Improved Analytical Technologies for Detection of Foodborne Toxins and Their Metabolites

CRIS 5010-42000-049-00D

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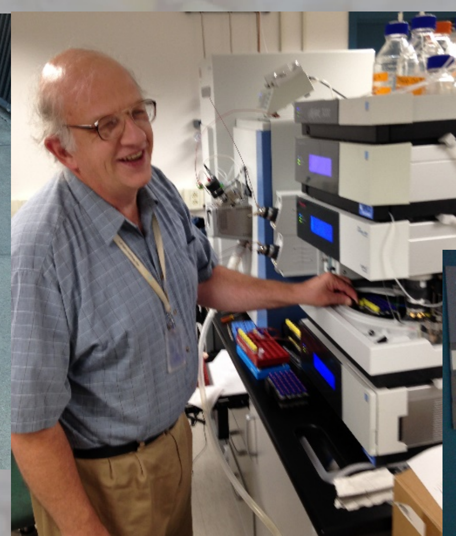
Chris Maragos



Mark Busman

Mike Appell

Monitoring: the first (and last) line of defense

- Supports efforts to mitigate toxin production
- Supports efforts to determine toxicological relevance
  - Hazard assessment
  - Trace-back causation
- Reduces exposure
  - Improves food safety
  - Improves feed safety
- Facilitates trade

Goals of this CRIS

- Improve Methods for Detection of Toxins
  & Related Metabolites
- Reduce Exposure to Toxins
  & Related Metabolites

Objectives

- Improved Detection Methods

Objective 1: Improve detection of foodborne toxins through development of novel technologies based upon biosensor platforms and new component materials.

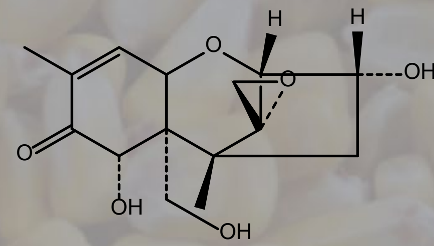
Objective 2: Improve detection of foodborne toxins through development of direct detection technologies based upon novel mass-spectrometric platforms

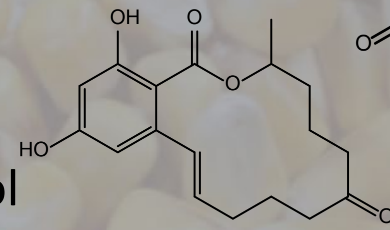
Objective 3: Improve the ability to detect and measure “masked” mycotoxins and biomarkers of mycotoxin exposure in commodities and foods

- Reduce Exposure

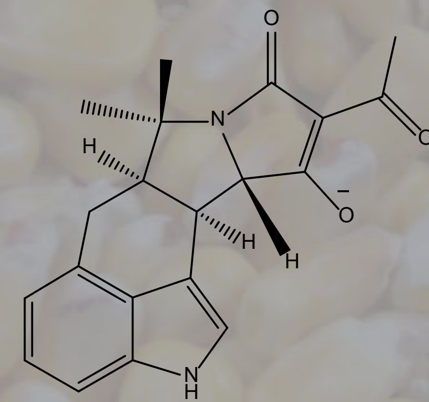
Objective 4: Improve toxin detection methods and reduce exposure through the development and application of synthetic materials.

Recent Accomplishments
(mile high view)

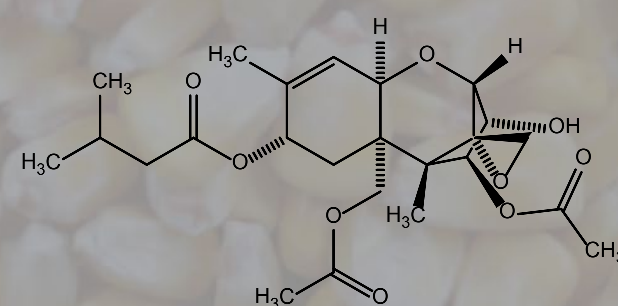




- **Objective 1 (Biosensors)**
  - iSPR biosensor for T-2 toxin, Zearalenone (ZEA) & Deoxynivalenol (DON) in maize
  - Biolayer Interferometry Sensor for DON in wheat dust (Sanders)
  - Antibodies & immunoassays for Cyclopiazonic acid (CPA) in maize & cheese
  - Rare Earth Luminescence assay for CPA

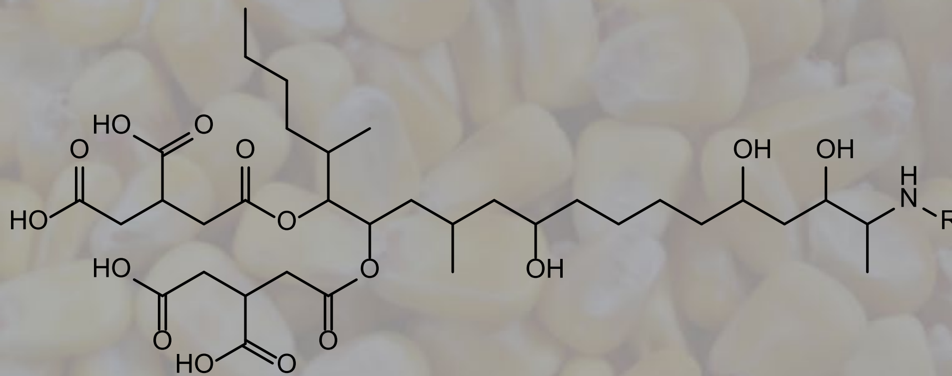


- **Objective 2 (Novel Mass Spec Methods)**
  - Laser-Assisted Ambient ionization-mass spectrometry aflatoxin B_1

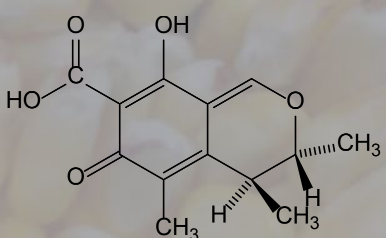


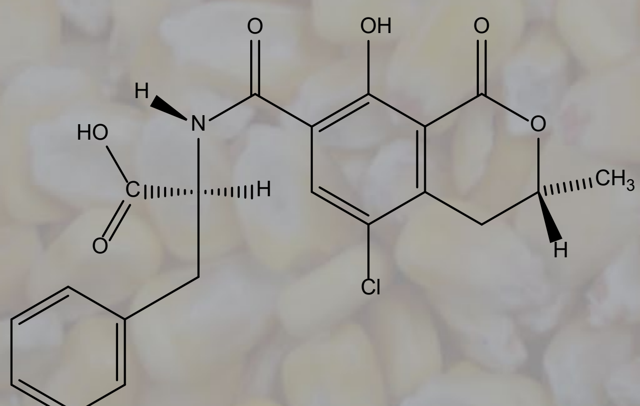
Recent Accomplishments
(mile high view) (2)

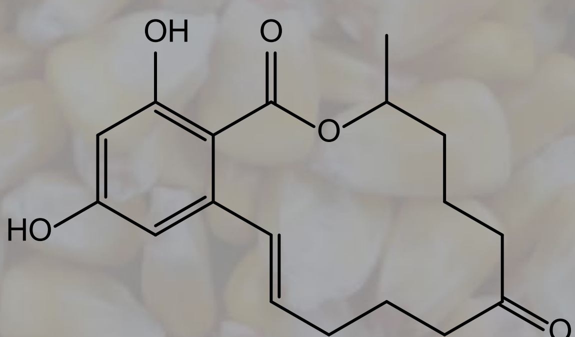
- **Objective 3 (Masked Mycotoxins)**
  - Antibodies & Immunoassays for masked fumonisins
  - LC-HRMS for masked fumonisins



- **Objective 4 (Reducing Exposure)**
  - Molecularly imprinted polymers (MIPs) for citrinin
  - “Nanosponges” for ochratoxin A (OTA)
  - Computational modeling of zearalenone (ZEN), CIT, & trichothecenes



