http://www.cnb.csic.es/~meml

Engineering bacteria for the Environment: tools, strategies and some lessons from the past

Víctor de Lorenzo

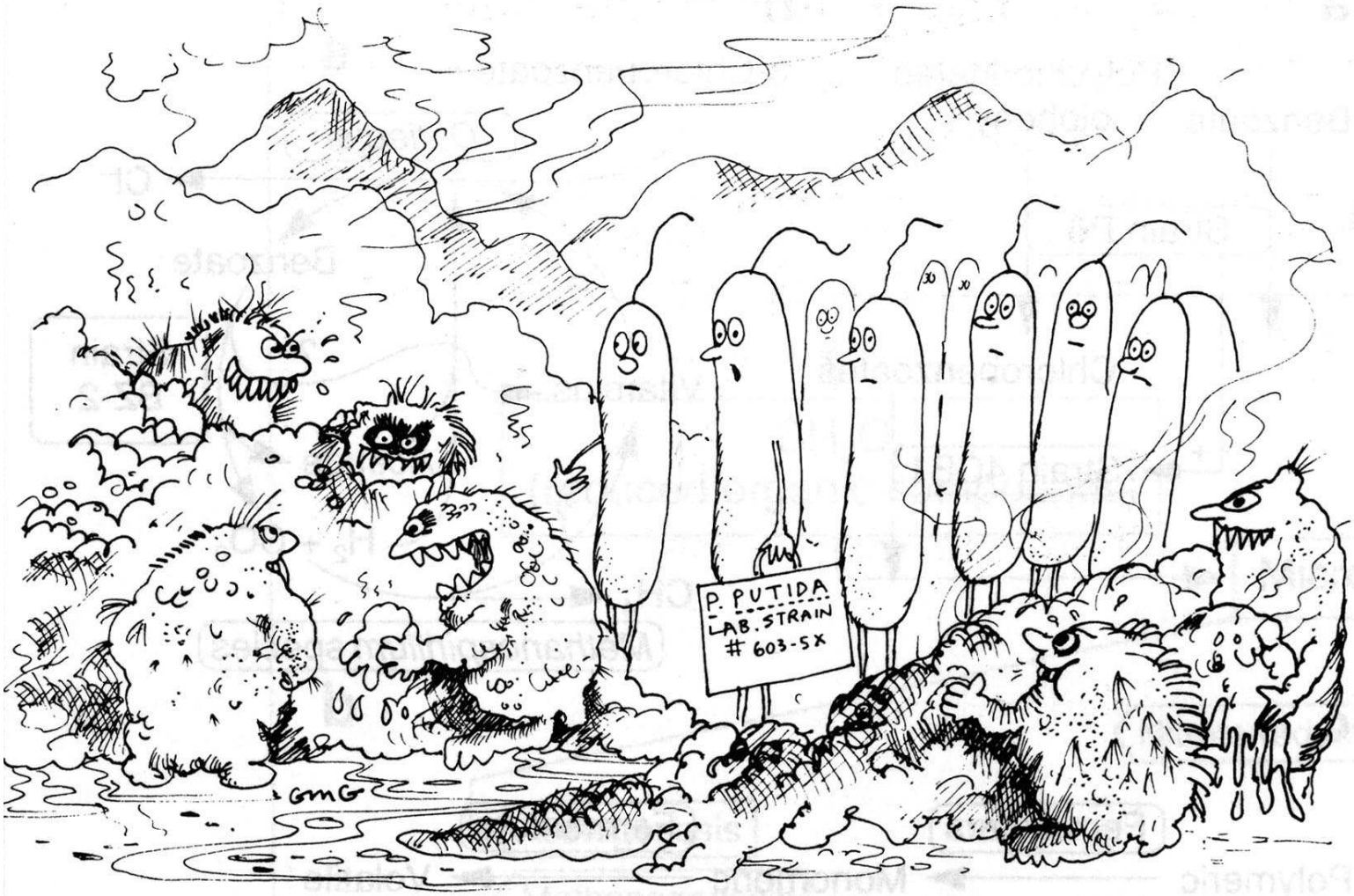
Patent application:

Microorganisms having multiple compatible degradative energy-generating plasmids and preparation thereof

- Filed in 1972
- Supreme Court ruling in 1980
- Patent #425944 issued in 1981

Environmental uses of GMOs:

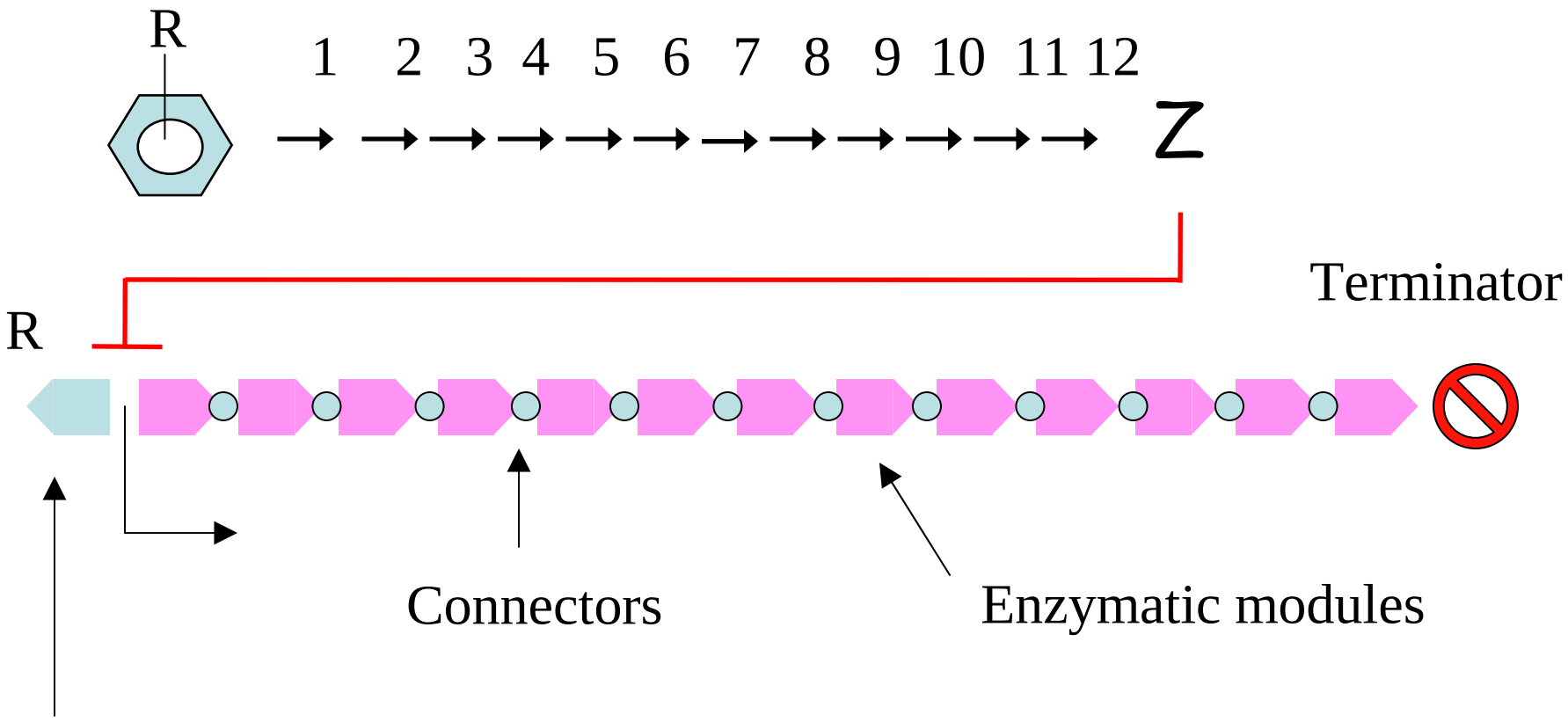
- *In situ* biodegradation
- Bio-transformation
- Detection
- Immobilization

