HHS Public Access

Author manuscript

Mass Spectrom Rev. Author manuscript; available in PMC 2024 February 16.

Published in final edited form as:

Mass Spectrom Rev. 2024 ; 43(1): 106–138. doi:10.1002/mas.21824.

Biological Mass Spectrometry Enables Spatiotemporal 'Omics: From Tissues to Cells to Organelles

Leena R. Pade,

Kaitlyn E. Stepler,

Erika P. Portero,

Kellen DeLaney,

Peter Nemes*

[1]Department of Chemistry & Biochemistry, University of Maryland, 8051 Regents Drive, College Park, MD 20742

Abstract

Biological processes unfold across broad spatial and temporal domains, and measurement of the underlying molecular world is essential to their understanding. Interdisciplinary efforts advanced mass spectrometry (MS) into a tour de force for assessing virtually all levels of the molecular architecture, some in exquisite detection sensitivity and scalability in space-time. In this review, we offer vignettes of milestones in technology innovations that ushered sample collection and processing, chemical separation, ionization, and mass analysis to progressively finer resolutions in the realms of tissue biopsies and limited cell populations, single cells, and subcellular organelles. Also highlighted are methodologies that empowered the acquisition and analysis of multi-dimensional MS datasets to reveal proteomes, peptidomes, and metabolomes in ever-deepening coverage in these limited and dynamic specimens. In pursuit of richer knowledge of biological processes, we discuss efforts pioneering the integration of orthogonal approaches from molecular and functional studies, both within and beyond MS. With established and emerging community-wide efforts ensuring scientific rigor, reproducibility, and outreach to diverse stakeholders, spatiotemporal MS raises an exciting and powerful resource to address scientific questions at present and to ask new ones leading us to the future, to benefit society at large.

Keywords

mass spectrometry; proteomics; metabolomics; peptidomics; imaging

I. INTRODUCTION

Interdisciplinary efforts sculpted mass spectrometry (MS) into an indispensable tool in the molecular study of life processes (Fig. 1). Virtually all levels of the molecular architecture—

***Correspondence to**: Peter Nemes, Department of Chemistry & Biochemistry, University of Maryland, 8051 Regents Drive, College Park, MD 20742, USA. Phone: (1) 301-405-0373. nemes@umd.edu.

IV. CONTRIBUTIONS

All authors contributed to the writing and revision of this report.

from genes to transcripts to proteins to peptides to metabolites—can now be measured using this technology, some in exquisite detail. MS detection and quantification of proteomes, peptidomes, and metabolomes (‘omes) expanded the analytical toolbox of biology across the taxa, helping to generate and test new hypotheses. Over the last decades, technological advances broke down classical limitations in detection limits (sensitivity), speed, and scalability to enable analysis of limited amounts of materials. This review offers examples of innovations that extended MS-based ‘omics to study the spatiotemporal organization of diverse biological systems at the realms of the organism, organ, tissue, cell, and organelle.

MS Meets Research Needs.

Life processes respond to intrinsic and extrinsic events with complex and dynamic molecular changes across broad spatial and temporal dimensions, and measuring these perturbations is key to their understanding. Systems biology promotes holistic understanding, in part, by characterizing all types of molecules and their potential interactions from organismal to subcellular scales. Vast signal amplification via polymerase chain reaction (PCR) facilitated next-generation sequencing of minute amounts of transcripts and genes, thus leading single-cell sequencing to become quasi-routine. Subcellular RNA-seq, for example, recently uncovered transcriptomic differences between the cell body (soma) and dendrites in individual GABAergic and glutaminergic neurons during biological functions (Perez et al., 2021), raising the question of subcellular heterogeneity for functional molecules downstream of transcription. Without technologies capable of amplifying whole proteomes to metabolomes, information from sequencing is often used as a proxy for the proteome.

However, the relationship between mRNA expression, protein production, and metabolism is complex in dynamic systems (Liu et al., 2016). Post-transcriptional and -translational regulation as well as protein turnover rates contribute to widely variable correlation between transcription and translation. This relationship is further complicated by processes occurring heterogeneously in space and time, as was recently found during cell culturing (Vogel et al., 2012), signal transduction between cells and neurons, differentiation of stem cells and developing organisms (Lombard-Banek et al., 2016,Peshkin et al., 2015), cancer invasion and metastasis (Saadatpour et al., 2015), and responses to drug treatments (Tian et al., 2004). Similarly, bioactive peptides and metabolites, which also carry out important physiological functions, are the results of interconnected molecular pathways, complicating their predictions from information upstream. Elucidating these molecular relationships over space-time is important for understanding states of health and disease, but this goal requires direct detection of proteins, peptides, and metabolites.

MS, especially high-resolution MS (HRMS), emerged as the technology of choice to bridge this knowledge gap. It integrates exceptional molecular specificity with a capability for quantification, typically without the need for functional probes. Readers interested in the fundamentals of MS and HRMS, ranging from ion generation to mass analysis, are referred to recent reviews (Feng et al., 2008). From tissues and cell cultures, hundreds to tens of thousands of biomolecules are routinely assessed in discovery studies with temporal resolution, with near-complete coverage of proteomes (Geiger et al., 2012,Hebert et al., 2014). For example, HRMS on two- (2-D) and three-dimensional (3-D) tissue cultures of

human embryonic stem cells (hESCs) and induced pluripotent stem cells helped delineate molecular pathways critical for cell differentiation, cellular repair, and degeneration (Arnold et al., 2022,Cervenka et al., 2021,Sperber et al., 2015). Quantitative HRMS on proliferating neuronal stem cells revealed proteomic alterations between cellular and secreted proteomes in response to the microenvironment that would be complicated to predict from the transcriptome (Cervenka et al., 2021). HRMS quantified ~2,800 proteins and their temporal expression profiles at progressive stages of differentiation, offering a glimpse into molecular mechanisms toward developing next-generational cell-based therapies such as cell grafting (Cervenka et al., 2021). Similarly, metabolic profiling of naïve and primed hESCs revealed alterations on epigenetic dynamics underpinning cell fate determination (Sperber et al., 2015).

The advent of high-sensitive HRMS propelled ‘omics to dynamic and microscale biological systems. This group of technologies integrated existing approaches and invented new ones to accomplish trace-level sensitivity, sufficient to probe molecules at physiological concentrations. In doing so, they also allowed for scaling the spatial and temporal resolution of analysis to the specific biological systems and questions at focus. Figure 1 conceptualizes broad spatio-temporal domains that contemporary HRMS is able to address. With sample collection capable of spatiotemporal scalability, high-sensitivity HRMS opened even more investigative possibilities. Rapid sampling enabled HRMS to assist studies on fast biochemical processes, including those underpinning cellular processes. For example, methods of sampling with duty cycles lasting minutes to seconds can collect cellular materials even within a ~15–45 min cell cycle in cleavage-stage embryos, as shown from the South African clawed frog (*Xenopus laevis*) in Figure 1. Sampling over minutes to hours brought molecular studies to important physiological processes, including the maintenance of circadian cycles, feeding, and response to stress (see examples later). Additionally, scalability in physical dimensions raised the possibility of capturing spatial information on the distribution of molecules in specimens on the order of millimeter to microns. Mass spectrometry imaging (MSI) played an important role by enabling the mapping of proteins, peptides, and metabolites across biological tissues, supporting studies on exploring their functional roles and aiding drug discovery. Profiling and imaging HRMS were recently adapted to subcellular structures. Chemical labeling enhanced spatial resolution to the domain of interacting molecules, thus vastly enhancing knowledge of the biochemistry underlying biology, such as signaling between neurons in the brain.

These various advancements expanded spatiotemporal HRMS to further support basic and translational studies. Next-generation separations, enhanced analyzers, and intelligent data acquisition strategies facilitated molecular identifications and quantification from minute amounts of materials. Thousands of proteins and metabolites can now be measured, even from single cells. For targeted proteins, heavy-element tagged antibodies extended HRMS to MSI and single-cell resolution. Novel mathematical algorithms helped processing complex primary data in depth. User-friendly software packages and community-wide efforts to freely share them aided adoptability across laboratories needing sensitive ‘omics, raising an opportunity to support application in basic biological and translational studies. Molecular information from these measurements holds answers to existing questions and support new questions and hypotheses, which in turn require further advances in all aspects of

technology and method development. Efforts in pursuit of sensitivity, scalability, and ‘omic coverage progressively expand our technological toolbox, thus accelerating the scientific cycle of exploration and discovery. This review highlights technological and methodological milestones in the development of HRMS enabling spatiotemporal ‘omics.

II. SENSITIVE HRMS FOR SPATIO-TEMPORAL ‘OMICS

Spatiotemporal HRMS builds on the general workflow of MS-based ‘omics. The use of MS in systems biology was recently reviewed elsewhere (Feng et al., 2008). For spatiotemporal analysis, the interconnected steps of the analytical pipeline are presented in Figure 2. During sampling, specimens, such as tissues and cells, are collected and lysed to release their contents by a variety of methods and technologies, with each tailoring to specific benefits. The lysate is typically enriched for the biomolecules of interest, such as proteins or metabolites, then subjected to various sample clean-up/purification steps to render compatibility for instrumental analysis. Various technologies are used for separation in solution, such as liquid chromatography (LC) and capillary electrophoresis (CE) or the gas phase, such as ion mobility spectrometry (IMS). The biomolecules are next ionized, detected, identified, and quantified by HRMS. An array of fragmentation (tandem/multistage MS) and analyzer-detector systems provide complementary speed and spectral resolution, ranging from quadrupoles to time-of-flight (TOF), orbitrap, and Fourier-transform ion cyclotron resonance (FTICR). The primary data from these measurements are processed and analyzed, usually with specialized MS bioinformatics software packages supporting identifications of proteins, peptides, metabolites, and their posttranslational modifications as well as relative or absolute quantification. Statistical and multivariate models from chemometrics allow for systematic evaluations of the observed effects between different experimental conditions under study. The results are interpreted in relation to canonical knowledge and the biological context. In successful investigations, the specifics of each step of the workflow are matched to the goals of the study, usually according to the following considerations (recall Fig. 2). Following sections focus on technologies that advanced spatiotemporal analysis in different biological specimens.

A. Sample Collection

The type of question and biological system at hand *de facto* determines the method of sampling. In tissues displaying heterogeneous biochemistry, such as brains and kidney, local sampling with accuracy ensures contamination-free analysis from neighboring cell populations, thus aiding the interpretation of results. Likewise, sample collection with single-cell resolution unmasks differences between single cells (cell heterogeneity) within the same tissue, which are otherwise obscured due to signal averaging during cell pooling. As exemplified in Figure 3, various strategies were developed to collect and purify tissue biopsies, small populations of cells, single cells, or subcellular fractions of interest with different speeds and operational conditions.

i. From Biopsies to Single Cells—Manual dissection is simple and can have high accuracy. Surgical tools, ranging from sharp forceps to tungsten needles to eyebrow knives, are readily adaptable to excising distinct regions of tissues. With sufficient finesse, this

approach also enables isolation of limited cell populations and individual cells from broad types of biological models. Dissection techniques, for example, enabled isolation of spatially distinct colon tumor biopsies from humans (Arnadottir et al., 2020), brain tissues from the mouse (Zhou et al., 2019), large single cells (Onjiko et al., 2015,Zhang et al., 2022) and small cell populations (Baxi et al., 2018) from developing *X. laevis* embryos, neurons from *A. californica* (Hanson et al., 2009), and distinct regions of nervous tissue from *Callinectes sapidus* (Hu et al., 2021). Needle-based biopsies, such as core needle (Satpathy et al., 2020) or fine needle (Piga et al., 2021) aspiration, were also used to collect small tissues or cells from humans. Labeling with dyes or fluorescent reporters enhances the accuracy of dissections under a microscope. Figure 3 demonstrates one such example, where identified cholinergic neurons from *A. californica* were dissected, permitting transcriptomic analysis to study aging (Moroz et al., 2013).

Spatially resolved sampling reveals information on the whereabouts of the biomolecules. With narrow focusing and adjustable power (fluence), lasers naturally became a method of choice in this research field. As illustrated in Figure 3, laser capture microdissection (LCM) concentrates infrared or ultraviolet light to lift off small tissue areas to groups of cells to single cells (e.g., 3–5 μm in size), with a capability for resolutions down to micrometers (Simone et al., 1998). LCM-based HRMS facilitated biological studies on genes, proteins, and metabolites in broad contexts including cancer biopsies (Shen et al., 2021) and recently single-cell proteomics (Zhu et al., 2018). The technology was recently reviewed elsewhere (von Eggeling et al., 2020). Because the dimensions of laser ablation are scalable, LCM can access subcellular structures. Representative examples include analysis of growth cones of a retinal ganglion cell (Zivraj et al., 2010), soma and dendrites of single neurons (Perez et al., 2021), and invadosomes coordinating motility of normal and cancer cells (Ezzoukhry et al., 2018). Dissection by LCM enables accurate isolation of tissue areas that is scalable to even smaller regions. For example, LCM with LC-MS profiled lipids between cell bodies and synapses of *Drosophila* neurons (Hebbar et al., 2014). Likewise, HRMS of optic tectum and telencephalon regions in the zebrafish brain identified over 400 proteoforms with heterogeneous distribution, including many with important activities for visual and motor functions and neuropeptide signaling (Lubeckyj et al., 2022). These approaches provide an effective snapshot of the ‘omic state of the specimen at a given time.

For smaller cell populations or dispersed cells, alternative strategies enhance the fidelity of sampling. Flow cytometry-based techniques, such as fluorescence-activated cell sorting (FACS), were employed in several studies to purify heterogenous cell populations (Chappell et al., 2016,Chen et al., 2020,Sergent-Tanguy et al., 2003). As illustrated in Figure 3, FACS enables accurate and high-throughput purification of cell populations to single cells based on fluorescent markers. Although specialized cytometers are able to sort particles ranging from 0.2 μm–150 μm in diameter, commercial FACS instruments usually have a low-pass limit of ~20 μm cell diameter (Davey et al., 1996). Required expertise, expense, and technical limitations for larger cells culminated into alternative methods. In magnetic-activated cell sorting, for example, antibody-conjugated magnetic beads of several nanometers in diameter can be used to recognize surface markers before pulling down specific types of cells and subcellular organelles (Miltenyi et al., 1990). Microfluidics renders added benefits in sample collection with minimal manual intervention. Cell sorting in microfluidic chips employs

orthogonal mechanisms for isolation, ranging from acoustic waves to radiation forces to hydrodynamic flow to microfiltration and cellular immobilization. These and other emerging technologies were recently reviewed elsewhere (Zhang et al., 2021). It follows that these approaches can be extended to collecting single cells. However, due to mixing during processing, information on the spatial origin of the cells is lost, which may be important in the context of biology.

ii. Subcellular Organization—For targeted subcellular analysis, distinct organelles may be isolated using various approaches. Sequential lysis of cellular compartments and density-based centrifugation are well-established for cells and cell types from multiple biological models. Organelles such as nuclei, mitochondria, peroxisomes, lysosomes, and the Golgi apparatus can be separated based on their size, shapes, and surface charges (Tharkeshwar et al., 2018). Similar centrifugation-based strategies allow for the isolation of exosomes from myeloid-derived suppressor cells involved in cancer tumor signaling (Burke et al., 2014) and from *Bacillus subtilis* at different developmental stages (Kim et al., 2016). Organelles with similar densities, however, may pose challenges for high-purity isolation.

Biochemical approaches and bioinformatics provide alternatives for subcellular analyses. Subcellular labeling HRMS was the topic of a previous review (Lee et al., 2010). Isotope tagging (LOPIT) and hyperLOPIT precluded the need for absolute organelle purification by taking a multipronged approach, in which organelles were partially separated into multiple fractions using multistep density centrifugation for multiplexed quantification via LC-HRMS, followed by deep machine learning to extract information on the subcellular localization of detected proteins (Geladaki et al., 2019). The accuracy of organelle fractionation is improved in MS-compatible buffers by reducing chemical interferences. For example, radio-immunoprecipitation assay (RIPA) buffer with ethylene glycol and phase transfer surfactants aided isolation of cytoplasmic, nuclear, and organelle fractions from small populations of cells without the need for traditional proteomic cleanup steps (Masuda et al., 2020).

Integration of orthogonal methods of isolation enhances capacity, and so molecular specificity and accuracy. In fluorescence-activated organelle sorting (FAOS), subcellular targets are fluorescently labeled, e.g., using antibodies for two-step purification via ultracentrifugation and FACS. This strategy enabled purification of synaptosomes without neuronal and glial contaminants, identifying novel proteins released in glutaminergic synapses (Biesemann et al., 2014). Magnetic isolation using differentially coated superparamagnetic iron oxide nanoparticles (SPIONs) helped purify endosomes during the study of Niemann-Pick disease, a lysosomal storage disorder, by pulling down endosomes enriched with dimercaptosuccinic acid-coated SPIONs and plasma membranes adhering to aminolipid-coated SPIONs (Tharkeshwar et al., 2017).

MSI techniques such as micro- and nano-probe HRMS with bioinformatics emerged for precision organelle analysis, precluding the need for physically isolating microscopic organelles. By tightly focusing beams of projectiles, from ions to photons (recall Fig. 2), spatial ‘omics recently opened the door to subcellular ‘omics, as was the focus of a recent review (Seydel 2021). High-definition multiplex ion beam imaging (HD-MIBI) secondary

ion MS (SIMS) offered a 30-nm resolution, sufficient to localize cisplatin enrichment in nuclear speckles with exclusion from chromatin regions, providing new information on subcellular microenvironments for next-generation drug development (Rovira-Clave et al., 2021). Spatial single nuclear metabolomics (SEAM) integrated TOF-SIMS with novel computational data analysis modules (Fig. 3) to identify and localize single nuclei with variable metabolic patterns in healthy and fibrotic liver tissue (Yuan et al., 2021). In the 3D OrbiSIMS, the coupling of TOF-SIMS with an ultra-high-resolution orbitrap empowered high-speed 3D molecular imaging, providing information on the subcellular localization of lipids and neurotransmitters in the hippocampus and single macrophages (Passarelli et al., 2017). Similarly, using image-guided matrix-assisted laser desorption ionization (MALDI) coupled to an ultra-high-resolution mass analyzer, neuropeptides and lipids were identified from 0.5–2 μm vesicles in *A. californica* to reveal distinct metabolic subpopulations within these organelles (Castro et al., 2021). These and other advancements enabling subcellular and single-organelle spatial ‘omics analyses will continue to inform us of molecular profiles among cells and within their components and in turn help uncover ‘omic mechanism underlying health and disease states.

In situ sampling can take a molecular snapshot of the tissues or cells under innate conditions with minimal external perturbations during collection. As illustrated in Figure 3, fast photochemical oxidation of proteins (FPOP) method utilizes laser-induced photolysis of hydrogen peroxide to irreversibly label proteins in their native state within a cell, thus enabling studies on protein structure and folding and protein-protein interactions (Espino et al., 2019). Pioneering a new approach in epitope mapping, oxidative labeling was successfully used to map antibody-binding regions of the protein serine protease thrombin (Jones et al., 2011). Such information is essential to develop monoclonal antibody-based therapeutics that target specific proteins.

iii. In Situ/Vivo—Chemical labeling enables isolation of molecular partners in interactions. A plethora of labeling techniques allow for tracing biomolecules in subcellular compartments, some *in vivo*. Bioorthogonal reactions, for example, use a wide array of click chemistry to anchor specific biomolecules without disturbing the intracellular native environment. Such molecular handles were used to label proteins, lipids, glycans, nucleic acids, and enzymes, and for targeted delivery of small molecules, reviewed in (Bird et al., 2021). Bioorthogonal noncanonical amino acid tagging (BONCAT), Figure 3, enabled tracing of newly synthesized proteins by incorporating biotin-tagged noncanonical amino acids in the nascent proteins with follow-up affinity purification or imaging by fluorescence microscopy (Landgraf et al., 2015). The approach recently allowed for tracing tumor cell proteome in ductal adenocarcinoma in the mouse (Azizian et al., 2021). BONCAT was recently used to find local translation for 42 proteins in the axoplasm during regeneration of the sciatic nerve following injury in rats (Di Paolo et al., 2021). Alternatively, proximity labeling using ascorbate peroxidase, APEX or APEX2 (Lam et al., 2015), or biotin ligase, BioID (Roux et al., 2012), localized enzymatic activity to map their proteomic environment in organelles enabling proteomic studies of synaptic clefts of excitatory and inhibitory neurons (Loh et al., 2016). Isotope tracing adds a dynamic component to these studies via spatial fluxomics. Glutamine metabolism was monitored in mitochondria and the cytosol

of cancers to elucidate metabolome remodeling (Lee et al., 2019). Alternatively, subcellular fractionation with activity-based protein profiling (ABPP) monitored proteomic changes in secretory pathways in the endoplasmic reticulum in response to cellular stress (Bechtel et al., 2020).

Sampling *in vivo* extends HRMS 'omics to assess the molecular dynamics underlying physiological states. Figure 3 shows examples of *in vivo* probes capable of minimally invasive sampling. Microdialysis has a long history in the research of neuropeptides, neurotransmitters, and metabolites with ca. millimeter resolution in live biological models. This strategy was used to access molecular information on physiologically relevant timescales (e.g., from minutes to hours) in functional, behavioral, and clinical studies. Representative examples helped decipher the roles and spatiotemporal dynamics of neuropeptides and their role in feeding using crabs (Schmerberg et al., 2013,Schmerberg et al., 2015), decision-making and drug response in rats (Zestos et al., 2017), and the tumor-promoting impact of radiation in glioblastoma using human xenografts in mice (Gupta et al., 2020), as well as clinically for cerebral metabolic analysis of human patients with aneurysmal subarachnoid hemorrhage among others (Helbok et al., 2017).

Real-time biopsies with rapid chemical analysis opened exciting new capabilities for health research (Fig. 3). Liquid biopsies enabled the sampling of circulating cancer cells for diagnosis (Abouleila et al., 2019) or the assessment of effectiveness during therapy (Bensen et al., 2021). In the clinical setting, desorption electrospray ionization (DESI) and MasSpec Pen (Fig. 3) gained recognition for its ability to downscale microextraction into < 10 μL of water droplet on tissue regions of interest, even in morphologically complex samples (Brown et al., 2018,Zhang et al., 2017). With fast sample transfer and HRMS detection, the approach aided cancer diagnoses of ovarian, lung, thyroid, and breast biopsies *ex vivo*, and recently also *in vivo*, thus empowering surgical applications with a new tool.

Microsampling confined to capillaries and nanochannels downscales sampling to the realm of cells and organelles. Whole cells, portions of a cell, or subcellular contents may be aspirated using precision-fabricated capillaries, usually with assistance from a translation stages and microscopy. The introduction of ambient ionization and ultra-sensitive HRMS technologies ushered the extension of capillary microsampling to endogenous and exogenous metabolites in broad types of single cells, as was recently reviewed elsewhere (Yang et al., 2017,Zhang et al., 2018,Zhu et al., 2021). Early on, for single cells, live-video MS with direct ESI detected metabolites from single adherent embryonic fibroblasts (mouse) and plant cells (Fujii et al., 2015,Tsuyama et al., 2008), T-probe (Fig. 3) profiled metabolic perturbations in HeLa cells on anticancer treatment (Liu et al., 2018), and laser ablation electrospray ionization (LAESI) surveyed secondary metabolites in *Allium cepa* cells under guidance by optical microscopy (Taylor et al., 2021). The live cell analysis device (LCAD) exchanged capillaries to nanofabricated channels, where localized electroporation facilitated tyrosine phosphatase extraction from cells during proliferation and hydrogen peroxide treatment in human triple-negative breast cancer cells with protein detection enabled by self-assembled monolayers for matrix-assisted laser desorption/ionization (SAMDI) MS (Mukherjee et al., 2020).

