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Proteomics in the context of omics sciences: evaluation of tissue-specific responses in biomedical research

Proteomika w kontekście nauk omicznych: analiza odpowiedzi tkankowo specyficznych w badaniach biomedycznych

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Abstract

Introduction and objective: Advances in omics sciences, including genomics, proteomics, and metabolomics, have transformed biomedical research by enabling multi-layered profiling of biological systems. Within this framework, the present study applied advanced proteomic approaches to investigate tissue-specific responses to experimental interventions, focusing on fluorine-containing drug administration and selenium-based dietary supplementation. The main aim was to elucidate molecular mechanisms underlying these interventions and assess the potential of proteomic signatures for biomarker discovery. **Materials and methods:** Experimental models included Wistar rats exposed to cinacalcet, a fluorinated pharmaceutical, and lambs supplemented with selenium compounds combined with other nutrients. Liver, brain, and cardiac tissues underwent high-resolution LC-MS/MS analysis under a label-free quantification workflow. Data were processed with MaxQuant and Perseus for statistical evaluation, while functional annotation and protein–protein interaction analyses were performed using the STRING database to identify key pathways and interaction clusters. **Results:** Proteomic profiling identified thousands of proteins with distinct alterations across tissues. Cinacalcet induced pronounced changes in liver proteins related to xenobiotic metabolism, lipid regulation, ion transport, and inflammation, while brain analysis revealed alterations in chromatin-associated proteins with potential roles in cognitive regulation. Selenium supplementation modulated protein expression in cardiac tissue, particularly in proteins linked to cytoskeletal organisation, adhesion, and muscle development. Protein–protein interaction analysis revealed distinct functional clusters reflecting coordinated molecular responses. **Conclusions:** This study highlights the value of proteomics in elucidating tissue-specific adaptations to pharmacological and nutritional interventions. Integration with other omics offers powerful opportunities for biomarker discovery, drug safety assessment, and precision medicine.

Keywords: proteomics, label-free quantification, protein–protein interactions, drug safety, omics sciences

Streszczenie

Wprowadzenie i cel: Postępy w naukach omicznych, obejmujących genomikę, proteomikę i metabolomikę, zrewolucjonizowały badania biomedyczne poprzez umożliwienie wielowarstwowego profilowania układów biologicznych. W tym kontekście w niniejszej pracy przedstawiono zaawansowane podejścia proteomiczne do analizy odpowiedzi tkankowo specyficznych na interwencje eksperymentalne, w tym na podawanie leków zawierających fluor oraz suplementację diety związkami selenu. Głównym celem była charakterystyka mechanizmów molekularnych leżących u podstaw tych interwencji oraz ocena potencjału sygnatur proteomicznych w identyfikacji biomarkerów. **Materiał i metody:** Zwierzęta laboratoryjne obejmowały szczury ze szczepu Wistar eksponowane na cynakalcet (fluorowany produkt farmaceutyczny) oraz jagnięta suplementowane związkami selenu w połączeniu z innymi składnikami odżywczymi. Tkanki wątroby, mózgu i serca poddano analizie proteomicznej metodą wysokorozdzielczej spektrometrii mas LC-MS/MS w trybie *label-free*. Dane przetwarzano z wykorzystaniem oprogramowania MaxQuant i Perseus w celu oceny statystycznej, natomiast adnotacje funkcjonalne oraz analizy interakcji białko–białko przeprowadzono przy użyciu bazy STRING w celu identyfikacji głównych szlaków i klastrów interakcji. **Wyniki:** Profilowanie proteomiczne pozwoliło zidentyfikować tysiące białek wykazujących zróżnicowane zmiany w poszczególnych tkankach. Cynakalcet wywoływał wyraźne zmiany w zakresie białek wątrobowych związanych z metabolizmem ksenobiotyków, regulacją lipidową, transportem jonów oraz procesami zapalnymi, natomiast analiza mózgu ujawniła zmiany w białkach związanych

z chromatyną, potencjalnie uczestniczących w regulacji funkcji poznawczych. Suplementacja selenem modulowała ekspresję białek w tkance sercowej, szczególnie z białkami powiązanymi z organizacją cytoszkieletu, adhezją komórkową oraz rozwojem mięśni. Analiza interakcji białko–białko wykazała odrębne klastry funkcjonalne odzwierciedlające skoordynowane odpowiedzi molekularne. **Wnioski:** Niniejsza praca podkreśla znaczenie proteomiki w wyjaśnianiu tkankowo specyficznych adaptacji do interwencji farmakologicznych i żywieniowych. Integracja z innymi naukami omicznymi stwarza szerokie możliwości w zakresie odkrywania biomarkerów, oceny bezpieczeństwa farmakoterapii oraz rozwoju medycyny precyzyjnej.

Słowa kluczowe: proteomika, analiza proteomiczna bez znaczników izotopowych, interakcje białko–białko, bezpieczeństwo leków, nauki omiczne

INTRODUCTION

The rapid development of omics sciences, such as genomics, transcriptomics, proteomics, metabolomics, and, more recently, metallomics, has profoundly transformed the landscape of biomedical research and personalised medicine. These integrative disciplines enable comprehensive profiling of biological systems at multiple molecular levels, offering unprecedented insight into the mechanisms of disease and variability in therapeutic responses. One of the key objectives of personalised medicine is to tailor therapeutic strategies to the individual molecular profile of the patient. This approach relies on the identification of specific genetic variants, transcriptomic signatures, and proteomic alterations that may influence disease progression and drug sensitivity. Proteomics, in particular, has emerged as a powerful tool for elucidating dynamic changes in protein abundance, post-translational modifications, and interaction networks that underlie pathological processes and treatment outcomes. In parallel, metallomics, a rapidly growing branch of omics research, focuses on the role of metals, especially those being present at trace level in biological systems. Elemental signatures reflecting the distribution and concentration of various elements in tissues and fluids are increasingly recognised as potential diagnostic biomarkers and indicators of disease progression or therapeutic efficacy. Aberrations in elemental's profile may influence key biochemical pathways, modulate enzymatic activity, and affect redox balance, thereby contributing to disease aetiology and therapeutic response. The integration of omics information, especially proteomic and metallomic data, provides a more holistic view of the molecular environment in health and disease. This combined approach not only improves the identification of candidate biomarkers but also enhances our understanding of complex molecular interactions that determine clinical efficacy and safety of therapies.

As illustrated in Fig. 1, the cascade of omics sciences represents a hierarchical and interconnected framework, where each layer of molecular information builds upon the preceding one. Genomics forms the foundational level, defining the static blueprint of genetic information. Epigenomics, positioned directly below genomics, focuses on heritable but reversible chemical modifications to DNA and histone proteins that regulate gene accessibility and activity without altering the underlying genetic code. These epigenetic marks, influenced by environmental factors, disease states, and lifestyle, add a crucial regulatory dimension to the static genome. Transcriptomics translates the combined genomic and epigenomic landscape into dynamic gene expression profiles, while proteomics captures the functional protein profiles that executes and regulates cellular processes. Metabolomics reflects the downstream biochemical activities and metabolic fluxes, offering a real-time snapshot of physiological status. Metallomics, positioned as both a complementary and integrative branch, intersects with multiple levels of the cascade by modulating enzymatic functions, structural protein stability, and signalling pathways through metal-dependent mechanisms. **This layered architecture emphasises that no single omics discipline can fully capture biological complexity; instead, it is the integration across the cascade that enables a truly systems-level understanding of health and disease.** As omics data become increasingly incorporated into clinical decision-making, they are reshaping the principles of diagnostics, prognosis, and treatment design. The shift from population-based interventions toward precise, individualised therapies represents a milestone in contemporary

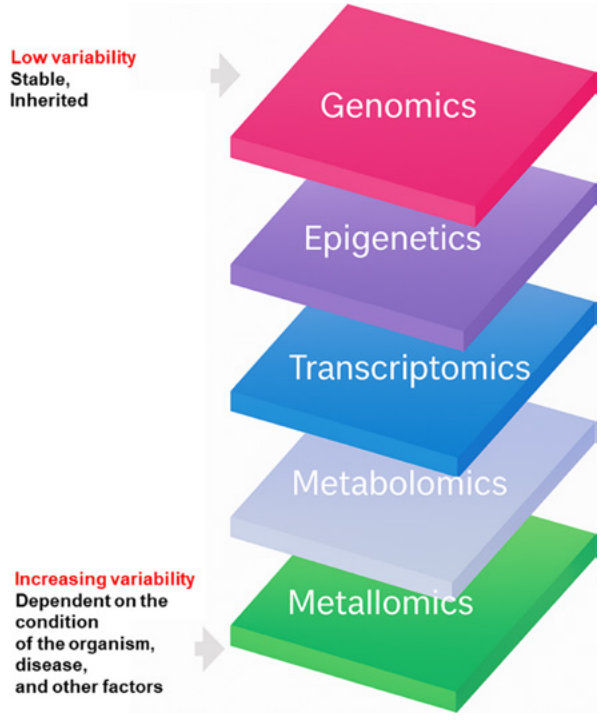

Fig. 1. Cascade of omics sciences

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medicine, with the potential to improve treatment outcomes, reduce adverse drug reactions, and optimise healthcare resource utilisation.

