

What are the limits of (Synthetic) Biology?



Prof. Vitor B. Pinheiro
Department of Pharmacy

Synthetic Biology in context

- Depending on interpretation the concept of Synthetic Biology, the field is over 100 years old.
- As early as in the dawn of molecular biology, Synthetic Biology was already envisioned.

The work on restriction nucleases (...) has led us into the new era of synthetic biology where not only existing genes are described and analyzed but also new gene arrangements can be constructed and evaluated.

Wacław Szybalski

Synthetic Biology

Biology **for synthesis**

Biology **as a tool**

Alternative Biology

Synthesising Biology

Biology **as data**

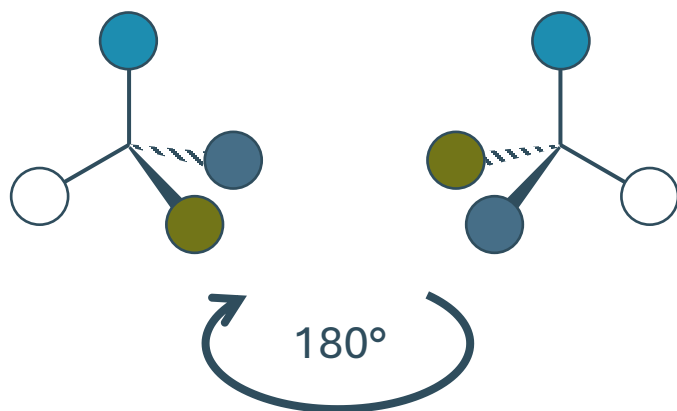
- More systematic
- Bottom-up
- Nature as a starting point

“What I cannot create, I cannot understand.”

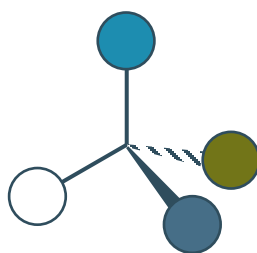
Richard Feynman

Biology vs Chemistry

Rotation



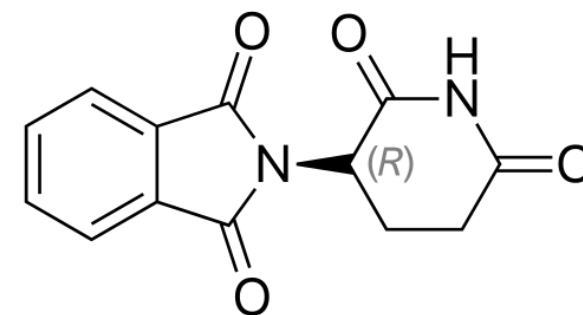
Chirality



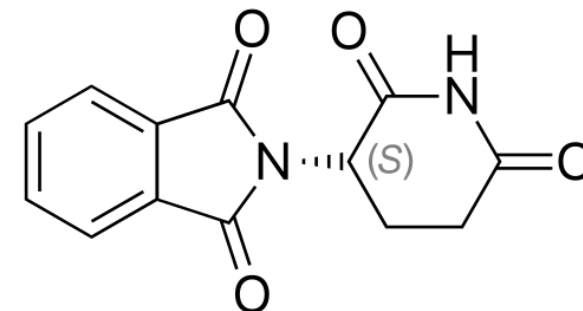
- Lack of symmetry in a molecule creates a chiral centre
 - Handedness (enantiomer)

- Handedness matters in biology
 - Thalidomide

‘wonder drug’
for morning
sickness



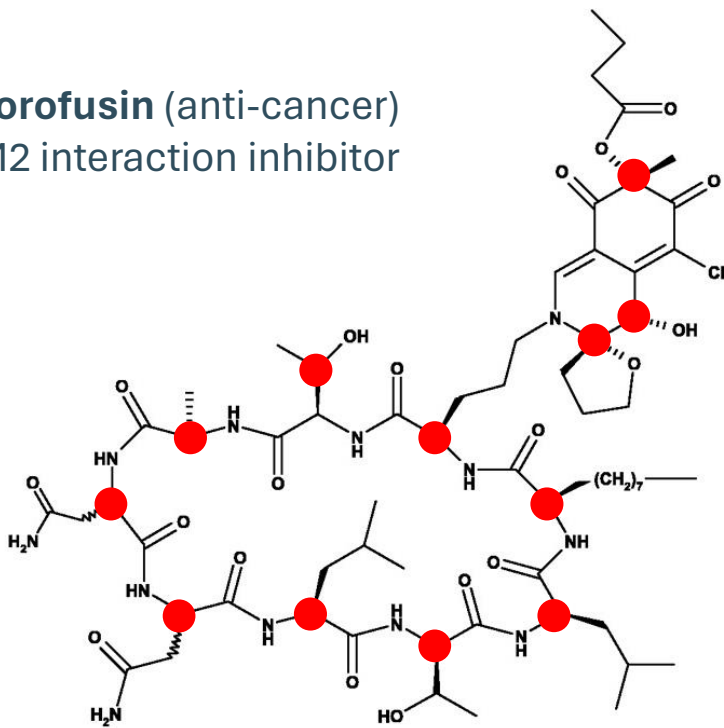
Induces foetal
development
defects



Biology vs. Chemistry

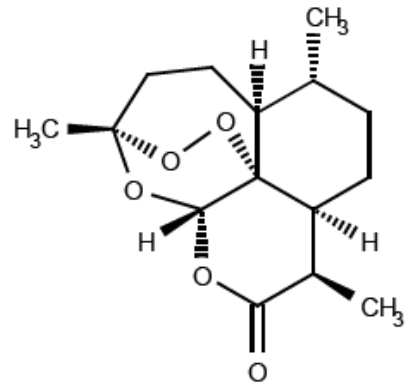
- Bioactive compounds (in general) have multiple chiral centres

