Plant Synthetic Biology: a New Platform for Industrial Biotechnology?

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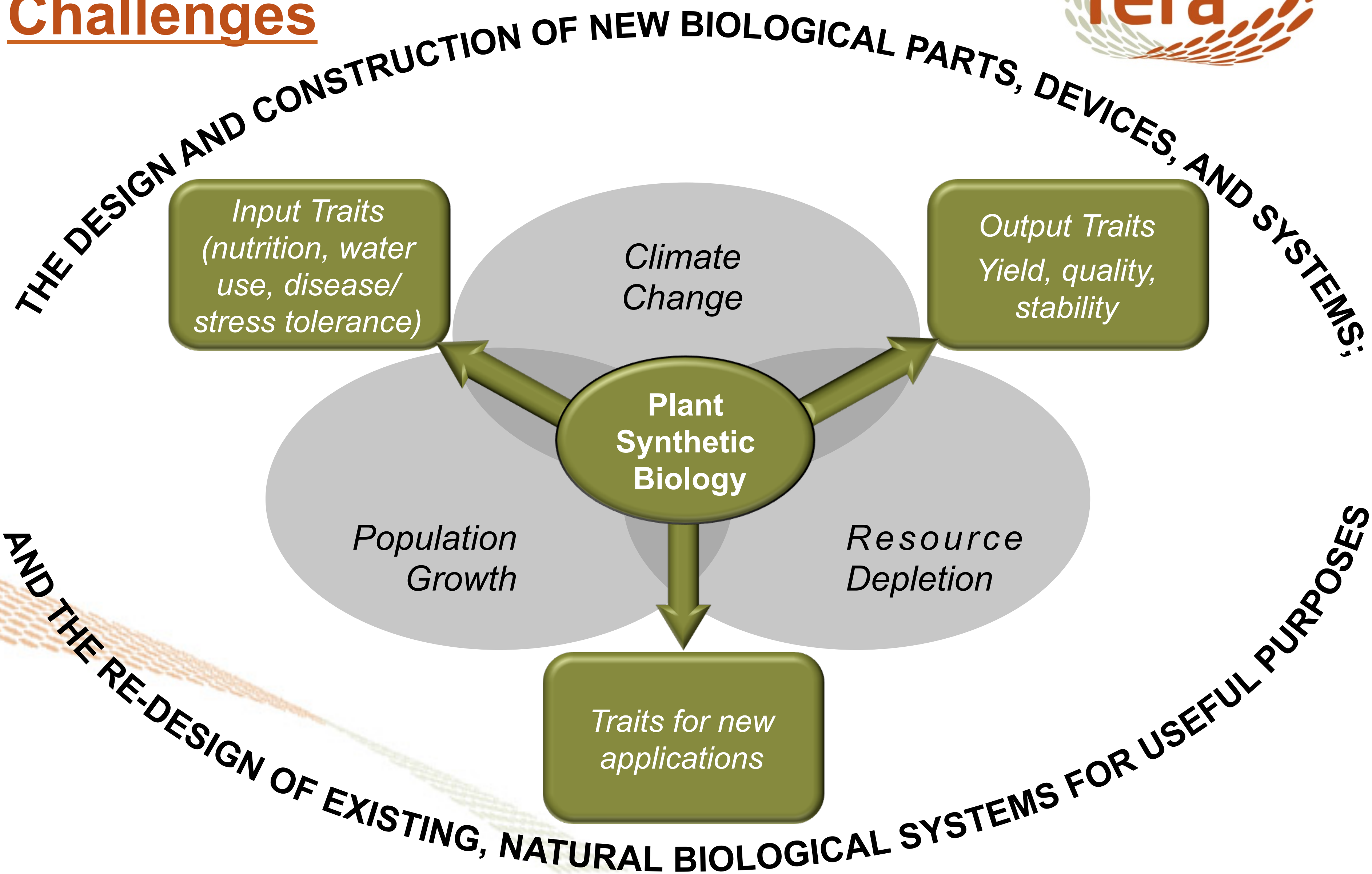
Outline

- Synthetic Biology and Biotechnology
- Plants and Biorefining
- Metabolic Engineering of Plant Feedstocks using Synthetic Biology Approaches
- Synthetic Biology Governance

Plant Synthetic Biology and Global Challenges



SB definition is from the Royal Academy of Engineering report "Engineering Synthetic Biology: scope, applications and implications", 2009

THE INDEPENDENT

Thursday 14 June 2007

Blair vs The Independent

OUR READERS ANSWER BACK – A LETTERS SPECIAL, IN EXTRA

Scientists challenge major review of global reserves and warn that supplies will start to run out in four years' time

