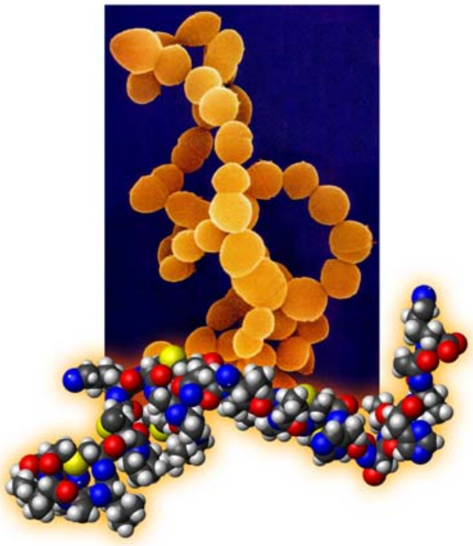

Synthetic Biology in Food & Health

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Chair for Systems & Synthetic Biology

Wageningen University

HELMHOLTZ ZENTRUM FÜR INFEKTIONSFORSCHUNG

Disclaimer: Synthetic Biology in the Food field is not about square tomatoes…

….nor about magic food or ingredients!

Its is about helping us to more effectively promote health and nutrition

Joseph Tart/EHP, Shutterstock

How?

Up to a large extent, by bringing the ethos, methodologies and expertise within the various disciplines in Synthetic Biology and the engineering paradigm (forward engineering, abstraction levels, standardisation, modeling and design) to biology, much as in applications to:

White Biotechnology & biopharmaceuticals (Panke), Environment (de Lorenzo), Energy (Cherry/Willems) and Health (Weber), NanoMaterials (Dawson)

Opportunities and applications of Synthetic Biology in the Food field

1. Metabolites, health products and processing aids

2. Probiotics, microbial communities

3. Plants, plant-derived products and feedstocks

4. Downstream processing of (food-)waste

1. Metabolites, health compounds, processing aids

- Nutraceuticals
- Food ingredients (including fermentation products)
- Metabolites, enzymes
- Food preservatives
- Flavors and fragrances
- Biosensors (eg. artificial nose)
- Etc.

Nutraceuticals

•vitamins & supplements

•resveratrol (antioxidant from red grape products)

•soluble dietary fiber products (e g. psyllium seed husk for reducing hypercholesterolemia)

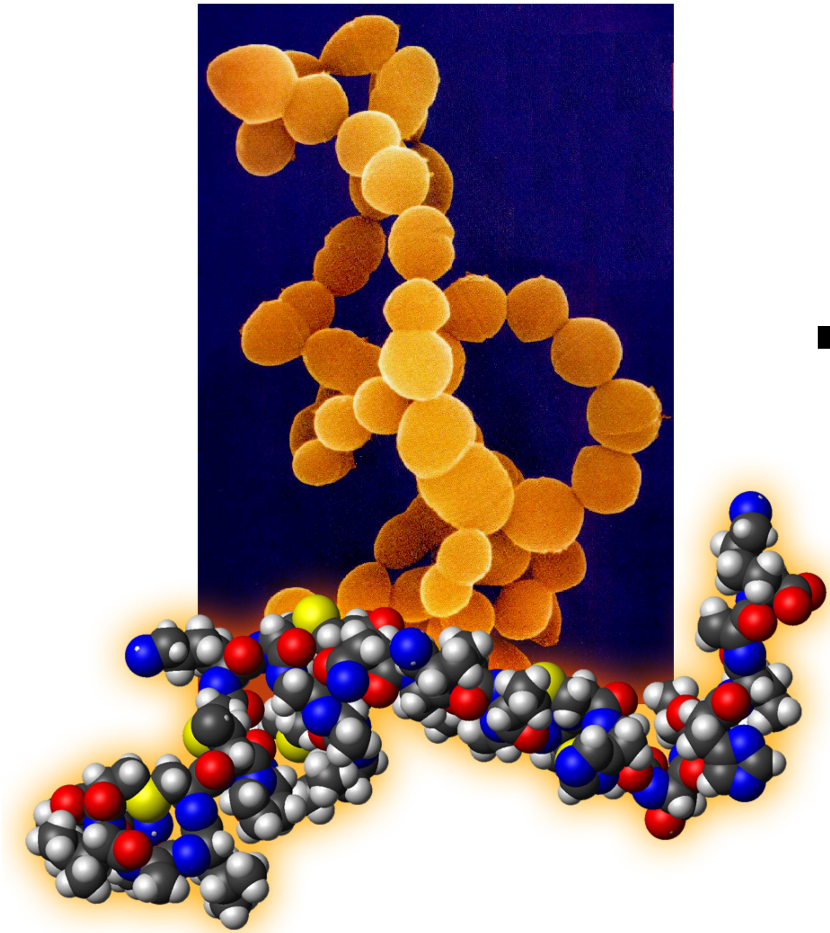
•glyconutrients (specific carbohydrates and sugars)

• sulforaphane (in broccoli, as a cancer preventative)

•flavonoids (alpha-linolenic acid from Chia seeds, beta-carotene from marigold petals, anthocyanins from berries)

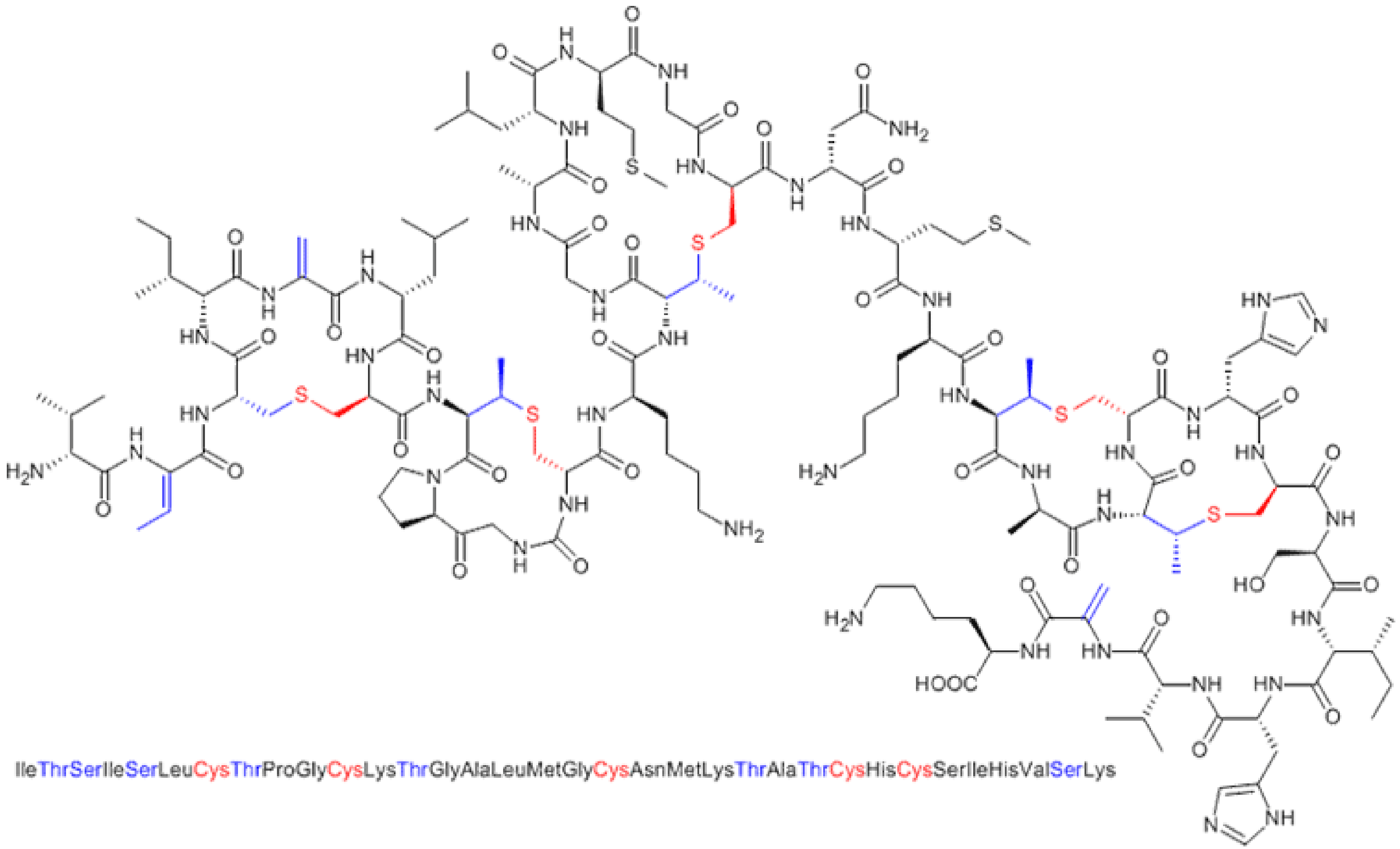
•isoflavonoids (from clover or soy, related to arterial health)

•Example food preservative: Nisin



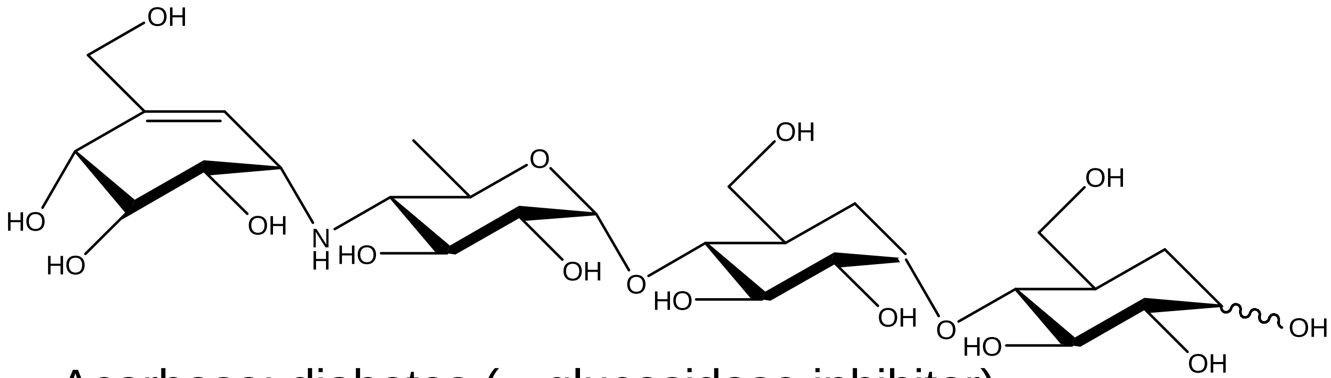
natural antimicrobial agent (peptide) with activity against a wide variety of undesirable food borne (pathogenic) bacteria

•Example food preservative: Nisin

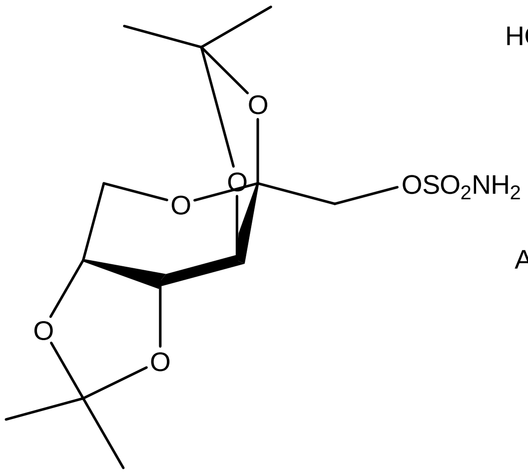


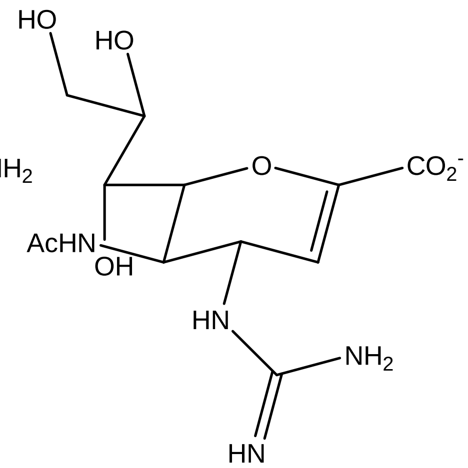
Family of lantibiotics with Complex structure, low volumetric productivity in natural fermentations