To detect deeper into single-cell ‘omes, microsampling can be combined with high-efficiency separations prior to *in situ/vivo* HRMS. By aspirating of <5% of the total cell volume using a pulled borosilicate capillary, proteomic and metabolomic cell heterogeneity was found in embryos of *X. laevis* and zebrafish (Lombard-Banek et al., 2019,Onjiko et al., 2017,Portero et al., 2019,Saha-Shah et al., 2019). Capillary microsampling facilitated integration of HRMS ‘omics with functional biology. Patch-seq has a history of leveraging this strategy for sequencing mRNAs from neurons after their electrophysiological characterization (Cadwell et al., 2016), as was recently reviewed elsewhere (Lipovsek et al., 2021). Likewise, after eavesdropping on electrical activity using a patch pipette, neurotransmitters in the mouse (Aerts et al., 2014) and the American cockroach (Neupert et al., 2018), and recently also proteins in the mouse (Choi et al., 2022) were measured in identified single neurons in the central nervous systems of these model organisms. Multi-step capillary microaspiration also enabled *in vivo* analysis of dynamic reorganization in the metabolome and proteome of single identified blastomeres in the live *X. laevis* embryo, which successfully developed to a functioning tadpole with indistinguishable behavior from wild-type siblings in a background color preference assay (Lombard-Banek et al., 2021). Accessing such information on molecular interactions in space-time offers an important piece of the puzzle towards the holistic understanding of molecular mechanisms underlying biological functions and disease states.

B. Sample Preparation

Sample preparation can make or break studies requiring trace detection, and those addressing spatiotemporal processes are no exception. Specimens encompassing few cells, single cells, or subcellular organelles provide only micro- to picogram quantities of starting materials (Ctortecka et al., 2021,Zhang et al., 2018). Processing such limited amounts poses nontrivial challenges in handling of miniscule volumes without solvent evaporation and with minimal-to-no losses for proteins, peptides, and metabolites due to surface adsorption (Yang et al., 2021). Advances in small-scale sample preparation was the topic of several reviews (Hu et al., 2021,Kelly 2020,Matula et al., 2020,Qin et al., 2022,Taylor et al., 2021,Yang et al., 2021). The following section highlights developments that ushered HRMS to spatiotemporal ‘omics from small tissue biopsies down to the single-cell and subcellular level.

i. Proteomics—HRMS provides critical information on proteomes during states of health and disease. Methods and technologies enabling bottom-up, middle-down, and top-down proteomics are able to identify and quantify broad types of proteins, assess their structure, gauge their interactions, and determine modifications during biological processes in space and time. Deep proteome coverage is an enabling feature of bottom-up proteomics, which is the most widely used of the three workflows. In this approach, proteins are enzymatically digested to peptides, which can be efficiently ionized and sequenced to pinpoint their source proteins using advanced bioinformatics. Thousands of different proteins can be identified at once using this approach, even from limited sample amounts. Complex spatiotemporal processes were studied using this approach at the organismal to subcellular levels in development (Baxi et al., 2021,Yang et al., 2020), neuroscience (Hobson et al., 2022,Velasquez et al., 2019), and various disease states (Vo et al., 2007,Waas

et al., 2020). The information from these measurements provides the foundation for hypothesis-driven and functional studies.

HRMS-based proteomics executes multiple analytical steps with each able to affect sensitivity. Lysis, extraction, reduction, alkylation (of disulfide bonds), enzymatic digestion, and desalting are routine stages of sample processing requiring care for limited samples. Simplification of the workflow was recently found to improve performance. For single-cell measurements, for instance, MS-compatible detergents and mechanical lysis methods (i.e., freezing/thawing or laser pulses) and exclusion of reduction and alkylation eliminated the need for downstream clean-up, reduced nonspecific adsorptive losses, and dilution (Lombard-Banek et al., 2019,Schoof et al., 2021). Select strategies enabling high-fidelity proteomics are presented in Figure 4 and discussed further.

“One-pot” strategies confined the analytical steps to a single location. For amount-limited samples, immobilization of molecular processing and reduction or elimination of sample transfers proved particularly beneficial. For example, gel-aided sample preparation (GASP) (Fischer et al., 2015), suspension trapping filter (S-Trap) (HaileMariam et al., 2018), and filter-aided sample preparation (FASP) (Wisniewski et al., 2009) used gels and filter membranes, respectively, to reduce sample transfer steps and improve coverage from hundreds of micrograms of protein. By miniaturizing and confining sample processing using a multi-well plate, micro-FASP was able to identify ~3,400 proteins from ~200 ng of yolk-free proteome from single blastomeres that were isolated from early-stage *X. laevis* embryos (Zhang et al., 2020). Single-pot solid-phase-enhanced sample preparation (SP3) demonstrated similar capabilities for comparable protein amounts from 1,000 HeLa cells and *Drosophila* embryos by immobilizing proteins and peptides on carboxylate-coated paramagnetic beads throughout sample preparation (Hughes et al., 2014).

Microsampling *in situ* provided a potential to streamline the analytical workflow and explore potentials toward their automation. As illustrated in Figure 4, single-cell proteomics by MS (SCoPE-MS) limited sample processing to one pot, allowing for multiplexed protein quantification among single cells (Budnik et al., 2018). Capillary-based systems may serve as alternative substrates for single-cell sampling and processing. As shown in Figure 4, The integrated proteome analysis device (iPAD) directly supplied a suspension of living cells (iPAD-100) for online cell lysis, protein digestion, and peptide desalting and trapping to a 10-port valve-based system on a commercial nanoLC, identifying ~600–650 proteins from a single injection of 100 cells (~10 ng protein) using nanoLC-HRMS (Chen et al., 2015). In pursuit of cell heterogeneity, iPAD-1 (Fig. 4) used an ultranarrow-bore analytical column to identify ~270 proteins from a single HeLa cell (220 pg proteome) in 1 h of analysis with nanoLC-HRMS (Shao et al., 2018). These and other simplified devices make it easier for other laboratories to adopt these technologies.

Microfabricated devices present a natural setting for “one-pot” workflows toward sensitivity and advanced measurement throughput. ProteoCHIP used polytetrafluoroethylene (PTFE)-based microfluidics to automate single-cell isolation and small-volume handling, enabling the processing of up to 192 samples simultaneously for LC-MS (Ctortecka et al., 2022). As shown in Figure 4, nanodroplet processing in one pot for trace samples (nanoPOTS)

miniaturized the processing of 100 cells to a single mammalian cell onto nano-wells on microfabricated glass chips using nanoliters of reagents for deep proteomics (Zhu et al., 2018,Zhu et al., 2018). Using LCM to dissect ~100 μm squares from mouse tissues, the approach helped uncover spatial differences between uterine cell types during pregnancy in preparation for blastocyst implantation (Piehowski et al., 2020). NanoPOTS also enabled proteomics on single sensory hair cells measuring ~0.6–1 pL in volume, facilitating the study of cell differentiation in chicken embryos (Zhu et al., 2019). Nested nanoPOTS (N2) arrayed multiple wells to enhance measurement throughout for multiplexed quantification using nanoLC-HRMS (Woo et al., 2021). Recent efforts sought to further refine one-pot sample preparation strategies to make the setups cost effective and allow scalable sample preparation, as was recently reviewed in (Slavov 2022). Automated preparation in one pot for trace samples (autoPOTS), the latest upgrade of nanoPOTS, now allows automated processing of 1–500 cells using only commercially available instrumentation for sample processing, making it widely adaptable (Liang et al., 2021).

Similar strategies also emerged for top-down proteomics. Detection of intact proteins provides insights into proteoforms and PTMs (Catherman et al., 2014). Fewer analytical steps than its bottom-up sibling, miniaturized formats for detergent removal and desalting (Nickerson et al., 2021,Yu et al., 2021), and “one-pot” sample preparation approaches (Dagley et al., 2019,Zhou et al., 2020) extended top-down proteomics to limited sample amounts as low as hundreds to tens of nanograms. As mentioned earlier, analyte recovery can be enhanced by eliminating the routines of sample handling and desalting. Freeze-thaw lysis and non-ionic surfactant-based extraction from ~200 brain cells enabled spatial top-down proteomics for ~400 proteoforms between the optic tectum and telencephalon regions of the zebrafish brain (Lubeckyj et al., 2022). Further advancements in technology raise exciting potentials to leverage proteoforms as biomarkers of diseases (Smith et al., 2021).

ii. Peptidomics—While analyzing enzymatically digested peptides (i.e., bottom-up proteomics) provides information about the proteomic content of a cell or biological sample, endogenous peptides, such as neuropeptides, can provide additional insight into key biological processes, particularly related to the nervous and neuroendocrine systems. These peptides are synthesized from larger proteins via enzymatic cleavage, and their biological activity is often distinct from the preprohormone or precursor-related peptides (Hook et al., 2018). Many neuropeptides contain similar sequences that may vary by only one or a few amino acids, but their biological functions could be identical or largely different (DeLaney et al., 2018). Furthermore, neuropeptides are thought to be the most diverse class of signaling molecules, are present in low abundances *in vivo*, and prone to rapid degradation. As such, analyzing endogenous peptides in space and time presents unique challenges and requires a rapid, sensitive workflow with specificity capable of differentiating between similar isoforms. MS emerged as the gold standard for profiling neuropeptides, as it is capable of rapidly detecting neuropeptides at sub-attomole levels and can identify peptides without prior knowledge (Phetsanthad et al., 2021).

Peptidomics consists of peptide extraction, purification via molecular weight cutoff filtration, and desalting prior to MS analysis. A critical aspect of this process is deactivation of proteolytic enzymes to prevent peptide degradation and achieve highly sensitive

peptidomics analyses, which can be accomplished by flash-freezing the tissue immediately after dissection, heat stabilization, or acidification. Acidification is particularly effective for deactivating proteases when the acid is in an organic solvent (e.g., acidified methanol or acidified acetonitrile), as while proteases are deactivated by the low pH, large proteins are precipitated from the solution. For example, acidified methanol was recently used to successfully extract neuropeptides from single mollusk neurons, enabling sequencing of peptides in the cytoplasm and nucleus of the neurons and identification of differences in peptide abundances based on subcellular location (Zhang et al., 2018). In MALDI-MS workflows, 2,5-dihydroxybenzoic acid (DHB) was found ideal for peptide extraction, as it served as both the extraction buffer and the matrix (Romanova et al., 2008,Wang et al., 2009). Though other stabilizing agents such as EDTA and protease inhibitor cocktails can also be added to the extraction buffer to improve peptide stability (Chen et al., 2009,Kwok et al., 2006,Onorato et al., 2019), MS compatibility is important to consider, particularly with limited samples, so as to reduce additional cleanup steps and sample losses.

Peptidomics from limited samples may also benefit from enrichment methods. Affinity-enhanced magnetic beads offered one such approach to improve detection for low-abundance peptides (Schmerberg et al., 2013,Vocat et al., 2020). The incorporation of magnetic beads coated with NPY02 antibodies during sample preparation led to femtomolar sensitivity and quantitation of neuropeptide Y in human plasma (Vocat et al., 2020). Considerations of sample recovery for the desalting step are similar as in bottom-up proteomics workflows. Additionally, solid-phase extraction (Bardsen et al., 2019) and on-plate droplet desalting for MALDI-MS (Wang et al., 2017,Yoon et al., 2018) improved the sensitivity of neuropeptide analyses. Solid-phase extraction was also brought online to seamlessly clean up neuropeptide samples prior to LC-MS (Axel et al., 2017).

Alternatively, MSI strategies are popular for direct measurement and spatial localization of bioactive peptides, particularly in limited samples; sampling with a focused laser beam requires minimal sample preparation yet yields a sufficient amount of analytes for ionization and detection. Development of validated protocols made sample preparation for MSI straightforward and robust (Chatterji et al., 2013,DeLaney et al., 2022,Reyzer et al., 2013). They predominantly involve sectioning of frozen or embedded tissues or dissection of cells; however, careful steps need to be undertaken to maintain sample integrity and reproducibility within measurements. To remove interfering contaminants and reduce signal suppression, washing or desalting steps are common prior to freezing. By stretching the tissue, the spatial resolution of MSI was improved to enable localization of neuropeptides in individual neurons of *A. californica* (Zimmerman et al., 2011). Similarly, electroblotting through trypsin containing membrane was found to enhance tryptic digestion directly in tissues to improve protein identification without compromising their spatial location (Andrews et al., 2021). Employing a secondary step of ionization on the expanding MALDI plume improved ion yields, boosting the sensitivity of spatial imaging of the tryptic peptides(McMillen et al., 2021).

iii. Metabolomics—High-sensitivity MS-based metabolomics can be streamlined to deliver high reproducibility facilitating both absolute and relative quantification of metabolites. Common sample preparation protocols in metabolomics include quenching

of enzymatic activity, analyte extraction, purification, and desalting, and/or chemical derivatization prior to separation. To so deepen metabolite coverage, detection sensitivity can be improved by tailoring the chemical composition, pH, and temperature of the extraction solvent to match the chemical properties of the compound classes of interest (e.g., amino acids, lipids, nucleotides). For example, a discovery study in *E. coli* culture reported that polar solvents (e.g., water, ethanol) in combination with high temperature effectively extracted hydrophobic and hydrophilic metabolites as compared to less polar solvents (e.g., acetone) (Yanes et al., 2011). Several advances for cellular and subcellular sample preparation were developed, including optimized extraction solvent mixtures (Onjiko et al., 2016), on-column preconcentration or sample stacking (Kawai et al., 2019,Liao et al., 2020,Liu et al., 2014), and microfluidic devices (Wu et al., 2004). Recent technologies in droplet microfluidics were reviewed elsewhere (Matula et al., 2020).

Detection sensitivity benefits from sample cleanup. These strategies aim to reduce ion suppression while minimizing the risk of concentration dilution to help distinguish analyte signals from chemical interference. Recently, six targeted metabolites were detected in MCF-7 cells without lipid interference using simplified microextraction, which used the same pulled glass capillary to dispense a droplet of solvent onto the targeted cell for selective extraction and sampling by aspiration (Zhang et al., 2016). Alternatively, an on-probe derivatization step was reported to selectively enhance the signal of target metabolites, thus reducing ion suppression (Cao et al., 2020). Simplified extraction methods can also be employed to broaden the types of metabolites detectable in single cells and subcellular organelles, as was accomplished for polar and non-polar metabolites in single cells using analyte partitioning within a microcapillary (Hamilton et al., 2017). The single-step method successfully decreased ion suppression during lipidomics by MALDI-MS.

As with other ‘omic areas, droplet-based microfluidic chips allow limited sample handling by confining single cells in low-volume droplets to preserve the intact cell. These approaches were the focus of several comprehensive reviews (Feng et al., 2015,Gao et al., 2013,Joensson et al., 2012,Liu et al., 2019,Matula et al., 2020,Shang et al., 2017). A major advantage of microfluidic methods is the resulting high throughput that enables the rapid interrogation of hundreds of cells in a single experiment (Cahill et al., 2019,Zhang et al., 2016). For example, by combining single-cell printer (SCP) technology with liquid-vortex capture (LVC)-MS (Fig. 4), a recent study demonstrated direct analysis of metabolites in single cells at a rate of ~30 cells/s (Cahill et al., 2019). LVC-MS has also been combined with LCM to enable matrix-free spatially resolved direct analysis of metabolites from 20–40 μm tissue sections (Cahill et al., 2016). An important aspect to consider when using microfluidics for cell isolation is the possible perturbation to the cell’s native metabolome due to external factors, such as mechanical forces.

Techniques enabling *in situ* or minimally intrusive cellular or subcellular isolation can assist in these challenges. Recent methods using, for example, sharpened or dual-barrel capillaries, reproducibly targeted and aspirated picoliter-to-nanoliter sample volumes from neurons (Aerts et al., 2014,Choi et al., 2022), *Allium cepa* cells (Huang et al., 2021,Saha-Shah et al., 2016), and *X. laevis* embryonic cells (Lombard-Banek et al., 2021,Onjiko et al., 2017,Saha-Shah et al., 2019). Likewise, a single-lysosome mass spectrometry (SLMS)

platform was recently reported which combines patch-clamp recording and induced nanoESI (InESI)-MS to enable electrophysiological profiling followed by direct metabolic analysis of individual enlarged lysosomes within ~80 s (Zhu et al., 2021). The same glass micropipette used as a recording electrode served as the electrospray emitter for subsequent ESI-MS analysis. Further advances aiming to extract and measure mitochondrial, cytosolic, and nuclear metabolites can open new avenues for investigating metabolic regulation within these subcellular compartments. Overall, the fast sampling afforded by these and other capillary probes help minimize metabolic changes and stress arising from endogenous metabolism, transitions in cell cycle, or metabolic degradation, thus providing a more precise view of the cell's metabolic state (Onjiko et al., 2017).

Integration of HRMS with light microscopy, fluorescent labeling, and MSI opened the possibility of high-throughput single-cell and -organelle metabolomics. SpaceM, an open-source method for *in situ* single-cell metabolomics, demonstrated the detection of ~100 metabolites from more than 1,000 individual cells per hour to classify cell types with high accuracy (~91%) (Rappez et al., 2021). Microscopy-guided MALDI allowed for profiling over 30,000 individual rat cerebellar cells, revealing cellular heterogeneity (Neumann et al., 2019). Image-guided MALDI HRMS employing a FTICR mass spectrometer enabling surveying lipid and peptide heterogeneity between 0.5- to 2-μm-diameter dense-core and lucent vesicles in *A. californica* cells (Castro et al., 2021). As shown in Figure 4, a liquid microjunction (LMJ) probe further enabled coupling optically guided MALDI with CE MS to extend the metabolic coverage from single pancreatic islet cells (Comi et al., 2017).

Minimization or elimination of chemical matrix for the purpose of sampling or ionization facilitated measurement throughput and reduced chemical interferences. In the vacuum, nano laser probe-based LDI achieved a resolution of 300 nm and was able to capture the localization of anticancer drugs within organelles of a HeLa cell (Meng et al., 2020). NIMS (Woo et al., 2008) and silicon NAPA (Korte et al., 2019) used specialized substrates to promote desorption and ionization for high-resolution spatial imaging of tissues, cells, and organelles. SIMS has a demonstrated history of imaging small biomolecules with ~650 yoctomole sensitivity (Greving et al., 2011) with resolutions on the order of a few tens of nanometer, as was reviewed recently (Massonnet et al., 2019). Early on, for example, the technology found changes in lipid domains in mating *Tetrahymena thermophila* (Kurczy et al., 2010). NAPA imaged triglycerides from tissues, which are otherwise difficult to ionize (Fincher et al., 2020). To facilitate chemical identifications, a TOF instrument equipped with dual C_{60}–SIMS and MALDI increased the impact area of molecular-ion projectiles (Lanni et al., 2014), while matrix-enhanced SIMS used a matrix as energy sink, allowing for profiling metabolites and neuropeptides in tissues (Potocnik et al., 2017) and single cells with a high 6 s/cell measurement throughput (Do et al., 2017). These and other developments in SIMS and MALDI were the focus of recent reviews (Lanni et al., 2012,Zhu et al., 2022).

Ambient MS brought spatial and temporal imaging to the ordinary environment. Live single-cell video-MS (Mizuno et al., 2008), DESI, and LAESI MS provided convenient detection of primary and secondary metabolites in scalable resolution from tissues to cells, as was reviewed (Nemes et al., 2010,Wiseman et al., 2006,Zhang et al., 2018). LAESI MS pioneered spatial interrogations of tissues from plants and mouse brain sections in 2

(Nemes et al., 2010,Stopka et al., 2019) and 3 dimensions (Nemes et al., 2009). Sequential tissue sectioning with ambient MSI on each section allowed DESI (Eberlin et al., 2010) and nanoDESI (Lanekoff et al., 2015) to reconstruct the 3-D distribution of metabolites in the mouse brain. The ability to spatially resolve metabolic signatures in tissues to cells promises to drive new biological discoveries and applications, for example, in the study of cancer cell populations and their microenvironment. Moreover, the development of methods to measure subcellular metabolic signatures offers an avenue for new biological insights.

iv. Multi-‘Omics—While individual ‘omics analyses can provide insight into specific types of biomolecules in various samples, simultaneously characterizing multiple classes of biomolecules in the same samples can provide a deeper and more complete picture of biological pathways. Furthermore, measuring the levels of multiple biomolecules across space and time is necessary to better understand dynamic biological processes, such as development, which would not be captured by a single-time snapshot (Lopes et al., 2020). Protein, peptide, and metabolite levels also change as a result of intrinsic and extrinsic factors (i.e., conditions of sample collection), such that simultaneous collection of multiple biomolecules from the same samples is favorable to enhance the fidelity, robustness, and reproducibility of analyses, particularly on limited samples.

Several methods were developed to enable sample processing for multi-omic studies on the same sample. Representative examples range from simple molecular weight cutoff filtration for proteomics and peptidomics (Zougman et al., 2008) to multi-phase partitioning via simultaneous metabolite, protein, lipid extraction (SIMPLEX) (Coman et al., 2016). A more recent tip-based extraction, simultaneous trapping (SiTrap), was developed to allow proteomics and metabolomics on tens of micrograms of proteins from the same cell or tissue (Zougman et al., 2020). With further miniaturization, this method could be advantageous for multi-‘omic analysis of limited samples, as fewer transfer steps help to minimize sample handling and reduce analyte loss compared to phase-based extractions. Although recent efforts introduced methods to collect samples from the same tissue or cell for dual proteomics and metabolomics(Lombard-Banek et al., 2021,Pace et al., 2021), simultaneous extraction techniques for multiple biomolecules to enable multi-‘omics from limited samples have yet to be developed. Continued advancements in this area are necessary to facilitate spatiotemporal multi-‘omics of small populations of cells, single cells, and subcellular components, which will make new biological discoveries possible.

C. Separation

To facilitate molecular detection in the collected specimens, chemical separation usually precedes HRMS measurement. Separation provides another layer of compound-dependent information, the time of separation, to aid molecular identifications. It also helps minimize or eliminate chemical interferences during ionization and detection, thus improving sensitivity and the confidence of molecular identifications. Further, by spreading the chemical complexity of the sample over time, separation enhances the efficiency of duty cycle utilization during tandem and multi-stage MS, which in turn deepens the detectable and quantifiable portion of the ‘omes. This section highlights approaches, shown in Figure 5,

that facilitate spatiotemporal studies on limited specimens, ranging from cell populations to single cells to organelles, using some form of separation.

i. Liquid Chromatography—As a result of extensive research in stationary phase chemistries and engineered systems capable of withstanding high pressures, automation, and commercialization, LC became the mainstream separation technology for HRMS. Virtually all types of proteins, peptides, metabolites, lipids, and drug molecules can be separated using high-performance LC, or HPLC (Aydogan et al., 2020,Broeckhoven et al., 2021). Reversed-phase liquid chromatography (RPLC) employing packed-bed stationary phases with C_{18} chemistries (Fig. 5) is efficient in resolving peptides and hydrophobic metabolites including lipids, whereas C_4 or C_8 chemistries were found to work well for intact proteins (Catherman et al., 2014). The development of hydrophilic-interaction LC (HILIC) extended analyses to small and polar molecules, including neuropeptides, amino acids, and glycans (Aydogan et al., 2020,Mihailova et al., 2008).