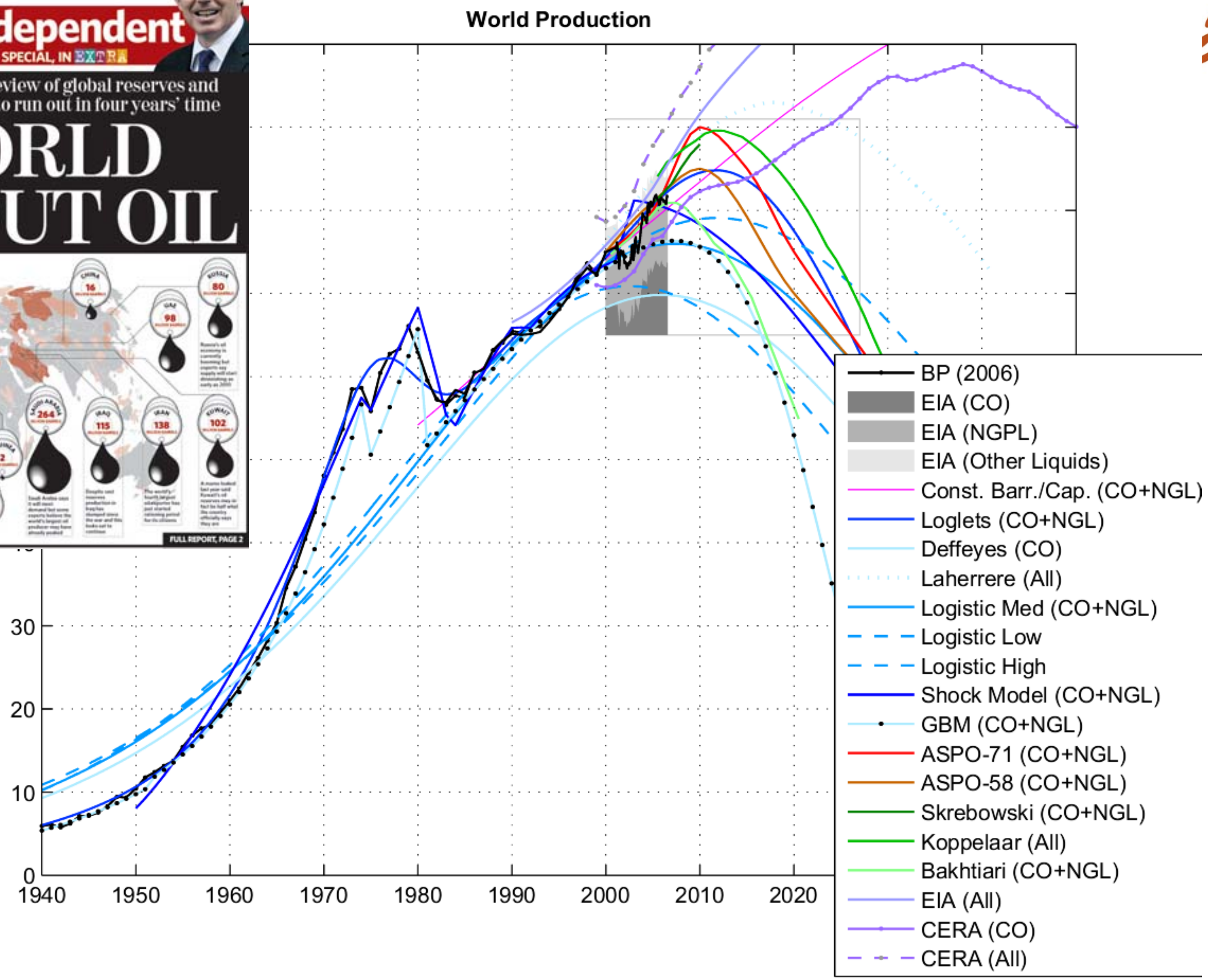
A WORLD WITHOUT OIL

What oil nations say they have left

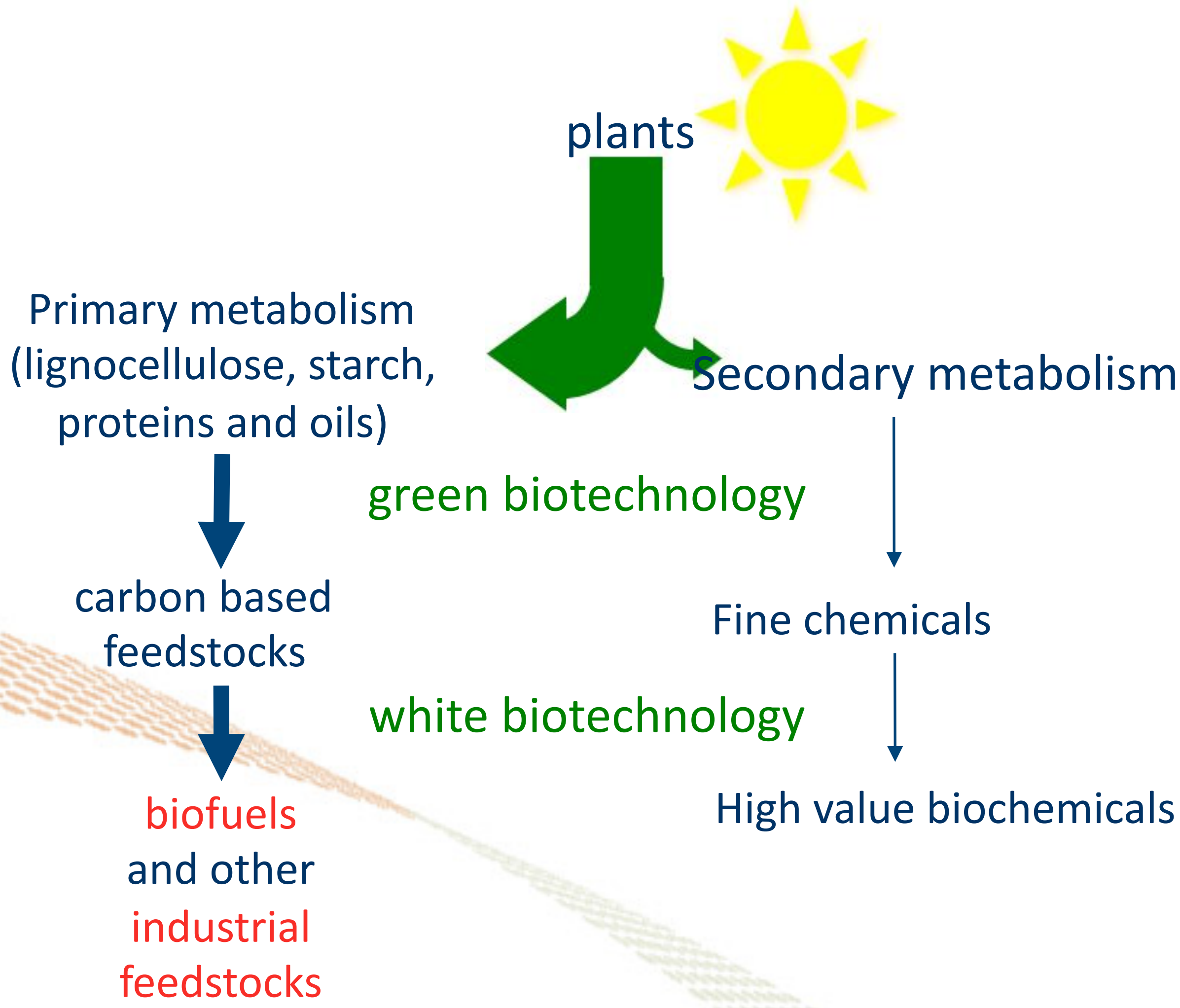
CANADA 17, UK 4, CHINA 16, RUSSIA 80, UAE 98, ALGERIA 12, UNITED STATES 30, LIBYA 42, MEXICO 13, VENEZUELA 80, NIGERIA 36, SAUDI ARABIA 264, IRAQ 115, IRAN 138, KUWAIT 102, E. GUINEA 2, ANGOLA 9, BRAZIL 12

KEY
Major oil fields

FULL REPORT, PAGE 2



From Black to Green Feedstocks- Chemurgy: Replacing Oil with Plants

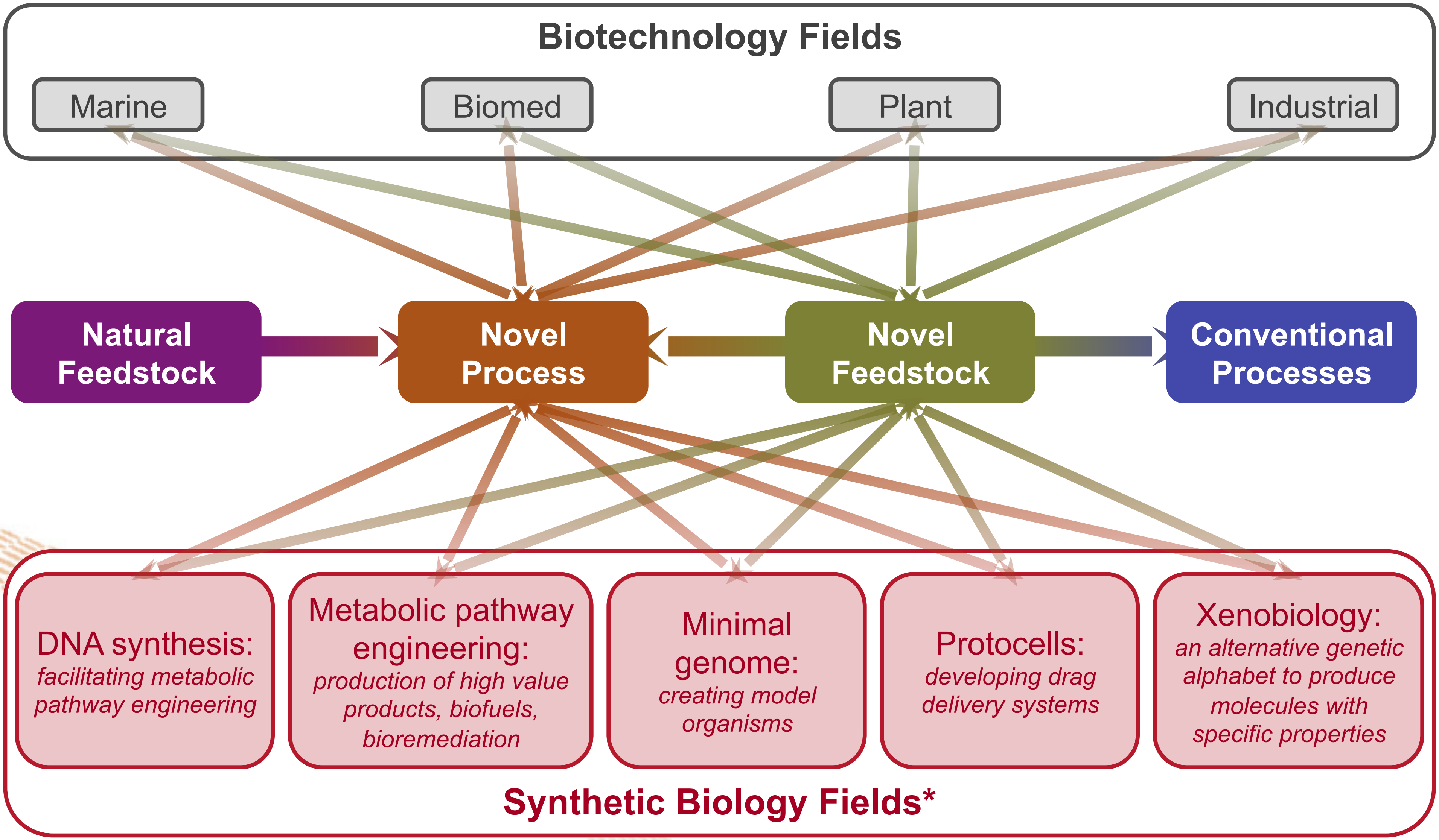


Oil Replacement: *Plant Biorefining*

Primary Product

'Value Added' Products

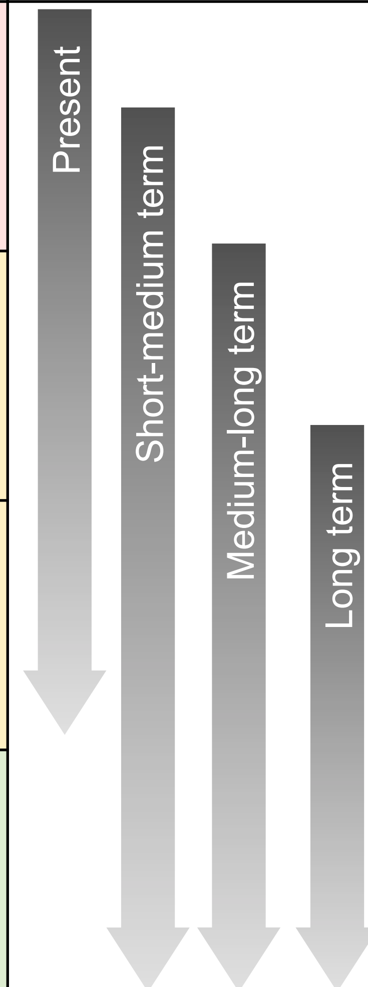
Synthetic Biology: *New Biotechnology Platforms*



