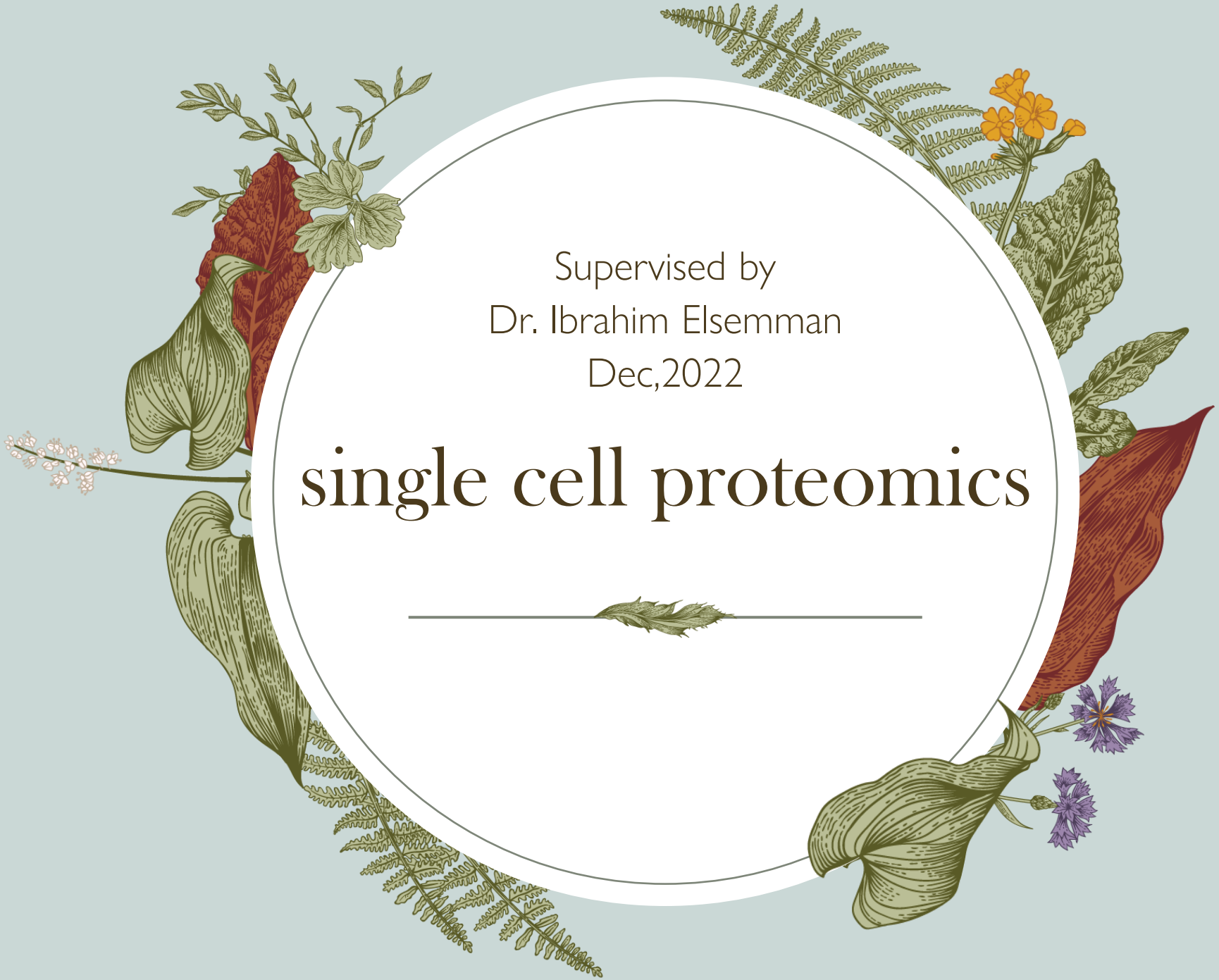


Supervised by
Dr. Ibrahim Elsemman
Dec,2022

single cell proteomics

Agenda

Introduction

Single-cell proteomics by mass spectrometry (SCoPE-MS) is a recently introduced method to quantify multiplexed single-cell proteomes. While this technique has generated great excitement, the underlying technologies (isobaric labeling and mass spectrometry (MS)) have technical limitations with the potential to affect data quality and biological interpretation. These limitations are particularly relevant when a carrier proteome, a sample added at 25–500× the amount of a single-cell proteome, is used to enable peptide identifications. Here we perform controlled experiments with increasing carrier proteome amounts and evaluate quantitative accuracy, as it relates to mass analyzer dynamic range, multiplexing level and number of ions sampled. We demonstrate that an increase in carrier proteome level requires a concomitant increase in the number of ions sampled to maintain quantitative accuracy. Lastly, we introduce Single-Cell Proteomics Companion (SCPCompanion), a software tool that enables rapid evaluation of single-cell proteomic data and recommends instrument and data analysis parameters for improved data quality.

