

CLINICAL METAPROTEOMICS WORKFLOW TO STUDY HOST-MICROBIOME DYNAMICS

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CLINICAL METAPROTEOMICS WORKFLOW TO STUDY HOST-MICROBIOME DYNAMICS

- ***MICROBIOME RESEARCH***
- ***MASS SPECTROMETRY DATA ANALYSIS***
- ***METAPROTEOMICS RESEARCH***
- ***GALAXY BIOINFORMATICS PLATFORM***
- ***CLINICAL METAPROTEOMICS WORKFLOW***
- ***CLINICAL METAPROTEOMICS: COVID-19 PANDEMIC***
- ***CLINICAL METAPROTEOMICS: CYSTIC FIBROSIS***
- ***CLINICAL METAPROTEOMICS: ORAL CANCER***
- ***METAPROTEOMICS EDUCATION***

MICROBIOME RESEARCH

Microbiome IN NUMBERS

100 Trillion

sybiotic microbes live in and on every person and make up the human microbiota

The human body has more microbes than there are stars in the milky way

95%

of our microbiota is located in the GI tract

150:1

The genes in your microbiome outnumber the genes in our genome by about 150 to one

The surface area of the **GI tract** is the same size as 2 tennis courts

You have

1.3X

more microbes than human cells

>10,000

Number of different microbial species that researchers have identified living in and on the human body

2kg

The gut microbiota can weigh up to 2Kg

ap
Microbiome
Ireland

Interfacing Food & Medicine

The microbiome is more medically accessible and manipulable than the human genome

90%

It is thought that of disease can be linked in some way back to the gut and health of the microbiome

5:1

Viruses:Bacteria in the gut microbiota

2.5

The number of times your body's microbes would circle the earth if positioned end to end



Each individual has a unique gut **microbiota**, as personal as a fingerprint



<https://worldmicrobiomeday.com/resources/>



<https://www.nature.com/articles/d41586-020-00193-3>

MICROBIOME

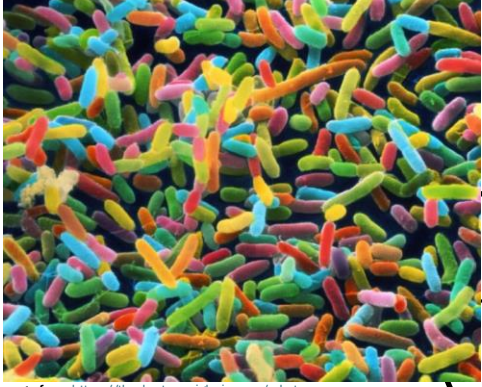
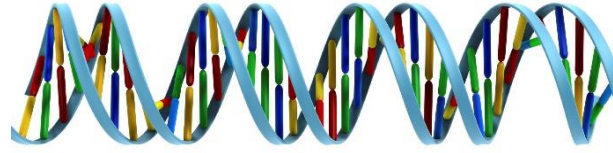


Image from <https://thedoctorweighs.in.com/what-everyone-should-know-about-the-infant-microbiome/>

Potential to unravel the mechanistic details of microbial interactions with host / environment by analyzing the functional dynamics of the microbiome.



METAGENOMICS

TAXONOMY

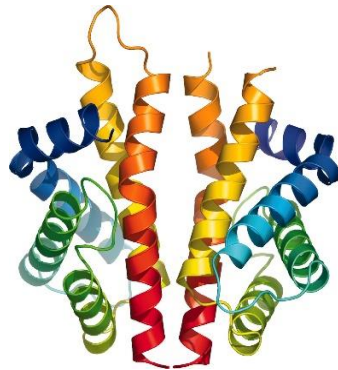
function



METATRANSCRIPTOMICS

TAXONOMY

function

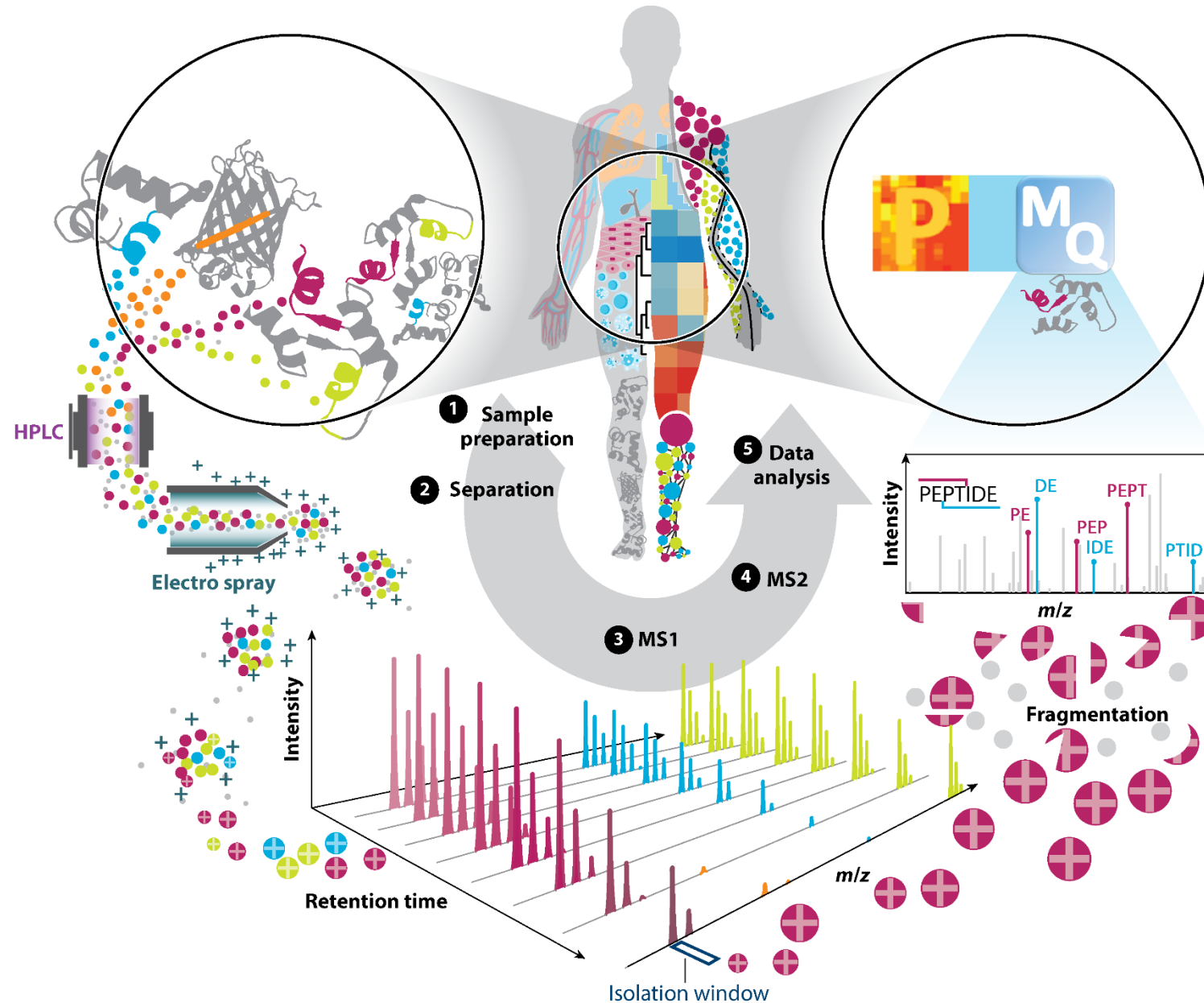


METAPROTEOMICS

TAXONOMY

FUNCTION

MASS SPECTROMETRY AND PROTEOMICS

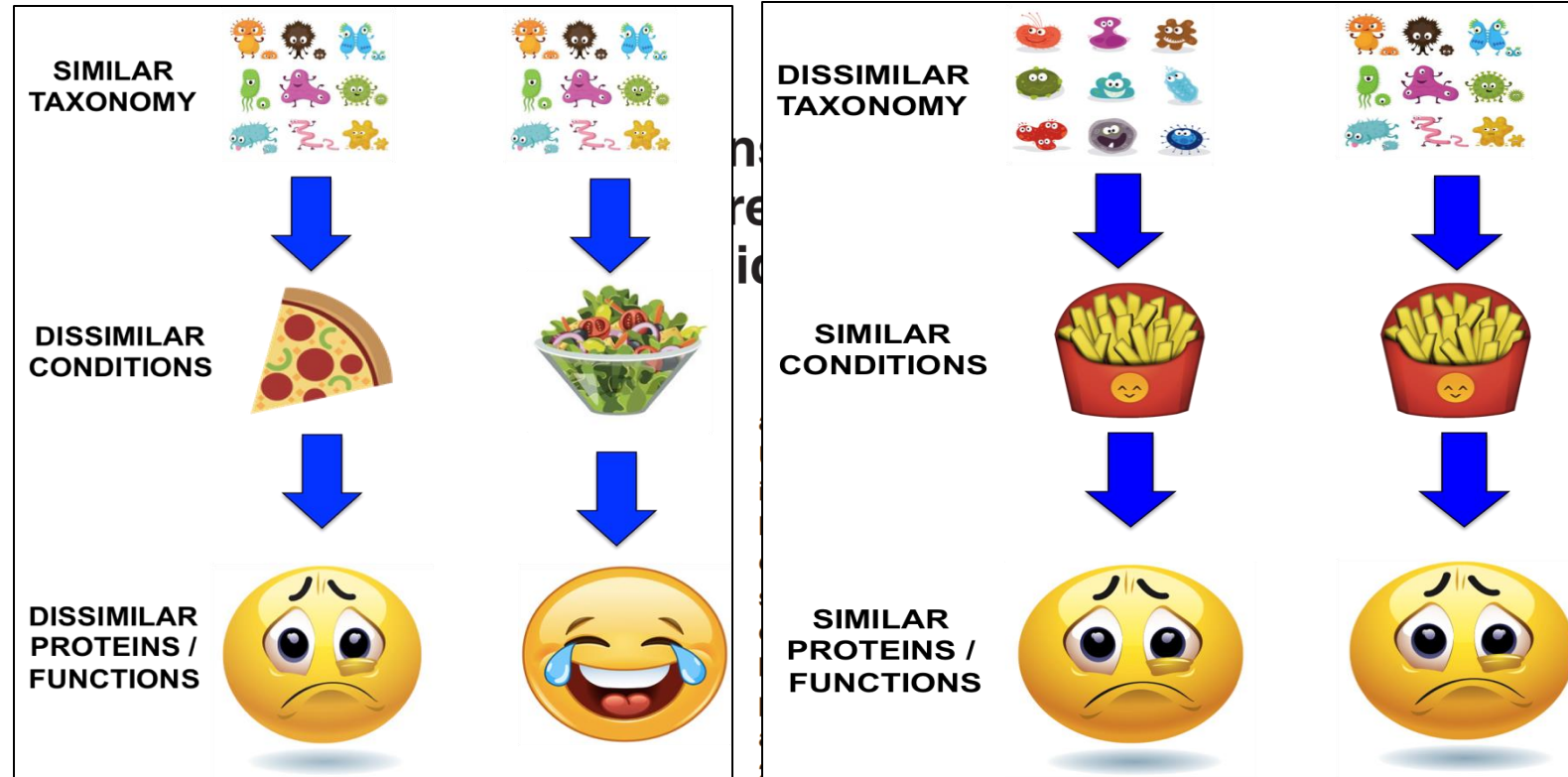


METAPROTEOMICS

Bond and Wilmes 2004

“The large-scale characterization of the entire protein complement of environmental microbiota at a given point in time”

Environ. Microbiol. 6, 911–920.



Bond and Wilmes 2015

“Through the application of metaproteomics to different microbial consortia over the past decade, we have learnt much about key functional traits in the various environmental settings where they occur.”

Proteomics. doi:10.1002/pmic.201500183.

METAPROTEOMICS ANALYTICAL CHALLENGES

SINGLE-ORGANISM PROTEOMICS



METAPROTEOMICS



SEARCH DATABASE

SIZE

SMALL TO MEDIUM SIZE (10 K TO
100K SEQUENCES)

LARGE (1 MILLION AND ABOVE)

COMPLEXITY

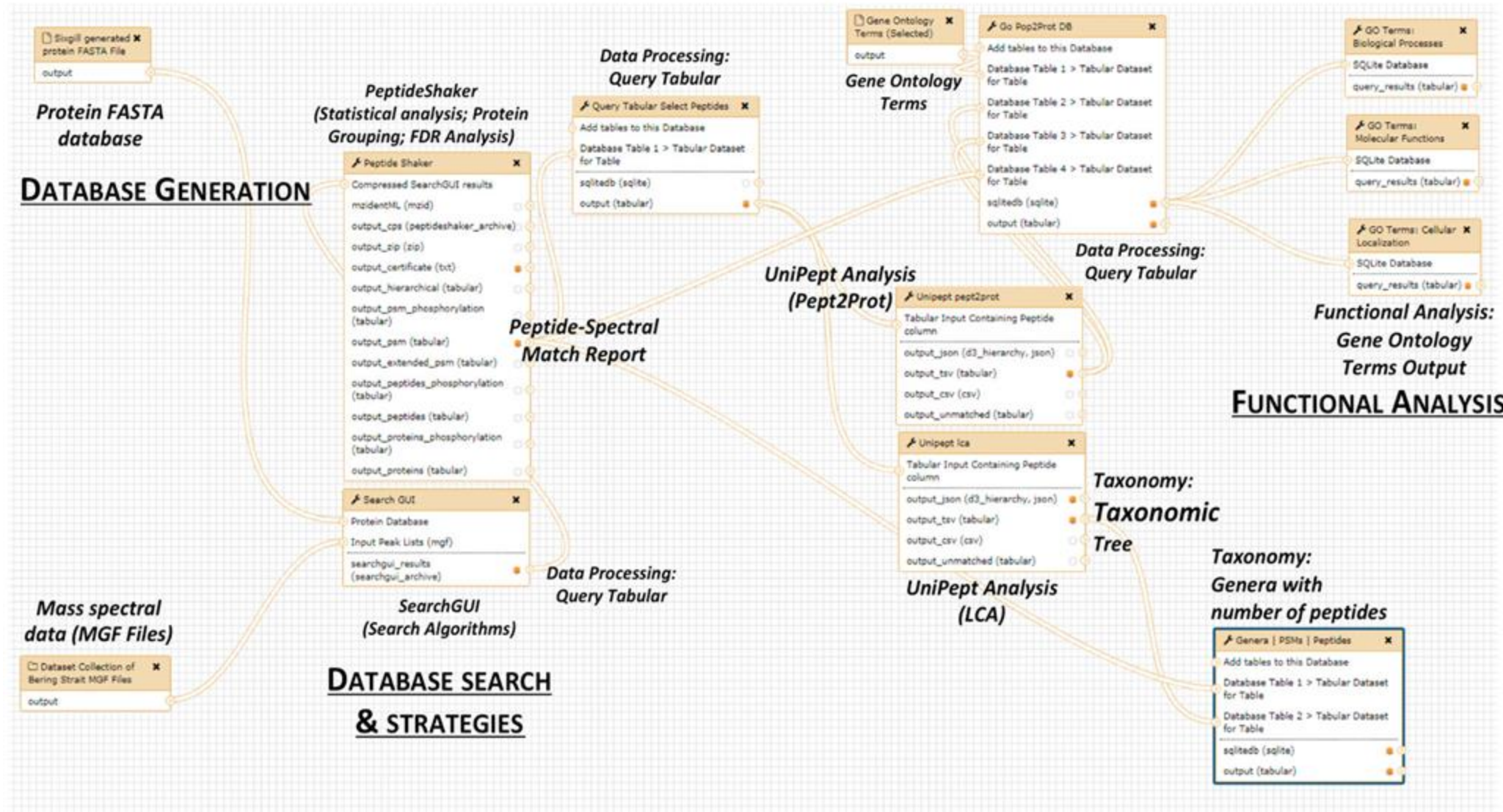
SINGLE + CONTAMINANTS

MULTI-ORGANISM DATABASE WITH HOMOLOGOUS PROTEINS

Disparate tools and
multiple processing
steps.

