COMPUTATIONAL DESIGN OF CATALYTICALLY ACTIVE TIM BARREL XYLANASES

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TIM barrel enzymes perform five out of six Enzyme Commission (EC) reaction classes, and are therefore one of the most promising class of enzymes for computational design. Up until now, chimeric TIM barrels were made out of half barrels and catalytic activity was installed using laborious rounds of random mutagenesis, selection and rational design, whereas a recently described de novo designed TIM barrel lacked the elaborate loop conformations necessary to install catalytic activity, and indeed did not show activity.

In order to design catalytically active TIM barrel glycoside-hydrolase 10 family xylanases, we sampled diverse protein backbone conformations, in some cases generating completely new combinations of backbones at all eight beta-alpha blades, and optimized the sequence for both protein stability and catalytic activity. We then selected structurally diverse, low energy subset of the designs for further characterization. Designs had <60% sequence identity to natural xylanases, incorporating many insertions and deletions in loop regions. All designs expressed well and 30% showed catalytic activity, the most active one having $K_{cat}/K_m = 10^6$.

The design process samples extensively the sequence-structure space and the designs can serve as a library for altered enzyme selectivity. Moreover, our algorithm is general and robust, and can be applied to other TIM barrel enzyme families having a few dozen solved structures and to other modular protein folds.