Human prolyl endopeptidase (hPEP) has several functions in the brain, one of which is the degradation of neuropeptides. This is significant for diseases like Alzheimer's, which is known to be caused (at least partially) by neural plaques such as amyloid-β; hPEP plays a role in generating the sticky amyloid plaques as part of its protease function. hPEP is purported to be inhibited by peptides smaller than 30 amino acids. Due to the structural similarity between hPEP and a natural glutenase, spPEP (Sunn pest prolyl endopeptidase), we hypothesize that hPEP could have a second mechanism of binding similar to that of spPEP which binds larger peptides. We are using spPEP as a control due to its 99% structural identity based on modeling and its ability to bind larger substrates, specifically gluten proteins, up to several hundred amino acids in length. This second mechanism occurs in the active site between the two lobes of the spPEP enzyme. Using peptides of varying length, we are investigating if hPEP can bind larger substrates in the same way as spPEP.

A peptide found in αS1-casein with a size of 18 amino acids is a known inhibitor of the hPEP; this inhibitor sequence is being used in αS1-casein peptides of varying length; specifically, we are using the 18-mer, 28-mer, and a 38-mer. The focus of this study is to optimize the purification process of the isolated recombinant peptides using Casein peptide 9, a 68-mer, expressed as a fusion to SUMO protein in *E. coli.* The 68-mer was chosen due to its large size making it easier to visualize upon purification. Presumably, the smaller recombinant peptides will express and purify using the same protocol developed in this study. The recombinant hPEP is also being expressed and purified during this project, which will be used with the inhibitors to investigate its kinetics to see if it does bind larger peptides than 30 amino acids in length.