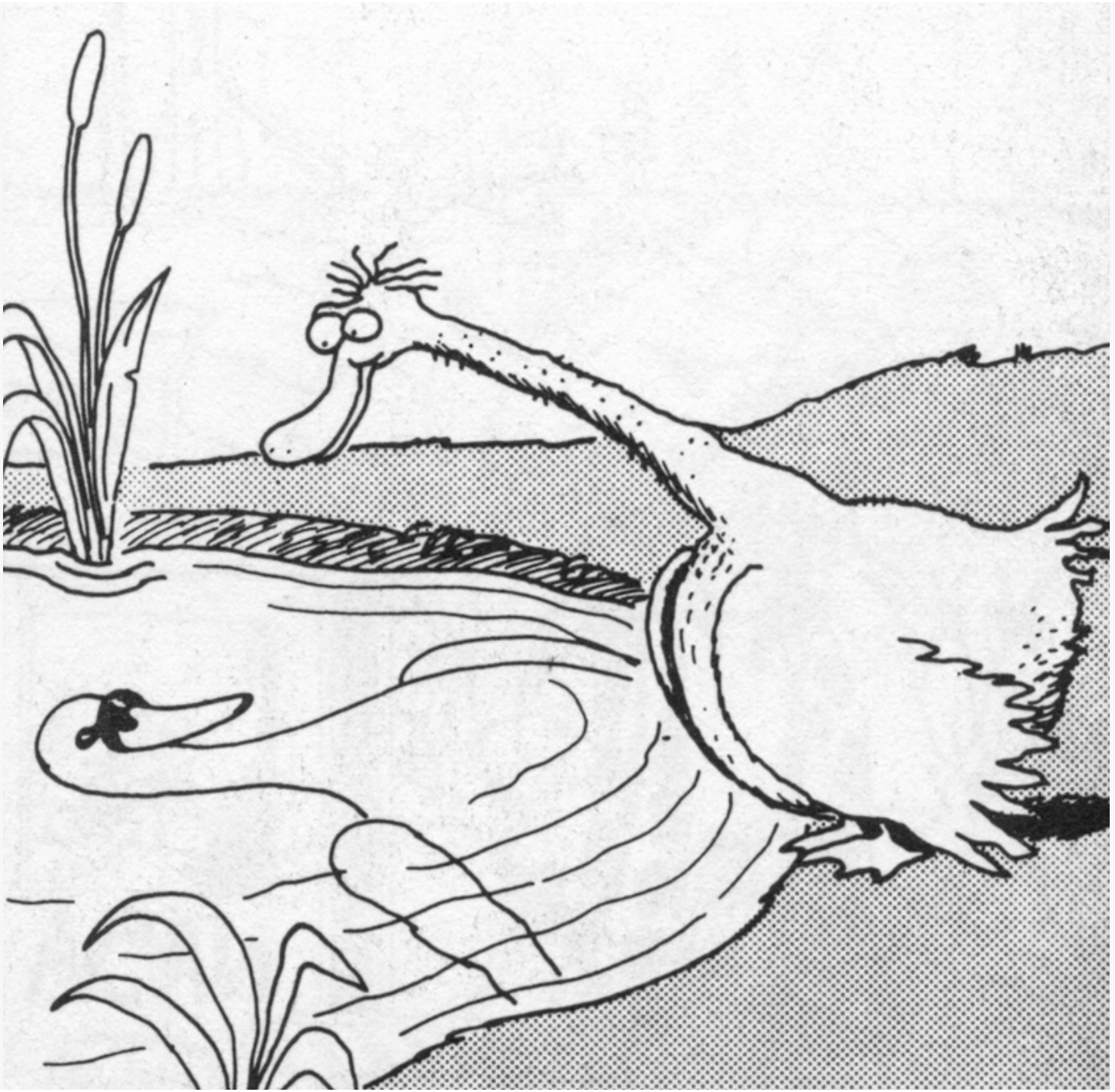


"Oh dear! I didn't realize 'in the field' would be like this!
We should have stayed in the laboratory."

The engineer's view of pathway assembly…

Automated pathway design? Automated pathway construction?





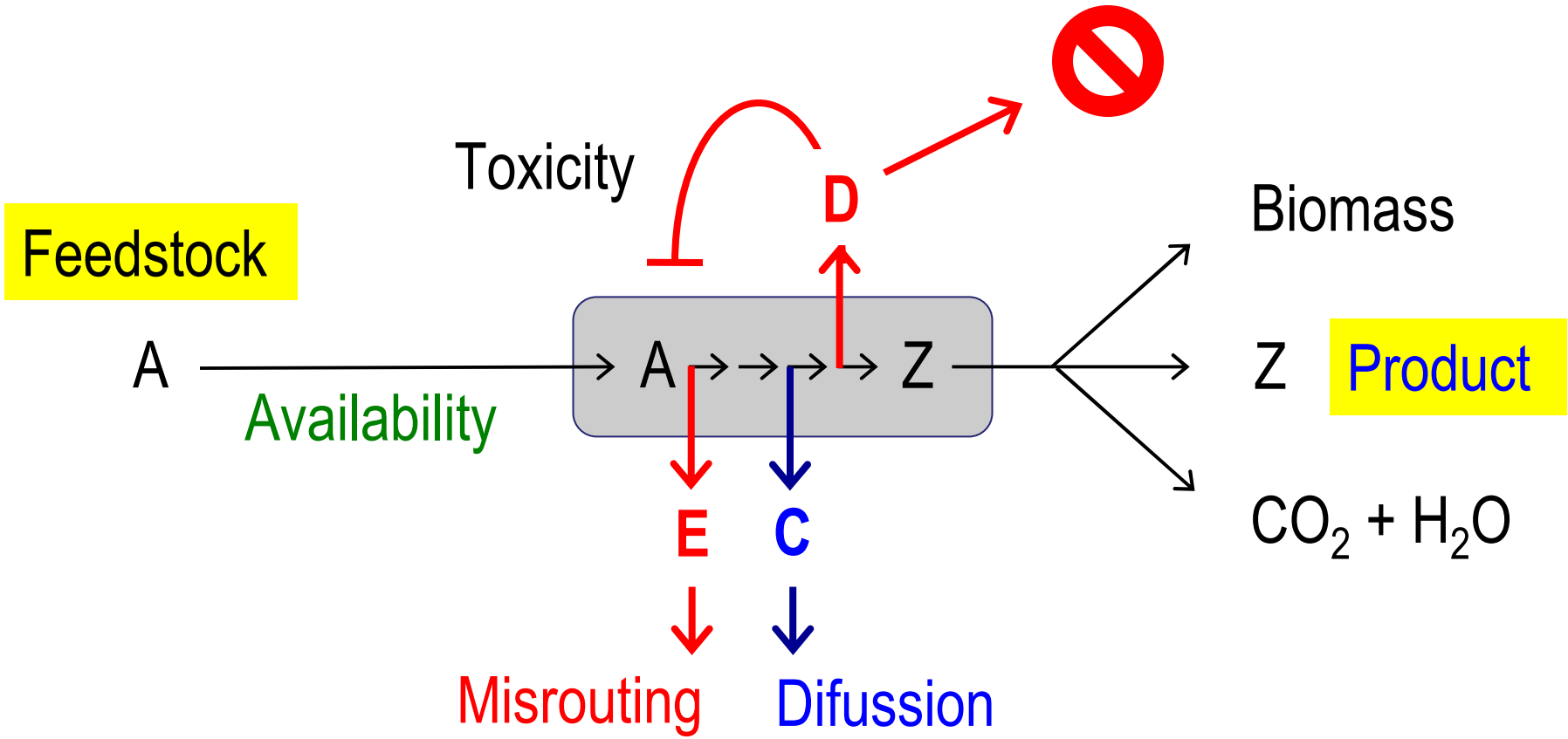
But we are not doing very well …

- Biological components are context-dependent
- Subject to Darwinian evolution
- Interact with metabolites & chemicals
- Combination originates emergence

"... Engineers hate complexity. I hate emergent properties. I like simplicity. I don't want the plane I take tomorrow to have some emergent properties while it is flying..."