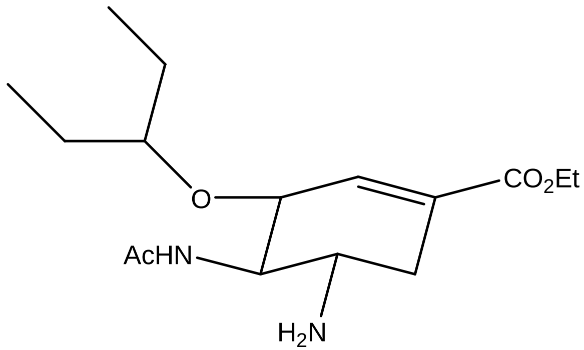
Novel types of therapeutic molecules



Acarbose; diabetes (α-glucosidase inhibitor)







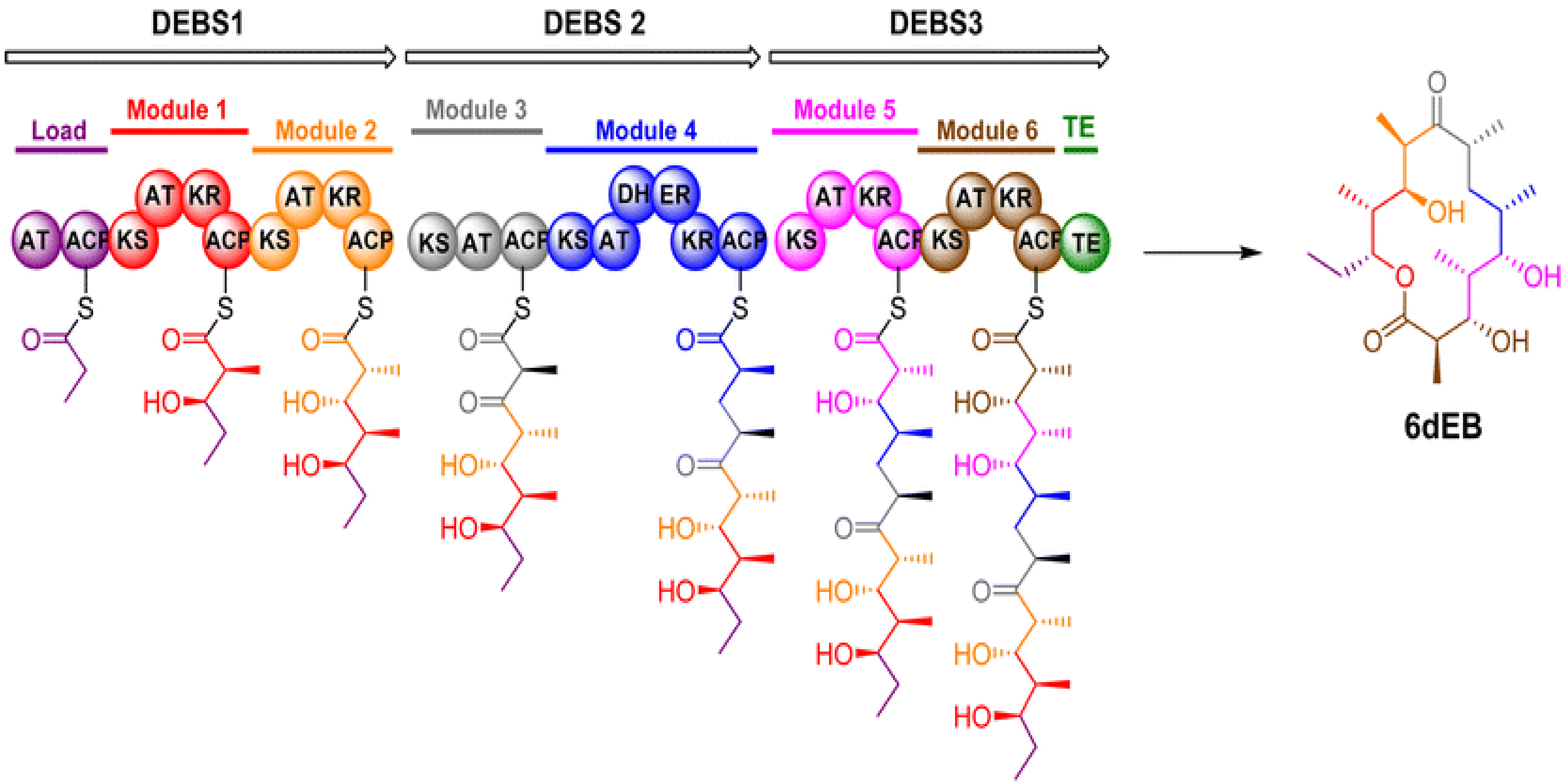
Topiramate
Anticonvulsant

Zanamivir
Neuraminidase inhibitor

Oseltamivir
Neuraminidase inhibitor

S. Panke ETH

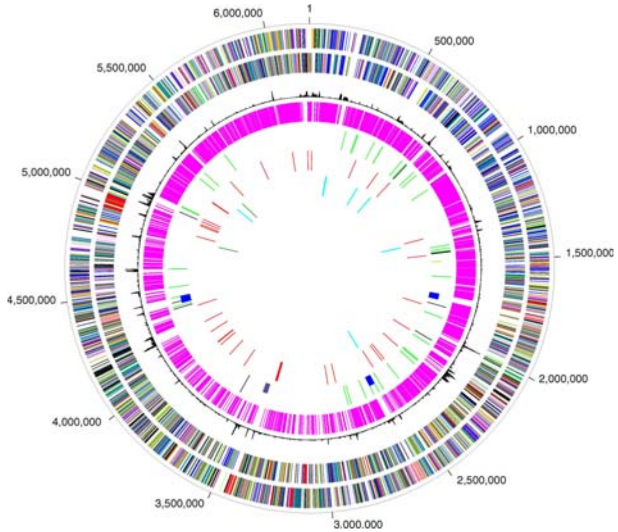
Novel antibiotics and cytostatics from re-engineering polyketides

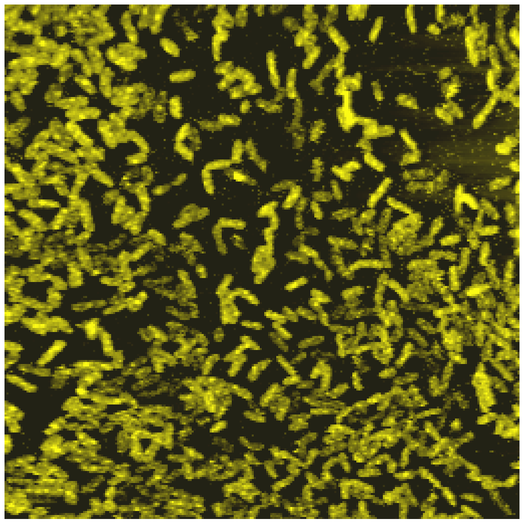


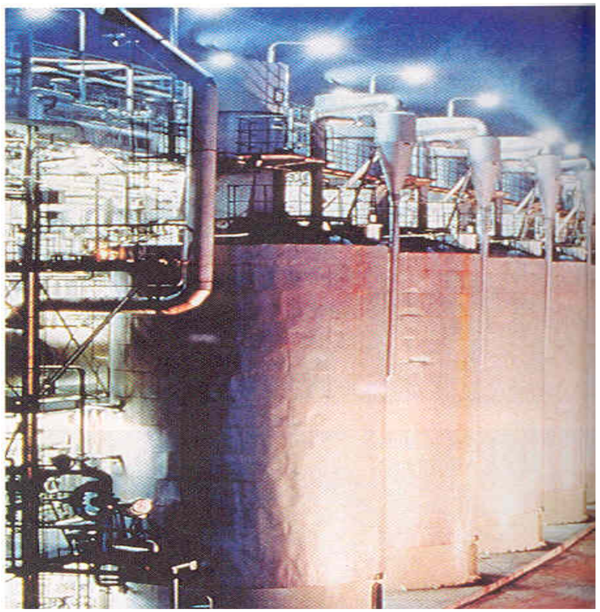
This modularity can be exploited for easy recombination of modules leading to novel antibiotics:

Menzella *et al.*, Nature Biotechnology 23:1171

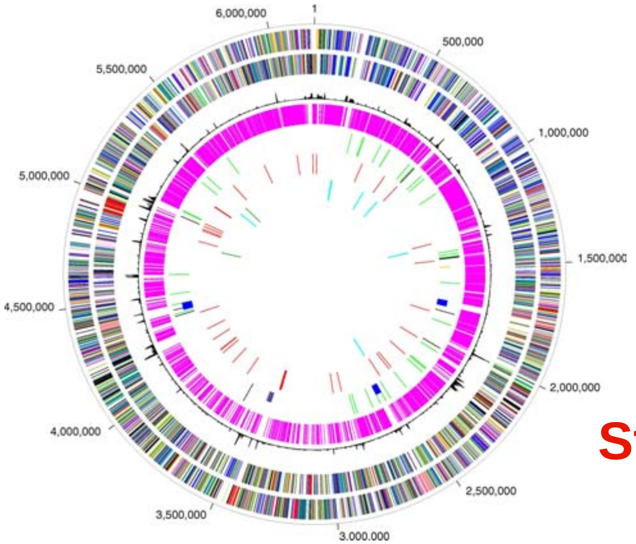
Ideally, a microbial factory from scratch



