AK Biokatalyse

biocat

Materials Valley e. V. Workshop

// Biokatalyse //

24.04.2014

BASF SE Feierabendhaus, Ludwigshafen Hessen

Donnerstag den 24. Aprli 2014

Biocatalytic processes in cellular systems

Synthetic Biology and Biocatalysis

NEDILJKO BUDISA

Berlin Institute of Technology/TU Berlin

Department of Chemistry

Biocatalysis Group

Müller-Breslau-Straße 10

D-10623 Berlin

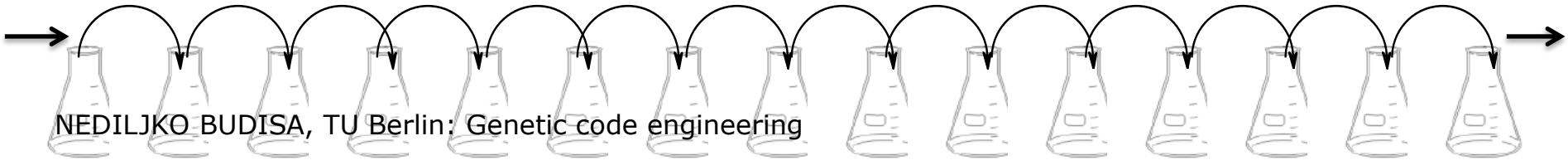
www.biocat.tu-berlin.de

Talk structure

- BIOCATS IN BERLIN
- CODE ENGINEERING: WHY & HOW?
- APPLICATIONS
- CURRENT PROBLEMS (AND SOLUTIONS) IN THE FIELD
- BIOORTHOGONAL CHEMISTRIES AND ARTIFICIAL METABOLISM
- VISION
- COOPERATION OPPORTUNITIES

Biocatalysis Group, Institute of Chemistry
Berlin Institute of Technology (TU Berlin), Germany

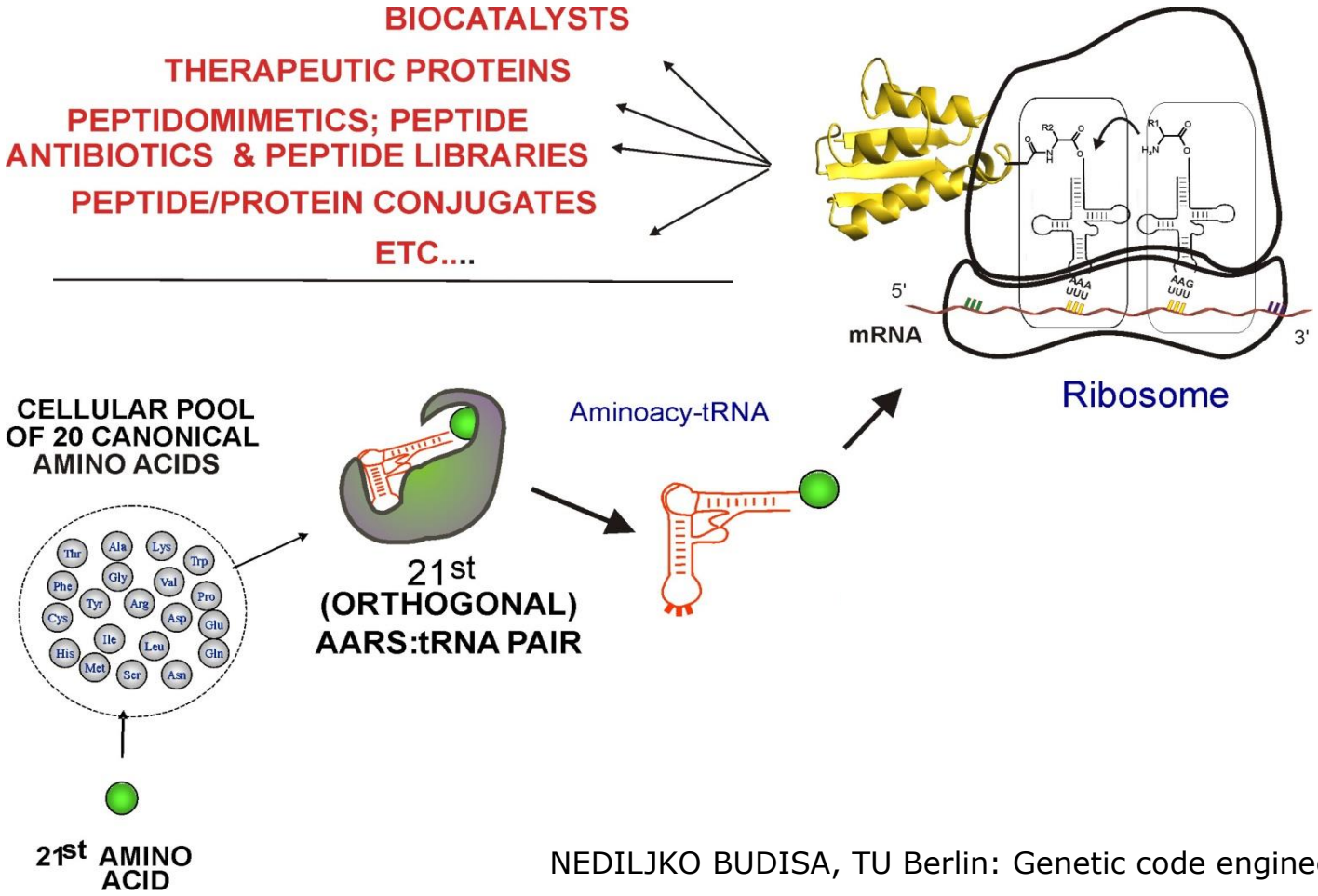
Group leader: Prof. Dr. Nediljko Budisa Web: www.biocat.tu-berlin.de



Biocat Group in Berlin - Our Mission

TO EXPAND THE SCOPE OF PROTEINS SYNTHESIS

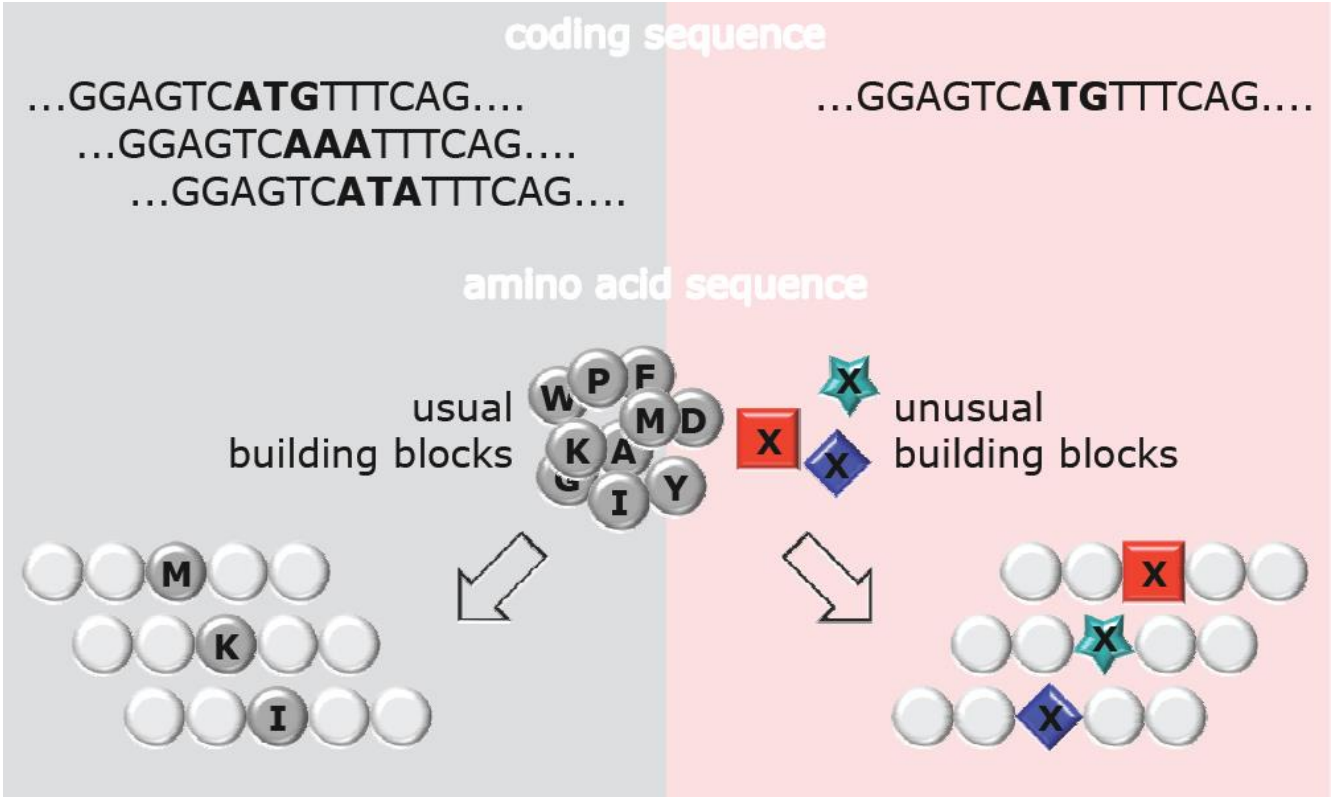
(Genetic Code Engineering & Expansion)



