

CLINICAL METAPROTEOMICS WORKFLOW TO STUDY HOST-MICROBIOME DYNAMICS

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Google Scholar: z.umn.edu/pjgs

PubMed: z.umn.edu/pjagtappreferences



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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

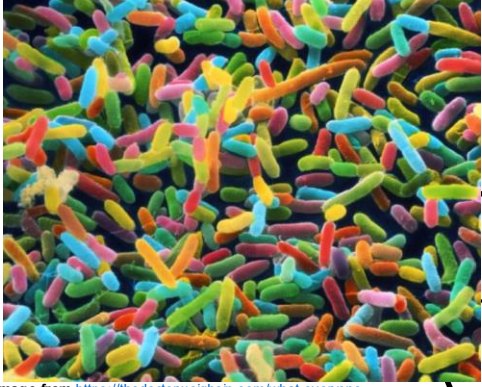
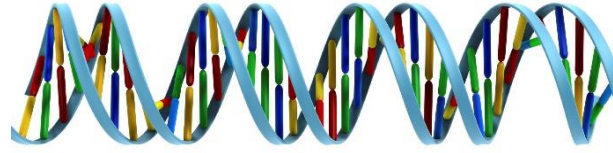


Image from <https://thedoctorwithsin.com/what-everyone-should-know-about-the-infant-microbiome/>

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



METAGENOMICS

TAXONOMY

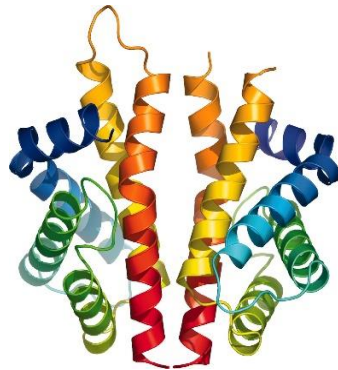
function



METATRANSCRIPTOMICS

TAXONOMY

function

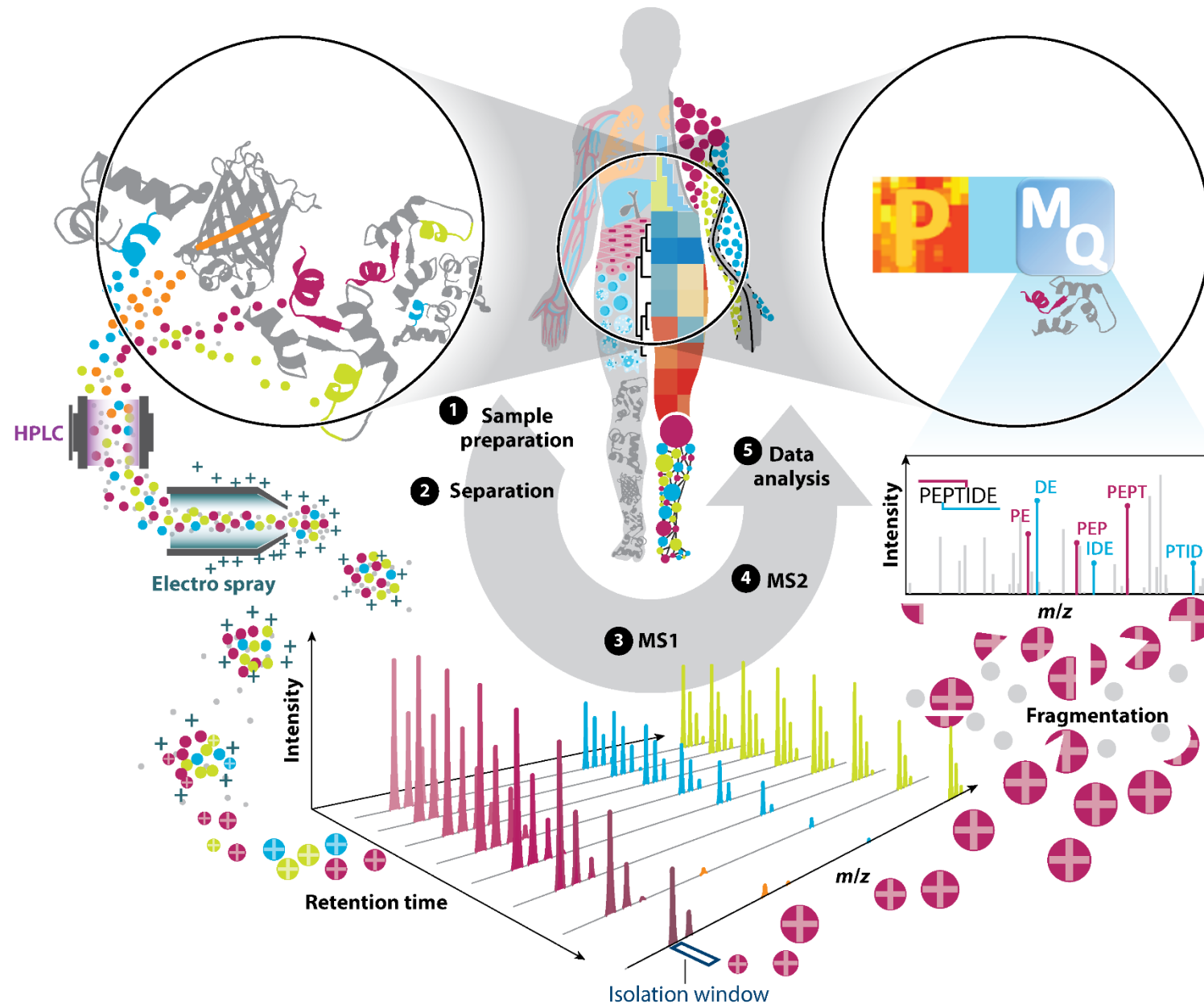


METAPROTEOMICS

TAXONOMY

FUNCTION

MASS SPECTROMETRY AND PROTEOMICS

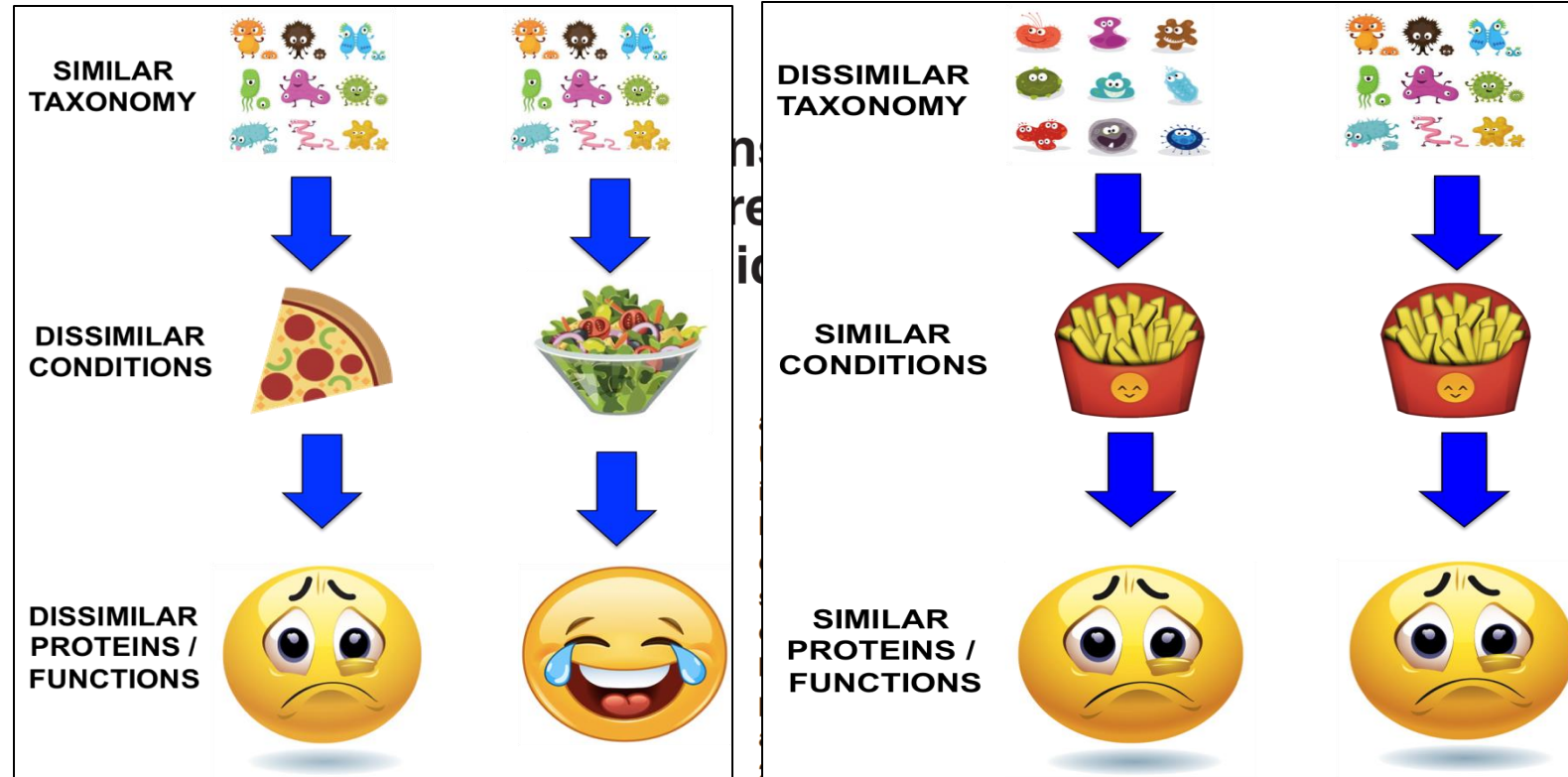


METAPROTEOMICS

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

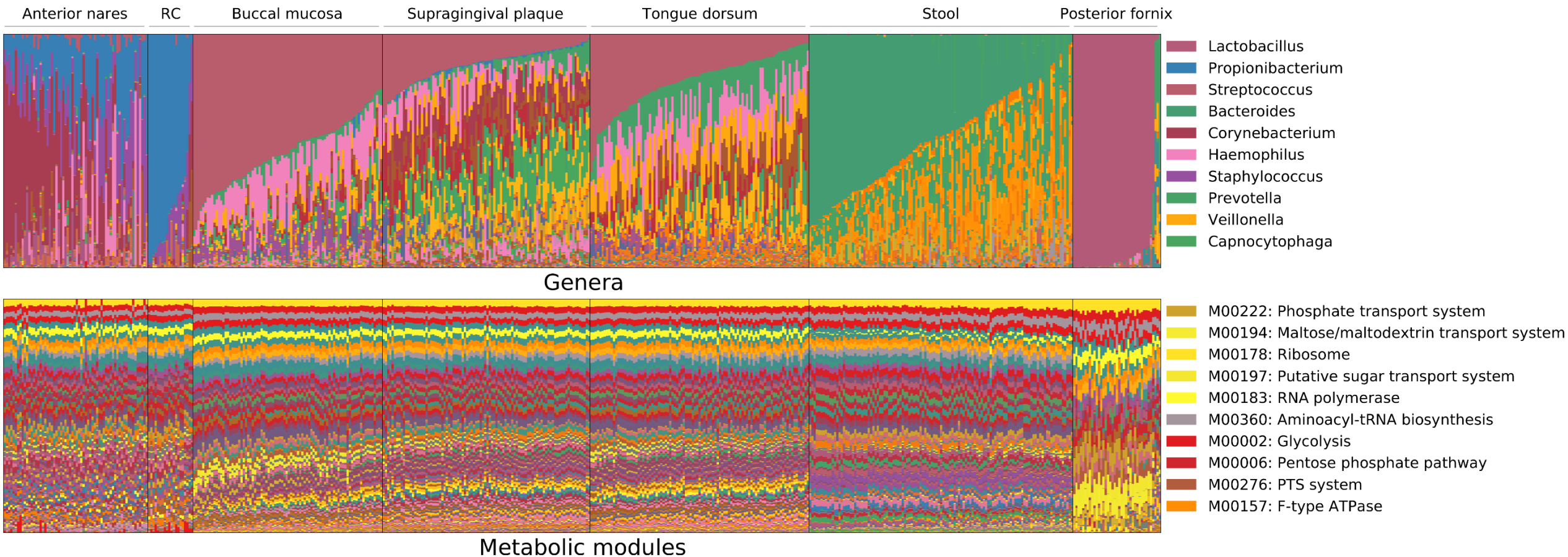
SINGLE + CONTAMINANTS

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

MICROBIAL TAXA VARY WHILE METABOLIC PATHWAYS REMAIN STABLE WITHIN A HEALTHY POPULATION



Human Microbiome Project Consortium (2012). Nature. 486(7402):207-214. doi: 10.1038/nature11234.

CLINICAL METAPROTEOMICS WORKFLOW TO STUDY HOST-MICROBIOME DYNAMICS

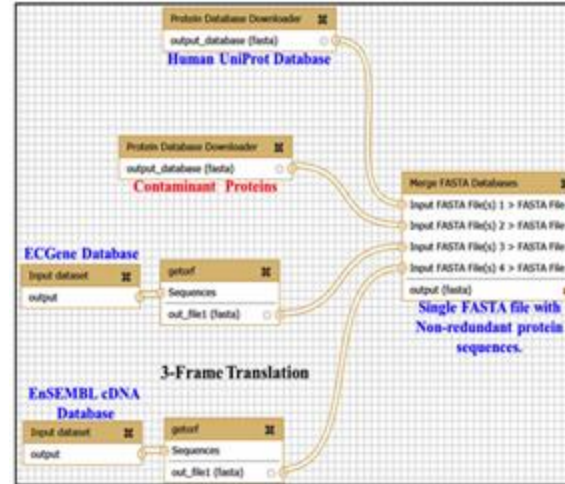
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Defining Workflows and Histories

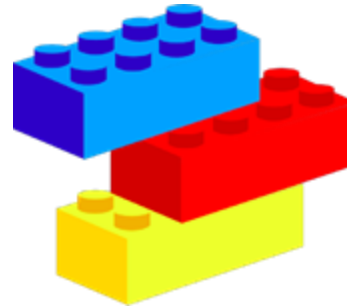
Workflows: Multiple software tools used in a sequential manner for an analysis



Single software tool
in Galaxy



Integrated workflow using
multiple, connected tools



History: recorded, complete analysis
(workflow + all data and results)



