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Toward standardization and a concerted vision for platelet proteomics research: communication from the SSC of the ISTH

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All authors wrote the manuscript.

Conflicts of Interest

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Abstract

Over the past three decades, omics technologies have revolutionized our understanding of platelet molecular content and organization, enabling the systematic analyses of platelet physiology. Among these approaches, proteomics has been especially significant in discovering as well as validating molecular mechanisms of platelet function in health and disease. However, several conceptual and practical challenges continue to limit the full utility of platelet proteomics tools and data. Methodological and analytical inconsistencies remain a key concern, with biological and technical variables exerting substantial influence on study outcomes and interpretation. These issues are compounded by the rapid pace of proteomics tool development and dataset collection, outstripping efforts to standardize best practices and ensure consensus as platelet proteomics consolidates itself as a tool for research even outside the Thrombosis and Hemostasis field.

In this communication from the International Society on Thrombosis and Haemostasis (ISTH) Scientific and Standardization Committee (SSC), we highlight recent advances in platelet proteomics studies, and we identify where collective efforts can strengthen experimental design, execution, and analysis. As a practical recommendation, we encourage platelet biologists to recognize current discrepancies and advance efforts to standardize and customize methods and reporting practices, including blood collection, platelet isolation, data acquisition, and data interpretation. By aligning protocols and ensuring detailed reporting, the field can more effectively integrate proteomics findings and accelerate our understanding of platelet biology.

Keywords

Biomarkers; Blood Platelets; Mass spectrometry; Physiology; Proteomics; Thrombosis; Hemostasis

1. Introduction

Platelets serve as the primary cellular mediators of hemostasis, while also supporting diverse aspects of health and disease. The multifaceted contributions of platelets to vascular biology, inflammation, immunity and other areas, arise, in large part, from platelet interactions with their milieu via a repertoire of cell surface receptors, coupled to their ability to

store, mobilize, uptake and release diverse biologically active modulators in a given physiologic context. Deciphering the molecular mechanisms by which platelets transduce environmental and physiological stimuli into functional cellular responses is a cornerstone of platelet biology research [1]. Historically, these topics have been pursued through meticulous studies of individual platelet responses with specific assays at the biochemical and cellular level. While these approaches remain critical to understanding the biology of platelets, recent studies of the molecular mechanisms of platelet function have been revolutionized by unbiased, high-throughput approaches that identify and quantify protein dynamics in platelets under specific physiological and pathological conditions [2–5]. To this end, proteomics tools, especially mass spectrometry (MS), have quantified the specific protein contents of platelets, defined secretomes, mapped out intracellular signaling systems underlying platelet function, and are expected to continually fuel discovery as well as systematic and functional studies of platelets [6–12].

The promises and pitfalls of proteomics strategies have long been recognized by researchers as well as other members of the greater International Society on Thrombosis and Haemostasis (ISTH) community – many of whom implement and also develop proteomics methods to address questions in the hemostasis and thrombosis fields [2, 4, 7, 13]. Nearly 20 years ago, the ISTH Scientific and Standardization Committee (SSC) subcommittee on Platelet Physiology published a prescient report on “Mapping the platelet proteome”, summarizing early accomplishments, as well as noting technical and conceptual limitations around the application of proteomics tools to platelet studies [14]. While many technical issues have been resolved or accounted for since that time, other barriers to understanding platelet proteomes persist or have evolved and grown in complexity. Here, we consider how existing proteomics tools and concepts may now best be utilized amongst researchers from the perspective of the SSC subcommittees of the ISTH Models of Thrombosis and Hemostasis, Platelet Physiology and Predictive and Diagnostic Variables in Thrombotic Disease. Given the diversity and complexities of planning, executing, validating, and interpreting large-scale platelet proteomics experiments, thorough and complete standardization between platelet proteomics scientists is still not likely possible, nor practical. Nonetheless, the platelet, hemostasis, thrombosis, and proteomics research communities are all likely to benefit from consensus guidelines in recognition of how specific experimental, analytical, and conceptual variables can affect platelet proteomics experiments, and how such variables should be accounted for, discussed and, perhaps most importantly, reported.

2. Platelet proteomics studies from past to present

When the ISTH Platelet Physiology SSC subcommittee overviewed efforts to map the platelet proteome in 2005, a total of 93 manuscripts were indexed on “platelet proteomics” in PubMed. At present, there are over 2,000 manuscripts indexed, and the application of proteomics to platelet studies is steadily increasing, even outside the Thrombosis and Hemostasis field (Figure 1). Most of the earliest proteomics studies of platelets followed the advent of two-dimensional electrophoresis (2-DE) [15]. The earliest applications of 2-DE in platelet biology included studies identifying abundant and dynamically regulated cytosolic proteins, and membrane glycoproteins in platelets in quiescent and activated states [16].

Eventually, 2-DE studies unmasked many of the dynamic, post-translational modifications (PTMs) that progress platelet activation programs, namely reversible tyrosine, serine, and threonine phosphorylation [17, 18]. As MS-based proteomics methods gradually replaced 2-DE in the early 2000s, higher resolution and broader dynamic ranges of proteins were detected, albeit at higher operational costs and more complex data analysis.

