

The Protein Structure Initiative (PSI) Centers have determined over 3,500 protein structures. Greater than 1,600 have unknown functions and many more, although annotated with bioinformatics approaches, have not been functionally characterized. For instance, TM0312 is annotated as an oxidoreductase; however, this is a large class of enzymes involved in many different cellular pathways. As a result, functional annotation provides little insight into the role the enzyme plays in the cell.

TM Number	PDB ID	Putative function
TM0312	1zh8	oxidoreductase
TM0423	1kq3	glycerol dehydrogenase
TM0436	1vj0	alcohol dehydrogenase, zinc containing
TM0920	1o2d	alcohol dehydrogenase, iron containing
TM1097	1vlv	ornithine carbamoyltransferase
TM1131	1vp4	aminotransferase, putative
TM1255	1o4s	aspartate aminotransferase
TM1478	1o0x	methionine aminopeptidase
TM1698	2qb3	aspartate aminotransferase

In an effort to determine the function of proteins which have not been previously investigated, the University of Virginia Chemistry undergraduates with a Biochemistry specialization teamed up with the Joint Center for Structural Genomics (JCSG). Table 1 lists the nine proteins that the undergraduates investigated in Spring 2009. The students researched and designed an enzymatic assay that was best suited to investigate the enzyme that was assigned to them.

The following is a summary of the results from one 10 week semester.

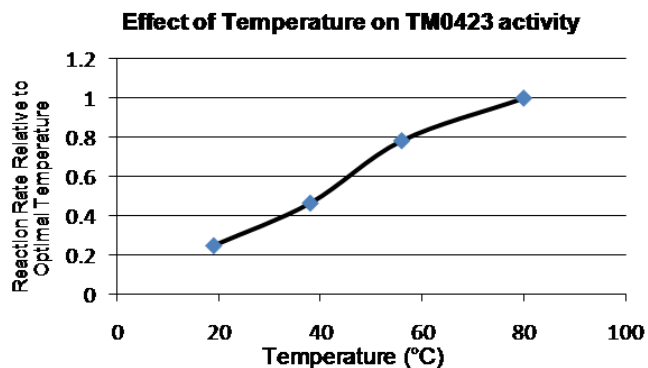


Figure 1. Temperature dependence of the glycerol dehydrogenase activity observed for TM0423

Activity increased with an increase in temperature and over the range investigated maximal activity was at the growth temperature of *T. maritima* (80°C).

The activity of **TM1097** was confirmed to be an ornithine carbamoyltransferase which catalyzes the transfer of the carbamoyl group of the carbamoyl phosphate metabolite to the 5-amino group of L-ornithine to produce L-citrulline and inorganic

Based on structural similarity to other enzymes, **TM0423** is predicted to be a glycerol dehydrogenase (GDH) that catalyzes the oxidation of glycerol into glycerone with simultaneous reduction of NAD^+ to NADH . In addition to its glycerol substrate and NAD^+ cofactor, it is presumed that the enzyme is dependent on zinc. GDH allows most thermophiles to utilize glycerol as a carbon source under anaerobic conditions. The activity (at 25°C and pH 8) was confirmed with a K_M of 19 mM for glycerol. In addition, the temperature dependence of activity was

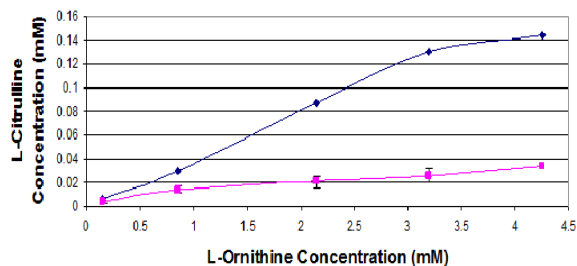


Figure 2. Ornithine carbamoyltransferase activity at 37°C (blue) and 55°C (pink).

phosphate. Figure 2 shows the production of citrulline with varying amounts of ornithine using a fixed point assay. The temperature dependence of the activity was attempted; however, a drop in activity with an increase in temperature was observed and more experiments are needed to investigate the substrate stability, in particular carbamoyl phosphate, at higher temperatures.