The Galaxy Interface

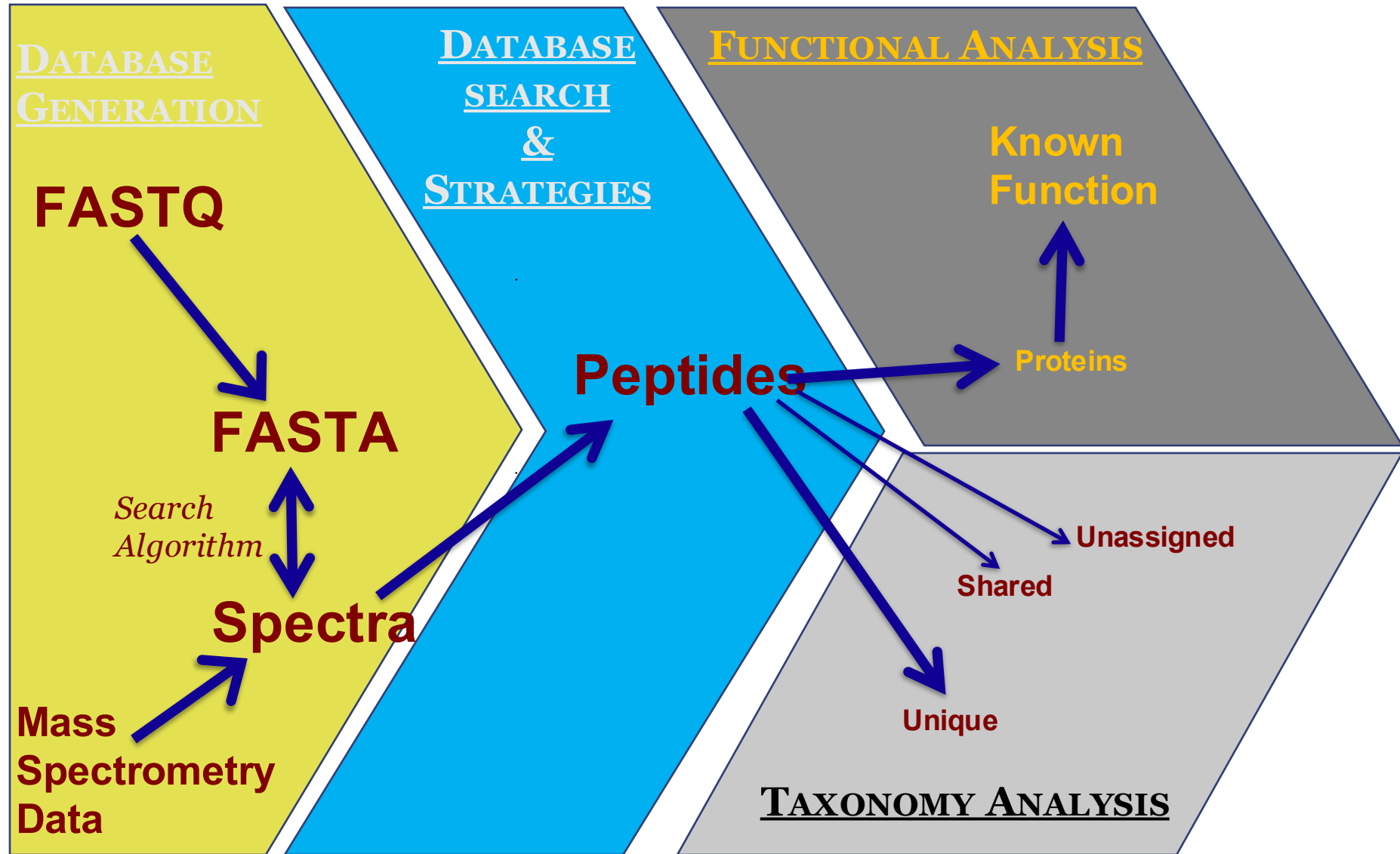
Main viewing window
(workflow development, results visualization etc)

Tools

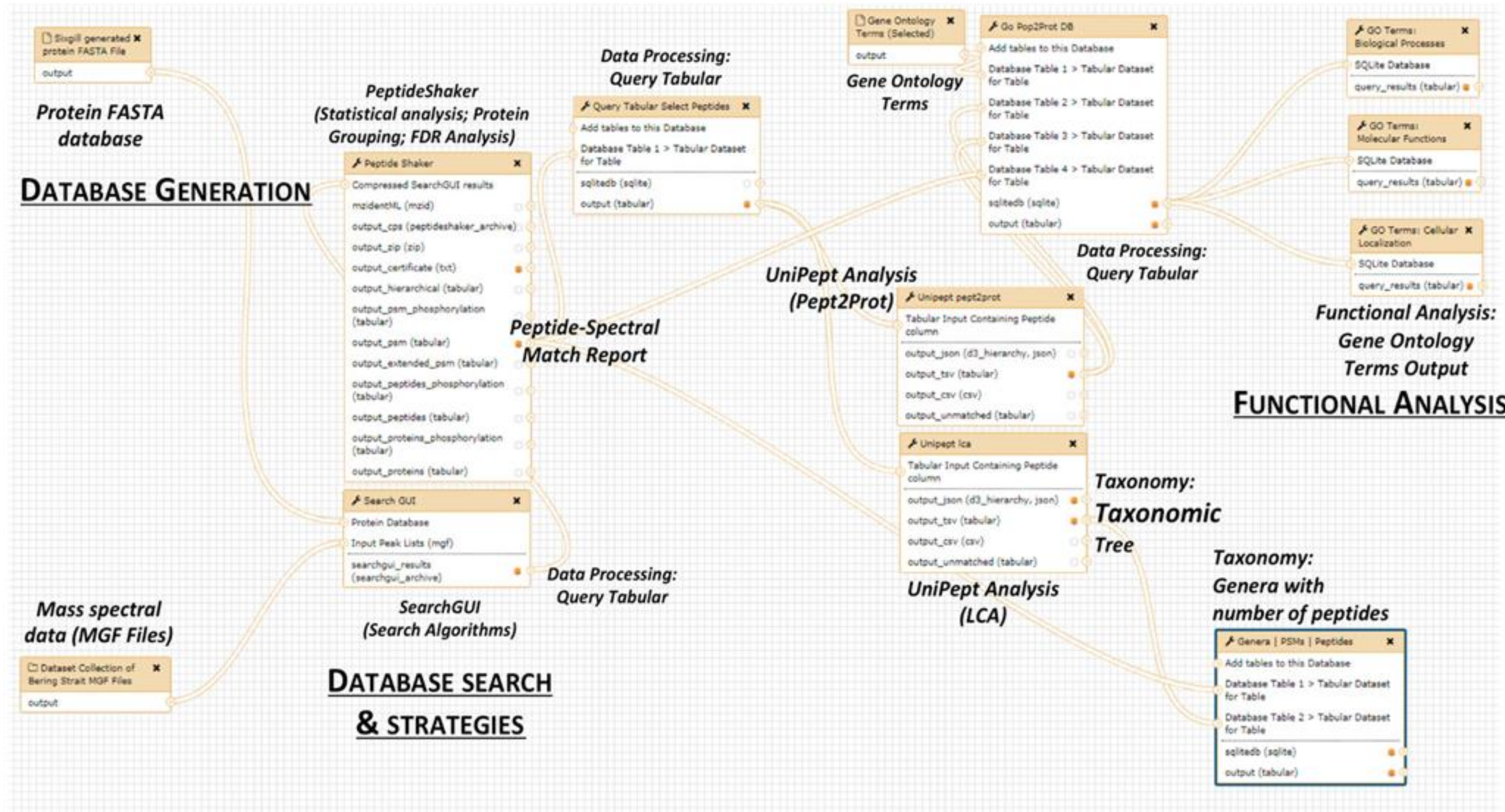
History

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 this panel. 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 this central area. On the right is a 'History' panel showing a list of workflow steps, such as 'imported: Test1 Workflow for human proteogenomics' and '33: BLAST-P Filtered Peptide Report'. 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'.

METAPROTEOMICS WORKFLOW



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.

Metaproteomics Publications

PROTEOMICS

Jagtap P *et al* (2012) Deep metaproteomic analysis of **human salivary supernatant**. Proteomics. 12(7):992-1001. doi: 10.1002/ pmic.201100503.

Microbiome

Rudney JD *et al* (2015) Protein relative abundance patterns associated with **sucrose-induced dysbiosis** are conserved across taxonomically diverse oral microcosm **biofilm models of dental caries**. Microbiome. 3:69. doi: 10.1186/s40168-015-0136-z.

SCIENTIFIC
REPORTS
nature research

Afiuni-Zadeh S *et al* (2018) Evaluating the potential of residual Pap test fluid as a resource for the metaproteomic analysis of the **cervical-vaginal microbiome**. Sci Rep. ;8(1):10868. doi: 10.1038/s41598-018-29092-4.



ATS Journals

AJRCMB

Jagtap PD *et al* (2018) **BAL Fluid Metaproteome** in Acute Respiratory Failure. Am J Respir Cell Mol Biol. 59(5):648-652. doi: 10.1165/rcmb.2018-0068LE.

ACS Publications
Most Trusted. Most Cited. Most Read.

Journal of
proteome
research

Thuy-Boun PS *et al* (2021). Metaproteomics Analysis of **SARS-CoV-2-Infected Patient Samples** Reveals Presence of Potential Coinfecting Microorganisms. J Proteome Res. 20(2):1451-1454. doi: 10.1021/acs.jproteome.0c00822

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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

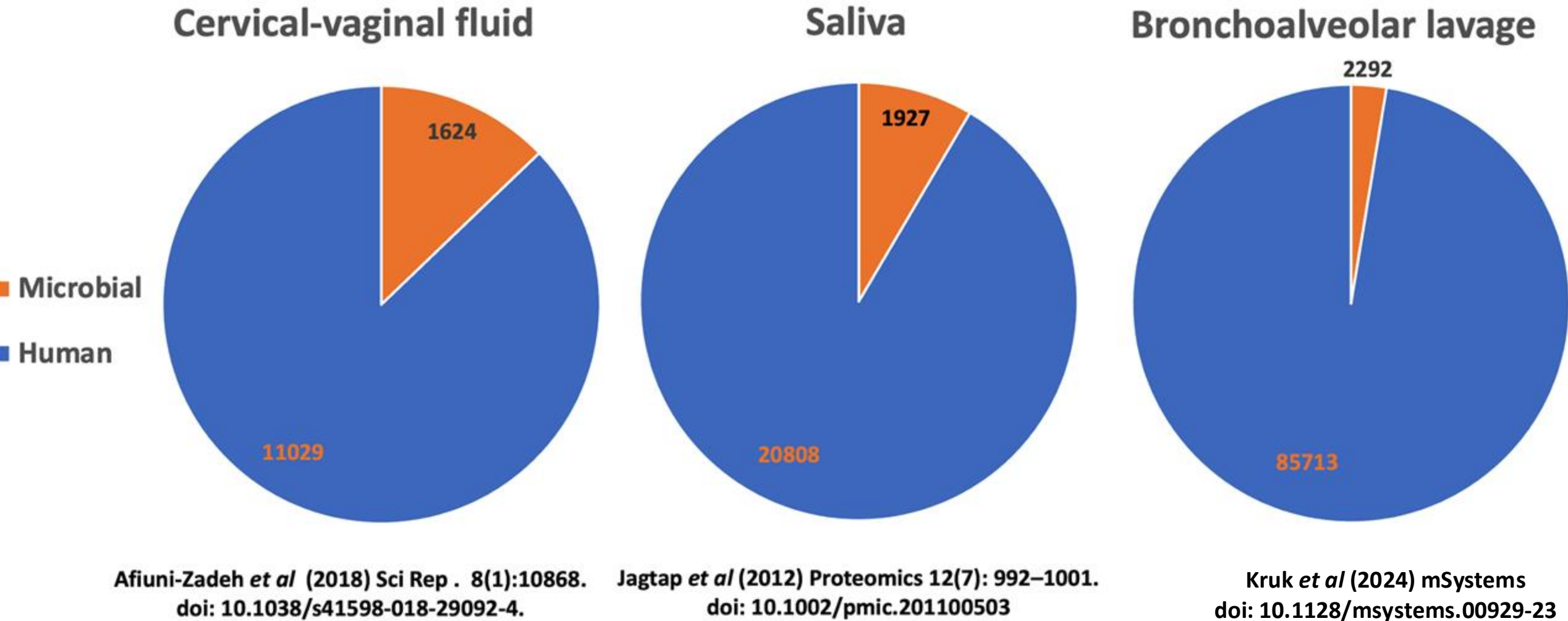
⁶ Department of Pediatrics, Division of Pulmonary and Sleep Medicine, Seattle Children's Hospital, Seattle, WA, USA



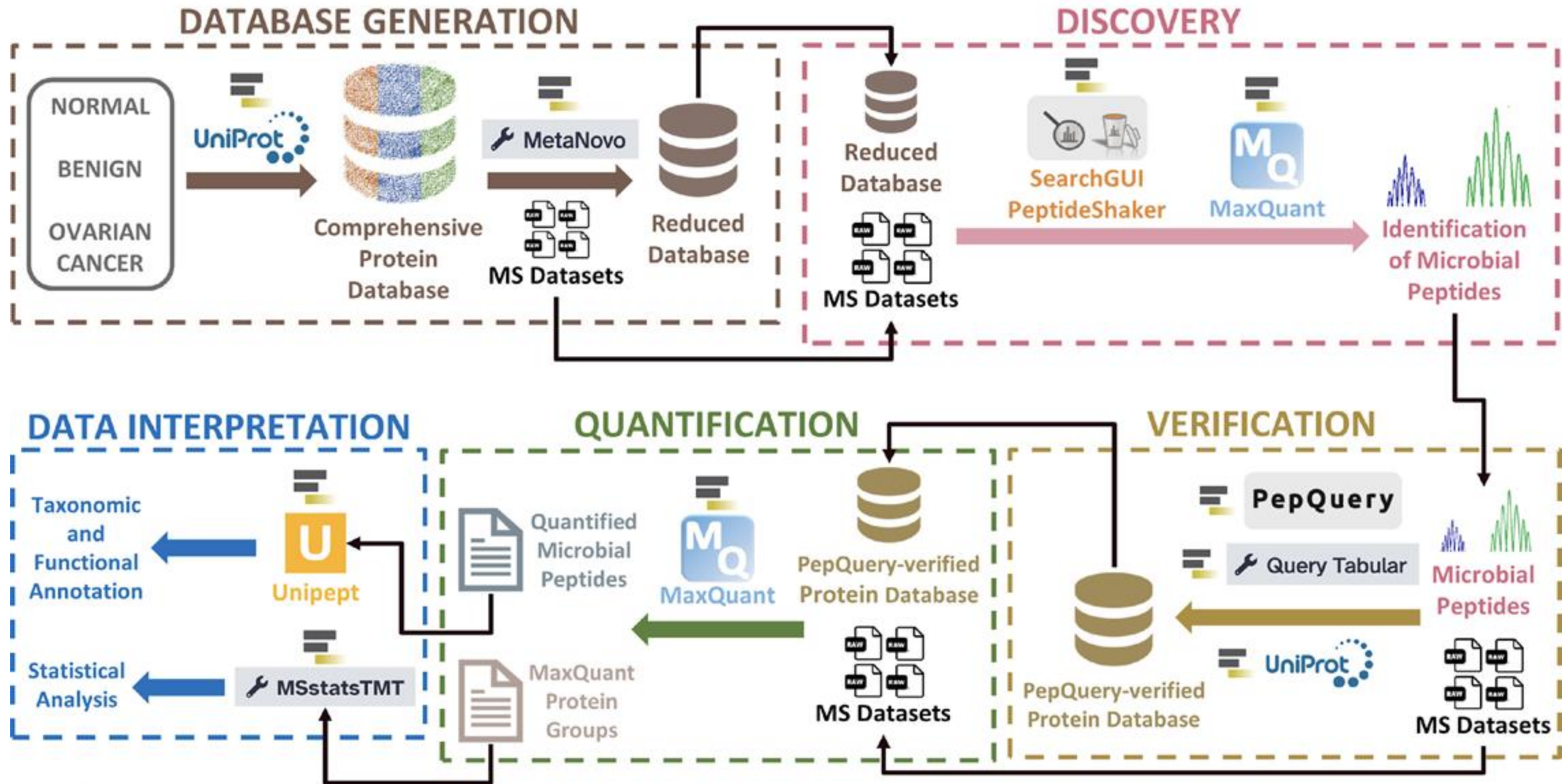
Do K et al (2024). A novel clinical metaproteomics workflow enables bioinformatic analysis of host-microbe dynamics in disease. mSphere <https://doi.org/10.1128/msphere.00793-23>



