

Cavitands, Liquid Crystals and Antibodies: Materials for Integrated Chem/Bio. Sensing

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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

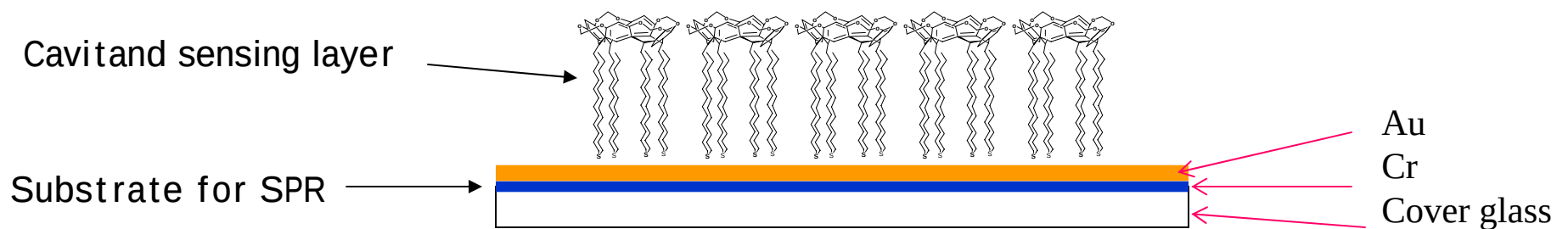
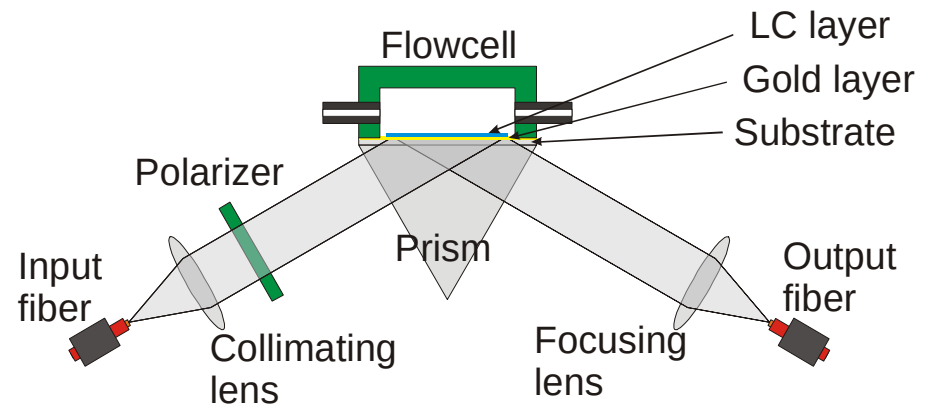


Approach

Technique - SPR spectroscopy

Sensing with - Cavitanths 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.

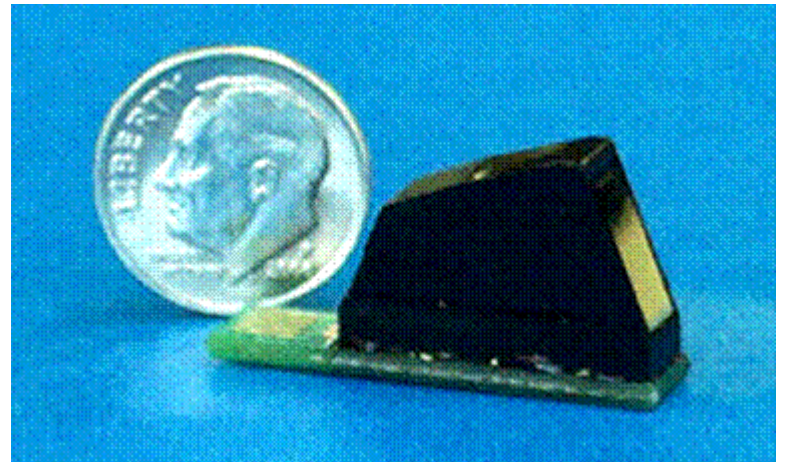
- Common optical and data processing platform
- Different front ends for chem. and bio. samples
- Isolated channels for chem. and bio. sensing layers



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

CBW Agents Detectable with SPR

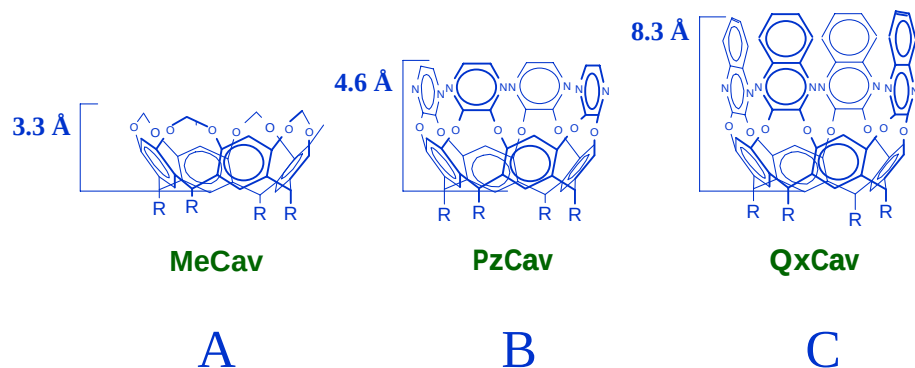
Type of Agent	Examples of Agents of Interest	Current Direct Detection level	Amplification/ verification
Protein toxins	SEB Ricin Botulinum toxin,	100 pM (2.8 ppb) 20 nM (64 ppb; current level) <50 nM (750 ppb, current level)	Yes (2.8ppt) Not yet done Yes
Spores	<i>B. Anthracis</i> , BG spores (<i>simulant</i>)	~10 ⁵ cfu/ml (prelim)	Not yet done
Viruses	Small pox, Marburg, Ebola, Encephalitis, Hemorrhagic fever Flu (as a model system)	~10 ⁹ pfu (prelim expts)	Yes (prelim)
Microbial cells	<i>Y. pestis</i> <i>F. tularensis</i> <i>E. Coli</i>	In progress ~5x10 ⁴ cfu/ml (prelim) In progress	Yes Not yet done Yes
CW (organics)	VX Soman Sarin tabun DPMP(<i>stimulant</i>) Domoate Cortisol DNP	Antibodies tested at ECBC 50 nM (15 ppb) 750 pM (271 ppt) ~1 uM (340 ppb)	

C. Furlong et al, U. Washington

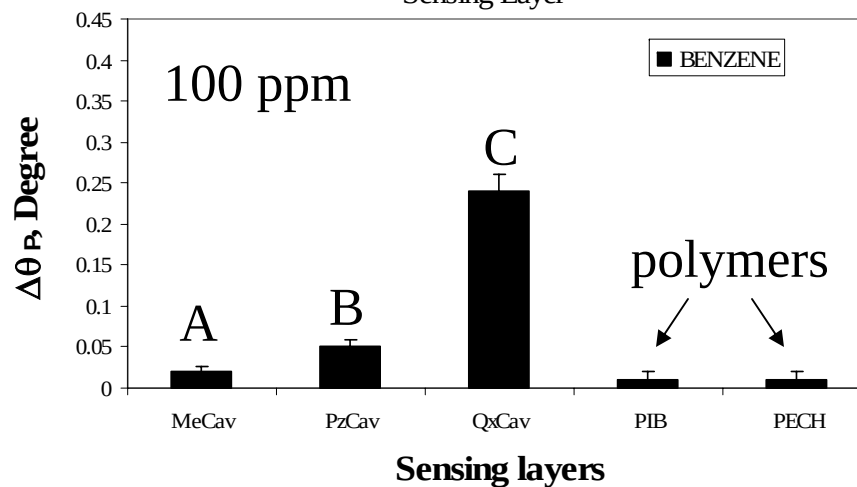
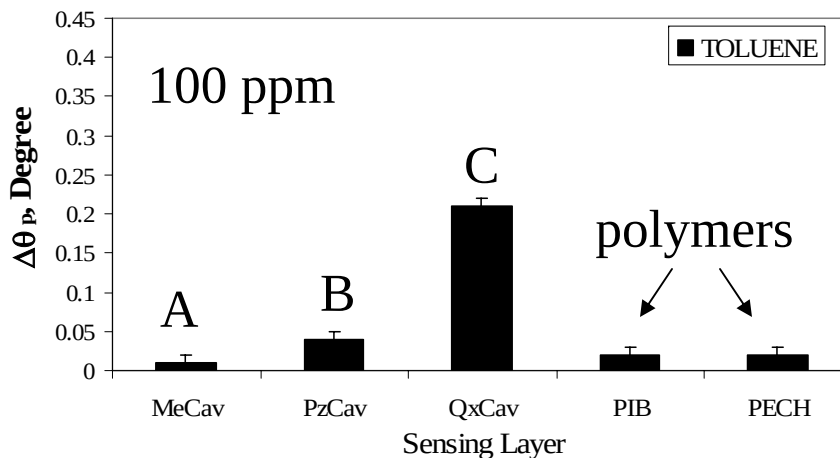


