

Classification of Multi-Domain Glycoside Hydrolases to Aid in the Enzymatic Production of Biofuels from Biomass

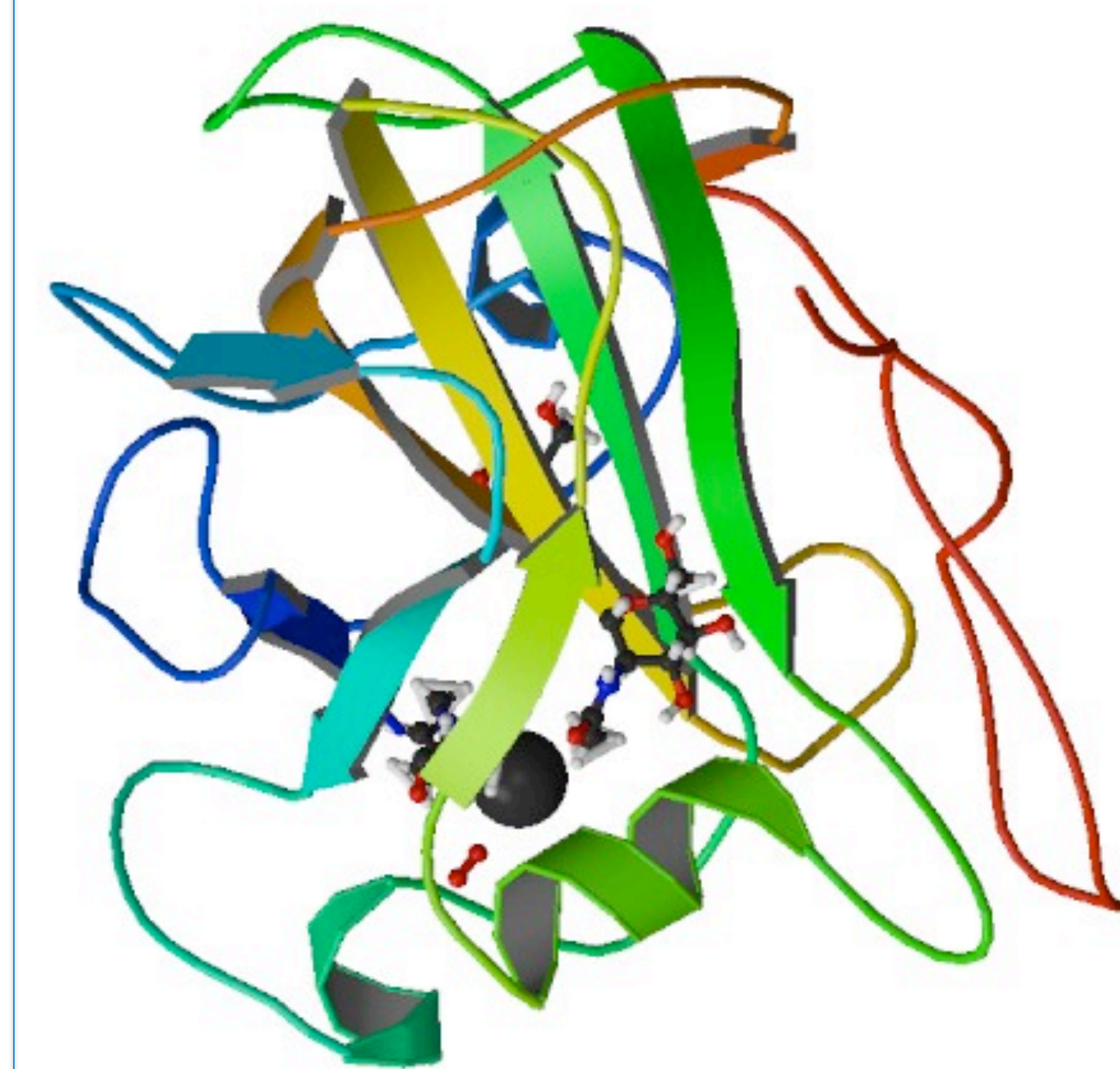
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