**Synthetic Biology – Update 2013, Anticipating developments in synthetic biology; COGEM topic report*

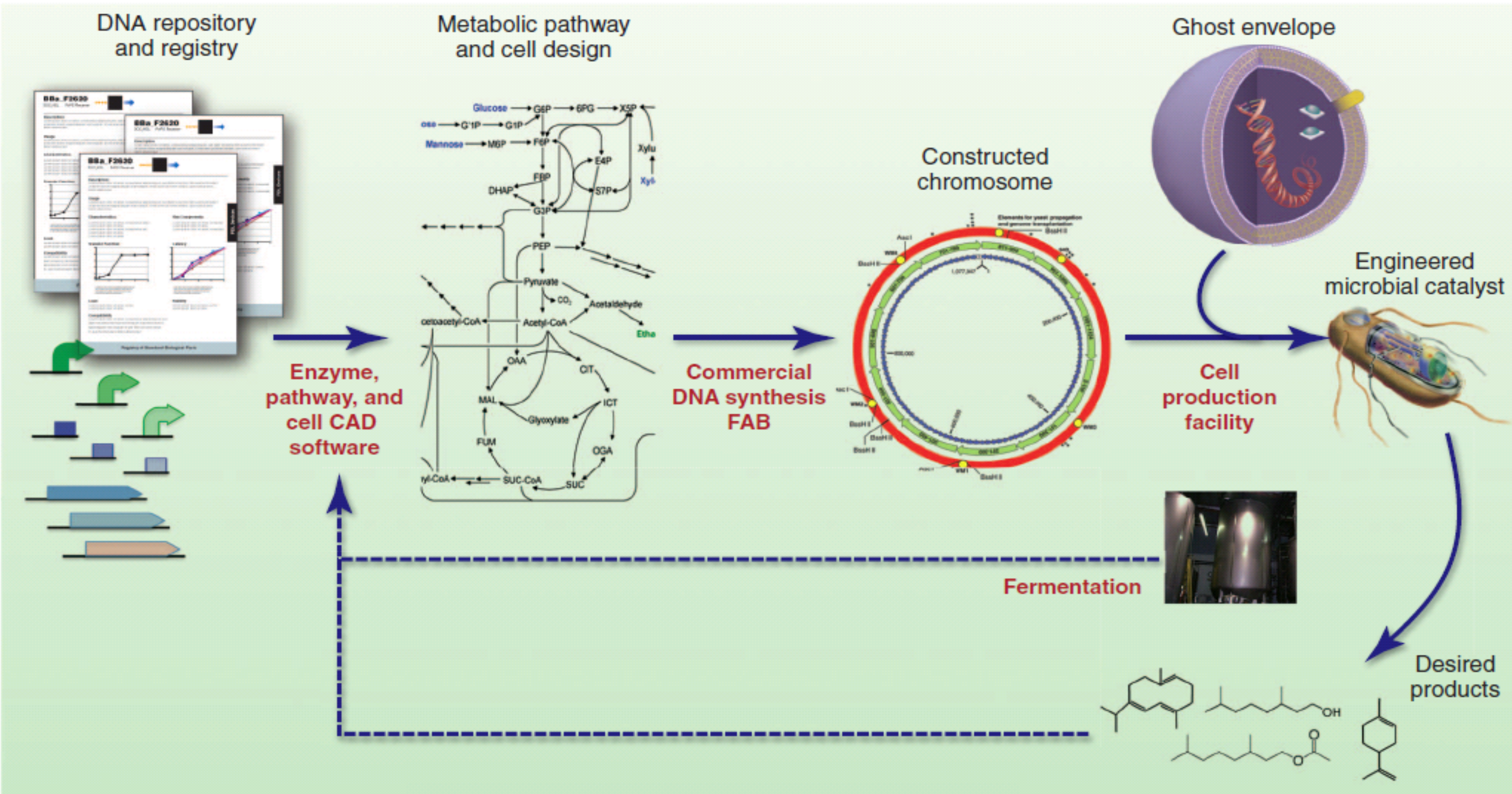
Application Routes

Feedstock	Technology	Developments	Issues	Products	R&D,D Challenges
Natural biocrops	**Conventional** biorefinery	Improvements are limited	Resolved	• Limited yield • Limited selectivity	
Natural biocrops	**Novel** biorefinery	Novel SB microorganisms	• Biosecurity & biosafety could be addressed • Public opinion	• Limited yield due to feedstock availability • Improved process selectivity	
Novel biocrops	**Conventional** biorefinery	Novel SB input and output traits	• Biosecurity • Biosafety • Repetition of GMO	• Improved yield • Limited process electivity due to technological capabilities	
Novel biocrops	**Novel** biorefineries	SB microorganisms and SB feedstock	• Biosecurity • Biosafety • Policy framework	• Improved yield • Improved selectivity	



Microbial metabolic engineering leads the way in SynBio

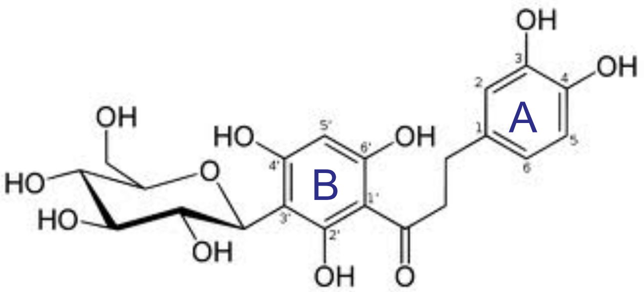
"One can envision a future when a microorganism is tailor-made for production of a specific chemical from a specific starting material, much like chemical engineers build refineries and other chemical factories from unit operations..."



Keasling, J.D.: Manufacturing molecules through metabolic engineering. Science , 2010

