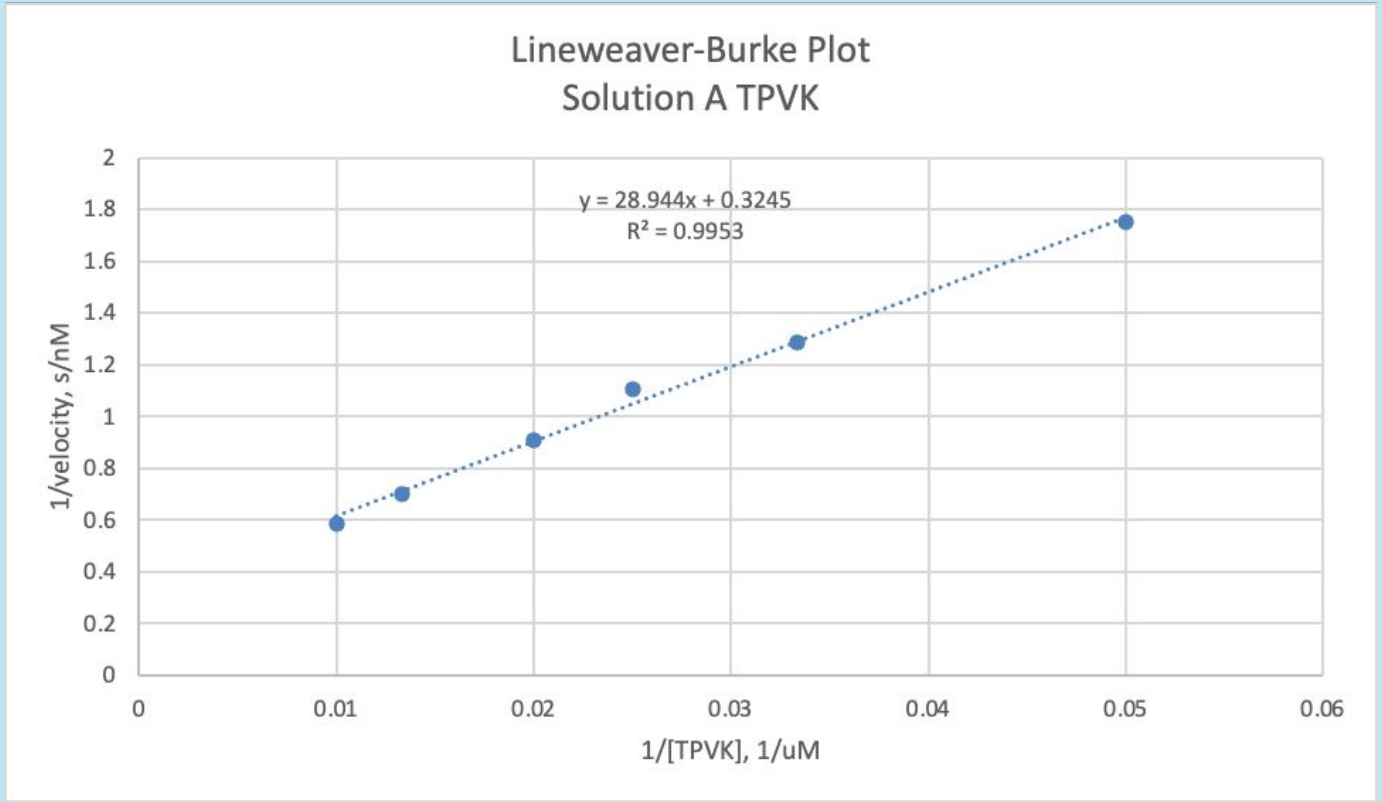
Creating The AMC Standard Curve

- We created serial dilutions of AMC
- We used the fluorescence values on the standard curve to turn the fluorescence values into a concentration.