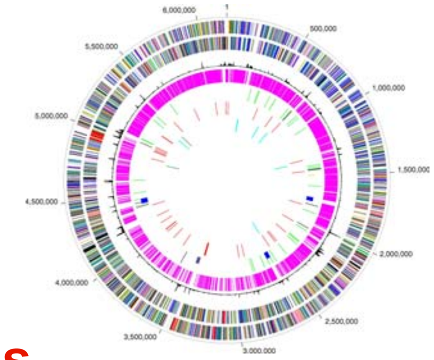




Top-down: simplifying & using existing systems

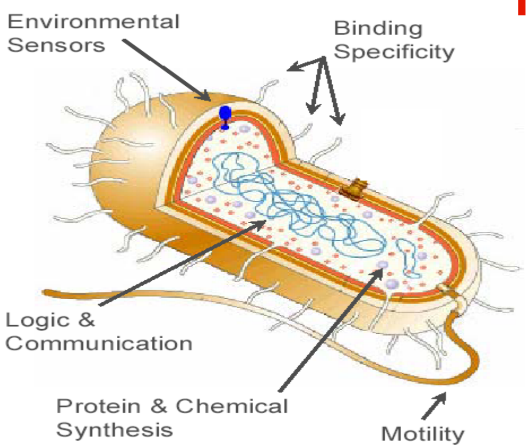


Streamline

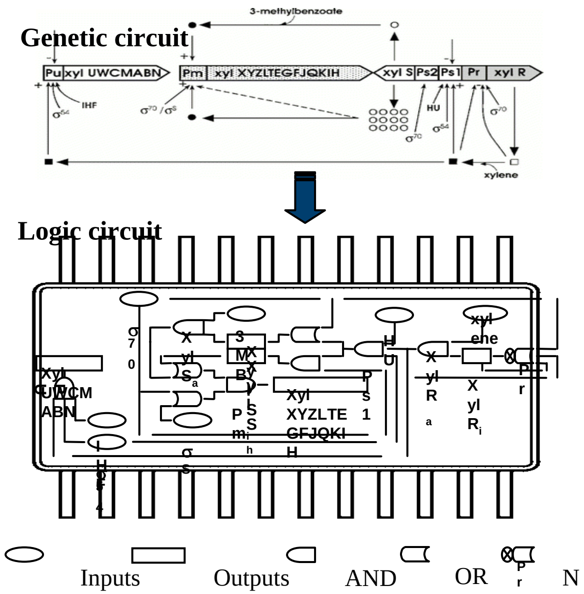


Streamlined genome as a chassis

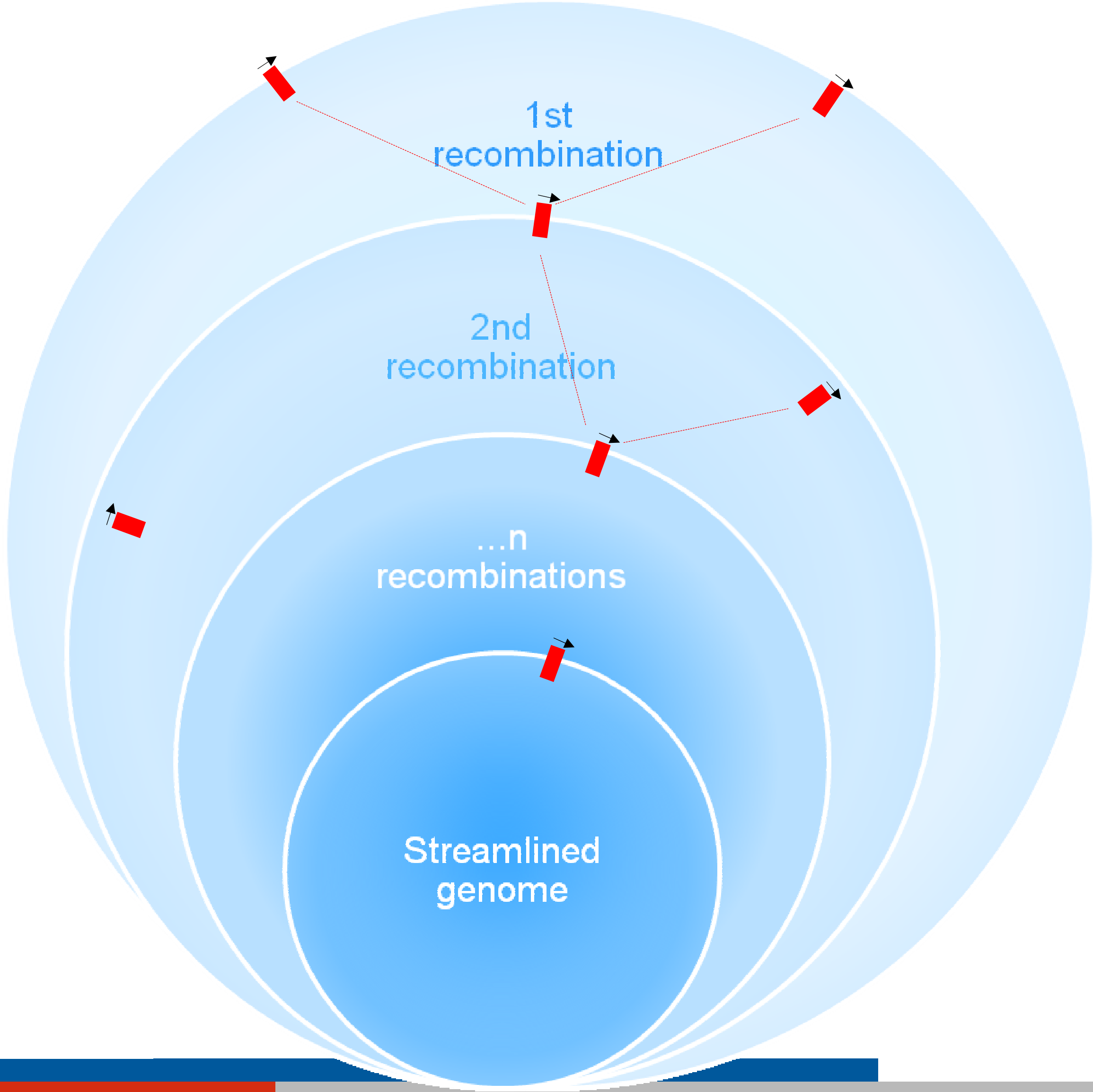
Reduced complexity – improved orthogonality



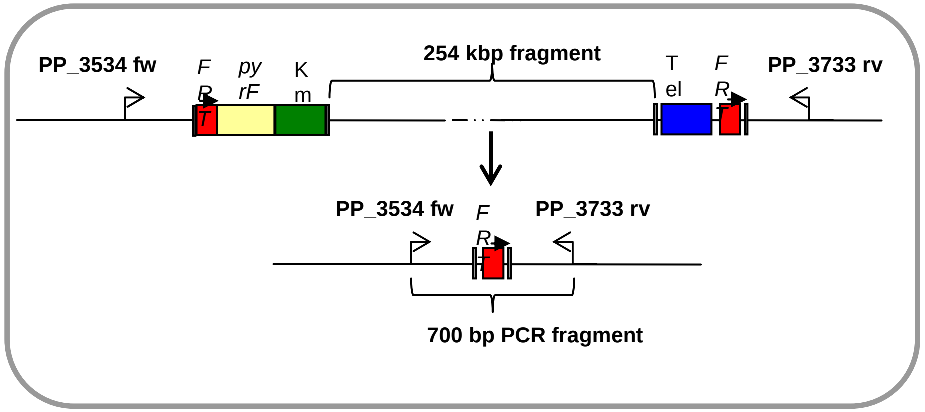
Reprogramme



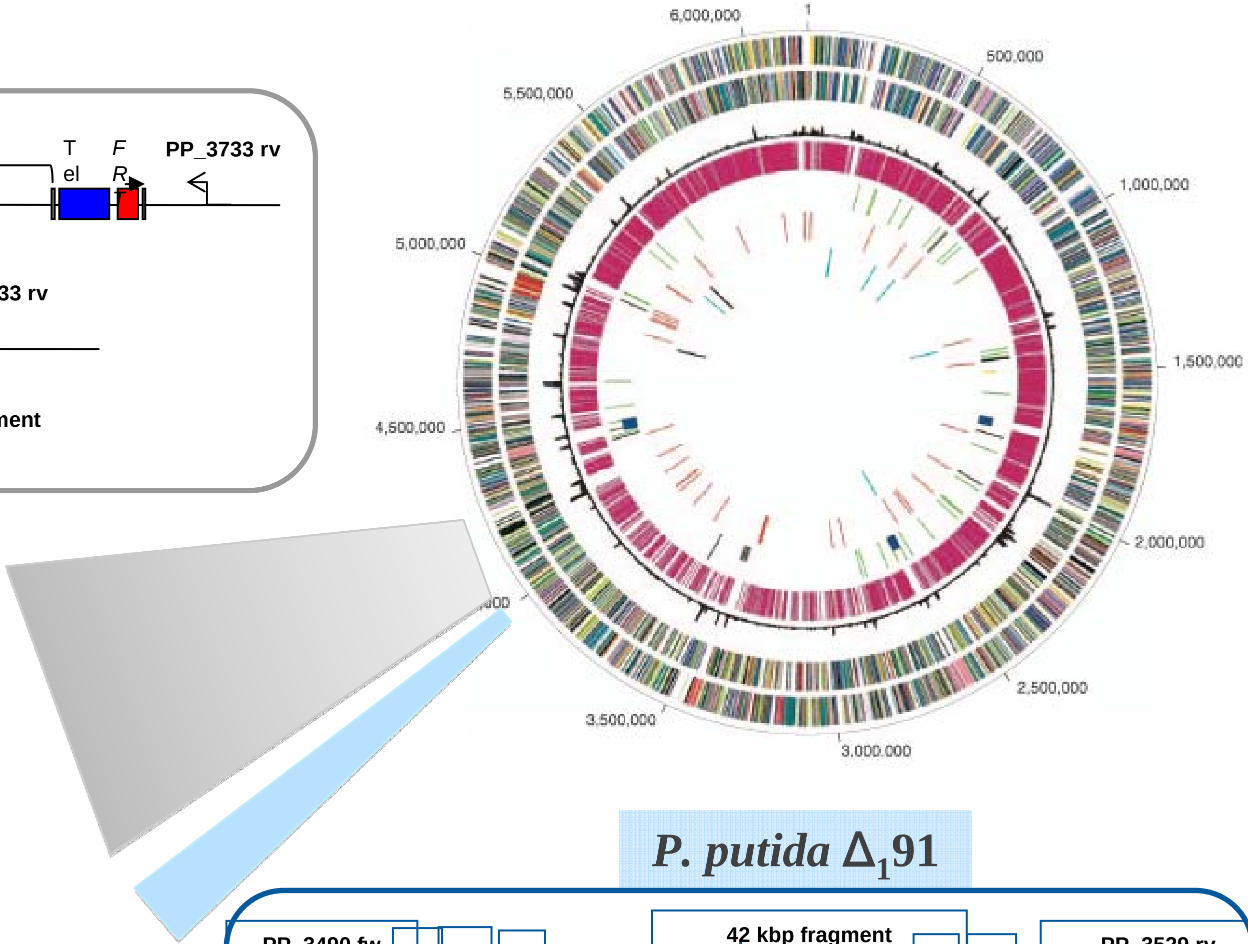
Random transposon mutagenesis: Each site-specific recombination between two FRT recognition sites leaves a single FRT behind in the genome. After several recombination several FRT sites are positionned randomly in the genome and offer a higher probability for successful recombinations and deletions



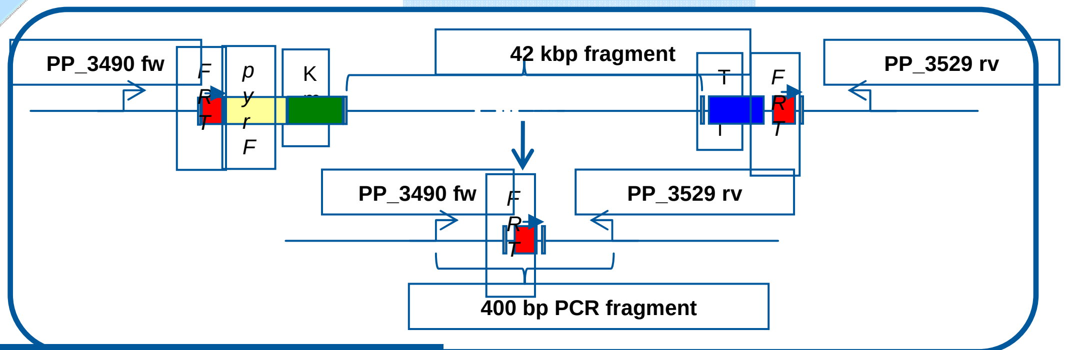
Reduction of the genome of *Pseudomonas putida*



P. putida $\Delta_1 407$



P. putida $\Delta_1 91$



What do we get from the genome sequence?

