What are the limits of biology?

What is unique about biology?

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Synthetic Biology

Alternative Biology

Synthesising Biology

Biology for synthesis

Biology as a tool

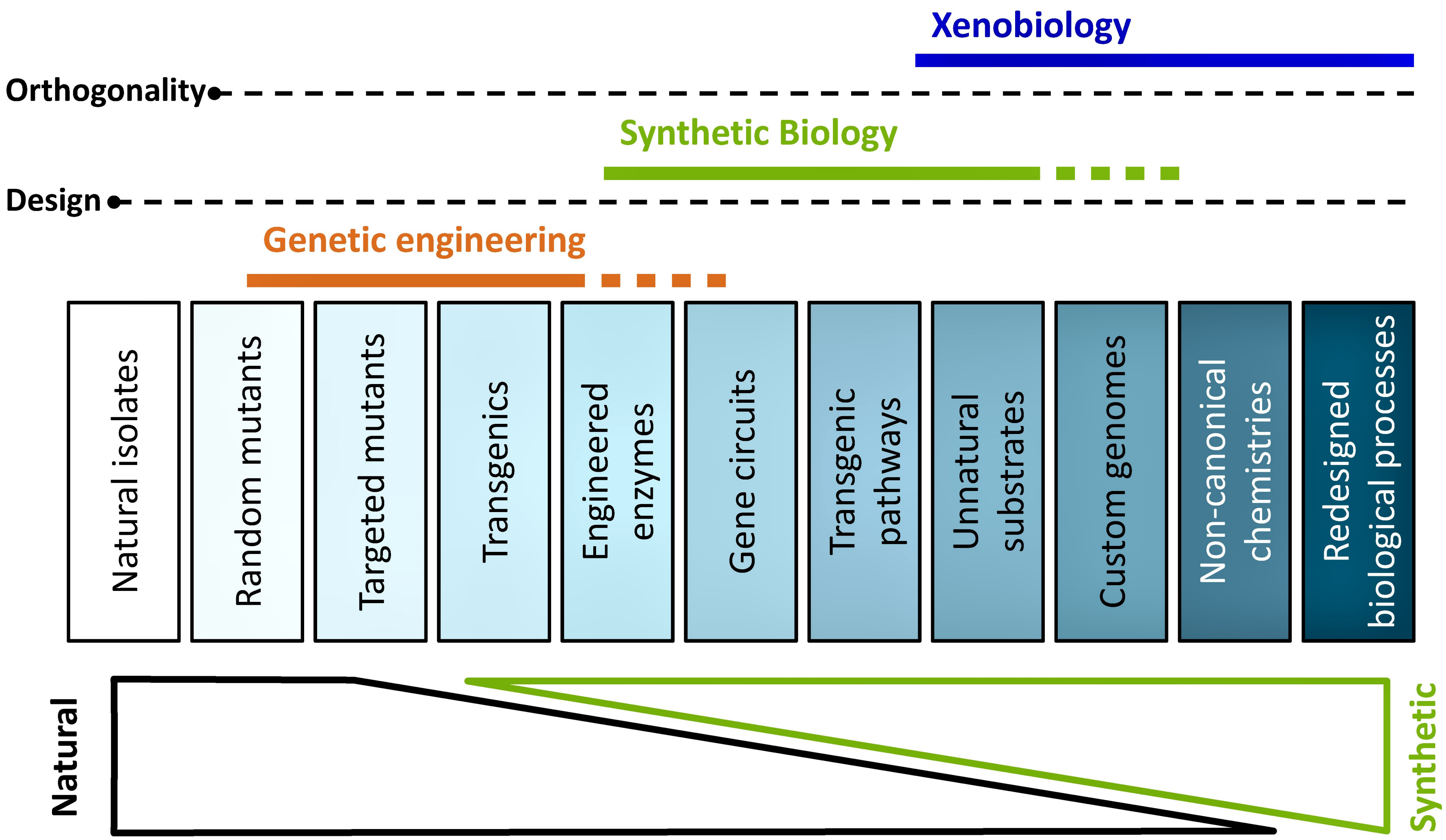
Biology as data

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

"What I cannot create, I cannot understand."

