

POINT OF VIEW

The Proteome Is the Prescription: From Static Stratification to Longitudinal Precision Therapeutics

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Precision medicine has invested two decades in genomic stratification, and that investment has been valuable: germline sequencing defines a landscape of biological potential, whereas serial tumor genotyping in oncology provides a partially dynamic record of clonal evolution. Yet stratification at diagnosis, or even periodic repeat genotyping, incompletely describes what a patient's cells are doing under a specific treatment at a particular time point. I argue that quantitative proteomics, enabled by advances in data-independent acquisition (DIA), dia-PASEF on trapped ion mobility instruments, and high-resolution Astral platforms, is positioned to become a dynamic functional layer within precision therapeutics, complementing rather than replacing genomics. I propose the Proteomic Pathway Response Index (PPRI), a patient-referenced metric summarizing serial proteomic change relative to a prespecified therapeutic target state and intended to report downstream pathway response rather than target occupancy or clinical benefit. The PPRI extends a simpler fixed-baseline pathway index that my colleagues and I previously built and piloted in bladder cancer, with molecular alerts that coincided with subsequent clinical assessments in six longitudinally monitored patients. I outline how high-frequency microsampling paired with DIA could support a proteomic N-of-1 framework for longitudinal treatment monitoring and, following prospective validation, contribute to dose adaptation or rational combination strategies. I also describe the analytical, clinical, regulatory, and implementation requirements that would need to be met before such measurements could inform prescribing. I close with a ten-year research agenda organized around testable milestones rather than a finished roadmap.

Keywords: quantitative proteomics, data-independent acquisition, dia-PASEF, precision medicine, prescriptomics, biomarker validation, sustainability by design

Genomics defines biological potential; it does not fully describe current therapeutic state

The genomic architecture of precision medicine is not wrong, in my view. Germline sequence defines a relatively stable landscape of biological potential: the protein variants a patient can produce, the metabolic enzymes whose function may be altered, and the pharmacogenomic variants that may influence drug disposition. In oncology, serial tumor genotyping and circulating tumor DNA extend this framework by tracking clonal evolution, copy-number alterations, and treatment-selected resistance mutations over time. The cancer genome is therefore not static in the way germline sequence is, and other molecular layers, including the epigenome and transcriptome, are also dynamic.

What genomic data, including serial genomic measurements, do not establish by themselves is the functional state of a pathway at a specific treatment time point. A resistance mutation detected in circulating tumor DNA indicates that a clone carrying that alteration has expanded; it does not tell us whether the

intended drug target remains detectable at the expected abundance, whether abundance changes consistent with bypass-pathway activation have emerged, or whether a dose modification made two weeks earlier has altered the relevant protein signature. These are different kinds of information, and I think proteomic measurements are particularly well positioned to contribute to them.

Quantitative proteomics is distinctive not because it is the only dynamic molecular modality. Transcriptomics, metabolomics, phosphoproteomics, epigenomics, and spatial omics can all report context-dependent biological change. Its particular value, I think, lies in measuring many of the molecular entities directly targeted by therapeutics while simultaneously capturing downstream alterations in cellular state that may lie mechanistically closer to treatment action than genomic variation alone.^{1,12} Conventional abundance proteomics does not provide a complete readout of pathway activity, which may also depend on phosphorylation, localization, protein complex formation, conformation, and other regulatory processes. Nevertheless, the combination of proximity to therapeutic targets and responsiveness to treatment is what I want to build the rest of this argument on.

A fundamental limitation must also be stated at the outset, because it does not disappear no matter how good the assay becomes: proteomics reports the molecular state of the sampled compartment, which may not correspond directly to the state of the disease-driving tissue. Plasma, urine, and dried-blood proteomes may reflect systemic inflammation, hepatic acute-phase responses, renal filtration and tubular handling, immune-cell composition, tissue injury, treatment toxicity, hydration, or vascular permeability. None of these is equivalent to measuring pathway activity within a solid tumor, a brain lesion, or another inaccessible site of disease.

Such specimens may nevertheless provide clinically valuable information at different biological levels. Some signals may reflect systemic treatment response, others may be organ-proximal, and only a subset may validly report processes occurring within the disease tissue itself. The relationship between a sampled biofluid and the relevant pathological compartment must therefore be established for each disease, specimen type, biomarker set, and intended use rather than assumed. A patient-referenced trajectory of the kind I propose below may reduce interindividual variability, but it does not, by itself, resolve the problem of tissue representativeness.

DDA and DIA: two acquisition strategies with different sampling properties

In data-dependent acquisition, the mass spectrometer selects the most abundant precursor ions in real time and fragments them one at a time. It is intuitive, well-validated, and remains the workhorse of discovery proteomics, but it is inherently stochastic. Which precursors get selected varies from run to run, particularly for lower-abundance species, producing the missing-value problem that has long complicated cross-sample comparisons and limited the quantitative completeness of conventional DDA in large, longitudinal clinical cohorts.

Data-independent acquisition inverts this logic: instead of selecting individual precursors, the instrument co-isolates and fragments populations of precursor ions within sequential or multiplexed m/z windows that collectively cover a predefined acquisition range. Recent head-to-head evaluations support what has become a working consensus: DIA generally provides substantially greater quantitative completeness and, in many workflows, improved sensitivity and quantitative precision relative to conventional DDA.² Quantitative completeness is the most secure of these advantages; precision differences are workflow-dependent.

