

### A bioinformatics approach to the structural and functional analysis of the glycogen phosphorylase protein family Jieming Shen<sup>1,2</sup> and Hugh B. Nicholas, Jr.<sup>3</sup>

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#### Abstract

The enzyme glycogen phosphorylase catalyzes the breakdown of glycogen into glucose subunits. In this study, we utilized computational methods to examine 282 sequences in the glycogen phosphorylase protein family. Specifically, we multiple integrated sequence alignments obtained from a variety of different algorithms a single refined alignment using the into GeneDoc program and identified twenty highly conserved motifs using MEME. A phylogenetic tree, constructed using the PHYLIP suite software, SeqSpace, and GEnt, provided insight into the patterns of evolutionary descent for the protein family and organized sequences into various subfamilies based on distinctive sequence characteristics. Preliminary analysis of the tree identified twelve major subfamilies as well as brain (B), liver (L) and muscle (M) isozymes within vertebrates in the Metazoan subfamily. Visualization of the conserved and variable features among subfamily members through VMD revealed their structural and functional significance to protein activity.

#### Introduction

Cross entropy analysis of enzyme subfamilies provides insights into the positions of residues responsible for the evolutionary adaptations of that particular subfamily. Knowing such positions aids in the development of a phylogenetic profile for a protein family, which can indicate the pathways and physiological processes in which the proteins appear. Glycogen phosphorylase is one of several enzymes expressed differentially during the development of the ventral furrow in drosophila embryo. A cross entropy analysis was conducted on the phosphorylase family in order to learn more about the enzyme's role in this physiological event. Each subunit of the dimer contains an active site for the phosphorolysis of glycogen, a bound pyridoxal phosphate cofactor at catalytic site, a glycogen binding site, an allosteric effector site near the interface between the two subunits, and an interface region.

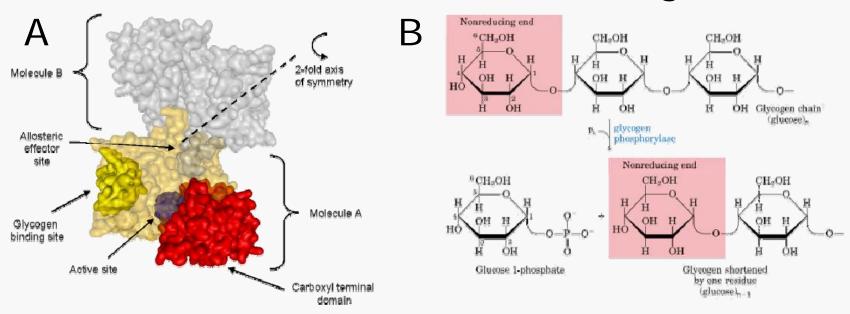
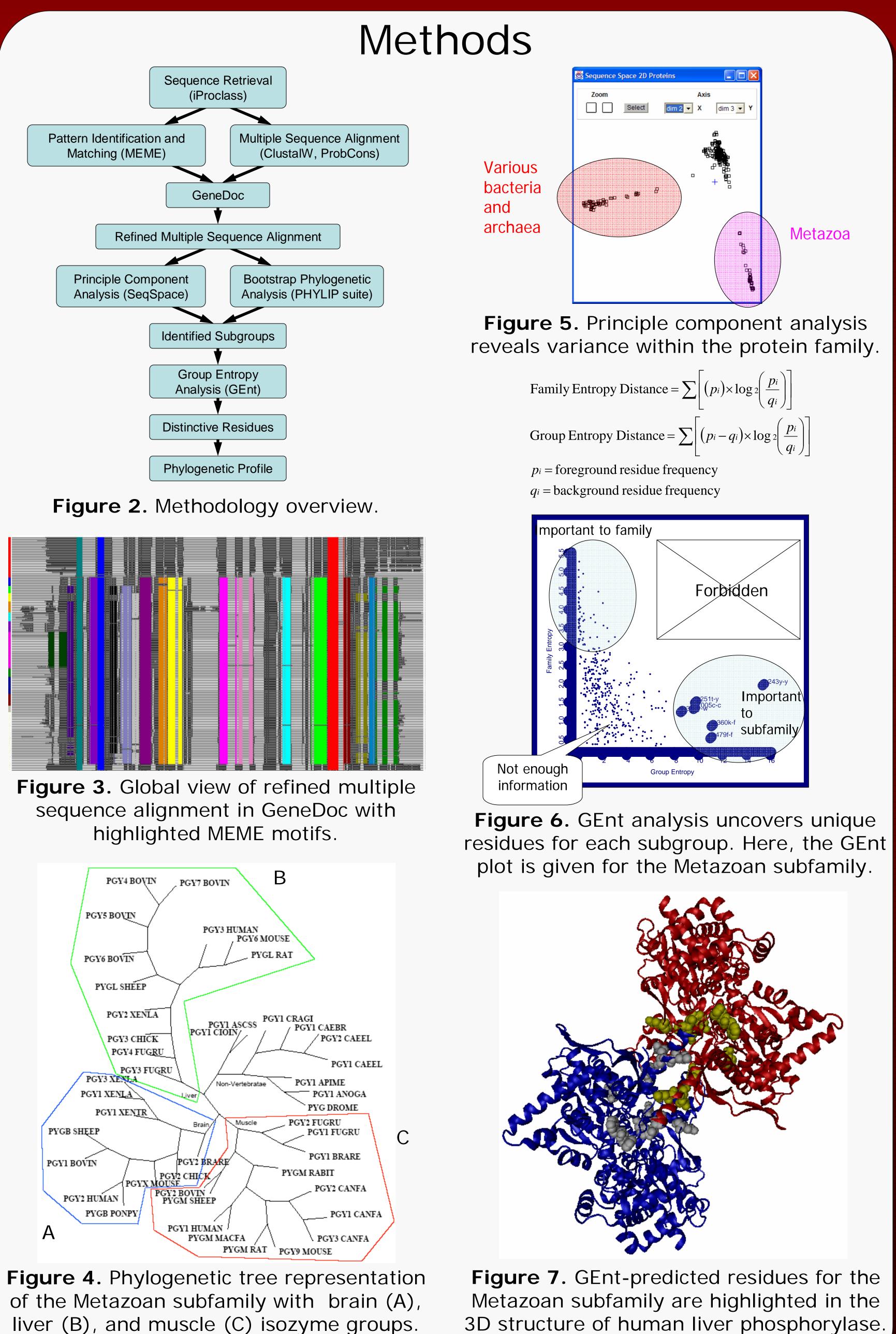


Figure 1. A 3D representation of glycogen phosphorylase with functional residues noted (A). Glycogen phosphorylase removes glucose monomers from linear chains of glycogen (B).



#### Results & Conclusions

In the Metazoan subfamily, our cross entropy analysis was able to identify residue 155, known to be important in allosteric control of the enzyme. Other predicted residues, in Metazoa as well as other subfamilies, have a high probability of reflecting important adaptations of the species in the subfamilies to their environments. Future research would involve performing site-directed mutagenesis experiments to test the GEntpredicted residues and determine their contribution to protein function. Additional computation work involving quantum mechanics and molecular mechanics can be conducted to identify more properties of the conserved residues, motifs, and GEnt-predicted residues.

Our phylogenetic analysis allowed us to trace a possible evolutionary path of the glycogen phosphorylase protein family.

In addition, we noticed that ProbCons and T-COFFEE, both consistency-based MSA methods, did not perform as well as ClustalW. This observation can probably be attributed to the large number of sequences and long sequence length of the dataset.

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