UNIVERSITY of MARYLAND
SCHOOL OF PHARMACY
MASS SPECTROMETRY CENTER

Mass Spectrometry Center: Empowering Drug Discovery and Development

ICTR Enrichment Seminar
April 14, 2026

Maureen A. Kane, Ph.D.

Executive Director; School of Pharmacy Mass Spectrometry Center

Professor; Department of Pharmaceutical Sciences

University of Maryland, Baltimore

https://www.pharmacy.umaryland.edu/centers/massspec/

UMB SOP Mass Spectrometry Center Mission and Vision

UNIVERSITY of MARYLAND SCHOOL OF PHARMACY MASS SPECTROMETRY CENTER

The **UMB SOP MS Center** is an **Organized Research Center** with an emphasis on collaborative research

Our **vision** is to accelerate discovery by providing investigators, on campus and beyond, access to cutting-edge technologies in mass spectrometry

Our **mission** is to lead or integrate into the research project teams with the goal of conducting high quality analytical science, publishing novel research, and obtaining sustainable funding

The MS Center:

- Supports a diverse set of **collaborators**, and partners
- Bioanalytical chemistry, technology development, data science, and biomedical research
- Provide integrated mass spectrometry-based tools that deliver biological insight, not just data

- Typically find that the best quality research comes from our analytical team **optimizing processes for individual project goals** and value working closely in a true collaborative effort

- **Training** graduate students, postdoctoral fellows, and staff scientists in mass spectrometry-based analyses relevant to pharmaceutical science including drug discovery and drug development.

UMB SOP Mass Spectrometry Center

UNIVERSITY of MARYLAND
SCHOOL OF PHARMACY
MASS SPECTROMETRY CENTER

Key Metrics

- **Organized Research Center** with an emphasis on collaborative research
- Multi-million dollar facility
- **>$17M** in instrumentation
- **>$2.5 M** in facilities upgrades
- **19** Mass Spectrometers of varying function
- Annually supporting research towards **$110+ Million in total funding** to the UMB Campus over the past 5 years
- Utilized by **80+** different investigators from UMB
- Utilized by **20+** external universities/companies
- Lead or contribute to **>181 papers** (2020-2024)

Mass Spectrometry Center History

Pre2009: MS Facility has several instruments
2009: SOP investment in new instrumentation begins
2010: Construction of current SOP facilities begins
2011: Current facilities open as SOP MS Facility
2013: named Waters Center of Innovation
2014: UMB Organized Research Center designation established
2018: Additional renovations expand footprint
2022: Continued SOP investment in instrumentation

Project breakdown (2020-2024):

- 70 Awards
- 41 award PIs

Owning School (totals for 2020-2024):

- 41 SOP- $44,369,250.66 total
- 26 SOM - $63,403,478.18 total
- 1 SON - $2,684,997.00 total
- 1 Office of the President - $50,000
- **All 70 awards in total: $110,507,725.84**

ICTR: Institute for Clinical & Translational Research

UNIVERSITY of MARYLAND
SCHOOL OF PHARMACY
MASS SPECTROMETRY CENTER

- **ICTR**

- **Investigator Resources**
- **ICTR Cores of Services**
- *The UMB ICTR Brings Together Experts Across Campus To Provide Free Hours of Fundamental Research Services to the UMB Faculty**

- **ICTR Drug Discovery and Development Core**

- **Mass Spectrometry Center**

Examples of resources available through ICTR

- The MSC will offer a free consultation and subsidized proteomic studies of up to 12 samples* where the person time and 5% of the hourly instrument use charges will be subsidized**.

- Experiments for other analysis techniques may also be possible up to the **25 ICTR-supported hours of service**, but require an ICTR Resource Request submission and consultation to determine scope and feasibility.

- For experiments requiring specialized reagents, reagents will be charged at cost.

- For **UMB faculty**, use your UMID to log in to the **ICTR Resource Request** webpage to access the link to the application (developed in ***REDCap***).

Set up a consultation to discuss your experimental goals, scope, and feasibility!

UNIVERSITY of MARYLAND
SCHOOL OF PHARMACY
MASS SPECTROMETRY CENTER

Today's Goals:

- Give an overview of the types of questions you can ask using mass spectrometry-based analyses
- Highlight various capabilities of the MS Center
- Highlight MS Center collaborations and publications

Mass Spectrometry fueling Drug Discovery and Drug Development at UMB

UNIVERSITY of MARYLAND
SCHOOL OF PHARMACY
MASS SPECTROMETRY CENTER

Drug Discovery

- Proteomics
- Metabolomics
- Mass Spectrometry Imaging

Drug Development

- Quantitative LC-MS/MS
  - Drug quantification
  - Pre-clinical screening assays
  - PK/PD

Mass Spectrometry fueling Drug Discovery and Drug Development at UMB

UNIVERSITY of MARYLAND
SCHOOL OF PHARMACY
MASS SPECTROMETRY CENTER

Drug Discovery

- Proteomics
- Metabolomics
- Mass Spectrometry Imaging

Drug Development

- Quantitative LC-MS/MS
  - Drug quantification
  - Pre-clinical screening assays
  - PK/PD

PROTEOMICS OVERVIEW – DIFFERENT TYPES OF ANALYSIS

BIOLOGICAL SAMPLES

Cells | Plasma CSF | Tissues

PROTEIN EXTRACTION & ENRICHMENT

Affinity Purification | Nano-particle

PROTEIN DIGESTION

(e.g., Trypsin / Lys-C)

GLOBAL PROTEOMICS (DISCOVERY)

GOAL:
- Unbiased profiling of the entire proteome
- No a priori hypothesis
- Dynamic range exploration

WORKFLOW

Data-Dependent (**DDA**) | Data-Independent (**DIA**)

OUTPUT & EXAMPLE DATA

Volcano Plot | Heat-Map

TARGETED PROTEOMICS

GOAL:
- High-precision quantification
- Testing a hypothesis
- Validation of biomarkers

WORKFLOW

Heavy Standards

Multiple Reaction Monitoring (**MRM**) | Parallel Reaction Monitoring (**PRM**)

OUTPUT & EXAMPLE DATA

Absolute/Relative Quantification
Area under the curve for each peptides

POST-TRANSLATIONAL MODIFICATION

GOAL:
- Enrichment and identification of modified peptides / proteins
- Investigating signaling cascades or disease states

WORKFLOW

ENRICH METHODS: PHOSPHO- : **IMAC / TiO_2** | GLYCO- : **HILIC / MAX**

OUTPUT & EXAMPLE DATA

Phospho: GAAAQETEL**S**V**S**AEL

Glyco: VPTSSW**N**ISSELNK

PTM Site Mapping

CHEMICAL PROTEOMICS

GOAL:
- Map small molecule-protein interactions
- Deconvoluting drug targets
- Mechanism of action

WORKFLOW

REACTIVE PROBE → ENRICHMENT
BIOTINYLATED DRUG → STREPTAVIDIN

OUTPUT & EXAMPLE DATA

Target Binding Curve | Competition Assay

Binding Affinity | Concentration

SINGLE-CELL & SPATIAL PROTEOMICS

GOAL:
- Ultra-sensitive & high-resolution
- Distinguish heterogenous cells
- Cell-type specific proteome discovery

WORKFLOW

S.C Isolation / LMD-Section → High-throughput Sample prep → LC-MS

OUTPUT & EXAMPLE DATA

Cell-type Mapping | Topography Stack

PROTEOMICS OVERVIEW – DIFFERENT TYPES OF ANALYSIS

BIOLOGICAL SAMPLES

Cells | Plasma CSF | Tissues

PROTEIN EXTRACTION & ENRICHMENT

Affinity Purification | Nano-particle

PROTEIN DIGESTION

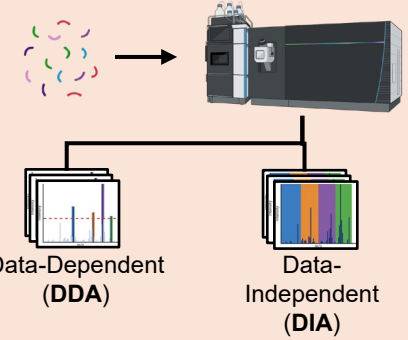
(e.g., Trypsin / Lys-C)

GLOBAL PROTEOMICS (DISCOVERY)

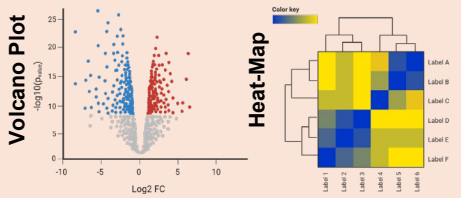
GOAL:

- Unbiased profiling of the entire proteome
- No a priori hypothesis
- Dynamic range exploration

WORKFLOW



OUTPUT & EXAMPLE DATA



❑ Discovery proteome

- Large-scale protein identification/quantification
  - ✓ Tissues, tissue regions
  - ✓ Fluids (plasma, serum, CSF)
  - ✓ Cell culture, isolated/sorted cells
  - ✓ Lysosomal fractionation analysis
  - ✓ Pull down products analysis
  - ✓ Nanoparticle enrichment proteomics
  - ✓ Membrane proteome enrichment analysis
  - ✓ Exosome analysis

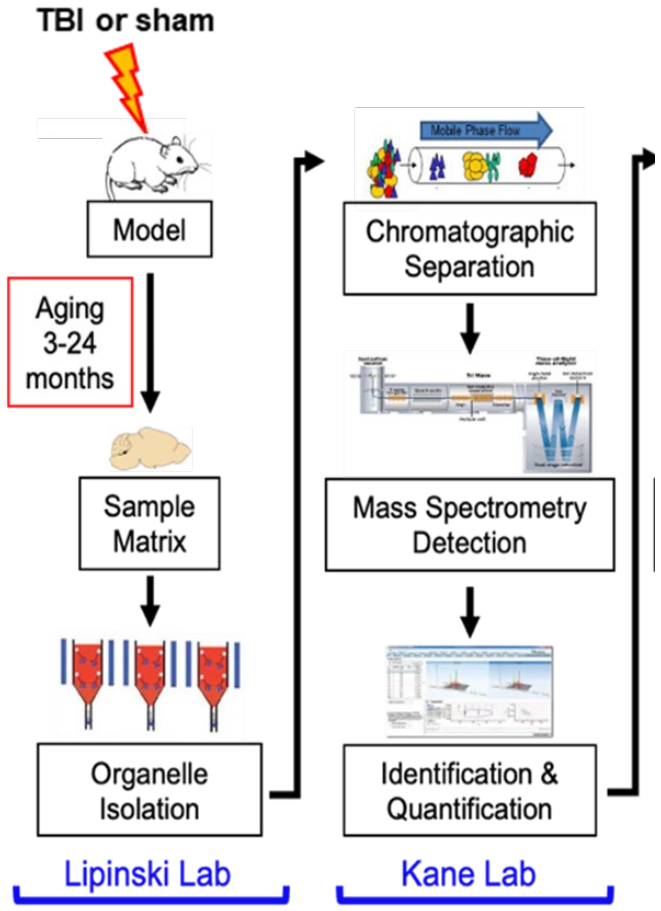
❑ Bioinformatics analysis

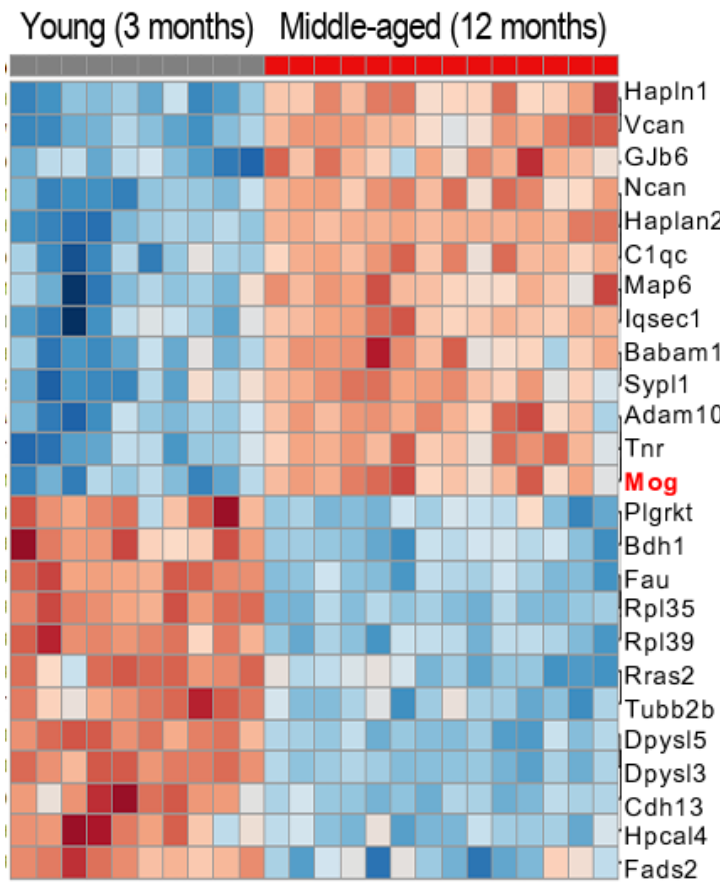
- Pathway analysis of Omics data
  - ✓ Comparative pathway network analysis
  - ✓ Upstream regulator analysis
  - ✓ Downstream effect analysis
  - ✓ Biomarker identification
- Omics data annotation and classification
  - ✓ Biological process
  - ✓ Molecular function
  - ✓ Cellular component
  - ✓ Protein-protein interaction network analysis
  - ✓ Functional enrichment analysis

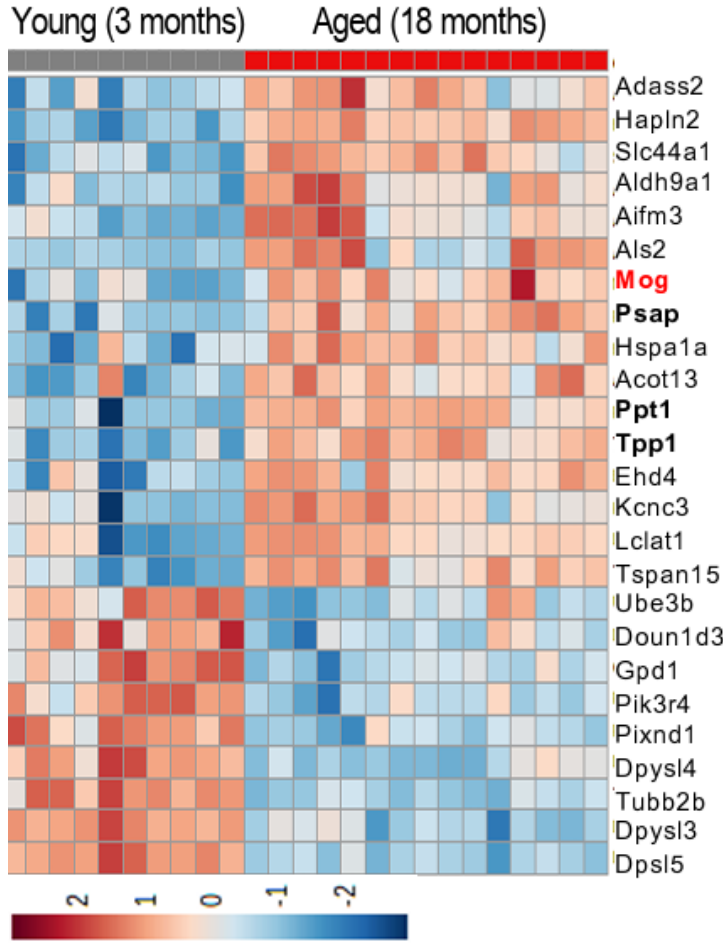
Neurotrauma
Global proteomics
Isolated lysosomes

❑ Marta Lipinski Lab, SOM

Lysosomal multi-omics reveals altered sphingolipid catabolism as driver of lysosomal dysfunction in the aging brain

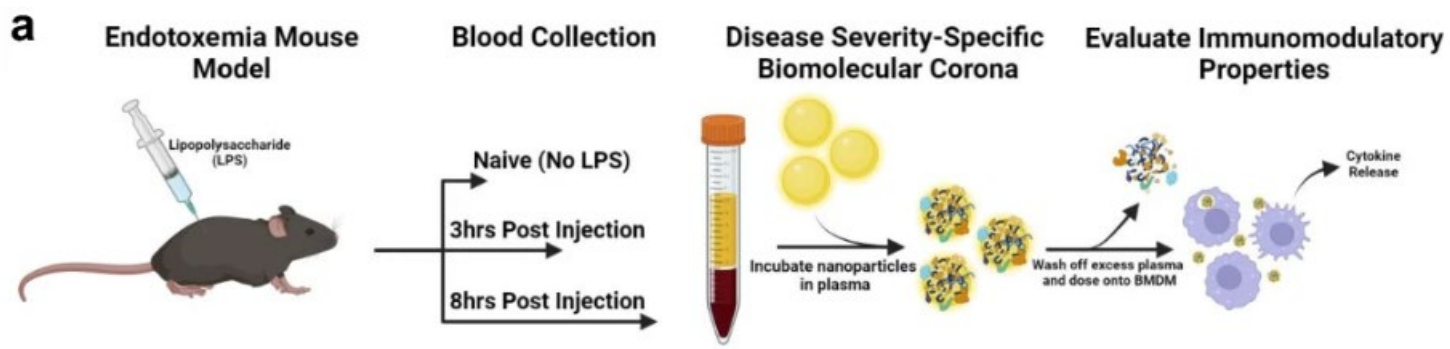


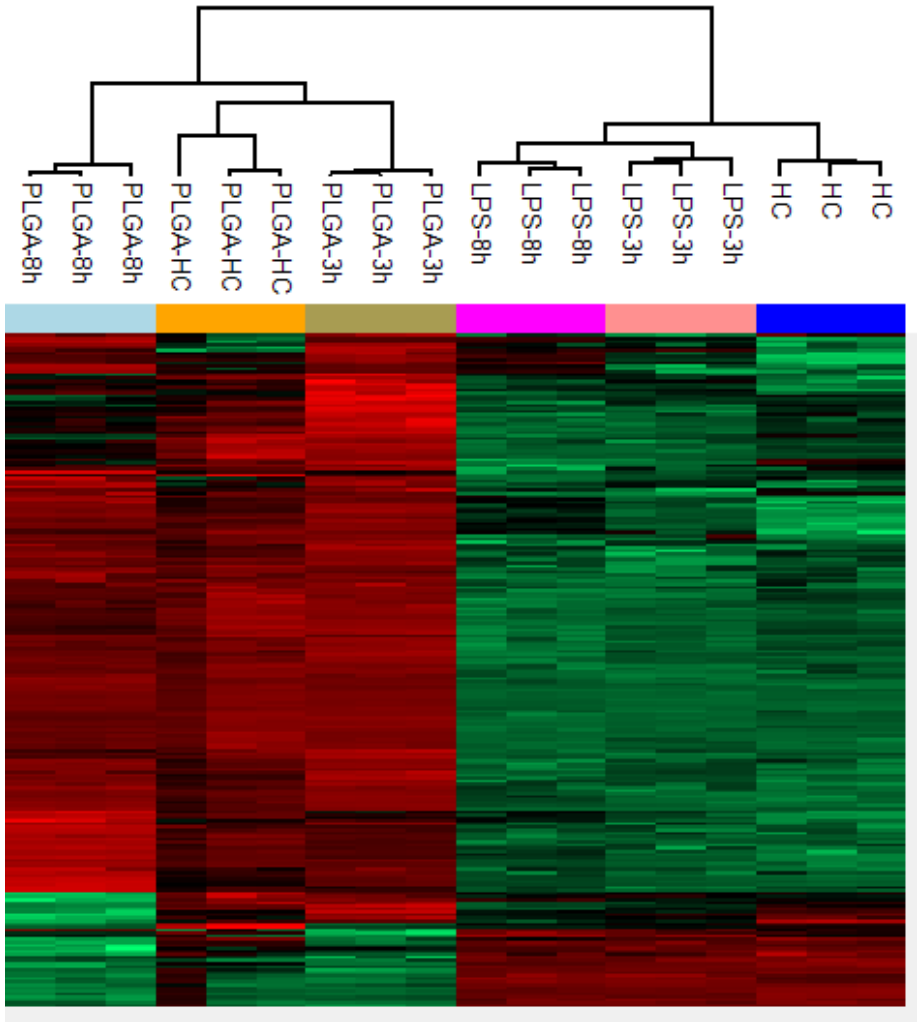




(***doi:*** *10.1101/2025.09.10.675421*)

❑ Ryan Pearson Lab, SOP





Inflammatory disease progression shapes nanoparticle biomolecular corona-mediated immune activation profiles.

Shaw JR, Caprio N, Truong N, Weldemariam M, Tran A, Pilli N, Pandey S, Jones JW, **Kane MA**, **Pearson RM.**

Nat Commun. 2025 Jan 22;16(1):924.

doi: 10.1038/s41467-025-56210-4.

