Microfluidic Systems for Bioreporting, Separations, Vibrational Spectroscopy, & Microcantilever Sensing of EDCs

(EPA – 83274001)

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Background

→ Nanomechanical Bio-MEMS Sensing

→ SERS in Environmental Analysis

→ Electrophoretic Separations in Environmental Analysis

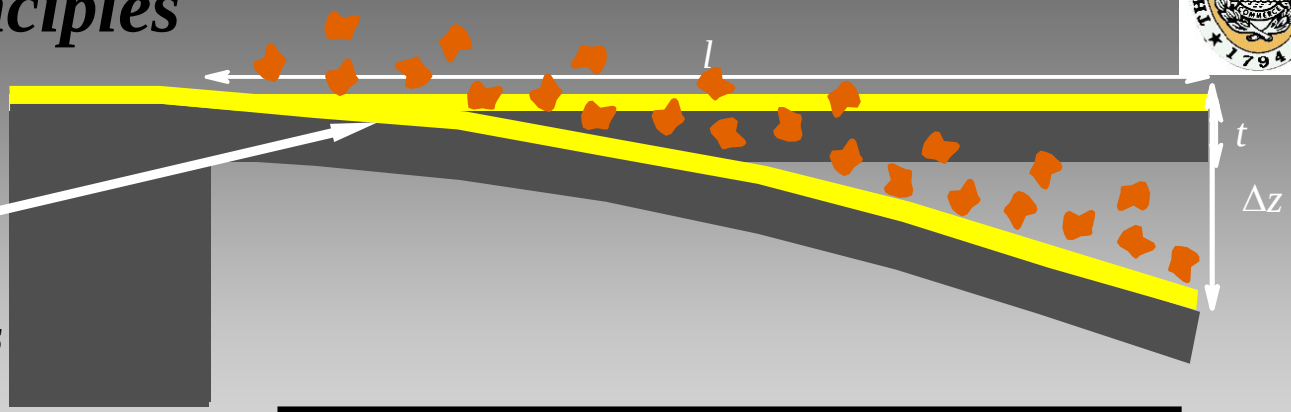
→ Concept of an Integrated Fluidic Platform for EDC Analysis

→ Future Work

→ Nanomechanical Bio-MEMS Sensing

Measurement principles

Nanostructuring the MC surface prior to MRP coating greatly enhances responses



Nanostructures

Cavitand receptors

Thin organic films

Biodfinity phases

MC-based measurements

$$\Delta z = 3l^2(1-\nu)\,\Delta\sigma / Yt^2$$

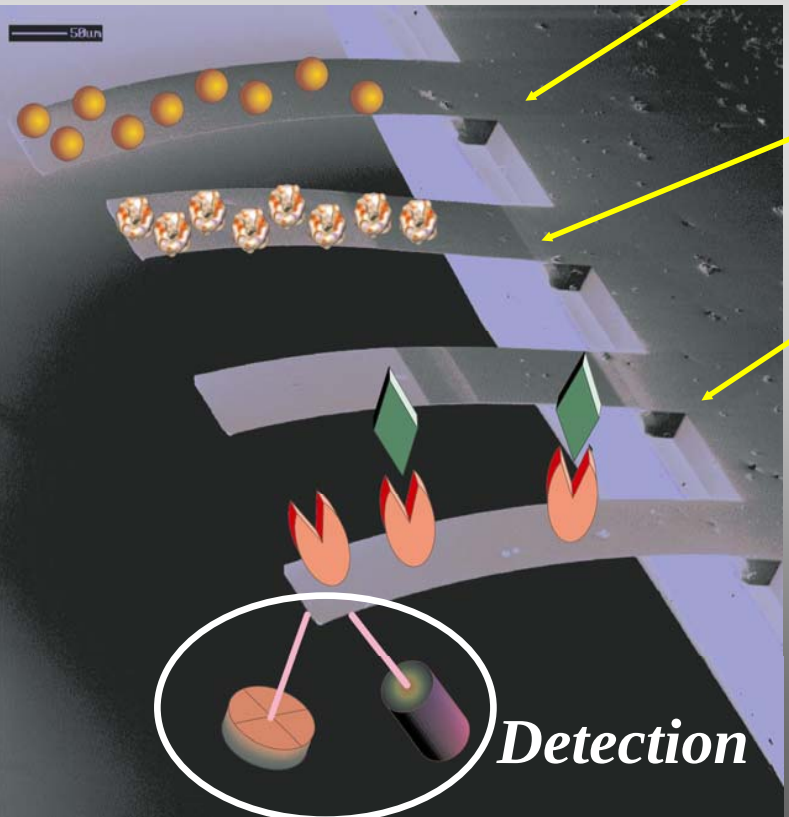
Δz – tip displacement

Δσ – differential stress change

l & t – length & thickness

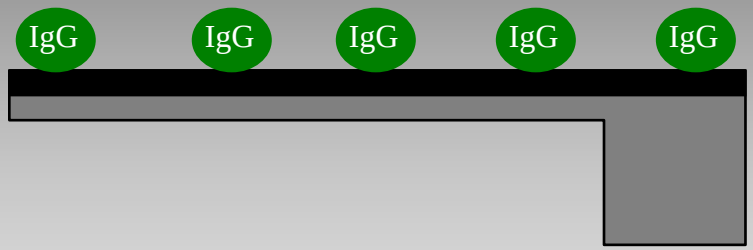
Y- Young's modulus

ν - Poisson's ratio

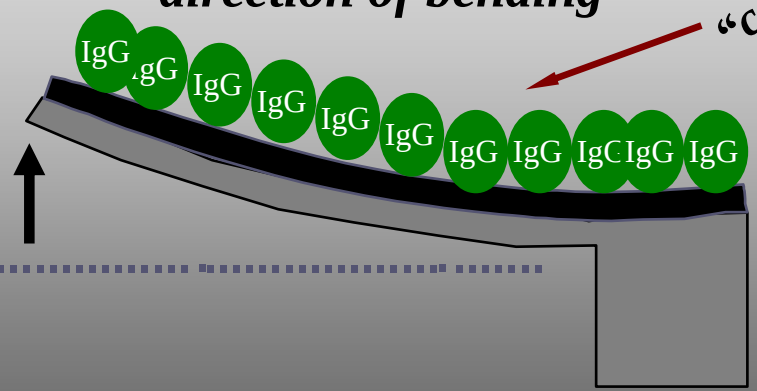


Analytes – Extremely versatile (metals, VOCs, proteins, drugs, pollutants, DNA, etc.)

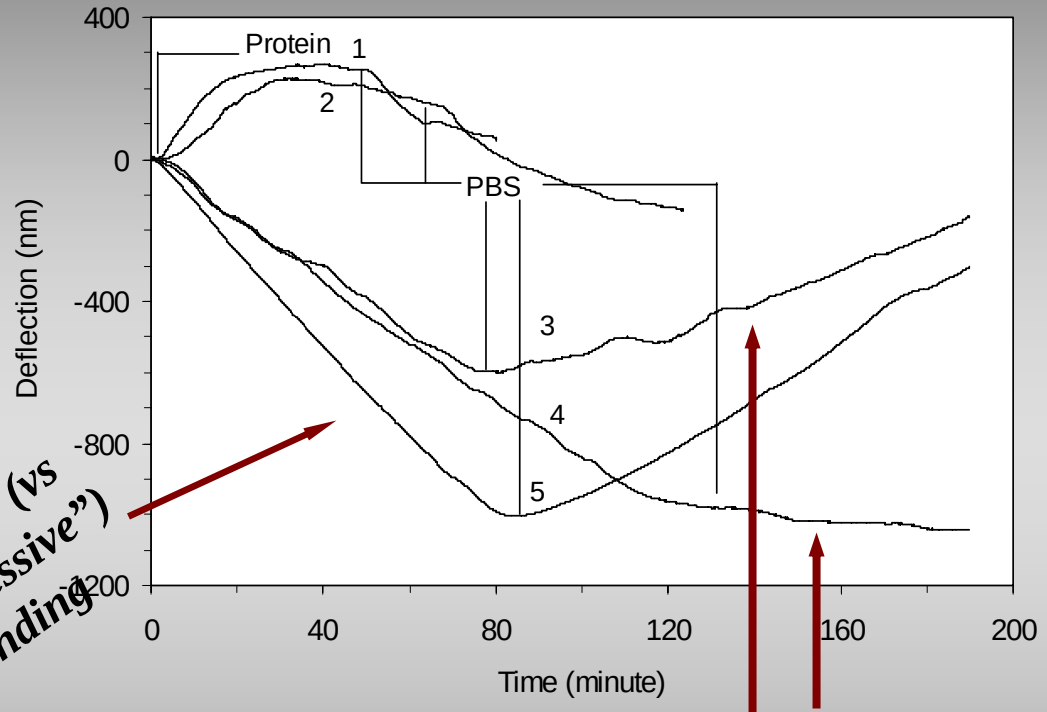
Non-specific and Specific Binding of Proteins (Toward Bioaffinity Approach to Selectivity in nanomechanical sensing of EDCs)



Following initial exposure (above), protein-protein & protein-surface interactions and charge effects can influence magnitude and direction of bending



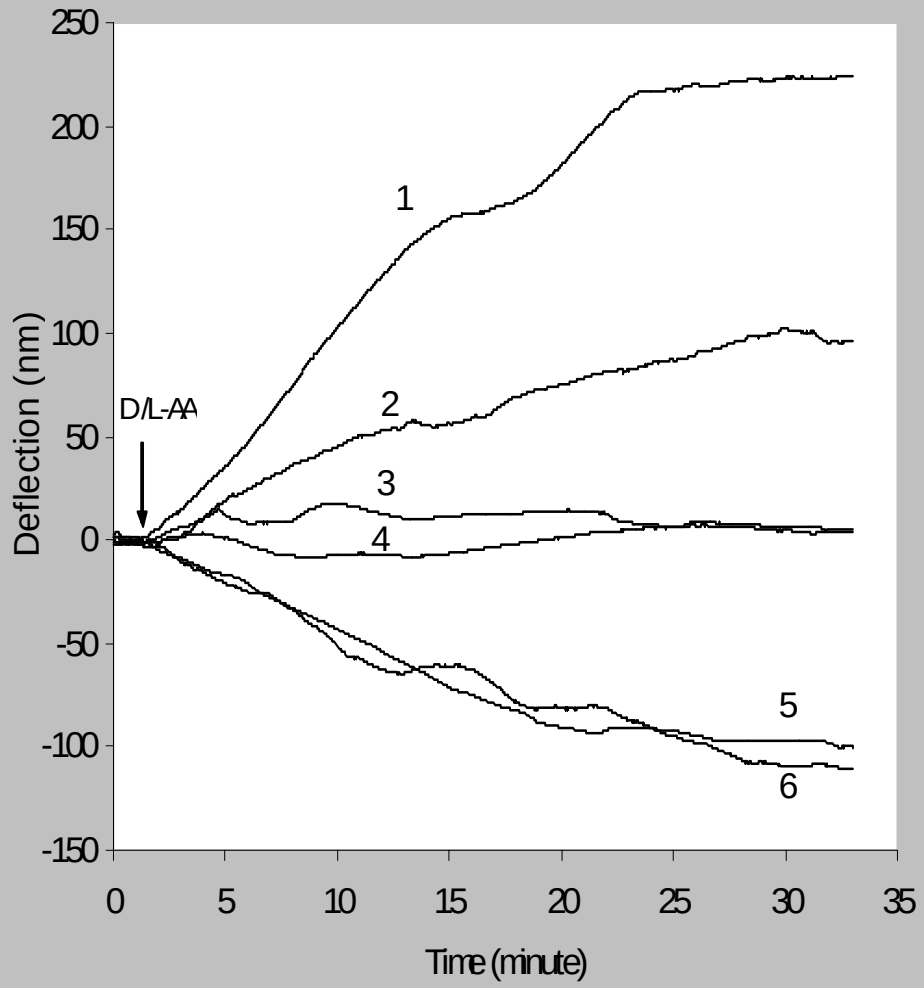
"tensile" (vs "compressive") bending



Stable receptors require anchoring; e.g. Glutaraldehyde or Protein A

2 – IgG on smooth surface
3 – IgG on nanostructured surface
4 – IgG on functionalized, nanostructured surface

Chiral Discrimination Using Ab-Mediated Cantilever Nanomechanics



1 – 50 mg/L L-tryptophan (anti-L-AA)

2 – 50 mg/L L-phenylalanine (anti-L-AA)

3 – 50 mg/L D-tryptophan (anti-L-AA)

4 – 50 mg/L D-phenylalanine (anti-L-AA)

5 – 50 mg/L L-phenylalanine (IgG)

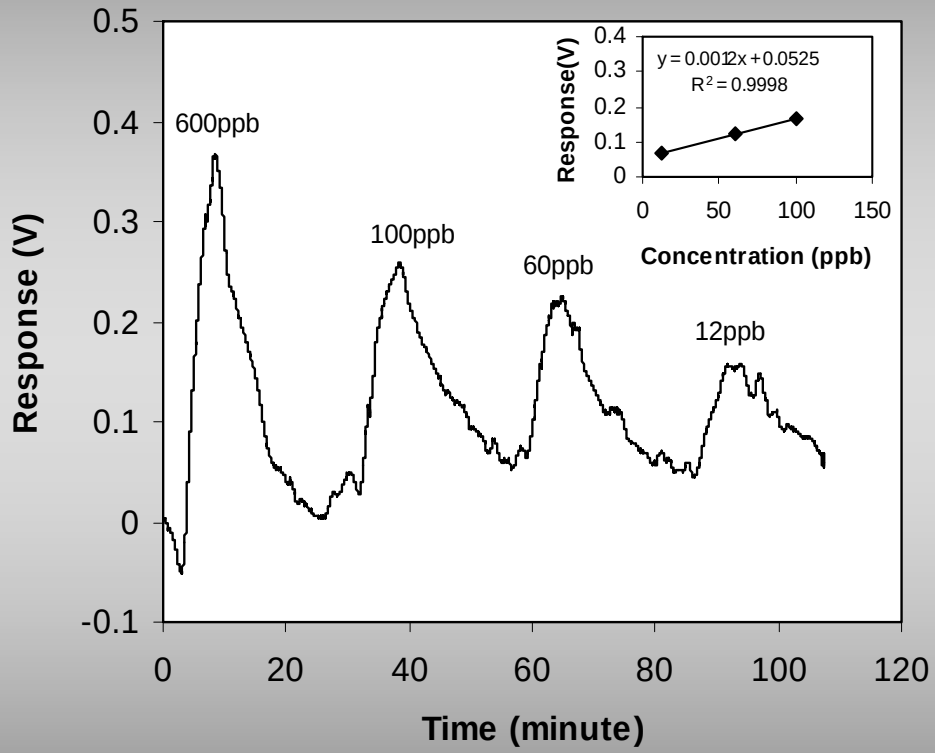
6 – 50 mg/L D-phenylalanine (IgG)

→ *3 & 4 no response with "wrong" Ab (exceptional selectivity)*

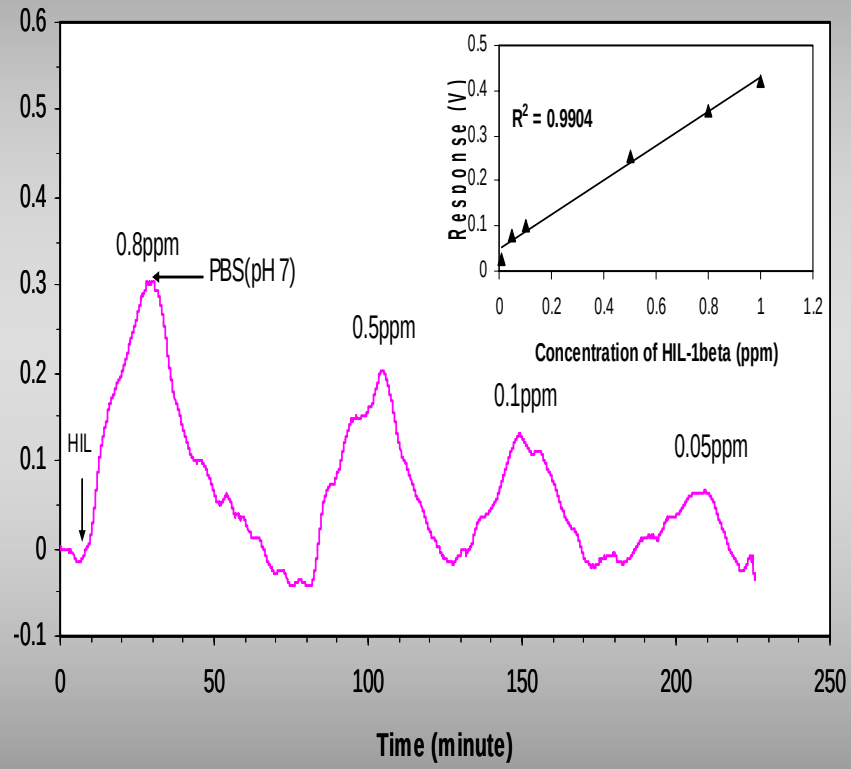
→ *Linear calibration and > 99% ee capability*