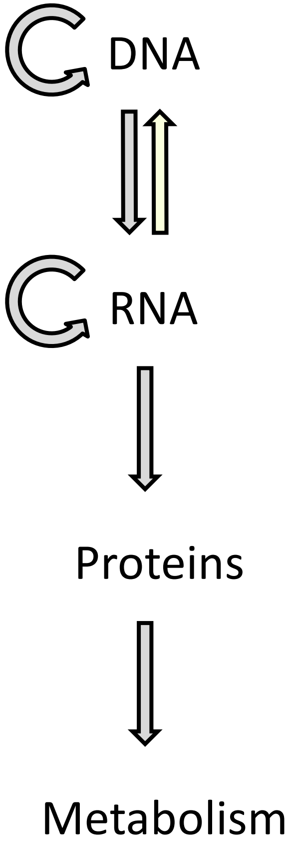
Richard Feynman

Synthetic Biology is a continuum

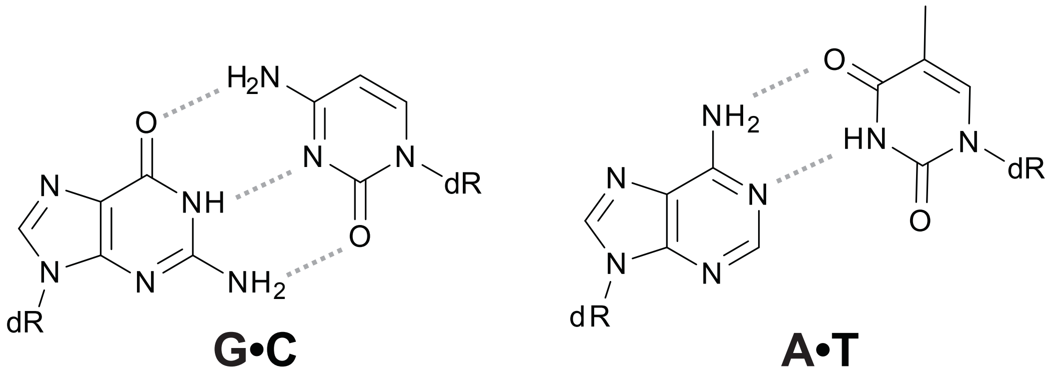


adapted from de Lorenzo (2010) **Bioessays**
10.1002/bies.201000099

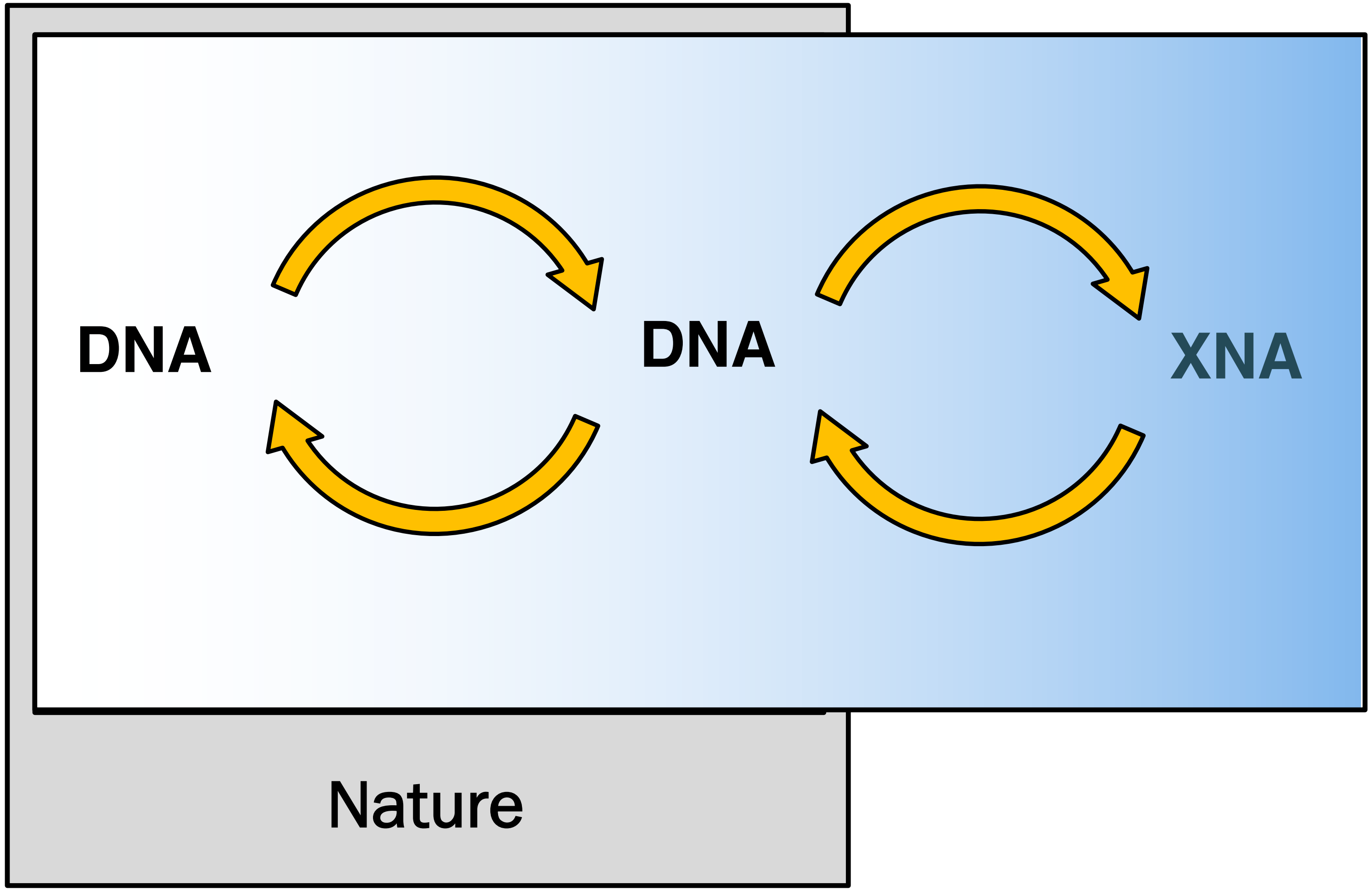
Information transfer in biology – The central dogma