Chlorofusin (anti-cancer)
P53/MDM2 interaction inhibitor

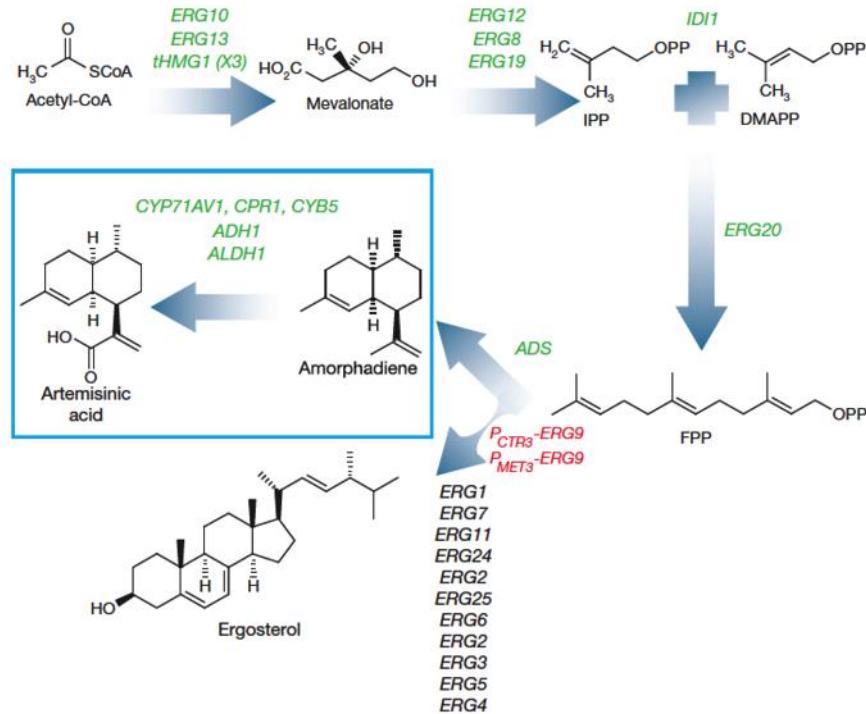


- If chemistry can't deliver enantiospecificity then each synthetic step limits yields to 50%
 - Per chiral site!
- Biology on the other hand is exceptional at making reactions enantioselective and enantiospecific.

Biological synthesis of artemisinin



- Anti-malarial drug
- Extracted from *Artemisia annua*



- Amorphadiene
 - Intermediate in the artemisinin synthesis
 - Natural yeast metabolite
- Strategy
 - Overexpression of yeast enzymes
 - Heterologous expression of *A. annua* enzymes
 - Downregulation of yeast pathways (side reactions)
 - Chemical conversion post extraction

Spider silk – a ‘super material’

We study silk proteins spun by spiders to determine what gives them their incredible properties.



**Bolt
Threads**

We develop proteins inspired by these natural silks by using bioengineering to put genes into yeast.



We produce the protein in large quantities through fermentation, using yeast, sugar, and water.

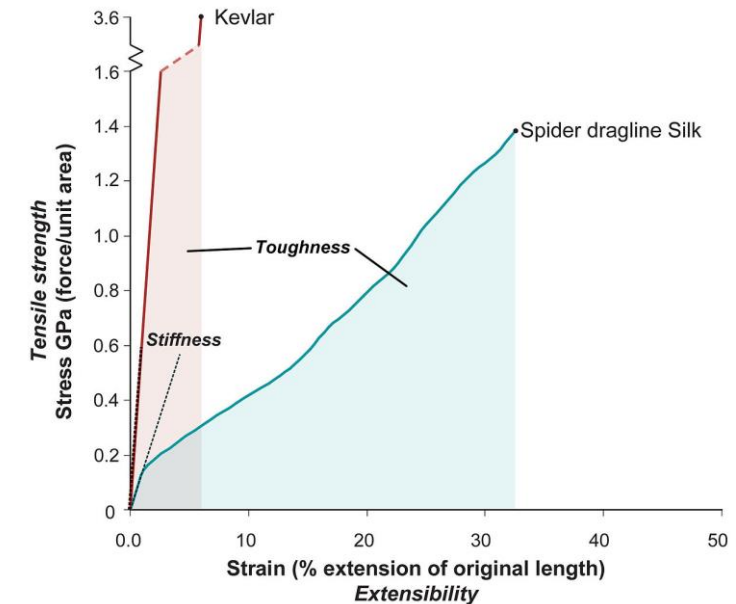


We isolate and purify the silk protein, then spin it into fibers, similar to fibers like rayon and acrylic.

We knit these fibers into fabrics and garments.



- Tensile strength comparable to high-grade steel
- Can be stretched up to 5 times its natural length

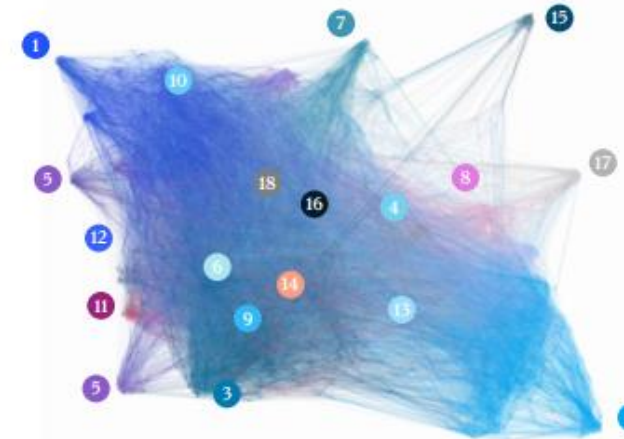


Biology for Synthesis in Europe

42 Synbio Startups Leading in Europe



Europe Bio Revolution ecosystem, n = 4,487 companies



Ecosystem of companies based on similar company descriptions

Cluster name	Share of total, %	Share of total, %	
1 Therapeutics and pharmaceuticals	15	10 Gene-based	4
2 Biofuels and renewable energy	14	11 Genomic analysis (health and nonhealth)	3
3 Bioinformatics	9	12 Pharmaceutical manufacturing	2
4 Biomaterials and biochemicals	9	13 Crop-related technology	2
5 Pharmaceutical services, drug development, and drug discovery	9	14 Biosensor and medical devices	2
6 Molecular diagnostics	9	15 Biotechnology services	2
7 Cell-based	8	16 Microbiome	2
8 Microalgae	5	17 Plant-based meat and food	1
9 Synthetic biology	5	18 Fertility and prenatal screening	1

Note: Figures may not sum to 100% because of rounding.
Source: McKinsey Growth Analytics; McKinsey Strategy & Corporate Finance Insights

McKinsey & Company

<https://www.eusynbios.org/synbiobrewery>



SynbioBrewery



Say hello to **SynbioBrewery** – the ultimate platform for change-makers like you to shape the world through science and creativity! 🌱🔬

At its core, SynbioBrewery is a dynamic **hackathon community platform** where individuals passionate about synthetic biology can **connect, form teams to solve challenges**, and access free **entrepreneurship** training sessions.

Each year a thematic focus will be selected, ranging from **environmental remediation, sustainable energy, healthcare and climate change mitigation**. Challenges will be meticulously crafted and hosted in our platform, allowing you to unleash your creativity to develop innovative solutions with SynBio.

