Synthetic Biology: from parts to modules to therapeutic systems

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Synthetic biology in different chassis

- bacterial
- mammalian

Just adapt parts
Different parts & devices

degree of similarity?

Different engineering principles
Fundamental questions to address

- How is mammalian synthetic biology the same and how different than bacterial & yeast synbio?
- How can efforts in mammalian synthetic biology help other chassis?
- What are potentially transformative applications?

Different engineering principles?

- Robustness
- Regulation complexity
- Crosstalk
- Genetic stability
- Multicellularity
- Part toolbox
Synthetic biology applications

- **Bioenergy production**
  - biodiesel
  - hydrogen
  - methane
  - ...

- **Microbial biochemical synthesis**
  - artemisinin
  - other pharmaceuticals

- **Environmental applications**
  - environmental remediation
  - toxin sensing
  - explosive sensing

- **Biomedical applications**
  - cancer therapeutic agents
  - artificial tissue homeostasis
  - programmed tissue regeneration
  - artificial immune system
From idea to implementation
Overview

• Basic modules
  – Digital, analog, multicellular

• Larger scale system design
  – RNAi based cancer therapy
  – Artificial tissue homeostasis for β cells
Library of parts and devices (partial list)

**Regulation**

**Cell-cell communication**

**Reporters**

**Interface with cell & environment**

- **Regulation**
  - Diagrams showing various regulatory mechanisms.
  - Examples include DNA, mRNA expression, and Ste5 recruitment.

- **Cell-cell communication**
  - AHL (acyl-homoserine lactone) signaling pathways.
  - Examples include LuxR-GFP reporter constructs.

- **Reporters**
  - Various reporter constructs for different applications.

- **Interface with cell & environment**
  - Holin protein-mediated transport mechanisms.
  - Clockwise rotation for biological processes.
  - Dark sensor and repellent mechanisms.
Modules implemented in Weiss lab

**Ultrasensitive Switch**

\[ \text{EYFP} = \text{NOT(NOT(aTc))} \]

- **Sender:** send(AHL)
- **Receiver:** If (AHL = medium) then express (RFP)

**Ring-like spatial patterns**

\[ \text{aTc} \]

**Pulse Generator**

- **Sender:** send(AHL)
- **Receiver:** Transient GFP expression
BioCompiler: A tool chain for synthetic biology

High Level Description
- High level simulator

Formal Description
- Coarse chemical simulator

Abstract Genetic Regulatory Network
- Detailed chemical simulator

DNA Parts Sequence

Assembly Instructions
- Testing

Cells

Modular architecture also open for flexible choice of organisms, protocols, methods, ...

Jake Beal, BBN
Doug Densmore, Bu
Jon Babb, MIT
Noah Davidsohn, MIT
A biocompiler example

(yellow (not (cyan (AHL))))

if (AHL is high)
  produce Cyan
else
  produce Yellow

High Level Description

Formal Description

Abstract Genetic Regulatory Network

DNA Parts Sequence

Assembly Instructions

Cells

Testing

AHL

LuxR

CFP

B

EYFP

pHef1a

LuxR

pLux

CFP

pTre

LacI

mirff4 pHef1a-LacO1Oid

EYFP

4xff4

Aspirate

Dispense

20 μl "Work" (Col. 1, Row 2)

20 μl "Work" (Col. 1, Row 3)

20 μl Water free dispense "Work" (Col. 2, Row 2)

20 μl Water free dispense "Work" (Col. 1, Row 3)
BioCompiler Optimization

Copy Propagation

Dead Code Elimination

Dead Code Elimination
(def sr-latch (s r)
  (letfed+ ((o boolean (not (or r o-bar)))
             (o-bar boolean (not (or s o))))
    o))

(green (sr-latch (aTc) (IPTG)))

Jake Beal, BBN
Aaron Adler, BBN
Fusun Yaman, BBN

(see poster)

More complex circuit: feedback latch

Unoptimized: 15 functional units, 13 transcription factors

Final Optimized:
5 functional units
4 transcription factors

Dead Code Elimination
BioCompiler capabilities

Combinatorial logic

\[
\text{2-bit adder}
\]

\[
\text{Spatial}
\]

\[
\text{State}
\]

Beal, Bachrach, SCW (2008)
Cancer therapy: decipher the transcriptome

• **Problem:** Most existing cancer therapies are not specific enough and result in significant collateral damage.

