# Systems Biotechnology for Industrial Microbiology

**Dr Jens O Krömer**

# Background

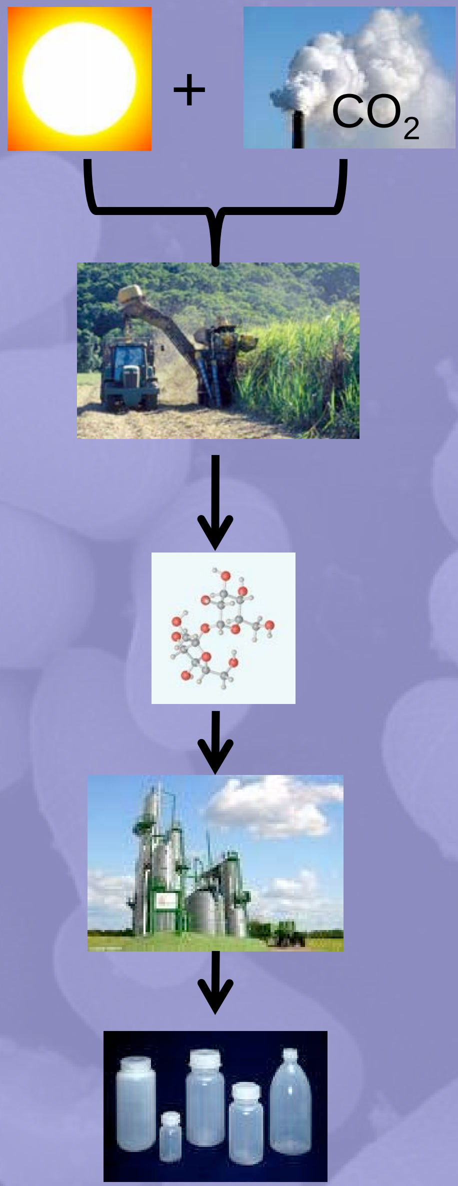


The global chemical industry grew by approximately 5 % p.a. to around **US$ 3.35 trillion** in 2007

95 % of the feedstocks come from petroleum and natural gas (rising prices, decreasing availability)

It is predicted that the current **3-4 %** renewable feedstocks (2009) will increase to **17 %** by 2025 (A D Little).

# Background




**Queensland's sugar industry:**

- **harvesting sunlight and $CO_2$**
- **highly productive,**
- **dependent on volatile sugar prices.**

**Sugar cane produces a highly fermentable bio-feedstock: SUCROSE**

**Biobased chemical feedstocks are a large opportunity for value addition in Biorefineries**

# Systems Biotechnology

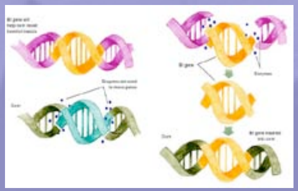
Genome Scale FBA
Thermodynamic
Extreme pathways / elementary modes

