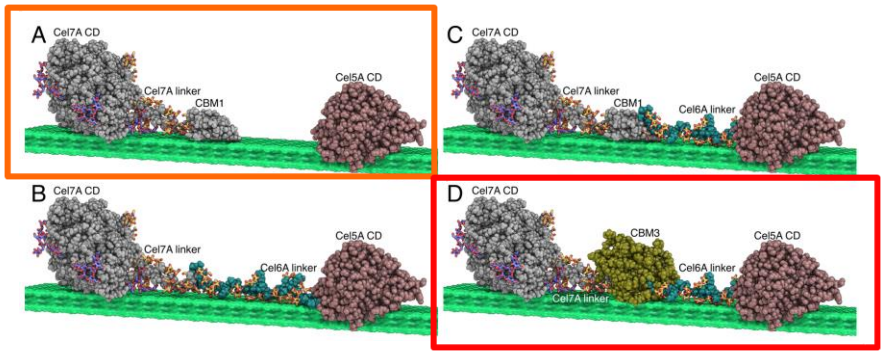
Synthetic Fungal Multifunctional Cellulases for Enhanced Biomass Conversion: New Design Principles for Superior Biomass Degrading Enzymes

Background

- Significant effort has been expended toward the discovery and/or engineering of improved single catalytic domain cellulases for conversion of biomass to fermentable sugars.
- An alternative to this approach is to use promising multi-catalytic domain enzymes found in some anaerobic bacteria and produce them in traditional industrial hosts.
- Most if not all relevant bacterial enzymes of this type do not express well in fungi. Therefore, developing a systematic understanding of how to construct multifunctional enzymes that can be expressed in commercial fungal hosts while retaining high specific activity is crucial for developing the next generation of enzymes for biomass deconstruction.

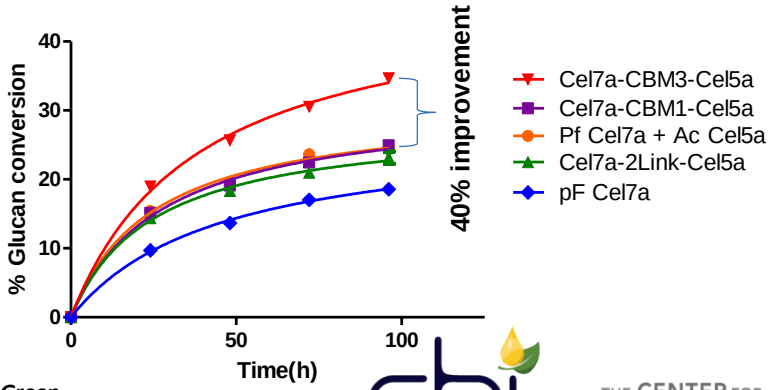
Approach

- Multifunctional cellulolytic enzymes, such as Cel7A from *Caldicellulosiruptor bescii*, show extremely high cellulolytic activity. CelA is comprised of a GH9 and a GH48 catalytic domain connected by three type III cellulose-binding modules (CBMs) that unfortunately do not express well if at all in fungal hosts. Here, we engineered several multifunctional enzymes inspired from the architecture of CelA using components known to express well in the industrially relevant fungus *T. reesei*.



Outcomes

- All multifunctional enzymes showed improved activity over Cel7A.
- One construct showed a 40% increase in performance compared to equal mixtures of free enzymes.
- Several constructs showed equal activity to binary mixtures of Cel7A and Cel5A illustrating that simply linking two cellulases will not necessarily result in superior enzymatic activity.
- All multifunctional enzymes tested had different meso-scale deconstruction mechanisms of cellulose indicating that tuning enzymes for biomass deconstruction is possible.



Significance

- This is the first example of a fungal expressible multifunctional enzyme with significantly enhanced performance.
- The meso-scale deconstruction mechanism of the best chimeric construct is unique and has never been observed for natural variants.
- This result provides insight and proof-of-concept for a new strategy for highly effective commercial cellulolytic enzymes.

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