



Aalto University
School of Chemical
Technology

Synthetic biology

(Course CHEM-E8125), spring 2019

Introduction

Prof. Merja Penttilä

Synthetic biology is now possible - Conceptually and technologically new way of doing bio

- Synthetic Biology is the design and construction of new biological functionalities not found in Nature, and
- Use of novel tools and concepts that make biology engineerable

From repetitive trial-and-error biology to
design and a controlled way of building biological systems

Biotechnology is the key enabling technology
for our future, and
Synthetic Biology is its cutting edge.

Impact on all fields of biotech (industrial, medical, plant, bionanotech)

1970
1980
1990
2000
2010
2020

Restriction enzymes
First recombinant DNA molecules
DNA Sequencing technique

PCR (polymerase chain reaction)

First gene expression arrays
Yeast genome published

Human genome published

First minimal genome bacteria

First bacteria with synthesized genome
New-to-nature genetic code
Synthetic yeast chromosome

Synthetic yeast

Gene technology
Gene function



Genome data
Inventory, Potential



Omics-technologies

Bioinformatics
Mathematical modelling

Systems biology
Molecular networks
responsible for
cell function



Engineering principles
De novo design & synthesis

Metabolic engineering
Quantitative measurements

Synthetic biology
De novo & Redesign



New products, sensors, nano devices,...

Enablers of synbio

- Fast and cheap genome sequencing and DNA synthesis
- Computer-based design and automated cell construction
- Biology as an engineering science; engineering principles
- Genome-editing tools (CRISPR)

Human design of standardised modular biological parts and devices that can be assembled in desired combinations to functional controllable systems.



“What I can not build,
I can not understand”

Physicist Richard Feynman

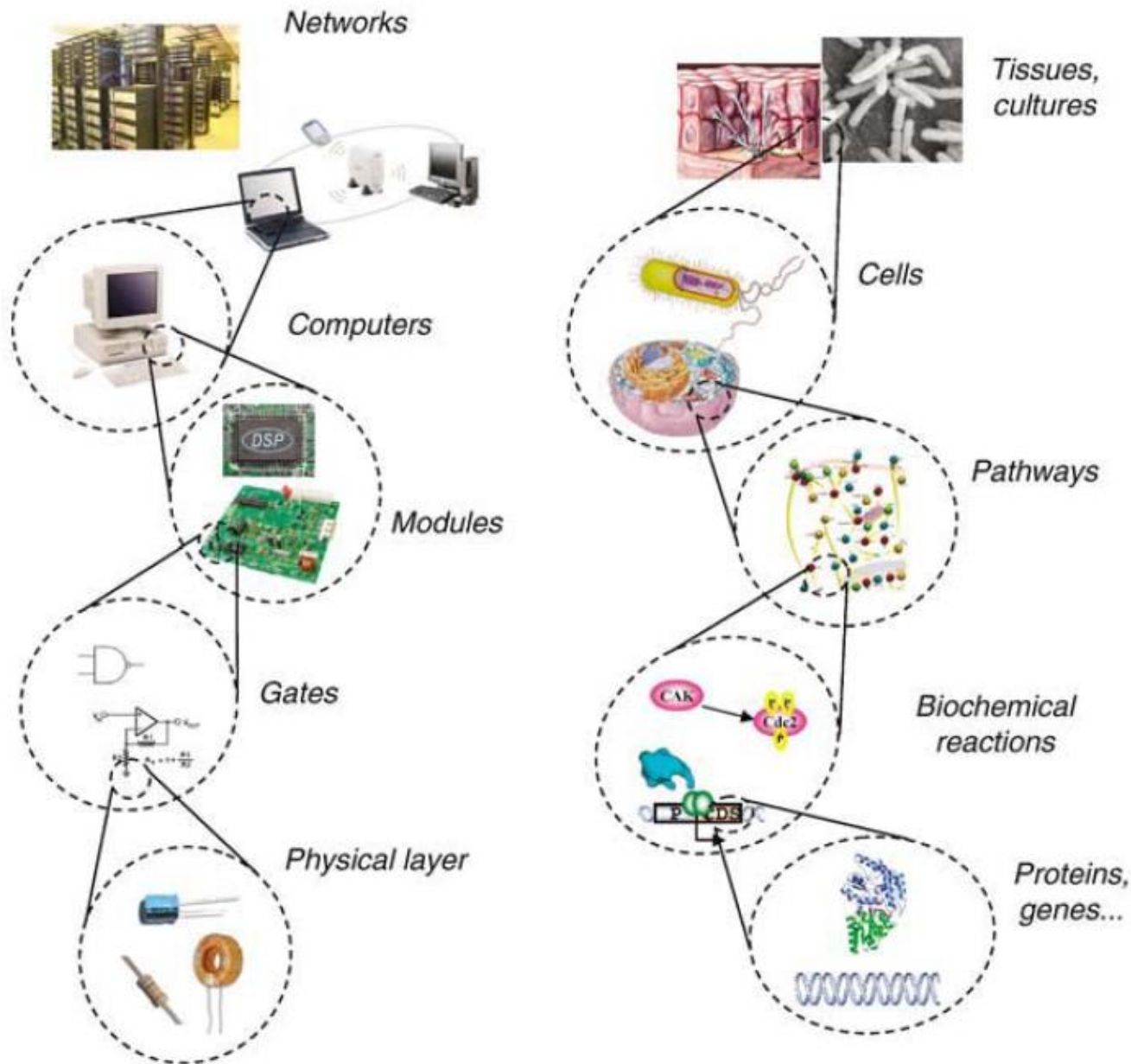


Synthetic biology

- Construction of designed biological operational units
 - model-based
 - quantifiable
 - programmable
 - standardized
 - orthogonal
- Synthetic genes, proteins, production pathways, intracellular control circuits, cell organelles, production hosts (chassis) ...
- DNA as an engineering platform universal for life (as we know it)