In light of the recently strengthened collaboration between the University of Warsaw and the Military Institute of Medicine, including the joint establishment of a Faculty of Medicine, there is a growing need to promote interdisciplinary approaches and highlight the research potential of integrated molecular sciences. Multi-omics technologies, by offering high-resolution insight into the biological complexity of health and disease, represent a cornerstone of modern biomedical innovation and translational research. In this context, the present work aims to introduce and explore the practical applications and analytical capabilities of proteomics as a central pillar of omics research within the broader framework of molecular medicine. By focusing on proteomic methodologies, we seek to illustrate how detailed analyses of protein expression patterns and interaction networks can enhance our understanding of pathophysiological mechanisms and support the development of individualised therapeutic strategies.

INSTRUMENTAL METHODS FOR PROTEIN IDENTIFICATION

The essence of biological research lies in the fundamental role of proteins in sustaining vital functions, a fact recognised even in the early stages of scientific development. The term "protein", derived from the Greek word *proteios* meaning "primary", was first introduced by Berzelius in 1838[1] underscoring the importance of this class of compounds. Proteins are essential components of all tissues, including skin, muscles, nerves, and connective tissue. They form the structural basis of enzymes, hormones, and antibodies. The vast diversity of proteins present in cells makes understanding their interactions and modifications crucial for elucidating the mechanisms governing biological systems. Proteomics is a scientific discipline concerned with the comprehensive analysis of the proteome, defined as the complete set of proteins present in a given cell, tissue, or organ. The term was introduced by Marc Wilkins in 1994, combining "protein" and "genome". In the following years, there was a dynamic development of proteome research methodology, and the importance of these achievements was confirmed by subsequent Nobel Prizes, which will be discussed further below.

The proteome is dynamic and exhibits a complexity that exceeds that of the genome, as illustrated in Fig. 2.

It is worth noting that the human genome contains approximately 20,000 protein-coding genes, with each gene capable of producing multiple protein isoforms, subject to various post-translational modifications or interactions with other molecules. Transcriptomic studies, enabled by the development of DNA microarray technology, provide information

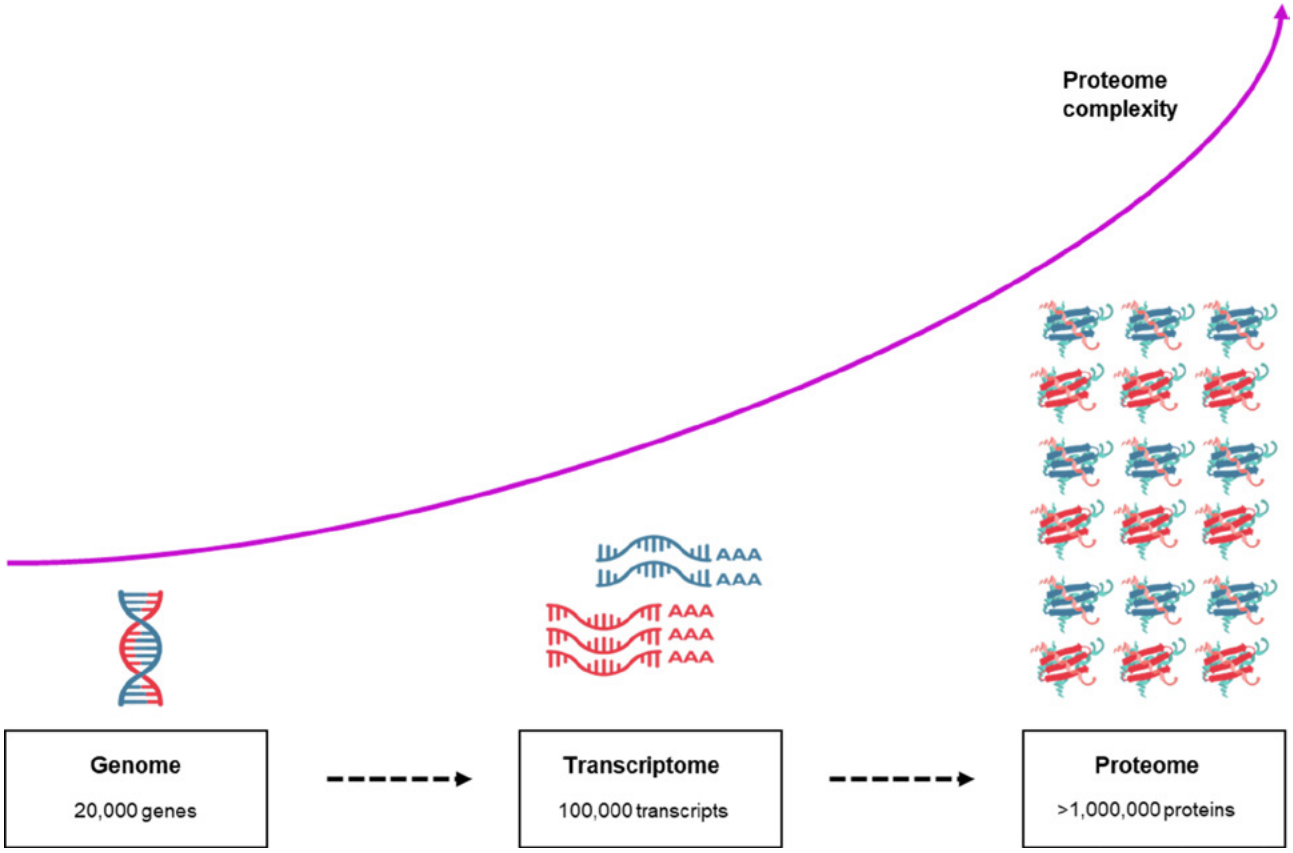

Fig. 2. Graphical representation of proteome complexity

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on gene expression at the mRNA level. While they indicate genomic targets for protein synthesis, they do not yield complete insights into protein function. Proteomics bridges the gap by translating genetic information into the functional domain, thereby enabling a deeper understanding of physiological and pathological processes. Without doubt, proteomic research plays a pivotal role in drug discovery by identifying protein targets. The integration of proteomic data with genomic, transcriptomic, and metabolomic findings facilitates a more comprehensive understanding of molecular-level biological processes. The identification of protein amino acid sequences is of paramount importance in scientific research. The first protein to be sequenced was insulin, using the pioneering method developed by Frederick Sanger, for which he was awarded the Nobel Prize in Chemistry in 1958. Sanger later received a second Nobel Prize in 1980 for developing the DNA sequencing method. Despite improvements such as Pehr Edman's enzymatic sequencing method, and the automation of the process by Stanford Moore and William Stein, these techniques still had limitations. Sequencing was relatively slow, requiring about one hour per cycle, and ineffective for peptides exceeding 30 amino acid residues. Moreover, it could only be applied to purified single protein samples, demanded relatively large quantities of material (about 100 pmol), and was unsuitable for proteins lacking a free N-terminal amino group[2]. In the 1970s, two-dimensional gel electrophoresis[3] was employed in proteomic studies, but this method also faced significant limitations. Its resolution was insufficient to fully separate numerous proteins within complex samples, and the requirement for known protein standards hindered the identification of previously unknown proteins. Nevertheless, advances in molecular biology heightened the demand for large-scale protein identification to discern differences between functional states of organisms.

