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Structure-based Metabolic Engineering and Synthetic Biology for Efficient Strain Development

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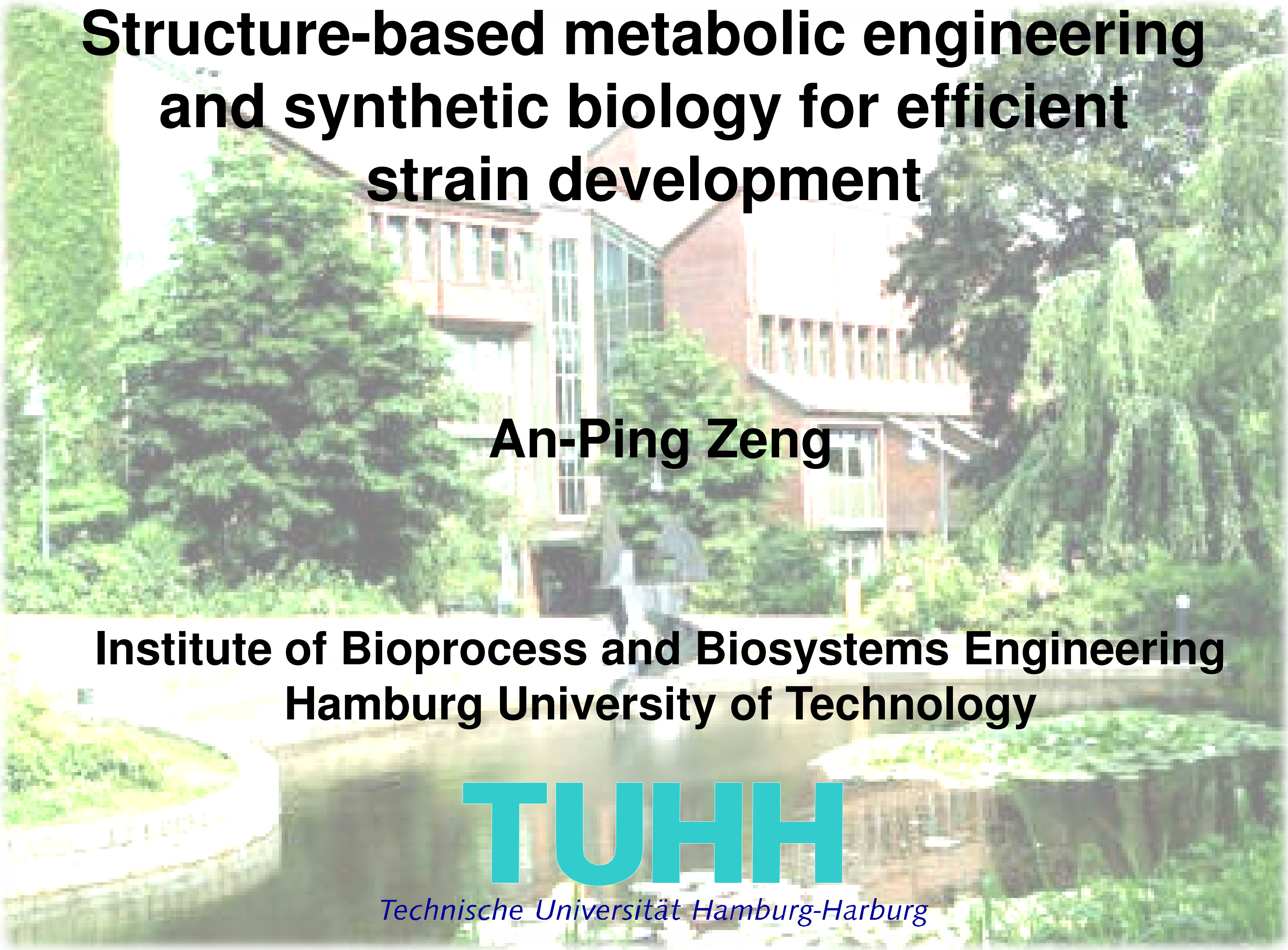

Structure-based metabolic engineering and synthetic biology for efficient strain development

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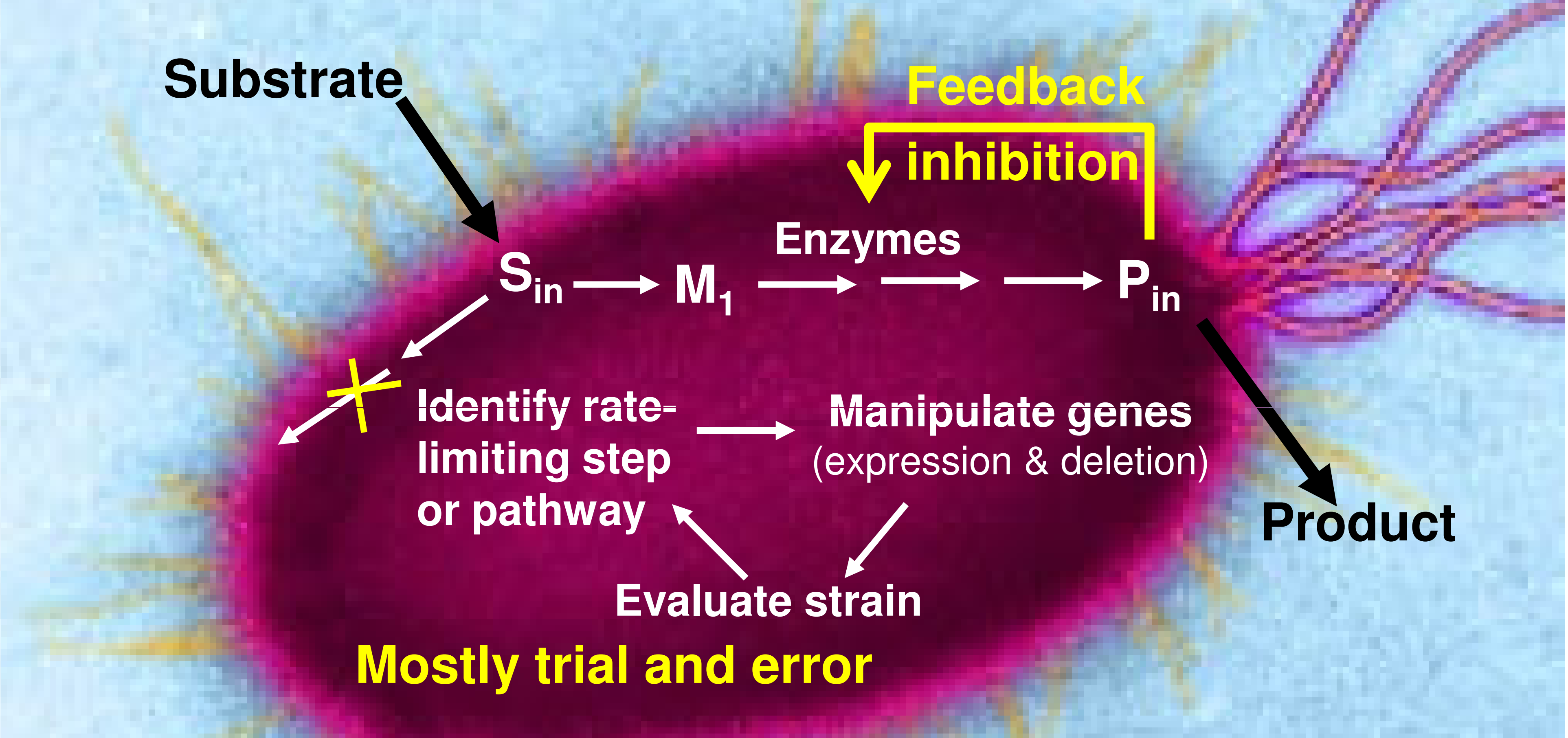
Our research areas:

1.Design of biocatalysts
2.Systems Biology / Modeling
3.Integrated bioprocesses / Biorefinery

Present praxis of metabolic engineering and its limitation in strain development



Substrate

Feedback inhibition

Enzymes

S_{in} → M_1 → → → P_{in}

Product

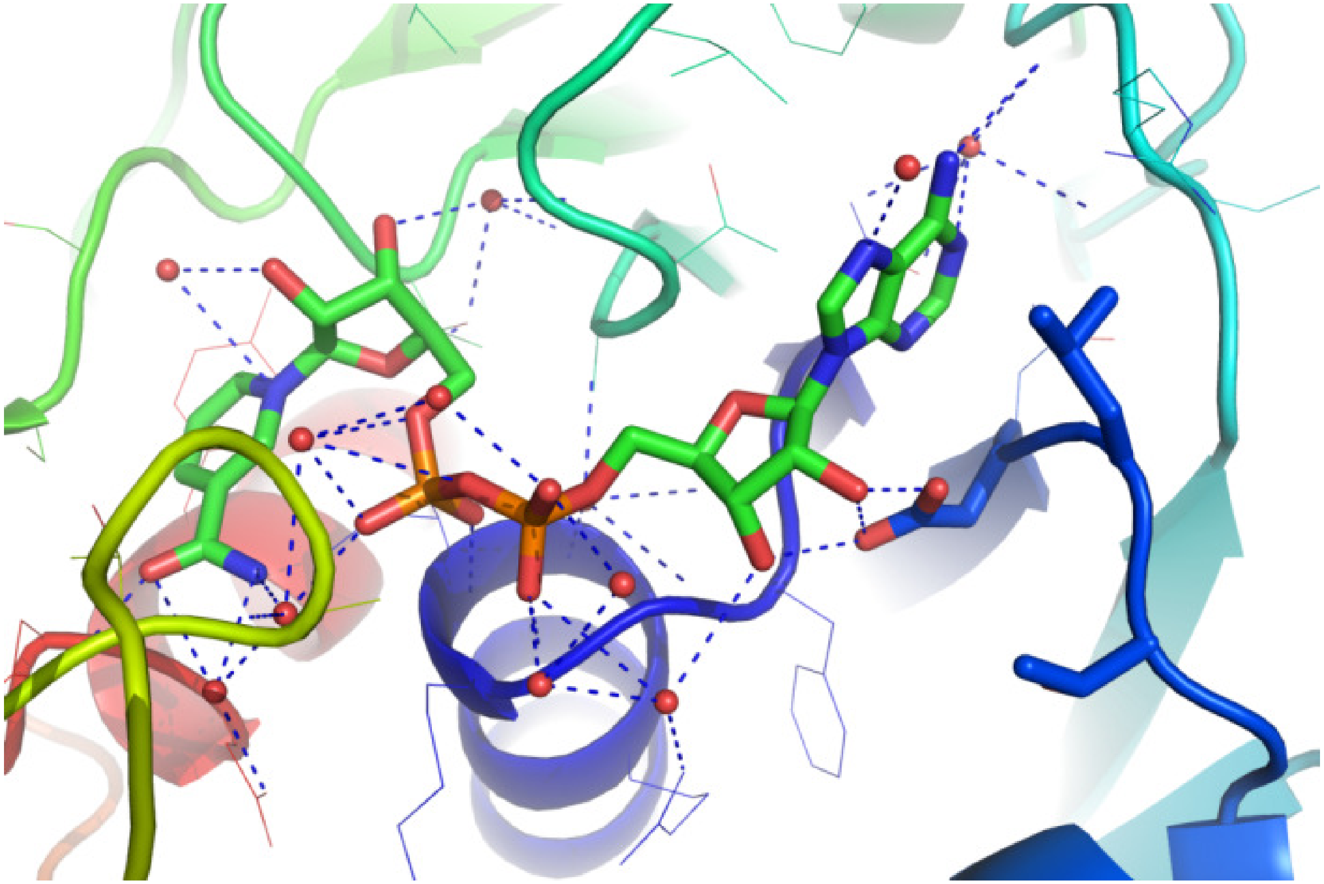
Identify rate-limiting step or pathway → Manipulate genes (expression & deletion) → Evaluate strain