Combining orthogonal mechanisms of separation enhances trace-sensitive analyses. Higher theoretical plate numbers resulting from the hyphenation of multiple dimensions of separation deepens the molecular depth of detection. The multidimensional protein identification technology (MudPIT) recognized these fundamental advantages for bottom-up proteomics early on by supplementing strong cation exchange (SCX) with reversed-phase chromatography (Schirmer et al., 2003). To reduce salt load from SCX, a modification of the approach used RPLC chemistries in both dimensions, first to fractionate at high pH (e.g., pH 8–10), then to separate each fraction at the conventional low pH (e.g., pH 2–3). High pH fractionation doubled the overall depth of the detectable phosphoproteome from mouse cell lines compared to SCX, identifying over 30,000 phosphopeptides (Batth et al., 2014). For limited cell populations, for example, this approach recently yielded 70% improvement in the coverage of the measurable proteome, allowing for the molecular characterization of the developing neural ectoderm during embryogenesis (Baxi et al., 2018). Fractionation using stage tips (Kim et al., 2019) or nano-liter volumes (Reubsaet et al., 2020) further aided in reducing sample loss for deeper molecular coverage.

Recent advances in high performance LC (HPLC) extended HRMS to ever-decreasing amounts of materials (Fig. 5). To reduce analytical requirements to few micrograms to nanograms of proteomes, new-generational columns enhanced the peak capacity of partition chromatography by packing narrow-bore columns (inner diameters < 50 μm) with small silica particles (< 5 μm). These columns operate at nanoflow rates (< 50 nL/min), thus concurrently reducing sample dilution and increasing ionization efficiency towards ultrasensitive detection. Recently, 362–874 protein groups became identifiable from a single HeLa cell (< 150 pg) using flow rates of ~20 nL/min through an in-house-packed nanoLC column with 20 μm inner diameter (Cong et al., 2020). With 10–100-fold improvement in sensitivity over narrow-bore packed columns (~15–30 μm inner diameter), these continuous developments in HPLC were recently adopted to microfluidic systems capable of automating lossless sample preparation, such as nanoPOTS (Williams et al., 2020), to address the challenge of protein loss. Similarly, metabolomic coverage also benefited from reducing the flow rate from the analytical (0.5 mL/min) to the microflow (8 μL/min) regime, especially for sample-limited studies (Geller et al., 2022).

New-generation LC columns overcame challenges associated with packing narrow columns with robust performance. As illustrated in Figure 5, open tubular, monolithic, and micro pillar array columns (μPAC) reduced band broadening and increased peak capacities using flow rates decreased to nanoliters to subnanoliters per minute (Desmet et al., 2020). The resulting enhancements in separation improved sensitivity and reproducibility for trace-level proteomics and peptidomics (Aydogan et al., 2020,Broeckhoven et al., 2021,Yi et al., 2017) as well as metabolomics (Maes et al., 2014,Turiak et al., 2018,Zemenova et al., 2017). Capillary monolithic columns with 12 nL/min flow rate enabled identification of over 2,300 proteins from 1 ng of HeLa digest (Gregus et al., 2020). Flows on the order of picoliters per minute through narrow open tubular columns with ~2 μm inner diameter recently elevated identifications to ~1,000 proteins from 75 pg of *Shewanella oneidensis* tryptic digest, which was equivalent to half the amount obtained from a single HeLa cell (Xiang et al., 2020). Monolithic capillary columns were used to spatially profile the proteome and phosphoproteome of the plasma and the vacuolar membrane in *Oryza sativa* (Whiteman et al., 2008). Alternatively, fabricated monolithic capillaries were used for solid phase extraction and concentration to peptides released from spatially distinct regions of *A. californica* brain tissue followed by MALDI analysis (Iannacone et al., 2009).

Improvements in LC-ESI interfaces played an equally important role in advancing the sensitivity of analyses. In the conventional ESI interfaces, the LC eluent is fed through a metal emitter or pulled glass capillary, and sufficient potential difference is established between the spray tip and the mass spectrometer to form a stable electrospray. Electrosprays, however, can operate in different ways (Nemes et al., 2007), and transitions between these modalities can impact performance and the higher-order structure of molecules (Nemes et al., 2008). Figure 5 shows the impact the onset of different spraying regimes has on spray dynamic and MS ion signal when the ESI potential was adjusted. The relative ion signals, stability, and chemical composition of the electrospray are all impacted by alterations in the spraying regime. As illustrated, the cone-jet regime generates small droplets continuously, thus maximizing ionization efficiency (Nemes et al., 2007). The interface called UniSpray (Fig. 5) places an impactor rod of high voltage between the grounded capillary tip and inlet of the MS to facilitate electrospray ionization of the primary electrosprayed droplets, thus boosting S/N for neuropeptides (Bongaerts et al., 2020). Following the scaling laws of ESI, emitter tips with submicron-diameters (e.g., ~0.6 μm) were recently demonstrated to improve protein and oligonucleotide sensitivity by efficiently reducing their formation of clusters and adducts (Kenderdine et al., 2018).

ii. Capillary Electrophoresis—While LC is a powerful separation method for complex biological samples, separation with CE can offer distinct advantages. Unlike HPLC, CE separates charged compounds in solution based on differences in their electrophoretic mobilities. With typical sample consumption on the order of a few nanoliters, this technology is a natural fit for volume- and mass-limited samples. Several methods allow for efficient pre-concentration of analytes via in-column sample stacking (Osbourn et al., 2000). Field-amplified sample stacking (FASS) and exquisite separation power permit efficient differentiation of trace amounts of proteins, peptides, and metabolites, as was recently reviewed in multiple studies (DeLaney et al., 2019,Drouin et al., 2021,Lombard-Banek et

al., 2019,Zhao et al., 2012). With large-volume sample stacking (LVSS) using a dynamic pH junction, a 10-fold enhancement in sensitivity (Fig. 5) enabled the detection of 5,700 proteoforms in the *Escherichia coli* proteome (McCool et al., 2018) and peptides from the renin-angiotensin system in the paraventricular nucleus of the mouse central nervous system (DeLaney et al., 2022). An international study found CE robust and reproducible among 13 different laboratories (Drouin et al., 2020), projecting this technology to complement chromatography-based HRMS for spatiotemporal ‘omics.

Ultrasensitive measurements also benefited from the innovation of specialized CE ion sources for HRMS. The development of low sheath-flow and sheathless interfaces supported the coupling of CE with electrospray ionization (ESI) HRMS, reviewed elsewhere (Gomes et al., 2019,Lindenburg et al., 2015). Figure 5 highlights representative CE-ESI interfaces that enabled stable and sensitive CE-ESI operation using blunt-tip and tapered-tip metal as well as microfabricated borosilicate and fused silica emitters. These custom-built CE-HRMS instruments extended these measurements to attomole to zeptomole to yoctomole levels of metabolites and peptides from limited samples and broad types of biological models (Bonvin et al., 2012,Choi et al., 2017,DeLaney et al., 2022,Hirayama et al., 2018,Moini 2007,Peuchen et al., 2017,Vermeire et al., 2020).

Microsampling and custom-built CE-HRMS platforms enabled spatiotemporal profiling of important biomolecules. Using micropunch sampling, the technology recently permitted characterization of nearly all the angiotensin peptides of the renin-angiotensin system in the subfornical organ and paraventricular nucleus of the mouse brain with a 30 zmol (~16,000 copies) of limit of detection, raising a potential to facilitate future studies on thirst and water homeostasis and regulation of the neuroendocrine system to stress (DeLaney et al., 2022,Lombard-Banek et al., 2019). Further, microdissection and capillary microsampling extended ultrasensitive CE-HRMS to hundreds of different metabolites (Aerts et al., 2014,Onjiko et al., 2015) and up to ~2,000 different proteins in single neurons in the central nervous system of the mouse (Choi et al., 2022) as well as blastomeres in *X. laevis* and zebrafish embryos (Lombard-Banek et al., 2021,Lombard-Banek et al., 2019,Lombard-Banek et al., 2016). Results from these studies revealed previously unknown molecular differences between identified embryonic cells and reorganization of the single-cell proteome and metabolome during formation of cell lineages (Lombard-Banek et al., 2019,Onjiko et al., 2017). Microdialysis combined with CE has enabled *in vivo* study of cerebral metabolism of peptide E in rodent brain (Behrens et al., 2010).

Information from these studies supported the generation of hypotheses and led to biological discoveries. CE-HRMS metabolomics on dissected identified neurons revealed distinct metabolomes in *A. californica* neurons and their regional composition (Lapainis et al., 2009,Nemes et al., 2013). CE-HRMS was key in the discovery of noncanonical cell fate-altering molecules and mechanisms of embryonic patterning (Onjiko et al., 2015). Most recently, the technology enabled the characterization of ~700 proteins and ~150 metabolites from ~10 nL aspirates from single identified blastomeres in cleavage-stage *X. laevis* embryos, enabling *in vivo* proteomics in live embryos (Lombard-Banek et al., 2021). Using top-down HRMS, the technology also enabled detection of ~400 different proteoforms in distinct regions of the zebrafish brain, containing ~250 cells (Lubeckyj et al., 2022).

CE-HRMS aided temporal studies on the expression of N- glycans and N-glycosylated peptides during vertebrate embryonic development, revealing extensive reorganization of the N-glycome expression during neurulation indicating their importance in neural development (Qu et al., 2020).

Integration of CE with orthogonal HRMS technologies opened exciting new directions in basic research. CE-HRMS with CE modes (Xu et al., 2012) and LC fractionation (Yang et al., 2019) deepened detection of proteomes. Coupling high pH fractionation followed by CE analysis enabled identification of 6,500 proteins from 500 ng of protein from a human cell line. When coupled with ion mobility detection, optical isomers became distinguishable in single-cell bodies isolated from *A. californica* pleurin neurons (Mast et al., 2021). With MALDI-MS, CE enabled rapid fingerprinting of individual pancreatic islet cells with single-cell resolution, enabling comparison of deep metabolic profiles between the different cell types (Comi et al., 2017).

Further automation and miniaturization through microfluidic chips raised the possibility of ultrasensitive measurements. Microfluidic CE systems further expand HRMS to ultrasensitive analysis by performing critical steps of the analytical workflow, from sample lysis to separation, in a single platform with minimal intervention and, in turn, reducing sample loss. For example, an integrated microfluidic device enabled the analysis of α and β subunits of hemoglobin in 12 human erythrocytes per minute (Mellors et al., 2010). High throughput provided by this platform was used to analyze intracellular levels of metabolites in a large number of neuronal cells under different physiological conditions (Li et al., 2016). Such automated systems complement fast separation permitted by CE to enable high throughput analysis of large single cell populations, as was reviewed elsewhere (Abraham et al., 2019).

iii. Ion Mobility Spectrometry—Gas-phase ion separation provides speed and added resolution to aid molecular detection. With ion mobility separations taking less than 100 milliseconds, ion mobility can be nested into most liquid-phase separations prior to HRMS detection. Most IMS techniques yield information to enable the calculation of collision cross sections (CCS), which serves as a compound-dependent information to aid molecular identifications. This approach enabled the elucidation of compound classes with large molecular diversity, including D- and L-amino acid containing peptides (Fouque et al., 2017) and neuropeptides of the renin-angiotensin system in the paraventricular nucleus of the mouse central nervous system (DeLaney et al., 2022). IMS facilitated a broad range of investigations, ranging from proteins to small metabolites to lipids to glycans, as was reviewed elsewhere (Burnum-Johnson et al., 2019).

IMS techniques combined with other separation techniques, such as HPLC, to help distinguish isomers, isobars, and conformers. As a dual separation device and charge-state filter, field asymmetric waveform IMS FAIMS increased unique peptide identifications by ~30% and doubled the number of quantifiable peptides over non-IMS experiment from HEK293 cells due to a 10-fold enhancement in ion signal abundance and improved utilization of MS/MS duty cycles (Pfammatter et al., 2018). FAIMS was employed to filter out singly charged ions and improve detection of peptides from single HeLa cells

processed using nanoPOTS, providing over two-fold improvement compared to without FAIMS (Cong et al., 2020). Trapped IMS (TIMS) enabled regio-specific profiling of novel D-amino acid-containing peptides in the *A. californica* central nervous system (Mast et al., 2020). IMS also aids deepening molecular coverage from complex biological samples by providing an additional dimension of separation, the collision cross section (CCS), and reducing chemical noise and isobaric interferences. HPLC with TIMS-MS provided orthogonal separation to facilitate speciation for over ~1,400 lipids and capture their spatial distribution in mouse brain tissues (Chen et al., 2022). HPLC with traveling wave IMS (TWIMS) was used to identify metabolic changes during colorectal cancer progression (Williams et al., 2015). Distinct metabolic profiles were captured to differentiate colorectal cancer from non-malignant tissues, and across different stages of cancer progression.

IMS provides separation in cases when classical solution-based separations are restrictive or not feasible. Such is the case in most ambient ionization experiments, where LC- or CE-based separation would hinder analytical throughput and complicate sample processing. For example, capillary microsampling coupled with TWIMS-MS enabled neuropeptide measurements from cytoplasmic and nuclear components of single neurons from *Lymnaea stagnalis*, yielding previously unavailable data for functional biological experiments on their roles (Zhang et al., 2018). Similarly, dual-probe microsampling with drift-tube IMS-MS reported 73 lipids and 79 metabolites from live mammalian microglial cells (Domenick et al., 2020). Further, IMS boosts the reproducibility and confidence of identifications and quantification in MSI (Sans et al., 2018). LAESI with IMS imaged the distribution of isobaric primary and secondary metabolites, such as flavonoids, in plant species and lipids and metabolites in mouse brain sections (Li et al., 2015). MALDI with TIMS MS enabled spatial mapping of isobaric and isomeric lipids in crustacean tissues (Fu et al., 2020). Recently, ambient ionization using liquid extraction surface analysis with TWIMS allowed imaging of intact proteins from mouse kidney tissue (Hale et al., 2020).

D. Mass Analysis and Detection

Spatial HRMS ‘omics profits from a still-expanding array of technological innovations. Development of specialized ion optics enabling efficient ion collection and transfer (e.g., ion funnel) as well as systems integrating new-generation mass analyzers, digitizers, and ion detectors (e.g., TOF, orbitrap, FTICR, SIMS) ushered in a new era of sensitivity (detection limit), speed, and spectral resolution for limited specimens. A subset of developments is shown in Figure 6. High-repetition lasers and fast electronics enabled record 50 pixels/s data acquisition rates in MALDI-TOF, sufficiently high to enable fast 2- and even 3-D imaging. In a recent example, 3D-MALDI-TOF MS (Fig. 6) revealed differences between the lipid architecture of metastatic and non-metastatic medulloblastoma tumors, yielding new information on disease progression (Paine et al., 2019). Customized nano-flow ESI (nanoESI) ion sources on tims-TOF MS (Fig. 6) quadrupled the detectable ion signal, deepening proteome coverage to > 3,900 proteins in 1 ng of protein from single HeLa cells (Brunner et al., 2022).

Higher spectral resolution from orbitrap and FTICR instruments supported molecular identifications and coverage. Late-generation orbitrap tribrid mass spectrometers increased

the detectable proteome from a single HeLa cell by ~36% and ~20% at the protein and peptide level, respectively, compared to the earlier orbitrap designs (Cong et al., 2020). Additionally, transferring identification based on FAIMS filtering (TIFF) permitted identification of ~1,700 proteins from single HeLa cells using varying compensation voltages along with retention time and accurate mass (Woo et al., 2022). A 3D SIMS instrument equipped with an orbitrap analyzer (Fig. 6) permitted high-resolution spatial imaging with high-accuracy spectral information, revealing lipid dysregulation during macrophage differentiation (Suvannapruk et al., 2022). Microscopy-guided MALDI with FTICR MS (Fig. 6) enabled profiling ca. 500 lipids from 30,000 cells from the rat cerebellum, finding previously unknown molecular cell heterogeneity in the tissue in record time (Neumann et al., 2019). *In situ* microsampling by fiber-optic LAESI, or f-LAESI (Fig. 6), with 21 T FTICR MS tracked metabolite responses in single plant cells upon bacterial infection (Samarah et al., 2020). These and other advancements continue to expand the capabilities of spatial measurements down to the building block of life, the cell.

Spatiotemporal ‘omics naturally benefited from advances in tandem MS with increasing throughput, sensitivity, and specificity. An intelligent DDA method monitored the elution order of peptides based on prior discovery measurements to target tandem MS events, resulting in 80% more protein identifications than the standard (Bailey et al., 2014). BoxCar DDA leveraged multiple narrow mass-to-charge (*m/z*) windows to improve ion accumulation during MS scans, realizing an order of magnitude improvement in quantification (Meier et al., 2018). A DDA ladder employed a nested exclusion series of abundant peptide ions to reduce MS/MS redundancy, allowing identification of 35% more proteins and quantification of 415 proteins from ~5 ng of protein digest (approx. 10 neurons) from the mouse nervous system (Choi et al., 2021). In parallel, instruments with multiple fragmentation cells opened the door to using structurally diagnostic fragment ions to trigger sequential or multiplexed dissociation toward deeper sequence coverage. For example, electron transfer dissociation and higher-energy collisional dissociation (EThcD) discovered 4 different N-linked and 14 distinct O-linked glycosylated neuropeptides as well as 91 novel neuropeptides in identified crustacean neural tissues (Cao et al., 2020). The advent of data-independent acquisition (DIA) remedied limited tandem MS duty cycles during DDA, approximately doubling the number of neuropeptides in limited neural tissues (DeLaney et al., 2019). This approach detected ~1,500 proteins from single cells in the developing *X. laevis* embryo (Saha-Shah et al., 2019).

Spatial HRMS provides several strategies for quantification (recall Fig. 2). Both label-free quantification (LFQ) and label-based quantification were adapted to limited specimens in the context of spatiotemporal biology. LFQ uses spectral counts, peak intensities, or peak areas to estimate the abundance of proteins, peptides, and metabolites without barcoding (Dupree et al., 2020,Lai et al., 2013). A reduction in sample processing steps aids analytics on trace amounts of samples, as was reviewed recently (Slavov 2021). Chemical derivatization helps analytical throughout and reproducibility, and when performed online, enabled low-picomolar detection limits for peptides in microdialysates continuously collected from rat brains (Wilson et al., 2018). To enhance throughput, label-based quantification may integrate stable isotopes during sample preparation *in vivo* (metabolic labeling) or *in vitro* (chemical labeling) to enable multiplexing, as was reviewed elsewhere (Arul et al., 2019).

Label-based quantification became quasi routine for high-throughput quantification across broad spatial and temporal processes, lately even for single cells. Reductive methylation (DeLaney et al., 2021) and DiLeu labeling (Sauer et al., 2021) aided neuropeptidomics. The carrier approach pioneered the use of tandem mass tags (Thermo Fisher Scientific) in abundance to boost tandem MS on isobarically tagged protein digests from single cells (Slavov 2022), while usage of a sacrificial protein helped reduce nonspecific protein losses during sample preparation (Slavov 2021). SCoPE-MS (recall Fig. 4) is one such method, which distinguished cancer cell types at the single-cell level and characterize cell-to-cell heterogeneity during embryonic stem cell differentiation (Budnik et al., 2018). The new-generation SCoPE2 further downscaled volumes, automated sample preparation, increased throughput, and optimized MS data acquisition and analysis to quantify ~1,000 proteins per TMT experiment with only one hour of LC separation (Specht et al., 2021). Integration of carrier-based approaches with newer instrumentation advancements further compounded the demonstrated improvements in proteome coverage and quantification from single cells. For example, FAIMS and real-time search in both MS/MS and synchronous precursor selection (SPS)-MS^3 methods on an orbitrap mass spectrometer recently facilitated higher proteome coverage and more accurate quantification in single acute myeloid leukemia cells by lengthening ion trap times, thus boosting sensitivity (Furtwängler et al., 2022). Combining small-window DIA acquisition with a TMT tagging yielded high-throughput multiplexing analysis of trace samples (Ctortecka et al., 2022). Additional improvements in efficient duty cycle utilization, sensitivity, and real-time search capabilities made possible by continued technological advances will further enhance performance of these carrier-based quantification methods for proteomics analysis of limited samples.

E. Data Analysis

Bioinformatics plays a critical and enabling role for spatiotemporal HRMS. This field of HRMS yields challenging data, not only in terms of complexity and size, but also metadata to assess in relation to space and time. In Figure 7, representative examples are grouped based on their abilities to access these dimensions of information. General steps for data analysis include raw data processing (feature extraction or identification), data preprocessing, statistical analysis (quantitative information), and gene ontology annotations (qualitative information). Data from imaging microscopy (optical to electron) and functional tests, including electrophysiology and behavioral assays, complement chemical data from MS ‘omics with information on the biological phenotype. Analysis of such complex data poses several challenges such as handling big datasets, integrating orthogonal information obtained by combining different data acquisition techniques, accuracy of identification, and data processing speeds. Here we highlight select examples of software packages that helped address these challenges.

The first step of data analysis includes processing raw files obtained from MS analysis to identify proteins, peptides, or metabolic features. With ever-decreasing sample amounts to achieve spatial resolution at cellular and subcellular level, distinguishing true signal from noise is a fundamental challenge. New and next-generation bioinformatics aided the processing of low ion signals that could be mistaken for noise (false negative) or noise that could be mistaken for signal (false positive). The *Trace* software package employed

machine learning on CE-HRMS datasets to create signal images from *m/z* and separation time data. These signal images effectively distinguish true signal from noise, boosting the surveying of molecular features to a record 95% confidence in single-cell metabolomics (Liu et al., 2019). NPFimg recently extended the approach to metabolomics based on gas chromatography-MS (Jirayupat et al., 2021). OffsampleAI employed artificial intelligence for spatial metabolomics to recognize background interfering ions during MSI experiments. A machine learning tool was trained using ~2,300 manually curated ion images to recognize background ions, thus improving metabolite identification from tissues (Ovchinnikova et al., 2020).

In parallel, a series of software developments provided ever-increasing speed, accuracy, and depth of coverage of the analyzed ‘omes. To improve identifications from complex convoluted DIA datasets, newer machine learning tools, such as DeepMass (Tiwary et al., 2019), accurately predicted peptide fragment spectra to build spectral libraries *in silico*, essentially eliminating restrictions on laborious experimental reference libraries from precious, rare, or limited specimens. Similarly, PRESnovo, a *de novo* sequencing tool developed for neuropeptidomics, directly predicted peptide sequences (DeLaney et al., 2020), leading to the identification of 13 novel, putative neuropeptides from *Carcinus maenas* and 77 from *Cancer borealis* brain tissues. Most recently, several software tools were developed to overcome limitations in computationally intense DIA and ion mobility data, which were recently discussed elsewhere (Allison et al., 2020,Rosenberger et al., 2017,Szymanska et al., 2016,Zhang et al., 2020).

As in other areas of HRMS, scientific rigor calls for stringent quality controls not just during measurements but also during data processing. After extracting qualitative (molecular identifications) and quantitative (abundance or intensities) information, data preprocessing uses normalization and advanced data checks to disentangle biological variations from artifacts (Slavov 2022). Programs such as SCPCompanion helped credential the quality of single-cell proteomics data (Cheung et al., 2021) and suggest optimization of instrument parameters based on the preliminary data. In addition, single-cell proteomics readout of expression (SCeptre) and NOREVA provided normalization strategies to assist with reducing non-biological variations in multiplexed single-cell ‘omics (Li et al., 2017,Schoof et al., 2021). SpectralAnalysis was adapted to multiple instruments and provides a framework from data preprocessing to multivariate analysis within a single software package (Race et al., 2016).

