

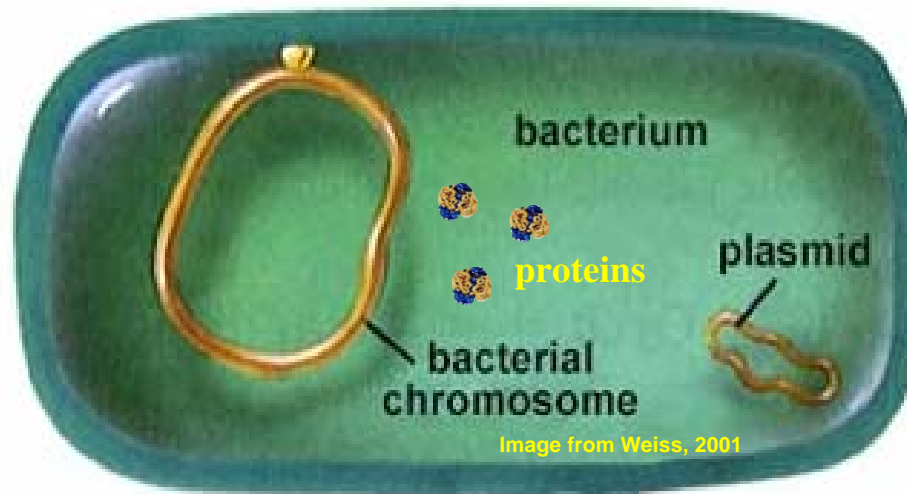
Programmable Bacterial Catalysts

Vítor Martins dos Santos

Division of Microbiology
Helmholtz Centre for Infection Research
Braunschweig, Germany

MISSION OF METABOLIC ENGINEERING

To understand microbial cellular behaviour [from a Systems Biology perspective] and to translate this insights into relevant biotechnological applications



Ideal biocatalyst: design from scratch?

Engineering framework: Interplay circuits of interest and genome

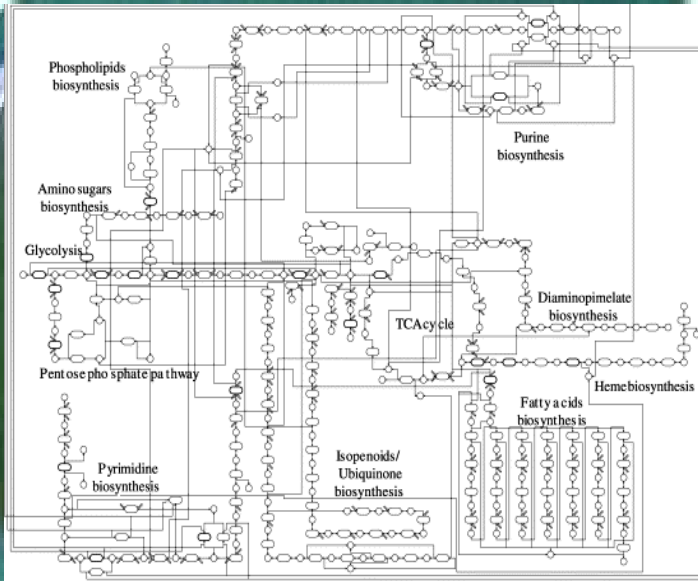
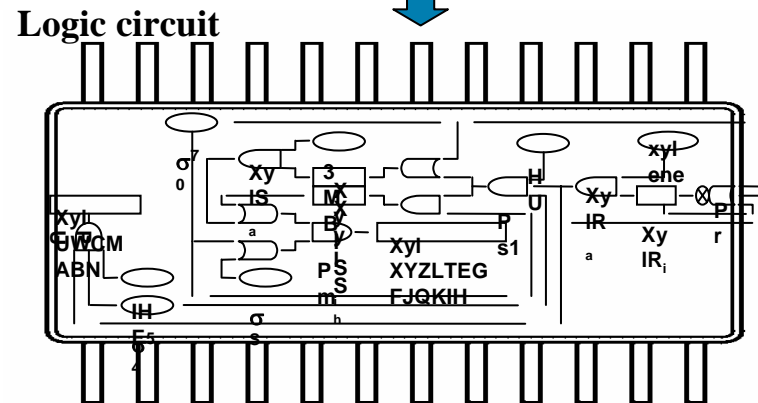
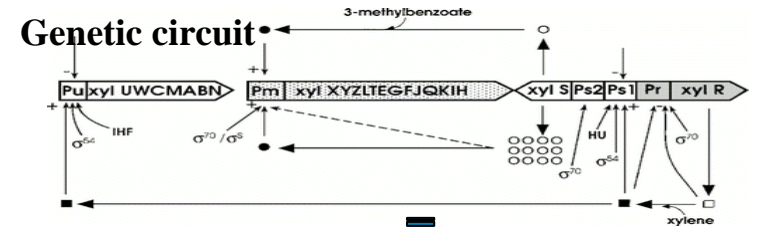


Image from Weiss, 2001



Aim is to have an integrated bacterial blueprint to enable (re-)programming

GOALS: PROBACTYS

To construct of a functioning, **streamlined bacterial cell** devoid of much of its genome and endowed with a series of coordinated, newly **assembled genetic circuits** for the **production of aromatic derivatives** that would include synchronized behaviour, **low-temperature biocatalysis**.

By achieving such constructs as a **proof-of-principle**, it is aimed at establishing a solid, rational framework for the engineering of cells **performing effectively biocatalytic processes**, in this case **production of aromatic derivatives**

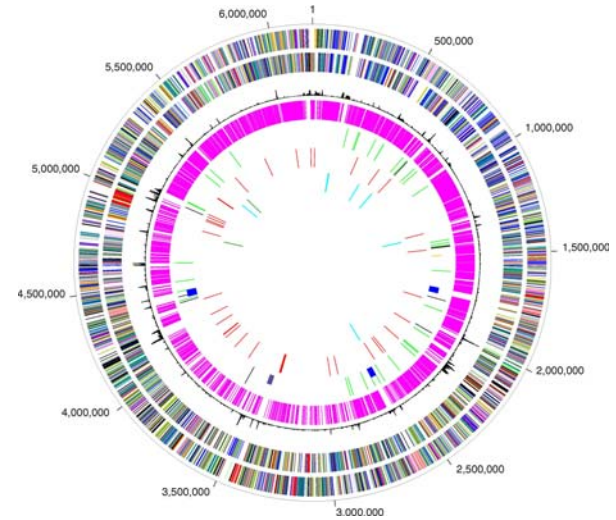
The global work strategy

- 1) develop a basic interrogation **scaffold** (blue print);
- 2) genome **minimization** (random ; directed)
- 3) **engineering** the different **genetic circuits** of choice (Biotransformation parts list etc.);
- 5) assessment of the **interplay of constructed circuits** with the template genome
- 6) **re-programme** for specific biocatalytic conditions : cold shock
- 7) **use of the model framework** and **constructed** cells for the **production of high-added value products** from halogenated aromatics

Pseudomonas putida as cell factory

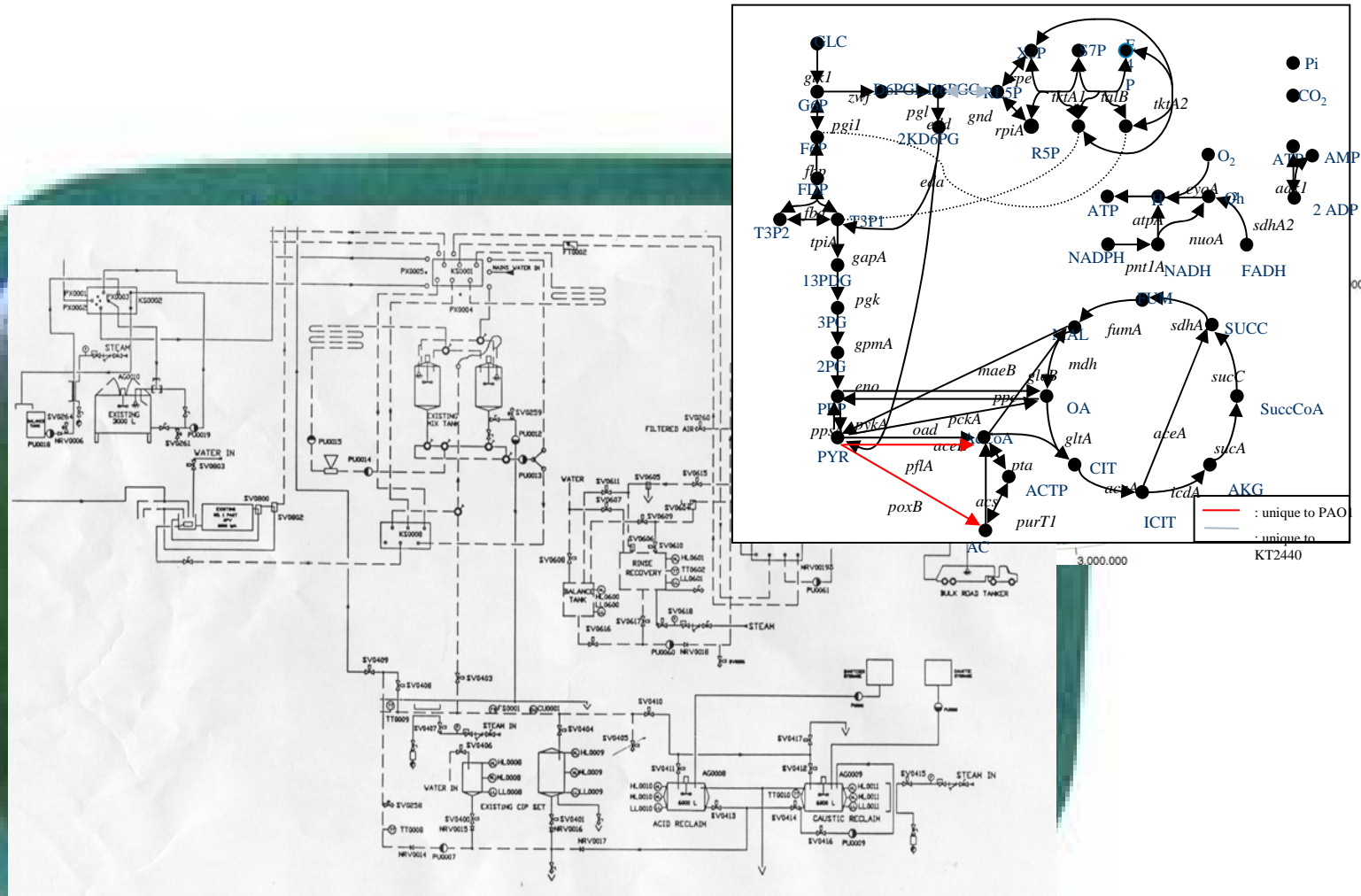
Pseudomonas putida KT2440

- Paradigm of ubiquitous copiotrophic soil bacteria
- Great metabolic diversity
- Certified biosafety strain
- Preferred host for the design of novel degradation/biocatalytic processes
- Large range of applications:
Agricultural, environmental, industrial



- Biotransformation of a wealth of chemicals, in particular aromatic and aliphatic derivatives
- Resistance to many stresses (eg. solvents)
- Genetically amenable for engineering
- Excellent host for heterologous expression

Towards a microbial blueprint:



What 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, fdol, 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, nuol, 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), *maeB*(Fraenkel, 1996)
Alternative Carbon Source *adhC, adhE, agaY, agaZ, aldA, aldB, aldH, araA, araB, araD, bgIX, cpsG, deoB, deoC, fruK, fucA, fucl, 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, ygiG, ygiH, alaB(Reitzer, 1996), *dapC*(Greene, 1996), *pat*(McFall

an Newman, 1996), *pr*(McFall and Newman, 1996), *sad*(Berlyn et al., 1996), *Methylthioadenosine nucleosidase*(Glansdorff, 1996), *5-Methylthioribose kinase*(Glansdorff 1996), *5-Methylthioribose-1-phosphate isomerase*(Glansdorff, 1996), *Adenosyl homocysteinase*(Matthews, 1996), *L-Cysteine desulfhydrase*(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, pyrl, tdk, thyA, tmk, udk, udp, upp, ushA, xapA, yicP, CMPglycosylase*(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), *nadF*(Penfound and Foster, 1996), *nadG*(Penfound and Foster, 1996), *panE*(Jackowski, 1996), *pncA*(Penfound and Foster, 1996), *pncC*(Penfound and Foster, 1996), *thiB*(White and Spenser, 1996), *thiD*(White and Spenser, 1996), *thiK*(White and Spenser, 1996), *thiL*(White and Spenser, 1996), *thiM*(White and Spenser, 1996), *thiN*(White and Spenser, 1996), *ubiE*(Meganathan, 1996),

ubiF(Meganathan, 1996), *Arabinose-5-phosphate isomerase*(Karp et al., 1998), *Phosphopantothenate-cysteine ligase*(Jackowski, 1996), *Phosphopantothenate-cystein decarboxylase*(Jackowski, 1996), *Phospho-pantetheine adenylyltransferase*(Jackowski, 1996), *DephosphoCoA kinase*(Jackowski, 1996), *NMN glycohydrolase*(Penfound and Foster, 1996)

Lipid Metabolism *accA, accB, accD, atoB, cdh, cdsA, cls, dgkA, fabD, fabH, fadB, gpsA, ispA, ispB, pgpB, pgsA, psd, pssA, pgpA*(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, murl, rfaC, rfaD, rfaF, rfaG, rfaI, rfaJ, rfaL, ushA, glmM*(Mengin-Lecreulx and van Heijenoort, 1996), *lpcA*(Raetz, 1996), *rfaE*(Raetz, 1996), *Tetraacyldisaccharide 4' kinase*(Raetz, 1996), *3-Deoxy-D-manno-octulosonic-acid 8-phosphate phosphatase*(Raetz, 1996)

