

# **Sensing Superfund Chemicals with Recombinant Systems**

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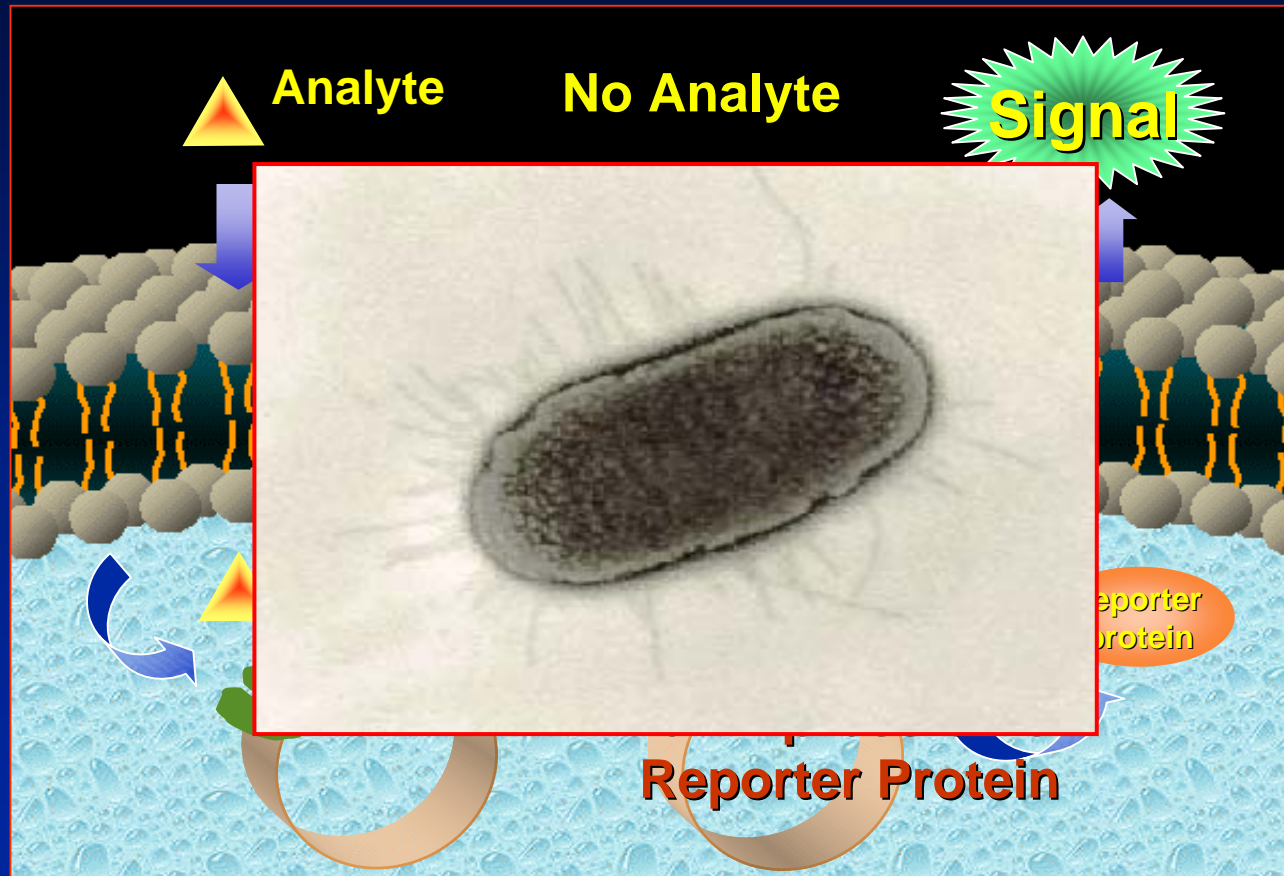


# Molecular Recognition in Analytical Chemistry

- Proteins
- Cells
- High Throughput Screening

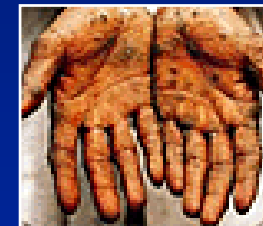
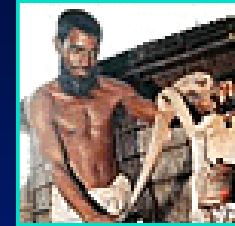


# •Whole Cell-Based Sensing Systems



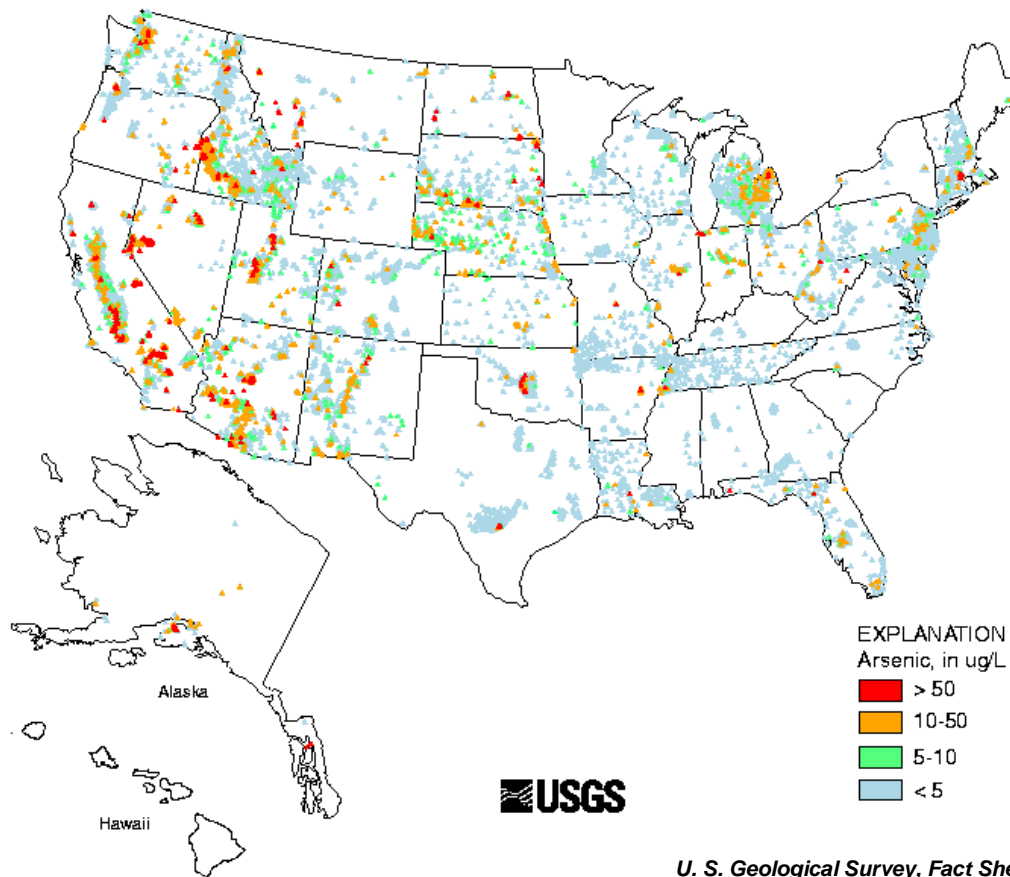
# Arsenic Poisoning

- Applications
  - Agriculture
  - Treatment for diseases
  - Industrial uses
- Long exposure to low doses of arsenic
  - Skin hyperpigmentation and cancer
  - Other cancers
  - Inhibition of cellular enzymes



*New Bangladesh Disaster: Wells that Pump Poison... New York Times November 10, 1998*