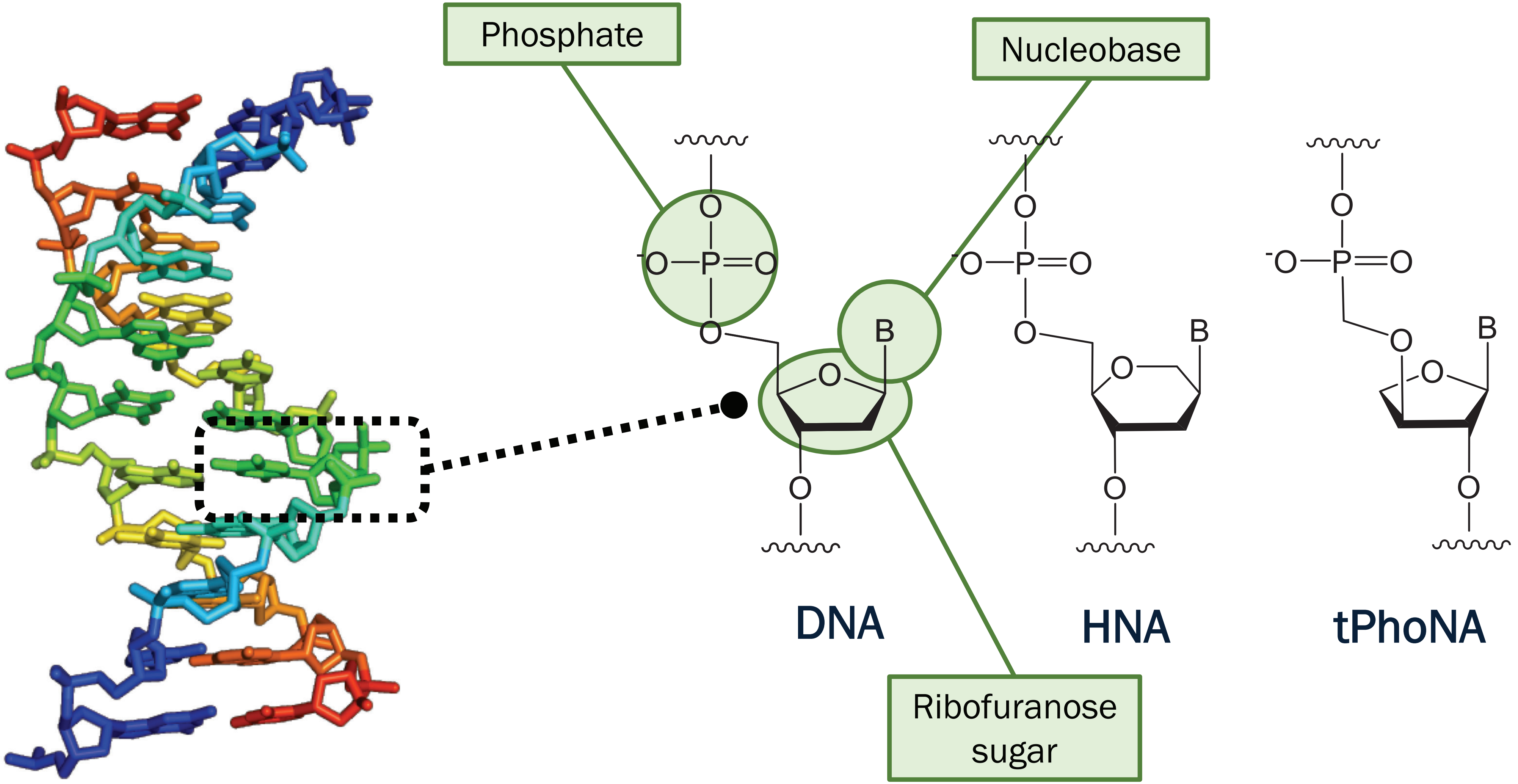
- Information storage and propagation are essential for life
- Central Dogma – information only accessible from DNA and RNA in biological systems
- Propagation is viable because of the efficient and unambiguous base pairing



