

„Okolje“ v kontekstu sintezne biologije:

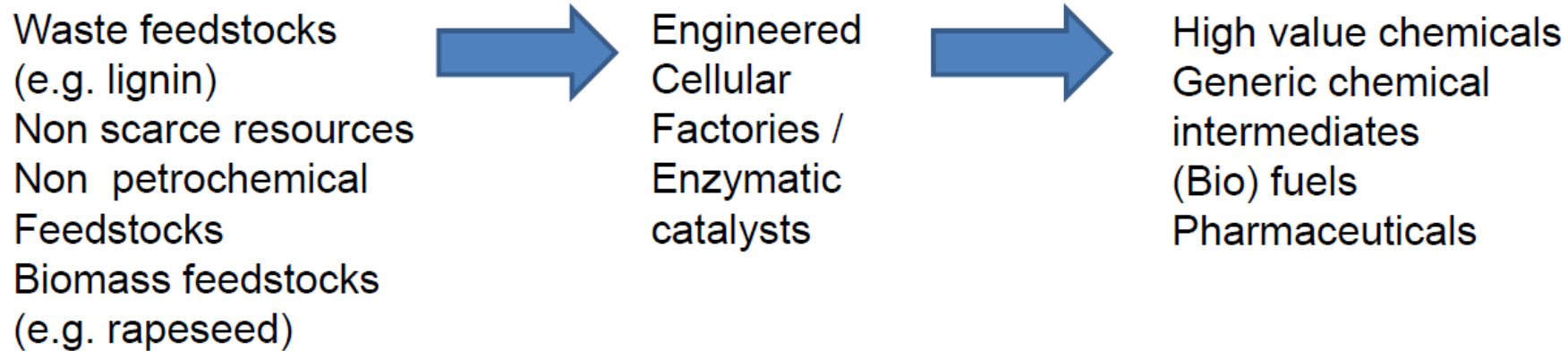
- prispevek k zmanjšanju onesnaževanja
- okoljski senzorji
- bioremediacija
- sintezno okolje / inženiring ekosistemov
- GM rastline z vezji za večjo odpornosti proti okoljskim dejavnikom

RAEng (2009) Visions (10 and 25 years), as these relate to the environment

1. Advanced biofuels
2. Carbon capture e.g. by the development of artificial leaf technology
3. Engineer new types of environmentally – friendly pesticides
4. Substitution of petroleum- based products with biologically engineered substitutes
5. Synthetic biotechnology processes and protocols for the synthesis of fine chemicals industrial-based enzymes and pharmaceuticals.

Technology Strategy Board / RCUK Industrial Feasibilities Competition

1. Sustainable manufacturing and energy production



Closed loop approaches, eco-efficiency, less reliance on non renewable sources, yield improvement and reduced use of toxic chemicals

2. Novel (e.g. engineered phage, sterile vector) approaches for human and livestock antibiotics, pest control, disease control

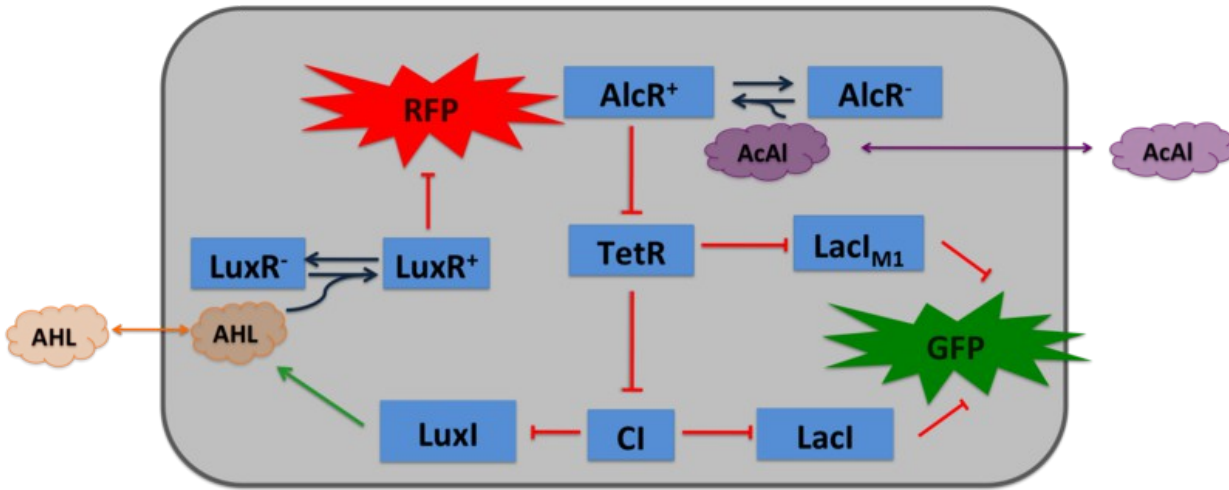
potential for reduction in pesticide use, and potential for impacts / interactions of engineered organisms released into the environment

Examples of how synthetic biology, promised or developed at even modest scales, could significantly affect the Aichi Biodiversity Targets.

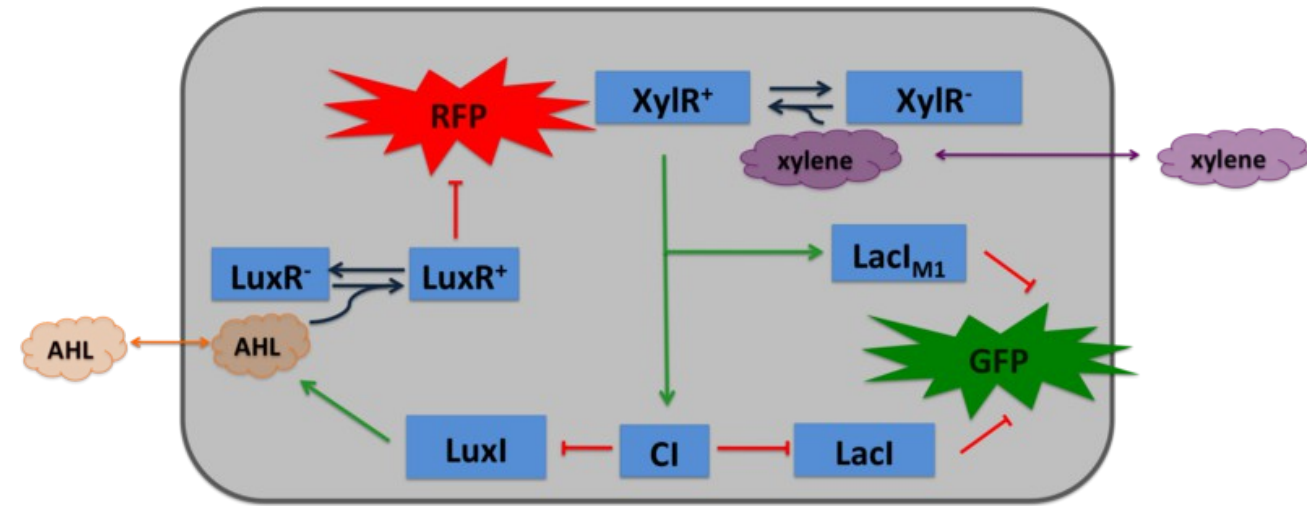
Aichi Strategic Goal	Examples of Potential Impact of Synthetic Biology
<p>A. Address the underlying causes of biodiversity loss by mainstreaming biodiversity across government and society. (Targets 1–4)</p>	<ul style="list-style-type: none"> • Peoples' awareness of biodiversity may be affected by an ability to artificially transform organism genomes, eroding understandings of what "nature" is (1) • Transition to sustainable production and consumption (which protects biodiversity) may be promoted (4) • Proposed synthetic biology solutions might move policymakers away from addressing underlying causes for biodiversity loss (4)
<p>B. Reduce the direct pressures on biodiversity and promote sustainable use. (Targets 5–10)</p>	<ul style="list-style-type: none"> • Synthetic traits in organisms may promote invasive capabilities (or novel organisms may be invasive) (9) • Synthetic organisms might improve potential for ecological restoration or creation (9) • The potential for synthetic organisms in the agricultural production sectors might foster "sustainable intensification" and "land sparing" to reduce land conversion and increase protection of wild habitats (6 and 7) • Industrial uses created by synthetic biology might drive significant land use change towards feedstock production (7 and 8)
<p>C. To improve the status of biodiversity by safeguarding ecosystems, species, and genetic diversity. (Targets 11–13)</p>	<ul style="list-style-type: none"> • Novel organisms might affect the integrity of protected areas (11) • Recreated extinct species may create credits to species lists, allowing natural species extinctions to occur while meeting targets to arrest species extinctions (12) • "Moral hazard" may reduce society's willingness to support measures to conserve endangered species (12) • Synthetic biology capability may make <i>ex situ</i> conservation more attractive relative to <i>in situ</i> with impacts on support for existing protected areas (13)
<p>D. Enhance the benefits to all from biodiversity and ecosystem services. (Targets 14–16)</p>	<ul style="list-style-type: none"> • Synthetic life forms could replace "nature's services" for clean water, clean air, etc., thereby removing the ecosystem services justification for nature conservation (14, 15) • Synthetic biology may extend private ownership of genetic material in ways that restrict access for public benefit (16)
<p>E. Enhance implementation through participatory planning, knowledge management, and capacity building. (Targets 17–20)</p>	<ul style="list-style-type: none"> • Since biological knowledge based on synthetic biology is both different and much more restricted than knowledge for biodiversity conservation, fundamental inequities may prevent the desired coherent, participatory actions for conservation (18 and 19)
<p>There are 20 Targets grouped under five Strategic Goals agreed to by 193 countries that are Parties to the CBD in 2010. Individual target numbers are indicated in parentheses under each example. The full list of targets can be found at http://www.cbd.int/sp/targets/. doi:10.1371/journal.pbio.1001530.t001</p>	