Applying synthetic biology in biorefining to make artificial bioactives

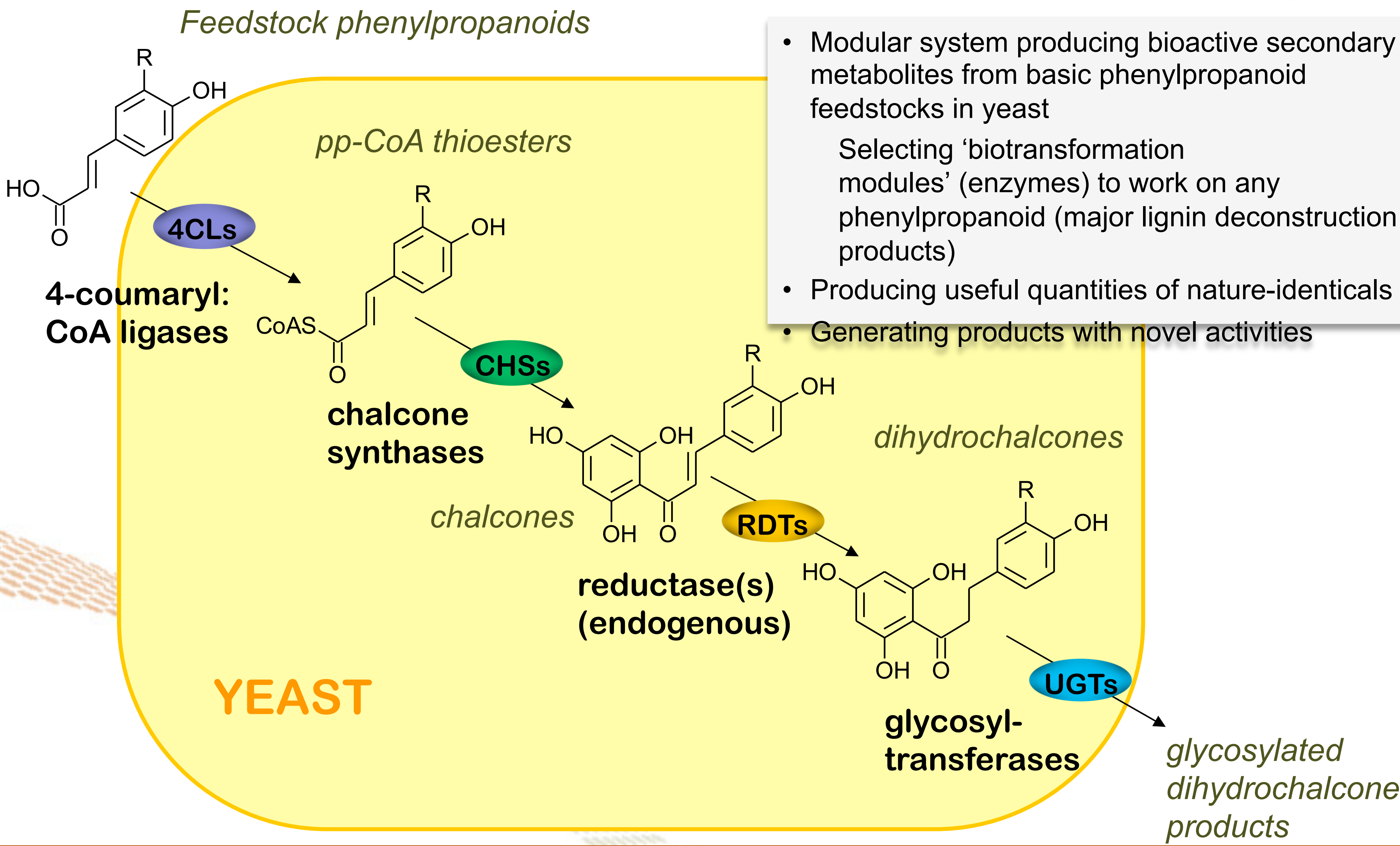
Glycosylated dihydrochalcones



- Take cheap and abundant by-products of ethanol fermentation (ferulic acid)
- Biotransform using modular synthetic pathways constructed from plants and yeast
- Naringin dihydrochalcone: 500x sweeter than sucrose
- Aspalathin: antioxidant from Rooibos (redbush tea)
- Neohesperidin dihydrochalcone: 2000x sweeter
  - used in food, pharma and animal feed industries

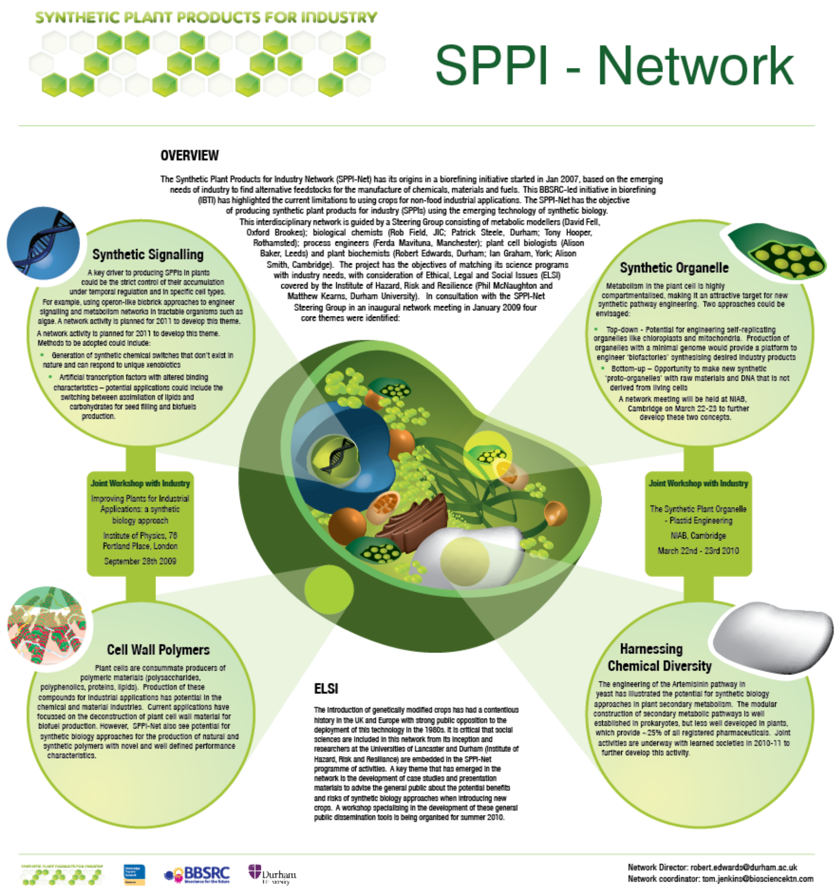
Pathway to artificial sweetners

- Modular system producing bioactive secondary metabolites from basic phenylpropanoid feedstocks in yeast
  - Selecting 'biotransformation modules' (enzymes) to work on any phenylpropanoid (major lignin deconstruction products)
- Producing useful quantities of nature-identicals
- Generating products with novel activities



Synthetic Plant Products for Industry

- 2008 formed an RC-funded network of academics and industrialists to identify potential applications for synbio in plants
- Influenced thinking in the private sector
- Stimulated funded projects



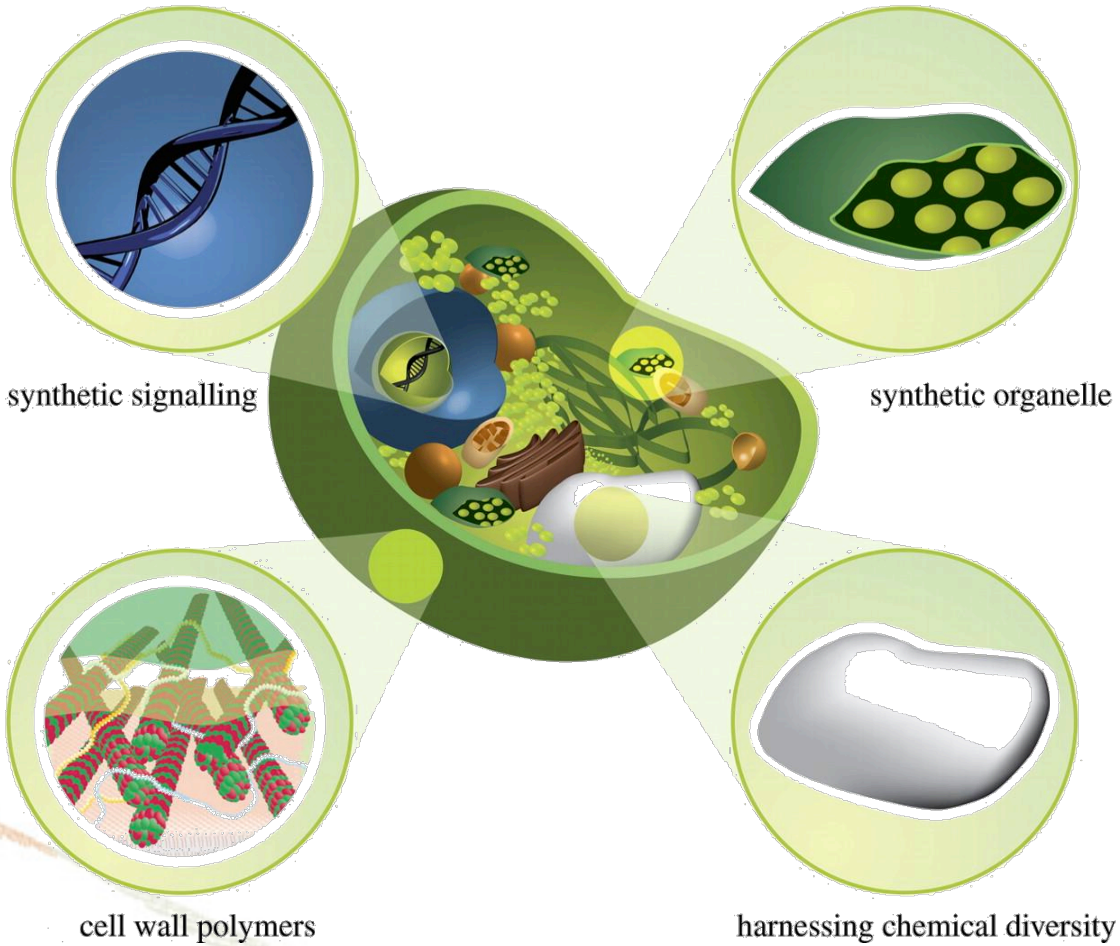
Why use Synthetic Biology in Plants ?