Mass spectrometry (MS), whose origins trace back to the turn of the 20th century when Joseph John Thomson built the first mass spectrometer[4] became a cornerstone of analytical chemistry in the 1980s. The advent of commercially available mass analysers enabled elemental and small-molecule analysis. Early challenges in analysing complex biological systems stemmed largely from difficulties in efficiently ionising proteins, nucleic acids, and complex carbohydrates. Initial ionisation techniques often caused extensive molecular fragmentation within the ion source and required large sample amounts. A breakthrough occurred in 1988 with the introduction of two novel ionisation methods: MALDI (matrix-assisted laser desorption/ionisation) and ESI (electrospray ionisation). These approaches resolved the ionisation issues associated with macromolecules, revolutionising MS applications in analytical sciences. This period also saw rapid development of new mass analysers, tandem MS systems, and advanced separation techniques. In recognition of these achievements, John Fenn, Koichi Tanaka, and Kurt Wüthrich were awarded the 2002 Nobel Prize in Chemistry. MALDI and ESI remain cornerstone techniques for ionising macromolecules. Another milestone was the development of the Orbitrap mass analyser by Alexander Makarov in 1999[5] which significantly expanded MS applications in proteomics. The Orbitrap offers high resolving power (up to 500,000 FWHM) and exceptional mass accuracy, reaching 1 ppm[6]. Today, MS is widely applied in large-scale protein identification. However, it is not a universal solution for all proteomic objectives; selecting the appropriate experimental techniques, measurement strategies, and bioinformatic tools is critical for obtaining meaningful results in each proteomic study.

PROTEIN IDENTIFICATION USING HIGH-RESOLUTION MS

High-resolution MS-based proteomics generally follows a multi-step workflow: (I) sample preparation; (II) chromatographic separation of proteins or peptides; (III) MS analysis of individual components and (IV) data interpretation using protein sequence databases and bioinformatics software. It should be highlighted that in proteomic protocols, two principal analytical strategies are used: top-down and bottom-up approaches[7].

In top-down proteomics, intact proteins or large fragments retaining their full amino acid sequence are analysed directly by MS without prior proteolysis. This approach preserves the complete molecular information of proteins, enabling not only the identification of previously unknown proteins but also the comprehensive characterisation of proteoforms, that is, all molecular variants of a protein encoded by a single gene, including those generated by genetic variation, alternative splicing, and post-translational modifications (PTMs)[8,9]. Such detailed information cannot be fully captured by peptide-based methods, making top-down proteomics uniquely suited for studies of protein heterogeneity, structural biology, or drug–protein interactions at the proteoform level. However, several analytical challenges limit its widespread application. The restricted *m/z* range of mass analysers, the decreased ionisation efficiency of intact proteins, and the insufficient resolving power of current separation techniques for highly complex protein mixtures often constrain top-down workflows to single proteins, model systems, or relatively simple biological matrices[9–11]. Nonetheless, recent advances in high-resolution Orbitrap and FT-ICR mass spectrometers, improved ion fragmentation methods (e.g., electron capture dissociation, electron transfer dissociation), and novel separation technologies such as capillary electrophoresis and multidimensional chromatography are steadily expanding the applicability of this approach, suggesting a growing role for top-down proteomics in biomedical and pharmaceutical sciences[10,12].

Whereas, the bottom-up strategy, also referred to as "shotgun proteomics", relies on the analysis of peptides generated from proteins through proteolytic digestion, typically using enzymes such as trypsin. In this approach, proteins are

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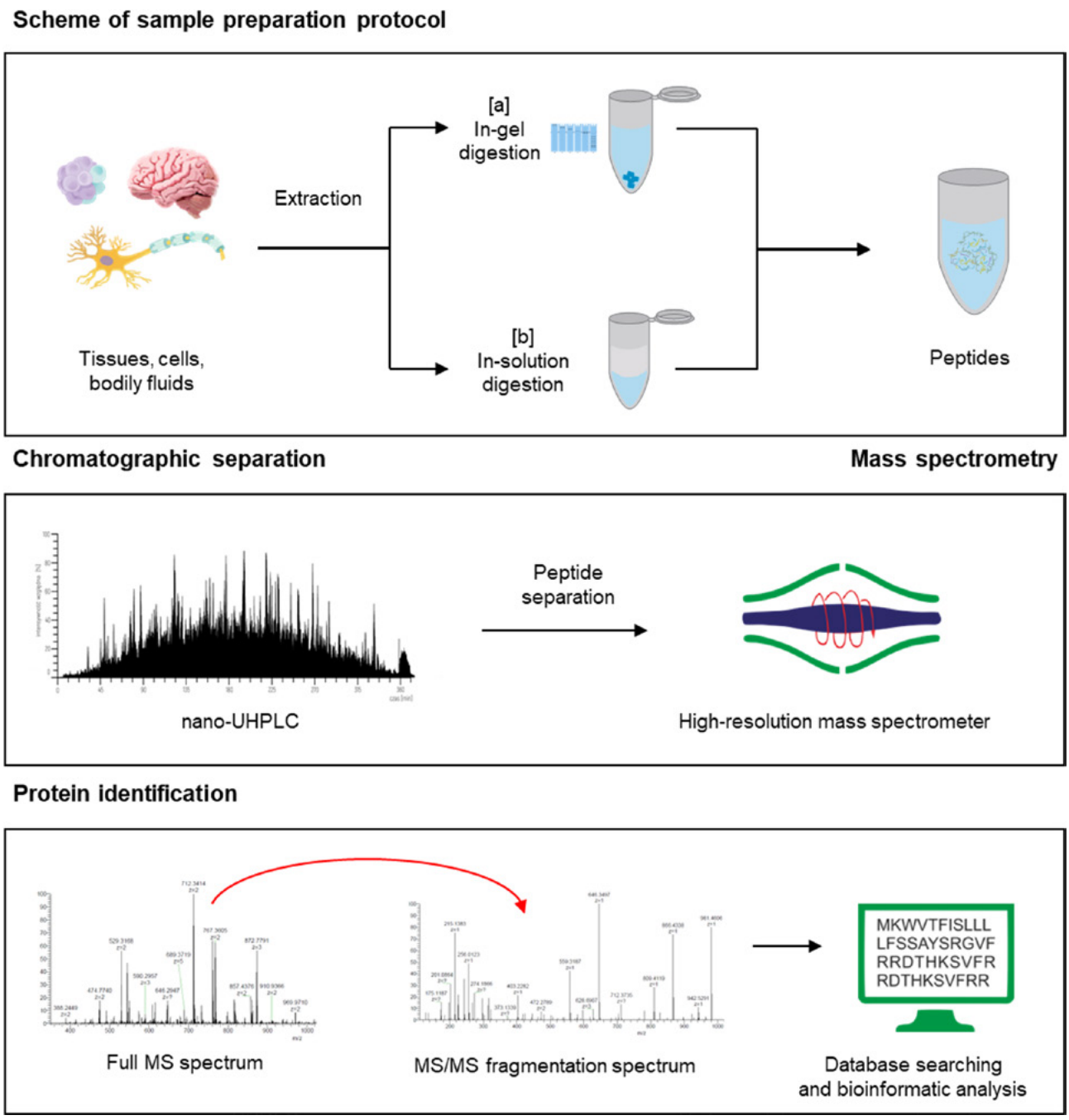

Fig. 3. Sample preparation steps for proteomic analysis in the bottom-up approach

inferred from the identification of one or more unique peptide sequences (proteotypic peptides), which serve as reliable molecular signatures for specific proteins. Fig. 3 shows the bottom-up proteomics workflow, from sample preparation and protein digestion to MS analysis and bioinformatic data interpretation.

