1 Contemporary Proteomics Technologies: A Comprehensive Review of Discovery, Quantitation, Molecular Context, Regulatory State, and Proteoform Biology

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1.1 Abstract

Proteomics has evolved from broad protein cataloging into a mature analytical discipline that now underwrites mechanistic biology, translational biomarker research, perturbational pharmacology, and therapeutic protein characterization [1, 2, 3]. The modern field is not organized around one dominant mass spectrometric paradigm, but around a family of technologies that solve different evidentiary problems: discovery-scale coverage, cohort-scale reproducible quantification, recovery of low-abundance regulatory states, mapping of interaction context, and preservation of intact proteoform and native complex information [4, 5, 6, 3]. Data-independent acquisition (DIA) transformed discovery proteomics by reducing stochastic precursor selection and turning large studies into reusable digital proteome maps rather than one-time identification exercises [4, 5]. Quantitative frameworks including label-free analysis, tandem mass tag (TMT) multiplexing, SILAC, and targeted verification workflows transformed proteomics into a measurement science capable of supporting comparative biology and translational adjudication rather than descriptive protein listing alone [7, 8, 9]. PTM-centered and interaction-centered proteomics shifted the field toward regulatory state and molecular context, while top-down, ion-mobility, and native mass spectrometry forced a conceptual upgrade from generic protein labels toward proteoforms, local neighborhoods, and intact-state biology [10, 11, 12, 3]. This review argues that the deepest logic of contemporary proteomics is not technological accumulation but evidentiary design: the best proteomics programs are those that match the analytical regime to the dominant uncertainty of the biological question while preserving traceability, auditability, and molecular honesty at every stage [1, 13].

1.2 Keywords

Proteomics; mass spectrometry; DIA; TMT; SILAC; targeted proteomics; phosphoproteomics; glycoproteomics; AP-MS; BioID; TurboID; ion mobility; top-down proteomics; proteoforms; Olink; SomaScan; affinity proteomics; biomarker discovery

1.3 Introduction

Mass spectrometry-based proteomics now occupies a central role in molecular biology because proteins are the most immediate large-scale readout of cell state, signaling, drug response, and biochemical function [1, 2]. Unlike genomic or transcriptomic measurements, proteomic data can reveal abundance, modification state, interaction context, turnover, and in some cases intact molecular architecture, all of which may be essential for mechanistic interpretation [14, 3]. The field has therefore expanded from exploratory protein identification toward a layered analytical system that supports reproducible quantitative measurement, discovery in complex clinical matrices, pathway-oriented perturbation analysis, and increasingly structural and proteoform-aware characterization [1, 7, 3].

Historically, modern proteomics emerged from the convergence of several older traditions: protein chemistry, electrophoretic separation, soft-ionization mass spectrometry, database search methods, and later high-throughput genomics-era expectations for scale and system-level comparability. Early bottom-up proteomics became broadly practical only after peptide-friendly ionization methods such as electrospray and MALDI were coupled to improving tandem mass spectrometers and searchable sequence databases, allowing peptide spectra to be turned into protein identifications at meaningful scale [15, 14]. In that sense, proteomics did not arise as a single invention, but as a synthetic field created when instrument physics, computation, and molecular biology matured enough to support one another.

This evolution reflects a simple reality: no single proteomics workflow solves all biological questions. Discovery-scale proteomics must contend with stochastic sampling, crowded peptide mixtures, and broad dynamic range [4, 16]. Quantitative proteomics must control normalization, missingness, and statistical inference strongly enough to support comparative and translational interpretation [7, 2]. PTM-centered proteomics must selectively recover rare modified peptides from overwhelming unmodified backgrounds [10]. Interaction proteomics must distinguish bona fide associations from nonspecific carryover and transient spatial proximity from stable complex formation [6, 11]. Structural and top-down approaches must preserve information about intact proteoforms and assemblies that is otherwise fragmented away in peptide-centric workflows [17, 3]. The resulting field architecture is summarized conceptually in Figure 1.

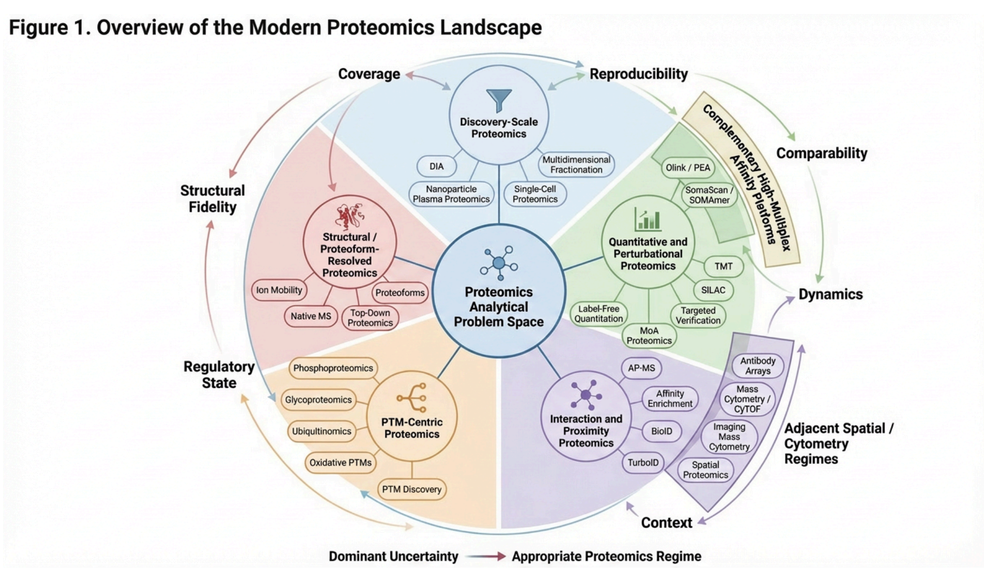

Figure 1. Overview of the modern proteomics analytical landscape.

Figure 1. Overview of the modern proteomics analytical landscape. A conceptual map of contemporary proteomics organized by the dominant uncertainty each method family is designed to reduce. Discovery-scale, quantitative, interaction-centered, PTM-centered, and structural/proteoform-resolved methods are shown as complementary evidence layers rather than interchangeable services.

The deeper implication is that proteomics has become a discipline of evidence design rather than instrument use alone. Investigators must decide whether the main barrier to knowledge is incomplete coverage, inadequate reproducibility, weak quantitation, poor access to low-abundance or modified species, lack of interaction context, or loss of intact molecular-state information [1, 2]. Those decisions determine the most appropriate workflow architecture. In that sense, proteomics should not be described as a list of tools, but as a structured way of matching analytical regime to biological uncertainty [1, 3].

For these reasons, this review is organized by analytical function rather than by service-style capability list. Each section asks the same set of questions: what biological or analytical problem the method solves, how it is implemented, why investigators choose it over alternatives, and what important work it has enabled. That structure is intentional because contemporary proteomics is best understood not as a menu of isolated technologies, but as a coordinated methodological ecosystem in which different tools resolve different evidentiary bottlenecks [1, 2]. It also reflects how advanced proteomics programs actually operate: discovery, quantitation, interaction mapping, PTM analysis, and structural characterization are brought to bear on the same biological problem from complementary angles rather than as isolated service modules [1, 2, 3].

1.3.1 A conceptual framework for reading the proteomics field

One of the reasons proteomics is often misunderstood outside specialist circles is that workflows are frequently described by instrument brand, acquisition mode, or assay label rather than by the uncertainty they are meant to reduce [1, 2]. A more useful framework is to ask which dimension of biological ignorance is dominant. If the unknown is broad composition, discovery-scale workflows dominate. If the unknown is comparative magnitude, quantitative workflows dominate. If the unknown is interaction state, affinity and proximity workflows dominate. If the unknown is regulatory chemistry, PTM-centered workflows dominate. If the unknown is intact structural or proteoform state, native, ion-mobility, and top-down methods dominate [3, 6, 10].

This framework matters because it turns proteomics from a pile of methods into a logic of evidence selection. It also makes clear why no serious proteomics platform can be evaluated simply by the number of techniques on its menu. What matters is whether the platform can move from one evidentiary regime to another as the biological question evolves from exploration to quantitation to mechanism to verification [1, 2]. The field has matured precisely because it now contains enough specialized methods to make that movement possible.

1.3.2 Scope and limits of this review

This review is intentionally centered on mass spectrometry-based proteomics and adjacent enrichment or interaction strategies rather than on immunoassay-only or array-only protein analysis systems [2, 14]. That choice reflects both the current architecture of the field and the practical reality that MS-based approaches remain the most general-purpose route to large-scale, unbiased, modification-aware protein analysis [1, 10]. At the same time, this review does not claim that all biologically useful protein measurements must be MS-based. High-multiplex affinity platforms such as Olink proximity extension assays and SomaLogic/SomaScan aptamer-based proteomics have become major practical components of translational protein measurement, especially in large-cohort plasma and cerebrospinal-fluid studies, and they are treated below as complementary regimes rather than as peripheral footnotes [18, 19, 20]. The core argument is therefore not that MS is the only valid protein-analysis substrate, but that MS-based proteomics provides the deepest shared reference architecture from which narrower or more deployment-oriented platforms can often be interpreted and benchmarked [9, 13, 18].

1.3.3 Historical turning points in modern proteomics

Several turning points transformed proteomics from a technically impressive niche into a general analytical language for biology. Early tandem mass spectrometry workflows and database-driven peptide identification made it possible to move from isolated protein characterization toward scalable identification programs [15, 14]. Quantitative frameworks then changed the field's ambition by showing that proteomics could compare states, not merely name components, with later standardization work pushing the field closer to clinically meaningful measurement [7, 13]. Phosphoproteomics marked another decisive shift because it brought system-scale signaling biology into the proteomics domain and established the principle that protein-state chemistry could be measured globally rather than piecemeal [21, 22].

Later transitions were equally important. Interaction proteomics expanded from purified-complex logic into high-throughput AP-MS and then into proximity labeling, changing what it meant to measure protein context [23, 11, 6]. DIA changed what it meant for discovery proteomics to be reproducible across cohorts, shifting the field toward more durable, re-interrogable data structures [5, 4]. Top-down and proteoform-centered work formalized the idea that the true molecular object of interest is often not the generic protein but the intact proteoform [24, 3]. Single-cell proteomics then imported the heterogeneity challenge from the single-cell omics revolution and forced proteomics to operate at a new scale of analytical scarcity [25, 26].

Seen together, these turning points show that proteomics developed by repeatedly confronting its own blind spots. Each major methodological wave arose because a previous generation of methods had become good enough to reveal what it still could not see.

1.3.4 Chronology of field-shaping innovations

It is useful to name the major phases of the field explicitly because proteomics did not mature linearly; it matured by stacking analytical revolutions that solved different categories of ignorance [15, 1]. A first phase was the identification era, in which soft ionization, tandem MS, and database search made peptide-centric proteome-scale identification feasible in the first place [15, 14]. A second phase was the quantitation era, in which isotopic, isobaric, and calibration-driven methods turned proteomics from a cataloging discipline into a comparative measurement discipline [7, 13, 8]. A third phase was the state-and-context era, in which phosphoproteomics, glycoproteomics, AP-MS, and proximity labeling extended the field from abundance toward regulation and network architecture [21, 11, 10, 6]. A fourth phase is the current integrative era, defined by cohort-scale reproducibility, single-cell sensitivity, proteoform resolution, and FAIR computational reuse [5, 25, 3, 1].

Seen through this chronology, the most important developers are often not simply the inventors of one assay, but the investigators who changed the evidentiary grammar of the field. Early proteome-scale vision was shaped by the work of Aebersold, Mann, Yates, and others who helped establish peptide-centric proteomics as a scalable analytical program rather than a one-protein-at-a-time craft [15, 14]. Quantitative maturation was driven by groups that made stable-isotope logic, multiplexing, and later quantitative standardization operationally routine enough to support larger biological claims [7, 13, 8]. The interaction turn was accelerated by investigators who recognized that purification logic and neighborhood logic were not the same thing, thereby giving AP-MS and BioID/TurboID distinct conceptual identities rather than treating them as mere variants of “protein interaction analysis” [23, 11, 6]. The proteoform turn, associated strongly with the work of Kelleher and others, reframed the field around the molecular reality that a gene product is not a sufficient substitute for an intact biological protein species [24, 3].

This history matters because it teaches a methodological habit: each major proteomics advance began by declaring that the current dominant readout was too lossy for the biological question now being asked. The future of the field will likely continue in the same pattern. Methods will advance not because novelty is desirable for its own sake, but because the current level of molecular compression will again become biologically inadequate.

1.4 Discovery-Scale Proteomics

Discovery-scale proteomics is used when the relevant biology is not yet narrowed to a small panel of known targets and the investigator needs broad, information-rich profiling across tissues, cells, or biofluids [4, 5]. The central difficulties in this regime are incomplete precursor sampling, matrix complexity, low-abundance signal loss, and the need to compare many samples without sacrificing reproducibility [16, 27]. Methods in this class include DIA, multidimensional fractionation, nanoparticle-assisted biofluid enrichment, and increasingly low-input or single-cell workflows [4, 28, 25]. Their relationship to one another is illustrated in Figure 2.

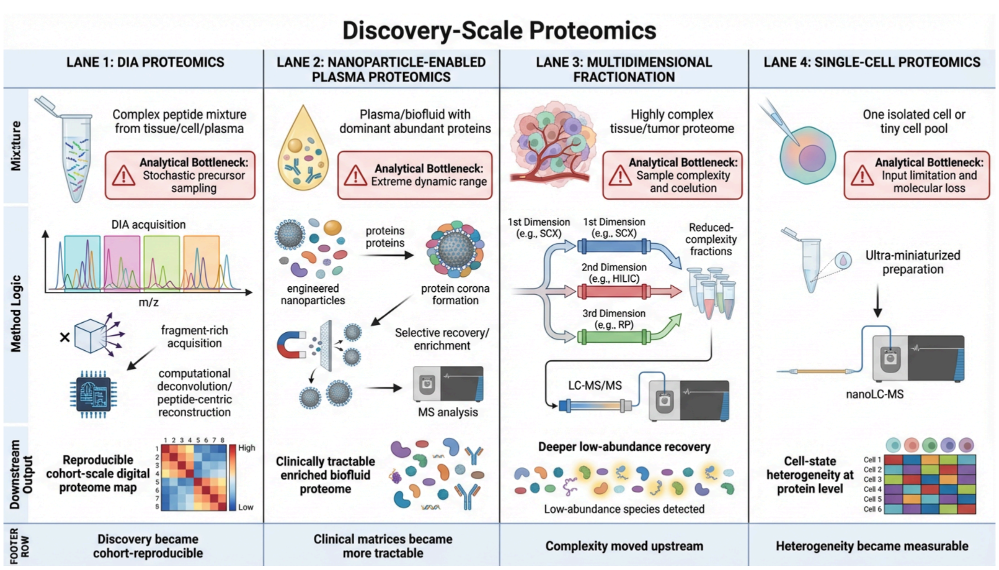

Figure 2. Discovery-scale proteomics workflows.

Figure 2. Discovery-scale proteomics workflows. Schematic comparison of DIA, nanoparticle-enabled plasma proteomics, multidimensional fractionation, and single-cell proteomics. The figure highlights the primary analytical bottleneck addressed by each

workflow: stochastic sampling, dynamic range, sample complexity, and input limitation.

Discovery-scale methods are often the first stage in a broader proteomics program because they establish the searchable molecular landscape from which later quantitative or targeted work proceeds [5, 2]. Their success is therefore judged not simply by how many proteins they identify, but by whether they preserve enough breadth and reproducibility to support downstream narrowing into mechanism, verification, or translational follow-up [4, 16]. In practical terms, discovery proteomics is not an end point but the first map of a still only partly known molecular terrain.

