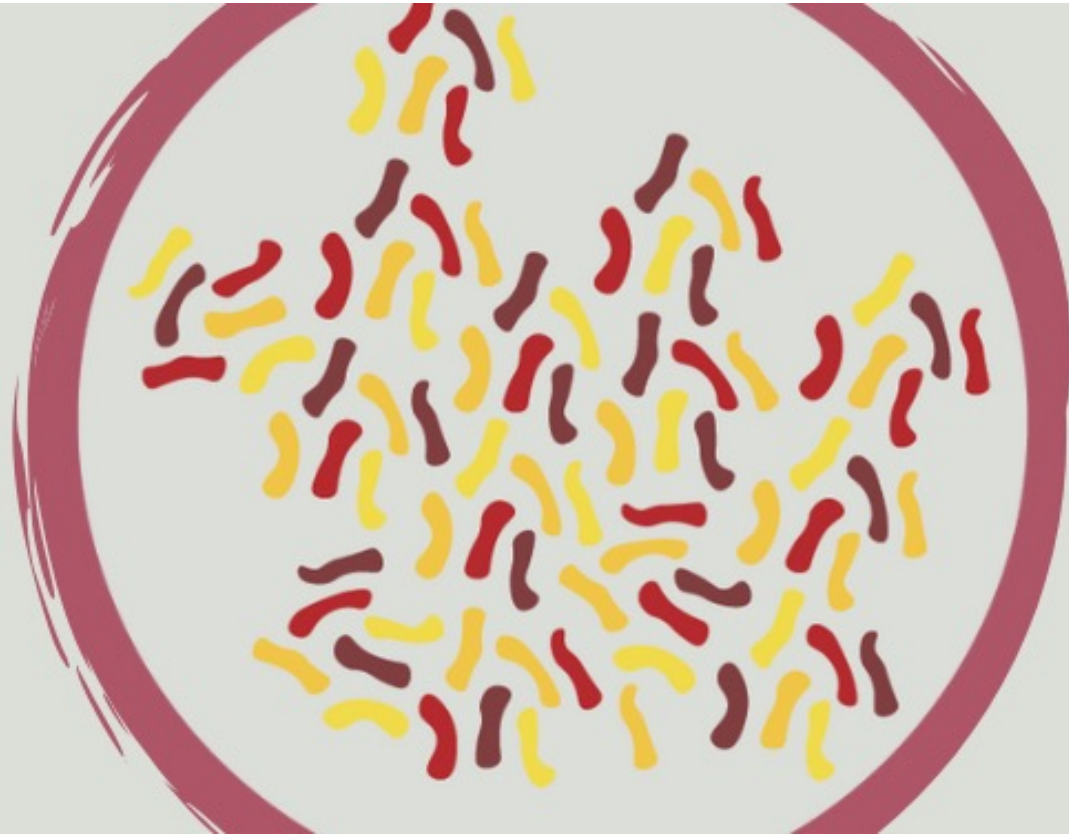


ENRICHMENT OF MICROBIAL PROTEINS FOR MASS SPECTROMETRY-BASED CLINICAL METAPROTEOMICS STUDIES



Microbiome and Cancer Symposium

2022 SEED FUNDS RECAP

Beverly Wuertz, Monica Kruk, Tim Griffin, Frank Ondrey and Pratik Jagtap

MICROBIOME

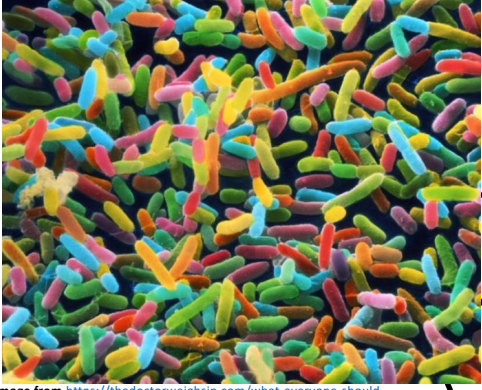
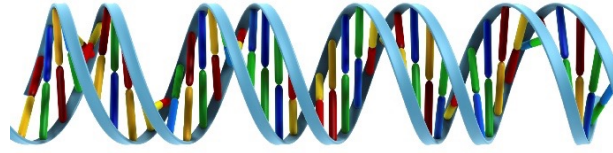


Image from <https://thedoctoryeighsin.com/what-everyone-should-know-about-the-infant-microbiome/>

