Mass Spectrometry-based bottom-up proteomics and its applications in cancer research: an overview

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Researcher Live Event
28.07.2022

Synopsis:

Sample preparation

SCX | SPE I IP (robot)

MS data acquisition

MS/MS Spectra

Orbitrap

TOF: Time of Flight

Ion mobility
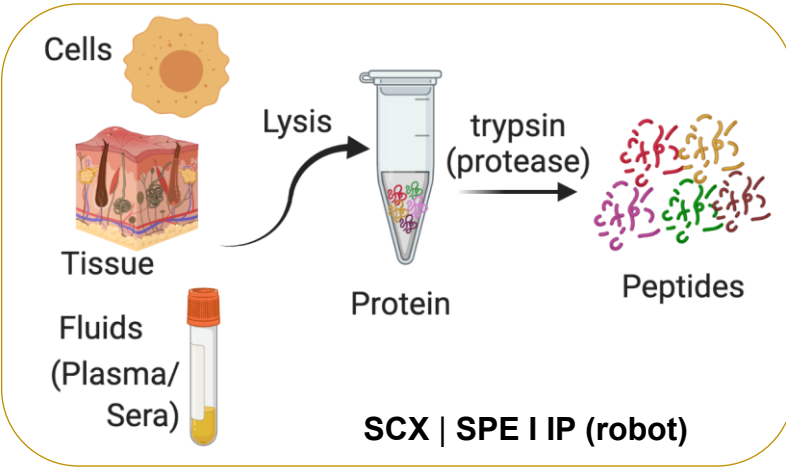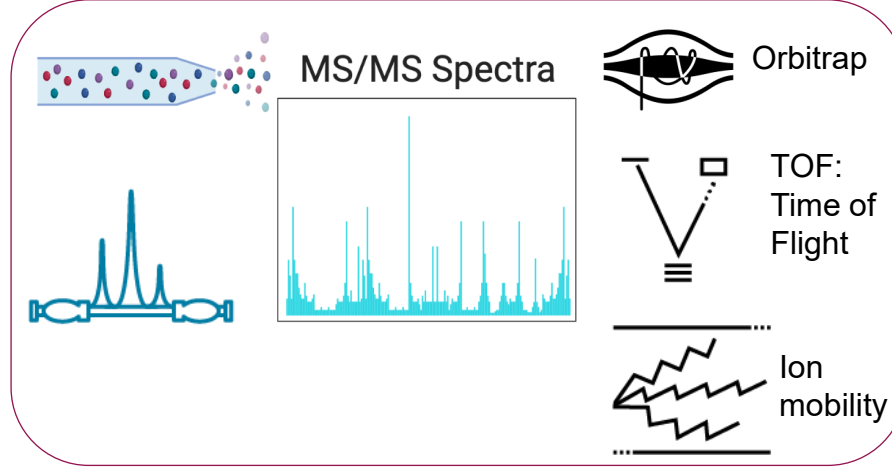
Enrichment analysis/ Data visualization

Perseus | R Studio

MS data extraction

UniProt

protein IDs

MSFRAGGER

MaxQuant | DIA-NN | Spectronaut
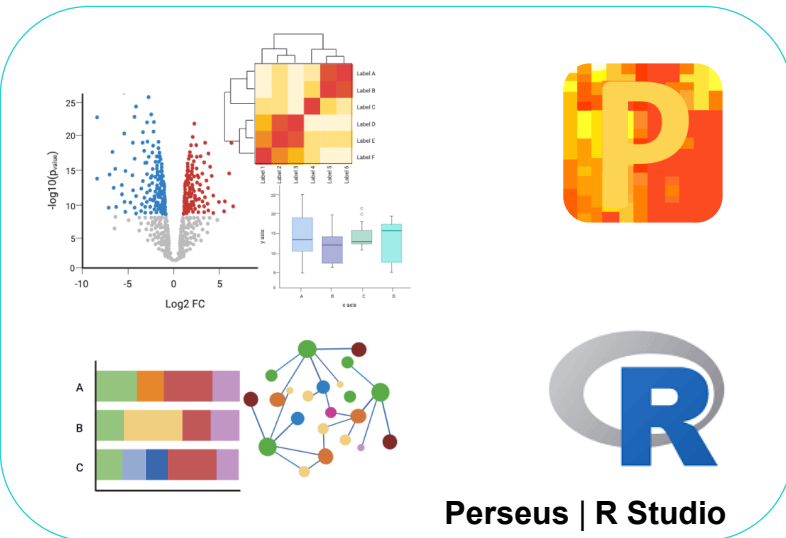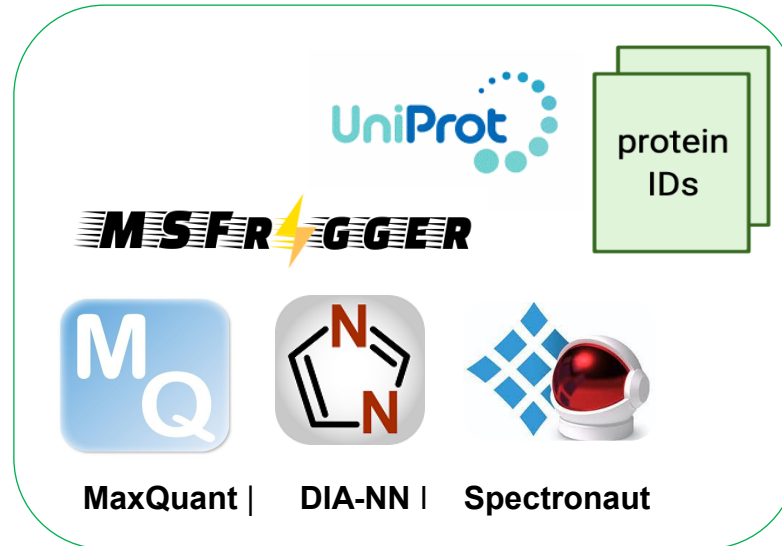
Application in cancer biology

Protein Expression Profiling Identifies Key Proteins and Pathways Involved in Growth Inhibitory Effects Exerted by Guggulsterone in Human Colorectal Cancer Cells

Rari Leo [1], Lubna Therachiyil [1,2], Sivaraman K. Siveen [1], Shahab Uddin [1], Michal Kulinski [1], Joerg Buddenkotte [1,3], Martin Steinhoff [1,3,4,5,6] and Roopesh Krishnankutty [1,*]

[1] Translational Research Institute, Academic Health System, Hamad Medical Corporation, Doha 3050, Qatar; RLeo@hamad.qa (R.L.); LTherachiyil@hamad.qa (L.T.); SSivaraman@hamad.qa (S.K.S.); SKhan34@hamad.qa (S.U.); MKulinski@hamad.qa (M.K.); JBuddenkotte@hamad.qa (J.B.); MSteinhoff@hamad.qa (M.S.)
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check for updates

Abstract: Colorectal cancer (CRC) is a leading killer cancer worldwide and one of the most common malignancies with increasing incidences of mortality. Guggulsterone (GS) is a plant sterol used for treatment of various ailments such as obesity, hyperlipidemia, diabetes, and arthritis. In the current study, anti-cancer effects of GS in human colorectal cancer cell line HCT 116 was tested, potential targets identified using mass spectrometry-based label-free shotgun proteomics approach and key pathways validated by proteome profiler antibody arrays. Comprehensive proteomic profiling identified 14 proteins as significantly dysregulated. Proteins involved in cell proliferation/migration, tumorigenesis, cell growth, metabolism, and DNA replication were downregulated while the protein with functional role in exocytosis/tumor suppression was found to be upregulated. Our study evidenced that GS treatment altered expression of Bcl-2 mediated the mitochondrial release of cytochrome c which triggered the formation of apoptosome as well as activation of caspase-3/7 leading to death of HCT 116 cells via intrinsic apoptosis pathway. GS treatment also induced expression of p53 protein while p21 expression was unaltered with no cell cycle arrest. In addition, GS was found to inhibit NF-kB signaling in colon cancer cells by quelling the expression of its regulated gene products Bcl-2, cIAP-1, and survivin.