1.4.1 Data-independent acquisition proteomics

DIA has become one of the dominant discovery frameworks because it addresses a classic weakness of data-dependent acquisition: stochastic precursor selection [9, 4]. In DDA, the instrument typically fragments the most intense precursors visible during each survey scan, which can produce excellent identifications but inconsistent peptide recovery across large sample series [9]. DIA instead cycles across predefined m/z windows and fragments all ions within each window, producing a more systematic and reproducible record of the sample [4, 5]. The resulting mixed spectra are computationally demanding, but advances in scan speed, mass accuracy, library construction, and deconvolution tools have made DIA highly effective for cohort-scale proteomics [5, 16].

Historically, DIA grew out of dissatisfaction with the sampling instability of DDA and from earlier ideas about parallel fragmentation and systematic windowed acquisition. What became important was not just the concept of fragmenting more ions, but the realization that large comparative studies required a more deterministic acquisition logic than classical top-N precursor selection could provide [9, 5]. The rise of DIA therefore reflects a field-wide demand for cohort-compatible proteomics rather than a mere instrument upgrade.

Method design is central to DIA performance. Variable window width, chromatographic peak width, cycle time, fragment ion density, and retention-time alignment all determine how recoverable peptide-level evidence will be from the mixed spectra [5, 16]. In mature DIA workflows, acquisition and informatics are inseparable; a poorly designed acquisition plan cannot be rescued fully by software, and an excellent acquisition run can still be analytically wasted by weak deconvolution or library mismatch [4, 5]. This is one reason why the best DIA publications read as integrated analytical-system papers rather than as instrument-only reports.

Investigators choose DIA because its strengths align with translational and systems-level biology: broad coverage, improved run-to-run consistency, and the ability to reanalyze archived digital proteome maps as methods improve [5, 4]. This reusable “digital proteome” concept is one of DIA’s major conceptual advantages because it makes discovery data less perishable than earlier acquisition paradigms [5]. Its limitations are equally important. Window design, chromatography, and software quality all strongly affect performance, and poor DIA implementations can shift uncertainty from acquisition into hidden analysis artifacts [16, 5].

High-impact applications reflect these strengths. DIA has been used in disease-state profiling, tumor classification, and cohort-scale plasma or tissue studies because these settings require both proteome breadth and cross-sample consistency [29, 30]. The broader importance of DIA is that it helped shift proteomics from a partly opportunistic discovery technology toward a more repeatable measurement framework suitable for larger biological programs [9, 5].

1.4.2 Plasma and nanoparticle-enabled biofluid proteomics

Plasma proteomics is especially valuable for translational research because it offers clinically relevant, minimally invasive access to disease biology, but it is analytically difficult because a few abundant proteins dominate the matrix and obscure lower-abundance signals [27, 28]. Nanoparticle-assisted proteomics emerged as one response to this problem. In these workflows, proteins adsorb onto engineered particle surfaces and form a protein corona that can selectively enrich analytes otherwise hard to detect in unenriched plasma [28]. The method therefore acts as a biochemical interface that reshapes the accessible proteome before MS analysis rather than merely increasing sensitivity in a generic way [28].

The historical background here is the long struggle to make clinically realistic biofluids analytically useful without destroying their biological meaning through overly aggressive depletion or excessive preprocessing. Nanoparticle-corona methods emerged from the intersection of nanomedicine, colloid science, and proteomics, where investigators realized that protein adsorption behavior could be exploited analytically rather than treated only as a nuisance phenomenon [31, 28]. That shift in perspective is part of what made the approach influential.

The value of this class of methods lies in selective access. The investigator is not merely processing plasma more deeply but changing which parts of plasma can be observed at all [28]. That makes the approach particularly attractive in biomarker discovery, host-response profiling, and liquid-biopsy settings where low-abundance signals are most likely to matter biologically [30, 32]. Similar logic applies to urinary proteomics, extracellular-vesicle proteomics, and other biofluid strategies in which analytical tractability is itself one of the major barriers to insight [27, 33].

However, the enrichment is selective and therefore biased by surface chemistry and matrix behavior, which means interpretation depends on understanding what the enrichment step did to the sample representation [28]. This is one reason why biofluid proteomics must be evaluated as a complete system, not simply by identification count. Reproducibility, capture behavior, recovery chemistry, and biological interpretability all matter together [28, 27]. In practical terms, this means that nanoparticle and other selective-biofluid workflows are most persuasive when accompanied by benchmarking against matrix effects, contamination sources, and study-to-study comparability rather than by raw depth claims alone [34, 35].

1.4.3 Multidimensional separation and deep fractionation

Not all complexity can be solved computationally. In tissue proteomics, tumor heterogeneity studies, and other highly crowded matrices, front-end fractionation remains essential because it reduces coelution, ion suppression, and fragment ambiguity before the peptides reach the instrument [14, 16]. High-pH fractionation, LC-LC workflows, and related multidimensional strategies are used when deeper separation changes the biological answer rather than simply expanding the identification list [14].

The principle is straightforward: if too many analytes enter the instrument at once, the system loses information through competition and ambiguity. Orthogonal fractionation moves some of that complexity upstream, creating cleaner subsets and increasing the likelihood that low-abundance or analytically crowded species become measurable [14]. This is especially important in tissue proteomics and highly heterogeneous tumor samples where subtle regulatory proteins may be the true biological signal [14, 29].

These methods cost throughput and increase data-handling burden, but they remain powerful when complexity itself is the dominant analytical bottleneck [14]. In that sense, multidimensional workflows should be understood as strategic rather than routine tools: they are justified when more separation changes the biological inference, not merely when it increases the length of the protein list [14].

1.4.4 Single-cell proteomics

Single-cell proteomics addresses an even more extreme discovery problem: how to recover meaningful protein-level information from one cell or a very small number of cells [25, 26]. Its biological motivation is that bulk measurements average away heterogeneity that may be central to cancer state, immune activation, developmental trajectories, or tissue microenvironments [36, 25]. Methodologically, single-cell proteomics depends on low-loss handling, miniaturized preparation, sensitive nanoLC-MS, and computational pipelines tolerant of sparse data and variable recovery [25, 26].

Single-cell proteomics emerged historically after single-cell transcriptomics made cellular heterogeneity impossible to ignore. Once RNA measurements demonstrated how much biological structure was lost in bulk averaging, the pressure on proteomics was obvious: could protein-level measurement reach comparable cellular granularity while retaining the unique functional advantages of direct proteomic observation? The field's answer has been gradual rather than sudden, driven by low-input chemistry, microhandling innovation, and instrument sensitivity gains rather than by one single enabling invention [25, 26].

The technical challenge is not just sensitivity in the abstract, but total molecular preservation from the moment the cell is isolated. Sample loss, adsorption, digestion efficiency, and transfer fidelity all become dominant variables because there is so little starting material [25]. This means single-cell proteomics is as much a workflow-engineering discipline as it is a mass-spectrometric one [26]. It is also increasingly a computation discipline, because sparse observations and modality integration become central to interpretation [26, 36].

It is chosen when heterogeneity itself is the biological object of study, despite the significant technical burden involved [36]. High-value applications include tumor-state mapping, immune-cell heterogeneity, and developmental-state resolution, where the key biology resides in differences between cells rather than in bulk averages [36, 25]. The field remains technically demanding, but it has already changed what proteomics is expected to reveal about cellular populations [26].

1.4.5 Benchmarking, reproducibility, and translational readiness in discovery proteomics

Discovery methods are often compared by coverage depth alone, but that is an inadequate standard for translationally relevant work [1, 27]. A useful discovery workflow must also preserve interpretability across time, sample classes, operators, and downstream reanalysis contexts [5, 1]. For that reason, contemporary benchmarking in discovery proteomics increasingly asks not only how many proteins were observed, but how stable the recovery was across cohorts, how sensitive the system is to preanalytical variability, and whether the resulting data can support later verification and statistical modeling [16, 37]. The most important implication is practical: the best discovery platform is not the one that produces the longest protein list in a single heroic run, but the one that generates sufficiently rich and sufficiently stable evidence for the next stage of the research program [5, 1].

1.4.6 Representative high-impact discovery use cases

The power of discovery-scale proteomics is easiest to appreciate in applications where breadth and reproducibility changed what could be asked biologically. DIA has been used in clinically oriented biomarker discovery programs where cohort consistency was essential, including lung-cancer biomarker work in bronchoalveolar lavage fluid and serum-based pediatric vascular-disease studies in which 4D-DIA enabled systematic differential profiling across structured cohorts [38, 39]. In those settings, the value of DIA was not just depth but the ability to compare many samples under one acquisition logic and identify candidate biology that could later move into verification workflows [38, 39].

Plasma and nanoparticle-enabled discovery methods have likewise enabled applications that would have been difficult to execute using conventional unenriched plasma proteomics alone. Recent work using multiplexed nanoparticle corona strategies showed that deep plasma profiling can be improved in both precision and depth, while follow-on studies evaluated contamination sensitivity and sample-class behavior directly rather than relying on depth claims in isolation [40, 35]. Other studies extended these ideas to organ-derived protein recovery, menstrual-fluid vesicle analysis, and kidney-associated protein detection in plasma, illustrating how selective enrichment creates new analyte spaces rather than merely making old ones more sensitive [41, 42].

Single-cell discovery proteomics offers an even clearer example of how a method changes the question space. Tumor heterogeneity studies, circulating tumor cell work, and multimodal spatially aware cancer proteomics have all demonstrated that clinically relevant protein-state diversity can be hidden by bulk averaging and must sometimes be measured at single-cell or cell-resolved scale to become intelligible [43, 44, 45]. These applications matter because they show that single-cell proteomics is not only a technical frontier but a biological one: it makes heterogeneity itself a first-class object of proteomic measurement.

1.4.7 Common failure modes in discovery-scale proteomics

Discovery proteomics fails most often when investigators confuse breadth with reliability. In DIA, one common trap is to assume that systematic acquisition guarantees systematic truth; in reality, poor window design, weak chromatographic separation, and inadequate spectral interpretation can make the data reproducibly wrong rather than reproducibly right [16, 5]. In biofluid enrichment workflows, another trap is to interpret selective enrichment as neutral sampling, thereby forgetting that the method itself imposes a biochemical filter on what becomes visible [28, 31]. In single-cell proteomics, the most common trap is overinterpreting sparse, fragile observations as if they had the same robustness as bulk measurements, when in fact missingness and handling sensitivity remain dominant sources of uncertainty [26, 25].

The practical lesson is that discovery claims should be graded by stability and downstream translatability, not just by the novelty or size of the protein list. A discovery result that cannot be reproduced, contextualized, or narrowed into a verification framework is often analytically less valuable than a shallower but cleaner result [1, 37].

1.4.8 Seminal transition: from opportunistic discovery to cohort-scale discovery

One of the most important conceptual changes in proteomics was the shift from discovery as a mostly opportunistic identification exercise toward discovery as a reproducible, cohort-aware analytical framework. Early shotgun proteomics was revolutionary because it could identify many proteins at once, but modern discovery proteomics is revolutionary for a different reason: it can do so repeatedly across structured biological series with enough stability to support comparative inference [9, 5]. That transition, more than any single instrument feature, is what made proteomics genuinely scalable in translational research.

1.4.9 Discovery papers that changed the field's standards

Some discovery-oriented papers mattered less because of the specific biomarker or disease domain they addressed and more because they changed what the field thought discovery proteomics was allowed to claim. Methodological work on DIA helped establish that reproducibility across cohorts could itself be a primary deliverable, not merely a secondary virtue of a deep run [5, 4]. That changed how investigators designed large sample-series studies: instead of asking only how many proteins could be identified, they began asking whether the same proteomic evidence structure could survive across longitudinal sampling, multi-condition contrasts, and revisitation by improved computational tools [5].

Plasma and other biofluid studies made a parallel contribution by showing that clinical realism could be treated as an analytical design target instead of a nuisance constraint. Enrichment-centered and matrix-aware discovery papers in plasma, urinary proteomics, and extracellular-vesicle analysis demonstrated that the problem was not simply insufficient instrument sensitivity, but mismatched workflow architecture for hostile clinical matrices [28, 27, 33]. This repositioned discovery proteomics as a discipline of matrix-specific engineering rather than generic deep profiling.

Single-cell discovery papers shifted standards again by demonstrating that heterogeneity itself could be the object of proteomic discovery. Once that became credible, the field could no longer treat bulk averages as a default neutral summary of biology [25, 26, 36]. The real historical importance of these papers is that they altered the default unit of discovery, from the proteome of a pooled sample toward the proteome of a structured population or even a single cell. That is not just a technical extension. It is a change in what proteomics is understood to be for.

1.5 Quantitative and Perturbational Proteomics

Quantitative proteomics became necessary when the field moved beyond simple protein detection and began asking comparative, translational, and mechanistic questions that required stronger measurement discipline [7, 2]. The core issue is that protein signal intensity is shaped by sample handling, ionization efficiency, missingness, normalization, and peptide-to-protein inference, meaning that quantitative proteomics is an experimental-design and statistical problem as much as an instrument problem [2, 37]. This is why quantitative workflows are defined as much by calibration and analysis strategy as by acquisition mode [13, 2]. The major quantitative design options are summarized in Figure 3.

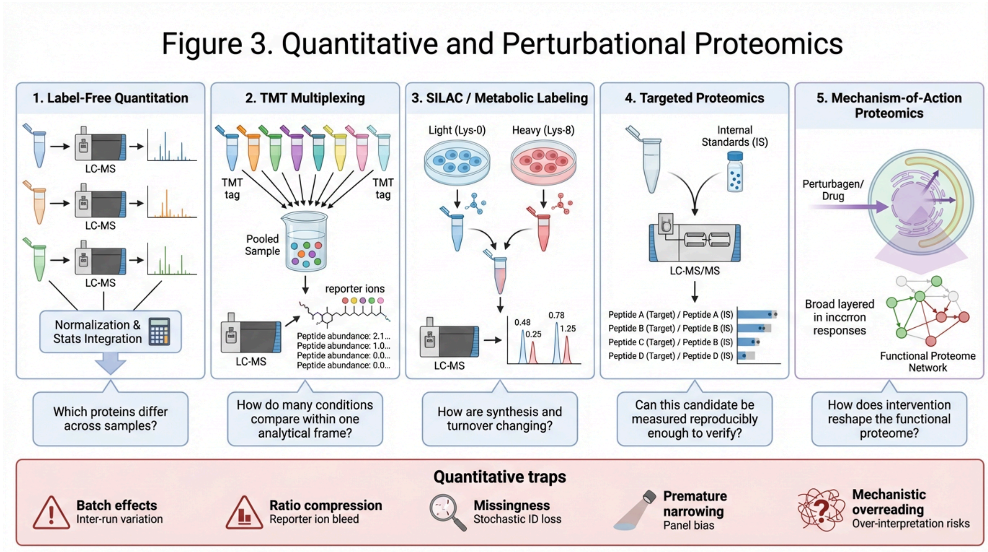

Figure 3. Quantitative and perturbational proteomics design space.

Figure 3. Quantitative and perturbational proteomics design space. Comparative framework for label-free quantification, TMT multiplexing, SILAC or metabolic labeling, targeted verification workflows, and mechanism-of-action proteomics. The figure emphasizes the relationship between quantitative design and the biological questions of comparability, turnover, verification, and functional response.

