«АГРИ» - 20
«Генетика» - 50

AGRI/Genetika

НАПРАВЛЕННОЕ ИЗМЕНЕНИЕ ВНУТРИКЛЕТОЧНЫХ ПОТОКОВ УГЛЕРОДА С ПОМОЩЬЮ РЕДАКТИРОВАНИЯ ГЕНОМА ДЛЯ РЕШЕНИЯ ЗАДАЧ МЕТАБОЛИЧЕСКОЙ ИНЖЕНЕРИИ

Л.И. Голубева, А.А. Крылов, С.В. Машко

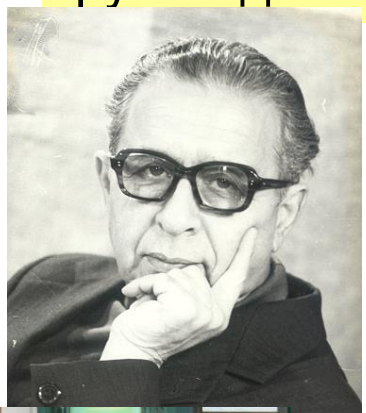
ЗАО «Научно-исследовательский институт Аджиномото-Генетика» («АГРИ»)

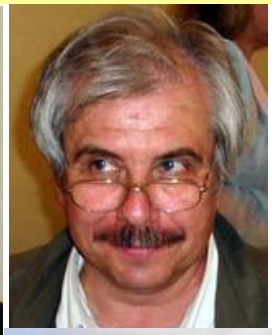
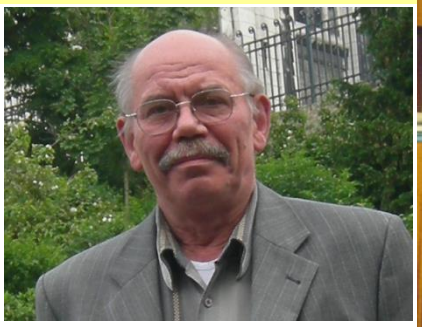
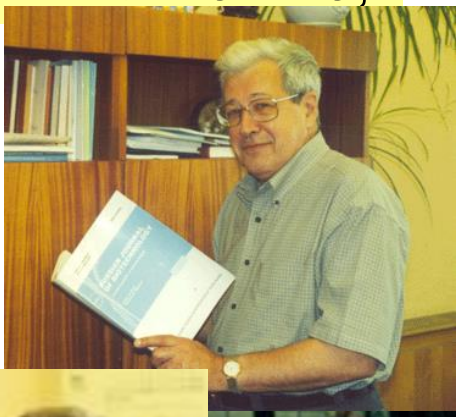
Октябрь 04, 2018

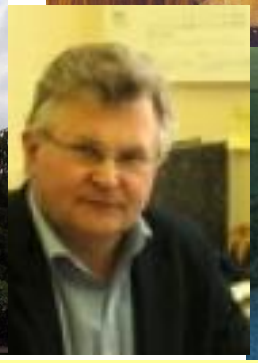
ГосНИИГенетика в развитии биотехнологии в СССР/России

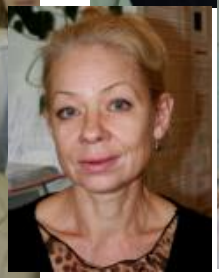
Основа биотехнологического производства АК – штаммы-продуценты.

В СССР работы по генетической селекции продуцентов АК начались со II-половины 60-х годов XX века во «ВНИИгенетика» и проводились под руководством и с непосредственным участием: проф. С.И. Алиханяна,



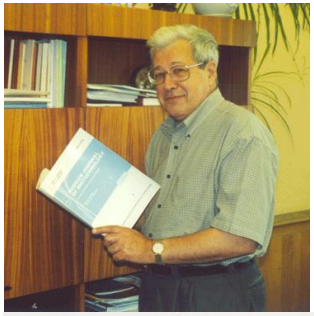




проф. Н.И. Ждановой, позднее – Академиков РАН, проф. В.Г. Дебабова и Н.К. Янковского, проф. Ю.И. Козлова и их многочисленных последователей и учеников и в Генетике, и в АГРИ

AGRI's history (Data & "Promoters")

1970	1-st contact between specialists of Genetika & Ajinomoto (Dr. Zhdanova & Dr. Shio);
1978	1-st Recombinant Thr-producer – Genetika (Profs. Debabov & Kozlov);
1982	Licensing of Thr-strain from Genetika to Ajinomoto;
1990 – 1998	Contract-base research on AA and NA in Genetika;
1995 – 1997	Feasibility study on Joint Research Institute;
1998/05	Contract-base research with different form;
1998/06/01	Ajinomoto-Genetika Project (AGP) in Genetika started;
1998/07/27	Direction of the Russian Prime Minister of establishment of Ajinomoto-Genetika Research Institute (AGRI);
1998/11	Signing constituent documents;
1998/12/08	Start of AGRI's activity;
2000/01/26	AGRI obtained accreditation as a Scientific organization;
2003/04/30	Share purchase agreement between Ajinomoto & Genetika was entered in;
2003/05/22	Ajinomoto become a sole shareholder of AGRI;
2005/12	Ajinomoto decided to construct a new building for AGRI;
2008/11	AGRI continued its activity in the new building



Метаболическая инженерия: 1991 - Рождение термина

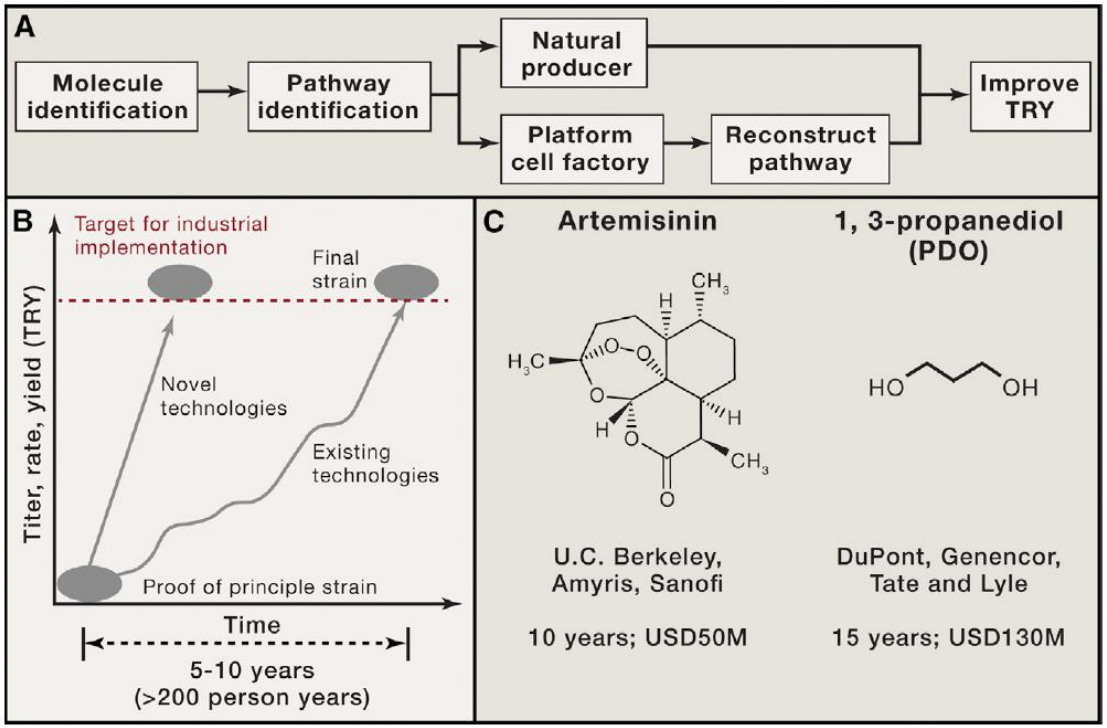
"Metabolic engineering is the improvement of cellular activities by manipulation of enzymatic, transport, and regulatory functions of the cell with the use of recombinant DNA technology" [1].

Here we define "*metabolic engineering* as the directed *improvement* of product formation or *cellular* properties through the modification of specific biochemical reactions or introduction of new ones with the use of *recombinant DNA technology*" [2].

"*Metabolic engineering* can be defined as purposeful modification of *cellular* metabolism using *recombinant DNA* and other molecular biological techniques. Metabolic engineering considers metabolic and cellular system as an entirety and accordingly allows manipulation of the system with consideration of the efficiency of overall bioprocess, which distinguishes itself from simple genetic engineering " [3].

1. Bailey JE (1991) Toward a Science of Metabolic Engineering. *Science* **252**(5013):1668-1675.
2. Stephanopoulos G (1999) Metabolic Flaxes and Metabolic Engineering. *Metab Eng* **1**: 1-11.
3. Lee et al. (2009) Metabolic engineering of microorganisms: general strategies and drug production. *Drug Discovery Today* **14(**1/2):78-88.

ME-2018: Главные успехи за ≈ 30 лет



Cell *164*, March 10, 2016 1185

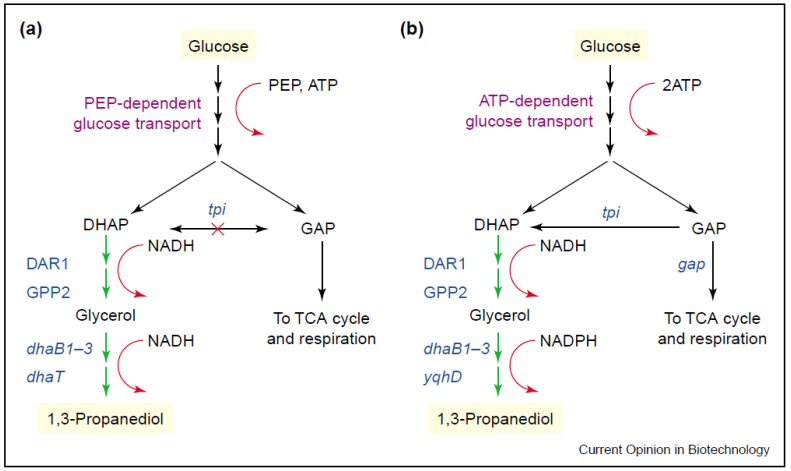


Table 1. Some Success Stories of Metabolic Engineering

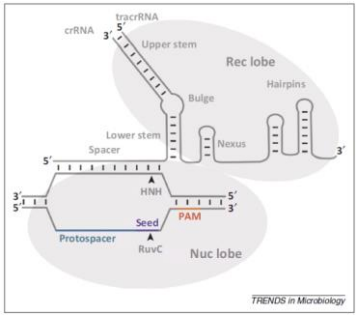
Chemical	Application	Cell Factory	Companies
Lysine	feed additive (>1 million tons/year)	*Corynebacterium glutamicum*	Evonik, ADM, CJ, Ajinomoto
1,3-Propanediol	chemical building block, e.g., for production of materials, cosmetics, and food ingredients	*Escherichia coli*	Dupont and Tate&Lyle joint venture
7-ADCA	precursor for the broad-spectrum antibiotic Cephalexin	*Penicillium chrysogenum*	DSM
1,4-Butanediol	chemical building block, e.g., for production of Spandex	*Escherichia coli*	Genomatica
Artemisinic acid	anti-malarial drug	*Saccharomyces cerevisiae*	Sanofi Aventis (process developed by Amyris)
Isobutanol	advanced biofuel	*Saccharomyces cerevisiae*	Gevo, Butamax





Specific Advantages of the Current Producer Strain Breeding

1. **Amazing improvement of bacterial chromosome editing and synthetic biology tools. (Recombineering, BioBricks, MAGE, CRISPR and etc.)**



2. **Broaden implementation of mathematic approaches, computer modeling and design in Met Eng.**

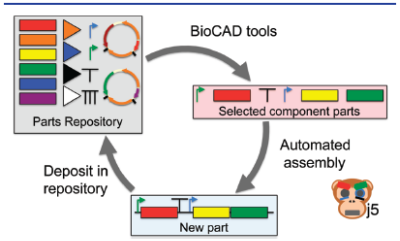
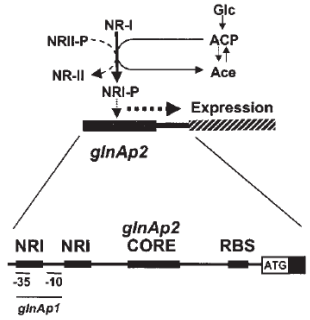


Figure 5. j5 DNA assembly design automation as part of an integrated Synthetic Biology design-implement-assay cycle.

