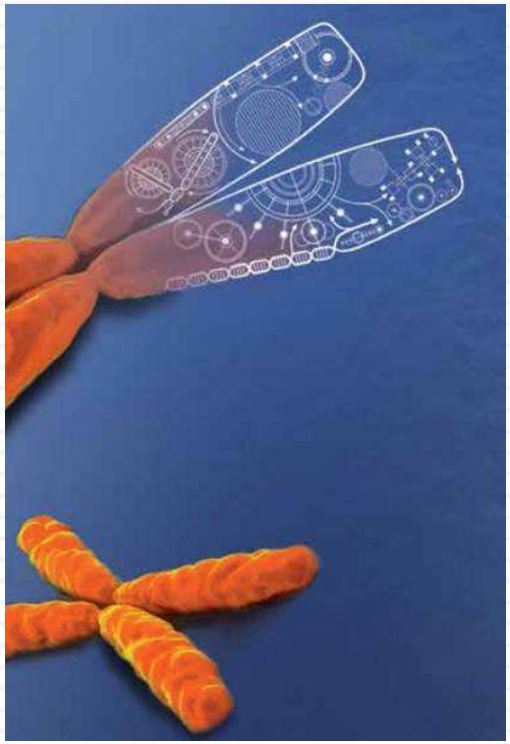
CENTRE OF BIOLOGICAL ENGINEERING

University of Minho
School of Engineering

Tailor-made Biotechnological solutions: from lab to business

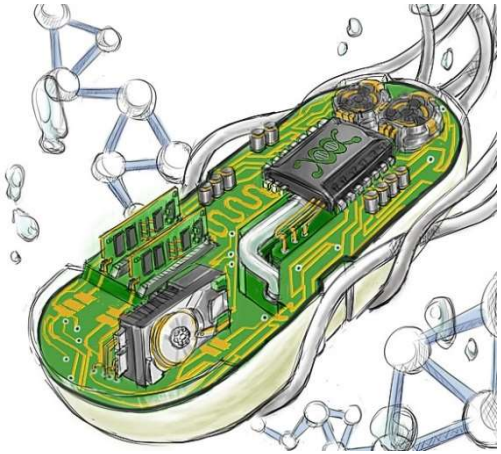


Lígia Rodrigues

University of Granada, Granada, 16 March 2017

Outline

- Industrial biotechnology
- An expanding toolbox for industrial biotechnology
- Tailor-made biotech solutions
- Innovation & entrepreneurship
- The Biotech business
- The path from lab to business



Industrial Biotechnology: What is it?

- Use of enzymes and microorganisms to make biobased products in sectors such as chemicals, food and feed, detergents, paper and pulp, textiles and bioenergy (e.g. biofuels or biogas)
- It uses renewable raw materials (from land and sea) and is one of the most promising, innovative approaches towards lowering greenhouse gas emissions
- Its application makes significant contributions towards mitigating the impacts of climate change in these and other sectors
- It can improve industry's performance and product value

Industrial Biotechnology: What is it?

- It may have a larger impact on the World than health care and agricultural biotechnology
- It offers businesses a way to reduce costs and create new markets while protecting the environment (pollution prevention and resource conservation)
- Many of its products do not require the lengthy review times that drug products must undergo → it's a quicker, easier pathway to the market
- New industrial processes can be taken from lab study to commercial application in two to five years, compared to up to a decade for drugs

Industrial Biotechnology: Priorities

- **Circular bioeconomy** → EU bioeconomy ~ €2 trillion. Biorefinery concept: production and use of renewable resources, and the use of waste to make value added products, bio-based products and bioenergy

- **Climate change** → Reduce dependency on fossil resources thus reducing greenhouse gas emissions throughout the value chain

- **Consumer benefits** → More sustainable processes and products reducing carbon footprint. New products with novel properties

- **Jobs & Growth** → Industrial biotechnology employs over 22 million people, making up around 9% of the EU's total workforce. Potential to add more value and competitiveness to sectors ranging from primary production right through to consumer goods

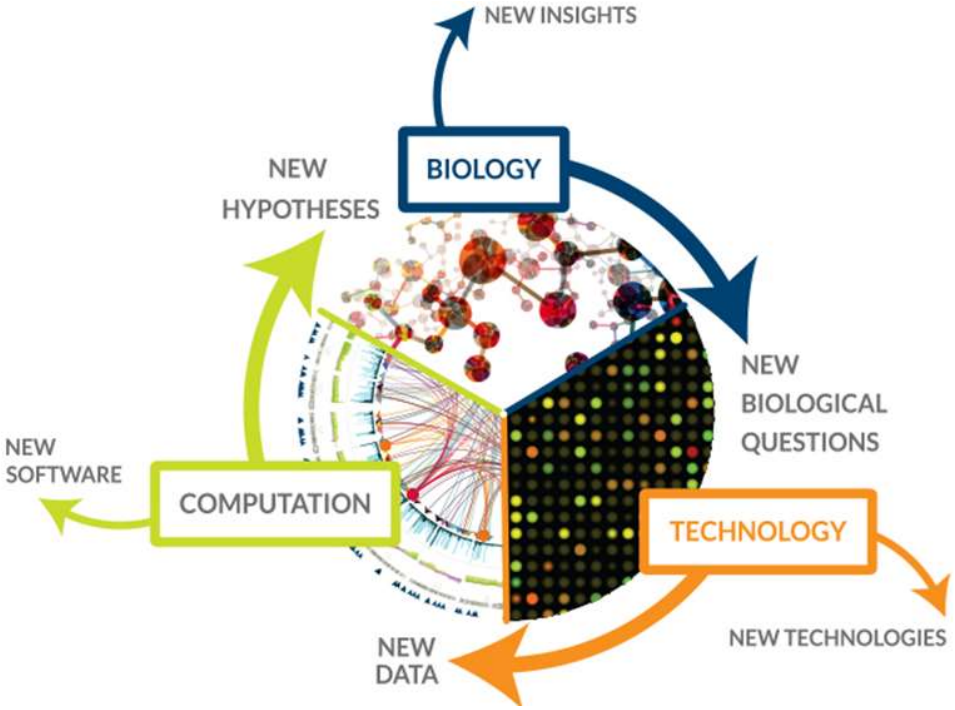
Industrial Biotechnology: Priorities

- **Innovation** → Industrial biotechnology (IB) offers innovative solutions for resource and energy-efficient processes while simultaneously bringing economic and social benefits (e.g. mitigation of carbon emissions; bioremediation; reducing waste; greener mining)

- **Sustainable biomass** → Using renewable biomass to make products that have been traditionally fossil-carbon based holds great potential benefits for the EU including the ability to re-grow feedstock year on year, rather than relying on imported, finite fossil carbon, creating jobs in rural, coastal and deindustrialised areas, and boosting economic growth whilst minimising our CO_2 emissions

Industrial Biotechnology: Opportunities

- Industrial biotechnology involves working with Nature to maximize and optimize existing biochemical pathways that can be used in manufacturing

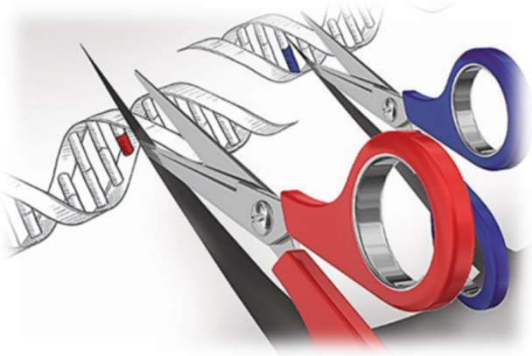


The industrial biotechnology revolution relies on the study of detailed information derived from the cell: genomics, proteomics and bioinformatics

- New techniques can be applied to a large number of microorganisms ranging from bacteria, yeasts, and fungi to marine diatoms and protozoa

Industrial Biotechnology: Opportunities

- Companies use specialized techniques to find and improve the enzymes nature
- Genomics is helping researchers capitalize on the wealth of genetic diversity in microbial populations
- Researchers first search for enzyme-producing microorganisms in the natural environment and then use DNA probes to search at the molecular level for genes that produce enzymes with specific biocatalytic capabilities
- Once isolated, such enzymes can be identified and characterized for their ability to function in specific industrial processes
- These can be further improved with advanced biotechnology techniques (e.g. synthetic biology)

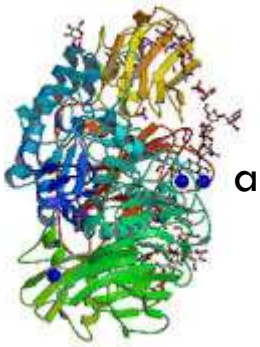


Toolbox for Industrial Biotechnology

Protein Engineering

Rational Design

Directed Evolution



Wild-type enzymes not always suitable for industrial processes

Engineer/optimize enzyme activity, selectivity on non-natural substrates, thermostability, tolerance to organic solvents, enantioselectivity and substrate/product inhibition toward commercially viable enzymatic processes

