



BioDetection Systems

# 9th Biodetectors Meeting 2016 Lausanne

## State of the Art Biodetectors from BDS

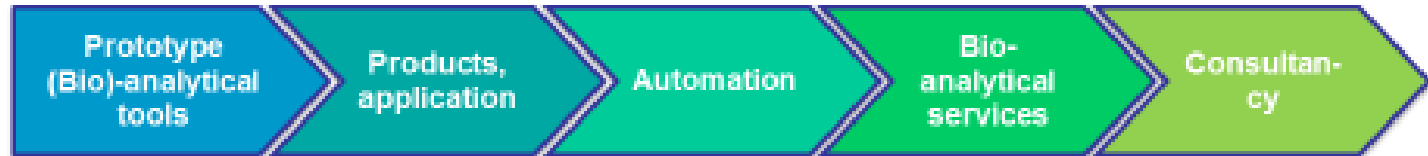
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CEO of BDS & MLS, Amsterdam  
Professor of Environmental Toxicology & Ecogenomics,  
VU University Amsterdam**



# Core activity: Develop and apply novel bioassays for health & safety assessment

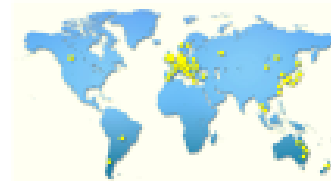


Safety assessment    Authenticity testing    Screening for bioactive compounds



Projects, partnerships

Demonstration studies



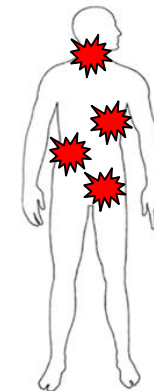
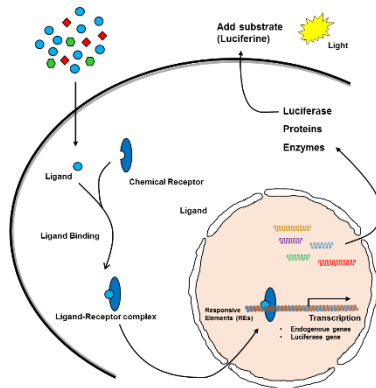
Novel bioanalytical solutions

## Safety assessment:

- ❖ Food,
- ❖ Feed,
- ❖ Water,
- ❖ Health
- ❖ Environment
- ❖ Chemicals
- ❖ Consumer products

## Key Benefits:

- ❖ High predictivity of health related-effects
- ❖ Good estimate of total effect from mixtures
- ❖ Can predict unknown effects of chemicals
- ❖ Can discover unknown chemicals in matrices
- ❖ Level of precision similar to instrumental methods
- ❖ Low cost, high capacity, easy to operate

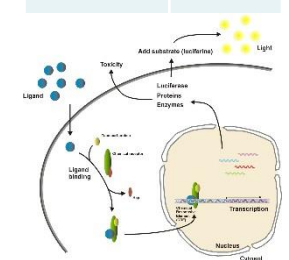


## Best Usage:

- ❖ Most valuable tool for (human) biomonitoring
- ❖ Powerful screening tool for safety assessment e.g food, water
- ❖ Good *in vitro* alternative for chemical safety assessment

Nuclear receptors			Signaling pathways			Controls		
name	status	cell	name	status	cell	name	status	cell
DR CALUX	✓	U2OS						
PAH CALUX	✓	all						
ER CALUX	✓	all						
ERalpha CALUX	✓	all						
ERbeta CALUX								
ERalpha CALUX								
ERbeta CALUX								
AR CALUX								
PR CALUX								
GR CALUX								
TR CALUX								
RAR CALUX								
PPARγ1 CALUX								
PPARγ2 CALUX								
PPARα CALUX	✓	U2OS	STAT CALUX	✓	U2OS			
PPARδ CALUX								
LXR CALUX								
PXR CALUX								
VDR CALUX								
MR CALUX	✓	U2OS						

- Acute toxicity
- Oxidative stress
- AhR pathway
- Endocrine effects/EDCs
- obesogens
- Reproductive effects
- Genotoxicity/carcinogenicity
- Metabolism
- etc



**CALUX: n=28**  
**Agonist/antagonist: 25x2=56 assays**



# BDS AOP-based detection methods are applied in a wide range of sectors

Environment  
technology



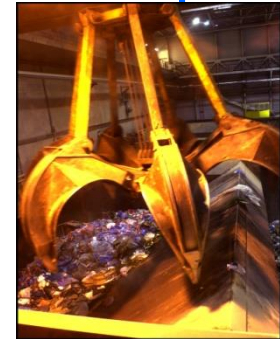
Human  
epidemiology



Medicin



Technique and  
processes



Quality  
Management



Feed



Food



Pharma





# What are new developments within BDS regarding bioassays/applications?

## OECD currently identified urgent needs & gaps in EDC testing:

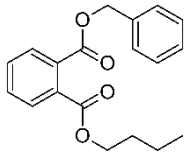
- Incorporation of metabolism into *in vitro* (EDC) assays
- Thyroid *in vitro* assays
- PPAR alfa and gamma assays
- AhR based assay, like DR-CALUX for OECD validation

## *BDS innovation priorities 2015 related to EDCs*

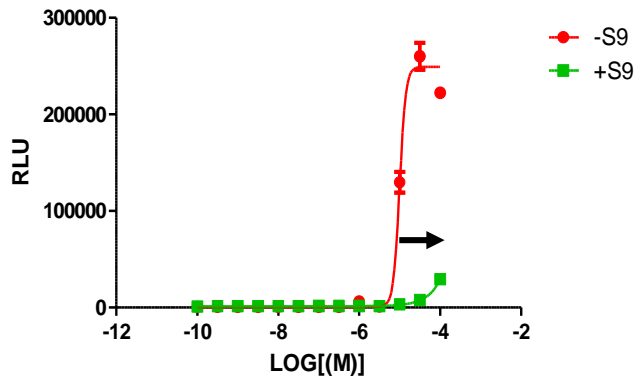
- Development dual metabolism – CALUX reporter system
- Continue validation of ER, AR for OECD
- Validation/applications of TTR-TR $\beta$  CALUX
- H295R based CALUX for steroidogenesis
- Validation of DR-CALUX assay for OECD
- PPARalfa, -beta, -gamma CALUX panel for obesity/metabolic syndrome

