Synthetic Biology: applications

Emrah Nikerel,
DSM Biotechnology Center

DSM – key activity areas

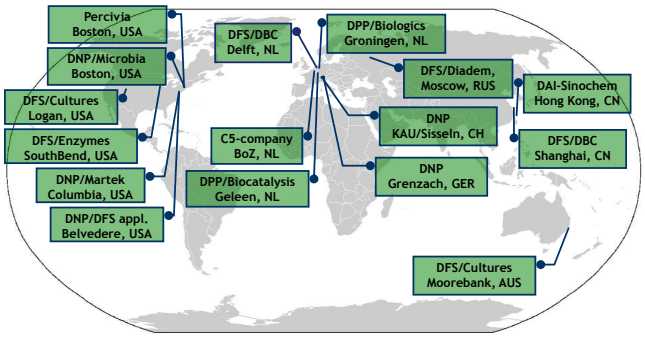
Health
Advanced, cost-effective health and medical innovations, and healthier food and beverages, to meet the needs of a growing and ageing global population

Nutrition
World's leading producer of vitamins and nutritional ingredients meeting the growing need for more nutritious and more sustainable food and animal feed

Materials
Enabling lighter, stronger, more advanced and more sustainable performance materials

DSM's 22,000 employees deliver annual net sales of about € 9 billion

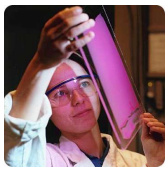
The DSM Biotechnology Network



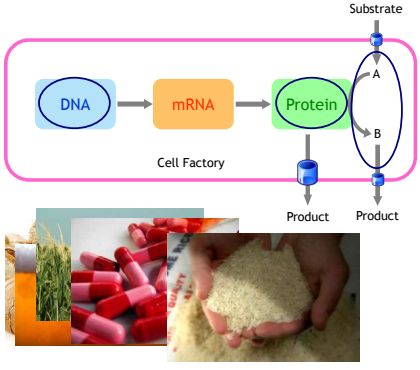
DSM biotechnology center, Delft

DSM Biotechnology Center Delft

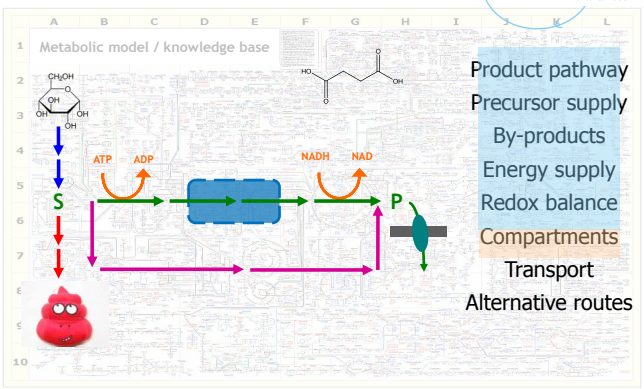
- Established in 2009 by merging of R&D Departments of DSM Food Specialties and DSM Anti-Infectives
- Applying biotechnology in products and processes
- Approx. 500 scientists & technicians located in Delft
- Serves DSM own products in the food, pharma and white biotechnology.
- Contract development and manufacturing services (DSM BioSolutions)



Cell as a factory – examples @DSM



Pathway & Strain



Examples of Products produced by Biotechnology

Metabolites
- vitamins, pharmaceuticals, chemicals (*e.g.* antibiotics, citric acid, arachidonic acid)

Proteins
- Enzymes (*e.g.* PreventASe™, Panamore™, Maxiren™ , Maxilact™,.....

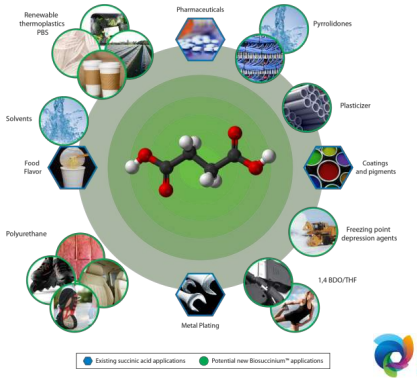
Biomass
- Yeast Extracts, cultures e.g.Maxarome™, Delvo-Yog™

DSM BRIGHT SCIENCE. BRIGHTER LIVING.

Slide 8

Biosuccinium™ succinic acid

a Versatile Building Block for Multiple Applications



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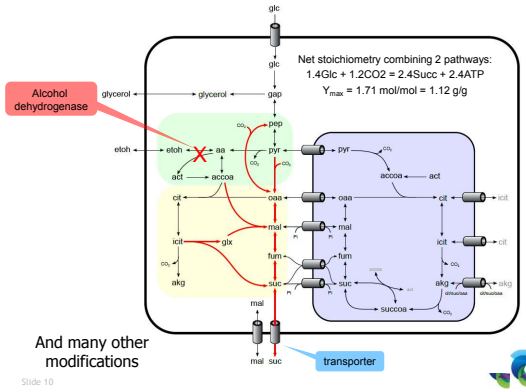
Slide 9

S. cerevisiae Metabolic Engineering Strategy

Introduction of red. TCA cycle, glyoxylate shunt and export



From **<1 g/L** to **High product concentrations**

And many other modifications

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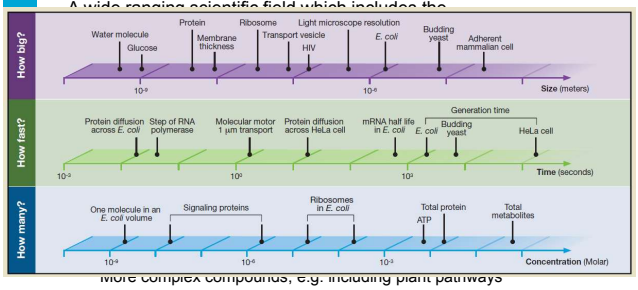
Slide 10

What is it all about with Biotechnology

A wide ranging scientific field which includes the



More complex compounds, e.g. including plant pathways

How Old Is Biotechnology ?