- SEARCH ALGORITHMS BEING DEVELOPED TO ADDRESS LARGE AND COMPLEX DATABASE SEARCHES
- PROTEIN GROUPING AT MULTI-ORGANISM LEVEL
- IDENTIFICATION STATISTICS AFFECTED BY LARGE DATABASES
- TAXONOMY BASED ON UNIQUE PEPTIDE IDENTIFICATIONS
- FUNCTIONAL ANALYSIS BASED ON PROTEINS IDENTIFIED

SOLUTION: GALAXY BIOINFORMATICS PLATFORM



Software tools can be used in a sequential manner to generate analytical workflows that can be reused, shared and creatively modified.

The Galaxy Interface

Main viewing window
(workflow development, results visualization etc)

Tools

History

The screenshot displays the GalaxyP web interface. On the left is a 'Tools' panel with a search bar and a list of tool categories including CORE TOOLS, PROTEOMICS, and NGS. A red arrow points from the 'Tools' label to the 'Send Data' tool in the CORE TOOLS section. The main workspace features a green 'Welcome to GalaxyP' message, a 'GalaxyP' logo, and an 'Updates' section. A red arrow points from the 'Main viewing window' label to the central workspace area. On the right is a 'History' panel showing a list of workflow steps, such as '33: BLAST-P Filtered Peptide Report' and '32: BLAST-P Filtered Peptides'. A red arrow points from the 'History' label to this panel. The top navigation bar includes links for 'Analyze Data', 'Workflow', 'Shared Data', 'Visualization', 'Help', and 'User', along with a memory usage indicator 'Using 35.5 GB'.

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- *CLINICAL METAPROTEOMICS: ORAL CANCER*
- *METAPROTEOMICS EDUCATION*

CLINICAL METAPROTEOMICS WORKFLOW TO STUDY HOST-MICROBIOME DYNAMICS

Katherine Do¹, Subina Mehta¹, Surbhi Bihani², Monica E. Kruk¹, Aryan Gupta², Kevin Murray¹, Andrew Rajczewski¹, Reid Wagner³, Dechen Bhuming¹, Kristin Boylan⁴, Amy Skubitz⁴, Theresa Laguna^{5,6}, Sanjeeva Srivastava², Timothy Griffin¹, Pratik Jagtap¹

¹ Biochemistry, Mol. Biology and Biophysics, University of Minnesota, Minneapolis, USA

² Indian Institute of Technology Bombay, Mumbai, India

³ Minnesota Supercomputing Institute, University of Minnesota, Minneapolis, USA

⁴ Department of Laboratory Medicine and Pathology, University of Minnesota, Minneapolis, MN, USA

⁵ Department of Pediatrics, University of Washington School of Medicine, Seattle, WA, USA

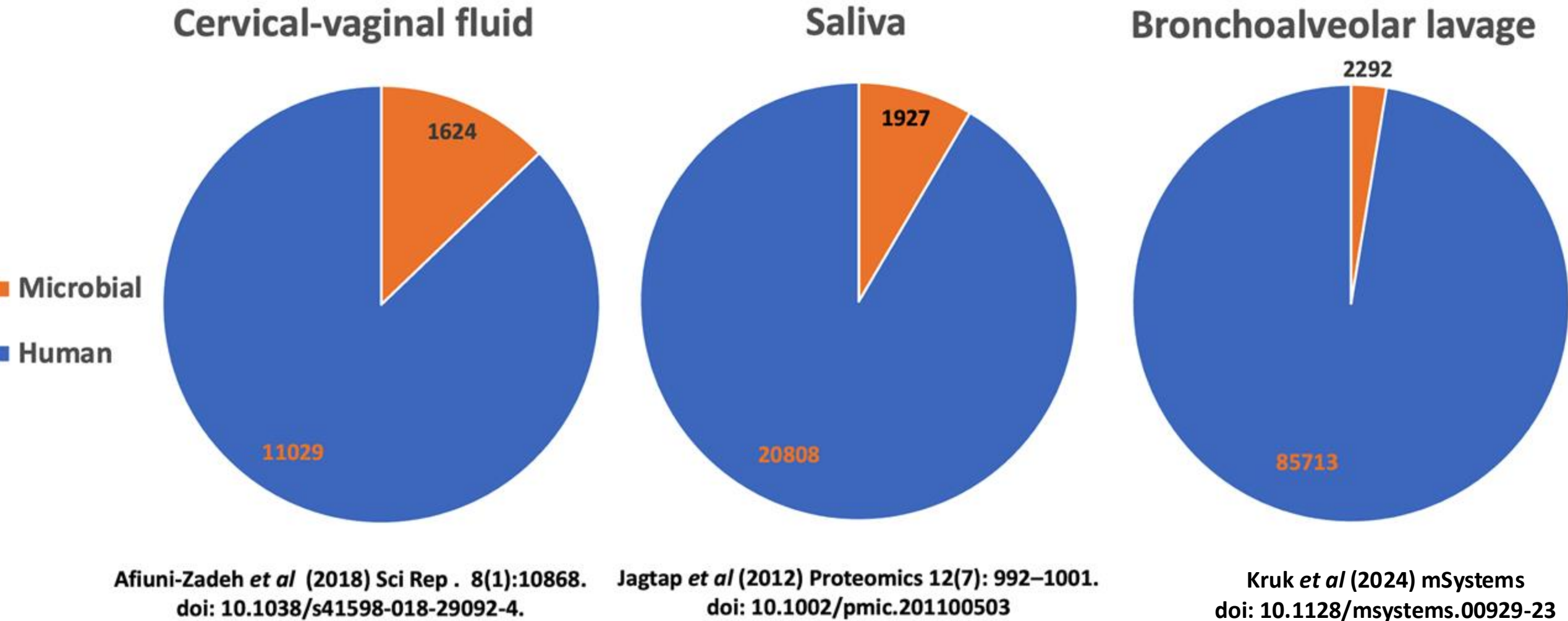
⁶ Department of Pediatrics, Division of Pulmonary and Sleep Medicine, Seattle Children's Hospital, Seattle, WA, USA



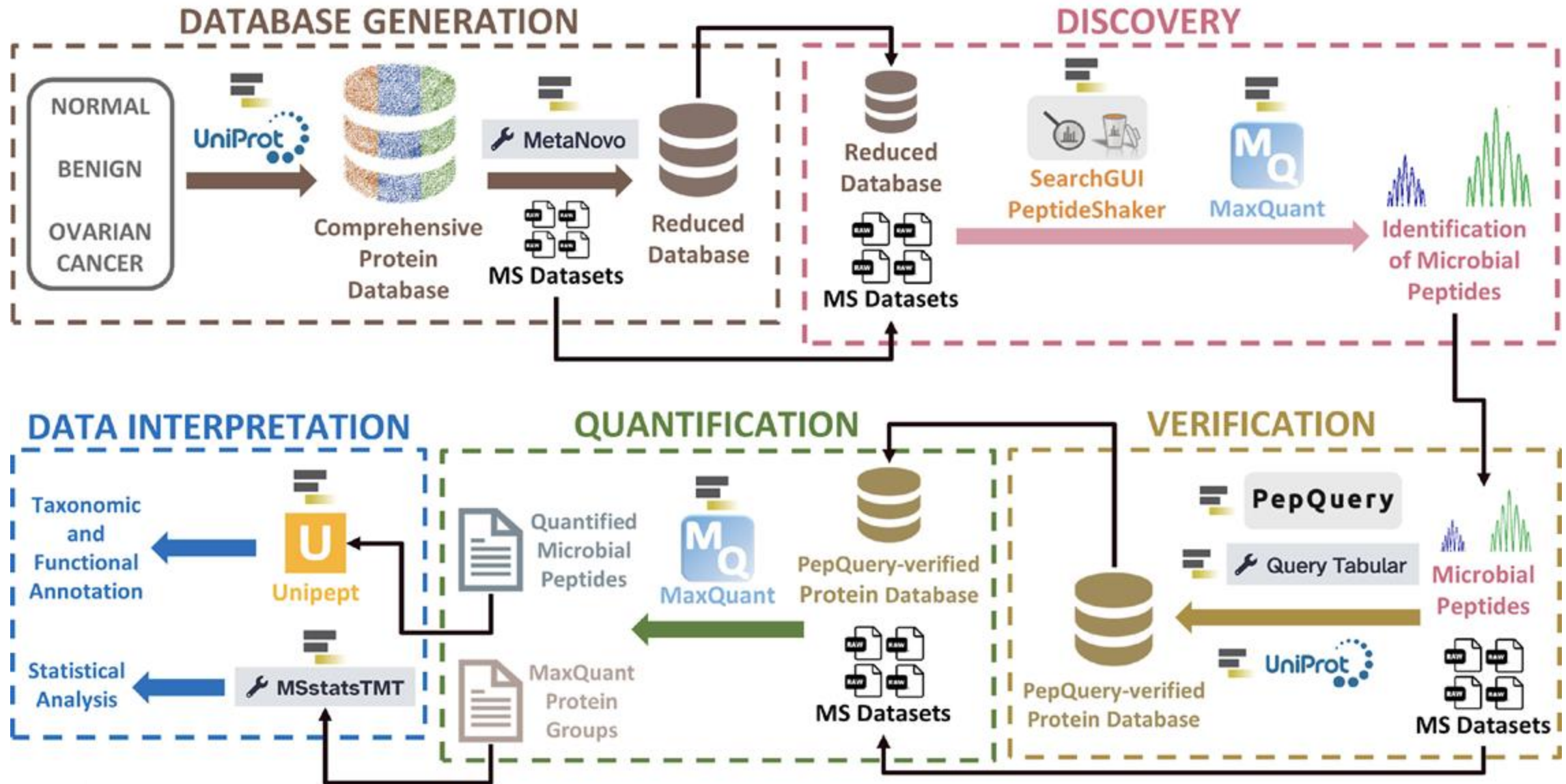
Do K et al (2024). A novel clinical metaproteomics workflow enables bioinformatic analysis of host-microbe dynamics in disease. mSphere <https://doi.org/10.1128/msphere.00793-23>



