LOSCHMIDT LABORATORIES

Syntetická biologie
- moderní přístup pro modifikace

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Brno   **6.9.2017**

BIOTECHNOLOGIE

- využití vědeckých a inženýrských principů při zpracování surovin a materiálů biologickými činiteli - organizmy, buňkami nebo jejich částmi - s cílem získat užitečné produkty

- Poprvé použil Karl Ereky 1917

BIOTECHNOLOGIE

- Červené – medicínské
- Bílé – industriální
- Zelené – zemědělské
- Modré – akvatické
- Černé – bioterorismus
- Šedé – environmentální

BIOTECHNOLOGIE

- Klasické

  - Přírodní kmeny organizmů

  - Pivo, víno, kynuté pečivo, antibiotika…

- Molekulární = moderní

  - Využívají geneticky upravené organizmy

# Construction of Biologically Functional Bacterial Plasmids *In Vitro*

(R factor/restriction enzyme/transformation/endonuclease/antibiotic resistance)
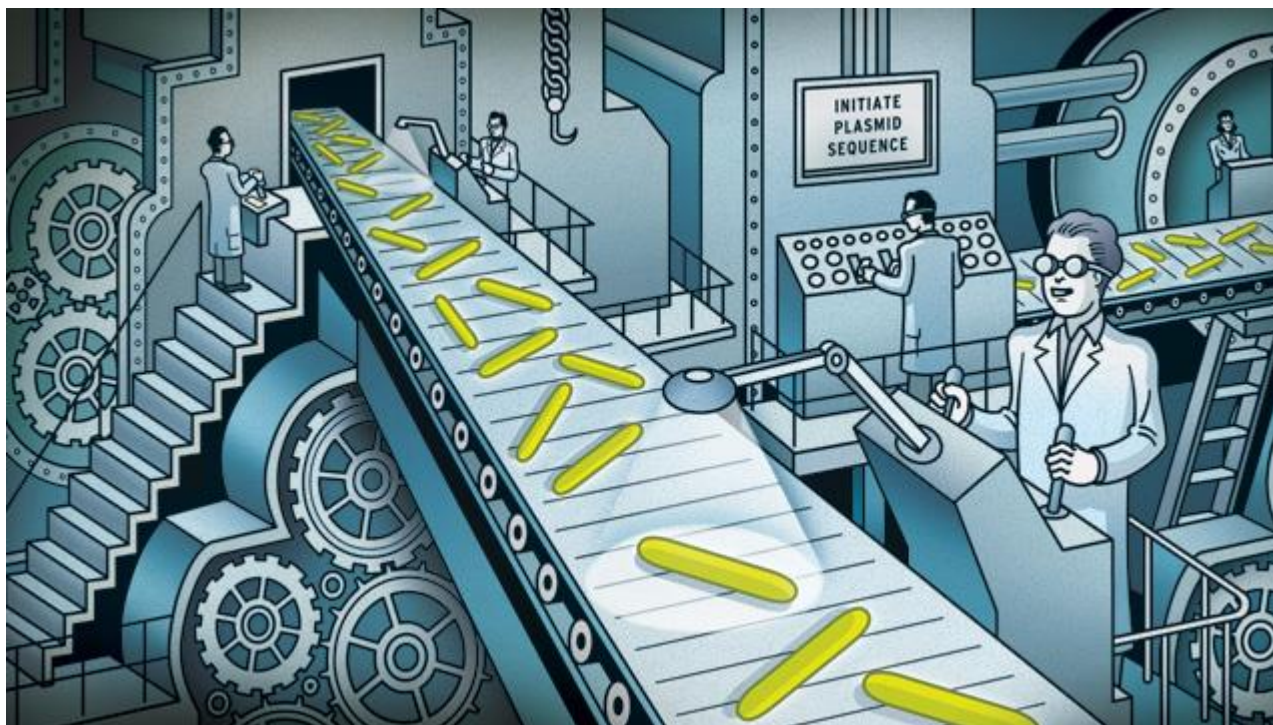
STANLEY N. COHEN*, ANNIE C. Y. CHANG*, HERBERT W. BOYER†, AND ROBERT B. HELLING†

* Department of Medicine, Stanford University School of Medicine, Stanford, California 94305; and † Department of Microbiology, University of California at San Francisco, San Francisco, Calif. 94122

- První konstrukce funkčních fragmentů DNA
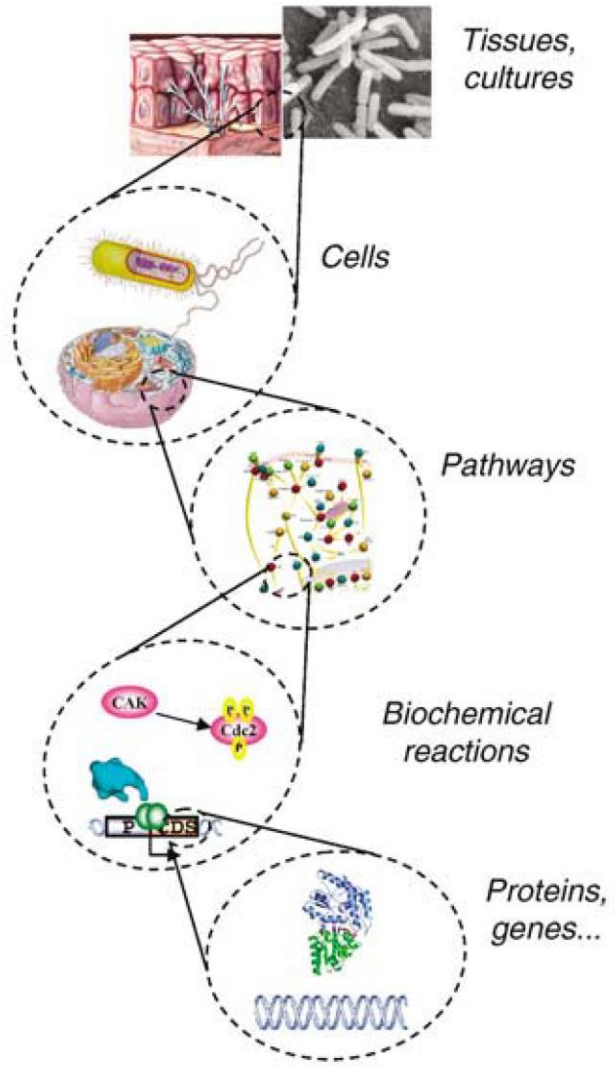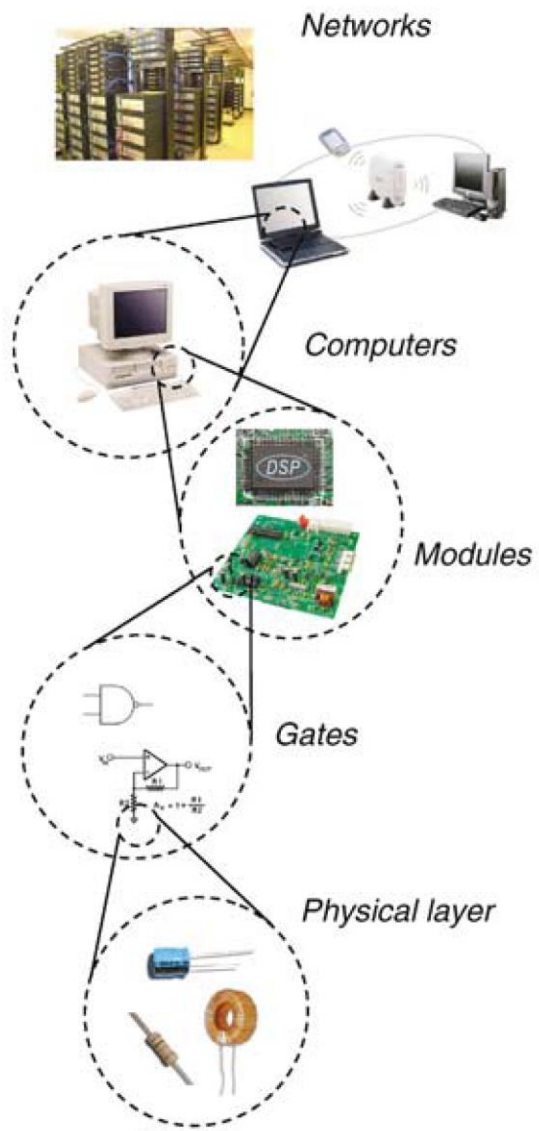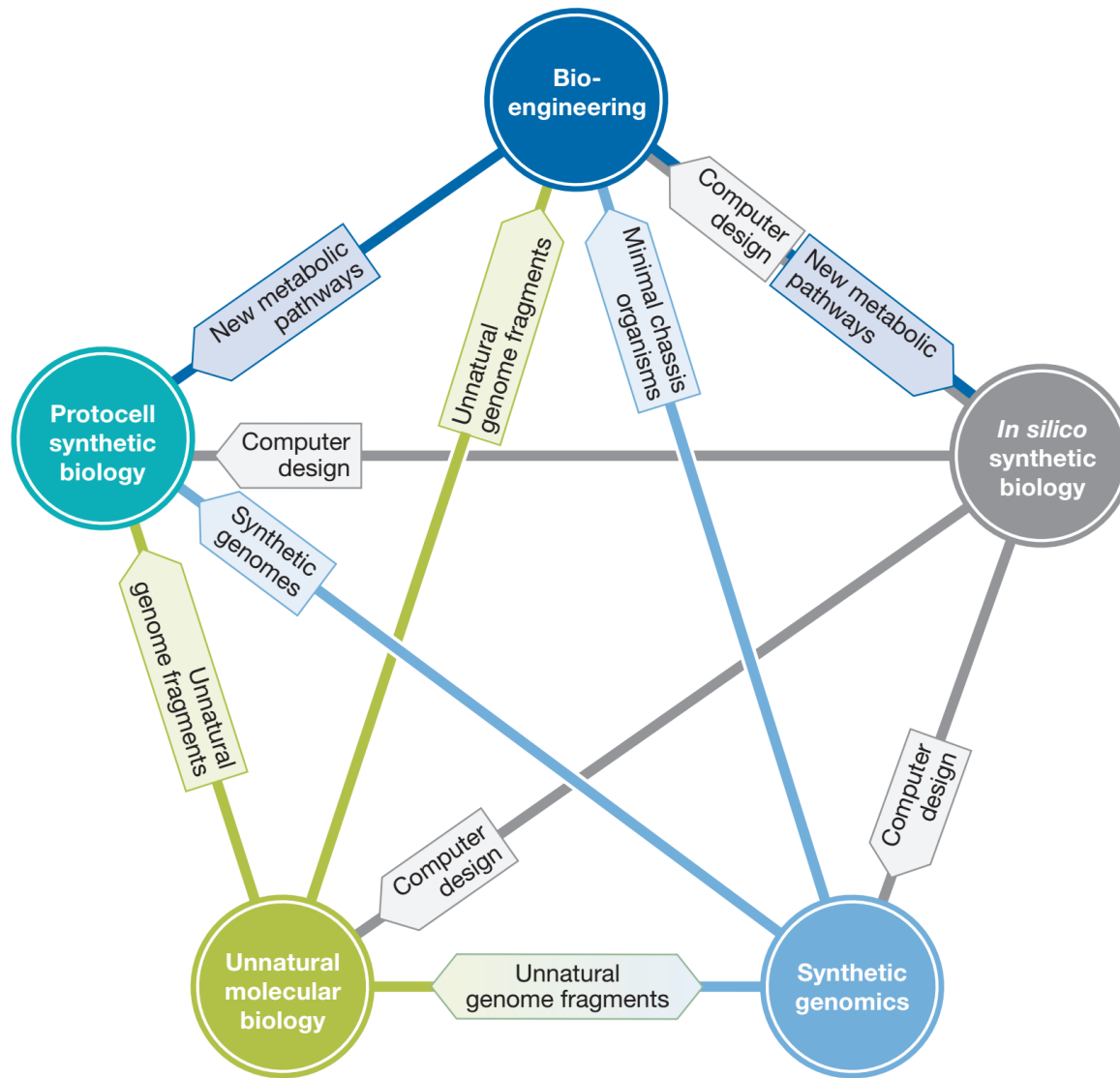
- Základ pro moderní přístupy
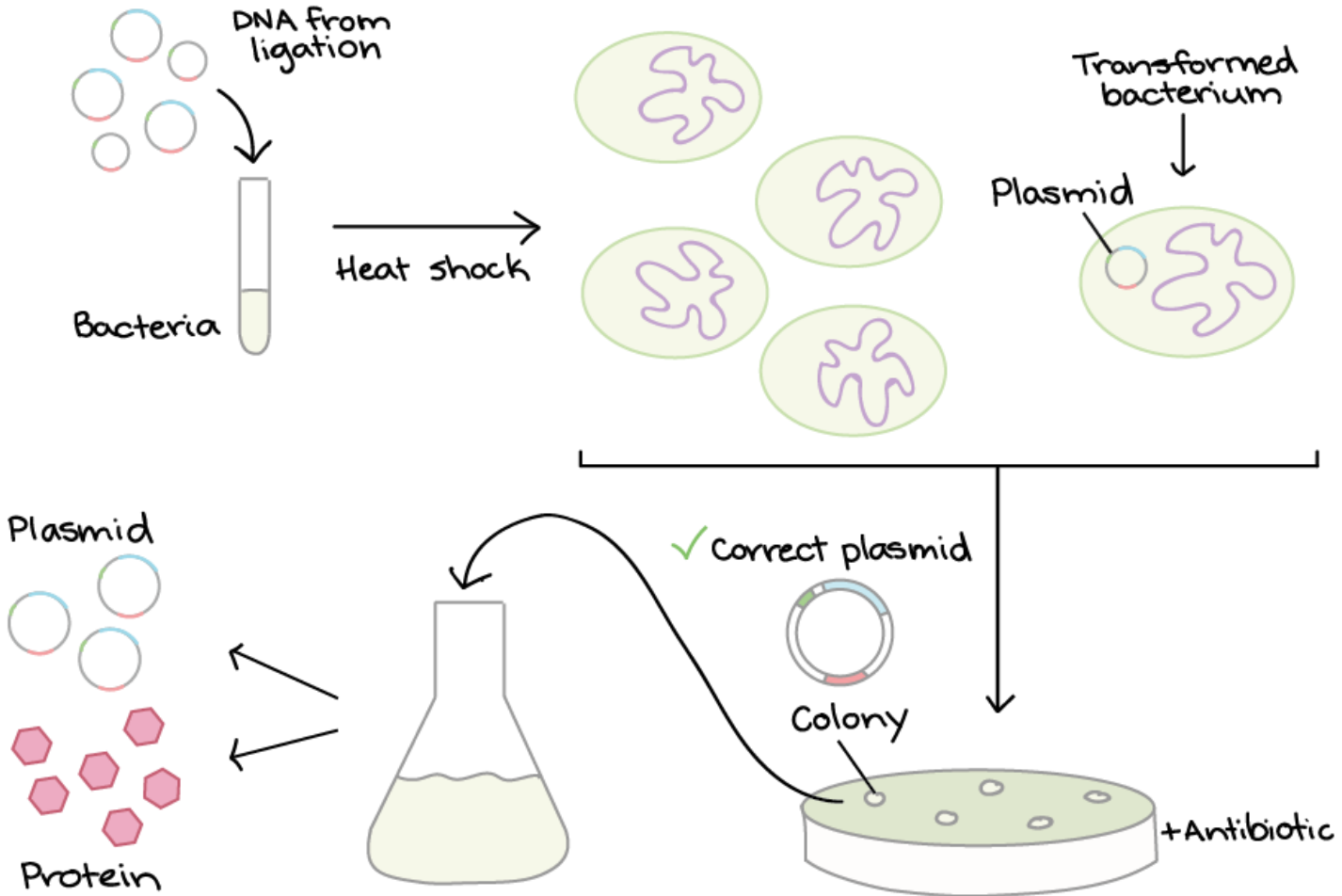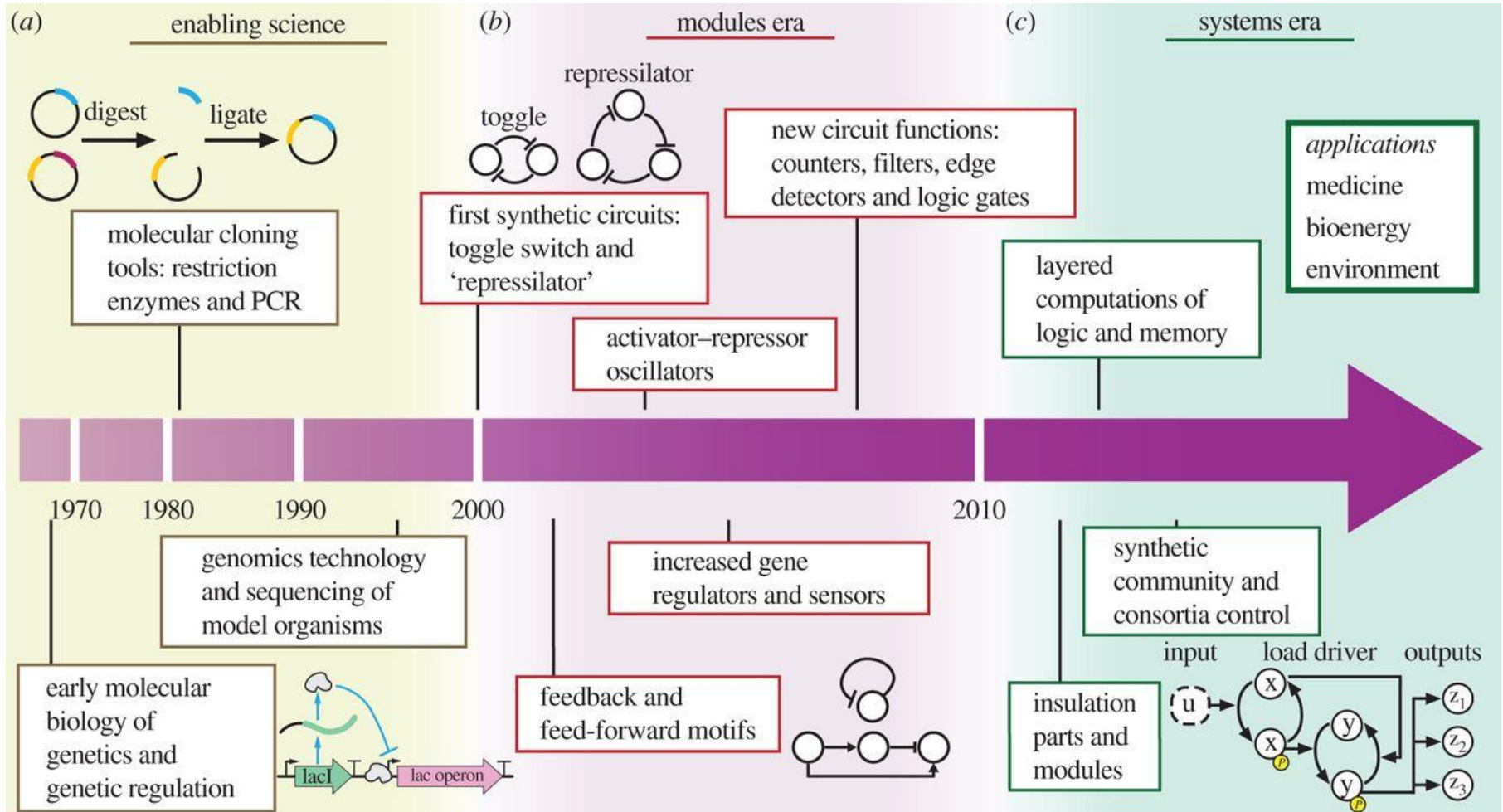
SYNTETICKÁ BIOLOGIE

Syntetická biologie

- Moderní odvětví molekulárních biotechnologií

- Kombinuje znalosti fyziologie a mol. biologie

- Aplikace inženýrských principů a přístupů pro pochopení fungování živých systémů

- Logika vztahů mezi jednotlivými komponentami živých systémů
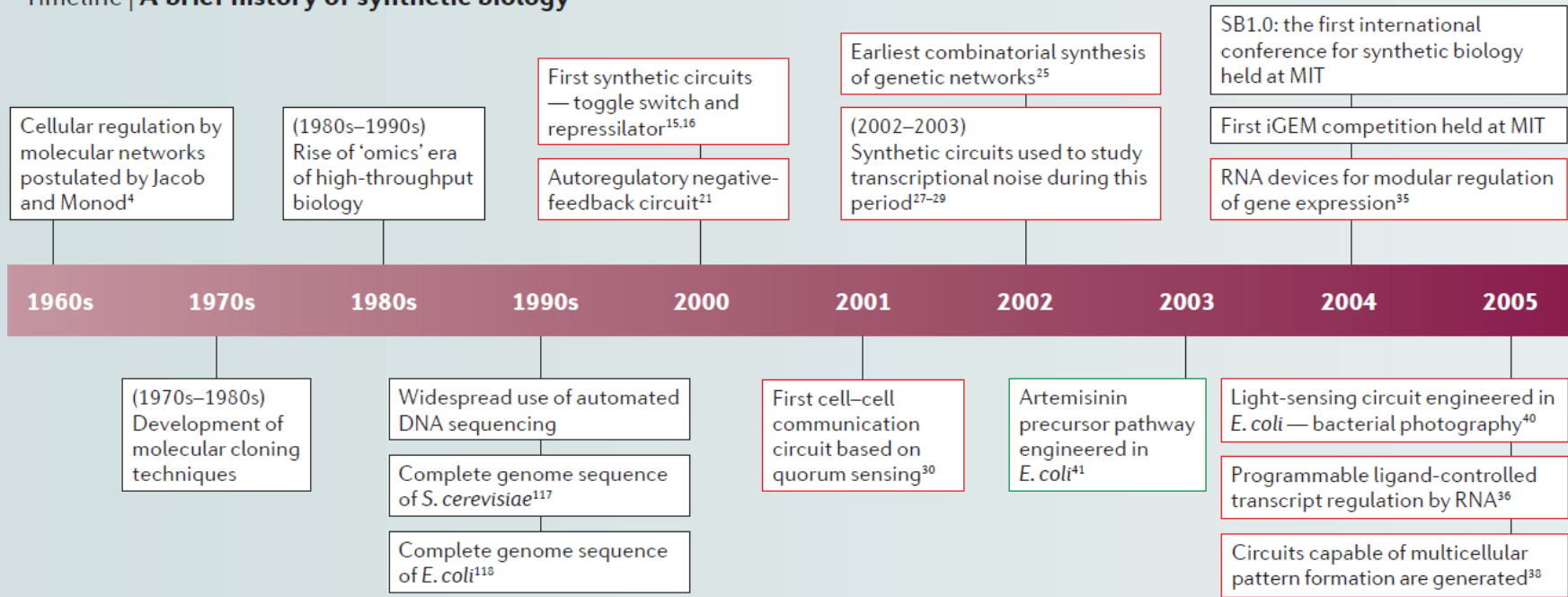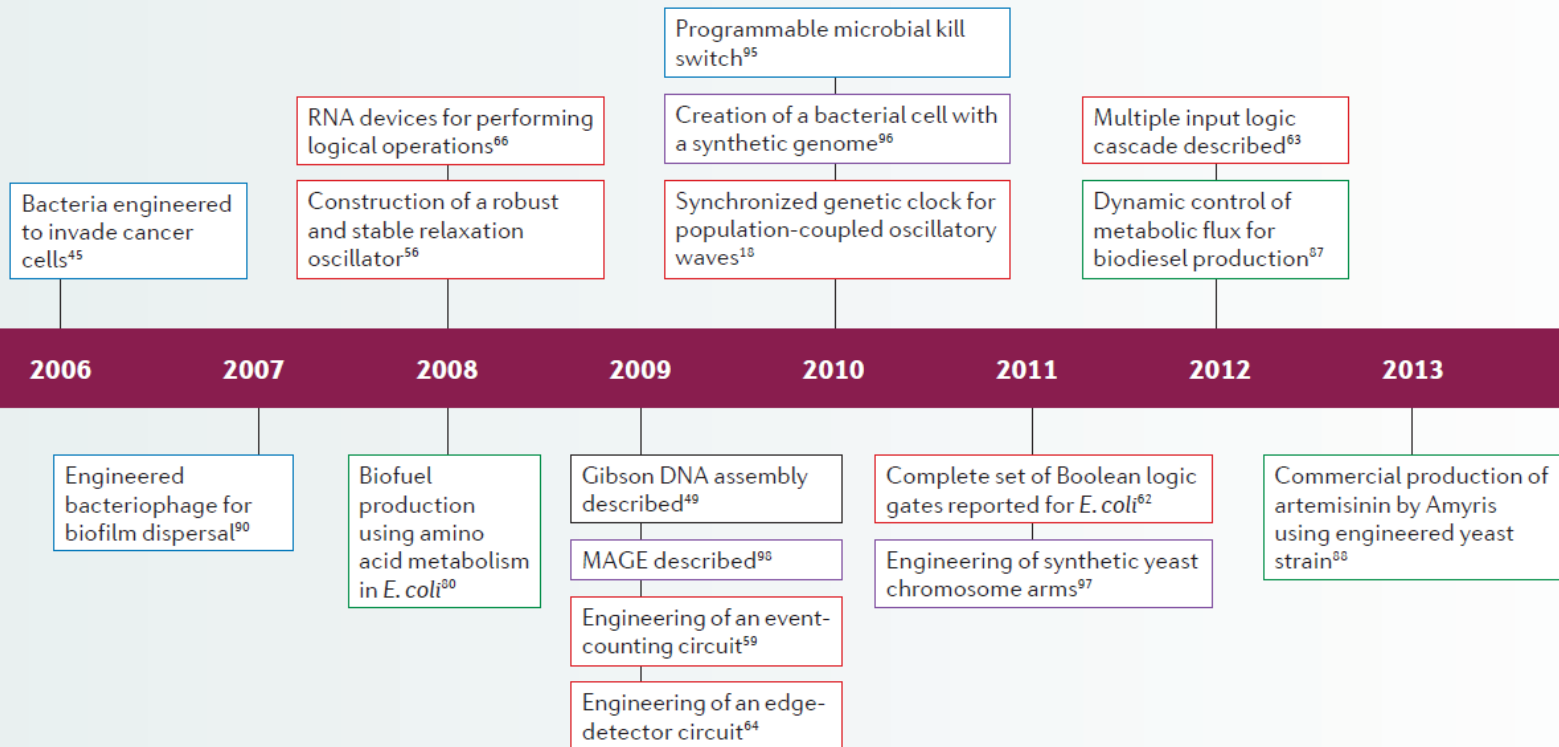
- Vyvolává obavy a strach z možného zneužití

Networks

Computers

Modules

Gates

Physical layer

Tissues, cultures

Cells

Pathways

Biochemical reactions

Proteins, genes...

Historie syntetické biologie

Historie syntetické biologie

Timeline | **A brief history of synthetic biology**

Cellular regulation by molecular networks postulated by Jacob and Monod[4]

(1980s–1990s) Rise of 'omics' era of high-throughput biology

First synthetic circuits — toggle switch and repressilator[15,16]

Autoregulatory negative-feedback circuit[21]

Earliest combinatorial synthesis of genetic networks[25]

(2002–2003) Synthetic circuits used to study transcriptional noise during this period[27–29]

SB1.0: the first international conference for synthetic biology held at MIT

First iGEM competition held at MIT

RNA devices for modular regulation of gene expression[35]

1960s   1970s   1980s   1990s   2000   2001   2002   2003   2004   2005

(1970s–1980s) Development of molecular cloning techniques

Widespread use of automated DNA sequencing

Complete genome sequence of S. cerevisiae[117]

Complete genome sequence of E. coli[118]

First cell–cell communication circuit based on quorum sensing[30]

Artemisinin precursor pathway engineered in E. coli[41]

Light-sensing circuit engineered in E. coli — bacterial photography[40]

Programmable ligand-controlled transcript regulation by RNA[36]

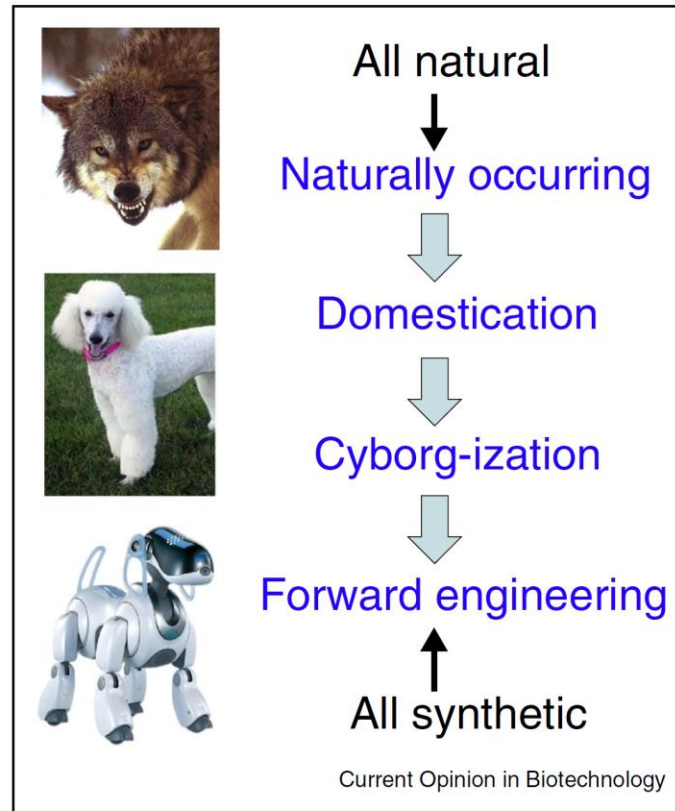Circuits capable of multicellular pattern formation are generated[38]

Key to coloured boxes: technical or cultural milestones (black); circuit engineering (red); synthetic biology in metabolic engineering (green); therapeutic applications (blue); whole genome engineering (purple). E. coli, Escherichia coli; iGEM, International Genetically Engineered Machine; MAGE, multiplex automated genome engineering; MIT, Massachusetts Institute of Technology; SB1.0, Synthetic Biology 1.0; S. cerevisiae, Saccharomyces cerevisiae.