3. **Application of Robotics for High-Throughput (HT) Cloning and Screening Assays.**

Robots And Microbes: Zymergen Raises $44 Million From Big VCs

4. **Broad introduction of Dynamic metabolic control strategy instead of Static approach.**



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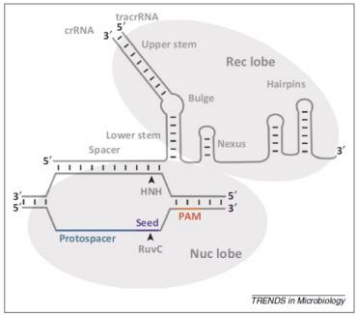

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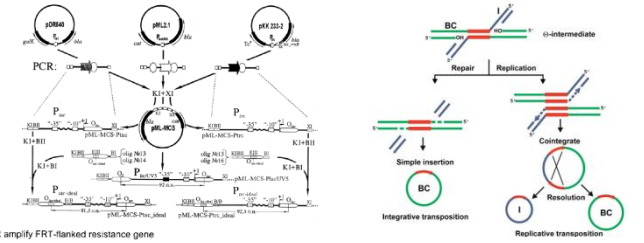
Improvement of chromosome editing tools

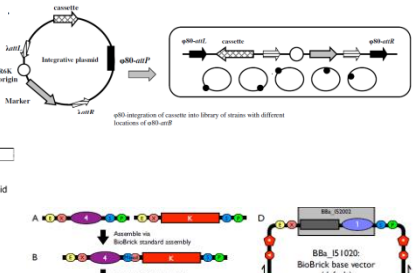
Cloning genes in multi-copy plasmids;
Mu-driven random integration/amplification

Gene 1981, **13:**37-46; **Gene** 1991, **97:**259-266



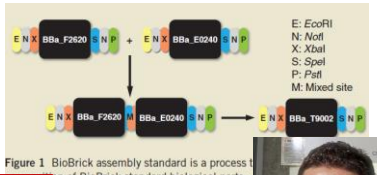
Red/RecET-driven Recombineering;
Targeted site-specific integration/amplification

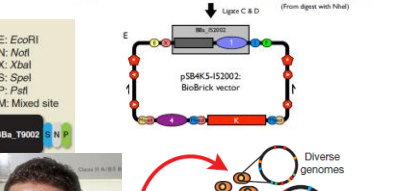
PNAS 2000, **97:**6640-6645; **BMC Biotechnol** 2008, **8:**63



Automation design from BioBricks;
Targeted genes/genomes synthesis and cloning

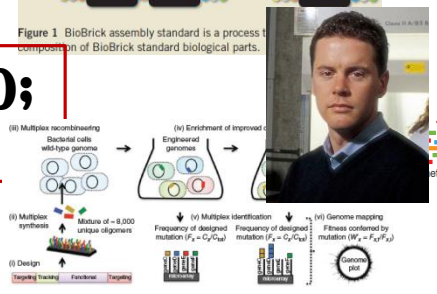
Nat Biotechnol 2008, **26:**787-793; **BMC J Biol Eng** 2008, **2:**5





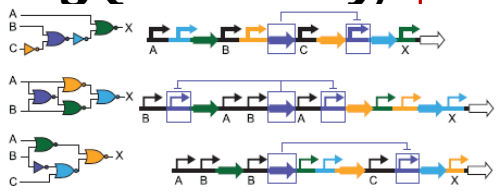
Multiplex automated genome engineering (MAGE);
Trackable multiplex recombineering (TRMP)

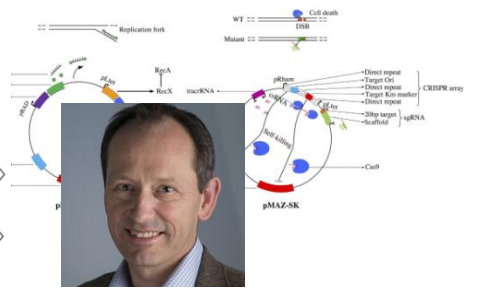
Nature 2009, **460:**894-898; **Nat Biotechnol** 2010, **28:**856-862



CRISPR optimized MAGE recombineering (CRMAGE);
Genetic circuit design automation

Sci Rep 2016, 6:19452; **Science** 2016, **352:** aac7341





Summary for *E. coli* tools

1) Редактирование генома с пом. Recombineering по Datsenko & Wanner идет в *E. coli* очень эффективно и дальнейшее CRISPR/Cas9-зависимое улучшение возможно, но не обязательно;

Recombineering = **Recombi**nation mediated genetic engi**neering**

2) Эффективность Recombineering может быть повышена еще примерно на 2 порядка (до 10^4 клонов на выжившие после электропорации 10^6 клеток) использованием pSIM-плазмид и протоколов DL Court

PROTOCOL

206 | VOL.4 NO.2 | 2009 | NATURE PROTOCOLS

Recombineering: a homologous recombination-based method of genetic engineering

Shyam K Sharan[1], Lynn C Thomason[2], Sergey G Kuznetsov[1] & Donald L Court[3]

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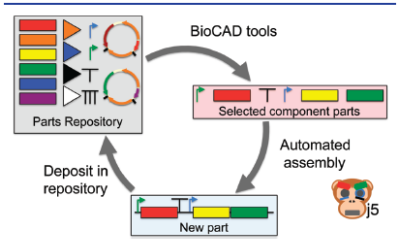

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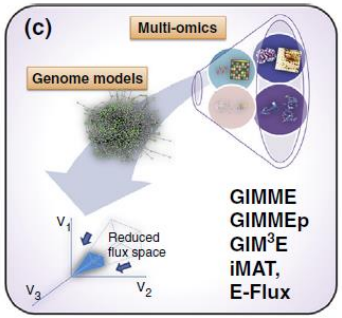
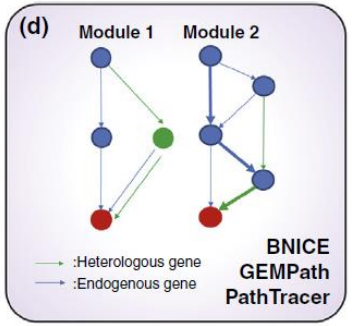
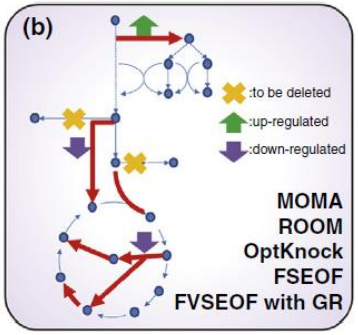
Selected computational tools for Met Eng

Name	Description	Reference
Whole-genome metabolic models databases		
MetaCyc/BioCyc	Metabolic pathways database	Caspi et al. (2012)
KEGG	Metabolic pathways database	Kanehisa et al. (2012)
BRENDA	Enzyme functional data database	Schomburg et al. (2013)
ENZYME	Enzyme database	Bairoch (2000)
REACTOME	Pathway database	Croft (2013)
MetRxn	Integrated database of genome-scale metabolic models	Kumar et al. (2012)
BiGG	Genome-scale models database	Schellenberger et al. (2010)
BioModels	Biological models database	Li et al. (2010)
Whole-genome metabolic models reconstruction		
KAAS	Automated genome annotation and pathway reconstruction	Moriya et al. (2007)
Pathways Tool	Integrated tool for prediction and comparative analyses of	Karp et al. (2002) and Dale et al. (2
PathoLogic	pathway/genome databases	
SEED	Automated reconstruction of genome-scale models	Devoid et al. (2013)
CaNOE	Automated reconstruction of genome-scale models	Smith et al. (2012)
Pathway search tools		
FMM	Search pathways in KEGG between source and target metabolites	Chou et al. (2009)
BNICE	Retrosynthesis-based pathway search	Hatzimanikatis et al. (2005)
MetaHype	Retrosynthesis-based hypergrah pathway enumeration	Carbonell and Fichera (2012)
Metabolic flux analysis		
MetaTool	Elementary flux mode analysis	Kamp and Schuster (2006)
Copasi	Simulation and analysis of biochemical networks	Hoops et al. (2006)
FluxAnalyzer	Pathway and flux analysis of metabolic networks	Klamt et al. (2003)
COBRA PYCOBRA	Constraints-based metabolic flux analysis of genome-sc models	Schellenberger et al. (2011) and Eb
SurreyFBA	Constraints-based metabolic flux analysis of genome-sc models	Gevorgyan and Bushell (2011)
OptFlux	Integrated interface for constraints-based metabolic flux analysis	Rocha et al. (2010)
OptKnock	Constraints-based analysis of optimal knockouts	Burgard (2003)
OptGene	Constraints-based analysis of optimal knockouts	Patil et al. (2005)
OptStrain	Constraints-based analysis of optimal knockins	Pharkya et al. (2004)
Integrated frameworks for the design of metabolic pathways		
OptForce	In silico platform for the design of heterologous pathways. Ranking based on FBA and K_M	Chatsurachai et al. (2012)
	Optimization procedure to identify genetic manipulations leading to target overproduction	Ranganathan et al. (2010)
	Retrosynthesis-based framework that rank pathways based on similarity and thermodynamics feasibility	Cho et al. (2010)
	Retrosynthesis-based framework that searches for best fit for non-natural substrates through molecular modeling	Brunk and Neri (2012)
RetroPath	Retrosynthesis-based framework that ranks pathways based on pathway efficiency, FBA and toxicity	Carbonell et al. (2013c)





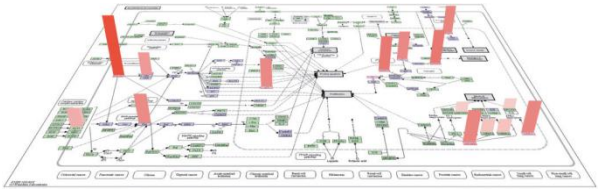


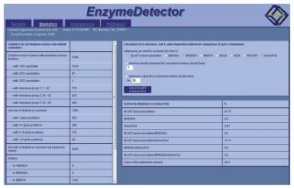
J Biotechnol 2014, **192:**302-313

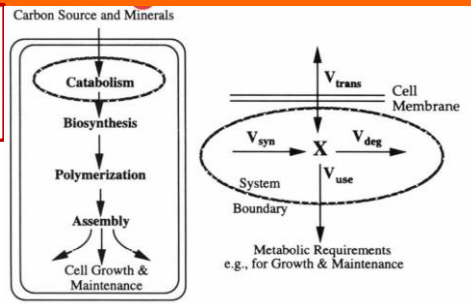
Imlementation of mathematic approaches in Met Eng

Flux Balance Analysis (FBA);
Whole genome databases (KEGG, BRENDA *etc.*)

Bio/Technology 1994, **12:**994-998; **NAR** 2012, **40:**D109-D114







Dynamic Flux Balance

$$\frac{dX}{dt} = S \cdot v - b$$

$v = fn(X,...)$

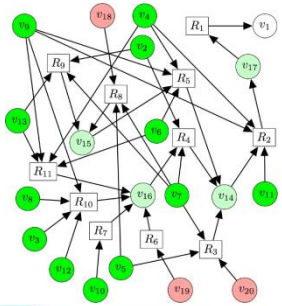
X = Metabolite Concentrations
S = Stoichiometric Matrix
v = Reaction Fluxes
b = Net Transport Out

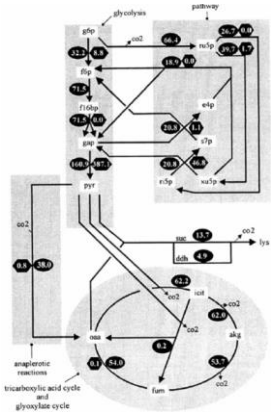
Steady State

$$S \cdot v = b$$

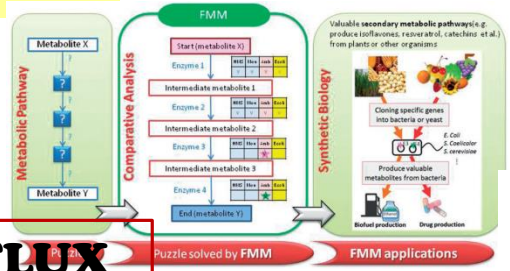
Unknown Metabolic Fluxes **v**

Initiation of ^{13}C-MFA based on NMR/MS data;
Pathway search tools (FMM, BNICE *etc.*)



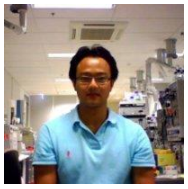


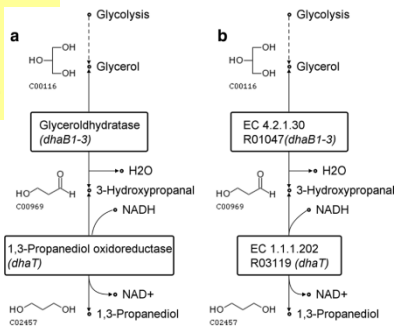
Biotech Bioeng 1996, **49:**111-129; **Bioinf** 2002, **18(S1):**S225-S232

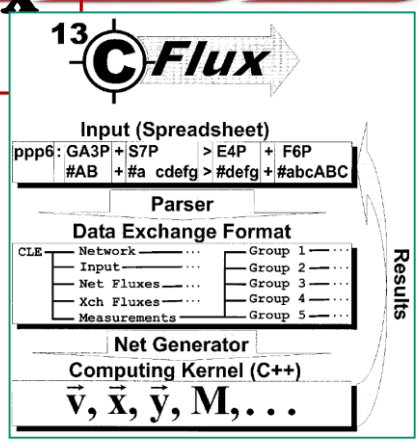


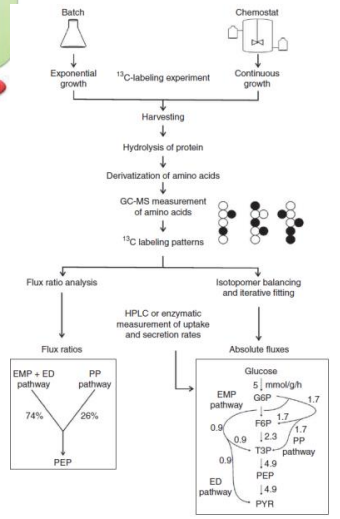
Establishment of ^{13}C-MFA, 13CFLUX; OpenFLUX
Platform for design of heterologous pathways

Metab Eng 2001, **3:**265-283; **Nat Protoc** 2009, **4:**878-892; **BMC Bioinf** 2012, **13:**93





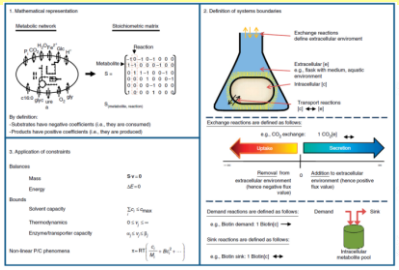




Imlementation of mathematic approaches in Met Eng

Genome-scale metabolic modeling (GSMM);
High precision COMPLETE-(^{13}C)-MFA;
D-Taylor: Automated analysis and design of DNA sequences;
J5 DNA assembly design automation software;
OptForce – Genetic manipulations for overproduction

Nat Protoc 2010, **5:**93-121; **PLoS Comput Biol** 2010, **6:**e1000744; **ACS Synth Bi**
2012, 1:14-21; **Metab Eng** 2013, **20:**49-55; **Bioinformatics** 2014, **30:**1087-1094



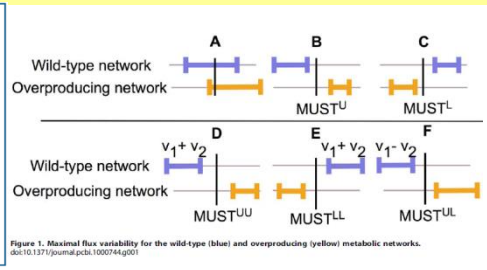
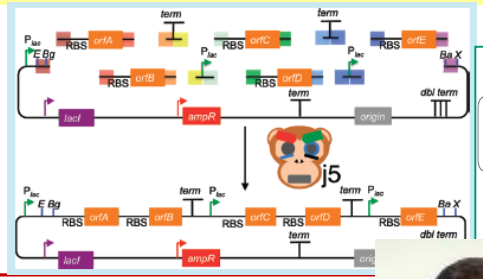
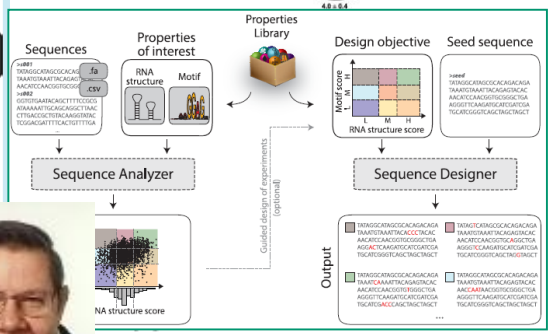


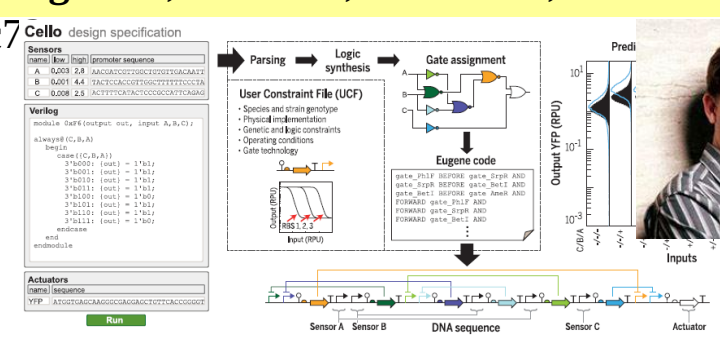
Figure 1. Maximal flux variability for the wild-type (blue) and overproducing (yellow) metabolic networks.

