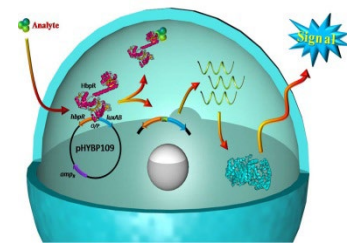


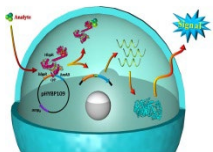


Sensing Superfund Chemicals with Recombinant Systems

Amol Date, Kendrick Turner, Patrizia Pasini,
Leonidas G. Bachas and Sylvia Daunert

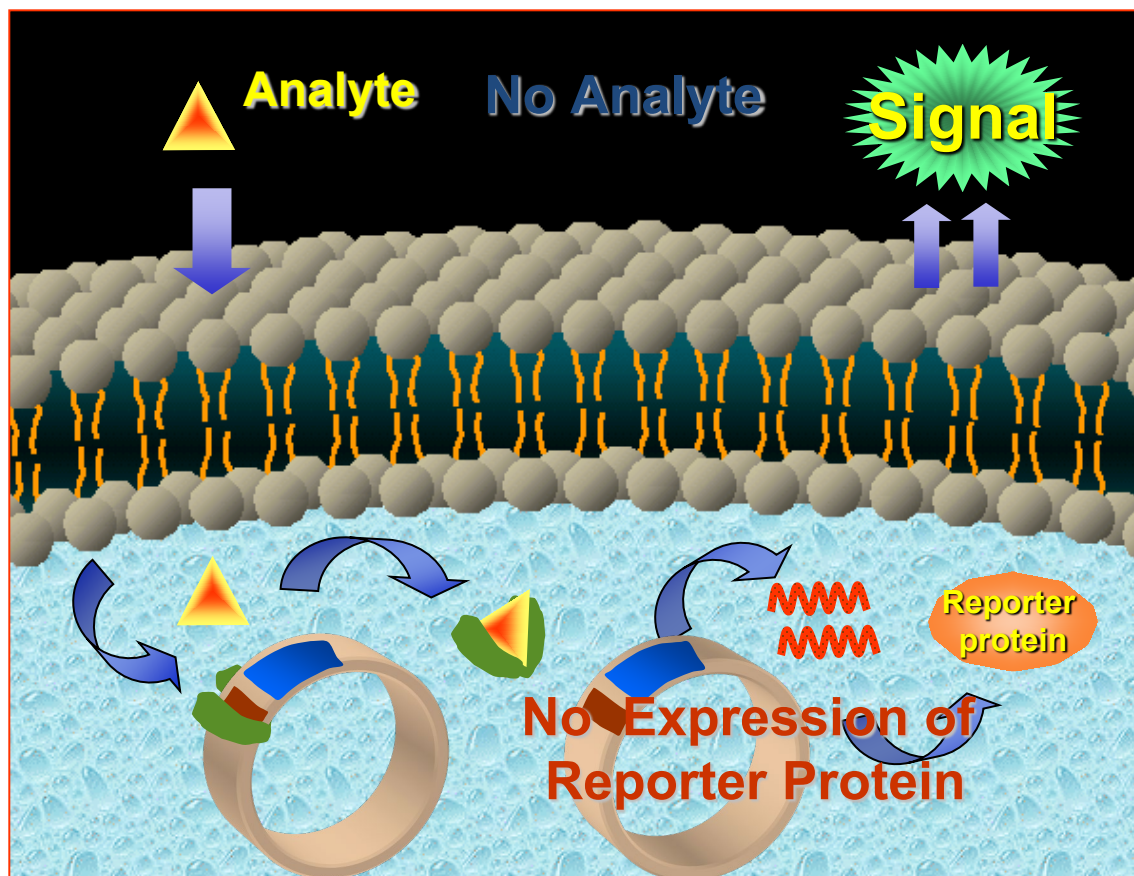
Department of Chemistry
University of Kentucky
Lexington, KY 40506

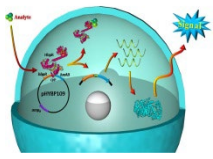




Whole Cell Biosensor

- Cell death-based
- Reporter gene-based

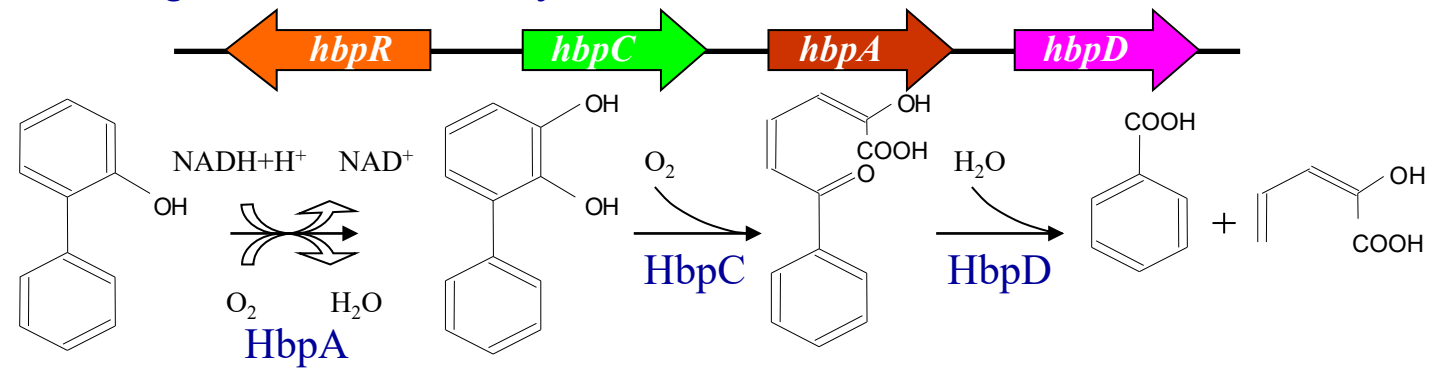




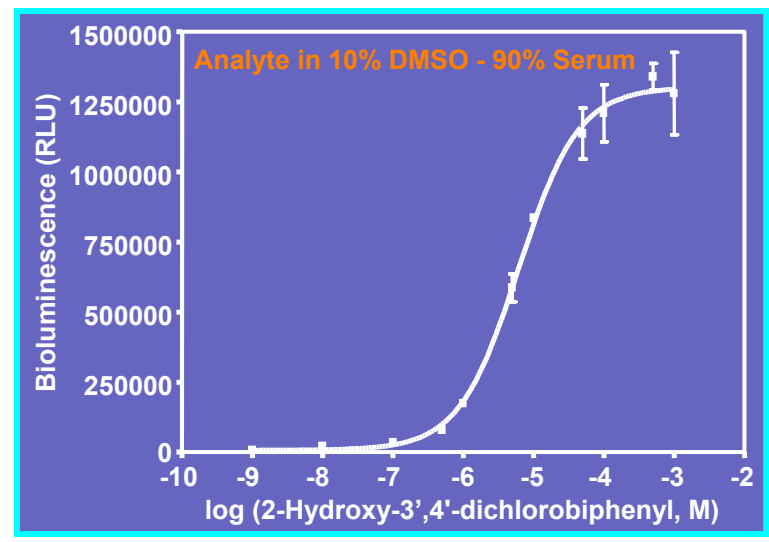
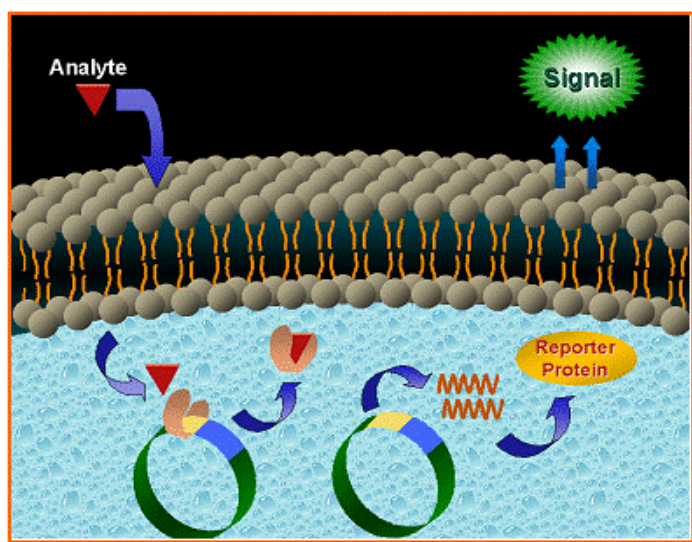
Whole Cell Biosensors for Hydroxy-PCBs

