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Review Article

Proteomics

Advances in Single-cell Proteomics

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Abstract:

Nowadays, single-cell proteomics emerges and applied for some critical realm both scientifically and medically. In single-cell analysis, biological variability can be attributed to individual cells, their specific state, and the ability to respond to external stimuli, which are determined by protein abundance and their relative alterations. Mass spectrometry (MS)-based proteomics (e.g., SCoPE-MS and SCoPE2) can be used as a non-targeted method to detect molecules across hundreds of individual cells. To achieve high-throughput investigation, novel approaches in Single-Cell Proteomics (SCP) are needed to identify and quantify proteins as accurately as possible. Many novel techniques associated single-cell proteomics gradually emerge in a sooner future along with the development of science and medicine. Herein, the research progress of this field has been intensively discussed. Moreover, the current bottleneck of the application of single-cell proteomics is also discussed.

Keywords: Single-Cell Proteomics, Research Progress, Current Challenge, Prospective.

INTRODUCTION

Exploring the Micro World

In the vast landscape of life sciences, cells as fundamental units of life have always been central to research. Traditional proteomics studies typically focus on cell populations, which can reveal overall protein expression patterns but fail to capture subtle differences between cells. However, numerous studies demonstrate that even within seemingly homogeneous cell populations, significant heterogeneity exists in protein expression among individual cells (Kelly, R.T. ., 2020). This heterogeneity plays a crucial role in processes such as development and disease progression.

Single-cell proteomics emerged as a breakthrough in this context, transcending conventional research limitations to

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analyze the proteome at the cellular level. Traditional bulk tissue analysis averages all the differences between cell diversity presented in most of the biological/ biomedical samples, whereas single-cell analysis allows the characterization of each individual cell, studying- at the single cell level-its genomics, transcriptomics, proteomics, metabolomics, and cell-cell interactions. This analysis enables the discovery and classification of unknown cell states (Slavov, N., 2020). This technology has opened a new window into the microscopic world, not only filling the gap in single-cell protein analysis within life sciences but also providing unprecedented perspectives for understanding biological processes and disease mechanisms. From revealing tumor cell heterogeneity to exploring unique signaling pathways between neurons, single-cell proteomics is increasingly becoming a driving force for advancing life sciences and medicine, guiding us to unravel the mysteries of the microscopic biological realm. New approaches and technologies for experimental design, sample preparation, data acquisition and data analysis have enabled the measurement of several thousand proteins in small subpopulations of cells and even in single mammalian cells (Derks J, *et al.*, 2022; Choi SB, *et al.,* 2022). These developments open exciting new opportunities for biomedical research (Figure 1).

WHAT IS SINGLE-CELL PROTEOMICS

Single-cell proteomics, a cutting-edge branch of proteomics, focuses on the qualitative and quantitative analysis of all proteins within individual cells. In multicellular organisms, cellular functions are not static but regulated by multiple factors. Even cells within the same tissue may exhibit vastly different protein expression patterns, with intercellular heterogeneity being the root cause of many biological phenomena. Complex biological processes are based on dynamic interactions between individual cells, involving in many cases multiple cell types as well as different states and susceptibilities (Carlota Arias-Hidalgo, *et al.,* 2022).The core objective of this technology is to comprehensively analyze protein expression levels, modification states, interactions, and subcellular localization within single cells, thereby providing deeper insights into cellular functions and regulatory mechanisms during specific physiological or pathological conditions. Proteomics aims to identify, characterize, and quantify all the protein isoforms in a cell, tissue, organ, or organism of interest (Perkel J.M, *et al.,* 2021). Global proteome measurements based on Mass Spectrometry (MS) and/

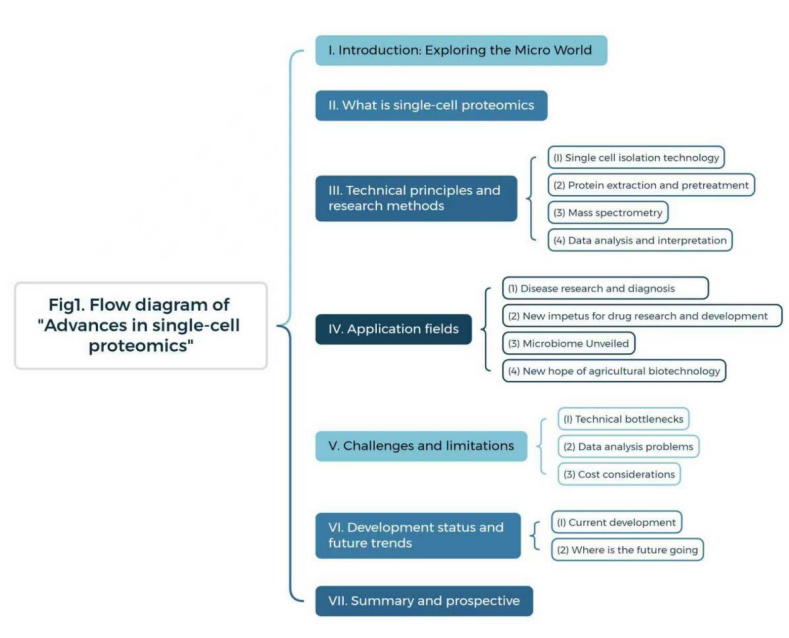

Figure 1: Flow diagram of advances in single-cell proteomics

or tandem mass spectrometry (MS/MS), which is used to improve the specificity of the mass spectrometer coupling two analyzers by using a collision cell) have been performed with biological samples that comprise thousands or millions of cells. This provides a quantitative protein expression profile but does not account for heterogeneity within the sample (Kelly, R.T. ., 2020).Currently, from a technical implementation perspective, single-cell proteomics integrates a series of advanced technologies, high-content data sets of single-cell genomic and transcriptomic data can be generated. For instance, researchers nowadays will be able to integrate single-cell mRNA and proteomic measurements (Marx, V. *et al.,* 2019). The abundance and role of many proteins are regulated by PTMs and degradation that cannot be inferred from genomic and transcriptomic approaches, making proteomics essential for determine protein patterns relevant to disease diagnosis and/or drugs response, among others. Furthermore, genomic and transcriptomic sequencing cannot directly explain protein localization and protein-protein interactions, which are critical for numerous signaling pathways (Franks, A.*et al.,* 2017; Liu, Y.S. *et al.,* 2016).

In the single-cell isolation phase, microfluidic technology, fluorescence-activated cell sorting (FACS), and Laser Capture Microscopy (LCM) are commonly employed to ensure precise extraction of individual cells from complex cell populations, providing purified samples for subsequent analyses. Taking microfluidic technology as an example, it enables manipulation of single cells within microchannel systems on microchips, achieving efficient capture and separation with advantages such as high throughput and minimal sample consumption. Protein extraction and purification require gentle yet efficient methods to prevent protein degradation and modification while maximizing protein information acquisition from extremely limited single-cell samples.