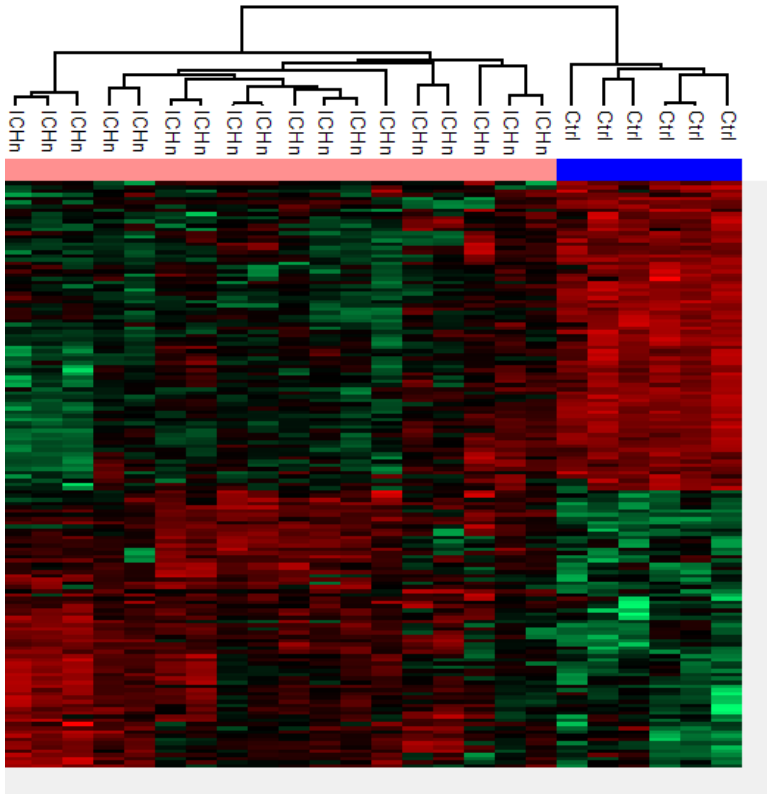
PMID: 39843415

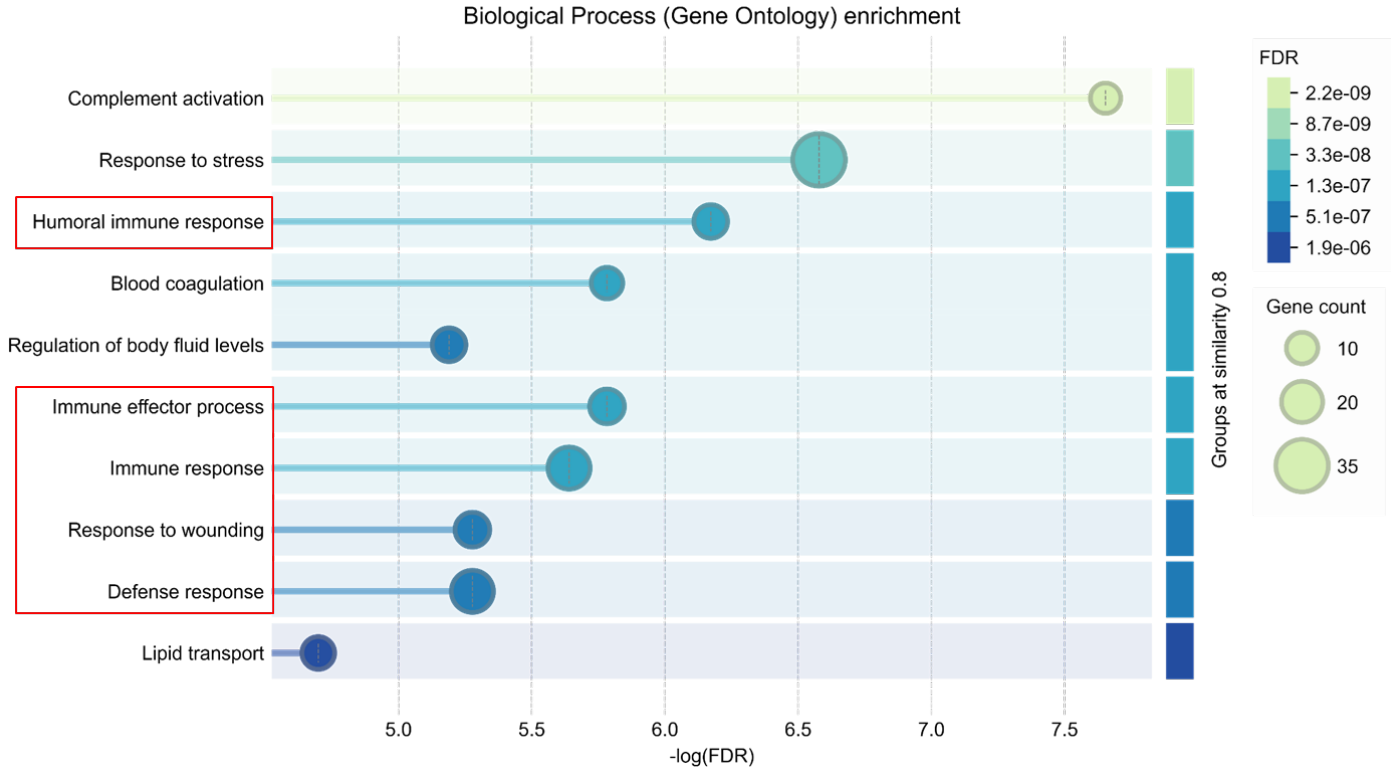
❑ Prajwal Ciryam Lab, SOM

Brain Injury Study - Subarachnoid Hemorrhage Inflammatory Proteome

- Identify biomarkers for early brain injury and delayed cerebral ischemia (DCI) in CSF using proteomics

Key Approach
- NP-enriched DIA-LC-MS/MS to increase low-abundance protein coverage



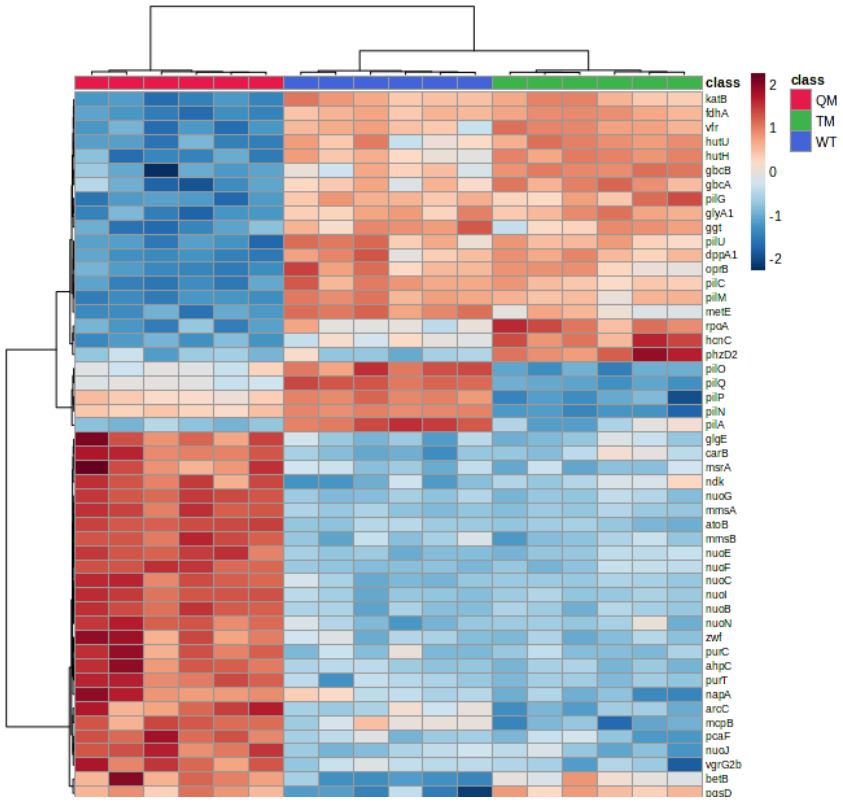


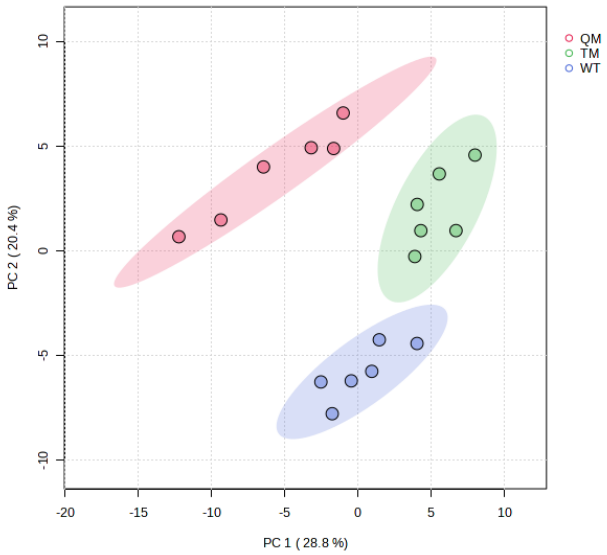
Unpublished, manuscript in preparation

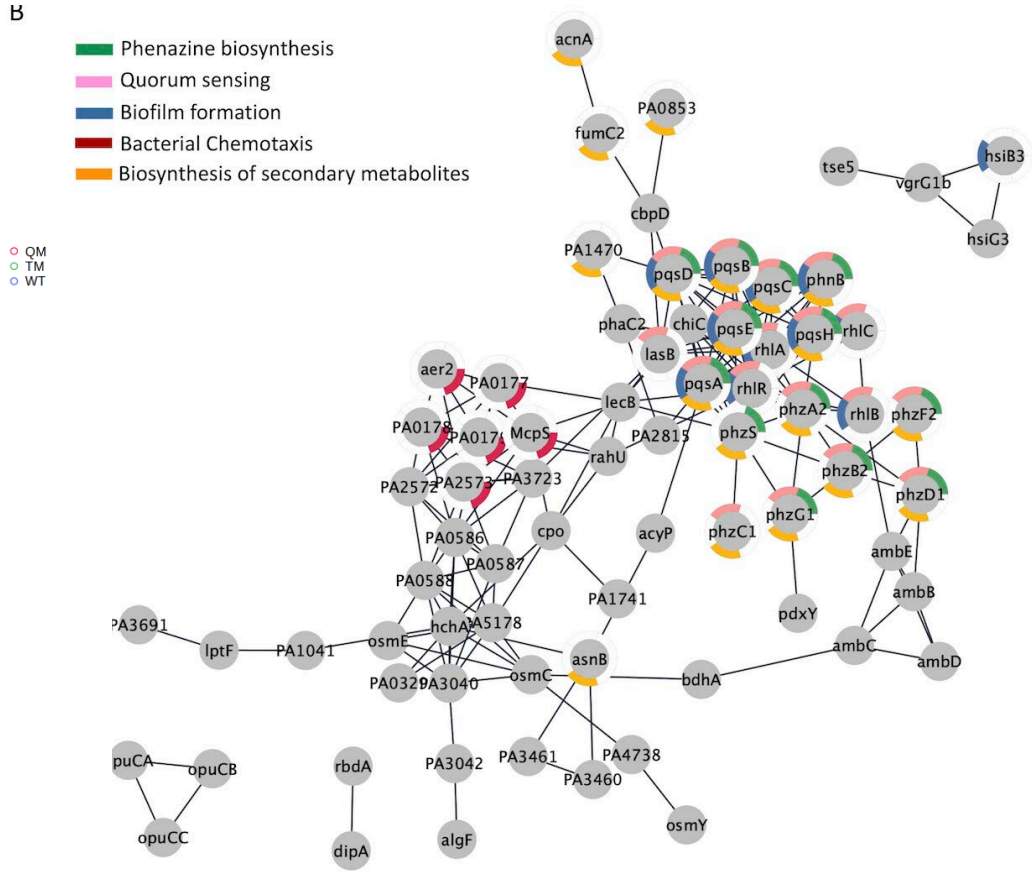
❑ Angela Wilks Lab, SOP

Investigation of heme and iron regulated pathways of *Pseudomonas aeruginosa*

Comparative proteomics of PAO1 versus the allelic strains lacking a functional heme oxygenase (*hemO*in) or one reengineered to produce BVIXα (*hemO*α)



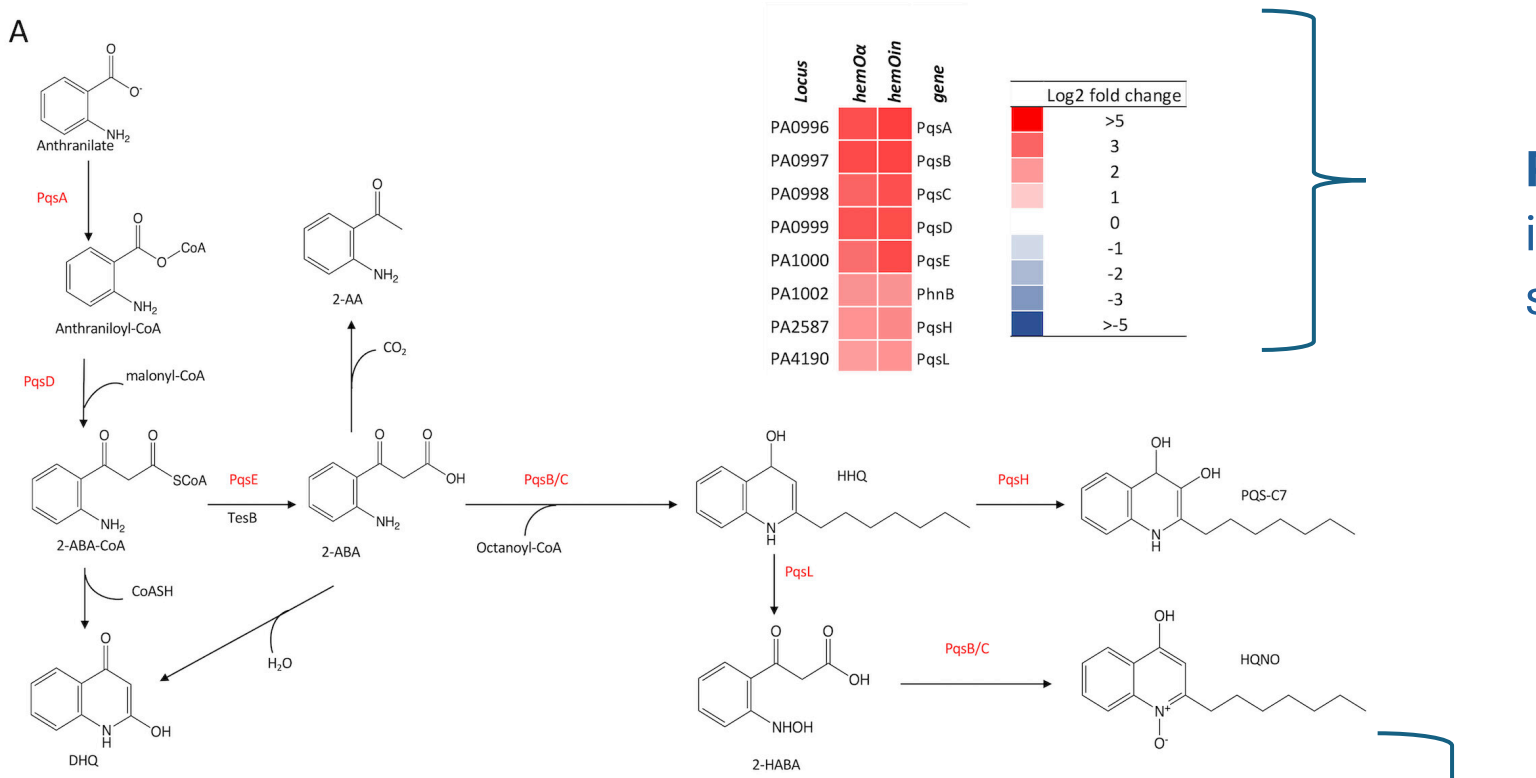




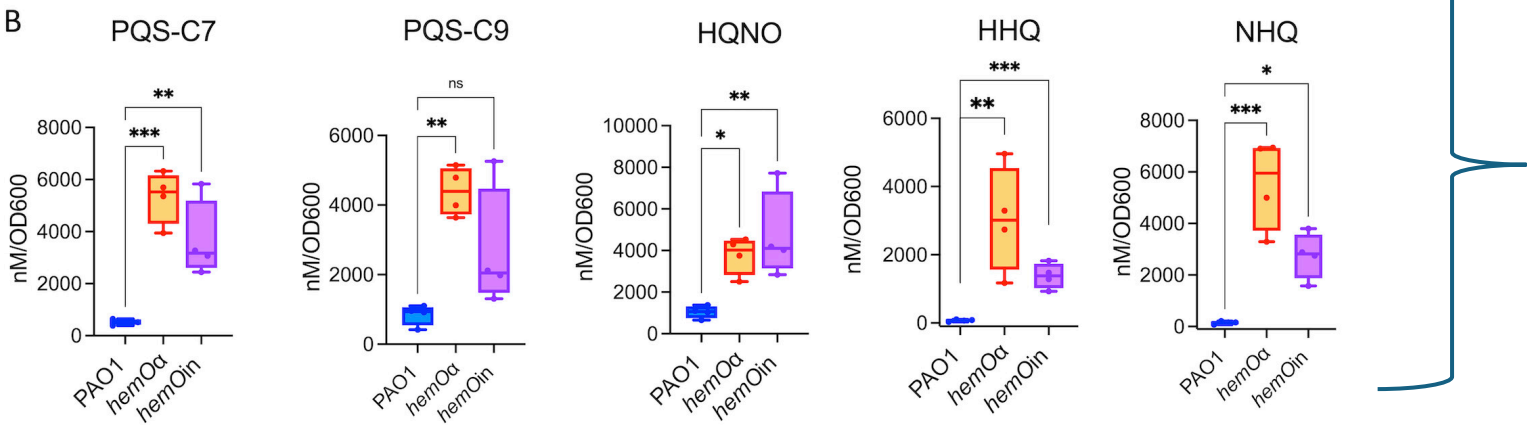
Pseudomonas aeruginosa heme metabolites biliverdin IXβ and IXδ are integral to lifestyle adaptations associated with chronic infection.
Shahzad S, Krug SA, Mouriño S, Huang W, **Kane MA, Wilks A**.
mBio. 2024 Mar 13;15(3):e0276323.
doi: 10.1128/mbio.02763-23. Epub 2024 Feb 6.
PMID: 38319089

Integration of proteomic and metabolomic data yields biological insight

UNIVERSITY of MARYLAND SCHOOL OF PHARMACY MASS SPECTROMETRY CENTER



Proteomic results = changes in quinolone-based quorum-sensing system PQS



Targeted metabolomics of PQS biosynthesis pathway

Shahzad S, Krug SA, Mouriño S, Huang W, Kane MA, Wilks A.mBio. 2024 Mar 13;15(3):e0276323. PMID: 38319089

PROTEOMICS OVERVIEW – DIFFERENT TYPES OF ANALYSIS

BIOLOGICAL SAMPLES

Cells

Plasma
CSF

Tissues

PROTEIN EXTRACTION & ENRICHMENT

Affinity Purification

Nano-particle

PROTEIN DIGESTION

(e.g., Trypsin / Lys-C)

TARGETED PROTEOMICS

GOAL:

- High-precision quantification
- Testing a hypothesis
- Validation of biomarkers

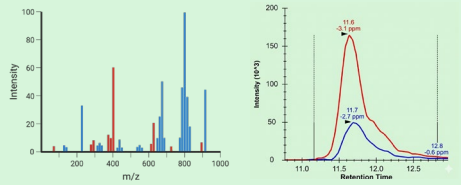
WORKFLOW

Heavy Standards

Multiple Reaction Monitoring (**MRM**)

Parallel Reaction Monitoring (**PRM**)

OUTPUT & EXAMPLE DATA



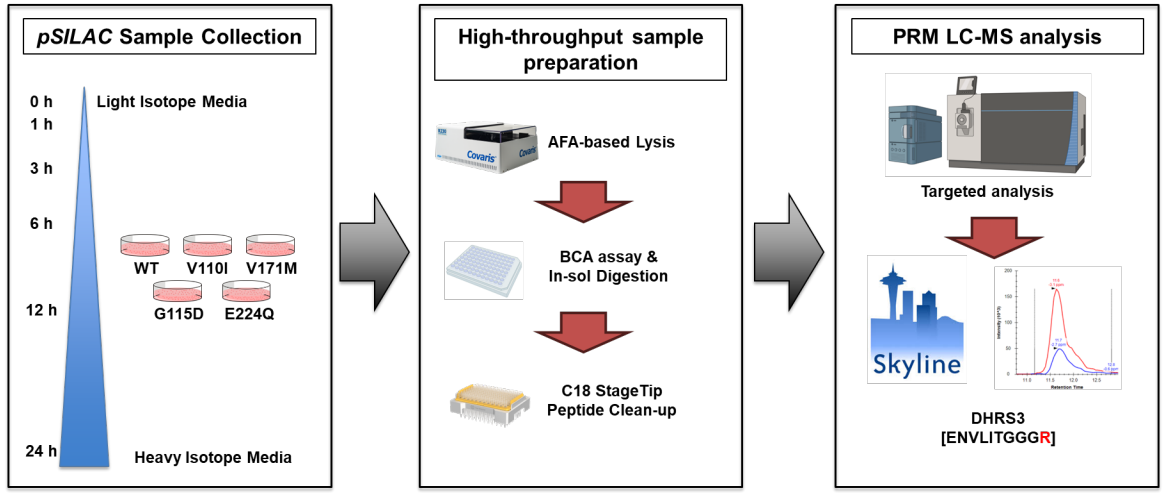
Absolute/Relative Quantification

Area under the curve for each peptides

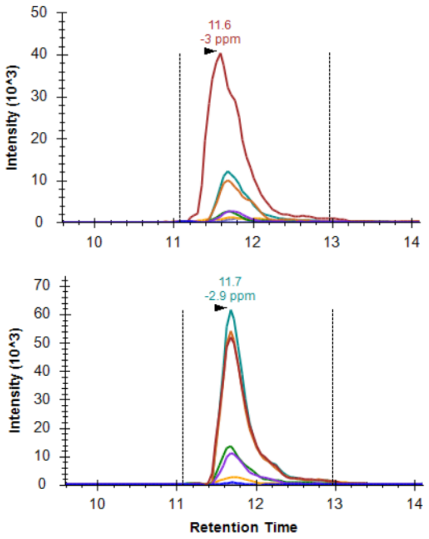
Protein Dynamic Half-life – DHRS3 Variants

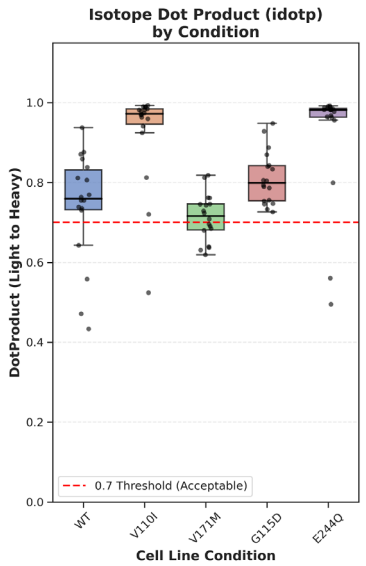
Targeted proteomics
Stable isotope labeling to determine half-life