Toolbox for Industrial Biotechnology

Metabolic Engineering

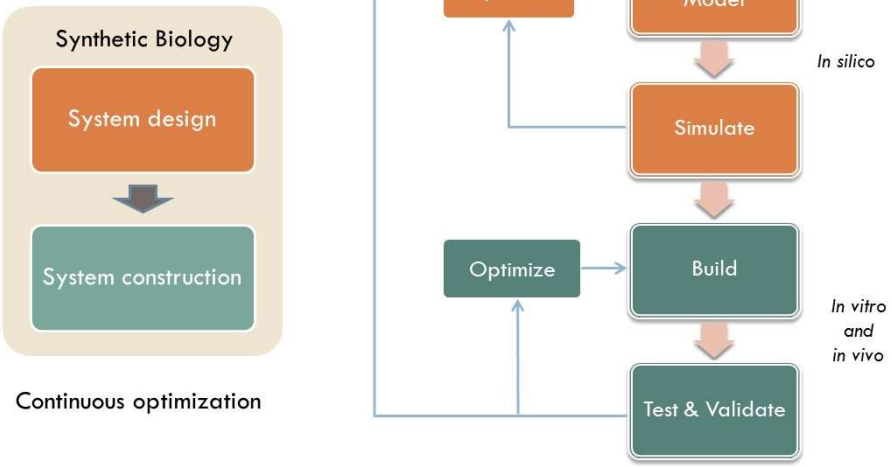
- Redirects precursor metabolic fluxes, changes protein cellular levels, fine-tunes gene expression and controls gene expression regulation in several hosts through manipulation of enzymatic, transport and regulatory functions in the cell

Metabolic engineering to produce large amounts of valuable metabolites that are difficult to extract from their natural sources, and too complex or expensive to produce via chemical synthesis is an attractive option

Toolbox for Industrial Biotechnology

Synthetic Biology

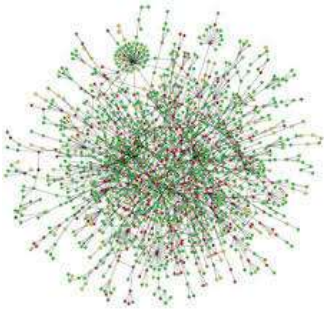
- Design and construction of biological systems that do not exist in nature and/or reconstruction of already existing natural systems using engineering approaches



Novel synthetic networks at the level of transcription, translation, and signal transduction by manipulating and stringing together modular biological components such as promoters, repressors, and RNA translational control devices

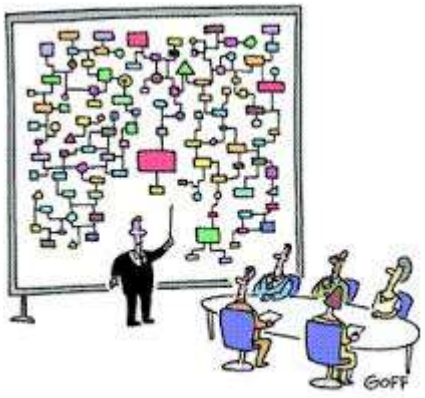
- Combined with metabolic engineering, synthetic biology provides tools to build synthetic pathways for the production of biofuels, chemicals and pharmaceuticals

Toolbox for Industrial Biotechnology



Systems Biology

- Entire complex cellular networks are analysed and optimized for application in the development of strains and bioprocesses
- Complex cellular networks involve the integration of non-linear gene, protein, and metabolite interactions across multiple metabolic and regulatory networks via computer simulation



"And that's why we need a computer."

- "Omics" (genomics, transcriptomics, proteomics, metabolomics, fluxomics) → reconstruction of genome-scale metabolic models
- *In silico* methods used in metabolic flux analysis (MFA) to solve a high-dimensional set of non-linear differential equations
- "Omics" + 'in *silico*' → successful examples of systems metabolic engineering
- However, more information embedded in large-scale genome-wide data and computational simulation results still not fully explored

Toolbox for Industrial Biotechnology

Downstream Processing

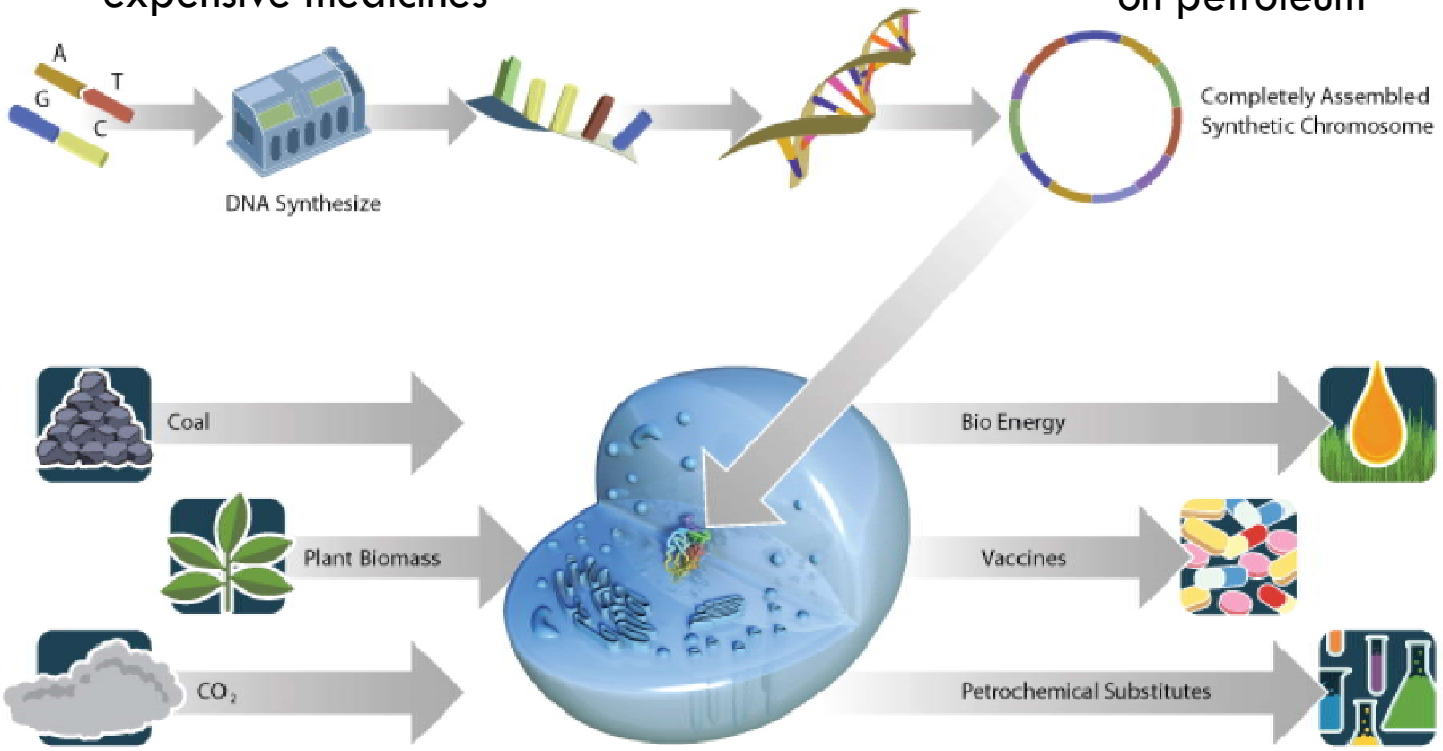
- Scaling-up enzyme-catalysed reactions from the laboratory benchtop to industrial scale is a multidisciplinary effort (sterilization, rheology, mixing, agitator design, enzyme immobilization, fluidization, heat transfer, mass transfer, separation and purification, surface phenomena, hydrodynamics, modelling, and instrumentation and process control)

  - Bioprocesses are mainly batch-wise and typical bioreactors include stirred-tank bioreactors and airlift reactor systems
  - Product recovery and purification is the major cost in downstream bioprocessing (e.g. extraction by distillation or liquid–liquid extraction, chromatographic methods (adsorption) and membrane separation)
  - Immobilization overcomes enzyme instability in industrial processes and allows the enzyme to be recycled, making it suitable for continuous processes

Tailor-made Biotech Solutions

Treat diseases more efficiently and create new, better and less expensive medicines

Develop new energy sources, cheaper and less polutant than the fuels based on petroleum



Detect and treat air, soil and water polution

Tailor-made Biotech Solutions

Chemical Industry

Crude oil

Petroleum Refinery

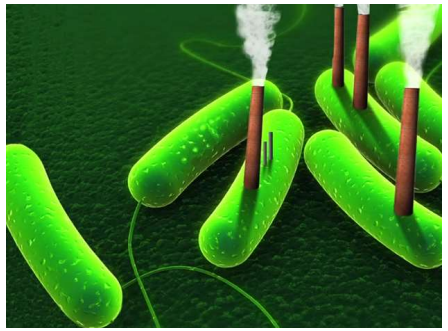
Fuels

Gasoline, aviation fuel, kerosene, fuel oil

Petrochemicals

Olefins and aromatics for polymers, resins, adhesives, detergents, fibers, lubricants