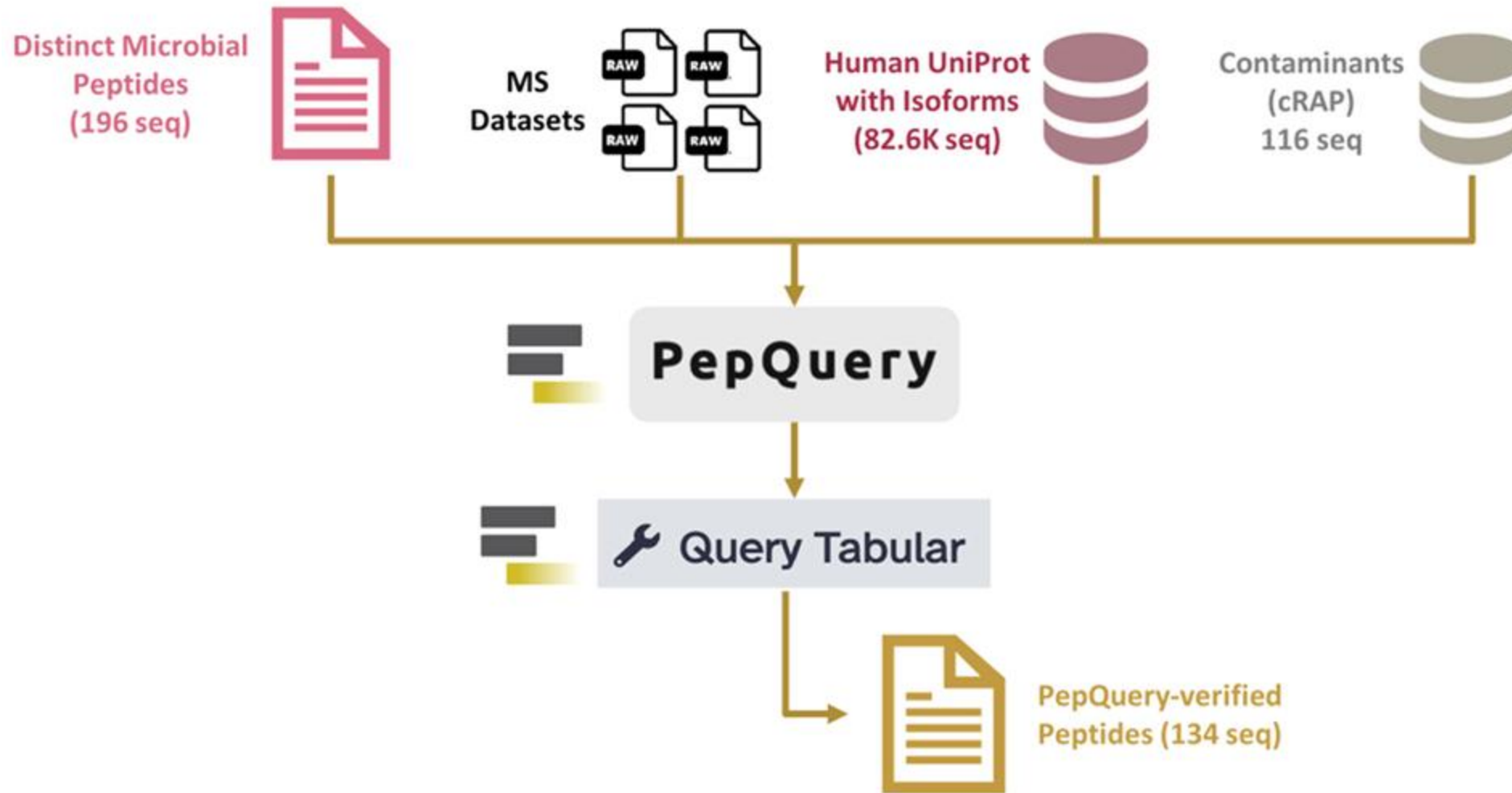
CLINICAL METAPROTEOMIC STUDIES DETECT LIMITED NUMBER OF MICROBIAL PEPTIDES



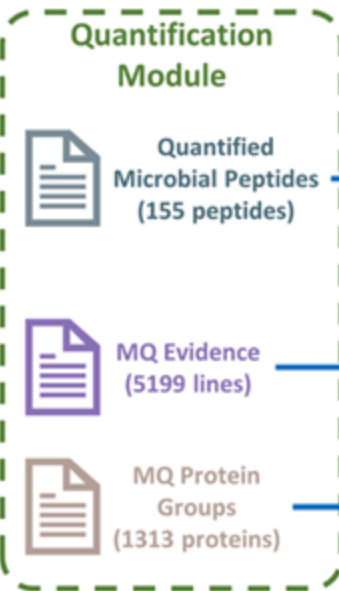
OVERVIEW OF CLINICAL METAPROTEOMICS WORKFLOW



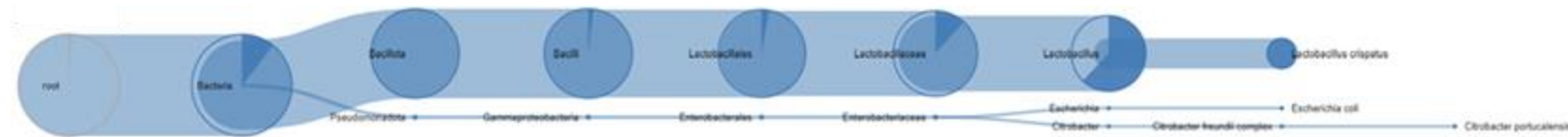
VERIFICATION AND VERIFIED DATABASE GENERATION MODULE



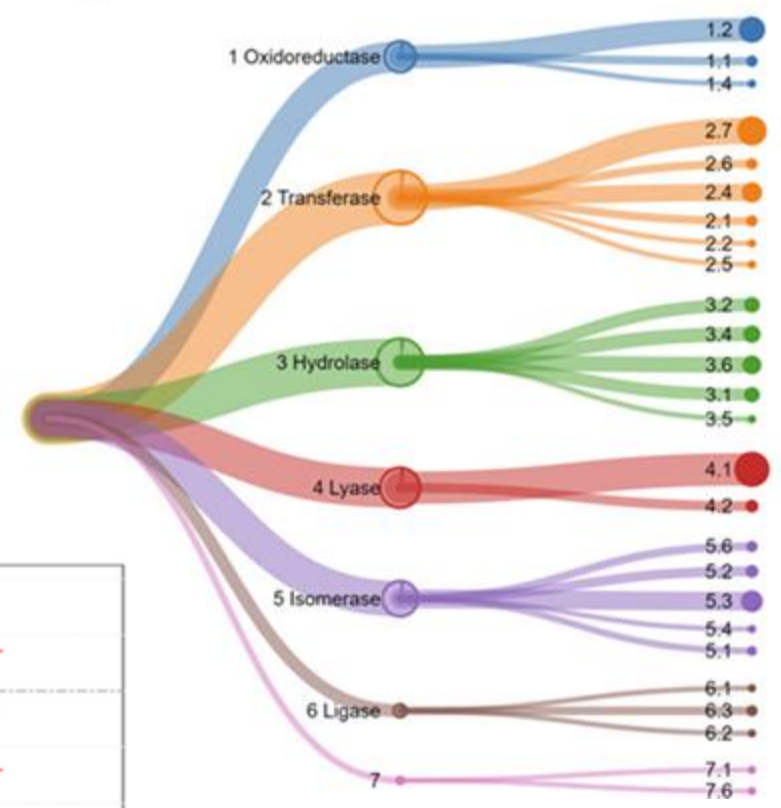
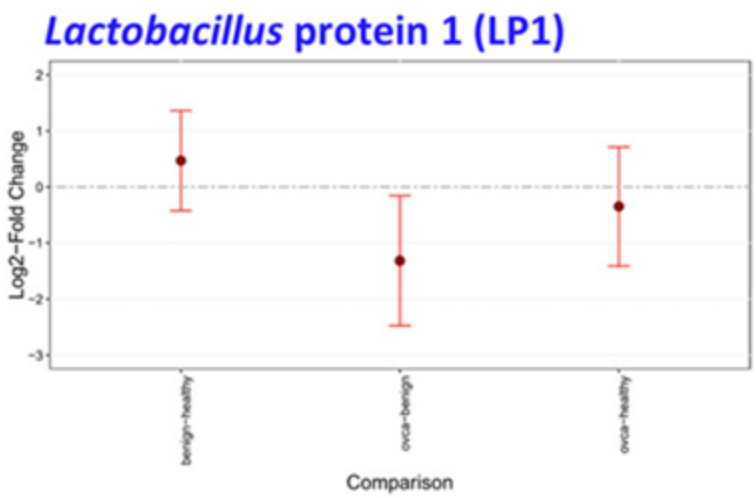
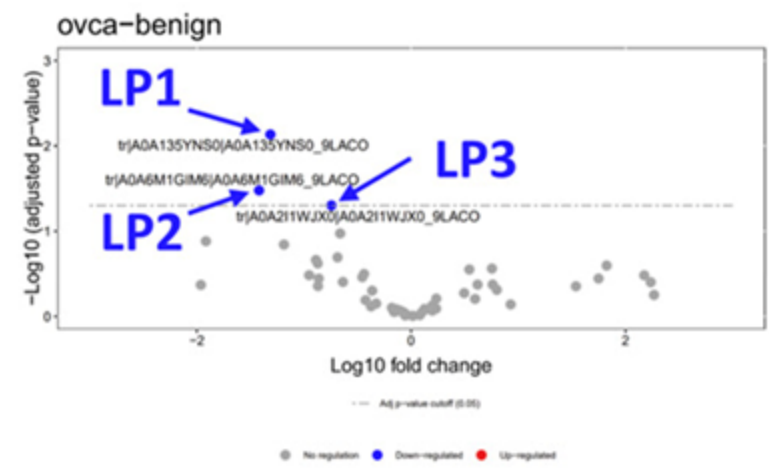
DATA INTERPRETATION MODULE



Taxonomic and Functional Annotation



Statistical Analysis



The workflow modules, training data and documentation are available via the Galaxy Training Network.
<https://training.galaxyproject.org/training-material/learning-pathways/clinical-metaproteomics.html>

GALAXY TRAINING NETWORK

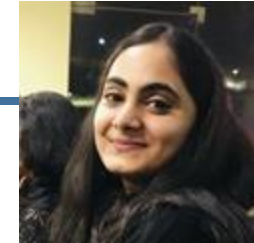


<https://galaxyproject.org/events/gcc2021/training/>

<https://training.galaxyproject.org/training-material/topics/proteomics/>

CLINICAL METAPROTEOMICS: COVID-19 PANDEMIC

Bihani S *et al* (2023) Metaproteomic Analysis of Nasopharyngeal Swab Samples to Identify Microbial Peptides in COVID-19 Patients. J Proteome Res 22(8):2608-2619.
doi: 10.1021/acs.jproteome.3c00040.



Surbhi Bihani



IIT BOMBAY

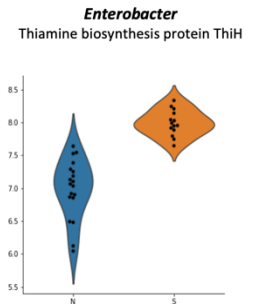
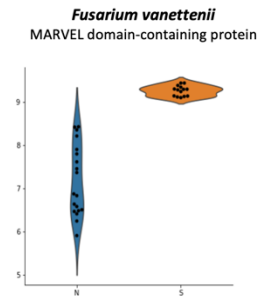
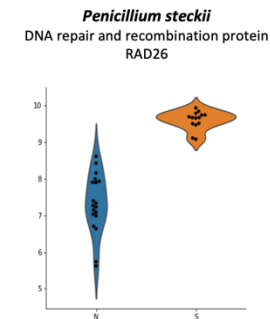
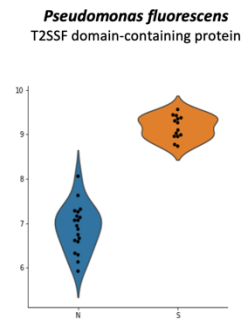
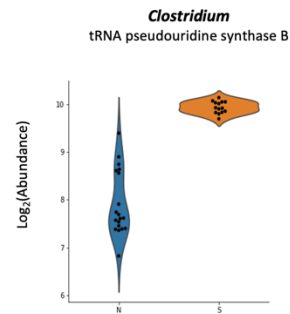
**Nasal Swabs
from COVID-19
patients**



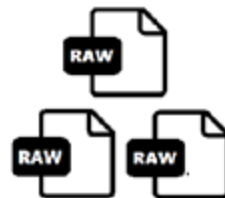
**Sample
Collection**



**Sample
processing**

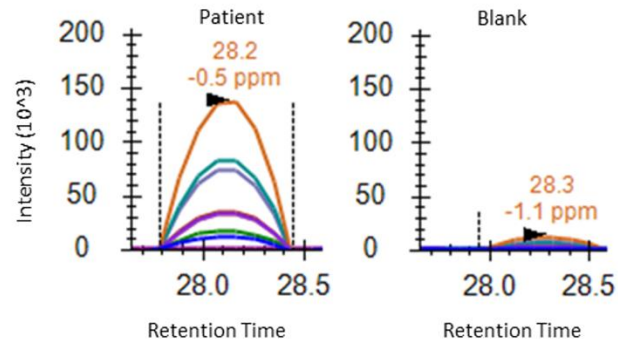


Data Acquisition



TARGETED ANALYSIS OF POTENTIAL PATHOGENS IN COVID PATIENTS

QGIQNIPVVSQTGK (*Streptococcus parasanguinis*)

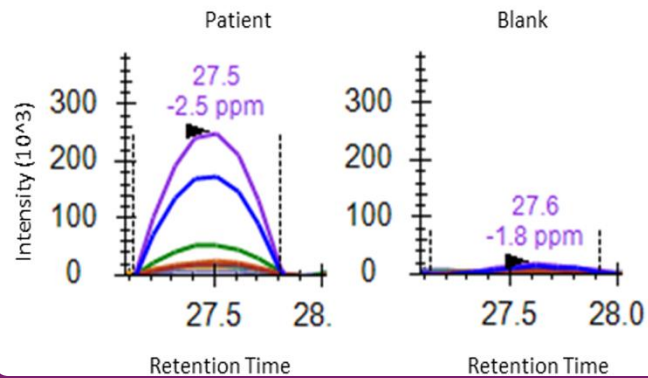


Streptococcus parasanguinis

Dominant isolate of dental plaque

Opportunistic pathogen associated with subacute endocarditis

GLFGDELCK (*Burkholderia cenocepacia*)

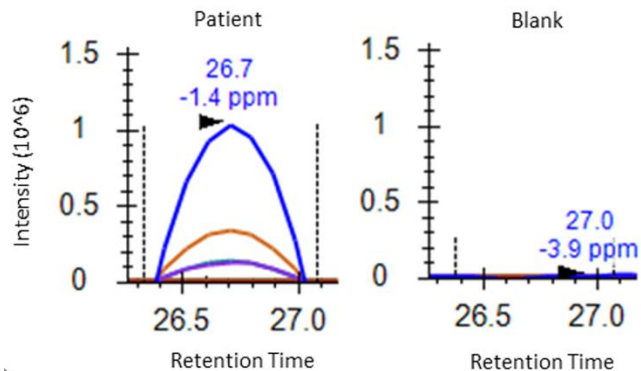


Burkholderia cenocepacia

Opportunistic pathogen

Cause systemic infections in immunocompromised individuals including cystic fibrosis patients.

IVDQEGAIVPK (*Cryptococcus floricola*)

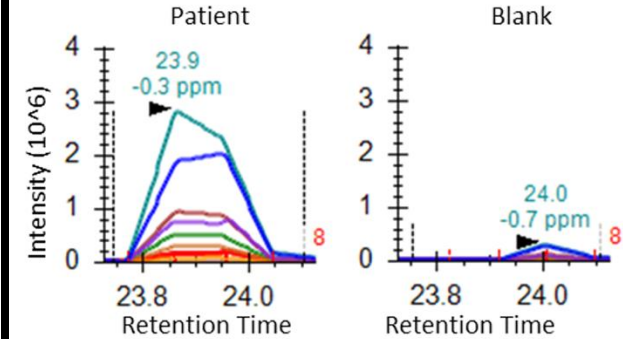


Cryptococcus floricola

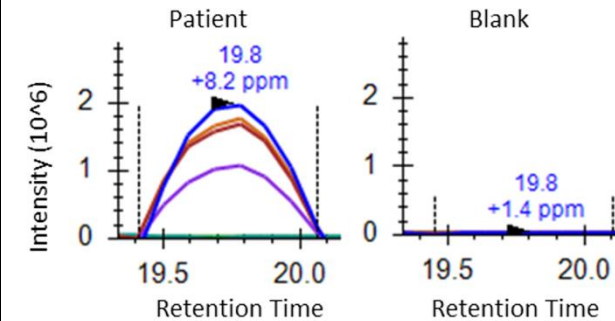
Infect immunocompromised hosts

Infection initiates in the lungs.

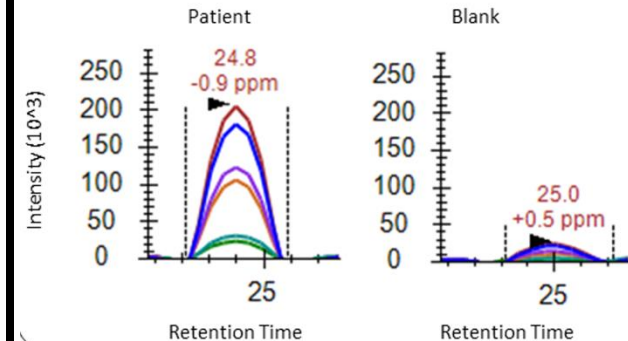
ICVLHGDK (*Clostridium sp.*)



DETDDQFEK (*Staphylococcus sp.*)



TPTLVSDLR (*Pseudomonas sp.*)



CLINICAL METAPROTEOMICS: CYSTIC FIBROSIS

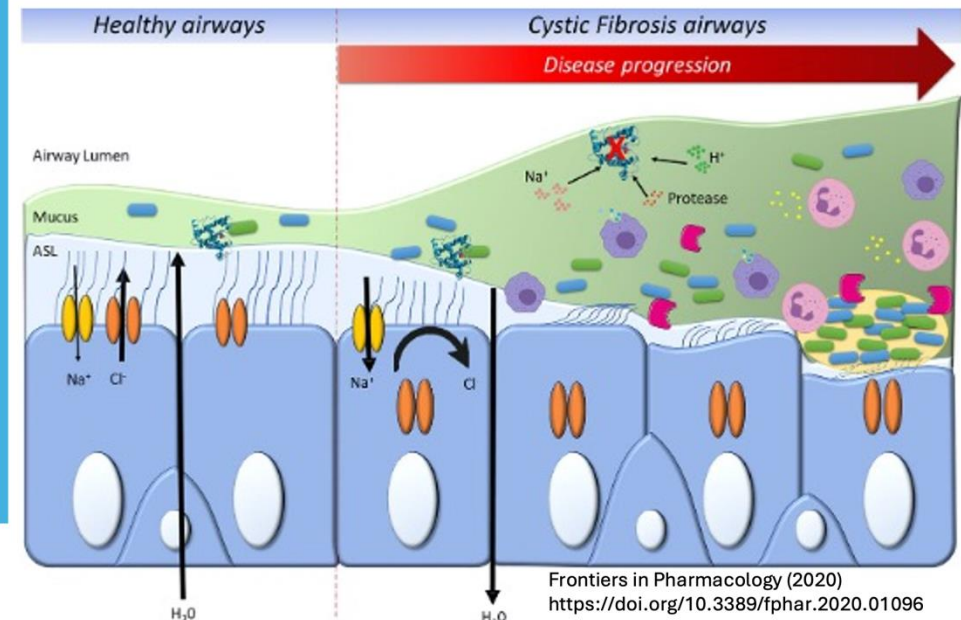
Kruk M *et al* (2024) An integrated metaproteomics workflow for studying host-microbe dynamics in bronchoalveolar lavage samples applied to cystic fibrosis disease. mSystems (<https://doi.org/10.1128/msystems.00929-23>).

CYSTIC FIBROSIS AND THE MICROBIOME

- Life-shortening Mendelian disease.
- Mutations in the CF transmembrane conductance regulator (CFTR) gene, which encodes an epithelial anion channel.
- Abnormal anion transport across epithelia of secretory glands including lung.



- Thick mucus which predisposes patients to chronic bacterial infections and airway inflammation.
- Progressive and irreversible airway damage.
- Reduced quality and length of life in CF.