NEDILJKO BUDISA, TU Berlin: Genetic code engineering

Our Mission. Congeners vs. Mutants

NEDILJKO BUDISA, TU Berlin: Genetic code engineering



B. Wiltschi

MUTANTS - CLASSICAL SITE-DIRECTED MUTAGENESIS & GUIDED EVOLUTION

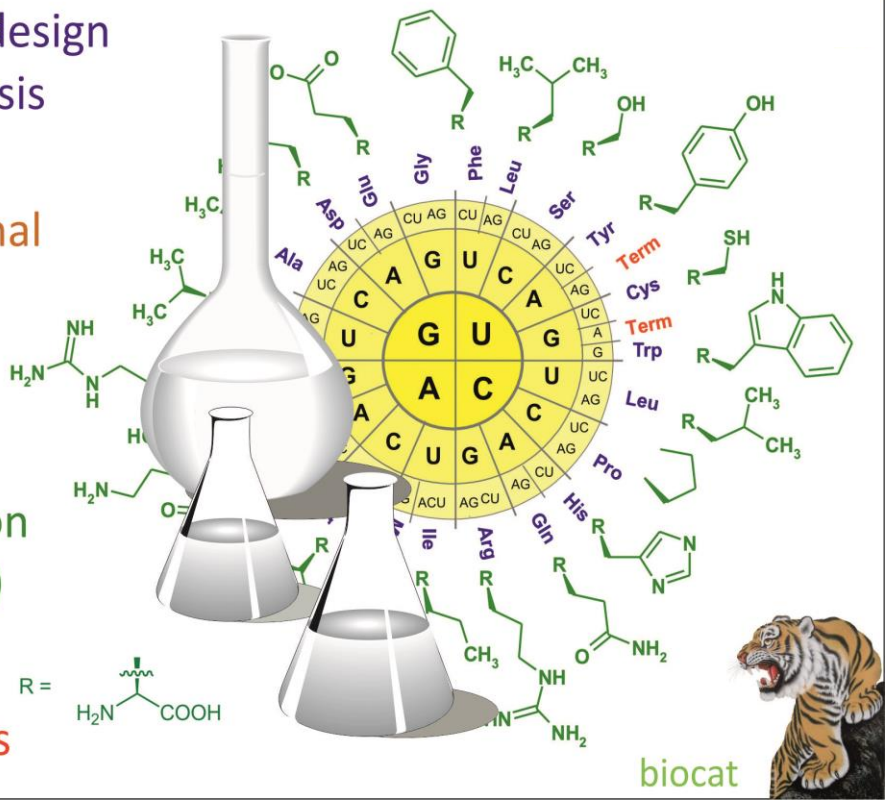
CONGENERS -THE SAME GENE SEQUENCE AS WT BUT CONTAINS A FRACTION OF SYNTHETIC AMINO ACIDS

Biocat Group in Berlin- Research overview

SYNTHETIC BIOLOGY OF PROTEINS AND CELLS

(at the interface of chemistry and biology)

- Alloprotein (congeneric proteins) design & engineering in protein biosynthesis
  (Polypetide based bio/nano-materials)
- Chemical control of posttranslational modifications of proteins
  (Bioorthogonal transformations)
- Metabolic engineering
- Genetic code engineering/expansion (orthogonalization/reprogramming)
- Visions: A new chemistry of life
  Designer proteins and cells



NEDILJKO BUDISA, TU Berlin: Genetic code engineering

Topic

CODE ENGINEERING

WHY & HOW?

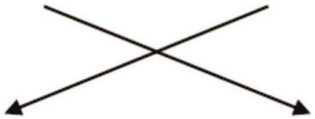
APPLICATIONS?

The Big Picture: Flow of Information in Biology

State of the art

GENETIC CODE RE-DESIGN

at the level of:



a) Informational polymers (e.g. nucleic acids)

- Creation of novel "letters" (e.g. noncanonical coding units) by:

→ New/different **combinations** of "letters" or coding units (e.g. quadruplets, pentaplets)

→ New/different **chemical make-up** of informational polymers (e.g. XNA)

b) Catalytic polymers (e.g. proteins)

- Building blocks "monomers" (e.g. amino acids) :

→ **Reduction**

→ **Substitutions** (engineering) of canonical building blocks with noncanonical ones (e.g. noncanonical amino acids, ncAAs)

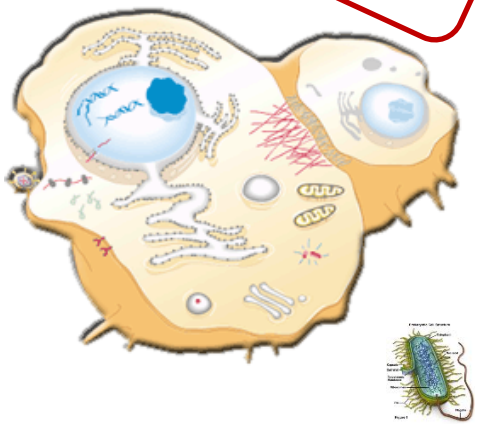
→ **Expansions** of standard repertoire with noncanonical building blocks (e.g. ncAAs)

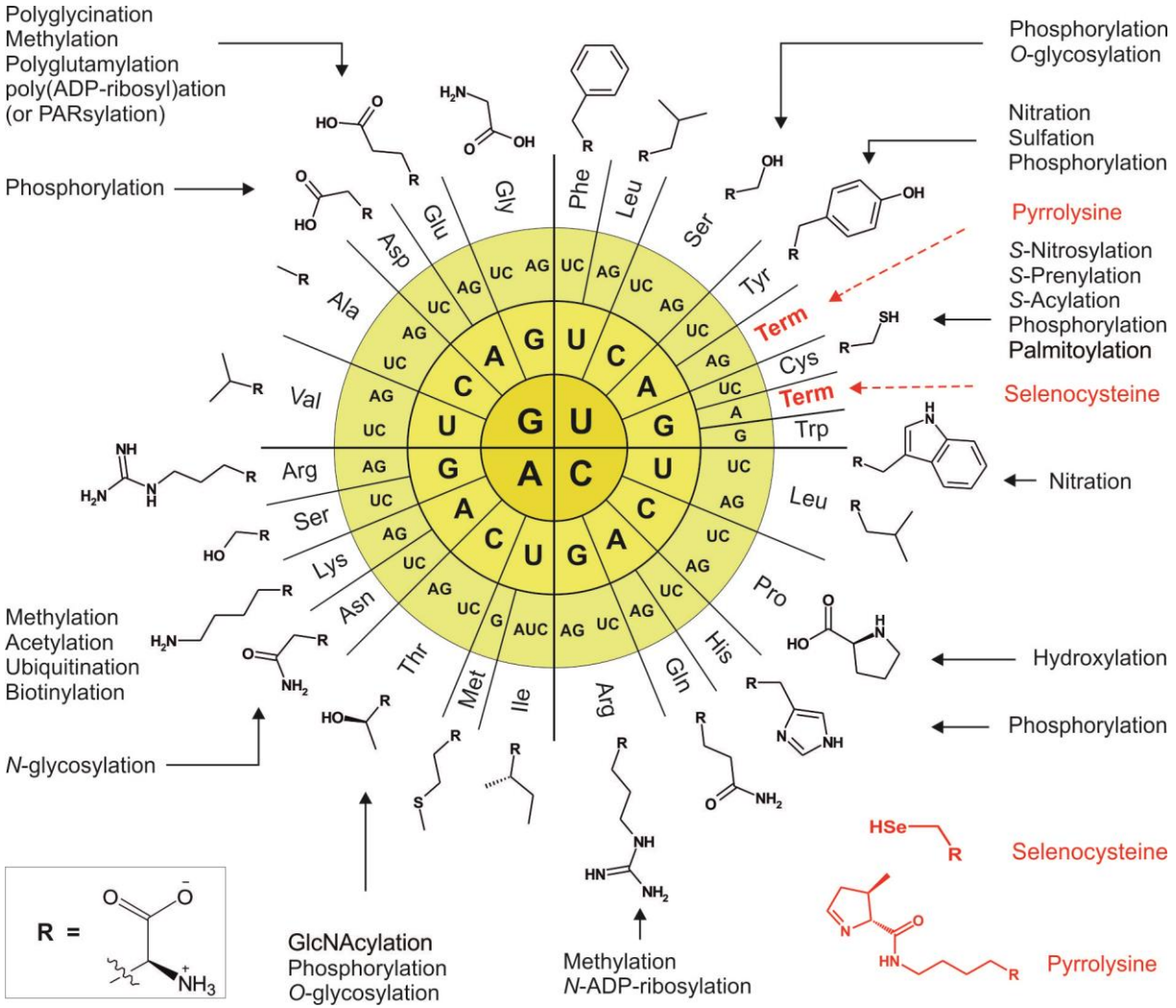
N. Budisa: Xenobiology, New-to-Nature Synthetic Cells and Genetic Firewall *Current Organic Chemistry*, **18**, (2014) In press .

WHY CODE ENGINEERING?

