

Cell-free prototyping enables in vivo implementation of optimized reverse β -oxidation pathways in heterotrophic and autotrophic bacteria

Bastian Vögeli¹, Luca Schulz¹, Shivani Garg², Katia Tarasava³, James M. Clomburg^{2,3}, Seung Hwan Lee³, Aislinn Gonnot², Elamar Hakim Mouilly^{4,5}, Blaise R. Kimmel^{4,5}, Loan Tran², Hunter Zeleznik², Steven D. Brown², Séan D. Simpson², Milan Mrksich^{4,5}, Ashty S. Karim¹, Ramon Gonzalez^{*3}, Michael Köpke^{*2}, Michael C. Jewett^{*1}

¹Department of Chemical and Biological Engineering and Center for Synthetic Biology, Northwestern University, Evanston, IL, USA;

²LanzaTech Inc., Skokie, IL, USA;

³Department of Chemical, Biological, and Materials Engineering, University of South Florida, Tampa, FL, USA

⁴Department of Biomedical Engineering, Northwestern University, Evanston, IL, USA;

⁵Department of Chemistry, Northwestern University, Evanston, IL, USA

*Correspondence to: ramongonzale@usf.edu, michael.koepke@lanzatech.com, and m-jewett@northwestern.edu

Supplementary Information

Supplementary Figures

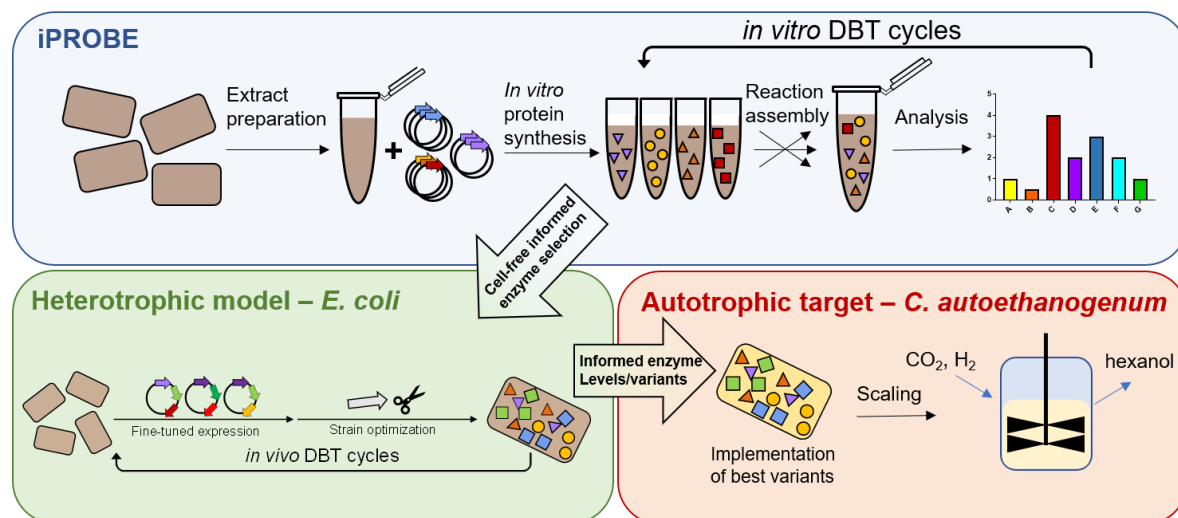


Figure S1. Scheme of the overall workflow for the project. Information gained in each system benefits all the other systems. iPROBE led to the identification of selective r-BOX variants. *E. coli* strain optimization led to the generation of r-BOX optimized CFPS extracts.