The Omics Cascade
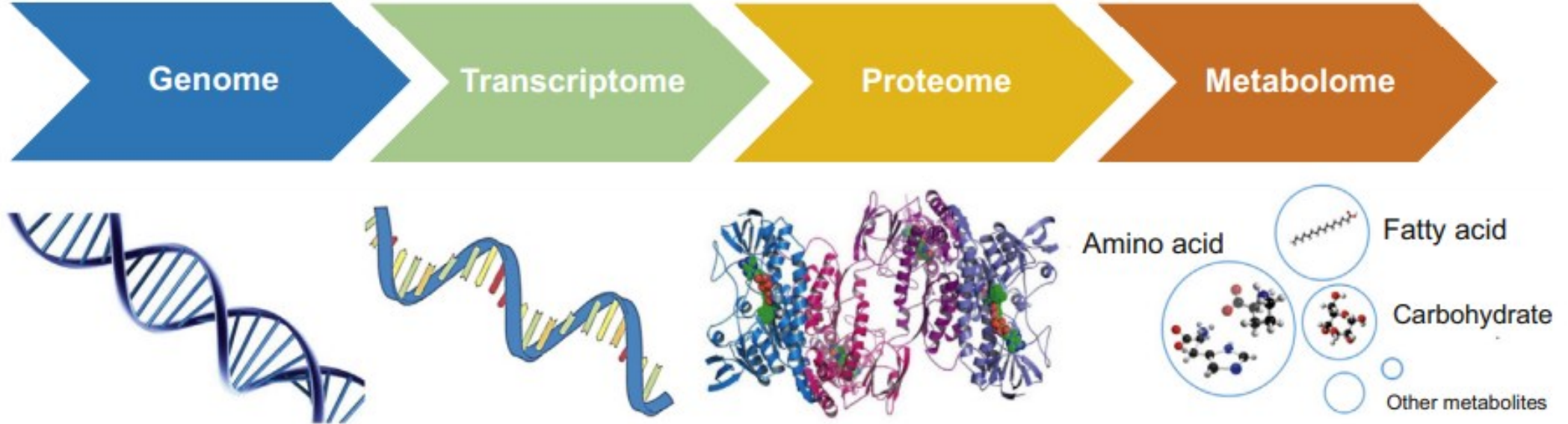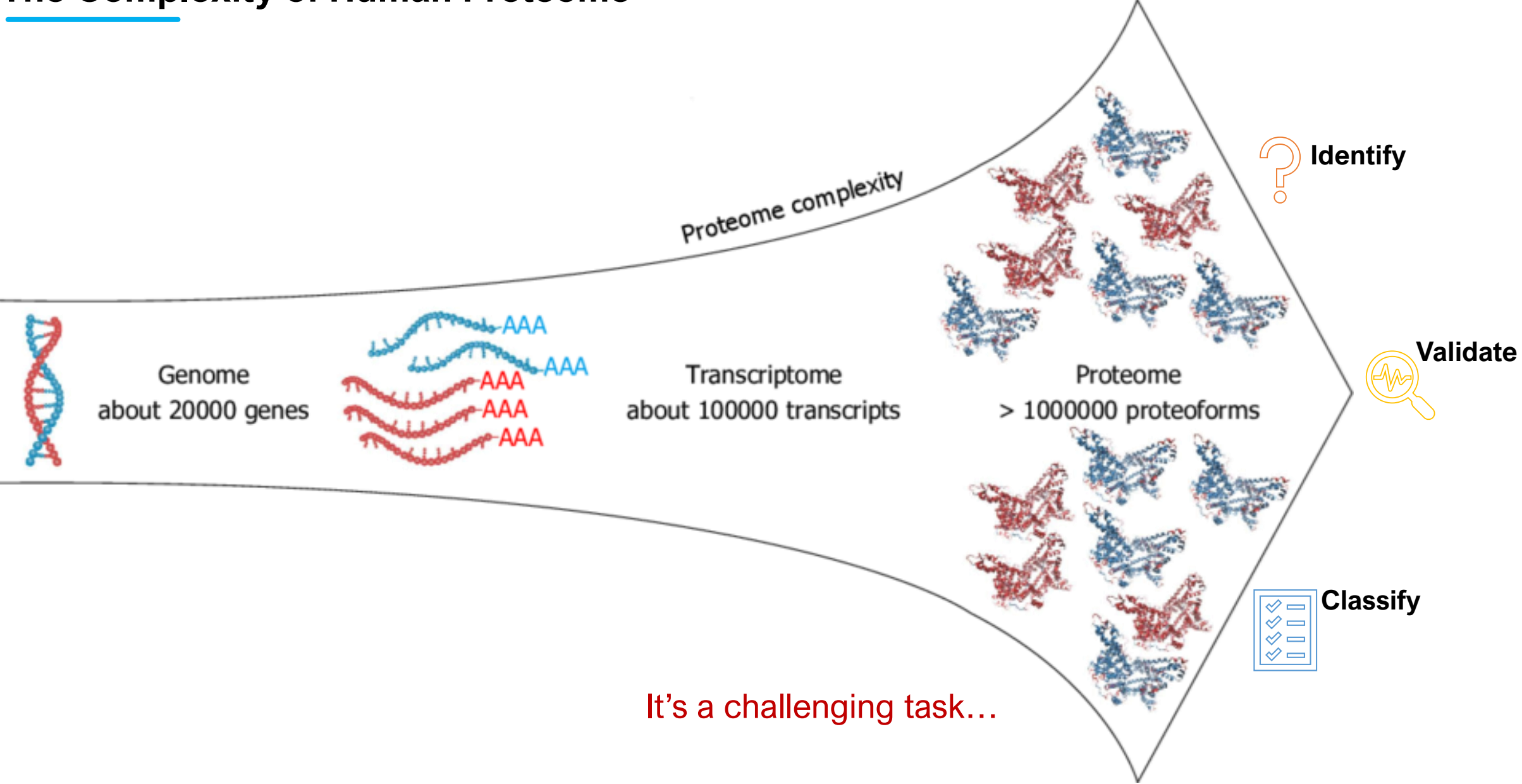

Proteome & Proteomics

'Proteome' forms the <u>entire set of proteins</u> expressed by an <u>organism</u>, <u>tissue</u> or <u>cell</u> at a <u>given condition</u>, at a <u>given time</u>.

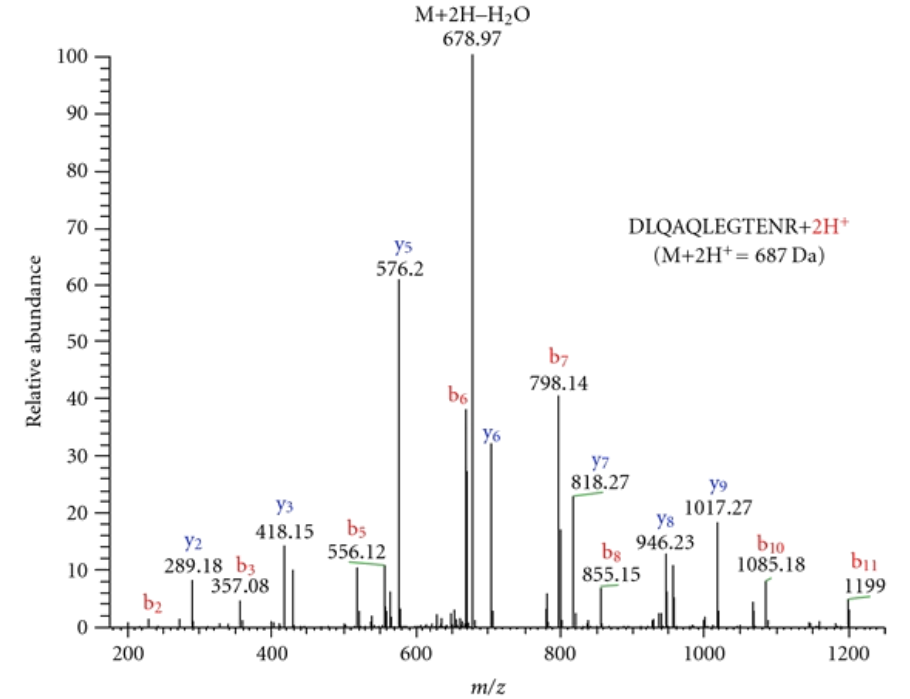**"Proteomics"** is a large-scale comprehensive study of a **<u>specific</u> 'Proteome'**.

The Complexity of Human Proteome

Genome
about 20000 genes

Transcriptome
about 100000 transcripts

Proteome
> 1000000 proteoforms

Proteome complexity

Identify

Validate

Classify

It's a challenging task…

Bennike S. et al., 2014

Mass spectrometry (MS)-based Proteomics

- **Mass Spectrometry** is a technique for detection and resolution of a sample of ions by their <u>mass-to-charge ratio (*m/z*)</u>.

b - ions

							y - ions
b_1	P		E	P	T	I	D	E	y_6
b_2	P	E		P	T	I	D	E	y_5
b_3	P	E	P		T	I	D	E	y_4
b_4	P	E	P	T		I	D	E	y_3
b_5	P	E	P	T	I		D	E	y_2
b_6	P	E	P	T	I	D		E	y_1

PEPTIDE

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Types of Proteomics Approach

Top-down proteomics

Protein mixture (Mass ≤ 50 kDa) → Separation of proteins → MS analysis of intact protein (≤ 50 kDa) → MS (Intact protein mass) → MS/MS (Protein sequence)

Middle-down proteomics

Protein (mixture) (No mass limit) → Digestion → Separation of peptides → MS analysis of peptides (~2000 – 20000 Da) → LC-MS (Intact peptide masses) → MS/MS (Peptide sequences)

Bottom-up proteomics

Protein (mixture) (No mass limit) → Digestion → Separation of peptides → MS analysis of peptides (~500 – 3000 Da) → LC-MS (Intact peptide masses) → MS/MS (Peptide sequences)

MS-based Proteomics: Read out

Proteome

Mass spectrometer

List of Proteins

Pros:

- High throughput
- Comprehensive analysis
- Sensitive
- Accurate

Cons:

- Labor intense
- Workflow optimizations
- Dynamic range

Shirle M. et al., 2012

Dynamic range of plasma proteins:

span >10 orders of magnitude in abundance.

Limit of detection

MS Sample Preparation:

Source

Protein extraction

Cells

Tissue

Fluids (Plasma/ Sera)

Lysis

Protein

Tryptic digestion

trypsin (protease)

Peptides

Sample clean-up

Manual

Plates (96-well)

Vials

Automate

Liquid handlers

Commercial systems

Agilent

Agilent Bravo system

PREOMICS

Preon system

Thermo SCIENTIFIC

AccelerOme

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MS Measurement Technologies:

Separation

HPLC

Electro spray ionisation (ESI)