### Methods:

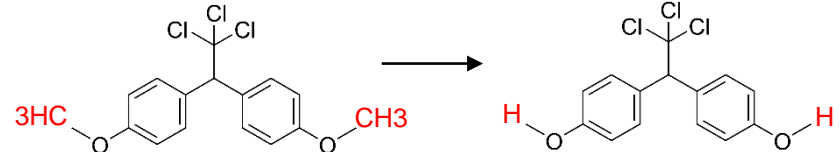
- Expose ERα-CALUX cells to compound
- Add S9 mix (0.03%), including cofactors for Phase I (P450s)
- Incubate for 24h
- Lyse & measure



butyl benzyl phthalate

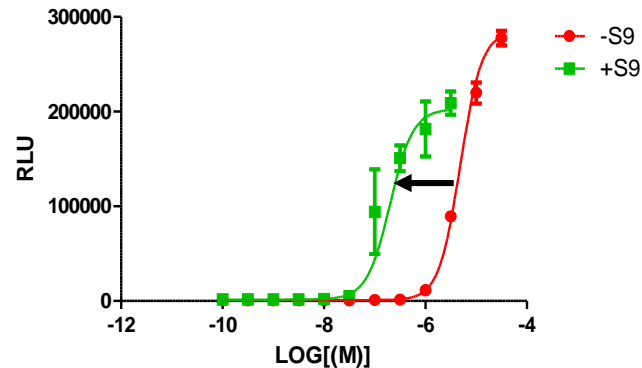


Butyl benzyl phthalate:  
almost completely degraded by S9 mix



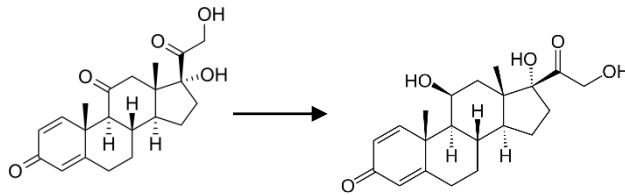
methoxychlor

HPTE



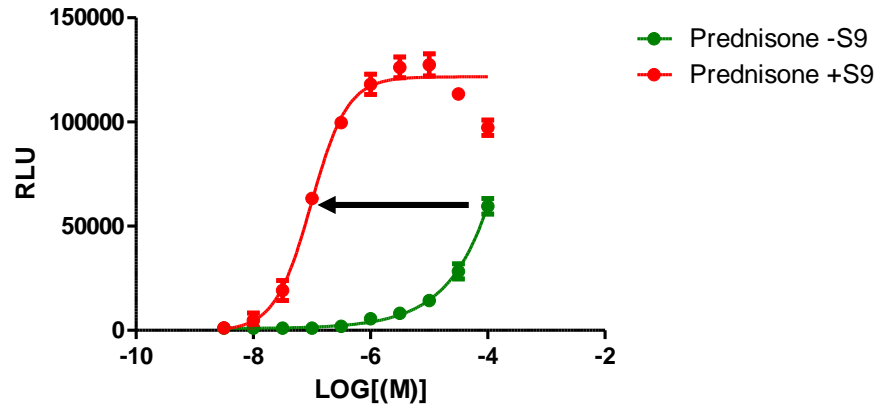
Methoxychlor:  
Converted into more estrogenic metabolite HPTE by S9 mix

GR-CALUX



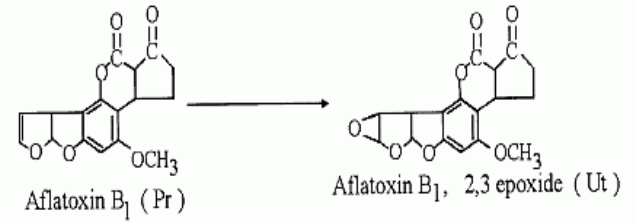
Prednisone

Prednisolone

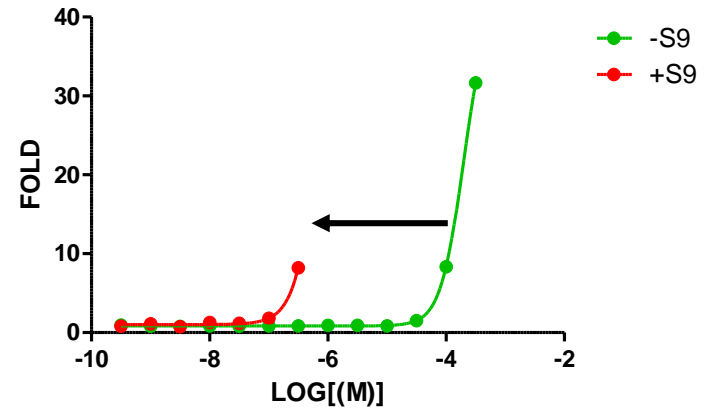


Prednisone:  
Converted into more active glucocorticoid  
Prednisolone by S9 mix

p53-GENTOX



Aflatoxine B1

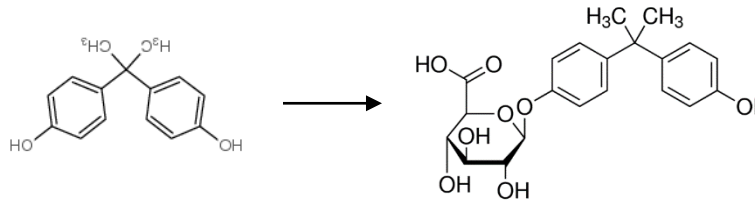


Aflatoxin B1:  
Converted into DNA-damaging adduct by S9 mix

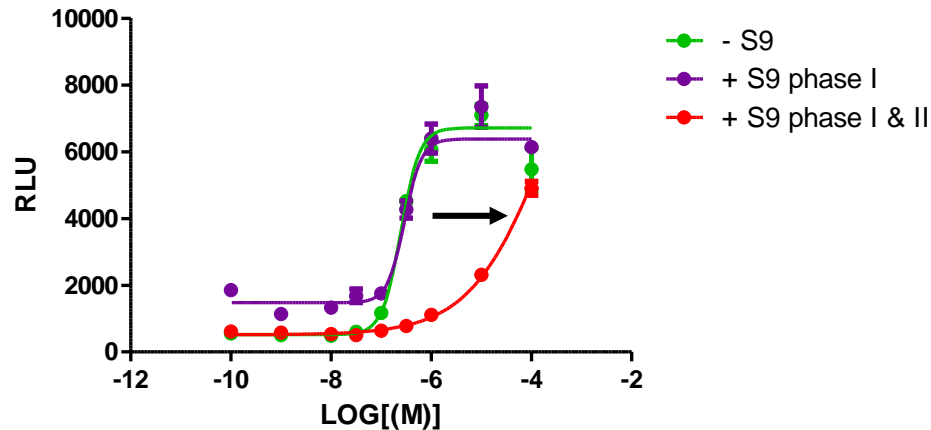


### Methods:

- Expose ERα-CALUX cells to compound
- Add S9 mix (0.03%), including cofactors for Phase I (P450s) and Phase II (SULTs, UGTs, GSTs)
- Incubate for 24h
- Lyse & measure