10,000 BC Domesticating Crops

6,000 BC Brewing Beer

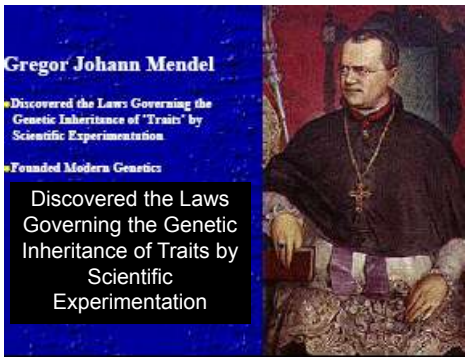
Domesticating Animals 8,000-9,000 BC

4,000 BC Leavening Bread

1880's Production of Vaccines

1940's Production of Antibiotics

1980's Use of genetically modified organisms



How Old is Modern Biotechnology?

Modern Biotechnology

- Molecular Biology
  - microbiology
    - biochemistry
  - cell biology
- Molecular Genetics
- Genetic Engineering: Moving a gene from one organism to another
  - chemical engineering
  - biomanufacturing

And what about Biotech apps ?


Impossible inventions

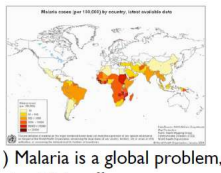
Just over a century ago, heavier-than-air flight was deemed outlandish. The Wright brothers soon proved that wrong. In fact, history is littered with ideas that defied conventional wisdom. *New Scientist* celebrates the impossible technologies in our everyday lives

Smartphones

- If you were to build an iPhone using components from the mid 1980s…
- Battery 5 times as large
- Antenna sticking out
- GPS receiver hefty backpack and batteries
- Motion sensor was mechanical
- Two film camera's
- Processor match Cray X-MP

Slide Drew End

Most projects are Herculean.



1) Malaria is a global problem, artemisinin offers a cure.

2) Jay Keasling's team spent $25M to make artemisinin via biotechnology.

3) But artemisinin resistance is already occurring.

Must we always spend many years and $25M for each pressing biotech project?

32

We need new tools.

Slide Drew End

1973

Construction of biologically functional bacterial plasmids in vitro

Cohen et al., PNAS, 1973

1985

Cloning and expression of the human erthropoietin gene

Lin et al., PNAS, 1985

2006

Production of the antimalarial drug precursor artemisinic acid in engineered yeast

Ro et al., Nature, 2006

Purification and use of *EcoRI* restriction endonuclease ...

After converting the *Bst*EII site into an *Bam*HI site ... the fragment was inserted into the unique BamHI site

... the amplified product was cleaved with *Spe*I and *Hind*III...

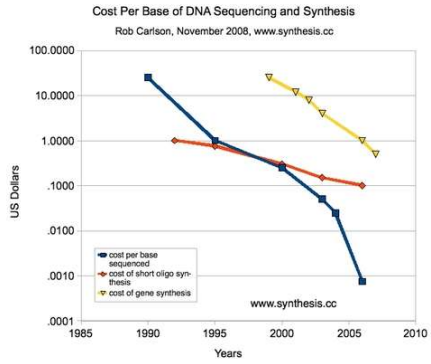
Much of rDNA basics unchanged past 30+ years

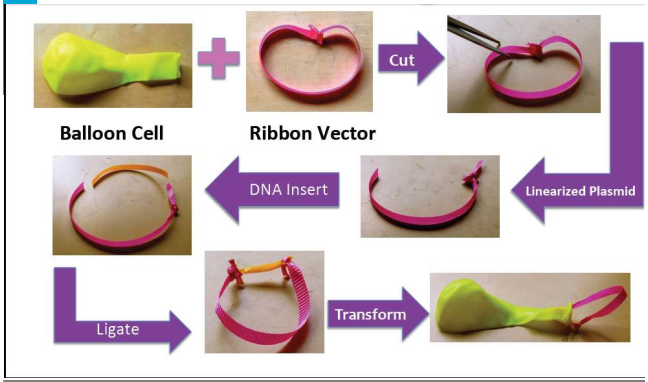
SB Technology drivers

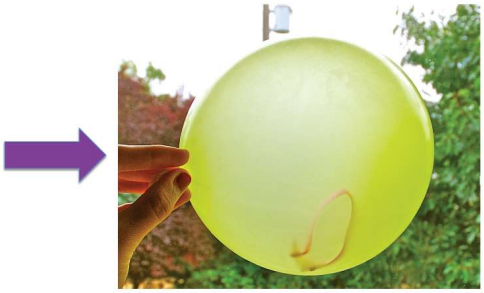
Purchase the full report >

GENOME SYNTHESIS AND DESIGN FUTURES: IMPLICATIONS FOR THE U.S. ECONOMY

James Newcomb, Robert Carlson, Steven Aldrich
Bio Economic Research Associates, 2007







Completed Cell with New Vector!

