# Food Safety: A "Sense-able" Approach

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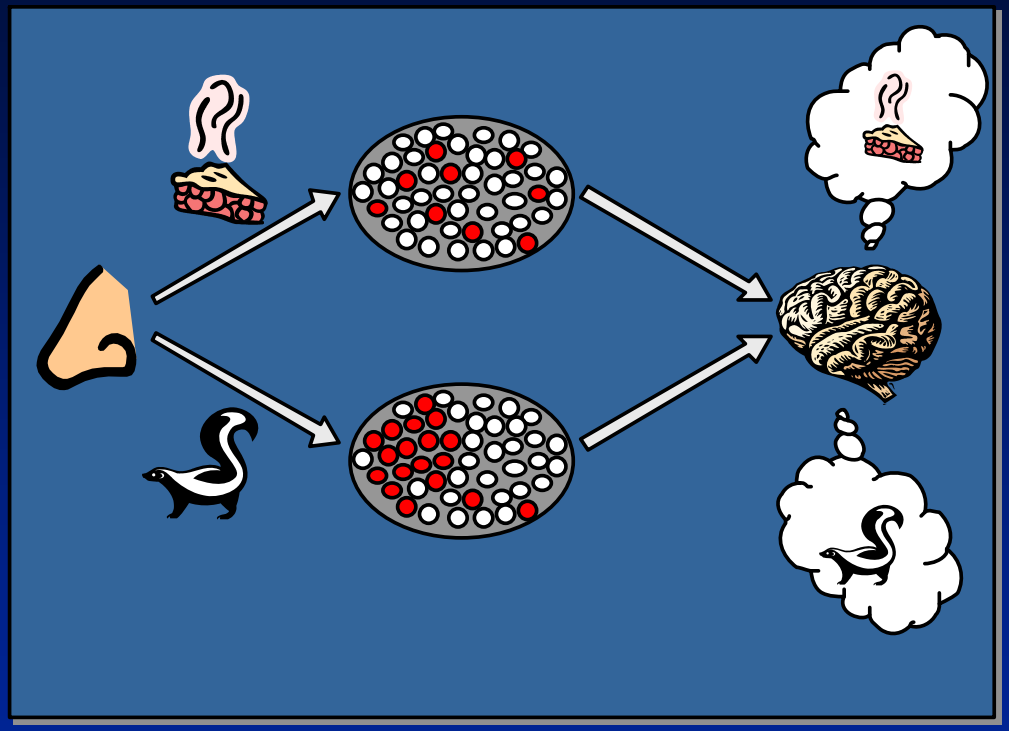
# Overview

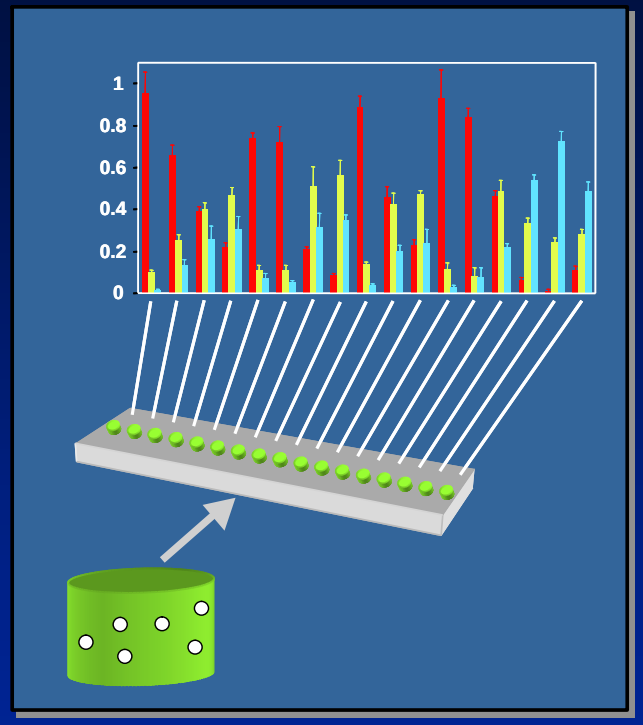
- Living in a world filled with potential pathogens is risky
- Nature has solved the problem of coexisting with pathogens in different ways
- Leveraging the experiments of nature can improve food safety

# A Sense-able Approach: SMELL

- Opportunistic feeders use smell to find and interrogate food

# Array-Based Chemical Sensing





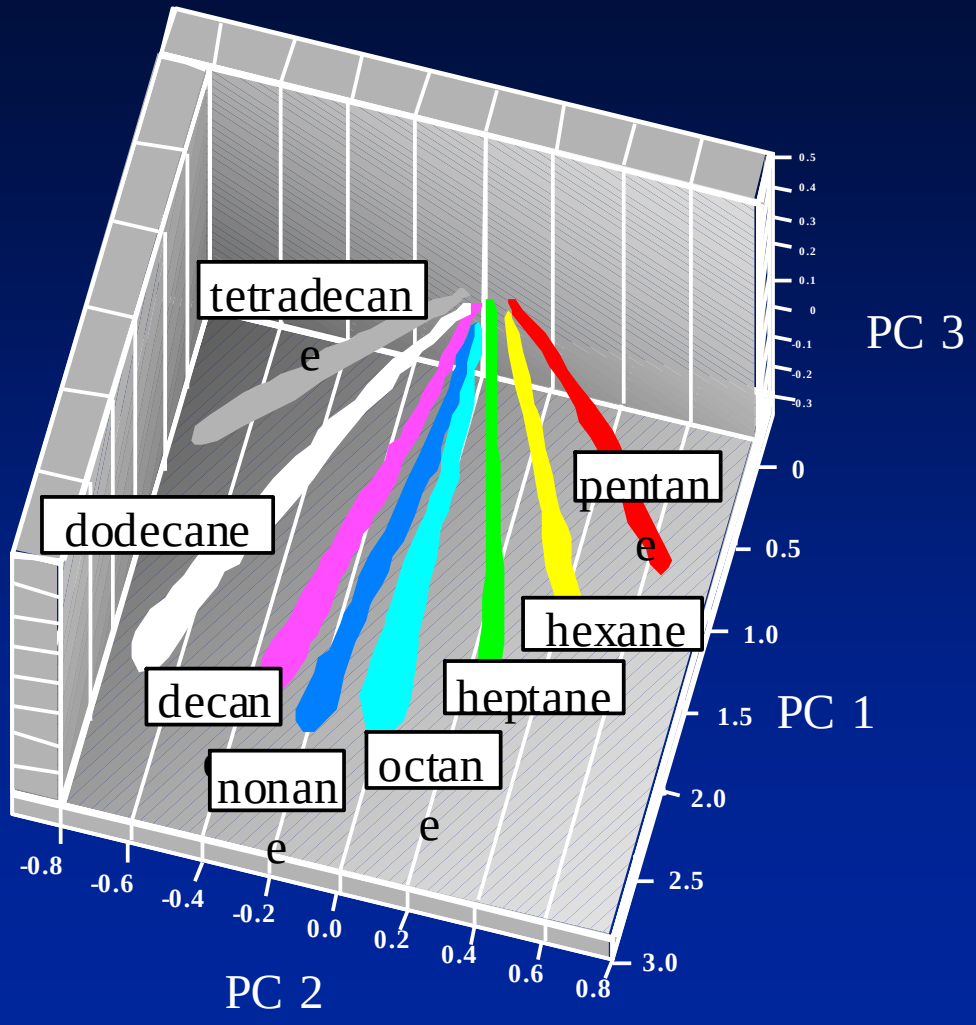

In mammalian olfaction, arrays of receptors respond differently to different odors, creating unique fingerprints that can be analyzed by the brain