Chemometrics is pivotal for the interpretation of metadata from spatiotemporal HRMS (recall Fig. 2). This field of research offers various solutions for statistical and multivariate analyses. For example, volcano plots are widely used to examine molecular differences between spatially or temporally distinct sample types. Unsupervised and supervised dimensionality reduction and clustering algorithms such as principal component analysis (PCA), linear discriminant analysis (LDA), t-distributed stochastic neighbor embedding (t-SNE), hierarchical cluster analysis (HCA), heatmaps, k- and fuzzy-mean clustering are broadly adaptable to exploring systematic molecular differences between samples, such as types of tissues or cells. Figure 7 presents representative examples on spatially profiling to imaging proteins between sciatic nerves dissected from the rat using unsupervised HCA with

LC-HRMS (Di Paolo et al., 2021), neuropeptides in dense core vesicles in *A. californica* neurons using unsupervised PCA/t-SNE with 2-D MALDI MSI (Castro et al., 2021), and chlorophyll and secondary metabolites in areas of the variegated *A. squarrosa* leaves using light-ion image correlation with 3-D LAESI MSI (Nemes et al., 2009). Pearson correlation analysis and LC-HRMS permitted comparison of temporal expression between transcription and translation at the level of whole developing *X. laevis* embryos (Peshkin et al., 2015), single differentiating embryonic stem cells (Budnik et al., 2018). Live stereomicroscopy and micro-probe CE-MS used fuzzy means cluster analysis to find reorganization of the single-cell proteome as cells formed a neural tissue-fated clone in the live *X. laevis* embryo (Onjiko et al., 2017). STRING was used to predict functional association networks on proteins that were quantified in single dopaminergic neurons in the mouse brain after electrophysiology using patch CE-MS (Choi et al., 2022). Software packages like MetaboAnalyst (Pang et al., 2021), Perseus (Tyanova et al., 2016), Orange (Demsar et al., 2013), and IOAT (Wu et al., 2021) provide user-friendly platforms for performing such analyses. EXIMS (Wijetunge et al., 2015) is another such tool developed to recognize spatial distribution patterns in MSI datasets.

A broadening number of software tools enable systems biology for spatiotemporal HRMS. Apart from classical tools from chemometrics, freeware programs, such as MetaboAnalyst, helped integrate transcriptomic to proteomic to metabolomic datasets, with added capabilities for enrichment studies and analyses of pathway networks. STATegra, another open-source tool, merged and identified important features from multi-‘omics data in a stepwise manner (Planell et al., 2021). Similarly, Mergeomics tailored to extracting information on key biological pathways and networks, with demonstrated utility in aiding the understanding of disease progression (Ding et al., 2021). These and other statistical and data visualization pipelines help generate and test new hypotheses at the levels of proteomes, peptidomes, and metabolomes in biological processes occurring in space and time.

III. CONCLUSIONS

Interdisciplinary efforts molded HRMS ‘omics into a powerful tool to advance basic and translational research and also understanding of biological processes occurring in space and time. These developments are the result of still rapidly progressing interdisciplinary and collaborative efforts innovating essentially all steps of biological HRMS. Inventions of specialized strategies, equipment, and miniaturization to collect and process samples with speed and high fidelity is pivotal for studies on limited and dynamic biological systems. Advances in chemical separations, ionization, ion transfer and accumulation, and HRMS detection increase detection sensitivity and measurement throughput, thus ushering ‘omics to increasingly finer spatial and temporal resolutions. Continued developments in data acquisition and user-friendly software packages to handle the resulting complex data promise to further deepen the detectable and quantifiable portion of proteomes, peptidomes, and metabolomes from trace amounts of specimens. Grand challenges in the acquisition, integration, and distribution of complex data from spatiotemporal multi-‘omics pose grant challenges and exciting opportunities to move science forward toward a holistic understanding of life processes.

Sustaining the scientific momentum is as important as using them to best benefit society at large. Collection of proven protocols, formulation of community standards and best practices, and deposition of primary and secondary data into public data repositories—such as pioneering works in metabolomics (Sansone et al., 2007), proteomics (Taylor et al., 2008), MS informatics (Binz et al., 2008), and soon in single-cell analyses—is pivotal for stringent, reproducible, and rigorous science. Investigators continue to break down classical boundaries in bioanalyses to reach ever-deeper into the molecular world. In parallel, it is equally important to make these developments available and adaptable to broad users, including but not limited to investigators in the basic life sciences, translational research, pharmaceutical industry, and healthcare. Furnishing methods with versatility and compatibility across disciplines, automation, and cost-efficiency, are necessary and proven paths toward addressing pressing biological questions at present and to pose new ones to continue leading us to the future.

ACKNOWLEDGEMENTS

This work was sponsored in part by the National Institute of Health (award no. 1R35GM124755 to P.N.), the National Science Foundation (award no. IOS-1832968 to P.N.), the University of Maryland–National Cancer Institute Partnership Program (seed award to P.N.), and the COSMOS Club Foundation (research award to L.R.P).

AUTHOR BIOGRAPHIES

Leena R. Pade is a Ph.D. candidate in Chemistry at the University of Maryland, College Park. She is a fellow under the National Cancer Institute–University of Maryland Partnership for Integrative Cancer Research. She received her master's degree in Pharmaceutical Analysis at National Institute of Pharmaceutical Education and Research, Hyderabad (India) in 2018. Her research in the Nemes Laboratory focuses on developing microanalytical proteomic approaches to elucidate molecular mechanisms underlying the differentiation of embryonic cells and stem-like cell populations to neural and craniofacial tissues during vertebrate embryonic development.

Kaitlyn E. Stepler earned her Ph.D. in Chemistry from Vanderbilt University in 2021 under the mentorship of Dr. Renã A. S. Robinson. Her dissertation research used mass spectrometry-based proteomics to understand racial disparities in Alzheimer's disease. Dr. Stepler is currently a postdoctoral associate at the University of Maryland, College Park in Dr. Peter Nemes' laboratory, where her research focuses on understanding various aspects of embryonic development using proteomics.

Erika P. Portero obtained her Ph.D. in Chemistry from the University of Maryland, College Park under the mentorship of Dr. Peter Nemes. She developed single-cell mass spectrometry tools and methodologies to investigate metabolic reorganization during vertebrate embryogenesis. Dr. Portero is presently a postdoctoral fellow at the Dana-Farber Cancer Institute and the Harvard Medical School in Dr. Nika Danial's laboratory, where she investigates the metabolic characteristics and associated vulnerabilities in pediatric gliomas.

Kellen DeLaney obtained her Ph.D. in Chemistry from the University of Wisconsin-Madison, WI where she developed mass spectrometry techniques to study neuropeptides in

crustacean model organisms in the laboratory of Dr. Lingjun Li. As a postdoctoral associate, she focused on using capillary electrophoresis with ion mobility mass spectrometry to study endogenous peptides in brain tissue in Dr. Peter Nemes' laboratory at the University of Maryland, College Park.

Peter Nemes holds a Ph.D. from the George Washington University (advisor: Dr. Akos Vertes), where he (co)-invented laser ablation electrospray ionization for ambient mass spectrometry and 3-dimensional imaging. As a postdoctoral associate with Dr. Jonathan V. Sweedler (University of Illinois, Urbana–Champaign, IL), he developed mass spectrometry instruments and methods for analytical neuroscience. Research in the Nemes Laboratory, currently at the University of Maryland, College Park, builds ultrasensitive mass spectrometry technologies to study proteo-metabolomic mechanisms governing single-cell processes in biological models of (neuro)developmental biology and neuroscience. Results from the group led to the finding of previously unknown molecular differences between identified cells and neurons as well as the discovery of noncanonical effectors of cell fate decisions and mechanism controlling the patterning of the vertebrate body plan and organismal behavior.

VI. REFERENCES

Abouleila Y, Onidani K, Ali A, Shoji H, Kawai T, Lim CT, Kumar V, Okaya S, Kato K, Hiyama E, Yanagida T, Masujima T, Shimizu Y, Honda K. 2019. Live single cell mass spectrometry reveals cancer-specific metabolic profiles of circulating tumor cells. Cancer Sci. 110:697–706. [PubMed: 30549153]

Abraham DH, Anttila MM, Gallion LA, Petersen BV, Proctor A, Allbritton NL. 2019. Design of an automated capillary electrophoresis platform for single-cell analysis. In: Allbritton NL, Kovarik ML, Editors. Enzyme Activity in Single Cells. London: Academic Press Ltd-Elsevier Science Ltd. p 191–221.

Aerts JT, Louis KR, Crandall SR, Govindaiah G, Cox CL, Sweedler JV. 2014. Patch clamp electrophysiology and capillary electrophoresis-mass spectrometry metabolomics for single cell characterization. Anal. Chem 86:3203–3208. [PubMed: 24559180]

Allison TM, Barran P, Benesch JLP, Cianferani S, Degiacomi MT, Gabelica V, Grandori R, Marklund EG, Menneteau T, Migas LG, Politis A, Sharon M, Sobott F, Thalassinos K. 2020. Software requirements for the analysis and interpretation of native ion mobility mass spectrometry data. Anal. Chem 92:10881–10890. [PubMed: 32649184]

Andrews WT, Bickner AN, Tobias F, Ryan KA, Bruening ML, Hummon AB. 2021. Electroblotting through enzymatic membranes to enhance molecular tissue imaging. J. Am. Soc. Mass Spectrom 32:1689–1699. [PubMed: 34110793]

Arnadottir SS, Mattesen TB, Vang S, Madsen MR, Madsen AH, Birkbak NJ, Bramsen JB, Andersen CL. 2020. Transcriptomic and proteomic intra-tumor heterogeneity of colorectal cancer varies depending on tumor location within the colorectum. PLoS One 15:18.

Arnold PK, Jackson BT, Paras KI, Brunner JS, Hart ML, Newsom OJ, Alibeckoff SP, Endress J, Drill E, Sullivan LB, Finley LWS. 2022. A non-canonical tricarboxylic acid cycle underlies cellular identity. Nature 603:477–481. [PubMed: 35264789]

Arul AB, Robinson RAS. 2019. Sample multiplexing strategies in quantitative proteomics. Anal. Chem 91:178–189. [PubMed: 30525468]

Axel M, Ewelina K, Jenny-Maria B, Leif K. 2017. An online SPE LC-MS/MS method for the analysis of antibiotics in environmental water. Environ. Sci. Pollut. Res 24:8692–8699.

Aydogan C, Rigano F, Krcmova LK, Chung DS, Macka M, Mondello L. 2020. Miniaturized LC in molecular omics. Anal. Chem 92:11485–11497. [PubMed: 32867499]

Azizian NG, Sullivan DK, Nie LT, Pardo S, Molleur D, Chen JJ, Weintraub ST, Li YL. 2021. Selective labeling and identification of the tumor cell proteome of pancreatic cancer in vivo. J. Proteome Res 20:858–866. [PubMed: 33289385]

Bailey DJ, McDevitt MT, Westphall MS, Pagliarini DJ, Coon JJ. 2014. Intelligent data acquisition blends targeted and discovery methods. J. Proteome Res 13:2152–2161. [PubMed: 24611583]

Bardsen K, Gjerstad MD, Partinen M, Kvivik I, Tjensvol AB, Ruoff P, Omdal R, Brede C. 2019. Considerably lower levels of hypocretin-1 in cerebrospinal fluid is revealed by a novel mass spectrometry method compared with standard radioimmunoassay. Anal. Chem 91:9323–9329. [PubMed: 31260261]

Batth TS, Francavilla C, Olsen JV. 2014. Off-line high-pH reversed-phase fractionation for in-depth phosphoproteomics. J. Proteome Res 13:6176–6186. [PubMed: 25338131]

Baxi AB, Lombard-Banek C, Moody SA, Nemes P. 2018. Proteomic characterization of the neural ectoderm fated cell clones in the Xenopus laevis embryo by high-resolution mass spectrometry. ACS Chem. Neurosci 9:2064–2073. [PubMed: 29578674]

Baxi AB, Pade LR, Nemes P. 2021. Mass spectrometry based proteomics for developmental neurobiology in the amphibian Xenopus laevis. In: Sokol SY, Editor Amphibian Models of Development and Disease. San Diego: Elsevier Academic Press Inc. p 205–231.

Bechtel TJ, Li C, Kisty EA, Maurais AJ, Weerapana E. 2020. Profiling cysteine reactivity and oxidation in the endoplasmic reticulum. ACS Chem. Biol 15:543–553. [PubMed: 31899610]

Behrens HL, Li LJ. 2010. Monitoring neuropeptides in vivo via microdialysis and mass spectrometry. In: Soloviev M, Editor Peptidomics: Methods and Protocols. Totowa: Humana Press Inc. p 57–73.

Bensen RC, Standke SJ, Colby DH, Kothapalli NR, Le-McClain AT, Patten MA, Tripathi A, Heinlen JE, Yang ZB, Burgett AWG. 2021. Single cell mass spectrometry quantification of anticancer drugs: proof of concept in cancer patients. ACS Pharmacol. Transl. Sci 4:96–100. [PubMed: 33615163]

Biesemann C, Gronborg M, Luquet E, Wichert SP, Bernard V, Bungers SR, Cooper B, Varoqueaux F, Li LY, Byrne JA, Urlaub H, Jahn O, Brose N, Herzog E. 2014. Proteomic screening of glutamatergic mouse brain synaptosomes isolated by fluorescence activated sorting. EMBO J. 33:157–170. [PubMed: 24413018]

Binz PA, Barkovich R, Beavis RC, Creasy D, Horn DM, Julian RK, Seymour SL, Taylor CF, Vandenbrouck Y. 2008. Guidelines for reporting the use of mass spectrometry informatics in proteomics. Nat. Biotechnol 26:862-862. [PubMed: 18688233]

Bird RE, Lemmel SA, Yu X, Zhou QA. 2021. Bioorthogonal chemistry and its applications. Bioconjugate Chem. 32:2457–2479.

Bongaerts J, Segers K, Van Oudenhove L, Van Wanseele Y, Van Hulle M, De Bundel D, Mangelings D, Smolders I, Vander Heyden Y, Van Eeckhaut A. 2020. A comparative study of UniSpray and electrospray sources for the ionization of neuropeptides in liquid chromatography tandem mass spectrometry. J. Chromatogr. A 1628:14.

Bonvin G, Veuthey JL, Rudaz S, Schappler J. 2012. Evaluation of a sheathless nanospray interface based on a porous tip sprayer for CE-ESI-MS coupling. Electrophoresis 33:552–562. [PubMed: 22451047]

Broeckhoven K, Desmet G. 2021. Advances and innovations in liquid chromatography stationary phase supports. Anal. Chem 93:257–272. [PubMed: 33300336]

Brown HM, Pirro V, Cooks RG. 2018. From DESI to the MasSpec Pen: ambient ionization mass spectrometry for tissue analysis and intrasurgical cancer diagnosis. Clin. Chem 64:628–630. [PubMed: 29378688]

Brunner AD, Thielert M, Vasilopoulou C, Ammar C, Coscia F, Mund A, Hoerning OB, Bache N, Apalategui A, Lubeck M, Richter S, Fischer DS, Raether O, Park MA, Meier F, Theis FJ, Mann M. 2022. Ultra-high sensitivity mass spectrometry quantifies single-cell proteome changes upon perturbation. Mol. Syst. Biol 18:15.

Budnik B, Levy E, Harmange G, Slavov N. 2018. SCoPE-MS: mass spectrometry of single mammalian cells quantifies proteome heterogeneity during cell differentiation. Genome Biol. 19:12. [PubMed: 29386051]

Burke M, Choksawangkarn W, Edwards N, Ostrand-Rosenberg S, Fenselau C. 2014. Exosomes from myeloid-derived suppressor cells carry biologically active proteins. J. Proteome Res 13:836–843. [PubMed: 24295599]

Burnum-Johnson KE, Zheng X, Dodds JN, Ash J, Fourches D, Nicora CD, Wendler JP, Metz TO, Waters KM, Jansson JK, Smith RD, Baker ES. 2019. Ion mobility spectrometry and the omics: Distinguishing isomers, molecular classes and contaminant ions in complex samples. Trends Anal. Chem 116:292–299.

Cadwell CR, Palasantza A, Jiang XL, Berens P, Deng QL, Yilmaz M, Reimer J, Shen S, Bethge M, Tolias KF, Sandberg R, Tolias AS. 2016. Electrophysiological, transcriptomic and morphologic profiling of single neurons using Patch-seq. Nat. Biotech 34:199–203.

Cahill JF, Kertesz V, Weiskittel TM, Vavrek M, Freddo C, Van Berkel GJ. 2016. Online, absolute quantitation of propranolol from spatially distinct 20-and 40-mu m dissections of brain, liver, and kidney thin tissue sections by laser microdissection-liquid vortex capture-mass spectrometry. Anal. Chem 88:6026–6034. [PubMed: 27214103]

Cahill JF, Riba J, Kertesz V. 2019. Rapid, untargeted chemical profiling of single cells in their native environment. Anal. Chem 91:6118–6126. [PubMed: 30955322]

Cao QJW, Yu Q, Liu Y, Chen ZW, Li LJ. 2020. Signature-ion-triggered mass spectrometry approach enabled discovery of N- and O-linked glycosylated neuropeptides in the crustacean nervous system. J. Proteome Res 19:634–643. [PubMed: 31875397]

Cao YQ, Zhang L, Zhang J, Guo YL. 2020. Single-cell on-probe derivatization-noncontact nanocarbon fiber ionization: unraveling cellular heterogeneity of fatty alcohol and sterol metabolites. Anal. Chem 92:8378–8385. [PubMed: 32420735]

Castro DC, Xie YR, Rubakhin SS, Romanova EV, Sweedler JV. 2021. Image-guided MALDI mass spectrometry for high-throughput single-organelle characterization. Nat. Meth 18:1233–1238.

Catherman AD, Skinner OS, Kelleher NL. 2014. Top down proteomics: facts and perspectives. Biochem. Biophys. Res. Commun 445:683–693. [PubMed: 24556311]

Cervenka J, Tyleckova J, Skalnikova HK, Kepkova KV, Poliakh I, Valekova I, Pfeiferova L, Kolar M, Vaskovicova M, Pankova T, Vodicka P. 2021. Proteomic characterization of human neural stem cells and their secretome during in vitro differentiation. Front. Cell. Neurosci 14:20.

Chappell J, Boward B, Dalton S. 2016. Expanding the utility of FUCCI reporters using FACS-based ‘omics analysis. In: Turksen K, Editor Embryonic Stem Cell Protocols. Totowa: Humana Press Inc. p 101–110.

Chatterji B, Pich A. 2013. MALDI imaging mass spectrometry and analysis of endogenous peptides. Expert Rev. Proteomics 10:381–388. [PubMed: 23992420]

Chen L, Yang RR, Kwan T, Tang C, Watt S, Zhang YM, Bourque G, Ge B, Downes K, Frontini M, Ouwehand WH, Lin JW, Soranzo N, Pastinen T, Chen L. 2020. Paired rRNA-depleted and polyA-selected RNA sequencing data and supporting multi-omics data from human T cells. Sci. Data 7:7. [PubMed: 31959765]

Chen Q, Yan GQ, Gao MX, Zhang XM. 2015. Ultrasensitive proteome profiling for 100 living cells by direct cell injection, online digestion and nano-LC-MS/MS analysis. Anal. Chem 87:6674–6680. [PubMed: 26061007]

Chen RB, Ma MM, Hui LM, Zhang J, Li LJ. 2009. Measurement of neuropeptides in crustacean hemolymph via MALDI mass spectrometry. J. Am. Soc. Mass Spectrom 20:708–718. [PubMed: 19185513]

Chen X, Yin YD, Luo MD, Zhou ZW, Cai YP, Zhu ZJ. 2022. Trapped ion mobility spectrometry-mass spectrometry improves the coverage and accuracy of four-dimensional untargeted lipidomics. Anal. Chim. Acta 1210:9.

Cheung TK, Lee CY, Bayer FP, McCoy A, Kuster B, Rose CM. 2021. Defining the carrier proteome limit for single-cell proteomics. Nat. Meth 18:76–83.

Choi SB, Munoz-Llancao P, Manzini MC, Nemes P. 2021. Data-dependent acquisition ladder for capillary electrophoresis mass spectrometry-based ultrasensitive (neuro)proteomics. Anal. Chem 93:15964–15972. [PubMed: 34812615]

Choi SB, Polter AM, Nemes P. 2022. Patch-clamp proteomics of single neurons in tissue using electrophysiology and subcellular capillary electrophoresis mass spectrometry. Anal. Chem 94:1637–1644. [PubMed: 34964611]

Choi SB, Zamarbide M, Manzini MC, Nemes P. 2017. Tapered-tip capillary electrophoresis nano-electrospray ionization mass spectrometry for ultrasensitive proteomics: the mouse cortex. J. Am. Soc. Mass Spectrom 28:597–607. [PubMed: 27853976]

Coman C, Solari FA, Hentschel A, Sickmann A, Zahedi RP, Ahrends R. 2016. Simultaneous metabolite, protein, lipid extraction (SIMPLEX): a combinatorial multimolecular omics approach for systems biology. Mol. Cell. Proteomics 15:1453–1466. [PubMed: 26814187]

Comi TJ, Makurath MA, Philip MC, Rubakhin SS, Sweedler JV. 2017. MALDI MS guided liquid microjunction extraction for capillary electrophoresis-electrospray ionization ms analysis of single pancreatic islet cells. Anal. Chem 89:7765–7772. [PubMed: 28636327]

Cong YZ, Liang YR, Motamedchaboki K, Huguet R, Truong T, Zhao R, Shen YF, Lopez-Ferrer D, Zhu Y, Kelly RT. 2020. Improved single-cell proteome coverage using narrow-bore packed nanoLC columns and ultrasensitive mass spectrometry. Anal. Chem 92:2665–2671. [PubMed: 31913019]

Ctortecka C, Hartlmayr D, Seth A, Mendjan S, Tourniaire G, Mechtler K. 2022. An automated workflow for multiplexed single-cell proteomics sample preparation at unprecedented sensitivity. bioRxiv:2021.2004.2014.439828.

Ctortecka C, Krssakova G, Stejskal K, Penninger JM, Mendjan S, Mechtler K, Stadlmann J. 2022. Comparative proteome signatures of trace samples by multiplexed data-independent acquisition. Mol. Cell. Proteomics 21:11.

Ctortecka C, Mechtler K. 2021. The rise of single-cell proteomics. Anal. Sci. Adv 2:84–94.

Dagley LF, Infusini G, Larsen RH, Sandow JJ, Webb AI. 2019. Universal solid-phase protein preparation (USP3) for bottom-up and top-down proteomics. J. Proteome Res 18:2915–2924. [PubMed: 31137935]

Davey HM, Kell DB. 1996. Flow cytometry and cell sorting of heterogeneous microbial populations: the importance of single-cell analyses. Microbiol. Rev 60:641–696. [PubMed: 8987359]

DeLaney K, Buchberger AR, Atkinson L, Grunder S, Mousley A, Li LJ. 2018. New techniques, applications and perspectives in neuropeptide research. J. Exp. Biol 221:16.