Mostly trial and error

◆ Not efficient for protein-level limitation

◆ Often Yes/No changes/lack of dynamic control

Design and control of bioreactions at atomic level and from first principle



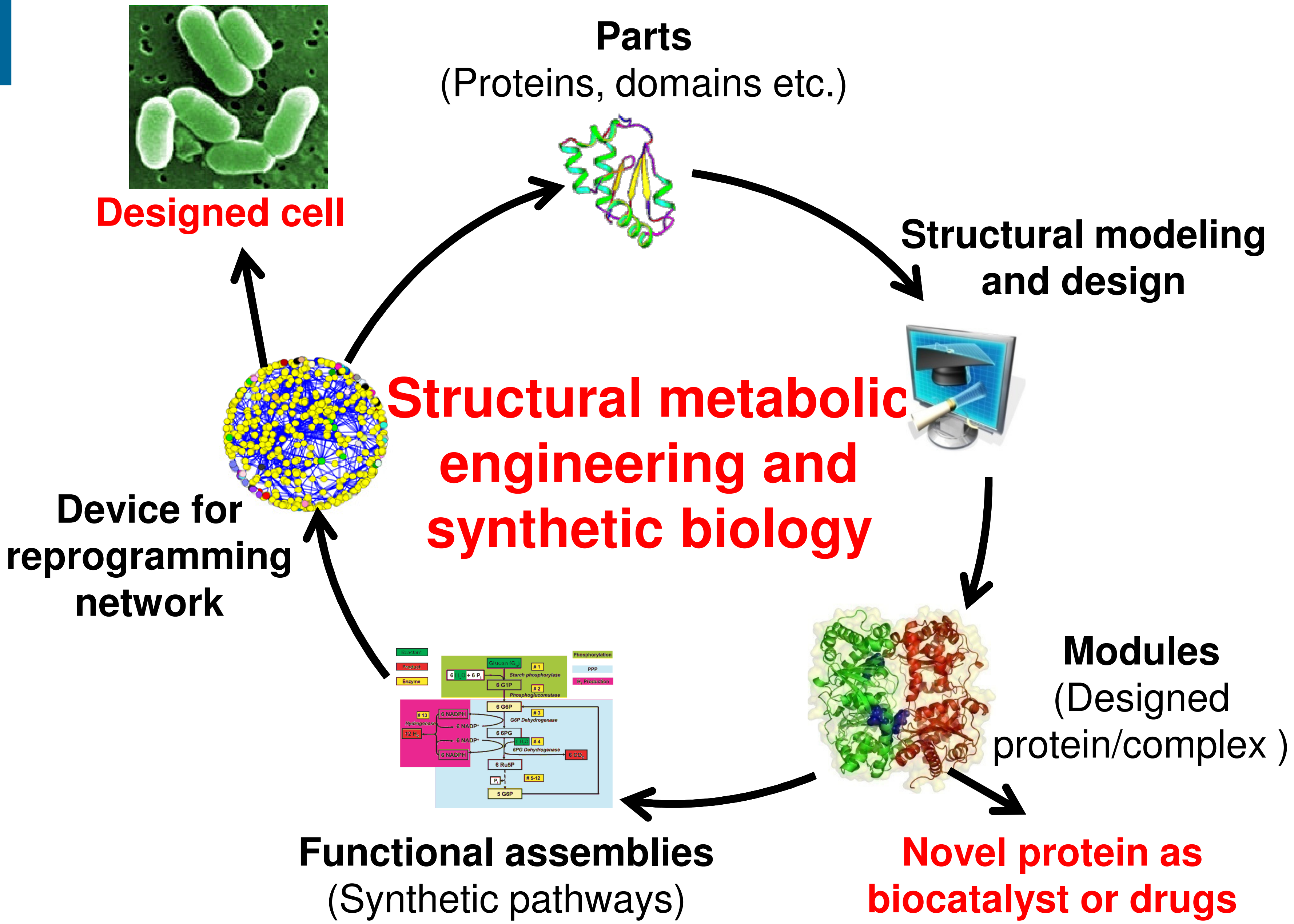
Make use of the potentials in structural biology and computing

World’s most brilliant X-ray and light sources for structural study at DESY/Hamburg

Beyond the static structure

also „see“ dynamics at fs (10^{-15}s)!

Petra III European free-electron laser facilities



Designed cell

Parts
(Proteins, domains etc.)

Structural modeling and design

Structural metabolic engineering and synthetic biology

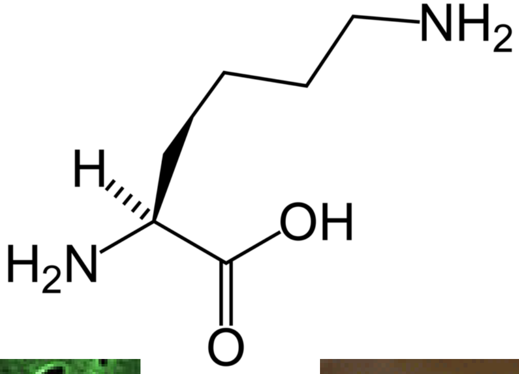
Device for reprogramming network

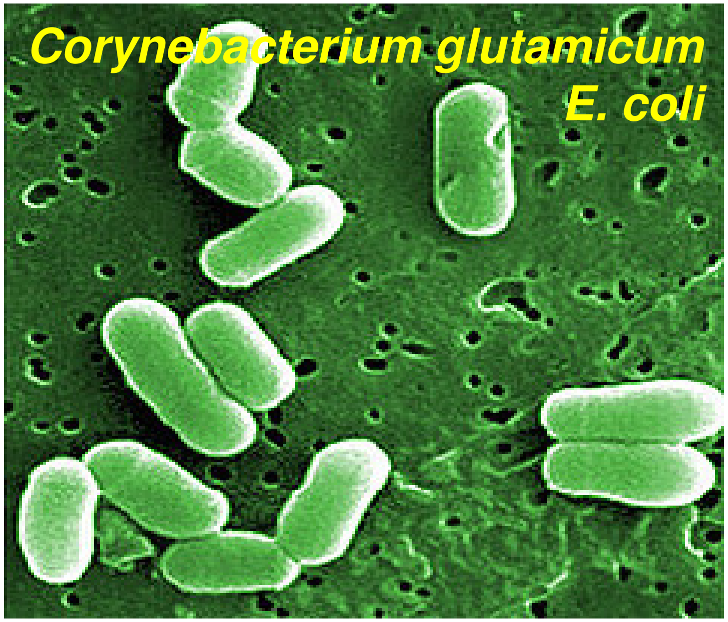
Modules
(Designed protein/complex)

Novel protein as biocatalyst or drugs

Functional assemblies
(Synthetic pathways)

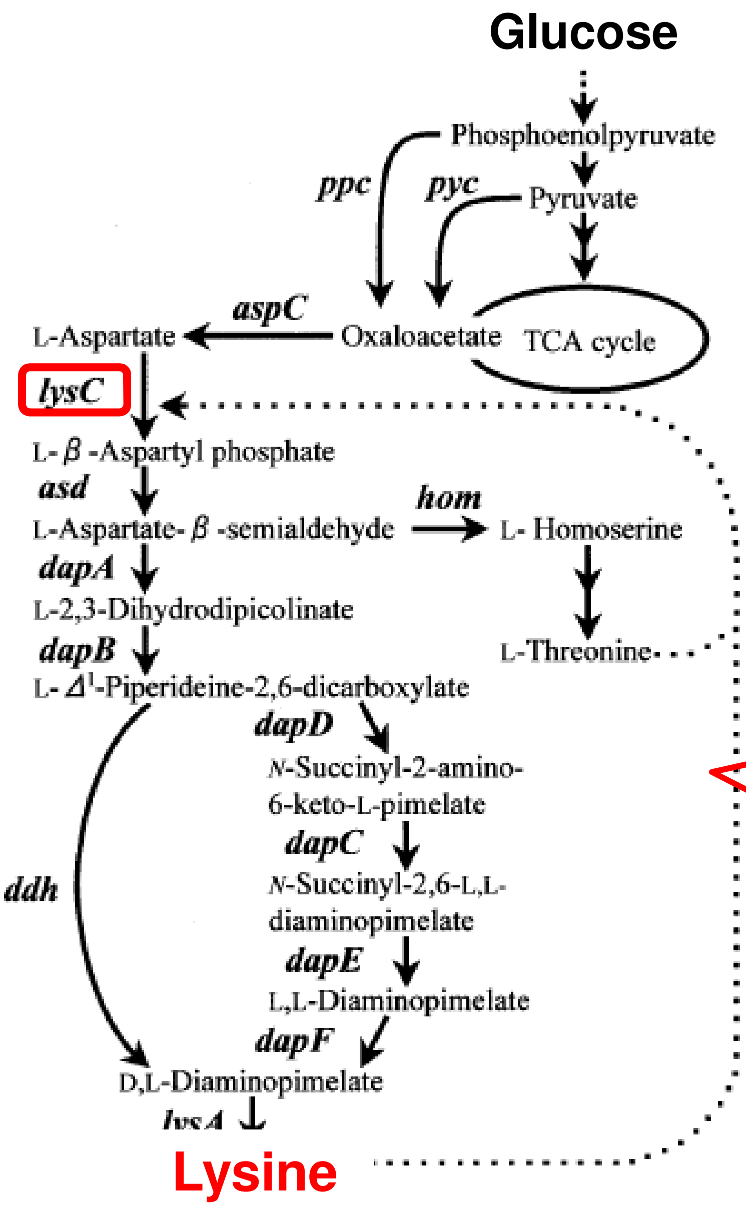
Example: Strain development for amino acid production




Corynebacterium glutamicum
E. coli


Lysine: > 1.5 Mio. t/y

Problem: Allosteric feedback inhibition



Example: Aspartokinase (*lysC*)

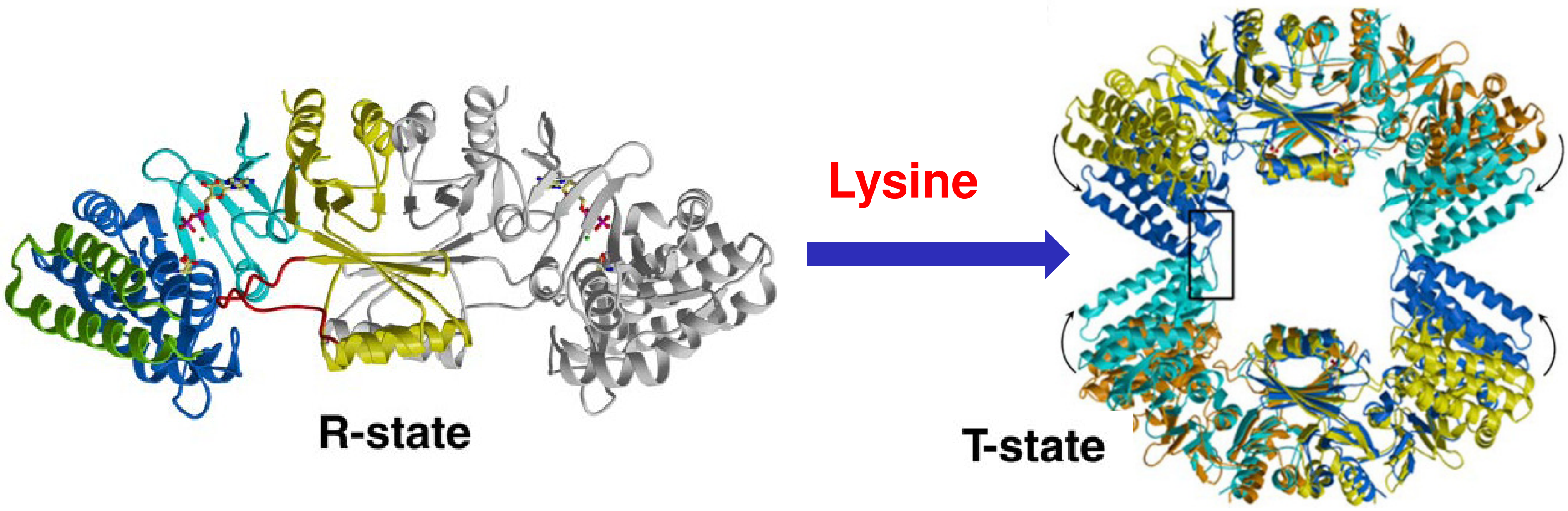
- **catalyse the first and limiting step of synthesis of aspartate family amino acids**

Allosteric inhibition

A general problem limiting product yield and titer in many industrial bioprocesses!

How to combat allosteric inhibition?

Traditional view of allostery: Binding of an effector and conformational change of the enzyme



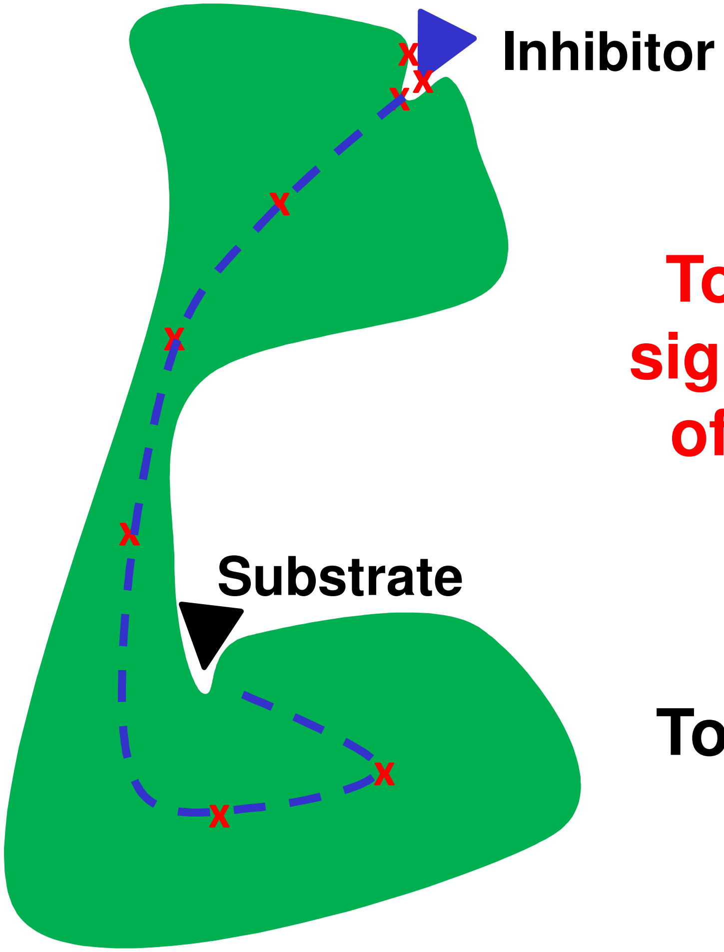
Solution:
Mutation of the enzyme to avoid effector binding !

