**Proteomics Lecture and Study Guide**

Suggested reading:

Review on spatial proteomics (study guide on introductory section):

Andreas Mund, Andreas-David Brunner, Matthias Mann. Unbiased spatial proteomics with single-cell resolution in tissues Mol Cell 2022 Jun 16;82(12):2335-2349. PMID: 35714588

<https://pubmed.ncbi.nlm.nih.gov/35714588/>

Short review article on spatial proteomics:

<https://pubmed.ncbi.nlm.nih.gov/35590074/>

More in depth technical reading:

Mund et al. Deep Visual Proteomics defines single-cell identity and heterogeneity Nature Biotechnology. 2022 Aug;40(8):1231-1240. PMID: 35590073

<https://pubmed.ncbi.nlm.nih.gov/35590073/>

Thierry M Nordmann et al. Spatial proteomics identifies JAKi as treatment for a lethal skin disease. Nature 2024 Nov;635(8040):1001-1009 PMID: 39415009

<https://pubmed.ncbi.nlm.nih.gov/39415009/>

Topic Review: Protein/Peptide Sequencing with Mass Spectrometry

Hanno Steen 1 , Matthias Mann. The ABC's (and XYZ's) of peptide sequencing Nat Rev Mol Cell Biol. 2004 Sep;5(9):699-711. PMID: 15340378

<https://pubmed.ncbi.nlm.nih.gov/15340378/>

Landmark Study: First proteome organization of an organism

Krogan et al., Global landscape of protein complexes in the yeast Saccharomyces cerevisiae Nature 2006 Mar 30;440(7084):637-43. PMID: 16554755

<https://pubmed.ncbi.nlm.nih.gov/16554755/>

Classic review (excellent overview):

Gstaiger M, Aebersold R. Applying mass spectrometry-based proteomics to genetics, genomics and network biology. Nat Rev Genet. 2009 Sep;10(9):617-27. PMID: 19687803

<https://pubmed.ncbi.nlm.nih.gov/19687803/>

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I have selected this article since it represents a snapshot of the state of the proteomics field in 2024/2025. The paper elegantly puts some important high-level aspects of the field in context. The introductory paragraphs are an excellent summary of both state-of-the art approaches and concepts, as well as containing references to foundational ideas, principles, and work that defines the proteomics field.

The senior author is Matthias Mann, who is recognized as one of the world authorities and top innovator in proteomics. Dr. Mann earned his PhD at Yale University advised by John Fenn. Dr. Mann’s PhD work established fundamental technologies that gave rise to modern proteomics. This work led to Dr. Fenn sharing the 2002 Nobel Prize in Chemistry for the development of methods for identification and structure analyses of biological macromolecules.

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I will annotate the introduction highlighting key concepts that will map to the lecture slides. Some lecture topics will be covered in greater depth than others. This annotated guide to the review will help both the expert and novice glean the important concepts that I hope to summarize in the lecture. First, read the first page of the article, then re-read each paragraph below with my annotated comments.

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This first paragraph puts proteomics in the context of single-cell biology, which is on point for the review. However, there are some interesting ideas that help set the stage for proteomics in general. Complexity: This comes up repeatedly and is the defining challenge of all “omics”. The molecules of life (DNA, RNA, Protein) are made of simple chemical building blocks and in general “omics” technologies sequence these building blocks in the order they are arranged in genomes, transcriptomes, and proteomes. However, all the building blocks are arranged in precisely defined polymers and these polymers are found as complex mixtures in huge quantities in cells. You can take a moment to imagine how this complexity is compounded when you consider how individual cells are organized (nucleus, cytoplasm, organelles), then how cells are organized into tissues (lining of the stomach), tissues to organs, organs to systems (kidneys and heart attached to the vasculature), systems attached to scaffolds (organs attached to the skeleton surrounded by the skin), and finally defining an entire organism like a human. So, think about the human genome and appreciate that each cell has a genome that is contained within this complexity. The same is true for RNA and Proteins. Omics technologies can tell you the sequence of the biological molecules, but the experimental design and the choice of methodology will contain the information that links all the sequence information to the biological context.

The introduction also relates the omics field to that of microscopy where there have been similar advances in tools and techniques that have provided increased resolution of complex systems. If you are unfamiliar with omics technologies, imagine how photography developed from whole organism to tissue, to cell, to subcellular, and to now near atomic resolution.

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This paragraph sets the stage for single cell proteomics. However, the ability to study a single cells proteome is based on many years of development in the proteomics field that allowed proteins to be directly measured in a mass spectrometer. Proteomics is a blend of methods that have established how proteins are collected, processed, and introduced into a mass spectrometer. In parallel, there has been intense development of mass spectrometry technologies that have allowed the direct measurement of proteins one at a time and in complex mixtures. The field was initially dominated by collecting proteins from mixtures of cells and looking at the extracted proteins “all at once” which destroys all the organizational information. In this paragraph, the concept of “targeting” for proteomics is compared to the use of antibodies which are a single molecule reagent that can bind and label a protein. Antibodies, in general, can provide a direct “targeted” readout of a single protein in its original context. Hower the vast majority of antibody and proteomics measurements are a combined signal derived from hundreds to millions of single protein/antibody complexes or “peptides” as is the case for mass spectrometry-based proteomics. The reference to CyTOF is to acknowledge one of the first single cell “proteomics” technologies. In CyTOF, a spectrometer is used but is fundamentally different than the type of MS based proteomics that is most common.

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A reference to the foundational work done by Fenn, Mann and others. This sentence acknowledges the parallel efforts to develop more powerful mass spectrometers.

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This section is a reference to advances in the mass spectrometers used for proteomics. The changes in technology are multi-fold and are beyond the scope of this course. I have a lecture slide that plots some similar advances made a decade ago. Two example references are listed for further reading if you are interested.



Hebert AS, et a, Coon JJ. **The one hour yeast proteome**. *Mol Cell Proteomics*. 2014 PMID: 24143002

M P Washburn, D Wolters, J R Yates, **Large-scale analysis of the yeast proteome by multidimensional protein identification technology** Nature Biotechnology. 2001 Mar;19(3):242-7. PMID: 11231557

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This paragraph is important to help frame the state of the art in single cell omics. The “depth” is an important term that is used frequently but rarely put in absolute terms. The take home message is that no single cell RNA seq or proteomics experiment is comprehensive in depth. This means that only a fraction, typically the most abundant, proteins and RNAs are quantified. Nevertheless, the current “depth” has been considered significant enough to extract vast amount of information from single cell transcriptomes and proteomes and therefore more and more studies are being initiated. One very recent example from the same authors (Nordmann et al, Nature 2024; PMID:39415009) is a glimpse of the future of this very exciting direction in the proteomics field.

Finally, the subtle reference to oligo-nucleotide amplification is important since this frames the biggest difference in nucleic acid based omics and proteomics. The ability to use polymerases and PCR techniques to amplify and make exact copies of the genome or transcriptome is the basis for most DNA/RNA omics studies. This allows for amplification of the raw material and can vastly increase the sensitivity of omics techniques. Proteomics has no similar method, and a major challenge is to increase sensitivity of detection (quantification) with only the amount of protein directly extracted from the biological sample. This was the fundamental barrier to single cell proteomics, and is still a major limitation in the proteomics field. Bar coding approaches are emerging that couple antibody detection to DNA barcoding (PMID: 30078711) as well as alternative methods for HTS protein sequencing (bioRxiv 2024.12.31.630920). It is possible that emerging technologies will replace MS based proteomics.