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11/17/04
**Report Documentation Page**

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Standard Form 298 (Rev. 8-98)  
Prescribed by ANSI Std Z39-18
Outline

- Technology Gaps
- Goal, approach
- Cavitands for chemical vapor sensing
- Liquid Crystals for sensitivity amplification
- Label-free antibodies for SPR biosensing
- Conclusions
Technology Gaps

- **Chemical detectors**
  - limited sensitivity - still use canines for bomb detection
  - high false alarm rates - limited specificity
  - slow response time - due to sensing material used

- **Biological Detectors**
  - need reagents, labels, not real time

- **Separate Detectors**
  - for chem. and bio. threats increase logistical burden/cost
Goal

Develop an integrated SPR chem/bio detector with

False Alarm Rate < $10^{-3}$ for chem. and < $10^{-8}$ for bio.

Sensitivity - below permissible exposure level (PEL) for chem.
  - LOD of 100 organisms/liter of air for bacteria/viruses
  - LOD of 10 nanograms/liter of air for toxins

Response time - < 1 minute for chem. and < 10 min for bio.

Military and civilian need

Chem/Bio detectors with high sensitivity, specificity, stability, and speed in portable format for airborne threats
Approach

Technique - SPR spectroscopy

Sensing with - Cavitands for chem. threats
- Liquid Crystals for chem. threats
- Antibodies for bio. threats

- Sensitive optical transduction technique - part in $10^7$ refractive index changes can be measured
Integration of chem/bio.

- Common optical and data processing platform
- Different front ends for chem. and bio. samples
- Isolated channels for chem. and bio. sensing layers

Top View of SPR substrate
Sensor Components:
TI Spreeta 2000 (3-channel)

- Spreeta 2000 SPR components developed in collaboration with UW
- Miniaturized, robust, high performance devices
- Inexpensive: ~$4 in large quantity
- Excellent manufacturing capabilities and quality control.

Spreeta 2000 SPR sensing chip
<table>
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<tr>
<th>Type of Agent</th>
<th>Examples of Agents of Interest</th>
<th>Current Direct Detection level</th>
<th>Amplification/ verification</th>
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<tr>
<td>Protein toxins</td>
<td>SEB, Ricin, Botulinum toxin, <em>B. Anthracis</em>, <em>B. Anthracis</em> spores (simulant)</td>
<td>100 pM (2.8 ppb) 20 nM (64 ppb; current level) &lt;50 nM (750 ppb, current level)</td>
<td>Yes (2.8 ppt) Not yet done Yes</td>
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<td>Spores</td>
<td>Small pox, Marburg, Ebola, Encephalitis, Hemorrhagic fever Flu (as a model system)</td>
<td>~10⁵ cfu/ml (prelim) ~10⁹ pfu (prelim expts)</td>
<td>Not yet done Yes (prelim)</td>
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<td>Viruses</td>
<td><em>Y. pestis</em>, <em>F. tularensis</em>, <em>E. Coli</em></td>
<td>In progress ~5x10⁴ cfu/ml (prelim) In progress</td>
<td>Yes Not yet done Yes</td>
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<td>Microbial cells</td>
<td><em>VX</em>, Soman, Sarin, tabun, <em>DPMP</em> (stimulant) Domoate, Cortisol, DNP</td>
<td>Antibodies tested at ECBC 50 nM (15 ppb) 750 pM (271 ppt) ~1 uM (340 ppb)</td>
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<td>CW (organics)</td>
<td><em>VX</em>, Soman, Sarin, tabun, <em>DPMP</em> (stimulant) Domoate, Cortisol, DNP</td>
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*C. Furlong et al, U. Washington*
Cavitand Selectivity


- Cavitand with deepest cavity shows largest response
- Selectivity for aromatic vapors confirmed
- PECH and PIB polymers for comparison

A B C

3.3 Å 4.6 Å 8.3 Å
MeCav PzCav QxCav

TOLUENE

100 ppm

Sensing Layer

Copolymers

BENZENE

100 ppm

Sensing layers
Concentration Dependence of Signal

- Saturation due to analyte “filling” of cavitands
- QxCav completely encloses benzene molecule within cavity
Materials Processing: Spin Coated vs Self-Assembled Cavitands

