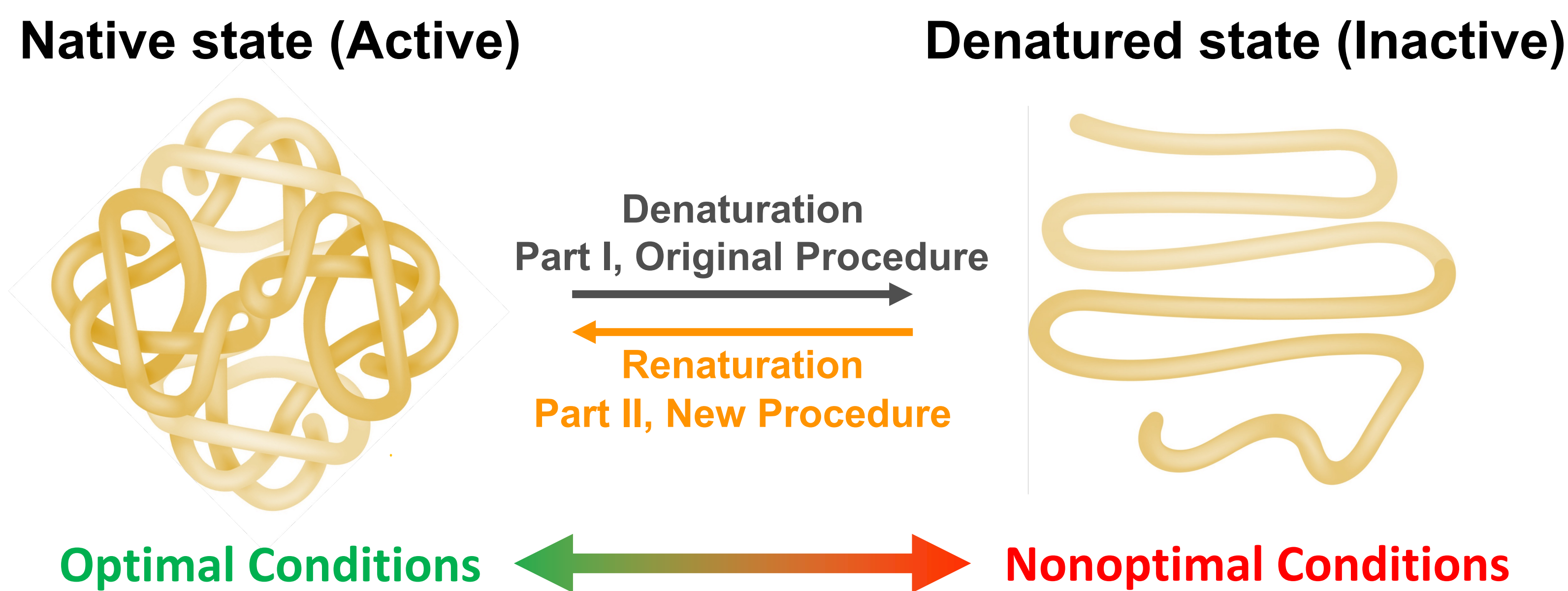


Exploring Enzyme Renaturation In An Introductory Biology Lab Course

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Learning Objectives

Denaturation exercises are often used in introductory-level biology labs to demonstrate the relationship between enzyme structure and activity. However, few of our first year students were grasping the concept that loss of functional form is what causes enzyme activity to decrease when conditions are pushed outside of an enzyme's optimal range.



Turnip peroxidase renatures after return to optimal conditions, reinforcing the conceptual link between form and function. In our updated lab procedure, students manipulate pH to induce both denaturation *and renaturation*, using the initial rate of reaction to determine at what acidity levels the enzyme loses and regains its three-dimensional structure.

Procedure

Step 1. Prepare reaction

Reagents:
Turnip extract (Peroxidase source)
 H_2O_2 (Substrate)
Guaiacol (Colorimetric reagent)

Part I

Combine reagents in pH 5 buffer (Trial 1), pH 7 buffer (Trial 2), and pH 9 buffer (Trial 3).

Proceed to Steps 2 and 3, then repeat entire procedure starting with Part II.

Part II

Combine reagents in pH 9 buffer to denature, then add 100 μ L sodium citrate pH 5 to restore optimal conditions for peroxidase activity (Trial 1). Repeat twice more using student-determined volumes of sodium citrate (Trials 2-3).

