Purdue-UAB

Pennington-Rutgers

UIC

Technologies and experimental approaches in the NCCAM/ODS Botanicals Centers

Stephen Barnes, PhD

Purdue-UAB Botanicals Center for Age-Related Disease

West Lafayette, IN and Birmingham, AL

Center for Botanical Lipids
AT WAKE FOREST UNIVERSITY HEALTH SCIENCES

Wake Forest-Harvard

Memorial-Sloan-Kettering

Iowa State

Challenges in NCCAM Botanicals Centers

How to bring systematic research to a complex problem

A botanical is a complex mixture

↓ *Reductionist approach*

Identify the active agent and study it using *in vitro, molecular,* cellular and *in vivo* models

A disease is complex process

↓ *Reductionist approach*

Identify the target and study it using *in vitro,* molecular and cellular models

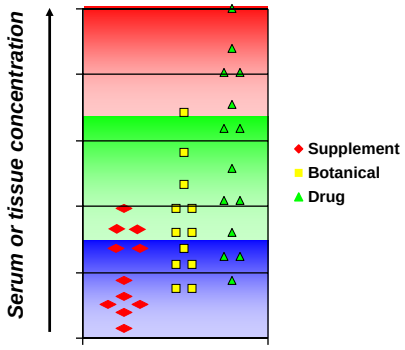
Pharmaceuticals versus botanicals or supplements

Target specific	What is the target?
Are they absorbed?	Are they absorbed?
Metabolism?	Metabolism?
Pharmacokinetics?	Pharmacokinetics?
Tissue levels?	Tissue levels?
Effective doses?	Effective doses?
Toxic doses?	Toxic doses?
Pharmacogenomics?	Nutrigenomics?

From no effect to toxicity

Addition of exogenous compounds can prevent the imbalance of metabolites associated with chronic disease

- **However, even the most targeted of xenobiotics hit other unintended targets**



Note the very wide range of concentration with each mode - due to genetic variation in response

Targets and assays of botanicals

Phyto*estrogen*?

In vitro receptor binding assay

Agonist or antagonist?

Reporter gene (luciferase) assay using a cell system

Agonist or antagonist?

Reporter gene (luciferase) assay *in vivo* using nude mice

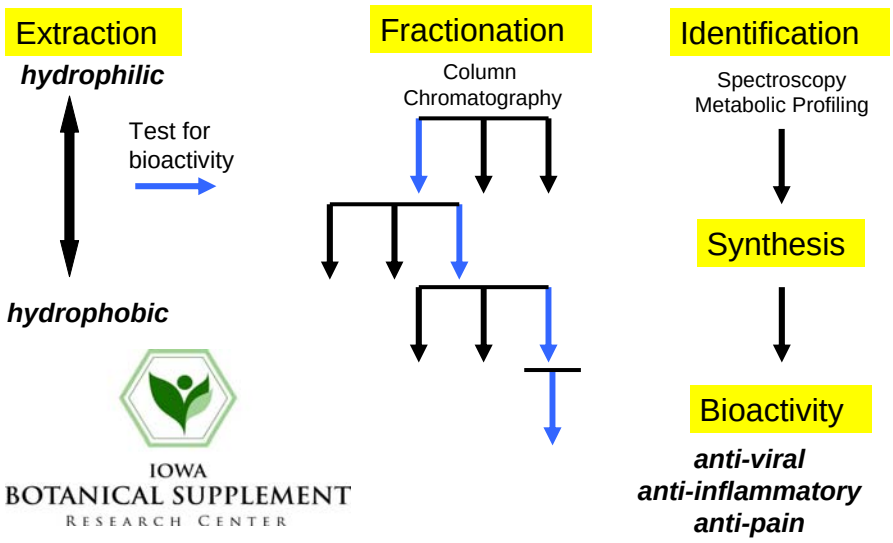
Agonist or antagonist?