Historie syntetické biologie

Bacteria engineered to invade cancer cells[45]

RNA devices for performing logical operations[66]

Construction of a robust and stable relaxation oscillator[56]

Programmable microbial kill switch[95]

Creation of a bacterial cell with a synthetic genome[96]

Synchronized genetic clock for population-coupled oscillatory waves[18]

Multiple input logic cascade described[63]

Dynamic control of metabolic flux for biodiesel production[87]

2006 | **2007** | **2008** | **2009** | **2010** | **2011** | **2012** | **2013**

Engineered bacteriophage for biofilm dispersal[90]

Biofuel production using amino acid metabolism in E. coli[80]

Gibson DNA assembly described[49]

MAGE described[98]

Engineering of an event-counting circuit[59]

Engineering of an edge-detector circuit[64]

Complete set of Boolean logic gates reported for E. coli[62]

Engineering of synthetic yeast chromosome arms[97]

Commercial production of artemisinin by Amyris using engineered yeast strain[88]

Stav vývoje syntetické biologie

All natural
↓
Naturally occurring
⇩
Domestication
⇩
Cyborg-ization
⇩
Forward engineering
↑
All synthetic

Current Opinion in Biotechnology

The way towards full predictability of bioengineered systems. The process shows some resemblance to the historical roadmap to domesticate wild animals for the sake of increasing productivity, ease of handling and response to human instructions. Cyborg-ization is the stage where the systems at stake combine naturally-occurring and engineered/synthetic traits.

Strach z neznámého

Artificial cells

Like biological cells, artificial cells function with content retained inside to:
- act on outside permeant molecules
- release products of interaction

Enzymes
Haemoglobin
Cells, stem cells
Tissues (islets)
Microorganisms
Genetically engineered cells
Recombinant enzymes
Biotechnological products
Magnetic materials
Adsorbents
Immunosorbents

Antibodies
White blood cells
Tryptic enzymes

Living organisms

Reducing complexity

Top-down   Stripping or replacing the genomes

Artificial cells

Increasing complexity

Bottom-up   Assembling

Non-living components

Bezbuněčná syntetická biologie
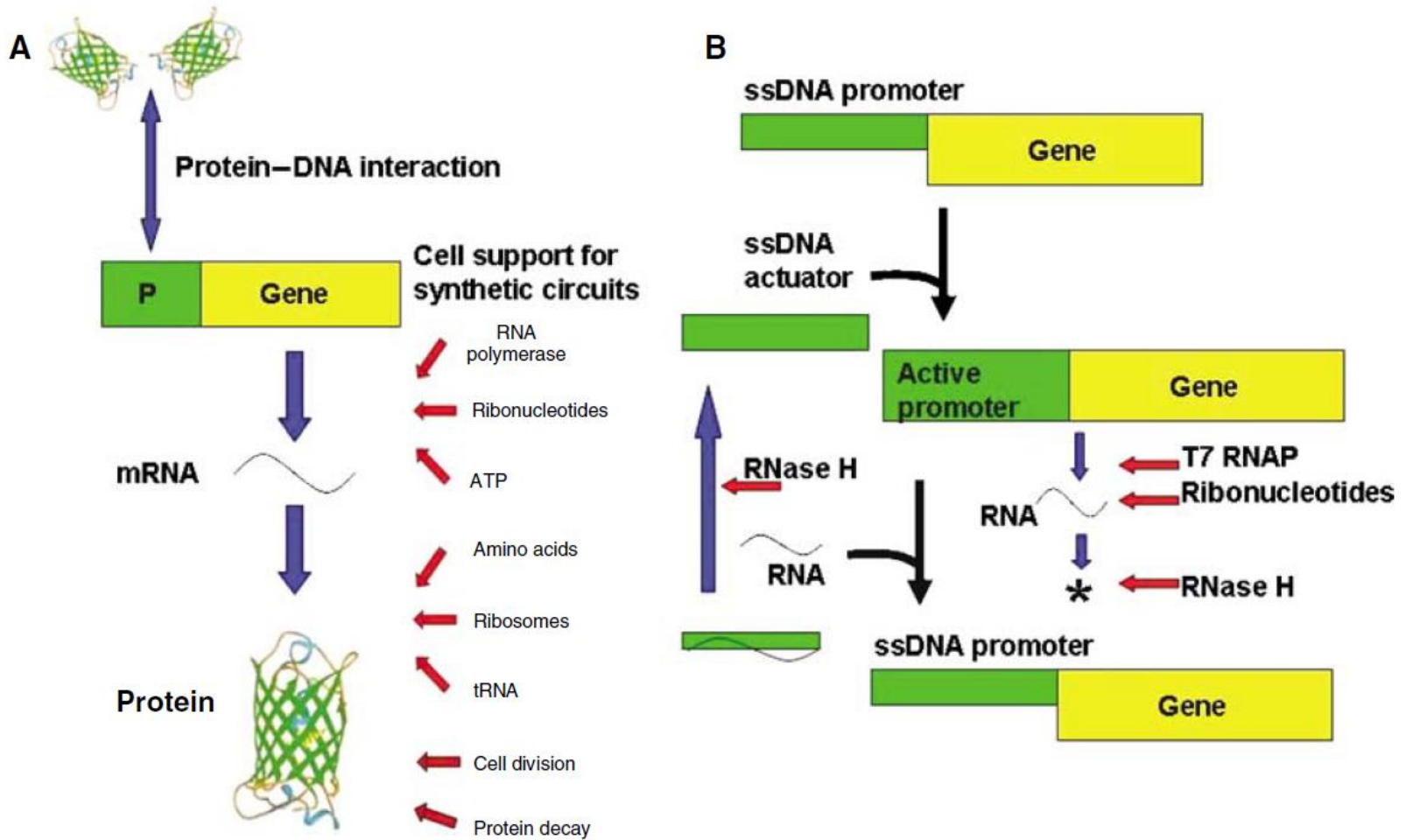
Workflow syntetické biologie
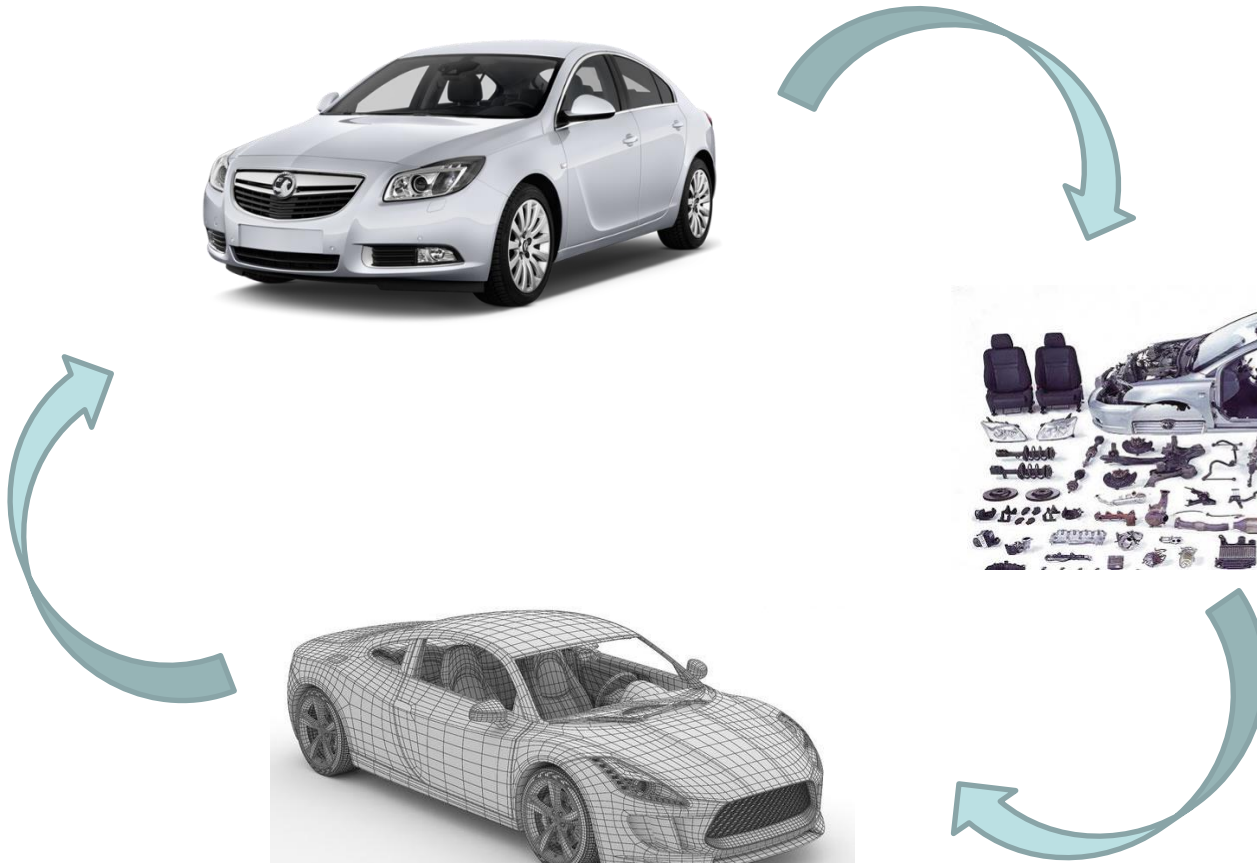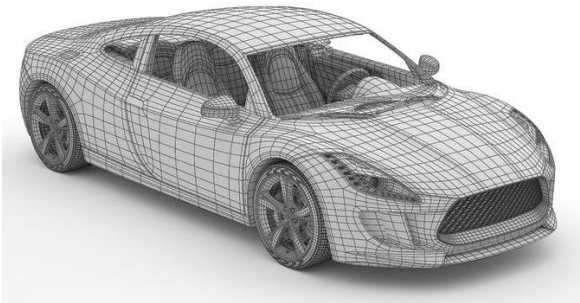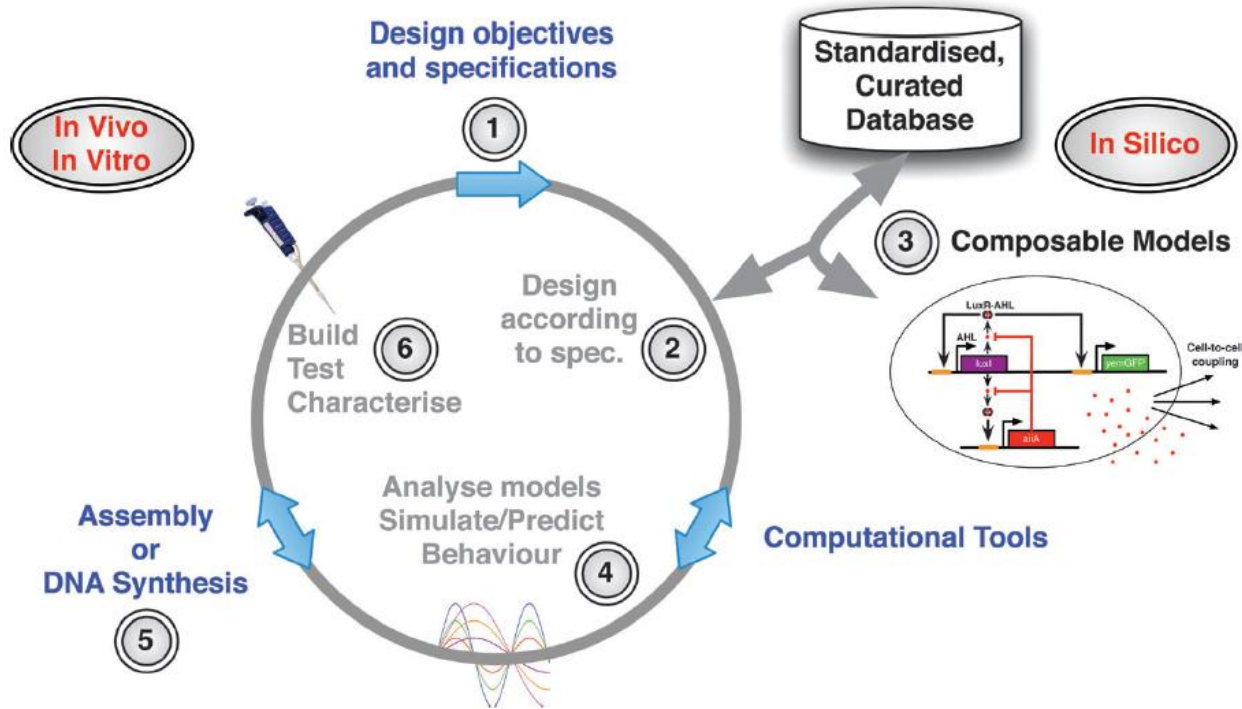
Fig. 3 The engineering design cycle (inspired by Chandran *et al.*).[121] Design starts *in silico* and proceeds iteratively along the cycle with the following workflow: (1) The design begins by the definition of the design objectives, *i.e.*, what dynamical phenotypic behaviour is sought after? with what properties (*e.g.*, robustness, yield, time response)? under which constraints (*e.g.*, upper limits on output variability, chassis and environment specifics)? (2) Based on these design specifications, different possible designs are envisioned.[43,122] These designs differ by the choice of components or parts used, and the way these parts are interconnected. (3) For each design a set of models is constructed, ideally by using a library of composable models for the parts and considering the interconnection rules imposed by the considered design. (4) Model-based analysis and simulations are performed to assess *in silico* the performance of each design with respect to the initial design specifications. At this stage, *in silico* analysis and optimisation allows for the search for parameter values leading to the desired behaviour. Furthermore, using robustness analysis, different models can be assessed with respect to their ability to withstand structural and dynamic perturbations. This typically leads to *in silico* iterations whose goal is to eventually select a subset of design candidates for the wet-lab implementation. (5) The candidate *in silico* designs are "translated/compiled" into DNA sequences for *in vitro* or *in vivo* wet-lab implementation. (6) The *in vitro* or *in vivo* implementation is tested and characterised to yield biological data that are then fed back into the model, thereby closing the engineering design loop.

Build
DNA design &
Synthesis

Test
Automated genome and
plasmid
engineering
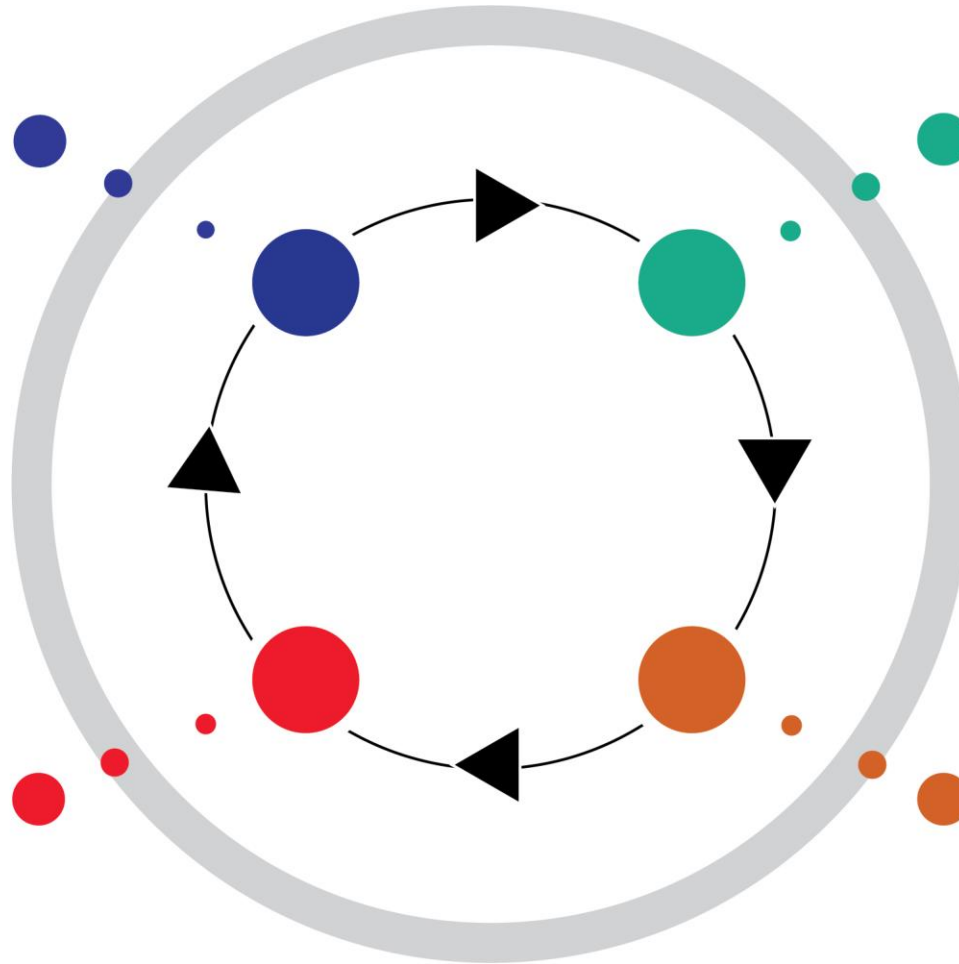
(Re)Design
Bioinformatic analysis
Modeling

Learn
Assesment and
enrichment of
engineered biological systems

Current Opinion in Chemical Biology
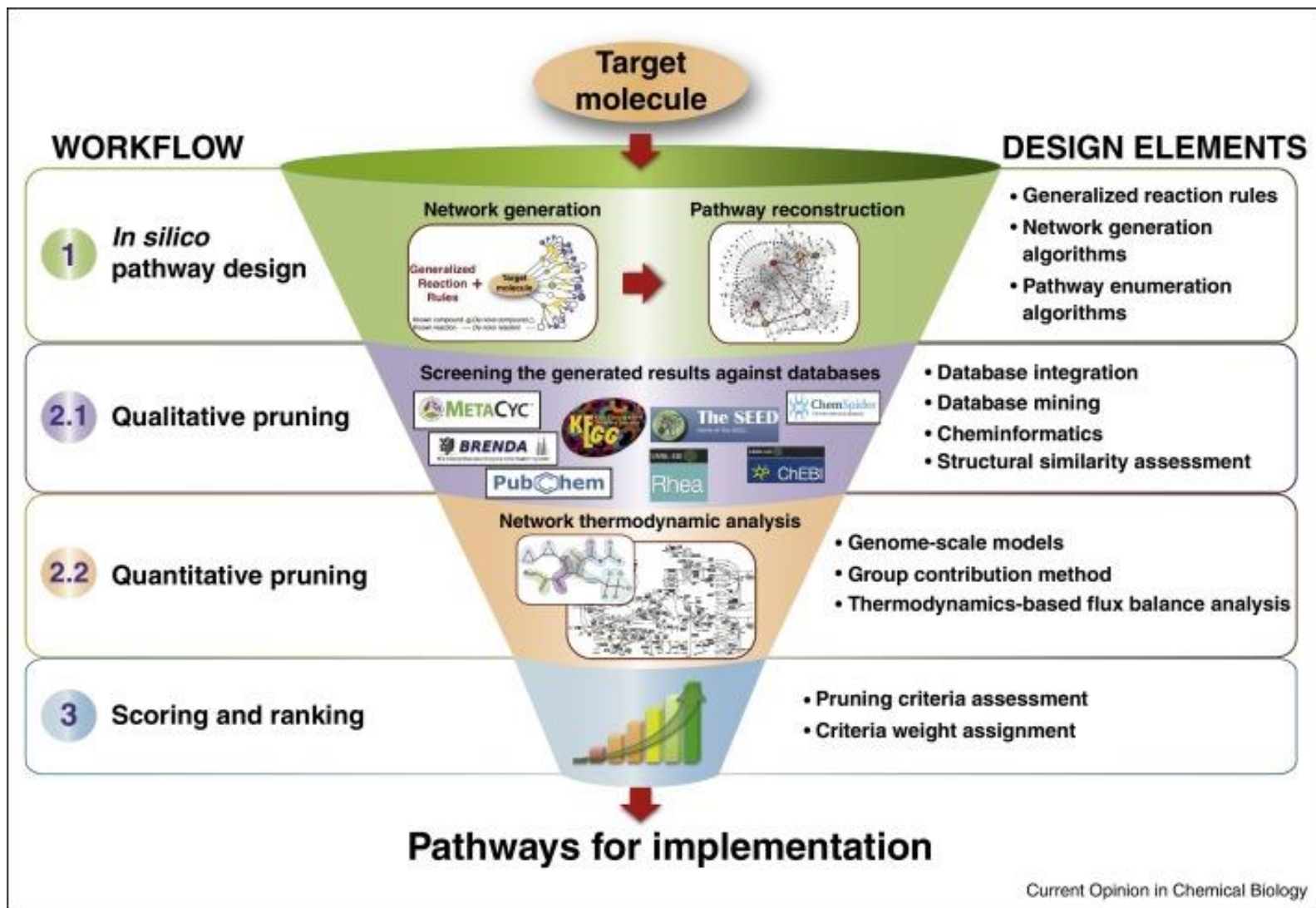
Tvorba genetických okruhů

promotore (start)

STOP

Gene di interesse

Plasmide
(scheda madre)
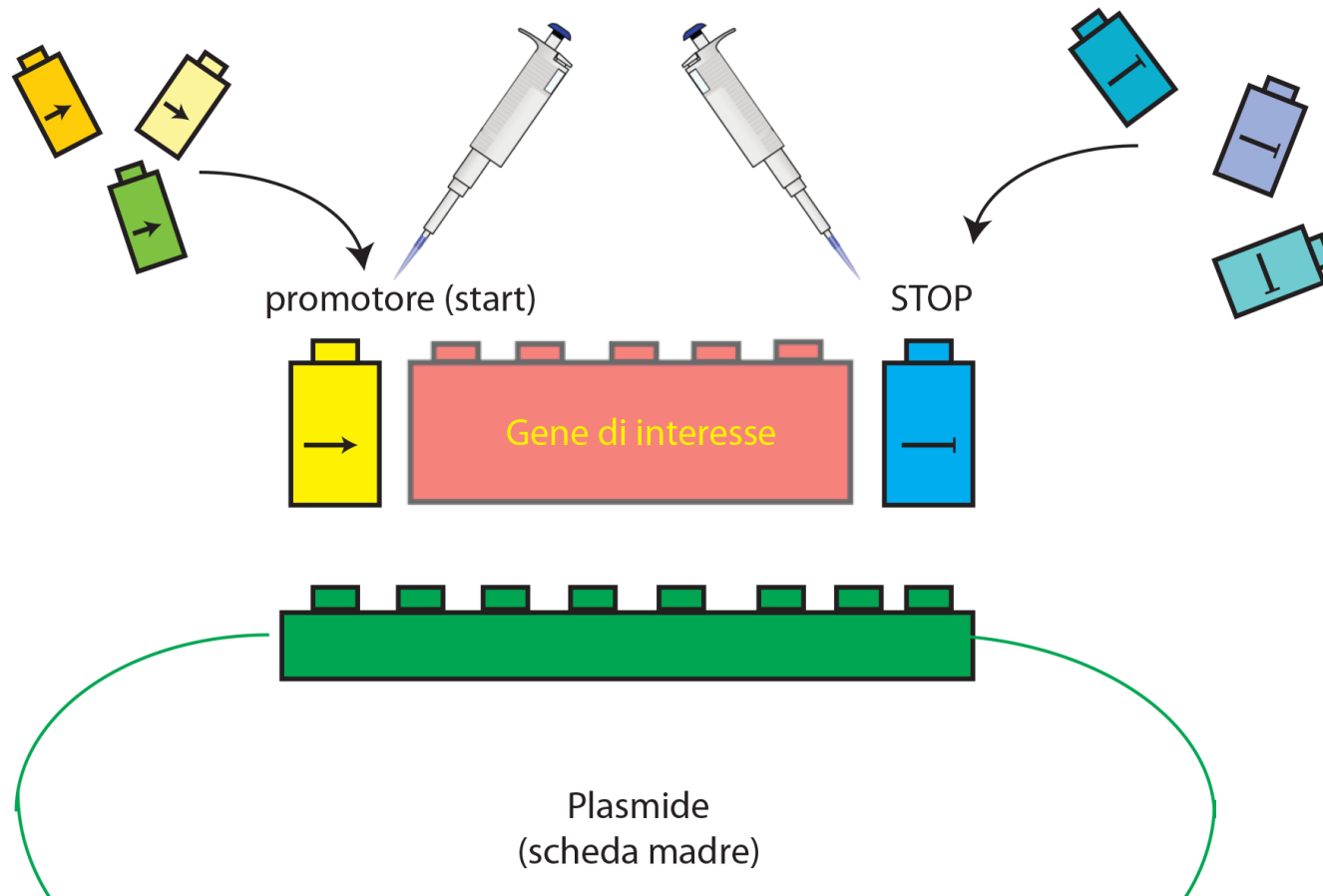
Synthetic Biology Open Language

|			
⌐→	promoter	⌐→	primer binding site
⬠	cds	|	restriction site
⌒	ribosome entry site	][	blunt restriction site
⊤	terminator	⌐	5' sticky restriction site
☐	operator	⌐	3' sticky restriction site
▣	insulator	═	5' overhang
✕	ribonuclease site	═	3' overhang
↓	rna stability element	=	assembly scar
✕	protease site	✕▭	signature
↓	protein stability element	▭	user defined
○	origin of replication		