Cavitand Selectivity

Feresenbet, E.; Dalcanalet, E.; Dulcey, C.; Shenoy, D.K.,
Sensors and Actuators B, 97, 211 (2003)

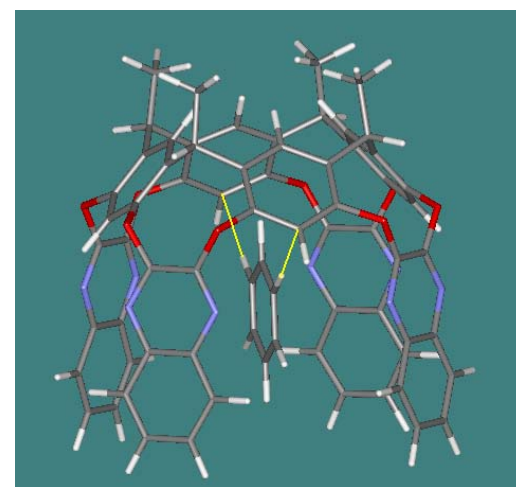
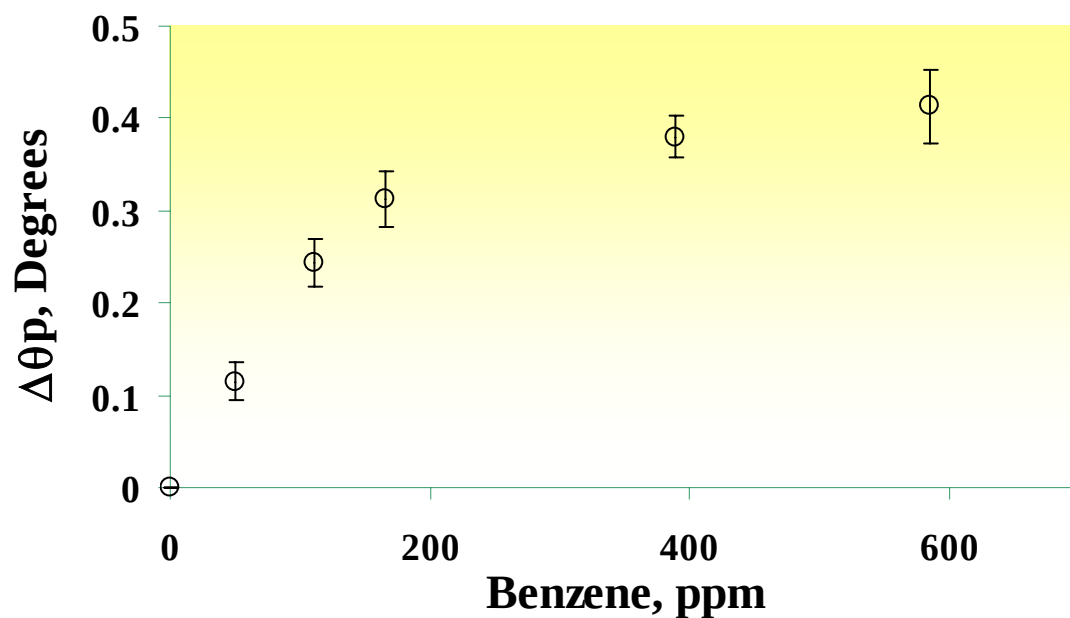


- Cavitand with deepest cavity shows largest response
- Selectivity for aromatic vapors confirmed
- PECH and PIB polymers for comparison





Concentration Dependence of Signal



QxCav-Benzene complex

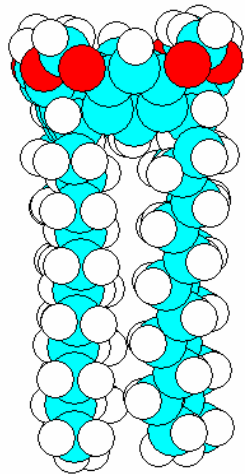
- Saturation due to analyte “filling” of cavitands
- QxCav completely encloses benzene molecule within cavity





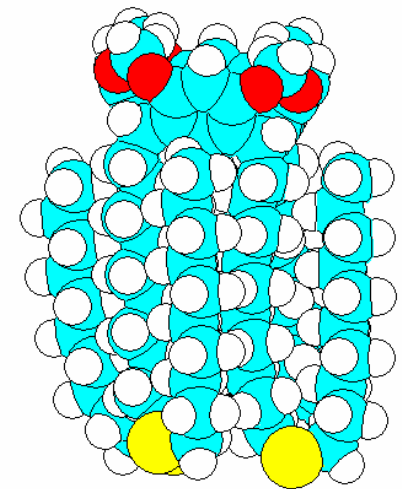
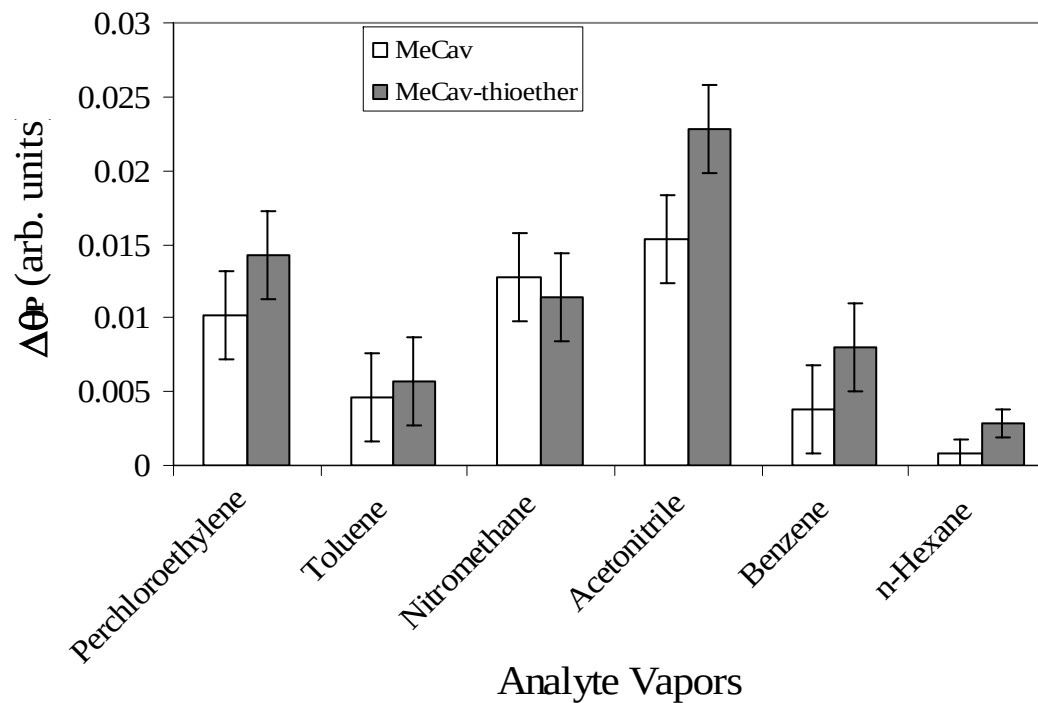
Materials Processing: Spin Coated vs Self-Assembled Cavitands