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



METAGENOMICS

TAXONOMY

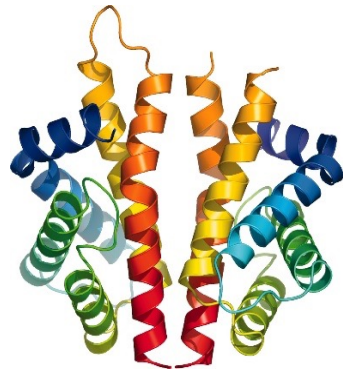
function



METATRANSCRIPTOMICS

TAXONOMY

function



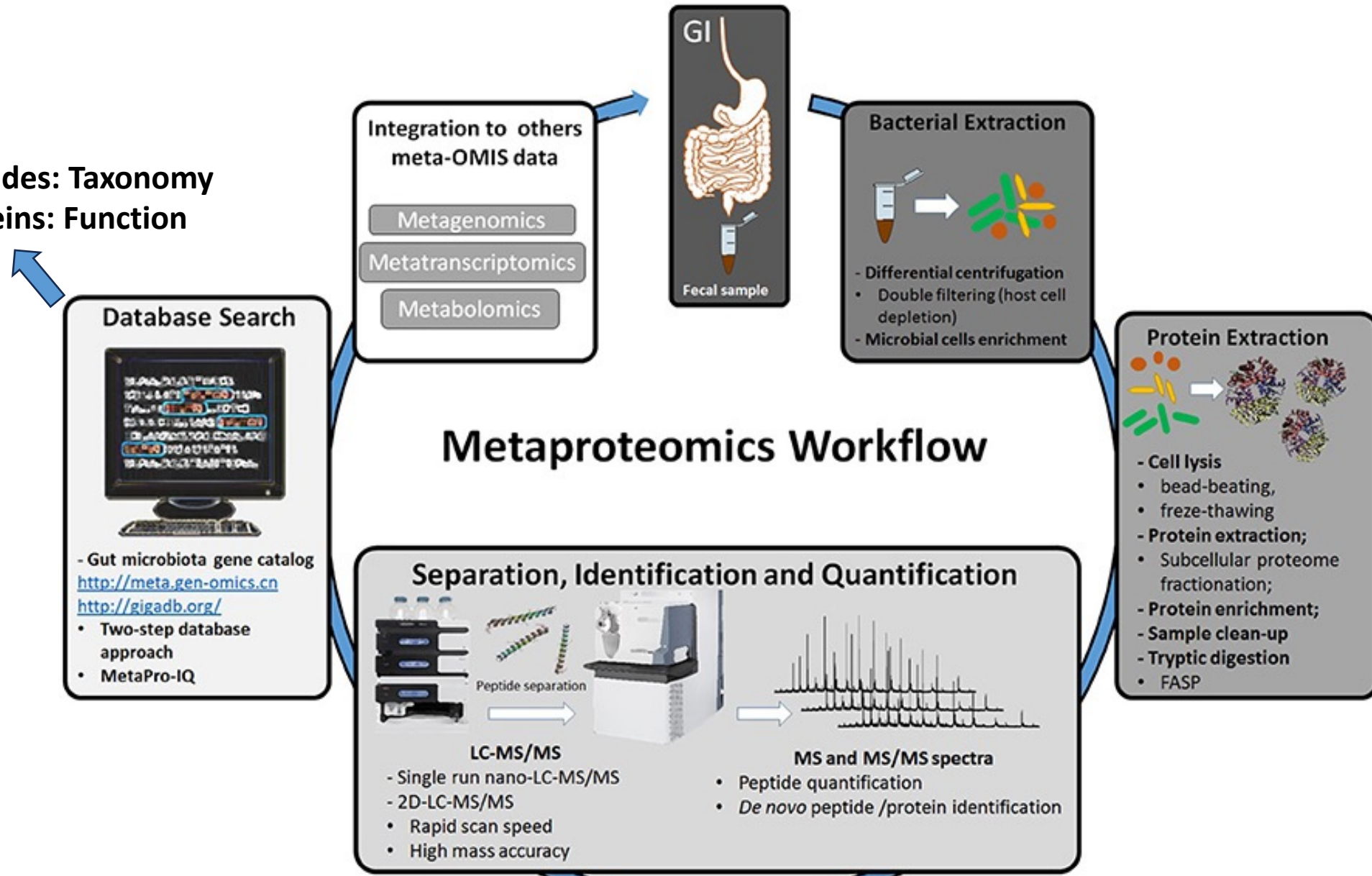
METAPROTEOMICS

TAXONOMY

FUNCTION

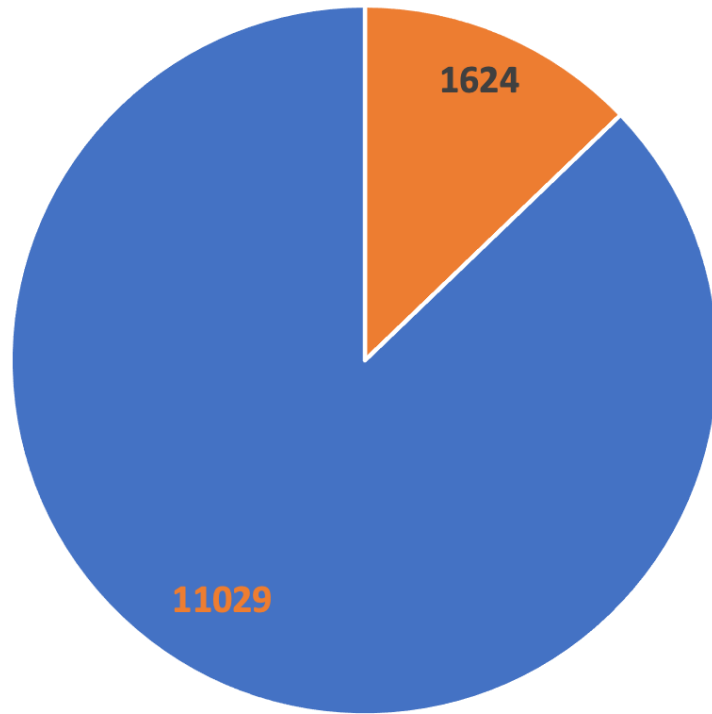
METAPROTEOMICS: MICROBIOME ANALYSIS USING MASS SPECTROMETRY

Microbial Peptides: Taxonomy
Microbial Proteins: Function



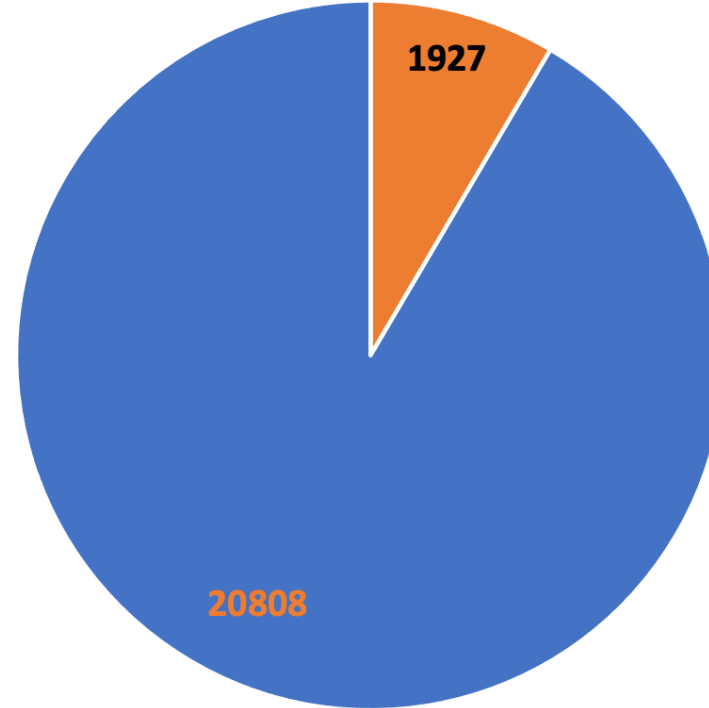
CLINICAL METAPROTEOMIC STUDIES DETECT LIMITED NUMBER OF MICROBIAL PEPTIDES

Cervical-vaginal fluid



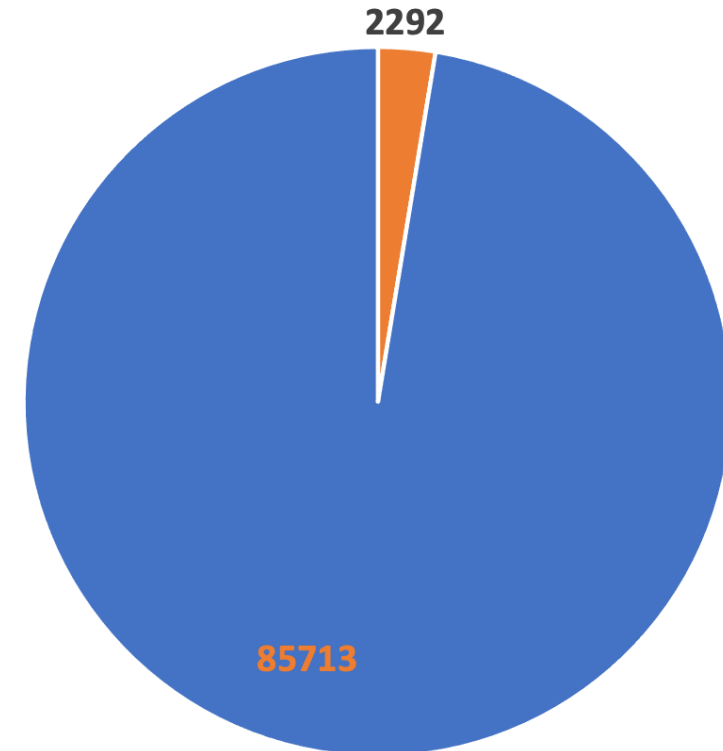
Afiuni-Zadeh *et al* (2018) *Sci Rep* . 8(1):10868.
doi: 10.1038/s41598-018-29092-4.