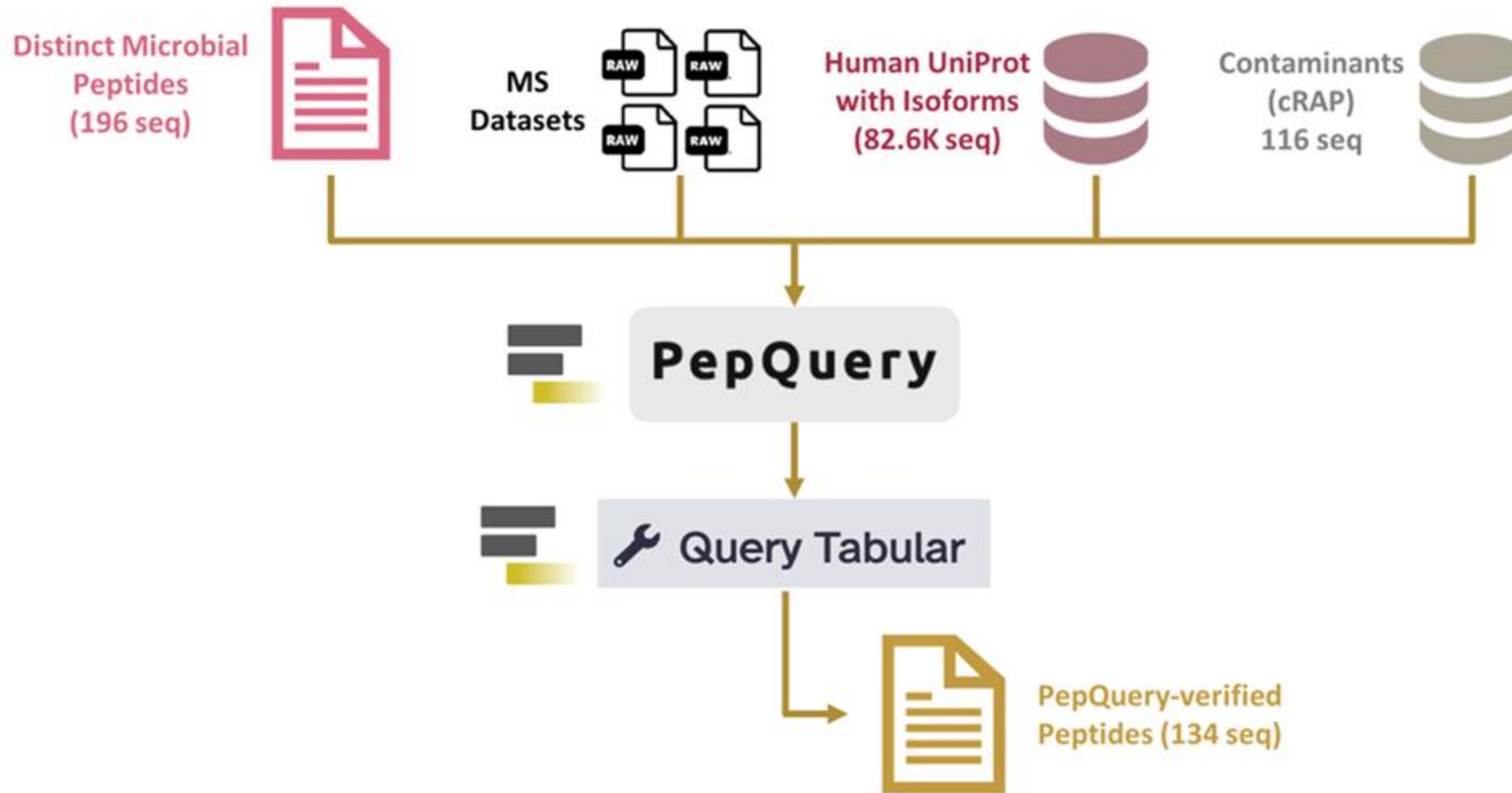
CLINICAL METAPROTEOMIC STUDIES DETECT LIMITED NUMBER OF MICROBIAL PEPTIDES



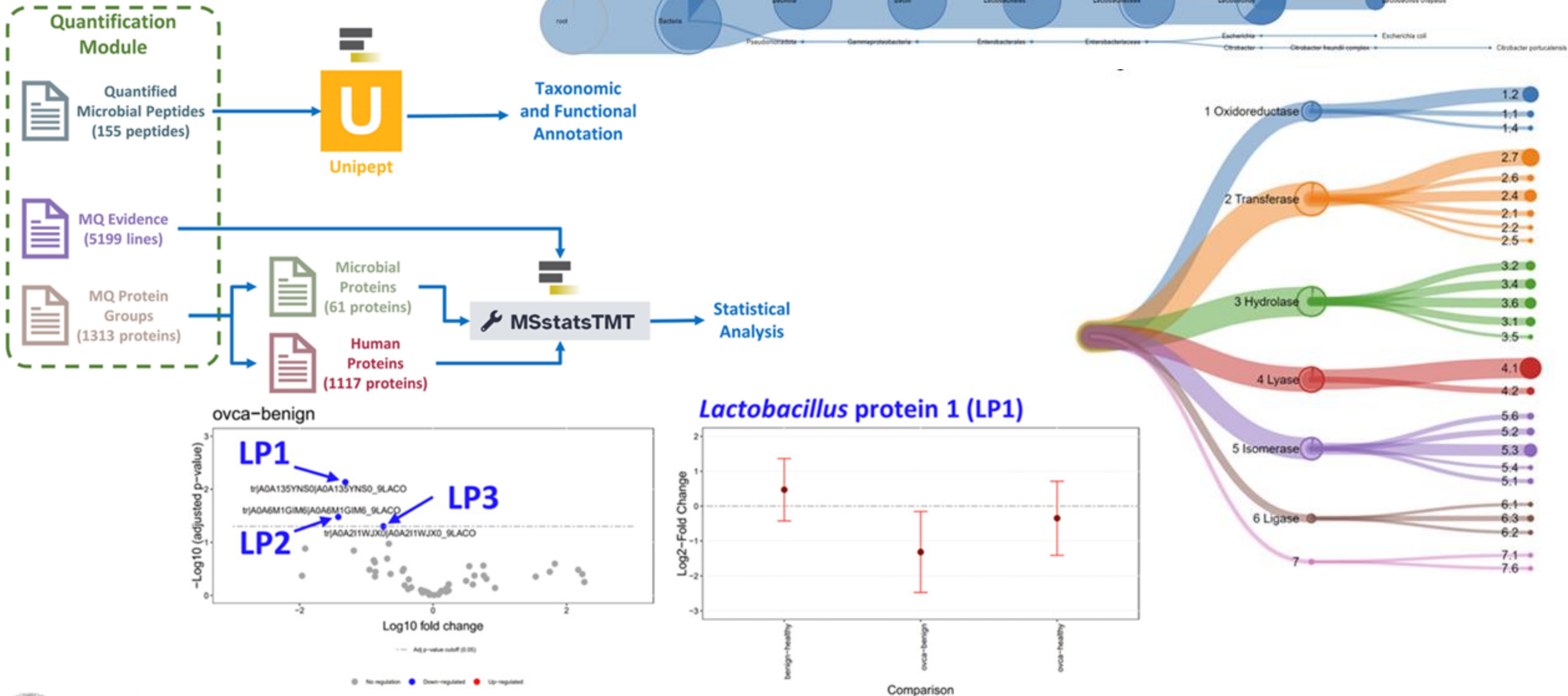
OVERVIEW OF CLINICAL METAPROTEOMICS WORKFLOW



VERIFICATION AND VERIFIED DATABASE GENERATION MODULE



DATA INTERPRETATION MODULE



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

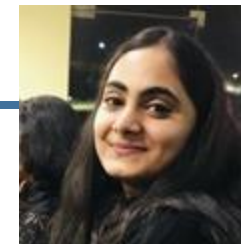


<https://galaxyproject.org/events/gcc2021/training/>

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

CLINICAL METAPROTEOMICS: COVID-19 PANDEMIC

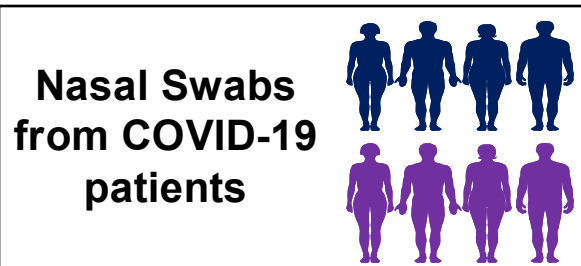
Bihani S *et al* (2023) Metaproteomic Analysis of Nasopharyngeal Swab Samples to Identify Microbial Peptides in COVID-19 Patients. J Proteome Res 22(8):2608-2619.
doi: 10.1021/acs.jproteome.3c00040.



Surbhi Bihani



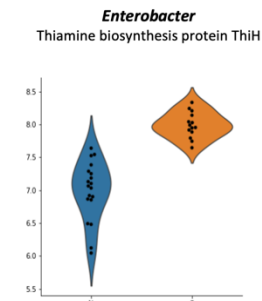
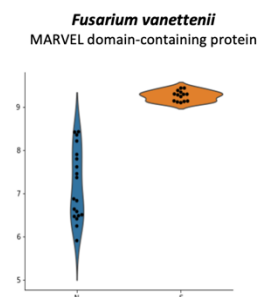
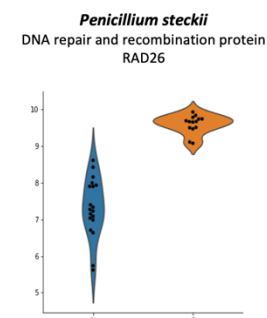
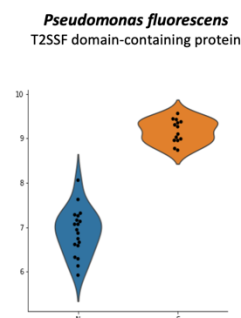
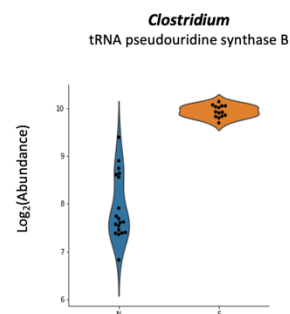
IIT BOMBAY



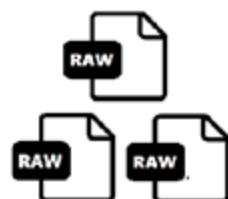
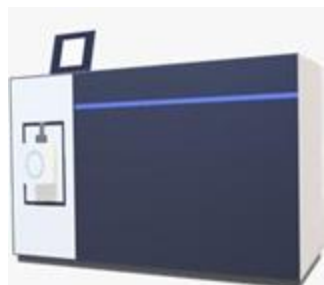
Sample
Collection



Sample
processing

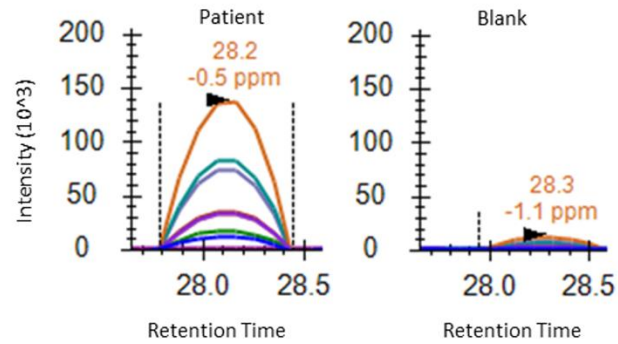


Data Acquisition



TARGETED ANALYSIS OF POTENTIAL PATHOGENS IN COVID PATIENTS

QGIQNIPVVSQTGK (*Streptococcus parasanguinis*)

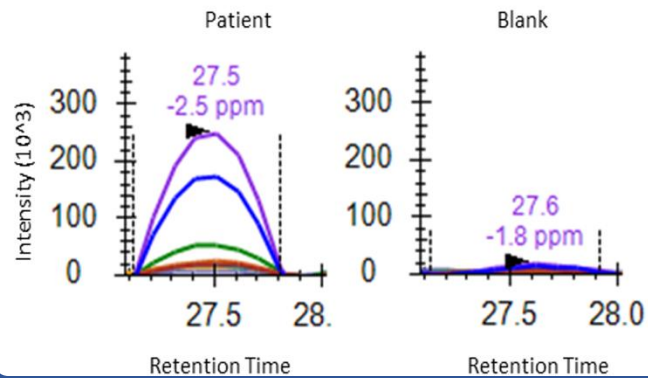


Streptococcus parasanguinis

Dominant isolate of dental plaque

Opportunistic pathogen associated with subacute endocarditis

GLFGDELCK (*Burkholderia cenocepacia*)

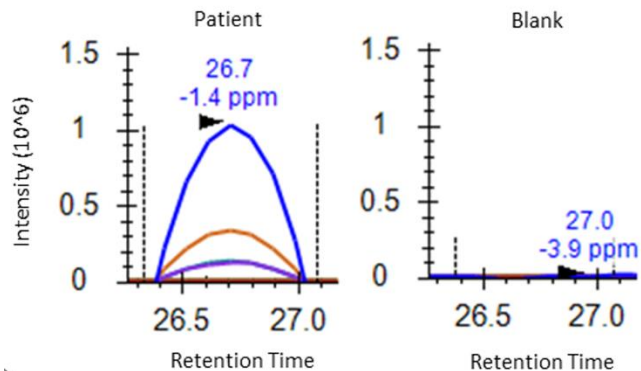


Burkholderia cenocepacia

Opportunistic pathogen

Cause systemic infections in immunocompromised individuals including cystic fibrosis patients.

IVDQEGAIVPK (*Cryptococcus floricola*)

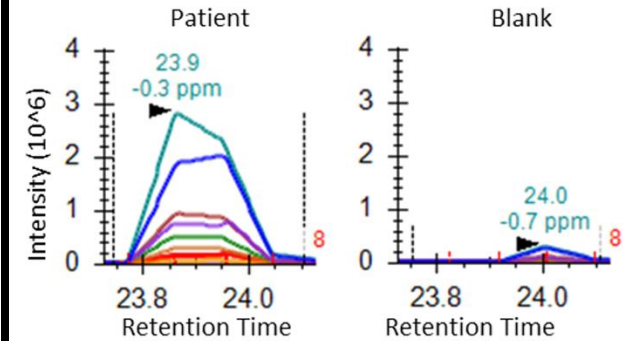


Cryptococcus floricola

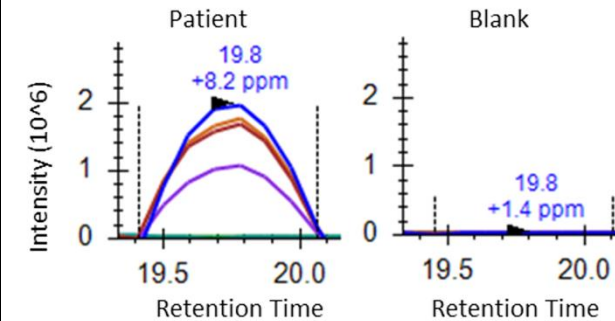
Infect immunocompromised hosts

Infection initiates in the lungs.