# Arsenic contamination in the USA

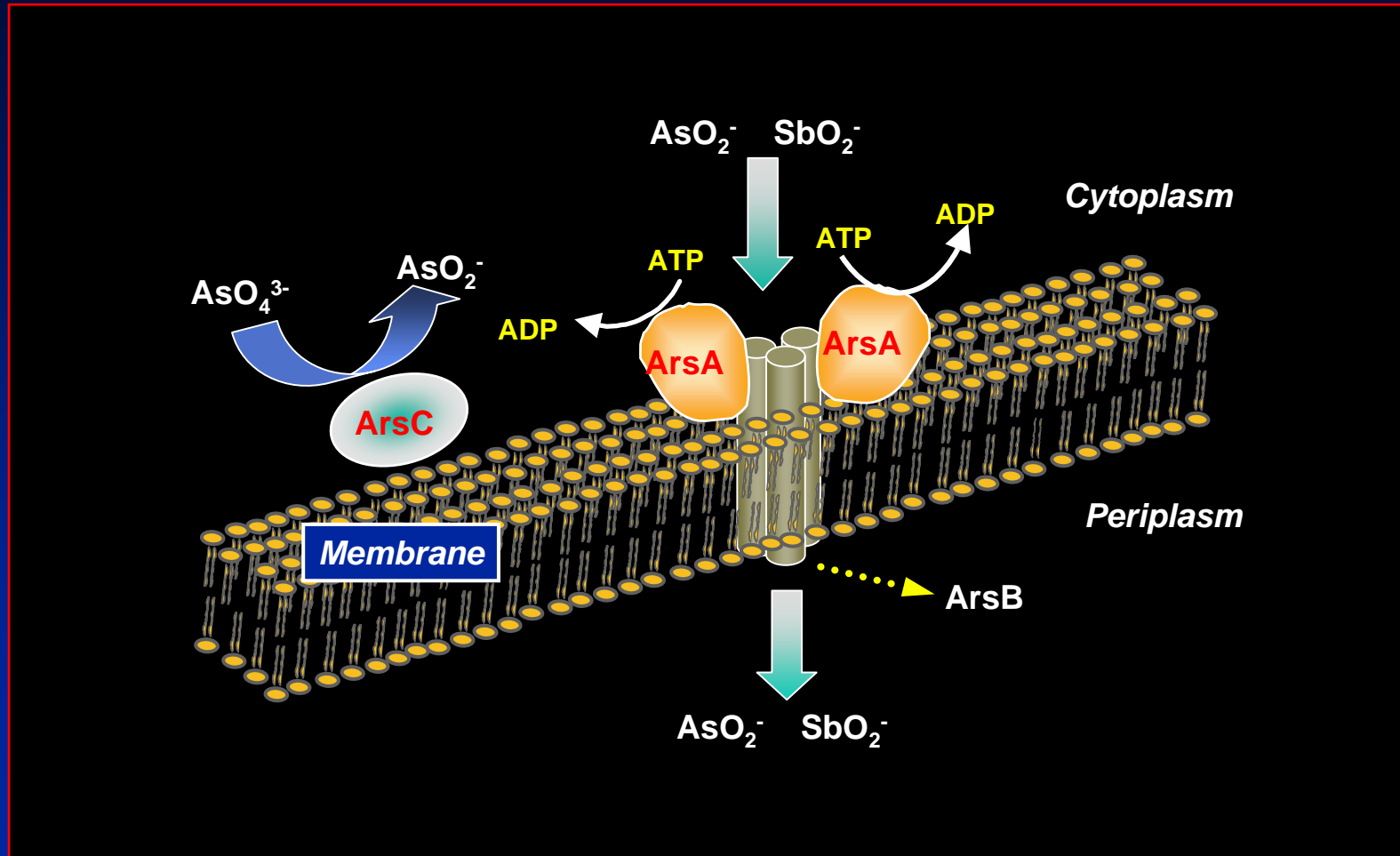


U. S. Geological Survey, Fact Sheet FS 063-00, May 2000

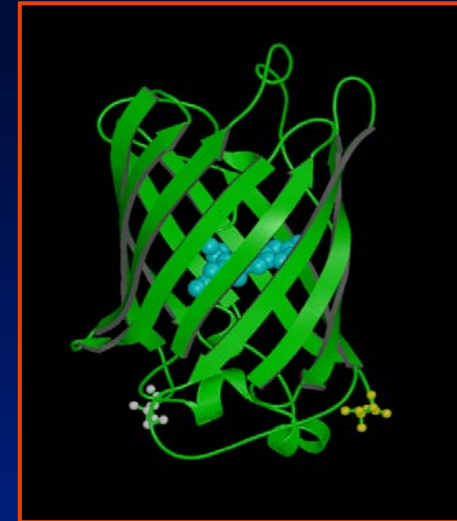
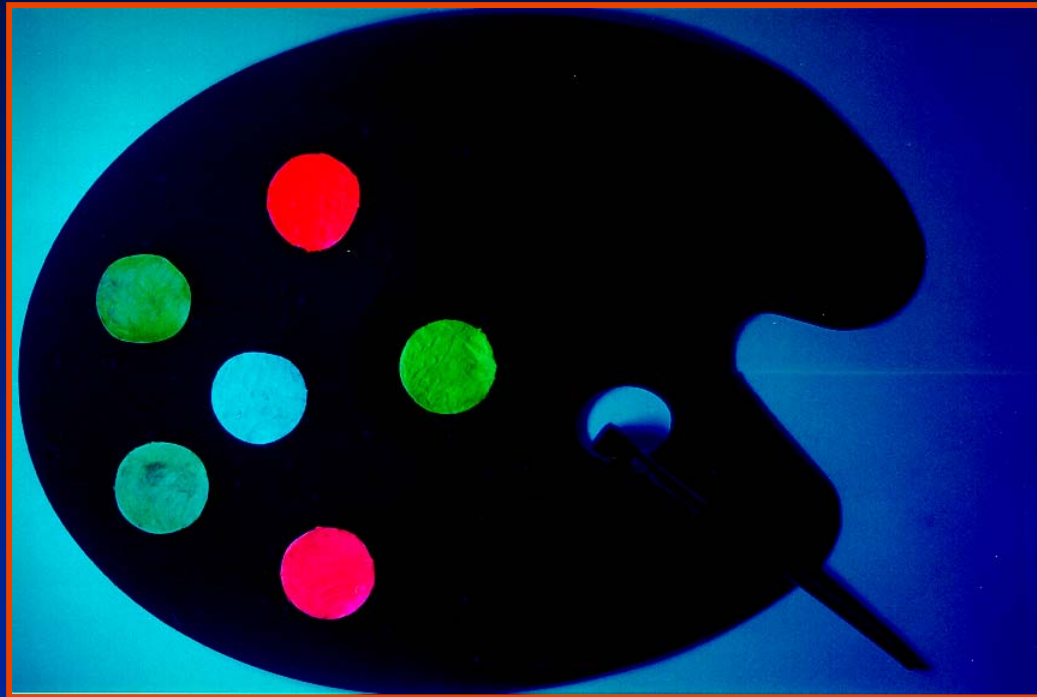
## Arsenite Resistance in *E. coli*



# Schematic Representation of the Antimonite/Arsenite Pump



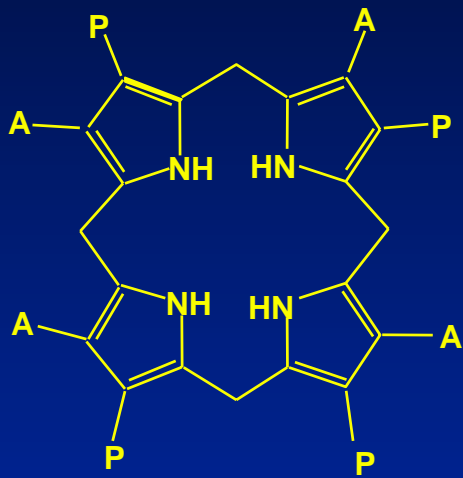
# Fluorescent Reporter Proteins in Array Detection



Protein	Excitation $\lambda_{\max}$	Emission $\lambda_{\max}$
GFP	395 (470)	509
EGFP	488	509
BFP	380	440
GFPuv	395	509
YFP	513	527
CFP	433	475
CobA	357	605
RFP	558	583

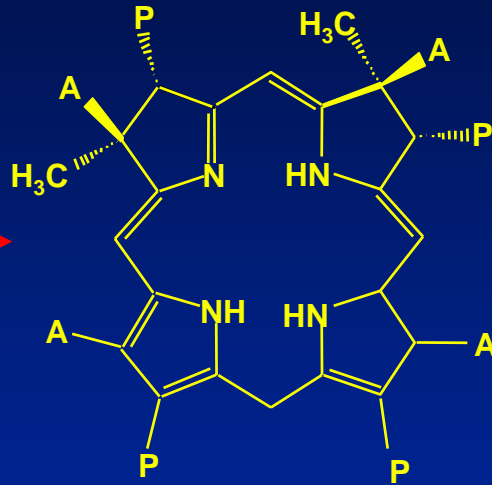


# Production of fluorescent porphyrinoid compounds



urogen III

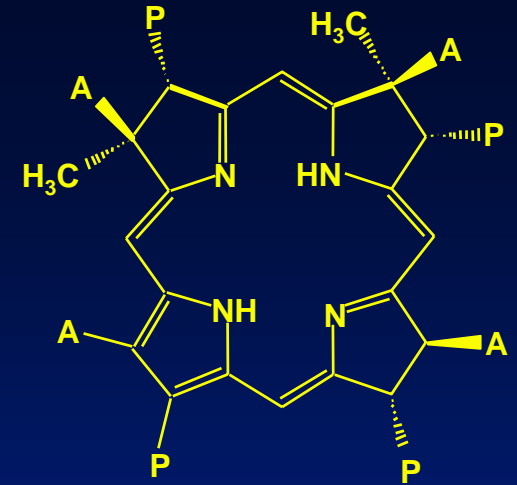
UMT  
SAM



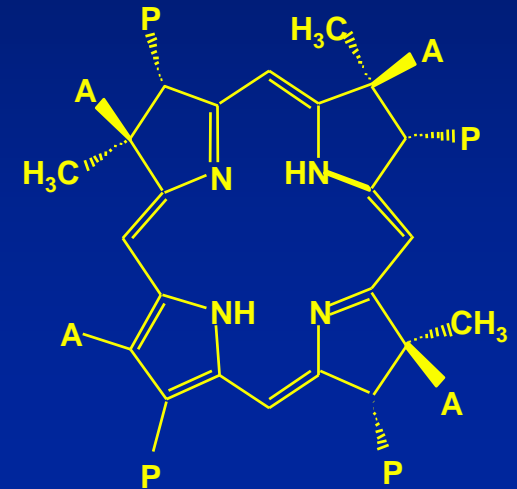
Dihydrosirohydrochlorin  
(Precorrin-2)