Calibration Using Antibody Functionalized MCs

Toxin A

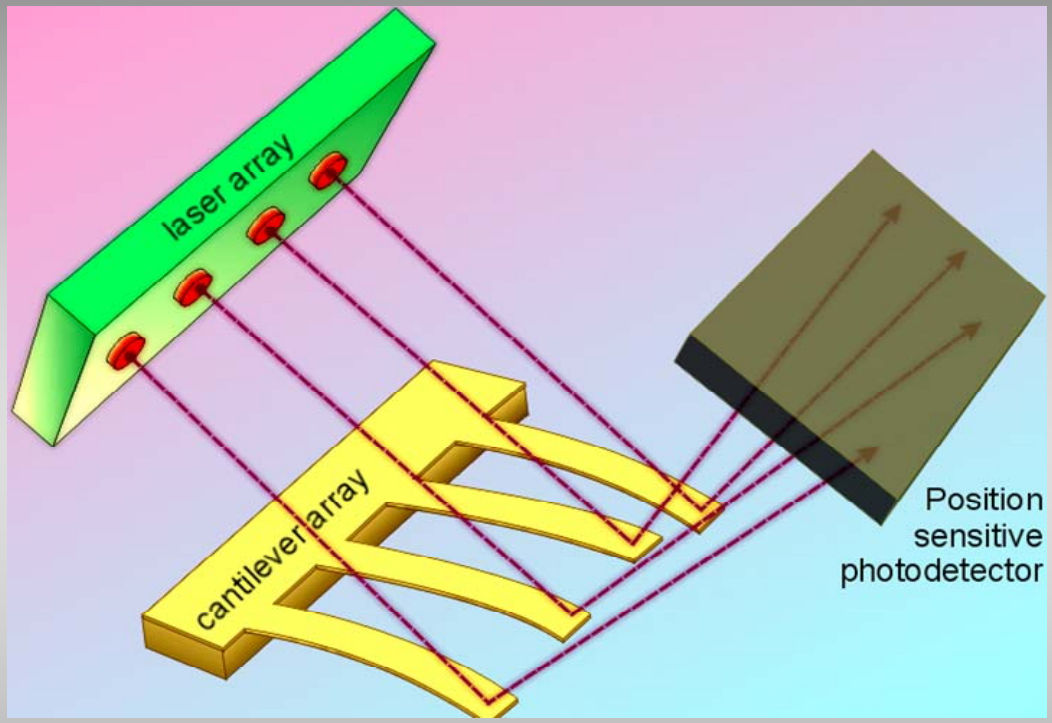


Cytokine

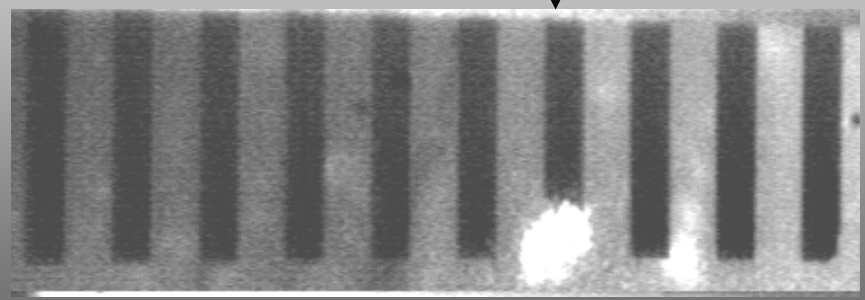


→ *Low ppb concentrations can be detected*

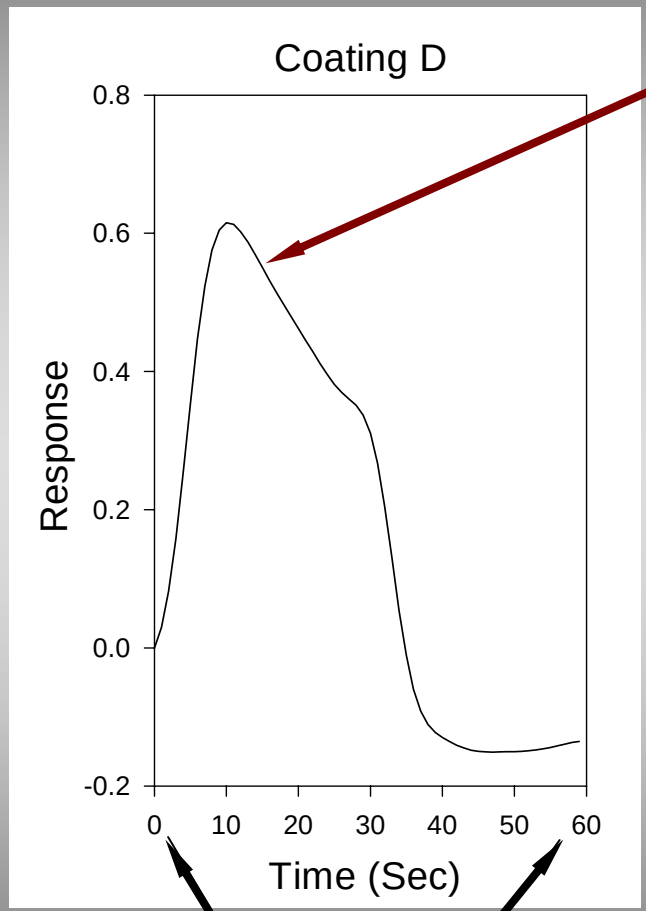
Distributed Selectivity Approach via Differentially Coated, VCSEL-Interrogated MCAs



Laser array pitch matches that of MC array; lasers – 850 nm, 5 mW typically scanned over MC array in less than one second



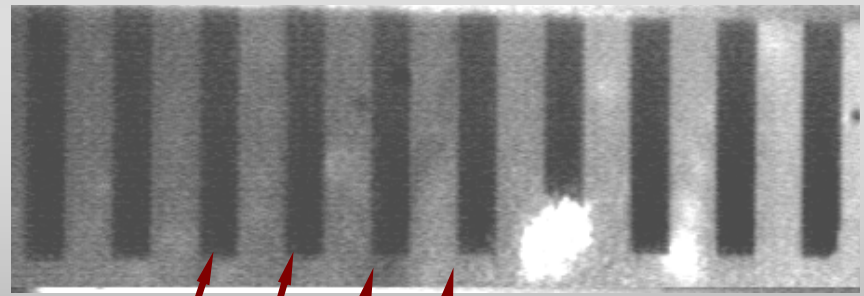
Distributed Selectivity Response Signatures (Signatures of EDCs should be possible)



Data @ one/sec

Both Equilibrium & kinetic phenomena influence shape following 30 sec injection

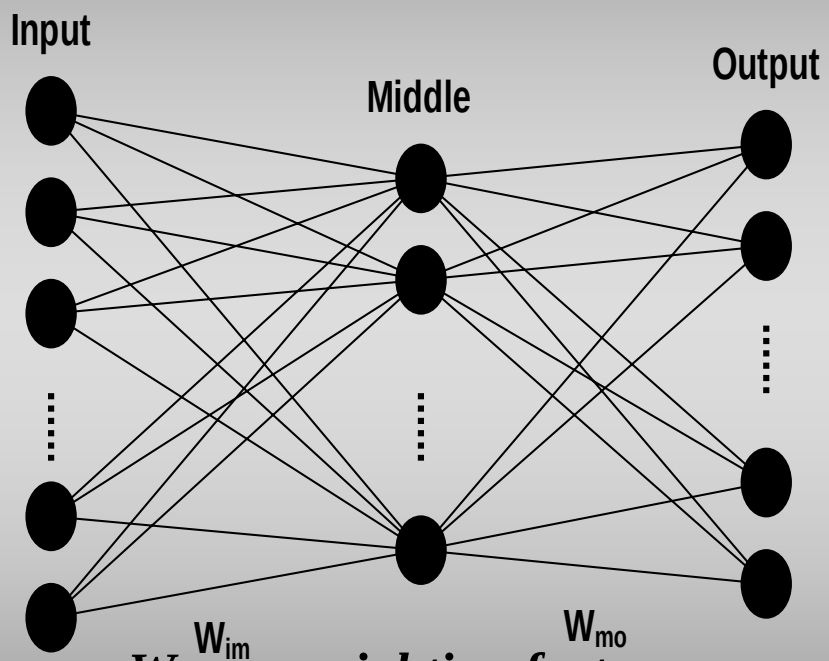
Each MC in the array produces a characteristic response that depends on the MRP & analyte



GC phase 1
Polymer phase 1
CD macrocycle
Calix macrocycle
Etc.

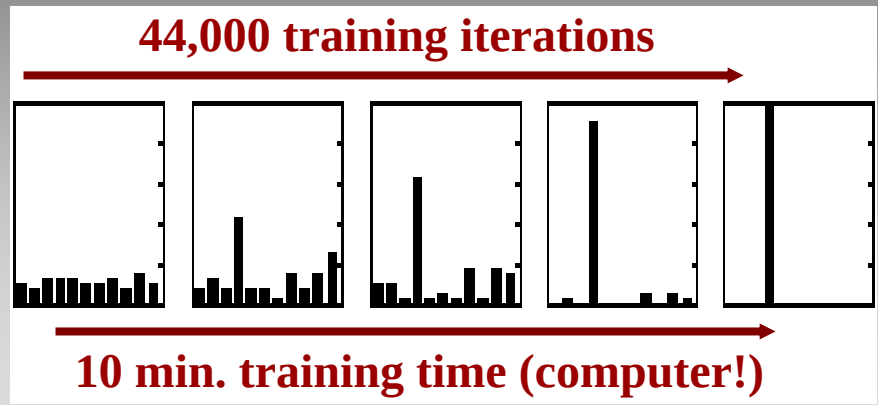
Distributed Selectivity & ANN Training & Performance

ANN 3-Layer Feed-Forward, Back-Propagation Diagram



Ws are weighting factors

ANN Training For Dioxane (4th analyte)



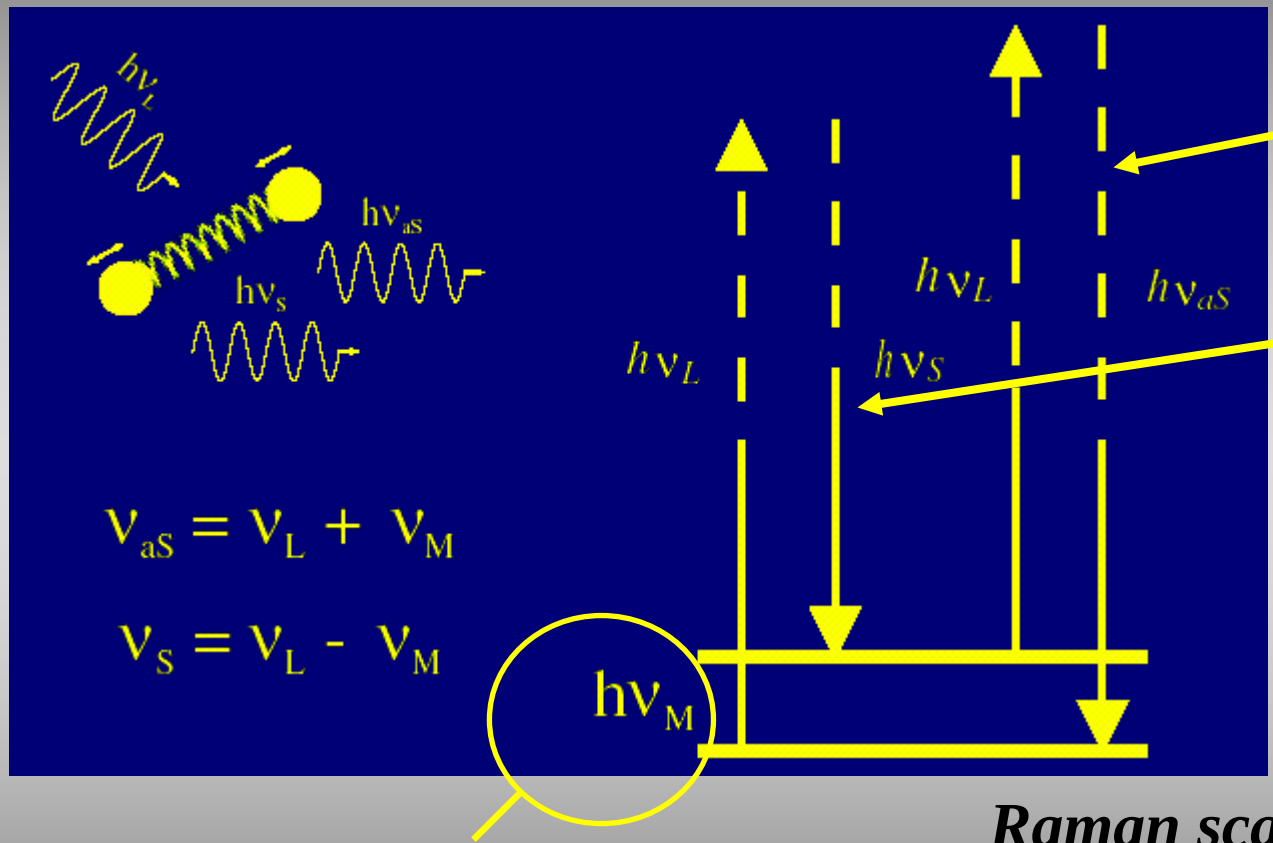
→ ***Five snapshots of training showing zeroing-in on 1.0 probably that analyte is DIOXANE***

→ ***Most components in 10 analyte test suite identified with > 95%***

→ ***Does not identify $CHCl_3$ for which not trained***

→SERS in Environmental Analysis

The Optical Process



Raman scattering is an information rich process but, unfortunately, it is also very inefficient

SERS - Why

→ *Small inherent cross sections of the normal Raman process lead to poor sensitivity, however Raman signals can be greatly enhanced by SERS and resonance effects (enhancements > 10^{12} have been reported in rare cases)*

→ *Commonly used optical methods fluorescence & absorbance are "information poor" while Raman methods provide detailed vibration information (unambiguous identification when needed)*

SERS - Why Not

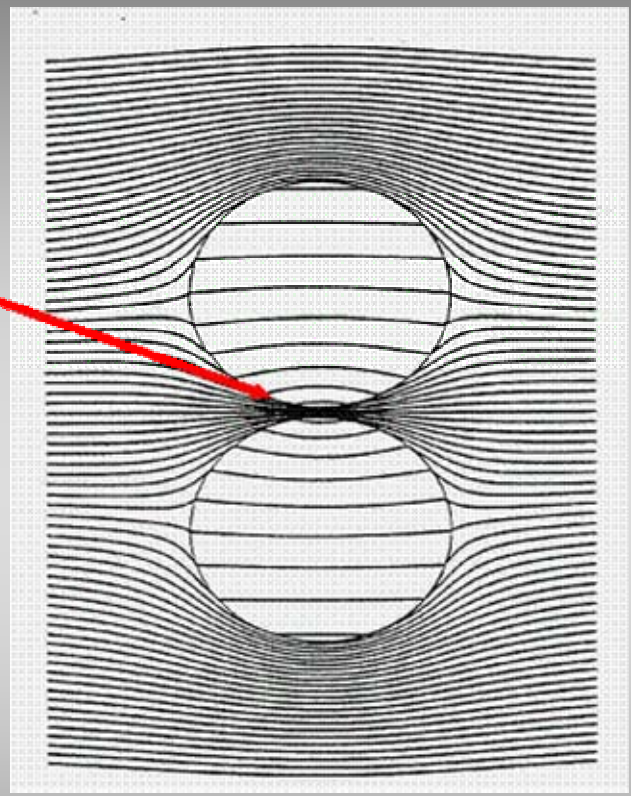
- → *Limited qualitative & quantitative reproducibility*
- → *Limited dynamic range*
- → *High sensitivity is not very uniform*

} *surface/ nanostructure/ phenomenon*

SERS – What is it

→ I_{Raman} *is proportional to product of EM field and polarizability of molecule*

Potential "Hot Spot"



→ *Electromagnetic model ("E") -> Radiation-induced polarization in nanometer-dimensioned precious metals enhances field (E) experienced by proximal analytes; metal type (Ag & Au), dimensions and shape, and spatial arrangement are very important*

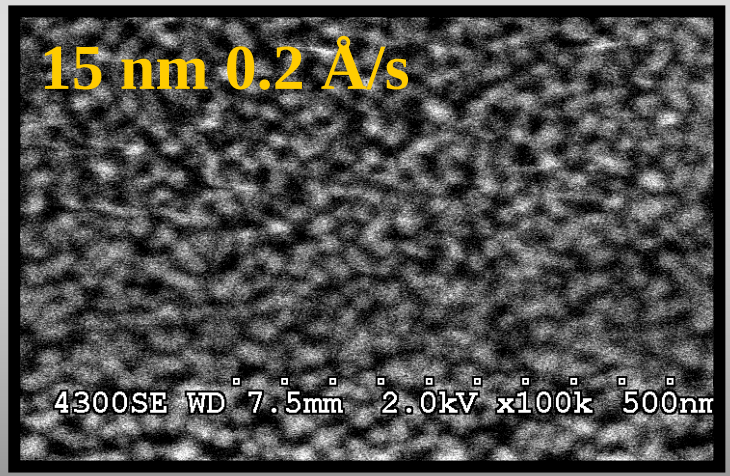
→ *Closely spaced metal nanospheres (or other shapes) can generate large enhance-ments of incident fields due to concerted plasmon field effects.*

SERS Project Goals

By technique and substrate advances, address these analytical limitations and apply to EDCs

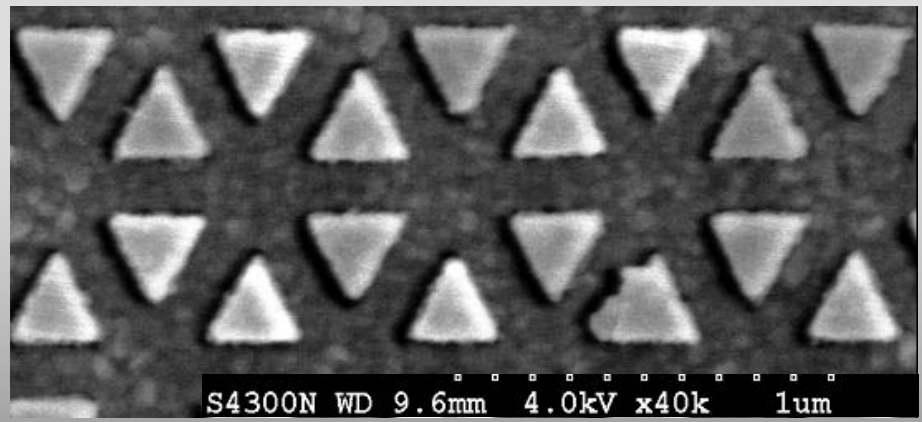
Our Advanced substrates are metal – polymer nano-composites substrates formed by vapor depositing Ag or Au onto:

Moldable PDMS Films



→ ***Spontaneous generated substrates with random morphologies***

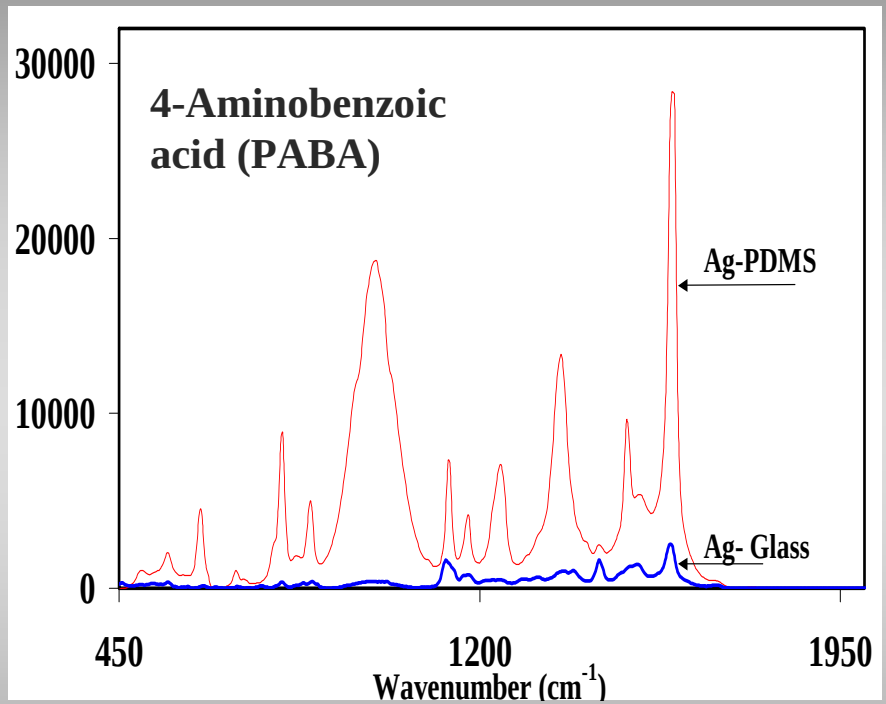
E-Beam Resist Pillars



→***Lithographically prepared substrates with controllable morphologies***

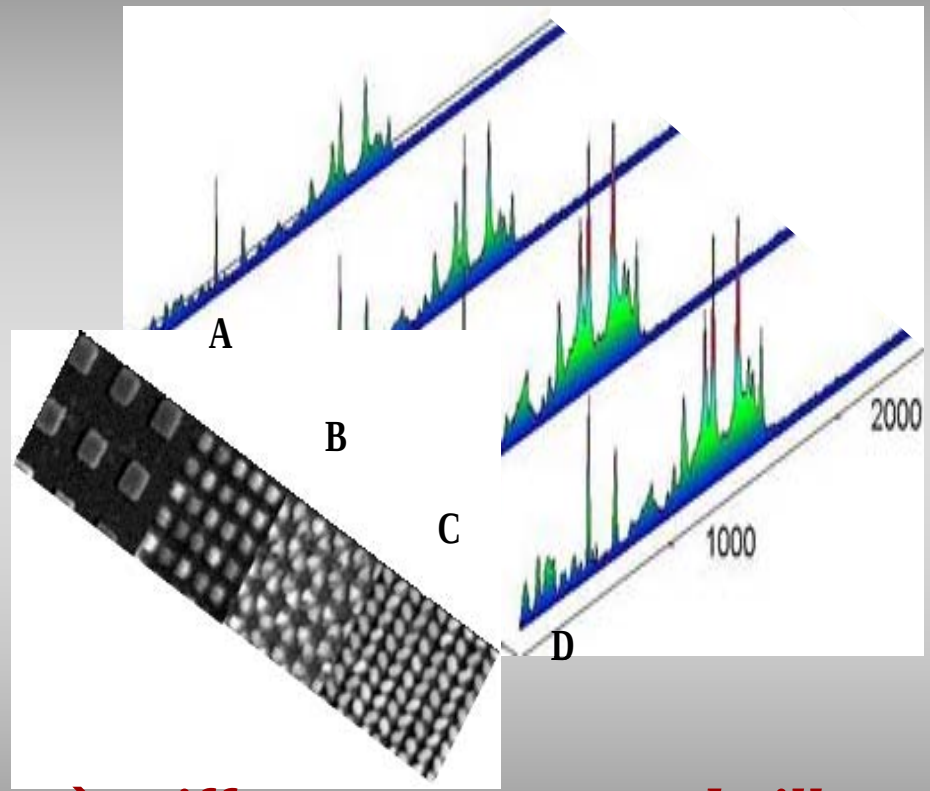
Comparison of SERS Substrates

Ag-PDMS



→ *Our substrates significantly out-perform traditional Ag islands on glass*

Ag-E-Beam Pillars

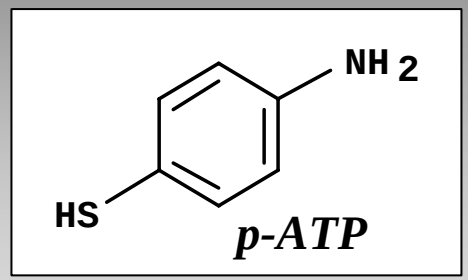


→ *Different EBL-created pillar patterns yield variations in SERS activity*

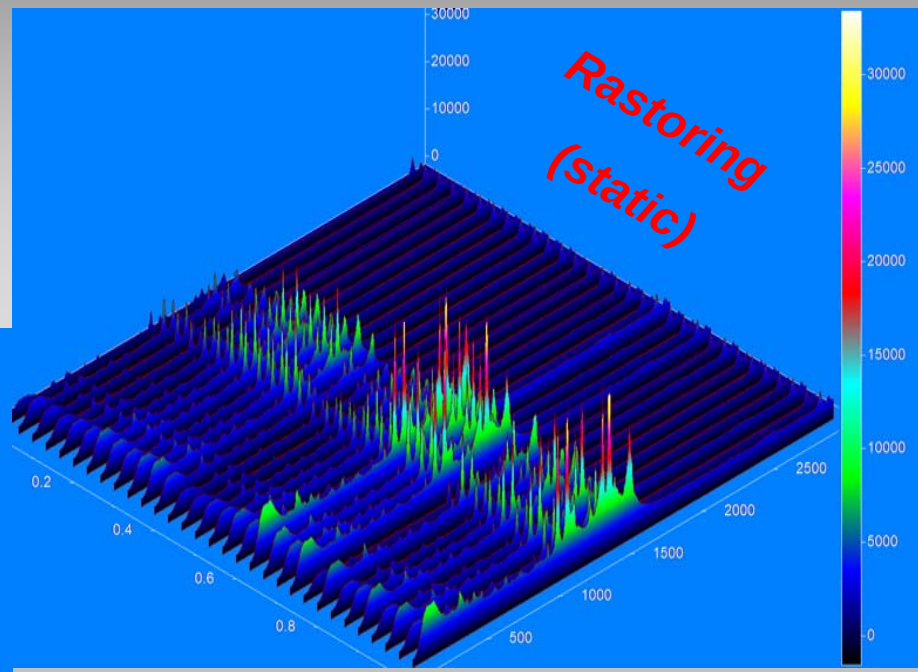
SERS "On-The-Move" Improves Performance

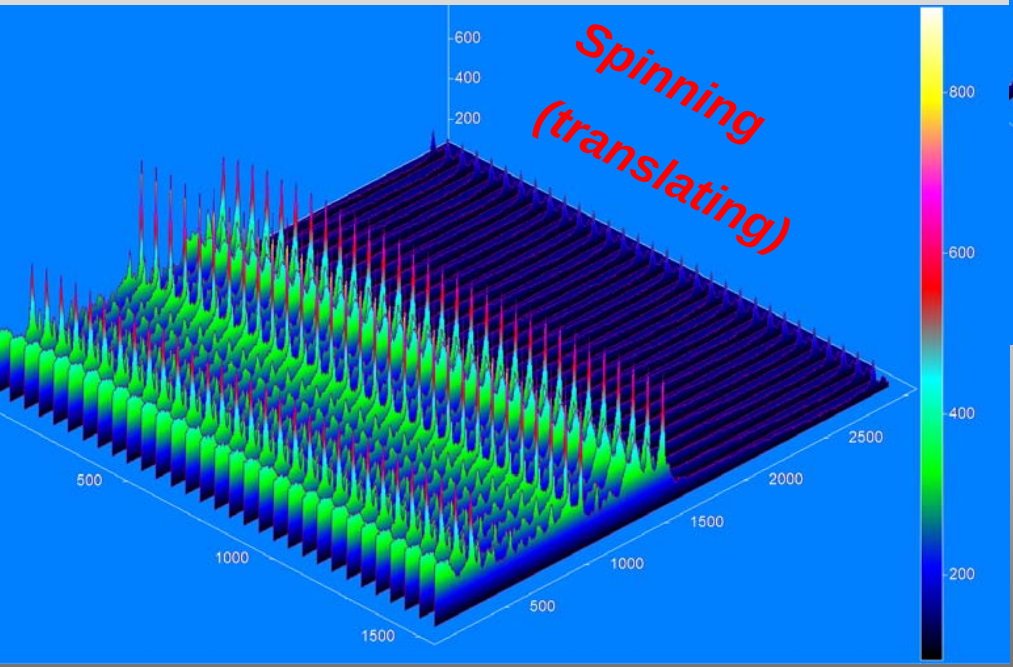
Translating *Stationary*

SERS -Mapping



STT-SERS

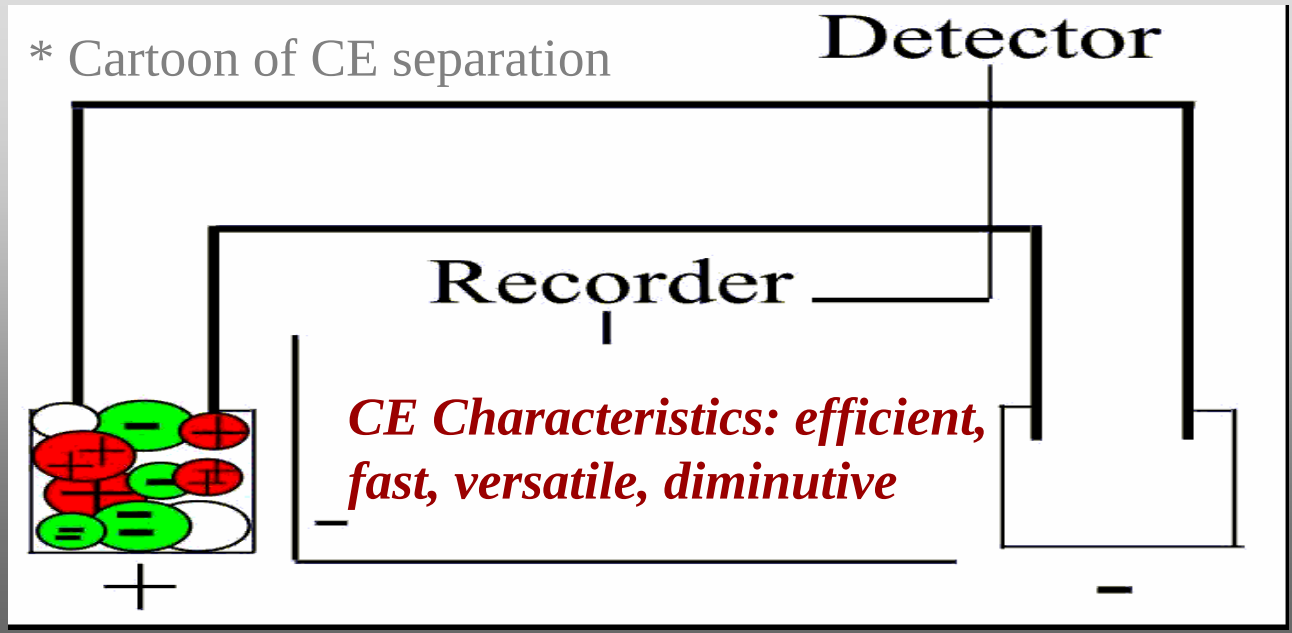




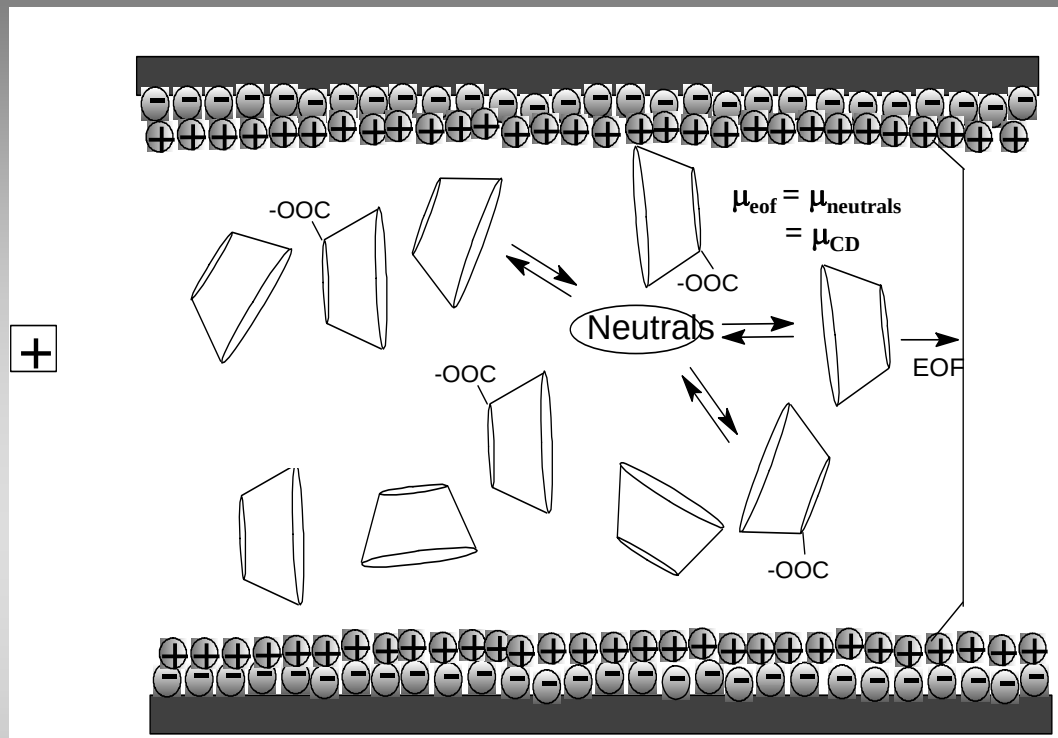
Reproducibility:
Intra-Well RSD: <7%
Inter-Well RSD: <10%

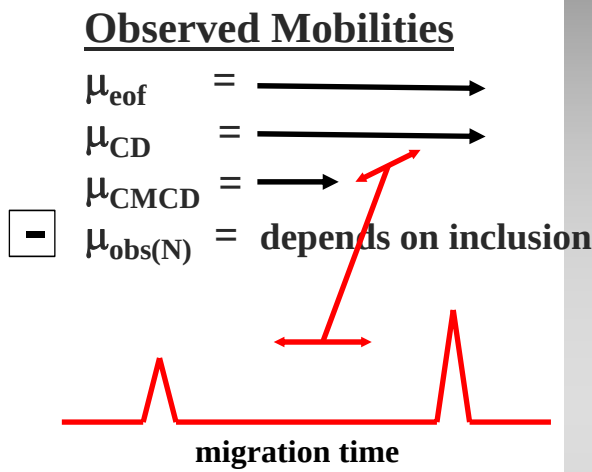
→ Electrophoretic Separations (CE) in Environmental Analysis

- → *Separation technique based on the mobility differences in an electric field for ions injected into narrow-bore capillaries*
- → *Mobility scales roughly with charge to mass ratios of the ions*
- → *Running buffer is electrokinetically pumped through the capillary*
- → *Neutrals can be separated based on differential association with charged running buffer additives (e.g., CDs)*
- → *Capillary format can be changed to a lab-on-a-chip platform using lithographic techniques*



Capillary Electrophoretic Separations of Neutrals by "CDCE"



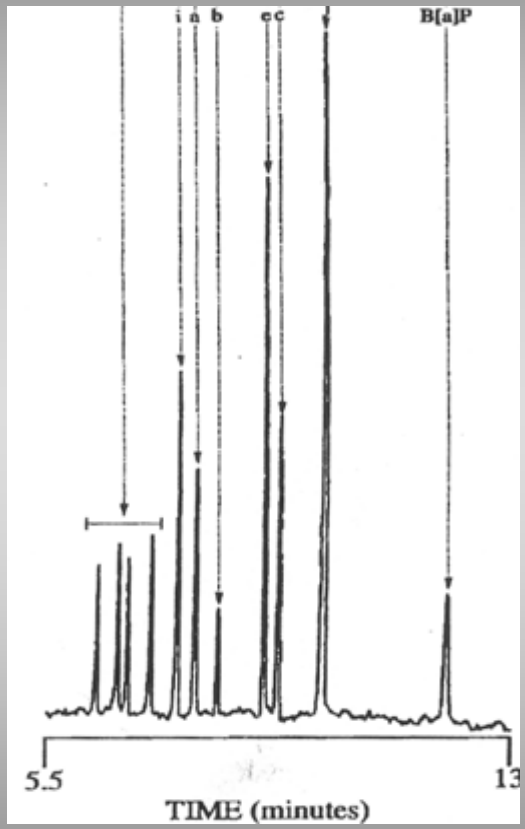


Wide windows very desirable

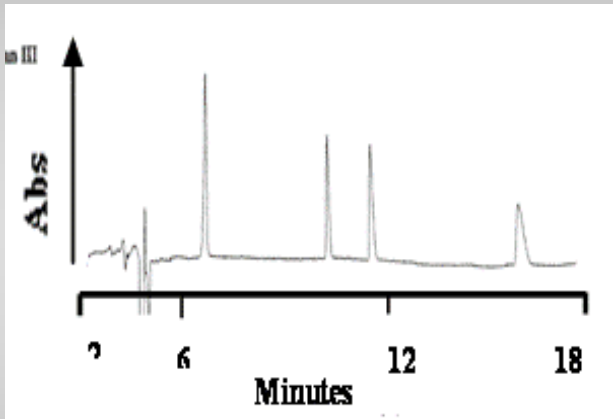
→ *Inclusion in the cavity depends on the chemical and physical properties of both the CD and Analyte.*

→ *There are several dozen different commercial types of CDs and we have also chemically modified native CDs; hence a plethora of CD "cocktails" can be tailored to meet EDC separation challenges*