It is well known that Mass spectrometry serves as a pivotal technique in single-cell proteomics for protein identification and quantification. By enzymatically digesting proteins into peptides and measuring their mass-to-charge ratios (m/z), this method enables precise protein identification and quantitative analysis. Furthermore, antibody-based immunoassays such as flow cytometry and Western blotting are extensively employed in single-cell proteomics research. These techniques specifically recognize and detect target proteins, providing robust support for investigating

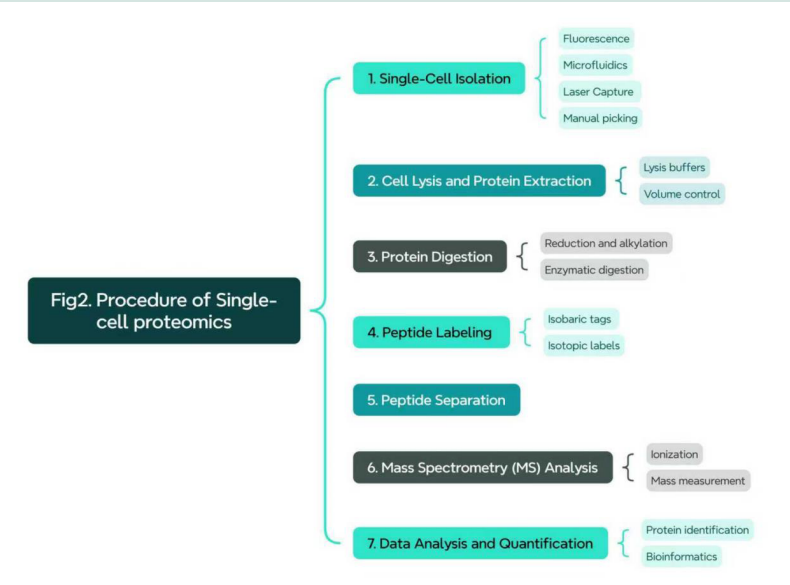

Figure 2: Procedure of single-cell proteomics.

their expression patterns and functional roles within individual cells.

TECHNICAL PRINCIPLES AND RESEARCH METHODS

The procedure of Single-cell proteomics is shown in Figure 2, which is detailed described as follows:

Single cell Isolation Technology

Single-cell isolation serves as the foundational step in single-cell proteomics research, where precise cell acquisition is critical for subsequent analyses. Single-cell isolation, which is crucial for single-cell analysis, ensures reliable cell isolation and quality control for further downstream analyses. Microfluidic chips are small lightweight systems that facilitate efficient and high-throughput single-cell isolation and real-time single-cell analysis on- or off-chip. Therefore, most current single-cell isolation and analysis technologies are based on the single-cell microfluidic technology. Traditional single-cell isolation techniques include limiting dilution (T. Bonnefoix, *et al.,* 1996), laser capture microdissection (LCM) (M. R. Emmert-Buck *et al.,* 1996), micromanipulation (H. Babbe, *et al.,* 2000;J. Fröhlich *et al.,* 2000), and fluorescence-activated cell sorting (FACS)(W. A. Bonner *et al.,* 1972). Although these are well-established techniques, they have both advantages and disadvantages. Owing to its simplicity, low cost, and good cell activity, limiting dilution has been widely used for decades to isolate monoclonal cells for antibody production (H. Gratzner, *et al.,* 1982; Y. Sayama, *et al.,* 2021). However, owing to the separation principle of continuous equal division, obtaining single cells has statistical randomness and low efficiency. According to the Poisson distribution, the maximum efficiency of single-cell separation was lower than 37%. Furthermore, microscopic imaging is necessary to determine the location of single cells, and the throughput is limited by the well plate (J. Harcourt, *et al.,* 2020). LCM, because of its ease of manipulation and in situ isolation, is often used to isolate specific pure cells for subsequent analyses. However, manual selection and isolation processes severely limit throughput and samples often require fixation, which compromises cell integrity and activity. Studies that require cell viability (e.g., proteins and exosomes) cannot be performed. In particular, when contact-based cell extraction methods are used, it cannot be determined whether adjacent cell debris is transferred to the matrix along with target cells (F. Fend, *et al.,* 2000). Micromanipulation is similar to LCM, and the targeted isolation of specific cells under a microscope is one of the main advantages of this technique. In addition, this method is applicable to various cell types and can selectively isolate live cells, including prokaryotic cells, from suspensions (M. Hohnadel *et al.,* 2018) with the advantages of flexible operation and good cell activity. However, the manual process of obtaining single cells using this method limits single-cell throughput, and additional observations of the target location are required to determine whether single cells are successfully transferred. Although the development of mechanical automation has made automated micromanipulation an alternative to manual work, its serial processing still limits single-cell throughput (Z. Lu, *et al.,* 2010).The advent of Fluorescence Activated Cell Sorting (FACS) has greatly increased single-cell throughput. Such systems can generate up to 100000 droplets per second and analyze up to 70000 cells per second. They are often used to isolate heterogeneous cell samples and have increasing prospects for diagnosis and treatment (N. Navin, *et al.,* 2011; L. A. Herzenberg, *et al.,* 2002). However, FACS also has certain limitations: the systems are typically bulky, mechanically complex, and expensive, and can only be used to analyze cells at fixed time points (S. M. Prakadan, *et al.,* 2017), cells must be in suspension, which leads to the loss of tissue structure and function in the adherent state (*R. R. Jahan-Tigh, et al., 2012*). In addition, the sorting shear force and detection laser can cause damage to cell activity (*M. Mollet, et al., 2008*), the most important limitation is that the cells isolated by FACS must be at a certain concentration, and the total number of rare cells cannot be processed. Over the past few years, numerous mature microfluidic chips have been developed for single cell isolation and analysis. Polydimethylsiloxane (PDMS) is the most widely used microfluidic material owing to its good light transmittance and low biological toxicity (*A. M. Unger, et al., 2000*).However, no single-cell chip can guarantee all excellent indicators simultaneously because some indicators are in conflict. When ensuring high-activity

single-cell isolation, it is often necessary to increase throughput and efficiency as much as possible while ensuring the space and activity of cell isolation. However, owing to the existence of the Poisson distribution, the isolation efficiency is insufficient, and certain cells are lost. In contrast, improving isolation efficiency requires reducing the physical size, which further increases cell throughput but inevitably reduces the space and cell activity indicators.

Flow cytometers (Han Y, *et al.,* 2015) employ laser or fluorescence labeling techniques to track and identify cells. When cells in the sheath fluid form a single-cell stream, laser excitation triggers fluorescence signals that are converted into electrical signals through detectors like photomultiplier tubes or avalanche photodiodes. By setting parameters such as fluorescence intensity and scattering light, these systems enable targeted cell sorting. Their advantages include high throughput (capable of processing thousands or even millions of cells simultaneously) and exceptional precision. However, these instruments come with significant costs, require strict sample preparation standards, and are susceptible to interference from factors like cellular aggregation and fluorescence compensation.

Micro-manipulation involves manually selecting individual cells using precision tools like microneedles and micropipettes under microscopic observation. This technique, renowned for its operational precision, is particularly effective for isolating rare or structurally unique cells. However, it heavily relies on the experiential expertise of technicians, resulting in low throughput and time-consuming procedures. The droplet method utilizes microfluidic chips to encapsulate single cells within microscopic droplets, which function as independent reaction units. This approach effectively minimizes intercellular contamination while enabling automated operations and increased throughput. Nevertheless, it requires advanced equipment and reagents, with strict control needed for the stability and uniformity of droplet generation.

The magnetic bead separation method (González Fernández C, *et al.,* 2020), based on antigen-antibody specificity, couples magnetic beads with specific antibodies. After incubation with cells under an applied magnetic field, the labeled beads separate target cells. This technique is relatively simple and minimally damaging to cells, making it commonly used for isolating specific cell types like immune cells. However, its specificity depends on antibody quality, and potential nonspecific binding may affect purity. Laser confocal scanning microscopy enables single-cell isolation by focusing laser beams on specific sample layers to acquire three-dimensional cellular images through layered scanning. This method excels in identifying and selecting target cells for studying subpopulations and cell-microenvironment interactions. Nevertheless, it requires complex equipment, high costs, and presents challenges in data analysis and processing.