Central Metabolism (EMP, PPP, TCA cycle, Electron transport)

aceA, aceB, aceE, aceF, ackA, acnA, acnB, acs, adhE, agp, appB, appC, atpA, atpB, atpC, atpD, atpE, atpF, atpG, atpH, atpI, cydA, cydB, cydC, cydD, cyoA, cyoB,
cyoC, cyoD, dld, eda, edd, eno, fba, fbp, fdhF, fdnG, fdnH, fdnI, fdoG, fdoH, fdoI, frdA, frdB, frdC, frdD, fumA, fumB, fumC, galM, gapA, gapC_1, gapC_2, glcB, glgA
glgC, glgP, glk, glpA, glpB, glpC, glpD, gltA, gnd, gpmA, gpmB, hyaA, hyaB, hyaC, hybA, hybC, hycB, hycE, hycF, hycG, icdA, lctD, ldhA, lpdA, malP, mdh, ndh,
nuoA, nuoB, nuoE, nuoF, nuoG, nuoH, nuoI, nuoJ, nuoK, nuoL, nuoM, nuoN, pckA, pfkA, pfkB, pflA, pflB, pflC, pflD, pgi, pgk, pntA, pntB, poxB, ppc, ppsA, pta, pur
pykA, pykF, rpe, rpiA, rpiB, sdhA, sdhB, sdhC, sdhD, sfcA, sucA, sucB, sucC, sucD, talB, tktA, tktB, tpiA, trxB, zwf, pgl(Fraenkel, 1996*), mae*B(Fraenkel, 1996)

Alternative Carbon Source ***adhC, adhE, agaY, agaZ, aldA, aldB, aldH, araA, araB, araD, bglX, cpsG, deoB, deoC, fruK, fucA, fucI, fucK, fucO, galE, galK, galT, galU, gatD, gatY, glk, glpK,gntK, gntV, gpsA, lacZ, manA, melA, mtlD, nagA, nagB, nanA, pfkB, pgi, pgm, rbsK, rhaA, rhaB, rhaD, srlD, treC, xylA, xylB***

Amino Acid Metabolism ***adi, aldH, alr, ansA, ansB, argA, argB, argC, argD, argE, argF, argG, argH, argI, aroA, aroB, aroC, aroD, aroE, aroF, aroG, aroH, aroK, aroL, asd, asnA, asnB,aspA, aspC, avtA, cadA, carA, carB, cysC, cysD, cysE, cysH, cysI, cysJ, cysK, cysM, cysN, dadA, dadX, dapA, dapB, dapD, dapE, dapF, dsdA, gabD, gabT, gadA,gadB, gdhA, glk, glnA, gltB, gltD, glyA, goaG, hisA, hisB, hisC, hisD, hisF, hisG, hisH, hisI, ilvA, ilvB, ilvC, ilvD, ilvE, ilvG_1, ilvG_2, ilvH, ilvI, ilvM, ilvN, kbl, ldc***
leuA, leuB, leuC, leuD, lysA, lysC, metA, metB, metC, metE, metH, metK, metL, pheA, proA, proB, proC, prsA, putA, sdaA, sdaB, serA, serB, serC, speA, speB, speC,
***speD, speE, speF, tdcB, tdh, thrA, thrB, thrC, tnaA, trpA, trpB, trpC, trpD, trpE, tynA, tyrA, tyrB, ygjG, ygjH, ala*B**(Reitzer, 1996*), dap*C(Greene, 1996*), pa*t(McFall an
Newman, 1996*), pr*r(McFall and Newman, 1996*), sa*d(Berlyn et al., 1996*), Methylthioadenosine nucleosida*se(Glansdorff, 1996*), 5-Methylthioribose kina*se(Glansdorf 1996*), 5-Methylthioribose-1-phosphate isomera*se(Glansdorff, 1996*), Adenosyl homocysteina*se(Matthews, 1996*), L-Cysteine desulfhydra*se(McFall and Newman, 1996*), Glutaminase* A(McFall and Newman, 1996*), Glutaminase* B(McFall and Newman, 1996)

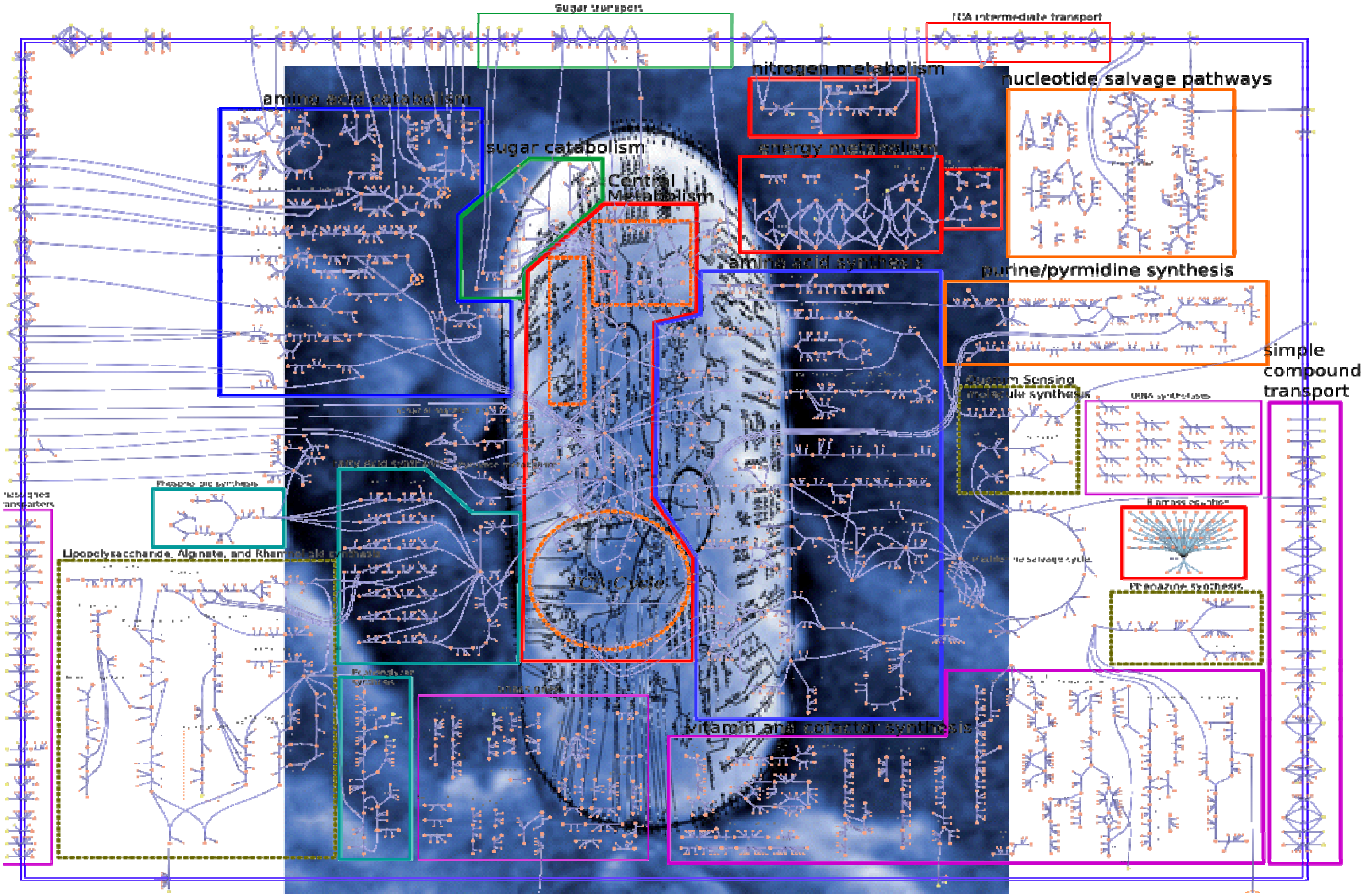
Purine & Pyrimidine Metabolism ***add, adk, amn, apt, cdd, cmk, codA, dcd, deoA, deoD, dgt, dut, gmk, gpt, gsk, guaA, guaB, guaC, hpt, mutT, ndk, nrdA, nrdB, nrdD, nrdE, nrdF, purA, purB, purC,purD, purE, purF, purH, purK, purL, purM, purN, purT, pyrB, pyrC, pyrD, pyrE, pyrF, pyrG, pyrH, pyrI, tdk, thyA, tmk, udk, udp, upp, ushA, xapA,*** *yicP, CMPglycosyla*se(Neuhard and Kelln, 1996)

Vitamin & Cofactor Metabolism ***acpS, bioA, bioB, bioD, bioF, coaA, cyoE, cysG, entA, entB, entC, entD, entE, entF, epd, folA, folC, folD, folE, folK, folP, gcvH, gcvP, gcvT, gltX, glyA, gor, gshA,gshB, hemA, hemB, hemC, hemD, hemE, hemF, hemH, hemK, hemL, hemM, hemX, hemY, ilvC, lig, lpdA, menA, menB, menC, menD, menE, menF, menG, metF, mutnadA, nadB, nadC, nadE, ntpA, pabA, pabB, pabC, panB, panC, panD, pdxA, pdxB, pdxH, pdxJ, pdxK, pncB, purU, ribA,***
ribB, ribD, ribE, ribH, serC, thiC, thiE, ththiG, thiH, thrC, ubiA, ubiB, ubiC, ubiG, ubiH, ubiX, yaaC, ygiG, nadD(Penfound and Foster, 1996*), nad*F(Penfound and Foster, 1996*), nad*G(Penfound and Foster,1996*), pan*E(Jackowski, 1996*), pnc*A(Penfound and Foster, 1996*), pnc*C(Penfound and Foster, 1996*), thi*B(White and Spenser, 1996*), thi*D(White and Spenser, 1996) *thi*K(White and Spenser, 1996*), thi*L(White and Spenser, 1996*), thi*M(White and Spenser, 1996*), thi*N(White and Spenser, 1996*),*
*ubi*E(Meganathan, 1996*),*
*ubi*F(Meganathan, 1996*), Arabinose-5-phosphate isomera*se(Karp et al., 1998*), Phosphopantothenate-cysteine liga*se(Jackowski, 1996*), Phosphopantothenate-cystein decarboxyla*se(Jackowski, 1996*), Phospho-pantetheine adenylyltransfera*se(Jackowski, 1996*), DephosphoCoA kina*se(Jackowski, 1996*), NMN glycohydrola*se(Penfound and Foster, 1996)

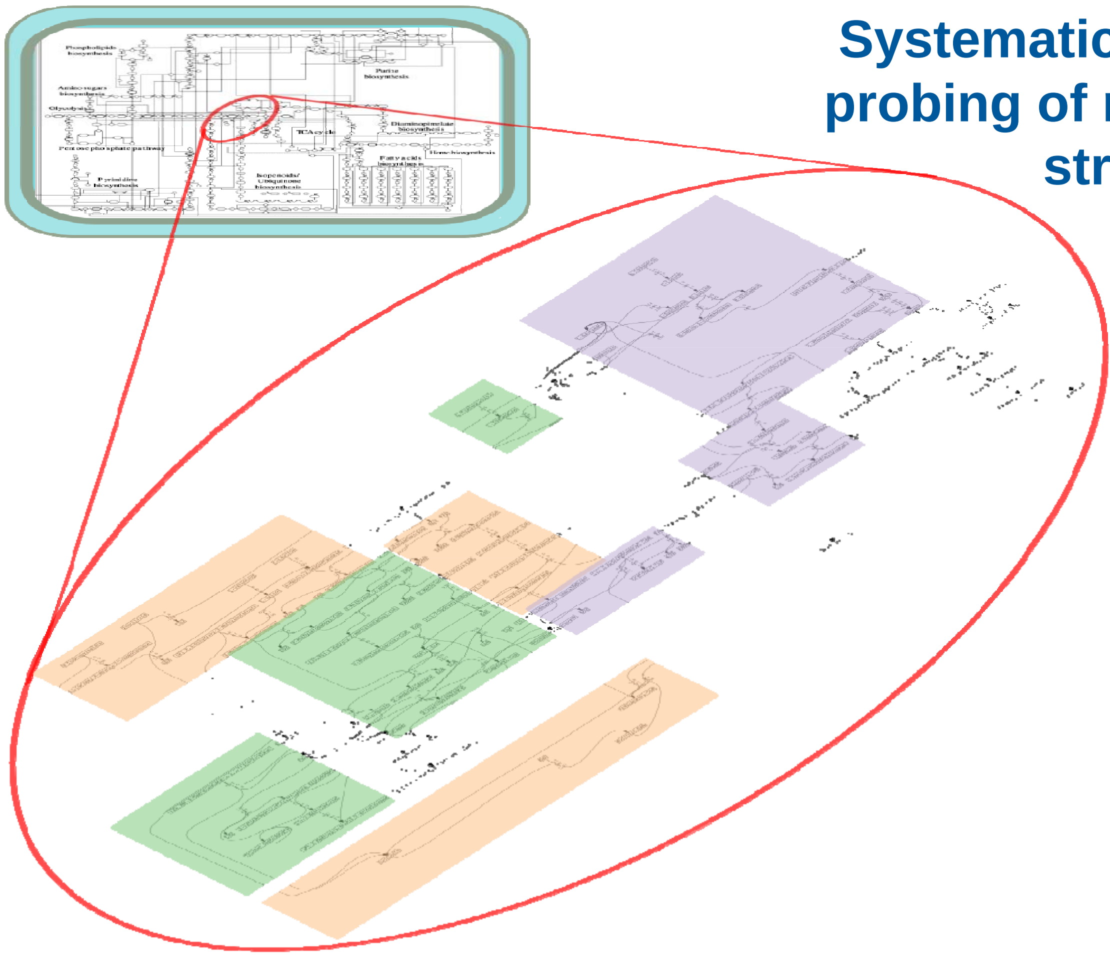
Lipid Metabolism ***accA, accB, accD, atoB, cdh, cdsA, cls, dgkA, fabD, fabH, fadB, gpsA, ispA, ispB, pgpB, pgsA, psd, pssA, pgp*A**(Funk et al., 1992)