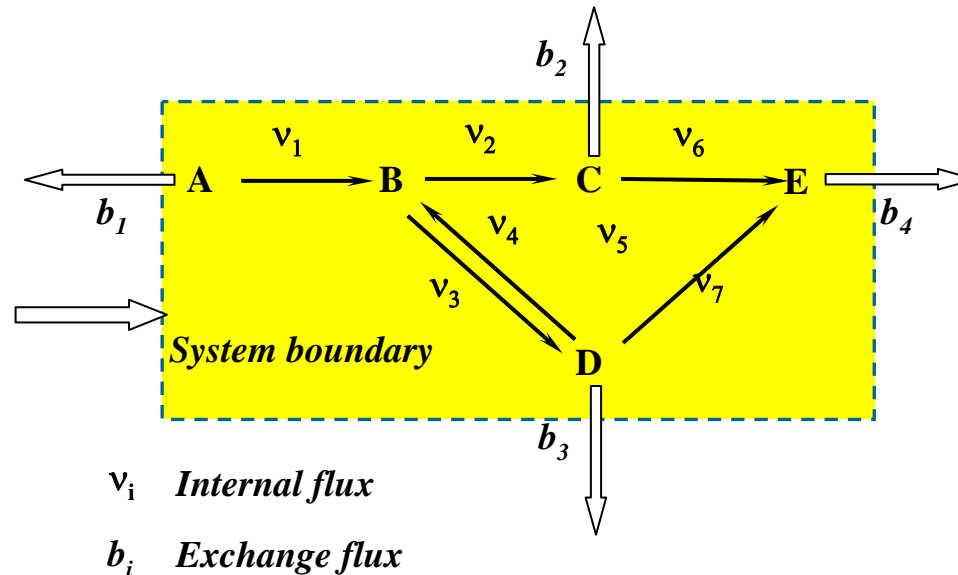
Transport Processes *araE, araF, araG, araH, argT, araP, artI, artJ, artM, artP, artQ, brnQ, cadB, chaA, chaB, chaC, cmtA, cmtB, codB, crr, cycA, cysA, cysP, cysT, cysU, cysV, cysW, cysX, cysY, cysZ, dhaA, dhaB, dhaC, dhaD, dhaE, dhaF, dhaG, dhaH, dhaI, dhaJ, dhaK, dhaL, dhaM, dhaN, dhaO, dhaP, dhaQ, dhaR, dhaS, dhaT, dhaU, dhaV, dhaW, dhaX, dhaY, dhaZ, dhaAA, dhaAB, dhaAC, dhaAD, dhaAE, dhaAF, dhaAG, dhaAH, dhaAI, dhaAJ, dhaAK, dhaAL, dhaAM, dhaAN, dhaAO, dhaAP, dhaAQ, dhaAR, dhaAS, dhaAT, dhaAU, dhaAV, dhaAW, dhaAX, dhaAY, dhaAZ, dhaBA, dhaBB, dhaBC, dhaBD, dhaBE, dhaBF, dhaBG, dhaBH, dhaBI, dhaBJ, dhaBK, dhaBL, dhaBM, dhaBN, dhaBO, dhaBP, dhaBQ, dhaBR, dhaBS, dhaBT, dhaBU, dhaBV, dhaBW, dhaBX, dhaBY, dhaBZ, dhaCA, dhaCB, dhaCC, dhaCD, dhaCE, dhaCF, dhaCG, dhaCH, dhaCI, dhaCJ, dhaCK, dhaCL, dhaCM, dhaCN, dhaCO, dhaCP, dhaCQ, dhaCR, dhaCS, dhaCT, dhaCU, dhaCV, dhaCW, dhaCX, dhaCY, dhaCZ, dhaDA, dhaDB, dhaDC, dhaDD, dhaDE, dhaDF, dhaDG, dhaDH, dhaDI, dhaDJ, dhaDK, dhaDL, dhaDM, dhaDN, dhaDO, dhaDP, dhaDQ, dhaDR, dhaDS, dhaDT, dhaDU, dhaDV, dhaDW, dhaDX, dhaDY, dhaDZ, dhaEA, dhaEB, dhaEC, dhaED, dhaEE, dhaEF, dhaEG, dhaEH, dhaEI, dhaEJ, dhaEK, dhaEL, dhaEM, dhaEN, dhaEO, dhaEP, dhaEQ, dhaER, dhaES, dhaET, dhaEU, dhaEV, dhaEW, dhaEX, dhaEY, dhaEZ, dhaFA, dhaFB, dhaFC, dhaFD, dhaFE, dhaFF, dhaFG, dhaFH, dhaFI, dhaFJ, dhaFK, dhaFL, dhaFM, dhaFN, dhaFO, dhaFP, dhaFQ, dhaFR, dhaFS, dhaFT, dhaFU, dhaFV, dhaFW, dhaFX, dhaFY, dhaFZ, dhaGA, dhaGB, dhaGC, dhaGD, dhaGE, dhaGF, dhaGG, dhaGH, dhaGI, dhaGJ, dhaGK, dhaGL, dhaGM, dhaGN, dhaGO, dhaGP, dhaGQ, dhaGR, dhaGS, dhaGT, dhaGU, dhaGV, dhaGW, dhaGX, dhaGY, dhaGZ, dhaHA, dhaHB, dhaHC, dhaHD, dhaHE, dhaHF, dhaHG, dhaHH, dhaHI, dhaHJ, dhaHK, dhaHL, dhaHM, dhaHN, dhaHO, dhaHP, dhaHQ, dhaHR, dhaHS, dhaHT, dhaHU, dhaHV, dhaHW, dhaHX, dhaHY, dhaHZ, dhaIA, dhaIB, dhaIC, dhaID, dhaIE, dhaIF, dhaIG, dhaIH, dhaII, dhaIJ, dhaIK, dhaIL, dhaIM, dhaIN, dhaIO, dhaIP, dhaIQ, dhaIR, dhaIS, dhaIT, dhaIU, dhaIV, dhaIW, dhaIX, dhaIY, dhaIZ, dhaJA, dhaJB, dhaJC, dhaJD, dhaJE, dhaJF, dhaJG, dhaJH, dhaJI, dhaJJ, dhaJK, dhaJL, dhaJM, dhaJN, dhaJO, dhaJP, dhaJQ, dhaJR, dhaJS, dhaJT, dhaJU, dhaJV, dhaJW, dhaJX, dhaJY, dhaJZ, dhaKA, dhaKB, dhaKC, dhaKD, dhaKE, dhaKF, dhaKG, dhaKH, dhaKI, dhaKJ, dhaKL, dhaKM, dhaKN, dhaKO, dhaKP, dhaKQ, dhaKR, dhaKS, dhaKT, dhaKU, dhaKV, dhaKW, dhaKX, dhaKY, dhaKZ, dhaLA, dhaLB, dhaLC, dhaLD, dhaLE, dhaLF, dhaLG, dhaLH, dhaLI, dhaLJ, dhaLK, dhaLL, dhaLM, dhaLN, dhaLO, dhaLP, dhaLQ, dhaLR, dhaLS, dhaLT, dhaLU, dhaLV, dhaLW, dhaLX, dhaLY, dhaLZ, dhaMA, dhaMB, dhaMC, dhaMD, dhaME, dhaMF, dhaMG, dhaMH, dhaMI, dhaMJ, dhaMK, dhaML, dhaMM, dhaMN, dhaMO, dhaMP, dhaMQ, dhaMR, dhaMS, dhaMT, dhaMU, dhaMV, dhaMW, dhaMX, dhaMY, dhaMZ, dhaNA, dhaNB, dhaNC, dhaND, dhaNE, dhaNF, dhaNG, dhaNH, dhaNI, dhaNJ, dhaNK, dhaNL, dhaNM, dhaNN, dhaNO, dhaNP, dhaNQ, dhaNR, dhaNS, dhaNT, dhaNU, dhaNV, dhaNW, dhaNX, dhaNY, dhaNZ, dhaOA, dhaOB, dhaOC, dhaOD, dhaOE, dhaOF, dhaOG, dhaOH, dhaOI, dhaOJ, dhaOK, dhaOL, dhaOM, dhaON, dhaOO, dhaOP, dhaOQ, dhaOR, dhaOS, dhaOT, dhaOU, dhaOV, dhaOW, dhaOX, dhaOY, dhaOZ, dhaPA, dhaPB, dhaPC, dhaPD, dhaPE, dhaPF, dhaPG, dhaPH, dhaPI, dhaPJ, dhaPK, dhaPL, dhaPM, dhaPN, dhaPO, dhaPP, dhaPQ, dhaPR, dhaPS, dhaPT, dhaPU, dhaPV, dhaPW, dhaPX, dhaPY, dhaPZ, dhaQA, dhaQB, dhaQC, dhaQD, dhaQE, dhaQF, dhaQG, dhaQH, dhaQI, dhaQJ, dhaQK, dhaQL, dhaQM, dhaQN, dhaQO, dhaQP, dhaQQ, dhaQR, dhaQS, dhaQT, dhaQU, dhaQV, dhaQW, dhaQX, dhaQY, dhaQZ, dhaRA, dhaRB, dhaRC, dhaRD, dhaRE, dhaRF, dhaRG, dhaRH, dhaRI, dhaRJ, dhaRK, dhaRL, dhaRM, dhaRN, dhaRO, dhaRP, dhaRQ, dhaRR, dhaRS, dhaRT, dhaRU, dhaRV, dhaRW, dhaRX, dhaRY, dhaRZ, dhaSA, dhaSB, dhaSC, dhaSD, dhaSE, dhaSF, dhaSG, dhaSH, dhaSI, dhaSJ, dhaSK, dhaSL, dhaSM, dhaSN, dhaSO, dhaSP, dhaSQ, dhaSR, dhaSS, dhaST, dhaSU, dhaSV, dhaSW, dhaSX, dhaSY, dhaSZ, dhaTA, dhaTB, dhaTC, dhaTD, dhaTE, dhaTF, dhaTG, dhaTH, dhaTI, dhaTJ, dhaTK, dhaTL, dhaTM, dhaTN, dhaTO, dhaTP, dhaTQ, dhaTR, dhaTS, dhaTT, dhaTU, dhaTV, dhaTW, dhaTX, dhaTY, dhaTZ, dhaUA, dhaUB, dhaUC, dhaUD, dhaUE, dhaUF, dhaUG, dhaUH, dhaUI, dhaUJ, dhaUK, dhaUL, dhaUM, dhaUN, dhaUO, dhaUP, dhaUQ, dhaUR, dhaUS, dhaUT, dhaUU, dhaUV, dhaUW, dhaUX, dhaUY, dhaUZ, dhaVA, dhaVB, dhaVC, dhaVD, dhaVE, dhaVF, dhaVG, dhaVH, dhaVI, dhaVJ, dhaVK, dhaVL, dhaVM, dhaVN, dhaVO, dhaVP, dhaVQ, dhaVR, dhaVS, dhaVT, dhaVU, dhaVV, dhaVW, dhaVX, dhaVY, dhaVZ, dhaWA, dhaWB, dhaWC, dhaWD, dhaWE, dhaWF, dhaWG, dhaWH, dhaWI, dhaWJ, dhaWK, dhaWL, dhaWM, dhaWN, dhaWO, dhaWP, dhaWQ, dhaWR, dhaWS, dhaWT, dhaWU, dhaWV, dhaWW, dhaWX, dhaWY, dhaWZ, dhaXA, dhaXB, dhaXC, dhaXD, dhaXE, dhaXF, dhaXG, dhaXH, dhaXI, dhaXJ, dhaXK, dhaXL, dhaXM, dhaXN, dhaXO, dhaXP, dhaXQ, dhaXR, dhaXS, dhaXT, dhaXU, dhaXV, dhaXW, dhaXX, dhaXY, dhaXZ, dhaYA, dhaYB, dhaYC, dhaYD, dhaYE, dhaYF, dhaYG, dhaYH, dhaYI, dhaYJ, dhaYK, dhaYL, dhaYM, dhaYN, dhaYO, dhaYP, dhaYQ, dhaYR, dhaYS, dhaYT, dhaYU, dhaYV, dhaYW, dhaYX, dhaYY, dhaYZ, dhaZA, dhaZB, dhaZC, dhaZD, dhaZE, dhaZF, dhaZG, dhaZH, dhaZI, dhaZJ, dhaZK, dhaZL, dhaZM, dhaZN, dhaZO, dhaZP, dhaZQ, dhaZR, dhaZS, dhaZT, dhaZU, dhaZV, dhaZW, dhaZX, dhaZY, dhaZZ*

The stoichiometric matrix as a metabolic map

Genetic content

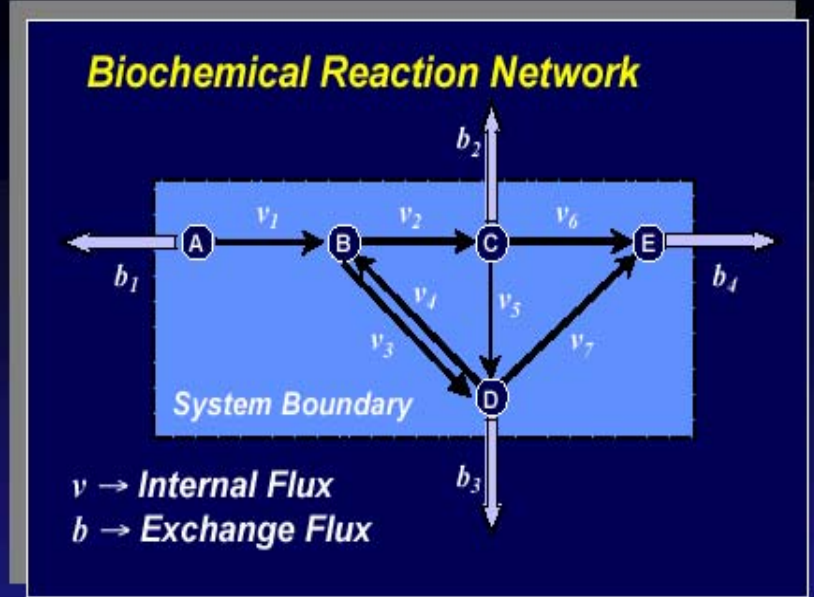
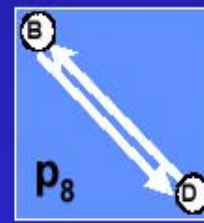
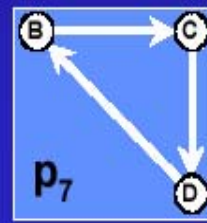
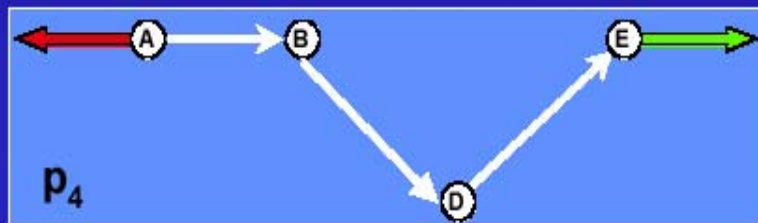
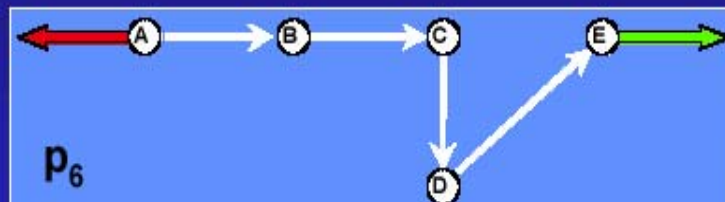
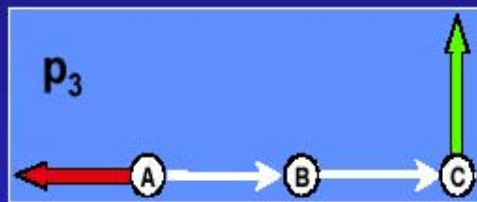
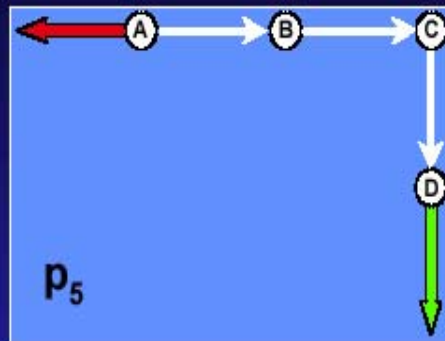
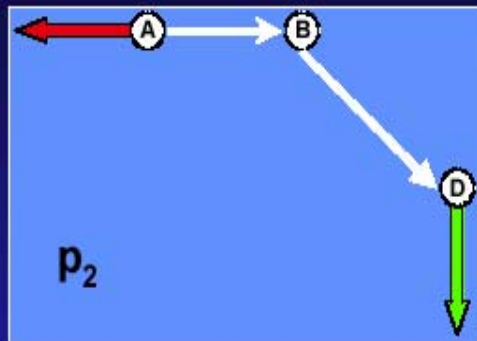
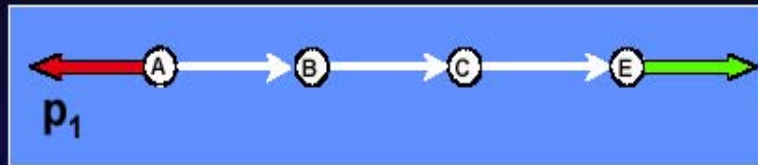
<u>gene</u>	<u>enzyme</u>	<u>flux</u>	<u>rxn</u>
<i>mglA, maglB</i>	galactose transporter	v_1	$A \rightleftharpoons B$
<i>galT</i>	uridyl transferase	v_2	$B \rightleftharpoons C$
<i>galK</i>	galactokinase	v_3	$B \rightleftharpoons D$