Step 2. Record enzyme activity via spectrophotometry

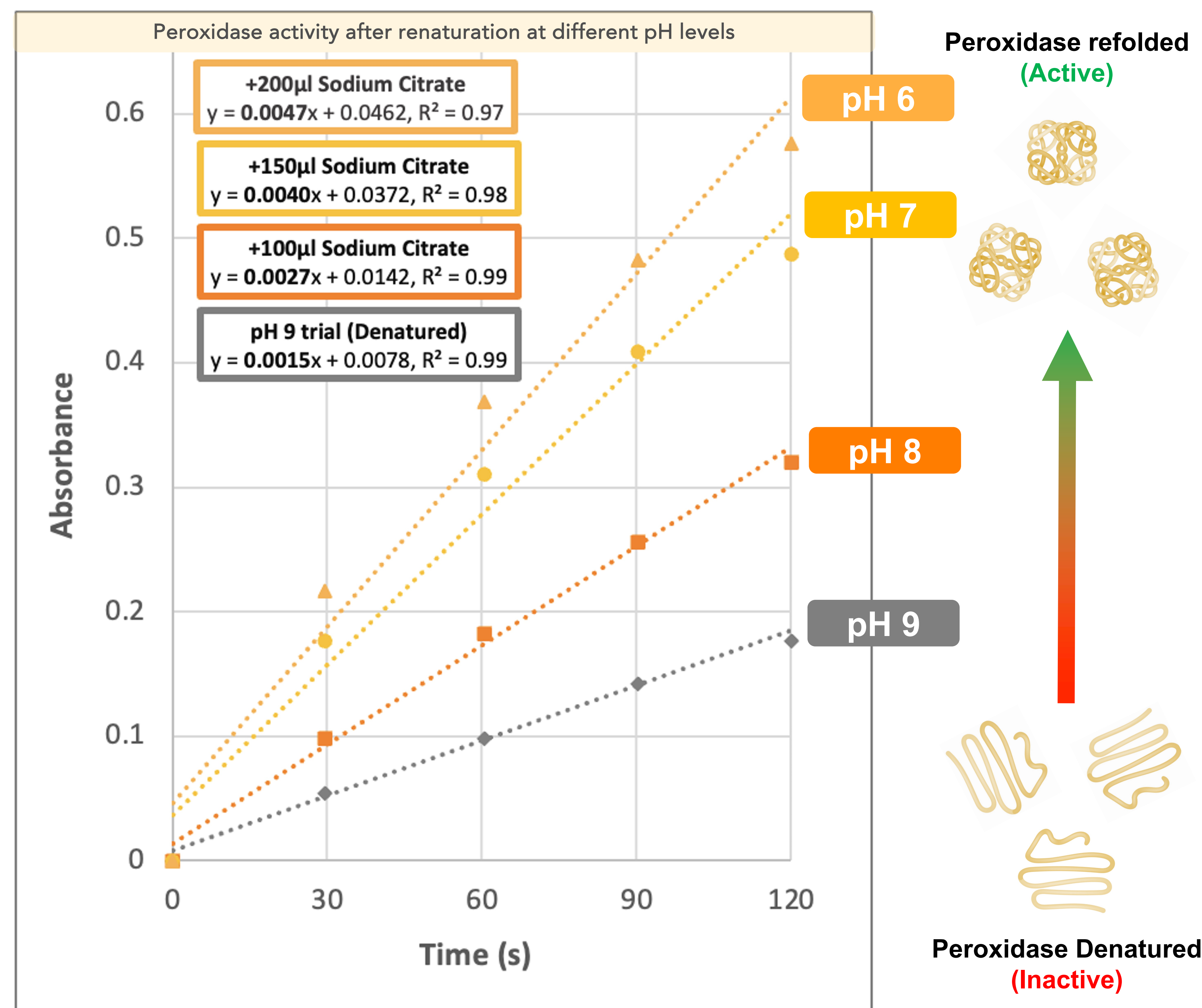
Collect absorbance readings at time 0 and at 30-second intervals for 2 minutes (5 readings total). Then, measure final pH of solution.

Step 3. Find initial rate via linear regression

Slope of absorbance over time for each trial approximates initial enzyme rate, which is approximately linear and consequently easier to grasp for first year students than a more complex model of enzyme kinetics.