DeLaney K, Cao WF, Ma YD, Ma MM, Zhang YZ, Li LJ. 2020. PRESnovo: prescreening prior to de novo sequencing to improve accuracy and sensitivity of neuropeptide identification. J. Am. Soc. Mass Spectrom 31:1358–1371. [PubMed: 32266812]

DeLaney K, Hu MZ, Hellenbrand T, Dickinson PS, Nusbaum MP, Li LJ. 2021. Mass spectrometry quantification, localization, and discovery of feeding-related neuropeptides in Cancer borealis. ACS Chem. Neurosci 12:782–798. [PubMed: 33522802]

DeLaney K, Jia D, Iyer L, Yu Z, Choi SB, Marvar PJ, Nemes P. 2022. Microanalysis of brain angiotensin peptides using ultrasensitive capillary electrophoresis trapped ion mobility mass spectrometry. Anal. Chem.:in print

DeLaney K, Li LJ. 2019. Data independent acquisition mass spectrometry method for improved neuropeptidomic coverage in crustacean neural tissue extracts. Anal. Chem 91:5150–5158. [PubMed: 30888792]

DeLaney K, Phetsanthad A, Li LJ. 2022. Advances in high-resolution MALDI mass spectrometry for neurobiology. Mass Spectrom. Rev 41:194–214. [PubMed: 33165982]

DeLaney K, Sauer CS, Vu NQ, Li LJ. 2019. Recent advances and new perspectives in capillary electrophoresis-mass spectrometry for single cell “omics”. Molecules 24:21.

Desmet G, de Beeck JO, Van Raemdonck G, Van Mol K, Claerebout B, Van Landuyt N, Jacobs P. 2020. Separation efficiency kinetics of capillary flow micro-pillar array columns for liquid chromatography. J. Chromatogr. A 1626:11.

Di Paolo A, Farias J, Garat J, Macklin A, Ignatchenko V, Kislinger T, Silveira JS. 2021. Rat sciatic nerve axoplasm proteome is enriched with ribosomal proteins during regeneration processes. J. Proteome Res 20:2506–2520. [PubMed: 33793244]

Ding J, Blencowe M, Nghiem T, Ha SM, Chen YW, Li GY, Yang X. 2021. Mergeomics 2.0: a web server for multi-omics data integration to elucidate disease networks and predict therapeutics. Nucleic Acids Res. 49:W375–W387. [PubMed: 34048577]

Do TD, Comi TJ, Dunham SJB, Rubakhin SS, Sweedler JV. 2017. Single-cell profiling using ionic liquid matrix-enhanced secondary ion mass spectrometry for neuronal cell type differentiation. Anal. Chem 89:3078–3086. [PubMed: 28194949]

Domenick TM, Vedam-Mai V, Yost RA. 2020. Design and implementation of a dual-probe microsampling apparatus for the direct analysis of adherent mammalian cells by ion mobility-mass spectrometry. Anal. Chem 92:12055–12061. [PubMed: 32786468]

Drouin N, Ramautar R. 2021. Capillary Electrophoresis-Mass Spectrometry for Metabolomics: Possibilities and Perspectives. In: Simionato AVC, Editor Separation Techniques Applied to Omics Sciences: From Principles to Relevant Applications. Cham: Springer International Publishing Ag. p 159–178.

Drouin N, van Mever M, Zhang W, Tobolkina E, Ferre S, Servais AC, Gou MJ, Nyssen L, Fillet M, Lageveen-Kammeijer GSM, Nouta J, Chetwynd AJ, Lynch I, Thorn JA, Meixner J, Lossner C, Taverna M, Liu S, Tran NT, Francois Y, Lechner A, Nehm R, Banni GAD, Nasreddine R, Colas C, Lindner HH, Faserl K, Neususs C, Nelke M, Lammerer S, Perrin C, Bich-Muracciole C, Barbas C, Gonzalvez AL, Guttman A, Szigeti M, Britz-McKibbin P, Kroezen Z, Shanmuganathan M, Nemes P, Portero EP, Hankemeier T, Codesido S, Gonzalez-Ruiz V, Rudaz S, Ramautar R. 2020. Capillary electrophoresis-mass spectrometry at trial by Metabo-ring: effective electrophoretic mobility for reproducible and robust compound annotation. Anal. Chem 92:14103–14112. [PubMed: 32961048]

Dupree EJ, Jayathirtha M, Yorkey H, Mihasan M, Petre BA, Dark CC. 2020. A critical review of bottom-up proteomics: the good, the bad, and the future of this field. Proteomes 8:26. [PubMed: 32971853]

Eberlin LS, Ifa DR, Wu C, Cooks RG. 2010. Three-dimensional vizualization of mouse brain by lipid analysis using ambient ionization mass spectrometry. Angew. Chem., Int. Ed 49:873–876.

Espino JA, Jones LM. 2019. Illuminating biological interactions with in vivo protein footprinting. Anal. Chem 91:6577–6584. [PubMed: 31025855]

Ezzoukhry Z, Henriet E, Cordelieres FP, Dupuy JW, Maitre M, Gay N, Di-Tommaso S, Mercier L, Goetz JG, Peter M, Bard F, Moreau V, Raymond AA, Saltel F. 2018. Combining laser capture microdissection and proteomics reveals an active translation machinery controlling invadosome formation. Nat. Commun 9:11. [PubMed: 29296021]

Feng XJ, Liu BF, Li JJ, Liu X. 2015. Advances in coupling microfluidic chips to mass spectrometry. Mass Spectrom. Rev 34:535–557. [PubMed: 24399782]

Feng XJ, Liu X, Luo QM, Liu BF. 2008. Mass spectrometry in systems biology: an overview. Mass Spectrom. Rev 27:635–660. [PubMed: 18636545]

Fincher JA, Korte AR, Dyer JE, Yadavilli S, Morris NJ, Jones DR, Shanmugam VK, Pirlo RK, Vertes A. 2020. Mass spectrometry imaging of triglycerides in biological tissues by laser desorption ionization from silicon nanopost arrays. J. Mass Spectrom 55:8.

Fischer R, Kessler BM. 2015. Gel-aided sample preparation (GASP)-A simplified method for gel-assisted proteomic sample generation from protein extracts and intact cells. Proteomics 15:1224–1229. [PubMed: 25515006]

Fouque KJD, Garabedian A, Porter J, Baird M, Pang XQ, Williams TD, Li LJ, Shvartsburg A, Fernandez-Lima F. 2017. Fast and effective ion mobility-mass spectrometry separation of D-aAmino-acid-containing peptides. Anal. Chem 89:11787–11794. [PubMed: 28982001]

Fu TT, Oetjen J, Chapelle M, Verdu A, Szesny M, Chaumot A, Degli-Esposti D, Geffard O, Clement Y, Salvador A, Ayciriex S. 2020. In situ isobaric lipid mapping by MALDI-ion mobility separation-mass spectrometry imaging. J. Mass Spectrom 55:6.

Fujii T, Matsuda S, Tejedor ML, Esaki T, Sakane I, Mizuno H, Tsuyama N, Masujima T. 2015. Direct metabolomics for plant cells by live single-cell mass spectrometry. Nat. Protoc 10:1445–1456. [PubMed: 26313480]

Furtwängler B, Üresin N, Motamedchaboki K, Huguet R, Lopez-Ferrer D, Zabrouskov V, Porse BT, Schoof EM. 2022. Real-time search-assisted acquisition on a Tribrid mass spectrometer improves coverage in multiplexed single-cell proteomics. Mol. Cell. Proteomics 21.

Gao D, Liu HX, Jiang YY, Lin JM. 2013. Recent advances in microfluidics combined with mass spectrometry: technologies and applications. Lab Chip 13:3309–3322. [PubMed: 23824006]

Geiger T, Wehner A, Schaab C, Cox J, Mann M. 2012. Comparative proteomic analysis of eleven common cell lines reveals ubiquitous but varying expression of most proteins. Mol. Cell. Proteomics 11:1–11.

Geladaki A, Britovsek NK, Breckels LM, Smith TS, Vennard OL, Mulvey CM, Crook OM, Gatto L, Lilley KS. 2019. Combining LOPIT with differential ultracentrifugation for high-resolution spatial proteomics. Nat. Commun 10:15. [PubMed: 30604768]

Geller S, Lieberman H, Belanger AJ, Yew NS, Kloss A, Ivanov AR. 2022. Comparison of microflow and analytical flow liquid chromatography coupled to mass spectrometry global metabolomics methods using a urea cycle disorder mouse model. J. Proteome Res 21:151–163. [PubMed: 34843255]

Gnann C, Cesnik AJ, Lundberg E. 2021. Illuminating Non-genetic Cellular Heterogeneity with Imaging-Based Spatial Proteomics. Trends in Cancer 7:278–282. [PubMed: 33436349]

Gomes FP, Yates, III. 2019. Recent trends of capillary electrophoresis-mass spectrometry in proteomics research. Mass Spectrom. Rev 38:445–460. [PubMed: 31407381]

Gregus M, Kostas JC, Ray S, Abbatiello SE, Ivanov AR. 2020. Improved sensitivity of ultralow flow LC-MS-based proteomic profiling of limited samples using monolithic capillary columns and FAIMS technology. Anal. Chem 92:14702–14712. [PubMed: 33054160]

Greving MP, Patti GJ, Siuzdak G. 2011. Nanostructure-initiator mass spectrometry metabolite analysis and imaging. Anal. Chem 83:2–7. [PubMed: 21049956]

Gupta K, Vuckovic I, Zhang S, Xiong YN, Carlson BL, Jacobs J, Olson I, Petterson XM, Macura SI, Sarkaria J, Burns TC. 2020. Radiation induced metabolic alterations associate with tumor aggressiveness and poor outcome in glioblastoma. Front. Oncol 10:19. [PubMed: 32064233]

HaileMariam M, Eguez RV, Singh H, Bekele S, Ameni G, Pieper R, Yu YB. 2018. S-trap, an ultrafast sample-preparation approach for shotgun proteomics. J. Proteome Res 17:2917–2924. [PubMed: 30114372]

Hale OJ, Sisley EK, Griffiths RL, Styles IB, Cooper HJ. 2020. Native LESA TWIMS-MSI: spatial, conformational, and mass analysis of proteins and protein complexes. J. Am. Soc. Mass Spectrom 31:873–879. [PubMed: 32159346]

Hamilton JS, Aguilar R, Petros RA, Verbeck GF. 2017. DAPNe with micro-capillary separatory chemistry-coupled to MALDI-MS for the analysis of polar and non-polar lipid metabolism in one cell. J. Am. Soc. Mass Spectrom 28:918–928. [PubMed: 28251574]

Hanson JN, Motala MJ, Heien ML, Gillette M, Sweedler J, Nuzzo RG. 2009. Textural guidance cues for controlling process outgrowth of mammalian neurons. Lab Chip 9:122–131. [PubMed: 19209344]

Hebbar S, Schulz WD, Sauer U, Schwudke D. 2014. Laser capture microdissection coupled with on-column extraction LC-MS^n enables lipidomics of fluorescently labeled Drosophila neurons. Anal. Chem 86:5345–5352. [PubMed: 24820458]

Hebert AS, Richards AL, Bailey DJ, Ulbrich A, Coughlin EE, Westphall MS, Coon JJ. 2014. The one hour yeast proteome. Mol. Cell. Proteomics 13:339–347. [PubMed: 24143002]

Helbok R, Kofler M, Schiefecker AJ, Gaasch M, Rass V, Pfausler B, Beer R, Schmutzhard E. 2017. Clinical use of cerebral microdialysis in patients with aneurysmal subarachnoid hemorrhage-state of the art. Front. Neurol 8:25. [PubMed: 28220104]

Hirayama A, Abe H, Yamaguchi N, Tabata S, Tomita M, Soga T. 2018. Development of a sheathless CE-ESI-MS interface. Electrophoresis 39:1382–1389. [PubMed: 29493797]

Hobson BD, Choi SJ, Mosharov EV, Soni RK, Sulzer D, Sims PA. 2022. Subcellular proteomics of dopamine neurons in the mouse brain. eLife 11.

Hook V, Lietz CB, Podvin S, Cajka T, Fiehn O. 2018. Diversity of neuropeptide cell-cell signaling molecules generated by proteolytic processing revealed by neuropeptidomics mass spectrometry. J. Am. Soc. Mass Spectrom 29:807–816. [PubMed: 29667161]

Hu MZ, Helfenbein K, Buchberger AR, DeLaney K, Liu Y, Li LJ. 2021. Exploring the sexual dimorphism of crustacean neuropeptide expression using callinectes sapidus as a model organism. J. Proteome Res 20:2739–2750. [PubMed: 33872031]

Hu R, Li Y, Yang YH, Liu ML. 2021. Mass spectrometry-based strategies for single-cell metabolomics. Mass Spectrom. Rev:1–28.

Huang LS, Fang ML, Cupp-Sutton KA, Wang Z, Smith K, Wu S. 2021. Spray-capillary-based capillary electrophoresis mass spectrometry for metabolite analysis in single cells. Anal. Chem 93:4479–4487. [PubMed: 33646748]

Hughes CS, Foehr S, Garfield DA, Furlong EE, Steinmetz LM, Krijgsveld J. 2014. Ultrasensitive proteome analysis using paramagnetic bead technology. Mol. Syst. Biol 10:14.

Iannacone JM, Ren SF, Hatcher NG, Sweedler JV. 2009. Collecting peptide Release from the brain using porous polymer monolith-based solid phase extraction capillaries. Anal. Chem 81:5433–5438. [PubMed: 19485405]

Jirayupat C, Nagashima K, Hosomi T, Takahashi T, Tanaka W, Samransuksamer B, Zhang GZ, Liu JY, Kanai M, Yanagida T. 2021. Image processing and machine learning for automated identification of chemo-/biomarkers in chromatography mass Spectrometry. Anal. Chem 93:14708–14715. [PubMed: 34704450]

Joensson HN, Svahn HA. 2012. Droplet microfluidics-a tool for single-cell analysis. Angew. Chem., Int. Ed 51:12176–12192.

Jones LM, Sperry JB, Carroll JA, Gross ML. 2011. Fast photochemical oxidation of proteins for epitope mapping. Anal. Chem 83:7657–7661. [PubMed: 21894996]

Kawai T, Ota N, Okada K, Imasato A, Owa Y, Morita M, Tada M, Tanaka Y. 2019. Ultrasensitive single cell metabolomics by capillary electrophoresis-mass spectrometry with a thin-walled tapered emitter and large-volume dual sample preconcentration. Anal. Chem 91:10564–10572. [PubMed: 31357863]

Kelly RT. 2020. Single-cell proteomics: progress and prospects. Mol. Cell. Proteomics 19:1739–1748. [PubMed: 32847821]

Kenderdine T, Xia ZJ, Williams ER, Fabris D. 2018. Submicrometer nanospray emitters provide new insights into the mechanism of cation adduction to anionic oligonucleotides. Anal. Chem 90:13541–13548. [PubMed: 30351906]

Kim H, Dan K, Shin H, Lee J, Wang JI, Han D. 2019. An efficient method for high-pH peptide fractionation based on C18 StageTips for in-depth proteome profiling. Anal. Meth 11:4693–4698.

Kim Y, Edwards N, Fenselau C. 2016. Extracellular vesicle proteomes reflect developmental phases of Bacillus subtilis. Clin. Proteomics 13:8. [PubMed: 27095950]

Korte AR, Morris NJ, Vertes A. 2019. High throughput complementary analysis and quantitation of metabolites by maldi- and silicon nanopost array-laser desorption/ionization-mass spectrometry. Anal. Chem 91:3951–3958. [PubMed: 30786207]

Kurczy ME, Piehowski PD, Van Bell CT, Heien ML, Winograd N, Ewing AG. 2010. Mass spectrometry imaging of mating Tetrahymena show that changes in cell morphology regulate lipid domain formation. Proc. Natl Acad. Sci. U. S. A 107:2751–2756. [PubMed: 20133641]

Kwok R, Tobe SS. 2006. Hemolymph clotting in crustaceans: implications for neuropeptide extraction from invertebrate hemolymph. Peptides 27:590–596. [PubMed: 16330126]

Lai X, Wang L, Witzmann FA. 2013. Issues and applications in label-free quantitative mass spectrometry. Int. J. Proteomics 2013:756039. [PubMed: 23401775]

Lam SS, Martell JD, Kamer KJ, Deerinck TJ, Ellisman MH, Mootha VK, Ting AY. 2015. Directed evolution of APEX2 for electron microscopy and proximity labeling. Nat. Methods 12:51–54. [PubMed: 25419960]

Landgraf P, Antileo ER, Schuman EM, Dieterich DC. 2015. BONCAT: metabolic labeling, click chemistry, and affinity purification of newly synthesized proteomes. Methods Mol. Biol 1266:199–215.

Lanekoff I, Burnum-Johnson K, Thomas M, Cha J, Dey SK, Yang PX, Conaway MCP, Laskin J. 2015. Three-dimensional imaging of lipids and metabolites in tissues by nanospray desorption electrospray ionization mass spectrometry. Anal. Bioanal. Chem 407:2063–2071. [PubMed: 25395201]

Lanni EJ, Dunham SJB, Nemes P, Rubakhin SS, Sweedler JV. 2014. Biomolecular imaging with a C60-SIMS/MALDI dual ion source hybrid mass spectrometer: instrumentation, matrix enhancement, and single cell analysis. J. Am. Soc. Mass Spectrom 25:1897–1907. [PubMed: 25183225]

Lanni EJ, Rubakhin SS, Sweedler JV. 2012. Mass spectrometry imaging and profiling of single cells. J. Proteomics 75:5036–5051. [PubMed: 22498881]

Lapainis T, Rubakhin SS, Sweedler JV. 2009. Capillary electrophoresis with electrospray ionization mass spectrometric detection for single-cell metabolomics. Anal. Chem 81:5858–5864. [PubMed: 19518091]

Lee WD, Mukha D, Aizenshtein E, Shlomi T. 2019. Spatial-fluxomics provides a subcellular-compartmentalized view of reductive glutamine metabolism in cancer cells. Nat. Commun 10:14. [PubMed: 30600315]

Lee YH, Tan HT, Chung MCM. 2010. Subcellular fractionation methods and strategies for proteomics. Proteomics 10:3935–3956. [PubMed: 21080488]

Li B, Tang J, Yang QX, Li S, Cui XJ, Li YH, Chen YZ, Xue WW, Li XF, Zhu F. 2017. NOREVA: normalization and evaluation of MS-based metabolomics data. Nucleic Acids Res. 45:W162–W170. [PubMed: 28525573]

Li H, Smith BK, Mark L, Nemes P, Nazarian J, Vertes A. 2015. Ambient molecular imaging by laser ablation electrospray ionization mass spectrometry with ion mobility separation. Int. J. Mass Spectrom 377:681–689.

Li XT, Zhao SL, Hu HK, Liu YM. 2016. A microchip electrophoresis-mass spectrometric platform with double cell lysis nano-electrodes for automated single cell analysis. J. Chromatogr. A 1451:156–163. [PubMed: 27207575]

Liang YR, Acor H, McCown MA, Nwosu AJ, Boekweg H, Axtell NB, Truong T, Cong YZ, Payne SH, Kelly RT. 2021. Fully automated sample processing and analysis workflow for low-input proteome profiling. Anal. Chem 93:1658–1666. [PubMed: 33352054]

Liao HW, Rubakhin SS, Philip MC, Sweedler JV. 2020. Enhanced single-cell metabolomics by capillary electrophoresis electrospray ionization-mass spectrometry with field amplified sample injection. Anal. Chim. Acta 1118:36–43. [PubMed: 32418602]

Lindenburg PW, Haselberg R, Rozing G, Ramautar R. 2015. Developments in interfacing designs for CE-MS: towards enabling tools for proteomics and metabolomics. Chromatographia 78:367–377.

Lipovsek M, Bardy C, Cadwell CR, Hadley K, Kobak D, Tripathy SJ. 2021. Patch-seq: past, present, and future. J. Neurosci 41:937–946. [PubMed: 33431632]

Liu JX, Aerts JT, Rubakhin SS, Zhang XX, Sweedler JV. 2014. Analysis of endogenous nucleotides by single cell capillary electrophoresis-mass spectrometry. Analyst 139:5835–5842. [PubMed: 25212237]

Liu RM, Pan N, Zhu YL, Yang ZB. 2018. T-Probe: an integrated microscale device for online in situ single cell analysis and metabolic profiling using mass spectrometry. Anal. Chem 90:11078–11085. [PubMed: 30119596]

Liu YF, Chen XY, Zhang YQ, Liu J. 2019. Advancing single-cell proteomics and metabolomics with microfluidic technologies. Analyst 144:846–858. [PubMed: 30351310]

Liu YS, Beyer A, Aebersold R. 2016. On the dependency of cellular protein levels on mRNA abundance. Cell 165:535–550. [PubMed: 27104977]

Liu ZC, Portero EP, Jian YR, Zhao YJ, Onjiko RM, Zeng C, Nemes P. 2019. Trace, machine learning of signal images for trace-sensitive mass spectrometry: a case study from single-cell metabolomics. Anal. Chem 91:5768–5776. [PubMed: 30929422]

Loh KH, Stawski PS, Draycott AS, Udeshi ND, Lehrman EK, Wilton DK, Svinkina T, Deerinck TJ, Ellisman MH, Stevens B, Carr SA, Ting AY. 2016. Proteomic analysis of unbounded cellular compartments: synaptic clefts. Cell 166:1295–1307. [PubMed: 27565350]

Lombard-Banek C, Choi SB, Nemes P. 2019. Single-cell proteomics in complex tissues using microprobe capillary electrophoresis mass spectrometry. In: Allbritton NL, Kovarik ML, Editors. Enzyme Activity in Single Cells. London: Academic Press Ltd-Elsevier Science Ltd. p 263–292.

Lombard-Banek C, Li J, Portero EP, Onjiko RM, Singer CD, Plotnick DO, Al Shabeeb RQ, Nemes P. 2021. In vivo subcellular mass spectrometry enables proteo-metabolomic single-cell systems

biology in a chordate embryo developing to a normally behaving tadpole (X. Laevis). Angew. Chem., Int. Ed 60:12852–12858.

Lombard-Banek C, Moody SA, Manzin MC, Nemes P. 2019. Microsampling capillary electrophoresis mass spectrometry enables single-cell proteomics in complex tissues: developing cell clones in live Xenopus laevis and zebrafish embryos. Anal. Chem 91:4797–4805. [PubMed: 30827088]

Lombard-Banek C, Moody SA, Nemes P. 2016. Single-cell mass spectrometry for discovery proteomics: quantifying translational cell heterogeneity in the 16-cell frog (Xenopus) embryo. Angew. Chem. Int. Ed 128:2500–2504.

Lombard-Banek C, Reddy S, Moody SA, Nemes P. 2016. Label-free quantification of proteins in single embryonic cells with neural fate in the cleavage-stage frog (Xenopus laevis) embryo using capillary electrophoresis electrospray ionization high-resolution mass spectrometry (CE-ESI-HRMS). Mol. Cell. Proteomics 15:2756–2768. [PubMed: 27317400]

Lombard-Banek C, Yu Z, Swiercz AP, Marvar PJ, Nemes P. 2019. A microanalytical capillary electrophoresis mass spectrometry assay for quantifying angiotensin peptides in the brain. Anal. Bioanal. Chem 411:4661–4671. [PubMed: 30953113]

Lopes A, Magrinelli E, Telley L. 2020. Emerging roles of single-cell multi-omics in studying developmental temporal patterning. Int. J. Mol. Sci 21:18.

Lubeckyj RA, Sun LL. 2022. Laser capture microdissection-capillary zone electrophoresis-tandem mass spectrometry (LCM-CZE-MS/MS) for spatially resolved top-down proteomics: a pilot study of zebrafish brain. Mol. Omics 18:112–122. [PubMed: 34935839]

Maes K, Van Liefferinge J, Viaene J, Van Schoors J, Van Wanseele Y, Bechade G, Chambers EE, Morren H, Michotte Y, Heyden YV, Claereboudt J, Smolders I, Van Eeckhauta A. 2014. Improved sensitivity of the nano ultra-high performance liquid chromatography-tandem mass spectrometric analysis of low-concentrated neuropeptides by reducing aspecific adsorption and optimizing the injection solvent. J. Chromatogr. A 1360:217–228. [PubMed: 25145562]

Marginean I, Kelly RT, Page JS, Tang KQ, Smith RD. 2007. Electrospray characteristic curves: In pursuit of improved performance in the nanoflow regime. Anal. Chem 79:8030–8036. [PubMed: 17896826]

Massonnet P, Heeren RMA. 2019. A concise tutorial review of TOF-SIMS based molecular and cellular imaging. J. Anal. At. Spectrom 34:2217–2228.