CYSTIC FIBROSIS DATASETS



BALF from
Cystic Fibrosis
or
Disease Control
Samples



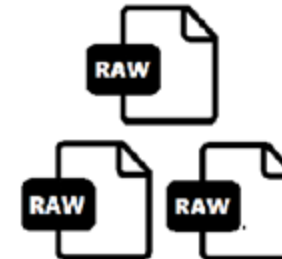
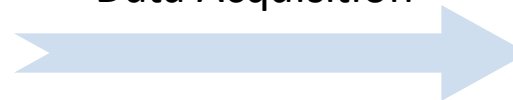
Sample
Collection



Sample
processing



Data Acquisition

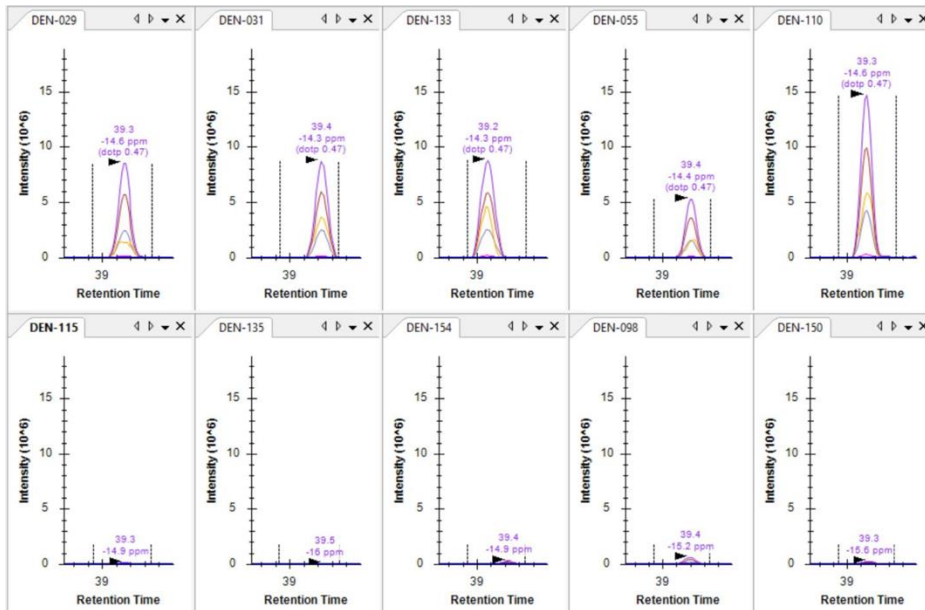


Monica E. Kruk

MICROBIAL PEPTIDES FROM CYSTIC FIBROSIS PATIENTS

DWLDSLQQR

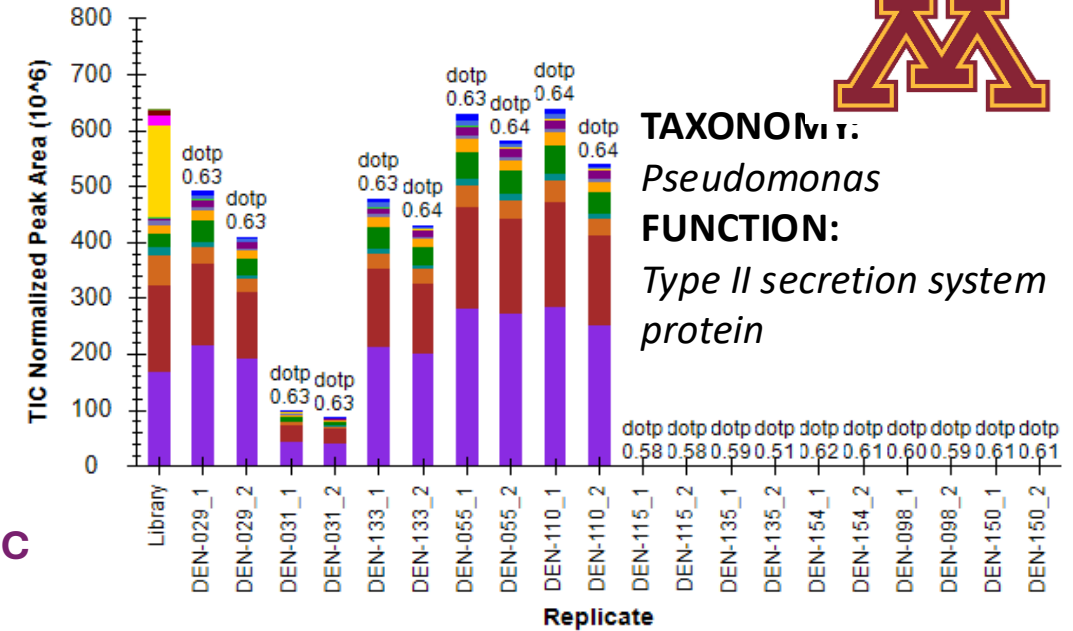
CF



DC

CF

DC



TAXONOMY:

Pseudomonas

FUNCTION:

Type II secretion system protein

MKIGNLGGAYR



CF

TAXONOMY:

Streptococcus agalactiae

FUNCTION:

DNA recombination enzyme

DC

VLGARGHR



CF

TAXONOMY:

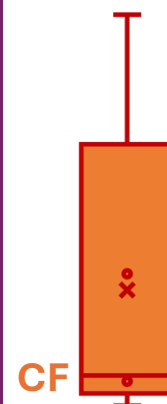
Bacteria (UniPept)

FUNCTION:

Membrane kinase activity
Nucleoside triphosphate hydrolase (A0A119BW38)

DC

AALGAYDLR



CF

TAXONOMY:

Unassigned (UniPept)

FUNCTION:

Uncharacterized protein (A0A1A2YS21)

DC

ATVEELHLEGI



CF

TAXONOMY:

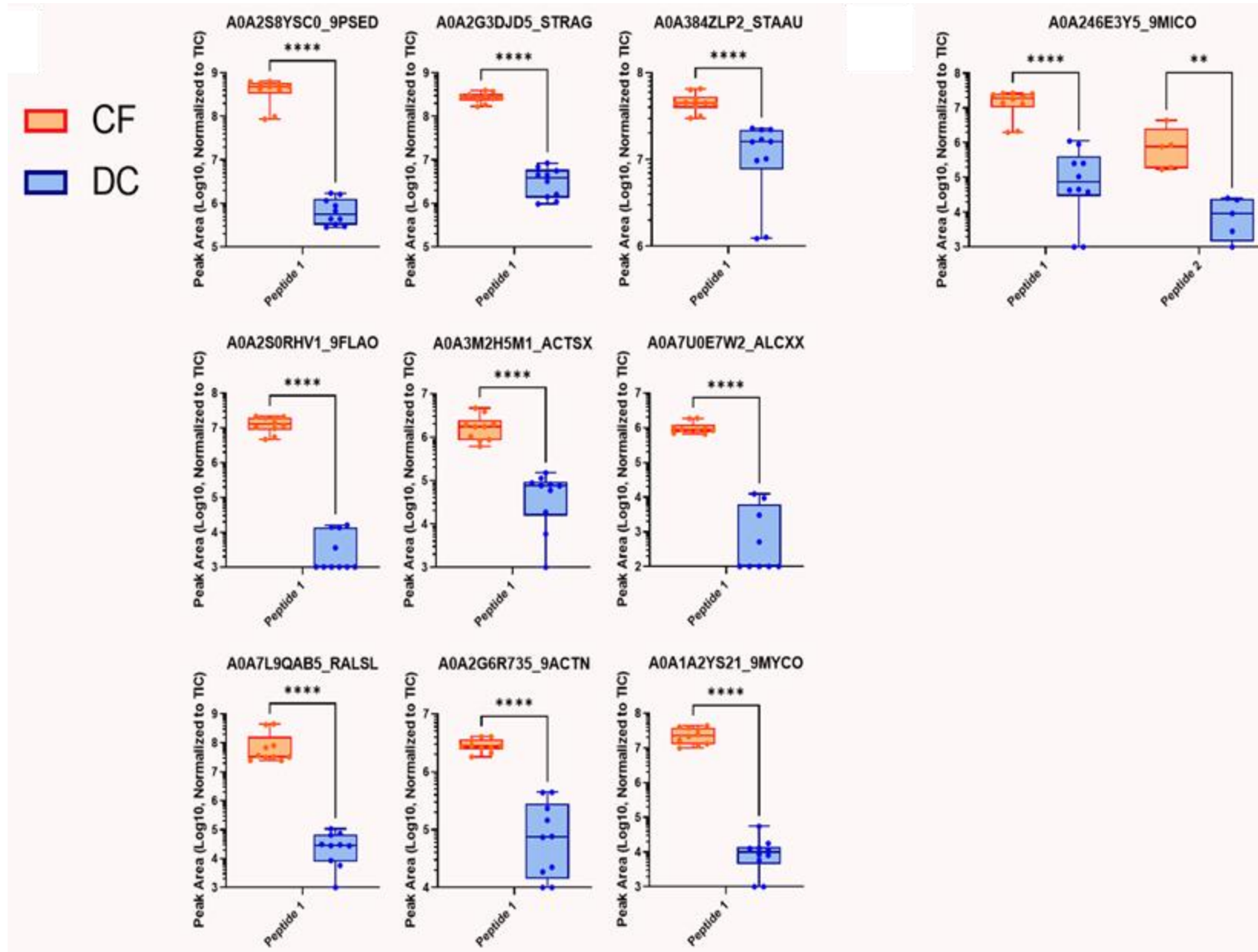
Unassigned (UniPept)

FUNCTION:

Uncharacterized (A0A1H9MU03)

DC

MICROBIAL PEPTIDES FROM CYSTIC FIBROSIS PATIENTS

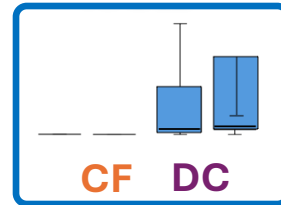


TARGETED ASSAY FOR HOST-MICROBIAL PROTEIN DYNAMICS IN CLINICAL CF SAMPLES

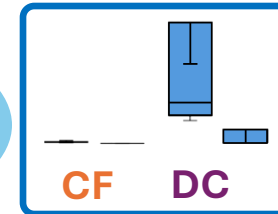


- Lipoprotein particle remodeling
- Cholesterol transport
- Phospholipid efflux.

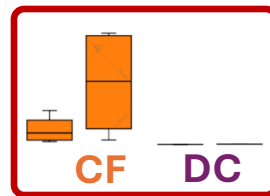
APOC2



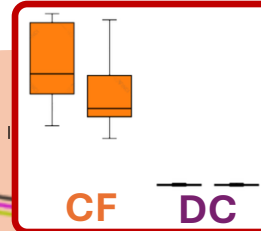
APOE



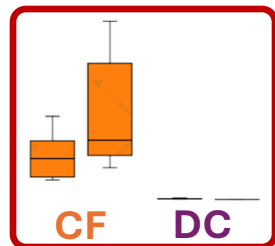
S100A9



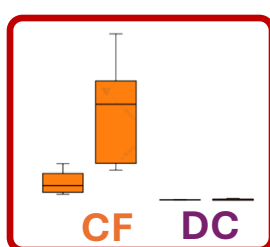
MPO



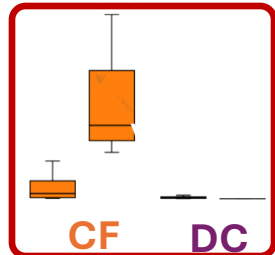
MMP8



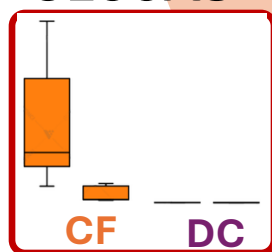
FGR



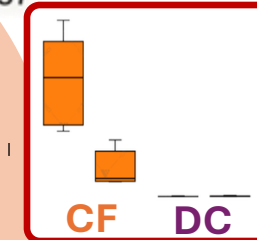
S100A12



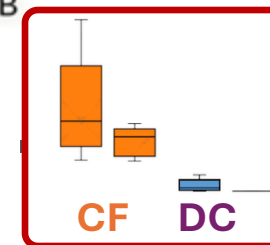
S100A8



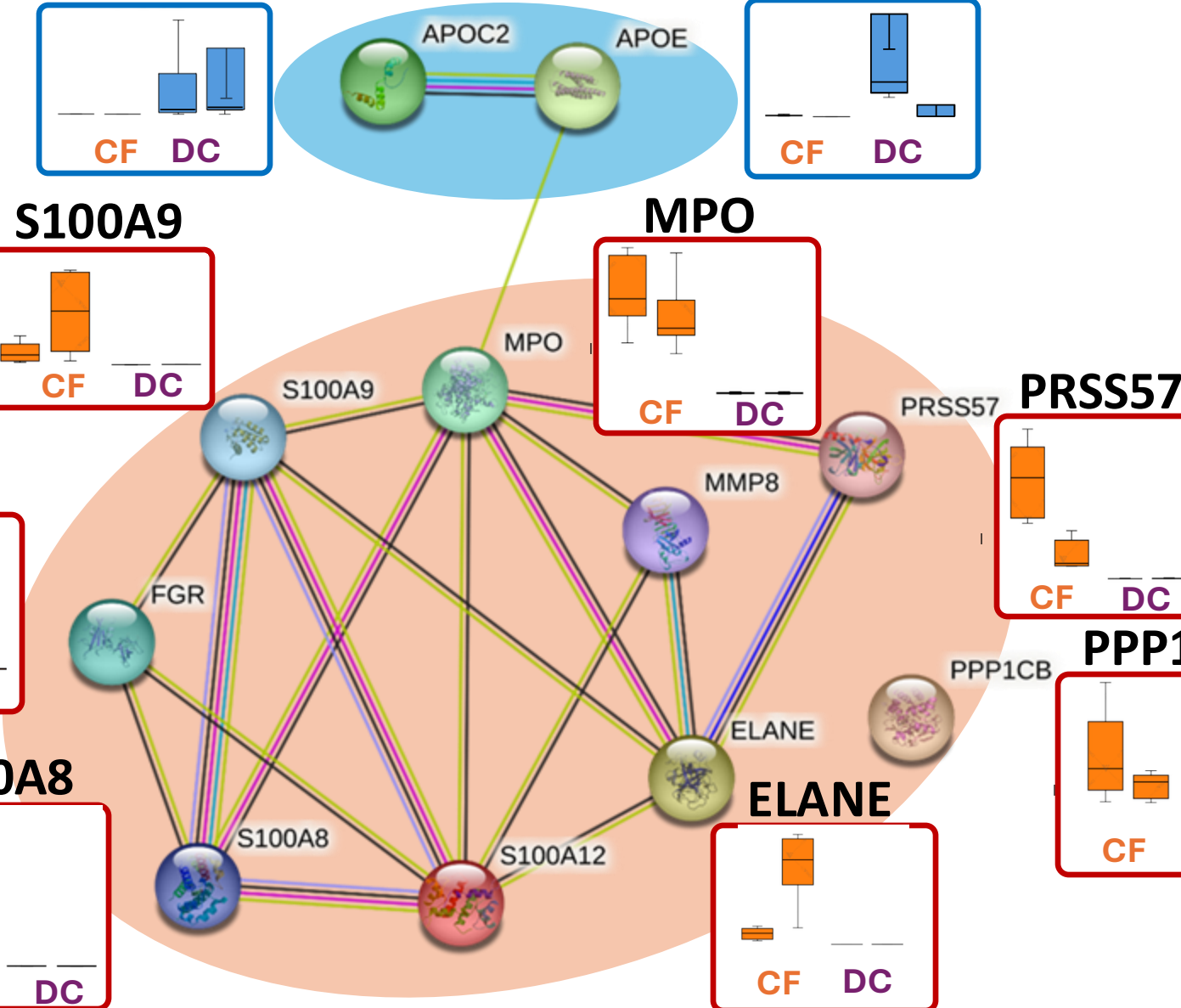
PRSS57



PPP1CB



- Inflammatory response
- Leukocyte migration
- Neutrophil degranulation
- Cytokine production
- Defense response to bacteria



CLINICAL METAPROTEOMICS

- We have developed a MS-based Galaxy-driven bioinformatics workflow for processing of microbial and host proteins, generating verified microbial peptide candidates suitable for targeted analysis within individual patient samples.
- We have utilized this workflow to detect and validate microbial peptides during a) co-infection during the COVID-19 pandemic (Bihani *et al* 2023, JPR); b) CF disease progression studies by comparing it with disease control (Kruk *et al* 2024, mSystems (In Press)) and c) Ovarian cancer studies (Mehta *et al*, work in progress).
- We hope that the workflow availability through Galaxy Training Network will help users detect differentially expressed host and microbial proteins in disease state.