Key concepts in Synthetic Biology

- Abstraction, Standardization: allows non-biologists to work with cells.
- Great example of initiative: parts registry database, iGEM projects.

Synthetic biology application examples: iGEM projects

- The availability of the SB technology drives not only academia, industry, but also education, small enterprises, backyard labs etc.
- iGEM: international Genetically Engineered Machine competition:
  Yearly , student competition
  students come up with their own ideas, concepts, and realize them over summer

FOOD WARDEN

It's rotten and you know it!

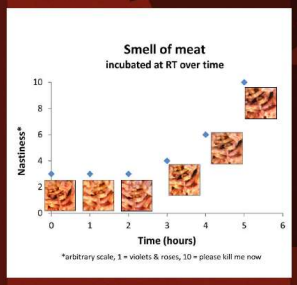
iGEM Groningen 2012
Renske van Raaphorst

MAIN GOAL

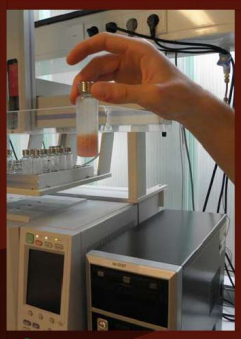
- Detect and show the moment that meat starts to spoil, using *Bacillus subtilis*

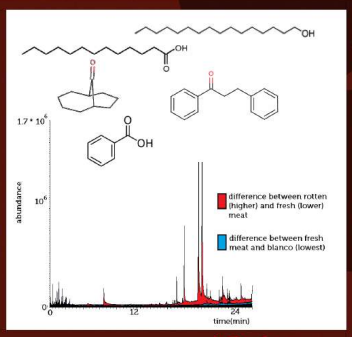
WHAT IS SPOILED MEAT?

- No clear definition!
  - Smell, taste, color
- One guideline (EU)
  - Amount of bacteria present in meat



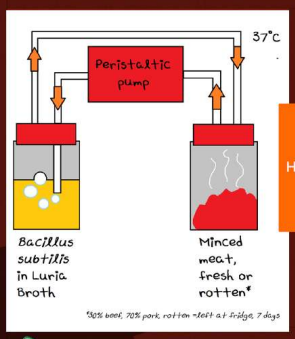
SPOILED MEAT VOLATILES

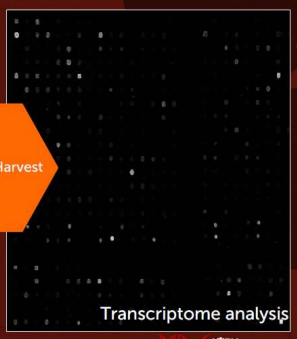




Example spectrum of rotten/fresh meat. (CH_2Cl_2)

RESPONSE TO SPOILED MEAT

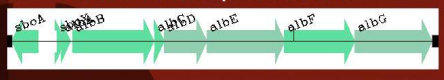


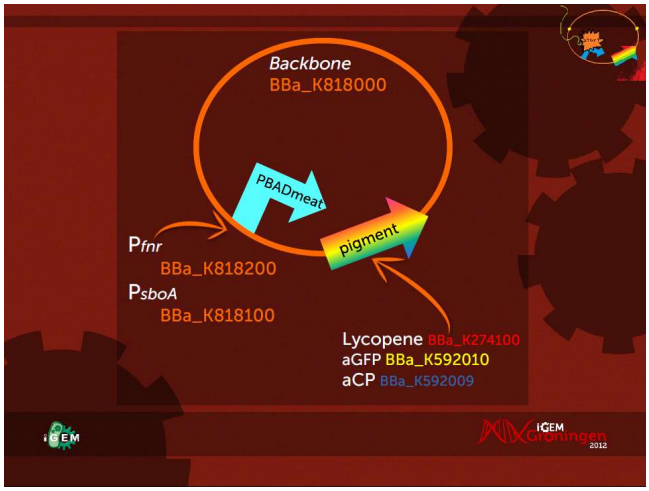


RESULTS

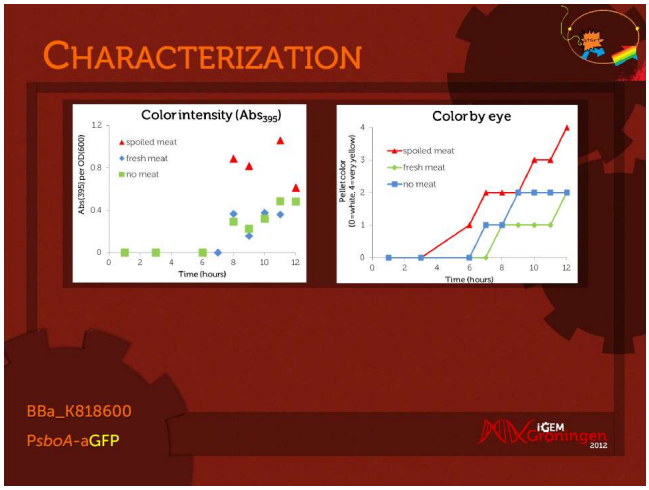
- P*fnr*
  - Nitrate metabolism

- P*sboA*
  - Biosubtilosin production




Backbone
BBa_K818000
PBADmeat
pigment
Pfnr
BBa_K818200
PsboA
BBa_K818100
Lycopene BBa_K274100
aGFP BBa_K592010
aCP BBa_K592009