The process can be artificially reproduced using arrays of chemiresistive sensors

*Caltech / Next Dimension Technologies*

## Detection and Quantification of Chemically Similar Species

Sensor responses are typically linear with concentration and unique for each chemical analyte



# A Sense-able Approach: VISION

- Hunters use "tracks" to follow and find prey, while avoiding predators
- The "tracks" laid down by bacteria can be used to confirm where the bugs are hiding
- Near-IR spectroscopy approach detects and quantifies bacterial metabolites (tracks)

# Differentiation of Oral Bacteria using Near Infrared Spectroscopy (NIRS)

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## ABSTRACT

The differentiation of oral bacteria traditionally requires time consuming, labor-intensive processes including growth of the microorganism, microscopic examination, and/or DNA extraction and amplification of genes specific to a particular bacterial species or strain. NIRS provides information on biochemical composition of bacterial cell wall components. Photon energies associated with infrared spectrum between wavelengths 1100 and 2500 nm probe vibrational states of covalently bonded atoms, with each molecule having its own characteristic vibrational energies. Bacterial cell wall and cell-wall associated polysaccharides are characteristic of each strain and are responsible for the generation of an NIRS spectrum unique to each strain. OBJECTIVE: NIRS was evaluated as a method for identification of oral bacterial species METHODS: The vibrational infrared intensities emitted by *S. sanguis*, *S. mutans* and *A. viscosus* were captured using an NIR spectrophotometer and the data were analyzed using Principal Component and Linear Discriminant analysis. RESULTS: NIRS was successfully used in this study to differentiate *S. sanguis*, *S. mutans* and *A. viscosus*. The system could identify these individual genus/species grown as micro-colonies on blood agar plates or as biofilms on hydroxyapatite discs. Growth conditions for the bacteria did influence the spectral pattern, but did not impact the ability to differentiate the microorganisms. CONCLUSIONS: NIRS was found to be a rapid method for identification and differentiation of oral bacteria at the genus/species level and does not require destruction of the sample. The current system was used to establish proof-of-principle for the potential application of this technique to generate spectral fingerprints of major oral pathogens and provide utility for the characterization of *in vivo* biofilm composition in oral diseases.

Supported by UK Center for Oral Health Research.

## INTRODUCTION

- Periodontal disease is one of the most common chronic illnesses in humans. Microorganisms associated with periodontal disease have been traditionally identified based on morphological or staining characteristics of the bacteria.
- More recently, culture independent molecular methods have been developed although they require the removal of the bacteria from the *in vivo* biofilm ecology.
- Identification of microorganisms comprising bacterial consortia that play a critical role in periodontal disease requires the development of new diagnostic tools. One such tool is based on using vibrational spectroscopic techniques.
  - Differentiation of microorganisms using vibrational spectroscopy is based on the absorption of infrared energy by the cellular components of the microorganisms.
  - Cellular composition of each bacterial species is unique resulting in the absorption spectra that is a fingerprint for that particular species.
  - Vibrational spectroscopy has been used to differentiate bacteria on a genus, species and strain level.
  - Vibrational spectroscopy is rapid, accurate and requires minimal sample handling, hence, has a great potential in clinical diagnosis of periodontal disease.
- Raman spectroscopy and Infrared (mid-Infrared and near-Infrared) spectroscopy are complementary vibrational spectroscopy techniques
  - Each gives unique spectral information
  - Each has been used to differentiate bacteria at the species/strain level.
  - Near-Infrared spectroscopy (NIRS) offers certain advantages over Raman; being non-destructive, sensitive (high signal-to-noise ratio), and with an acquisition time <1 min.
  - Infrared spectroscopy has the potential for *in vivo* evaluation of disease-associated biofilms.
- The objective of this study was to apply NIR spectroscopy to differentiate individual oral bacteria.
  - The microorganisms selected included different genera/species and pigmented/non-pigmented strains within a species

## MATERIALS AND METHODS

- **Bacterial strains:** The following bacterial strains were used in this study *Eikenella corrodens* ATCC 23834, *Streptococcus mutans* ATCC 25175, *Streptococcus sanguis* ATCC 10556, *Fusobacterium nucleatum* ATCC 25586, *Actinomyces viscosus* ATCC 43146, *Actinomyces naeslundii* ATCC 49340, *Prevotella intermedia* ATCC 25611, *Porphyromonas gingivalis* ATCC 381. On each blood agar plate, a 500 µl of liquid bacterial culture was spread and incubated under the appropriate growth conditions for approximately 1-3 days. An uninoculated plate was also included as a control. *P. intermedia* ATCC 25611 and *P. gingivalis* ATCC 381 pigmented biofilms were grown for approximately 10 days.
- **Spectrometer:** A InfraAlyzer 500 (Bran + Luebbe, Elmsford, New York) was used to collect spectral data. Monochromatic near-Infrared light is supplied by a tungsten lamp and the absorption spectra were collected between 1100-2500 nm wavelength range at 2 nm intervals.
- **Data acquisition:** NIR spectra of biofilms from blood agar plates were collected for 10 bacterial specimens representing 8 species. We also included a pigmented and nonpigmented form of 2 species. For each microorganism, seven blood agar plates were scanned including a control. Each plate was scanned three times at three different locations.
- **Statistical analysis:** The raw NIR spectra were preprocessed by multiplicative scatter correction (MSC). To evaluate classification of bacteria, a combination of linear discriminant analysis and BEST distances were used. A principal component (PC) transformation was done after MSC to reduce the number of variables. Seven PCs retained 100% variance. A 7 PC model using a 3 SD cutoff was chosen for CVA analysis. From the 7 PC model, 6 canonical variables were significant for discrimination. All data analysis was done with Matlab 7.1 (MathWorks, Natick, MA).

## RESULTS

Figure 1. A second-derivative NIR spectra for each of the ten oral bacteria. The absorption spectra shown for each organism is in the range between 1100-2500 cm-1 wavelength. The spectral patterns identify multiple wavelengths and signal amplitudes that differentiate genera, species and pigmentation across the isolates.

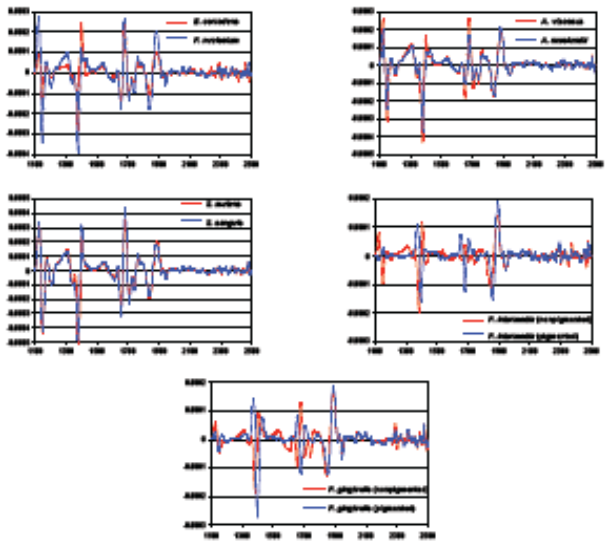


Figure 2. Clustering of ten oral bacteria based on first and second canonical variate (CV1 and CV2) using principal component analysis-canonical variate analysis (PCA-CVA) of second-derivatized NIR spectra of biofilms. CVA uses the principal components to minimize the variance within an isolate and maximize the variance between isolates. CVA was able to differentiate all ten bacteria belonging to different genera (eg. *E. corrodens* and *F. nucleatum*) and species (eg. *S. sanguis* and *S. mutans*). Pigmented and non-pigmented forms of *P. gingivalis* and *P. intermedia* clustered closely but well spaced.

