Synthetic Microbiology

Is the Future of Infection Carbon-based?
James T. Griffith, Ph.D., CLS (NCA)
- Chancellor Professor Emeritus
- Dept. of Medical Laboratory Science
- University of Massachusetts
- Managing Partner

Forensic DNA Associates
Orthogonal: Complete isolation of artificial life from “evolved” life

Xenobiology: Make something so totally different that it CANNOT interact with “earth life”
“Evolutionary biology” has combined in many varied ways to create a complex net of genetic variations.
- **Green** = Life forms to date
- **Pink** = SynBio
**Synthetic Biology:** According to the National Academy of Sciences

- The application of engineering principles in order to design and construct new biological parts, devices and systems and to re-design existing natural biological systems for useful purposes.
Our level of manipulation almost amounts to “tinkering” with individual genetic parts.
Synthetic Biology:

- At the end of the day, “synthetic biology” is the same as “evolved biology” except that in synthetic biology;
  - Humans choose particular bio-capabilities
  - Insert them into a “biological chassis”
- Eventually it may be difficult to tell them apart as synthetic life interacts with the evolved biosphere.
A hint that all this is not only possible, but practiced came from a recent article suggesting that remnants of retroviruses that entered the human genome millions of years ago can regulate some innate immune responses.

These viral sequences have previously been linked to controlling early mammalian development and formation of the placenta.

It is now established that one such endogenous retrovirus in human cells can also regulate the interferon response, which helps organisms quickly respond to infections.

Endogenous retroviruses have ends (LTRs), that are optimized to have regulatory sequences in just 300 to 400 base pairs of DNA.
There are at least 27 transposable elements that likely originated from the long repeats at the ends of retroviral sequences. One such element, known as MER41, comes from a virus that invaded the genome approximately 45 - 60 million years ago;

- Present-day human cells contain interferon-inducible binding sites
- Endogenous retroviral elements make up about 8% of the human genome

Dendrogram of various classes of endogenous retroviruses
Synthetic Biology:

- Biology + Engineering
  - Systematic design
    - Computational modeling
    - Molecular parts
    - Standardized measurements

- All of this is novel in the world of “reprogramming cellular systems”
Past 40 years of Genetic Engineering:
- Recombinant DNA (Ligation, RE’s)
- Polymerase Chain Reaction (PCR)
- DNA Sequencing (increasing speed, reducing costs)

Synthetic Biology
- Automated DNA Construction/Printing - $$$
- Standardization
- Abstraction

Synthetic Biology aims to put the “Engineering” in Genetic Engineering
Synthetic Microbiology

- **1960s**
  - Understanding of the genetic code
  - Central Dogma of molecular biology
  - DNA encodes RNA, RNA encodes Proteins

- **1970s**
  - Manipulation / transfer / cloning
  - beginning of “genome revolution”

- **2000s**
  - Sequence the human genome (HGP)
2015

- Complete genomic sequences for most classes of “evolutionary organisms”
- Genetic Sequencing: Reading DNA
- Genetic Engineering: Cutting & Pasting
- SynBio: Writing / Programming new DNA
  - Create new genetic machines from scratch
  - Gain new insights about how life works
TODAY

- Makes it possible to think about “shuffling the deck of genomic cards”
- These data sets are in “web browser” form accessible from anywhere in the world on your cell phone.
  - BioBricksPartsRegistry.org
NEXT

- Should not be a surprise that we now are headed toward:
  - Specifications
  - Mathematical modeling
  - Standardization
  - Prototyping
  - Genome system design
- Bottom - Up
  - Design & Build
    - Synthetic
    - Protocells
  - Use basic chemicals & biochemical building blocks from scratch

- Top – Down
  - Conceptual framework
    - Engineering
    - Systemic design
  - Build new biologic systems via;
    - Integration to robust “bio-parts” into existing system
    - Uses extensive mathematical modeling
Current GMO (Genetically Modified Organism)
- Corn, Rice, etc.
- Usually 1 modified gene