Kombinace BioBricks
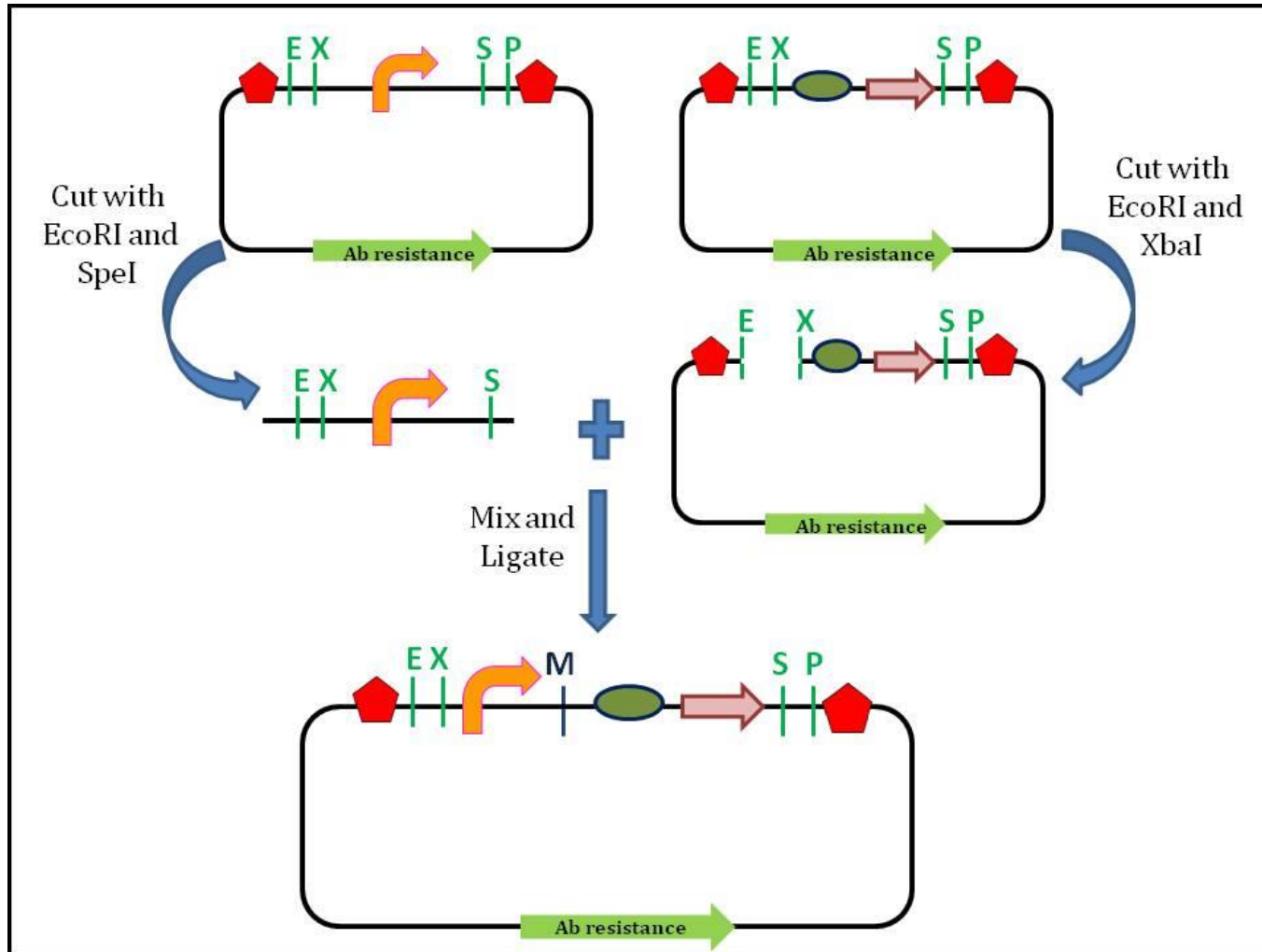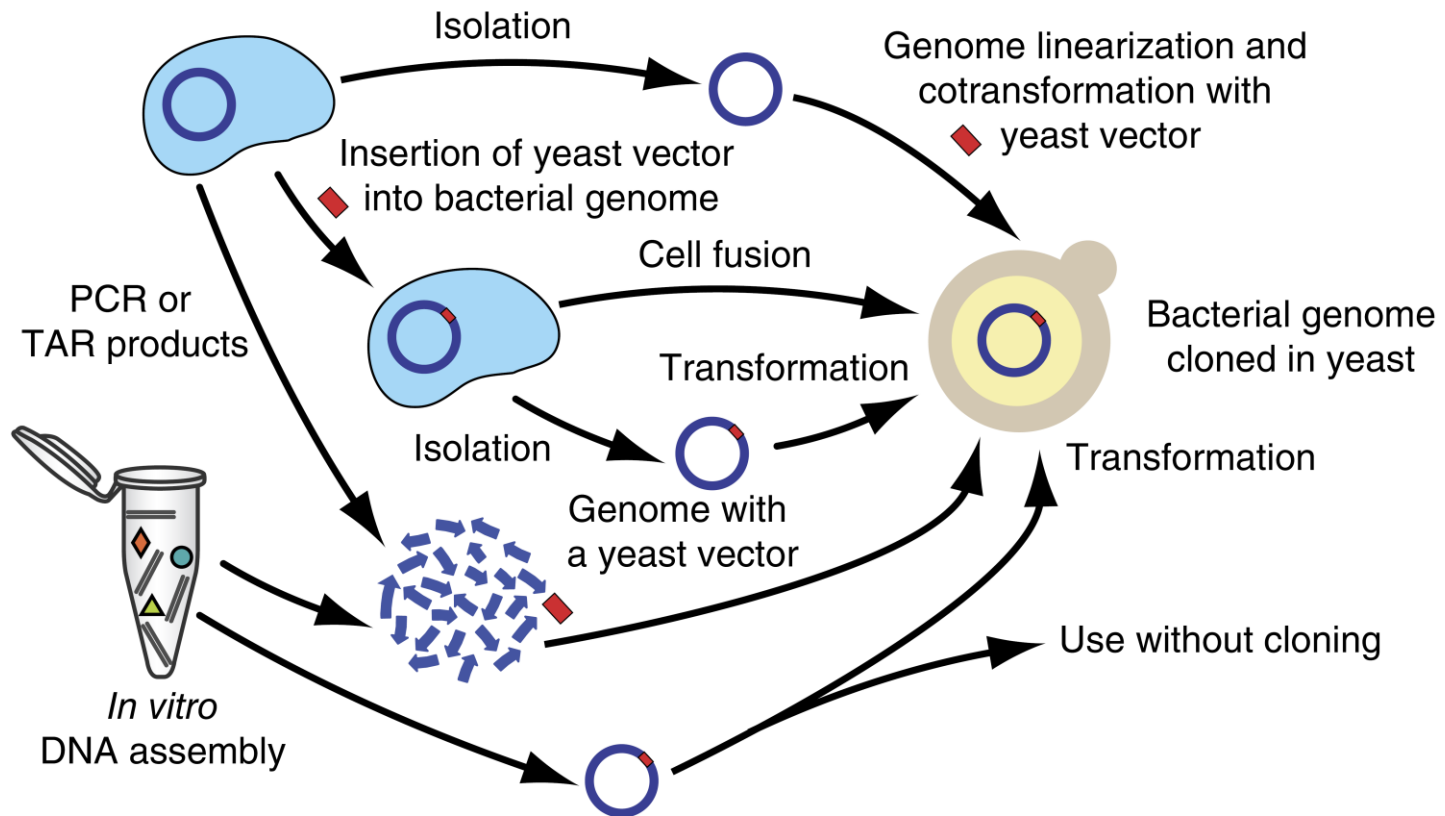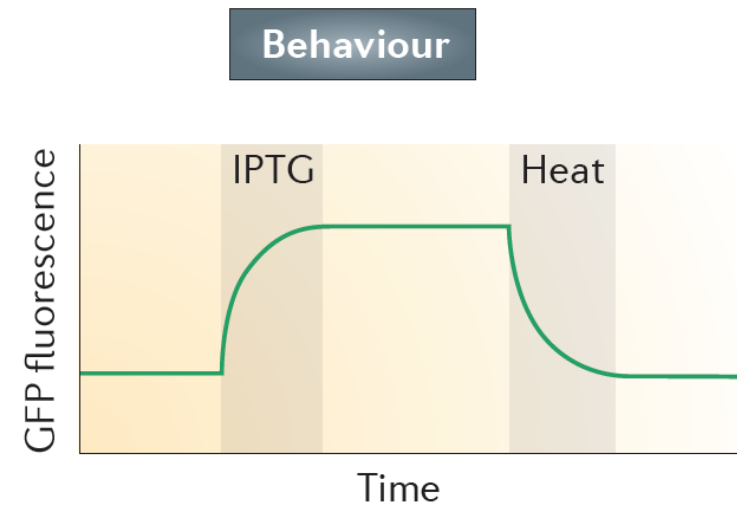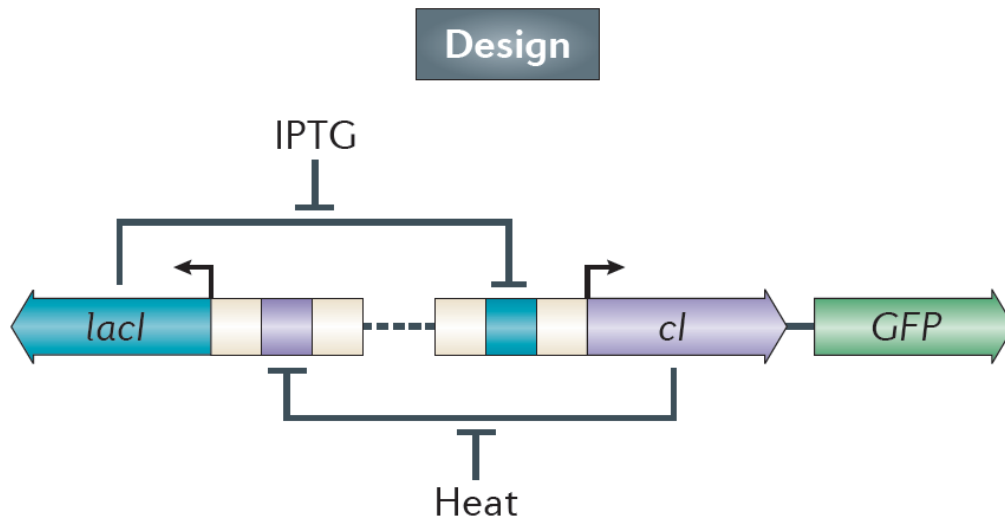
Možnosti skládání genomu

Figure 2 │ Options for genome assembly. TAR, transformation-associated recombination.

Vypínač

- Kombinace dvou navzájem se reprimujících promotorů

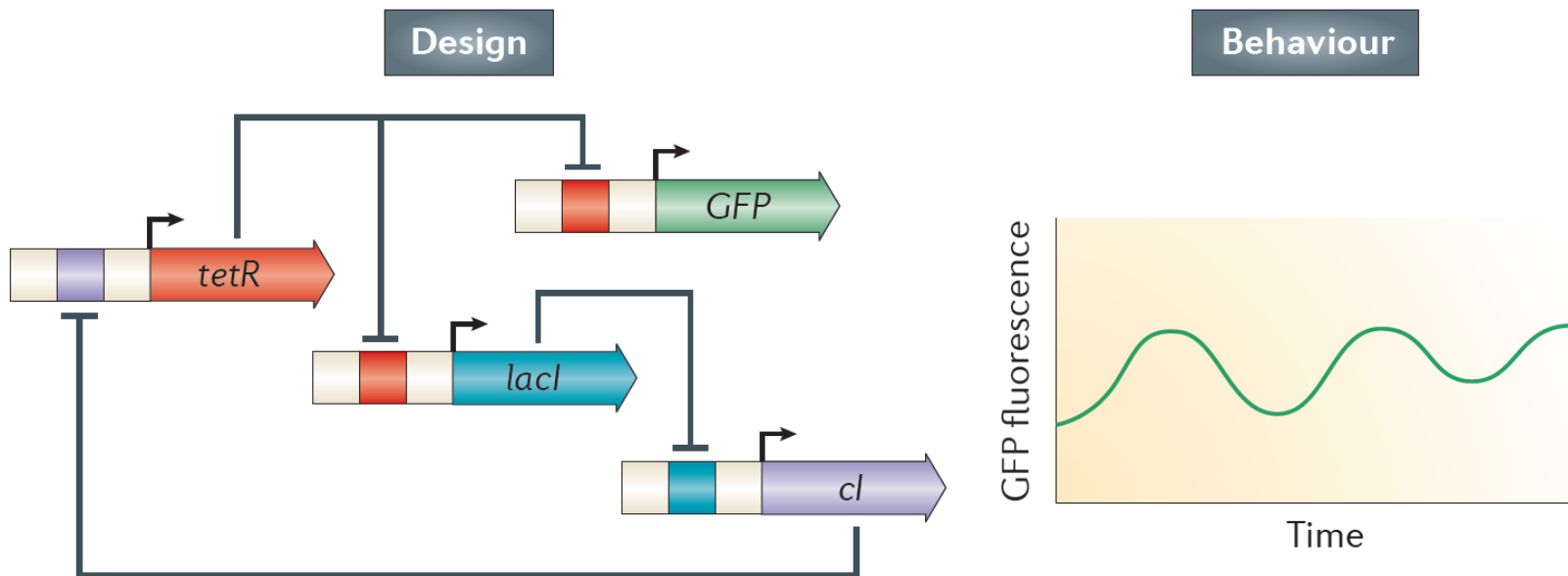- Regulace exprese proteinů on/off

a Toggle switch

Repressilátor

- Kombinace tří dvojic represor-promotor

- Úroveň exprese signálního proteinu osciluje, časem se ale sníží a zmizí v šumu
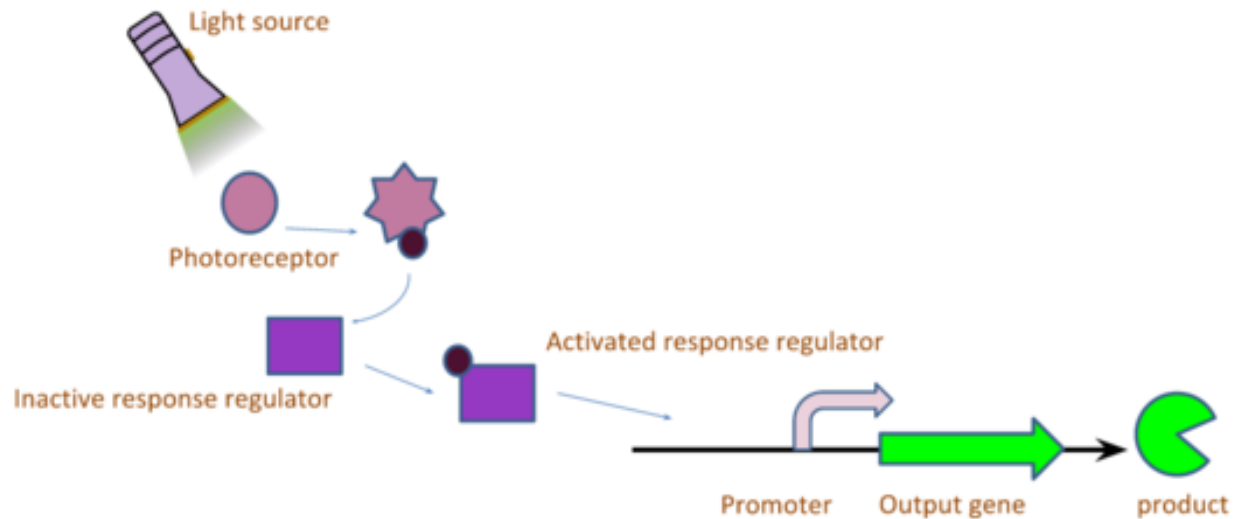

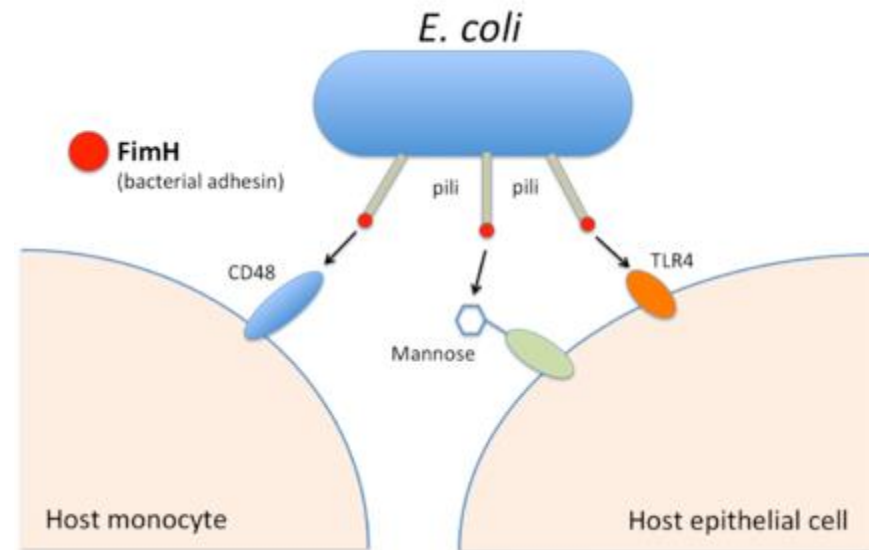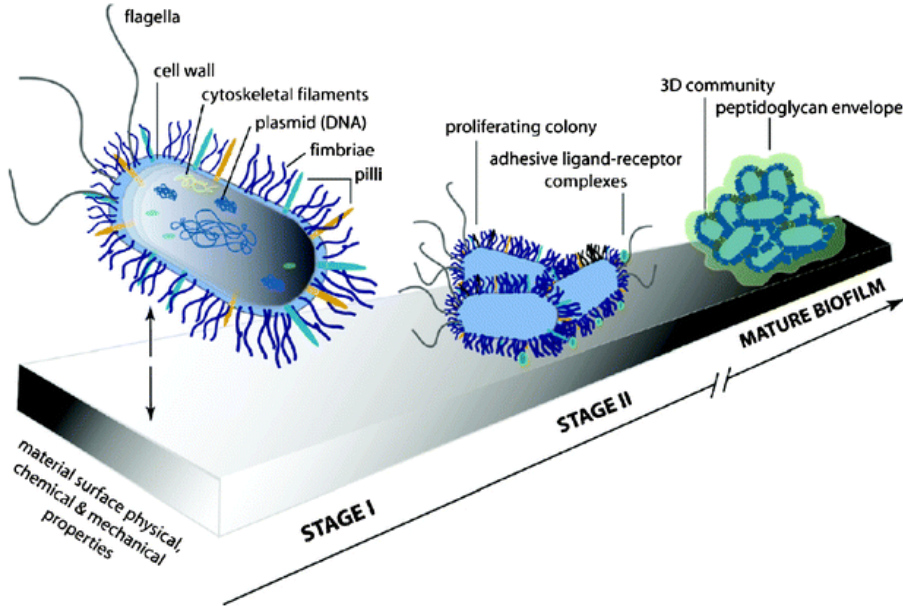**b** Repressilator

Transkripční kaskády
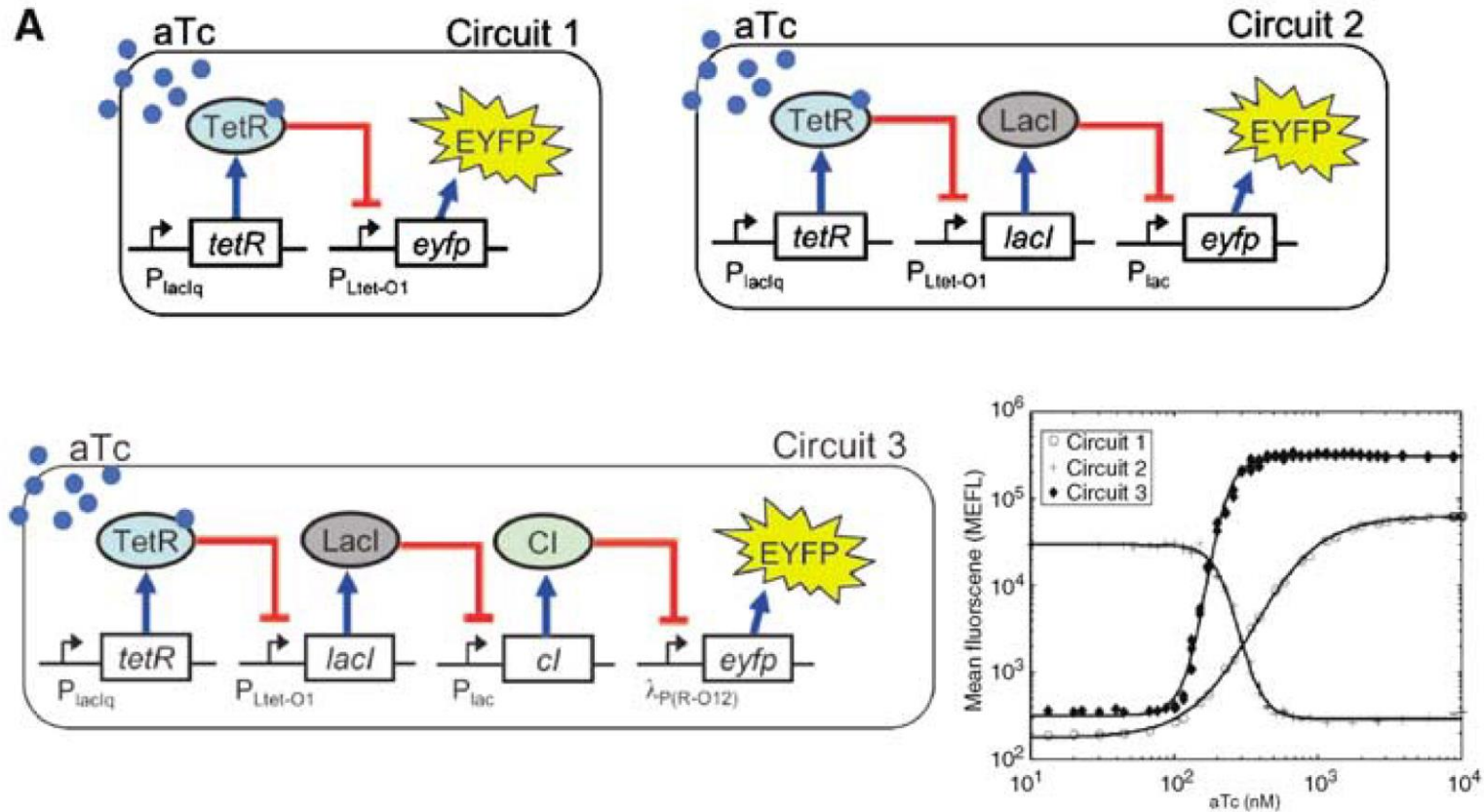
Indukce exprese světlem

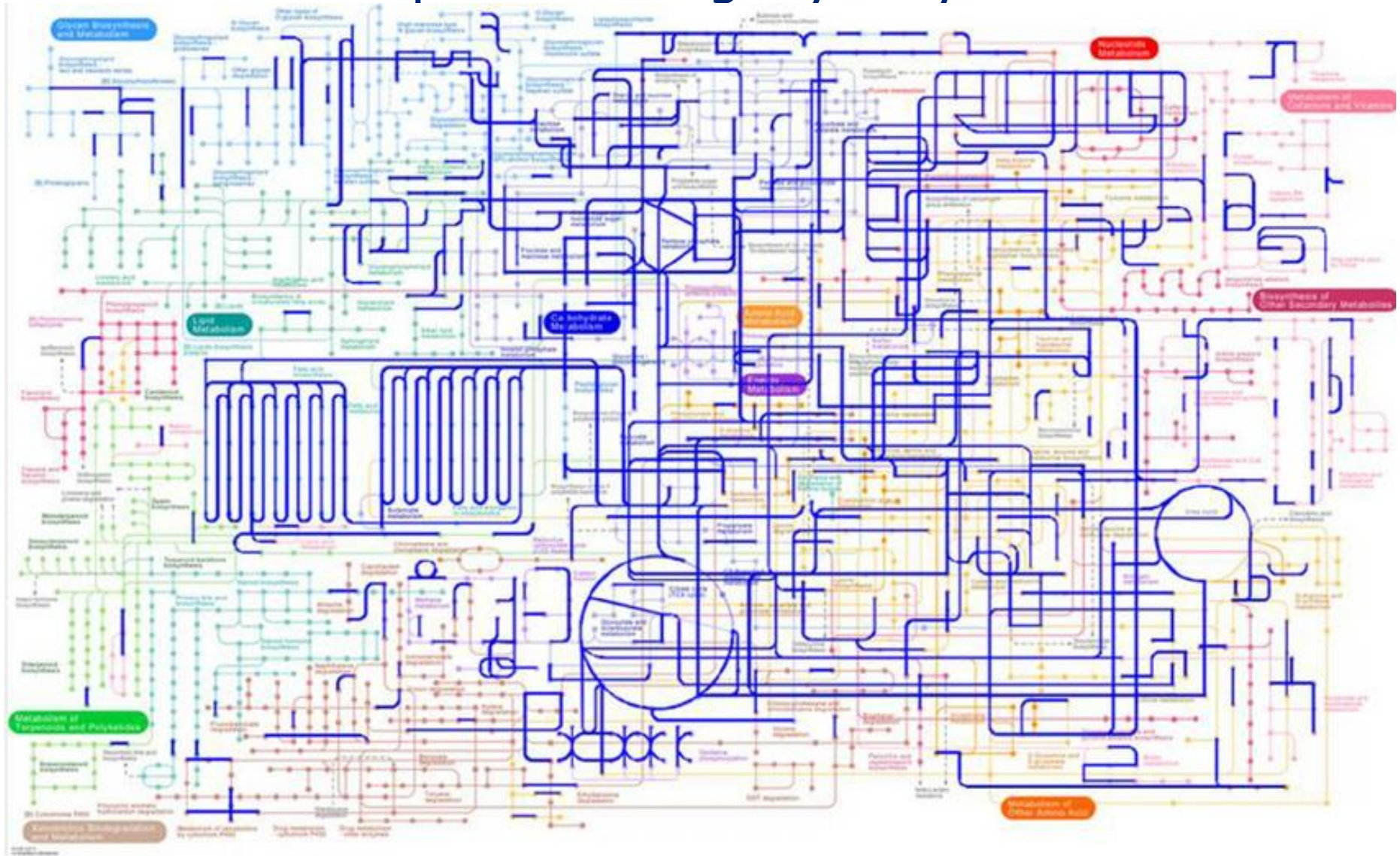- Receptor vnímá ozáření, spustí kaskádu vedoucí k expresi cílového genu
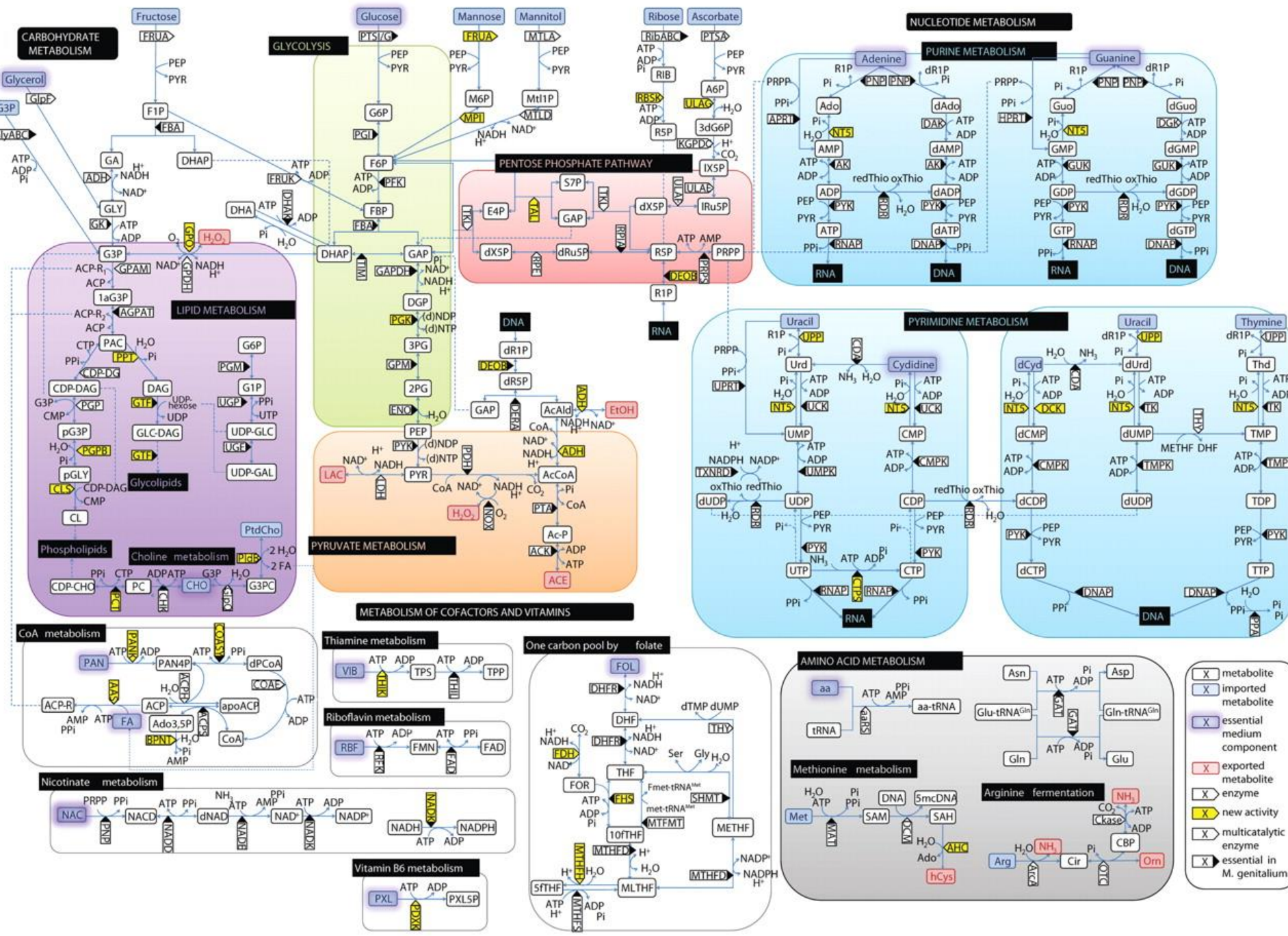
Indukce exprese dotykem

- Exprese se spouští při adhezi na povrch nebo při interakci s jiným organizmem nebo buňkou
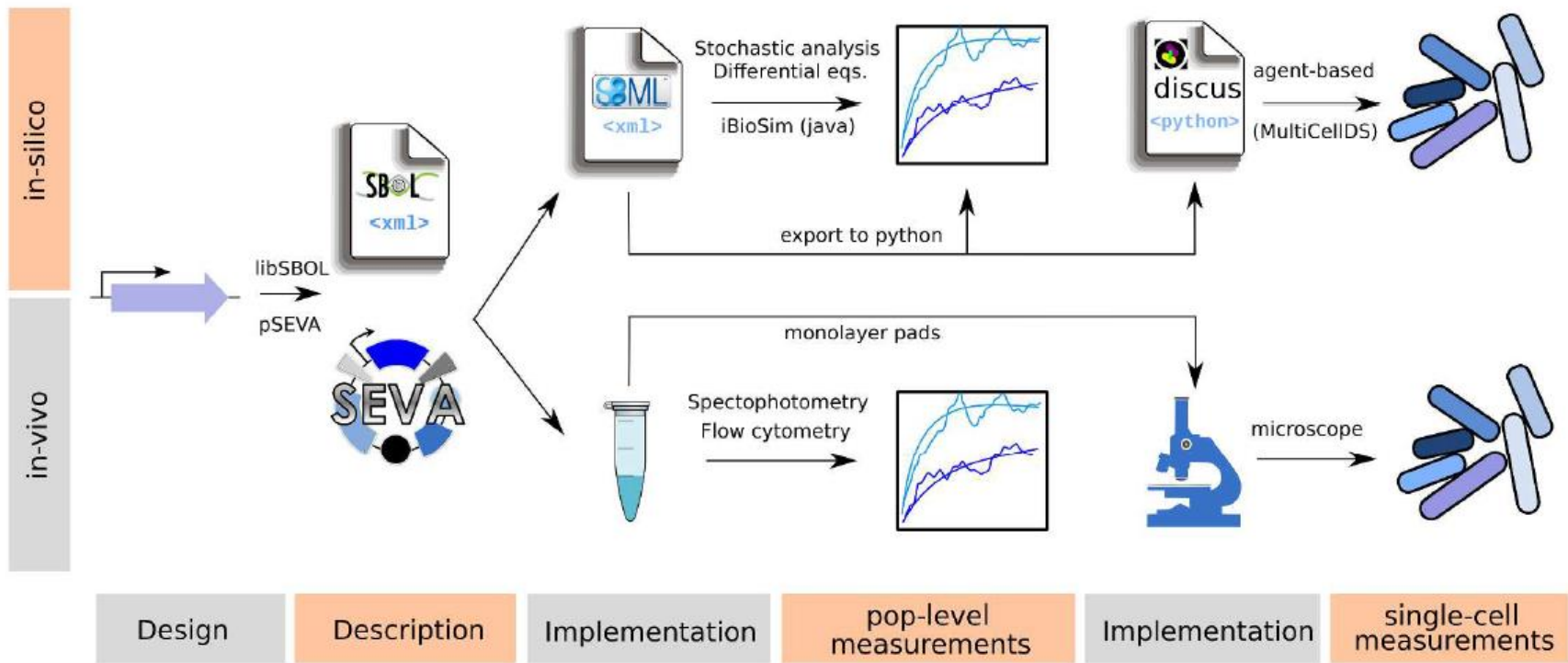

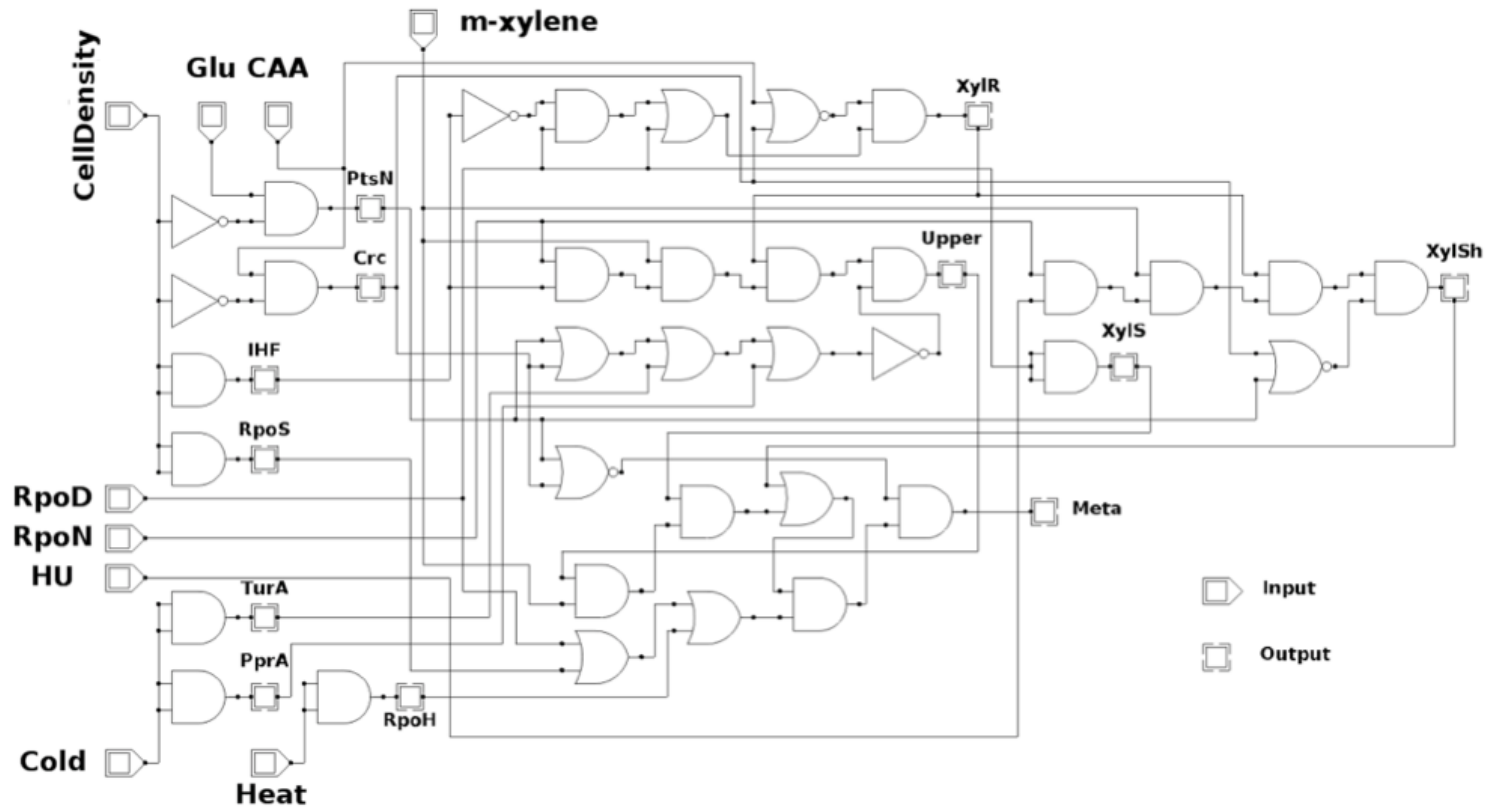- V budoucnosti: adhesiny a la carte

Komplexita biologických systémů

Komplexní model metabolické dráhy

RBS Calculator v2.0

tunable control of the translation initiation rate

Design Methods

RBS Calculator
  Predict: RBS Translation Rates
  Design: RBS Sequences
  Design: RBSs with Constraints

RBS Library Calculator
  Predict: RBS Library
  Optimize: Search Mode
  Optimize: Genome Editing

Operon Calculator
  Predict: Operon Expression[Physics-only]
  Predict: Operon Expression[Annotated][New]
  Design: Multi-CDS Operons[New!]