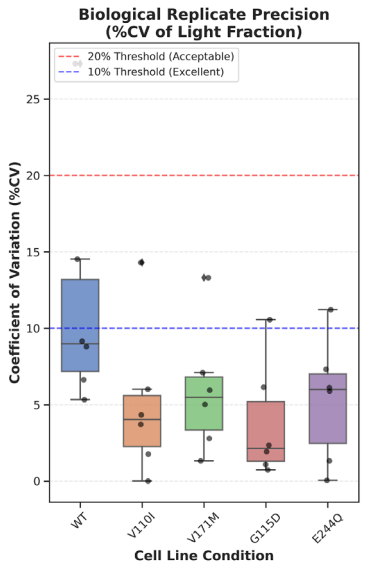
Workflow

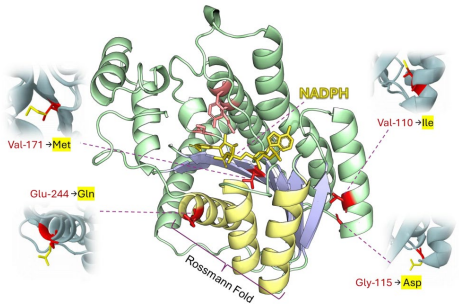


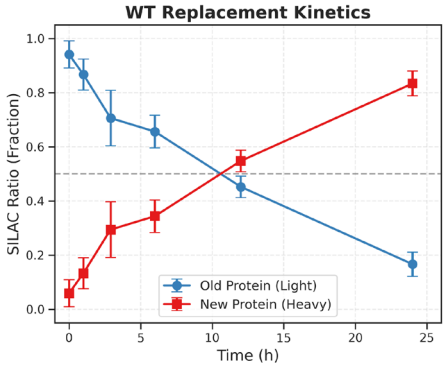
Light & Heavy XICs

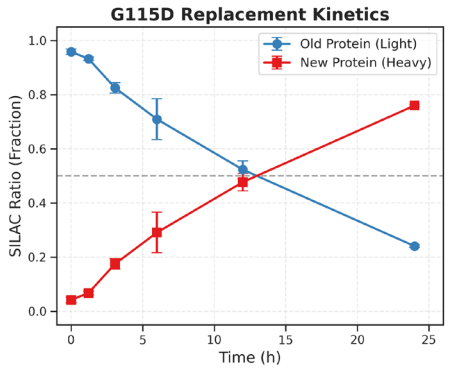


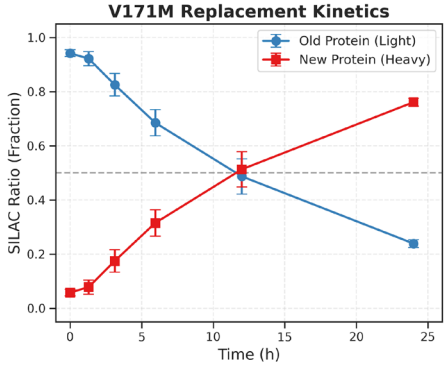


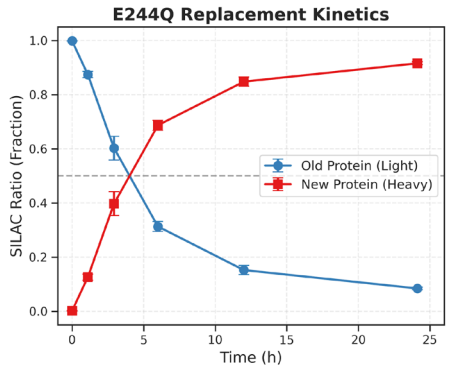


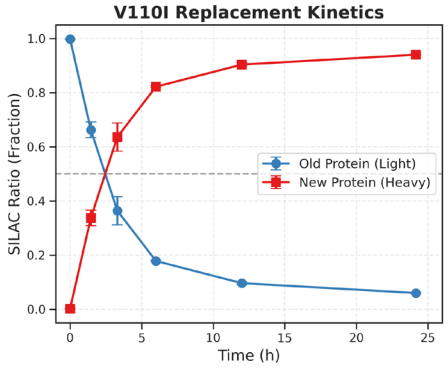




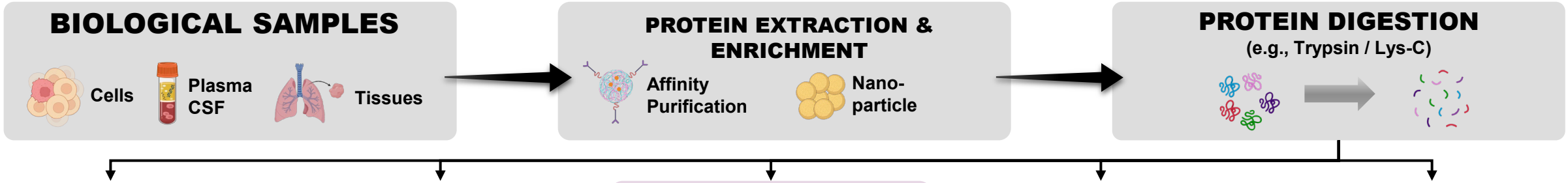








PROTEOMICS OVERVIEW – DIFFERENT TYPES OF ANALYSIS



❑ Post-translational modifications (PTMs)

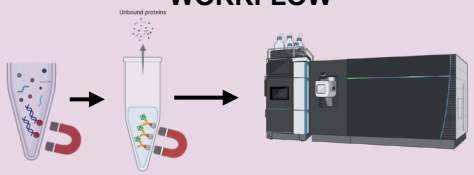
- Large-scale PTM identification/quantification
  - ✓ Phosphorylation
  - ✓ Glycosylation
  - ✓ Biotynilation
  - ✓ Histone modification
  - ✓ Methylation
  - ✓ Glutamylation

POST-TRANSLATIONAL MODIFICATION

GOAL:

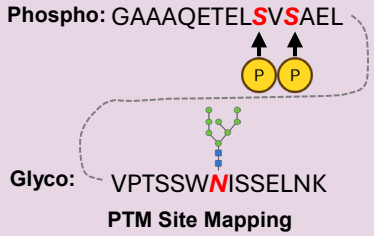
- Enrichment and identification of modified peptides / proteins
- Investigating signaling cascades or disease states

WORKFLOW



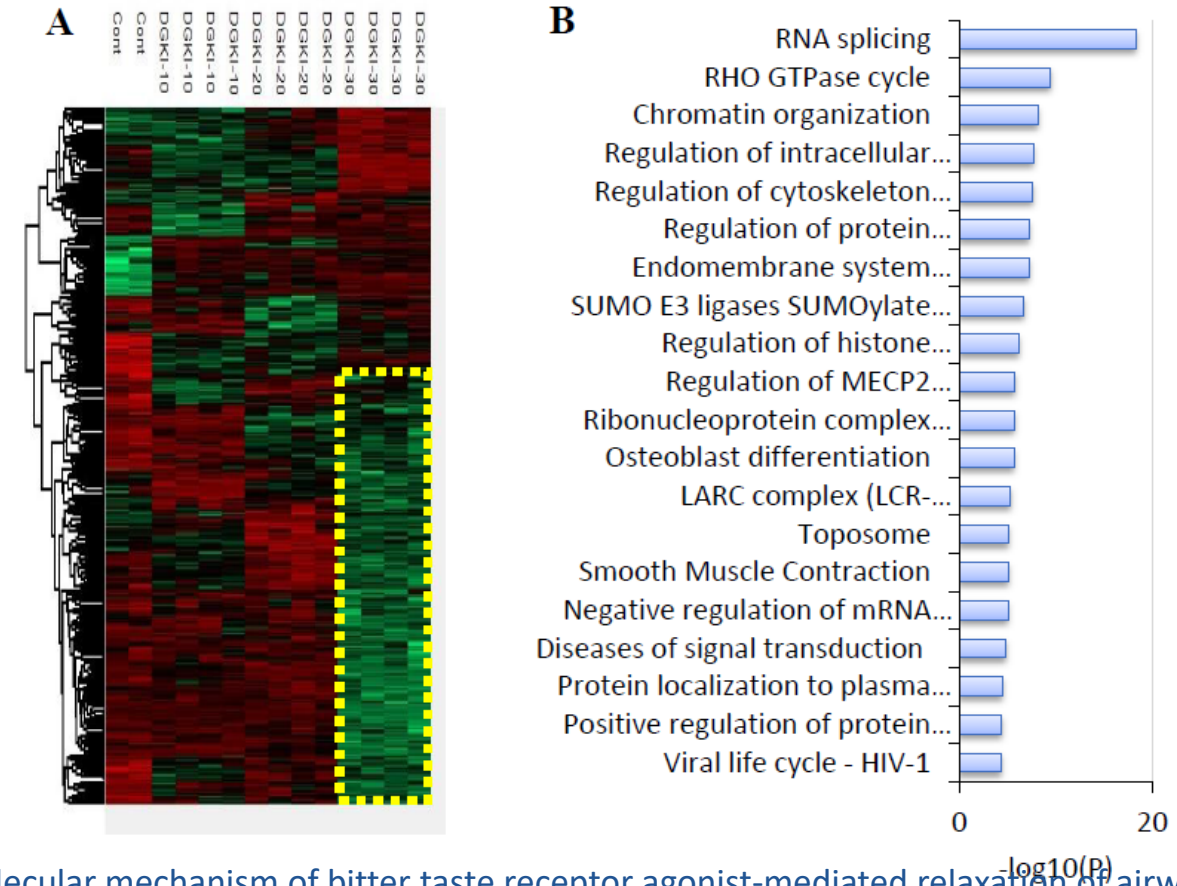
ENRICH METHODS
- PHOSPHO- : **IMAC** / **TiO_2**
- GLYCO- : **HILIC** / **MAX**

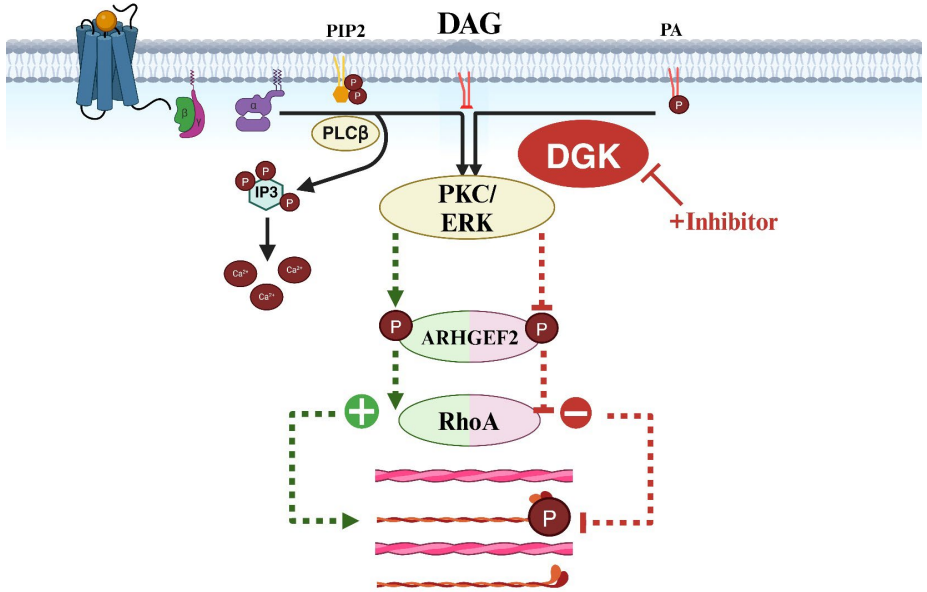
OUTPUT & EXAMPLE DATA



❑ Deepak A. Deshpande Lab, Thomas Jefferson University (TJU)

Phosphoproteomics analysis - Targeting DAG Metabolism to Modulate Contractile Signaling and Function in Airway Smooth Muscle



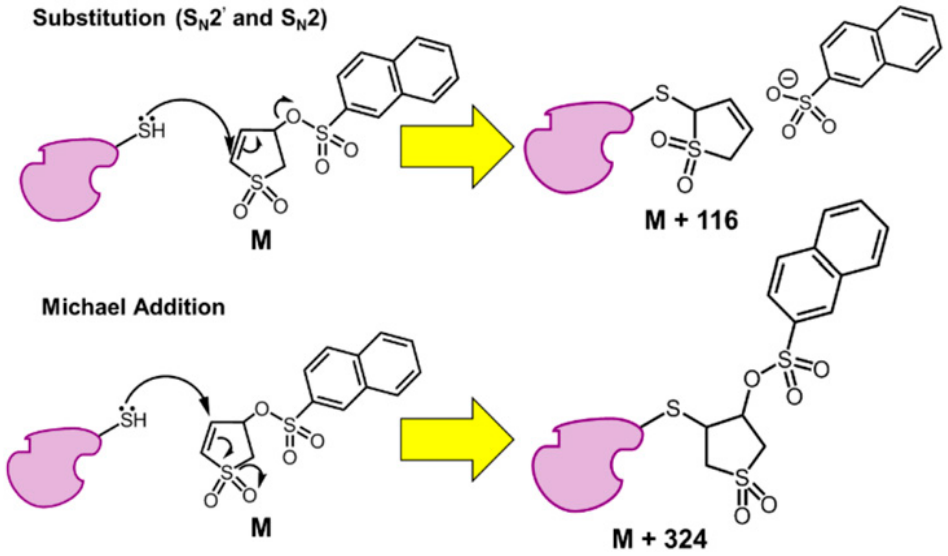


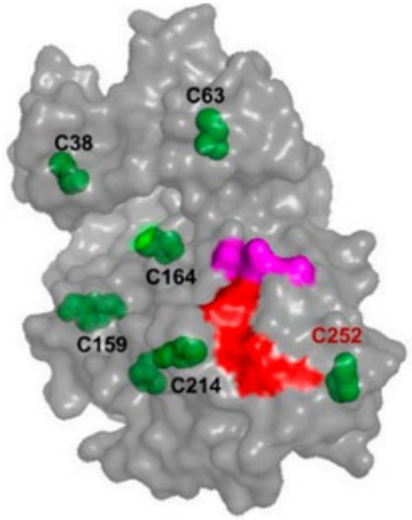
Molecular mechanism of bitter taste receptor agonist-mediated relaxation of airway smooth muscle.
Conaway S Jr, Huang W, Hernandez-Lara MA, **Kane MA,** Penn RB, **Deshpande DA.**
FASEB J. 2024 Jul 31;38(14):e23842.
doi: 10.1096/fj.202400452R.
PMID: 39037554

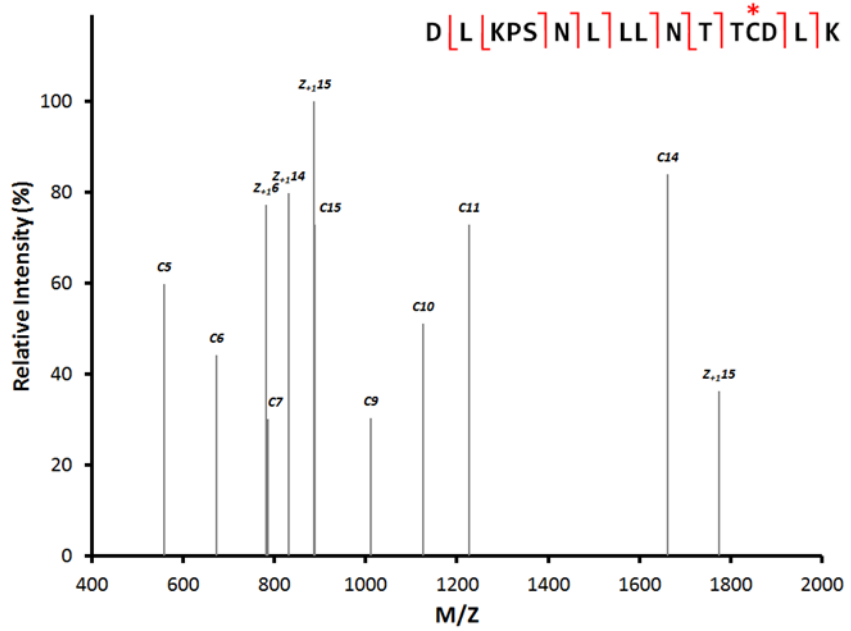
PTM analysis
ID of covalent modification
Subsequent global proteomics and phosphoproteomics

❑ Paul Shapiro Lab, SOP

Identifying compound covalent binding site of ERK2





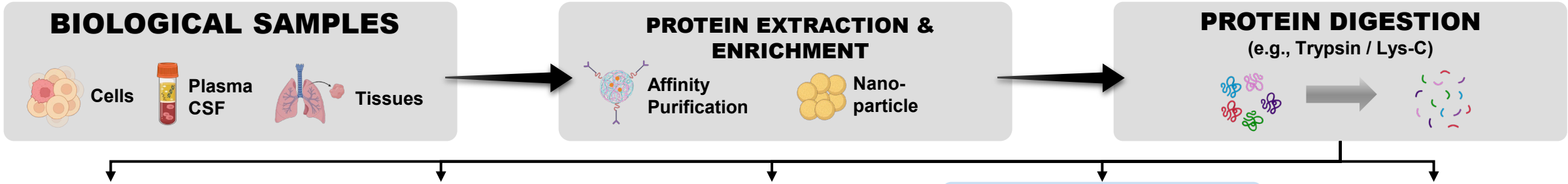


Mechanistic Analysis of an Extracellular Signal-Regulated Kinase 2-Interacting Compound that Inhibits Mutant BRAF-Expressing Melanoma Cells by Inducing Oxidative Stress.
Martinez R 3rd, Huang W, Samadani R, Mackowiak B, Centola G, Chen L, Conlon IL, Hom K, **Kane MA**, **Fletcher S, Shapiro P.**
J Pharmacol Exp Ther. 2021 Jan;376(1):84-97.
PMID: 33109619

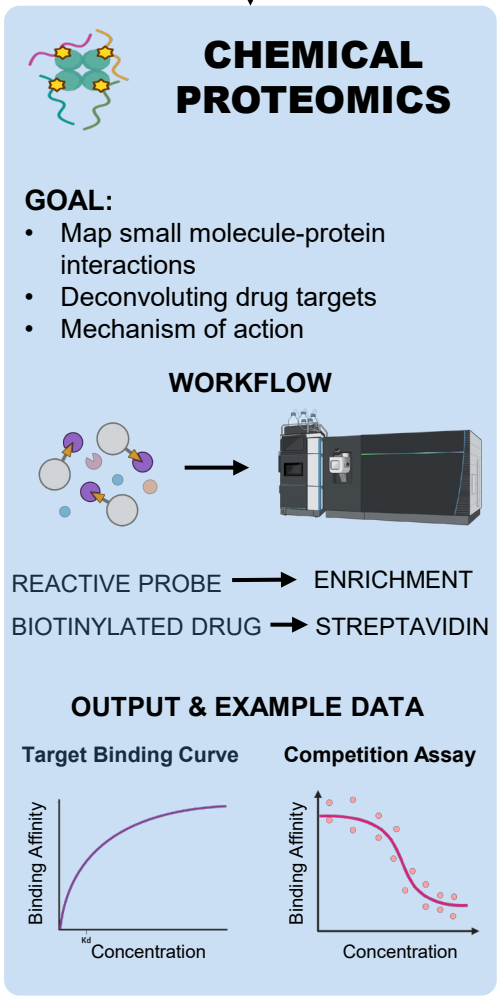
Effects of ATP-competitive and function-selective ERK inhibitors on airway smooth muscle cell proliferation.
Defnet AE, Huang W, Polischak S, Yadav SK, **Kane MA, Shapiro P, Deshpande DA**.
FASEB J. 2019 Oct;33(10):10833-10843.
PMID: 31266368

Targeted Inhibition of Select Extracellular Signal-regulated Kinases 1 and 2 Functions Mitigates Pathological Features of Asthma in Mice.
Shah SD, Nayak AP, Sharma P, Villalba DR, Addya S, Huang W, **Shapiro P, Kane MA, Deshpande DA.**
Am J Respir Cell Mol Biol. 2023 Jan;68(1):23-38.
PMID: 36067041

PROTEOMICS OVERVIEW – DIFFERENT TYPES OF ANALYSIS



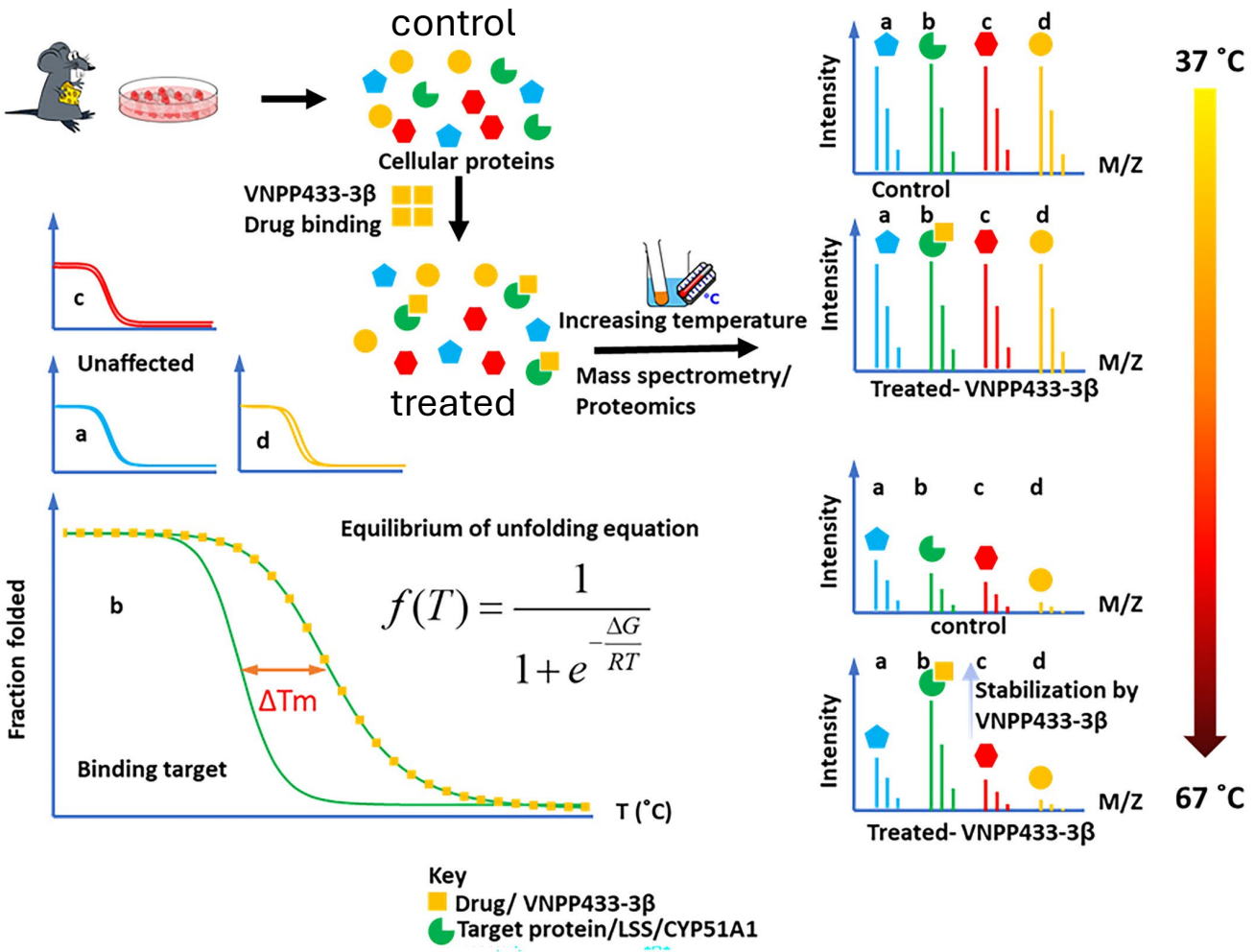
- Identification of drug targets
  - Thermal proteome profiling (TPP)
- Identification of interacting proteins by proximity labeling
  - Bio ID / Turbo ID
- Identification of transported proteins via chemical labeling
  - DiDBiT



Chemical proteomics
Thermal proteome profiling (TPP)
ID of drug target

❑ Vincent C. O. Njar Lab, SOM

Thermal proteome profiling and proteome analysis using high-definition mass spectrometry



Identification of drug target engagement via an increase or decrease in thermal stability

Thermal proteome profiling and proteome analysis using high-definition mass spectrometry demonstrate modulation of cholesterol biosynthesis by next-generation galeterone analog VNPP433-3β in castration-resistant prostate cancer.