Extending biology through directed evolution

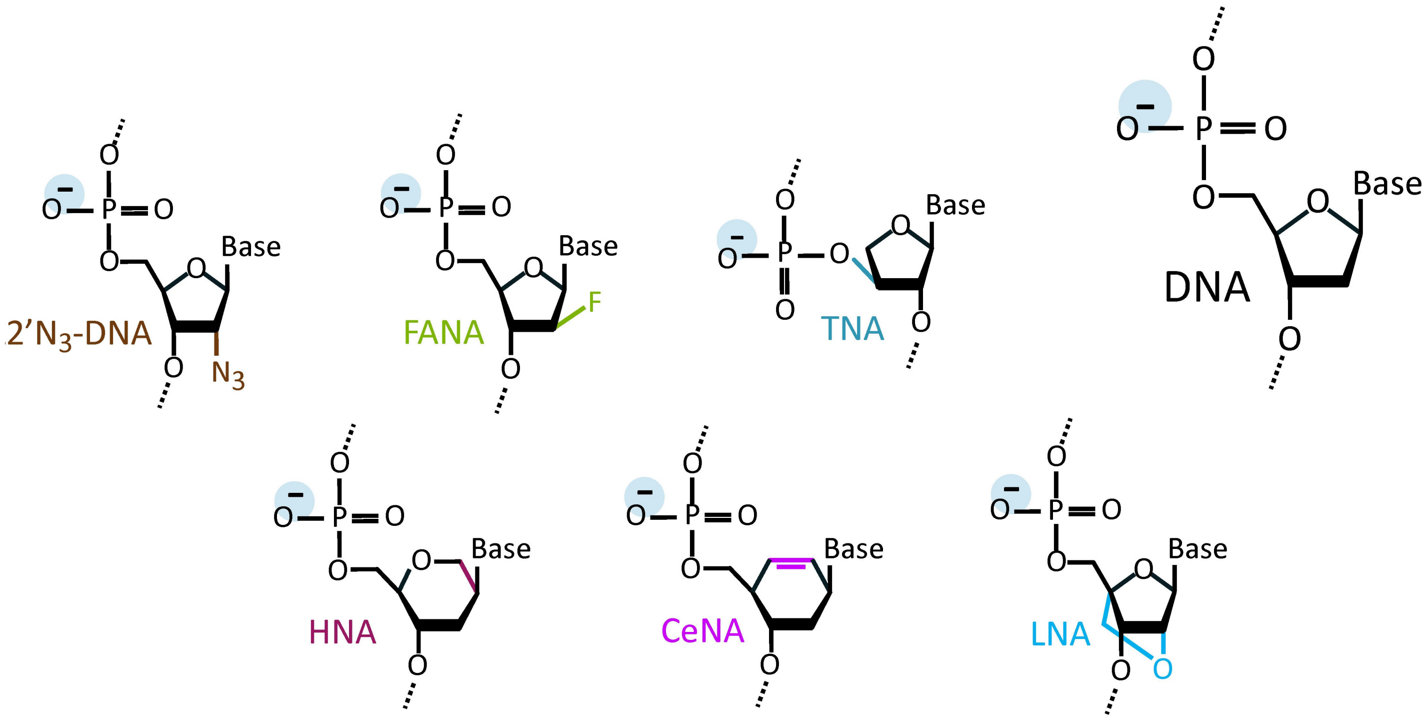


Xenobiotic nucleic acids (XNAs)



Synthetic genetic systems (DNA → XNA → DNA)

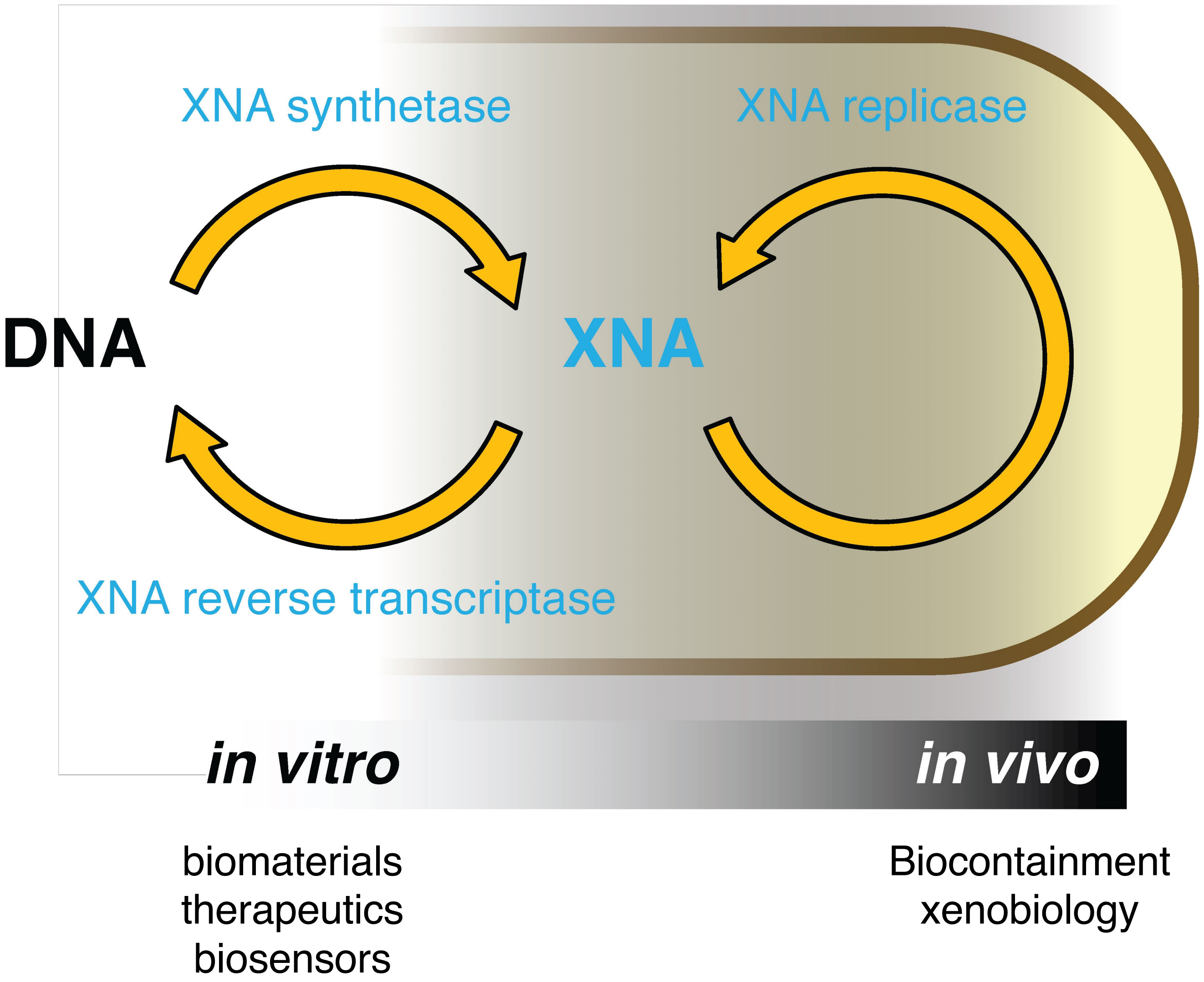
- **Synthesis and recovery of information** from synthetic backbone establishes a **synthetic genetic system**



