Biosafety: What xenobiology has to offer

MEACB meeting– 24.11.17

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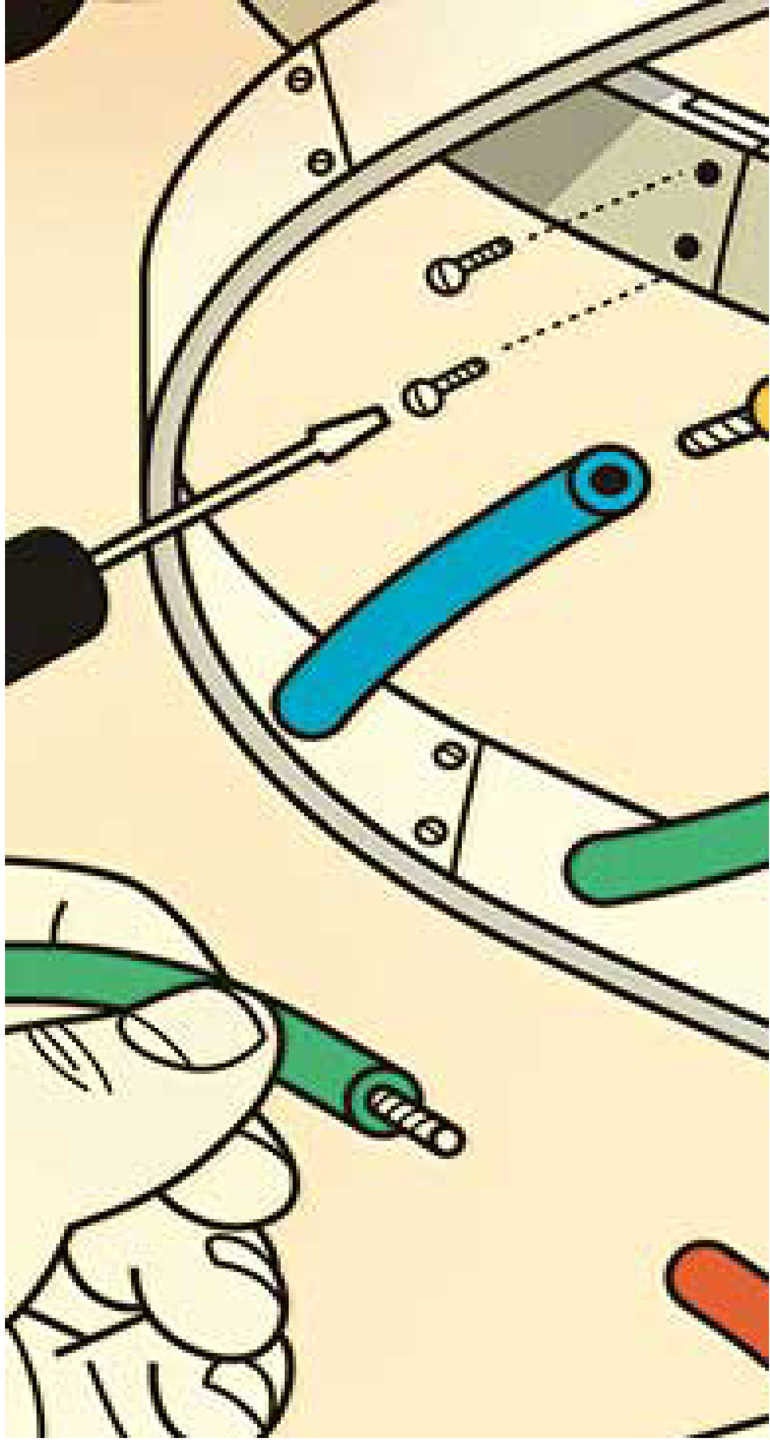


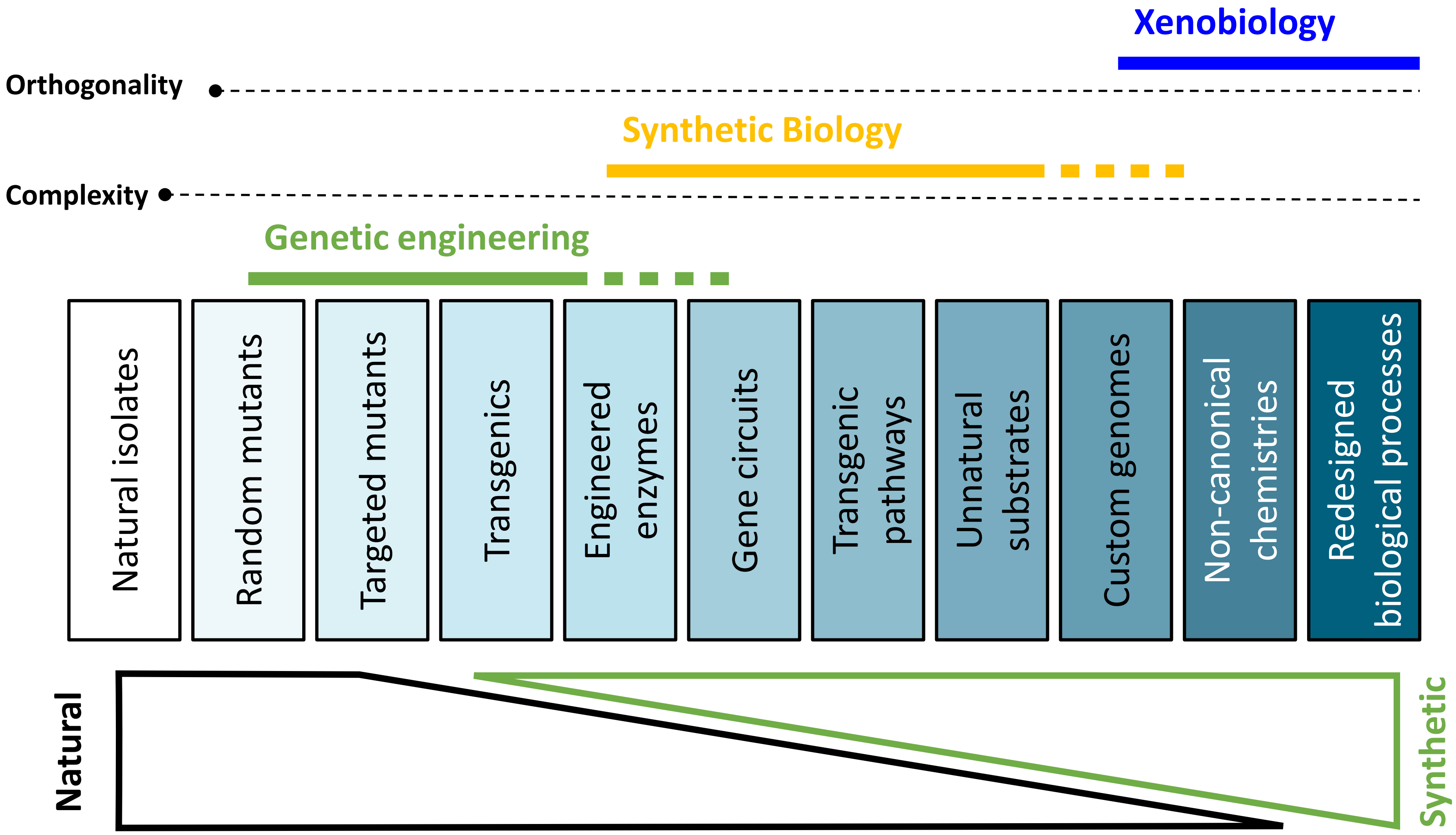
Synthetic Biology

- Traditional scientific approach:
  - Real systems are **complex**
  - Minimise complexity to make system tractable – **top-down approach**
- Synthetic Biology
  - Biology as engineering – **bottom-up approach**
  - Can **complex systems be assembled from parts**?
  - Can we **redesign biological systems**?

"What I cannot create, I cannot understand."

Richard Feynman

Redesigning biological systems

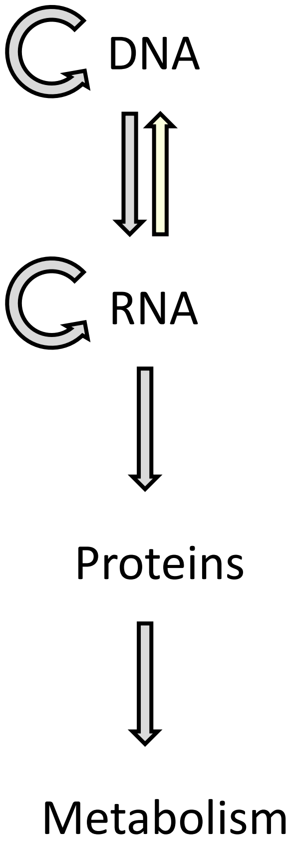


adapted from de Lorenzo (2010) **Bioessays**
10.1002/bies.201000099

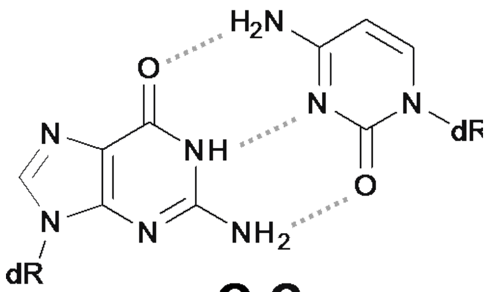
Orthogonality

- It results in a system that **cannot interact with nature** or that can co-exist with natural processes without affecting them.
- As a biosafety tool, it aims at enhancing **containment** – a **biological firewall**.
- Orthogonality can be achieved in a number of different routes and some can be added in **parallel**.