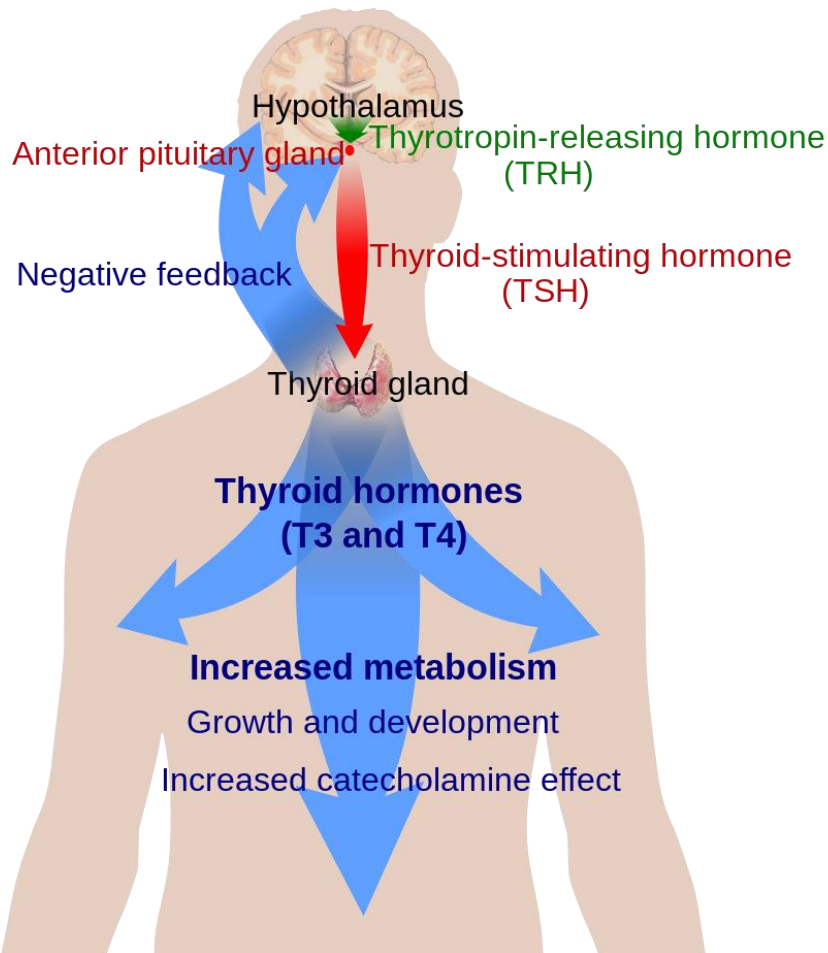


**Bisphenol A**



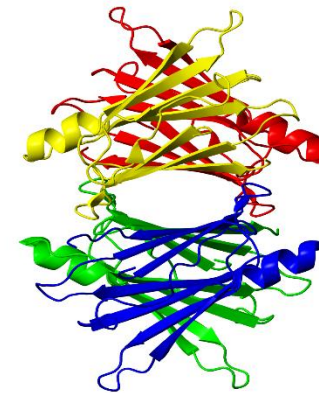
Bisphenol A:  
 Not affected by Phase I metabolism  
 Conjugated by phase II metabolism

## Thyroid system



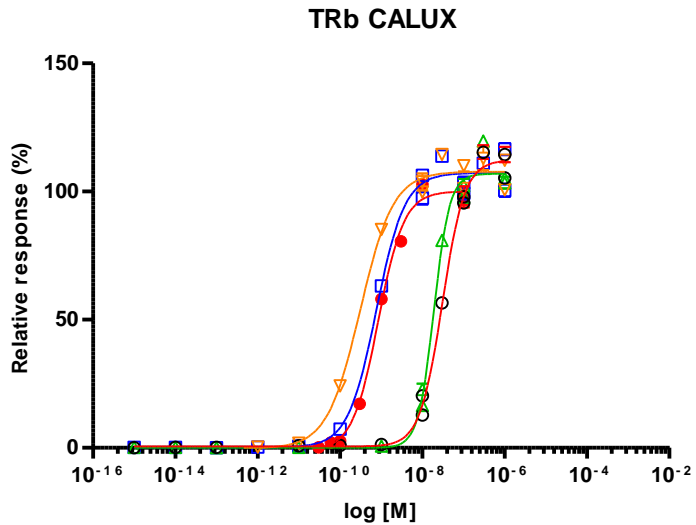
## Thyroid hormone transport

- TBG
- TTR
- albumin

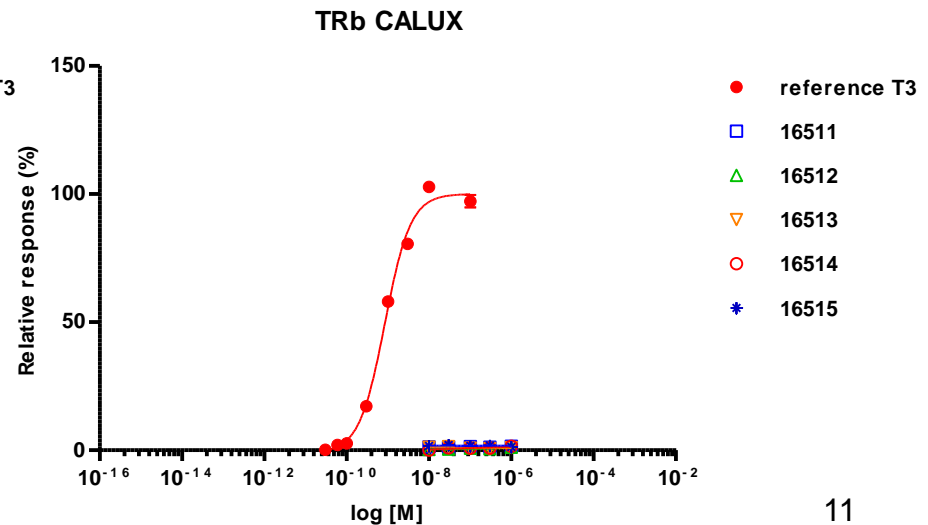
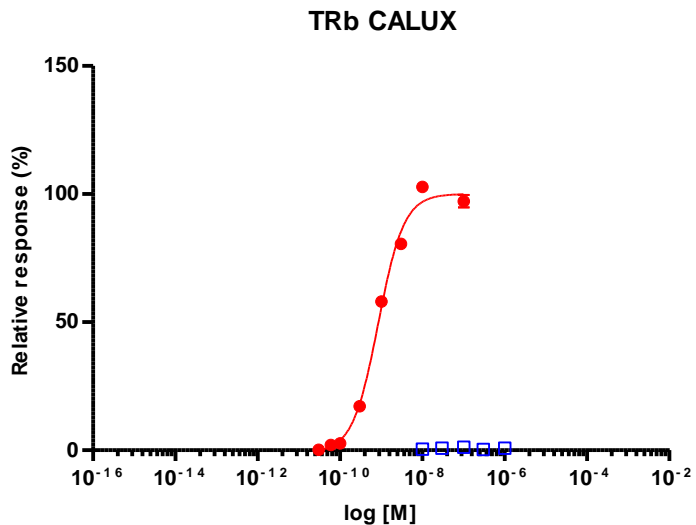