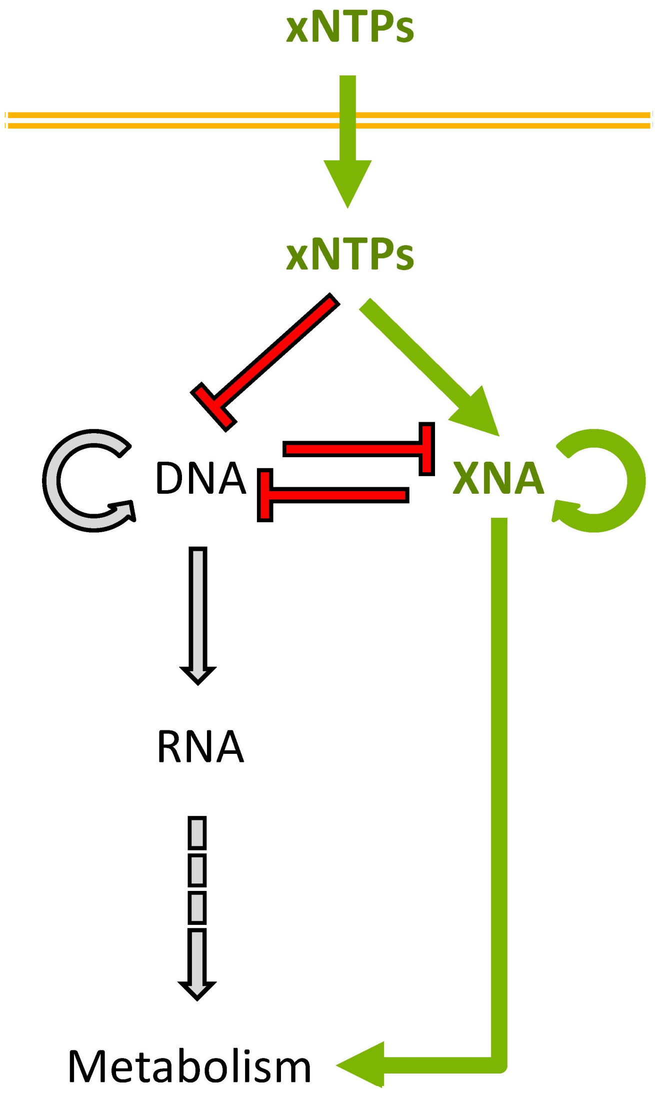
Genetic system	Aggregate misincorporation error (x 10^{-3})
CeNA	4.31
FANA	5.03
ANA	5.81
HNA	7.54
DNA	8.30
TNA	48.5
LNA	52.8