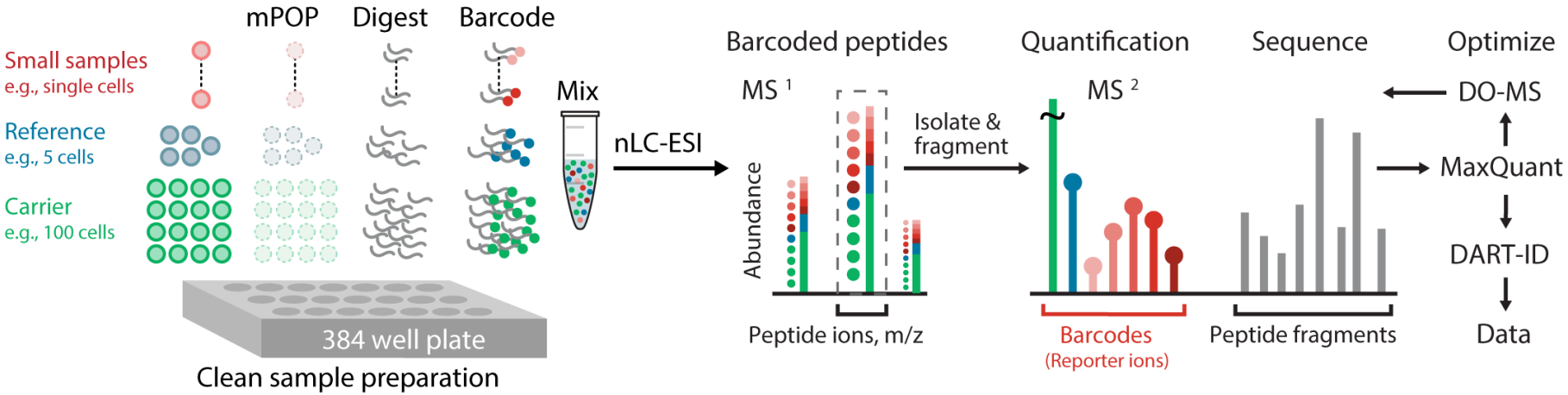
Single-cell proteomics techniques can identify large numbers of proteins expressed within thousands of individual cells at a given point in time (snapshot). Such techniques have the potential to provide insight into the processes of disease development, progress, and treatment effects, as well as be a biomarker for disease diagnosis and prognosis.

Single-cell mass spectrometry (MS) can now be used to measure tens to hundreds of proteins, metabolites, and lipids in individual cells.

The field of single-cell proteomics is bringing change to how we infer proteins from cellular mRNA levels. It's early days, but it's not a distant dream to be able to tally the proteins in single cells. Proteins are tougher to work with than RNA or DNA, for example they're stickier.

Quantification of single-cell proteomes (SCP) could complement transcriptomics by monitoring protein abundance, post-translational modification status and regulation. While analysis of proteins in single cells is generally performed with antibody-based technologies, MS-based SCP is capable of quantifying single cell proteomes in an unbiased manner.

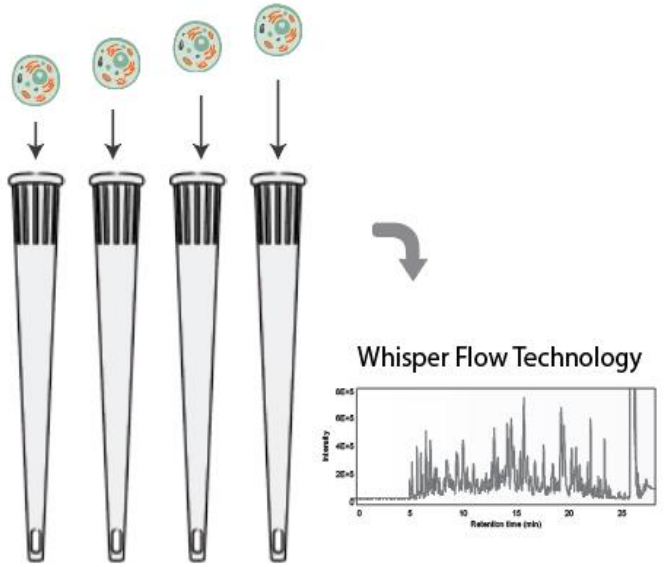
Single Cell ProtEomics by Mass Spectrometry (SCoPE2)



Primary Information

Single cell proteomics is a field in rapid development, enabled by significant technological improvements over the last years and it presents a key contribution to answer fundamental biological questions about heterogeneity within complex systems.

