Platelet Proteomics and its Applications to Study Platelet-Related Disorders

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Abstract

Proteomics is a rapidly evolving research approach in parallel with genomics, utilizing advanced technologies in protein separation, identification, quantification, and bioinformatics. We have reviewed the different proteomic techniques that have been used successfully to analyze platelets in humans over time. Using more advanced technologies, an increasing number of platelet proteins have been identified and this by investigating the resting platelet proteome and that of activated platelets using diverse agonists. It is also possible to analyze platelet subproteomes including that of granules, microparticles, and membrane proteins. Furthermore, experiments can be designed that specifically study changes in the phosphorylation, glycosylation, or palmitoylation profiles of platelet proteins. These studies have generated extensive protein databases of >5000 proteins for platelets under normal physiological conditions that are also useful to study disease. Examples of proteomic studies that were designed to study platelet-related bleeding disorders and cardiovascular diseases, but also other complex disorders where platelets can be used as a model cell, are discussed.

Introduction to Platelet Proteomics

Proteomics is the large-scale study of proteins encoded by a genome under specific conditions and at a given time (Wilkins et al. 1996) and has also been used to study platelets and platelet-related diseases. Because of recent advances, especially in mass spectrometry (MS), proteomics now enables the measurement of multiple properties for thousands of proteins simultaneously, such as their abundance, modifications, subcellular localization, and protein–protein interactions (Larance and Lamond 2015). Platelets are estimated to contain about 5000 different proteins, spanning a wide abundance range and with different post-translational modifications (Burkhart et al. 2012). The platelet proteome is highly dynamic as some proteins are secreted upon platelet activation and the proteome itself changes with age (Cini et al. 2015) or disease state (Macaulay et al. 2005). In addition, the capability of platelets to absorb plasma proteins further increases the proteome variability and complexity. Platelets are anuclear, having no DNA and only limited amounts of mRNA, which makes proteomic research an attractive alternative for gene expression studies. Platelets are readily available in living organisms and in relatively high amounts; therefore proteomes can also be applied to analyze platelets from children starting from small volumes of blood. As for other cell types and organisms, knowledge of the proteome is expected to be more useful than information on the transcriptome as proteins are believed to be the main effectors of gene functions. It has been estimated that the 20,000 human genes translate into one million different proteoforms due to alternatively splicing and posttranslational modifications (Brett et al. 2002). Furthermore, several

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studies have revealed a poor correlation between mRNA and protein profiles in lower organisms (Gygi et al. 1999a, b), and this has also been described for platelets (Burkhart et al. 2012; Londin et al. 2014). This discrepancy might be explained by a rapid decay of mRNAs in response to various stimuli and posttranslational protein ubiquitination leading to protein degradation. Moreover, a disease state may be determined by translocation of proteins to different cellular compartments rather than by changes in transcript levels. In addition, several technical issues can explain the imperfect correlation between transcriptomic and proteomic data. For example, the current transcriptomic techniques provide only limited information on alternative splicing, whereas proteomics can detect separate protein isoforms encoded by these variants. Given all these considerations, proteomics represents an efficient tool to gain deeper insights into the molecular mechanisms regulating unknown platelet functions and platelet-related diseases. We, and others, published extensive reviews on the application of proteomics in the field of platelet research, mainly focused on the commonly used methodologies and the key achievements in the elucidation of platelet-related diseases (Di Michele et al. 2012a, b; Zufferey et al. 2012; Rotilio et al. 2012). Here, we summarize recent updates for the main contemporary proteomic strategies for platelet research in humans and the most important studies that applied proteomics to unravel the molecular mechanisms underlying platelets in physiological ("Proteomic Methods Used for Platelet Research" and "Proteomic Strategies to Decipher Platelet Biology") and pathological conditions ("Platelet Proteomics to Gain Insights in Human Diseases").

Proteomic Methods Used for Platelet Research

Gel-Based Proteomics

Gel-based methods, including conventional 2DE (bidimensional electrophoresis) and DIGE (differential in-gel electrophoresis), are commonly used for platelet proteomics. In 2DE, proteins are first separated based on their isoelectric point along a pH gradient by isoelectric focusing and then based on their apparent molecular mass in a polyacrylamide gel by SDS-PAGE (Rogowska-Wrzesinska et al. 2013). Proteins are visualized by in-gel staining, matched in different 2DE gels, quantified, and proteins of interest are excised for further identification by MS. 2DE was first used to study the platelet proteome in the 1970s, identifying some most abundant proteins and membrane glycoproteins (Clemetson et al. 1979) and later for the identification of proteins with changed levels upon platelet storage (Snyder et al. 1987). This method also contributed to generate the proteome reference maps of quiescent platelets (Gravel et al. 1995; Marcus et al. 2000; O'Neill et al. 2002; Garcia et al. 2004a, b), activated platelets (Claeys et al. 2005; Shai et al. 2012), and platelet subproteomes including microparticles (Shai et al. 2012; Garcia et al. 2005), cytosol, and microsomes (Claeys et al. 2005). 2DE holds intrinsic limitations such as the difficulty to analyze hydrophobic, extremely charged, and very small or large proteins, as well as having a limited reproducibility among gels and the presence of multiple proteins in a single spot. Combined with restricted sample loading and the scarce sensitivity of protein stains, all of this hampers the detection of low-abundant proteins. Some of these disadvantages were overcome using DIGE, which allows improved reproducibility, higher quantification accuracy, and reduced time/costs. With DIGE, two samples and one internal standard are loaded together on the same gel after labeling with different fluorescent dyes (Cydye-2, Cydye-3, and Cydye-5), each with specific excitation/emission wavelengths (Unlu et al. 1997). The internal standard is a pool of equal amounts of all the samples used to facilitate accurate spot matching across gels, minimizing inter-gel variability, and allowing to calculate the abundance of each protein spot as a ratio to its corresponding spot in the internal standard, leading to the measurement of subtle changes in protein abundance with high statistical confidence. DIGE has been used to study platelet proteomes under basal or activated conditions (Cini et al. 2015; Baumgartner et al. 2013; Winkler et al. 2008; Veitinger et al. 2012).

Finally, 1D SDS-PAGE can be used in combination with MS in the so-called GeLC-MS approach (Shevchenko et al. 2006). The underlying workflow involves SDS-PAGE, in-gel protein staining, slicing of each lane into discrete gel bands, in-gel protein digestion, peptide purification, and LC-MS/MS analysis. GeLC-MS was successfully used to study human platelet proteomes in resting and stimulated conditions (Piersma et al. 2009; Qureshi et al. 2009; van den Bosch et al. 2014; Ambily et al. 2014), during storage (Dzieciatkowska et al. 2015a; Schubert et al. 2012a; b), or to analyze specific subproteomes including the immunoproteasome (Klockenbusch et al. 2014) and palmitoylome (Dowal et al. 2011).