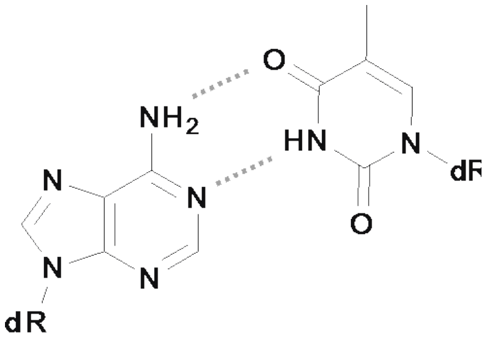
Information transfer in biology – The central dogma



- 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

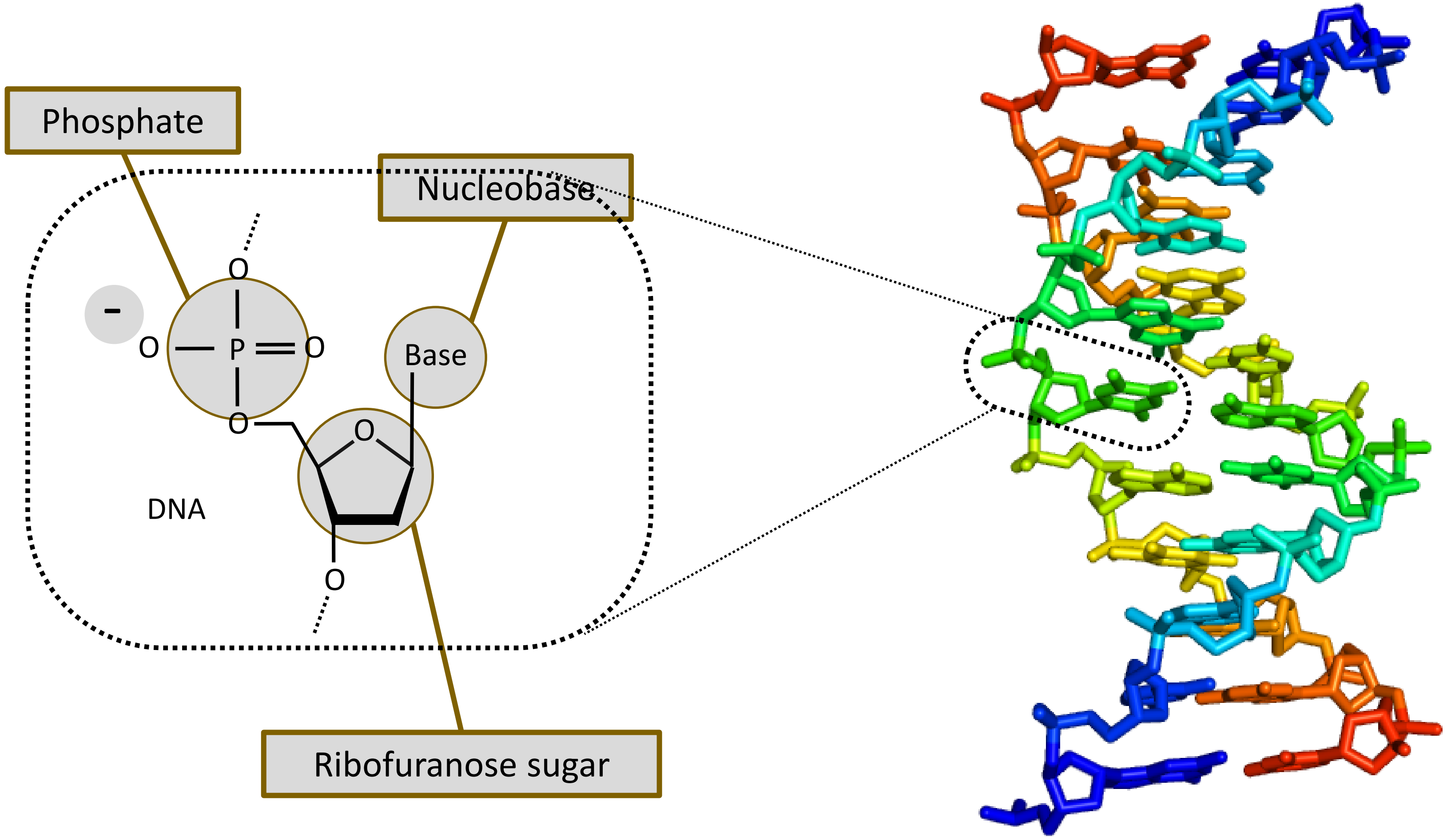


G•C



A•T

Nucleic acid Structure and Function



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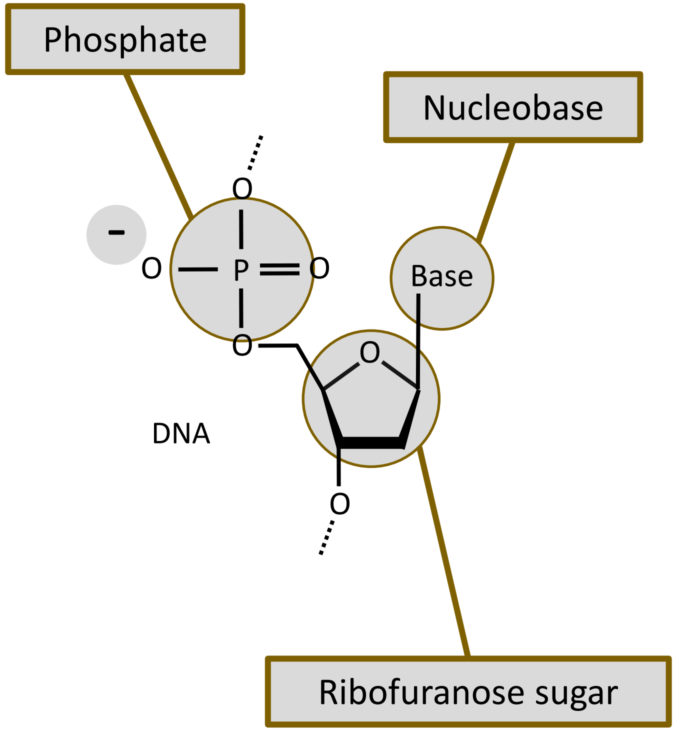
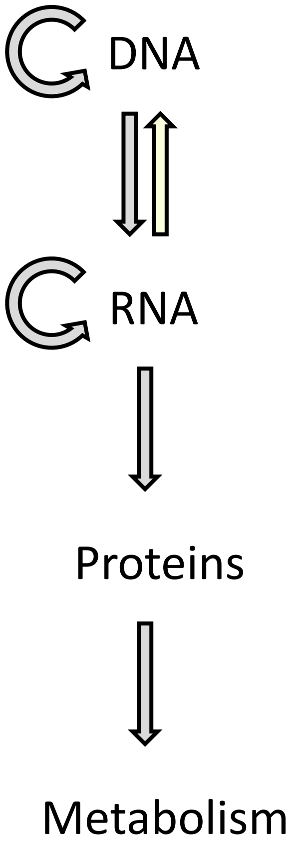


- **All three** chemical moieties contribute to nucleic acid chemical properties, structure and **function**
- Modification in any of the moieties generates a synthetic (or **x**enobiotic) **n**ucleic **a**cid (**XNA**)
  - **Range of compatibility** with natural systems
  - Range of **chemical and biological stability**

Information transfer in biology – The central dogma



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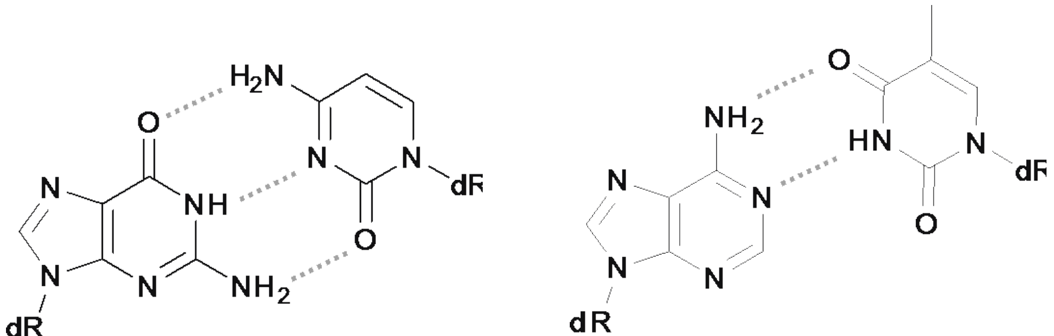
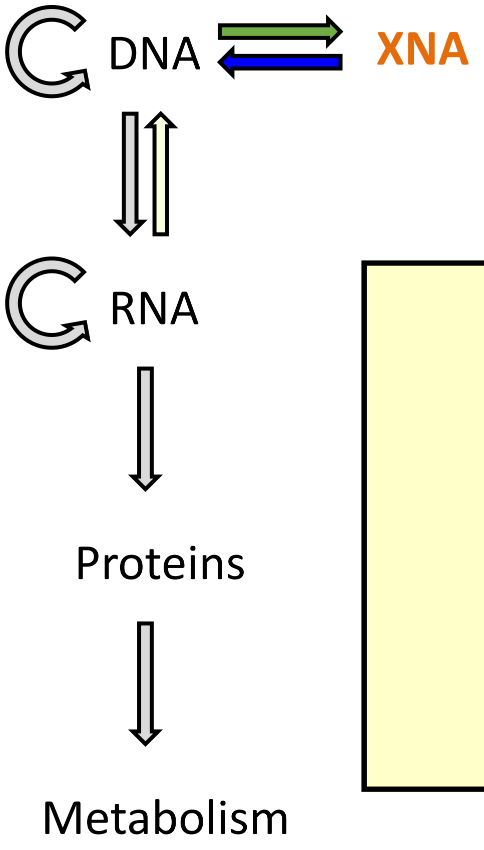