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Biology as a tool

- Tools have specialisms and limitations

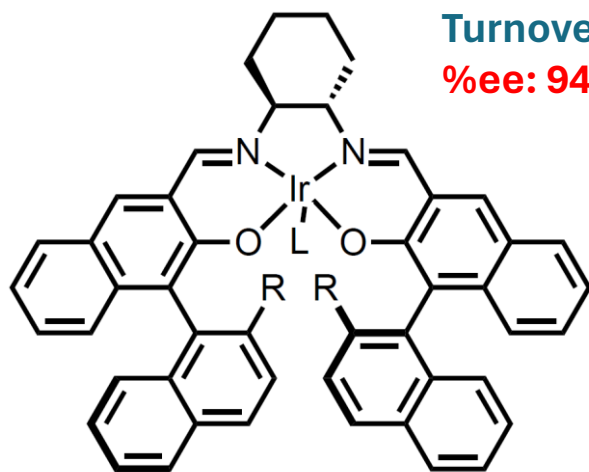


Biology as a tool

- **Tools have specialisms and limitations**
- Biology is limited by what is possible, not what is natural
 - **Extensive not thorough**
 - Invention ($0 \rightarrow \delta$) is rare
 - Optimisation ($\delta \rightarrow 1$) can be very efficient
 - Optimisation leads to **natural-to-natural discrimination**

Extensive not thorough

- Biology can be engineered to reach other chemical reactions
- Carbon – Silicon bonds are difficult to make chemically

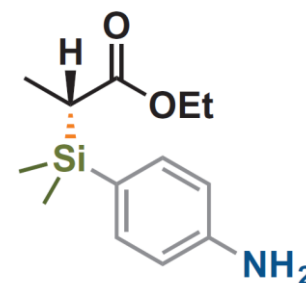
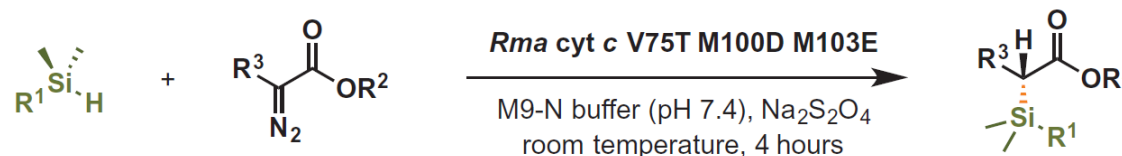


Dichloromethane
-30°C for 24h

Turnover #: 24-50

%ee: 94-99%

- C-Si bonds are not naturally made but can be accessed by engineering cytochrome P450

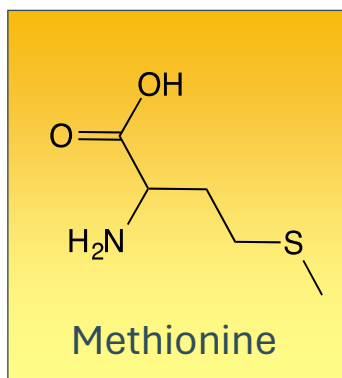


22: 6080 TTN, >99% ee
8210 TTN, >99% ee

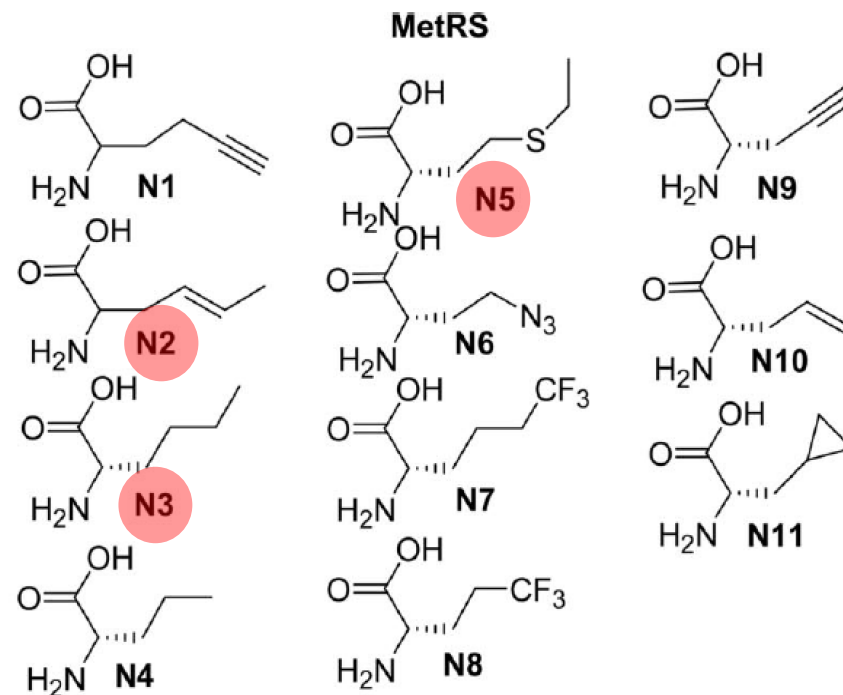
Kan et al. (2016) **Science**
DOI: 10.1126/science.aah6219

Natural-to-natural discrimination

- *E. coli* aaRS *in vitro* shown to incorporate unnatural substrates
 - 13 of 20 aaRS
 - Significant range of chemical modifications



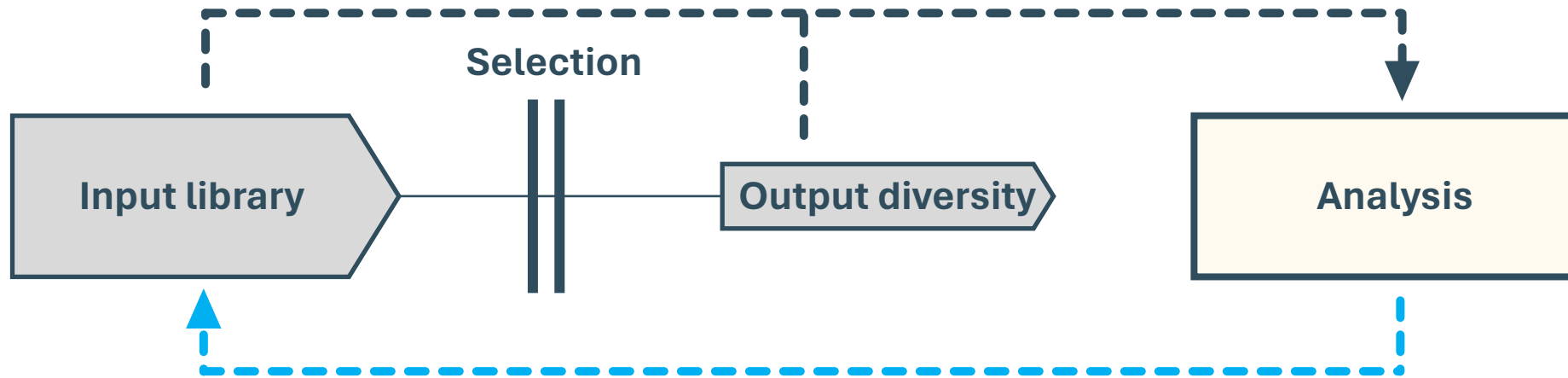
Hartman et al. (2006) **PNAS**
10.1073pnas.0509219103