Random mutation over 50 years identified 6 (reported) targets for lysC

- **Random Mutation:** e.g. UV-Light, X-ray
- **Selection:** Analoge Chemicals, Auxotrophic

Time-consuming and many other disadvantages!
Patenting

Conventional approach identified mainly mutations on the protein surface

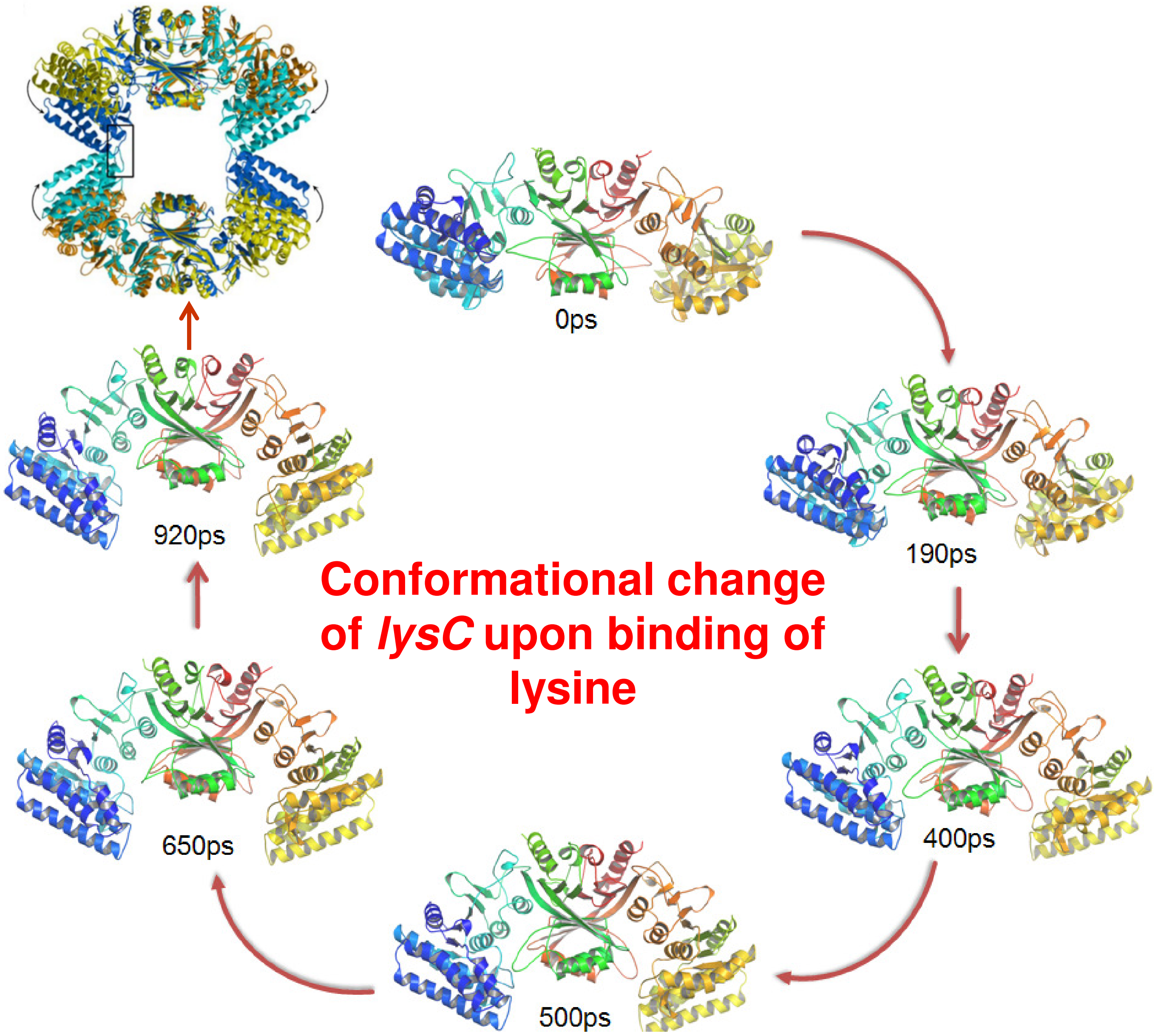


Our new approach:
To identify and modify the signal transduction pathway of conformational change

Key:
To find the residue-residue interaction network

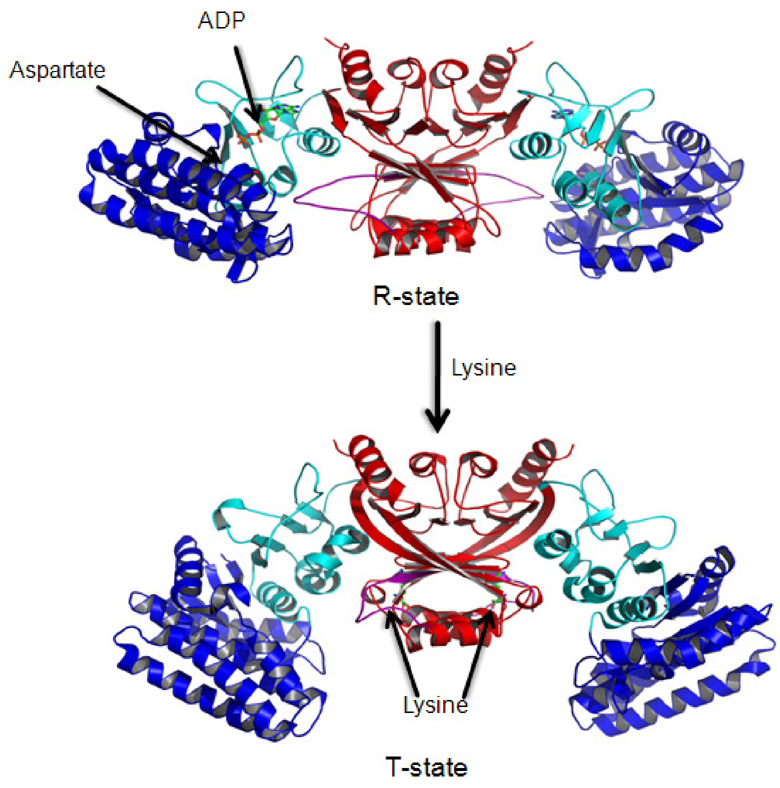
Z. Chen et al. Appl. Environ. Microbiol. 2011; Z. Chen et al. J. Biotechnol 2011

Targeted molecular dynamic simulation

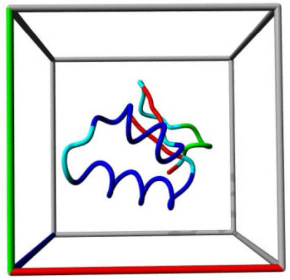


Conformational change of *lysC* upon binding of lysine

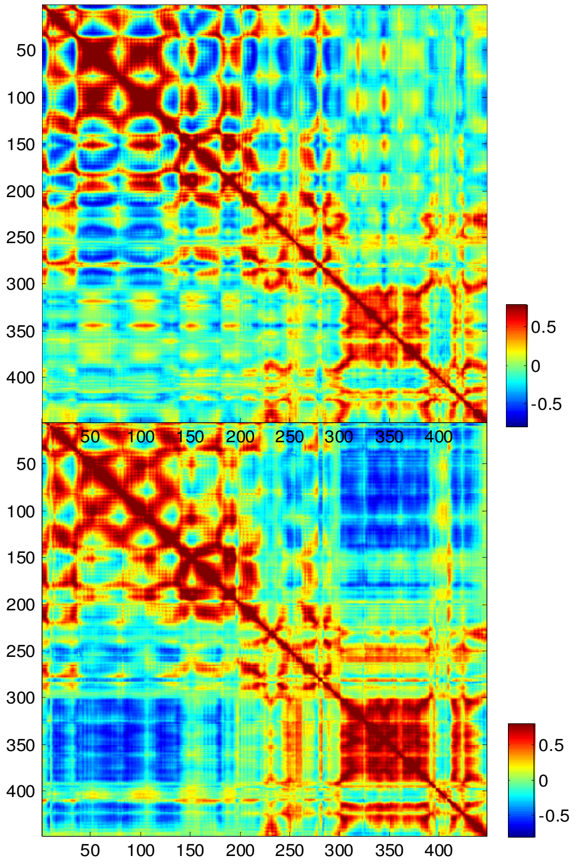
Mapping the patterns of atomic motion to dynamic residue interaction network