A direct comparison of DDA and DIA on the same next-generation instrument found substantially greater peptide and protein coverage for DIA, with only moderate correlation between the two acquisition modes' quantitative outputs (~ 0.79), a reminder that depth and precision are related but distinct axes of comparison.⁷ This is a real and useful advance, but it should not be treated as a solved problem. Contemporary comparative studies and broader assessments of the field continue to identify acquisition strategy, preprocessing, spectral-library-based or library-free data-analysis workflows, false-discovery-rate control, normalization, and cross-study comparability as active sources of variability.^{2,7} DIA's advantage over DDA is well established within a single, well-controlled pipeline; whether that advantage survives intact

across different laboratories, platforms, specimen types, and multi-year longitudinal collection is a separate, and still open, empirical question.

dia-PASEF and Orbitrap Astral: converging capability gains, not a solved instrumentation problem

dia-PASEF, parallel accumulation–serial fragmentation combined with DIA on trapped ion mobility instruments, adds ion mobility as an additional gas-phase separation dimension and exploits the structured correlation between peptide m/z and mobility to coordinate precursor isolation with ion release from the trapped ion mobility device.¹ This coordination increases ion utilization by aligning quadrupole isolation with the mobility-dependent release of peptide precursors, and it is now being extended into demanding applications such as HLA immunopeptidomics, where it has been shown to improve peptidome coverage at lower sample amounts and enable quantification of less abundant peptide species, including neoantigens.³

That same underlying separation principle has continued to be engineered for greater sensitivity and throughput rather than superseded by a different one: higher-generation trapped ion mobility instruments have substantially increased the number of reproducibly quantified plasma precursors relative to earlier-generation ion-mobility instruments,¹⁴ and lower-input configurations of the same instrument family have pushed sensitivity toward sub-nanogram and single-cell inputs.¹⁵ In parallel, a second engineering trajectory, narrow-window DIA on Orbitrap platforms, and the Orbitrap Astral mass spectrometer, which pairs a quadrupole and Orbitrap analyzer with an asymmetric-track lossless analyzer for fast, high-resolution MS2 acquisition — has pursued the same goal of comprehensive, low-input proteome coverage through a different mass-analyzer design.^{4,5,6}

Reported depth figures in the range of ten thousand or more plasma proteins, or several thousand proteins from single cells, are frequently the product of depletion, immunoaffinity- or nanoparticle-based enrichment, increased sample input, or specific protein-inference and identification criteria.⁷ In optimized workflows, including depletion, enrichment, or other forms of plasma prefractionation, Astral- and ion-mobility-based DIA have produced markedly expanded proteome coverage, and that is genuinely useful technical progress. It should not be conflated with routine, direct, clinically validated analysis of neat plasma in a rapid workflow. Single-cell figures in particular need to distinguish proteins identified in at least one cell from median proteins per cell, from proteins quantified consistently across cells, and from carrier-assisted versus fully label-free approaches, distinctions the underlying literature is itself still working to standardize.¹⁵

Nor is it accurate to say instrumentation has stopped being a limiting factor. It is no longer the sole limiting factor. Plasma's enormous dynamic range, sample-preparation robustness, chromatographic stability and carryover, computational analysis, cross-platform harmonization, clinical interpretation, and regulatory validation are now, collectively, at least as consequential as instrument sensitivity for whether any of this becomes clinically deployable. Framed as engineering capability, ion mobility separation, ion-utilization efficiency, duty cycle, scan speed, quantitative completeness, and sample economy, rather than as a competition between named instrument families, the honest summary is this: two independently engineered platform lines are converging on the same target, which is a reasonable basis for optimism about where the technology is heading, and a poor basis for claiming the destination has already been reached.

From protein lists to a patient-referenced pathway metric: the Proteomic Pathway Response Index

Depth and reproducibility are necessary but not sufficient. Precision medicine requires translating a quantitative protein signature into an actionable therapeutic hypothesis, and the literature offers real, if still preliminary, evidence that proteomics can do this. Pathway-resolved quantitative proteomics of tumor cells under combination treatment has revealed proteome-level rewiring of apoptosis, metabolism, and antigen-presentation pathways not visible from genomic profiling alone.⁹ In pancreatic and neuroblastoma models, quantitative proteomic profiling of drug-treated cells has characterized synergistic combination effects at the pathway level, with functional assays confirming superior efficacy over monotherapy.^{9,10} Quantitative proteomics has been proposed as a predictive biomarker platform for antibody–drug conjugates, where target protein abundance and pathway context shape efficacy in ways genomic alterations alone do not

predict,⁸ and multi-omic integration of proteomic and transcriptomic data has been used to nominate drug-repurposing candidates matched to pathway-level disease signatures, candidates the original authors were explicit still require experimental and clinical validation.¹¹

This evidence establishes association and functional plausibility, not a route from measurement to prescription. Standard, global, abundance-based proteomics measures relative protein abundance; it does not, on its own, measure catalytic activity, receptor or target occupancy, phosphorylation state, proteolytic activation, conformational state, subcellular localization, complex formation, or flux through a pathway. An abundance-based pathway score cannot by itself demonstrate that a drug has engaged its target, and equivalence between protein abundance and pathway activity cannot be inferred from abundance measurements alone. A defensible framework has to keep at least five distinct quantities separate: target abundance; target engagement or molecular occupancy; proximal pharmacodynamic effect; downstream pathway response; and clinical response. These can, and often do, disagree with one another, and a useful proteomic metric has to say which of them it is actually reporting.