G•C A•T

Expanding the central dogma

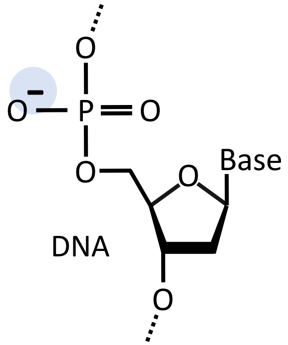


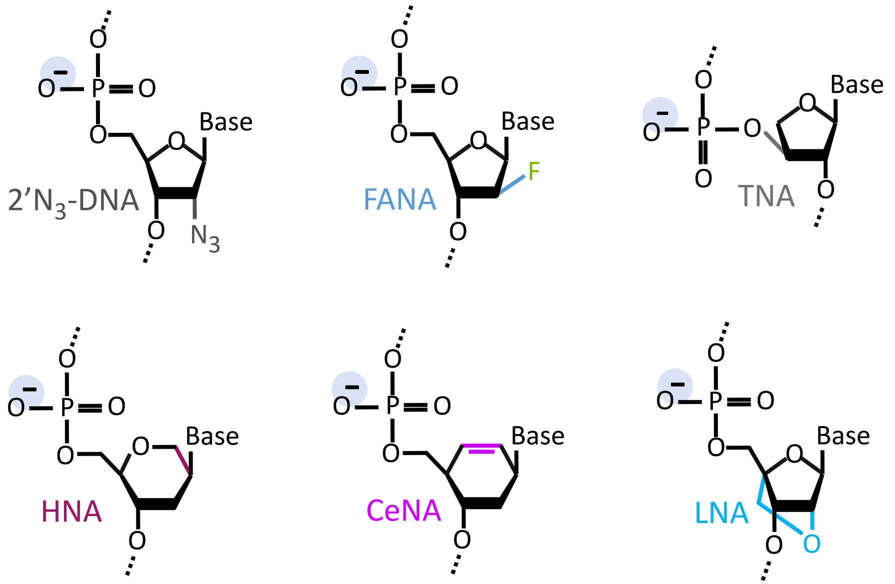
- DNA and RNA are the only genetic polymers in biology
- **Can there be other genetic polymers?**

- Why have biological processes we can observe settled on their current arrangement?
- Is the natural setup an intrinsic limitation or a frozen accident?
- **Can we try something different?**

Synthetic genetic systems (DNA → XNA → DNA)

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

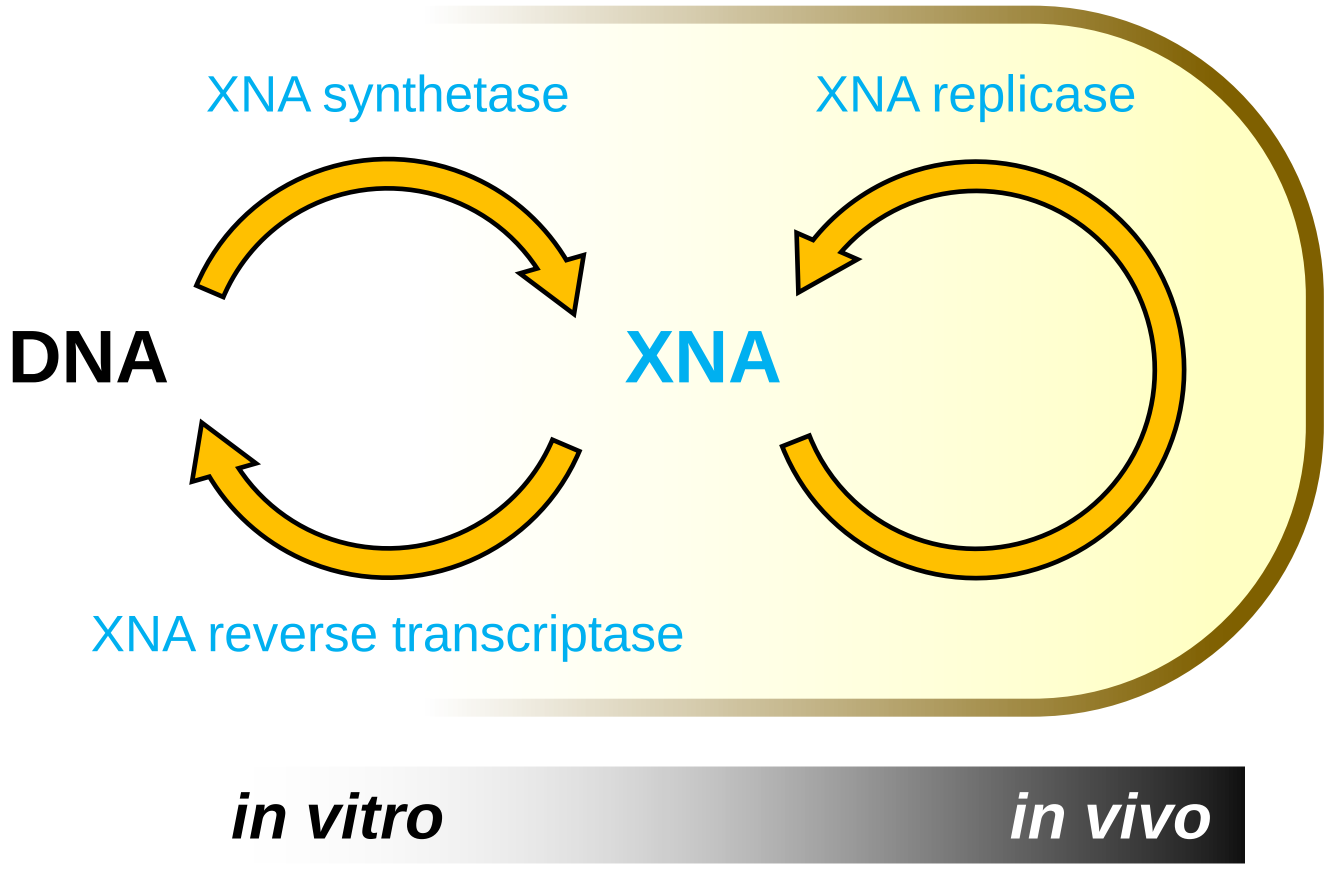




Genetic system	Aggregate misincorporation error (x 10^{-3})
CeNA	4.31
FANA	5.03
ANA	5.81
HNA	7.54
DNA	8.30
TNA	48.5
LNA	52.8