MD without effector

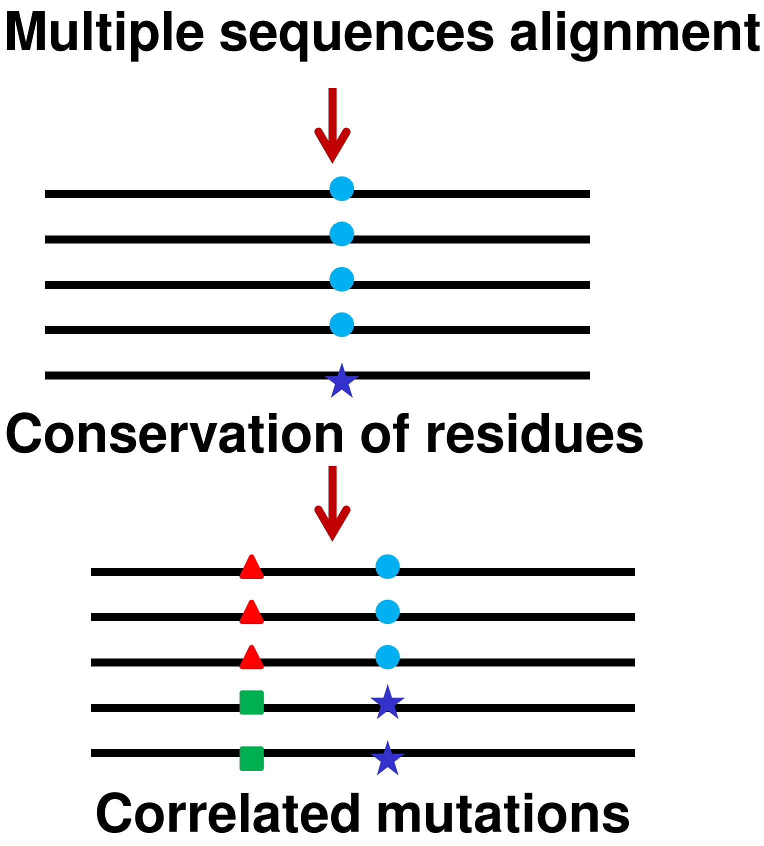


MD with effector



Correlated coefficient matrix

Co-evolutionary analysis is also very useful to study allostery

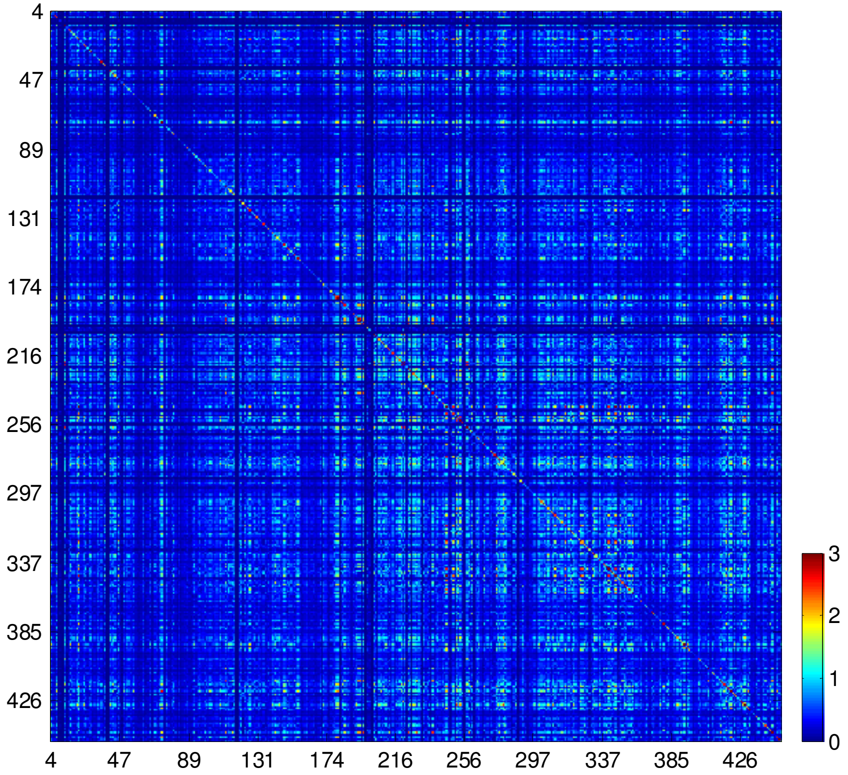


Multiple sequences alignment

Conservation of residues

Correlated mutations

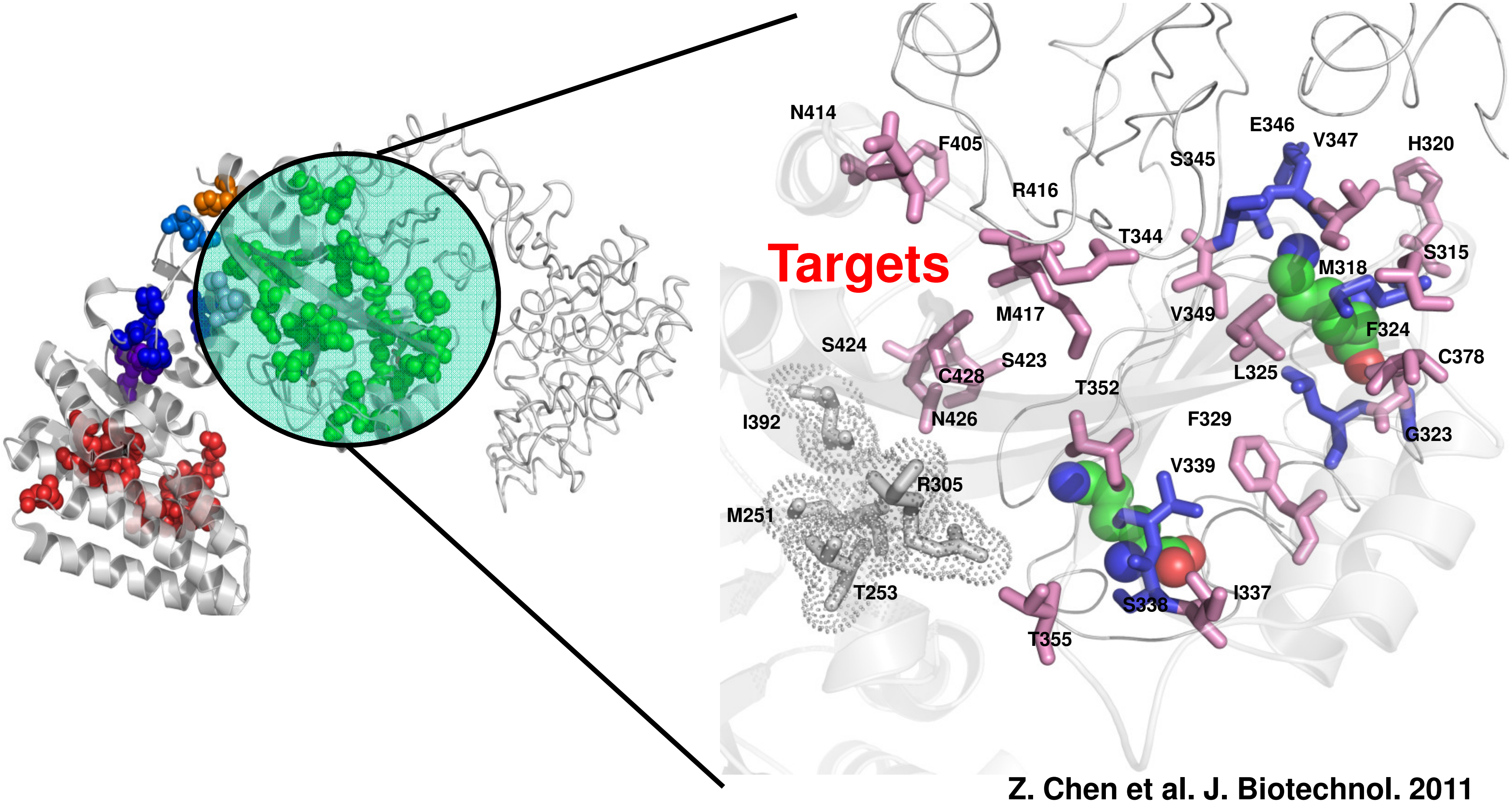
Correlated mutation matrix



-Structurally or functionally relevant
-Adopted to import new function or regulation

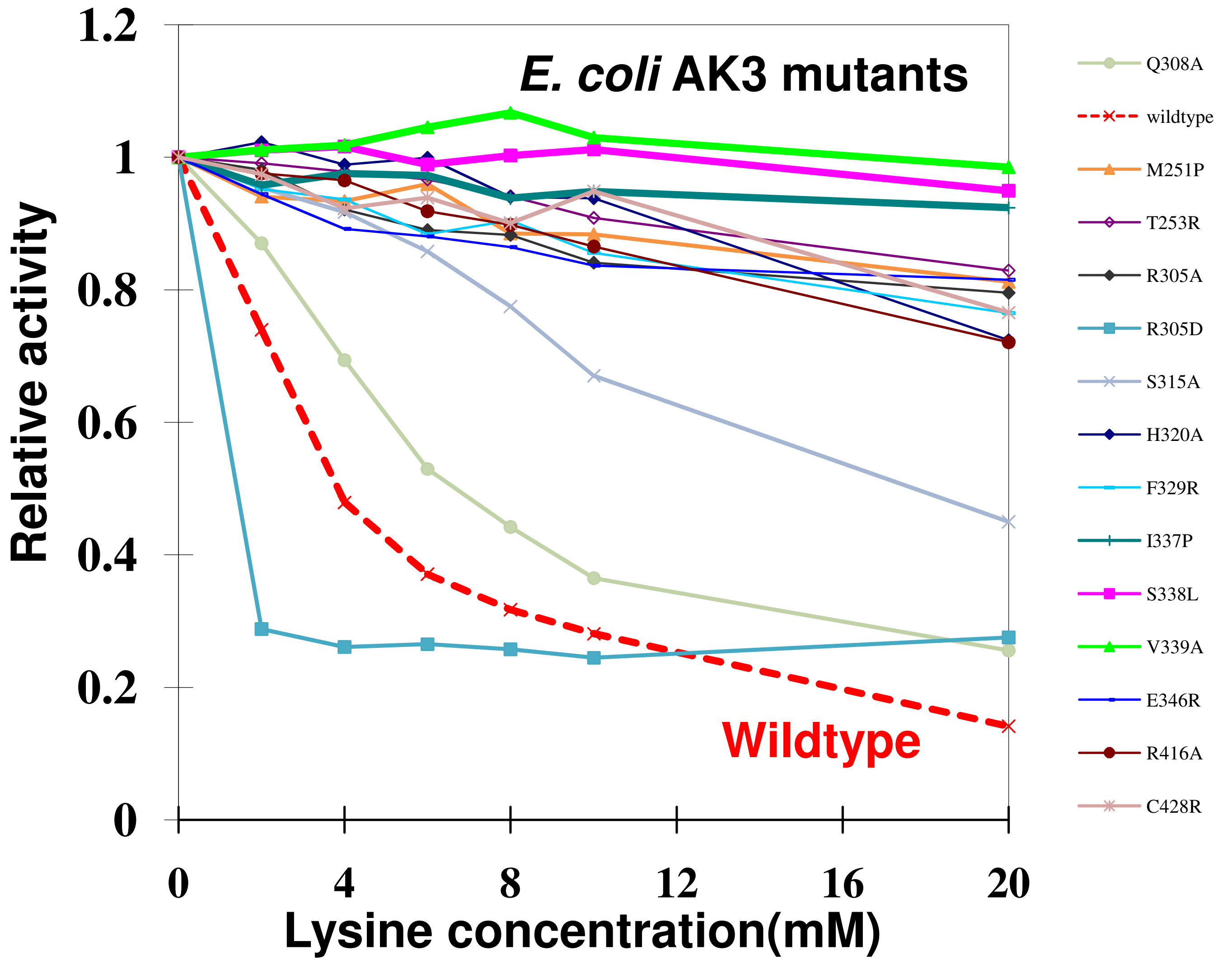
Evolutionary and MD analyses revealed signaling network of AK3 at atomic level

All the previous 6 mutations from the last decades also found!

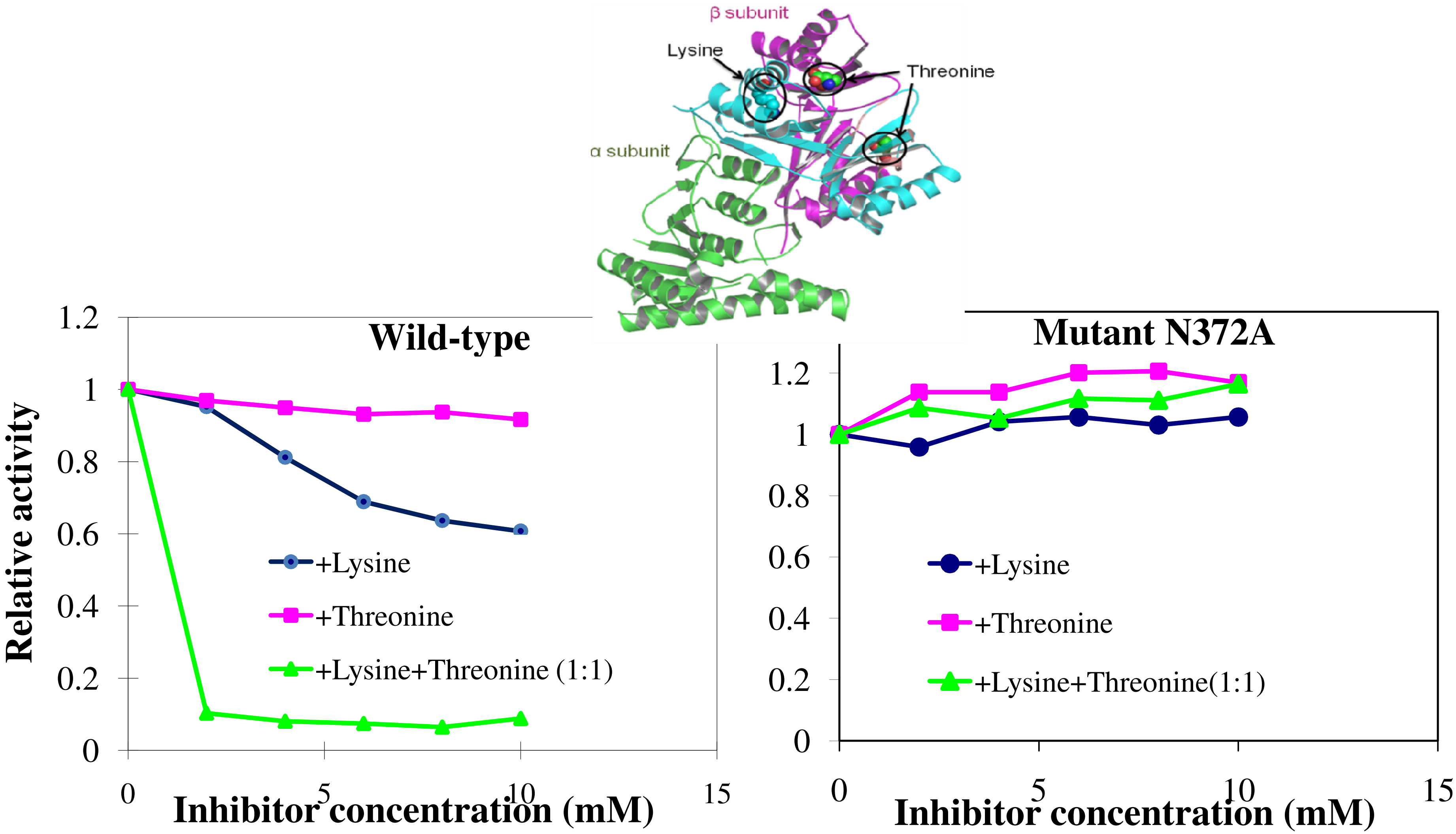


Z. Chen et al. J. Biotechnol. 2011

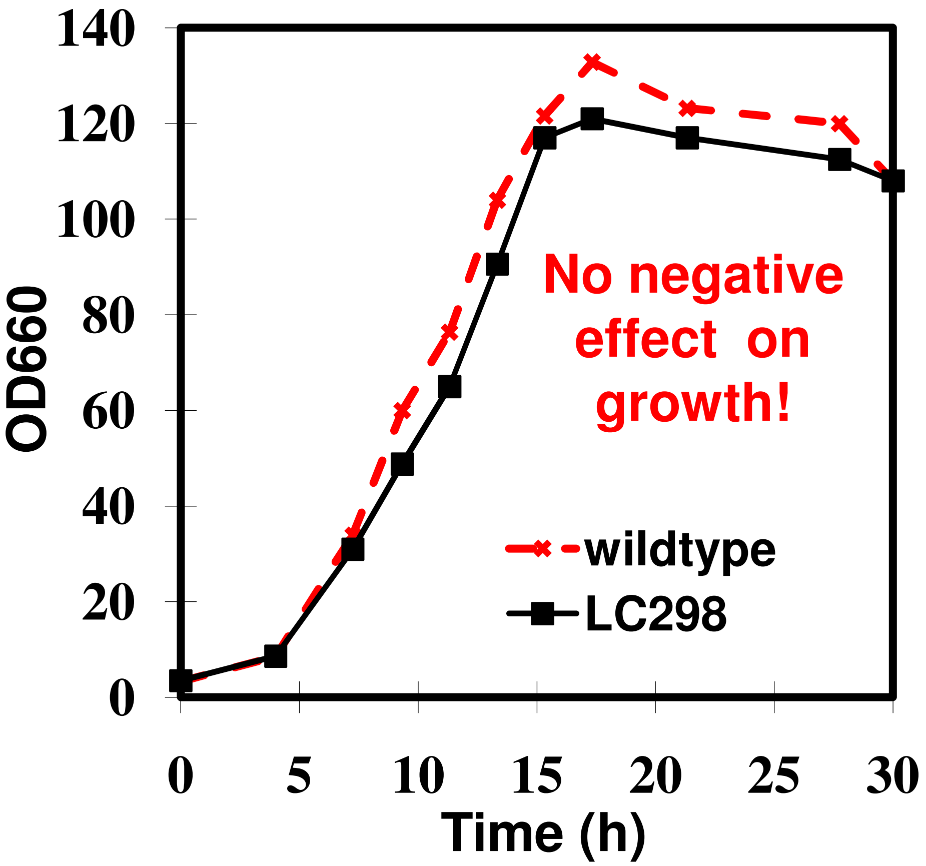
30 mutations predicted and experimentally confirmed with reduced feedback inhibition