(www.edge.org; Engineering Biology: a talk with Drew Endy, 2008)

In vivo/in situ catalysis in reality…



Seven propositions on how:

Sythetic Biology can (and should!) capitalize on past experiences (incl. failures) in designing GMOs for environmental release

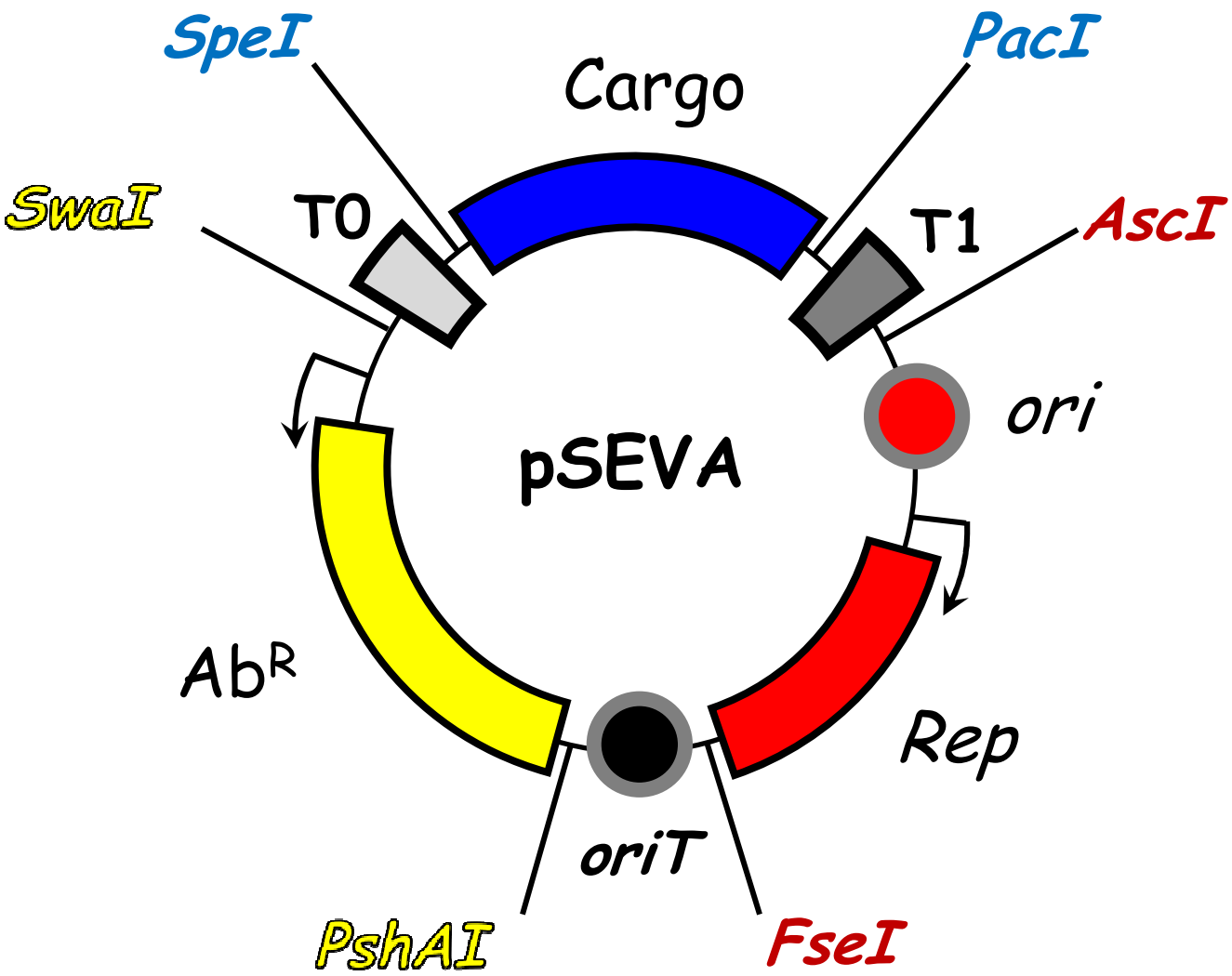
Proposition #1

Standards are feasible for a limited number of simple biological objects and functions but not (yet) for those for which we still ignore fundamental facts

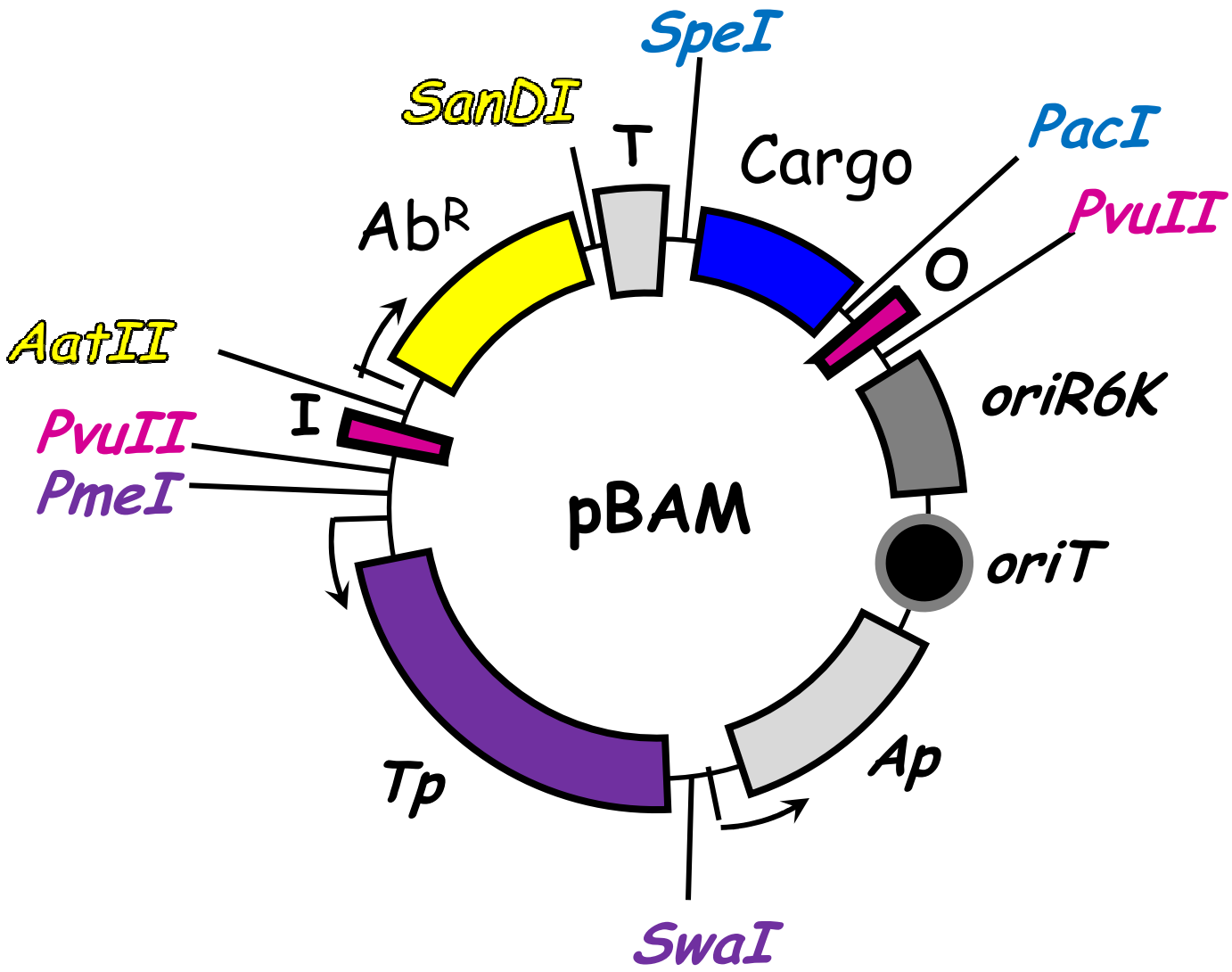
E.g. start by standarizing the very tools