Pathway Map Calculator
  Predict: Create Pathway Maps[New]

Riboswitch Calculator[NEW]

Title
[_____]

Pre-Sequence [?]   Protein Coding Sequence [?]
[_____]   [_____]

Target Translation Initiation Rate [?]   [_____] Proportional scale (0 to 100,000+) ☐ Goal: Maximize

Select a Free Energy Model [?]
[Version 2.0 ▾]

Organism or (16S rRNA) [?] (start typing)
[_____]

Submit
Design Jobs: 1 queued, 5 currently running   For Non-Commercial Use Only. Click here for commercial usage.

Updates & Tips

Have a Question? Our Documentation, Publications, and References may have your answer!

When using these results, please reference A. Espah Borujeni, A.S. Channarasappa, and H.M. Salis, "Translation rate is controlled by coupled trade-offs between site accessibility, selective RNA unfolding and sliding at upstream standby sites", *Nucleic Acid Research*, 2013 and H.M. Salis, E.A. Mirsky, C.A. Voigt, *Nat. Biotech.*, 2009

a

30S

50S

70S

mRNA

Initial ΔG_{tot} Final

b

Initial state

Final state

30S complex

AUUCCUCCA

UCCUUUCUAGAGA AAGA CAGGGGGAC UAGUCAUCG AUG CGUCAUUCACAGUACUCAUCUACCGUAUCACG

Standby site   **Shine Dalgarno**   **Spacing**   **Start**

ΔG_{mRNA}

$\Delta G_{standby}$ $\Delta G_{mRNA:rRNA}$ $\Delta G_{spacing}$ ΔG_{start}
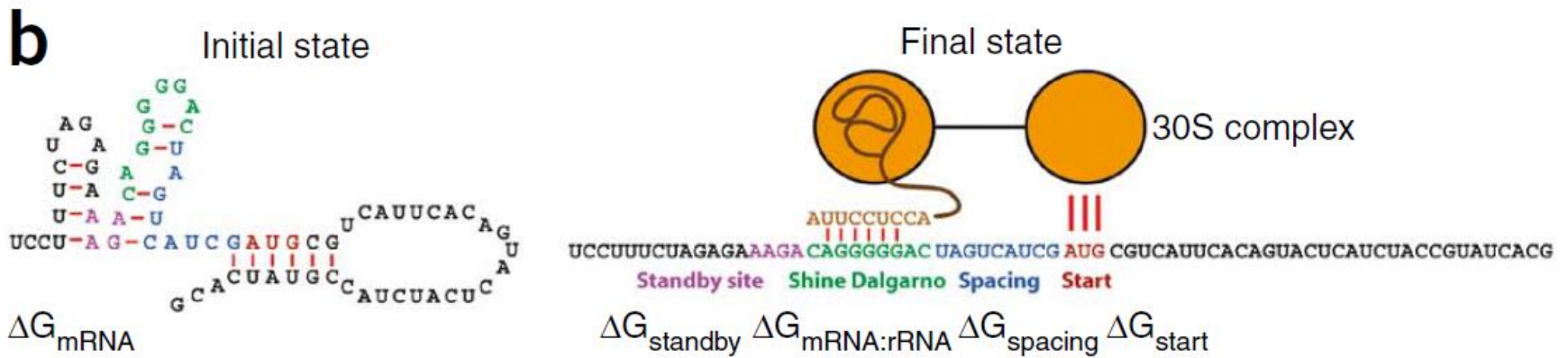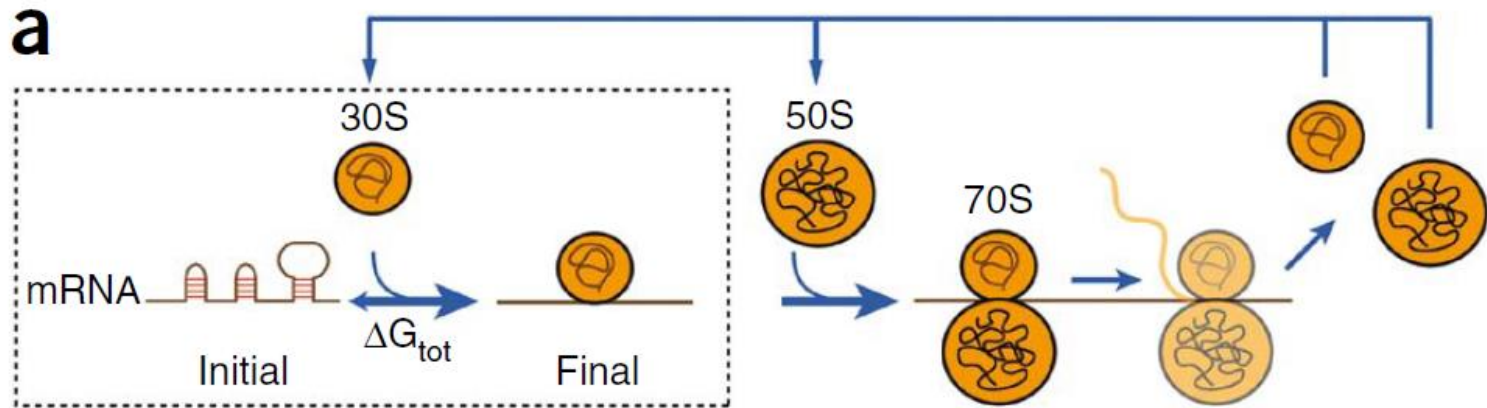
Figure 2 A ribosome binding site design method. (**a**) Reverse engineering. The method predicts the relative translation initiation rate (red) of an RBS upstream of a given protein coding sequence (blue). The ΔG_{tot} is the free energy change before and after the 30S ribosomal complex assembles on the mRNA. Equation (1) predicts a linear relationship between the log protein fluorescence and the predicted ΔG_{tot}. (**b**) Red fluorescence protein reporter expression driven by 28 natural or existing RBSs compared to predicted ΔG_{tot} calculations. Error bars are s.d. of six measurements performed on two different days. Linear regression $R^2 = 0.54$ with slope $\beta = 0.45 \pm 0.05$. (**c**) Histogram of the distribution of error in the predicted ΔG_{tot}, denoted by $|\Delta\Delta G|$, of the sequences in **b**. The average of this distribution is 2.11 kcal/mol. (**d**) Forward engineering. A simulated annealing optimization algorithm iteratively mutates an RNA sequence until a target ΔG_{tot} is found. (**e**) RFP expression driven by 29 synthetic RBSs compared to the predicted ΔG_{tot} calculations. Error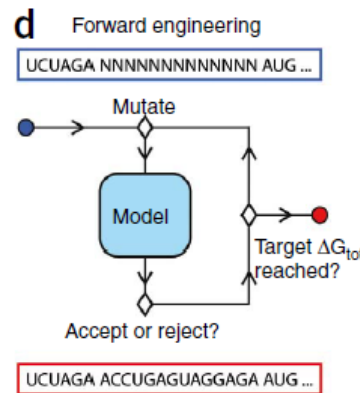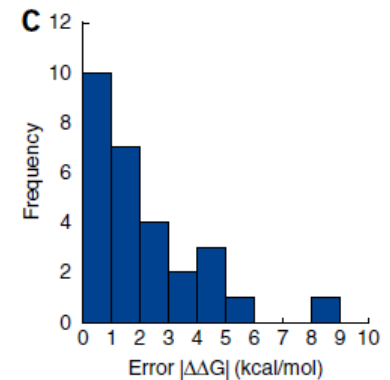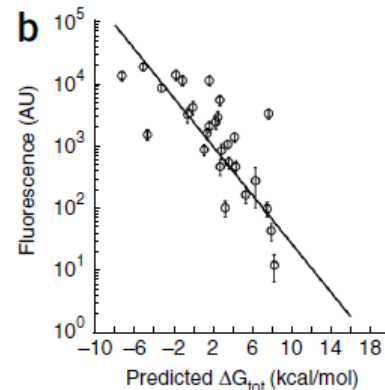 bars are s.d. of at least five measurements performed on two different days. Linear regression $R^2 = 0.84$ with slope $\beta = 0.45 \pm 0.01$. (**f**) Histogram of the distribution of the error, $|\Delta\Delta G|$ from **e**. The average of the distribution is 1.82 kcal/mol and fits well to a one-sided Gaussian distribution (red line) with s.d. $\sigma = 2.44$ kcal/mol.
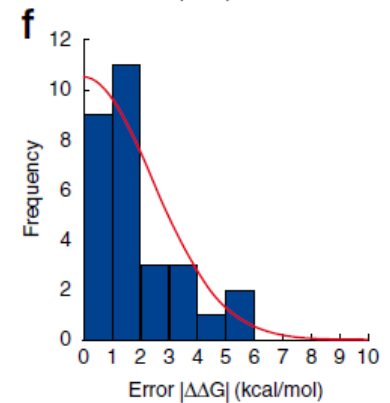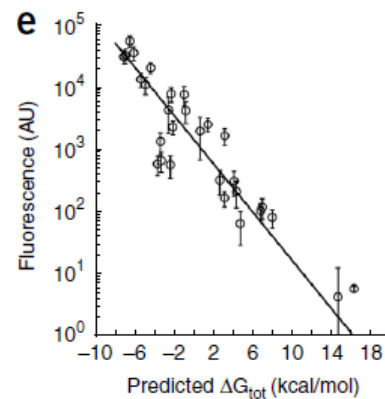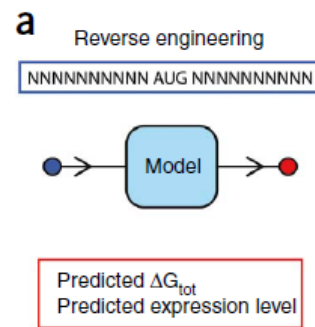
Genomové inženýrství

- **Multiplex automated genome engineering (MAGE)**
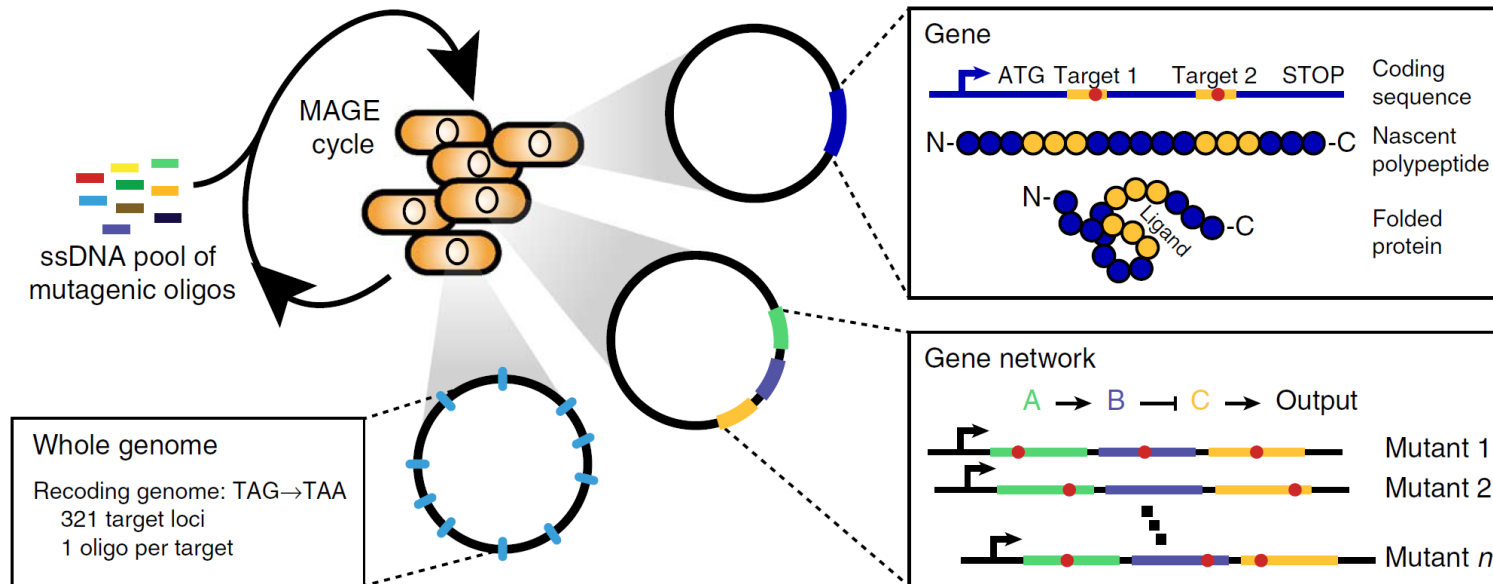
Figure 1 | Multiplex automated genome engineering (MAGE) processes and applications. MAGE can be used to generate many mutations in genes (targets in orange), gene networks (targets in green, orange and purple) or full genomes (targets in blue). Depending on the number of ssDNAs targeted to each locus, MAGE can be used to edit the bacterial chromosome (one oligo per locus) or to generate combinatorial diversity (>1 oligo per locus). Combinatorial diversity can be explored in a population of cells that have incorporated different mutations (red) at different targets. Populations undergoing MAGE mutagenesis can be cycled iteratively until the desired level of diversification or recoding is achieved.

Genomové inženýrství

- ■ Multiplex automated genome engineering (MAGE)
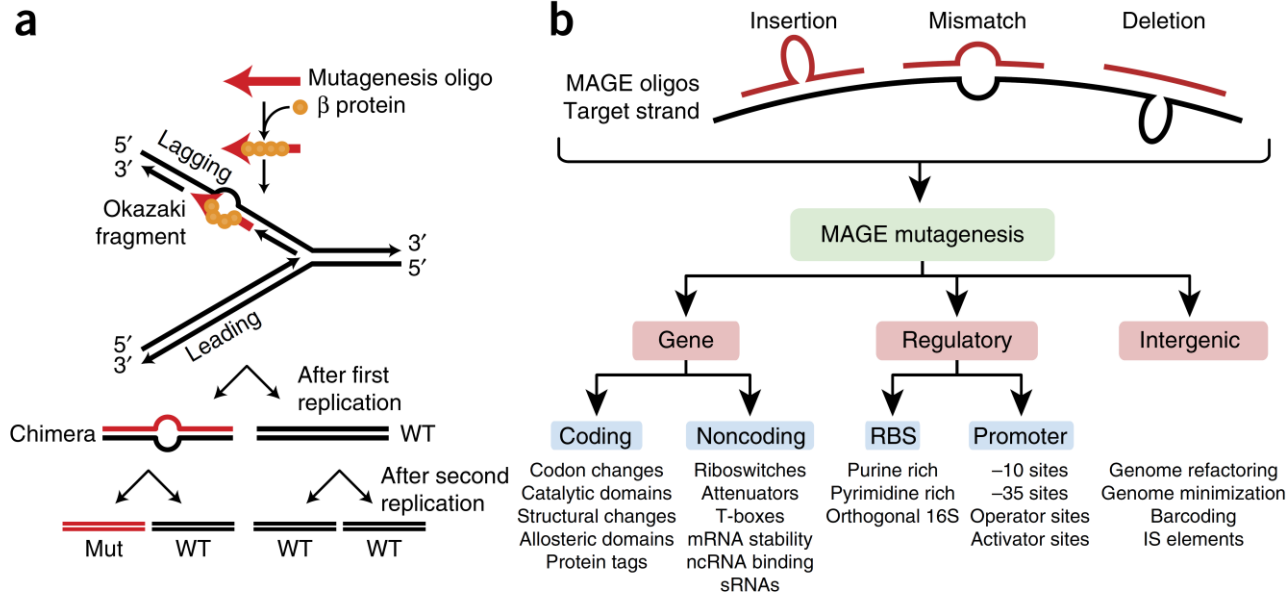
Figure 2 | Proposed mechanism and categories of MAGE-based mutagenesis. (a) The mutagenesis oligo is bound by β-protein and anneals to the lagging strand of the replication fork, between discontinuous Okazaki fragments. At the end of replication, one wild-type (WT) chromosome and one chimeric chromosome exist. During the second round of replication, two strands of the chimeric chromosome segregate into a fully WT and a fully mutant (mut) chromosome. (b) MAGE is capable of introducing insertion, deletion or mismatch mutations. By targeting coding, regulatory or intergenic regions, these mutations can be used to modify transcription rates, translation rates, mRNA stability, enzyme activity and so on. RBS, ribosome binding sites.

Genomové inženýrství
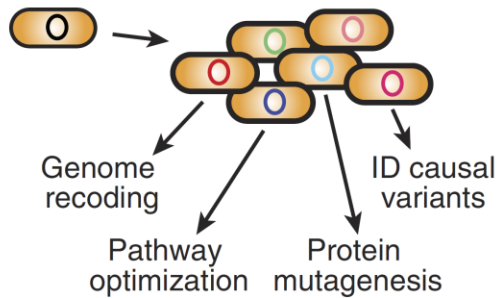
- Multiplex automated genome engineering (MAGE)
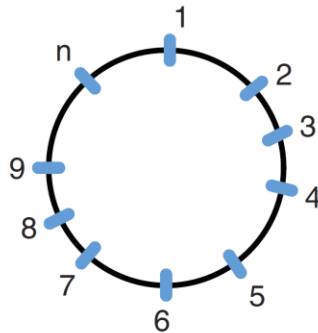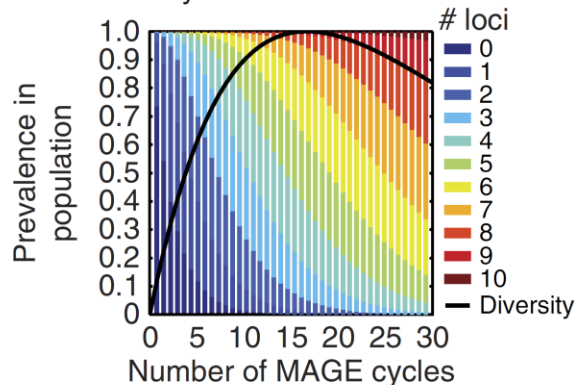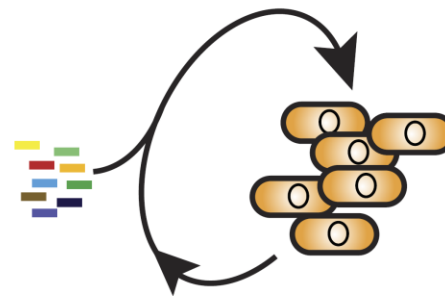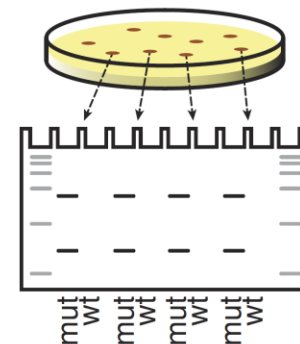
CRISPR-Cas9 EDITACE GENOMU
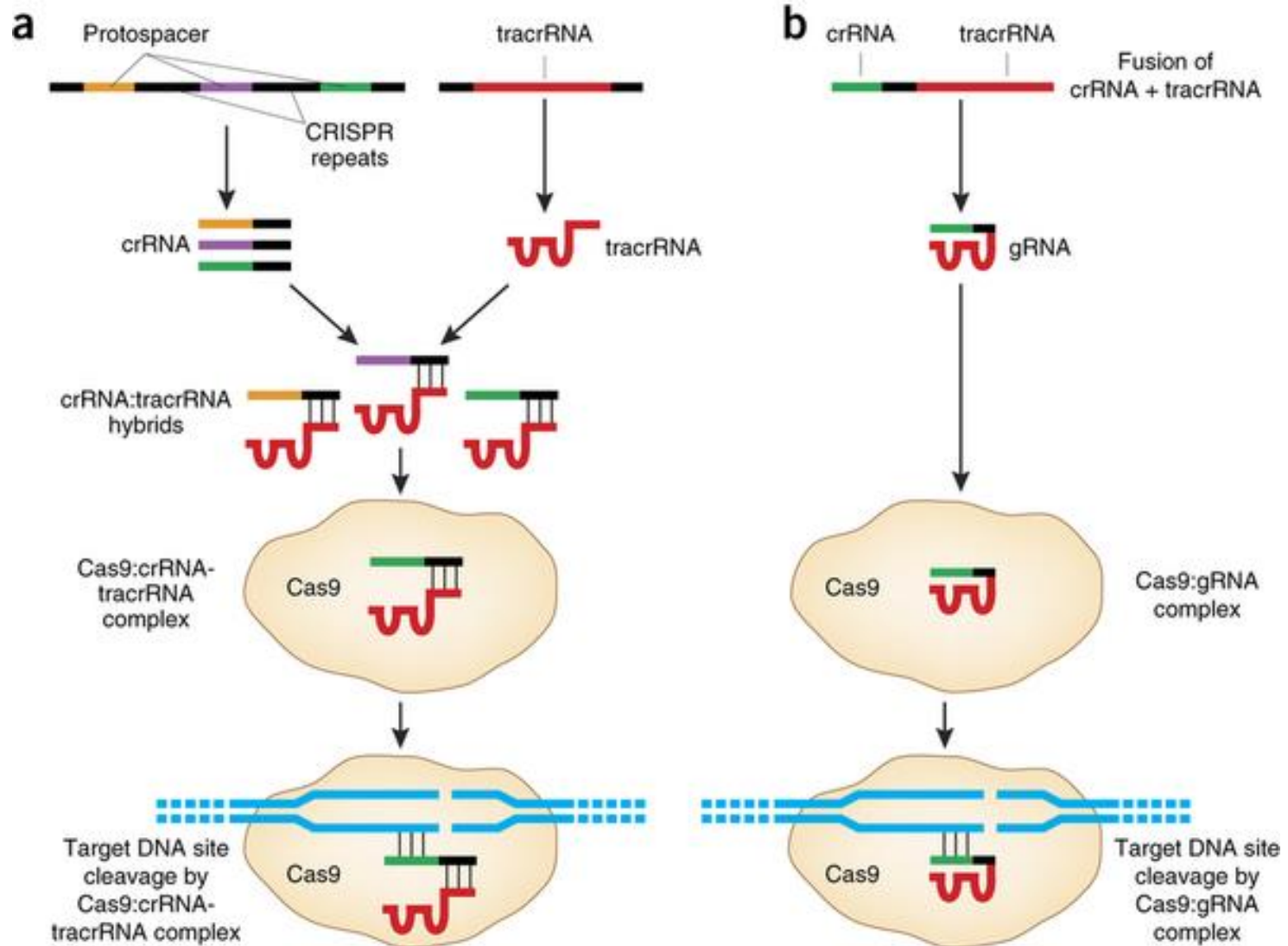
Escherichia coli

- Nejvýznamnější organizmus v biotechnologiích

- Řada dostupných kmenů

- Přes 60 let intenzivního výzkumu

- Znalost vektorů, promotorů, …

- Desítky let výzkumu fungování operonů

- Známé genetické manipulace

Escherichia coli

- Patogenní kmeny

- Organizmus není příliš vhodný pro environmentální aplikace

- Dobrý růst v laboratorních podmínkách a průmyslových bioreaktorech
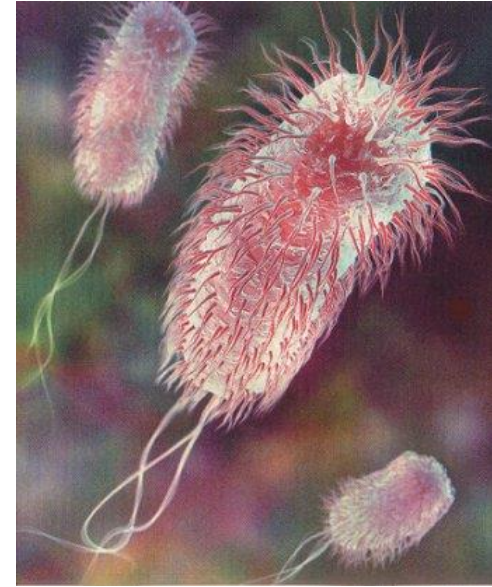
Pseudomonas putida

- Půdní bakterie

- Rezistentní vůči mnoha abiotickým faktorům

- Přítomna na kontaminovaných místech

- Vzrůstající význam v molekulárních biotechnologiích

- Genomové inženýrství pro vylepšení organizmu
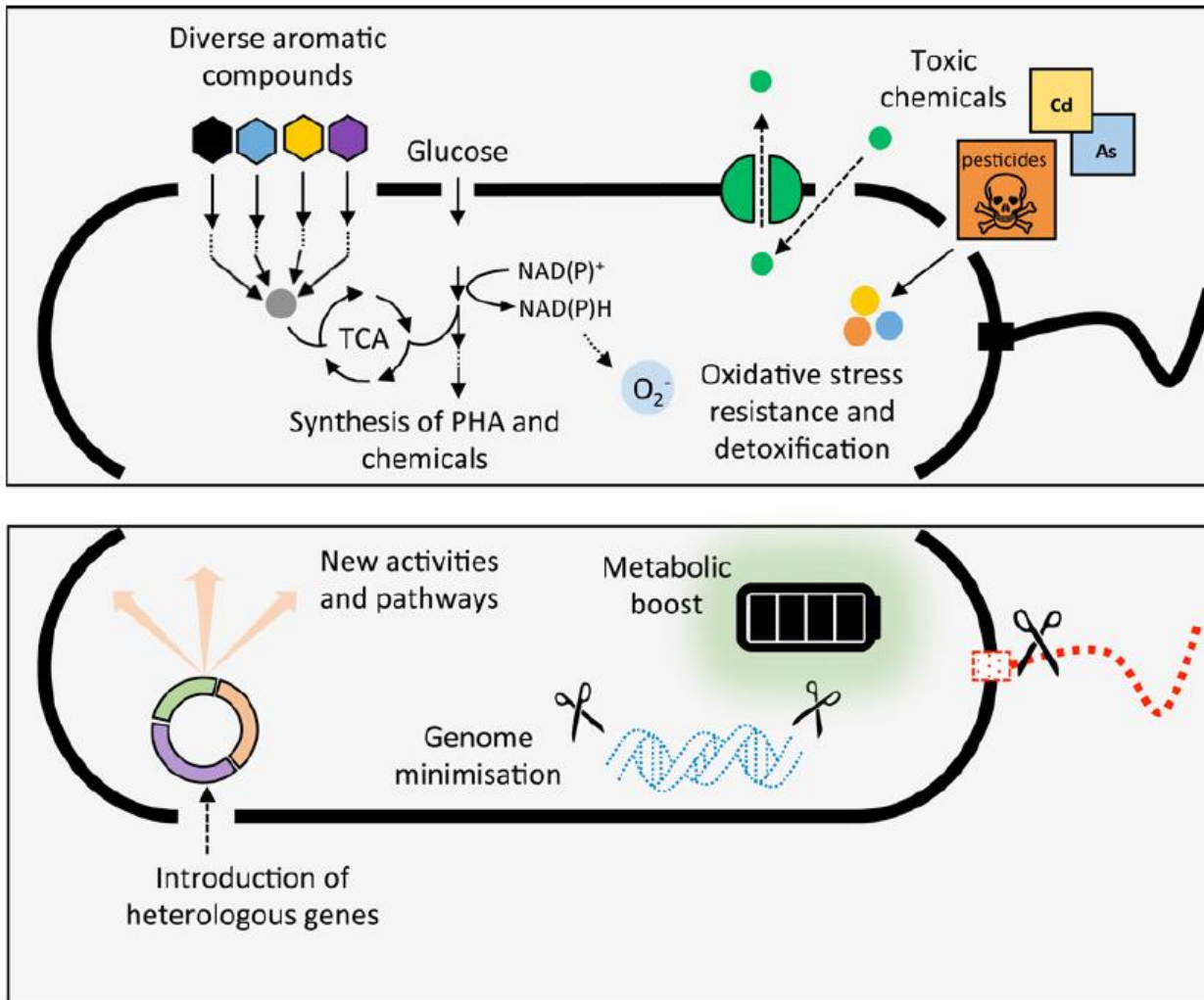
Figure 3. *Pseudomonas putida* as a suitable chassis for multiple applications in synthetic biology

putida is naturally endowed with outstanding capabilities for biodegradation, biotransformation, bioplastic production, detoxification and ress survival (upper panel). The strain KT2440 has been upgraded further using synthetic biology with a dedicated set of molecular tools d genome editions to boost its metabolic capabilities further (lower panel).