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

From synthetic biology to xenobiology

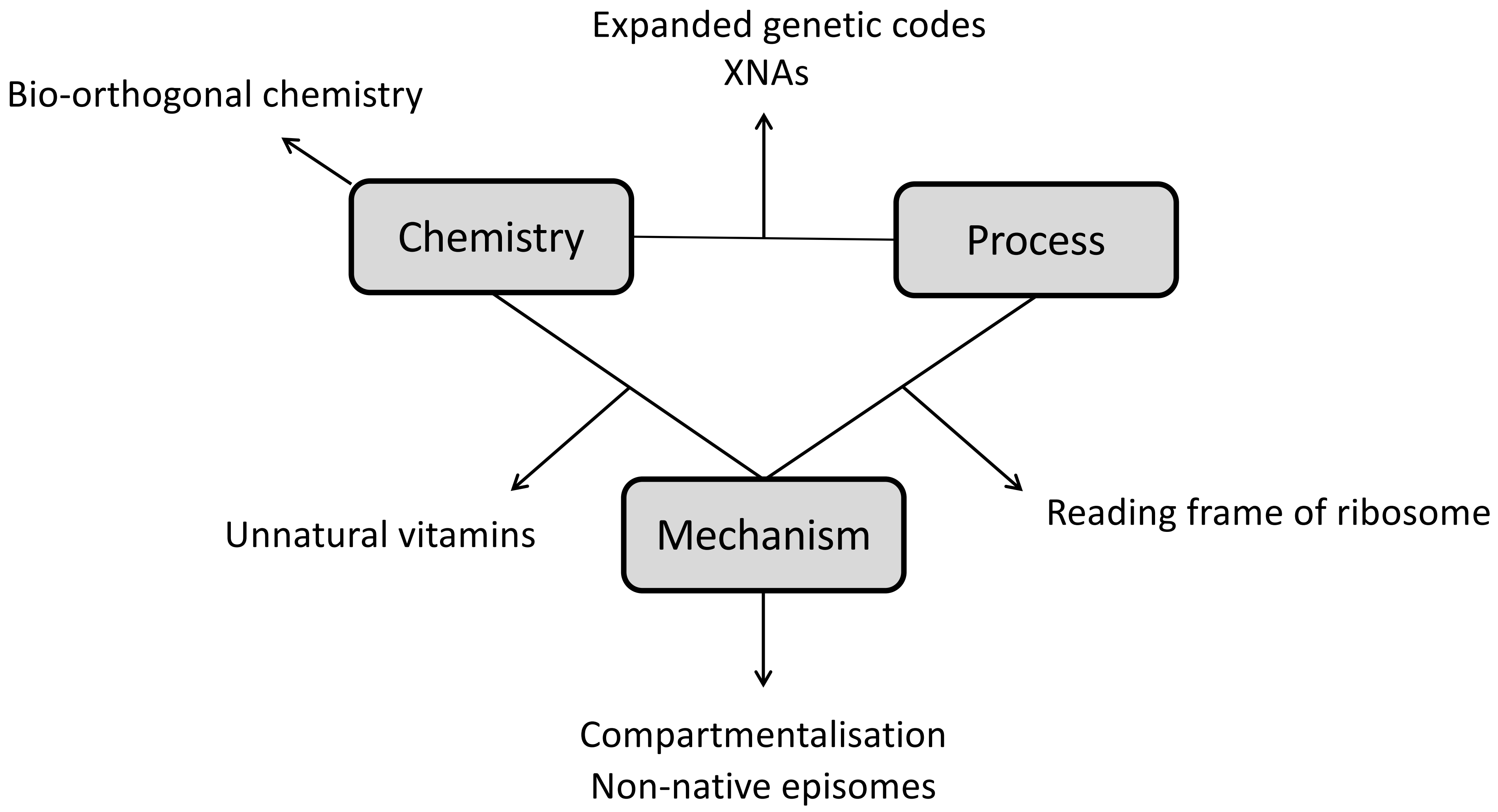


Challenges to introducing XNA *in vivo*



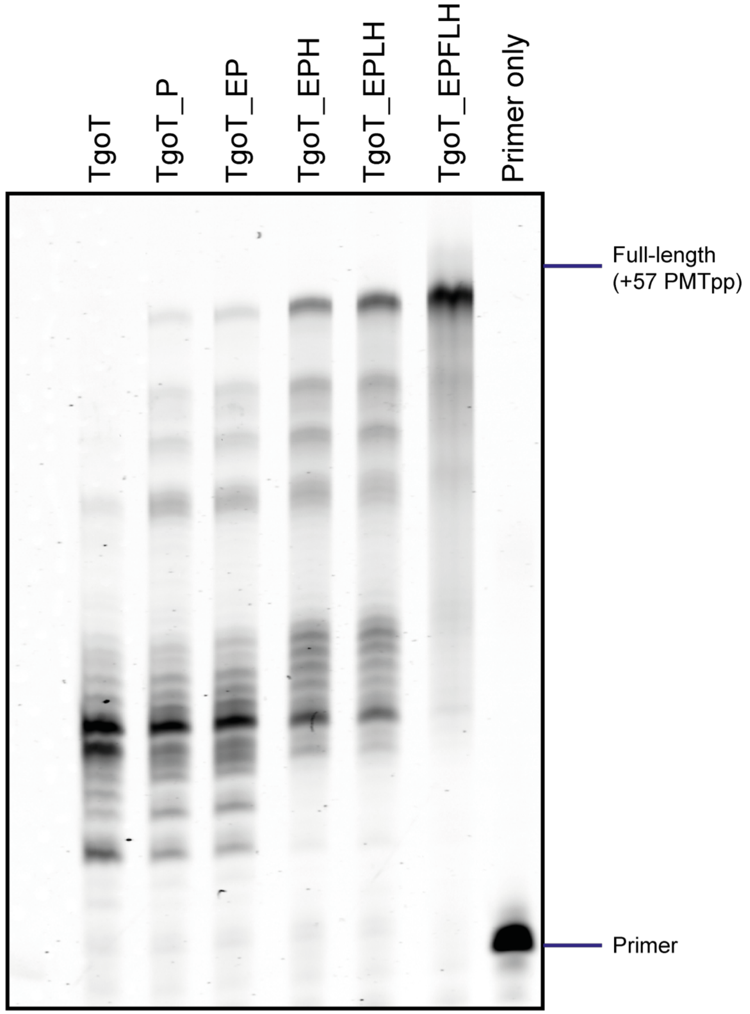
- XNA chemistry (nucleosides, nucleotides and polymers) must not be toxic to the cell.
- XNA nucleotides must be delivered to (or activated in) the cell
- XNA nucleotides cannot be incorporated by natural polymerases
- XNA replicase cannot incorporate dNTPs or rNTPs
- XNA needs to be replicated and maintained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

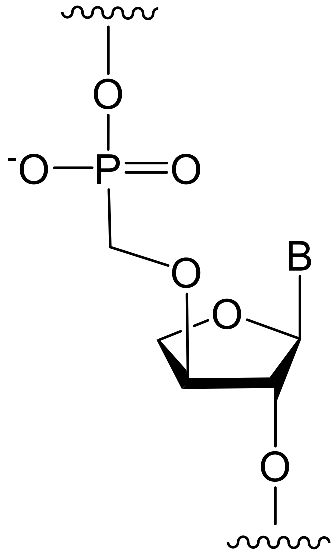
Sources of orthogonality



Towards polymerase rational design

Mutation rationale	Enzyme	Full-length PMT synthesis
Increased template modification tolerance (**V93Q**) Exonuclease defficient (**D141A, E143A**) Increased incorporation of unnatural substrates (**A485L**)	**TgoT** V93Q, D141, E143A, A485L	N.D.
Enhances synthesis of sugar-modified nucleic acids	**TgoT_P** L403P	1.1%
Shifts the exonuclease:polymerase sampling balance towards the polymerase function	**TgoT_EP** H147E	1.5%
Mutations at E664 have been reported as essential for RNA, TNA and 2'-5' DNA synthesis	**TgoT_EPH** E664 $\xrightarrow{X}$ H	6.0%
Increases polymerase activity, enabling RT function and enhancing XNA synthesis	**TgoT_EPLH** I521L	9.4%
Targeted to affect Y409 positioning seeking better stacking against the threosyl in PMTpp	**TgoT_EPFLH** L408 $\xrightarrow{\phi}$ F	20%