INVESTIGATING KEY HOST, MICROBIAL AND VARIANT PEPTIDES FOR DETECTION OF ORAL CANCER USING ADVANCED MULTI-OMICS METHODS.

Pratik Jagtap¹, Ruben Shrestha², Beverly Wuertz³, Monica Kruk⁴, Subina Mehta¹, Alvaro Sebastian Vaca Jacome², Matthew Willetts⁴, Frank Ondrey³, Timothy Griffin¹

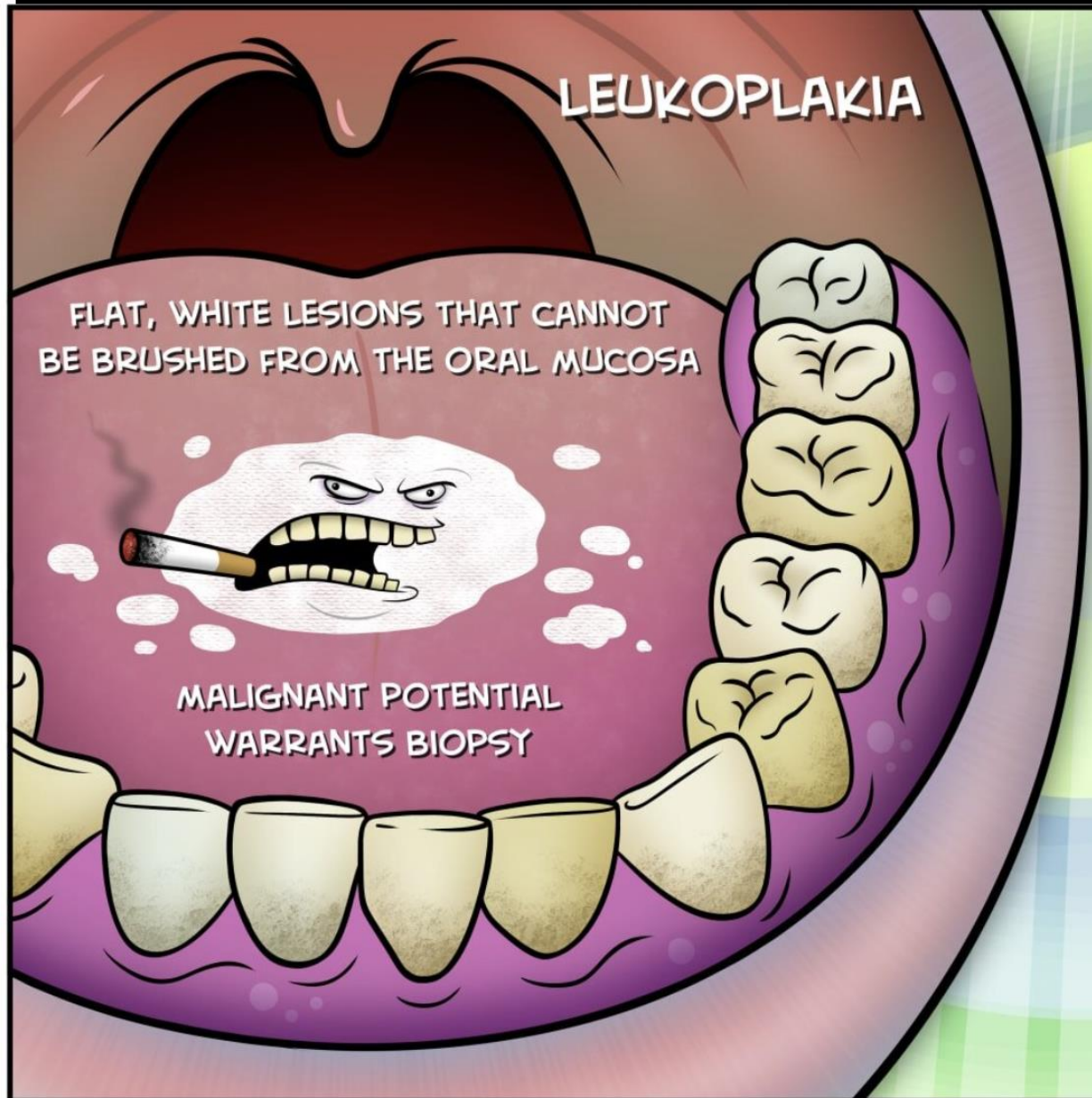
¹Biochemistry, Molecular Biology and Biophysics, University of Minnesota, Minneapolis, USA

²Bruker Scientific LLC, San Jose, CA;

³Otolaryngology Department, University of Minnesota, Minneapolis, Minnesota;

⁴Bruker Scientific, LLC, Billerica, MA

ORAL LEUKOPLAKIA: ORAL CANCER RISK



BACKGROUND

- * PAINLESS, SLOW-GROWING LESION on MUCOUS MEMBRANES of ORAL CAVITY
- * POTENTIAL PRECANCEROUS CONDITION

CAUSES



- * HEAVY SMOKING
- * CHEWING TOBACCO
- * EXCESSIVE ALCOHOL USE

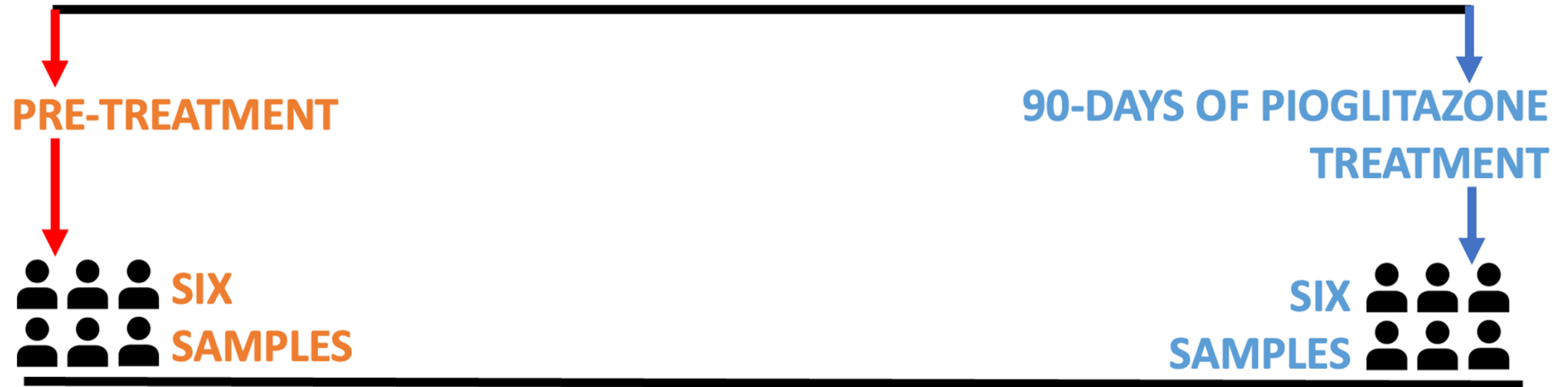


- * POOR ORAL HEALTH
- * LONG-TERM TRAUMA to ORAL CAVITY
- * ADVANCED AGE
- * HPV INFECTION



EXPERIMENTAL WORKFLOW

ORAL LEUKOPLAKIA



ORAL RINSE SAMPLES

Enrichment

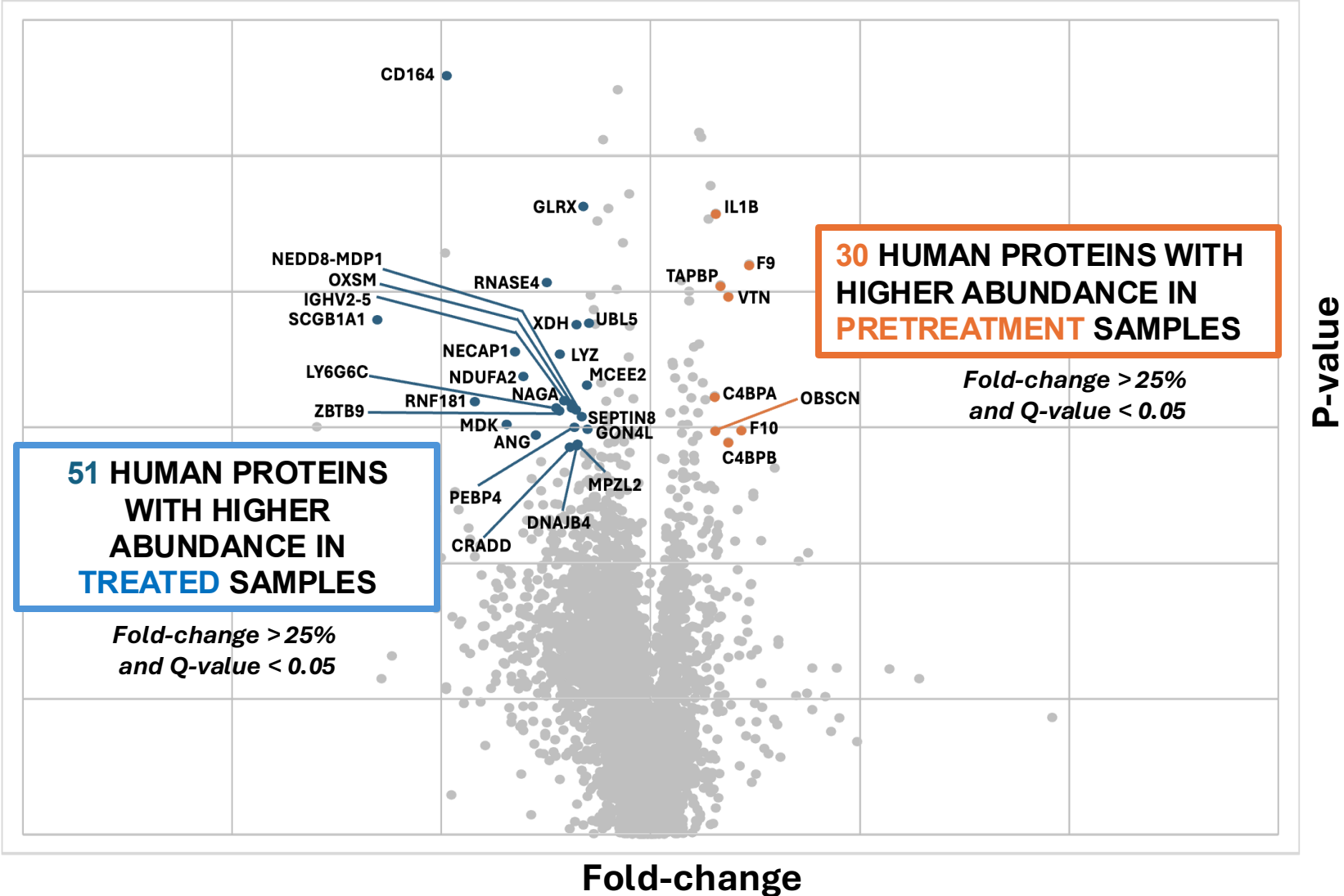
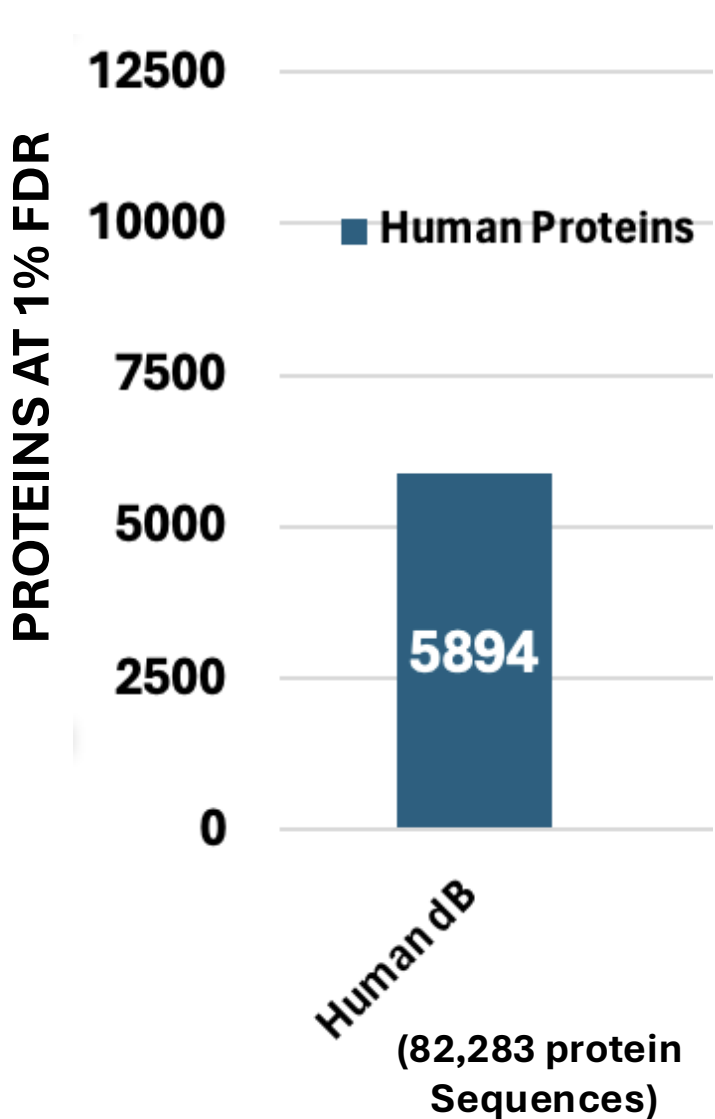
Mass spectrometry using DIA-PASEF