Compared with top-down proteomics, bottom-up workflows require more complex sample handling and typically comprise several steps: (I) collection of biological material (cells, tissues, organs such as brain); (II) protein extraction and purification; (III) reduction of disulfide bonds followed by alkylation to stabilise sulfhydryl groups; (IV) enzymatic proteolysis into peptides; (V) peptide separation typically by liquid chromatography (LC); (VI) MS analysis, where peptide fragmentation spectra are generated and computationally matched to protein databases and (VII) bioinformatic analysis of the resulting spectra, including protein identification, quantitative evaluation, and interpretation of biological functions. This strategy offers significant advantages in terms of proteome coverage, sensitivity, and compatibility with highly complex samples, enabling the identification and quantification of thousands of proteins in a single experiment.

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However, it inherently sacrifices information about intact proteoforms, making it less suitable for the comprehensive study of combinatorial PTMs or structural isoforms.

Sample preparation is a critical determinant of data quality in bottom-up proteomics, as each step of the workflow influences the digestion efficiency, thus peptide recovery and the reliability of subsequent protein identification and quantification. To ensure solubilisation of protein containing items, chaotropic agents are used to disrupt hydrogen bonding and hydrophobic interactions, thereby preventing aggregation. Tissue or cell lysis is performed in buffers containing strong reducing agents. During cell lysis, endogenous proteases are released, potentially leading to nonspecific proteolysis and structural disruption. To mitigate this, protease inhibitors such as phenylmethylsulfonyl fluoride (PMSF) or PMSF in combination with ethylenediaminetetraacetic acid (EDTA) are commonly employed. Reduction of disulfide bonds is typically carried out using dithiothreitol (DTT), β-mercaptoethanol, or tris(2-carboxyethyl)phosphine (TCEP). The resulting sulfhydryl groups are most often alkylated with iodoacetamide; however, due to potential side reactions, acrylamide or iodoacetic acid is increasingly used as an alternative. Enzymatic digestion is most commonly performed with modified trypsin, which is stabilised against autolysis. Digestion can be conducted either within a polyacrylamide gel matrix (in-gel digestion) or in solution (in-solution digestion, also known as the shotgun approach). Following digestion, peptides are typically purified by solid-phase extraction. Analytical separation is then performed using nano-flow liquid chromatography coupled to a high-resolution mass spectrometer, such as nano-UHPLC-ESI-(Orbitrap)MS/MS. Protein identification is based on MS/MS fragmentation spectra of peptides, which are matched to theoretical spectra generated from protein sequence databases[13–15]. Open-source software packages such as MaxQuant, OpenMS, and Viper, as well as commercial solutions including Mascot and PEAKS, are widely used for database searching. Many major mass spectrometer manufacturers also provide proprietary software for proteomic data analysis, such as Progenesis QI for Proteomics (Waters) and Proteome Discoverer (Thermo Fisher Scientific). While database searching is the predominant strategy in proteomic workflows, it is inherently limited when unknown proteins are present in the sample. In such cases, an alternative *de novo* sequencing approach is employed, in which peptide fragmentation spectra are directly interpreted to assign amino acid sequences without prior database reliance.

Taken together, top-down and bottom-up proteomics become complementary rather than competing approaches. Bottom-up approaches remain the method of choice for large-scale, high-throughput proteomic profiling, while top-down strategies provide unparalleled detail in the characterisation of proteoforms and their modifications. The integration of both approaches within a multi-omics framework offers the most complete representation of the proteome, bridging global coverage with molecular specificity and thereby enhancing our understanding of protein function in health, disease, and therapeutic intervention.

Quantitative analysis in proteomics

In analytical chemistry, absolute quantification refers to the determination of the accurate concentration of an analyte in a sample, typically expressed in molar or mass units, using a calibration curve generated from known standards. Absolute quantification of each protein in a complex biological sample would require the availability of a pure reference standard for every target protein that the condition that remains largely unfeasible, and in many cases unattainable, within large-scale proteomics[16–18]. This is due to the vast complexity of the proteome, the dynamic range of protein abundances spanning several orders of magnitude, and the difficulty of producing isotopically or chemically identical reference standards for all proteins of interest. Consequently, most quantitative proteomic studies rely on relative quantification, in which changes in protein abundance between conditions are measured without determining their exact absolute concentrations.

In molecular biology, many major discoveries have been made by diversity assessment between functional states of a biological system. One example is the discovery of protein ubiquitination, which was based on comparing the status of cells under stress with that of cells under healthy conditions[19,20]. In such studies, the exact protein concentration is often less important than the information on how the abundance of a given protein changes in response to a specific factor. MS-based quantitative proteomics requires a carefully designed strategy that accounts for both technical limitations of the measurement systems and the economic aspects of the experiment[21]. Ionisation efficiency in typical ion sources can be affected by the chemical environment; for ESI, factors such as sample salinity, solvent volatility, and surface tension are particularly important. These effects can be minimised through pre-fractionation[22] and/or sample purification[23], which reduce the presence of interfering components. Quantitative proteomics methods can be divided into two main categories: those that use stable isotope labelling and those that are label-free (label-free quantification, LFQ)[24]. Stable isotope labelling involves introducing a specific marker that alters the isotopic composition of the sample, enabling the mass analyser to distinguish between signals from different sample components. A key advantage of isotope labelling is that signals from labelled and unlabelled analytes are measured simultaneously, which helps eliminate variability caused by ionisation efficiency, solvent composition changes, or fluctuations in instrument performance. Despite their popularity and well-developed protocols, isotope-labelling methods have several important limitations: (I) the need for additional sample preparation steps during the experiment; (II) high cost of labelling reagents; (III) variability in labelling efficiency depending on sample preparation reproducibility and (IV) limits on the number of samples that can be analysed simultaneously in a single experiment[25–27]. Due to these limitations,

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and thanks to advances in instrumentation and computational algorithms, alternative label-free methods for protein/peptide quantification are increasingly used. In such workflows, mass spectra are recorded for peptides as they elute from the chromatographic column, providing complete information on each peptide, including retention time, signal intensity, and *m/z* value. Selected precursor ions can then be fragmented to obtain detailed *m/z* and intensity data for all fragment ions. Bioinformatic analysis of these datasets allows full quantitative characterisation of each peptide in the sample, based on peak area, signal intensity, and *m/z* values for the peptide and its fragments detected in the MS/MS spectrum. Currently, most bioinformatics tools for quantitative proteomics follow the general methodology illustrated in Fig. 4.

In label-free proteomics experiments, a large number of samples are analysed independently using an LC–MS/MS system, generating raw data. These data are then processed through specialised protocols and algorithms which, by referencing protein sequence databases, extract information on the number and identity of proteins present. The results obtained from different samples are compared to generate a quantitative data matrix for multiple proteins. Proteins whose expression levels change under specific conditions can then be identified through statistical and validation analyses. Recent technological advances have provided researchers with access to vast proteomic datasets, which require advanced computational algorithms to extract biologically meaningful information. One notable solution is the MaxLFQ algorithm, introduced by Matthias Mann in 2014 as part of the MaxQuant software package[28]. This algorithm processes LC–MS/MS data by identifying features corresponding to individual peptides, then compares these features across samples to construct an intensity matrix assignable to specific peptides. These intensity values are summed to estimate the relative abundance of proteins in each sample. MaxLFQ incorporates several statistical tools, including delayed normalisation and a maximal peptide ratio extraction method, to improve the accuracy of relative protein quantification. The quality of proteomic analysis depends strongly on the software used. While the literature contains numerous reviews of bioinformatics tools for quantitative proteomics[25–27], objective performance comparisons between these tools remain scarce. Currently, widely used platforms include MaxQuant (with the MaxLFQ algorithm), Perseus, and Proteome Discoverer, all of which are continuously developed and updated by international teams of experts.