Saliva



Jagtap *et al* (2012) *Proteomics* 12(7): 992–1001.
doi: 10.1002/pmic.201100503

Bronchoalveolar lavage

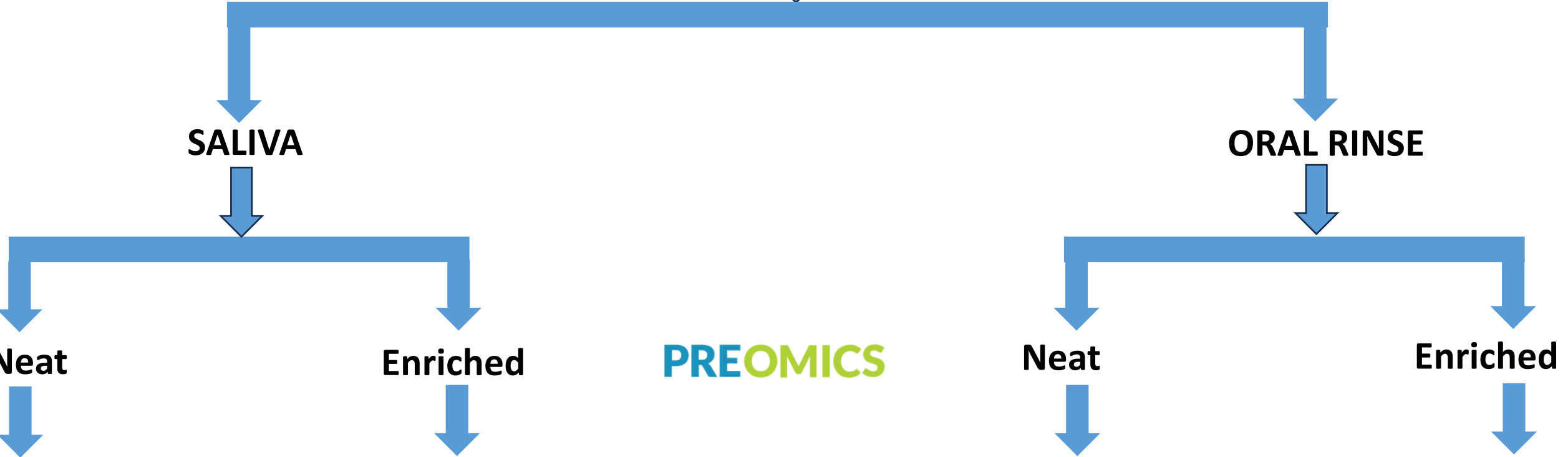
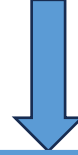


Kruk *et al* (2023) Submitted

■ Microbial
■ Human

MASS SPECTROMETRIC ANALYSIS OF LOW-ABUNDANCE PROTEOME

ORAL CANCER SAMPLE



SALIVA

ORAL RINSE

PREOMICS

Neat

Enriched

Neat

Enriched

MASS SPECTROMETRY USING DIA-PASEF METHOD USING TimS TOF INSTRUMENT

Saliva

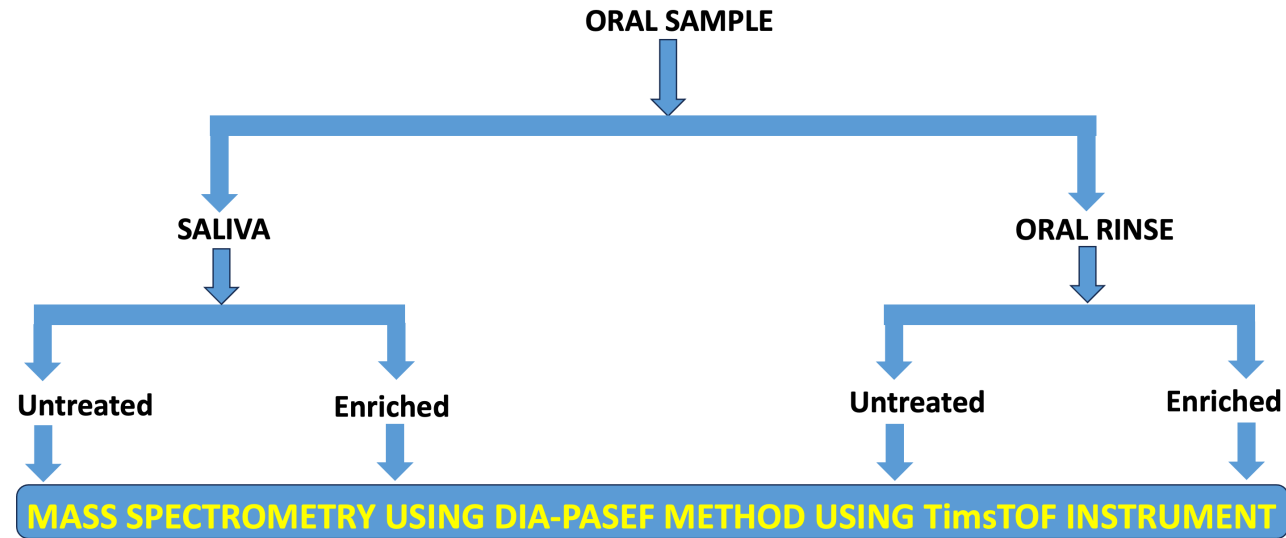
Saliva
ENRICH

Oral Rinse

Oral Rinse
ENRICH



MASS SPECTROMETRIC ANALYSIS OF LOW-ABUNDANCE PROTEOME



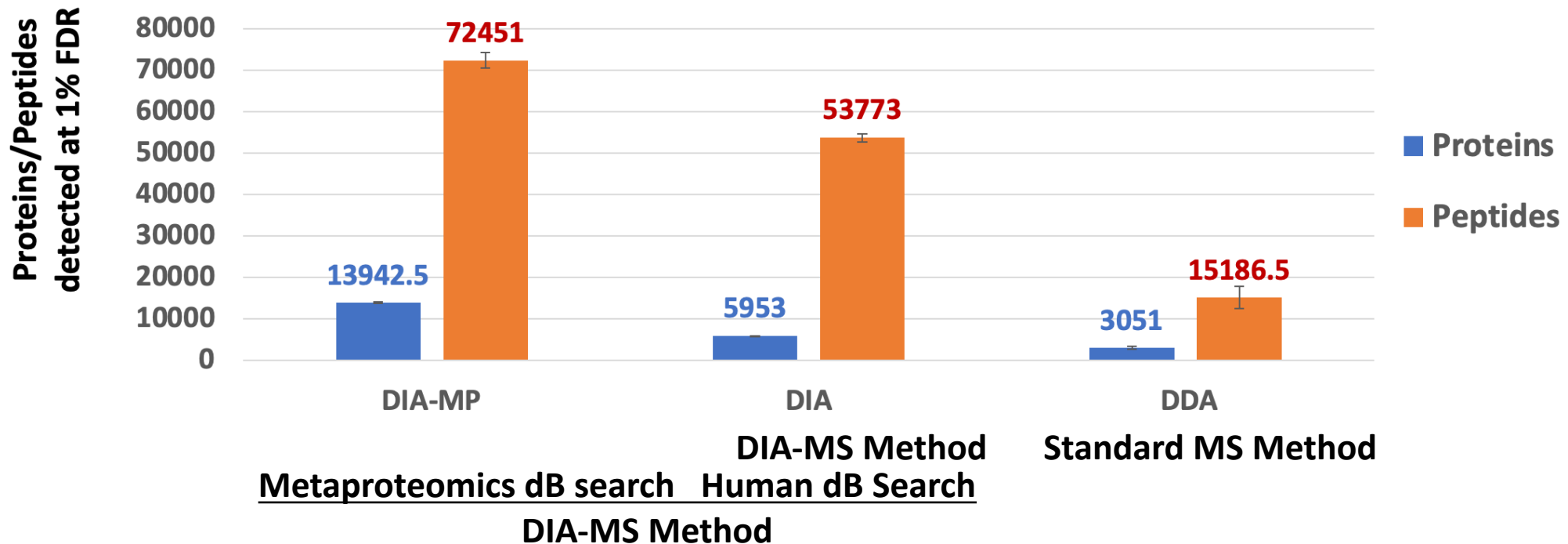
QUESTIONS:

- How do oral rinse sample proteins compare with neat saliva samples?
- Does enrichment affect number of detected proteins?
- Does enrichment lead to an improvement in the detection of number of microbial proteins?
- Does the detection of microbial taxonomy and functions improve upon enrichment?

