

Protein Design: New Approaches and Their Applications in The Development of Industrial Biocatalysts

Z. Chen, S. Rappert, D. Frank, R. Bommareddy, and A-P. Zeng

Introduction

Protein engineering has become one of the key technologies for the success in industrial biotechnology with regard to its important role in biocatalysis and strain development.¹ Rational protein design aims to construct novel enzymes with desired activity, stability, specificity and selectivity based on sequence, structure and biochemical properties of enzymes. These enzymes could be further assembled into functional modules or integrated into the cellular network of microbes to construct novel industrial bioprocesses or new strains¹. Toward this goal, our group has made large efforts in the method development as well as in the application of protein design. Two strategies are developed and well used in our practice: statistical evolutionary approach to analyze sequence-structure-function relationship within and between protein families, and molecular modeling to study proteins dynamics and their interactions with ligands/substrates. Based on these methods, protein design has been successfully applied to rewire the allosteric interaction network of enzymes in metabolic engineering and to design enzymes with new substrate/cofactor spectra and new functions (four EU patent applications since 2010). Together with other approaches of synthetic biology, we propose a new framework of structural synthetic biology for the construction of novel industrial biocatalysts (Fig.1).¹

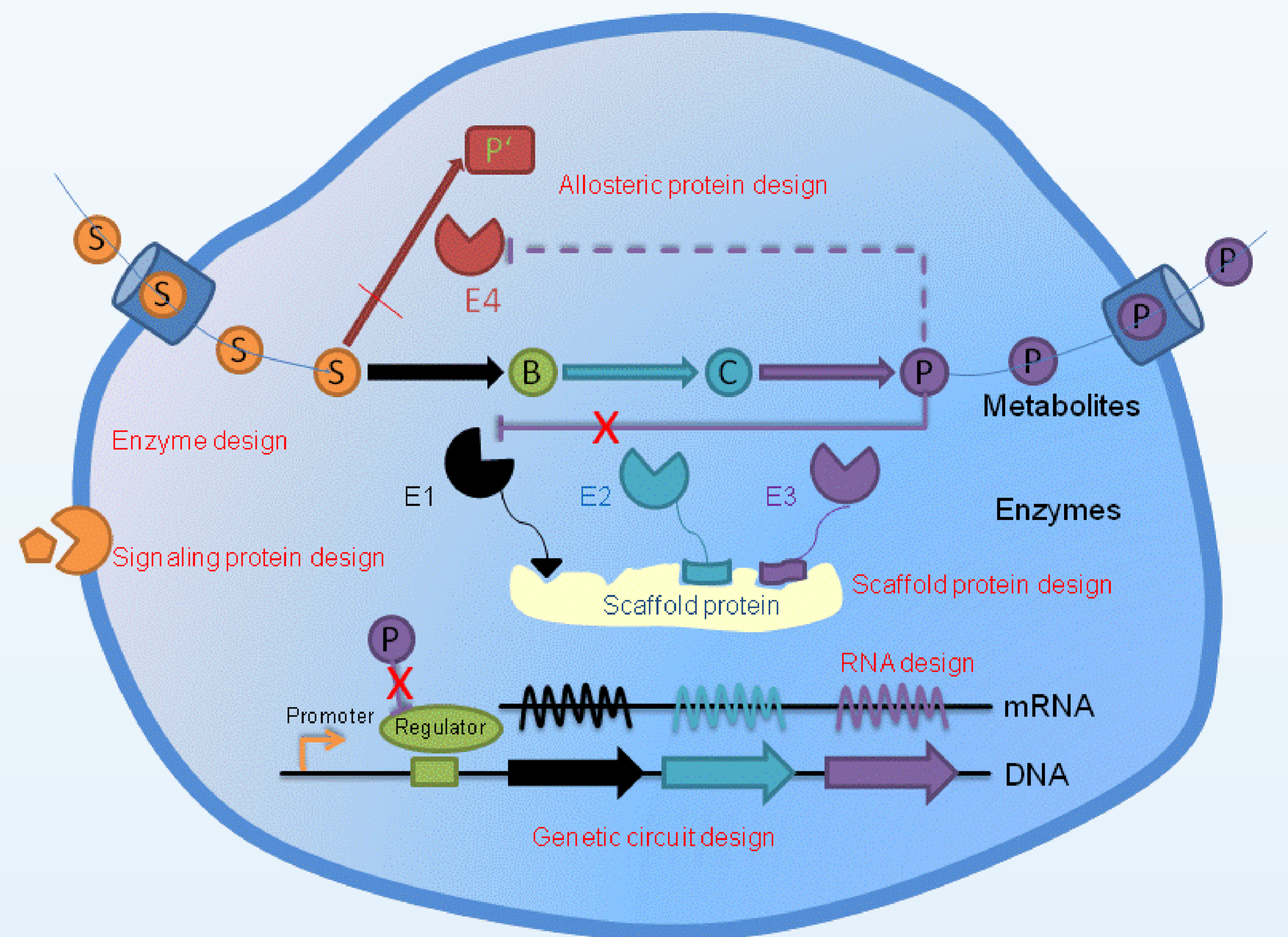


Fig.1 Application of protein design for the development of industrial biocatalysts.