BIOLOGICAL RELEVANCE OF PROTEOMIC DATA: BIOINFORMATICS TOOLS

Understanding the biological significance of proteomic data requires not only the identification and quantification of proteins but also the integration of this information

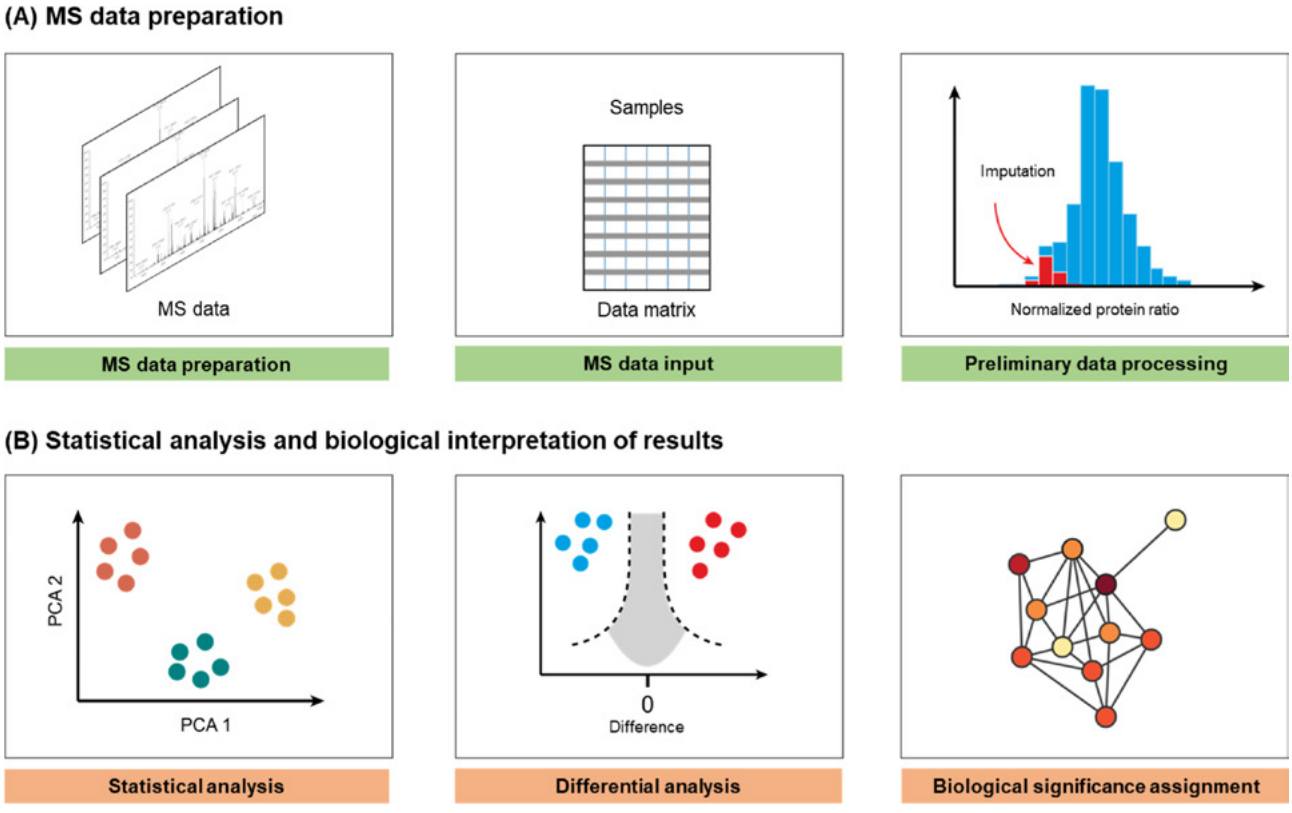

Fig. 4. Methodology of bioinformatic analysis in quantitative proteomics using MaxQuant and Perseus software, comprising: **A.** *preparation of MS data, and* **B.** *statistical analysis and biological interpretation of the obtained results*

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into a functional context. The complexity of proteomes, driven by diverse expression patterns, post-translational modifications, and molecular interactions, demands advanced computational approaches for data interpretation. Bioinformatics enables the transition from raw experimental output to biologically meaningful insights, linking protein profiles with cellular processes, signalling pathways, and interaction networks. Proteomics is a key approach for studying biological systems, particularly in functional genomics, enabling detailed investigation of protein expression, post-translational modifications, and molecular interactions. Bioinformatics tools are essential for the interpretation of large, complex datasets generated in proteomic studies. Processing, statistical analysis, and validation of these datasets, which vary depending on experimental strategies such as sample preparation protocols and MS parameters, are critical for understanding the molecular basis of biological processes. Bioinformatics supports experimental design, data storage and management, quality control, and statistical evaluation of experimental results[29,30]. Most proteins operate within interaction networks rather than in isolation. Understanding their function often requires analysis in the context of associated partners. Protein–protein interaction (PPI) analysis is central to elucidating cellular processes, providing insight into protein relationships and their roles in multiprotein complexes[31]. Protein interaction networks include all protein-coding genes in a genome, defining functional connections that can be studied at levels ranging from individual proteins to complete proteomes. Effective PPI analysis depends on accurate input data and reference to experimentally validated interaction datasets. Several open-access databases support the exploration of PPI networks and functional inference based on direct or indirect interactions. Widely used resources include STRING (Search Tool for the Retrieval of Interacting Genes/Proteins; string-db.org)[32] and BioGRID (Biological General Repository for Interaction Datasets; thebiogrid.org)[33]. STRING integrates data from multiple sources, including curated databases and literature mining, and groups proteins with similar sequences. It provides interaction confidence scores and visualises relationships as network graphs, facilitating interpretation. The platform also predicts new interactions based on sequence similarity and genomic context analysis. Because it aggregates data from varied sources, the possibility of false positives must be considered in interpretation. BioGRID offers similar visualisation and interaction modelling capabilities but focuses mainly on experimentally confirmed human PPIs, with limited data for other species. STRING covers a broader range of organisms, including humans, animals, plants, fungi, and bacteria. Different data processing and interpretation protocols in the two resources can lead to variation in results, making cross-comparison advisable. STRING is widely used due to its extensive dataset, user-friendly interface, and structured data presentation. Interaction data are organised by type and supported by evidence from other databases and publications. Integration with additional resources such as the Gene Ontology (GO)[34] and the Kyoto Encyclopedia of Genes and Genomes (KEGG)[35], enhances its value as a comprehensive platform for functional protein analysis.

CASE STUDIES: APPLICATIONS OF PROTEOMICS IN BIOMEDICAL RESEARCH

Proteomic analysis of tissues following administration of fluorine-containing drugs

Fluorine-containing pharmaceuticals represent one of the most significant achievements of modern medicinal chemistry. The introduction of fluorine atoms into active pharmaceutical compounds has become a widely adopted strategy because of their unique electronic and steric properties, which can profoundly influence pharmacological performance[36]. Fluorine, as the most electronegative element, can stabilise metabolic "hot spots" on drug molecules, preventing rapid enzymatic degradation and thus increasing metabolic stability. Moreover, the presence of fluorine atoms enhances lipophilicity and membrane permeability, resulting in improved oral bioavailability. These benefits have led to the successful development of fluorinated drugs across diverse therapeutic areas including oncology (fluoropyrimidines), neurology (fluoxetine, paroxetine), cardiology (atorvastatin), endocrinology (cinacalcet), and infectious diseases (antiviral fluoroquinolones)[36]. Despite these clinical successes, the incorporation of fluorine is not without concern. Increasing reports point to possible long-term adverse effects of fluorinated compounds, which may emerge only after months or years of therapy[37]. Potential risks include bioaccumulation, off-target interactions, disruption of enzymatic pathways, oxidative stress, and covalent modifications of macromolecules. Indeed, the biological consequences of embedding fluorine within bioactive structures remain insufficiently characterised, especially in terms of molecular-level mechanisms in mammalian tissues. From a pharmacovigilance perspective, understanding both the therapeutic benefits and potential liabilities of fluorinated drugs is crucial, particularly for agents administered chronically or in polypharmacy contexts. In this regard, proteomics offers a powerful tool: by enabling large-scale identification and quantification of proteins, as well as mapping post-translational modifications and PPI, it provides unique insight into drug-induced molecular alterations that extend beyond conventional toxicological endpoints.