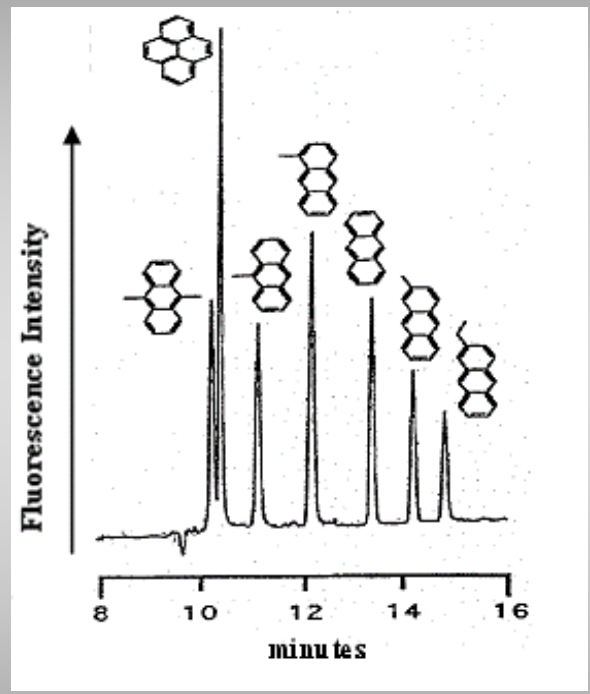
Examples of Prior Pseudo-phase CE Separations of Environmentally Significant Mixtures



mixture of ten mycotoxins



mixture of PCBs



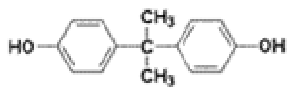
alkyl substituted PAHs

Conceptual Design & Early Progress

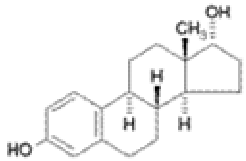
→ Some Targeted EDCs in Our Work

Previously tested with Bioreporters

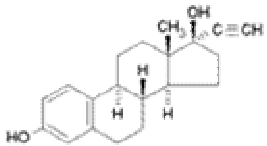
Bisphenol A



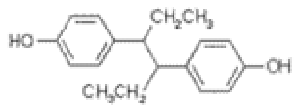
17α-estradiol



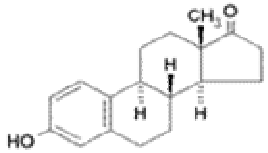
17α-ethynylestradiol



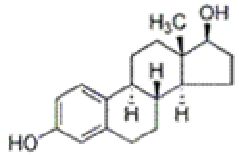
Diethylstilbestrol



Estrone

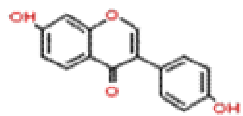


17β-estradiol

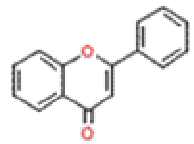


Promising SERS responders

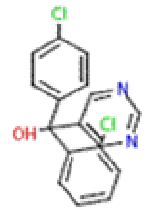
Daidzein



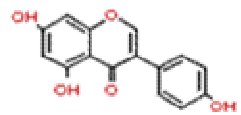
Flavone



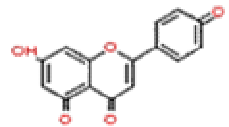
Fenarimol



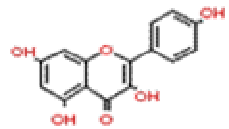
Genistein



Apigenin

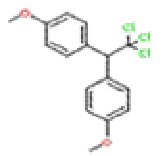


Kaempferol

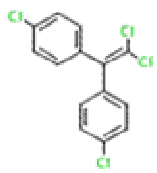


Bisphenol B

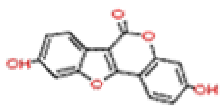
p, p'-methoxychlor



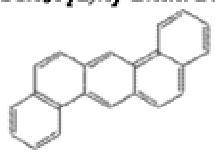
p,p'-DDE



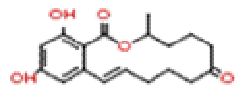
Coumestrol



Dibenzo[a,h]-anthracene



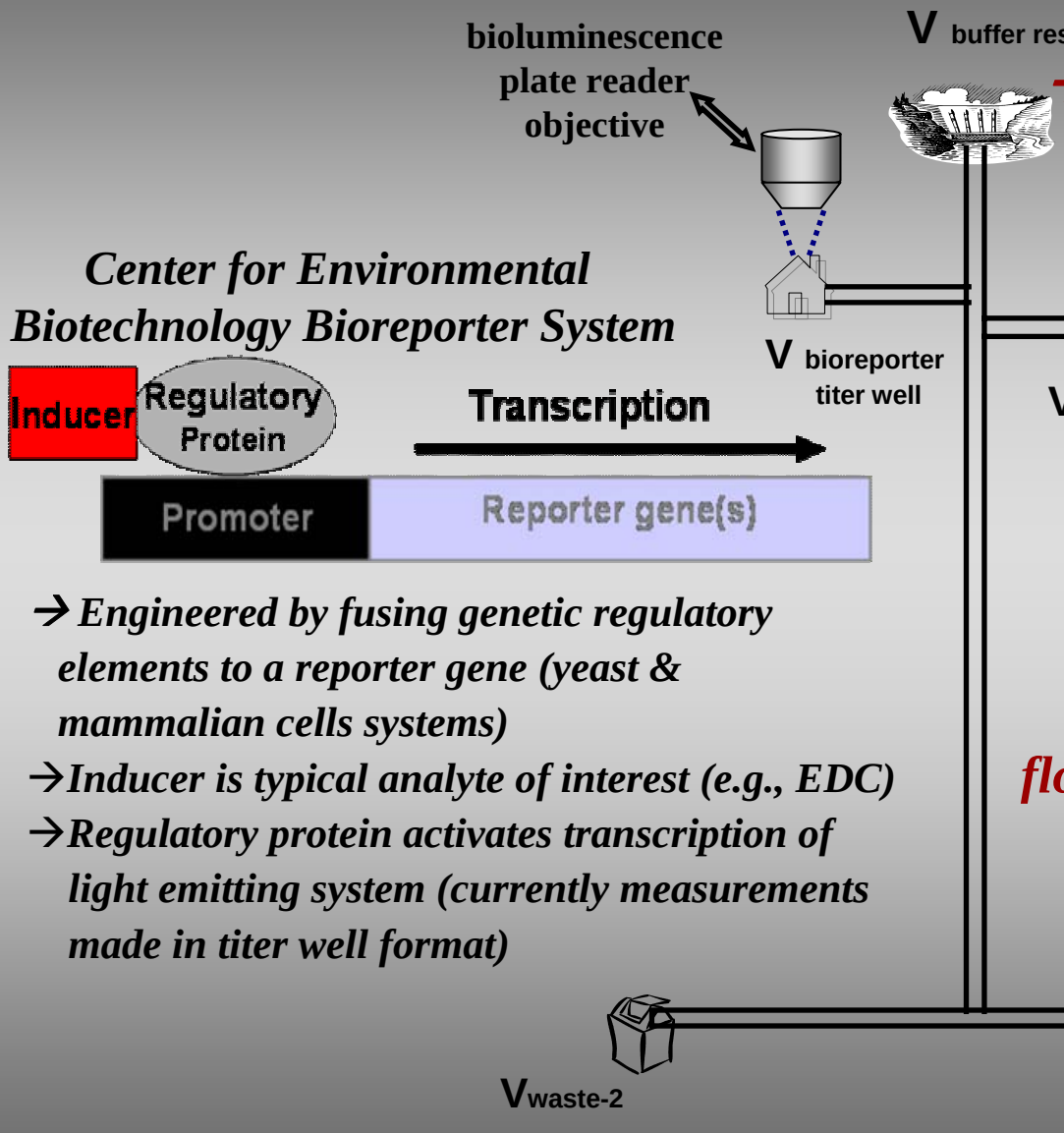
Zearalenone



Used in our prior electrokinetic separations

EPA STAR EDC-'06

Conceptual Design & Early Progress



→ *Channels (typically 50x100 μm) created in PDMS using photolithography; footprint size of microscope slide*

→ *PDMS has been used to house living cells*

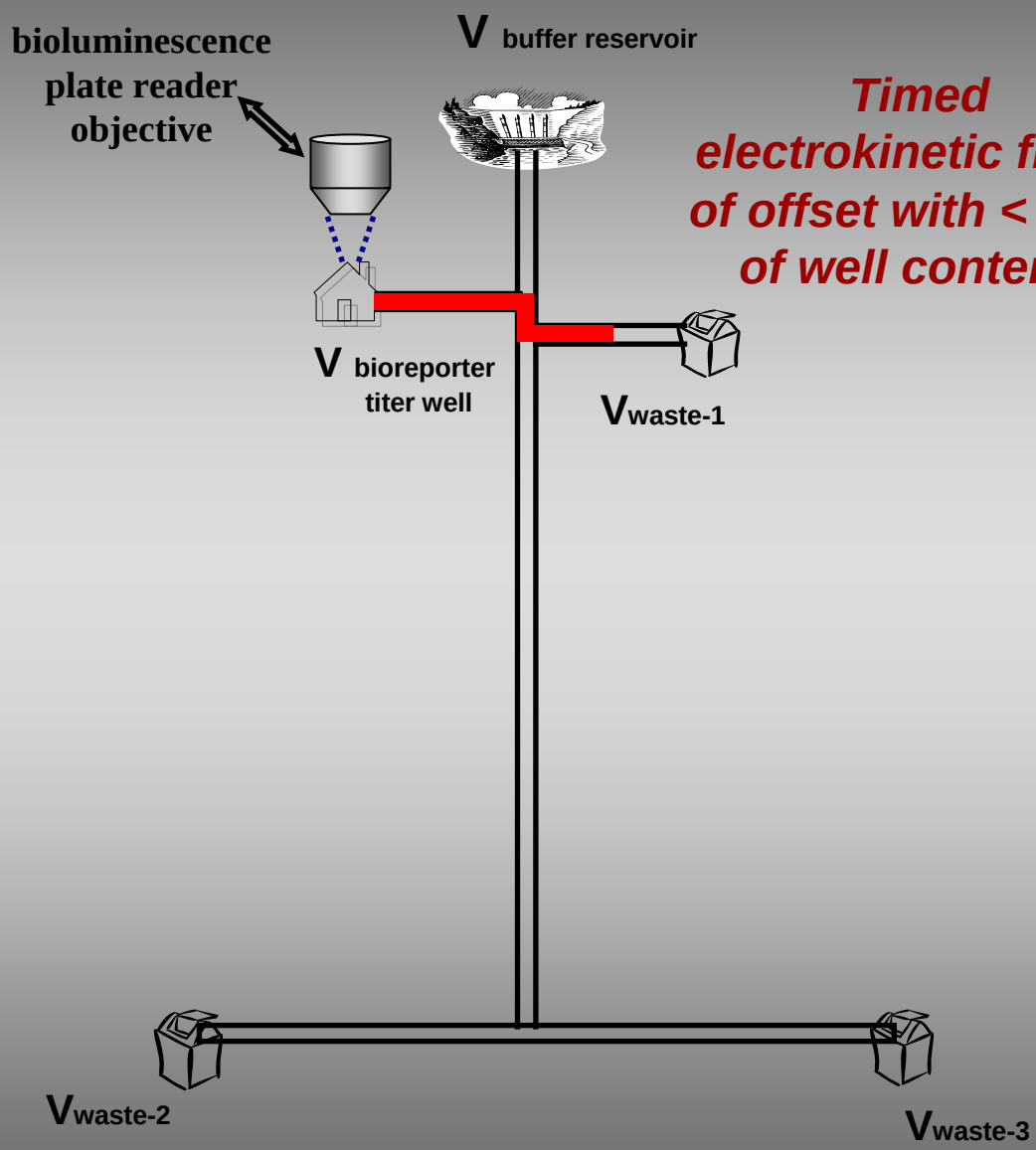
→ *Suitable manipulation of applied voltages (Vs) allows flow control for sampling, injection, and separation/detection*

→ *Engineered by fusing genetic regulatory elements to a reporter gene (yeast & mammalian cells systems)*

→ *Inducer is typical analyte of interest (e.g., EDC)*

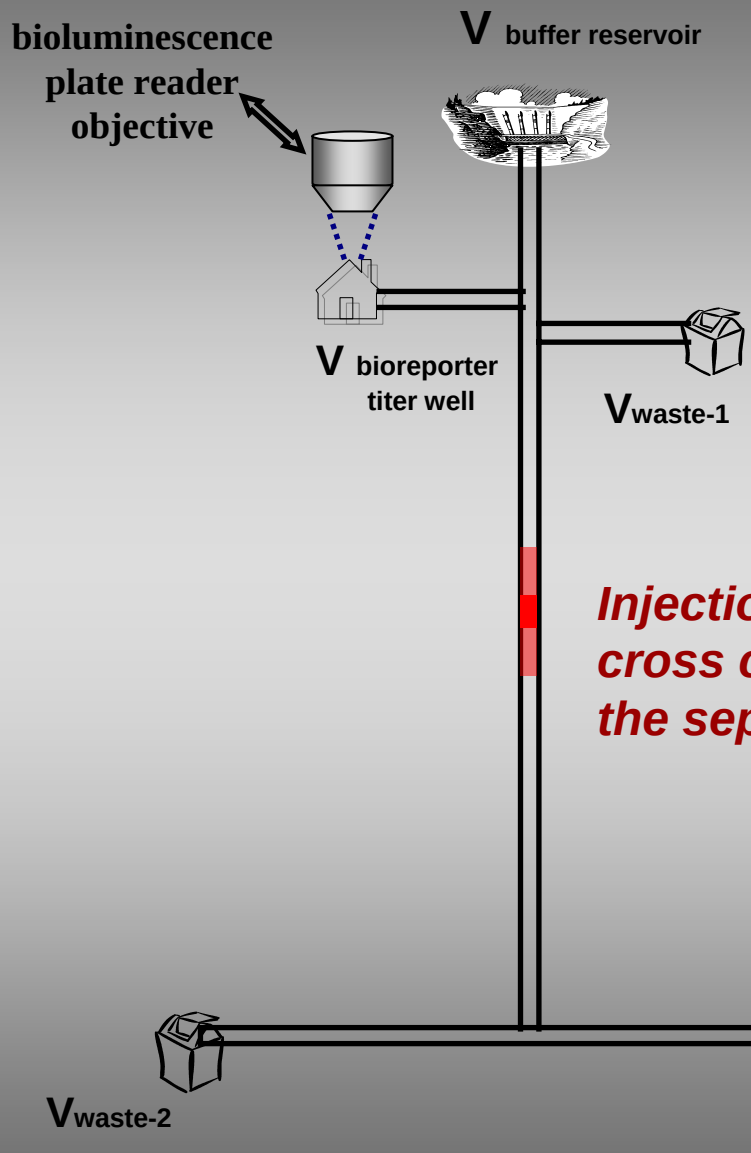
→ *Regulatory protein activates transcription of light emitting system (currently measurements made in titer well format)*