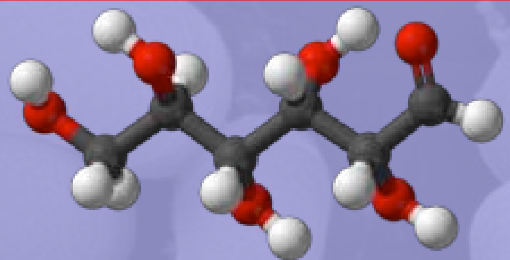
**Modelling**

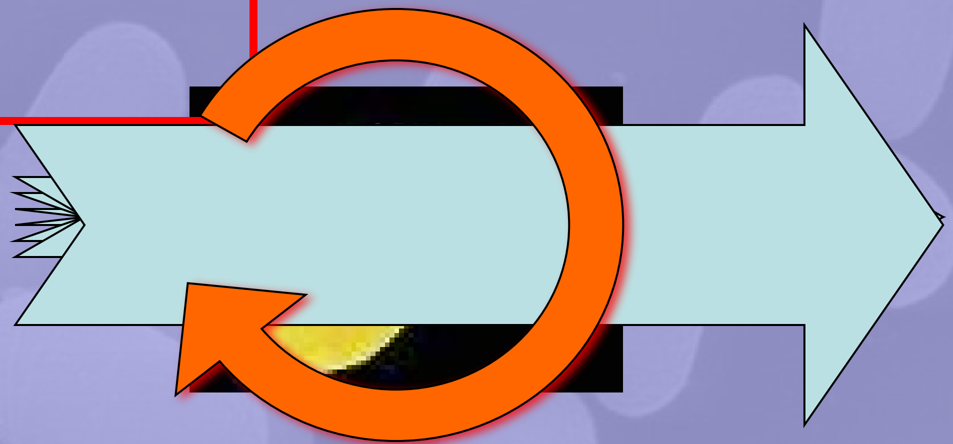
KO / KI

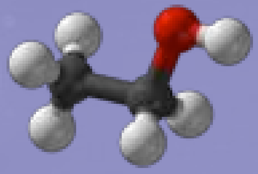
Overexpression

**Manipulation**



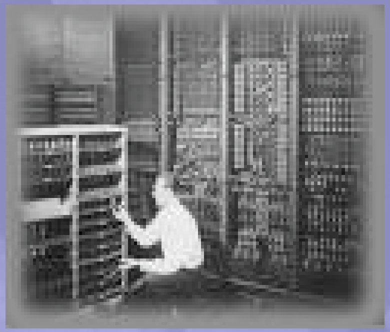






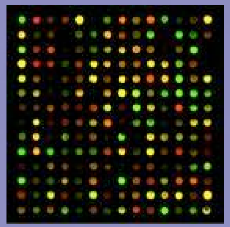
**Mine**

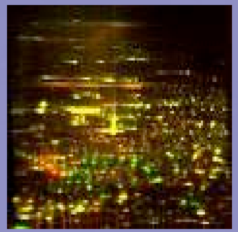
**Measure**

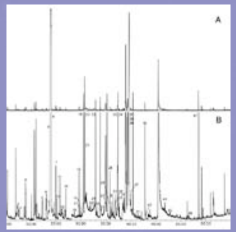


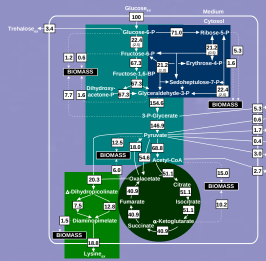
Omics data integration

Transcriptomics Proteomics Metabolomics Fluxomics





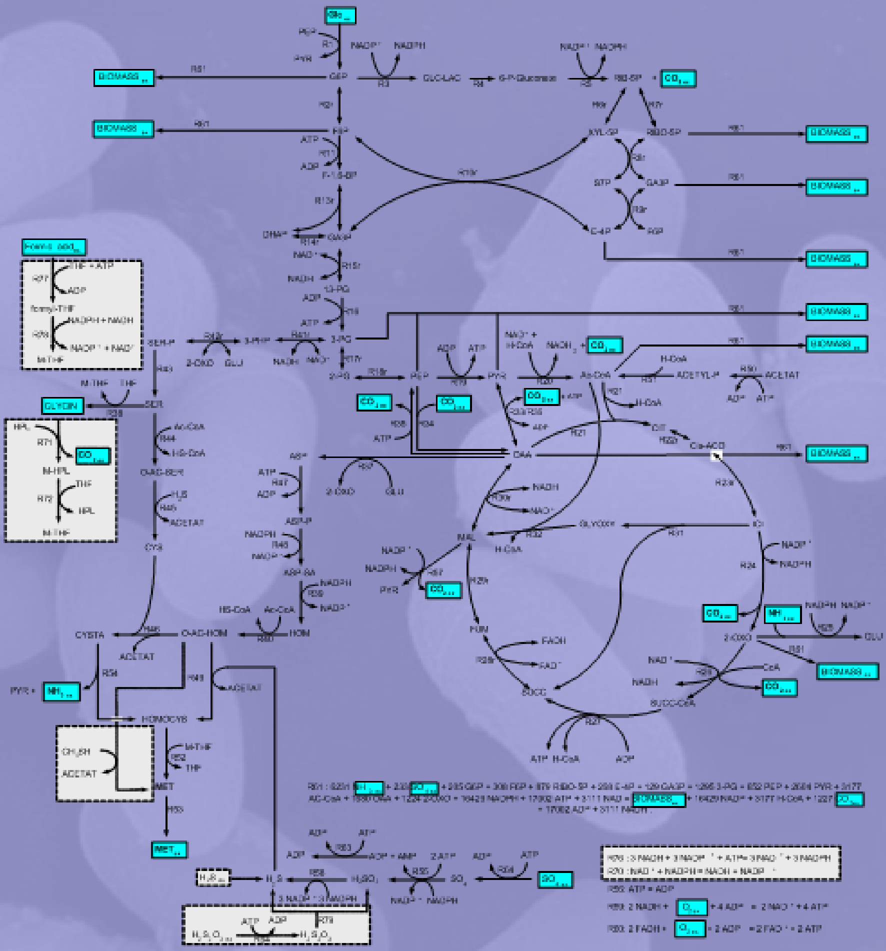


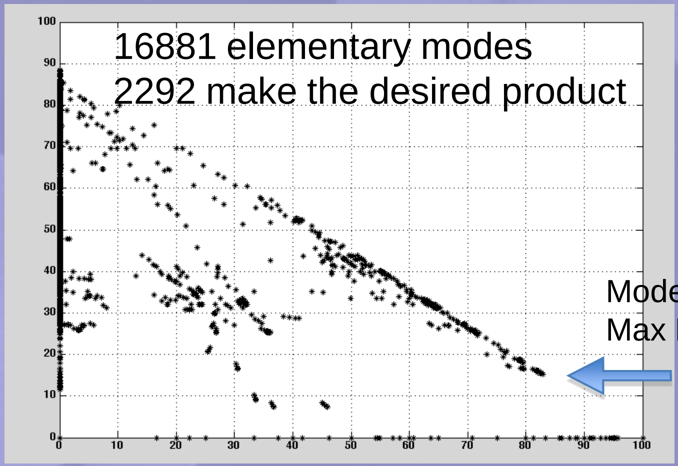


# Modelling

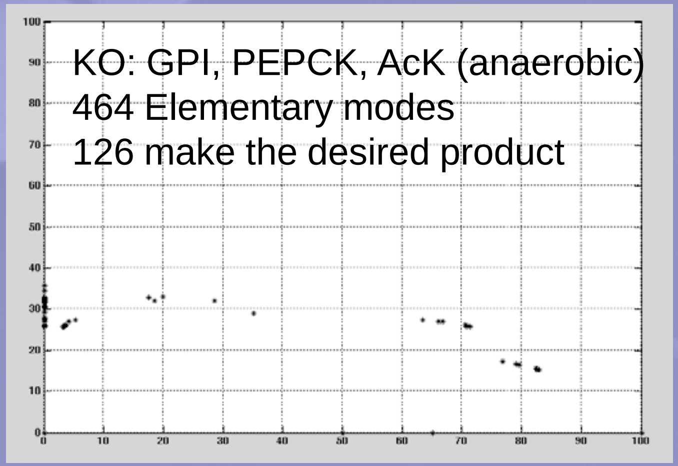


## Exploring the stoichiometric capacity of metabolic networks






Mode #11048
Max P with X>0




## Optimization of production strain and process *in-silico*

# Systems Biotechnology



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**Thermodynamic**