Mass Spectrometer

MS/MS Spectra

LC Column

Capillary column
Commercial

Technologies

Orbitrap
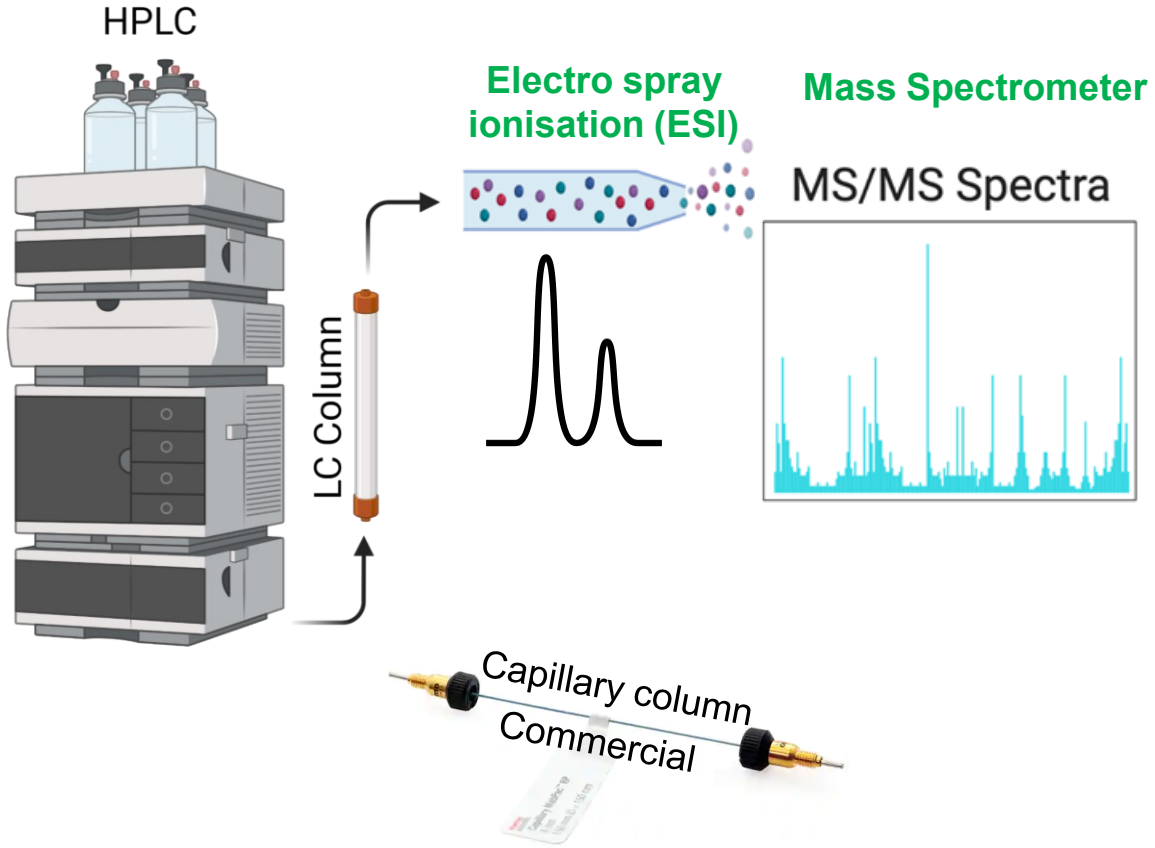
TOF: Time of Flight

Ion trap
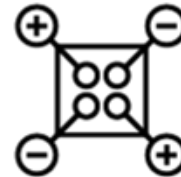
Ion mobility

Commercial systems

Fusion Lumos

Thermo SCIENTIFIC

Agilent

Agilent 6550

Qtrap 6500

SCIEX

BRUKER

tims TOF Pro
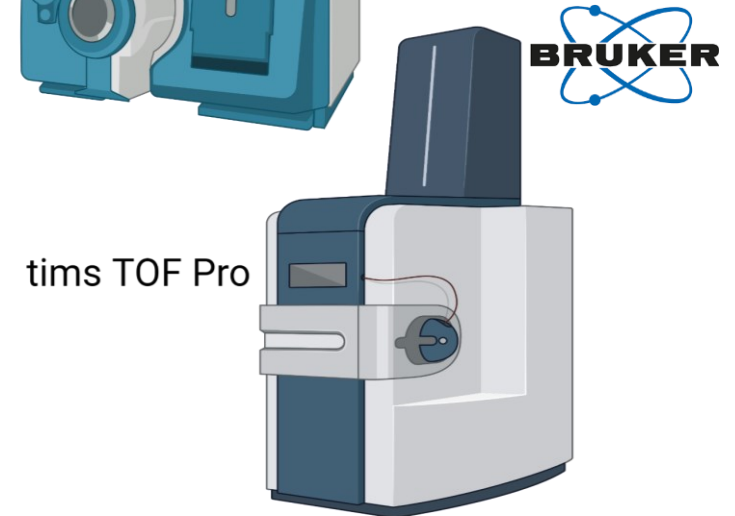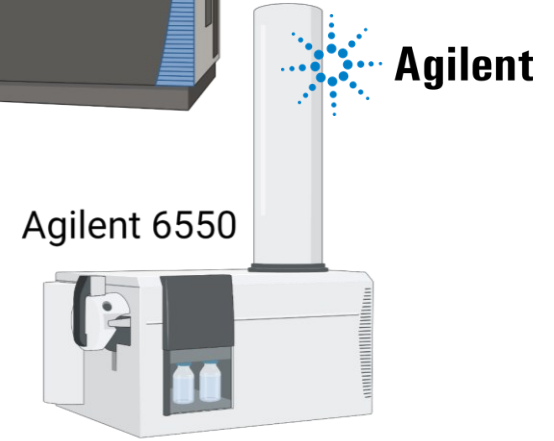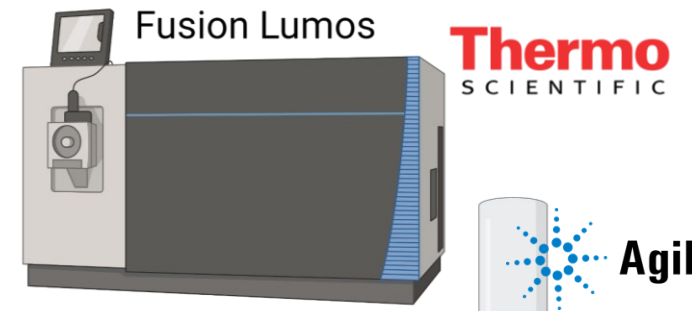
Proteomics: Improving MS acquisitions for in-depth coverage
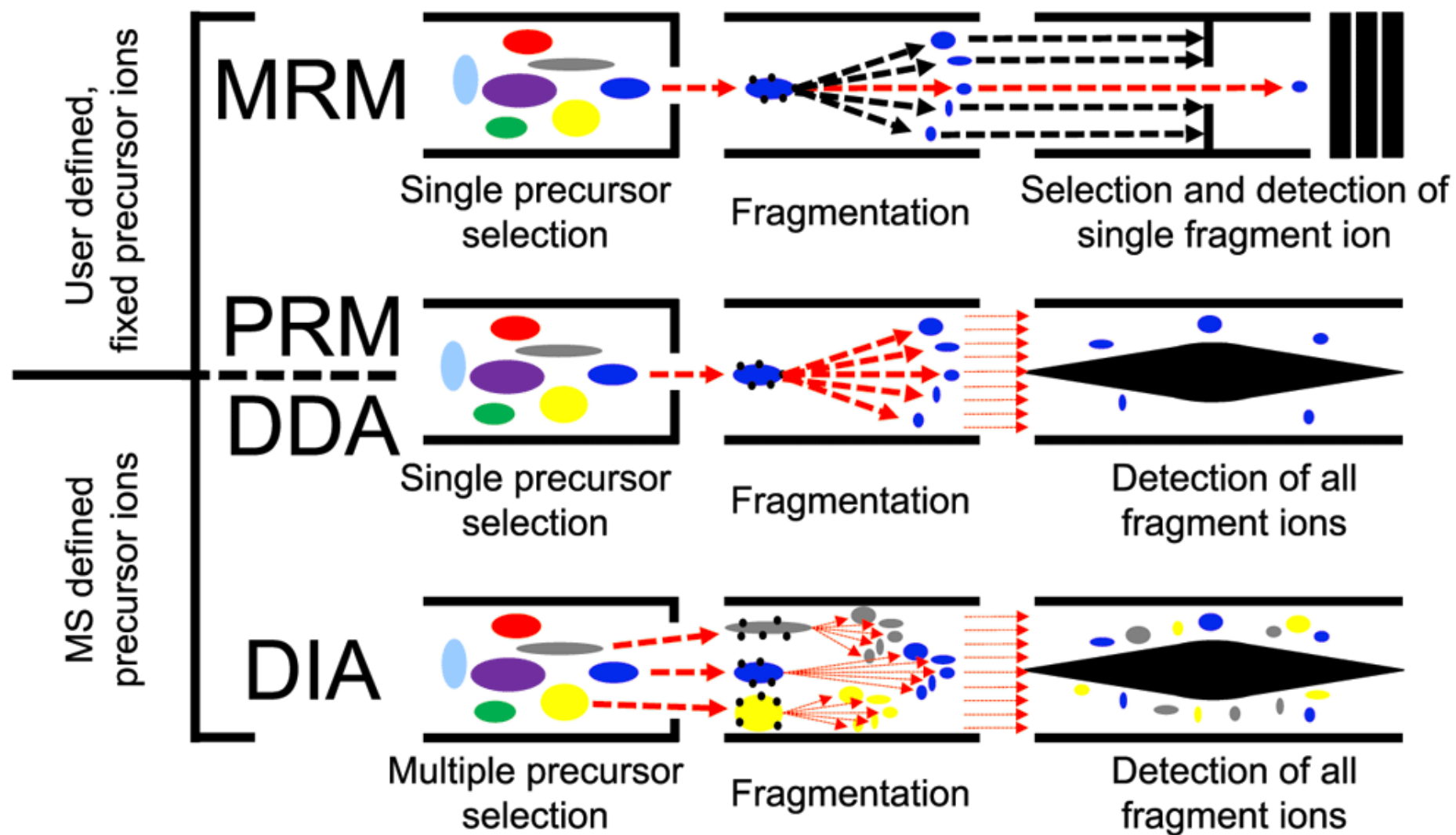
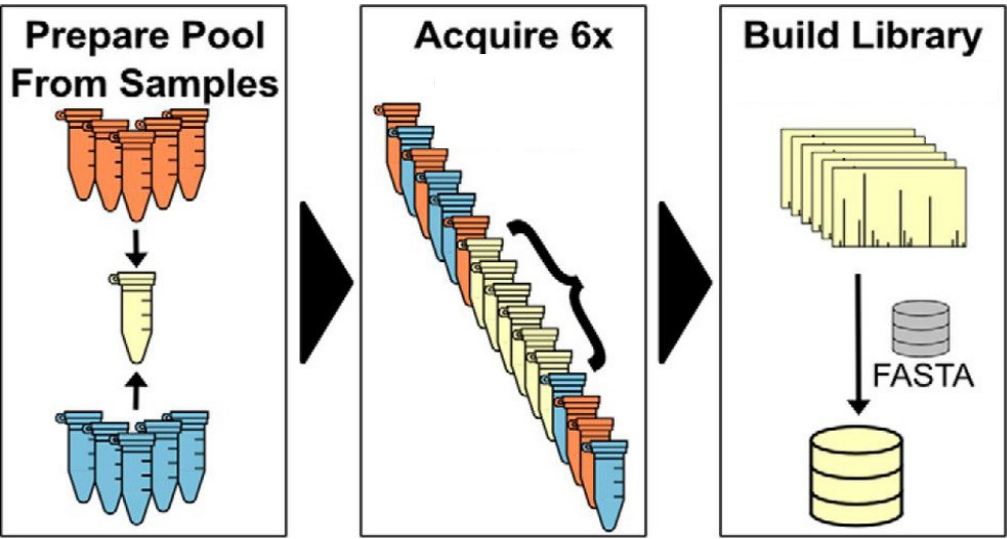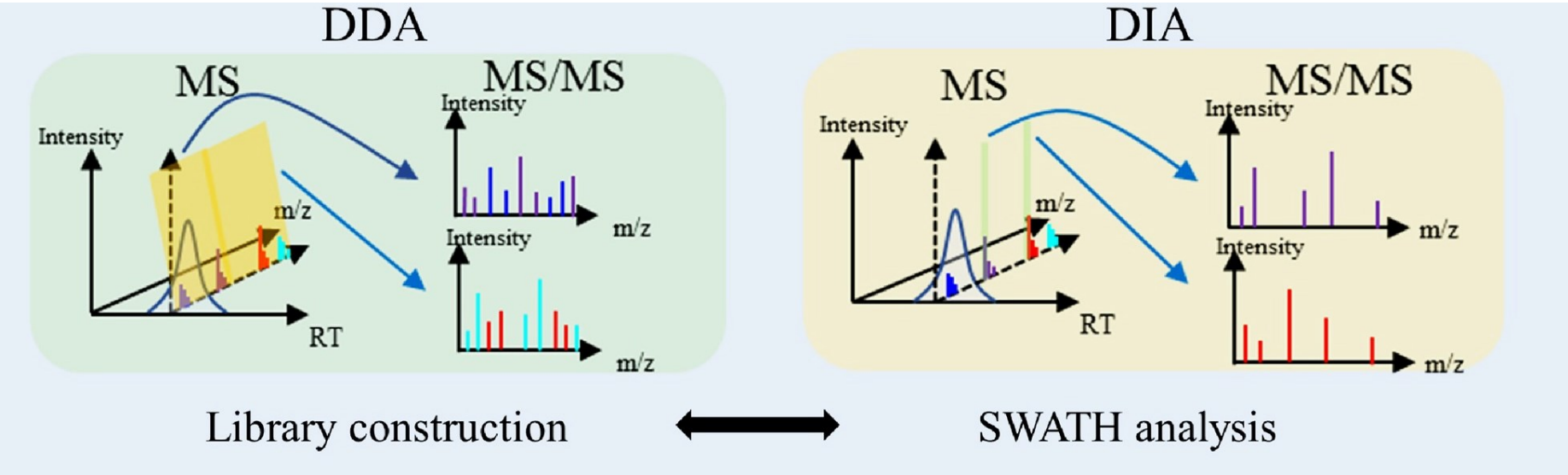# Improving MS acquisitions for in-depth proteome coverage:
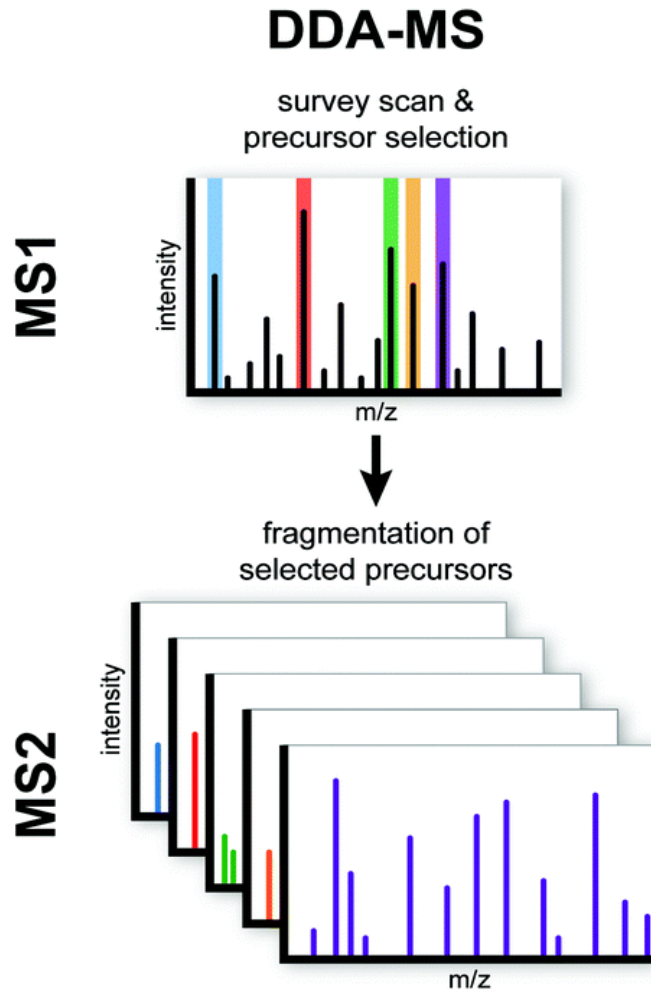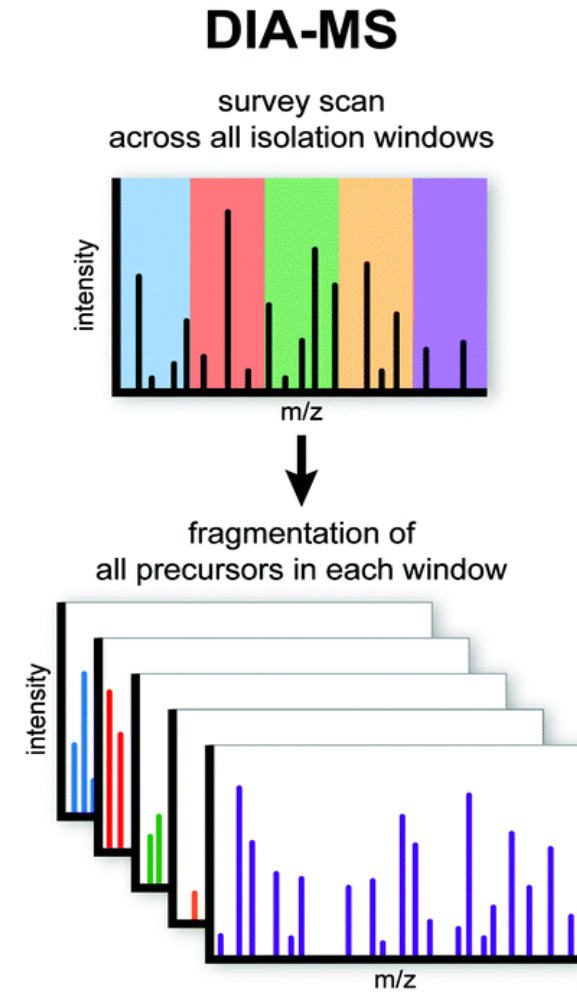
DIA is spectral library dependent