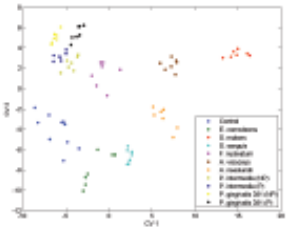


Table 1. Classification results for each bacterial isolate derived from cross-validation analysis

| Group | Correct Classifications | Accuracy (%) | Precision (%) | Recall (%) |
|---|---|---|---|---|
| Control | 7 (n=10) | 98.28 | 100 | 70 |
| *E. corrodens* | 8 (n=7) | 98.75 | 100 | 85.714 |
| *S. mutans* | 8 (n=7) | 98.75 | 100 | 85.714 |
| *S. sanguis* | 5 (n=7) | 97.5 | 100 | 71.429 |
| *F. nucleatum* | 4 (n=7) | 96.25 | 100 | 57.143 |
| *A. viscosus* | 6 (n=7) | 98.75 | 100 | 85.714 |
| *A. naeslundii* | 7 (n=7) | 100 | 100 | 100 |
| *P. intermedia* (NP) | 4 (n=7) | 96.25 | 100 | 57.143 |
| *P. intermedia* (P) | 6 (n=7) | 98.75 | 100 | 85.714 |
| *P. gingivalis* (NP) | 5 (n=7) | 97.5 | 100 | 71.429 |
| *P. gingivalis* (P) | 7 (n=7) | 100 | 100 | 100 |
| GLOBAL | 83 (n=80) | 98.088 | 100 | 76.36 |

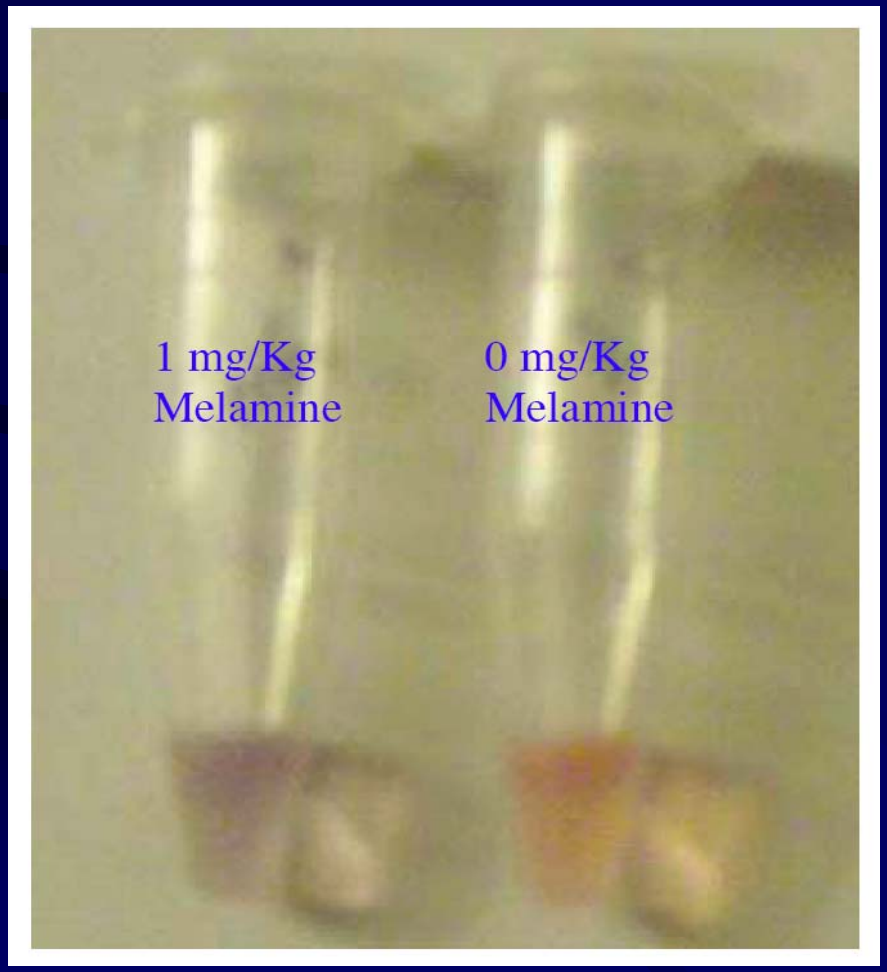
Accuracy = (TP + TN) / (TP + TN + FP + FN)
Precision = TP / (TP + FP)
Recall = TP / (TP + FN)
Where TP = True Positive, TN = True Negative, FP = False Positive and FN = False Negative.

These results demonstrate the utility of NIRS analysis in differentiating oral bacteria as mono-cultures. Additional studies will test NIRS on multispecies biofilm composites.

## SUMMARY AND CONCLUSIONS

- NIR spectral patterns were able to differentiate oral bacteria, including different genera and species
- NIR spectral patterns were able to differentiate pigmented and non-pigmented variants within a species for both *P. gingivalis* and *P. intermedia* that have been associated with disease.
- The NIRS technique has the potential to provide a new tool to evaluate *in vivo* biofilm composition to provide an innovative method for monitoring microbial changes associated with periodontal disease