Standard European Vector Architecture

SEVA plasmids



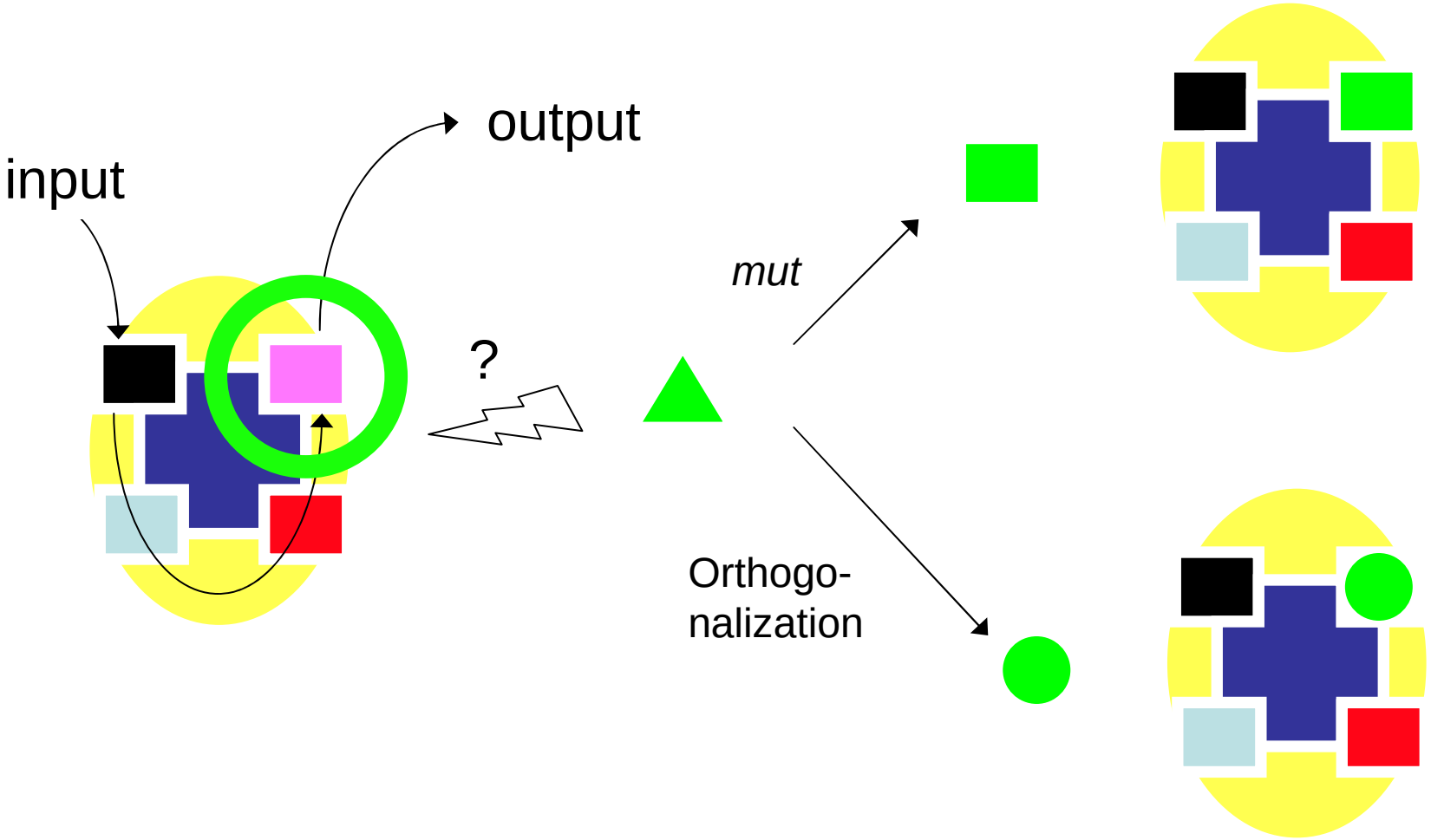
SEVA transposon vectors



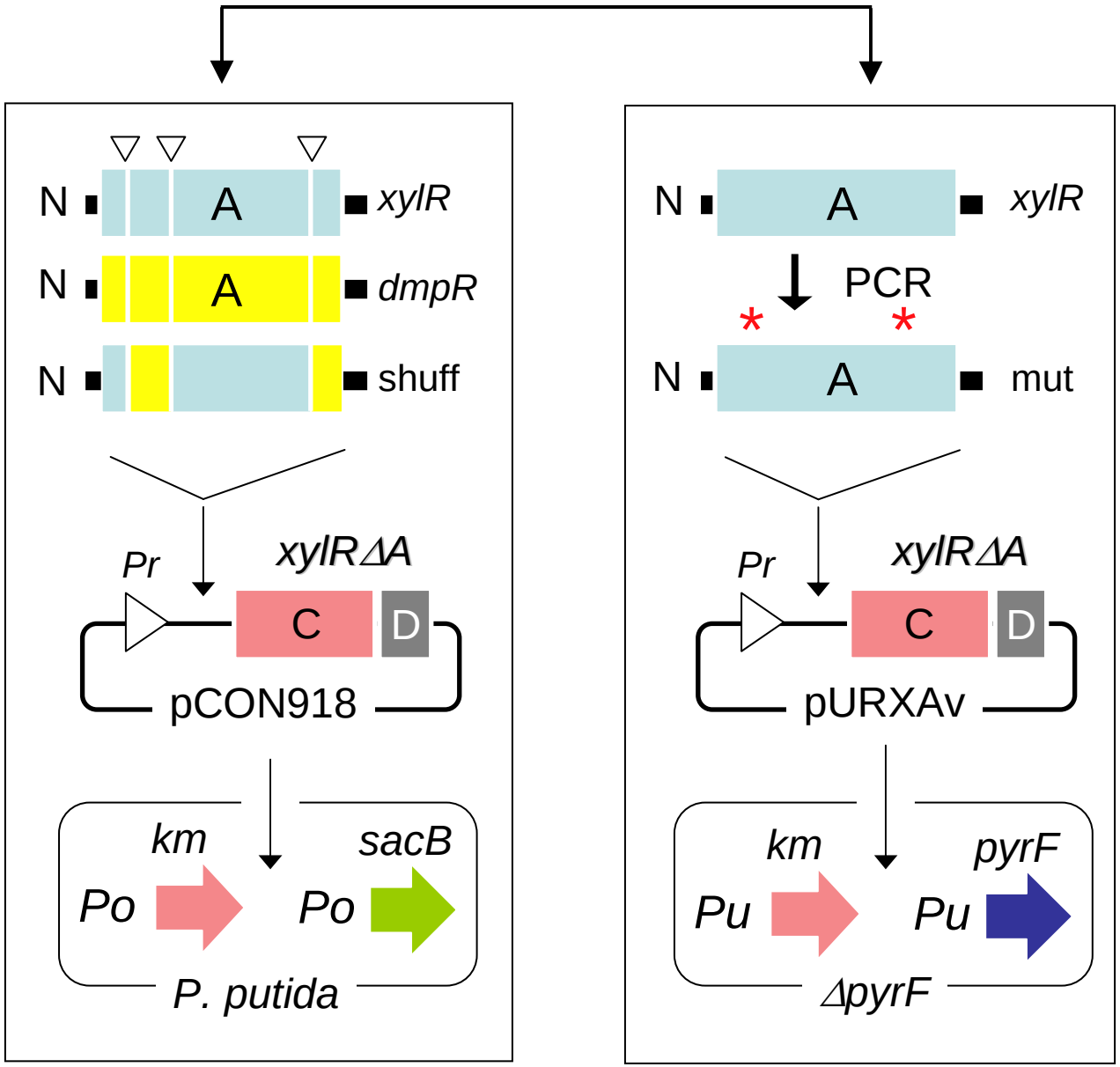
Proposition #2

Darwinian selection operates at every level of any biological system. Make an alliance with evolution rather than supress it or ignore it.

