

Pre-class preparation

Please answer the following questions.

1. What is the rate-limiting step in a typical enzyme reaction described by the Michaelis-Menten equation?
2. Why is it not possible to neglect the concentration of the ES intermediate in Michaelis-Menten kinetics?
3. We derived the equation

$$v_o = \frac{V_{max}[XAOH]}{(K_M + [XAOH])}$$

which is valid at a fixed concentration of $[H_2O_2]$. Please write an analogous equation that holds at constant $[XAOH]$.

4. Given extinction coefficient of 2, 4, 6-trichlorophenol, what is the highest concentration of this substrate that can reasonably be studied? Consider the fact that substrate absorbance at 312 nm should be less 1.5.
5. Britten Chance first presented horseradish peroxidase kinetics in an article published in 1950. He described three forms of HRP. The ferric form of the protein, the resting state of the enzyme, has a brownish color. After adding H_2O_2 alone he observed that the protein solution turned a pale green color. If he added substrate to this form the solution turned red. As the reaction proceeded to completion and all of the H_2O_2 was consumed, the solution became brown again. What are the green and red forms of HRP in terms of the peroxidase reaction scheme.
6. In the experiment conducted in class, we will use a short-time approximation (method of initial rates). That means that we will only measure the change in absorbance of product formation for a few seconds. This has the advantage that the change is linear. Why do we use this approach? Why not fit the entire kinetic trace to a function?