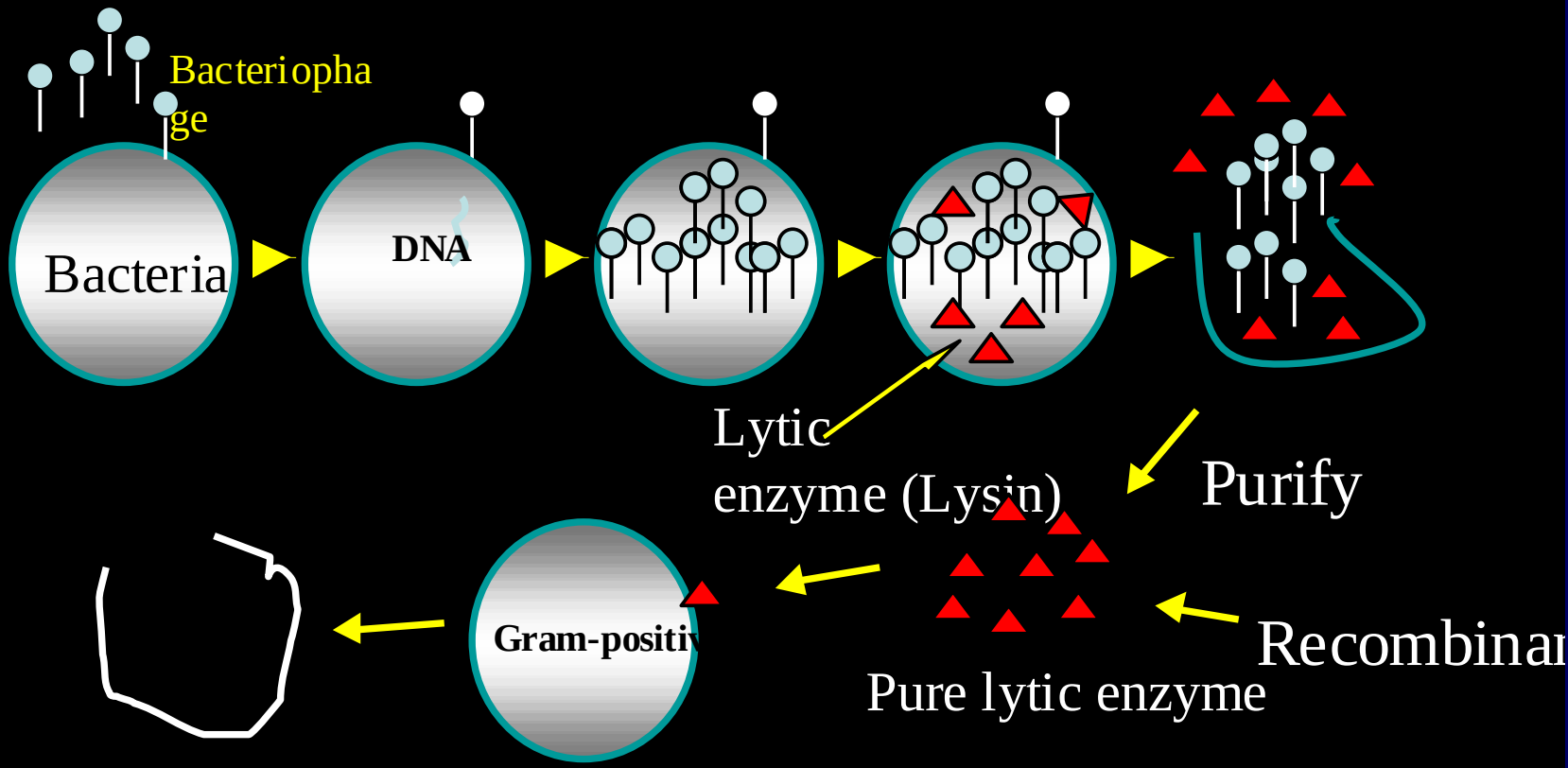
- Engineered nanoparticles complex melamine in milk, causing color change



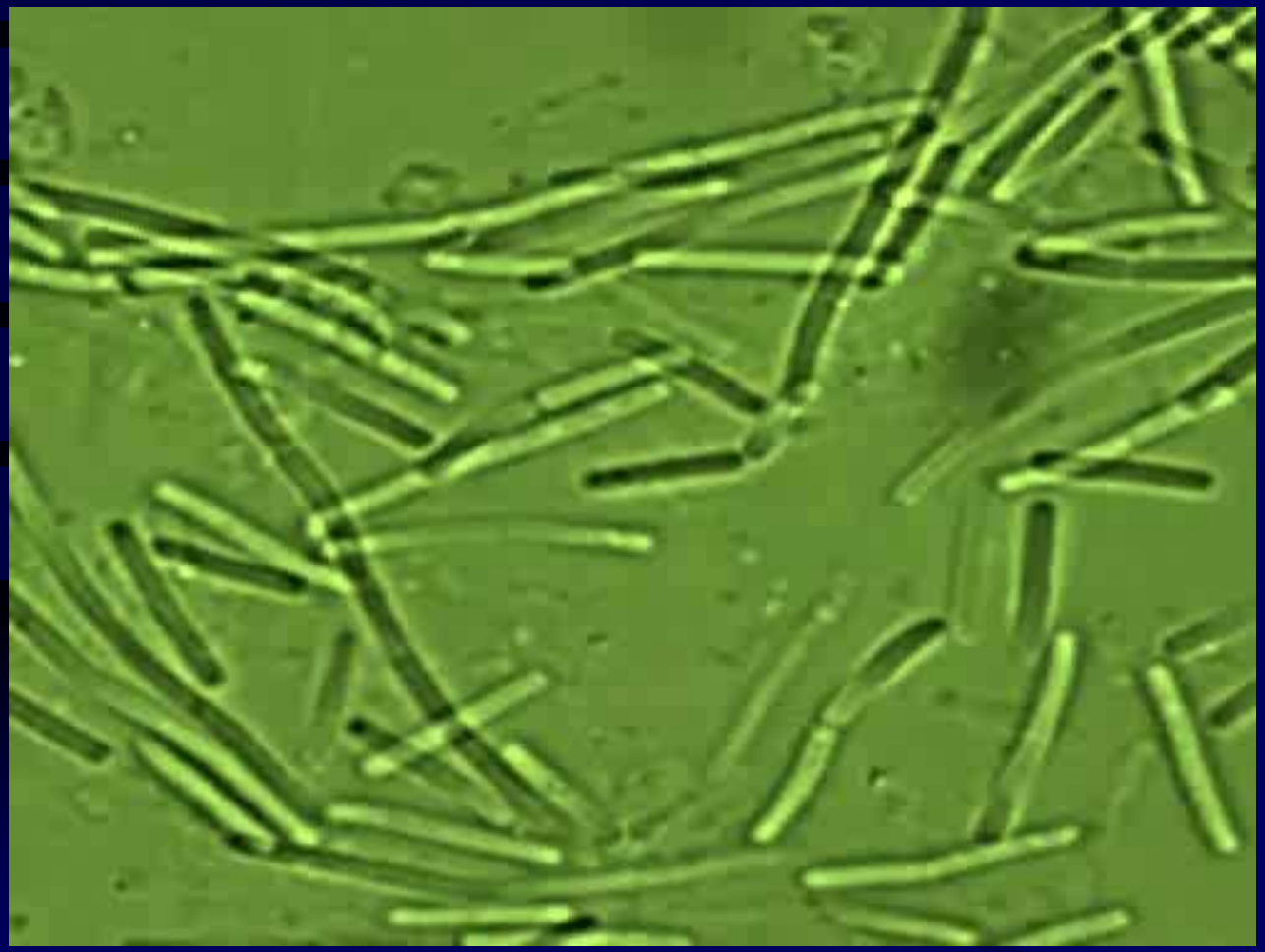
# A Sense-able Approach: TASTE

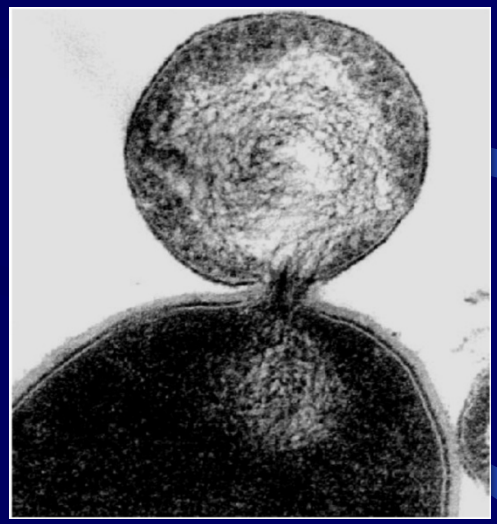
- Bitter and other chemosensory percepts warn against food dangers and spoilage
- The "taste" of a bacteria tells bacteriophages which microbes to attack
- The chemosensory discrimination of phages results in a high level of specificity