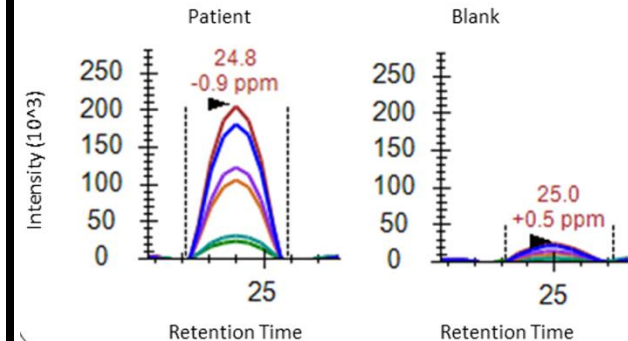
ICVLHGDK (*Clostridium sp.*)



DETDDQFEK (*Staphylococcus sp.*)



TPTLVSDLR (*Pseudomonas sp.*)



CLINICAL METAPROTEOMICS: CYSTIC FIBROSIS

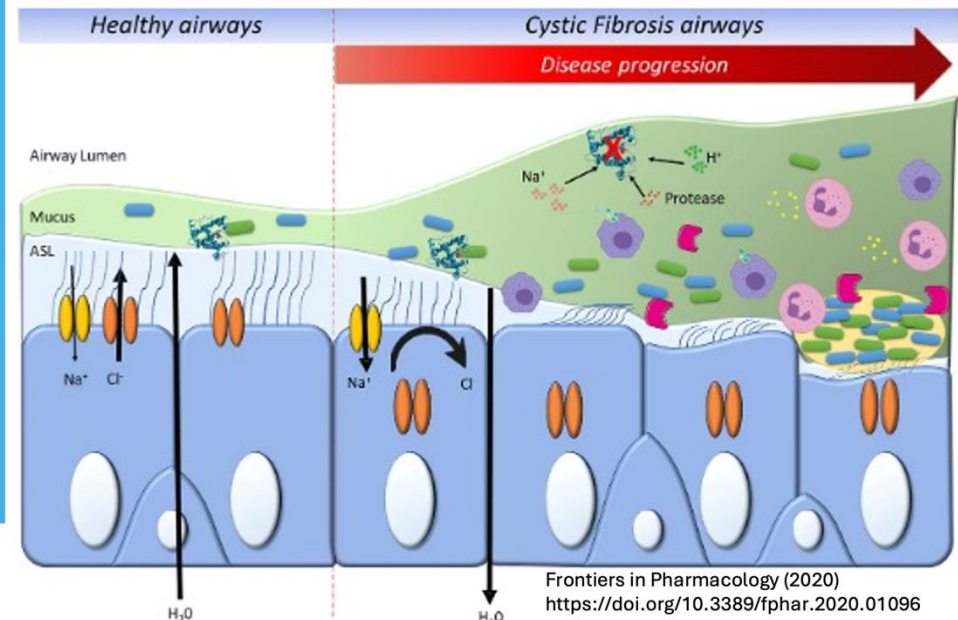
Kruk M *et al* (2024) An integrated metaproteomics workflow for studying host-microbe dynamics in bronchoalveolar lavage samples applied to cystic fibrosis disease. mSystems (<https://doi.org/10.1128/msystems.00929-23>).

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.



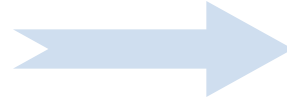
CYSTIC FIBROSIS DATASETS



BALF from
Cystic Fibrosis
or
Disease Control
Samples



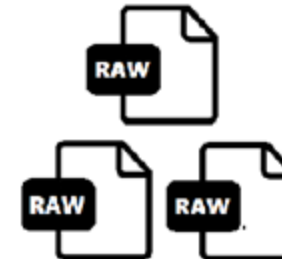
Sample
Collection



Sample
processing

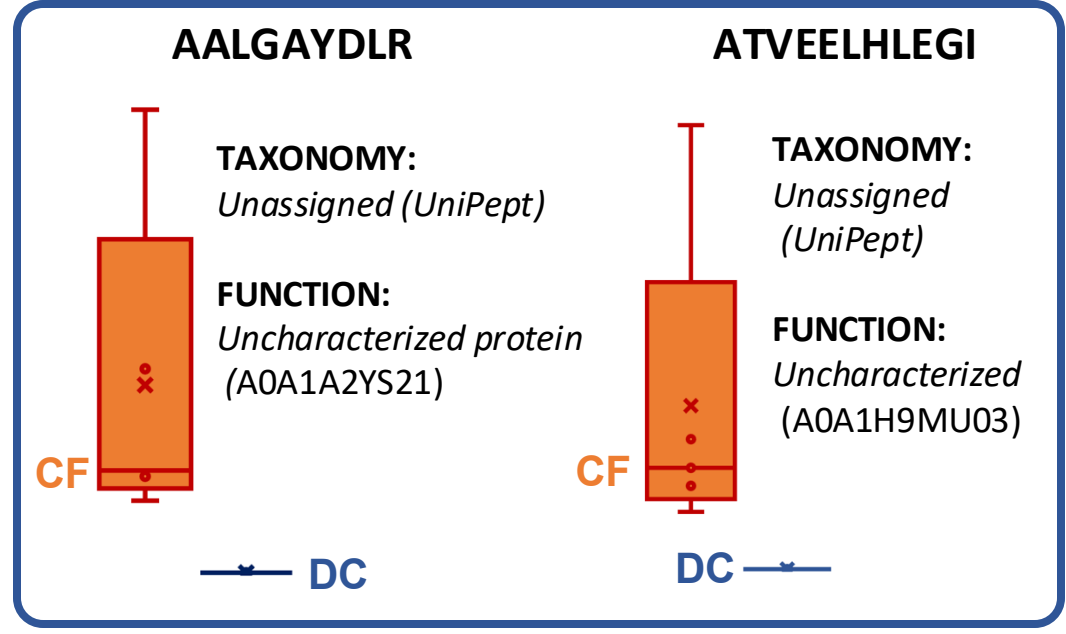
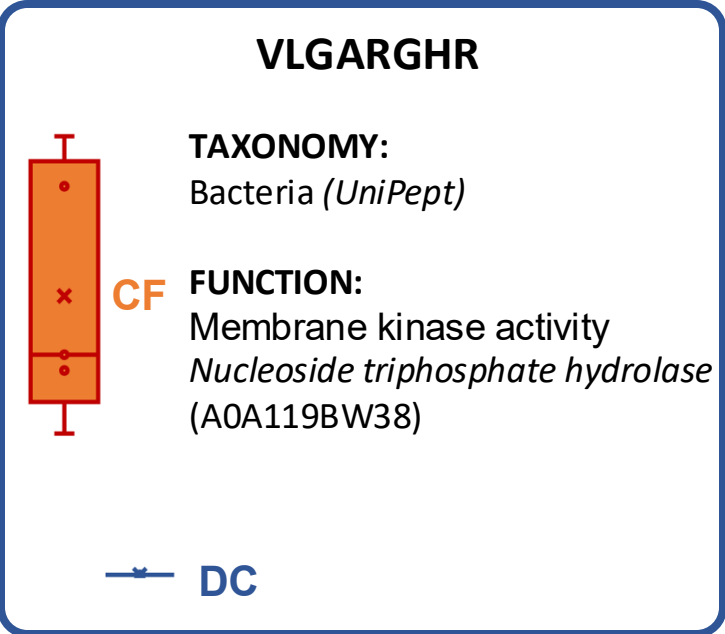
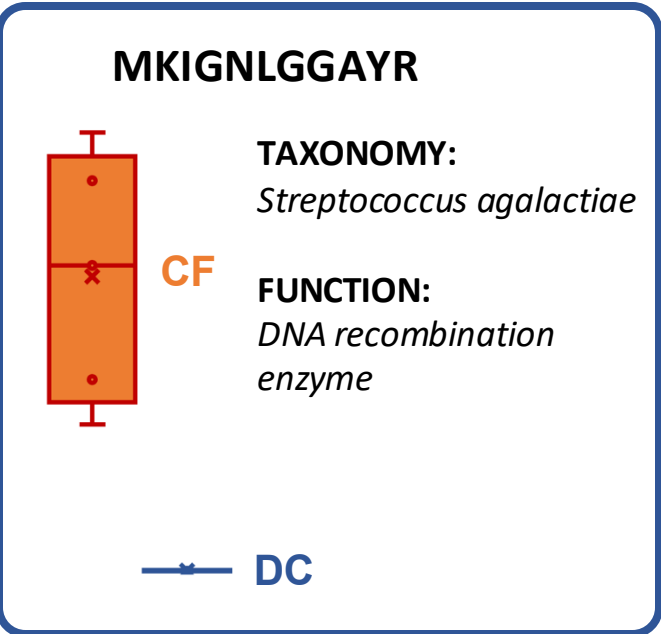
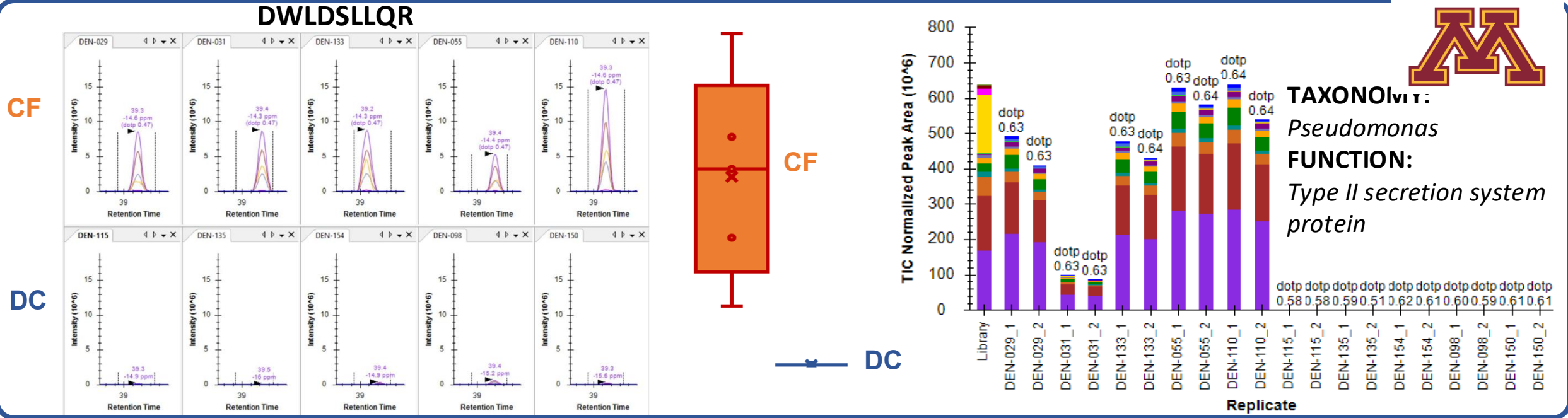


Data Acquisition

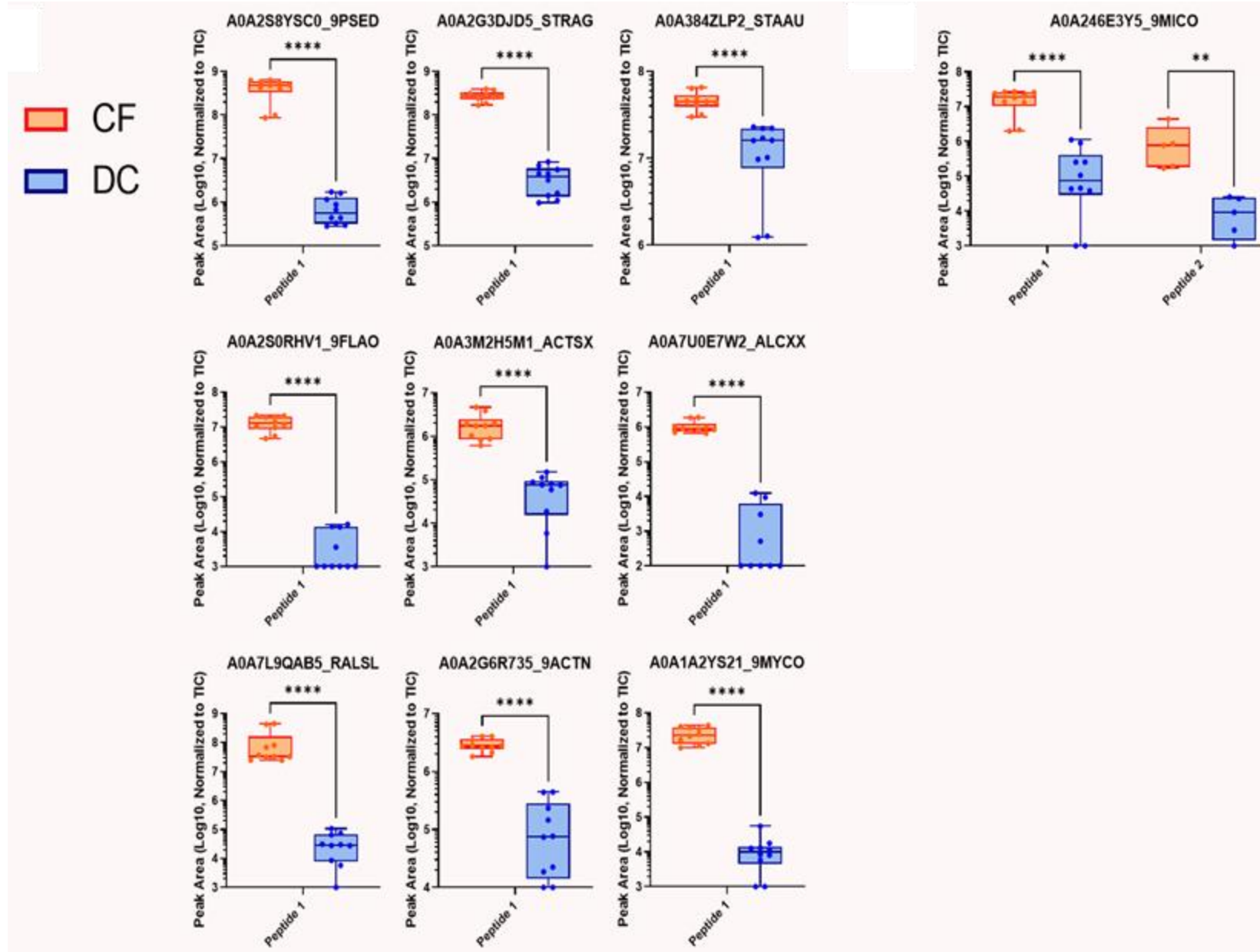


Monica E. Kruk

MICROBIAL PEPTIDES FROM CYSTIC FIBROSIS PATIENTS



MICROBIAL PEPTIDES FROM CYSTIC FIBROSIS PATIENTS

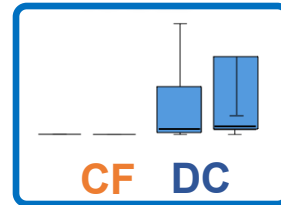


TARGETED ASSAY FOR HOST-MICROBIAL PROTEIN DYNAMICS IN CLINICAL CF SAMPLES

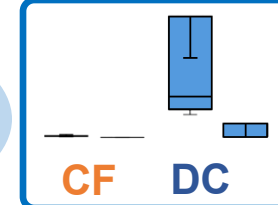


- Lipoprotein particle remodeling
- Cholesterol transport
- Phospholipid efflux.