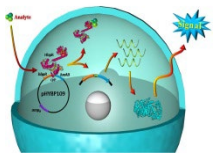
Daunert Group

Degradation Pathway of Strain *Pseudomonas azelaica* HBP1



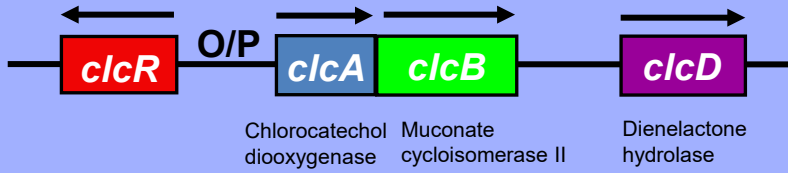
Construction of reporter plasmid



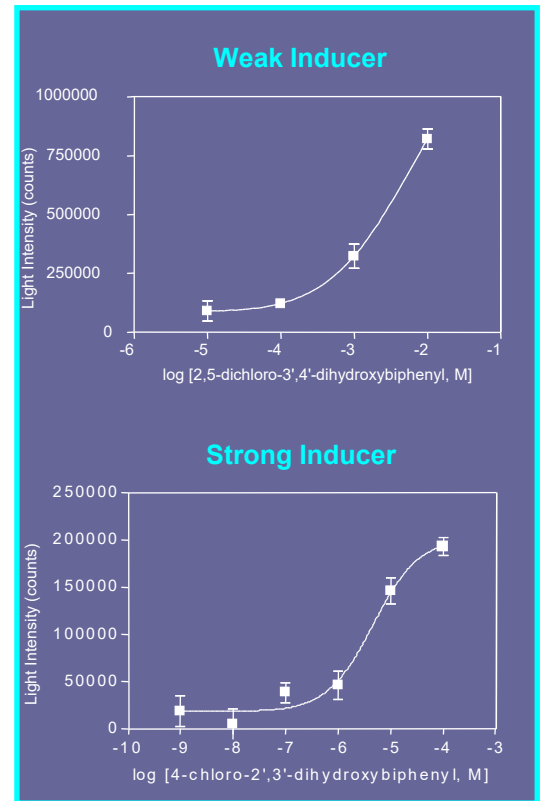
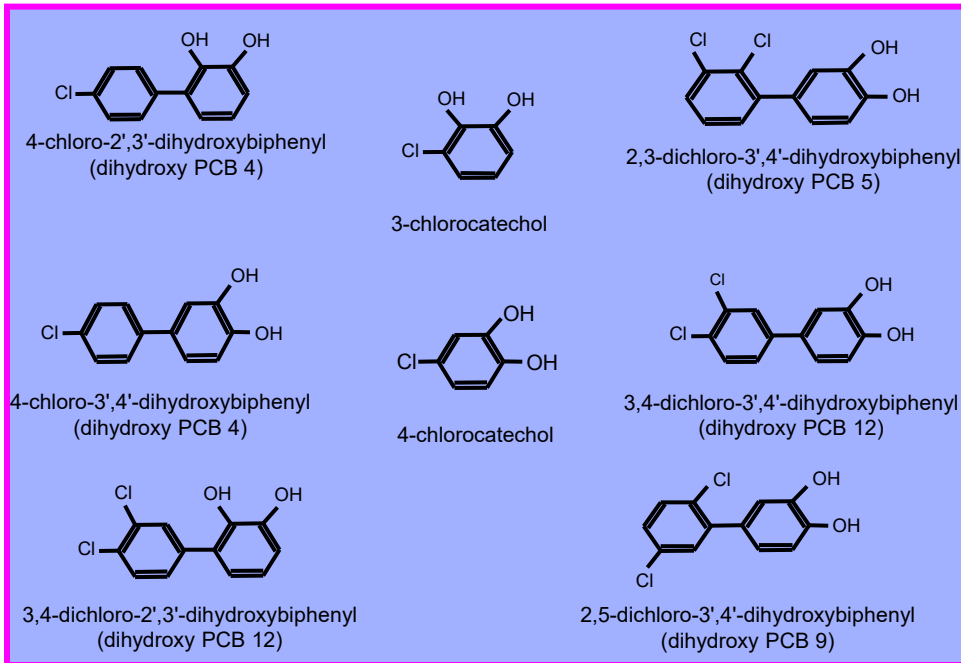
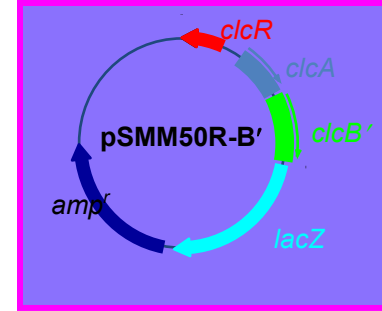
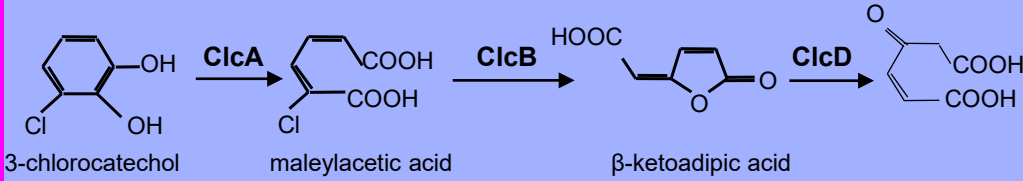


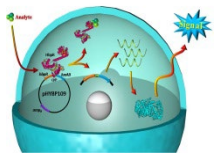
Whole Cell Biosensors for Hydroxy-PCBs

Degradation Pathway of *Pseudomonas putida*



Chlorocatechol dioxygenase Muconate cycloisomerase II Dienelactone hydrolase

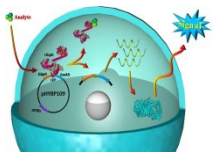




Binding Proteins Based Biosensors

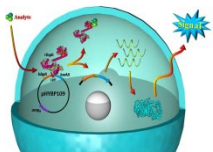
- Single polypeptide chain
- Two globular domains connected by short polypeptides
- Ligand-binding is in the cleft between the two globular domains

QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.