The first comprehensive, quantitative analysis of the human platelet proteome by MS was described by Burkhart *et al.* in 2012 [9]. This study determined copy number estimates and quantified the rank-order abundances of more than 3,700 unique proteins in human platelets, and provided peptide-level evidence for the presence of about 4,190 total proteins in the human platelet proteome. Since that time, continual advancements in proteomics methods and upstream sample fractionation have enabled the characterization of specific platelet compartments or subproteomes, including the plasma membrane surfaceome [19], α -granule proteome [20], secretome [10], and platelet-derived extracellular vesicle (EV) proteomes [21]. Advances in peptide enrichment and detection methods have also specified regulated phosphorylation sites on platelet proteins, allowing for the characterization of signalosomes and pathways driving platelet responses downstream of key platelet receptors, including GPVI [12], CLEC-2 [22], and P2Y₁/P2Y₁₂ [11]. Several other varieties of PTMs have also been identified in platelets [23], including acetylation [24], methylation [25], ubiquitinylation [26], glycosylation [27], and palmitoylation [28]. Now, a range of different MS-based experimental approaches can routinely quantify relative levels of thousands of proteins and PTM-containing peptides (*e.g.*, phosphopeptides) in platelet samples, poising the field for a revolution in the systematic understanding of platelet and cell function in general [2, 5–7].

In the translational realm, proteomics has detailed platelet-based molecular signatures associated with *in vivo* systemic disease [29–32], as well as *ex vivo* platelet storage lesion in the context of transfusion medicine [33]. Proteomics studies of platelets also support the notion that platelets can reflect specific health states or responses to treatment in a manner informative to biomarker discovery, and to the development of targeted and specific antiplatelet therapies [4, 13, 34, 35]. Yet proteomics markers in platelets unique to specific disease states remain to be elucidated [2, 4, 6], as only tens of studies have measured changes in platelet proteins or proteomic features in specific human disease conditions. For instance, the transfer of neutrophil proteins (*e.g.*, S100A8/9) to platelets is a common proteomic feature of platelets in many disease states, including cardiovascular disease [36] and COVID-19 [37]. Platelet proteomes differ qualitatively through development and in disease, and proteins involved in platelet metabolism appear to vary, as a common feature, in a range of conditions [38, 39]. Promising, but more limited examples of baseline alterations in platelet signaling in health and disease are also apparent through phosphoproteomic analyses of platelets in Scott syndrome [40], obesity [41], metabolic conditions (*e.g.*, pseudohypoparathyroidism) [42], and aging [43]. As basic, translational, and clinical studies of platelet proteomes progress, methodologies and analysis platforms are likely to increase in complexity and diverge. Thus, acknowledgement of methodological and analytical differences is imperative for adequate interpretation, understanding, discussion, and ultimately, clinical translation of platelet proteomics tools, as discussed below.

3. Sample preparation and preanalytical variables in platelet proteomics studies

It has long been recognized that preanalytical variables affect proteomics results, especially methodological procedures used in preparing blood components and other biological samples for experimentation. The relationship between sample processing methodologies and downstream proteomics results has been of recognized and of growing interest to platelet proteome studies for around two decades [14, 44–46]. Despite considerable inter-laboratory similarities amongst platelet preparation methodologies, several preanalytical challenges limit the interpretation and impact of many platelet proteomics studies. A number of *ex vivo* factors, such as choice of blood anticoagulants, storage time, and processing temperature, among others, can affect the platelet proteome [33, 44, 47]. Likewise, standardization efforts apart from the ISTD, including the Human Proteome Organization (HUPO) Plasma Proteome Project, reciprocally demonstrate that blood processing procedures can have specific effects on the proteomes of other blood cells and fluids, often in a manner involving platelet activation, secretion and/or endocytosis. The purity of the target sample or fraction of study is a key aspect in platelet proteomics studies, and contaminant proteins resulting from inadequate depletion of white and red blood cells, and plasma proteins from platelet preparations may also impact downstream analyses [47, 48]. Thus, bearing all of these in mind, a specific choice during the processing methodology must not be assumed as standard method, and should be reported.

Here, we highlight key areas where standardization has been, and is, increasingly recognized to be relevant in platelet, plasma, and other proteomics studies, and we recommend where discussion and consensus should be prioritized to benefit the ongoing and future expansion and utility of platelet proteomics analyses. Ultimately, there is not likely to be an agreement or complete adoption of several of these specific steps for all research groups. Nonetheless, individual research teams should be encouraged to maintain their own consistent platelet preparation workflows to avoid introducing artefactual effects based solely on intra-experimental differences in blood extraction and sample handling. Accordingly, blood collection and processing steps should, at a minimum, be consistent for all samples throughout an entire experiment; ideally, each step should be reported, and proteomics data should be accompanied with assays addressing platelet purity (e.g., using a hematology analyzer or residual white blood cell counter, or by flow cytometry quantification of platelets, leukocytes and erythrocytes in the sample) and baseline function quality controls (flow-cytometry analysis of platelet surface, degranulation and activation markers in quiescent and stimulated platelets, when experimentally required) (see Table 1 and Figure 2).