Conceptual Design & Early Progress



Timed electrokinetic filling of offset with < 1 μL of well contents

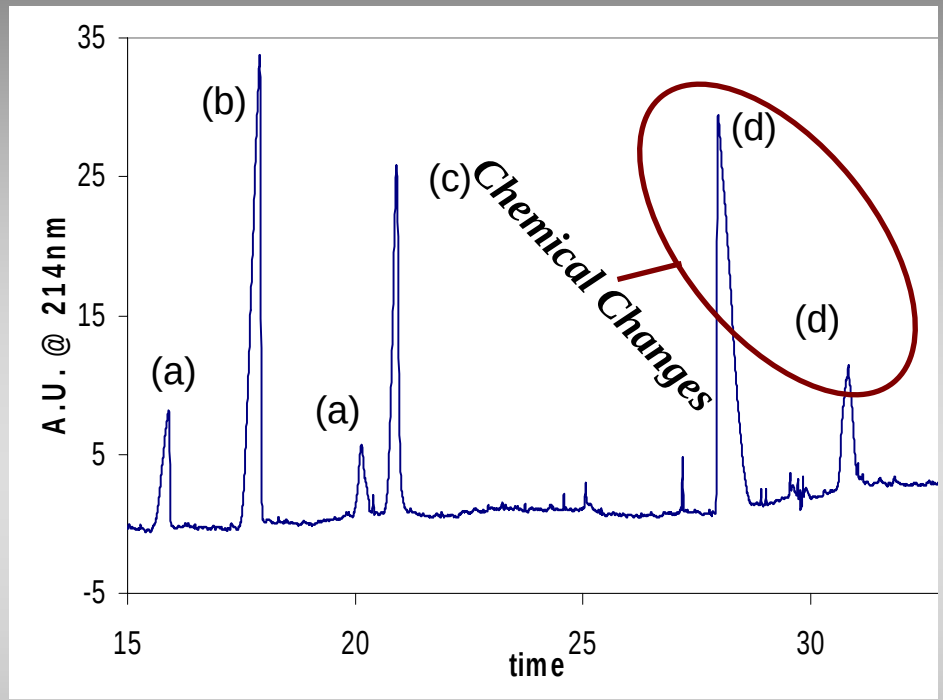
Conceptual Design & Early Progress

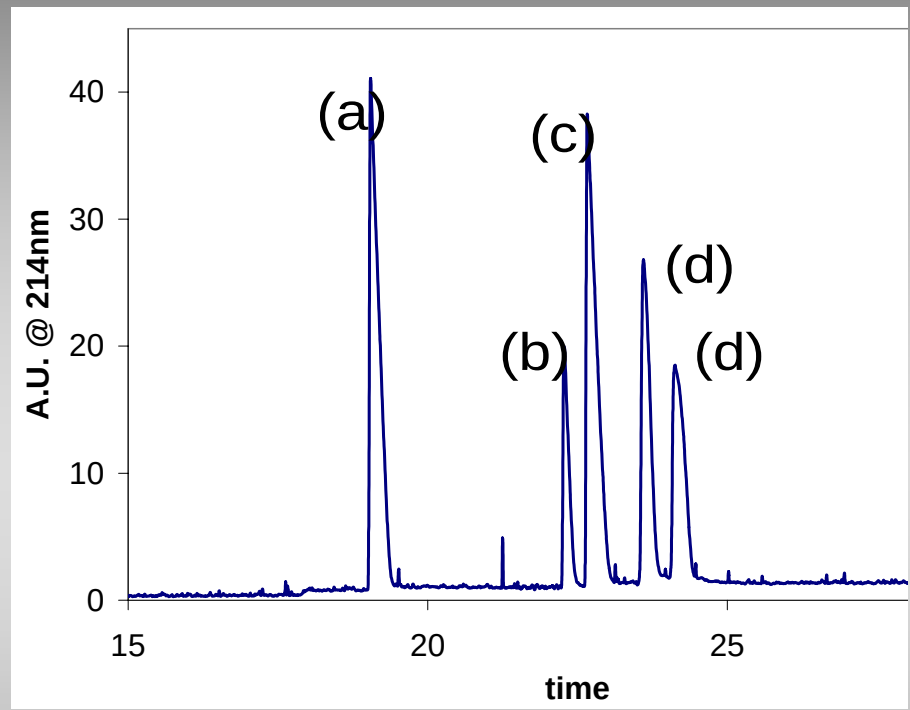


Injection performed, cross channels cleared, the separation proceeds

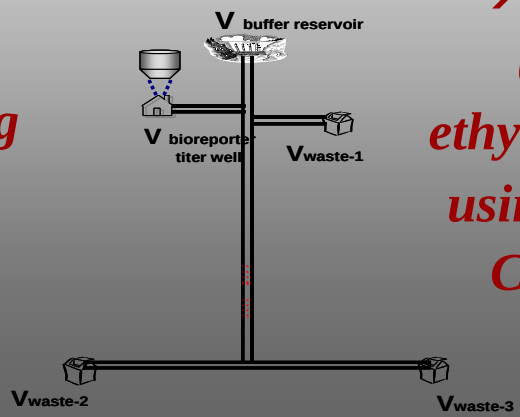
Conceptual Design & Early Progress

Example CE separation of EDCs



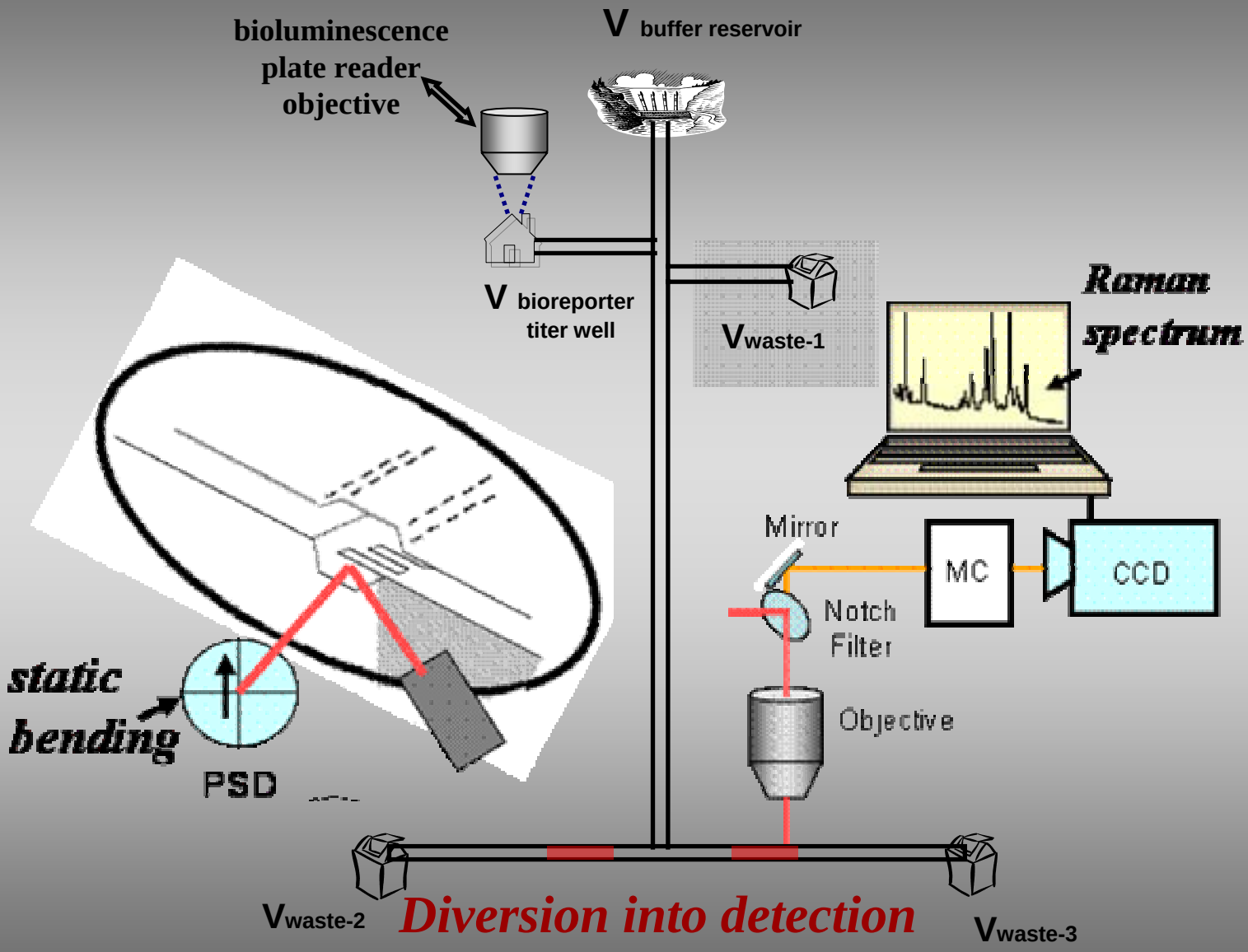


→*Separation of kaempferol (a), genistein & daidzein (b), apigenin (c) and DES (d) using a running buffer with β-CD & SDS pseudo-phases.*



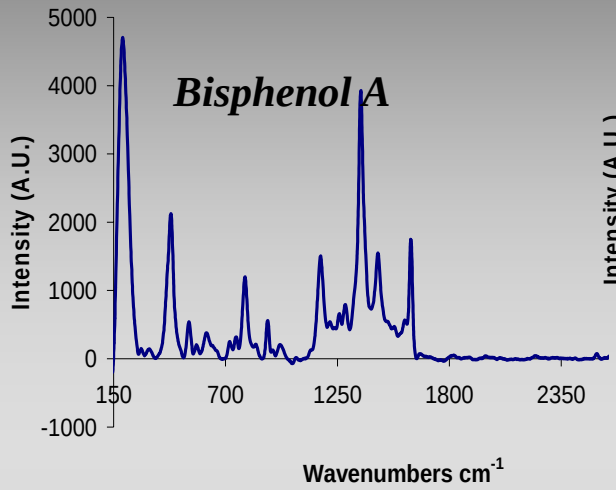
→*Separation of bisphenol A (a), 17b-esradiol (b), 17 a-ethynylestradiol (c) and DES (d) using a running buffer with β-CD & SDS pseudo-phases.*

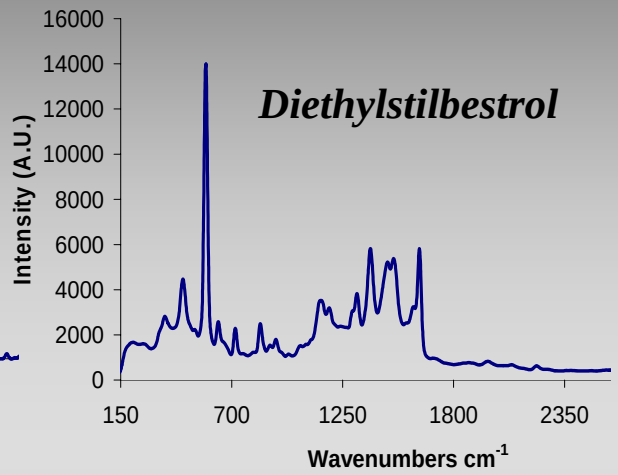
Conceptual Design & Early Progress

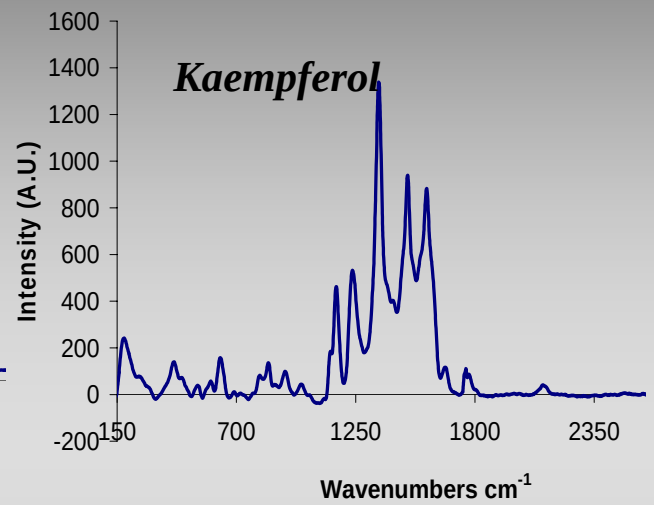


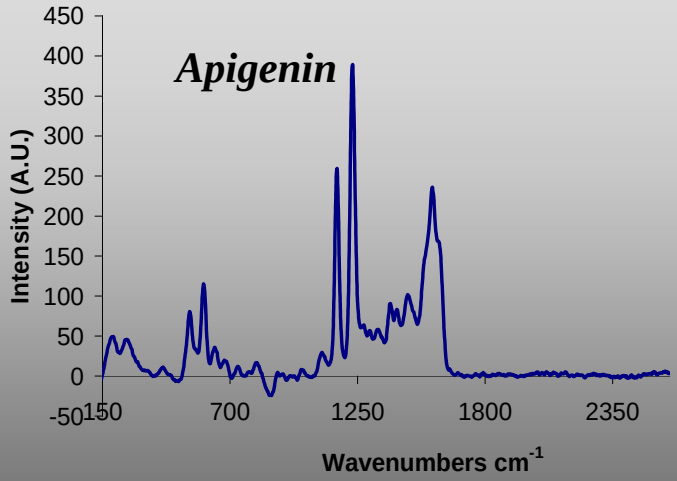
Conceptual Design & Early Progress

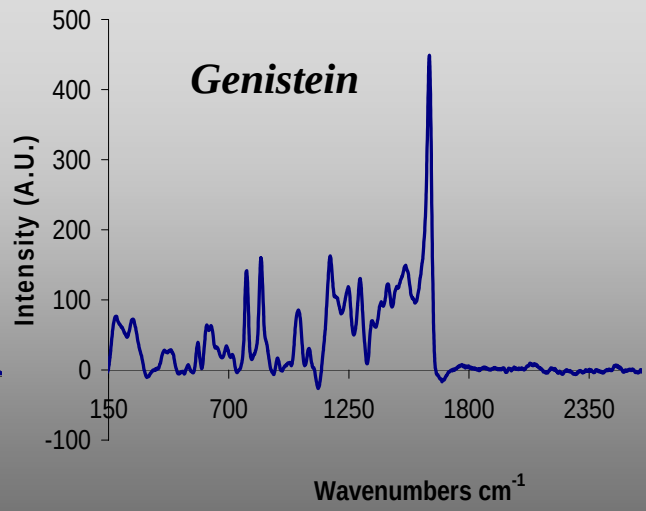
Distinctive SERS Spectra of EDCs using Ag-PDMS substrates

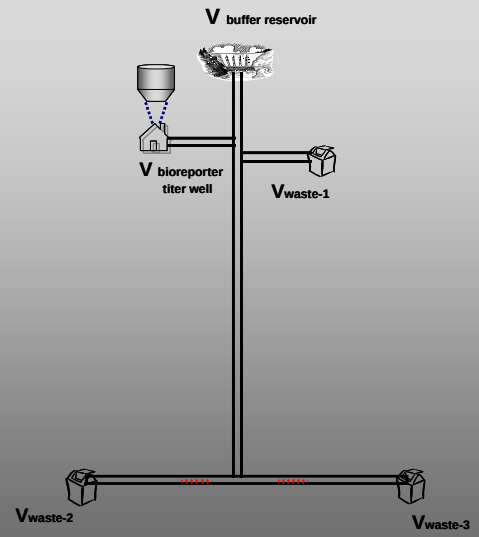








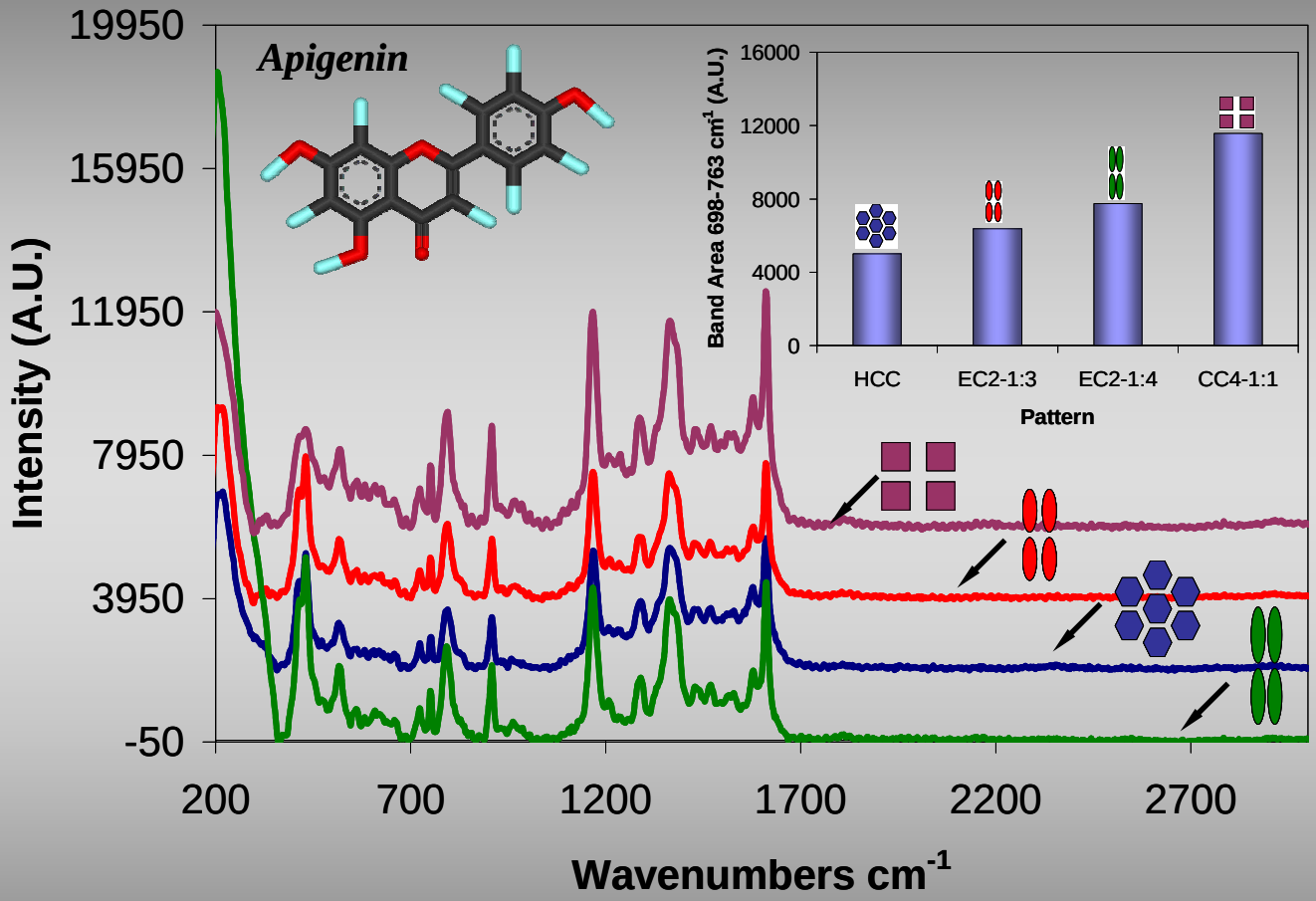




→ *Bands unique to each EDC are evident*

Conceptual Design & Early Progress

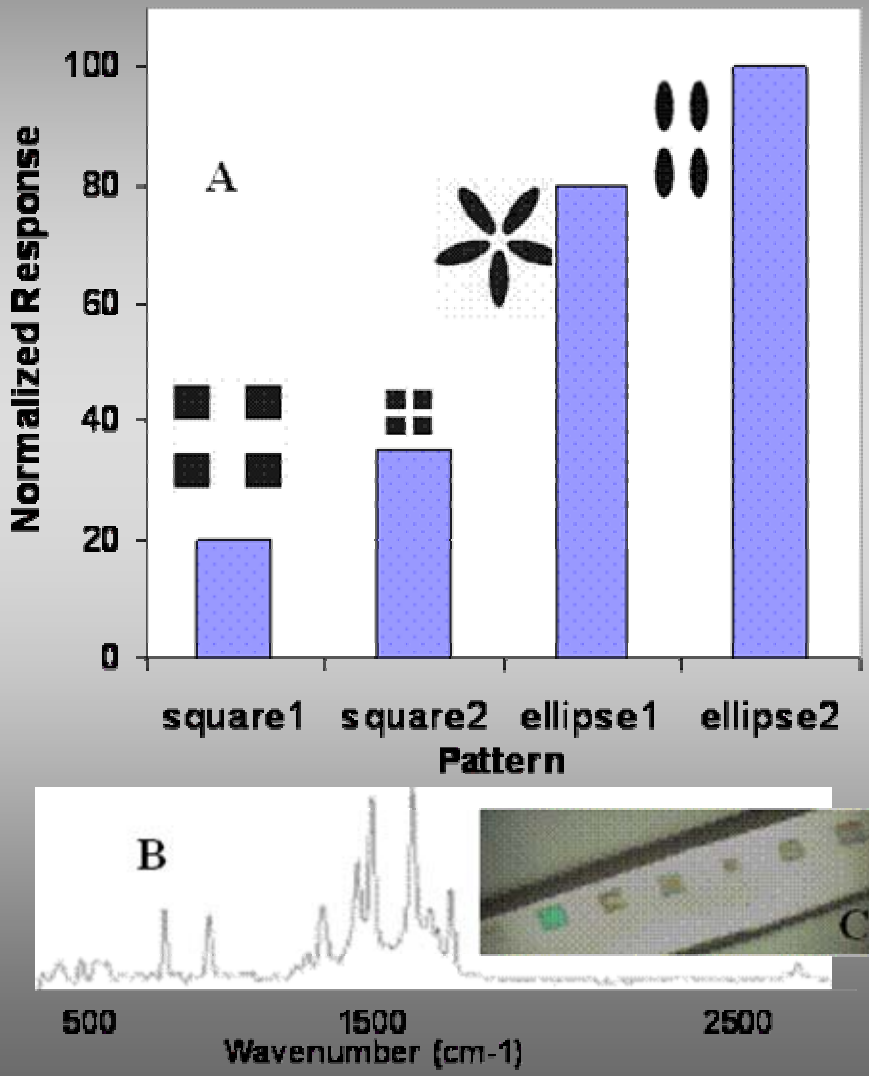
SERS Spectra of EDCs using EBL nanofabricated substrates



→ *25 nm of Ag was deposited onto nano-dimensional hexagon, square, and elliptical (2 aspect ratio) pillars; Apigenin SERS activity greatest on square pattern*

Conceptual Design & Early Progress

SERS using EBL nanofabricated substrates (in channel)