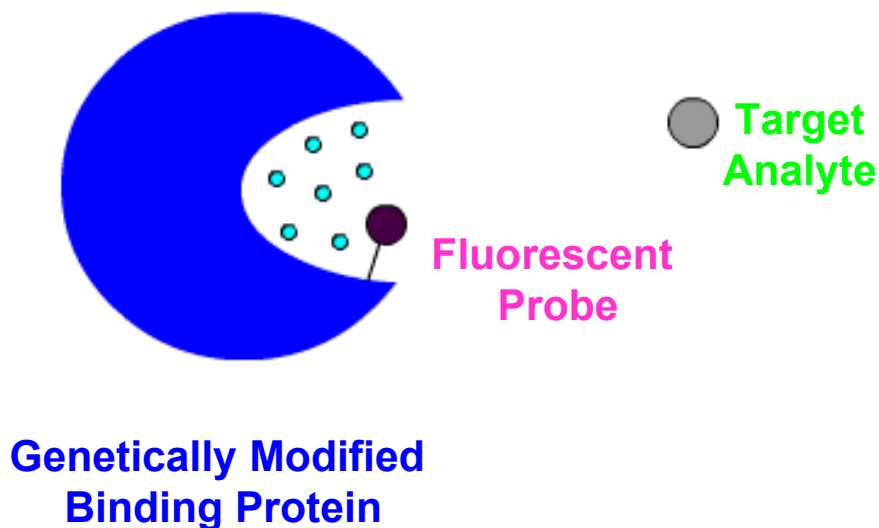
Venus Flytrap





Sensing Analytes with Genetically Engineered Hinge-Motion Proteins

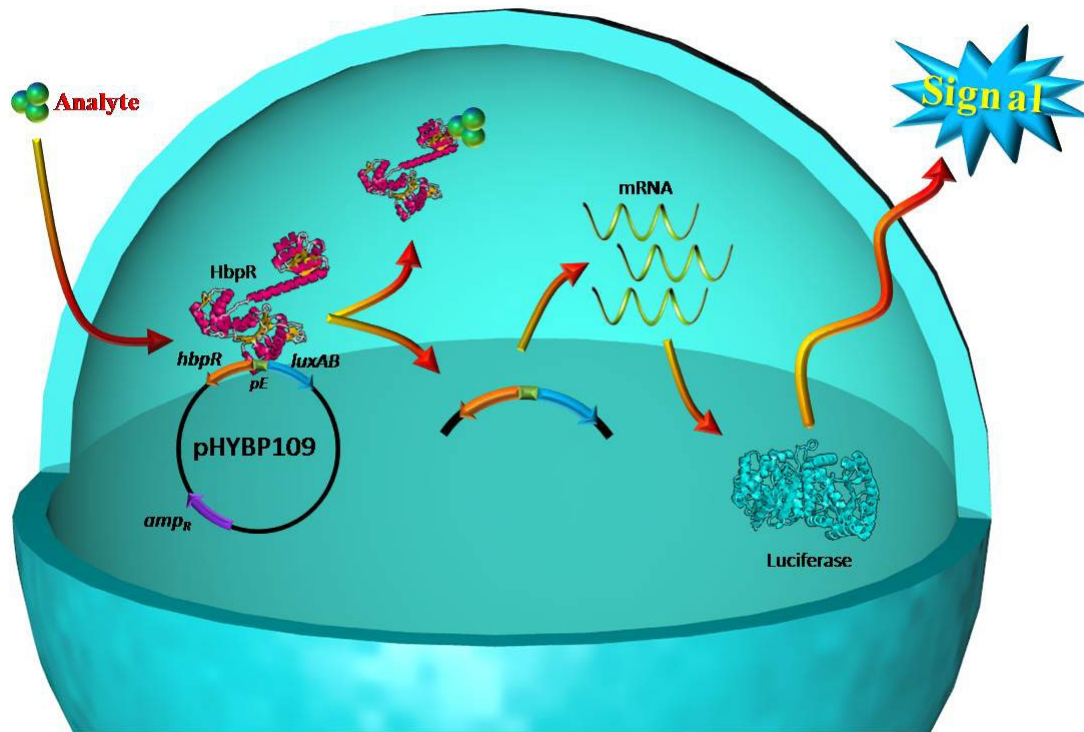
Reagentless Biosensors



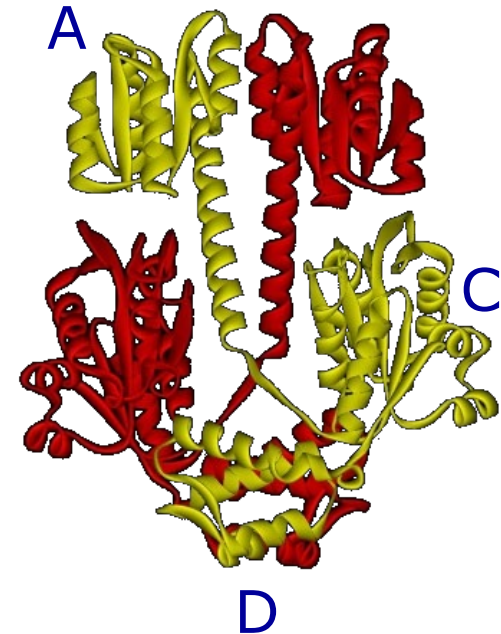
- Recombinant Proteins
- No substrates needed
- Random and/or site-selective modification with fluorophore
- Highly Selective and Sensitive
- Miniaturization/HTPS