SALIVA AND ORAL RINSE PROTEINS /PEPTIDES DETECTED

Comparison of DIA-PASEF (Metaproteome search) with DIA-PASEF (Human search) with DDA-MS search

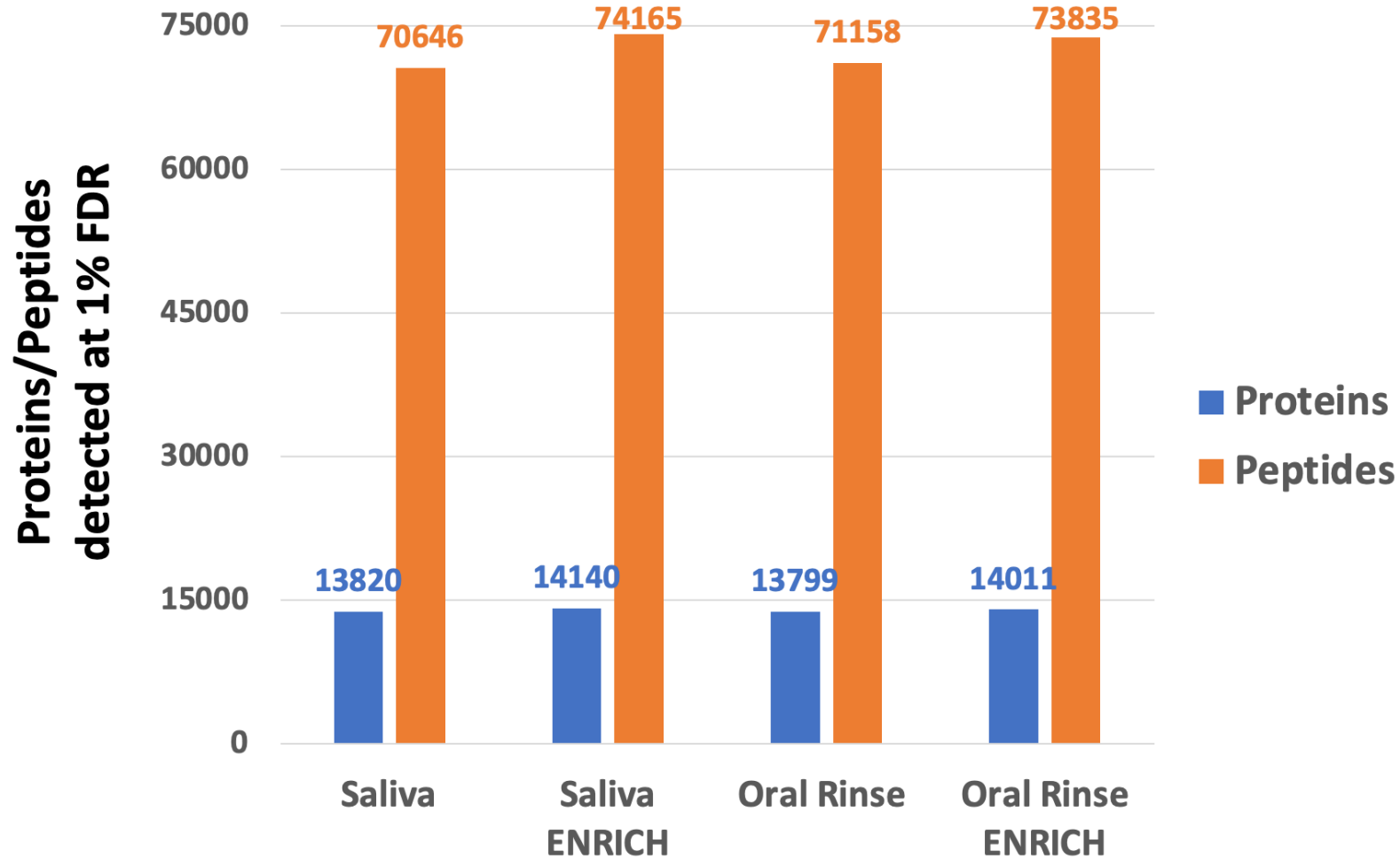
Metaproteomics dB search vs Human dB Search vs DDA-MS



- ***DIA-PASEF detects more peptides and proteins as compared to the DDA-PASEF method.***
- ***Search against microbial protein database detects ~8000 microbial proteins and ~18500 microbial peptides (on an average).***