Gel-Free Proteomics

Gel-based methods are progressively replaced by so-called bottom-up MS-based proteomic methods that analyze peptide mixtures derived from isolated proteomes. These techniques require lower amounts of material, which is particularly important when studying rare platelet-related diseases, platelets from young children, or subproteomes. The first step in gel-free analysis typically consists of digesting the proteome with a specific protease. This is

followed by a chromatographic separation of the obtained peptides coupled to MS for analysis, subsequent data processing, and peptide identification.

The pioneering method is multidimensional protein identification technology (MudPIT) which combines orthogonal peptide separations prior to MS/MS analysis, thereby maximizing sensitivity and resolution (Washburn et al. 2001). The main advantage of MudPIT over gel-based methods is that it largely avoids protein solubilization problems as proteins are cut into peptides that are easier to solubilize. MudPIT was used to analyze the basal platelet proteomes (Finamore et al. 2010), the thrombin-induced secretome (Coppinger et al. 2004), the serine hydrolase subproteome (Holly et al. 2013), and the effects of dysregulated miRNA on thrombocythemic platelets (Xu et al. 2012).

The exponentially modified protein abundance index (emPAI) approach is widely used and assumes that abundant peptides are more often detected, but also considers that bigger proteins and proteins with many peptides in the m/z-range for efficient MS analysis will generate more observed peptides (Ishihama et al. 2005). The emPAI method was successfully employed when studying protein networks associated with platelet glycoprotein VI stimulation (Wright et al. 2011).

Combined fractional diagonal chromatography (COFRADIC) is a gel- and label-free proteomic method (Gevaert et al. 2002). Given its versatility, several COFRADIC protocols were developed, each allowing for the enrichment of a specific set of peptides (Gevaert et al. 2003; Stes et al. 2014; Staes et al. 2011). COFRADIC relies on three steps: (1) a first RP-HPLC fractionation of the digested proteome, (2) a chemical or enzymatic reaction of single or combined peptide fractions that modifies the chemical structure of selected peptides that will then obtain altered chromatographic properties, and (3) a series of RP-HPLC fractionations identical to the first one during which the altered peptides are isolated for LC-MS/MS analysis. COFRADIC was applied for studying the human platelet proteome together with GeLC-MS/MS and MudPIT, revealing that the use of complementary techniques allows for more comprehensive proteome mapping (Martens et al. 2005; Lewandrowski et al. 2009).

Labeling methods rely on the incorporation of stable isotopes, i.e., ^{2}H, ^{13}C, ^{15}N, and ^{18}O by metabolic (SILAC), chemical (ICAT, iTRAQ, or TMT), or enzymatic reactions. In stable isotope labeling by amino acids in culture (SILAC), cells are grown separately in media supplemented with heavy or light labeled essential amino acids, such as lysine and arginine (Ong et al. 2002). Although SILAC is typically only applied to cells that can be expanded in vitro, it was also successfully used for studying platelet proteomes (Kruger et al. 2008; Zeiler et al. 2014). In isobaric tags for relative and absolute quantitation (iTRAQ), quantification occurs using MS/MS spectra rather than MS spectra (Gygi et al. 1999a, b). iTRAQ makes use of an amine-reactive reagent available in different isotopic variants to label peptides at their primary amines. iTRAQ was used to analyze the proteome of human platelets focusing on inter- and intra-biological variations (Burkhart et al. 2012; Vaudel et al. 2012) and platelets stimulated with ADP, thrombin, collagen, TRAP, and/or iloprost (Beck et al. 2014; Cimmino et al. 2015). Furthermore, iTRAQ-based gel-free proteomics, combined with DIGE- and ICAT-based proteome analysis, was applied to detect changes in platelet protein levels during storage of blood products (Thon et al. 2008). Similar to iTRAQ, tandem mass tags (TMTs) employ amine-reactive chemicals to label peptides (Thompson et al. 2003), and up to ten different isobaric tags are available (Werner et al. 2014). TMT labeling was applied to study protein changes in platelets from subjects sensitive or resistant to aspirin (Floyd et al. 2014). Enzymatic labeling is based on peptide labeling with oxygen-18 isotopes (Yao et al. 2001). Stable enzymatic labeling of tryptic peptides in combination with COFRADIC was applied to analyze platelets (Staes et al. 2004).

Proteomic Strategies to Decipher Platelet Biology

General Considerations for Experimental Design of Platelet Proteomic Studies

Most proteomic studies are feasible with about 5 mL of blood allowing the isolation of 10^8 platelets, corresponding to 1 mg of proteins (Burkhart et al. 2012). For comparative proteomic studies, different groups should contain age- and sex-matched donors. Indeed, a DIGE study reported a quantitative variation of 18 % in platelet proteomes of a group of 56–100 years old volunteers, probably due to age differences (Winkler et al. 2008). Also another study detected important differences in the platelet proteome of children and adults using DIGE coupled to LC-MS/MS (Cini et al. 2015). Moreover, higher levels of signaling cascade proteins have been found by 2DE and MS in male compared to female donors (Eidelman et al. 2010). Significant gender differences in platelet proteins have also been detected in a recent study using QconCAT-based MRM, not only in fresh blood from donors but also in apheresis platelet concentrates following varying storage times (Dzieciatkowska et al. 2015b). Finally, inter- and intra-subject variation among the human platelet proteome was measured in four healthy donors and three different blood samples from one donor and revealed that the proteome showed about 15 % of quantitative variation among donors (Burkhart et al. 2012). These studies

highlight the usefulness of including biological replicates in the experimental setup to counterbalance for the intrinsic variability of samples. In addition, also some other factors might be considered when choosing platelet donors, such as medication use (such as aspirin), lifestyle, and dietary habits. Furthermore, cigarette smoking was also found to differentially regulate the levels of several platelet proteins (Della Corte et al. 2012).