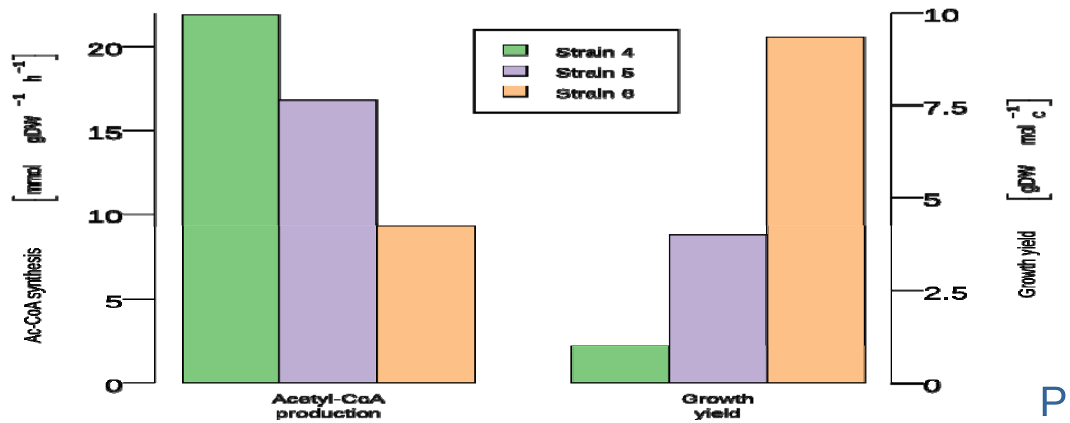
Cell Wall Metabolism ***ddlA, ddlB, galF, galU, glmS, glmU, htrB, kdsA, kdsB, kdtA, lpxA, lpxB, lpxC, lpxD, mraY, msbB, murA, murB, murC, murD, murE, murF, murG, murI, rfaC, rfaD,rfaF, rfaG, rfaI, rfaJ, rfaL, ushA, glm*M**(Mengin-Lecreulx and van Heijenoort, 1996*), lpc*A(Raetz, 1996*), rfa*E(Raetz, 1996*), Tetraacyldisaccharide*

P. putida (815 genes, 877 reactions, Puchalka et al., PLoS Comput. Biol, 2008))



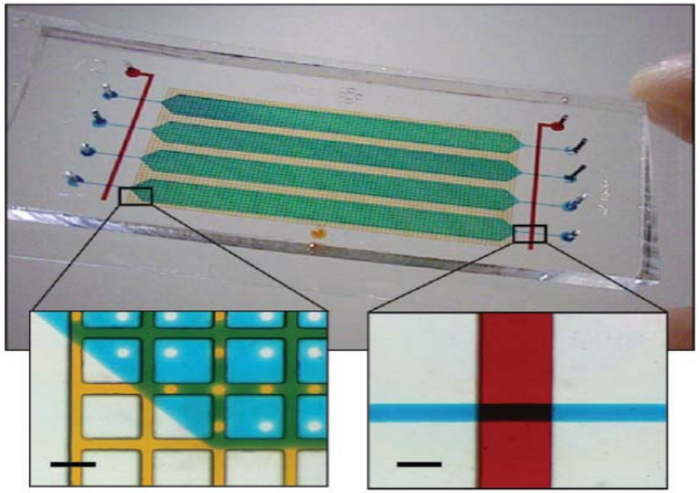
Systematic computational probing of re-programming strategies



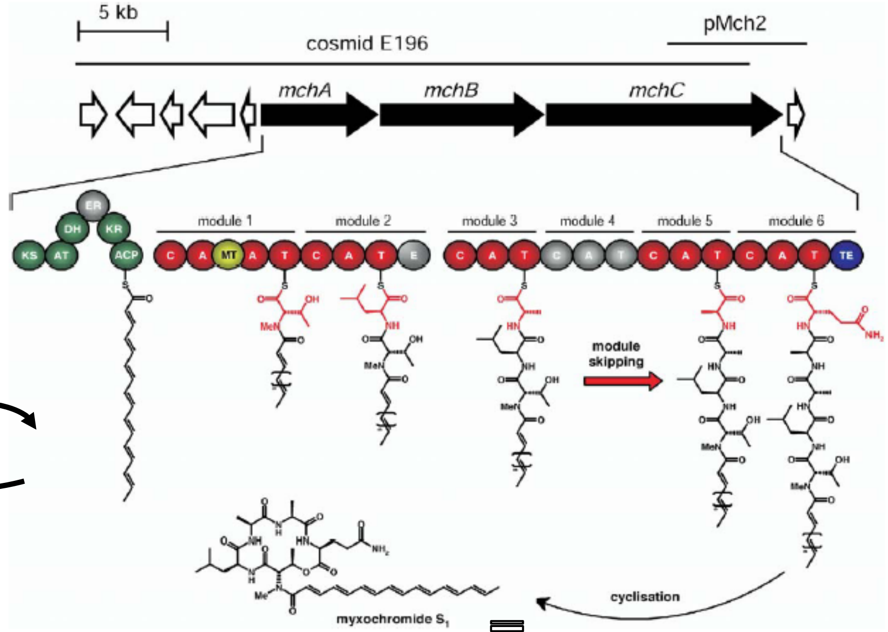


PLoS Comp. Biol, 2008

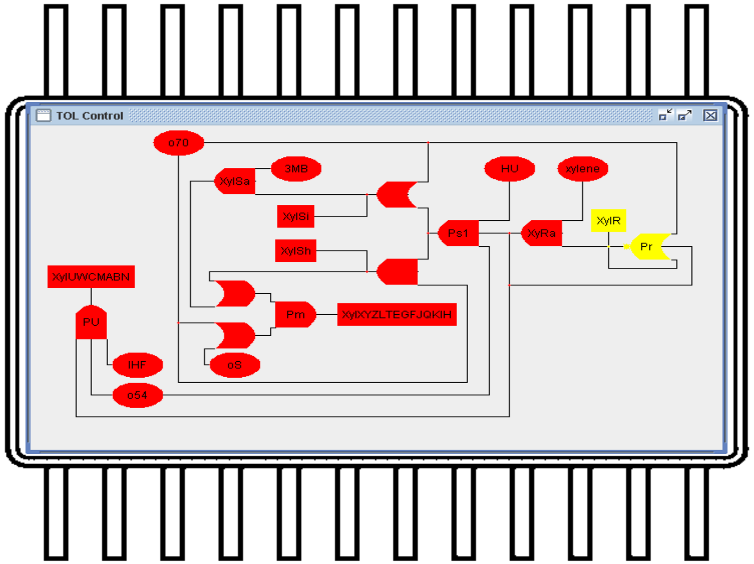
(Streamlined) Bacterial Chassis



PKS Genetic & Biochemical circuit



Logic Circuit (PKS-on-a-chip)



INPUTS OUTPUTS AND OR NOR

Biosensors and artificial noses for the detection and production fragrances and flavors

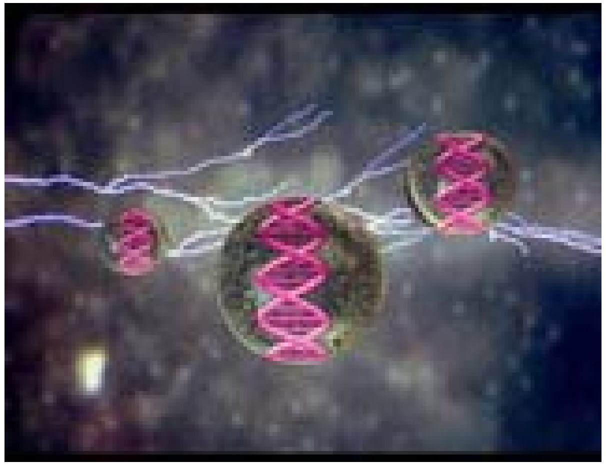
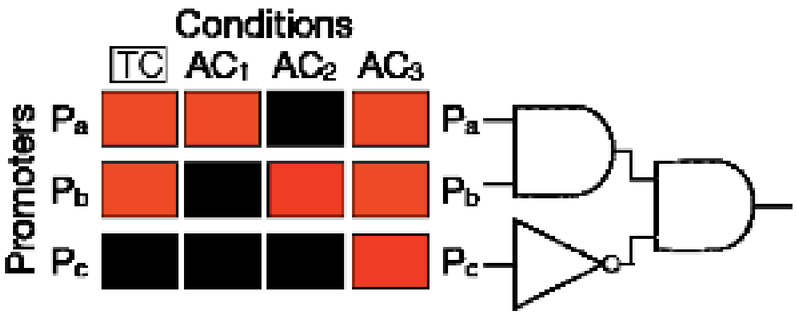