# Only very few chemicals act directly on the Thyroid Receptor

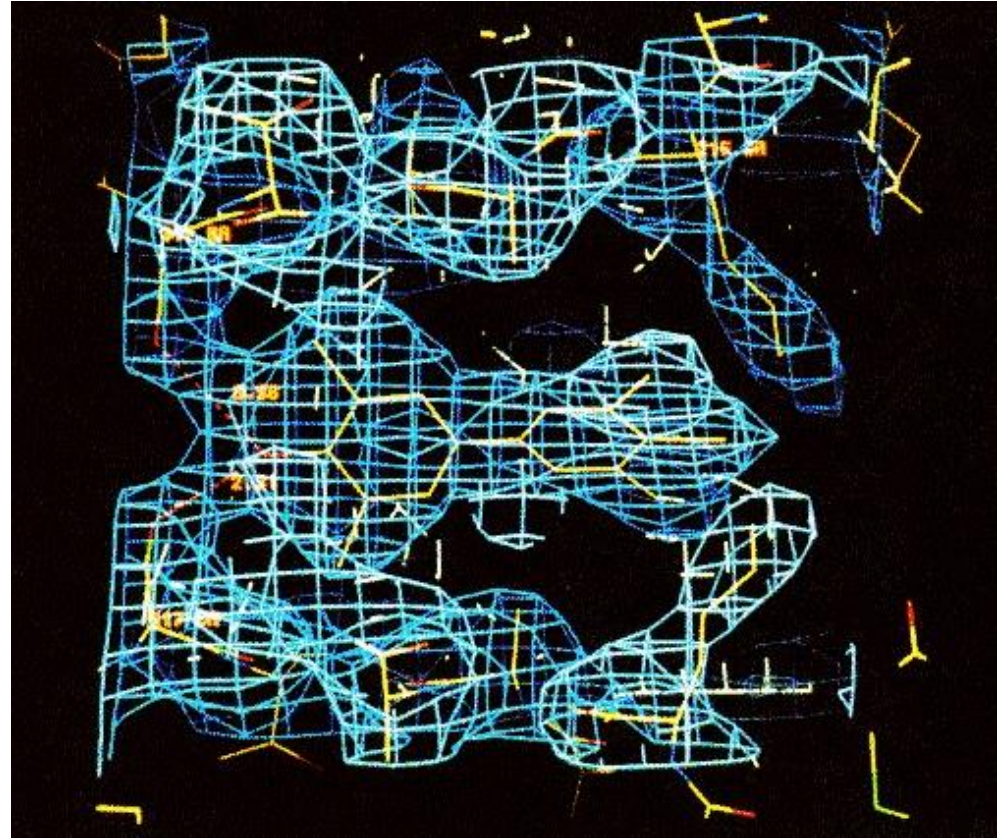
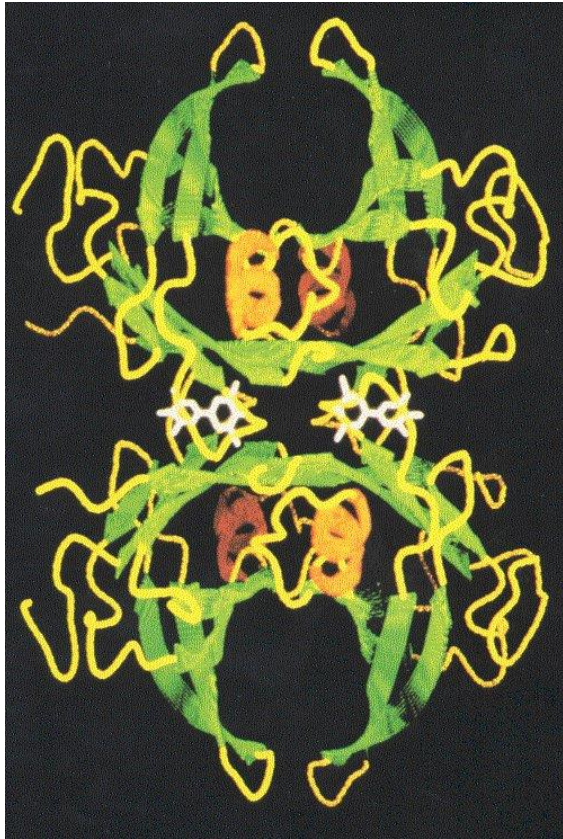
## Example results: TR $\beta$ CALUX



Sample ID*	Compound	CAS	
1	16506	Triiodothyronine (T3)	6893-02-03
2	16507	Thyroxine (T4)	51-48-9
3	16508	TRAC (T3-like analogue)	51-24-1
4	16509	TETRAC (T4-like analogue)	67-30-1
5	16510	Amiodarone	19774-82-4
6	16511	Pentachlorophenol (PCP)	87-86-5
7	16512	Ethylene thiourea (ETU)	96-45-7
8	16513	2,2,4,4-tetrahydroxybenzophenone	131-55-5
9	16514	Methimazole	60-56-0
10	16515	Solvent only (negative control)	

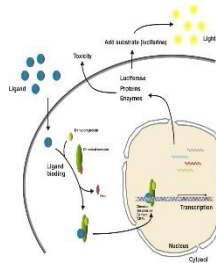
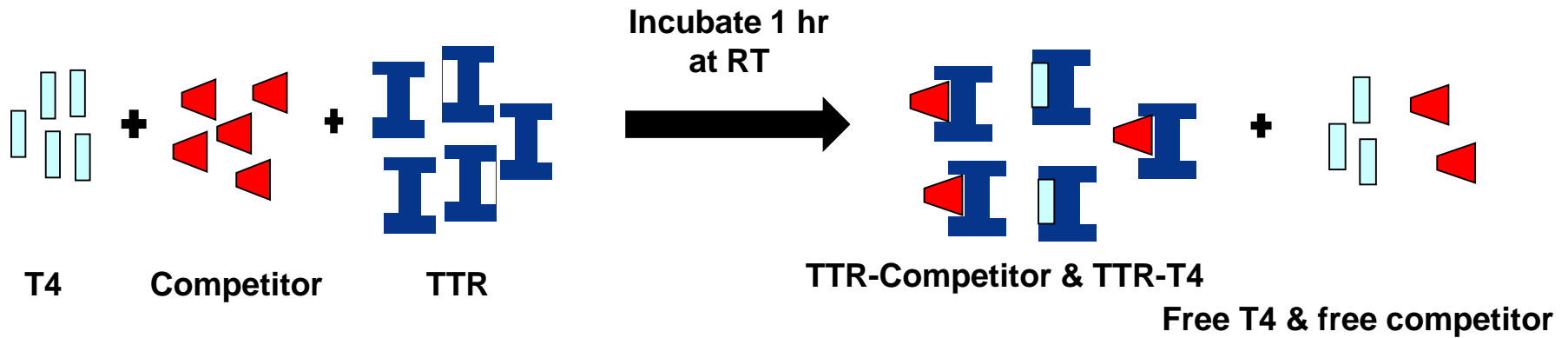