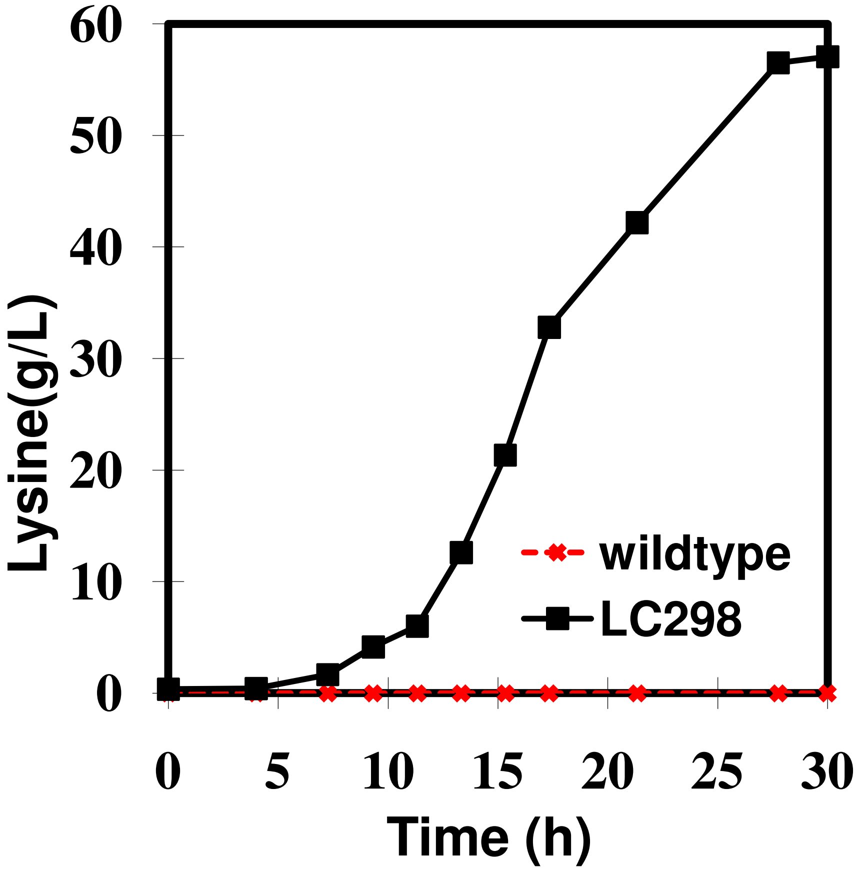
Predictions also applied to aspartokinase from *C. glutamicum*



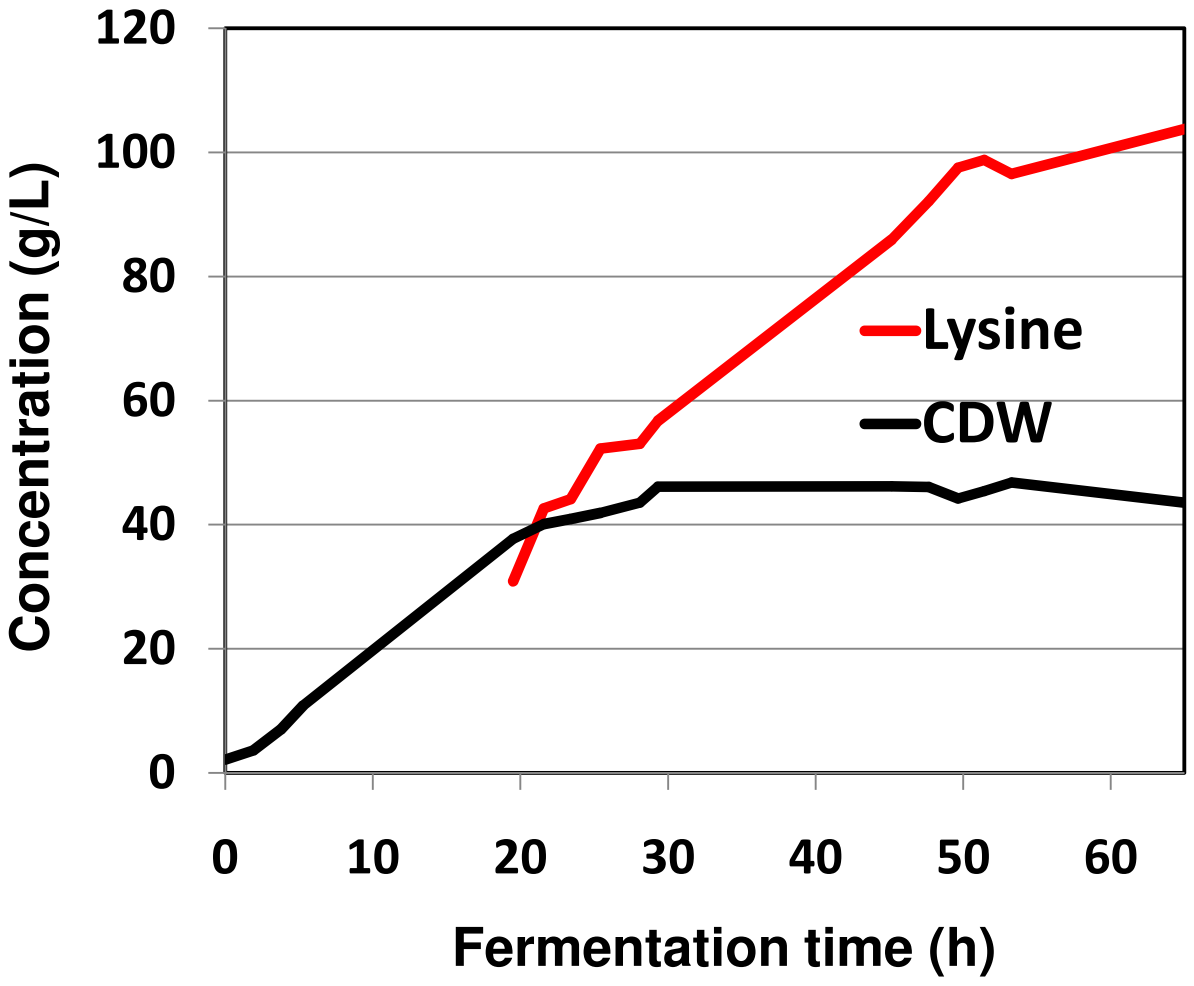
Wildtype vs. point mutation of lysC for lysine production in *C. glutamicum*



No negative effect on growth!

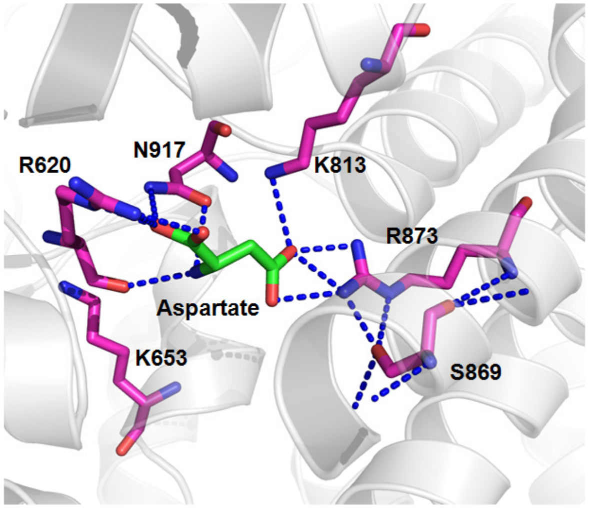


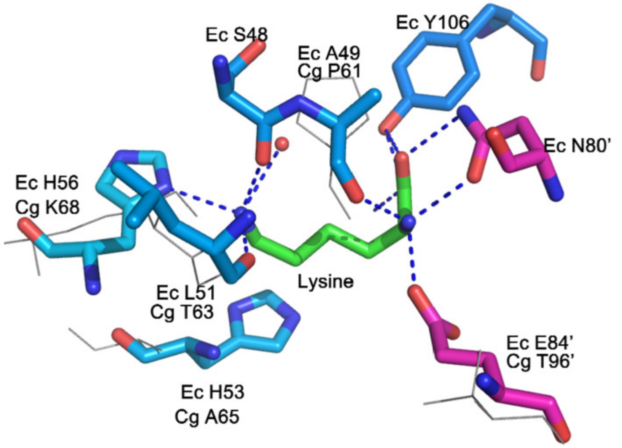
From „zero to hero“ with a single mutation and in a minimal medium



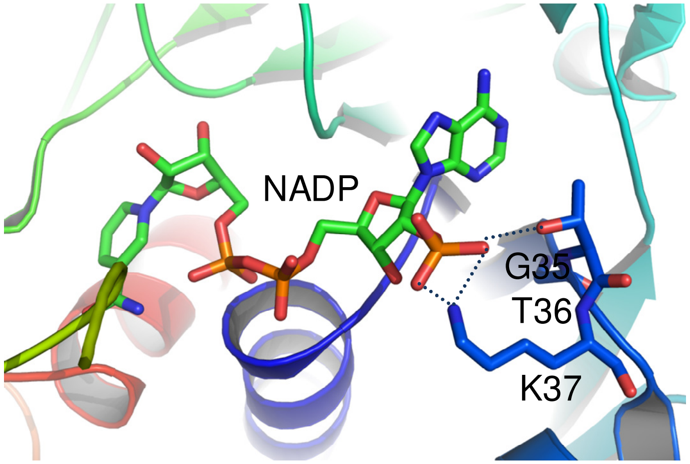
Systematic rational design of pathways

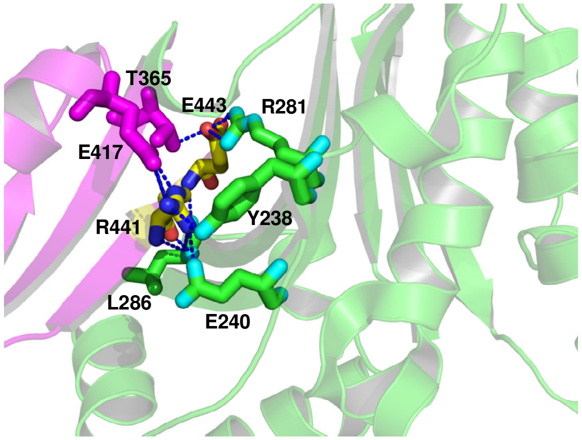
Enhance precursor availability





Increase NADPH supply

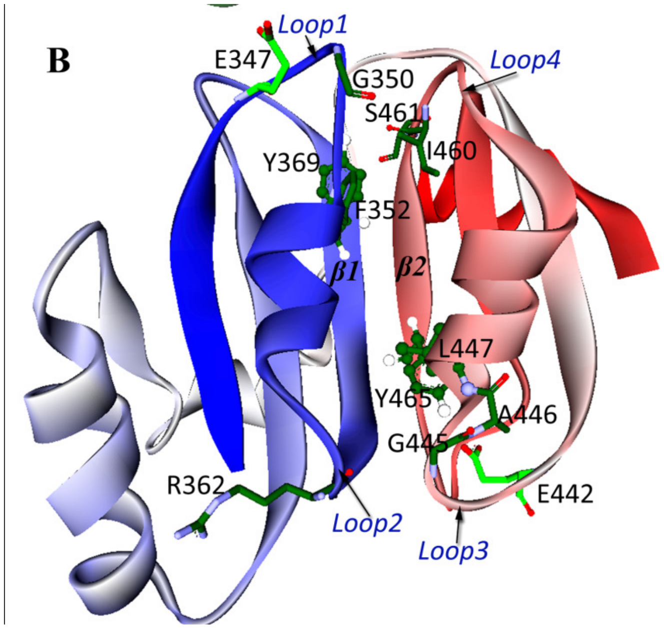


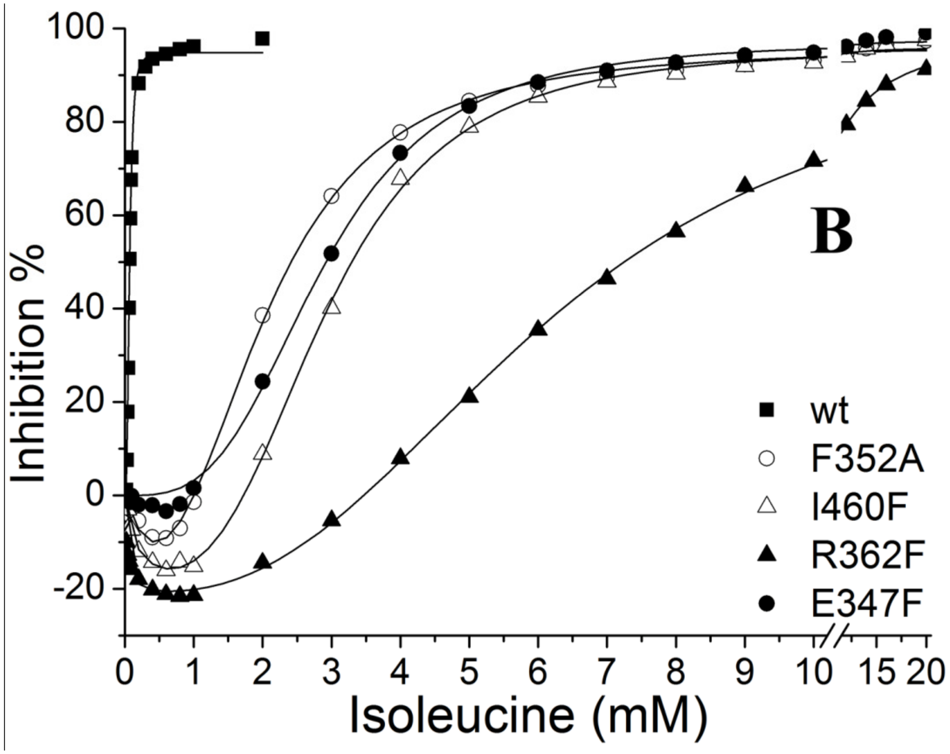


On the way beyond 150 g/L by design in short time!