Hu, A. & Noble S.W., 2016
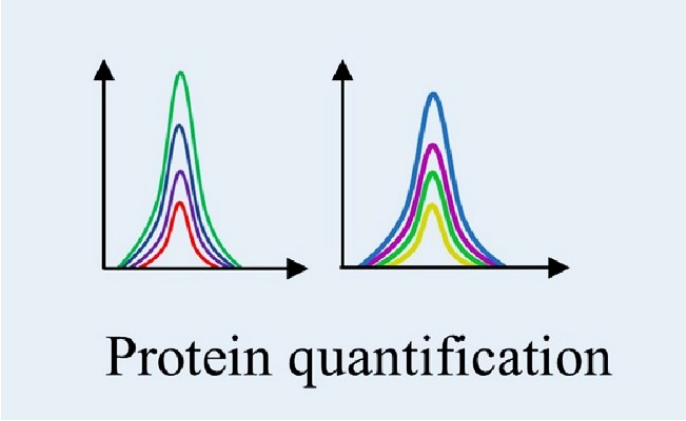
directDIA: Spectral library free DIA

- **Label Free proteome Quantification (LFQ)** with the high quantitative precision
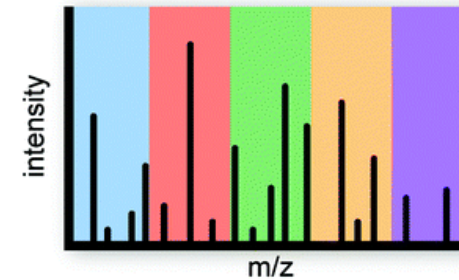

DIA-MS

Acq. Methods:
- DIA segments (m/z isolation windows)
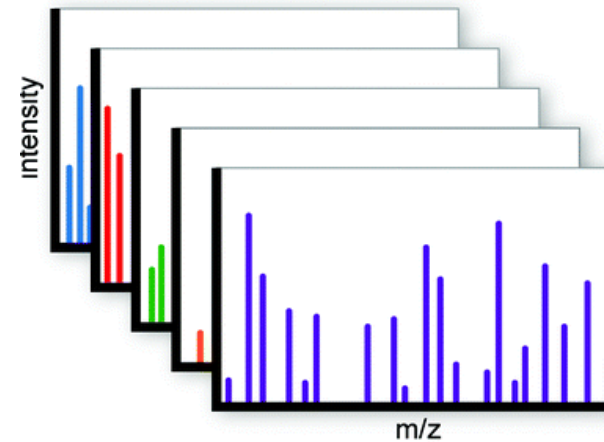- Scan range: 350-1650 Th
- ~70 segments (dynamic)

survey scan across all isolation windows

- "pseudo-spectra" derived from directDIA runs
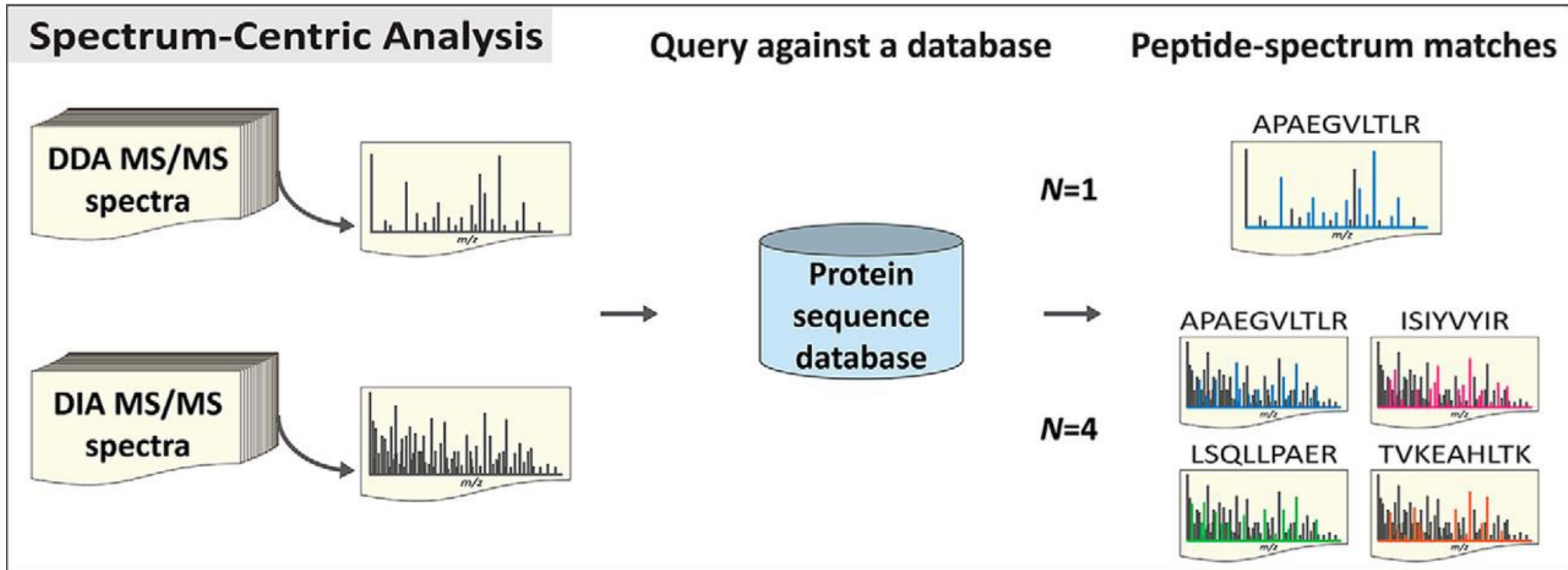- avoid the use of spectral libraries