Mast DH, Checco JW, Sweedler JV. 2020. Differential post-translational amino acid isomerization found among neuropeptides in aplysia californica. ACS Chem. Biol 15:272–281.

Mast DH, Liao HW, Romanova EV, Sweedler JV. 2021. Analysis of peptide stereochemistry in single cells by capillary electrophoresis-trapped ion mobility spectrometry mass spectrometry. Anal. Chem 93:6205–6213. [PubMed: 33825437]

Masuda T, Sugiyama N, Tomita M, Ohtsuki S, Ishihama Y. 2020. Mass spectrometry-compatible subcellular fractionation for proteomics. J. Proteome Res 19:75–84. [PubMed: 31599158]

Matula K, Rivello F, Huck WTS. 2020. Single-cell analysis using droplet microfluidics. Adv. Biosyst 4:28.

McCool EN, Lubeckyj RA, Shen XJ, Chen DY, Kou Q, Liu XW, Sun LL. 2018. Deep top-down proteomics using capillary zone electrophoresis tandem mass spectrometry: identification of 5700 proteoforms from the Escherichia coil proteome. Anal. Chem 90:5529–5533. [PubMed: 29620868]

McMillen JC, Gutierrez DB, Judd AM, Spraggins JM, Caprioli RM. 2021. Enhancement of tryptic peptide signals from tissue sections using MALDI IMS postionization (MALDI-2). J. Am. Soc. Mass Spectrom 32:2583–2591. [PubMed: 34515472]

Meier F, Geyer PE, Winter SV, Cox J, Mann M. 2018. BoxCar acquisition method enables single-shot proteomics at a depth of 10,000 proteins in 100 minutes. Nat. Meth 15:440–448.

Mellors JS, Jorabchi K, Smith LM, Ramsey JM. 2010. Integrated microfluidic device for automated single-cell analysis using electrophoretic separation and electrospray ionization mass spectrometry. Anal. Chem 82:967–973. [PubMed: 20058879]

Meng YF, Cheng XL, Wang TT, Hang W, Li XP, Nie W, Liu R, Lin Z, Hang L, Yin ZB, Zhang BL, Yan XM. 2020. Micro-lensed fiber laser desorption mass spectrometry imaging reveals subcellular distribution of drugs within single cells. Angew. Chem., Int. Ed 59:17864–17871.

Mihailova A, Malerod H, Wilson SR, Karaszewski B, Hauser R, Lundanes E, Greibrokk T. 2008. Improving the resolution of neuropeptides in rat brain with on-line HILIC-RP compared to on-line SCX-RP. J. Sep. Sci 31:459–467. [PubMed: 18266260]

Miltenyi S, Muller W, Weichel W, Radbruch A. 1990. High-gradient magnetic cell separation with MACS. Cytometry 11:231–238. [PubMed: 1690625]

Mizuno H, Tsuyama N, Harada T, Masujima T. 2008. Live single-cell video-mass spectrometry for cellular and subcellular molecular detection and cell classification. J. Mass Spectrom 43:1692–1700. [PubMed: 18615771]

Moini M. 2007. Simplifying CE-MS operation. 2. Interfacing low-flow separation techniques to mass spectrometry using a porous tip. Anal. Chem 79:4241–4246. [PubMed: 17447730]

Moroz LL, Kohn AB. 2013. Single-neuron transcriptome and methylome sequencing for epigenomic analysis of aging. In: Tollefsbol TO, Editor Biological Aging: Methods and Protocols, 2nd Edition. Totowa: Humana Press Inc. p 323–352.

Mukherjee P, Berns EJ, Patino CA, Moully EH, Chang LQ, Nathamgari SSP, Kessler JA, Mrksich M, Espinosa HD. 2020. Temporal sampling of enzymes from live cells by localized electroporation and quantification of activity by samdi mass spectrometry. Small 16:10.

Nemes P, Barton AA, Vertes A. 2009. Three-dimensional imaging of metabolites in tissues under ambient conditions by laser ablation electrospray ionization mass spectrometry. Anal. Chem 81:6668–6675. [PubMed: 19572562]

Nemes P, Goyal S, Vertes A. 2008. Conformational and noncovalent complexation changes in proteins during electrospray ionization. Anal. Chem 80:387–395. [PubMed: 18081323]

Nemes P, Marginean I, Vertes A. 2007. Spraying mode effect on droplet formation and ion chemistry in electrosprays. Anal. Chem 79:3105–3116. [PubMed: 17378541]

Nemes P, Rubakhin SS, Aerts JT, Sweedler JV. 2013. Qualitative and quantitative metabolomic investigation of single neurons by capillary electrophoresis electrospray ionization mass spectrometry. Nat. Protoc 8:783–799. [PubMed: 23538882]

Nemes P, Woods AS, Vertes A. 2010. Simultaneous imaging of small metabolites and lipids in rat brain tissues at atmospheric pressure by laser ablation electrospray ionization mass spectrometry. Anal. Chem 82:982–988. [PubMed: 20050678]

Neumann EK, Ellis JF, Triplett AE, Rubakhin SS, Sweedler JV. 2019. Lipid analysis of 30,000 individual rodent cerebellar cells using high-resolution mass spectrometry. Anal. Chem 91:7871–7878. [PubMed: 31122012]

Neupert S, Fusca D, Kloppenburg P, Predel R. 2018. Analysis of single neurons by perforated patch clamp recordings and MALDI-TOF mass spectrometry. ACS Chem. Neurosci 9:2089–2096. [PubMed: 29906100]

Nickerson JL, Baghalabadi V, Rajendran S, Jakubec PJ, Said H, McMillen TS, Dang ZH, Doucette AA. 2021. Recent advances in top-down proteome sample processing ahead of MS analysis. Mass Spectrom. Rev:1–39.

Onjiko RM, Moody SA, Nemes P. 2015. Single-cell mass spectrometry reveals small molecules that affect cell fates in the 16-cell embryo. Proc. Natl Acad. Sci. U. S. A 112:6545–6550. [PubMed: 25941375]

Onjiko RM, Morris SE, Moody SA, Nemes P. 2016. Single-cell mass spectrometry with multi-solvent extraction identifies metabolic differences between left and right blastomeres in the 8-cell frog (Xenopus) embryo. Analyst 141:3648–3656. [PubMed: 27004603]

Onjiko RM, Portero EP, Moody SA, Nemes P. 2017. In situ microprobe single-cell capillary electrophoresis mass spectrometry: metabolic reorganization in single differentiating cells in the live vertebrate (Xenopus laevis) embryo. Anal. Chem 89:7069–7076. [PubMed: 28434226]

Onorato JM, Xu C, Chen XQ, Rose AV, Generaux C, Lentz K, Shipkova P, Arthur S, Hennan JK, Haskell R, Myers MC, Lawrence RM, Finlay HJ, Basso M, Bostwick J, Fernando G, Garcia R, Hellings S, Hsu MY, Zhang RG, Zhao L, Gargalovic P. 2019. Linking (Pyr)(1)apelin-13 pharmacokinetics to efficacy: stabilization and measurement of a high clearance peptide in rodents. Anal. Biochem 568:41–50. [PubMed: 30605634]

Osbourn DM, Weiss DJ, Lunte CE. 2000. On-line preconcentration methods for capillary electrophoresis. Electrophoresis 21:2768–2779. [PubMed: 11001283]

OuYang CZ, Liang ZD, Li LJ. 2015. Mass spectrometric analysis of spatio-temporal dynamics of crustacean neuropeptides. BBA-Proteins Proteomics 1854:798–811. [PubMed: 25448012]

Ovchinnikova K, Kovalev V, Stuart L, Alexandrov T. 2020. OffsampleAI: artificial intelligence approach to recognize off-sample mass spectrometry images. BMC Bioinform. 21:11.

Pace CL, Simmons J, Kelly RT, Muddiman DC. 2021. Multimodal mass spectrometry imaging of rat brain using IR-MALDESI and nanoPOTS-LC-MS/MS. J. Proteome Res 21:713–720. [PubMed: 34860515]

Paine MRL, Liu JB, Huang DN, Ellis SR, Trede D, Kobarg JH, Heeren RMA, Fernandez FM, MacDonald TJ. 2019. Three-dimensional mass spectrometry imaging identifies lipid markers of medulloblastoma metastasis. Sci. Rep 9:10. [PubMed: 30626881]

Passarelli MK, Pirkl A, Moellers R, Grinfeld D, Kollmer F, Havelund R, Newman CF, Marshall PS, Arlinghaus H, Alexander MR, West A, Horning S, Niehuis E, Makarov A, Dollery CT, Gilmore IS. 2017. The 3D OrbiSIMS-label-free metabolic imaging with subcellular lateral resolution and high mass-resolving power. Nat. Methods 14:1175–1183. [PubMed: 29131162]

Perez JD, Dieck ST, Alvarez-Castelao B, Tushev G, Chan ICW, Schuman EM. 2021. Subcellular sequencing of single neurons reveals the dendritic transcriptome of GABAergic interneurons. eLife 10:26.

Peshkin L, Wuhr M, Pearl E, Haas W, Freeman RM, Gerhart JC, Klein AM, Horb M, Gygi SP, Kirschner MW. 2015. On the relationship of protein and mRNA dynamics in vertebrate embryonic development. Dev. Cell 35:383–394. [PubMed: 26555057]

Peuchen EH, Zhu GJ, Sun LL, Dovichi NJ. 2017. Evaluation of a commercial electro-kinetically pumped sheath-flow nanospray interface coupled to an automated capillary zone electrophoresis system. Anal. Bioanal. Chem 409:1789–1795. [PubMed: 27981343]

Pfammatter S, Bonneil E, McManus FP, Prasad S, Bailey DJ, Belford M, Dunyach JJ, Thibault P. 2018. A novel differential ion mobility device expands the depth of proteome coverage and the sensitivity of multiplex proteomic measurements. Mol. Cell. Proteomics 17:2051–2067. [PubMed: 30007914]

Phetsanthad A, Vu NQ, Yu Q, Buchberger AR, Chen ZW, Keller C, Li LJ. 2021. Recent advances in mass spectrometry analysis of neuropeptides. Mass Spectrom. Rev:1–45.

Piehowski PD, Zhu Y, Bramer LM, Stratton KG, Zhao R, Orton DJ, Moore RJ, Yuan J, Mitchell HD, Gao Y, Webb-Robertson B-JM, Dey SK, Kelly RT, Burnum-Johnson KE. 2020. Automated mass spectrometry imaging of over 2000 proteins from tissue sections at 100-μm spatial resolution. Nat. Commun 11:8. [PubMed: 31911630]

Piga I, Capitoli G, Clerici F, Mahajneh A, Brambilla V, Smith A, Leni D, L'Imperio V, Galimberti S, Pagni F, Magni F. 2021. Ex vivo thyroid fine needle aspirations as an alternative for MALDI-MSI proteomic investigation: intra-patient comparison. Anal. Bioanal. Chem 413:1259–1266. [PubMed: 33277997]

Planell N, Lagani V, Sebastian-Leon P, van der Kloet F, Ewing E, Karathanasis N, Urdangarin A, Arozarena I, Jagodic M, Tsamardinos I, Tarazona S, Conesa A, Tegner J, Gomez-Cabrero D. 2021. STATegra: multi-omics data integration - a conceptual scheme with a bioinformatics pipeline. Front. Genet 12:12.

Portero EP, Nemes P. 2019. Dual cationic-anionic profiling of metabolites in a single identified cell in a live Xenopus laevis embryo by microprobe CE-ESI-MS. Analyst 144:892–900. [PubMed: 30542678]

Potocnik NO, Fisher GL, Prop A, Heeren RMA. 2017. Sequencing and identification of endogenous neuropeptides with matrix-enhanced secondary ion mass spectrometry tandem mass spectrometry. Anal. Chem 89:8223–8227. [PubMed: 28753276]

Qin SY, Zhang YX, Tian Y, Xu FG, Zhang P. 2022. Subcellular metabolomics: Isolation, measurement, and applications. J. Pharm. Biomed. Anal 210:14.

Qu YY, Dubiak KM, Peuchen EH, Champion MM, Zhang ZB, Hebert AS, Wright S, Coon JJ, Huber PW, Dovichi NJ. 2020. Quantitative capillary zone electrophoresis-mass spectrometry reveals the N-glycome developmental plan during vertebrate embryogenesis. Mol. Omics 16:210–220. [PubMed: 32149324]

Race AM, Palmer AD, Dexter A, Steven RT, Styles IB, Bunch J. 2016. SpectralAnalysis: software for the masses. Anal. Chem 88:9451–9458. [PubMed: 27558772]

Rappez L, Stadler M, Triana S, Gathungu RM, Ovchinnikova K, Phapale P, Heikenwalder M, Alexandrov T. 2021. SpaceM reveals metabolic states of single cells. Nat. Meth 18:799–805.

Reubsaet L, Sweredoski MJ, Moradian A, Lomenick B, Eggleston-Rangel R, Garbis SD. 2020. Nano volume fractionation strategy for dilute-and-shoot injections in off-line loss-less proteomic workflows for extensive protein identifications of ultra-low sample amounts. J. Chromatogr. A 1609:10.

Reyzer ML, Caprioli RM. 2013. Imaging mass spectrometry of proteins and peptides. In: Desiderio DM, Nibbering NMM, Editors. Mass Spectrometry for Drug Discovery and Drug Development. John Wiley & Sons, Inc.

Romanova EV, Rubakhin SS, Sweedler JV. 2008. One-step sampling, extraction, and storage protocol for peptidomics using dihydroxybenzoic acid. Anal. Chem 80:3379–3386. [PubMed: 18321135]

Rosenberger G, Bludau I, Schmitt U, Heusel M, Hunter CL, Liu YS, MacCoss MJ, MacLean BX, Nesvizhskii AI, Pedrioli PGA, Reiter L, Rost HL, Tate S, Ting YS, Collins BC, Aebersold R. 2017. Statistical control of peptide and protein error rates in large-scale targeted data-independent acquisition analyses. Nat. Methods 14:921–927. [PubMed: 28825704]

Roux KJ, Kim DI, Raida M, Burke B. 2012. A promiscuous biotin ligase fusion protein identifies proximal and interacting proteins in mammalian cells. J. Cell Biol 196:801–810. [PubMed: 22412018]

Rovira-Clave X, Jiang SZ, Bai YH, Zhu BK, Barlow G, Bhate S, Coskun AF, Han GJ, Ho CMK, Hitzman C, Chen SY, Bava FA, Nolan GP. 2021. Subcellular localization of biomolecules and drug distribution by high-definition ion beam imaging. Nat. Commun 12:18. [PubMed: 33398000]

Saadatpour A, Lai SJ, Guo GJ, Yuan GC. 2015. Single-cell analysis in cancer genomics. Trends Genet. 31:576–586. [PubMed: 26450340]

Saha-Shah A, Esmaeili M, Sidoli S, Hwang H, Yang J, Klein PS, Garcia BA. 2019. Single cell proteomics by data-independent acquisition to study embryonic asymmetry in Xenopus laevis. Anal. Chem 91:8891–8899. [PubMed: 31194517]

Saha-Shah A, Green CM, Abraham DH, Baker LA. 2016. Segmented flow sampling with push-pull theta pipettes. Analyst 141:1958–1965. [PubMed: 26907673]

Samarah LZ, Khattar R, Tran TH, Stopka SA, Brantner CA, Parlanti P, Velickovic D, Shaw JB, Agtuca BJ, Stacey G, Pasa-Tolic L, Tolic N, Anderton CR, Vertes A. 2020. Single-cell metabolic profiling: metabolite formulas from isotopic fine structures in heterogeneous plant cell populations. Anal. Chem 92:7289–7298. [PubMed: 32314907]

Sans M, Feider CL, Eberlin LS. 2018. Advances in mass spectrometry imaging coupled to ion mobility spectrometry for enhanced imaging of biological tissues. Curr. Opin. Chem. Biol 42:138–146. [PubMed: 29275246]

Sansone SA, Fan T, Goodacre R, Griffin JL, Hardy NW, Kaddurah-Daouk R, Kristal BS, Lindon J, Mendes P, Morrison N, Nikolau B, Robertson D, Sumner LW, Taylor C, van der Werf M, van Ommen B, Fiehn O. 2007. The metabolomics standards initiative. Nat. Biotechnol 25:844–848.

Satpathy S, Jaehnig EJ, Krug K, Kim BJ, Saltzman AB, Chan DW, Holloway KR, Anurag M, Huang C, Singh P, Gao A, Namai N, Dou YC, Wen B, Vasaikar SV, Mutch D, Watson MA, Ma C, Ademuyiwa FO, Rimawi MF, Schiff R, Hoog J, Jacobs S, Malovannaya A, Hyslop T, Clauser KR, Mani DR, Perou CM, Miles G, Zhang B, Gillette MA, Carr SA, Ellis MJ. 2020. Microscaled proteogenomic methods for precision oncology. Nat. Commun 11:16. [PubMed: 31911602]

Sauer CS, Li LJ. 2021. Mass spectrometric profiling of neuropeptides in response to copper toxicity via isobaric tagging. Chem. Res. Toxicol 34:1329–1336. [PubMed: 33706502]

Schirmer EC, Yates JR 3rd, Gerace L. 2003. MudPIT: A powerful proteomics tool for discovery. Discov. Med 3:38–39. [PubMed: 20704860]

Schmerberg CM, Li LJ. 2013. Mass spectrometric detection of neuropeptides using affinity-enhanced microdialysis with antibody-coated magnetic nanoparticles. Anal. Chem 85:915–922. [PubMed: 23249250]

Schmerberg CM, Liang ZD, Li LJ. 2015. Data-independent MS/MS quantification of neuropeptides for determination of putative feeding-related neurohormones in microdialysate. ACS Chem. Neurosci 6:174–180. [PubMed: 25552291]

Schoof EM, Furtwangler B, Uresin N, Rapin N, Savickas S, Gentil C, Lechman E, Keller UAD, Dick JE, Porse BT. 2021. Quantitative single-cell proteomics as a tool to characterize cellular hierarchies. Nat. Commun 12:15. [PubMed: 33397905]

Sergent-Tanguy S, Chagneau C, Neveu I, Naveilhan P. 2003. Fluorescent activated cell sorting (FACS): a rapid and reliable method to estimate the number of neurons in a mixed population. J. Neurosci. Meth 129:73–79.

Seydel C. 2021. Single-cell metabolomics hits its stride. Nat. Methods 18:1452–1456. [PubMed: 34862499]

Shang LR, Cheng Y, Zhao YJ. 2017. Emerging droplet microfluidics. Chem. Rev 117:7964–8040. [PubMed: 28537383]

Shao X, Wang XT, Guan S, Lin HZ, Yan GQ, Gao MX, Deng CH, Zhang XM. 2018. Integrated proteome analysis device for fast single-cell protein profiling. Anal. Chem 90:14003–14010. [PubMed: 30375851]

Shen SC, Li J, Huo SA, Ma M, Zhu XY, Rasam S, Duan XT, Qu M, Titus MA, Qu J. 2021. Parallel, high-quality proteomic and targeted metabolomic quantification using laser capture microdissected tissues. Anal. Chem 93:8711–8718. [PubMed: 34110778]

Simone NL, Bonner RF, Gillespie JW, Emmert-Buck MR, Liotta LA. 1998. Laser-capture microdissection: opening the microscopic frontier to molecular analysis. Trends Genet. 14:272–276. [PubMed: 9676529]

Slavov N. 2021. Single-cell protein analysis by mass spectrometry. Curr. Opin. Chem. Biol 60:1–9. [PubMed: 32599342]

Slavov N. 2022. Scaling up single-cell proteomics. Mol. Cell. Proteomics 21:7.

Smith LM, Agar JN, Chamot-Rooke J, Danis PO, Ge Y, Loo JA, Pasa-Tolic L, Tsybin YO, Kelleher NL, Consortium Top-Down P. 2021. The human proteoform project: defining the human proteome. Sci. Adv 7:8.

Specht H, Emmott E, Petelski AA, Huffman RG, Perlman DH, Serra M, Kharchenko P, Koller A, Slavov N. 2021. Single-cell proteomic and transcriptomic analysis of macrophage heterogeneity using SCoPE2. Genome Biol. 22:27. [PubMed: 33419460]

Sperber H, Mathieu J, Wang YL, Ferreccio A, Hesson J, Xu ZJ, Fischer KA, Devi A, Detraux D, Gu HW, Battle SL, Showalter M, Valensisi C, Bielas JH, Ericson NG, Margaretha L, Robitaille AM, Margineantu D, Fiehn O, Hockenbery D, Blau CA, Raftery D, Margolin AA, Hawldns RD, Moon RT, Ware CB, Ruohola-Baker H. 2015. The metabolome regulates the epigenetic landscape during naive-to-primed human embryonic stem cell transition. Nat. Cell Biol 17:1523–1535. [PubMed: 26571212]

Stopka SA, Samarah LZ, Shaw JB, Liyu AV, Velickovic D, Agtuca BJ, Kukolj C, Koppenaal DW, Stacey G, Pasa-Tolic L, Anderton CR, Vertes A. 2019. Ambient metabolic profiling and imaging of biological samples with ultrahigh molecular resolution using laser ablation electrospray ionization 21 Tesla FTICR mass spectrometry. Anal. Chem 91:5028–5035. [PubMed: 30821434]

Suvannapruk W, Edney MK, Kim D-H, Scurr DJ, Ghaemmaghami AM, Alexander MR. 2022. Single-cell metabolic profiling of macrophages using 3D OrbiSIMS: correlations with phenotype. Anal. Chem.:in print

Szymanska E, Daviesc AN, Buydens LMC. 2016. Chemometrics for ion mobility spectrometry data: recent advances and future prospects. Analyst 141:5689–5708. [PubMed: 27549384]

Taylor CF, Binz PA, Aebersold R, Affolter M, Barkovich R, Deutsch EW, Horn DM, Huhmer A, Kussmann M, Lilley K, Macht M, Mann M, Mueller D, Neubert TA, Nickson J, Patterson SD, Raso R, Resing K, Seymour SL, Tsugita A, Xenarios I, Zeng R, Julian RK. 2008. Guidelines for reporting the use of mass spectrometry in proteomics. Nat. Biotechnol 26:860–861. [PubMed: 18688232]

Taylor MJ, Lukowski JK, Anderton CR. 2021. Spatially resolved mass spectrometry at the single cell: recent innovations in proteomics and metabolomics. J. Am. Soc. Mass Spectrom 32:872–894. [PubMed: 33656885]

Taylor MJ, Mattson S, Liyu A, Stopka SA, Ibrahim YM, Vertes A, Anderton CR. 2021. Optical microscopy-guided laser ablation electrospray ionization ion mobility mass spectrometry: ambient single cell metabolomics with increased confidence in molecular identification. Metabolites 11:15.

Tharkeshwar AK, Gevaert K, Annaert W. 2018. Organellar omics-a reviving strategy to untangle the biomolecular complexity of the cell. Proteomics 18:12.

