

Abstract

Look around you. Engineers have designed and created most of the advanced technologies and complex devices around us today. However, no engineer has yet been able to come close in either the design or creation of a system that rivals the intricacies and complexities of biological systems. Even the simplest living biological systems, single cellular microbes, accomplish tasks that are not even imaginable to the current engineer.

Understanding and harnessing the power of biological systems is a major engineering challenge. The first step is to reverse engineer the innards of simple microbial systems. But we will not know we have completely understood the system until we can forward engineer synthetic organisms and create artificial life. This is undoubtedly a difficult endeavor and brings many moral, ethical, and practical implications. There are many questions that need to be asked and not many easy answers. In the process of studying Nature, we hope to learn much about engineering complexity and, perhaps, even understand this thing we call life.

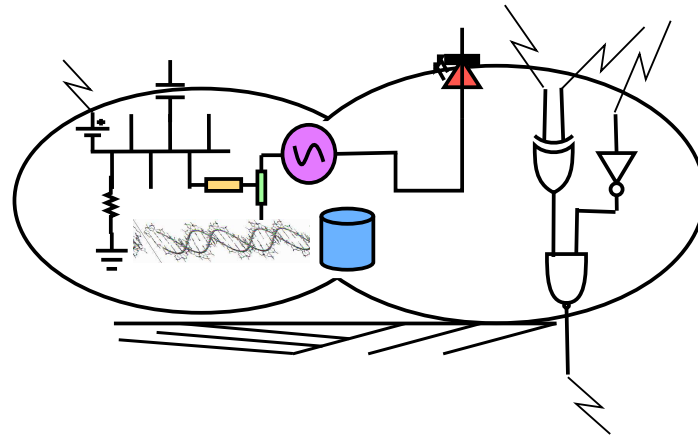
The Next Step in Bioevolution: Engineering the Microbial World

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Dangerous Ideas Seminar

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<http://syntheticbiology.org/>

Pretty picture slide

Bacteria really exist!



What am I doing here?

Cells can already multiply and divide. Can we teach them to add and subtract?

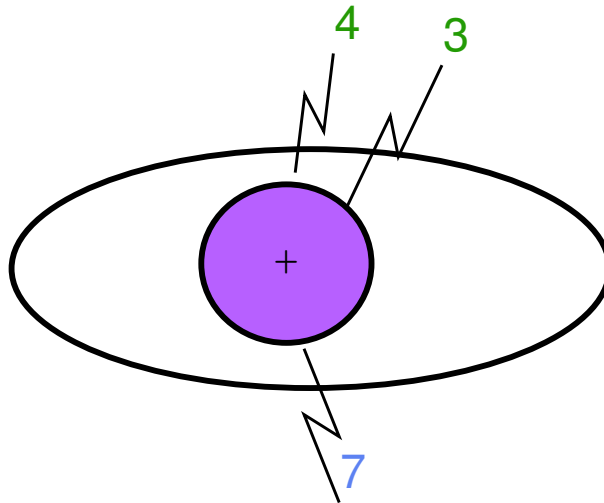
Focus on *engineering* and not just science

Reverse engineering existing, working systems

Forward engineering new (working?) systems

Questions that no one can answer but we should all ask

▶ *Can we? Should we? Will we?*



O Bacterium, My Bacterium!

Bacteria live the happy life.

Bacteria are simplest organisms that can survive autonomously

Tools for manipulating bacteria

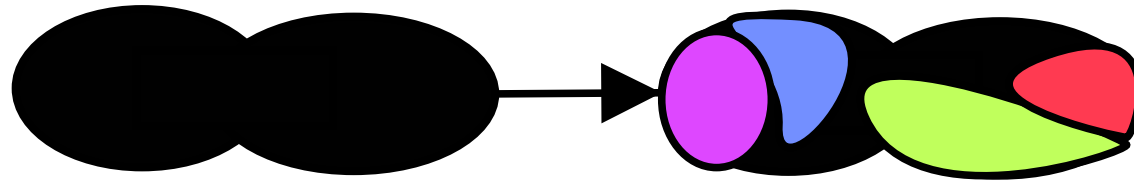
- DNA sequencing becoming faster and cheaper
 - ▷ *Around 100 complete microbial genomes sequenced*
- DNA synthesis becoming faster and cheaper
- Understanding of the intricacies of many genetic networks

