Product Inhibition: Interpretation of kinetic experiments can be complicated by the fact that the reactions can be reversed. Even if the catalytic conversions of the reverse steps have too high an activation energy to actually proceed, the products, which obviously have some structural resemblance to the reactants could inhibit the enzyme as they could compete with reactants for binding to the enzyme. In contrast to studying enzyme inhibition using varying concentrations of substrate at different fixed concentrations of inhibitor, the concentration of products produced by an enzyme are constantly increasing over the time course of the reaction. This suggests one immediate reason that most kinetic parameters are determined by initial rate methods in which the inhibitor-product has not yet build to a sufficient concentration to alter the rate of conversion of substrate to product. Product inhibition can occur in single substrate reactions as well.

Dead End Inhibition: How do added inhibitors affect the double reciprocal plots of multisubstrate reactions? Let's consider a special case of inhibitors call dead-end inhibitors. These reversible inhibitors bind to a form of the enzyme and inhibit product formation but do not participate in the reaction. It would be represented on a Cleland diagram as a vertical line coming off the the horizontal line which represent different enzymes forms (E, EA, EAB, E'Q, EP, EQ, etc) that lead to product formation. A quick inspection of Cleland diagrams lead to two simple rules that helps in the interpretation of double reciprocal plots in the presence of different fixed and nonsaturating concentrations of dead-end inhibitors (I) in multisubstrate reactions (when one substrate S is varied):

1. Slope changes when:
   • I and S bind to the same form of the enzyme (for example E binds both S and I) OR
   • I binds to a form of E (on the horizontal line) which is connected to the form that S binds, and I binds first (for example, I binds to E and then S binds).

2. Y Intercept changes when:
   • I and S bind to different forms of the enzyme unless I binds first and the binding of I and S are in rapid equilibrium.

The rules predict 1/v vs 1/S plots for simple competitive inhibition (S and I bind to the same enzyme form, E) and uncompetitive (S binds to E followed by binding of I to ES). If the slope changes and the y intercept doesn't, that's competitive inhibition. If the y intercept changes and the slope remains constant, that is uncompetitive inhibition. It works also for mixed inhibition where I binds to E (the same form as S binds to), which changes the slope, AND also binds to EA (a different form of the enzyme than S binds to which is E), which changes the y intercept.

These same rules apply for product inhibition. Consider the rapid equilibrium ordered bibi reaction above when the concentration of the other substrate is around its Kx value:

• Ia (an inhibitor that resembles A) and A both bind to E and EB, so the inhibition is competitive as the slope changes but not the Y intercept
• Ia and B both bind to the same enzyme form (E) so the slope should change, but Ia also binds to EB (to which B can not bind) so the Y intercept would change, which when combined would give either uncompetitive or noncompetitive.

Contributors
• Prof. Henry Jakubowski (College of St. Benedict/St. John's University)