**Extreme pathways / elementary modes**

**Modelling**

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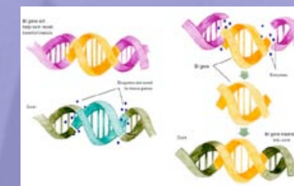
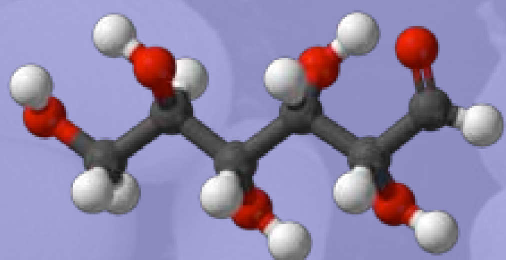
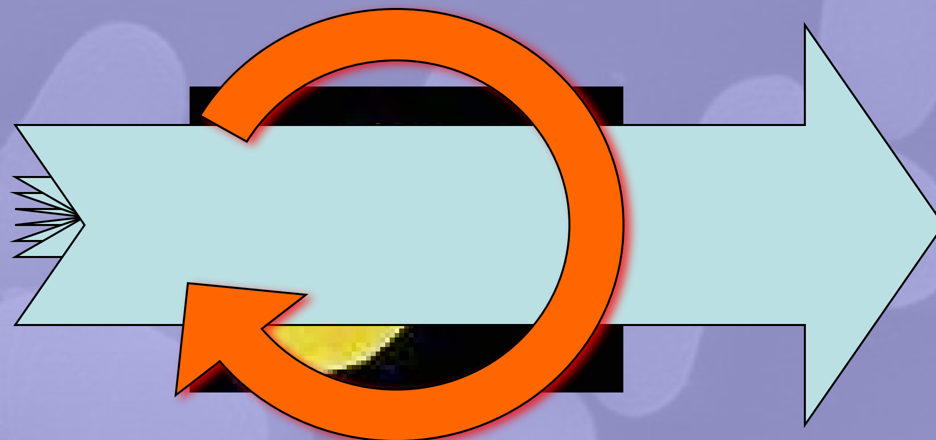
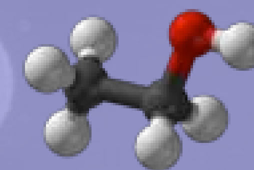
**Manipulation**







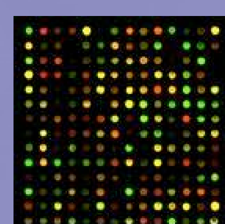


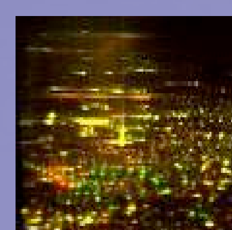
**Mine**

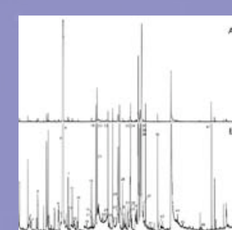
**Measure**

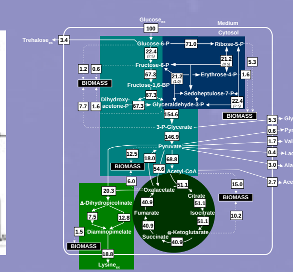
**Omics data integration**

**Transcriptomics** **Proteomics** **Metabolomics** **Fluxomics**









# Systems Biotechnology



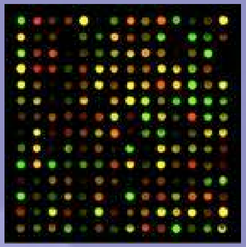
## Genomics

ATGCTAGCGTAGTCGTAGAGATAGTCTAT
ATATGATAGTAGCTGCTCGCTATGCTGAT
CGATCGGATGCGTAGCGTTGCGCTCCAT
GCTAGCGTAGTCGTAGAGATAGTCTATAT
ATGATAGTAGCTGCTCGCTATGCTGATCG
ATCGGATGCGTAGCGTTGCGCTCCATGCT
AGCGTAGTCGTAGAGATAGTCTATATATG
ATAGTAGCTGCTCGCTATGCTGATCGATC
GGATGCGTAGCGTTGCGCTCCATGCTAG
CGTAGTCGTATGATCGATCGGATGC