# What are Phage Lytic Enzymes?

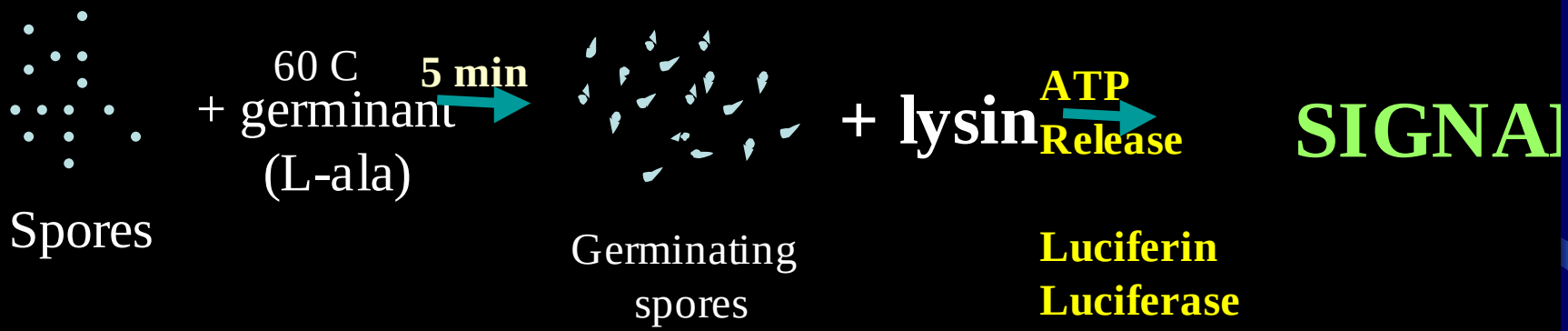


# *Bacillus cereus (*RSVF*)* treated with PlyG Lysin



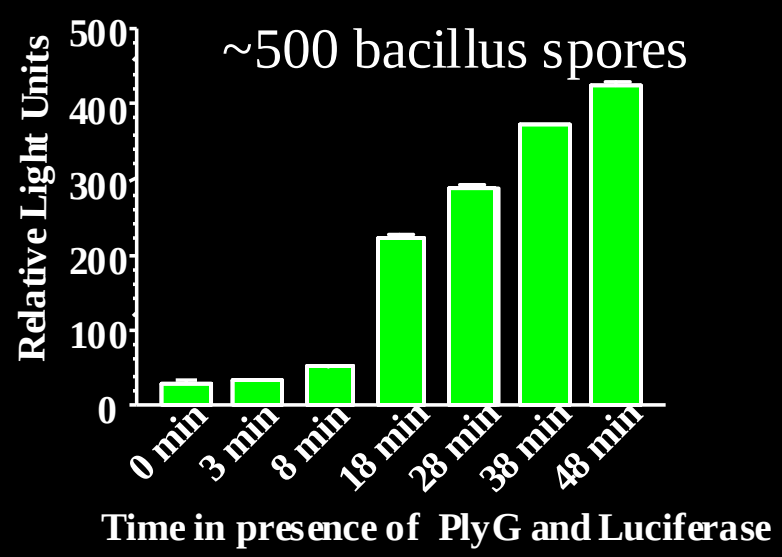


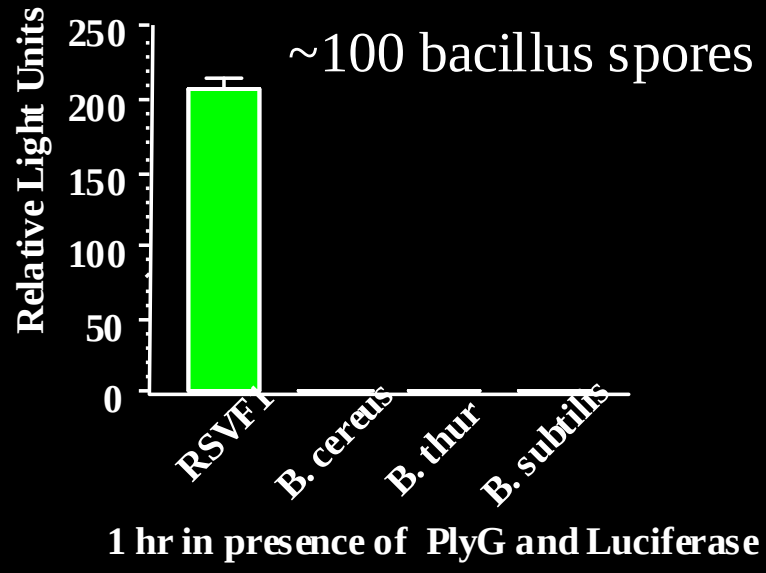
# Use of PlyG Lysin for Rapid Identification of *B. anthracis* Spores