Obviously, protocols for sample preparation are also a non-negligible source of variability. For example, the analytical variation of a single sample of platelet proteins within and between four laboratories each using its own 2DE protocol was measured, and the coefficient of variation for each of the matched spots after automatic and subsequent manual matching ranged between 5 and 60 % (de Roos et al. 2008). A 2DE study also revealed changes in the platelet proteome in terms of composition rather than yield depending on the precipitation method used to purify proteins, namely, ethanol versus trichloroacetic acid (Zellner et al. 2005). The type of anticoagulant (acid citrate dextrose, heparin, EDTA, etc.) used for blood collection is of fundamental importance, as these substances can induce changes by interacting with specific platelet proteins (Capila and Linhardt 2002). Particular attention must be given to obtaining highly purified platelet fractions, due to possible contamination mainly from plasma and other blood cells. The origin of plasma proteins found in platelet proteomes, however, is still a matter of debate as it is not clear if they derive from the sample preparation method or are actually located within platelets given their surface-connected open canalicular system and its continuous exchange with plasma components. A shotgun proteome analysis of the cytoplasmic, microparticle, and secreted fractions of TRAP-activated platelets supports the platelet origin of some plasma proteins identified in platelet proteomes, thus excluding methodological artifacts (Veitinger et al. 2012). Concerning contamination with other blood cells, a protocol for isolating highly pure platelets preparations is recommended, leading to platelet preparations with less than 1 leukocyte per 10^6 platelets and less than 1 erythrocyte per 10^4 platelets, beyond <1.5 % by volume of plasma per platelet (Burkhart et al. 2012; Gambaryan et al. 2010). In this context, particularly useful are freely available protein databases of purified blood cells (including T cells, monocytes, neutrophils, erythrocytes, and platelets) obtained by both 2DE and shotgun proteomics, which may be used as references (Haudek et al. 2009).

Proteomics of Resting Platelets

Since the first attempts to characterize the human platelet proteome in the late 1970s by 2DE (Clemetson et al. 1979), a huge number of such proteome studies have followed that have identified a growing number of proteins. A combination of 2DE, immunoblotting, and N-terminal sequencing identified about 25 platelet proteins (Gravel et al. 1995). The cytosolic fraction of platelets was analyzed in a successive study using 2DE combined with MS, leading to the identification of more than 200 proteins (Marcus et al. 2000). Two 2DE studies in combination with improved first dimension separation identified more than 500 and 300 different proteins, respectively (O'Neill et al. 2002; Garcia et al. 2004a, b). COFRADIC gave a further impulse to the development of a detailed platelet proteome map of about 650 platelet proteins, including several proteins not previously detected, and hydrophobic membrane proteins (Gevaert et al. 2003). Another study based on 2DE and shotgun proteomics contributed to the compilation of the platelet proteome in basal conditions by identifying about 100 unique proteins (Finamore et al. 2010). Shotgun proteomics, based on peptide separation by OFFGEL fractionation and RP chromatography coupled to LC-MS/MS detection, was also applied to platelet proteomics and led to the identification of more than 1300 proteins (Krishnan et al. 2011). Finally, the most comprehensive proteome database of human platelets consists of more than 4000 unique (phospho)proteins and has been obtained by iTRAQ and TiO_2 enrichment for the phosphorylated fraction (Burkhart et al. 2012).

Proteomics of Activated Platelets

Upon activation by endothelial cell damage or inflammatory events, platelets release a high number of proteins into the circulation, the so-called platelet secretome or releasate, which is involved in the regulation of primary hemostasis, but also in coagulation, inflammation, angiogenesis, wound healing, and some other processes (Golebiewska and Poole 2015). Numerous proteomic studies have attempted to characterize the platelet secretome using different methods. Moreover, different agonists have been used to activate platelets and induce the protein releasate, such as ADP, thrombin, TRAP, collagen, and arachidonic acid. These agonists have been used alone or in combination, influencing the composition of platelet secretome (Rogowska-Wrzesinska et al. 2013). A concentration step on reverse-phase chromatographic beads of diluted peptide mixtures following in-gel digestion of 2DE-separated proteins and MALDI-TOF-MS analysis was applied to the proteome of platelets activated by thrombin with the identification of several proteins that translocate to the cytoskeleton fraction (Gevaert et al. 2000). In another study, a combination of 2DE and MudPIT resulted in a comprehensive characterization of the secretome of thrombin-activated platelets, leading to more than 300 identified proteins, many of which were

described for the first time in platelets, such as secretogranin III, cyclophilin A, and calumenin (Coppinger et al. 2004). Another MudPIT-based analysis led to the identification of 82 proteins differentially expressed in the platelet secretome upon stimulation with thrombin (McRedmond et al. 2004). In activated platelets, these proteins include chemokines, signaling molecules, and histones, as well as coagulation factors and receptors involved in thrombosis. Another study aimed at characterizing the proteome of thrombin-stimulated platelets using DIGE coupled to MALDI-TOF-MS (Della Corte et al. 2008). By comparing the secretome of unstimulated and stimulated platelets, several differentially secreted proteins were identified, including laminin A, a nuclear protein described for the first time as released by platelets. A recent study using DIGE and LC-MS/MS focused on the difference in the platelet secretome following stimulation with thrombin or collagen (Vélez et al. 2015). Thirty-eight proteins differentially present between these conditions were identified, and this relatively high number of protein differences confirms the influence of the type of agonist used for platelet stimulation on the composition of the secretome. The analysis of the platelet proteome in resting or TRAP-activated conditions by 2DE led to the identification of 31 regulated proteins (Garcia et al. 2004a, b). Among these, eight were not known to be present in platelets, including the adapter downstream of tyrosine kinase 2 (DOK-2). The number of proteins identified in the secretome of TRAP-stimulated platelets dramatically increased when using a GeLC-MS/MS approach, based on 1D SDS-PAGE and LTQ-FT MS with the identification of 716 proteins (Piersma et al. 2009). About 40 % of these proteins were identified for the first time in platelet releasates.

Platelets were also stimulated with less common agonists such as GPIV-activating collagen receptor peptide (CRP) (Wright et al. 2011). In this study, different platelet subcellular compartments were isolated using ultracentrifugation to reduce the overall complexity. The majority of differentially expressed proteins after CRP stimulation were low abundant and involved in signaling. In addition, more than half of the 663 identified proteins were not previously known to be present in platelets. An accurate quantitative proteomic strategy based on stable isotope dimethylation, SCX, and MS analysis was used to compare the platelet secretome following PAR1 or PAR4 stimulation or in resting conditions (van Holten et al. 2014). The differential release of pro- and anti-angiogenic growth factors by PAR1 and PAR4 might be important for the regulation of angiogenesis, though no large differences in protein abundance could be detected that confirmed such a hypothesis.