DETECTION OF SPOILED MEAT
BBa_K818600
PsboA-aGFP

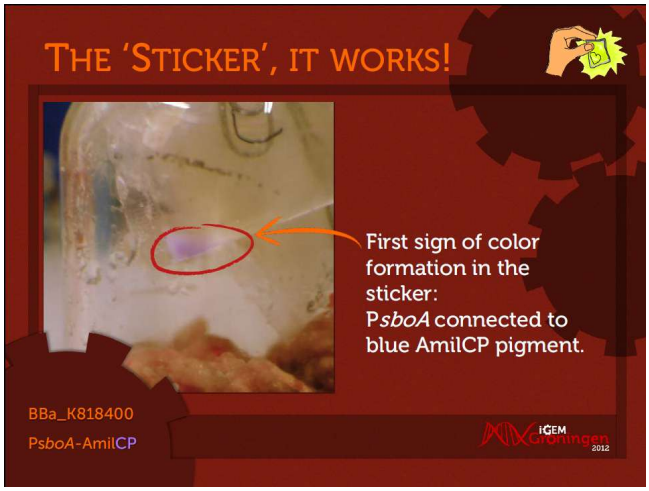

CHARACTERIZATION
Color intensity (Abs395)
spoiled meat
fresh meat
no meat
Time (hours)
Color by eye
Time (hours)
BBa_K818600
PsboA-aGFP


From construct to product:
FOOD WARDEN
Safe
Reliable
User friendly

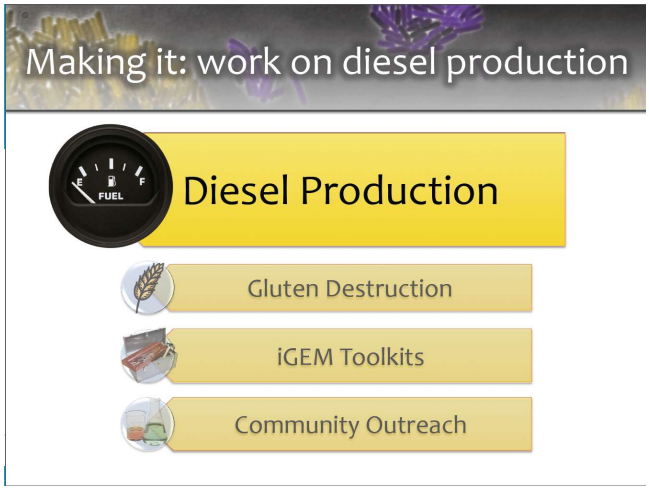

THE 'STICKER'
Break!

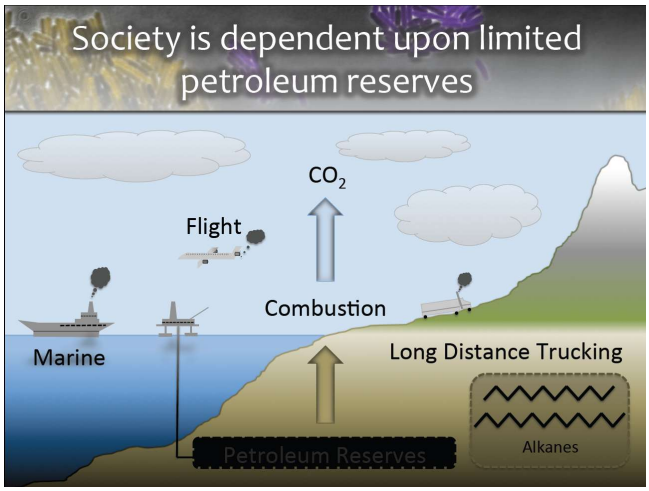

THE 'STICKER'
Safe
TPX®, FDA approved
Nanopores (1-10 nm)
User friendly
Reliable
Timing of bacterial growth: modeling

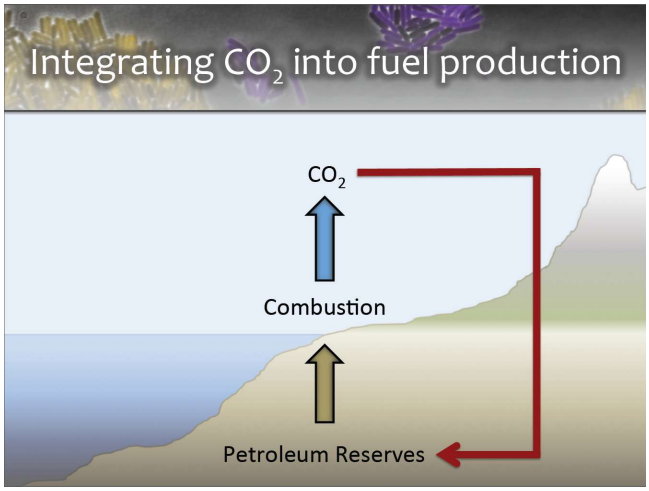

THE 'STICKER'
iGEM
iGEM Groningen 2012


THE 'STICKER', IT WORKS!
First sign of color formation in the sticker:
PsboA connected to blue AmilCP pigment.
BBa_K818400
PsboA-AmilCP
iGEM Groningen 2012