Chen et al. *J. Biotechnol.* 2011; AEM. 2011; Geng et al. AMB, 2012, Zeng et al. Patent applications

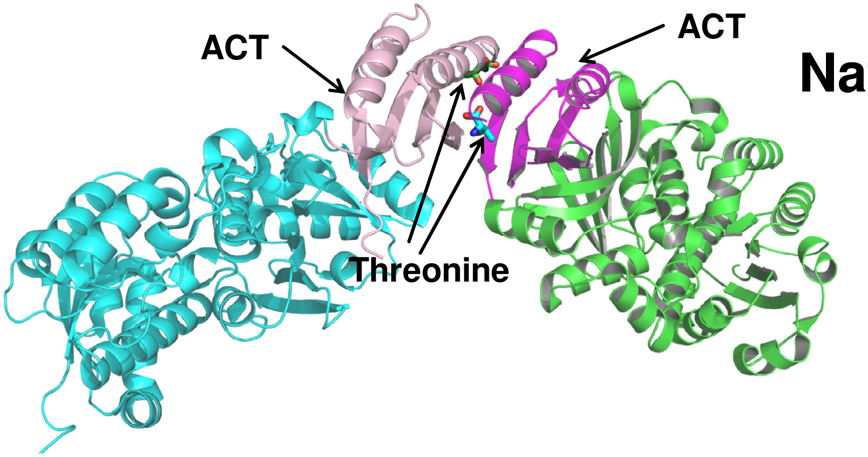
Reengineering of threonine deaminase for theronine production in *E.coli*





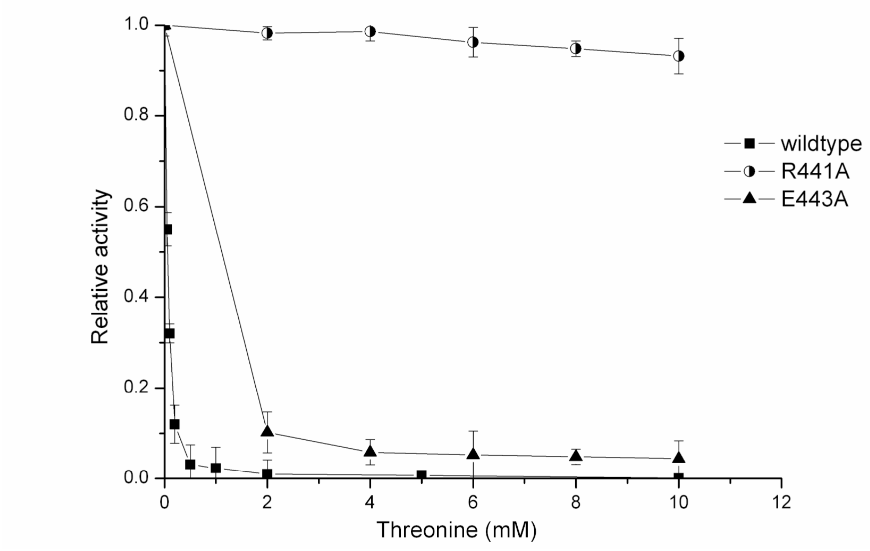
L. Chen et al. Appl Microb Biotechnol 2012

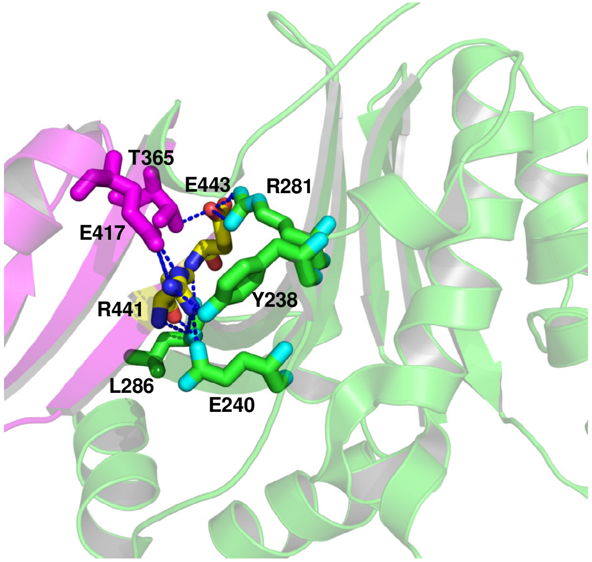
Redesign of homoserine dehydrogenase



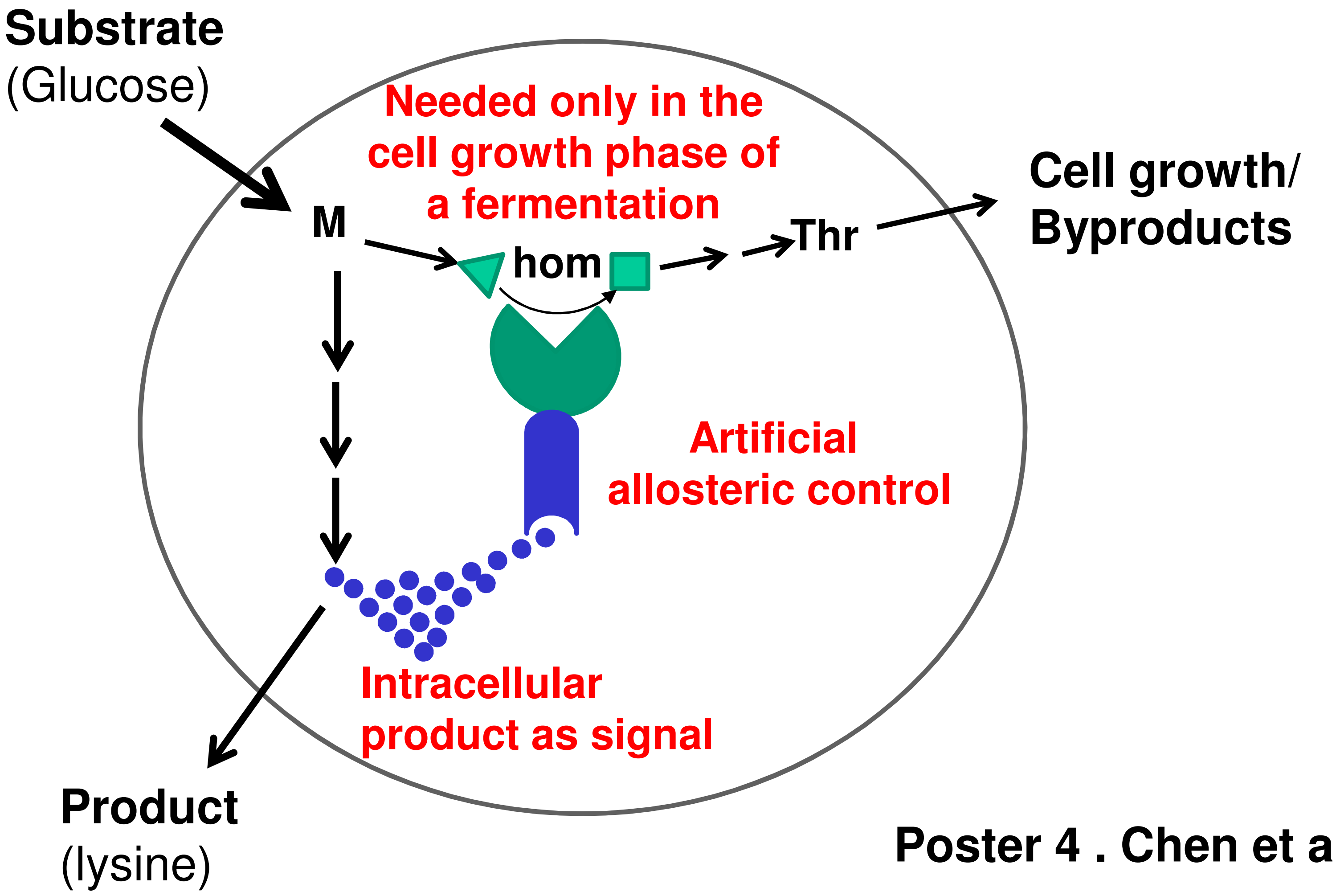
Natively inhibited by threonine

Design based on predicted allosteric signal transduction between catalytic domain and regulatory domain





Synthetic protein for dynamic and self-regulation of biosynthesis



Substrate
(Glucose)

Needed only in the cell growth phase of a fermentation

Cell growth/
Byproducts

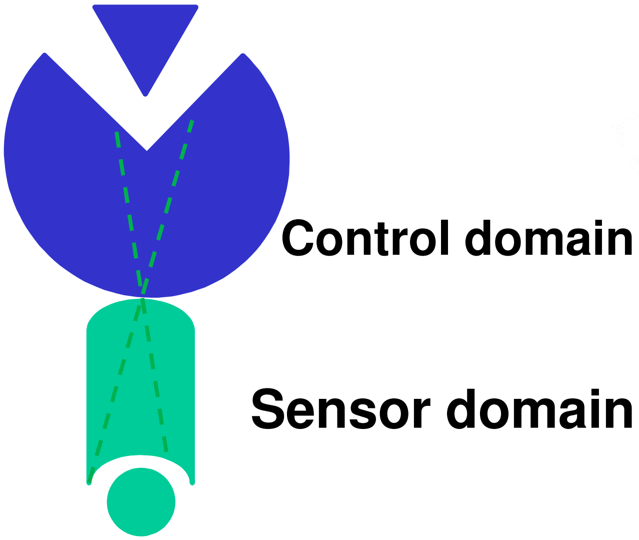
Artificial allosteric control

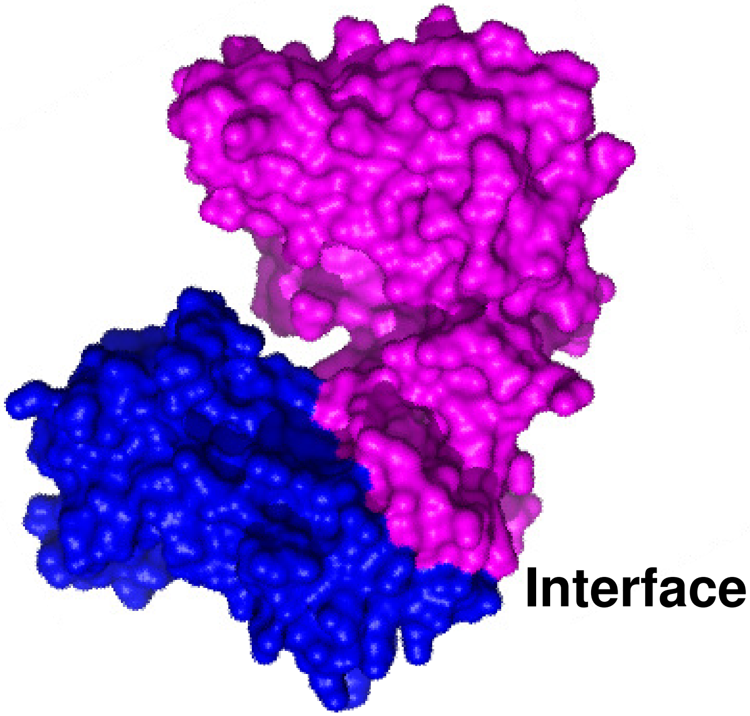
Intracellular product as signal

Product
(lysine)

Poster 4 . Chen et al.