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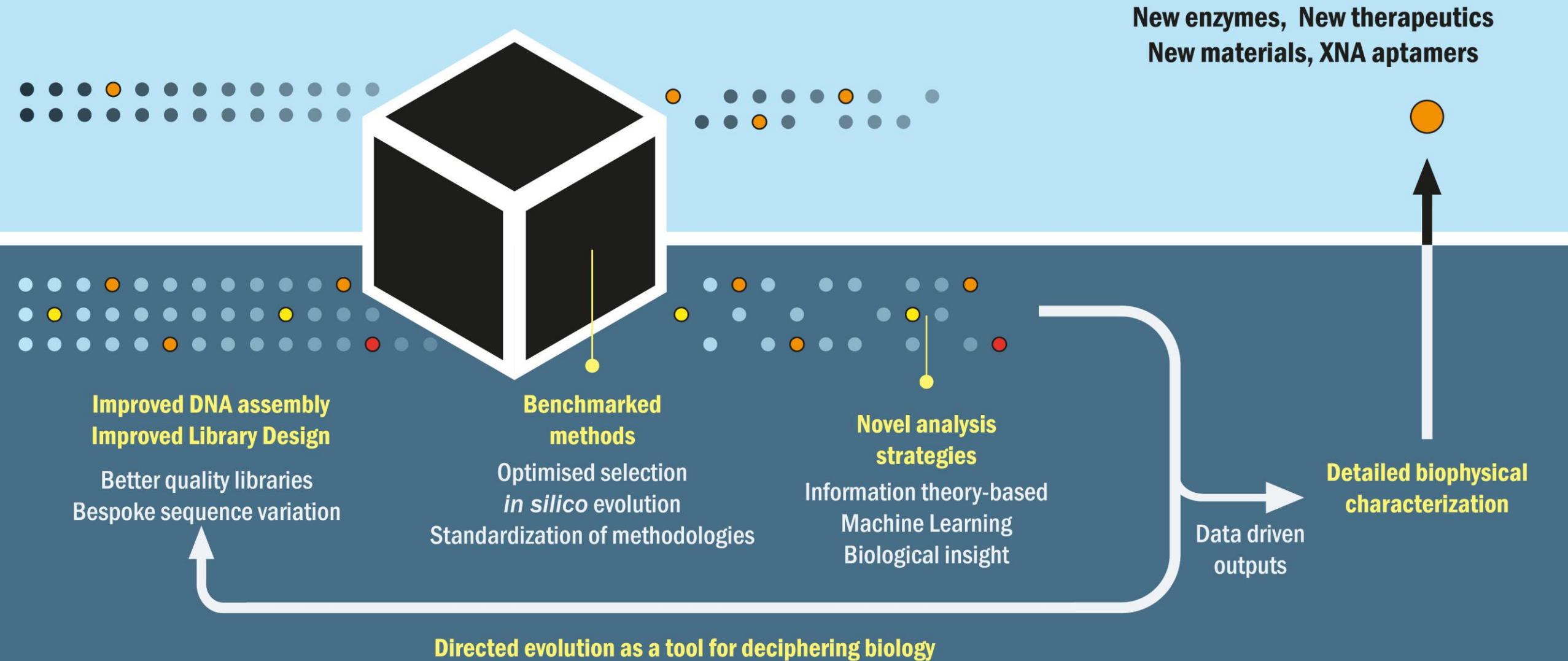
Evolution can be harnessed as a tool for Synthetic Biology



- Directed evolution as an engineering cycle
 - **Design, build, test and learn** but up to 10^{14} at a time in parallel
- **Bypasses knowledge gaps**
 - Reaches beyond biology
 - Refractory to incomplete or incorrect knowledge
- Highly versatile but technically challenging
 - Constrained by **target knowledge, library, selection method and data analysis**.

Tizei *et al.* (2016) **Biochemical Soc. Transactions**
10.1042/BST20160076

Synthetic Biology through Directed Evolution



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Synthesising Biology

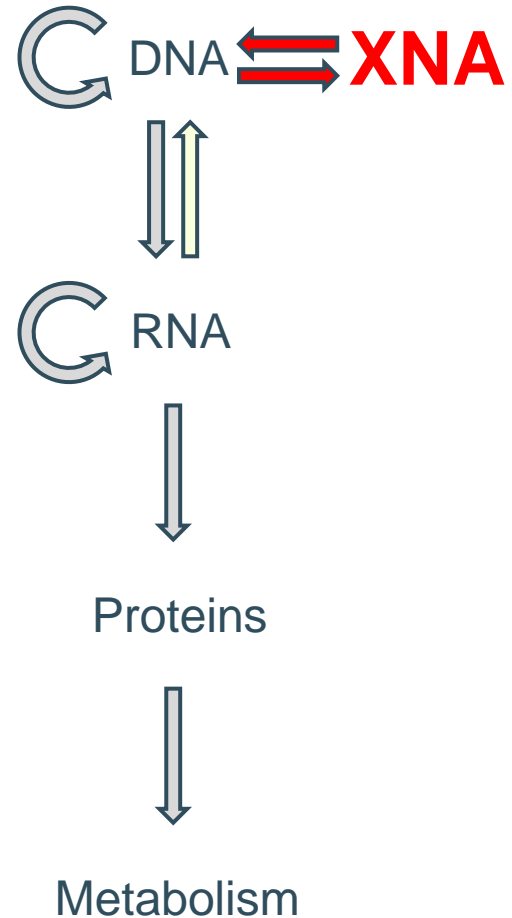
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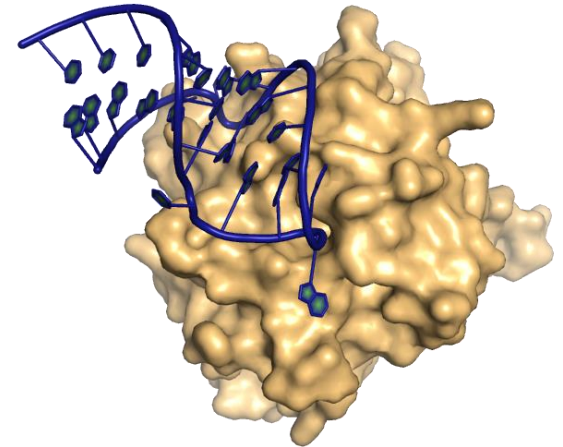
Alternative Biology (Xenobiology)