Results and Discussion

As one of the practical examples, we demonstrated the successful application of protein design in the development of amino acid producing strains covering several aspects of metabolic engineering, such as deregulation of the allosteric inhibition of bottleneck enzymes, enhancement of precursor supply, construction of novel NADPH generation pathway, reduction of byproduct formation etc.^{2,3} The promising results demonstrate the high potential of protein design for industrial bioprocess development.

1) Construction of novel NADPH generation pathway (Fig.2)

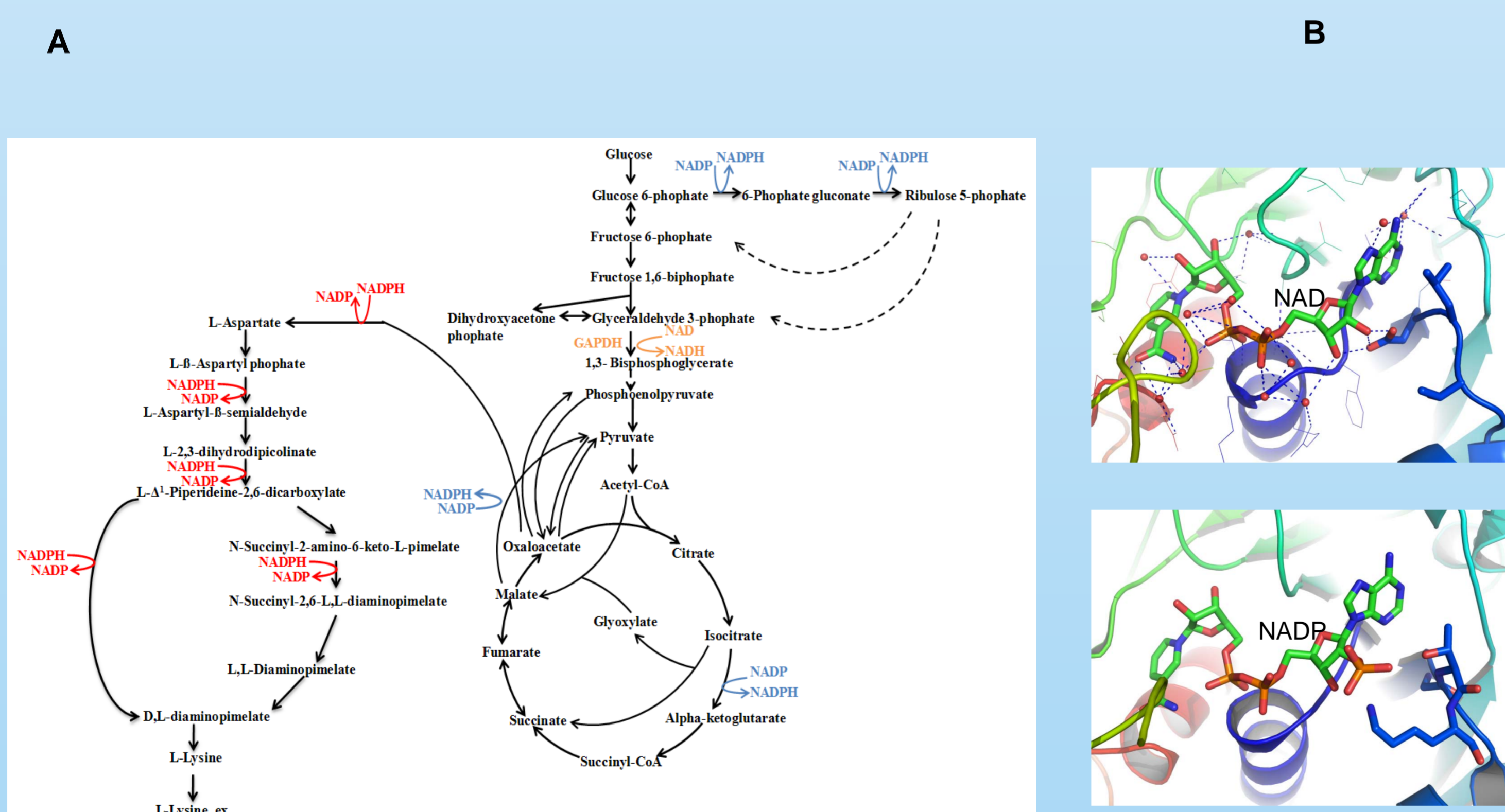


Fig.2 Construction of novel NADPH generation pathway. (A) The generation and consumption of NADPH in bacteria. A novel NADPH generation pathway was constructed by the conversion of the coenzyme specificity of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) from NAD to NADP. (B) A successful design of NADP-dependent GAPDH.