3.1. Understand how blood collection affects the platelet proteome.

In general, phlebotomy and blood collection procedures show only minor differences between most platelet research groups. Still, it remains to be determined whether and how several choices in blood collection protocols prior to preparing washed or purified platelets affect platelet function and their proteomes. Variables around how blood is drawn from healthy human donors, patients, or even animal models of choice, should all be

considered in the platelet proteomics study design, including site of blood collection (*e.g.*, venipuncture *vs* port draw), types of needles, syringes, collection tubes, and anticoagulants. For example, a recent quantitative, high resolution MS study demonstrates that differences in *ex vivo* blood anticoagulation (*e.g.*, citrate, ethylenediaminetetraacetic acid -EDTA- and heparin) have specific, although minimal, effects on the platelet proteome composition [44]. Citrate anticoagulants (*e.g.*, sodium citrate, acid citrate dextrose) are generally preferred for blood collection by most platelet groups for biochemical and proteomics experiments. However, the use of other anticoagulants (*e.g.*, EDTA, heparin), is not *per se* contraindicated for proteomics studies, particularly for specific multi-omics workflows [49], or for anticoagulant-focused studies (*e.g.*, platelet proteome in heparin-induced thrombocytopenia). Accordingly, it is critical that the same anticoagulant is used consistently to prepare all samples throughout a specific platelet proteomics study. Other related studies show that preanalytical variations such as drawing blood into a syringe *vs* a vacuum tube can affect the proteome and molecular composition of plasma and blood samples [47, 50, 51]; however, it is not well documented whether these and other sample collection factors (*e.g.*, fasting) may impact the results of platelet proteomics experiments. Accordingly, medications affecting platelets (*e.g.*, antihypertensive, anti-inflammatory and anti-platelet drugs) should be excluded unless their administration is standard-of-care in the study population (*e.g.*, acute myocardial infarction patients), or when such agents are the subject of a study. In those cases, controls should be carefully designed to avoid biases, by including not only healthy untreated individuals as control group, but also a control group of patients receiving the same medication (if possible), either for the treatment of another condition or at a different pathophysiological state than the study cohort.

3.2. Effects of blood sample storage and transport on the platelet proteome.

Sample environment, transport, and storage conditions each generally influence the results of proteomics experiments [52], and likely impact platelet proteomics studies. The time between blood collection and processing to prepare platelets and other blood components for analyses can have profound effects on proteomics studies and should be explicitly stated. For instance, platelets harvested from anticoagulated blood samples that remain unprocessed for 1 h *vs* 24 h lose several granule proteins over time through secretion, while internalizing some plasma proteins through endocytosis [44]. Likewise, complementary studies of plasma proteomes note blood storage time-dependent increases in the presence of platelet specific proteins [47]. Accordingly, all samples for a given platelet proteomics study should be processed in the same timeframe to avoid effects of extended blood storage times. For example, it is not ideal to compare platelet proteomes from control blood samples that are collected and immediately processed in a laboratory, relative to experimental blood samples that are shipped from a collaborating clinical site with several hours of preprocessing travel time. Protocols for preparing washed platelets for biochemical and cell biological studies (including proteomics studies) comprise gentle mixing of anticoagulated blood samples by manual inversion. If the logistics of the study impede immediate processing of the sample, slow mechanical rotation to avoid sedimentation shall be considered; however, the impact of maintaining whole blood or washed platelet samples with or without agitation remains to be specified at the proteome level, with minimal impact at the platelet functional level [53]. Likewise, a number of studies indicate that maintaining whole blood and washed platelet

samples at room temperature preserves platelet responses better than storing them at 30°C [53–56]; however, the ideal temperature for maintaining platelet proteomes has not yet been specifically studied.

3.3. Understand how blood processing affects the platelet proteome.

Platelet recovery and purification through a series of differential centrifugation steps likely affects the platelet proteome, and intuitively, it is expected that washing cycles should be kept to a minimum to avoid platelet activation. However, it is not clear if increased washing steps, duration or buffer volume can reduce levels of plasma and other proteins associated with platelets, without undesired platelet activation and subsequent alterations in the platelet proteome. Low speed centrifugation (*e.g.*, $200 \times g$, without braking) of anticoagulated blood to obtain platelet-rich plasma (PRP) is generally recommended, followed by the collection of the upper portion of the PRP fraction (leaving approximately one fourth of the volume above the interphase behind). Moreover, it is not known whether platelet proteomes significantly differ if platelets are prepared by differential centrifugation, gel filtration, acoustophoretic-based or other methods [44, 57, 58]. A recent study compared centrifugation and leukocyte depletion methods in preparing platelets for transcriptomics analyses and found differences at the RNA level. This ISTH SSC Communication concluded that “teams should consider the relative advantages and disadvantages of these platelet isolation techniques to determine their preferred method of platelet isolation” [59], underscoring the impact of different sample preparation procedures on omics studies. Finally, the use of modified HEPES/Tyrodé's buffer is typically recommended for platelet washing and collection, where buffer components and their concentrations should likewise be reported [12, 44].