1.5.1 Label-free and absolute quantitative frameworks

Label-free quantification remains attractive because it avoids labeling constraints and is broadly usable across complex sample types, including clinical materials [7, 2]. It is chosen when design flexibility and sample scalability matter more than pooled multiplex control. Its limitations are sensitivity to run-to-run variability and a heavier dependence on normalization and statistical treatment [2, 37]. Absolute quantitative strategies go further by using calibration or internal standards to anchor signal to more interpretable abundance scales, which becomes especially important in biomarker verification and translational measurement [13].

The conceptual importance of absolute or semi-absolute frameworks is that they shift proteomics closer to a clinical measurement model. Instead of only asking whether a protein is higher or lower, the investigator can begin asking how much is present and whether that amount is meaningful across studies or models [13]. This matters in biomarker validation, systems biology, and therapeutic monitoring, where relative difference alone may not be enough [2, 13].

1.5.2 TMT and multiplexed proteomics

TMT-based proteomics solves a practical comparative problem: how to analyze many samples in one pooled experiment while minimizing between-run variance [8]. Peptides from different samples are labeled with isobaric tags that separate at the reporter-ion level after fragmentation, enabling multiplexed relative quantification [8]. TMT is chosen for cohort studies, perturbation series, and comparative phosphoproteomics because it raises throughput and improves cross-sample analytical control [8]. It is particularly effective when the scientific value of the experiment depends on ranking many conditions against one another under tightly shared analytical conditions, such as time series, dose series, or multigroup clinical discovery sets [46, 47].

The historical roots of multiplexed isobaric tagging lie in the desire to preserve the comparative strength of pooled measurement while avoiding the low throughput of serial single-sample runs. Early tandem-mass-tag and related reporter-ion strategies changed proteomics because they transformed multiplexing from a specialized trick into a core design principle for comparative biology [15, 8]. Their continued importance reflects the fact that modern biological studies increasingly ask comparative questions over many states rather than binary comparisons over a few.

The method's power lies in pooled analytical comparison, but that power comes with a known cost. Reporter-ion quantification is susceptible to co-isolation interference and ratio compression, meaning multiplexing performance depends strongly on isolation strategy, sample complexity, and data filtering [8]. In practice, this means TMT is best used when the design benefits of multiplexing outweigh the costs of a more interference-sensitive quantification model [8].

1.5.3 SILAC and metabolic labeling

SILAC and related metabolic-labeling approaches are chosen when the biology is fundamentally dynamic and the investigator needs to distinguish protein synthesis, degradation, or turnover rather than merely endpoint abundance [2]. By incorporating isotopically labeled amino acids into proteins during growth, these workflows allow direct comparative analysis of labeled and unlabeled peptide populations [2]. SILAC is especially useful in signaling and mechanism-of-action studies because it converts proteomics from a static census into a dynamic response measurement [2].

Historically, SILAC was one of the major steps by which proteomics escaped purely descriptive abundance logic and became capable of tracking flux, turnover, and state transitions. Its intellectual importance exceeds its sample-class limitations because it helped establish that proteomics could be a dynamic science of protein behavior rather than only a snapshot science of protein presence [2].

The key strength of metabolic labeling is interpretive. A protein abundance change can arise from altered synthesis, altered degradation, or both, and label-free snapshots often cannot cleanly separate those possibilities [2]. SILAC-class methods are valuable precisely because they tie protein measurement more directly to proteome flux and remodeling [2]. Their limitation is that they are best suited to experimentally labelable systems rather than arbitrary clinical specimens [2].

1.5.4 Targeted quantitation and verification workflows

Targeted proteomics becomes necessary when discovery candidates must be converted into reproducible, narrower assays suitable for verification, biomarker follow-up, or pharmacodynamic monitoring [9, 2]. SRM, MRM, PRM, and related guided approaches deliberately sacrifice proteome breadth in exchange for specificity, quantitative discipline, and the ability to focus on predefined analytes with internal standards or controlled transitions [9]. They are chosen not because they are "better discovery," but because they are better measurement tools once the discovery question has narrowed [2].

This is the bridge between candidate generation and translational utility. Many discovery studies fail to produce downstream impact because they identify too many candidates too loosely. Targeted quantitation imposes analytical narrowing and makes smaller numbers of claims more rigorously testable [9, 13]. In biomarker work and clinical follow-up, that narrowing is often exactly what is needed [9]. It also creates a practical decision boundary in proteomics programs: once the question is no longer “what changes?” but “can we measure this specific thing reliably enough to act on it?”, targeted methods usually become more appropriate than broad discovery runs [48, 49].

1.5.5 Mechanism-of-action and perturbational proteomics

Proteomics is especially powerful in perturbational settings because many drugs and biological interventions exert functionally important effects at the protein, PTM, or network level rather than solely at the transcript level [14, 2]. Mechanism-of-action proteomics therefore combines quantitative profiling with pathway or state interpretation to identify how a perturbation reshapes the proteome as a system [14]. Its strength lies in revealing functional consequence rather than only nominal target presence; its main challenge lies in distinguishing target-proximal effects from downstream compensatory changes [14].

This is one reason why mechanism-of-action studies often combine multiple proteomic regimes rather than relying on one. A compound may alter bulk abundance, phosphosite state, interaction architecture, or proteoform balance simultaneously, and no single analytical layer fully captures that complexity [50, 6, 3]. Perturbational proteomics is therefore less a single method than a design philosophy for reading functional response [14, 2].

1.5.6 Statistical rigor, cohort structure, and why quantitative design fails

The technical machinery of quantitative proteomics is only as good as the experimental design it serves [37, 2]. Cohort imbalance, missingness, preanalytical variability, sample pooling errors, and inappropriate normalization can all distort otherwise sophisticated workflows [37, 51]. This is why quantitative proteomics increasingly depends on integrated statistical frameworks, batch-aware modeling, and explicit quality-control logic rather than on instrument output alone [37]. In a mature translational setting, quantitative proteomics succeeds when acquisition chemistry, cohort structure, and statistical modeling are designed together rather than sequentially [13, 2].

1.5.7 Representative high-impact quantitative and perturbational use cases

The most instructive quantitative studies are those in which method choice clearly served the biological question. TMT-based phosphoproteomics and multiplexed cohort designs have become influential because they allow many signaling states to be compared in one analytical frame, making them well suited for comparative oncology and pathway-response experiments where sample-to-sample consistency is critical [46, 47]. Related work in plasma proteomics and multi-condition disease studies illustrates how multiplexed or carefully normalized quantitative designs can support clinically structured comparisons that would be difficult to stabilize through ad hoc label-free analysis alone [51, 52].

Targeted quantitative proteomics shows its value most clearly in verification and clinical-adjacent studies. Urinary biomarker discovery in preterm infants, perioperative bleeding-risk studies, and endocrine-metabolic intervention work all demonstrate the logic of narrowing broad candidates into more disciplined analyte panels once the biological question becomes measurement-oriented rather than exploratory [53, 54, 55]. Similarly, biomarker verification in oncology and related translational settings has repeatedly depended on targeted designs because the clinical question demands tighter measurement control than a broad discovery workflow alone can provide [56, 57].

Mechanism-of-action and perturbational proteomics gains force when abundance and regulatory-state data are interpreted together. Drug-response studies, pathway-remodeling experiments, and systems pharmacology designs increasingly use a combination of quantitative abundance profiling, PTM-state analysis, and sometimes interaction mapping to separate target-proximal effects from downstream adaptation [14, 50]. The broader lesson is that quantitative proteomics is most powerful when it functions as an evidentiary bridge between discovery and mechanism rather than as a numerically improved version of discovery alone.

1.5.8 Common failure modes in quantitative proteomics

Quantitative proteomics most commonly fails when technical precision is mistaken for inferential validity. A highly reproducible assay can still answer the wrong question if the cohort is poorly constructed, the normalization scheme is inappropriate, or the analyte panel reflects discovery noise rather than biologically stable targets [37, 13]. In TMT workflows, multiplexing can tempt investigators into overconfidence when ratio compression and co-isolation interference remain active distortions [8]. In label-free workflows, statistical flexibility can become a liability if batch effects, missingness, or preanalytical heterogeneity are under-modeled [37].

Targeted verification has its own failure mode: narrowing too early to a candidate set that was never stable enough to deserve verification in the first place. The result is a beautiful targeted assay built on a weak discovery premise. The more mature alternative is staged narrowing: broad discovery, quantitative stability testing, mechanistic contextualization where appropriate, and only then verification or deployment [9, 2].

1.5.9 Seminal transition: from differential expression to proteomic measurement science

Quantitative proteomics became a true measurement science when the field stopped treating fold-change lists as self-validating and began emphasizing calibration, cohort design, standardization, and analytic comparability [7, 13]. That shift matters historically because it marks the point where proteomics became suitable for higher-stakes translational use. The intellectual breakthrough was not simply that proteins could be measured quantitatively, but that proteomic quantitation had to be engineered as a disciplined system rather than assumed from instrument output alone.

1.5.10 Landmark quantitative use patterns and what they taught the field

Some of the most important lessons in quantitative proteomics came not from method papers alone but from the kinds of studies that methods made newly credible. Multiplexed phosphoproteomics showed that high-condition comparative designs could be executed at a scale suitable for pathway analysis rather than anecdotal signaling observation, but it also exposed how ratio compression and experimental structure could distort conclusions if not explicitly managed [46, 47, 8]. Label-free and calibrated workflows in cohort-oriented studies demonstrated that useful translational quantitation depends at least as much on normalization discipline and differential-expression modeling as on acquisition depth itself [37, 51]. Targeted verification work taught an equally important lesson from the opposite direction: when candidate space is already narrowed, assay discipline matters more than discovery breadth, and it is usually better to make a smaller number of claims with higher calibration confidence than to preserve exploratory flexibility that the study no longer needs [48, 49, 9].

Mechanism-of-action proteomics provided a different kind of education. It showed that quantitative change is not automatically mechanistic change and that the strongest perturbational interpretations usually emerge when abundance, pathway, and time-structured response data constrain one another rather than when any one layer is overread in isolation [14, 50]. In practice, this is what moved quantitative proteomics beyond "differential expression with proteins" into a more serious explanatory science. The field learned that measurement precision is valuable not as an aesthetic achievement, but because mechanistic claims become brittle when the quantitative substrate underneath them is poorly disciplined.

1.5.11 Quantitative controversies: relative confidence, absolute meaning

Quantitative proteomics still carries a tension between what is analytically repeatable and what is biologically interpretable. Relative quantitation can be exceptionally stable and still leave open the question of whether a measured difference has any transferable meaning outside the immediate cohort, platform, or preparation regime that produced it [7, 13]. This is why the field repeatedly returns to debates about calibration, comparability, and standardization: not because relative quantitation is weak, but because translational programs often demand a more portable kind of truth than relative ranking alone can provide [13, 2].

There is also a deeper epistemic issue. Highly optimized quantitative pipelines can create a false sense that numerical precision and biological specificity naturally rise together. They do not. One can measure the wrong analyte, the wrong peptide surrogate, or the wrong cohort contrast with great reproducibility [37, 2]. The best quantitative proteomics papers therefore treat statistics, calibration, and assay design as protections against self-deception rather than as mere technical polish. That stance is part of what differentiates measurement science from numerically elaborate pattern reporting.

1.6 Protein Interaction, Affinity Enrichment, and Proximity Labeling Proteomics

Interaction-centered proteomics was developed because abundance-only workflows cannot reliably answer questions about context, assembly, local protein environment, or target engagement [6, 11]. Many of the most important biological problems are relational rather than merely quantitative: which proteins bind, which complexes assemble, which transient interactions appear under stress, and which proteins enter the local neighborhood of a signaling node or organelle [6]. Interaction proteomics addresses those questions by imposing biochemical or enzymatic selectivity before mass spectrometric readout [58, 11]. The conceptual difference between these interaction strategies is summarized in Figure 4.

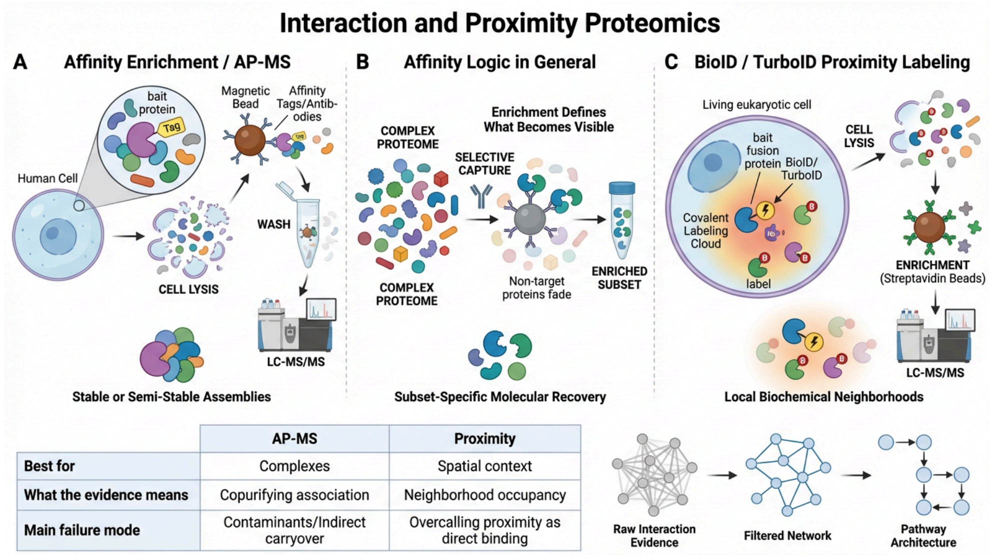

Figure 4. Interaction and proximity proteomics.

Figure 4. Interaction and proximity proteomics. Comparison of affinity enrichment, AP-MS, and proximity-labeling approaches such as BioID and TurboID. Stable complex capture, local neighborhood labeling, and systems-level interactome interpretation are distinguished as different evidentiary modes rather than mere technical variants.

1.6.1 Affinity enrichment and AP-MS

Affinity enrichment is one of the central architectural moves in interaction proteomics because it reduces complexity by selectively capturing a biologically meaningful subset of molecules before MS analysis [58]. In AP-MS, a bait protein or complex is isolated under controlled conditions and associated proteins are identified by LC-MS/MS, allowing reconstruction of interaction neighborhoods and pathway assemblies [6, 23]. AP-MS is chosen when the question concerns stable or semi-stable assemblies, but it requires strong false-positive control because sensitive instruments do not distinguish automatically between true interactors and proteins that copurify nonspecifically [23]. Quantitative control strategies and careful experimental design are therefore essential [23].

The historical lineage of AP-MS comes from classical biochemistry and complex purification, but mass spectrometry transformed those traditions by letting investigators identify associated proteins at scale rather than one at a time. The method became especially important once researchers realized that the problem was no longer only complex recovery, but also false-positive control in an era of very sensitive detection [23, 6]. That tension still defines the field today.

Its main value is that it makes physical association analytically tractable at proteome scale. Its main weakness is that preservation and purification are both imperfect: harsh conditions can eliminate biologically real weak interactions, while permissive conditions can increase nonspecific recovery [6, 23]. Good AP-MS design is therefore as much about choosing the right biochemical compromise as it is about mass spectrometric depth [6].

1.6.2 Proximity labeling

Proximity labeling methods such as BioID and TurboID address a different problem: many biologically important associations are too transient, weak, or spatially restricted to survive conventional purification [11]. By enzymatically labeling proteins that come into the bait's local environment in living cells, these methods allow investigators to capture local interactomes and organellar neighborhoods that are difficult to preserve biochemically [11]. They are chosen when spatial or transient context matters more than classical stable pull-down logic, especially in membrane biology, organelle proteomics, and signaling microenvironments [11].

Historically, proximity labeling became important when researchers working on membrane systems, organelles, and transient signaling recognized that many biologically relevant interactions were being systematically lost by purification-based methods. BioID and later faster-labeling variants changed the problem definition from "what survives biochemistry?" to "what existed near the bait in the living cell?" [11]. That was a conceptual advance as much as a technical one.