TM0312, TM0436, and TM0920 were the most difficult to characterize due to the large number of highly variable substrates. The most successful result was for **TM0920** for which alcohol dehydrogenase (ADH) activity was observed for substrates 2-propanol and 1,3-propanediol. Activity was NADP(H) specific, a characteristic of Fe-containing ADHs in other hyperthermophiles. The students identified a side reaction due to the oxidation of iron (involved in catalysis) that interfered with the spectrophotometric enzymatic assay and were able to design their blank appropriately. A K_M of 31 mM and 23 mM and k_{cat} of 23 min^{-1} and 30 min^{-1} were determined for 2-propanol and 1,3-propanediol, respectively. These values are relatively high compared to other ADH enzymes and the experiments should be carried out in anaerobic conditions to minimize inactivation due to oxidation of the iron, which has been observed previously.

Investigations of **TM1478** aminopeptidase activity were difficult due to the solubility and stability of the substrate L-methioninet-*p*-nitroanilide (L-Met-*p*-NA). Although activity could not be quantitatively determined, the activity was confirmed and a Cu^{2+} metal cofactor was determined to be essential (not Co^{2+} or Fe^{2+}) No metal cofactors were observed in the crystal structure; however, most characterized methionyl aminopeptidases are known to utilize Co^{2+} and possibly Mg^{2+} (and in some cases are inhibited by Cu^{2+}). In addition, activity was observed for L-Leu-*p*-NA indicating that there may be broad specificity of the enzyme.

The structure of **TM1255** was determined by the JCSG at 1.90 Å resolution. Two covalently bound pyridoxal 5'-phosphate (PLP) molecules (believed to be a critical cofactor to the activity of the enzyme) were observed in the crystal structure. AspATs catalyze reversible transamination reactions for the degradation of most amino acids, converting L-aspartate and 2-oxoglutarate to oxaloacetate and L-glutamate by a ping-pong bi-bi mechanism. Of the three putative aspartate aminotransferases investigated only **TM1255** was confirmed to have activity. Figure 3 shows the HW plots for both substrates aspartate and oxoglutarate. The K_M

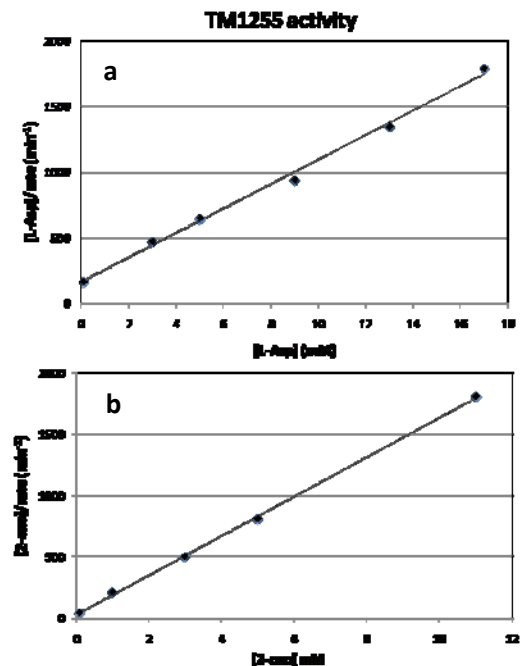


Figure 3. Hanes-Wolfe plots for both TM1255 substrates, L-aspartate (a) and 2-oxoglutarate (b).

was determined to be 0.3 mM and 0.16 mM and k_{cat} 8.3 s⁻¹ and 0.1 s⁻¹, respectively. These data with error analysis are currently being prepared for a “For the Record” in Protein Science.

Future directions

Based on the results and students’ experiences during the past Spring semester, there are a few changes that will be made to improve the course. The students were limited in material. The UVA Chemistry Department and the College of Arts and Sciences Dean’s office have provided additional funds for equipment and materials. Scientifically, many of the students could not investigate temperature dependence of their enzymatic activity because the colorimetric assay used a coupled enzyme, which was not stable at high temperatures. Therefore, we are preparing and characterizing several common enzymes used in these coupled assays (e.g., lactate and malate dehydrogenase).

The results of the first 10 week semester dedicated to this endeavor demonstrate that publishable results can be obtained by undergraduate teams working in an upper division classroom under the direction of UVA research professors. We are continuing this program and will run this undergraduate classroom laboratory each Spring. In addition, we have hired a teaching/research postdoctoral fellow that will work with undergraduates throughout the year to investigate PSI proteins using methods that extend beyond assays used in the classroom.