Synthetic Biology – Trends and Updates

EC WORKSHOP ON SYNTHETIC BIOLOGY - FROM SCIENCE TO POLICY AND SOCIETAL CHALLENGES

Luxembourg 9th Dec 2015

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WHAT IF?

1. Drink
Synthetic E. chromi bacteria are ingested as a probiotic yoghurt.

2. Colonise
Colonising the gut, the E. chromi keep watch for the chemical markers of disease.

3. Monitor
If a disease is detected, the bacteria secrete an easily-read colour signal, visible in faeces.

E. chromi - cheap, personalised disease monitoring from the inside out.

E. chromi, 2009 University of Cambridge iGEM team
RESEARCH ARTICLE

A Forward-Design Approach to Increase the Production of Poly-3-Hydroxybutyrate in Genetically Engineered Escherichia coli

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Abstract

Biopolymers, such as poly-3-hydroxybutyrate (P(3HB)) are produced as a carbon store in an array of organisms and exhibit characteristics which are similar to oil-derived plastics.
WHAT IF?

Gluconacetobacter xylinus

Gluconacetobacter xylinus + yeast

Suzanne Lee

Aqualose 2014 Imperial College iGEM team  http://2014.igem.org/Team:Imperial
Genetic engineering of the cellulose-producing bacterium Komagataeibacter rhaeticus for production of novel biomaterials.
Florea et al 2015 in press
WHAT IF?

Viruses

Bacteria

Parasites

Infector Detector 2007 Imperial College iGEM team http://2007.igem.org/Imperial/Infector_Detector/Introduction
Paper-Based Synthetic Gene Networks

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http://dx.doi.org/10.1016/j.cell.2014.10.004

SUMMARY

Synthetic gene networks have wide-ranging uses in reprogramming and rewiring organisms. To date, there has not been a way to harness the vast potential of these networks beyond the constraints of a laboratory or in vivo environment. Here, we present an in vitro paper-based platform that provides an alternate, versatile venue for synthetic biologists to operate and a much-needed medium for the safe deployment of engineered gene circuits beyond the lab. Commercially available cell-free systems are freeze dried onto paper enabling the inexpensive, sterilizable transport of gene circuits. Earlier studies in the area of in vitro synthetic biology and cell-free systems have made important contributions to our understanding of fundamental molecular biology and biochemistry and, more recently, in the study of molecular switch dynamics and complex gene circuits (Hong et al., 2014; Karzbrun et al., 2014; Sun et al., 2014; Takahashi et al., 2014). These efforts, however, have focused on solution-phase reactions using fresh from frozen cell-free systems and often in liposomes with the goal of assembling artificial cells (Kuruma et al., 2009; Kobori et al., 2013). These solution-phase reactions are not stable or practical for handling outside of the lab and therefore miss the opportunity to leverage the abiotic and sterile nature of these systems.
WHAT IF?

Carbon feedstocks
- Petroleum
- Coal
- Natural gas

Building blocks
- Ethylene / propylene / butadiene
- Benzene / toluene / xylene

Value-added chemicals
WHAT IF?

Biomass feedstocks

CO₂

Cellulosic crops

Lignocellulosic waste

Building blocks

Ethylene / propylene / butadiene

Benzene / toluene / xylene

Value-added chemicals

CO₂

Cellulosic crops

Lignocellulosic waste

Building blocks

Value-added chemicals
S. cerevisiae secreting farnesene/biodiesel

~112K bases added
~41K bases removed
~450 single nucleotide changes
~1.25% of the genome!

DNA as a programmable material
WHAT IF?