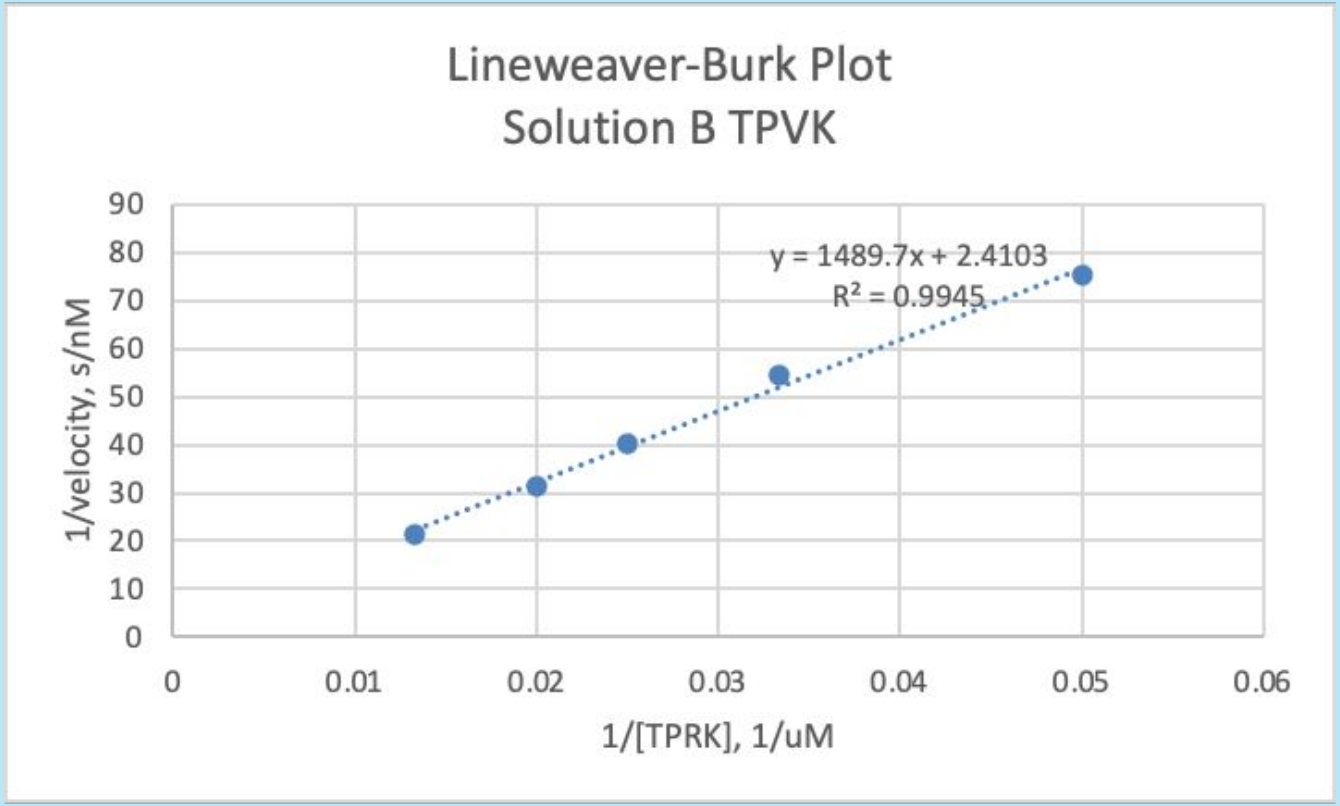


Results!!