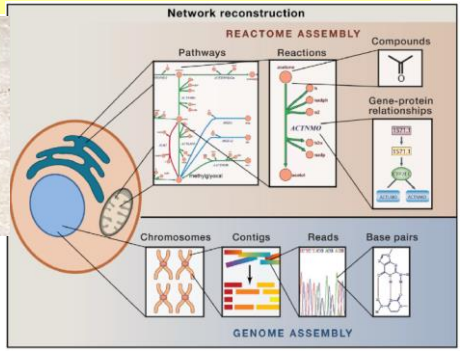


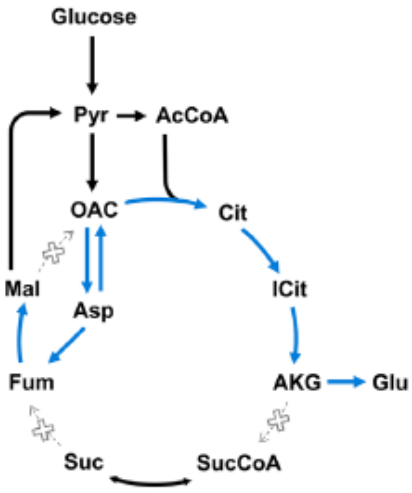


Validation of GSMM due to ^{13}C-MFA;
Using GSMM-based COBRA to predict capabilities;
Genetic programming using Cello

Metab Eng 2014, **26:**23-33; **Cell** 2015, **161:**971-987; **Science** 2016, **352:**aac7







Specific Advantages of the Current Producer Strain Breeding

1. Amazing improvement of bacterial chromosome editing and synthetic biology tools. (Recombineering, BioBricks, MAGE, CRISPR and etc.)

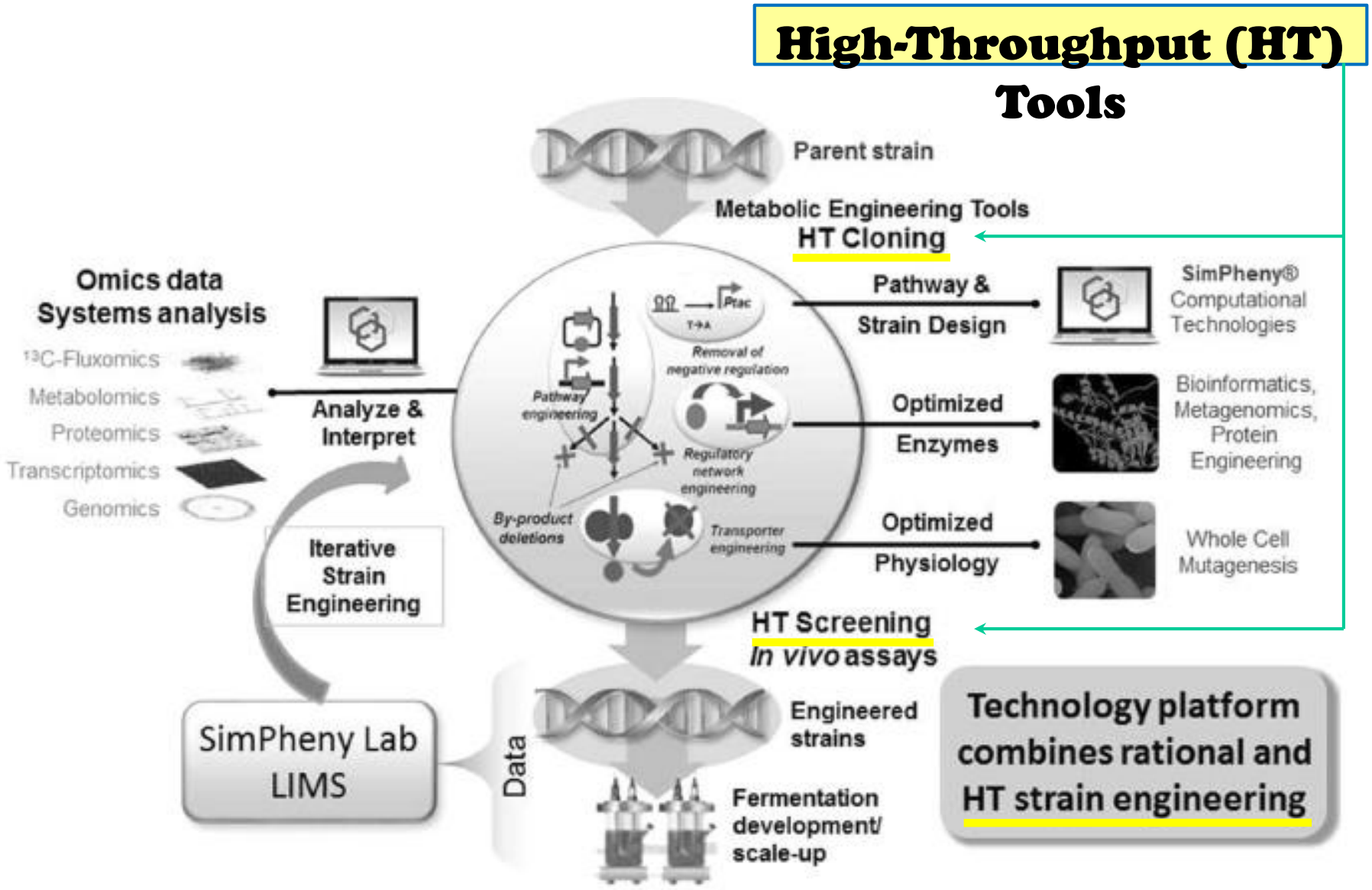
2. Broaden implementation of mathematic approaches, computer modeling and design in Met Eng.

3. **Application of Robotics for High-Throughput (HT) Cloning and Screening Assays.**


Robots And Microbes: Zymergen Raises $44 Million From Big VCs

4. Broad introduction of Dynamic metabolic control strategy instead of Static approach.

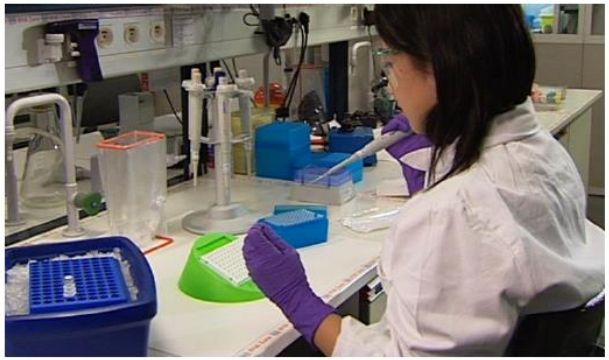
Current systems-based platform for strain breeding



J Ind Microbiol Biotechnol 2015, **42:**349-360

Amyris' Industrial Revolution

Traditional biotechnology



"Artisan" approach to biology
- Limited capacity
- Limited data acquisition
- Error-prone, reproducibility problems

AMYRIS industrialized Synthetic Biology

Scale-down, standardize, & automate
- 1000X capacity gain
- Automated data acquisition
- Economies of scale

Total Quality Management
- Continuous improvement
- Perfect reproducibility
- Only ask each question ONCE!

Edwards Deming, Kaoru Ishikawa

AMYRIS

Specific Advantages of the Current Producer Strain Breeding

1. Amazing improvement of bacterial chromosome editing and synthetic biology tools. (Recombineering, BioBricks, MAGE, CRISPR and etc.)

2. Broaden implementation of mathematic approaches, computer modeling and design in Met Eng.

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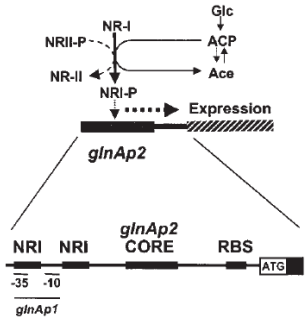
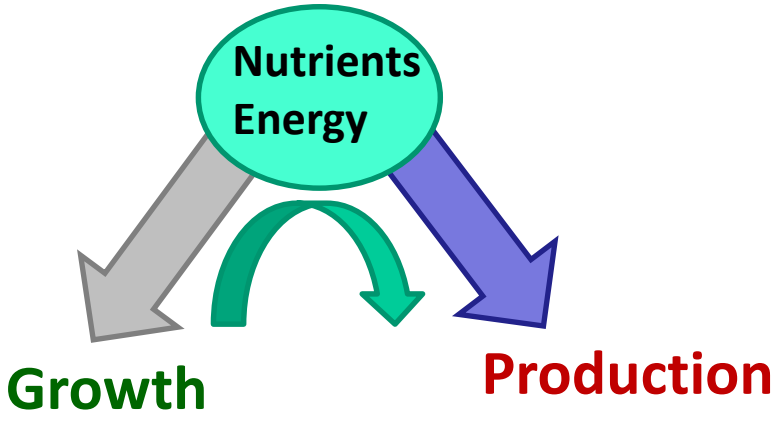
4. **Broad introduction of Dynamic metabolic control strategy instead of Static approach.**

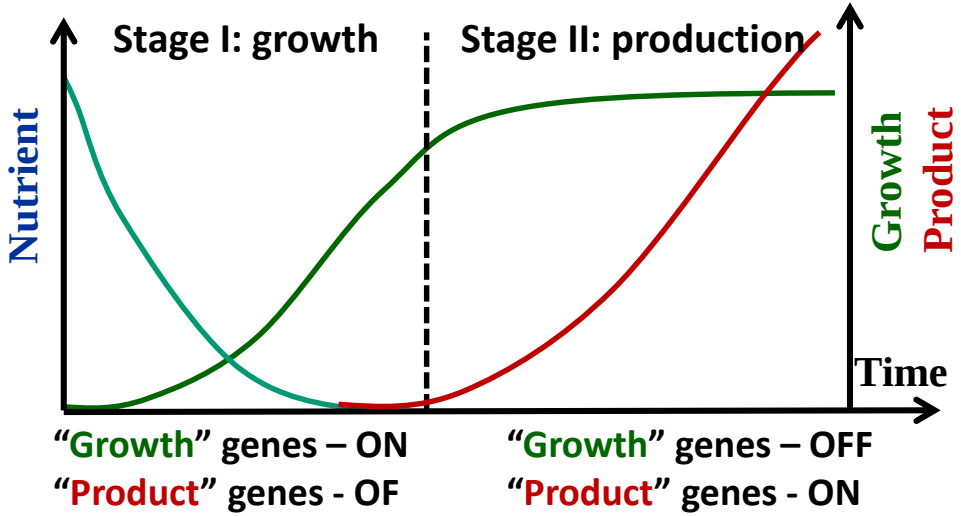


Dynamic Metabolic Control: General Principle

The main aim of metabolic engineering is improvement of product formation or cellular properties through the targeted manipulation of cellular metabolism.
Traditional strategy of gene deletion/overexpression could let to undesired strain properties like growth retardation.



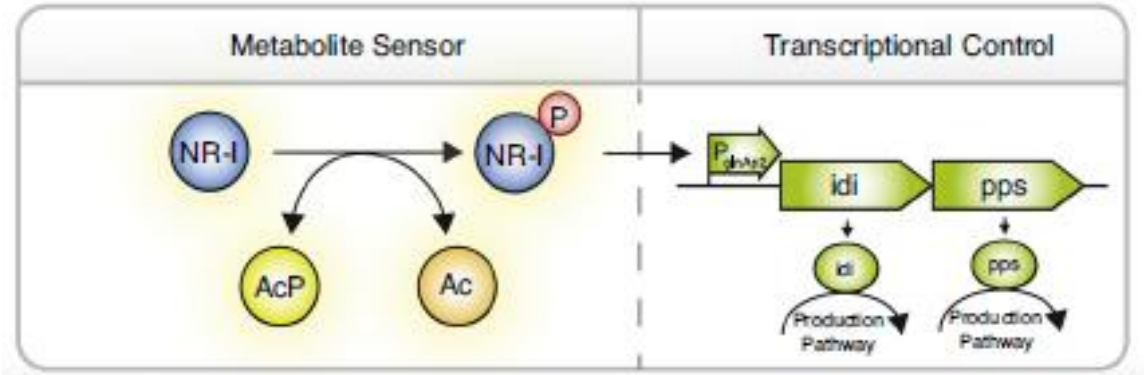
Two stage fermentation:



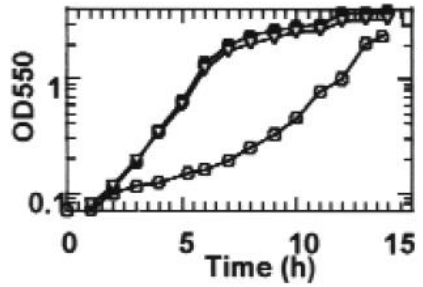
Growth is restricted by nutrient limitation;
Regulated gene switch ON/OFF system provide resource re-distribution to product synthesis at competition points.