Search against Human + Microbiome + Novel Proteoforms

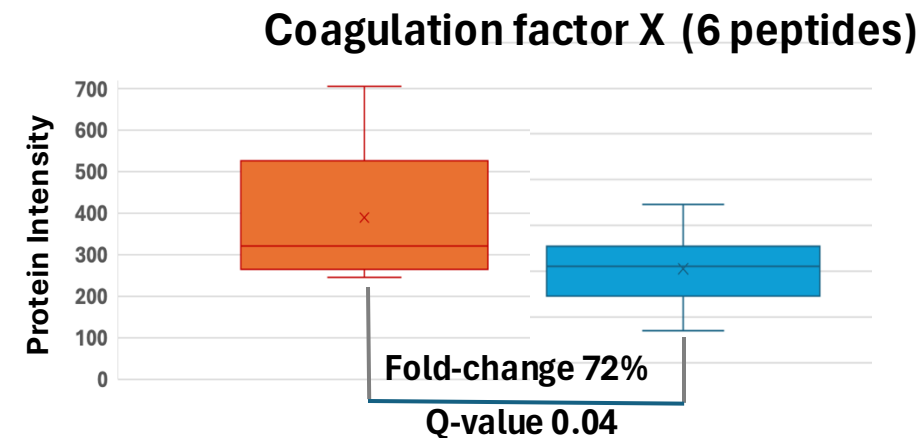
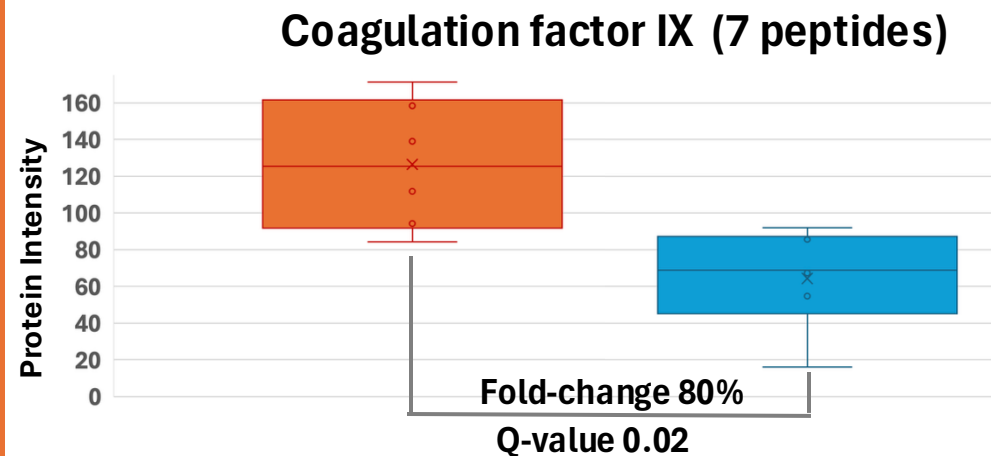
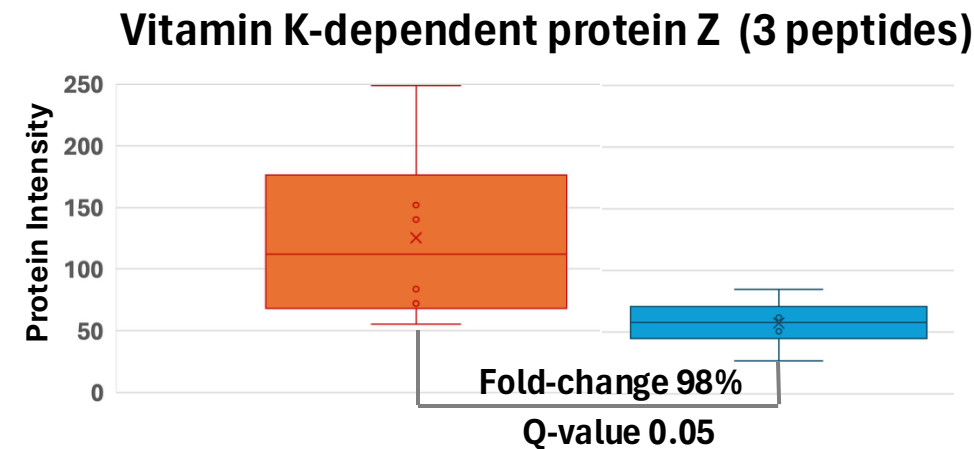
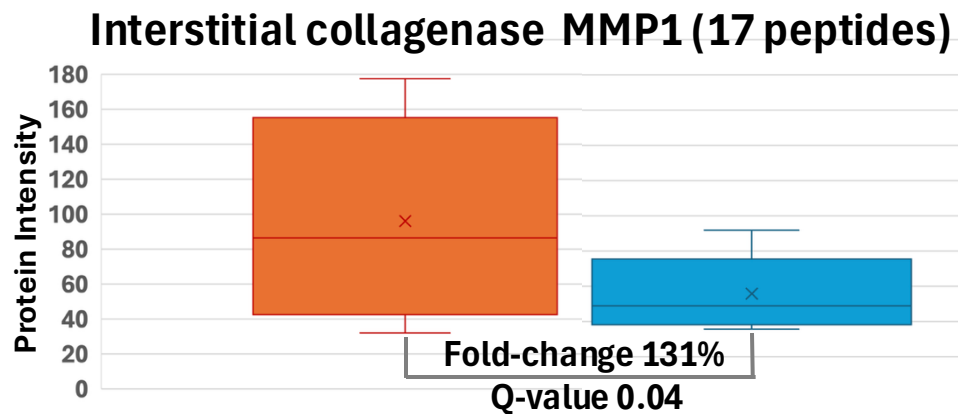
PO



PROTEINS DETECTED AND DIFFERENTIALLY ABUNDANT PROTEINS



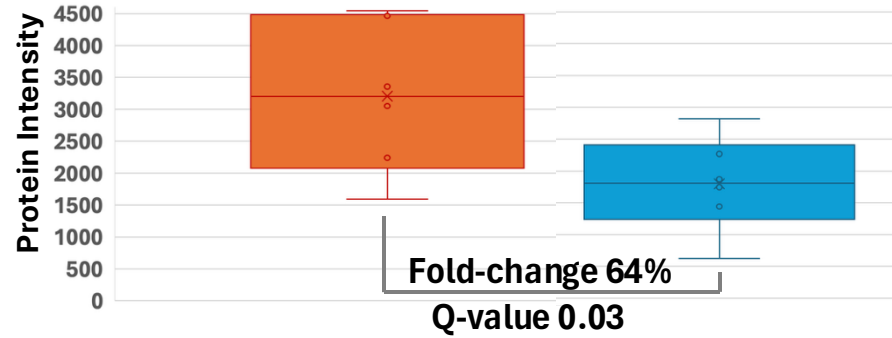
MMP1 & COAGULATION CASCADE: DOWNREGULATED AFTER TREATMENT



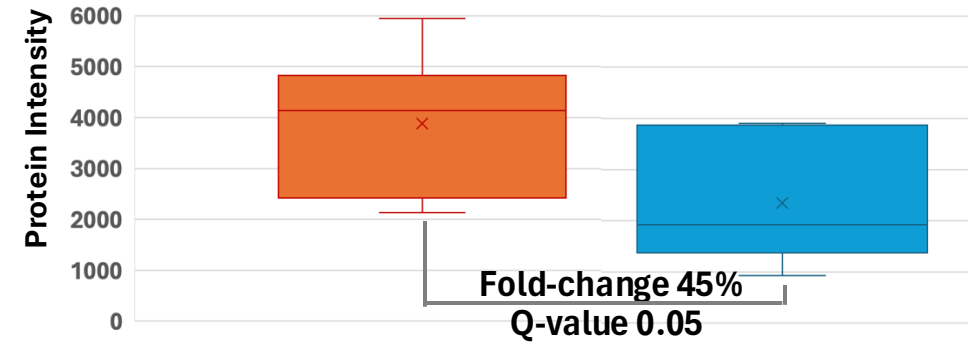
Coagulation Cascade

COMPLEMENT CASCADE: DOWNREGULATED AFTER TREATMENT

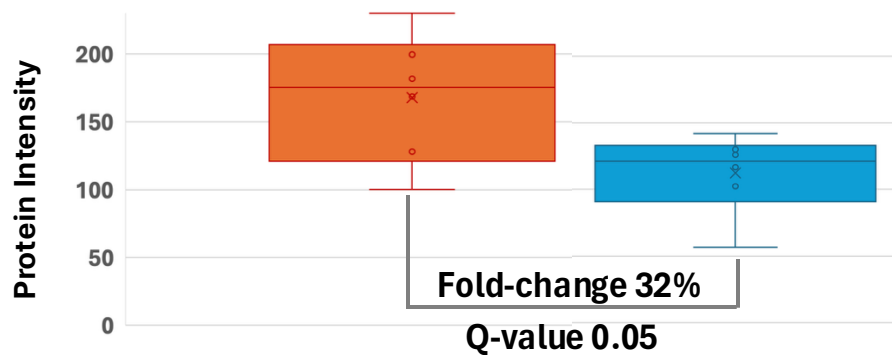
VITRONECTIN (12 peptides)



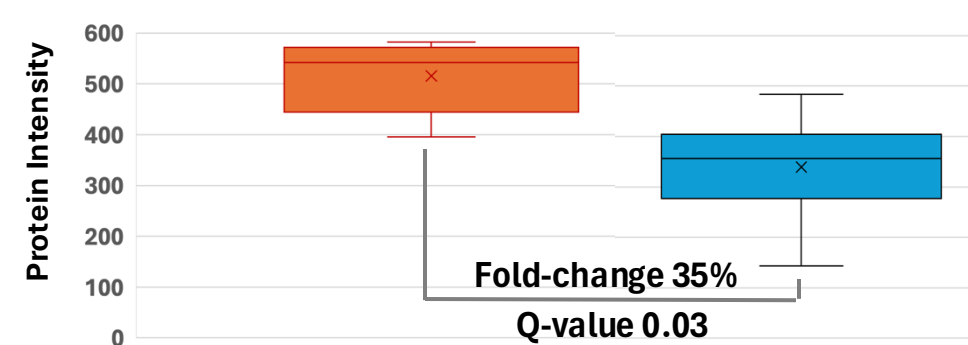
C4b-binding protein alpha chain (18 peptides)



Carboxypeptidase B2 (9 peptides)



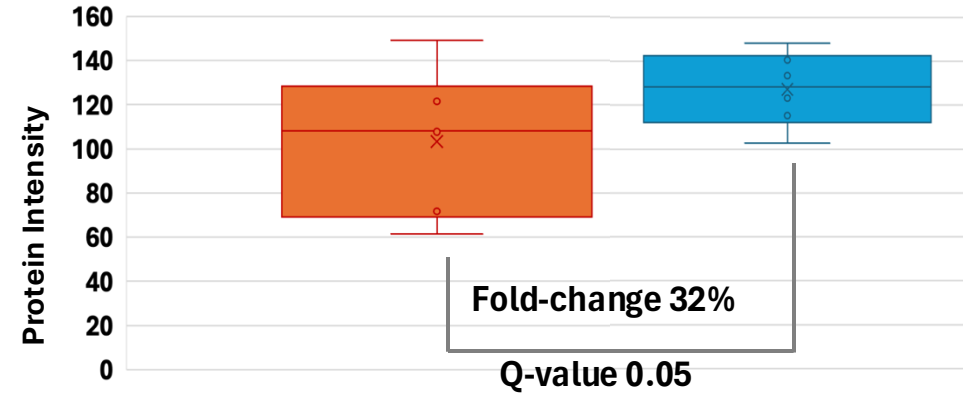
Complement component C8 beta chain (18 peptides)



Regulation of Complement cascade

APOPTOSIS: UPREGULATED AFTER TREATMENT

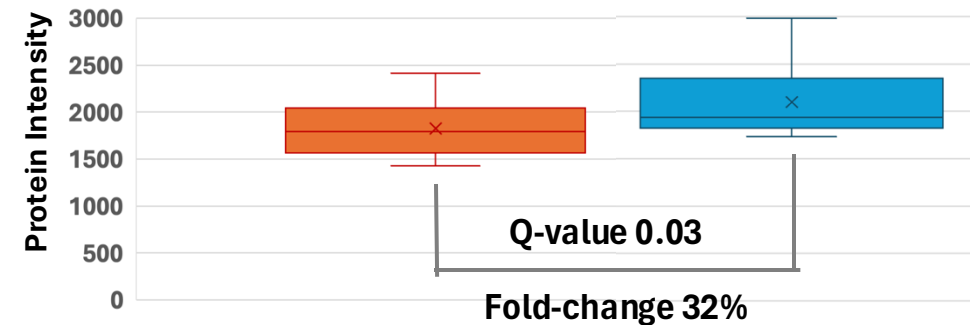
DESMOGLEIN-2 (16 peptides)



- Component of intercellular desmosome junctions mediating cell-cell adhesion.
- Involved in apoptotic pathway.
- Prognostic marker in renal cancer, pancreatic cancer, lung cancer, head and neck cancer, colorectal cancer and cervical cancer.

- An adaptor protein that is composed of two protein-protein interaction domains
- Functions as key mediator in apoptosis and inflammation via the activation of caspases.
- Prognostic marker in renal cancer

Apoptosis-associated speck-like protein containing a CARD (13 peptides)