What quantitative proteomics may be particularly well positioned to report is the fourth of these, downstream pathway response, inferred from a prespecified multivariate pattern of patient-referenced abundance changes across selected pathway components, and that is worth formalizing, provided it is named and defined honestly. Contemporary DIA provides one promising measurement framework for this, given its quantitative completeness and retrospective data accessibility, though the underlying construct is not specific to any single acquisition method. I propose calling it the Proteomic Pathway Response Index (PPRI), deliberately avoiding the term ‘occupancy,’ which already has a specific pharmacological meaning, the fraction of a target bound by a ligand, that abundance-based proteomics does not measure and should not imply.

This is not an entirely untested idea. In earlier work, my colleagues and I introduced the differential Personal Pathway Index (dPPi), a patient-referenced, longitudinal pathway score computed from urine label-free quantitative proteomics in bladder cancer, built around seven hallmark-of-cancer pathways and referenced throughout to each patient’s own baseline sample.¹⁹ The underlying study compared urine proteomes from 30 patients, 13 with recurrence or progression and 17 without, and then followed six of these patients longitudinally, for as long as 62 months, computing a dPPi at each clinical time point. The resulting dPPi alerts were concordant with subsequently documented recurrence, progression, and, in one patient, a benign cystitis that did not require surgical intervention, at the evaluated time points across that six-patient series.¹⁹ We explicitly acknowledged that this was a small, non-independent longitudinal pilot, and that the approach needed replication in a larger population before any clinical claim could be made.¹⁹ What the study does establish is that a patient-referenced, fixed-baseline, pathway-level proteomic score is not merely a theoretical construct, it has already been built, and it has already produced clinically concordant alerts in a small, real longitudinal human series. PPRI extends that conceptual logic to drug- and combination-specific pathway modulation. Most importantly, PPRI retains the patient-specific pretreatment baseline while adding an independently defined therapeutic target state: a treatment-response index must ask not only how the patient has changed from where they started, but whether the observed trajectory is moving toward the state the intervention is intended to produce. PPRI and the proteomic N-of-1 framework described in the **Serial Microsampling and a Proteomic N-of-1 Framework** section are original proposals put forward in this article; they are conceptual starting points, not validated clinical or analytical endpoints, and require the validation sequence described in the **Validation Pathway this Would Actually Require** section before any clinical application.

Conceptually, for a given patient p , pathway, and treatment time t , PPRI compares the patient’s current pathway-level protein vector to two reference points: the patient’s own pretreatment baseline, and an independently defined therapeutic target state for that pathway and indication. These are not the same thing, since a pathway’s pretreatment state may represent severe, unwanted disease biology, and a drug may be intended to move the pathway toward a new state rather than simply back to where it started. Here, $\mathbf{p}_t$ denotes a prespecified normalized representation of the pathway proteins, for example, log-transformed

protein abundances expressed relative to a patient-specific pretreatment reference, rather than necessarily conventional population z-scores. One deliberately simplified candidate formulation is:

$$PPRI(p, t) = \frac{1 - d(z_{p,t} \text{ } z_{reference})}{[d(z_{p,0} \text{ } z_{reference}) + \epsilon]}$$

where $z_{p,0}$ is the patient's pretreatment pathway vector, $z_{p,t}$ is the vector at time t , $z_{reference}$ is the prespecified therapeutic target state, d is a weighted distance metric whose weights could incorporate analytical precision and biological relevance, and ϵ is a small regularization term. This expression is illustrative; it is not proposed as the final computational definition of PPRI. It exposes five requirements for method development. Denominator stability: $d(z_{p,0} \text{ } z_{reference})$ approaching zero makes an unregularized ratio unstable, so ϵ would need prespecification and sensitivity analysis rather than ad hoc insertion. Indication- and treatment-specific definition of the reference state: a single universal target is unlikely to exist, and the reference would instead need to be derived from mechanistic models, responding-cohort data, or experimentally defined perturbation states, each carrying its own causal assumptions. Preservation of directional and component-level information: a scalar distance can obscure which proteins are driving movement and in which direction, so directional pathway structure would require additional modeling, signed features, pathway topology, or a directional model, rather than weighting alone. Propagation of analytical and biological uncertainty: a clinically interpretable PPRI would need to be reported with a confidence or credible interval, not as a bare point estimate. And separation of temporal association from causal treatment effect: natural disease fluctuation, concomitant medication, cell-composition change, or toxicity could all produce an apparently favorable scalar movement, a distinction that matters especially for an N-of-1 framework with no concurrent control arm. None of this is solved; I am naming it as an open, tractable measurement-science problem, not presenting a finished instrument.

Serial Microsampling and a Proteomic N-of-1 Framework

The pilot study described in the ***From protein lists to a patient-referenced pathway metric: the Proteomic Pathway Response Index*** section supports the feasibility of using fixed-baseline, serial urine proteomes, collected during ordinary clinic visits, to produce alerts concordant with subsequent clinical assessment in a small, disease-surveillance context.¹⁹ It does not establish whether serial, patient-referenced proteomic monitoring can be extended to drug- or combination-specific titration decisions, to other specimen types, or from occasional clinic visits to something closer to routine, high-frequency collection, questions I want to leave open for the remainder of this section.