Synthetic biology, analogy to electrical engineering

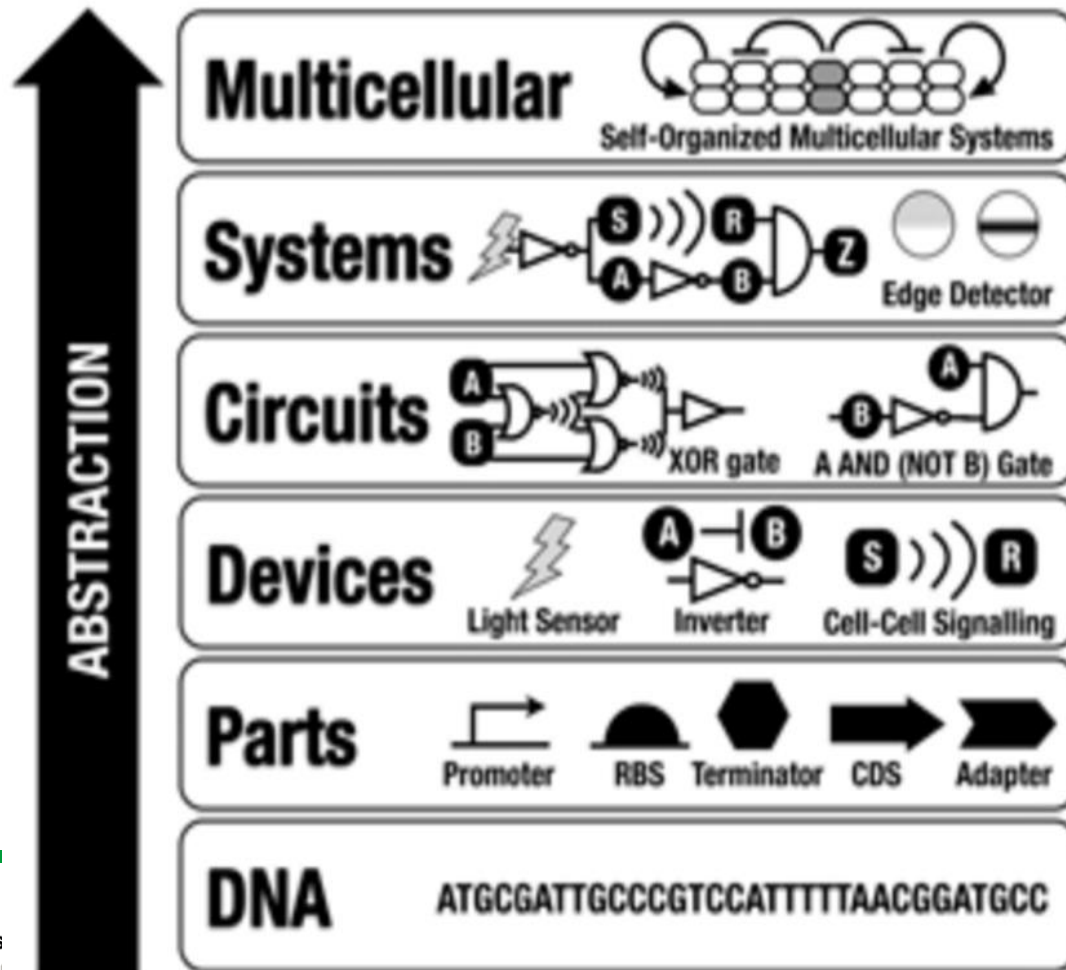


Human design of **standardised** modular biological parts and devices that can be assembled in desired combinations to functional controllable systems (BUT, does not need to be standardised only, e.g. laboratory evolution)

A mindset change needed & new interdisciplinary teaching

A practical definition of synthetic biology

Making biology easier to engineer: “Engineering of biology remains complex because we have never made it simple” (Thomas F Knight, MIT/Gingko Bioworks)



Biobricks

MIT & Berkeley & Harvard & UCSF & Imperial College & ETH ...

Register of standard biological parts – BioBricks™

[Promoters](#)

[Ribosome Binding Sites](#)

[Protein domains](#)

[Protein coding sequences](#)

[Translational units](#)

[Terminators](#)

[DNA](#)

[Plasmid backbones](#)

[Primers](#)

[Protein generators](#)

[Reporters](#)

[Inverters](#)

[Receivers and senders](#)

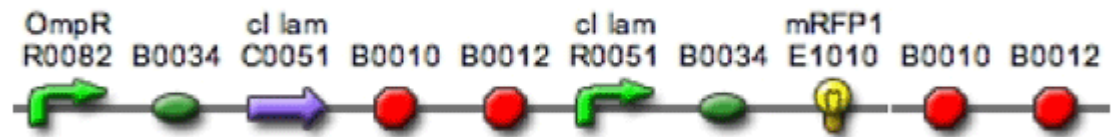
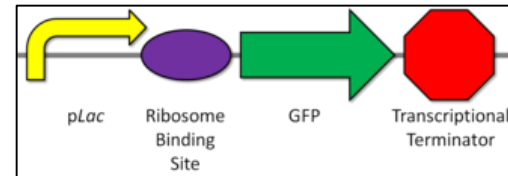
[Measurement devices](#)

[Cell death](#): Parts involved in killing cells.

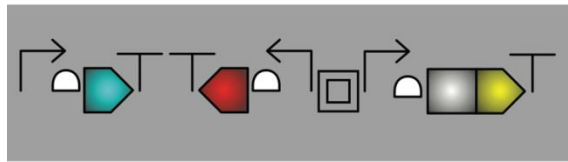
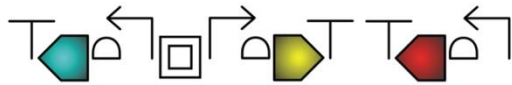
[Coliroid](#): Parts involved in taking a bacterial photograph.






















[Chassis](#)

[Assembly standards](#)



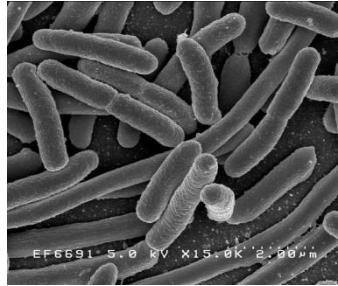
SBOL, synthetic biology open language



 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	

Chassis

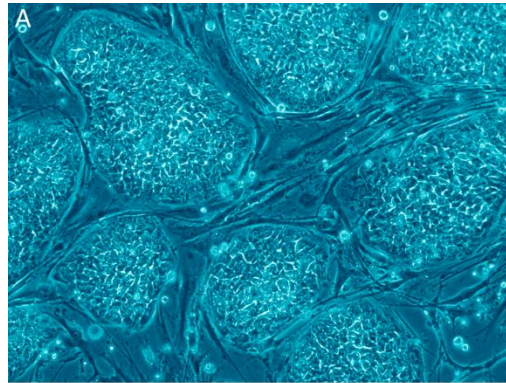
Bacterium
Escherichia coli



Yeast
Saccharomyces cerevisiae



Human embryonic
stem cells



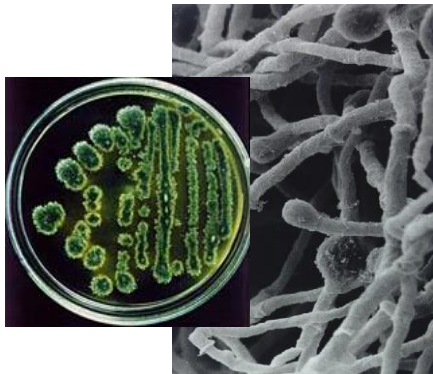
Cyanobacterium
Synechocystis



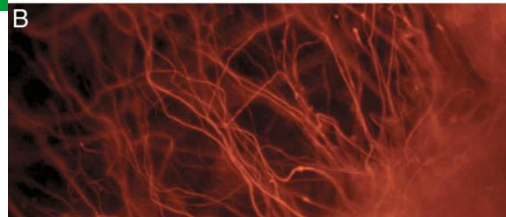
Plant
Arabidopsis thaliana



Filamentous fungus
Trichoderma reesei



Human nerve cells



Synthetic biology is revolutionising Biotechnology and is needed for sustainable breakthroughs in Bioeconomy

Gene technology Systems biology

Synthetic biology

1st generation Cell factories

Raw materials and sugars naturally used by the microbe (e.g. starch, glucose, lactose)



- Use of natural organisms, no genetic engineering
- Strain improvement by random mutagenesis with chemicals and radiation



Organism specific products e.g.

- alcohol and flavour compounds with wine yeasts
- mixture of acetone, butanol and ethanol (ABE) with bacteria

2nd generation Cell factories

Broadened substrate range (e.g. (hemi)cellulose, xylose)



- Transfer of combination of genes found in nature to the production organism
- Strain improvement by targeted gene expression and inactivation



New traits/products in old hosts e.g.