Protein-Based Biosensing System for Hydroxylated-PCBs



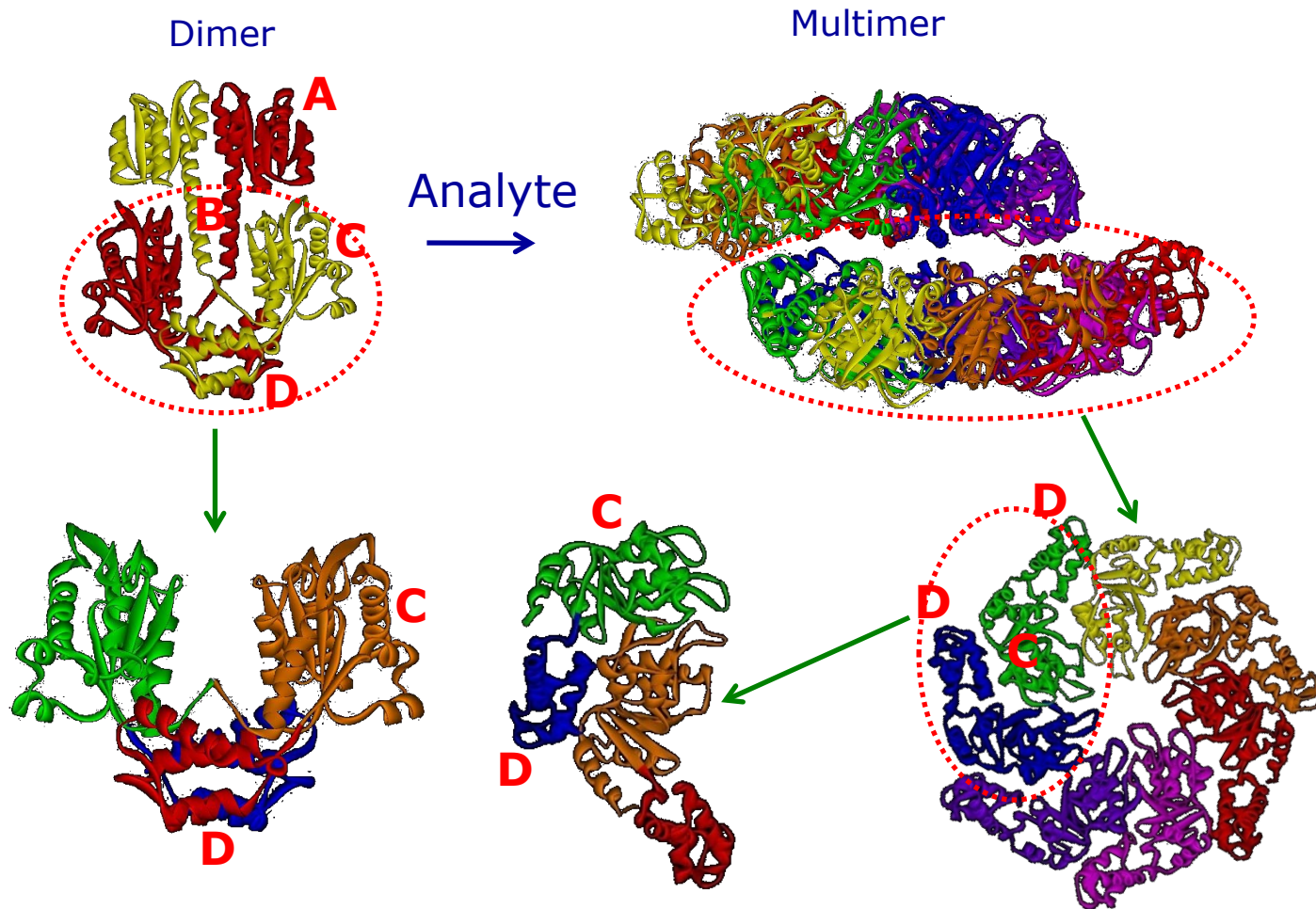
NtrC1





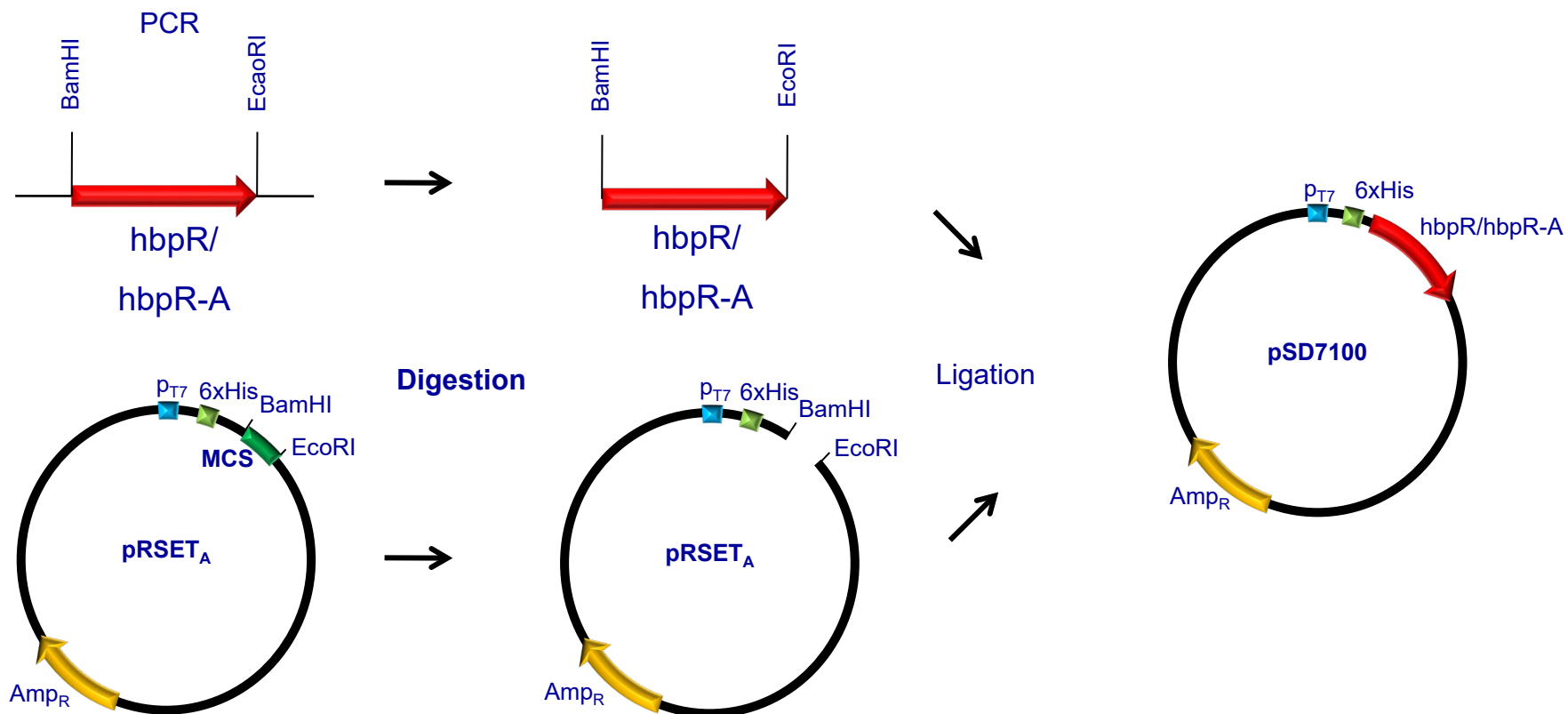
Protein-Based Biosensing System for Hydroxylated-PCBs