Some proteomic studies also investigated the effect of different agonists simultaneously on the platelet proteome. 2DE combined with LC-MS/MS was used to identify 144 platelet proteins with changing levels following activation with arachidonic acid, collagen, and thrombin (Majek et al. 2010). A more recent study analyzed the platelet secretome following thrombin and collagen treatment using a reversed releasate approach (Wijten et al. 2013). Contrary to classical studies on platelet secretomes, which may also detect proteins derived from cell lysis, in this case the protein levels in the platelets after stimulation were accurately quantified following stable isotope labeling, assuming that the released proteins are less abundant in the platelet proteome. Importantly, of the about 4500 platelet proteins quantified, only 124 were found to be secreted, spanning a concentration range of $\geq$5 orders. These proteins included several novel low-abundant proteins and well-known proteins such as thrombospondin and von Willebrand factor.

Analysis of Platelet Subproteomes

Platelet Granules

To reduce the complexity of analyzing total proteomes, the isolation of organelles prior to analysis is often used. Organelle isolation can be achieved via density gradient centrifugation, immunopurification, or free-flow electrophoresis (Zufferey et al. 2012). However, assessment of possible contamination from other organelles is recommended prior to analysis using organelle markers via immunoblotting, electron microscopy, or enzymatic assays. Platelets contain three types of secretory granules, alpha granules, dense granules, and lysosomes, each characterized by a different number per platelet, content, morphology, and response to stimuli (Rendu and Brohard-Bohn 2001). The proteome of alpha granules isolated by sucrose gradient ultracentrifugation was extensively characterized by GeLC-MS/MS, leading to the identification of 219 proteins (Maynard et al. 2007). Not surprisingly, the majority of these were already described in platelet secretomes, while 44 proteins were novel. The proteome of dense granules was analyzed using two proteomic methods: 2DE coupled to MALDI-TOF-MS and LC-MS/MS analysis (Hernandez-Ruiz et al. 2007). Overall, 40 proteins were identified, and most of them, such as actin-associated proteins, glycolytic enzymes, and regulatory proteins, were not previously known to reside in these organelles. More recently, a proteomic analysis carried out by subcellular fractionation on a sucrose gradient and MS analysis led to the most comprehensive characterization of the platelet granule proteome, identifying over 800 proteins (Zufferey et al. 2014).

Platelet Microparticles

In the most comprehensive analysis of platelet microparticles so far, almost 600 proteins were identified

(Garcia et al. 2005). About 65 % of these proteins have been described for the first time, suggesting that these organelles have a unique protein composition. The proteome of platelet microparticles was also analyzed in a subsequent study using 2DE and LC-MS/MS, in which the microparticles were separated by gel filtration in four classes based on their size (from approximately 100 nm to greater than 500 nm), revealing major differences in protein composition (Dean et al. 2009). In particular, mitochondrial proteins were mostly present in the largest microparticles, whereas alpha granule proteins were more found in the smallest microparticle fractions. The platelet microparticles proteomes were different depending on the stimulus used to activate platelets as found by another proteomic study using 2DE (Shai et al. 2012). Twenty-six proteins were differentially expressed between shear- and thrombin-activated platelets, mainly involved in signaling pathways. The proteome of microparticles from ADP-stimulated platelets was also extensively characterized by shotgun proteomics (Capriotti et al. 2013). This approach led to the identification of more than 600 proteins, 40 % of which were described in platelet microparticles for the first time.

Platelet Plasma Membrane

Despite a major role for membrane receptors and associated proteins for platelet function, they are relatively poorly represented in proteomics studies. This is mainly due to the characteristics of these proteins, such as their low abundance, hydrophobicity, and the reduced accessibility of trypsin to transmembrane domains, which hamper both gel- and MS-based proteomics. Therefore, specific methods are required to improve plasma membrane protein identification. The first study focusing on the platelet plasma membrane proteome used pre-fractionation over a sorbitol gradient to remove high abundant cytoskeletal proteins and contaminants prior to plasma membrane isolation and GeLC-MS/MS analysis (Moebius et al. 2005). In addition, 1D SDS-PAGE separation was performed both by conventional SDS-PAGE and benzyldimethyl-n-hexadecylammonium chloride/SDS separation to improve protein resolution on gel. Almost 300 proteins could be identified, the majority of which were plasma membrane proteins. In a subsequent study, three different techniques were used for the enrichment of platelet plasma membrane proteins before LC-MS/MS analysis, namely, lectin affinity chromatography, biotin/NeutrAvidin affinity chromatography, and free-flow electrophoresis (Senis et al. 2007). A total of 136 membrane proteins were identified, many of which previously not known as platelet membrane proteins. An interesting comparative analysis of the membrane proteomes from platelets in control and thrombin-activated conditions was carried out by NeutrAvidin affinity chromatography, prior to protein separation by liquid-phase IEF and SDS-PAGE and analysis by FT-ICR MS to identify 88 differentially expressed proteins (Tucker et al. 2009). Another study identified 182 membrane proteins using a different proteomic approach (Qureshi et al. 2009). Membrane proteins were first precipitated via ultracentrifugation, resuspended in a glucopyranoside/guanidium buffer and subsequently analyzed by LC-MS/MS. The most extensive proteomic study on platelet plasma membrane proteins led to the identification of 1282 proteins, which were also relatively quantified via an emPAI-based method (Lewandrowski et al. 2009). The power of this study resides in the use of three different proteomic approaches, GeLC-MS/MS, MudPIT, and N-terminal, methionine, or cysteine COFRADIC, all in combination with aqueous two-phase partitioning.

Proteomics to Analyze Platelet Posttranslational Modifications

Platelet Phosphoproteome

Phosphoproteomics is particularly useful for studying cellular signaling events. 2DE and radioactive labeling were used to identify phosphoproteins upon thrombin activation (Immler et al. 1998). Several protein spots showing a significant increase or decrease in phosphorylation could be detected, in particular different myosin isoforms. Another study was also conducted on thrombin-stimulated platelets, focusing on tyrosine phosphorylation, which plays a central role in platelet activation (Maguire et al. 2002). Proteins containing phosphorylated tyrosine were immunoprecipitated using a monoclonal antibody, separated by 2DE, and the resulting protein spots differentially found between platelets in control and activated conditions were identified by immunoblotting and MALDI-TOF-MS. Further development of modern and more resolving techniques for phosphoprotein/peptide enrichment and MS analysis significantly contributed to shed light on signal-dependent activation responses (Zahedi et al. 2006). For example, two different approaches, treatment with ^{32}P followed by 2DE using different pI ranges and autoradiography or immunoprecipitation coupled to GeLC-MS/MS analysis, led to the identification of 55 phosphoproteins and some in vivo phosphorylation sites (Marcus et al. 2003). In another work, using IMAC-based phosphopeptide enrichment and SCX chromatography coupled to LC-MS/MS, 564 phosphorylation sites belonging to almost 280 proteins were identified in resting platelets (Zahedi et al. 2008). An extensive analysis of the proteome and phosphoproteome of platelets in resting conditions, with particular attention to the integrin signaling pathway, was undertaken by IMAC and GeLC-MS/MS (Qureshi et al. 2009). This led to the identification of more than 1500 proteins, including 262 phosphoproteins. Another strategy for phosphoprotein identification is based on the