3.4. Effects of platelet inhibitors on platelet proteome.

Inhibitors of platelet activation are often introduced into specific steps of washed platelet preparations to limit platelet activation. However, the effect of prostaglandin (PGE1/PGE2), prostacyclin (PGI2), apyrase, aspirin, and other inhibitors on the results of platelet proteomics analyses is not well specified. While it is unlikely that these agents will significantly affect the analysis of platelet proteomes, some inhibitors may alter the outcome of post-translational protein modifications (*e.g.*, phosphorylation, acetylation) in the context of platelet activation and intracellular signaling studies. It should also be noted that some inhibitors exert transient actions and can be eliminated over time by metabolism (*e.g.*, PGI2), or by washing platelets (*e.g.*, apyrase), while others are irreversible (*e.g.*, aspirin) [4, 11].

3.5. Effects of different lysis methods on the platelet proteome.

Sample lysis collection buffers can impact how proteins are solubilized, digested, and ultimately detected by MS methods [60]. The effects of specific detergents and proteases (apart from trypsin), as well as how the use of protease and phosphatase inhibitors affects platelet proteomics experiments are not well documented.

3.6. Recognize and define contaminants from intrinsic and experimental sources.

Investigators should note that many proteins that are considered as contaminants from external (*e.g.*, hair and skin proteins, such as keratins) or intrinsic (*e.g.*, blood cell and plasma proteins) sources are often filtered out of MS results, especially when collaborating with MS facilities with limited experience in hematologic studies. Overall, it is still unclear how the identification of several such proteins in platelet samples may reflect the biology of interest, or contamination, especially as platelets can incorporate erythrocyte, leukocyte, plasma, and other proteins through physiological as well as artefactual processes from sample processing. For example, fibrinogen and coagulation factor V – both abundant and physiologically relevant components of the platelet proteome and platelet secretome – are incorporated into megakaryocytes and platelets from the plasma [61, 62]. Accordingly, defining true platelet *vs* plasma proteins and proteomes remains conceptually elusive. To this end, microscopy and high-throughput imaging analyses offer promise in visualizing proteins within platelets to better determine if they are structural, vesicular, or surface-attached proteins of the platelet proteome [63].

Altogether, artifacts from preanalytical variables can undermine the biological interpretation and cross-validation of platelet proteomics data. The optimization and standardization of sample handling to avoid platelet activation and contamination with other blood components is critical not only for obtaining unbiased, biologically informative results, but also for reproducible clinical translation of platelet proteomics [8]. While it is not feasible or practical for all researchers around the globe to use identical instruments and reagents, and to strictly adhere to specific steps of a universal sample preparation method, we recommend that scientists recognize how preanalytical variables can impact proteomics experiments, and conduct and report their own processes and results accordingly. Researchers may also establish permissive windows within their platelet preparation protocols, especially when not all of the recommended procedures and conditions can be met: for example, when samples are sent from a long distance, or when the experimental setup requires it [53].

Further consideration of standardization around platelet sample collection, processing and analysis will also likely help to bring other related efforts into the realm of platelet proteomics studies. For example, proteomics studies of platelets for transfusion may use specific donors, collection protocols (*e.g.* plateletpheresis) or storage conditions [33]. Accordingly, platelet concentrates may not be an ideal source of platelets for other mechanistic studies of platelets (or as independent controls), as they are normally pooled from different donors, and platelets alter their quality over time due to the platelet storage lesion. Likewise, the use of platelets purchased from commercial sources for MS experiments with unspecified isolation and storage protocols may not be as appropriate and their use should be avoided, unless they are the specific subject of the study [65].

Overall, we generally recommend that researchers prepare all experimental and control sample replicates within a single proteomics experiment under the same conditions, and to report experimental conditions in detail.

4. Mass spectrometry data acquisition and analysis

The acquisition, analysis, and interpretation of platelet proteomics data are each dependent on available analytical tools, resources, and investigator expertise. Analytical platforms involved in acquiring platelet proteomics data (e.g., liquid chromatography systems, mass spectrometers) offers distinct technical capabilities and limitations; however, researchers must also face the challenge of balancing costs with the need for sensitive and comprehensive detection, particularly in large-scale clinical studies aimed at biomarker discovery. For instance, multi-dimensional fractionation techniques, which can enhance deep proteome detection, may not be feasible due to limited sample amounts or resource constraints. Methods for the accurate quantitation of proteomes and corresponding analysis pipelines also change over time. For example, label-free quantification (LFQ), multiplexed isobaric labeling techniques (e.g., tandem mass tags [TMT]), and data independent acquisition (DIA) are now widely used. Each method presents its own advantages and drawbacks, and experimental design plays a crucial role in achieving the best outcomes for specific research questions.