The conceptual shift here is important. Proximity labeling is not asking exactly the same question as AP-MS. AP-MS asks what remained physically associated through purification. Proximity labeling asks what occupied the bait's local biochemical neighborhood in the living system [11]. That makes it especially useful when microenvironment, topology, and transient signaling geography matter as much as stable binding [11].

1.6.3 Interaction proteomics as systems biology

Recent work increasingly frames interaction proteomics as part of systems biology rather than as a niche specialty because interaction state often defines pathway logic more directly than bulk abundance does [6, 59]. Interaction data can be layered with abundance proteomics, phosphoproteomics, or perturbation designs to reveal how networks are rewired by disease, stress, or therapeutic intervention [6, 59]. Its importance therefore lies not just in naming interactors, but in turning protein lists into functional network models [6].

1.6.4 Choosing between AP-MS and proximity labeling

One of the most important practical distinctions in interaction proteomics is whether the investigator needs evidence of stable physical association or evidence of local biochemical neighborhood [6, 11]. AP-MS is generally better aligned to the former, particularly when the goal is to recover relatively persistent assemblies under biochemically preserved conditions [23, 6]. Proximity labeling is better aligned to the latter, especially when the relevant biology is transient, membrane-proximal, spatially constrained, or difficult to preserve through purification [11, 60]. In practice, many high-value interaction studies use both logics serially or comparatively, because the strongest network interpretations are often those supported by more than one interaction modality [6, 11].

1.6.5 Representative high-impact interaction-proteomics use cases

The biological value of interaction proteomics is most obvious when it resolves organizational questions that abundance profiling cannot answer. Recent proximity-labeling studies have been used to define unique subcellular proteomes, deepen neuronal and glial proteomic context, and map receptor-proximal neighborhoods that are difficult to preserve through conventional purification [61, 62, 60]. These studies show that local proteomic geography can itself be biologically informative, especially in signaling and membrane contexts where neighborhood is part of mechanism.

AP-MS and affinity-centered designs remain particularly important when the question concerns more stable complexes or higher-confidence interaction assemblies. Improvements in false-positive control, high-throughput interactome logic, and affinity workflow design have made these methods increasingly useful for converting complex pull-down outputs into pathway models and functional modules rather than mere interactor lists [6, 23, 58]. In practice, the high-impact contribution of interaction proteomics is not only that it names associated proteins, but that it reveals how protein function is embedded in relational architecture.

1.6.6 Common failure modes in interaction proteomics

Interaction proteomics often fails when investigators overclaim the meaning of proximity or purification. AP-MS can overstate specificity when contaminants or indirect associations are insufficiently controlled, while proximity labeling can be misread as direct binding when it really reports local neighborhood occupancy [23, 11]. Another failure mode is to treat interaction lists as conclusions rather than as structured evidence requiring network interpretation, perturbational comparison, and orthogonal context [6, 59].

The central discipline in this area is definitional honesty. If the method captures stable copurification, it should be interpreted as such. If it captures local proximity, that is a different but equally valuable evidentiary statement. Much confusion in the literature comes from blurring these categories rather than from the methods themselves [11, 6].

1.6.7 Seminal transition: from complexes to neighborhoods

Historically, interaction proteomics first matured around stable complex recovery and only later expanded into neighborhood-aware logic through proximity labeling. That transition matters because it widened the field's effective target space from "what remains attached after purification?" to "what shares local biochemical territory with the bait in living cells?" [11, 6]. It represents a conceptual broadening of what counts as interaction evidence, not just a technical refinement.

1.6.8 Landmark interaction case studies and their methodological lessons

The interaction-proteomics literature is especially instructive because landmark studies often succeed by being methodologically modest about what their evidence actually means. High-throughput AP-MS programs demonstrated that large interaction maps are useful only when they are paired with contaminant control, comparative context, and network logic capable of distinguishing constitutive architecture from condition-specific rewiring [6, 23]. Proximity-labeling studies of receptor neighborhoods, organelle-localized proteomes, and cell-type-specific subcellular environments taught a complementary lesson: local proteomic geography can be biologically decisive even when direct stable binding is not the right abstraction for the question [60, 61, 62].

These studies mattered because they corrected a recurring simplification in molecular biology: the idea that interaction can be represented adequately as a yes-or-no edge between two proteins. Interaction proteomics instead forced the field to recognize a spectrum ranging from stable physical assemblies to dynamic spatial neighborhoods, each of which may be the correct explanatory unit depending on the biology [11, 6]. The methodological lesson is not merely to pick a tool, but to decide what kind of relational claim the biology justifies and then choose the assay whose evidentiary semantics match that claim.

1.6.9 Interaction controversies: what counts as an interaction?

Interaction proteomics remains one of the clearest areas where the field's language can outrun its evidence. A purified complex is not the same thing as a direct binary interaction. A proximity-labeled protein is not the same thing as a stable binding partner. A reproducible neighborhood is not the same thing as a mechanistically privileged edge in a pathway model [23, 11, 6]. Yet the literature often compresses these distinctions under the single word "interaction," which can make very different evidentiary claims appear more interchangeable than they really are.

The strongest work in this area succeeds by narrowing the claim to the method rather than stretching the method to the claim. AP-MS is strongest when it says something disciplined about copurifying assemblies under explicit biochemical conditions [23, 6]. Proximity labeling is strongest when it maps local context or spatially constrained proteomic environments rather than implying direct binding where none was actually tested [11, 60]. The controversy, then, is not whether one method is better than the other. It is whether the field is willing to let method semantics constrain biological rhetoric. That willingness is one of the best indicators of maturity in interaction proteomics.

1.7 PTM-Centric Proteomics

PTM-centered proteomics is indispensable because proteins are often regulated more directly by chemical state than by total abundance [10, 50]. Phosphorylation, glycosylation, ubiquitination, oxidation, nitrosylation, and many other modifications alter signaling, localization, activity, turnover, and interaction state across essentially all areas of biology [10]. PTM proteomics exists to make these functionally decisive states experimentally accessible. The logic of PTM-state analysis and enrichment is summarized in Figure 5.

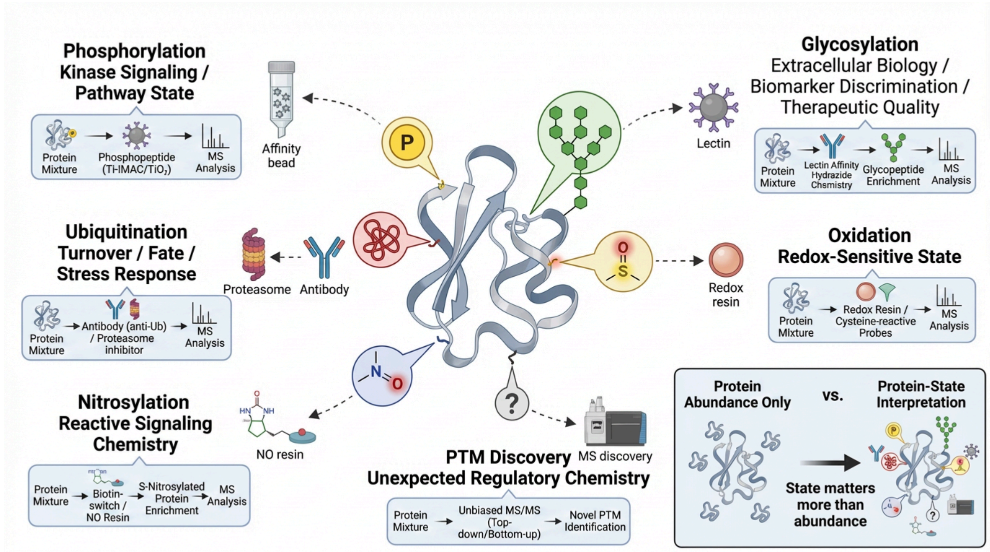

Figure 5. PTM-centric proteomics.

Figure 5. PTM-centric proteomics. Overview of phosphorylation, glycosylation, ubiquitination, oxidation, nitrosylation, and broader PTM discovery workflows. The figure emphasizes selective enrichment, regulatory interpretation, and the transition from PTM listing to mechanism.

The main analytical challenge is that modified peptides are usually rare, chemically diverse, and embedded in overwhelming unmodified background [10]. As a result, PTM proteomics is often an enrichment problem before it is a detection problem. The method succeeds when the enrichment chemistry, chromatographic behavior, and computational interpretation are well matched to the modification class in question [10, 63].

1.7.1 Phosphoproteomics

Phosphoproteomics is one of the most influential PTM workflows because phosphorylation encodes kinase activity, signaling state, and pathway response in disease and normal physiology [50, 64]. It typically requires selective phosphopeptide enrichment followed by quantitative MS and increasingly sophisticated computational analysis of phosphosite-level networks [10, 50]. Investigators choose phosphoproteomics when the goal is to understand pathway activation, treatment response, or kinase-network remodeling, especially in oncology and signaling biology [64, 65].

Phosphoproteomics has a particularly deep historical significance because it represents the moment when proteomics moved into mainstream signaling biology. Before large-scale phosphoproteomics, phosphorylation was typically studied site by site or pathway by pathway. The advent of enrichment-driven phosphoproteomics made it possible to observe signaling architectures at system scale, which fundamentally altered how pathway biology could be studied [21, 22, 50].

The biological importance of phosphoproteomics lies in its immediacy. Phosphorylation often changes more rapidly and more directly than total abundance, making phosphosite data particularly valuable in perturbational studies and mechanism-of-action analysis [50, 64]. This is one reason why phosphoproteomics is frequently paired with quantitative and perturbational designs rather than used as a standalone descriptive layer [50].

1.7.2 Glycoproteomics and N-glycosylation mapping

Glycoproteomics is chosen when investigators need to understand extracellular biology, immune recognition, secreted or membrane-associated proteins, or therapeutic protein quality attributes [63]. Because glycans influence folding, trafficking, and functional recognition, glycopeptide and glycoprotein mapping is central to biomarker discovery, vaccine work, and biopharmaceutical characterization [63]. The analytical burden is substantial because glycopeptides are heterogeneous and often low-abundance, which is why selective enrichment chemistries remain central to the field [63].

The historical development of glycoproteomics reflects the meeting of proteomics with glycobiology, a domain whose chemical complexity long resisted simple large-scale MS analysis. As enrichment materials, fragmentation strategies, and computational interpretation improved, glycoproteomics moved from specialized methodological niche toward a central translational tool, especially in extracellular biology and therapeutic characterization [66, 67, 63].

The translational importance of glycoproteomics is unusually high because glycans affect both disease-state biology and therapeutic performance [63]. In this sense, glycoproteomics bridges mechanistic biology and product characterization more directly than many other proteomic subfields do [63]. This dual relevance is one reason glycoproteomics often appears both in disease-biomarker programs and in therapeutic-protein quality assessment, even though those are otherwise quite different use cases [68, 69].

1.7.3 Ubiquitination, oxidation, nitrosylation, and PTM discovery

Ubiquitination proteomics matters because protein degradation, trafficking, and stress response are heavily governed by the ubiquitin system [10]. Oxidation and nitrosylation proteomics matter because redox state influences signaling, damage response, and disease-associated chemistry, but these measurements are particularly sensitive to handling artifacts and stabilization challenges [70]. PTM discovery methods, including open or differential spectral strategies, extend this work by identifying unexpected modifications that conventional predefined search spaces would miss [71, 72, 73]. This shift from PTM confirmation toward PTM discovery is important because it acknowledges that the proteome’s chemically functional landscape is richer than most targeted search pipelines assume [72].

This discovery logic is especially important in disease biology, chemical biology, and stress physiology, where noncanonical or poorly anticipated modifications may carry important functional meaning [72, 73]. In that sense, PTM discovery pushes proteomics away from confirmation bias and toward broader chemical openness [71].

1.7.4 From PTM lists to regulatory mechanism

One of the hardest interpretive problems in PTM proteomics is that a long list of modified sites is not yet a mechanism [50, 10]. The field increasingly relies on network inference, pathway context, localization information, and perturbational comparison to determine which modification events are likely to be regulatory, causal, or disease-relevant [50, 65]. This is particularly obvious in phosphoproteomics, where identifying thousands of sites is now relatively routine but understanding which of those sites matter biologically remains the harder task [50]. The same principle applies to glycoproteomics and ubiquitinomics: analytical detection is necessary, but functional contextualization is what turns detection into explanatory biology [63, 10].

1.7.5 Representative high-impact PTM use cases

The impact of PTM-centric proteomics becomes clear in applications where molecular state is closer to the biology than total abundance. Phosphoproteomics has been central to cancer-signaling analysis, kinase-network interpretation, and immunotherapy-related pathway work because it captures functional regulatory transitions that often precede or exceed bulk protein-level shifts [65, 74, 64]. Noncanonical phosphorylation studies further show that the PTM landscape remains incompletely charted and that new regulatory chemistry continues to emerge when the search space is expanded thoughtfully [75].

Glycoproteomics provides a similarly strong case for PTM-specific analysis. Tissue and fluid biomarker studies, bladder-cancer detection work, and clinical cancer glycoproteomics all demonstrate that glycoform-specific information can provide disease discrimination and biological specificity that generic abundance measurements may miss [68, 76, 69]. The recurring lesson is that PTM-aware methods are often not an optional refinement; they are the only analytical layer that exposes the biology that actually matters.

1.7.6 Common failure modes in PTM proteomics

PTM proteomics often fails when enrichment efficiency is mistaken for biological completeness. Modified-peptide capture can be powerful while still being selective in ways that underrepresent important subclasses of the PTM landscape [10, 63]. Another trap is to equate the number of observed sites with mechanistic depth, when the real challenge is determining which modification events are functionally relevant and under what context they become meaningful [50, 65].

For oxidation and nitrosylation studies, analytical artifact is an especially serious concern, because the chemistry under investigation can be perturbed by sample handling itself [70]. For PTM discovery workflows, expanded search space can create false-confidence if downstream validation does not keep pace with the openness of the detection strategy [72, 73]. The discipline of PTM proteomics is therefore not just detection, but controlled interpretation under chemically and biologically complex conditions.

1.7.7 Seminal transition: from protein abundance to protein state

PTM proteomics marks one of the clearest moments when proteomics stopped being mainly a protein census and became a state-sensitive regulatory science. The key insight was that biological function often lives in modification state rather than in total protein amount, and that large-scale proteomics would remain mechanistically shallow until it could observe that state directly [21, 10]. This shift remains one of the defining conceptual achievements of the field.

1.7.8 Landmark PTM case studies and why they changed expectations

Large-scale phosphoproteomics changed expectations most dramatically in systems where pathway dynamics, therapeutic response, or oncogenic rewiring could not be inferred adequately from bulk abundance. Cancer-associated phosphosite network studies, kinase-centered inference workflows, and perturbational phosphoproteomic analyses showed that proteomics could move from descriptive regulation catalogs toward pathway-active mechanistic models with therapeutic relevance [50, 65, 74]. These studies did not matter simply because they found many sites. They mattered because they demonstrated that protein-state measurements could reorganize how signaling biology was reasoned about.

Glycoproteomics produced a parallel shift in clinically oriented fields. Tissue and fluid studies showed that disease discrimination sometimes lives more clearly in glycoform-specific patterns than in unguided protein abundance summaries, especially in extracellular and cancer-associated biology [68, 76, 69]. PTM-discovery workflows, meanwhile, taught a more destabilizing lesson: even a mature proteomics field may still be searching a space that is too narrow to capture functionally important chemistry [71, 72, 73]. Together these case studies reset the field's expectations. A "complete" proteomic analysis that ignores regulatory state is often not complete in the way biology cares about most.