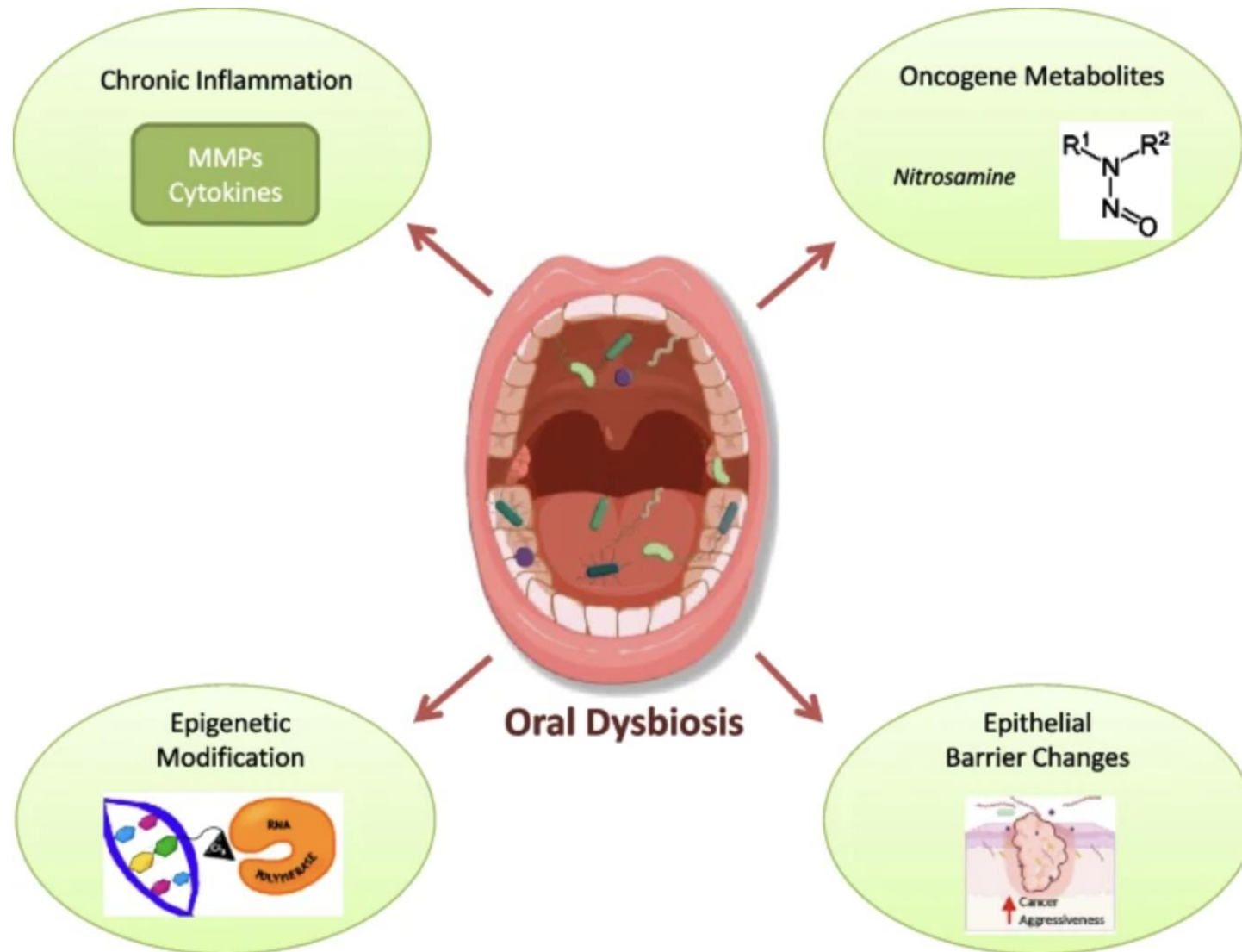


CANCER RELEVANCE OF DIFFERENTIALLY ABUNDANT PROTEINS

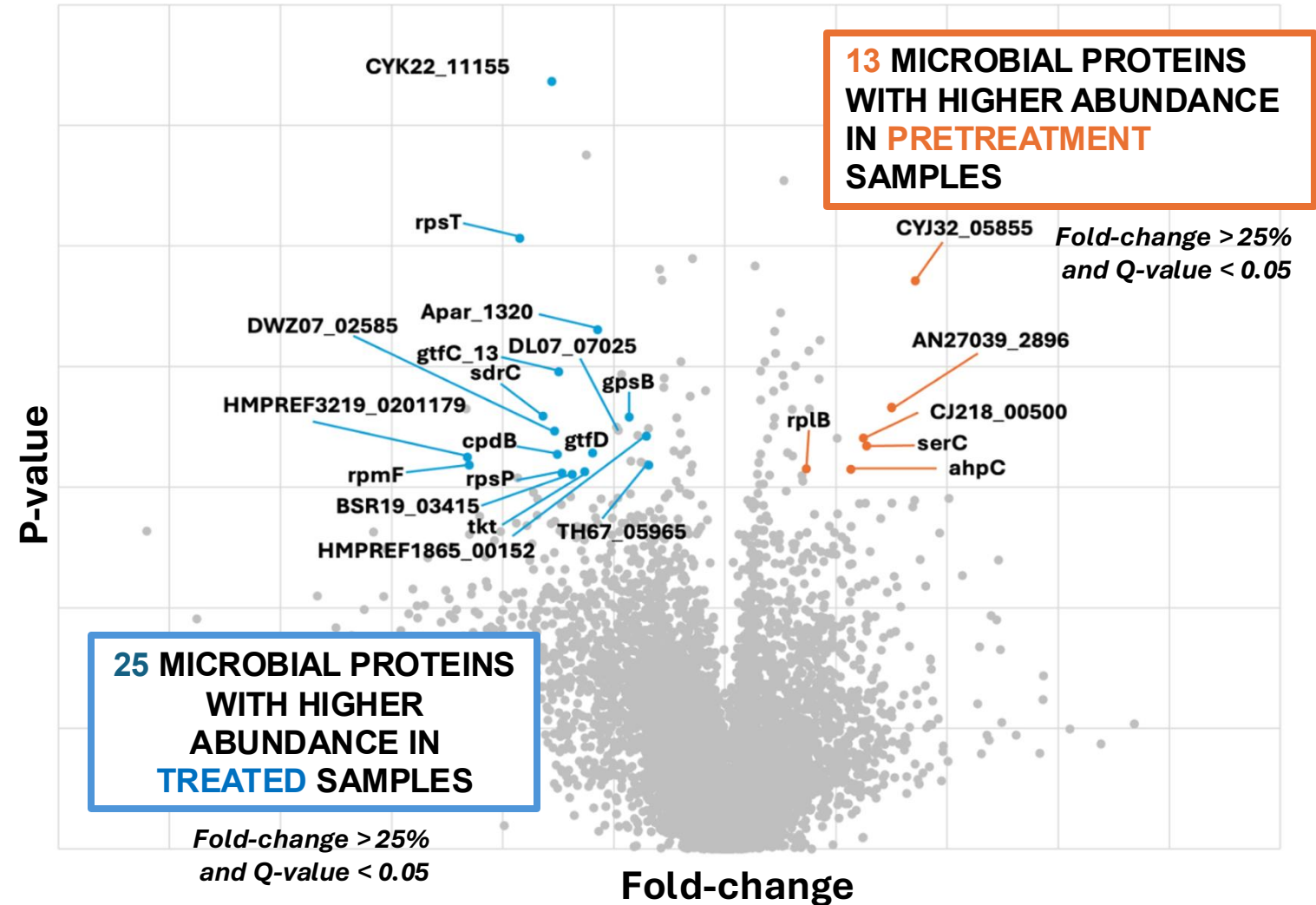
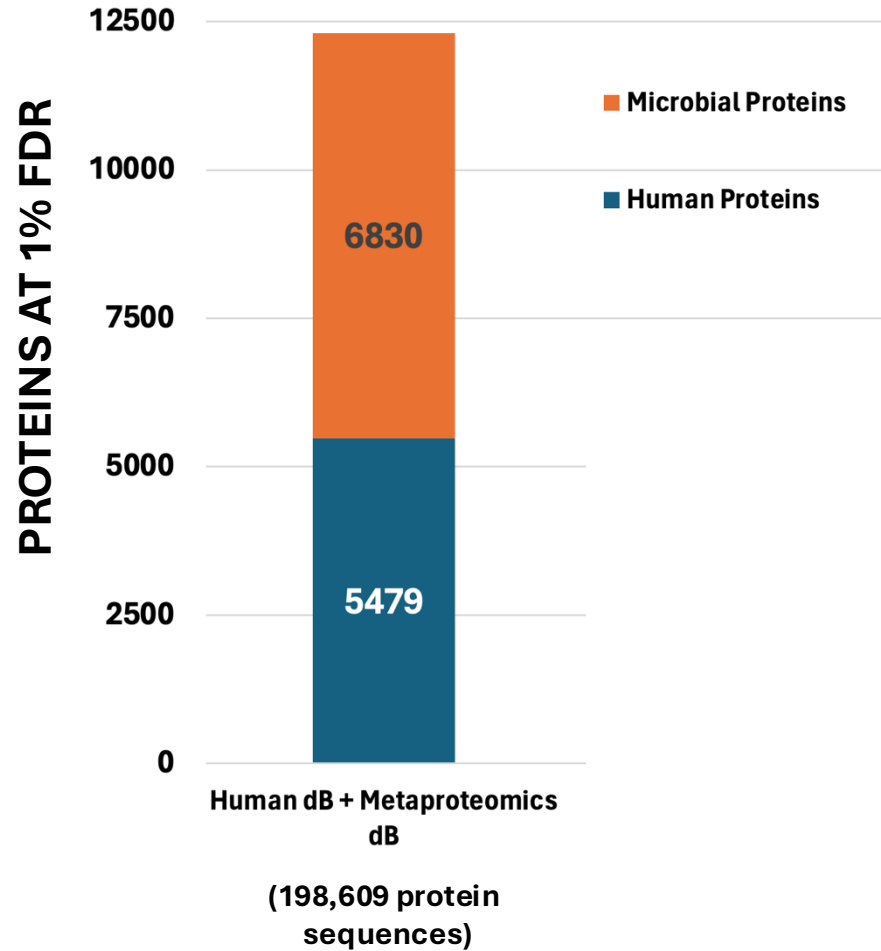
	PROTEIN DESCRIPTION	RENAL	STOMACH	HEAD & NECK	PANCREATIC	ENDOMETRIAL	BREAST	UROTHELIAL	PROSTATE	LIVER	LUNG	COLORECTAL	CERVICAL	GLIOMA	THYROID	ORAL
HIGHER ABUNDANCE IN TREATED SAMPLES	Cathepsin D															
	Lysozyme C															
	Golgi reassembly-stacking protein 2															
	Receptor-type tyrosine-protein phosphatase S															
	Sialate O-acetyltransferase															
	Plexin-B3															
	Glycerol-3-phosphate phosphatase															
	Cystatin-D															
	Serine hydroxymethyltransferase, cytosolic															
	Di-N-acetylchitinase															
	Desmoglein-2															
	Chitinase-3-like protein 2															
	Apoptosis-associated speck-like protein containing a CARD															
	Xanthine dehydrogenase/oxidase															
HIGHER ABUNDANCE IN PRE-TREATMENT SAMPLES	Carboxypeptidase B2															
	C4b-binding protein alpha chain															
	Vitronectin															
	Ferritin light chain															
	Cytochrome P450 4F3															
	Complement component C8 beta chain															
	Cytochrome c oxidase subunit 4 isoform 1, mitochondrial															
	Mitochondrial adenyl nucleotide antiporter SLC25A24															
	Coagulation factor X															
	Interstitial collagenase															
	Coagulation factor IX															
	Signal peptidase complex catalytic subunit SEC11															