# Most thyroid-based EDCs act on TH transport & metabolism steps

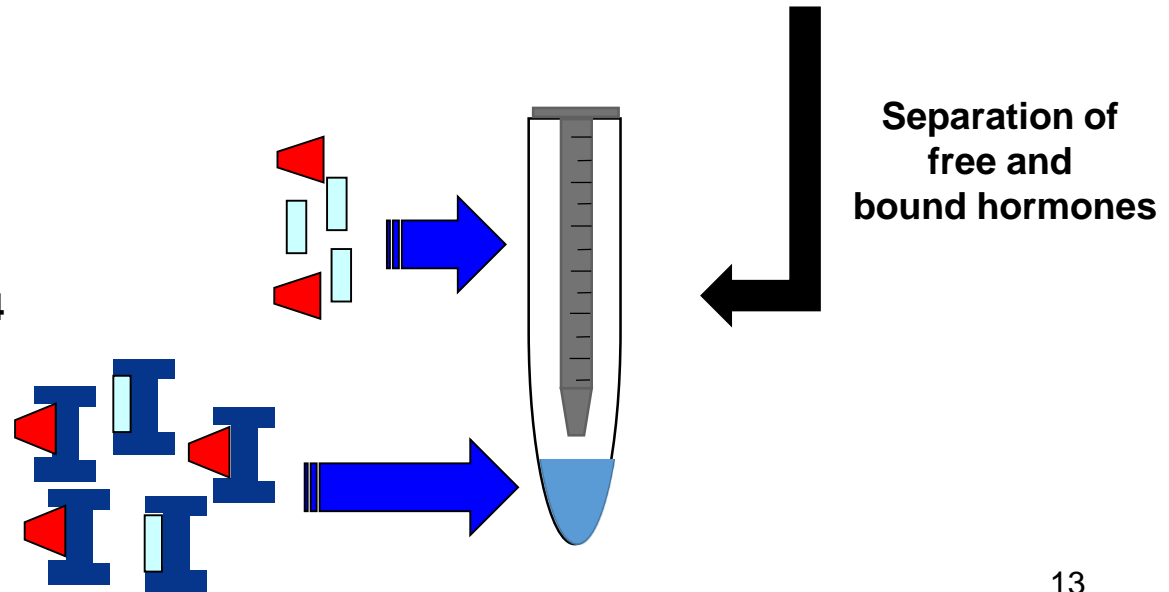


**X-Ray crystallographic analysis of hydroxy-PCB nested in the T4-binding pocket on TTR Ghosh et al., Acta Cryst. (2000) D56, 1085-1095**

# Development of label-free (TTR-)TR $\beta$ CALUX assay (Available mid 2016)



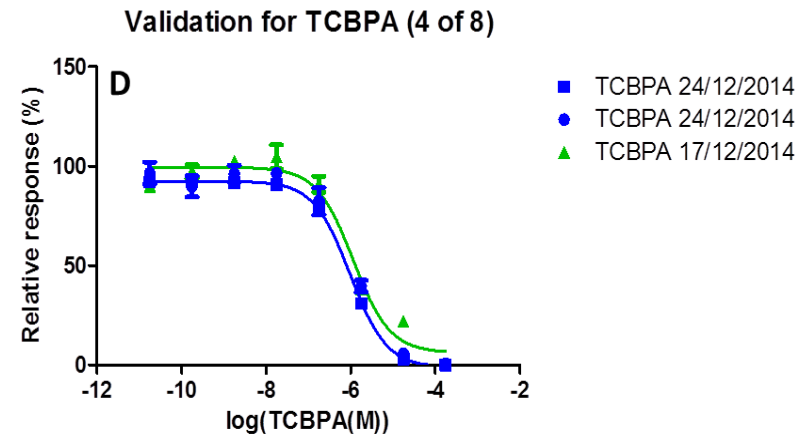
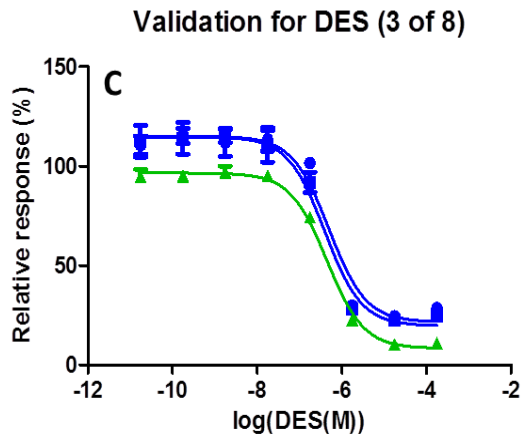
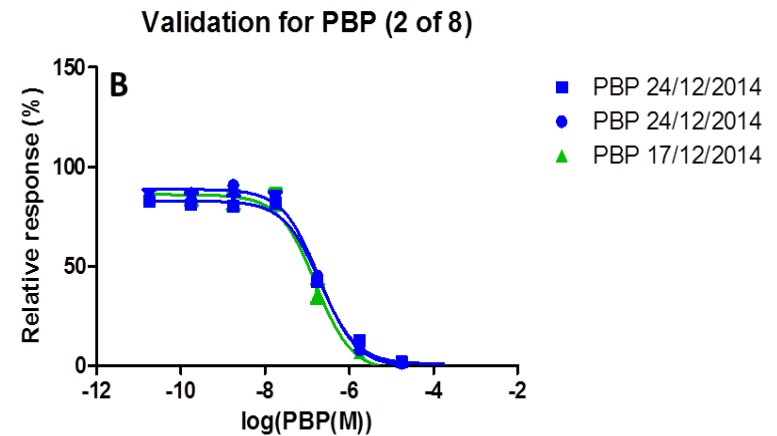
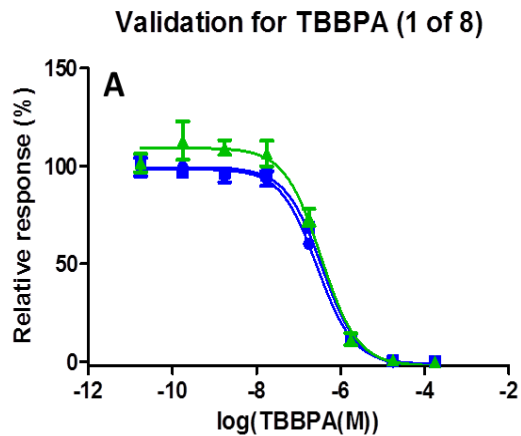
TR $\beta$  CALUX incubation (overnight) and subsequent analysis of bound T4