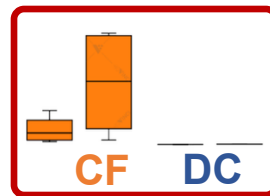
APOC2



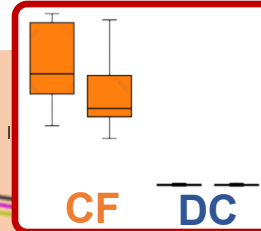
APOE



S100A9

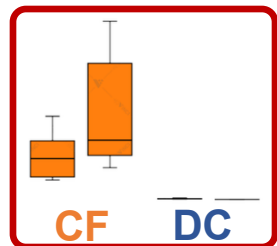


MPO

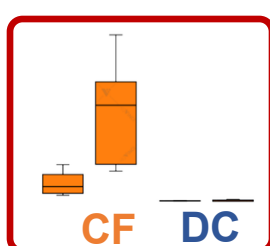


- Inflammatory response
- Leukocyte migration
- Neutrophil degranulation
- Cytokine production
- Defense response to bacteria

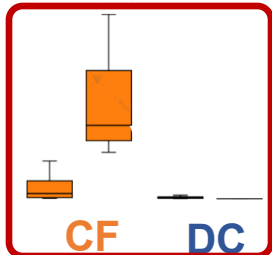
MMP8



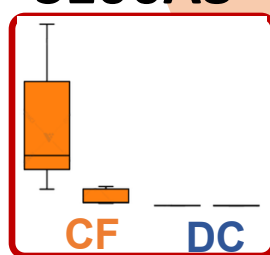
FGR



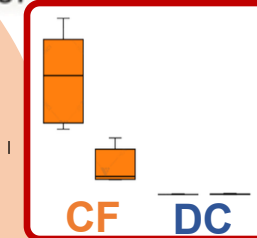
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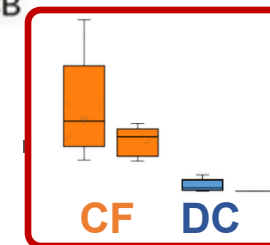
S100A8



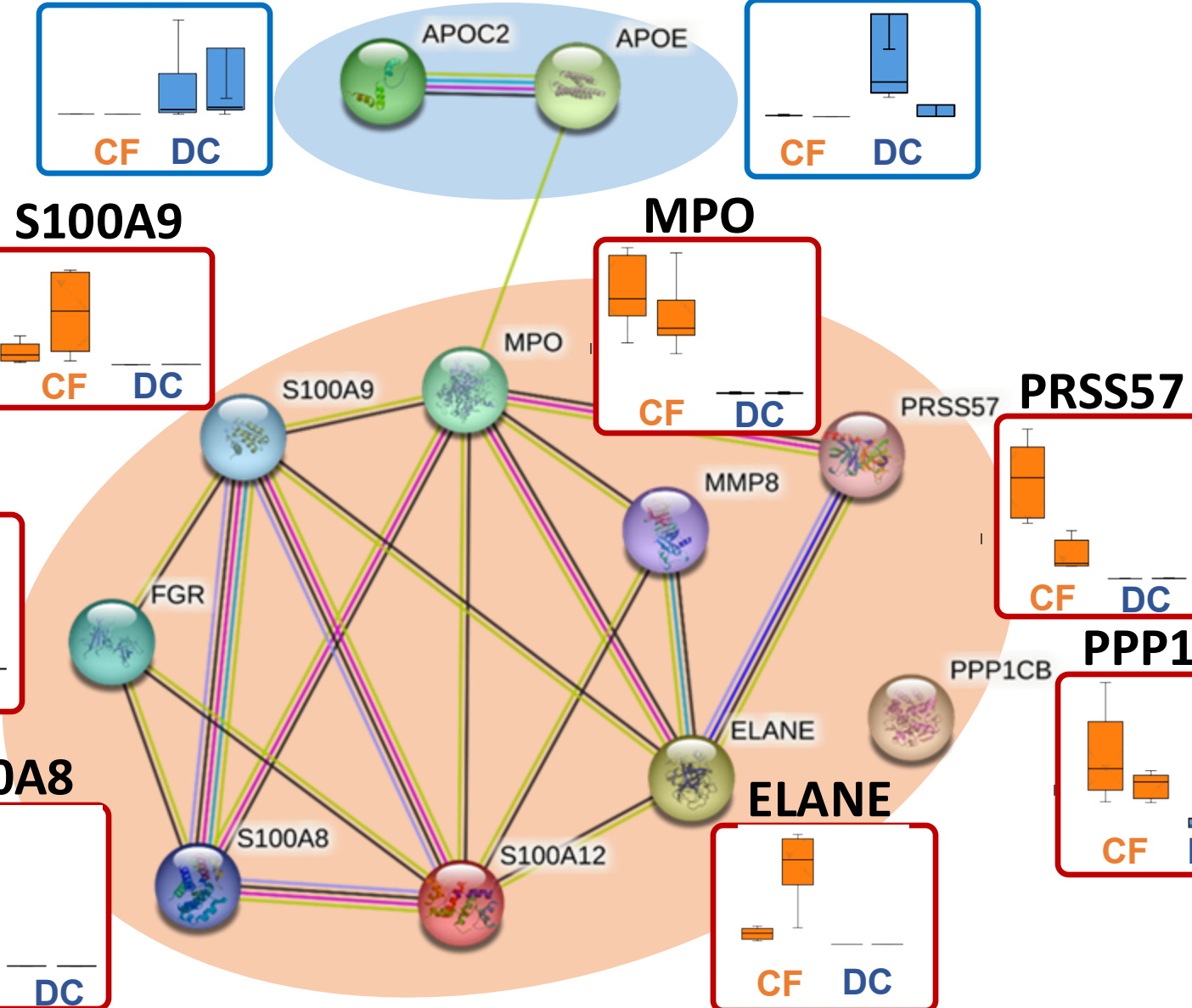
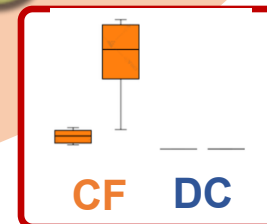
PRSS57



PPP1CB



ELANE



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 co-infection during the COVID-19 pandemic.
- The workflow was also used to generate a promising microbial and host peptide panel for application to CF disease progression studies by comparing it with disease control (DC).
- We intend to use this workflow in other clinical metaproteomic studies to detect differentially expressed host and microbial proteins in disease state.

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

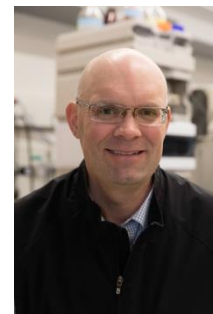
Pratik Jagtap¹, Ruben Shrestha², Beverly Wuertz³, Monica Kruk⁴, Subina Mehta¹, Alvaro Sebastian Vaca Jacome², Matthew Willetts⁴, Frank Ondrey³, Timothy Griffin¹

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

²Bruker Scientific LLC, San Jose, CA;

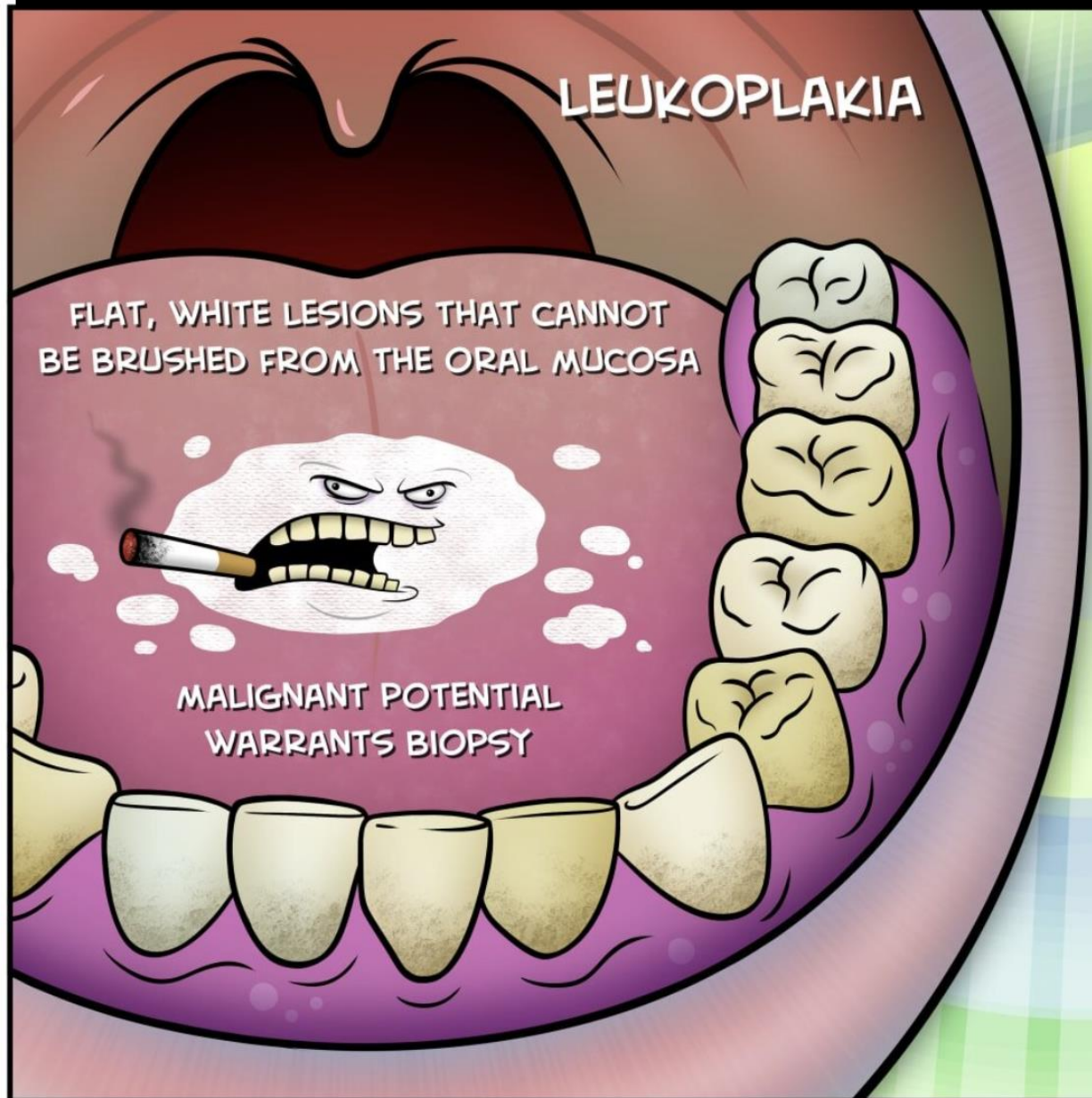
³Otolaryngology Department, University of Minnesota, Minneapolis, Minnesota;

⁴Bruker Scientific, LLC, Billerica, MA



WORK NOT PUBLISHED YET

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

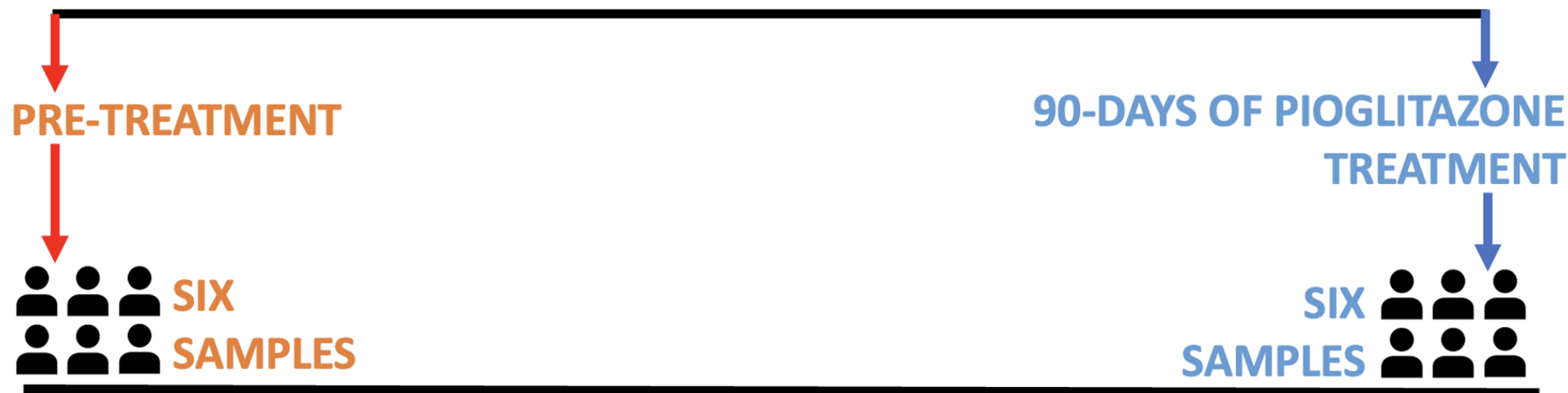


- * POOR ORAL HEALTH
- * LONG-TERM TRAUMA to ORAL CAVITY
- * ADVANCED AGE
- * HPV INFECTION



EXPERIMENTAL WORKFLOW

ORAL LEUKOPLAKIA



ORAL RINSE SAMPLES

Enrichment

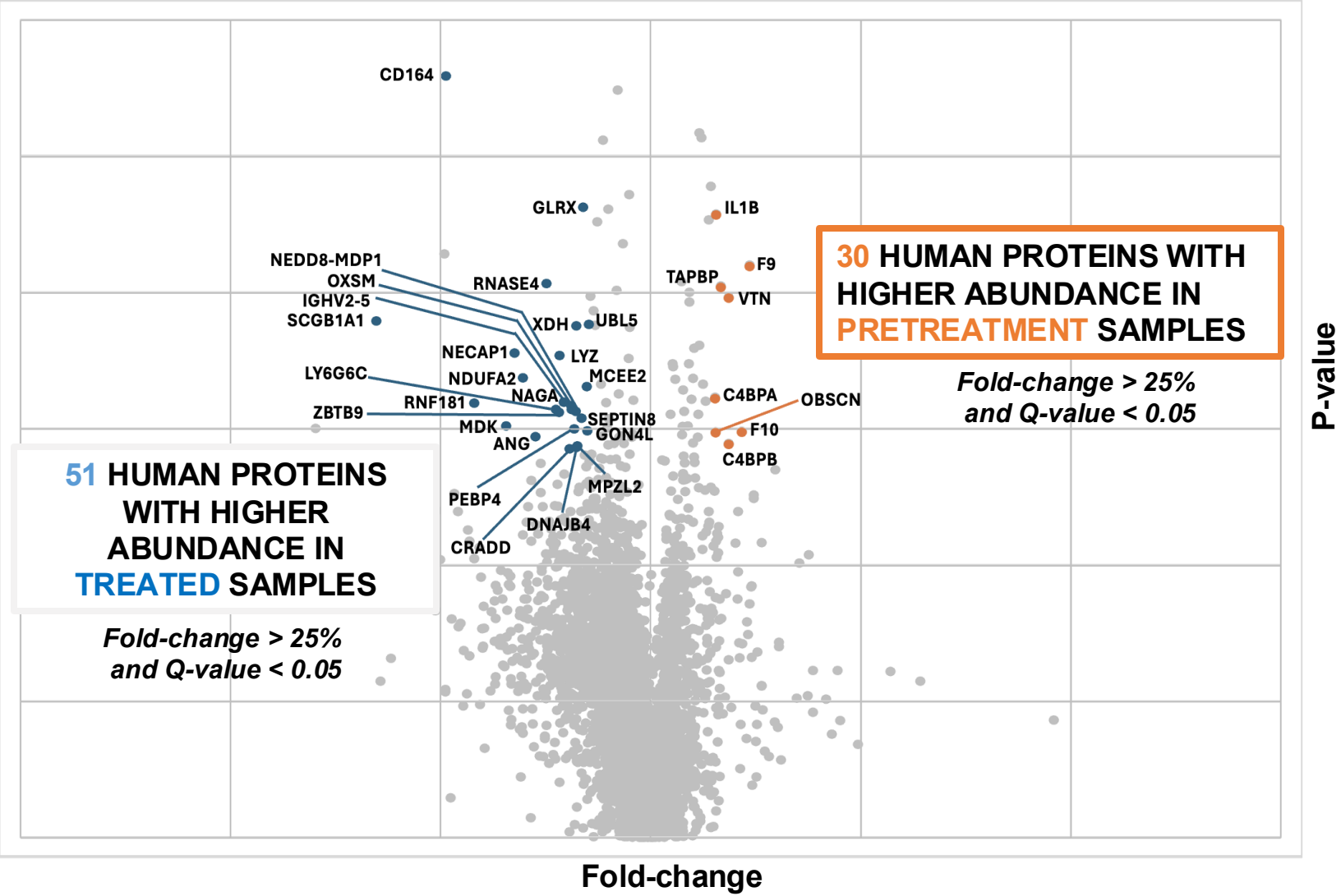
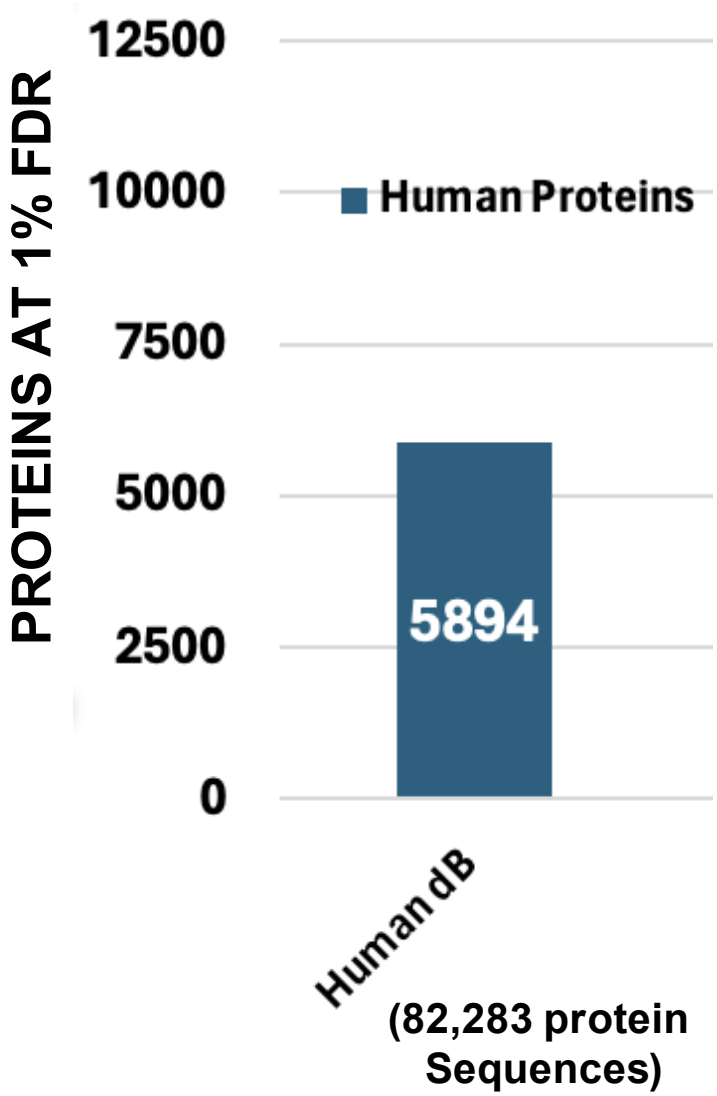
Mass spectrometry using DIA-PASEF