To address this knowledge gap, we performed a proteomic study[14] in collaboration with the Medical University of Warsaw and the Warsaw University of Life Sciences, focusing on the fluorine-containing drug cinacalcet. Wistar rats were treated for 7 or 21 days, and liver and brain tissues were collected for analysis alongside untreated controls. Protein extracts were prepared, enzymatically digested, and analysed using nano-UHPLC coupled to an Orbitrap

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Fusion™ Tribrid™ mass spectrometer, with label-free quantification in MaxQuant and statistical analysis in Perseus. In total, ~2,200 proteins in brain and ~2,500 in liver were identified, and principal component analysis showed clear separation of drug-treated and control groups, confirming therapy-dependent proteomic changes. After 21 days, 13 proteins were differentially abundant in liver and one in brain; functional classification indicated that most were involved in enzymatic regulation, catalytic activity, and molecular binding. STRING-based PPI analysis revealed two principal clusters associated with metal ion transport, lipid metabolism, inflammatory pathways, and chromatin organisation. As an illustrative example, Fig. 5 presents a graphic interpretation of PPIs for proteins whose expression changed after cinacalcet exposure, generated using the STRING database (v11.5).

Notably, hepatic alterations suggested impaired detoxification (e.g. glutathione S-transferases) and adaptive metabolic responses, while the only brain protein altered, histone H1.0, pointed to potential epigenetic modulation relevant to long-term neurocognitive outcomes. Importantly, evidence of fluorination-related amino acid modifications was also detected, supporting the hypothesis that fluorinated drugs can covalently alter protein stability and function. The key achievement of this study lies in demonstrating organ-specific responses to fluorinated therapy, revealing how proteomics can uncover mechanistic insights, early biomarkers of toxicity, and functional PPI networks. Overall, proteomics emerges as a critical approach for evaluating the safety of fluorinated drugs, bridging the gap between molecular pharmacology and pharmacovigilance, and guiding the rational development of safer therapeutics.

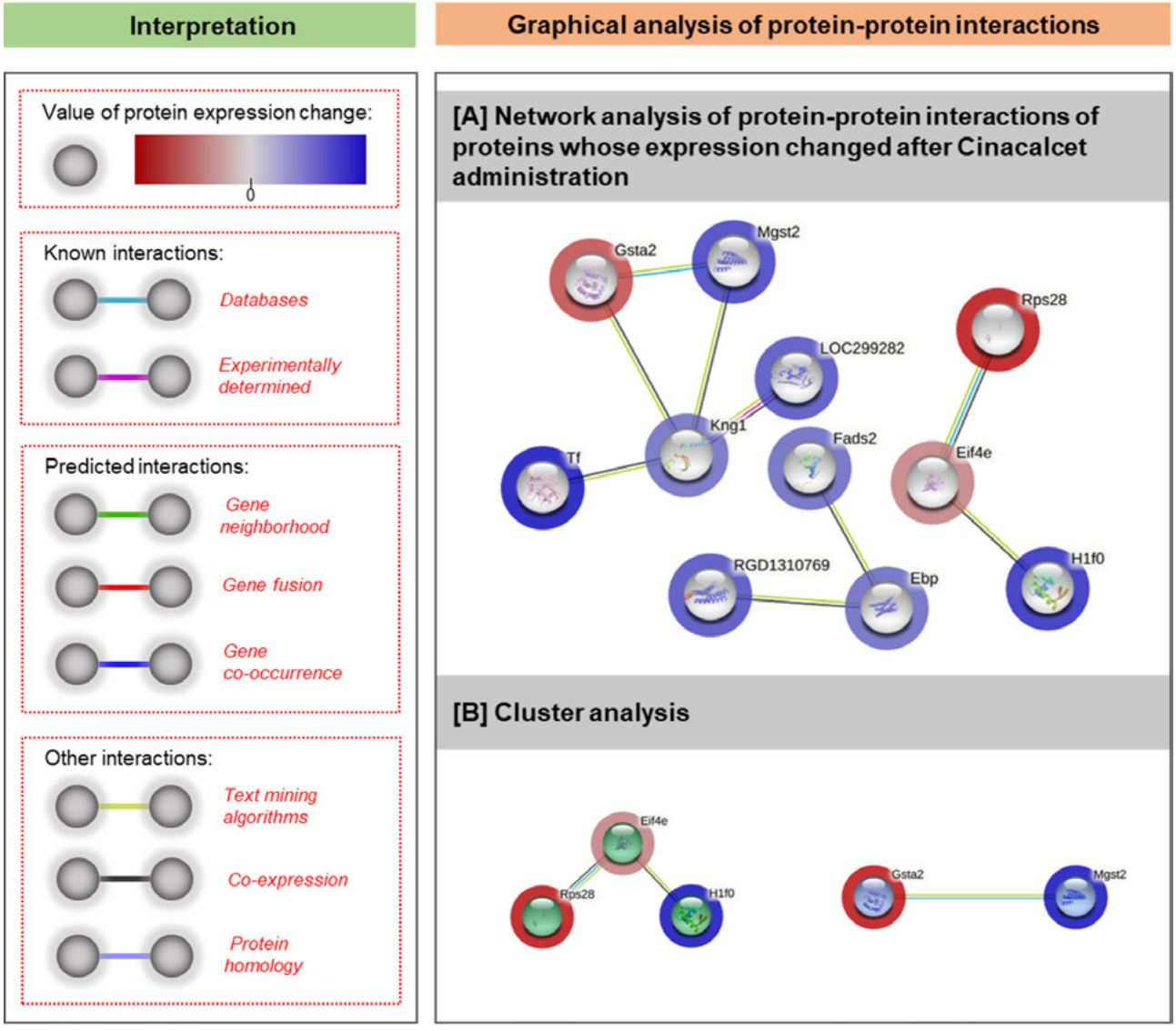

Fig. 5. Graphic interpretation of PPI for proteins whose expression changed after exposed to fluorinated drug. The data were generated using the STRING database, version 11.5

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Identification of fluorine-related protein modifications

Proteomics not only enables the profiling of global protein expression but also allows the detection of chemical modifications induced by drug metabolism, including post-translational modifications (PTMs) and, as recently demonstrated[16], fluorination events arising from fluorine-containing drug administration. This capacity is particularly important in the context of fluorinated pharmaceuticals, since the incorporation of fluorine into biomolecules can alter protein stability, activity, and molecular interactions in ways that remain poorly understood. Previous evidence[14] has shown that metabolism of fluorinated compounds can lead to the release of fluoride ions or trifluoroacetic acid, yet it has remained unclear whether fluorine can directly bind to proteins *in vivo*. To explore this question, proteomic analysis of rat liver and brain tissues following prolonged administration of cinacalcet was conducted, with particular attention to the identification of fluorine-related modifications. Using high-resolution LC–MS/MS combined with stringent data validation, fluorination of specific amino acid residues was confirmed in a subset of proteins. These modifications were absent in control tissues, providing direct evidence that therapeutic exposure to fluorinated drugs may result in covalent incorporation of fluorine into proteins. The analysis revealed fluorination events in proteins with diverse biological roles. In the liver, modifications were identified in phosphofructokinase/fructose-2,6-bisphosphatase 1, a key regulator of glycolytic flux; mitochondrial carbamoyl-phosphate synthase, central to the urea cycle and nitrogen metabolism; and β-spectrin, a cytoskeletal component critical for membrane organisation[15]. In the brain, fluorinated peptides were detected in β-spectrin as well as in dihydropyrimidinase-related protein 4, involved in neuronal development and apoptosis; tRNA phosphotransferase 1, associated with RNA processing; and prominin-2, a protein linked to endocytosis and membrane remodelling. In most cases, alanine residues were fluorinated, while in one instance a phenylalanine residue carried the modification. These results carry several important implications. First, they provide molecular-level evidence that fluorinated drugs can alter the proteome not only by shifting expression patterns but also by introducing new covalent modifications. Second, the functional spectrum of the affected proteins indicates that such modifications may influence energy metabolism, detoxification, structural stability, RNA biology, and neuronal processes. Finally, the discovery of protein fluorination highlights the capacity of proteomics to uncover drug-induced chemical alterations alongside conventional post-translational modifications such as phosphorylation, oxidation, or acetylation. This ability to map both expression changes and chemical modifications within PPI networks is crucial for understanding potential long-term consequences of therapy with fluorinated agents. In summary, the identification of fluorinated peptides in liver and brain tissues demonstrates for the first time that prolonged treatment with fluorine-containing drugs can result in stable covalent protein modifications. This observation raises important questions regarding the persistence, reversibility, and functional outcomes of such modifications, and emphasises the role of proteomics as a comprehensive platform for evaluating molecular safety profiles of fluorinated therapeutics.