oxidation

UMT  
SAM



sirohydrochlorin



trimethylpyrrocorphin

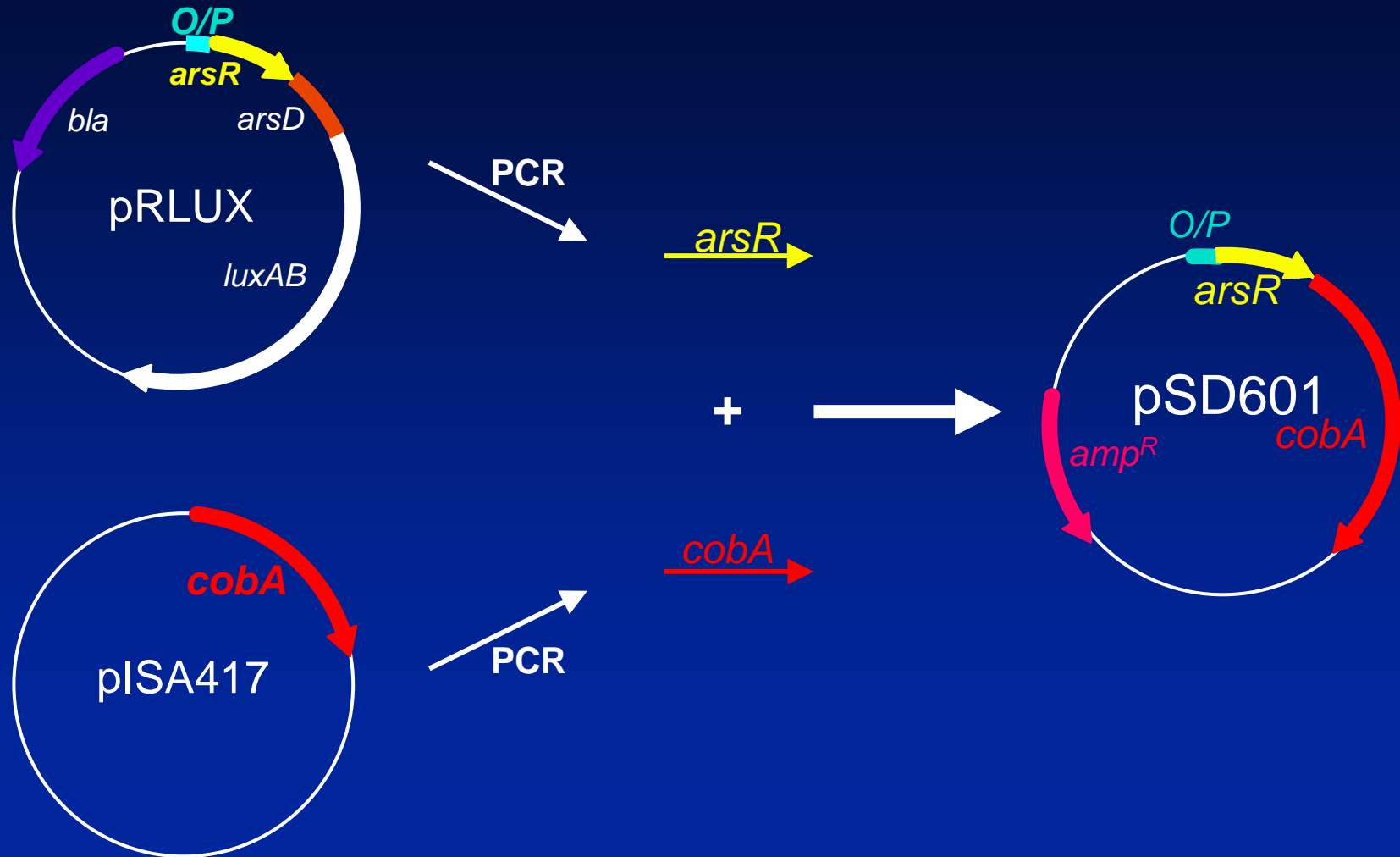
A-CH<sub>2</sub>COOH

P-CH<sub>2</sub>CH<sub>2</sub>COOH

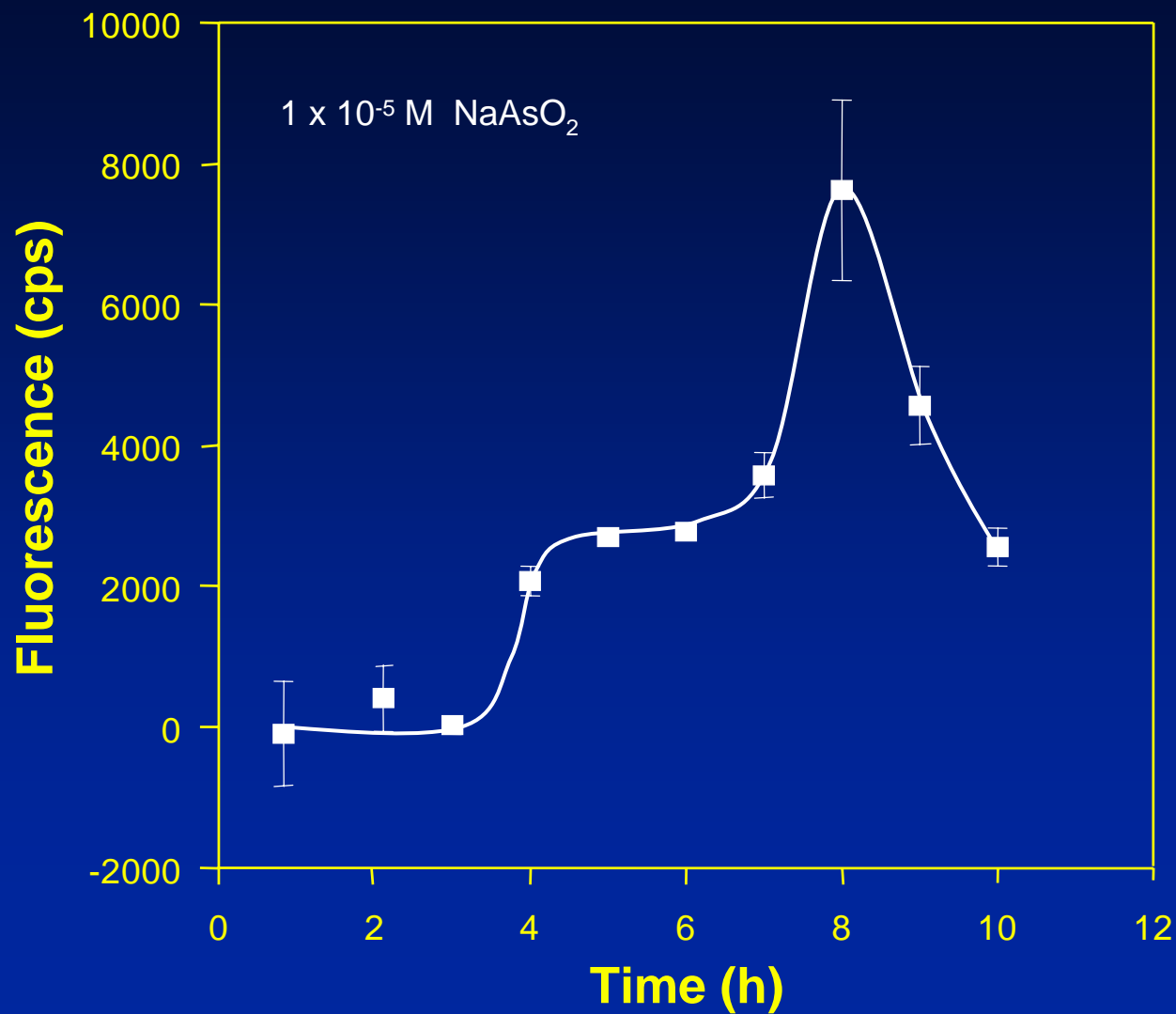
SAM - S-adenosyl-L-methionine

UMT- uroporphyrinogen methyltransferase III

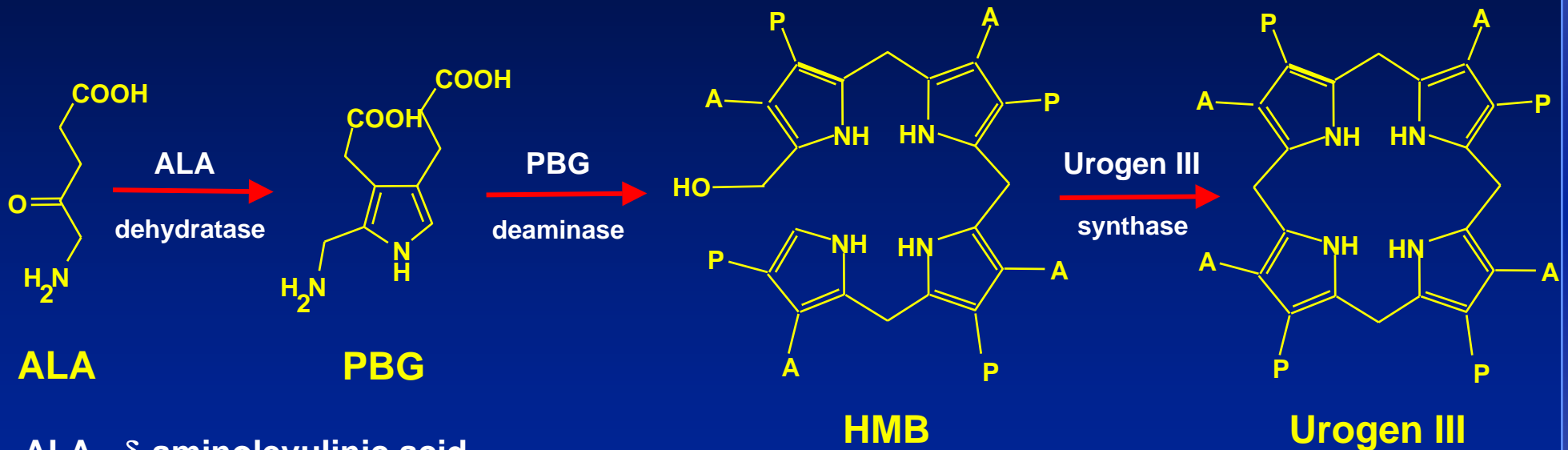
# Construction of pSD601 plasmid



# Time Study for Arsenite



# ALA in Urogen Pathway



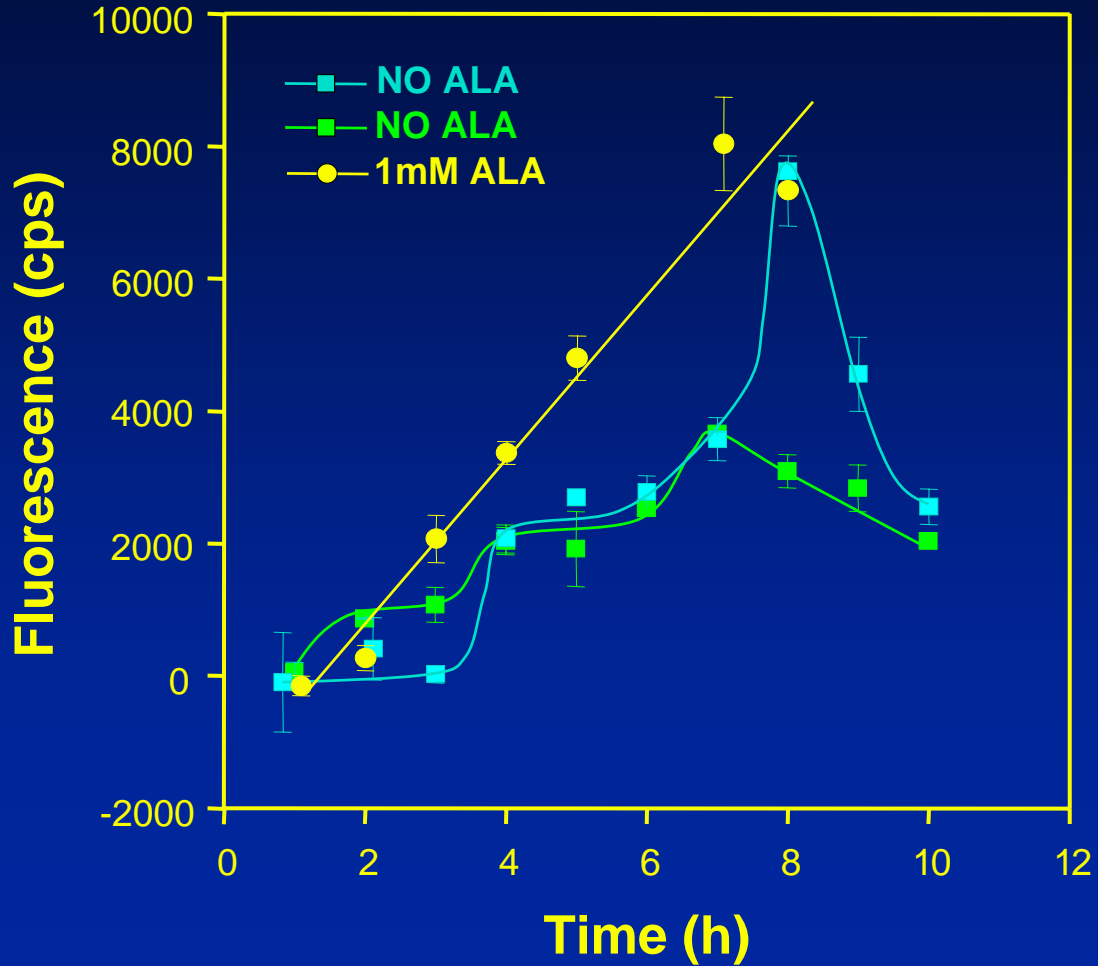
ALA -  $\delta$ -aminolevulinic acid