SALIVA AND ORAL RINSE PROTEINS /PEPTIDES DETECTED

DIA-PASEF dataset analysis against host + microbial proteins

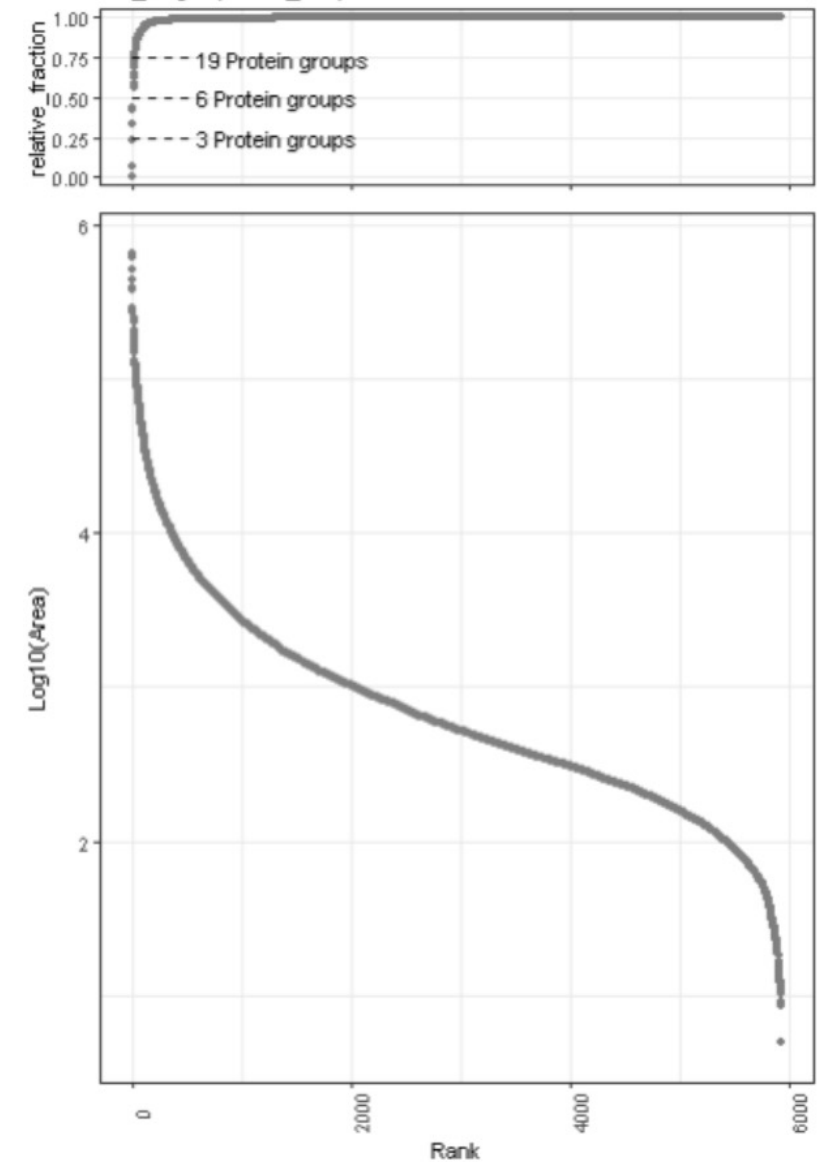
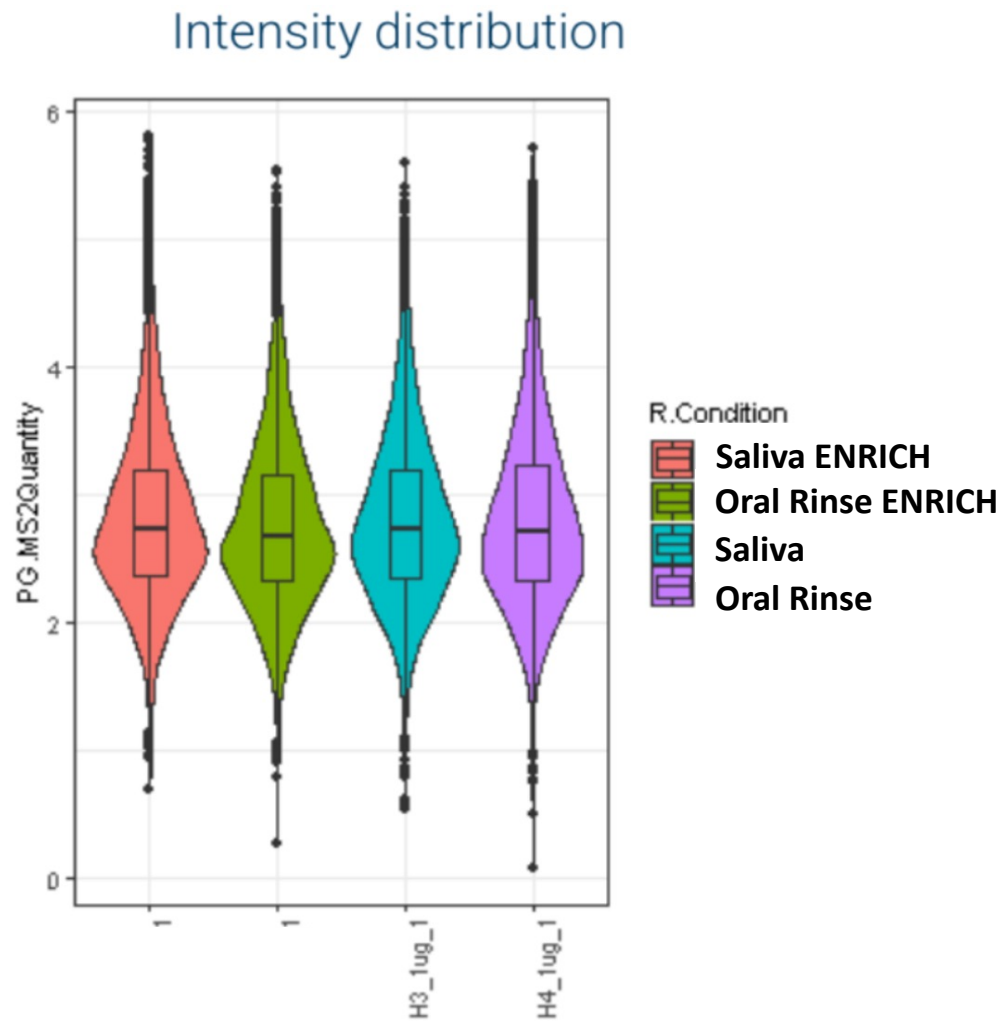


- *Protein detections for the oral rinse are comparable with neat saliva samples.*
- *Enrichment detects slightly more proteins and peptides in both oral rinse and saliva samples.*

