Like protein 1, an unweighted webserver consensus approach was used to record the total number of appearances of residues listed as functional sites. The webservers ConSurf, POOL, I-TASSER, PredictProtein (which uses Prosite for its functional site prediction), Evolutionary Trace, Swiss-Prot, and SMART were queried for functional site information. Notably, SMART returned no hits. Unlisted in the figure is the Catalytic Site Atlas (CSA), which when queried, only listed Tyr7 as a functional residue. We omitted this result from the table due to lack of information. The sum totals of appearances of functional residues are highlighted in blue. We also used experimentally determined functional sites listed in Cardoso et al (2003) as our gold standard. From our MSA prediction, we were able to obtain predictions for half of the residues with a webserver prediction total greater than or equal to 4 (Y7, W8, Q67). However, it is notable that our MSA prediction yielded many false positives (predicted by our manual MSA but not by the literature, residues R18, L21, P56, Y57) and false negatives (not predicted by our manual MSA but was predicted by the literature, residues G12, L13, W41, K45, N54, L55, S68, D101, R103, S107, Y111, Q204, Q207). This may be the result of error in our MSA, as masking to produce results from the MSA is very subjective and can easily affect conservation and identity scores of residues, changing predictions of functional residues. Additional webservers in our prediction may have been advantageous since we only sampled 8 webservers and removed results from one (CSA) from the data comparison. Our results indicate that manual prediction of functional residues using an MSA can be difficult and error-prone, depending on the skill of whoever is performing masking and analysis of the MSA. Furthermore, our gold standard for functional site identification may either lack or have an excess of information. It is possible that Cardoso et al (2003) have not identified some residues critical to catalytic activity, and/or have identified sites as important but are really not essential to catalytic function. Future work may include a consensus approach through the literature to best identify functional sites to be used as a gold standard.