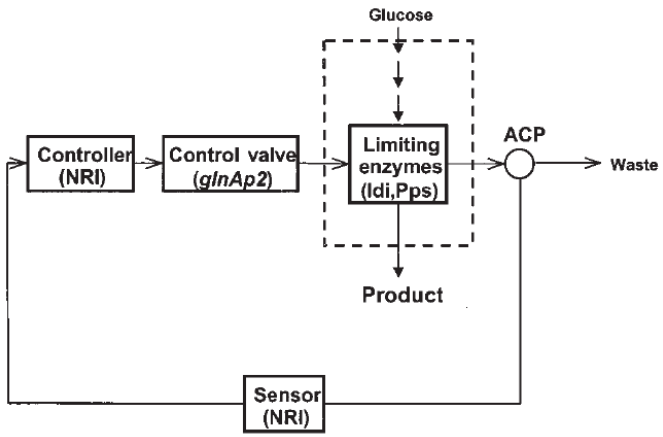
First Application of Dynamic Metabolic Control

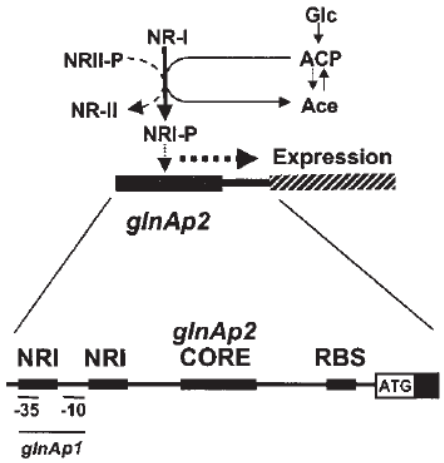
Farmer WR, Liao JC (2000) Improving lycopene production in *Escherichia coli by engineering* metabolic control. *Nat Biotechnol* **18**:533-537

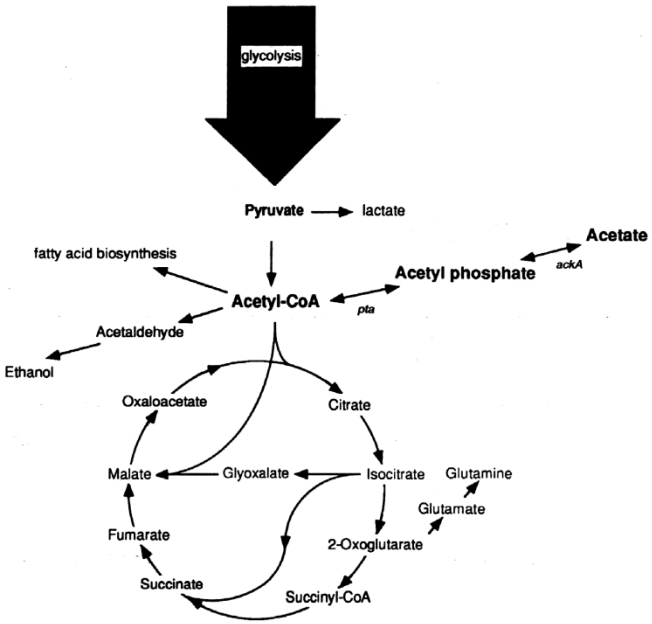


Acetate (Ac) concentration is sensed using the acetyl-phosphate (AcP)-dependent NR-I protein. ACP phosphorylates NR-I, allowing it to activate the P_{glnAp2} promoter. This promoter drives the expression of two genes required for the lycopene production pathway, in the presence of excess acetate.









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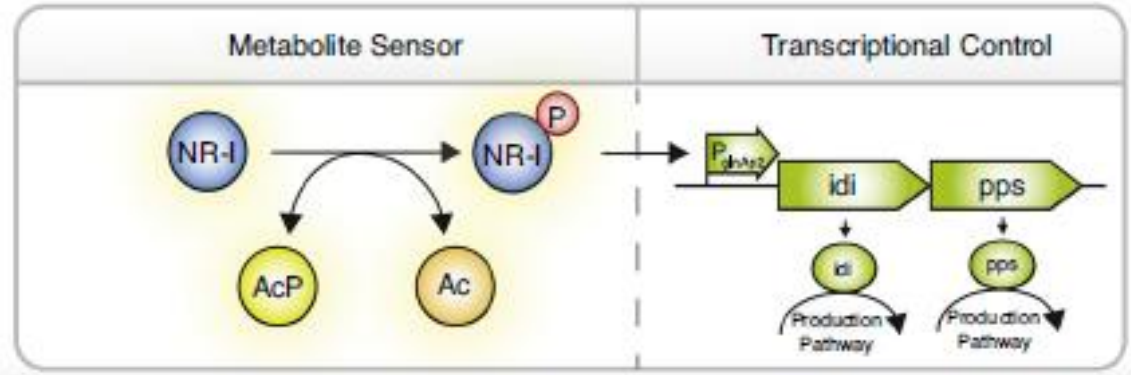

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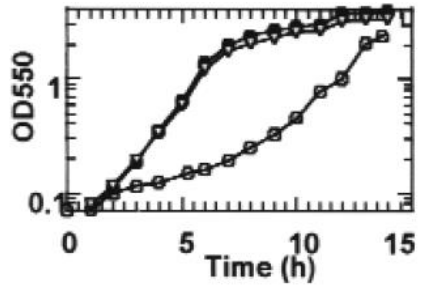
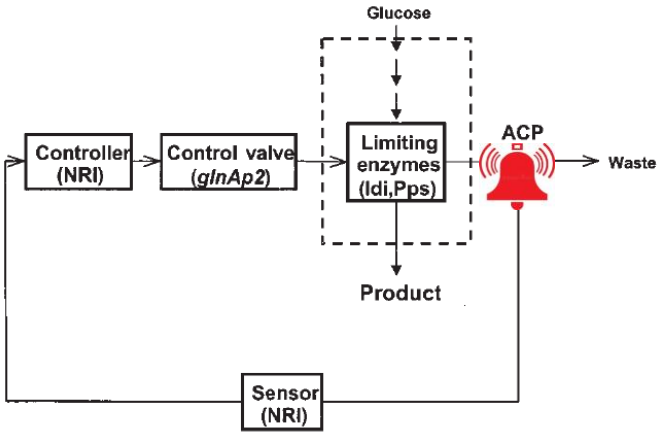
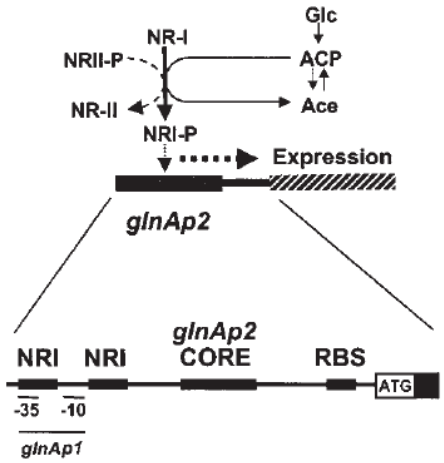
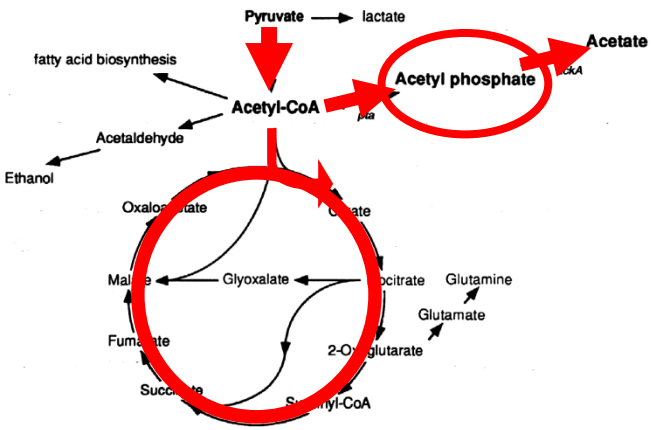








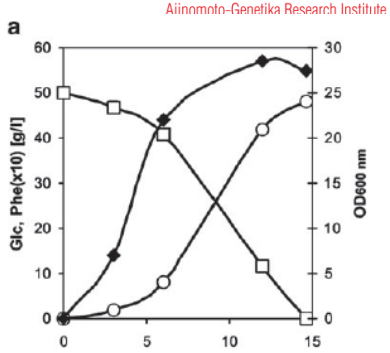
Basic Research in AGRI (Dynamic metabolic control)

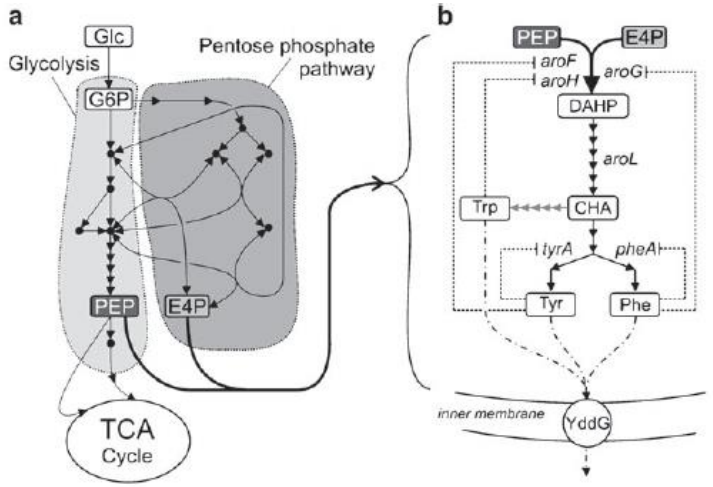
AGRI
Ajinomoto-Genetika Research Institute

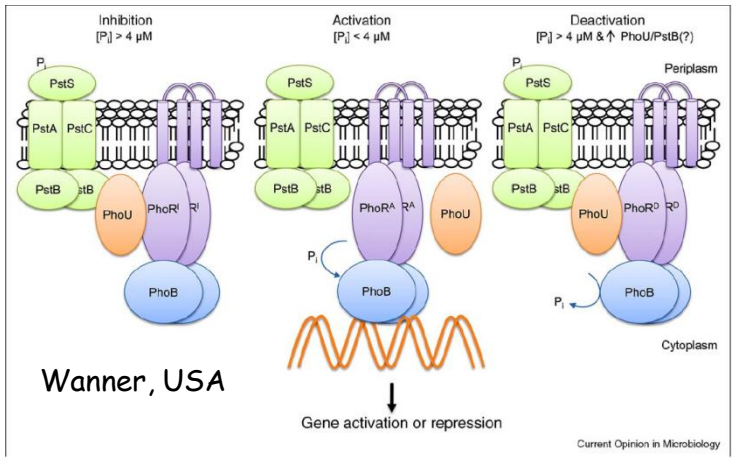
Pho regulon promoter-mediated transcription of the key pathway gene *aroG*^{Fbr} improves the performance of an L-phenylalanine-producing *Escherichia coli* strain

Vera G. Doroshenko · Irina S. Tsyrenzhapova · Alexander A. Krylov · Evgeniya M. Kiseleva · Vladimir Yu. Ermishev · Svetlana M. Kazakova · Irina V. Biryukova · Sergey V. Mashko

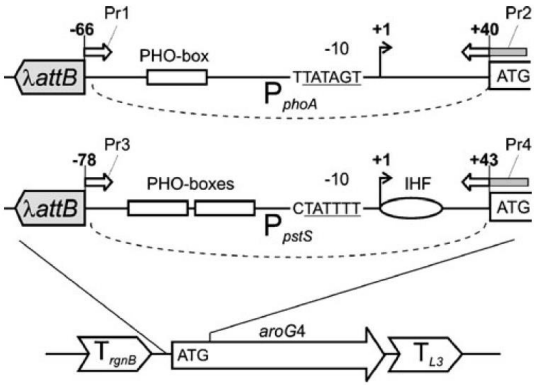
Appl Microbiol Biotechnol (2010) 88:1287–1295
DOI 10.1007/s00253-010-2794-x







Wanner, USA



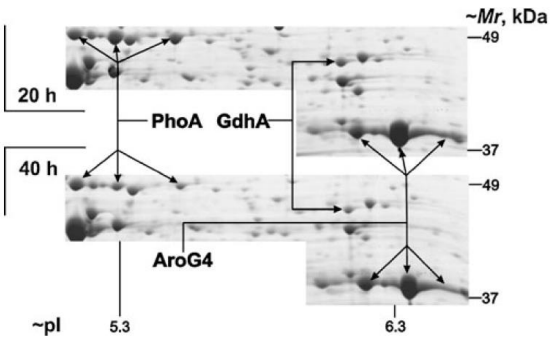
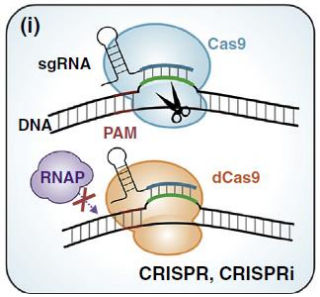


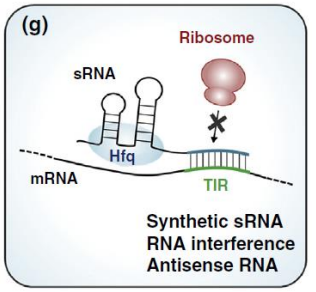
Fig. 3 Typical time profiles of DV269(Δ*tyrA*) cultivated in batch conditions. **a** Glc consumption (*open squares*), cell growth (*solid diamonds*), Phe accumulation (*open circles*). **b** DAHP synthase specific activity measured in the absence (*solid columns*) or in the presence of 5 mM Phe (*open columns*). **c** P_i consumption (*open squares*) and PhoA activity (*solid diamonds*). Standard deviations of each curve (three experiments) did not exceed 10%. *Error bars* indicate the standard deviations of three replicates

Conditional Silencing for Dynamic Metabolic Control

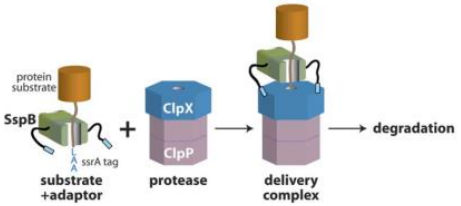
The desirable silencing could be achieved due to genetic circuit, where the target gene:



1) Would be tanscriptionally repressed (LacI-like TALE, CRISPR-dCas9, etc.);
2) Cis-acting Riboswitch in the 5'-UTR of mRNA;
3) Cis-element in the 5'-UTR of mRNA interacting with small *trans*-acting RNA :
   3.1. antisense RNA (asRNA);
   3.2. parallel complementary RNA (pRNA);
   3.3. trans-acting Riboswitch.
   These interactions could result in transcription activation/termination, modification of mRNA stability and mRNA translation efficiency.