University of Illinois-Chicago

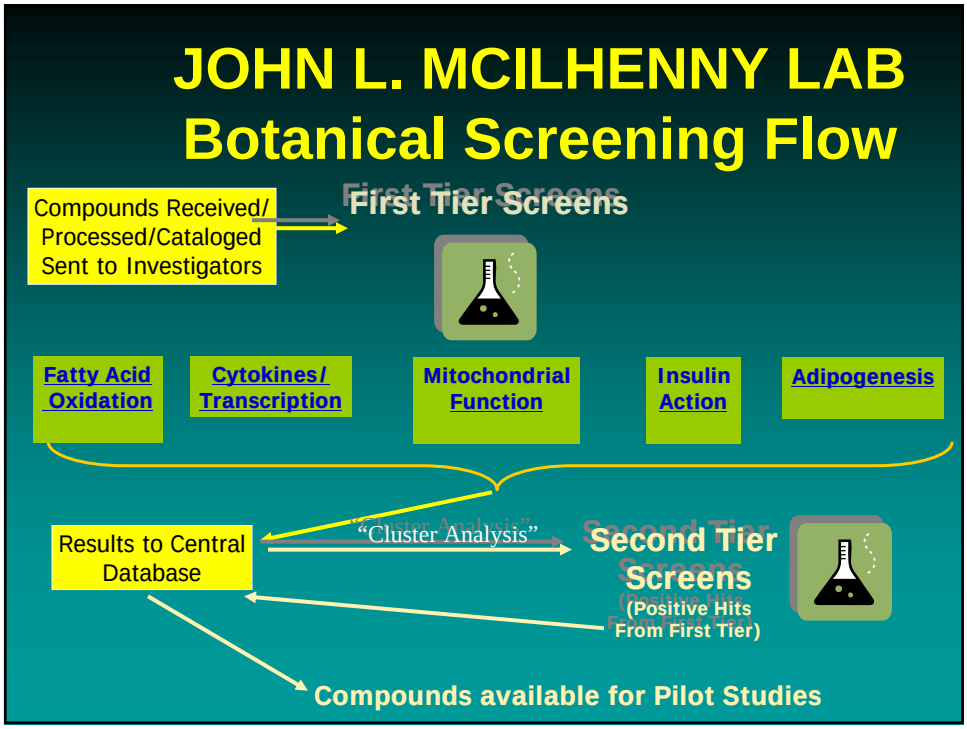
Advantage of this approach is that the compound can be administered orally and it must get to the target tissue.

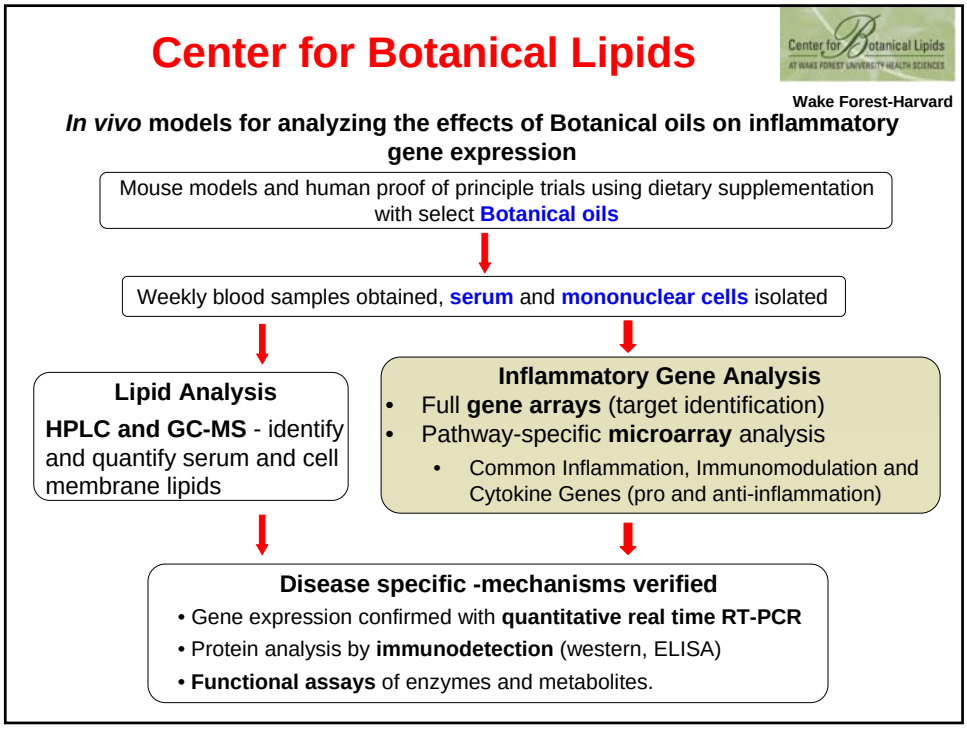
Could be used for any receptor or particular element of a signaling pathway