In the microscopic realm of life sciences, cell separation technology serves as the key to unlocking deeper research. Traditional methods like density gradient centrifugation and magnetic bead sorting, while instrumental in early biological studies, exhibit inherent limitations. Density gradient centrifugation's resolution is constrained – separating cells with similar densities resembles identifying twins, making precise isolation challenging. Its operation remains prone to human interference, resulting in poor reproducibility. Magnetic bead sorting, though specific, risks compromising cell viability and functionality through residual beads, akin to introducing contaminants that impair precision. With limited throughput and processing capacity, it struggles to meet large-scale cell sorting demands, much like a small water pipe failing to handle substantial flow.

To overcome these technological bottlenecks, developing integrated microfluidic platforms (Gebreyesus ST, *et al.,* 2022) has emerged as a cutting-edge innovation. These platforms achieve large-scale parallel processing through integrated and miniaturized fluid manipulation technologies, essentially condensing a massive industrial facility into a precision compact laboratory. This breakthrough significantly enhances experimental efficiency and accuracy. With their compact size, operational flexibility, cost-effectiveness, and adaptability to integrate multiple functional modules, they enable automation of complex biological and chemical

processes. In biomedical applications, they enable full-process automation from sample collection to result analysis, improving diagnostic accuracy and efficiency. For chemical synthesis and analysis, they allow precise control of reaction conditions, accelerating research into novel materials and pharmaceutical development. For instance, microfluidic technology facilitates operations such as cell culture, gene sequencing, and protein analysis, driving advancements in personalized medicine and precision healthcare.

The adoption of novel separation technologies has emerged as a crucial pathway for advancement. Traditional cell sorting methods, which rely on specific surface markers and employ fluorescent labeling or antibodies, often cause cellular damage that compromises subsequent research outcomes. In contrast, emerging label-free or minimally invasive techniques—such as fluid dynamics-based sorting using microfluidic chips and dielectric electrophoresis—separate cells by leveraging their inherent physical properties like size, shape, and dielectric constant. These methods achieve high-purity isolation without external markers, minimize cellular disturbance and damage, and enable precise sorting in a «quiet» environment. This approach provides researchers with samples that closely resemble natural biological conditions, significantly enhancing the authenticity of downstream studies.

Protein Extraction and Pretreatment

Protein extraction from single cells employs various methods (Andrew Leduc, *et al.,* 2022). Physical techniques such as ultrasonic disruption utilize high-frequency vibrations to break cell membranes and release proteins, offering simplicity and efficiency but potentially causing denaturation. The freeze-thaw method involves rapid freezing and thawing, causing cellular rupture under temperature changes. While requiring minimal equipment, it results in relatively lower protein yields. Chemical methods employ surfactants like sodium dodecyl sulfate (SDS) to disrupt cell membranes and organelle structures for effective protein solubilization, though this may compromise subsequent analyses. Alkaline treatment requires precise pH control during cell lysis to prevent structural damage. Biological approaches utilize enzymatic hydrolysis: lysozyme for bacterial cell wall disruption, cellulase and pectinase for plant cell walls. These methods demonstrate strong specificity with minimal protein degradation, yet face challenges including elevated enzyme costs and varying optimal conditions across different enzymes.

In protein pretreatment, impurity removal is critical. Nucleases (e.g., DNase and RNase) can degrade nucleic acids to prevent co-precipitation with proteins that may interfere with analysis. Polysaccharides can be removed using enzymes like cellulase or pectinase, or separated through ethanol precipitation and acetone precipitation. Lipids may be extracted with organic solvents (e.g., chloroform or methanol) or processed via ultracentrifugation and gel filtration chromatography. Ultrafiltration is commonly used for protein concentration, where centrifugal force or pressure allows small molecules to pass through ultrafiltration membranes while proteins are retained, offering a simple and mild procedure. However, this method struggles to produce high-concentration protein powder. Digestion steps typically employ trypsin to break proteins into peptides, facilitating subsequent mass spectrometry analysis. Precise control of digestion conditions is essential to ensure peptide quality and reproducibility.

Mass Spectrometry

MS can accurately measure thousands of proteins and their Post-Translational Modifications (PTMs) in a single run, which provide more comprehensive information at the proteomic level. In matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF) (Li S, *et al.,* 2015), protein samples are mixed with a matrix to form a co-crystal. When laser irradiated, the matrix absorbs energy and transfers it to the proteins, causing them to undergo desorption and ionization. The resulting charged ions are accelerated by an electric field into a time-of-flight mass analyzer, where separation and detection are achieved through the relationship between ion flight time and mass-to-charge ratio. This technique enables rapid determination of protein molecular weights, making it suitable for preliminary identification and fast screening of proteins. While it offers fast analysis speed and relatively low requirements for sample

purity, its resolution and sensitivity remain limited when detecting low-abundance proteins.

Liquid chromatography-mass spectrometry (LC-MS) combines the high-efficiency separation capabilities of liquid chromatography with the high-sensitivity detection of mass spectrometers. Samples are first separated through a liquid chromatography column, with components entering the mass spectrometer in sequence based on their retention times. The ions are then ionized at the source and analyzed by the mass analyzer and detector. Electrospray ionization (ESI), the commonly used ion source, charges sample molecules under an electric field, causing solvent evaporation to produce gaseous ions. This method is particularly suitable for analyzing polar and thermally unstable proteins. Atmospheric Pressure Chemical Ionization (APCI), on the other hand, generates ions through chemical ionization processes, making it more appropriate for analyzing moderately polar and nonpolar compounds. LC-MS enables comprehensive analysis of complex protein mixtures, facilitating protein identification and quantification. Widely applied in single-cell proteomics research, it can detect low-abundance proteins while providing rich structural information. However, the instrument is expensive, analysis time is relatively long, and data processing remains complex.

In the realm of protein research, protein extraction and mass spectrometry analysis (Dhabaria A, *et al.,* 2015) are two critical components. However, traditional methods remain as outdated as horse-drawn carriages, struggling to meet the high-speed demands of modern scientific research. Conventional protein extraction techniques such as salting-out and organic solvent precipitation suffer from severe protein loss – akin to constant cargo loss during precious goods transportation – leading to inefficient extraction and insufficient protein quantities for subsequent studies. Moreover, these methods often fail to remove impurities, resulting in low-purity proteins similar to sand mixed into pure water, which compromises protein quality and analytical accuracy. Regarding mass spectrometry analysis, traditional techniques demonstrate limited resolution and sensitivity. When dealing with low-abundance proteins or complex protein mixtures, they prove inadequate – like using a low-resolution camera to capture intricate patterns without capturing details, making precise protein identification and quantification impossible. This limitation significantly restricts in-depth research into protein functions and mechanisms of action.

To achieve breakthroughs in protein analysis, developing innovative low-loss, ultra-sensitive sample preparation methods has become imperative. Scientists have developed microfluidic-based protein extraction technology that utilizes microfluidic chips with intricate fluid channels and reaction zones, enabling efficient protein extraction and enrichment. This technology offers advantages such as compact size, ease of operation, minimal sample consumption, high separation efficiency, rapid analysis, and integration/automation capabilities, allowing precise manipulation and processing of samples at micro/nano scales. For instance, microfluidic chips enable rapid extraction and separation of proteins from cell lysates, significantly enhancing extraction efficiency and purity. The use of novel protein precipitants and separation media reduces loss during extraction while improving recovery rates, providing higher-quality samples for subsequent mass spectrometry analysis.