enrichment of phosphopeptides via titanium dioxide chromatography and LC-MS/MS after a prior enrichment of plasma membrane proteins in an aqueous two phase (Premsler et al. 2011). In yet another study, protein pulldown using SH2 domains and LC-MS/MS analysis were used to investigate the phosphotyrosine state of platelets upon ADP activation (Schweigel et al. 2013). To study the phosphorylation events mediating platelet activation by collagen, a comparative phosphoproteomic study was carried on phosphotyrosine proteins in resting and CRP-activated platelets (Bleijerveld et al. 2013). This approach was based on immunoprecipitation of phosphotyrosine peptides and stable isotope labeling MS and allowed to identify more than 200 phosphotyrosine sites with an altered phosphorylation status. Also prothrombotic oxidized phospholipids in addition to thrombin were used as agonists for platelet stimulation (Zimman et al. 2014). Here, proteins from stimulated platelets were digested by trypsin, and phosphotyrosine peptides were enriched by immunoprecipitation followed by IMAC. The nonprecipitated proteins were fractionated by SCX, and the resulting fractions further enriched for phosphopeptides using TiO_2 beads. Finally, phosphopeptides were identified and quantified by LC-MS/MS. Interestingly, one study focused on the characterization of the signaling pathways involved in platelet inhibition rather than platelet activation (Beck et al. 2014). Human platelets were treated with iloprost, a stable analog of prostacyclin, which is the most important physiological inhibitor of platelet activation acting on the cAMP/PKA signaling cascade. Using iTRAQ and TiO_2 phosphopeptide enrichment, about 300 phosphopeptides modulated by iloprost were detected.

Platelet Glycoproteome

Studies have shown that platelets possess efficient glycosyltransferase machinery, with more than 200 glycosyltransferases and several substrates (Wandall et al. 2012). The first extensive proteomic study on platelet glycoproteome was performed using concanavalin A affinity chromatography and hydrazide chemistry for enriching glycopeptides, after which they were deglycosylated by N-glycosidase F and analyzed by LC-MS/MS (Lewandrowski et al. 2006, 2009). Concanavalin A, one of the most common lectins in glycoproteomic studies, selectively binds N-glycoproteins. This approach identified 41 glycoproteins and 70 different glycosylation sites. In a subsequent study, the same group focused on the glycosylation sites of platelet membrane proteins (Lewandrowski et al. 2007). The membrane fraction was enriched via aqueous two-phase partitioning in a polyethylene glycol/dextran polymer system, the obtained proteins were digested by trypsin, and the glycopeptides were purified by SCX chromatography prior to N-glycosidase F deglycosylation and MS analysis. By applying this method, almost 150 glycosylation sites on 79 different proteins could be identified, 75 % of which were annotated as plasma membrane proteins. The same group also introduced another strategy for glycopeptide enrichment based on electrostatic repulsion hydrophilic interaction chromatography (Lewandrowski et al. 2008). Importantly, this method might allow resolving different protein isoforms, because of the different interactions of glycopeptides with the stationary phase. By identifying 125 glycosylation sites on 66 different proteins, this work largely contributed to the compilation of an extensive glycosylation site database for human platelet proteins.

Platelet Palmitoylome

Protein palmitoylation, the covalent attachment of long chain fatty acids to cysteines, has an important role in platelet biology, being involved in the regulation of platelet activation and thrombi formation (Sim et al. 2007). A first attempt to characterize the platelet palmitoylome was performed by enriching the platelet membrane protein fraction by acyl-biotinyl exchange chemistry and LC-MS/MS analysis and led to the identification of 215 palmitoylated proteins (Dowal et al. 2011). These proteins included already known palmitoylated proteins, but also 103 new putative palmitoylated ones.

Platelet Proteomics to Gain Insights in Human Diseases

Platelet Proteomics to Study Platelet-Related Bleeding Disorders

Rare inherited platelet-related bleeding disorders are a highly heterogeneous group of disorders that can be caused by abnormal platelet numbers, morphology, and function (Freson et al. 2014). Many genes have been identified as a cause for these disorders, though several patients with an expected platelet-related bleeding disorder still do not receive a genetic diagnosis (Westbury et al. 2015). However, in recent years, next-generation sequencing has offered the potential to improve the diagnosis for such patients. However, in parallel to such genetic studies, proteomics has been used with success to gain insights in such platelet-related bleeding disorders. In 2007, changes in α-granule proteins were quantified for a patient with gray platelet syndrome (GPS) compared to a healthy control (Maynard et al. 2007). GPS is an inherited bleeding disorder caused by a reduced number of α-granules and macrothrombocytopenia. A linear sucrose gradient method was used to isolate α-granules, and proteins were separated by 1D SDS-PAGE and identified by LTQ-FT MS. About 586 proteins were identified, and it was found that soluble, biosynthetic cargo proteins were severely

reduced or undetected in GPS platelets, whereas the packaging of soluble, endocytic cargo proteins was only moderately affected, supporting the defect in α-granule incorporation of proteins synthesized in megakaryocytes. Another bleeding disorder that affects α-granules is Quebec platelet disorder (QPD), characterized by high levels of urinary plasminogen activator (uPA) within platelets. The platelet proteome of four members of the same QPD family was compared to that of two healthy donors using 1D SDS-PAGE gels and LC-MS/MS analysis (Maurer-Spurej et al. 2008). Three α-granule proteins, fibrinogen, multimerin, and thrombospondin-1, were downregulated in patients' platelets, and it was speculated that this was due to degradation by platelet-derived uPA. Combined DIGE and MALDI-ToF/ToF were used to compare the platelet releasate of eight patients with storage pool disease and bleeding symptoms to that of nine unrelated healthy controls (Di Michele et al. 2011). This more advanced technology identified 60 and 14 protein spots that varied in the technical and biological replicate studies, and most proteins are cytoskeleton-related. It was hypothesized that the dense granule defect in these storage pool disease cases might be due to an underlying cytoskeleton defect. A similar proteomic design was used to analyze platelet proteins from patients with dominant macrothrombocytopenia (Karmakar et al. 2015). Patients have altered levels of actin-binding proteins, peroxiredoxin 2, protein disulfide isomerase, and transthyretin that might be associated with the structural changes of their platelets.