Methods like DIA and TMT-labeling offer improvements over label-free, data dependent acquisition (DDA) proteomics methods regarding sensitivity, detection depth, and reproducibility. Interestingly, a recent study of neonate and adult platelets implementing DIA indicates increased depth and sensitivity in quantitative assessments of platelet proteomes [38]. Nonetheless, technical questions remain around whether and how low microgram sample loads and shortened LC gradients have the dynamic range to quantify and compare thousands of protein features across multiple samples. In contrast, multiplexed TMT labeling allows for more starting material to be extensively fractionated in comparable instrument time and has demonstrated deep, quantitative profiling of platelet proteomes [12, 44].

A critical but, perhaps overlooked aspect of analyses of platelet proteomics data is the choice of the protein sequence collection that defines the search space for almost all data analysis methods. The UniProt resource is the most widely used source of protein FASTA files [64]. These sequence collections range from minimally redundant (one protein per gene) to more complete collections with alternatively spliced forms and other sequence variations. The different FASTA file choices exist to address different experimental design questions, and some trial-and-error data analyses may be required to determine suitable choices. One protein per gene FASTA files (with minimal peptide redundancy) are better starting points for quantitative analyses.

Open source, freely available, or proprietary data analysis pipelines (designed to streamline and improve reliability) are frequently updated to handle the ever-changing data complexity. It can be difficult to find optimal approaches to data analyses, with ongoing community debates about experimental designs, acquisition methods, instrument settings, spectral library generation, FASTA file choices, quality control methods, data normalization and imputation methods, and statistical modeling. Accordingly, collaboration with analytical instrument experts and proteomics data analysis specialists may be beneficial. While these topics remain specialized areas of discussion, and beyond the scope of many researchers, it

is essential to understand the importance of depositing raw data into public repositories (e.g., PRIDE [65]) while also comprehensively documenting data acquisition settings and analysis methods (e.g., metadata) to ensure transparency and reproducibility of platelet proteomics studies. All these crucial steps will determine the results, and interpretation of them, and transparency is necessary for the validation and reutilization of data.

The biological interpretation and translation of platelet proteomics datasets also offers challenges and questions in efforts to standardize platelet proteomic analyses, as there are a variety of computational and systems biology approaches available. Individual proteins and protein modifications (e.g. phosphorylation), or sets of proteins of interest from platelet proteomics studies are all generally queried with gene set enrichment tools, biological pathway databases, and integrated platforms (e.g., Reactome [66], STRING [67]). A typical approach to data analysis (for any omics type, especially proteomics) with these tools treats sets of genes as pathways, and then detects overrepresented pathways in differential proteomics results.

Other computational methods consider connections between proteins of interest in a dataset, including the direction of the change connections between proteins or other features (e.g., phosphorylation sites) in pathway databases. Apart from detecting differentially activated kinases (e.g., KSEA [68]), interpreting these network results is not always straightforward, as it is not clear how to test them experimentally. This gap is addressed by methods that factor in causality while detecting subgraphs (e.g., CausalPath [69]). For instance, by considering the semantics of pathway relations and their site specificities, CausalPath has mapped out how phosphorylation changes in platelet phosphoproteomics datasets likely cause other observed changes, resulting in a network composed of testable hypotheses. Further discussion and consensus are needed within the platelet biology community to standardize how these and other pathway analysis tools can best be used to summarize and report the results of platelet omics studies, as well as how results around specific platelet-related pathways can be best controlled with meaningful statistical thresholds. As of today, the platelet community necessitates curated databases specific to platelets, especially for platelet-centric studies.

5. Moving forward: translation, application, and integration of proteomics data throughout the platelet research community

Proteomics tools have evolved rapidly over the past decade, fueling an expansion of data around mechanisms of platelet function. However, despite advances in uncovering and understanding the proteomic-level intricacies of platelets, the translation of this knowledge into new models of platelet physiology, and actionable clinical outcomes is not straightforward. The vast amount of proteomics data available has yet to conclusively demonstrate how specific proteomics features and additional omics layers (e.g., transcriptomics, metabolomics) correlate with or influence particular aspects of platelet phenotype and function. This gap underscores a critical need for further work to bridge the divide between laboratory discoveries and clinical applications, where cooperation and standardization of key aspects of workflows will be essential. We recommend the inclusion

of experimental details and procedures outlined in Table 1, which is meant as a checklist that summarizes essential items that should be included when reporting platelet proteomics data. Some of these issues must be a priority to develop projects and initiatives to best specifically address the long-standing issues and future potential discrepancies of platelet proteomics, including:

1. platelet isolation (e.g., how does blood draw procedure, anticoagulation, storage time, motion while awaiting processing, transport, temperature and other preanalytical variables affect platelet proteome?);
2. proteome sample preparation and analysis (e.g., how should platelet samples be lysed, enriched for PTMs and sub-proteomes, digested, and ultimately analyzed to collect proteomic data?);
3. data analysis and reporting (e.g., how should platelet proteomics experiments be analyzed, interpreted and shared?). Are platelet-specific databases for targeted pathway analysis of demand?

