FIGURES

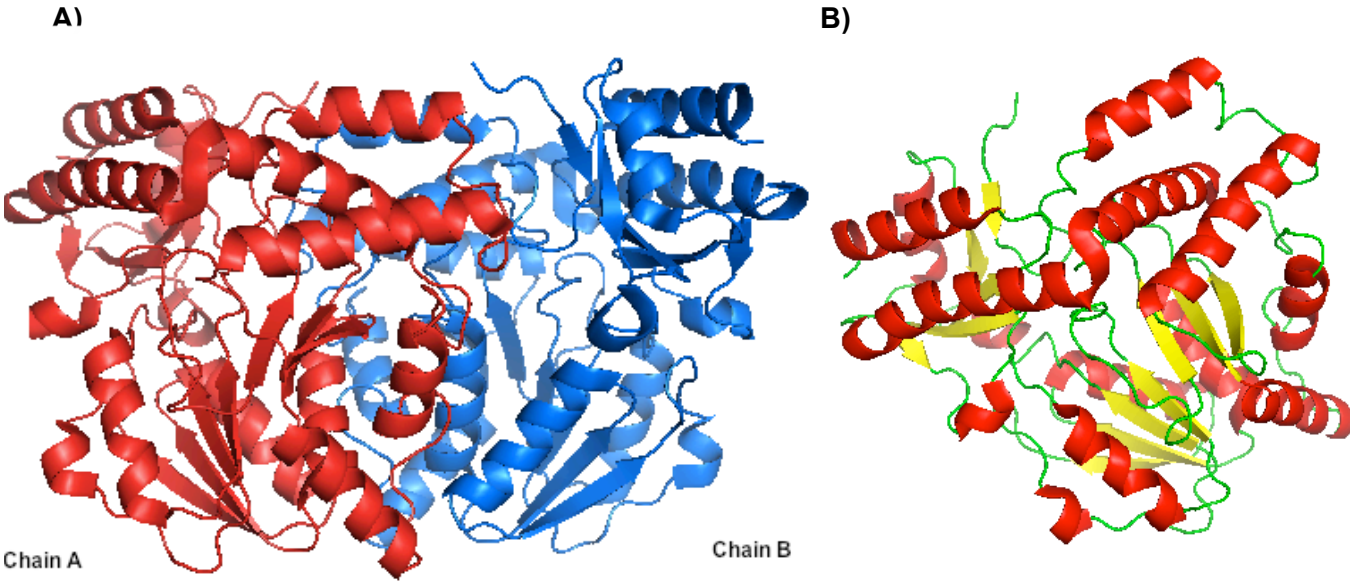


Figure 1: Overall Structure and Secondary Structure of ChPSAT. A) *Assumed Biological Assembly of ChPSAT.* The biological homodimeric state of ChPSAT was constructed in PyMol from the crystallized asymmetric subunit chain A, represented in red. The identical chain B is depicted in blue. **B)** *Secondary Structure of ChPSAT Asymmetric Unit.* Alpha helices are depicted in red, beta sheets in yellow, and loops in green. The seven-stranded parallel beta sheet characteristic of the α-family of aminotransferases is visible towards the center of the molecule.