Bacteria are everywhere (10^{30} bacteria on Earth vs. 10^{10} humans)

You have as many bacterial cells in your gut (10^{14}) as you have total human cells

Reverse engineering and simplifying existing organisms

Nature gets an A for effort but an F for design.



Many working organisms to use to compare and to take apart

Understand functionality of parts by breaking the system

Remove complex parts that we don't understand

- E. coli is most well studied organism but over a third of genome codes for proteins of unknown function
- Study complexity by adding back one piece at a time
 - ▷ *Some pieces will not be added back*

Minimal living organisms can help define life

Great things can be reduced to small things, and small things can be reduced to nothing.

What is necessary for life? What does it mean to be living?

- Can we optimize the code? All genomes can be compressed.
- What is the smallest working program?

<i>Species</i>	<i>Base pairs (bp) of DNA</i>
Amoeba dubia	670Gbp
Amoeba proteus	290Gbp
Lilium longiflorum (lily)	90Gbp
Human	3.4Gbp
Eschericia coli	4.6Mbp
Mesoplasma florum	860 Kbp
Mycoplasma genitalium	580Kbp
Nanoarchaea equitans	500Kbp
Human immunodeficiency virus 1	9.8Kbp
Single gene	0.5Kbp

No relationship between complexity and genome size (or number of genes)

Stripping down to the bare essentials

Creating the foundations for our chassis and power supply.

1. Start with a working system (Mesoplasma florum)

▷ *Non-pathogenic, convenient to grow, small genome*

2. Rip out a piece and make it simpler

▷ *Hope the system still works...*

▷ **...and if not, fix it and learn new biology**

3. Go to 2

4. Characterize thoroughly what remains

5. End with a not so happy cell but happy engineers

Would we get a different “minimal” organism if we start with a different system or rip out different pieces?

Code rewrite and audit

The code is 3.5 billion years old; it's time for a rewrite.

- Remove spaghetti code
- Eliminate unnecessary non-coding regions
- Standardize and characterize regulatory regions
- Reclaim codon usage in proteins (eliminate redundancy)
- Add debugging hooks and comments!

```
/* Copyright (c) God
```

```
Major revisions:
```

```
0 create prototype
```

```
30 functional RNA and proteins
```

```
90 added DNA. major release!
```

```
100 standardize genetic code
```

```
140 new feature: fermentation
```

```
300 respiration developed */
```

```
/* FIXME: Redundant code */
```

```
TGAAGTCGGTTCCTTCAATGGTTAAAAACAAAGGCTTACTGTGCGCAGAGGAA
```

```
/* Unreachable code! */
```

```
CCCATCTAGCGGCTGGCGTCTTGAATGCTCGGTCCTTTGTCATTCCGGATTAGTGTCTATCACCC
```

Tools needed for forward engineering of synthetic systems

A Scientist discovers that which exists. An Engineer creates that which never was.

Debugging code

- Add `bio_printf`
- Can we comment out or remove debug code when not needed?