Tharkeshwar AK, Trekker J, Vermeire W, Pauwels J, Sannerud R, Priestman DA, te Vruchte D, Vints K, Baatsen P, Decuypere JP, Lu HQ, Martin S, Vangheluwe P, Swinnen JV, Lagae L, Impens F, Platt FM, Gevaert K, Annaert W. 2017. A novel approach to analyze lysosomal dysfunctions through subcellular proteomics and lipidomics: the case of NPC1 deficiency. Sci. Rep 7:20. [PubMed: 28154414]

Tian Q, Stepaniants SB, Mao M, Weng L, Feetham MC, Doyle MJ, Yi EC, Dai HY, Thorsson V, Eng J, Goodlett D, Berger JP, Gunter B, Linseley PS, Stoughton RB, Aebersold R, Collins SJ, Hanlon WA, Hood LE. 2004. Integrated genomic and proteomic analyses of gene expression in mammalian cells. Mol. Cell. Proteomics 3:960–969. [PubMed: 15238602]

Tiwary S, Levy R, Gutenbrunner P, Soto FS, Palaniappan KK, Deming L, Berndl M, Brant A, Cimermancic P, Cox J. 2019. High-quality MS/MS spectrum prediction for data-dependent and data-independent acquisition data analysis. Nat. Meth 16:519–525.

Tsuyama N, Mizuno H, Tokunaga E, Masujima T. 2008. Live single-cell molecular analysis by video-mass spectrometry. Anal. Sci 24:559–561. [PubMed: 18469458]

Turiak L, Toth G, Ozohanics O, Revesz A, Acs A, Vekey K, Zaia J, Drahos L. 2018. Sensitive method for glycosaminoglycan analysis of tissue sections. J. Chromatogr. A 1544:41–48. [PubMed: 29506752]

Velasquez E, Martins-de-Souza D, Velasquez I, Carneiro GRA, Schmitt A, Falkai P, Domont GB, Nogueira FCS. 2019. Quantitative subcellular proteomics of the orbitofrontal cortex of schizophrenia patients. J. Proteome Res 18:4240–4253. [PubMed: 31581776]

Vermeire PJ, Van Schepdael A, Petersen NJ. 2020. Development of a novel sheathless CE-ESI-MS interface via a CO2 laser ablated opening. Talanta 214:8.

Vo TD, Palsson BO. 2007. Building the power house: recent advances in mitochondrial studies through proteomics and systems biology. Am. J. Physiol.-Cell Physiol 292:C164–C177. [PubMed: 16885397]

Vocat C, Dunand M, Hubers SA, Bourdillon N, Millet GP, Brown NJ, Wuerzner G, Grouzmann E, Eugster PJ. 2020. Quantification of neuropeptide Y and four of its metabolites in human plasma by micro-UHPLC-MS/MS. Anal. Chem 92:859–866. [PubMed: 31790196]

Vogel C, Marcotte EM. 2012. Insights into the regulation of protein abundance from proteomic and transcriptomic analyses. Nat. Rev. Genet 13:227–232. [PubMed: 22411467]

von Eggeling F, Hoffmann F. 2020. Microdissection-an essential prerequisite for spatial cancer omics. Proteomics 20:10.

Waas M, Kislinger T. 2020. Addressing cellular heterogeneity in cancer through precision proteomics. J. Proteome Res 19:3607–3619.

Wang JH, Jiang XY, Sturm RM, Li LJ. 2009. Combining tissue extraction and off-line capillary electrophoresis matrix-assisted laser desorption/ionization Fourier transform mass spectrometry for neuropeptide analysis in individual neuronal organs using 2,5-dihydroxybenzoic acid as a multi-functional agent. J. Chromatogr. A 1216:8283–8288. [PubMed: 19473662]

Wang S, Xiao CS, Li Y, Ling L, Chen XS, Guo XH. 2017. A surface pattern on MALDI steel plate for one-step in-situ self-desalting and enrichment of peptides/proteins. J. Am. Soc. Mass Spectrom 28:428–433. [PubMed: 28058591]

Whiteman SA, Nuhse TS, Ashford DA, Sanders D, Maathuis FJM. 2008. A proteomic and phosphoproteomic analysis of Oryza sativa plasma membrane and vacuolar membrane. Plant J. 56:146–156. [PubMed: 18557835]

Wijetunge CD, Saeed I, Boughton BA, Spraggins JM, Caprioli RM, Bacic A, Roessner U, Halgamuge SK. 2015. EXIMS: an improved data analysis pipeline based on a new peak picking method for exploring imaging mass spectrometry data. Bioinformatics 31:3198–3206. [PubMed: 26063840]

Williams MD, Zhang X, Park JJ, Siems WF, Gang DR, Resar LMS, Reeves R, Hill HH. 2015. Characterizing metabolic changes in human colorectal cancer. Anal. Bioanal. Chem 407:4581–4595. [PubMed: 25943258]

Williams SM, Liyu AV, Tsai CF, Moore RJ, Orton DJ, Chrisler WB, Gaffrey MJ, Liu T, Smith RD, Kelly RT, Pasa-Tolic L, Zhu Y. 2020. Automated coupling of nanodroplet sample preparation with liquid chromatography-mass spectrometry for high-throughput single-cell proteomics. Anal. Chem 92:10588–10596. [PubMed: 32639140]

Wilson RE, Jaquins-Gerstl A, Weber SG. 2018. On-column dimethylation with capillary liquid chromatography tandem mass spectrometry for online determination of neuropeptides in rat brain microdialysate. Anal. Chem 90:4561–4568. [PubMed: 29504751]

Wiseman JM, Ifa DR, Song QY, Cooks RG. 2006. Tissue imaging at atmospheric pressure using desorption electrospray ionization (DESI) mass spectrometry. Angew. Chem., Int. Ed 45:7188–7192.

Wisniewski JR, Zougman A, Nagaraj N, Mann M. 2009. Universal sample preparation method for proteome analysis. Nat. Meth 6:359–362.

Woo HK, Northen TR, Yanes O, Siuzdak G. 2008. Nanostructure-initiator mass spectrometry: a protocol for preparing and applying NIMS surfaces for high-sensitivity mass analysis. Nat. Protoc 3:1341–1349. [PubMed: 18714302]

Woo J, Clair GC, Williams SM, Feng S, Tsai C-F, Moore RJ, Chrisler WB, Smith RD, Kelly RT, Paša-Tolić L, Ansong C, Zhu Y. 2022. Three-dimensional feature matching improves coverage for single-cell proteomics based on ion mobility filtering. Cell Syst. 13:426–434.e424. [PubMed: 35298923]

Woo J, Williams SM, Markillie LM, Feng S, Tsai CF, Aguilera-Vazquez V, Sontag RL, Moore RJ, Hu DH, Mehta HS, Cantlon-Bruce J, Liu T, Adkins JN, Smith RD, Clair GC, Pasa-Tolic L, Zhu Y. 2021. High-throughput and high-efficiency sample preparation for single-cell proteomics using a nested nanowell chip. Nat. Commun 12:1. [PubMed: 33397941]

Wu HK, Wheeler A, Zare RN. 2004. Chemical cytometry on a picoliter-scale integrated microfluidic chip. Proc. Natl Acad. Sci. U. S. A 101:12809–12813. [PubMed: 15328405]

Xiang PL, Zhu Y, Yang Y, Zhao ZT, Williams SM, Moore RJ, Kelly RT, Smith RD, Liu SR. 2020. Picoflow liquid chromatography-mass spectrometry for ultrasensitive bottom-up proteomics using 2-μm-i.d. open tubular columns. Anal. Chem 92:4711–4715. [PubMed: 32208662]

Xu X, Liu K, Fan ZH. 2012. Microscale 2D separation systems for proteomic analysis. Expert Rev. Proteomics 9:135–147. [PubMed: 22462786]

Yanes O, Tautenhahn R, Patti GJ, Siuzdak G. 2011. Expanding coverage of the metabolome for global metabolite profiling. Anal. Chem 83:2152–2161. [PubMed: 21329365]

Yang LW, George J, Wang J. 2020. Deep profiling of cellular heterogeneity by emerging single-cell proteomic technologies. Proteomics 20:12.

Yang YY, Huang YY, Wu JH, Liu N, Deng JW, Luan TG. 2017. Single-cell analysis by ambient mass spectrometry. Trends Anal. Chem 90:14–26.

Yang ZC, Shen XJ, Chen DY, Sun LL. 2019. Improved nanoflow RPLC-CZE-MS/MS system with high peak capacity and sensitivity for nanogram bottom-up proteomics. J. Proteome Res 18:4046–4054. [PubMed: 31610113]

Yang ZC, Sun LL. 2021. Recent technical progress in sample preparation and liquid-phase separation-mass spectrometry for proteomic analysis of mass-limited samples. Anal. Meth 13:1214–1225.

Yi L, Piehowski PD, Shi TJ, Smith RD, Qian WJ. 2017. Advances in microscale separations towards nanoproteomics applications. J. Chromatogr. A 1523:40–48. [PubMed: 28765000]

Yoon S, Park S, Kim MS, Lee CY. 2018. Concomitant desalting and concentration of neuropeptides on a donut-shaped surface pattern for MALDI mass spectrometry. Chem. Commun 54:5688–5691.

Yu DH, Wang Z, Cupp-Sutton KA, Guo YT, Kou Q, Smith K, Liu XW, Wu S. 2021. Quantitative top-down proteomics in complex samples using protein-level tandem mass tag labeling. J. Am. Soc. Mass Spectrom 32:1336–1344. [PubMed: 33725447]

Yuan ZY, Zhou QM, Cai LS, Pan L, Sun WL, Qumu SW, Yu S, Feng JX, Zhao HS, Zheng YC, Shi ML, Li S, Chen Y, Zhang XR, Zhang MQ. 2021. SEAM is a spatial single nuclear

metabolomics method for dissecting tissue microenvironment. Nat. Methods 18:1223–1232. [PubMed: 34608315]

Zemenova J, Sykora D, Freislebenova A, Maletinska L. 2017. LC-MS/MS analysis of lipidized analogs of prolactin-releasing peptide utilizing a monolithic column and simple sample preparation. Bioanalysis 9:1319–1328. [PubMed: 28901165]

Zestos AG, Kennedy RT. 2017. Microdialysis coupled with LC-MS/MS for in vivo neurochemical monitoring. Aaps J. 19:1284–1293. [PubMed: 28660399]

Zhang FF, Ge WG, Ruan G, Cai X, Guo TN. 2020. Data-independent acquisition mass spectrometry-based proteomics and software tools: a glimpse in 2020. Proteomics 20:12.

Zhang JL, Rector J, Lin JQ, Young JH, Sans M, Katta N, Giese N, Yu WD, Nagi C, Suliburk J, Liu JS, Bensussan A, DeHoog RJ, Garza KY, Ludolph B, Sorace AG, Syed A, Zahedivash A, Milner TE, Eberlin LS. 2017. Nondestructive tissue analysis for ex vivo and in vivo cancer diagnosis using a handheld mass spectrometry system. Sci. Transl. Med 9:11.

Zhang L, Vertes A. 2018. Single-cell mass spectrometry approaches to explore cellular heterogeneity. Angew. Chem. Int. Ed 57:4466–4477.

Zhang LW, Khattar N, Kemenes I, Kemenes G, Zrinyi Z, Pirger Z, Vertes A. 2018. Subcellular peptide localization in single identified neurons by capillary microsampling mass spectrometry. Sci. Rep 8:10. [PubMed: 29311571]

Zhang XC, Wei ZW, Gong XY, Si XY, Zhao YY, Yang CD, Zhang SC, Zhang XR. 2016. Integrated droplet-based microextraction with esi-ms for removal of matrix interference in single-cell analysis. Sci. Rep 6:9. [PubMed: 28442706]

Zhang YY, Zheng TT, Wang L, Feng L, Wang M, Zhang ZC, Feng HH. 2021. From passive to active sorting in microfluidics: a review. Rev. Adv. Mater. Sci 60:313–324.

Zhang ZB, Dubiak KM, Huber PW, Dovichi NJ. 2020. Miniaturized filter-aided sample preparation (MICRO-FASP) method for high throughput, ultrasensitive proteomics sample preparation reveals proteome asymmetry in Xenopus laevis embryos. Anal. Chem 92:5554–5560. [PubMed: 32125139]

Zhang ZB, Dubiak KM, Shishkova E, Huber PW, Coon JJ, Dovichi NJ. 2022. High-throughput, comprehensive single-cell proteomic analysis of Xenopus laevis embryos at the 50-cell stage using a microplate-based MICROFASP System. Anal. Chem 94:3254–3259. [PubMed: 35143156]

Zhao SS, Zhong XF, Tie C, Chen DDY. 2012. Capillary electrophoresis-mass spectrometry for analysis of complex samples. Proteomics 12:2991–3012. [PubMed: 22888086]

Zhou MW, Uwugiaren N, Williams SM, Moore RJ, Zhao R, Goodlett D, Dapic I, Pasa-Tolic L, Zhu Y. 2020. Sensitive top-down proteomics analysis of a low number of mammalian cells using a nanodroplet sample processing platform. Anal. Chem 92:7087–7095. [PubMed: 32374172]

Zhou XH, Xiao W, Su ZY, Cheng JH, Zheng CY, Zhang ZJ, Wang YQ, Wang L, Xu BH, Li SP, Yang XF, Hoi MPM. 2019. Hippocampal proteomic alteration in triple transgenic mouse model of Alzheimer's disease and implication of PINK 1 regulation in donepezil treatment. J. Proteome Res 18:1542–1552. [PubMed: 30484658]

Zhu GZ, Shao YL, Liu YX, Pei T, Li LJ, Zhang DT, Guo GS, Wang XY. 2021. Single-cell metabolite analysis by electrospray ionization mass spectrometry. Trac-Trends Anal. Chem 143:18.

Zhu HY, Li QQ, Liao TP, Yin X, Chen Q, Wang ZY, Dai MF, Yi L, Ge SY, Miao CJ, Zeng WP, Qu LL, Ju ZY, Huang GM, Cang CL, Xiong W. 2021. Metabolomic profiling of single enlarged lysosomes. Nat. Meth 18:788–798.

Zhu XP, Xu TY, Peng C, Wu SH. 2022. Advances in MALDI mass spectrometry imaging single cell and tissues. Front. Chem 9:27.

Zhu Y, Clair G, Chrisler WB, Shen Y, Zhao R, Shukla AK, Moore RJ, Misra RS, Pryhuber GS, Smith RD, Ansong C, Kelly RT. 2018. Proteomic analysis of single mammalian cells enabled by microfluidic nanodroplet sample preparation and ultrasensitive nanoLC-MS. Angew. Chem., Int. Ed 57:12370–12374.

Zhu Y, Dou MW, Piehowski PD, Liang YR, Wang FJ, Chu RK, Chrisler WB, Smith JN, Schwarz KC, Shen YF, Shukla AK, Moore RJ, Smith RD, Qian WJ, Kelly RT. 2018. Spatially resolved

proteome mapping of laser capture microdissected tissue with automated sample transfer to nanodroplets. Mol. Cell. Proteomics 17:1864–1874. [PubMed: 29941660]

Zhu Y, Piehowski PD, Zhao R, Chen J, Shen YF, Moore RJ, Shukla AK, Petyuk VA, Campbell-Thompson M, Mathews CE, Smith RD, Qian WJ, Kelly RT. 2018. Nanodroplet processing platform for deep and quantitative proteome profiling of 10–100 mammalian cells. Nat. Commun 9:10. [PubMed: 29295980]

Zhu Y, Scheibinger M, Ellwanger DC, Krey JF, Choi D, Kelly RT, Heller S, Barr-Gillespie PG. 2019. Single-cell proteomics reveals changes in expression during hair-cell development. eLife 8:26.

Zimmerman TA, Rubakhin SS, Sweedler JV. 2011. MALDI mass spectrometry imaging of neuronal cell cultures. J. Am. Soc. Mass Spectrom 22:828–836. [PubMed: 21472517]

Zivraj KH, Tung YCL, Piper M, Gumy L, Fawcett JW, Yeo GSH, Holt CE. 2010. Subcellular profiling reveals distinct and developmentally regulated repertoire of growth cone mRNAs. J. Neurosci 30:15464–15478. [PubMed: 21084603]

Zougman A, Pilch B, Podtelejnikov A, Kiehntopf M, Schnabel C, Kurnar C, Mann M. 2008. Integrated analysis of the cerebrospinal fluid peptidome and proteome. J. Proteome Res 7:386–399. [PubMed: 18052119]

Zougman A, Wilson JP, Roberts LD, Banks RE. 2020. Detergent-free simultaneous sample preparation method for proteomics and metabolomics. J. Proteome Res 19:2838–2844. [PubMed: 31743035]

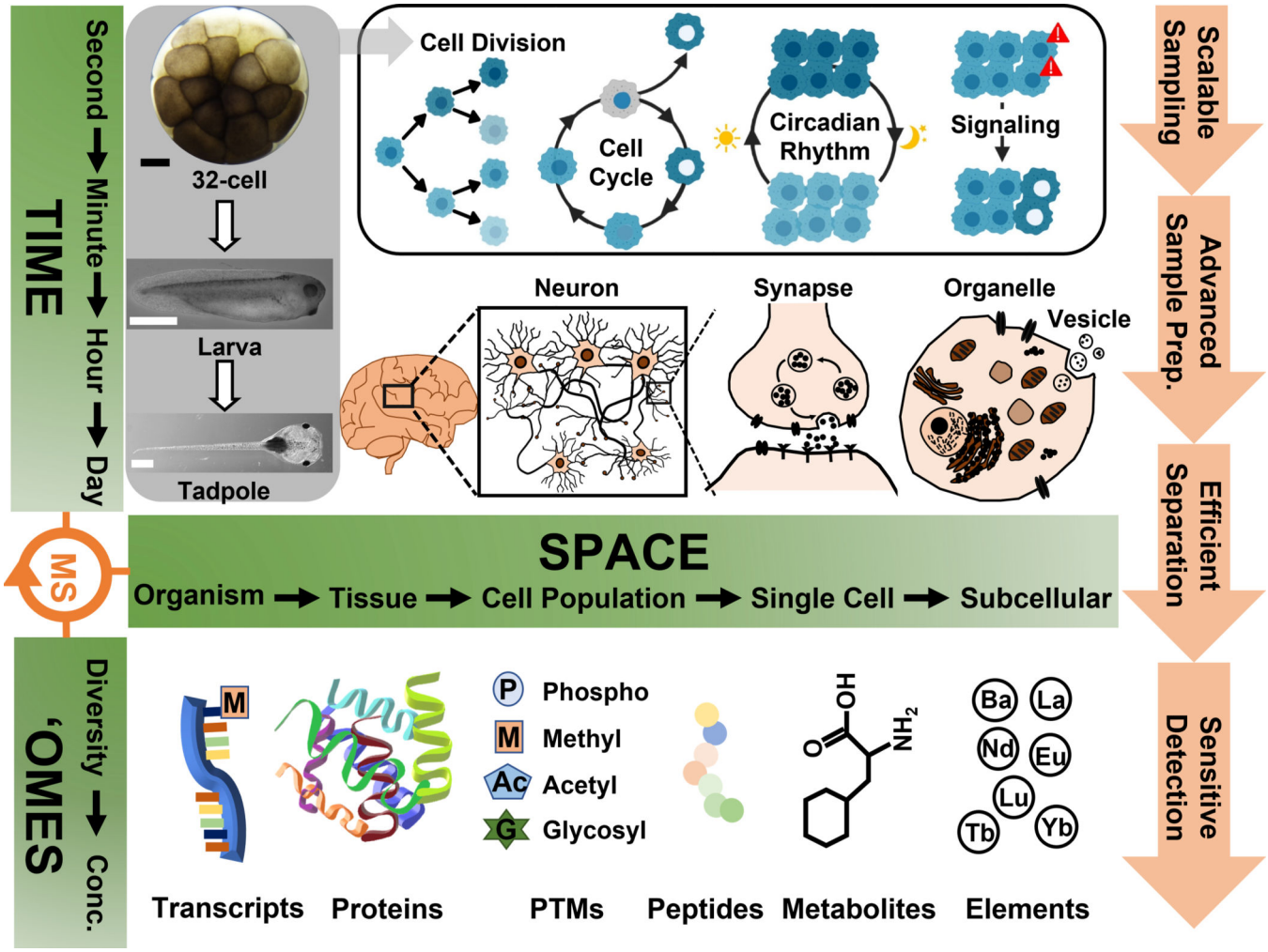

Figure 1.

Understanding biological processes hinges on the analysis of molecular heterogeneity that they orchestrate over broad spatial and temporal domains. Recent technological innovations and the development of specialized methodologies in sample collection and preparation, chemical separation, and HRMS analysis and detection provide sufficient sensitivity and spatiotemporal resolution for studying molecular processes at the levels of transcriptomes, proteomes, peptidomes, and metabolomes ('omes) in ever-deepening information content. Information from these studies fuel basic and translational research forward. Representative examples are highlighted from whole organismal to subcellular studies. Adapted with permission from (Gnann et al., 2021,Lombard-Banek et al., 2021).

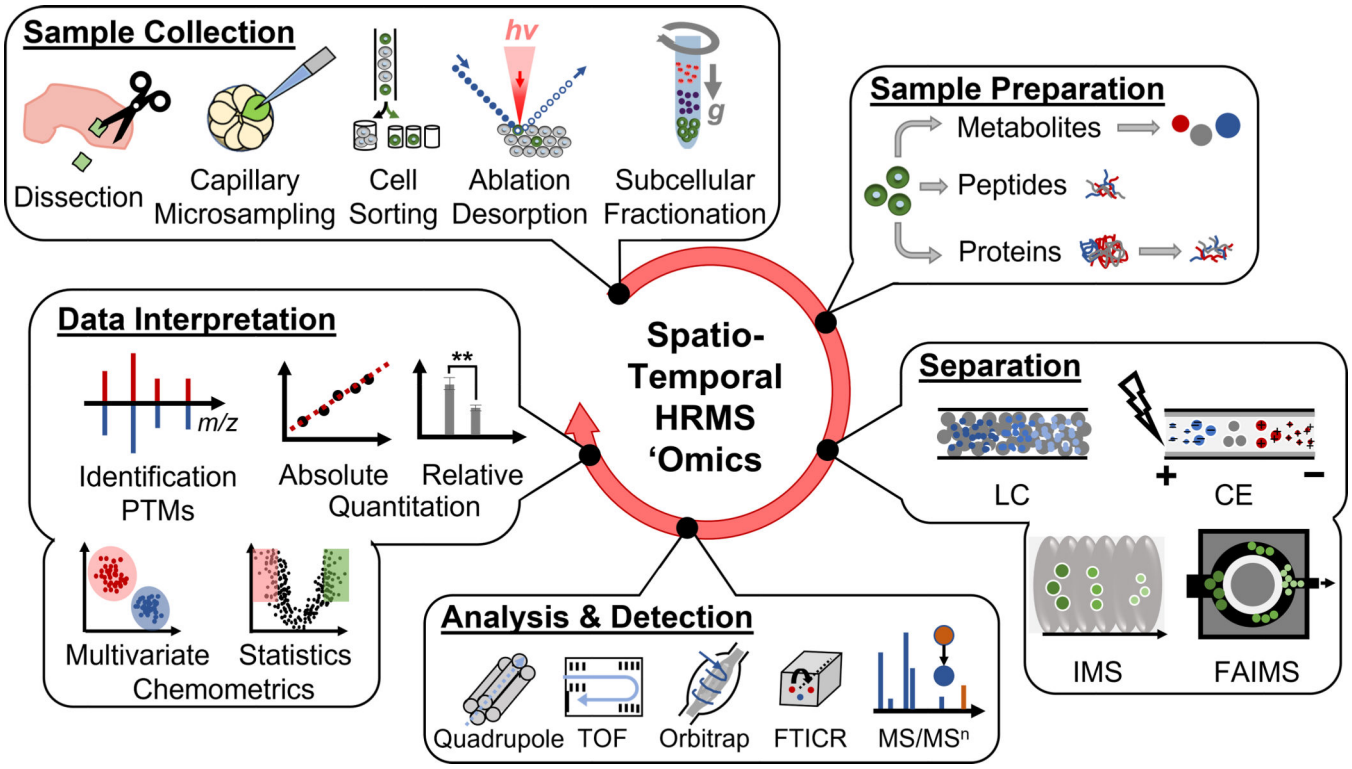

Figure 2.