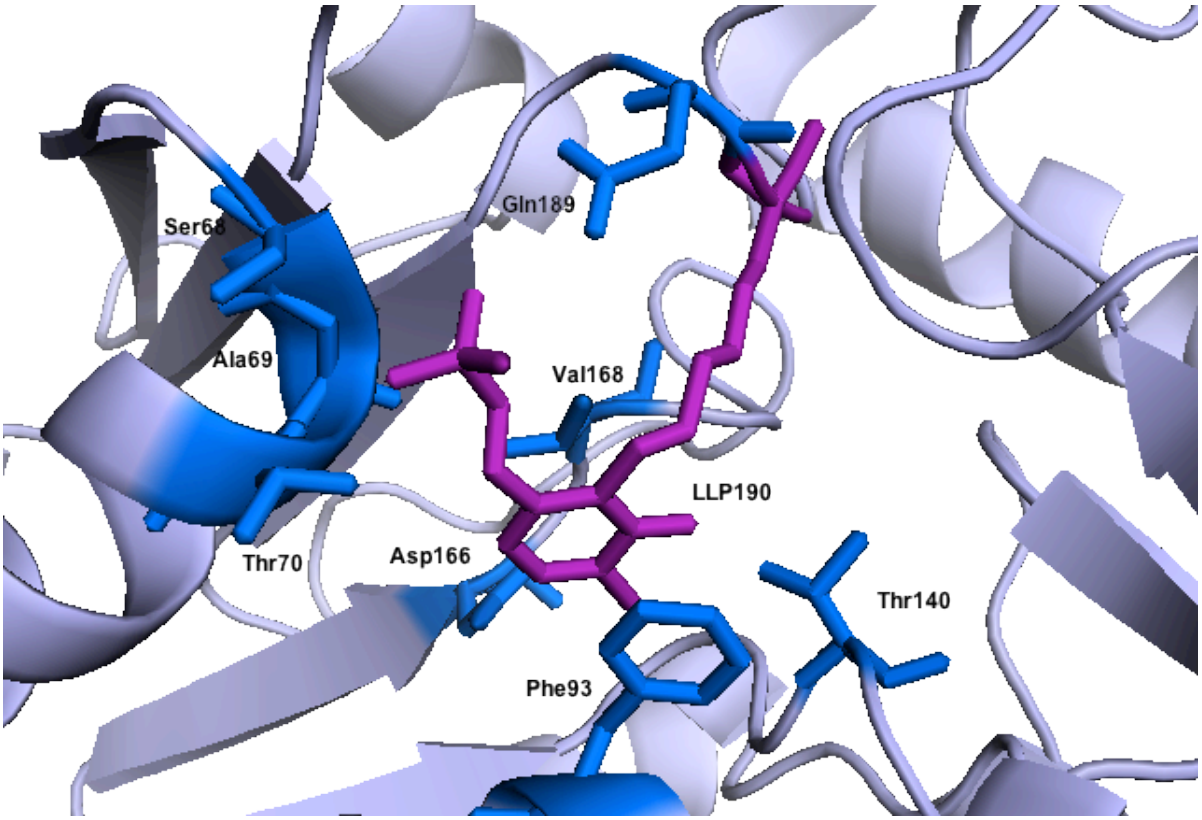
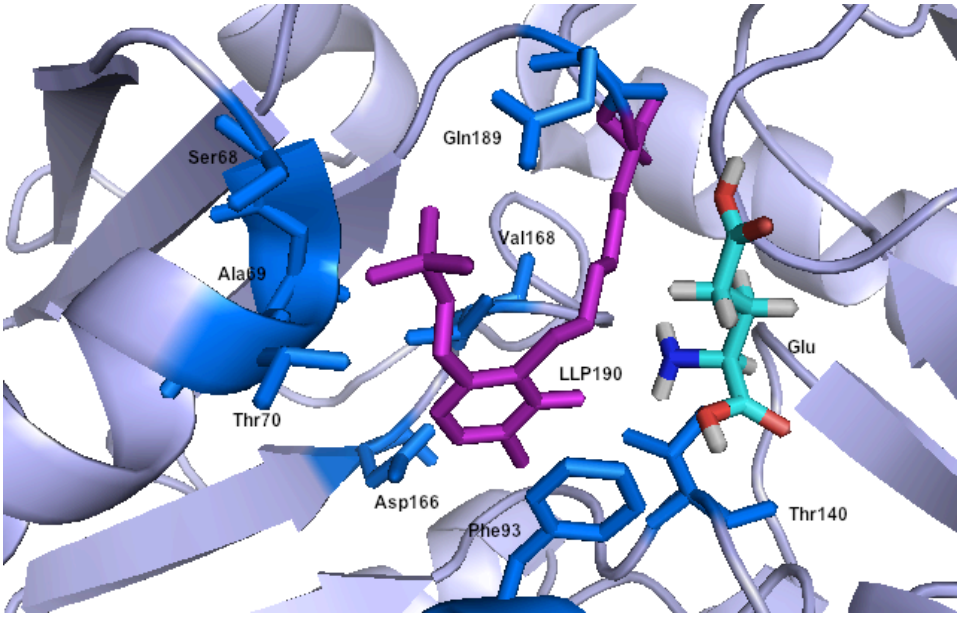


Figure 2: Active Site Representation of ChPSAT. Active site residues were determined based on sequence alignment with enzymes of the AAT_I superfamily using the NCBI database. ChPSAT was crystallized with LLP190 in the active site. 2-Lysine (3-hydroxy-2-methyl-5-phosphonooxymethyl-pyridin-4-ylmethane), or LLP, is the Schiff base intermediate formed when the epsilon amine of catalytic lysine 190 attacks the carbonyl carbon of pyridoxal-5'-phosphate. The orientation of phenylalanine 93 in the active site indicates favorable π-stacking interactions with the LLP190 carbon ring.