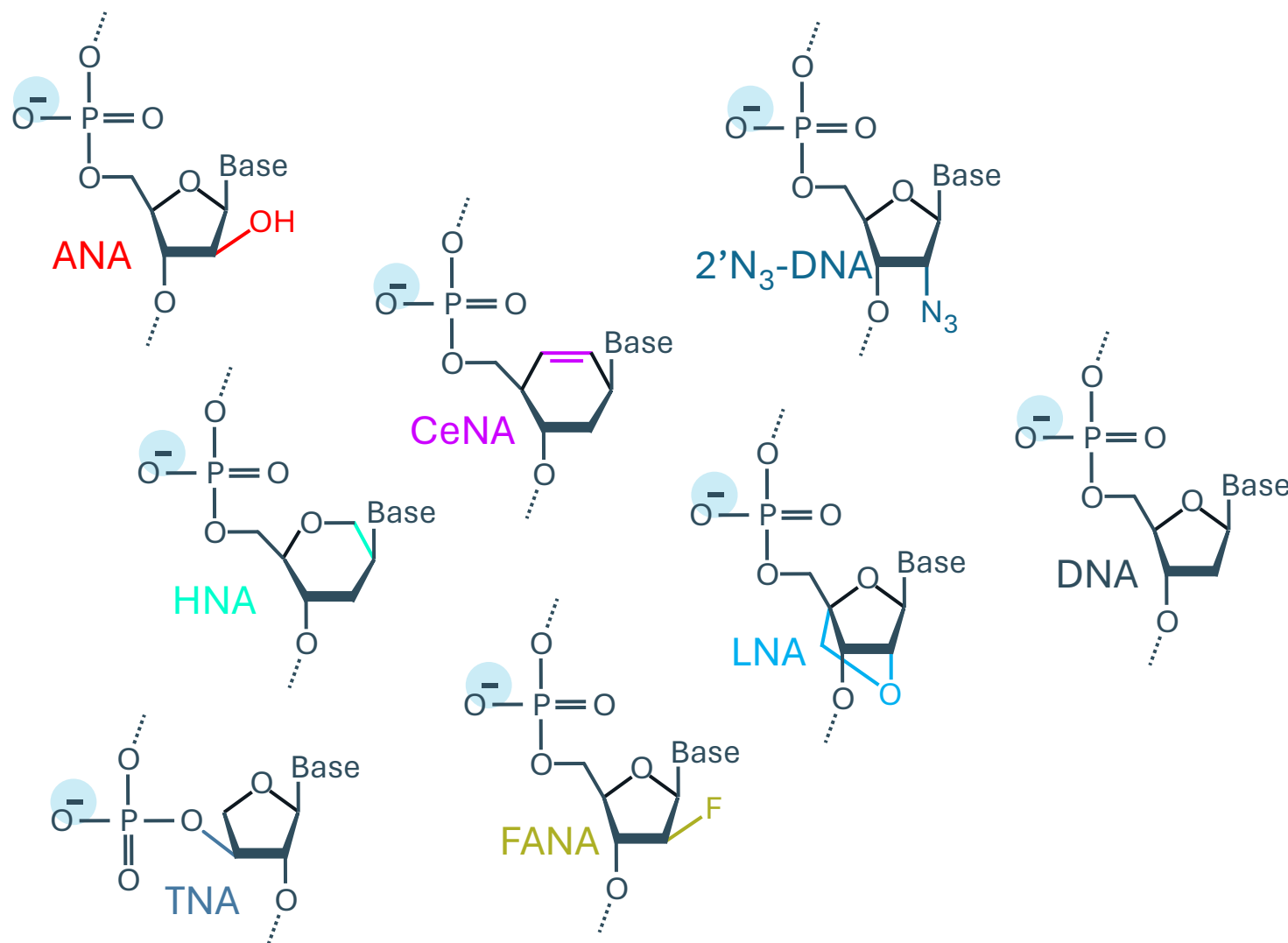
- Information **storage** and **propagation** are essential for life.
- DNA and RNA are the only genetic polymers in biology
- **While uniquely suited, are they unique?**

in vitro

XNA aptamers, XNA biomaterials



Synthetic genetic polymers

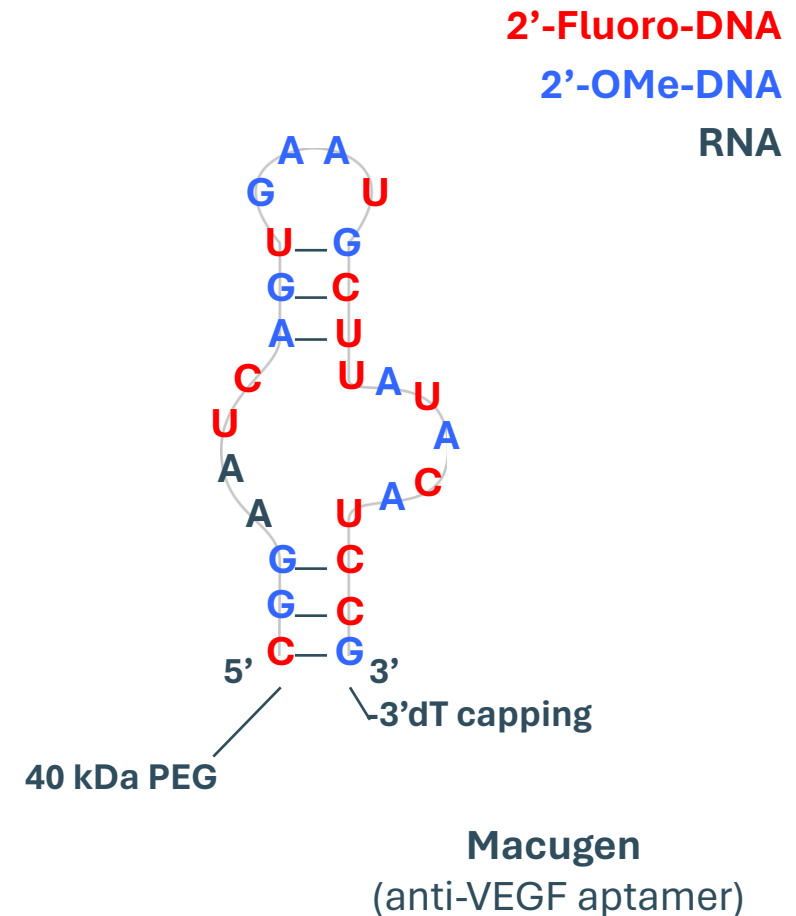


Genetic system	Aggregate misincorporation error (x 10 ⁻³)
CeNA	4.31
FANA	5.03
ANA	5.81
HNA	7.54
DNA	8.30
TNA	48.5
LNA	52.8