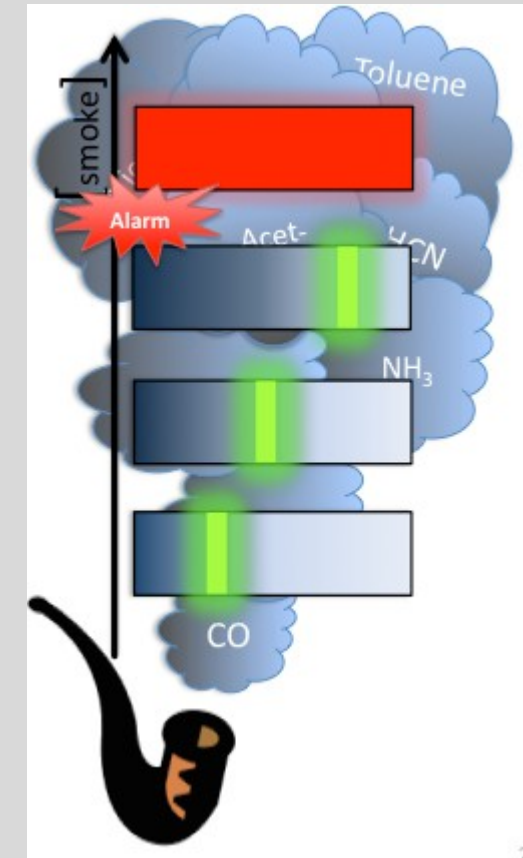
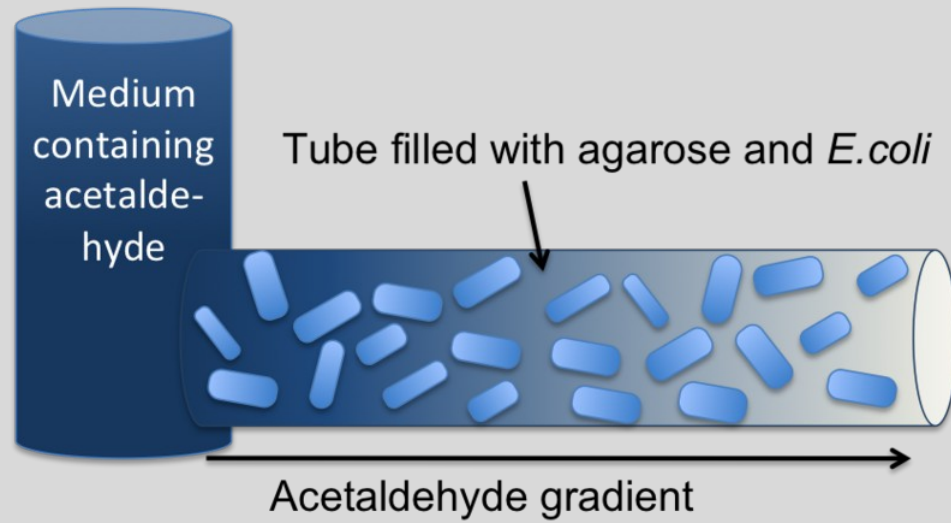
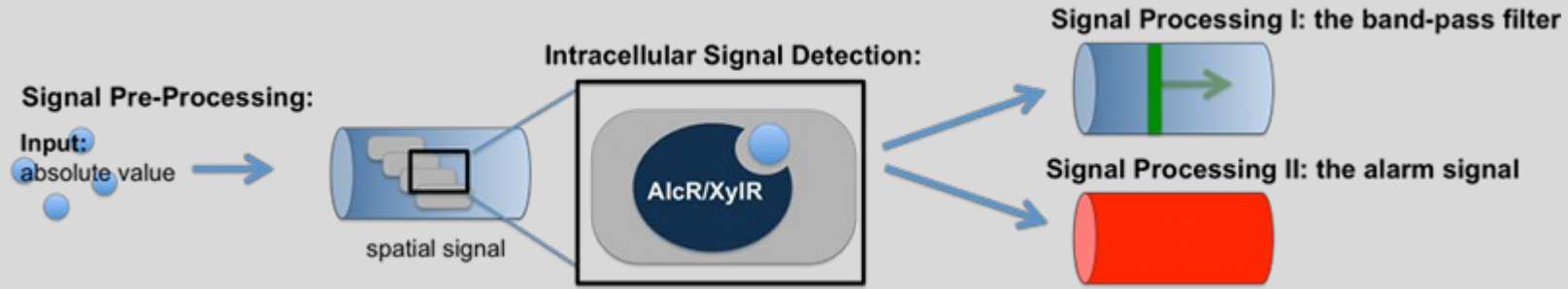
Okoljski senzorji

Inženirsko zasnovani mikroorganizmi se odzivajo na dražljaje iz okolja in v prisotnosti dražljaja sintetizirajo nek reporter. Dražljaji so lahko težke kovine, sestavine kompleksnih mešanic (npr. acetaldehid in ksilen v cigaretnem dimu, arabinoza v gojišču) itd.