A)



B)

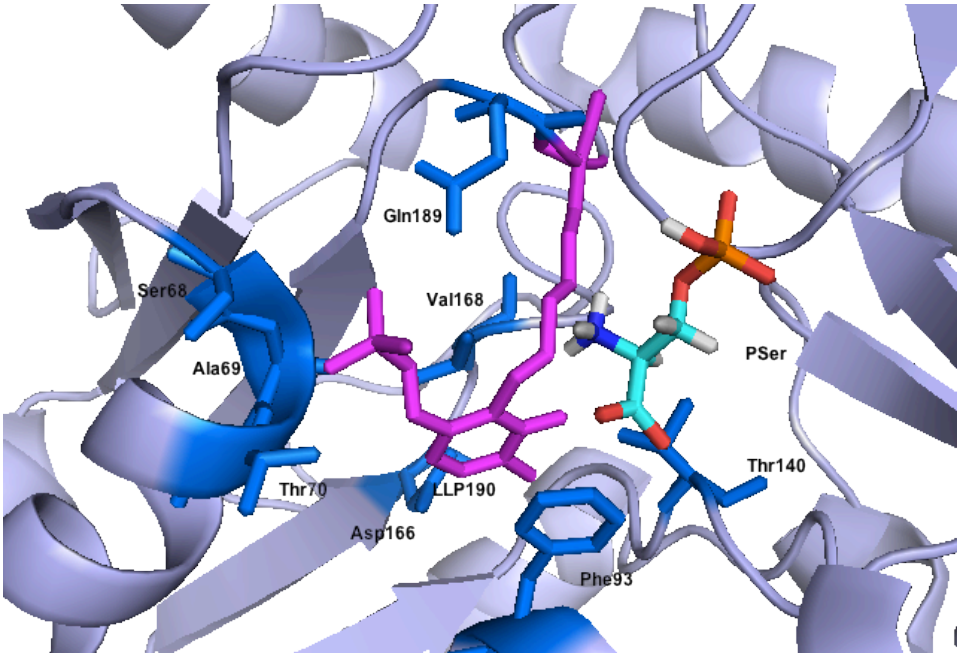


Figure 3: Patch Dock Solutions for ChPSAT Active Site. Distance constraints between the amino group of the substrate and the carbonyl carbon in the aldehyde group from pyridoxal-5'-phosphate in LLP were used to narrow down possible solutions and ensure that the ligand was in the vicinity of the active site. LLP190 is shown in magenta and the ligands are colored by atom type, with nitrogen in blue, oxygen in red, phosphorous in orange, carbon in cyan and hydrogen atoms in white. **A)** *Docking with Glutamate*. Solution 1 was chosen based on its highest score for geometric shape complementarity, 2020, and an interface area of 228.40. **B)** *Docking with Phosphoserine.* Solution 1 was chosen, which had the higher score, 2188, out of the two possible solutions and an area of 273.40.