QUANTITATIVE ACCURACY AND DYNAMIC RANGE



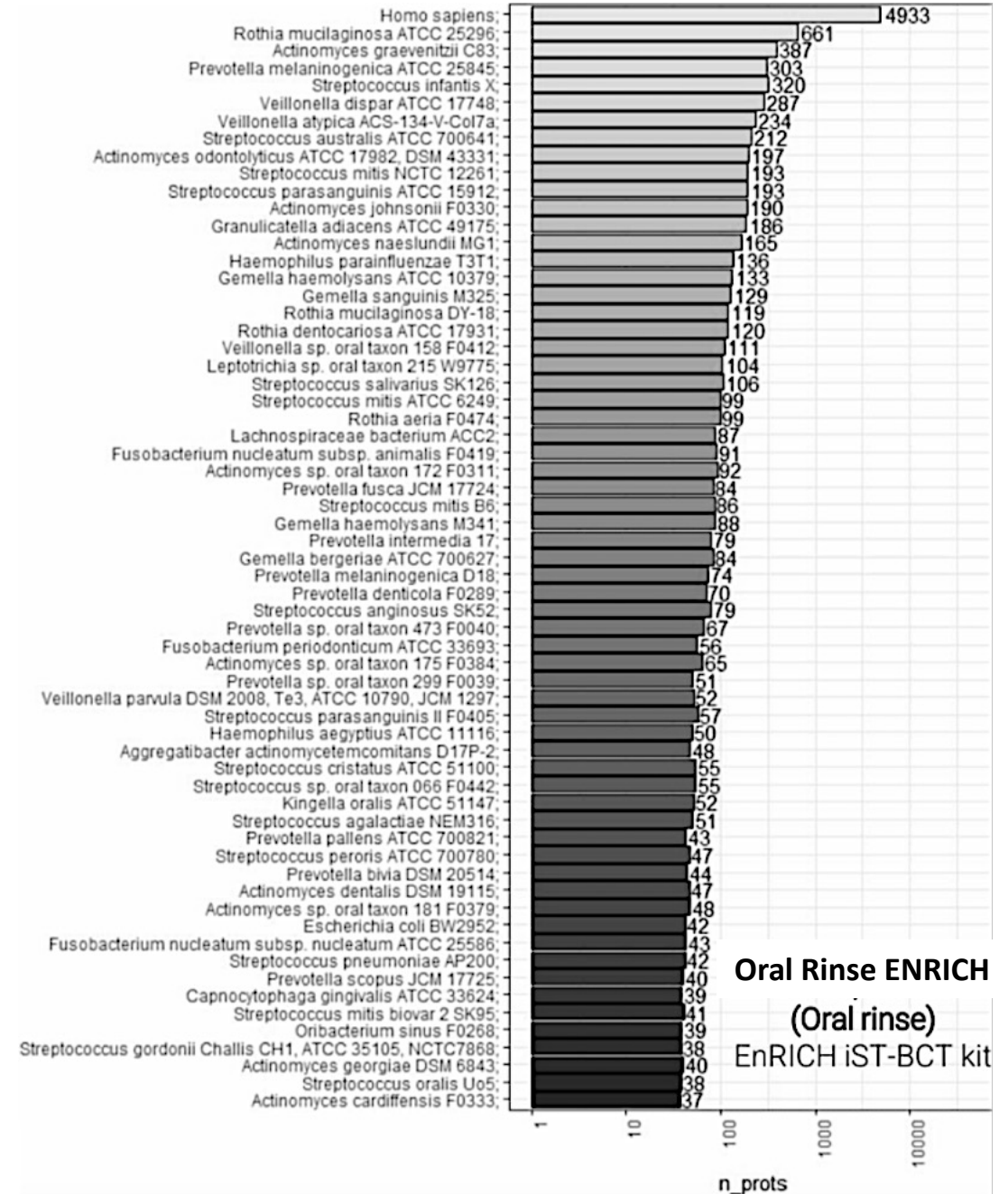
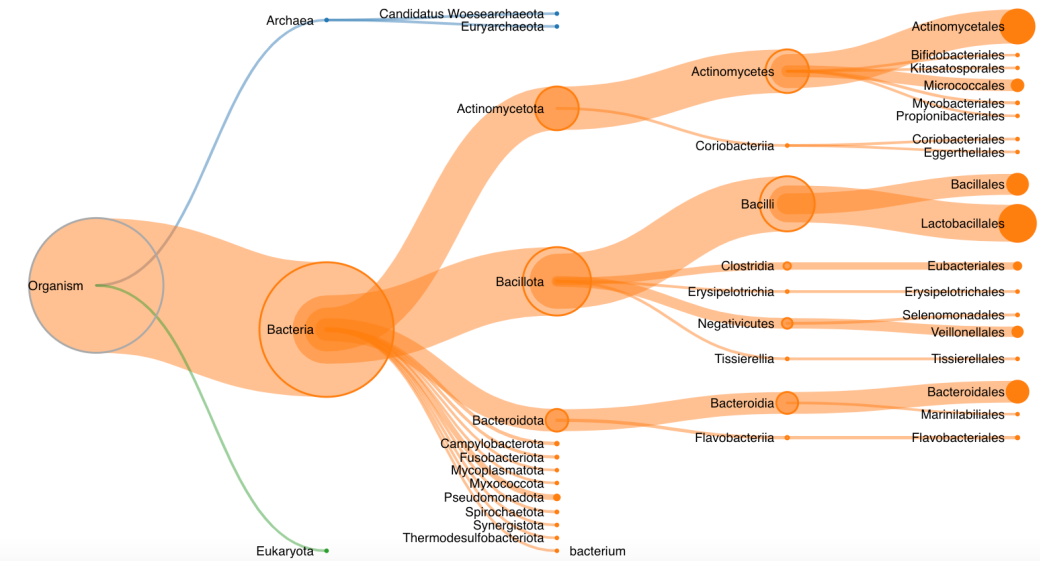
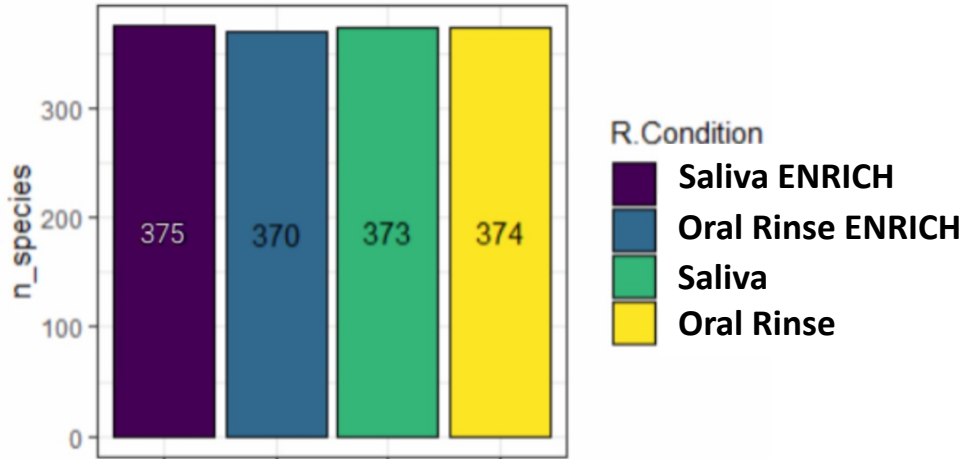
TimstoF-HT



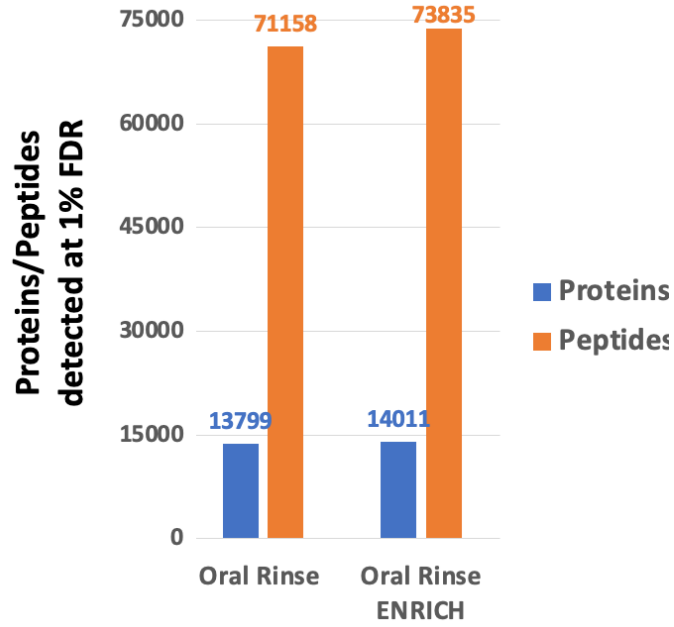
DIA-PASEF data exhibited an excellent quantitative accuracy and a broad dynamic range (almost 5 log-fold).

DIA-PASEF ANALYSIS DETECTS ~370 TAXONOMIC SPECIES

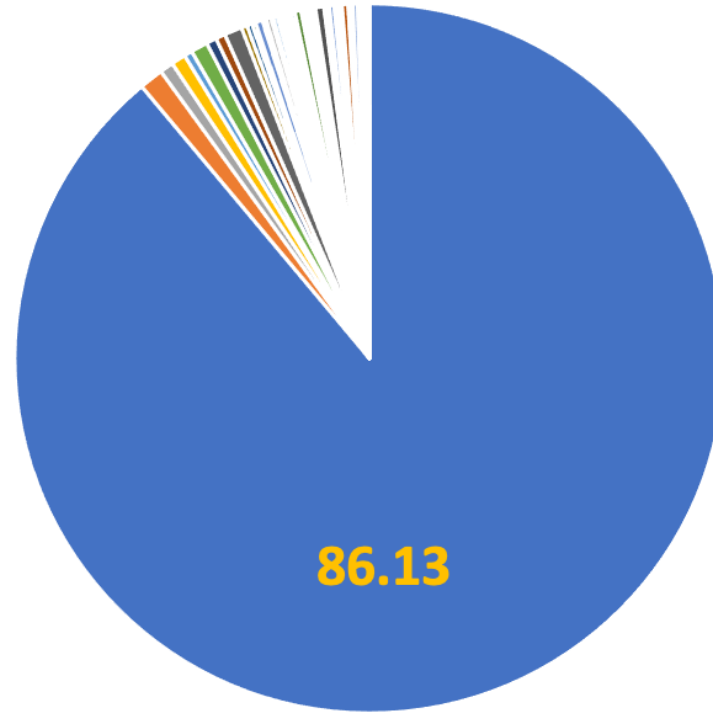
Number of organisms identified per MS run