**Genetic potential of an organism**

***Which reactions are available?***

## Transcriptomics

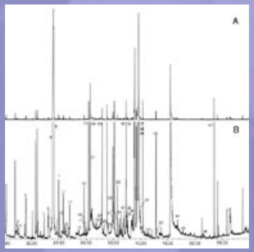


## Proteomics

**Gene expression**

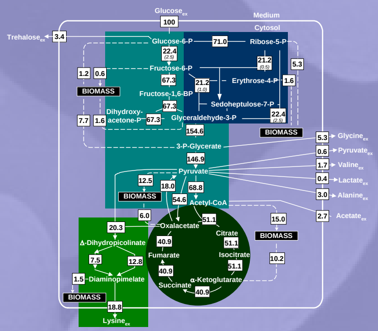
***Which reactions do we need under the conditions studied?***

## Metabolomics



**Substrate – product concentrations**

***Given the thermodynamics, is a reaction reversible?***



## Fluxomics

**quantitative representation of metabolic phenotype**

# Methionine



Example:

Industrial production of Methionine

# Methionine



Methionine is an essential sulphur-containing amino acid used as supplement in animal feed



Current world market is 800-900 000 t p.a. Around 1.8 Bn $US market size.

Production as a racemic mixture in chemical synthesis using hazardous chemicals such as acrolein and cyanide

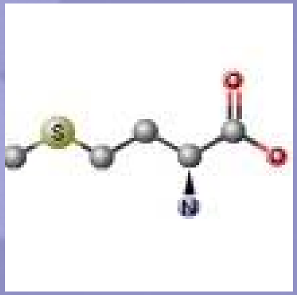
Purification of the product requires large amounts of organic solvents.

# Methionine



Advantages of a biotechnological production:

- Pure L-methionine would be obtained




- No need for hazardous feedstocks
- No elaborous purification
- Production from renewable resources

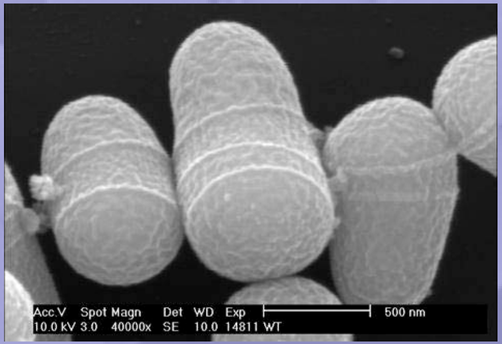
# Methionine



## *Corynebacterium glutamicum*

Most important organism for the industrial production of L-glutamate and L-lysine