Image Todd Rider,MIT

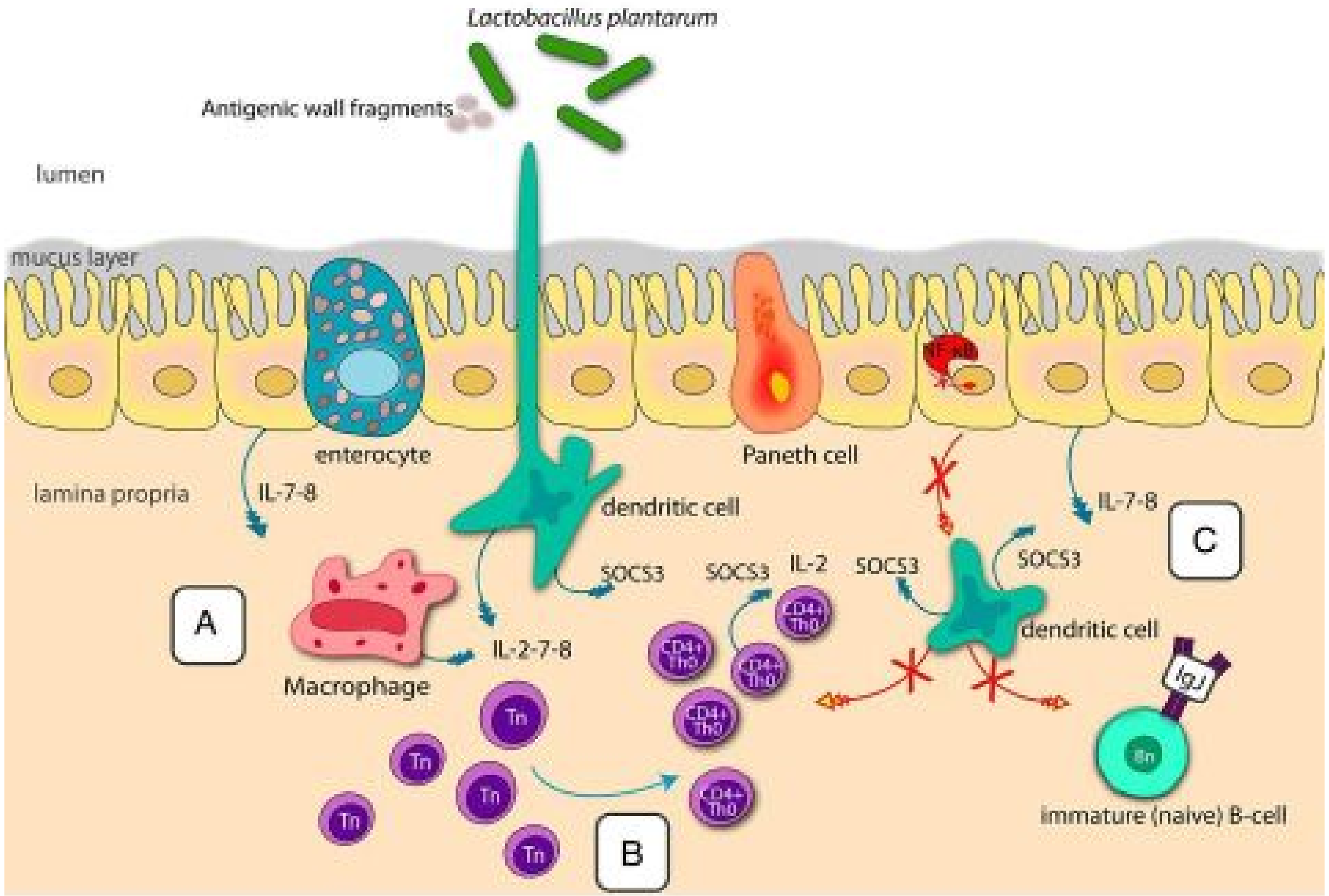


2. Probiotics and nutrigenomics

A growing number of health problems, from inflammatory bowel disease to obesity and even autism have been linked to disruptions in human-associated microbiota or alterations of the intimate cross-talk between these microbes and human cells.

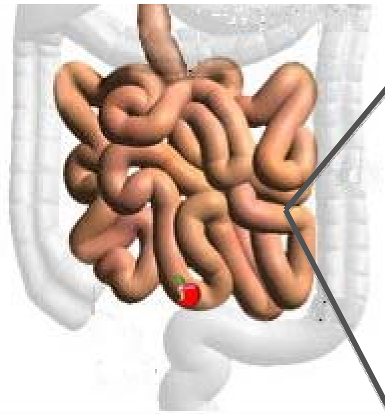
Probiotics are dietary supplements of live microorganisms thought to be healthy for the host organism.

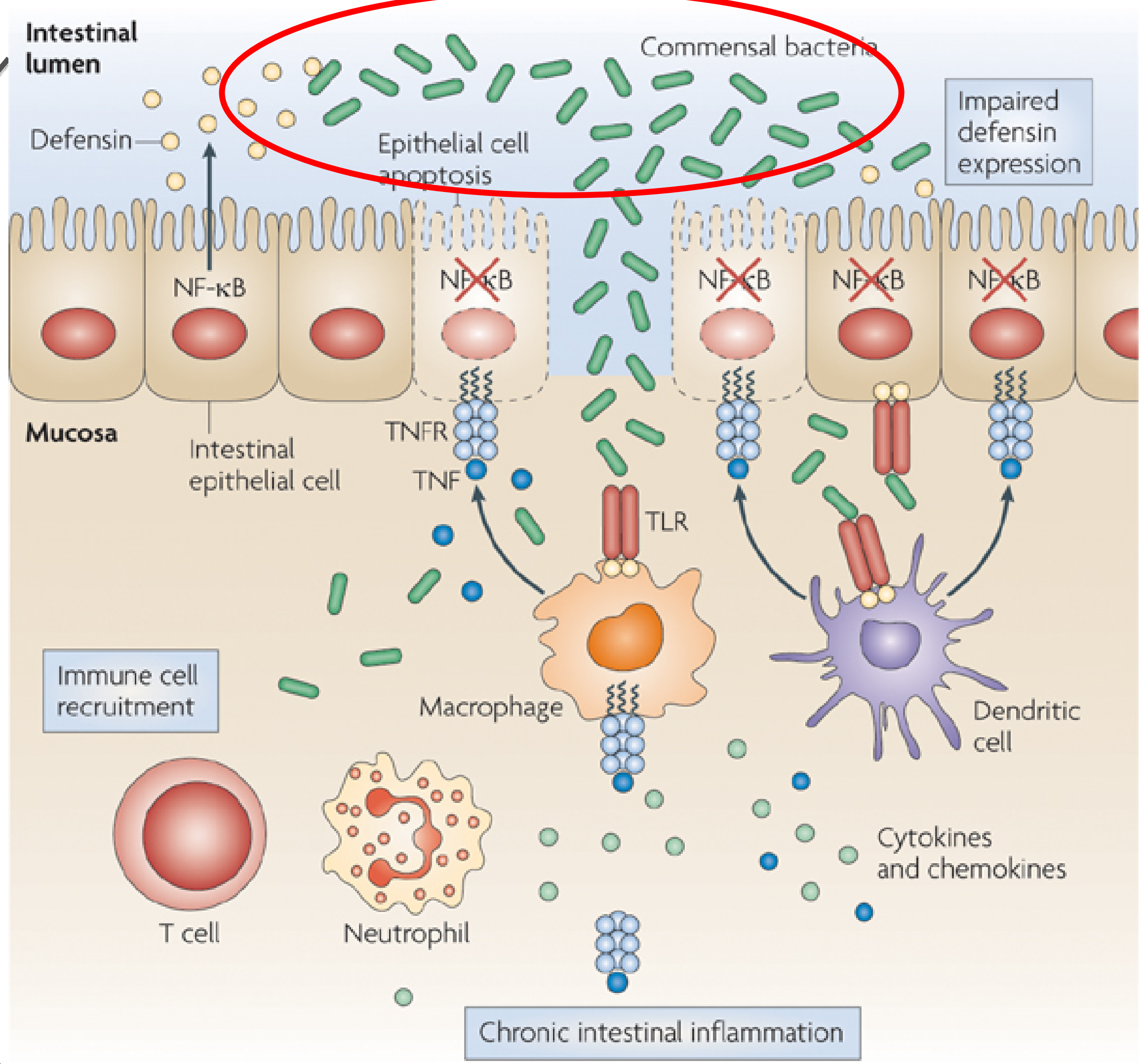
Eg. of probiotics-induced modulation of microbial-mammalian interactions



Differential NF-κB pathway induction (immune response) by *L. plantarum* in the duodenum of healthy humans correlating with immune tolerance, Van Baarlen et al, PNAS, 2009

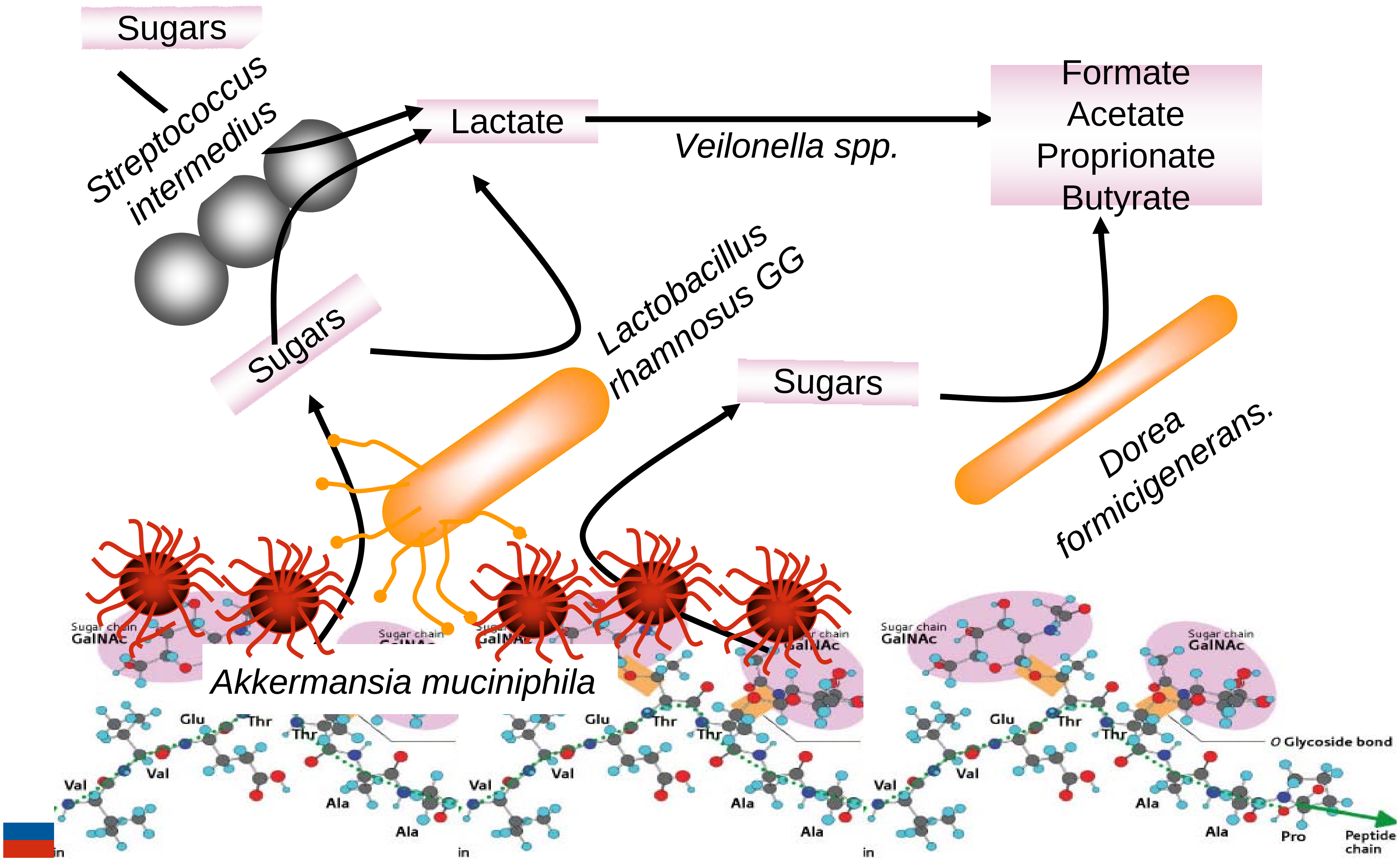
The small intestine is the primary organ in response to nutrients & food components





Nature Reviews | Immunology

A forward engineering approach combining systems & synthetic biology to understand and re-programming of gut flora