Single cell proteomics as a robust and standardized application

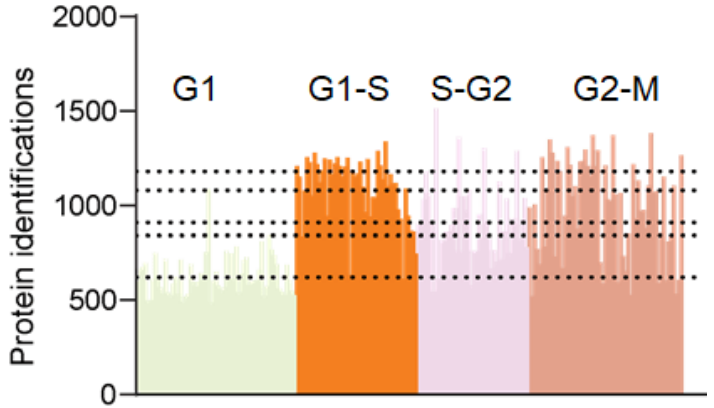


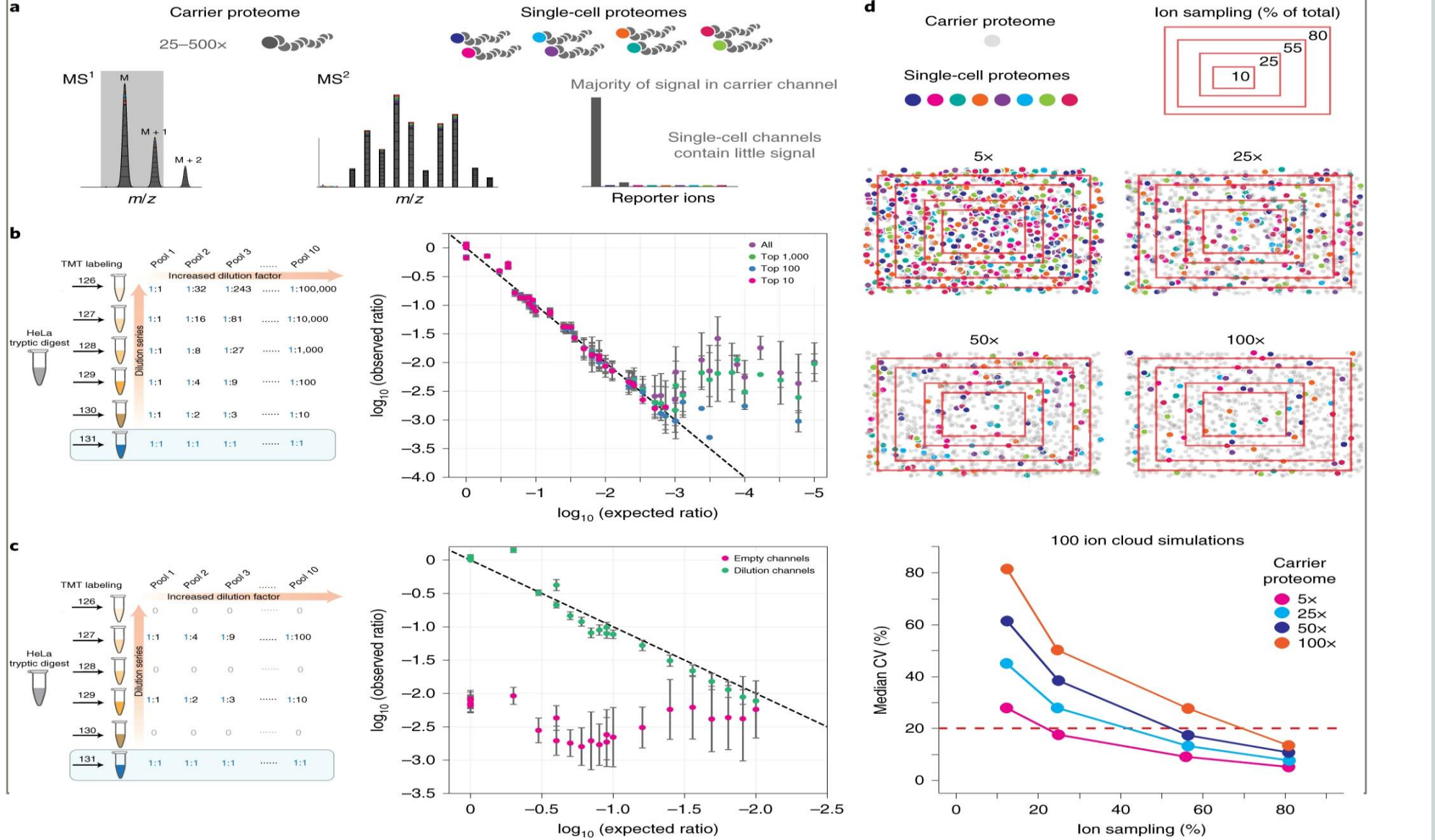
Single cell proteomics is an emerging technology with a lot of promise but there are still several challenges to overcome. A great deal of effort has been made in how to re-think sample preparation workflows to be automated and involve as few steps as possible. Evotips support handling and preservation of single cell digests.

