Synthetic Biological Systems

2. Synthetic Life and Genome Engineering
The construction of synthetic organisms

Synthesising biological life will be a 21st Century Grand Challenge

Designer Organisms

Synthetic Genomes

The Minimal Cell

Designer Organisms
Fascinating big-ticket projects

Making the minimal cell
- Bottom-up approach to build from parts
- Top-down approach to reduce natural cells

Building the first synthetic organism
- J. Craig Venter Institute

Re-factoring a genome
- Re-write the genetics of a cell to suit our needs
- “Genomic Engineering”
Genome Engineering

Full synthesis
- Transform
- Assemble
- JCVI-1.1 580 kb
- Synthetic M. genitalium genome in yeast
- Built for easy redesigns

Minimization
- E. coli K-12
- MDS12, 41, 42, 43
- Eliminate unstable DNA elements

Genome fusion
- Synoecytosis sp. PCC6003 (3.5/3 kb)
- Bacillus subtilis 168 BEST7303 (<4.215 kb)
- Gene tools in one, metabolism in the other

Refactoring
- Redefine connections for module independence

MAGE
- Continuously evolving cell populations
- Many small changes for metabolic optimization or new genetic codes

In vitro
- Synthetic subsets of living systems
- Energy regeneration
- Translation factor (IF, EF, RF, RR)
The first synthetic organism

The 1st synthetic organism – life made from a chemically synthesized genome

tRNA gene synthesized – Nobel Prize for Khorana
Phage/Virus genomes synthesized – synthesis of polio virus 2002
Viruses re-factored – separate each gene in a human-designed logical way

Next... Bacteria
A big two-part project by the J Craig Venter Institute
Part 1: Can a complete DNA genome be synthesized from chemicals 2008
Part 2: Can a cleaned DNA genome boot-up a cell 2007
Synthetic Organism = Parts 1 and Parts 2 combined DOING
The first synthetic organism: (2) Booting-up

AIM: Genome A into Cell B → turn Cell B into Cell A
A: M.mycodies  B: M.capricolum  *different but compatible biology

C Lartigue et al. Science 2007

Comparable to nuclei-switch experiments in In Vitro Fertilisation
Genomes are fragile to handle in the lab – maintain in agarose plugs

How to get DNA into cell B? – incredibly inefficient, requires cell fusions (no cell wall)

What happens to genome of cell B? – doesn’t have antibiotic resistance
Verify with sequencing, proteomics and phenotyping

Itaya et al.: fused complete genome of Synechocystis into B.subtilis - silencing
Holt Lab: build H.influenzae genome as BACs in E.coli - incompatible
The first synthetic organism: (1) Synthesis

AIM: Synthesize $\sim 10^4$ DNA 50-base oligomers and assemble into a complete error-free 582970 bp \textit{M. genitalium} genome (watermarks)

1. Companies synthesise 101 pieces of 5 to 7 kb from overlapping oligos (e.g. Blue Heron and GeneArt)
2. 101 pieces recombined using \textit{in vitro} enzymes to make 24 big pieces
3. 24 big pieces maintained in BACs in \textit{E. coli} and recombined to make even bigger pieces
4. Big pieces all inserted into yeast and whole circular genome is made by recombination using native yeast genetics (using a YAC)
5. DNA sequencing used to check fidelity throughout process

DG Gibson \textit{et al.} Science 2008

2010... Where’s the synthetic organism? – restriction enzymes, methylation
Genome Engineering Stages and Technologies

1. Synthesis  
   traditional oligo chemistry, on-chip polymerisation, cell factories

2. Joining  
   chemical, ligation, polymerisation, recombination

3. Assembly  
   serial, heirarchical, parallel, pooling

4. Error control  
   selection, tuning, repair, purification, sequencing
The minimal cell: top-down approach

Smallest natural genomes = 500 genes 500000 bps of DNA (e.g. *M. genitalium*)
But... not all genes are required for lab-based growth

How many essential genes?
1. **Compare DNA** throughout nature to identify essential genes
   Estimates: 50 to 380
2. **Knock-out (delete) genes** of small genomes to see what is needed
   Estimate: 430