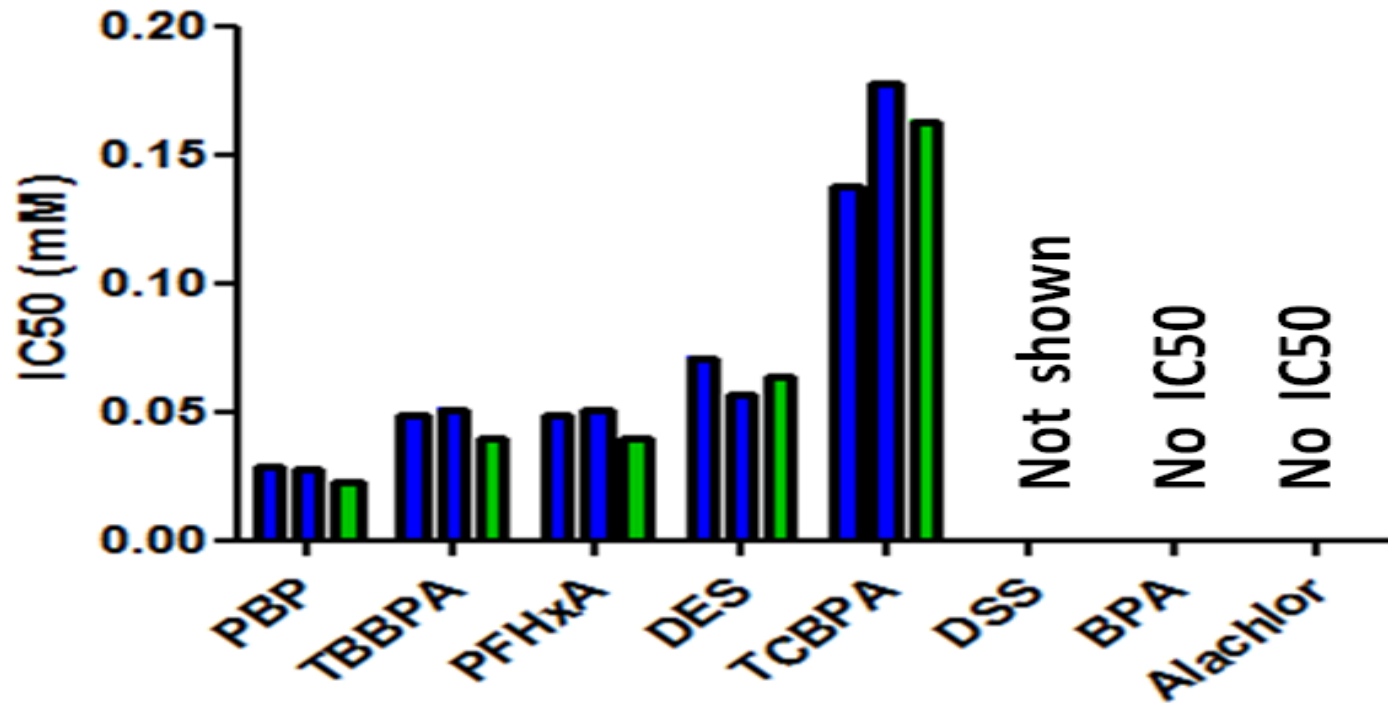
- 150  $\mu$ l eluate
- 500  $\mu$ l exposure medium



# Several examples of TTR-binding inhibition by halogenated phenolic compounds (1)



# Several examples of TTR-binding inhibition by halogenated phenolic compounds (3)





# What are new developments within BDS regarding bioassays/applications?

## BDS innovation priorities: non-EDC applications

- Continue robot-based CALUX methods (endocrine, carcinogenic)
- CALUX panel bioactivity screening panel design (plant, biobased)
- Develop application CALUX panel for Mycotoxins
- Decision support tool Chemical safety assessment by bioassays
- CALUX panel application for biomass valuation & soil quality
- NGS-based authenticity test development (oil, meat, TBD)
- Luc-based bacterial reporters