Ultra sensitive single-cell proteomics

One of the greatest goals of single-cell proteomics is to reach the needed sensitivity and maintain the performance throughout the lifetime of a project.

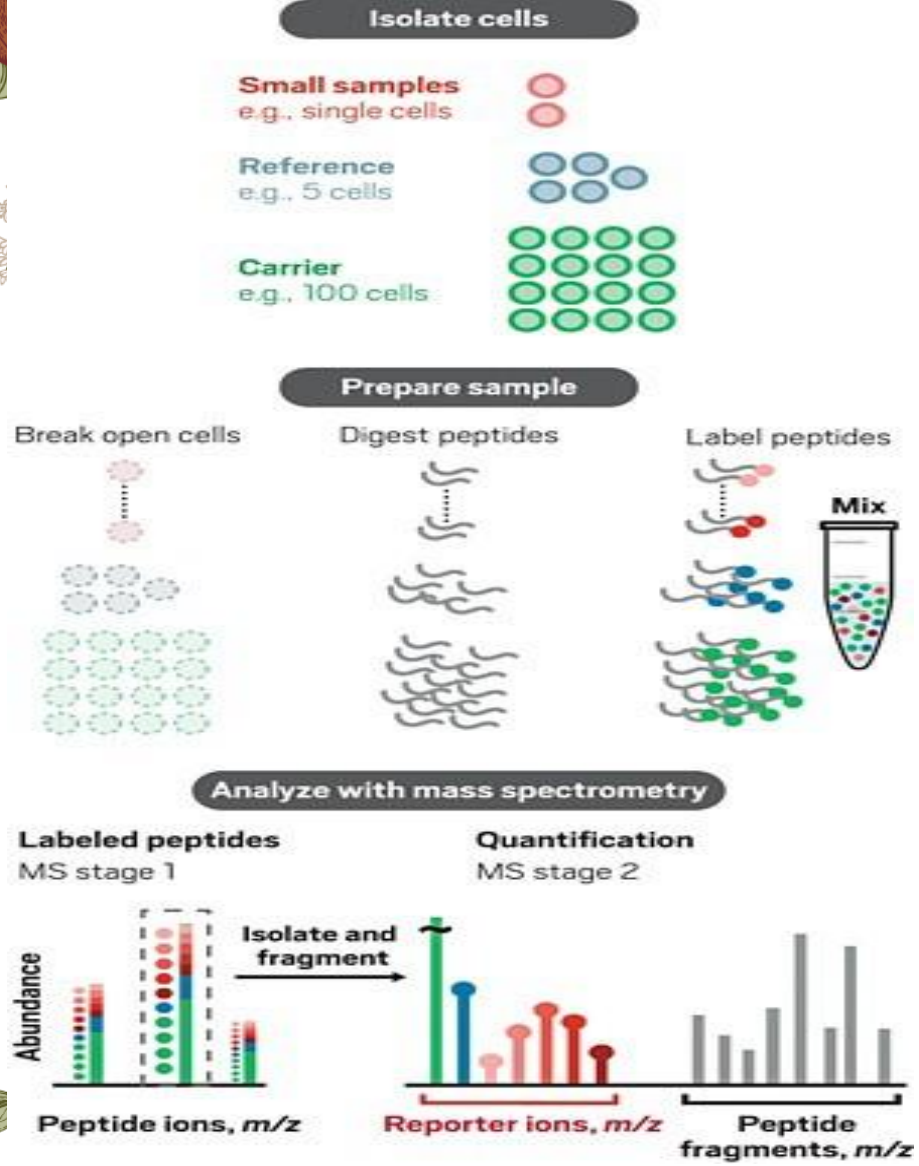
Proteomics is the study of how individual cells, and their environments change during the cell cycle.