• **Goal:** Develop an adaptable, effective and highly specific cancer therapy that evaluates internal cell state using combinatorial logic

• **Approach:**
  (1) a “smart virus” infects a cell,
  (2) computes whether the transcriptome is indicative of cancer, and if so,
  (3) decides to destroy the cell

• **Note:** Also, non-clinical applications

Zhen Xie
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ETH

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ETH
HeLa cell classifier

**High level behavior:**
Detect (and kill) HeLa cells

**Bio-program:**
HeLa = $miR-21 \land (miR-17 + miR-30a) \land \neg miR-141 \land \neg miR-142 \land \neg miR-146$

$$
\begin{align*}
\uparrow & \quad miR-21 \\
\uparrow & \quad miR-17 + miR-30a \\
\downarrow & \quad miR-141 \\
\downarrow & \quad miR-142 \\
\downarrow & \quad miR-146
\end{align*}
$$

$A \land B = A \text{ and } B$

$\neg A = \text{ NOT}(A)$
HeLa-Low microRNA sensors

A HeLa-Low sensor: $\sim\text{miR-X}$

Combining multiple HeLa-Low sensors: $\sim\text{miR-141} \land \sim\text{miR-142} \land \sim\text{miR-146a}$

$A \land B = A \text{ and } B$

$\sim A = \text{ NOT}(A)$
HeLa-high sensors

HeLa-High sensor: *miR-X*  
Combining: *miR-21 ∧ (miR-17 + miR-30a)*
Full classifier circuit

\[ miR-21 \land (miR-17 + miR-30a) \land \neg miR-141 \land \neg miR-142 \land \neg miR-146a \]
Verifying all 32 input combinations

<table>
<thead>
<tr>
<th></th>
<th>2H/3H (−)</th>
<th>1H (−)</th>
<th>2H/3H (+)</th>
<th>1H (−)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4L</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>5L</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>6L</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
</tbody>
</table>

Used HeLa cells
- Transfected 4L/5L/6L to emulate indicated levels
- Disrupted microRNA sites for 1H/2H/3H as indicated in the four panels
HeLa classifier full circuit and subcircuits
Selective killing with transfected circuit
What’s next?

Therapy?

Classifier circuit

Cell type identification

Complexity

Delivery

Robustness

Construction

Integration

Classifier circuit

Construction

Robustness

Delivery

Complexity

Cell type identification

iPS Cells

Mesoderm (Middle Layer)

Endoderm (Internal Layer)

Ectoderm (External Layer)

Cardiac Muscle

Tissue Cell of the Kidney

Smooth Muscle (In Gut)

Lung Cell (Aerosol Cell)

Pancreatic Cell

iPS Cells of Epithelium

Neuron Cell

Pigment Cell
Tissues by design

ES or iPS cell → population of cardiomyocytes

Fundamental question in tissue engineering:
Can we create and maintain large scale spatially defined functional tissues?
Directed differentiation with cell fate regulators

- Sox17
- MyoD
- Cdx2
- Ppar-γ
- Mash1
- Ngn1
- Gata4
- Directed
- differentiation
- with
- cell fate
- regulators
Artificial tissue homeostasis for β cells

- 7.8% of the US population has diabetes
- In Diabetes Type I (10% of diabetics), auto-immune response (slowly) kills insulin-producing pancreatic β cells.

**Goal:**
Maintain population level of β cells using auto-regulated differentiation of ES cells that counter-balances auto-immune attacks.

Complex system with 22 components

Design with ‘known’ modules!
A self-timed genetic program for β cell differentiation

High level behavior:

“Self-timed, multi-step β cell differentiation”

Bio-program:

input: DOX
if (DOX)
  express(Endo-CFR)
if (Endoderm)
  express(Islet-CFR)
if (Islet)
  express(GFP)
A self-timed genetic program for β cell differentiation

Module A: constitutive expression
- pEF1a → rtTA

Module B: inducible with Dox (to start the circuit)
- pTRE → gata4

Module C: active in endoderm
- phAFP → ngn3 2A pdx1 2A dsRed

Module D: active in pancreatic (beta-like) cells
- phIns → egfp

iPS/ES → Gata4 → Endoderm → Pdx1, Ngn3 → Pancreatic Beta-like Cells
A self-timed genetic program for β cell differentiation

**Gata4 (-Dox)**

**Gata4 (+Dox)**

**Sox17 (+Dox)**

**BF**

**GFP**

**Dox**

**Hef1α**

**rtTA**

**TRE**

**Endo-CFR**

**GFP**

Endogenous differentiation pathways
A self-timed genetic program for β cell differentiation

Endogenous differentiation pathways
Mean FACS values

Clone #

BF + all  MIP  AFP  Glut2
Insulin-production assay with C-peptide antibodies

Program 1

GATA4
Ngn3
Dox

Program 2

GATA4
Ngn3
Pdx1
Dox/aTc

Neg. ctrl

CCE

Pos. ctrl

Beta TC6
Teaching stem cells a new language

Mammalian Sender

Mammalian Receiver

AHL Dosage Response

AHL (uM)

GFP FLR (A.U.)