Biomass
(glucose and other simple sugars)



Bio-Refinary

Biofuels

Bioethanol, butanol, propanol

Value added Biochemicals

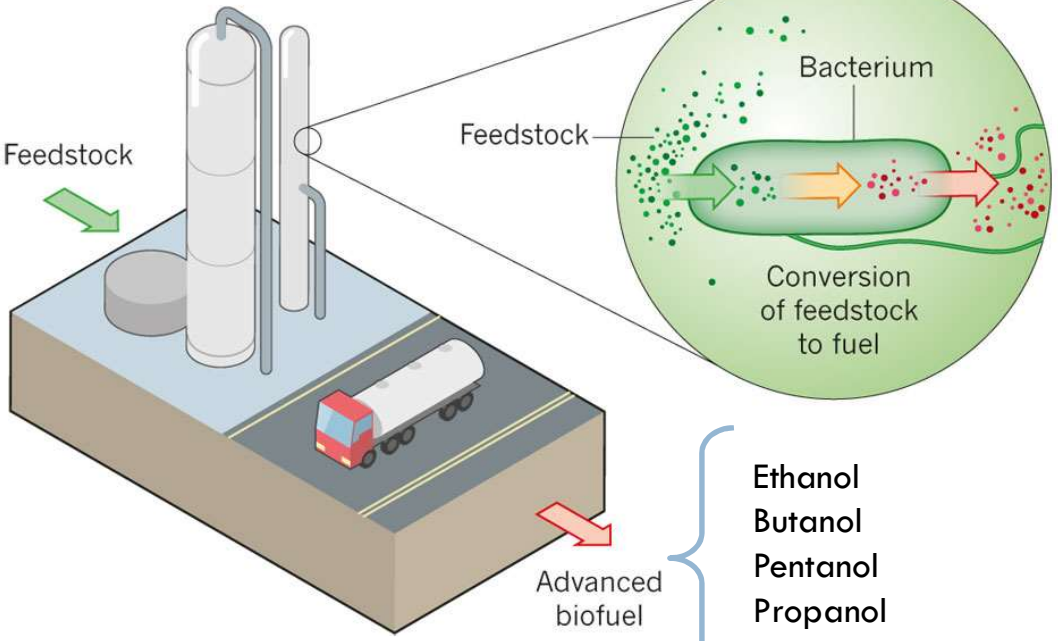
Ex: glucaric acid, 3-hydroxy-c-butyrolactone

Tailor-made Biotech Solutions

- **Biofuels:** competitive if production costs are ≤ drilling and refining costs of oil
- Routes implemented need to be highly efficient
- Raw material has to be low cost and not "food"

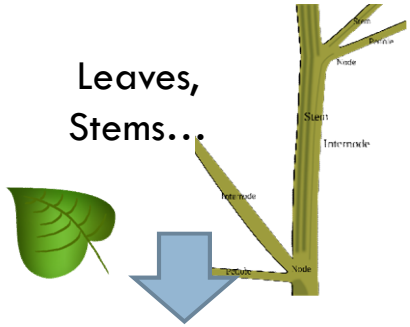
Chemical Industry

Glucose
Xylose
Arabinose
Rhamnose
Mannose
Galactose
Lignocellulose



Ethanol
Butanol
Pentanol
Propanol
4-methyl-pentanol

Leaves, Stems…



Cellulose

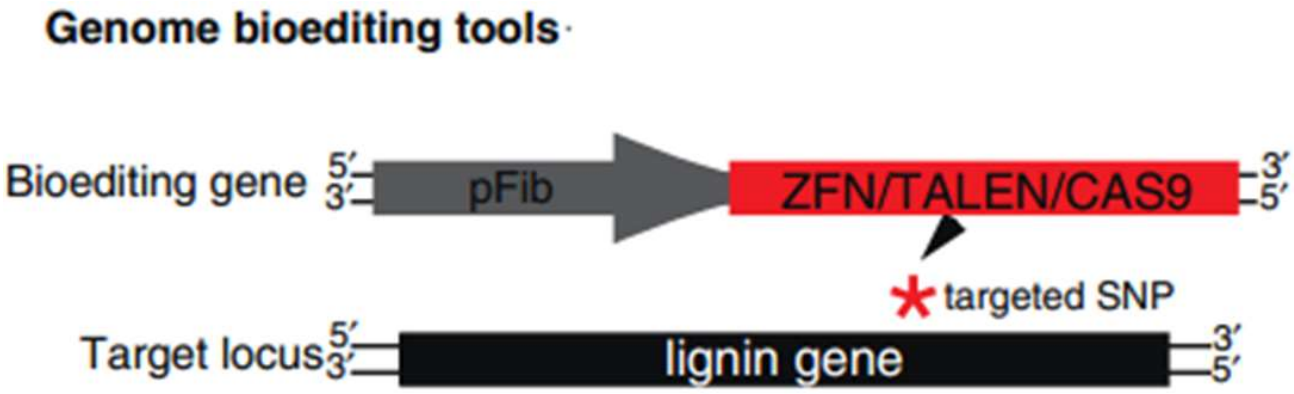
Lignin

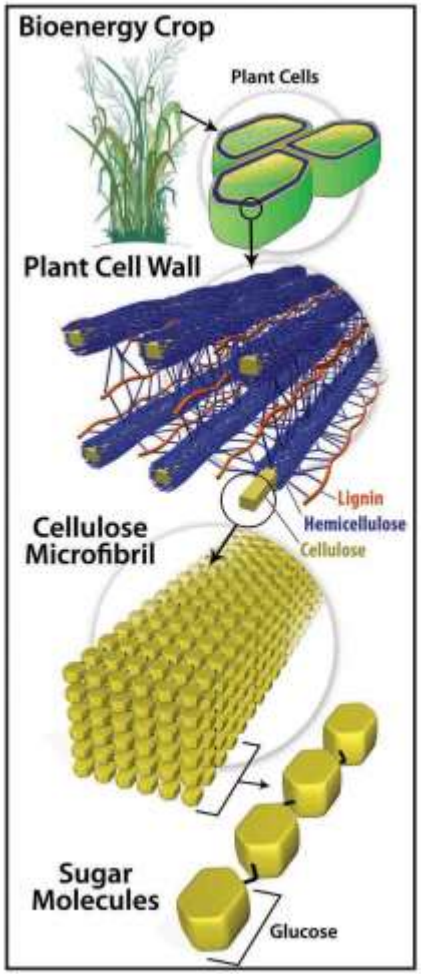
Degradation

Tailor-made Biotech Solutions

Chemical Industry

- Deep reductions of lignin cause growth defects and often correlate with the loss of vessel integrity that adversely affects water and nutrient transport in plants.
- Using a fiber-specific promoter to drive the expression of enzymes designed to recognize a lignin biosynthetic gene would repress lignin biosynthesis only in fiber cells without affecting the lignification of vessel cells.





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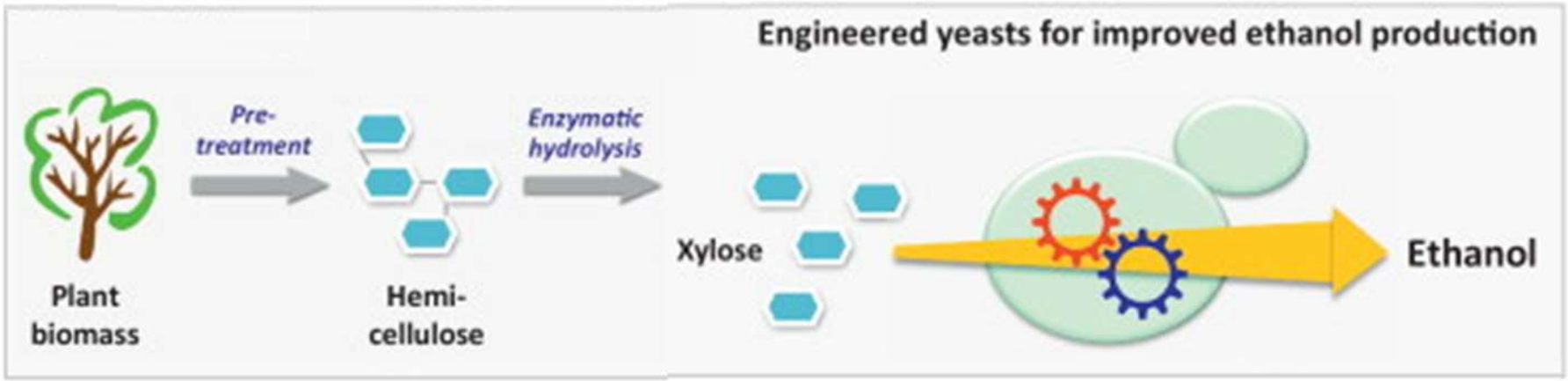
Chemical Industry