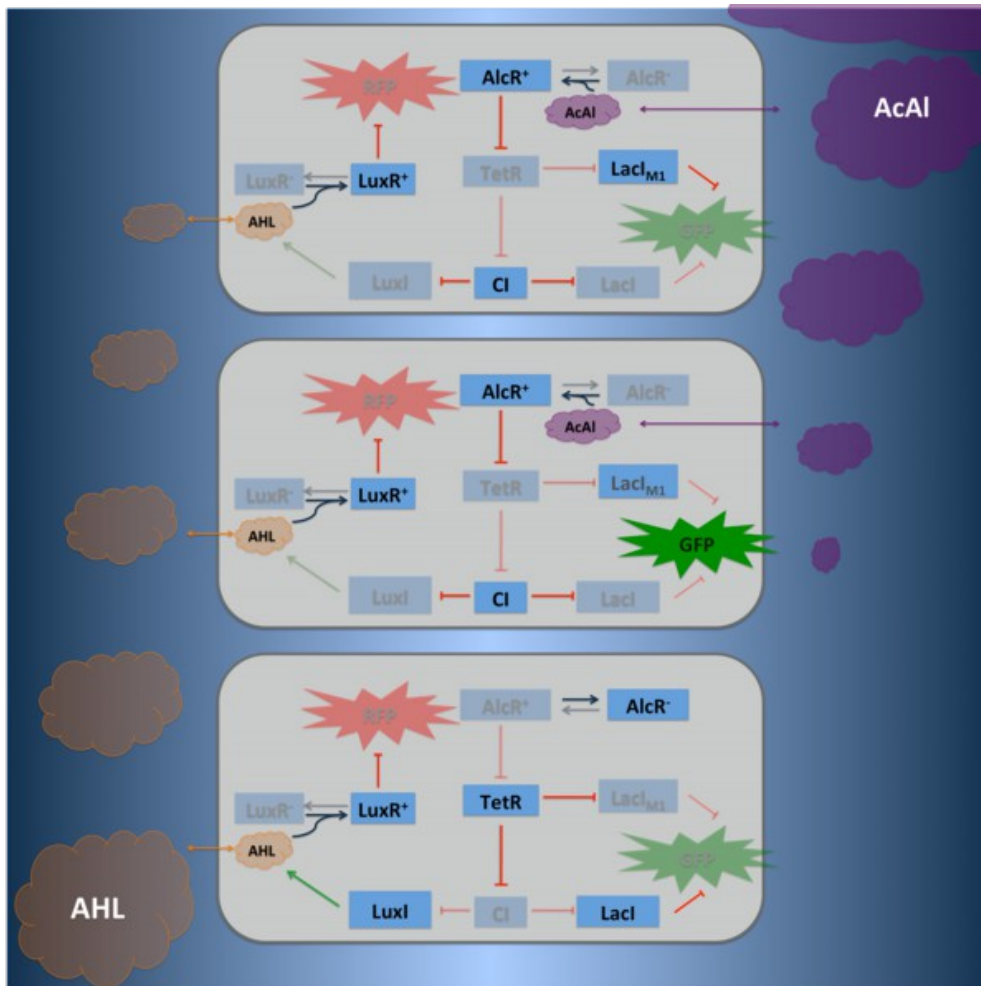
SmoColi, iGEM 2011, ETH Zürich



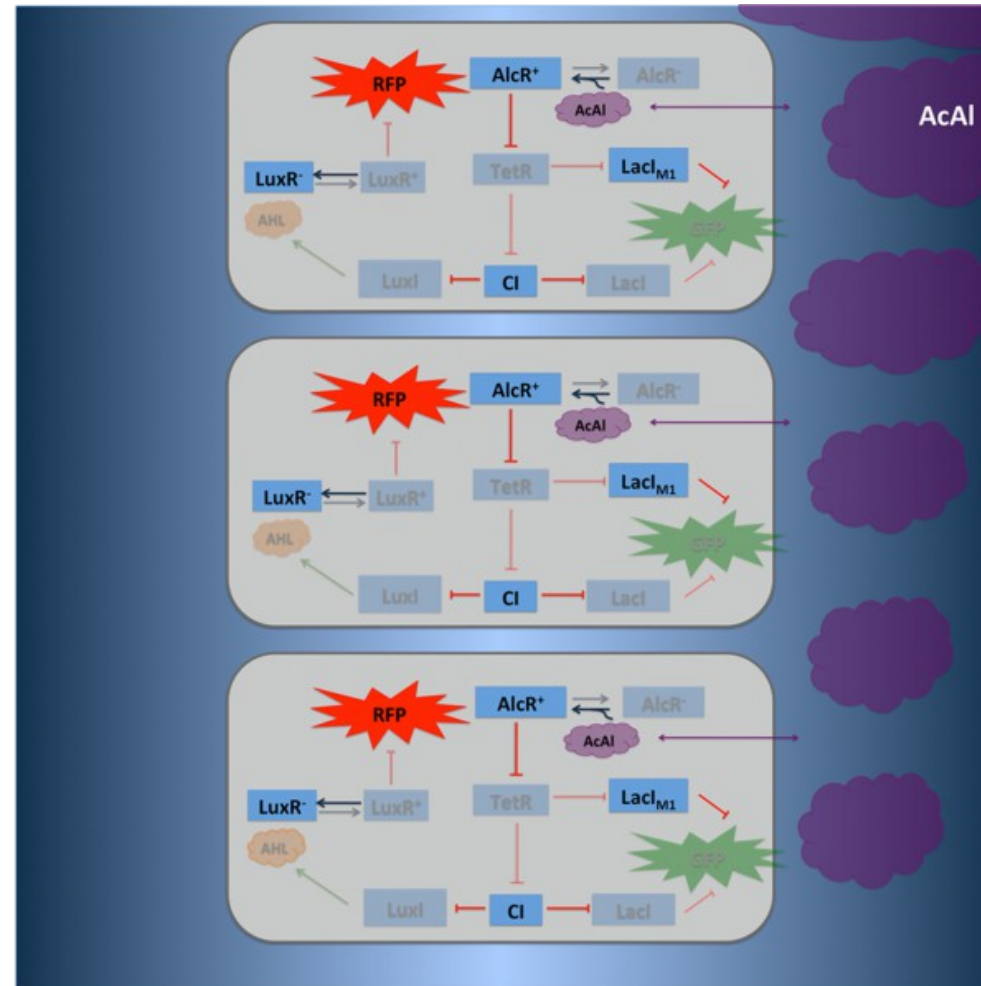


Pasovnoprepustni filter

Senzorski sistemi se lahko odzivajo različno na različne koncentracije dražljaja. Za izvedbo je treba v vezje vključiti pasovnoprepustni filter (band pass filter), ki uvede različen odziv glede na raven dražljaja.



konc. acetaldehida je srednja – GFP



konc. acetaldehida je visoka – RFP

AlcR: receptor za acetaldehid; po vezavi na operator deluje kot represor

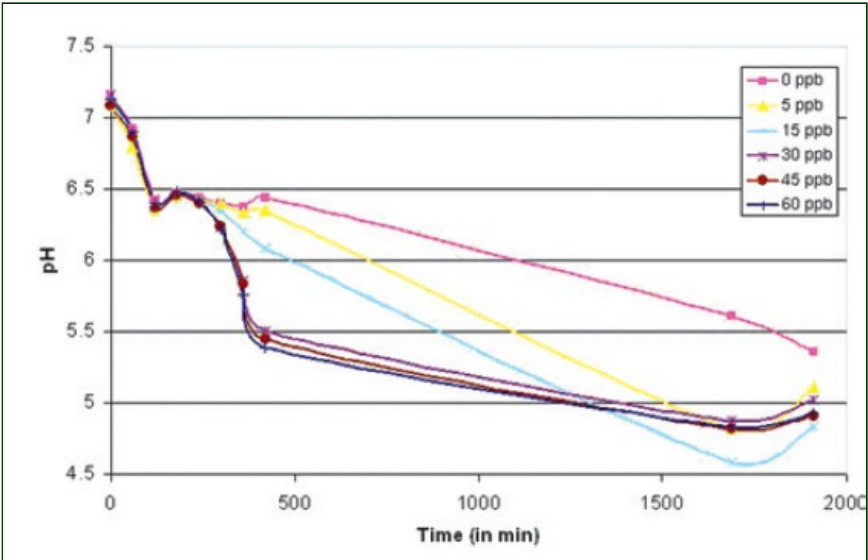
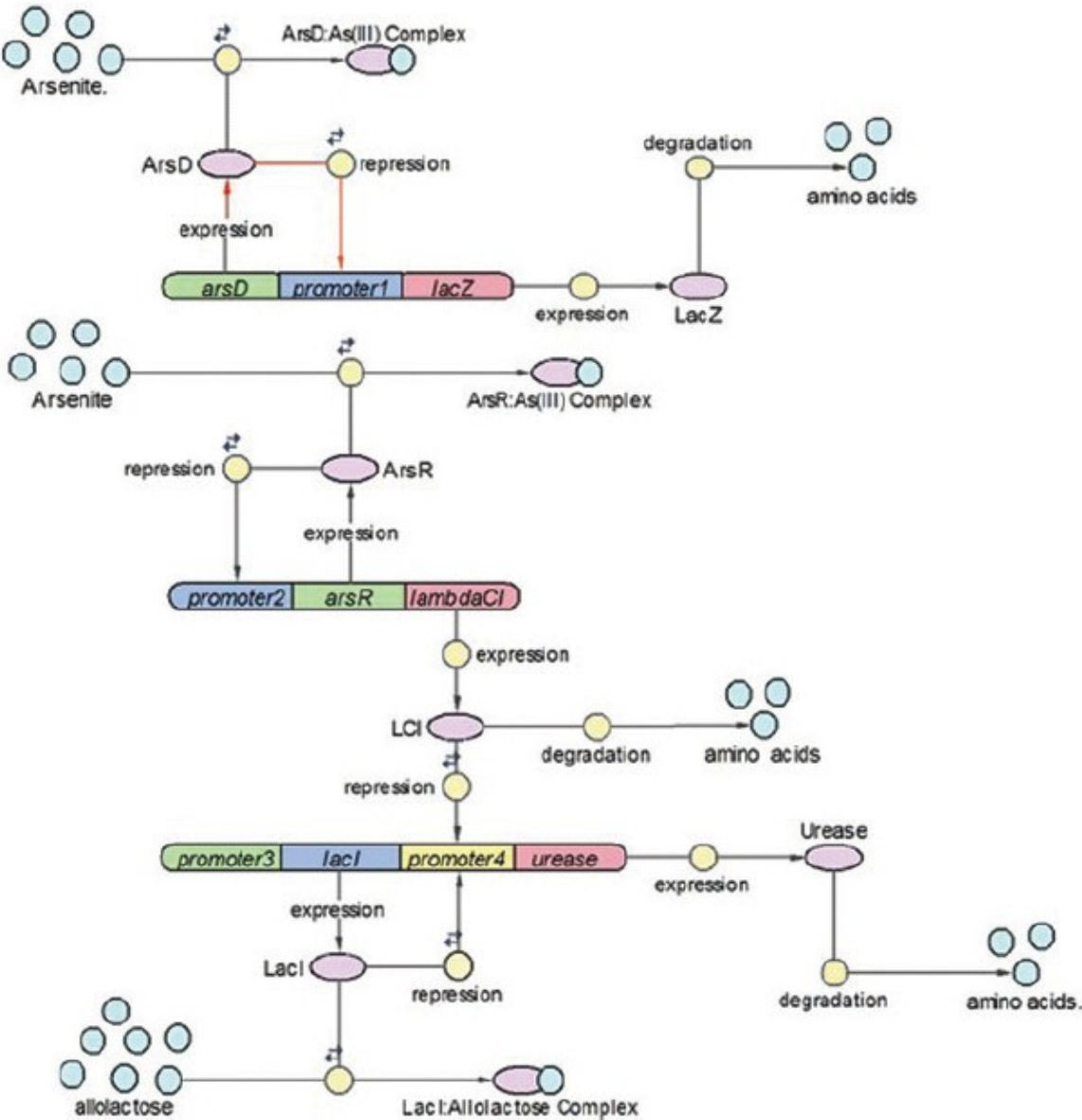
TetR: deluje kot inverter za sicer negativni signal

CI uravnava izražanje AHL sintaze (LuxI) – samo pri zelo nizkih konc. acetaldehida.