Events

Fluorescence (A.U.)

neg. ctrl

LuxIm + ACPm + Citxm

LuxR + GFP

PHEF1

LuxR + DsRed

PHEF1

Citx

PHEF1

ACP

PHEF1

Luxl

PHEF1

Luxl

PHEF1

Luxl

PHEF1

Luxl

PHEF1

Citx

PHEF1

Citx

AHL
Microscope observations

+ AHL

- AHL

BF | Red | Green
Information processing: toggle switch

Toggle Switch Bistability
HIGH - LOW

Toggle Switch Bistability
LOW - HIGH
Module: population_control()
{
    if (state == uncommitted)
        send(AHL)
    if ((state == uncommitted) &&
        (Tat > threshC) &&
        (AHL > threshUC))
        growth_arrest()
}

Module: run_oscillator()
{
    if ((state == uncommitted) &&
        (Tat < threshC) &&
        (oscillator > threshosc))
        state = committed
    if (state == committed)
        send(Tat)
}

Module: differentiate()
{
    input: DOX
    if (DOX)
        express(Endo-CFR)
        if (Endoderm)
            express(Islet-CFR)
            if (Islet)
                express(GFP)
}
System architecture 1

Using phenomological model with ODE’s
System architecture 2: add Switch

Main idea:
Fast feedback that reports on \textit{decision} to differentiate and decouples lengthy differentiation process.
Simulation of artificial tissue homeostasis

Blue = uncommitted
Red  = committed
System architecture 3: add oscillator

Main idea:
Exploit inherent biological noise to break symmetry

Influence of rate constants on system performance (S/N)

System dynamics
System 3: Oscillator module phenotypes

Important question in system design and construction:

*How do you optimize individual modules prior to system integration?*

Relate module phenotypes to overall system performance!

Influence of oscillator phenotypes on system performance
Simulation of artificial tissue homeostasis

Blue = uncommitted
Red = committed
Artificial tissue homeostasis gene network

\[ I1 \rightarrow R1 \rightarrow R2 \rightarrow R3 \rightarrow A2 \rightarrow GAF \]

\[ A1 \rightarrow R4 \rightarrow R5 \rightarrow R6 \rightarrow R7 \]

\[ Rec1 \rightarrow Gata4 \rightarrow Ngn3 \rightarrow Pdx1 \rightarrow AFP \]
Artificial tissue homeostasis gene network

Diagram showing interactions between genes including Rec1, GAF, AI1, A1, I1, R2, R1, A2, R4, I2, R3, R5, R6, R7, Ngn3, Pdx1, Gata4, and AFP.
Artificial tissue homeostasis gene network

- Oscillator
- Uncommitted Pop.
- Committed Population
- Differentiation
- Toggle Switch
- A1, A2, R1, R2, R3, R4, R5, R6, R7
- AI1, AI2, J1, J2
- Rec1, GAF, Rec2
- Ngn3, Pdx1, Gata4, AFP, Gata4
Main idea:
Rapid lateral inhibition to throttle commitment process during toggle switch transition
Summary

• Implemented a variety of basic modules
  – Digital cascades, pulse generator, band detect, population control, communication, toggle switch

• Pursuing applications
  – Programmed tissue regeneration, artificial tissue homeostasis, artificial immune system, RNAi-based cancer therapy

• Exploring system design principles and technologies
  – Hybrid circuits, multicellular, larger scale systems-level integration
  – Computational tools for unknown environments and imprecise information
Outlook for synthetic biology design

**Today:**
- model-guided design using approximations from simulations
- initial part characterization
- working small modules
- iterative construct/debug cycle

**Future:**
- better understanding of circuit interaction with cellular context
- predictive design & construction of large circuits

**Soon:**
- end-to-end (high level design to automated DNA assembly)
- composition rules for small circuits
- orthogonal part libraries that minimize interaction with cellular context
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