Everything argued so far still assumes proteomics happens the way it has for twenty years: a venous draw, a clinic visit, a batched run weeks later. Microsampling devices that collect dried plasma or blood from a fingerstick, designed for patient-centric, at-home, higher-frequency collection, have been validated against targeted proteomic panels.¹⁸ A 2025 study used frequent longitudinal dried-blood microsampling and a limited, targeted circulating-protein panel to track proteome dynamics in a mouse model of type 1 diabetes, identifying disease markers early enough, in principle, to support timely intervention.¹⁷ That is a genuine and useful proof of principle for the idea that frequent microsampling can capture longitudinal protein dynamics. It is not a demonstration that untargeted, high-depth DIA proteomics can deliver equivalent performance from serial human microsamples in routine clinical care, and it should not be read as one.

Clinical translation would also require control of microsampling-specific preanalytics, including hematocrit effects, collection volume and spot spreading, extraction recovery, drying and storage conditions, transport stability, hemolysis, and differences between capillary and venous blood composition, as well as temporal effects of food intake, exercise, and circadian rhythm. Higher sampling frequency does not automatically produce better biological information if these sources of variance are comparable in magnitude to the treatment-associated signal PPRI is meant to detect.

What I consider defensible is the following extrapolation, clearly labeled as an extrapolation: pairing a validated microsampling device with a high-sensitivity DIA workflow could, in principle, substantially reduce the sample-amount and access barriers that have kept proteomics confined to occasional, clinic-anchored venous draws. I will call the resulting concept, once, as a metaphor, not a technical description, a proteomic Holter monitor: not continuous sensing, but a structured cadence of low-volume, patient-collected microsamples, perhaps weekly or after each treatment cycle, feeding a DIA pipeline that returns a PPRI trajectory instead of a single snapshot. High-frequency longitudinal proteomic monitoring, or serial proteomic monitoring, is the accurate description of what this would be, and I use that description for the remainder of this argument. Continuous glucose monitoring changed diabetes management by shifting decisions from quarterly HbA1c checks to daily titration; serial, patient-referenced proteomic monitoring could plausibly do something analogous for combination oncology and immunology regimens, but the comparison is aspirational, not a claim that the enabling technology is already in place.

If such a system were analytically and clinically validated, the rationale is straightforward on at least three fronts: pharmacodynamic monitoring, assessing whether treatment is producing the expected proximal or downstream molecular response rather than merely achieving adequate systemic exposure; early resistance detection, where proteomic signatures of pathway reactivation or bypass signaling might precede radiographic or genomic evidence of relapse; and toxicity monitoring, tracking off-target proteome perturbation that correlates with adverse events before they become clinically manifest. None of these applications is conceptually new. What would be new is the measurement stability to make them routinely reliable, and that stability, as argued in the ***DDA and DIA: two acquisition strategies with different sampling properties*** section, is a real but not yet fully generalized advance.

The validation pathway this would actually require

None of the above becomes clinically usable through analytical performance alone, and it is worth spelling out the steps instead of gesturing at ‘standardization’ as a single undifferentiated task. Analytical validity comes first, and what must be harmonized is performance, reporting, and interpretation, not any single vendor’s software: harmonized specimen-handling procedures, reference materials, system-suitability criteria, acquisition controls, peptide-identification and protein-inference rules, normalization procedures, missing-data policies, model-locking procedures, reporting conventions, and external quality-assessment schemes, together with metrological traceability where achievable and demonstrated interlaboratory comparability using commutable reference materials, so that a PPRI computed on one pipeline means the same thing computed on another’s. ICH Q2 principles remain relevant to defined analytical procedures, but they do not by themselves address the full validation problem posed by an untargeted, multivariate proteomic pipeline coupled to a computational clinical score.

Clinical validity comes second: demonstrating that a PPRI trajectory actually correlates with a clinically meaningful state, response, resistance, or toxicity, across independent cohorts, specimen types, and treatment contexts, which is a substantially larger and slower undertaking than analytical harmonization. Clinical utility comes third, and is the highest bar: demonstrating, ideally in a prospective trial, that acting on a PPRI value changes a prescribing decision in a way that improves outcomes relative to not measuring it. Regulatory strategy and intended-use definition should nonetheless inform assay and software development from the outset, even though formal authorization, reimbursement, and laboratory-developed-test or in vitro diagnostic classification require the evidentiary package built across all three steps.

Because PPRI would be a multivariate computational output, not a single-analyte assay, validation would also have to separately address the underlying measurement itself, peptide identification and protein quantification, pathway membership and weighting, the locked score calculation, the clinical decision threshold, and the clinical interpretation, and would have to address feature locking, pathway-definition versioning, software updates, missing-feature handling, calibration drift, and the risk that changes in the analytical pipeline alter the score independently of any underlying biology.