Advancing high-resolution, high-sensitivity, and high-speed mass spectrometry technologies remains crucial. Take multidimensional separation technology as an example: it integrates multiple separation modes (such as liquid chromatography, gas chromatography, capillary electrophoresis, etc.) to perform multi-stage protein separation, significantly enhancing both separation efficiency and identification accuracy. Through multidimensional liquid chromatography-mass spectrometry (MALPMS), researchers can conduct more comprehensive and precise analysis of complex protein mixtures, uncovering more low-abundance proteins and protein isoforms. Ion Mobility Mass Spectrometry (IMMS) measures ion migration patterns in electric fields and buffer gases to obtain structural and morphological information, further improving the resolution and selectivity of mass spectrometry. This technique distinguishes structurally similar proteins and peptides, providing richer data for structural analysis and functional studies of proteins.

Data Analysis and Interpretation

Data reproducibility and evaluation can be performed at several levels of increasing difficulty, namely, repeating, reproducing and replicating (Laurent Gatto, *et al.,* 2023).Repeating a computational experiment or an analysis simply consists of using the exact same data, code, software and environment (typically the same computer), assuming that these are still available. Reproducing an experiment or analysis is an attempt by a different person that will mimic the original setup by downloading data and code, without necessarily having access to the same software environment. Replication represents a further challenge in which the results are to be obtained using new code, implementation and/or software; it is only possible with extensive and detailed description of the performed analyses. This description must include the versions of all software and databases used as well as all search parameters, ideally saved as structured documents, for example, xml.

Protein mass spectrometry data analysis begins with protein identification. This process involves comparing experimental data with protein databases to identify proteins in samples through analysis of peptide mass-to-charge ratios and fragment ion information. Quantitative analysis employs two methods: non-labeled and labeled quantification. Non-labeled quantification measures peptide signal intensity across samples, offering simplicity but with accuracy affected by experimental conditions. Labeling quantification methods like stable isotope labeling introduce isotopic tags that create characteristic peaks in mass spectra, enhancing quantification precision. Subcellular localization analysis utilizes bioinformatics tools to predict cellular compartments (e.g., mitochondria, nucleus, or cell membrane) based on amino acid sequence features and modification sites, providing spatial insights for understanding protein functions.

Functional pathway annotation maps identified proteins to established biological pathway databases such as KEGG (Kyoto Encyclopedia of Genes and Genomes), analyzing biological processes and signaling pathways involving proteins. This approach uncovers functional connections between proteins and reveals cellular physiological and pathological mechanisms. In single-cell proteomics research, data analysis acts as the central hub that bridges experimental data with biological significance. Through deep mining of massive complex datasets, it enables the revelation of cellular functional states, intercellular differences, and molecular mechanisms underlying disease development at the single-cell level, providing crucial support for life science research and clinical applications.

APPLICATION FIELDS

Disease Research and Diagnosis

Single-cell proteomics plays a pivotal role in cancer research. Given the high heterogeneity of tumor tissues, distinct cancer cells exhibit significant variations in protein expression. Through proteomic analysis of single tumor cells, researchers can precisely identify cancer cell subpopulations with stem-like characteristics. These cells often demonstrate enhanced proliferative and metastatic capabilities, serving as a critical driver of tumor recurrence and metastasis. For instance, in breast cancer studies, single-cell proteomics has revealed that certain cancer cells exhibit high expression of specific signaling pathway proteins involved in cell invasion and migration. This discovery provides key therapeutic targets for developing anti-metastatic strategies. Furthermore, the technology uncovers interaction mechanisms between tumor cells and immune cells. By analyzing protein expression changes across different cell types in the tumor microenvironment, researchers gain insights into molecular mechanisms of immune evasion, thereby optimizing immunotherapy regimens. Recent advances in MS-based proteomics have enabled direct, in depth, and quantitative analysis of the expression levels of various cancer-related proteins, as well as their cancer-specific proteoforms, and proteins that fluctuate with cancer initiation and progression in cell lines and tissue samples.In the study of Yoshimi Haga (Yoshimi Haga, *et al.,* 2023), it has been proposed that deep proteomic profiling provides clinically useful information in various aspects, including understanding the mechanisms of cancer development and progression and discovering targets for diagnosis and drug development. Furthermore, it is expected to make a significant contribution to the promotion of personalized medicine. In this review, recent advances

and impacts in MS-based clinical proteomics are highlighted with a focus on oncology.

Neurodegenerative diseases such as Alzheimer's and Parkinson's are closely associated with abnormal protein aggregation and modification within neurons. Single-cell proteomics enables detailed analysis of pathological cells by detecting changes in protein modifications like phosphorylation and ubiquitination, along with alterations in protein interaction networks. Research shows that in Alzheimer's patients' brains, excessive phosphorylation of tau protein in neurons leads to neurofibrillary tangles, impairing normal cellular functions. By detecting these abnormal protein changes at early disease stages through single-cell proteomics technology, we can provide evidence for early diagnosis and intervention, potentially enabling early detection and treatment of neurodegenerative disorders.

In cardiovascular diseases, single-cell proteomics provides insights into protein expression changes in cardiomyocytes and vascular endothelial cells during pathological states. During myocardial infarction, cardiomyocytes undergo a series of stress responses. Proteomic studies have revealed significant alterations in protein expression related to energy metabolism and apoptosis. By analyzing these changes, researchers can elucidate the pathogenesis of myocardial infarction and identify potential therapeutic targets, such as proteins involved in cardiomyocyte repair and regeneration, thereby providing theoretical support for developing novel cardiovascular drugs. Additionally, this technology can be used to assess disease prognosis by predicting disease progression and treatment efficacy based on characteristic protein profiles in patients' cardiomyocytes.

In the study of Yuanyuan Shen (Yuanyuan Shen, *et al.,* 2024),_examination of CSF proteins in mutation carriers and non-carriers for autosomal dominant Alzheimer's disease uncovers early proteomic changes and generates a robust ADAD prediction model. The findings highlight the similarities and differences between ADAD and sporadic Alzheimer's disease, which can lead to personalized medicine for mutation carriers in ADAD genes.Biqing Zhu (Biqing Zhu, *et al.,* 2024) employed single nucleus transcriptomic studies and unbiased proteomic interrogation of the prefrontal cortex in PD so as to provide invaluable insight into the complex molecular and cellular pathobiology of late-stage PD. It is clear that these datasets are a valuable resource to the scientific research community for assessment of the underlying pathogenesis of PD. It is also a tool to evaluate overarching neurodegenerative disorder dysfunctions through cross comparisons with analogous datasets obtained from diseases, including movement disorders with cognitive decline, the AD spectrum, and other tauopathies. Such investigations may lead to a better mechanistic understanding of disease processes as well as provide viable targets for therapeutic intervention.

New Impetus for Drug Research and Development

In the early stages of drug development, single-cell proteomics can help identify potential therapeutic targets. By analyzing proteomic differences between disease-related cells in both normal and pathological states, researchers can pinpoint key proteins closely associated with disease progression that serve as viable drug candidates. For instance, in lung cancer research, scientists utilized single-cell proteomics to discover abnormally elevated expression of specific kinase proteins in cancer cells. These kinases are crucial for cancer cell proliferation and survival. Building on this discovery, targeted inhibitors targeting these kinases were developed, providing a novel approach for precision therapy in lung cancer.

Before clinical trials, drugs must undergo comprehensive safety and efficacy evaluations. Single-cell proteomics enables analysis of drug effects at the cellular level by detecting changes in protein expression, modifications, and interactions within cells post-treatment, providing deeper insights into drug mechanisms. Research has shown that certain medications may cause side effects on healthy cells while treating diseases. Single-cell proteomics analysis can reveal the molecular mechanisms behind these adverse effects, offering guidance for optimizing drug structures and reducing side effects. For instance, during the development of an antibiotic, researchers

discovered its impact on protein synthesis and metabolic pathways in liver cells. Based on this finding, structural adjustments were made to effectively reduce the drug's hepatotoxicity.