Likewise, scientific journal editors and peer reviewers should be cognizant of these variables and champion the rigorous reporting of methodologies, as well as the deposition of raw data, metadata, software code and workflows in public repositories.

Looking forward, the integration of multi-omics data represents a promising yet challenging frontier in platelet research and clinical translation [2, 3, 5]. Questions remain about which layers of transcriptomics, proteomics, metabolomics, and other omics data sets are most pertinent for a comprehensive understanding of platelets in various health and disease states, and how to link the findings to specific (patho)-physiologic processes [3, 5]. Systematic models of platelet function will continue to evolve through the concerted interpretation, validation and incorporation of newly generated data, and will likely advance via the adoption and integration of orthogonal methodologies, reagents and emerging/developing omics technologies [70]. For example, single-cell proteomics [71] could significantly refine our understanding of platelet heterogeneity and function, offering new perspectives on platelet behavior in health and disease [6, 72]. “Top-down” proteomics approaches that profile intact proteoforms [73], and fractionation methods that assess protein complex assemblies (including protein:metabolite interactions) are also likely to aid in the evolution of systems models of mechanisms of platelet function [74]. Leveraging these advanced methodologies will also provide opportunities to overcome current limitations and propel platelet proteomics towards meaningful clinical applications [75].

The SSC subcommittees of the ISTH are continually faced with challenges in studying, understanding, communicating, and, ultimately, translating findings from state-of-the-art scientific models to the clinical management of thrombotic and hemostatic disorders. A major goal remains in predicting thrombotic events, balancing the bleeding risk of anticoagulant therapy, and personalizing treatments of various diseases. Platelets, as per their nature, stand as an untapped reservoir of protein biomarkers in a range of health and disease states, and the application of - proteomic technologies for their unbiased discovery has highlighted its suitability in this endeavor. However, the complexity and costs associated with implementing these methods pose significant challenges for their routine use

in diagnostic labs. Resulting applications of MS data will most likely involve developing more accessible and cost-effective methods such as ELISAs and flow cytometry, once key biomarkers are established. Other high-throughput platforms (*e.g.*, Luminex, Olink, SomaScan) can also offer a means to measure several known protein analytes of interest from platelets in a multiplex manner. These antibody- or aptamer-based technologies, however, lack the specificity of mass spectrometry, and thus are limited in the information they provide. Nonetheless, these more practical techniques are expected to facilitate standardized and more targeted measurements within clinical settings, enhancing the utility of proteomics discoveries. Despite these challenges, pioneering clinical studies have already revealed promising proteomic signatures from platelets in cardiovascular disease [30, 37, 42], infection [31], metabolic disorders [43, 57], cognitive impairment [32] and cancer [36]. As the field of platelet proteomics continues to mature, and systems biology tools are further integrated into biomarker discovery, we anticipate that platelet proteomics will produce transformative diagnostic and prognostic tools for clinicians worldwide, poised to revolutionize clinical practice in hemostasis and beyond.

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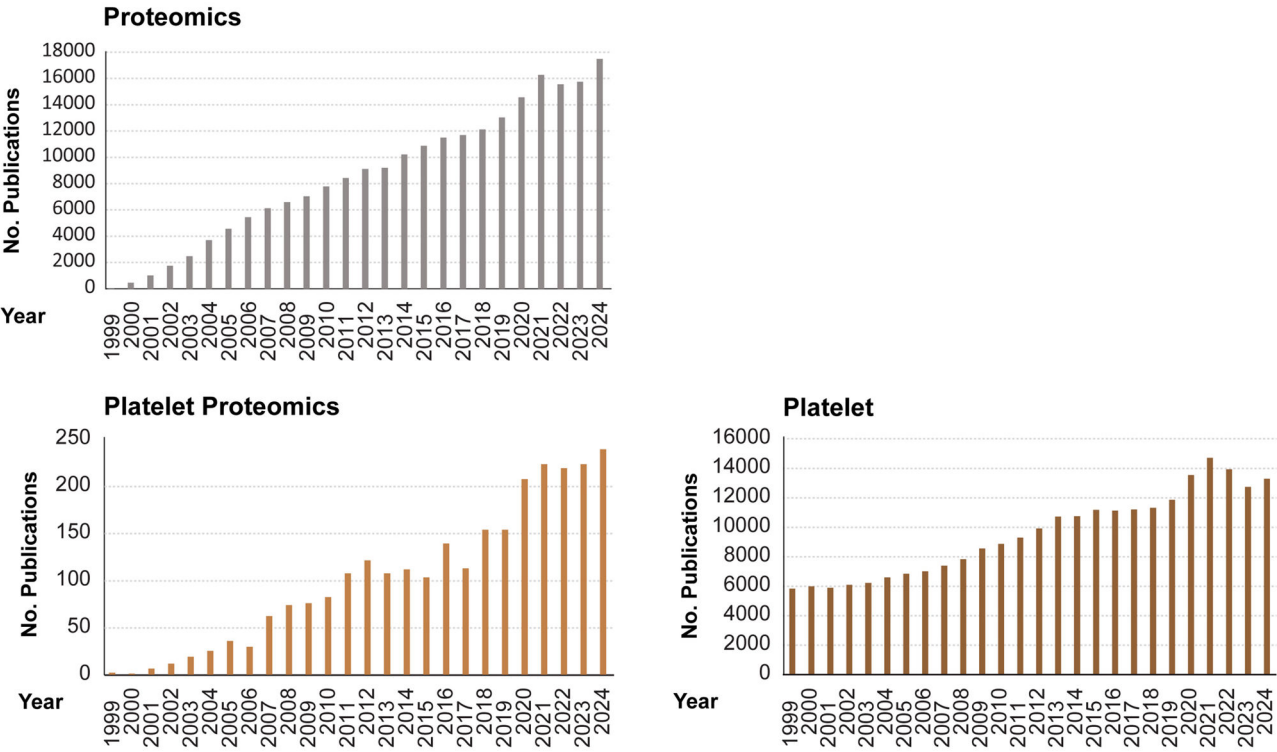