CO- AND POST-TRANSLATIONAL MODIFICATIONS

State of the art





Engineering point of view:

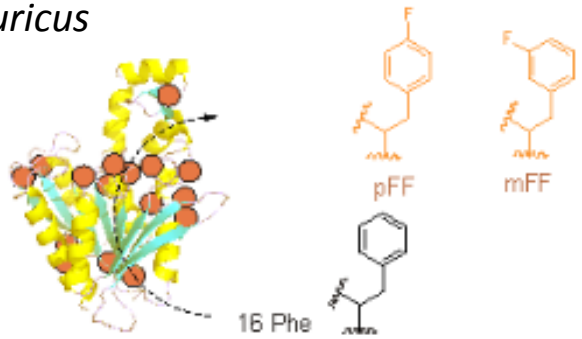
- PTM machinery
  - highly complex (compartmentalization!)
- large quantities of homogeneous product?
- recognition features - easily destroyed

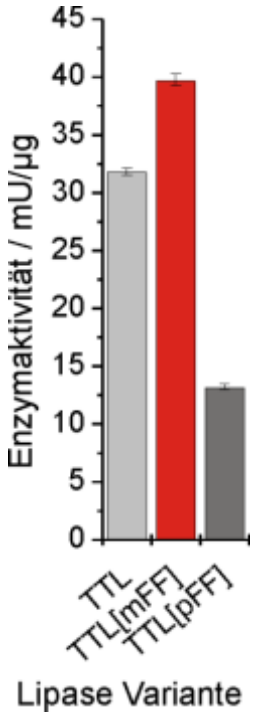
Example: improved lipase (TTL)

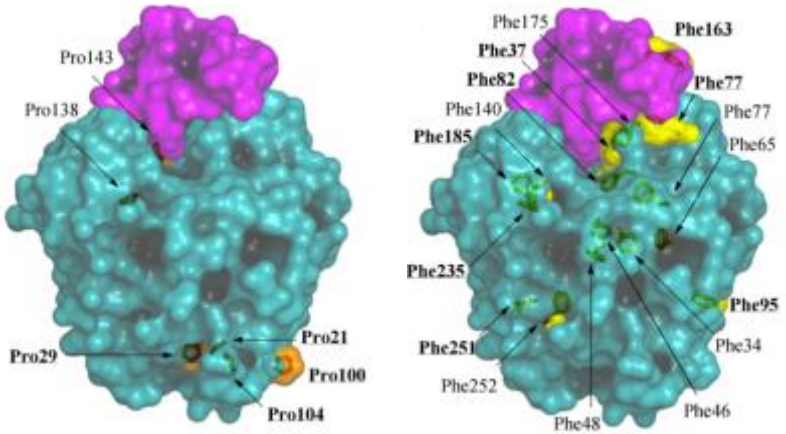
Novel

Phenylalanine → *meta*-Fluorphenylalanine Global Exchange in TTL (Lipase form *Thermoanaerobacter thermohydrosulfuricus* SOL1)

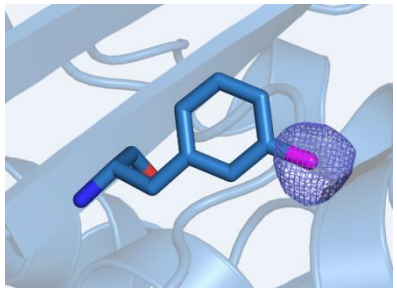
→ Congener is ~ 25% active







Structural data:



Spyros D. Chatziefthimiou
Willmanns Group at EMBL Hamburg (2014)

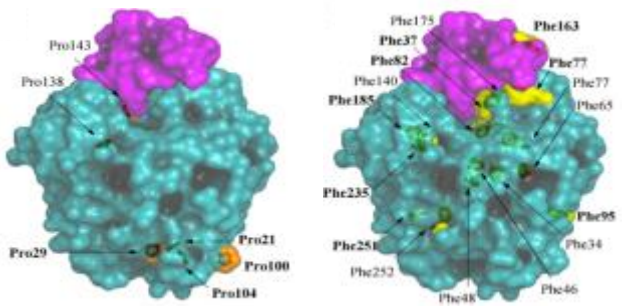
Hoesl *et al*. Lipase Congeners Designed by Genetic Code Engineering. ChemCatChem, 3, 213-221 (2011).

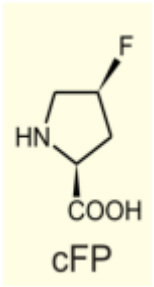
Example: improved lipase (TTL) in organic solvents

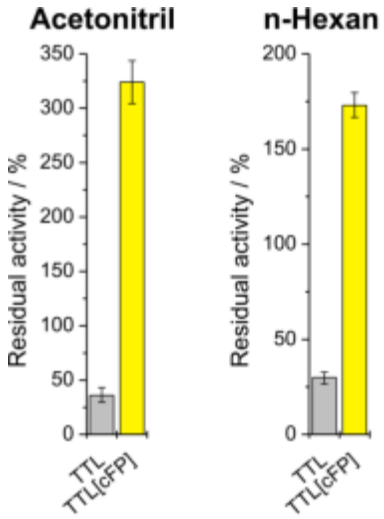
Novel

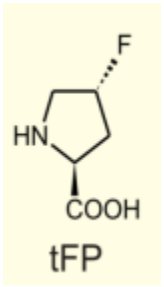
Prolin → Fluoroproline
Exchange in TTL:

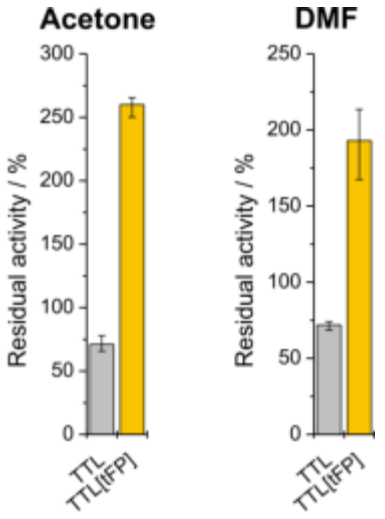
→ Congeners with increased resistance to 90% of solvent







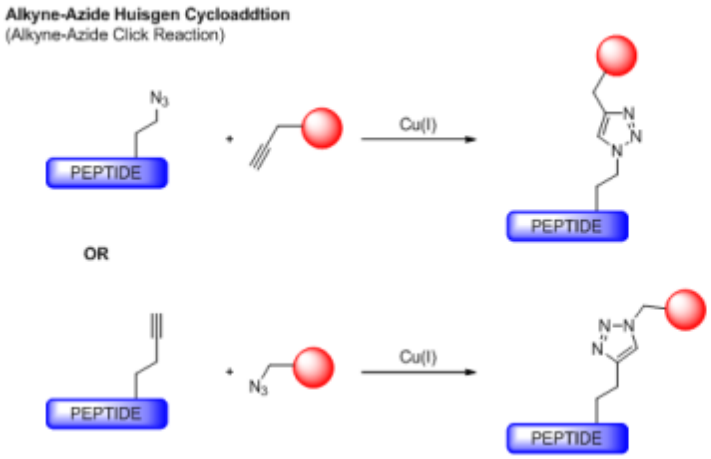


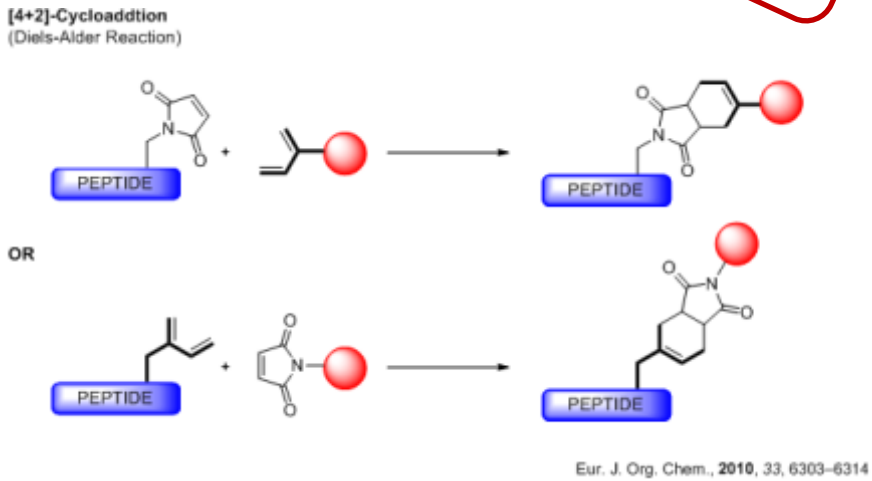


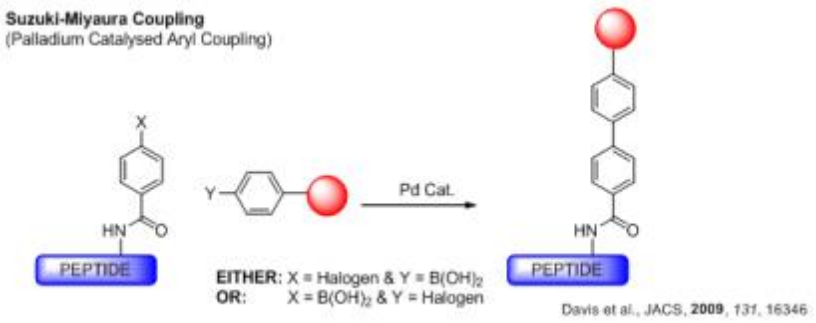
Acevedo-Rocha *et al.* Non-canonical amino acids as a useful synthetic biological tool for lipase-catalysed reactions in hostile environments *Catal. Sci. Technol.*, 3, 1198-1201 (2013)

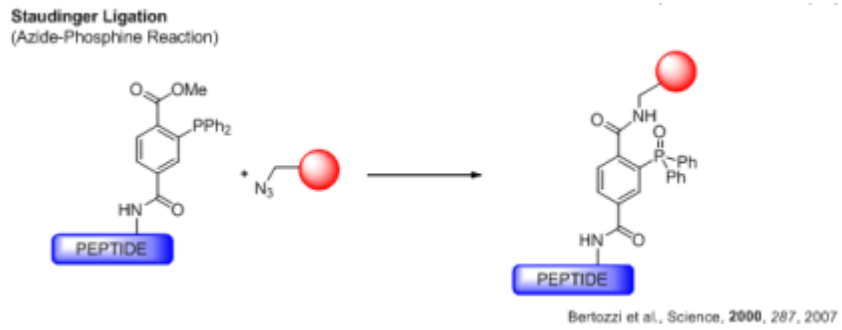
Congeners for bio-orthogonal coupling chemistries