PBG - Porphobilinogen

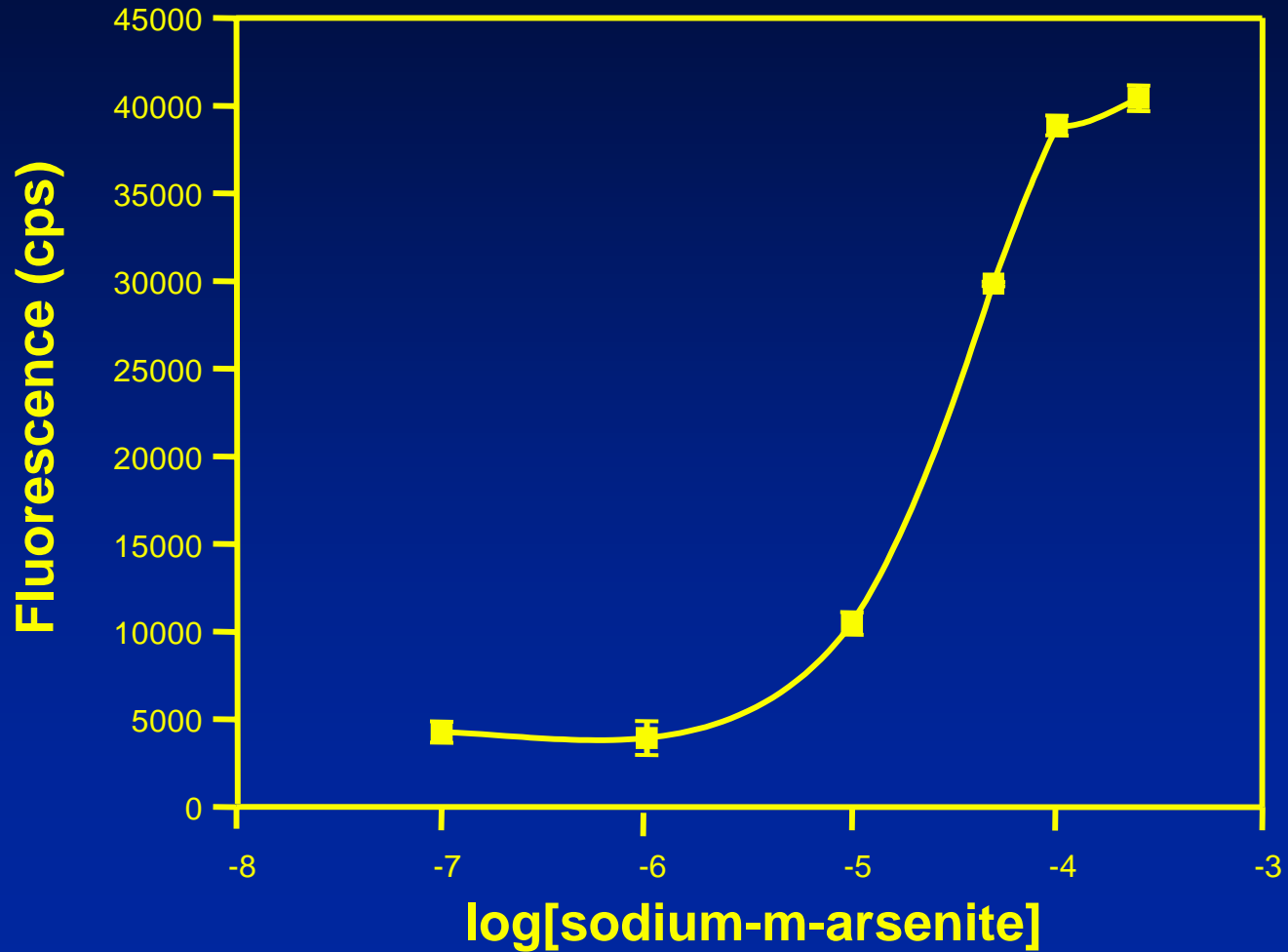
HMB- Hydroxymethylbilane

Urogen- Uroporphyrinogen

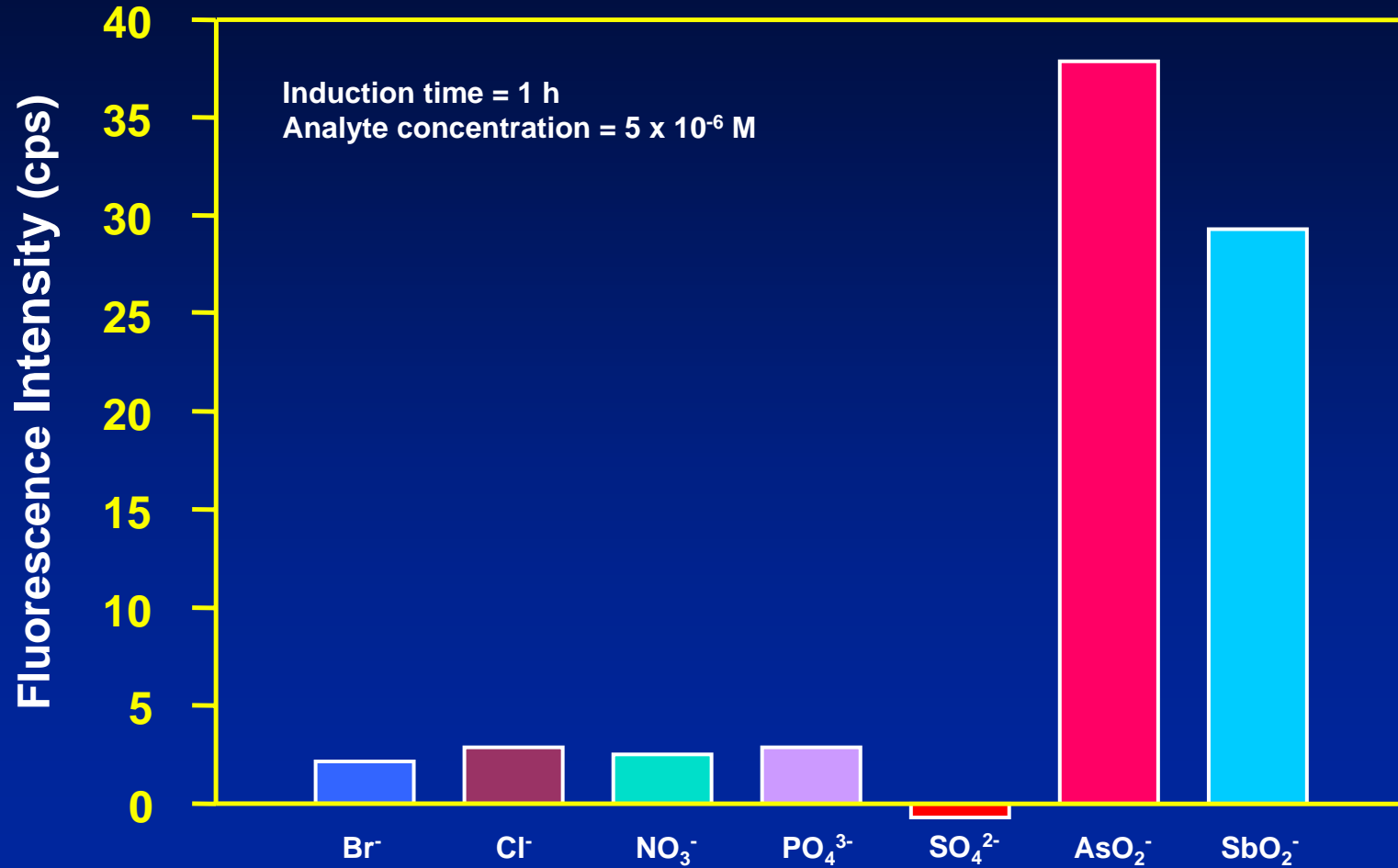
# Time Study of Arsenite with ALA



# Calibration Plot with ALA



# Selectivity Study



## Field Challenges

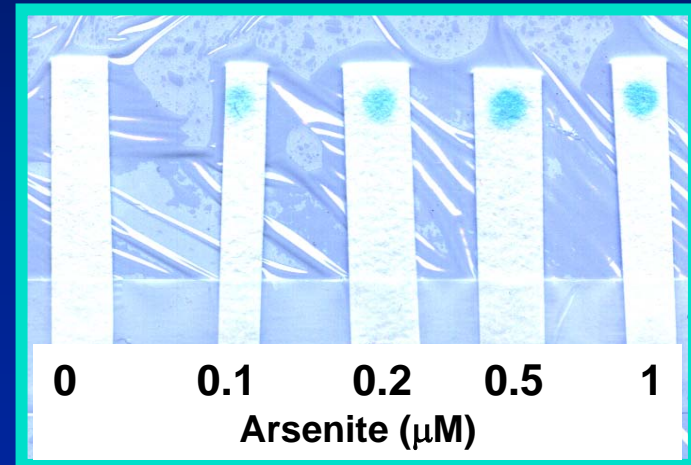
- Conventional analytical techniques
- Field-kits
- Background signal
- Viability of the cells
  - Freeze drying
  - Strips ( $\beta$ -galactosidase)