1.7.9 PTM controversies: abundance-first thinking versus state-first biology

PTM proteomics continues to expose a conceptual tension in the broader field: many investigators still organize biological interpretation around protein abundance and treat PTM information as an advanced overlay rather than as a primary explanatory layer [10, 50]. Yet in signaling biology, stress adaptation, immune response, and therapeutic mechanism, regulatory state is often more proximal to function than total abundance ever will be [64, 74]. This means that abundance-first reasoning can be scientifically conservative in the worst sense: it feels stable, but it risks centering the wrong molecular variable.

The strongest PTM papers have therefore changed the field not only by adding methods, but by reversing the hierarchy of interpretation. They implicitly argue that a protein's regulatory chemistry is not a refinement of its abundance profile. In many cases it is the actual biological event of interest. That claim remains disruptive because it demands that proteomics be judged less by how comprehensively it inventories proteins and more by how faithfully it reconstructs active molecular states.

1.8 Ion Mobility, Native Mass Spectrometry, and Top-Down / Proteoform-Resolved Proteomics

Bottom-up proteomics is exceptionally powerful, but it reconstructs proteins indirectly from peptides and therefore partially collapses intact molecular state [3, 77]. Ion mobility, native mass spectrometry, and top-down proteomics were developed to restore different aspects of that missing structural information. These methods matter because the biologically relevant molecular entity is often not the abstract protein name, but the proteoform, assembly, or conformational state [3, 1]. Their complementary roles are summarized in Figure 6.

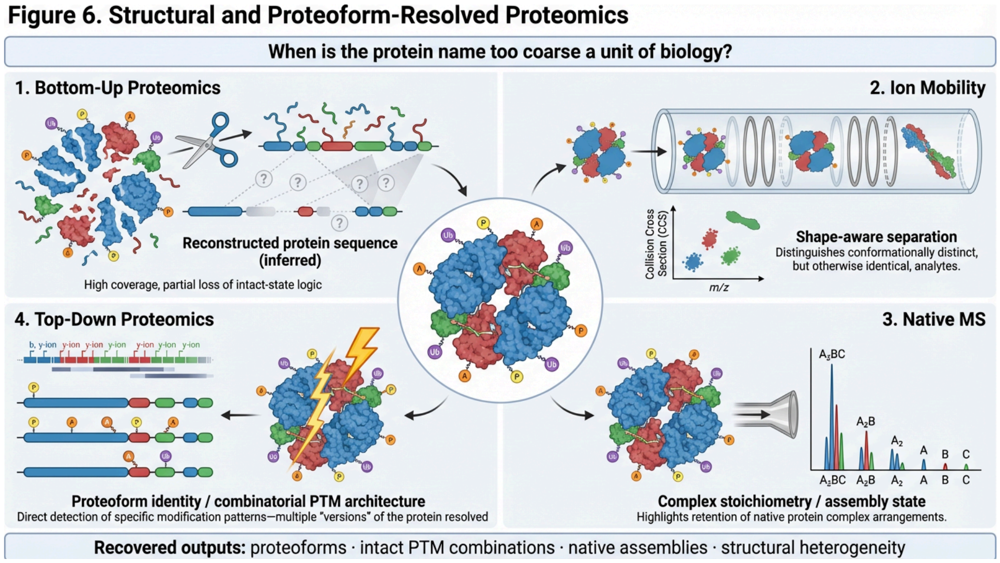

Figure 6. Structural and proteoform-resolved proteomics.

Figure 6. Structural and proteoform-resolved proteomics. Conceptual relationship between ion mobility, native mass spectrometry, and top-down proteomics. The figure illustrates what bottom-up workflows compress and what intact-state methods recover, including conformational separation, complex stoichiometry, and proteoform-specific information.

1.8.1 Ion mobility mass spectrometry

Ion mobility adds an orthogonal separation dimension by discriminating ions according to gas-phase mobility, which reflects size, shape, and charge state as well as m/z [12]. It is chosen when analyte overlap or structural ambiguity limits interpretability in conventional LC-MS workflows and is particularly useful in deep discovery and proteoform-aware analyses [12, 77]. Its value lies in resolving crowded analytical spaces in ways that m/z-only separation cannot [12].

1.8.2 Native mass spectrometry

Native MS preserves aspects of noncovalent assembly and intact complex state that denaturing peptide-centric workflows disrupt [17, 77]. It is chosen when stoichiometry, ligand association, or complex architecture is itself the central biological question [17]. Its value is structural fidelity; its cost is technical complexity and lower general throughput compared with standard bottom-up workflows [17].

1.8.3 Top-down proteomics and proteoforms

Top-down proteomics analyzes intact proteins directly, allowing detection of truncations, sequence variants, combinatorial PTM states, and other proteoform-level features that bottom-up inference often compresses or fragments apart [3, 77]. It is chosen when intact molecular identity matters, including signaling proteoforms, therapeutic-protein characterization, and studies where multiple protein states coexist under one gene product label [3]. These methods are analytically demanding, but they restore the actual functional molecular unit of biology: the proteoform rather than the abstract protein name [3, 1].

Historically, top-down proteomics emerged as a corrective to the success of bottom-up proteomics. As peptide-centric approaches became dominant, it became increasingly clear that some of the most important molecular information was being inferred only indirectly or lost entirely through digestion. Top-down methods therefore developed not in opposition to bottom-up proteomics, but as a response to its epistemic limits [24, 3]. The modern proteoform concept crystallized that concern into a field-level agenda.

The broader point is that structural and proteoform-aware workflows solve a real epistemic problem in proteomics. They reduce the gap between the molecular form that exists in the sample and the molecular abstraction reported in the output [3, 77]. That is why these methods matter even if they are not always the highest-throughput option [3].

1.8.4 Proteoforms, therapeutic characterization, and the next maturity step for proteomics

Proteoform-aware analysis is one of the clearest signs that proteomics is maturing beyond abundance-centric thinking [1, 3]. In therapeutic characterization, for example, intact-state heterogeneity is not a minor detail but often the core analytical concern, because truncations, glycoforms, assembly variants, or by-products can directly affect function, safety, or manufacturability [78, 79]. In disease biology, proteoform logic matters because two molecular species produced from the same gene may participate in very different regulatory or pathological states [3, 77]. This is why top-down and related structural workflows are increasingly important not just as specialist methods, but as signals of where the whole field is headed conceptually.

1.8.5 Representative high-impact structural and proteoform use cases

The importance of structural and proteoform-resolved proteomics is particularly clear in applications where intact state is inseparable from interpretation. Nanomedicine studies of protein corona composition, bispecific-antibody characterization, and glycoprotein heterogeneity analyses all show that proteoform-aware workflows can reveal manufacturing by-products, intact molecular diversity, and combinatorial structural states that would be difficult to reconstruct from peptide-centric readouts alone [80, 78, 79]. In disease biology, proteoform-sensitive studies in muscle disease and related systems indicate that intact-state characterization can illuminate functional molecular phenotypes that are otherwise partly hidden behind gene-centric labels [81, 3].

These use cases reinforce a broader point: structural proteomics is not only about elegance or completeness. It is about analytical honesty. When biological or therapeutic function depends on intact molecular form, a workflow that collapses that form too early may be answering a simpler question than the one the biology is actually asking.

1.8.6 Common failure modes in structural and proteoform-resolved proteomics

Structural and top-down proteomics can fail by overpromising generality. These methods are uniquely powerful for intact-state questions, but they are often lower throughput, more technically delicate, and less universally deployable than peptide-centric workflows [3, 77]. Another common trap is to invoke proteoform language without truly measuring proteoforms, instead inferring intact-state claims from fragmented evidence that does not actually preserve the relevant combinatorial molecular structure [3].

The stronger path is to be explicit about what level of molecular state was truly observed: peptide, site, domain, intact proteoform, or native complex. This may sound obvious, but much of the confusion in structural proteomics arises when these levels are rhetorically blurred in ways that exceed the evidence actually generated [3, 17].

1.8.7 Seminal transition: from proteins to proteoforms

The move from protein-centric language to proteoform-centric language is historically one of the field's most profound conceptual upgrades. It acknowledged that many biologically important molecular entities are not well represented by a single gene-product label and that intact-state diversity is often functionally decisive [3, 1]. Top-down and related structural methods gave that realization analytical form. Whether or not they become the dominant workflow class, they have already changed how the field thinks.

1.8.8 Landmark proteoform and structural case studies

The value of structural and proteoform-aware proteomics becomes clearest in contexts where peptide-level summaries are not just incomplete but actively misleading. Therapeutic-protein characterization studies have shown that intact-state analysis can reveal exchange by-products, glycoform heterogeneity, and assembly variation that would be difficult to reconstruct confidently from bottom-up fragments alone [78, 79]. Protein-corona and nanomedicine-oriented analyses have likewise demonstrated that structurally aware workflows can expose heterogeneity in adsorbed protein states that matters for biological interpretation and material behavior, not merely for analytical elegance [80, 77].

Disease-centered work reinforces the same point from another direction. When proteoform diversity shapes the phenotype, a gene-centric protein label can become too coarse to support explanation, and the analytical question must move upward toward intact molecular state rather than downward toward more peptide fragments [81, 3]. Landmark structural studies therefore changed the field less by promising universal replacement of bottom-up proteomics than by proving that some of the most important biological and translational questions were being asked at the wrong level of molecular abstraction.

1.8.9 Why structural proteomics still feels "future tense" and why that is misleading

Structural and proteoform-resolved proteomics is still often described as if it belongs mainly to the future of the field, while bottom-up proteomics is treated as the fully realized present. That framing is misleading [3, 77]. The real issue is not temporal maturity but analytical fit. In many therapeutic-characterization and proteoform-centric disease questions, intact-state methods are already the correct present-tense tools because peptide-centric compression answers a systematically simpler question than the biology is asking [78, 79, 81].

What gives structural proteomics its "future" reputation is partly operational: lower throughput, higher technical burden, and narrower deployment compared with mainstream bottom-up workflows [3]. But that should not be confused with scientific optionality. The strongest structural papers already prove that when intact molecular form is functionally decisive, abstraction to the generic protein level is not merely incomplete.

It can be categorically misleading. In that sense, structural proteomics is not waiting to become relevant. It is already relevant wherever molecular integrity, assembly, or proteoform identity is the real explanatory unit.

1.9 Translational Synthesis and Practical Framework for Method Selection

The major proteomics technologies matter together because real biological and translational questions rarely fit cleanly into only one analytical lane [1, 2]. A plasma biomarker study may require nanoparticle enrichment for discovery, DIA for cohort-scale reproducibility, and targeted verification for follow-up [28, 5, 9]. A therapeutic mechanism study may require quantitative abundance profiling, phosphoproteomics, AP-MS or proximity labeling, and perhaps proteoform-aware analysis if intact molecular state shapes function [50, 6, 3]. This is why contemporary proteomics is best understood as a modular analytical architecture in which the right technology is chosen because it resolves the dominant evidentiary bottleneck of the biological problem at hand [1]. The integrative logic of this modular architecture is summarized in Figure 7.

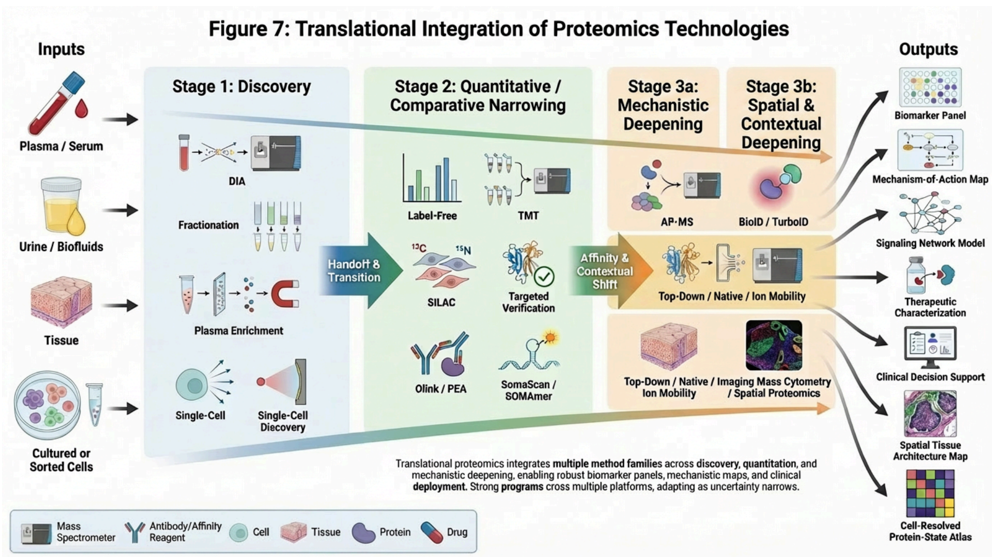

Figure 7. Translational integration of proteomics technologies.

Figure 7. Translational integration of proteomics technologies. A practical workflow map showing how discovery, quantitative, interaction, PTM, structural, affinity-panel, and spatial methods may be combined in biomarker discovery, mechanism-of-action studies, therapeutic characterization, and translational research programs.

From a practical standpoint, method choice can be organized around a small set of primary questions. If the priority is broad reproducible profiling across many samples, DIA or similarly cohort-friendly discovery methods are usually appropriate [4, 5]. If the priority is quantitative comparability across many conditions in one experiment, TMT or other multiplexing regimes may be preferable [8]. If the priority is dynamic turnover or biosynthetic remodeling, SILAC-class approaches become attractive [2]. If the priority is pathway state, phosphoproteomics or other PTM-centered workflows are usually necessary [50]. If the priority is context or assembly, AP-MS or proximity labeling may be more informative than bulk abundance change [6, 11]. If the priority is intact molecular state, top-down or native methods may be required despite their greater technical burden [3, 17].

Another useful framing is to ask where irreducible uncertainty resides in the experiment. If uncertainty is mostly about what is there, discovery methods dominate. If uncertainty is about whether a signal is quantitatively robust, quantitative and targeted methods dominate. If uncertainty is about where the protein is acting or with whom it is associated, interaction methods dominate. If uncertainty is about regulatory state, PTM methods dominate. If uncertainty is about molecular form, structural and proteoform-aware methods dominate. This is a simple heuristic, but it captures why method choice in proteomics is really evidence-design logic rather than instrument preference alone.

The same framework is useful in disputes about capability or analytical scope. A serious proteomics platform should not be described simply by the list of technologies it can nominally access. It should be described by the classes of questions it can answer, the evidentiary limitations it can overcome, and the kinds of translational or mechanistic claims it can support responsibly. That is a higher bar than "having the workflow," but it is the bar that matters when proteomics is being used to drive real biological or strategic decisions.

1.9.1 Comparative decision matrix

The following matrix summarizes the dominant use logic of the major proteomics method families discussed in this review.

Dominant question	Primary method families	Core strength	Principal liability
What is broadly present across many complex samples?	DIA, multidimensional fractionation, biofluid enrichment	Breadth plus cohort-scale discovery	Coverage can be mistaken for stability
Can we compare many states precisely?	TMT, label-free quantitative proteomics, calibrated quantitation	Comparative and translational measurement	Statistical and design fragility
How does the proteome change over time or under perturbation?	SILAC, perturbational profiling, phosphoproteomics	Dynamic and mechanistic response readout	Interpretation can outrun causality
Which proteins interact or share local context?	AP-MS, affinity enrichment, BioID, TurboID	Context and network architecture	Proximity and purification can be overclaimed
Which regulatory states matter?	PTM proteomics, phospho/glyco/ubiquitin workflows, PTM discovery	Direct access to protein-state biology	Enrichment bias and mechanistic overinterpretation
Which intact molecular forms are present?	Top-down, native MS, ion mobility, proteoform analysis	Structural and intact-state fidelity	Lower throughput and greater technical burden

This matrix is intentionally reductive, but it captures the central design intuition of the field: method choice is best driven by the dominant uncertainty of the question, not by prestige hierarchy or instrument novelty.