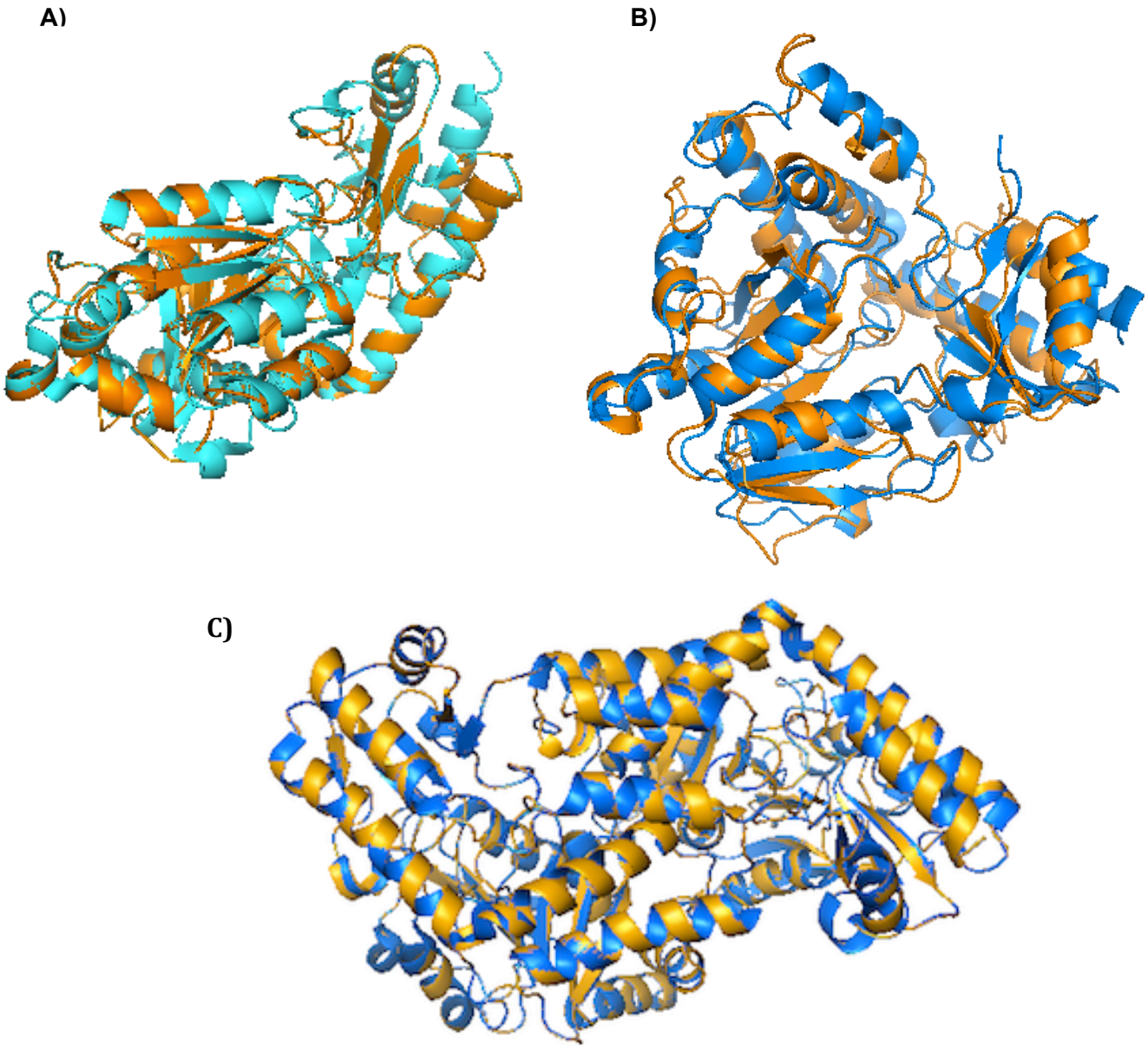


Figure 4: Homology Modeling with ChPSAT. Homologs with high and low sequence similarity to ChPSAT were identified using NCBI BLAST. Sequences were entered into Swiss-Model and the proposed structure models were superimposed in PyMol. **A)** *Low Homology Template: PSAT from Microscilla marina with Template 2kbw.* The low homology protein phosphoserine aminotransferase from *Microscilla marina* had a nearly identical structure to its template alanine: glyoxylate aminotransferase from yeast (pdb ID = 2kbw) **B)** *Low Homology Model: Microscilla marina with ChPSAT.* The low homology protein shares much of the same structural features as ChPSAT, but is not fully aligned. The QMEAN Z-Score was -4.97 indicating a model of poor quality; sequence identity was 20.68% and E-value was 4.20e-45. **C)** *High Homology Model: PSAT from Cytophaga aurantiaca with ChPSAT*. The phosphoserine aminotransferase from *Cytophaga aurantiaca* was nearly identical when superimposed with its template, ChPSAT. The QMEAN Z-Score was -0.33; E-value was 0.00 with 94.46% sequence identity.

Table 1: Semester Timeline

Week		
1	Prepare protein for kinetics assays	Prepare media and buffers
2		Protein expression
3		Protein purification and dialysis
4		Protein quantification & optimization of enzyme concentration for kinetics assays
5	Kinetics Assays	Determination of kinetic parameters
6		
7		pH dependence of kinetic parameters
8		
9		Temperature dependence of kinetic parameters
10		
11		Final assays
12		