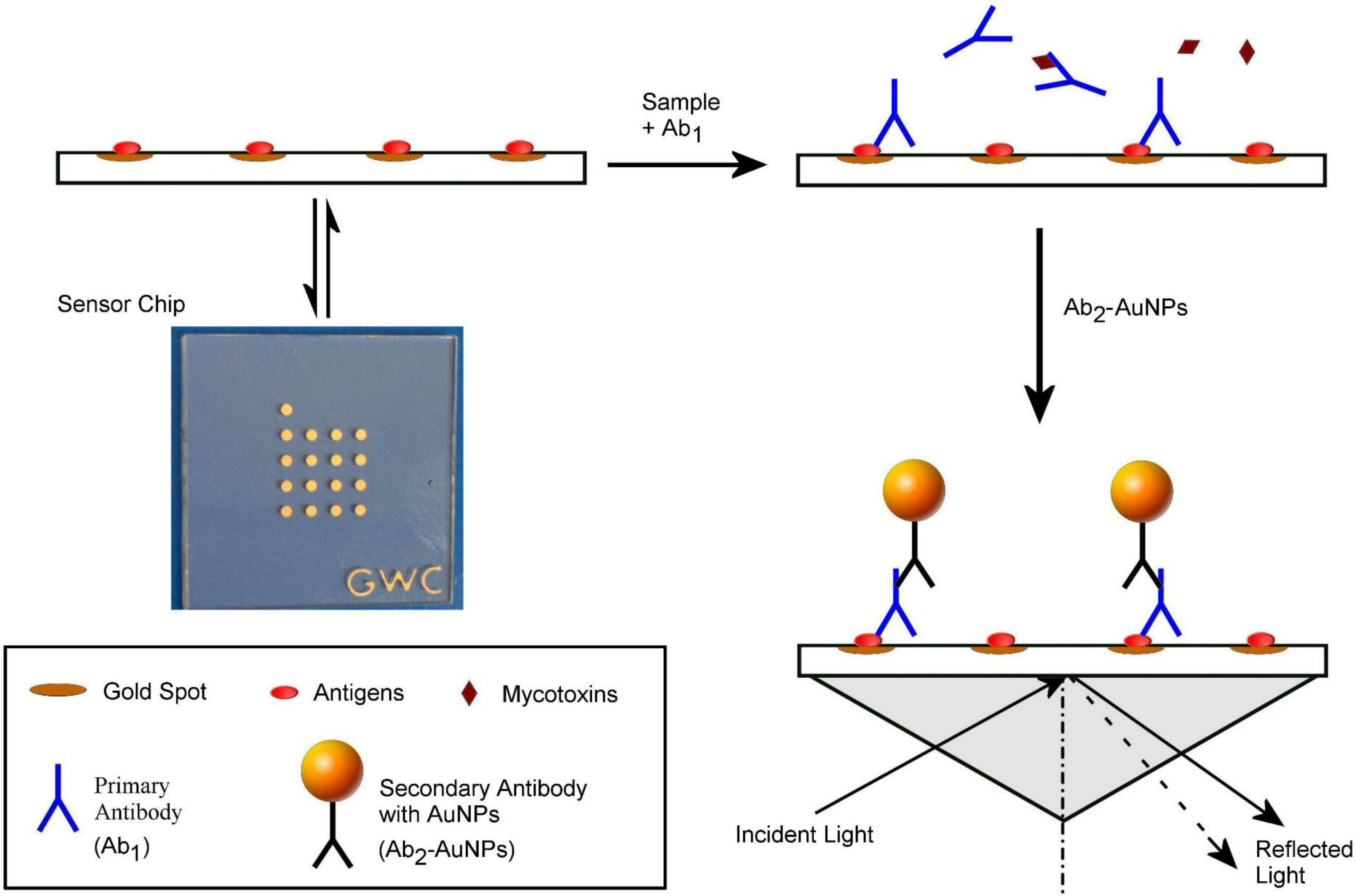




Objective 1:
Novel Biosensors

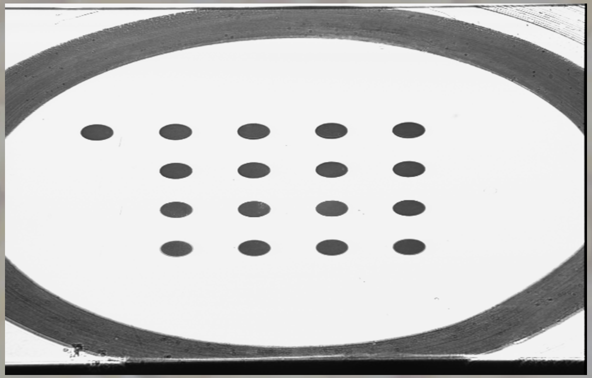
Md Zakir Hossain

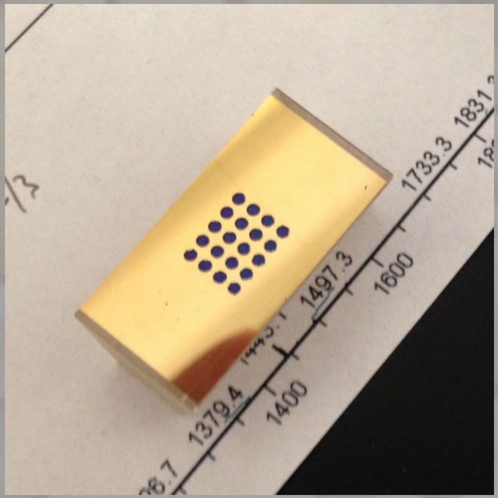
Imaging Surface Plasmon Resonance (iSPR)



iSPR Biosensors

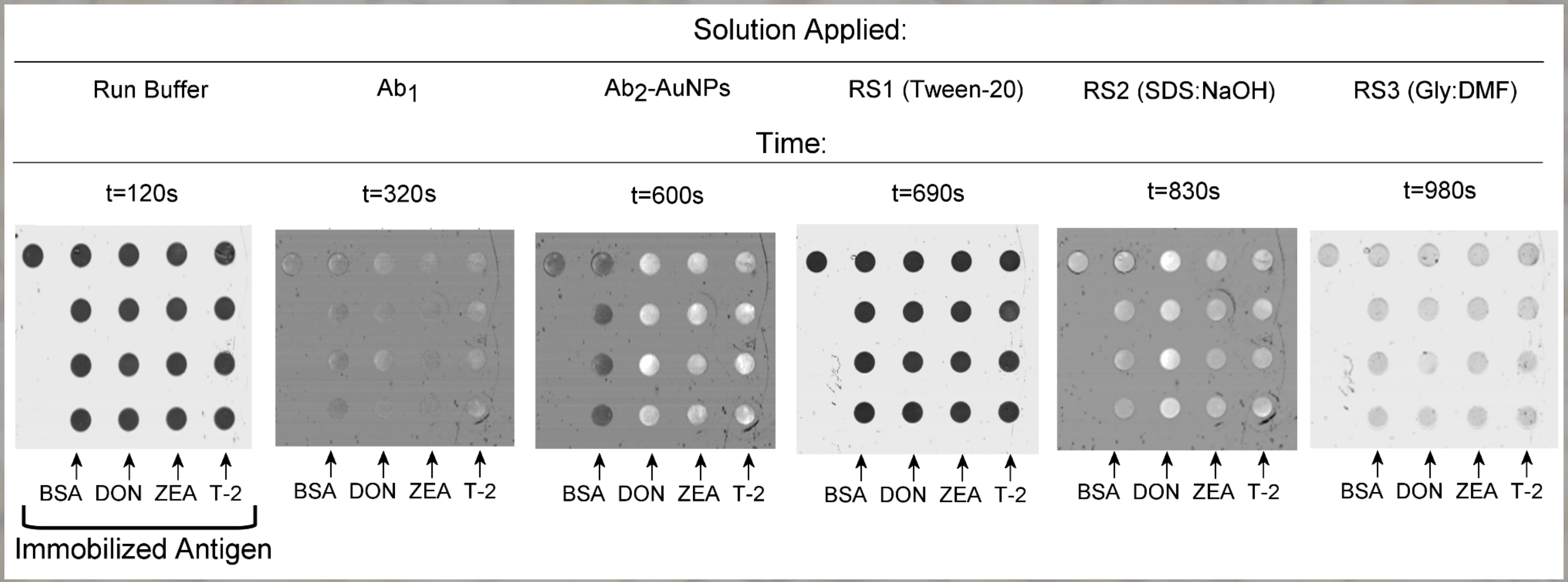
- Multiplexing: one sample extract, one chip,
  multiple tests