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

Therapeutic aptamers

- Limited chemical and biological stability
- Nucleic acids can be chemically modified
 - Most modifications are poor polymerase substrates, **hindering directed evolution**
- Highly charged backbone hinders cellular uptake
 - Aptamers can only target extracellular or cell surface targets



Towards XNA aptamer therapeutics

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- **XNA genetic materials**

- **Custom chemical and biological stability**
- **Compatible with directed evolution**
- **Access different chemical diversity**



Due to multiple issues with early aptamer technology, Somalogic is one of the last companies that still work on aptamers

Davies et al. (2012) **PNAS**
10.1073/pnas.1213933109

50 shades of ligase

1. Optimise reaction

- DNA ligase as a “good engine”
- Forcing reaction conditions

Kestemont, Renders et al. (2018) **Chem. Comm.**
10.1039/C8CC02414F

2. Rational design

- Bioinformatics-driven design

Vanmeert et al. (2019) **NAR**
10.1093/nar/gkz551

3. Cell-display

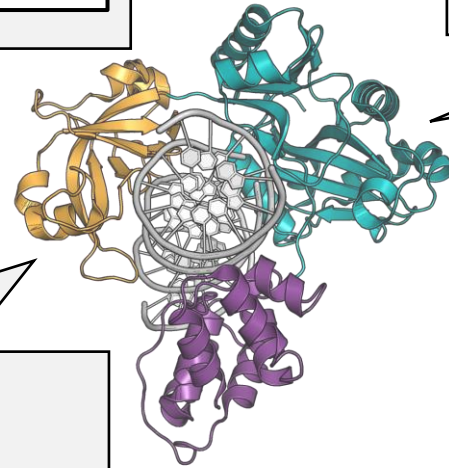
- Suitable for mesophilic ligase engineering
- Isolated from cellular machinery

Csibra et al. (2020) **Chem. Biochem.**
10.1002/cbic.202000203

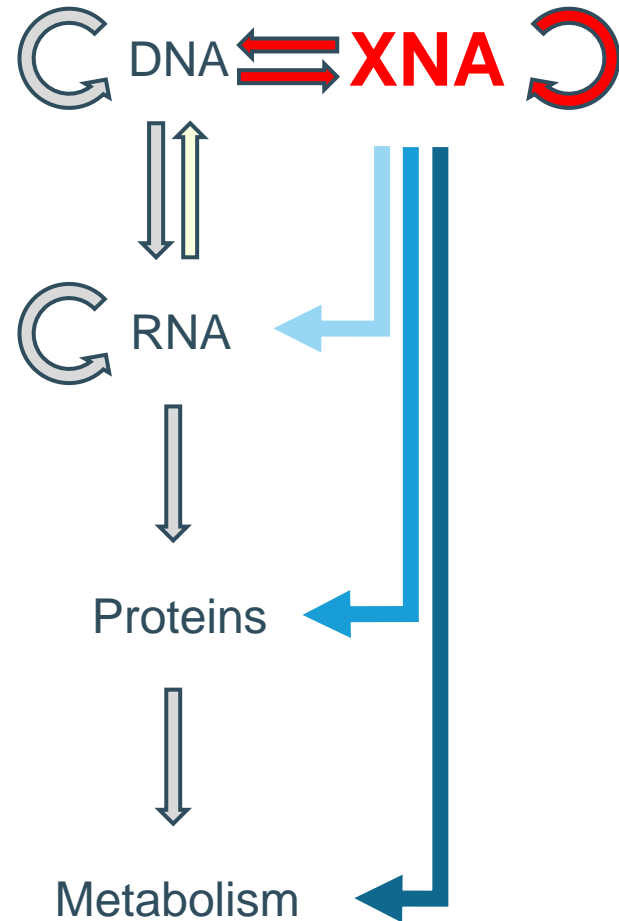
4. Compartmentalised ligase self-tagging

- Parallels with polymerase engineering
- Greatest flexibility and larger library sizes
- Better suited for thermophilic ligases

Kestemont et al. (*in preparation*)



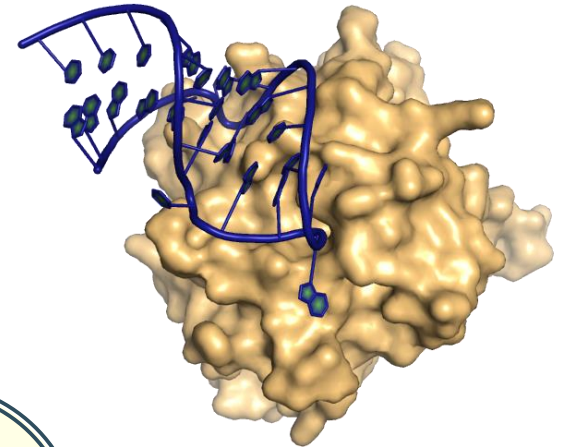
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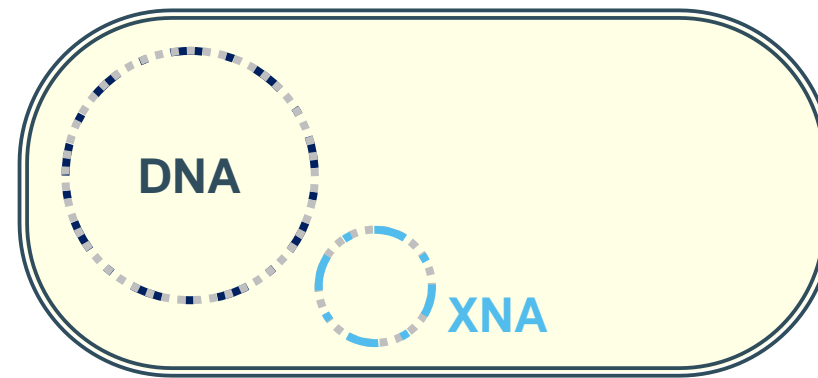
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in vivo

