Whole-cell biochips for toxicity detection

(and a few words on related issues)

"Biomedical applications of micro- and nanotechnologies"
Rumanian Academy, Bucharest
Dec 4, 2007

Shimshon Belkin

Institute of Life Sciences
The Hebrew University of Jerusalem

From our own lab:

Rachel Rozen
Rami Pedahzur

Itay Benovich
Eran Sagi
Navit Hever
Yaki Davidov

- Biosensors $\Rightarrow$ Whole-cell biosensors
- Toxicity bioassays
- Genetic engineering of microbial biosensors
- Can microbial reporters sense bacterial toxins?
- The panel approach
- What do we do with the numbers?
- Cell immobilization and storage
- Examples:
  - Fiber optics
  - Whole-cell biochips

biosensor

The coupling of a biological material with a microelectronic system or device to enable rapid, accurate, low-level detection of various substances in body fluids, water and air.

Biological material?

Any biological entity displaying the desired degree of specificity:

- Enzymes and their substrates
- Antibodies and their antigens
- Receptors and their targets
- Nucleic acids and their complementary sequences

Whole-cell biosensors:
The "biological material" is an intact, living, functioning cell

"Our" whole cell biosensors:

* Bacterial cells
* Genetically modified
* Bioluminescent or fluorescent
* Tailored to respond to global stress factors

How can one detect the presence of toxic compounds in a sample?

The alternative approach:

Toxicity bioassays

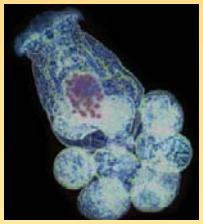
* The question asked is not "what does the sample contain"?, but rather "how toxic is the sample"?

TOXICITY BIOASSAYS

Standard toxicity bioassays:

Exposure of a test organism under standard conditions to the tested sample, usually at several concentrations, and quantification of the negative effects.



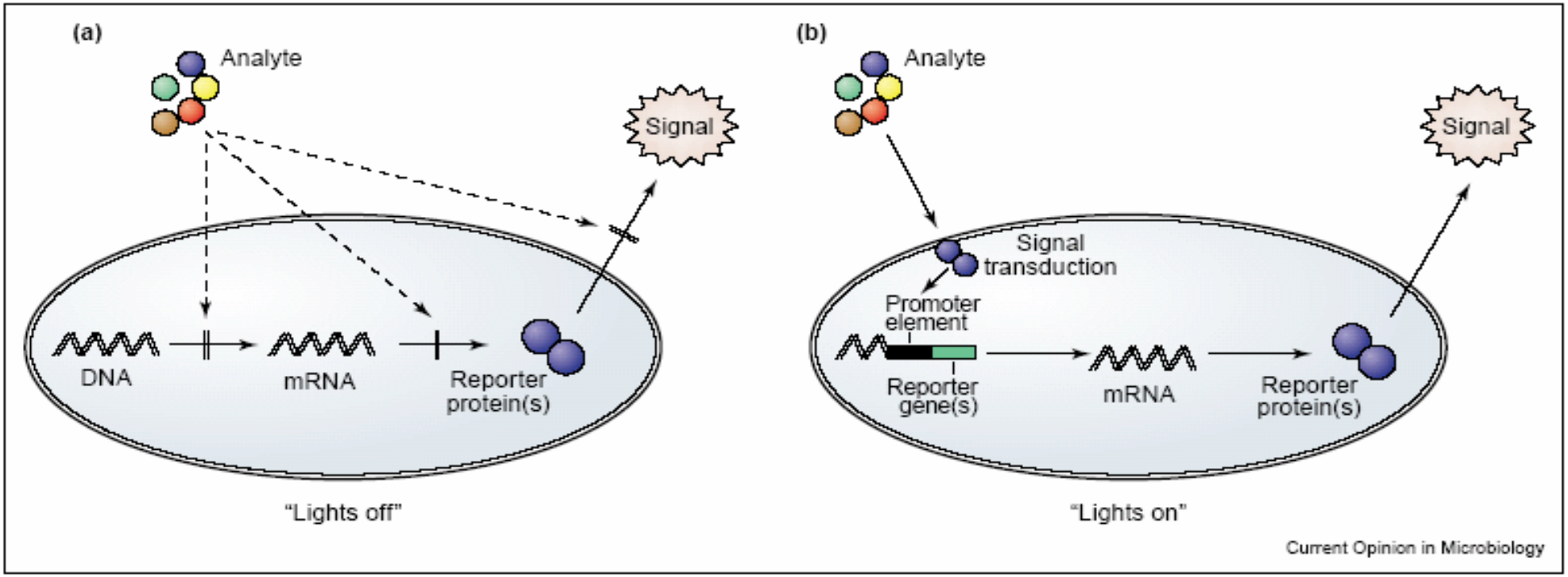



Our solution:

Rather than use whole animals, we will genetically engineer live cells to emit a signal in the presence of toxicants


Cell-based toxicity biosensors

Two approaches in the design of whole-cell reporters:



Three elements in a whole-cell biosensor:

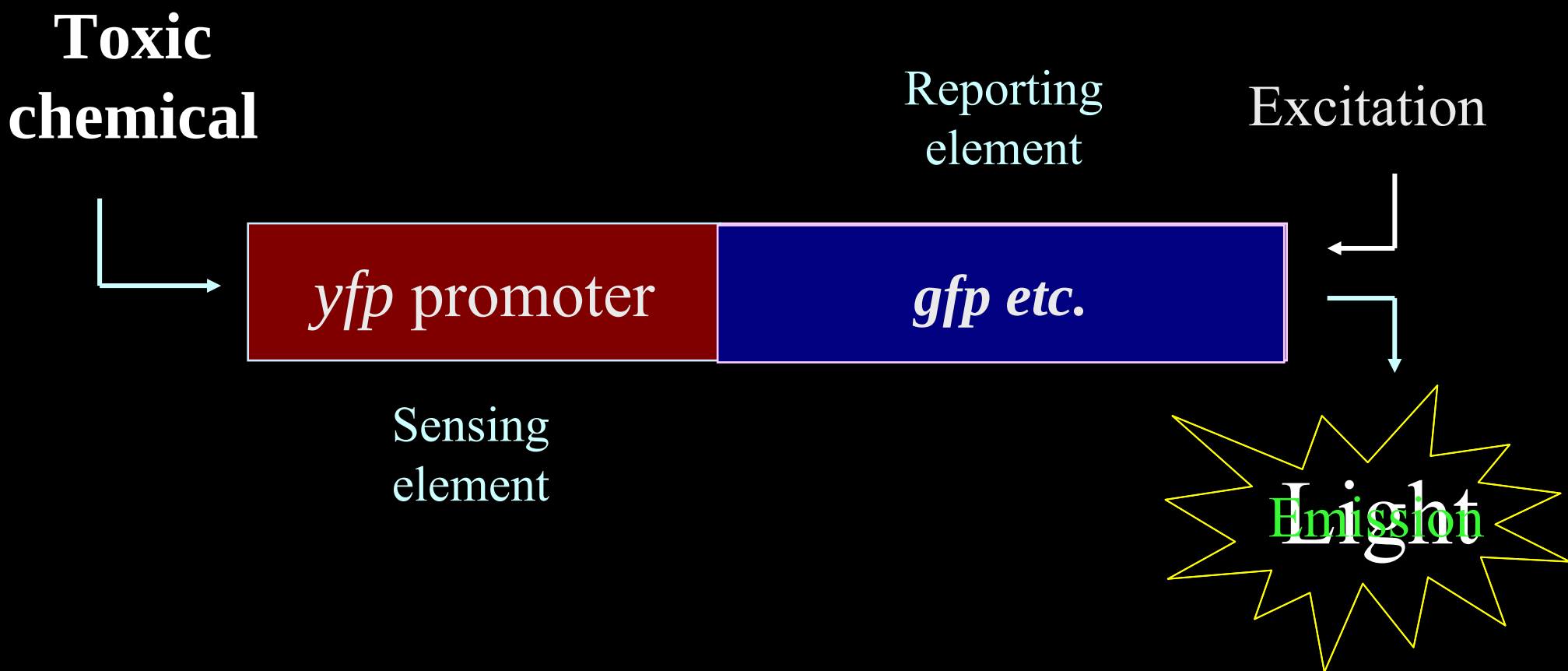
- Sensing element — A promoter of a gene involved in the response to the desired target
- Reporting element — A gene or a group of genes the products of which can be monitored quantitatively
  - Enzyme activity (*lux* – bioluminescence)
  - Presence (*gfp* etc. – fluorescence)
- Host cell — Convenience & relevance

A practically unlimited spectrum of options for whole-cell biosensors engineering

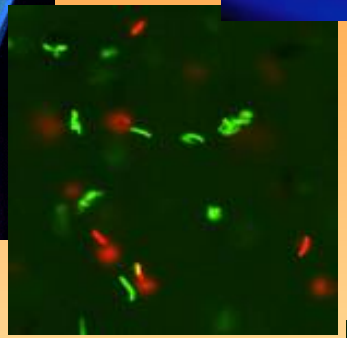
Reporter strains in our laboratory

* Toxicity and general stress
* Genotoxicity
* Oxidative stress
* Heat shock and protein damage
* Osmotic and marine stress
* Starvation and nutrient stress
* Cyanobacterial nutrient sensors
* Halogenated aromatics
* Halogenated aliphatics
* Assimilable organic carbon
* Promoter screening libraries