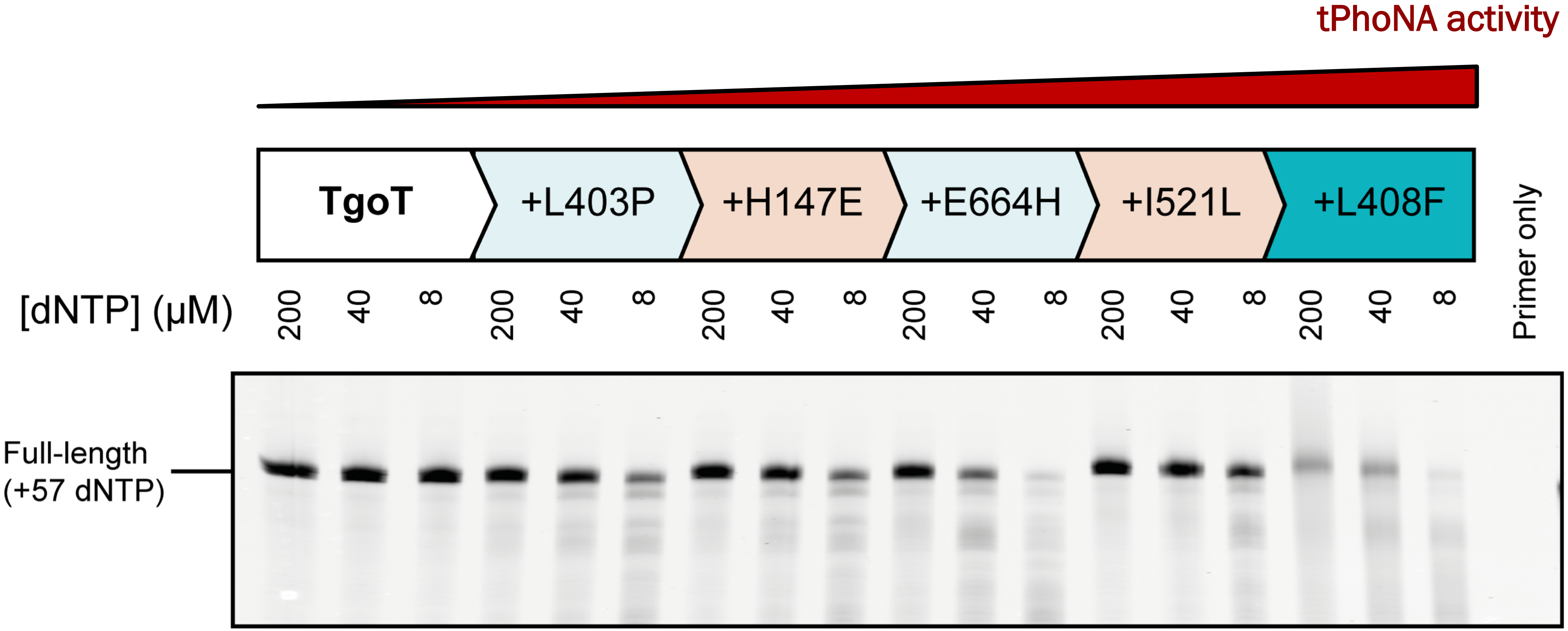




tPhoNA

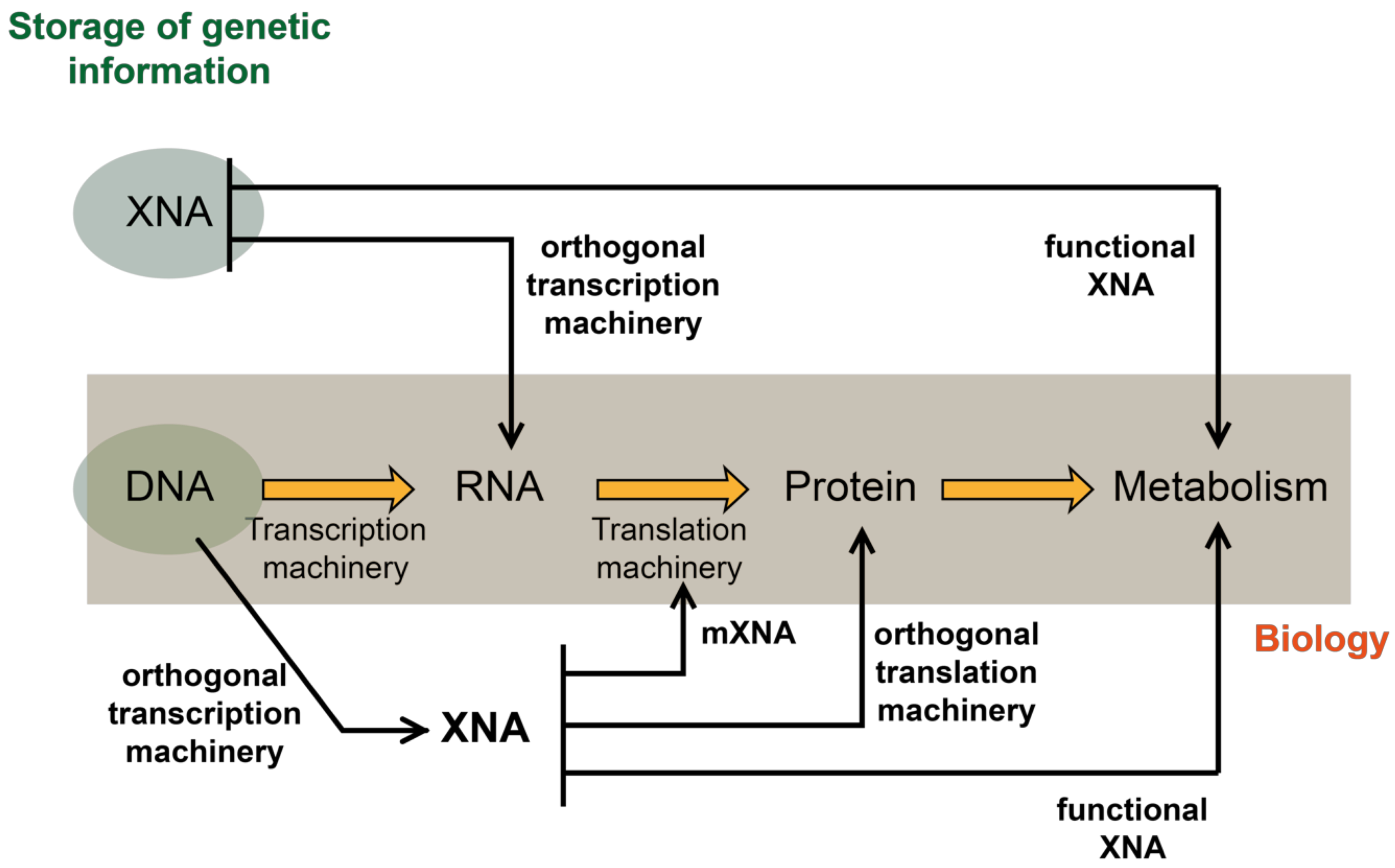
Liu and Cozens et al. (2018) **JACS**
10.1021/jacs.8b03447