- human insulin with yeast
- high levels of industrial enzymes with fungi
- 2nd generation bioethanol (C5 fermenting *Saccharomyces* yeast)

3rd generation Cell factories

Tailor-made carbon and energy use (e.g. waste CO₂, methanol, glycerol)



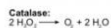
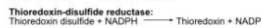
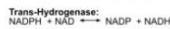
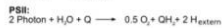
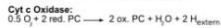
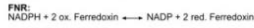
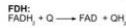
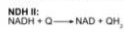
- Design of new-to-nature genes, gene combinations and synthetic metabolic reaction pathways, and ultimately entire cells
- Strain improvement by rapid automated gene assembly and transformation, combined with very high-through-put screening



Novel products in tailor-made hosts e.g.

- biotech production of current petrochemicals
- novel jet fuels produced with yeast
- engineered spider silk for superior materials

Mathematical models of cellular metabolism



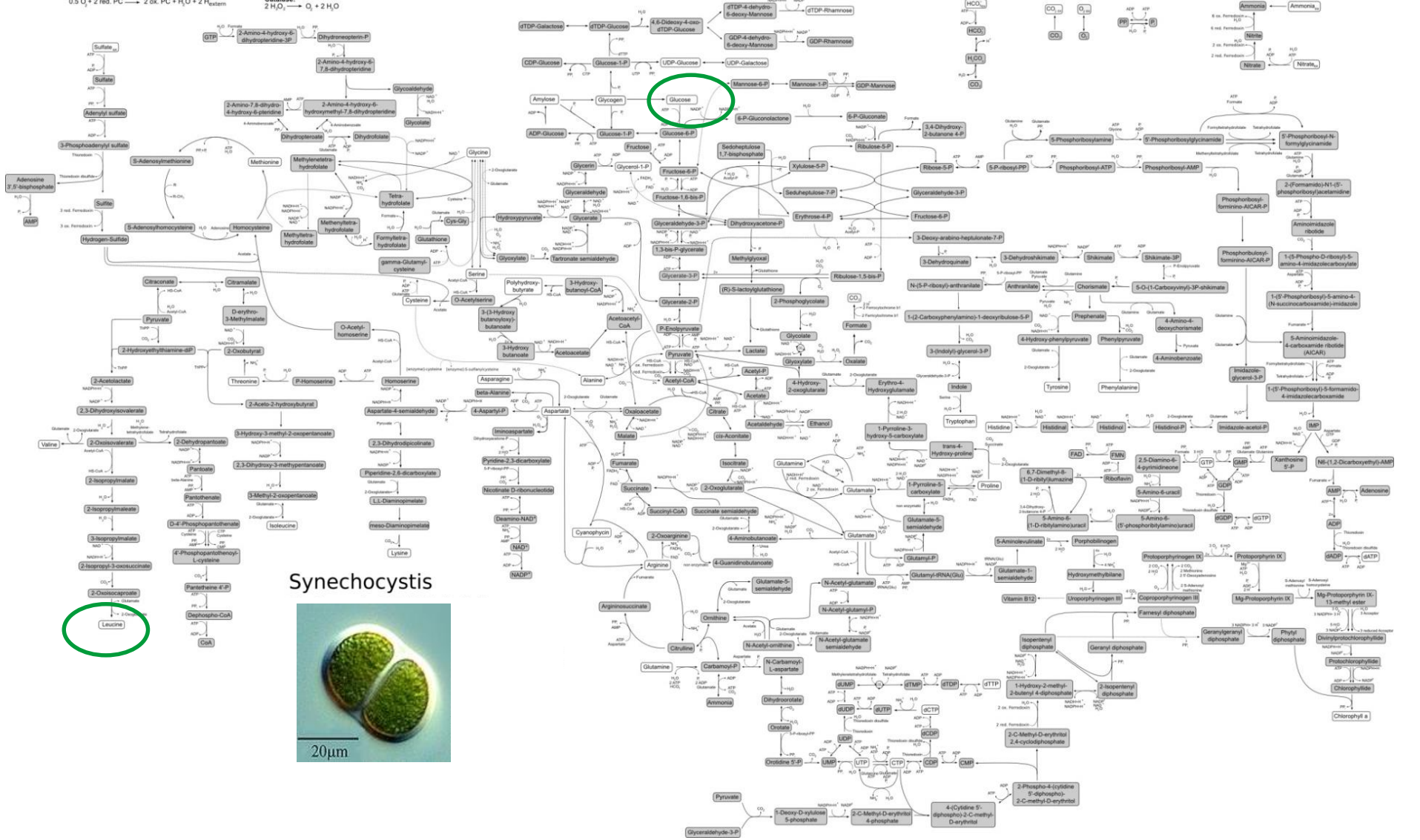
0.018 methionine + 0.039 phenylalanine +
 0.054 threonine + 0.009 tryptophan + 0.076 valine +
 0.053 arginine + 0.014 histidine + 0.101 alanine +
 0.098 aspartate + 0.132 glutamate +
 0.007 cysteine + 0.086 glycine + 0.038 proline +
 0.054 serine + 0.033 tyrosine + 2 GTP + ATP + 2 H₂O
 \Rightarrow protein + 2 GDP + AMP + PPI + 2 P_i

\Rightarrow RNA + PPI

JDNA: 0.279 dTTP + 0.222 dCTP + 0.222 dGTP +
 0.279 dATP
 \Rightarrow DNA + PPI

Synthesis of Biomass

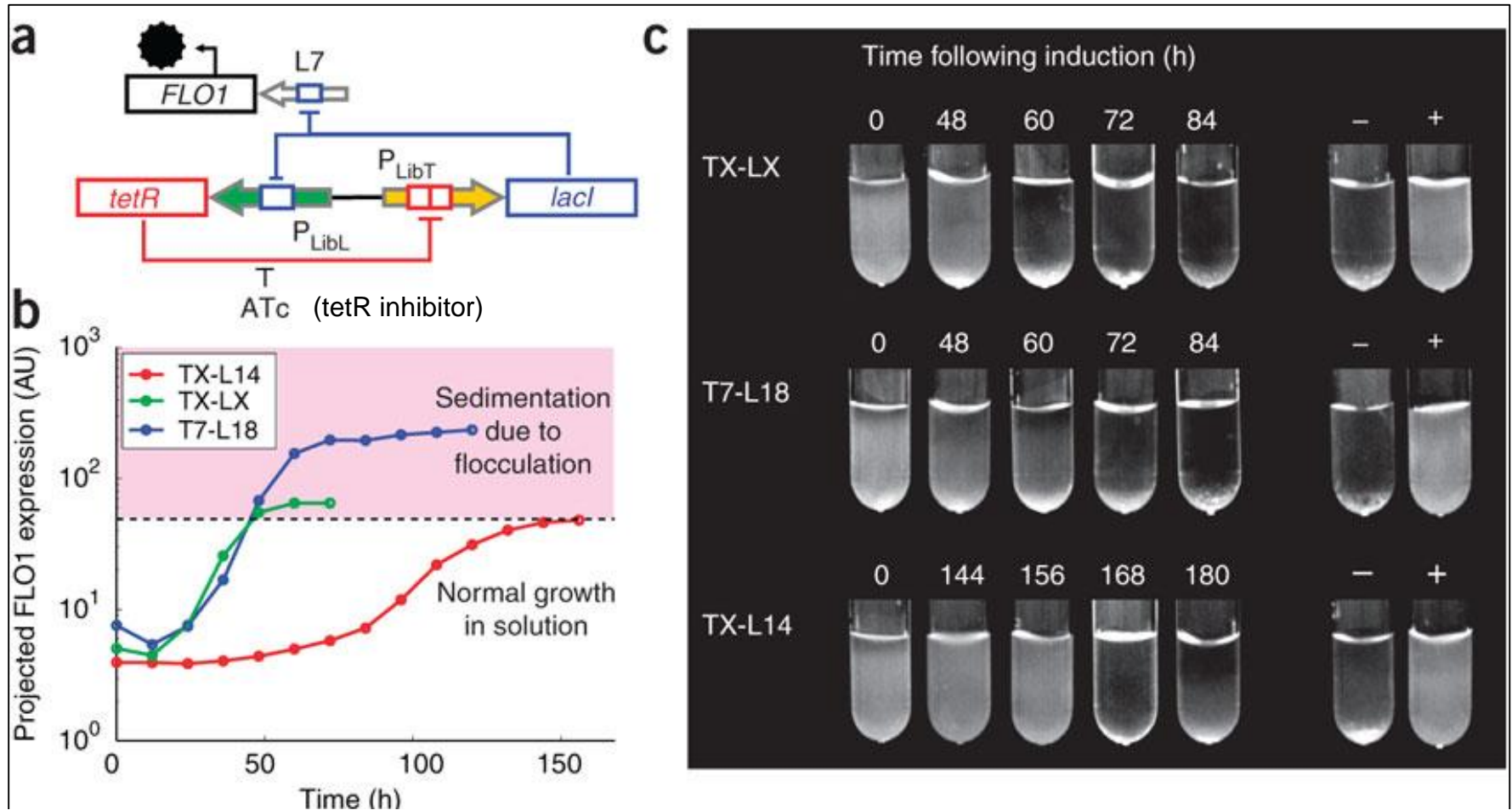
JBio: 0.8404 protein + 0.1230 carbohydrate +
 0.0182 lipid + 0.0130 RNA + 0.0039 DNA +
 0.0016 chlorophyll
 \Rightarrow 1 Biomass



