SAND2016-4624PE

Enzyme Engineering for Biofuels

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Mission Statement

The harsh conditions of biorefinery sugar production are often unsuitable for the optimal activity of natural enzymes. Therefore, we are engineering key cellulolytic and ligninolytic degradation enzymes to improve their performance under biorefinery relevant conditions, such as high temperature and ionic liquid presence. Our goal is to develop enzyme mixtures that maximize biomass loadings and minimize the enzyme dose required to produce monomeric sugars and lignin fragments for biological upgrading to fuels and chemicals.

Enzyme Engineering

Our enzyme engineering approaches span from generation of random mutant libraries and directed evolution to targeted enzyme design using structure- and biochemical-based rationale.

Engineering a thermophilic endoglucanase for improved activity on ionic liquid pretreated biomass.

- Cel5A from *Thermotoga maritima* had high temperature and IL tolerance but lower than desired activity.
- Libraries of random mutations were screened for enhanced activity in the presence of IL.
- The optimal mutation profile displayed a 33% increased activity (Figure 1).

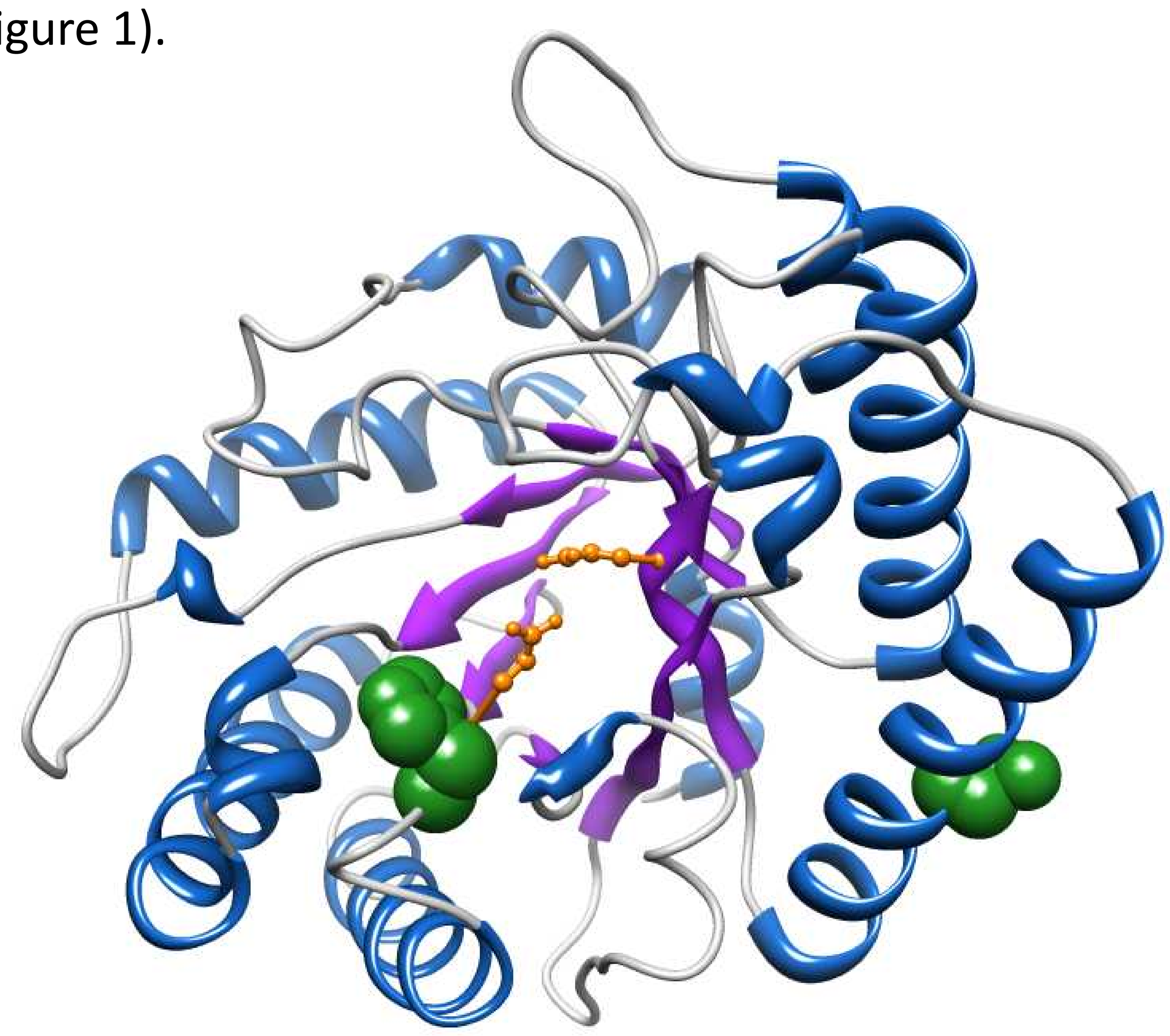


Figure 1. Positions of activity enhancing mutations of Cel5A_*Tma* in the crystal structure. The H138R and N236D mutations yielded a 22% and a 30% increase in specific activity on [C_2mim][OAc] pretreated switchgrass, respectively. The double mutant (H138R + N236D) displayed a 33% increase in specific activity on [C_2mim][OAc] pretreated switchgrass. The catalytic residues of Cel5A_*Tma* are depicted in orange and H138 and N236 are shown in green.

Engineering a thermophilic β-glucosidase for increased ionic liquid tolerance.

- The β-glucosidase from *Thermosipho africanus* has high activity that decreases rapidly in IL.
- Directed evolution is being used to improve IL tolerance.
- Several mutants have improved IL-50, enabling enhanced biomass processing at up to 20% IL load (Figure 2).