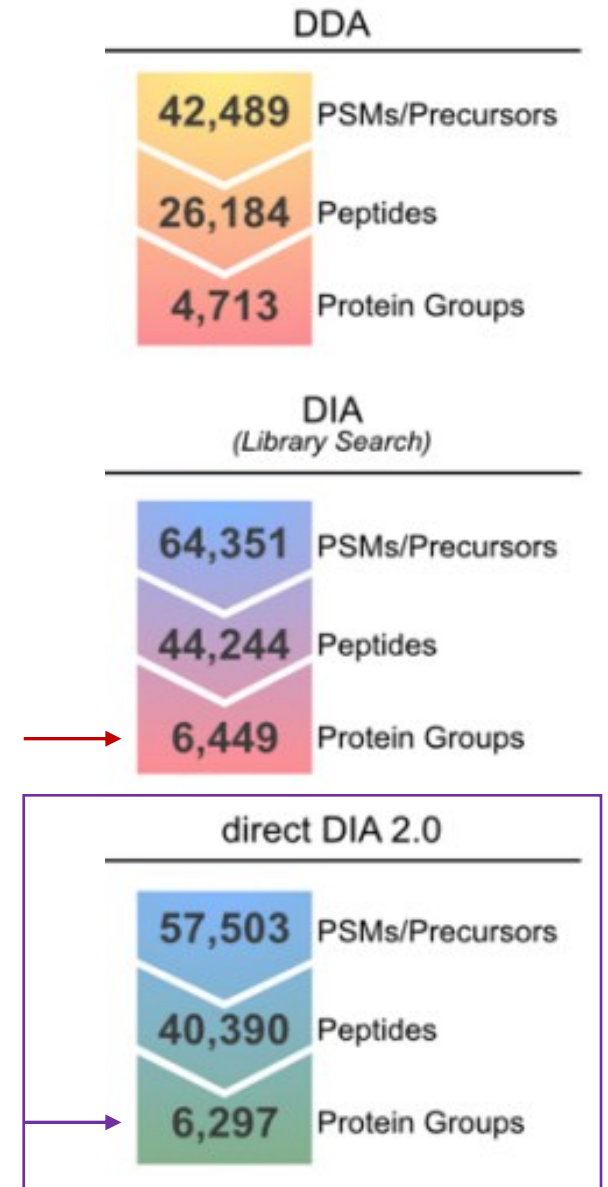fragmentation of all precursors in each window

directDIA:

Spectra searched in a **spectrum-centric approach** analogous to conventional DDA searches

- Each MS/MS spectrum (DDA/DIA) is queried against a protein sequence database
- Peptides that yield the **best scoring** *N* statistically significant PSMs are assigned to the corresponding MS/MS spectrum
- Typically, N is **one** for a DDA spectrum and **multiple** for a DIA spectrum

directDIA: in-depth coverage with < instrument time

- **Reproducible** and **precise quantification** of thousands of proteins in a single sample **without** the need for DDA based **spectral libraries**

- **Simple workflow** for label-free proteome quantification

- **Significant savings in instrument time** while maintaining high quantitative precision

- **High reproducibility** at the same level as the targeted analysis of DIA data using spectral libraries

DDA
- 42,489 PSMs/Precursors
- 26,184 Peptides
- 4,713 Protein Groups

DIA
(Library Search)
- 64,351 PSMs/Precursors
- 44,244 Peptides
- → 6,449 Protein Groups

direct DIA 2.0
- 57,503 PSMs/Precursors
- 40,390 Peptides
- → 6,297 Protein Groups

Mehta D. et al, 2020

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MS data Extraction/ Database search tools

List of Proteins

Database Accession #	Protein Name
P10809	60 kDa heat shock protein, mitochondrial
P08238	Heat shock protein HSP 90-beta
P07900	Heat shock protein HSP 90-alpha
P14625	Endoplasmin
Q12931	Heat shock protein 75 kDa, mitochondrial
P14618	Pyruvate kinase PKM
P49327	Fatty acid synthase
P13639	Elongation factor 2
P06733	Alpha-enolase
P11021	78 kDa glucose-regulated protein
P11142	Heat shock cognate 71 kDa protein
P0DMV8	Heat shock 70 kDa protein 1A
P63261	Actin, cytoplasmic 2
P06576	ATP synthase subunit beta, mitochondrial
P07437	Tubulin beta chain
P68371	Tubulin beta-4B chain

MaxQuant

DIA-NN

Spectronaut

Protein database

protein IDs

MS Search | Spectrum Summary | Build TIC | Workflows | Too

Data Visualisation/ Enrichment Analysis

Data Visualisation tools
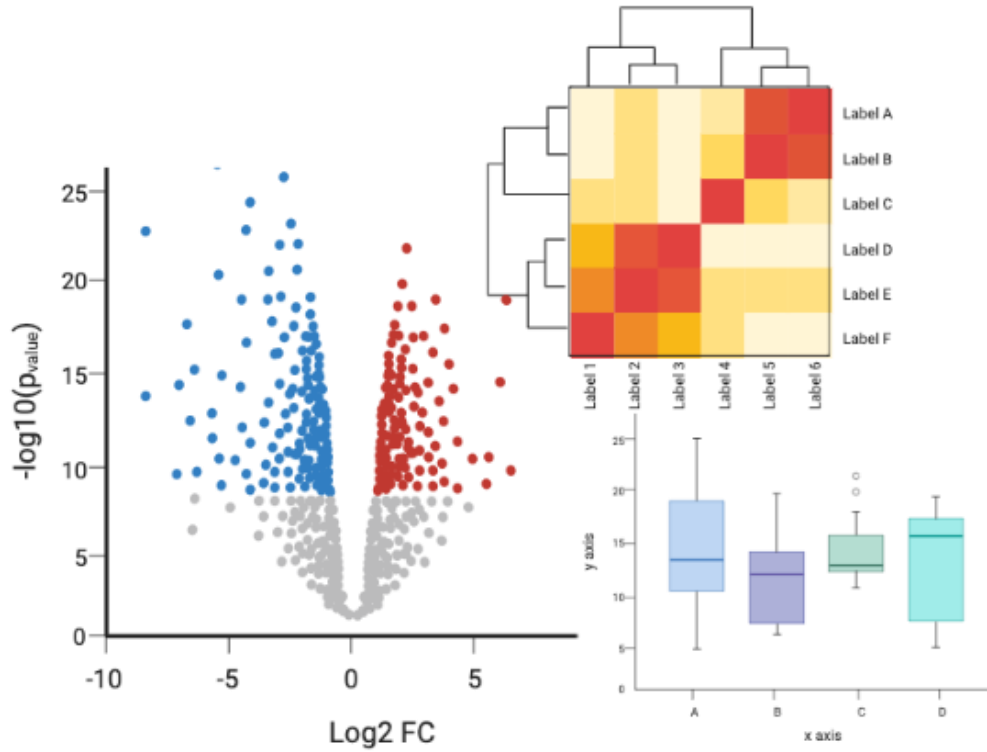
Enrichment Analysis/
Predicted Protein-Protein Interaction

Perseus

R/ R Studio

cancers

MDPI

Protein Expression Profiling Identifies Key Proteins and Pathways Involved in Growth Inhibitory Effects Exerted by Guggulsterone in Human Colorectal Cancer Cells

Rari Leo [1], Lubna Therachiyil [1,2], Sivaraman K. Siveen [1], Shahab Uddin [1](ID), Michal Kulinski [1], Joerg Buddenkotte [1,3], Martin Steinhoff [1,3,4,5,6] and Roopesh Krishnankutty [1,*](ID)

[1] Translational Research Institute, Academic Health System, Hamad Medical Corporation, Doha 3050, Qatar; RLeo@hamad.qa (R.L.); LTherachiyil@hamad.qa (L.T.); SSivaraman@hamad.qa (S.K.S.); SKhan34@hamad.qa (S.U.); MKulinski@hamad.qa (M.K.); JBuddenkotte@hamad.qa (J.B.); MSteinhoff@hamad.qa (M.S.)

[2] Department of Pharmaceutical Sciences, College of Pharmacy, Qatar University, Doha 2713, Qatar

[3] Department of Dermatology and Venereology, Hamad Medical Corporation, Doha 3050, Qatar

[4] Department of Medicine, Weill Cornell Medicine-Qatar, Qatar Foundation-Education City, Doha 24144, Qatar

[5] Department of Medicine, Weill Cornell Medicine, 1300 York Avenue, New York, NY 10065, USA

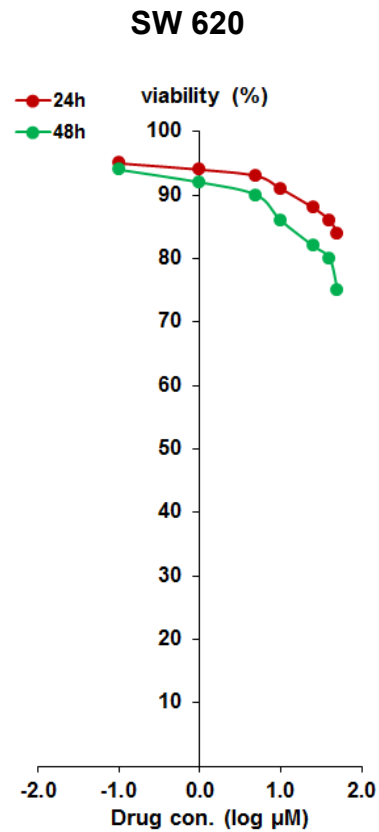[6] College of Medicine, Qatar University, Doha 2713, Qatar

[*] Correspondence: rkrishnankutty@hamad.qa; Tel.: +974-4439-0971

Guggulsterone (GS) inhibits proliferation of HCT 116 cells

A

HCT 116

- 24h
- 48h
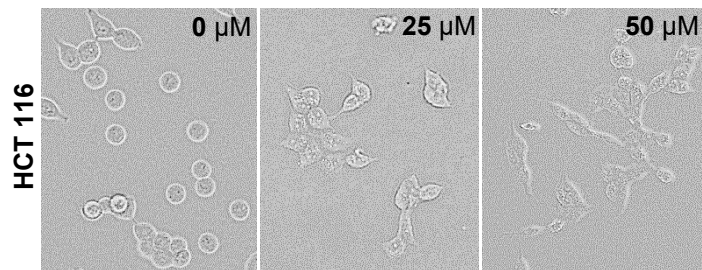
viability (%)