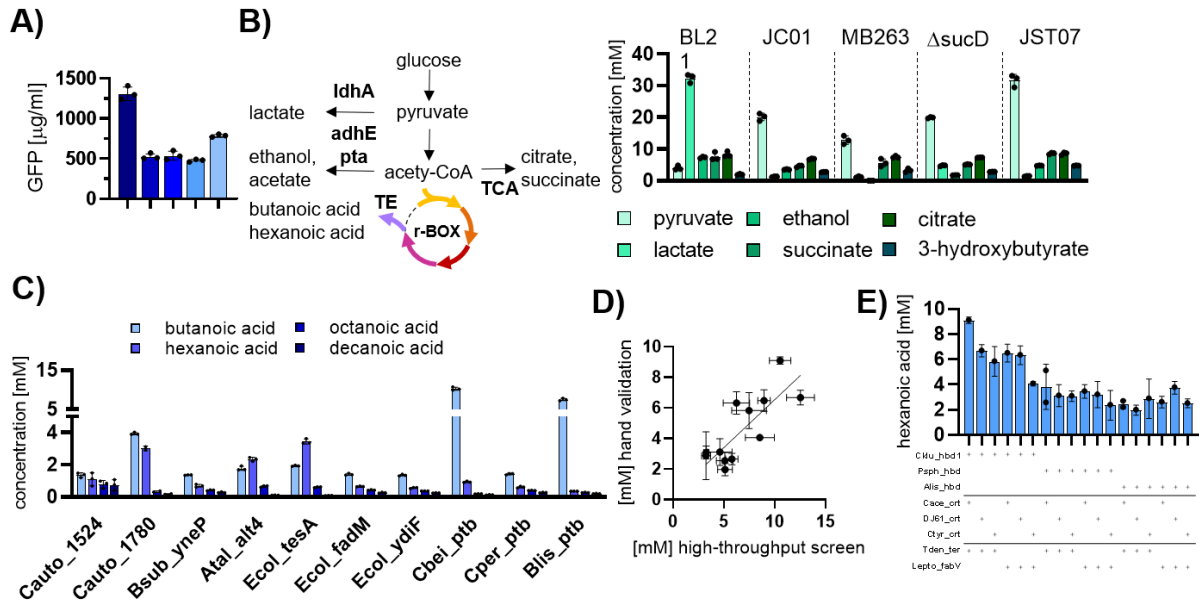


Figure S2. (A) Cell-free protein synthesis yields. (B) Side product quantification for all extracts using 0.3 μM of Cn_bktB, Ck_hbd, Ca_crt, Td_ter and 0.15 μM of Ec_tesA. (C) Screen of different thioesterases or a combination of a butyrate kinase (DJ052_buk) and phosphate butyryltransferases at 0.15 μM keeping all core r-BOX enzymes the same as in S1B. Ec_tesA shows the highest specificity for hexanoic acid, Cb_ptb and Bl_ptb show high specificity for butanoic acid. (D) Select variants (top 15 combinations) measured both in the automated plate-based assay at 4 μL volume and in (E) hand-pipetted assays at 30 μL volume in Eppendorf tubes. The set shows a positive correlation with a slope of 0.63 ± 0.10 and an R^2 of 0.59. Data represent n=3 independent experiments, with the standard deviation shown.