- Current studies have largely concentrated on re-engineering microbes
- Similar approaches in multicellular eukaryotes clearly more challenging but possible if a step-wise approach adopted
- Crops have been selected for food production and are generally inefficient feedstocks for biorefining (zero waste)
- Particularly useful for the small number of major crops we rely on

Output Traits: *Tailored Content*

- *Synthetic chemical switches that do not exist in nature and can respond to unique xenobiotics*
- *Artificial transcription factors with altered binding characteristics (eg. switching between assimilation of lipids and carbohydrates)*

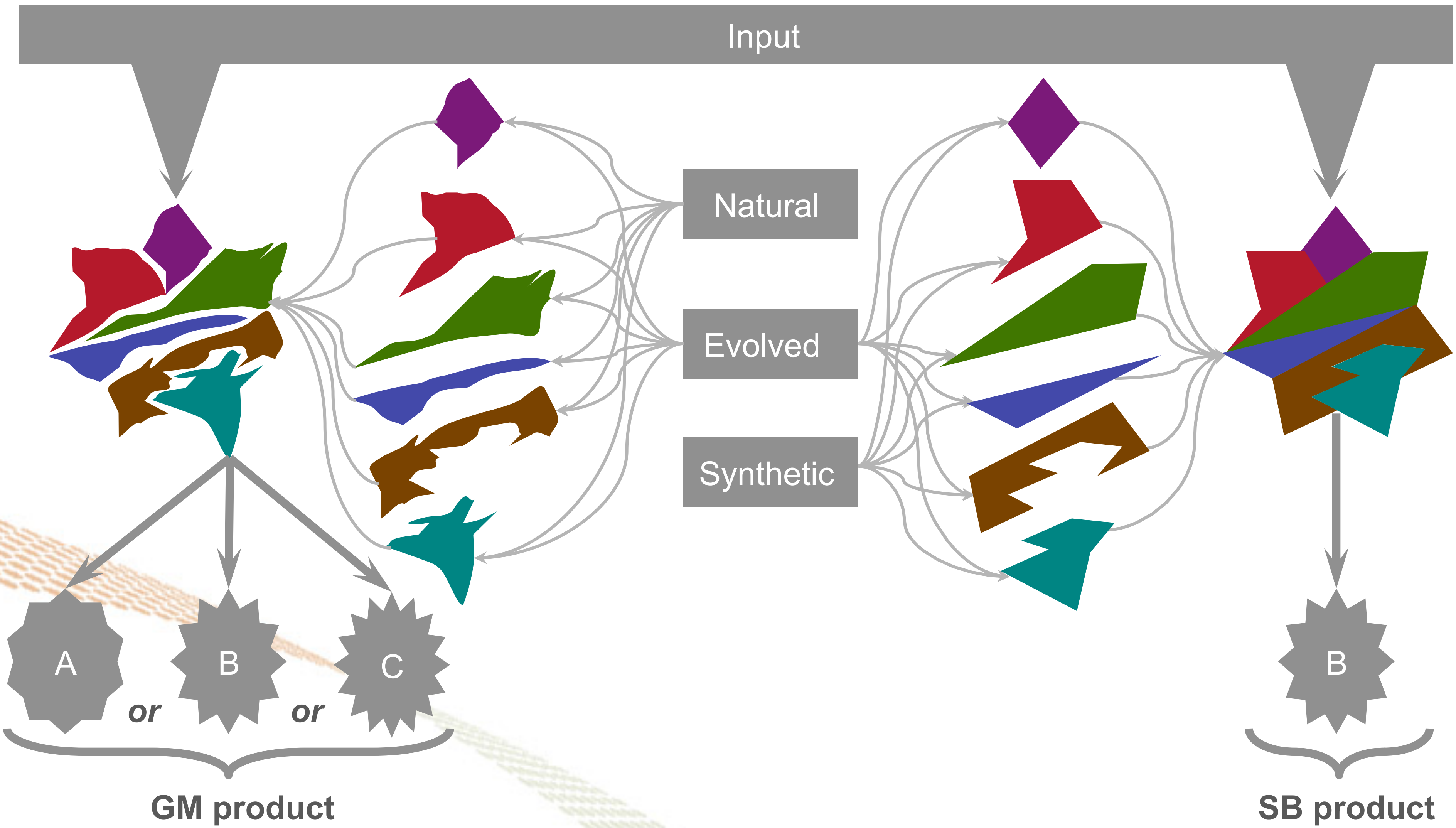
- *Production of natural polymers with novel and well defined properties*



- *Organelles with a minimal genome as a platform to engineer "biofactories" synthesising desired industry products*
- *Production of new synthetic "proto-organelles"*

- *The modular construction of secondary metabolic pathways is well established in prokaryotes, but less developed in plants which provide ca. 25% of all registered pharmaceuticals*

Engineering metabolism a clear leader



Adapted from: E. Leonard, D. Nielsen, K. Solomon, K. J. Prather; Engineering microbes with synthetic biology frameworks , Trends in Biotechnology, 2008

Biorefining- Synthetic biology & input and output metabolism

Plant Traits

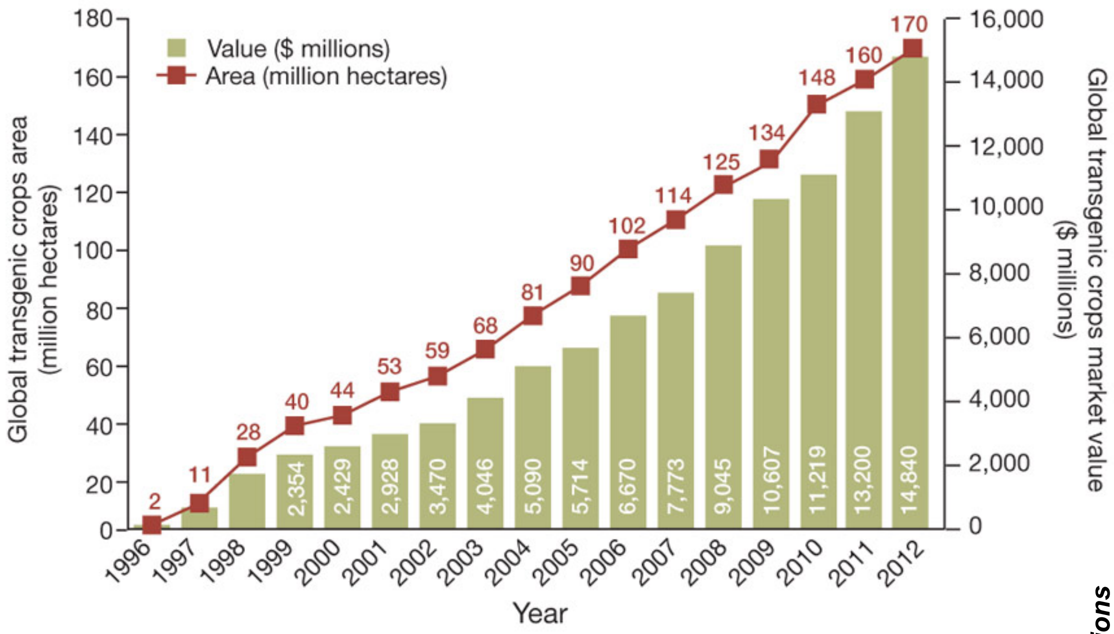
Input: *alter inputs needed in production*			**Output:** *alter the harvested product*		
Fixation of N, C, nutrients & plant form	New crop protection agents	Metabolism for resilience	More usable biomass	Shift to higher value products	New synthetic traits

