RESNICK INSTITUTE
science + energy + sustainability

RESEARCH HIGHLIGHTS

From the Resnick Sustainability Institute
Graduate Research Fellows at the
California Institute of Technology

Better Enzymes for Biofuels and Green Chemistry: Solving the Cofactor Imbalance Problem

Jackson Cahn

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Global Significance

For millenia, mankind has used microbes to produce simple chemicals of value, including ethanol, acetic acid, and lactic acid. With the growth of modern molecular biology techniques, engineering new microbial strains to produce more complex compounds of interest is increasingly being viewed as a sustainable alternative to traditional industrial chemistry due to lower energy requirements and a lack of toxic solvents or heavy metal catalysts. However, particularly in the area of fuels, these methods must produce sufficient yeilds to compete in terms of cost. As more and more novel biosynthetic pathways are constructed, a need has arisen for tools to rapidly and reliably optimize enzymes for new metabolic contexts.

Because of the complexity of protein structure and function, protein engineering typically functions from the ground up with each project. This project represents the first attempt to provide a general solution to a recurring protein engineering challenge. In doing so, it draws on many disciplines including molecular biology, structural biology, biochemistry, bioengineering, structural bioinformatics, and chemical engineering.

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Project Summary

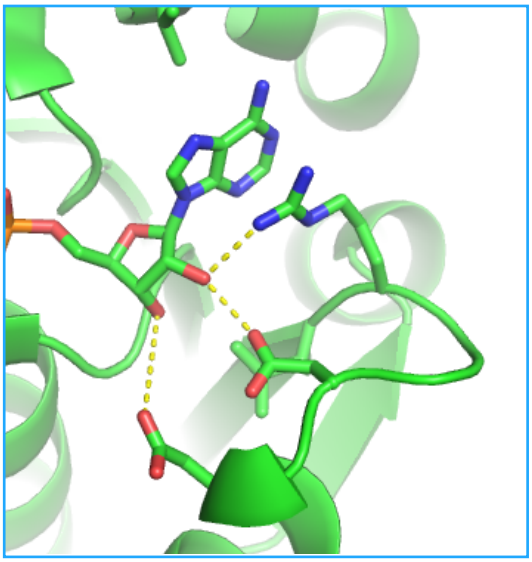
While each biosynthetic pathway faces its own productivity hurdles, certain problems recur frequently, and one of the very common ones is cofactor balance. Nature uses a pair of distinct molecules, NADH and NADPH, which are known as redox cofactors, to transport and store electrons for chemistry. For efficiency, it is essential that any new pathway balance the production and consumption of each cofactor, which can be challenging when enzymes from diverse sources are placed into new metabolic contexts.

Learning from past efforts, in combination with biophysical and structural insight, we have sought to develop a reliable minimal procedure reversing the cofactor specificity of any enzymatic binding site. Testing this procedure on a structurally and functionally diverse set of industrially relevant enzymes has demonstrated its broad applicability and reliability.

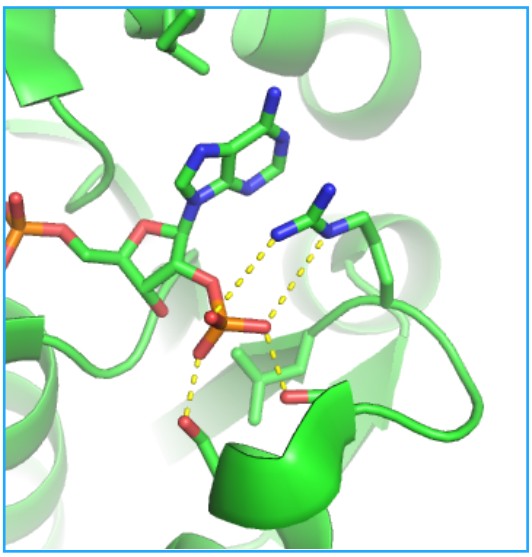
This method is now being developed into an easy-to-use digital tool so that it can be of broad use to the industrial and academic bioengineering communities.

Potential Impact

If successful, this research would significantly accelerate the process of optimizing the function of newly designed biosynthetic pathways, speeding the transition between the lab and the marketplace. It would also provide a model for the development of further semi-rational protein engineering approaches to drive industrial biocatalysis forward.



An NADPH binding pocket.



The same pocket with three mutated amino acids binding to NADH.