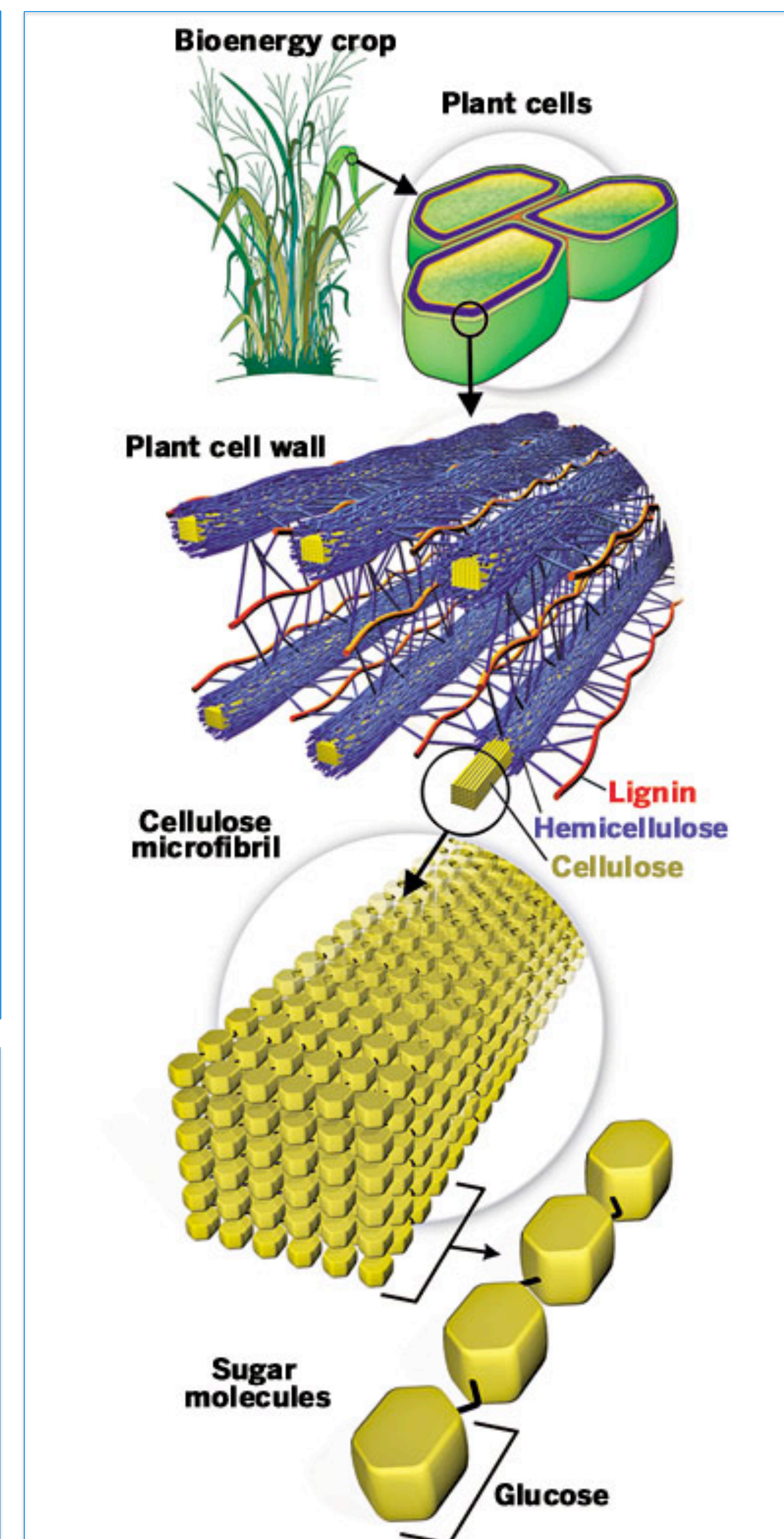
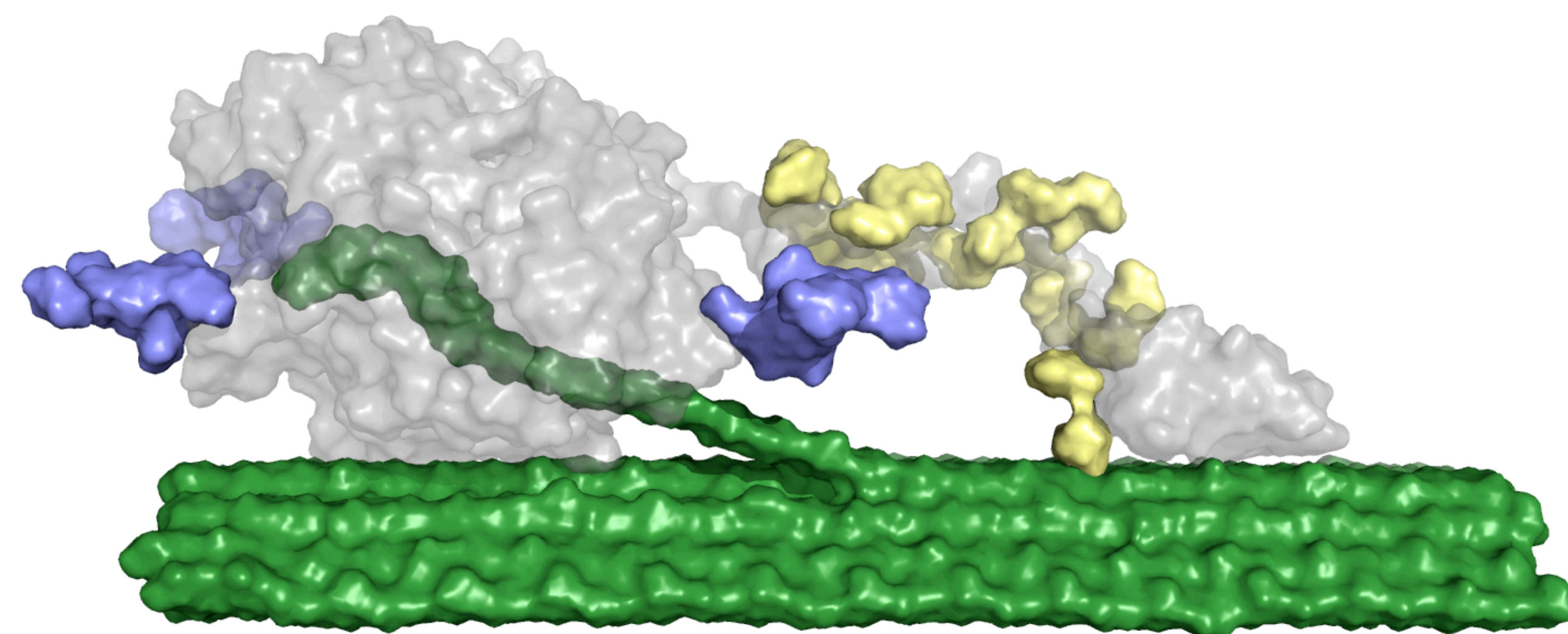
Abstract