A

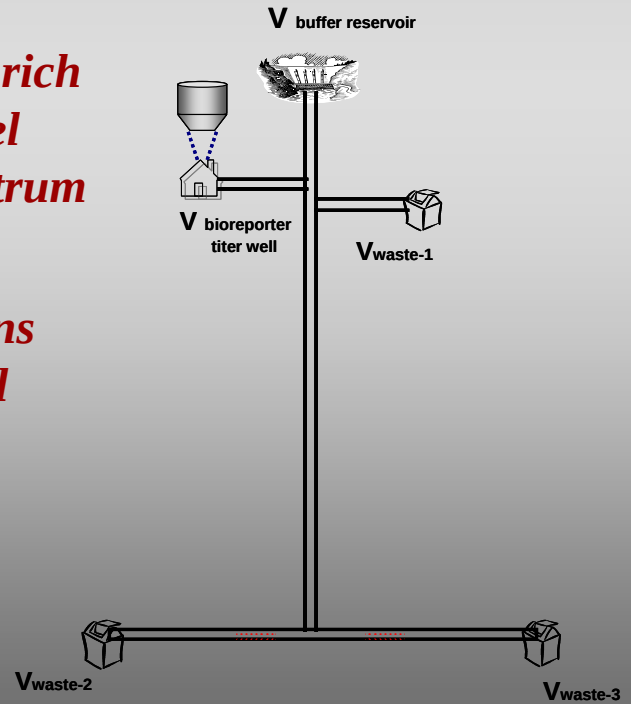
EBL nanopillar morphologies resorufin band intensities

B

Information-rich in-channel Raman spectrum

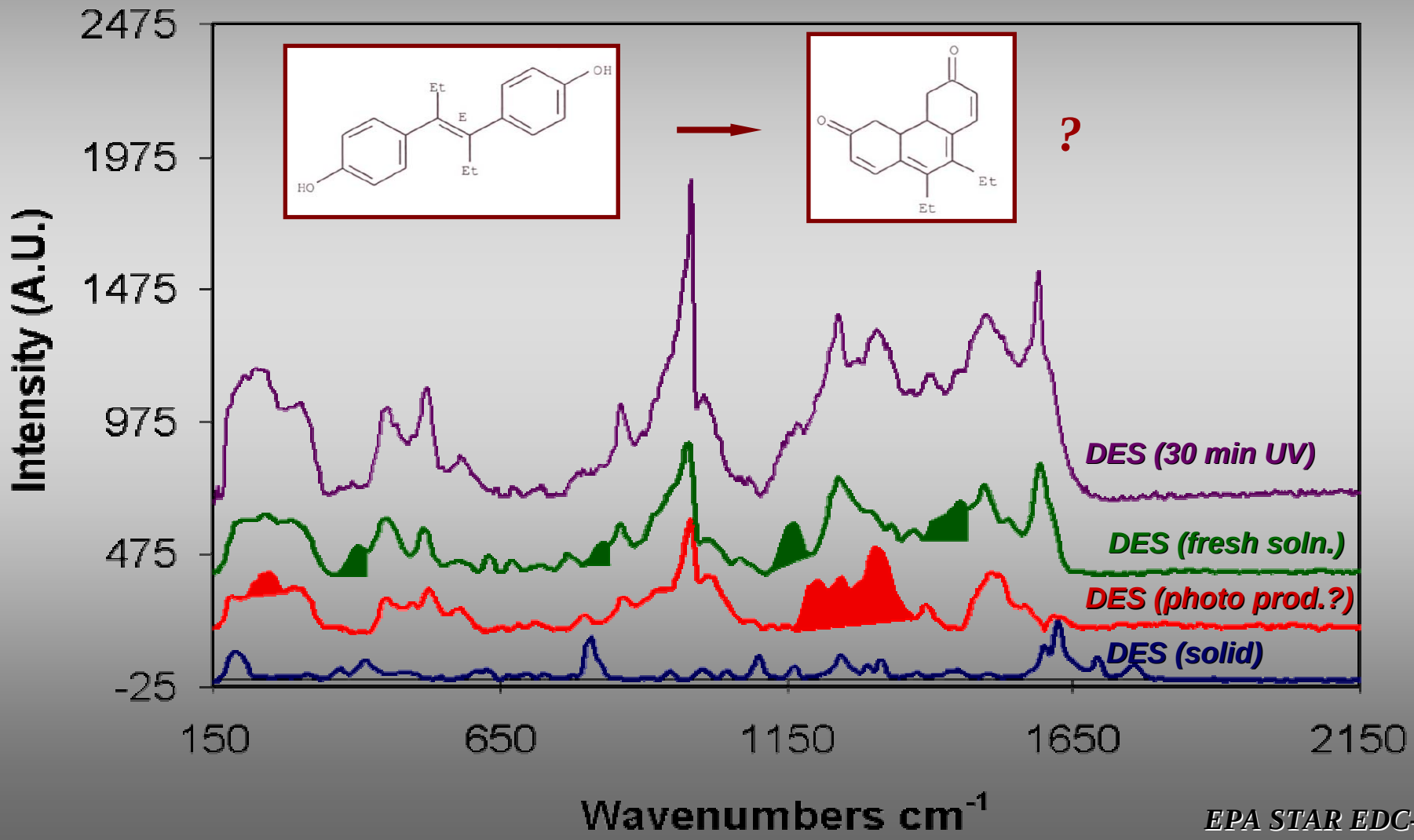
C

EBL patterns in channel



Conceptual Design & Early Progress

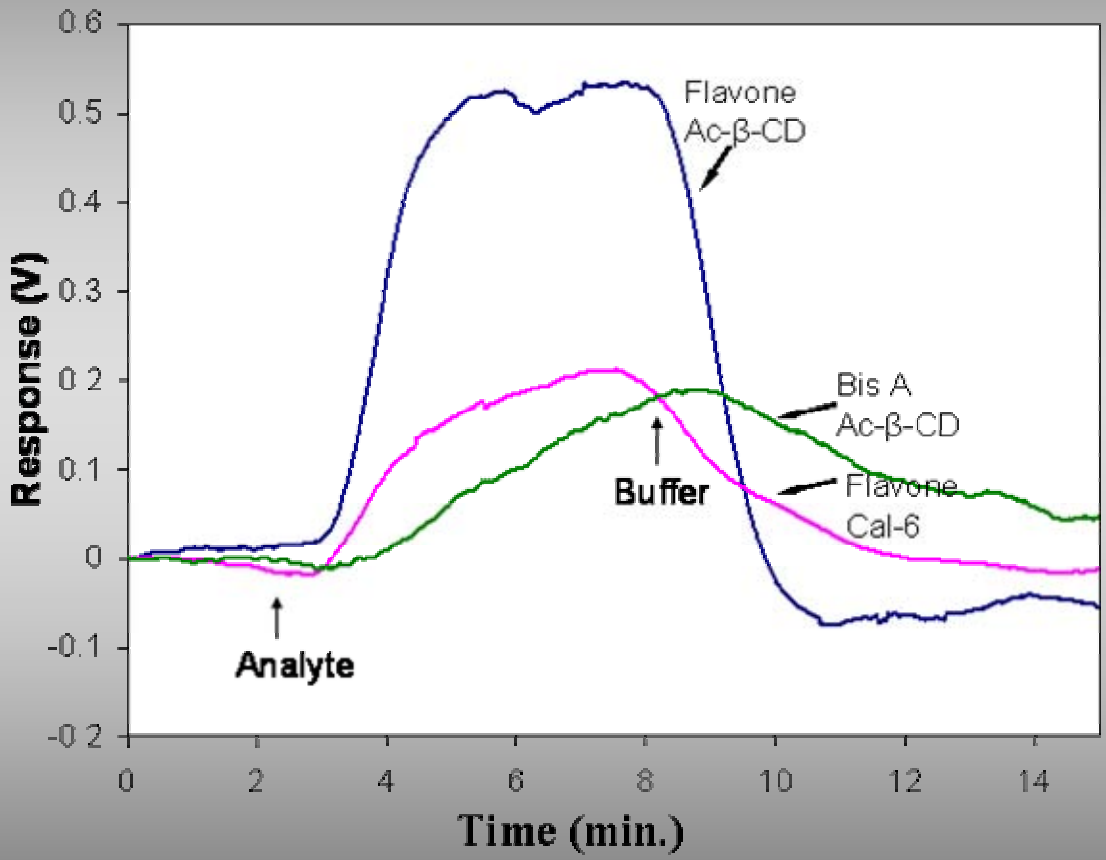
SERS Studies of EDC Chemical Fate Using EBL Nanofabricated Substrates

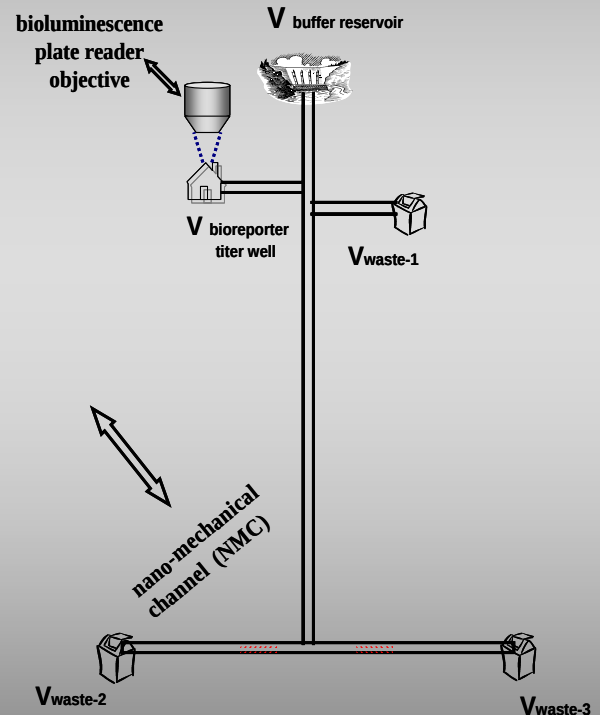


Conceptual Design & Early Progress

Nanomechanical sensing of EDCs

MCA functionalized with macrocyclic receptors



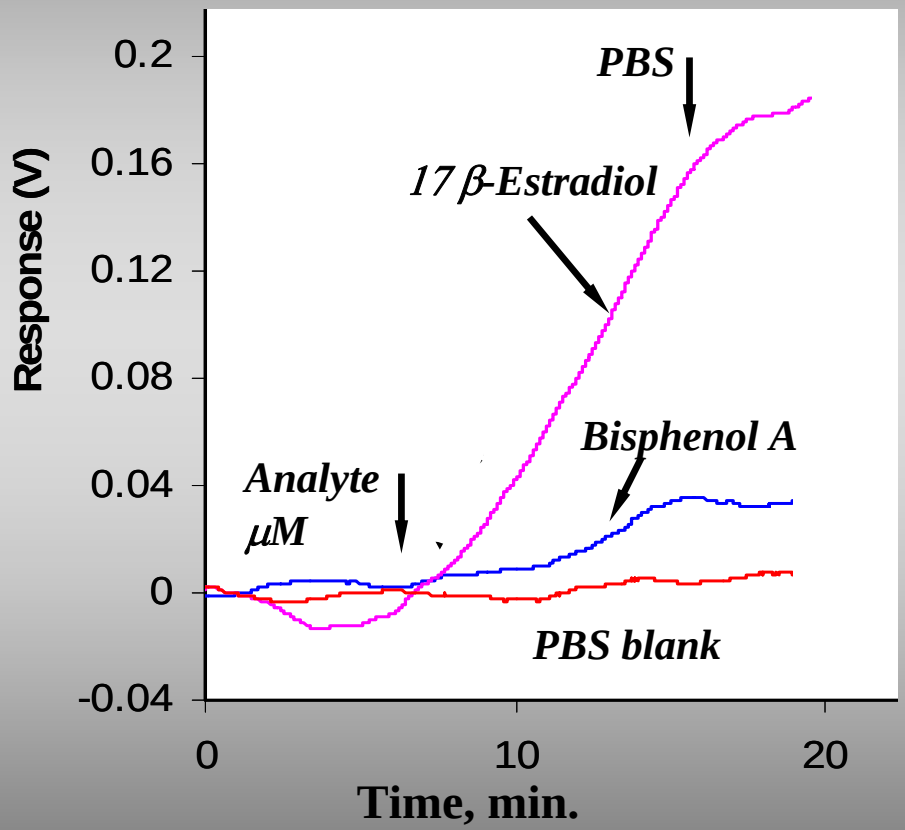


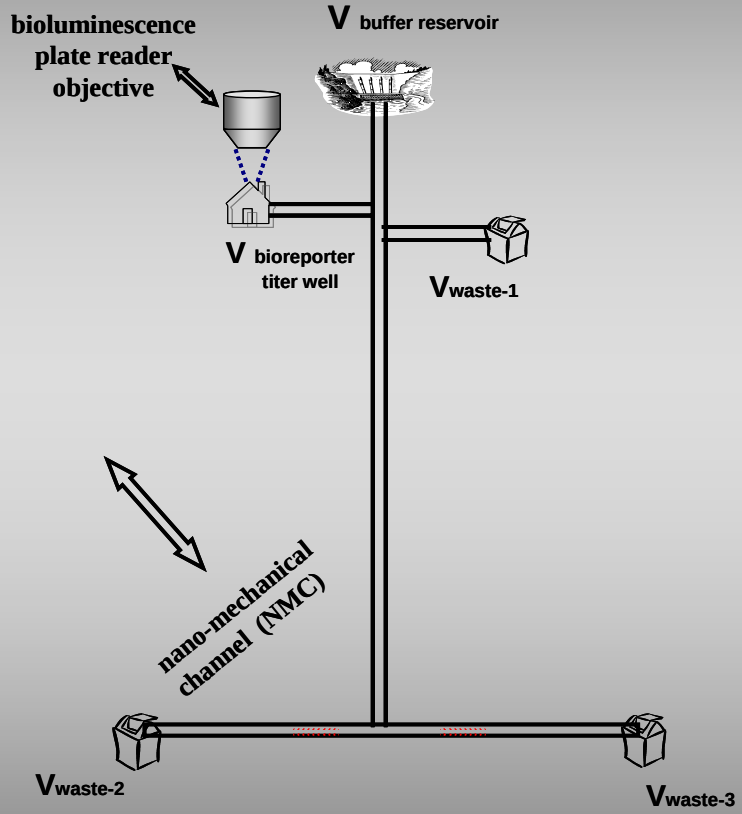
→ *Different equilibrium & kinetic responses based on analyte & MRP; distributed selectivity with pattern recognition possibilities*

Conceptual Design & Early Progress

Nanomechanical sensing of EDCs

Responses to EDCs for MC with an Immobilized h-ER-β on surface





→*Further bioaffinity approach studies are being performed (literally as I speak)*

Future Work (next 12 month period):

- → *Continue surveying targeted EDCs for nanomechanical and SERS responses*
  - → *MCA modifications (phases and nano structuring) to enhance responses & selectivity*
  - → *Advanced SERS substrates to enhance sensitivity*
  - → *Fate studies of DES and other EDCs*
- → *Develop conditions for on-chip separations for various target EDC combinations*
- → *Perform correlation studies involving mating our advanced methods with bioluminescence reporting in titer well format*
- → *Work toward integrating the individual analytical components and field portability*

Key Members of Research Team:

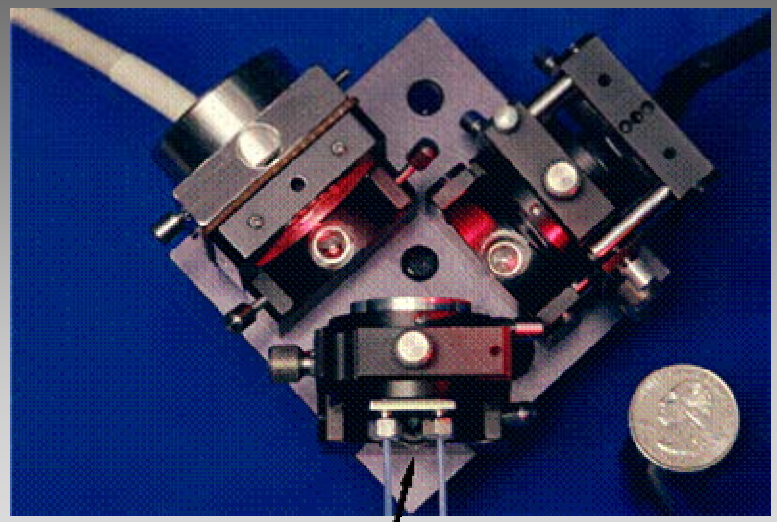
UT-Chemistry – Maggie Connatser, Kasey Hill, Jenny Oran
Pampa Dutta, Marco DeJesus
UT-CEB – Gary Sayler, John Sanseverino, Melanie Eldridge

Questions?