AHL difundira v sosednje celice in se tam veže na receptor LuxR, ki je hkrati represor za sintezo RFP.

AHL proizvajajo samo celice na tisti strani zelenega pasu, kjer je konc. acetaldehida nizka, na na koncu takih celic sploh ne bo več, zato represija RFP usahne in sinteza RFP steče.

Visokoobčutljivi senzor za arzen



Bioremediacija – saniranje onesnaženja v okolju z uporabo naravnih in inženirsko pripravljenih organizmov (običajno mikrobov). Molekule, ki predstavljajo onesnaženje, pretvorijo do končnih razgradnih produktov.

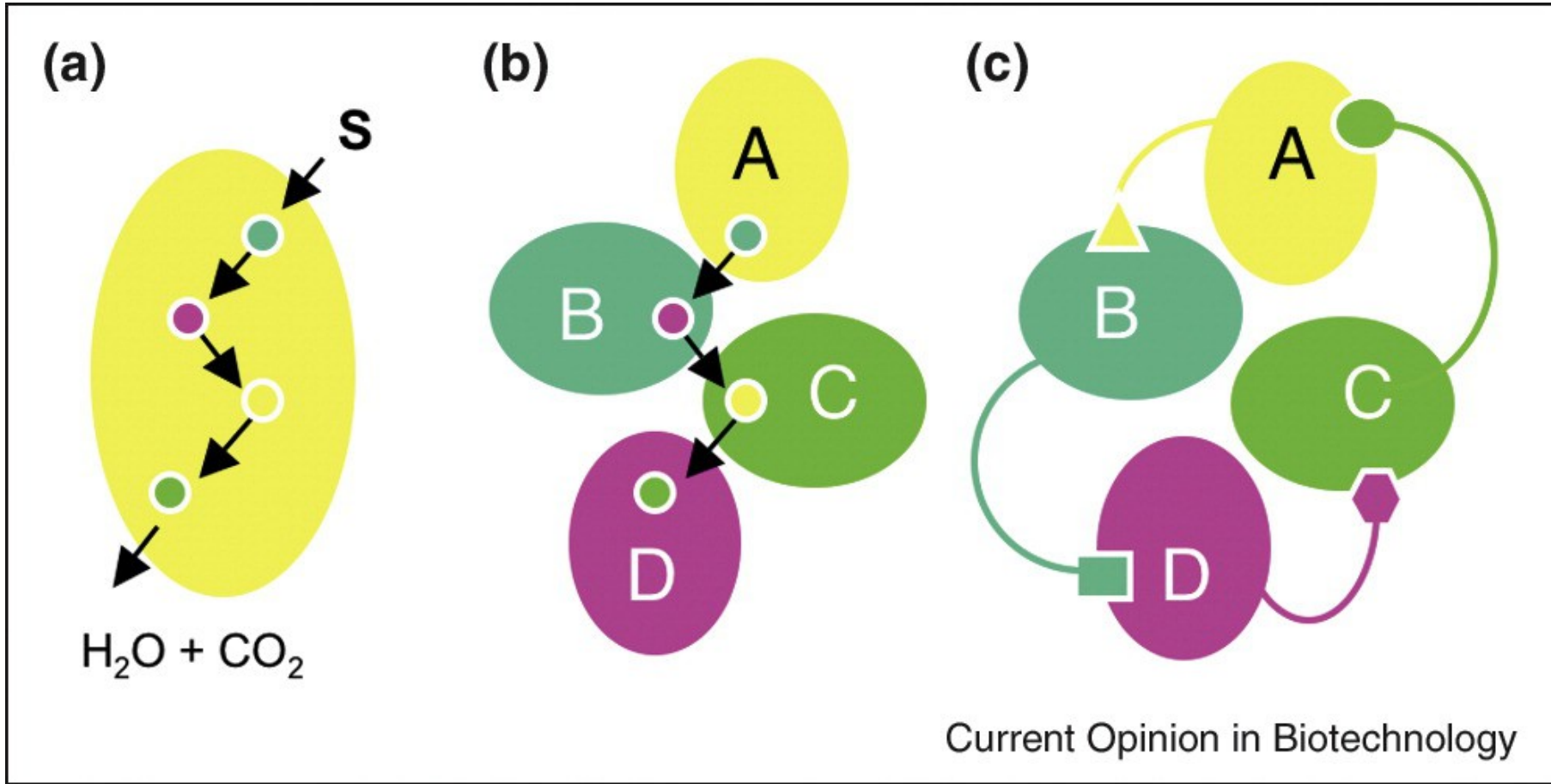
Kritične molekule v okolju: toksini, ksenobiotiki.

Primeri onesnaženj: minska polja, razlitja nafte, sproščanje izotopov, odlagališča težkih kovin

Izhodišče za razvoj primernih organizmov je pogosto *Pseudomonas*.

Cilj sintezne biologije je pripraviti mikroorganizme, ki bodo obstojni v degradiranem okolju, bodo samostojno zaznavali prisotnost polutanta ali toksina in na kontroliran način razgrajevali neželene snovi. Če bi bili neopazno vključeni v okolje, bi lahko v njem bili stalno prisotni.

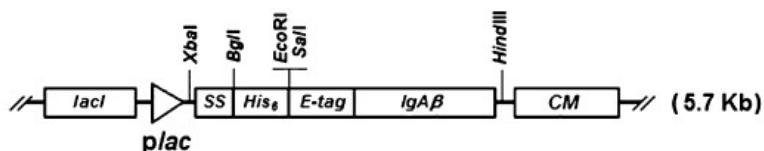
V naravi sicer obstajajo organizmi, ki lahko razgrajujejo kompleksne snovi, a običajno delujejo počasi in je njihov spekter pretvorb sorazmerno ozek. Z uvedbo novih razgradnih poti, odstranjevanjem stranskih reakcij in optimizacijo pretokov intermediatov bi lahko pripravili zmogljivejše seve.



Synthetic Phytochelatin Surface Display in *Cupriavidus metallidurans* CH34 for Enhanced Metals Bioremediation

A

pHEβ



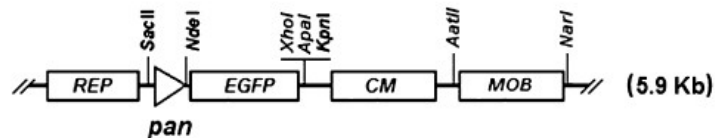
B

pCM1



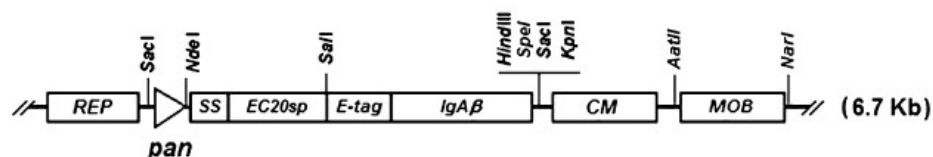
C

pBB-panEGFP



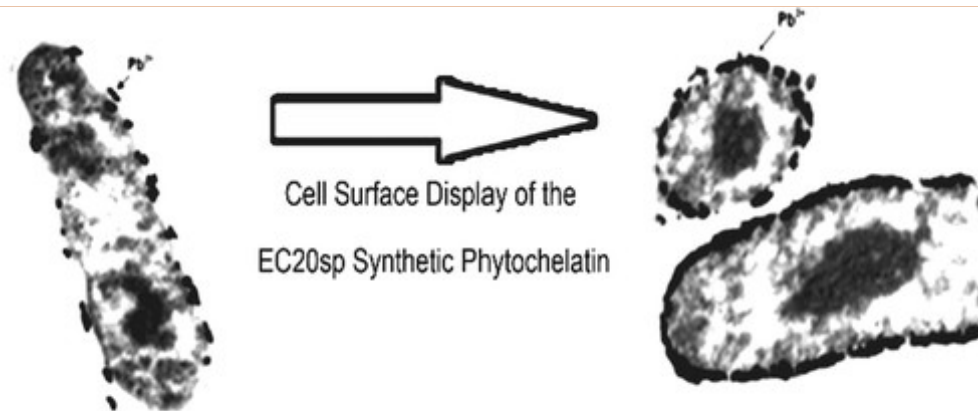
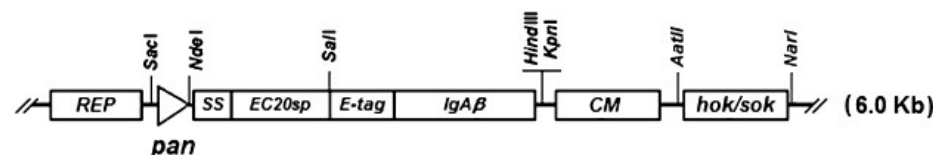
D