Overcoming resistance to implantation of new activities in a *Pre-existing network*

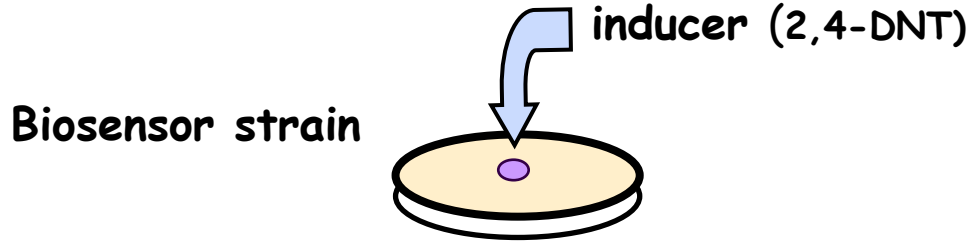


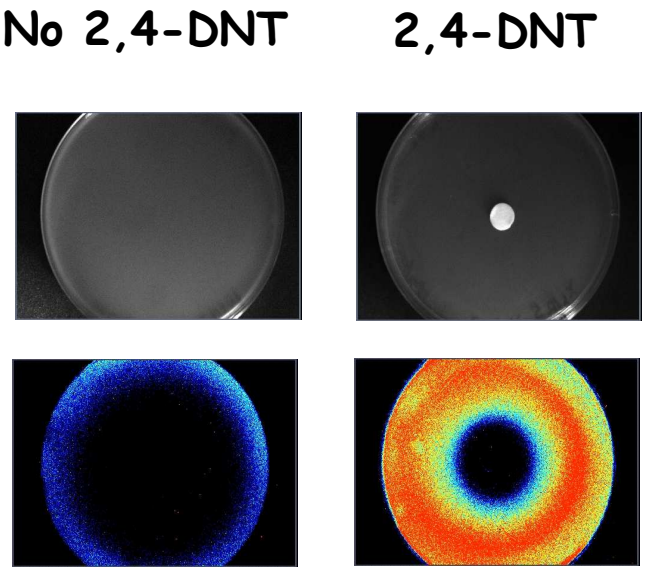
Generation of diversity and selection of 2,4 DNT A domain responders



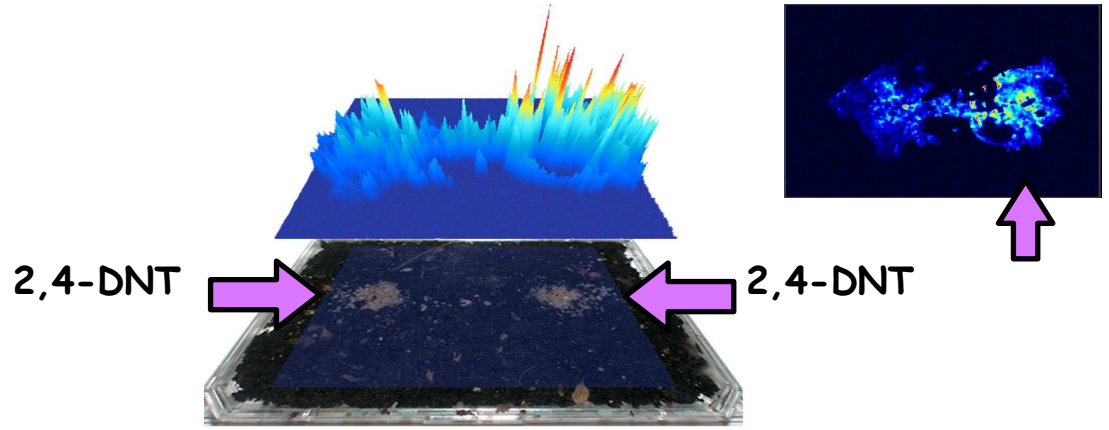
2,4 DNT Biosensor strains

In vitro





In vivo (soil microcosm)

