ENGINEERING ENZYMES WITH NON-CANONICAL ACTIVE SITE FUNCTIONALITY

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The combination of computational enzyme design and laboratory evolution provides an attractive platform for the creation of protein catalysts with new function. To date, designed mechanisms have relied upon Nature's alphabet of 20 genetically encoded amino acids, which greatly restricts the range of functionality which can be installed into enzyme active sites. Here, we have exploited engineered components of the cellular translation machinery to create a protein catalyst which operates via a non-canonical catalytic nucleophile. We have subsequently shown that powerful laboratory evolution protocols can be readily adapted to allow optimization of enzymes containing non-canonical active site functionality. Crystal structures obtained along the evolutionary trajectory highlight the origins of improved activity. Thus our approach merges beneficial features of organo- and biocatalysis, by combining the intrinsic reactivities and greater versatility of small molecule catalysts with the rate enhancements, reaction selectivities and evolvability of proteins.

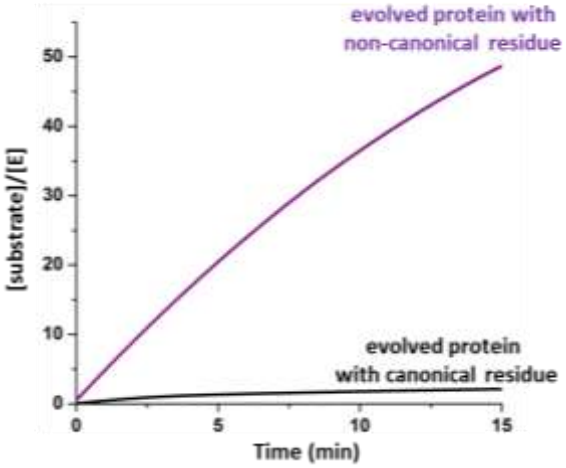

Figure 1 – Reaction profile showing the most evolved variant. Replacement of the non-canonical amino acid with a canonical residue abolishes catalytic activity