Microbial chemical factories are sustainable biomanufacturing platforms that complement traditional petrochemical industries by using renewable feedstocks. However, there are few economical methods to convert plant biomass into sugars that can be more readily used by existing microbial platforms without the production of toxic byproducts. An alternative strategy is the use of non-model microbes that are adapted to the direct use of crude, untreated biomass and engineer these microbes for biomanufacturing. In my lab, we focus on one such class of organisms, early-diverging anaerobic fungi (phylum Neocallimastigomycota), due to their robust degradation of untreated plant feedstocks in the herbivore digestive tract and their novel biosynthetic capabilities. To effectively leverage these abilities, however, a synthetic biology engineering toolbox is required. My lab isolates novel anaerobic fungi, characterizes them, and pursues a number of plasmid-, genomic-, and epigenomic- strategies for tool development to domesticate anaerobic fungi for biotech. We have identified several gene regulatory elements from transcriptomic and genomic resources to drive gene expression for the first time in either the nucleus or cytoplasm of anaerobic fungi. Validation studies with flavin-based fluorescent reporters and selection markers confirm sequence dependent increases in gene expression in a controllable manner. These tools enable library-based screening approaches to construct the first stable anaerobic fungal plasmid and the introduction of CRISPR-based endonucleases for genomic integration. We have also pioneered epigenetic regulation of anaerobic fungi and identified several chemical inhibitors that can perturb the epigenome of anaerobic fungi with concurrent phenotypic changes in biomass-degrading enzyme secretion. Efforts are currently underway to capitalize on systems biology methods to rapidly elucidate and expand parts available for engineering applications. In parallel, we explore how emerging systems biology knowledge of anaerobic fungi may be leveraged in existing microbial platforms. Our growing toolbox of genetic and epigenetic strategies can be readily generalized to other non-model systems, and will soon enable direct biomanufacturing in anaerobic fungi.