Novel







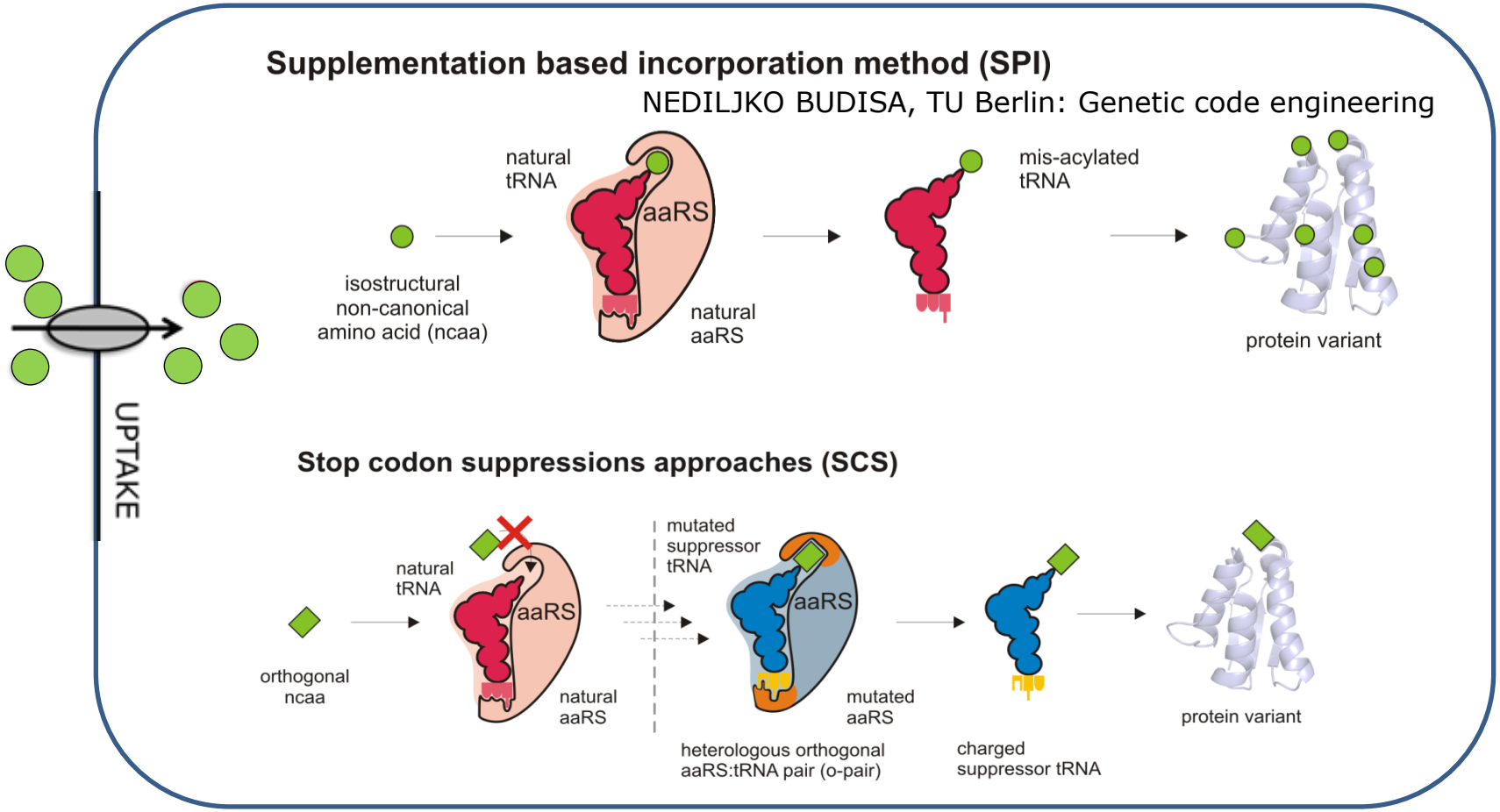


Artner, L. A., Merkel, L., Bohlke, N., Beceren-Braun, F., Weise, C., Dernedde, J., Budisa, N, Hackenberger, C. P. R. (2012). *Chem. Commun* **48**, 522 – 524

Strategies to expand the genetic code

State of the art

NEDILJKO BUDISA, TU Berlin: Genetic code engineering

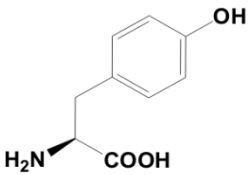


Orthogonal translation

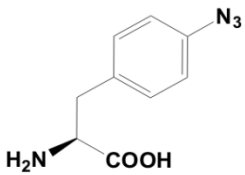
Novel

Currently available orthogonal pairs & Cell permeable amino acids

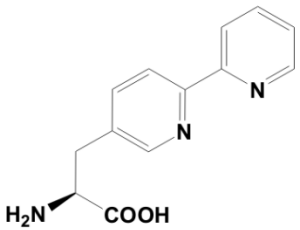
Methanocaldococcus jannaschii
TyrRS System (Peter Schultz)



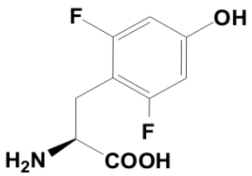
Tyrosin



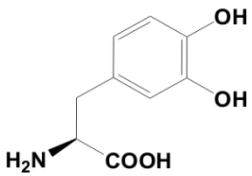
Azidophenylalanin



Bipyridylalanin

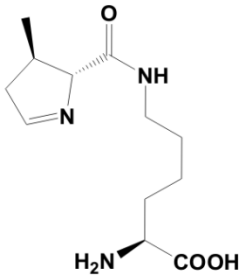


2,6-Difluoro-Tyrosin

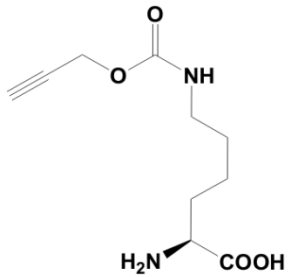


DOPA

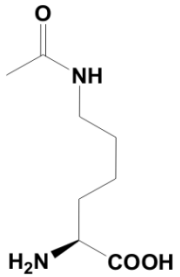
Methanosarcina sp.
PylRS System



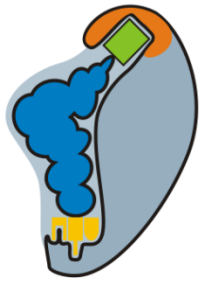
Pyrrolysin

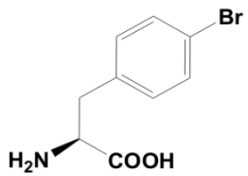


Alkin-Pyl-Analog



Keto-Pyl-Analog





4-Bromophenylalanin

NEDILJKO BUDISA, TU Berlin: Genetic code engineering

Topic

CURRENT PROBLEMS (AND SOLUTIONS) IN THE FIELD

BIOORTHOGONAL CHEMISTRIES AND ARTIFICIAL METABOLISM

General problems and issues

Issues

- Scale of production
- Metabolic engineering

(ncAAs should be synthetized from simple precursors)

- Quality of o-pairs/engineered enzymes
- **Evolution and selection procedures (robust strains)**
- Paradigm shift: single proteins -> Proteomes
- Societal and industrially relevant problems

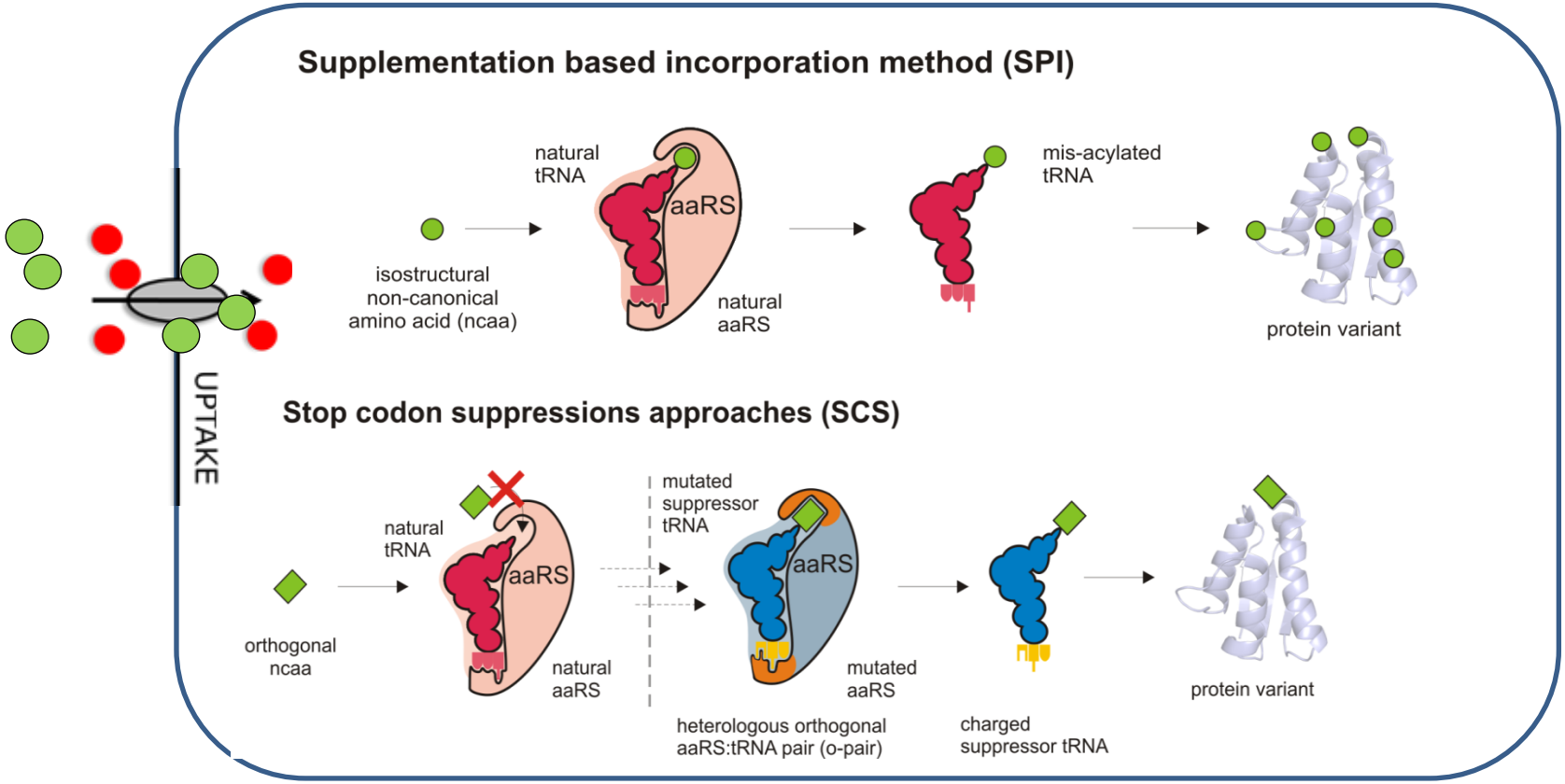
N. Budisa: Xenobiology, New-to-Nature Synthetic Cells and Genetic Firewall
Current Organic Chemistry, **18**, (2014) In press .