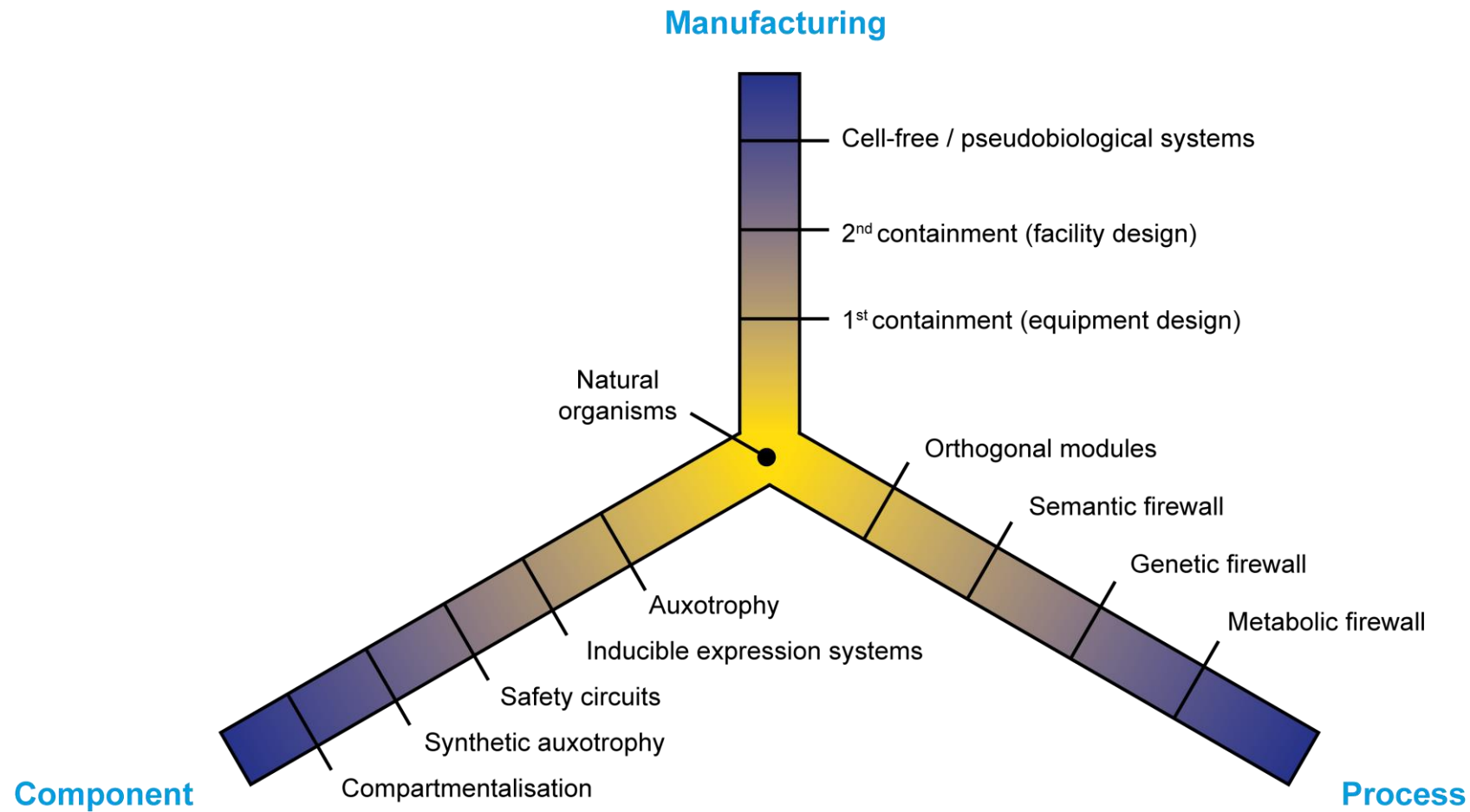


Orthogonality

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



Routes to safe bioprocessing



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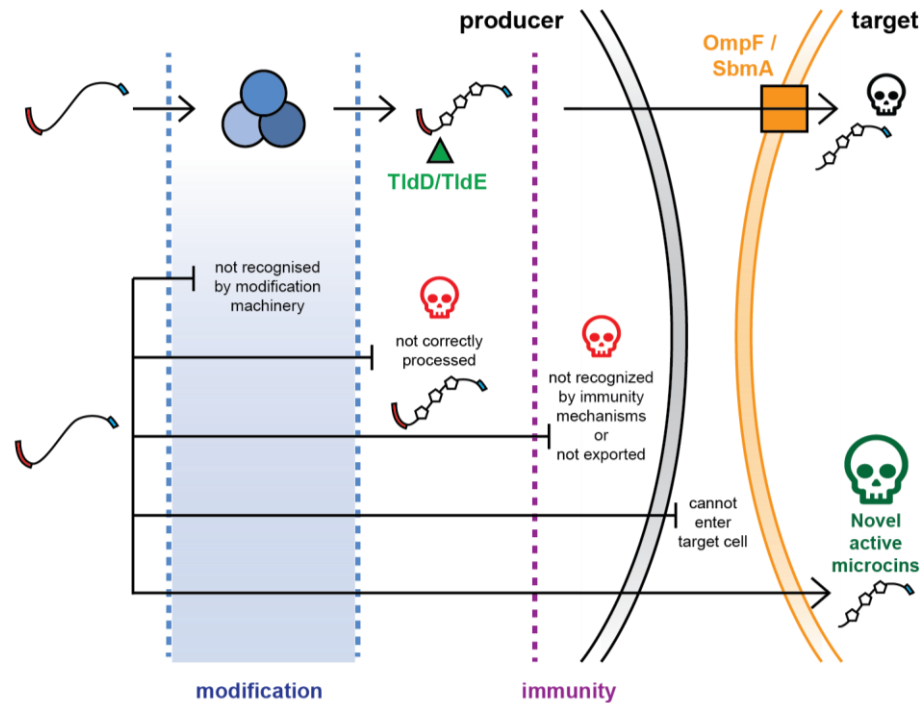
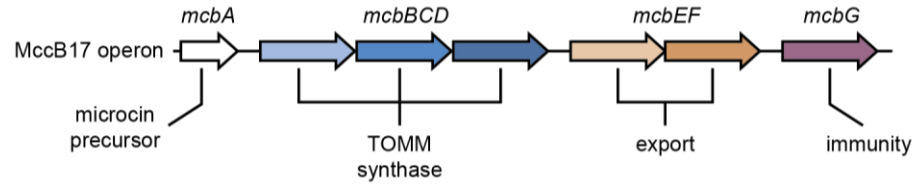
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Directed evolution of new microcins