Proteomic analysis of tissue responses to dietary supplementation

Diet plays a pivotal role in shaping the molecular environment of living organisms, directly influencing gene expression, protein synthesis, and metabolic regulation. Nutritional interventions can therefore alter proteomic landscapes with functional consequences for tissue development, stress adaptation, and organismal homeostasis. Proteomic technologies offer a powerful approach to study these processes, enabling large-scale identification and quantification of proteins and their interaction networks in response to specific dietary regimens[38]. Selenium is an essential micronutrient with well-documented roles in redox balance, antioxidant defence, and thyroid hormone metabolism[39–41]. While organic selenium molecules, such as selenomethionine, are more bioavailable and frequently studied in nutritional research, inorganic selenium compounds remain widely used in livestock supplementation due to their availability and economic efficiency. The present study[13], conducted in collaboration between the University of Warsaw, and the Institute of Genetics and Animal Biotechnology, Polish Academy of Sciences, investigated the effects of inorganic selenium supplementation on proteomic changes in lamb cardiac tissue. Heart tissue samples from control and selenium-supplemented animals were analysed using a label-free LC–MS/MS workflow with high-resolution Orbitrap detection. Despite attempts to directly identify selenoproteins by including Se-cysteine and Se-methionine as variable modifications, unambiguous detection proved challenging. Therefore, additional verification was carried out to confirm the correctness of peptide identification. As shown in Fig. 6, manual verification of the isotope pattern was performed for a peptide from sucrose synthase protein containing-selenium.

Manual checking of isotope patterns and fragmentation spectra is important because automated search engines can sometimes assign peptides incorrectly, especially in complex samples or when rare amino acids such as seleno-residues are present. Such verification reduces the risk of false identifications and increases confidence in the final proteomic results. Instead, the study focused on quantitative differences in protein expression between experimental groups. More than 2,300 proteins were identified in total, and 40 of them showed statistically significant expression changes after supplementation (FDR = 1%, adjusted $p < 0.01$). Functional annotation using Gene Ontology revealed that these proteins were associated with cytoskeletal

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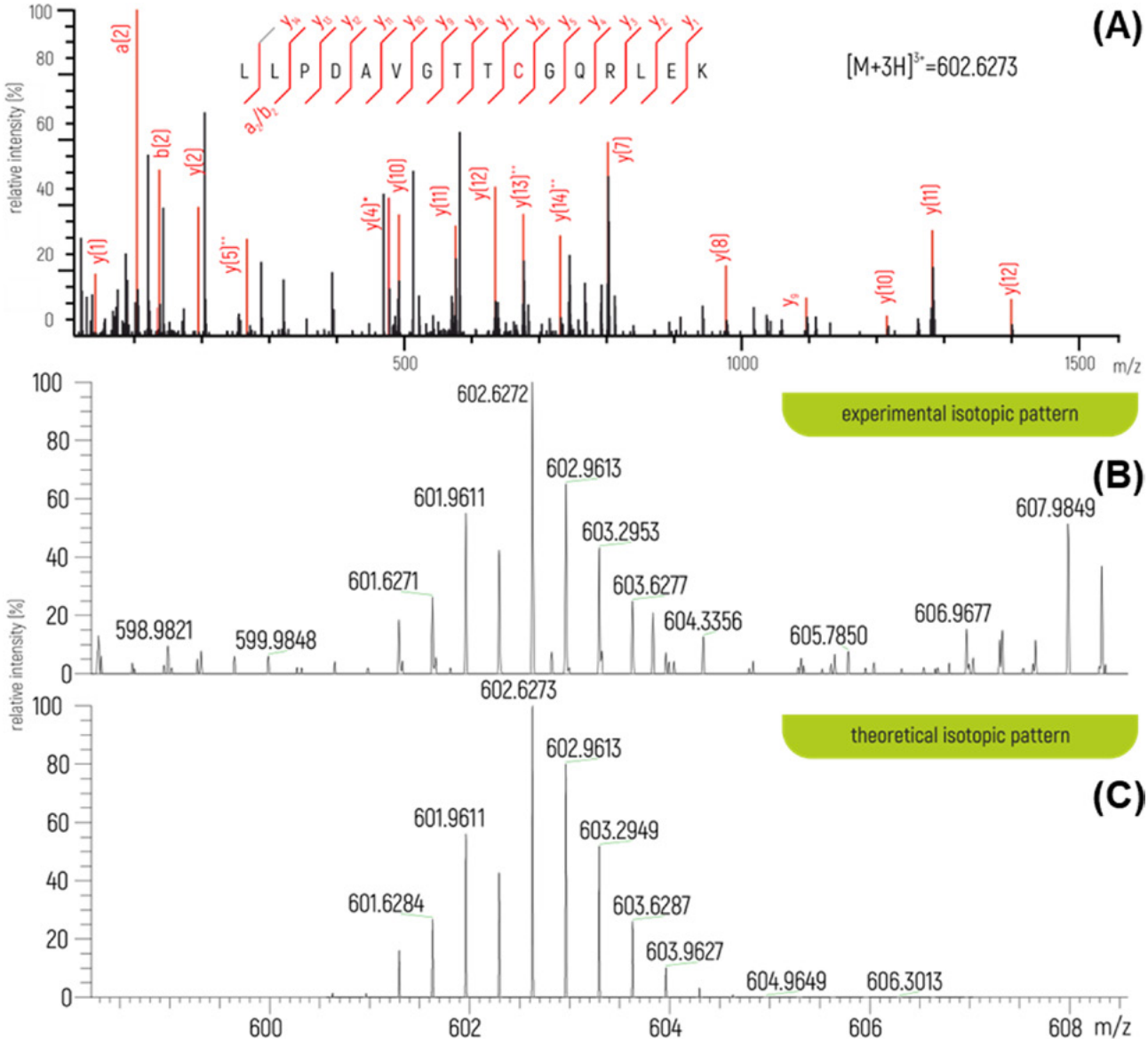

Fig. 6. Manual verification of the isotope pattern for a selected peptide form protein contains-selenium: **A.** *fragmentation spectrum of the LLPDAVGTTCGQRLEK peptide evaluated using the Mascot program;* **B.** *experimental isotope pattern obtained for the LTCGQDAVGT peptide;* **C.** *theoretical isotope pattern calculated for the LLPDAVGTTCGQRLEK peptide*

organisation, membrane repair, cardiac development, and actin filament–based motility, suggesting that selenium may play an indirect but substantial role in regulating cellular architecture and contractile properties. PPI analysis with the STRING database (v11.5) further demonstrated two distinct functional clusters: the first included proteins related to cell adhesion and cardiac development, while the second was enriched in myosin superfamily members such as myosin light chain 4, myosin heavy chains 9 and 10, and myosin IC, which are critical for intracellular transport, actin-based motility, and repair of damaged membranes. These findings underline the importance of selenium in maintaining cytoskeletal dynamics and tissue integrity. Interestingly, earlier reports did not observe significant effects of selenium supplementation on myosin expression in skeletal muscle[9], highlighting possible tissue-specific differences in response to dietary selenium. The present results are, however, consistent with other studies showing that selenium-enriched diets support cytoskeletal remodelling and stress resilience[42]. Taken together, these results provide strong evidence that inorganic selenium supplementation, despite its lower bioavailability compared to organic forms, induces detectable proteomic changes with potential implications for cardiac physiology. By reshaping protein expression and interaction networks, selenium influences pathways essential for structural maintenance and cellular stress responses, underscoring the utility of proteomics for understanding the molecular consequences of dietary interventions.