# Addressing Environmental Analysis with Self-Contained Kits

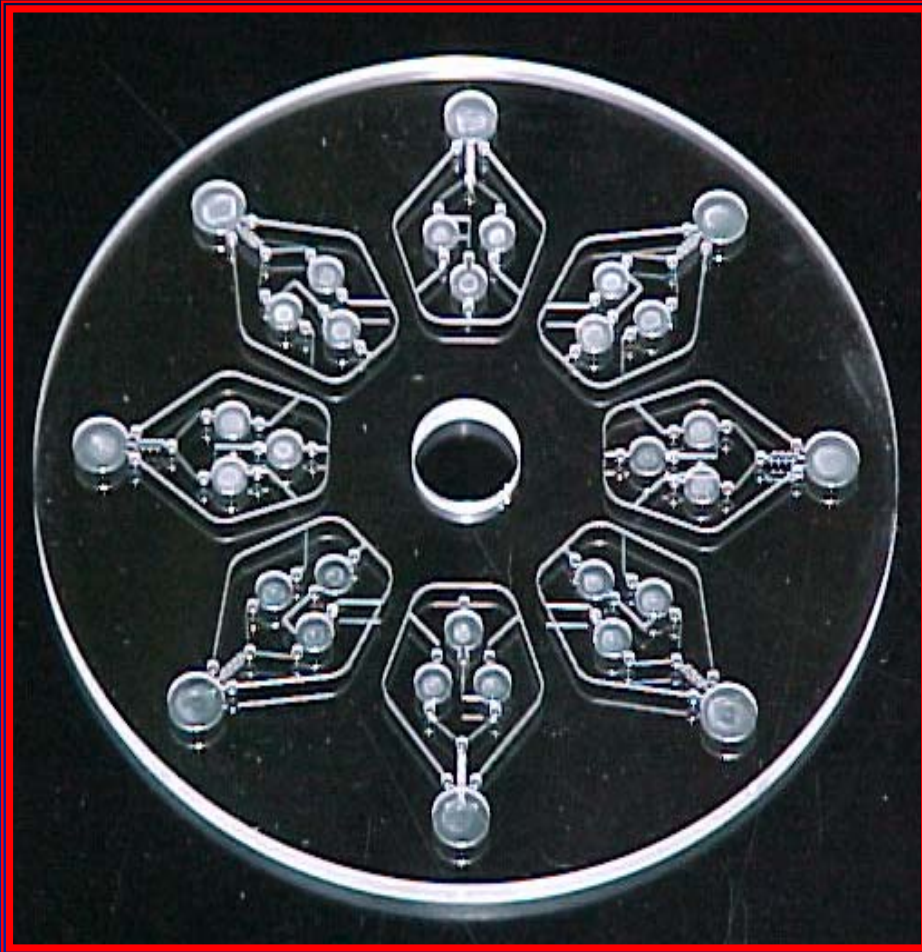


Lyophilized Reagents



Strip Sensors

# Centrifugal Microfluidic Platform for Micro-Total Analysis Systems



**Low power and space requirements**

**Less reagent and sample consumption**

**Easy disposal**

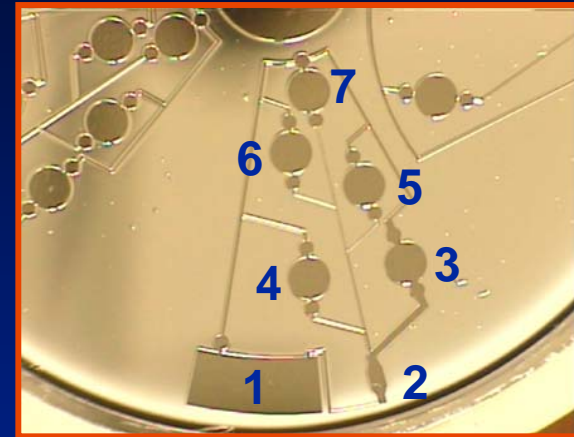
**Short analysis time**

**Integrate washing, sample preparation,  
and calibration**

# Prototype Compact Disc

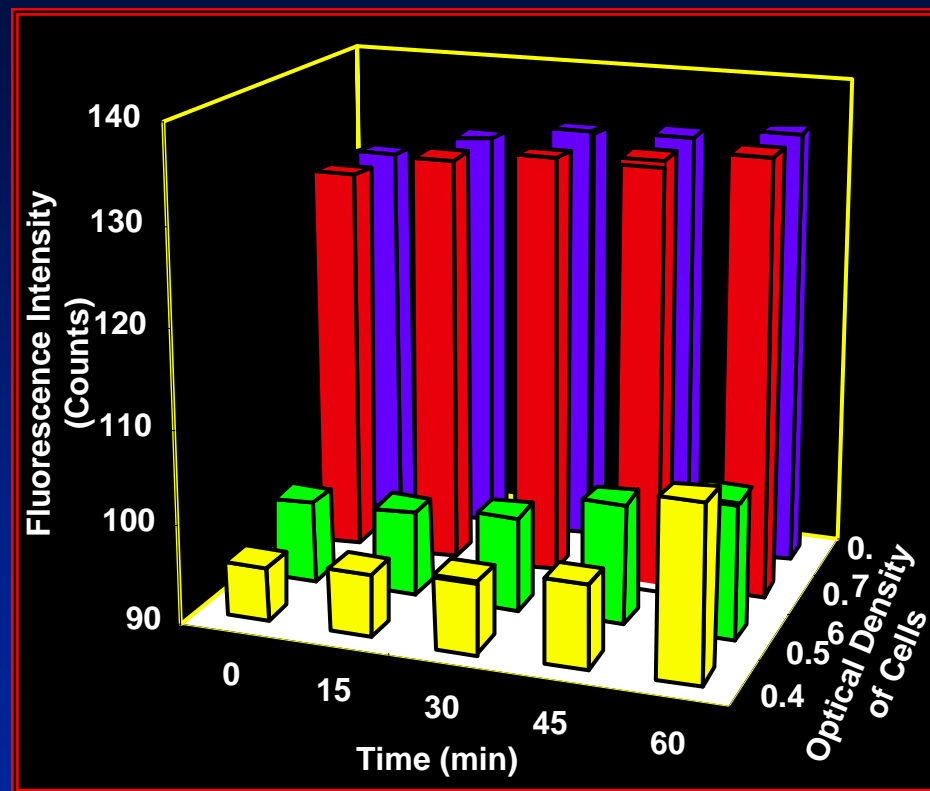
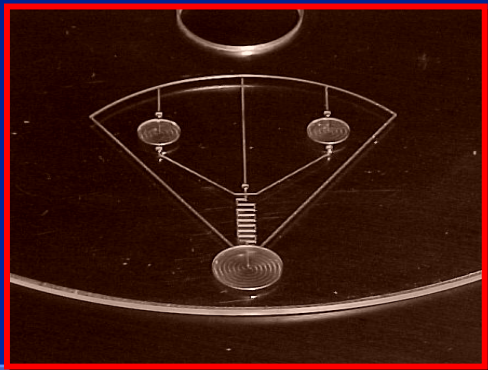
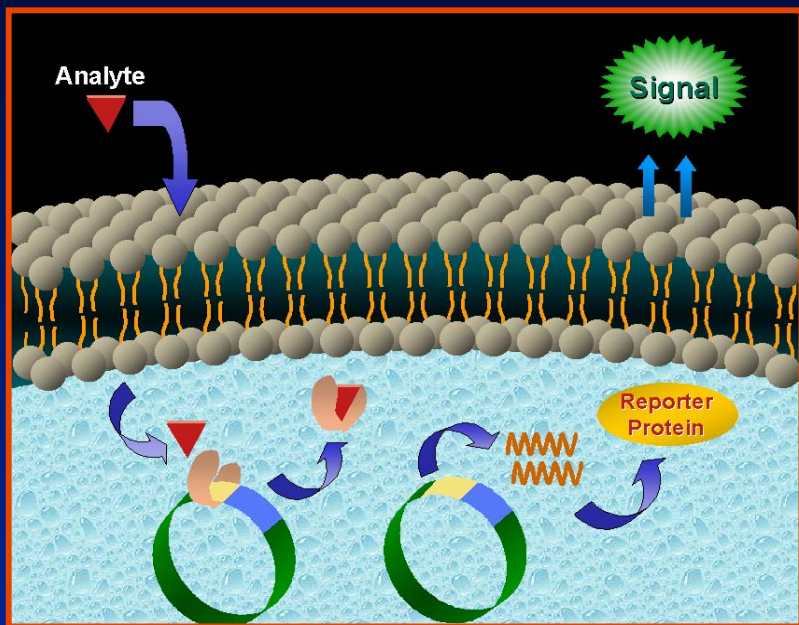


Prototype CD for four simultaneous analyses



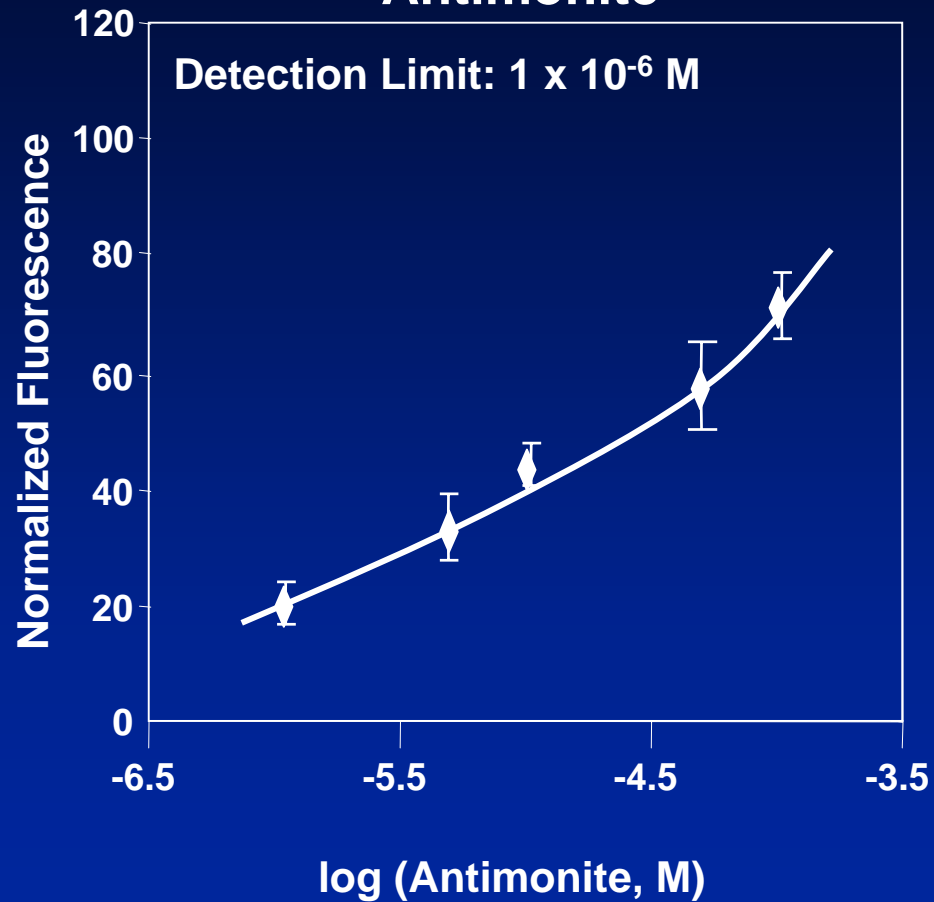
1. Waste Reservoir
2. Optode
- 3-7. Solution Reservoirs