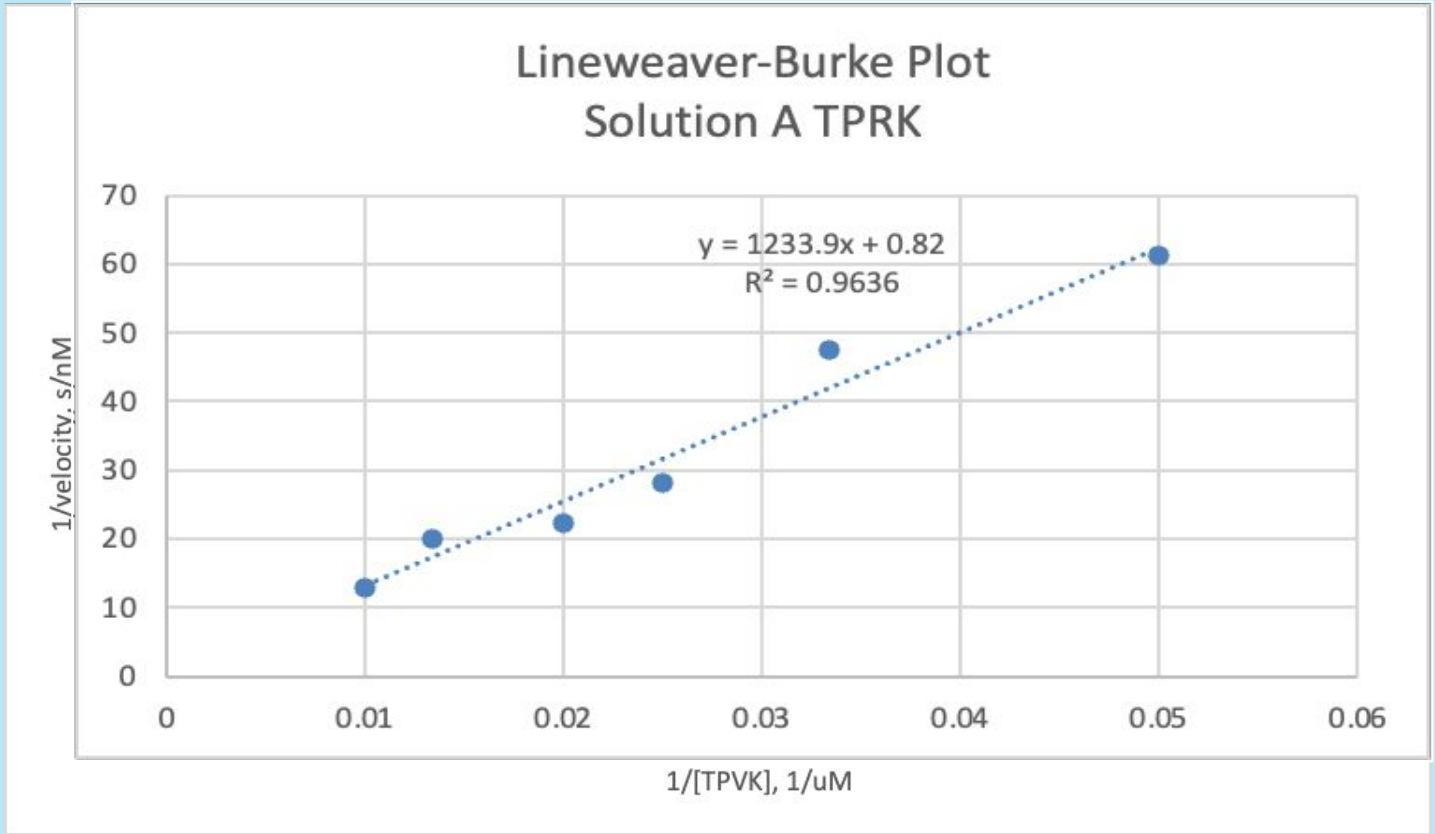
Results and Data from Solution A TPVK



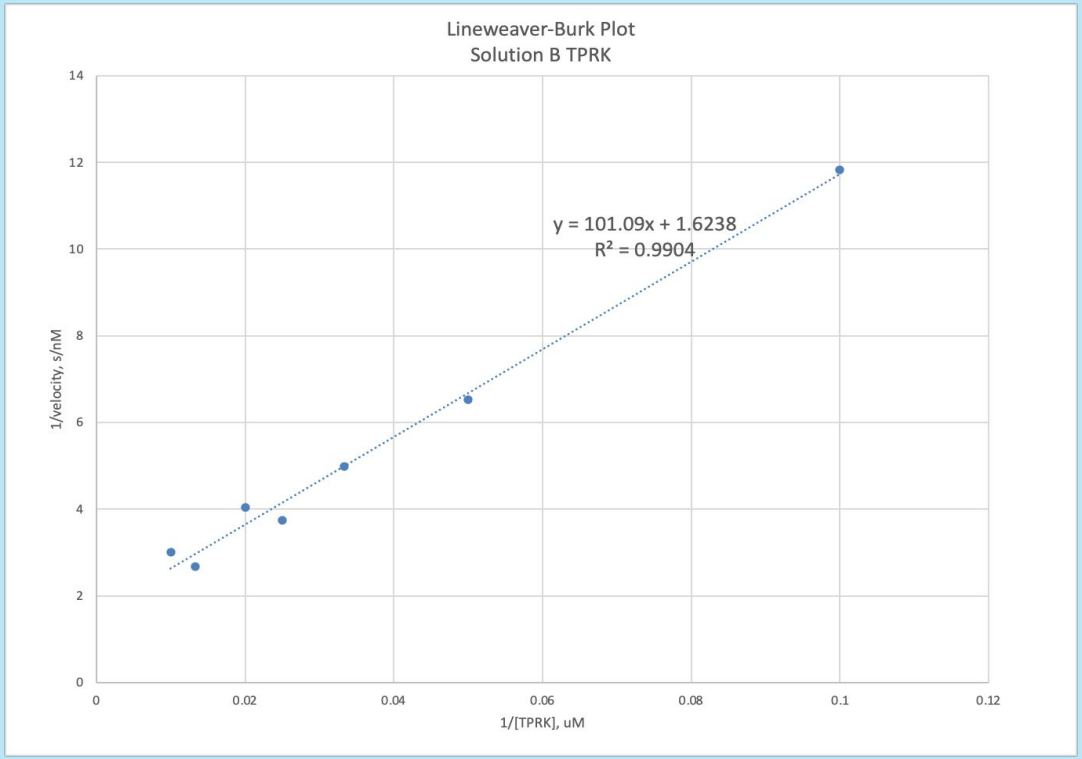
Results and Data from Solution B TPVK



Results and Data from Solution A TPRK



Results and Data from Solution B TPRK



Conclusion:

		Km (nM)	Vmax (nM/s)
Solution A - **Papin**	TPVK	89.35	3.02
	TPRK	1504	1.22
Solution B - **Bromelain**	TPVK	618.06	0.41
	TPRK	62.1	0.62

- We took the slopes for each concentration to apply out Michaelis Menten Analysis to compare the Vmax and Km of each reaction to determine the identity of solution A and B based off their reaction rate.