Strategy of iterative fractionation and testing for the presence of bioactive constituents from *H. perforatum* and *Echinacea spp.*



IOWA BOTANICAL SUPPLEMENT RESEARCH CENTER


JOHN L. MCILHENNY LAB
Botanical Screening Flow
Compounds Received/ Processed/Cataloged Sent to Investigators
First Tier Screens
Fatty Acid Oxidation
Cytokines/ Transcription
Mitochondrial Function
Insulin Action
Adipogenesis
Results to Central Database
"Cluster Analysis"
Second Tier Screens
(Positive Hits From First Tier)
Compounds available for Pilot Studies


Center for Botanical Lipids
Wake Forest-Harvard
In vivo models for analyzing the effects of Botanical oils on inflammatory gene expression
Mouse models and human proof of principle trials using dietary supplementation with select Botanical oils
Weekly blood samples obtained, serum and mononuclear cells isolated
Lipid Analysis
HPLC and GC-MS - identify and quantify serum and cell membrane lipids
Inflammatory Gene Analysis
Full gene arrays (target identification)
Pathway-specific microarray analysis
Common Inflammation, Immunomodulation and Cytokine Genes (pro and anti-inflammation)
Disease specific -mechanisms verified
• Gene expression confirmed with quantitative real time RT-PCR
• Protein analysis by immunodetection (western, ELISA)
• Functional assays of enzymes and metabolites.

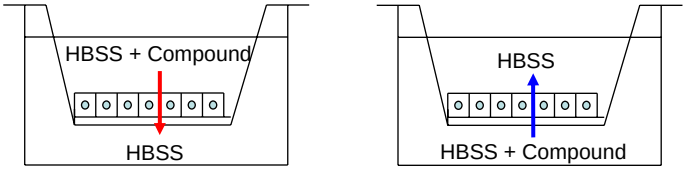
Mass spectrometry and botanicals research

Mass spectrometry is an essential tool to:

- **establish the qualitative and quantitative content of a botanical**
- **ensure quality control of a botanical preparation or subfraction**
- **to identify and quantify the metabolites of specific botanical compounds in the blood, urine and other physiologically relevant fluids and tissues**

Real-time screening of metabolites in an *in vitro* small intestine (Caco-2 cells)

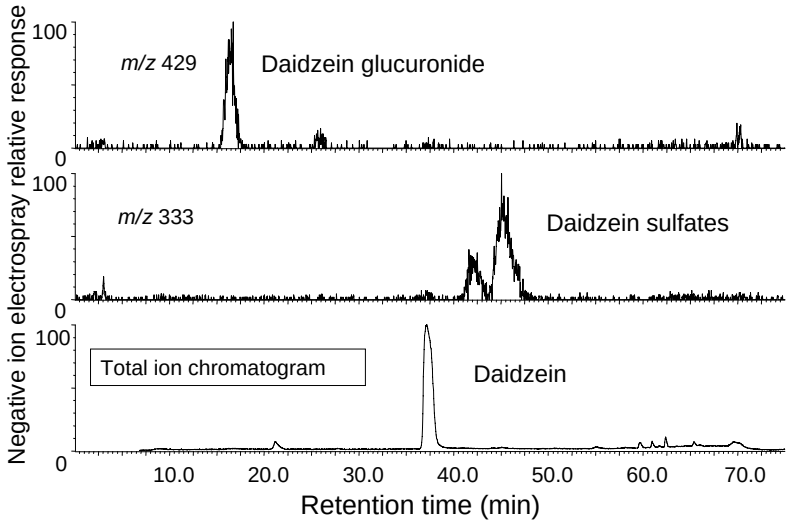
- **Uptake of compounds across intestinal mucosa is determined by a several processes.**
- **Facilitated transport (i.e., P-glycoprotein) can be probed using specific inhibitors and measuring transport rates in opposite directions**
- **Use of mass spectrometry enhances the amount of information available concerning substrate metabolism and allows multiple compounds to be studied simultaneously**