Search against Human + Microbiome Protein sequences dB

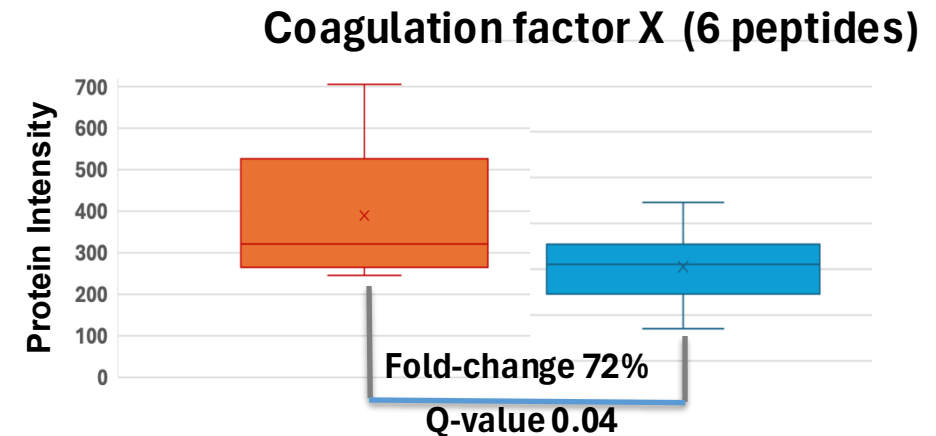
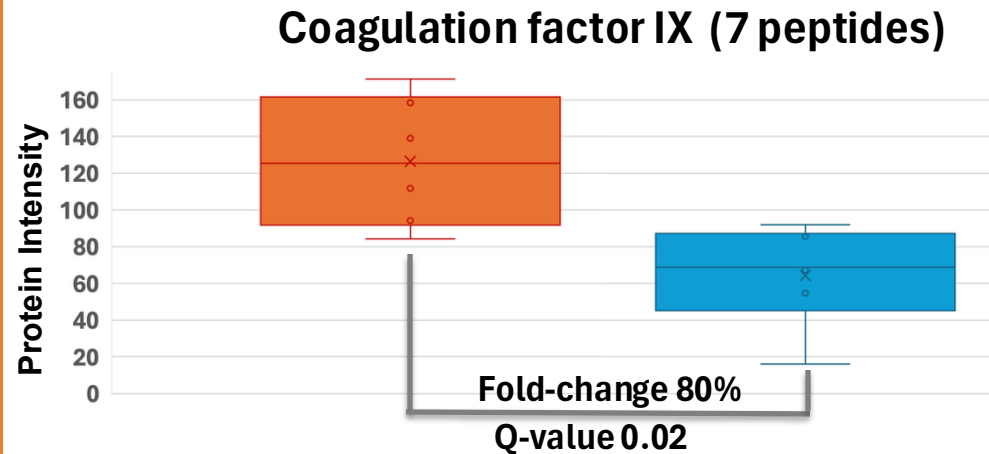
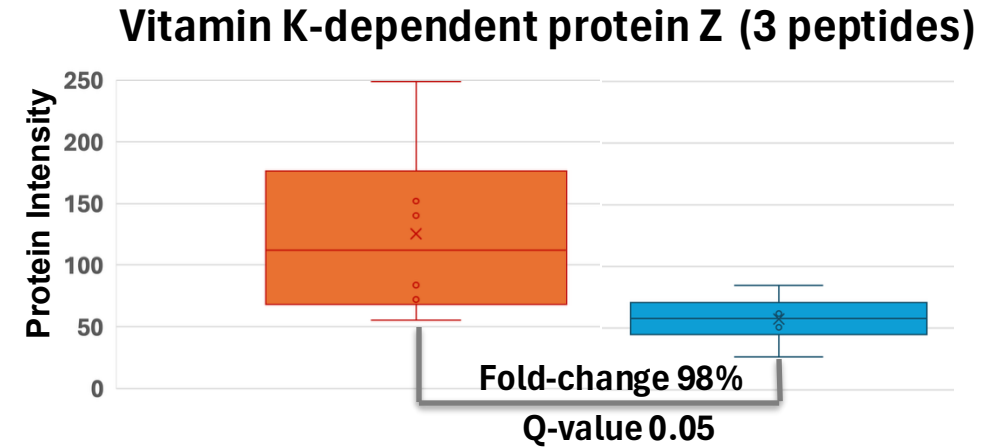
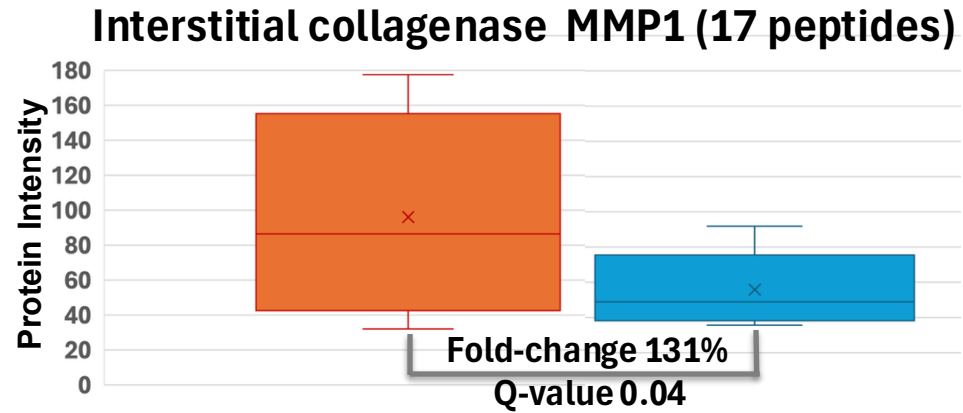
PO



PROTEINS DETECTED AND DIFFERENTIALLY ABUNDANT PROTEINS



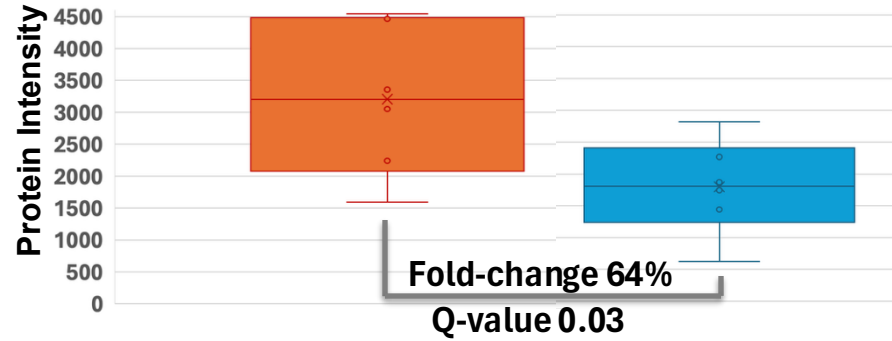
MMP1 & COAGULATION CASCADE: DOWNREGULATED AFTER TREATMENT



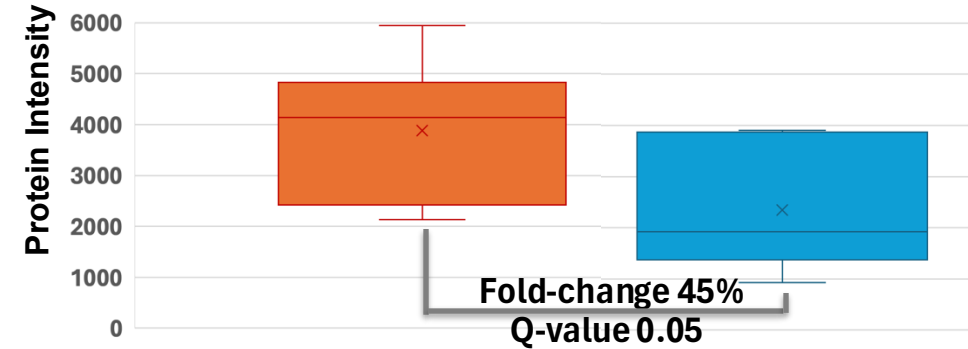
Coagulation Cascade

COMPLEMENT CASCADE: DOWNREGULATED AFTER TREATMENT

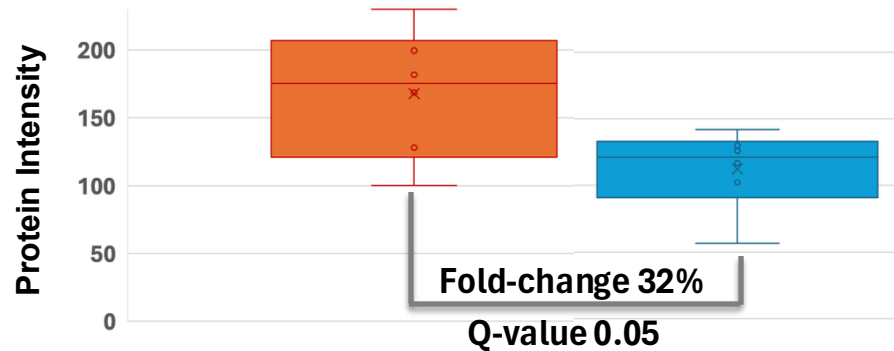
VITRONECTIN (12 peptides)



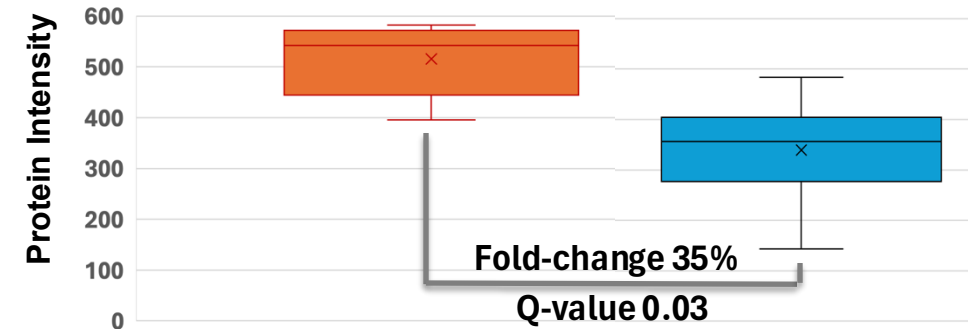
C4b-binding protein alpha chain (18 peptides)



Carboxypeptidase B2 (9 peptides)



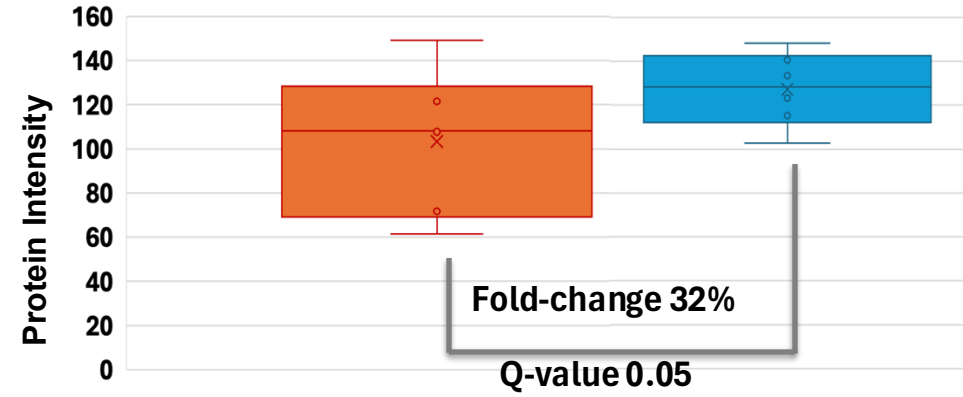
Complement component C8 beta chain (18 peptides)



Regulation of Complement cascade

APOPTOSIS: UPREGULATED AFTER TREATMENT

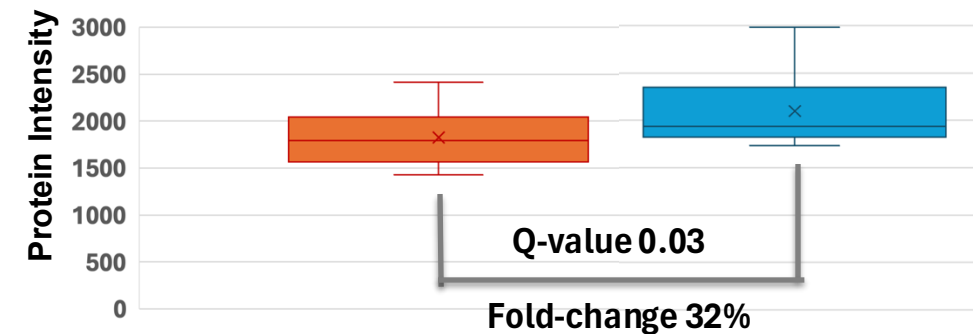
DESMOGLEIN-2 (16 peptides)



- 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.