ENRICHMENT (SLIGHTLY) INCREASES THE PROPORTION OF MICROBIAL CONTENT

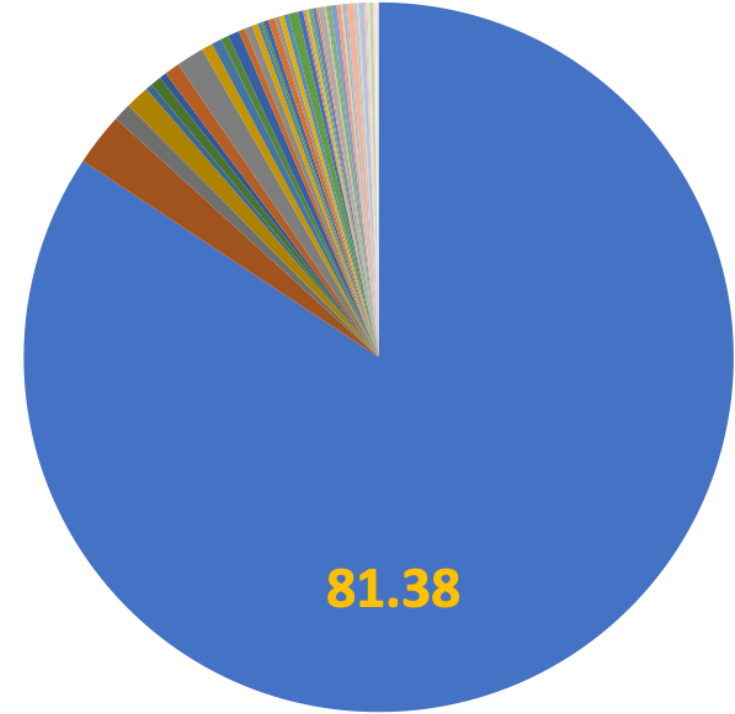


Oral Rinse Percent Intensity



- Homo sapiens
- Rothia mucilaginosa ATCC 25296
- Actinomyces graevenitzii C83
- Streptococcus infantis X
- Prevotella melaninogenica ATCC 25845
- Veillonella atypica ACS-134-V-Col7a

Oral Rinse Enriched Percent Intensity

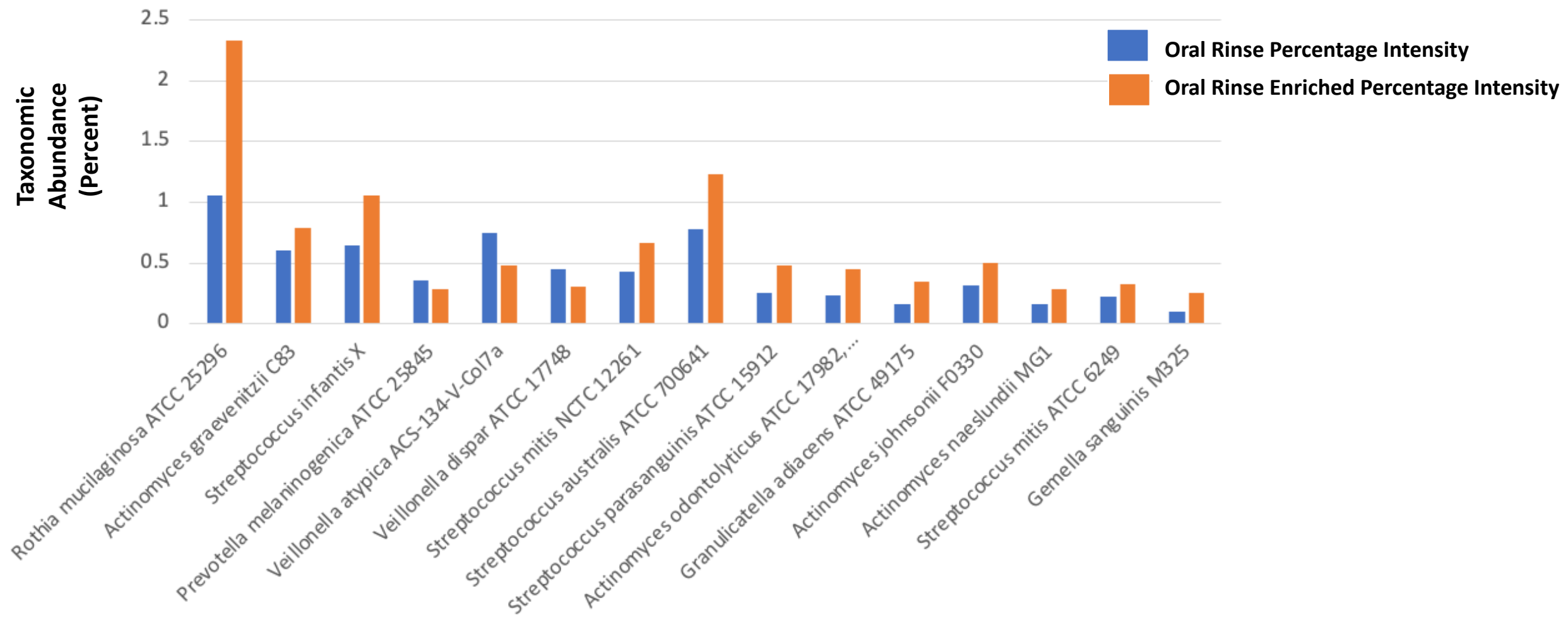


- Homo sapiens
- Rothia mucilaginosa ATCC 25296
- Actinomyces graevenitzii C83
- Streptococcus infantis X
- Prevotella melaninogenica ATCC 25845
- Veillonella atypica ACS-134-V-Col7a

Enrichment results in detection of more taxonomic groups.