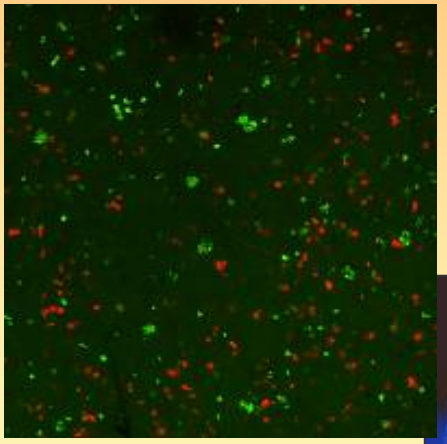
BIOLUMINESCENT GENOTOXICITY (DNA DAMAGE) SENSOR

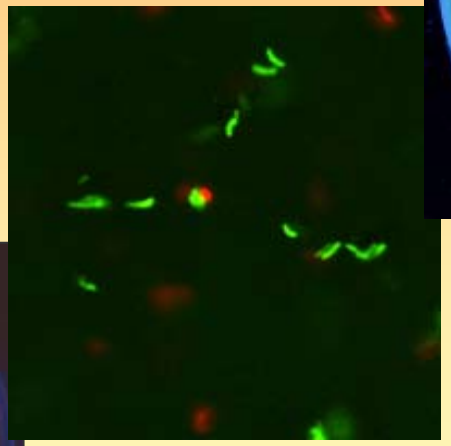



HUJI

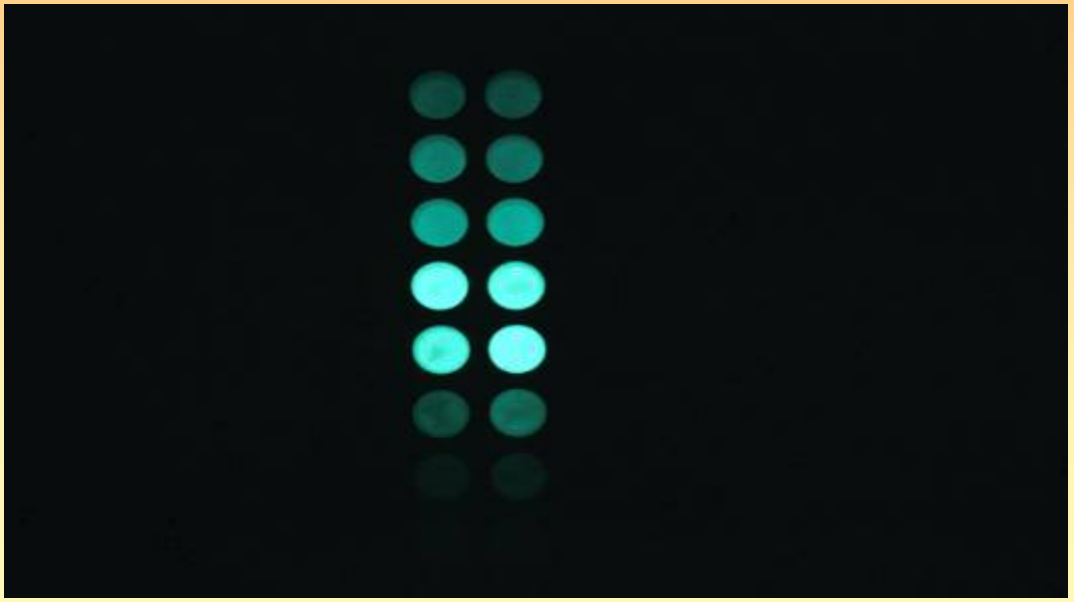



HUJI

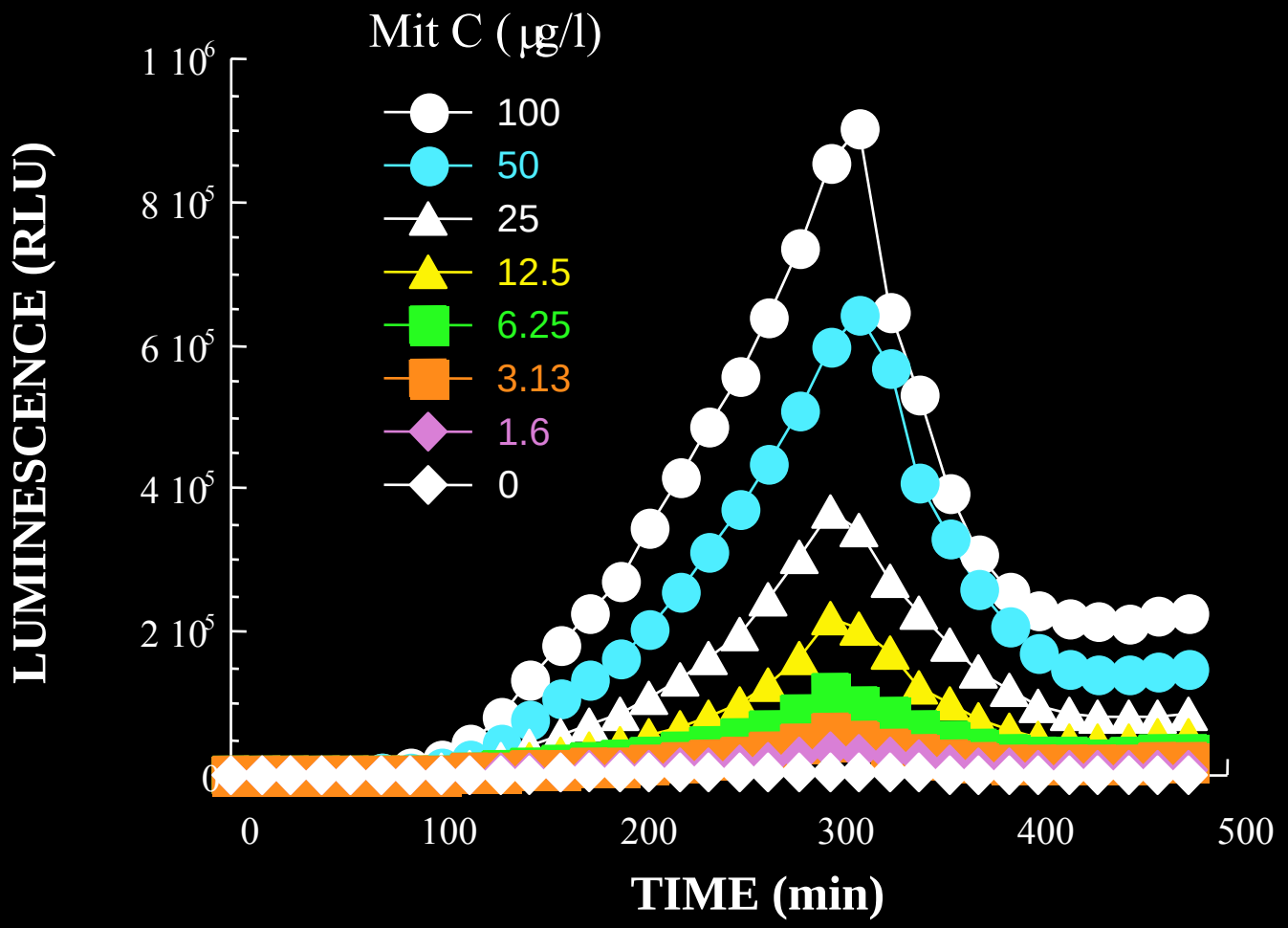




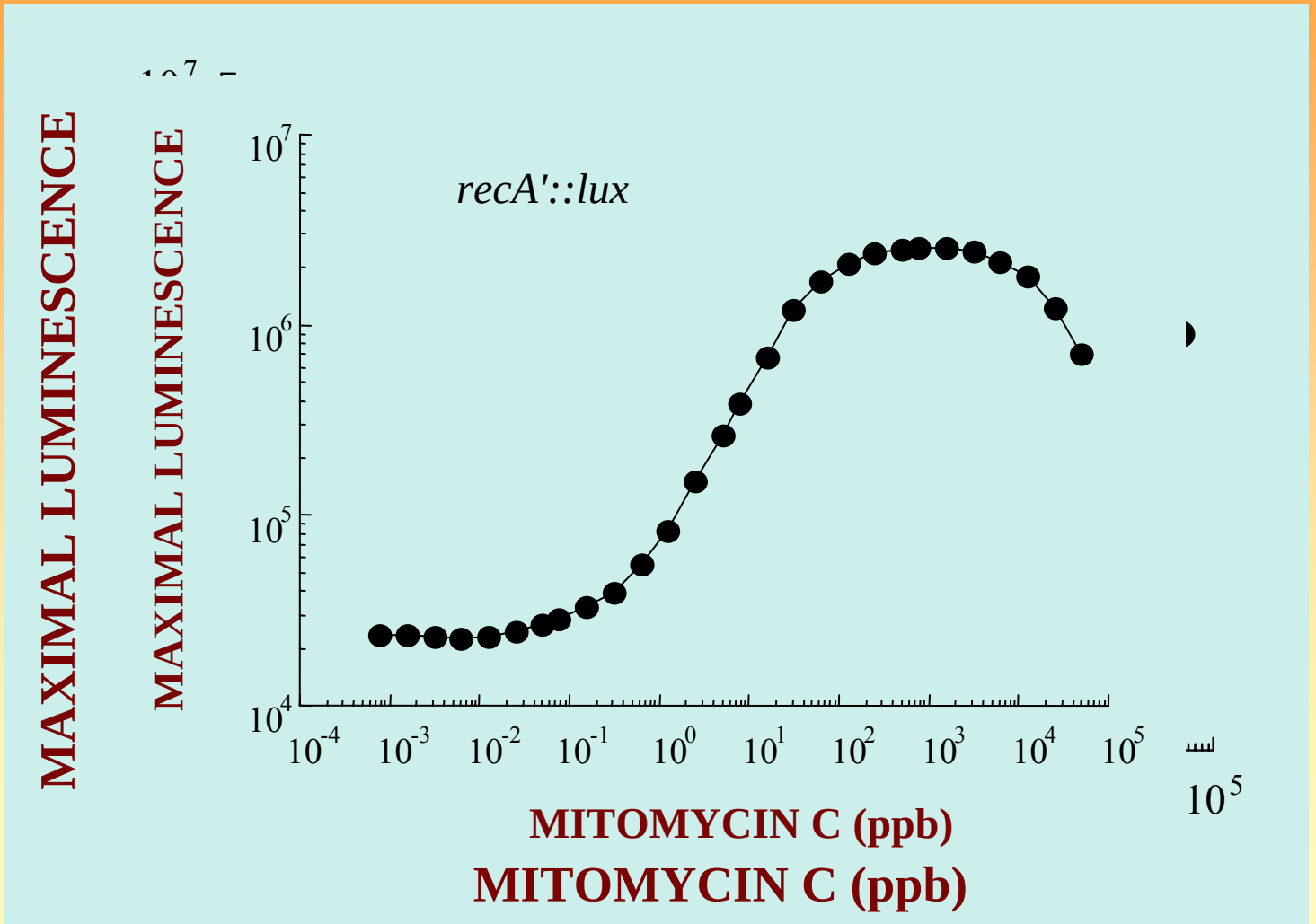

6



Exposure of strain DPD3063 to mitomycin C: kinetics of light development



DPD3063: CHROMOSOMAL recA'::lux RESPONSE TO MITOMYCIN C

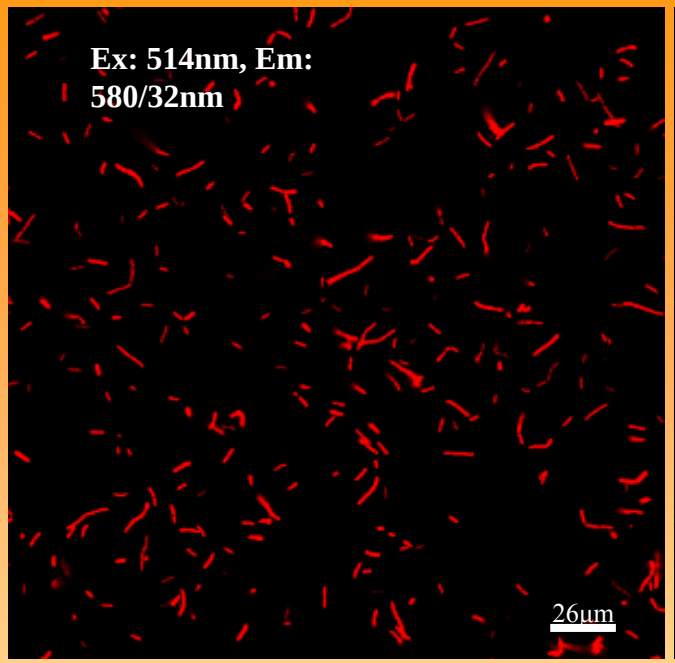


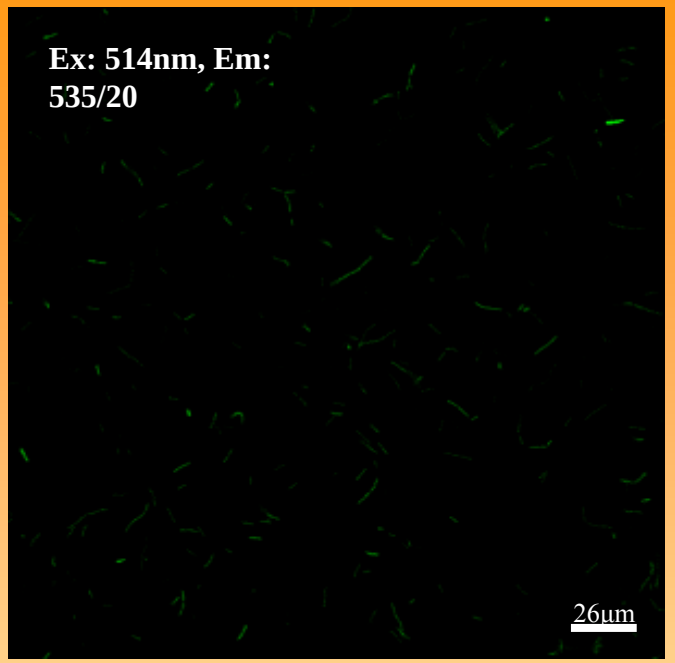
A "dual action" fluorescent microbial sensor: strain NHEX-R

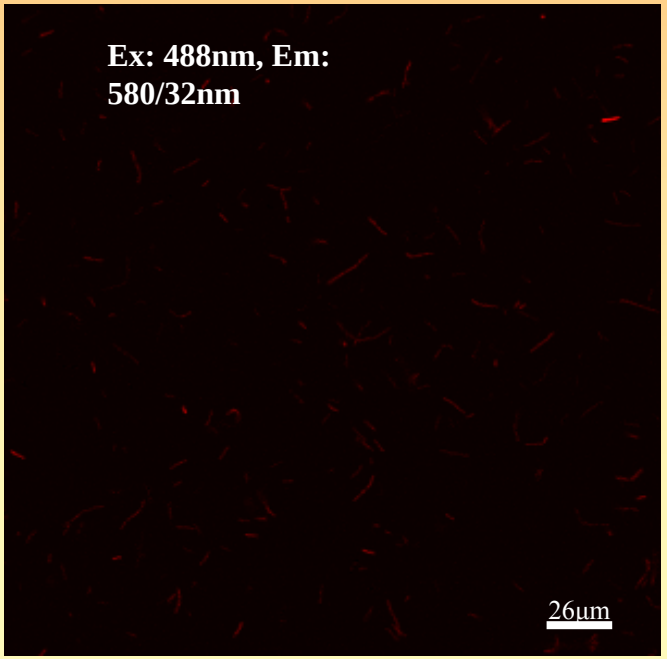
Reports on **cytotoxicity** by red fluorescence:
a *grpE'*::DsRedExpress fusion

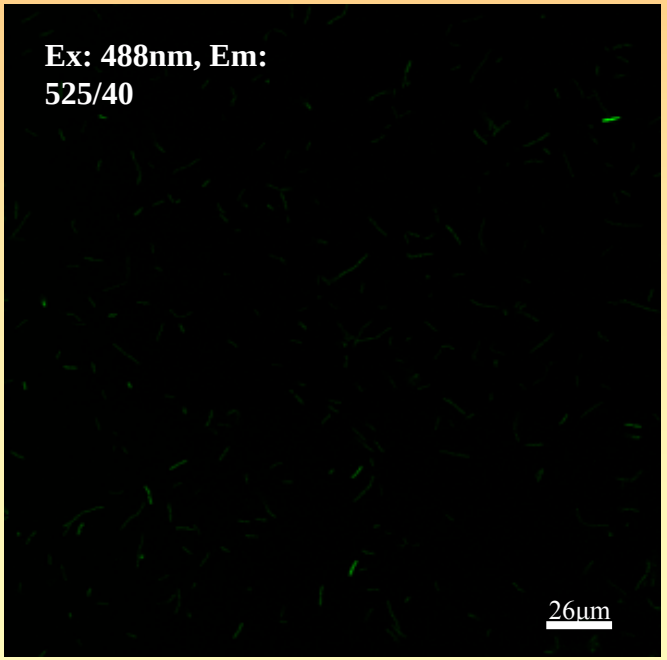
Reports on **genotoxicity** by green fluorescence:
a *recA*'::EGFP fusion

"Dual action" strain NHEX-R: Induction with ethanol

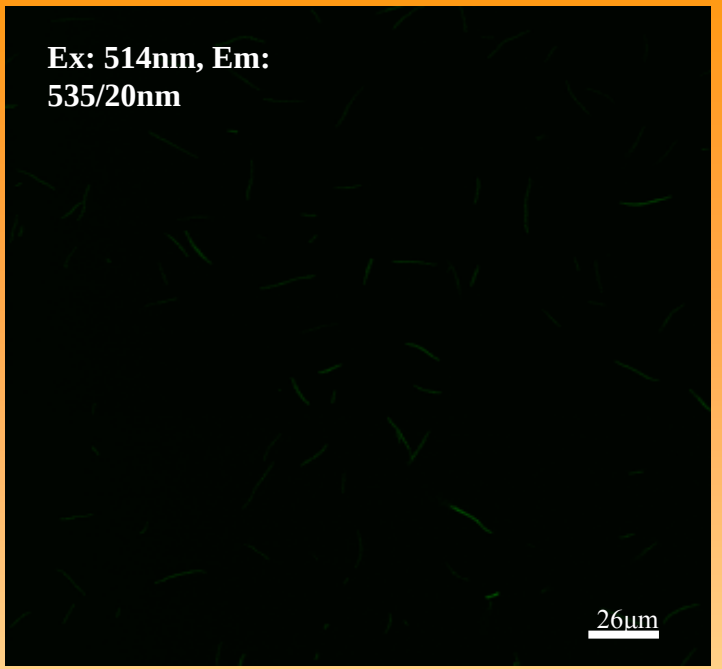






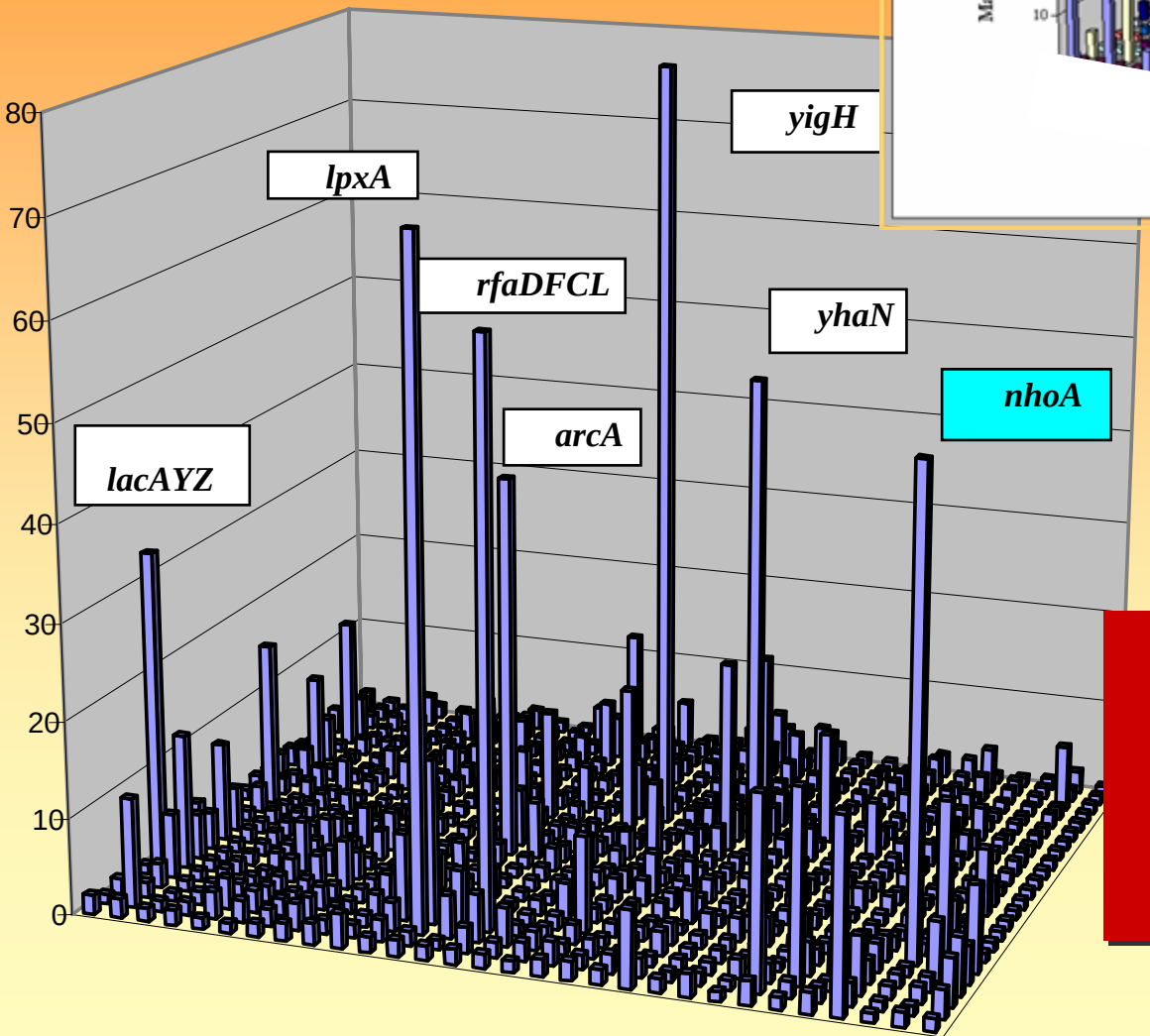


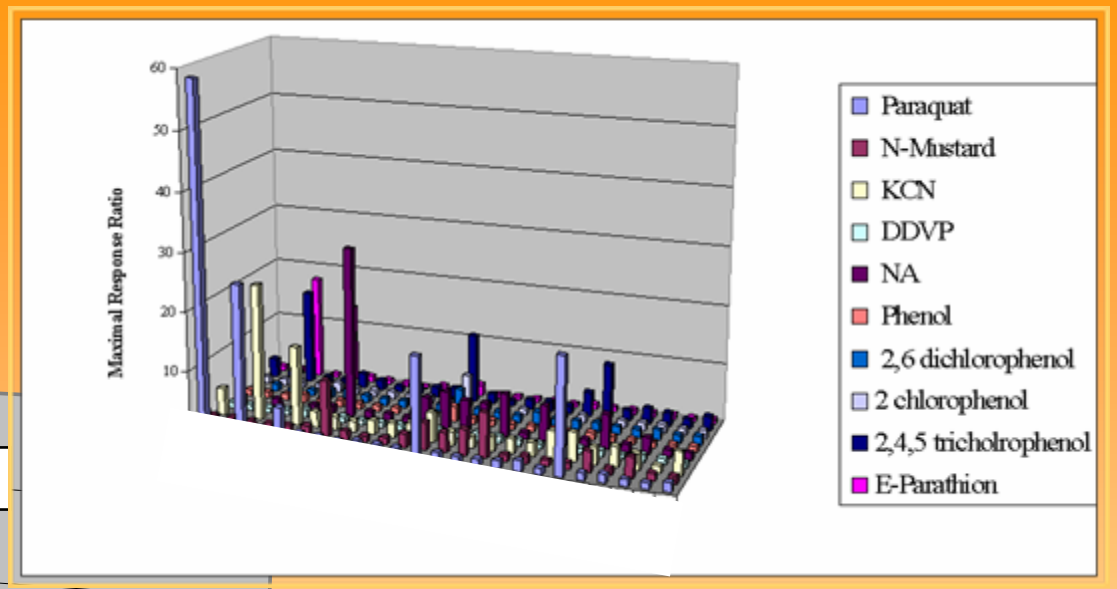
"Dual action" strain NHEX-R: Induction with nalidixic acid



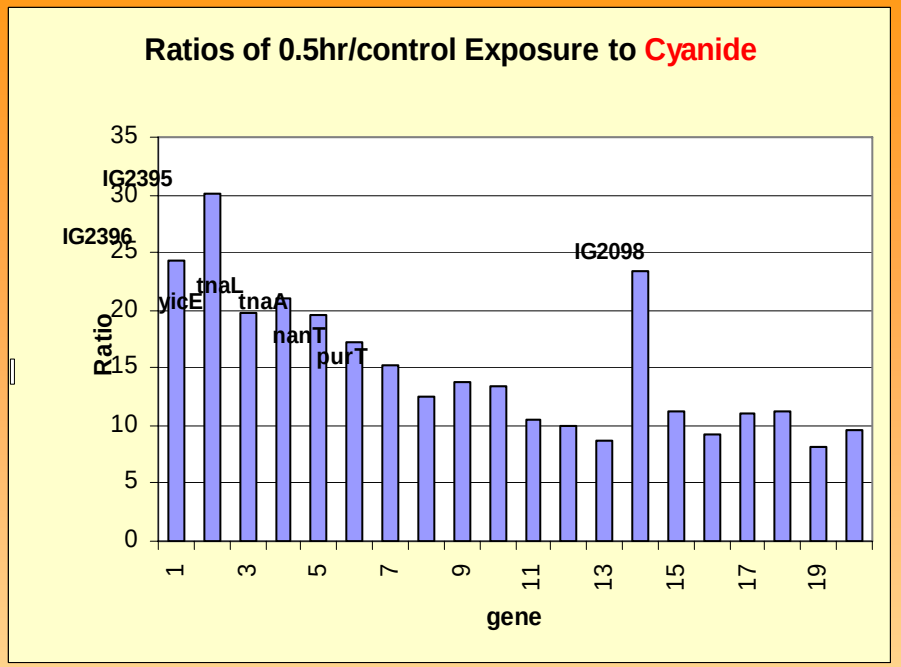
We have shown that microbial reporters can be employed for the detection of numerous chemical toxins.