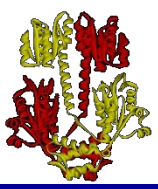
Conformational Change of NtrC1



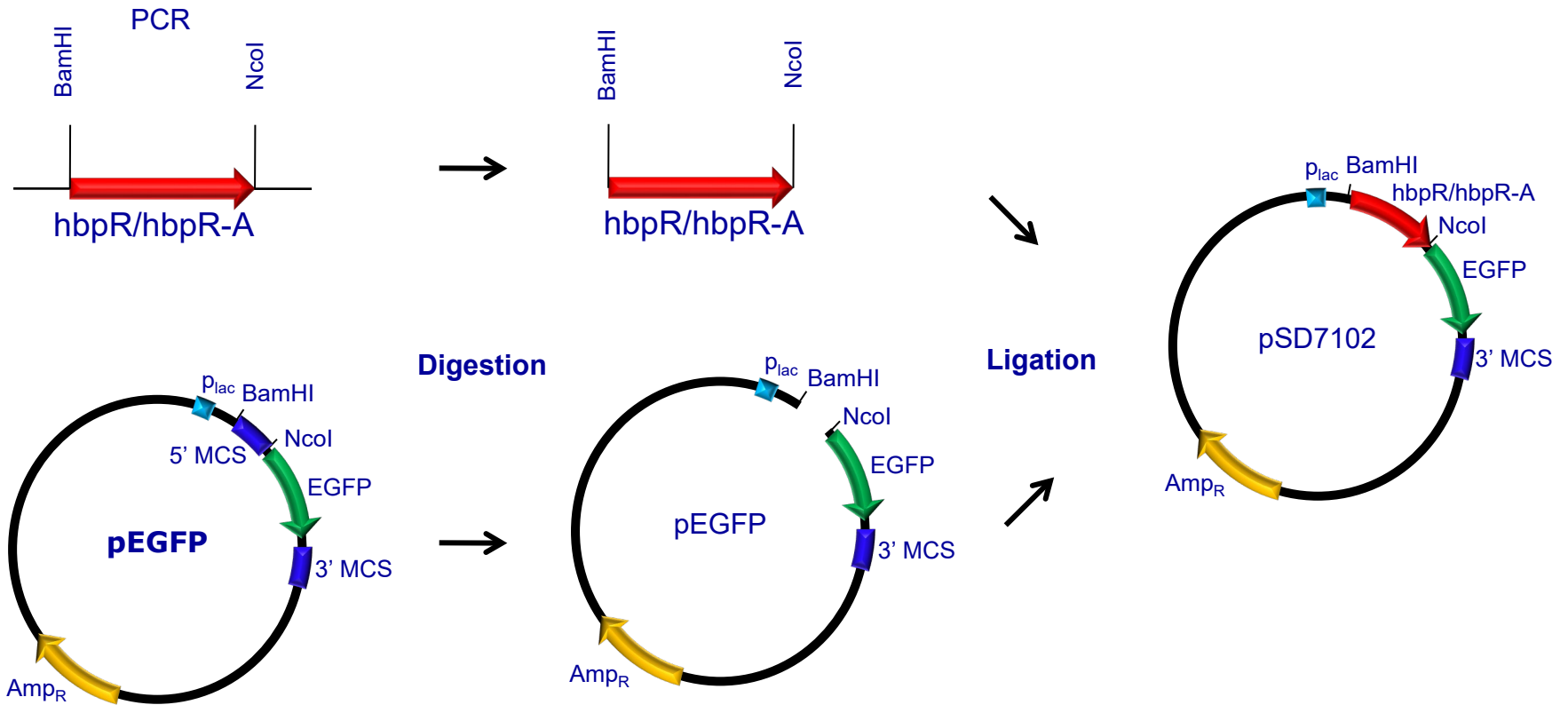


Construction of *hbpR* and *hbpR-A* Expression Plasmids





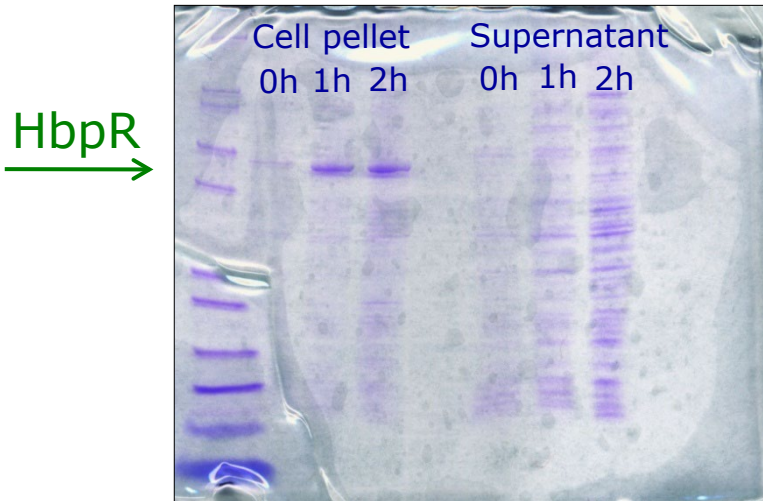
Construction of *hbpR-EGFP* and *hbpR-A-EGFP* Plasmids





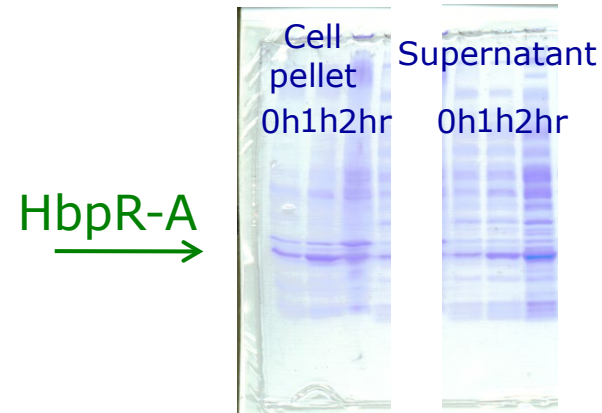
Protein Expression of HbpR and HbpR-A

HbpR



Expression as inclusion bodies in cell pellet, will attempt to denature/renature

HbpR-A

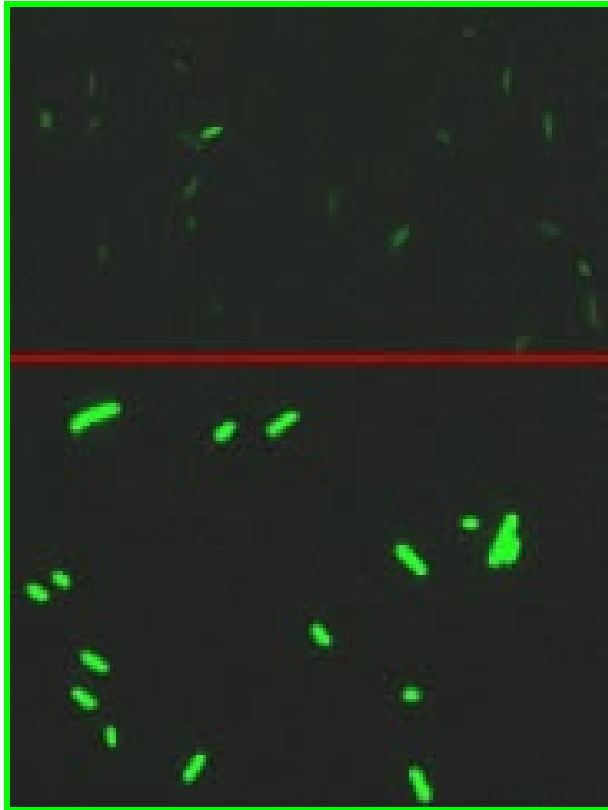


Expression as soluble protein!



HbpR-A-EGFP Fluorescence Emission

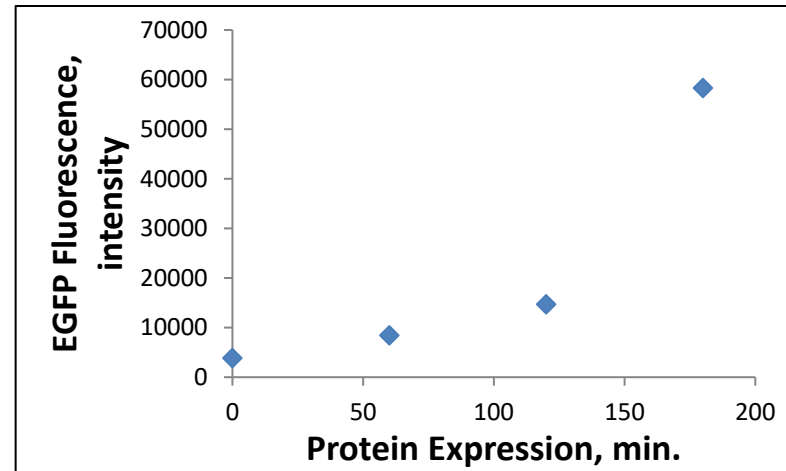
Reporter: GFP



HbpR-A-EGFP

EGFP Fluorescence Intensity,
Intact Cells, 24h Induction

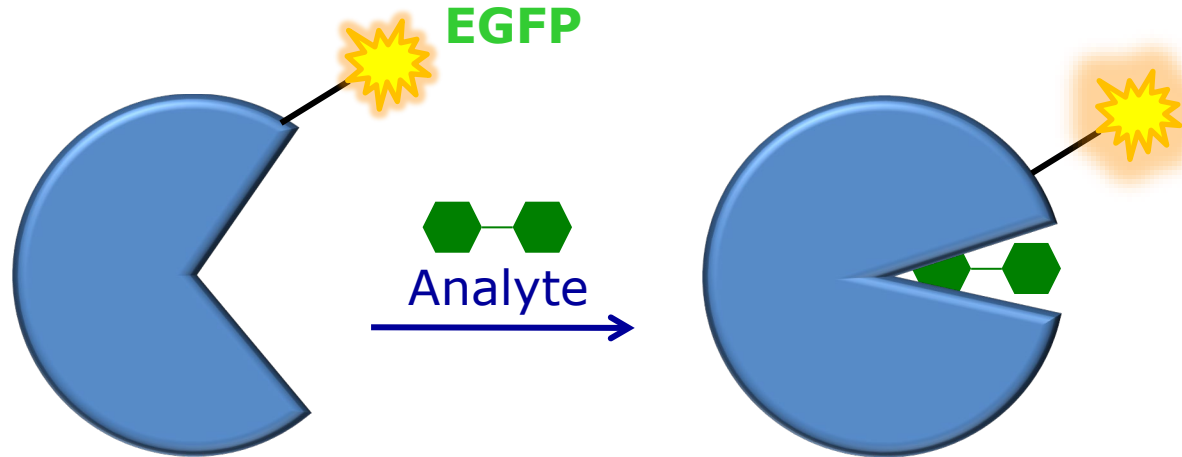
Negative Control	HbpR-A-EGFP
1.2×10^4	5.9×10^4



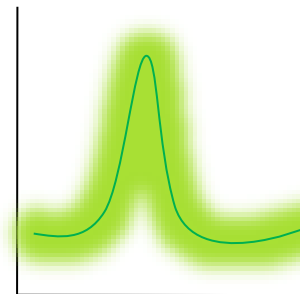
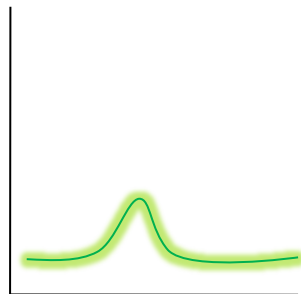
Nature, October 2003;
<http://www.nature.com/nsu/030929/030929-7.html>