# Incorporation of Whole-cell Sensing System for Arsenite/Antimonite on the CD Platform

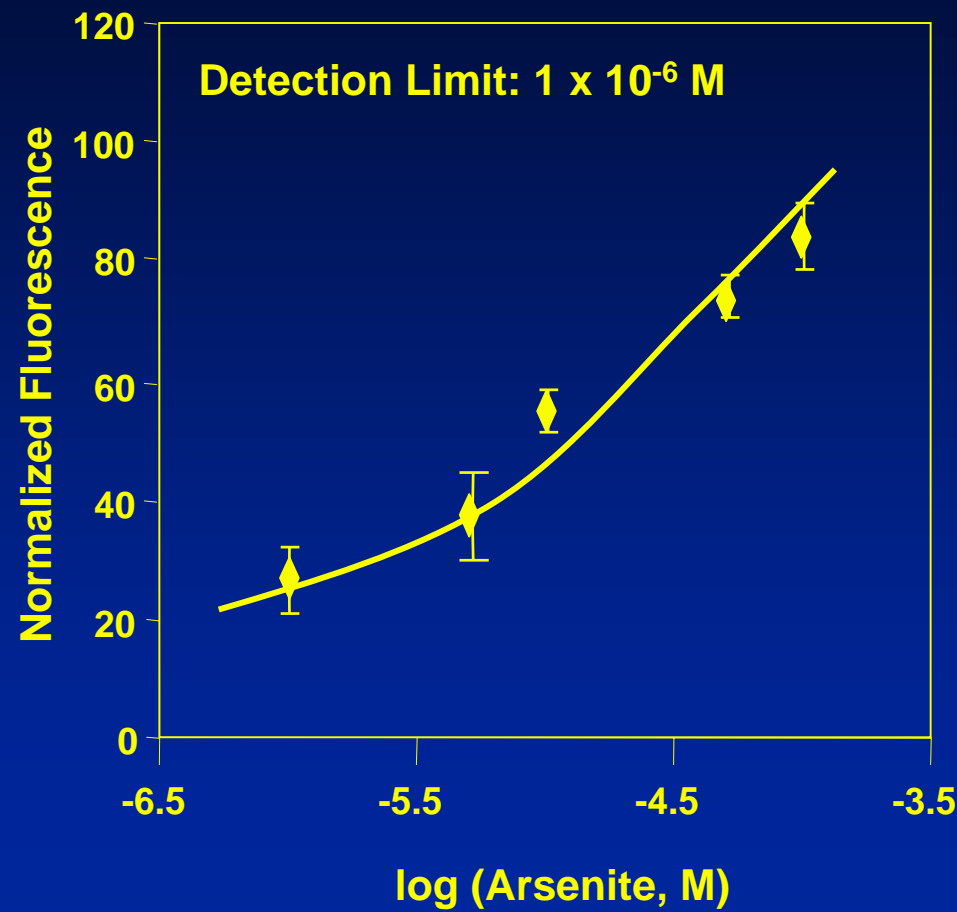


# Calibration Curves

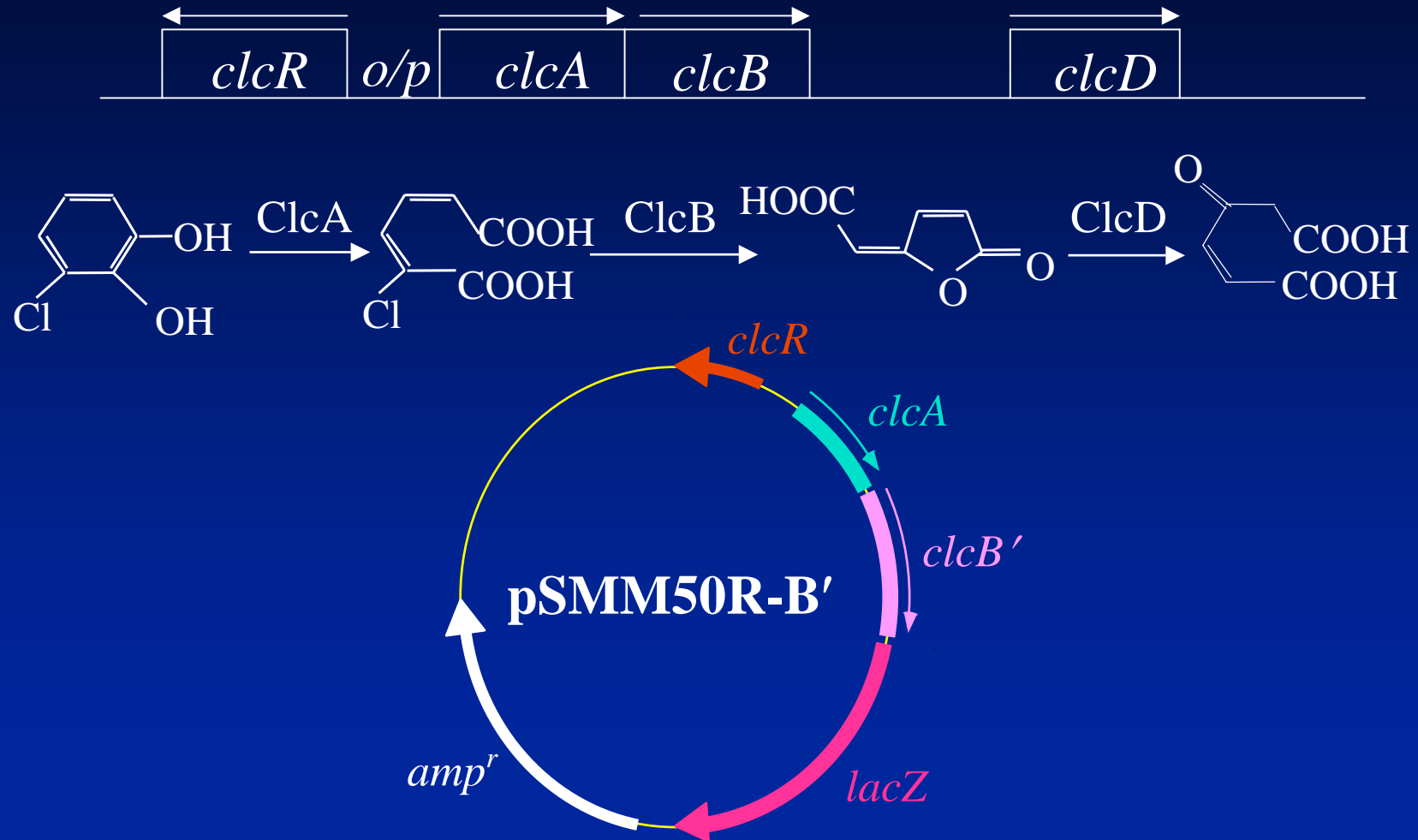
## Antimonite



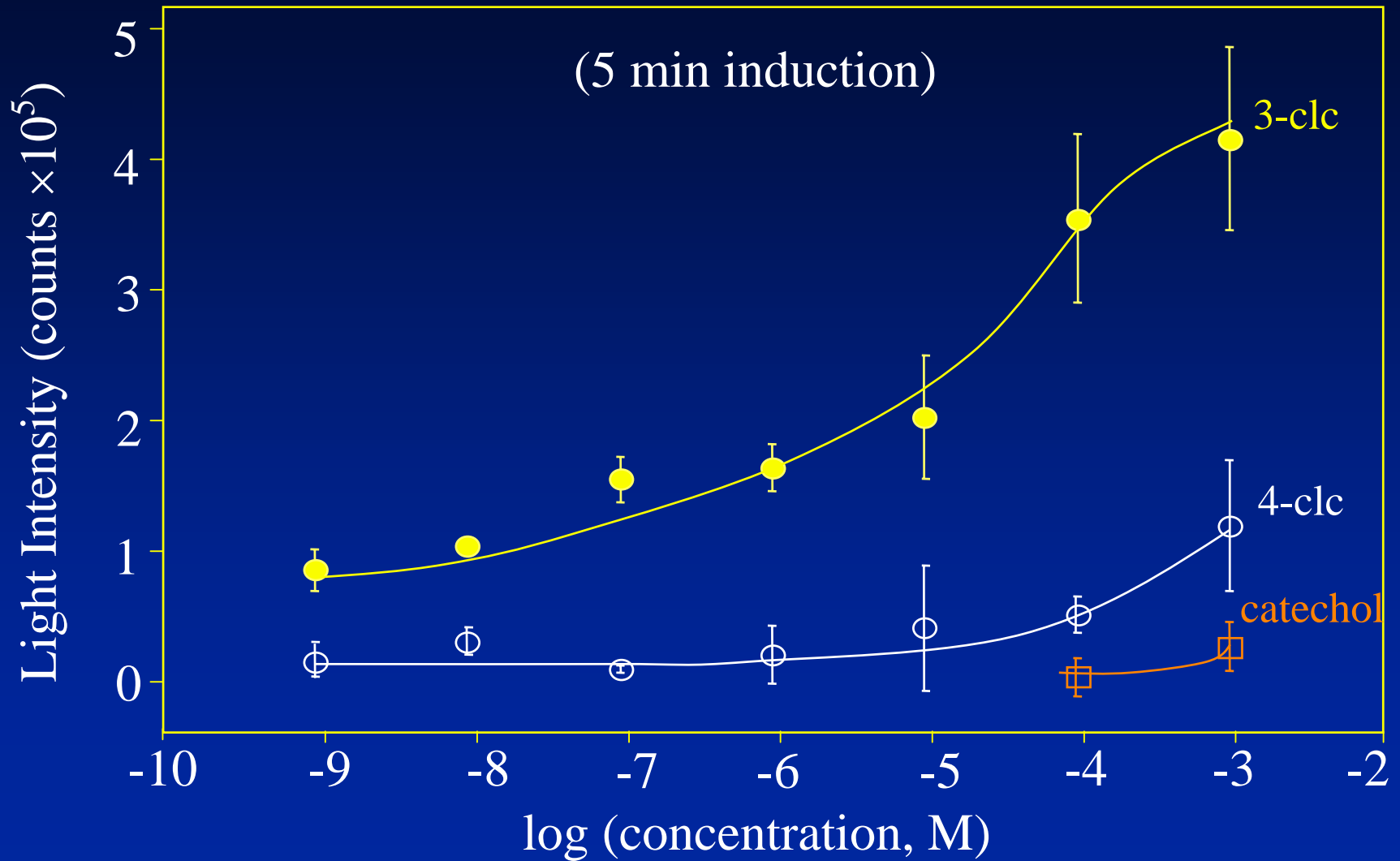
## Arsenite



# Clc Operon and 3-chlorocatechol Pathway

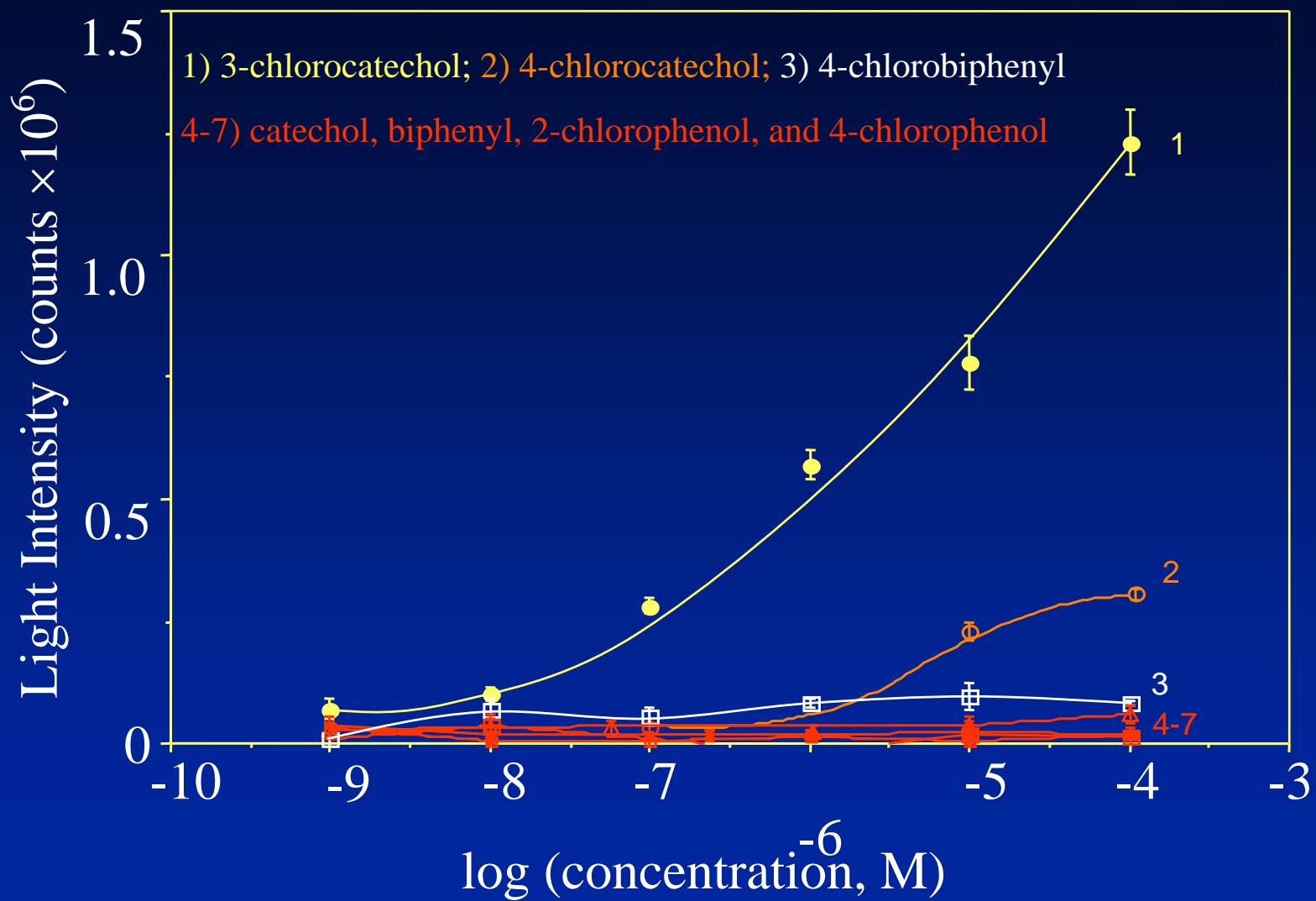


# Calibration Curves





# Selectivity Study





# Challenges in Environmental Sample Analysis of Chlorocatechol

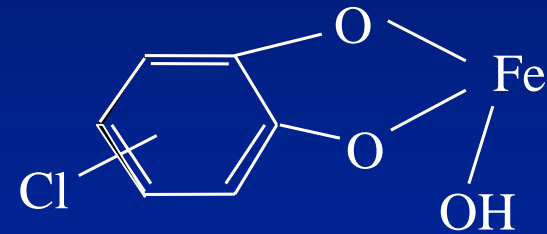
## 1. Extraction Method

- Free Chlorocatechol

Can be extracted by organic solvent

- Bound Chlorocatechol

Difficult to be extracted

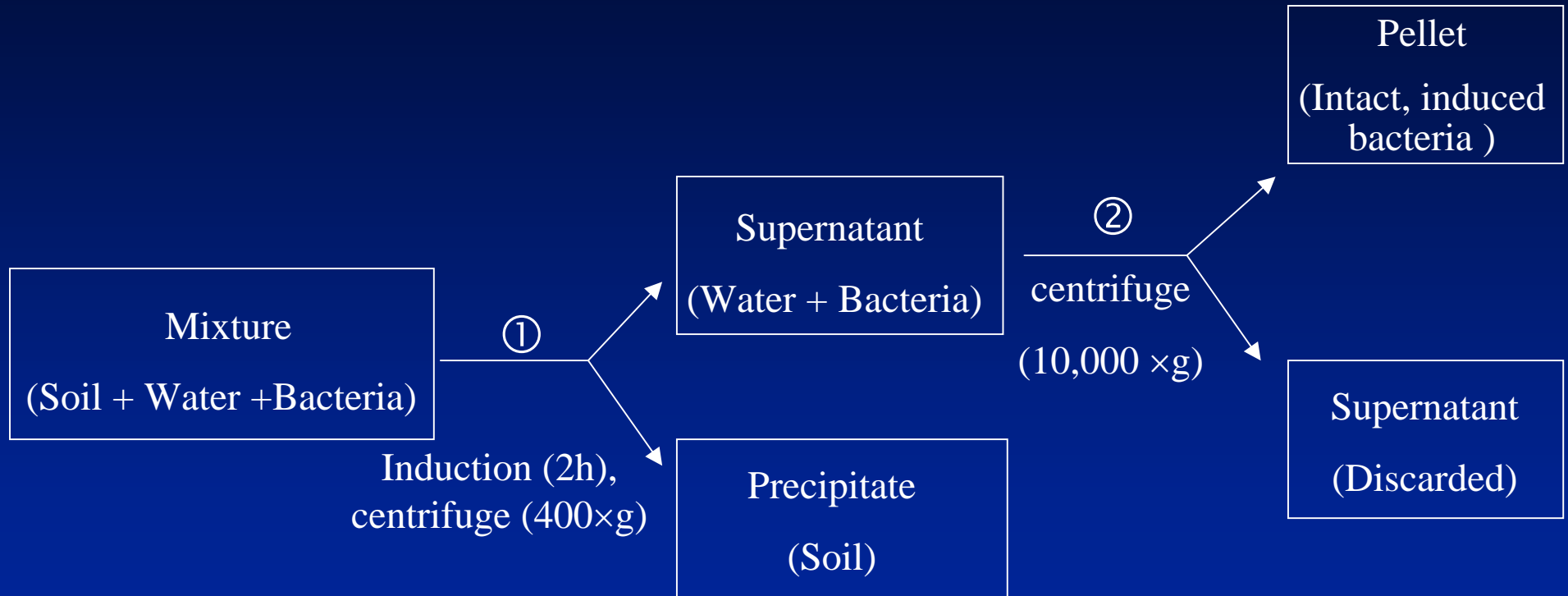


## 2. Matrix Effect

## Selected Chemical Characteristics of Soils

Soils	Organic carbon (%)	Electrical conductivity ( $\mu\text{mhos}\cdot\text{cm}^{-1}$ )	pH	Oxalate extractable		
				P	Al	Fe