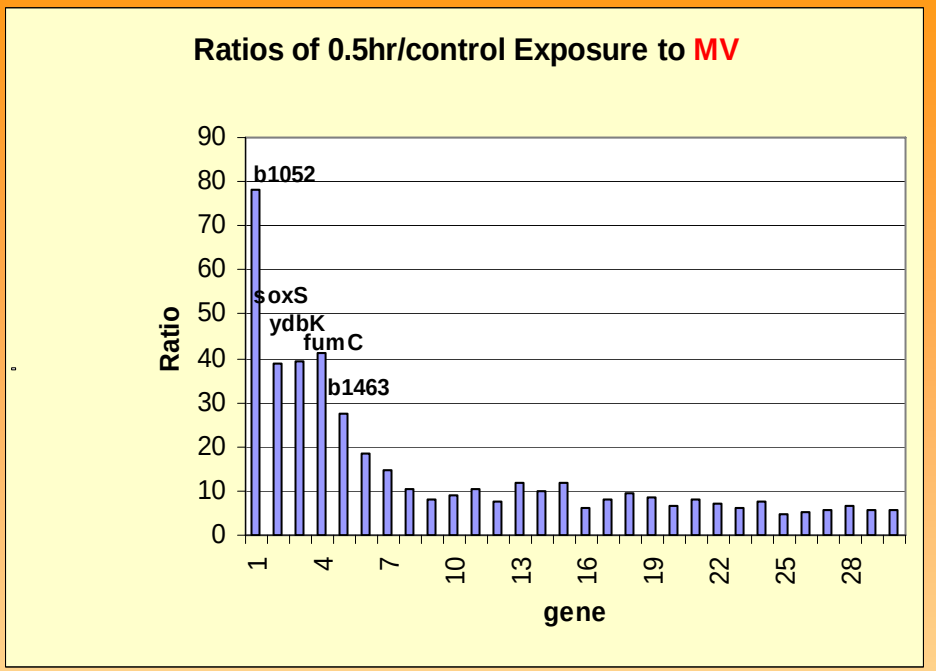
Can they also sense **biological** toxins?

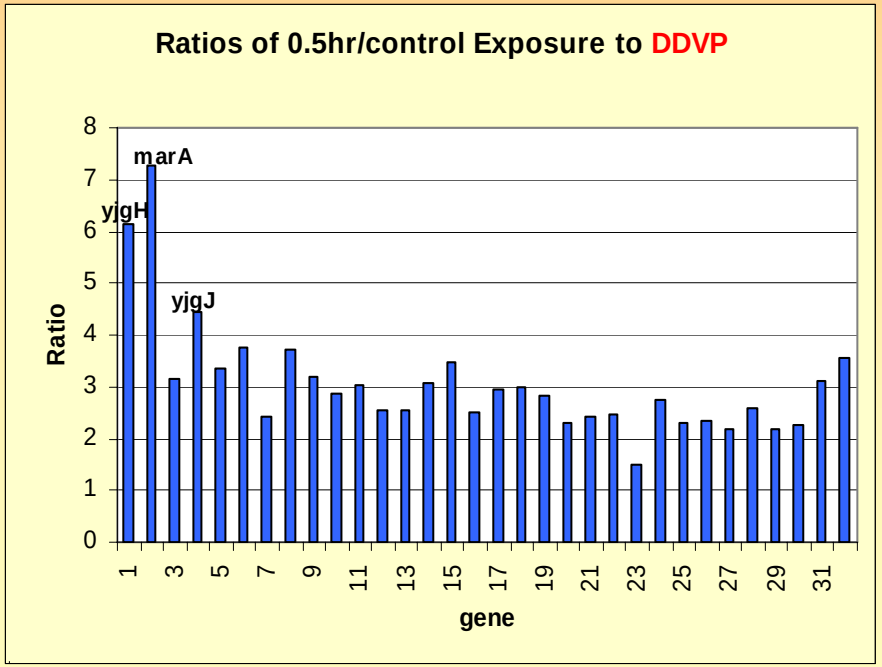




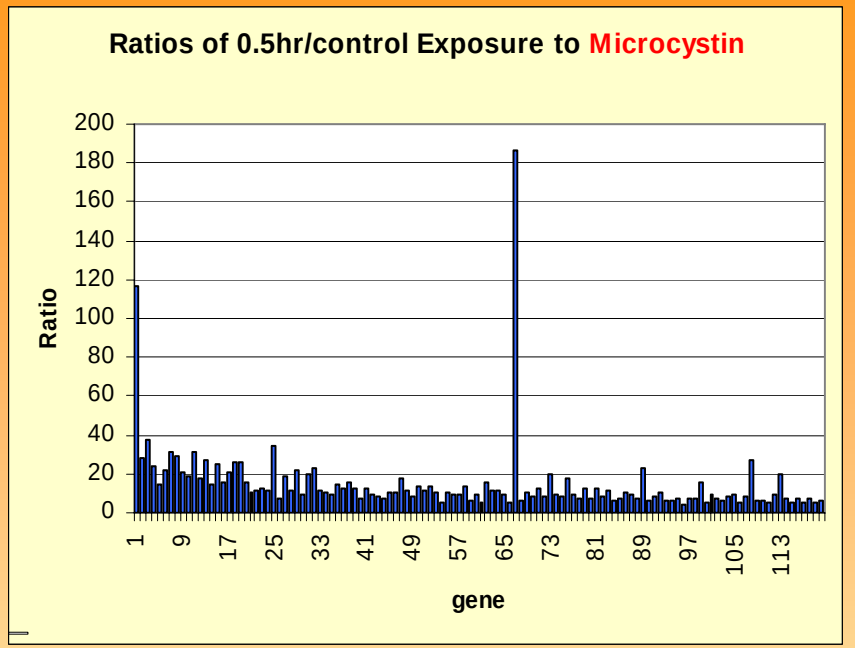
Yes, with the correct choice of sensing elements!

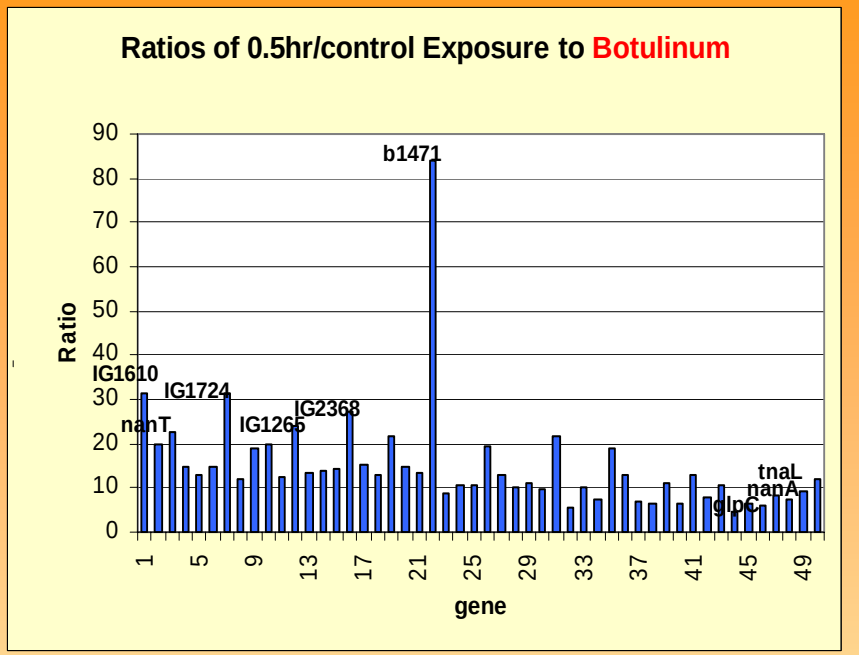

Ratios of 0.5hr/control Exposure to Cyanide
Ratio
gene
IG2395
IG2396
yicE
tnaL
tnaA
nanT
purT
IG2098

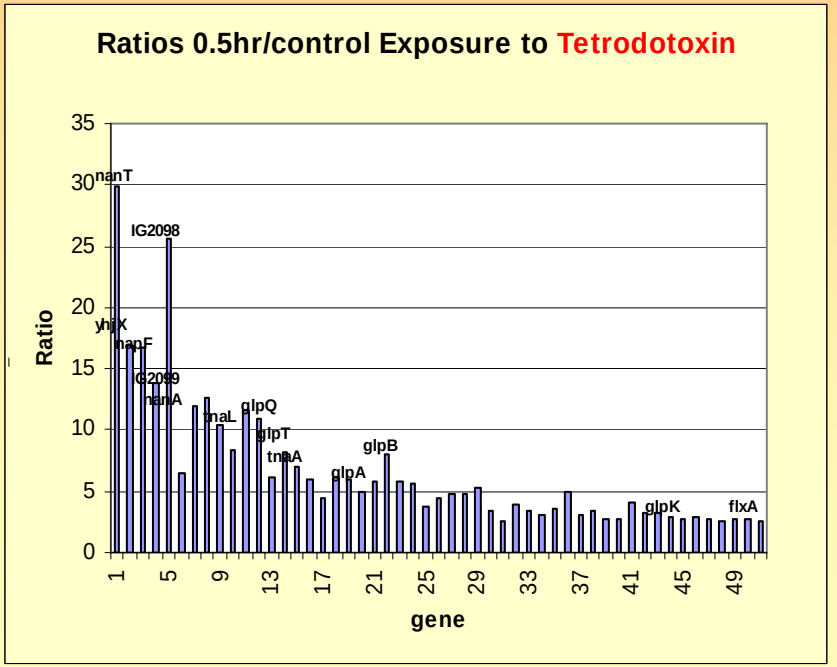

Ratios of 0.5hr/control Exposure to MV
Ratio
gene
b1052
soxS
ydbK
fumC
b1463

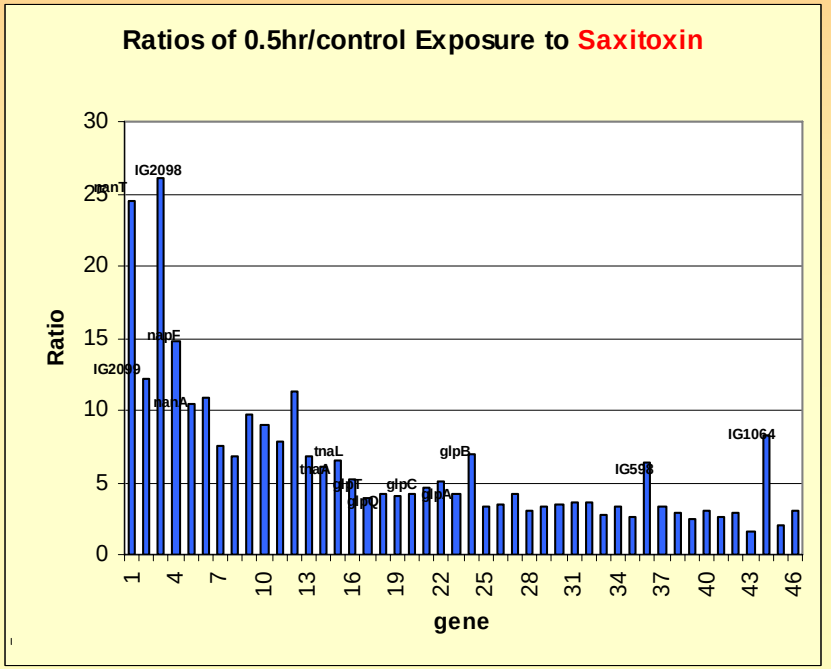

Ratios of 0.5hr/control Exposure to DDVP
Ratio
gene
marA
yjgH
yjgJ

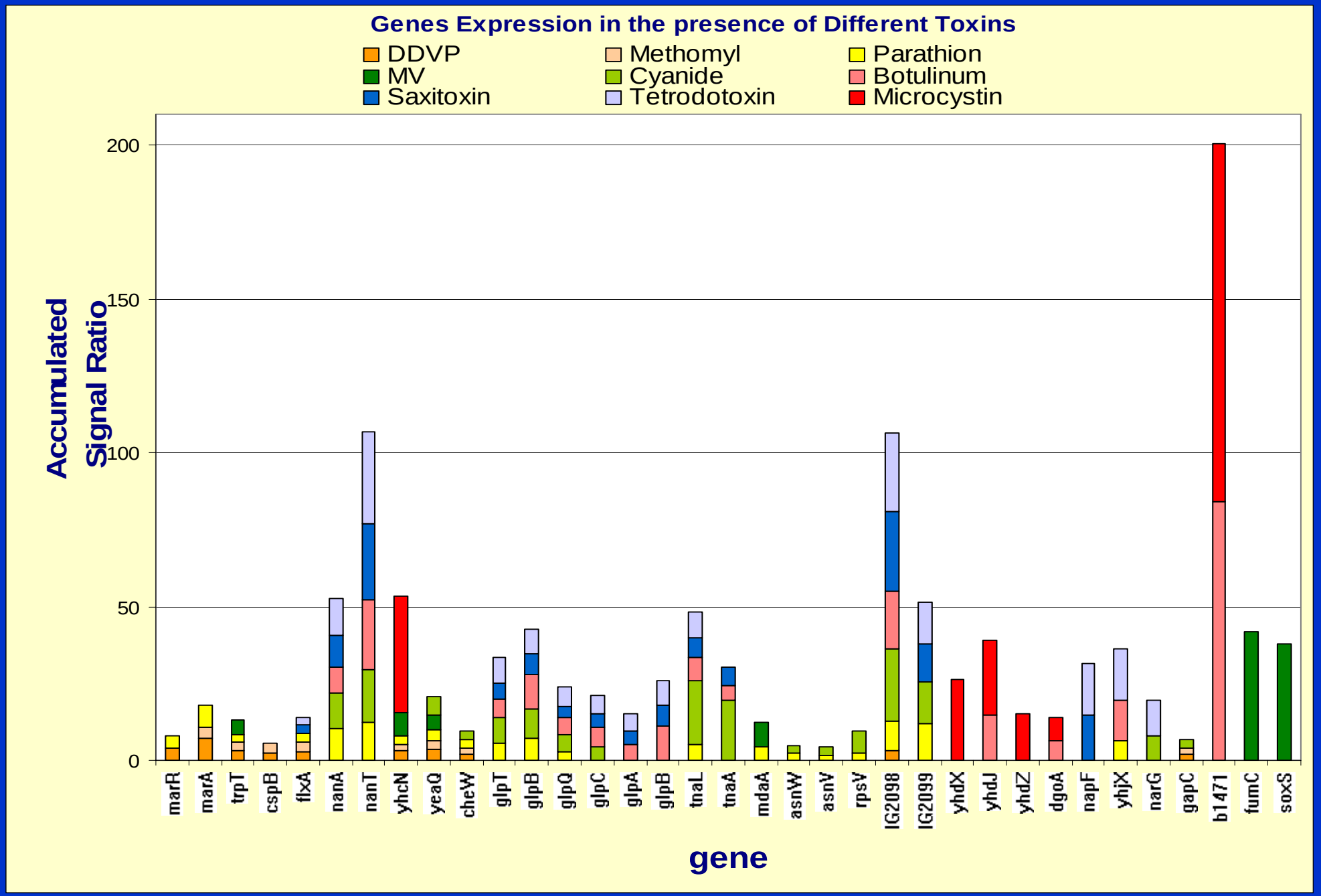
The search for toxicant responsive promoters