Synthetic Organism (SO)
- Totally custom designed genome
- e.g. 2006 Synbio Rice = Disease, Flood & Stress tolerant
- By 2013 – 4 million farmers
  - Philippines
  - Bangladesh
  - India
Some Specifics

- Comparison between the past 30 years (single-cell technology) and today (SynBIO)
Global Market

- $16 B by 2018
- Pharmaceuticals
- Diagnostic tools
- Chemicals
- Energy products (BioFuels)
Stimuli

- Energy constraints (India, AC)
- Water constraints (12 Biggest Cities)
- Squeeze on cultivable land (next slide)
- Greenhouse gas emissions / Climate remediation
- Cost of health care / disease
Univ. Minnesota (Global Landscapes Initiative)

- Agriculture takes up 40% Ice-free land on Earth
- Accounts for 70% of human water use
- By 2050 there will be 2 Billion more eaters
- Climate change will ↓ crop yields 10-40%
- Food (GMO now) is not Insulin (synthetic HUMAN, 1978)
  - May be some cultural barriers
  - Of course if you are starving, that may fade.
Current Examples

① Food & flavorings Fermented with SynBio yeast
② Muufri (start-up) Animal-free milk
③ Bay-Area Biohackers Vegan cheese
  ■ “crowd-funded”
④ Evolva (Swiss) Saffron
  Vanillin
  Stevia
⑤ Solazyme MicroAlgae “Butter”
  Protein-rich flour
  Vegan protein
Vanillin

- Billions of SynBio combos screened to get
  - Sugar
  - Electricity
  - Water
  - GMO yeast

- NOTE: Much of our current Vanilla is made from petroleum
Friends of the Earth
- SynBio is an extreme form of genetic engineering

Woodrow Wilson Center (Synthetic Biology Project)
- Requisite testing almost MUST involve environmental release in species diversity of “evolved organisms”

U.Cal. Davis
- Each “gene” needs to be tested in “confined field trials”
- Make scientific prognostics regarding co-evolution thereafter
- ? Pollinators
USDA

- Authority is likely “inadequate”

- Example;
  - Kickstart Project to make “glowing plants” as a sustainable natural lighting
  - Fireflies > Mustard Plant > Laser-print DNA > coat on metal particles > Gene-gun shoot into seeds > 600,000 seeds produced in 1st run
  - Did it, legal (wouldn't be in EU)

Source: ZME Science, Mark Zimmer
Technology Needed

- **PCR**
- **1983** Kary Mullis
- MAKE COPIES

Source: ABM, abmgood.com
Technology Needed

- **Restriction Enzyme Cloning**
- **1973 Herbert Boyer, Stanley Cohen**
- **BIOBRICKS, ASSEMBLY**

Source: *Registry of Standard Biological Parts*, parts.igem.org
Technology Needed

- **BioBricks**
- **2003** Tom Knight
- **DENSE CONSTRUCTION** = MAJOR PIECES OF SYNBIO

Source: *Bacterial Crowding Circuit*, biobricks.foundation.org

University of Massachusetts
Technology Needed

- Gel Electrophores
- 1962 (acrylamide gels, sucrose 1930s)
- Ornstein and Davis
- SAMPLE ANALYSIS


University of Massachusetts
Technology Needed

- **Sequencing**
- 1974
- Frederic Sanger
- **CONFIRM ID OF CLONED DNA, ERROR CHECK**

Technology Needed

- **Southern Blot**
- 1975
- Edwin Southern
- **COPY NUMBER DETERMINATION**

Source: http://askabiologist.asu.edu/southern-blotting
Technology Needed

- **Real Time PCR**
- **1993**
- **R. Higuchi**

**BEST COPY NUMBER MEASURE**

Technology Needed

- **Northern Blotting**
- **1977**
- James Alwin, David Kemp, George Stark
- **DETECT AND QUANTIFY RNA**

Technology Needed

- **Microarray**
- 1995
- T.D. Shalon
- ASSAY EXPRESSION CHANGES IN LARGE NUMBERS OF GENES