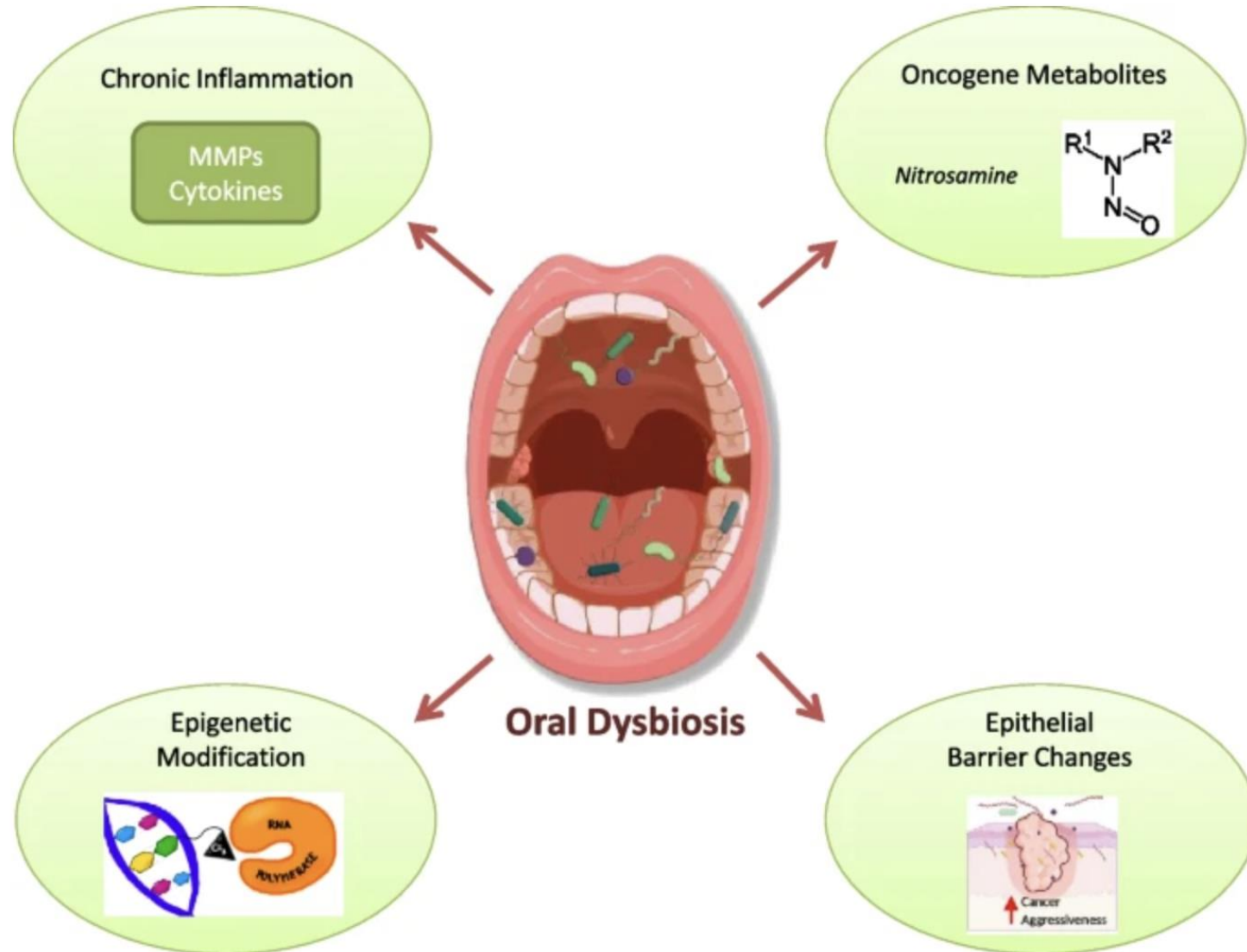
- An adaptor protein that is composed of two protein-protein interaction domains
- Functions as key mediator in apoptosis and inflammation via the activation of caspases.
- Prognostic marker in renal cancer

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

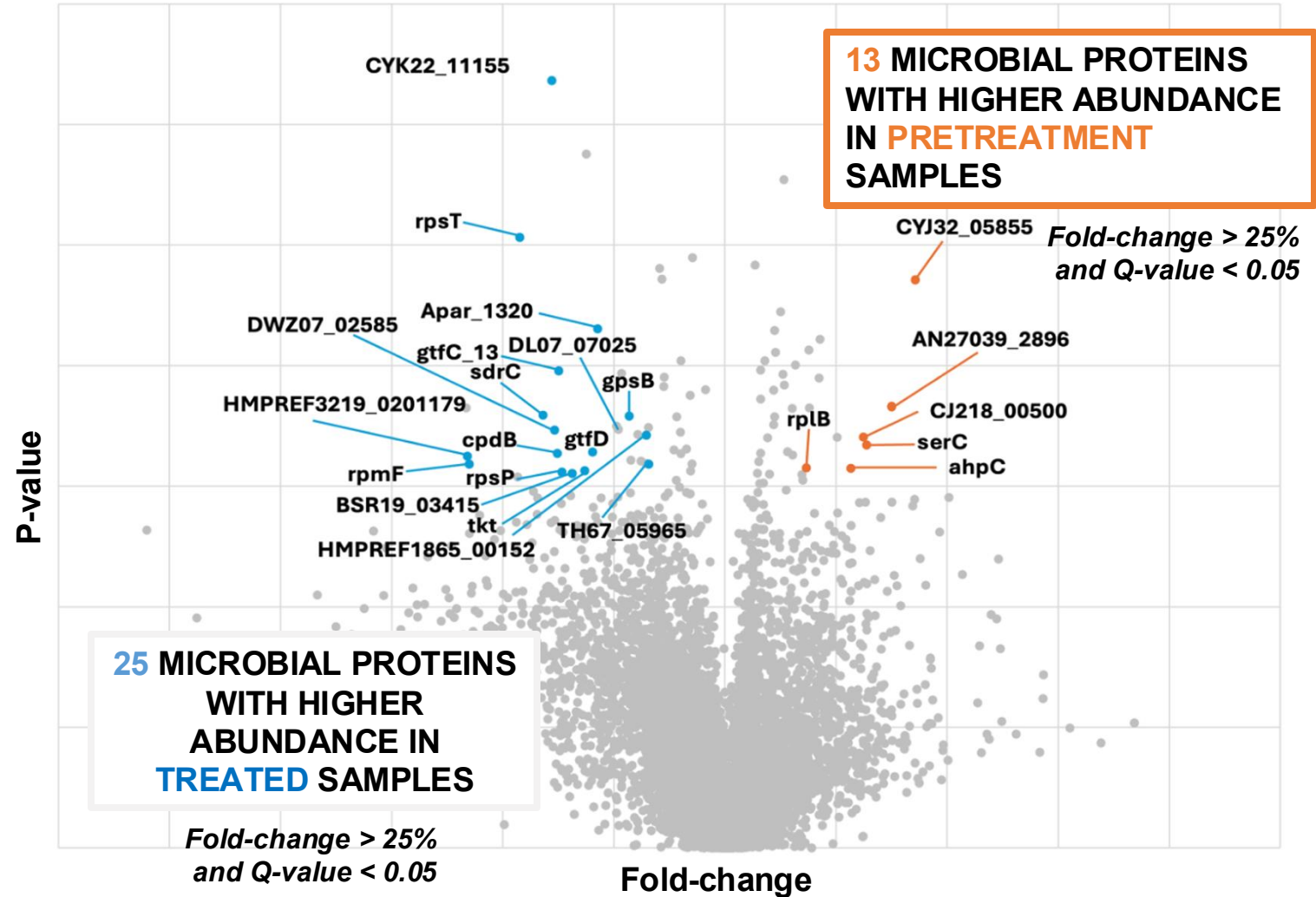
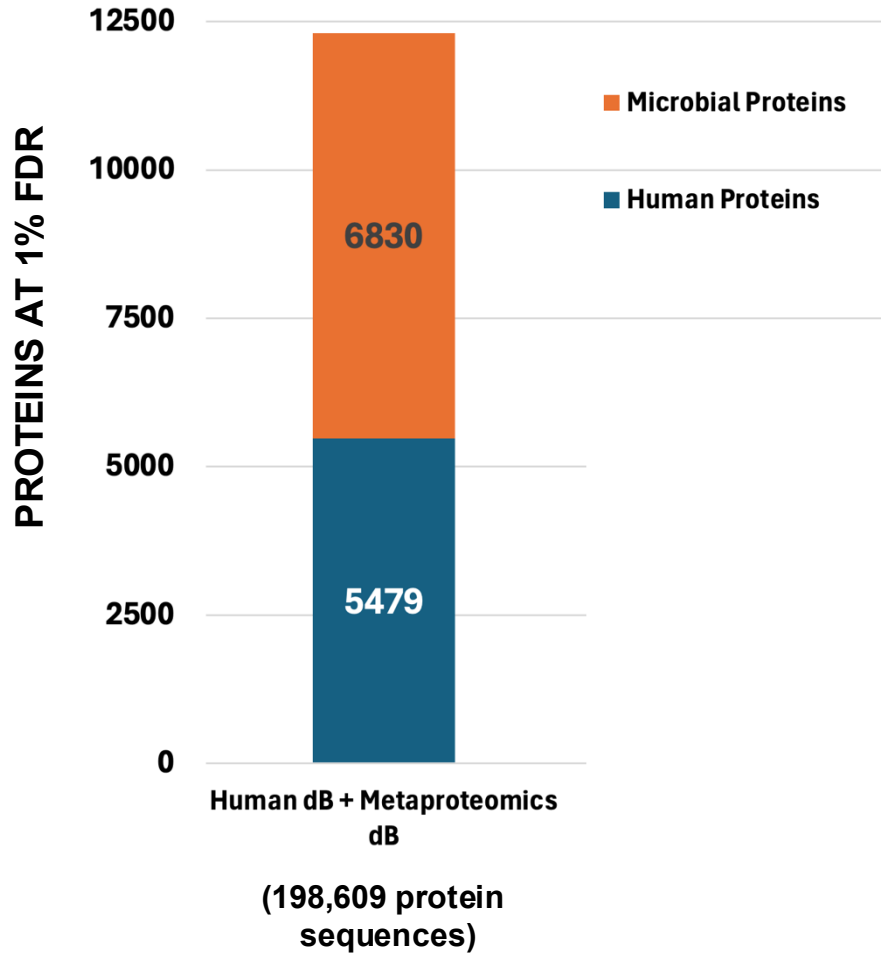