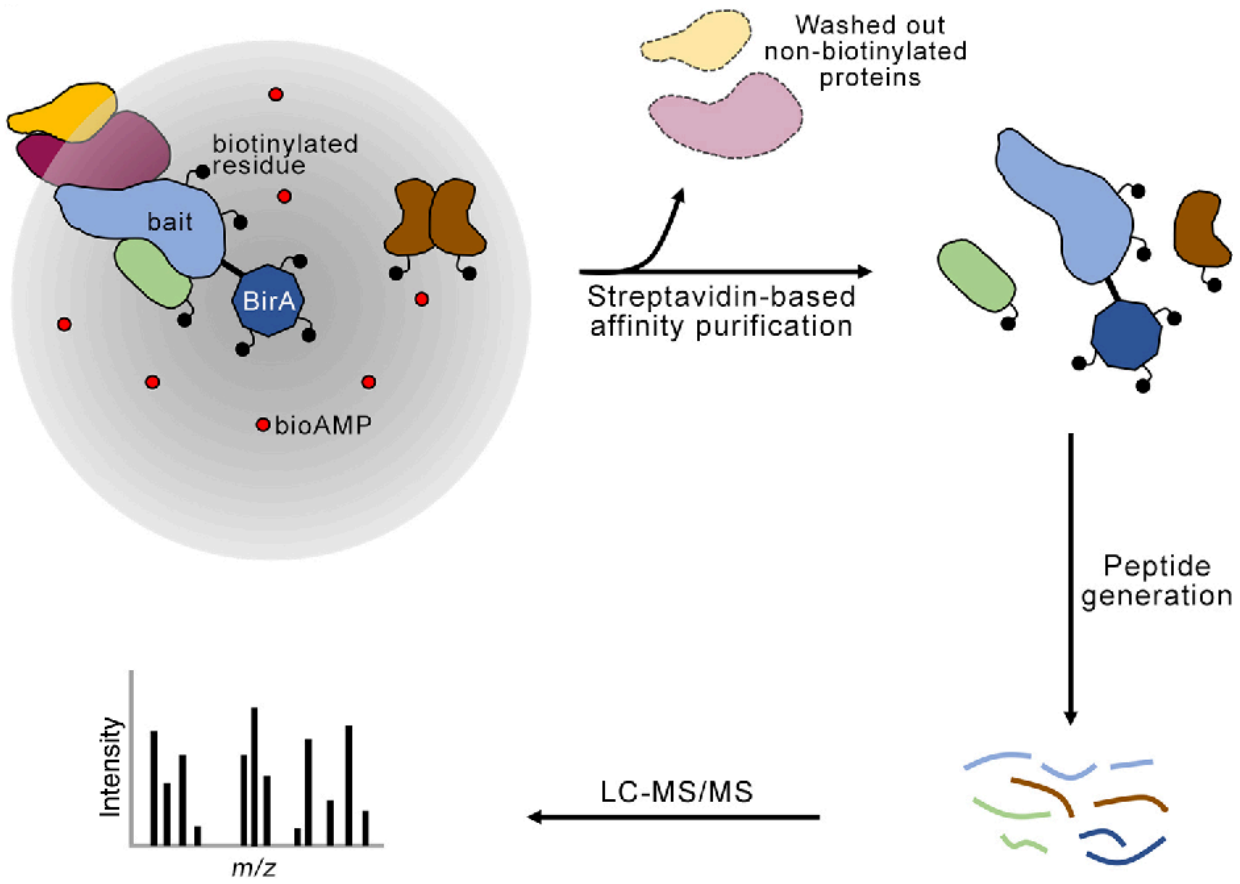
Thankan RS, Thomas E, Weldemariam MM, Purushottamachar P, Huang W, **Kane MA**, Zhang Y, Ambulos N, Wang BD, Weber D, **Njar VCO.**

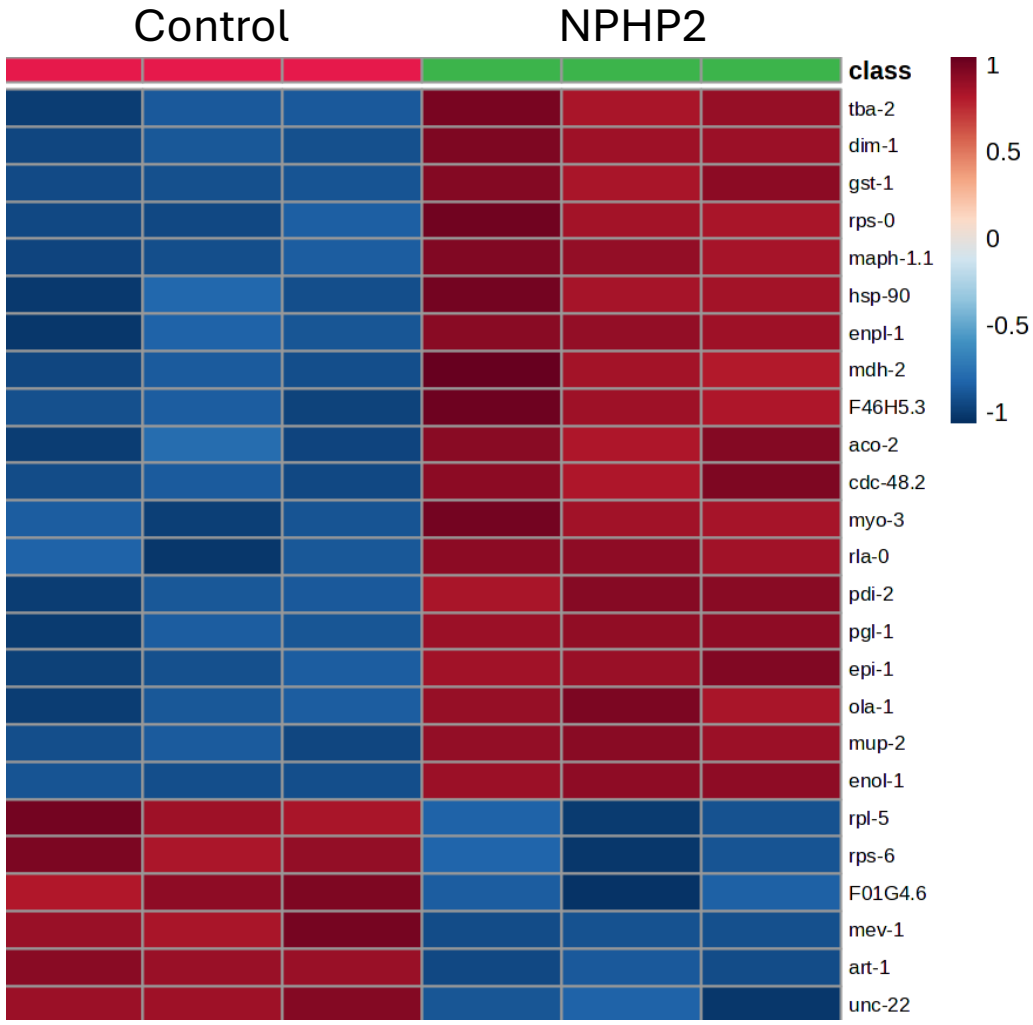
Mol Oncol. 2025 Aug;19(8):2292-2309.

PMID: 40007440

❑ Bruce Vogel Lab, SOM

Identification of biotinylated proteins using proximity labeling – BioID/TurboID





- An enzyme fused to a protein of interest to covalently attach a reactive label to nearby proteins in a living cell.

❑ Marc Simad Lab, SOM

- Aim - changes in the **transportome in spinal cord axons** after injury ± drug treatment.
- **NHS-biotin labeling method -** to label spinal cord axons through intracortical injection of NHS-biotin

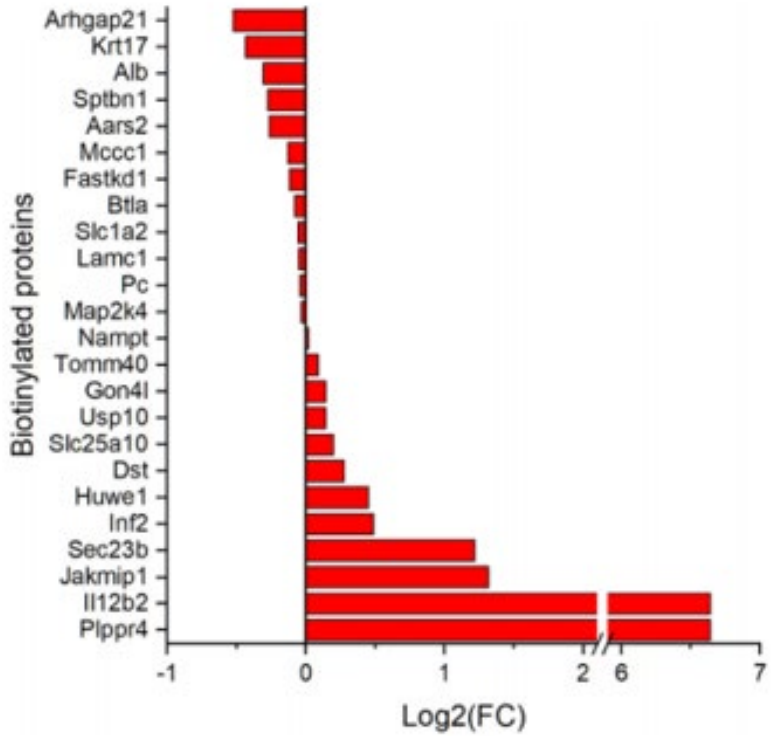
Transportome analysis using NHS biotin labeling method

- Proteomic changes in the transportome in spinal cord axons

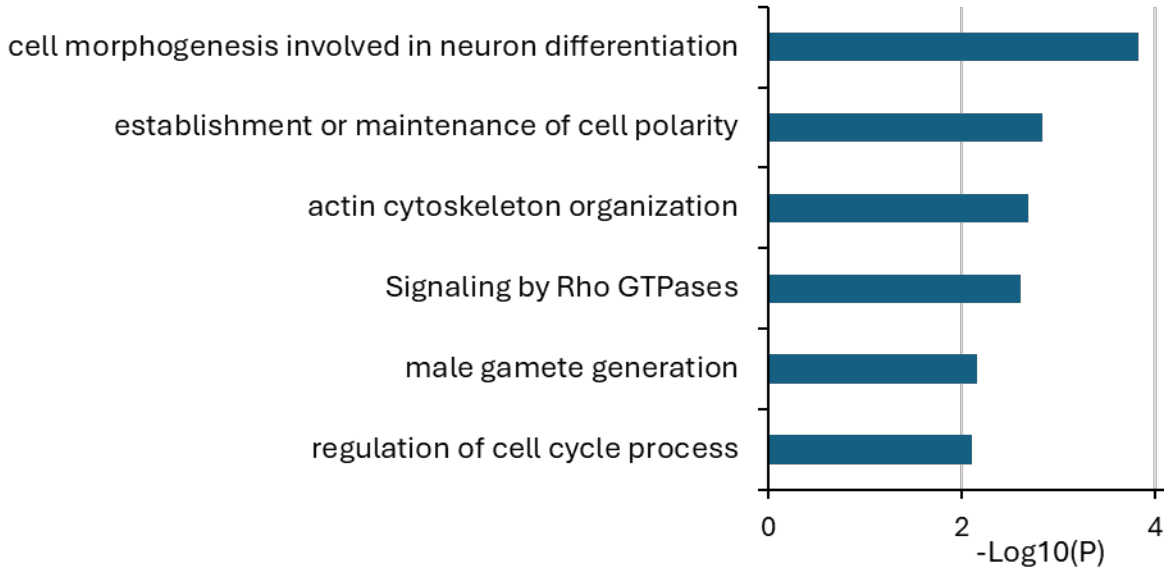
DiDBiT - Direct Detection of Biotin-containing Tags

direct detection of biotin-tagged newly synthesized peptides

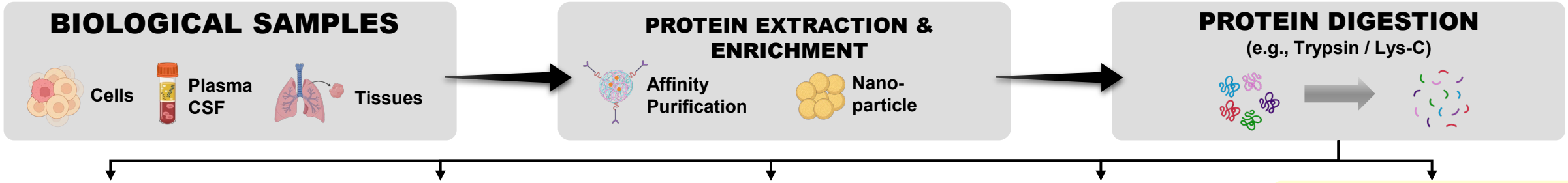
- As described in: PMID: 25117199, PMID: 31412257



GO – biotinylated proteins



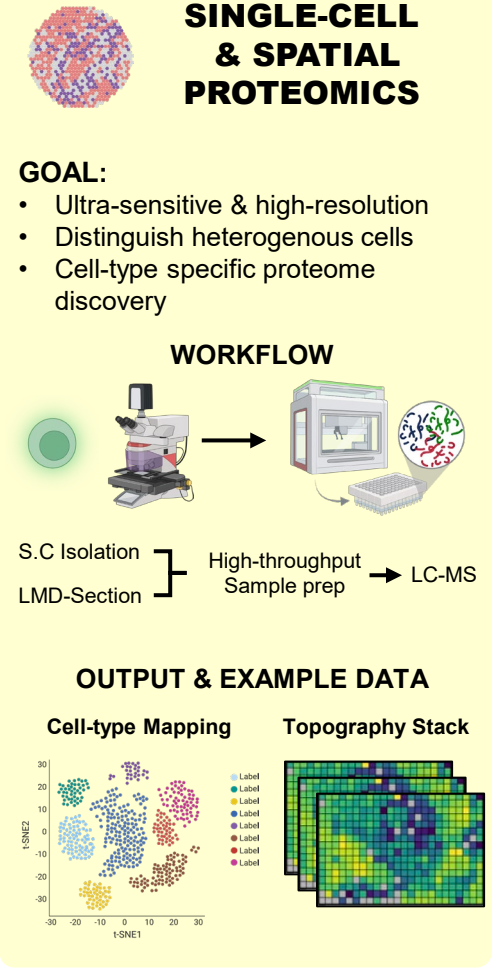
PROTEOMICS OVERVIEW – DIFFERENT TYPES OF ANALYSIS



Spatial protein signatures

Methods of spatial isolation
- Physical dissection
- Laser capture microdissection
- Single-cell (low cell input) / sorted cells

Useful for sample types:
- Tumors
- Lesions (burns, wounds)
- Tissue regions



Localized Cutaneous Radiation Injury in Porcine CRI Model – Dose Dependent

Background

- The zone of coagulation contains dead tissue as a result of direct injury.
- Adjacent to this area in the zone of stasis tissues suffer hypoperfusion owing to the vasoconstriction of vessels in response to the injury, and although not directly damaged, these tissues are vulnerable to ischemia, infection and necrosis, and initial burn wounds may expand and deepen.
- Global proteomics to discover the injury maker proteins with various dose rate per wound

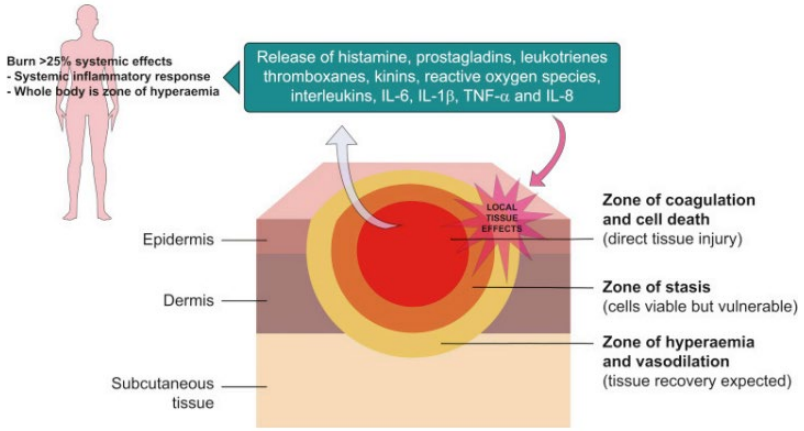
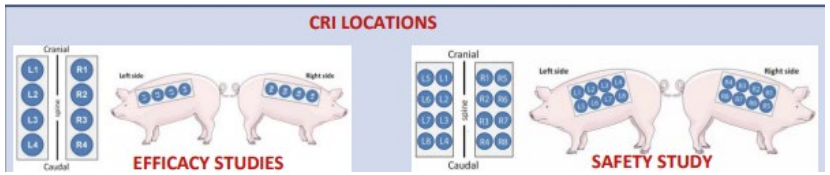
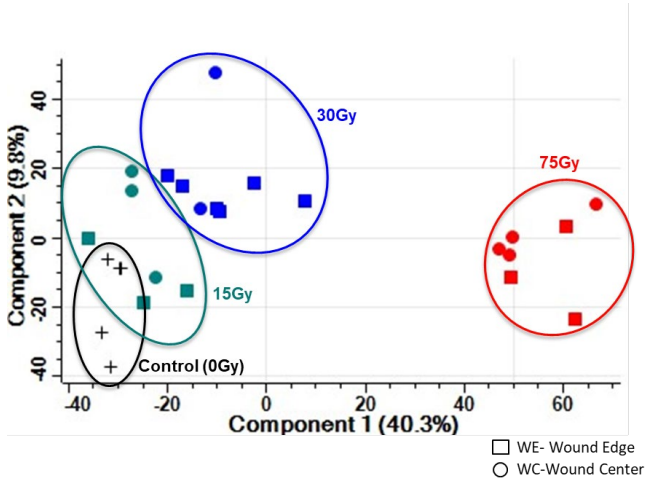
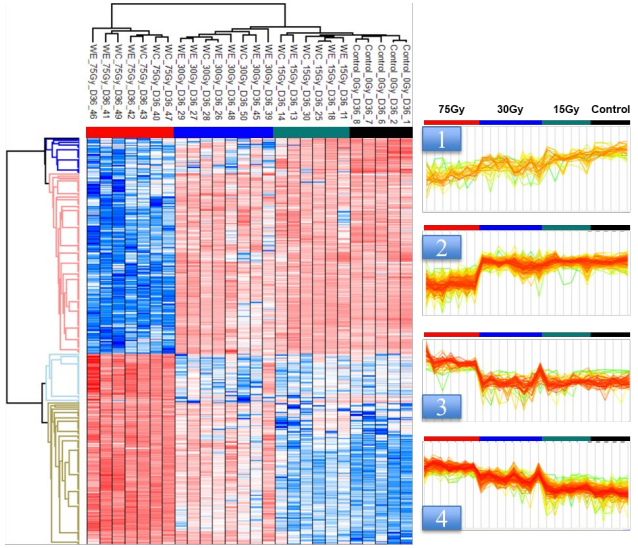
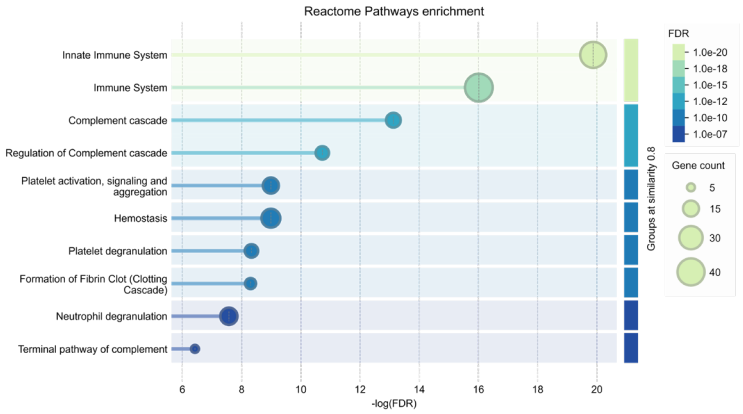


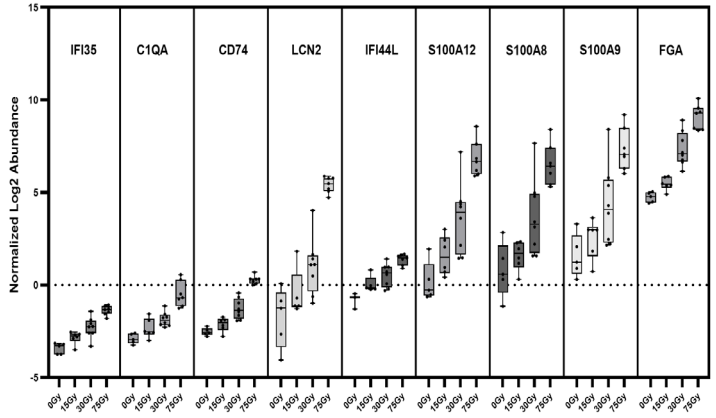
Image: BJA Educ. 2021 Dec 21;22(3):94–103. doi: 10.1016/j.bjae.2021.10.001