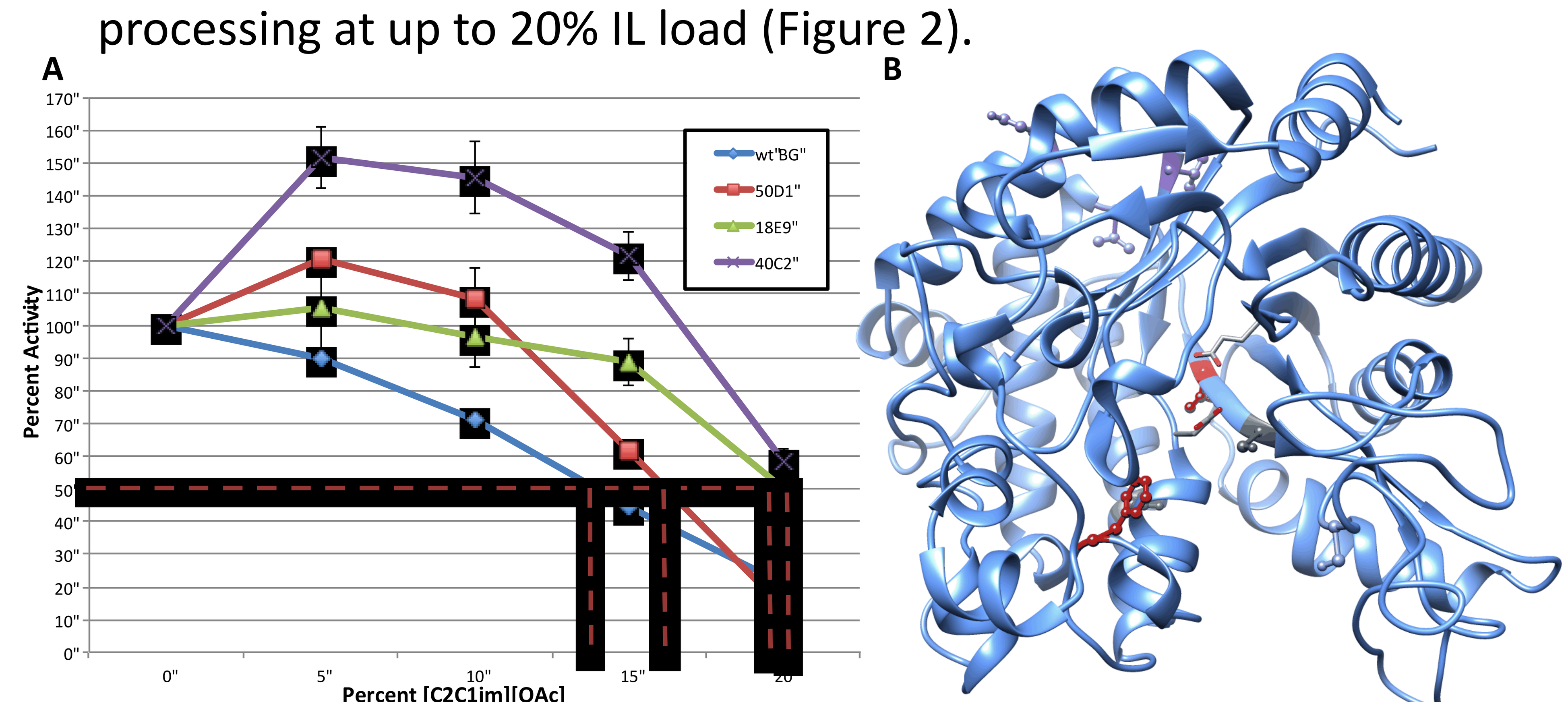
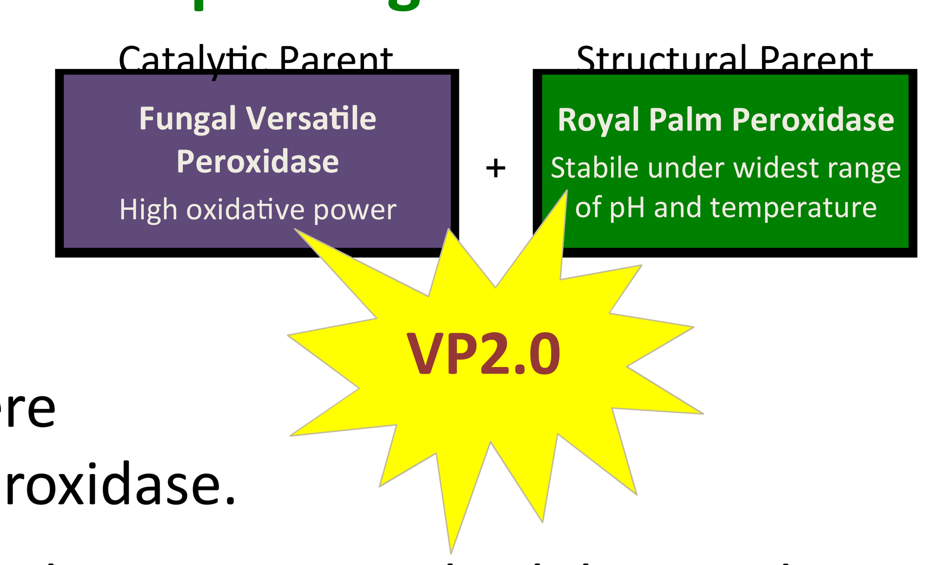


Figure 2. Positions of IL-50 enhancing mutations. (A) Several mutations were identified that enhanced the ability of the enzyme to function under higher IL concentrations. Successful engineered candidates exhibited higher IL-50 values, defined as the concentration of IL that leads to a 50% reduction in activity (IL-50). (B) Key mutations are depicted on the homology-modeled enzyme structure. Residue colors correspond to the legend in (A).

Targeted engineering a plant-fungal hybrid peroxidase for activity over broader temperature and pH ranges.

- High redox potential Fungal Versatile Peroxidases function under a fairly narrow range of conditions.
- 16 amino acid point mutations were introduced into the Royal Palm Peroxidase.
- A broader functional range equals greater applicability and a reduction in total enzyme required.



