



## A consensus-guided approach yields a heat-stable alkane-producing enzyme and identifies residues promoting thermostability

Tabinda Shakeel<sup>1</sup>, Mayank Gupta<sup>1</sup>, Zia Fatma<sup>1</sup>, Rakesh Kumar<sup>1</sup>, Raubins Kumar<sup>1</sup>, Rahul Singh<sup>1</sup>, Medha Sharma<sup>1</sup>, Dhananjay Jade<sup>1</sup>, Dinesh Gupta<sup>1</sup>, Tasneem Fatma<sup>2</sup> and Syed Shams Yazdani<sup>1\*</sup>

### + Author Affiliations

\* Corresponding author; email: [shams@icgeb.res.in](mailto:shams@icgeb.res.in)

Author contributions: T.S. and S.S.Y. conceptualization; T.S., M.G., Rakesh Kumar, D.J., and S.S.Y. data curation; T.S., M.G., Rakesh Kumar, D.J., and D.G. software; T.S., Z.F., Rakesh Kumar, R.S., D.J., and S.S.Y. formal analysis; T.S., Raubins Kumar, and S.S.Y. validation; T.S., M.G., Rakesh Kumar, D.G., and S.S.Y. visualization; T.S., Z.F., R.S., M.S., and S.S.Y. methodology; T.S. writing-original draft; Z.F., Raubins Kumar, R.S., M.S., D.G., T.F., and S.S.Y. investigation; D.G., T.F., and S.S.Y. supervision; D.G. and S.S.Y. funding acquisition; D.G. and S.S.Y. project administration; D.G. and S.S.Y. writing-review and editing; S.S.Y. resources.

### Abstract

Aldehyde-deformylating oxygenase (ADO) is an essential enzyme for production of long-chain alkanes as drop-in biofuels, which are compatible with existing fuel systems. The most active ADOs are present in mesophilic cyanobacteria, especially *Nostoc punctiforme*. Given the potential applications of thermostable enzymes in biorefineries, here we generated a thermostable (Cts)-ADO based on a consensus of ADO sequences from several thermophilic cyanobacterial strains. Using an *in silico* design pipeline and a metagenome library containing 41 hot-spring microbial communities, we created Cts-ADO. Cts-ADO displayed a 3.8-fold increase in pentadecane production on raising the temperature from 30°C to 42°C, whereas ADO from *N. punctiforme* (Np-ADO) exhibited a 1.7-fold decline. 3D structure modeling and molecular dynamics simulations of Cts- and Np-ADO at different temperatures revealed differences between the two enzymes in residues clustered on exposed loops of these variants, which affected the conformation of helices involved in forming the ADO catalytic core. In Cts-ADO, this conformational change promoted ligand binding to its preferred iron, Fe<sub>2</sub>, in the di-iron cluster at higher temperature, but the reverse was observed in Np-ADO. Detailed mapping of residues conferring Cts-ADO thermostability identified four amino acids, which we substituted individually and together in Np-ADO. Among these substitution variants, A161E was remarkably similar to Cts-ADO in terms of activity optima, kinetic parameters, and structure at higher temperature. A161E was located in loop L6, which connects helices H5 and H6, and supported ligand binding to Fe<sub>2</sub> at higher temperatures, thereby promoting optimal activity at these temperatures and explaining the increased thermostability of Cts-ADO.

aldehyde deformylating oxygenase long-chain alkane thermostable enzyme  
enzyme mutation biofuel protein engineering enzyme mechanism  
molecular modeling structure-function molecular dynamics

Received October 26, 2017.

Accepted April 9, 2018.

Published under license by The American Society for Biochemistry and Molecular Biology, Inc.

### Recommended for you

Crystal Structure of PnpCD, a Two-subunit Hydroquinone 1,2-Dioxygenase, Reveals a Novel Structural Class of Fe<sup>2+</sup>-dependent Dioxygenases  
Tiandi Wei et al., Journal of Biological Chemistry

The Molecular Structure of Hyperthermostable Aromatic

A Basic Helix-Loop-Helix Transcription Factor Essential for Cytochrome P450 Induction in Response to Alkanes in Yeast *Yarrowia lipolytica*  
Setsu Yamagami et al., Journal of Biological Chemistry

Structural Basis of Autoinhibition and Activation of the DNA-targeting ADP-ribosyltransferase Pierisin-1



I A]  
COLI

Excha  
with e  
meet  
your f

Share  
success  
Connect  
other

REG

ASI  
ANNUA  
SAN DEC

- Aminotransferase with Novel Substrate Specificity from *Pyrococcus horikoshii***  
Kazuaki Harata et al., Journal of Biological Chemistry
- Deoxyhypusine Hydroxylase Is an Fe(II)-dependent, Heat-repeat Enzyme**  
**IDENTIFICATION OF AMINO ACID RESIDUES CRITICAL FOR Fe(II) BINDING AND CATALYSIS**  
Yeon Sook Kim et al., Journal of Biological Chemistry
- A Thermally Sensitive Loop in Clostridial Glutamate Dehydrogenase Detected by Limited Proteolysis**  
Suren Aghajanian et al., Journal of Biological Chemistry
- The Energy Transduction Mechanism of Na,K-ATPase Studied with Iron-catalyzed Oxidative Cleavage**  
Rivka Goldshleger et al., Journal of Biological Chemistry
- Takashi Oda et al., Journal of Biological Chemistry
- Engineering Bifunctional Laccase-Xylanase Chimeras for Improved Catalytic Performance**  
Lucas F. Ribeiro et al., Journal of Biological Chemistry
- Engineering Hyperthermostability into a GH11 Xylanase Is Mediated by Subtle Changes to Protein Structure**  
Shaun Healey et al., Journal of Biological Chemistry
- Conserved Bases in the TΨC Loop of tRNA Are Determinants for Thermophile-specific 2-Thiouridylation at Position 54**  
Naoki Shigi et al., Journal of Biological Chemistry

---

Powered by

Advertisement