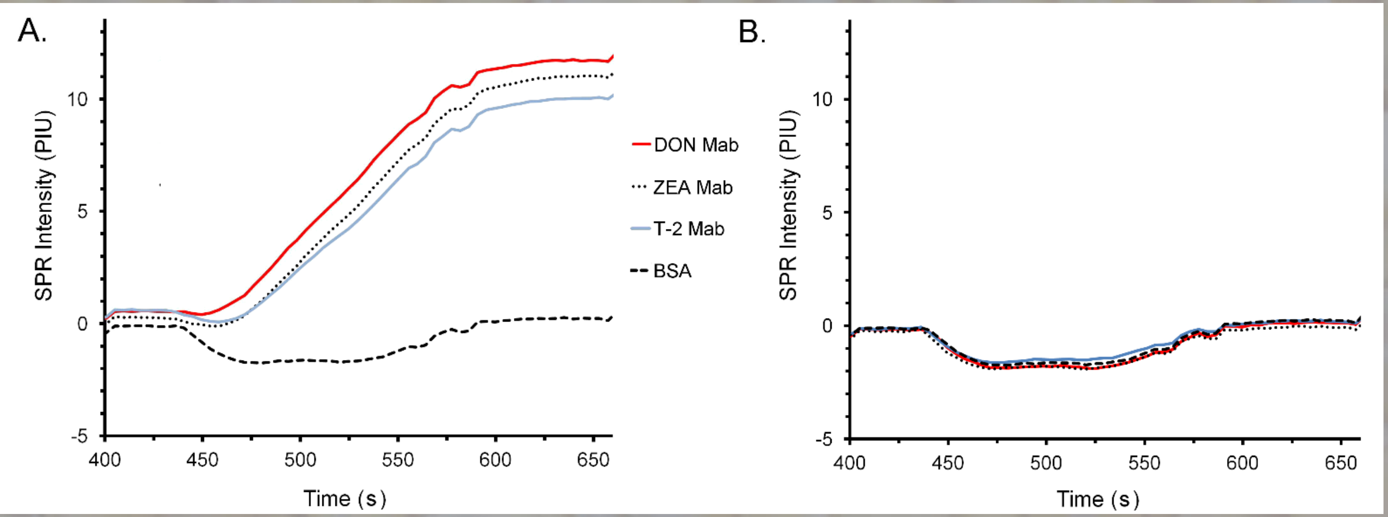




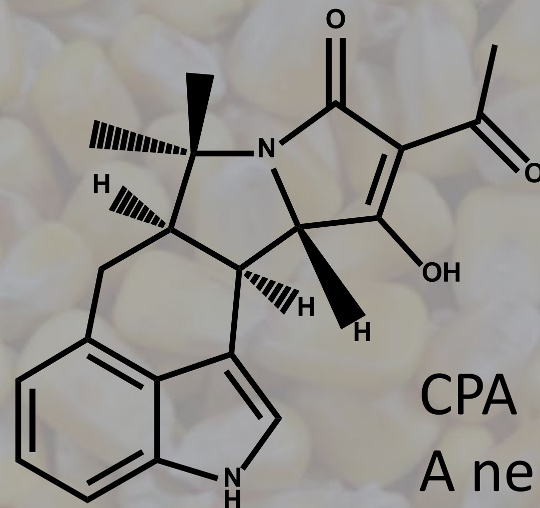
- Reusable chips
- Fairly rapid for quantification
- Can be made more rapid for qualitative testing
- Significant potential for automation