FAVORABLE PROGNOSIS VALUE
 UNFAVORABLE PROGNOSIS VALUE
 HIGHER ABUNDANCE

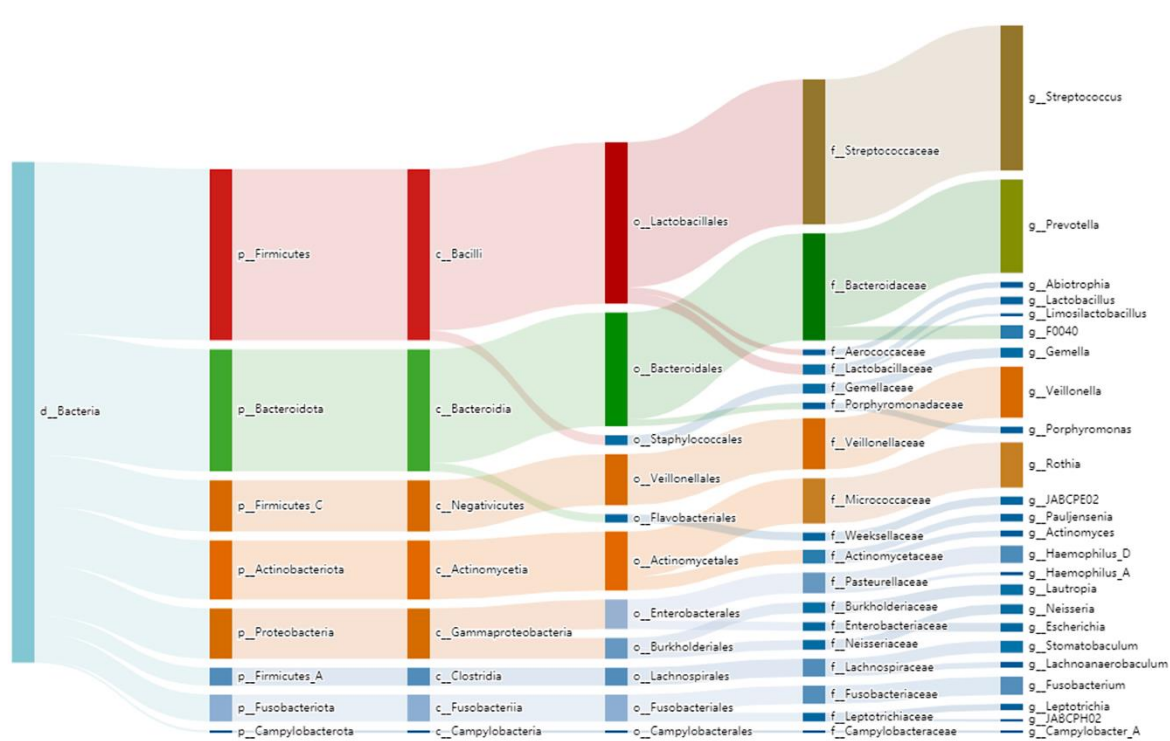
ROLE OF BACTERIA IN ORAL CANCER DEVELOPMENT



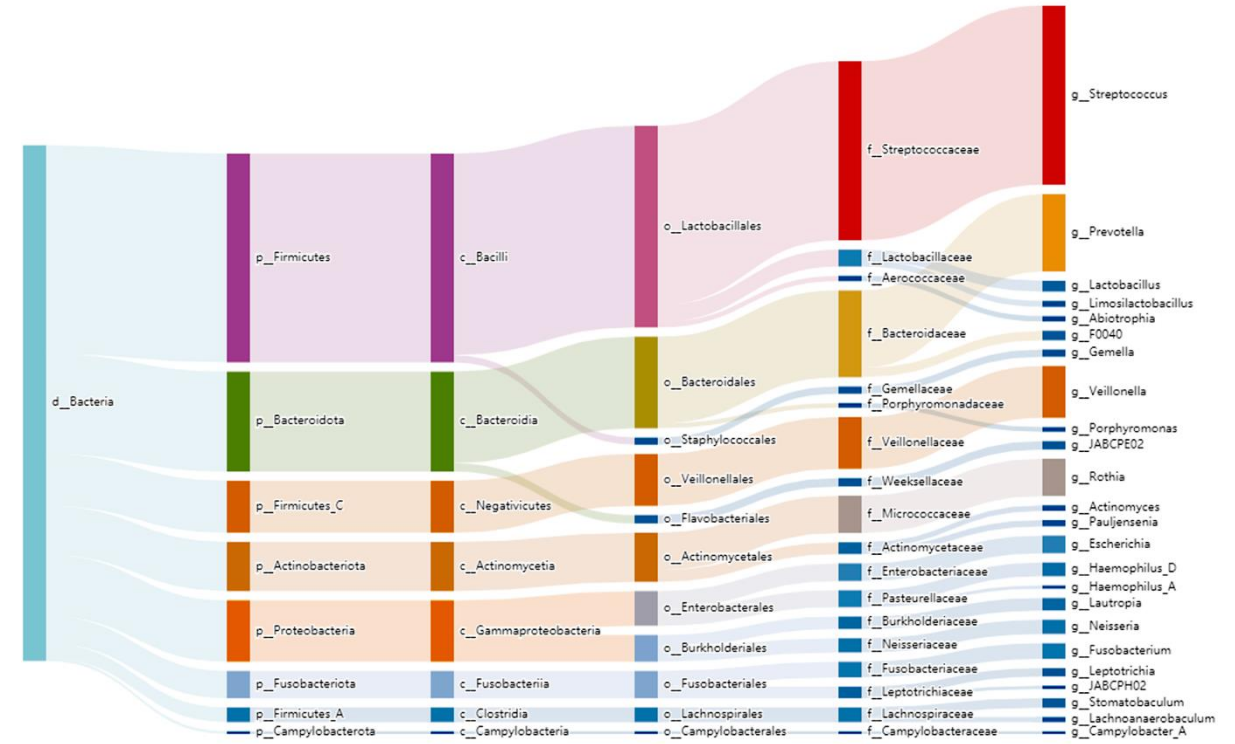
MICROBIAL DATABASE SEARCH RESULTS



MICROBIAL TAXONOMY OUTPUTS



Genera detected in pretreated samples



Genera detected in treatment samples



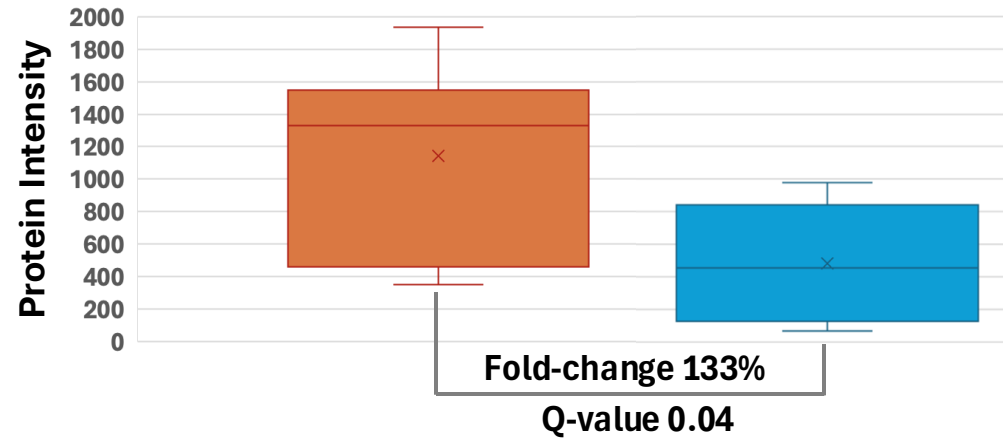
MetaLab and



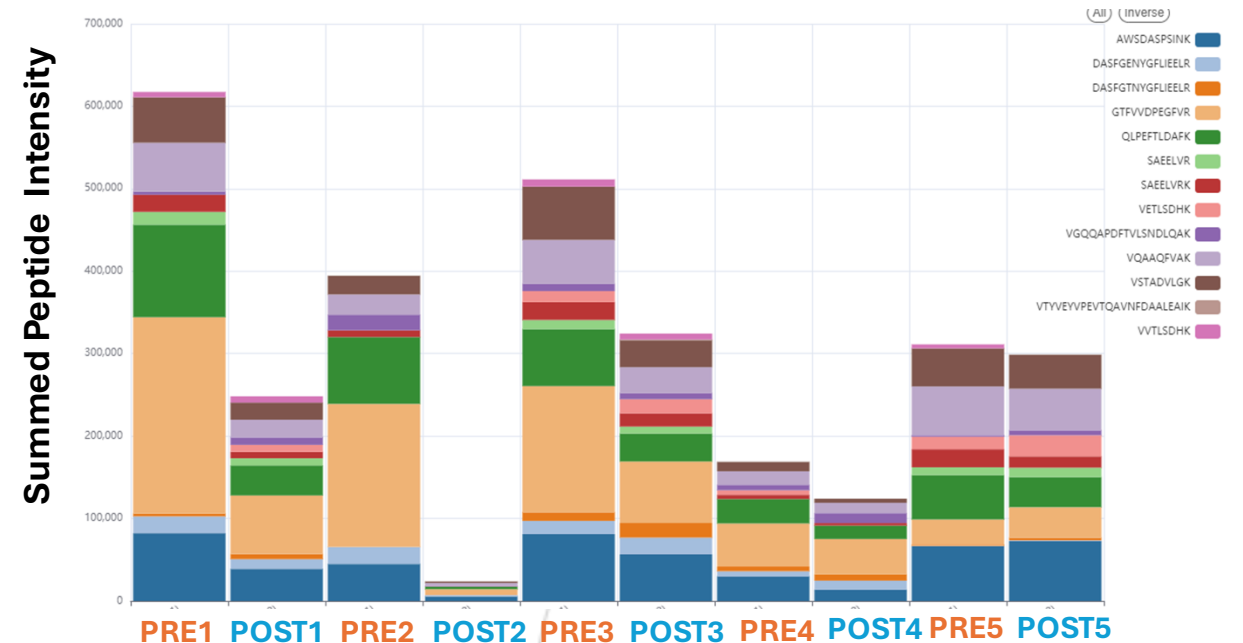
analysis by Kai Cheng, Qing Wu (Daniel Figeys Lab, University of Ottawa)

MICROBIAL PROTEINS DOWNREGULATED AFTER TREATMENT

Alkyl hydroperoxide reductase C (11 peptides)



- Responsible for the detoxification of reactive oxygen species.
- Survival under environmental stresses or during infection.



In this study, the protein was expressed by *Veillonella* genus.

Veillonella



MetaLab

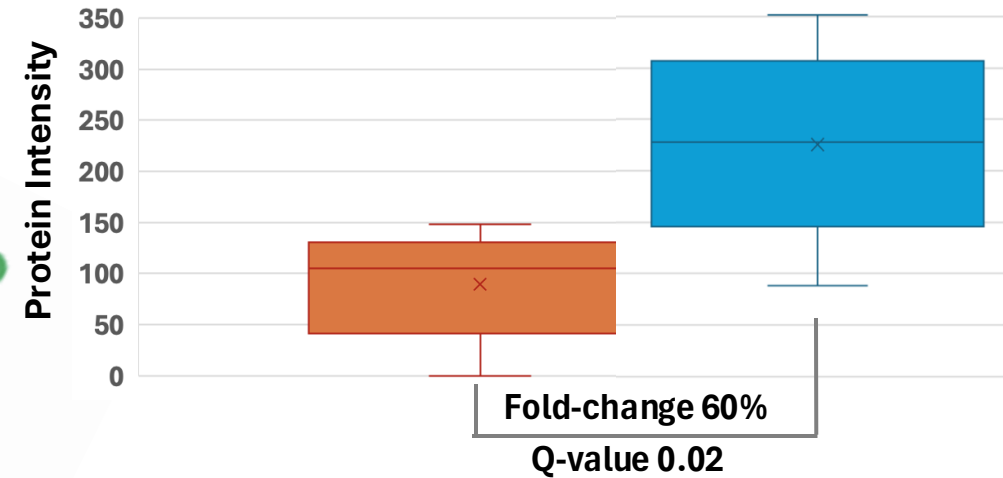


analysis by Kai Cheng, Qing Wu (Daniel Figeys Lab, University of Ottawa)

MICROBIAL PROTEINS UPREGULATED AFTER TREATMENT

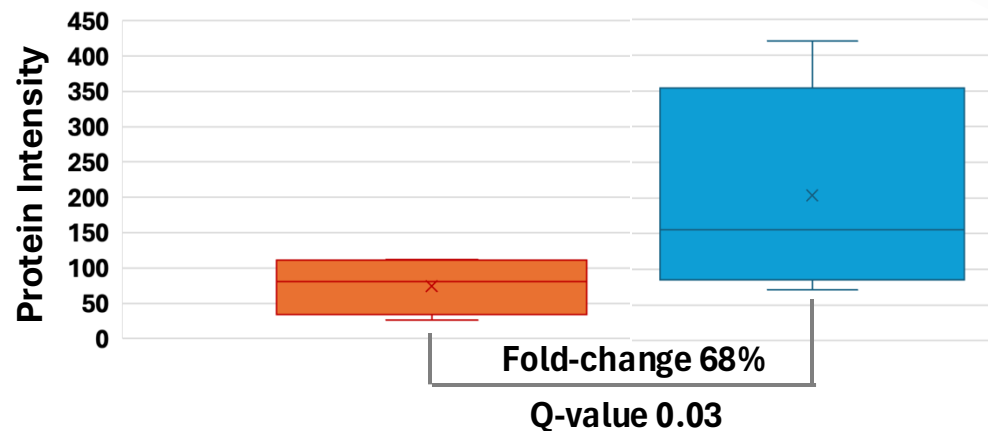
- Glucosyltransferase that catalyzes the transfer of glucosyl residues to dextran polymer.
- Involved in biofilm formation.

Dextranucrase (61 peptides)



Streptococcus salivarius

serine-type D-Ala-D-Ala carboxypeptidase (21 peptides)



- Involved in bacterial cell wall synthesis by mediating peptidoglycan cross-linking.

DIFFERENTIALLY ABUNDANT MICROBIAL PROTEINS

PROTEIN DESCRIPTIONS	UNIPROTIDS	% CHANGE	QVALUE	# UNIQUE PEPTIDES
Alkyl hydroperoxide reductase C	E4LFM1	133.47	0.04	11
Dextranucrase	E9DP35, J7SIV6, J7TRB0	-62.27	0.02	61
Serine-type D-Ala-D-Ala carboxypeptidase	A0A2A5QD93	-67.91	0.03	21
Serine protease	E3H076	-44.89	0.05	4
Uncharacterized protein	A0A2S7ZRS7	-46.03	0.01	5
RND transporter, HAE1 family	E1L728	-37.59	0.03	5
Ribosomal subunit protein S16	A5VKN7;C8P927	-64.94	0.03	3
Glutamate--ammonia ligase	Q5M2N1	-58.15	0.04	7
Elongation factor Ts	A0A0X8K315	-46.57	0.03	8

ORAL CANCER DATASET: CONCLUSIONS AND FUTURE WORK

- **Several human, microbial and variant proteins were detected to be differentially abundant in pretreatment and treated samples.**
- **Pathways such as coagulation and complement cascade were downregulated and apoptotic pathways were upregulated after treatment.**
- **Microbial functions associated with glucosyltransferase activity were upregulated and oxidative stress functions were downregulated after treatment.**
- **Peptides associated with differentially abundant human and microbial will be used for targeted analysis.**

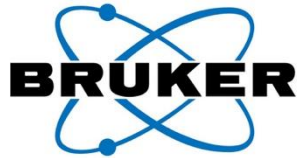
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UNIVERSITY OF MINNESOTA
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COLLEGE of BIOLOGICAL SCIENCES

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- **Subina Mehta**
- **Reid Wagner**



uOttawa

- **Kai Cheng**
- **Qing Wu**
- **Daniel Figeys**



CLINICAL METAPROTEOMICS WORKFLOW TO STUDY HOST-MICROBIOME DYNAMICS

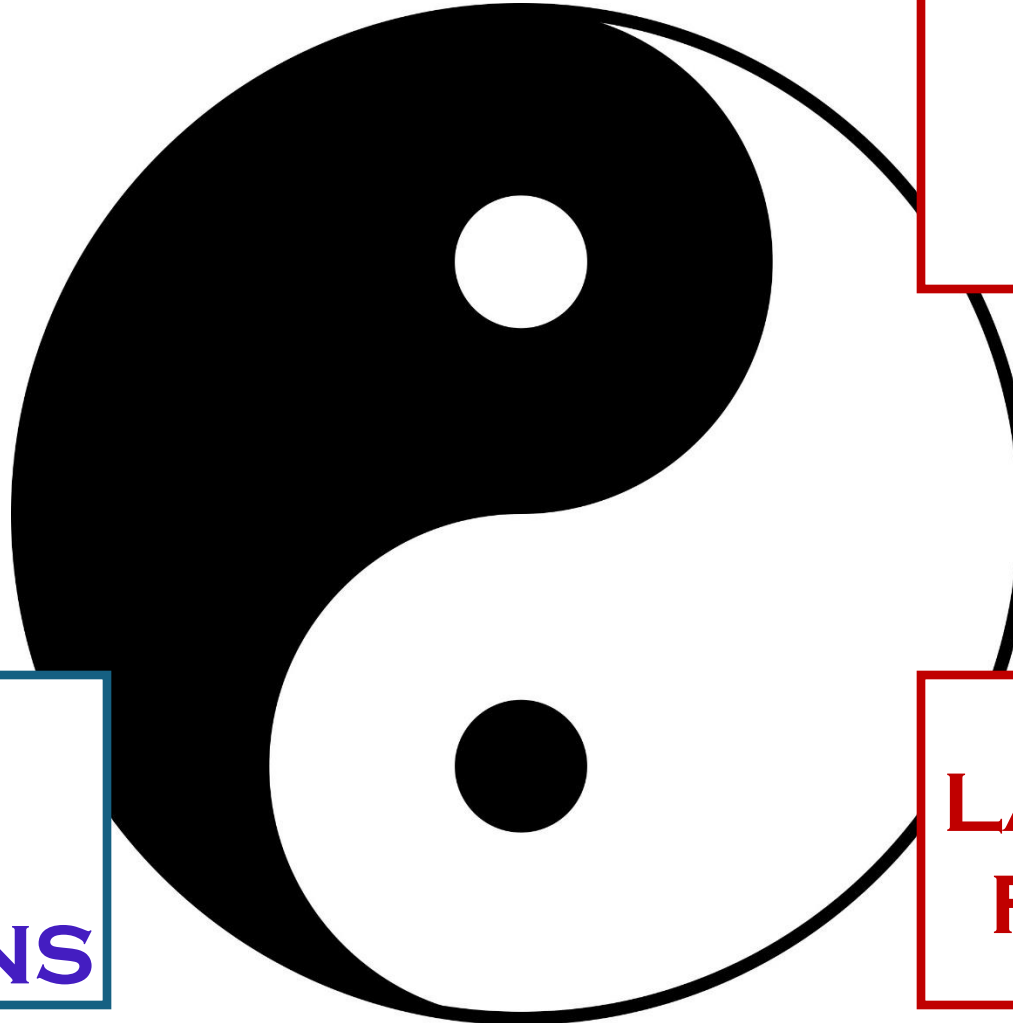
- *MICROBIOME RESEARCH*
- *MASS SPECTROMETRY DATA ANALYSIS*
- *METAPROTEOMICS RESEARCH*
- *GALAXY BIOINFORMATICS PLATFORM*
- *CLINICAL METAPROTEOMICS WORKFLOW*
- *CLINICAL METAPROTEOMICS: COVID-19 PANDEMIC*
- *CLINICAL METAPROTEOMICS: CYSTIC FIBROSIS*
- *CLINICAL METAPROTEOMICS: ORAL CANCER*
- ***METAPROTEOMICS EDUCATION***

METAPROTEOMICS: STRENGTHS & CHALLENGES

**FUNCTIONAL
COMPOSITION**

**HOST-
MICROBIOME
INTERACTION**

**QUANTIFYING
BIOMASS
CONTRIBUTIONS**



**PROTEIN
EXTRACTION**

**VARIABILITY &
ABUNDANCE**

**LARGE DATABASES
FALSE POSITIVES**

MARCH 2017
ABRF @ SAN DIEGO

JUNE 2017
ASMS @ INDIANAPOLIS

JUNE 2017
IMS @ SARDINIA

JULY 2017
GCC @ MONTPELLIER

JUNE 2019
ASMS @ ATLANTA

JULY 2019
GCC @ FREIBURG

WORKSHOPS

<http://galaxyp.org/workshops/>

MARCH 2020
ABRF @ PALM SPRINGS

MARCH 2020
LORENTZ @ LEIDEN

JUNE 2020
ASMS @ HOUSTON

JULY 2020
GCC @ TORONTO

FEBRUARY 2021
SMORGASBORD @ GLOBAL

MAY 2021
ELIXIR @ GHENT

JULY 2021
GCC @ GHENT

NOVEMBER 2021
ASMS @ PHILADELPHIA

NOVEMBER 2021
ASM-IUSSTF @ CHANDIGARH

MARCH 2022
TAPAS @ GLOBAL

JULY 2022
GCC @ MINNEAPOLIS

JULY 2022
IMTECH@ CHANDIGARH & IIT-BOMBAY @MUMBAI

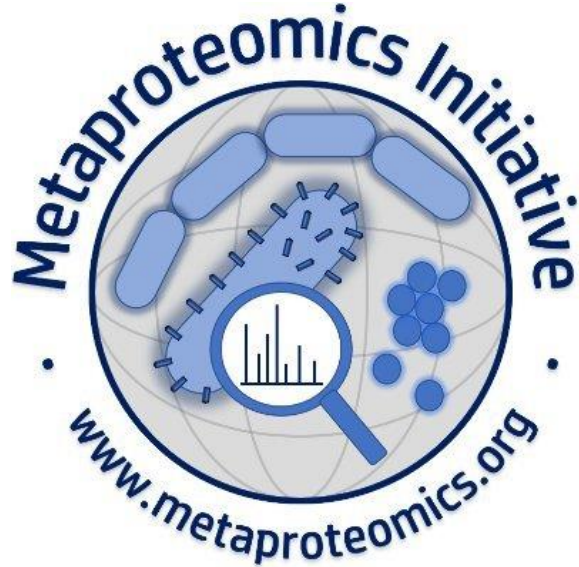
AUGUST 2022
IMSC @ MAASTRICHT

APRIL 2023
IMS @ AVIGNON

MAY 2023
SMORGASBORD @ GLOBAL

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QUESTIONS?