Biochemical reaction network



After Palsson, 2000

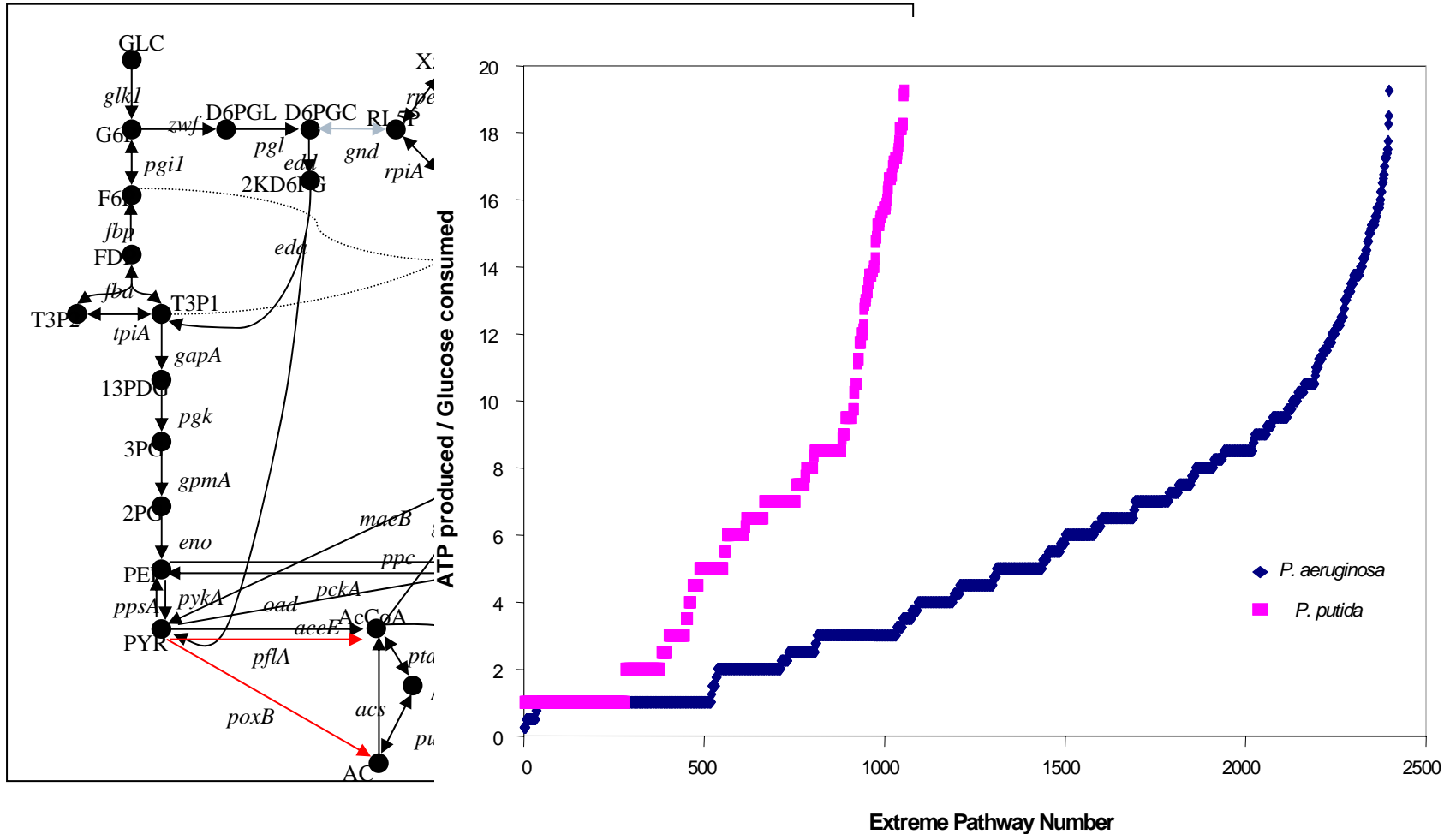
Extreme Pathways



7 internal fluxes
 + 4 exchange fluxes
 $n = 11$ fluxes
 $m = 5$ metabolites

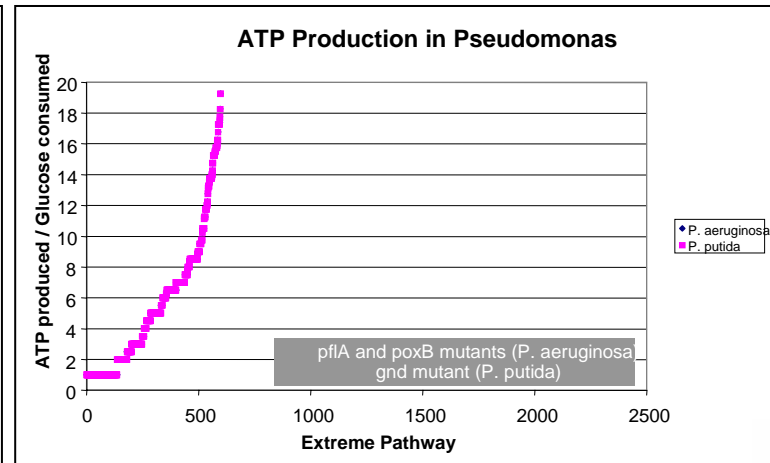
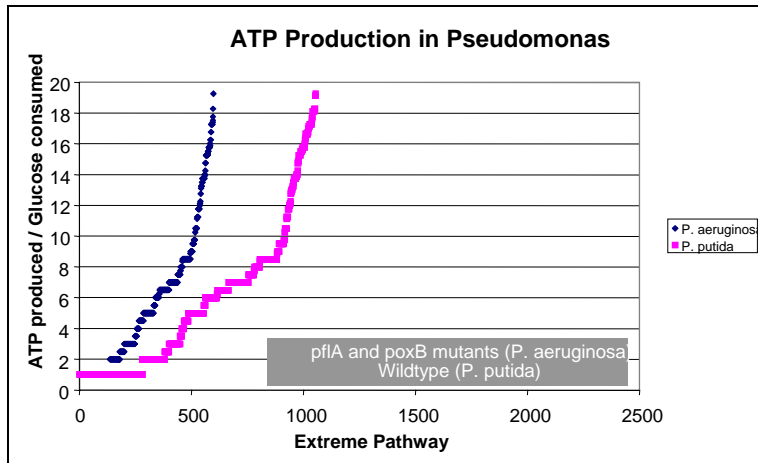
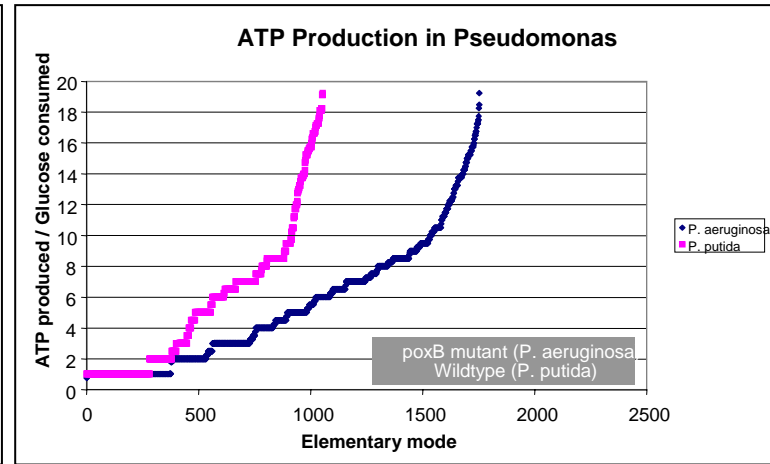
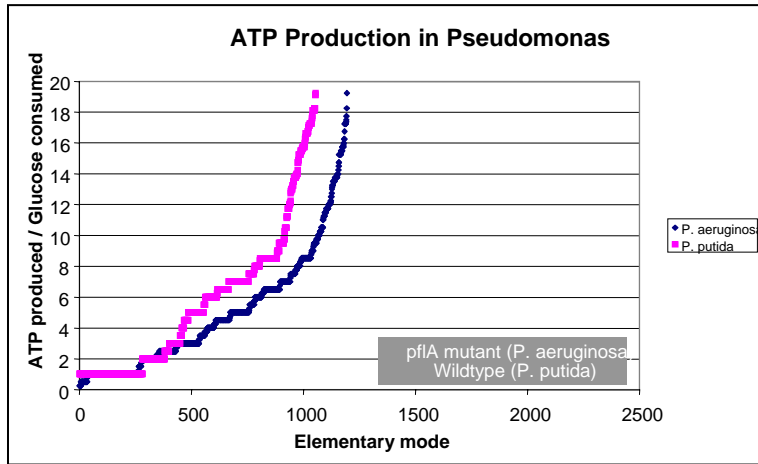
- stoichiometric matrix (5 x 11)
- dimension of null space = 6
- # of extreme pathways = 8

In silico genotype-phenotype relationship of *P. putida* KT2440 and *P. aeruginosa* PAO1



Emergent property of the system, not predictable from linear comparison of gene lists

In silico mutants assist rational experimental design



Emergent properties

An apparent small genetic difference can be strongly amplified

Emergent properties not evident from gene list only

Ps. aeruginosa* shows more redundancy than *Ps. Putida

But,

Is this real?

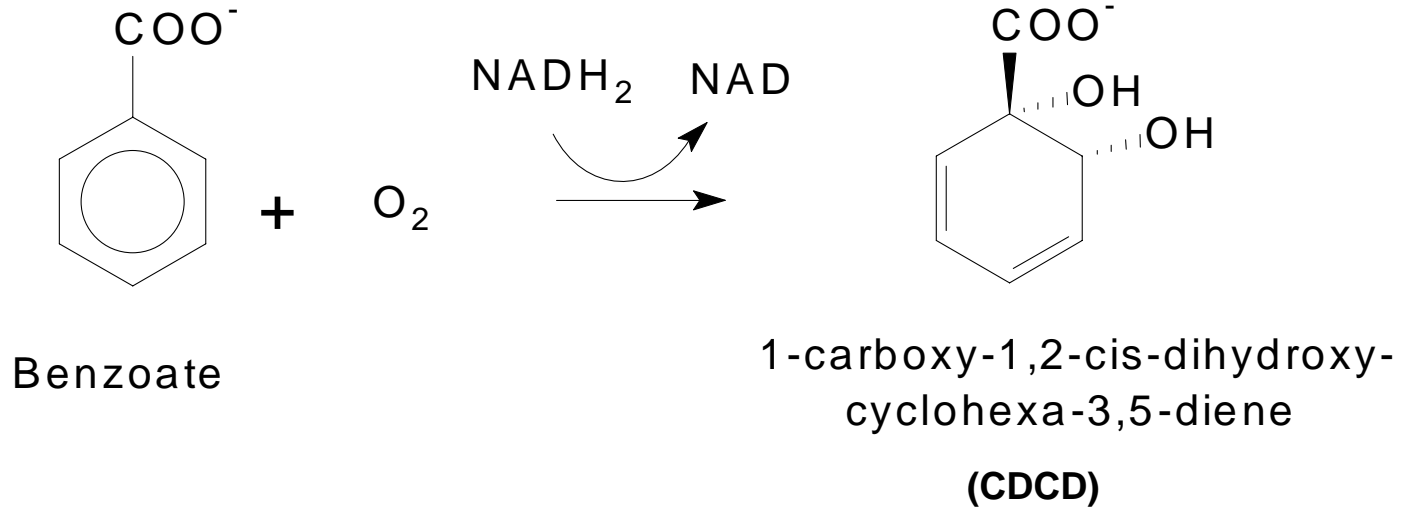
What is true biological meaning?

Experimental testing with transposon mutants

Sample	Transposon flanking region in KT2440 genome	Comparison to annotated <i>P. aeruginosa</i> PA01		
		flanking region found in gene list	PA No.	E.C. No.
AUX28	3-methyl-2-oxobutanoate hydroxymethyltransferase	ORF		2.1.2.11
AUX29	lipoate synthase	ORF	3996	
AUX46	catabolite repression control protein	ORF	5332	3.1.11.2
AUX14	Tryptophan synthase beta chain	ORF 00127	0036	4.2.1.20
AUX108	phosphoribosylaminoimidazole carboxylase, catalytic subunit	ORF 00363	5426	4.1.1.21
AUX30	orotate phosphoribosyltransferase	ORF 00434	5331	2.4.2.10
AUX73	acetylglutamate kinase	ORF 00438	5323	2.7.2.8
AUX13	Phosphomannomutase AlgC	ORF 00439	5322	5.4.2.8
AUX25	N-acetylglutamate synthase	ORF 00607	5204	2.3.1.1
AUX27	FAD binding domain	ORF 00650		
AUX59	dihydroxy-acid dehydratase	ORF 00694	353	4.2.1.9
AUX125	dihydroxy-acid dehydratase	ORF 00694	353	4.2.1.9
AUX126	dehydroxyaciddehydratase	ORF 00694	353	4.2.1.9
AUX3	Homoserine-O-Acetyl-Aminotransferase	ORF 00743	O390	2.3.1.31
AUX90	homoserine O-acetyltransferase	ORF 00743		2.3.1.46
AUX113	Aspartate carbamyltransferase	ORF 00911		
AUX78	adenosylhomocysteinase	ORF 00943		3.3.1.1
AUX4	S-adenosyl-L-homocysteine hydrolase	ORF 00948	O432	3.3.1.1
AUX76	adenosylhomocysteinase	ORF 00948		
AUX84	adenosylhomocysteinase	ORF 00948		3.3.1.1
AUX12	(Probable) phosphoserine phosphatase	ORF 01053	4960	3.1.3.3
AUX64	histidyl-tRNA synthetase	ORF 01085	3802	6.1.1.21
AUX23	phosphoribosylamine--glycine ligase	ORF 01203	4855	6.3.4.13
AUX95	phosphoribosylamine--glycine ligase	ORF 01203	4855	6.3.4.13
AUX122	lipoate-protein ligase B	ORF 01238		
AUX119	Mutation zwischen 2 ORFs	kein ORF vorhanden (vorher ORF 01239)		
AUX41	dihydrodipicolinate reductase	ORF 01370	4759	1.3.1.26
AUX50	dihydrodipicolinate reductase	ORF 01370	4759	1.3.1.26

Production of high-added value chemicals

Benzoate 1,2-dioxygenase



- precursor for many industrially relevant enantiopure derivatives
- difficult to synthesize chemically

