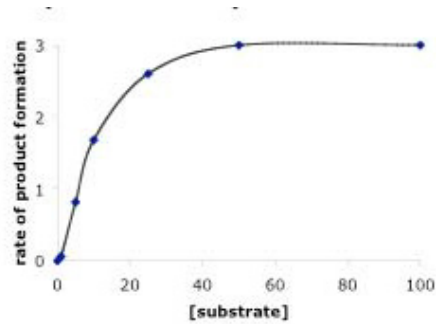


## Introduction

How can one study and understand a protein? Broadly speaking proteins are examined structurally, biochemically and genetically, and over the next few weeks you will explore these complementary approaches for one enzyme, beta-galactosidase. As you'll see, the three approaches can be intelligently combined to understand the activity and interactions of proteins at the molecular level as well as to rationally change the enzyme to change its properties.

For the visual folks among us, perhaps the most informative approach to understanding proteins is to elucidate their three-dimensional structures. Structures can be derived from cryoEM, NMR or X-ray diffraction patterns of the purified protein (more on protein purification later). The protein's shape may suggest how other molecules can or can't interact. For example, does the structure have a narrow cleft that would restrict interactions with molecules above a certain size? Structures also reveal exquisite detail. Is a particular amino acid exposed on the surface of the protein or is it buried deep in a hydrophobic core? Does it interact with a metal or water molecule? Is the active site a shallow or deep crevice of the protein? Are there rigid portions of the structure and more flexible areas? Critically important is the idea that structural information may lead to predictions for the protein's mechanism of action, and to testable hypotheses that examine these predictions. In lab you will have the chance to look at several proteins using a program called "[Protein Explorer](#)", examining the three-dimensional structures from all angles and identifying the location of particular amino acids. This will help you generate some predictions for how their modification could affect protein activity.

A second informative approach to understanding protein activity is the biochemical one. In such experiments, the protein in question is isolated from other proteins and then tested with other purified proteins or molecules under controlled conditions. This approach is particularly useful when studying enzymes, since the amount of product formed from an enzyme-catalyzed reaction can be measured and then compared to other enzymes or the same enzyme under different conditions. As you'd predict, an enzyme's efficiency depends on the experimental set-up. It's possible to reduce a reaction's efficiency by decreasing temperature since this lessens thermal movement, leading to fewer productive collisions between enzyme and substrate. Another way to reduce the amount of product formed is to decrease substrate concentration, making it harder for the enzyme and substrate to find one another in solution. Clearly reaction conditions must be specifically and clearly defined if enzymes are to be compared. When substrate for an enzymatic reaction is limiting, addition of more substrate will increase the amount of product formed until the substrate saturates the enzyme. After that point, further addition of substrate does not increase the amount of product that is formed in a given time since all the enzymes are fully occupied with substrate. This reaction pattern is a hallmark of enzyme-catalyzed reactions that was first mathematically characterized by Maud Menten and Leonor Michaelis (pictured below) and then further refined by Hans Lineweaver and Dean Burk. You will be performing such an analysis of purified beta-galactosidase in lab, measuring the protein's concentration and its enzymatic activity.



"Image of Maud Menten removed due to copyright restrictions."

"Image of Leonor Michaelis removed due to copyright restrictions."

A third powerful approach to understanding a protein is to genetically modify it. Indeed, genetic changes that affected beta-galactosidase activity played a seminal role in Francois Jacob and Jacques Monod's operon model for regulated gene expression. Changing the DNA that encodes a protein can help us understand the protein's role in a cell. Even knowing that a particular change will kill the cell tells you it's an important protein for the cell (or organism's) normal life. Genetic changes that affect protein activity can be less dramatic and can lead to molecular models for the mechanism of action of the normal and mutated protein. Given that mis-expression and mutations in proteins underlie many health issues, such an understanding is of more than academic interest. Recall that in the first experimental module, you genetically-modified a protein-encoding gene (GFP) by changing the DNA that encoded it. In this module you will modify a protein (beta-galactosidase) at the level of translation, by introducing a non-canonical amino acid. How this change affects the enzyme's expression and activity is something you will try to predict based on structural studies and then something you will measure through biochemical techniques.

Today's lab has several parts: First, you will become familiar with beta-galactosidase using the Protein Explorer program to examine the enzyme's three dimensional structure, considering where un-natural amino acids could be introduced and what the effects might be. You will also learn how to measure a protein's concentration using a "BioRad" assay, and determine its enzymatic activity using a modified "Miller" assay (also generically called a beta-gal assay). Before you leave lab you will induce expression of the modified enzyme so you can study it next time.