Greater efficiency and resilience in production in a changing environment

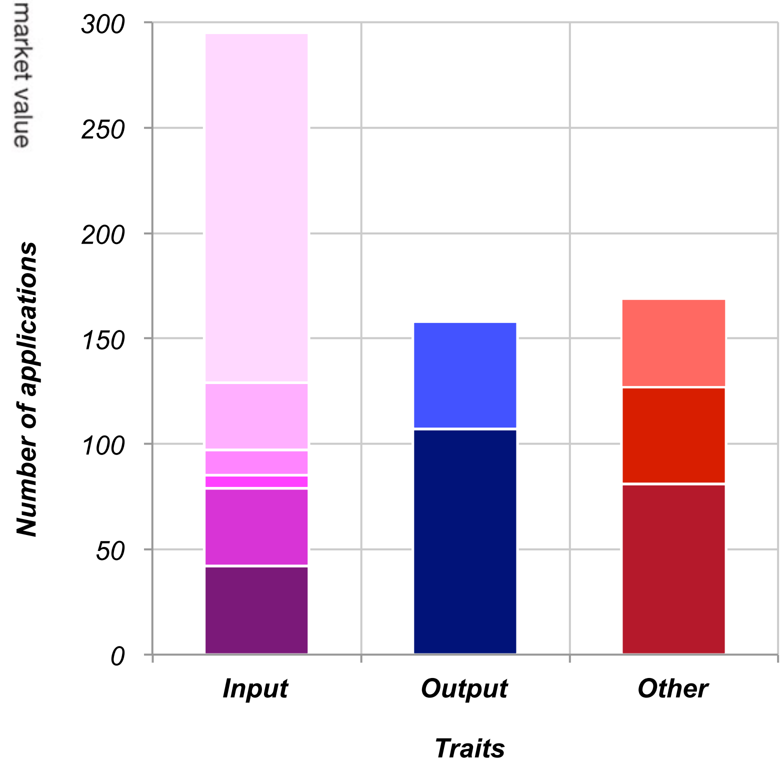
Moving toward zero waste and seamless interface with existing chemical industries

Input & Output Traits: *State of Play*

*Transgenic crops**



Plant biotechnology patent applications (USPTO, 2011)



- *other polynucleotide sequence*
- *transformation methods*
- *regulatory sequences*
- *altered phenotype*
- *altered content*
- *stress tolerance and/or yield*
- *other pathogen resistance – includes bacterial, viral and fungal pathogens*
- *nematode resistance*
- *stacked (HT+IR)*
- *insect resistance*
- *herbicide tolerance*

Plant Synthetic Biology: *Four examples of engineering metabolism*

- ✓ *Engineer novel **"smart" plants** with desirable traits which cannot be achieved through conventional breeding*
- ✓ *First generation extreme GM with increasing sophistication in metabolic engineering*

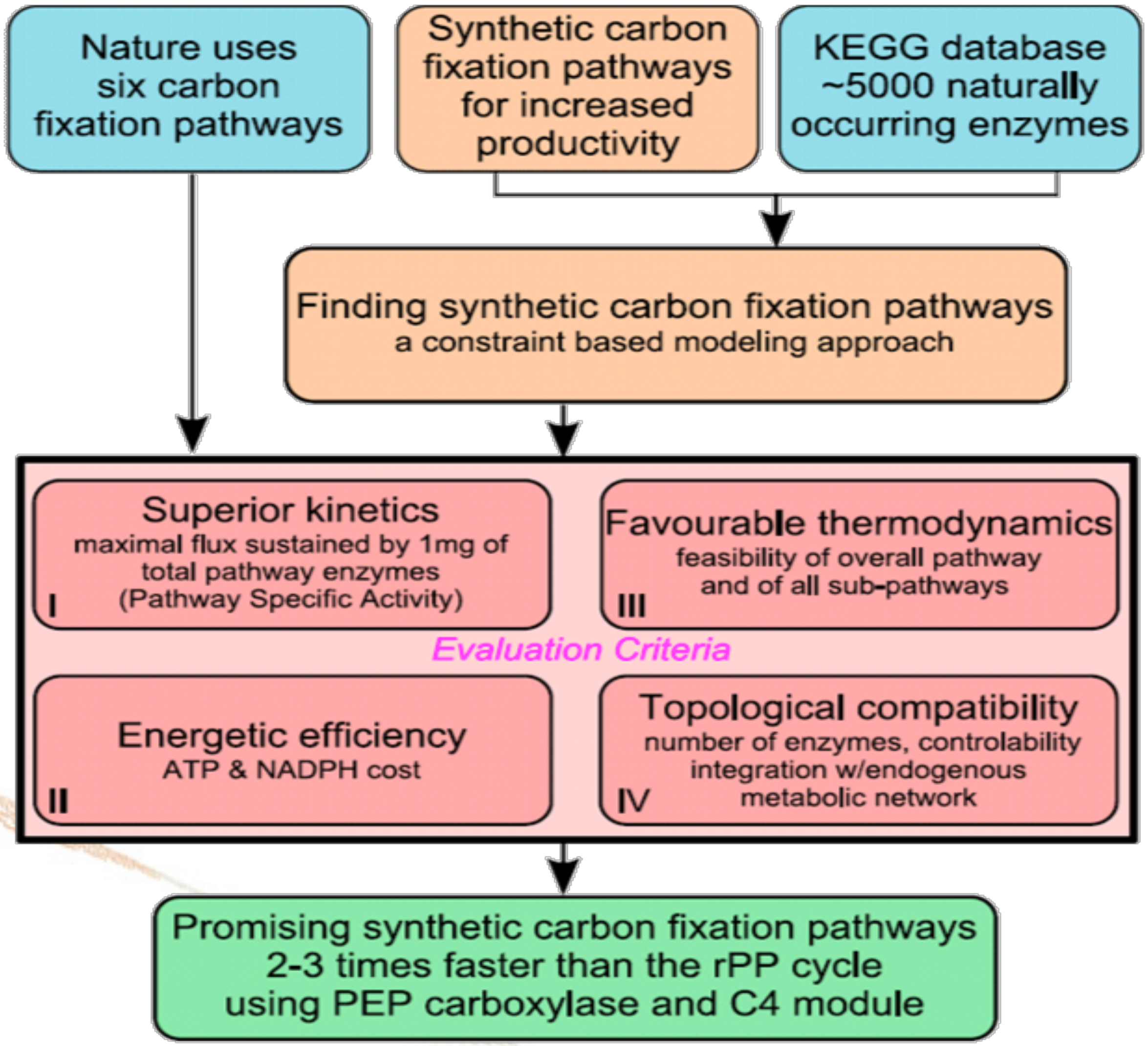
Input Traits

- ✓ Carbon fixation

Output Traits

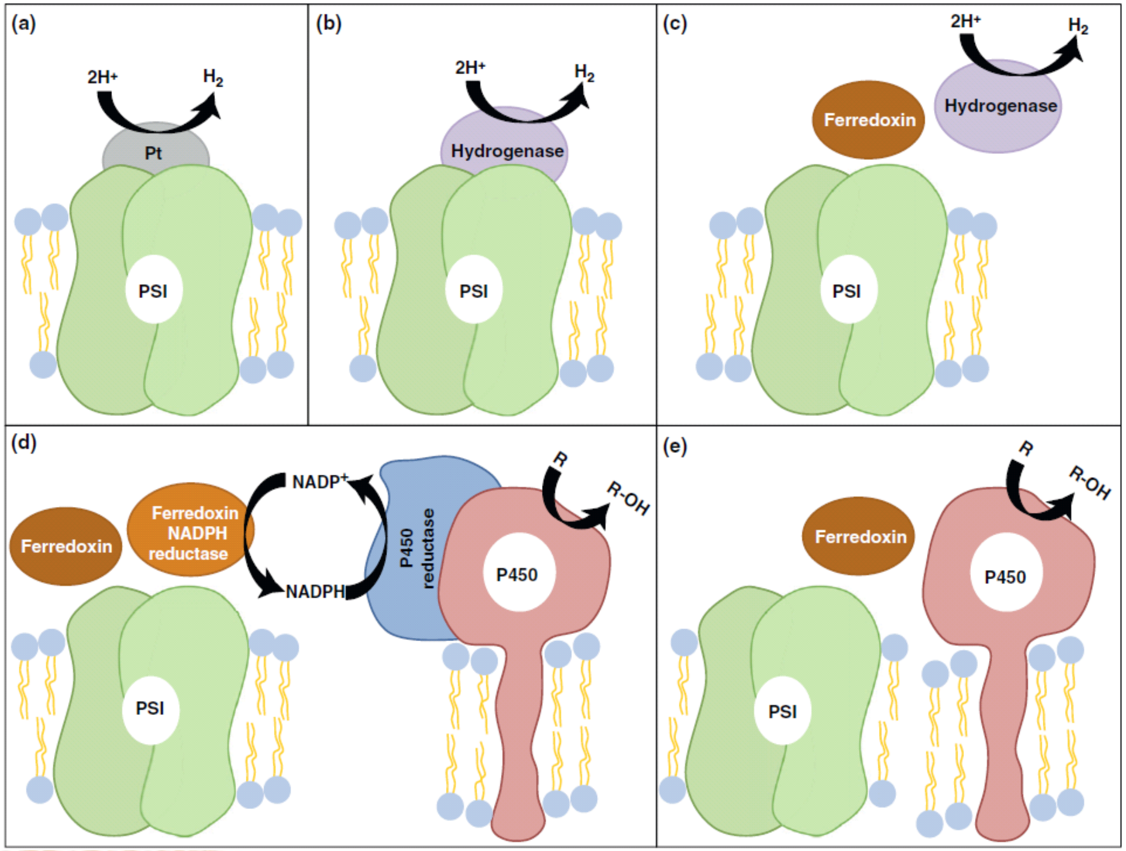
- ✓ Light driven secondary metabolism for tailored content:
  - pharmaceuticals
  - value added products
- ✓ Cell wall
- ✓ Biofuels