Eau de Yeast

GenSpace'

ActiveDry

YEAST

Almond  Coconut  Banana  Orange  Vanilla  Strawberry  Pineapple

Tie-dye shirt
DIY-Bio, Biohackers and the Growth of Community Labs
WHAT IF?
Engineered bacteria to detect and kill cancer cells

Engineered E. coli

Colorectal cancer

Healthy colon

Cell Systems
Programming a Human Commensal Bacterium, Bacteroides thetaiotaomicron, to Sense and Respond to Stimuli in the Murine Gut Microbiota

Graphical Abstract

Authors
Mark Minev, Alex C. Tucker, Christopher A. Voigt, Timothy K. Lu
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In Brief
The development of genetic parts to precisely program the human commensal gut bacterium Bacteroides thetaiotaomicron lays the foundation for microbiome engineering.

Highlights
- We develop sets of genetic parts for a human commensal bacterium
- Promoter and repressible sensors control gene expression over a 10,000-fold dynamic range
- Orthogonal, inducible sensors enable synthetic genetic memory and CREDRI
- Genetic circuits respond to stimuli in a complex mouse gut microbiota

Minev et al., 2016, Cell Systems 1, 50-71
July 20, 2015 © 2015 Elsevier Inc.
http://dx.doi.org/10.1016/j.cels.2015.06.001
WHAT IF?

Engineered phage and bacteria to target pathogens

bacteriophage  →  Attacking bacterium  →  Bacteria cell lyses and dies

Engineered E. coli

Engineered bacteriophage to treat infectious diseases

(adapted from Science 333: 6047 (2011); http://www.sciencephoto.com/)
Engineered bacteriophage targeting gene networks as adjuvants for antibiotic therapy

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Edited by Arnold L. Demain, Drew University, Madison, NJ, and approved February 3, 2009 (received for review January 16, 2008)

Antimicrobial drug development is increasingly lagging behind the evolution of antibiotic resistance, and as a result, there is a pressing need for new antibacterial therapies that can be readily designed and implemented. In this work, we engineered bacteriophage to overexpress proteins and attack gene networks that are not directly targeted by antibiotics. We show that suppressing the SOS network in E. coli with engineered bacteriophage enhances killing by quinolones by several orders of magnitude in vitro and significantly increases survival of infected mice in vivo. In addition, we demonstrate that engineered bacteriophage can enhance the killing of antibiotic-resistant bacteria, persisters cells, and biofilms, reduce the number of antibiotic-resistant bacteria that arise from an antibiotic-treated population, and act as a strong adjuvant for other bacterioidal antibiotics (e.g., aminoglycosides and β-lactams). Furthermore, we show that engineering bacteriophage to target non-SOS gene networks and to overexpress multiple factors can produce effective antibiotic adjuvants. This work establishes a synthetic biology platform for the rapid translation and integration of identified targets into effective antibiotic adjuvants.

antibiotic adjuvants | antibiotic resistance | bacterial persistence | bacteriophage therapy | synthetic biology
WHAT IF?
Engineered T-cells to target cancer

Adoptive T Cell Transfer for Cancer Immunotherapy in the Era of Synthetic Biology

Michael Kalos, Carl H. June

DOI: http://dx.doi.org/10.1016/j.immuni.2013.07.002

T cell engineering as therapy for cancer and HIV: our synthetic future

Carl H. June and Bruce L. Lenardo

It is now well established that the immune system can control and eliminate cancer cells. Adoptive T cell transfer has the potential to overcome the significant limitations associated with vaccine-based strategies in patients who are often immune compromised. Application of the emerging discipline of synthetic biology to cancer, which combines elements of genetic engineering and molecular biology to create new biological structures with enhanced functionalities, is the subject of this overview. Various chimeric antigen receptor designs, manufacturing processes and study populations, among other variables, have been tested and reported in recent clinical trials. Many questions remain in the field of engineered T cells, but the encouraging response rates pave a wide road for future investigation into fields as diverse as cancer and chronic infections.
WHAT IF?
Systematic cellular reprogramming

Induced pluripotent stem cells

Cell therapy and regenerative medicine

Highly Efficient Reprogramming to Pluripotency and Directed Differentiation of Human Cells with Synthetic Modified mRNA

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Nature Methods | Brief Communication

Cas9 gRNA engineering for genome editing, activation and repression

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NATURE REVIEWS MOLECULAR CELL BIOLOGY