B
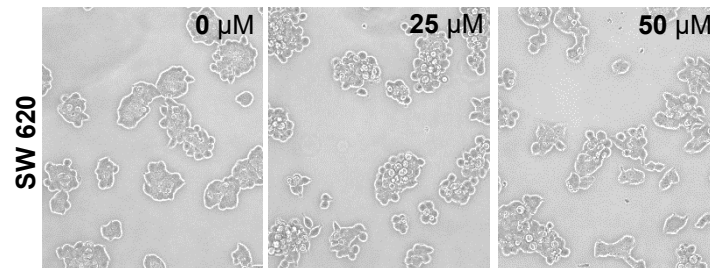
SW 620

- 24h
- 48h

viability (%)
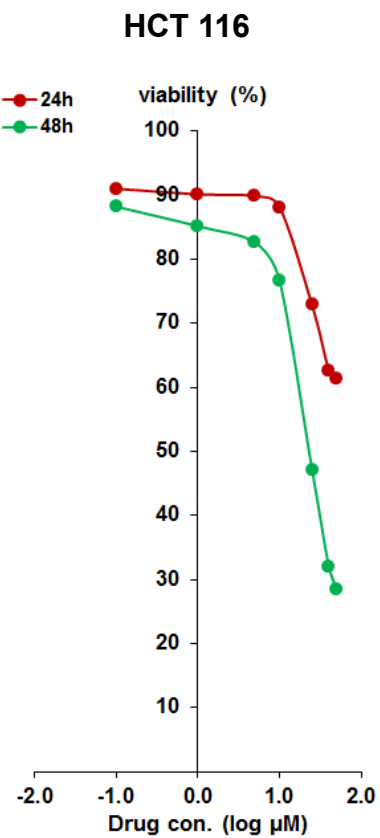
- GS treatment resulted in significant morphological changes in HCT 116 cells with loss of cell integrity as well as blebbing compared to the untreated intact cells.
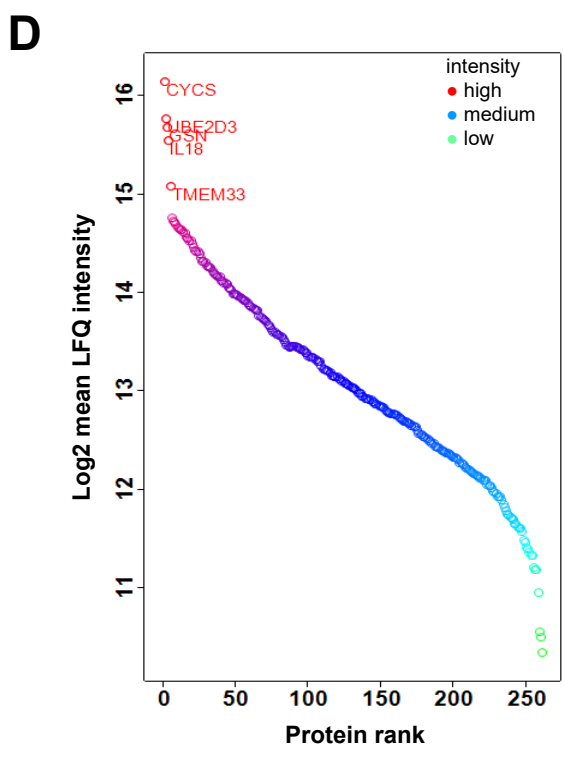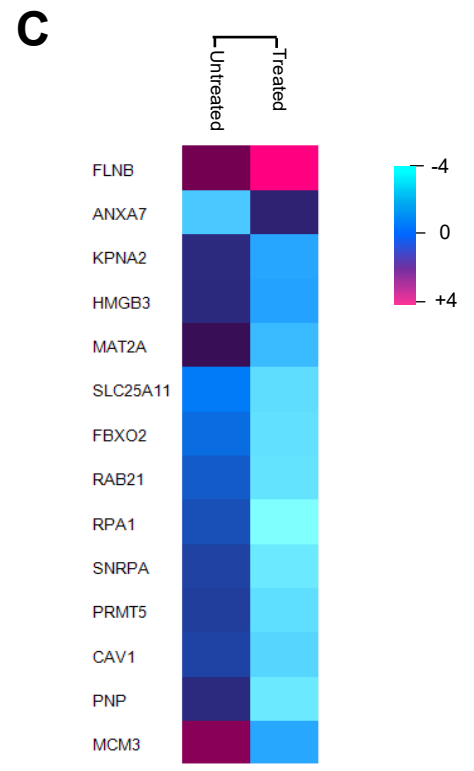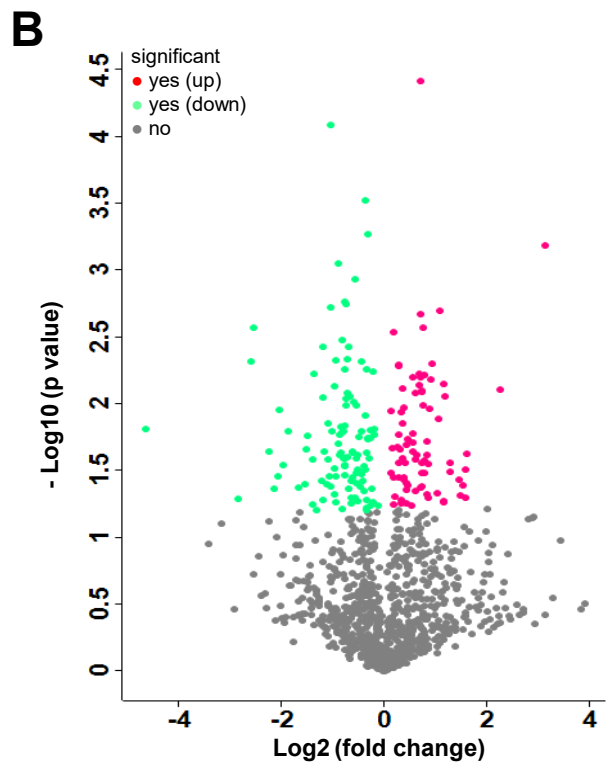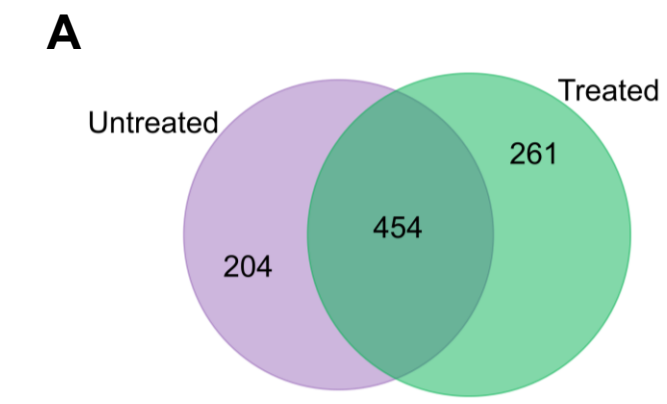
C

HCT 116

0 µM   25 µM   50 µM

D

SW 620
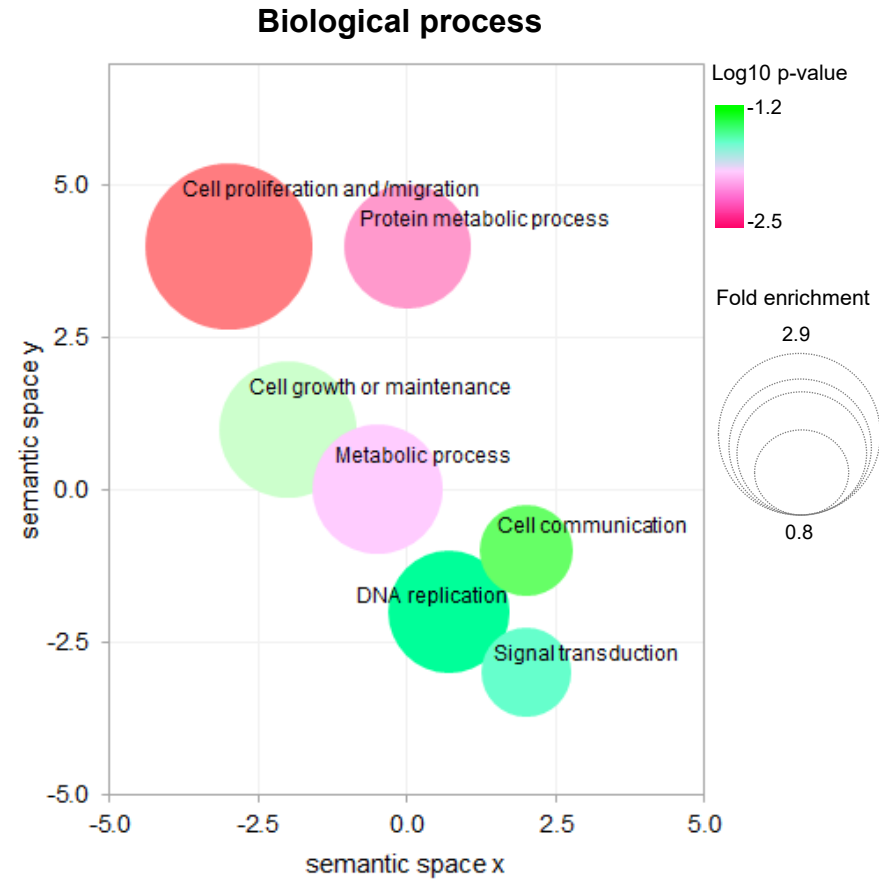
0 µM   25 µM   50 µM

Proteomics data interpretation and visualization

- The proteins significantly upregulated are red dots and downregulated are green dots, while the grey dots represent the proteins with unaltered expression. The *p*-value < 0.05 was used for this significance cutoff.