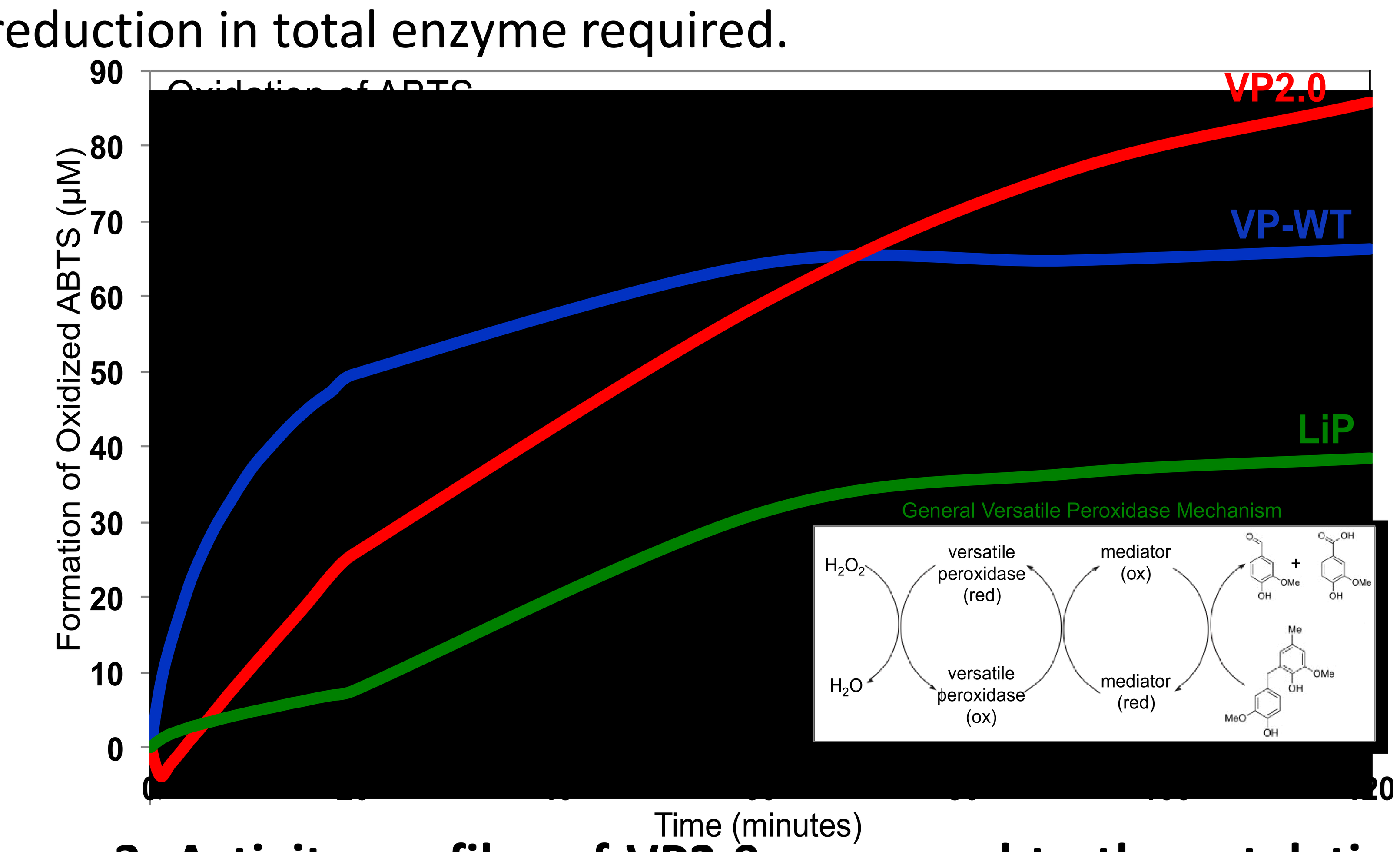


Figure 3. Activity profiles of VP2.0 compared to the catalytic parent enzyme and a lignin peroxidase positive control. VP2.0 has higher activity over longer time courses, suggesting it has an improved half-life.

Exceptional service in the national interest

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