Control circuits

- inputs, outputs, receivers, transmitters
- standardisation, reproducibility
- quantification & measurements
- mathematical modelling

Ellis et al. Nat Biotechnol. 2009; 27(5): 465–471. doi:10.1038/nbt.1536.



Control of yeast sedimentation (flocculation) with anhydrotetracycline (ATc) controllable expression circuit after product formation to aid product recovery and cell removal

Some examples of synthetic biology

- Technology: *In vitro* synthesis of designed DNA/genes & genome editing
- New genetic codes
- Unnatural aminoacids
- Synthesis of mirrow image polymers
- Novel enzyme catalysts
- Novel chemicals
- Molecular motors
- Nanorobots
- Energy generation (redesign of photosynthesis, biofuel cells)
- Virus assembly *in vitro* from parts
- Synthetic organelles
- Microbes for monitoring (odours, toxins etc, signal transmission)
- Minimalistic cells for production – chassis* (e.g. ~ 473 essential genes in *Mycoplasma*)
 - Assembly from parts and desired genes – novel genomes
 - Omittment of unnecessary reactions (->standard chassis) -> Addition of wanted ones
- "Normal" metabolic engineering

* asennuspohja

LETTERS

Encoding multiple unnatural amino acids via evolution of a quadruplet-decoding ribosome

Heinz Neumann^{1*}, Kaihang Wang^{1*}, Lloyd Davis¹, Maria Garcia-Alai¹ & Jason W. Chin¹

- Normally 61 codons (triplets of nucleotides) code for the 20 common amino acids (+ 3 codons for stop).
- Engineering of an orthogonal ribosome that reads quadrupled codons.
- Enables simultaneous incorporation of numerous (over 200) unnatural amino acids to proteins - novel properties – numerous applications.

The in vivo, genetically programmed incorporation of designer amino acids allows the properties of proteins to be tailored with molecular precision. The *Methanococcus jannaschii* tyrosyltransfer-RNA synthetase–tRNACUA (MjTyrRS–tRNACUA) and the *Methanosarcina barkeri* pyrrolysyl-tRNA synthetase–tRNACUA (MbPylRS–tRNACUA) orthogonal pairs have been evolved to incorporate a range of unnatural amino acids in response to the amber codon in *Escherichia coli*. However, the potential of synthetic genetic code expansion is generally limited to the low efficiency incorporation of a single type of unnatural amino acid at a time, because every triplet codon in the universal genetic code is used in encoding the synthesis of the proteome. To encode efficiently many distinct unnatural amino acids into proteins we require blank codons and mutually orthogonal aminoacyl-tRNA synthetase–tRNA pairs that recognize unnatural amino acids and decode the new codons. Here we synthetically evolve an orthogonal ribosome (ribo-Q1) that efficiently decodes a series of quadruplet codons and the amber codon, providing several blank codons on an orthogonal messenger RNA, which it specifically translates. By creating mutually orthogonal aminoacyl-tRNA synthetase–tRNA pairs and combining them with ribo-Q1 we direct the incorporation of distinct unnatural amino acids in response to two of the new blank codons on the orthogonal mRNA. Using this code, we genetically direct the formation of a specific, redox-insensitive, nanoscale protein cross-link by the bio-orthogonal cycloaddition of encoded azide- and alkyne-containing amino acids. Because the synthetase–tRNA pairs used have been evolved to incorporate numerous unnatural amino acids, it will be possible to encode more than 200 unnatural amino acid combinations using this approach. As ribo-Q1 independently decodes a series of quadruplet codons, this work provides foundational technologies for the encoded synthesis and synthetic evolution of unnatural polymers in cells.

Science 329:52- (2010)

Creation of a Bacterial Cell Controlled by a Chemically Synthesized Genome

Daniel G. Gibson,¹ John I. Glass,¹ Carole Lartigue,¹ Vladimir N. Noskov,¹ Ray-Yuan Chuang,¹ Mikkel A. Algire,¹ Gwynedd A. Benders,² Michael G. Montague,¹ Li Ma,¹ Monzia M. Moodie,¹ Chuck Merryman,¹ Sanjay Vashee,¹ Radha Krishnakumar,¹ Nacyra Assad-Garcia,¹ Cynthia Andrews-Pfannkoch,¹ Evgeniya A. Denisova,¹ Lei Young,¹ Zhi-Qing Qi,¹ Thomas H. Segall-Shapiro,¹ Christopher H. Calvey,¹ Prashanth P. Parmar,¹ Clyde A. Hutchison III,² Hamilton O. Smith,² J. Craig Venter^{1,2*}

We report the design, synthesis, and assembly of the 1.08-mega-base pair *Mycoplasma mycoides* JCVI-syn1.0 genome starting from digitized genome sequence information and its transplantation into a *M. capricolum* recipient cell to create new *M. mycoides* cells that are controlled only by the synthetic chromosome. The only DNA in the cells is the designed synthetic DNA sequence, including “watermark” sequences and other designed gene deletions and polymorphisms, and mutations acquired during the building process. The new cells have expected phenotypic properties and are capable of continuous self-replication.