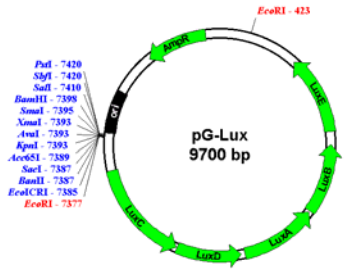


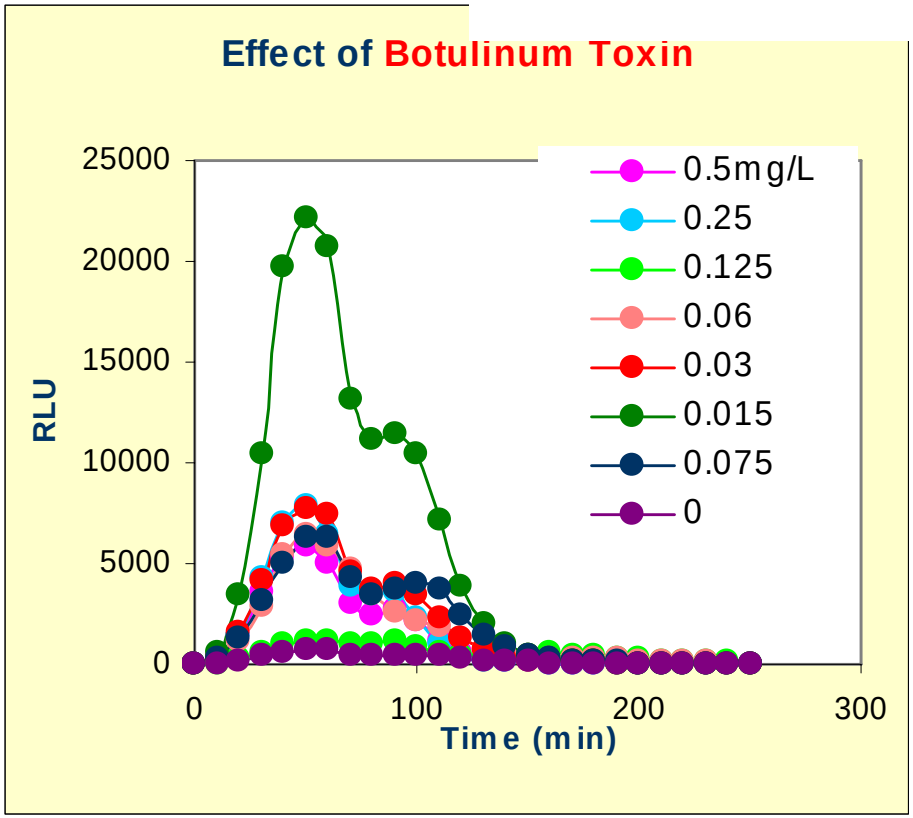


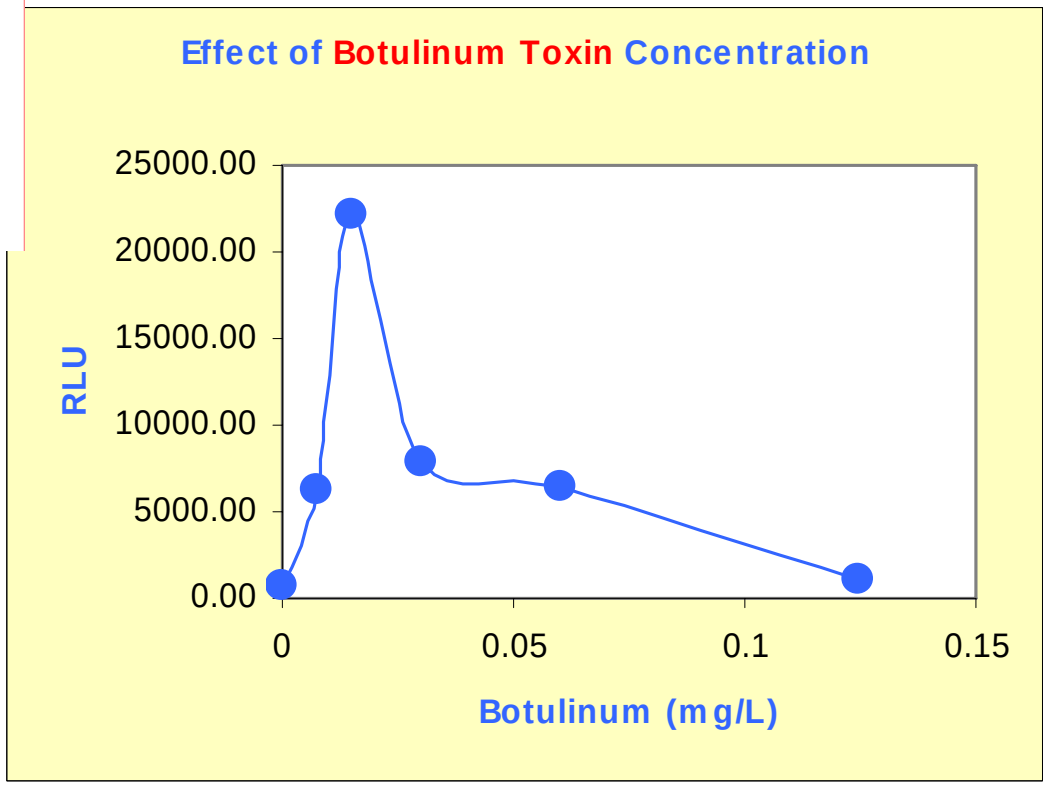



Genes Expression in the presence of Different Toxins
DDVP
Methomyl
Parathion
MV
Cyanide
Botulinum
Saxitoxin
Tetrodotoxin
Microcystin
Accumulated Signal Ratio
0
50
100
150
200
marR
marA
trpT
cspB
flxA
nanA
nanT
yhcN
yeaQ
cheW
glpT
glpB
glpQ
glpC
glpA
glpB
tnaL
tnaA
mdaA
asnW
asnV
rpsV
IG2098
IG2099
yhdX
yhdJ
yhdZ
dgoA
napF
yhjX
narG
gapC
b1471
fumC
soxS
gene

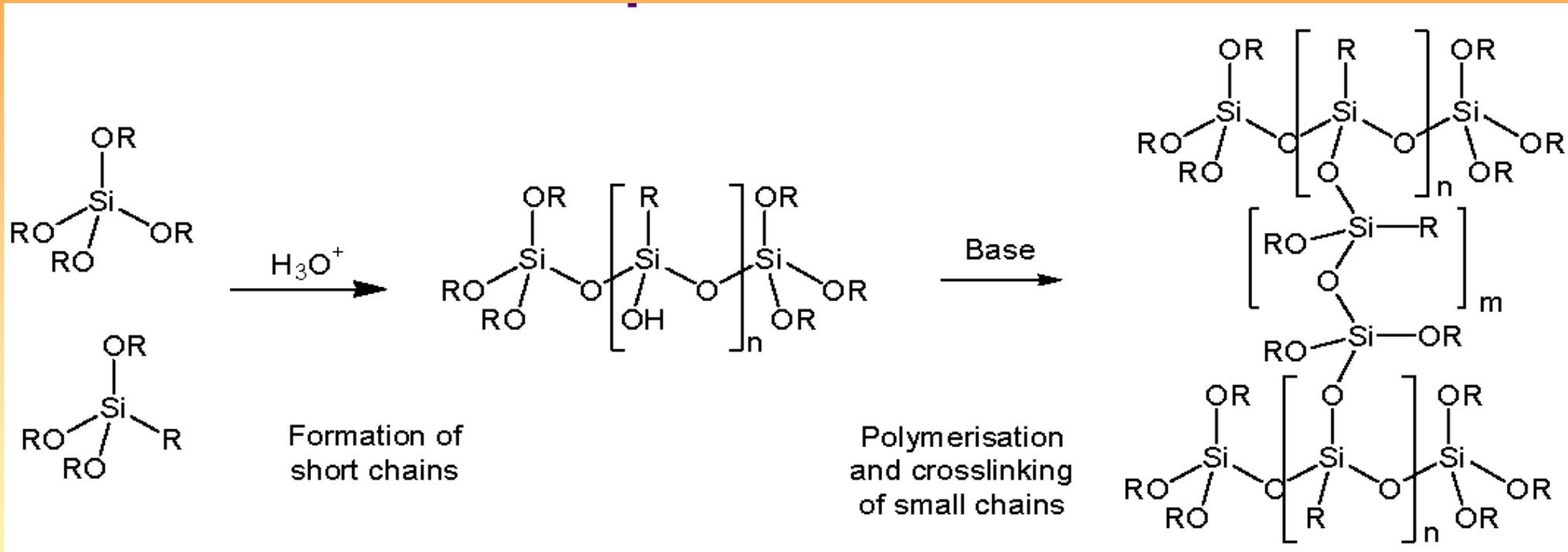
Preliminary responses to Botulinum toxin







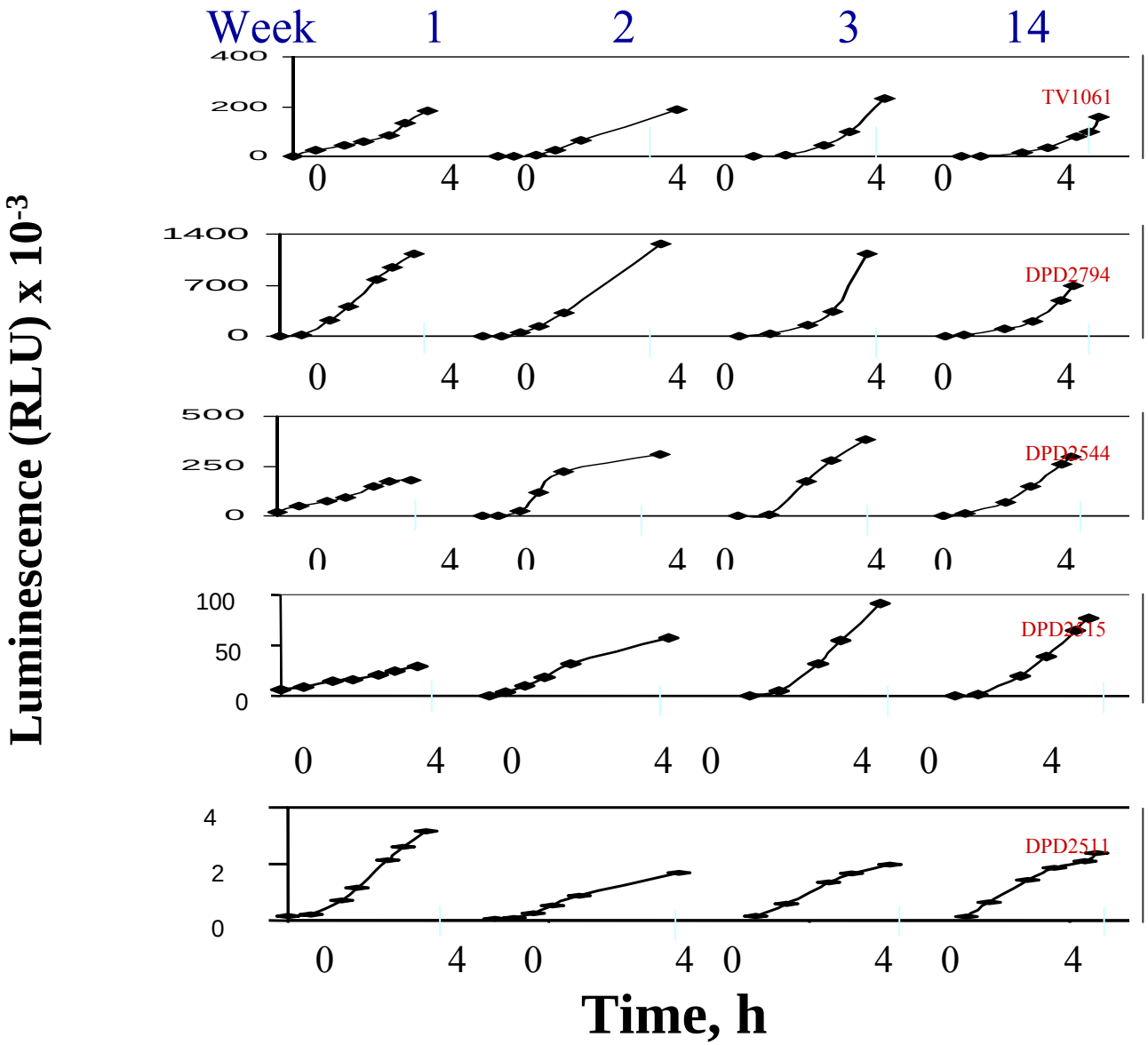
Cell immobilization and storage: the sol-gel option



e.g. tetramethylorthosilicate

O. Lev, R. Premkumar, D. Teseme, Y. Sharabi, Hebrew University

Stability and Repeatability



General Toxicity
2% Alcohol
grpE

Genotoxicity
Nalidixic acid 6 µM
recA

Fatty acids
0.5 mM Phenol
fabA

Oxidizers
3.9 mM Methyl viologen
micF

Peroxides
3.9 mM Hydrohen-peroxide
katG

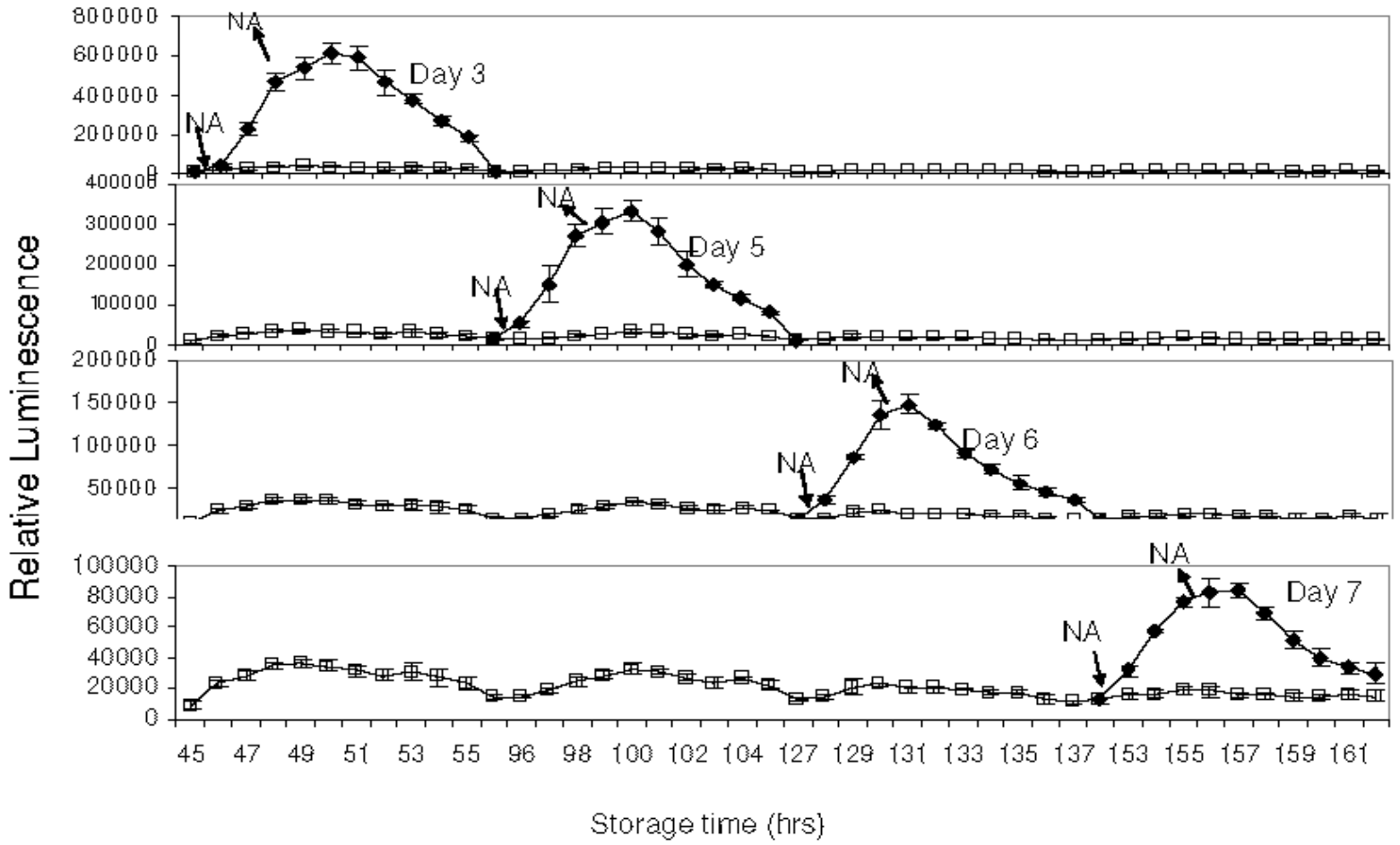
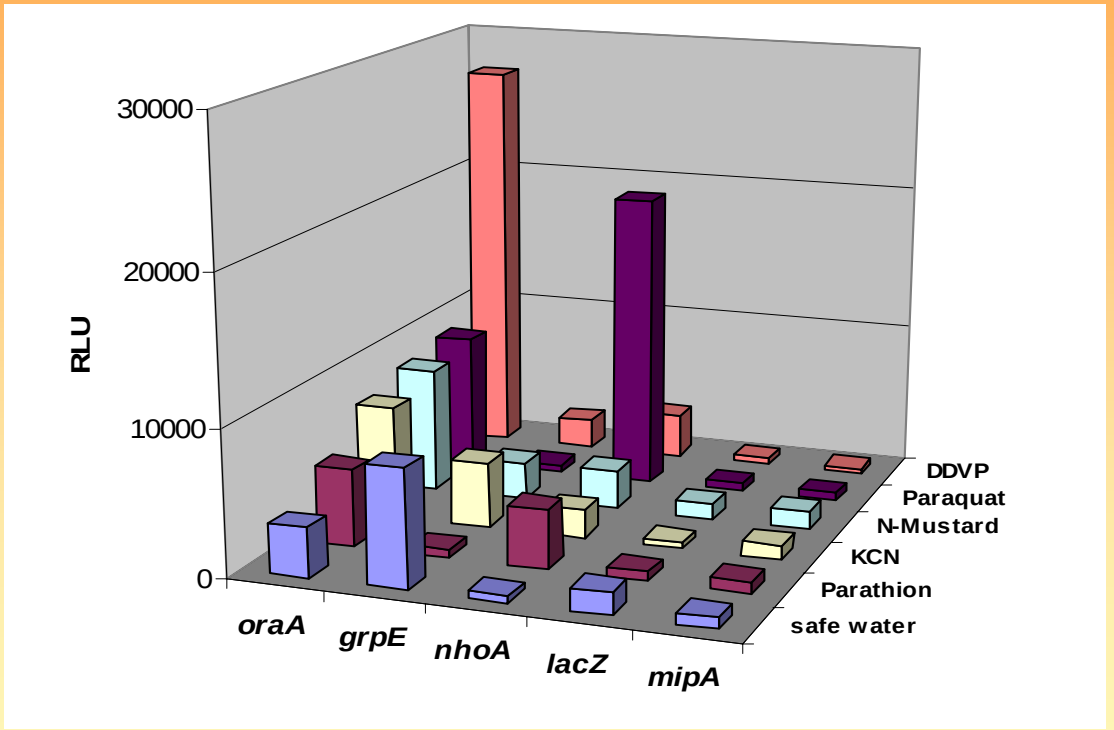
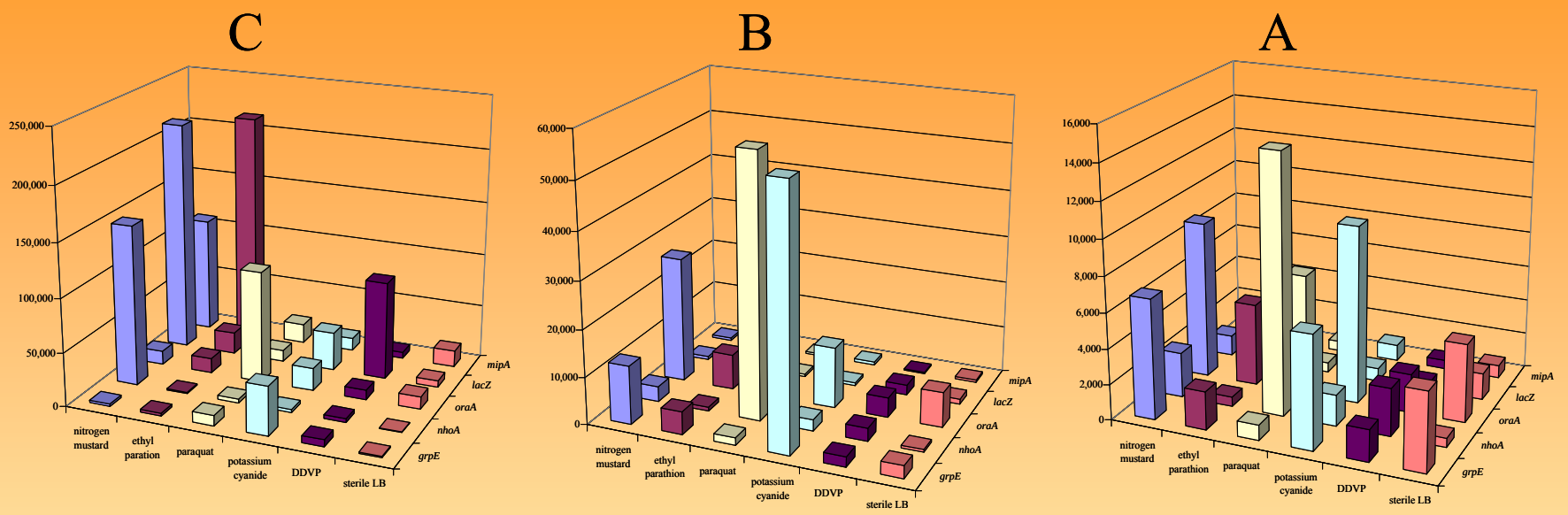