NEDILJKO BUDISA, TU Berlin: Genetic code engineering

Metabolic engineering ?

Issues

Solution of industrially relevant bio-production problems?



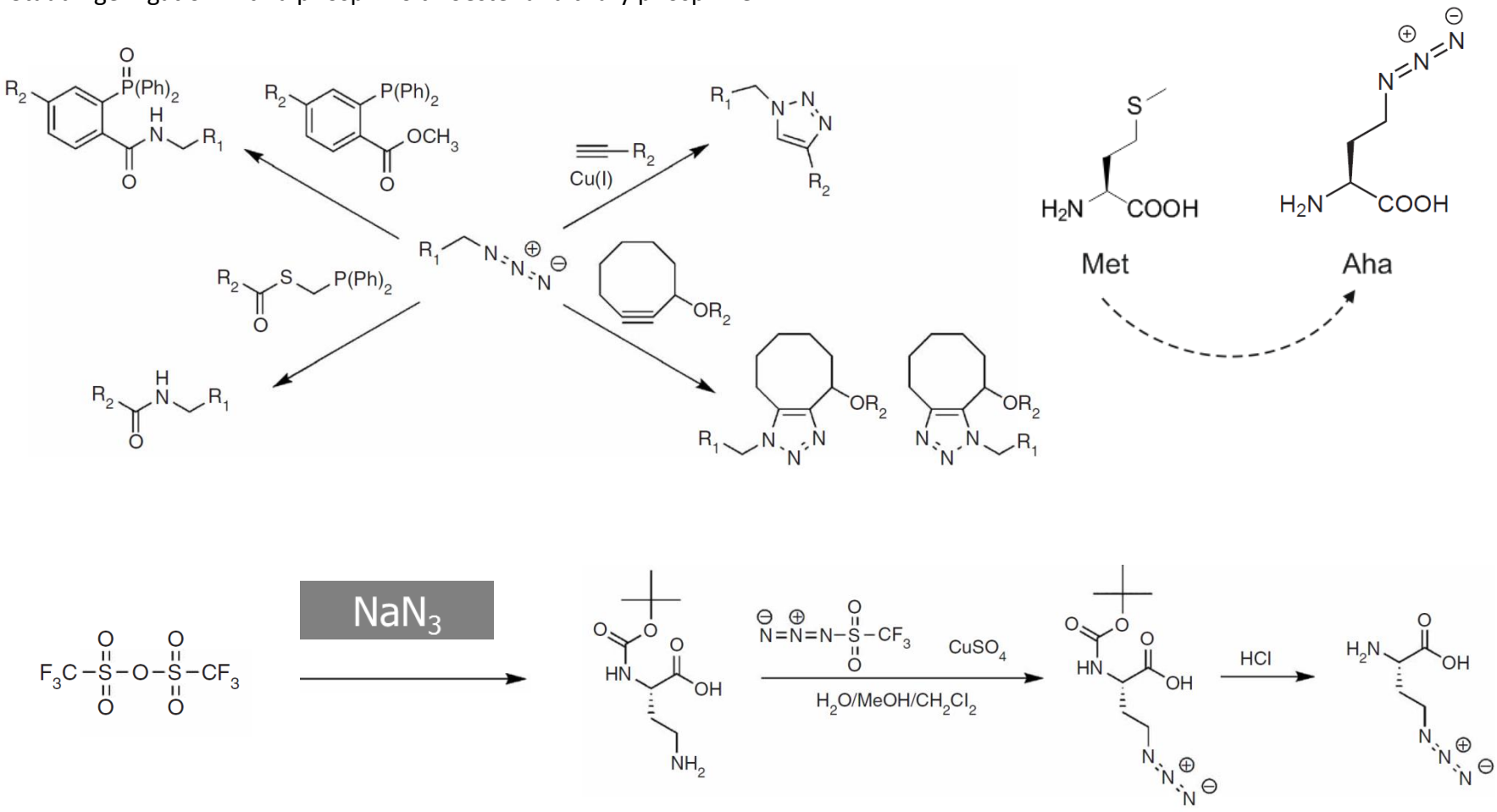
Metabolic engineering (ncAAs should be synthetized from simple precursors)

NEDILJKO BUDISA, TU Berlin: Genetic code engineering

Case study: functionalizable methionine analogue azidohomoalanine (Aha, 2-amino-4-azidobutanoic acid)

Novel

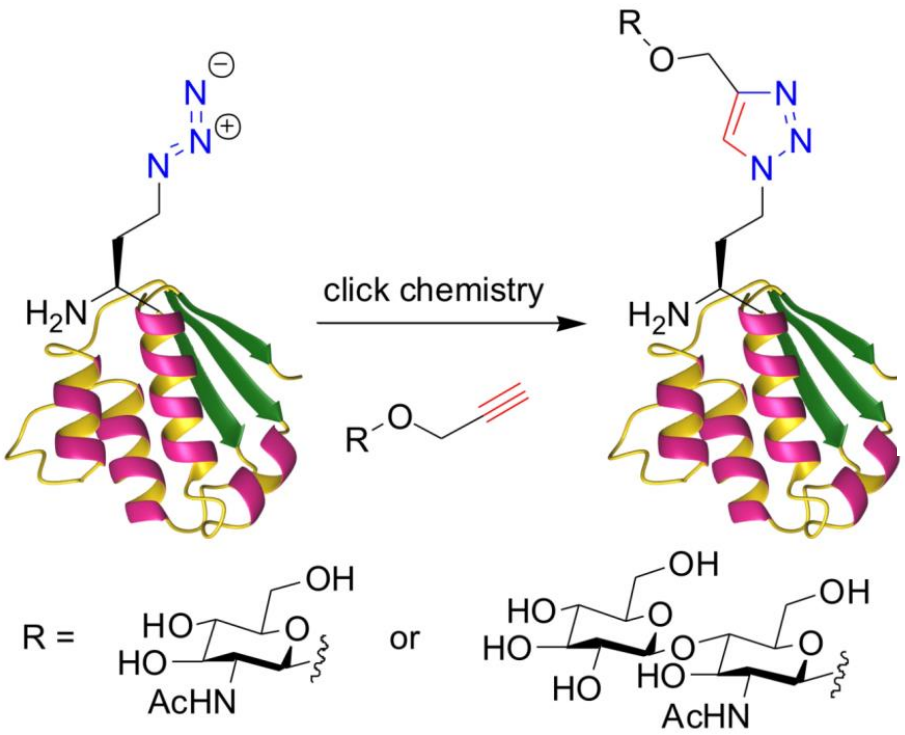
Link & Tirrell (2007) *Nature Protocols* **2**, 1879 – 1883:
Cu-induced azide–alkyne ligation, strain-promoted azide–alkyne ligation,
Staudinger ligation with a phosphino thioester and triarylphosphine.

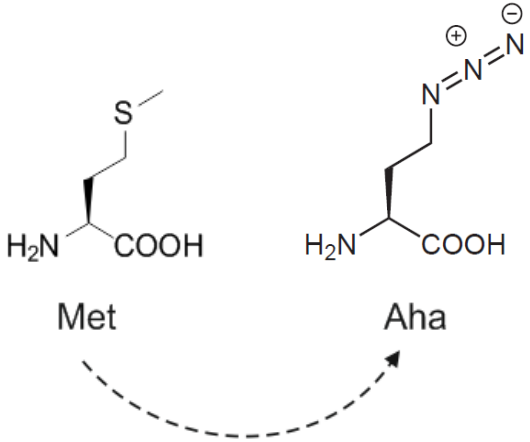


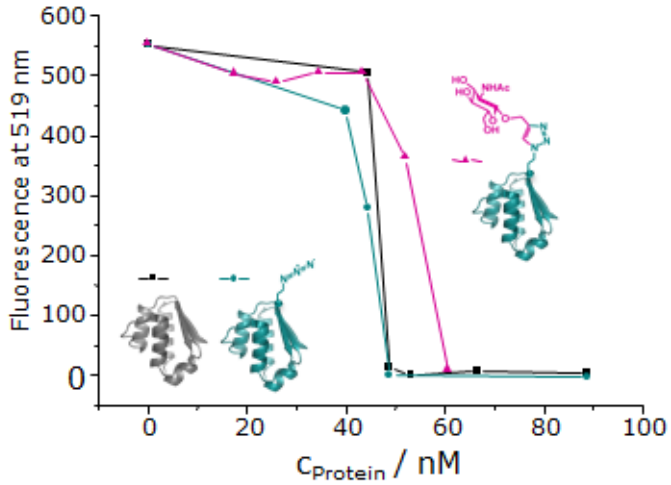
Step 1- azidification of side chain by copper-catalyzed diazo transfer; step 2 - deprotection of amine group.