Drug development is a time-consuming and costly process, making efficiency crucial. Single-cell proteomics technology enables high-throughput drug screening and evaluation by simultaneously analyzing the responses of numerous single cells to drug interventions, rapidly identifying potentially active molecules. Furthermore, this technology allows real-time monitoring of therapeutic effects, enabling timely adjustments to dosage and treatment plans based on dynamic cellular proteomic changes, thus accelerating drug development. In oncology research, applying single-cell proteomics helps rapidly identify drugs with specific cytotoxic effects against different tumor cell subpopulations, significantly enhancing both the precision and success rates of drug discovery.

Specifically, it has been summarized (Lin-hui Zhai, *et al.,* 2022) that proteomics technologies remarkably benefit Post-Translational Modifications (PTM)-associated drug research. For kinase inhibitors, phosphoproteomics provides a landscape of kinase network disturbance. For inhibitors against acetylation and methylation, global PTM analysis reveals a holistic view of altered histone marks, the acetylome and methylome, and systematically uncovers non-histone substrates. For inhibitors against ubiquitination, combined ubiquitinome and proteomics analysis can be used to monitor global protein turnover and identify substrates of molecular glue/PROTAC. These strategies benefit the delineation of drug mechanisms, potential off-target effects, and drug resistance mechanisms, as well as the development of synergistic drug combinations. Moreover, PTM crosstalk commonly exists in cells: cellular signaling pathways in kinase network interplay with each other, histone modifications exhibit meticulous crosstalks for epigenetic regulation, and ubiquitination can be modulated in an acetylation- or a phosphorylation-dependent manner. Therefore, targeting PTM crosstalk has emerged as a powerful strategy in drug research. Due to the ultra-depth landscape profiling and multiomics integration ability, proteomics technologies exhibit outstanding advantages in the study of PTM crosstalks in diseases and for the development of new drug therapies.

Microbiome Unveiled

Single-cell proteomics provides groundbreaking insights into microbial community composition and functionality. Natural microbial communities exhibit complex structures, comprising diverse species that perform distinct roles within ecosystems. This technology enables detailed proteomic analysis of individual microbial cells, identifying species and revealing their metabolic pathways and functions under specific environmental conditions. For instance, studies on soil microbiota have identified bacteria producing specialized proteins that facilitate nutrient cycling (e.g., nitrogen and phosphorus) in soil environments, offering crucial insights into material cycles and energy flows within terrestrial ecosystems.

Microorganisms exhibit complex interactions with each other and with their hosts. Single-cell proteomics provides insights into the molecular mechanisms of these interactions by analyzing protein expression changes in different microorganisms during co-culture or host-cell engagements, revealing processes such as signal transduction and material exchange. In studies of the human gut microbiome, researchers have identified protein-level interactions between probiotics and pathogens. Probiotics suppress pathogenic growth and virulence expression through secretion of specific proteins, thereby maintaining intestinal microbial balance. Further investigation of these interaction mechanisms could provide theoretical foundations for developing novel probiotic preparations and advancing prevention and treatment strategies for gastrointestinal disorders.

Microorganisms hold broad application potential across industrial production and environmental protection. Single-cell proteomics enables the identification of functional microbial strains and optimization of fermentation conditions and production processes. In biofuel production, this technology helps identify microorganisms capable of efficiently converting biomass into biofuels. By analyzing their proteomes, researchers can understand the expression

and regulatory mechanisms of key enzymes, thereby optimizing fermentation parameters to enhance both yield and quality of biofuels. For environmental protection, single-cell proteomics investigates microbial degradation mechanisms of pollutants, identifying highly effective biodegradable microorganisms that provide innovative biotechnological solutions for pollution control. By tracking the choreography of plaque microbiome structure, plaque metabolome, and host immune response during gingivitis onset and progression. Shi Huang (Shi Huang, *et al.,* 2021) unraveled a microbiome-defined suboptimal health (SoH) stage of gingivitis, i.e., the 24 to 72 h after a pause in oral hygiene. Although transient and asymptomatic, it has been approved that SoH is a crucial phase when the most intensive changes in plaque structure and metabolism as well as host immune factors take place, and it exhibits a microbial signature highly similar to that of periodontitis. Prevention or treatment of SoH would eliminate the risk of dramatically accelerated oral microbiome aging by avoiding full gingivitis development.

New Hope of Agricultural Biotechnology

Shijuan Yan (Shijuan Yan, *et al.,* 2022) summarized the recent progresses in Mass Spectrometry (MS)-based proteomics and metabolomics tools and workflows with a special focus on their applications to plant biology research using several case studies related to mechanistic understanding of stress response, gene/protein function characterization, metabolic and signaling pathways exploration, and natural product discovery. We also present a projection concerning future perspectives in MS-based proteomics and metabolomics development including their applications to and challenges for system biology. This review is intended to provide readers with an overview of how advanced MS technology, and integrated application of proteomics and metabolomics can be used to advance plant system biology research.

In crop growth and development research, single-cell proteomics reveals protein expression differences among cell types during growth, helping scientists gain deeper insights into the regulatory mechanisms of crop development. Taking rice as an example, its roots, stems, and leaves consist of various cell types that play distinct roles in nutrient absorption, photosynthesis, and substance transport. Through single-cell proteomics analysis, researchers discovered significant protein expression differences between root epidermal cells and cortical cells. Epidermal cells exhibit high expression of ion transport-related proteins to adapt to nutrient absorption in soil environments, while cortical cells are enriched with proteins associated with energy metabolism and cellular structure maintenance. These findings provide a theoretical foundation for optimizing crop growth through gene editing and other techniques, facilitating the development of more nutrient-efficient and environmentally adaptable crop varieties.

Crops face various threats from diseases and pests during growth, making the enhancement of their resistance to these challenges a crucial agricultural objective. Single-cell proteomics provides insights into protein changes within plant cells when exposed to pathogens, revealing key defense mechanisms. For instance, studies on wheat infected with rust pathogens revealed upregulated expression of defense-related proteins in leaf cells. These proteins are involved in plant hormone signaling and Reactive Oxygen Species (ROS) metabolism, triggering defensive responses. Building on these findings, molecular breeding techniques can be employed to boost expression of critical disease-resistant proteins, ultimately developing crop varieties with superior pest and disease tolerance.

Gene editing and molecular breeding are crucial tools in modern agricultural biotechnology, with single-cell proteomics providing robust support. During gene editing processes, it is essential to accurately assess the impact of genetic modifications on cellular proteomes, ensuring edited crops exhibit desired traits without adverse effects. Single-cell proteomics enables proteomic analysis of edited cells, detecting protein expression changes to evaluate gene editing efficacy. In molecular breeding, analyzing single-cell proteome differences across crop varieties helps identify protein markers associated with desirable traits, accelerating the development of superior cultivars. For instance, in corn molecular breeding, single-cell proteomics has been used to screen drought-resistant protein markers. Through marker-assisted selection, researchers can

rapidly develop new drought-tolerant corn varieties.

CHALLENGES AND LIMITATIONS

Technical Bottlenecks

In single-cell proteomics research, sample acquisition remains the foremost challenge (Ghosh G, *et al.*, 2024). When isolating single cells from complex tissues, researchers must simultaneously preserve cellular integrity and activity while ensuring isolation purity. For instance, in solid tumor tissues where cancer cells are densely interwoven with surrounding stromal cells and immune cells, Laser Capture Microscopy (LCM) technology, while enabling precise target cell acquisition, involves cumbersome procedures and low throughput, making it unsuitable for large-scale studies. Conversely, flow cytometry, though offering higher throughput, risks producing non-pure single cells due to nonspecific binding of surface markers. Furthermore, rare cell populations like Circulating Tumor Cells (CTCs) present extreme challenges in sample acquisition, with concentrations in blood typically ranging from a few to dozens per milliliter—a requirement that demands highly sophisticated techniques.