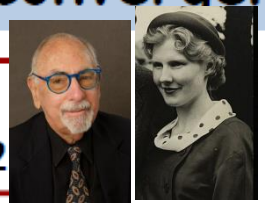


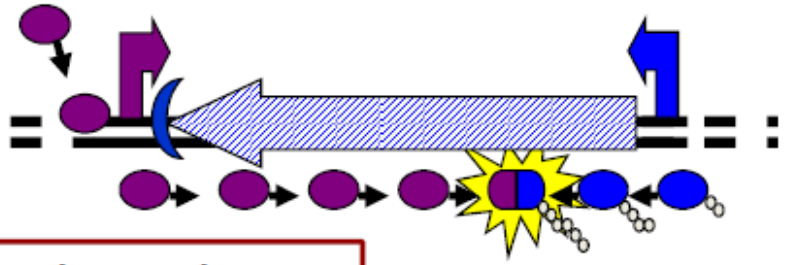
4) Target protein destabilization due to C- or N-terminus modification (SsrA-taggs).
5) Convergent transcription.



Gene silencing by convergent transcription: general principle

Ward and Murray, 1979;
Adhya and Gottesman, 1982

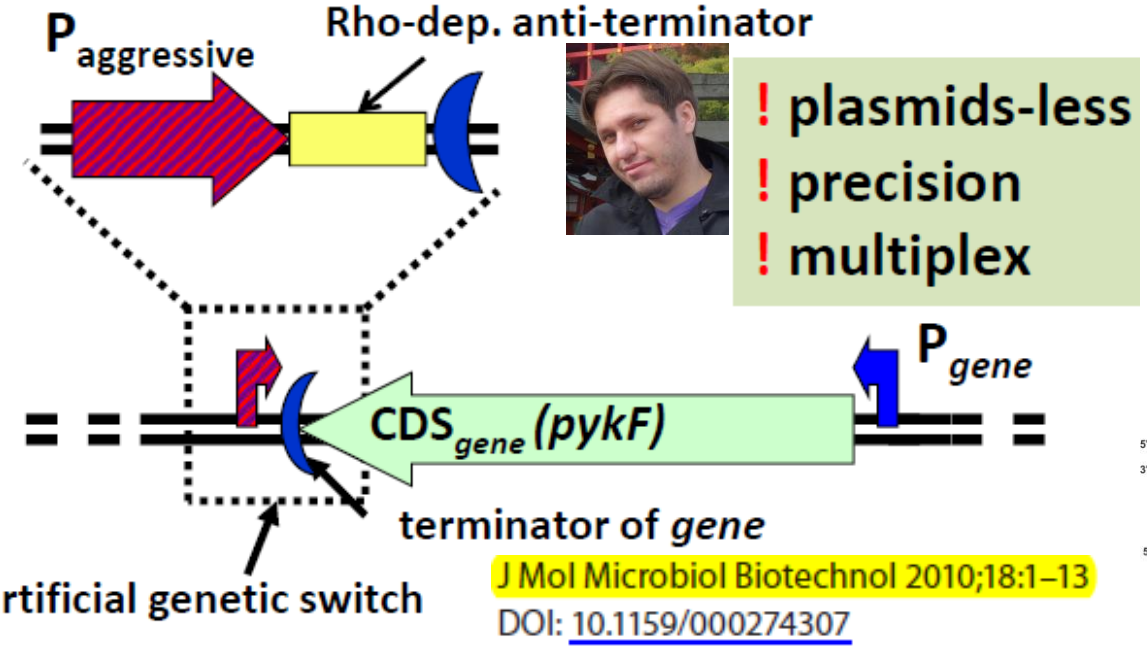




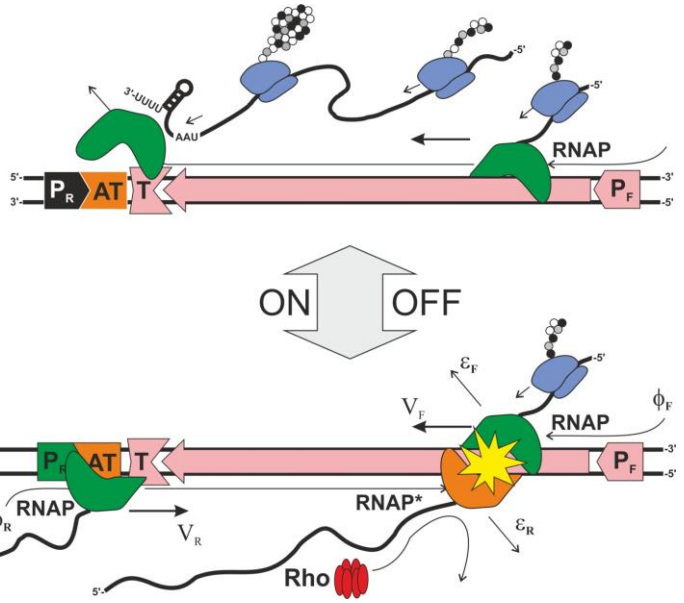
Krylov et al., 2010

The interruption of mRNA formation of the target gene through TI (transcriptional interference) as a result of the presence of two convergent promoters

Effective protection of the anti-sense RNA against Rho-dependent termination was confirmed

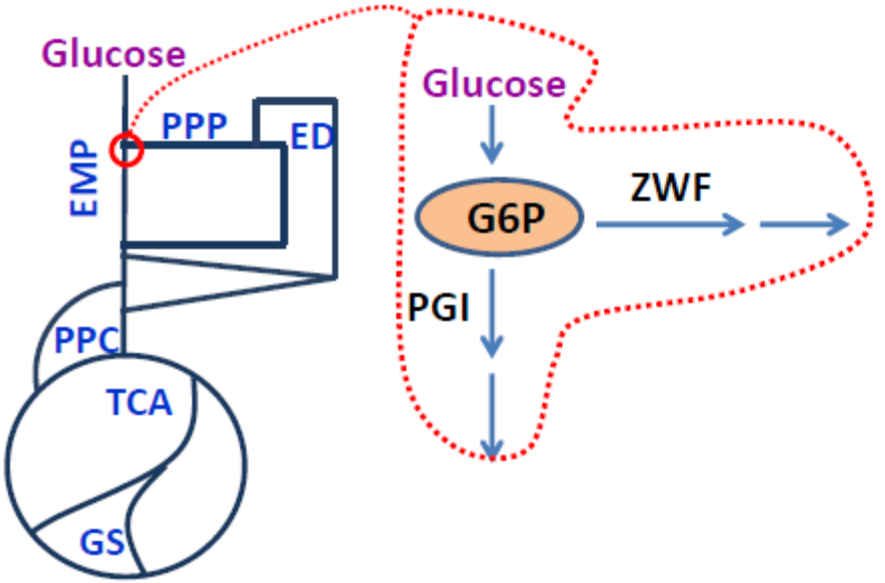


! plasmids-less
! precision
! multiplex

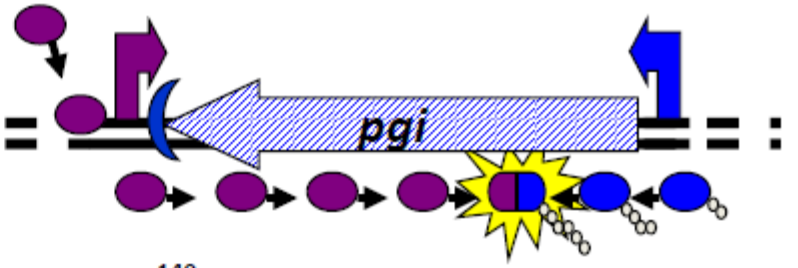


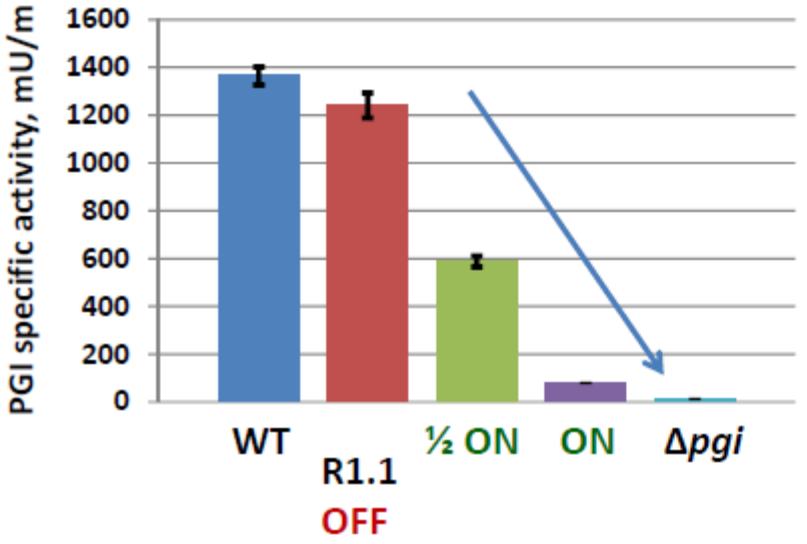
J Mol Microbiol Biotechnol 2010;18:1–13
DOI: 10.1159/000274307

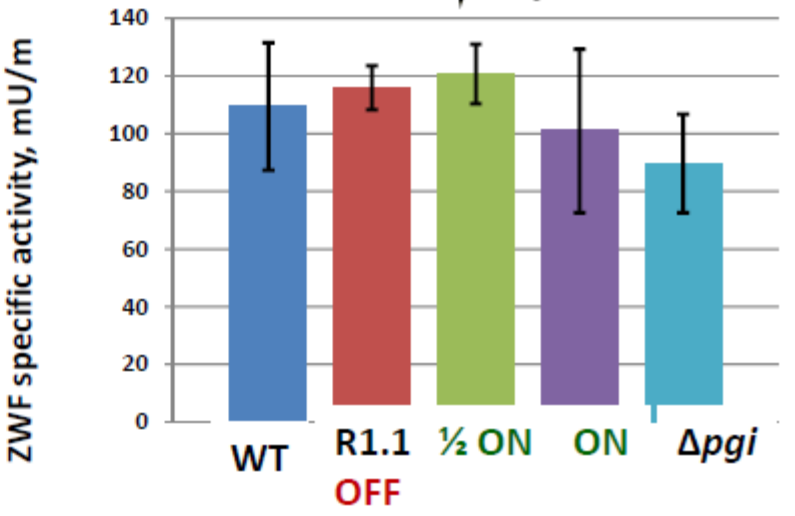
Gene silencing by convergent transcription: *pgi*-gene, as example



The transcription interference (TI) resulted in an interruption of target mRNA formation caused by the presence of two convergent promoters

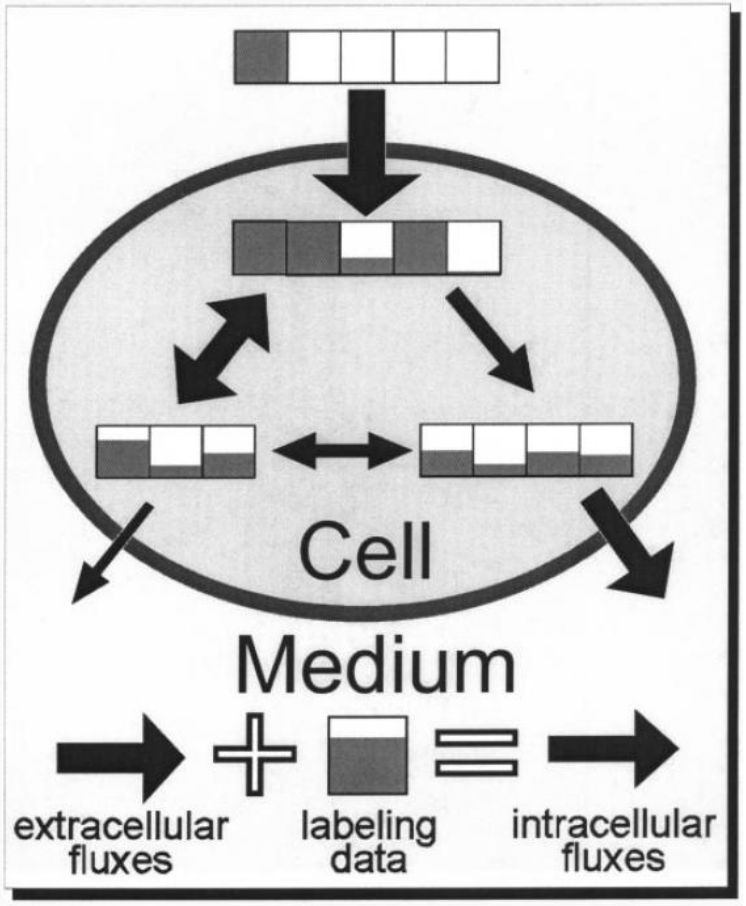




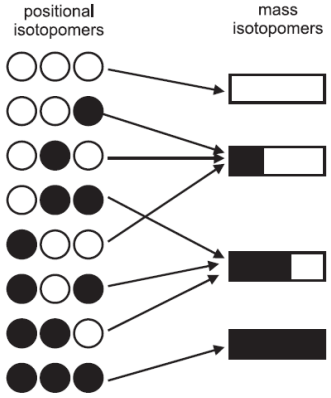


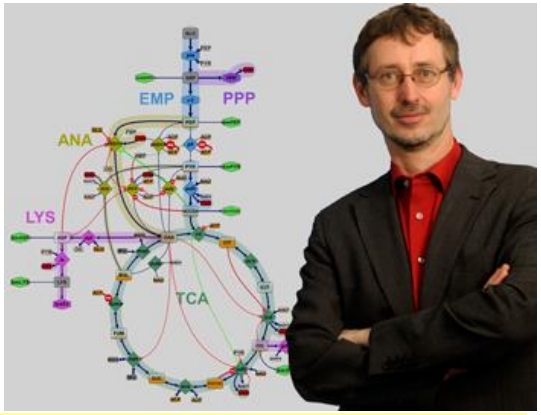
The effective *pgi*-silencing was achieved. What is about the flux rearrangement dependent on gene silencing?