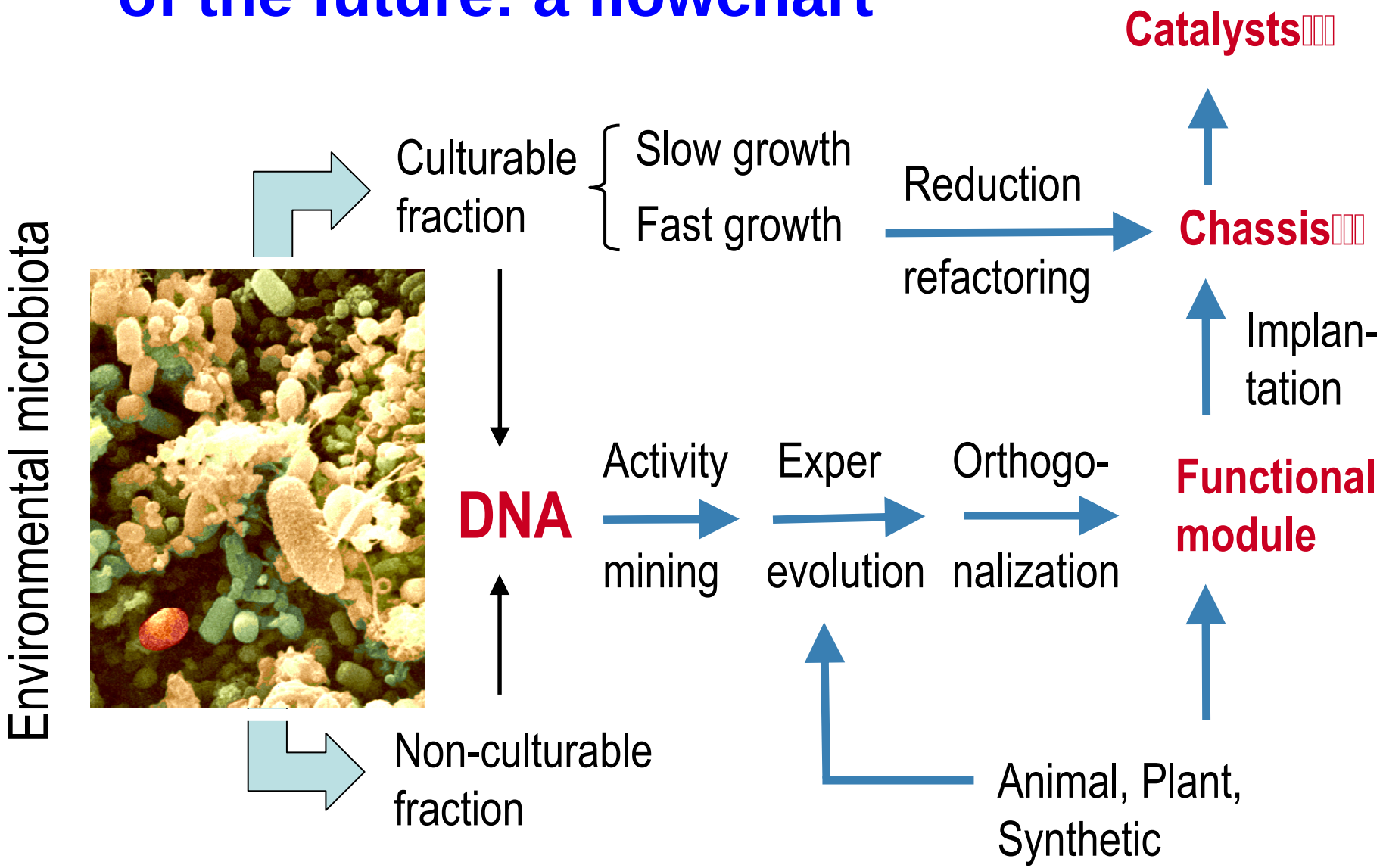


de las Heras A, et al. Environ Micro. 2008

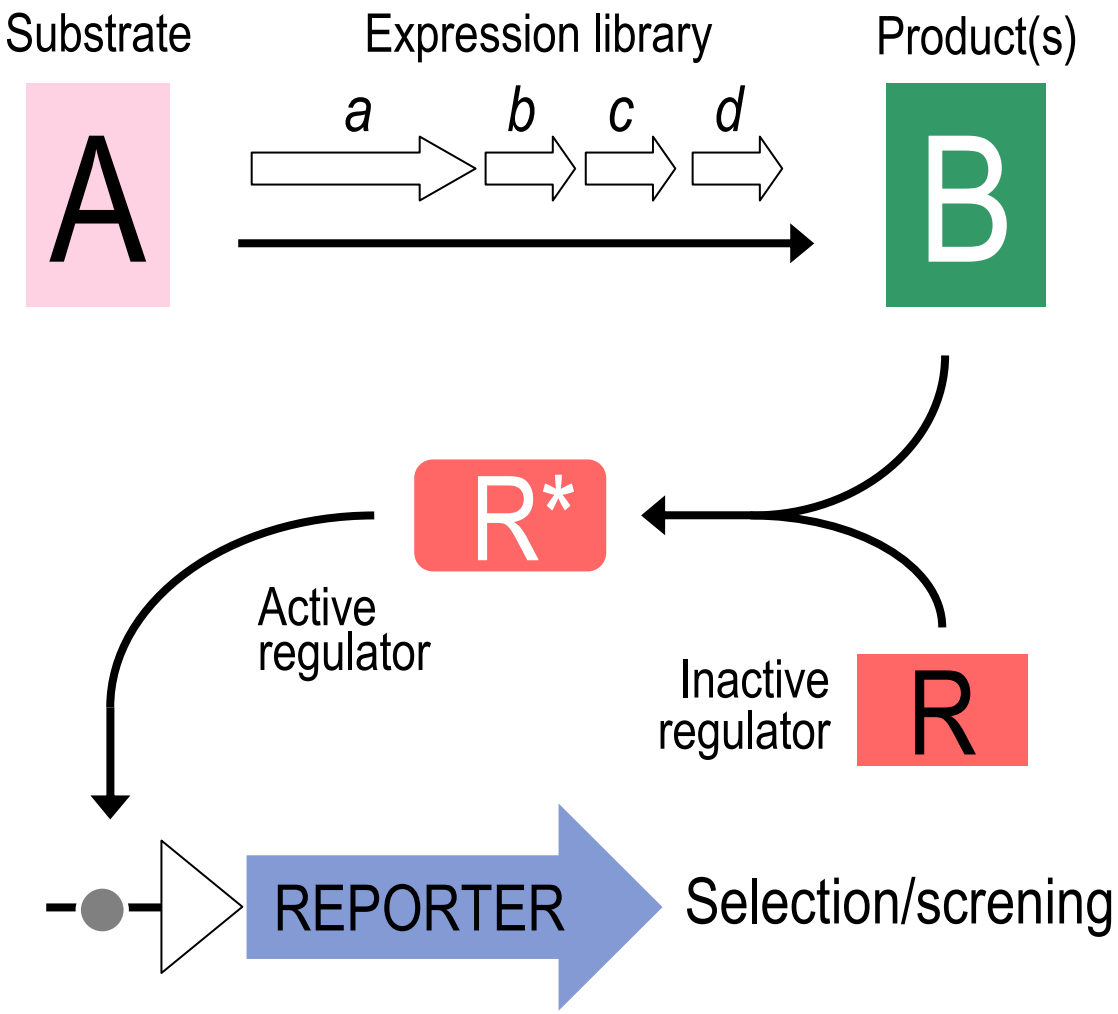
Proposition #3

Functions that we still miss in our engineered systems are to be found in environmental DNA (metagenome)

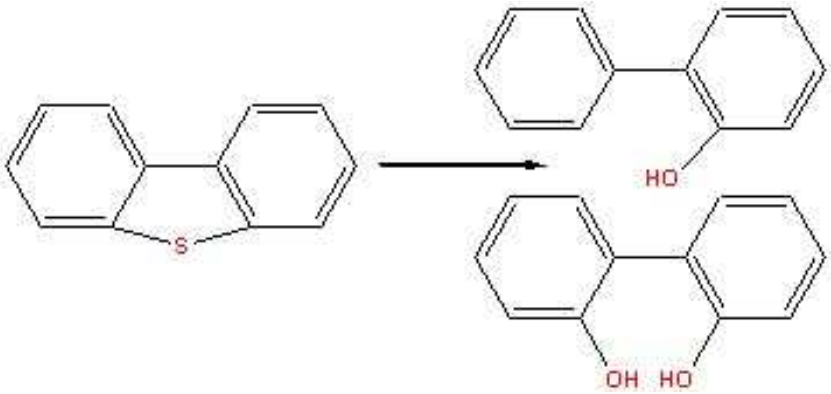
Environmental catalysts of the future: a flowchart