Katabolický represor GlpR v *P. putida*
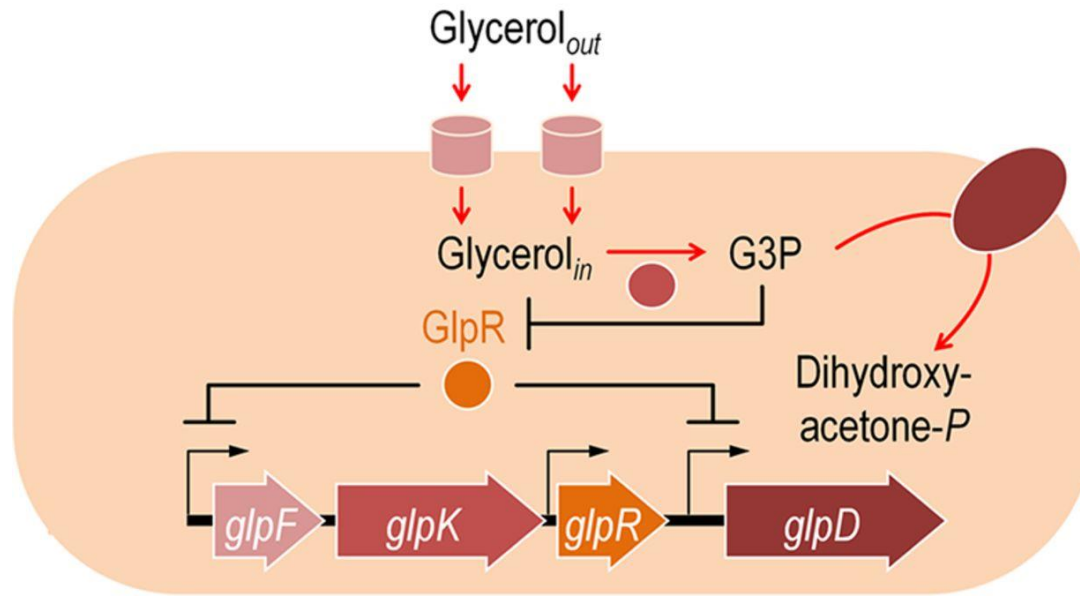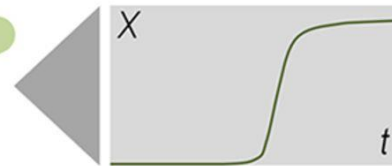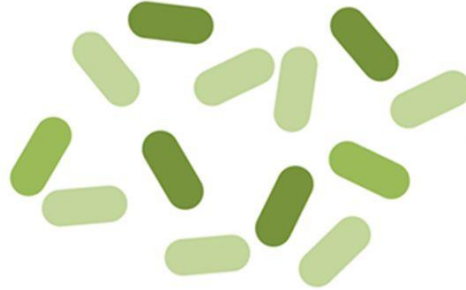
Katabolický represor GlpR v *P. putida*
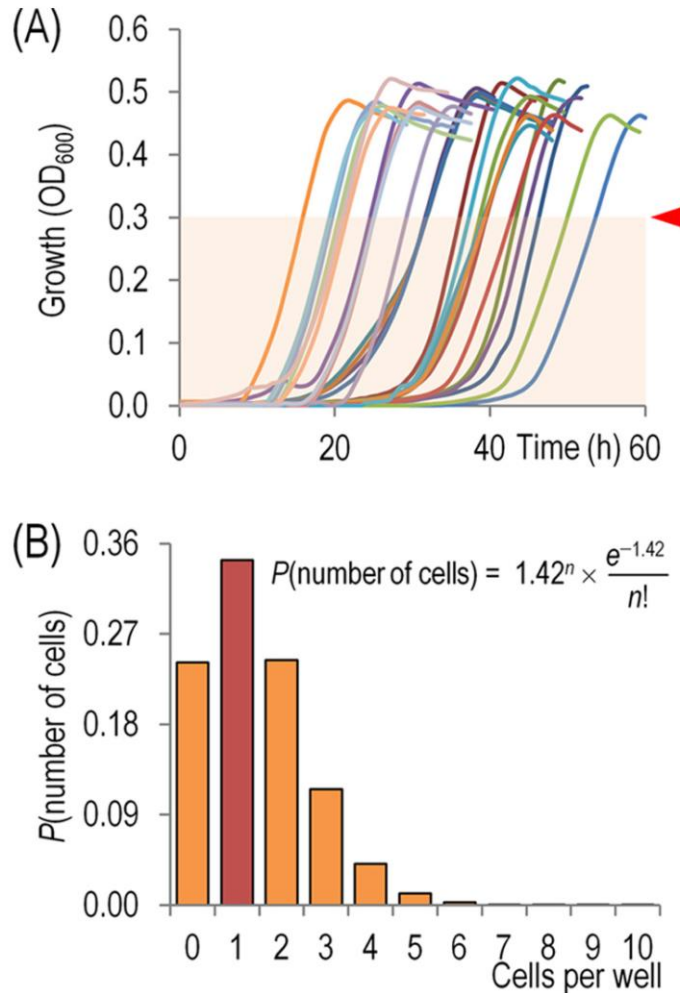
FIG 2 (A) Growth of *P. putida* KT2440 in single-cell batch cultures. Multiwell microtiter plates were inoculated with a highly diluted preculture previously developed in LB medium in order to start each culture from 1 cell per well. Cells were grown at 30°C in 200 μl of M9 minimal medium containing 40 mM glycerol with rotary agitation. The time needed to reach an optical density measured at 600 nm (OD_{600}) of 0.3 (mid-exponential phase of growth) is indicated by a red arrowhead for 50 independent cultures to illustrate the delay in growth initiation among individual wells. This parameter, termed time of metabolic response (t_{MR}), was further used to quantify the response of single-cell cultures. (B) Probability of inoculating a given number of cells in a particular well, as estimated by the Poisson probability distribution. After dilution and inoculation, and due to stochastic variations (e.g., small differences in the volume of the bacterial suspension transferred into each well), the number of cells per well (n) is not exactly known and may vary from well to well. The probability P of inoculating zero, one, two, or three cells per well is shown, indicating that the probability of inoculating a single cell is the most likely outcome.

Inducible systems for engineering heterologous gene expression in *Pseudomonas*[a]

TF[b]	Target promoter	Inducer(s)	Vector[c]		References
			Plasmid	Transp	
LacI[q]	*Plac*, *Ptac*, *Ptrc*	IPTG[d]	X	X	[43,107]
XylS	*Pm*	Benzoate, *m*-toluate	X	X	[54,108–111,112[••]]
XylR	*Pu*	Toluene, *m*-xylene, 3MBA[e]	X		[110,111]
NahR	*Psal*	Salicylate	X	X	[108,112[••]]
ChnR	*PchnB*	Cyclohexanone	X		[113,113a]
CprK	*PDB3*	CHPA[f]	X		[44[••],114]
AlkS	*PalkB*	*n*-Octane, DCPK[g]	X	X	[115,116]
RhaR	*PrhaB*	Rhamnose[h]	X		[112[••]]
AraC	*ParaB*	Arabinose[h]	X		[112[••]]
CymR	*Pcym*	*p*-Cumate	X		[117]
MekR	*PmekA*	Methyl ethyl ketone	X		[118]
HpdR	*PhpdH*, *PmmsA1*	3-Hydroxypropionic acid	X		[119]
MtlR	*PmtlE*	Mannitol	X		[120]
Alt[i] RNAP					
T7 RNAP	*PT7*	Various	X	X	[39,40,110,121,122]

[a] Not an exhaustive list, only most relevant expression devices for the sake of the article are listed.
[b] Transcription factor.
[c] System available in either a plasmid vector or a transposon vector as indicated.
[d] Isopropyl β-D-1-thiogalactopyranoside; intake mechanism into *Pseudomonas* unknown.
[e] 3-Methylbenzylalcohol.
[f] 3-Chloro-4-hydroxyphenyl acetic acid.
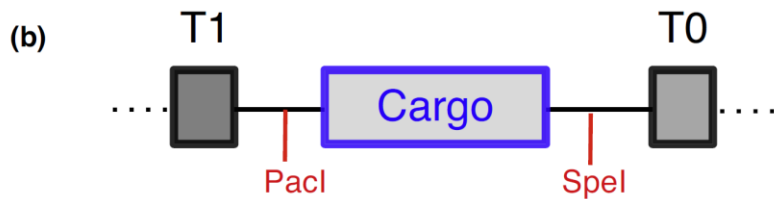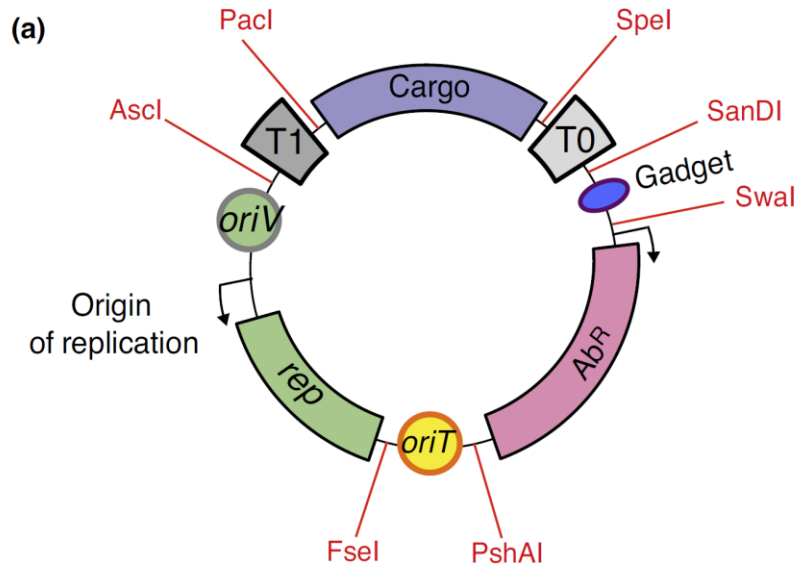[g] Dicyclopropyl ketone.
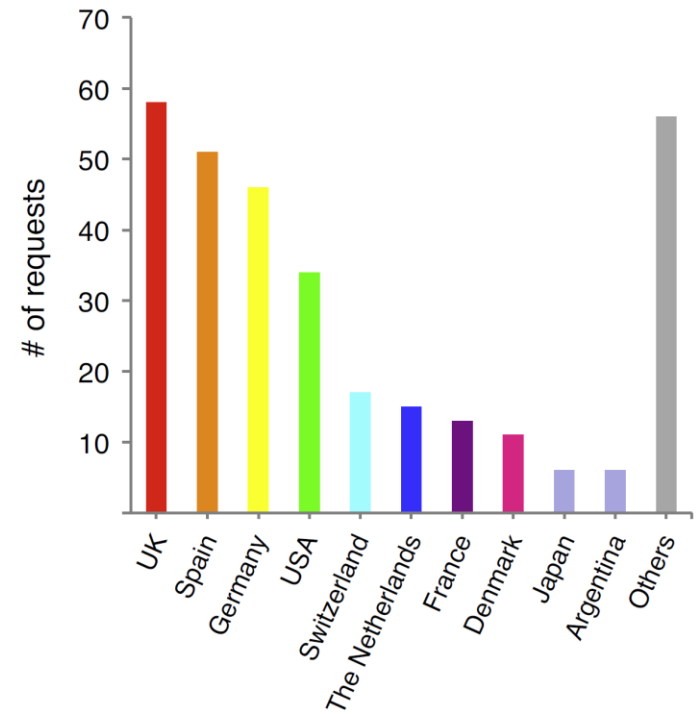[h] Intake mechanism into *Pseudomonas* unknown.
[i] Alternative.

Standardizace vektorů - SEVA

- Standard European Vector Architecture

Current Opinion in Biotechnology

Current Opinion in Biotechnology

No.	Ori V
1	R6K
2	RK2
3	pBBR1
4	pRO1600/ColE1
5	RSF1010
6	p15A
7	pSC101
8	pUC
9	pBBR322/ROP

No.	Antb
1	Ap
2	Km
3	Cm
4	Sm/Sp
5	Tc
6	Gm

No.	Cargo
1	MCS-default
2	*lacZα*-pUC19
3	*lacZα*-pUC18
4	*lacI^q*-Ptrc
5	*lacZ*
6	*luxCDABE*
7	*GFP*
8	*xylS-Pm*
9	*alkS-Palk*
10	*araC-Pbad*
11	*chnR-PchnB*
12	*cprK1-PDB3*
13	PEM7

No.	Gadget
α	*hok-sok*

pSEVA **X X X X**

Antibiotic	Replication	Cargo	Gadget
9 (A)	9 (C)	15 (-n)	α
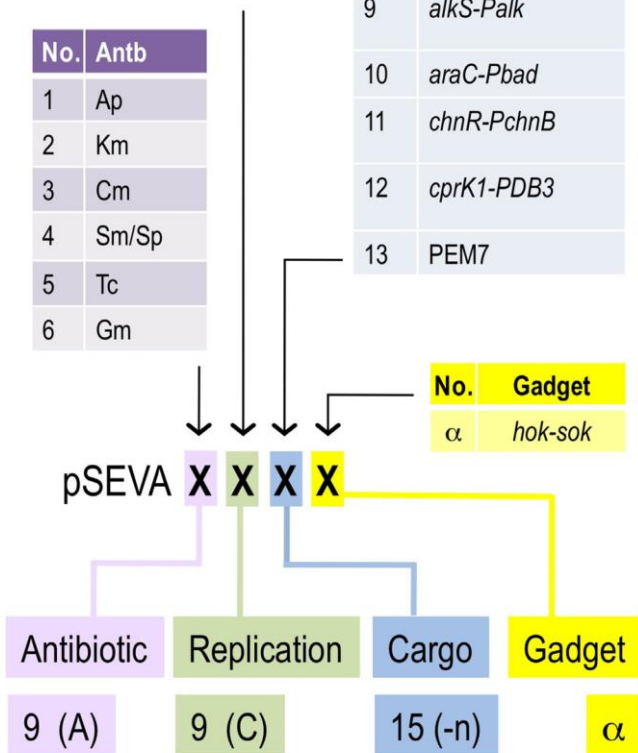
Figure 2. Expanded SEVA nomenclature. Vectors include four modules (antibiotic resistance marker, replication origin, cargo and gadget), which are represented in a code with four unequivocal positions. The *first* position is for antibiotic resistance markers, numbered 1 to 9 for the first nine and then 9B to 9Z for the next ones. The *second* position (the plasmid origin of replication) also receive a 1 to 9 code for the first variants and 9B to 9Z for those that follow. The cargo (*third* position), each cargo is assigned a sole number 1 to *n,* but the figure can be added with a capital letter in cases of variants of the same module. The *fourth* position is kept for the gadgets, which receive Greek letters (α to ω). See text for rationale and detailed explanation.

Cyanobakterie

- Fixace CO_2, fototrofní nebo heterotrofní

- Produkce barviv a biopaliv
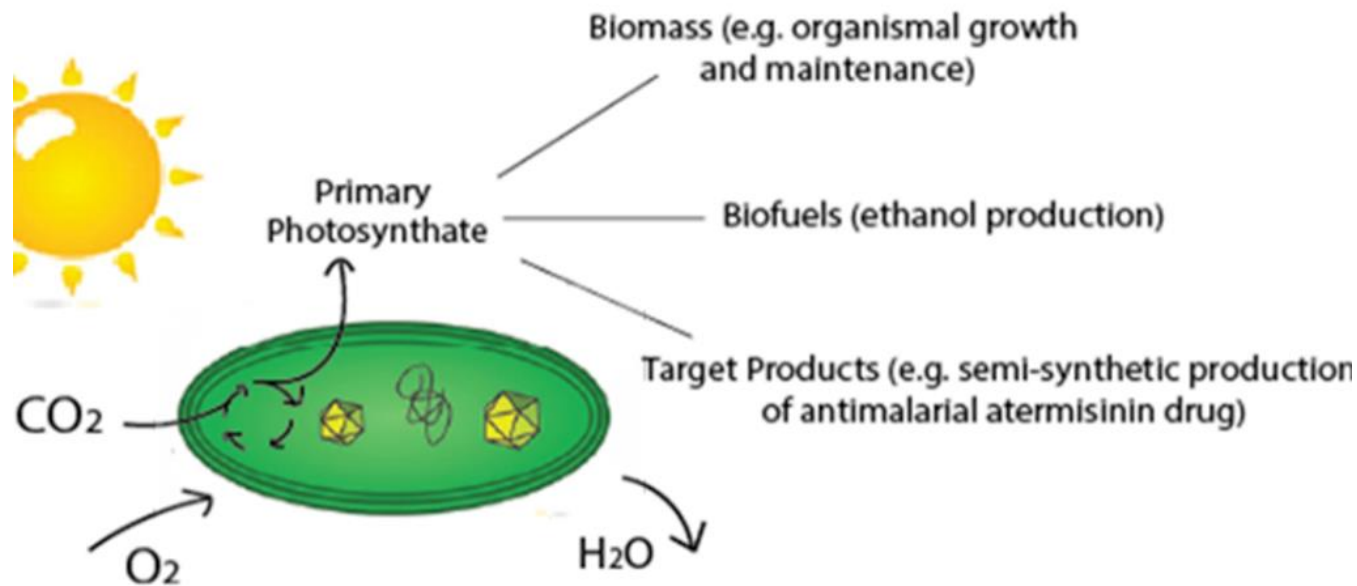
Cyanobakterie

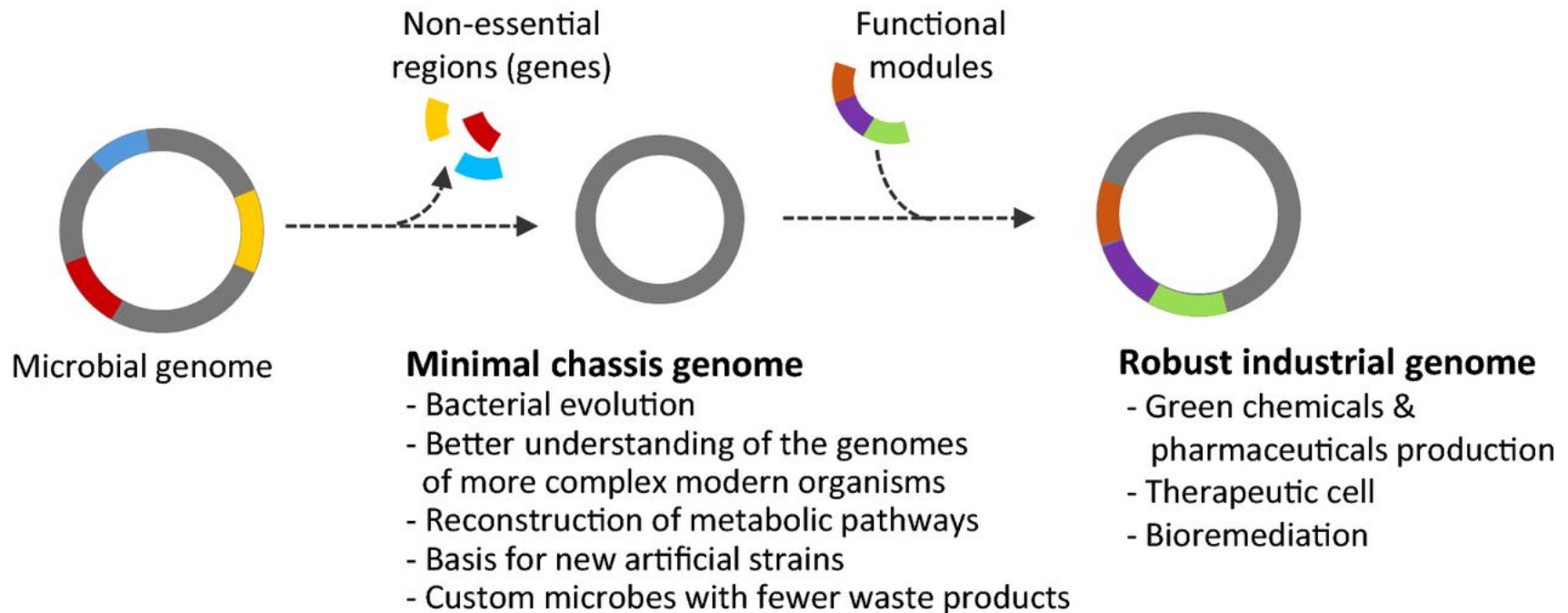| Strain	Genetic methods	Ideal growth temp (C)	Doubling time (h)	Metabolisms	Genome-scale models?	Notes	References
Synechocystis sp. PCC 6803	Conjugation, natural transformation, Tn5 mutagenesis, fusion PCR	30	6–12	Mixotrophic, autotrophic	Yes	Extensive systems biology datasets are available	Heidorn et al., 2011
Synechococcus elongatus PCC 7942	Conjugation, natural Transformation, Tn5 mutagenesis	38	12–24	Autotrophic	No	A model strain for the study of circadian clocks	Chen et al., 2012
Synechococcus sp. PCC 7002	Conjugation, natural transformation	38	3.5	Mixotrophic, autotrophic	Yes	Among the fastest-growing strains known	Xu et al., 2011
Anabaena variabilis PCC 7120	Conjugation, natural transformation	30	>24	Mixotrophic, autotrophic	No	Nitrogen-fixing, Filamentous	Zhang et al., 2007
Leptolyngbya sp. Strain BL0902	Conjugation, Tn5 mutagenesis	30	~20	Autotrophic	No	Filamentous, Grows well in outdoor photo-bioreactors in a broad range of conditions	Taton et al., 2012

Další organizmy v syntetické biologii

Table 1. Alternative chassis used in Synthetic Biology applications

Organism	Applications	Lifestyle
Clostridium acetobutylicum	Biofuels, flavouring, cosmetics, plasticisers	Anaerobic
Klebsiella spp.	Biofuel production	Facultatively anaerobic
Pseudomonas putida	Bioremediation, small molecules and bioplastics production	Aerobic
Streptomyces spp.	Antibiotic, secondary metabolite and protein production.	Aerobic
Shewanella oneidensis	Electricity production	Facultatively anaerobic
Geobacter sulfurreducens	Electricity production	Anaerobic
Bacteroides thetaiotaomicron	Control of gut microbiota	Anaerobic
Synechocystis spp.	Biofuels, small molecules production fixing CO_2	Photosynthetic
Deinococcus radiodurans	Bioremediation and small molecule production under stress	Aerobic
Mycoplasma spp.	Minimal genome engineering and vaccine	Facultative anaerobic

Current Opinion in Chemical Biology
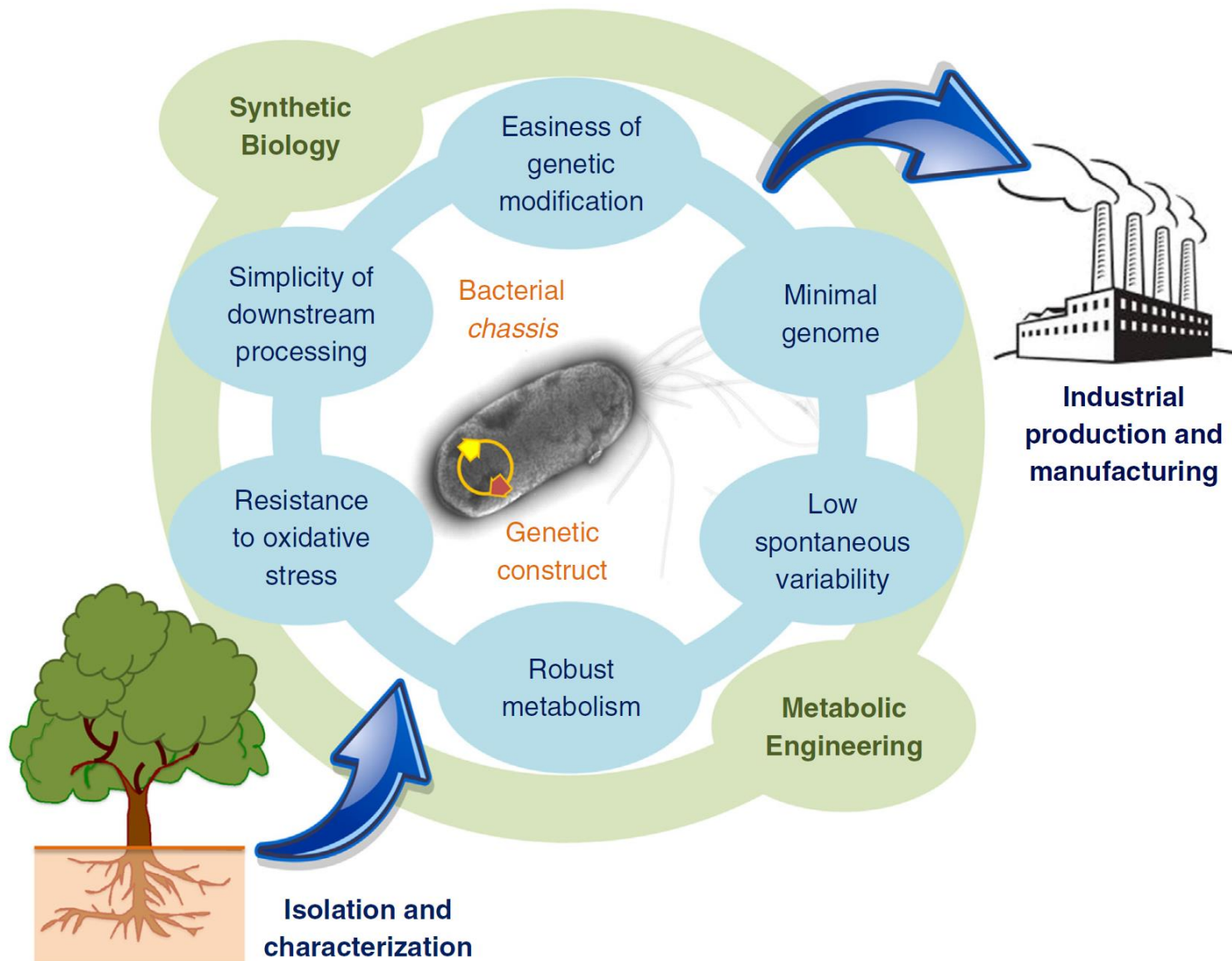
Minimální genom

- Genomy běžně používaných organizmů jsou zatíženy přítomností genů nevyužitelných v biotechnologiích

Non-essential regions (genes)

Functional modules

Microbial genome

Minimal chassis genome
- Bacterial evolution
- Better understanding of the genomes of more complex modern organisms
- Reconstruction of metabolic pathways
- Basis for new artificial strains
- Custom microbes with fewer waste products

Robust industrial genome
- Green chemicals & pharmaceuticals production
- Therapeutic cell
- Bioremediation