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Methodological validation and data interpretation in biomedical proteomics

In the rapidly expanding field of omics sciences, including genomics, transcriptomics, proteomics, metabolomics, and metallomics, the generation of large-scale datasets has become routine. However, the true value of these approaches lies not only in the ability to catalogue genes, proteins, or metabolites, but in the robust interpretation of the resulting data and the validation of analytical methods. In medical research, reliable interpretation of omics data is crucial for identifying biomarkers, understanding disease mechanisms, and developing personalised therapeutic strategies. Similarly, in nutrition, toxicology, and systems biology, validated workflows are indispensable for drawing meaningful conclusions about how environmental factors, dietary interventions, or pharmacological treatments shape molecular pathways. Proteomics, as the discipline linking genetic information with functional protein networks, plays a particularly central role in this framework. The complexity of proteomic datasets, often influenced by missing values, variable protein abundances, and experimental variability, necessitates the use of advanced bioinformatic tools, rigorous statistical frameworks, and standardised analytical procedures. Addressing these challenges ensures that proteomic research contributes not only descriptive catalogues of proteins but also actionable insights with translational relevance across diverse areas of biomedical science. Interpretation of label-free proteomics data is challenged by missing values (MVs), which can limit the detection of subtle yet biologically relevant changes. In our previous work[43] it demonstrated that advanced imputation strategies, particularly random forest (RF) and local least squares (LLS) methods, significantly improve MV handling, especially when preceded by data logarithmisation. The implementation of such approaches in future research will likely enhance sensitivity and reproducibility in detecting low-abundance or variably expressed proteins. This is particularly relevant in studies assessing dietary interventions or long-term therapeutic exposures, where changes in protein levels may be modest yet functionally significant. For example, proteomic analyses of tissues following supplementation with inorganic selenium have revealed distinct protein clusters involved in cytoskeletal organisation, cardiac development, and repair of cellular membranes. Even if such changes do not manifest as large shifts in expression, they may reflect adaptive responses with long-term physiological implications. Beyond statistical interpretation, robust validation of analytical workflows is essential to ensure the reproducibility and reliability of proteomic data. Gawor and Bulska[21] have proposed a standardised framework for high-resolution liquid chromatography–MS-based proteomics, integrating ISO/IEC 17025[44] and ISO 15189[45] requirements into every stage of the analytical pipeline. Applying such standardised protocols to preclinical and nutritional proteomics studies strengthens confidence in data quality and enables meaningful cross-study comparisons. This is particularly critical when proteomic results are to be translated into clinical or nutritional recommendations, such as defining biomarkers of dietary supplementation or evaluating the molecular safety of chronic therapies. Taken together, these considerations underscore the dual importance of methodological rigor and advanced statistical interpretation in proteomics. When combined, they allow researchers to move beyond descriptive protein inventories toward biologically coherent narratives that capture how therapeutic interventions or dietary factors reshape cellular networks. Whether in the context of pharmacological treatments or nutritional strategies such as selenium supplementation, integrating validated analytical standards with bioinformatic approaches enables a more reliable understanding of how proteomic alterations contribute to organismal physiology and disease risk.

As an example, Tab. 1 presents an example comparison[43] of true positives (TP) proteins identified using different imputation methods in two widely used protein identification pipelines, FragPipe and MaxQuant. This highlights how methodological choice directly impacts the number of reliably identified proteins and underscores the necessity of robust imputation strategies for meaningful biomedical interpretation.

The comparison presented in Tab. 1 demonstrates that FragPipe achieved the highest number of true positives when using the RF method (276), with LLS and BPCA performing only slightly worse. In contrast, MaxQuant yielded its best results with the SampMin method (283), which

Imputation method for proteins identification	Category	FragPipe	MaxQuant
LOD – imputation of lowest acquired intensity from entire dataset	Single-value	180	151
ND – imputation of a random value from the left tail of the normal distribution	Single-value	159	144
SampMin – imputation of lowest acquired intensity for a given protein	Single-value	223	283
***k*-NN** – *k*-nearest neighbours	Local-similarity	220	192
LLS – local least squares	Local-similarity	258	247
ALS – adaptive least squares	Local-similarity	176	144
RF – random forest	Local-similarity	276	254
SVD – singular value decomposition	Global-similarity	241	231
BPCA – Bayesian principal component analysis	Global-similarity	253	203
PPCA – probabilistic principal component analysis	Global-similarity	180	166

Tab. 1. Number of TP proteins identified by different imputation methods in FragPipe and MaxQuant

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stands out clearly from the other algorithms. The weakest performance in both pipelines was consistently associated with the LOD and ND methods, reflecting their limited ability to recover biologically relevant protein signals. These findings highlight that the choice of imputation algorithm has a profound influence on the number of proteins retained for downstream statistical analysis, underlining the necessity of careful methodological validation in biomedical proteomics.

Overall, the results clearly demonstrate that methodological choices at the stage of missing value imputation can substantially alter the outcome of proteomic analyses. FragPipe and MaxQuant, although both widely used and validated pipelines, respond differently to specific imputation approaches. These differences underline the fact that no single workflow is universally optimal; rather, the choice of both pipeline and imputation strategy must be aligned with the biological question and the expected scale of proteomic alterations. In studies requiring the detection of subtle, low-abundance protein changes, such as long-term pharmacological interventions, FragPipe combined with global imputation methods may provide higher sensitivity and reproducibility. In contrast, MaxQuant, supported by SampMin, may be more suitable in nutritional or toxicological applications where robust detection of more abundant proteins is required. Taken together, the integration of validated analytical standards with carefully selected bioinformatic approaches ensures that proteomic datasets yield not only comprehensive protein inventories but also biologically coherent and clinically relevant interpretations. This dual emphasis on methodological rigor and context-dependent data interpretation strengthens the translational potential of proteomics, enabling more reliable insights into how therapeutic strategies, dietary factors, or environmental exposures reshape molecular networks in health and disease.

CONCLUSIONS

The on-going advancement of omics sciences, including genomics, transcriptomics, proteomics, metabolomics, and metallomics, has fundamentally transformed biomedical research, enabling a systems-level perspective on health and disease. Within this integrative framework, proteomics holds a unique position as it directly reflects the dynamic functional state of biological systems, linking the static blueprint of the genome with real-time biochemical processes and cellular phenotypes. By mapping alterations in protein abundance, post-translational modifications, and interaction networks, proteomics provides insights into the molecular mechanisms underlying both physiological adaptation and pathological disruption. The investigations described in this work illustrate the power of proteomic strategies across diverse biological contexts. First, the analysis of liver and brain tissues following administration of fluorine-containing drugs demonstrated that pharmacological interventions can induce broad changes in protein expression related to xenobiotic metabolism, lipid regulation, ion transport, and inflammatory signalling, while also revealing potential chromatin-associated effects in the central nervous system. Second, high-resolution proteomic workflows allowed the unprecedented identification of fluorine-related protein modifications, showing that long-term exposure to fluorinated pharmaceuticals can lead to direct chemical alterations of proteins, with possible consequences for their stability and function. This finding opens new perspectives for studying drug–protein interactions beyond conventional metabolism and highlights the importance of structural proteomics in toxicological research. Third, proteomic profiling of cardiac tissues from animals supplemented with inorganic selenium revealed significant changes in proteins involved in cytoskeletal organisation, cardiac development, and membrane repair, with PPI analysis identifying functional clusters enriched in myosins and adhesion molecules. These results emphasise that even less bioavailable dietary forms of essential micronutrients can exert detectable molecular effects, underscoring the role of nutrition in modulating systemic proteomic responses. Collectively, these case studies confirm that proteomics is a versatile and sensitive tool capable of detecting drug-induced toxicity, nutrient-dependent adaptation, and novel chemical modifications of proteins, thereby providing a direct readout of the molecular phenotype. The integration of proteomic data with complementary omics layers, such as metabolomics for pathway flux, metallomics for trace element dynamics, or transcriptomics for gene regulation, will be crucial for constructing comprehensive models of therapeutic action, dietary impact, and environmental exposure. Ultimately, the ability of proteomics to capture molecular alterations at high resolution not only advances our understanding of biological complexity but also contributes to precision medicine by informing risk assessment, biomarker discovery, and the optimisation of individualised treatment and nutrition strategies.

Conflict of interest

The authors do not report any financial or personal connections with other persons or organisations which might negatively affect the content of this publication and/or claim authorship rights to this publication.

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Author contribution

Original concept of study; collection, recording and/or compilation of data; analysis and interpretation of data; writing of manuscript; critical review of manuscript; final approval of manuscript: AG, EB.

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