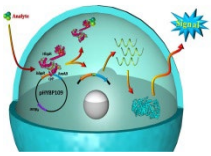


Biosensing System Based on HbpR-A



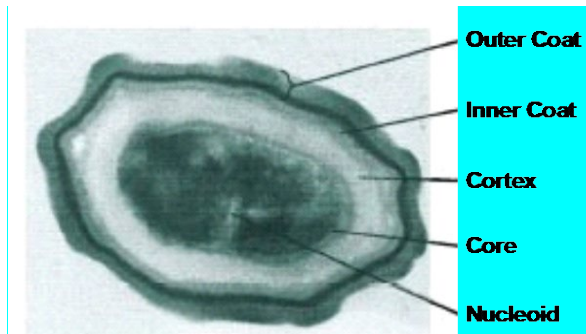
When the HbpR-A-EGFP fusion protein recognizes and binds the analyte, it changes its conformation, which in turn causes a change in the intensity of the fluorescence emission of EGFP





Bacterial Spores as Transport and Storage Vehicles of Living Biosensors

Electron micrograph of the cross section of a spore of *B. subtilis* (width: 1.2 μ m)

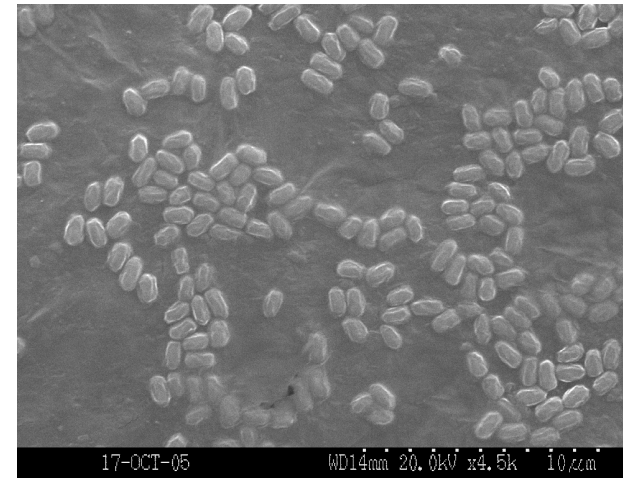


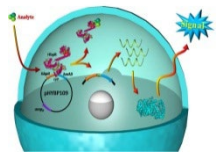
Adapted from Nicholson et al, 2000

Advantages

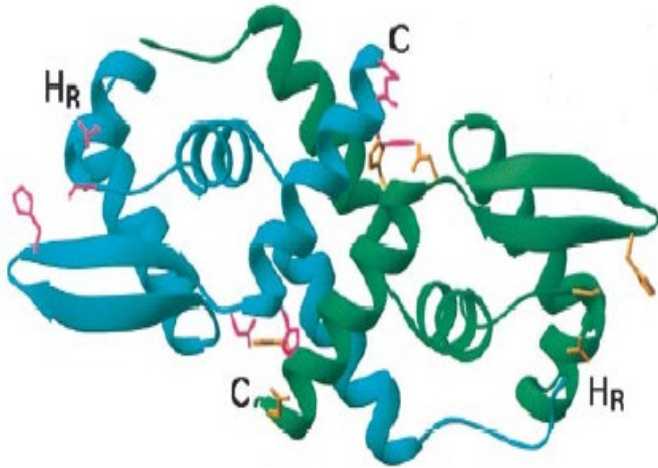
- Preserve DNA for long periods of time
- Stability under extreme conditions, i.e., heat/cold, humid/dry, pH, etc.
- Simple and economic production of spores

When subjected to stress, *Bacillus* form spores that lock the DNA into a dry metabolically inactive shell, thus preserving it for long periods of time, until conditions are suitable for regermination