Devanand K. Shenoy, Elias B. Feresenbet, Roberta Pinalli, Enrico Dalcanale; Langmuir 2003, 19, 10454

- Cavitand Morphology does not effect signal response
- Signal response normalized for thickness
Cavitands for DMMP

- Cavitand shows high selectivity for DMMP
- Cavitand shows good reversibility
Liquid Crystal-based Sensing

- Liquid Crystal (LC) perturbation causes **signal amplification**
- **Nematic order** probed by optical method
- Optical signal directly proportional to amount of vapor
LC film deposition

One side
Adhesive
Mylar

punch

5mm
100um

Liquid crystal molecule

Liquid crystal Drop (1ul)

Polyimide coated on gold

Cover Glass
LC alignment

A uniform planar orientation achieved
LC exposure to benzene vapors

E. Feresenbet, F. Taylor, T. M. Chinowsky, S. S. Yee, D.K Shenoy, 
Sensor Letters 2, 145-152, 2004

- Wavelength shift due to vapor exposure and resulting decrease in LC order
- Shift is reversible
Selectivity pattern of LC towards vapors

- LC shows differential response to chemical vapors
- Selectivity between isomers observed
LC exposure closer to phase transition

- Sensivity enhanced by two orders closer to phase transition
- Shift is reversible

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<tr>
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<tr>
<td>1</td>
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<tr>
<td>2</td>
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<td>3</td>
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Kinetics of Response

\[ \frac{d\lambda}{dt} = k\lambda^2 \]
\[ \lambda = \text{resonance wavelength} \ (\text{nm}) \]
\[ t = \text{time} \ (\text{min}) \]
\[ k = \text{rate constant} \]

- Second order kinetics describes data for vapor diffusion into LC
- Time response additional selectivity parameter

**SPR Biosensing**

- Neutravidin-Biotin mediated antibody immobilization

- Effect of blocking binding to thiol-biotin coated gold surface

- NeutrAvidin blocked by biotin binds poorly to the thiol biotin surface

- Effect on binding of biotinylated antibody

- Subsequent binding of the biotinylated antibody is also poor
Re-cycling the sensor surface

Neutravidin binding to different sensor surfaces

- Neutravidin binds as well after cleaning of the gold surface and re-application of the thiol biotin layer
SPR model immunoassay

Bt-Rabbit anti-Goat IgG binding Goat-IgG

Amplifier Donkey anti-Goat IgG binding

**SPR Shift (nm)**

**Time (min)**

- 0.0
- 0.5
- 1.0
- 1.5
- 2.0
- 2.5
- 3.0
- 3.5
- 4.0
- 4.5
- 5.0
- 5.5
- 6.0

**SPR Shift (nm)**

**Time (min)**

- 0.0
- 0.5
- 1.0
- 1.5
- 2.0
- 2.5
- 3.0
- 3.5
- 4.0
- 4.5
- 5.0
- 5.5
- 6.0

**Concentrations:**
- 0.01
- 0.1
- 1.0
- 10 ug/ml
Staphylococcal Enterotoxin B detection by SPR

SEB Direct Detection

Amplified Detection
Ricin detection by SPR

**Direct Detection of Ricin**

**Ab-Amplified Detection of Ricin**
Bacterial spore detection by SPR

**Direct detection of B. globigii**

- **SPR shift (nm)**
- **Time (min)**
- **0 spores/ml**
- **10^5 spores/ml**
- **10^6 spores/ml**
- **10^7 spores/ml**

**Ab-Amplified Detection of B. globigii**

- **SPR shift (nm)**
- **Time (min)**
- **0 spores/ml**
- **10^5 spores/ml**
- **10^6 spores/ml**
- **10^7 spores/ml**
Conclusions

- Cavitands show high selectivity for chemical vapors
- Cavitand film morphology does not affect signal response
- Liquid Crystal materials for sensitivity amplification
- Label-free, real-time SPR biosensing
- Integration of chem. and bio. approaches appears feasible
Team Members

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Funding: Naval Research Laboratory, Joint Services