Minimální genom

Major bacterial species subject to genome reduction efforts

Species	Purpose	Technology
Escherichia coli	Optimization of Cell Factories	Double-stranded break repair
	Genome structure and organization	Intra-genomic recombination
	Exploration of essential genes	Surrogate editing in yeast
	Determination of minimal genetic complement	Cas9-facilitated recombination
	Multiplexing genome editing	CRISPR/Cas9-based editing
	Codon emancipation	Multiplexed genome engineering
Mycoplasma genitalium	Programmable Cells	Chemical genome synthesis
Mycoplasma mycoides	Reaching the limits of live systems	Chemical genome synthesis
Bacillus subtillis	Increasing Cell Factory predictability	Forced intra-genomic recombination
Corynebacterium glutamicum	Improving genetic stability and Cell Factory productivity	Forced intra-genomic recombination
Pseudomonas putida	Enhancing heterologous expression and redox catalysis	Double-stranded break repair
	Improving genetic programmability	Transposon-mediated random deletions
Streptomyces avermitilis	Expression of heterologous pathways	Homologous recombination and/or Cre/loxP-based recombination
Vibrio natriegens	Optimization of SynBio chassis	Chemical synthesis, recombination, CRISPR/Cas9-based manipulations

Minimální genom

Design and synthesis of a minimal bacterial genome

Clyde A. Hutchison III,[1]*† Ray-Yuan Chuang,[1]†‡ Vladimir N. Noskov,[1] Nacyra Assad-Garcia,[1] Thomas J. Deerinck,[2] Mark H. Ellisman,[2] John Gill,[3] Krishna Kannan,[3] Bogumil J. Karas,[1] Li Ma,[1] James F. Pelletier,[4]§ Zhi-Qing Qi,[3] R. Alexander Richter,[1] Elizabeth A. Strychalski,[4] Lijie Sun,[1]|| Yo Suzuki,[1] Billyana Tsvetanova,[3] Kim S. Wise,[1] Hamilton O. Smith,[1,3] John I. Glass,[1] Chuck Merryman,[1] Daniel G. Gibson,[1,3] J. Craig Venter[1,3]*

We used whole-genome design and complete chemical synthesis to minimize the 1079–kilobase pair synthetic genome of *Mycoplasma mycoides* JCVI-syn1.0. An initial design, based on collective knowledge of molecular biology combined with limited transposon mutagenesis data, failed to produce a viable cell. Improved transposon mutagenesis methods revealed a class of quasi-essential genes that are needed for robust growth, explaining the failure of our initial design. Three cycles of design, synthesis, and testing, with retention of quasi-essential genes, produced JCVI-syn3.0 (531 kilobase pairs, 473 genes), which has a genome smaller than that of any autonomously replicating cell found in nature. JCVI-syn3.0 retains almost all genes involved in the synthesis and processing of macromolecules. Unexpectedly, it also contains 149 genes with unknown biological functions. JCVI-syn3.0 is a versatile platform for investigating the core functions of life and for exploring whole-genome design.
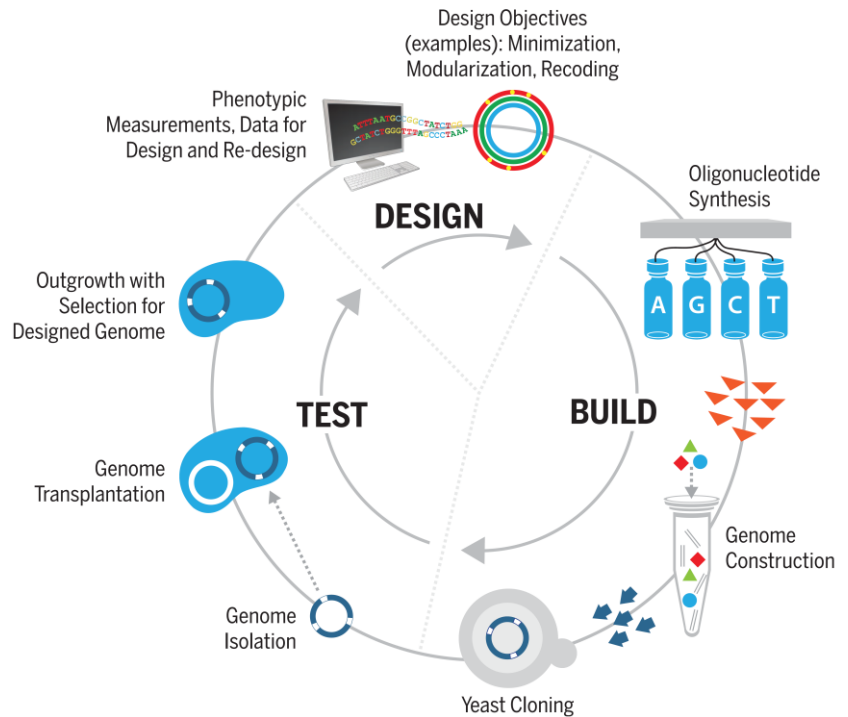

Fig. 1. The JCVI DBT cycle for bacterial genomes. At each cycle, the genome is built as a centromeric plasmid in yeast, then tested by transplantation of the genome into an *M. capricolum* recipient. In this study, our main design objective was genome minimization. Starting from syn1.0, we designed a reduced genome by removing nonessential genes, as judged by global Tn5 gene disruption. Each of eight reduced segments was tested in the context of a seven-eighths syn1.0 genome and in combination with other reduced segments. At each cycle, gene essentiality was reevaluated by Tn5 mutagenesis of the smallest viable assembly of reduced and syn1.0 segments that gave robust growth.

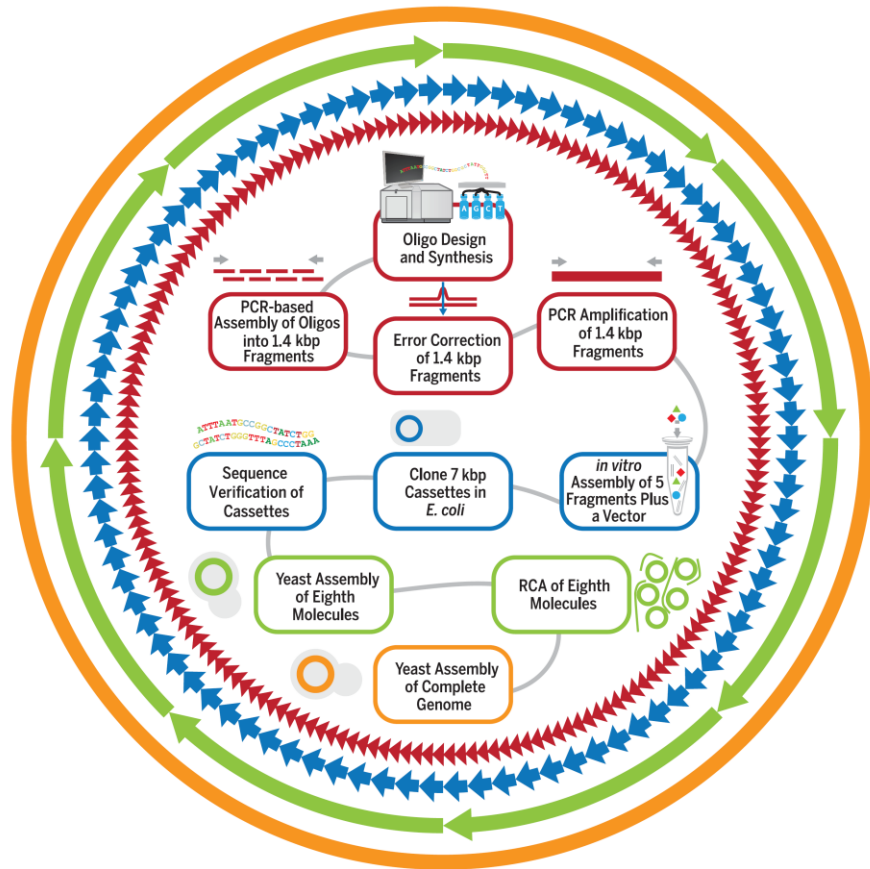# Konstrukce minimálního genomu

Fig. 2. Strategy for whole-genome synthesis. Overlapping oligonucleotides (oligos) were designed, chemically synthesized, and assembled into 1.4-kbp fragments (red). After error correction and PCR amplification, five fragments were assembled into 7-kbp cassettes (blue). Cassettes were sequence-verified and then assembled in yeast to generate one-eighth molecules (green). The eight molecules were amplified by RCA and then assembled in yeast to generate the complete genome (orange).
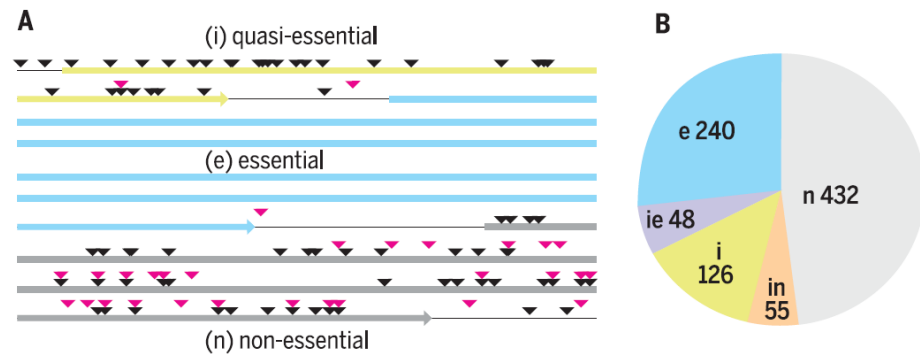

Fig. 3. Classification of gene essentiality by transposon mutagenesis. (A) Examples of the three gene classifications, based on Tn5 mutagenesis data. The region of syn1.0 from sequence coordinates 166,735 to 170,077 is shown. The gene MMSYN1_0128 (lime arrow) has many P0 Tn5 inserts (black triangles) and is an i-gene (quasi-essential). The next gene, MMSYN1_0129 (light blue arrow), has no inserts and is an e-gene (essential). The last gene, MMSYN1_0130 (gray arrow), has both P0 (black triangles) and P4 (magenta triangles) inserts and is an n-gene (nonessential). Intergenic regions are indicated by black lines. (B) The number of syn1.0 genes in each Tn5-mutagenesis classification group. The n- and in-genes are candidates for deletion in reduced genome designs.

Fig. 6. Partition of genes into four major functional groups.
Syn3.0 has 473 genes. Of these, 79 have no assigned functional category (Table 1). The remainder can be assigned to four major functional groups: (i) expression of genome information (195 genes); (ii) preservation of genome information (34 genes); (iii) cell membrane structure and function (84 genes); and (iv) cytosolic metabolism (81 genes). The percentage of genes in each group is indicated.
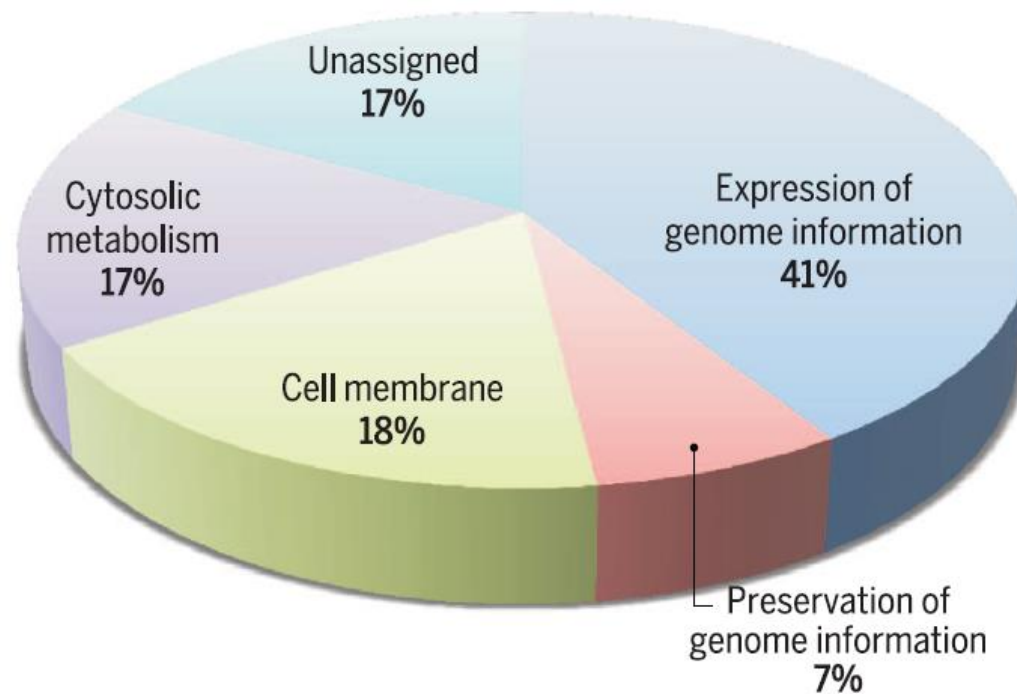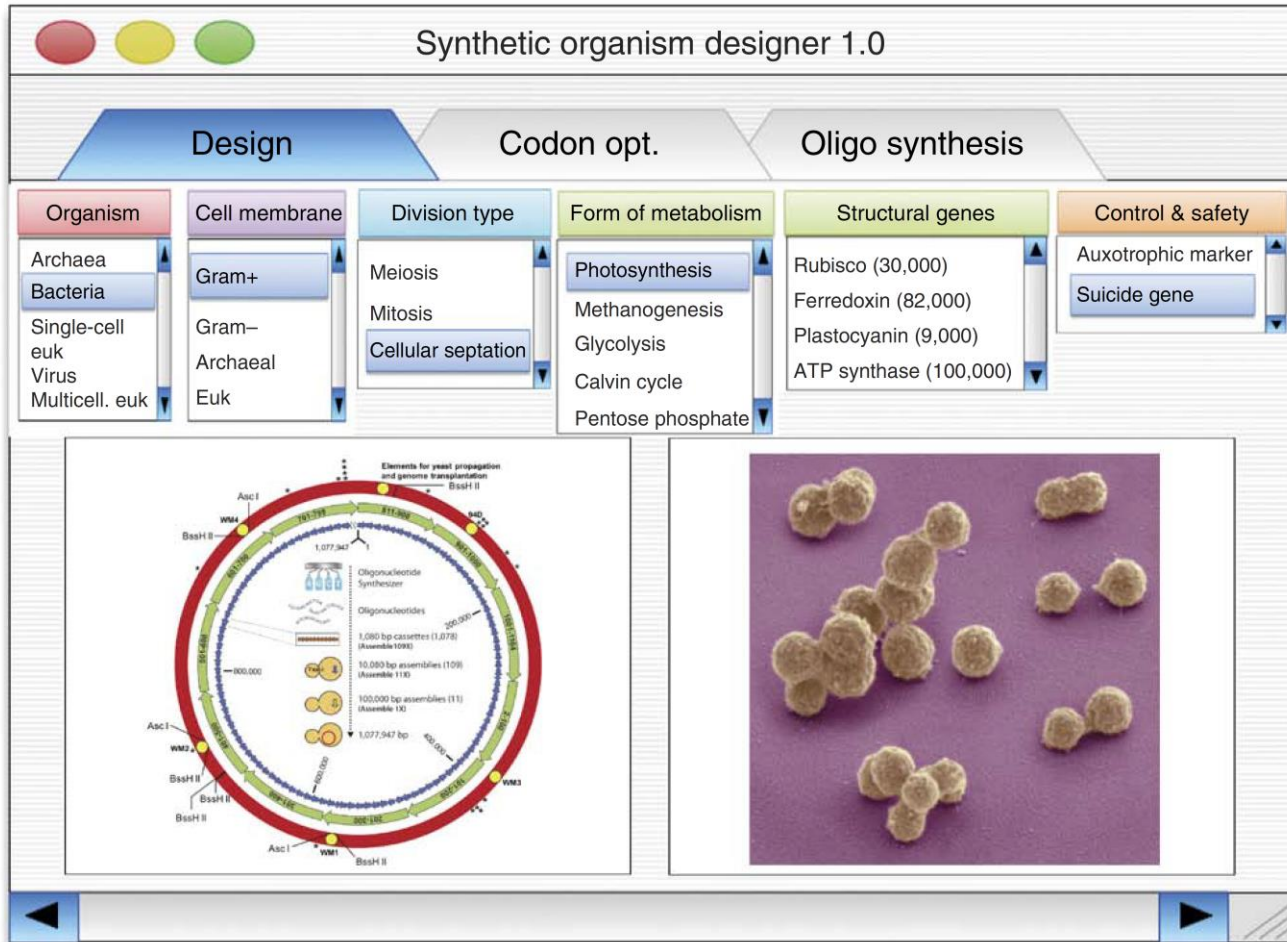
Budoucnost inženýrství organizmů

Figure 4 | A futuristic vision for designíng synthetic organisms on demand.

Aplikace syntetické biologie

- Bioremediace organických polutantů

- Biodegradace plastů

- Produkce farmak

- Produkce vzácných chemických látek

- Příprava biosenzorů

- Produkce biopaliv

- Personalizovaná medicína

Bioremediace

- Degradace organických polutantů

- Akumulace těžkých kovů v rostlinách

- Likvidace ropných havárií
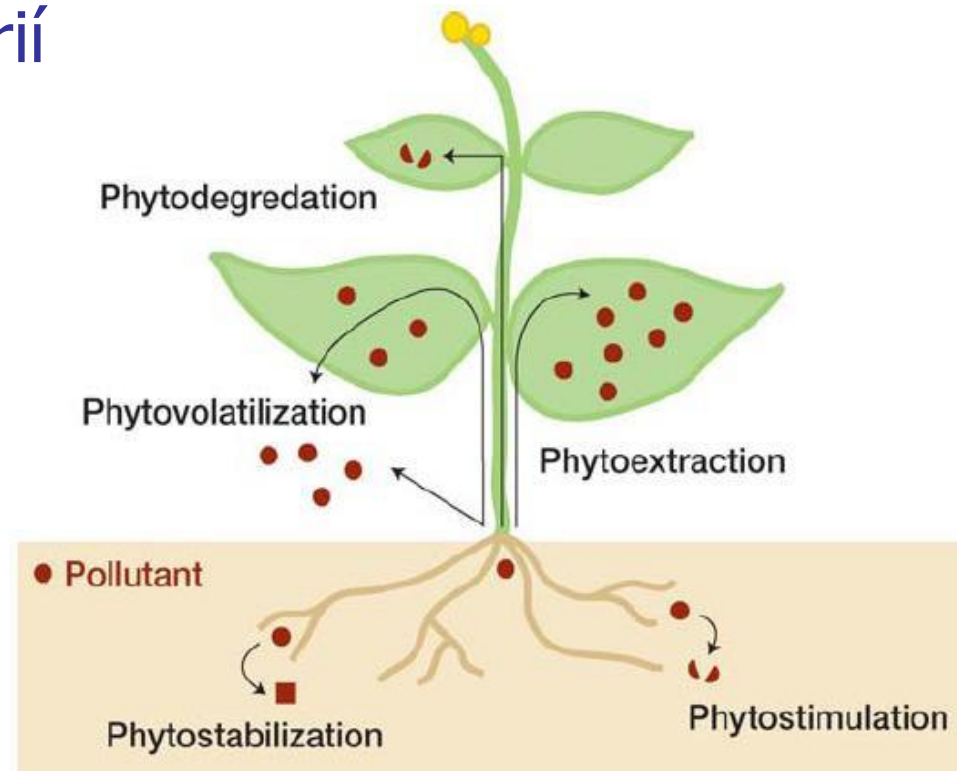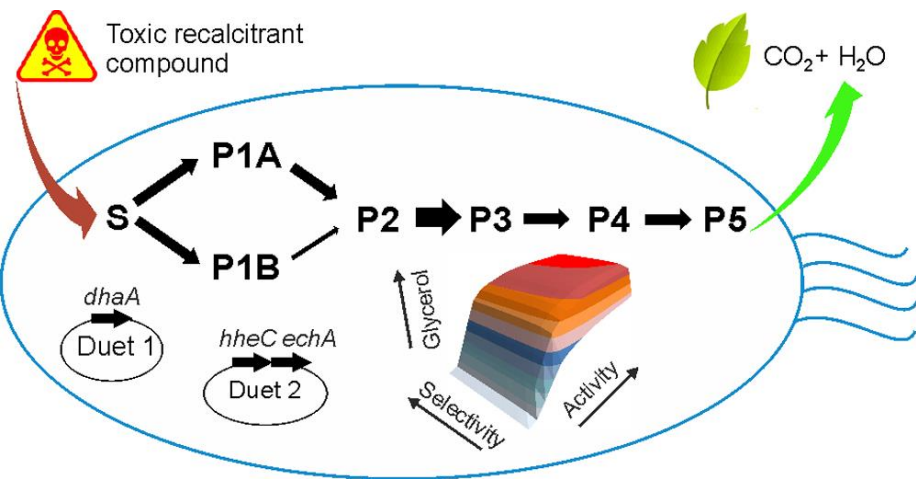
Degradace 1,2,3-trichlorpropanu
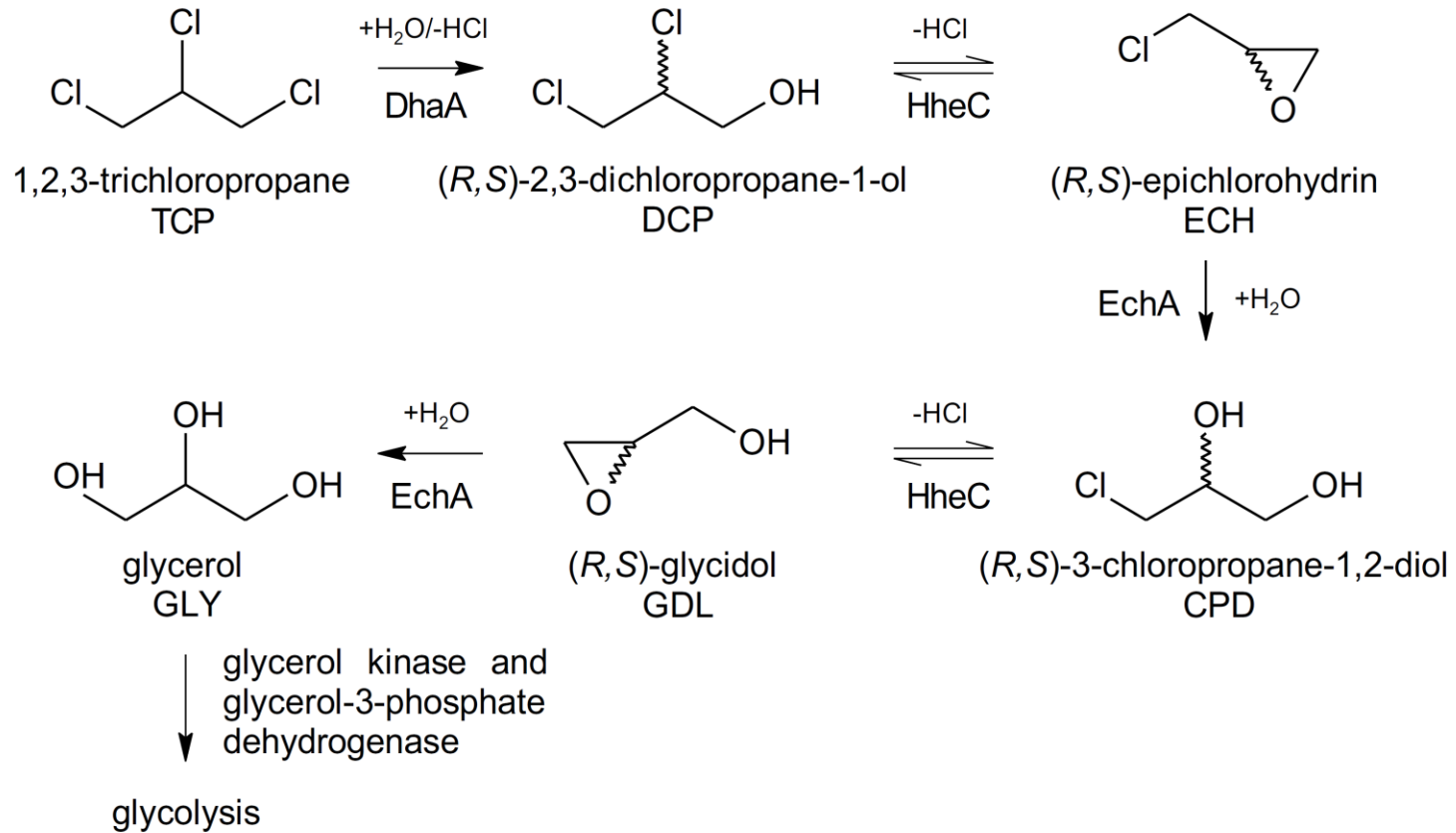
Scheme 1 Five-step biotransformation of 1,2,3-trichloropropane into glycerol by the enzymes of the synthetic biodegradation pathway. The pathway consists of haloalkane dehalogenase DhaA from *Rhodococcus rhodochrous* NCIMB 13064 [33] with the haloalcohol dehalogenase HheC and epoxide hydrolase EchA from *Agrobacterium radiobacter* AD1 [34, 35]. Computer-assisted protein engineering was used to improve activity of the haloalkane dehalogenase towards 1,2,3-trichloropropane, leading to the development of the 32-fold more active and 26-fold more efficient mutant DhaA31 [31]. Formed glycerol can be utilized in central catabolic pathways of the host cell
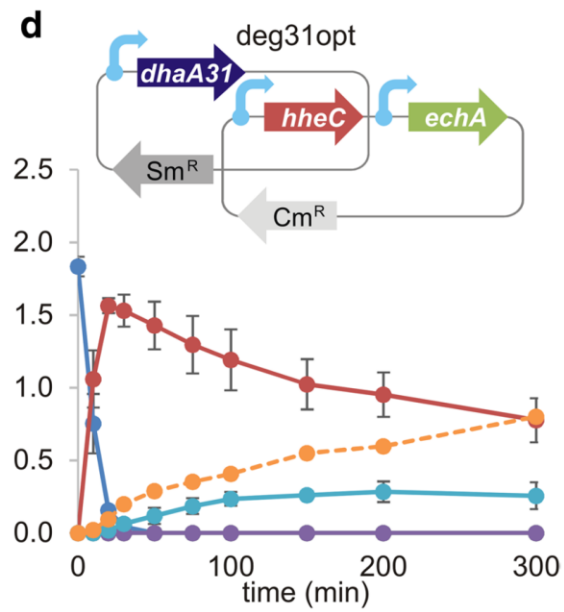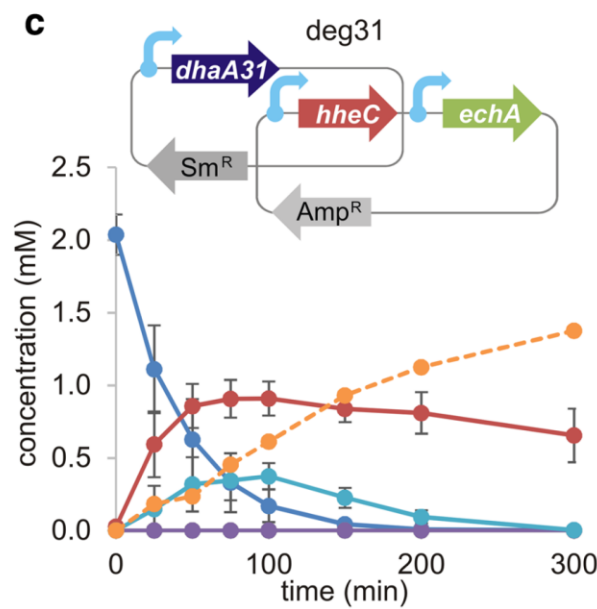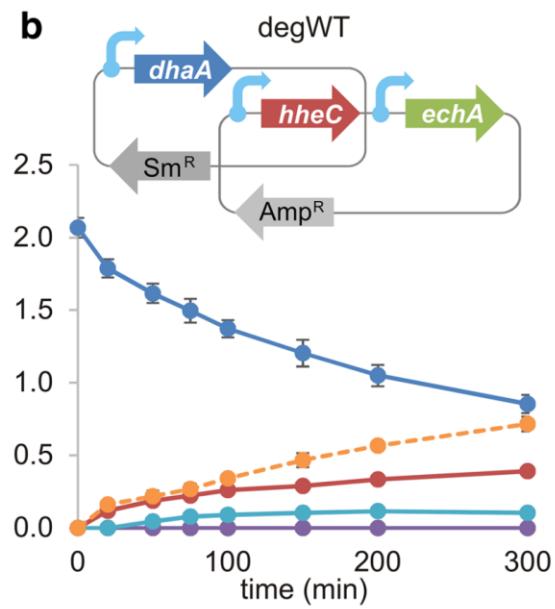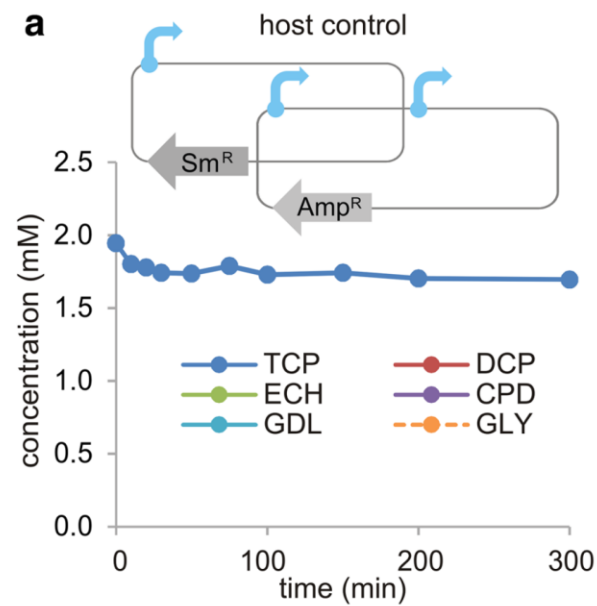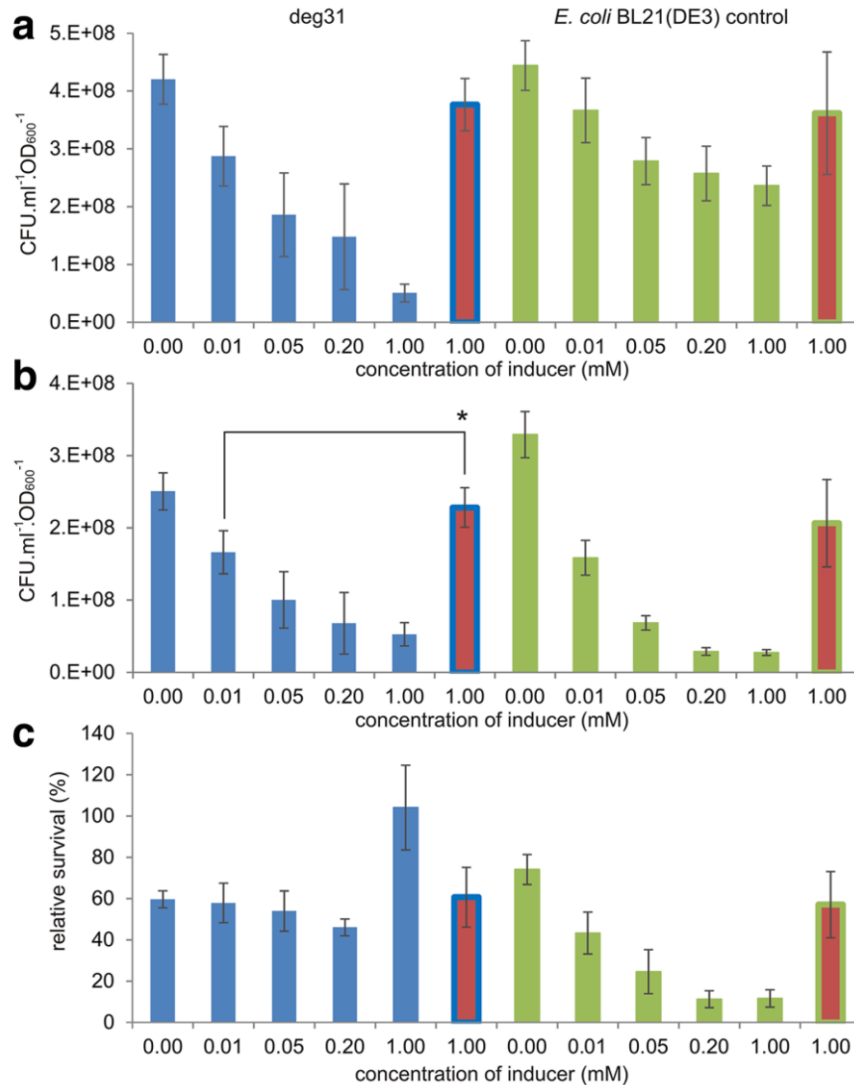
Efekt induktoru na viabilitu