Сущность ^{13}C-MFA



Важнейший вывод: Используя экспериментальную информацию об эффлюксах (внеклеточные потоки продуктов, субстратов, расход предшественников на биомассу и др.) и о распределении ^{13}C в метаболитах (изотопомеры – NMR, масс-изотопомеры – GC-MS (/MS)) можно методами математической регрессии установить параметры и рассчитать статистику внутриклеточных потоков для выбранной метаболической модели.





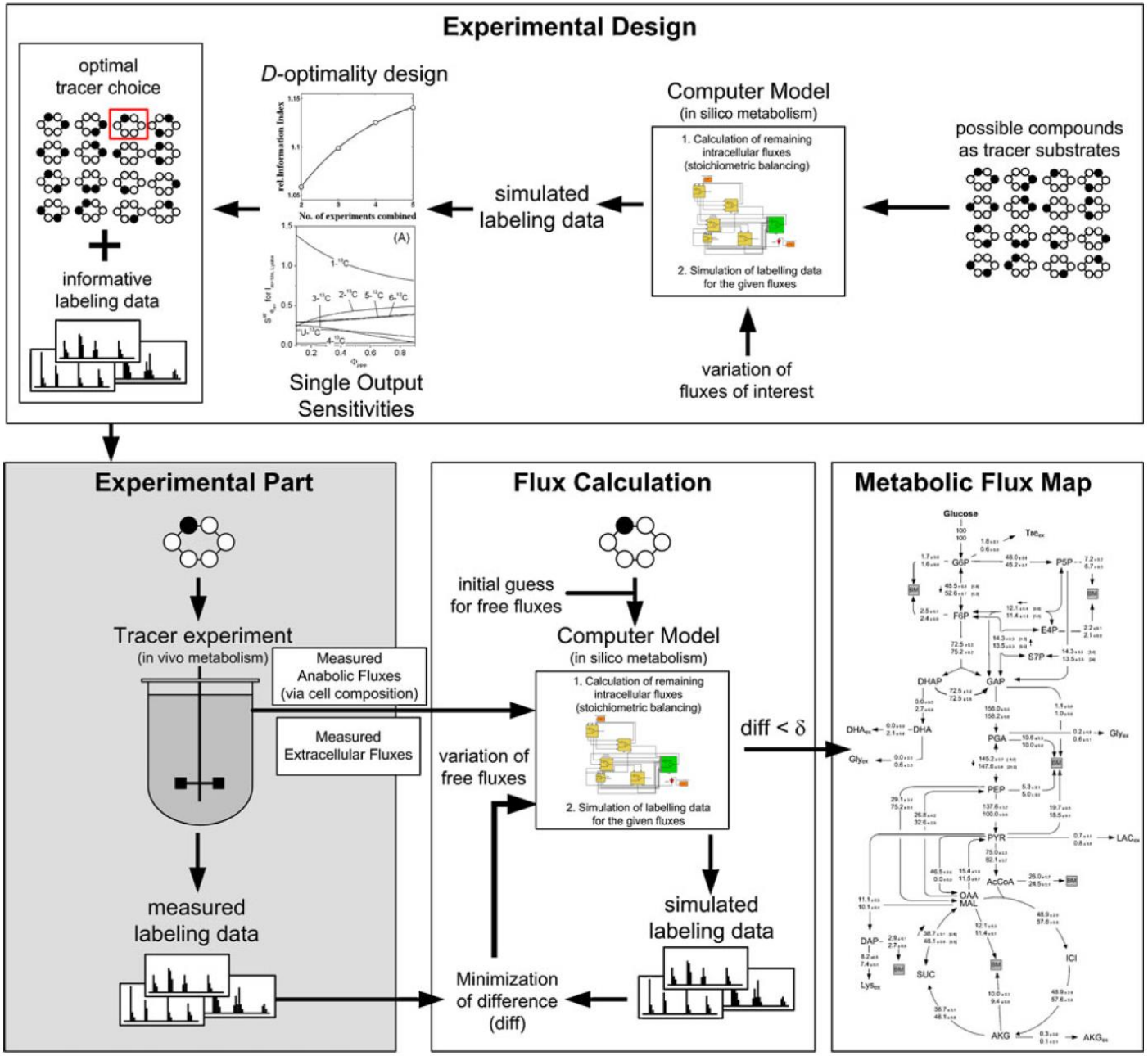
"The evaluation of carbon labeling experiment is one of the most complicated mathematical methods ever applied to biological systems".

Wiechert W (2001) "^{13}C Metabolic flux analysis". Metab Eng **3**:195-206.

Стадии эксперимента по ^{13}C-MFA

Metabolic fluxes and beyond—systems biology understanding and engineering of microbial metabolism

Michael Kohlstedt · Judith Becker · Christoph Wittmann



Appl Microbiol Biotechnol (2010) 88:1065–1075
DOI 10.1007/s00253-010-2854-2

Стадии эксперимента по ^{13}C-MFA

^{13}C Metabolic Flux Analysis

Step 1 — **Design isotopic labeling experiment**
- *In silico* simulations of isotopic labeling experiments
- Select optimal tracers & labeling measurements

Step 2 — **Isotopic labeling experiment**
- Culture cells with isotopic tracers
- Sample biomass, intra- and extracellular metabolites

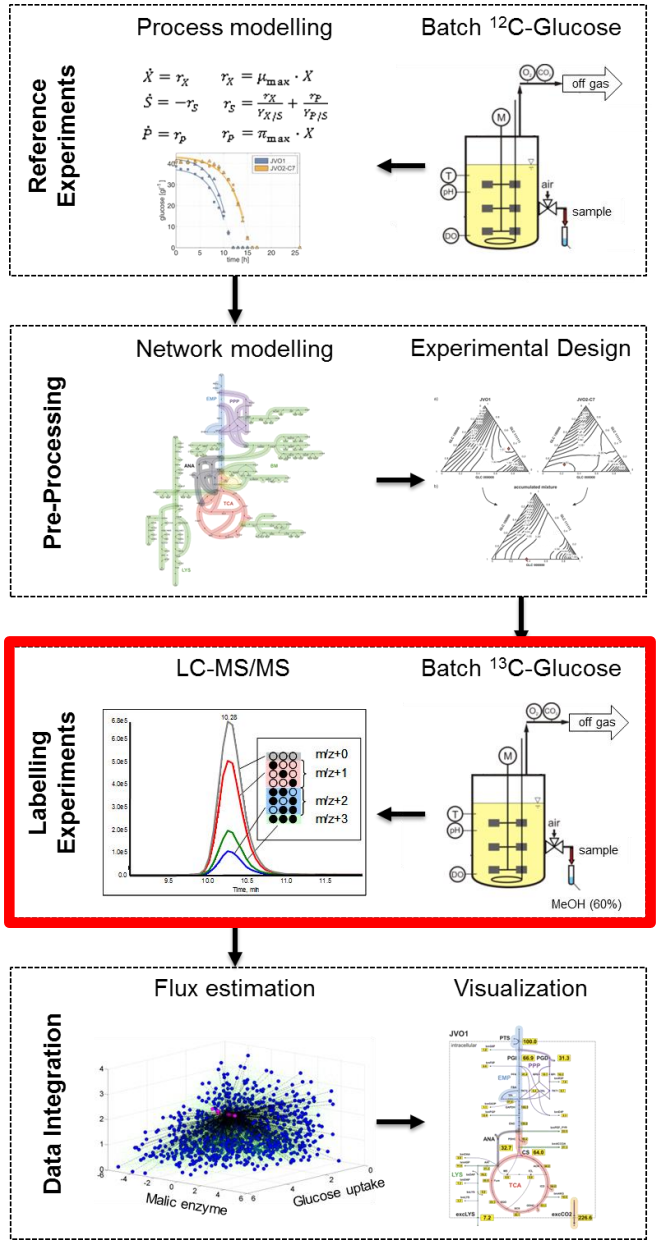
Step 3 — **Labeling measurements**
- Prepare samples for analysis (e.g. derivatization)
- MS, tandem MS, and/or NMR measurements

Step 4 — **^{13}C Metabolic flux analysis**
- Estimate metabolic fluxes (e.g. using Metran)
- Maximize fit between measurements and simulation

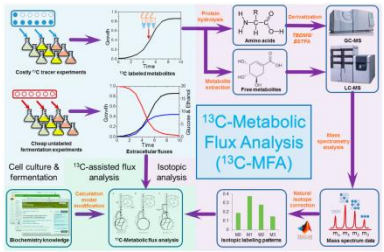
Step 5 — **Statistical analysis**
- Goodness-of-fit analysis
- Calculate nonlinear confidence intervals for fluxes