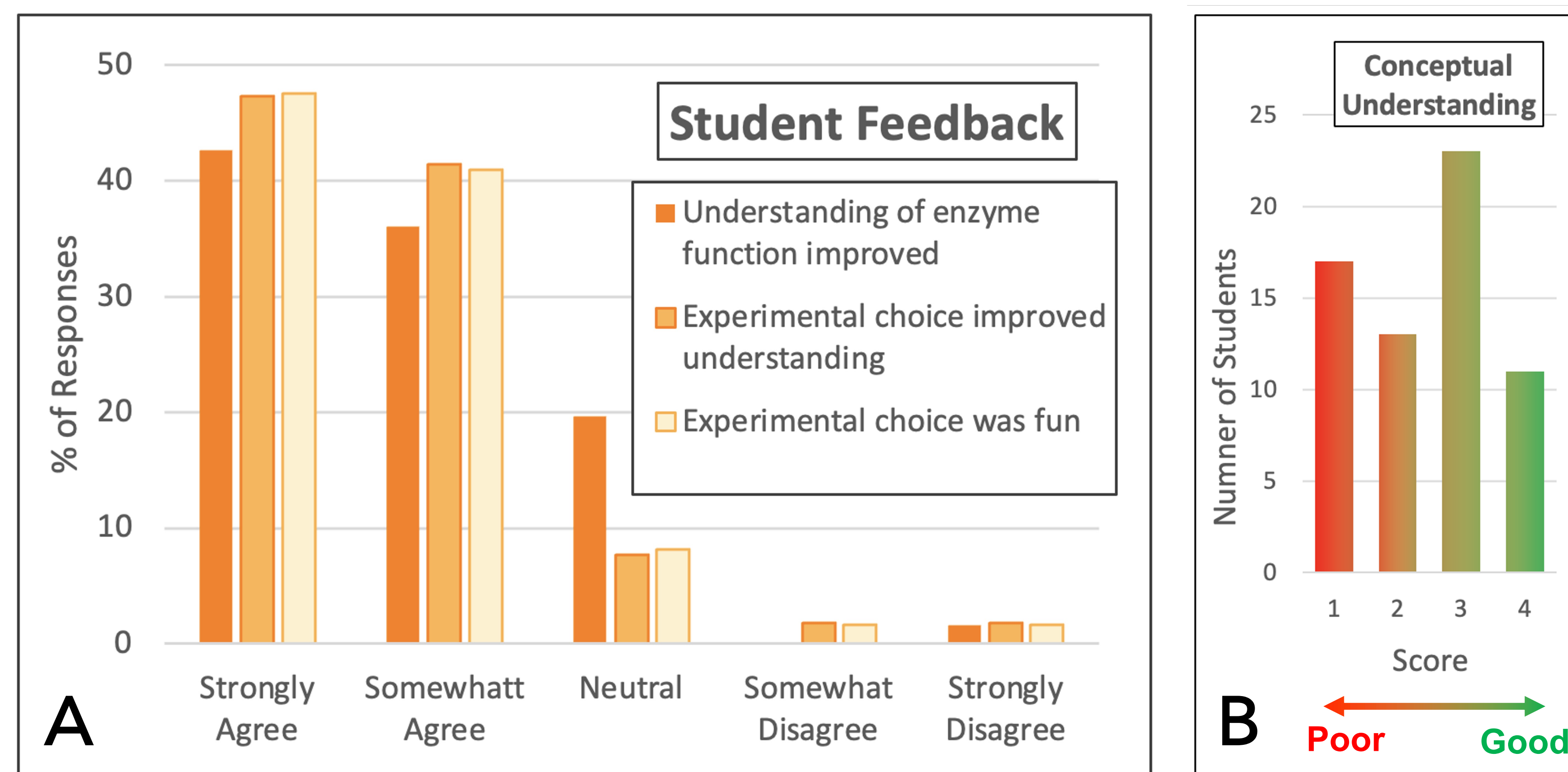
Expected Results: Enzyme will exhibit little to no activity at pH 9 because it is denatured. Activity will increase relative to pH 9 trial as volume of sodium citrate added is increased.

Results



In classroom trials, 20 out of 28 student pairs documented clear patterns of **denaturation and renaturation** (i.e. significant decrease in slope at pH 9 relative to pH 5 and pH 7 in Part I, and significant increase in slope upon addition of sodium citrate in Part II). Slope at each pH varied widely between pairs.

Student Reception and Learning Outcomes



A. Most students responded positively to the updated procedure and enjoyed choosing what volumes of sodium citrate to add in Part II.

B. In a blind assessment of the associated post-lab assignment, juniors and seniors who had taken the class previously reported that 34 of 64 students demonstrated understanding of the structure/function relationship in enzymes.