Biomass conversion to renewable biofuels provides an alternative to conventional fossil-fuel based transportation fuels and a means to reduce dependence on foreign oil. However, plant cell walls have evolved to be quite resistant to enzymatic deconstruction, a phenomenon generally termed biomass recalcitrance. As enzymes represent a substantial cost in biofuels production, there is significant impetus to understand and improve their efficiency in converting cell wall carbohydrates to fermentable sugars. Much research has been conducted on single "free" enzymes with one catalytic unit per protein and on the much larger, complexed "cellulosomes" with many tens of catalytic units per protein, but little work has been done on multi-domain enzymes that are an interpolation in size between free enzymes and cellulosomes. A bioinformatics study on multi-domain glycoside hydrolases was conducted to gather information on the various ways each family is found and organized in nature. GH61s, GH6s and GH7s in particular have been classified based on order of protein domain, catalytic domain, carbohydrate binding module, linker length, and origin. It is hoped that this will eventually become a complete database of multi-domain enzymes that will aid in the development of cost-effective methods of lignocellulosic biomass conversion.



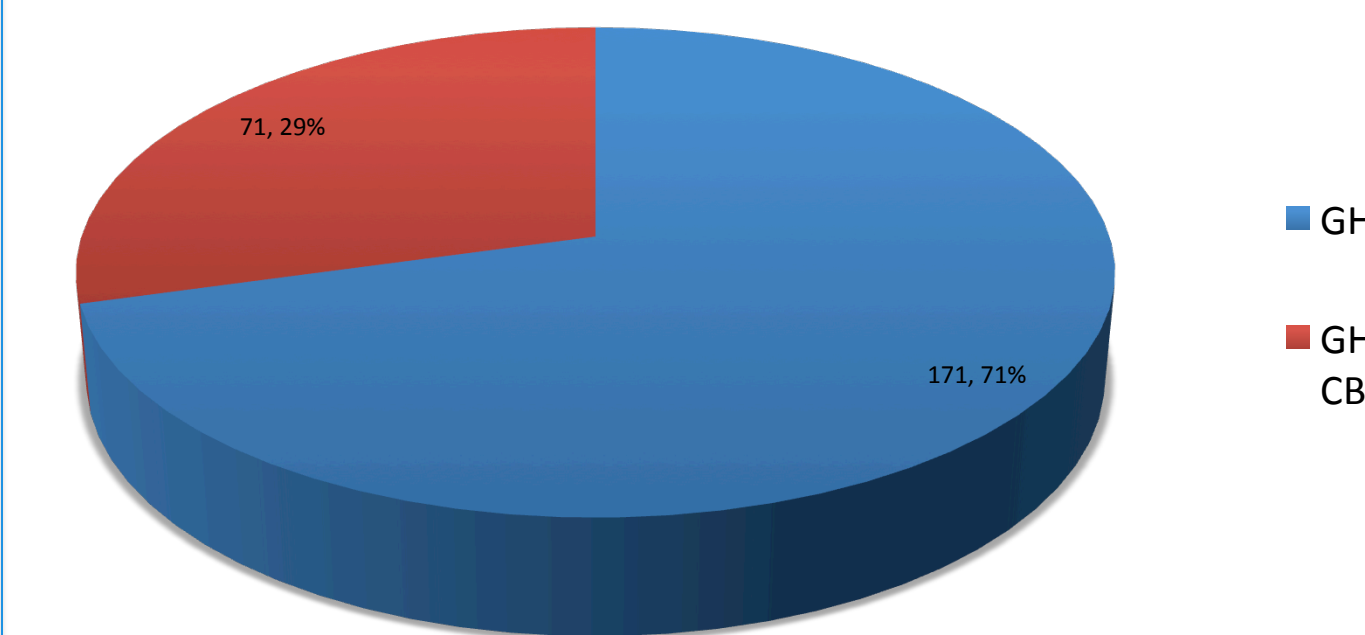
Glycoside hydrolases (GH), like the GH61 structure pictured (left, PDB ID= 4EIR), represent a group of enzymes that hydrolyze glycosidic bonds, breaking down cellulose and releasing glucose and other fermentable sugars. There are many different families of GHs, each identified with a number and each with a different mechanism or purpose. For example, GH6s are a highly conserved family that cleave the β -1,4 glycosidic bonds in cellulose making them traditional cellulases. However, GH61s exhibit weak glycosidase activity and are thought to enhance the breakdown of lignocellulose when paired with other cellulases.