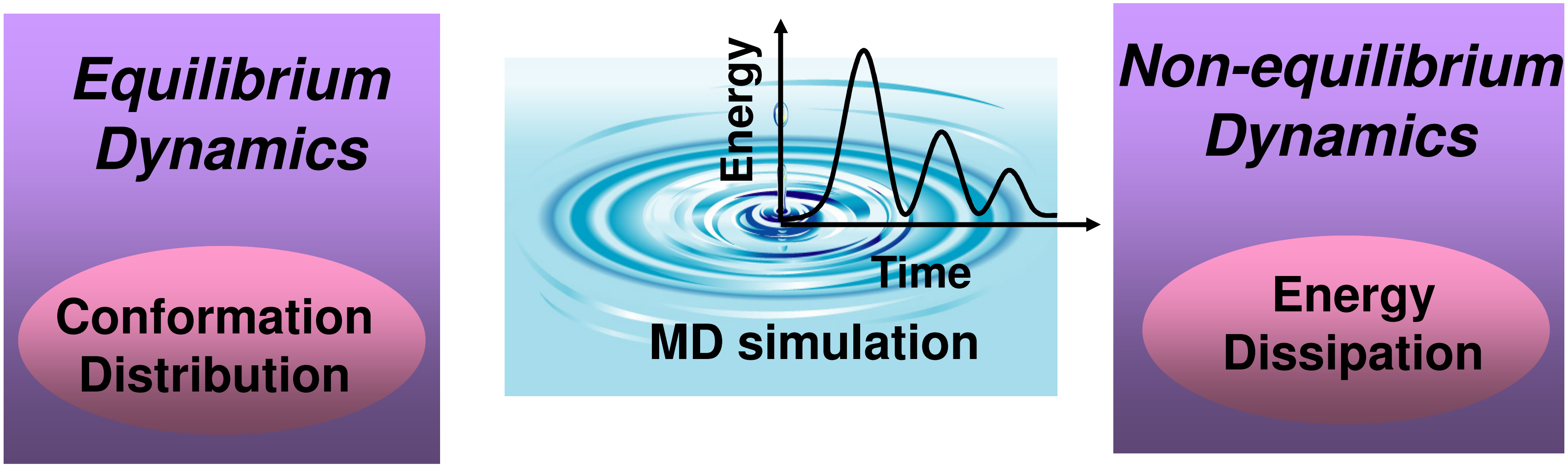
Modular design of artificial enzyme





- Redesign of signal molecule binding sites in the sensor domain
- Identify the (allosteric) signaling pathway
- Protein-protein docking to predict domain-domain interaction mode
- Design of specific linker to provide potential domain interface
- Combination of rational design with directed evolution

New concept for protein design: From structure via dynamic modules to function



Equilibrium Dynamics

Conformation Distribution

MD simulation

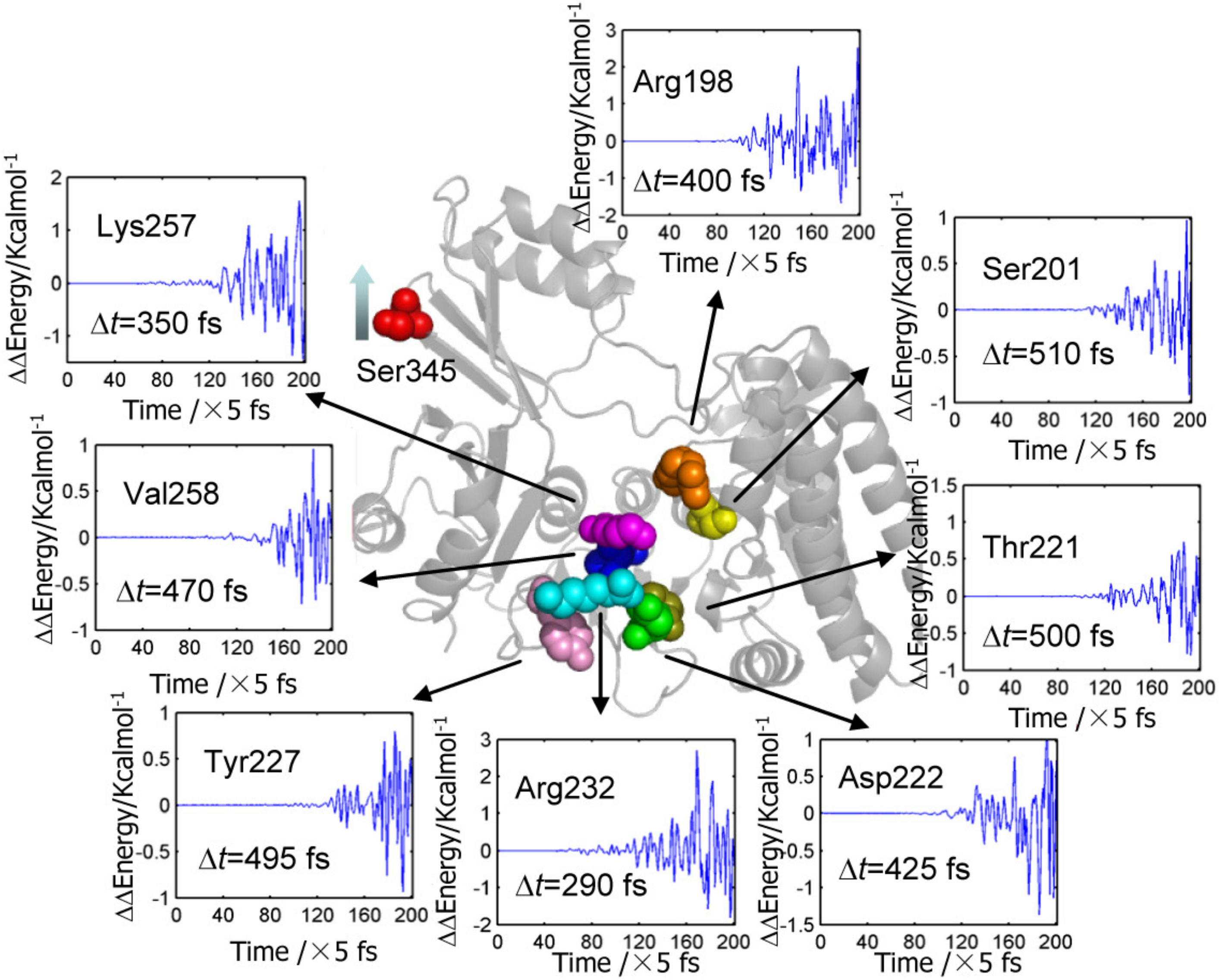
Non-equilibrium Dynamics

Energy Dissipation

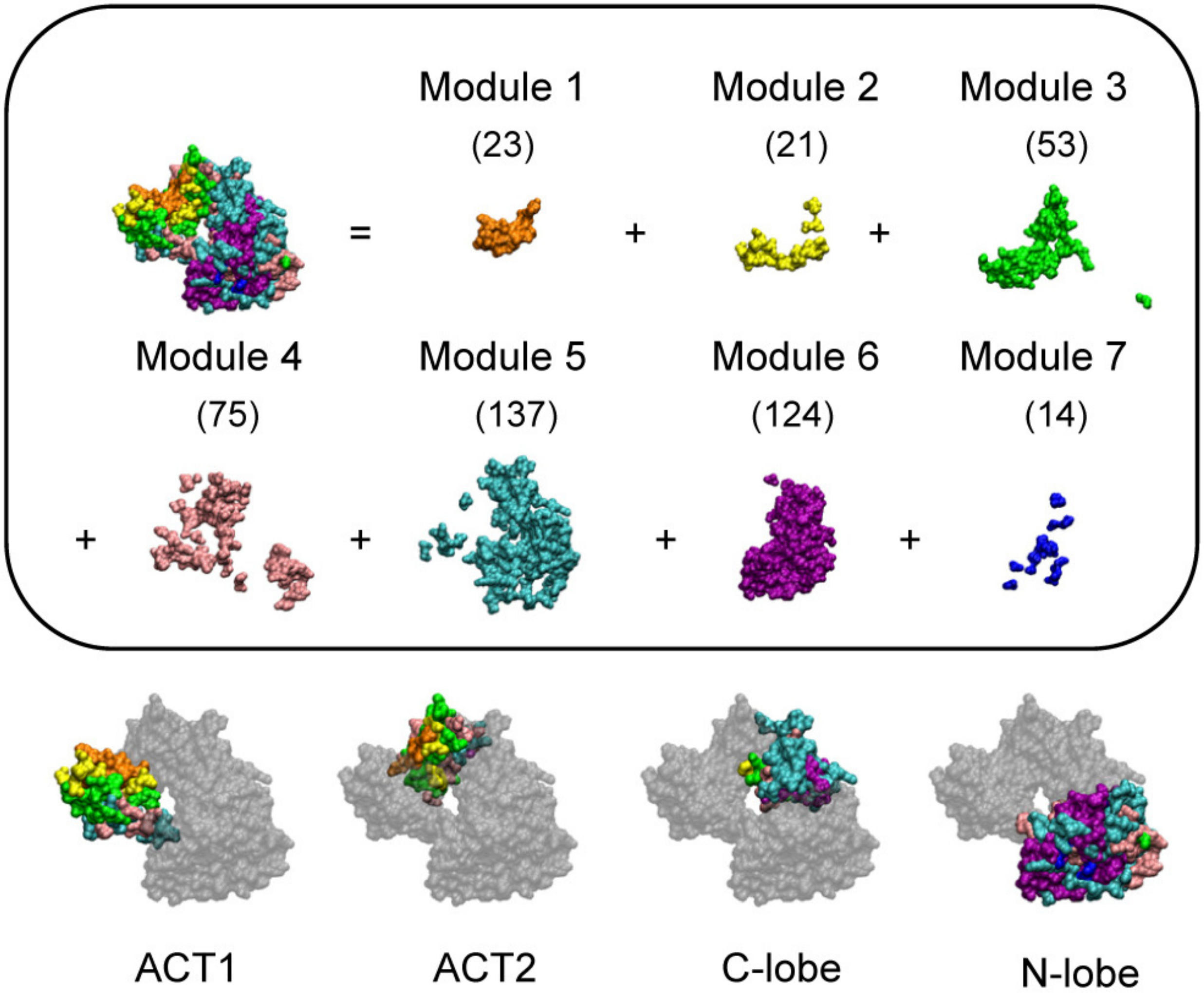
<u>Poster 191</u> Ma and Zeng. New Concept and Algorithms to Reveal Protein Dynamics and Intramolecular Interaction Network and Their Application for Protein Design

C. Ma. PLoS ONE 6(10): e26453. 2011
C.Ma. PLos One 7(2):e31529, 2012
C. Ma et al. Submitted. 2012

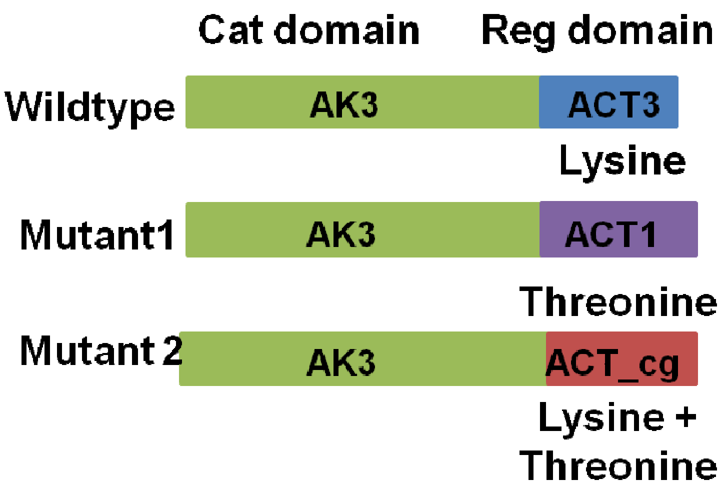
Energy dissipation of key residues in AK3