Platelet Proteomics to Study Cardiovascular Disease

Arterial thrombosis is a pivotal event in the development of cardiovascular disease, and platelets play a fundamental role in this process. Platelet proteomics has been applied to study this complex disease, and we have selected some examples. The first study was performed in 2008 using 2DE and MS and focused on patients with arterial thrombosis, primarily with ischemic stroke (Arias-Salgado et al. 2008). Most of the differences detected between groups were related to cytoskeletal changes, which supported the idea of preactivated platelets. 2DE and MS analysis of platelets from patients with non-ST segment elevation acute coronary syndrome (ACS) revealed 22 differentially expressed proteins compared to matched cases with chronic ischemic cardiopathy (Parguiña et al. 2010). Most of these proteins are interconnected as part of the network related to cell assembly and morphology and are predicted to participate in platelet activation via $\alpha_{IIb}\beta_3$ or GPVI receptors. Platelet proteins isolated from ACS patients admitted within 24 h of chest pain were compared to these from patients with stable coronary ischemic disease (Lopez-Farre et al. 2011). Different proteins involved in cytoskeleton, glycolysis pathway, and cellular-related antioxidant system were altered in the acute phase of the coronary event as identified via 2DE and MS. The platelet proteome of ST-elevation myocardial infarction (STEMI) patients was compared to that of stable chronic ischemic cardiopathy (CAD) patients again using 2DE and MS (Parguina et al. 2011). This study found 42 differentially expressed proteins with altered major signaling pathways that include proteins related to integrin, integrin-linked kinase, and GPVI signaling. The analysis of a coronary thrombus itself by proteomics using different techniques as 2DE with MS, 1-DE with LS-MALDI MS/MS, and 1-DE with LTQ-Orbitrap identified a total of 708 proteins (Alonso-Orgaz et al. 2014). Some of these proteins were co-expressed with the platelet marker CD41 and pointed out a potential activation of a focal adhesion pathway in platelets during thrombus formation. Proteomics of microvesicles isolated from plasma of STEMI versus CAD patients was performed by DIGE and MS (Vélez et al. 2014). About 102 proteins were identified that correspond to 25 unique differentially expressed proteins that have been linked to inflammation, infarction, and thrombogenesis. The most recent proteomic study used isolated thrombi from STEMI patients during percutaneous coronary intervention at different time points after onset of pain (3 or 6 h) (Ramaiola et al. 2015). Thrombi at 3 h were platelet-rich, while at 6 h leukocyte infiltration was noticed, and proteomic differences between these time points were mainly related to changes in the cell cytoskeletal-associated proteome.

Antiplatelet therapies are widely used to prevent myocardial infarction, stroke, and other cardiovascular events. Platelet proteomics proved also to be useful to study the biological effects of antiplatelet therapy and to study mechanisms of patients' resistance to such drugs. The platelet proteome was analyzed using 2DE for patients with stable angina undergoing percutaneous coronary intervention before angiography, 12 h after clopidogrel, and 24 h after the intervention (Volpi et al. 2012). Protein changes were detected associated with platelet activation and clopidogrel response, and most proteins belong to the cytoskeleton rearrangement, energetic metabolism, and oxidative stress functional classes. Platelet protein expression profiles from aspirin (ASA)-resistant and ASA-sensitive CAD patients were compared using 2DE and MS (Mateos-Caceres et al. 2010). Differences were found for proteins involved in energetic metabolism, cytoskeleton, oxidative stress, and cell survival, which might be due to their different ability to respond to ASA. Pre- and post-aspirin treatment platelet lysate samples (300 mg daily for 28 days) from two ASA-resistant and four ASA-sensitive healthy subjects were analyzed by GeLC-MS/MS (Floyd et al. 2014). Though the

groups presented with no detectable changes in the platelet proteome at baseline, 406 differential proteins were present after aspirin treatment with a marked increase in GPIIIa for ASA resistance. Recently, a manually curated biochemical reaction network of platelet metabolism was constructed using 33 proteomic datasets and 354 literature references (Thomas et al. 2014). The effect of ASA resistance was evaluated using constraint-based modeling, providing evidence for a redirection of glycolytic, fatty acid, and nucleotide metabolism toward eicosanoid synthesis and reactive oxygen species stress as validated by novel proteomic data. The availability of such a network will stimulate data-driven system analysis of platelet metabolism in order to gain insights into pathologies.

Platelet Proteomics to Study Other Disorders

Platelet proteomics has also been used to gain insights in diseases that are linked to platelets, such as for understanding thrombosis risks for phosphomannomutase 2 (PMM2) deficiency, sepsis, deep vein thrombosis (DVT), diabetes and uremia, inflammation in cystic fibrosis, and bleeding in myelodysplastic syndrome (MDS). Some of these proteomic studies are discussed as examples. A subproteomic analysis was performed for patients with the congenital disorder of glycosylation PMM2 that have an increased risk for thrombosis (de la Morena-Barrio et al. 2014). DIGE analysis of the N-glycoproteins however showed no quantitative or qualitative differences between patients and controls. Platelet 2DE profiles of septic patients versus healthy controls resulted in five differentially expressed proteins that include GPIX and GPIIb (Liu et al. 2014). Proteins isolated form plasma-derived microparticles were tagged with iTRAQ reagents and analyzed by 2DE with LS-MALDI MS/MS for nine patients with DVT and six healthy controls (Ramacciotti et al. 2010). The differentially expressed or depleted proteins are expected to influence thrombosis via inflammation, cell shedding, inhibition of fibrinolysis, and hemostatic plug formation. Platelets from 13 diabetic patients were analyzed before and 12 weeks after pioglitazone therapy using DIGE and LS-MS/MS (Randriamboavonjy et al. 2012). More than half of the differentially expressed protein spots identified were known calpain substrates and could be classified as cytoskeletal proteins and signaling molecules. This study suggests that diabetes-induced platelet dysfunction might be due to calpain activation. Platelet proteomics was performed for uremia patients with functional versus dysfunctional platelets as tested by PFA100 (Marques et al. 2010). 2DE and MS analysis showed changes in protein levels that might have occurred at the megakaryocyte level. Shotgun nUPLC-MSE and 2DE were used to compare platelet proteins between cystic fibrosis (CF) patients and healthy controls (Pieroni et al. 2011). CF is caused by mutations in the cystic fibrosis transmembrane conductance regulator that is expressed on platelets and related to changes in inflammation for which the proteomic study provided evidence of changes in integrin signaling. The platelet proteome of myelodysplastic syndrome (MDS) patients was analyzed with DIGE and showed lower levels of proteins that are important for integrin $\alpha_{IIb}\beta_3$ signaling (Fröbel et al. 2013). Impaired platelet aggregation might therefore explain the bleeding complications observed for MDS patients even at sufficient platelet counts.