1.9.2 Canonical multi-method workflow archetypes

Another way to understand the field is to examine the recurrent workflow archetypes that high-performing proteomics programs use in practice [1, 2]. A biomarker-discovery archetype often begins with clinically realistic matrices such as plasma or urine, uses enrichment or careful front-end preparation to make those matrices tractable, applies DIA or another reproducible discovery workflow to build a broad candidate space, and then transitions into targeted verification once the candidate list has stabilized statistically and biologically [28, 27, 5, 9]. A mechanism-of-action archetype often begins with quantitative perturbational profiling, then adds phosphoproteomics or other PTM-state layers to expose pathway response, and finally incorporates interaction or proteoform-aware readouts when abundance and signaling changes alone do not localize the mechanism sufficiently [50, 14, 6, 3]. A therapeutic-characterization archetype usually inverts that order by beginning from structural and intact-state questions, then using targeted or orthogonal peptide-level methods for confirmation and release-oriented robustness rather than discovery [78, 79, 3].

These archetypes are useful because they shift attention away from isolated technique ownership and toward program design. Most serious disputes in proteomics are not actually about whether a method exists. They are about whether the method was deployed at the right stage, on the right sample type, against the right uncertainty, with an adequate bridge into the next evidentiary regime. The strongest platforms are not those that can perform the largest number of assays in parallel, but those that can move cleanly from one evidentiary layer to another without losing traceability, calibration, or biological meaning [1, 2, 13].

1.9.3 How to read Figures 1 through 9 as one system

The figures in this review are intended to function as an integrated visual argument rather than as decorative sectional summaries. Figure 1 establishes the analytical problem space of the field as a whole, while Figures 2 through 6 unpack the five major method families one evidentiary regime at a time. Figure 7 then shows how those families are recombined in real translational programs instead of being used as isolated endpoints. Figure 8 adds the missing temporal dimension by showing how the field's conceptual center of gravity shifted from identification to quantitation to state, context, and proteoform logic. Figure 9 closes the loop by converting that history and method inventory into an evidence-design workflow for actual project planning.

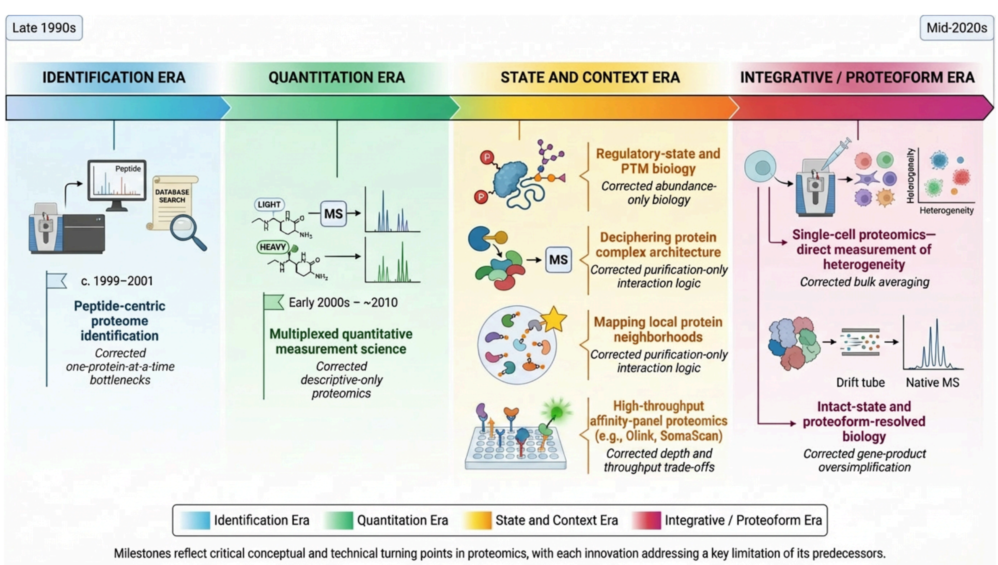

Figure 8. Historical evolution of the proteomics field.

Figure 8. Historical evolution of the proteomics field. A chronology-style figure showing the major conceptual transitions in proteomics: identification-era peptide-centric proteomics, quantitative or multiplexed measurement, PTM-centered regulatory-state analysis, interaction and proximity mapping, reproducible cohort-scale discovery, high-throughput affinity-panel proteomics, single-cell heterogeneity, and proteoform or native-state resolution. The emphasis is on how each transition arose to correct a specific blind spot in the prior dominant readout.

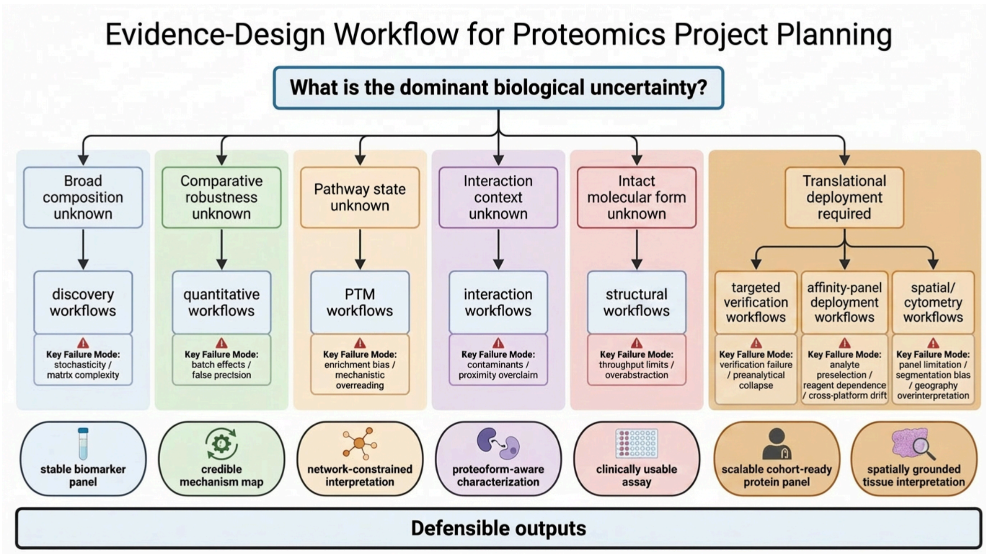

Figure 9. Evidence-design workflow for proteomics project planning.

Figure 9. Evidence-design workflow for proteomics project planning. A decision-oriented figure that begins with biological uncertainty and routes the investigator through the primary choice points of the field: discovery breadth, quantitative comparability, pathway-state analysis, interaction context, intact-state structure, verification discipline, and translational deployment. The figure makes explicit that method choice is driven by evidentiary bottleneck rather than by instrument novelty alone.

This progression matters because the manuscript's central claim is not simply that proteomics contains many useful tools. It is that the field has matured into a layered system for resolving different kinds of biological uncertainty. The figures are therefore meant to teach the reader how to think across regimes: from what can be discovered, to what can be measured, to what can be mechanistically localized, to what can be preserved structurally, and finally to what can be defended translationally. Read together, they are intended to function as a compact visual operating system for the manuscript rather than as a set of disconnected review panels.

1.9.4 Complementary affinity-based high-multiplex proteomics: Olink, SomaLogic, and the non-MS deployment layer

Any contemporary review that aims to be genuinely comprehensive must account explicitly for the rise of high-multiplex affinity-based proteomics platforms such as Olink proximity extension assay systems and SomaLogic/SomaScan aptamer-based platforms. These technologies occupy an important position in the field because they solve a different practical problem than classical discovery proteomics: not how to recover the deepest possible analyte space from first principles, but how to measure large protein panels reproducibly across substantial cohorts using comparatively small sample volumes and highly standardized workflows [18, 82, 83]. In that sense, they belong to the deployment and scalable-cohort layer of proteomics even when they are also used for exploratory biology.

Olink's proximity extension assay logic relies on paired antibody probes whose spatial colocalization on the target enables DNA-based readout, thereby combining immuno-recognition with multiplexed nucleic-acid quantification [82, 84]. This architecture has made Olink especially influential in plasma and serum studies where high sample throughput, small input volume, and panel reproducibility matter greatly, and it has been used both in biomarker discovery and in head-to-head comparisons with mass-spectrometry-based workflows [18, 85, 84]. The main strength of Olink-like platforms is disciplined panel-scale measurement under cohort conditions that are often operationally easier than unbiased MS discovery. The main limitation is that the analyte universe is preselected and the evidentiary substrate remains affinity-reagent dependent rather than discovery-open.

SomaLogic/SomaScan occupies a parallel but distinct lane. By using slow off-rate modified aptamers (SOMAmers) and related affinity logic, SomaScan has enabled very high multiplex protein measurement with broad panel breadth and substantial traction in biomarker-oriented and large-cohort translational studies [86, 87, 83]. It has also attracted unusually direct methodological scrutiny. Important papers on assay variability, platform caveats, and reproducibility have shown that the platform's power must be interpreted alongside issues of specificity, normalization, panel behavior, and the distinction between high-throughput signal and trustworthy analyte identity [88, 19, 89]. That scrutiny is healthy rather than embarrassing. It reflects the fact that affinity proteomics has become important enough that the field now argues seriously about its reliability standards.

The most productive way to place Olink and SomaScan is not to treat them as rivals to mass spectrometry in a simplistic winner-take-all sense. They are better understood as complementary regimes with different strengths and liabilities. Mass spectrometry remains stronger when openness, PTM awareness, proteoform reasoning, and analytical independence from predefined affinity panels are the central scientific needs [1, 3]. Olink and SomaScan are often stronger when the problem is large-scale, standardized, panel-based protein measurement in translational cohorts where throughput, reproducibility, and operational simplicity are at a premium [18, 89, 20]. Comparative studies in cerebrospinal fluid and plasma underscore that these platforms should be judged both by intra-platform reproducibility and by what kind of biological claims they can support when cross-platform agreement is only partial [20, 18].

The larger lesson is methodological pluralism with evidentiary clarity. A mature proteomics program should be able to say when affinity-based protein panels are the right tool, when MS-based discovery is indispensable, and when the two should be used in sequence rather than opposition. That is particularly important in biomarker science, where the optimal strategy is often to discover broadly using MS or multimodal workflows, then verify or scale with narrower but more operationally stable panel platforms where appropriate [9, 13, 18]. In other words, Olink and SomaScan belong in this review not as optional add-ons, but as major members of the modern proteomics ecosystem whose role becomes clearest when the field is viewed as an evidence-design system rather than a single-method competition.

1.9.5 Spatial proteomics, mass cytometry, and antibody-array lineages: adjacent regimes that matter

Proteomics also has important adjacent regimes that are sometimes omitted from review articles simply because they do not fit neatly into the classical LC-MS/MS discovery narrative. Antibody arrays and related protein-array technologies represent an older but still important lineage of multiplex protein measurement, particularly in contexts where predefined panel measurement, assay parallelism, or array-based screening is more practical than unbiased discovery [90, 91, 92]. These platforms historically helped establish the idea that protein measurement could be multiplexed at scale even before many current high-dimensional workflows matured, and they remain conceptually important because they form part of the ancestry of modern affinity-panel proteomics.

Mass cytometry and imaging mass cytometry occupy another crucial adjacent lane. By coupling antibody-based detection to metal isotopes and time-of-flight readout, CyTOF-class methods enabled high-dimensional single-cell and tissue-context protein analysis that is neither classical flow cytometry nor standard proteomics in the LC-MS sense [93, 94]. Imaging mass cytometry extends that logic into spatially resolved tissue architecture, making it possible to study immune organization, cellular neighborhoods, and histologic context with multiplex protein readouts embedded directly in tissue space [95, 96]. These methods matter because they solve a different problem than bulk molecular profiling: not simply what proteins are present, but where protein-marked cell states are located and how they are organized spatially.

Spatial proteomics more broadly now spans several partially overlapping regimes, including imaging mass cytometry, mass spectrometry imaging, and other spatially resolved protein-analysis strategies [97, 98, 99]. The unifying logic is that tissue geography is treated as biologically constitutive rather than as nuisance structure averaged away by homogenization. This is especially important in tumor microenvironments, immune organization, developmental systems, and organ pathology, where local cellular arrangement and regional molecular heterogeneity are themselves part of the mechanism [97, 95].

These platforms should therefore be understood as adjacent but not secondary. They do not replace open MS-based discovery, PTM proteomics, or proteoform-resolved workflows. They answer a different class of question: predefined high-dimensional panel measurement, cell-resolved protein phenotyping, and spatially situated tissue biology. In a fully mature proteomics ecosystem, they belong alongside MS-based and affinity-panel methods as part of the larger evidence-design toolkit. Their inclusion in this review is important precisely because a modern proteomics platform should know not only what it can measure deeply, but also what it can measure in place, in panels, and at the level of tissue organization.

1.10 Pre-analytical Design, Sample Preparation, and the Hidden Determinants of Proteomics Quality

One of the easiest ways to misunderstand proteomics is to think that analytical quality begins at the mass spectrometer. In reality, many of the most consequential determinants of proteomic quality arise earlier: biospecimen collection, anticoagulant choice, storage time, freeze-thaw history, depletion strategy, lysis chemistry, digestion efficiency, cleanup behavior, and sample randomization all shape what the instrument will be allowed to see

[27, 100, 31]. This is true in every part of the field, but it becomes especially acute in plasma, cerebrospinal fluid, extracellular-vesicle preparations, and low-input workflows where small handling differences can become large analytical distortions [100, 35].

The pre-analytical problem is particularly important in translational proteomics because the biological effect size of interest is often smaller than the variability introduced by handling [27, 51]. A workflow may be technically sophisticated at the acquisition stage and still underperform because the sample entered the instrument already compromised by hemolysis, blood contamination, uncontrolled clotting time, proteolysis, or inconsistent digestion chemistry [35, 100]. This is one reason why the strongest modern proteomics papers increasingly describe sample handling with the same rigor previously reserved for acquisition parameters [1, 100].

Sample preparation itself is not a neutral prelude to “the real analysis.” It is part of the analytical model. In nanoparticle-corona proteomics, preparation determines which proteins are even available for interpretation [31, 28]. In phosphoproteomics and glycoproteomics, enrichment chemistry determines which regulated species become visible and which are systematically missed [10, 63]. In top-down workflows, buffer chemistry and protein handling directly influence intact-state preservation [3, 17]. In single-cell proteomics, sample handling is so dominant that the distinction between “preparation” and “analysis” becomes almost artificial [25, 26].

This has two practical implications. First, method comparison without pre-analytical comparison is often misleading. A method may appear superior because it was paired with better specimen handling or a more stable preparation workflow rather than because its core analytical idea is better [100, 51]. Second, capability claims in proteomics should always be understood as workflow claims, not just instrument claims. A laboratory that can run a workflow but cannot control the specimen pathway into that workflow is likely to underperform relative to its nominal technical inventory [1, 27].

For a comprehensive review, this point matters because it reframes “technology” itself. The technology is not only DIA, TMT, BioID, or top-down MS. The technology is the complete chain that allows those methods to generate interpretable evidence. That chain begins earlier than many summaries admit, and it is often where otherwise excellent studies fail.