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



MeCav

Spin coated



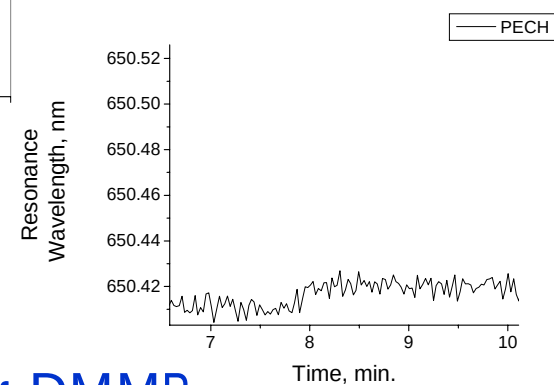
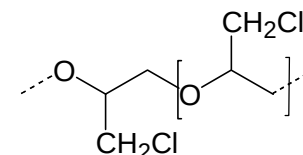
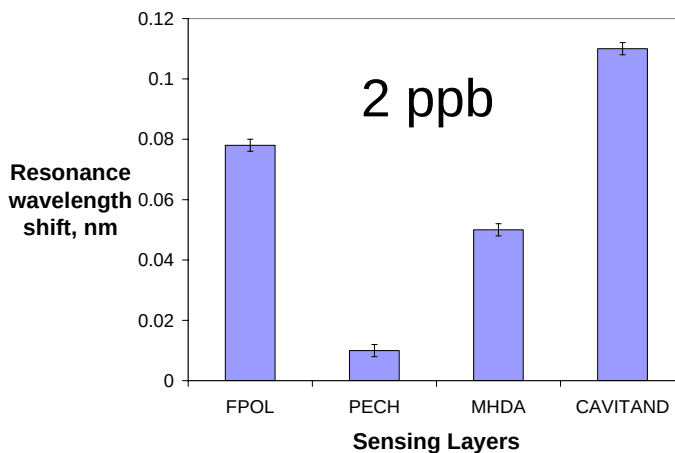
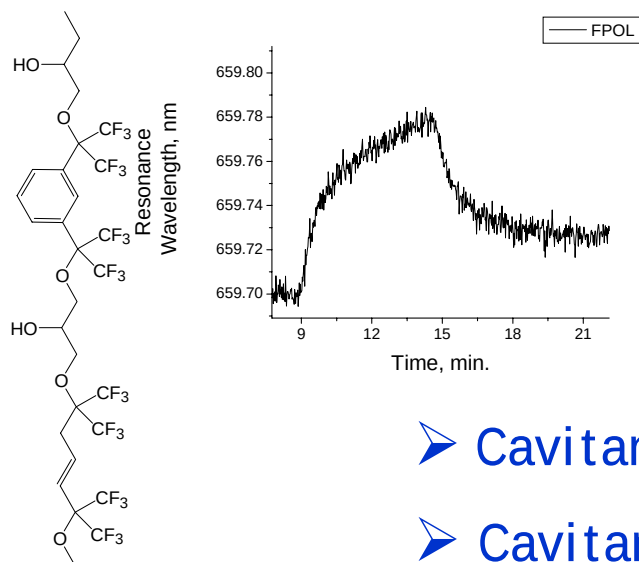
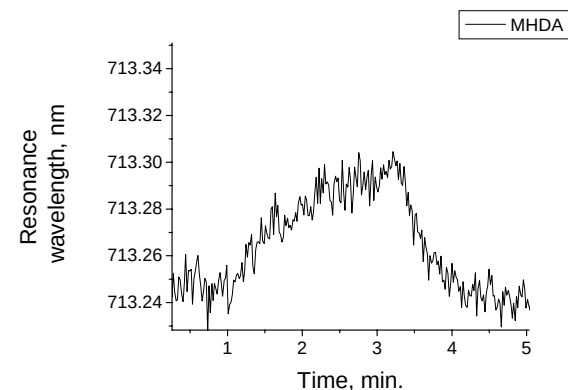
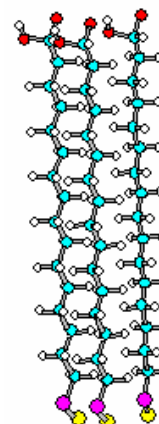
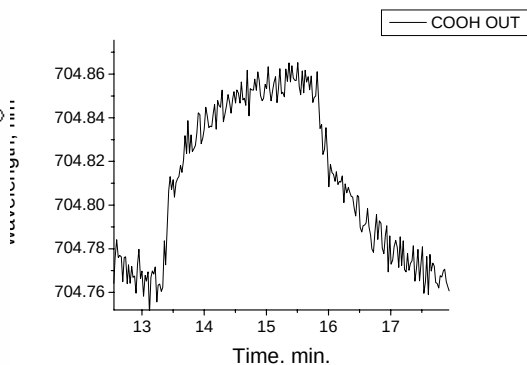
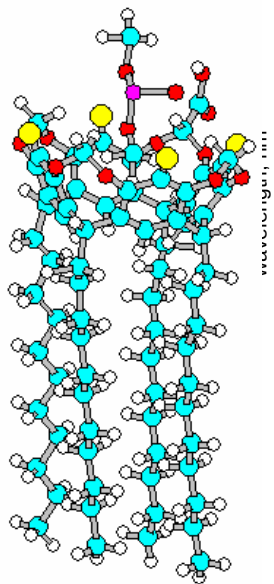
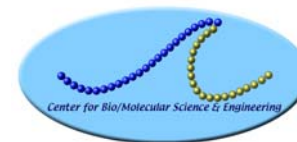
MeCav-thioether