Figure 5.1.12. *Sol-gel immobilized recA::lux cells, continuously exposed to a flowing buffer at room temperature, were subjected to a daily 2 hour dose of nalidixic acid (5 mg/l).*

The panel approach:
One reporter strain is not enough



Panel response pattern



30 min

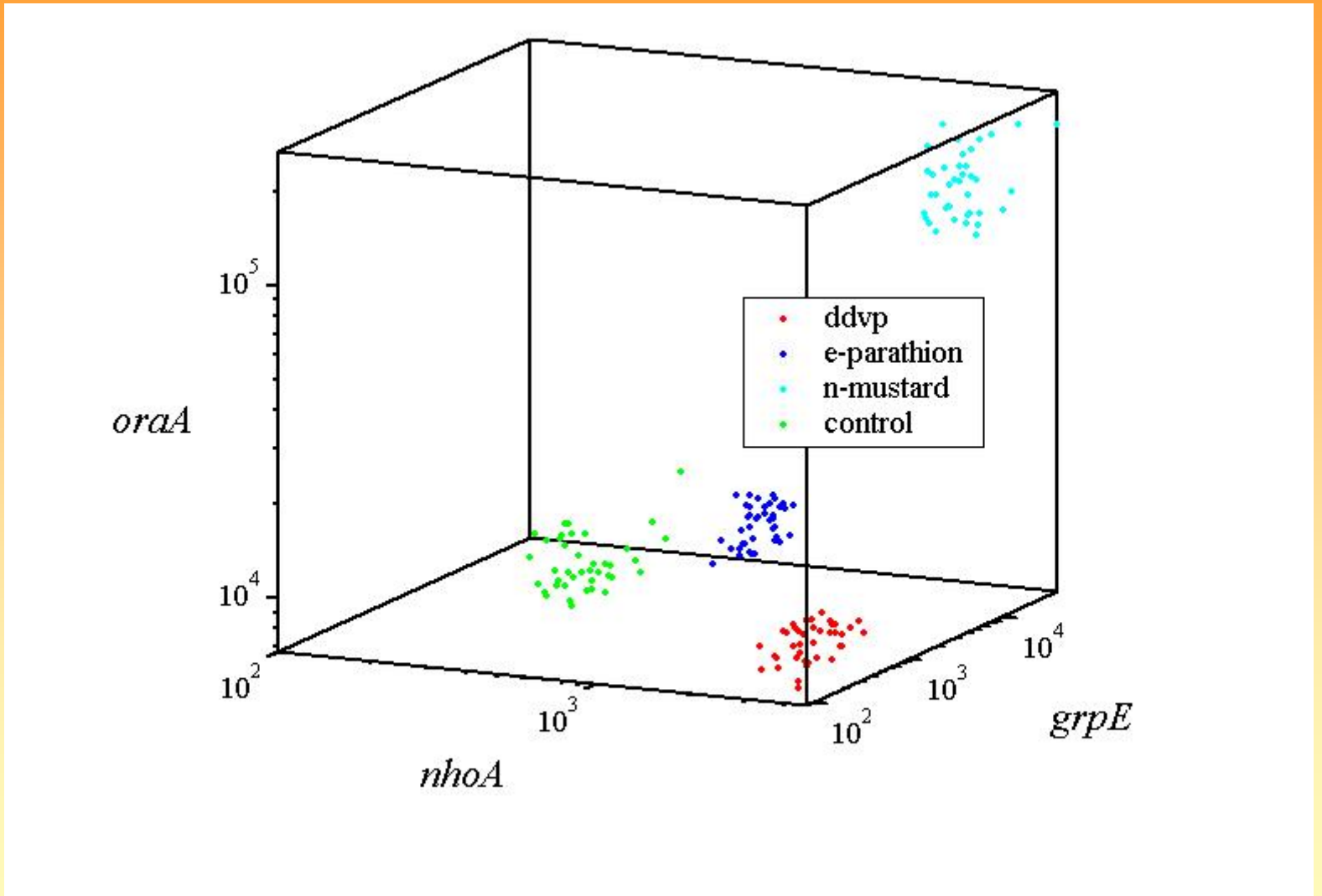
60 min

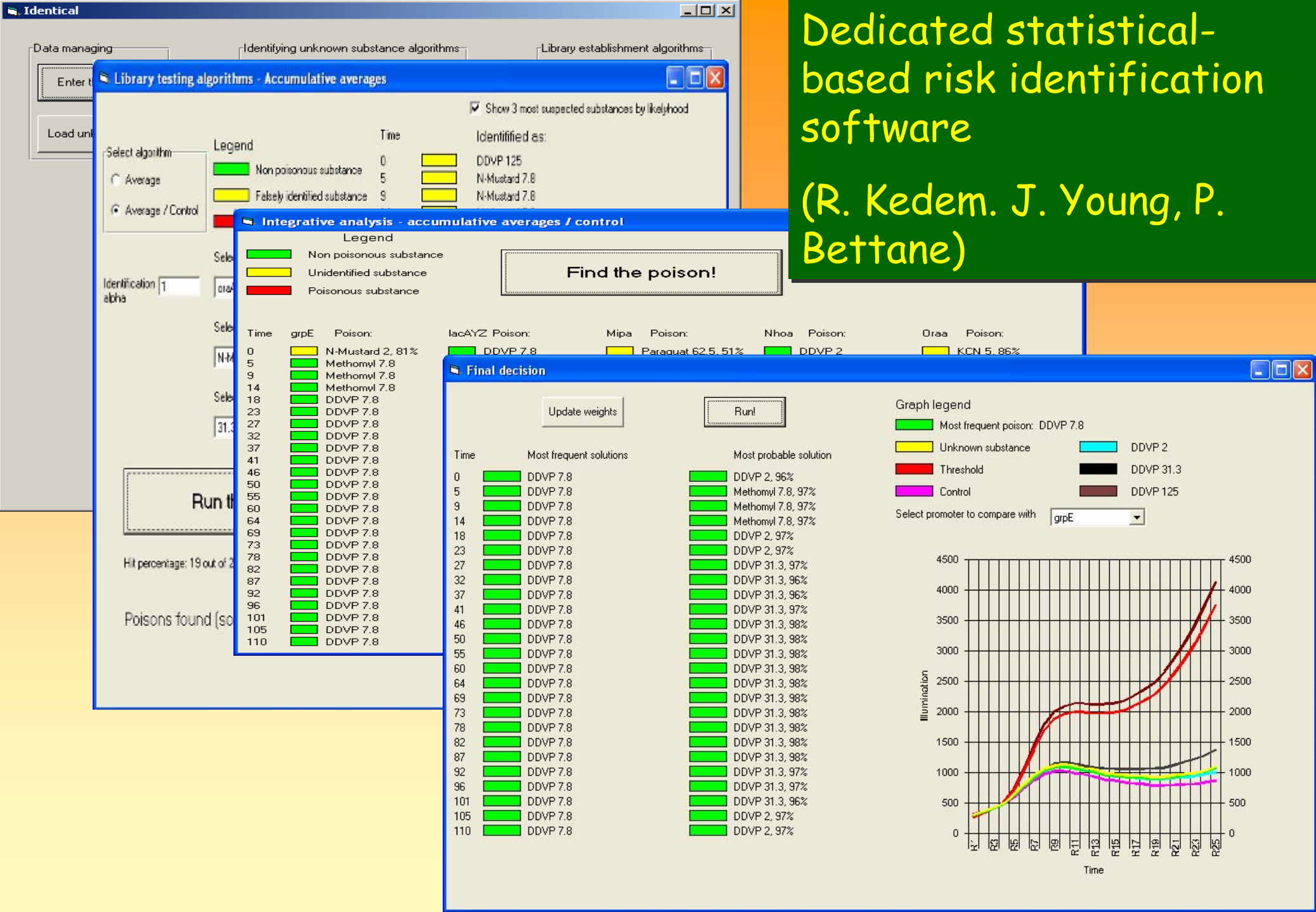
120 min

Pattern analysis/classification and toxicant identification:

- Statistical approaches
- Artificial neural networks
- Dedicated software

Bayesian decisions (T. Elad)





Dedicated statistical-based risk identification software

(R. Kedem. J. Young, P. Bettane)

How do we integrate our reporter cells into biosensors?

I. Immobilization onto the tips of optic fibers
II. Encapsulation in sol-gel matrices
III. Embedding in microtiter plates
IV. Integration into whole-cell biochips
V. Patterning on glass and other solid surfaces

How do we integrate our reporter cells into biosensors?

Incorporation into whole-cell biochips

Y. Shacham, TAU; D. Engelberg, HUJ; E. Rorman, MoH;
P. Bettane, B. Tadmor, IDF

Cell-based toxicity sensor chips

- Live cells, genetically engineered to emit a signal in the presence of toxicants
- Incorporated into a disposable biochip that provides:
  - Live cell maintenance
  - Microfluidics for sample introduction
- The biochip is inserted into a Toxicity Analyzer that contains:
  - Electronic control and operation circuits
  - Detection optics
  - Temperature control
  - Logic circuits and decision algorithms
  - Communication capacities

The original grand plan: three types of sensor cells

Bacterial systems (*E. coli*)

* Facilitated genetic manipulation
* Proven concept validity
* Limited relevance to human health

Human cell systems (liver, neuronal)

* Maximum human exposure relevance
* A much more complex technical challenge

Yeast cells (*S. cerevisiae*)

* Eukaryotic structure and function
* Relative facility in genetic manipulation

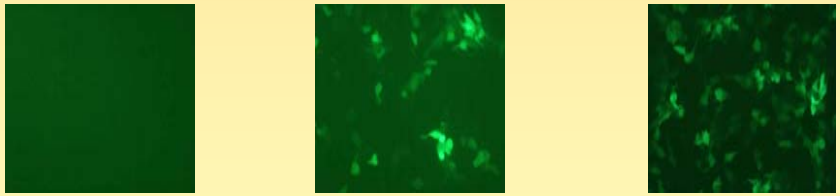
Feasibility of engineering human and yeast cell systems has been demonstrated, but performance is not yet in the required range

Human cell constructs

Clone # 43

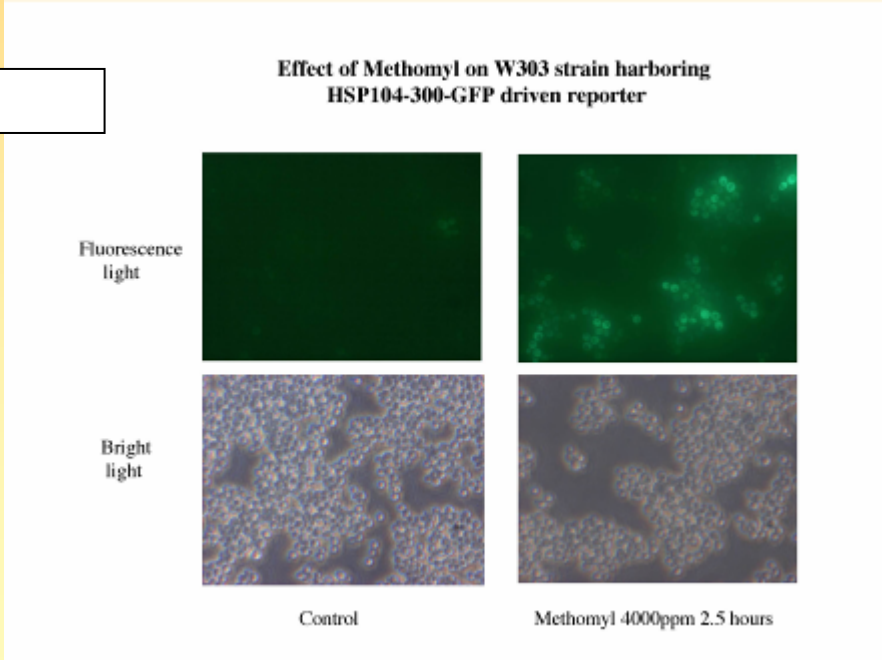
No Induction 0.03PPM Cd^{++} 20PPM DDVP Heat Shock

Clone # 31



No Induction 0.03ppm Cd^{++} Heat Shock

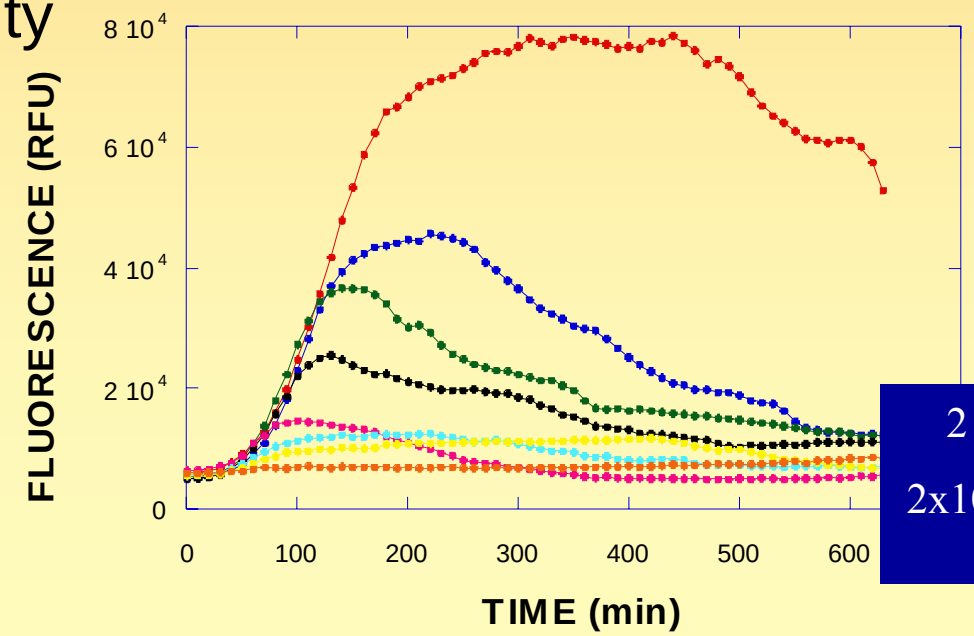
Yeast constructs



Using bacterial systems we demonstrated dose-dependent responses to all target compounds so far tested, at or close to the required detection thresholds