Fig. 4 Viability of *Escherichia coli* deg31 and host control strains after pre-induction with diverse concentrations of IPTG or 1 mM lactose, before and after incubation with TCP. **a** Viability of deg31 and *E. coli* BL21(DE3) with the empty pETDuet and pCDF plasmids as determined by plating of cells pre-induced with different concentrations of IPTG or 1 mM lactose (*red columns*) before incubation in phosphate buffer with TCP. **b** Viability of cells after incubation in buffer with 2 mM TCP. *Asterisks* denote significantly higher (at $P < 0.05$) cell count of deg31 pre-induced with 1 mM lactose when compared with the count of cells pre-induced with the lowest tested concentration of IPTG (0.01 mM). **c** Fraction of surviving cells calculated as the difference in the $CFUs.ml^{-1}.OD_{600}^{-1}$ before and after incubation with TCP. *Error bars* represent standard deviations calculated from at least four independent experiments. *CFU* colony forming units
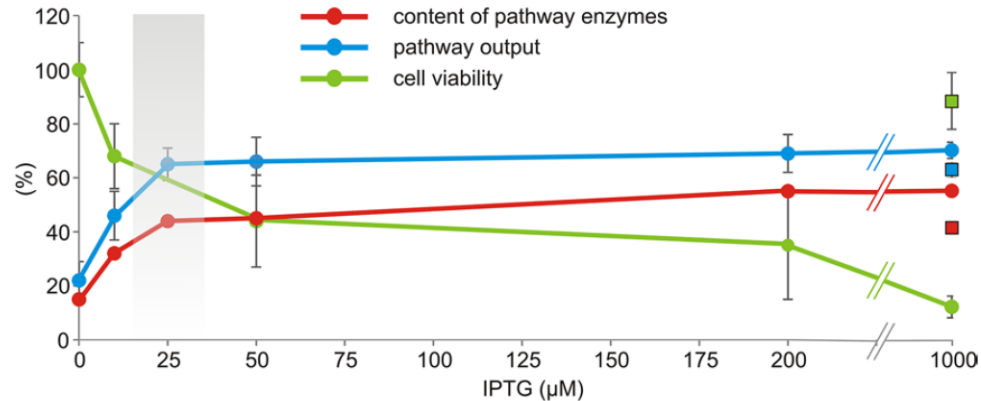
Optimalizace indukce

Fig. 5 Summarized effects of IPTG concentration on gene expression levels, pathway output, and cell viability in pre-induced *Escherichia coli* deg31 cells. Viability was determined by plating pre-induced deg31 cells resuspended in phosphate buffer before incubation with TCP. Pathway output was expressed as the theoretical conversion of TCP into glycerol at the end of 5 h degradation experiments with pre-induced, resting deg31 cells (see also Additional file 1: Fig. S5). The content of TCP pathway enzymes was estimated by analyzing cell-free extracts obtained from pre-induced cells by sodium dodecyl sulfate polyacrylamide gel electrophoresis (Additional file 1: Fig. S7 and Table S1). Two gels were analysed by densitometry and mean values are shown. *Error bars* represent standard deviations calculated from three independent experiments. Values determined for deg31 pre-induced with 1 mM lactose are indicated by *squares*

Bioremediace

- Halogenalkany

- Polychlorované bifenyly

- Rezidua farmakologických látek

- Těžké kovy

- Ropné produkty

- Plasty

Biosenzory/diagnostika

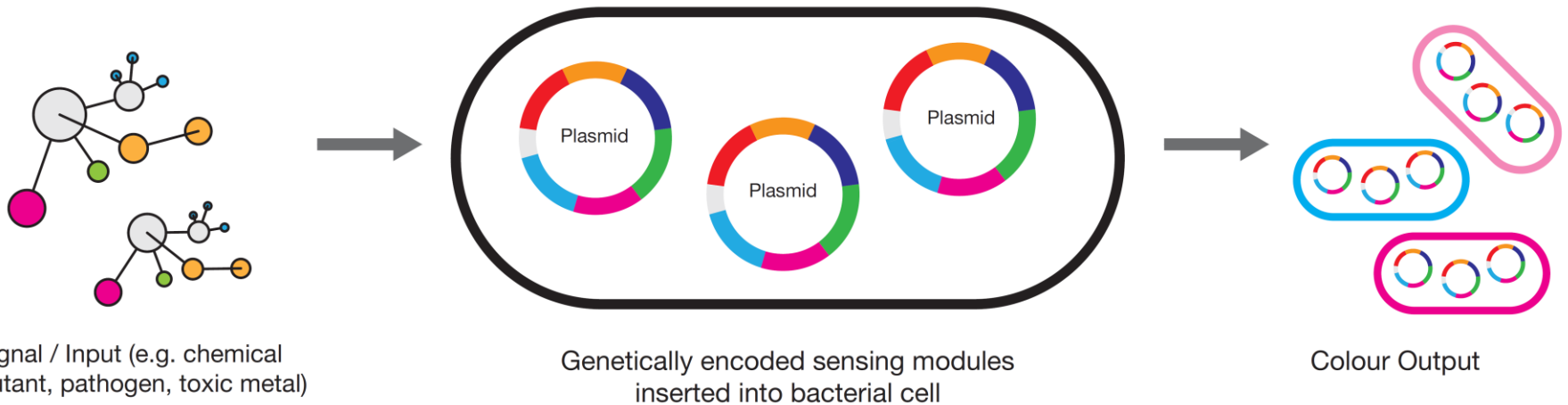- Exprese specifických genů při kontaktu s patogenem nebo chemickou látkou

Figure 1: Principle of whole-cell biosensors. Adapted from Goers, L et al (2013) 'Engineering Microbial Biosensors'. in Harwood, C, Wipat, A. (eds) Microbial Synthetic Biology. Academic Press: Burlington.
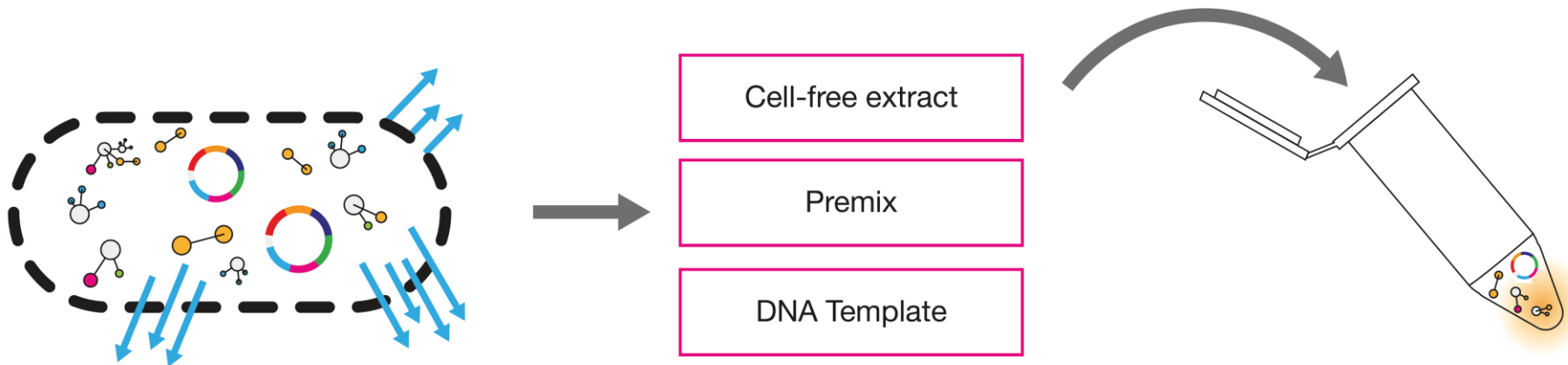
Biosenzory/diagnostika

- Bezbuněčné senzory

Figure 2: Cell-free biosensors made from lysed cells have three essential components: A cell-free extract containing machinery for transcription and/or translation; A premix containing buffers, energy and resources (cofactors, amino acids); and DNA templates from plasmids or PCR products. Adapted from presentation given by Paul Freemont. See also, Lu (2017) Cell-free synthetic biology: Engineering in an open world. Synthetic and systems biology. http://dx.doi.org/10.1016/j.synbio.2017.02.003
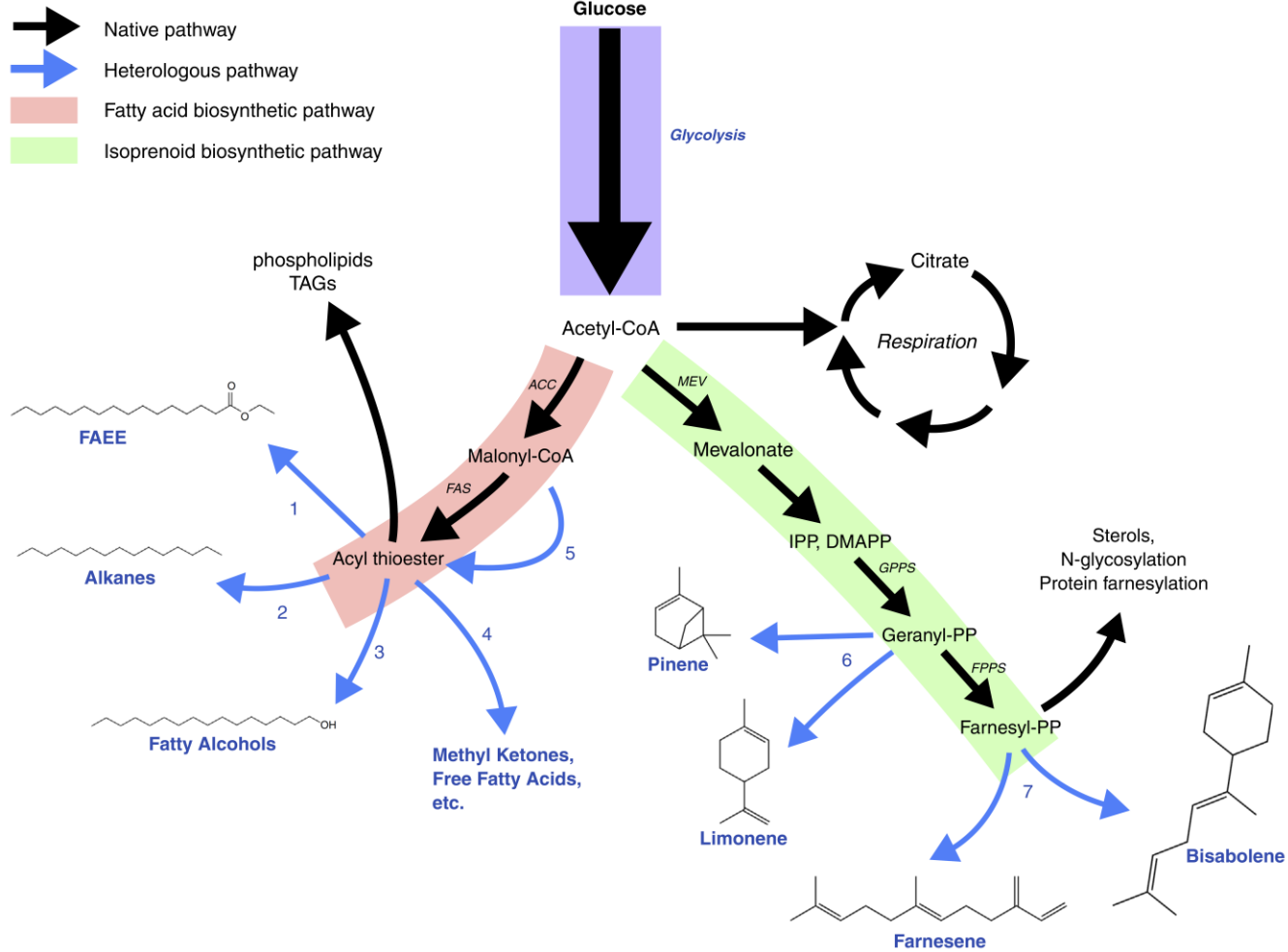
Biopaliva
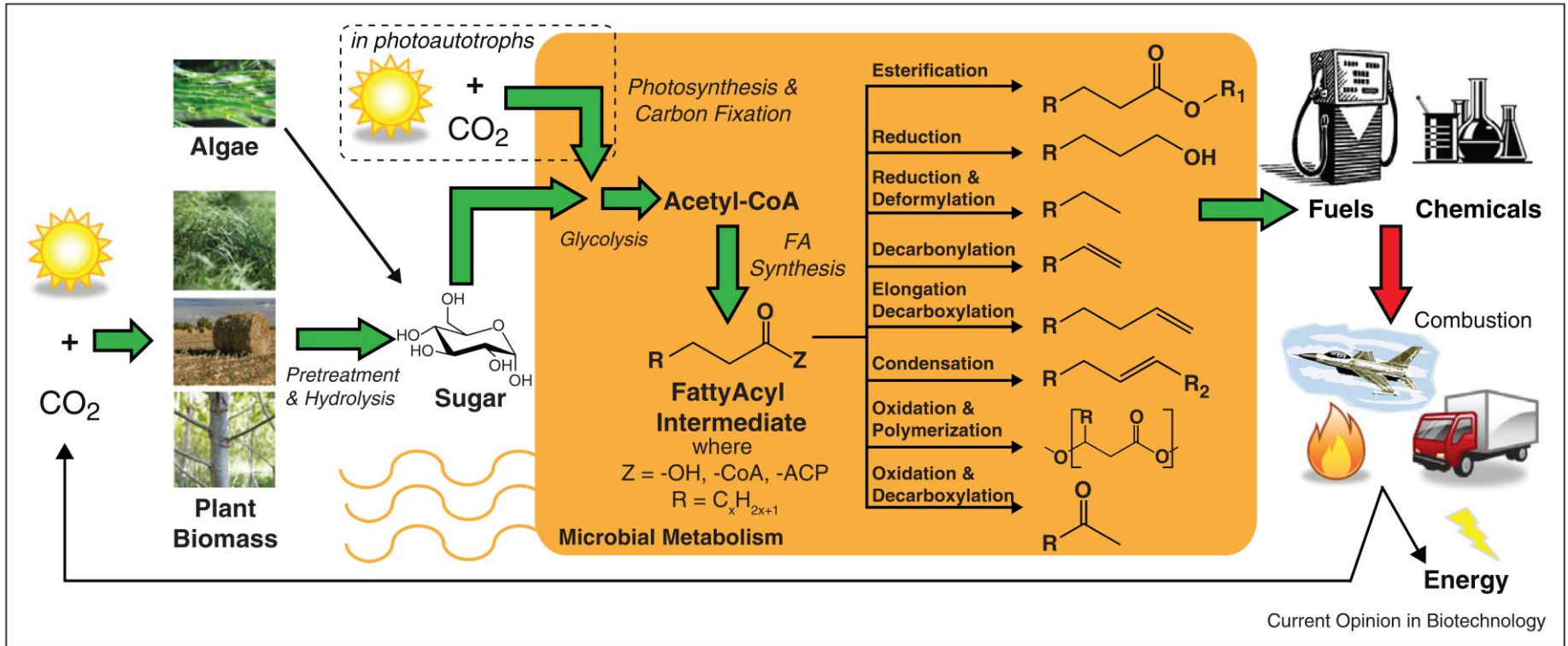
Sustainable production of fatty acid-derived fuels and chemicals. The process begins with the conversion of photosynthetic biomass (e.g. plants or algae) to fermentable intermediates that can be catabolized by cells to generate energy and the building blocks (e.g. acetyl-CoA) required to synthesize anabolic products, including fatty acids. Alternatively, these same chemicals can be derived directly from photosynthesis and carbon fixation. Acetyl-CoA is the direct precursor to fatty acid biosynthetic intermediates that are the starting point for a wide range of chemical products. These products can be used as fuels or chemicals. Upon combustion, the fatty acid-derived products release energy and carbon dioxide that can be recycled to close the carbon cycle.

Biodiesel
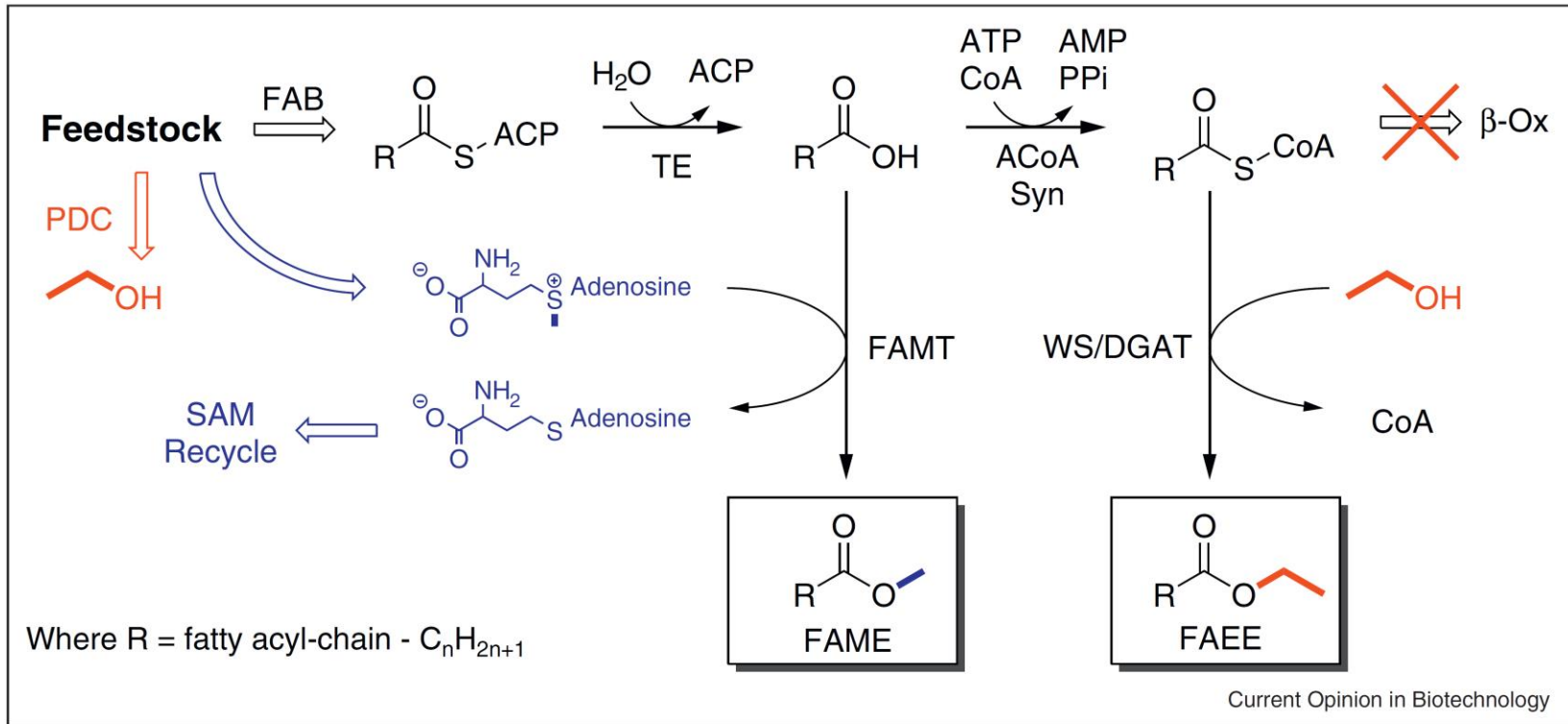
Metabolic pathways for synthesis of biodiesel. Microbes use sugars and other renewable feedstocks to produce a wide range of metabolites including ethanol, S-adenosylmethionine (SAM), and fatty acids. Fatty acid metabolism is used to produce long chain acyl-ACP thioesters where typically $R = C_{15}H_{31}-C_{17}H_{35}$. These acyl-chains which are natively incorporated into membrane lipids can be hydrolyzed by thioesterases (TE) to produce free fatty acids (FFAs). FFAs are the substrate of fatty acid O-methyltransferase (FAMT) which adds a methyl group from SAM to yield fatty acid methyl esters (FAME). Alternatively, FFA can be activated to acyl-CoA thioesters by acyl-CoA synthetases (ACoA Syn). These thioesters can be broken down for energy by the β-oxidation (β-ox) pathway that is often disrupted in engineered strains. Acyl-CoAs can be transesterified by wax ester synthase/acyl-CoA:diacylglycerol acyltransferases (WS/DGAT) using ethanol to yield fatty acid ethyl-esters FAEE. The by-products (e.g. CoA and S-adenosyl-homocysteine) are recycled by additional pathways in the cell.

Biopaliva

Current Opinion in Chemical Biology

Biopaliva

Table 1

Yields of lipid fuels produced in engineered *E. coli* and *S. cerevisiae*.

Biofuel	Max theoretical yield[a] (g/g glucose)	Host	Titer (g/L)	Yield (g/g glucose)	Percent of max theoretical yield[c]	Reference
FFA (C16)	0.37	*E. coli*	5.2	0.26	70%	[49]
		S. cerevisiae	2.2	0.11	30%	[35•]
FAEE (C18)	0.36	*E. coli*	1.5	0.075	21%	[50]
		S. cerevisiae	0.034	0.0017	<1%	[51]
Fatty alcohol (C16)	0.34	*E. coli*	3.8[b]	0.13	38%	[22••]
		S. cerevisiae	0.33	0.017	5%	[18]
Alkanes (C15)	0.30	*E. coli*	0.08[b]	0.0027	<1%	[14]
		S. cerevisiae	0.0037	0.00019	<1%	[52]
Bisabolene (C15)	0.27	*E. coli*	1.1[c]	0.11	41%	[53•]
		S. cerevisiae	1.0[d]	0.050	19%	[30]

Titers and yields of current laboratory-scale demonstrations of selected microbial biofuels produced in *E. coli* and *S. cerevisiae*. In general, experiments were performed using 2% glucose in shake flask fermentations.

[a] Maximum theoretical yields are calculated using an *in silico* optimization algorithm employing a whole genome-scale reconstruction [54,55].

[b] This example used 3% glucose rather than 2%.

[c] This example used 1% glucose rather than 2%.

[d] This example used 1.8% galactose and 0.2% glucose.

Produkce vodíku

- Heterocyty tvořící cyanobakterie

By **nitrogenase** catalysed reaction:

$$N_2 + 8\,H^+ + 8\,e^- + 16\,ATP \xrightarrow{\text{nitrogenase}} 2\,[NH_4]^+ + H_2 + 16\,ADP + 16\,P_i$$
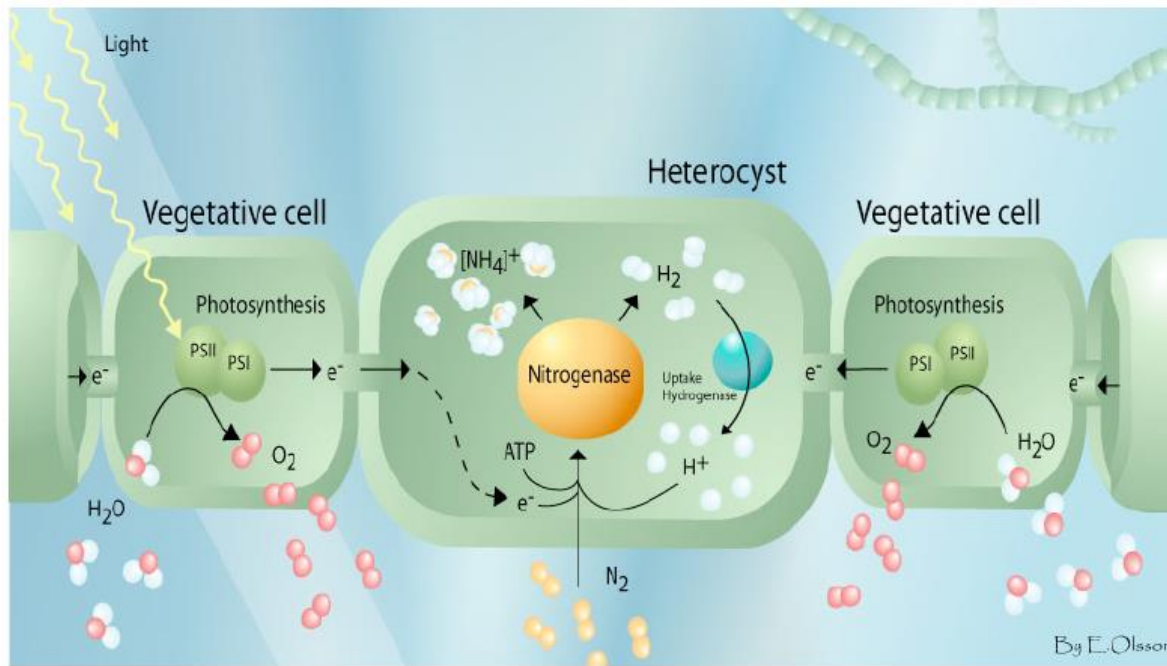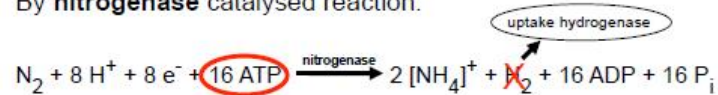
Produkce vodíku

- **Inženýrství cyanobakterií**
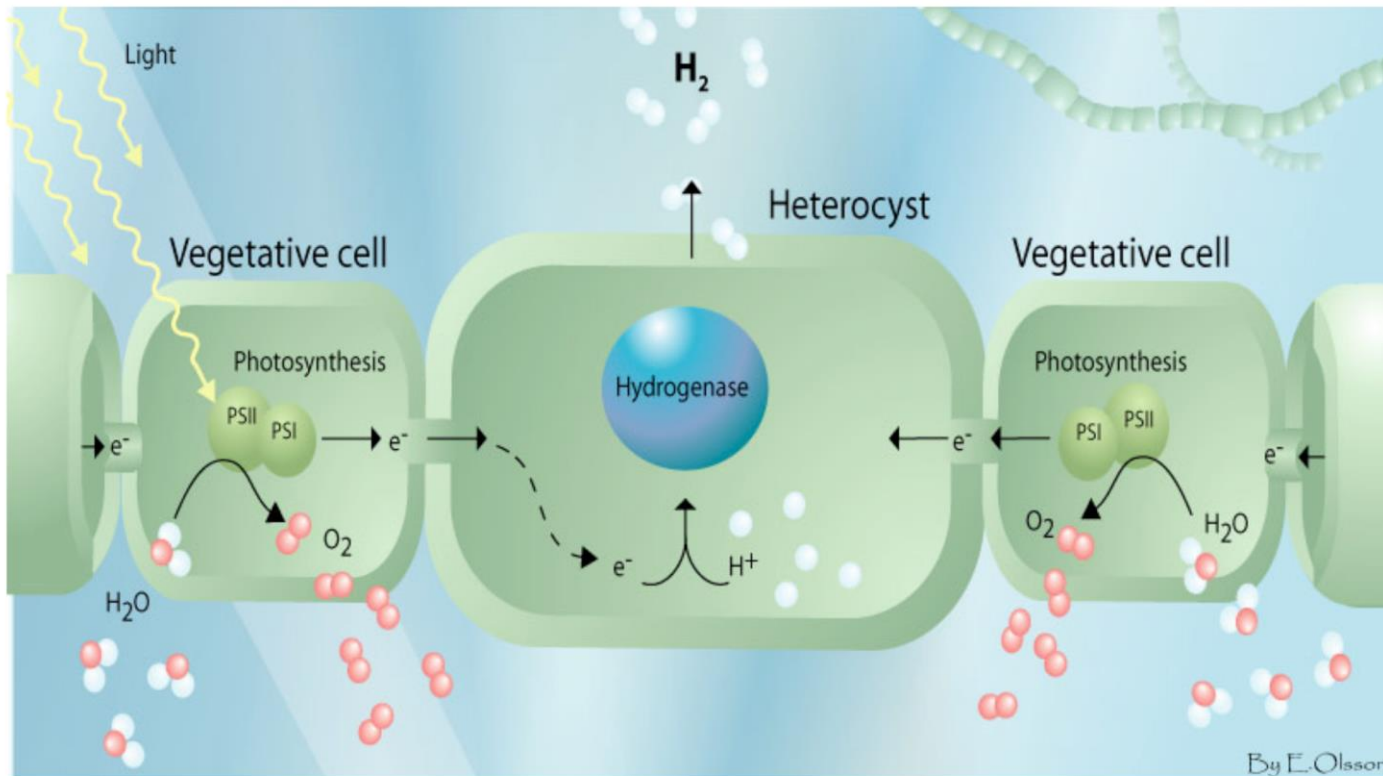
By **hydrogenase** catalysed reaction:
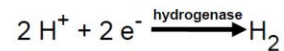
$$2\,H^+ + 2\,e^- \xrightarrow{\text{hydrogenase}} H_2$$