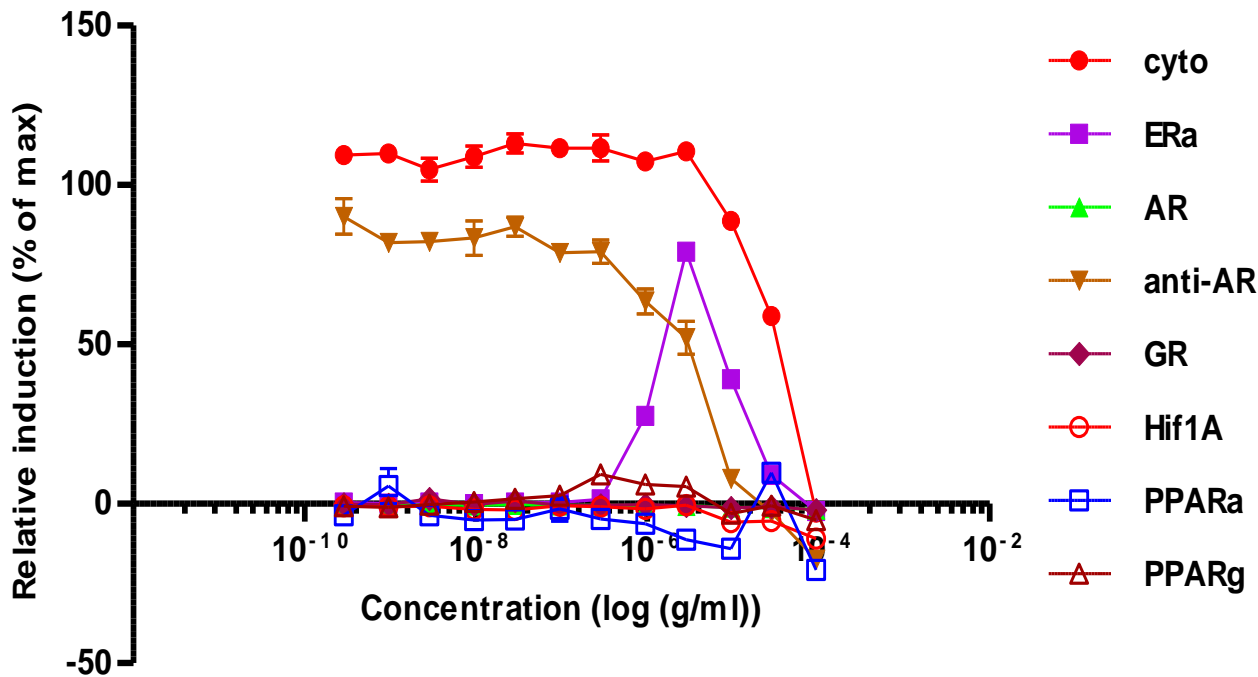


## Cleistocalyx Operculatus

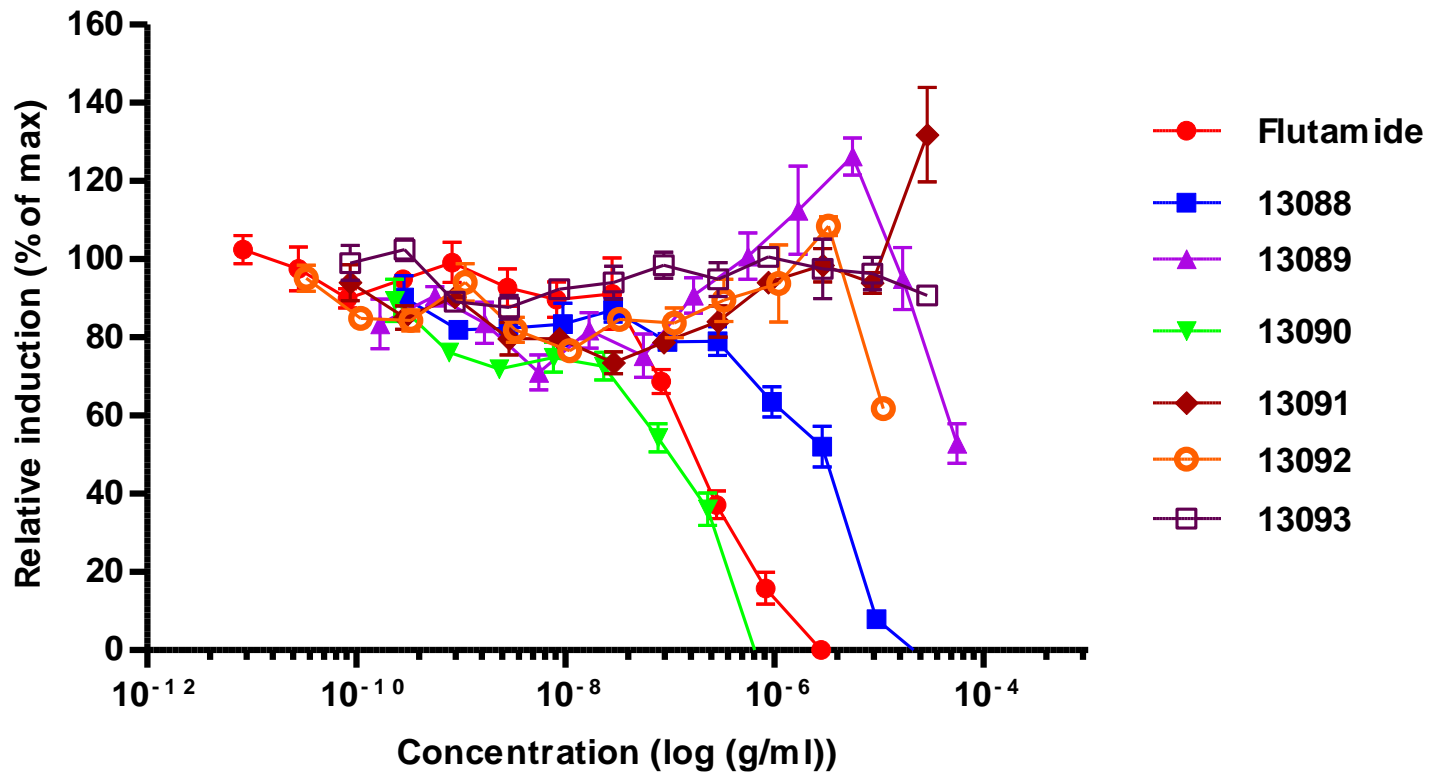
The leaves and buds of *C. operculatus* are harvested, dried, and brewed as an herbal tea in [Vietnam](#) known as "nước vôi" with stomachic properties. <sup>1</sup>



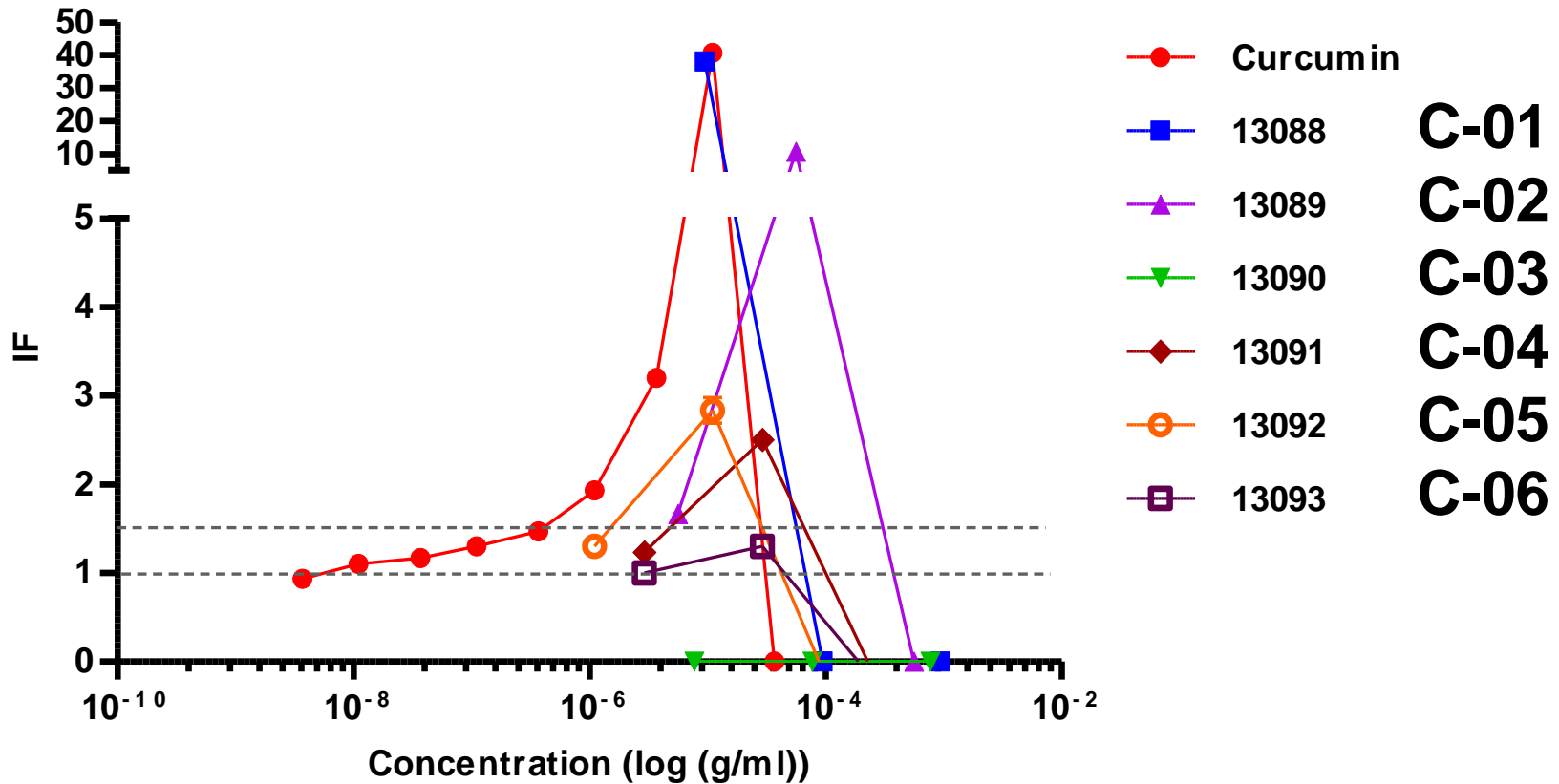
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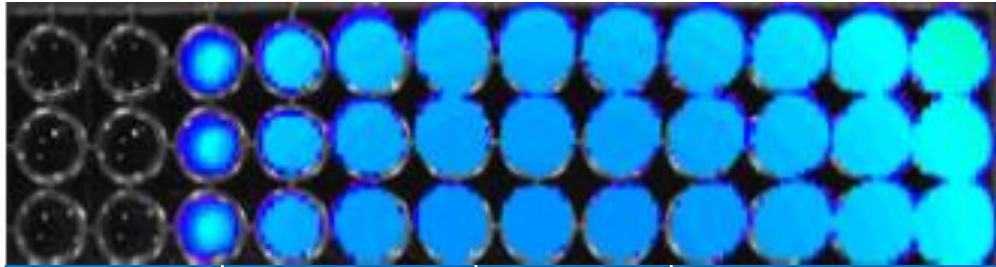
## anti-AR CALUX