# Detection of Bacillus Spores

## after 5 min exposure to L-ala as germinant

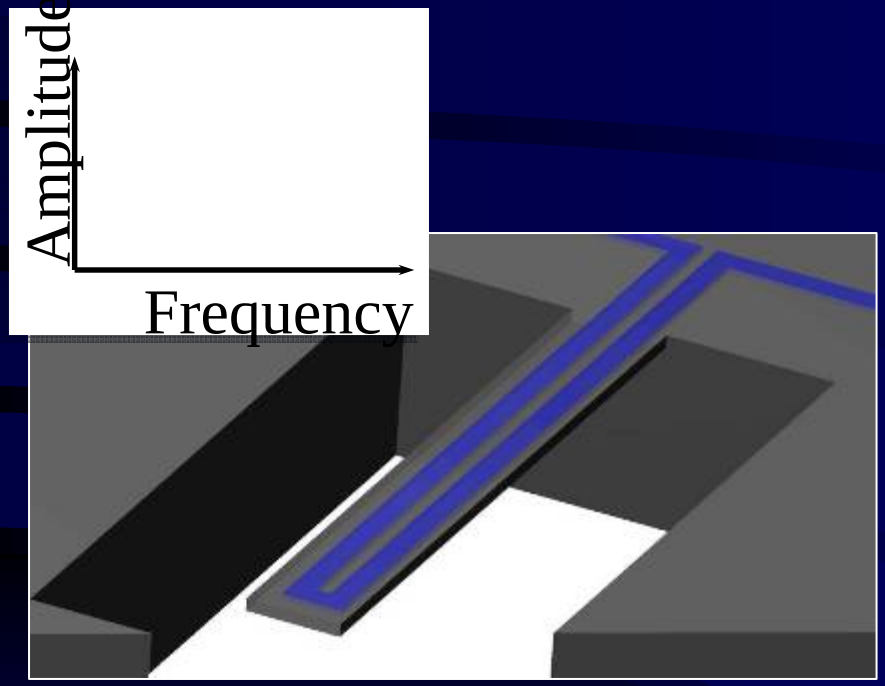


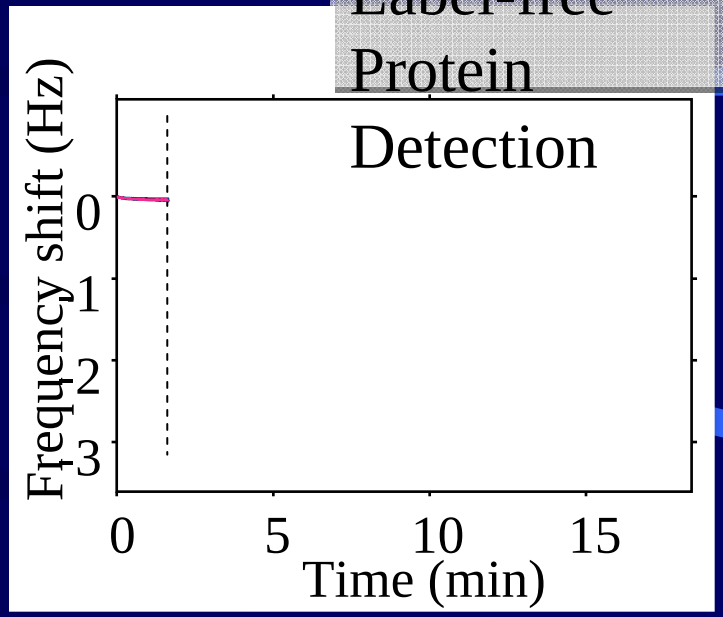



# A Sense-able Approach: TOUCH

- Ripe and nutritious fruit are selected both by sight and feel
- Pathogens and toxic proteins can be individually weighed using novel approaches

# Weighing Biomolecules, Single Cells, and Single Nanoparticles in Fluid




**Fluid flows through hollow resonator**

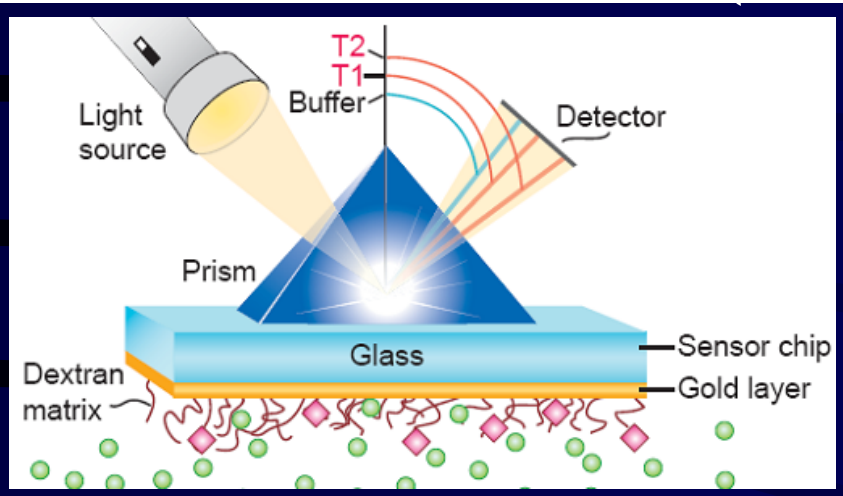
*Improves resolution by* ***$10^6$x***

*Nature* **446**, 1066-1069 (2007)

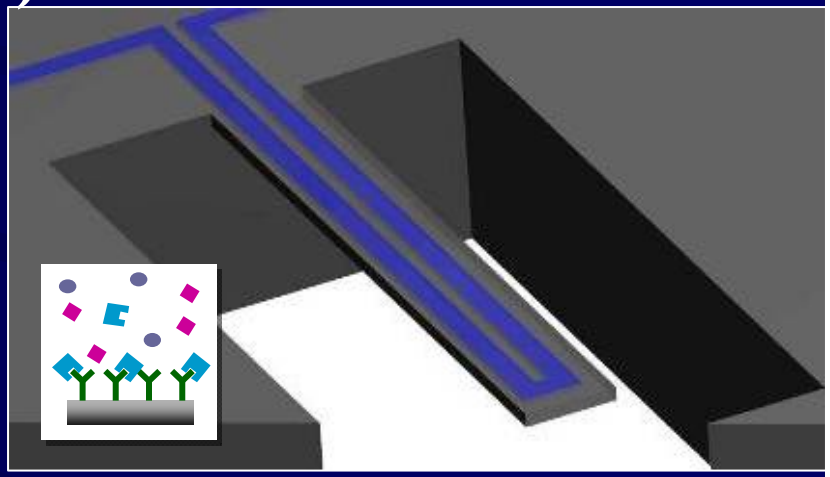
# Label-free Detection

- Enables simple, one-step assay
- Susceptible to nonspecific binding
- Limited sensitivity

Surface Plasmon Resonance (SPR)