Search:

- Biocatalysis processes of interest
- Cooperation partners

Contact

¹Prof. Dr. An-Ping Zeng
Hamburg University of Technology
Institute of Bioprocess- and Biosystems Engineering, Denickestr. 15, D-21073 Hamburg, Germany
Phone: ++49 / 40 / 42878-4183, e-mail: aze@tuhh.de

2) Deregulation of feedback inhibition of enzymes (Fig.3, Fig.4)

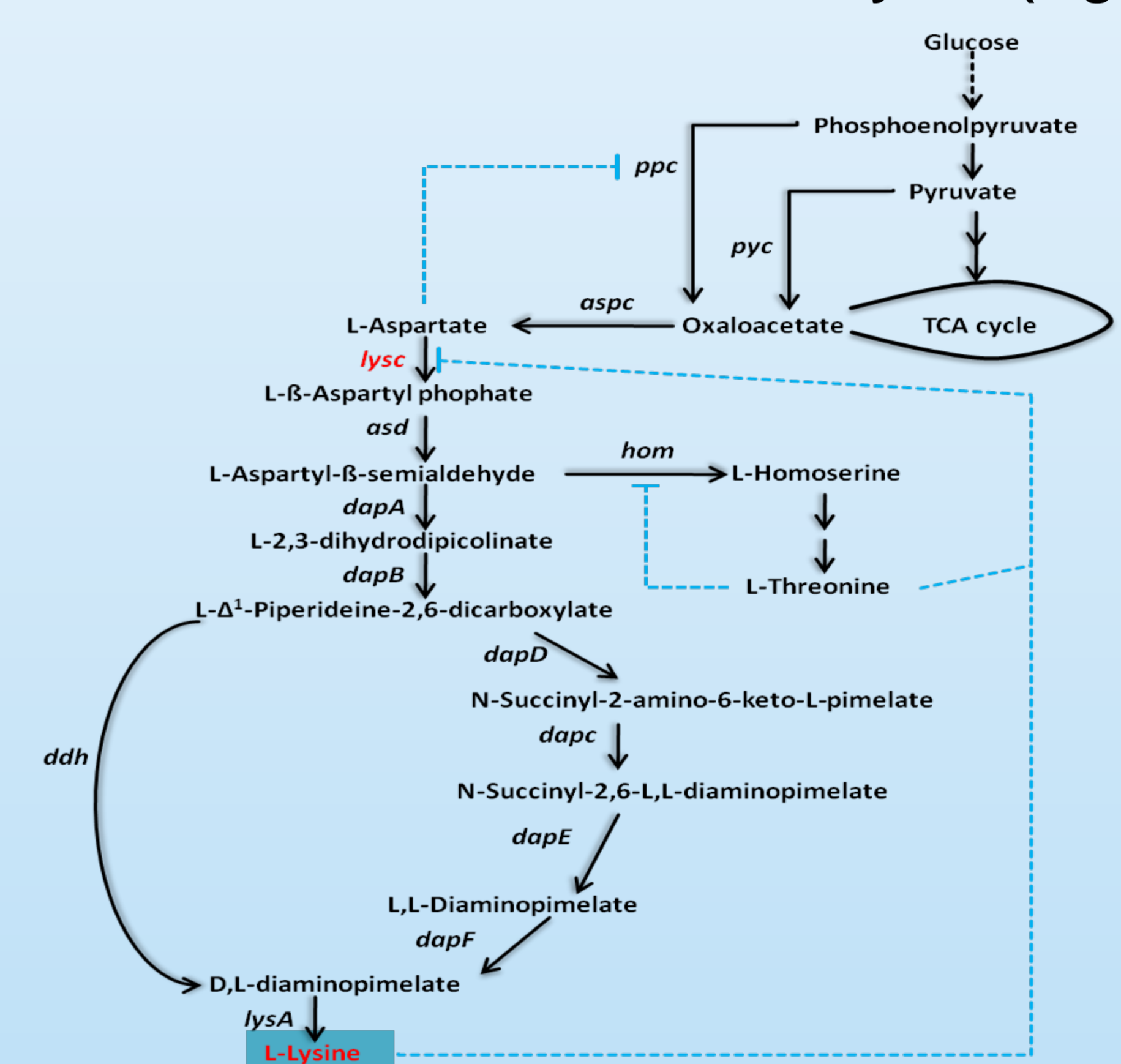


Fig.3 The feedback inhibition of enzymes for lysine synthesis

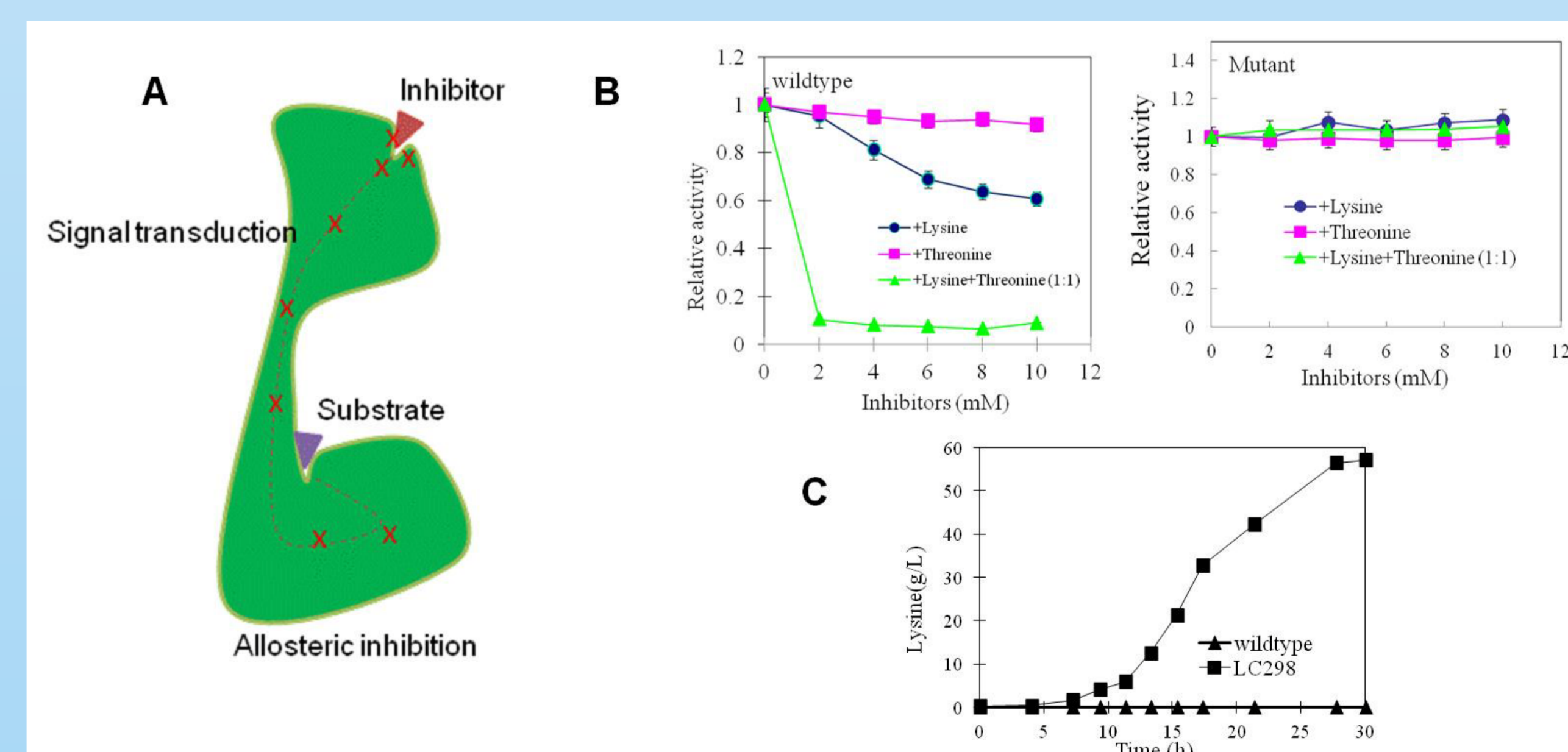


Fig.4 Deregulation the feedback inhibition of aspartokinase (*lysC*) and its impact for lysine production. (A) Rational deregulation of the feedback inhibition based on the destroy of inhibitor's binding sites or signal transduction pathway; (B) The inhibition profile of aspartokinase and its mutant. (C) Lysine production based on single point mutation.

Offer:

- Enzyme design and pathway design
- Strain development and characterization

References

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2. Chen Z, Rappert S, Sun J, Zeng AP. (2011) J. Biotechnol. 154, 248-254.
3. Chen Z, Meyer W, Rappert S, Sun J, Zeng AP. (2011) Appl. Environ. Microbiol. 77, 4352-4360.