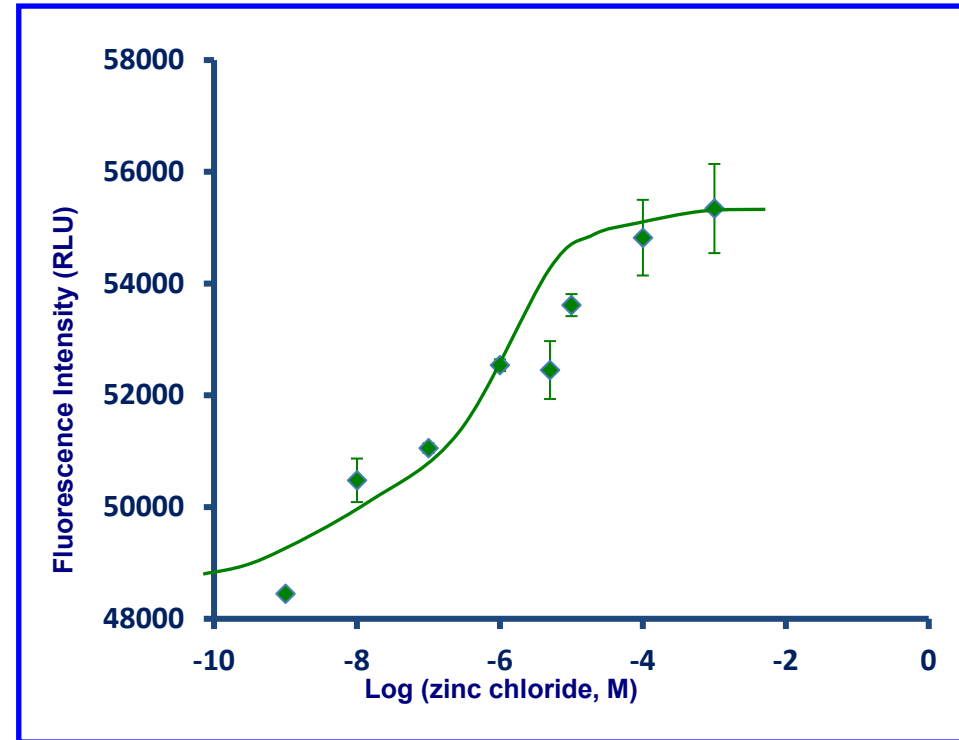


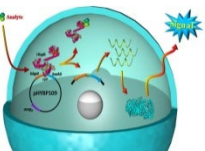


Zinc-Binding Protein SmtB/Sensing Zinc



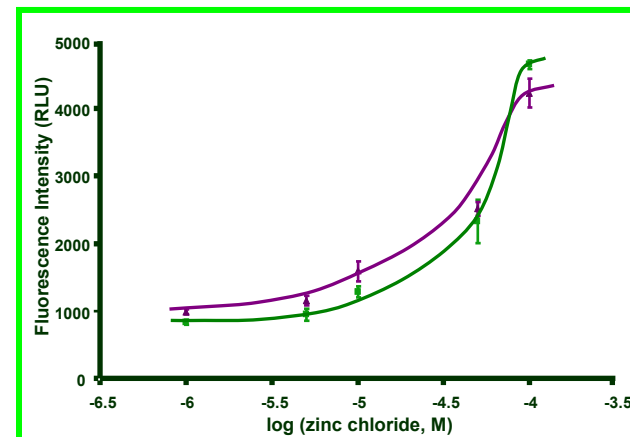
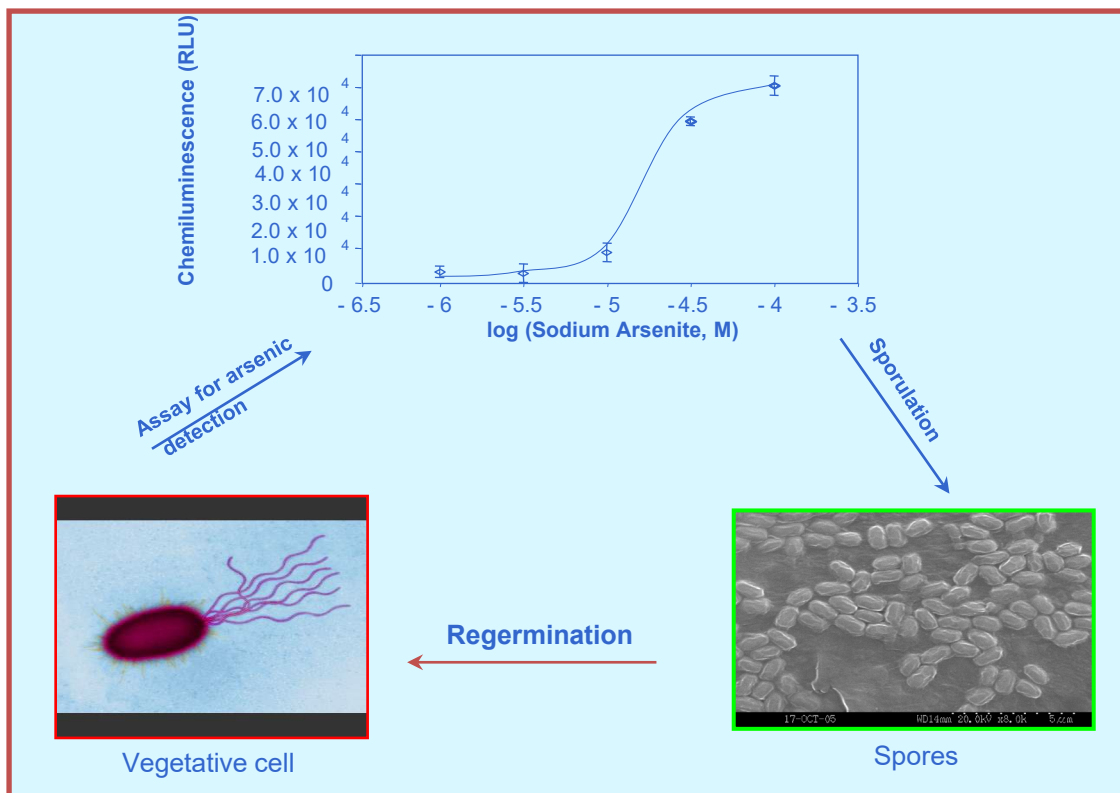
- There are 4 putative zinc binding sites
- Two sites are at the opposite sides of dimer formed by Cys61, Asp64, and His 97 in each monomer
- Other two sites are at the interface, formed by Asp104, His106 of one monomer and His117 and Glu120 of the other monomer



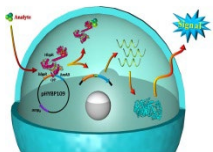


Dormant and Sensing Cycles

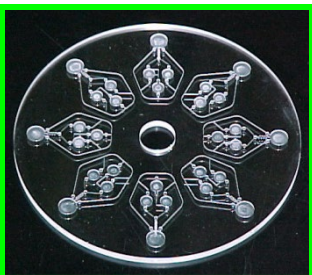
Response of *B. megaterium* Zinc Sensing Cells before and after Sporulation



Cycle #	Detection Limit (M)	Dynamic Range (M)
Before Sporulation	1x10 ⁻⁶	1x10 ⁻⁴ –1x10 ⁻⁶
Cycle 1	1x10 ⁻⁶	1x10 ⁻⁴ –1x10 ⁻⁶
Cycle 2	1x10 ⁻⁶	1x10 ⁻⁴ –1x10 ⁻⁶
Cycle 3	1x10 ⁻⁶	1x10 ⁻⁴ –1x10 ⁻⁶
After 8 Months Storage	1x10 ⁻⁶	1x10 ⁻⁴ –1x10 ⁻⁶

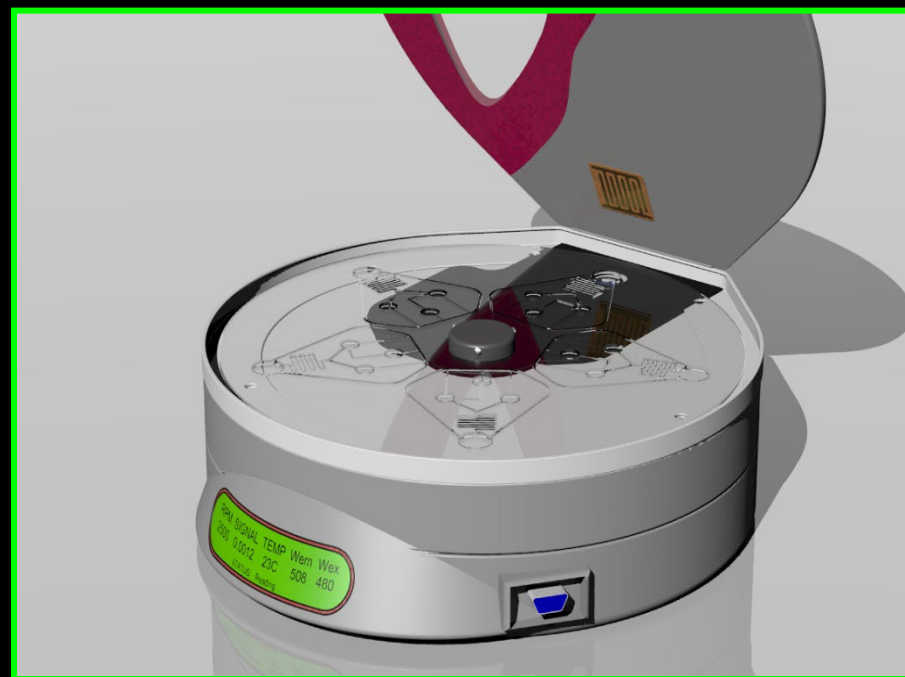


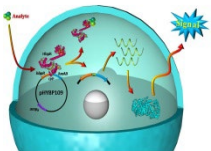
Miniaturization and Field Studies



Centrifugal Microfluidics Platform or Lab-on-a-CD

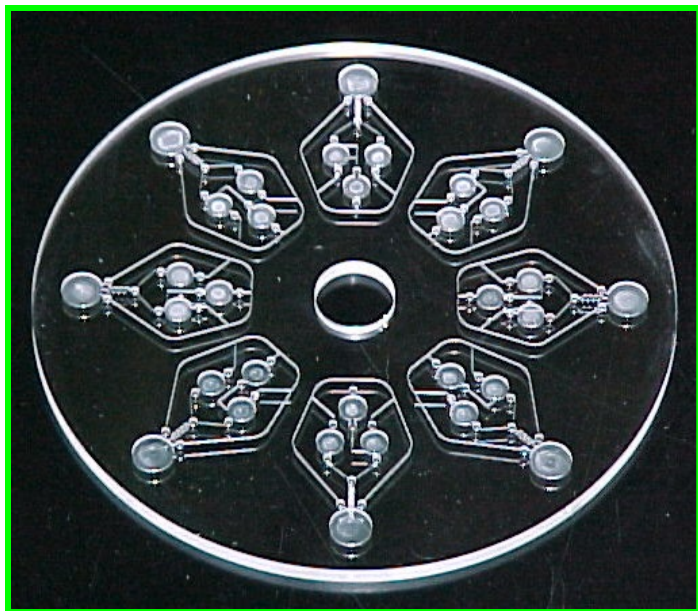
- Micro-Total Analysis System or μ -Tas
- Low power and space requirements
- Less reagent and sample consumption
- Portable
- Short analysis time
- High throughput multi-analyte detection
- Integrate washing, sample preparation and calibration