pCM2



E

pCM3

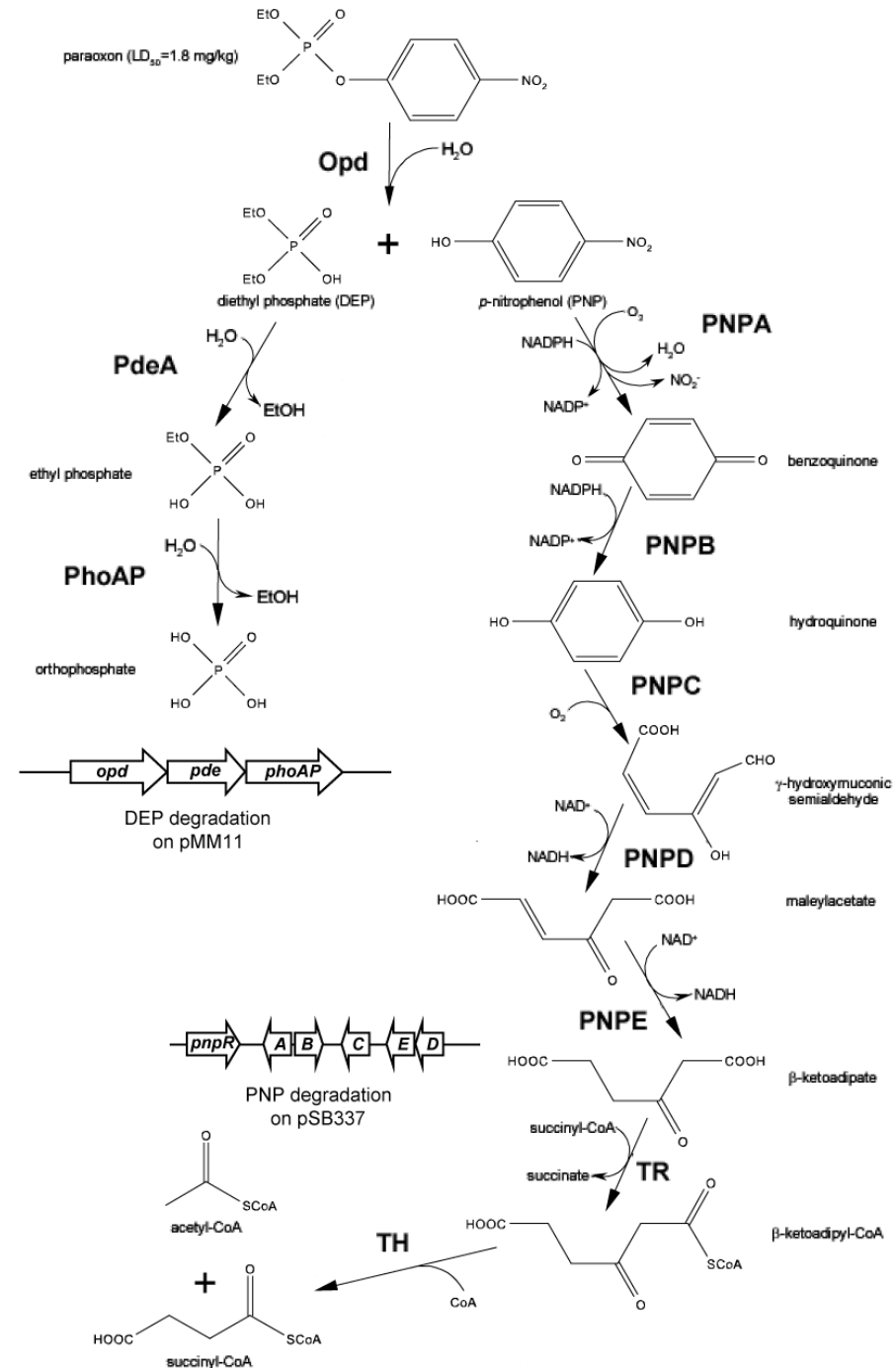


Control Cell binding Pb²⁺

Recombinant Cells binding increased amounts of Pb²⁺

- *C. metallidurans*: zelo odporna bakterija na težke kovine.
- Geni za odpornost so na kromosomu, megaplazmidu in 2 velikih plazmidih: sistemi za efluks → neprimerno za bioremediacijo.
- GS bakterije imajo na zunanji membrani rekombinantni fitokelatin.
- Sintezni gen za fitokelatin je za signalnim zaporedjem, na 3'-koncu pa je zapis za beta domeno avtotransporterja za prekursor IgA-proteaze.
- Promotor iz *B. subtilis* (*mrgA* oz. *pan*): delno inducibilen s kovinskimi ioni, a že visoka bazalna ekspresija.
- Povečana stabilnost plazmida z delecijo regije MOB oz. zamenjavo z regijo *hok/sok* iz *E. coli* (mehanizem za ubijanje celic, ki izgubijo plazmid).

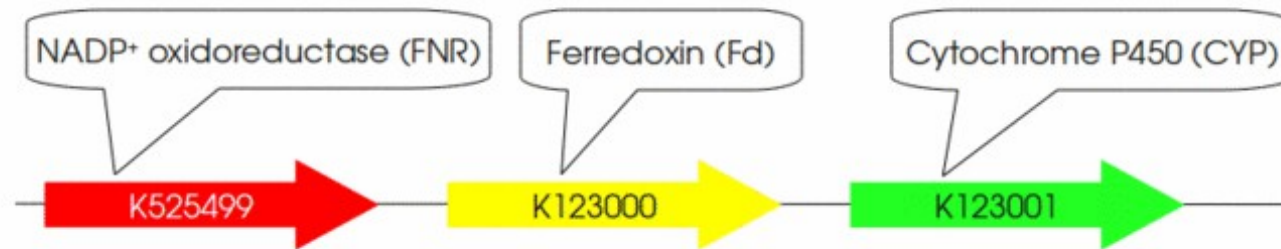
Razgradnja organofosfata paraoksona z bakterijo *Pseudomonas putida*, v katero so vključili operone iz 4 drugih mikrobov. Zmogljivost: 275 mg/l v 48 urah.



Bisphenol A degradation – reaction mechanism



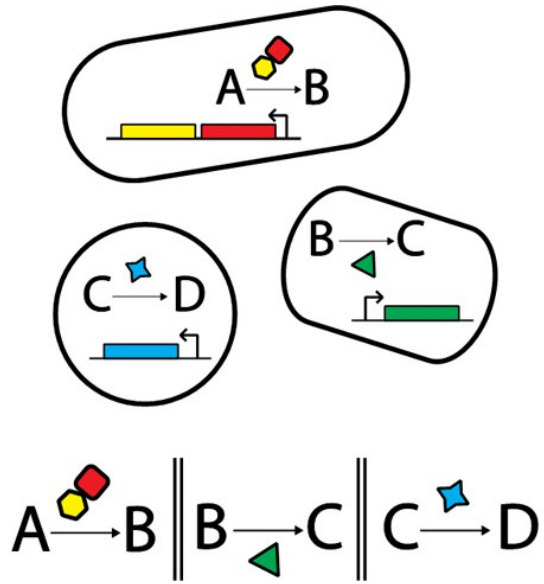
 Needed BioBricks and their functions



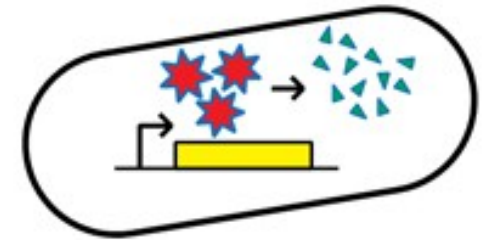
<http://2011.igem.org/Team:Bielefeld-Germany/Project/Description>

Suggested reaction mechanism of bisphenol A degradation and BioBricks needed for this reaction in vitro.