				(mg kg <sup>-1</sup> )		
Acid washed sand	0.02	12	6.1	8	32	54
Maury silt loam	3.3	53	5.0	704	1894	4126
Woolper	7.5	38	5.9	3203	3086	3203
Organic humus	15.3	1099	6.4	415	3575	1106

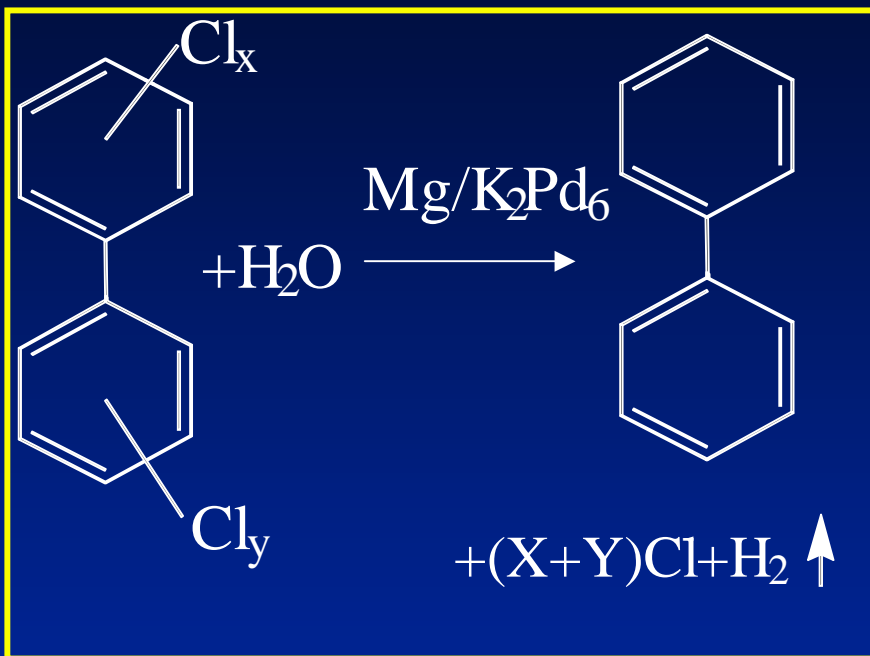
# Optimized Protocol for Soil Analysis



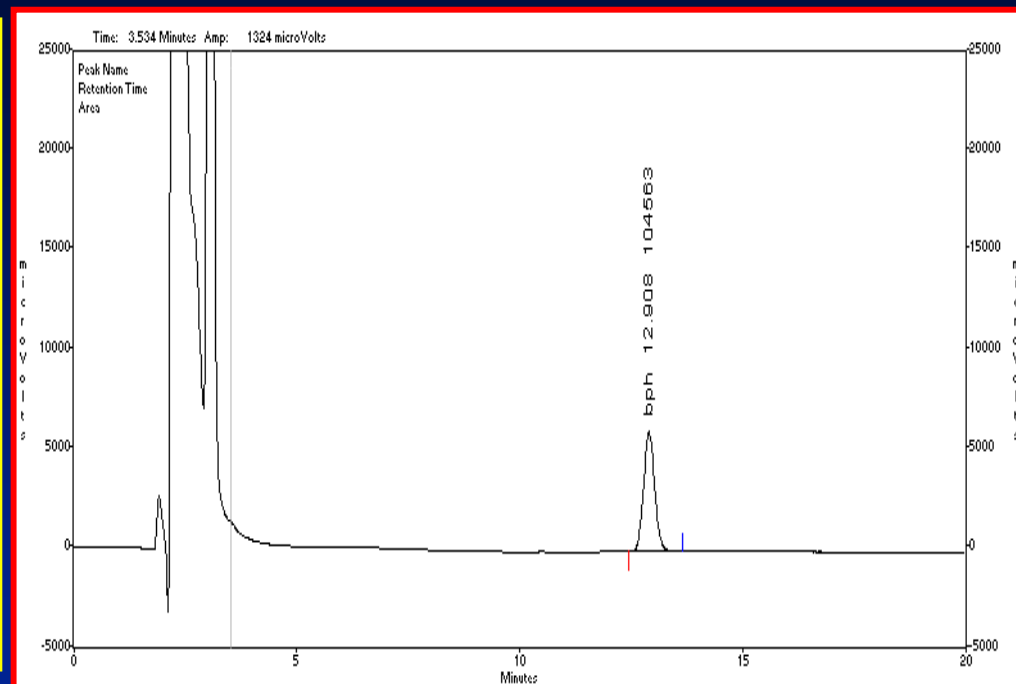
# Results of Soils

Sample matrix	HPLC		Bacterial sensing system	
	Theoretical Value (mg•kg <sup>-1</sup> )	Experimental ± SD (mg•kg <sup>-1</sup> )	Theoretical Value (mg•kg <sup>-1</sup> )	Experimental ± SD (mg•kg <sup>-1</sup> )
Sand	0.5	0.49 ± 0.02	0.5	0.51 ± 0.02
	2.0	2.03 ± 0.04	2.0	1.95 ± 0.08
			10	9.75 ± 0.55
			50	52.0 ± 2.6
Woolper	0.5	0.0 ± 0.0	0.5	0.52 ± 0.04
	2.0	0.0 ± 0.0	2.0	1.90 ± 0.18
			10	9.55 ± 0.75
			50	54.0 ± 4.5
Maury	0.5	0.0 ± 0.0	0.5	0.43 ± 0.08
	2.0	0.0 ± 0.0	2.0	2.24 ± 0.30
Organic potting soil	0.5	0.0 ± 0.0	0.5	0.43 ± 0.09
	2.0	0.0 ± 0.0	2.0	1.73 ± 0.28