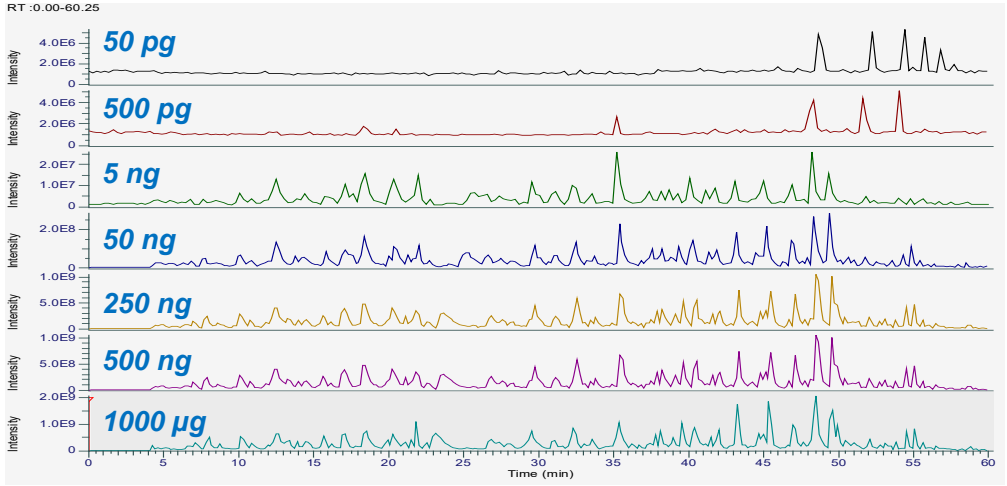




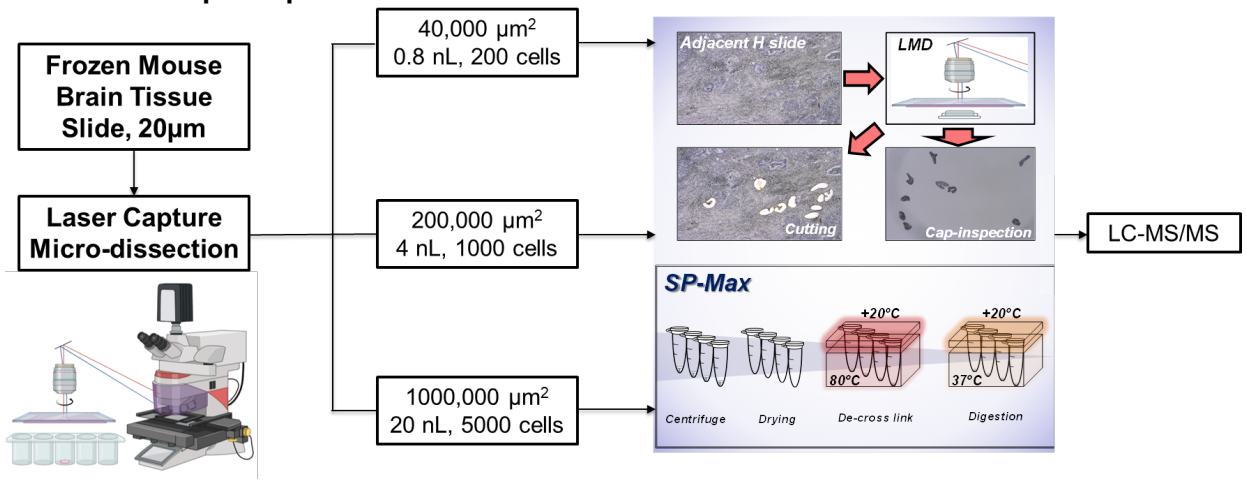


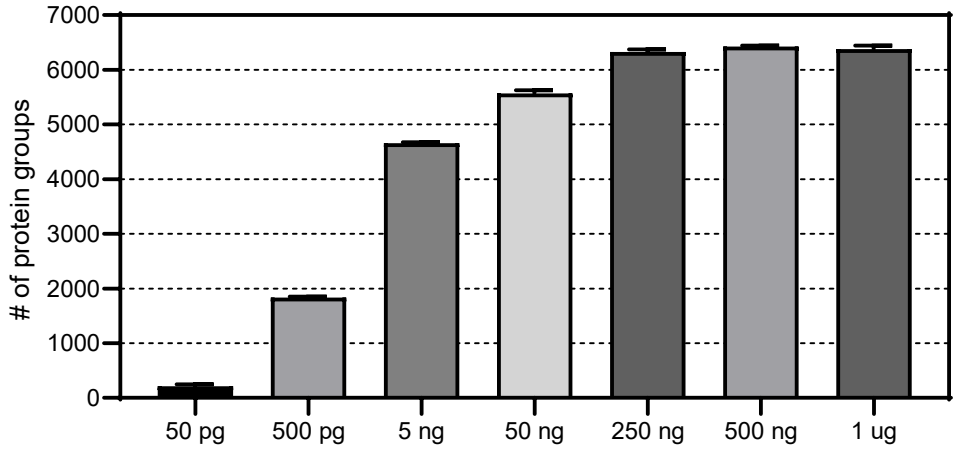
Low-Input Peptide Analysis & LMD-based Spatial Proteomics

Dynamics by HeLa peptide input amount from single-cell level to bulk level

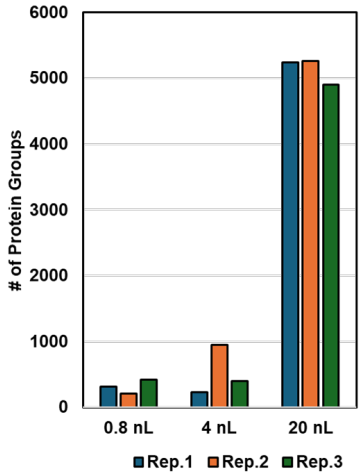


Workflow – Spatial proteomics

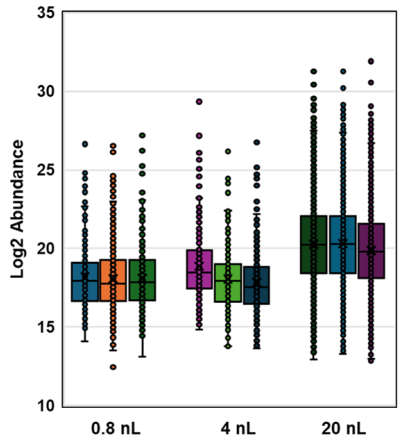




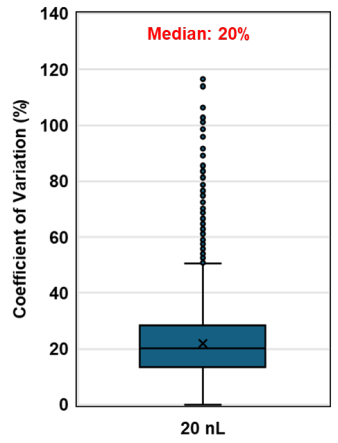
Identification



Quantification



Reproducibility



Mass Spectrometry fueling Drug Discovery and Drug Development at UMB

UNIVERSITY of MARYLAND
SCHOOL OF PHARMACY
MASS SPECTROMETRY CENTER

Drug Discovery

- Proteomics
- Metabolomics
- Mass Spectrometry Imaging

g Development

uantitative LC-MS/MS

- Drug quantification
- Pre-clinical screening assays
- PK/PD

Mass Spectrometry Imaging: Molecular Histology

UNIVERSITY of MARYLAND
SCHOOL OF PHARMACY
MASS SPECTROMETRY CENTER

MALDI-MSI
DESI-MSI

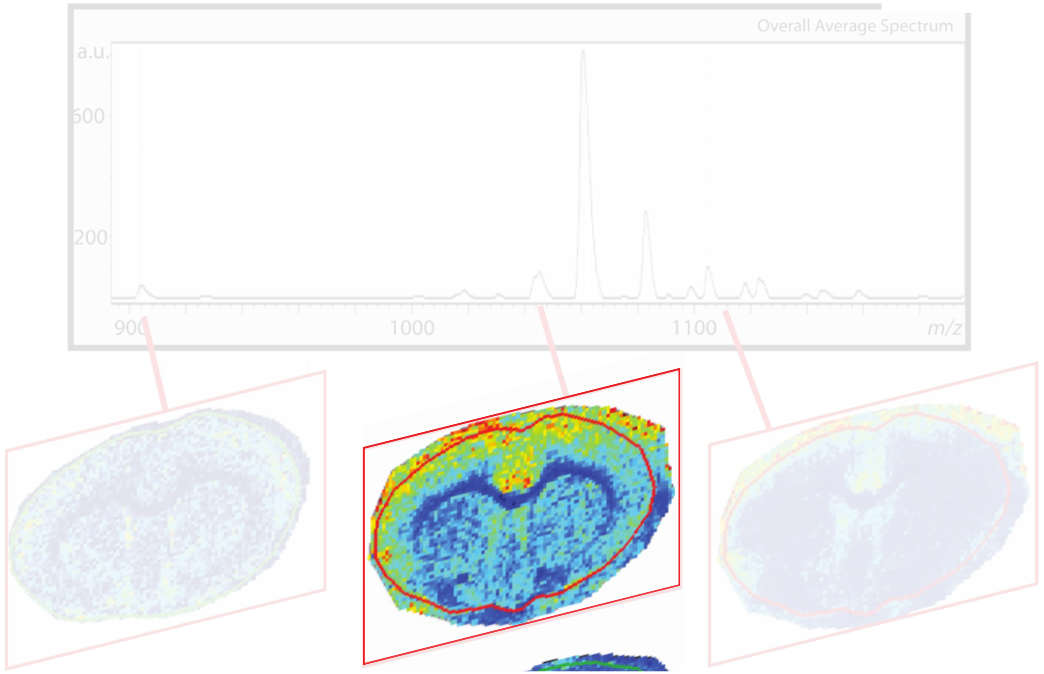
Combines mass spectrometry with histology-like information for the spatial distribution of molecules

A) Embedding and Sectioning **B) Matrix Application** **C) Instrumentation**

Spatially localize molecules

- Disease characterization
- ADME studies

- Drug molecules
- Metabolites
- Degradation products
- Biomarkers
- Markers of anatomical structure



D) Mass spectra and image display

MSI of tumors and lymph nodes

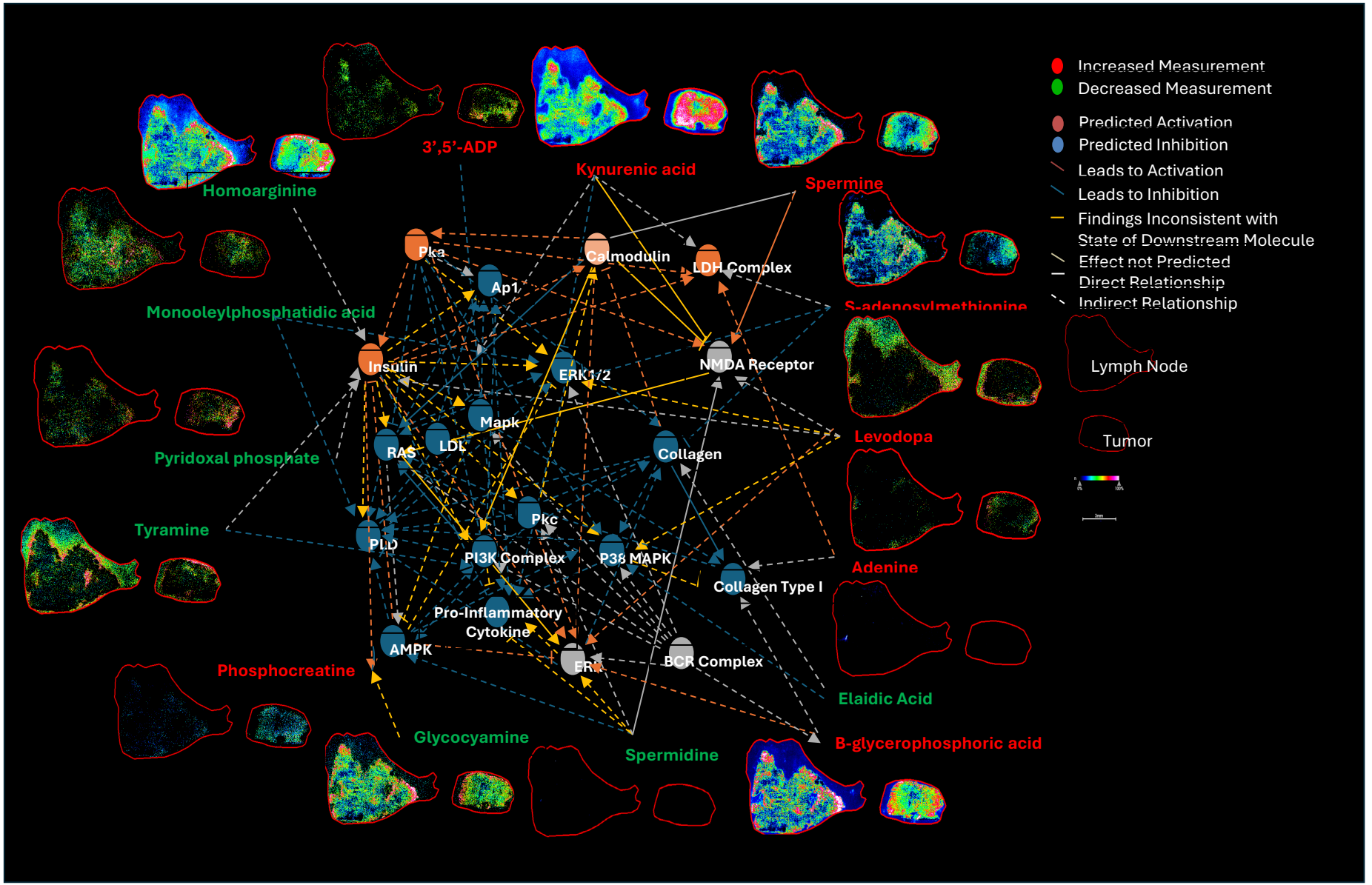
UNIVERSITY of MARYLAND
SCHOOL OF PHARMACY
MASS SPECTROMETRY CENTER

- Mass spectrometry imaging of immune response in human papillomavirus-associated vs. carcinogen-driven head and neck squamous cell carcinoma.

- MALDI MSI images of networked metabolites from IPA were generated and their relative intensities in immune cell, stromal cell, and tumor cell populations were assessed.

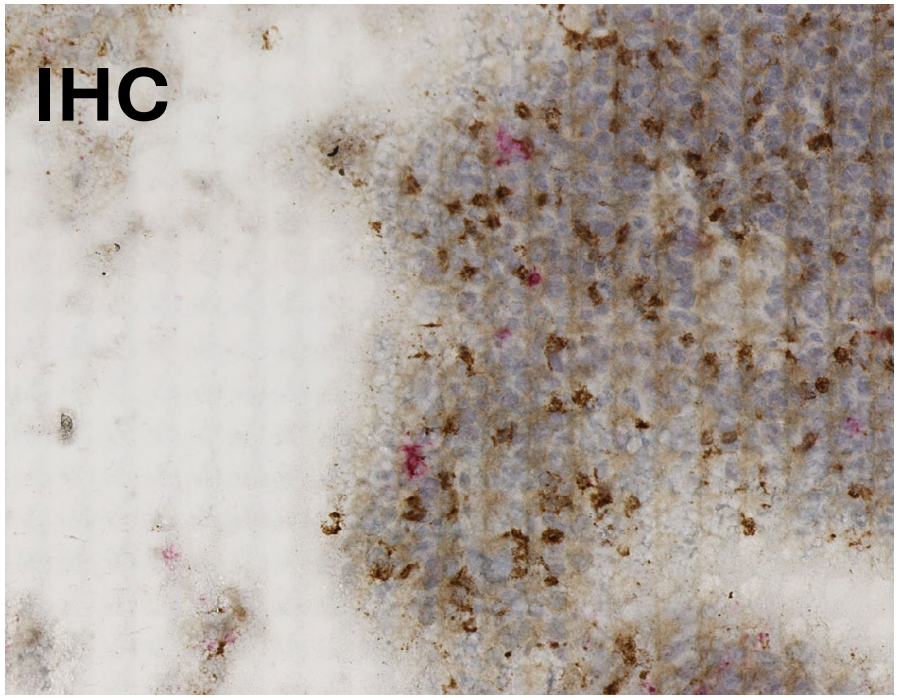
- Recent work from : **William Temple Andrews[1]**; Aleksandra Ogurtsova[2]; Mike Mikula[2]; Ogechi Nwankwoala[2]; Liz Engle[2]; Carole Fakhry[2]; R. Alex Harbison[2]; **Maureen Kane[1]**

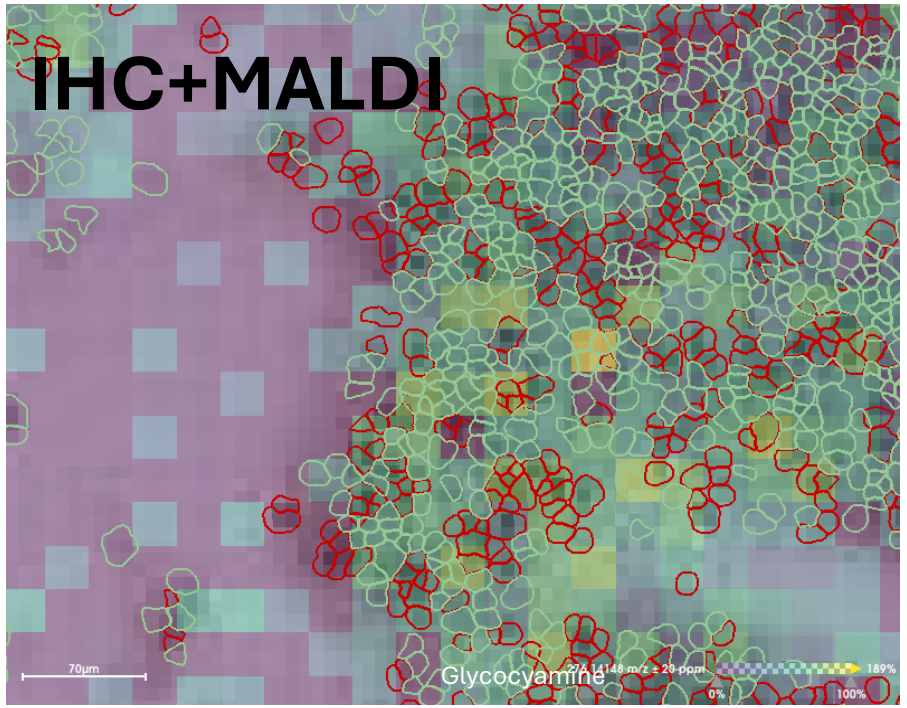
- [1]University of Maryland School of Pharmacy, Baltimore, MD; [2]Johns Hopkins Hospital, Baltimore, MD



MSI of tumors and lymph nodes

UNIVERSITY of MARYLAND
SCHOOL OF PHARMACY
MASS SPECTROMETRY CENTER





H&E staining was conducted on all samples after MALDI-MSI analysis.
MALDI slides were subjected to IHC staining for CD163, CD8, CD20, DAB, AE1 and AE2 for tumor immune response.

QuPath was used for cell detection and sorting cells into immune cell, stromal cell, and tumor cell populations based on their immunohistochemial staining.

Annotated cells were combined into one annotation per cell type and annotations were converted into regions in SCiLS Lab for analysis of each separate cell population.

Endocrine Disruptors in Skin

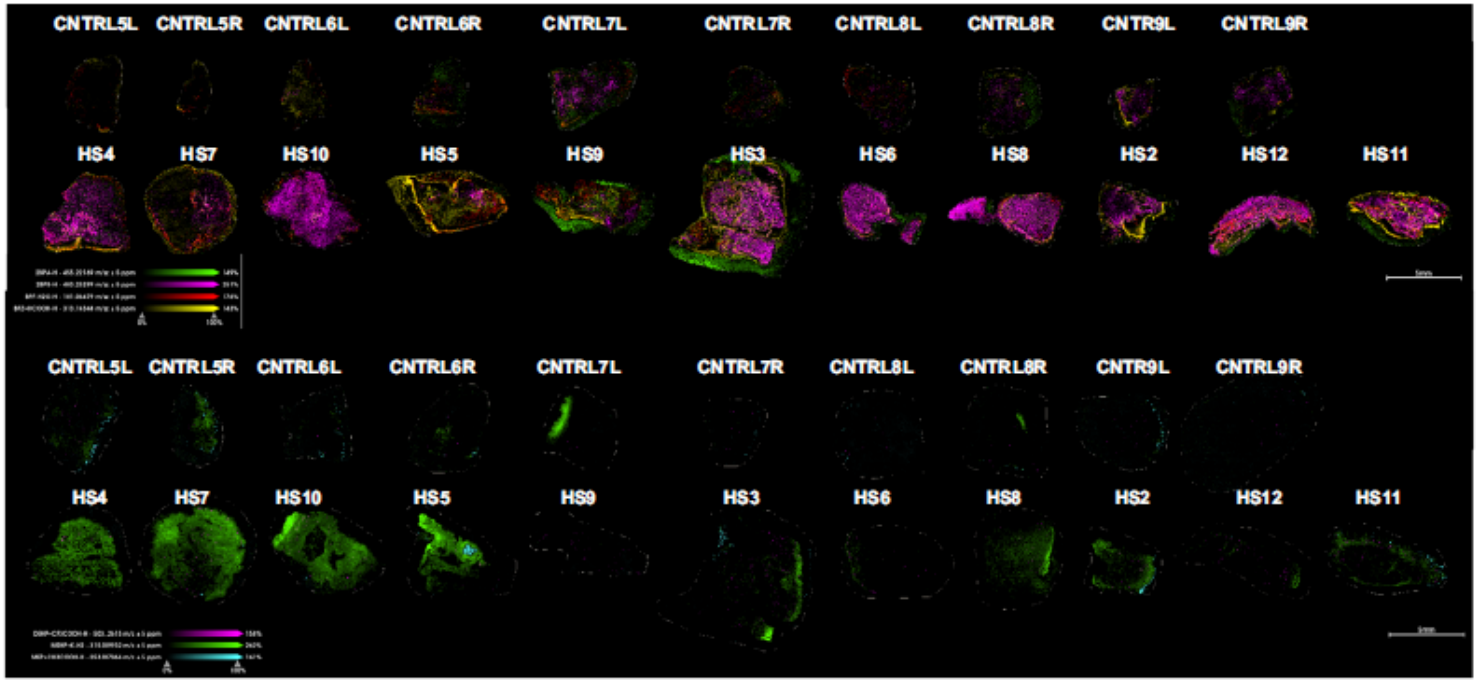
UNIVERSITY of MARYLAND
SCHOOL OF PHARMACY
MASS SPECTROMETRY CENTER

- HS skin contains high intensity of p-EDs compared to normal axillary controls as visualized by mass spectrometry imaging (MSI)

at the tissue level (**a**)

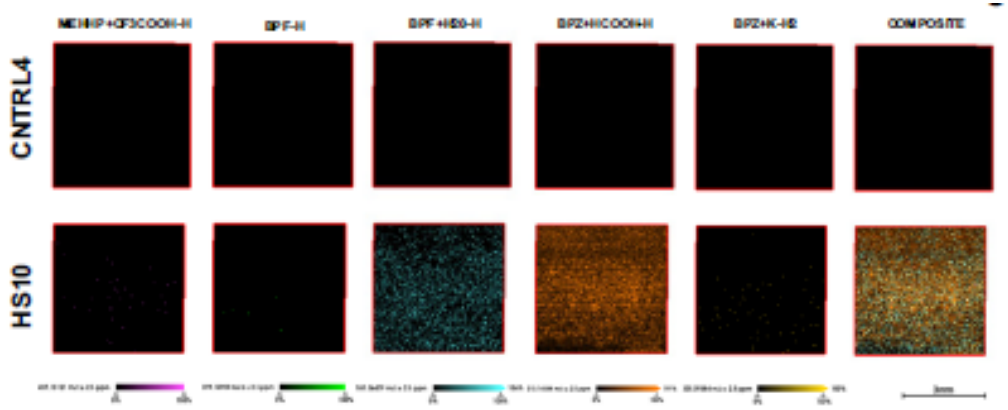
- top bisphenols
- bottom phthalates

a



in ex vivo cultured cells (**b**)

b



[Plastic associated endocrine disruptors reduce Nicastrin protein and potentiate inflammation in hidradenitis suppurativa skin disease.](#)
Williams KL, Badiei B, Reilly J, **Andrews W,** Minsky HB, Haddad NR, Martinez Pena EG, Sun M, Lee S, Li A, Curvin-Aquilla L, Johnson AY, Willis A, Kirby CS, van Ee A, Xue Y, Cox CA, Rajagopalan SP, Kang S, Kannan K, Caffrey J, Archer NK, **Kane M**, Garza LA.
Nat Commun. 2025 Nov 28;16(1):10755.
PMID: 41315355