Case study: glycosylation of barstar

Novel







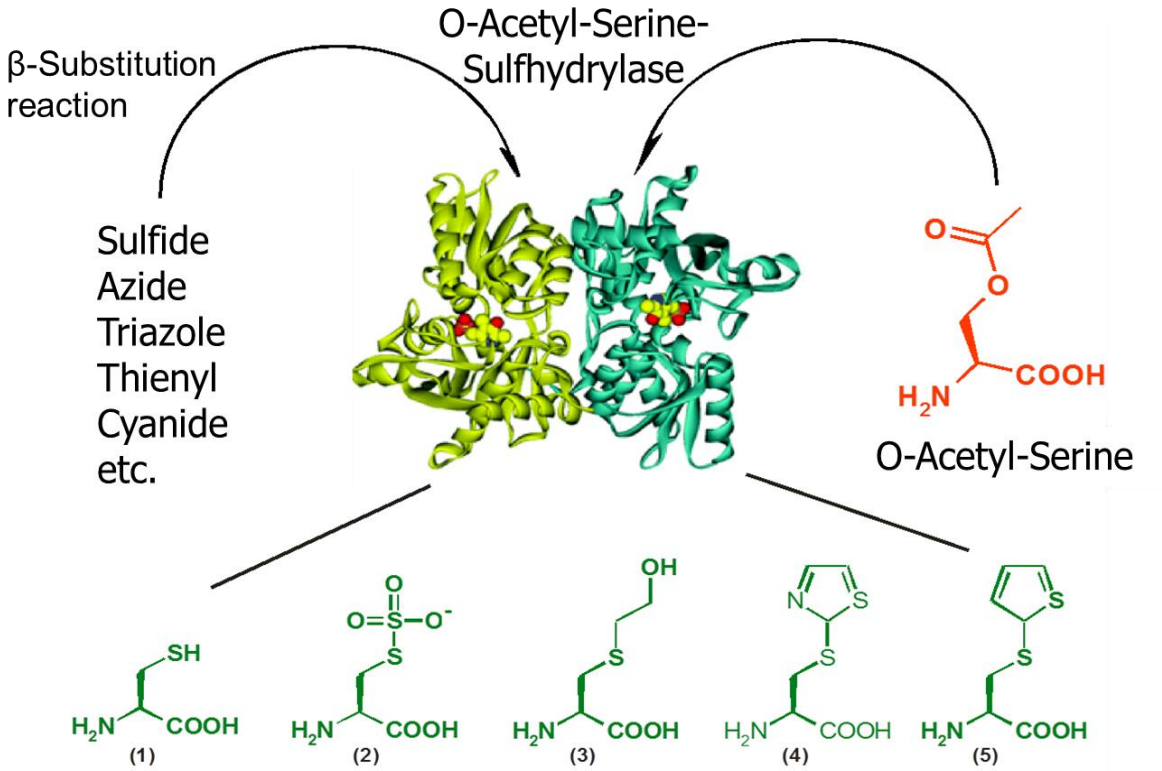
R = N-acetyl glucose (NAcGlu); or

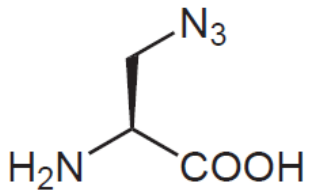
R = N-acetyl chitobiose (NacChi)

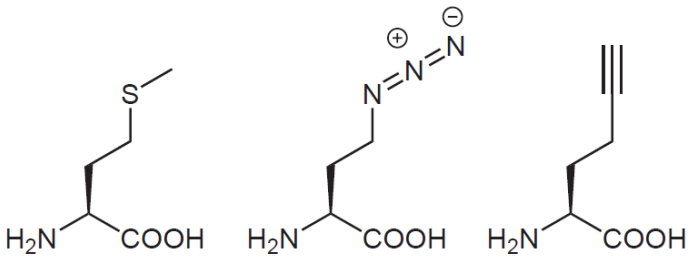
Merkel, L., Beckmann, H. S. G., Wittmann, V., Budisa, N. (2008). Efficient N-terminal Glycoconjugation of Proteins by the N-End Rule. *ChemBioChem* **9**, 1220-1224.

Metabolic engineering in *Escherichia coli*:
Intracellular biosynthesis of azidohomoalanine (Aha)?

Novel



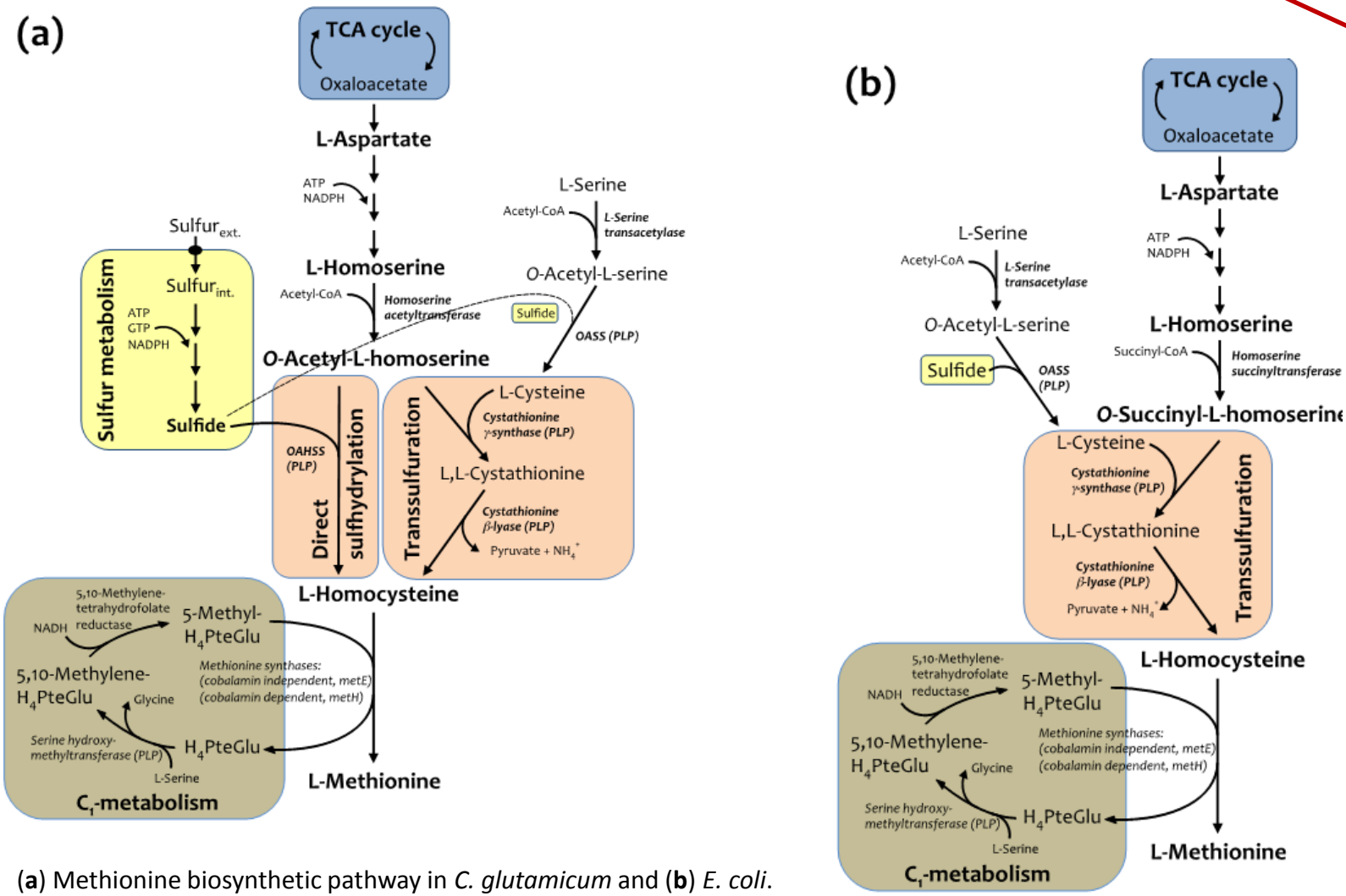




Maier, T.H. Semisynthetic production of unnatural L-alpha-amino acids by metabolic engineering of the cysteine-biosynthetic Pathway. *Nat. Biotechnol.* **2003**, *21*, 422–427.