Aerobic, non-sporulating, rod-shaped soil bacterium, ca. 2 x 0.5 µm, GRAS-status




Very promising organism for rational strain design of a methionine producer

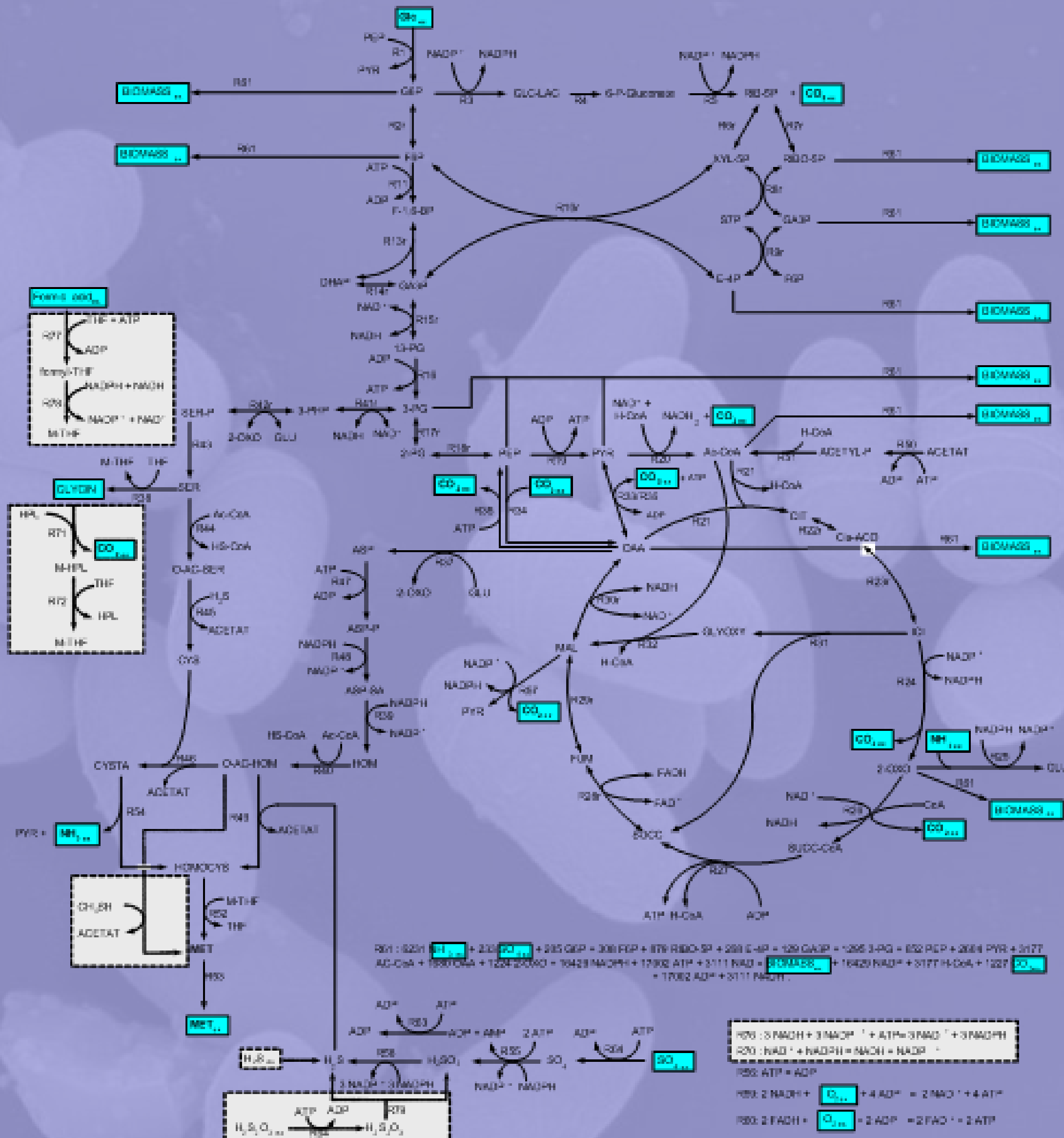
# Methionine



***Part 1***

## In-silico modelling

# Elementary Mode Analysis



Central carbon metabolism

Methionine biosynthesis

Anabolism

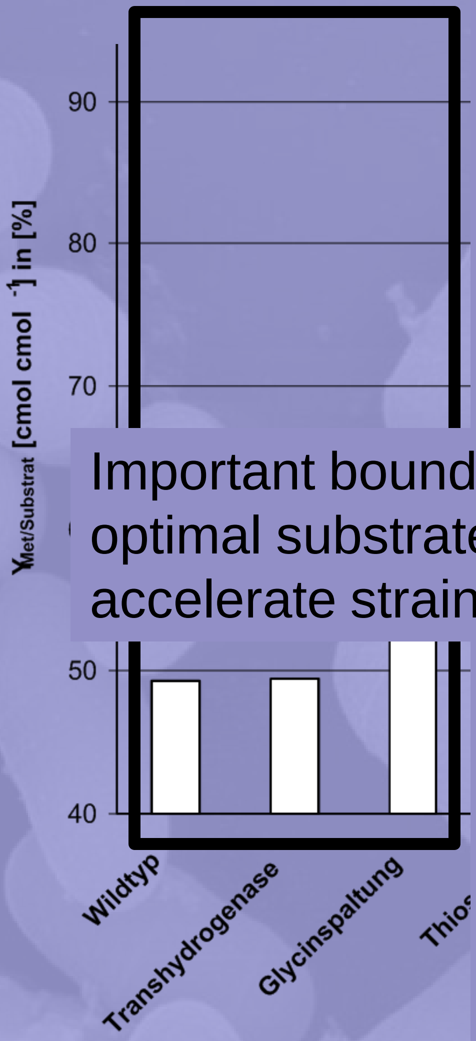
$C_1$- and sulphur metabolism

Energy and redox metabolism

# Elementary Mode Analysis

Genetic manipulation

Substrate choice




$Y_{Met/Glc}$ = 90,9 %

Important boundaries of the network will be defined and optimal substrate / process szenarios can be evaluated to accelerate strain development