Multiplex Biosensor for *Fusarium* toxins in wheat

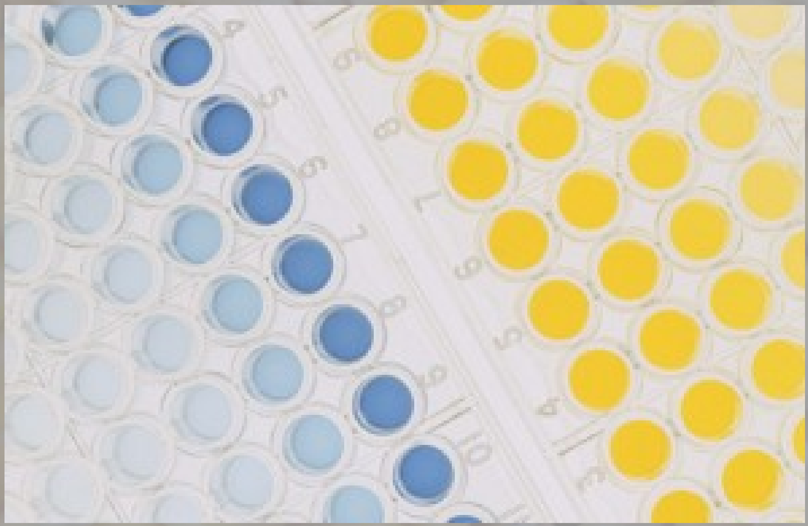


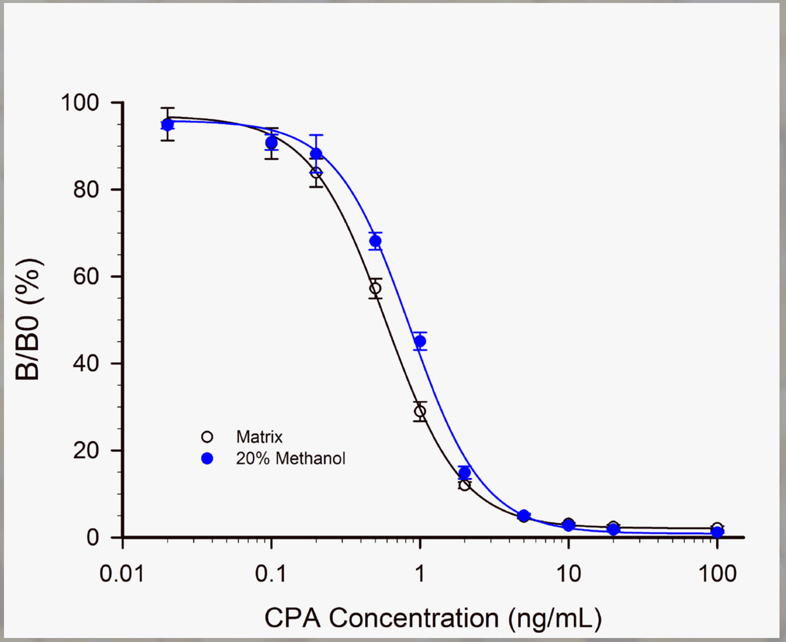


Immunoassays for CPA

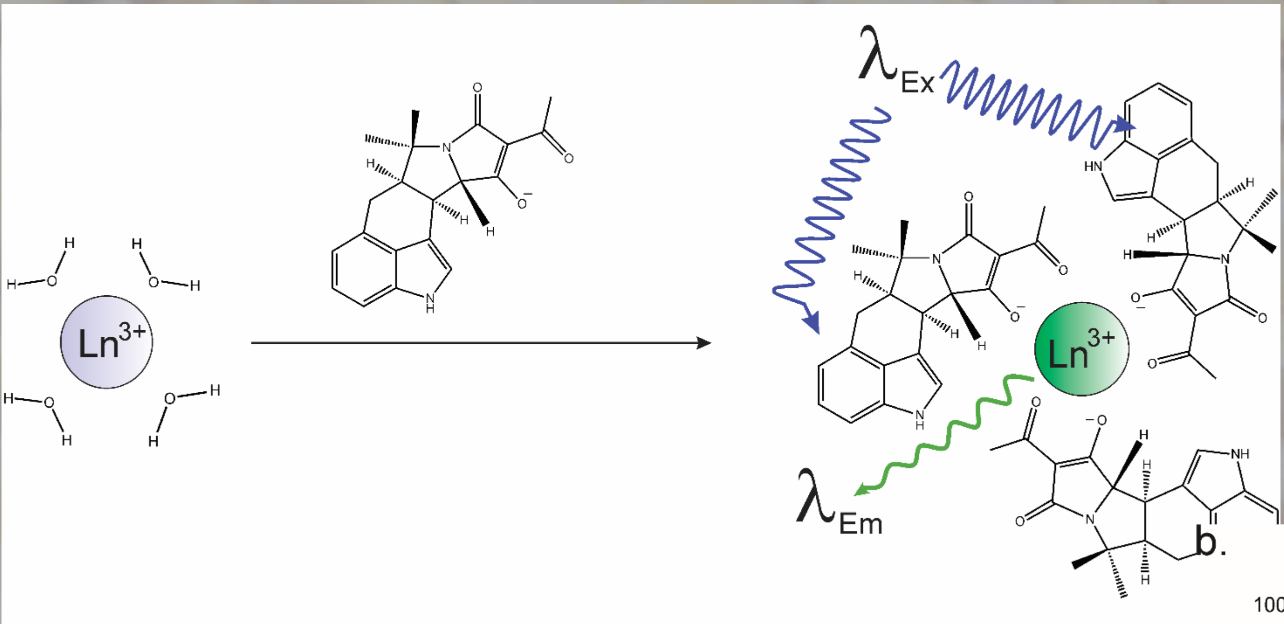


CPA
A neurotoxin

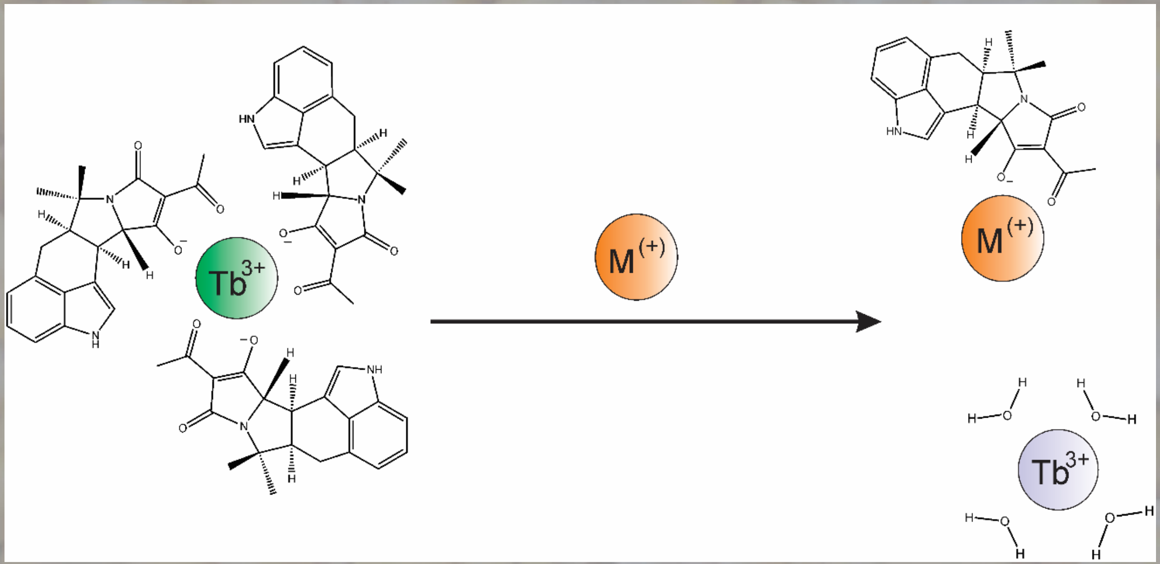


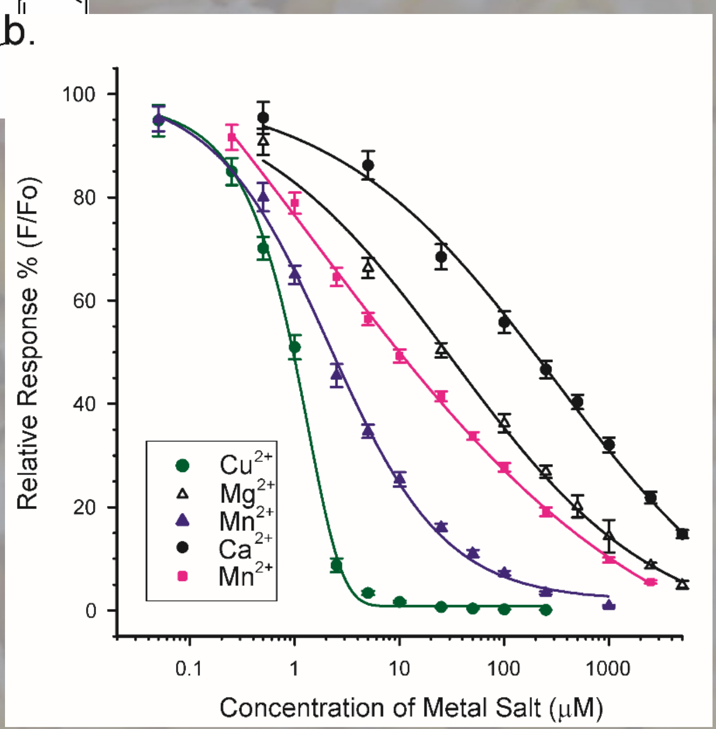


Luminescent Probes for CPA



(1) Detection of CPA
(2) Mechanism of Action

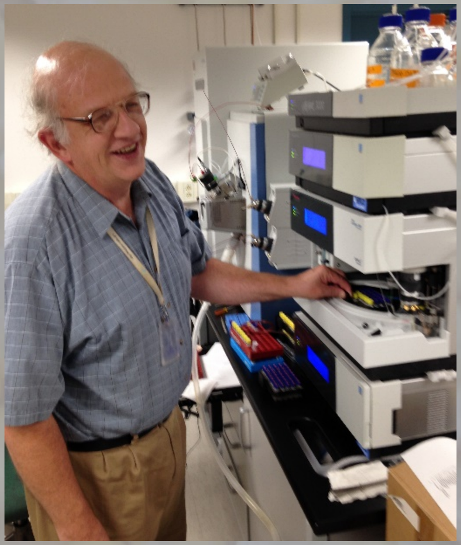




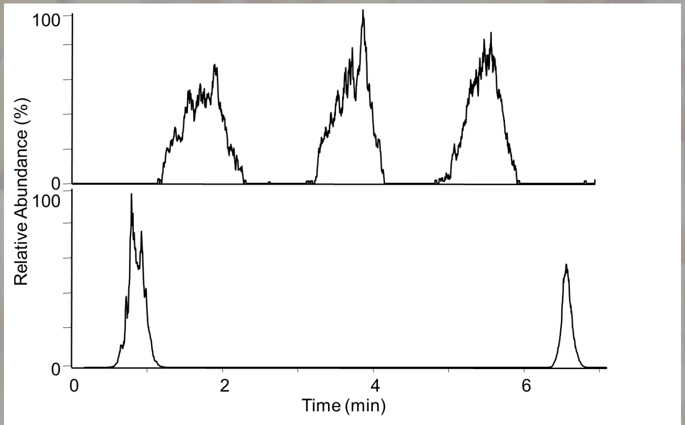
Objective 2:

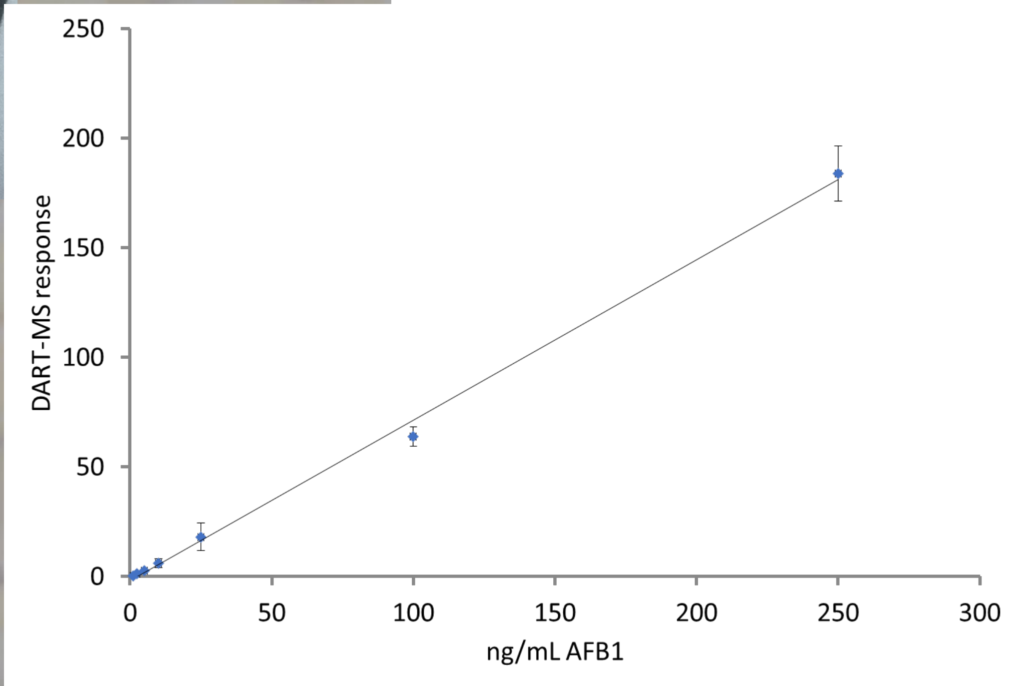
Novel Mass Spectrometric Methods

Ambient ionization MS of mycotoxins



Presentation of Small Surfaces to the DART-MS Interface



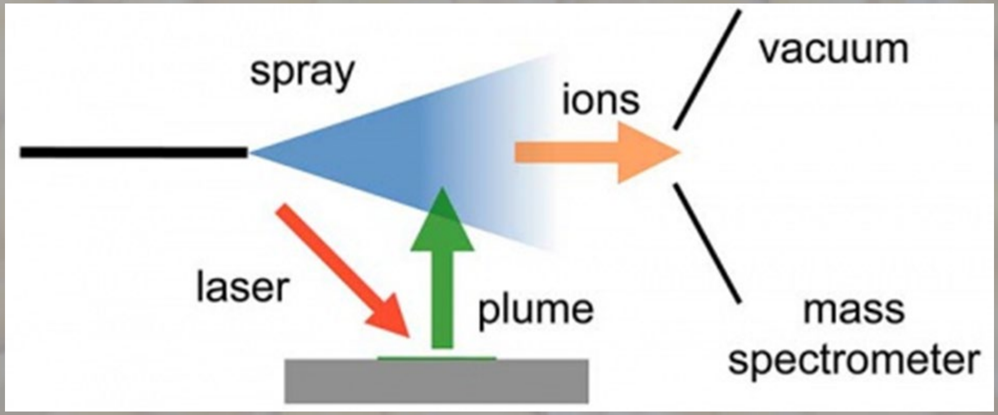


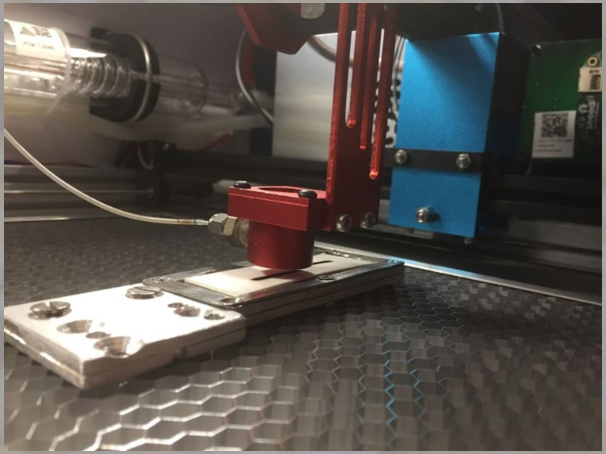
Advantages of Laser Assistance to Dart-MS

- allows analysis of difficult analytes
- allows spatial resolution of sampling
- applicable to imaging applications

Accomplishments for ambient ionization objective (laser assisted DART-MS)

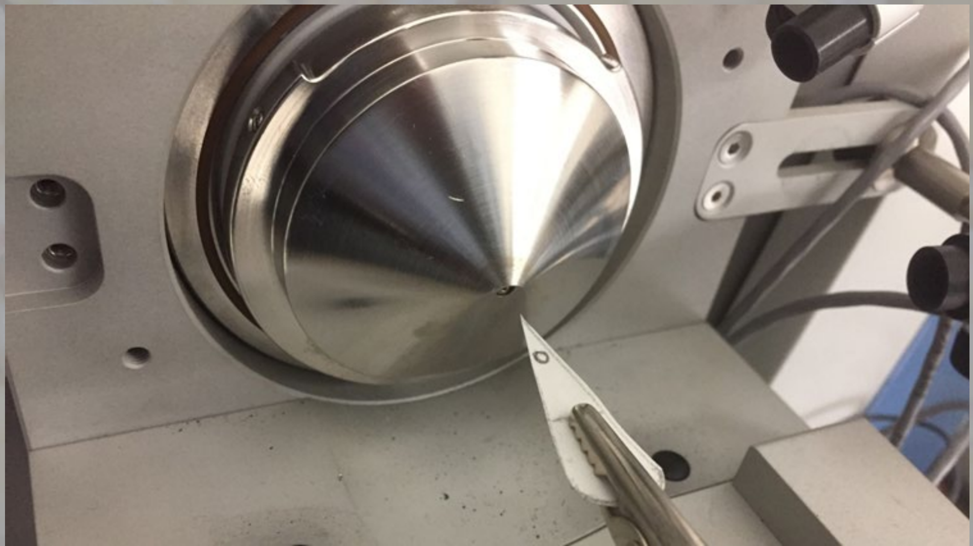
- construction and characterization of IR laser assisted DART-MS apparatus
- development of technique for IR laser assisted DART-MS analysis of AFB1 in maize extract
- development of technique for IR laser assisted DART-MS analysis of AFB1 from glass slides





Accomplishments for ambient ionization objective (multiplexed analysis)

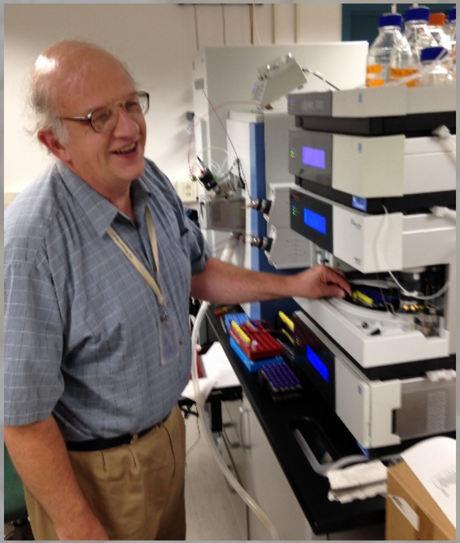
-development of technique for DART-MS multiplexed analysis of AFB1 and T-2 toxin from maize extract
-DART-MS and other ambient ionization methods compared for multiplexed analysis of 10 common Fusarium toxins in maize extract
-development of technique for paper spray-MS analysis of fumonisins