Less obvious are the platelet proteomic studies that have been designed to unravel disease mechanisms for neuropathologies. However, it is known that platelets do share common characteristics with neurons (Goubau et al. 2013). Again, examples of proteomic studies conducted for Alzheimer's disease (AD), but also for monogenetic neurological diseases, are discussed. The platelet proteome was analyzed by DIGE for 34 AD cases, 13 cases with vascular dementia, 15 Parkinson cases, and 49 healthy controls (Zellner et al. 2012). The study suggested that Mao-B platelet protein levels could be a biomarker for age-related dementia in AD. In addition, the platelet membrane proteome was determined for five AD cases versus controls using 1-DE and LC-MS/MS (Donovan et al. 2013). A total of 144 proteins were altered that represent secretory granule proteins, and it was suggested that platelets may serve as a source of blood-based biomarkers in neuropathologies. DIGE with MS identification was used to analyze platelets and fibroblasts from patients with alternating lemiplegia of childhood (AHC) due to *ATP1A3* mutations (Di Michele et al. 2013). A total of 93 proteins have a different expression of which seven were detected in both cell types, and this included lysosomal protein cathepsin. Functional validation studies showed that AHC might be associated with a defective regulation of apoptosis via the lysosomal cathepsin pathway.

Conclusion

The application of the proteomics and subproteomic approaches described above has provided an invaluable contribution to the elucidation of platelet physiological mechanisms. We have included a figure with the most important items discussed in this chapter (Fig. 1). Moreover, they also provided remarkable insights into the molecular basis underlying platelet-related diseases, though several challenges still remain to be addressed. However, we are confident that the integration of the most recent advances in MS-based techniques in platelet proteomic studies will allow further delineation of the mechanisms implicated in diseases and identify new biomarkers or drug targets.

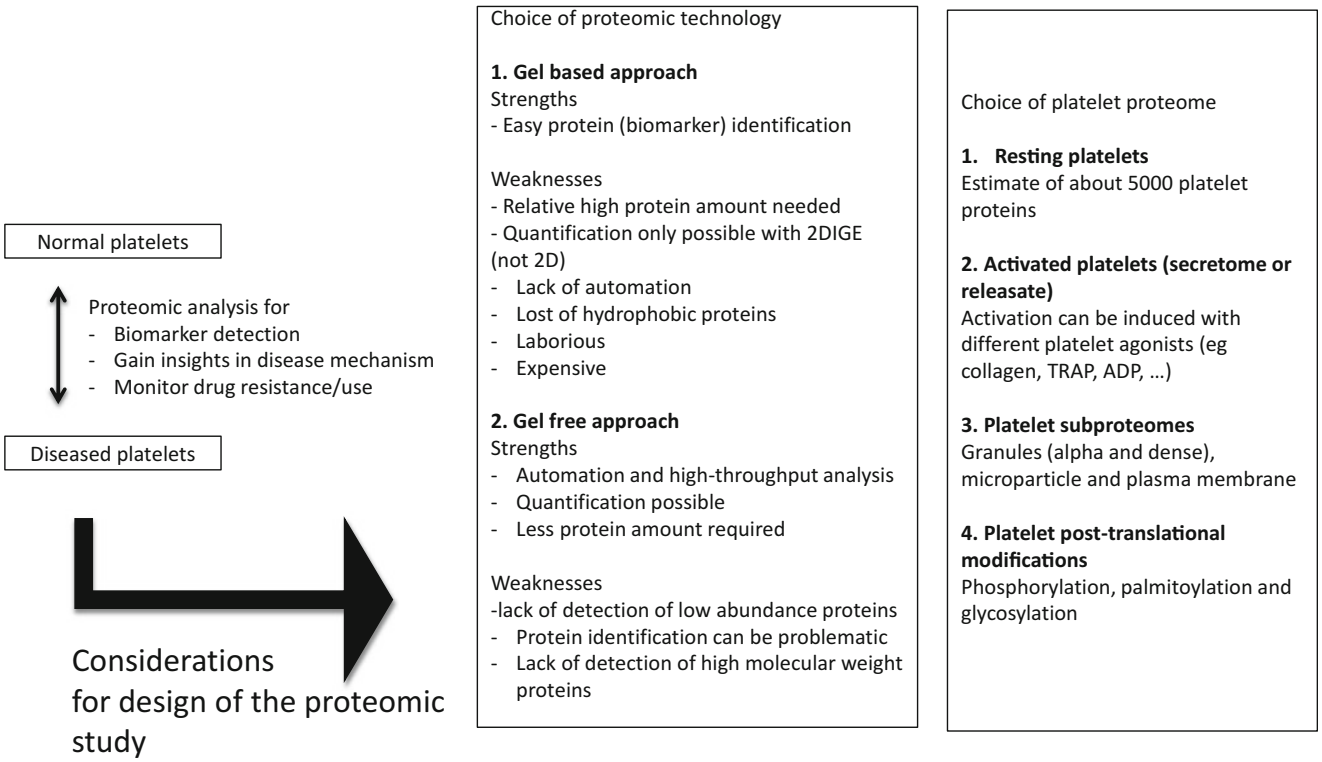

Fig. 1 Overview of proteomic technologies used in platelet research

Take-Home Messages

- Proteomics represents a powerful tool to study platelet proteins and the molecular mechanisms regulating unknown platelet functions and platelet-related diseases.
- Gel-based and gel-free proteomics technologies, both with their own advantages and limits, have been efficiently used to analyze platelets in physiological and pathological conditions.
- The experimental design of platelet proteomic studies should take into account the appropriate controls, technical/biological replicates, and sample preparation protocols.
- Proteomics can be applied to the study of (1) whole platelet proteome (in resting or activated condition); (2) platelet subproteomes (granules, microparticles, plasma membrane); and platelet PTMs (phosphoproteome, glycoproteome, palmitoylome).
- Platelet proteomics has been used to investigate platelet-related bleeding disorders and cardiovascular diseases, but also diseases less obviously linked to platelets.