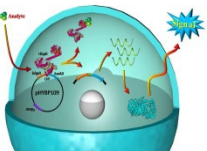


Sensing on the Lab-on-a-CD

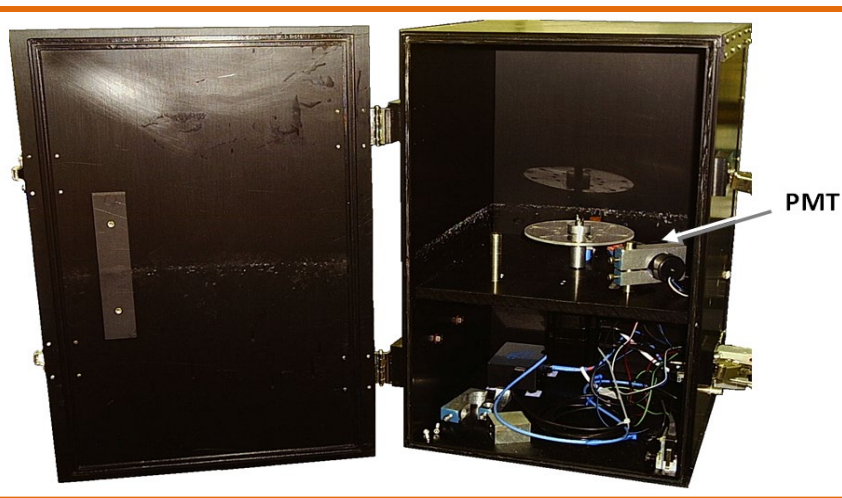
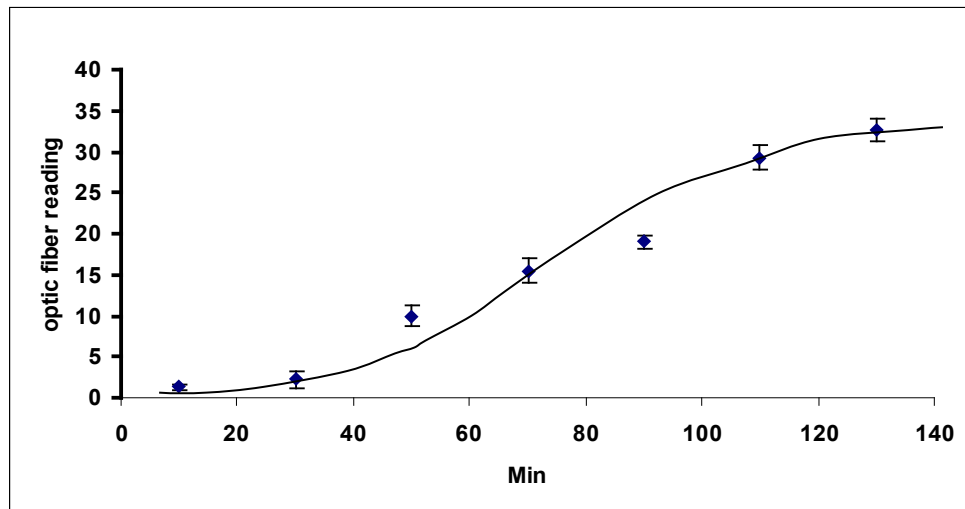
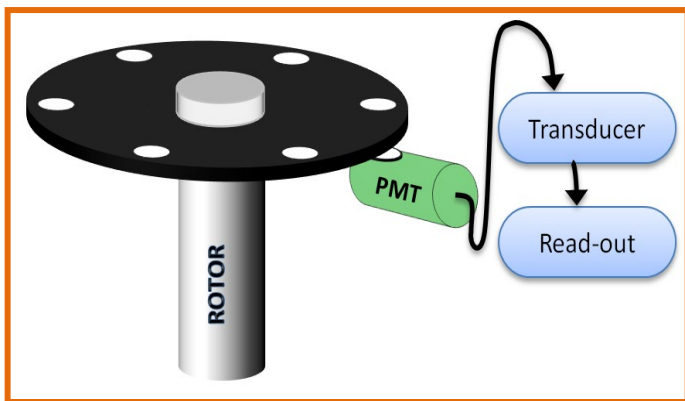
Microfluidics of Mixing Reagents



QuickTime™ and a YUV420 codec decompressor are needed to see this picture.

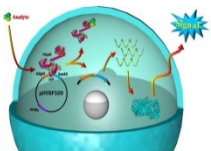


Germination Study of Spores on a Microfluidic Platform

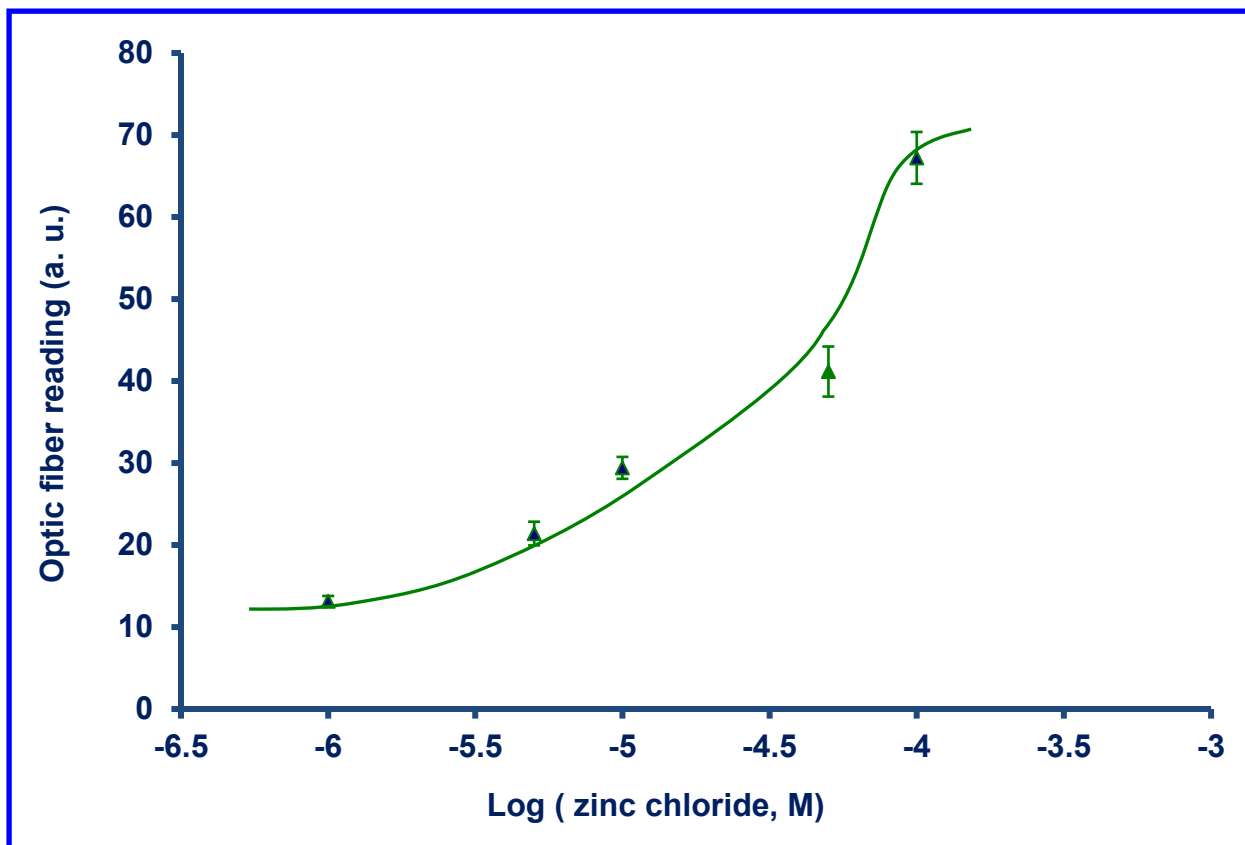


Actual Optical Density	0.2	0.3	0.4	0.5	0.7	0.8
Reading by Optic fiber	3.83	8.51	12.08	13.8	16.8	18.96

From the above data, the desired optical density of 0.7 is obtained after 90 minutes



Response to Zinc on the Lab-on-a-CD



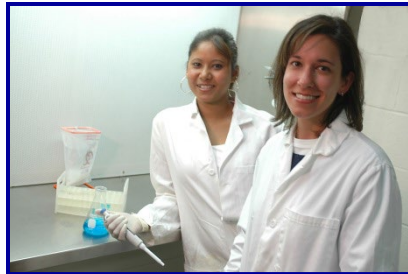


Special Thanks to:



The past and present
members of the Daunert Group

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University of Kentucky