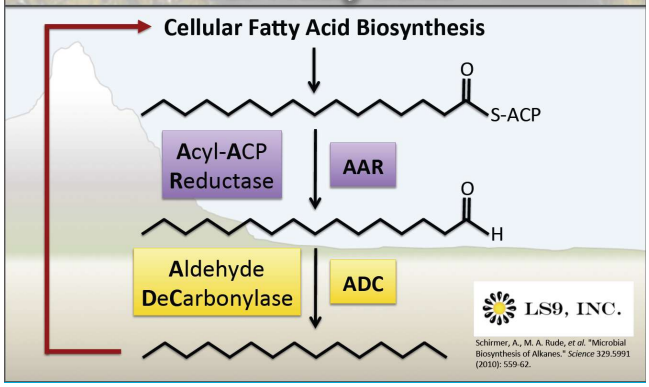

Make it or Break it:
Diesel Production
WASHINGTON
iGEM 2011
and Gluten Destruction
the Synthetic Biology Way


Making it: work on diesel production
E
F
FUEL
Diesel Production
Gluten Destruction
iGEM Toolkits
Community Outreach

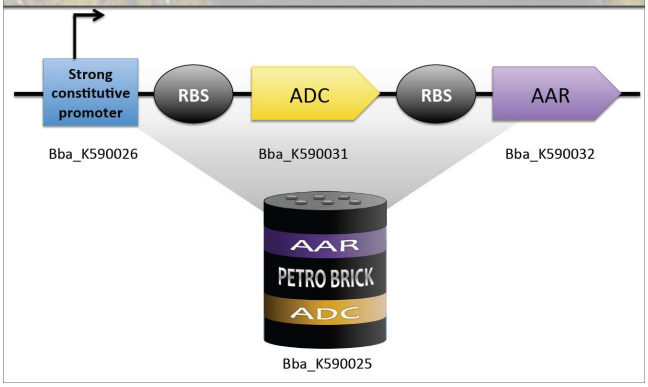

Society is dependent upon limited petroleum reserves
CO2
Flight
Combustion
Marine
Long Distance Trucking
Petroleum Reserves
Alkanes


Integrating CO2 into fuel production
CO2
Combustion
Petroleum Reserves

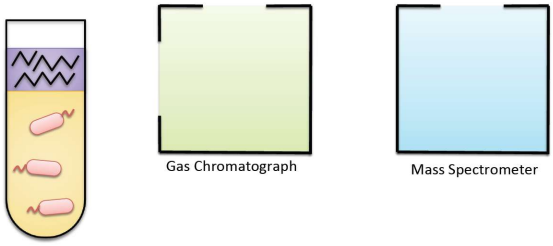
Microbial production of alkanes from fatty acids



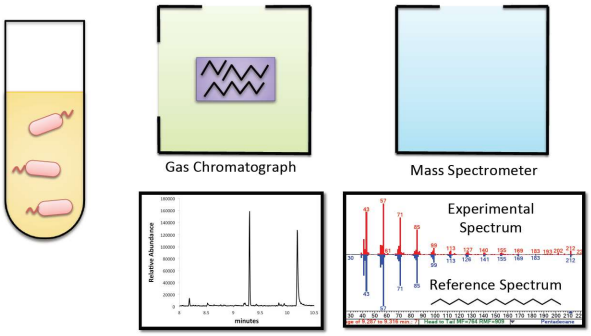
The basic alkane production pathway



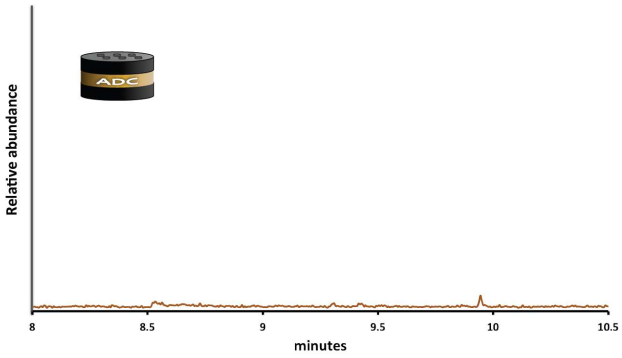
Analyzing alkane production with GCMS



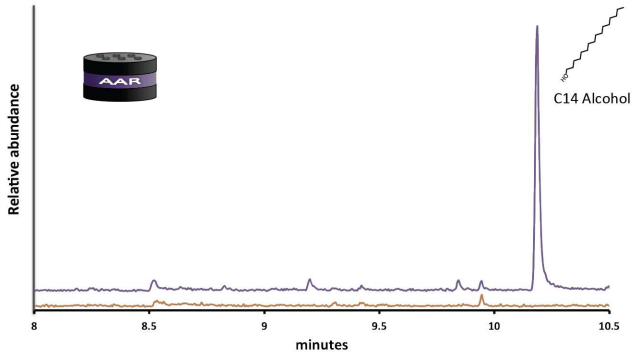
Analyzing alkane production with GCMS



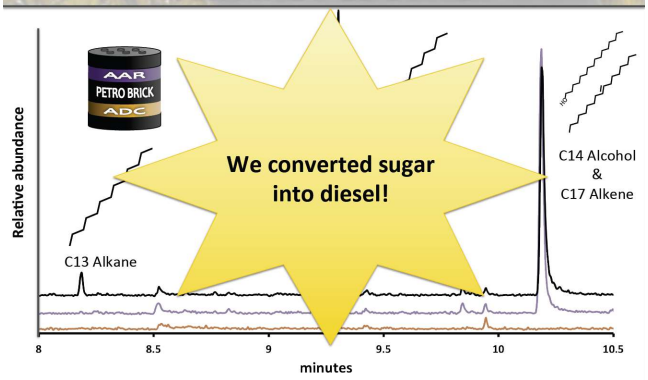
ADC expression alone is not sufficient for hydrocarbon production