Functional annotation and classification of dysregulated proteins by GS treatment
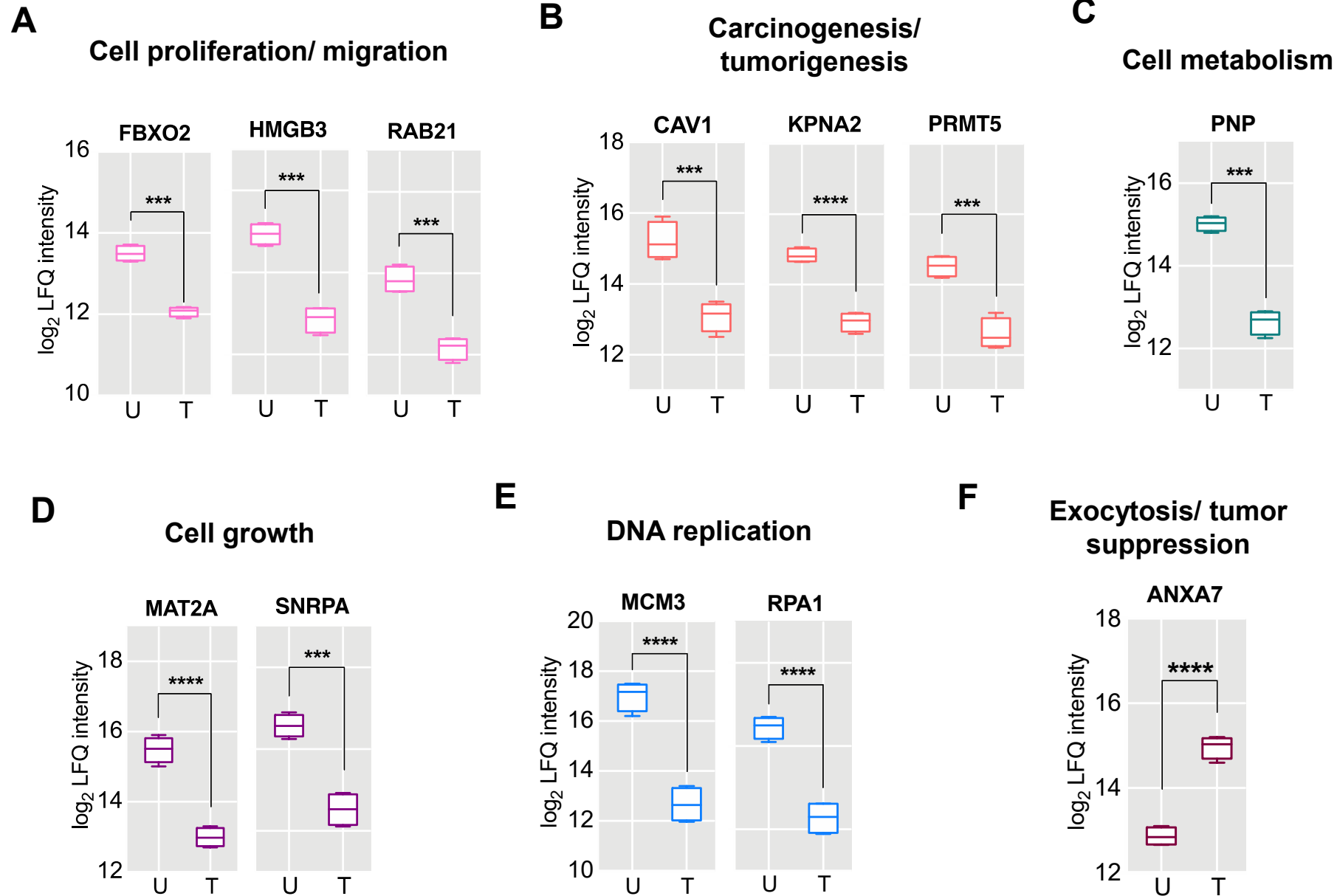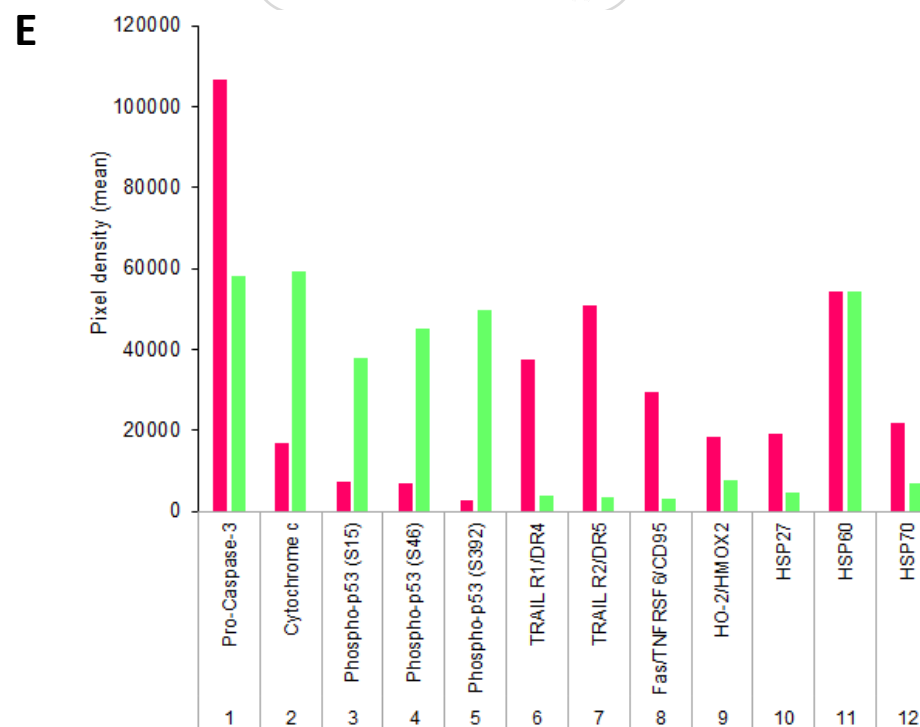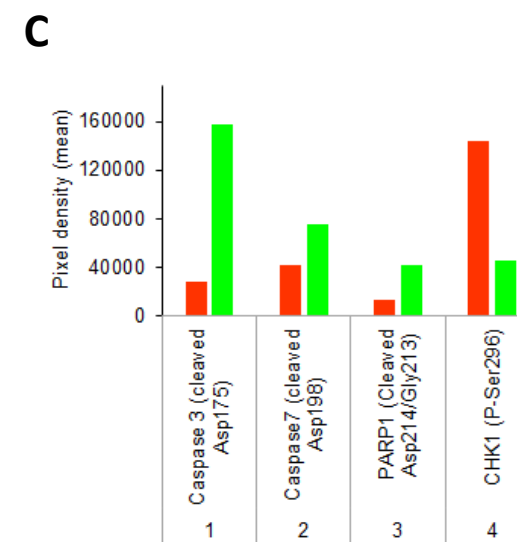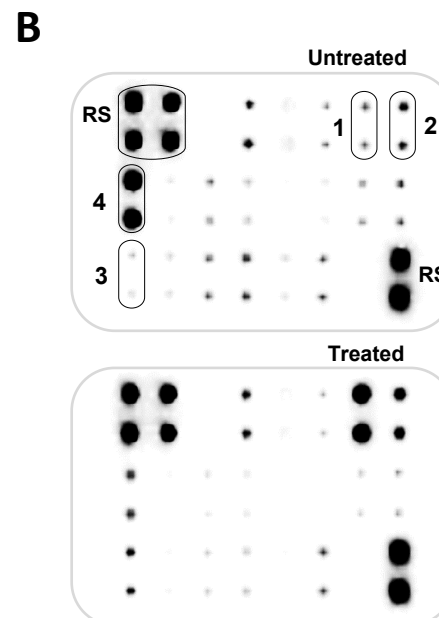

A

Cellular component

B

Biological process
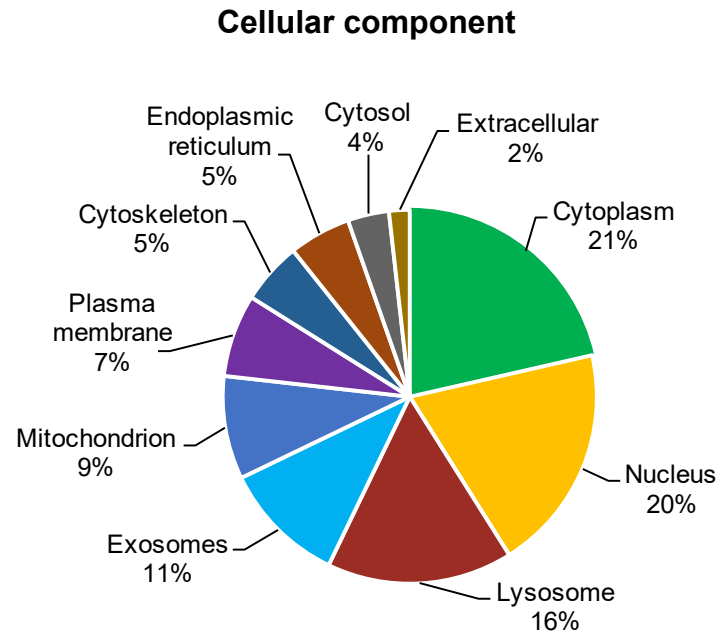
Proteomic signatures of GS treated HCT 116 cells