Metabolic engineering:
Intracellular biosynthesis of methionine

Novel

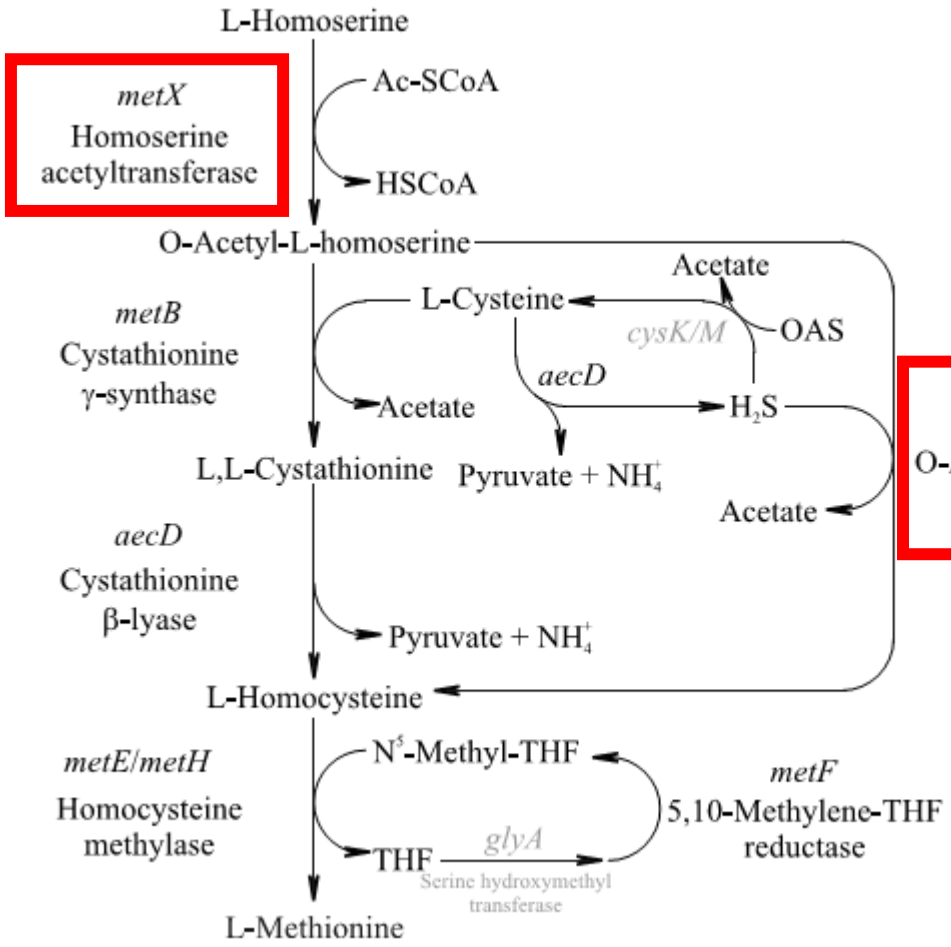


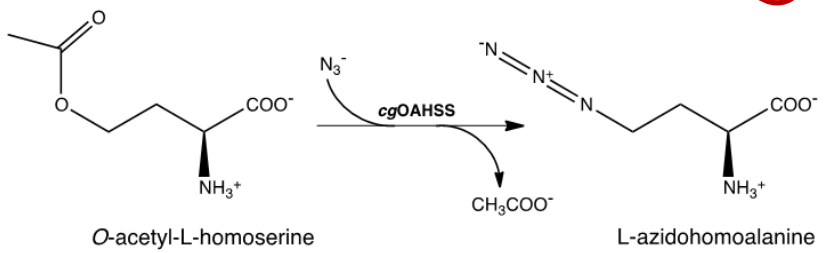
(**a**) Methionine biosynthetic pathway in *C. glutamicum* and (**b**) *E. coli*.

Metabolic engineering:

Intracellular biosynthesis of azidohomoalanine (Aha)

Novel

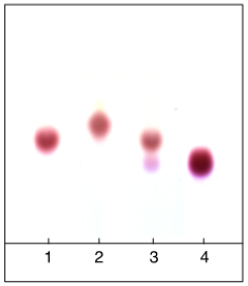


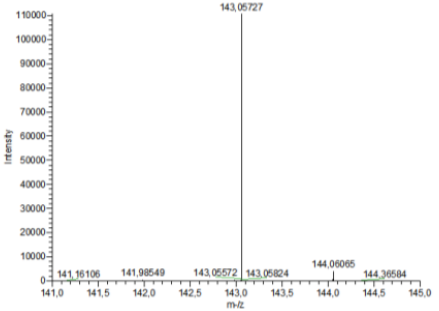


O-acetyl-L-homoserine

L-azidohomoalanine

In vitro biosynthesis of azidohomoalanine (Aha)



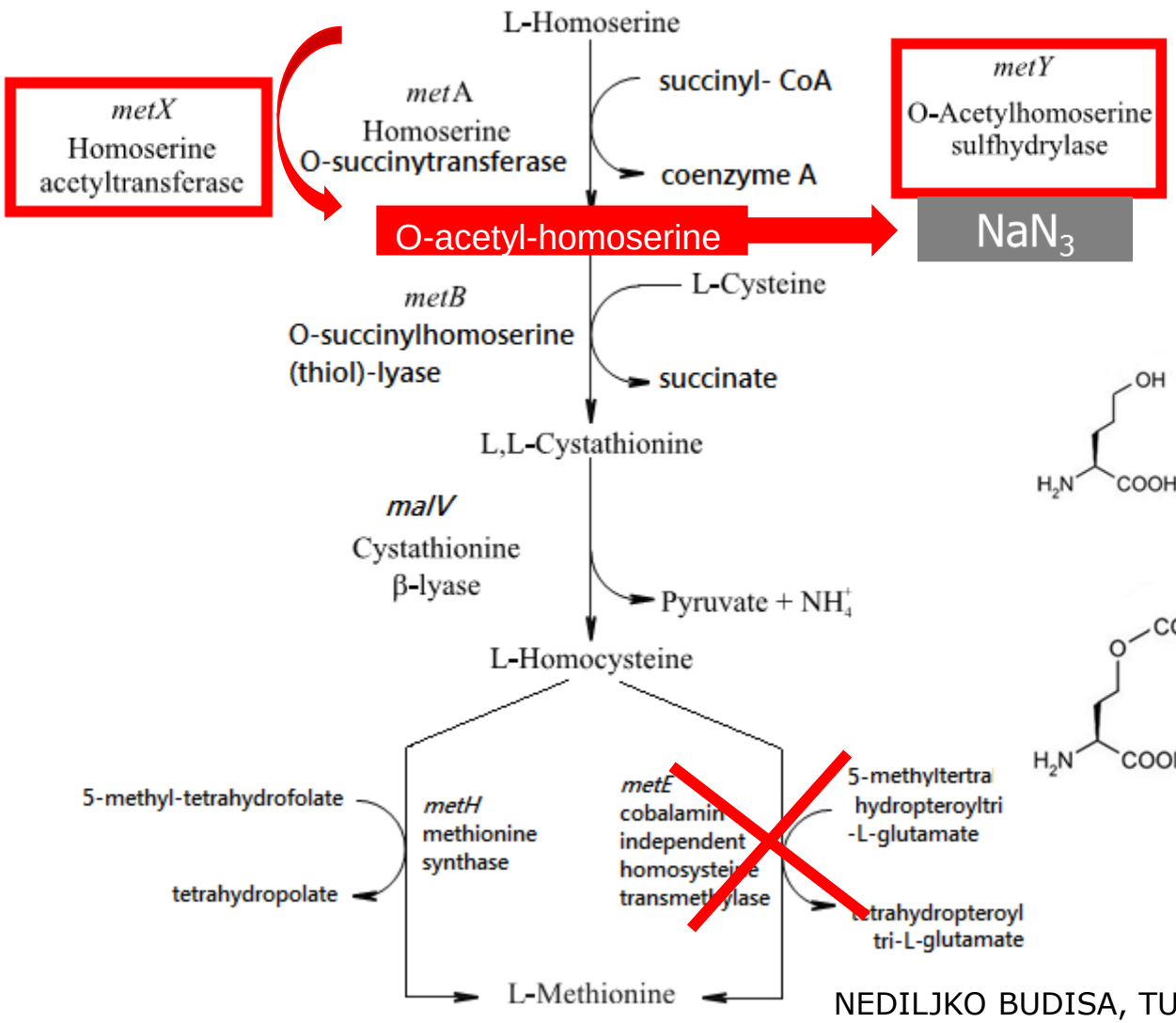


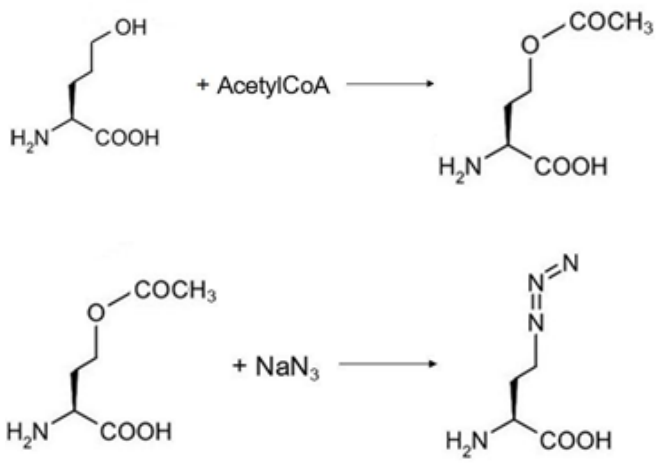
Methionine synthesis pathway in *Corynebacterium glutamicum*

Metabolic engineering:

Intracellular biosynthesis of azidohomoalanine (Aha)