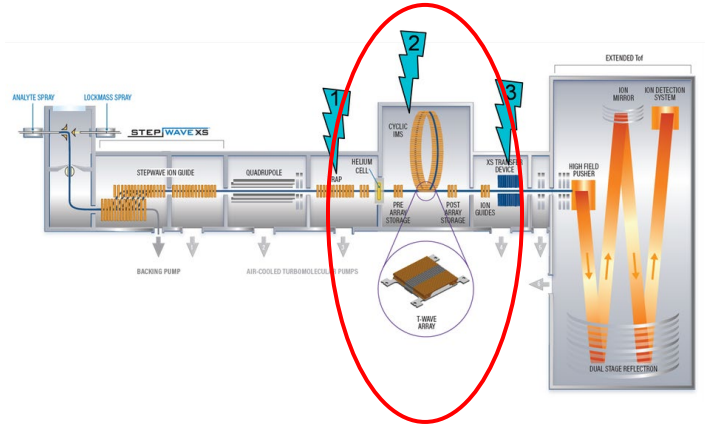
Imaging of lipids after TBI

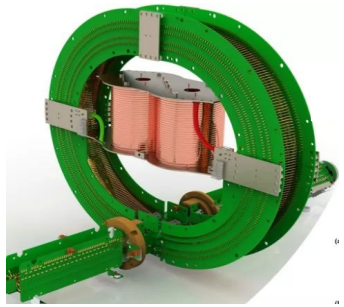
UNIVERSITY of MARYLAND
SCHOOL OF PHARMACY
MASS SPECTROMETRY CENTER

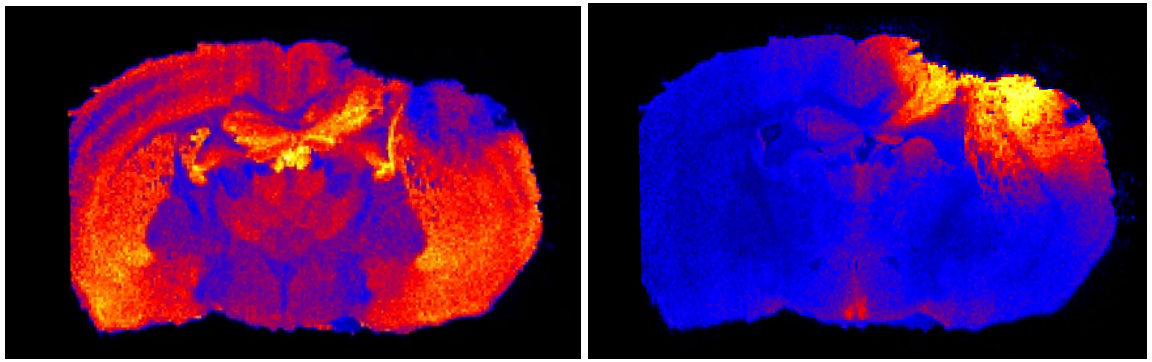
Incorporation of new technology

Waters CIMS Q-TOF
with DESI imaging source

Separation of isobaric lipids during MSI using cyclic ion mobility reveals distinct lipid distributions in brain after traumatic brain injury

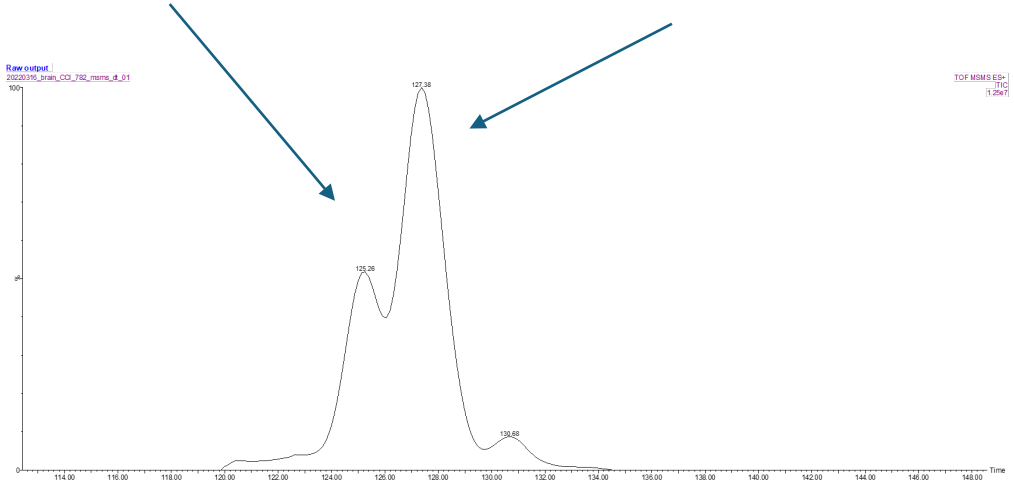






M+H PC 36:4 782.5694

M+Na PC 34:1 782.5670



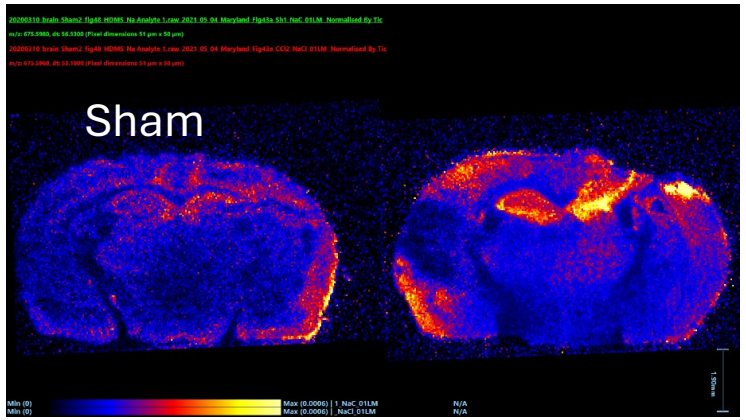
Recent work from : **Ludovic Muller**[1], Emmanuelle Claude[3], Niv Hedgekar[2], Chinmoy Sarkar[2], Marta M. Lipinski[2], **Maureen A. Kane**[1*]

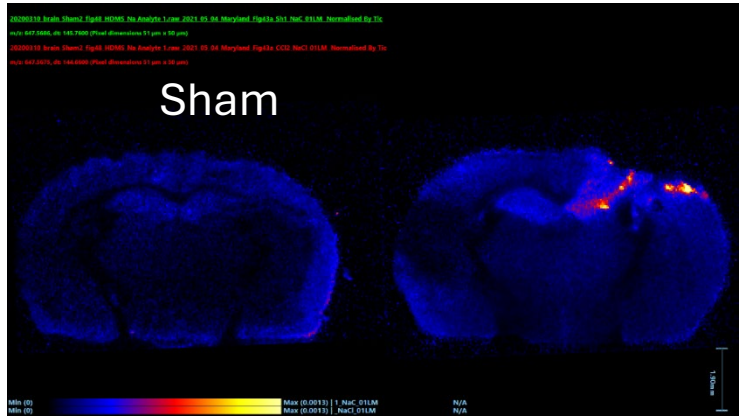
1. UMB School of Pharmacy; 2. UMB School of Medicine, 3. Waters *(manuscript in preparation)*

Imaging of lipids after TBI

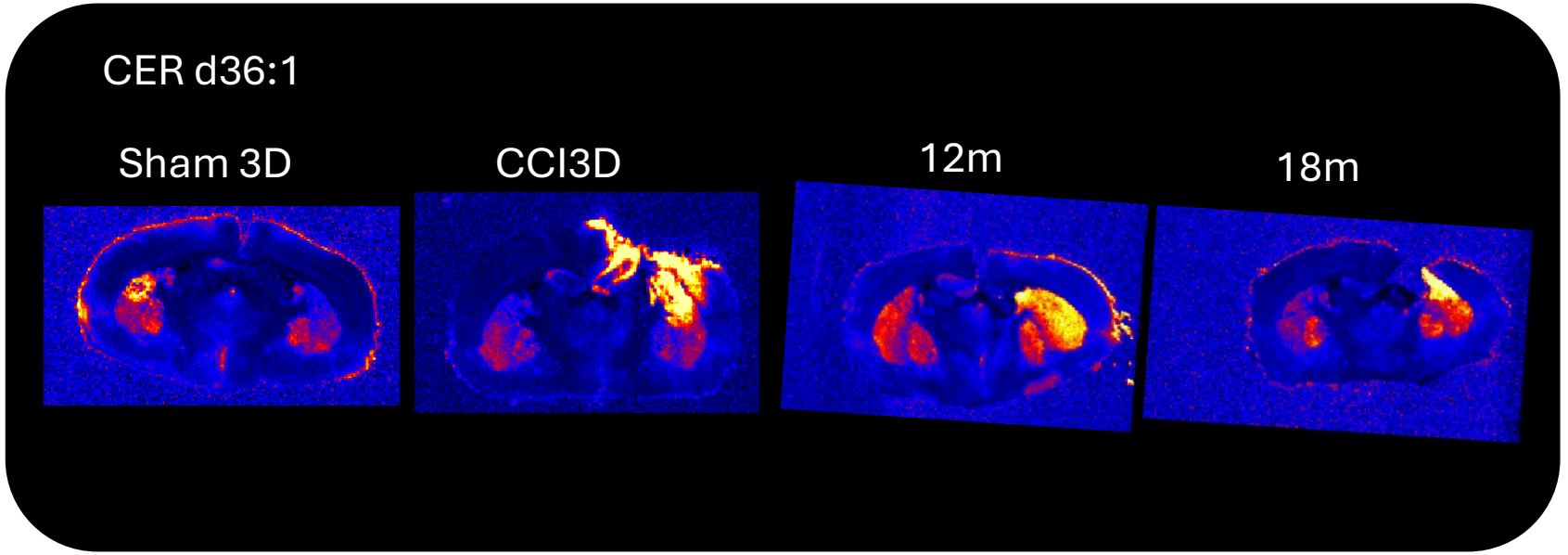
UNIVERSITY of MARYLAND
SCHOOL OF PHARMACY
MASS SPECTROMETRY CENTER

Cholesteryl esters





Longitudinal imaging of lipid distribution in brain after TBI

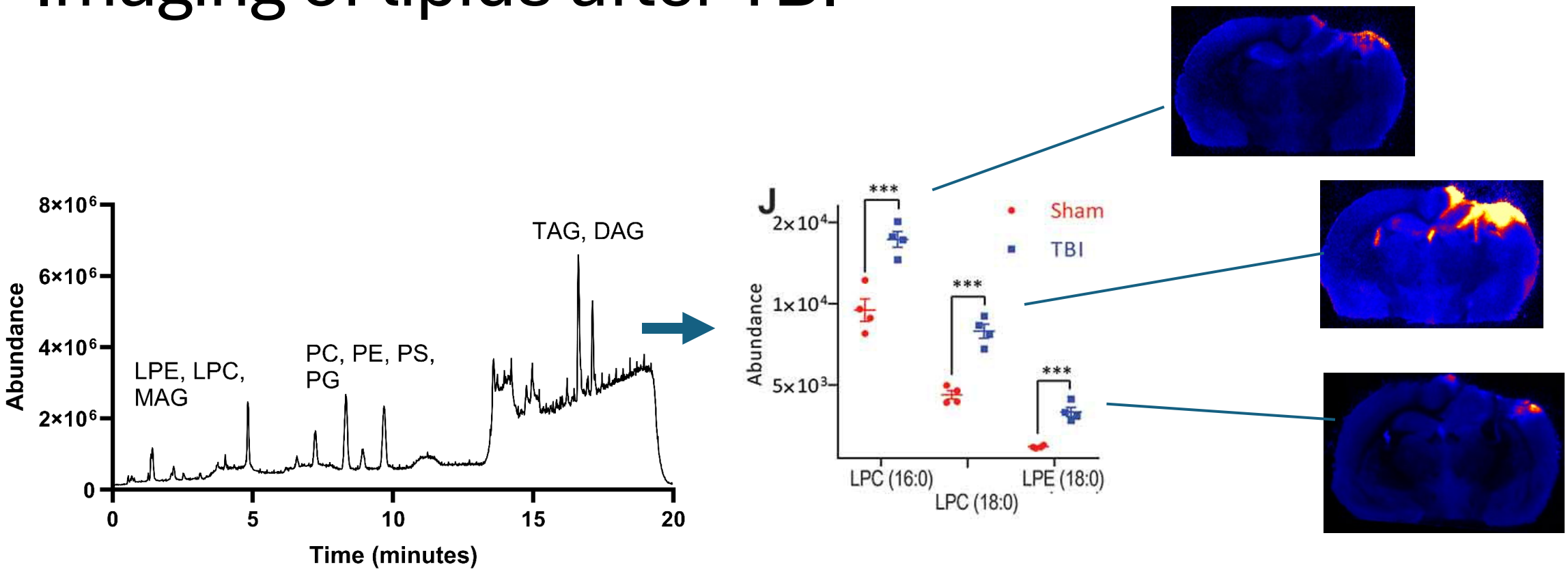


Inhibition of autophagy-lysosomal function exacerbates microglial and monocyte lipid metabolism reprograming and dysfunction after brain injury.
Mehrabani-Tabari AA, Hegdekar N, Herb BR, Kachi SA, Sarkar C, Thapa S, Nguyen DP, Morel Y, Weldemariam MM, Muller L, Temple Andrews W, Cortes-Gutierrez M, Fan X, Ayithan N, Pettyjohn-Robin O, Bustos S, Greer LK, Gore JT, **Kane MA,** Ament SA, Jones JW, **Lipinski MM.**
bioRxiv [Preprint]. 2025 Sep 8:2025.09.04.674092.
PMID: 40964307

In review

Integrating LC-MS/MS lipidomics with Imaging of lipids after TBI

UNIVERSITY of MARYLAND
SCHOOL OF PHARMACY
MASS SPECTROMETRY CENTER



PLA2G4A/cPLA2-mediated lysosomal membrane damage leads to inhibition of autophagy and neurodegeneration after brain trauma.
Sarkar C, Jones JW, Hegdekar N, Thayer JA, Kumar A, Faden AI, **Kane MA, Lipinski MM.**
Autophagy. 2020 Mar;16(3):466-485.
PMID: 31238788

Mass Spectrometry fueling Drug Discovery and Drug Development at UMB

UNIVERSITY of MARYLAND
SCHOOL OF PHARMACY
MASS SPECTROMETRY CENTER

Drug Discovery

- Proteomics
- **Metabolomics**
- Mass Spectrometry Imaging

Drug Development

- Quantitative LC-MS/MS
  - Drug quantification
  - Pre-clinical screening assays
  - PK/PD

Why Metabolomics?

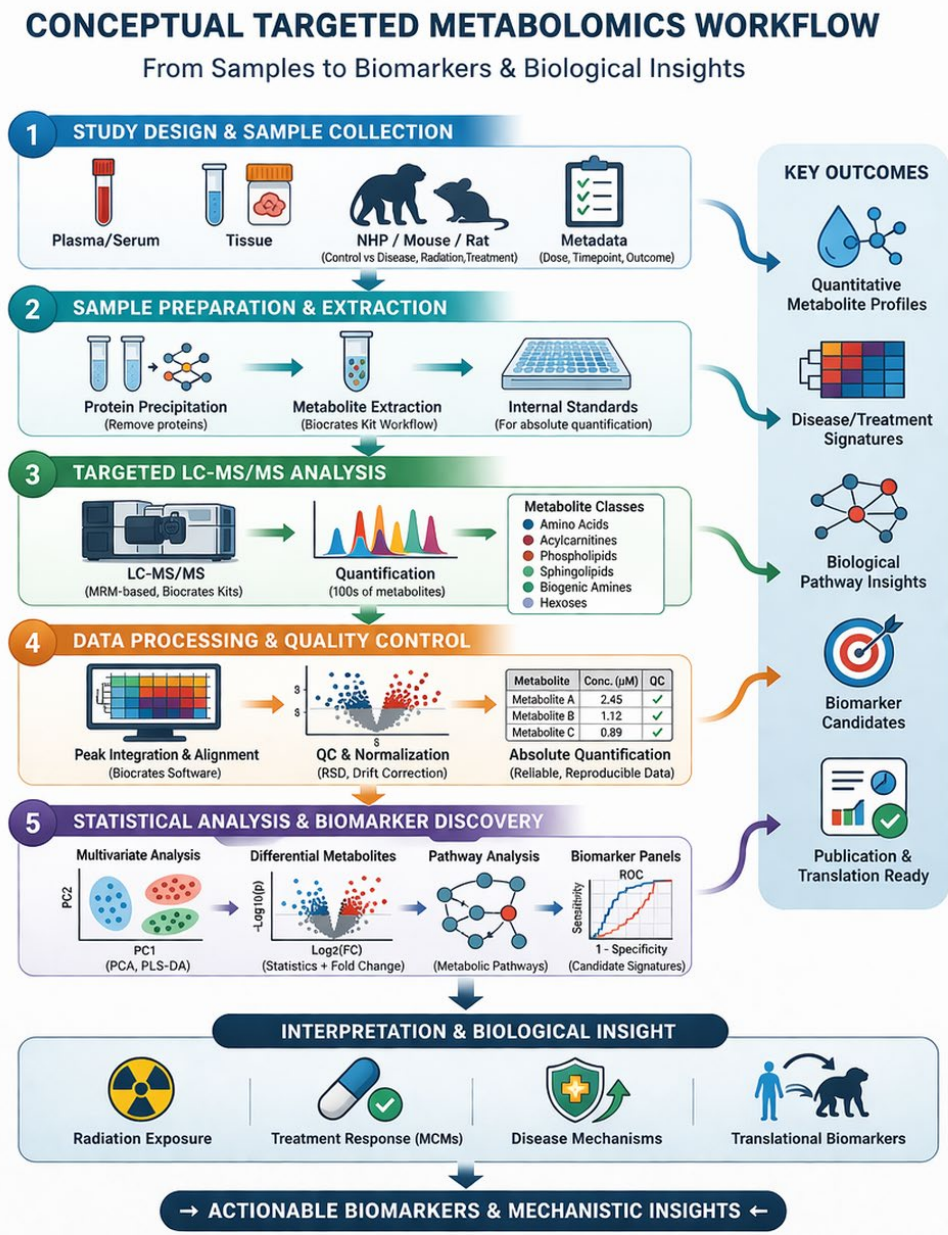
- Captures real-time biochemical changes
- Sensitive to disease, drug exposure, and environmental stress
- Enables biomarker discovery and mechanistic insights
- Translational relevance across species

- Metabolomics: typically molecules <1000 MW
  - Metabolites
  - Lipids

Targeted Metabolomics Workflow

- Targeted LC-MS/MS metabolomics
  - Biocrates Kit – commercially available, all-in-one
- Absolute quantification using internal standards
- High reproducibility and standardized assay
- All metabolites and lipids are identified
- Hundreds of metabolites and lipids spanning numerous metabolite & lipid classes

Biocrates panel	# biomarkers on panel
P180	226
MxP Quant 500	1492
MxP Quant 1000	1881



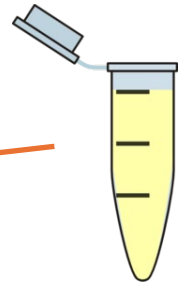
Biomarker discovery, development and multi-omic analysis of IR-induced injury

Mechanisms of Injury, Biomarker Identification and Characterization

Proteomics

Global, label-free proteomic analysis via LC-MS/MS

Huang et al. PMID: 32947488	Plasma, NHP
Huang et al. PMID: 34546219	Lung, NHP
Muller et al. PMID: 34546218	Lymph node, NHP
Zalesak et al. PMID: 34546217	Heart, NHP
Huang et al. PMID: 34546216	Kidney, NHP
Huang et al. PMID: 32947489	GI, NHP
Huang et al. PMID: 30652977	Lung, mouse
Huang et al. PMID: 30624357	GI, mouse
Yu et al. PMID: 34546221	Plasma, lung, heart, jejunum, NHP



Mass Spectrometry Imaging

(spatial metabolomics)

Muller et al. PMID: 34546218	Lymph node, NHP
Carter et. al. PMID: 32665567	Lung, NHP
Carter et al. PMID: 30681424	GI, NHP
Carter et al. PMID: 28871103	Lung, NHP
Carter et al. PMID: 26425906	Lung, NHP, + MCM

Metabolomics

Targeted metabolomic analysis via LC-MS/MS

Jones et al. PMID: 30681425	Plasma, mouse, M/F
Kumar et al. PMID: 34546220	Plasma, NHP
Zalesak et al. PMID: 34546217	Heart, NHP
Jones et al. PMID: 30624349	GI, plasma, mouse
Jones et al. PMID: 28971289	Lung, mouse, + MCM
Muller et al. PMID: 34546218	Lymph node, NHP
Jones et al. PMID: 27557409	Lung, mouse

Biomarkers: Pathway from Discovery to Validation

Well-Defined Models

NHP, Mouse, Rat, Swine

Discovery Omics

Metabolomics, lipidomics, proteomics

Identification of candidate biomarkers

Radiation injury-responsive
- *Tissue*
- *Plasma*

Validation of candidate biomarkers

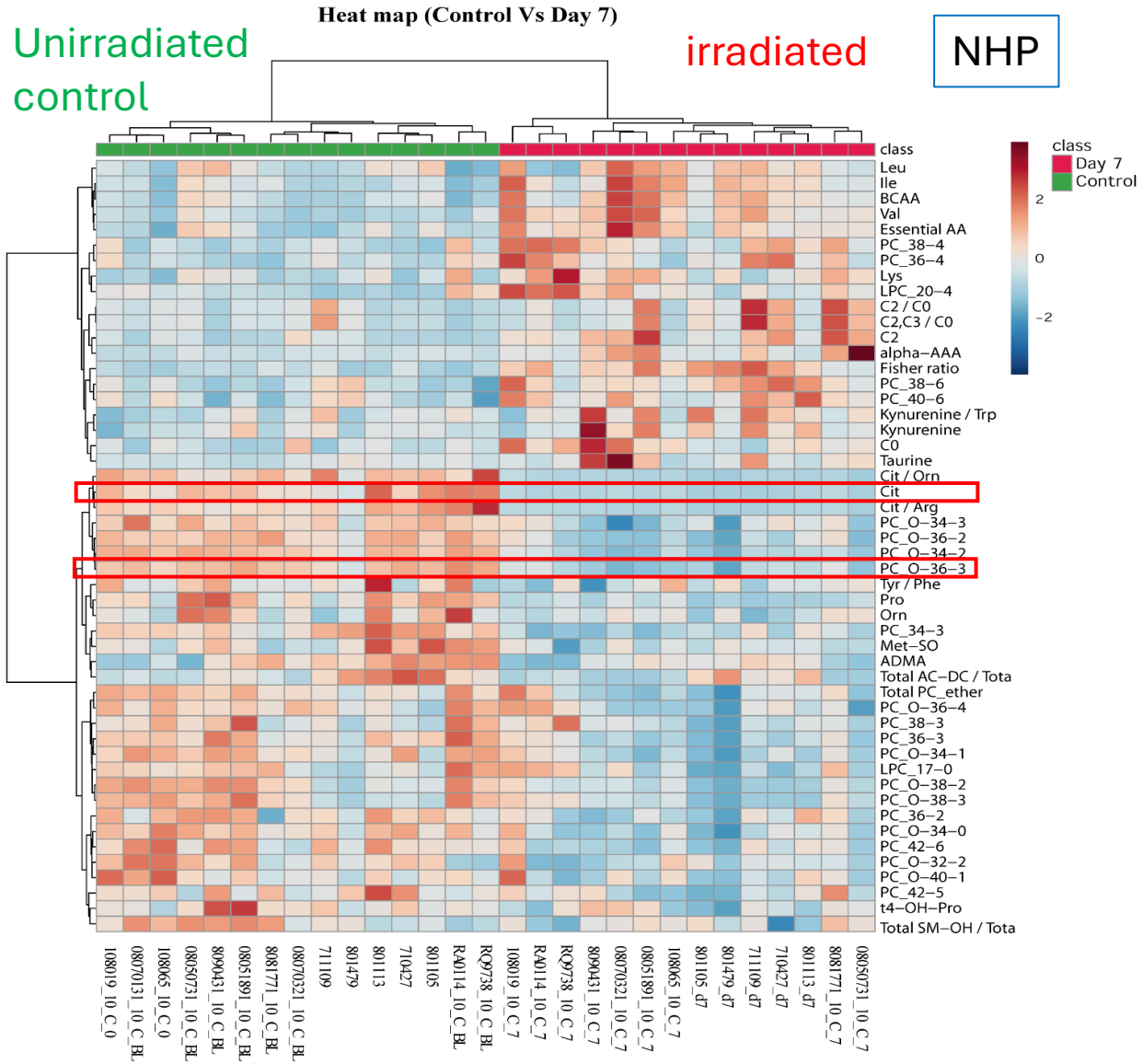
- *Cross species utility*
- *Circulating markers ability to inform on tissue damage*
- *Kinetics: time- and dose-dependency of change in abundance*
- ***Biomarker-Clinical Endpoint relationship: Ability to reflect syndrome and correlation with organ specific injury***