1.10.1 Sample-type-specific preanalytical hazards

Different proteomics matrices fail in different ways, and a serious review should make those hazards explicit rather than discussing “sample quality” only in generic terms [27, 100]. Plasma and serum are threatened by hemolysis, clotting variation, platelet activation, and subtle contamination events that can masquerade as biological signal if not modeled explicitly [35, 27]. Urine is vulnerable to collection timing, concentration variability, renal physiology, and storage-related compositional drift, all of which can distort comparative interpretation if the study design treats urine as a static matrix rather than a physiologically dynamic one [27]. Tissue proteomics is especially sensitive to ischemic delay, dissection bias, fixation or freezing heterogeneity, and the fact that macrodissected tissue often contains a mixture of cell states more diverse than the study labels admit [14, 29]. Single-cell proteomics inherits all of the above plus a far sharper dependence on cell isolation stress, transfer efficiency, and microscopic sample loss [25, 26].

The practical implication is that preanalytical rigor should be chosen in relation to matrix vulnerability. A protocol that is tolerable for cultured cells may be grossly inadequate for plasma biomarker work. A preparation chain that is acceptable for bulk-tissue discovery may be too lossy for single-cell proteomics. In other words, preanalytics is not a universal checklist. It is a matrix-specific risk model that must be engineered with the same care as acquisition design [27, 100, 25].

1.10.2 Digestion, depletion, enrichment, and the chemistry of bias

Sample preparation introduces not only noise but directionality. Depletion strategies can improve depth in plasma while also removing biologically coupled proteins that ride with the depleted fraction [27, 28]. Digestion chemistry can change peptide observability in sequence- and matrix-dependent ways, meaning that "protein abundance" is always partly a peptide-recovery problem [2, 37]. Enrichment steps in phosphoproteomics, glycoproteomics, and ubiquitin workflows make regulatory biology visible while simultaneously privileging the chemical subclasses best matched to the enrichment material [10, 63]. Even ostensibly neutral cleanup and desalting choices can shift which analytes survive into the instrument in low-input or poorly behaved matrices [25, 100].

For that reason, preparation chemistry should be described as a source of structured bias rather than merely "workflow optimization." High-end proteomics does not eliminate bias. It characterizes, constrains, and exploits bias in analytically honest ways. That is a more realistic and more scientifically mature description of what sample preparation actually does.

1.11 Computational Proteomics, Data Integration, and the Problem of Meaning

Proteomics has become computational not only because data volumes increased, but because many of the field's defining methods produce evidence that is uninterpretable without algorithmic mediation [5, 37]. DIA requires deconvolution and peptide-centric reconstruction from mixed fragment spectra [5, 4]. Phosphoproteomics requires pathway and kinase-network interpretation to distinguish important phosphosites from background abundance of phosphosite observations [50]. Top-down proteomics requires specialized intact-state identification and proteoform-resolved matching strategies [3, 101]. Single-cell proteomics requires models for sparse observations, noise, and multimodal integration that are qualitatively different from conventional bulk pipelines [26, 36].

The computational question is therefore not simply how to process larger files faster. It is how to convert proteomic observations into evidence that preserves uncertainty, supports biological reasoning, and remains reusable over time [1, 5]. This is one reason recent reviews place such strong emphasis on FAIR data structures, reproducibility standards, and interoperable evidence layers [1]. A proteomics result that cannot be reinterpreted, reweighted, or audited is analytically weaker than one that can, even if both came from equally advanced instruments.

In practice, proteomics computation now includes several distinct layers. One layer is analytical reconstruction: peptide identification, quantitative normalization, spectral alignment, deconvolution, and missing-value handling [2, 37]. A second layer is interpretive modeling: pathway analysis, kinase inference, network analysis, target-ranking, and proteoform grouping [50, 102, 59]. A third layer is translational synthesis: deciding which findings are robust enough to nominate as biomarkers, mechanism hypotheses, or assay targets [13, 14].

This is why high-end proteomics increasingly resembles a computational science of molecular evidence rather than a purely instrumental science of molecular detection. Even a seemingly straightforward review article now depends on structured retrieval, weighted evidence selection, subsection-level source discrimination, and sentence-level traceability. The same is true in experimental work. When the question is mechanistic or clinical, the difference between a useful proteomics data set and an impressive but unstable one is often computational architecture rather than raw analytical depth [1, 5].

Another consequence is that data integration has become central. Interaction proteomics gains explanatory power when layered with abundance and PTM-state data [6, 59]. Phosphoproteomics becomes more useful when coupled to perturbational or therapeutic-response context [50, 65]. Single-cell proteomics increasingly derives value from joint interpretation with transcriptomic or spatial measurements rather than from single-modality isolation [36]. In a modern review, this integrated computational layer is not ancillary; it is part of the field's core operating logic.

1.11.1 Spectral libraries, prediction models, and the changing boundary between measurement and inference

One of the most consequential changes in recent proteomics is that the boundary between direct measurement and computational inference has become increasingly fluid [5, 4]. DIA workflows originally depended heavily on empirical spectral libraries, but newer approaches increasingly exploit predicted spectra, predicted retention behavior, and hybrid evidence models that can recover peptide-level signals with less dependence on manually built reference libraries [5, 4]. This is analytically powerful because it expands what can be queried in the data; it is also epistemically delicate because stronger inference layers can make the resulting output look more deterministic than the underlying measurement truly was [5].

The best computational proteomics therefore does two things at once. It expands recoverable evidence through better models, and it preserves visibility into where model assumptions entered the evidentiary chain. This becomes especially important in review writing and translational work, where the difference between a directly observed feature, a strongly inferred feature, and a weakly rescued feature can matter materially for interpretation and downstream decision-making [1, 5].

1.11.2 Traceability, auditability, and why sentence-level evidence matters

Proteomics has reached the point where interpretive compression is often the main failure mode. The problem is no longer only that data are hard to collect, but that complex analytical chains can produce smooth-looking summaries that hide where uncertainty, recovery bias, enrichment selectivity, or computational inference entered the result [1, 2]. That is why sentence-level traceability is not merely a writing preference in a review of this kind. It mirrors what the field increasingly requires from experiments themselves: the ability to move from claim to evidence layer to analytic assumptions without losing the chain of reasoning [1, 13]. A mature proteomics culture should expect this not only from published data products but from its literature synthesis.

1.11.3 Missingness, imputation, and the politics of clean-looking data

One of the least glamorous but most consequential controversies in computational proteomics concerns missingness. Proteomics data sets often contain absent peptide or protein values for reasons that are analytically heterogeneous: low abundance, sampling instability, poor chromatographic behavior, matrix effects, incomplete digestion, enrichment selectivity, and software-level thresholding can all create “missing” observations that do not mean the same thing biologically [2, 37]. Yet downstream figures and statistical summaries frequently pressure those absences into clean matrices through imputation schemes or filtering rules whose assumptions are only partly visible to readers [37].

This is not a merely technical nuisance. Missingness decisions can reshape pathway rankings, compress cohort variance, and make a fragile candidate look stable enough to survive to the next stage of interpretation [2, 37]. The deepest lesson is that computational neatness is not the same as evidentiary honesty. Mature proteomics should prefer explicitly messy but interpretable uncertainty over cosmetically complete outputs that conceal how much of the final table was reconstructed statistically rather than measured directly [1, 2]. In review terms, this is why fields that celebrate “clean data” too aggressively often drift into overclaiming.

1.11.4 The debate over foundation models, prediction, and evidence rescue

A second live debate concerns how far prediction-driven proteomics should go before it begins to outrun what the instrument actually saw. Predicted spectra, predicted retention, proteoform grouping, and increasingly ambitious rescue models can transform weak or partial evidence into usable analytical output, especially in DIA and large-scale reanalysis settings [5, 4, 1]. This is one of the field’s great strengths. It allows proteomics to become more cumulative and more computationally powerful over time.

But the same trajectory creates a philosophical tension. The more successful rescue and prediction become, the easier it is to forget which parts of the result are anchored by strong direct evidence and which parts are mediated heavily by learned priors and model assumptions [5, 1]. The right response is not reactionary distrust of advanced computation. It is explicit evidentiary labeling. Future proteomics will be strongest when it can say, with technical precision, what was directly observed, what was strongly inferred, what was statistically imputed, and what was predicted into the analytical field by a model-informed retrieval layer. That degree of evidentiary bookkeeping is likely to become one of the real marks of maturity in the next generation of proteomics platforms.

1.12 Clinical Translation, Biomarker Science, and Why Proteomics Fails or Succeeds in the Real World

Proteomics has long been associated with biomarker discovery, but the history of the field shows that discovery alone is not enough [103, 57]. Many candidate-rich studies did not translate because they overestimated the value of initial differential signals, underestimated pre-analytical variability, or failed to build the measurement discipline needed for verification and deployment [9, 13]. The translational challenge is therefore not "can proteomics find differences?" but "can proteomics produce differences that survive replication, verification, matrix complexity, and clinical decision pressure?" [13, 27].

Biomarker-oriented proteomics succeeds when discovery, verification, and interpretability are built as one program rather than as disconnected stages. Discovery methods such as DIA or enriched plasma workflows are useful because they widen the candidate space [5, 28]. But those candidates become clinically meaningful only when they move into quantitative and targeted regimes where reproducibility, calibration, and statistical structure are stronger [9, 13, 48]. This is one reason why targeted proteomics remains strategically important even in an era of increasingly powerful discovery workflows: clinical programs need narrower, more defensible measurements than early discovery alone can provide [9, 49].

Mechanism also matters. A protein that differs statistically but lacks pathway coherence, PTM interpretation, or network context may be much harder to justify biologically than one that sits inside a coherent mechanistic model [50, 6]. This is why the most persuasive biomarker and translational proteomics programs increasingly combine abundance profiling with PTM-state analysis, interaction evidence, or proteoform-aware characterization rather than relying on one evidence layer alone [3, 63, 50]. In other words, clinical translation in proteomics increasingly rewards evidentiary convergence.

Proteomics also succeeds in the real world when it is honest about scope. Some workflows are excellent for early discovery but poor for direct clinical deployment. Others are analytically narrower but far more suitable for verification or regulated environments [2, 9]. A mature review should therefore resist the temptation to collapse all proteomics into one generic promise of "precision medicine." The field is powerful, but its real power lies in selecting the right evidence architecture for the stage of the translational problem [1, 13].

For that reason, a comprehensive review should treat biomarker discovery, assay verification, therapeutic characterization, and systems-level mechanism as related but distinct translational trajectories. Proteomics can serve all of them, but not with the same method and not under the same evidentiary assumptions. That distinction is one of the most important conceptual upgrades the field has undergone.

1.12.1 Biomarker discovery and verification are not the same analytical problem

One of the most persistent mistakes in translational proteomics is to treat biomarker discovery and biomarker verification as if they were merely different points on the same analytical continuum [103, 57]. In practice, they are distinct evidentiary regimes. Discovery tolerates broader search space, higher dimensionality, and a greater fraction of candidates that will ultimately fail. Verification demands narrower panels, tighter calibration, greater cohort discipline, and stronger handling of preanalytical variance [9, 13]. This is precisely why workflows often need to transition from DIA or enriched discovery logic into targeted quantitation rather than remaining in one method family throughout the life of the program [5, 48].

The implication for review writing is important: a fair assessment of a proteomics platform should not ask only whether it can discover candidates, but whether it has a credible route for reducing those candidates into a disciplined verification layer. Without that second lane, many biomarker programs remain impressive but operationally incomplete [9, 13].

1.12.2 Mechanism-of-action studies require convergent evidence

Therapeutic mechanism-of-action studies are often where proteomics shows its greatest conceptual power, because drugs and biologics rarely act only by changing transcript levels or one isolated target property [14, 50]. A persuasive proteomic mechanism study often requires convergence across several layers: abundance remodeling, phosphorylation-state shifts, interaction-network changes, and, in some cases, proteoform or intact-state evidence [50, 6, 3]. No one of these layers is always sufficient.

This is why the best perturbational proteomics studies rarely rely on a single assay identity. Instead, they build a response portrait in which different proteomic layers constrain one another. A phosphosite signal without pathway context may be ambiguous; an interaction shift without perturbational quantitation may be hard to rank; an intact-state change without comparative response data may be mechanistically suggestive but not explanatory. Convergent evidence turns these from isolated observations into mechanism [50, 14].

1.12.3 Therapeutic protein and biopharmaceutical characterization

Proteomics also occupies an unusual position in therapeutic characterization because some of the most important molecules in biomedicine are proteins whose safety, function, and manufacturability depend on intact-state detail [78, 79]. In such settings, proteomics is not only being used to study disease biology; it is being used to study the therapeutic objects themselves. This includes glycoform distribution, truncation state, assembly heterogeneity, process by-products, and other molecular features that may be functionally decisive but poorly represented by simplified peptide summaries [3, 78].

This translational lane is analytically distinct from both biomarker discovery and systems biology. It places a premium on structural and proteoform-resolved measurements, on robustness of sample handling, and on interpretive clarity around molecular heterogeneity [3, 79]. A comprehensive review of proteomics should therefore treat therapeutic characterization not as a niche afterthought but as one of the clearest demonstrations of why intact-state proteomics matters.

1.12.4 Clinical proteomics as decision support rather than technology display

The real measure of clinical proteomics is not whether a platform can deploy a sophisticated method, but whether that method changes the evidentiary quality of a clinical decision or translational claim [27, 13]. This requires more than good spectra. It requires stable sample handling, quantitative discipline, explicit uncertainty management, and a rationale for why the chosen analytical layer is the one closest to the biological or clinical decision point [1, 27]. In that sense, successful clinical proteomics is less about maximum technological display and more about choosing the minimum adequate complexity required to support the right inference.

1.12.5 Why the biomarker literature overpromises and how the best studies avoid it

Proteomic biomarker literature has often overpromised because early-stage discovery excitement was allowed to masquerade as verification-grade evidence [103, 57]. The classic failure pattern is familiar: a biologically plausible differential signal is detected in a limited cohort, elevated rhetorically into a clinical candidate, and then never survives the transition into larger, preanalytically messier, quantitatively stricter validation settings [9, 13]. This failure is not a trivial public-relations issue. It is one of the main reasons proteomics has sometimes been underestimated by outsiders despite being methodologically powerful.

The best studies avoid this trap by treating biomarker work as a staged evidentiary reduction process rather than as a one-step discovery-to-claim pipeline. They use discovery methods to widen search space, quantitative discipline to eliminate unstable candidates, targeted workflows to verify analytically tractable panels, and mechanistic or PTM-aware context to explain why the retained signals deserve confidence beyond nominal statistical association [5, 9, 50]. In other words, strong biomarker proteomics behaves less like mining and more like adjudication. It does not ask only what differs. It asks what differs reproducibly, what differs mechanistically, and what differs in a way that can survive contact with clinical reality.

1.12.6 Translational case patterns: what actually survives contact with the clinic

What survives translational pressure is usually not the most exotic proteomic result but the most disciplined one. Urinary biomarker programs in kidney disease, targeted plasma studies in perioperative bleeding and cardiovascular risk, and structured biomarker-verification efforts in oncology all suggest the same pattern: useful translational proteomics typically narrows aggressively, stabilizes preanalytics, and prefers a small defensible panel over a dramatic but fragile discovery signature [27, 53, 54, 56]. This can feel anticlimactic compared with broad discovery papers, but it is exactly what clinical usefulness demands.

Equally important are the cases that survive because they retain mechanistic support rather than only statistical separation. When phosphoproteomic or interaction-context evidence explains why a candidate panel behaves the way it does, the translational story becomes more durable because it is no longer just a pattern-recognition exercise [50, 6]. Likewise, glycoform-aware biomarker programs and proteoform-sensitive therapeutic-characterization studies tend to survive scrutiny better when they can show that the measured molecular feature is proximal to function rather than merely correlated with it [68, 76, 3]. The broader lesson is that translational proteomics lasts longer when it is mechanistically thick, not just analytically sharp.