Self-assembled

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



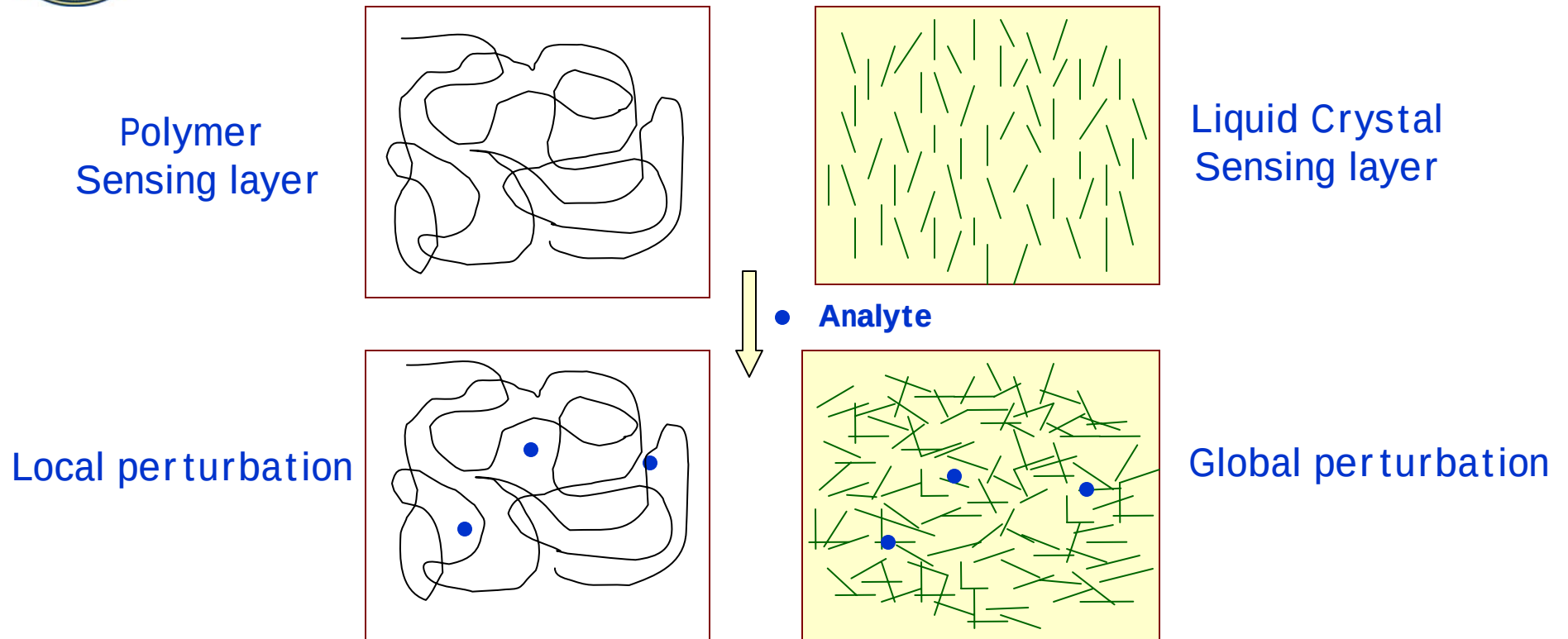
Cavittands for DMMP



- Cavitand shows high selectivity for DMMP
- Cavitand shows good reversibility



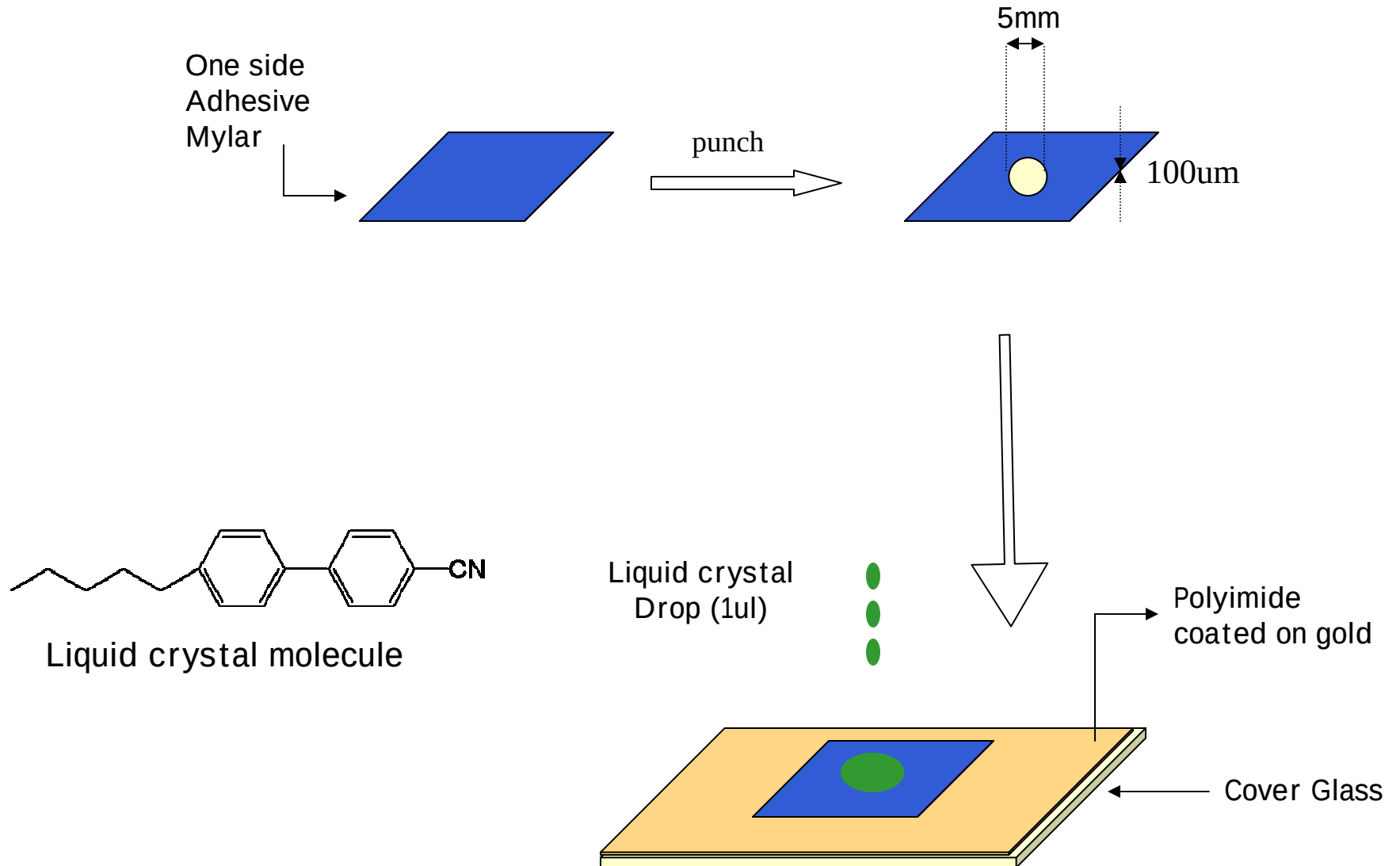
Liquid Crystal-based Sensing



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

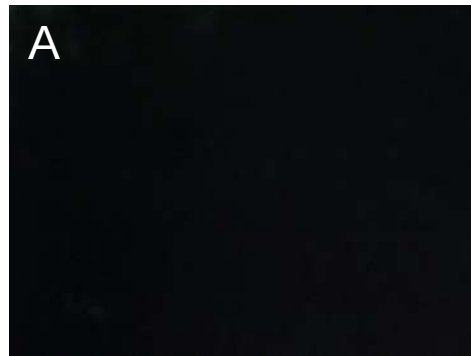
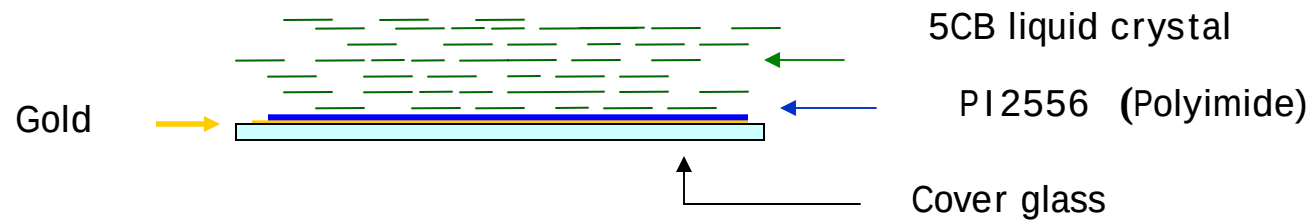


LC film deposition





LC alignment

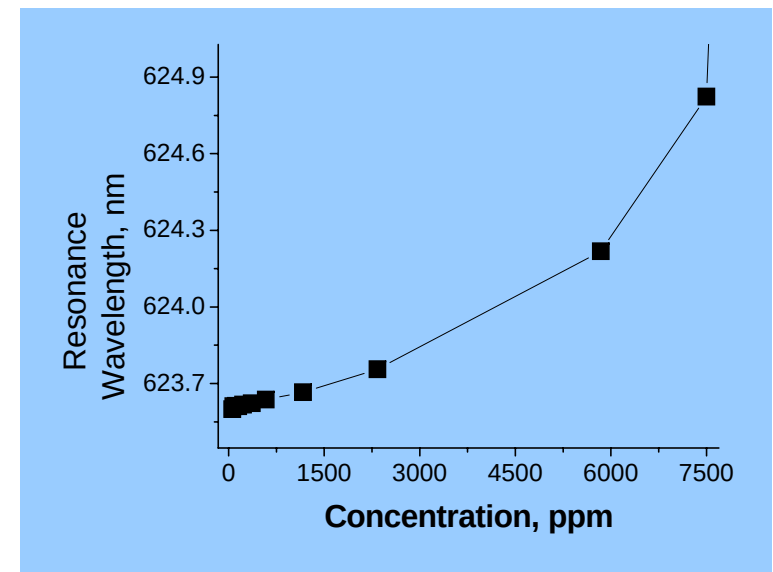
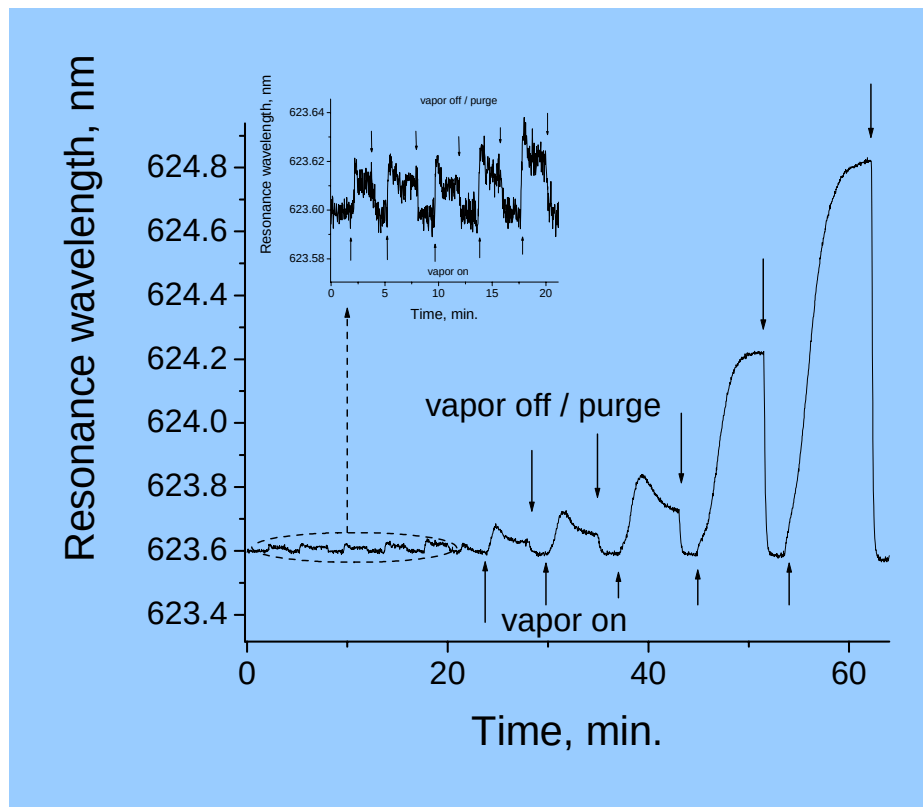