Pinheiro et al. (2012) **Science**
10.1126/science.1217622

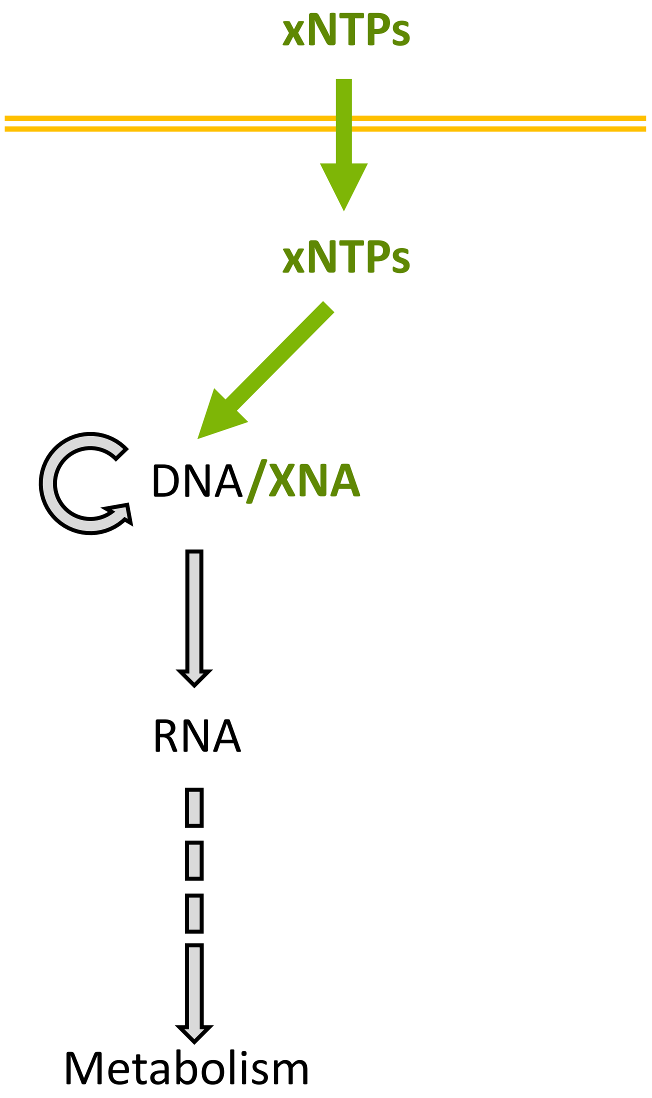
From synthetic biology to xenobiology



biomaterials
therapeutics
biosensors

Biocontainment
xenobiology

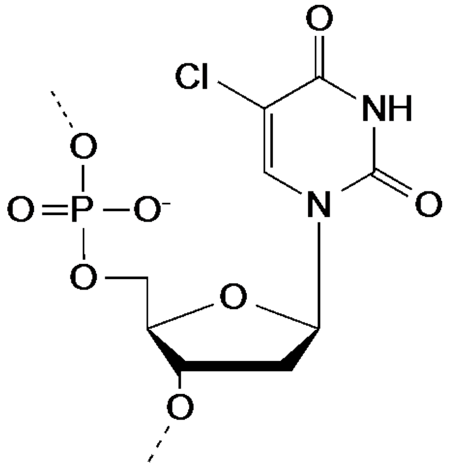
Challenges to introducing XNA *in vivo*



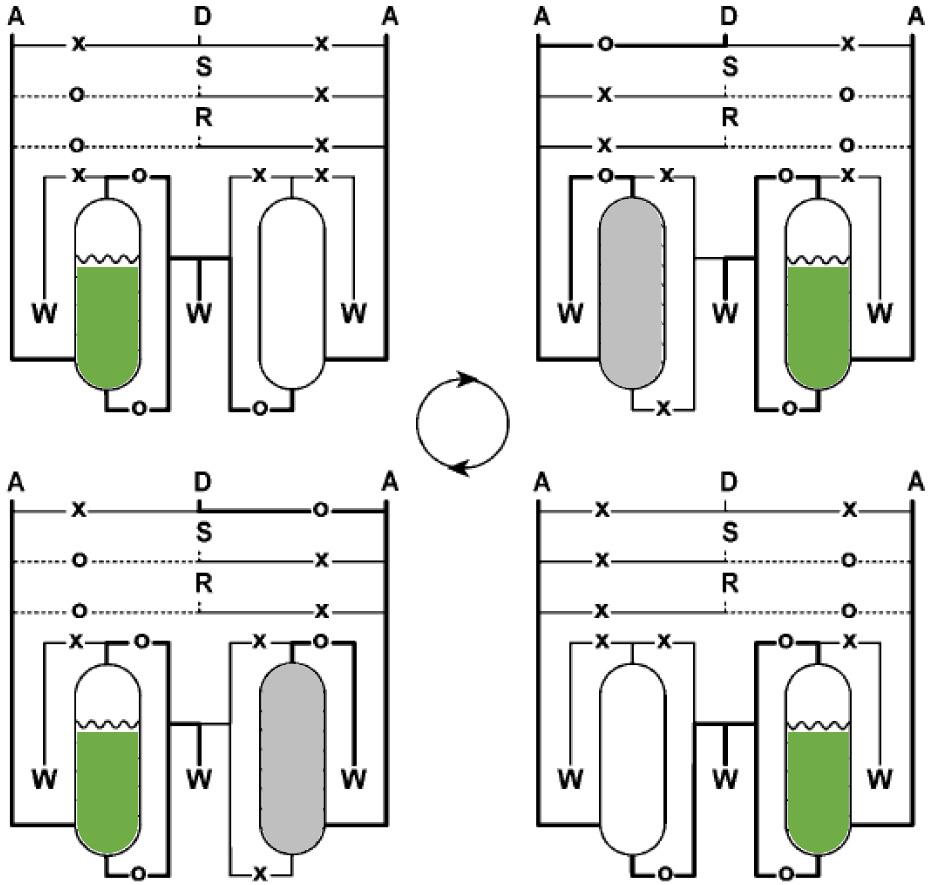
- XNA chemistry
- XNA precursor delivery
- XNA maintenance

XNAs *in vivo*

- Wholesale replacement of thymidine with chlorouridine
- Continuous growth automated *in vivo* selection



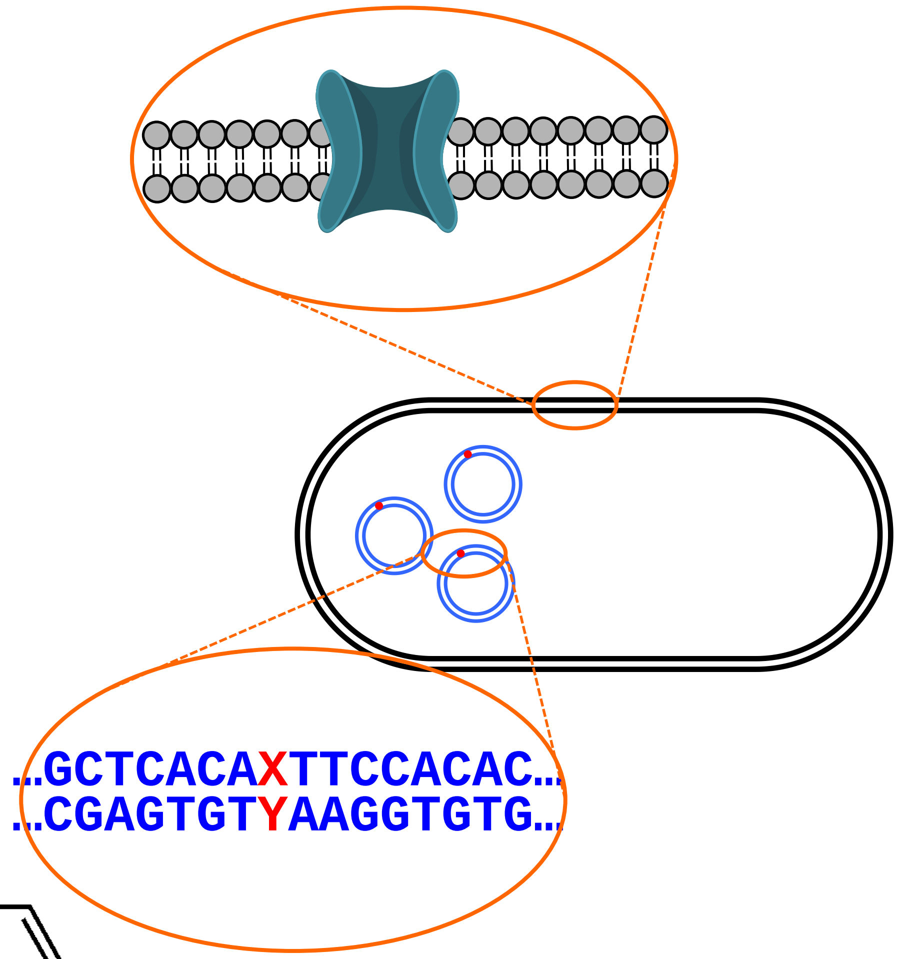
Chlorouridine

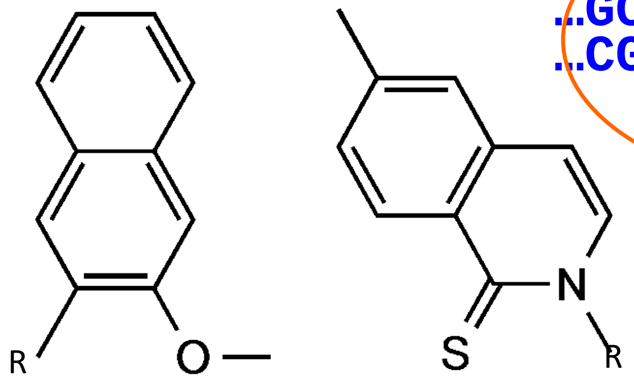


Marlière et al. (2011), **Angew. Chem. Int. Ed**
DOI: 10.1002/anie.201100535

XNAs *in vivo*

- Algal plastid triphosphate importer screened to identify xNTP importer
- Weak link between X•Y and cell metabolism made base-pair unstable ($t_{½} \approx 50$ h)

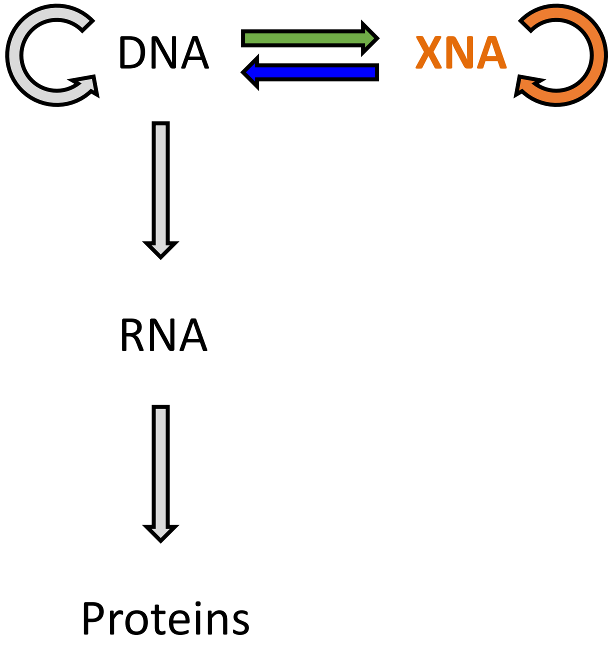




dNaM•d5SICS
(X•Y)