Correlation of plasma biomarker candidates with survival

Unirradiated control

irradiated

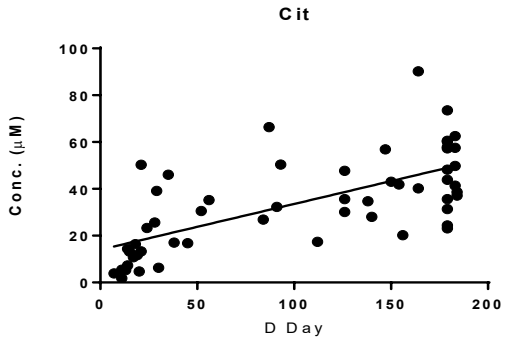
NHP



Pearson's correlation (r)

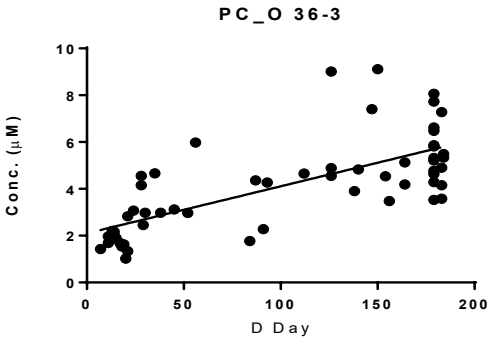
Correlation of GI-ARS candidate plasma biomarkers with survival

Metabolites	r	p value
PC_36-3	0.73	<0.0001
PC_O-36-3	0.7	<0.0001
Cit	0.68	<0.0001
PC_34-3	0.64	<0.0001
PC_O-36-2	0.61	<0.0001
Cit/Arg	0.59	<0.0001
Cit/Orn	0.59	<0.0001
PC_36-2	0.57	<0.0001
PC_O-32-2	0.52	<0.0001



Pearson r	
r	0.6827

P value	
P (two-tailed)	< 0.0001
P value summary	****
Significant? (alpha = 0.05)	Yes

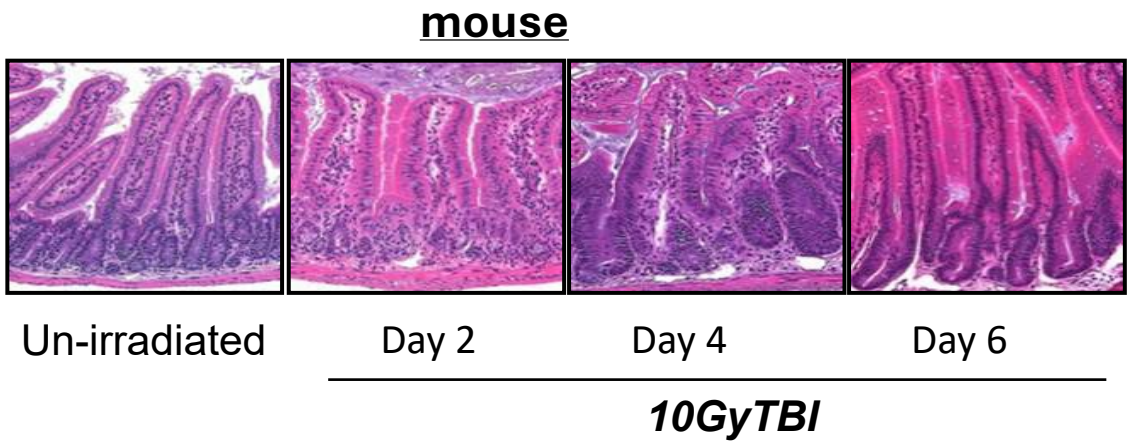


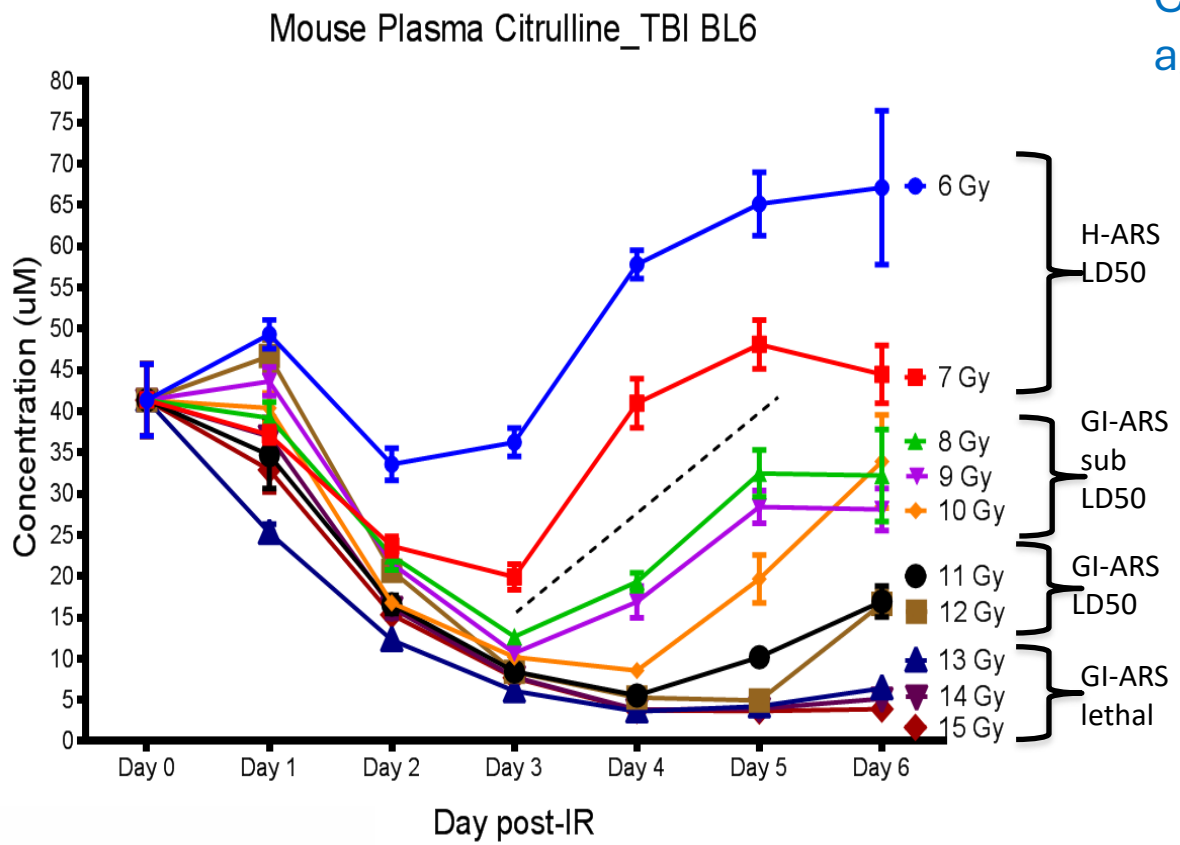
Pearson r	
r	0.7096

P value	
P (two-tailed)	< 0.0001
P value summary	****
Significant? (alpha = 0.05)	Yes

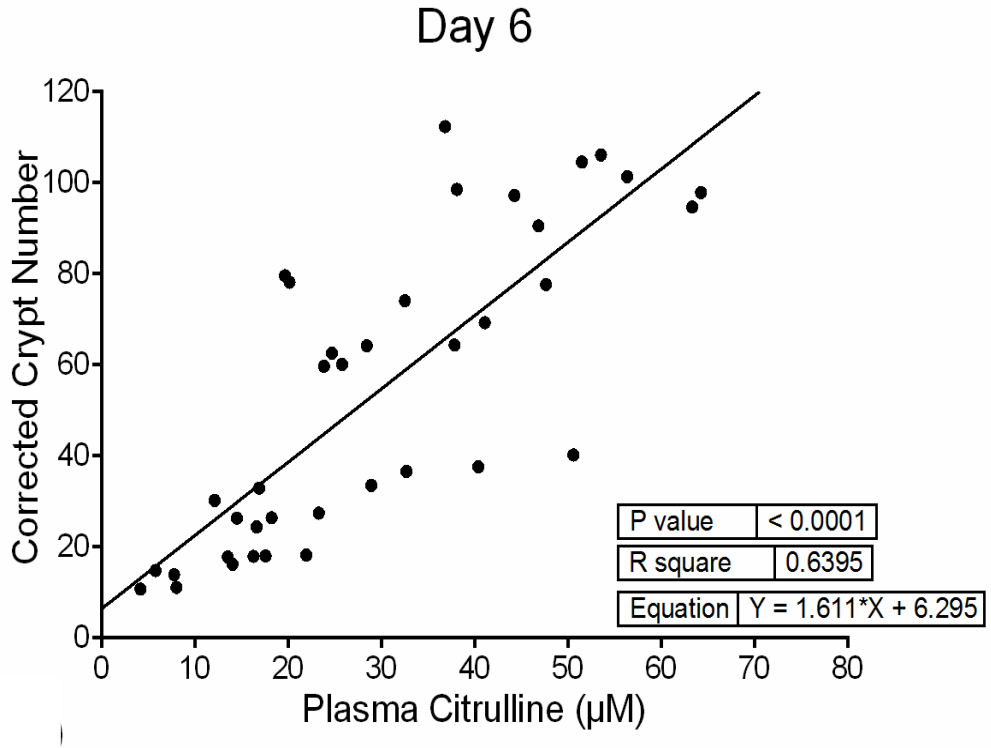
D7 irradiated data is from AXR23, n=5; AXR24, n=10 (n=15 total D7 irradiated).
Unirradiated / naïve, n=14. (10 Gy PBI/BM5 (AXR23) or 10 Gy PBI/BM2.5 (AXR24)).

Correlation of plasma biomarker with histological scoring





Correlation in mouse agrees with results in NHP

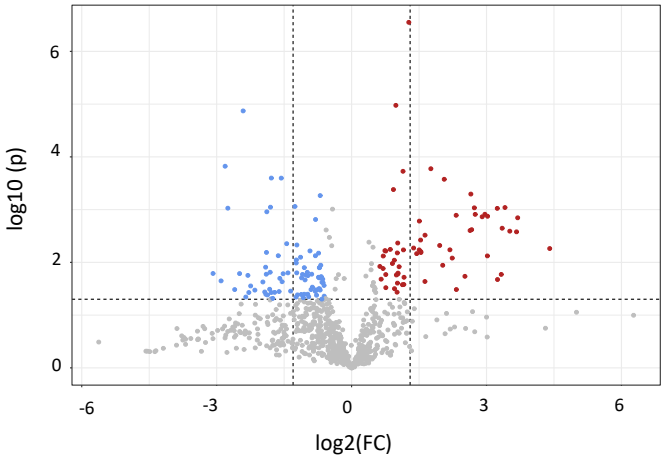


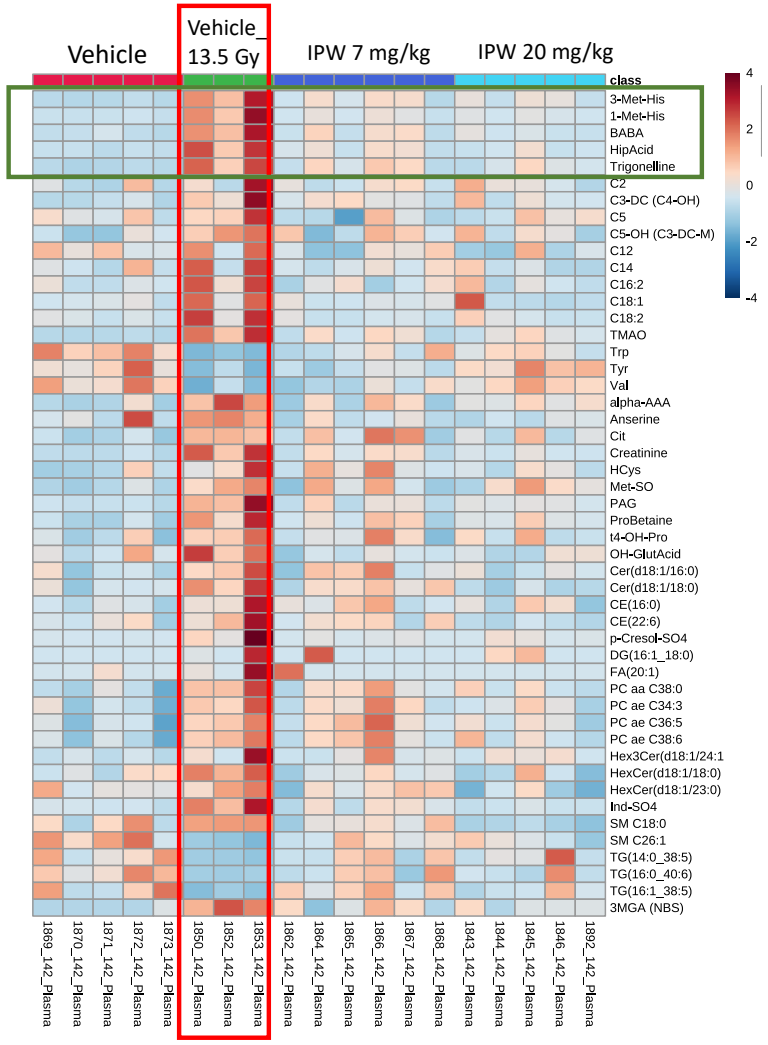
UNIVERSITY of MARYLAND
SCHOOL OF PHARMACY
MASS SPECTROMETRY CENTER

Biomarkers: Toxicity and Drug Efficacy

UNIVERSITY of MARYLAND SCHOOL OF PHARMACY MASS SPECTROMETRY CENTER

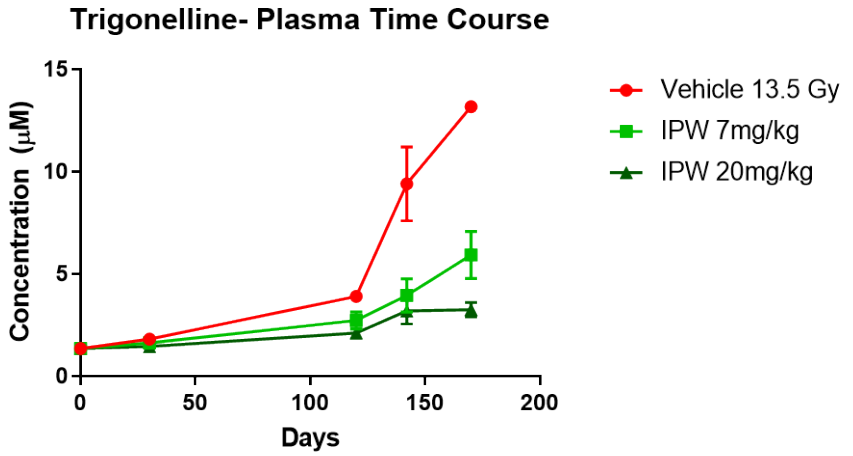
- *Biomarkers for toxicity and drug efficacy.* We have a robust biomarker program focused on identifying and validating biomarkers (towards FDA biomarker qualification) to be used in the drug development process for drug efficacy.





Metabolomic profile of rat PBI/BM8 model and identification of biomarkers with pharmacodynamic potential for IPW-5371

Example dose-responsive biomarker candidate identified from metabolomics



Recent work from: Swarnima Pandey1, Praveen Kumar1, Brian Fish3, Tom MacVittie2, Barry Hart4, **Maureen Kane1** 1UMB SOP Dept of Pharmaceutical Sciences, 2UMB SOM Radiation Oncology, 3Medical College of Wisconsin,4 Innovative Pathways

Manuscript in preparation

Mass Spectrometry fueling Drug Discovery and Drug Development at UMB

UNIVERSITY of MARYLAND
SCHOOL OF PHARMACY
MASS SPECTROMETRY CENTER

Drug Discovery

- Proteomics
- Metabolomics
- Mass Spectrometry Imaging

Drug Development

- Quantitative LC-MS/MS
  - Drug quantification
  - Pre-clinical screening assays
  - PK/PD

Targeted Metabolomics: Quantitative LC-MS/MS

Absolute quantitation

Targeted Metabolomics & Bioanalysis

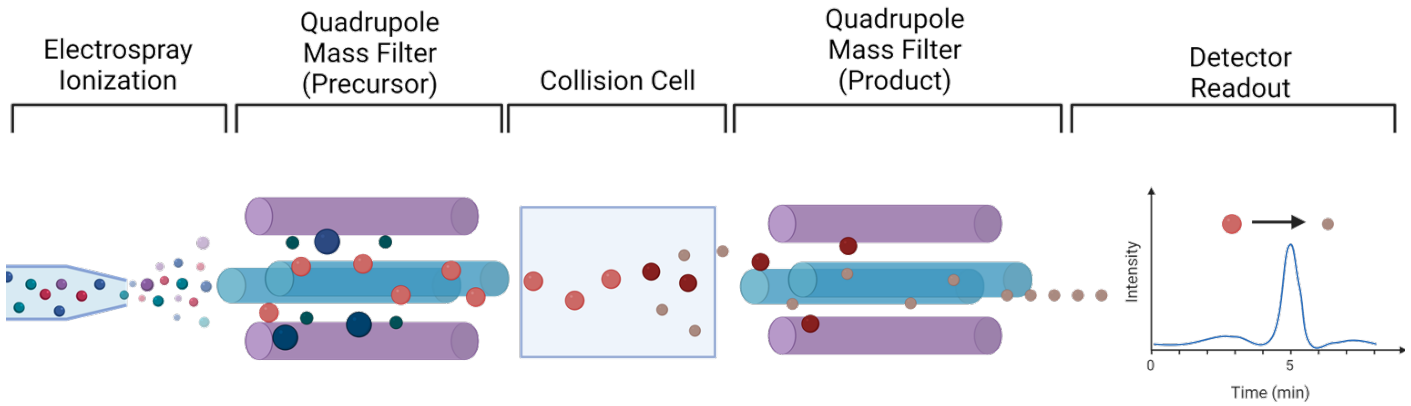
- ***Endogenous molecules***
- ***Biomarker candidates***
- ***Validation of 'omics hits***
- ***Quantification of drug in patient plasma***
- ***Patient PK profiles***

Assay Development

- *Sample preparation, chromatography, and MS detection scheme development and optimization*
- *Calibration curve to fit assay needs*

Assay Validation

- *QC samples used to validate assay*
- *Validation according to FDA Guidance*



Quantitation & Drug Analysis

Pharmaceutical Bioanalysis

UNIVERSITY of MARYLAND
SCHOOL OF PHARMACY
MASS SPECTROMETRY CENTER

Bioanalysis

- ***Patient PK profiles***
- ***Quantification of drug in patient plasma***
- ***Validation of 'omics hits & biomarker candidates***

Validation of a method for itraconazole and major metabolite hydroxyitraconazole for LC-MS/MS analysis with application in a formulation clinical study.
Krug SA, Coutinho AL, **Polli JE**, **Kane MA.**
J Pharm Biomed Anal. 2023 Sep 20;234:115505.
PMID: 37393691

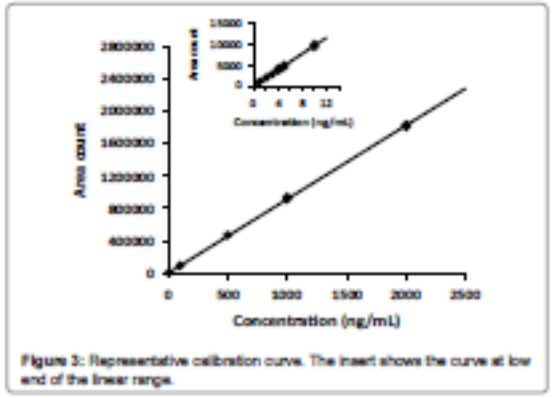


Figure 3: Representative calibration curve. The insert shows the curve at low end of the linear range.

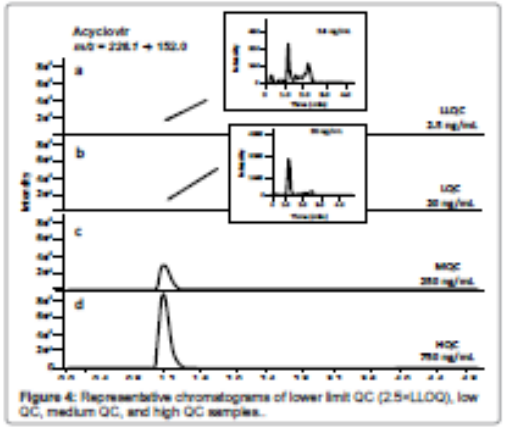


Figure 4: Representative chromatograms of lower limit QC (2.5=LLOQ), low QC, medium QC, and high QC samples.