The protein extraction process presents significant challenges. Given that individual mammalian cells contain only approximately 200-300 pg of protein, the extraction process is particularly vulnerable to losses caused by adsorption or degradation. Traditional methods like ultrasonic disruption combined with chemical lysis, while effective in cell disruption, often lead to protein denaturation and degradation due to harsh conditions (Findlay JBC, *et al.,* 2024). Although enzymatic digestion offers a milder approach, precise control of enzyme activity and specificity remains elusive, potentially compromising extraction efficiency. When extracting proteins from neural stem cells, the highly sensitive nature of these cells means even minor experimental disturbances can alter their proteomic profiles, making it extremely difficult to obtain complete and accurate protein information (Mansuri MS, *et al.*, 2024).

As a core technology in single-cell proteomics, mass spectrometry faces limitations in sensitivity and resolution. Analyzing individual cells' proteomes requires mass spectrometers to achieve exceptional sensitivity to detect trace protein signals. However, current mass spectrometry techniques often struggle with low-abundance proteins, where background noise frequently overshadows detection signals, compromising accuracy and reliability. When analyzing immune cells within tumor microenvironments, certain low-abundance proteins involved in immune regulation prove challenging to detect and quantify precisely, hindering deeper understanding of tumor immunological mechanisms. Additionally, the resolution constraints of mass spectrometers limit their ability to distinguish between similar proteins or protein isomers, making it difficult to accurately decipher protein structures and modification information. Gillet LC (Gillet LC, *et al.,* 2012) reported a method for qualitative and quantitative proteome probing of a sample in a single LC-MS/MS injection. This is achieved by the combination of a sequential windowed DIA method, generating exhaustive high specificity fragment ion map records, coupled with a postacquisition targeted data analysis strategy. This method permits quantification of (at least) as many compounds as those typically identified by regular shotgun proteomics with the accuracy and reproducibility of SRM across many samples. The method also provides new possibilities for data analysis, allowing quantification refinement and dynamic protein probing by iteratively re-mining the once-and-forever acquired data sets.

Data Analysis Problems

Single-cell proteomics generates massive data volumes. A single experiment may involve proteomic analysis of thousands of single cells, each containing expression profiles of thousands of proteins. This creates immense pressure on data processing and storage capacity. Traditional data processing tools and algorithms struggle to handle such large-scale, high-dimensional datasets, often resulting in slow processing speeds and frequent memory shortages. When analyzing proteomic data from 10,000 single cells, conventional software for differential expression analysis can take hours or even days to complete, severely impacting research efficiency. For instance, Harel M (Harel M, *et al.,* 2015) applied state of the art proteomics to study the micro particle proteome and developed the proteomics of micro particles with Super-SILAC Quantification

(PROMIS-Quan) method, which combines deep plasma micro particle coverage of more than 3200 proteins in a single run, with dual-mode relative and absolute Stable Isotope Labeling with Amino Acids in Cell Culture (SILAC) quantification. We demonstrated its utilization on samples of prostate cancer patients, and calculated the absolute amount of PSA, a well-known prostate cancer biomarker. This greatly elevated the efficiency of the data analysis during the procession of single-cell proteomics.

Single-cell proteomics datasets contain substantial noise from various sources, including impurities during sample preparation, mass spectrometry errors, and instrument background signals. This noise reduces the signal-to-noise ratio, making it challenging to accurately identify genuine protein expression differences. When analyzing single-cell proteomic data, false positives or false negatives may occur—mistaking noise signals for actual protein expression changes or overlooking weak but authentic differential expression proteins. Furthermore, systematic variations between experimental batches (batch effects) further complicate data standardization and integration, necessitating complex algorithms for correction and normalization. Shen X (Shen X, *et al.*, 2015) utilized an Experimental Null (EN)method that provided a straightforward, practical, and accurate alternative to statistics-based approaches for the development and evaluation of proteomic experiments and can be universally adapted to various types of quantitative techniques. This method can also be adapted to proteomic investigations based on isotope-labeling strategies. Furthermore, as the EN method experimentally measures the null distribution, it can also serve as a tool for the development of statistical efforts in the proteomic field.

The standardization and integration of single-cell proteomics data remain critical yet challenging aspects in data analysis, with no unified or effective methodology currently available (Ivanov AR, *et al.*, 2013). Variations in experimental platforms, technical workflows, and analytical approaches result in inconsistent data quality and characteristics, making direct comparisons and synthesis particularly difficult. When integrating single-cell proteomics data from different laboratories, poor comparability arises due to differences in sample processing and mass spectrometry conditions, necessitating complex data conversion and normalization procedures for effective consolidation. Furthermore, as single-cell proteomics is frequently combined with multi-omics data such as genomics and transcriptomics, the challenge lies in organically integrating these diverse datasets to uncover interrelated information—a major hurdle currently facing the field.

Interpreting single-cell proteomics data requires the integrated application of multidisciplinary knowledge from biology, bioinformatics, and statistics, demanding exceptional expertise from researchers. To accurately decipher biological significance, scientists must not only master proteomics principles and techniques but also be proficient in data analysis methods and possess a solid understanding of biological contexts. When analyzing tumor-specific single-cell proteomics, contextual knowledge of cancer development mechanisms and cellular signaling pathways is essential for interpreting correlations between protein expression changes and malignant tumor behaviors. However, the current shortage of interdisciplinary professionals has significantly hindered the comprehensive analysis and mining of such data.

As for the technical causes, the examples above mentioned are large challenge in the future development of single-cell proteomics, and it is needed intensive studies to break through the bottlenecks.

In the vast ocean of biomedical big data, data analysis resembles searching for precious treasures in an endless sea. However, traditional analytical methods are like outdated nautical charts that struggle to guide us accurately to our targets. Currently, biomedical data is experiencing explosive growth with diverse sources—including genome sequencing, proteomic analysis, clinical records—and complex data types ranging from structured to semi-structured and unstructured formats. These datasets, characterized by massive scale, high dimensionality, and abundant noise, pose significant challenges for analysis. Traditional approaches like statistical methods and basic bioinformatics tools often prove inadequate when handling such complex data. They struggle

to effectively filter out noise, much like how it's difficult to hear clear sounds in a noisy environment, compromising the accuracy and reliability of results. Additionally, they lack sufficient data normalization capabilities to standardize different sources and types of data, resulting in poor comparability—similar to shoes of different sizes being incomparable. Moreover, traditional methods fall short in integrating multi-omics data, failing to uncover inherent connections and synergistic effects between different data modalities, thus unable to reveal the fundamental nature of biomedical phenomena holistically.

To address these challenges, developing standardized and open-source bioinformatics tools has become crucial. These tools provide unified data processing workflows and algorithms, much like establishing universal maritime regulations that enable research teams to analyze data under consistent standards, thereby enhancing comparability and reliability of findings. They effectively filter out noise from various biomedical datasets through advanced filtering algorithms and data cleaning techniques, removing outliers and errors to establish a clean foundation for subsequent analyses. By applying normalization methods tailored to different data types, they convert data into comparable formats for comprehensive analysis. The development of open-source tools for multi-omics data integration enables coordinated analysis of genomic, proteomic, and metabolomics data, uncovering latent correlations and biological significance to deliver more holistic and profound insights for biomedical research.