Comment the code

- Annotation of the *M. florum* genome

Random variations in gene expression and chemical reactions

- Negative feedback to reduce variation and speed up time to reach steady state

Need a standard language like in circuit design

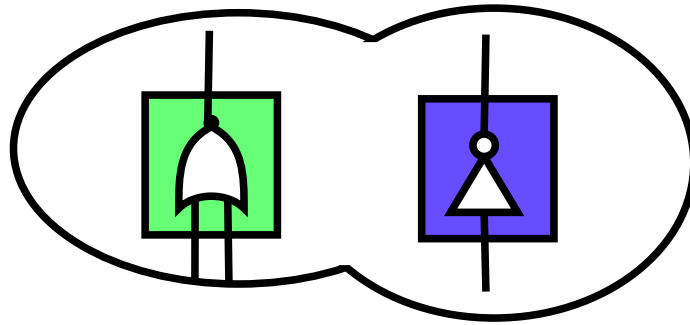
- Common parts and modularity
- Quantitative characterization and measurements of signals

BioBricks

The Legos of the Future

The TTL Data Book for biological systems

- Create standard components and characterize them
- Allow for connectivity and compatibility between modules



- Make standard logic gates

Eliminate the exceptions and make it always work (except when it doesn't)

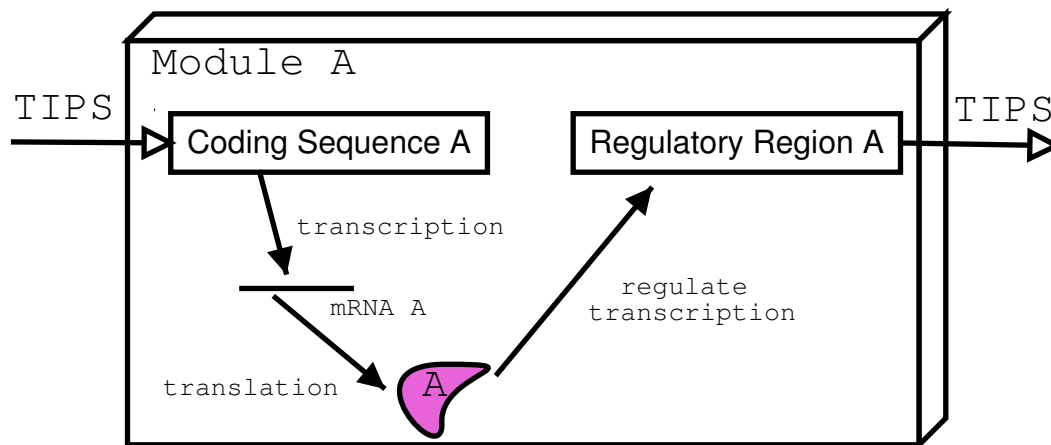
Standardized assembly and synthesis procedure

Standardizing Signal Measurements

It's time to digitize cells!

What should be used as the signal within a cell? What's a 1 or a 0?

- States of proteins (e.g. phosphorylation)
- total protein levels
- mRNA levels
- transcription initiations per second (TIPS)



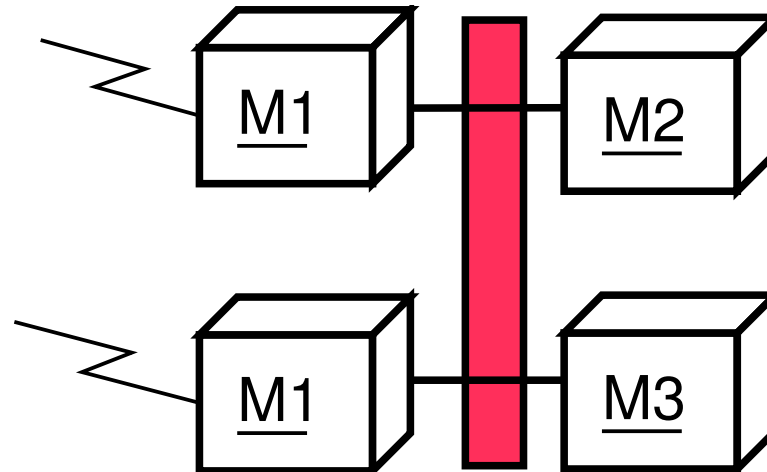
Utilizing Modularity

Modularity facilitates code reuse and is thus the number one priority for the lazy engineer.