Sustainability by design: from analytical efficiency to system-level value

Sustainability by design should accompany the validation pathway from its earliest stages, not be assessed only after a workflow has already been established. Analytical chemistry already has a mature vocabulary for this: green analytical chemistry codifies principles for reducing a method's environmental footprint, solvent and reagent consumption, waste generation, energy use, sample and reagent miniaturization, while white analytical chemistry evaluates analytical performance, environmental impact, and practical or economic feasibility as interconnected dimensions of method quality; greenness is not treated as a constraint that necessarily trades off against data quality.^{20,21} Although nano-flow chromatography limits mobile-phase consumption at the analytical column, the environmental footprint of large-scale proteomics also includes sample-preparation reagents, disposable plastics, enrichment consumables, instrument and gas-generator electricity, laboratory climate control, computational processing, data storage, and failed or repeated analyses. Solvent volume alone therefore provides an incomplete measure of analytical burden. This broader accounting also changes how chromatographic format should be evaluated. Nano-flow separations remain particularly well suited to very low sample inputs, including single-cell and near-single-cell workflows, where sensitivity is often the dominant constraint. For routine plasma, urine, and tissue-digest analysis at clinical scale, capillary- and microflow configurations may offer a more favourable compromise among sensitivity, chromatographic robustness, throughput, and operational complexity, although the optimal format remains dependent on sample amount, instrument sensitivity, and analytical objectives.^{22,23} Susceptibility to emitter blockage, the effectiveness of carryover-control procedures, and the degree of specialist intervention required may favour higher-flow formats in some settings; these are practical characteristics of a given chromatographic setup, not universal consequences of flow rate alone.

Solvent consumption per injection is therefore an insufficient primary sustainability metric for this architecture. The more relevant quantity is resource and energy use per analytically valid result meeting a prespecified intended-use performance standard. This is a proposed evaluation principle, not an already standardized metric with an agreed numerator, denominator, or system boundary. Such a metric should account for failed injections, repeat analyses, column and consumable lifetime, instrument utilization, computational processing and data-storage requirements, operator time, maintenance burden, and failure-associated resource use. Used in isolation, however, it can be improved without any genuine gain in clinical value, simply by reducing analytical scope, sensitivity, or clinical information content below what the intended use requires. Conversely, a narrower assay may represent a genuine efficiency gain if it preserves the analytical performance and clinical information required for the intended use. The metric is therefore only meaningful when interpreted alongside analytical scope, quantitative performance, and clinical information content. On this basis, a robust higher-flow method could plausibly outperform a more sensitive but less operationally stable nano-flow workflow, depending on the specimen, instrument, workflow configuration, and intended use.

The architecture proposed in this paper therefore presents a plausible sustainability hypothesis, but that hypothesis should be measured, not asserted. Microsampling could replace some venous collections with microlitre-scale sampling, potentially reducing biohazard waste and the logistical burden of repeated collection, although this benefit has not been established through formal life-cycle comparison. High-frequency monitoring could multiply the number of proteomic measurements performed per patient relative to episodic testing, and that added analytical burden must be evaluated against the procedures, visits, or ineffective treatments the monitoring system demonstrably changes. If prospectively shown to add sufficient clinical value, serial proteomic monitoring might eventually alter the timing or intensity of confirmatory procedures. In the bladder-cancer context described in the ***From protein lists to a patient-referenced pathway metric: the Proteomic Pathway Response Index*** section, this could include cystoscopy; however, the pilot study did not test whether dPPI could replace or reduce cystoscopy, so any such benefit remains hypothetical.

Similarly, proteome-informed treatment selection might reduce pharmaceutical and administration-related resource use if it were shown to prevent ineffective treatment exposure. Whether such savings would outweigh the analytical workflow's own material and energy requirements is not an analytical-performance question

alone; it requires formal life-cycle and health-system assessment. Any such assessment would need an explicit system boundary, for example, the analytical assay, laboratory workflow, patient-care episode, or full treatment pathway, because conclusions may change substantially depending on what is included. The net environmental balance could be favourable, neutral, or unfavourable depending on monitoring frequency, analytical configuration, clinical effectiveness, and what the assay actually displaces.

Sustainability by design should be built into analytical development, validation, and implementation from the outset and interpreted alongside analytical scope, quantitative performance, and clinical information content, not judged by resource consumption in isolation. Green and white analytical chemistry can provide the framework for defining assay-level metrics; assessing the complete architecture requires a broader, system-level evaluation involving life-cycle assessment specialists, clinicians, health economists, and health-system researchers.

Prescriptomics as the multidisciplinary implementation framework

In 2022, together with Capelo, Lodeiro, Dhir, and Kravchenko-Balasha, I proposed prescriptomics as a framework for integrating multi-omics data specifically to guide drug selection, dosing, effectiveness, safety, and rational combination therapy, not merely to integrate omics layers for diagnostic classification.¹³ Prescriptomics provides an appropriate implementation framework for the ideas developed above because none of them is adequately addressed as a single-omics problem. A PPRI will often be more interpretable when considered alongside genomic, transcriptomic, pharmacological, pathological, and clinical information that helps establish which pathways are relevant for a particular patient and treatment. The validation pathway described in the ***Validation Pathway this Would Actually Require*** section is likewise inherently multidisciplinary.

Prescriptomics will require institutional structure if it is to move from a conceptual framework to clinical practice. Training programs should begin defining and evaluating formal competencies in prescriptomic interpretation, spanning clinical pharmacology, molecular pathology, analytical proteomics, bioinformatics, biostatistics, and evidence-based therapeutic decision-making. These competencies are proposed here; they are not already established, and whether prescriptomics ultimately becomes a recognized subspecialty should depend on demonstrated clinical need and utility.