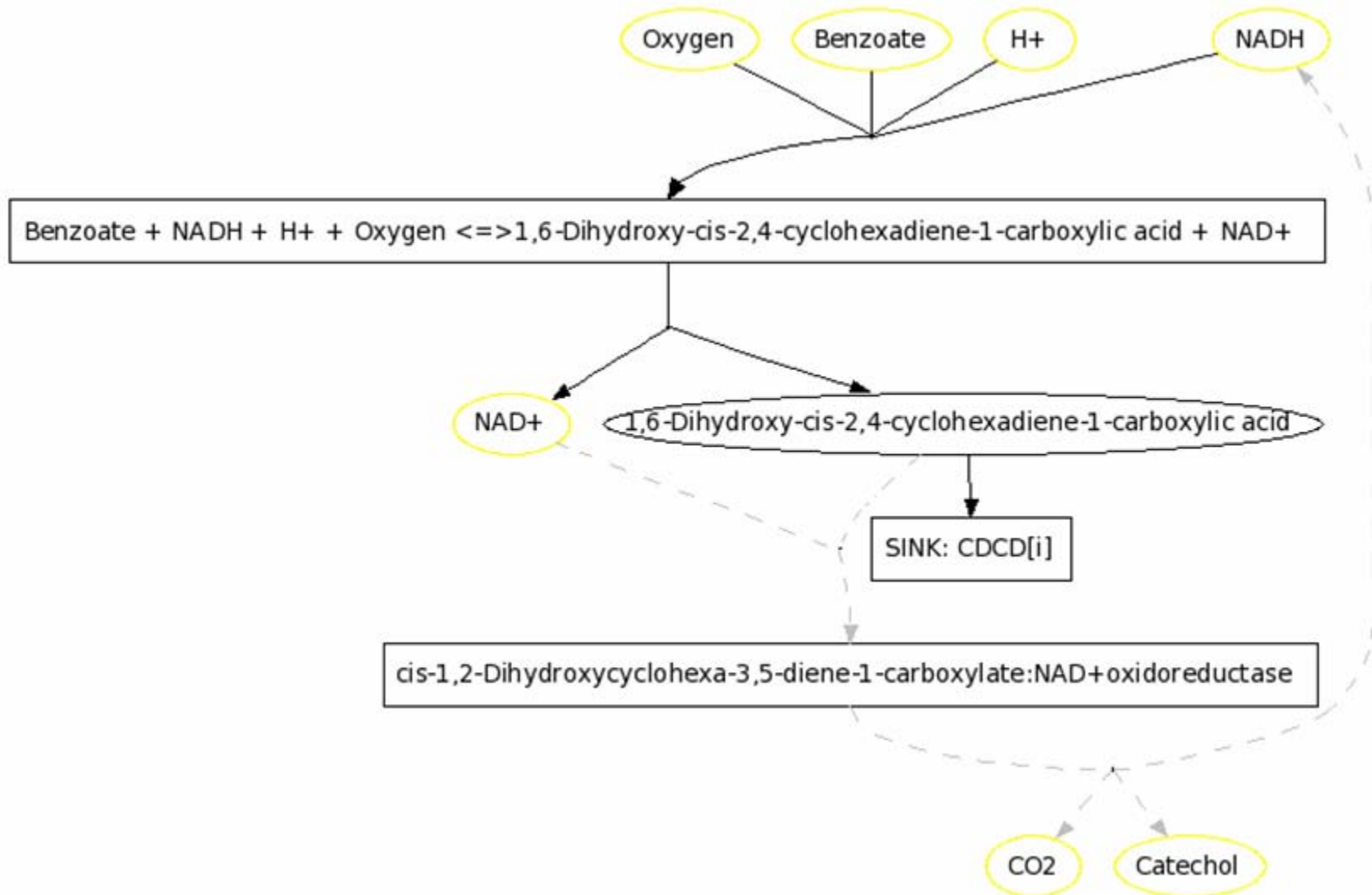


Attractive alternative: cofactor dependent biotransformation



Metabolic engineering.

Navigation example : Preliminary model of CDCD



Engineering framework: Interplay circuits of interest and genome

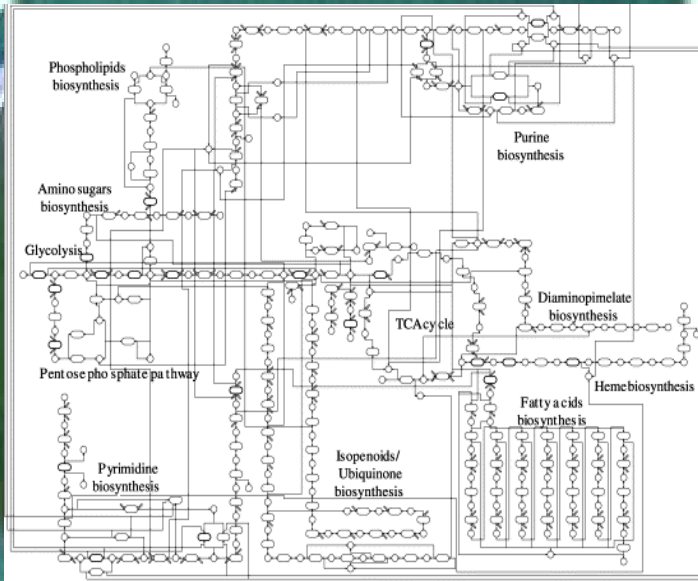
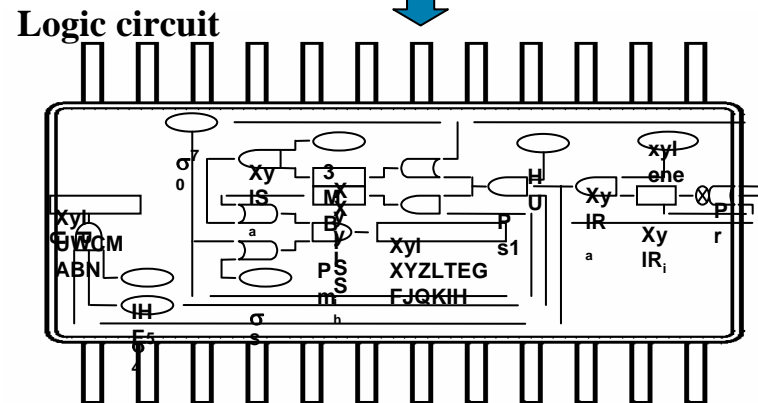
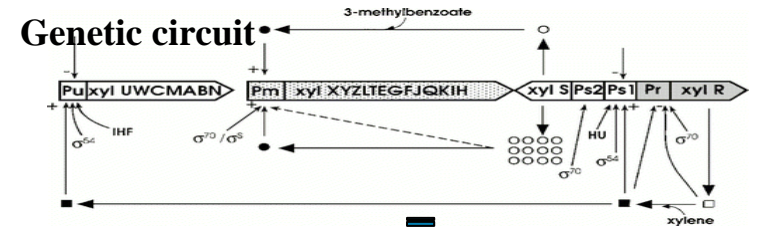
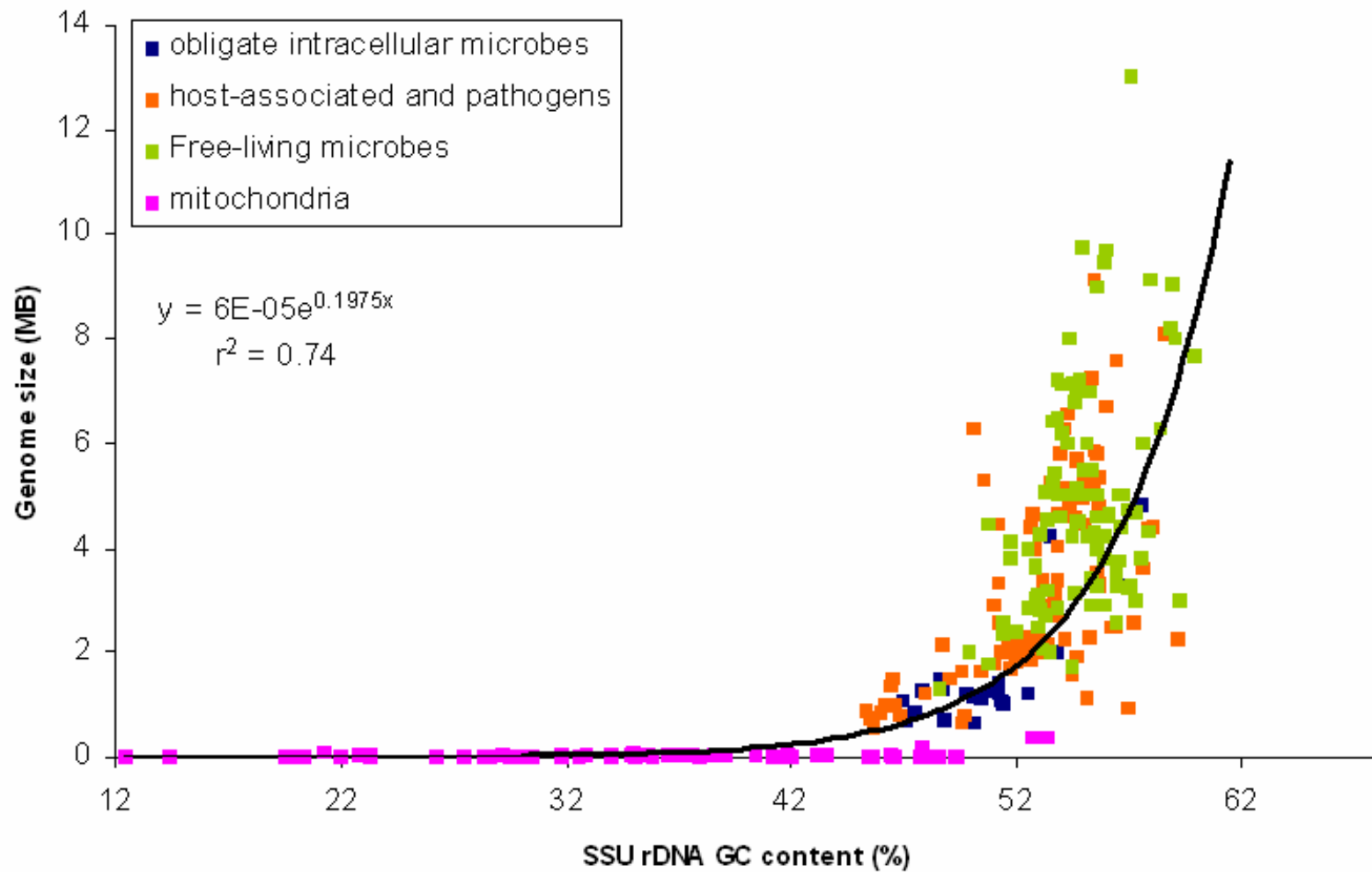


Image from Weiss, 2001



Aim is to have an integrated bacterial blueprint to enable (re-)programming

Minimisation: Genome size vs SSU rDNA GC content

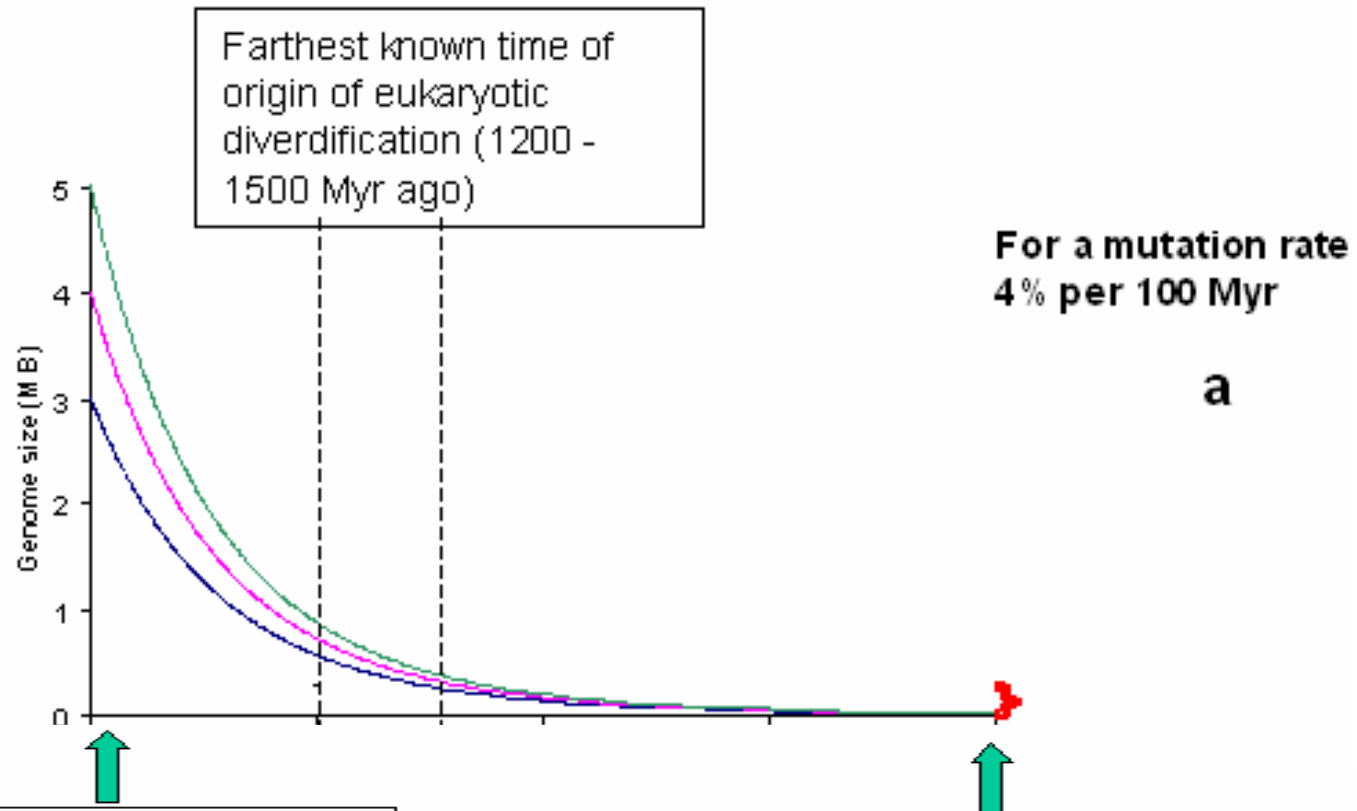


Bootstrapping to a timeline

Khachane et al., Mol. Biol. Evol, in press

$$\Delta GC \text{ per unit time} = m \times \frac{(\text{IV ratio} + \frac{1}{2})}{(\text{IV ratio} + 1)} \times (GC_f - GC_c)$$

$$\text{size}(t) = [0.00006m \cdot k] \cdot \text{size}(t-1) (1 - m \cdot k) \cdot e^{0.1975 \cdot m \cdot k \cdot GC_f}$$



2000 Myr ago:

Time-point at which pre-symbiotic ancestor of mitochondria makes a transition in its life-style from a free-living to an intracellular habitat.

Today:

Predicted genome size after 2000 Myr period (~0 - 0.1 MB). The genome size range of extant mitochondria (indicated by red curly brackets).