University of Massachusetts
- Use engineering principles to define part specifications
  - e.g. Think of a solution to a problem, then try to build it.
- RRI = Responsible Research & Innovation
In SynBio, this approach does not work very well without massive computer modeling (e.g. Evolva Vanilla)

It takes a LOT to get this far.
SynBIS

Synthetic Biology Information System

- Imperial College London

SynBio Design Cycle

- **SynBIOS**
- Registry of parts
  - Data about bio parts
  - Mets data
- Web-based, 4-layer architecture
  - Interface
  - Communication
  - Application
  - Database (SQL)
**SynBio Design Cycle**

- **SynBioS**
- Example of data storage hierarchy
- LacUV5
- A microbial promoter

SynBio Design Cycle

- **BioCAD**
- Interface between bio-data, gene capabilities, etc. and actually designing a new SynOrganism
SynBio Design Cycle

- **SynBIS**
- **BioCAD**

**SynBIS** – integrated BioCAD and modelling suite

**DNA Assembly**
- Robust automated DNA assembly methods
- Parts to Genes
- Genes to Pathways

**Characterisation (data for SynBIS)**
- *In vitro*
- *In vivo*
- Reference parts under different conditions

**Chassis (data for SynBIS)**
- *E. coli*
- *Yeast*
- *Bacillus subtilis*
- *Geobacillus*

**Synthetic Microbiology**

- **JCVI – Mycoplasma laboratorium**
  - *Mycoplasma genitalium* – fully synthetic self-replicating organism
  - Very slow generation time

- **Escherichia coli** K-12:
  - MG1655:
    - kBP: 4639
    - Genes: 4434
  - MDS 43:
    - kBP: 3931
    - Genes: 3691
- *E. coli* K-12
- 4,639,221 nucleotide pairs
- 1,546,407 codons
- 30,928 genes (MAX), @ 50 AA/ gene) = 4,000, 3,092, most likely (500 AA/ gene)
All declining:

- 2011
- 2014
You are looking for a “minimal cell” so as to have the least “other stuff” to deal with when you put your stuff in.
You are looking for a “minimal cell” so as to have the least “other stuff” to deal with when you put your stuff in.  

*Mycoplasma pneumoniae*
Researchers transplanted the genome of a *Mycoplasma capricolum* bacterium into *Mycoplasma mycoides* in 2007.

They later accomplished the same trick with a synthetic genome in 2010.

*Mycoplasma mycoides*

Source: *Science* J. Craig Venter Institute
- Make this;
- *Myc. mycoides, SynBio*
- Outer circle = 1 MBP, circular DNA genome.
  - Edited in several places
  - Includes water marks (WM)

## Minimal Cells

<table>
<thead>
<tr>
<th>Size</th>
<th>Organism</th>
<th>Base Pairs (MBP)</th>
<th>Genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1μ</td>
<td><em>E. coli</em></td>
<td>4.6</td>
<td>4,500</td>
</tr>
<tr>
<td>10μ</td>
<td></td>
<td>12.5</td>
<td>5,800</td>
</tr>
<tr>
<td></td>
<td>Eucaryotes</td>
<td>12</td>
<td>5,000</td>
</tr>
<tr>
<td></td>
<td>Some parasites</td>
<td>Much smaller</td>
<td>Rely on host</td>
</tr>
<tr>
<td>1μ</td>
<td>Smallest known free-living photosynthetic eukaryote</td>
<td>12.5</td>
<td>8,000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>144,000 bp</td>
<td>188</td>
</tr>
<tr>
<td>400 nm</td>
<td>Obligatory symbiont, smallest known living org.</td>
<td>490,000 bp</td>
<td>500</td>
</tr>
<tr>
<td>Size</td>
<td>Organism</td>
<td>Base Pairs (MBP)</td>
<td>Genes</td>
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<tr>
<td>------</td>
<td>-----------------------</td>
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</tr>
<tr>
<td>1μ</td>
<td><em>Myco. pneumoniae</em></td>
<td>816,000 bp</td>
<td>680</td>
</tr>
<tr>
<td></td>
<td><em>Myco. mycoides</em></td>
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<td></td>
<td><em>Myco. capricolum</em></td>
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<tr>
<td></td>
<td><em>Myco. genitalium</em></td>
<td>583,000 bp</td>
<td>482</td>
</tr>
</tbody>
</table>
Working backwards from *E. coli* K-12 MDS43

Why not just work forward from it?