Hospitals conducting serial or combination-therapy protocols could evaluate prescriptomic boards structured analogously to molecular tumor boards. Such boards would review a patient's longitudinal proteomic trajectory, pharmacological context, and relevant multi-omic evidence at treatment decision points, not only at diagnosis. Their implementation would require defined membership, evidentiary standards, reporting formats, procedures for communicating uncertainty, and clear clinical accountability. This proposal builds on an emerging international scientific community reflected in initiatives such as the Caparica Conference series on Prescriptomics and Precision Medicine, although community formation should not be confused with implementation of a validated clinical service.

Computational studies reviewed in Reference 16 suggest that integrated multi-omic models can improve drug-response prediction in some benchmark settings, although performance depends strongly on cohort construction, data-integration strategy, comparator selection, and external validation. More defensible near-term applications of peptide- or fragment-level DIA representations include feature representation, cross-run and cross-platform alignment, quality control, anomaly detection, and pathway-level response modeling. These are distinct tasks, and success in one does not imply success in the others. Algorithmic denoising must also be approached cautiously because a model may remove weak biological signal together with technical variation.

Missing-value recovery is particularly sensitive. An imputed value may appear biologically plausible while suppressing genuine measurement uncertainty and creating a false impression of quantitative continuity. Any model-generated value should therefore be reported as an estimate with explicitly propagated uncertainty, not treated as a direct measurement.

Evaluation of these models would require patient-level and site-level separation, prospective temporal validation, uncertainty calibration, and explicit testing against batch, instrument, and workflow confounding. Many omics machine-learning studies overestimate performance through non-independent validation in which samples, patients, batches, or centers leak across training and test sets. Raw DIA data also encode substantial technical domain structure arising from sample preparation, chromatography, acquisition settings, instrument platform, laboratory practice, and data-processing version. A model that has not been challenged against these sources of domain shift is not ready to inform prescribing, regardless of its performance on randomly partitioned data from a single site.

External validation alone would not be sufficient. Any model intended for clinical decision support would need to demonstrate incremental value beyond standard clinical variables, conventional biomarkers, and appropriately chosen simpler models. It would also require auditability, controlled versioning, performance-drift monitoring, uncertainty reporting, and defined human oversight.

A ten-year research agenda with testable milestones

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A ten-year research agenda with testable milestones

I want to end with specific, checkable commitments, not a general call for more research. Seven problems need to be addressed, each through a measurable milestone with prespecified success and failure criteria; the targets below are illustrative research targets, not validated thresholds.

Cross-platform transferability: Within the next several years, the field should be able to show that a PPRI, or an equivalent pathway-response score, computed independently on ion-mobility and Orbitrap/Astral platforms, achieves concordance above a prespecified, use-case-specific acceptance threshold defined before analysis. This should be tested not only on split aliquots of the same longitudinal sample set, which establish analytical comparability alone, but also through repeat sampling, preanalytical replication, and independent execution in separate laboratories, which together are needed to establish longitudinal measurement reliability and to partition platform, laboratory, preanalytical, and within-person biological variance. Failing to meet that threshold would be an informative result showing the index is not yet transferable, not a reason to relax the criterion afterward.

Analytical and clinical validation: Analytical performance specifications and a locked candidate pipeline should be established before definitive clinical validation begins, including prespecified limits for longitudinal precision, missingness, batch effects, calibration drift, carryover, and score stability, consistent with the analytical-validity-first sequence described in the ***Validation Pathway this Would Actually Require*** section, although earlier-phase clinical data may still be needed during development to define the score's features, weights, and thresholds ahead of that lock. Once locked, a prospective cohort should demonstrate that PPRI trajectories, computed under that harmonized pipeline, predict an independently assessed clinical endpoint, treatment response, resistance emergence, or toxicity, using prespecified effect measures, calibration analyses, confidence intervals, and external validation. The strongest version of this milestone is a prospective, temporal design in which the score measured at time t predicts an outcome occurring after t , evaluated with decision-curve or net-benefit analysis.

Biological specificity: A pathway score should reflect the intended therapeutic perturbation when that effect is expected to be observable in the sampled compartment, while remaining distinguishable from variation caused by inflammation, cell-composition shifts, circadian effects, renal or hepatic dysfunction, concomitant medication changes, acute intercurrent illness, and unrelated treatment toxicity. Without this, a technically reproducible index might still be clinically nonspecific.

Clinically actionable turnaround: Serial monitoring is only useful if results return fast enough to inform the next clinical decision. The acceptable turnaround threshold is not fixed; it must be defined in relation to

the specific decision being supported, potentially hours for selected urgent applications, days for resistance monitoring, or completion before the next treatment cycle for dose adjustment. A workflow that cannot report a PPRI value within the threshold appropriate to its intended use should be described as a research tool, not a monitoring platform, until it can.

Regulatory and reimbursement qualification: No such index should be described as guideline-adjacent until it has moved through the analytical-validity, clinical-validity, and clinical-utility sequence described in the **Validation Pathway this Would Actually Require** section. Regulatory qualification and reimbursement will require overlapping but distinct evidence packages: regulatory bodies focus on intended use, safety, analytical validity, software control, and clinical performance, while payers focus more on comparative benefit, cost-effectiveness, utilization, and budget impact. Regulatory engagement, intended-use definition, and payer evidence planning should nonetheless begin much earlier and run in parallel with that sequence, not be treated as a final step attempted only once clinical utility is established.