# Detection of PCBs Based on Dechlorination Followed by whole cell Sensing

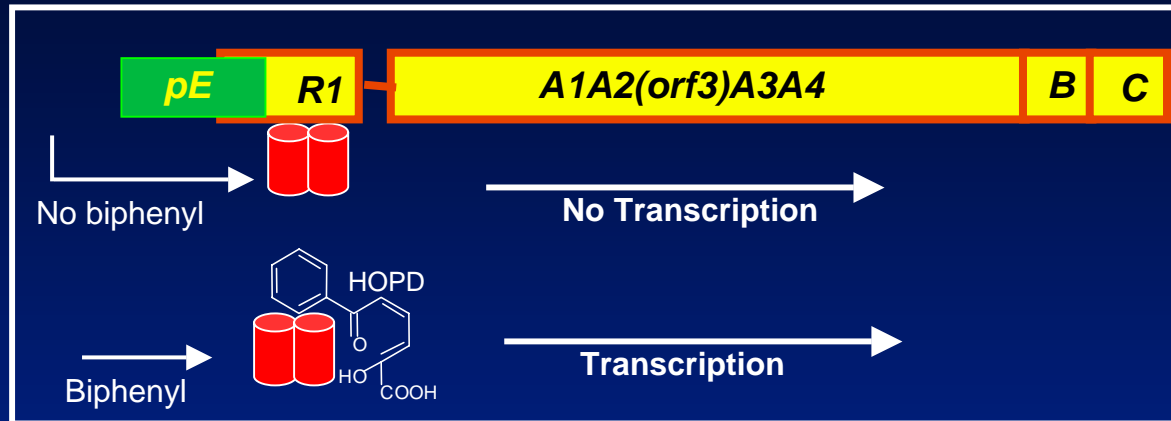


PCB Dechlorination Reaction



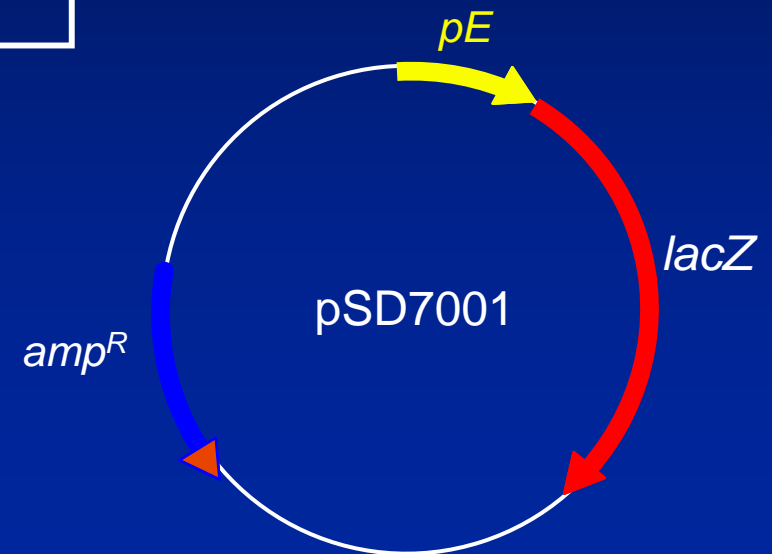
HPLC Chromatogram of Dechlorinated Product: Biphenyl

# Whole cell Sensing of Biphenyl Based on *bph* Operon from *P. pseudoalcaligenes* KF707

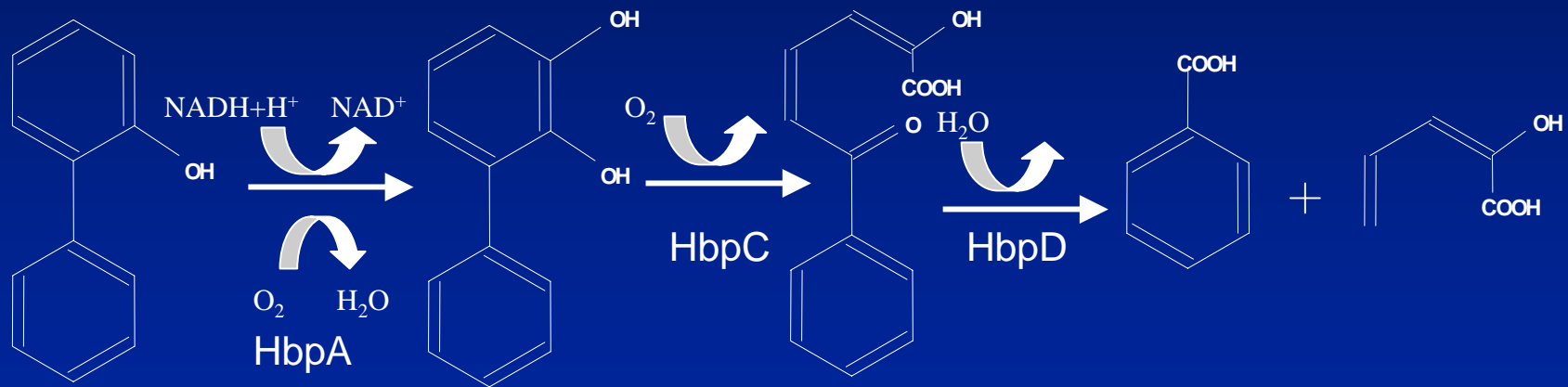
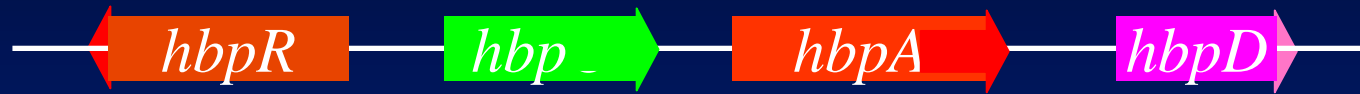


Bph operon of *P. pseudoalcaligenes* KF707

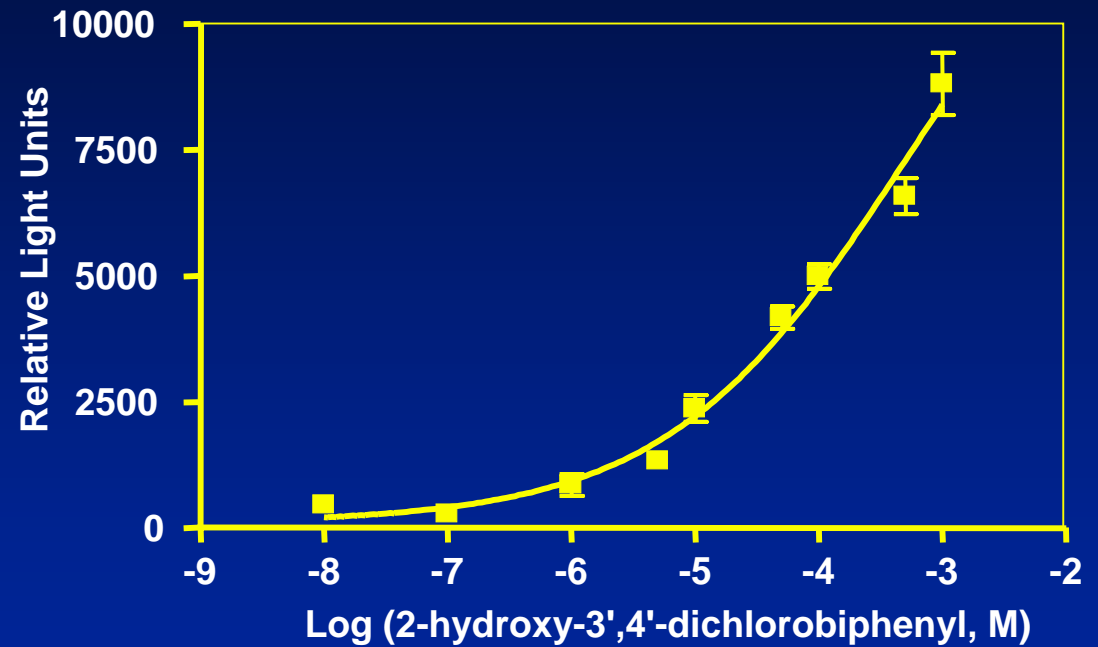
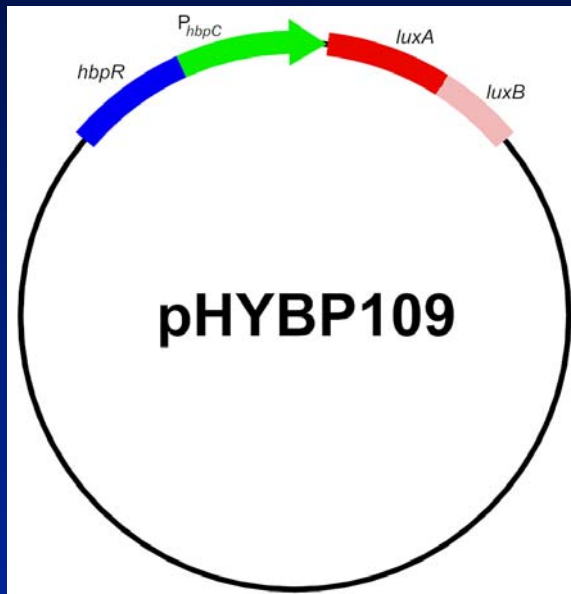
Biphenyl mol/L	Increase in Light Intensity (%)
$1 \times 10^{-5}$	$81.9 \pm 9.5$
$1 \times 10^{-6}$	$37 \pm 6.2$
$1 \times 10^{-7}$	$21 \pm 4.8$



# Degradation Pathway of Hydroxylated-Biphenyl in the strain *Pseudomonas azelaica* HBP1



# Whole Cell-Based Sensing System for Hydroxylated PCBS





## **Collaborators**

**Leonidas Bachas  
Marc Madou  
Barry Rosen  
Jan Roelof van der Meer**

## **Acknowledgments**

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