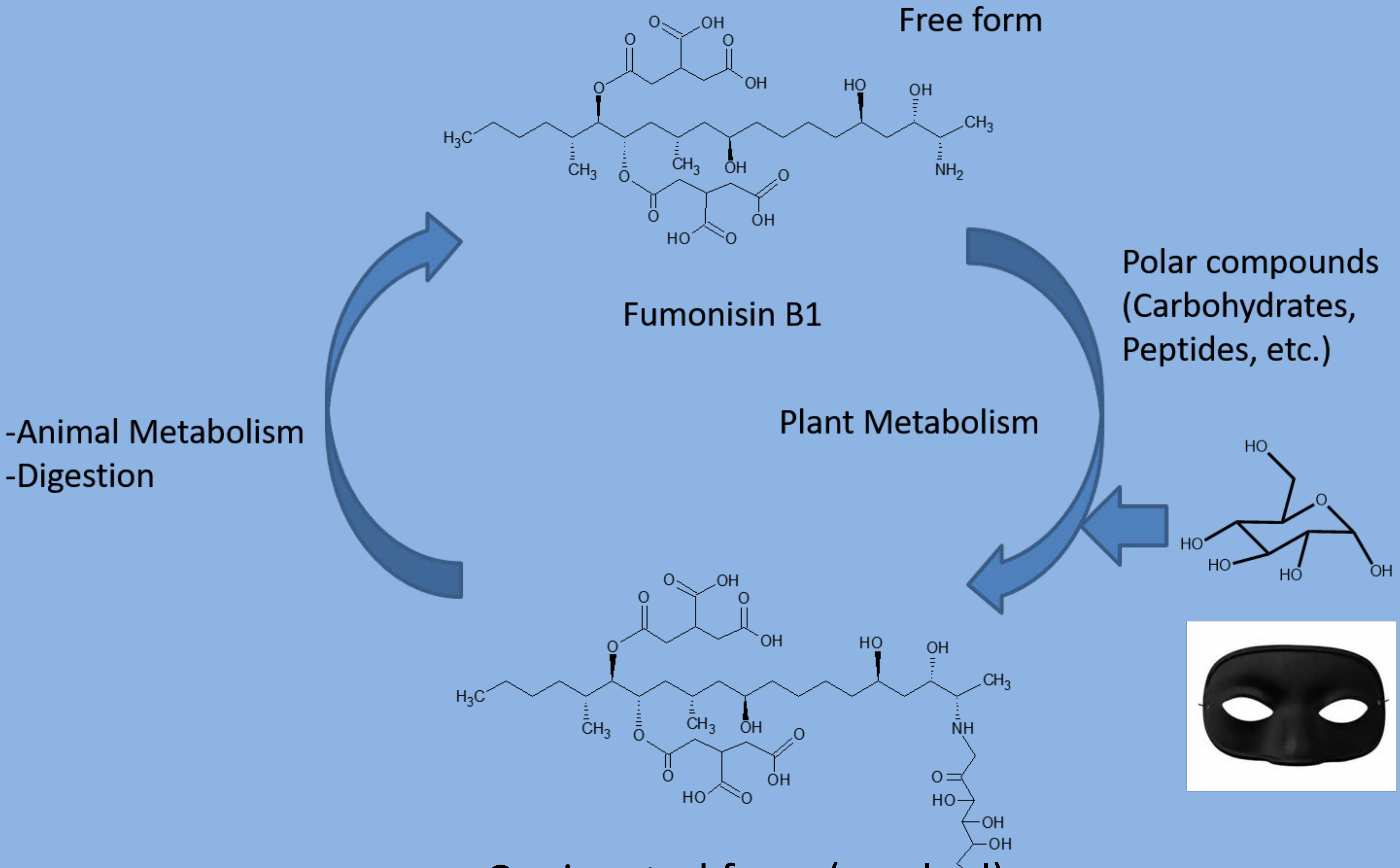
Objective 3

Detection of “Masked” Mycotoxins

Antibodies/Immunoassays/
Biosensors



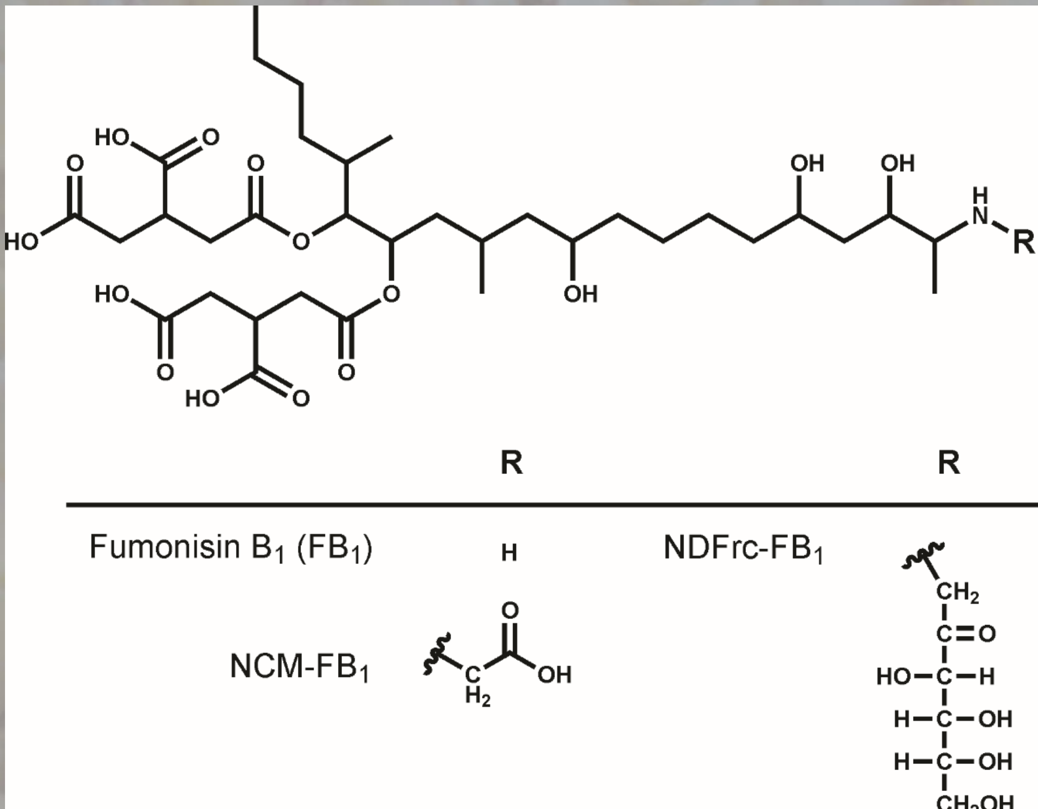
Mass Spec-Based Methods

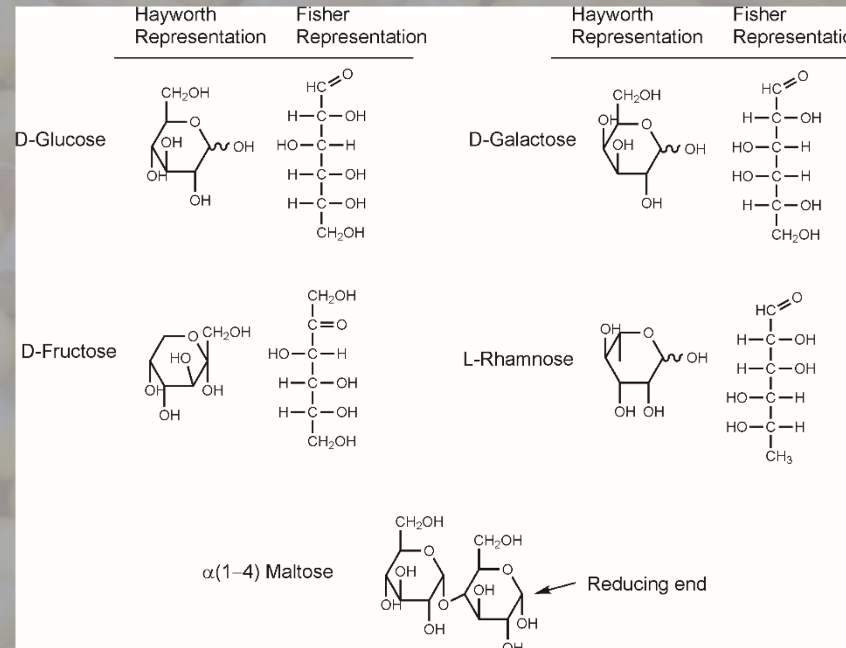


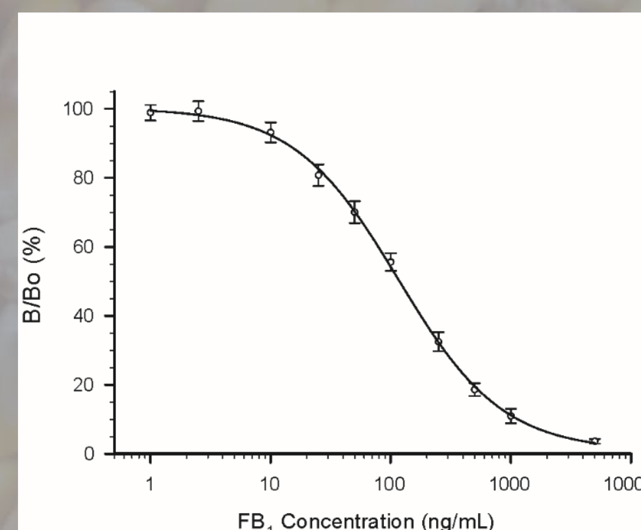
Conjugated form (masked)

(hidden from traditional monitoring techniques)