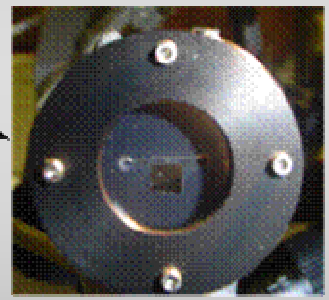
Key Members of Research Team:

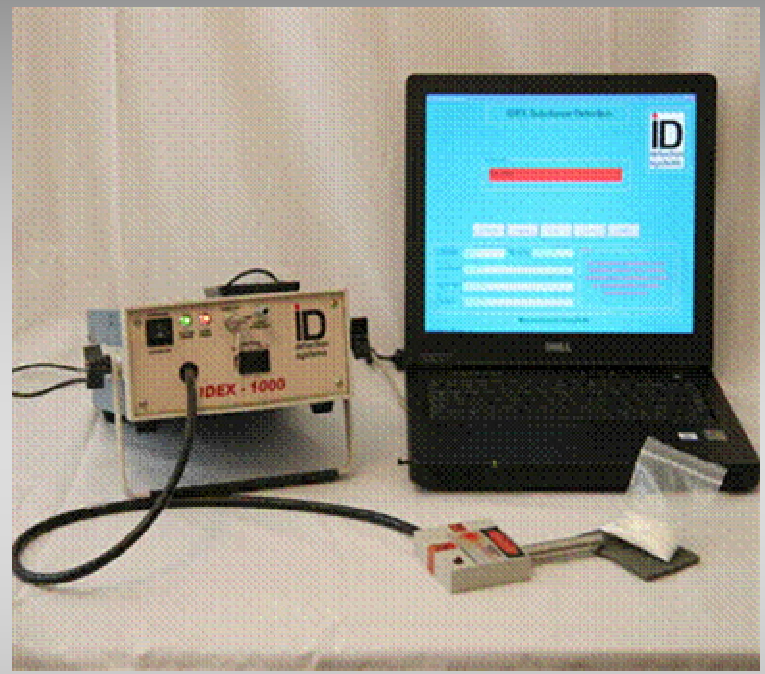
UT-Chemistry – Maggie Connatser, Kasey Hill, Jenny Oran
Pampa Dutta, Marco DeJesus
UT-CEB – Gary Sayler, John Sanseverino, Melanie Eldridge

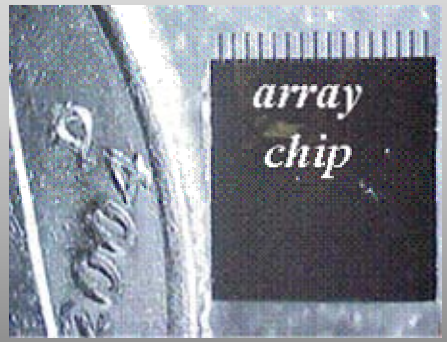
Field-Ready Potential for These Technologies



Flow Cell
~ 3 µL Vol

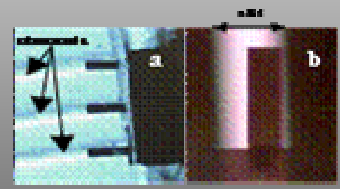






→ *Diminutive MCA Components*

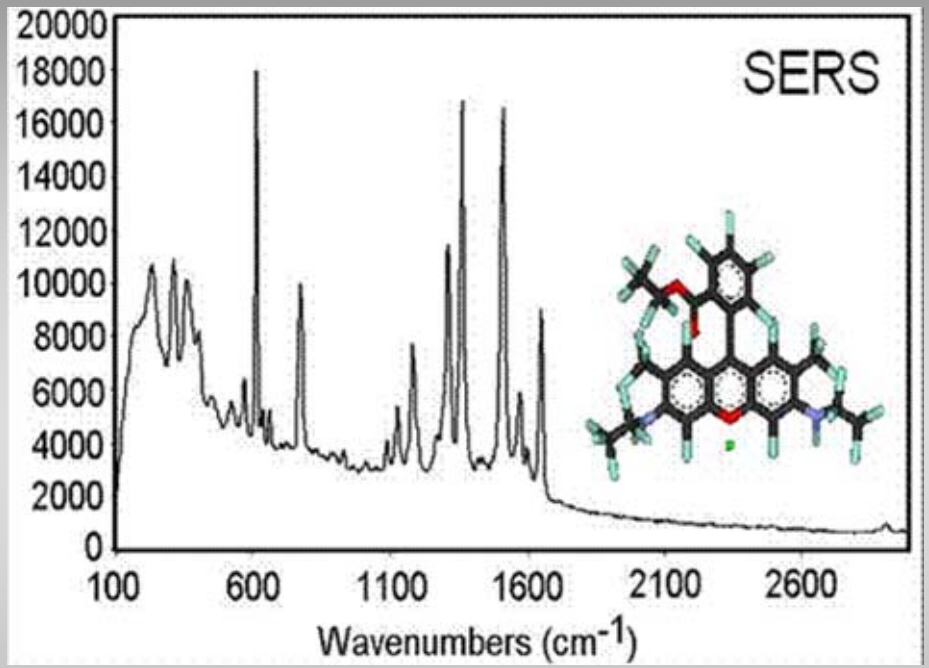
→ *Commercial Raman Spectrometer*



MCs can be differentially coated with µ-fluidic manifold (a) or PVD through a mask (b)

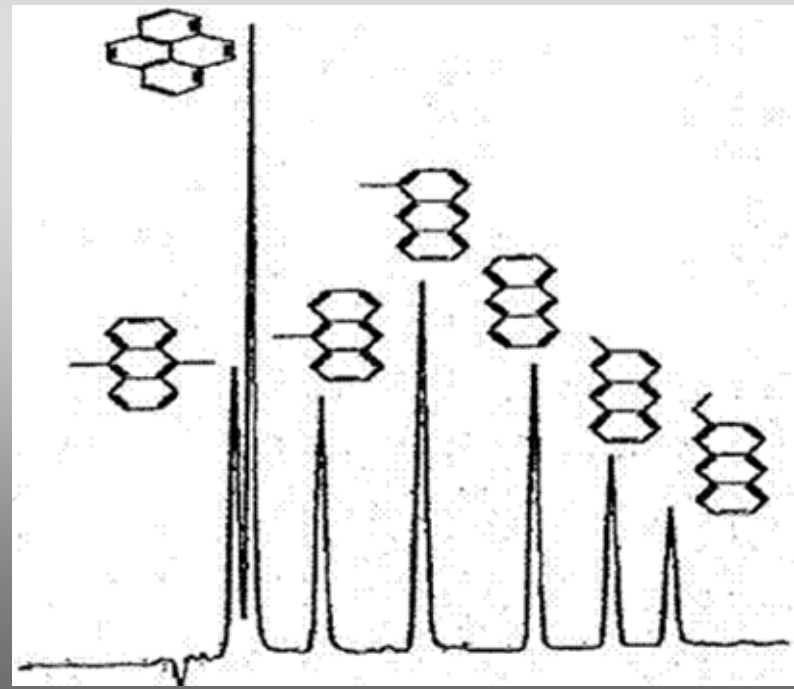
MCA extra

Key Issue - Selectivity
Achieving Selectivity Analysis



→ *Information rich spectroscopy:*
vibrational bands from Rhodamine6G seen in surface enhanced Raman spectrum provide analytical selectivity

→ *Chemical separations:*
selective interaction of substituted aromatics with CE cyclodextrin additives, and large plate counts, give rise to high selectivity



MCA extra

Achieving Selectivity In Analysis

With analyte sensitive sensors (e.g. MCs) these spectroscopic & chromatographic analytical attributes are not present. However, selectivity is attainable through the clever uses of molecular recognition phases (MRPs).

Two viable approaches

Use of highly selective MRPs with large binding constants:

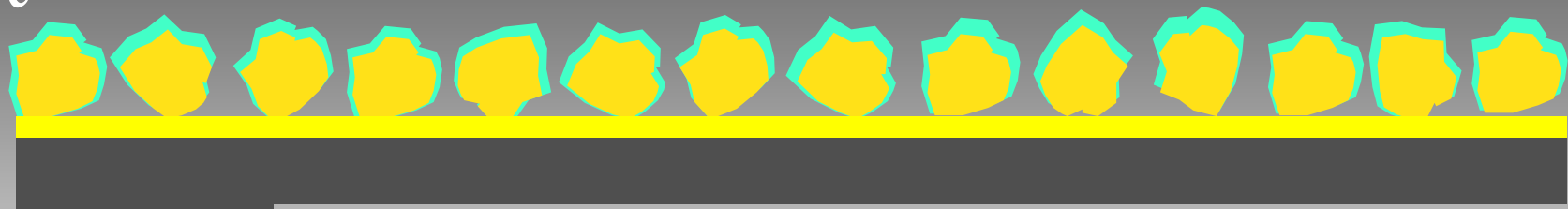
- *Bioaffinity*
- *Selective chelates for metal ions*
- *Issues of reversibility & robustness*

Use of moderately selective Phases in arrays for "distributed selectivity":

- *Based on response signatures*
- *Pattern recognition*
- *Issues of molecular recognition contrast and differential coating*

MCA extra

Nanostructured Interfaces Enhance Responses



Analyte	SAM (smooth)	SAM (nano)	17 nm (smooth)	17 nm (nano)	50 nm (nano)
2,3-DHN	290	4.8	31	0.99	0.025
2,7-DHN	250	36 X→ 7.5	39	39 X→ 1.0	0.039
Tolazoline	300	17	214	13	4.9
Benz. Acid	1500	140	250	42	18

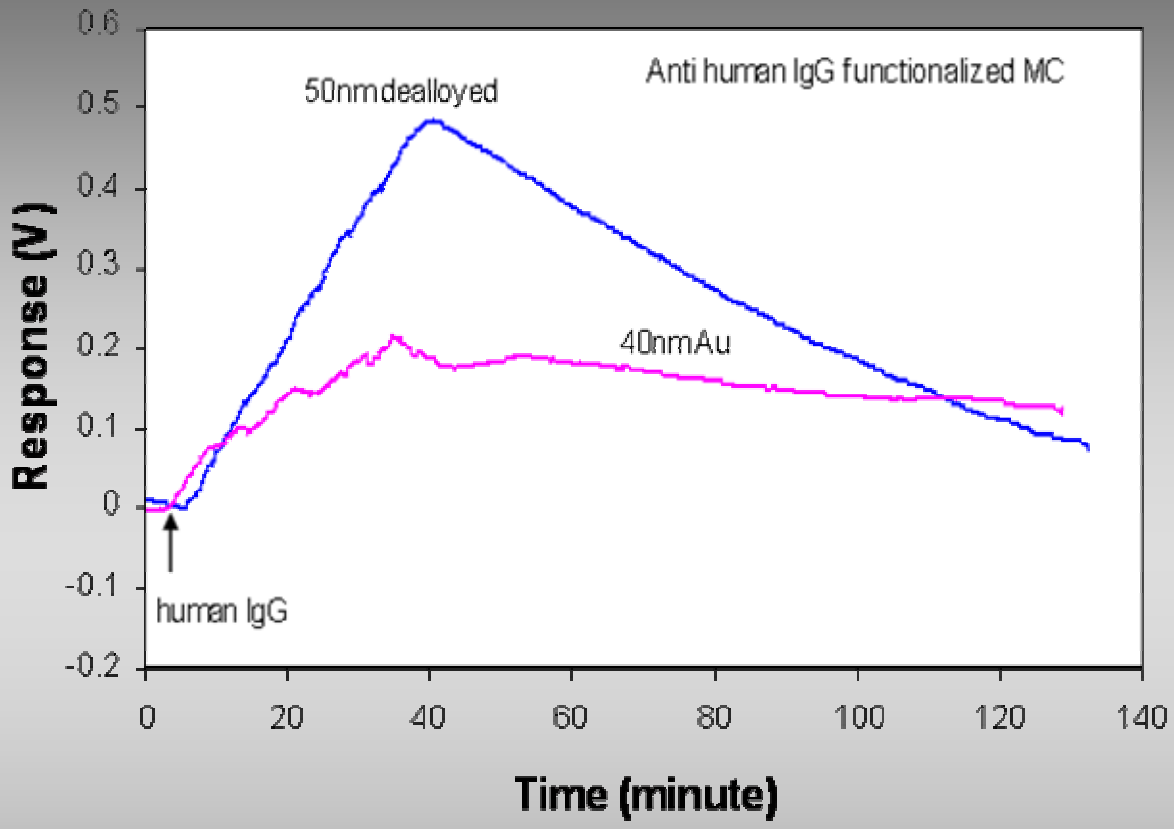
→*Example of the dramatic improvement in LODs (in ppm)*

with: - nanostructuring

- thicker films (phases are modified cyclodextrins)

MCA extra

Bioaffinity Interaction on Different Surfaces



Antihuman IgG - human IgG

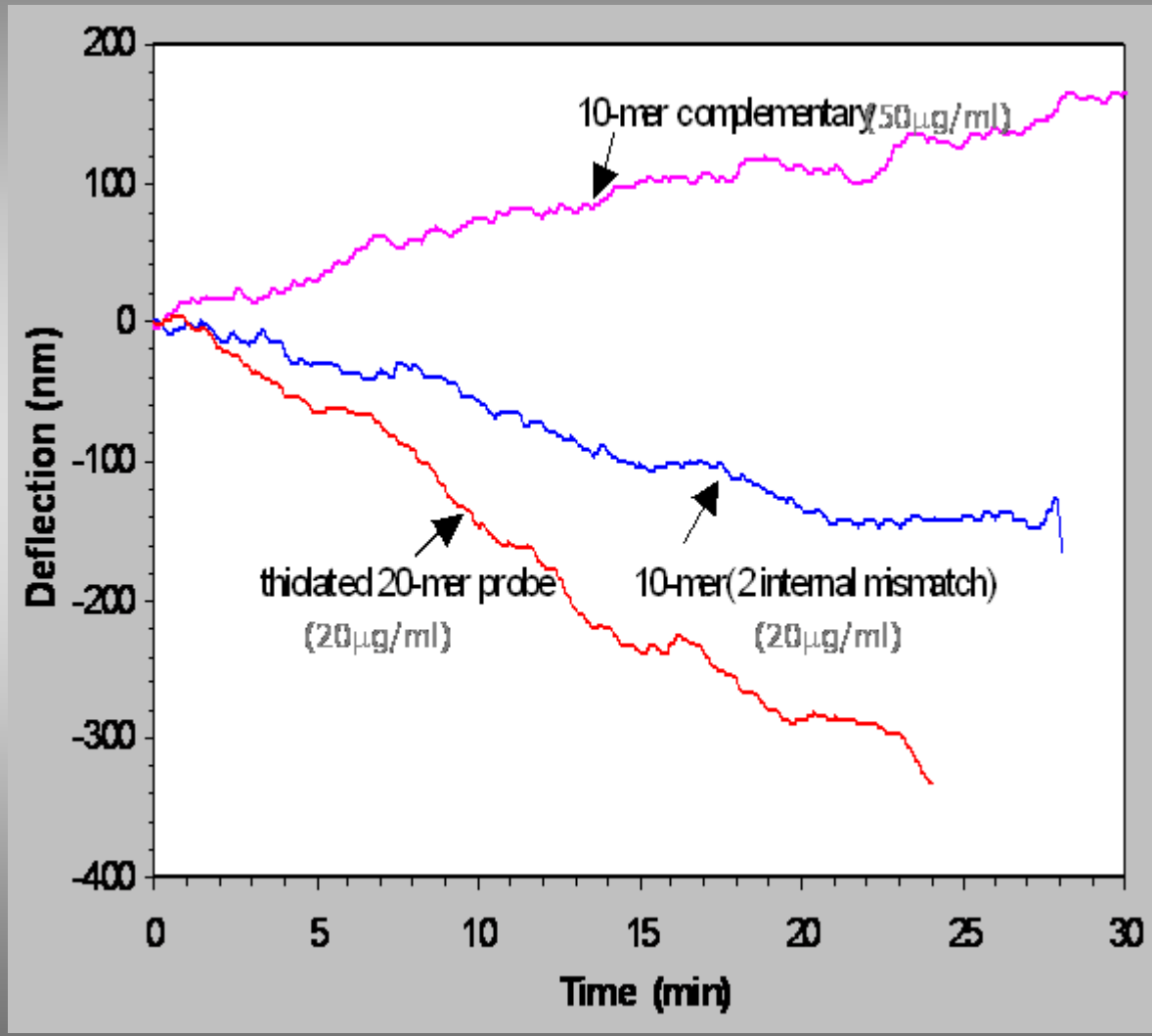
→***Our dealloyed surfaces not only provide larger signals, but also better reversibility. Studies are currently underway to investigate mechanism***

MCA extra

Cantilever Deflections Can Also be Used for DNA Hybridization Studies

→ *The direction of cantilever motion is opposite during immobilization and hybridization of ss-DNA on two different surfaces (dealloyed gold, smooth gold).*

→ *Inconsistencies maybe related to the complexity of surface charge, adsorbate-surface, adsorbate-adsorbate, and adsorbate rearrangement effects.*



MCA extra

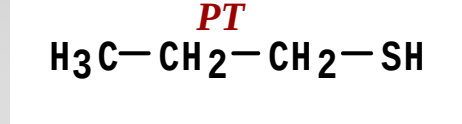
Metal Ion Detection

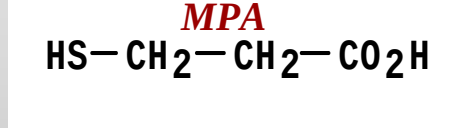
- *Reasons for heavy metal detection*
  - *Highly toxic*
  - *Bioaccumulation*
- *Traditional detection methods*
  - *Chromatography*
  - *Flow-injection systems*
  - *Atomic absorption*
  - *Electrochemistry*
  - *Sensors*

  (e.g., microcantilever MEMS)