- Dr. Jay Keasling, UCB
  - Roughly 95% of time and energy spent in synthetic biology research is identify and correcting for unintended interactions between metabolic byproducts.

Yes, but MDS43 is very minimal already...

- Uppsala iGEM 2011 – Show Color with Color
- Known color sensor genes (cph8, ccaS, YF1 = Red, Green, Blue)
- Color output systems
  - Chromoproteins – amilCP, amilGFP
  - mRFP1 (used native system for regulation – required gene knockout)

Want to eliminate as much “noise” as possible in genetic circuit design process.
Example architecture of SynBIO organism
**Theoretical Minimum**

- Estimated Size:
  - 151 genes
  - 113 kbp
- Lambda-red dsDNA Recombination
  - Bacteriophage proteins with engineered homologous DNA
- *Escherichia coli* K-12 MDS43

- So, where to begin?
  - Any suggestions?
  - Lactose Catabolism
  - Heat-shock/Osmotic Shock Response
  - Easy to quantify effects of gene deletion in the lab for minimal costs
  - True validation – Genome Sequencing
Synthetic Microbiology

- **Applications**
  - **Novel Materials**
    - Spider-silk, Teflon, Kevlar
  - **Fuels**
    - TAGs – *Rhodococcus opacus* PD630
  - **Plastics**
    - PHAs – *Ralstonia eutropha* H16
  - **Petroleum Substitutes**
    - OPX Bio – BioAcrylic
      - $less costly
      - Less (75%) greenhouse gas emissions
      - EDGE (Efficiency Directed Genome Engineering)
        - A microbial redesign process
Applications

Petroleum Substitutes

- Surfactants
  - Currently made from petrochemicals or seed oils (Palm & Coconut)
  - Equivalent to burning 3.6 Billion gallons of gasoline / year
  - IUCS (Columbia University)
    - Agricultural waste → Surfactants directly

- Rubber
  - Biosoprene
  - Instead of rubber from a rubber tree
  - DuPont & Goodyear

BioCosmetics

- Regenerate well-hydrated skin (No wrinkles)
  - Polylactic A. (PLA) - Currently made from Corn-syrup → Lactic A. → Link the short chains
  - Now can make it directly from \textit{E. coli} SynBIO
Applications

Personal Health:

- **BioSpray:**
  - Periodically apply to dissolve dead skin cells
  - Eliminates the need for shaving

- **Oral Wash:**
  - No need for brushing your teeth
  - No tarter, yellowing, halitosis, etc.

- **Indigestion:**
  - Infuse a microbial chassis with custom indigestion-blocking enzymes
  - Note: New England BioLabs currently sells ($235.00) a BioBrick Assembly Kit
Applications

Medicine:

- Microbiome engineering – *E. coli* synthetic circuits to trick *Vib. cholerae*
  - SynBIO *E. coli* makes signaling molecules preventing *Vib. cholerae* from producing toxins
  - SynBIO *E. coli* with modified T7 Bacteriophage, produces DspB that degrades Biofilms

- Cancer treatment –SynBIO *E. coli* with *inv* gene invades hypoxic human cells to shut off CTNNB1 gene known to be active in Colon Ca

- Enhancement of Infection Response

- Elimination of microbial toxins from food poisoning
  - Array #1 = South America
  - Array #2 = Africa
  - Array #3 = Asia

- Malaria
  - Artemisinin Project
    - Make Artemisinin (100% now from *Artemisia annua*), as a SynBIO pharmeceutical
Enhanced systems via removal of genes
Prokaryotic argonaute protein DNA-interference systems

- *Thermus thermophilus*
- *Rhodococcus opacus* PD630?
- *Ralstonia eutropha* H16?
Questions