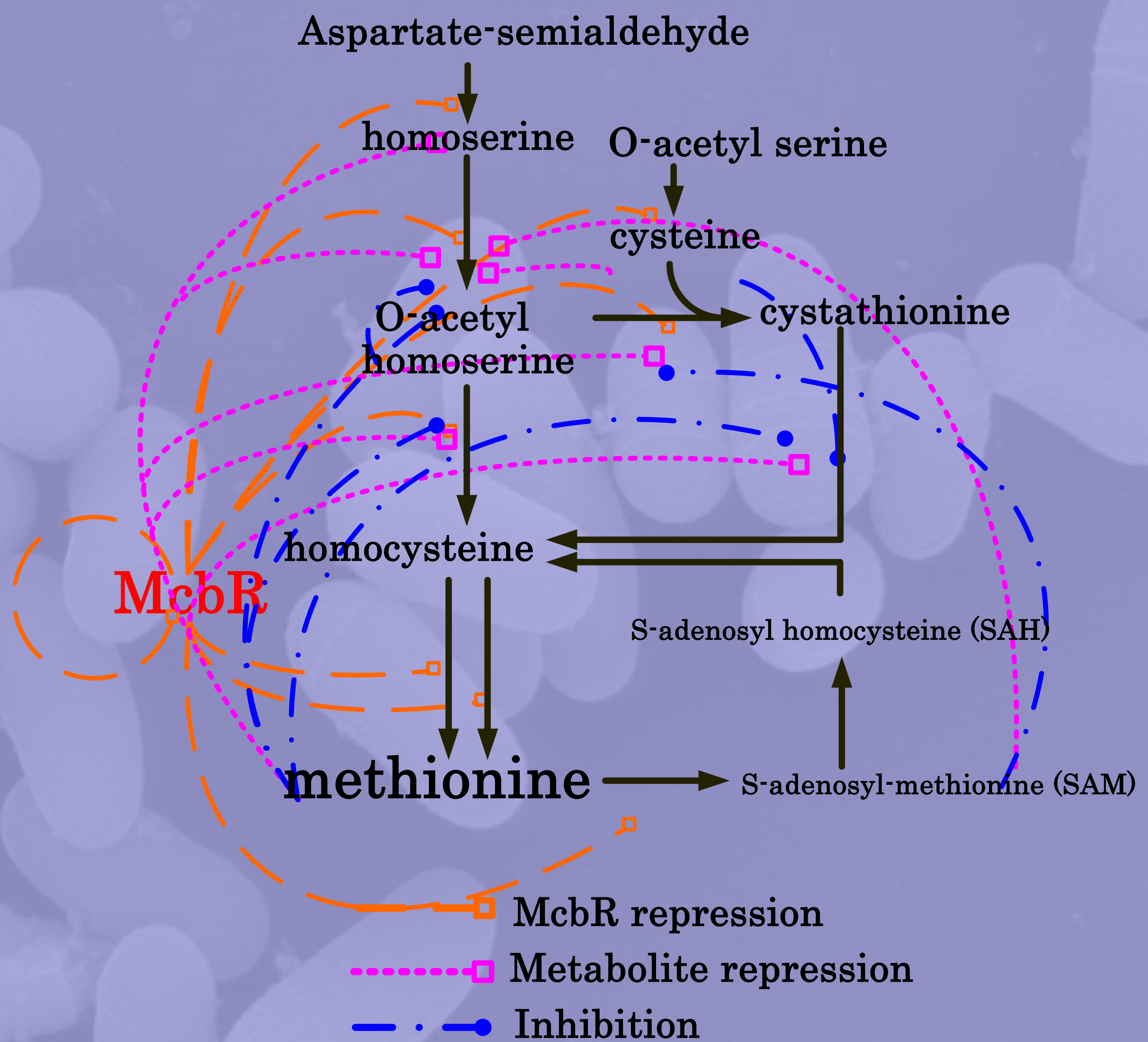
Krömer, J.O. et al. (2006). Metab. Eng. 8(4): 353-69.