Antibodies for NDFrc-FB_1

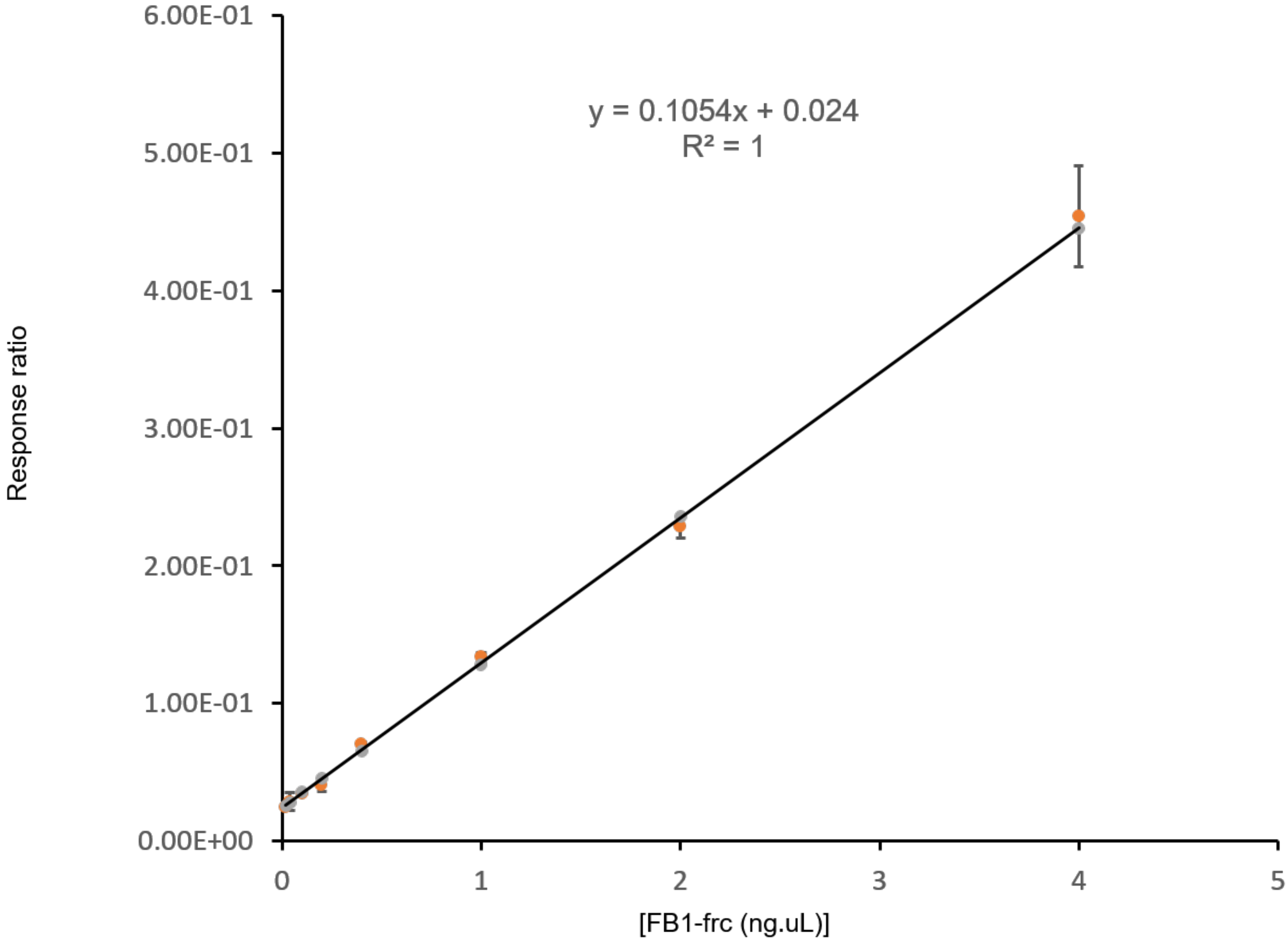




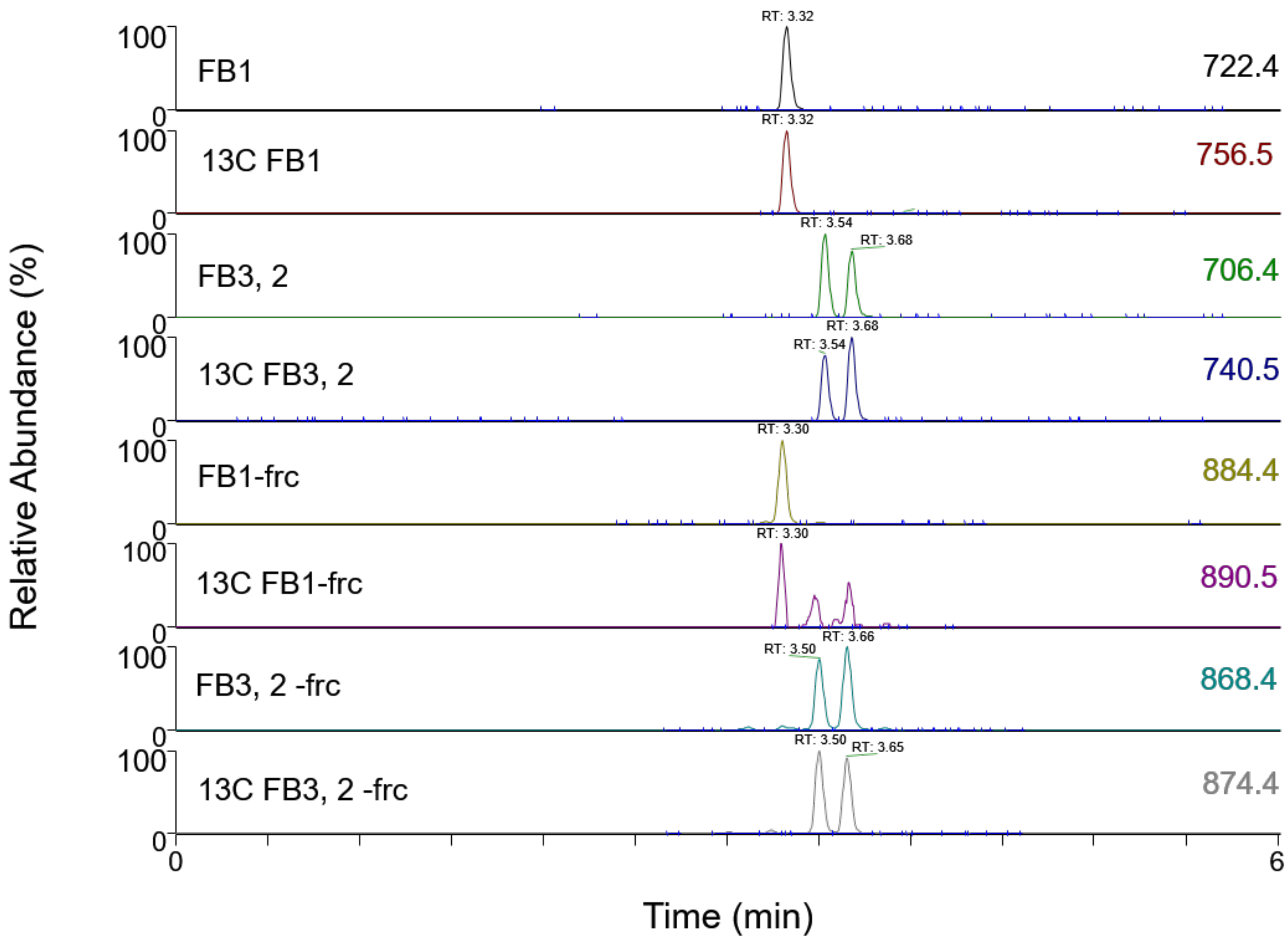


Application of Immunoaffinity columns and LC-MS for detection of masked fumonisins

Isotope ratio calibration curve: matrix standards



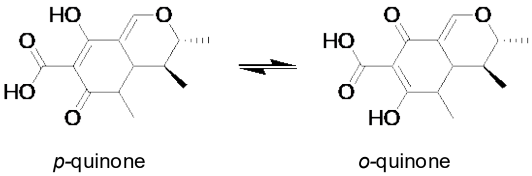
Naturally contaminated maize



Example analyses:

	FB1 (ppm)	FB1-frc (ppm)
Corn meal	0.22 (0.17)	0.54 (0.47)
Maize (artificially contaminated)	200.14 (32.06)	21.68 (2.02)
Maize (naturally contaminated)	10.26 (1.67)	3.37 (0.40)

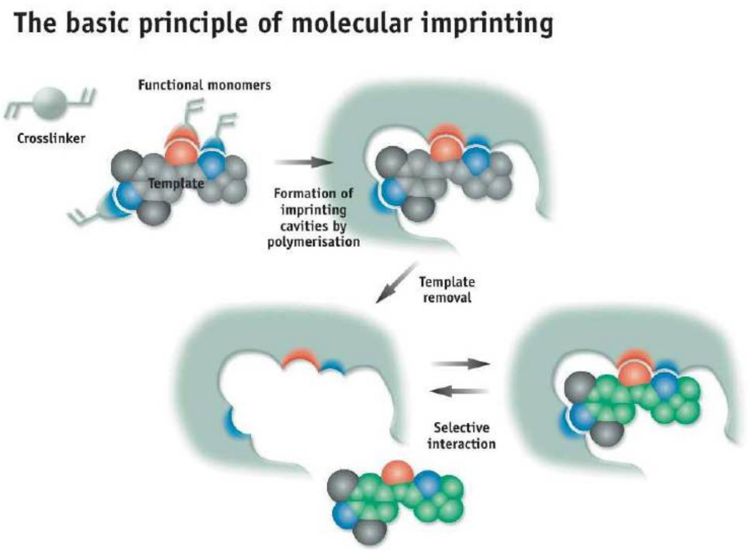
Synthetic Materials for Detection of Citrinin



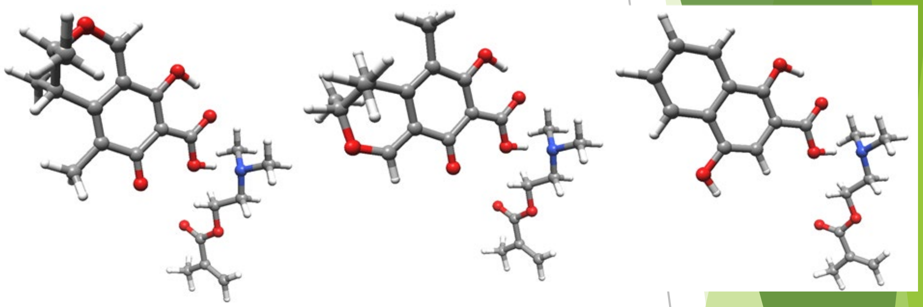
- Citrinin is a toxic contaminant of a number of agricultural commodities, including corn, grains, cheeses, and notably *Monascus*-fermented red rice
- Associated with kidney and liver damage, and often occurs with aflatoxins and ochratoxins
- EU has set a regulated limit of 2000 ppb for citrinin in *Monascus*-fermented rice products (supplements)
- Advisory limits for exposure in agricultural commodities have been issued for

  Japan (200 ppb) and EU (100 ppb)
- Developed an analytical method to improve determination of citrinin in corn using a novel synthetic material for sample clean-up
- Why synthetic materials?
  - economical
  - capability to incorporate chemical groups that do not exists in biological binding materials
  - solvent-tunable affinity that can overcome the limitations of current materials