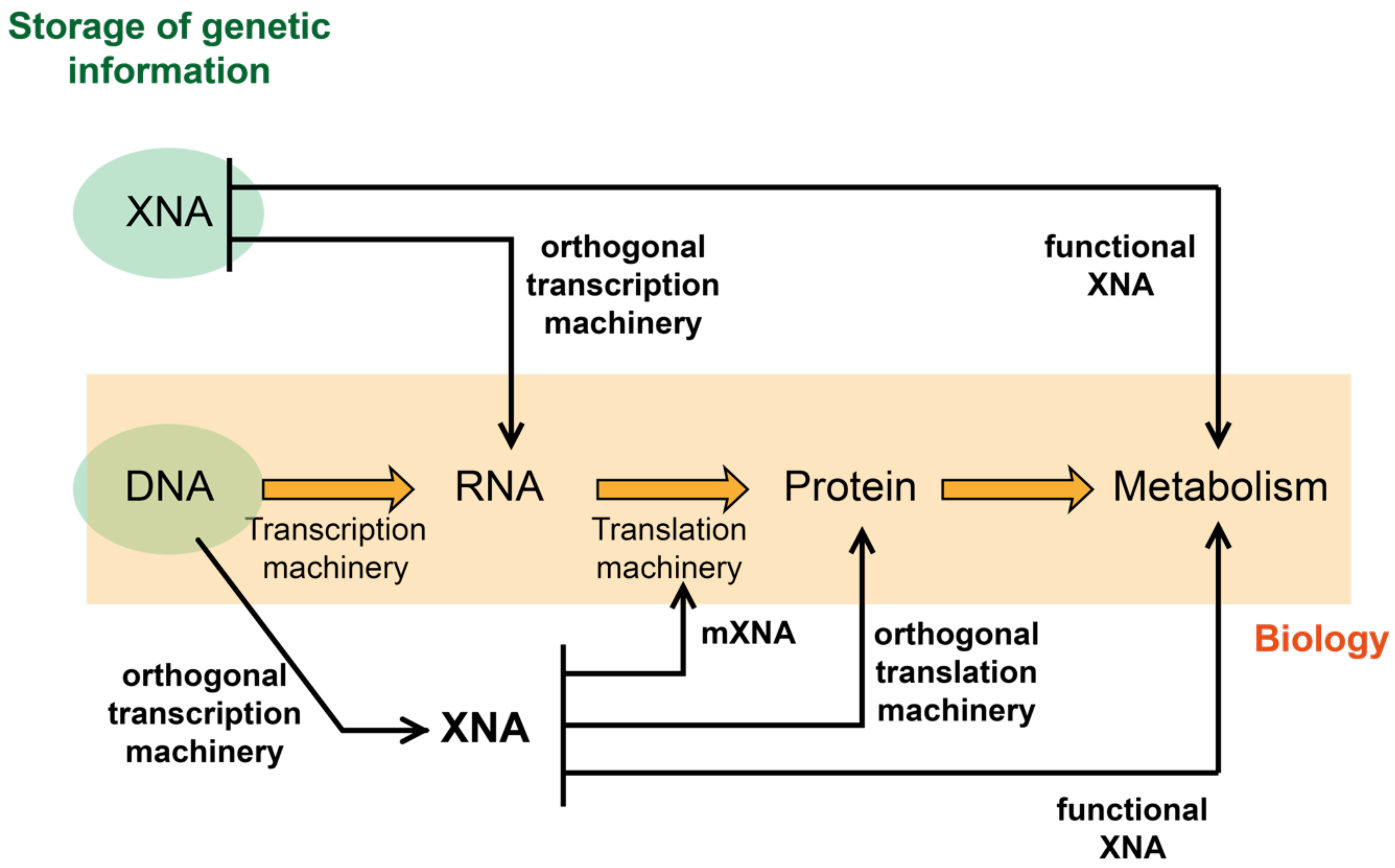
Malyshev et al. (2014), **Nature**
DOI: 10.1038/nature13314

From XNA to Xenobiology

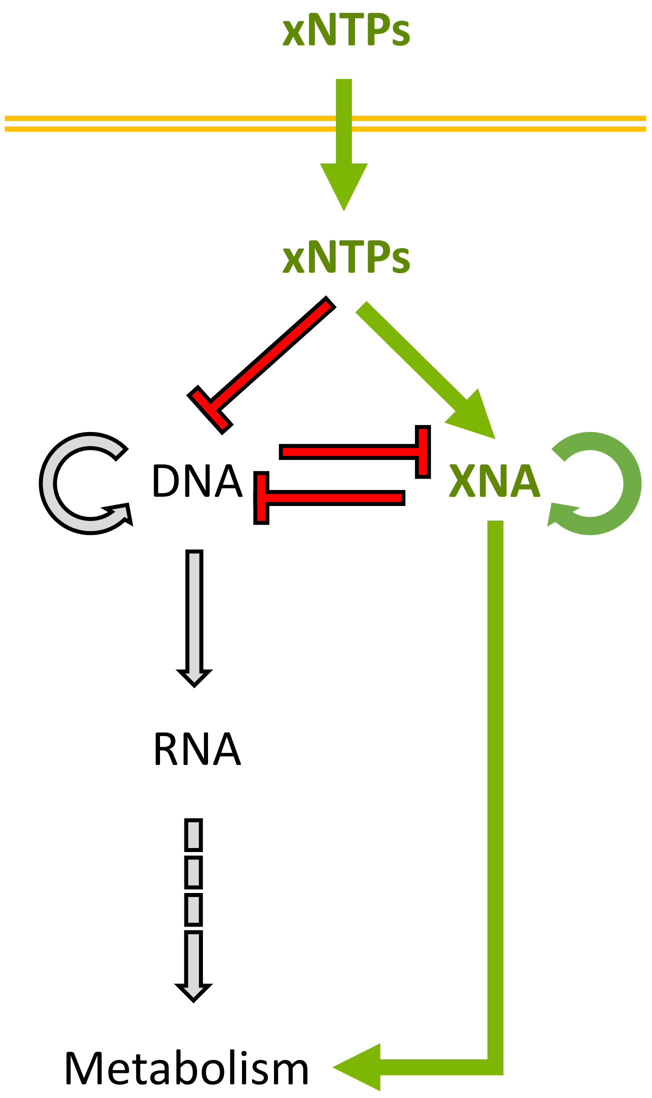


- XNA genetic material is the first step towards an XNA episome
  - XNA genetic element **stored independently** and **maintained stably** within an organism
- **Make information in XNA inaccessible to general biology**
- XNA maintenance *in vivo* depends on **XNA information** being required for **cell survival**
  - Link to metabolism
- Many viable topologies integrating XNA information to the cellular function

From XNA to Xenobiology

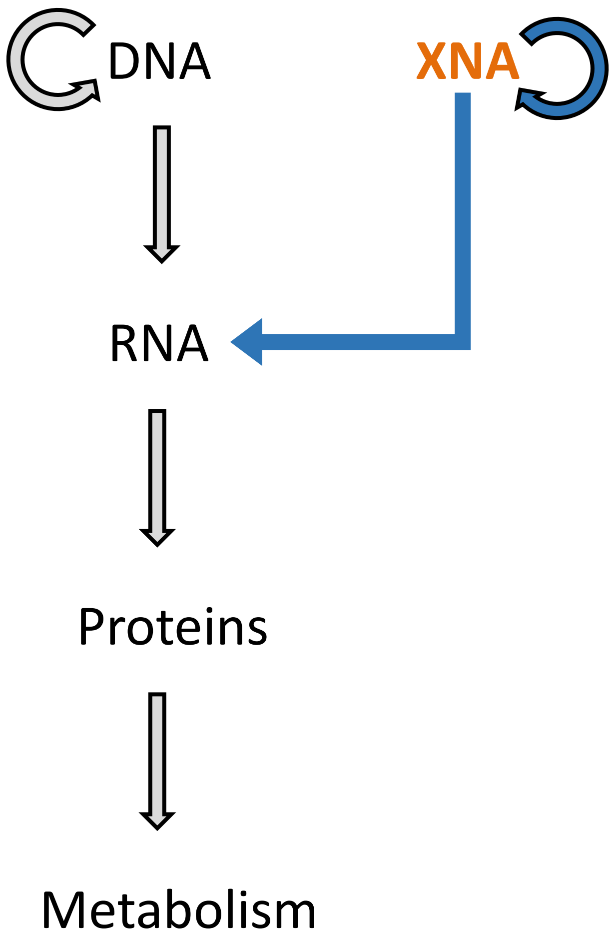


Challenges to introducing XNA *in vivo*



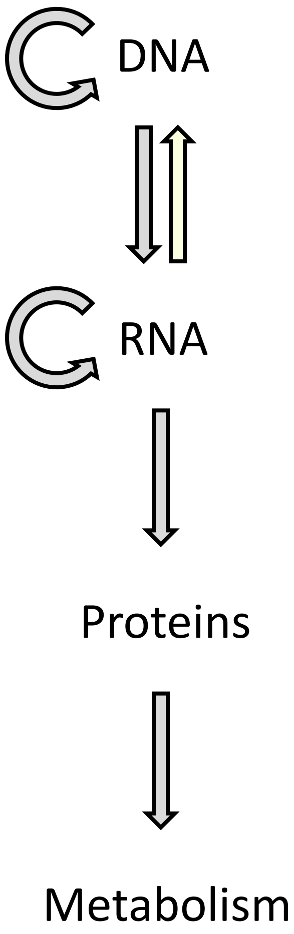
- XNA chemistry (nucleosides, nucleotides and polymers) must not be toxic to the cell.
- XNA nucleotides must be delivered to (or activated in) the cell
- XNA nucleotides cannot be incorporated by natural polymerases
- XNA replicase cannot incorporate dNTPs or rNTPs
- XNA needs to be replicated and maintained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