Figure 1. Publications indexed annually on PubMed.
Number of publications indexed on PubMed for the queries “proteomics”, “platelet proteomics” and “platelet” per year to the present (2024 still in progress). The search “platelet” was restricted to the years of the “platelet proteomics” search (1999-to date).

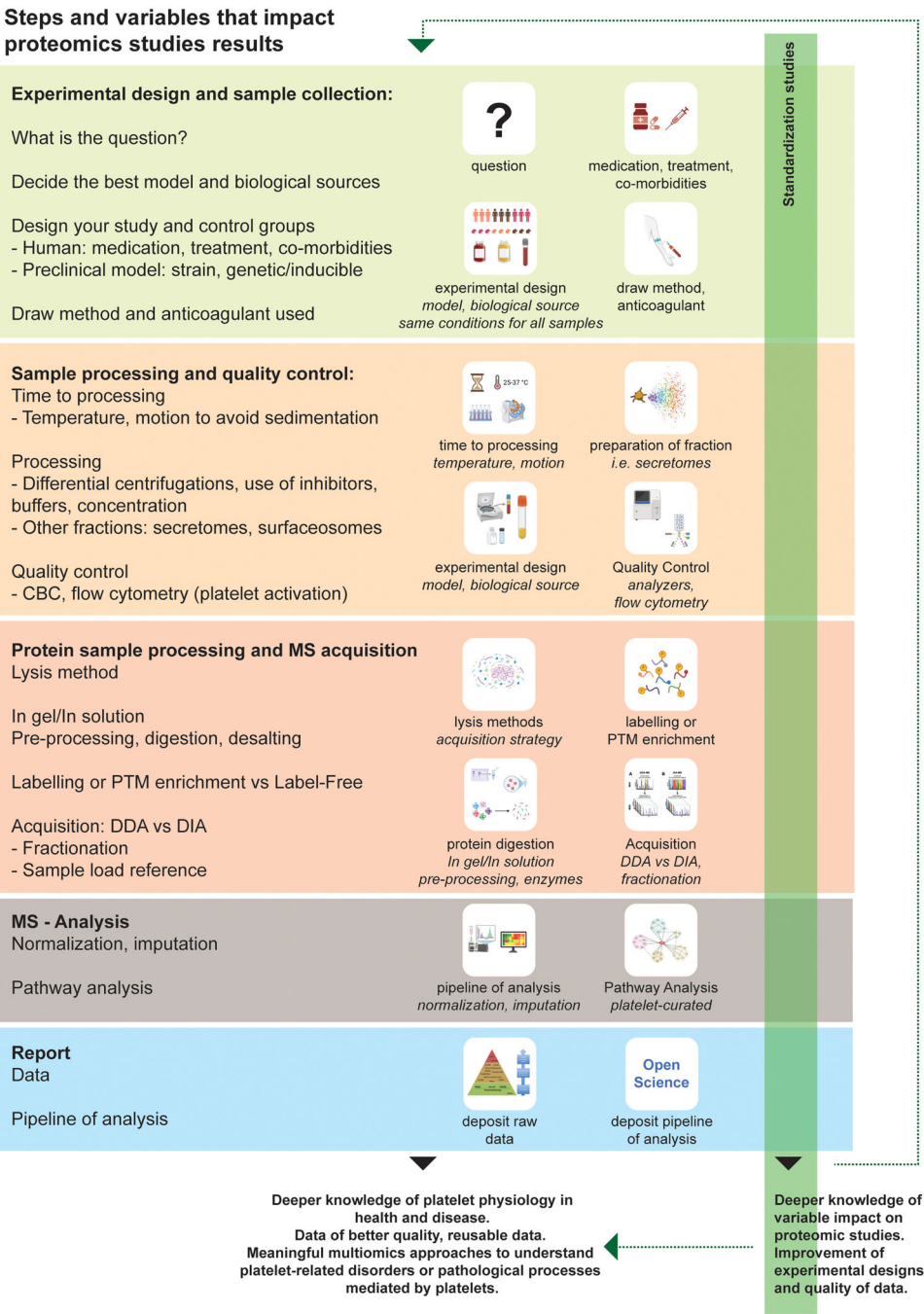


Figure 2. Variables that have an impact when performing platelet proteomics. Platelet preparation, processing, mass spectrometry and *in silico* analysis steps that may influence platelet proteomics shall be reported. Standardization studies of critical variable options will aid at decision-making in an experiment-wise manner. These improvements will lead to data of better quality, reusable, that will ultimately contribute to deepen our knowledge of platelet physiology, and platelet function in health and disease. Icons used in the figure were obtained from [Biorender.com](https://www.biorender.com).