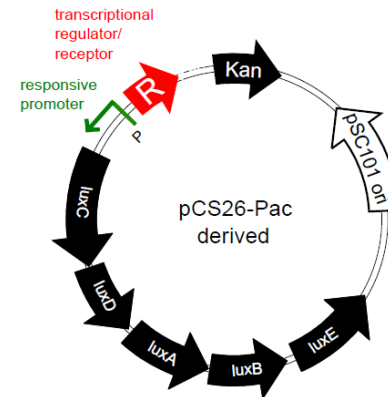
## Nrf2 CALUX



# Bacterial reporters for disease-suppressing compounds in soil extracts



Compounds	Promoter	Regulator	Strain
Phenazines	- <i>mexGHI-opmD</i> or -PA14-35160	<i>soxR</i>	<i>P. aeruginosa</i> PA14
2,4-DAPG	<i>phlACBDE</i>	<i>phlF</i>	<i>P. fluorescens</i> Pf-5
Pyoluteorin	<i>pltLABCDEFG</i>	<i>pltR</i>	<i>P. fluorescens</i> Pf-5 <i>Pseudomonas</i> M18
Quinolones	<i>pqsABCDE</i>	<i>pqsR</i> <i>mvfR</i>	<i>P. aeruginosa</i> PA14
Macrolides	<i>mph(A)mrx</i>	Mphr(A)	<i>E. coli</i> Tf481A



## Proof of principle

- ▲ *Photobacterium luminescens* luxCDABE operon (autonomous, real-time, stable @ 37°C)
- ▲ Low copy number plasmid
- ▲ *E. coli*

## Further optimization

- ▲ Chromosomal integration?
- ▲ Different host?
- ▲ Knockouts necessary?

Bjarnason *et al.* (2003) *J. Bact.*



# Bacterial reporter panel for Nature Mining (antibiotics, bioactivities, ecosystem services)

## Compound specific

Receptor mediated  
-phenazines  
-phloroglucinols  
-siderophores  
-macrolides  
-tetracyclines  
...

Sensor kinase signaling  
-cationic polypeptides  
-glycopeptides

## Mode of action

Inhibition of  
QS  
Replication  
Transcription  
Translation  
Cell wall synthesis  
Fatty acid synthesis

## Stress responses

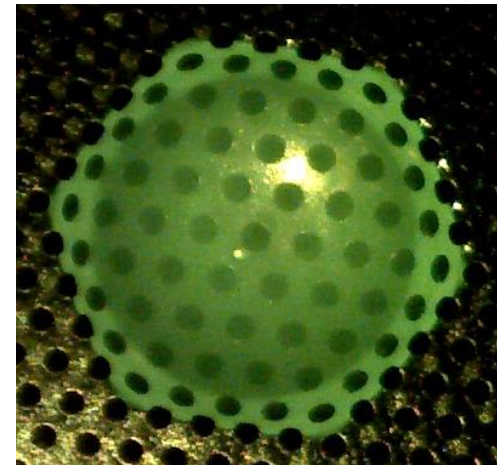
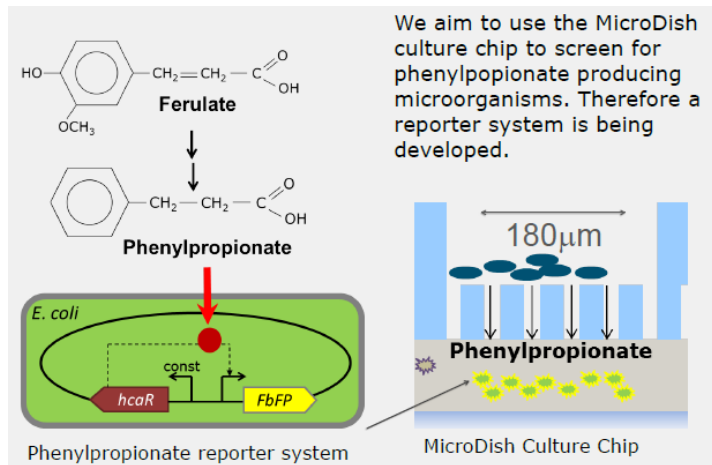
Cell cycle arrest  
Cell envelope  
Genotoxicity  
Oxidative  
Whole cell biosensor  
...



# Bacterial reporter based screens: Mining of ecosystems for lignin degraders

1. Development of (anaerobic) reporter assays for the detection of anaerobic lignin degraders. To be deployed under anaerobic conditions on an MDCC chip (rice straw enrichment).

**Lignin: focus on the intermediate product 3-phenylpropionic acid (3-PPA), degradation product of the lignin crosslink ferulic acid.**



## Take home messages

- **CALUX HTP-panel of assays with good predictions of *in vivo* effects available (e.g. endocrine disruption, reproductive toxicity, genotoxicity/carcinogenesis and acute toxicity);**
- **Modular *in vitro* metabolism step to CALUX reporter assays provides a good prediction of the bio-activation or degradation of compounds as occurs after *in vivo* exposure;**
- **Addition of protein-binding inhibition (TTR) step as a module to TRbeta reporter creates TH-EDC method with good predictability of *in vivo* action of compounds (e.g. halogenated phenolics) interfering with TH-metabolism;**
- **CALUX panel also useful for functionality screening of Beneficial's in plants, herbs;**
- **Luc-based microbial reporters for soil quality, food & biomass valuation available**





We are happy to discuss any options for future collaboration



**Thank you for your attention!**