Input Traits: *Synthetic Carbon Fixation Pathways*

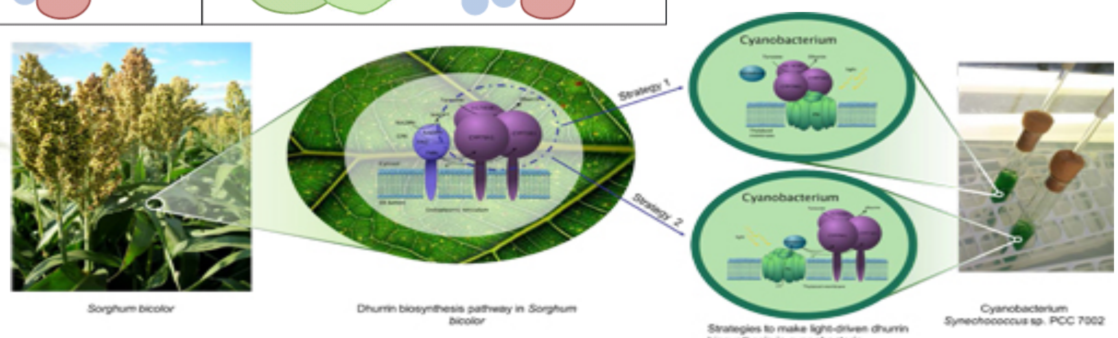


Bar-Even, A., Noor, E., Lewis, N.E., Milo, R., Design and analysis of synthetic carbon fixation pathways. Proceedings of the National Academy of Sciences of the United States of America , 2010

Output Traits: *PS I Redox*



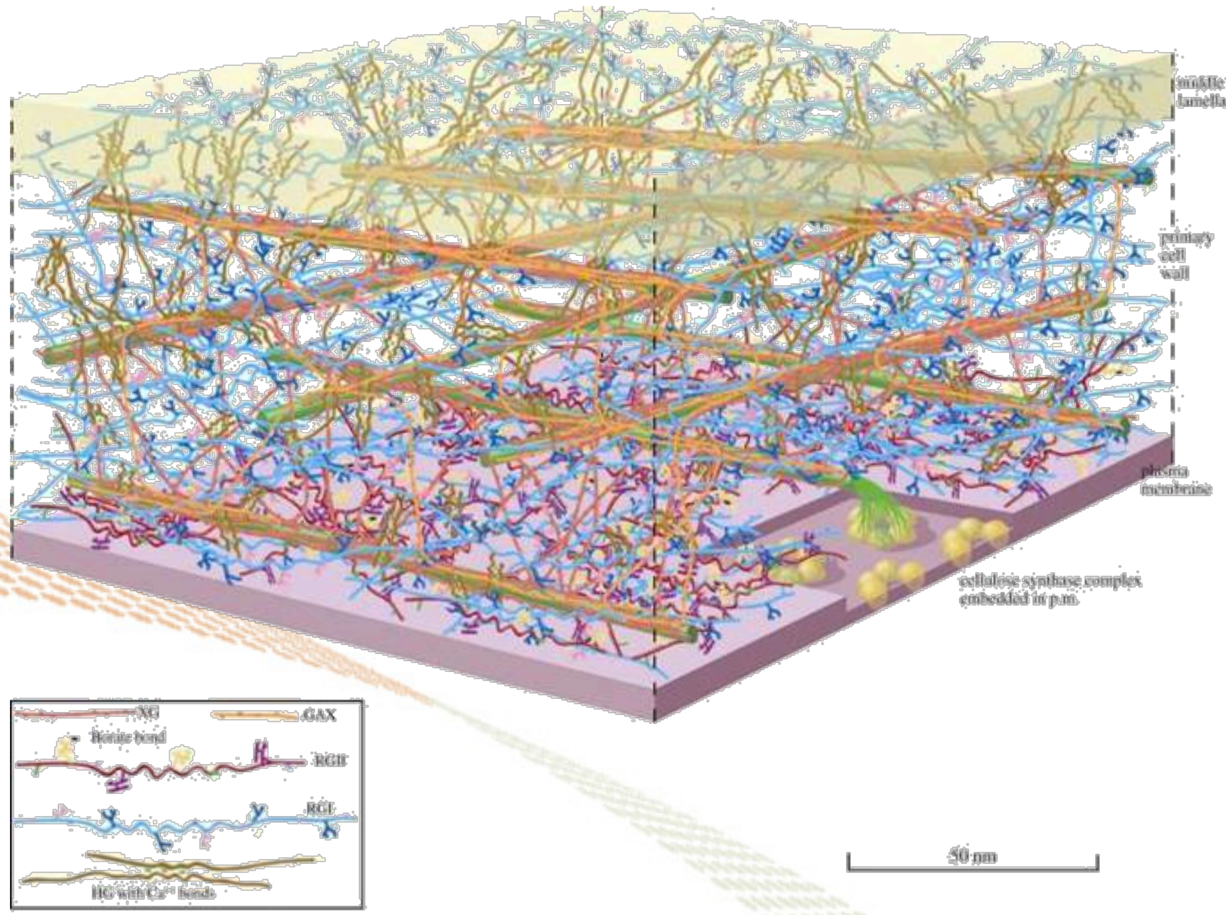
- coupling PSI directly to a cytochrome P450 to develop a system in which the enzymatic reaction of P450s is driven directly by the energy of solar light



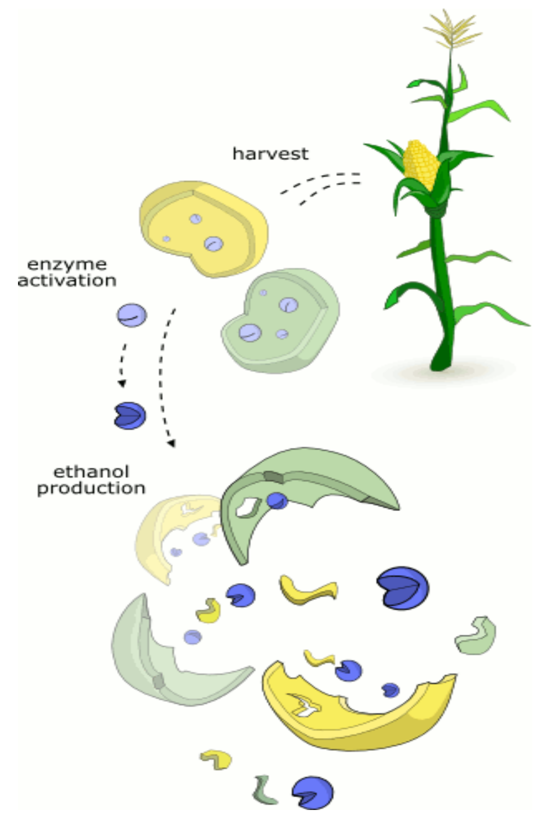
Jensen, K , Jensen, PE & Møller, BL, Light-driven chemical synthesis; Trends in Plant Science; 2012 and http://plen.ku.dk/english/research

Output Traits: Biofuels

- Plants engineered with glyco-hydrolases to assist fermentation post harvest
- Extensive engineering of plant cell wall with novel properties based on systems principles
- Real potential for increasing efficiency of bio-mass processing to biofuels and bulk chemicals