(Brinkmann-Chen, et al., 2013)

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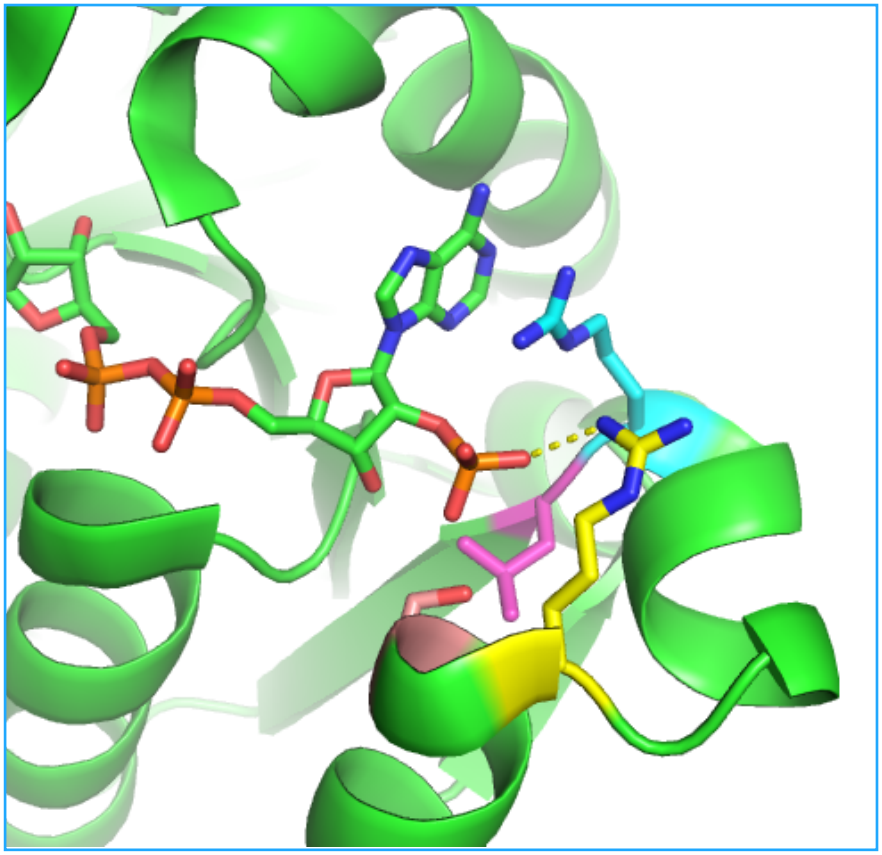
The Science

The goal of this project is to create a simple recipe for cofactor-switching any enzyme using these redox cofactors (NADH and NADPH). Specifically, such a recipe should require as few rounds of engineering as possible, with a minimum of mutants screened per round. The recipe should require minimal knowledge, experience, or skill on the part of the end user and yeild a highly-active final enzyme, with activity comparable to the wild-type enzyme.

The recipe we have developed is a semi-rational structure-guided approach, which identifies and prioritizes the residues of the phosphate-binding motif based on known engineering successes. The recipe then recommends a small degenerate-codon library to screen, with the goal of focusing on the sequence diversity most likely to generate motifs resembling those which bind NADH sucessfully. The recipe also identifies structural hotspots to mutate to provide compensatory stabilization for the switching-associated mutations.



The NADPH binding pocket of the EcIlvC enzyme, showing the distinct structural roles of the four amino acid residues composing the phosphate binding motif.

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Key Results

Our recipe, Cofactor Specificity Reversal – Structural Analysis and Library Design (CSR-SALAD), has been developed, and has been translated into an easy-to-use python applet. To demonstrate its broad applicability, we have successfully applied it to five unrelated oxidoreductase enzymes, including enzymes from three different fold superfamilies.

Future Steps

Having rigorously demonstrated the applicability of CSR-SALAD to oxidoreductases, we would like to see if it works for other NADPH-dependent proteins, starting with a Baeyer-Villiger monoxygenase. Before it can be broadly disseminated, we would like to build a web front-end for CSR-SALAD for ease of use, but in the mean time we have used it to give recommendations to a number of groups around the world. As their feedback arrives, it can be used to further tune the library design approach. Lastly, we would like to reverse the approach to design libraries to engineer NADH-dependent enzymes for NADPH utilization.

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Publications

- Cahn JKB*, Brinkmann-Chen S*, Spatzal T, Wiig JA, Buller AR, Einsle O, Hu Y, Ribbe MW, Arnold FH. Cofactor specificity motifs and the induced fit mechanism in Class I ketol-acid reductoisomerases. Biochemical Journal, Epub ahead of print.
- Brinkmann-Chen S*, Cahn JKB*, Arnold FH. Uncovering rare NADH-preferring ketol-acid reductoisomerases. Metabolic Engineering 26, 17-22. Nov 2014
- Brinkmann-Chen S, Flock T, Cahn JKB, Snow CD, Brustad EM, McIntosh JA, Meinhold P, Zhang L, Arnold FH. General approach to reversing ketol-acid redutoisomerase cofactor dependence from NADPH to NADH. Proceedings of the National Academy of the Sciences 110(27), 10946-10951. July 2013