Synthetic biology in mammalian cells: next generation research tools and therapeutics

Florian Lienert, Jason J. Lohmueller, Abhishek Garg & Pamela A. Silver

Affiliations | Corresponding author

Published online 17 January 2014

Gerontology

October 2015

Synthetic Biology: Rational Pathway Design for Regenerative Medicine

Davies J.A.
Centre for Integrative Physiology, University of Edinburgh, Edinburgh, UK
WHAT IF?

https://www.youtube.com/watch?v=ylSZN-1hhoY
So why is synthetic biology causing such a big fuss?

“Synthetic biology aims to design and engineer biologically based parts, novel devices and systems as well as redesigning existing, natural biological systems”
“SynBio is the application of science, technology and engineering to facilitate and accelerate the design, manufacture and/or modification of genetic materials in living organisms.”
Synthetic Biology is a rapidly growing field

Citations of papers containing key works synthetic biology 70256 - 47,000 papers since 2001
A growing community of student researchers – growth of iGEM
International Genetically Engineered Machine Competition

280 teams are registered for 2015
259 teams at the Jamboree
~15,000 iGEM alumni
Synthetic Biology has a powerful vision for merging engineering design practice into the construction of biology systems and cells at the genetic level.
In engineering systems, robustness and stability are achieved by

(1) System control
(2) Redundancy
(3) Modular design
(4) Structural stability
An engineering design framework for Synthetic Biology

(1) *System control* (feed-back/ feed-forward biological control networks)
(2) *Redundancy* (gene duplication/ multiple regulatory pathways)
(3) *Modular design* (evolutionary robust / multi-functional / compartmental)
(4) *Structural stability* (homeostasis)

Hypothesis – Are these also intrinsic features of complex natural living systems?

A systematic engineering framework for biological systems aims to test the hypothesis
Can we use Biology to Build new Biology? Can we learn about biology through design and construction?

- Biological systems are modular
- Biological function is primarily encoded in DNA
- Large knowledge base of genome sequences
- Large diversity of biological parts (genes/regulatory)
- Increased understanding of molecular / cell biology
- New technologies to synthesize and assemble DNA

However......
Standardising biology poses challenges

• Biology is not fully ‘plug and play’
  – Context dependency
  – Evolution, adaptation and natural selection
  – Non-predictive stochastic behaviour
  – Self assembly and emergent properties
  – Non-linear dynamical processes
  – Multi-scale interactions

• Living cells have constrained volumes and high concentrations of biochemical components
One approach to overcome biological complexity in engineering biology is the use of **Systematic Design**
What is Systematic Design?

Systematic design is founded on the following engineering principles

- Modularisation – interchangeable modules
- Standardisation – standard parts and processes
- Abstraction – reducing complexity

Systematic design aims to achieve Robustness and Reproducibility

Key requirement is interoperability
A systematic design framework using genetic parts that encode biological function

Modularity

Typical gene transcription module
- Ribosome binding site
- Protein coding sequence
- Terminator
- Transcription factor

Abstraction hierarchy

Systems

Devices

Parts

DNA

ATCGGTCAAGTGCCCT
A systematic **DESIGN CYCLE** for Synthetic Biology

- **Learn**
- **Design**
  - Specification
- **Build**
  - Parts
  - DNA Assembly
- **Test**
- **Devices & Systems**
  - Responsible Research & Innovation
Can we build new biological systems with standardised DNA Parts?
To enable forward engineering the synthetic biology field needs to develop standards.

The first standard thread Sir Joseph Whitworth 1841
How do we standardise the construction of living matter?

