What are the limits of biology?

What is unique about biology?
Synthetic Biology

Alternative Biology

Synthesising Biology

Biology for synthesis

Biology as a tool

Biology as data

• More systematic
• Nature as a starting point
• Biology has limitations and specialisms

“What I cannot create, I cannot understand.”

Richard Feynman
Synthetic Biology is a continuum

Orthogonality

Design

Genetic engineering

Natural isolates
Random mutants
Targeted mutants
Transgens
Engineered enzymes
Gene circuits
Transgenic pathways
Unnatural substrates
Custom genomes
Non-canonical chemistries
Redesigned biological processes

Natural

Synthetic

adapted from de Lorenzo (2010) Bioessays
10.1002/bies.201000099
Information transfer in biology – The central dogma

- Information **storage** and **propagation** are essential for life
- **Central Dogma** – information only accessible from DNA and RNA in biological systems
- Propagation is viable because of the efficient and unambiguous base pairing
Extending biology through directed evolution
Xenobiotic nucleic acids (XNAs)

Phosphate

Nucleobase

DNA

Ribofuranose sugar

HNA

tPhoNA
Synthetic genetic systems (DNA → XNA → DNA)

- Synthesis and recovery of information from synthetic backbone establishes a synthetic genetic system

<table>
<thead>
<tr>
<th>Genetic system</th>
<th>Aggregate misincorporation error (x 10⁻³)</th>
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<tbody>
<tr>
<td>CeNA</td>
<td>4.31</td>
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<tr>
<td>FANA</td>
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<tr>
<td>TNA</td>
<td>52.8</td>
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</tbody>
</table>

Pinheiro et al. (2012) *Science* 10.1126/science.1217622
From synthetic biology to xenobiology

- XNA synthetase
- XNA replicase
- XNA reverse transcriptase

**in vitro**
- biomaterials
- therapeutics
- biosensors

**in vivo**
- Biocontainment
- xenobiology
Challenges to introducing XNA *in vivo*

- XNA chemistry (nucleosides, nucleotides and polymers) must not be toxic to the cell.
- XNA nucleotides must be delivered to (or activated in) the cell.
- XNA nucleotides cannot be incorporated by natural polymerases.
- XNA replicase cannot incorporate dNTPs or rNTPs.
- XNA needs to be replicated and maintained (i.e. episome).
- Precise XNA information has to link to cell survival.
Sources of orthogonality

- Bio-orthogonal chemistry
- Expanded genetic codes
- XNAs
- Unnatural vitamins
- Reading frame of ribosome
- Compartmentalisation
  - Non-native episomes
Towards polymerase rational design

Liu and Cozens et al. (2018) JACS
10.1021/jacs.8b03447
First steps towards orthogonality?

Liu and Cozens et al. (2018) JACS
10.1021/jacs.8b03447
From XNA to Xenobiology

Storage of genetic information

XNA

DNA

RNA

Protein

Metabolism

Orthogonal transcription machinery

Functional XNA

Transcription machinery

Translation machinery

mXNA

Orthogonal translation machinery

Functional XNA

Biology
Alternative routes towards XNA *in vivo*

- Wholesale replacement of a natural nucleobase with an unnatural one

- Addition of new nucleobases to genome.
  - Malyshev et al. (2014), *Nature*  DOI: 10.1038/nature13314
Human risk of Xenobiology

• ‘Xeno’-organisms are still biological systems
  – As a class, broadly similar risks and hazards as posed by GMOs

• Additional considerations required depending on modification, its implementation and purpose:
  – **Input compounds** – e.g. XNA precursors – chemical toxicity of precursors, contaminants from precursor synthesis, abiotic precursor breakdown
  – **Intermediates and side reactions** – e.g. unnatural amino acids – biological modification or misuse of input compounds, pathway intermediates, truncation products, biologically accessible bypass alternatives
  – **Output compounds** – e.g. XNAs – biological activity or toxicity of intended products or molecules, and of their breakdown products by natural metabolic or environmental routes, co-option by cellular mechanisms
Key messages about the future of biology

• Chemistry is the limit of Biology – If it is chemically possible, it is biologically feasible.
• Biology is a powerful optimization engine. Implementation is technically challenging.
• Orthogonality (even if incomplete and part of a continuum) can be a regulatory tool for unknown or (yet) unquantifiable risks.

• Review tackling 20 emerging issues in biological engineering

Wintle et al. (2017) eLIFE 10.7554/eLife.30247

“The farther, the safer.”

Philippe Marliere
Routes to safe bioprocessing

- Bioprocessing focus
  - 1st containment (equipment design)
  - 2nd containment (facility design)
- Natural organisms
- Component focus
- Cell-free chassis
- Genome ablation
- Auxotrophy
- Inducible expression systems
- Genetic firewall
- Semantic firewall
- Metabolic containment
- Chassis focus