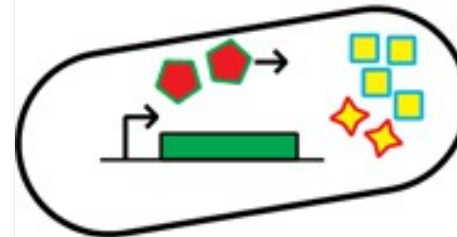
Načrtovanje bioloških združb



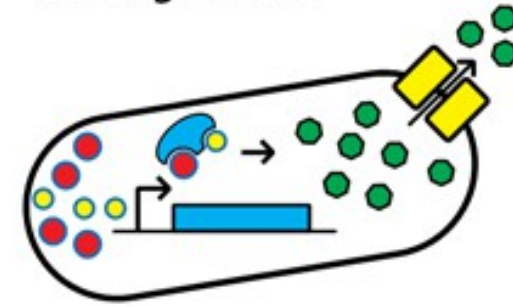
Bio-sensing



Bio-degradation

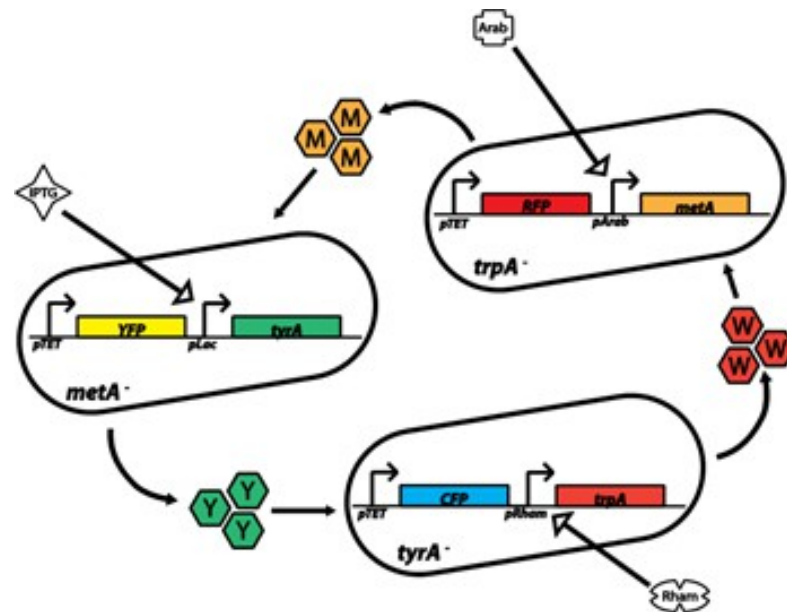


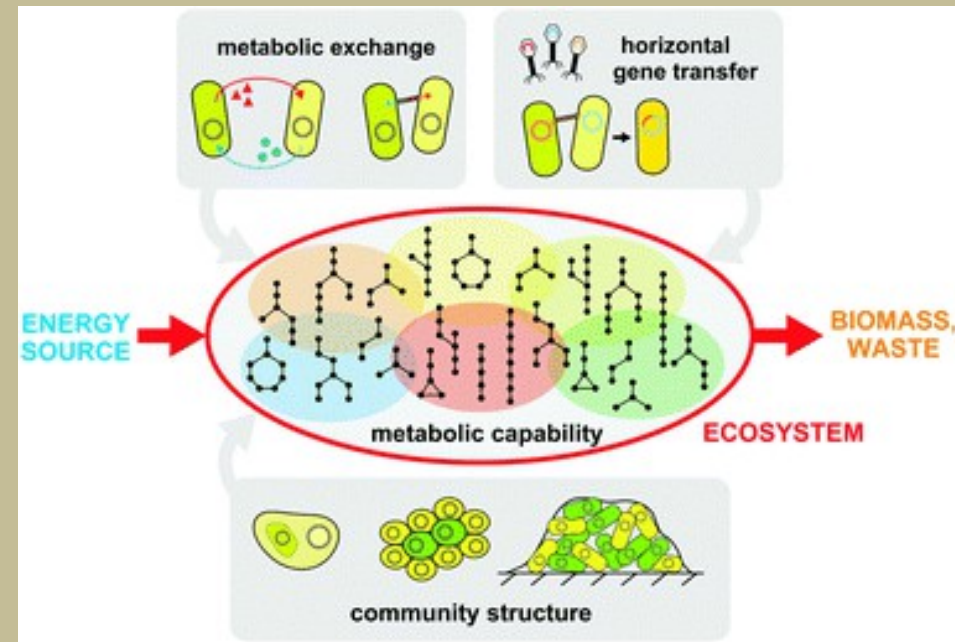
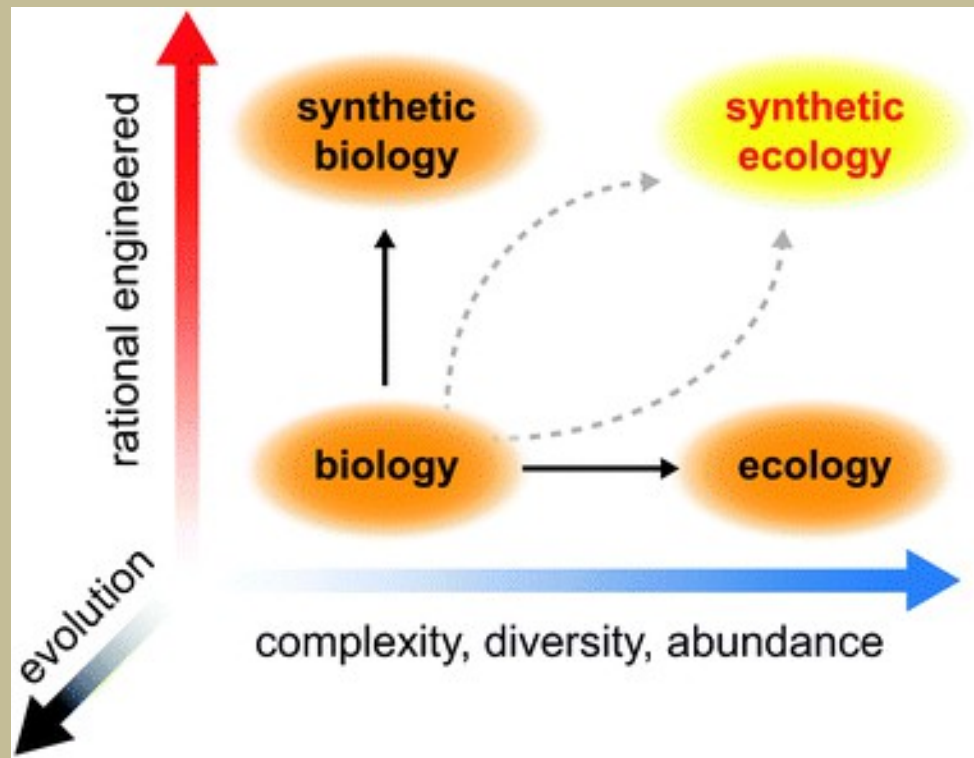
Bio-transformation