E. coli

B. subtilis

Bacillus megaterium
Standards development in synthetic biology

- Standard interchangeable biological parts

- Physical standards (DNA)
  - Assembly standards (may not be needed with increasing DNA synthesis)

- Functional standards
  - Standard culture conditions (media/temp/volume)
  - Standard measurements (e.g. Flow cytometry)
  - Standard strains of cell hosts or chassis

- Digital Information standards
  - SBOL
  - SBML
  - DICOM-SB
Different applications

Synthetic Biology Foundational Technology

- Chassis/Host cell Charact.
- Bio-CAD Design tools
- DNA Synthesis And Assembly
- Part / Device Charact.
- Genome editing / screens
Current Synthetic Biology research trends

• Engineering of biological systems
  – Refactoring and Redesigning
  – Genome editing
  – Genome construction
  – Automation, standards and tools
  – Deskilling and open source

• Creating alternative biological systems
  – exobiology/XNA
  – Artificial cell and Cell free systems
Synthetic Biology application trends

- Foundational tools
- Therapeutics & Novel Drug Delivery systems
- Agri-Science
- Fine Speciality Chemicals
- Bio-manufacturing processes
- Commodity chemicals
- Biomaterials
DESIGN
Available Bio-Design Tools

Pathway and circuit design
MATLAB: Simbiology http://www.mathworks.co.uk/products/simbiology/
OptCom http://maranas.che.psu.edu/software.htm
Cell designer http://www.celldesigner.org/
ProMoT http://www.mpi-magdeburg.mpg.de/projects/promot/
GenoCAD http://www.genocad.org/
Operon calculator https://salis.psu.edu/software/OperonCalculator_EvaluateMode

Biopart design
Rosetta. http://maranas.che.psu.edu/software.htm
NUPAC http://www.nupack.org/
RNA Designer http://www.rnasoft.ca/cgi-bin/RNAsoft/RNAdesigner/rnadesign.pl
mfold/UNAfold http://mfold.rna.albany.edu/
RBS Calculator https://salis.psu.edu/software
RBS Designer http://rbs.kaist.ac.kr
UTR designer http://sbi.postech.ac.kr/utr_designer

Miscellaneous
R2oDNA Designer http://r2odna.com/
SBOL http://www.sbolstandard.org/
SBOLv http://www.sbolstandard.org/visual
Part registries worldwide
BUILD

Parts
Costs of DNA synthesis is driving the field

Cost per base
- sequencing ~0.000001 $
- synthesis ~0.10 – 0.28 $

Rob Carlson  http://www.biodesic.com/
DNA assembly Standards

Interoperability

MODAL – Modular Overlap Directed Assembly with Linkers
(A. Casini et al NAR 2014a and 2014b)

BASIC - Biopart Assembly Standard for Idempotent Cloning

Long-overhang assembly No PCR!
Robust reactions easy to automate
Constructing Synthetic Yeast: **Sc2.0**

Design, Synthesise & Assemble a modified version of the *S. cerevisiae* genome 12 million bp and 16 chromosomes

www.syntheticyeast.org
www.syntheticyeastresource.com

Jef Boeke (NY Medical School)
A global synthetic biology project
Sc2.0: 16 chromosomes, 12 million bp
Total Synthesis of a Functional Designer Eukaryotic Chromosome

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TEST
Automation characterisation platform v1.0

C. Hirst, R. Kitney, G. Baldwin
Sample prep
Outgrowth and arrangement
Culture maintenance, sampling and measurement

- Cells kept at similar phase of growth
  - Ensure high quality data
  - Ensures cells are at an appropriate population for assay

- Growth and measurement separated
  - Reduces noise in data
  - Greatly reduces evaporation
Anderson 22x promoter characterisation

Single cell versus population measurements show high degree of correlation

C. Hirst, R. Kitney, G. Baldwin
Data Sheets for Parts and SynBIS
Automation characterisation platform v2.0 @Imperial College
Summary

• Automation and standardised metrology is accelerating the application of synthetic biology
• Data for part / device characterisation is being shared openly
• New chassis are being constructed e.g. Sc2.0
• Standards are being developed and shared
• Huge growth and interest by younger researchers
• Non-biologists are now doing synthetic biology e.g. Engineers
• Significant growth of community labs worldwide- see (www.biobuilder.org)