A Cell proliferation/ migration

B Carcinogenesis/ tumorigenesis

C Cell metabolism

D Cell growth

E DNA replication

F Exocytosis/ tumor suppression
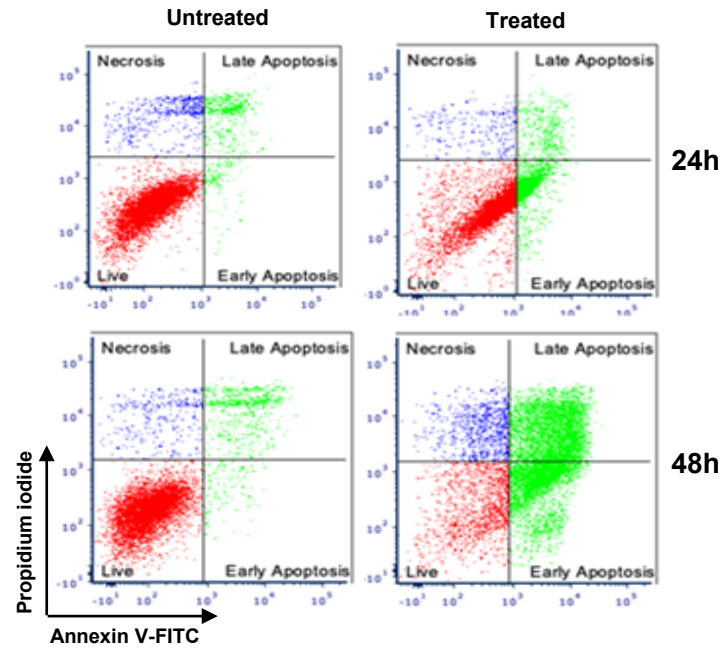
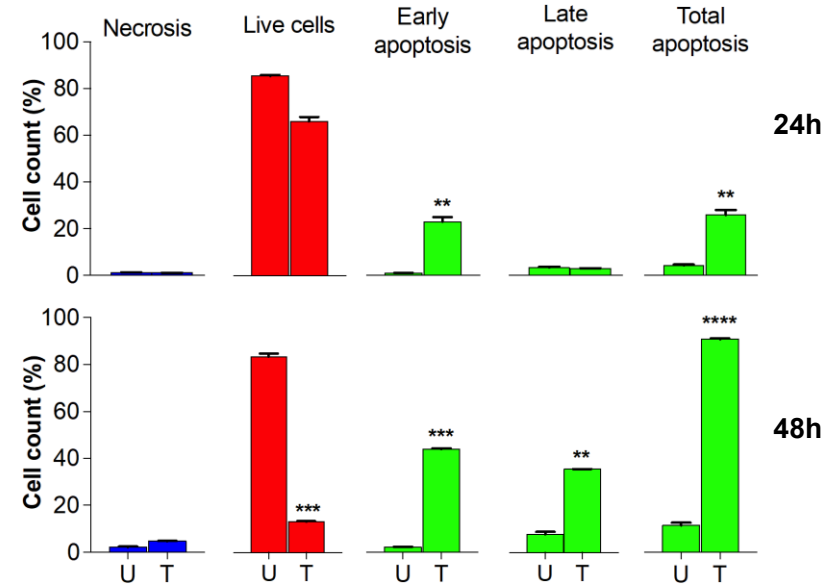# Biological pathways enriched by GS treatment and their validation by antibody arrays
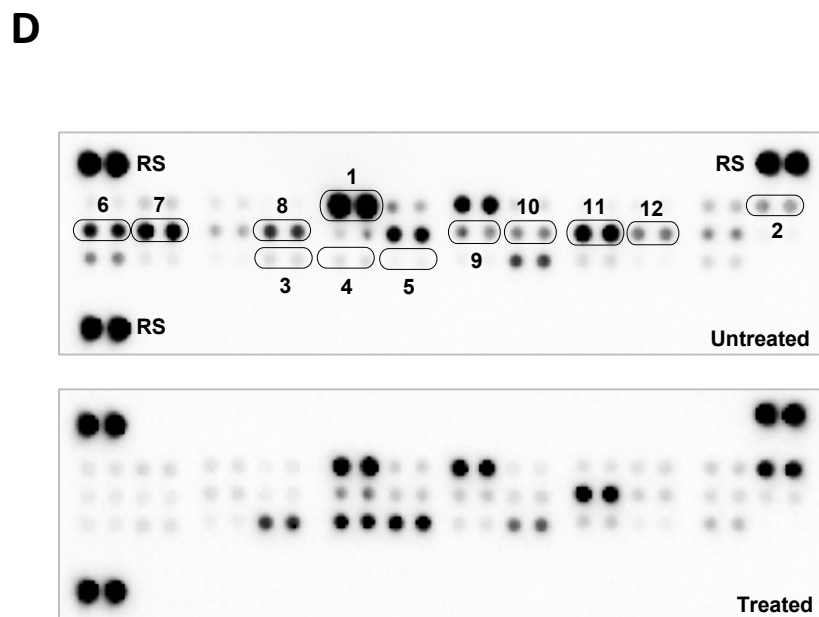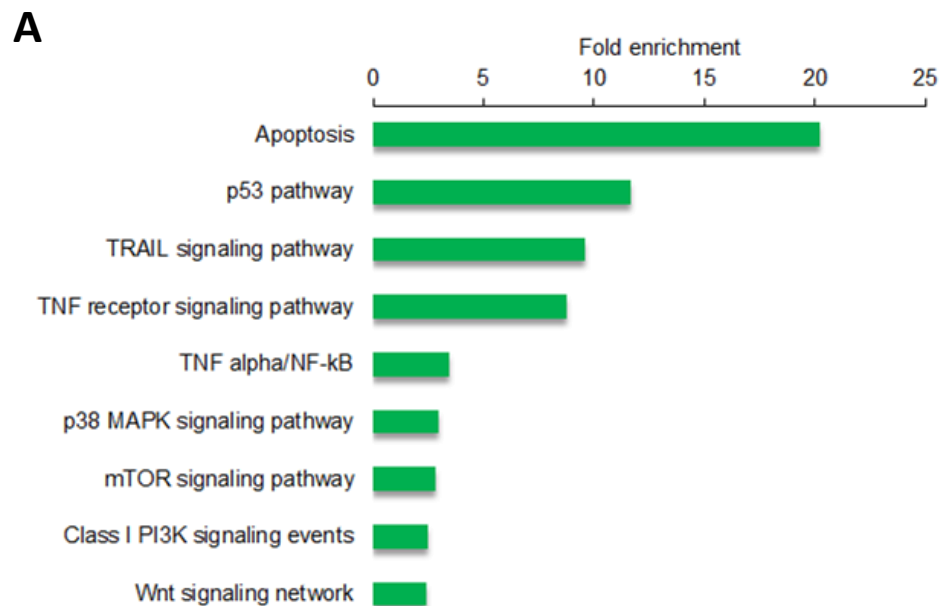
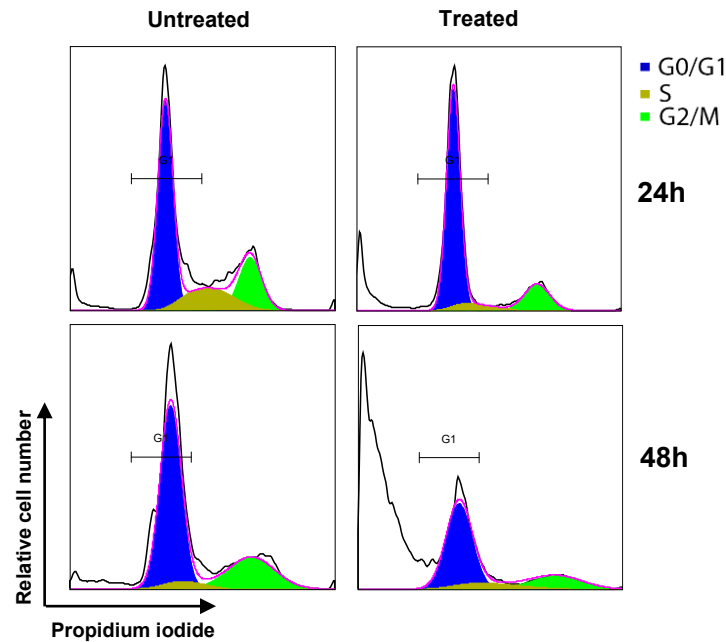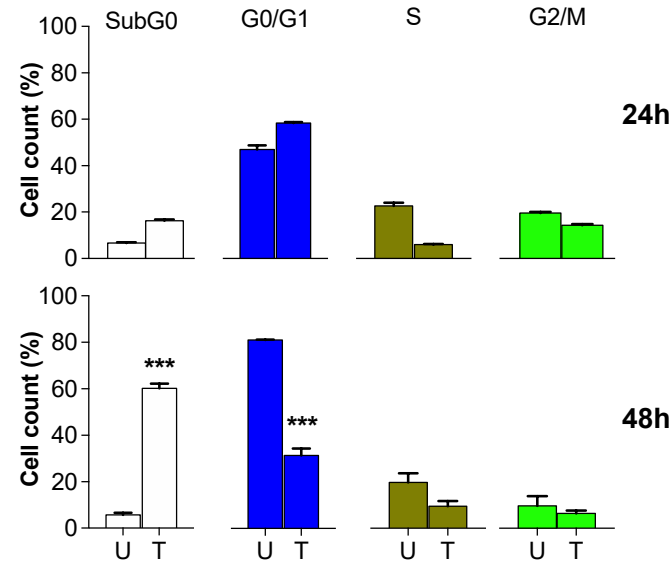# GS induced apoptosis in HCT 116 cells but no cell cycle arrest

Summary of the study:

- Guggulsterone treatment in HCT 116 cells lead to induction of p53-mediated intrinsic apoptosis resulting in significant cell death. It significantly reduced the cell proliferation and migration as well as inhibited the NF-kB signaling

- The LC-MS/MS based label-free proteomics approach facilitated comprehensive profiling of protein expression changes, thereby confidently identifying the proteomic signatures

- The data obtained from functional assays further enhanced the reliability of the GS targets we identified

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Acknowledgments

Current Trend in MS-based Proteomics: Single Cell Proteomics (SCP)

- Conventional bulk cell experiments mask the cell heterogeneity in the population
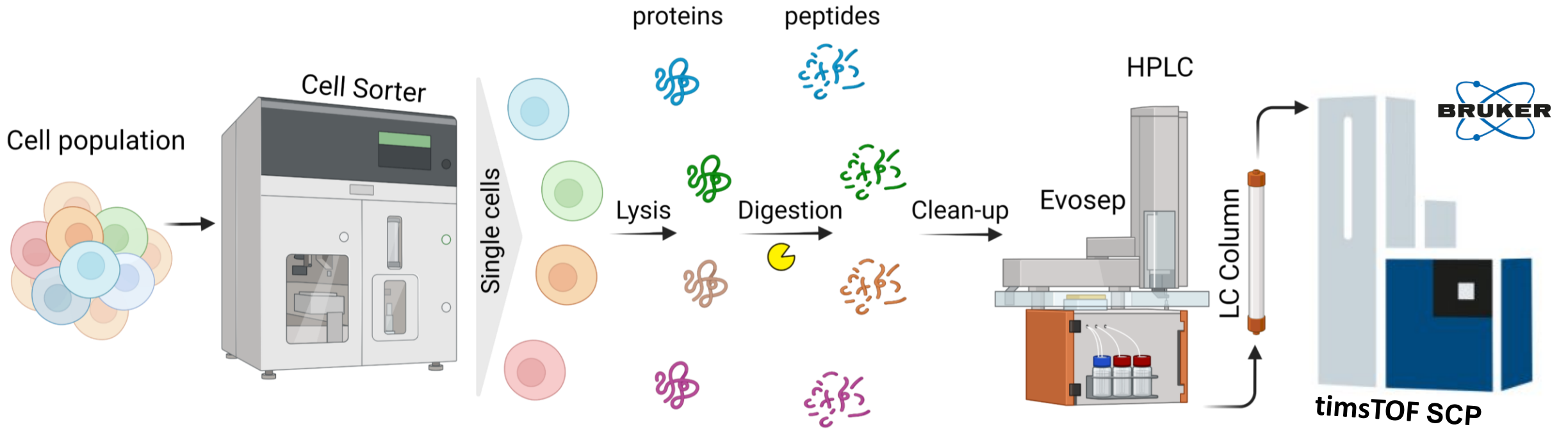
- Single-cell proteomic technologies will bring new insights into:
  signaling network regulation, cell heterogeneity, tissue architecture, disease diagnosis, and treatment monitoring.

Dr. Kristine Lennie
Managing Editor

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