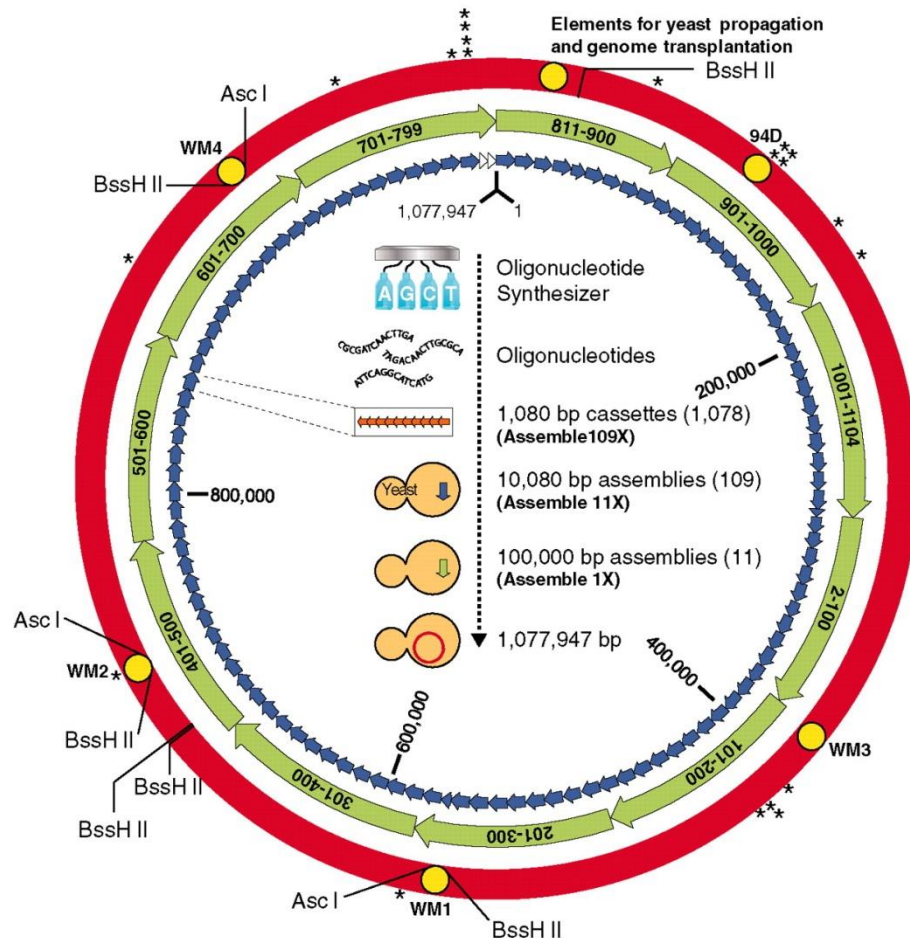
crude *M. mycoides* or *M. capricolum* extracts, or by simply disrupting the recipient cell’s restriction system (8).

We now have combined all of our previously established procedures and report the synthesis, assembly, cloning, and successful transplantation of the 1.08-Mbp *M. mycoides* JCVI-syn1.0 genome, to create a new cell controlled by this synthetic genome.

Synthetic genome design. Design of the *M. mycoides* JCVI-syn1.0 genome was based on the highly accurate finished genome sequences of two laboratory strains of *M. mycoides* subspecies *capri* GM12 (8, 9, 11). One was the genome donor used by Lartigue *et al.* [GenBank accession CP001621] (10). The other was a strain created by transplantation of a genome that had been cloned and engineered in yeast, YCpMmyc1.1- $\Delta type III res$ [GenBank accession CP001668] (8). This project was critically dependent on the accuracy of these sequences. Although we believe that both finished *M. mycoides* genome sequences are reli-

- Changing *Mycoplasma capricolum* cells to *M. genitalium* JCVI syn1.0 genome.
- A species changed into another with the use of a synthetic genome – “Synthetic cell”.
- One single nucleotide mistake (deletion in dnaA-DNA replication) caused a severe problem for the project.

Gibson et al. Science 329:52- (2010)



The assembly of a synthetic *M. mycoides* genome in yeast. A synthetic *M. mycoides* genome was assembled from 1078 overlapping DNA cassettes in three steps. In the first step, 1080-bp cassettes (orange arrows), produced from overlapping synthetic oligonucleotides, were recombined in sets of 10 to produce 109 ~10-kb assemblies (blue arrows). These were then recombined in sets of 10 to produce 11 ~100-kb assemblies (green arrows). In the final stage of assembly, these 11 fragments were recombined into the complete genome (red circle). With the exception of two constructs that were enzymatically pieced together in vitro (27) (white arrows), assemblies were carried out by in vivo homologous recombination in yeast. Major variations from the natural genome are shown as yellow circles. These include four watermarked regions (WM1 to WM4), a 4-kb region that was intentionally deleted (94D), and elements for growth in yeast and genome transplantation. In addition, there are 20 locations with nucleotide polymorphisms (asterisks). Coordinates of the genome are relative to the first nucleotide of the natural *M. mycoides* sequence. The designed sequence is 1,077,947 bp. The locations of the Asc I and BssH II restriction sites are shown. Cassettes 1 and 800-810 were unnecessary and removed from the assembly strategy (11). Cassette 2 overlaps cassette 1104, and cassette 799 overlaps cassette 811.

Genome editing - CRISPR

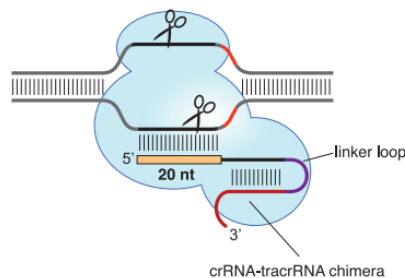
A Programmable Dual-RNA-Guided DNA Endonuclease in Adaptive Bacterial Immunity

Martin Jinek,^{1,2*} Krzysztof Chylinski,^{3,4*} Ines Fonfara,⁴ Michael Hauer,^{2†} Jennifer A. Doudna,^{1,2,5,6‡} Emmanuelle Charpentier^{4‡}

Clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated (Cas) systems provide bacteria and archaea with adaptive immunity against viruses and plasmids by using CRISPR RNAs (crRNAs) to guide the silencing of invading nucleic acids. We show here that in a subset of these systems, the mature crRNA that is base-paired to trans-activating crRNA (tracrRNA) forms a two-RNA structure that directs the CRISPR-associated protein Cas9 to introduce double-stranded (ds) breaks in target DNA. At sites complementary to the crRNA-guide sequence, the Cas9 HNH nuclease domain cleaves the complementary strand, whereas the Cas9 RuvC-like domain cleaves the noncomplementary strand. The dual-tracrRNA:crRNA, when engineered as a single RNA chimera, also directs sequence-specific Cas9 dsDNA cleavage. Our study reveals a family of endonucleases that use dual-RNAs for site-specific DNA cleavage and highlights the potential to exploit the system for RNA-programmable genome editing.

17 AUGUST 2012 VOL 337 SCIENCE — tracrRNA

Cas9 programmed by single chimeric RNA



www.sciencemag.org SCIENCE VOL 339 15 FEBRUARY 2013

RNA-Guided Human Genome Engineering via Cas9

Prashant Mali,^{1*} Luhan Yang,^{1,3*} Kevin M. Esvelt,² John Aach,¹ Marc Guell,¹ James E. DiCarlo,⁴ Julie E. Norville,¹ George M. Church^{1,2†}

www.sciencemag.org SCIENCE VOL 339 15 FEBRUARY 2013

Multiplex Genome Engineering Using CRISPR/Cas Systems

Le Cong,^{1,2*} F. Ann Ran,^{1,4*} David Cox,^{1,3} Shuailiang Lin,^{1,5} Robert Barretto,⁶ Naomi Habib,¹ Patrick D. Hsu,^{1,4} Xuebing Wu,⁷ Wenyan Jiang,⁸ Luciano A. Marraffini,⁸ Feng Zhang^{1†}

Cell Stem Cell

Brief Report

Functional Repair of CFTR by CRISPR/Cas9 in Intestinal Stem Cell Organoids of Cystic Fibrosis Patients

Gerald Schwank,^{1,2,7} Bon-Kyoung Koo,^{1,2,7,8} Valentina Sasselli,^{1,2} Johanna F. Dekkers,^{3,4} Inha Heo,^{1,2} Turan Demircan,¹ Nobuo Sasaki,^{1,2} Sander Boymans,¹ Edwin Cuppen,^{1,6} Cornelis K. van der Ent,³ Edward E.S. Nieuwenhuis,⁵ Jeffrey M. Beekman,^{5,6} and Hans Clevers^{1,2,*}

Cell Research (2015) 25:67-79.