Apical to Basolateral | **Basolateral to Apical**

Richard van Breeman, UIC

Reverse-phase LC-MS Analysis of Daidzein Metabolites after Incubation with Caco-2 Cells



Richard van Breeman, UIC

But mass spectrometry offers much more

Mass spectrometry beyond botanicals and their metabolites

Accelerator mass spectrometry
to measure ^{14}C or ^{41}Ca at sub-fmole levels in tissues and fluids

Qtof or Qtrap mass spectrometry
to identify post-translational modifications of proteins

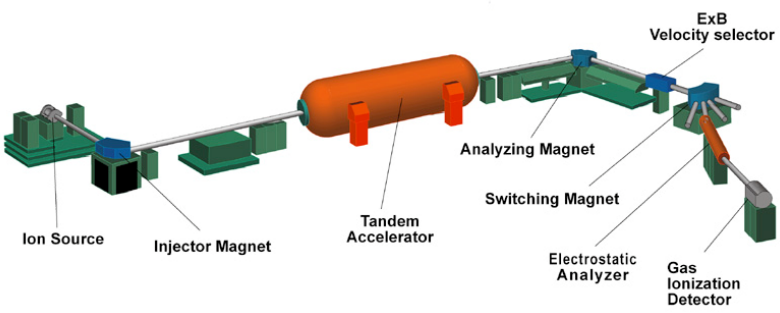
Triple quad mass spectrometry
to quantitatively measure peptides

MALDI-TOF mass spectrometry
to carry out peptide mass fingerprinting and identify proteins and peptides

MALDI-TOF mass spectrometry
For imaging and profiling proteins regionally in tissues

FT-ICR mass spectrometry
For high resolution analysis of proteins and protein structure

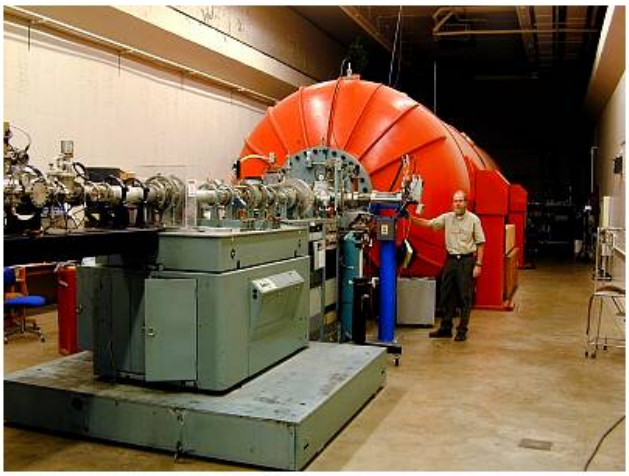
Purdue Rare Isotope Measurement Lab



Accelerator mass spectrometry for rare isotopes, ^{10}Be, ^{14}C, ^{26}Al, ^{36}Cl, ^{41}Ca, ^{129}I