- A uniform planar orientation achieved



LC exposure to benzene vapors

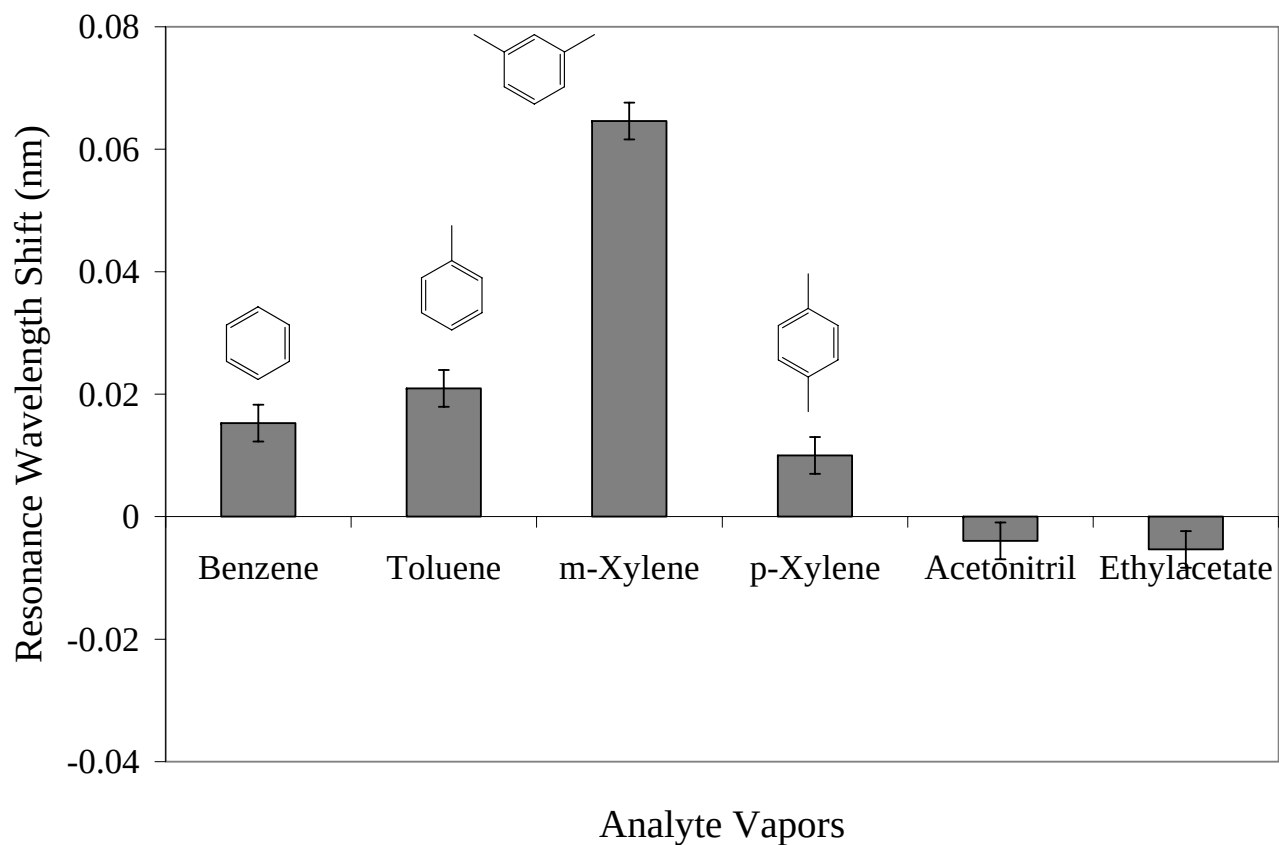
*E. Feresenbet, F. Taylor, T. M. Chinowsky, S. S. Yee, D.K Shenoy,
Sensor Letters 2, 145-152, 2004*



- Wavelength shift due to vapor exposure and resulting decrease in LC order
- Shift is reversible



Selectivity pattern of LC towards vapors

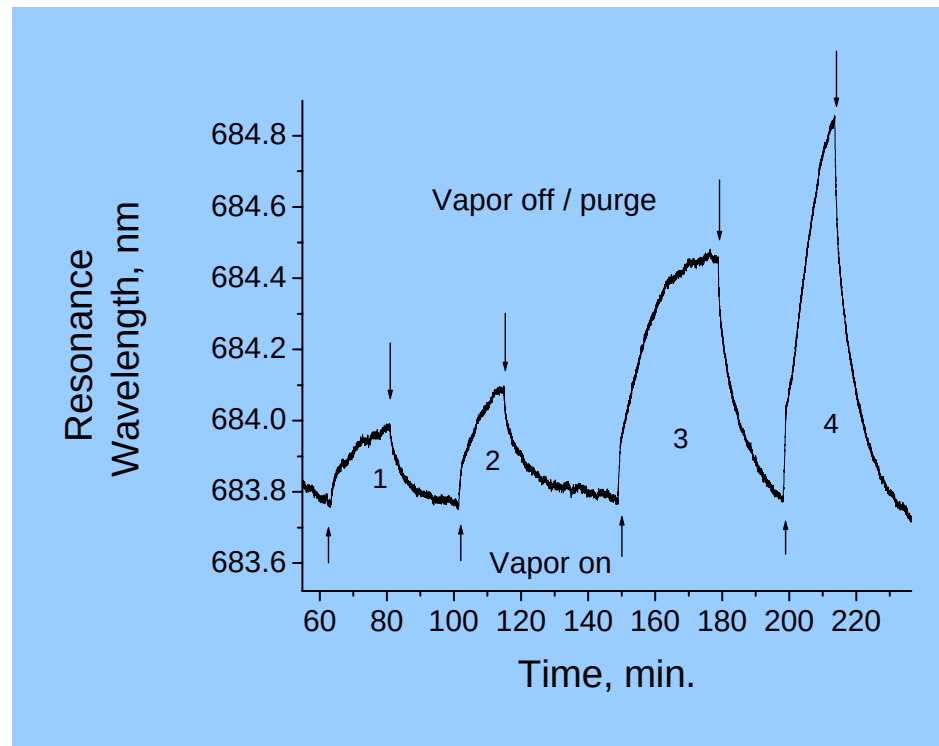


- LC shows differential response to chemical vapors
- Selectivity between isomers observed