Novel

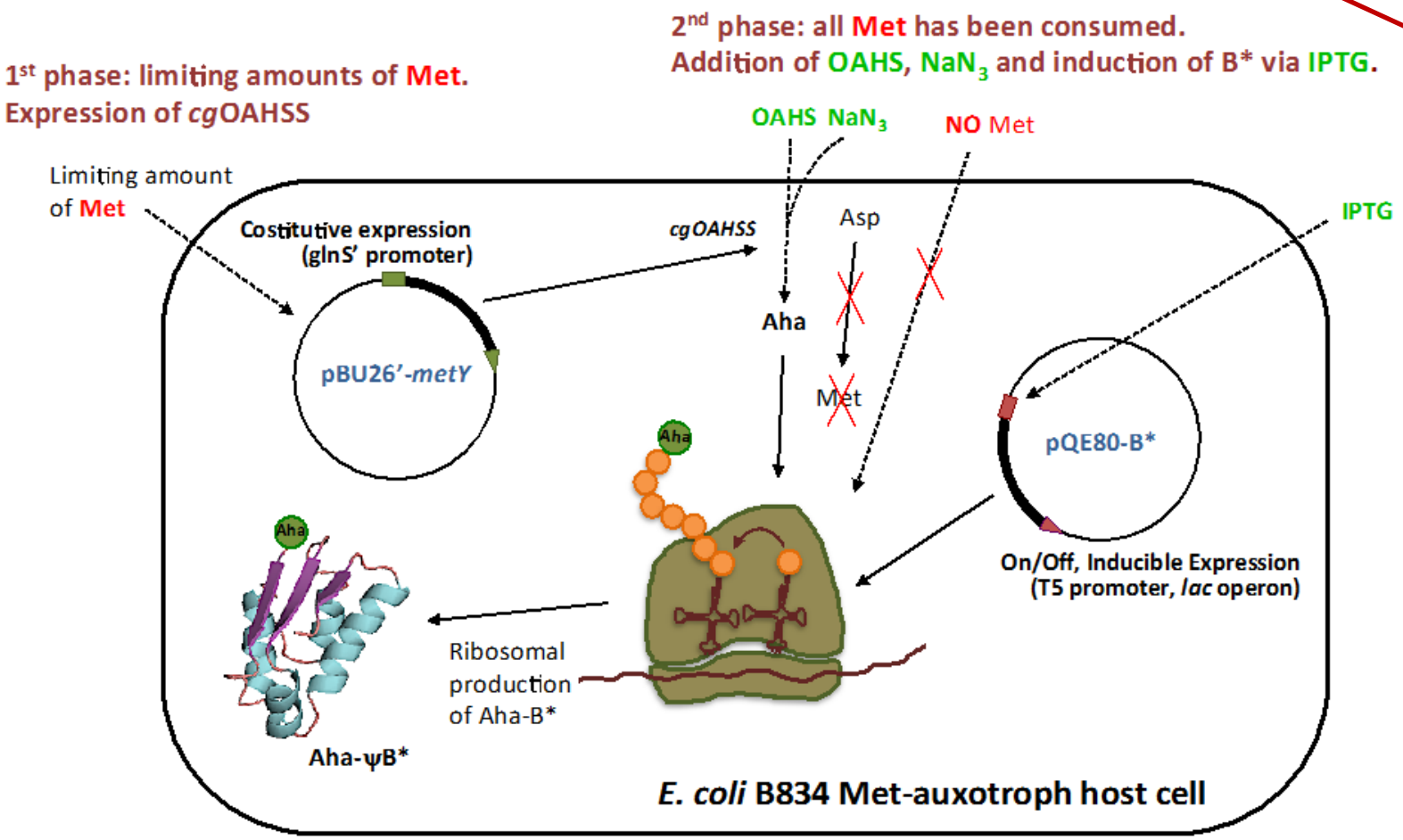




Methionine biosynthesis pathway in *E. coli*

General method for intracellular L-Aha production and incorporation into recombinant proteins

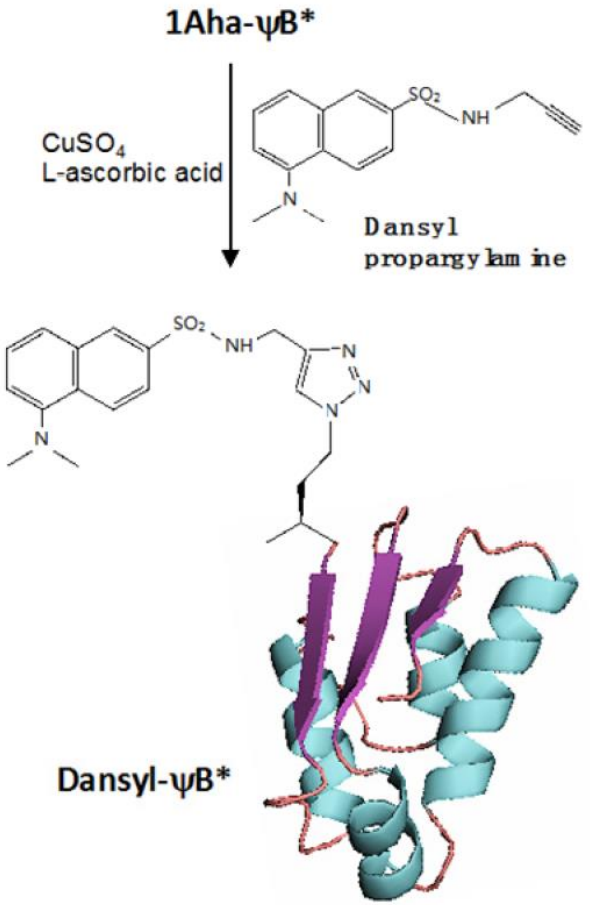
Novel

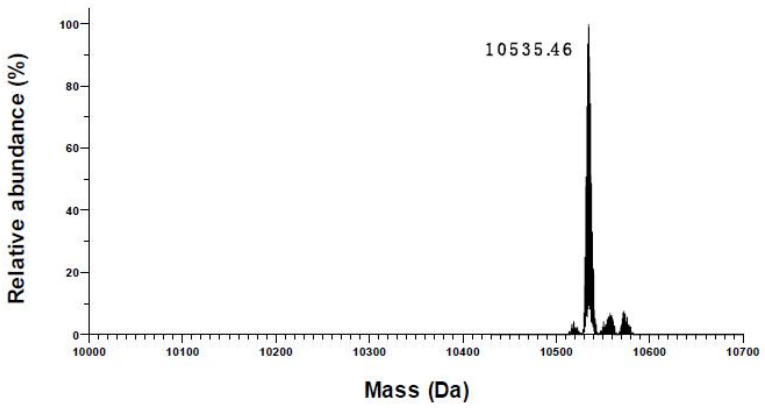


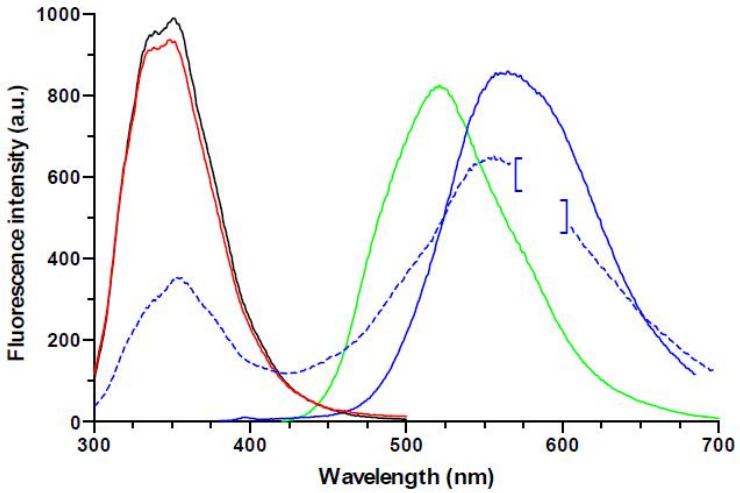
Ma Y, Biava H, Contestabile R, Budisa N, di Salvo ML (2014). Coupling Bioorthogonal Chemistries with Artificial Metabolism: Intracellular Biosynthesis of Azidohomoalanine and Its Incorporation into Recombinant Proteins.
Molecules **2014**, *19*, 1004-1022; doi:10.3390/molecules19011004

Aha-barstar: Click chemistry and fluorescence

Novel







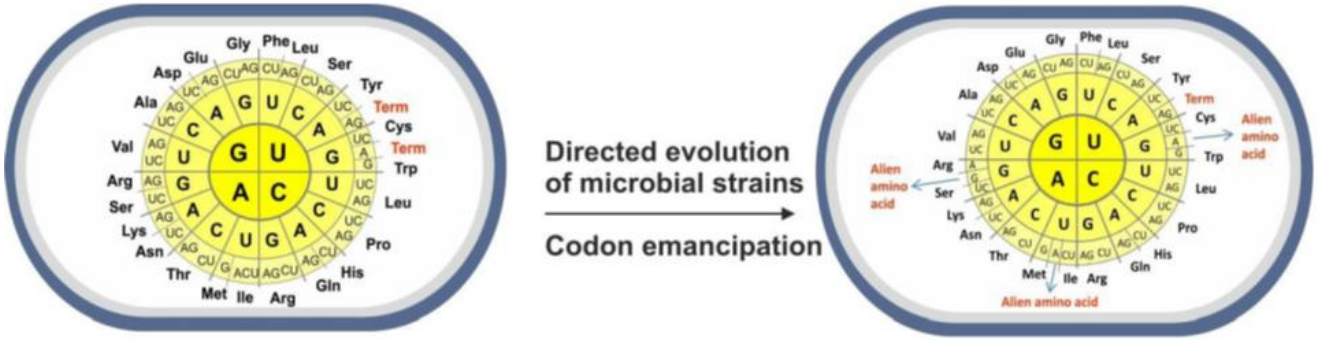
Ma Y, Biava H, Contestabile R, Budisa N, di Salvo ML (2014). Coupling Bioorthogonal Chemistries with Artificial Metabolism: Intracellular Biosynthesis of Azidohomoalanine and Its Incorporation into Recombinant Proteins.
Molecules **2014**, *19*, 1004-1022; doi:10.3390/molecules19011004

Topic

N. Budisa: Xenobiology, New-to-Nature Synthetic Cells and Genetic Firewall
Current Organic Chemistry, **18**, (2014) In press .

VISION

Why Synthetic Biology?



Why synthetic cells? Why new chemistry of life?

Novel

We need platforms with engineered genetic code for the transfer of numerous chemical reactions and processes from the chemical synthetic laboratory into the biochemistry of living cells.

- Living cells (microbes) → ready-made prefabricated production systems governed by a genetic program
- Synthetic organisms as an important emerging technology because nature's own versatility would allow the production of practically any imaginable substance
- biological organisms are akin to programmable manufacturing systems; by making small changes in their genetic program a bioengineer can effect big changes in their output

N. Budisa: Xenobiology, New-to-Nature Synthetic Cells and Genetic Firewall
Current Organic Chemistry, **18**, (2014) In press .

Biocat Group in Berlin- Research Topics

Drug Design
(Ribosome as a route to the diversity of small molecules)

Biomaterials (congeneric peptides and proteins)

Biocatalysis (biocatalytic processes in cellular systems)

Bioorthogonal conjugations (synthetic chemistry & in vivo chemistry)

Photobiology - chromophore design (synthetic chemistry & chemistry in vivo)

Biophysics - protein folding and stability (Engineering & Design)

Metabolic Engineering (reprograming intracellular amino acid syntheses)

Directed evolution and engineering of bacterial strains

Designer proteins and cells - alternative or novel chemistry of life

HERZLICHEN DANK FÜR IHRE AUFMERKSAMKEIT!!!