First steps towards orthogonality?

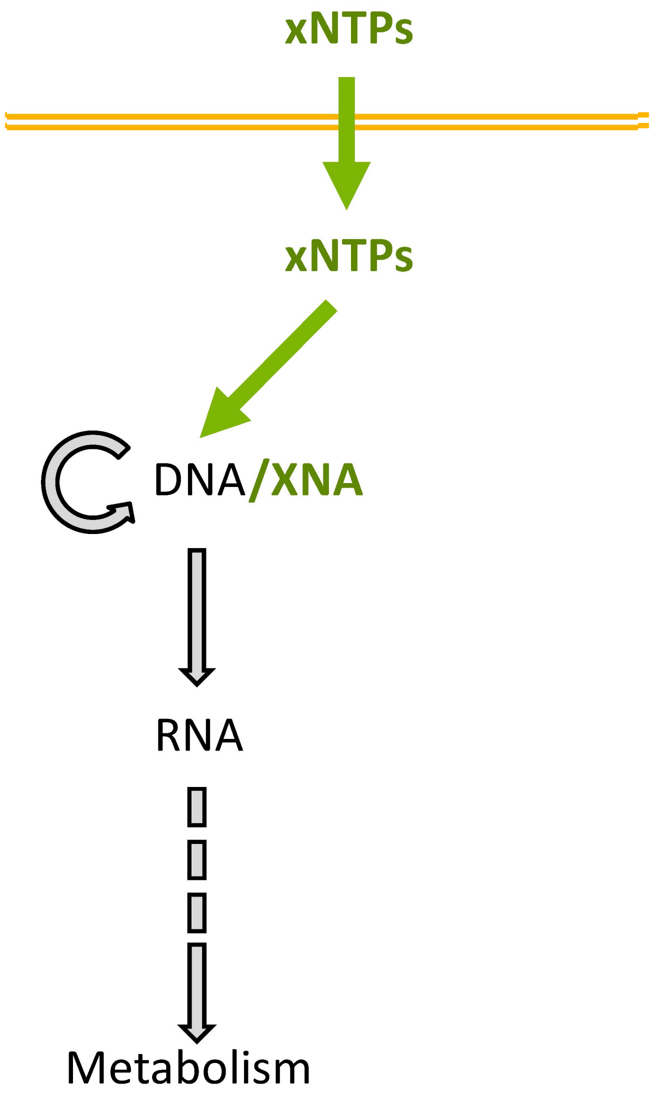


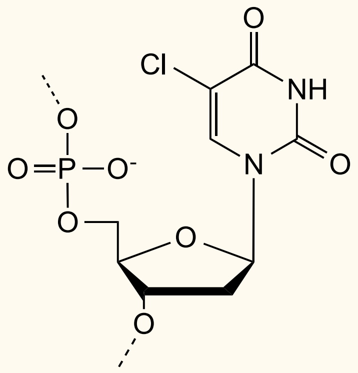
Liu and Cozens et al. (2018) **JACS**
10.1021/jacs.8b03447

From XNA to Xenobiology



Alternative routes towards XNA *in vivo*



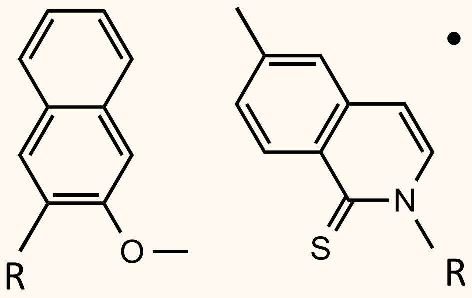


Chlorouracil

- Wholesale replacement of a natural nucleobase with an unnatural one

Marliere et al. (2011), **Angew. Chem. Int. Ed**

DOI: 10.1002/anie.201100535



dNaM•d5SICS

- 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