© 2015 IBCB, SIBS, CAS All rights reserved 1001-0602/15 \$ 32.00

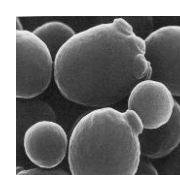
www.nature.com/cr



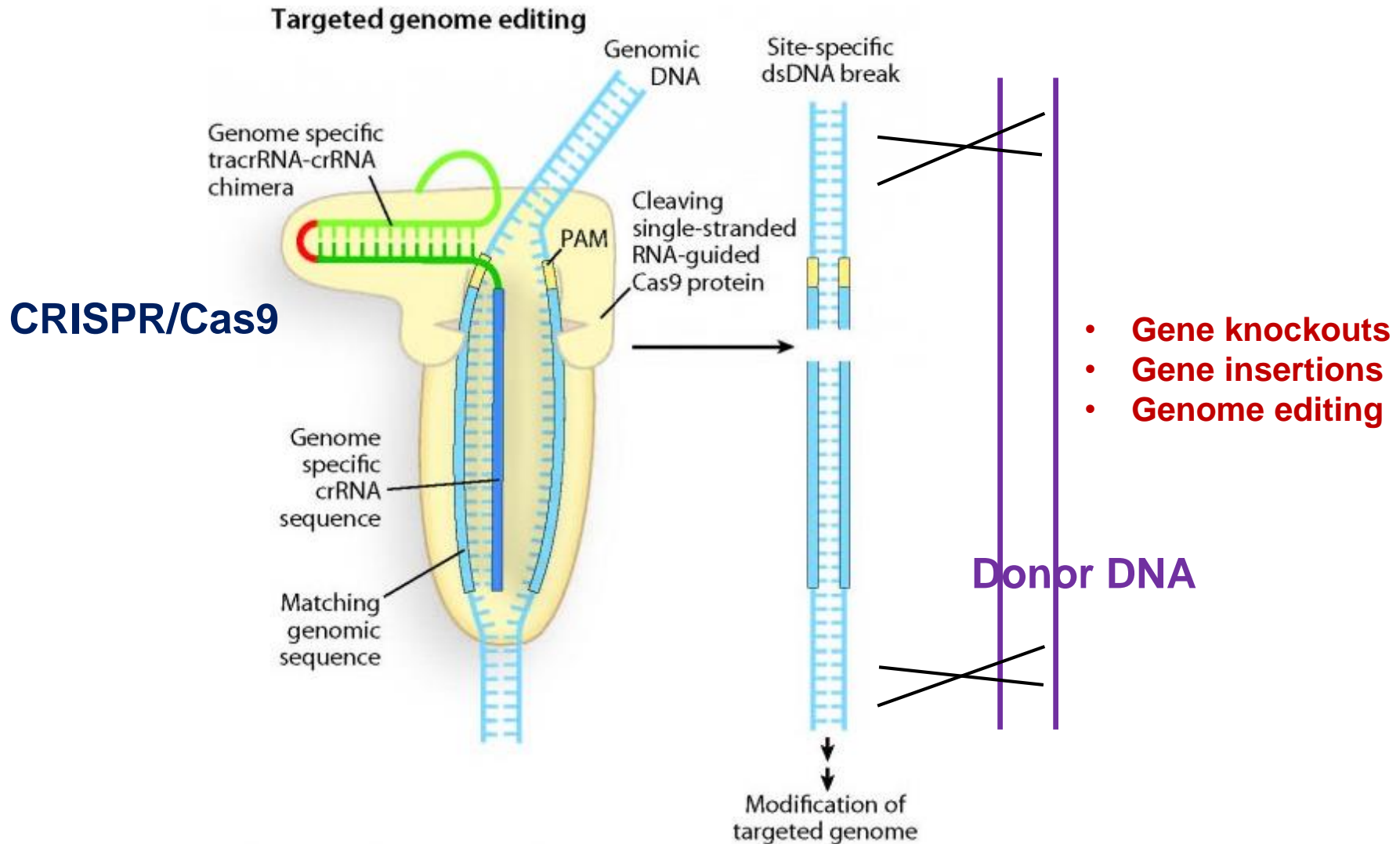
ORIGINAL ARTICLE

Correction of a genetic disease by CRISPR-Cas9-mediated gene editing in mouse spermatogonial stem cells

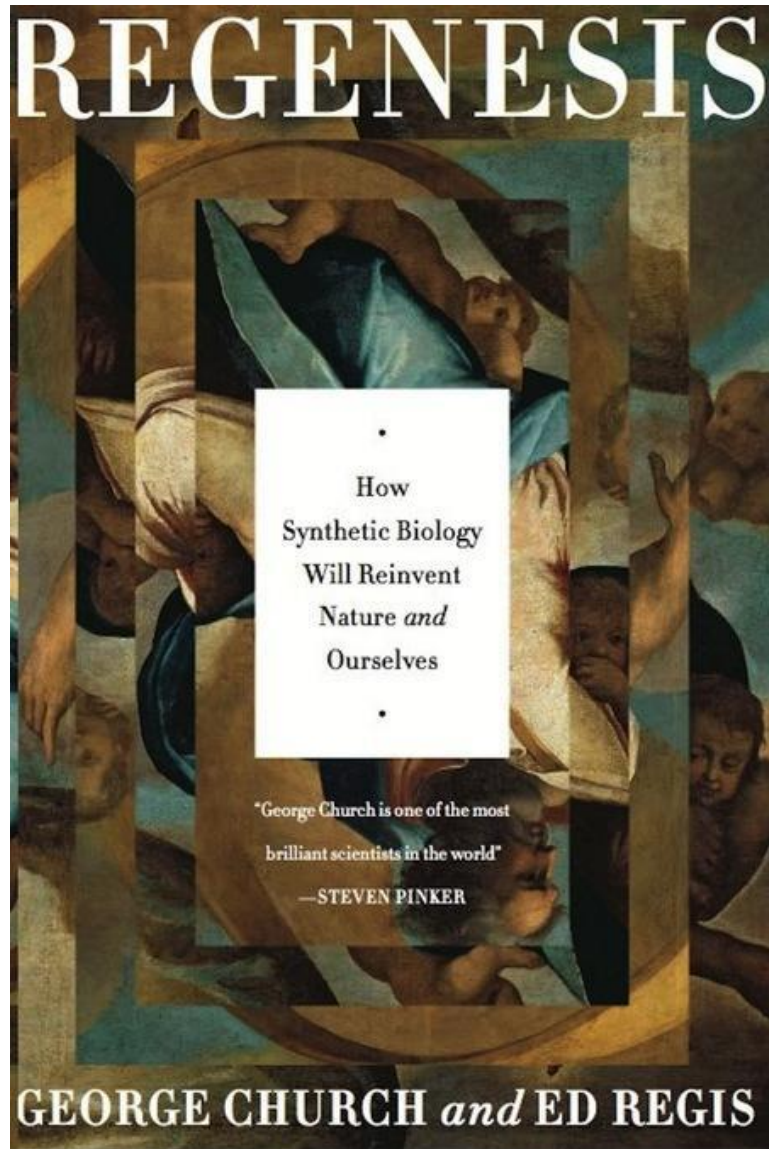
Yuxuan Wu^{1,2,*}, Hai Zhou^{1,2,3,*}, Xiaoying Fan^{4,*}, Ying Zhang^{2,5,*}, Man Zhang^{1,2,*}, Yinghua Wang^{1,2}, Zhenfei Xie^{1,2}, Meizhu Bai^{1,2,6}, Qi Yin^{1,2}, Dan Liang^{1,2}, Wei Tang⁷, Jiaoyang Liao^{1,2}, Chikai Zhou^{1,2}, Wujuan Liu^{1,2}, Ping Zhu¹, Hongshan Guo⁴, Hong Pan^{1,2}, Chunlian Wu³, Huijuan Shi⁸, Ligang Wu^{2,5}, Fuchou Tang⁴, Jinsong Li^{1,2,6}