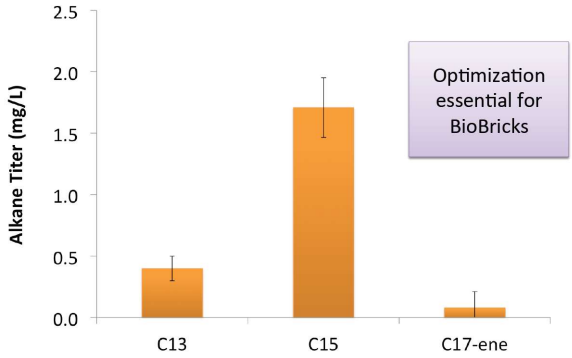
AAR expression results in production of a C14 alcohol



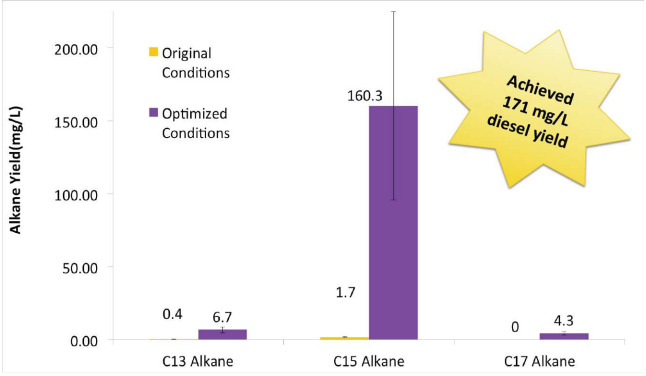
Alkane production from the PetroBrick



Initial alkane yield



Optimized alkane yield



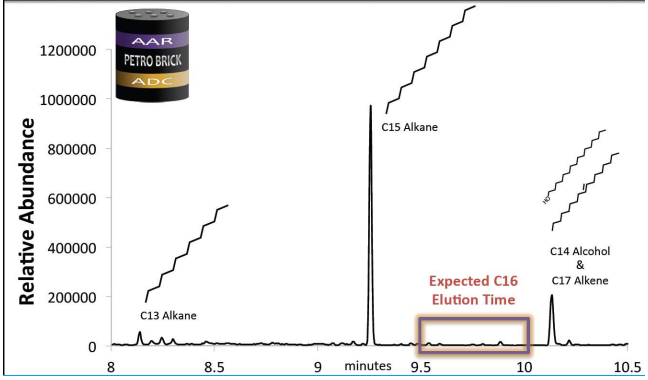
Filling Out the Alkane Profile Even Chain Length Production

Standard Fatty Acid 2 Carbon Starter Unit (*E. coli* FabH)

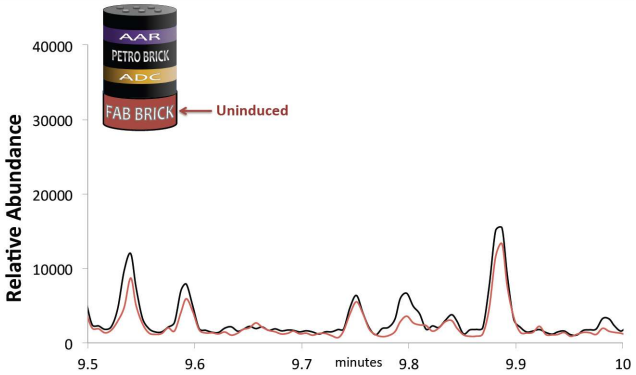
Alternative Fatty Acid 3 Carbon Starter Unit (*B. subtilis* FabH2)

- ✓ Synthesized FabH2 Gene
- ✓ Cloned into a 3K3-Lac Inducible Vector (aka the ***FabBrick***)