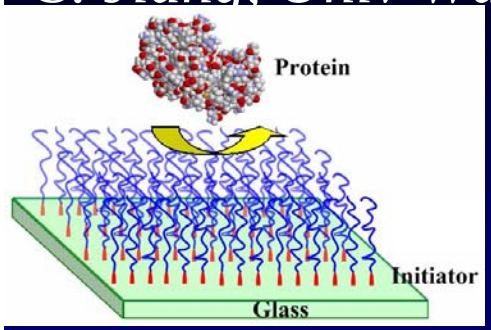



**Suspended Microchannel Resonator (SMR)**



**Ultra-low fouling surfaces**

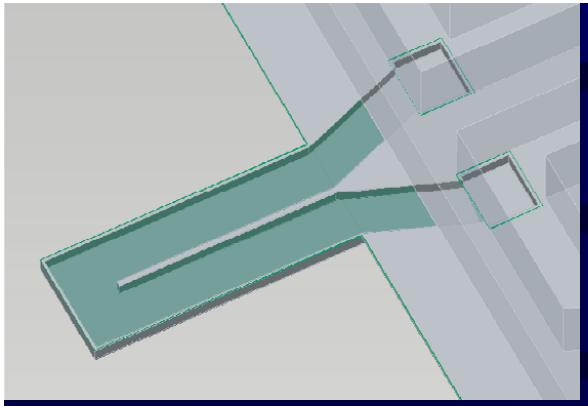
*S. Jiang, Univ Washington*



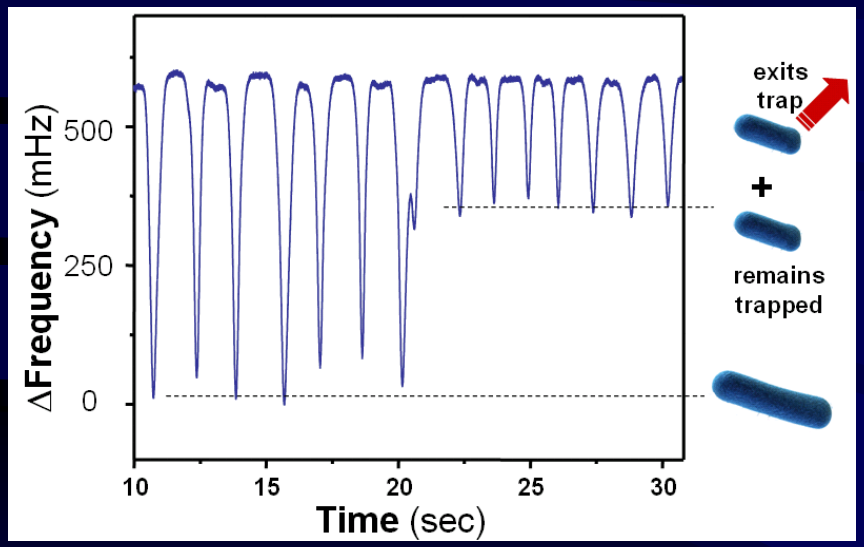

Reduces NSB by >100-fold more than ethylene glycol-based surfaces

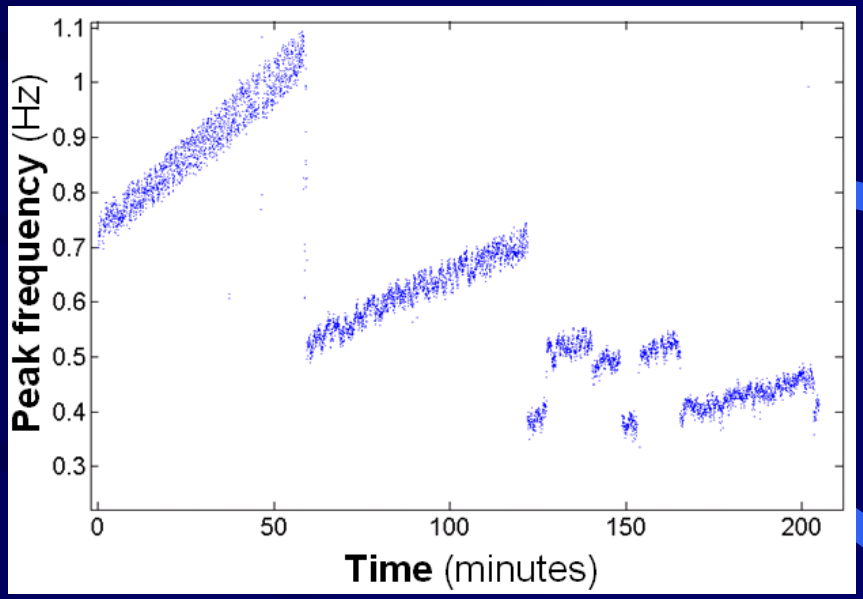
1st generation SMRs achieve sensitivity similar to SPR and could readily be improved another 10x

Direct detection of total mass in the μchannel (not just near the surface) enables detection of single cells