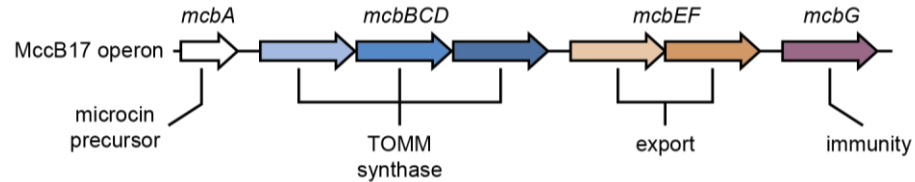


- Bottom-up approach to the natural machinery
 - Nature as a starting point

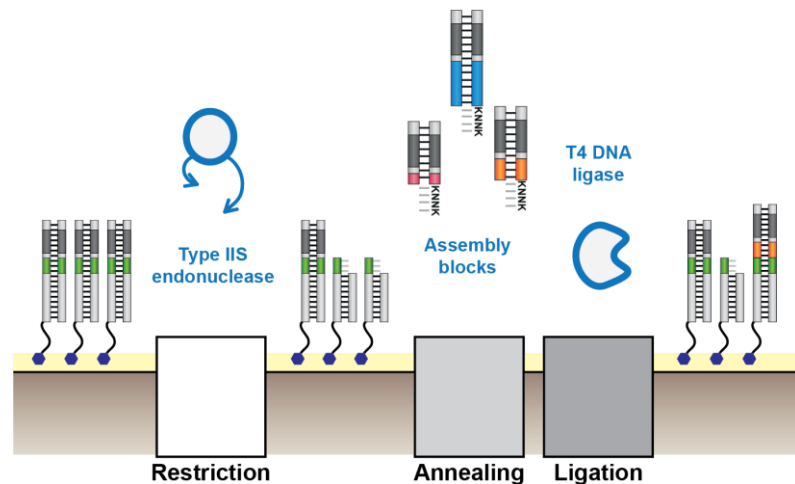
Alternative Biology

Withanage *et al.* (2023) **BioRxiv**
10.1101/2023.08.29.555279

Directed evolution of new microcins



- Bottom-up approach to the natural machinery
- Novel platforms for diversity generation
 - Variation in length and composition

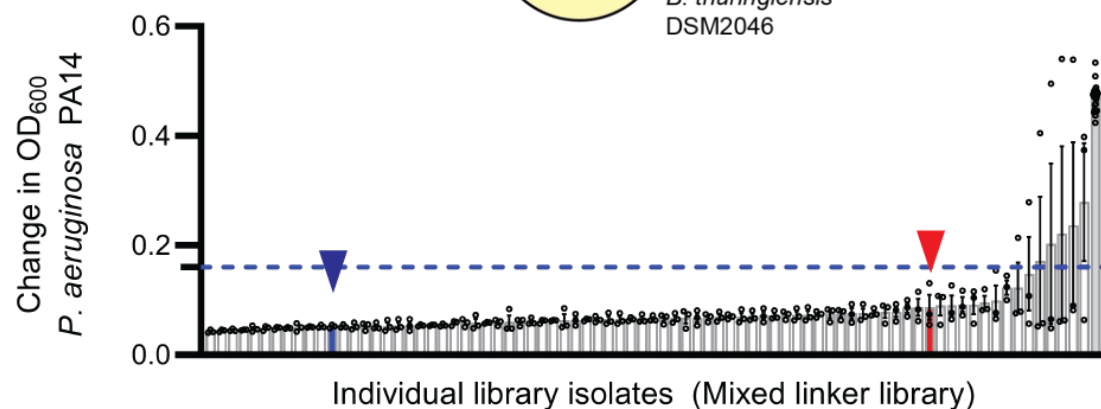
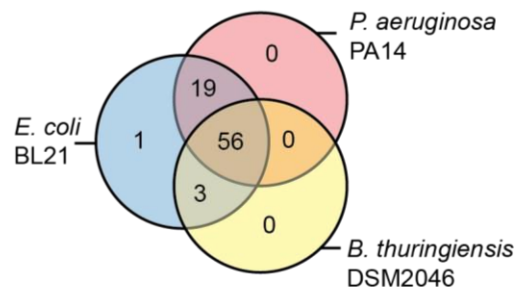
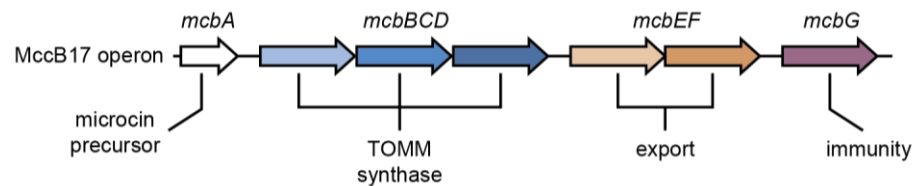


Alternative Biology

Biology as a tool

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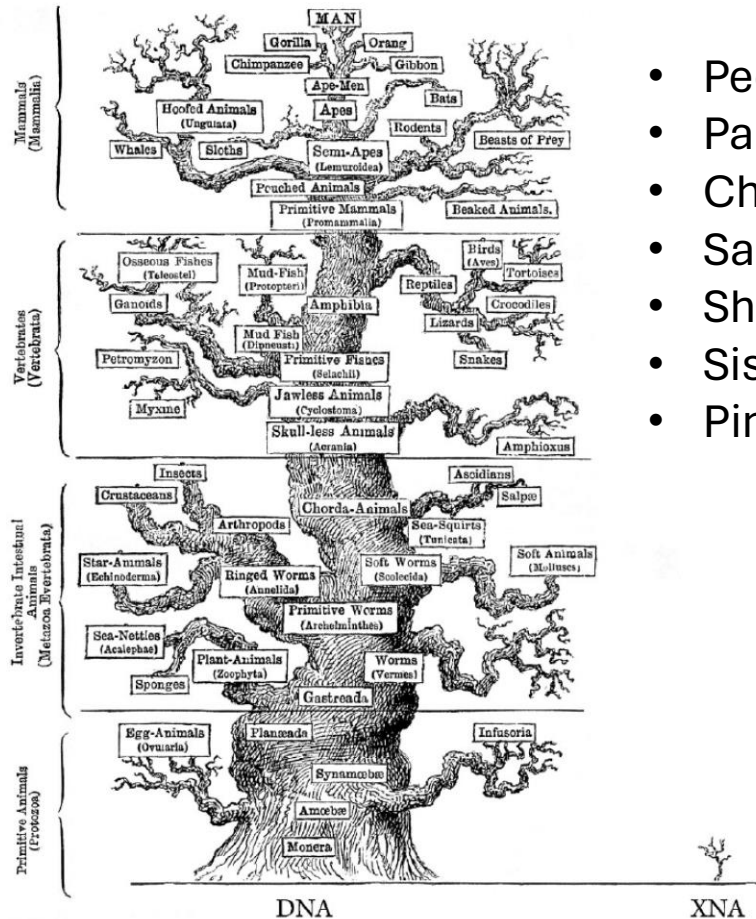


- Bottom-up approach to the natural machinery
- Novel platforms for diversity generation
- Identification of dense functional space

Alternative Biology
Biology for synthesis
Biology as a tool

Withanage *et al.* (2023) **BioRxiv**
10.1101/2023.08.29.555279

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- Phil Holliger

- POSSIBL consortium
- Arnout Voet
- Jeroen Lammertyn
- Syngulon
- Joleen Masschelein



Schmidt, M. (2010) **Bioessays**
10.1002/bies.200900147