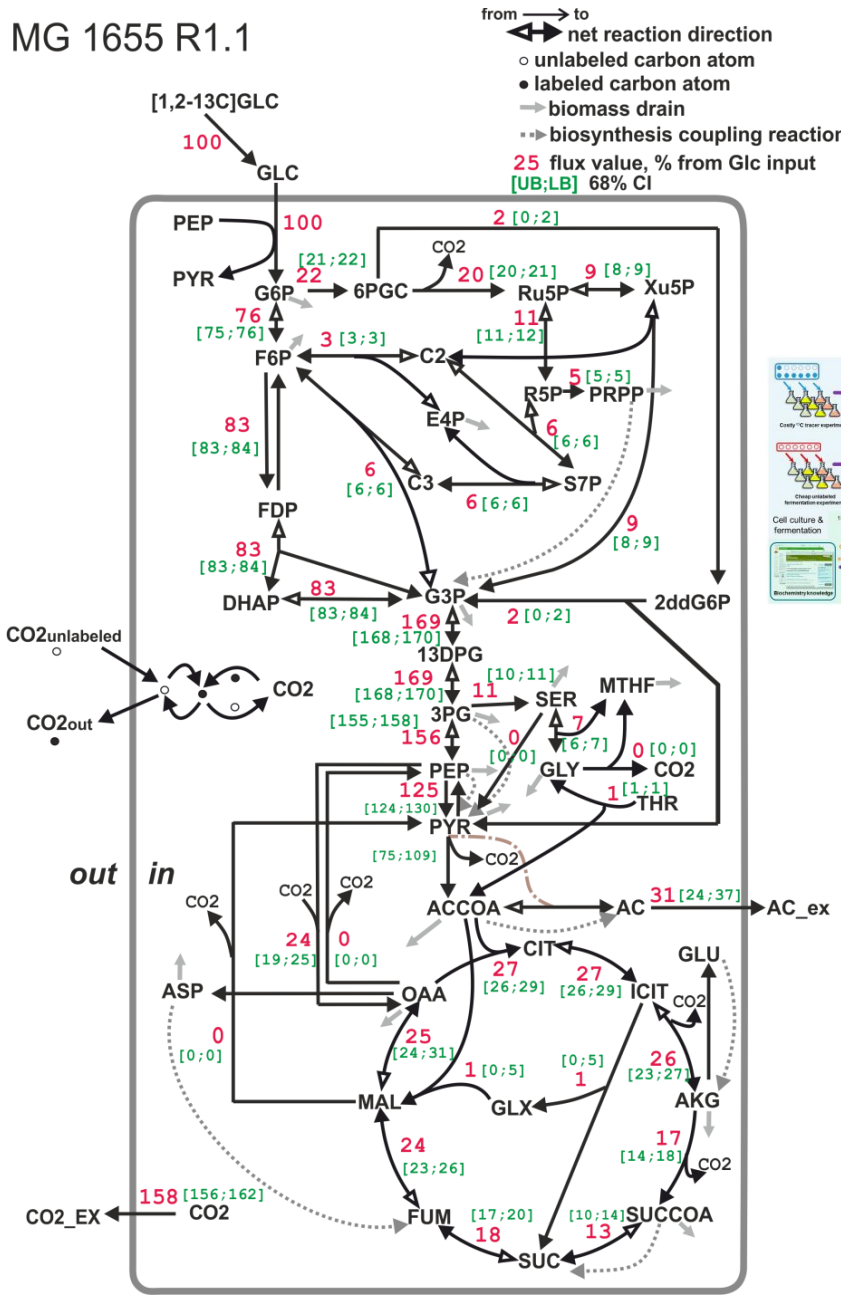
Current Opinion in Biotechnology

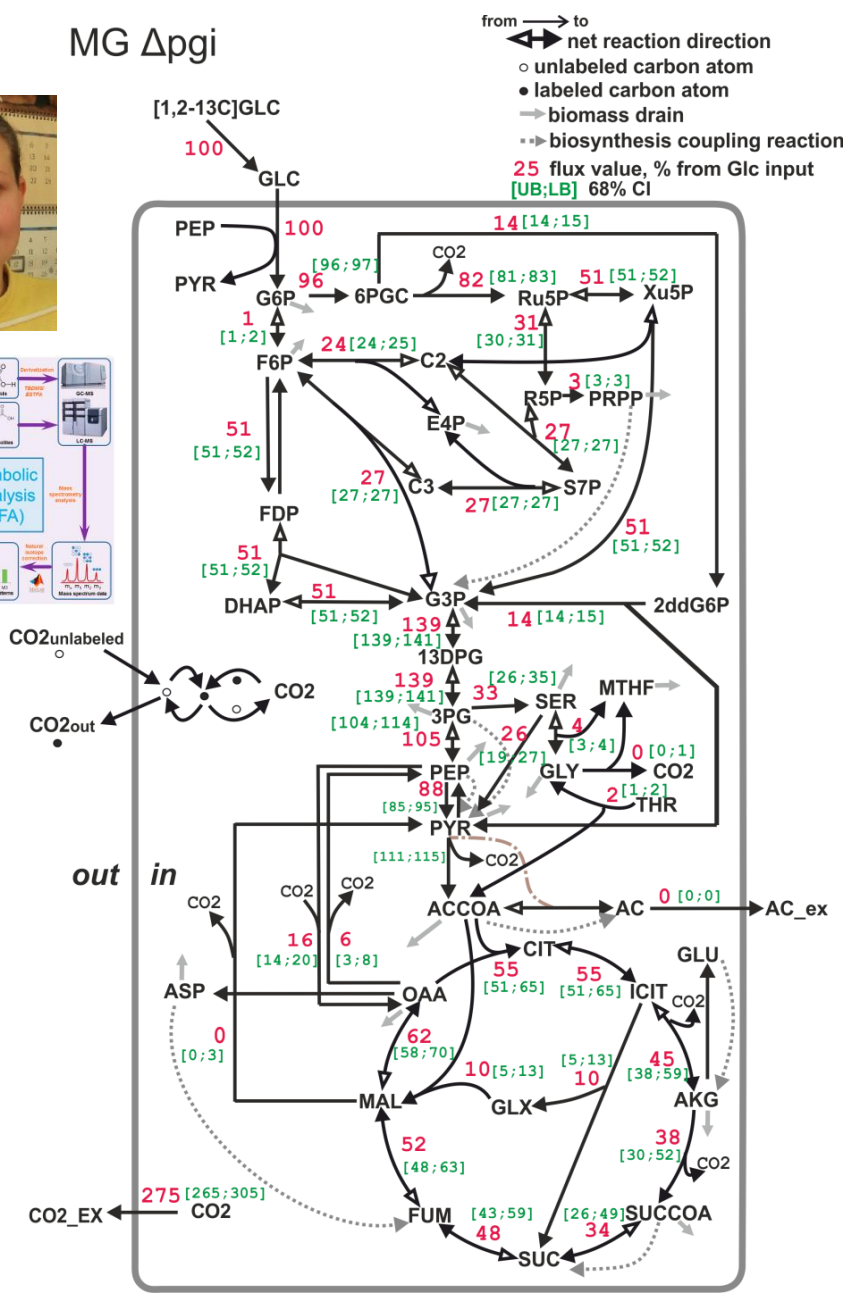
(2013) **24**(6): 1116-1121



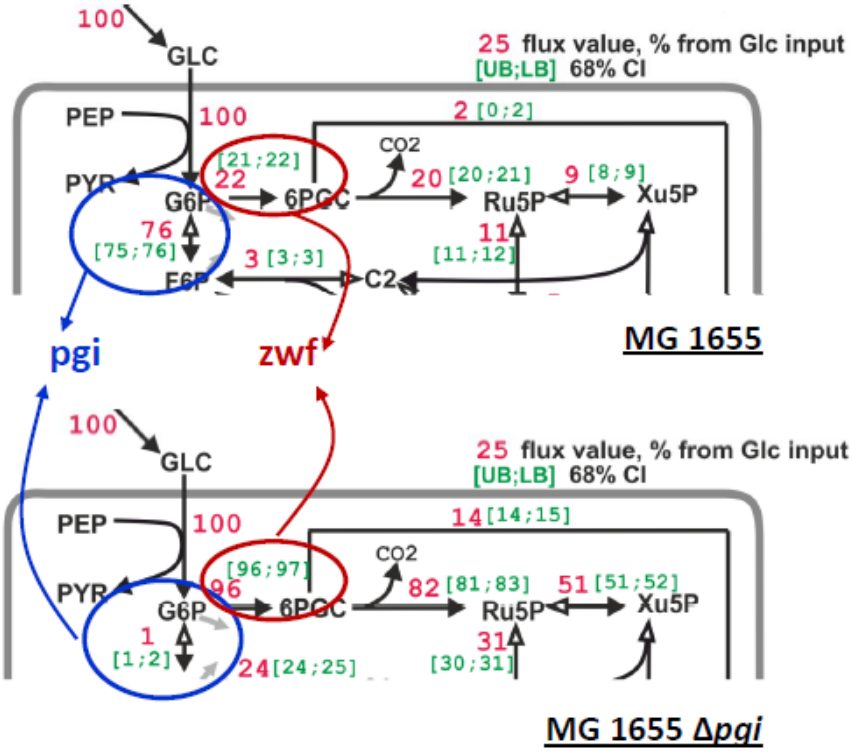
^{13}C-MFA штаммов с pgi-silencing







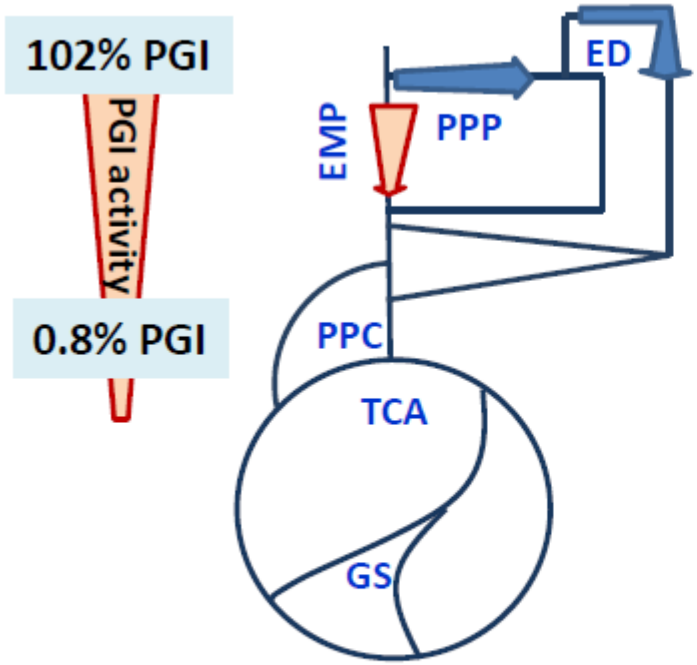
^{13}C-MFA штаммов с pgi-silencing

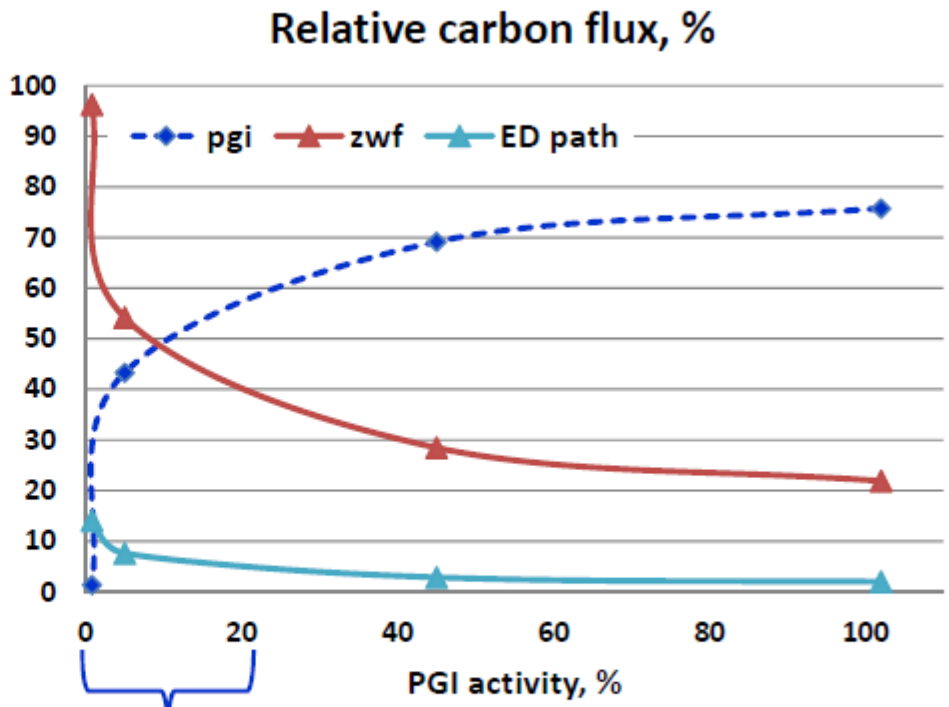


	[LB^{68}; UB^{68}]	
strain/reaction	MG 1655	MG Δ*pgi*
pgi	[75;76]	[1;2]
zwf	[21; 22]	[96; 97]

The flux through the inactivated PGI reaction is estimated near the zero value confirming accuracy of performed flux calculation.

^{13}C-MFA штаммов с pgi-silencing





Decrease of PGI activity led to increase a portion of a carbon utilized via oxPPP and activation of ED pathway.

Remarkable flux re-distribution is observed only at very low residual PGI activity

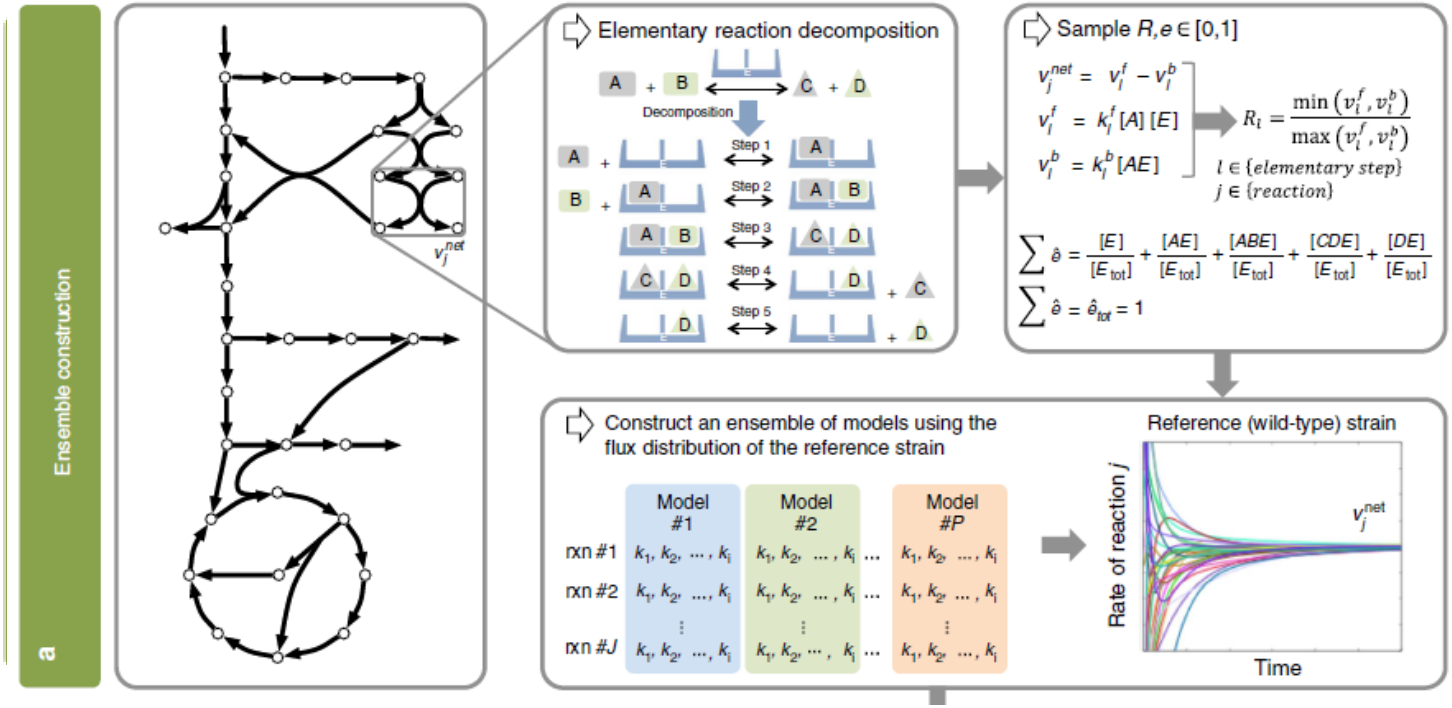
Получены интересные результаты о зависимости скорости роста клеток от потенциальной токсичности избытка NADPH. Дело в низкой активности ферментов obPPP : ингибирование до 40% NADPH для Zwf (*Cell Sytems* (2018) 6: 569–578) и низкой скорости потребления Glc.

«Кинетическое моделирование» pgi-silencing

Metabolic fluxes are the integral output of complex genetic and metabolic regulation, acting in the cell, which determine cellular phenotype.

In kinetic modeling approaches the attempts to predict flux distributions based on enzyme properties/regulation is undertaking in parallel with other flux analysis approach.