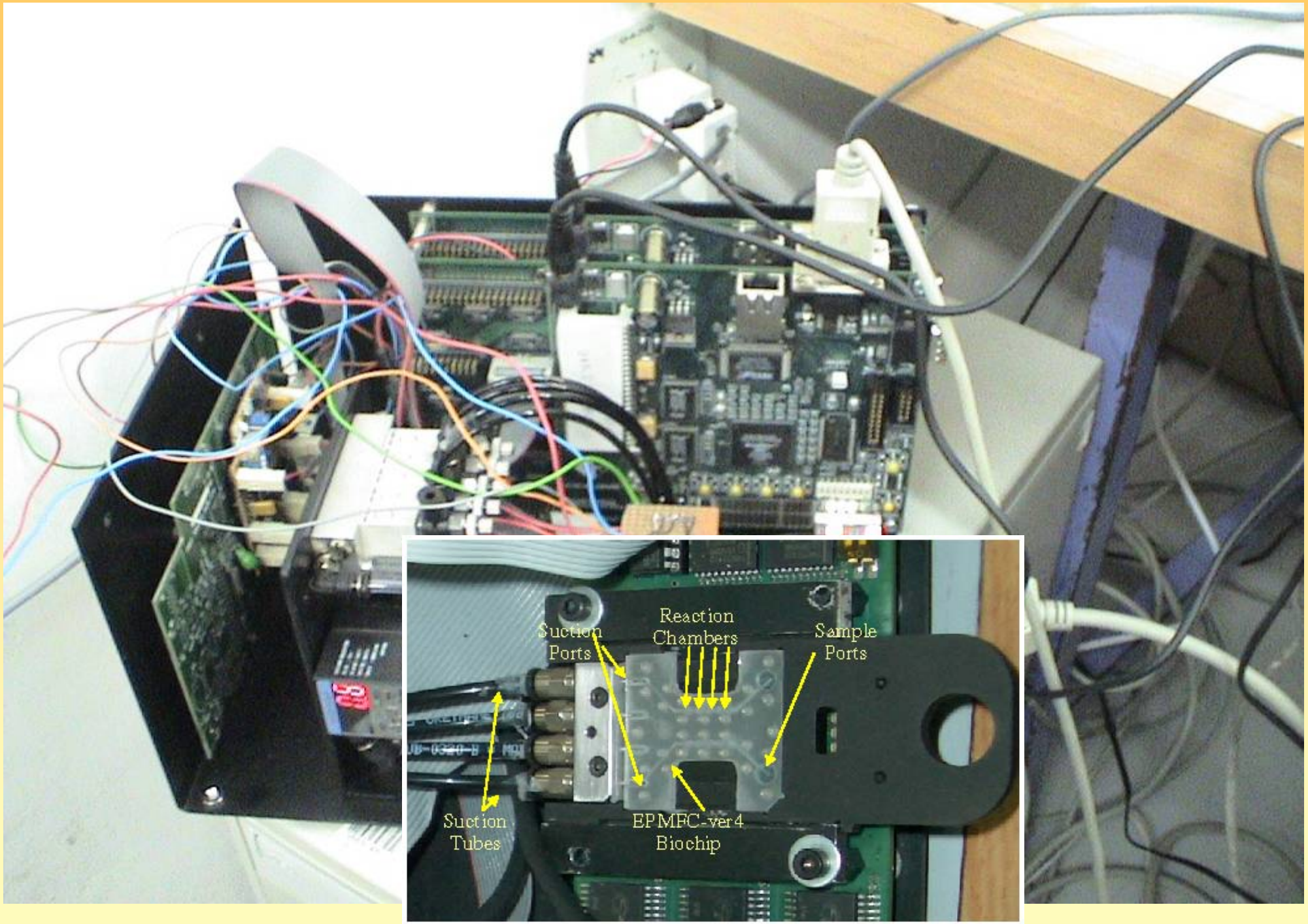
Obtaining a successful bacterial GFP induction in small volumes

- Absolute protection against drying
- Physiological condition of cells
- Appropriate cell density

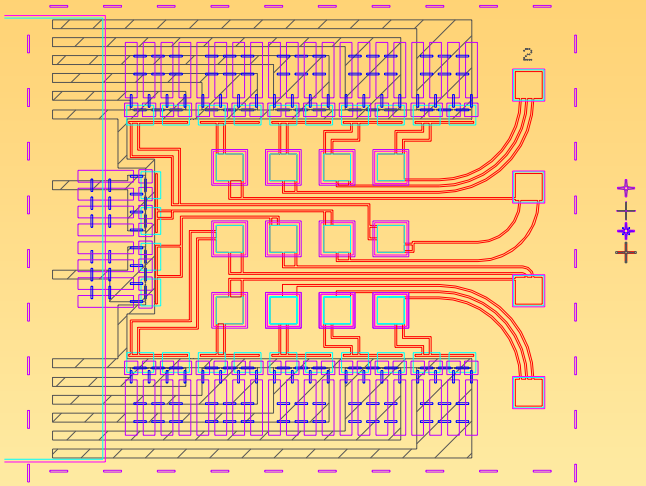


2 µl cell suspension

2x10^5 cells, *recA*::EGFP

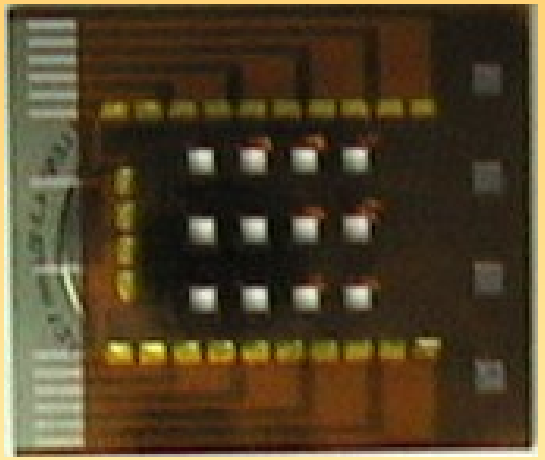

Suction
Ports
Reaction
Chambers
Sample
Ports
Suction
Tubes
EPMFC-ver4
Biochip

Putting the components together for a functional toxicity detection biochip

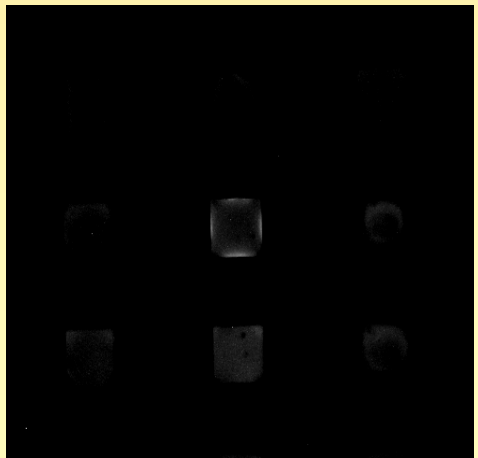


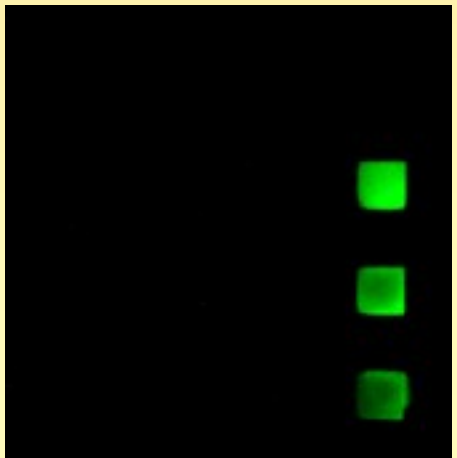
Chip design

Actual chip

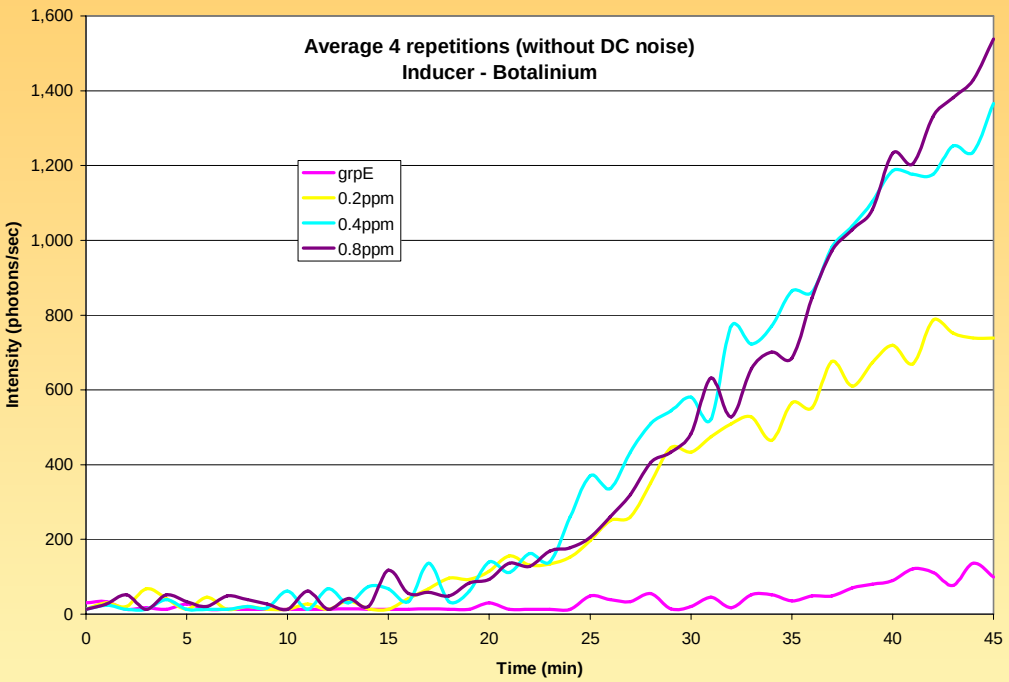


Background	Neutral sample	Toxic sample	Quantification





Putting the components together: on-chip botulinum toxin detection



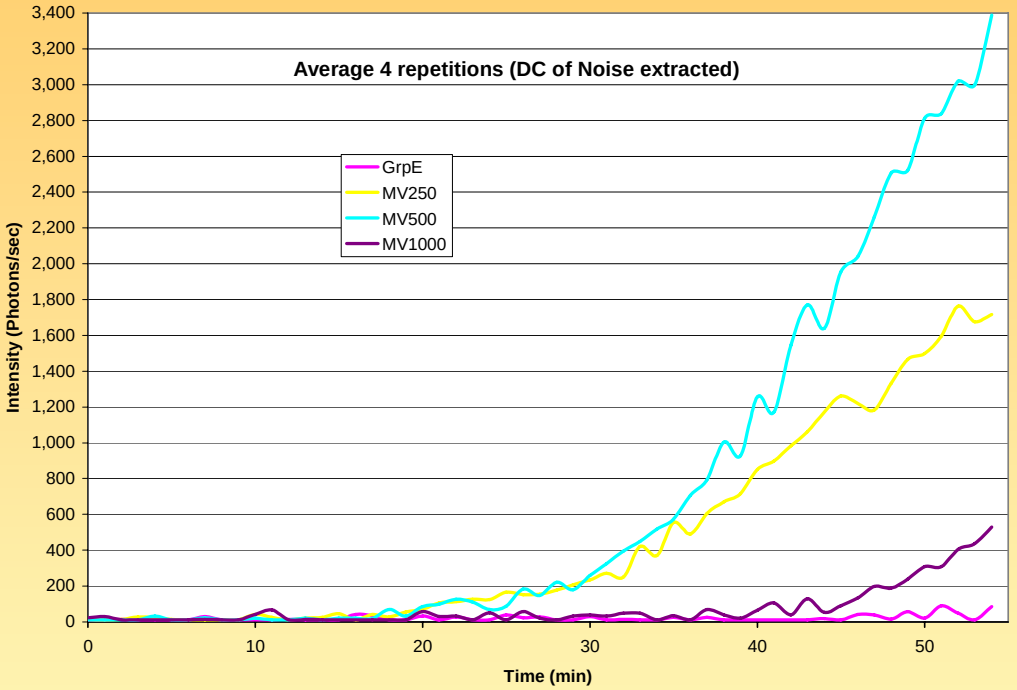


Figure 8.23. *Real time kinetics of on-chip light development in response to botulinum toxin (0.2, 0.4 & 0.8 mg/l, left panel) and paraquat (250, 500 & 1000 mg/l, right panel).*

IMT- Bucharest

National Institute for R&D in Microtechnologies

ISMB

Istituto Superiore Mario Boella

Toxichip

VigiCell

Health & Environment

Tel Aviv University

scienion

Tyndall

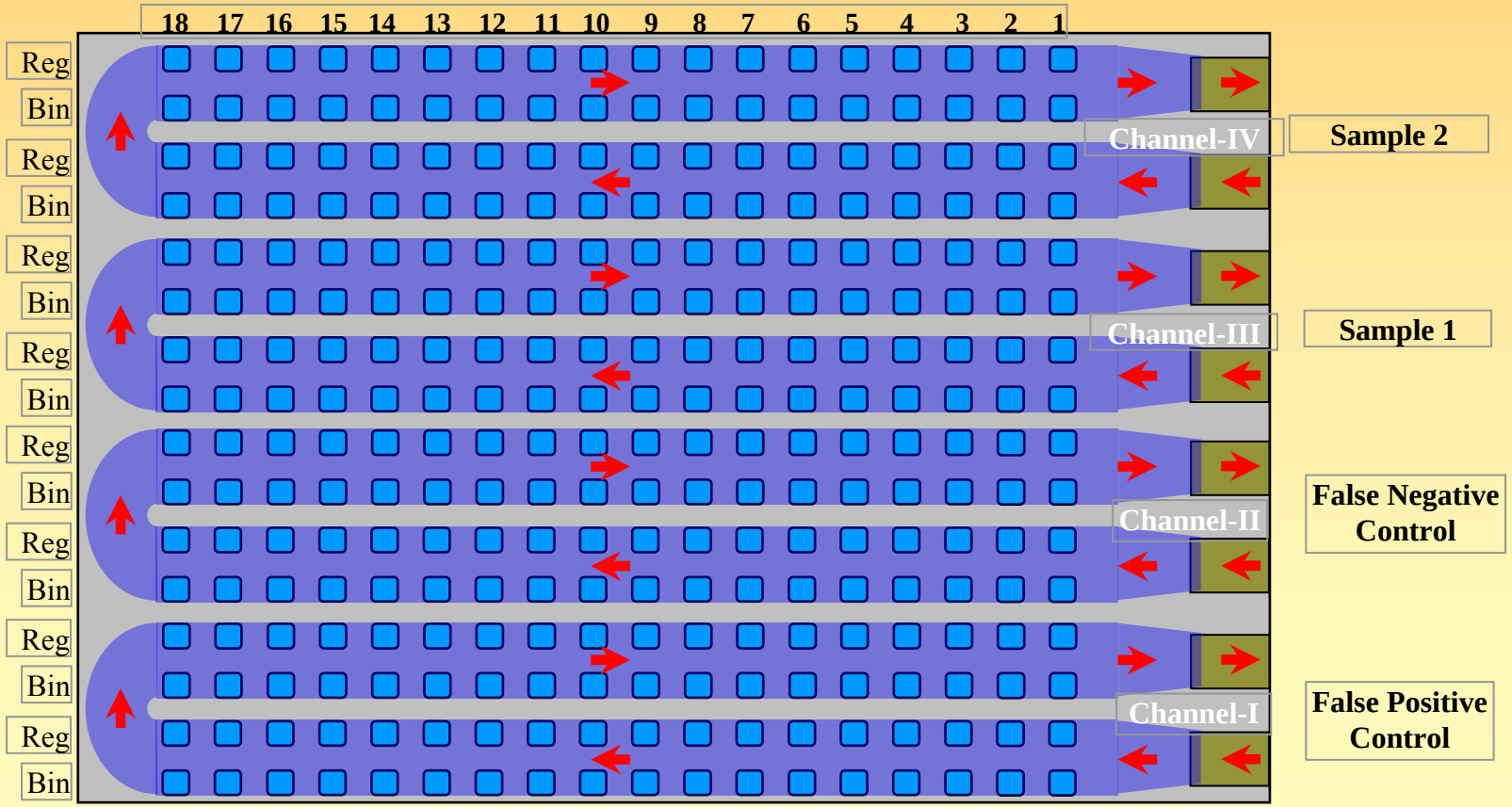
National Institute

EUROPEAN COMMISSION

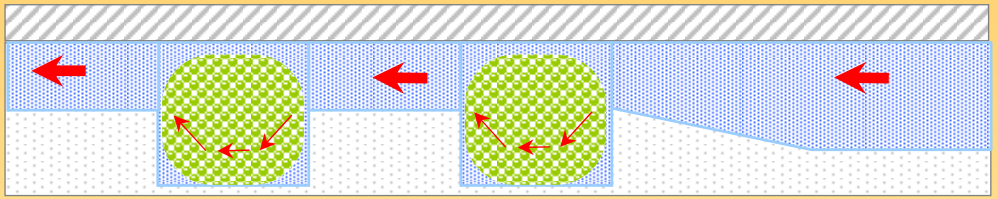
DIRECTORATE-GENERAL

Joint Research Centre

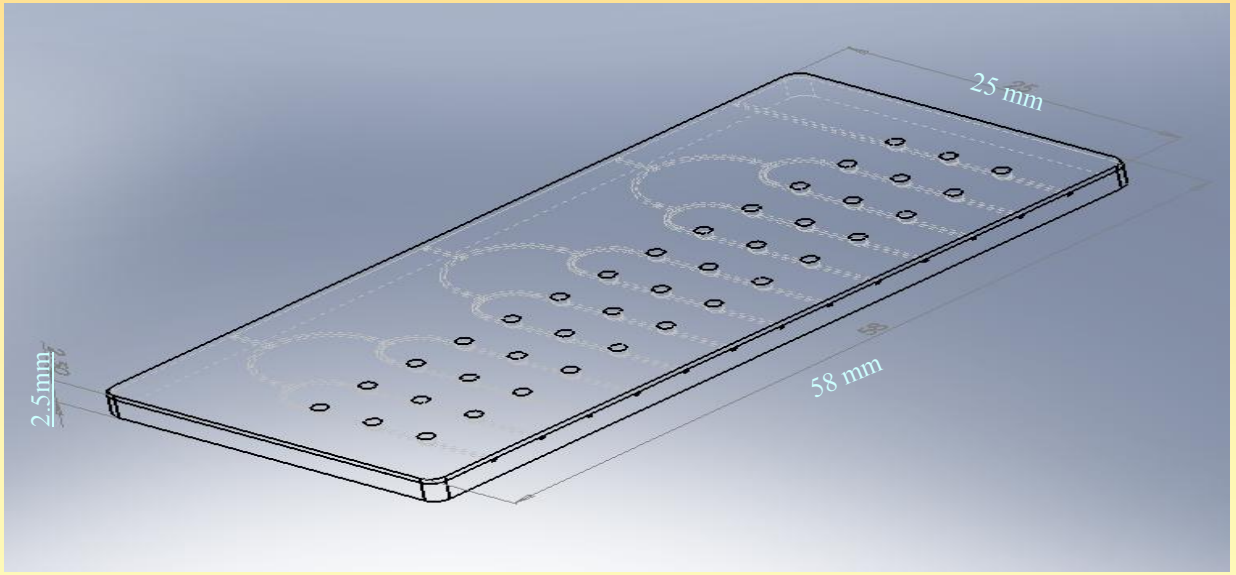
Flow-through whole-cell biochip: original design