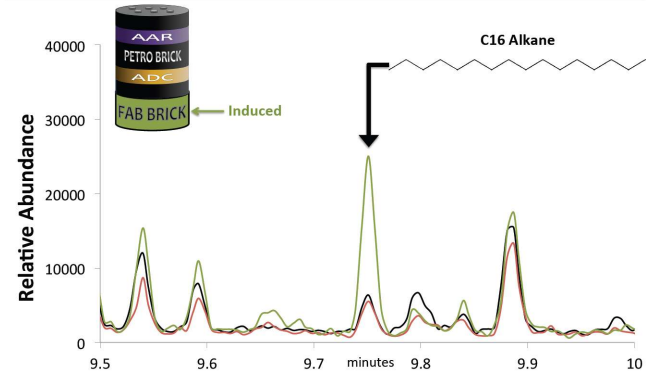
Looking for C16 Alkane Production



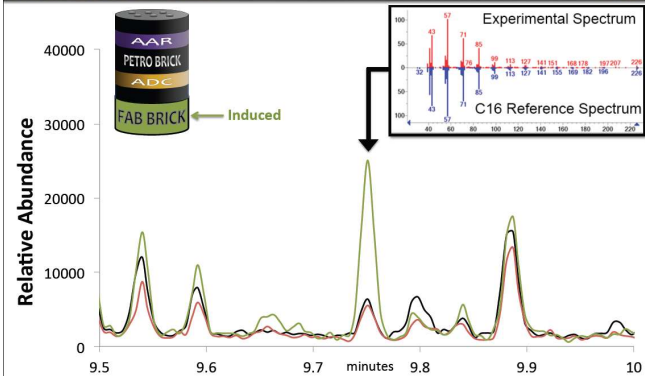
Looking for C16 Alkane Production



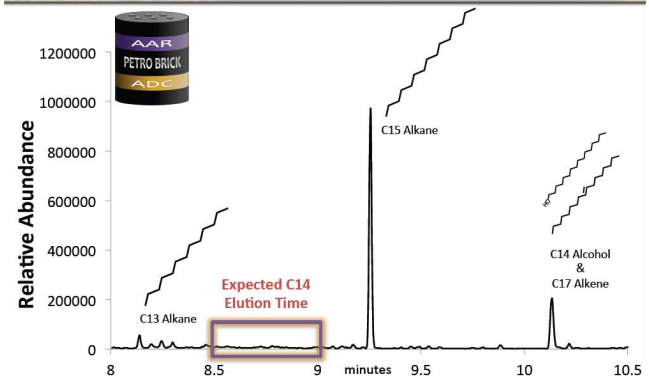
Looking for C16 Alkane Production



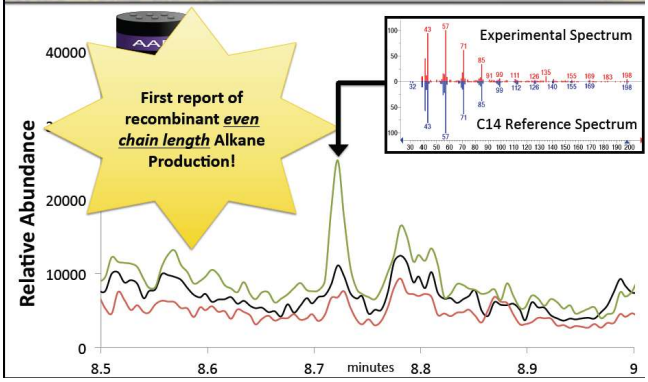
GCMS Confirms C16 Alkane Production



Looking for C14 Alkane Production



The Full n-Alkane Spectra is Complete

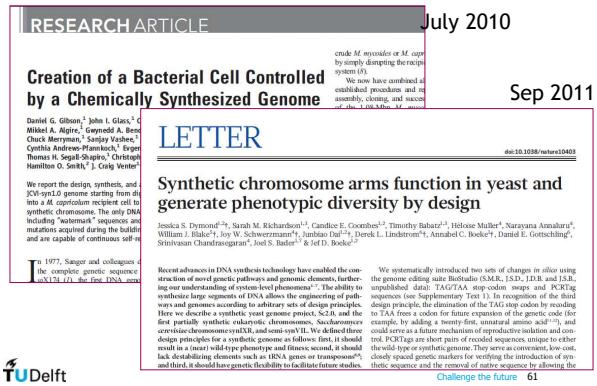


Take home messages

- SB applications enabled by technology, is the new era, both in applications, and conceptual thinking.
- It has a quite wide area of application
- Has its origins in different areas
  - Molecular Biology, Microbiology, Metabolic Engineering
  - Nanotechnology (esp. bottom-up approaches)
  - Information technology
  - Engineering
- SB is not only for biologists!!

Context project: programming life

From Synthetic Genome to a Synthetic Cell


RESEARCH ARTICLE

July 2010

Creation of a Bacterial Cell Controlled by a Chemically Synthesized Genome

Sep 2011

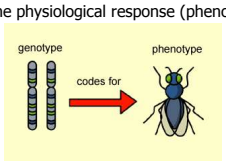
LETTER

doi:10.1038/nature10403

Synthetic chromosome arms function in yeast and generate phenotypic diversity by design

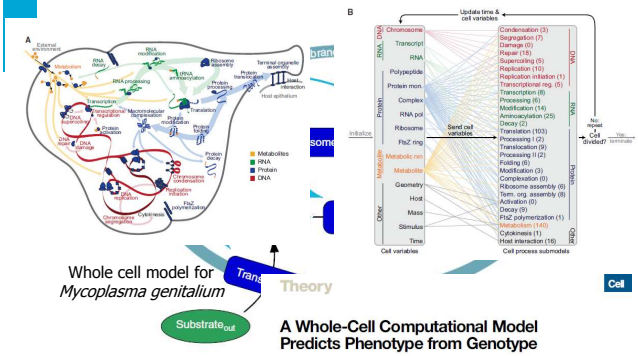
Context project: programming life

- In biotechnology applications typically,
  - We manipulate the DNA (genotype),
  - We observe the physiological response (phenotype)



- Predicting the phenotype from genotype is a great challenge.
  → One way to achieve this: whole cell models
  (simple, yet comprehensive)

Building virtual whole cells (concept)



A Whole-Cell Computational Model Predicts Phenotype from Genotype

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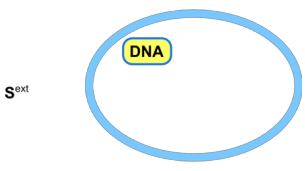
Building whole cell models

- Basic elements in a cell:
  - DNA,
  - Protein, ribosome,
  - Metabolism,
  - Transporters,
  - Cellular infrastructure (e.g. lipids)