Living systems are roughly modular

Modules cannot be simply reused within a cell

- Cannot isolate components
- Problem is that we don't have a generic insulating wire



- Will have undesired crosstalk

Will we run out of modules? How complex of a circuit is practical? Is this scalable?

Input/Output and Cellular Communication

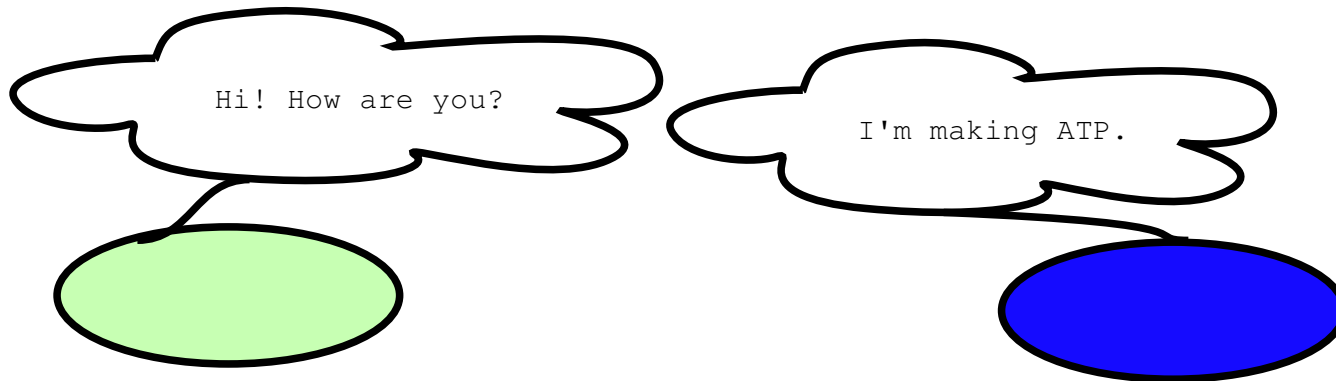
Can you hear me now? Good!

Need easy to use I/O interface

- Many two-component systems. Sensor/regulator pairs.
- Responsive to many environmental cues

Intercellular communication

- Quorum sensing
- Relies on diffusion and gradients



Clocking and syncing cells

- Some bacteria have circadian rhythms set by light

Light-based Input/Output

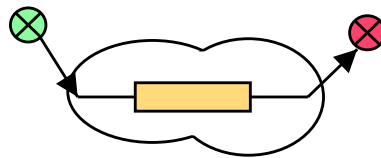
Cells already know SOS–Morse code should be easy.

Chemical inputs

- Chemicals can persist in environment for relatively long time
- Exact concentration of chemicals is difficult to control over time

Light input

- Important for life
 - ▷ *Many cells naturally respond to light*
- Easy to control
 - ▷ *Light can be switched on and off much faster than a chemical signal*
- Potentially information encoded in wavelength