ROLE OF BACTERIA IN ORAL CANCER

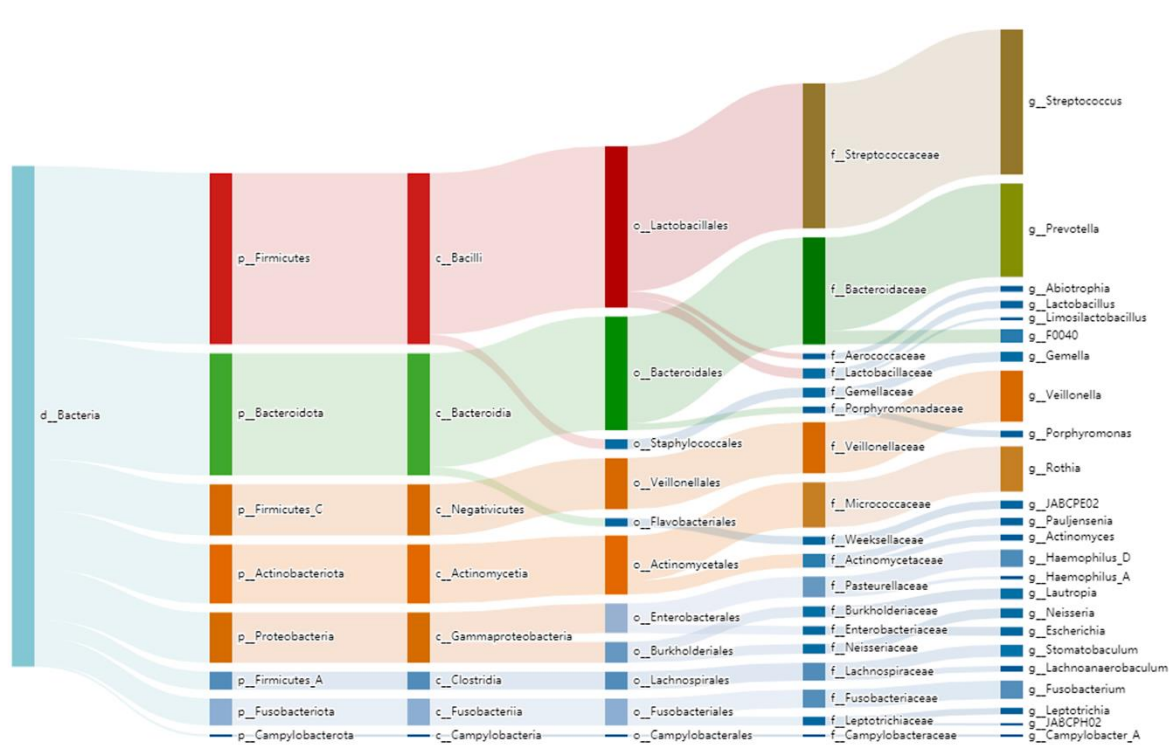
DEVELOPMENT



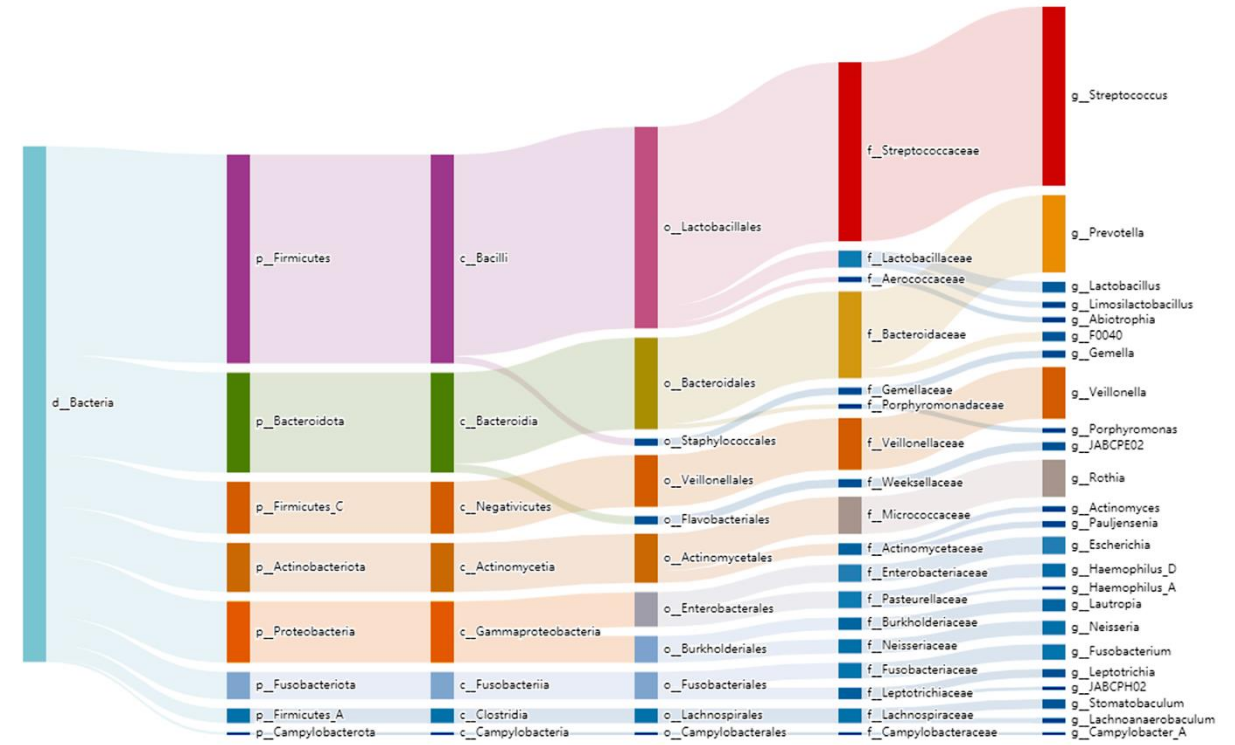
MICROBIAL DATABASE SEARCH RESULTS



MICROBIAL TAXONOMY OUTPUTS



Genera detected in pretreated samples



Genera detected in treatment samples



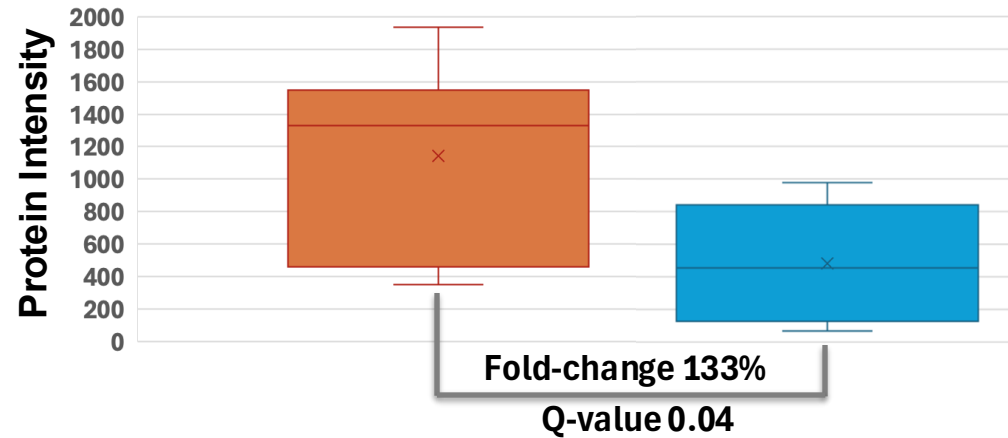
MetaLab and



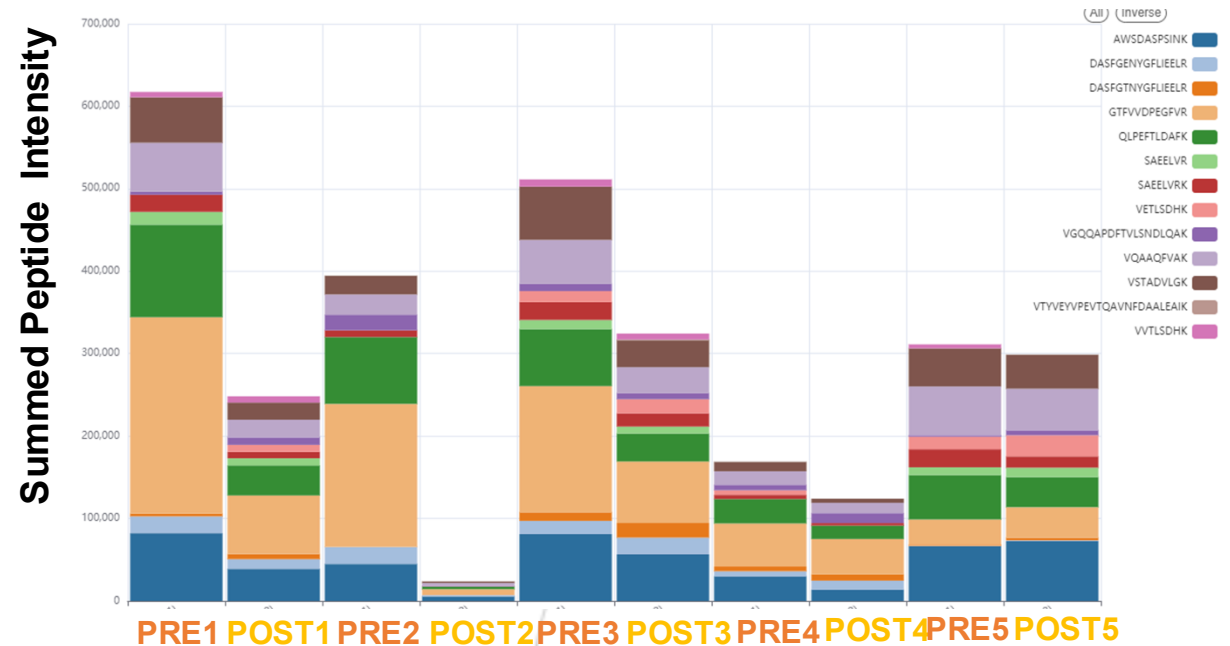
analysis by Kai Cheng, Qing Wu (Daniel Figeys Lab, University of Ottawa)

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 and

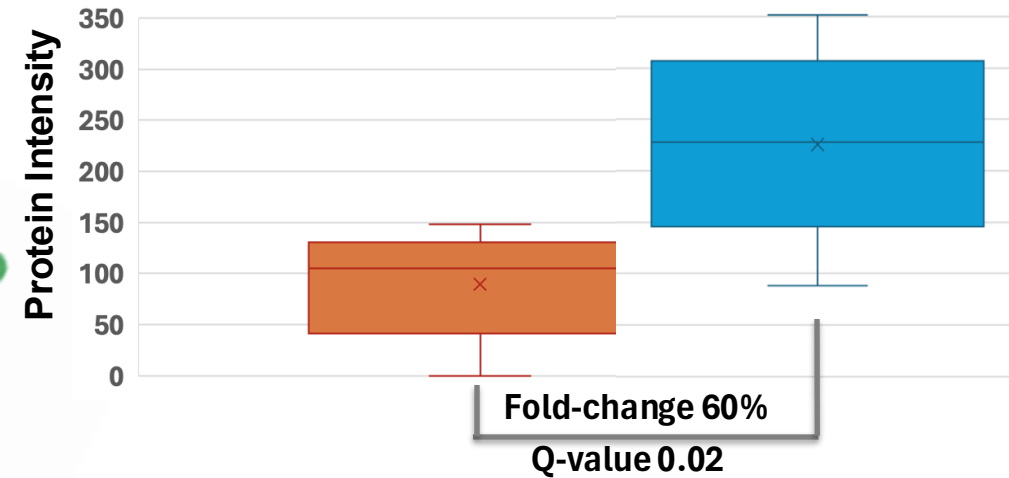


analysis by Kai Cheng, Qing Wu (Daniel Figeys Lab, University of Ottawa)

MICROBIAL PROTEINS UPREGULATED AFTER TREATMENT

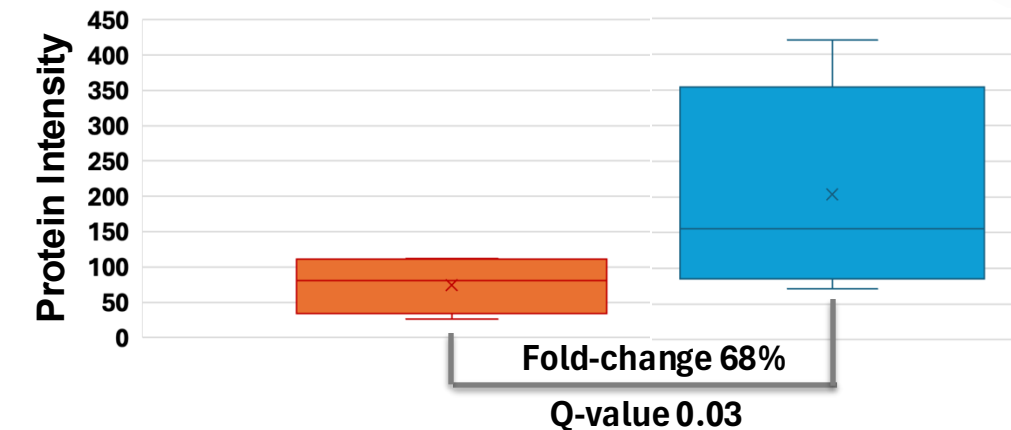
- Glucosyltransferase that catalyzes the transfer of glucosyl residues to dextran polymer.
- Involved in biofilm formation.

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.

PEPTIDES FOR TARGETED ANALYSIS

HUMAN PROTEINS	AFTER TREATMENT	
	UP-REGULATED	DOWN-REGULATED
	Peptides (for targeted analysis)	Peptides (for targeted analysis)
Desmoglein-2	16 (3)	
Apoptosis-associated speck-like protein containing a CARD	13 (6)	
Chitinase-3-like protein 2	14 (5)	
Cathepsin D	18 (10)	
Lymphocyte antigen 6 complex locus protein G6c	2 (2)	
Lysozyme C	8 (7)	
Alpha-N-acetylgalactosaminidase	5 (3)	
Receptor-type tyrosine-protein phosphatase S	21 (6)	
Interstitial collagenase MMP1		17 (1)
Coagulation factor IX		7 (2)
Coagulation factor X		6 (2)
Vitronectin		12 (4)
C4b-binding protein alpha chain		18 (4)
Carboxypeptidase B2		9 (3)
Complement component C8 beta chain		6 (4)
MICROBIAL PROTEINS		
Dextranucrase	61 (3)	
Serine-type D-Ala-D-Ala carboxypeptidase	21 (2)	
Glutamate--ammonia ligase	7 (2)	
Alkyl hydroperoxide reductase C		11 (5)

ORAL CANCER DATASET: CONCLUSIONS AND FUTURE WORK

- Several human, microbial 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 proteins will be used for targeted analysis.

CLINICAL METAPROTEOMICS WORKFLOW TO STUDY HOST-MICROBIOME DYNAMICS

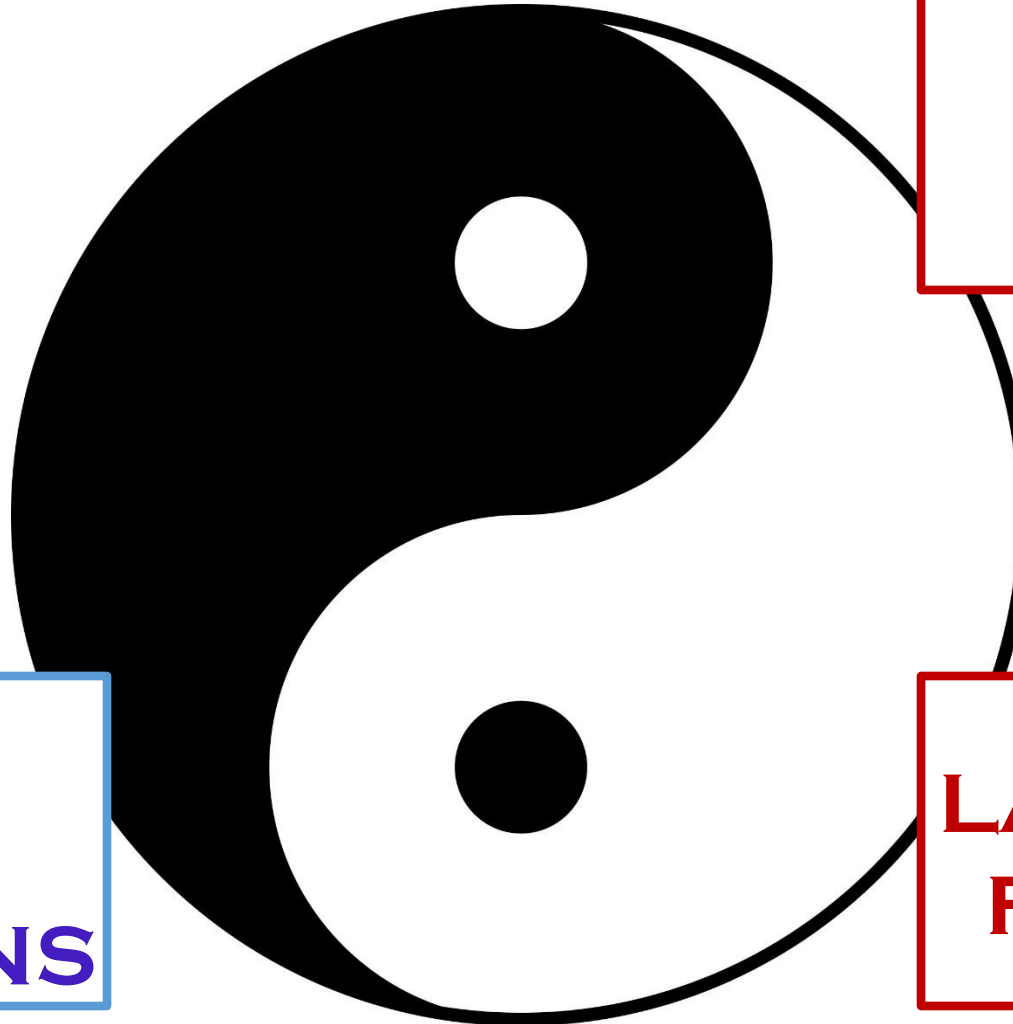
- *MICROBIOME RESEARCH*
- *MASS SPECTROMETRY DATA ANALYSIS*
- *METAPROTEOMICS RESEARCH*
- *GALAXY BIOINFORMATICS PLATFORM*
- *CLINICAL METAPROTEOMICS WORKFLOW*
- *CLINICAL METAPROTEOMICS: COVID-19 PANDEMIC*
- *CLINICAL METAPROTEOMICS: CYSTIC FIBROSIS*
- *CLINICAL METAPROTEOMICS: ORAL CANCER*
- ***METAPROTEOMICS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

JUNE 2023
ASMS @HOUSTON

GALAXY TRAINING NETWORK

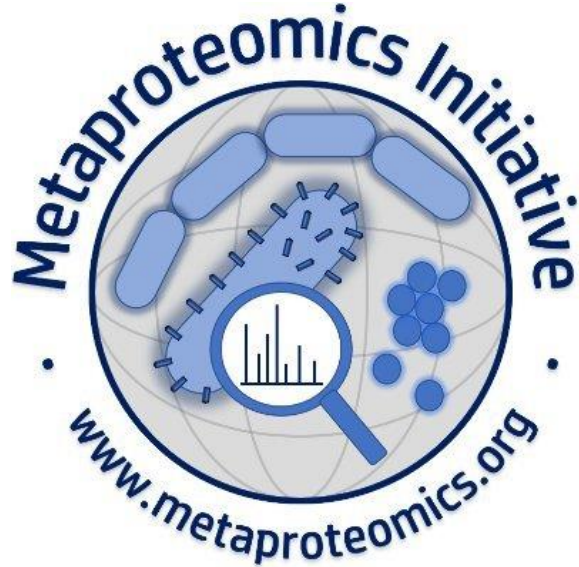


<https://galaxyproject.org/events/gcc2021/training/>

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

The Galaxy-P team seeks to continue integrating promising new software tools and workflows from leading laboratories into the Galaxy platform and encourages researchers to share their interests so that newer bioinformatics workflows can be made accessible via this resource.

EDUCATION PORTAL @ METAPROTEOMICS INITIATIVE



<https://metaproteomics.org/education/>

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ACKNOWLEDGMENTS

University of Minnesota

Timothy Griffin
Katherine Do
Subina Mehta
Monica Kruk
Andrew Rajczewski



Minnesota Supercomputing Institute
Reid Wagner

Brian Searle
Ohio State University

Magnus Øverlie Arntzen
NMBU, Oslo, Norway

Anshu Bharadwaj
*ImTech, Chandigarh
India*

Funding



Paul Piehowski
PNNL

Brook Nunn
U of Washington

Saskia Hiltmann

Magnus Palmblad
*Leiden,
Netherlands*

Thilo Muth
*Robert Koch
Institut, Germany*

Mak Saito
Woods Hole, MA

Jean Armengaud
*CEA Marcoule,
France*

Alessandro Tanca
*Porto Conte
Ricerche, Italy*

Sanjeeva Srivastava
*IIT, Mumbai
India*

Michael Shortreed
UW-Madison

Jeremy Fisher
Indiana University

Valdemir Carvalho
Fleury Group, Brazil

**Björn Gruening
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QUESTIONS?