From structural to dynamic modules revealed by the new method



Synthetic AK3 for response to different effectors

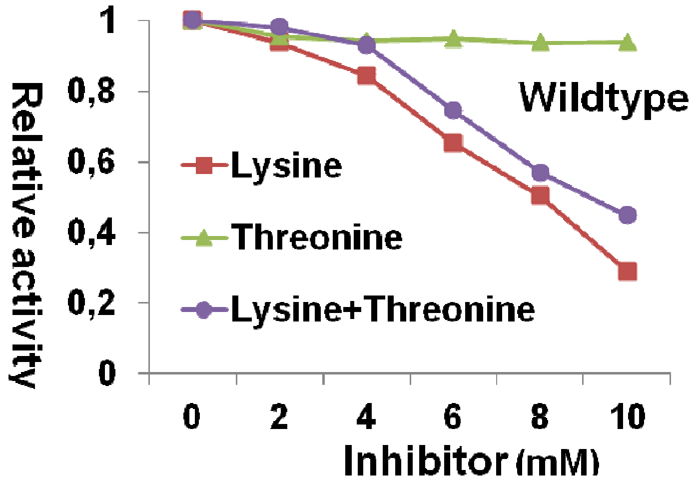


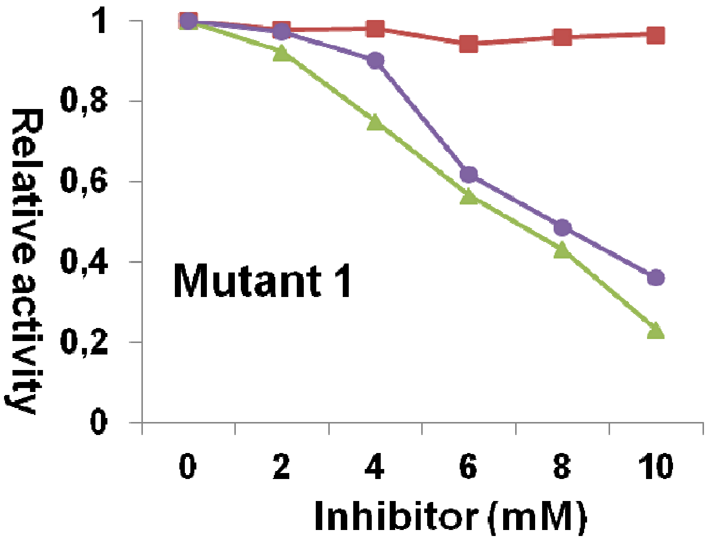
Cat domain **Reg domain**

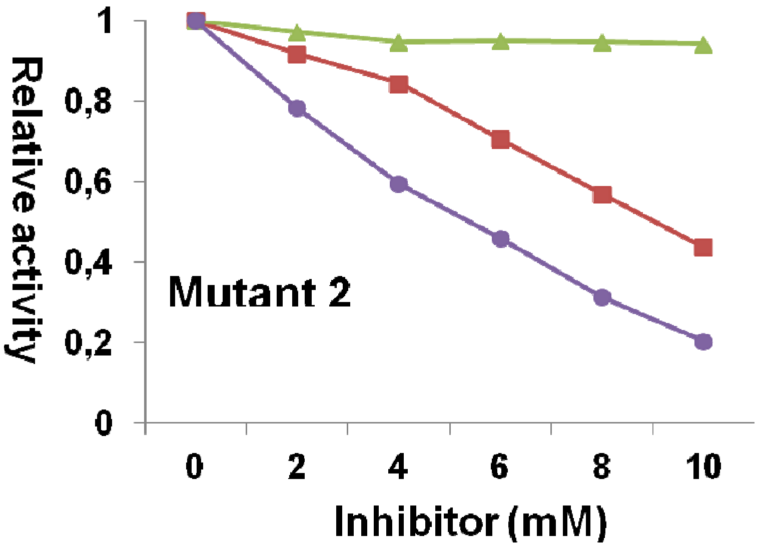
Wildtype AK3 | ACT3 — Lysine

Mutant1 AK3 | ACT1 — Threonine

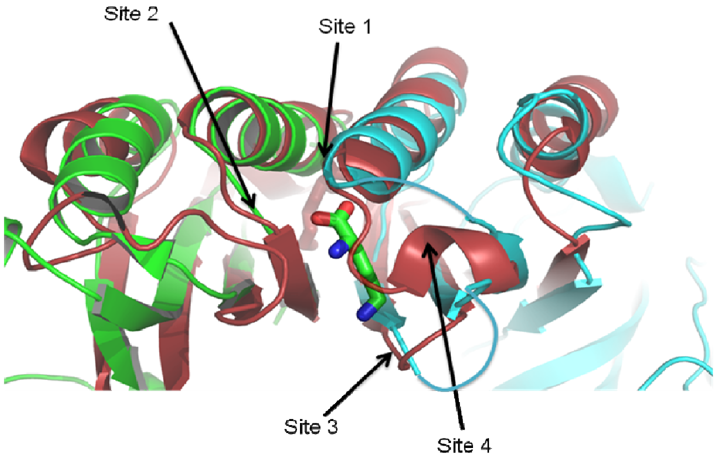
Mutant 2 AK3 | ACT_cg — Lysine + Threonine

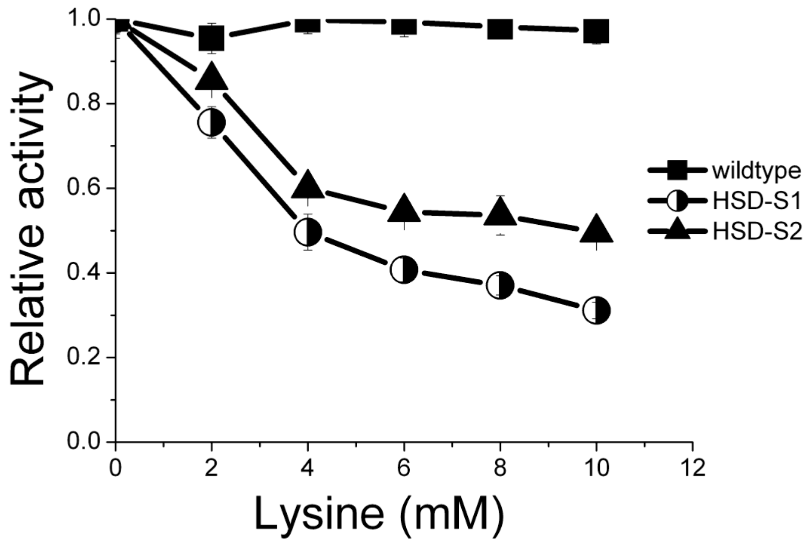


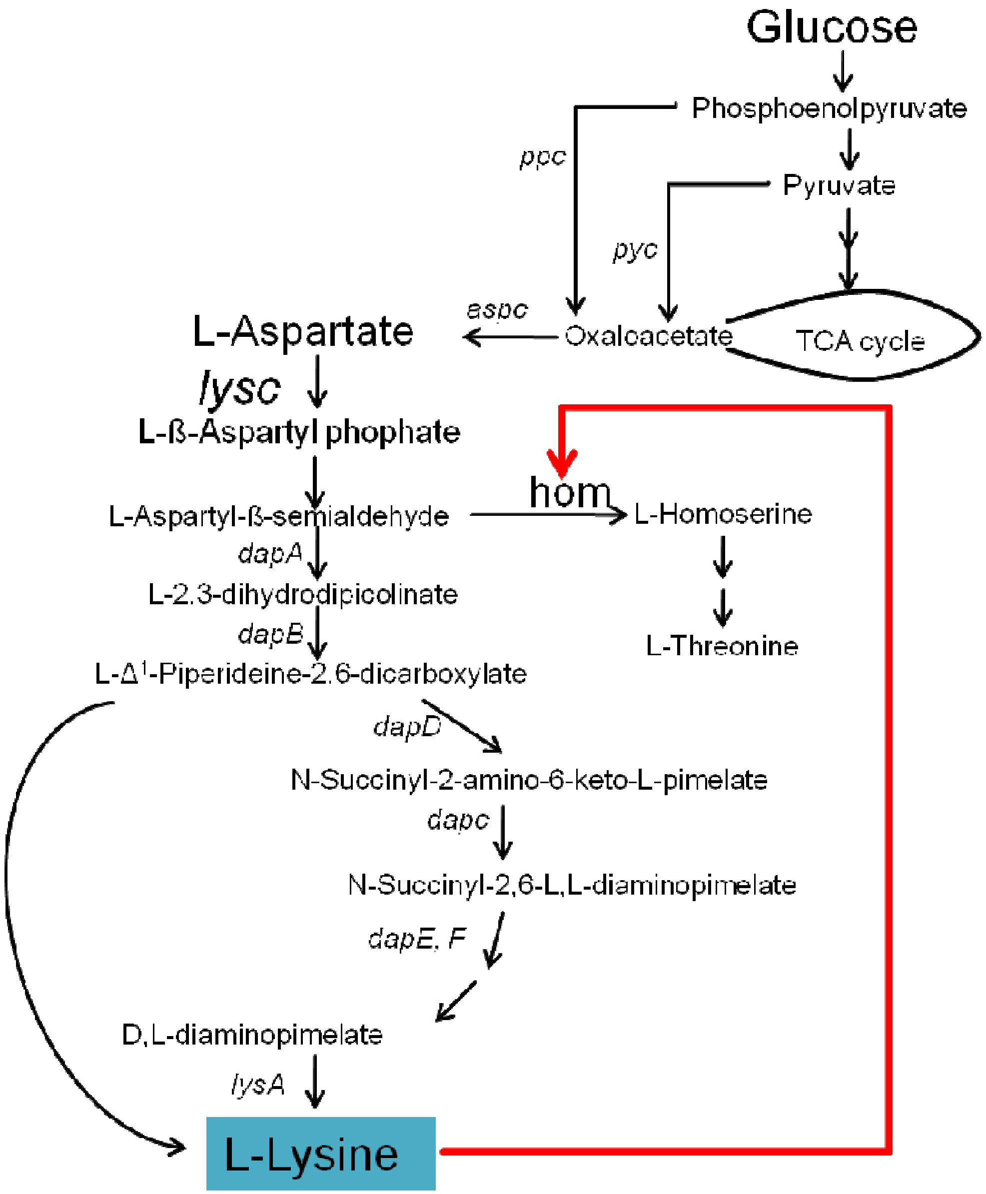




Design of non-native lysine responsive HSD







Glucose

Phosphoenolpyruvate

Pyruvate

ppc

pyc

aspc

Oxalcacetate

TCA cycle

L-Aspartate

lysc

L-ß-Aspartyl phophate

L-Aspartyl-ß-semialdehyde

hom

L-Homoserine

L-Threonine

dapA

L-2.3-dihydrodipicolinate

dapB

L-Δ^1-Piperideine-2.6-dicarboxylate

dapD

N-Succinyl-2-amino-6-keto-L-pimelate

dapc

N-Succinyl-2,6-L,L-diaminopimelate

dapE, F

D,L-diaminopimelate

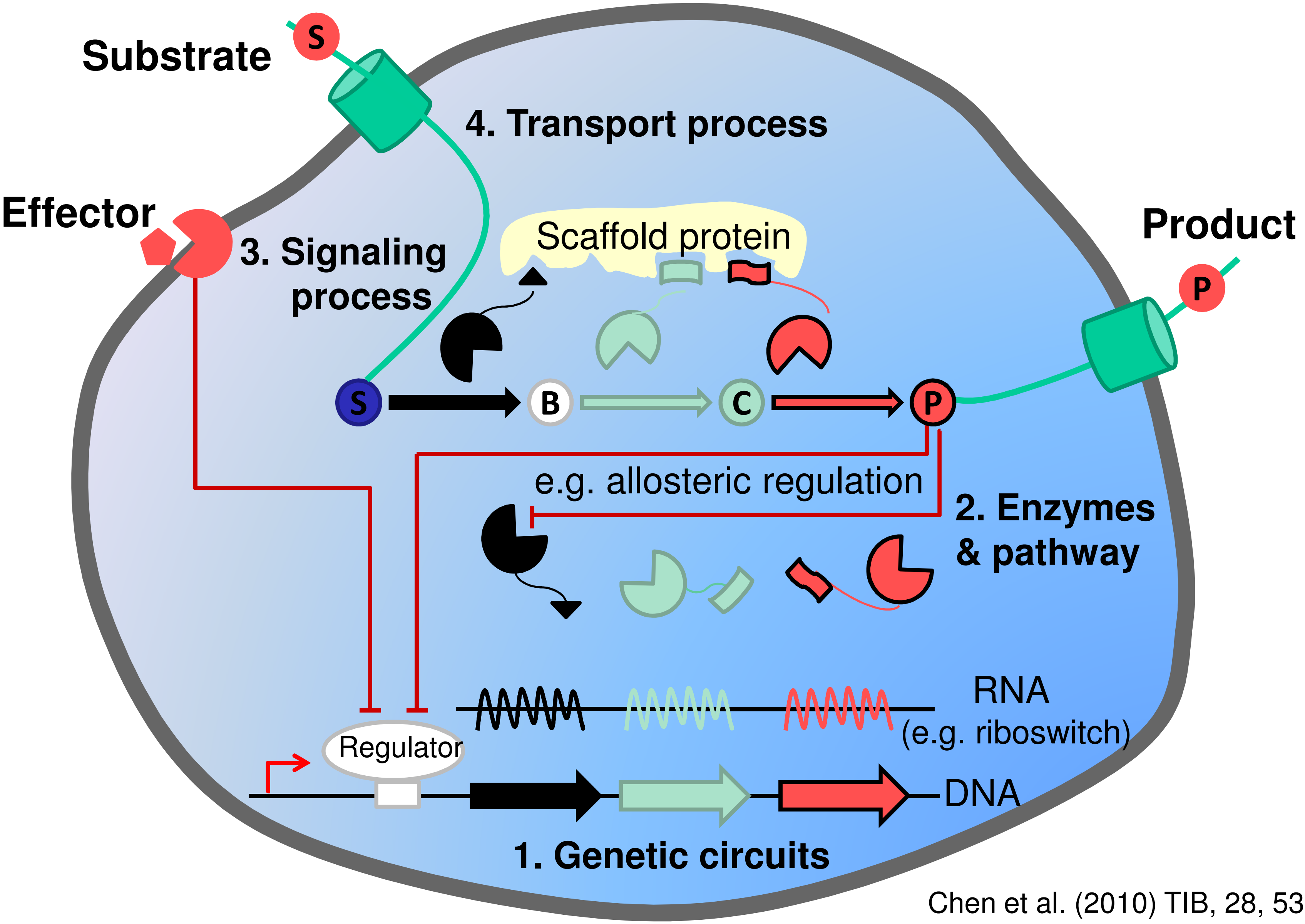
lysA

L-Lysine

Summary

- **The integration of MD and evolutionary analysis is a powerful and predictive appraoch for the re-design of allosteric regulation of enzymes of amino acid systhesis.**
  - For three different aspartokinases from *E.coli* and *C. glutamicum* 30 efficient mutation points have been successfully identified and experimenally verified.
- **Systematic structure-based design led to highly productive strain of L-lysine in a very short time.**
- **Synthetic protein designed for dynamic and self-regulation of biosynthesis**
  - New concept and algorithms for protein dynamics developed
- **Structure-based design provides new possibilities for a rational development of biocatalysts.**

Perspective: Structural ME and SynBio



Chen et al. (2010) TIB, 28, 534

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Dr. Sugima Rappert

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