Building a whole-cell from scratch

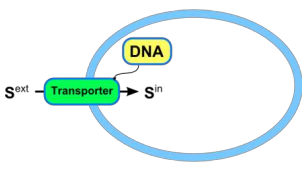


Objective: grow on Substrate available in the environment

Building a whole-cell from scratch

Transporter protein synthesis rate:
$k_{Tr} \cdot DNA$

Alternative transporters, have different capacity, or affinity, defined by k_{Tr} or $K_M^{tr,in}$



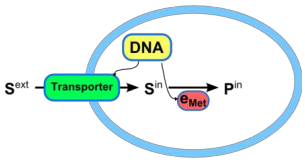
$$v_{transport,in} = v^{max} \cdot transporter \cdot \frac{S^{ext}}{S^{ext} + K_M^{tr,in}}$$

First thing to do: bring the substrate into the cell

Building a whole-cell from scratch

The synthesis rate of enzyme responsible for metabolism:

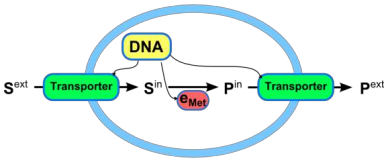
$k_{e_i} \cdot DNA$



$$v^{metabolism} = e^{met} \cdot \frac{S^{in}}{S^{in} + K_M^{met}}$$

Then, produce a valuable product, from the substrate, by metabolizing it

Building a whole-cell from scratch



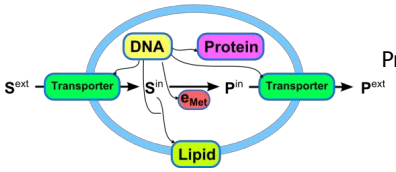
$$v^{transport,out} = transporter \cdot \frac{P^{in}}{P^{in} + K_M^{tr,out}}$$

Then, the product should be transported outside the cell

Building a whole-cell from scratch

Lipid synthesis rate:

$k_L \cdot DNA \cdot P^{in}$



Protein synthesis rate:

$k_p \cdot DNA \cdot P^{in}$

Cell growth rate:

$\mu = S^{in} \cdot Lipid \cdot Protein \cdot Cell$

The cell has also invest in it's *infrastructure,* for cell walls, proteins other than metabolism.

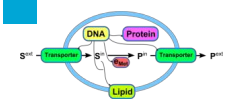
list of mathematical expressions for the whole cell model

$$\frac{dDNA}{dt} = v_{DNAsynth} \cdot \mu \cdot DNA$$
$$\frac{dLipid}{dt} = k_L \cdot DNA \cdot S_{int} - \mu \cdot L$$
$$\frac{dTransporter}{dt} = k_{Tr} \cdot DNA \cdot S_{int} - \mu \cdot Tr$$
$$\frac{de_i}{dt} = k_{e_i} \cdot DNA - \mu \cdot e_i - k_d \cdot e_i$$
$$\frac{dProtein}{dt} = k_p \cdot DNA - \mu \cdot P - k_d^p P$$
$$\frac{dSubs_{int}}{dt} = v_{tr} - v_{e_i} - v_{Lipid}$$
$$\frac{dSubs}{dt} = supply - v_{tr} \cdot Cell$$
$$\frac{dProd}{dt} = v_{e_i} - v_{tr}^{out} - v_{DNAsynt} - v_{ProtSynt}$$
$$\frac{dCell}{dt} = \mu(DNA, Lipid, Protein) \cdot Cell$$

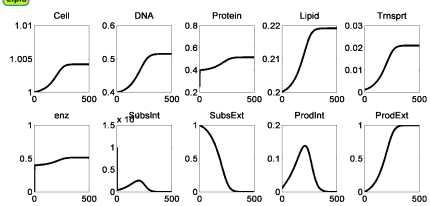
$$v_{DNAsynt} = k_{DNA} \cdot DNA \cdot Prod$$
$$v_{ProtSynt} = k_p \cdot DNA \cdot Prod$$
$$v_{tr}^{in} = k_{transport} \cdot Transporter \cdot \frac{S_{ext}}{S_{ext} + K_M}$$
$$v_{tr}^{out} = k_{transport}^{out} \cdot Transporter \cdot Prod$$

$$v_{e_i} = v^{max} \cdot e_i \cdot \frac{S_{int}}{S_{int} + K_M}$$
$$\mu = S_{int} \cdot Lipid \cdot Protein$$

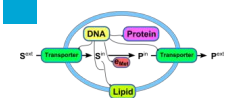
Example simulation of a whole cell, grown on substrate



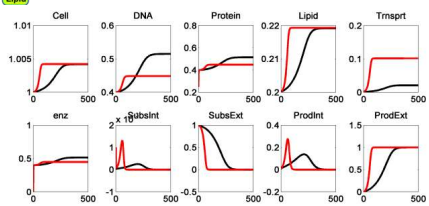
S is converted into P, cells grow



Example simulation of a whole cell, grown on substrate



What happens,
- if the transporter is under a stronger promoter?
- we synthetize DNA, expressing a better transporter?



What do I want?

- Whole-cell simulator:
  A software platform where we can simulate a phenotype response to changes into genotype.

- The software should be
  - Able to simulate the physiology over time, optimize for a selected output.
  - Modular, to test a variety of cellular components.
  - Scalable, as our knowledge increases more modules would be incorporated.