Genome editing - CRISPR



The CRISPR-Cas components consist of a **Cas9 endonuclease** and a designer genome targeting CRISPR **guide RNA (gRNA)**, thereby resulting in **a simple and versatile RNA-directed system to generate dsDNA breaks for genome targeting and editing.**



Gene-editing record smashed in pigs

Researchers modify more than 60 genes in effort to enable organ transplants into humans.

[Sara Reardon](#)

06 October 2015 *NATURE* | NEWS

Geneticist George Church has co-founded a company that is developing genetically modified pigs to grow organs for human transplant



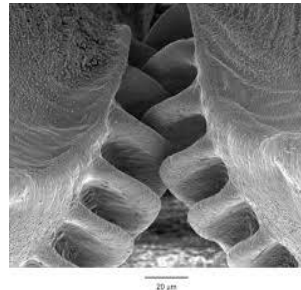
Biological organisms are excellent engineers

- Many functions manifest at single cell level
- The functions can be engineered and transferred to other organisms
- Cells have intrinsic synthesis power and the power of evolution – unlike in any other discipline

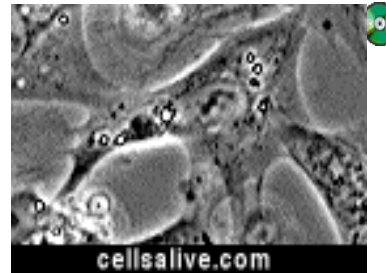
Fire fly



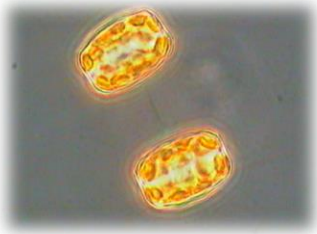
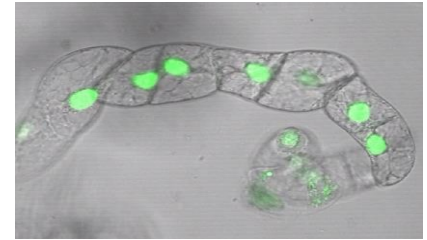
Lacewing fly's
jump mechanism



Heart cell



Cellulose forming
plant cells



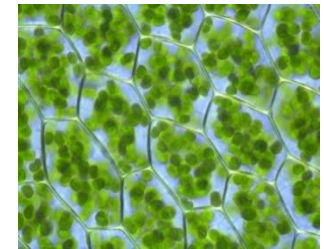
Fatty algal cells



Bacterial magnets



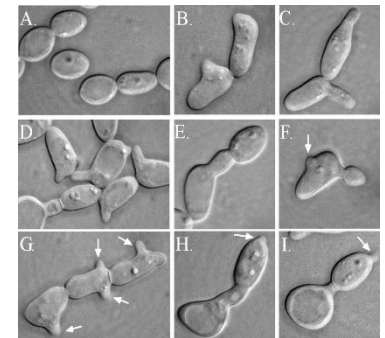
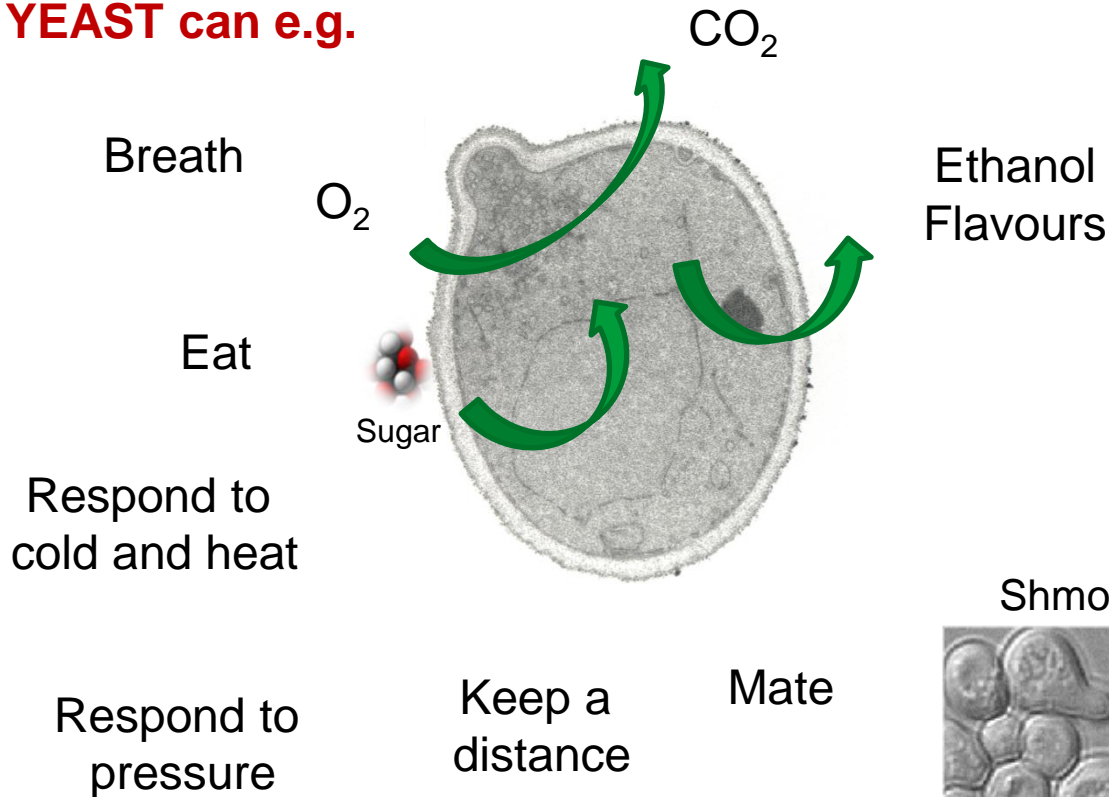
Spider silk