- *Take advantage of thiol-gold binding to form SAMs of monodentated ligands*

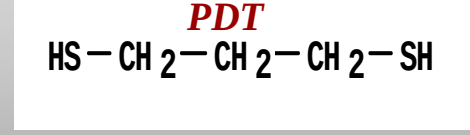
AET


PT


MPA


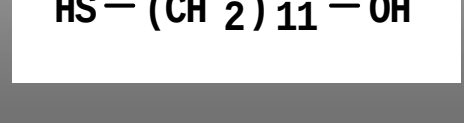
ODT

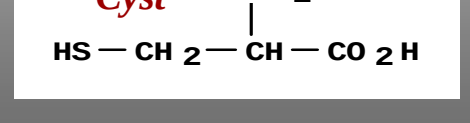

MUA


PDT


MP


OTD

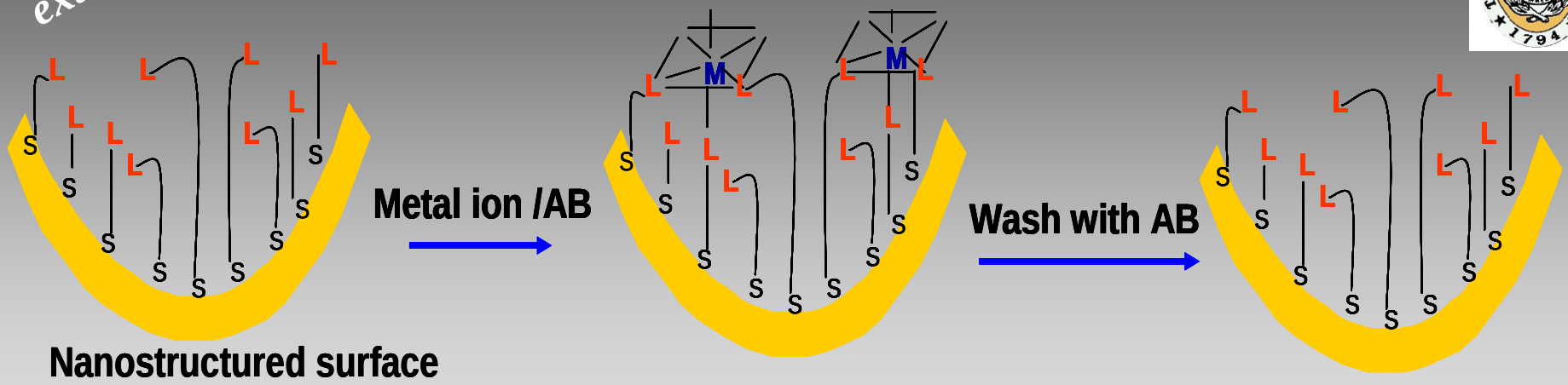

MUD


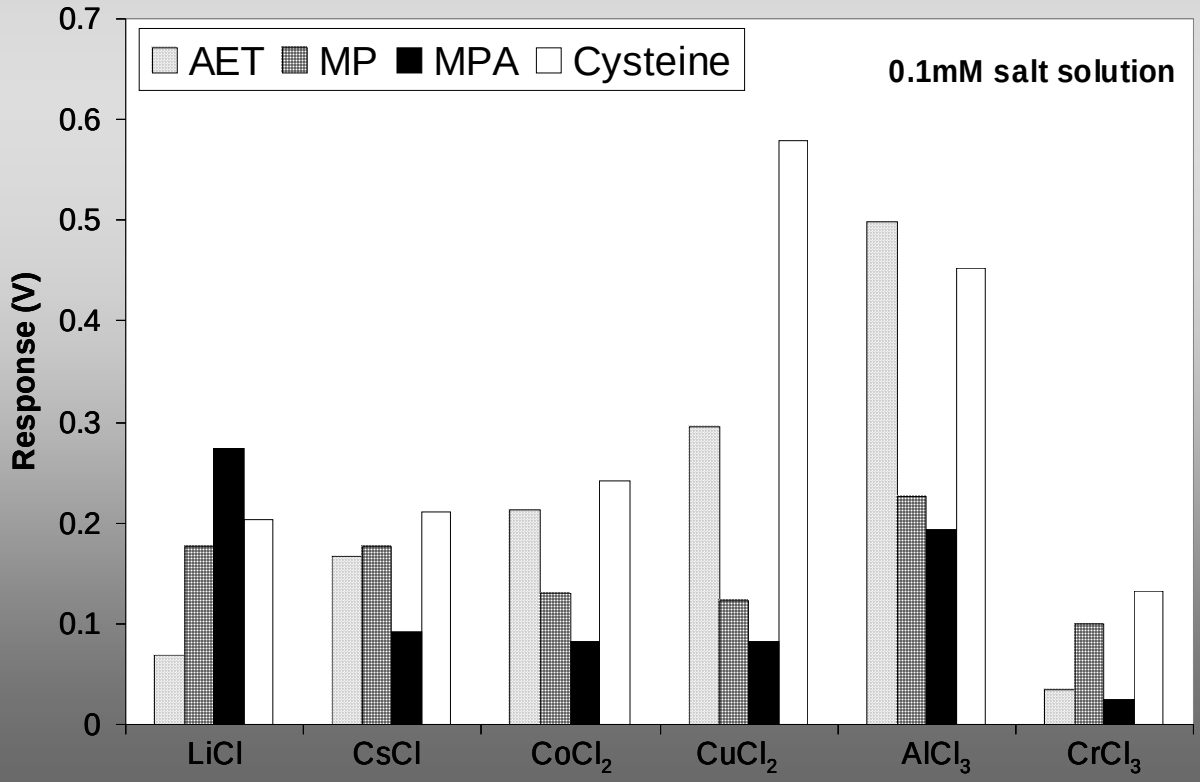
Cyst


EPA STAR EDC-'06

MCA extra

Metal-Ligand Interaction & MC Response



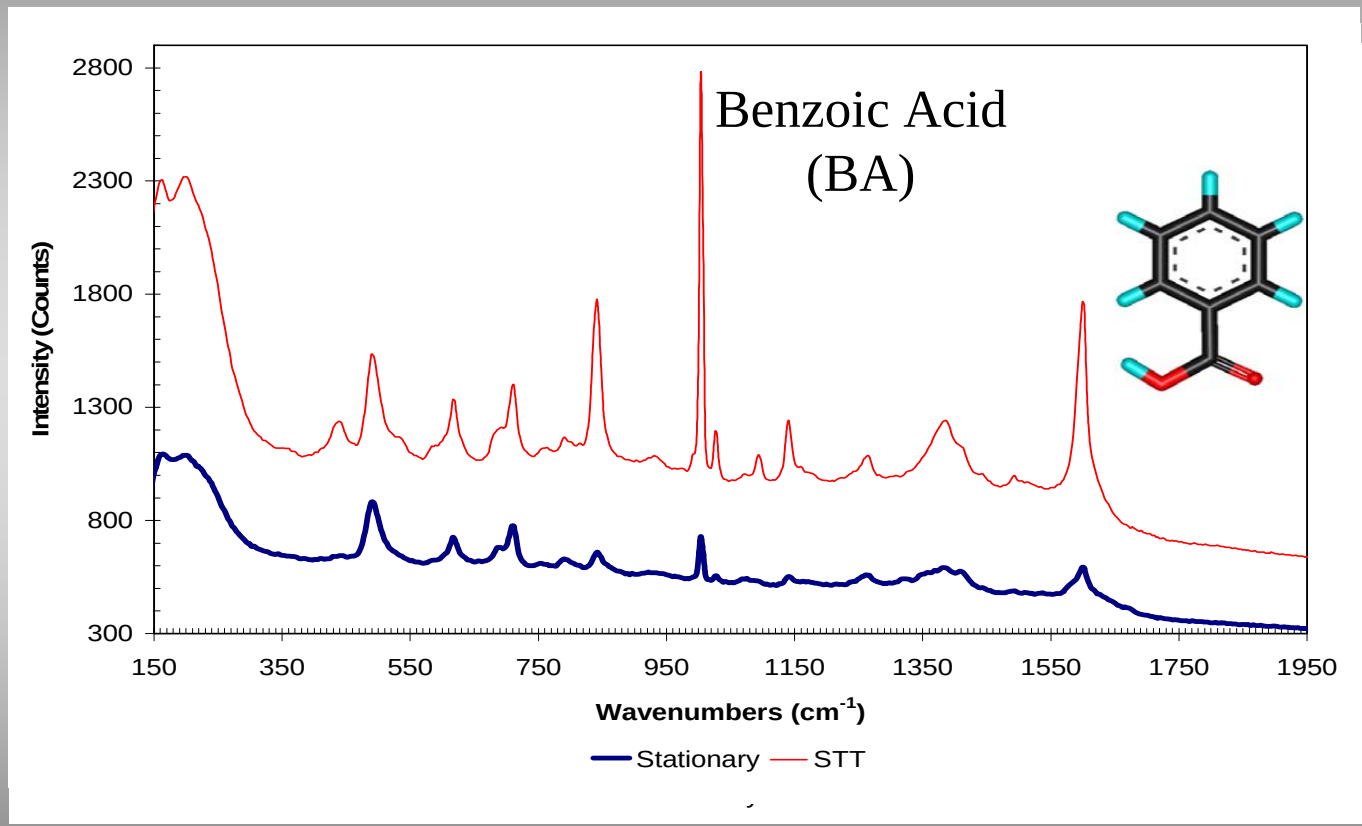


Selectivity patterns For MCs with SAMs of different monodentated ligands

SERS *extra*

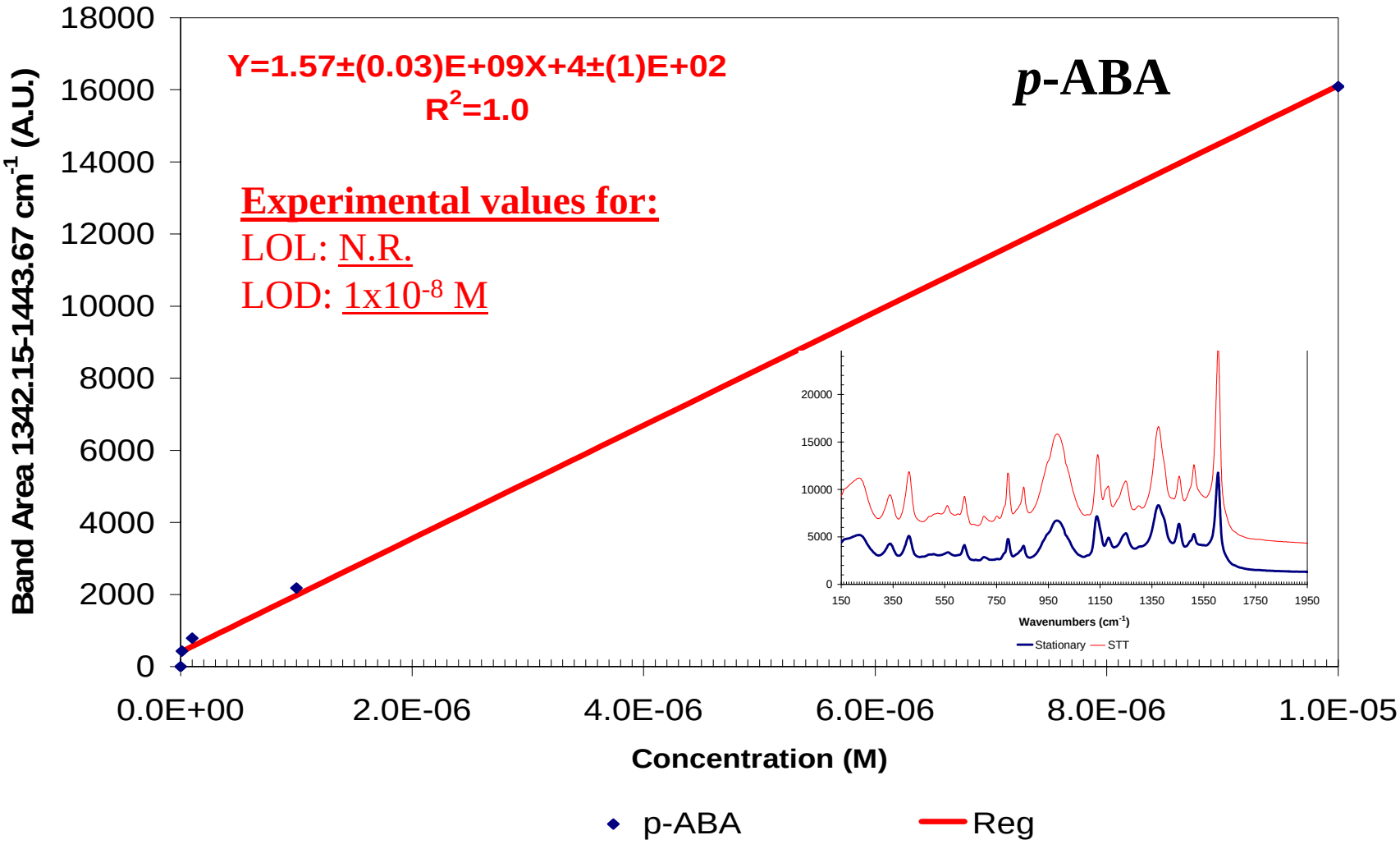
Sample Translation Technique;
Effect on Various Analytes

Samples centered on spinner then moved off-center a few hundred μm & spun at least several hundred RPM to yield STT spectra



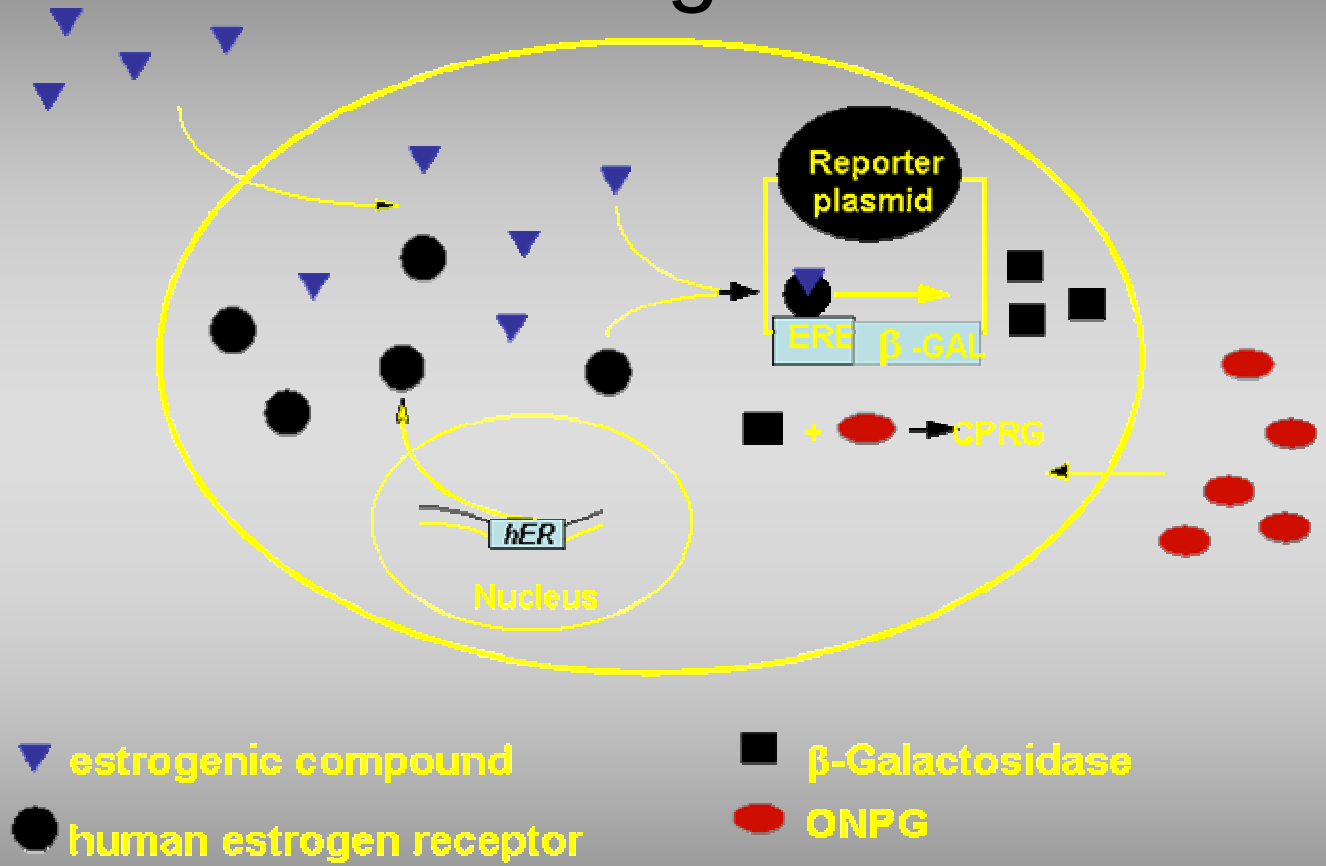
SERS *extra*

Quantitative Performance of STT-SERS With Ag-PDMS Substrates



Bio extra

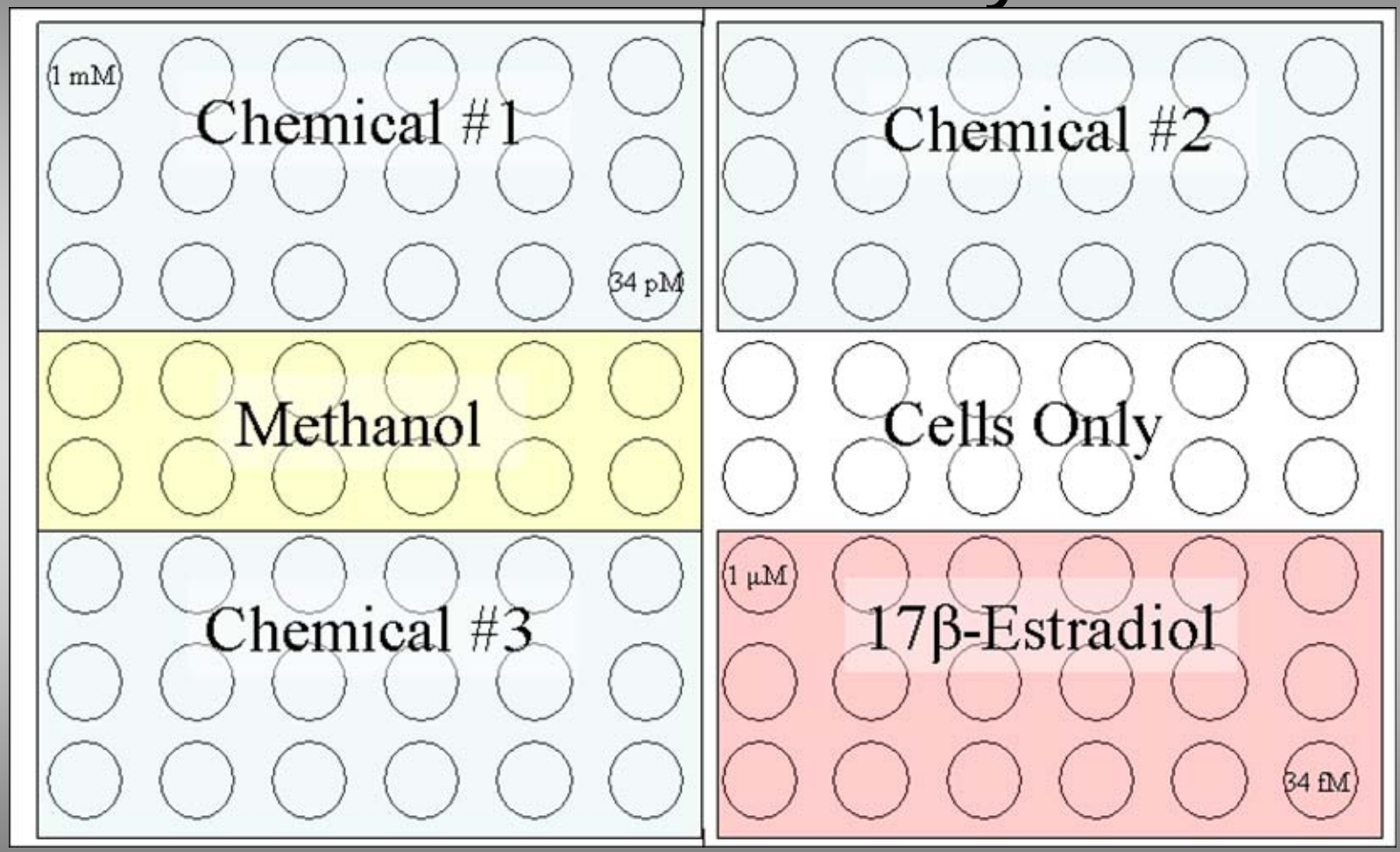
Saccharomyces cerevisiae Yeast Estrogen Screen



Routledge and Sumpter. 1996. Environ. Toxicol Chem. 15:241-248

Bio extra

Microtiter Plate Layout



Our EPA STAR work is focused on development of advanced, materials-based, integrated analytical methodologies for EDC analysis

Objectives:

→ ***Develop conditions for capillary then on-chip electrokinetic separations***

→ ***Optimize nanocomposite-based SERS substrate morphologies for greatest activity and survey responses***

→ ***Evaluate nanomechanical response signatures using functionalized MCAs***
- ***traditional molecular recognition phases***
- ***bioaffinity phases for EDCs***

→ ***Integrate bioreporter yeast conditions for total systems with bioluminescence-based screening of EDCs and the aforementioned advanced analytical technologies (initially in titer-plate to microfluidic configuration; ultimately a totally integrated platform)***