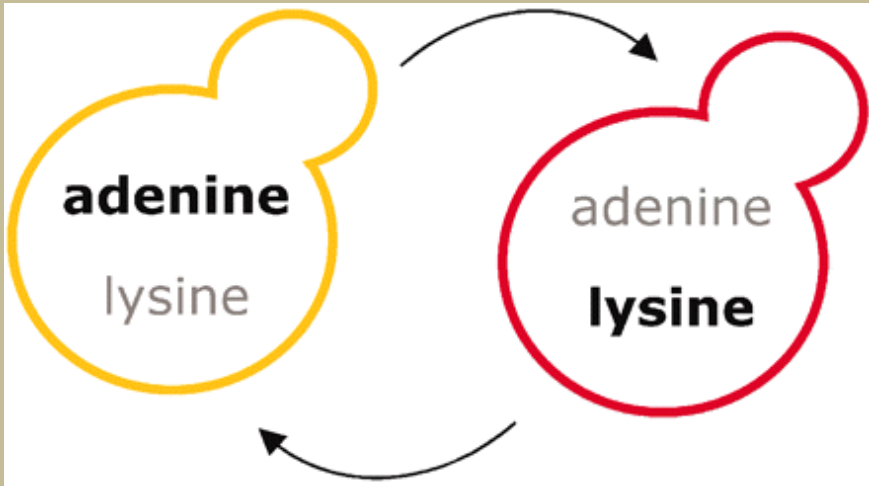


Bio-synthesis

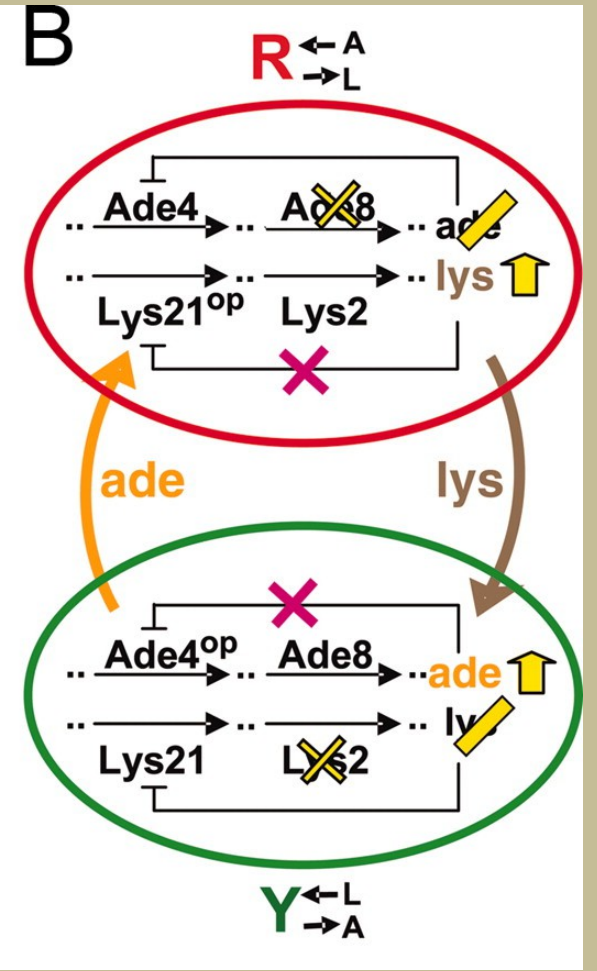
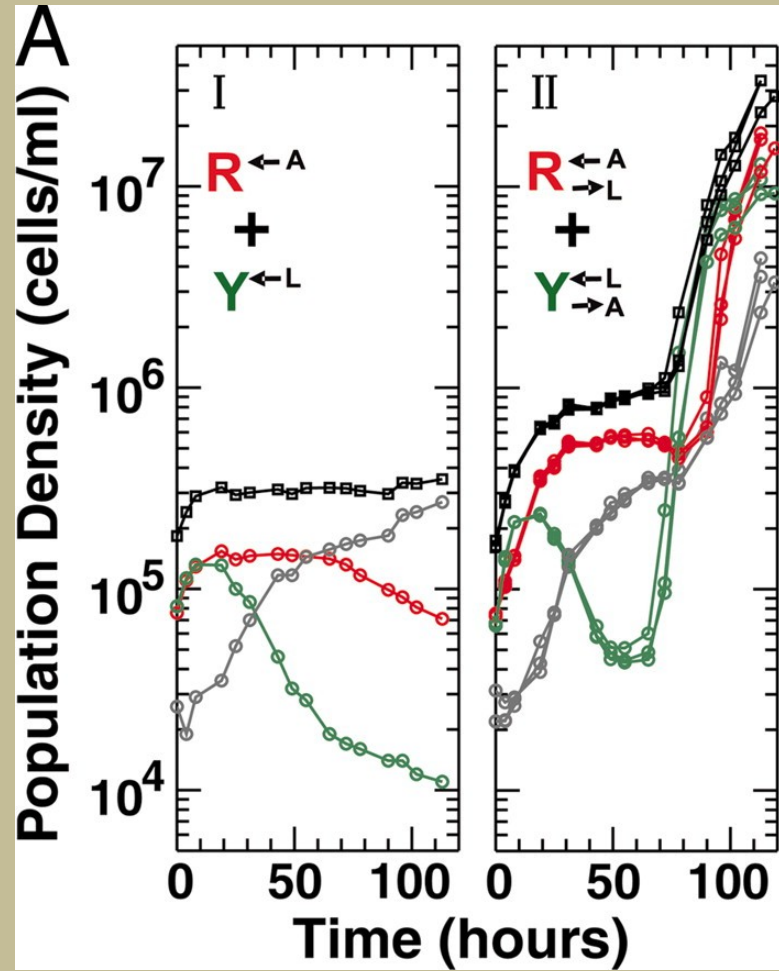
http://2012.igem.org/Team:British_Columbia