Implications and outlook

Most genome loss occur at early stages of evolution, possibly in chunks

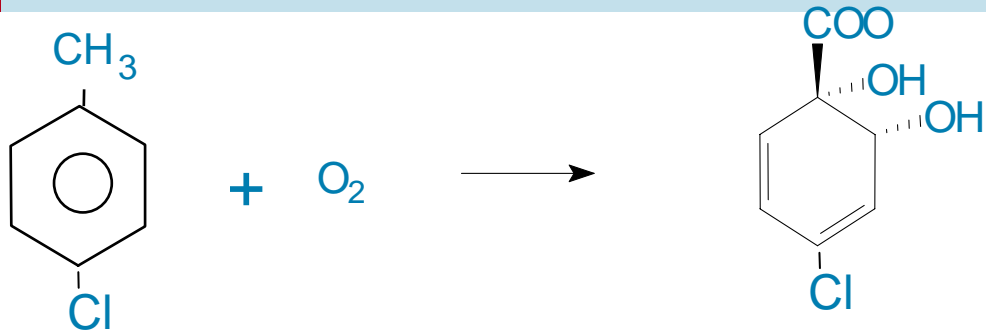


Rational dissection of functional networks (evolutionary patterns, constraint-based analysis) will provide candidate modules for directed deletion / expansion

Experimentally:

Global, transposon mutagenesis, random saturation with site-specific recombination sites and expression under strong selection processes, etc.

Circuit design for the production of fine chemicals



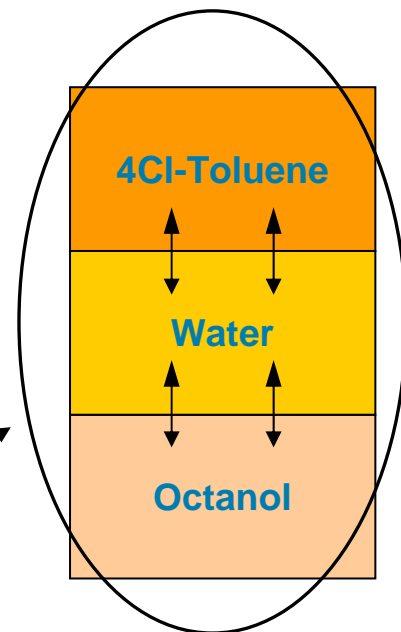
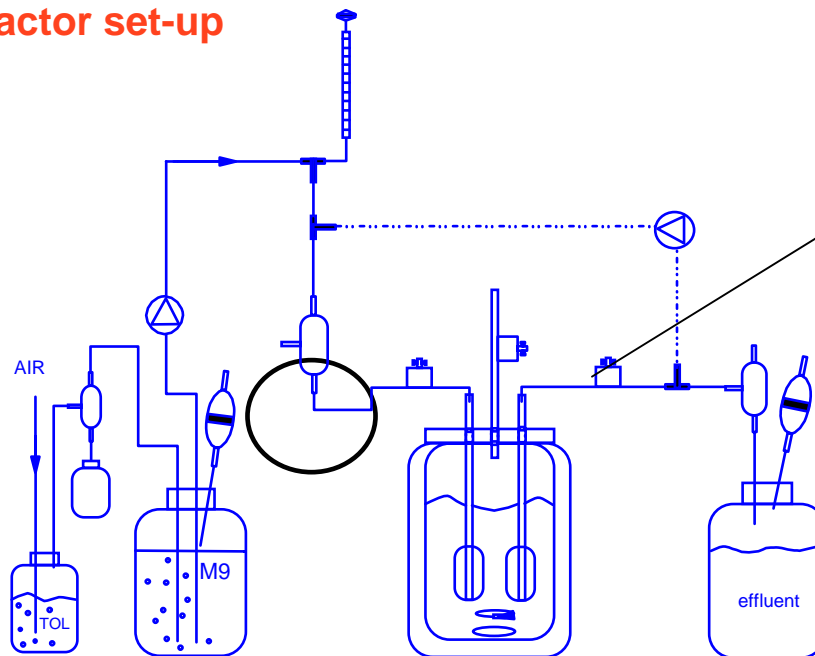
4-Cl toluene

cis-4-chlorodihydrodiol

- precursor for many industrially relevant enantiopure derivatives
- difficult to synthesize chemically

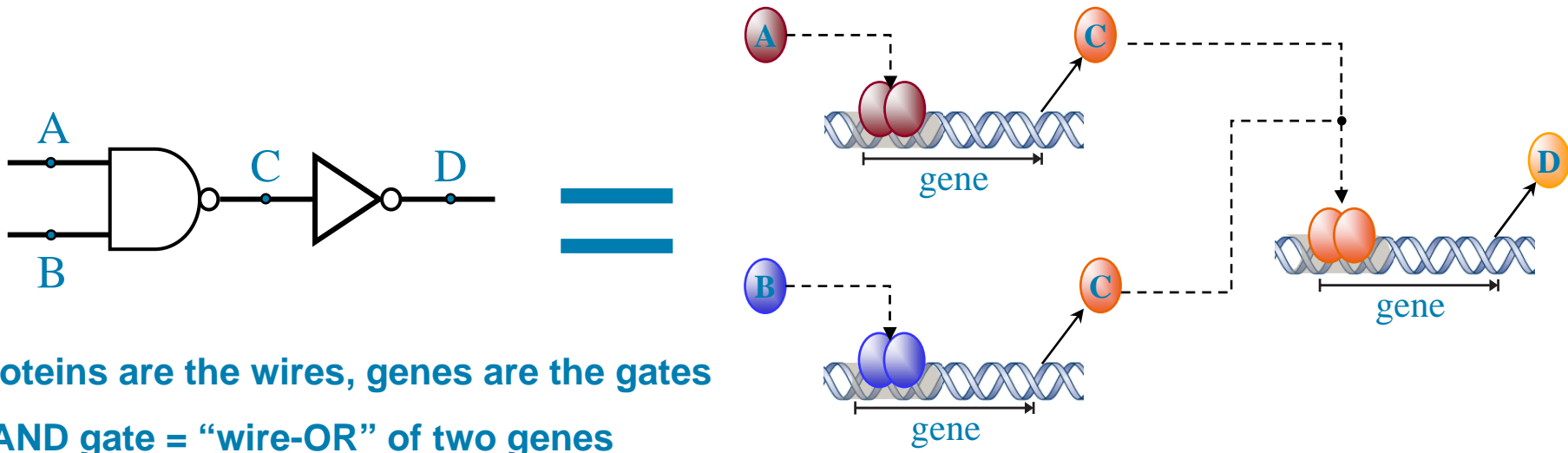
Attractive alternative: cofactor dependent biotransformation in two-phase systems

Reactor set-up



Logic genetic circuits based on inverters

With properly designed inverters, any (finite) digital circuit can be built

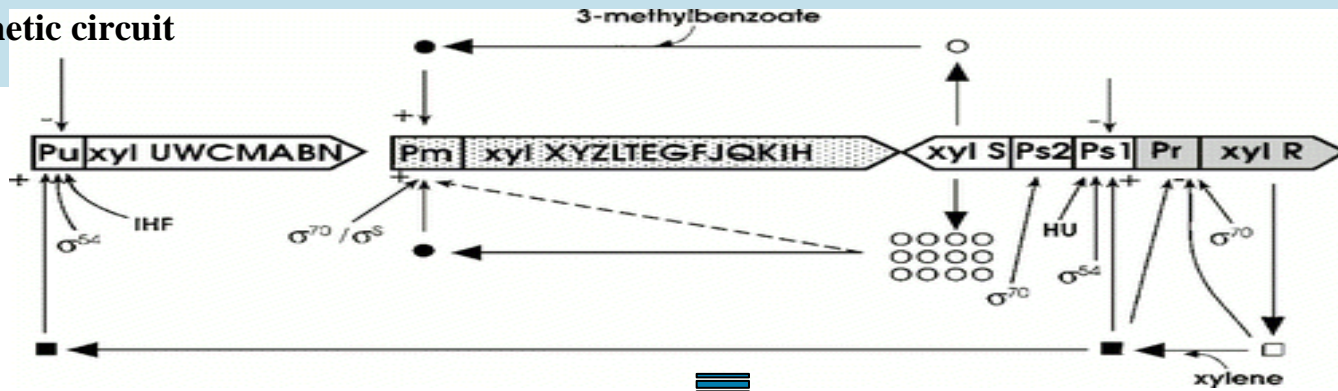


- Need a genetic circuit to combine *promoter* inputs
- Modular, interchangeable inputs and outputs
- Integrating more signals provides more environmental :

AND		
IN 1	IN 2	OUT
0	0	0
1	0	0
0	1	0
1	1	1

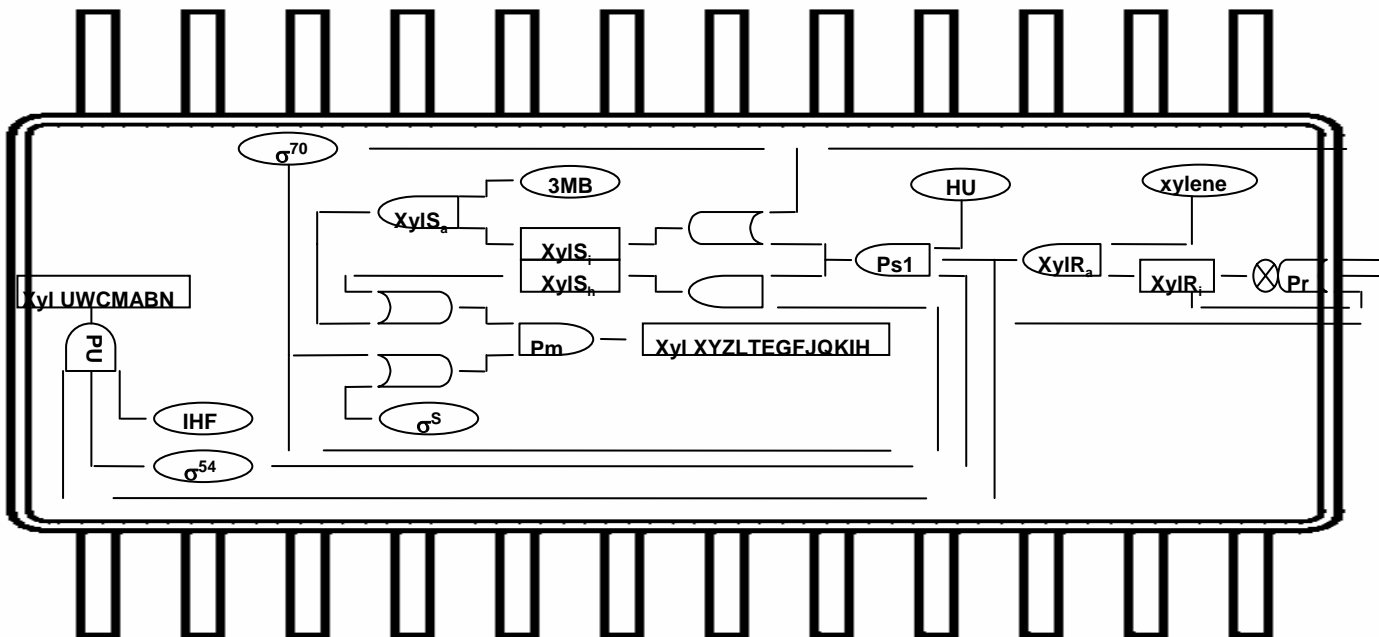
OR		
IN 1	IN 2	OUT
0	0	0
1	0	1
0	1	1
1	1	1