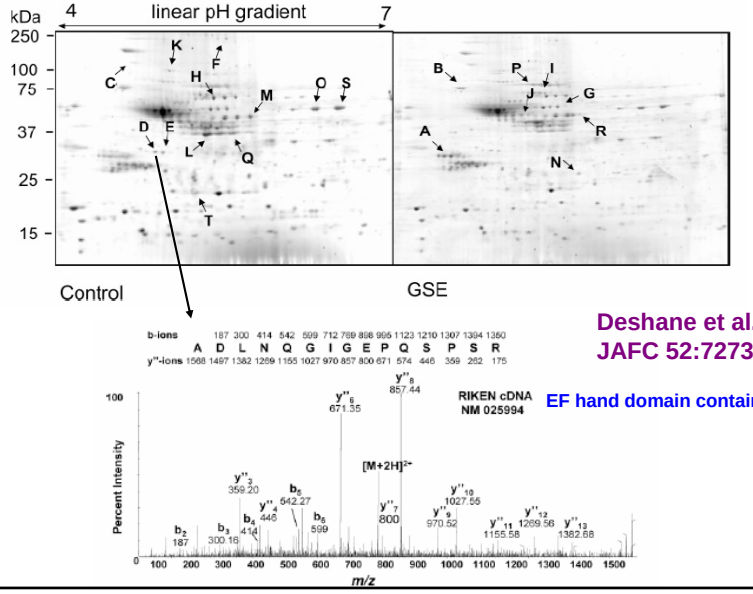
Mary Ann Lila (UIUC) in the Purdue-UAB Botanicals Center has synthesized radiolabeled polyphenols in plant cell culture

Accelerator in PRIME Lab



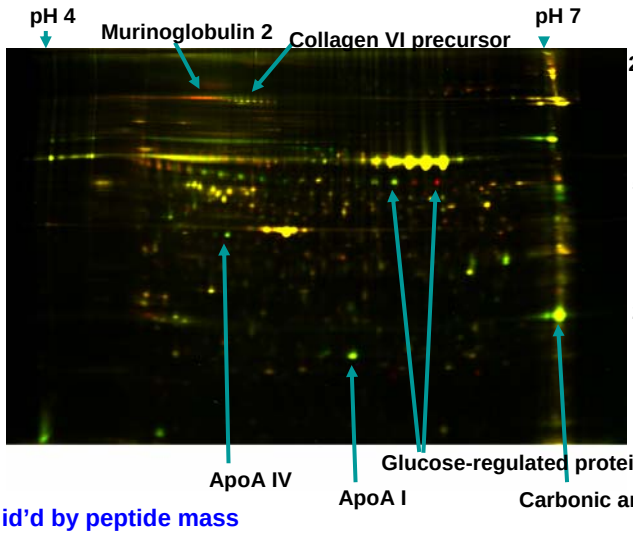
Dr. David Elmore next to the 10 MV accelerator

Proteomics in the brain - effect of grape seed extract



Deshane et al. (2004)
JAFC 52:7273

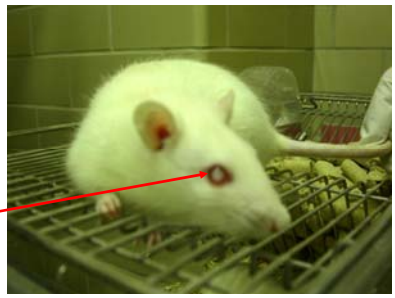
2D-DIGE analysis of polyphenol effects on rat mammary proteome



Proteins id'd by peptide mass fingerprinting and tandem MS

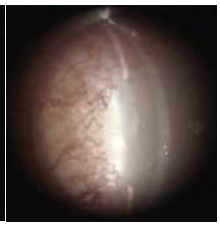
Helen Kim, UAB

The ICR/f rat - the enigmatic model of lens cataract disease

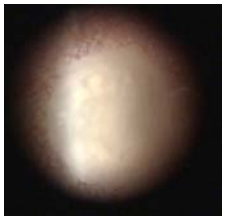


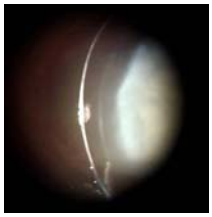
The ICR/f rat shown above has spontaneous cataracts by 70 days of age

Early cortical cataract



Late cortical cataract





Nuclear cataract

Summary

- The general structure of research on dietary supplements and botanicals is essentially the same as for natural products and combinatorial chemistry
- A key element in the progress of Botanicals Centers research has been in the rigorous analysis and quality control of the materials being used
- The breadth of dietary supplements research is amazing - each Center uses a diverse group of methodologies, many of which are state-of-the-art

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Ski Chilton


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