Multi-domain enzymes, like Cel7A (right), contain multiple protein domains attached by amino acid linkers of varying lengths. The carbohydrate binding module (CBM) bonds to the cellulose as the glycoside hydrolase (GH) deconstructs it into fermentable sugars.

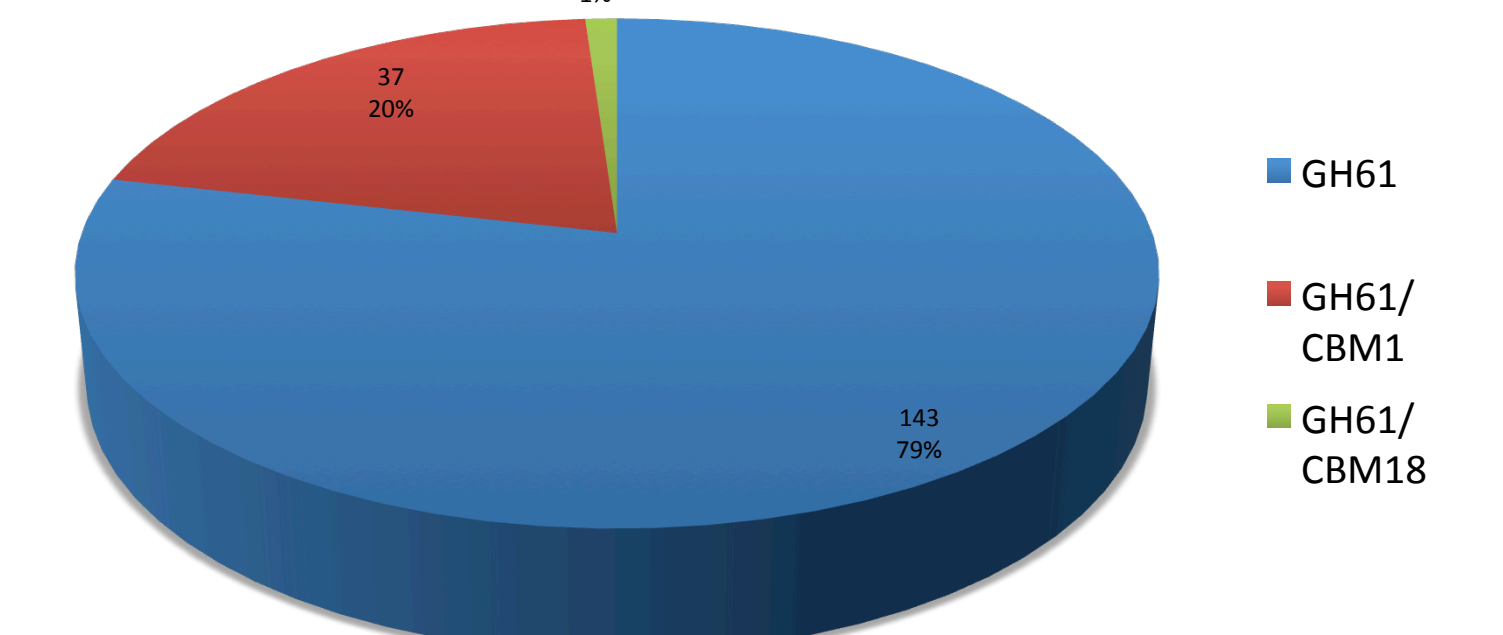


Biomass recalcitrance refers to the evolutionary resistance of plant cell walls to destruction by other organisms. Cellulose is a long chain of glucose molecules, which in itself is difficult to break down. Within a cell wall, cellulose microfibrils are encased in a layer of hemicellulose which is linked to lignin, forming a matrix of heteropolymers that is resistant to most low-cost treatment schemes. This has created a fundamental bottleneck in biofuels production.