Capturing activities from metagenomes

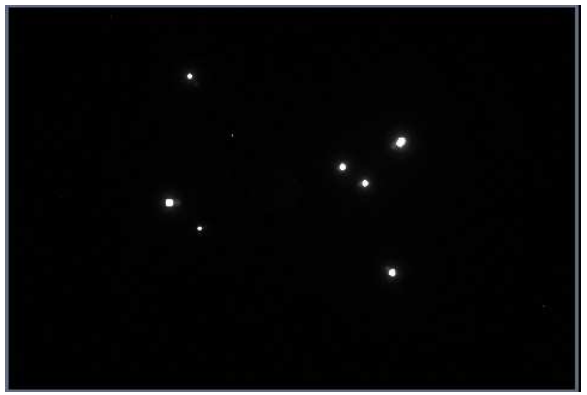


DBT biodesulphurization



HbpR

+DBT



pBG8

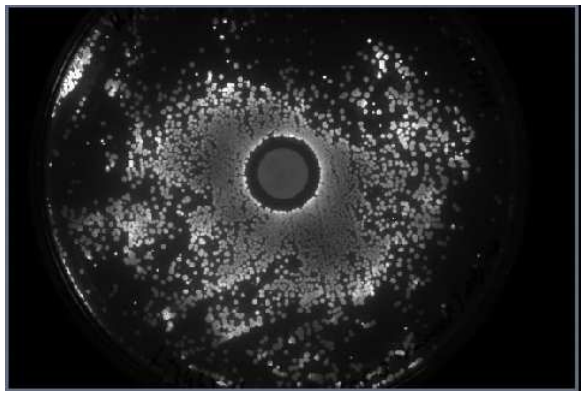
pBG8

+DBT

pVLT31

pESOX3

+DBT

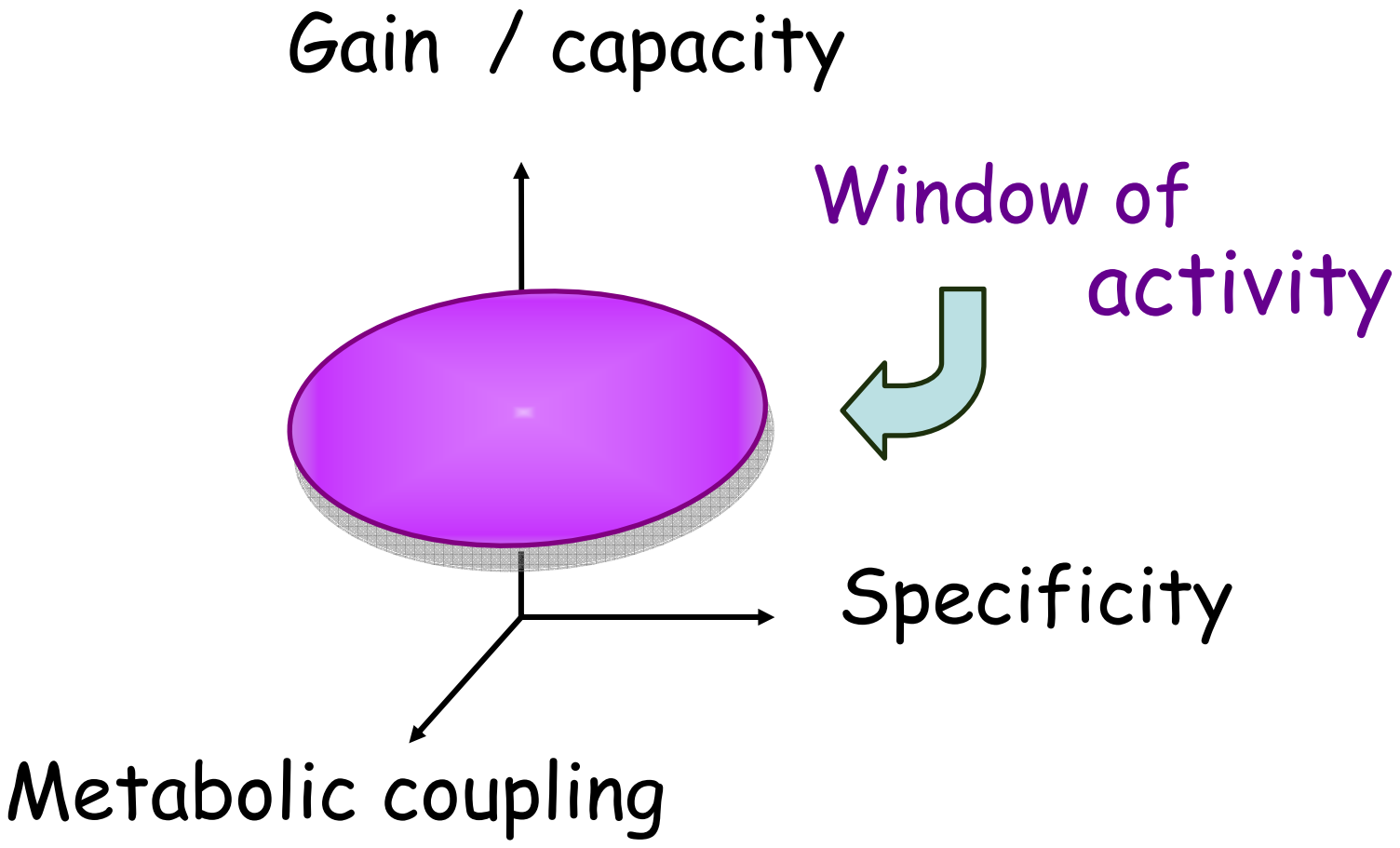


pESOX3

Proposition #4

Pursue inter-generational cooperation and make our engineered systems compatible with previous tools and resources

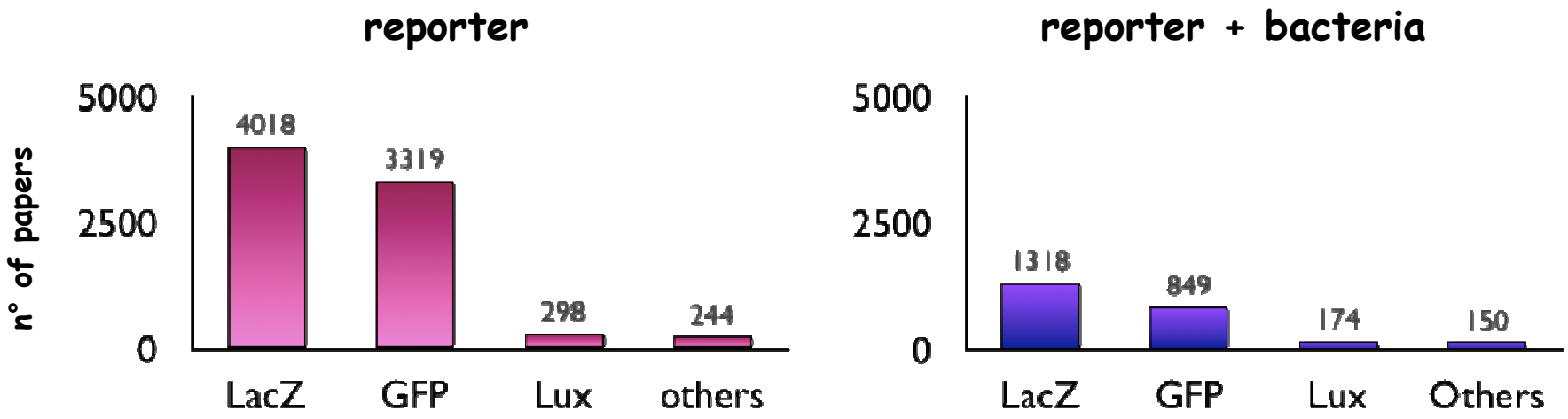
Definition of promoter activities in vivo



What reporter is best?

lux	*GFP*	*lacZ*
Non-invasive	Non-invasive	Invasive & Non-invasive
No excitation	Excitation	No excitation
Population	Population & Single cell	Population & Single cell
Costly	Non-costly	Non-costly
Days	Months	Months
Dimer	Monomer	Tetramer

Mostly, depends on the experiment settings



Evolution and Design of Biomolecular Systems

Concepts and strategies for systems and synthetic biology

October 18-20 (2009) Illetes-Mallorca (Spain)

Organisers
John McCarthy (Manchester Biocentre, UK);
Victor de Lorenzo (CNB-CSIC Madrid, Spain);
Virginia Cornish (U Columbia, USA);
Yoshi Nakamura (U Tokyo, Japan)

Speakers
Irene Chen (FAS Centre for Systems Biology, Harvard University, USA)
Phillip Holliger (LMB, Cambridge, UK)
Paul Freemont (Imperial College, London, UK)
Martin Fussenegger (ETH, Zűrich, Switzerland)
Rafael Giraldo (CIB, Madrid, Spain)
Tan Inoue (Kyoto University, Japan)
Richard Kitney (Imperial College, London, UK)
Vitor Martins dos Santos (HZI)
Steve Oliver (Cambridge University, UK)
Bernhard Palsson (University of California, San Diego, USA)
Luis Serrano (Centre for Genomic Regulation, Barcelona, Spain)
David Savage (Harvard University, MA, USA)
Stenbjörn Styring (Uppsala University, Sweden)
Daniel van der Lelie (Brookhaven, USA)
Peter Walde (ETH, Zűrich, Switzerland)
Ron Weiss (Princeton University, NJ, USA)

21-22 Oct satellite transcription sandpit
Defining promoter standards
(V. de Lorenzo, M. Buck, S. Busby
R. Gourse, H. Aiba, I. Golding, C. Gross, V. Rhodius and more)