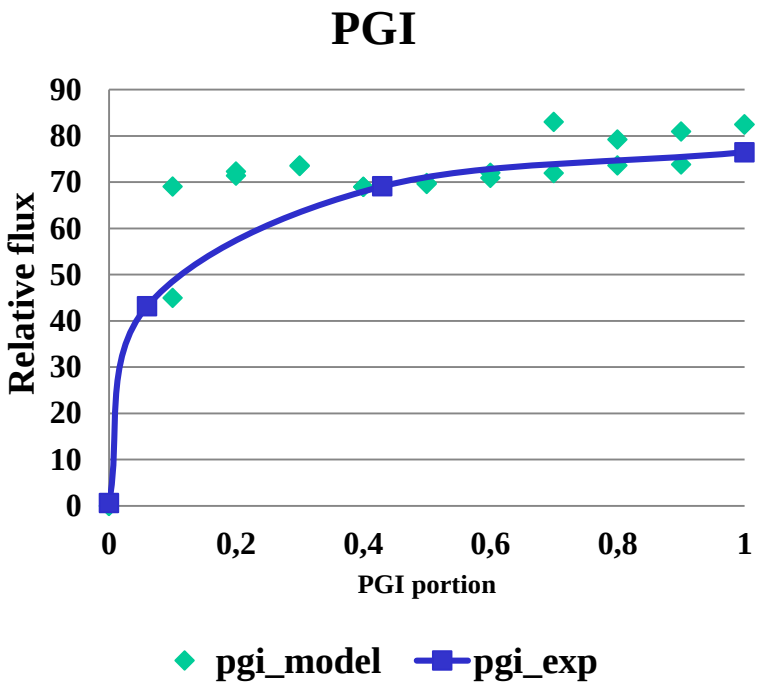
An approach named "metabolic ensemble modeling" (Tran L. M., 2008) which combines kinetic modeling and experimental data (growth parameters/^{13}C-MFA results) to quantitatively estimate values of kinetic parameters of each elementary reaction step has been developed.

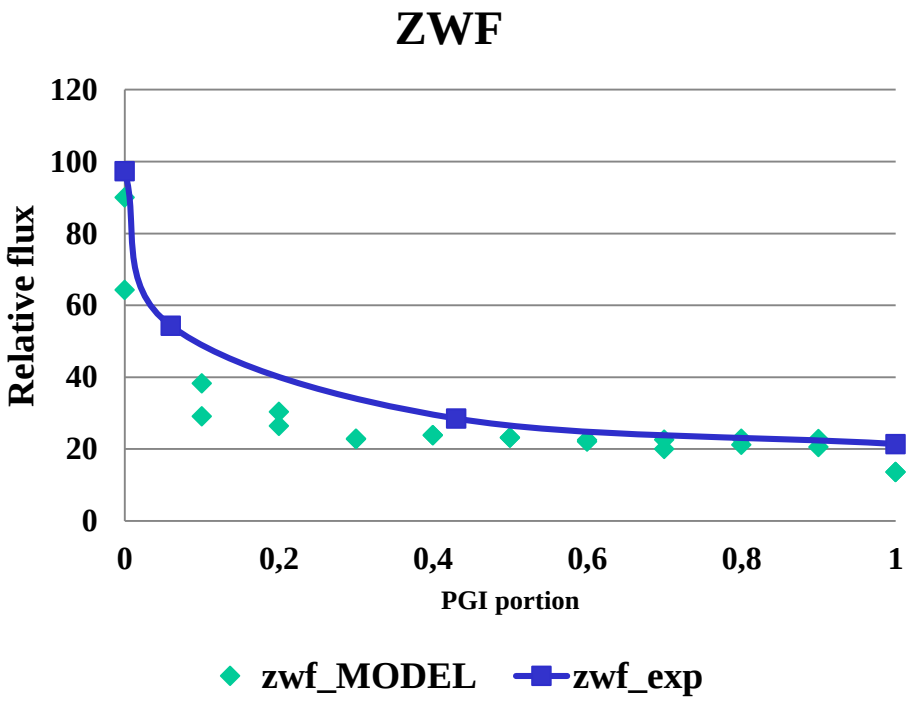


Adapted from Khodayari A., et al. 2016

Comparison of obtained results with kinetic modeling

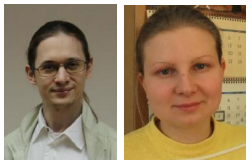
Recently develop *E. coli* kinetic metabolic model k-ecoli457 (Khodayari A, 2016) has been applied to quantitatively explain (predict) *pgi*-silencing effect.





Rather good agreement between experimental and *in silico* data for flux re-distribution at EMP/PP pathways branch point has been detected in preliminary evaluation.

Basic Research in AGRI (13C-MFA)



Steady-State ^{13}C-MFA and Ensemble Modeling-based Kinetic Model Consistently Characterized the Carbon Flux Rearrangements Resulted from *pgi* Gene Conditional Silencing Due to Regulated Convergent Transcription in Engineered *Escherichia coli* strains

Liubov Golubeva, Alexander Krylov, Mikhail Shupletsov, Mikhail Baboshin, Ekaterina Kovaleva and Sergey Mashko

Introduction: analysis of flux redistribution in cells resulted from knockout modification and/or heterologous gene expression gave new insight to pathway function and product synthesis limitations. However, much more less investigations relate to smooth gene expression perturbations.

Results: *pgi* gene silencing via convergent transcription

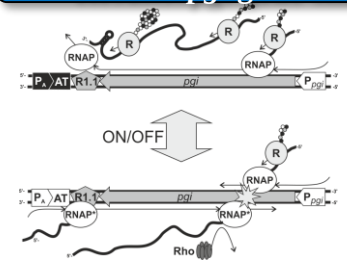


Figure_1. Genetic switch constructed for *pgi*-silencing based on convergent

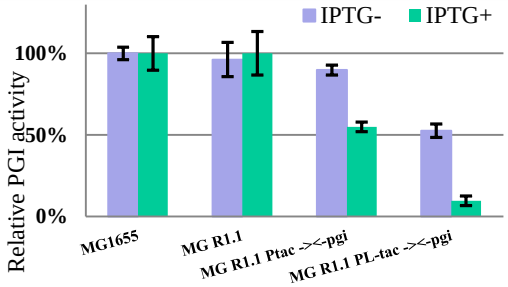


Figure_3. Efficiency of the *pgi*-silencing according to glucose-6-phospate isomerase activity.

Results: Intracellular carbon flux distribution analysis

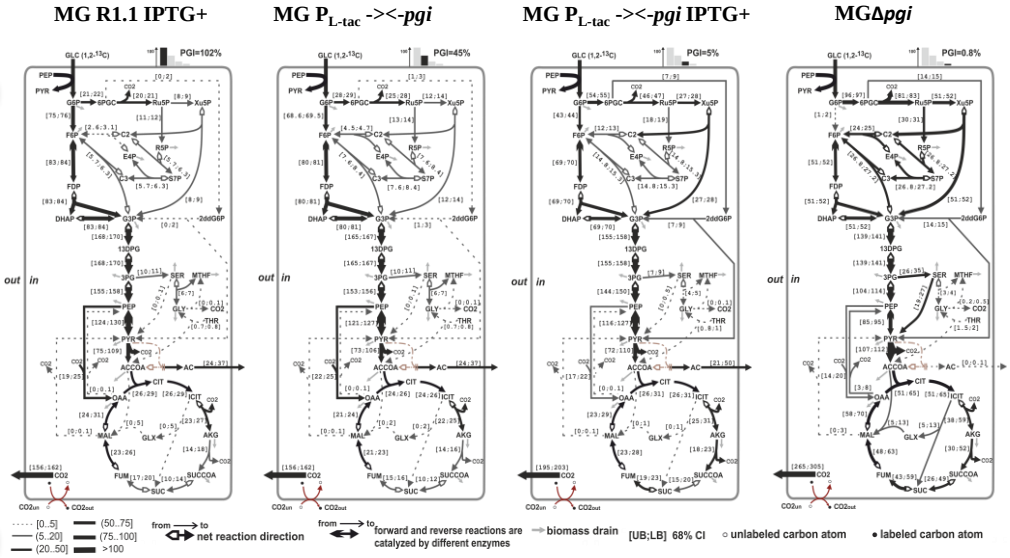


Figure_4. *In vivo* carbon flux distribution in central metabolism of the strains, possessed different level of PGI activity, estimated by ^{13}C-MFA.

Conclusions

- Step-by-step decrease of PGI activity was provided by IPTG-inducible convergent transcription;
- PGI activity decrease resulted in gradual carbon flux re-distribution in upper *E. coli* metabolism toward the PP pathway, predicted, also, by k-ecoli457 kinetic model based simulations except the observed ED pathway activation;
- Estimated carbon flux re-distribution resulted from smooth gene expression perturbation could be used for further improvement the kinetic metabolic models prediction capacity together with data obtained for disruption mutants.

Results: prediction of the *pgi*-silencing effect by genome-scale kinetic model of *E. coli* metabolism – k-ecoli457

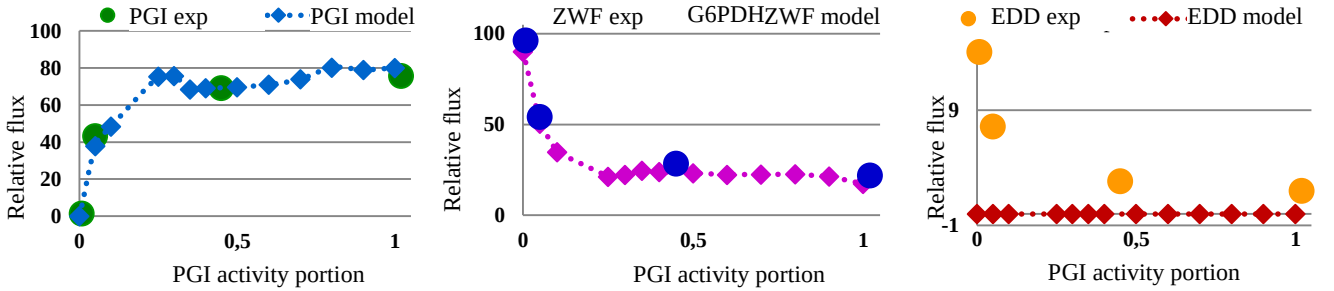


Figure_7. Flux distribution response to gradual PGI activity decrease measured experimentally and predicted by *E. coli* metabolism kinetic model k-ecoli457 [1].

Comparison of obtained results with kinetic modeling

- The recently developed genome scale kinetic model of *E. coli* metabolism – e-ecoli457–confirmed rather well the experimentally revealed effects at EMP/PP pathways branch point coupled with constancy of GDH flux and changing of glucose consumption rate.

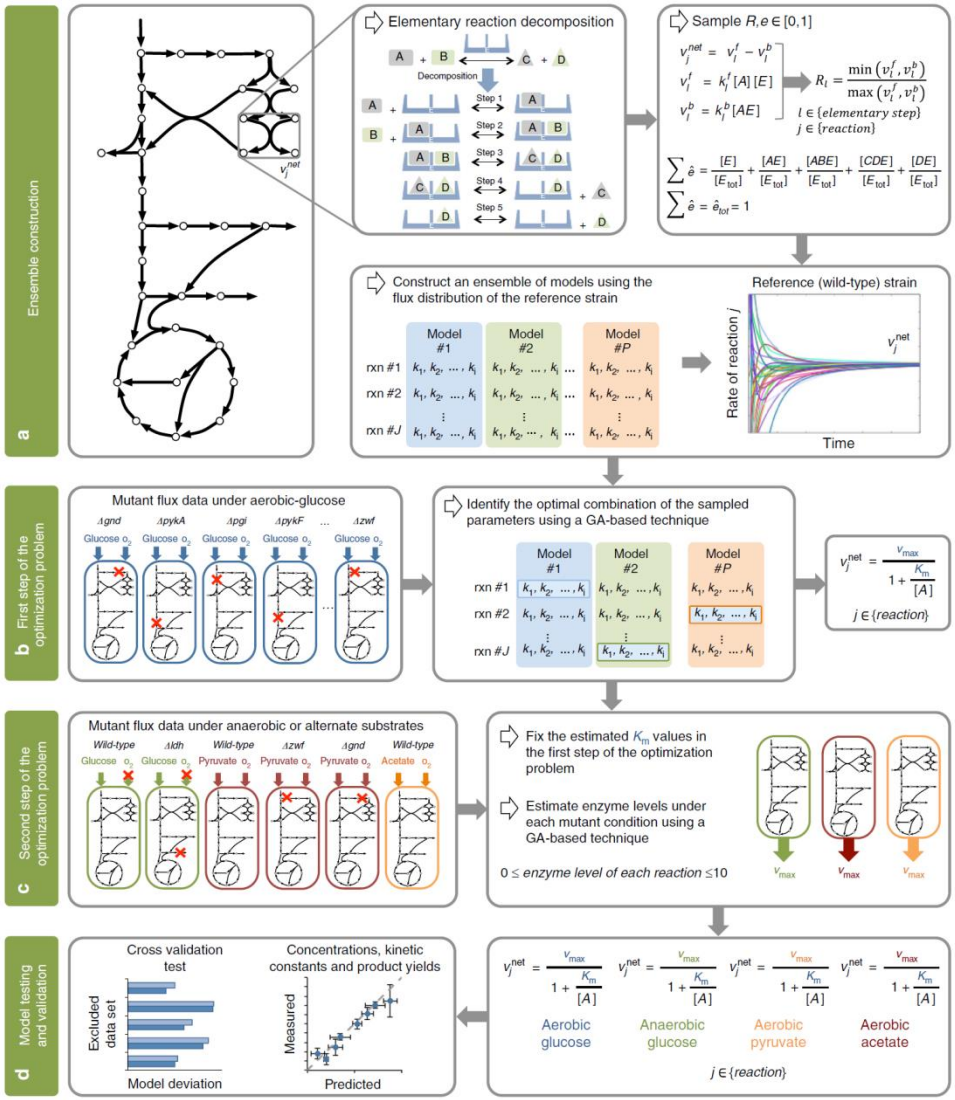
- On the other hand, the current kinetic model failed to predict ED pathway activation in *pgi* knock-out strain.

- Closed cooperation with the Prof. Maranas group could be helpful for improvement of the correlation between the experimentally obtained and proposed flux parameters for GS-based *E. coli* model and, finally, could significantly enhance the "predictive force" of the kinetic metabolic ensembled-based model .

Thank you for your attention!

"Metabolic ensemble modeling"

Khodayari A, Maranas CD (2016) *A genome-scale Escherichia coli kinetic metabolic model k-ecoli457 satisfying flux data for multiple mutant strains. Nat Communic* **17**:13806



Here, we introduce k-ecoli457, a genome-scale kinetic model of *Escherichia coli* metabolism that satisfies fluxomic data for wild-type and 25 mutant strains under different substrates and growth conditions. The k-ecoli457 model contains 457 model reactions, 337 metabolites and 295 substrate-level regulatory interactions.