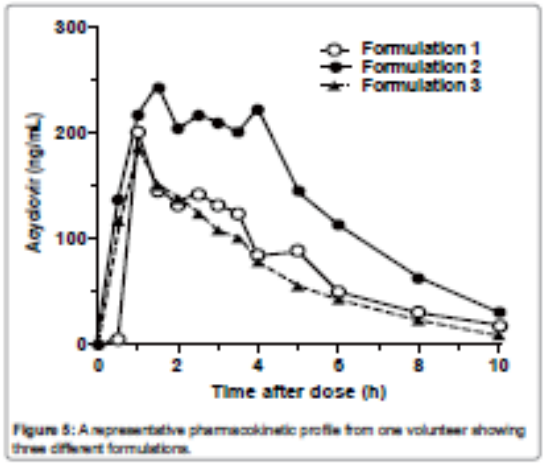
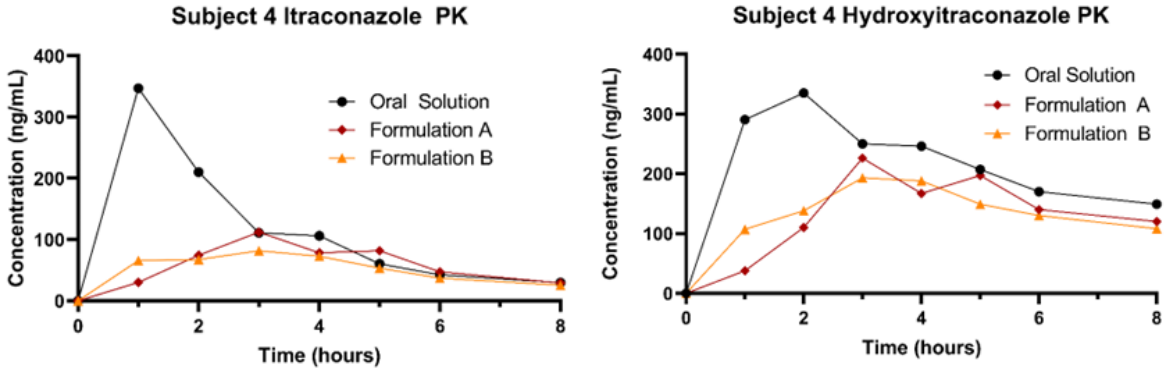


Figure 5: A representative pharmacokinetic profile from one volunteer showing three different formulations.

In Vitro-In Vivo Correlation Of Amorphous Solid Dispersion Enabled Itraconazole Tablets.
Coutinho AL, Adhikari A, Krug S, **Kane M**, Hollenbeck RG, Hoag SW, **Polli JE.**
Pharm Res. 2025 Mar;42(3):485-502.
PMID: 40069449

Figure 3: Different Formulations of 100 mg Itraconazole PK within the Same Subject for 0-8 hours.



Assay Development

- *Sample preparation, chromatography, and MS detection scheme development and optimization*
- *Calibration curve to fit assay needs*

Assay Validation

- *QC samples used to validate assay*
- *Validation according to FDA Guidance*

Pre-Clinical Screening Workflow for Drug Development
including Mass Spectrometry-Based Approaches

UNIVERSITY of MARYLAND SCHOOL OF PHARMACY MASS SPECTROMETRY CENTER

Can we apply typical TIER 1 workflows for metallodrug development?

-TIER 1 includes initial experiments for hundreds of analogs; we looked at ~15

Can the drug bind to the target?

- **K_d** – how well does the molecule bind with the target?
- **Solubility** – how can we dose *in vitro/in vivo*?

Can the drug reach the target?

- **Microsome Stability** – how fast is clearance? Too fast, drug will be metabolized before reaching desired target
- **Permeability** – can clearance occur and to what extent? Potential substrate liabilities?

Where does the drug go in vivo?

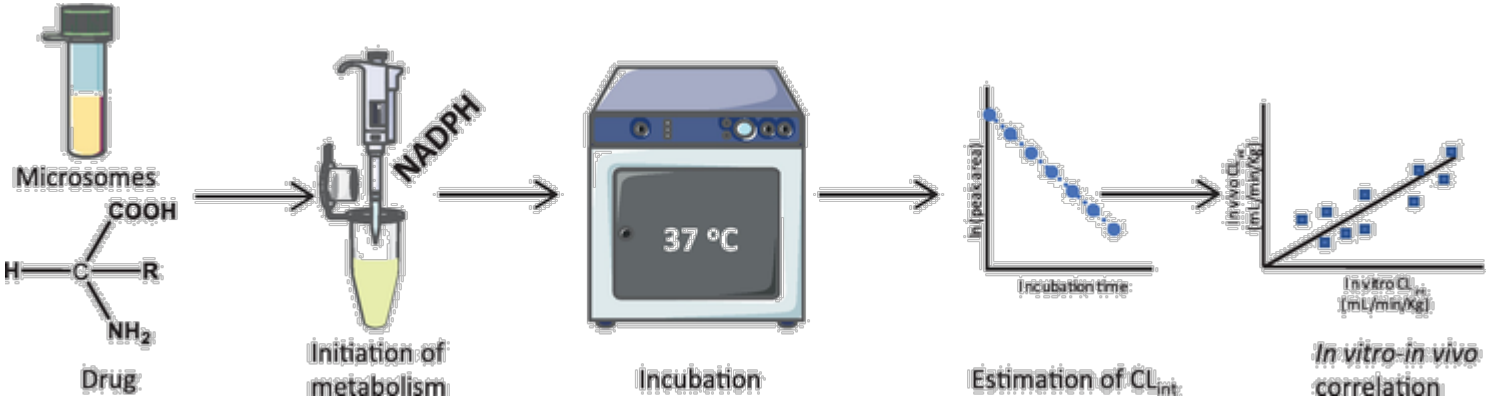
- **Bioaccumulation** that will inform tox studies
- How well does measured **in vitro clearance** correlate with **in vivo clearance**?

Preclinincal evaluation of GalSal analogs: Microsome Stability Overview

UNIVERSITY of MARYLAND SCHOOL OF PHARMACY MASS SPECTROMETRY CENTER

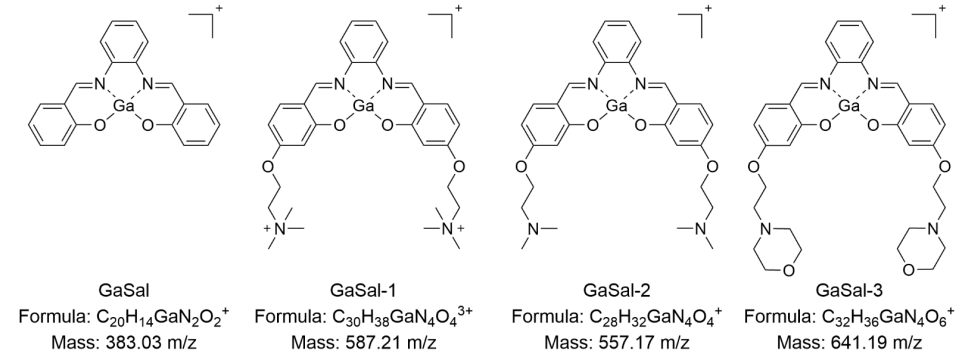
Microsomal Incubations can be used to predict clearance and metabolic pathway

- Scale up factors allow for prediction of dose in future studies
- Microsomes specifically are CYP enriched, Phase I metabolism only
- S9 has Phase I, Phase II, and other metabolic information (FMO, etc)



Screening metabolomics: Non-quantitative assay

- LC-MS/MS screening and look at decrease in area over time
- 1 hour max incubation
- Rapid elimination will impact drug efficacy



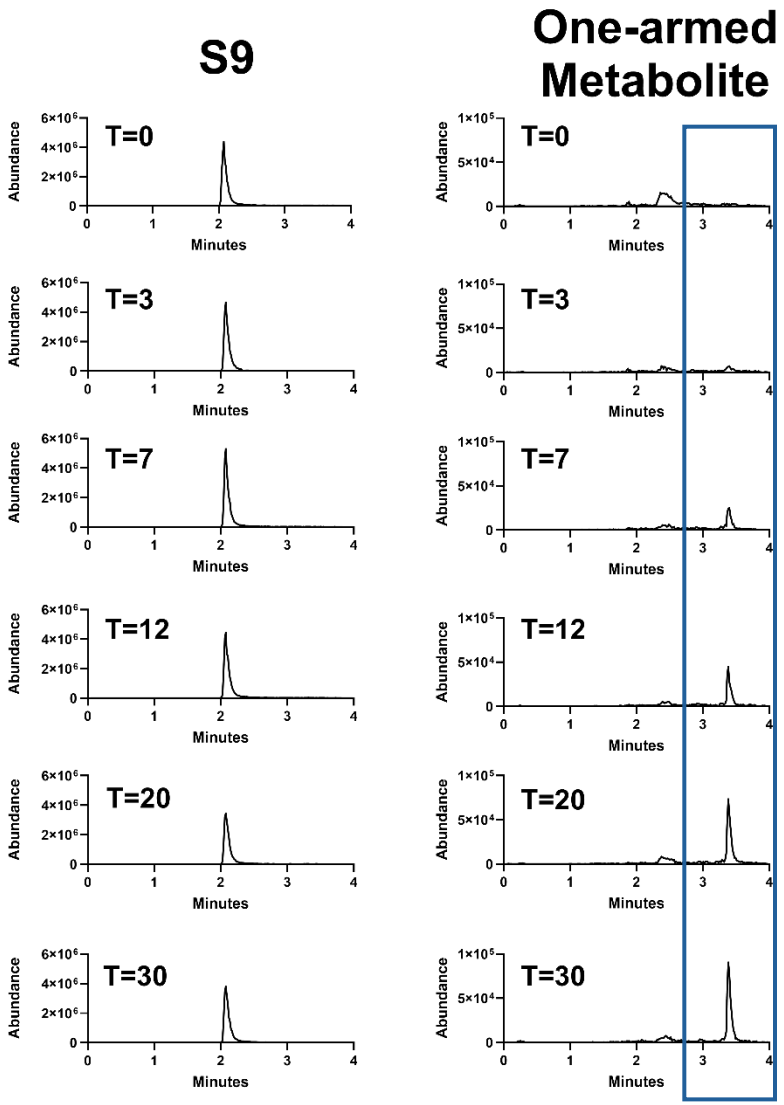
DOI: 10.5772/intechopen.108246

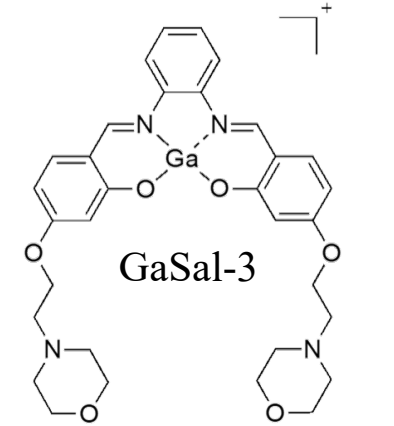
Microsomal Stability for GaSal analogs shows limited CYP mediated metabolism

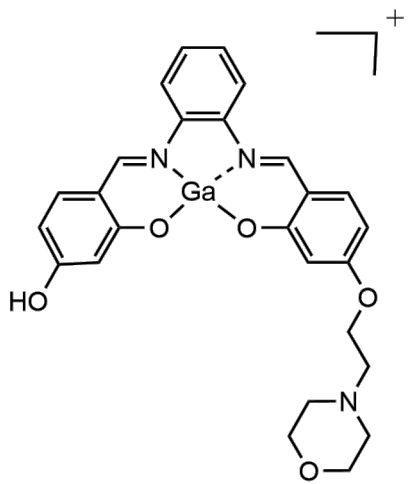
UNIVERSITY of MARYLAND
SCHOOL OF PHARMACY
MASS SPECTROMETRY CENTER

GaSal analogs do not undergo metabolism in microsomes

- Incubation with GaSal and rat liver microsomes shows that there is limited metabolism.
- When incubated in the S9 liver fraction, CLint ranges from 1.8-17.9 L/hr/kg for compounds tested
- Predicted metabolite formation in the S9 fraction from MetID studies shown on the right





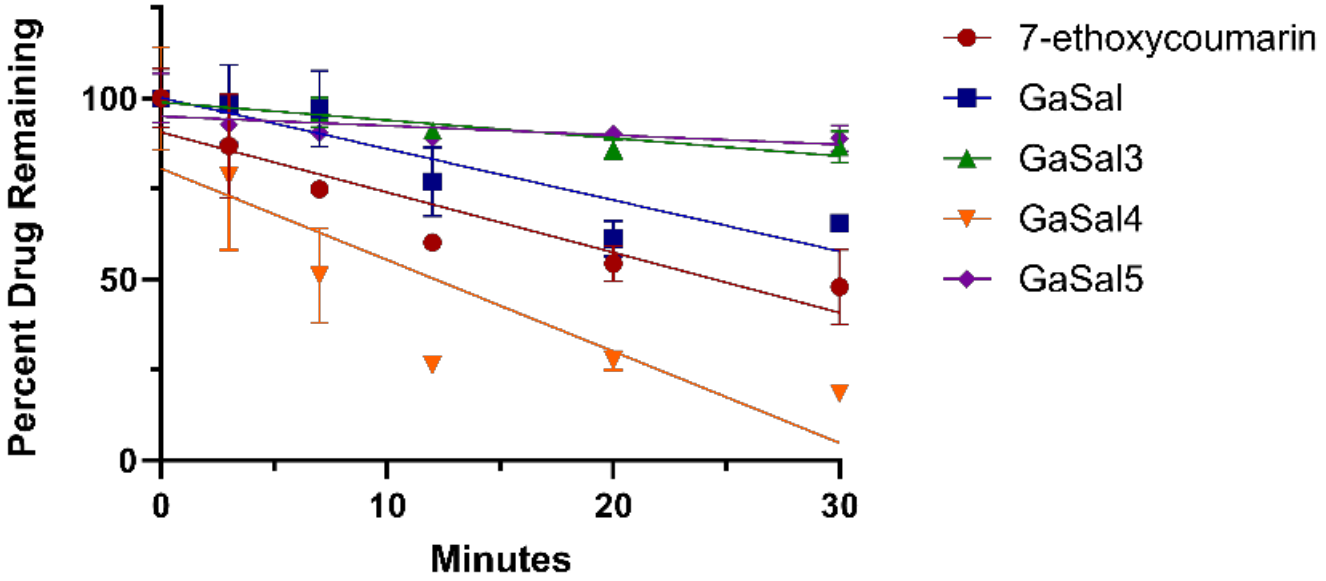


Metabolic intrinsic clearance predicted from S9 incubation

UNIVERSITY of MARYLAND SCHOOL OF PHARMACY MASS SPECTROMETRY CENTER

LC-MS/MS of GalSal compounds

- Different solubility arms influence clearance in S9 fraction



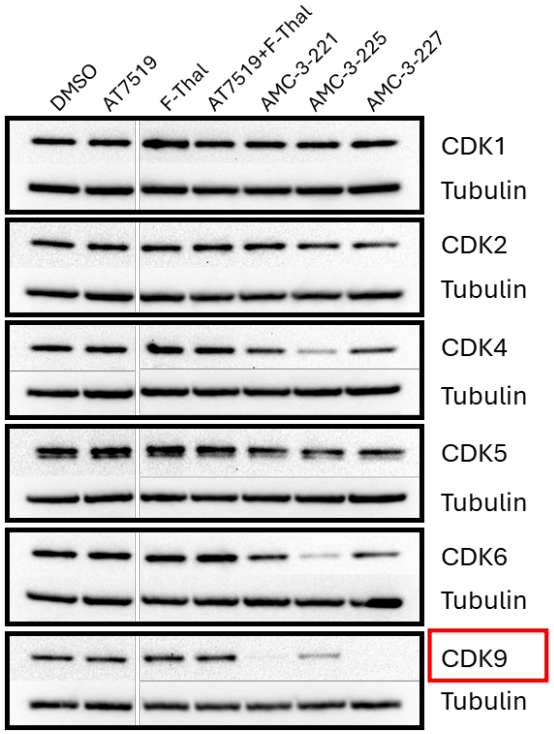
Compound	Elimination Slope	Half-life (hr)	Predicted CL_{int} (L/hr/kg)
7-ethoxycoumarin	-1.661±0.7044	0.42±0.13	11.49±2.71
GaSal	-1.414±0.6500	0.49±0.15	9.84±2.31
GaSal3	-0.4959±0.2703	1.40±0.50	3.45±0.91
GaSal4	-2.527±1.1340	0.27±0.08	17.87±4.08
GaSal5	-0.2594±0.2814	2.67±1.39	1.81±0.62

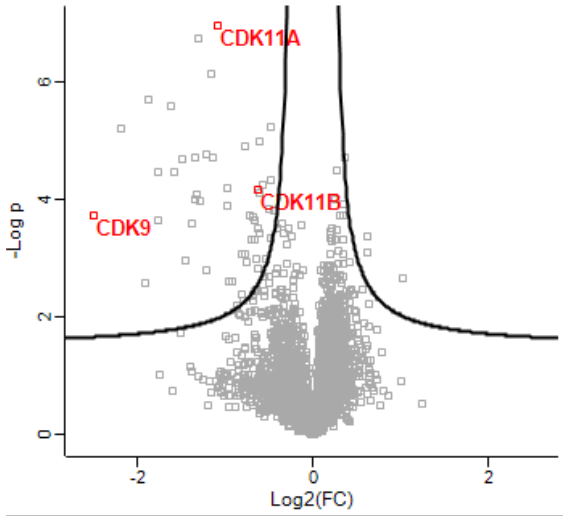
Application of preclinical absorption, distribution, metabolism, elimination in vitro techniques for the characterization and compound library optimization of novel antibiotic gallium salophen.
Krug SA, Frank A, Hwang L, Worth M, Johnson K, Rojas C, Muller L, Michel SLJ, Wilks A, Xue F, **Kane MA.**
Drug Metab Dispos. 2025 Jun;53(6):100080.
doi: 10.1016/j.dmd.2025.100080. Epub 2025 Apr 16.
PMID: 40449092

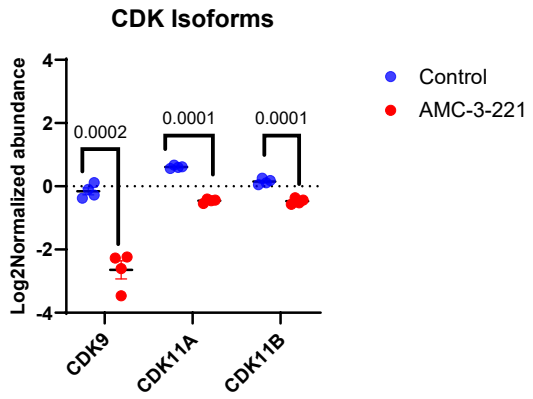
❑ Steven Fletcher Lab, SOP

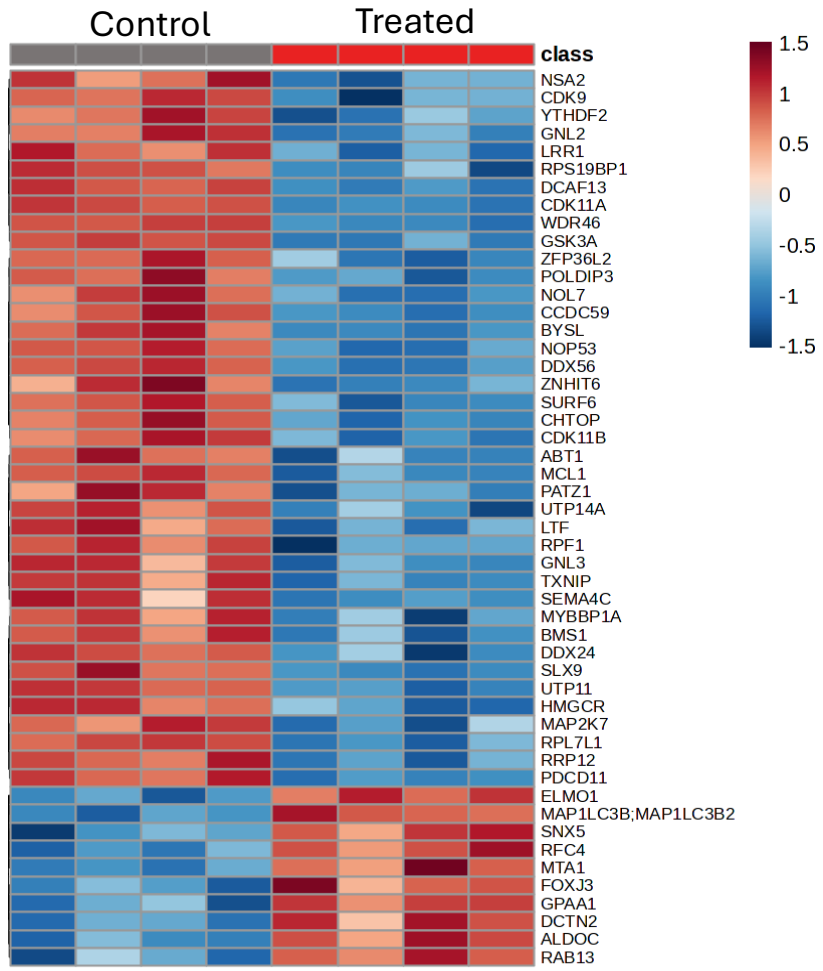
Using MS-based proteomics to validate small molecule target specificity

Validation of Western Blot Findings by Proteomics in CDK9 Inhibitor Analysis









Other MS Center Capabilities

UNIVERSITY of MARYLAND
SCHOOL OF PHARMACY
MASS SPECTROMETRY CENTER

- Metal analysis
  - ICP-MS
  - Metals in biology
  - Metal containing proteins

Sarah Michel: Dean SOP/Professor Pharmaceutical Sciences

- Hydrogen/deuterium exchange
  - Protein conformation / target engagement
  - Protein-protein interactions
  - Protein structure and dynamics

Daniel Deredge: Assistant Professor Pharmaceutical Sciences

- Oligonucleotide analysis
  - LC-MS/MS
  - Structural analysis
- Lipid structure and function

Jace Jones: Associate Professor Pharmaceutical Sciences

How to interact: MS Center

UNIVERSITY of MARYLAND
SCHOOL OF PHARMACY
MASS SPECTROMETRY CENTER

Get started with ICTR!

- Set up a consultation to discuss your experimental goals, scope, and feasibility!
- For **UMB faculty**, use your UMID to log in to the ICTR Resource Request webpage to access the link to the application (developed in ***REDCap***).

Grants/contracts

- Preliminary data, letters of support
- Funded projects – interact according to scope

General inquiries to: massspec@rx.umaryland.edu

https://www.pharmacy.umaryland.edu/centers/massspec/

Funding
- NIH
- FDA
- NSF
- UMB

MS Center Scientists
Mehari Weldemariam
Jongmin Woo
Temple Andrews, PhD
Jianshi Yu, PhD
Swarnima Pandey, PhD
Nagesh Pilli, PhD
Vanshika Patel

Samuel Krug
Nick Montes
Weiliang Huang, PhD
Ludovic Muller, PhD
Stephanie Zalesak-Kravec
Claire Carter, PhD
Jace W. Jones, PhD
Praveen Kumar, PhD
Pengcheng Wang, PhD
Tian Liu, MS