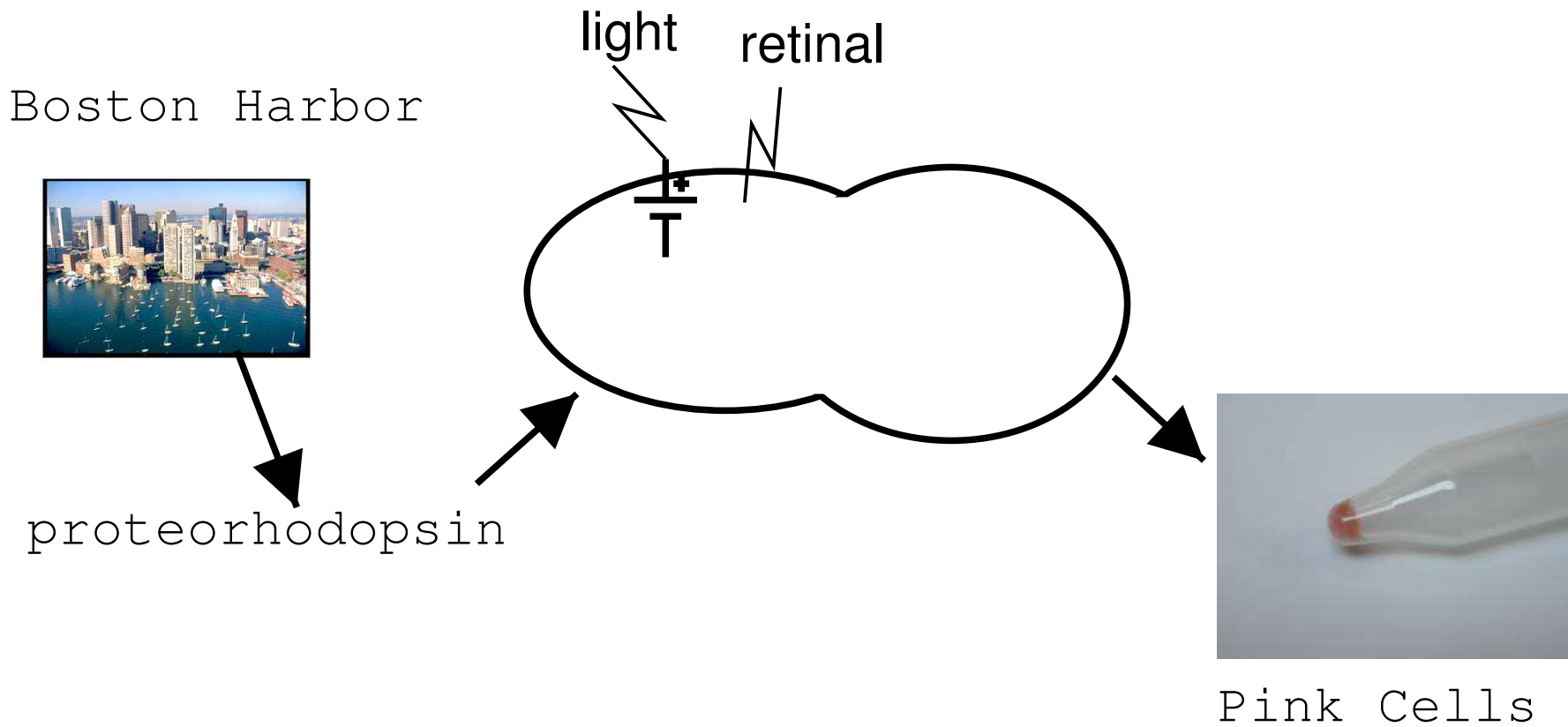
Light output

- Bioluminescence
- Fluorescence

Cellular Batteries

Runs on AAAAAAAAAAAAAAAAAAAAAA batteries.

Potential light powered organisms



Nanotechnology: The power of the tiny

Cells invented nanotechnology billions of years ago.

Largest

DNA length	10^{-3} (mm)
Human hair width	10^{-4}
Flagella length	10^{-5}
Cell diameter	10^{-6} (μm)
Transistor	10^{-7}
Flagella diameter	10^{-8}
Protein diameter	10^{-9} (nm)
atom size	10^{-10} (\AA)

Smallest

Combine artificial nanotechnology with the natural nanotechnology

Can we utilize cells as tools for nano-engineering?

- Cellular factories
- Ribosomal assembly lines
- Self assembly

Microbial Robots

Who wants a robot dog? I want a robot bacteria.

Autonomous

- Harvest energy from light
- Find food by following sugar gradients

Interact with the world

- Cells are responsive to environmental signals such as light, oxygen, temperature, pH, and chemicals such as food and sugars.
- Actuators and forces on order of pN-nN
- Bioremediation

Programmable and Cheap

- Self-replicating

Redirecting Evolution

Did God create us so we could become God ourselves?

Do we have the right to create life? Can we handle the responsibility?