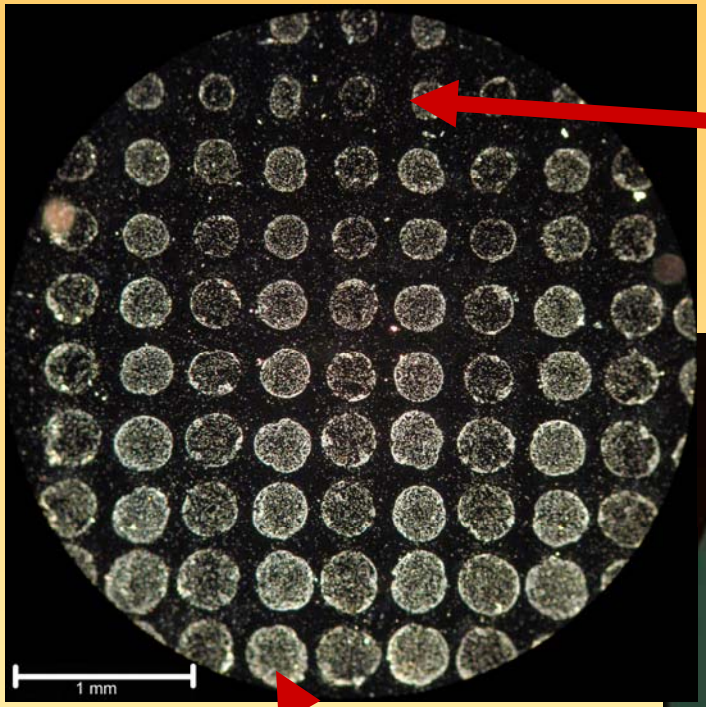
Flow-through whole-cell biochip: current design