LC exposure closer to phase transition

nematic $\longleftrightarrow$ isotropic
35.1°C



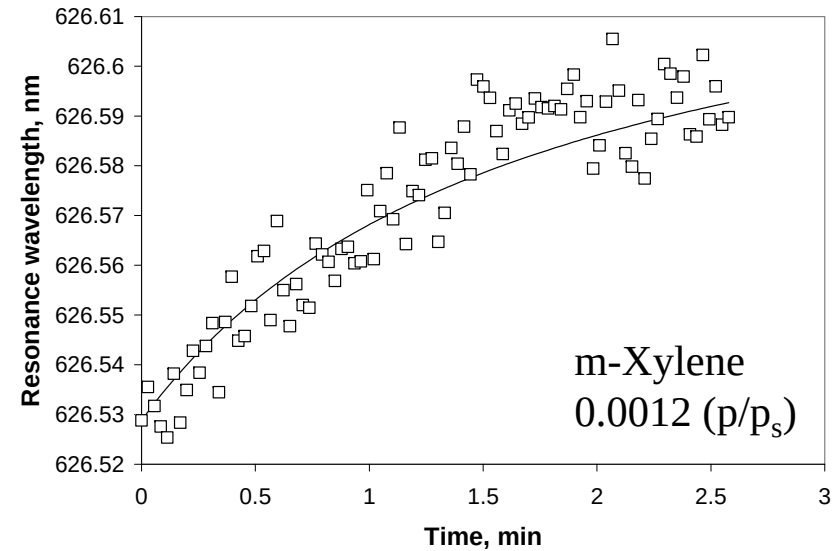
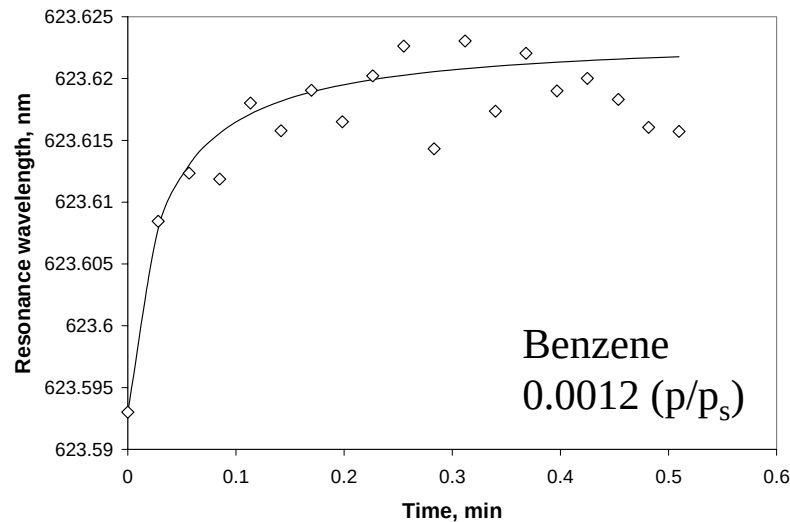
m-xylene

	ppb
1	270
2	380
3	680
4	850

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



Kinetics of Response



$$d\lambda/dt = k\lambda^2$$

λ = resonance wavelength (nm)

t = time (min)

k = rate constant

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

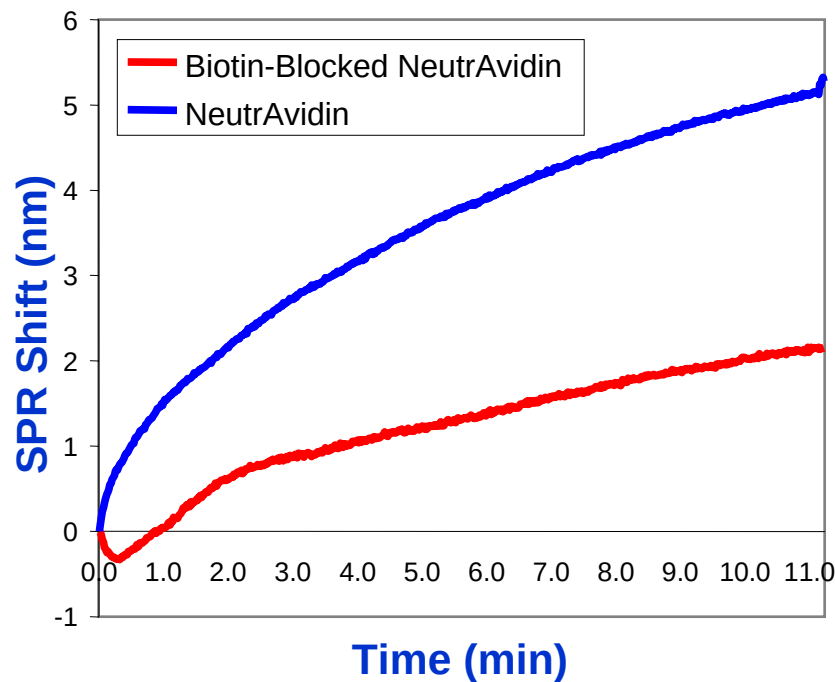
E. B. Feresenbet, F. Taylor, T. M. Chinowsky, S. S. Yee, and D. K. Shenoy, *Sensor Letters*, 2, 145-152, 2004



SPR Biosensing

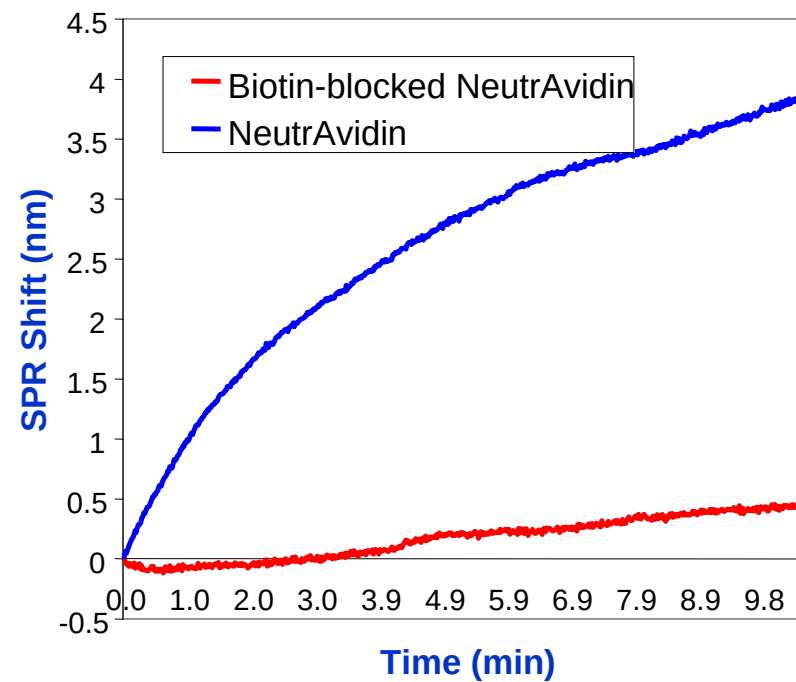
- Neutravidin-Biotin mediated antibody immobilization