Bacteria are ecologically extremely important

- Impact of mass destruction or creation of bacterial species is unknown

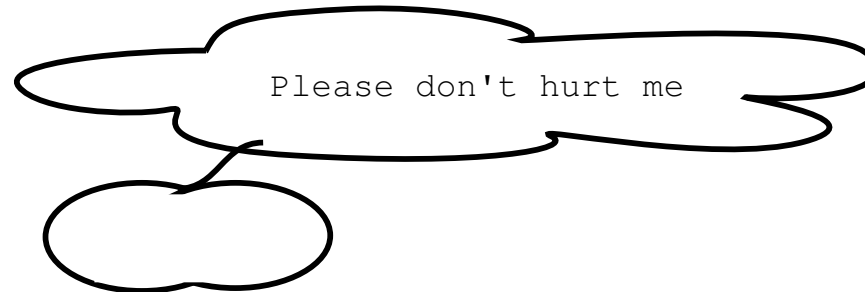
Human-directed evolution

- Can we do better than nature?
- Can we speed up evolutionary processes?



People for the Ethical Treatment of Bacteria (PETB)

Just say NO to mouthwash.



Reverse engineering biology may not be illegal (yet), but does that mean we should do it?

Are there any issues with enslaving and reprogramming living organisms to do our bidding?

At what point should ethics come in?

What if we used human rather than bacterial cells?

The Bad

Reality may be much weirder and scarier than science fiction.

Will we create a Frankensteinian monster? Probably not.

Do we really know what we're doing? Definitely not.

What if this turns into a real dangerous idea? Biological warfare?

- Doesn't take much to create something dangerous to ourselves

What if we lose control of cells we create?

Are we willing to risk all the bad things that *may* happen?

The Good

The future depends on many things, but mainly on us.

Scientific discovery

- Building a cell ourselves will give us insights into the mind of Mother Nature
- Lead to medical insights and discoveries

Cellular computing

- What will CelIOS 1.0 look like?
- Cellular memory looks to be a realistic possibility
 - ▷ *Being done with bacteriorhodopsin*
- Inherent parallelism in cells

Redefine life

- Understand what it means to be alive
- Is artificial life an oxymoron?
- Stimulate philosophical debate

It's a small world after all

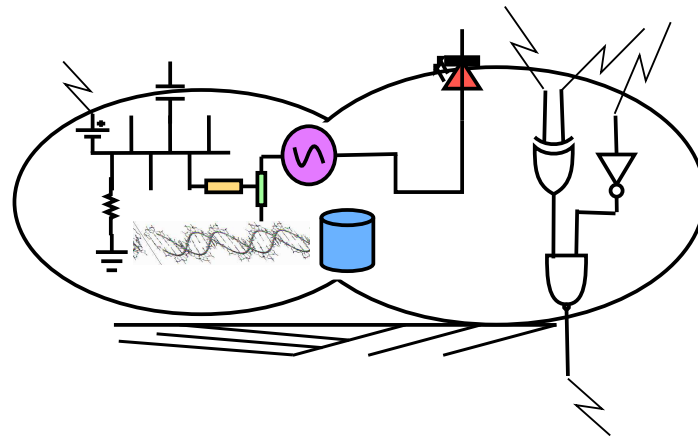
*It's a world of laughter, a world of tears,
It's a world of hopes and a world of fears.*

Potential of the micro-world to make a macro-impact

Hope that we can make creations as successful as nature

Hope that the technology is not put to destructive uses

Hope that I will be there for all of the fun!



People

Who are all these crazy people?

<http://syntheticbiology.org/>

Drew Endy	Biology and Biological Engineering
Tom Knight	AI Lab
Greg Fournier	AI Lab
Heather Keller	Endy Lab
Sriram Kosuri	Endy Lab
Randy Rettberg	AI Lab
Maya Said	EECS
Gerald Sussman	AI Lab
Samantha Sutton	Endy Lab
Vid Thayalan	Endy Lab