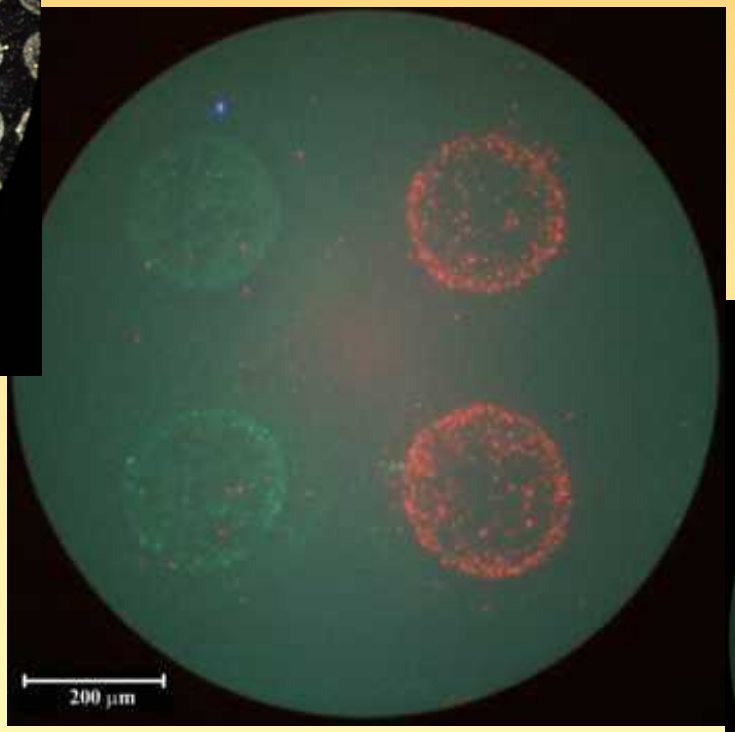
Complete 13 channel Biochip -Top View



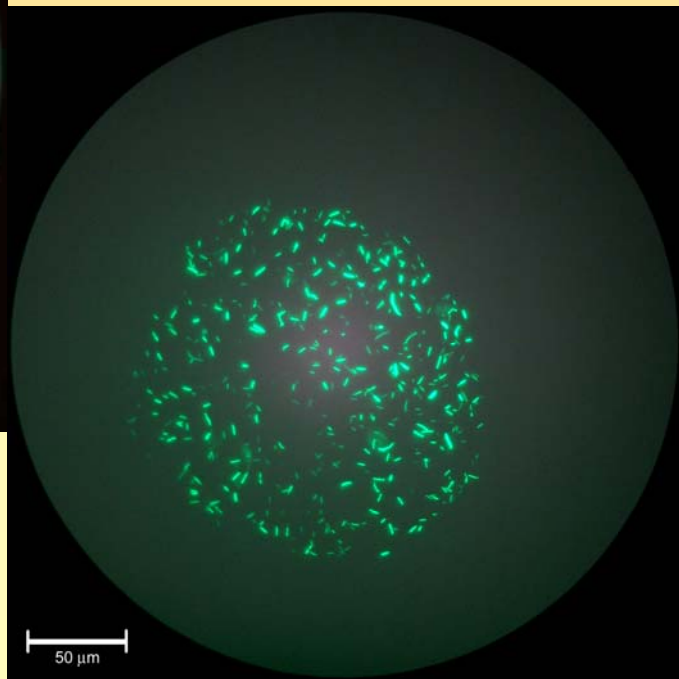
Cell patterning on a solid surface



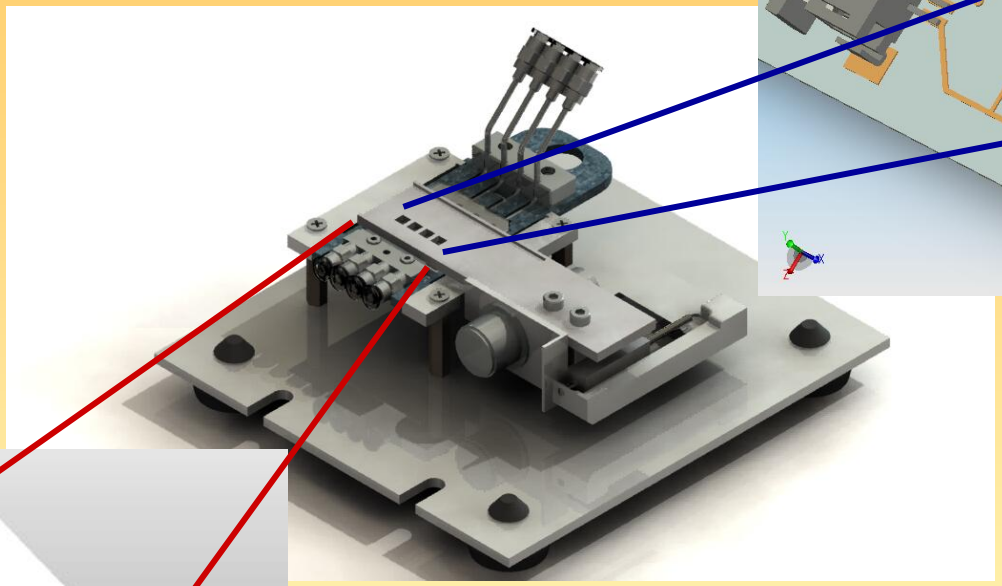
0.5 nl

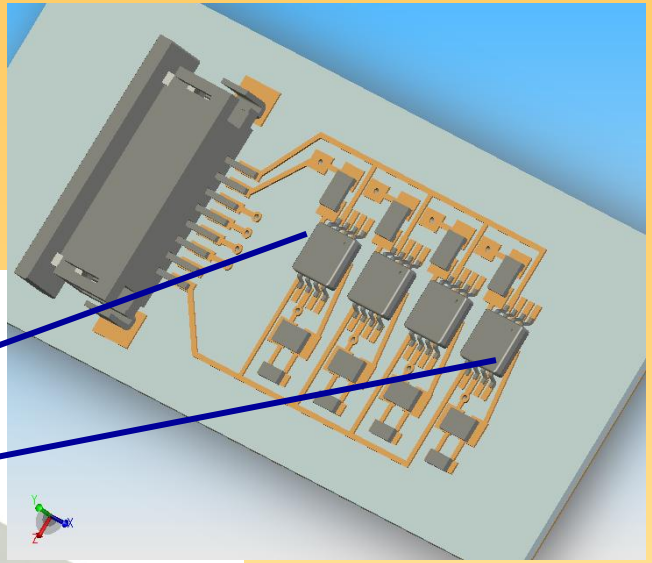


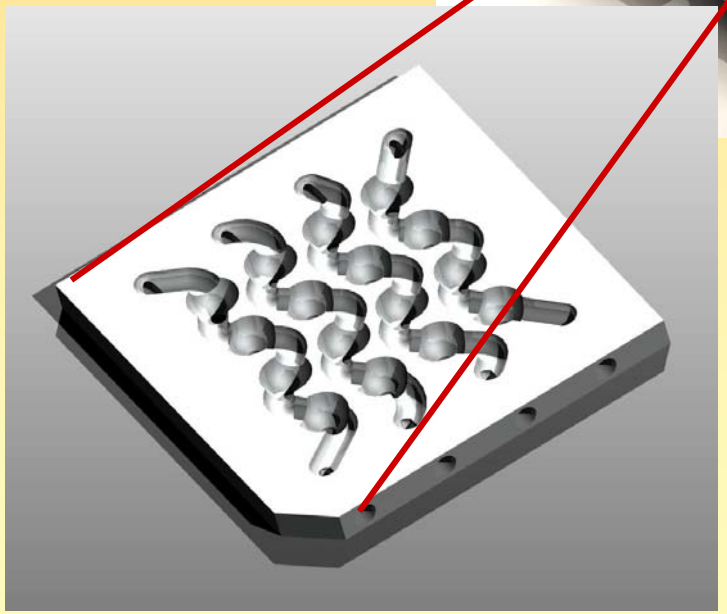
2.5 nl

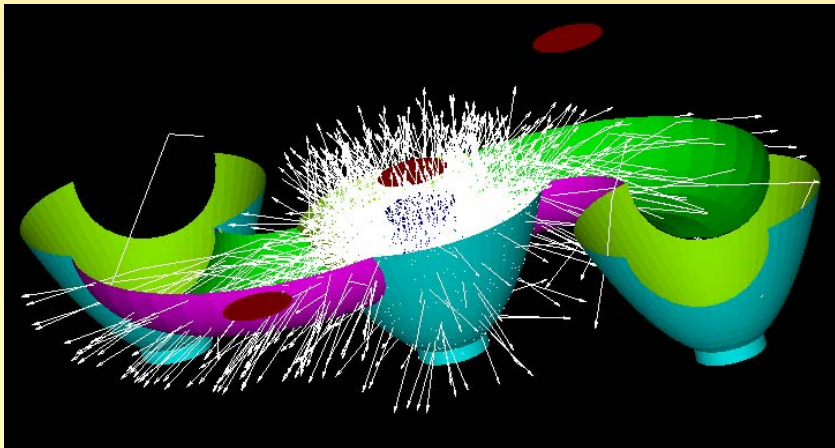


S. Yorish, Y. Shacham, H. Ben Yoav









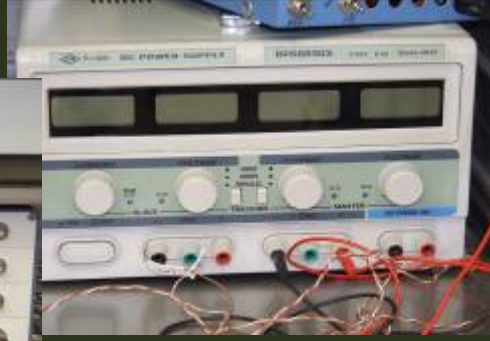
Two additional applications

1. Monitoring biological effects of RF radiation
2. Bioassays for nanoparticles toxicity

RF Radiation
Cellular Communication
Frequencies test system

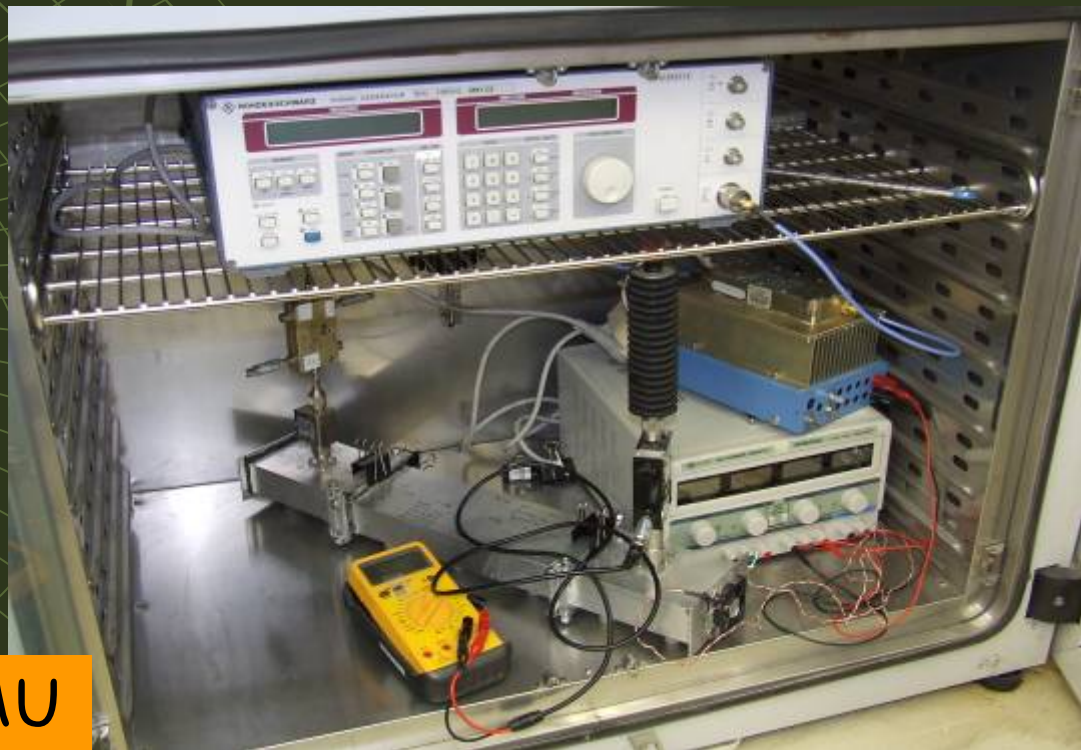
Amplifier

Frequency generator



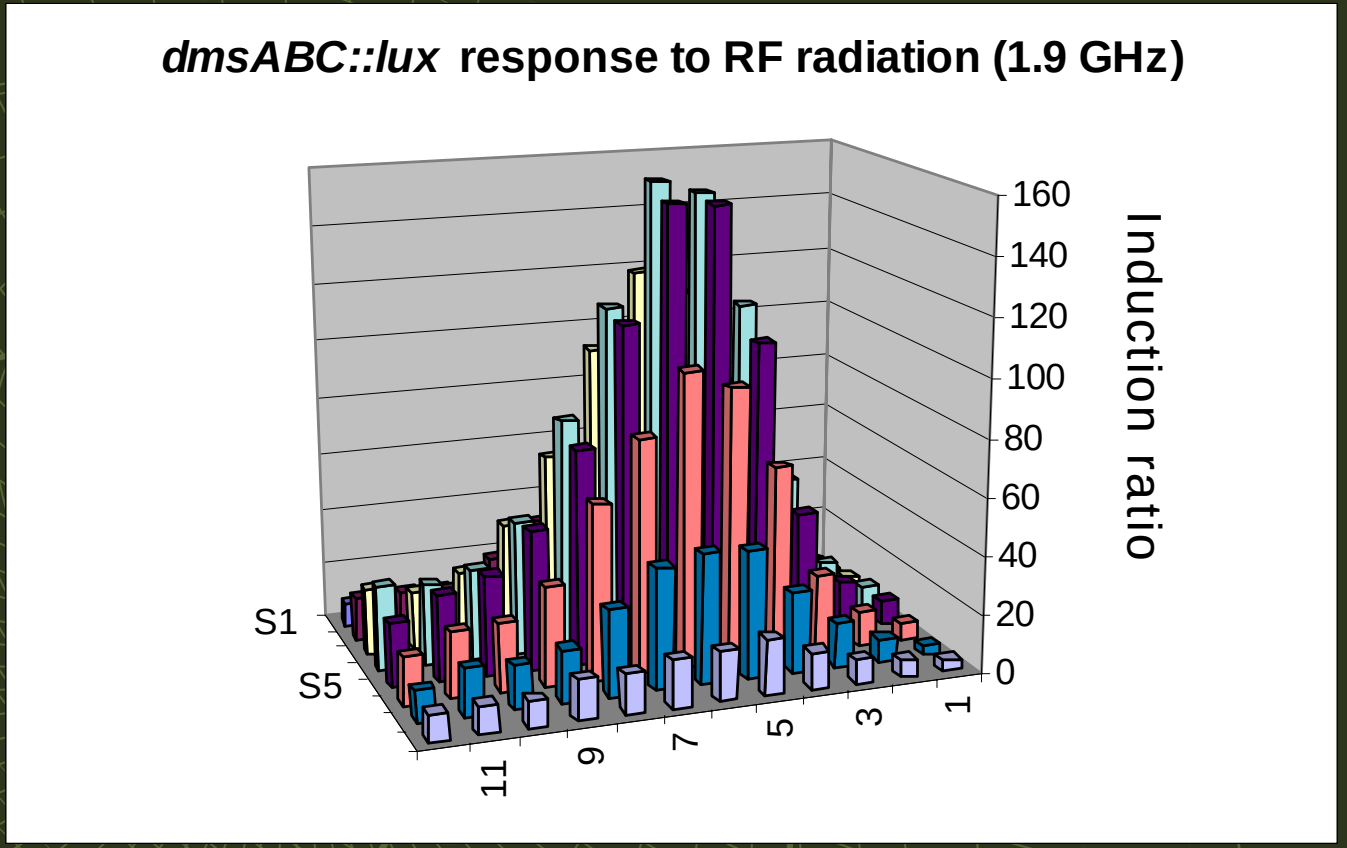
Power supply

Exposure chamber



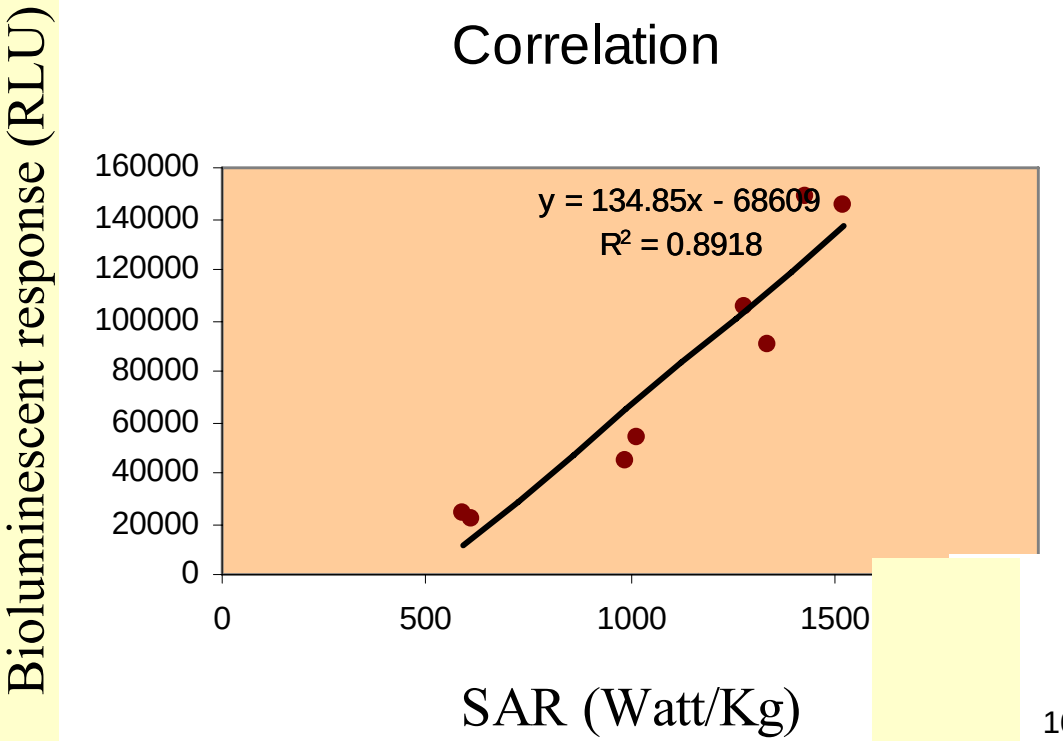
R. Korenstein, A. Abramovich, TAU

RF radiation at cellular communication frequencies: significant effects observed on sensor bacteria...



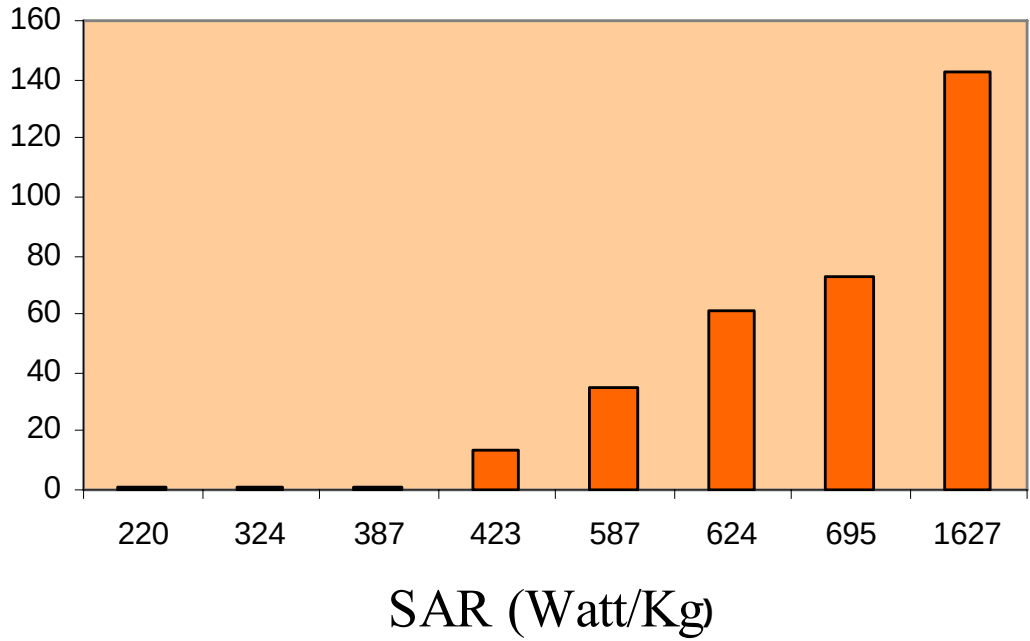
...but only using SAR values 100-1000 higher then in cellular phones

SAR/Bioluminescence Correlation

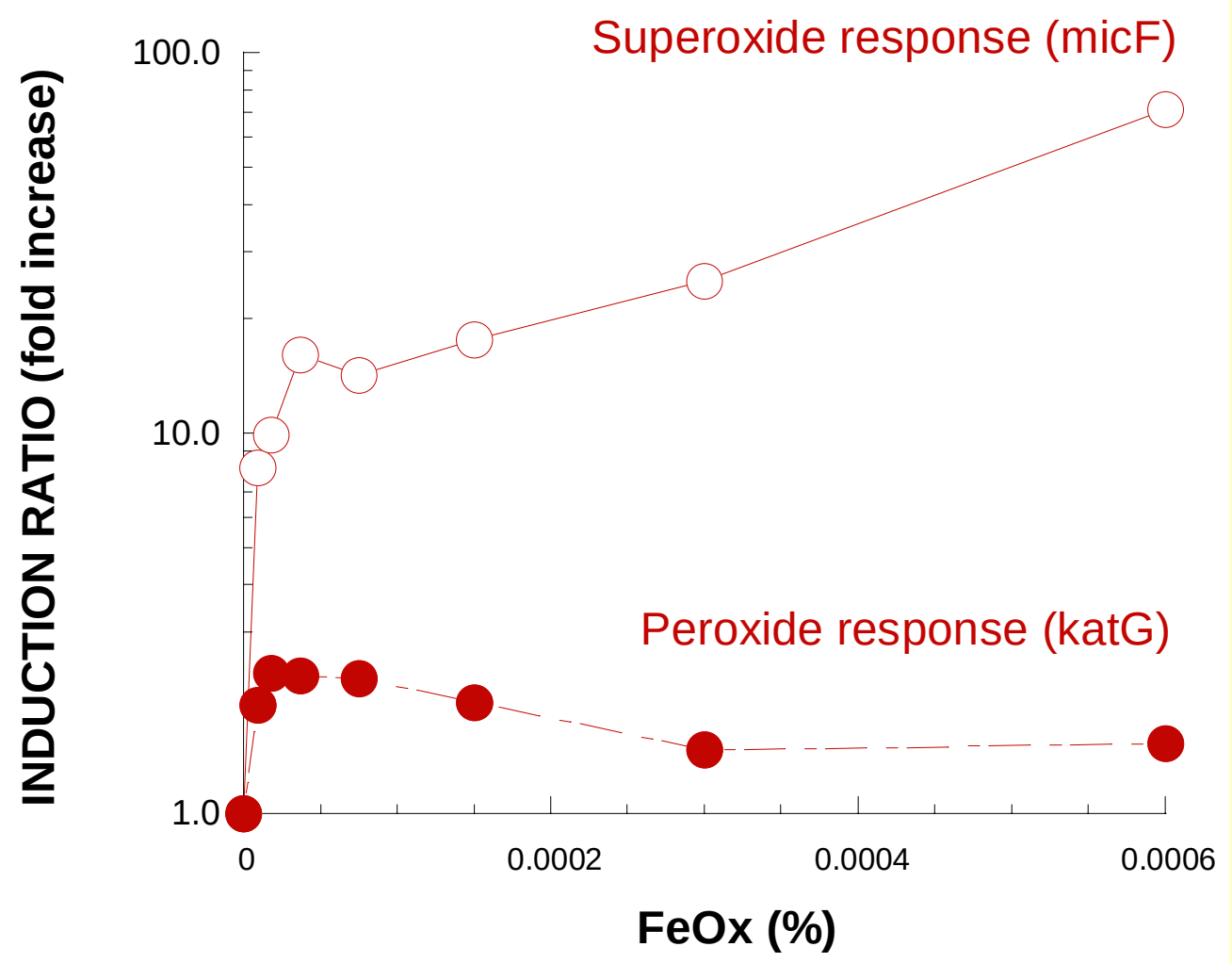


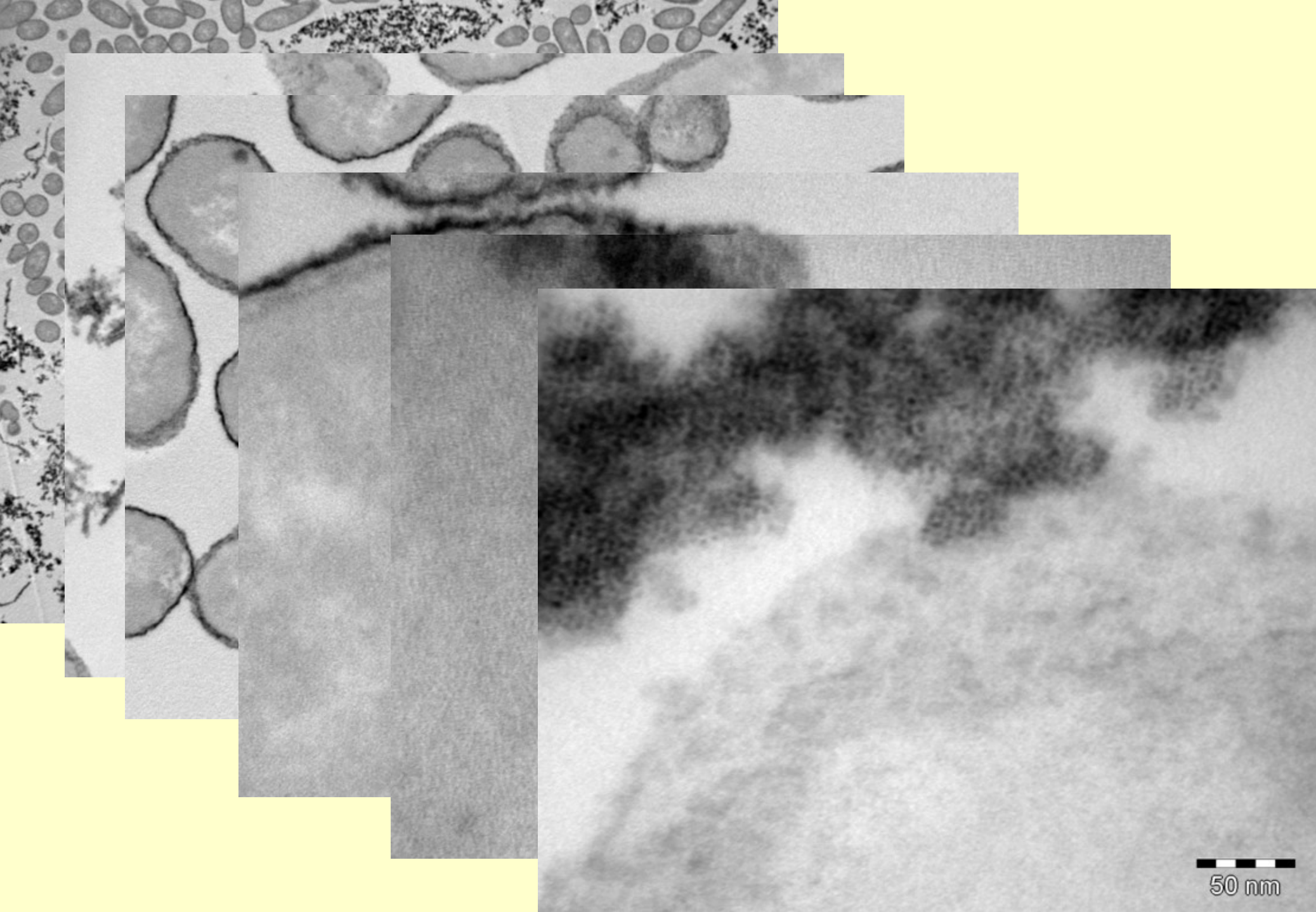
(SAR - Specific Absorbance Ratio)

Effect of total SAR on *dmsABC* induction



Strong oxidative effects of FeOx nanoparticles




50 nm

Summary

Reporter gene fusions are highly suitable for the construction of whole-cell reporters

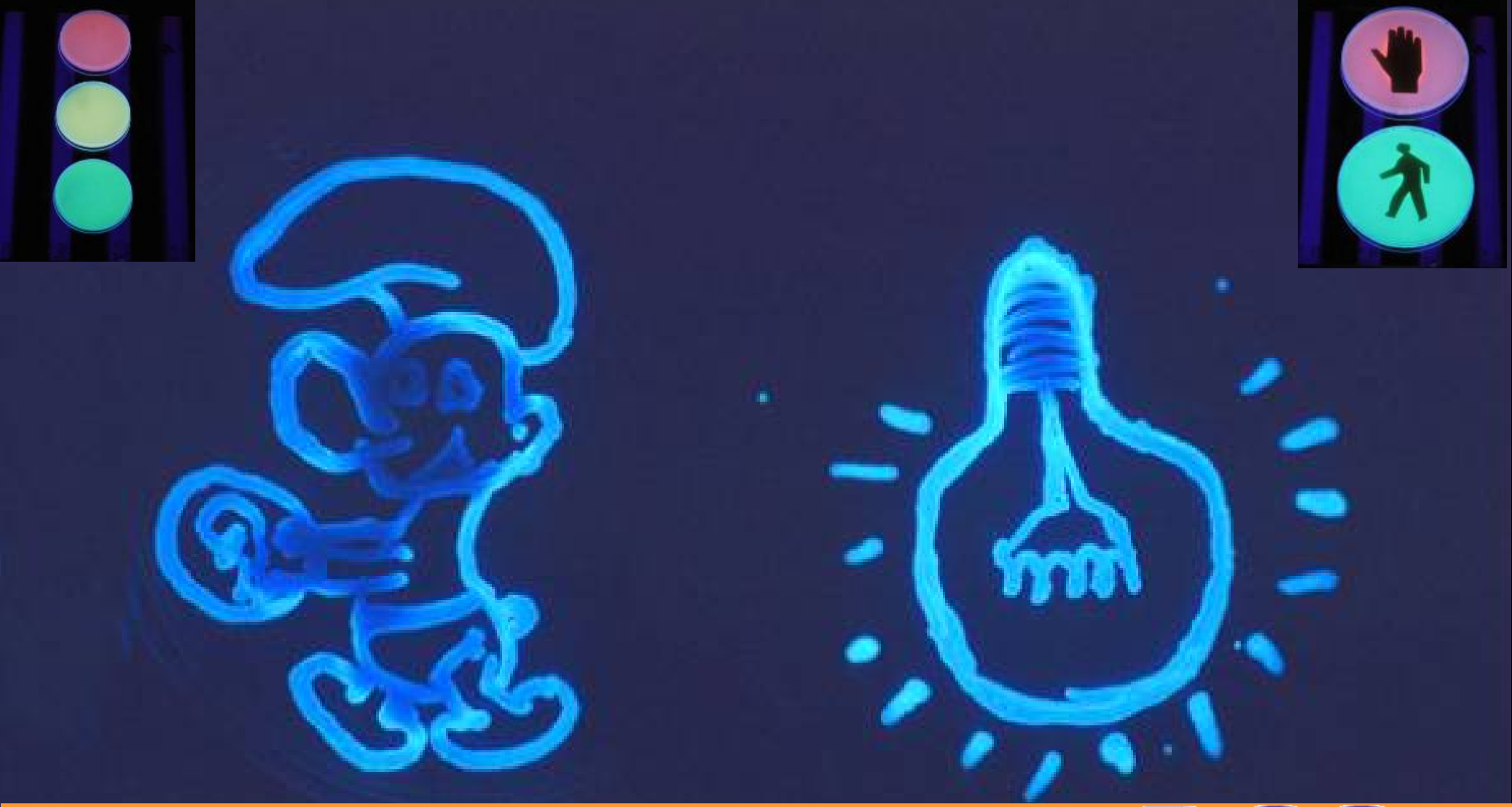
The approach allows real-time monitoring of cellular stress, and is thus attractive for the detection of toxic chemicals

We can also “tailor” the cells to report, in a dose dependent manner, the presence of biological toxins

Efficient long-term stabilization of the reporter cells was achieved by sol-gel encapsulation

First steps were taken towards the construction of a whole-cell reporter biochip and a dedicated toxicity analyzer

Preliminary results indicate that similar systems may be used to study the effects of RF radiation or nanoparticles on live cells



Funding: DARPA
Hebrew University
Israeli Ministry of Science
EU “Toxichip”