Institutional implementation: Prescriptomic review structures should be piloted and prospectively evaluated at selected centers over the coming years, generating publishable operational data on whether the prescriptomic review structure is feasible, reproducible, operationally sustainable, and capable of influencing decisions appropriately. A pilot that instead surfaces governance failures or logistical barriers would still constitute progress, since knowing why the structure does not work is as informative as knowing that it does.

Causal attribution and intervention testing: A PPRI trajectory that merely correlates with outcome is not sufficient grounds for a treatment decision. Once the score, threshold, treatment algorithm, and safety constraints have been prospectively locked, the field needs a trial or quasi-experimental design in which acting on the score, adjusting, escalating, or de-escalating treatment based on a PPRI threshold, is shown to change outcomes relative to standard care. A randomized biomarker-strategy trial is likely the strongest primary design for this purpose, since stepped-wedge alternatives remain vulnerable to secular changes in treatment and analytical performance over the study period. This milestone is what would convert PPRI from a predictive marker into an actionable clinical tool.

Above all, PPRI would need to add predictive or decision value beyond baseline clinical variables, genomics, and conventional biomarkers, not merely correlate with outcome. Absent that, a reviewer would be right to conclude the index is an elaborate summary of already measurable disease burden, not a new source of clinical information. I consider substantial progress across all seven milestones achievable within a decade, but achievable and already achieved are different claims. The proteome is not yet the prescription. Current analytical capability is sufficiently advanced to justify prospective testing of that role in narrowly defined clinical use cases, and the analytical chemistry community is particularly well positioned to lead the measurement and standardization work needed to determine whether that role can be realized.

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REFERENCES

- (1) Meier, F.; Brunner, A.; Frank, M.; Ha, A.; Bludau, I.; Voytik, E.; Kaspar-Schoenefeld, S.; Lubeck, M.; Raether, O.; Bache, N.; et al. diaPASEF: parallel accumulation–serial fragmentation combined with data-independent acquisition. *Nat. Methods* **2020**, *17*, 1229–1236. <https://doi.org/10.1038/s41592-020-00998-0>
- (2) Zou, X.; Wang, L.; Chen, Y.; Fu, H.; Gao, Y.; Liu, B.; Tan, M.; Zhai, L. In-depth analysis of data characteristics and comparative evaluation of DDA and DIA accuracy in label-free quantitative proteomics of biological samples. *Clin. Proteomics* **2025**, *23* (2). <https://doi.org/10.1186/s12014-025-09572-2>
- (3) Oliinyk, D.; Gurung, H.; Zhou, Z.; Leskoske, K.; Rose, C.; Klaeger, S. diaPASEF analysis for HLA-I peptides enables quantification of common cancer neoantigens. *Mol. Cell. Proteomics* **2025**, *24* (4), 100938. <https://doi.org/10.1016/j.mcpro.2025.100938>
- (4) Guzman, U.; Martinez-Val, A.; Ye, Z.; Damoc, E.; Arrey, T.; Pashkova, A.; Renuse, S.; Denisov, E.; Petzoldt, J.; Peterson, A. C.; et al. Ultra-fast label-free quantification and comprehensive proteome coverage with narrow-window data-independent acquisition. *Nat. Biotechnol.* **2024**, *42*, 1855–1866. <https://doi.org/10.1038/s41587-023-02099-7>
- (5) Hendricks, N.; Bhosale, S.; Keoseyan, A.; Ortiz, J.; Stotland, A.; Seyedmohammad, S.; Nguyen, C.; Bui, J.; Moradian, A.; Mockus, S.; Van Eyk, J. E. An inflection point in high-throughput proteomics with Orbitrap Astral: analysis of biofluids, cells, and tissues. *J. Proteome Res.* **2024**, *23* (9), 4163–4169. <https://doi.org/10.1021/acs.jproteome.4c00384>
- (6) Guzman, U.; Rykær, M.; Hendriks, I.; Stewart, H.; Denisov, E.; Hagedorn, B.; Petzoldt, J.; Kreutzmann, A.; Mueller, Y.; Arrey, T. N.; et al. Higher-throughput proteome profiling enabled by parallelized pre-accumulation and optimized ion processing in the Orbitrap Astral Zoom mass spectrometer. *Mol. Cell. Proteomics* **2026**, *25* (2), 101504. <https://doi.org/10.1016/j.mcpro.2025.101504>
- (7) Lopes, F. B. T. P.; Schlatzer, D.; Sudhadevi, T.; Harijith, A.; Ayati, M.; Koyutürk, M.; Chance, M. What does next-generation mass spectrometry offer for proteomics? A comprehensive platform comparison. *J. Proteome Res.* **2026**, *25* (4), 1929–1940. <https://doi.org/10.1021/acs.jproteome.5c01007>
- (8) McNamee, N.; Liu, J.; Poulos, R.; Aref, A.; Reddel, R. Predictive biomarkers of antibody–drug conjugate efficacy for solid tumors: current challenges and the potential role of quantitative proteomics. *Clin. Cancer Res.* **2026**, *32* (4), 661–673. <https://doi.org/10.1158/1078-0432.CCR-25-3103>
- (9) Rasam, S.; Lin, Q.; Shen, S.; Straubinger, R.; Qu, J. Highly reproducible quantitative proteomics analysis of pancreatic cancer cells reveals proteome-level effects of a novel combination drug therapy that induces cancer cell death via metabolic remodeling and activation of the extrinsic apoptosis pathway. *J. Proteome Res.* **2023**, *22* (12), 3780–3792. <https://doi.org/10.1021/acs.jproteome.3c00463>
- (10) Yu, P.; Kao, Y.; Chang, H.; Huang, C.; Hsu, W.; Huang, H.; Juan, H. Quantitative proteomics unveils the synergistic effects of combination drugs on cytoskeleton composition and autophagy-mediated cell death in neuroblastoma. *J. Proteome Res.* **2025**, *24* (7), 3578–3596. <https://doi.org/10.1021/acs.jproteome.5c00191>
- (11) Xu, T.; Ngan, D.; Zheng, W.; Huang, R. Systematic identification of cancer pathways and potential drugs for intervention through multi-omics analysis. *Pharmacogenomics J.* **2025**, *25*, 2. <https://doi.org/10.1038/s41397-025-00361-6>
- (12) Jiang, Y.; Wang, J.; Sun, A.; Zhang, H.; Yu, X.; Qin, W.; Ying, W.; Li, Y.; Chang, C.; Wang, X.; et al. The coming era of proteomics-driven precision medicine. *Natl. Sci. Rev.* **2025**, *12* (8), nwaf278. <https://doi.org/10.1093/nsr/nwaf278>
- (13) Capelo, J.; Lodeiro, C.; Dhir, R.; Kravchenko-Balasha, N.; Santos, H. Prescriptomics: the next frontier in medicine. *J. Integr. OMICS* **2022**, *12* (1). <https://doi.org/10.5584/jiomics.v12i1.211>