Some further claims on the effects Probiotics on health

Managing lactose intolerance

Prevention of colon cancer

Lowering cholesterol

Lowering blood pressure

Improving immune function and preventing infections

Helicobacter pylori

Antibiotic-associated diarrhea

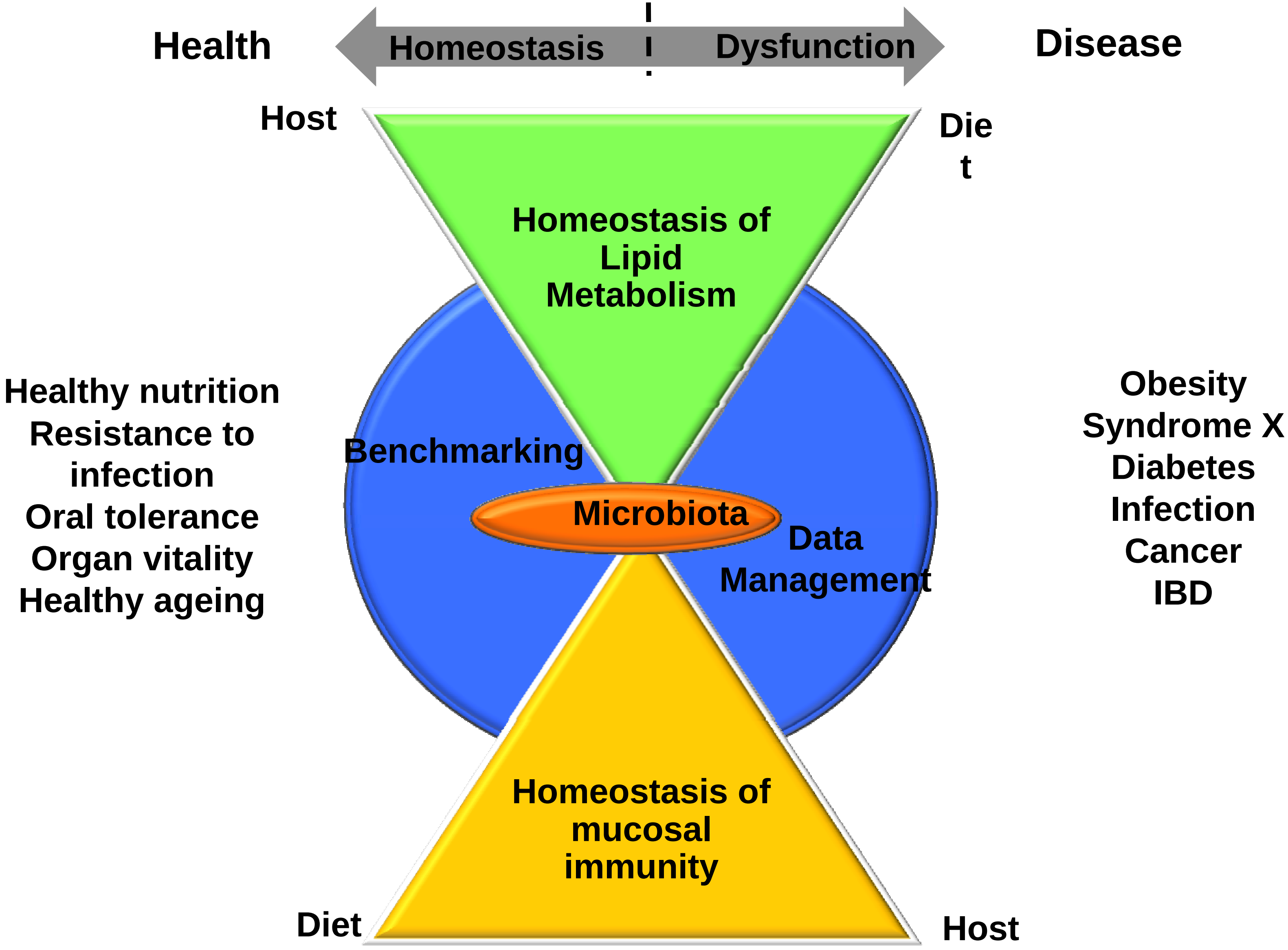
Reducing inflammation

Improving mineral absorption

Prevents harmful bacterial growth under stress

Irritable bowel syndrome and colitis

The Gatekeeper project at the Wageningen for Centre Biology on Food and Health



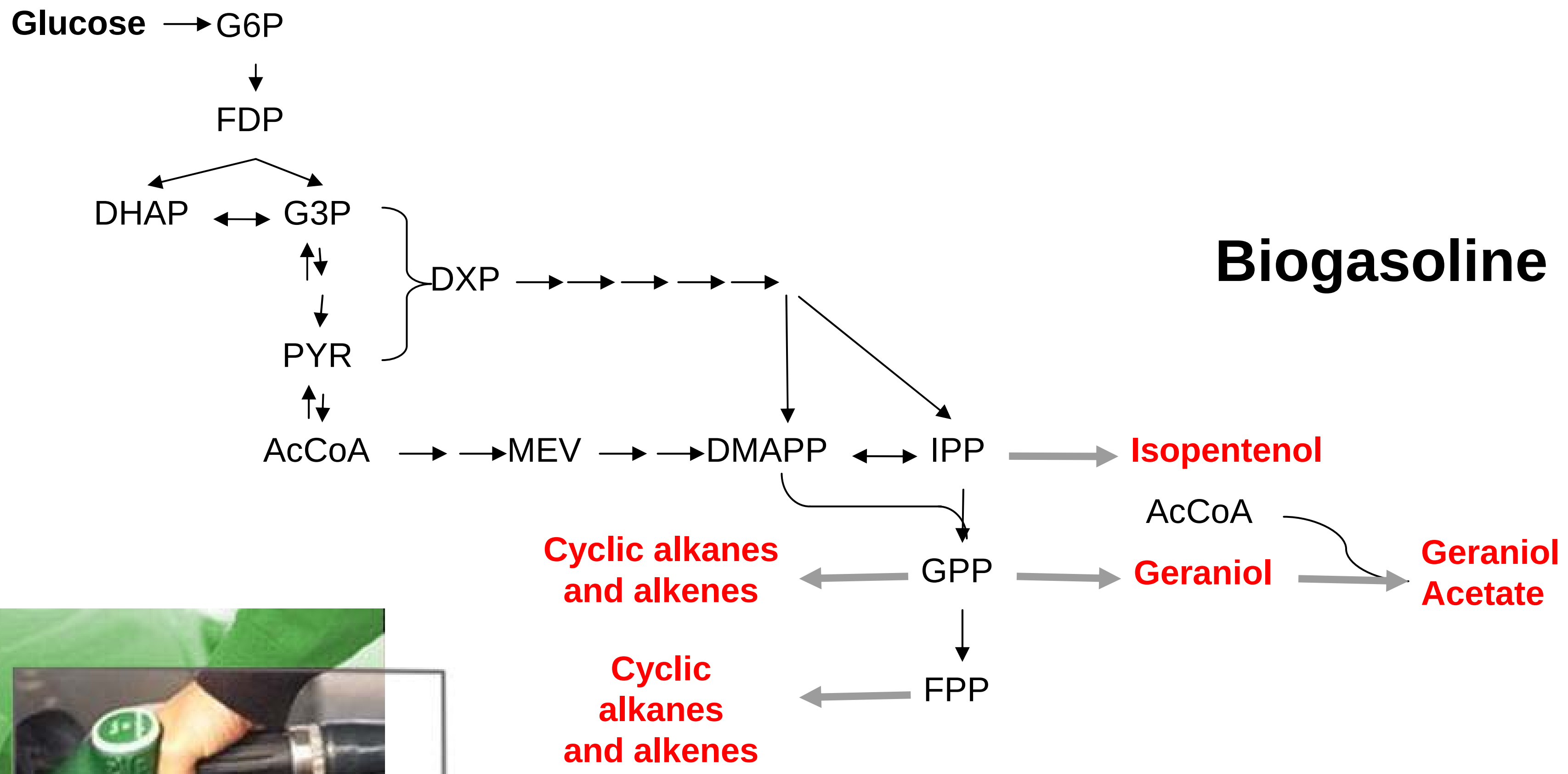
TIFN/NGI; Kluyver Centre; Nutrigenomics Consortium;
NCSB / NMC; MetaHIT; Human Metagenome Consortium; NUGO

3. Plants plant-derived materials for food and feedstocks

Courtesy of Dr Jim Haseloff

E.g. Nutrient-enriched plants, plant cellular re-programming, production of microbial starch from inedible waste materials, etc.

4. Downstream processing of food waste for use, eg. Biofuels



Biogasoline

Biodiesel

Bio Jet Fuel

Keasling lab, Amyris and other

Opportunities in Medical Synthetic Biology

- Re-programming stem cells

- Regenerative medicine

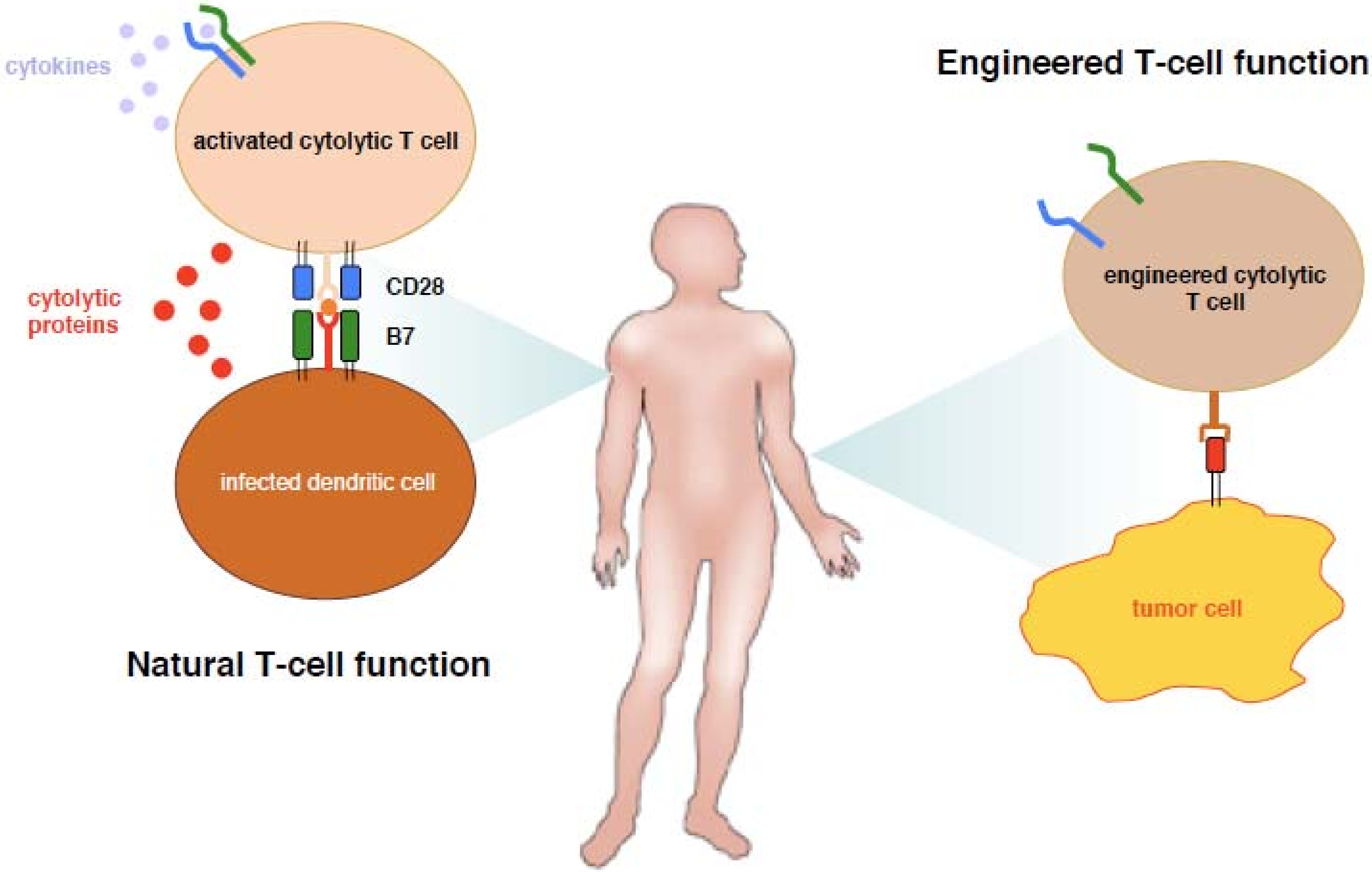
- Alternative processes of drug production