ENRICHMENT INCREASES THE TAXONOMIC PROPORTION OF MICROBIAL CONTENT

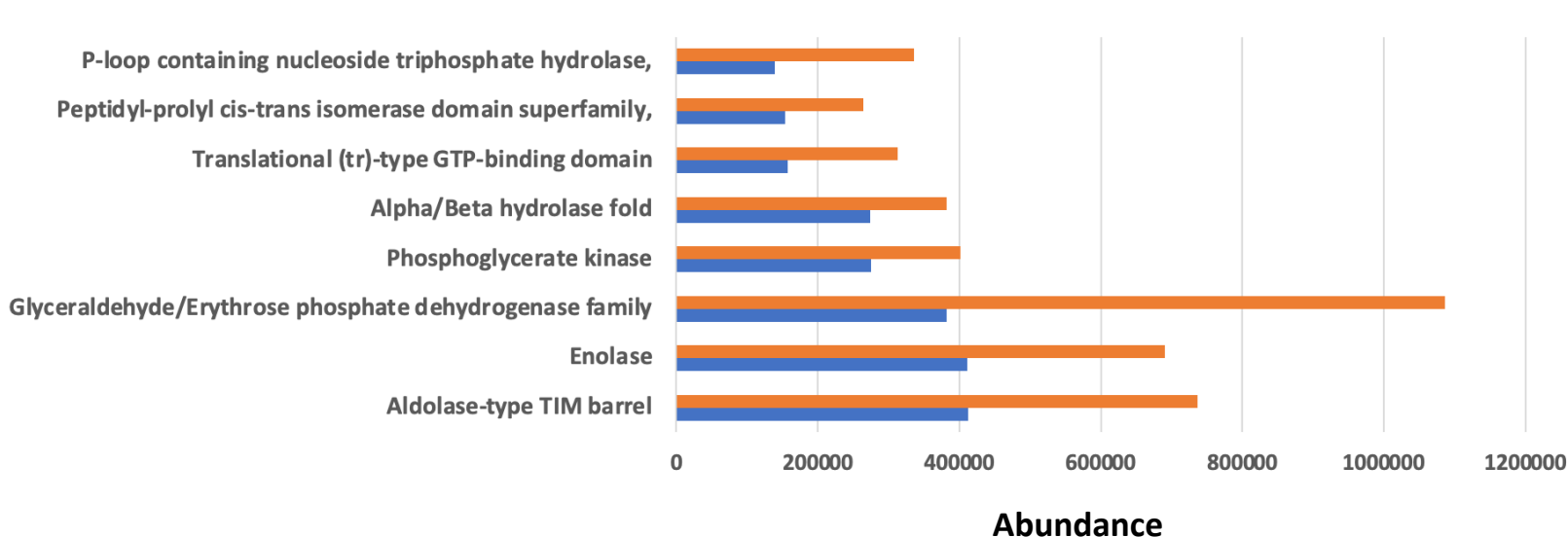
TOP FIFTEEN TAXONOMIC SPECIES



Enrichment allows for deeper taxonomic analysis of microbial proteins.

ENRICHMENT OF MICROBIAL FUNCTIONAL CONTENT

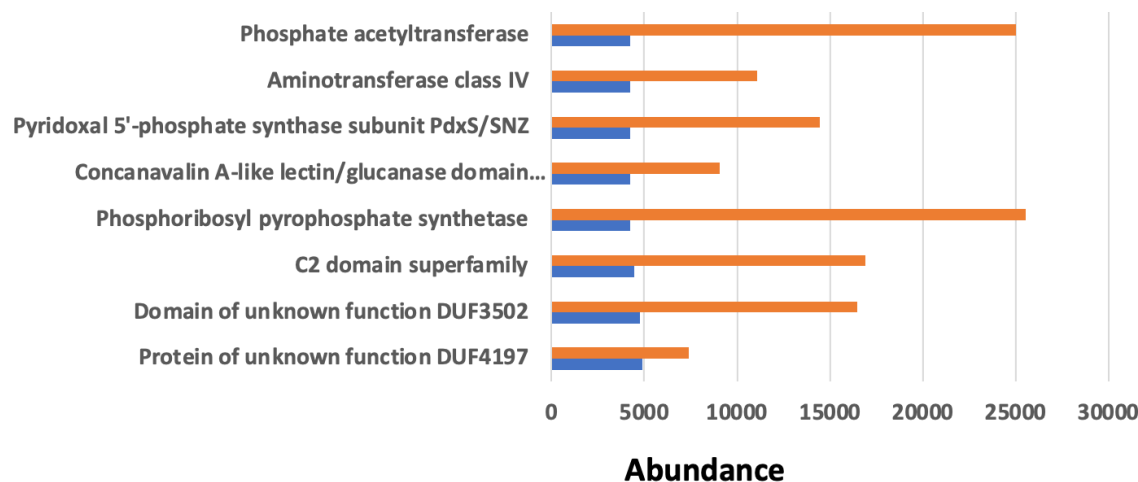
HIGH -ABUNDANCE PROTEIN FAMILIES



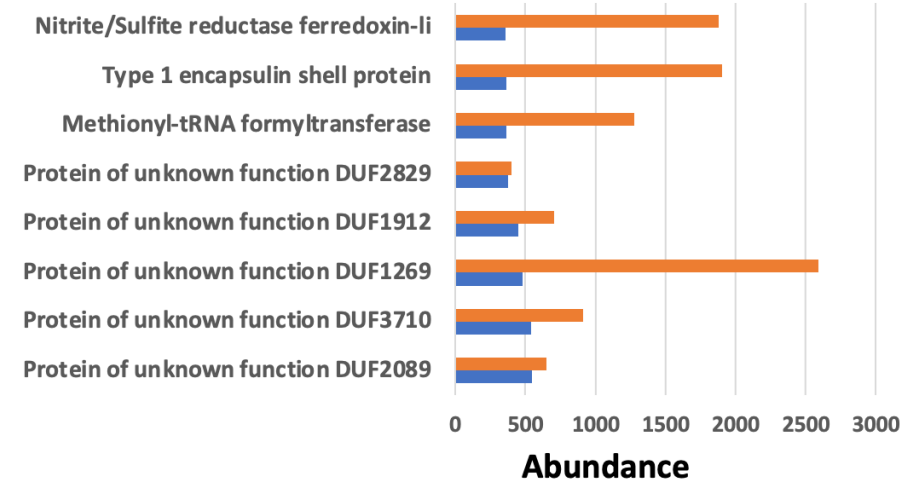
Oral Rinse Total Intensity
Oral Rinse Enriched Total Intensity

Enrichment allows for deeper functional analysis of microbial proteins.

MEDIUM ABUNDANCE PROTEIN FAMILIES



LOW-ABUNDANCE PROTEIN FAMILIES



SUMMARY

- **DIA-PASEF analysis on Human saliva and oral rinse samples detected ~14000 protein groups (~8000 microbial proteins), ~70,000 peptides (~18,500 microbial peptides) and ~370 microorganisms.**
- **DIA-PASEF data had an excellent proteome coverage, quantitative accuracy and low number of missing values (5%).**
- **Large dynamic range (almost 5 log-fold) was covered.**
- **Protein detections for the oral rinse are comparable with neat saliva samples.**
- **Enrichment detects slightly more proteins and peptides in both oral rinse and saliva samples.**
- **Enrichment allows for deeper taxonomic and functional analysis of microbial proteins.**

NEXT STEPS

- Oral rinse samples from oral cancer patients (before and after pioglitazone treatment) will be processed using the ENRICH kit.
- Perform 16S rRNA analysis and RNASeq analysis on pooled samples.
- Acquire DIA-PASEF MS data on Bruker TimsTOF instrument.
- Bioinformatic and statistical analysis using microbial database and host protein database to detect differentially expressed host proteins, microbial proteins and novel proteoforms.

ACKNOWLEDGEMENTS



MEDICAL SCHOOL

- Beverly Wuertz
- Frank Ondrey



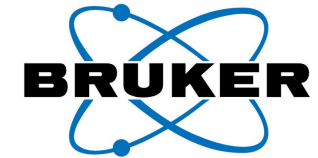
COLLEGE of BIOLOGICAL SCIENCES

- Monica Kruk
- Tim Griffin



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UNIVERSITY OF MINNESOTA



- Ruben Shreshta
- Sebastian Vaca
- Matt Willets
- Jon Lenz

PREOMICS

- Theresa Donnelly
- Measho Abreha

QUESTIONS?