- (14) Chan, J.; Flores-Campuzano, G.; Hu, Y.; Liu, M.; Marispini, M.; Mora, M.; Ramaswamy, S.; Ranjan, P.; Williams, P.; Zawada, R.; et al. timsTOF HT improves protein identification and quantitative reproducibility for deep unbiased plasma protein biomarker discovery. *J. Proteome Res.* **2024**, *23* (3), 929–938. <https://doi.org/10.1021/acs.jproteome.3c00646>
- (15) Li, S.; Li, S.; Liu, S.; Ren, Y. Mass spectrometry-based solutions for single-cell proteomics. *Genomics, Proteomics Bioinf.* **2025**, *23* (1), qzaf012. <https://doi.org/10.1093/gpbjnl/qzaf012>
- (16) Sada Del Real, K.; Swamy, V.; Arcagni, J.; Wang, E.; Rabadan, R.; Rubio, A. Foundation models and deep learning for cancer drug response prediction: a framework for data, metrics, and validation. *Brief Bioinform.* **2026**, *27* (3), bbag225. <https://doi.org/10.1093/bib/bbag225>
- (17) Parajuli, A.; Bendes, A.; Byvald, F.; Stone, V.; Ringqvist, E.; Butrym, M.; Angelis, E.; Kipper, S.; Bauer, S.; Roxhed, N.; Schwenk, J.; Flodström-Tullberg, M. Frequent longitudinal blood microsampling and proteome monitoring identify disease markers and enable timely intervention in a mouse model of type 1 diabetes. *Diabetologia* **2025**, *68*, 2277–2289. <https://doi.org/10.1007/s00125-025-06502-7>
- (18) Hober, A.; Henricsson, M.; Ruckh, T.; Davidsson, P.; Challis, B.; Miliotis, T. Quantitative assessment of a novel device designed for patient-centric sampling of dried plasma using targeted proteomics. *Anal. Chem.* **2025**, *97* (25), 12953–12962. <https://doi.org/10.1021/acs.analchem.4c05455>
- (19) Carvalho, L.; Capelo, J.; Lodeiro, C.; Dhir, R.; Campos, P. L.; López-Fernández, H.; Martins, G.; Medeiros, M.; Díaz, F.; Santos, H. Pathway-guided monitoring of the disease course in bladder cancer with longitudinal urine proteomics. *Commun. Med.* **2023**, *3*, 8. <https://doi.org/10.1038/s43856-023-00238-4>
- (20) Zou, Y.; Tang, W.; Li, B. Mass spectrometry in the age of green analytical chemistry. *Green Chem.* **2024**, *26* (9), 4975–4986. <https://doi.org/10.1039/D3GC04624A>
- (21) Pingili, D.; Awasthi, A.; Varshney, M. White analytical chemistry: a review of current developments. *J. Anal. Chem.* **2025**, *80* (6), 959–968. <https://doi.org/10.1134/S1061934825700327>
- (22) Tsiklauri, G.; Zheng, R.; Kabella, N.; Prokofeva, P.; Pynn, C.; Kuster, B. High-performance proteomics using nano-, capillary-, and microflow chromatographic separations. *J. Proteome Res.* **2025**, *24* (10), 4988–5000. <https://doi.org/10.1021/acs.jproteome.5c00327>
- (23) Sui, X.; Wu, Q.; Cui, X.; Wang, X.; Zhang, L.; Deng, N.; Bian, Y.; Xu, R.; Tian, R. Robust capillary- and micro-flow liquid chromatography–tandem mass spectrometry methods for high-throughput proteome profiling. *J. Proteome Res.* **2022**, *21* (10), 2472–2480. <https://doi.org/10.1021/acs.jproteome.2c00405>



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