XNA as a biosafety tool



- XNA as a **dead man's switch**
  - Synthetic precursors
  - XNA traits are isolated from biology
  - Cell's dependence on XNA limits its ecological impact
- Containment failure depends on **shortest evolutionary distance**
  - Current XNA RT is a **single mutation** away from a natural enzyme

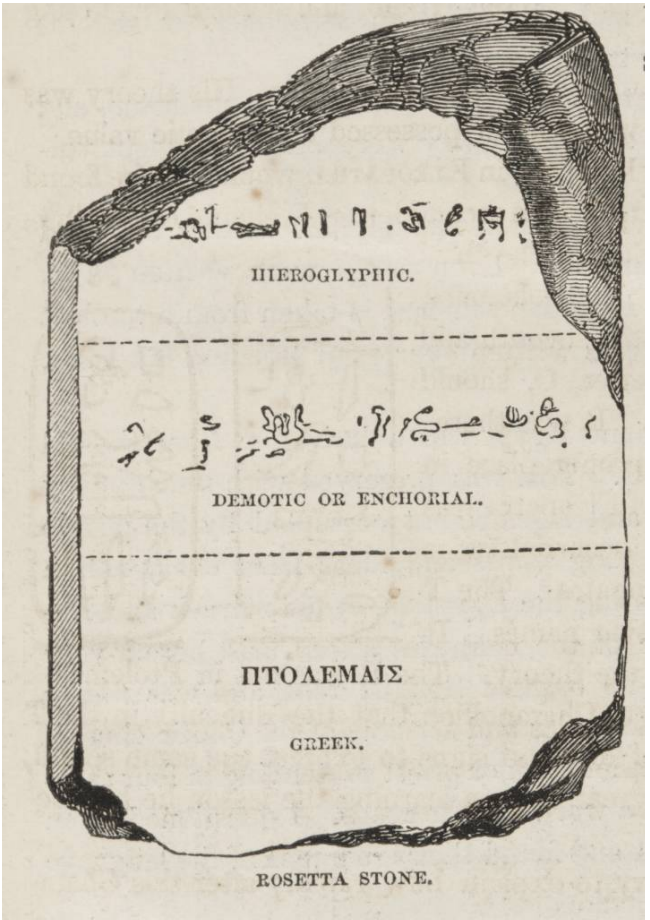
Information transfer in biology – The central dogma

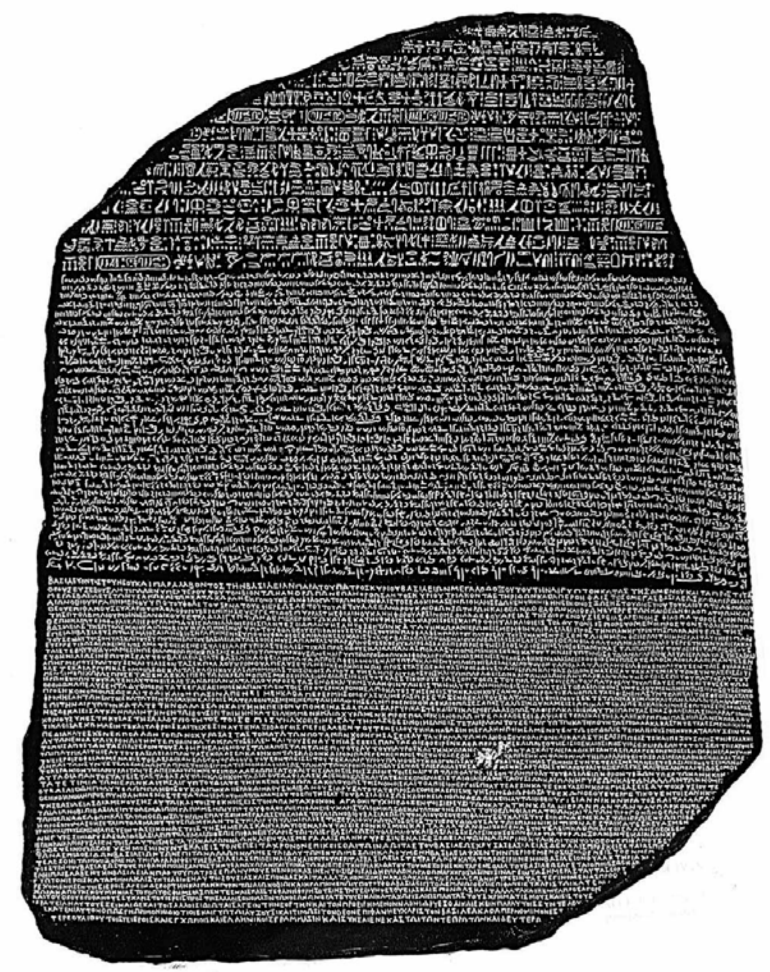


- Information **storage** and **propagation** are essential for life
- Information only **accessible** from DNA and RNA in biological systems
  - **The Central Dogma**
- There is a change in information media between RNA and proteins
  - **The Genetic code**

The genetic code

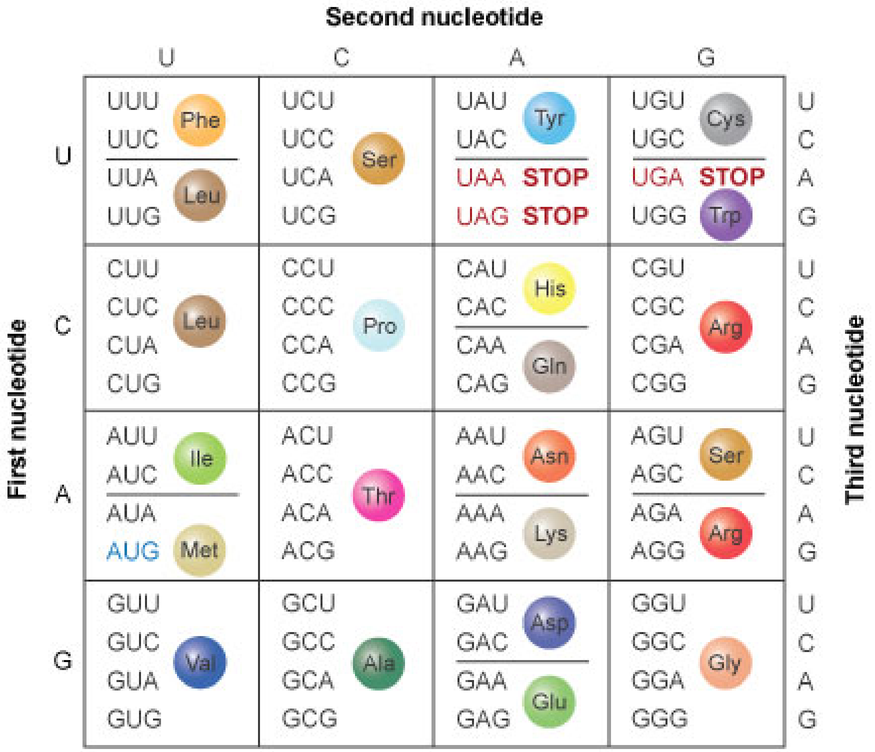
- Information in **RNA** is stored in **nucleotides** while information in **proteins** is stored in **amino acids**