Genetic circuit

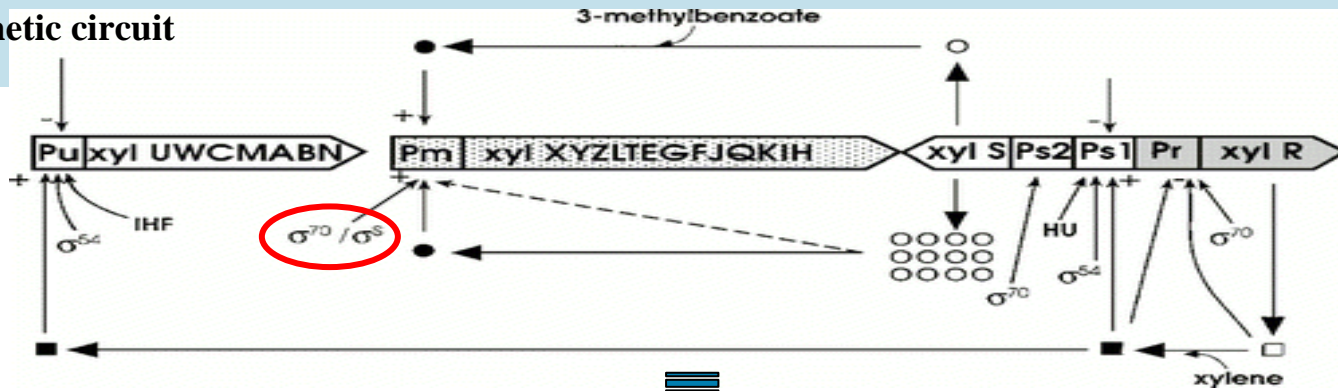


TOL-on-a-CHIP

Logic circuit

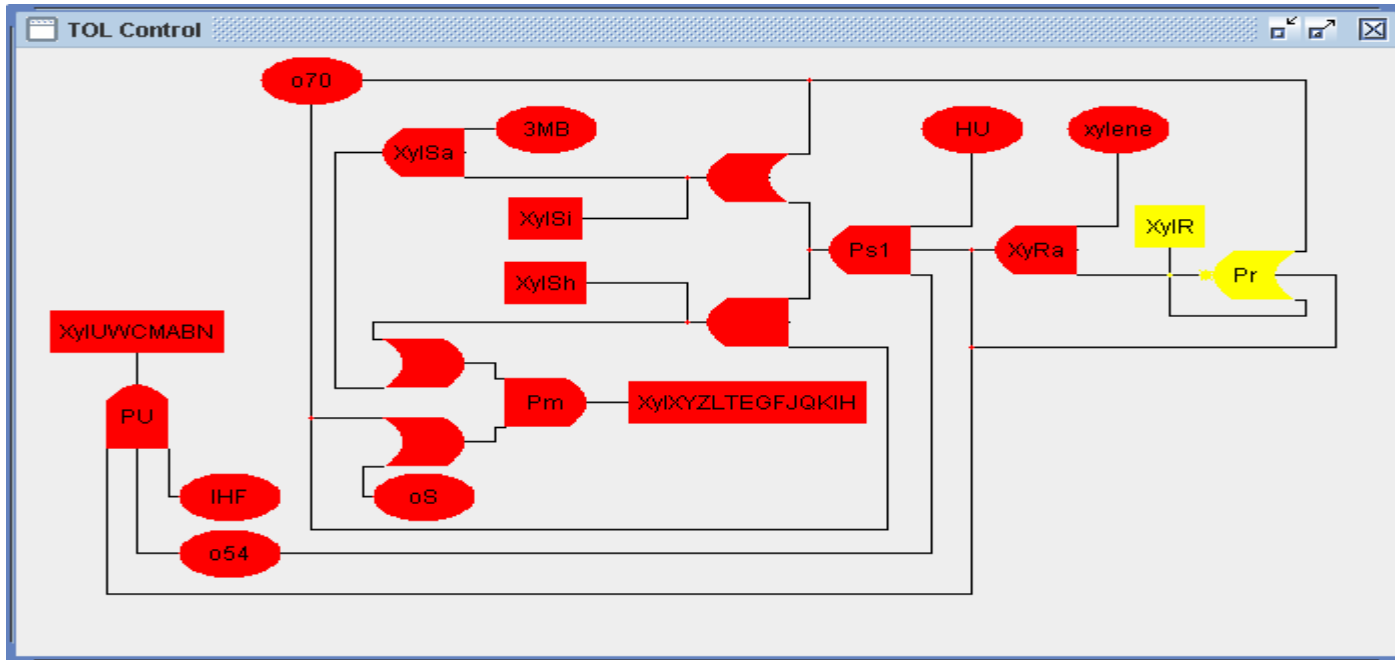


Genetic circuit



TOL-on-a-CHIP

Logic circuit



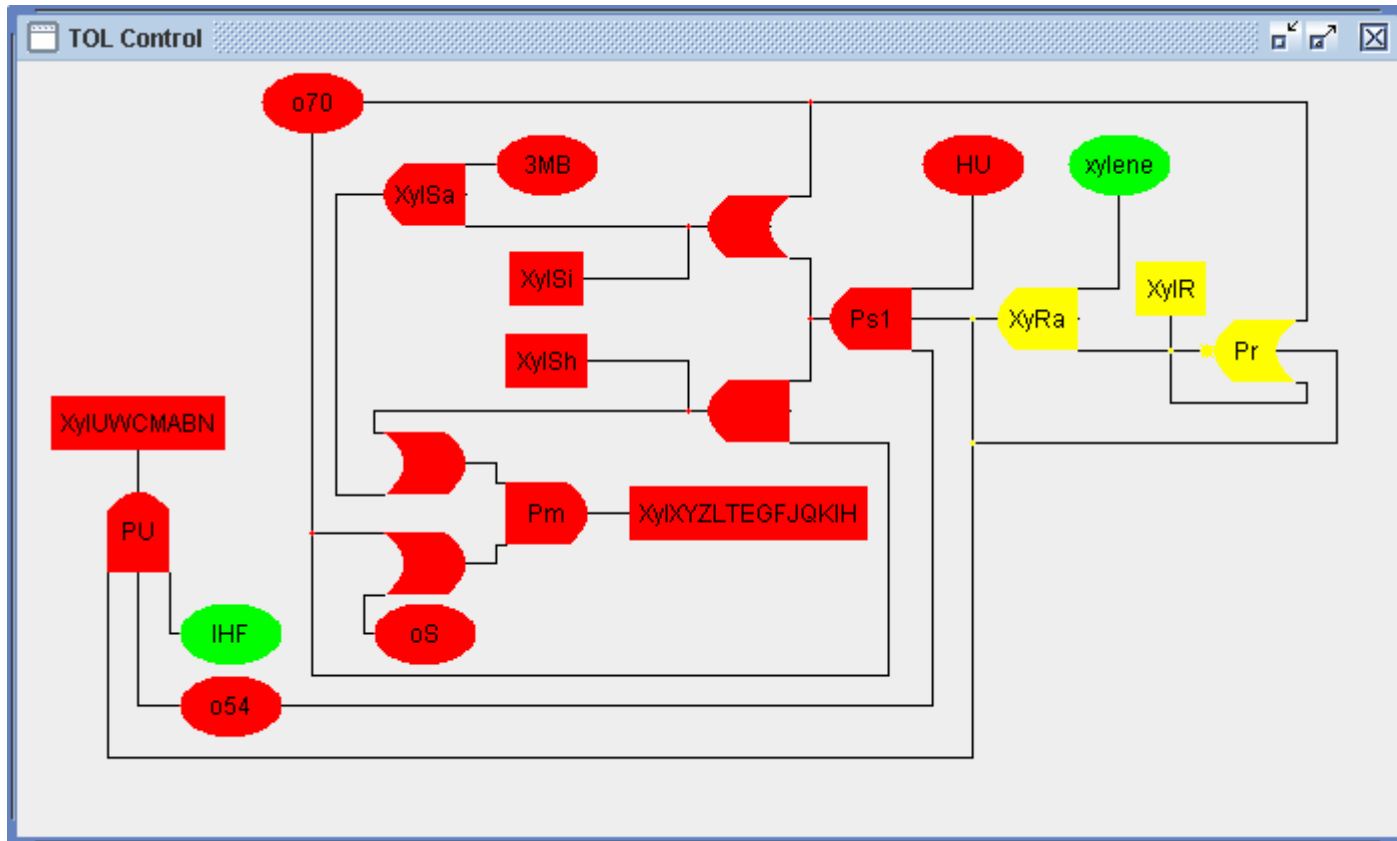
Inputs

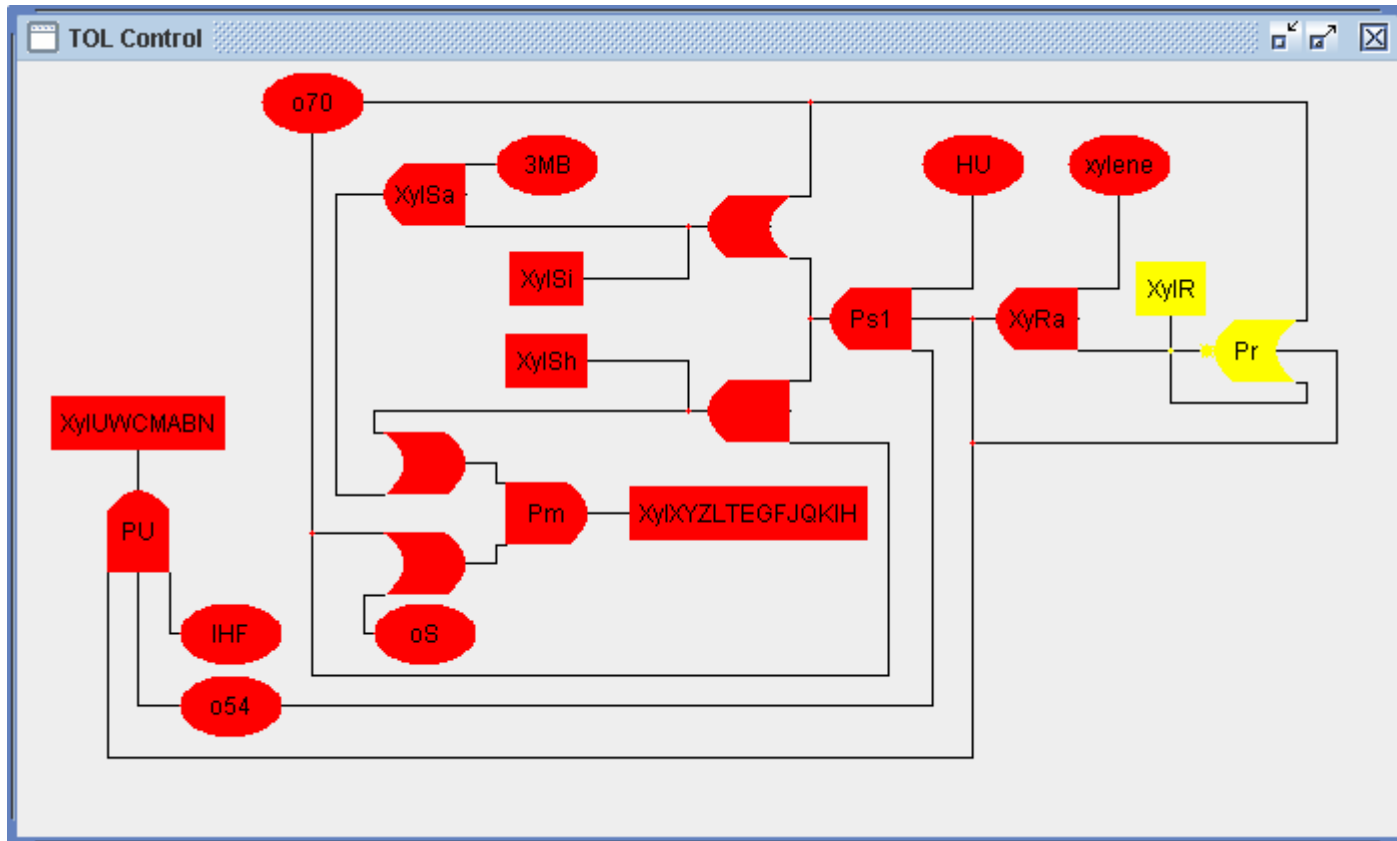
Outputs

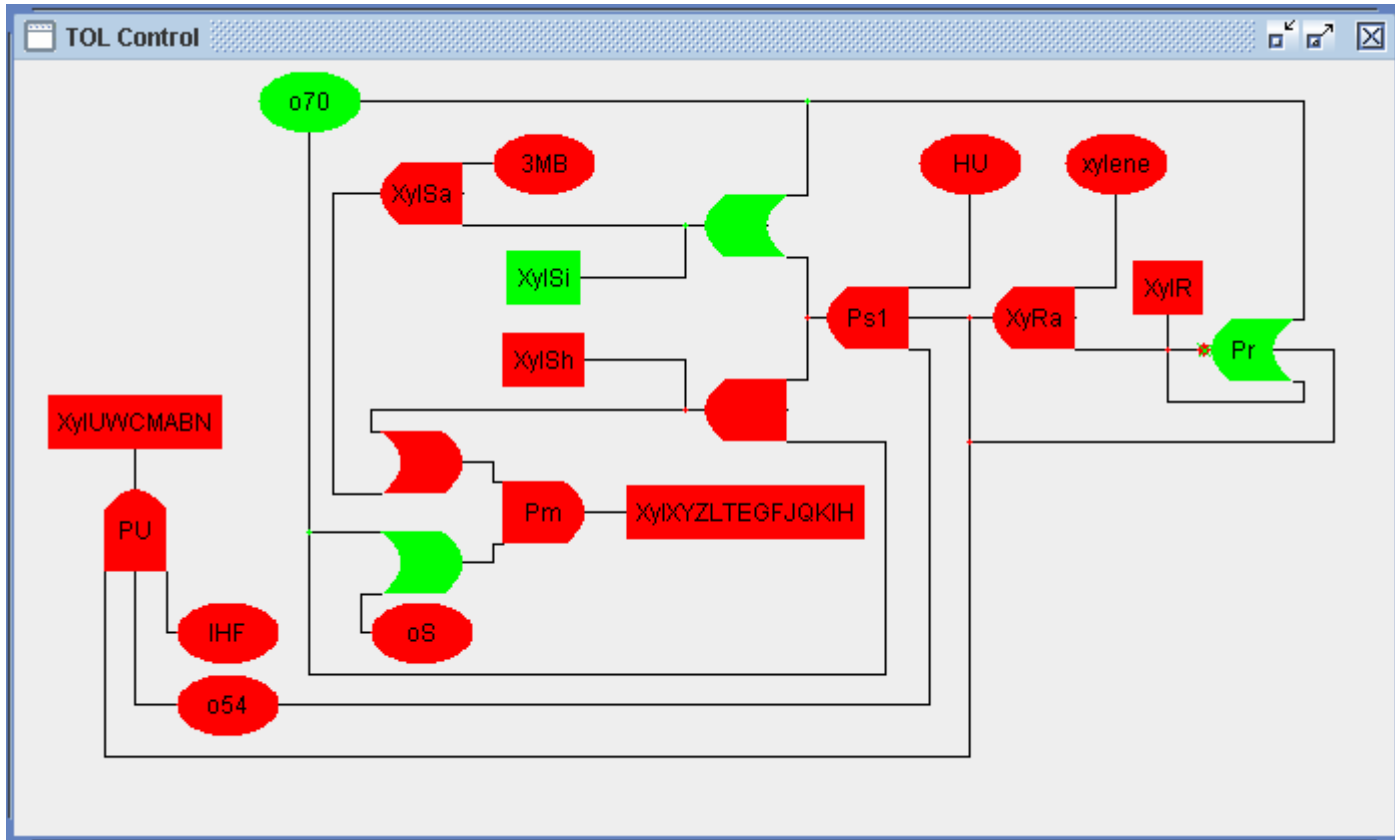
AND

OR

NOR







Model prediction of expression levels

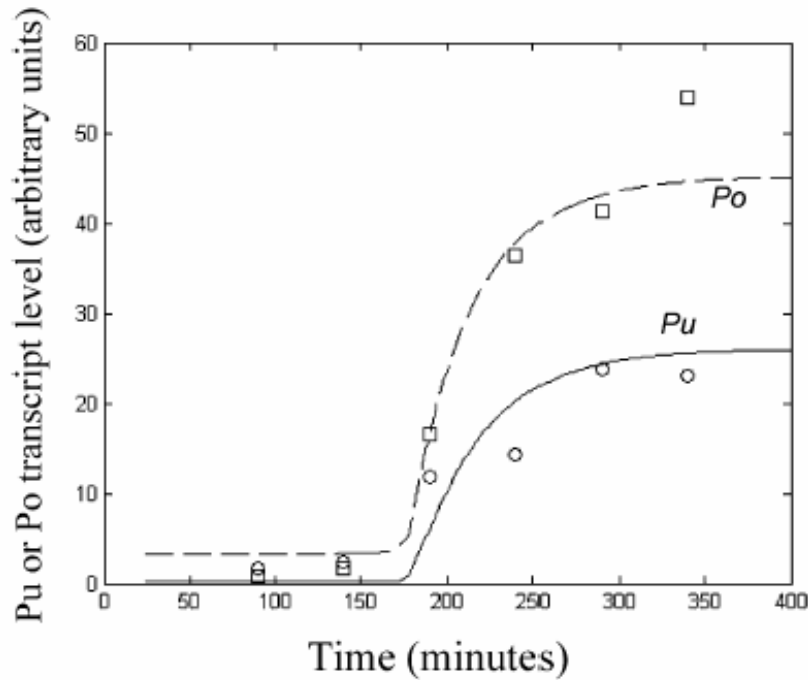


Fig. 1. Model simulation of transcription from σ^{54} -dependent promoters in wild-type cells.