- **Bioethanol:**
  - Not compatible with current fuel infrastructure for distribution and storage of fuel due to its corrosive nature and high hygroscopicity
  - Its energy content is only about 70% of the gasoline



Tailor-made Biotech Solutions

Chemical Industry

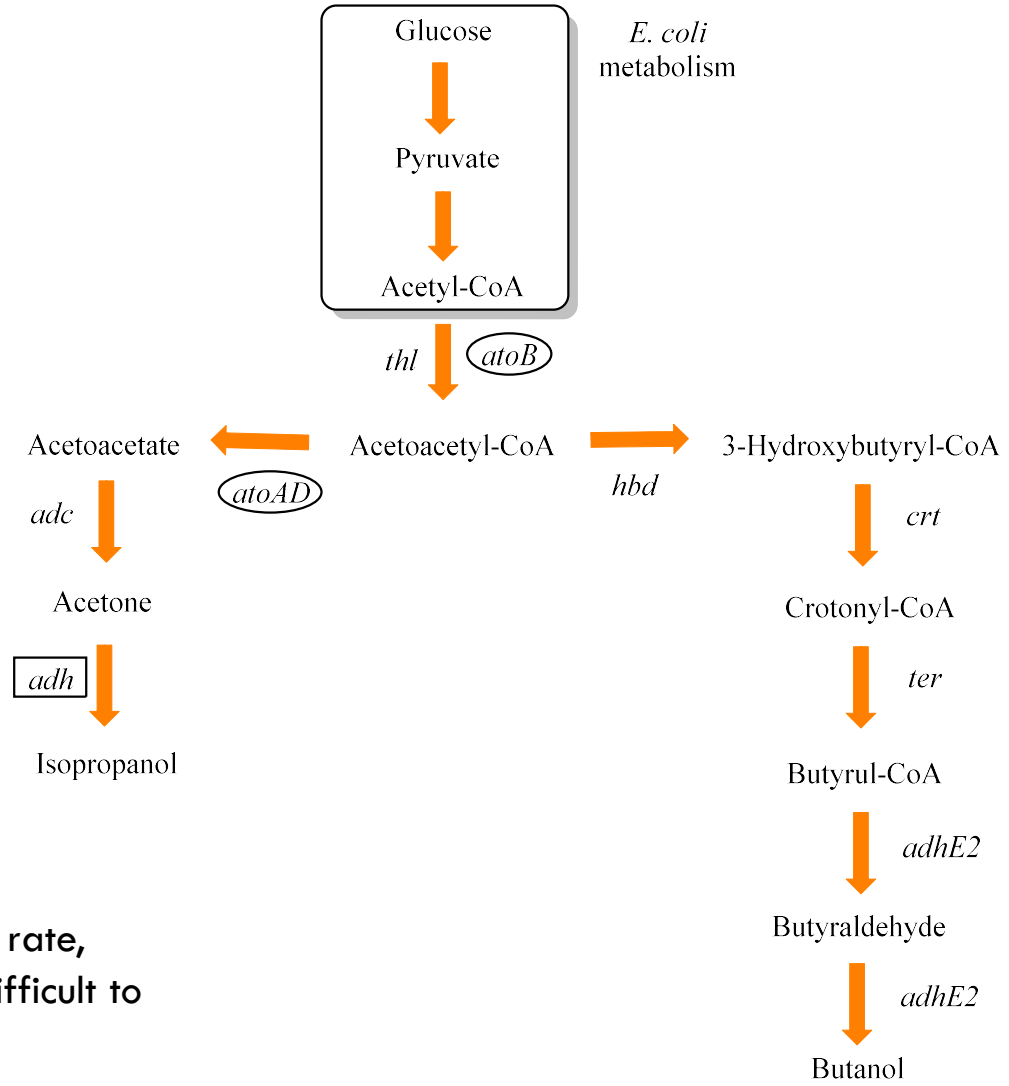
- **Alternative biofuels**
  Isopropanol & Butanol

- Better properties than Bioethanol

  (higher octane number, higher energy content, and lower water solubility)

- Produced by *Clostridium* species

  Anaerobic, slow growth rate, spore-forming cycles, difficult to genetically manipulate



Tailor-made Biotech Solutions

- **Naturally Replicating Rubber for Tires**
- Isoprene is a chemical used for the production of synthetic rubber

Chemical Industry

DUPONT®

GENENCOR®
A Danisco Division

Currently derived from petrochemical sources

working on the development of a reliable, high-efficiency fermentation-based process for the BioIsoprene™ monomer

Glucose

isoprene synthase enzyme
(rubber plant gene)

E. coli

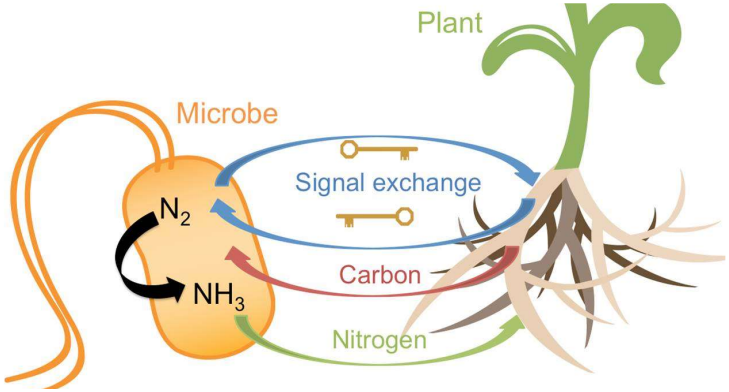
BioIsoprene™

Tailor-made Biotech Solutions

Environment

- Bioremediation (Degradation, removal or neutralization of pollutants)
  - Pesticides, radioactive compounds…
  - Unnecessary to dig and transport polluted soil to hazardous waste landfills

- Production of fertilizer
  - Atmospheric nitrogen fixation and conversion into ammonia



Tailor-made Biotech Solutions

Food Industry

- **Vanillin**
  - responsible for the characteristic aroma and vanilla flavour
  - Used in food and beverages
  - Limited supply
  - 1 kg vanillin / 500 kg vanilla beans
  - High extraction cost and low yield
  - 99 % chemically synthesized from lignin and petrochemical precursors

IFF International Flavors & Fragrances Inc. evolva

Glucose → *S. cerevisiae* + plant genes → Vanillin

- + real
- equivalent to natural vanillin

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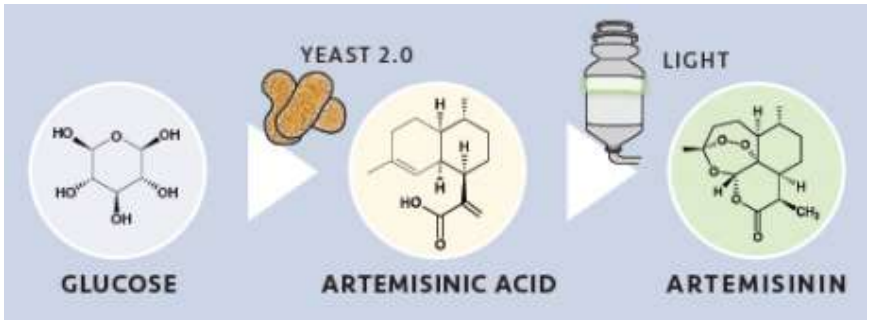
Pharmaceutical Industry

Artemisinin

Extraction from plant
- Low
- Slow (~10 months)
- Variable

Solution



E. coli or *S. cerevisiae* (~3 months)

Artemisia annua

Malaria

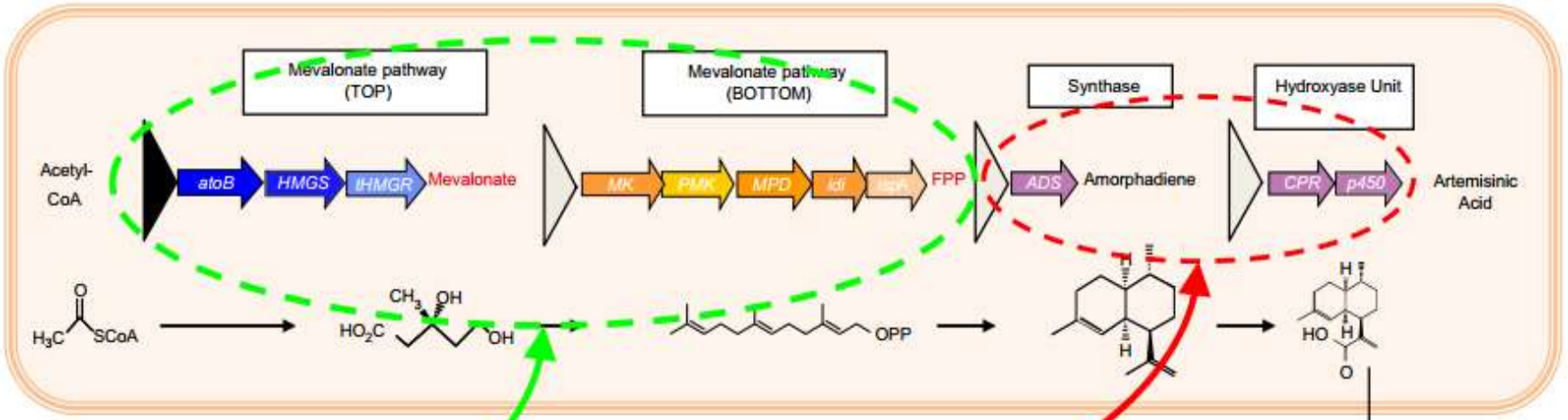
- > 200 million people annually are **infected**
- Almost 1 million **deaths** (90% in Africa)
- Caused by a parasite (*Plasmodium*) and transmitted by a mosquito