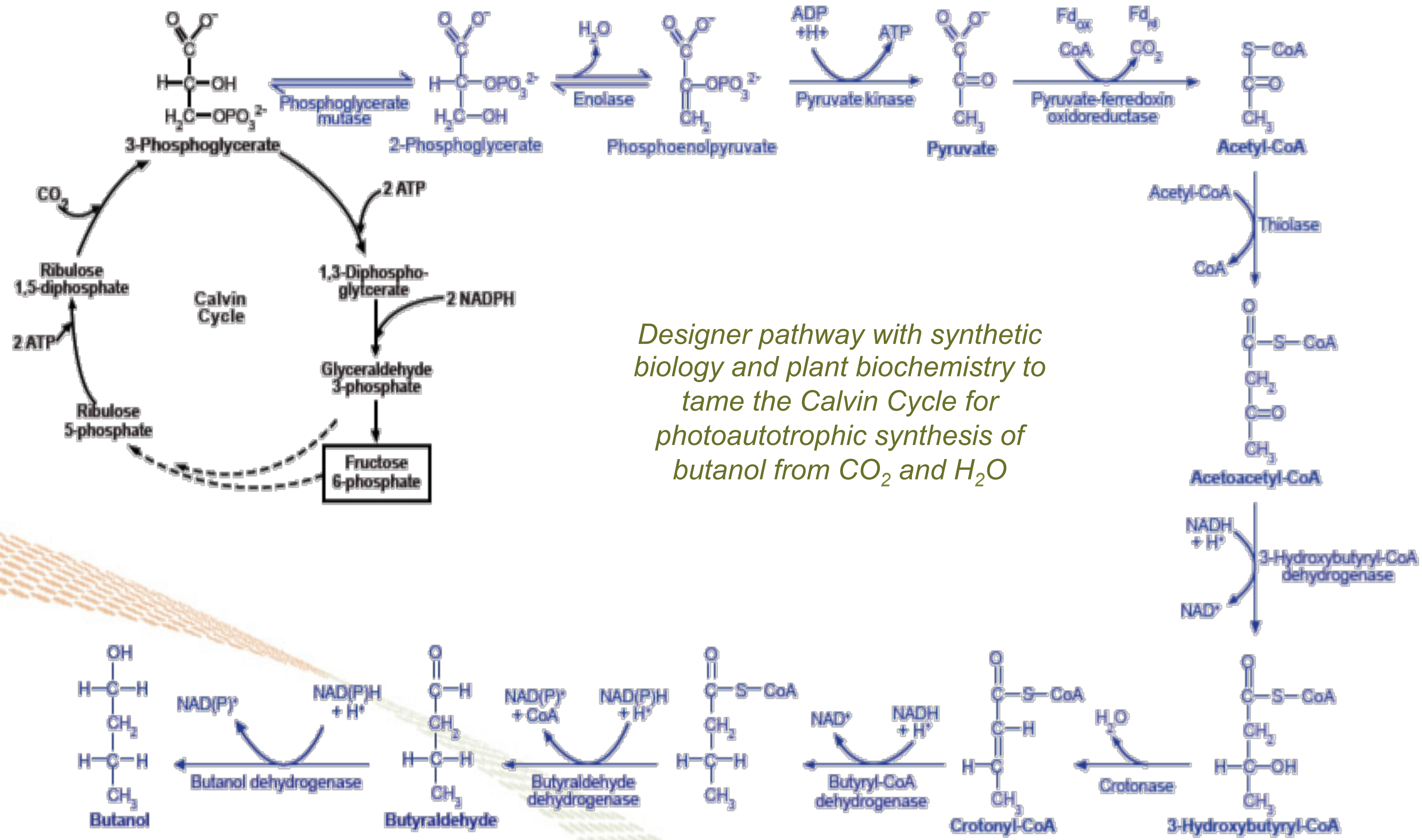


Self-digesting plants



Picture of cellulose: Somerville et al, Science, 2004

Output Traits: producing biofuels directly from sunlight in algae



Designer pathway with synthetic biology and plant biochemistry to tame the Calvin Cycle for photoautotrophic synthesis of butanol from CO_2 and H_2O

Plant synthetic biotechnology

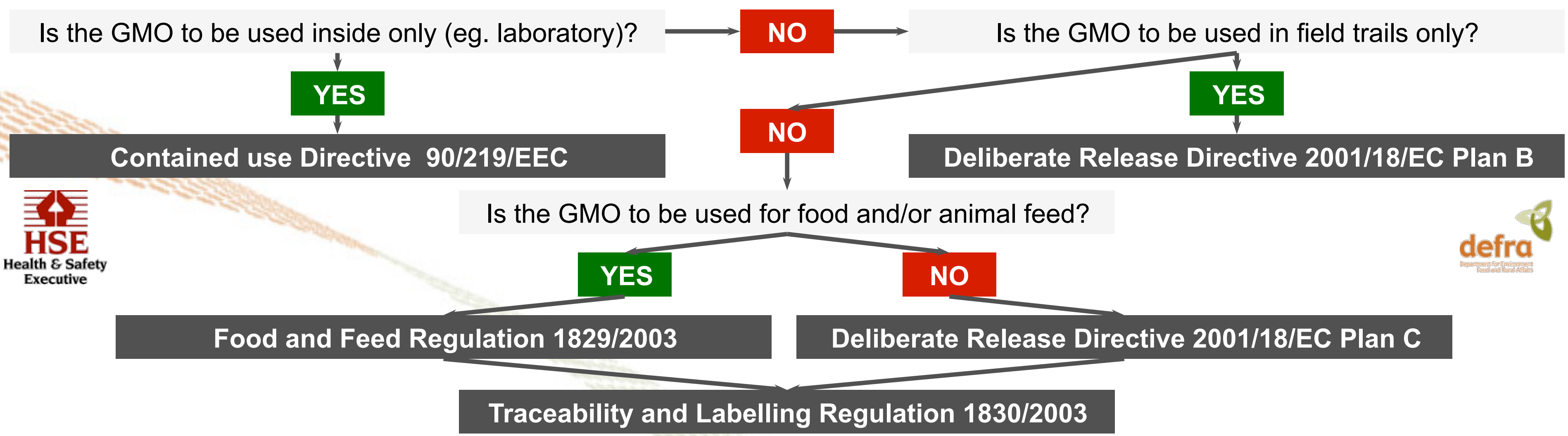
- First co-ordinated attempts to engineer plant metabolism using Synthetic Biology
- Combination of input and output trait modification
- Such new approaches needed to establish biorefining as a viable alternative to established chemical processes
- Are the right incentives in place ?- regulatory frameworks and public perception- plant GM has form !

Synthetic Biology: *Public Opinion and NGOs*

Regulation of Synthetic Biology Processes and Products

- Current regulations do not deal with synthetic biology *per se*
- Processes and products of synthetic biology are covered by Directives and Regulations that deal with GMO
- EU regulations tend to be stricter than their US counterparts, especially with respect to labelling and traceability requirements. The more stringent EU rules are attributed to public concern about the potential dangers of GMO
- The "goldilocks dilemma" for synthetic biology regulations:
  - must not be too precautionary (i.e. suppress innovation)
  - must not be too business friendly (i.e. facilitate unexpected risk)
- The regulatory process should require developers to consider unconventional and low probability risks as part of the scenario planning and risk mitigation process. The data required for a traditional risk appraisal may be lacking, in which case a precautionary approach seems appropriate whenever the potential risks are high

Structure of EU GMO regulations



The regulation of synthetic biology; A guide to United States and European union regulations, rules and guidelines, 2012

Science in retreat –neonicotinoids
Hazard Vs. Risk

Sets out restrictions on selling, supplying or storing pesticides and precautions to protect the health of humans, the environment, and particularly water, when using pesticides

- 1986
  - The Control of Pesticides **Regulations (SI 1986/1510)**
- 1991
  - The Plant Protection Products **Directive (91/414/EEC)**
- 1997
  - Control of Pesticides (Amendment) **Regulations (SI 1997/188)**
- 2005
  - The 91/414 Directive is implemented in the **UK** by the **Plant Protection Products Regulations** (PPPR)
- 2011
  - European legislation, **Regulation (EC) No 1107/2009**

Environment

+

Safety

~

Risk vs. Hazard
(endocrine disruptors only)
=

Cost increase in registration

Translating Plant Synthetic Biology Research: *from Lab to Field*

- Adopting synthetic biology in plant engineering is hard !
- Science drivers are sound- Engineering plant metabolism has clear public good and economic benefits (re: KBBE)
- GM history is not helpful but public dialogue exercises have been very useful
- EU regulatory frameworks re: Risk vs. hazard may be unable to resist co-ordinated lobbying from NGOs (eg: neonicotinoids)
- Science alone is not enough- Review and adjust policies/regulations/legislations to facilitate beneficial applications of SB
  - Consider 'public good' alongside biosafety and biosecurity
  - Create inventory and traceability of SB products

Acknowledgements

- SPPI-net core panel
- BBSRC/ TSB
- Bioscience KTN
- Fera emerging technologies SiG
- Elena Fesenko
- Mad Swiss Dude

'2 of your 5 a day in one'