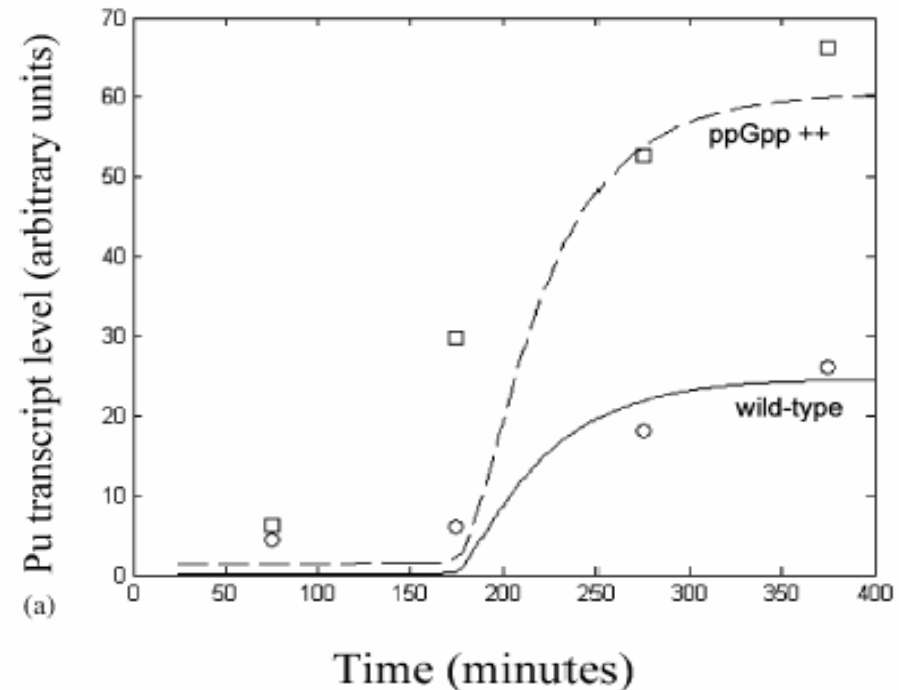
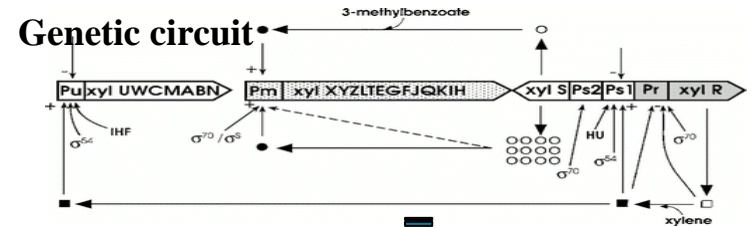
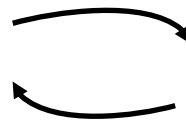
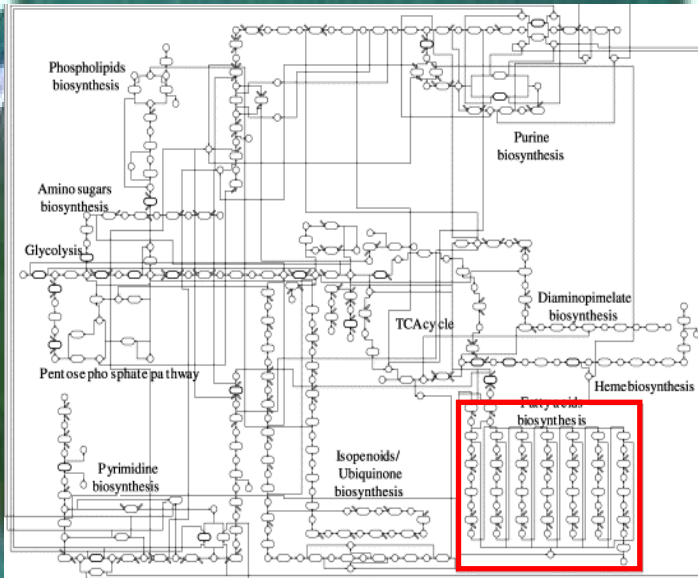


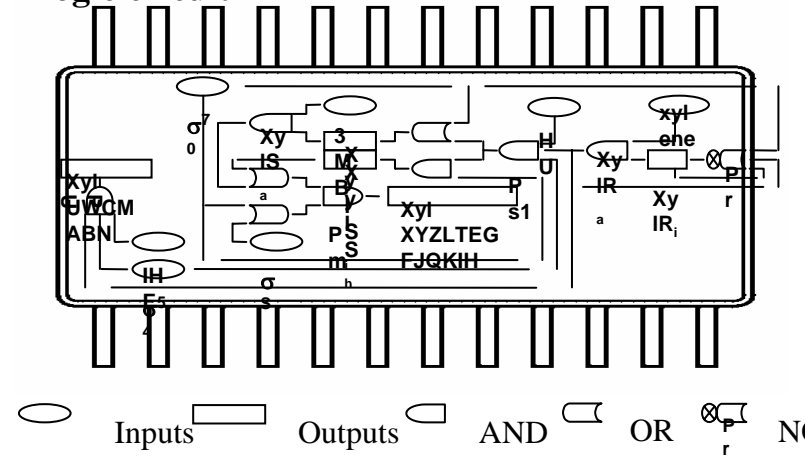
Fig. 2. Effect of σ^{54} overproduction on predicted expression curves of *Pu* (a) and *Po* (b). Solid lines, wild type; dashed line, normalized σ^{54}

Sze et al., 2002, Cases et al., 2001, van Dien et al., 2003

Interplay circuits of interest and genome



Logic circuit

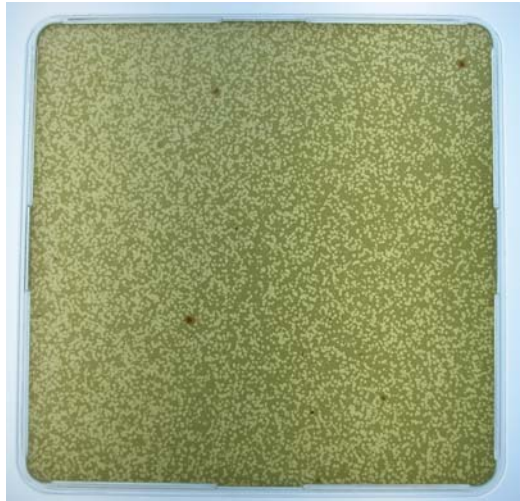


Aim is to have an integrated bacterial blueprint to enable (re-)programming

Niche advantages: importance of cold-adapted enzymes

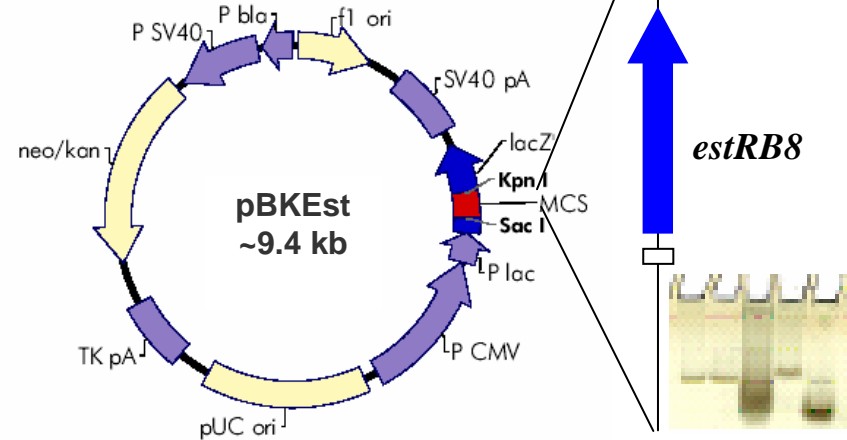
- **Biocatalysis at lower temperatures**
- **Higher enantioselectivities at low temperatures**
- **Lower energy consumption**
- **Bioremediation in cold environments**

An esterase from an expression library of the *O. antarctica* genome



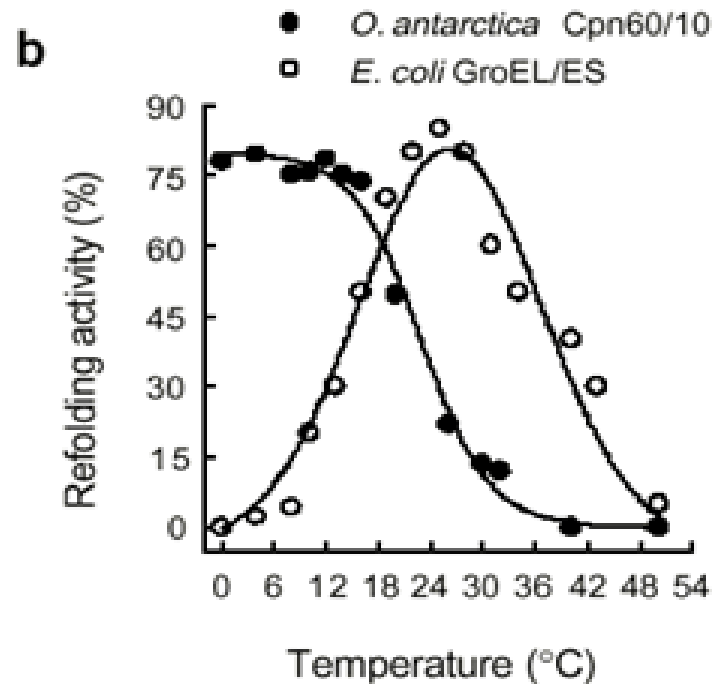
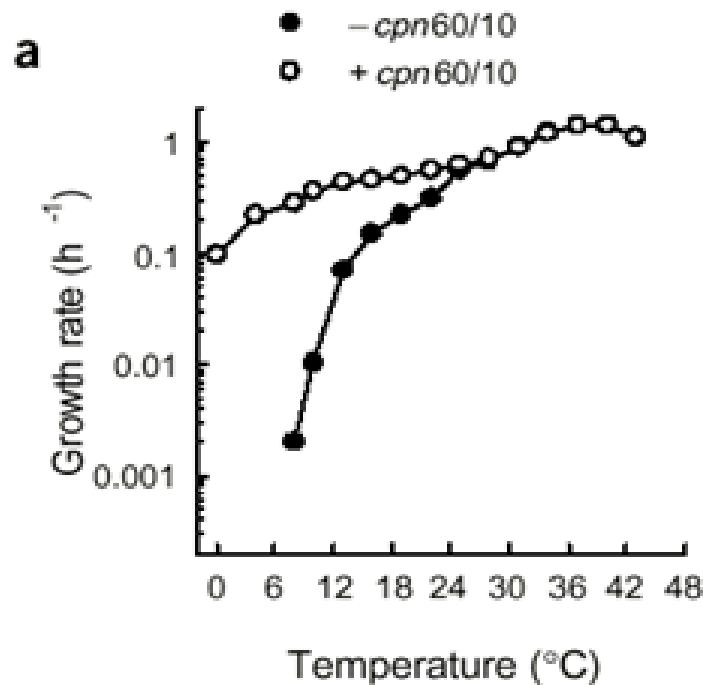
Screening of ca. 10,000 phage clones with chromogenic substrates

Subcloning,
→
hyperexpression



- Esterase EstRB8 obtained from an expression library of *O. antarctica*
- Exhibited low activity when expressed in *E. coli* at 30-37° C
- Reason? Misfolding? Problem with chaperones?
- Why? Chaperones are responsible for the folding and refolding of more than 20-30% of the proteins of the cell

Psychrophilic chaperonin provides advantage to e.coli



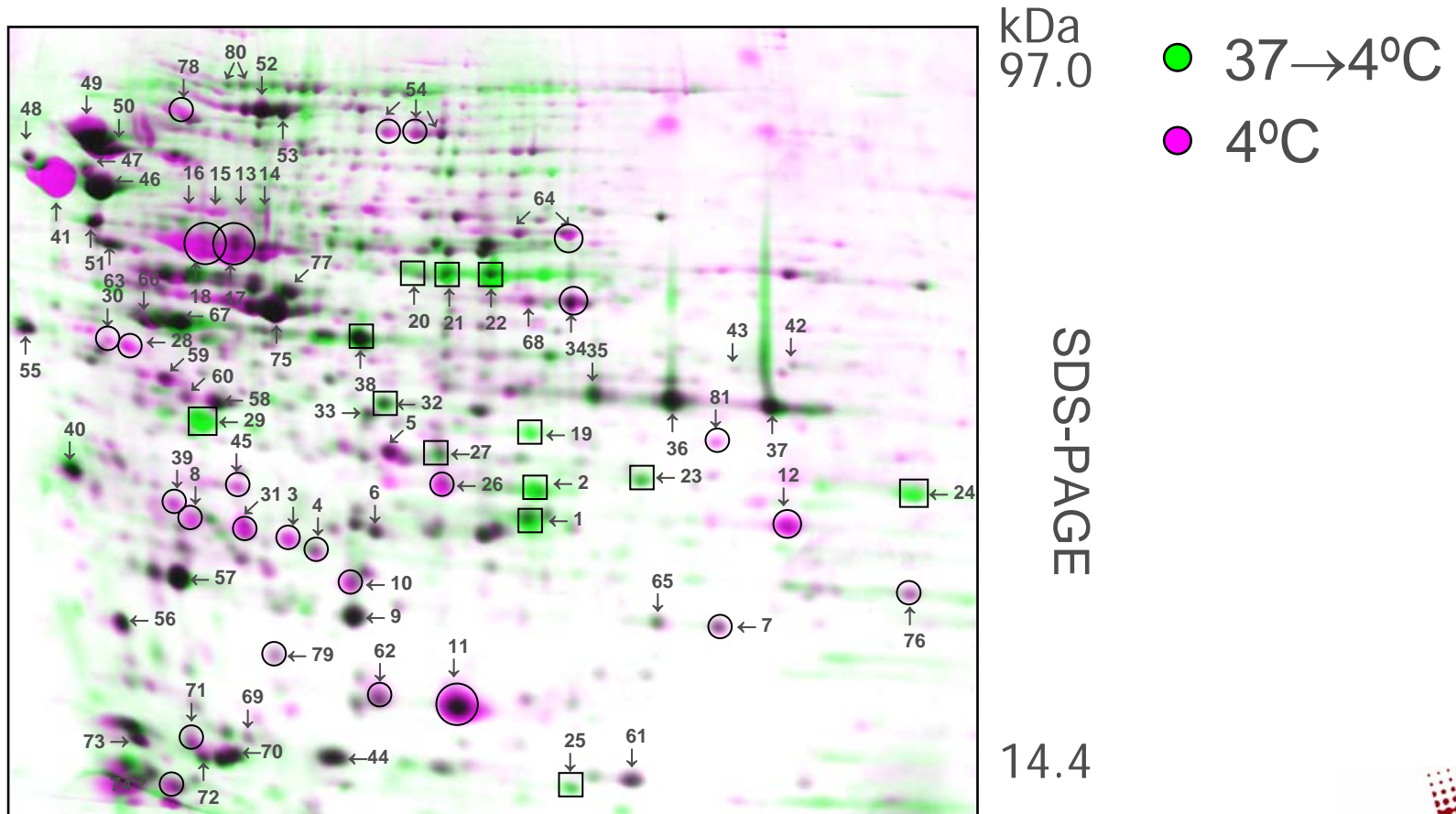
Expression of Cpn in *E. coli* lowers its minimum growth temperature from $7.5^{\circ}C$ to below $0^{\circ}C$ (theoretical minimum $-13.5^{\circ}C$!) and substantively improves its growth at temperatures below $20^{\circ}C$, which correlates with the low temperature optimum of *in vitro* refolding activity of Cpn

Questions:

Why does systems failure take place in *e. Coli* at low temperatures?

How does cpn protect *e. Coli* at low temperatures?