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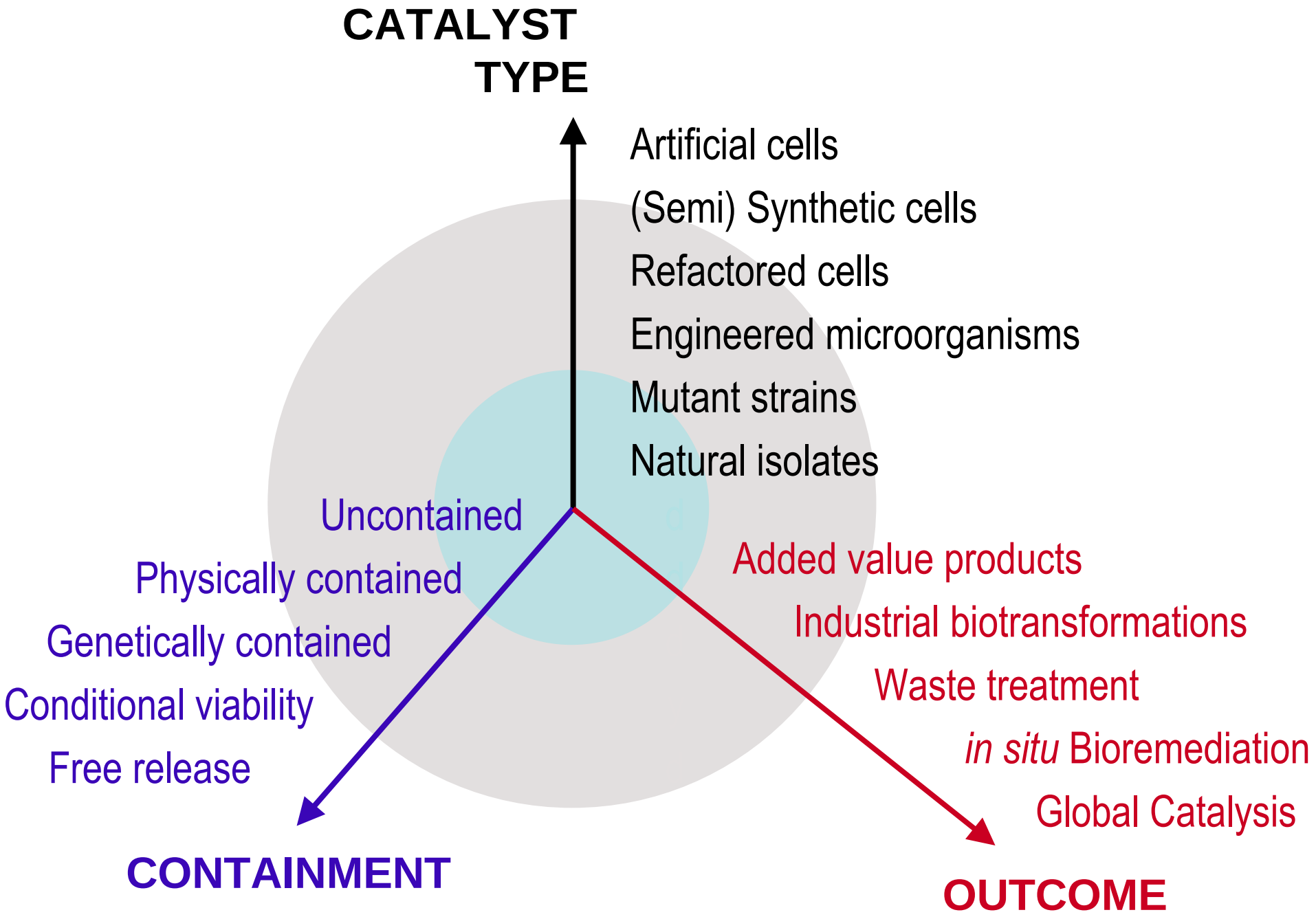
http://www. mib.ac.uk/sbmallorca

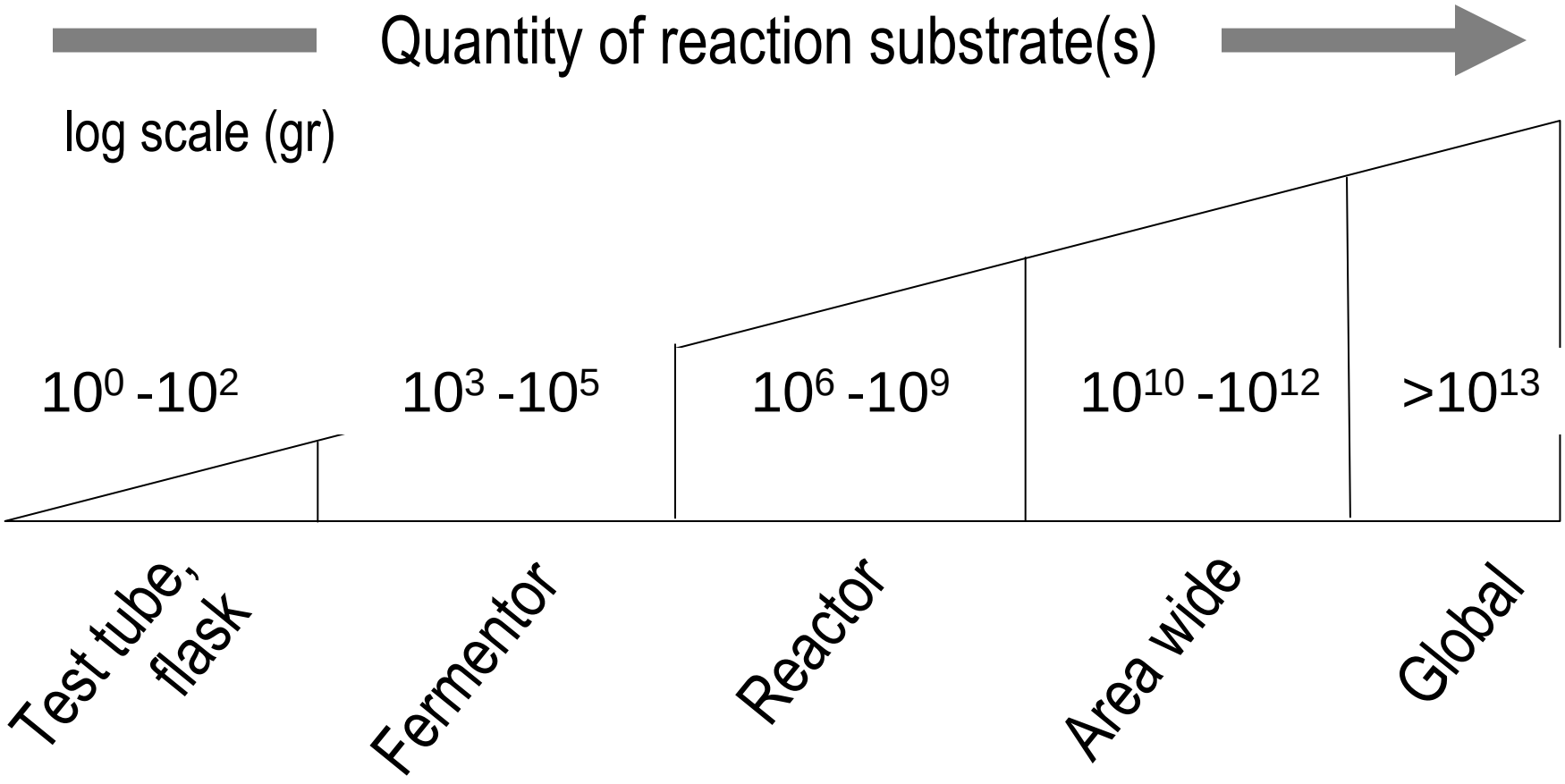
bbsrc · RSC Royal Society of Chemistry · The Royal Academy of Engineering · TARPOL Consortium · Emergence · CSIC

Proposition #5

Think BIG! For the first time we can entertain scenarios for engineering global biocatalysis interventions


CATALYST TYPE
Artificial cells
(Semi) Synthetic cells
Refactored cells
Engineered microorganisms
Mutant strains
Natural isolates
Uncontained
Physically contained
Genetically contained
Conditional viability
Free release
CONTAINMENT
Added value products
Industrial biotransformations
Waste treatment
in situ Bioremediation
Global Catalysis
OUTCOME

SIZE MATTERS



Corollary of Propositions 1-5

Bring Engineering into Biology,
but also
Bring Biology into Engineering

i.e. do not ignore Biology!