Produkce farmakologických látek

- Různé velikosti genových klastrů kódujících cílové molekuly
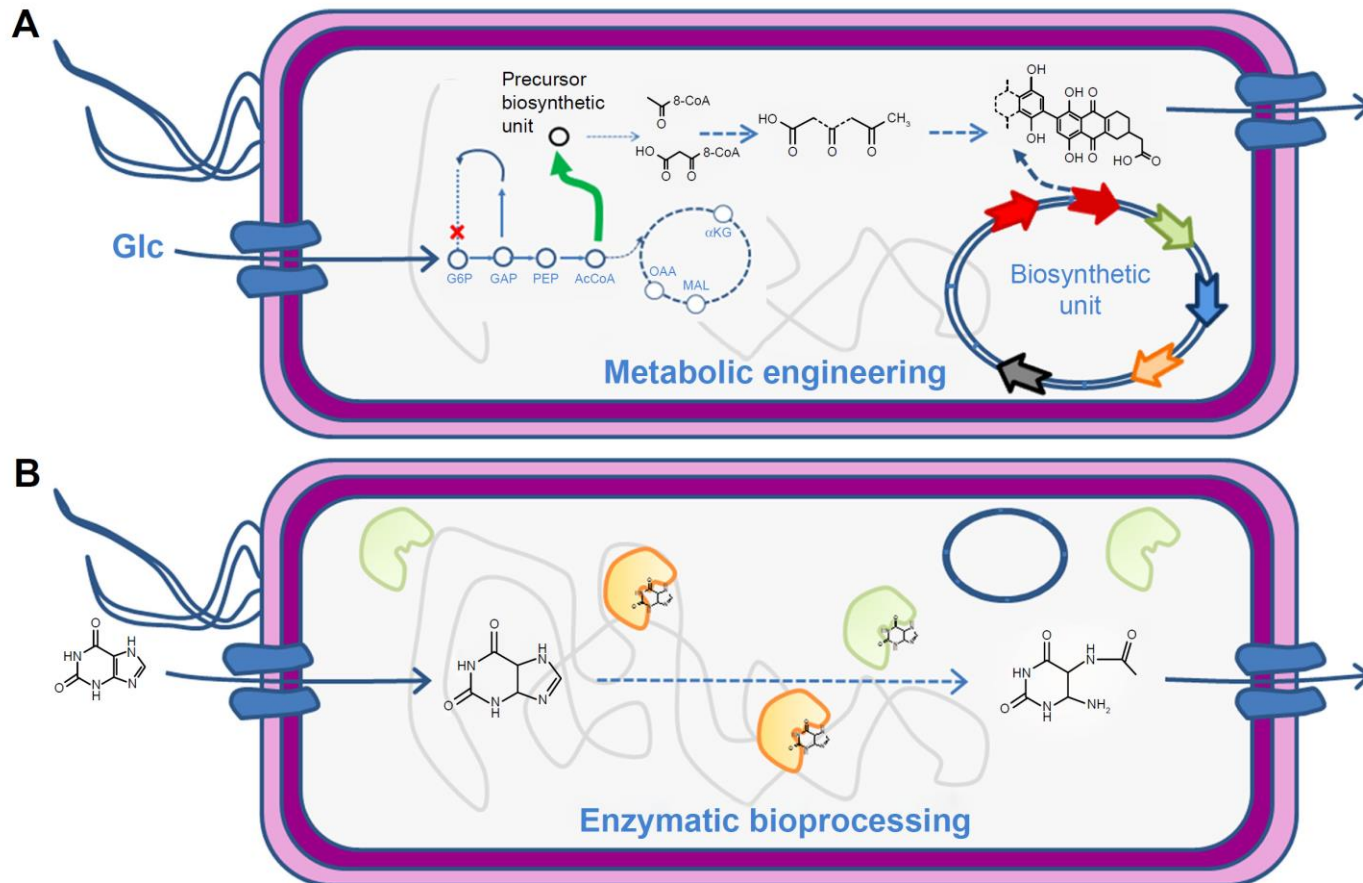
Příprava nových farmak

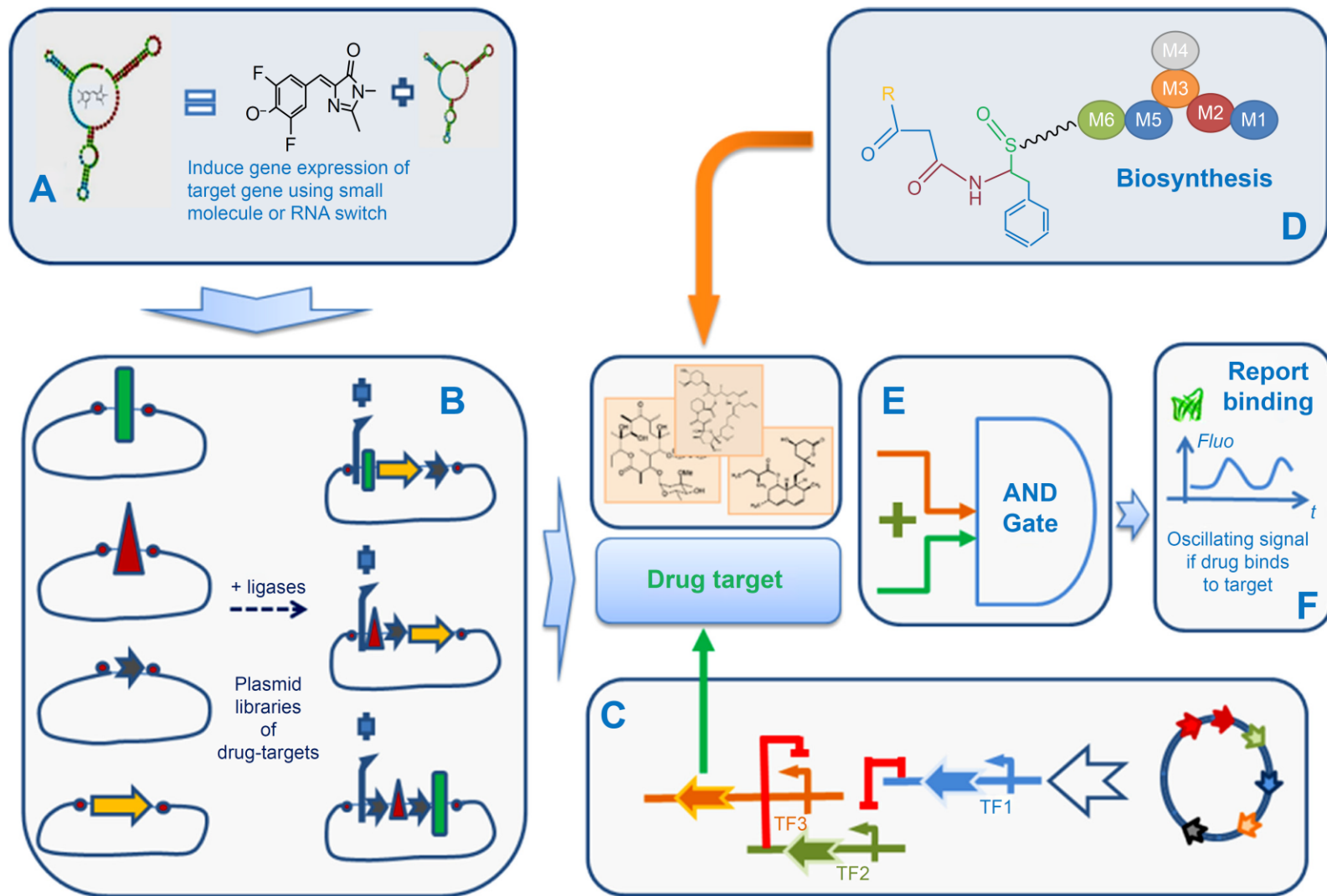- Metabolické inženýrství; úprava prekurzorů

Figure 5 Conceptual SB pipeline for drug screening in synthetic cell.

Notes: (**A**) A system to induce gene expression of protein target based on small molecule inducer and/or RNA-based switch for gene expression. (**B**) A combination of drug target from unique genes. (**C**) A genetic oscillator to focus readout on chosen drug target. (**D**) Generation of diverse drug candidate libraries from genetic shuffling of enzymatic modules. (**E**) A logical AND gate that gives output signal (**F**) if a drug candidate binds to biological target.

Abbreviations: SB, synthetic biology; Fluo, fluorescence signal; AND, logical AND gate; TF, transcription factor.

Quorum sensing v medicíně
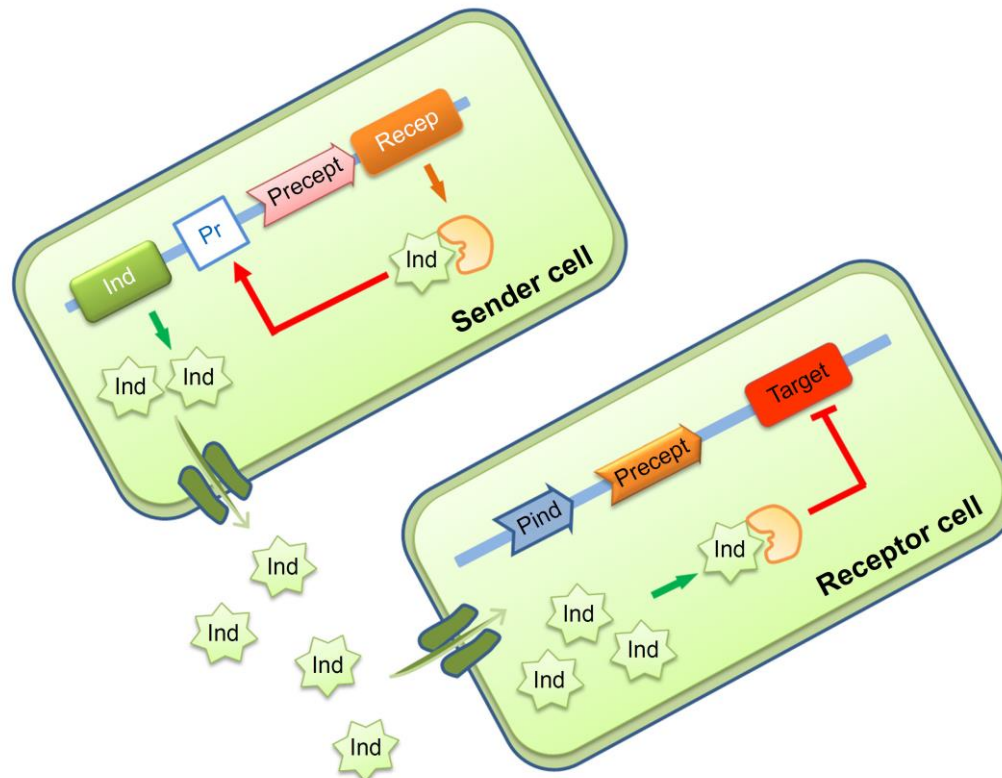
- Využití bakteriálního komunikačního systému

**Figure 6** Design of synthetic quorum sensing in consortium of bacteria.
Notes: The sender cell synthesizes a messaging molecule (inducer) that stimulates its receptor synthesized in the receiver cells. This complex triggers inhibition (or activation) of a target gene in a cell density-dependent manner.
Abbreviations: Ind, inducer; Pr, promotor; Precept, receptor's promoter.
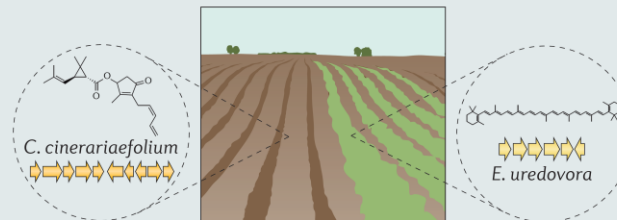
Produkce chemických látek

Current high-throughput and multiplexed genetic engineering strategies can be harnessed to develop applications for natural product producers outside the fermenter as well. This could have applications in environmental sensing (for example, by producing a small volatile metabolite in response to metal contamination in soils), or in the production of therapeutics by probiotic strains (for example, genetically engineering a probiotic strain to produce antibacterial compounds in response to a pathogen in the gastrointestinal tract)[142–144]. Other applications include the use of biocontrol agents to prevent the biofouling of marine surfaces[145], or creating hybrid biomaterials; for example, by expressing antibodies or natural product binding proteins in the membrane of bacterial magnetic nanoparticles[146] (see the figure). Natural products are already used extensively in agriculture for crop protection[147], and gaining fine-tuned control over the production dynamics either in soil microbial communities or in crop plants themselves could influence food production.
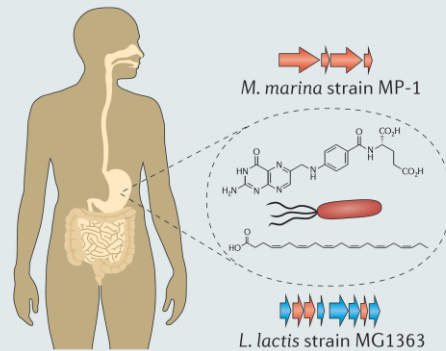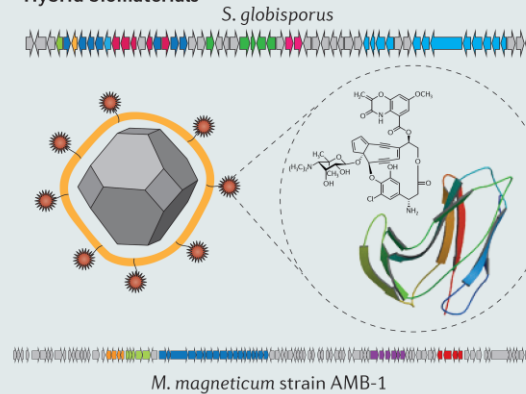
Antifouling agents

Z. verticillatum
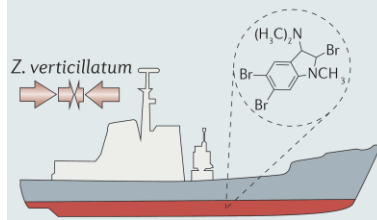
Crop protection and biofortification

C. cinerariaefolium

E. uredovora

Probiotic nutraceuticals

M. marina strain MP-1

L. lactis strain MG1363

Hybrid biomaterials

S. globisporus

M. magneticum strain AMB-1

C. cinerariaefolium, Chrysanthemum cinerariaefolium; E. uredovora, Erwinia uredovora; L. lactis, Lactococcus lactis; M. magneticum, Magnetospirillum magneticum; M. marina, Moritella marina; S. globisporus, Streptomyces globisporus; Z. verticillatum, Zoobotryon verticillatum.

Ochrana ekosystémů
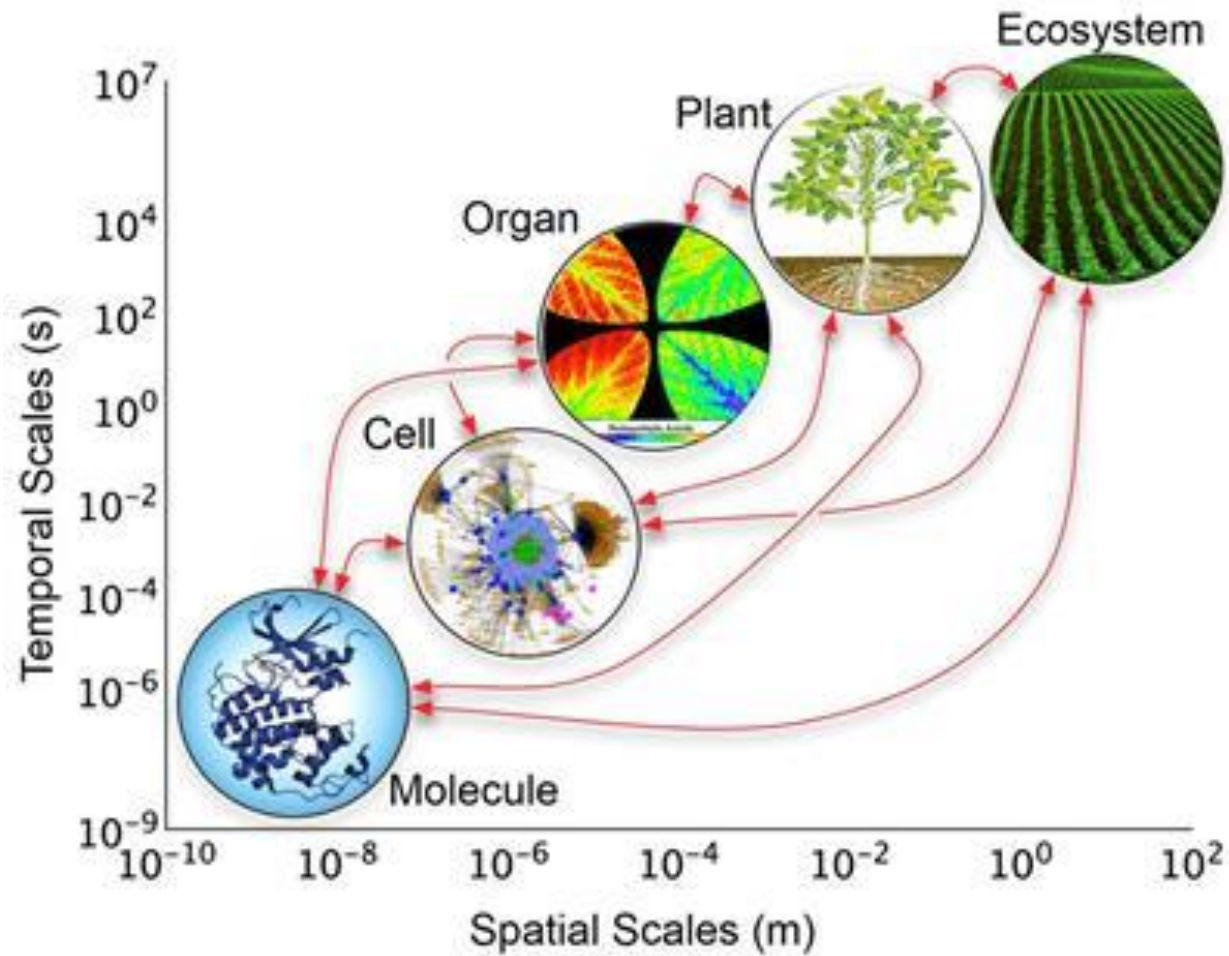
Astrobiologie

- 6 oblastí vesmírné syntetické biologie
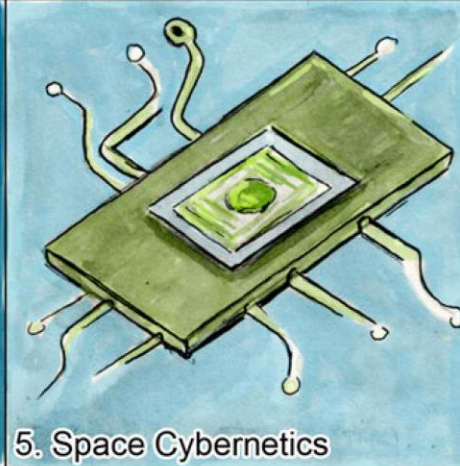

1. Resource Utilization
2. Manufacturing
3. Life Support
4. Space Medicine
5. Space Cybernetics
6. Terraforming
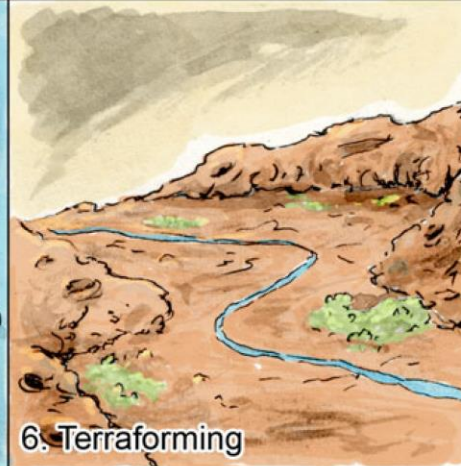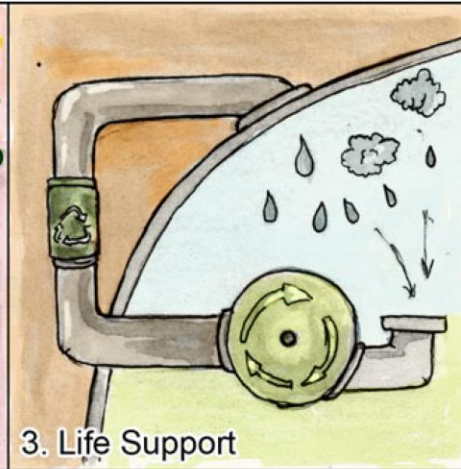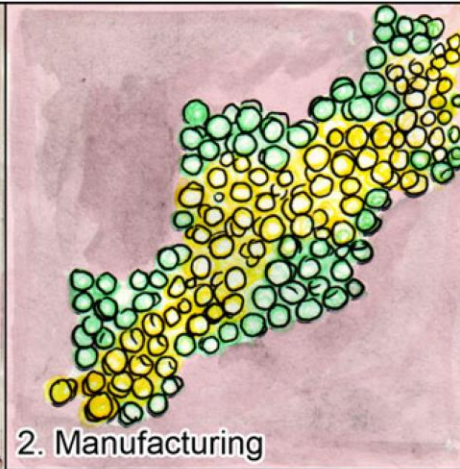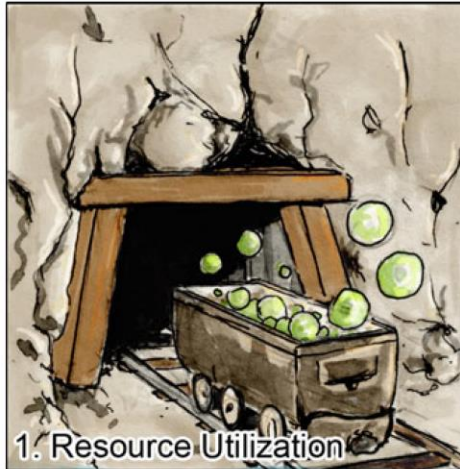
Astrobiologie

1. Resource utilization
    (a) Ensuring functionality in extreme environments.
    (b) Providing the capacity to harness three kinds of resources: wastes, volatiles and minerals.
    (c) Producing feedstocks for manufacturing processes and cell-based biomaterials for construction processes.
2. Manufacturing
    (a) Satisfying construction-related desires with adhesives to bind regolith, biocement and biopolymers.
    (b) Generating fuel for power and propulsion.
    (c) Revisiting abiotic manufacturing and construction technologies to leverage existing or synthetic biology capabilities.
3. Life support
    (a) Improving the biological management of waste, especially wastewater.
    (b) Treating, conditioning and recycling air, water and solid wastes through incorporating biology into traditionally inan-
        imate structures, e.g. creating a 'living' habitat.
    (c) Producing flavourful, texture-rich and nutritious food.
    (d) Providing nutrients, and assisting with the recycling of nutrients.
4. Space medicine and human health
    (a) Preventing disease and maintaining the human microbiome.
    (b) Manufacturing synthetic drugs to combat disease, radiation damage and the effects of reduced gravity.
    (c) Developing radiation-resistant, self-healing protective clothing and personal shielding.
5. Space cybernetics
    (a) Developing device-level biological control systems: biological sensors, actuators and controllers.
    (b) Designing biological control systems that are either completely composed of biological parts, or that partially integrate
        biological controllers and systems with abiotic sensors and actuators as a form of artificial life.
6. Terraforming
    (a) Paraterraforming with few multi-functional species that complete the carbon and nitrogen cycles.
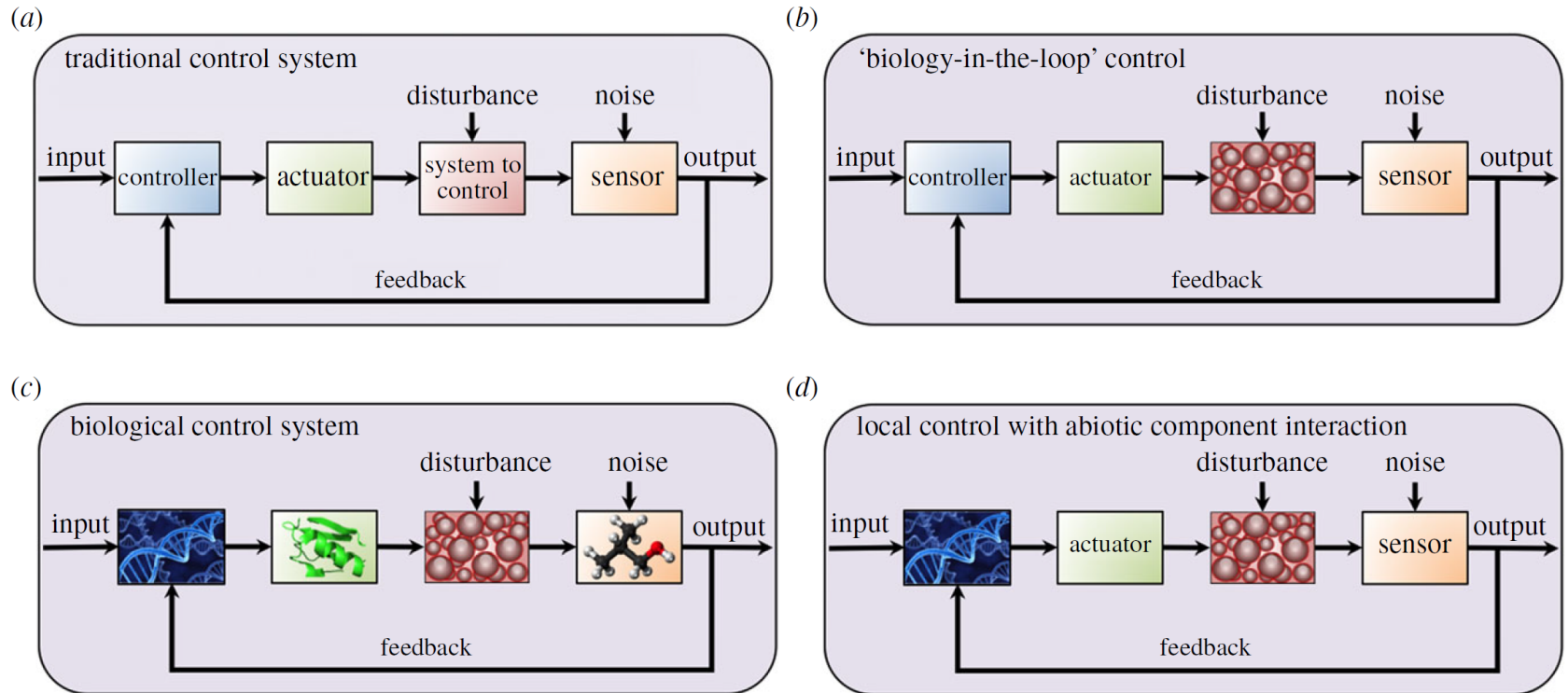
Astrobiologie

Figure 2. Synthetic biological control systems for use in space. (*a*) A traditional feedback control system consists of a controller, an actuator, a sensor and a system to be controlled, all arranged within a feedback loop. (*b*) 'Biology-in-the-loop' control refers to contemporary electromechanical (e.g. microfluidic or optical) techniques of externally controlling a biological system. (*c*) Challenge 5 moves towards a methodology that completely integrates biological controllers (perhaps based on gene regulatory networks), actuators (perhaps one or more proteins) and sensors (perhaps levels of chemicals of interest) with the biological system to be controlled (the control subchallenge). (*d*) Challenge 5 also includes the case where biological controllers and the systems to be controlled constitute separate biological subsystems that individually interact with abiotic sensors and actuators, all of which are part of a larger system, e.g. a hybrid robot (the artificial life subchallenge).

Co bude dál?

- Syntetické organizmy

- Drug delivery, léčba rakoviny

- Využití skleníkových plynů

- Biopaliva v primární produkci

- Změna legislativy ohledně využití GM mikroorg.
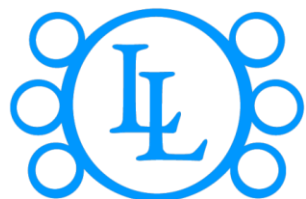
Děkuji za pozornost!

lukchrast@gmail.com

http://loschmidt.chemi.muni.cz/peg/