KEASLING LABORATORY

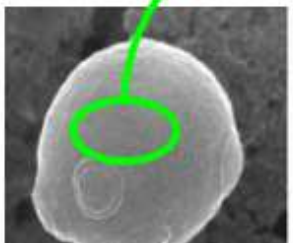
AMYRIS

Tailor-made Biotech Solutions

Pharmaceutical Industry



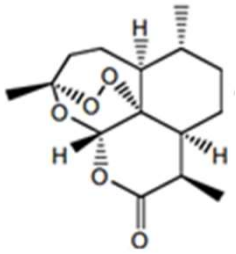
E. coli



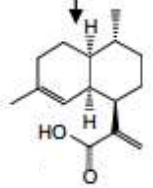
Saccharomyces cerevisiae

Artemisia annua

Chemical Synthesis



Artemisinin



Artemisinic Acid

SANOFI

Treat 200 millions people/ year

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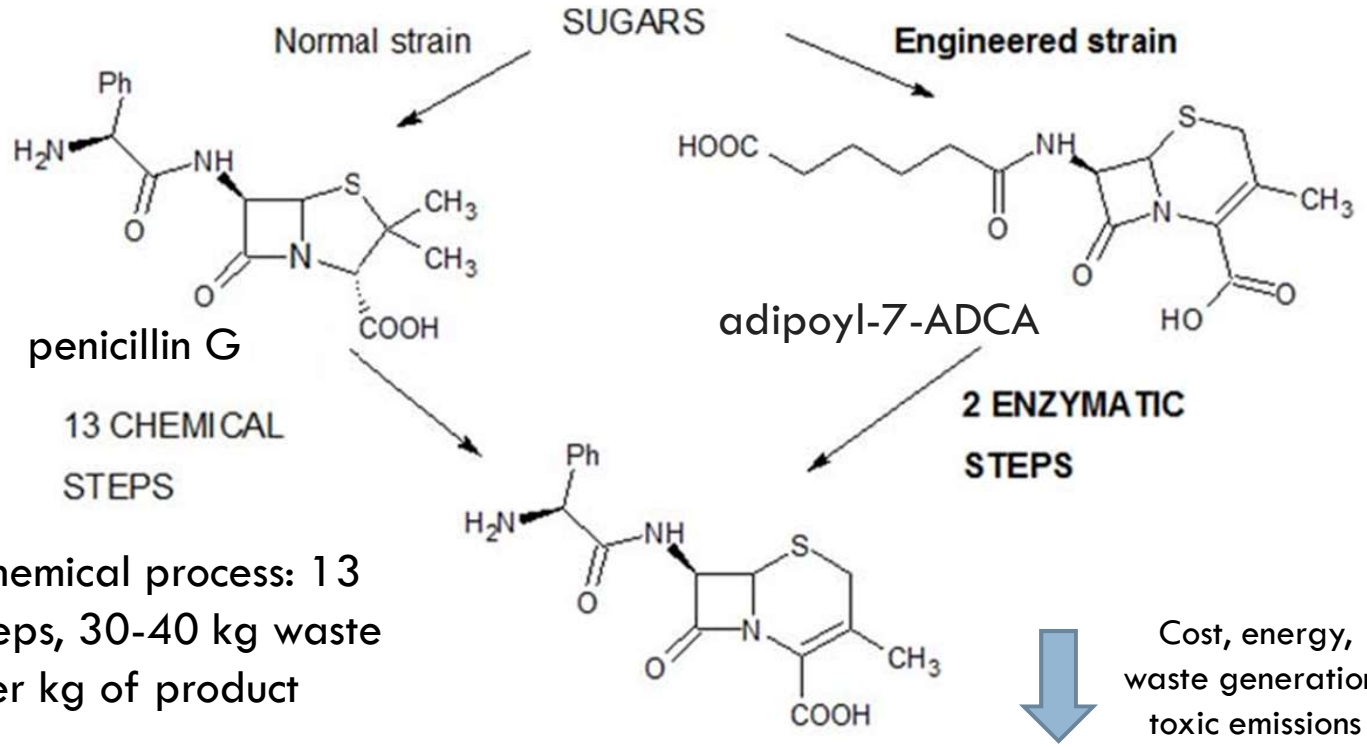
Pharmaceutical Industry

- **Commercial production of synthetic antibiotics**



Chemical process: 13 steps, 30-40 kg waste per kg of product

Cost, energy, waste generation, toxic emissions

penicillin-producing microbial strain (*Penicillium chrysogenum*)

+ optimized two enzyme-encoding genes

DSM
BRIGHT SCIENCE. BRIGHTI

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Vector Control

Control of insect borne diseases depends on the ability to control the mosquito populations that transmit and spread diseases

Malaria – *Anopheles gambiae*
Zika & Dengue – *Aedes aegypti*

Insects are being engineered to spread parasite resistance, anti-fertility, or conditional lethal genetic components across wild-type populations

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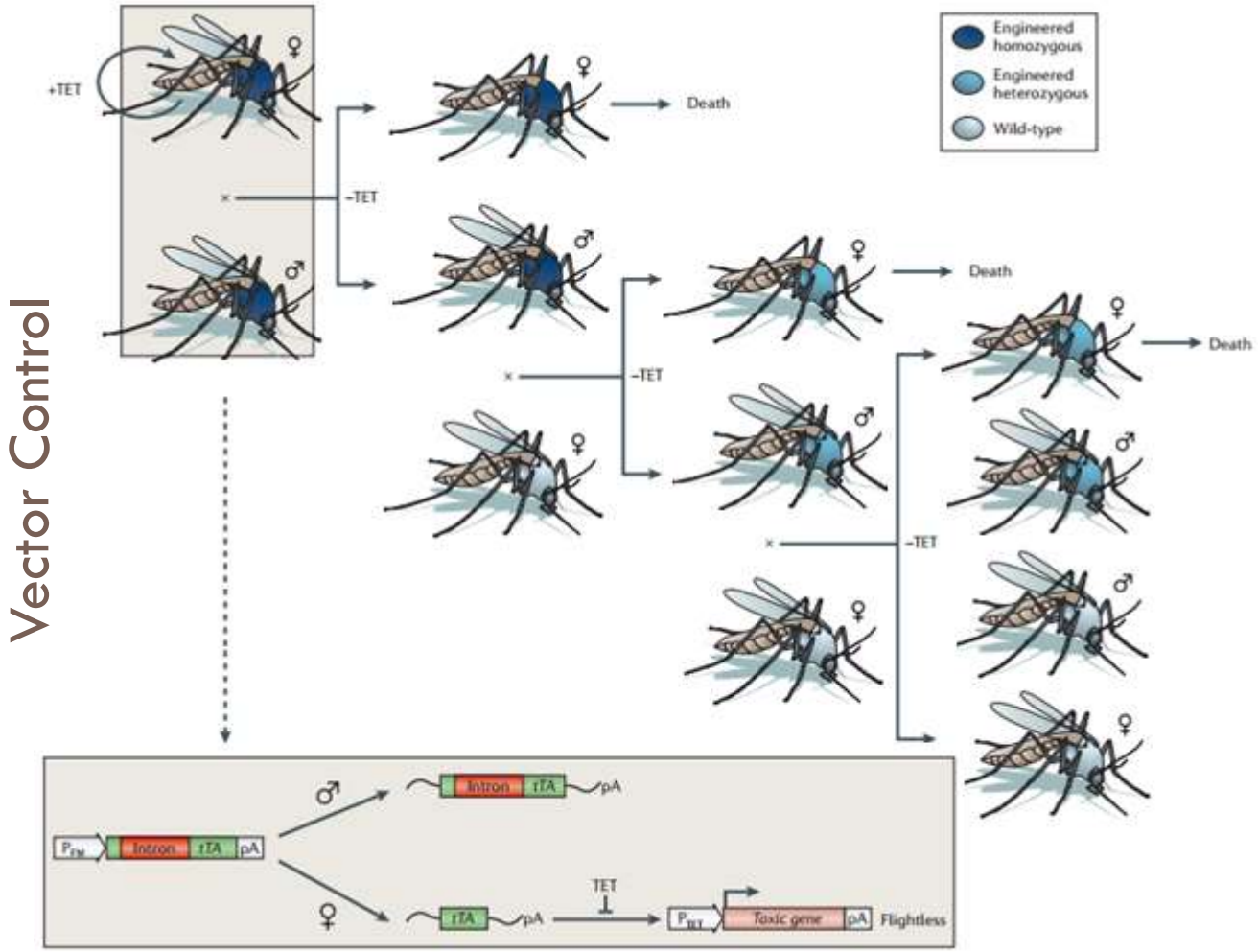