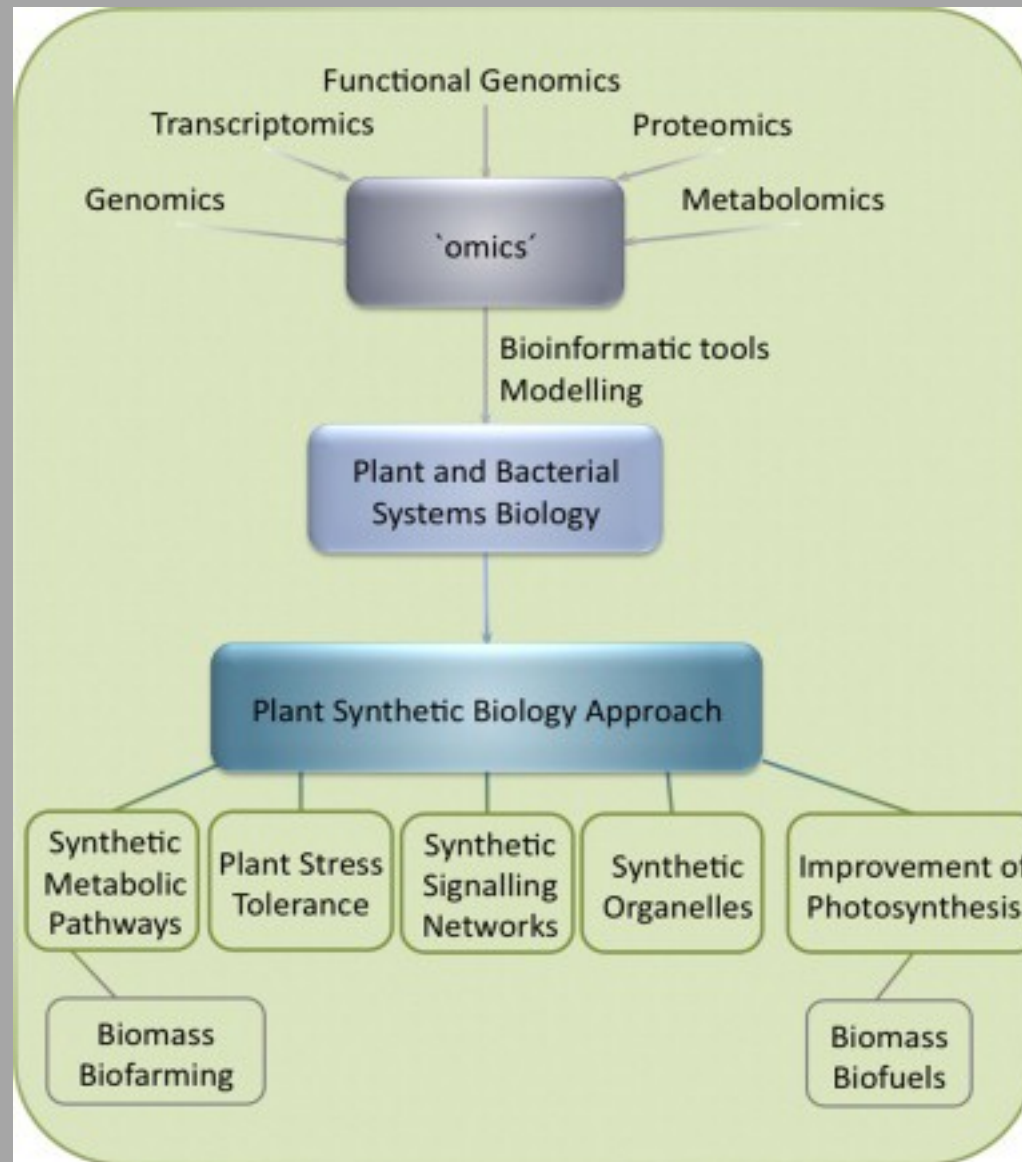




PNAS 104(6), 1741–1742



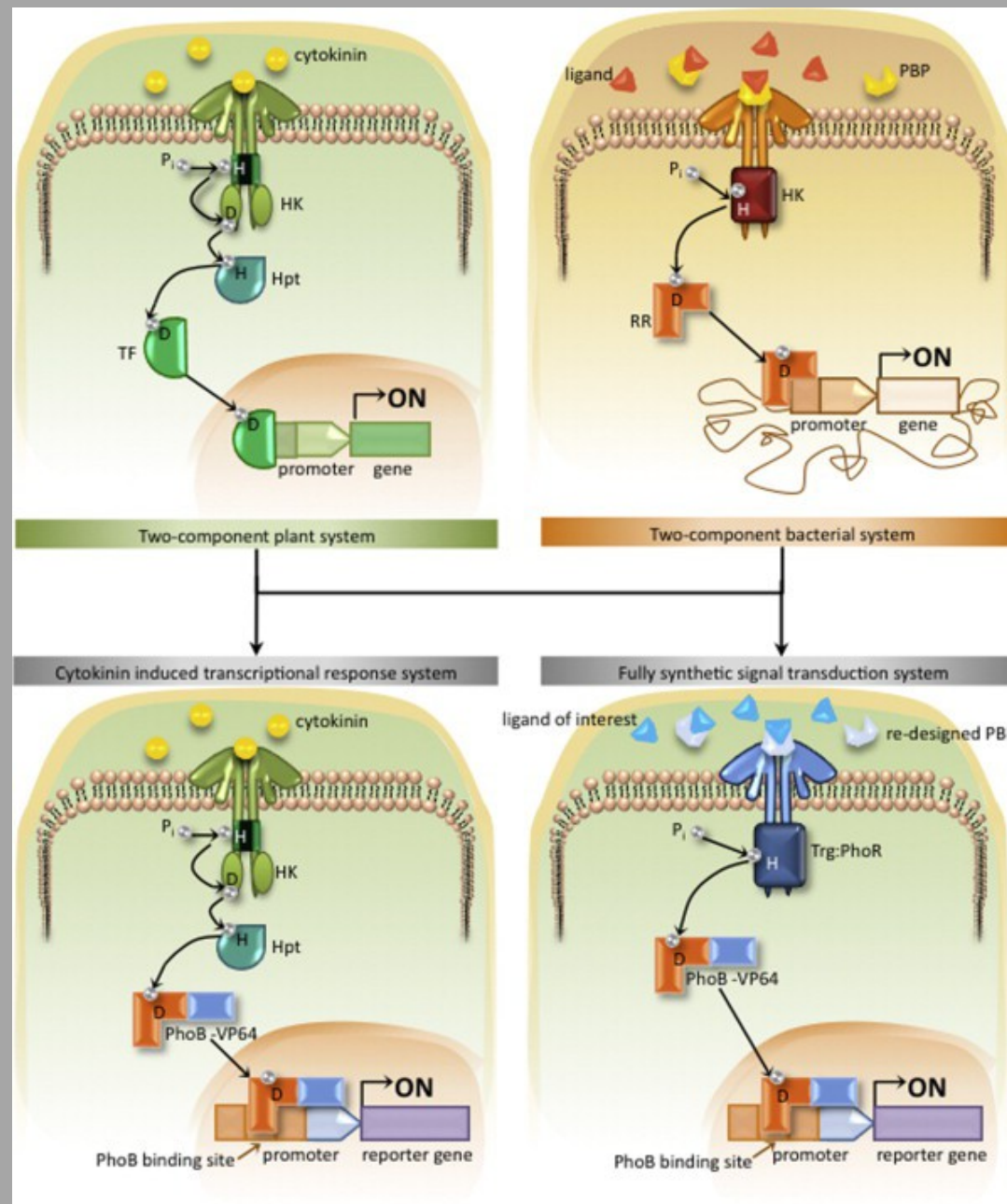
PNAS 104(6), 1877–1782



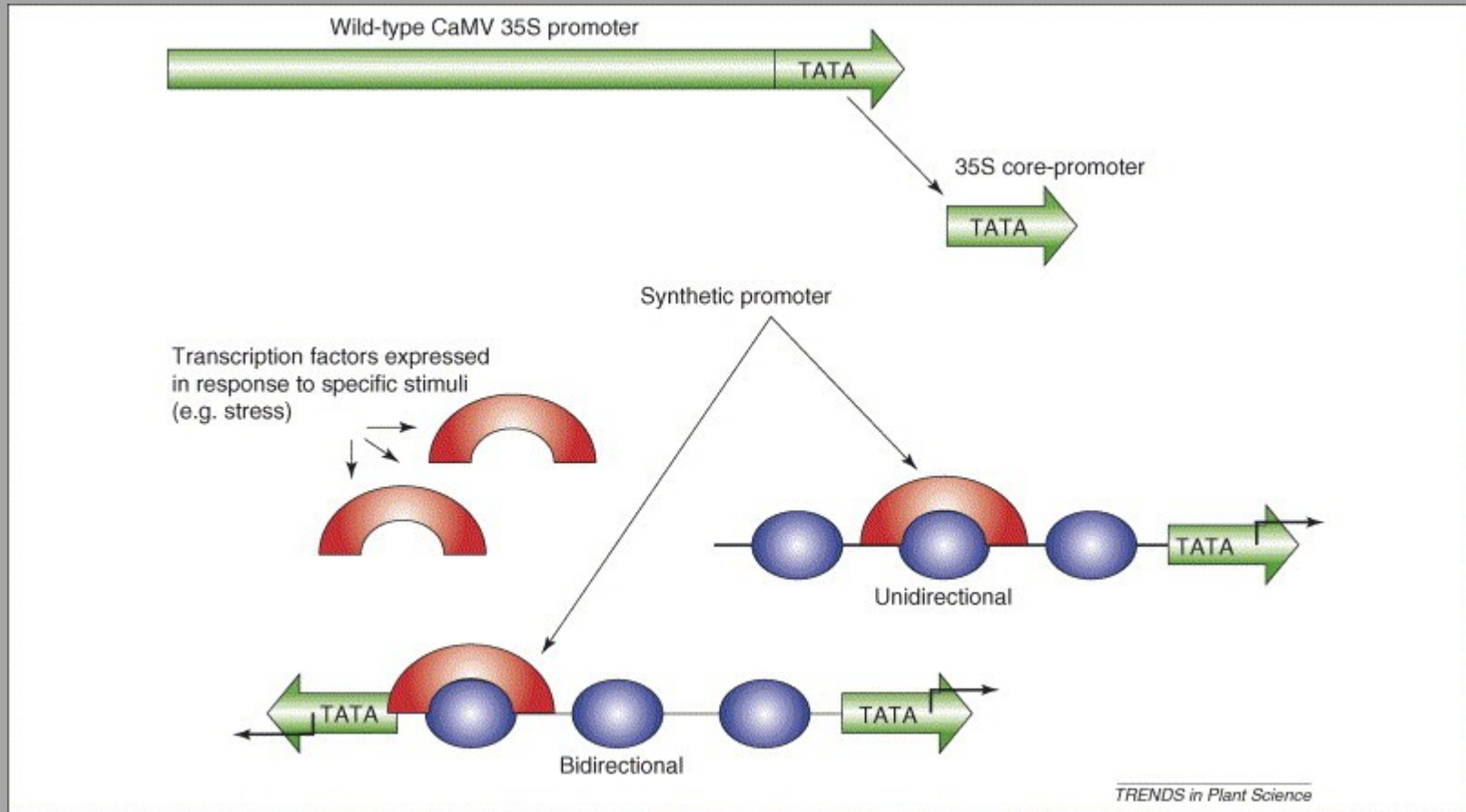
Cilji - večji prirast in rastline odporne proti:

- povečani slanosti tal
- prisotnosti težkih kovin
- suši
- ...

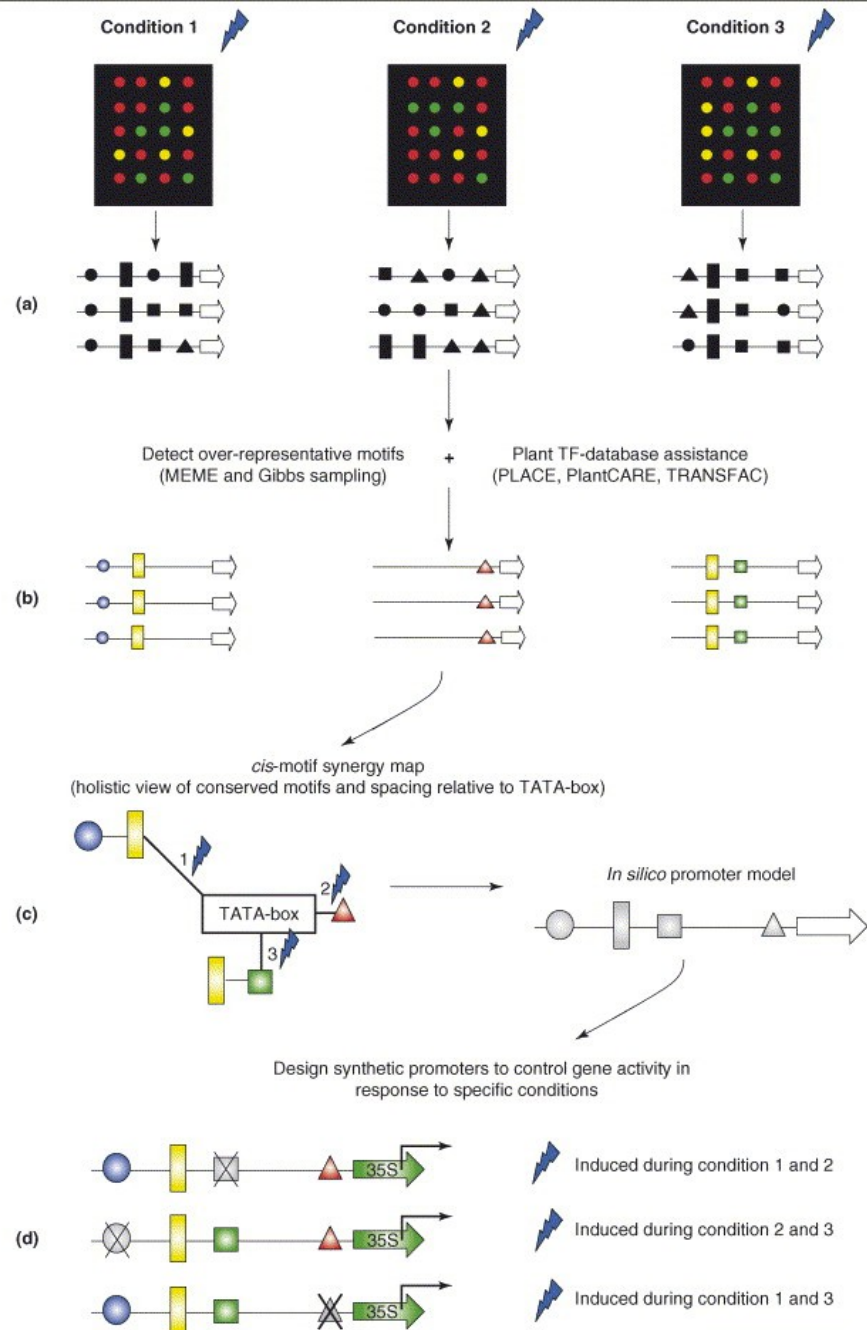
Dvokomponentni sintezni signalni sistemi



Sintezni promotorji za rastline na osnovi naravnih zaporedij



Kombinatorično inženirstvo *cis* motivov za natančno načrtovanje sinteznih promotorjev



Sintezni promotorji za načrtovanje rastlin, ki so odporne proti škodljivcem