The integration of machine learning (Fang H, *et al.*, 2025) and artificial intelligence has revolutionized data analysis. Machine learning algorithms can automatically identify patterns and features from vast datasets, much like how a skilled student quickly grasps key concepts to accurately classify and predict complex biomedical data. In disease diagnosis, training these models allows precise identification of specific diseases, their types, and severity levels through multidimensional data including genetic expression profiles, proteomic data, and clinical symptoms. Deep learning algorithms—such as Convolutional Neural Networks (CNNs) and Recurrent Neural Networks (RNNs)—demonstrate exceptional capabilities in feature extraction and model construction, effectively processing intricate spatial and temporal information. For medical imaging analysis, CNNs analyze X-rays, CT scans, and MRIs to detect lesions and characteristics, assisting doctors in diagnosis and treatment planning. Meanwhile, RNNs process biological sequences like DNA and protein chains to predict gene functions and protein structures, providing crucial support for biomedical research.

Cost Considerations

Single-cell proteomics research relies on advanced instruments such as high-precision mass spectrometers, single-cell sorting systems, and microfluidic chip platforms. These devices are prohibitively expensive, with state-of-the-art mass spectrometers costing millions to acquire. Combined with maintenance costs and supporting equipment, the financial burden on laboratories becomes substantial. For research institutions and labs with limited budgets, the prohibitive acquisition costs of these instruments significantly hinder advancement of single-cell proteomics studies.

Apart from this, the experimental process requires substantial consumption of reagents and consumables, including single-cell lysates, enzyme solutions, chromatography columns, and mass spectrometry matrices. The costs associated with these materials are significant. When conducting large-scale single-cell proteomics studies, expenses for reagents and consumables increase dramatically as experimental scale expands. A single proteomic analysis experiment involving 1,000 single cells could incur reagent and consumable costs reaching tens of thousands of yuan. For long-term, large-scale research projects, this represents a considerable financial commitment.

Single-cell proteomics experiments involve complex procedures requiring multiple steps including cell isolation, protein extraction, and mass spectrometry analysis. Each step demands strict adherence to experimental protocols and the technical expertise of researchers, necessitating specialized personnel and resulting in high labor costs. The entire workflow-from sample preparation to data analysis—requires significant time and effort. A skilled technician may

spend several weeks completing a batch of single-cell proteomics experiments and preliminary analyses. Furthermore, to ensure the accuracy and reliability of results, repeated experiments are required, which further increases both manpower and time investments.

Therefore, the prohibitive costs of single-cell proteomics technology have hindered its widespread adoption. For developing countries and research teams with limited resources, the high expenses make this technology less accessible, resulting in delayed development in these regions. Cost barriers also impede large-scale implementation of potential applications like clinical diagnostics and personalized medicine. While single-cell proteomics shows great promise for early cancer detection, its exorbitant price tag prevents it from becoming a routine clinical test, thereby limiting its practical value in early screening and diagnosis.

In scientific research, high costs act as a heavy burden that restricts the development and dissemination of studies, making it difficult for research achievements to shine like sunlight across broader fields. In biomedical research, advanced experimental equipment and reagents are prohibitively expensive—state-of-the-art mass spectrometers can cost millions or even tens of millions of yuan, deterring many research teams from pursuing such projects. Complex techniques like single-cell sequencing and spatial proteomics analysis require specialized technicians and intricate protocols, further driving up costs. As a result, these technologies remain accessible only in laboratories with substantial funding, limiting their application across wider scientific domains.

To break free from cost constraints, developing innovative and cost-effective microfluidic and mass spectrometry platforms has become crucial. By optimizing the design and manufacturing processes of microfluidic chips while adopting new materials and advanced processing techniques, we can reduce costs while enhancing performance and reliability. The development of integrated microfluidic chips that consolidate multiple experimental steps onto a single device reduces reagent and sample consumption, thereby lowering overall costs. In mass spectrometry platforms, miniaturized and portable instruments not only decrease equipment costs but also improve flexibility and operability, enabling broader application scenarios. Leveraging artificial intelligence and machine learning technologies for data analysis and processing, we can boost efficiency and accuracy in data interpretation while reducing reliance on specialized technical personnel, ultimately cutting research costs.

Collaborative sharing of protocols, datasets, and reference standards serves as a vital strategy to reduce costs and enhance research accessibility. Establishing open scientific resource-sharing platforms enables researchers to efficiently access and share experimental protocols, datasets, and benchmarks, thereby avoiding redundant efforts and resource waste. When developing new cell isolation technologies, research teams can leverage shared platforms to reference peers' experimental experiences and data, optimize their protocols, and reduce trial runs and costs. Collaboration between research institutions and enterprises also accelerates the translation of scientific achievements into practical applications. Companies can provide financial and technical support to help research institutions commercialize findings, while academic institutions can offer technological innovation and talent development programs for businesses, creating mutually beneficial partnerships.

DEVELOPMENT STATUS AND FUTURE TRENDS

Current Development

In recent years, single-cell proteomics has achieved remarkable progress in both technological and application aspects. Technological breakthroughs include continuous innovations in ultra-micro sample processing systems (Zhou W, *et al.,* 2021), such as nanoPOTS and ProteoCHIP, which have significantly reduced sample loss while enhancing reproducibility and processing efficiency. Cutting-edge high-resolution mass spectrometry platforms like Orbitrap Eclipse and Bruker timsTOF SCP, equipped with parallel accumulation, multi-dimensional separation, and enhanced ion transport technologies, have dramatically improved spectral sensitivity, making single-cell proteomic analysis a reality.

Label quantification and parallel analysis technologies

continue to be refined (Derks J, *et al.,* 2023). Elocentric labeling techniques like TMT enable simultaneous detection of multiple single-cell samples, enhancing both throughput and quantitative accuracy. In data processing and algorithm development, analytical methods for handling high missing-value and low-coverage datasets have been continuously optimized. The application of Bayesian modeling, deep learning embedding techniques, and sparse matrix completion algorithms has significantly improved the interpretability of single-cell protein data.

In expanding its applications, single-cell proteomics has demonstrated remarkable potential across multiple fields. In cancer research (Lei, Y, *et al.,* 2021), it is extensively utilized to analyze tumor microenvironments, investigate drug resistance mechanisms, and reveal immune evasion pathways. By examining the proteomes of individual tumor cells, researchers can gain deeper insights into cellular heterogeneity, providing precise evidence for personalized therapies. In stem cell and regenerative medicine (Ye J, *et al.,* 2024), this technology enables exploration of protein expression trajectories during differentiation processes, laying theoretical foundations for clinical stem cell applications. In neuroscience (Sun X, *et al.,* 2022), single-cell proteomics aids in studying neuronal functional states, signaling pathways, and metabolic changes, advancing our understanding of neurodegenerative disease mechanisms. In immunology, it helps decipher immune cell activation states and antigen recognition pathways, offering critical insights for developing targeted immunotherapies.

The Significance of Single-cell Proteomics

In summary, every advancement in bioanalytical technology carries profound significance. Innovations in cell isolation techniques have enabled us to obtain pure cells with greater precision, establishing a solid foundation for cellular-level research and ushering in a new era of single-cell studies. Upgrades in protein analysis have optimized the entire process from extraction to mass spectrometry, allowing deeper exploration of protein mysteries and revelation of molecular mechanisms in biological activities. Transformations in data processing have built intelligent systems that provide powerful tools for analyzing biomedical big data, driving progress in precision medicine and personalized healthcare. Breakthroughs in cost-effectiveness have broken financial barriers, enabling more research teams to conduct studies and promoting widespread application and sharing of scientific achievements. Explorations in spatial and epigenomics have expanded life science research horizons, providing comprehensive understanding of cellular and molecular functions through spatial and modification perspectives, offering novel targets and approaches for disease diagnosis and treatment. Interdisciplinary integration has bridged academic divides, accelerating knowledge sharing and innovative thinking while speeding up the translation of research outcomes from lab to clinic.