➤ 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

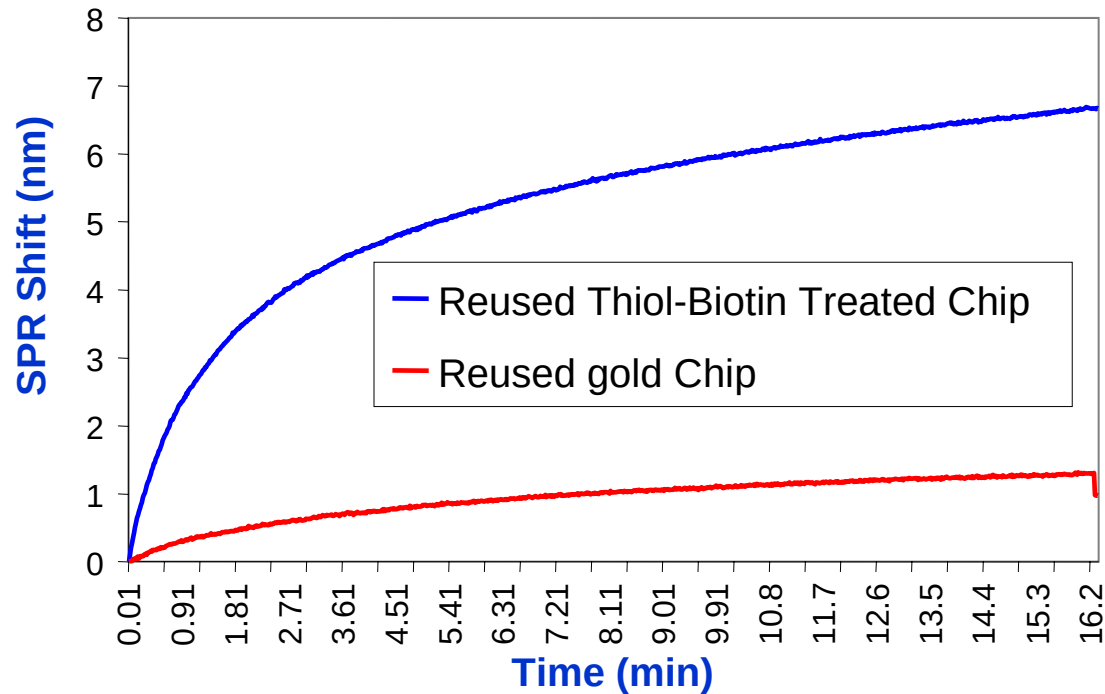


➤ Subsequent binding of the biotinylated antibody is also poor



Re-cycling the sensor surface

➤ Neutravidin binding to different sensor surfaces

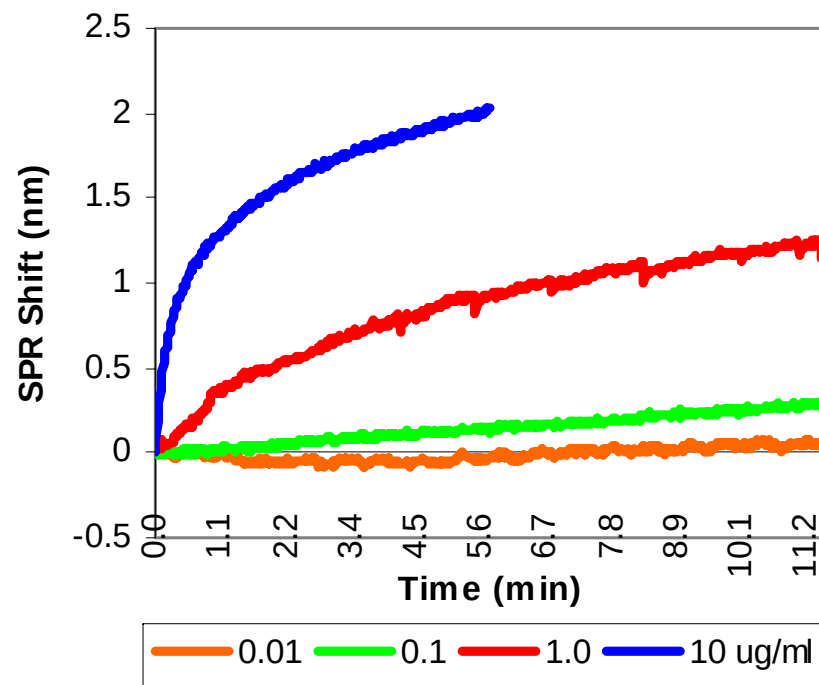


➤ Neutravidin binds as well after cleaning of the gold surface and re-application of the thiol biotin layer

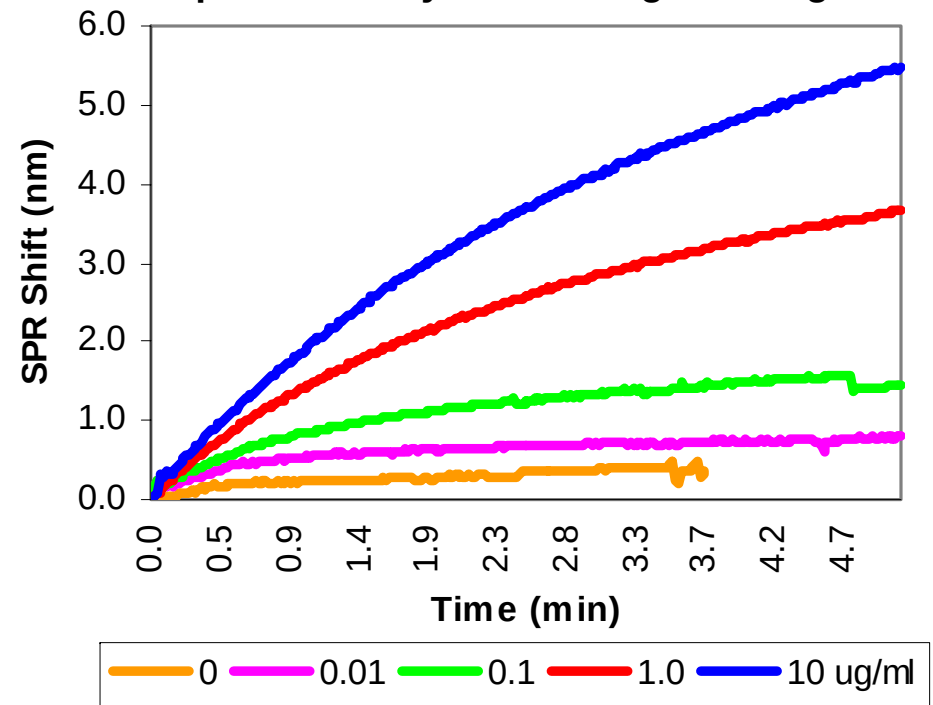


SPR model immunoassay

Bt-Rabbit anti-Goat IgG binding Goat-IgG



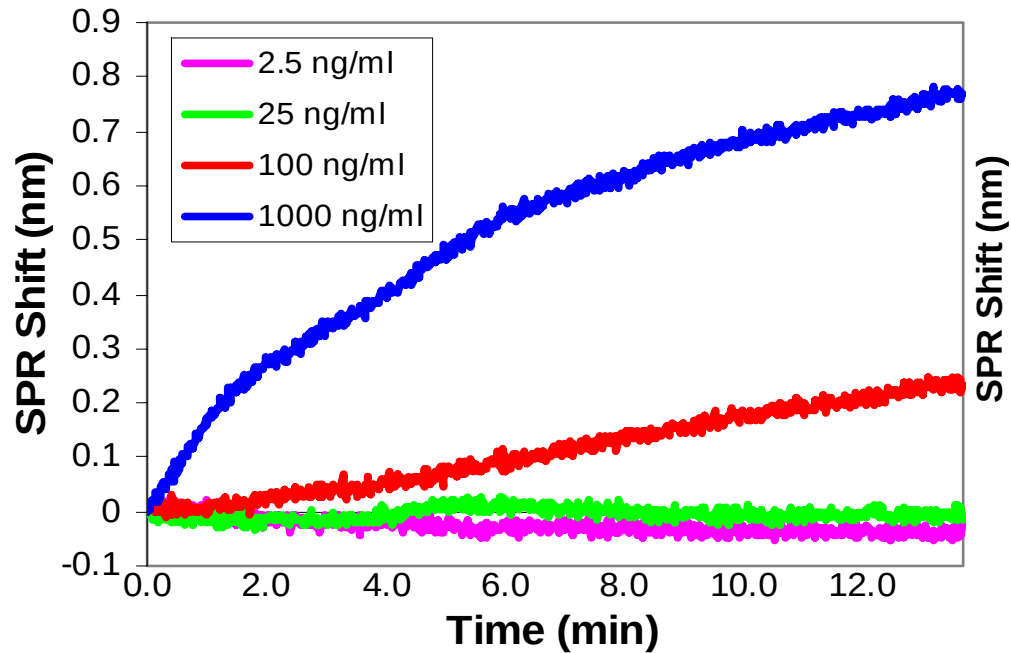
Amplifier Donkey anti-Goat IgG binding



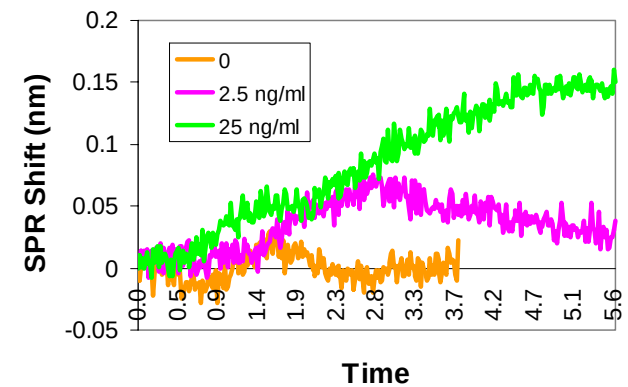
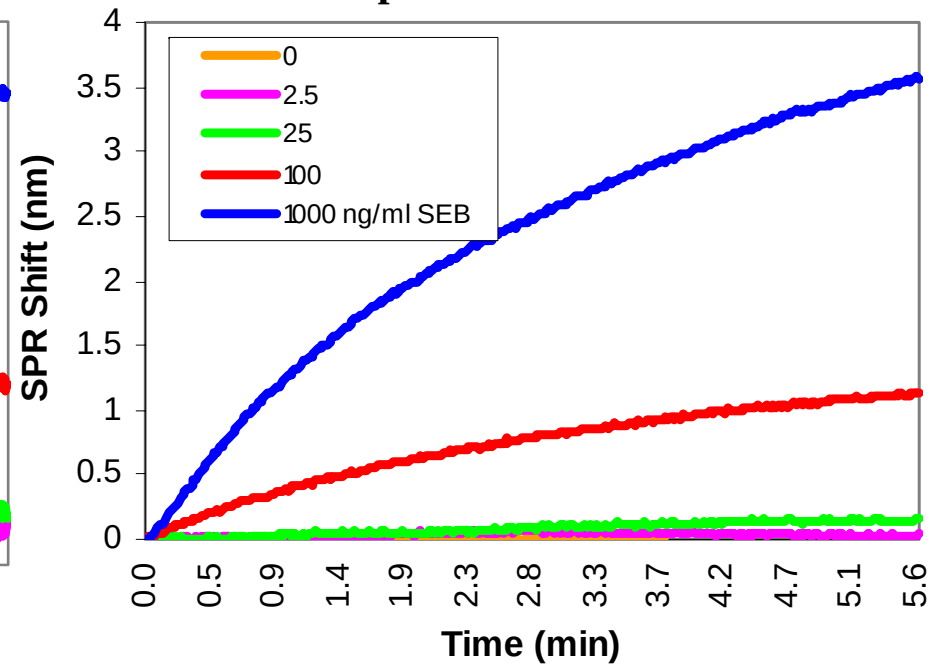