Table 1.

Checklist of recommended items to report for platelet proteomics studies.

Experimental steps and (pre)analytical variables	Current best practices, recommendations	Notes
Sample collection		
Blood donors, animal models	Use a range of multiple healthy donors for human studies; plan appropriate controls for studies of specific human (e.g., disease) populations and animal (e.g., mouse) models.	Report details on human subjects (e.g., number, ages, sex, health and medication status); report similar details and genetic background for mouse studies.
Blood draw	Venipuncture common for human samples; cardiac puncture and retroorbital collection common for mouse.	Ideal phlebotomy method for platelet proteome analysis remains to be specified; use and report same conditions for all samples.
Anticoagulation	Citrate widely used; heparin also common for murine blood.	Use same reagent for all study sample; different anticoagulants have subtle, significant effects on platelet proteome [44].
Processing time	Isolate platelets from all samples within the same time window after blood collection (ideally within 2 h).	When extended delays in blood processing are unavoidable, include controls with similar transport, storage and processing times.
Sample storage	Store all blood samples under the same conditions prior to platelet isolation, ideally at ambient temperature (25–30°C) with gentle agitation or manual inversion.	Report storage conditions (e.g., temperature, agitation/motion to avoid sedimentation, transport).
Centrifugation, washing	Whole blood spin (200 × g, no brake) to prepare PRP; wash and collect platelets (1000 × g); consider using reversible inhibitors (e.g., PGI ₂).	Report centrifugation forces, spin times, temperatures, braking conditions, wash steps, wash volumes, inhibitors (if used), etc.
Buffers	HEPES/Tyrod buffer common for platelet washing and resuspension.	Report buffer components, concentrations and supply sources.
Quality control (QC)	Flow cytometry or hematology analysis (e.g., CBC) of platelet, leukocyte, RBC content; flow cytometry analysis of platelet activation state (e.g., CD62P); Western blot analysis of subproteomes (e.g., cytosol, EVs, secretome).	Report results of QC experiments and specify antibody and reagents used for QC.
Sample prep		
Platelet lysis	Different platelet counts (0.2–1 × 10 ⁹ /ml), volumes, lysis buffers, detergents and (protease, phosphatase) inhibitors are used depending on assay goals.	Report platelet concentration, sample volume and lysis buffer components and reason for their selection.
Sample digestion	Trypsin protease, in-solution digestion is most common; in-gel digestion is appropriate in specific cases.	Specify proteases; for in-gel digest, specify if bands or whole lanes are collected.
Peptide reduction, alkylation, precipitation, clean-up	Several different reagents and commercial kits are used to prepare peptides for analysis.	Report peptide processing reagents and specific clean-up steps (e.g., S-trap).
Peptide enrichment, labeling	Specific peptides (e.g., PTMs) may be enriched with capture reagents; TMT labeling is common for quantitative multiplex analysis.	Report details, reagents. Kits used for peptide enrichment and labeling.
Sample fractionation	LC fractionation is common and recommended to enhance peptide detection.	Specify LC system and methods, fractionation numbers and collection times.
Sample analysis		

Experimental steps and (pre)analytical variables	Current best practices, recommendations	Notes
Spectrometer, acquisition method	For TMT labeling, FAIMS or SPS-MS3 are recommended.	Indicate MS instrument (e.g., Orbitrap) and analysis method (e.g., FAIMS, MS2, SPS-MS3).
Peptide search engine and related parameters, FASTA used	A low-redundant FASTA file is recommended (e.g., reviewed, canonical proteins).	Examples of open-source, free software: MaxQuant, FragPipe.
Data quality, transformation, normalization	Describe the data distribution and potential confounders.	
Normalization	Trimmed mean of M-values (TMM) normalization method in edgeR recommended for TMT experiments.	Specify normalization methods; when the data involves more than one plex, use internal reference scaling (IRS).
Zero values, missing values, imputation	Specify how null and missing values are normalized, statistically analyzed, and/or imputed.	Consider whether missing or null values are a result of disease or condition of study.
Statistical analysis, model building	Statistical testing in edgeR to determine BH corrected FDR values.	Many options are viable if reported appropriately.
Data sharing	Upload raw data files, metadata and analysis pipelines to public repository. Detail analyses with Jupyter notebooks.	Public repositories for data and code include ProteomeXchange (PRIDE), MASSive and GitHub.