1.12.7 The quiet economic truth of translational proteomics

There is also an economic reality that comprehensive reviews often understate: translational proteomics wins when it reduces uncertainty enough to justify its complexity. A workflow can be analytically brilliant and still fail to matter if it is too expensive, too fragile, too slow, or too interpretation-heavy for the stage of the translational program in which it is being asked to operate [13, 27]. This is one reason targeted assays, narrow verification panels, and high-discipline quantitative workflows remain strategically powerful even when broader discovery technologies continue to improve [9, 48].

The best translational platforms therefore behave like staged decision systems rather than like maximal-discovery machines. They use expensive breadth where breadth is actually needed, then contract toward cheaper, tighter, and more scalable evidence forms as the program matures. That logic is not glamorous, but it is one of the deepest reasons why some proteomics efforts become enduring translational assets while others remain technically admired but operationally marginal.

1.13 Field Controversies and Unresolved Debates

Comprehensive reviews become more useful when they state plainly where the field still argues with itself. Proteomics now has enough technical maturity that its central controversies are no longer about whether mass spectrometry can measure proteins at all. The controversies are about what level of molecular compression is still acceptable, how much computational rescue should be tolerated before evidence becomes too model-mediated, whether broader discovery really translates into better biomarker science, and when intact-state analysis is genuinely necessary rather than rhetorically invoked [1, 3, 5, 13].

One unresolved debate concerns breadth versus discipline. Large-scale discovery programs are scientifically intoxicating because they reveal the proteome as a wide search space, but the field's history shows that breadth alone does not guarantee mechanistic or clinical progress [5, 103]. Another concerns the meaning of "quantitative." For some investigators, relative stability across cohorts is enough; for others, translational seriousness requires much stronger calibration logic and narrower verification regimes [7, 13]. The field has not resolved this tension fully, because different use cases genuinely impose different burdens of proof.

There is also a live debate about what counts as the real molecular object of proteomics. Peptide-centric bottom-up workflows remain enormously effective, but proteoform-centered advocates are right that many biologically decisive states are not fully preserved by peptide-only abstractions [3, 77]. The field is therefore pulled between scale and fidelity: between methods that survey widely and methods that preserve more of the actual molecular entity being studied. This is not a problem to be solved once. It is likely to remain a defining design tension of proteomics for the foreseeable future.

Finally, there is a cultural controversy about evidence rhetoric itself. Proteomics papers often oscillate between cautious technical detail in Methods sections and overconfident biological storytelling in the Discussion. That gap is not trivial. It is part of why traceability, explicit uncertainty management, and staged evidentiary claims matter so much in the next generation of the field [1, 2]. A proteomics culture that can speak with precision about what was measured, what was inferred, and what remains unresolved will likely outperform one that still rewards maximal claims built on compressed evidence.

1.14 Future Directions

Several trajectories are likely to define the next phase of proteomics. First, the field will continue moving toward reproducible, reusable, and computationally durable data structures, especially in large-cohort and translational work [1, 5]. Second, low-input and single-cell methods will continue improving, making heterogeneity more routinely measurable at the protein level [25, 26]. Third, proteoform-aware and structural methods will become increasingly important as investigators demand intact-state information rather than only peptide-level proxies [3, 77]. Fourth, PTM discovery and open-search logic will likely expand the known functional chemistry of the proteome beyond the canonical modification classes currently emphasized [72, 73]. Fifth, multimodal integration will likely become less optional, because many of the strongest mechanistic inferences will come from combining proteomic layers rather than relying on one alone [36, 59].

The field's progress will depend not only on deeper instrumentation but on better integration of acquisition, enrichment, calibration, and computational interpretation. Proteomics becomes truly powerful when these are treated as parts of one analytical system rather than as loosely connected steps [1, 2, 5]. This is also why platforms such as PubMed-grounded review systems matter: the intellectual bottleneck is increasingly synthesis, traceability, and method selection rather than mere access to technology.

Two further directions deserve emphasis. One is standard-bearing clinical realism: future proteomics will be judged increasingly by whether methods survive ordinary biospecimen imperfection, multi-site reproducibility pressure, and verification-stage narrowing rather than by best-case discovery demonstrations alone [13, 27, 1]. The other is conceptual honesty about molecular objects. As the field deepens its commitment to proteoforms, local neighborhoods, PTM states, and single-cell heterogeneity, it will need to stop allowing coarse gene-product summaries to stand in for measurements that were never actually made at that level [3, 11, 25]. In practical terms, the future of proteomics is not only about deeper coverage. It is about reducing the mismatch between what biology cares about and what the analytical output still compresses.

Finally, proteomics will likely become more explicitly hierarchical in its reasoning. Discovery, quantitation, PTM state, interaction context, and intact-state characterization will not be competing endpoint modalities so much as successive layers of evidentiary refinement within one program [1, 2, 3]. The most advanced future platforms will therefore not be the ones that merely own all the methods. They will be the ones that can move between these layers without dropping reproducibility, traceability, or interpretive honesty as complexity rises.

1.15 Conclusion

Modern proteomics is no longer a single technical discipline defined by protein identification alone, but a coordinated evidence system spanning discovery-scale profiling, quantitative comparison, perturbational interpretation, interaction mapping, PTM-state analysis, and intact molecular characterization [1, 2, 3]. DIA and related discovery workflows changed what reproducible large-scale proteome mapping could mean [4, 5]. Quantitative frameworks and targeted methods changed what proteomics was allowed to claim in translational and mechanistic settings [7, 8, 9]. Interaction and proximity-labeling methods changed what counted as context [6, 11]. PTM-centered and proteoform-resolved workflows changed what the field accepted as the real molecular object of interest, shifting emphasis from generic proteins toward active states, local neighborhoods, and intact molecular forms [10, 3].

The deepest lesson is that proteomics has matured by repeatedly refusing its own simplifying assumptions. Each major advance arose when the field recognized that the current dominant readout was too compressed, too stochastic, too context-poor, or too mechanistically distant to answer the next class of biological questions honestly. The future of proteomics will almost certainly continue in that pattern. It will not be defined simply by more data, but by more explicit control over what kind of molecular reality the data actually preserve.

For that reason, the best comprehensive review is not one that merely lists methods or celebrates instrumentation. It is one that teaches how to design evidence. Proteomics becomes most powerful when methods are selected not for novelty, branding, or maximal apparent complexity, but because they resolve the actual uncertainty limiting the question while preserving traceability from claim to chemistry to computation. That is the standard by which serious proteomics should now be judged. It is also the standard that turns a long manuscript into something closer to a reference bible.

1.16 Methods and Traceability Statement

This article was constructed as a literature-grounded narrative review using a custom AI-assisted manuscript pipeline designed to preserve sentence-level traceability to the biomedical literature while maintaining human editorial control over topic selection, source adjudication, synthesis, and final wording. The workflow included structured retrieval, deduplication, subsection-level evidence packing, manuscript drafting, iterative revision, and citation-state management. Proprietary implementation details and internal orchestration logic are intentionally not disclosed here.

At the evidence level, the working manuscript was built around PubMed identifiers as the canonical source anchors for substantive scientific claims. Article metadata and abstract-level records traceable to the National Library of Medicine ecosystem were used as the core evidentiary substrate for literature organization and claim support. Formatted in-text citations were treated as a presentation layer generated after PMID-level source anchoring had already been established, allowing reversibility between human-readable citation formats and identifier-linked evidence states.

The literature workflow organized retrieved papers by analytical regime, methodological lineage, translational use case, and historical turning point. Source packs were then assembled at the subsection level so that drafting proceeded from dense, method-specific evidence clusters rather than from broad unspecialized summaries. The review was expanded iteratively against those curated packs to keep major methodological, historical, and comparative statements directly traceable to PubMed-linked bibliographic records and associated abstracts.

This manuscript is not presented as a formal systematic review or meta-analysis and should not be interpreted as such. Its purpose is to provide a comprehensive, traceable, high-fidelity narrative synthesis of the contemporary proteomics field using a custom AI-assisted literature pipeline in which all scientific content remains auditable against PMID-linked NLM abstract records and related bibliographic metadata, without disclosing proprietary pipeline internals or implementation-specific intellectual property.

1.17 Summary Tables

1.17.1 Table 1. Major proteomics method families

Method family	Primary analytical problem solved	Core workflow logic	Strengths	Main limitations	I
Discovery-scale proteomics	Broad composition unknown	DIA, fractionation, enrichment, or low-input workflows maximize searchable proteome space	Breadth, cohort-scale discovery, hypothesis generation	Dynamic range, missing low-abundance context, interpretation drift if discovery is overclaimed	B d p st
Quantitative and perturbational proteomics	Comparative magnitude or response unknown	Label-free, TMT, SILAC, targeted verification, and perturbation-linked quantification	Reproducible comparison, turnover logic, verification discipline	Normalization burden, ratio compression, assay narrowing	C b p v st
Interaction and proximity proteomics	Interaction context unknown	Affinity enrichment, AP-MS, BioID, TurboID, neighborhood labeling	Complex membership, local context, target-associated network logic	Contaminants, indirect interactions, proximity overclaim	S c d p
PTM-centric proteomics	Regulatory chemistry unknown	Selective enrichment of modified peptides plus computational interpretation	Direct access to signaling state and regulation	Enrichment bias, modification instability, overinterpretation of site lists	P g u re
Structural and proteoform-resolved proteomics	Intact molecular form unknown	Ion mobility, native MS, top-down or intact-state workflows	Proteoform fidelity, stoichiometry, conformational insight	Lower throughput, greater technical burden, narrower routine deployment	B cl p b c
Affinity-panel proteomics	Scalable predefined analyte measurement required	Olink, SomaScan, and related panelized affinity readouts	Throughput, cohort stability, low sample volume,	Preselected analyte universe, reagent dependence, cross-platform drift	L p st tr p

Method family	Primary analytical problem solved	Core workflow logic	Strengths	Main limitations	I
			deployment friendliness		
Spatial and cytometry regimes	Tissue geography or cell-resolved phenotype unknown	CyTOF, imaging mass cytometry, spatial proteomics, MSI	Spatial context, cell-state localization, multiplex tissue architecture	Panel dependence, segmentation bias, limited open discovery	T m ir ti

1.17.2 Table 2. Decision matrix for selecting the dominant proteomics regime

Dominant question type	Preferred regime	Why this regime fits	Common failure mode	Typical defensible output
What proteins or pathways broadly differ?	Discovery-scale proteomics	Maximizes open search space before narrowing	Treating breadth as proof	Candidate biology map, ranked targets, discovery signature
Are differences reproducible across conditions or cohorts?	Quantitative proteomics	Stronger comparability and statistical discipline	False precision from weak normalization	Comparative abundance model, response signatures
Can a candidate panel be verified or deployed?	Targeted or affinity-panel proteomics	Narrows to analytically tractable markers	Confusing deployment convenience with mechanistic depth	Stable biomarker panel, cohort-ready assay
What pathway state or signaling logic changed?	PTM-centric proteomics	Captures regulatory state rather than only abundance	Site lists without network interpretation	Kinase-state model, pathway activation logic
What proteins interact or share a local neighborhood?	Interaction or proximity proteomics	Resolves relational context directly	Calling proximity equivalent to direct binding	Complex map, neighborhood model, interaction hypothesis

Dominant question type	Preferred regime	Why this regime fits	Common failure mode	Typical defensible output
What intact molecular form actually exists?	Structural or proteoform-resolved proteomics	Preserves intact-state information compressed by bottom-up workflows	Overabstracting back to generic protein labels	Proteoform-aware characterization, stoichiometric complex view
Where in tissue or cell architecture is the biology located?	Spatial or cytometry regime	Embeds protein state in tissue geography	Segmentation and panel bias overinterpretation	Spatial atlas, cell-resolved tissue map

1.17.3 Table 3. PTM workflow comparison

PTM regime	Typical enrichment or capture	Main biological readout	Key interpretation risk	Strong translational mechanistic u
Phosphoproteomics	IMAC, TiO2, phosphopeptide enrichment	Kinase activity, signaling state, pathway rewiring	Treating phosphosite abundance as pathway truth without network context	Oncology signaling, drug-response mappi
Glycoproteomics	Lectins, hydrazide chemistry, boronate affinity, glycopeptide enrichment	Extracellular state, trafficking, immune recognition, therapeutic quality	Losing glycan structural context or overcalling biomarker specificity	Vaccine, cancer glycoform, biotherapeutic (
Ubiquitinomics	Signature-remnant enrichment, anti-ubiquitin capture	Protein fate, degradation logic, stress response	Confusing abundance change with regulated turnover	Proteostasis, targeted degradation, str biology
Oxidation or nitrosylation proteomics	Redox-reactive probes, resin capture, cysteine-directed chemistry	Redox-sensitive regulatory state or damage	Artifactual oxidation during handling	Neurodegenera inflammation, mitochondrial biology
PTM discovery	Open search, differential MS/MS, broad modification discovery logic	Unexpected regulatory chemistry	Inflated false positives or insufficient validation	Noncanonical I discovery, exploratory mechanism

1.17.4 Table 4. Platform comparison across open MS, panelized affinity, and spatial regimes

Platform class	Open discovery	Quantitative comparability	PTM or proteoform awareness	Spatial resolution	Best operational niche
DIA or DDA MS	High	Moderate to high with good design	Moderate for PTMs, low for intact proteoforms in standard bottom-up mode	None	Broad discovery, comparative profiling
TMT multiplexed MS	Moderate	High within multiplex	Moderate, especially with phospho workflows	None	Comparative multi-condition studies
Targeted MS	Low	High	Low to moderate, analyte dependent	None	Verification and deployable assays
Top-down or native MS	Low to moderate	Moderate	High	None	Proteoform and therapeutic characterization
Olink or PEA	None beyond predefined panel	High	Low	None	Large-cohort translational protein panels
SomaScan or SOMAmer	None beyond predefined panel	High	Low	None	Broad panelized cohort measurement
Antibody arrays	Low	Moderate	Low	None	Multiplex panel screening
CyTOF or imaging mass cytometry	None beyond panel	High within panel	Low	High	Cell-resolved tissue phenotyping
Spatial proteomics or MSI	Moderate depending on modality	Moderate	Moderate	High	Tissue geography and heterogeneity mapping

1.17.5 Table 5. Translational workflow archetypes

Translational objective	Recommended sequence of methods	Why this sequence works	Common pitfall
Biomarker discovery	Discovery-scale MS -> quantitative narrowing -> targeted or affinity-panel verification	Preserves openness early and discipline late	Treating discovery signatures as deployable assays
Mechanism-of-action	Quantitative proteomics -> PTM-centric or interaction context -> targeted follow-up	Links perturbation to pathway and network consequence	Confusing downstream compensation with direct mechanism
Therapeutic protein characterization	Structural or proteoform-resolved workflows -> glycoform or PTM analysis -> targeted QC	Keeps intact molecular form central	Overreliance on peptide summaries for intact therapeutics
Precision oncology or systems medicine	Discovery-scale cohort profiling -> PTM or interaction context -> selective clinical panelization	Combines cohort breadth with mechanistic thickening	Failing to narrow before translation
Spatial tissue biology	Discovery or panel selection -> spatial or cytometry deployment -> targeted orthogonal validation	Connects molecular candidates to tissue geography	Overinterpreting spatial correlations without biochemical support

1.18 Numbered Bibliography

Machine-readable citation libraries for this manuscript are provided alongside the paper at:

- `/Users/davidgraham/@Active2026/manuscripts/Proteomics_Review/proteomics_review_citation_library.json`
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1.19 Figure Prompt Appendix

Figure concepts are defined separately in:

- `/Users/davidgraham/@Active2026/manuscripts/Proteomics_Review/proteomics_review_figure_prompts.md`