General experimental workflow and representative technologies enabling spatiotemporal MS. Scalable sample collection and processing allows for probing biological specimens across broad dimensions in space and time. Separation in the liquid and/or gas phase reduces spectral complexity. High-resolution mass analyzers, tandem/multistage fragmentation, and efficient detector systems support molecular identifications in high specificity and quantification in high sensitivity. Development of advanced tools from bioinformatics and chemometrics facilitate the analysis and interpretation of MS data with spatiotemporal insights. Technological abbreviations are in the text.

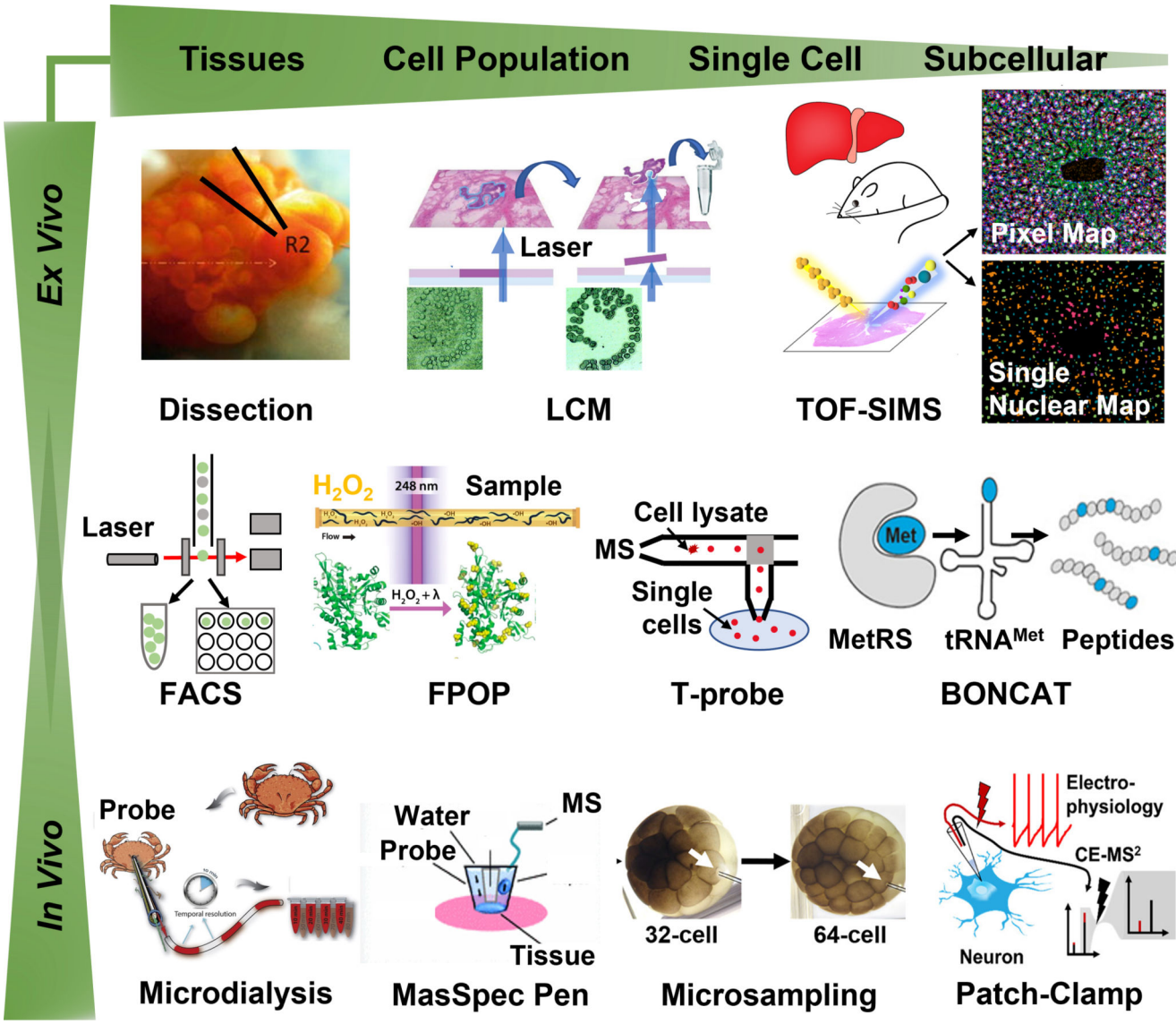

Figure 3.

Representative strategies enabling scalable spatiotemporal sampling. Diverse technologies enabled HRMS with scalable operation to assess ‘omics in broad types of biological systems and investigative contexts, ranging from *ex vivo* to *in situ* to *in vivo* analyses from small populations of cells to single cells to subcellular organelles. Technological abbreviations are in the text. Adapted with permission from (Azizian et al., 2021,Brown et al., 2018,Choi et al., 2022,Espino et al., 2019,Lombard-Banek et al., 2019,Moroz et al., 2013,OuYang et al., 2015,Simone et al., 1998,von Eggeling et al., 2020,Yuan et al., 2021).

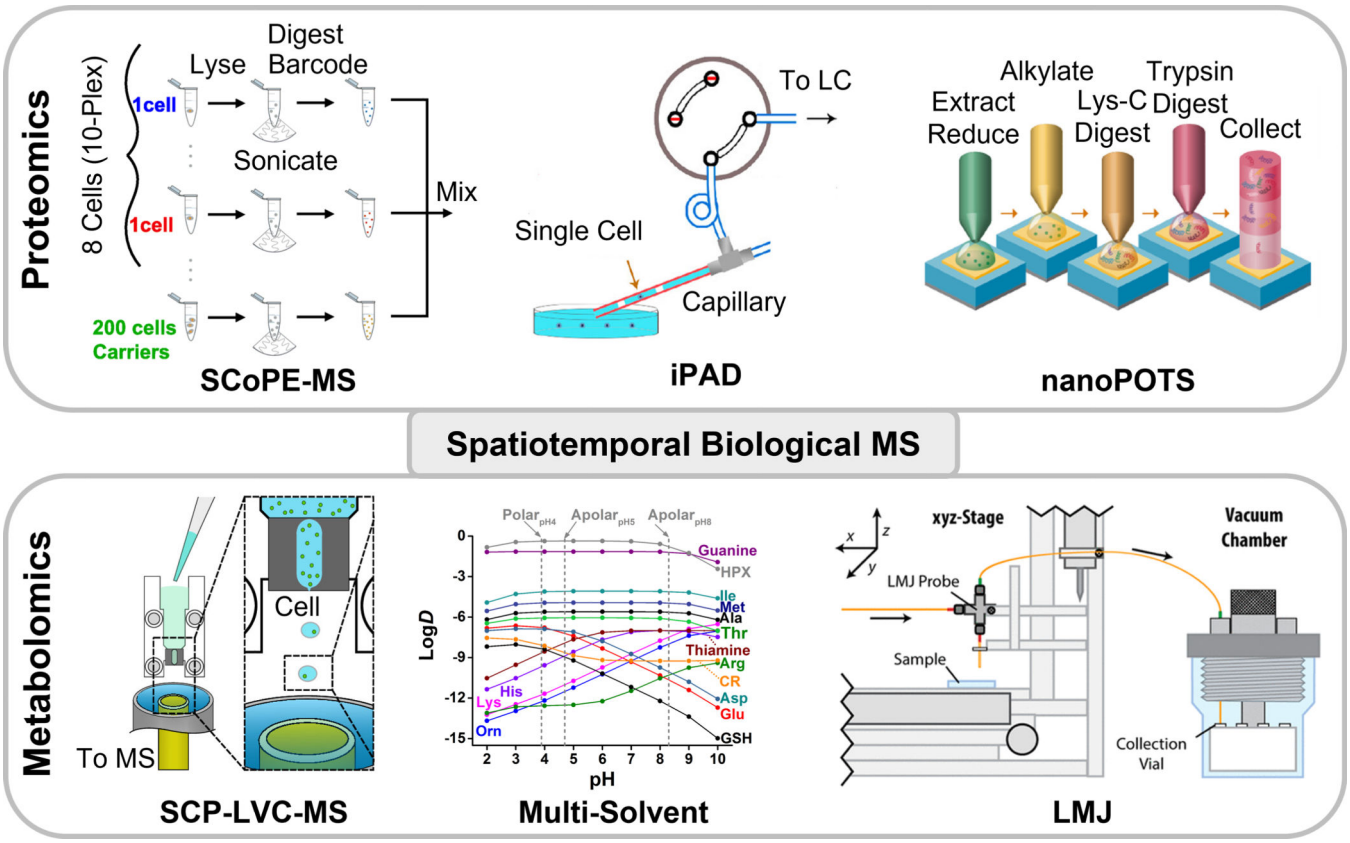

Figure 4.

Scalable and high-sensitive sample processing for metabolites and proteins enabling spatiotemporal biological MS. One-pot sample processing (SCoPE), direct cell capture and processing (iPAD), and miniaturization of sample processing steps (nanoPOTS) deepen the detectable proteome by reducing analyte losses. Direct cell capture (SCP-LVC), multi-solvent processing, and *in situ* metabolite extraction (LMJ) after MALDI imaging improve coverage of the single-cell metabolome. Technological abbreviations are in the text. Adapted with permission from (Budnik et al., 2018,Cahill et al., 2019,Comi et al., 2017,Kelly 2020,Onjiko et al., 2016,Zhang et al., 2020).

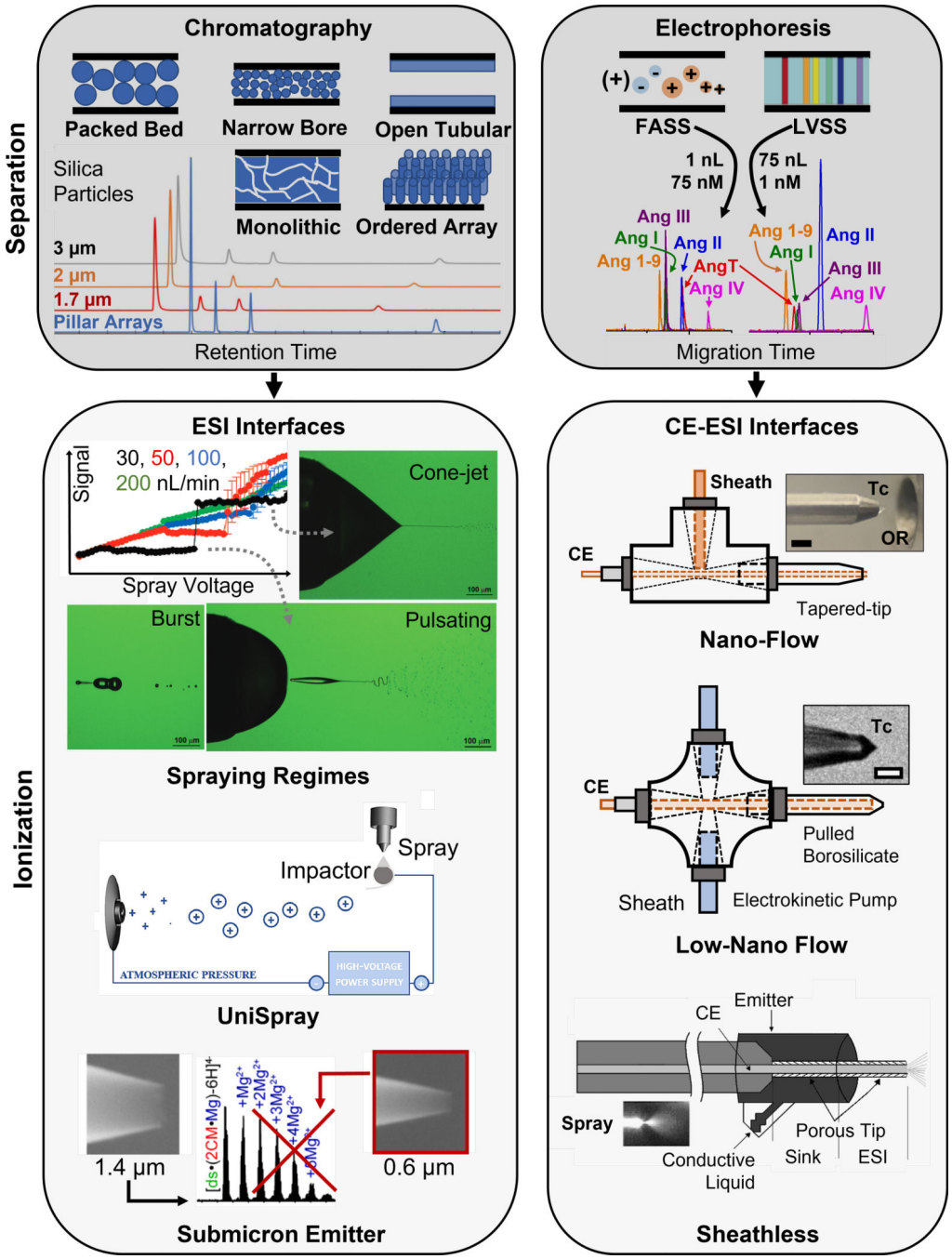

Figure 5.

Advances in separation and ionization aided the chemical analysis of limited amounts of materials. Reducing size of packing material, diameter of LC columns, newer generation columns minimize dispersion of molecules for high sensitivity during chromatography. Diverse stacking methods boost sensitivity during capillary electrophoresis. Specialized technologies and methods improve ion yields during electrospray ionization by controlling the spraying regime, charging of droplets, and reducing analyte dilution. Technological abbreviations are in the text. Key: OR, orifice of mass spectrometer; Tc, Taylor cone;

FASS, LVS abbreviations are in the text. Adapted with permission from (Bongaerts et al., 2020,Choi et al., 2017,DeLaney et al., 2022,Desmet et al., 2020,Kenderdine et al., 2018,Lombard-Banek et al., 2019,Lombard-Banek et al., 2019,Marginean et al., 2007,Moini 2007,Nemes et al., 2007).

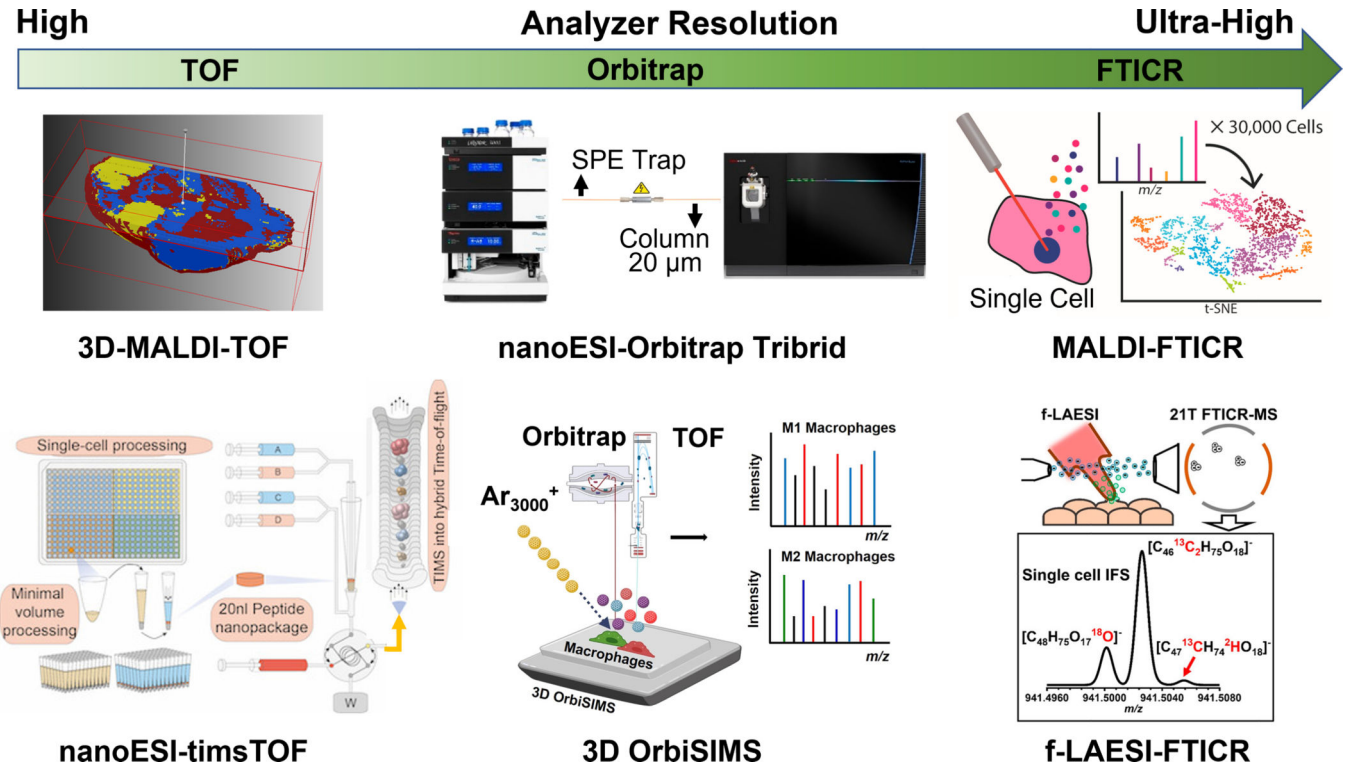

Figure 6.

High- to ultra-high resolution analyzers and instrumental configurations with enhanced ion transfer efficiency support spatiotemporal ‘omics. TOF analyzers are capable of fast operation, enabling spatial interrogation of molecular distributions in tissues and cells using focused laser beams (MALDI) and *in situ* ablation/ionization (LAESI). Increasing spectral resolution by orbitrap and FTICR analyzer-detector systems allow for the resolution of isobaric species with different spatial and temporal distributions. Abbreviations are in the text. Adapted with permission from (Brunner et al., 2022,Cong et al., 2020,Neumann et al., 2019,Paine et al., 2019,Samarah et al., 2020,Suvannapruk et al., 2022)

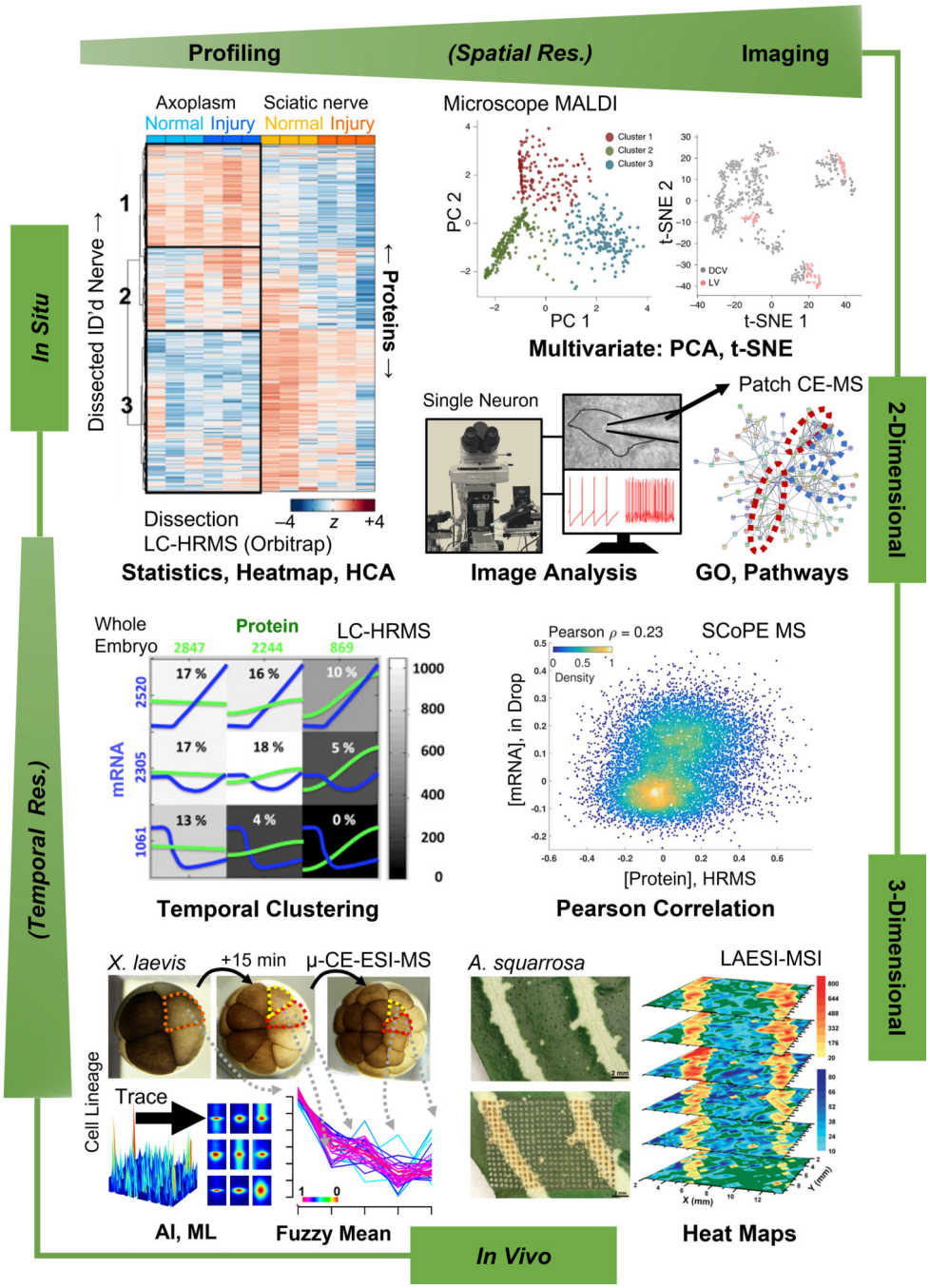

Figure 7.

Chemometrics plays a key role in analyzing metadata from spatiotemporal MS. Image analysis by optical microscopy and functional measurements, such as electrophysiology, help identify tissues and cells, guiding subsequent molecular characterizations to transcripts, proteins, peptides, and metabolites. Statistical and multivariate data analysis and knowledge bases are integral to interpreting molecular processes in specific biological contexts, as illustrated here for dissected rat sciatic nerves (HPLC), temporally developing whole *X. laevis* embryos (HPLC), and dense core vesicles in *A. californica* (MALDI). Working *in*

vivo, LAESI-MSI images chlorophyll and secondary metabolites in the variegated leaves of *A. squarrosa* and CE-HRMS enabled metabolomics-proteomics in single differentiating cells in *X. laevis* and neurons in the mouse brain. Models of artificial intelligence (AI) and machine learning assist in extracting trace-level signals (Trace) or uncovering relationships in the metadata. Technological abbreviations are in the text. Adapted with permission from (Budnik et al., 2018,Castro et al., 2021,Di Paolo et al., 2021,Liu et al., 2019,Nemes et al., 2009,Onjiko et al., 2017,Peshkin et al., 2015).