Testing *E. coli* to (cold) destruction: proteomics of systems failure in *E. coli* cells at 4° C and rescue by Cpn



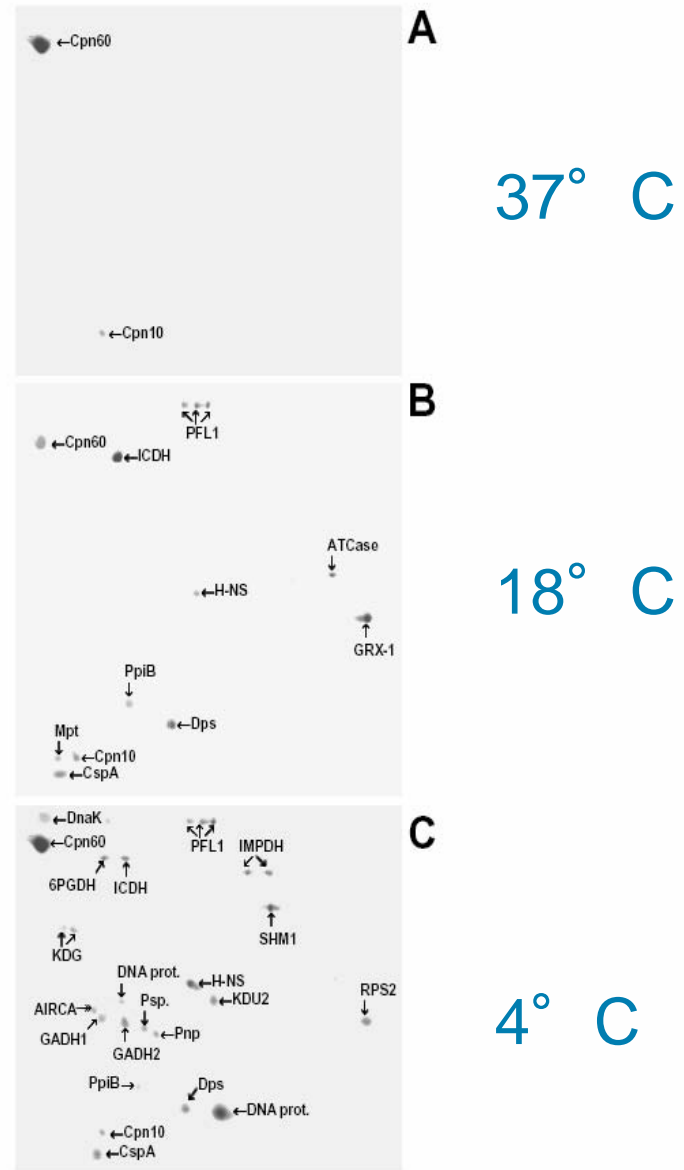
Why system's breakdown at low temperatures

- A range of housekeeping proteins, in particular nucleic acid metabolism and chaperone functions, are found at higher levels in Cpn⁺ *E. coli* cells at 4°C.
- Systems failure in Cpn⁻ *E. coli* cells at 4°C presumably results from cold inactivation of one or more of these
- Prevention of systems failure probably results from increased interactions of the key protein(s) with the Cpn chaperonins
- Can these interactions be captured by co-precipitation with Cpn?
- anti-Cpn60 antibody raised (anti-GroE antibody used as a control) and used to precipitate Cpn in cells grown at different temperatures

Co-precipitation with Cpn

Key housekeeping *E. coli* proteins
up-regulated at low temperatures
co-precipitate with Cpn
but not GroEL/ES

- DNA-binding proteins (Dps, H-NS, Rps2, Rps6, RbfA and Psp),
 - Molecular chaperones (CspA, ClpB, PpiB and Grx),
 - Proteins involved in energy metabolism (GADH, ICDH and 6-PGDH),
 - Nucleic acid metabolism (Pnp)
 - Polysaccharide degradation (KDU and KDG),
 - *de novo* purine biosynthesis pathway (AIRCA),
 - Amino acid biosynthesis (ATCase),
 - Pyruvate metabolism (PFL1) and acid resistance (GAD)
- all present at significantly higher levels (up to 114-fold)



HOW DOES *cpn* PROTECT *E. Coli* AT LOW TEMPERATURES?

- *Cpn* exhibits temperature-dependent double ring to single ring transitions of its barrel structure
- The single ring form dominating at low temperatures exhibits a greater hydrophobic patch surface
- The single ring form forms more stable associations with proteins fulfilling key cold sensitive functions whose inactivation leads to cellular systems failure
- This association protects these key functions and thereby prevents systems breakdown

Improved expression of important activities

- EstRB8 esterase activities expressed in *E. coli* grown at 4° C were orders of magnitude higher than those in *E. coli* grown at 37° C
- *In vitro*, the *O. antarctica* chaperonins did not promote re-folding of denatured EstRB8, so denatured EstRB8 seems not to be a substrate of Cpn
- the role of Cpn in obtaining high yields of active EstRB8 esterase is to allow growth of the host strain at 4° C, a temperature optimal for production and stability of the esterase

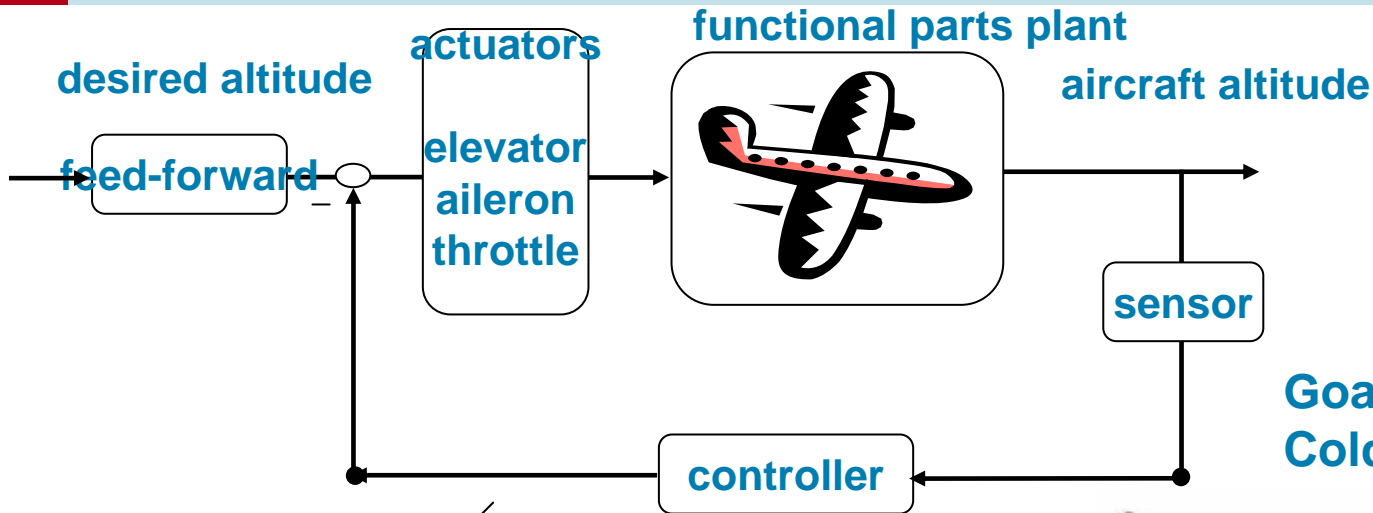
Applications of the cpn^+ *e.Coli* system

Functional expression/obtaining high yields of heterologous proteins, esp. thermosensitive proteins

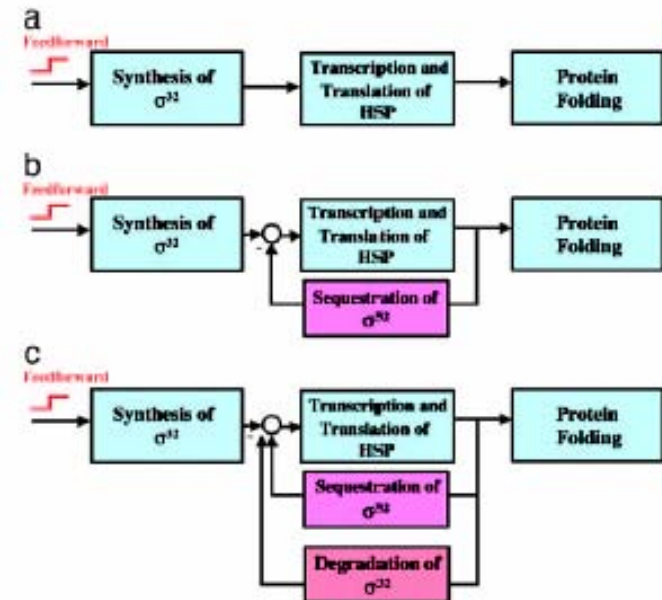
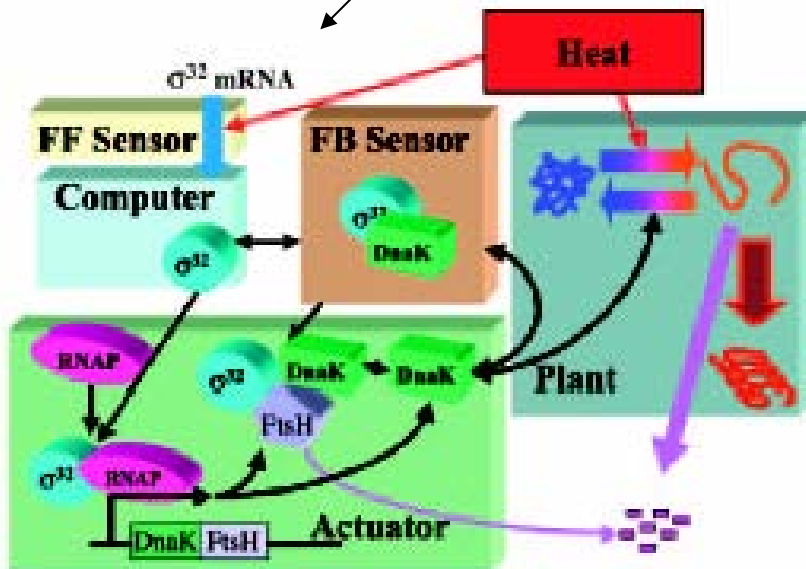
Functional expression of hydrophobic proteins

Re-folding of some denatured proteins and prevention of aggregation

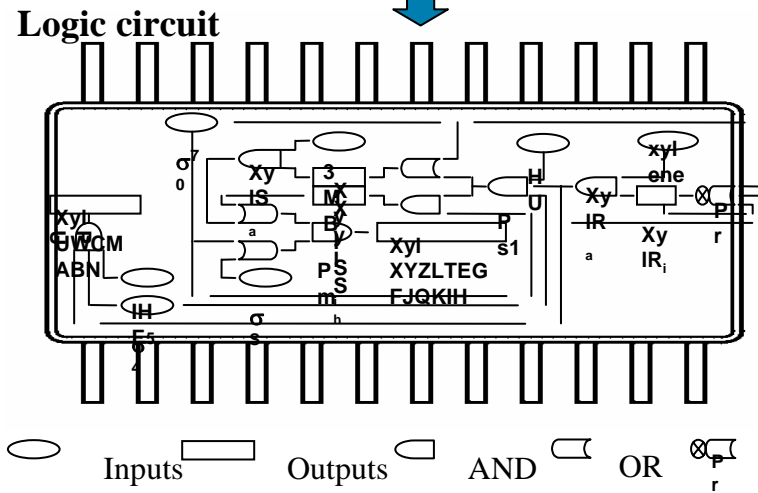
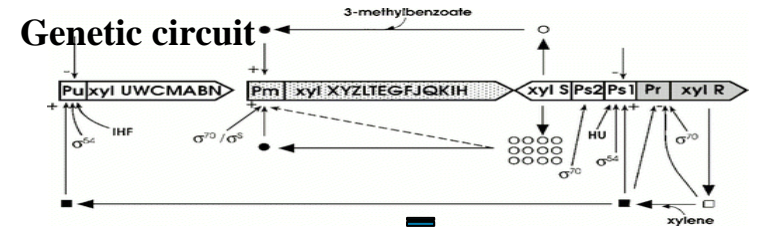
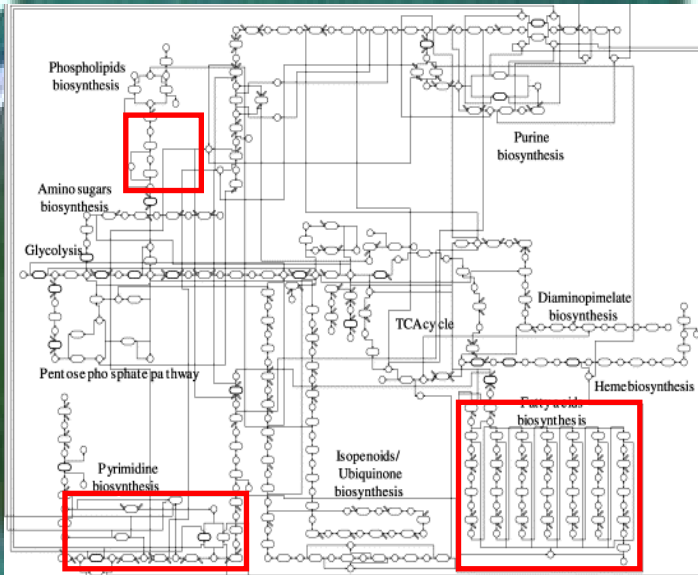
Reverse engineering of the cold network module



Goal: Obtain blueprint of Cold shock network



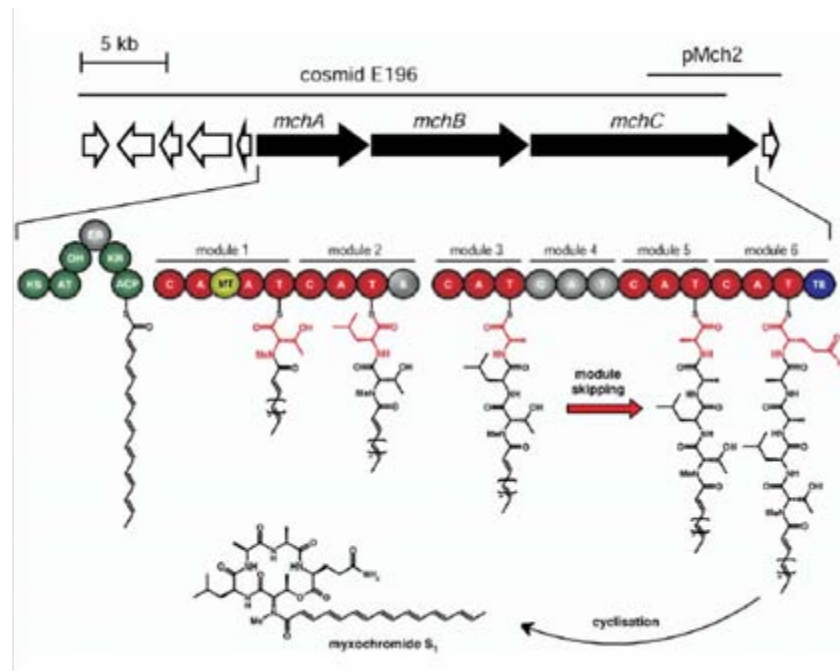
Interplay circuits of interest and genome



Aim is to have an integrated bacterial blueprint to enable (re-)programming

Outlook

- Modular description, design and construction of circuits for biocatalysis
- Explore the *Pseudomonas* „Chassis“ for heterologous expression of
- genes poorly expressed in traditional hosts and gene clusters
- from metagenomes



Acknowledgments:

HZI:

Peter Golyshin
Manuel Ferrer

Piotr Bielecki
Agata Bielecka
Taras Nechytyaylo
Amit Khachane
Miguel Godinho
Jacek Puchalka
Ken Timmis

PROBACTYS partners:

Victor de Lorenzo, CNB, ES
E. Pistikopoulos, Imperial College London, UK
Antoine Danchin, Institute Pasteur, FR
Jan de Bont, TNO, NL
Huanming Yang, Beijing Genome Centre, CHINA
Angel Cebolla, Biomedal, SP

External Collaborations:

J. Papin/M. Oberhardt Univ. Virginia, USA
J. Edwards Univ. Delaware, USA
B. Tümmler, MHH, DE