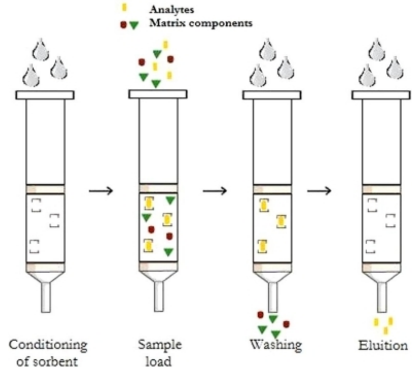
Preparation of Molecularly Imprinted Polymers (MIPs) for Citrinin



Rational design of materials



MISPE Analysis

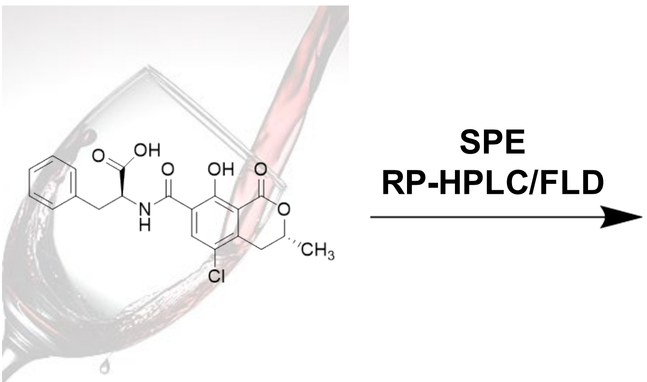


SUMMARY

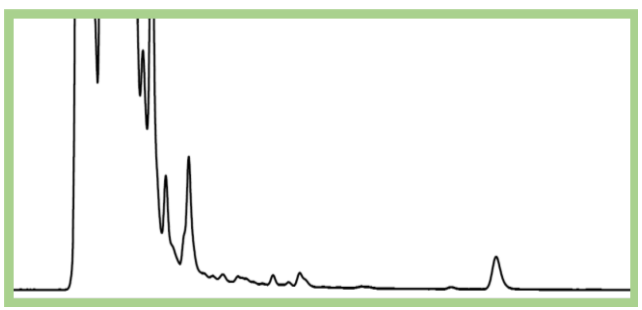
- **Deprotonation of citrinin is a major factor governing the shifts in fluorescence excitation maxima.**
- **A reliable method to detect citrinin in maize was developed using LC-fluorescence detection with MISPE sample clean up (LOD 0.01 $\mu g\ g^{-1}$; LOQ 0.03 $\mu g\ g^{-1}$).**
- **Significant recoveries of citrinin were obtained (82.3-91.5%) between 0.03 - 3 $\mu g\ g^{-1}$**

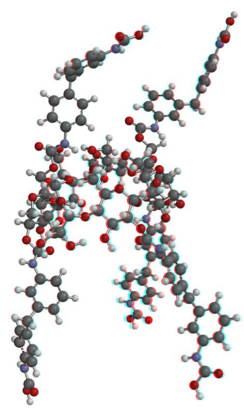
Preparation of “Nanosponges” for Sequestration of Ochratoxin A

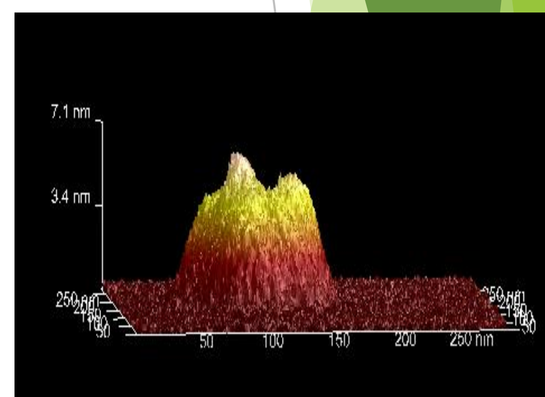
Ochratoxin A can contaminate a variety of commodities and foods, including fruit juices and wines.



Nanoscale features enable the novel β-cyclodextrin, polyurethane nanosponge material to selectively bind ochratoxin A in grape juice and wine during SPE sample clean-up.

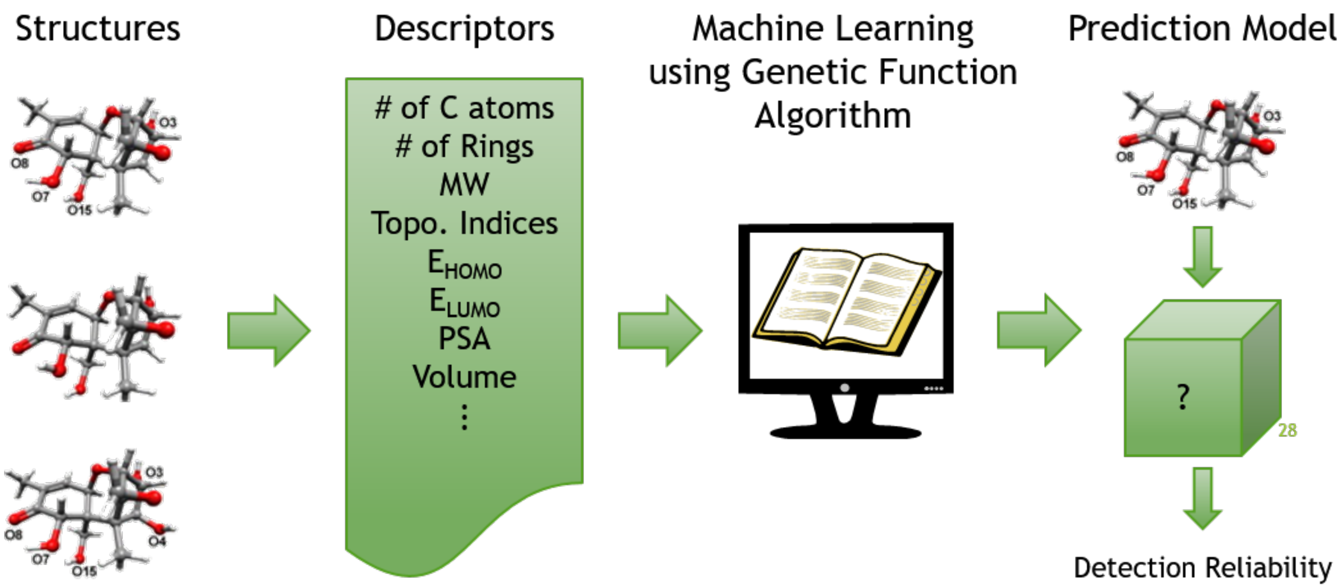






A liquid chromatography method using fluorescence detection (LC-FLD) was capable of detecting ochratoxin A levels below regulatory levels in grape juice and wine.

Use of Cheminformatics and Bioinformatics to Inform Detection Properties



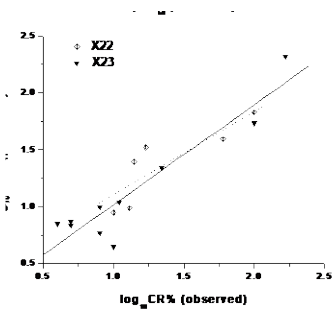
National Taiwan University

In collaboration with Prof. Yufeng Jane Tseng's group at National Taiwan University and Dr. Yi-shu Tu, a visiting scientist at NCAUR

Predictive Modeling of Cross-Reactivity Based Upon Structural Features

Trichothecenes

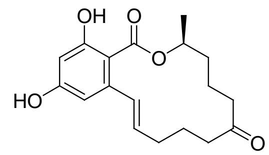
very large family of chemically related mycotoxins



False-positive detection is associated with spatial autocorrelation indices. **Trichothecenes with bond connectivity similar to DON can interfere with DON detection**

Zearalenone and analogs

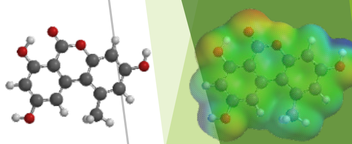
potent estrogenic metabolite produced by some *Fusarium* and *Gibberella* species



pH and the general structure of zearalenone must be conserved during fluorescence analysis to ensure accurate quantitation

Alternariol Alternariol monomethyl ether

European Commission has mandated the development of standardized methods for Alternaria toxins in foods



Quantum chemical analysis indicates UV and Fluorescence based detection require separation techniques to distinguish between alternariol and alternariol monomethyl ether

Future Projects

- More accurate determination of Ochratoxin A in oats
- Sample clean-up through green imprinted magnetic beads
- Incorporation of synthetic materials into additive manufacturing at NCAUR

Accomplishments (Non-Publication)

- MTAs – antibodies (old mechanism)
- MTRAs
- CRADA -Biosentinel
- Biological Material Commercial Evaluation Agreements – antibody producing cell lines
- Licensing Agreements ~30
- Testing kits based on these materials are provided by several manufacturers and are sold world-wide

Thanks for your attention!