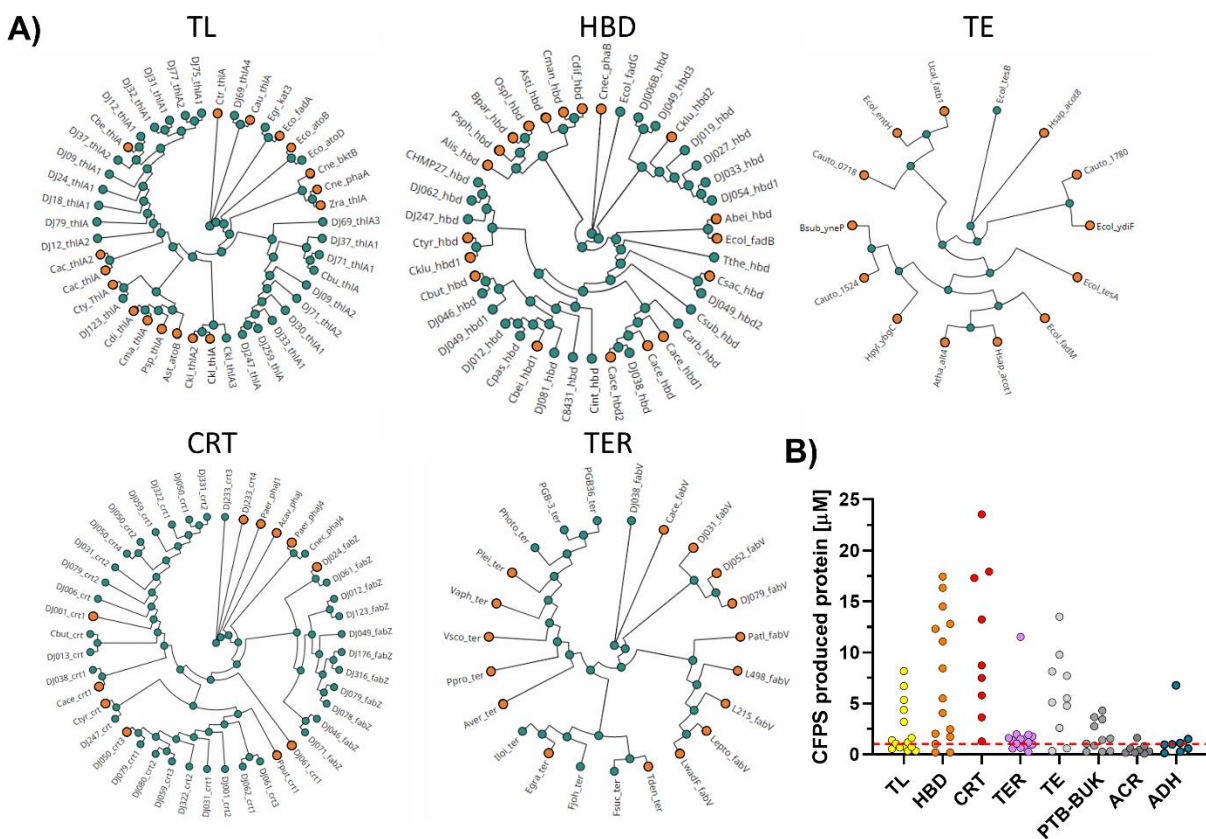


Figure S3. (A) Phylogenetic analysis of r-BOX enzymes using the MPI Tuebingen bioinformatics pipeline¹. Orange dots highlight the proteins included in our cell-free analysis. TL = thiolase, HBD = hydroxybutyryl-CoA dehydrogenase, CRT = crotonase, TER = trans enoyl-reductase, TE = thioesterase. **(B)** Soluble protein yields for the 100 selected rBOX enzymes classed by function. Red line indicates the cut-off for soluble protein classification. Detailed list of enzymes and expression yields in supplementary sheet FigureS2_enzyme list.

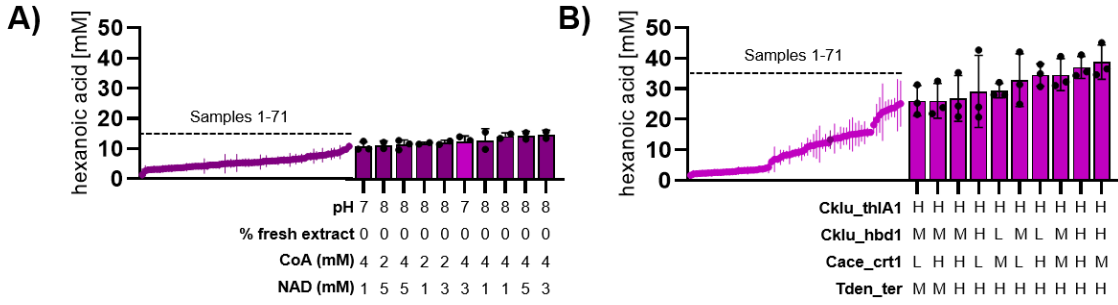


Figure S4. (A) Buffer, concentration of glycolytic enzymes and cofactor optimization for the best set of r-BOX enzymes from the screen. **(B)** Optimization of enzyme amounts by combinatorially testing all four enzymes at L = 0.05μM, M = 0.2μM and H = 0.6μM while keeping Ec_tesA at 0.10μM. Detailed results in supplementary sheet Figure2_screen. Data represent n=3 independent experiments, with the error bars showing standard deviation and mean shown.

References

- 1 Zimmermann, L. *et al.* A Completely Reimplemented MPI Bioinformatics Toolkit with a New HHpred Server at its Core. *J Mol Biol* **430**, 2237-2243, doi:10.1016/j.jmb.2017.12.007 (2018).