Looking ahead, bioanalytical technologies are poised for even more remarkable advancements. With continuous technological progress, we can confidently anticipate that cell separation techniques will achieve greater precision and efficiency, enabling the isolation of rare and trace cells to provide abundant resources for cellular therapies and regenerative medicine. Protein analysis technologies will make breakthroughs in resolution, sensitivity, and processing speed, allowing comprehensive and in-depth investigations into post-translational modifications that reveal their mechanisms in disease progression. Data analytics powered by artificial intelligence and machine learning will enable real-time analysis and prediction of biomedical big data, providing robust support for early diagnosis and prevention. Cost reduction and improved accessibility will expand access to these technologies across regions and fields, benefiting broader populations. Spatial and metabolomics will advance to deliver detailed spatial distribution maps of cells and molecules, along with dynamic protein modification processes, propelling life sciences research to new frontiers. Interdisciplinary collaboration will deepen through collaborative networks integrating biology, bioinformatics, engineering, and clinical medicine, collectively tackling major challenges in life sciences.

The future of bioanalytical technology is vast and full of possibilities. It will continue to drive advancements

in life sciences, provide robust support for addressing major health challenges facing humanity, and make indelible contributions to improving quality of life and promoting social development. Moving forward, we must persist in innovation, strengthen international collaboration and exchange, jointly explore new frontiers and applications of bioanalytical technologies, and ensure this field continues to benefit humankind.

Where is the Future Going

Looking ahead, single-cell proteomics is poised to advance in multiple critical directions. The key objectives for technological performance enhancement lie in achieving high-throughput and high-coverage capabilities. By improving ion implantation efficiency, enhancing Data-Independent Acquisition (DIA) strategies, and integrating ion mobility spectrometry, we can significantly boost throughput without compromising sensitivity. The deep integration of nanofluidic systems with chip-based analytical platforms will further propel single-cell proteomics toward automation and high-throughput processing, enabling it to meet the demands of large-scale research initiatives.

In advancing research dimensions, future single-cell proteomics will move beyond total protein quantification to comprehensively analyze protein modifications and functional states. The ongoing development of single-cell phosphoproteomics (scPhospho-proteomics) is expected to reveal critical nodes in dynamic cellular signaling pathways. Simultaneously, in-depth studies on protein-protein interactions and subcellular localization will significantly enhance both the depth and breadth of biological interpretations.

The integration of multi-omics and spatial information represents a significant trend. Single-cell proteomics will be combined with data from scRNA-seq, scATAC-seq, and other technologies to achieve comprehensive understanding across the entire chain from transcription to protein. By integrating spatial transcriptomics and tissue imaging data, we can map protein expression and functional networks within tissues, revealing cellular interactions and functional relationships at the spatial level. Monitoring cellular state transitions over time through dynamic proteomics analysis will help uncover protein regulation mechanisms, providing more complete and detailed molecular maps for systems biology research.

Clinical translation (Bader JM, *et al,* 2023) represents a pivotal direction for the future development of single-cell proteomics. With the enhancement of platform stability and maturation of data interpretation algorithms, its clinical application potential is becoming increasingly evident. In cancer treatment, analyzing single-cell protein status changes in tumor tissues enables drug resistance monitoring and efficacy evaluation, assisting physicians in formulating personalized treatment plans. Within immunotherapy, deciphering T-cell functional states and identifying key protein markers of immune activation/inhibition signals can predict treatment responses and improve therapeutic outcomes. As regulatory frameworks, standardized sample protocols, and data standards are further refined, single-cell proteomics is poised to become a cornerstone technology in precision medicine, making greater contributions to global health initiatives.

SUMMARY AND PROSPECTIVE

Single-cell proteomics, as a cutting-edge technology in life sciences, has opened new doors for our deep understanding of the microscopic world of cells. It breaks through the limitations of traditional proteomic research by revealing protein expression and functions at the single-cell level, providing crucial technical means to address numerous challenges in biology and medicine.

It has been anticipated that (Gatto L, *et al.,* 2023) the adoption of guidelines for performing and reporting single-cell proteomic studies by the scientific community and their promotion by journals and data archives is essential for establishing solid foundations for this emerging field. The suggested reporting standards will facilitate all levels of replication and thus promote the dissemination, improvement and adoption of single-cell technologies and data analysis. Sound data evaluation and interpretation will further promote the reuse of single-cell proteomic data and results outside of the laboratories that currently drive the domain and increase secondary added value of our experiments

and efforts. It is also hopeful in a sooner future that the initial guidelines offered here will evolve with the advancement of single-cell proteomic technologies, the increasing scale and sophistication of biological questions investigated by these technologies and the integration with other data modalities, such as single-cell transcriptomics, spatial transcriptomics, imaging, electrophysiology, prioritized MS approaches and post-translational-modification-level and proteoform-level (that is, top–down) single-cell proteomic methods. It has been expected that all community to discuss these guidelines and contribute to their evolution.

In the vast realm of life sciences, disciplinary barriers stand like invisible walls, hindering comprehensive breakthroughs and in-depth development (Vistain LF, *et al.,* 2021). Traditional biological research methods focus on exploring organisms' morphology, structure, and physiological functions, while bioinformatics emphasizes applying mathematical, statistical, and computer science approaches to analyze biological data. The lack of effective communication and collaboration between these fields makes it difficult for many studies to reveal the essence of life phenomena from multiple dimensions. Medical research concentrates on disease diagnosis and treatment, yet often overlooks innovative technologies and materials from engineering and materials science, leading to slow progress in medical device development and drug delivery systems. This interdisciplinary divide not only restricts knowledge sharing and integration but also impedes the collision and generation of innovative ideas, making it challenging for scientific achievements to achieve rapid translation from laboratory to clinical application.

To overcome this challenge, fostering interdisciplinary collaboration across biology, bioinformatics, engineering, and clinical medicine has become imperative. By organizing cross-disciplinary academic exchanges through regular symposiums, workshops, and joint research projects, we create platforms for researchers to share insights and collaborate on tackling major life science challenges, sparking innovative ideas. Establishing interdisciplinary teams that integrate expertise from diverse fields forms a powerful synergy for collaborative innovation. In cancer research, such cross-disciplinary teams combining biologists, medical scientists, bioinformaticians, and engineers can explore mechanisms of disease development, diagnostic methods, treatment strategies, and medical device innovations from multiple angles, achieving comprehensive solutions. Strengthening interdisciplinary talent development through cross-disciplinary courses and programs in universities and research institutions cultivates versatile professionals with multidisciplinary knowledge and skills, providing robust human capital support for cutting-edge interdisciplinary research.

A crucial aspect of advancing translational research lies in bridging scientific discoveries with clinical applications. This field focuses on translating basic scientific findings into practical medical products and treatment methods, offering innovative solutions to address real-world clinical challenges. To enhance this process, we should increase funding for translational research by establishing dedicated funds to support scientists in developing projects that bridge laboratory breakthroughs with clinical implementation. Building comprehensive translational research platforms is essential – these platforms integrate resources from academic institutions, healthcare organizations, and industry partners to achieve deep integration of industry, academia, and research. Academic institutions can deliver cutting-edge research, medical facilities provide clinical needs and trial sites, while enterprises offer financial and technical support. By strengthening intellectual property protection and incentive mechanisms for commercialization, we encourage researchers to actively pursue translational studies, transforming scientific achievements into tangible productivity and making greater contributions to global health.

Taken together, single-cell proteomics has a brilliant future for its application in more realm with more progressive development.

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CONFLICTS OF INTEREST

There exists not any conflict among the author and the Institute or any people.

DATA AVAILABILITY

Date of this review have all retrieved from the NCBI pubMed PMC.

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