EXPANDING THE ENZYMATIC TOOLBOX WITH DE NOVO PROTEIN DESIGN

Indrek Kalvet, Institute for Protein Design, University of Washington, Seattle, USA
ikalvet@uw.edu
David Baker, Institute for Protein Design, University of Washington, Seattle, USA

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Biocatalysis is often viewed as a more sustainable and selective alternative to chemical synthesis. Challenges in a more widespread adoption of biocatalytic methodologies stem from limited catalytic scope, and stability limitations of enzymes, among others. Protein engineering efforts have tremendously expanded the scope of accessible transformations by directed evolution, non-canonical amino acids, or binding synthetic cofactors, as well as helped optimize protein properties.[1,2] De novo protein design can further complement these efforts by providing access to highly stable proteins tailor-made for specific applications.[3]

This presentation will give an overview of our efforts in computationally designing new enzymes based on binding catalytic cofactors. From proof-of-principle studies in designing novel heme-binding proteins to enzymes utilizing fully synthetic metal complexes as their catalytic centers. We have succeeded in designing a hyperstable heme-binding protein with a reconfigurable active site, suitable for enabling a variety of catalytic reactions.[4] Through directed evolution we arrived at a relatively proficient peroxidases, while through computational redesign we were able to create enantiocomplementary enzymes for selective olefin cyclopropanation reaction. Deep learning-enabled generative protein design[5,6] has enabled us to further demonstrate how new hemoproteins can be designed by extensive backbone remodeling, yielding heme-binding proteins with fully customizable pocket shapes, sizes, and coordinating residues, optimizable for a large variety of catalytic applications.
This work establishes design principles for creating cofactor-binding enzymes with diverse functions and brings us a step closer to programming protein catalysts from scratch with little to no experimental intervention.

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