Distribution of GH7 Protein Domain Variations



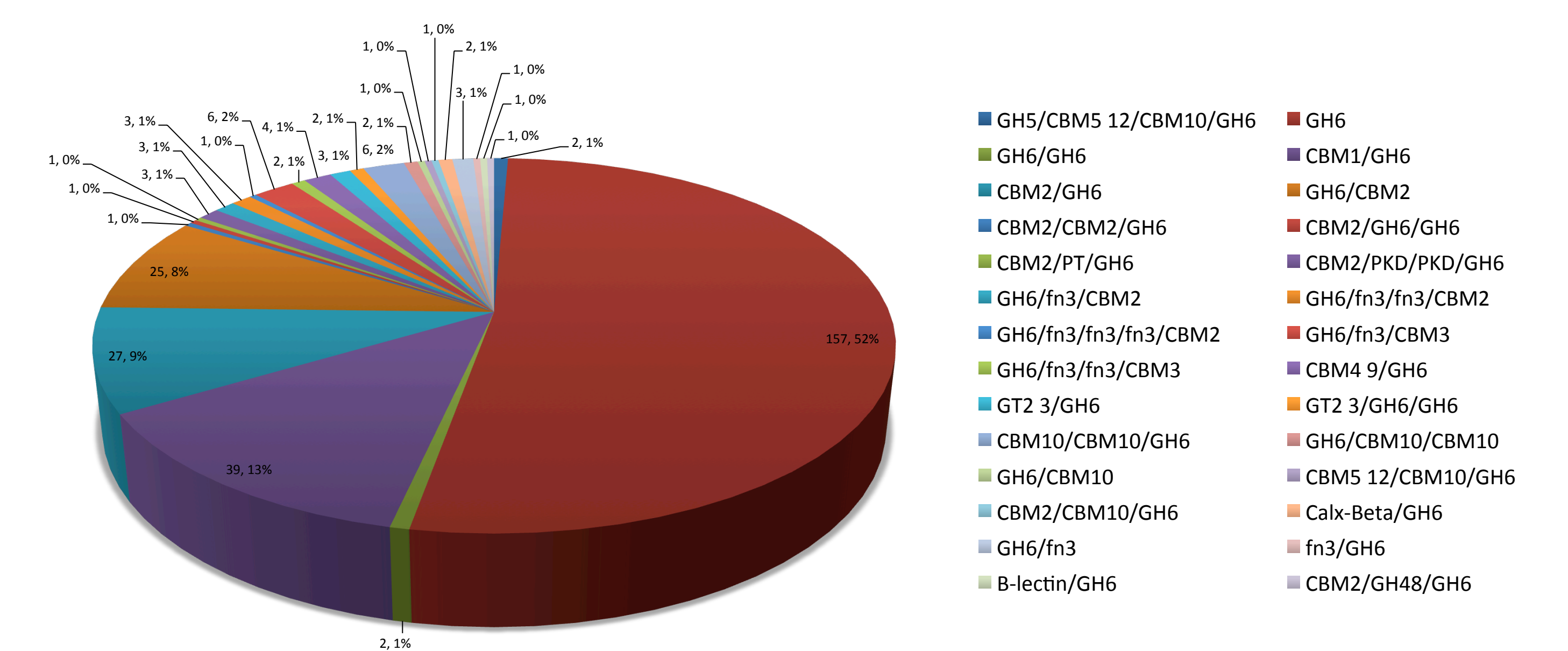
Distribution of GH61 Protein Domain Variations



Results

- GH7 and GH61 had very few (2-3) arrangements of protein domains
- GH6 had 30 variations of protein domain arrangement
- GH7 and GH61 were almost entirely found in eukaryotes
- GH6 were found in bacteria and eukaryotes, but specific arrangements were delineated by kingdom
- In general, linker lengths remained fairly constant within each arrangement

Distribution of GH6 Protein Domain Variations



Impact

This research will ideally lead to a database of multi-domain enzymes that will increase our understanding of their evolutionary benefits and eventually aid in the development of efficient and cost-effective lignocellulosic biomass conversion.



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