WO2007020295

## *Part 2*

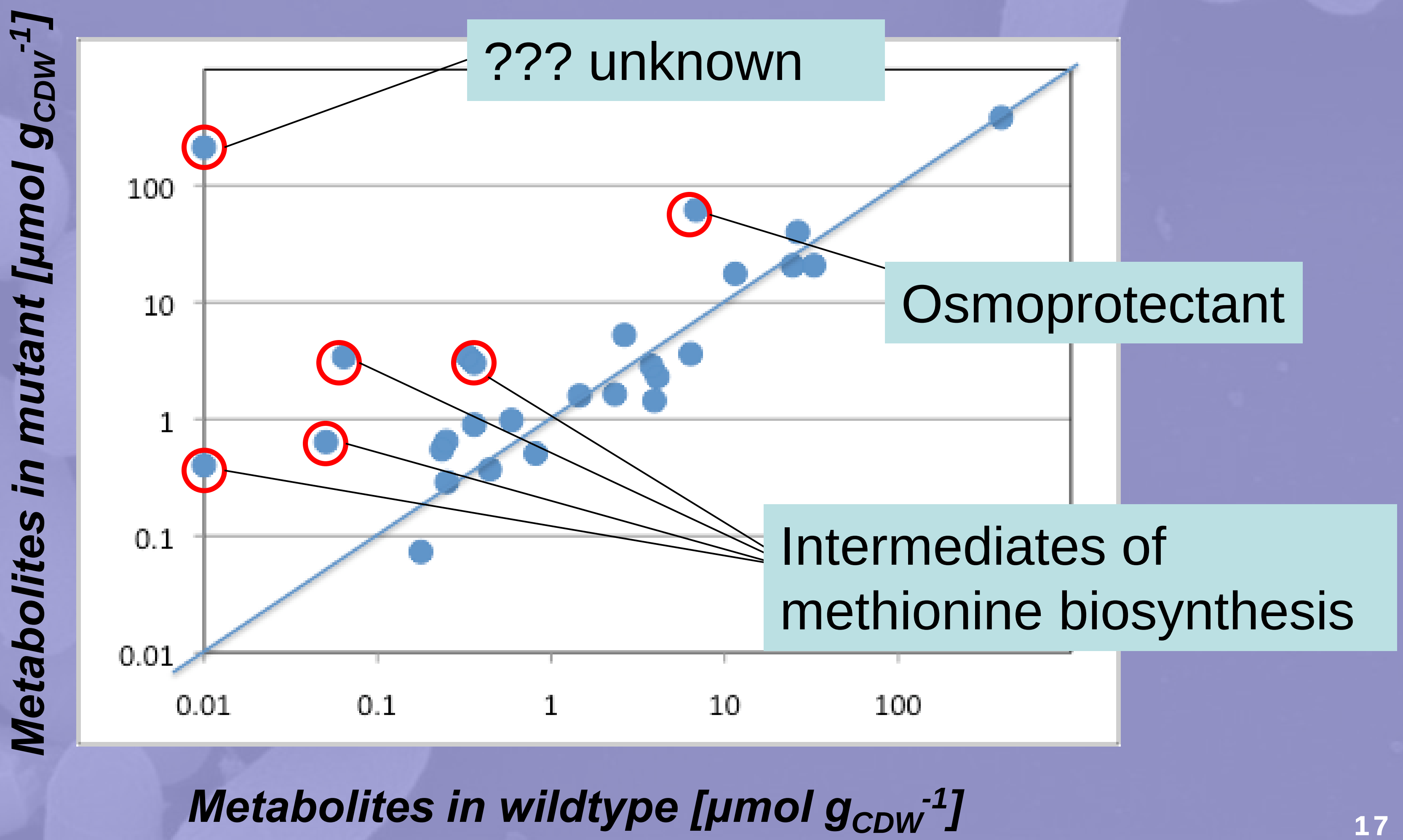
## Engineering of cells

# Metabolomics & Fluxomics



# Metabolomics

## *Effect of McbR knockout in Corynebacterium glutamicum*

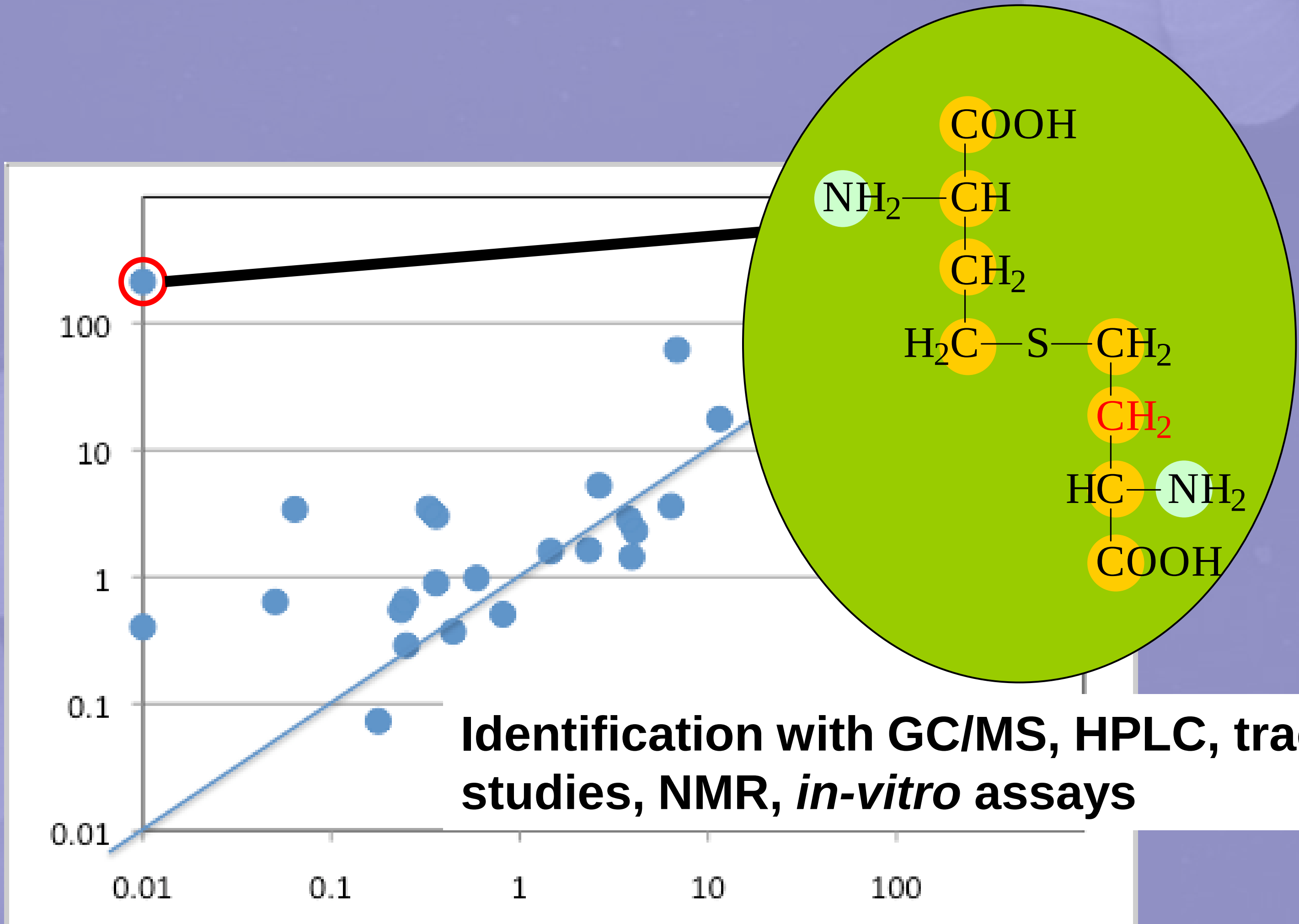


# Metabolomics



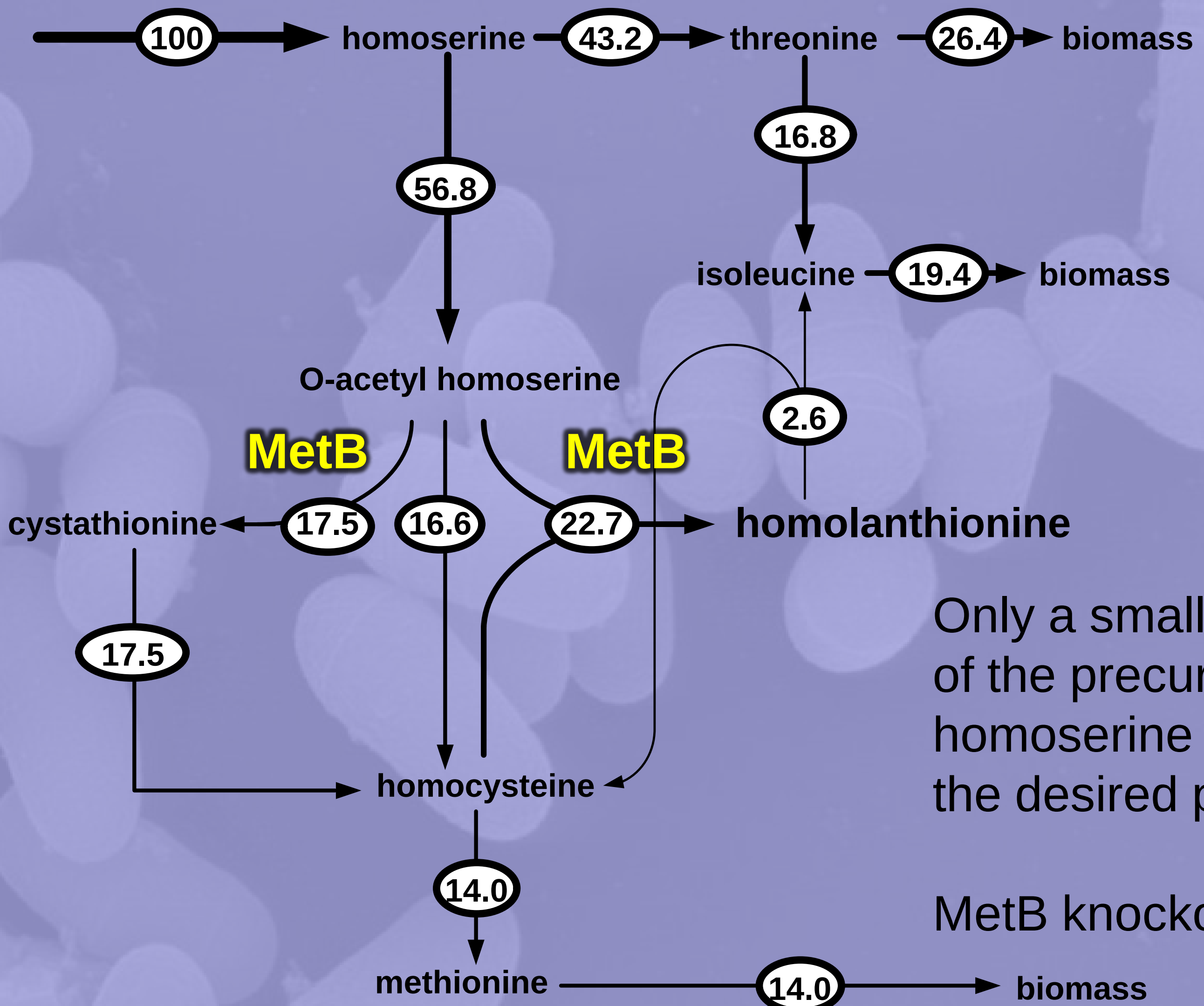
## *Corynebacterium glutamicum*

## homolanthionine




**Identification with GC/MS, HPLC, tracer studies, NMR, *in-vitro* assays**

# Metabolism of homolanthionine in *C. glutamicum*




Only a small fraction (14 %) of the precursor homoserine finally ends in the desired product

MetB knockout target

Krömer, J.O et al. (2006) J. Bacteriol. 188: 609-618. WO2007051725

# Summary



**Transcriptomics and proteomics indicated that all proteins in methionine biosynthesis were overexpressed, but no methionine was secreted.**

**Metabolomics and fluxomics could point to the activation of a previously unknown side route that drained vast amounts of precursor.**

**Combination of all ‘omics technologies with network analysis is the key to the development of new bio-catalysts. Random approaches did not work on methionine for decades.**

# Acknowledgements



**Elmar Heinzle**
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## Thank you for your attention