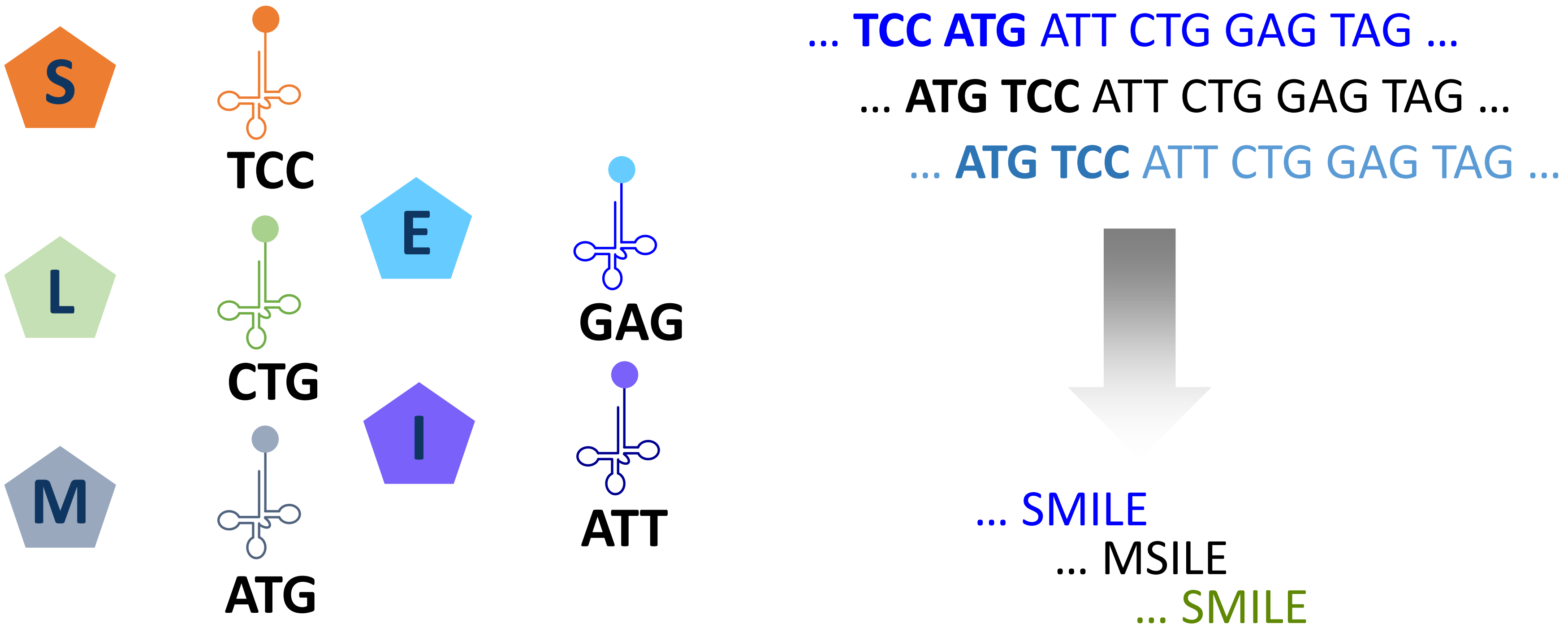


The genetic code

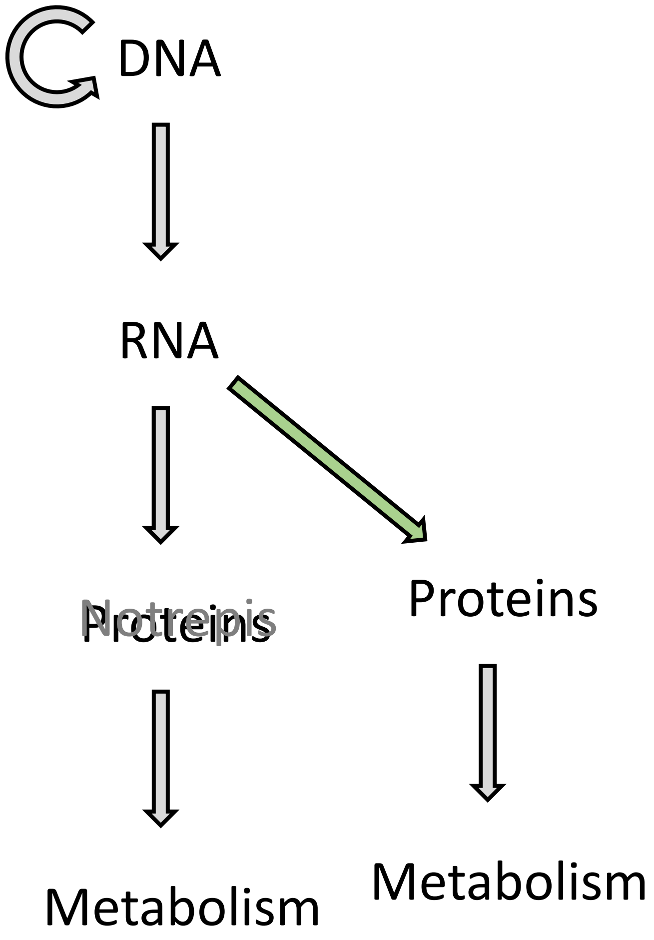
- Genetic code **emerged early** in evolution
- **Universal** bar a handful of exceptions
- **Can it be modified?**
- **Can it be made orthogonal?**



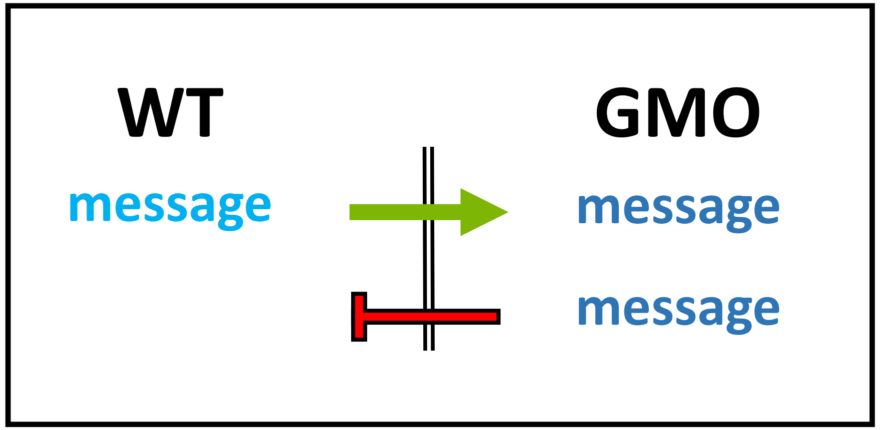
Rewriting the genetic code as a biosafety tool



Rewriting the genetic code as a biosafety tool



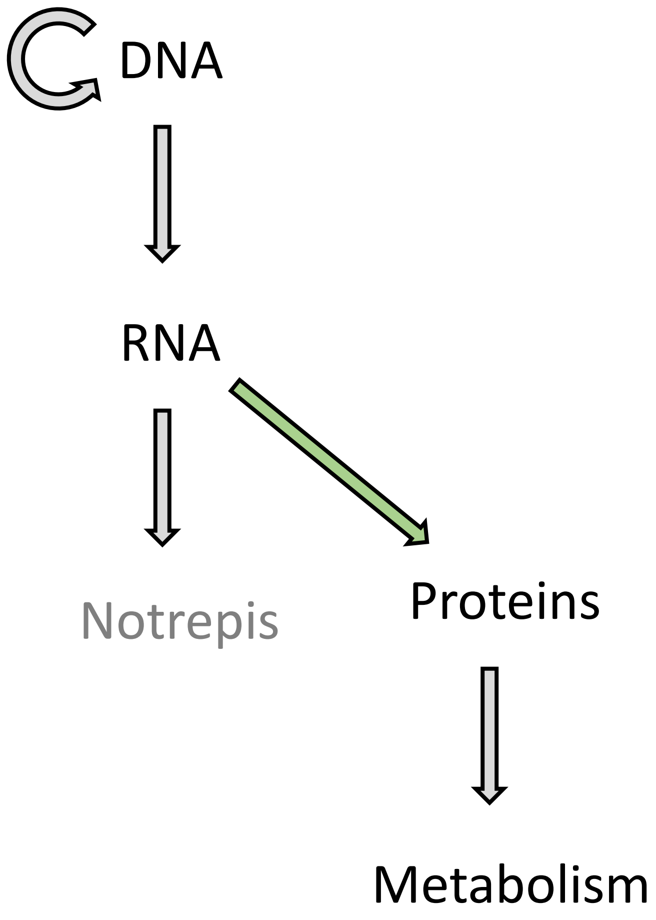
- A genetically recoded organism (GRO) dependent on unnatural amino acids
  - GRO ➔ Nature will not generate viable proteins
  - Nature ➔ GRO as is would still be viable



Mandell et al. (2015), **Nature**
DOI: 10.1038/nature14121

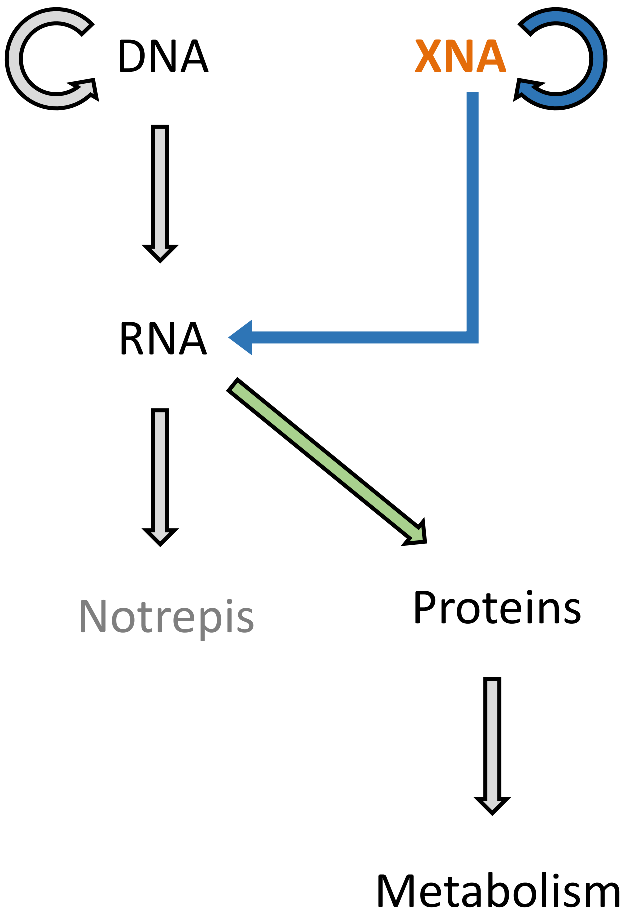
Rovner et al. (2015), **Nature**
DOI: 10.1038/nature14095