- Release of insects carrying a dominant lethal genetic system
- Diseases are only propagated by female mosquitoes
- Only engineered males are released
- The daughters are not able to fly and mate – occurs the reduction of wild-type
- The sons live and mate and only half of their offspring inherit the female-lethal gene

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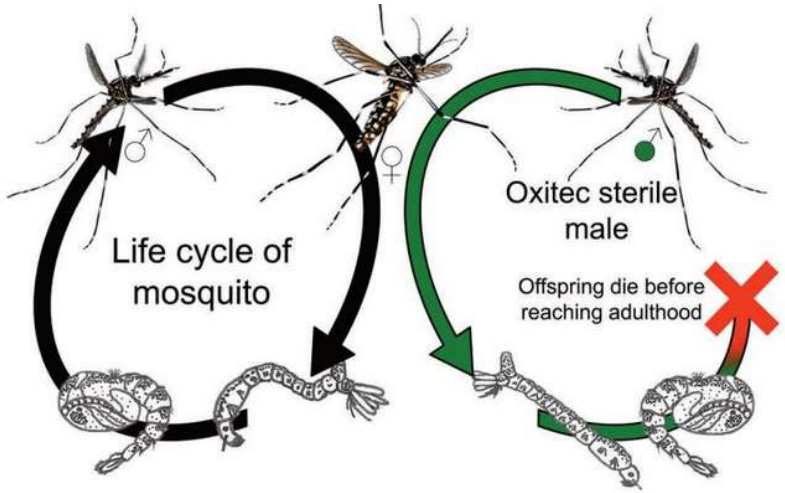
Vector Control

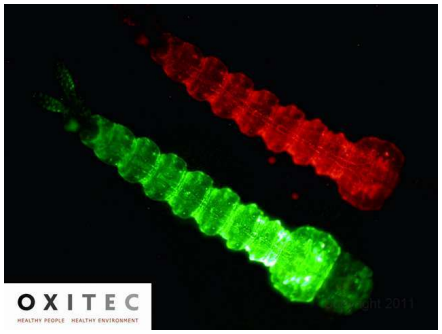
Very similar strategy with sterile male mosquitoes is already being used by Oxitec to control the transmission of dengue

OXITEC

Company genetically modifies insects



Trials across Brazil, Panama, Malaysia and the Cayman Islands all resulted in suppression of more than 90% the wild *A. aegypti* mosquito population.

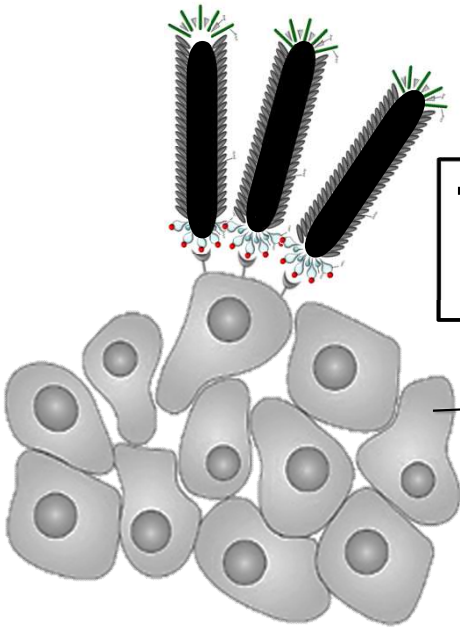


Oxitec's strains contain a heritable, fluorescent marker to distinguish them from wild insects and to help scientists with the management of pest control programs.

Tailor-made Biotech Solutions

Cancer Diagnosis & Treatment

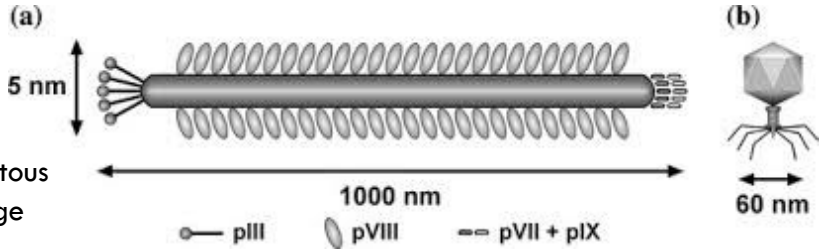
Engineered Bacteriophage for Cancer Diagnosis



Phage Display technology → **Selection method that favors the enrichment of specific binders from an initial library**

Identification of new targeting sequences towards cancer cells → **Identification of the correspondent target by reverse genetics and bioinformatic tools**

Novel Targets for Cancer Diagnosis and Treatment



M13 filamentous bacteriophage

T4 bacteriophage

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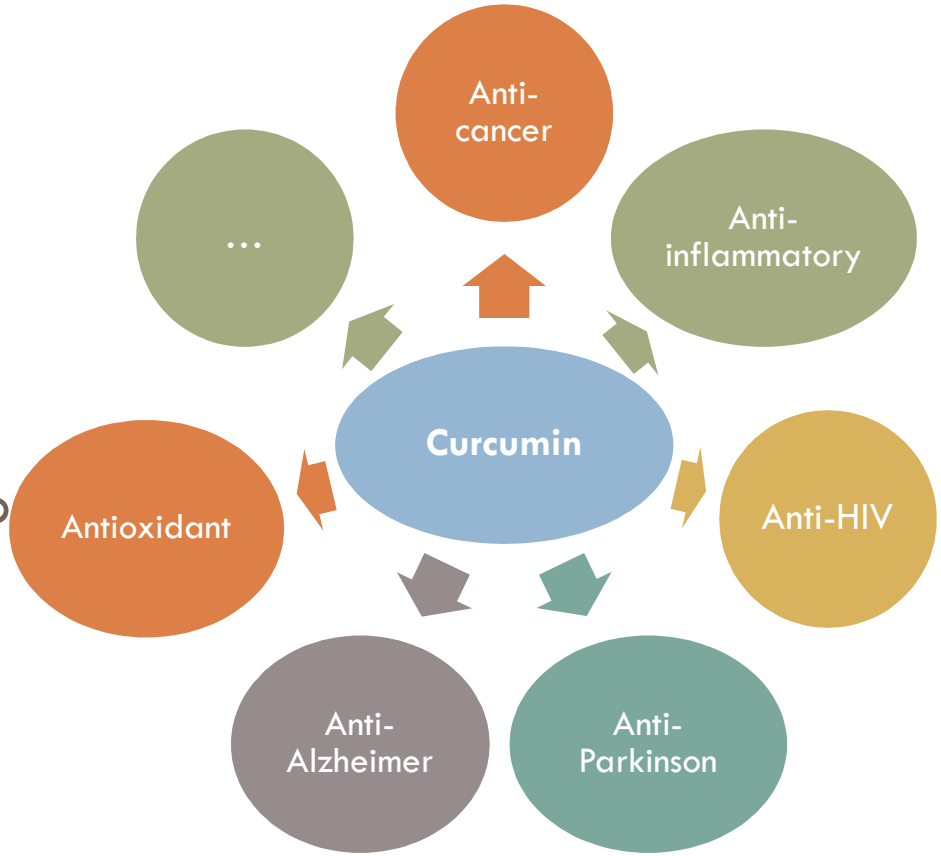
Tailor-made Biotech Solutions

Curcumin and other **curcuminoids** – phenolic compounds extracted from *Curcuma longa*