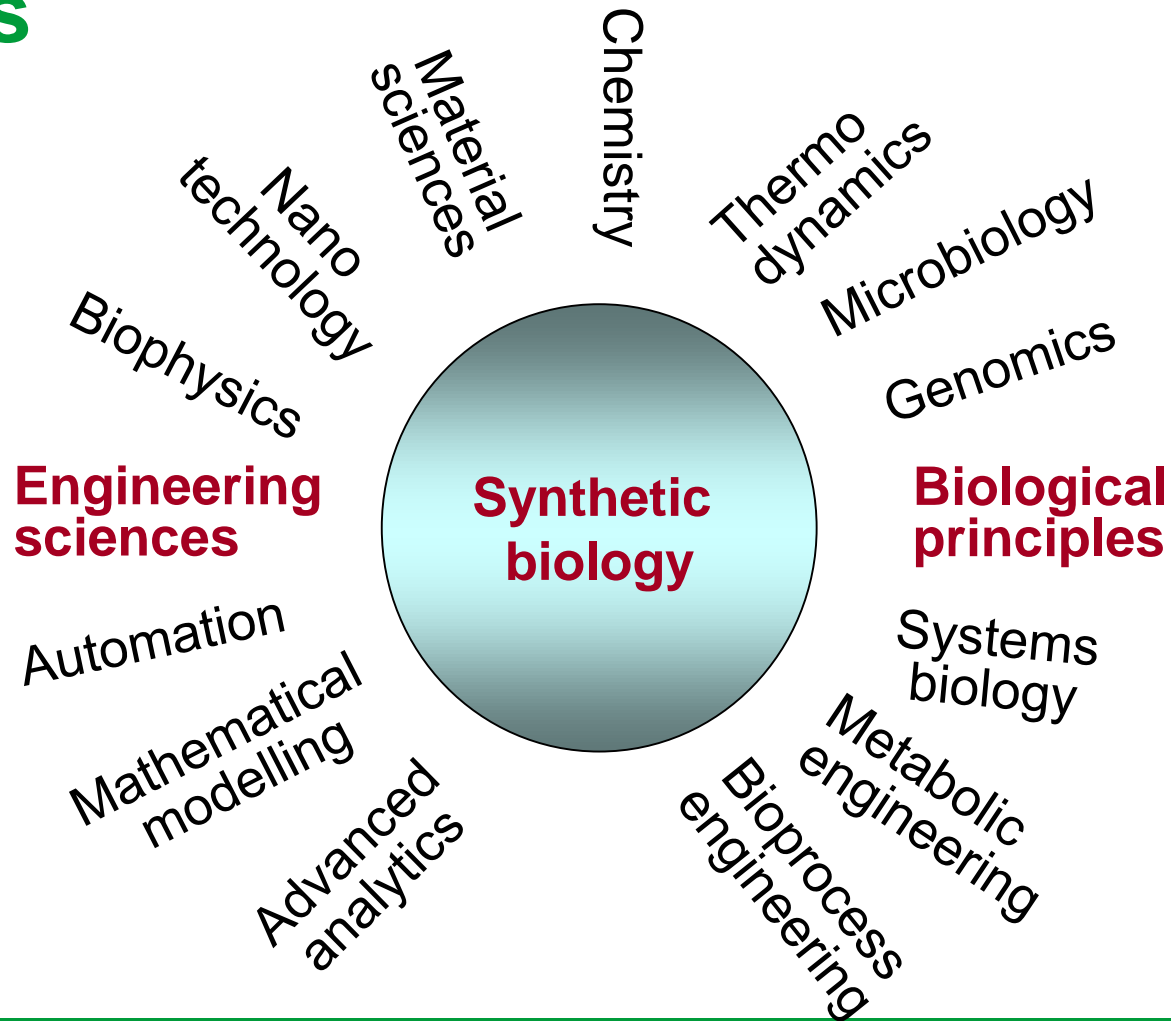
Light energy
capturing chloroplasts

Single cell organisms can have very "sophisticated" and varied functions

YEAST can e.g.



Synthetic biology relies on many disciplines



Synbio interests many

Scientists

- Interests many scientific fields, including physics, chemistry, nanosciences
- A dream job for a natural scientists (combination of biology and engineering sciences)

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DYI (do-it-yourself)

- Enabled by biobricks and standard protocols
- Garage biology (like ICT), activity in USA and Europe including associations



Croudfunded in Biocurious:
Glowing plants

BioCurious is the Hackerspace for Bio, Built in the Heart of Silicon Valley.

We are a community of scientists, technologists, entrepreneurs, and amateurs who believe that innovations in biology should be accessible, affordable, and open to everyone.

Located in Sunnyvale, CA, our co-working laboratory space and shared equipment is ideal for entrepreneurs, citizen scientists, hobbyists, and students.

Synbio interests many

Scientists

- Interests many scientific fields, including physics, chemistry, nanosciences
- A dream job for a natural scientists (combination of biology and engineering sciences)

DYI (do-it-yourself)

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Philosophers

- How we do science is changing, "from reading to writing", from examination of existing reality to design
- What is life? Natural?

Ethicists

- How far can we go? Can we engineer humans?
- Safety and need for new regulations?

Artists

- Experimenting with (conceptual) limits
- Biological living systems as art medium

Synbio interests many

[Laura Cinti: The Cactus Project](#) (2001) is a living artwork of cacti expressing human hair. The work explores a reproductive paradox in genetic engineering - the inherent sterility of transformation processes.



The work makes use of agrobacterium-mediated gene transfer to introduce hair keratin genes into cells of cacti. Transformed cells were regenerated as engineered transgenic cacti. The aim was to have human keratins expressed in cacti cells morphologically similar to hair and for these to be produced externally.

"[W]hy should we still call this hair human? Doesn't it belong now to the cactus and not in a trivial sense either? Or better still, doesn't it become just a standard biological part, to use a revealing phrase from the biological engineering laboratory at MIT? In this case, as in so many others, what makes it standard is not its elemental composition but its technical transmissibility: the apotheosis of Fordism put into service as an organizing principle for life?"

Paul Lewis, The Edge Effect: Art, Science, and Ecology in a Deleuzian Century in [An \[Un\]Likely Alliance: Thinking Environment\[s\] with Deleuze/Guattari](#)

Can you judge if this is real or not?

Artists

- Experimenting with (conceptual) limits
- Biological living systems as art medium



iGEM Jamboree Boston 2014



Course outline

- 25.2. **Introduction** to synthetic biology and the course
- 4.3. **Standardization, biobricks and chassis**
- 11.3. Presentations on homework and discussion
- 18.3. **Artificial Yeast Sc2.0**
- 20.3. 16-18, Design Factory: Companies pitch on their problems
- 25.3. Presentations on homework and discussion
- 1.4. **Modelling of circuits, oscillations and metabolism**
Elena Czeisler: Differential equations, SBOL
Paula Jouhten: Modelling of cellular chemistry
- 15.4. Presentations on homework and discussion
- 23.4. (Tue)
- 25.4. 16-19, Design factory: Mentoring
- 6.5. **Synbio as an enabler of applications in sustainable bioeconomy**
Production of fuels, chemicals and novel materials
- 13.5 **Ethics & safety, Conclusions**
- 20.5.
- 27.5 16-18, Design Factory: Final pitching & winners
- 31.5. **Exam**

Instructions

BioBuilder.org

Home work and oral presentations

1. Theme: Standardization, biobricks and chassis

2-3 groups to present

Design a sensor based on standard parts for input and parts for outputs + variations using the iGEM registry for standard parts and their assembly strategies.

http://parts.igem.org/Main_Page

2. Theme: Artificial Yeast Sc2.0

2-3 groups to present

Design a specific synthetic part of the yeast genome according to the rules laid out by the Sc2.0 consortium. Add your own ideas, discuss problems etc.

3. Theme: Modelling of circuits, oscillations and metabolism

2-3 groups to present

Everybody assign yourself to one group for home work (3 persons/group). Mix expertise. You can join a group which is not so close to your background.

Bring computers to classes, please!

Evaluation

Activity, Exam