a
Carrier proteome
25–500×
Single-cell proteomes
MS1
M
M + 1
M + 2
m/z
MS2
m/z
Majority of signal in carrier channel
Single-cell channels contain little signal
Reporter ions
b
TMT labeling
Pool 1
Pool 2
Pool 3
Pool 10
Increased dilution factor
126
127
128
129
130
131
HeLa tryptic digest
Dilution series
1:1
1:32
1:243
1:100,000
1:16
1:81
1:10,000
1:8
1:27
1:1,000
1:4
1:9
1:100
1:2
1:3
1:10
log10 (observed ratio)
log10 (expected ratio)
All
Top 1,000
Top 100
Top 10
c
Empty channels
Dilution channels
d
Carrier proteome
Single-cell proteomes
Ion sampling (% of total)
80
55
25
10
5×
25×
50×
100×
100 ion cloud simulations
Carrier proteome
Median CV (%)
Ion sampling (%)

a, Schematic of a SCoPE-MS experiment. b, HeLa tryptic digests were labeled with TMT and pooled together in defined ratios starting from a 1:1n series in the first pool, up to a 1:10n series in the tenth pool. Observed ratios relative to the TMT131 carrier channel were calculated and log transformed. The median (dot) and median absolute deviation (MAD, whisker) of the ratio distributions of the top n intensity-based peptides (n = 10, magenta; n = 100, blue; n = 1,000, teal; all, purple) were compared against their theoretical log ratio. c, HeLa tryptic digests were labeled with TMT and pooled together in defined ratios starting from a 1:1n series in the first pool up to a 1:10n series in the tenth pool. Here, every diluted channel (teal) was separated by an empty channel (magenta). The median (dot) and MAD (whisker) were calculated as described in b. d, Simulated cloud of 1,000 ions comprising eight equal single-cell populations and a carrier proteome population at 5×, 25×, 50× or 100×. Median CVs were calculated across the eight single-cell channels for 100 simulations and sampling levels of 10%, 25%, 55% or 80%.

single cell proteomics



Advantages and Disadvantages of single cell proteomics

Advantages of Single Cell proteomics :

1. Microorganisms have a high rate of multiplication
2. Microorganisms have high content of protein
3. Microorganisms can utilize a variety of carbon sources as major energy source, and some of the waste material can also be used as carbon source.
4. Microbial strains with high yield as well as good composition can be selected or produced and cultured in large quantity in laboratory conditions.
5. Microbial biomass production as single cell protein is independent of seasonal as well as climatic variations.

Disadvantages of Single Cell proteomics:

1-Many types of microorganisms produce some substances which are toxic to the human and also to the animals. Therefore, it has to be made sure that the produced microbial biomass does not contain any of these toxic substances.

2. Sometimes the microbial biomass when taken as diet supplement may lead to indigestion

3. Sometimes the microbial biomass when taken as diet supplement may lead to allergic reactions in humans.

4. The high nucleic acid content of many types of microbial biomass products Is also undesirable for human consumption as single cell protein. Sometimes this high level of nucleic acid content in microbial biomass will lead to kidney stone formation or gout.

5. The high nucleic acid content of many types of microbial biomass may lead to poor digestibility, gastrointestinal problem and also some skin reactions in humans.

6. The possibility of presence of toxins or carcinogenic compounds may lead to some serious health problems in humans as well as in animal stock.

7. Single cell protein production is a very expensive procedure as it needs high level of sterility control in the production unit or in the laboratory.

8. Single cell protein grown as animal feed on agricultural residues will be beneficial in the future economy of developing nations.

Applications of Single-Cell Protein

Applications of Single-Cell Protein:

1-Provides instant energy.

2-It is extremely good for healthy eyes and skin.

3-Provides the best protein supplemented food for undernourished children.

4-Serves as a good source of vitamins, amino acids, minerals, crude fibers, etc.

“Single cell proteomics is becoming a practical reality and we are now able to routinely measure large cohorts of single cell proteomes to reveal new biological information. The Evosep One has been pivotal to scale this robustly to thousands of real single cell measurements, making an ideal and standardized platform for future biomarker discovery work”

Professor Matthias Mann, Max-Planck Institute of Biochemistry,
Proteomics and Signal Transduction

Meet our team

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Nourhan Mohamed Ali

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Khloud Farouk Fouad

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Hend Khaled Lotfy

Team member

Salma Ahmed Nady

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Hala Khaled Mohamed

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Thank you

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