Rewriting the genetic code as a biosafety tool

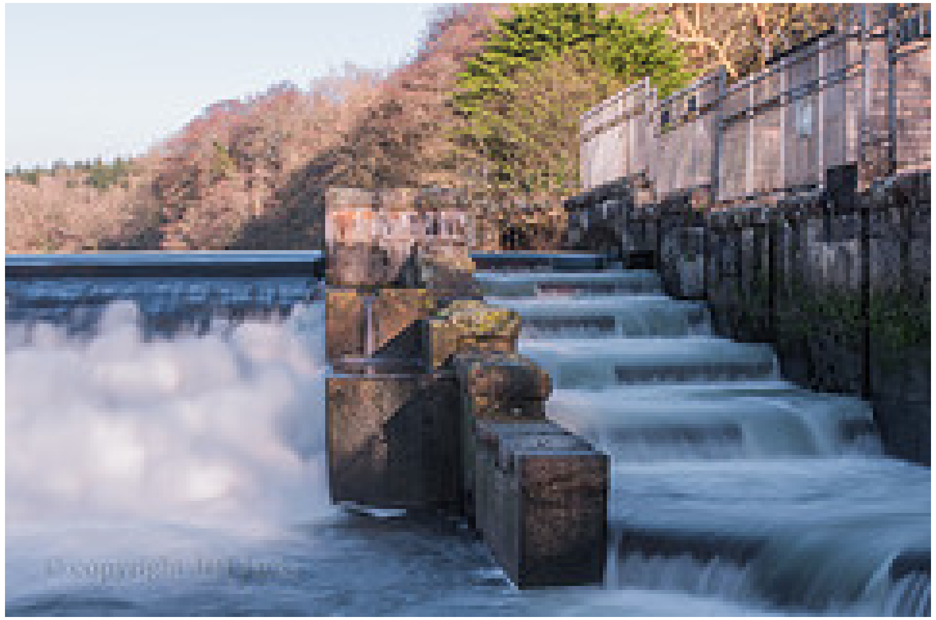


- A genetically recoded organism (GRO) dependent on unnatural amino acids
  - GRO ➔ Nature will not generate viable proteins
  - Nature ➔ GRO as is would still be viable
- A GRO operating with an incompatible code and made an auxotroph would be contained
  - **Semantic firewall**

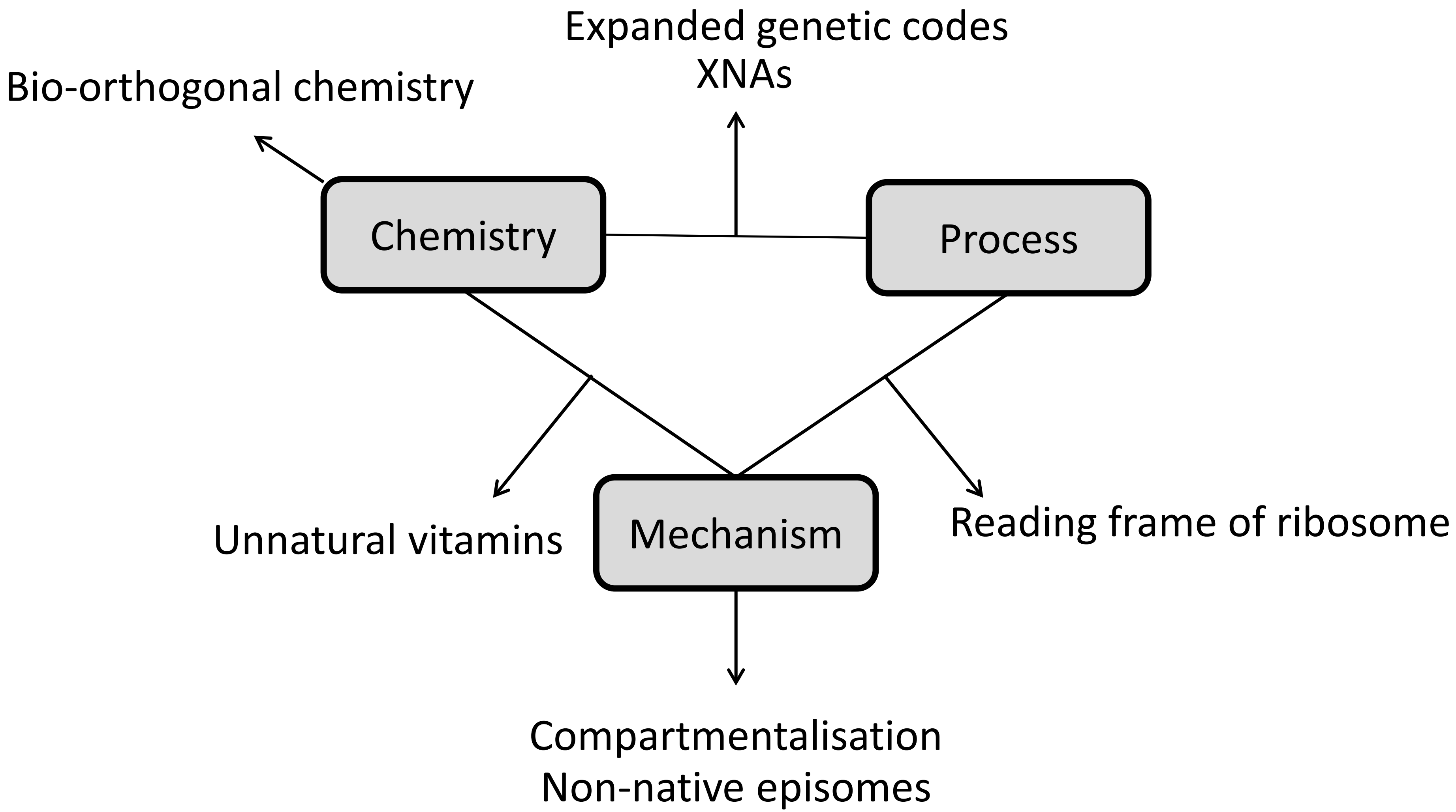
Xenobiology as a biosafety tool



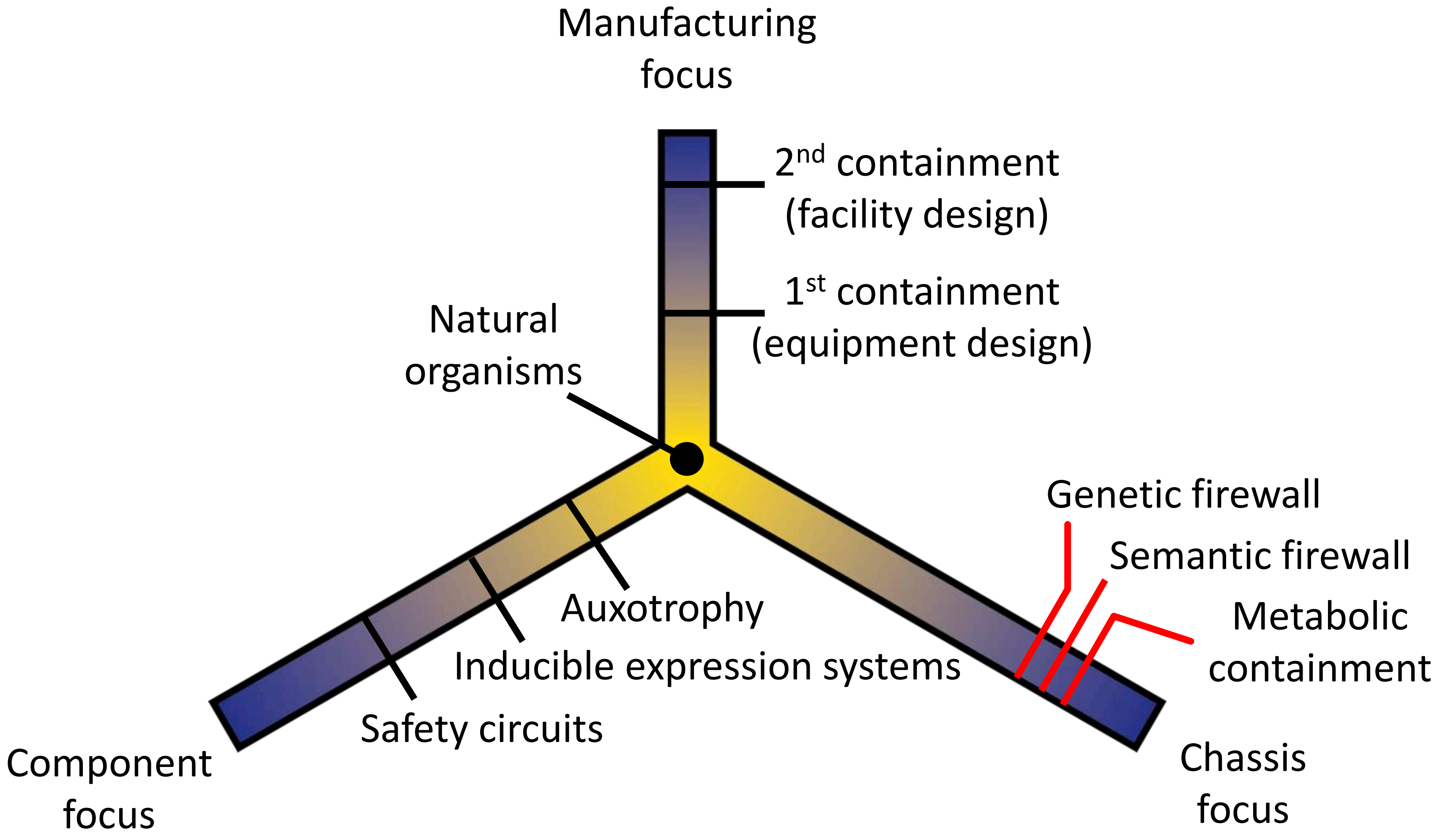
- Xenobiology systems are **additive** and can be systematically integrated to increase containment likelihood



Sources of orthogonality



Routes to safe bioprocessing



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

Project history

FP6 Orthosome (2006 – 2009) Herdewijn, Holliger and Marliere

Are XNAs viable genetic materials?

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Can XNA molecules catalyse chemical reactions?

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ERA-SynBio (2015 – 2018) Delarue, Herdewijn, Holliger, Marliere, Liu and Pinheiro

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Thank you for your attention!