Cancer Diagnosis & Treatment



Safe in high doses

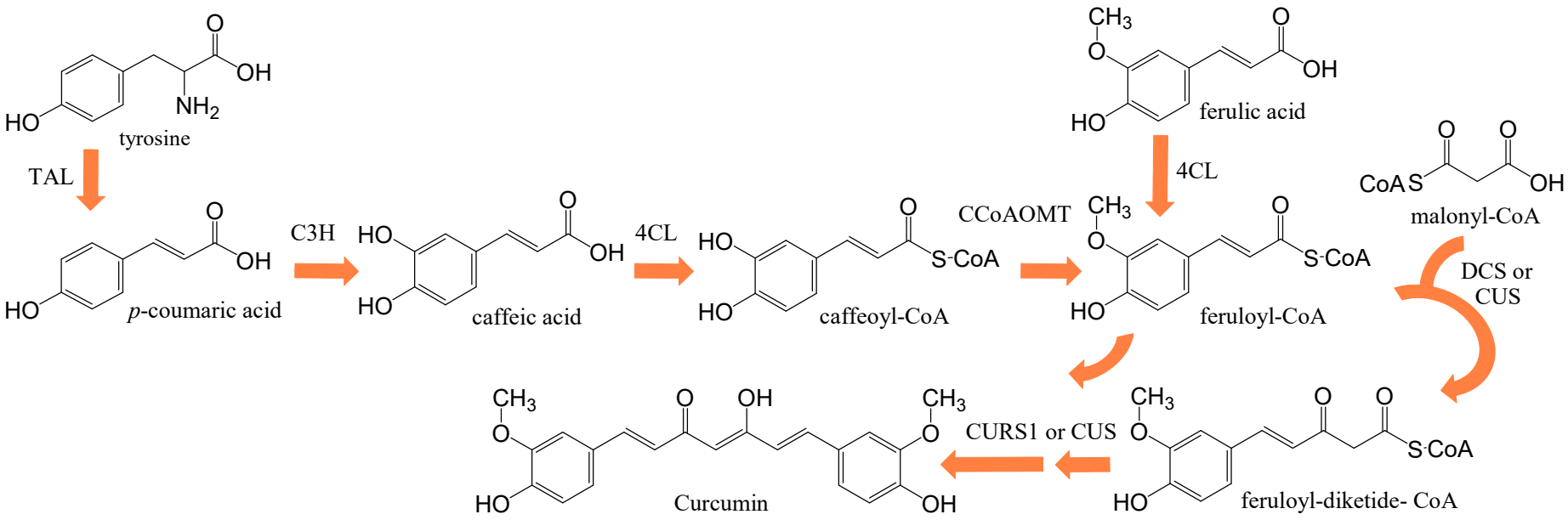
<u>Low bioavailability:</u>

Low absorption, fast elimination by the metabolism

Production *in situ*

Tailor-made Biotech Solutions

Cancer Diagnosis & Treatment



TAL, *Rhodotorula glutinis*
C3H, *Saccharothrix espanaensis*
4CL, *Arabidopsis thaliana*
CCoAOMT, *Medicago sativa*
CUS, *Oryza sativa*
DCS/CURS1, *Curcuma longa*

CENTRO DE ENGENHARIA BIOLÓGICA

Conclusions

- Great opportunities are still to be explored in Industrial Biotech
- Emergent tools and approaches hold great promise for future developments
- Novel technologies and products need to be scaled-up
- Economy of scale must be evaluated toward sustainability
- Society involvement is crucial to inform about the potential of new industrial biotech developments
- New businesses ougt to be created

Innovation & Entrepreneurship

KEY CONCEPTS

Creativity

Discovery

- Development of new ideas
  - Concepts
  - Knowledge

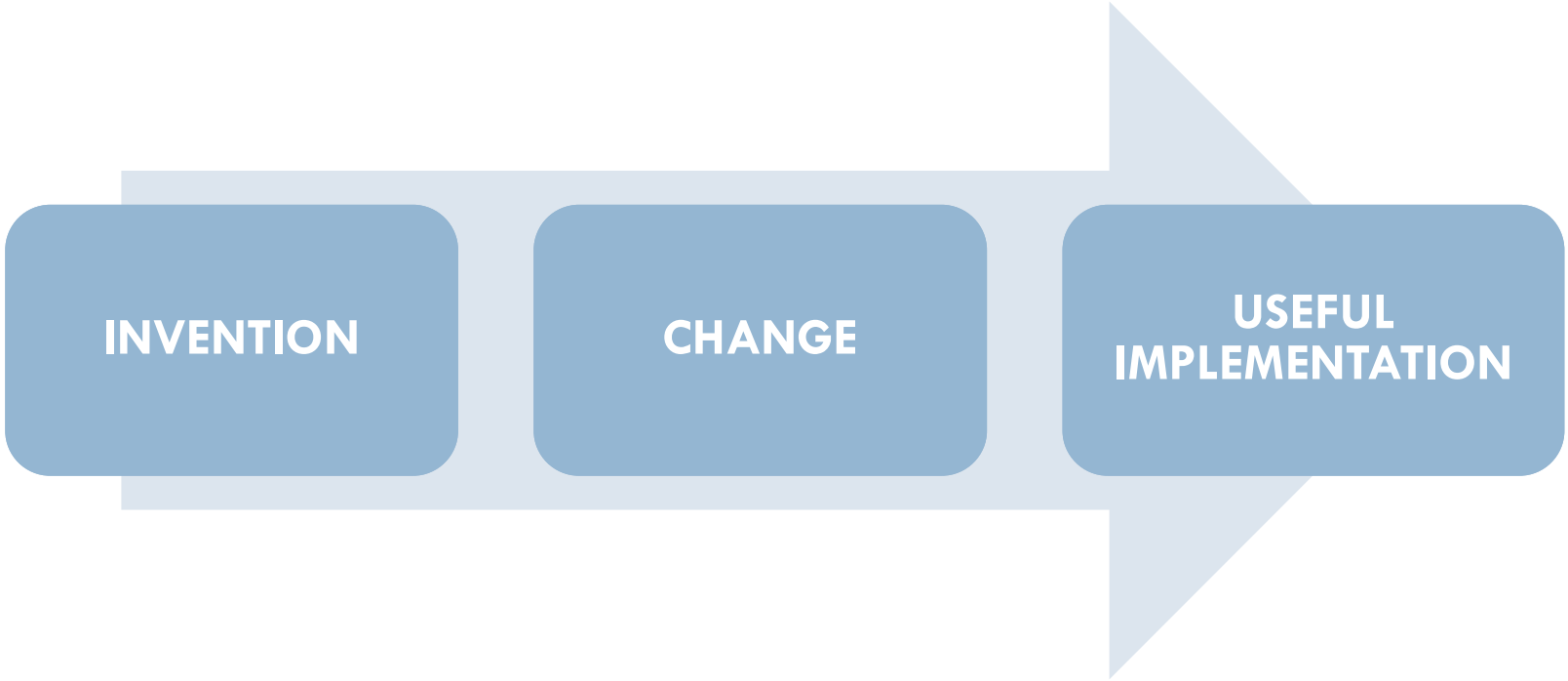
Invention

- Purposeful implementation of ideas
  - Technologies
  - Business models

Innovation

- Change made for comercial exploittaion of inventions
  - Products
  - Services

Innovation & Entrepreneurship



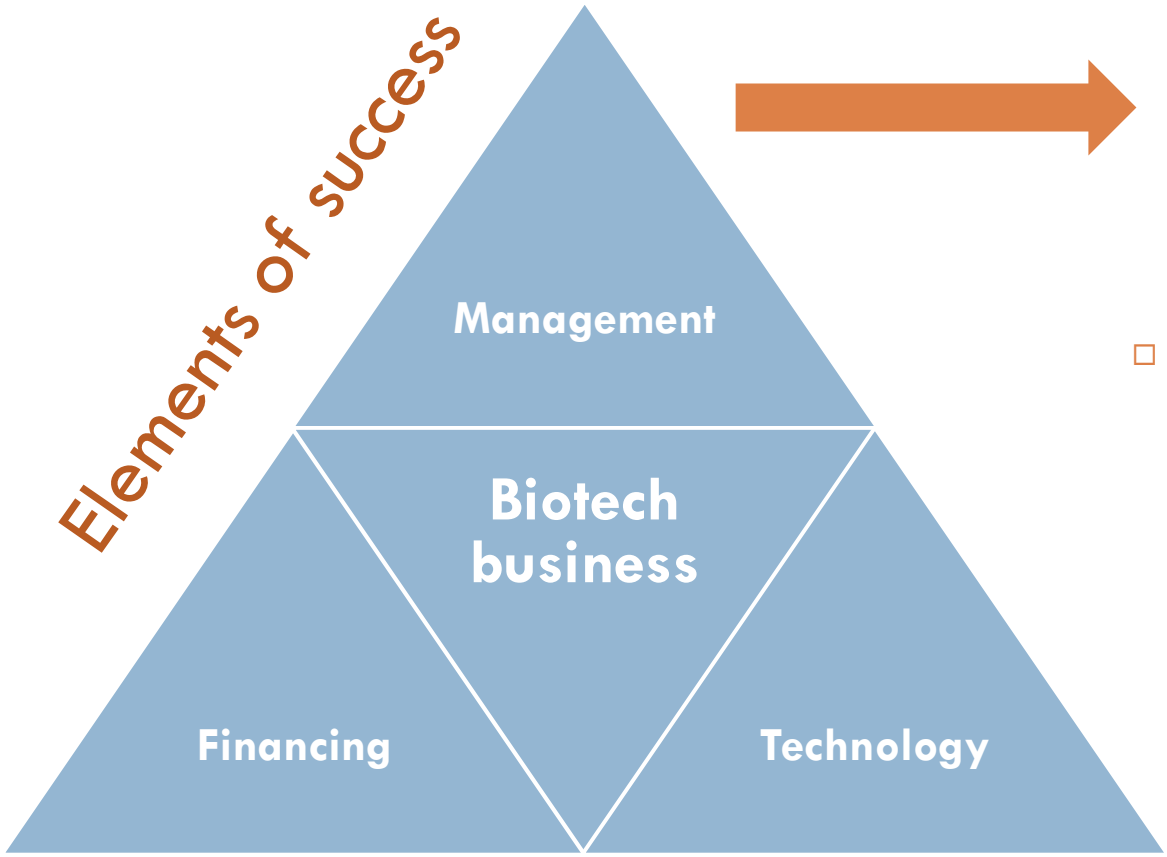
Innovation & Entrepreneurship

- Innovation → "The successful exploitation of new ideas":
  - New ideas - these might be for a new (or improved) product, process or service, or even a whole new business or business model
  - Exploitation - this indicates that the idea must be implementable and potentially value generating (i.e. innovation = invention + exploitation)
  - Successful - this implies that the innovation is actually adopted by the target audience (or indeed anyone else)