# *Measuring growth of single E.coli cells with a resonant microchannel*



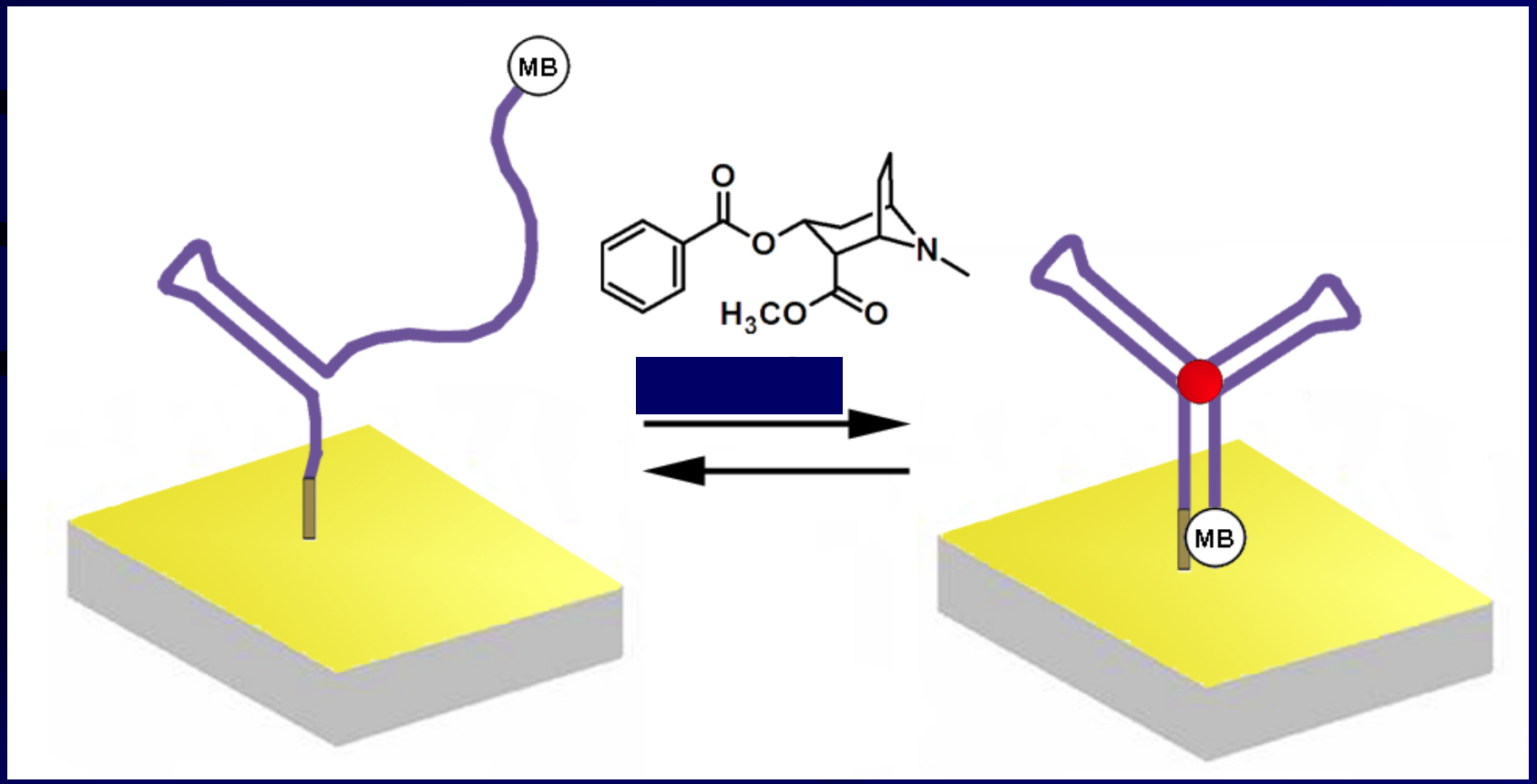

<u>Applications</u>

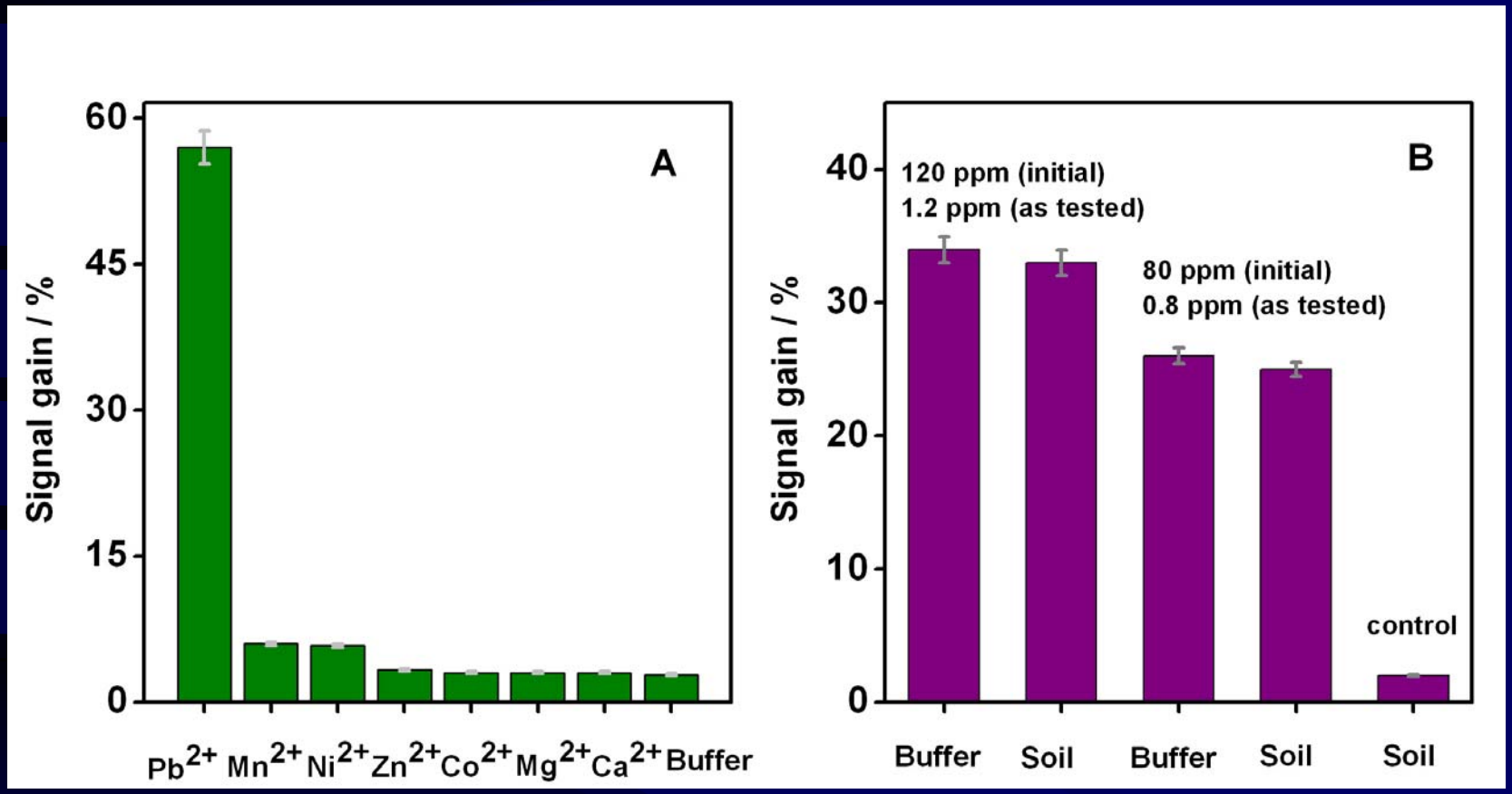
i) Development of anti-microbial peptides
ii) Identification of ‘injured’ bacteria
iii) Screening for drug resistant bacteria

# Developing Sense-able Systems as Integrated Tools

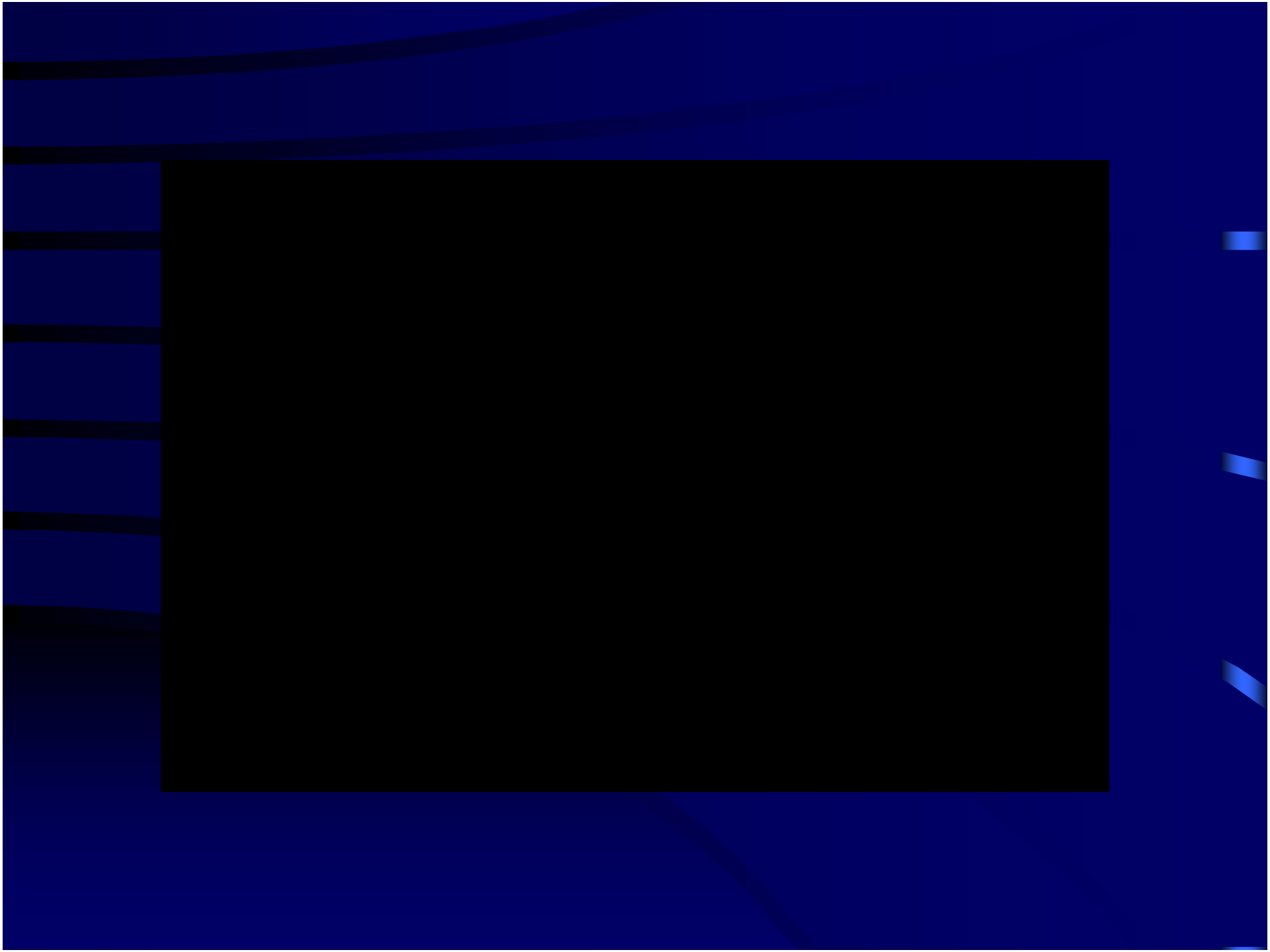
- A portable/hand-held laboratory on a slide can be adapted to many different environments (field, transport and processing)
- Rapid and reliable reporting on the fly

# Reagentless, Electrochemical Metal and Small Molecule Detection






Xiao, Y., Rowe, A.A. and Plaxco, K.W. (2007) "Electrochemical detection of parts per billion lead via an electrode-bound DNAzyme assembly." *J. Am. Chem. Soc.,* **129**, 262 – 263



# A Summary of Sense-able Approaches:

- Leveraging nature's solutions can create highly selective and sensitive tools
- Proof of principle studies demonstrate utility of approaches
- Many of the laboratory-based techniques can be adapted to manufacturing environments

# Food Safety:
# A "Sense-able" Approach