Around a quarter of genes identified by these screens have unknown function
How do we really know that a gene is essential and not just playing many roles in a network?
The minimal cell: bottom-up approach

“We know enough about a cell to identify the essential molecules and build our own from scratch” - hardcore synthetic biology

Biochemistry identifies the essential molecules that make cellular life

DNA $\rightarrow$ RNA $\rightarrow$ Protein

AC Forster & GM Church. Mol Sys Biol 2006
The minimal cell: bottom-up approach
The minimal cell: bottom-up approach

Just how many genes for a bottom-up minimal cell?
• Estimate: 151 genes = 38 RNAs + 113 proteins

<table>
<thead>
<tr>
<th>Function</th>
<th>Components</th>
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<tbody>
<tr>
<td>Basic DNA replication</td>
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</tr>
<tr>
<td>Chaperones</td>
<td>2</td>
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<tr>
<td>Ribosome</td>
<td>63</td>
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<td>RNA transcription</td>
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<td>21</td>
</tr>
<tr>
<td>tRNA modifiers</td>
<td>15</td>
</tr>
</tbody>
</table>

Would require all metabolites (eg. NTP) to be provided – no metabolism
Would have no control over compartmentalisation – no membrane synthesis

Really minimal cell – fragile in vitro system

Add metabolism, add lipid-synthesis for membranes, add proteins to control cell division, pores and transporters for sugar-import

Working minimal cell – capable of self-evolution
Existing *In Vitro* Transcription/Translation

**Cell-free systems**

**Existing use:**
- Protein synthesis for research and screening

**Planned use:**
- Microfluidic systems - e.g. lab-on-a-chip
- Fast mutation and evolution of DNA
Modeling with Genome Engineering

A computational platform to design genomes: needs large-scale bottom-up models

1. Model the central core life functions – Replication, Transcription and Translation
2. Model metabolic networks and enzymes involved
3. Add regulation: a global transcriptional model
4. Improve the models with *in silico* directed evolution
5. Use the models to choose the organisation of genes on the genome
6. Try building versions and testing these
Can we logically re-arrange a genome?

Add spacers, cut sites
Remove redundant DNA
Separate overlaps

- M13 phage
- T7 phage
- Yeast chromosome (part of the synthetic yeast genome project)
Why do genome engineering?

1. **Bottom-up synthetic biology**

- Adding genes and devices should be more predictable
- Creating a whole-cell model should be easier and allow better predictions of behaviour
- Provides a route to designing the chassis cell fit for a specific application
- Removal of unstable / recombination elements
Why do genome engineering?

2. Provides for safer synthetic biology

- Cell can be designed to only survive in lab conditions
- Cell could be made “orthogonal” so that its biology doesn’t interact with nature
- Examples: change codon usage or change stereochemistry
- Better predictability from bottom-up design
Why do genome engineering?

3. Custom synthesis of products

- Cells could be designed to produce non-natural proteins and sugars using synthetic building blocks
- Minimal cells would only use resources to make the desired products and so be more efficient
- Very cheap production of DNA could be engineered
- Synthesis of molecules that are toxic to produce in normal cells
Why do genome engineering?

4. Other areas

- Minimal cell gives us a chance to study the origins of cellular life and potentially exobiology
- Fast evolution can be engineered to rapidly produce new enzymes
- Minimal cells would be easier to integrate into life-on-a-chip systems – e.g. a small screening device that sequences DNA, then synthesizes all the proteins from that DNA and compares their affinity to an antigen
Further Reading

Genome Engineering – PA Carr and GM Church

Towards Synthesis of a Minimal Cell – AC Forster and GM Church
Molecular Systems Biology, Vol. 2 (22 August 2006)

Complete Chemical Synthesis, Assembly, and Cloning of a Mycoplasma genitalium Genome – DG Gibson et.al

Artificial assembly of a minimal cell – G Murtas

Towards the automated engineering of a synthetic genome – J Carrera, G Rodrigo and A Jaramillo
The minimal cell: bottom-up approach