Staphylococcal Enterotoxin B detection by SPR

SEB Direct Detection



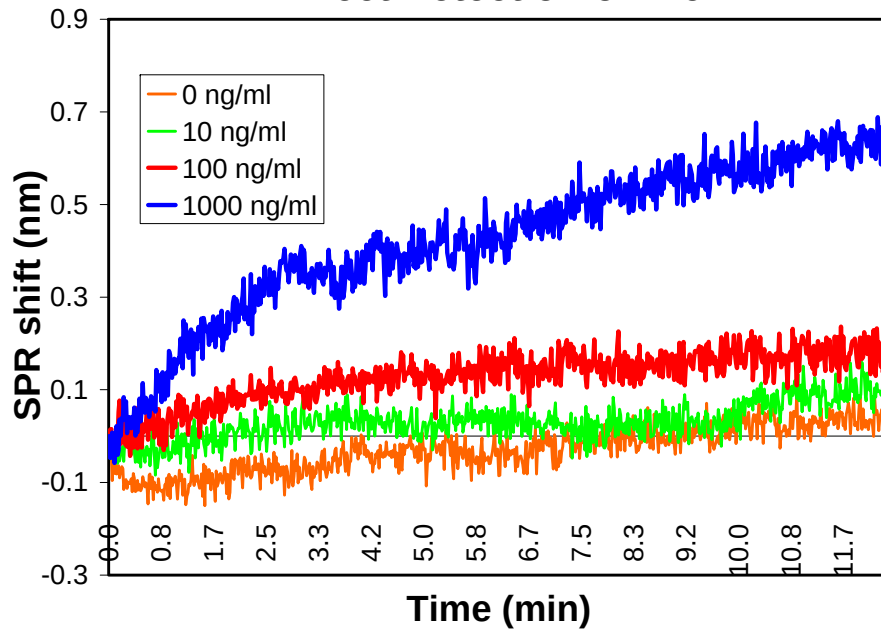
Amplified Detection



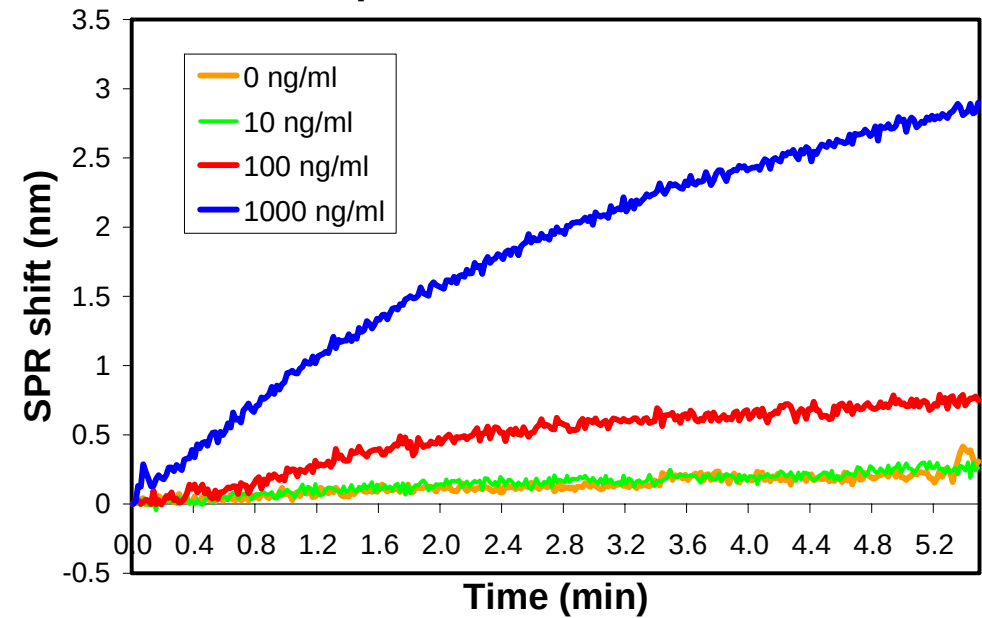


Ricin detection by SPR

Direct Detection of Ricin



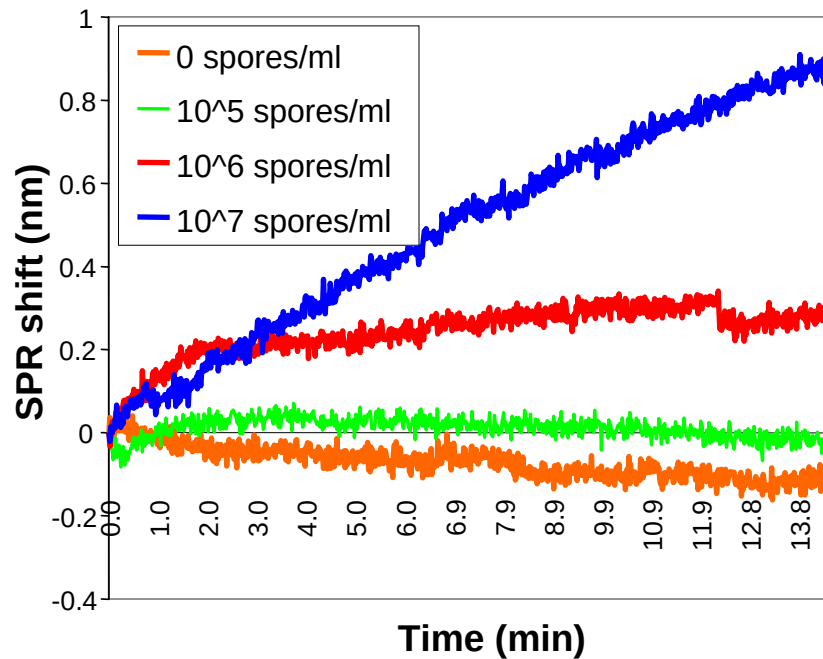
Ab-Amplified Detection of Ricin



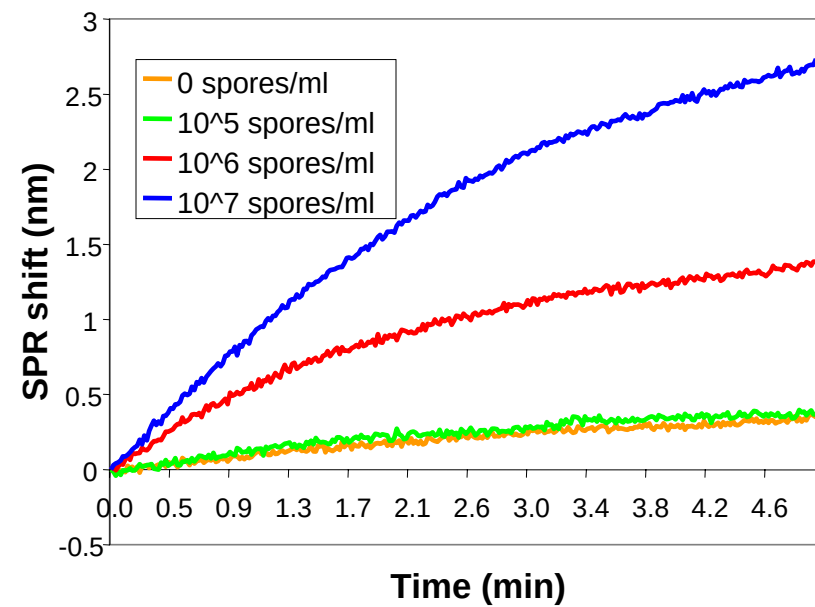


Bacterial spore detection by SPR

Direct detection of *B. globigii*



Ab-Amplified Detection of *B. globigii*





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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