Innovation & Entrepreneurship

- 'Entrepreneurship' is the act and art of being an entrepreneur or one who undertakes innovations or introduce new things, finance and business insight in an effort to transform innovations into economic goods (value)
- This may result in new organizations (start-up) or may be part of revitalizing mature organizations in response to a perceived opportunity (intrapreneurship/spin-offs)
- Many "high value" entrepreneurial ventures seek venture capital or angel funding (seed money) in order to raise capital to build the business
- Angel investors generally seek annual returns of 20-30% and more, as well as extensive involvement in the business

The Biotech Business



- Tasks:
  - Obtain financing and other resources
  - Manage expenditures
  - Align R&D with market needs
- Characteristics:
  - Early-stage: multifaceted individuals often with experience in R&D
  - Mature companies: management specialists

The Biotech Business – Application categories

Category	Red: Medicinal Biotech
Description	Drugs and other agents to treat, cure or prevent disease and products that assist in the diagnosis of diseases or measurement of critical factors in health and disease
Characteristics	High up-front development costs, FDA approval required prior to sale. High post-approval profit margins

Category	Green: Agricultural Biotech
Description	Products and applications related to livestock and crop production towards agricultural ends
Characteristics	Development costs are often similar to drugs, profits are often lower

Category	White: Industrial Biotech
Description	Modification or improvement of industrial processes or performing tasks previously served by industrial processes such as paper processing, bioremediation and synthesis of organic compounds
Characteristics	Reduced regulatory burden decreases development costs

The Biotech Business – Company Activities

Application Area	Commercial Activities	Deliverables
Red	Basic Research and Target Discovery	Products
	Applied Research	
Green	Clinical and Prototype Research	
White	Manufacturing	Services
	Sales and Distribution	

- Products:
  - Drugs
  - Tools
  - Reagents
  - …
- Services:
  - Contract research
  - Manufacturing
  - Optimization
  - Diagnostic services
  - …

The Biotech Business

- Biotech companies – High risk-high gain
- All exciting science in the World won't generate long-term value unless it is used to create products that bring a significant revenue to the company
- The discovery/development of a new drug is
  - Long: ~15 years
  - Arduous: several steps (clinical trials) to fullfill regulatory requirements
  - Expensive: average cost of single drug €500 million
  - For every 5000 compounds that emerge from discovery and animal testing (preclinical testing), only about 5 compounds perform well enough to move into human testing...and only 1 of those 5 makes it into the marketplace
  - Getting a product into the market doesn't guarantee long-term success

Take home message

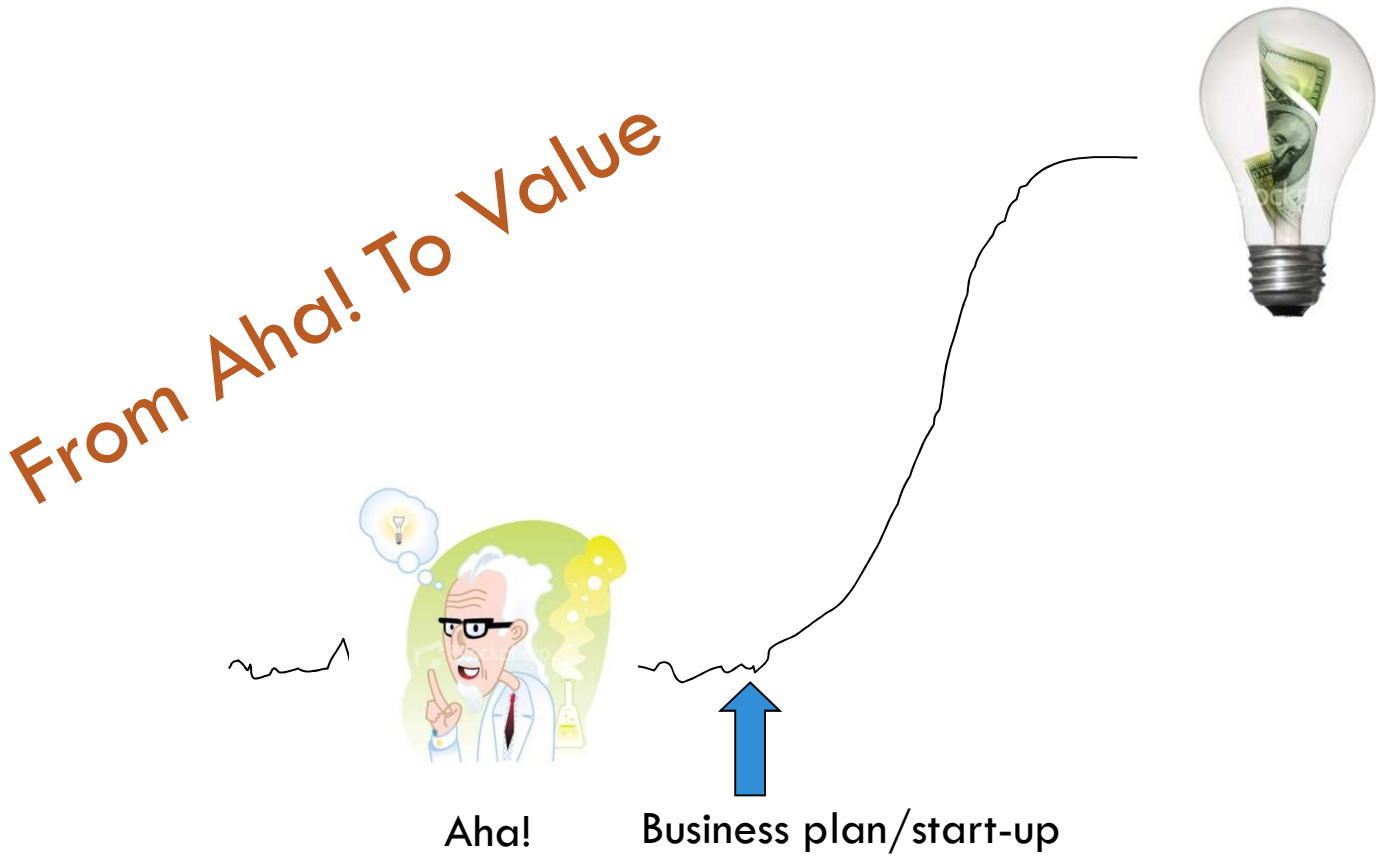
- What are the key elements to turn my research into business?
- What would be my “company” characteristics?
- What would be my “company” deliverables?
- Should I have concerns about regulatory issues?
- Protection of Intellectual property strategy?

The Path from Lab to Business

Aha!

I have something intriguing in the lab....now what?

The Path from Lab to Business



The Path from Lab to Business

Ask Questions

- Does it work yet?
- Is it special?
- Can we make it?
- Who cares?
- Who can help?
- Will they buy?

The Path from Lab to Business

Look into…

- IP strategy
- licensing models
- technology assessment
- customer contact
- market analysis/selection
- team building
- funding

The Path from Lab to Business

A contact sport

researchers

academics

potential customers

potential partners/investors

catalysts/mentors

TTO´s

successful/failed entrepreneurs

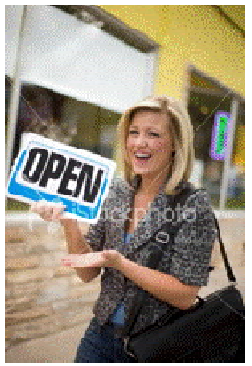
business associations

The Path from Lab to Business - Conclusion

What else?

Forget it

Partner



Start up

Back to the lab

License

‘Industrial Biotech Team’

CENTRE OF BIOLOGICAL ENGINEERING

University of Minho
School of Engineering

Thank you for your attention

Tailor-made biotechnological solutions: From Lab to Business

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