- New therapeutic methods (including *de novo* designed vaccines)

- Non-invasive diagnostics

- Engineering human immune cell responses
(which provide defenses against cancer, inflammation, autoimmune diseases…)

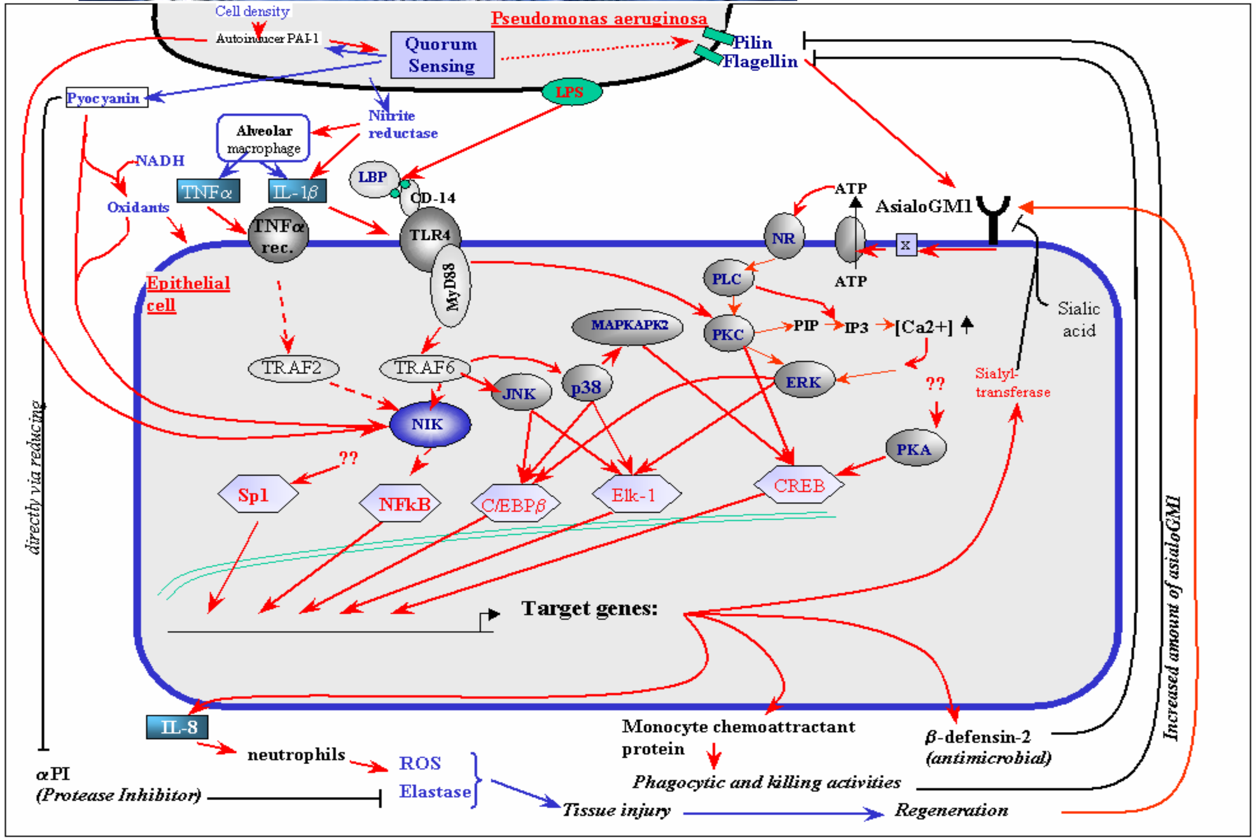
Cellular therapeutics



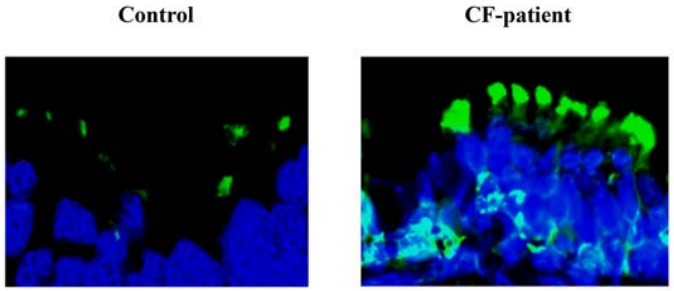
C. Smolke

Re-programming host-pathogen interactions

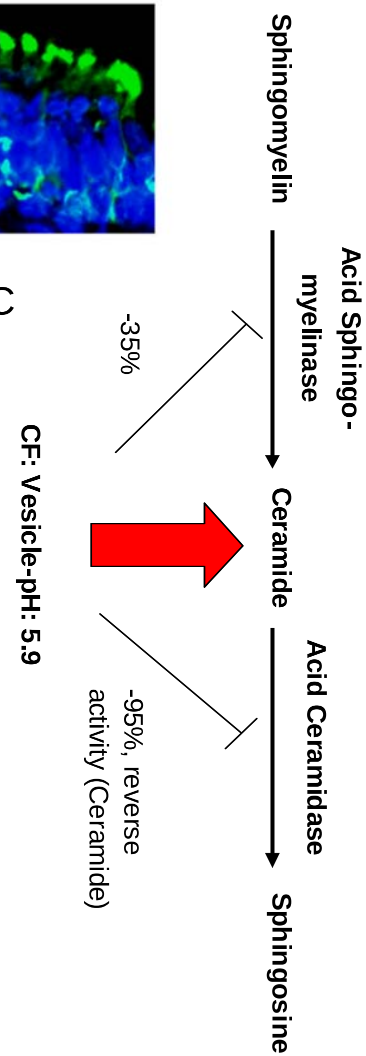
A



B



C



Longer-term perspective: tumor-killing bacteria

A Toolbox for Building Smart Delivery Agents

CIRCUITS

Signal Integrators

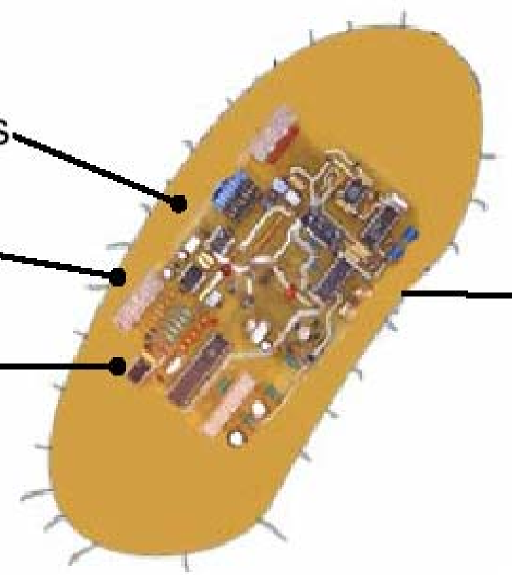
Switches

INPUTS

Environmental Signals

Touch / Adhesion

Exogenous Inducers (drugs/radiation)



OUTPUTS

Invade

Eat Malignant Cells

Deliver Therapeutic

Enzymatically convert prodrug

Suicide

CHASSIS

Benevolent

Acceptable immugenic properties

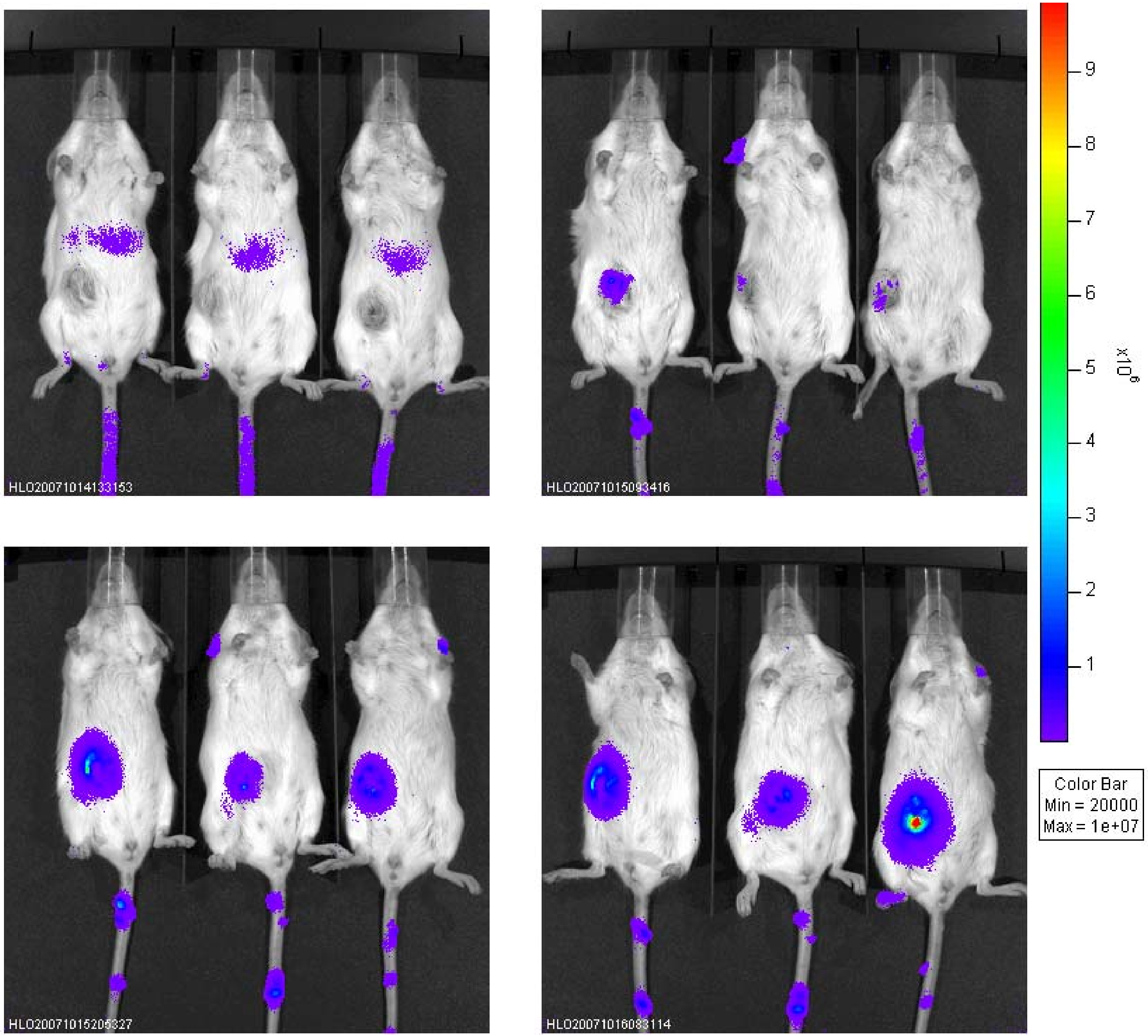
After Voigt, UCSF, 2005

Tumor mouse model (with S. Weiss, H. Loessner, S. Häussler)

30'	**24h**
36h	**48h**

PA01[pHL307]

intravenous dose ~ 5 x 10^6



Challenges in Synthetic Biology

Scientific:

•Orthogonality in Biological Systems

•Knowledge of intrinsic properties and functioning of the parts, devices and systems involved

•Accounting for evolution

Technological:

•Adapt current protocols for scope and scale in SynBio
•Availability of parts and devices

Organizational:

•Critical mass of practitioners adopting the „ethos“
•IP issues

Societal:

•Ethical, Legal, Social, Safety, Security, Governance

Summing up

Technological potential is vast, societal impact immense and growing and market opportunities substantial and diverse

An aging population and increased life expectancy is increasing awareness about good health and fueling the growth of demand for high quality food materials and nutrition strategies.

Synthetic Biology will play a pivotal role in meeting these and future demands

As with any other technological endeavour (SynBio or not), developments in SynBio for the food & health are to be tightly embedded in societal and regulatory context.

Disclaimer

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