The International Metaproteomics Initiative: Communicating and Advancing Metaproteomics Research

November 3, 2021
Philadelphia, PA
Workshop structure

5:45 PM: Introduction and Welcome: Tim Griffin (*University of Minnesota*)

5:55 PM: Metaproteomics@ASMS 2021: Pratik Jagtap (*University of Minnesota*)

6:00 PM: Critical Assessment of Metaproteome Investigation (CAMPI): Bob Hettich (*Oak Ridge National Laboratory*)

6:20 PM: Metaproteomics Initiative: Tim Van Den Bossche (*Ghent University*)

6:30 PM: Panel Discussion
   Panelists: Bob Hettich, Mak Saito (*Woods Hole Oceanographic Institution*), Pratik Jagtap, Tim Van Den Bossche, Tim Griffin

*Attendee questions and discussion*
Metaproteomics: Promise and challenge

- Characterization of functional state (beyond genomic functional potential)
- Taxa-function relationships
- Host-microbiome interactions
- Guide for small molecule identification
- Annotating genes/proteins of unknown function

- Complexity!
- Sample preparation and limited abundance
- Taxonomic assignment
- Proteins of unknown function

https://www.pnnl.gov/projects/soil-microbiome/research
Objectives of workshop

• Emphasize the value and success

• Outline challenges and solutions across critical aspects of metaproteomics: Experimental design, sample preparation, instrumentation, informatics

• Promote resources available to those engaging in metaproteomics

• Invite you to join the community (help wanted!)

• Answer your questions!
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METAPROTEOMICS @ ASMS2021

• 12 abstracts
• 5 sessions
• 4 talks
• 1 workshop

- Plant root association
- Sargasso Sea Surface Microbiome
- Spectral Library for DIA
- Quantitative Metaproteomics using DIA
- Microbial modulation of immune system
- Spinal cord injury sample analysis
- Closely-related species database construction
- COVID-19 co-infection
- CAMPI: Multi-lab comparison of workflows
- Chronic Kidney Disease
- Cystic Fibrosis BALF Samples
- Ocean Protein Portal
Critical Assessment of Metaproteome Investigation (CAMPI): A Multi-Lab Comparison of Established Workflows

Tim Van Den Bossche*1,2, Benoit J. Kunath*3, Kay Schallert*4, Stephanie S. Schäpe*5, Paul E. Abraham6, Jean Armengaud7, Magnus Ø. Arntzen8, Ariane Bassignani9, Dirk Benndorf4,10,11, Stephan Fuchs12, Richard J. Giannone6, Timothy J. Griffin13, Live H. Hagen8, Rashi Halder3, Céline Henry9, Robert L. Hettich6, Robert Heyer4, Pratik Jagtap13, Nico Jehmlich5, Marlene Jensen14, Catherine Juste9, Manuel Kleiner14, Olivier Langella15, Theresa Lehmann4, Emma Leith13, Patrick May3, Bart Mesuere1,16, Guylaine Miotello7, Samantha L. Peters6, Olivier Pible7, Udo Reich4,11, Bernhard Y. Renard12,17, Henning Schiebenhoefer12,17, Alexander Scryba18, Alessandro Tanca19, Kathrin Trappe12, Jean-Pierre Trezzi3,20, Sergio Uzzau19, Pieter Verschaffelt1,16, Martin von Bergen5, Paul Wilmes3, Maximilian Wolf4, and Lennart Martens*1,2 and Thilo Muth*21

Measurement considerations for metaproteomics

• **Sample selection/preparation**
  • How do we get microbial proteomes out of complex environmental matrices?
  • How do we make this method compatible with MS?

• **Metaproteome measurements**
  • High-performance, high-throughput LC-MS/MS for deep metaproteome measurements
  • “Figures of merit” considerations (*accuracy*, *resolution*, *sensitivity*, *dynamic range*)

• **Informatic considerations**
  • Metagenome / metaproteome match?
  • Protein inference from peptides?
  • Redundancy?
  • Normalization
  • Metabolic information?
All participants could use their choice of experimental and/or informatic workflow.
Complex LC-MS workflows and meta-omic search databases lead to more identifications.

REF = UniProtKB Reference Proteomes

REF = Integrated Gene Catalog (IGC)

While different bioinformatic pipelines show some differences on peptide level...
... these differences disappear at the protein group level
Taxonomic Assessment of the SIHUMIx sample

Taxonomic Assessment of the fecal sample
Summary of CAMPI results

• An array of experimental and informatic methods are available for metaproteomic analysis.

• Based on well-established workflows, we evaluated the influence of sample preparation, mass spectrometry acquisition, and bioinformatic analysis on two sample types.

• While bioinformatic pipelines contributed to variability in peptide identification, experimental approaches were the most important source of differences between analyses.

• Peptide-level differences largely converge at protein group level.

• While differences were observed for predicted community composition, similar functional profiles were obtained across workflows.
Special thanks to all collaborators!

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Why an Initiative?

• Need for better **communication** and **collaboration**
  • In the next years, major new analytical developments are likely to facilitate unprecedented insights into microbiome function and dynamics
  • Many metaproteomics researchers are working solitary in single-species proteomics labs, or in metagenomics/metatranscriptomics labs

• Need for better **education**
  • the microbiome community needs better education on the technical details and capabilities offered by metaproteomics, and easy access to experts

• Need for (better) **standardization**
  • efforts to standardize methodologies, will accelerate experimental and bioinformatic innovations
Mission and Vision statement

We promote dissemination of metaproteomics fundamentals, advancements, and applications through collaborative networking in microbiome research.

We aim to be the central information hub and open meeting place where newcomers and experts interact to communicate, standardize and accelerate experimental and bioinformatic methodologies in this field.
We’re going to stimulate collaboration via our Working Groups
Since our foundation in Feb. 2021, we’ve already gathered 120 enthusiastic metaproteomics researchers.

120 members from >50 labs in 15 countries (12 in Europe, Australia, Canada, and USA)*

* In this count, only the labs and countries are included of the researchers who want to have their lab listed on our website.
Some members were present at our International Metaproteome Symposium earlier this year.
We need you!

If you...

... Have environmental samples (gut, soil, biogas plants, ocean, dust, ...)

... Have “non-traditional” mass specs to run metaproteomics samples on

... Are just interested in this blooming research field

Become a member!
Becoming a member is really easy!

Want to stay up to date about the metaproteomics field and the Initiative? Become a member via [www.metaproteomics.org](http://www.metaproteomics.org)!

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info@metaproteomics.org
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