References

Alonso-Orgaz S, Moreno-Luna R, López JA, Gil-Dones F, Padial LR, Moreu J, de la Cuesta F, Barderas MG (2014) Proteomic characterization of human coronary thrombus in patients with ST-segment elevation acute myocardial infarction. J Proteomics 109:368–381

Ambily A, Kaiser WJ, Pierro C, Chamberlain EV, Li Z, Jones CI, Kassouf N, Gibbins JM, Authi KS (2014) The role of plasma membrane STIM1 and Ca(2+)entry in platelet aggregation. STIM1 binds to novel proteins in human platelets. Cell Signal 26:502–511

Arias-Salgado EG, Larrucea S, Butta N, Fernández D, García-Muñoz S, Parrilla R, Ayuso MS (2008) Variations in platelet protein associated with arterial thrombosis. Thromb Res 122:640–647

Baumgartner R, Umlauff E, Veitinger M, Guterres S, Rappold E, Babeluk R, Mitulovic G, Oehler R, Zellner M (2013) Identification and validation of platelet low biological variation proteins, superior to GAPDH, actin and tubulin, as tools in clinical proteomics. J Proteomics 94:540–551

Beck F, Geiger J, Gambaryan S, Veit J, Vaudel M, Nollau P, Kohlbacher O, Martens L, Walter U, Sickmann A, Zahedi RP (2014) Time-resolved characterization of cAMP/PKA-dependent signaling reveals that platelet inhibition is a concerted process involving multiple signaling pathways. Blood 123:e1–e10

Bleijerveld OB, van Holten TC, Preisinger C, van der Smagt JJ, Farndale RW, Kleefstra T, Willemsen MH, Urbanus RT, de Groot PG, Heck AJ, Roest M, Scholten A (2013) Targeted phosphotyrosine profiling of glycoprotein VI signaling implicates oligophrenin-1 in platelet filopodia formation. Arterioscler Thromb Vasc Biol 33:1538–1543

Brett D, Pospisil H, Valcarcel J, Reich J, Bork P (2002) Alternative splicing and genome complexity. Nat Genet 30:29–30

Burkhart JM, Vaudel M, Gambaryan S, Radau S, Walter U, Martens L, Geiger J, Sickmann A, Zahedi RP (2012) The first comprehensive and quantitative analysis of human platelet protein composition allows the comparative analysis of structural and functional pathways. Blood 120:e73–e82

Capila I, Linhardt RJ (2002) Heparin–protein interactions. Angew Chem Int Ed Engl 41:391–412

Capriotti AL, Caruso G, Cavaliere C, Piovesana S, Samperi R, Lagana A (2013) Proteomic characterization of human platelet-derived microparticles. Anal Chim Acta 776:57–63

Cimmino G, Tarallo R, Nassa G, De Filippo MR, Giurato G, Ravo M, Rizzo F, Conte S, Pellegrino G, Cirillo P, Calabro P, Ohman T, Nyman TA, Weisz A, Golino P (2015) Activating stimuli induce platelet microRNA modulation and proteome reorganisation. Thromb Haemost 114:96–108

Cini C, Yip C, Attard C, Karlaftis V, Monagle P, Linden M, Ignjatovic V (2015) Differences in the resting platelet proteome and platelet releasate between healthy children and adults. J Proteomics 123:78–88

Claeys D, Geering K, Meyer BJ (2005) Two-dimensional Blue Native/sodium dodecyl sulfate gel electrophoresis for analysis of multimeric proteins in platelets. Electrophoresis 26:1189–1199

Clemetson KJ, Capitanio A, Lüscher EF (1979) High resolution two-dimensional gel electrophoresis of the proteins and glycoproteins of human blood platelets and platelet membranes. Biochim Biophys Acta 553:11–24

Coppinger JA, Cagney G, Toomey S, Kislinger T, Belton O, McRedmond JP, Cahill DJ, Emili A, Fitzgerald DJ, Maguire PB (2004) Characterization of the proteins released from activated platelets leads to localization of novel platelet proteins in human atherosclerotic lesions. Blood 103:2096–2104

de la Morena-Barrio ME, Di Michele M, Lozano ML, Rivera J, Perez-Duenas B, Altisent C, Sevivas T, Vicente V, Jaeken J, Freson K, Corral J (2014) Proteomic analysis of platelet N-glycoproteins in PMM2-CDG patients. Thromb Res 133:412–417

de Roos B, Duthie SJ, Polley AC, Mulholland F, Bouwman FG, Heim C, Rucklidge GJ, Johnson IT, Mariman EC, Daniel H, Elliott RM (2008) Proteomic methodological recommendations for studies involving human plasma, platelets, and peripheral blood mononuclear cells. J Proteome Res 7:2280–2290

Dean WL, Lee MJ, Cummins TD, Schultz DJ, Powell DW (2009) Proteomic and functional characterisation of platelet microparticle size classes. Thromb Haemost 102:711–718

Della Corte A, Maugeri N, Pampuch A, Cerletti C, de Gaetano G, Rotilio D (2008) Application of 2-dimensional difference gel electrophoresis (2D-DIGE) to the study of thrombin-activated human platelet secretome. Platelets 19:43–50

Della Corte A, Tamburrelli C, Crescente M, Giordano L, D'Imperio M, Di Michele M, Donati MB, De Gaetano G, Rotilio D, Cerletti C (2012) Platelet proteome in healthy volunteers who smoke. Platelets 23:91–105

Di Michele M, Thys C, Waelkens E, Overbergh L, D'Hertog W, Mathieu C, De Vos R, Peerlinck K, Van Geet C, Freson K (2011) An integrated proteomics and genomics analysis to unravel a heterogeneous platelet secretion defect. J Proteomics 74:902–913

Di Michele M, Van Geet C, Freson K (2012a) Proteomics to unravel platelet-related diseases and identify novel anti-platelet drugs. Curr Med Chem 19:4662–4670

Di Michele M, Van Geet C, Freson K (2012b) Recent advances in platelet proteomics. Expert Rev Proteomics 9:451–466

Di Michele M, Goubau C, Waelkens E, Thys C, De Vos R, Overbergh L, Schyns T, Buyse G, Casaer P, Van Geet C, Freson K (2013) Functional studies and proteomics in platelets and fibroblasts reveal a lysosomal defect with increased cathepsin-dependent apoptosis in ATP1A3 defective alternating hemiplegia of childhood. J Proteomics 86:53–69

Donovan LE, Dammer EB, Duong DM, Hanfelt JJ, Levey AI, Seyfried NT, Lah JJ (2013) Exploring the potential of the platelet membrane proteome as a source of peripheral biomarkers for Alzheimer's disease. Alzheimers Res Ther 5:32

Dowal L, Yang W, Freeman MR, Steen H, Flaumenhaft R (2011) Proteomic analysis of palmitoylated platelet proteins. Blood 118: e62–e73

Dzieciatkowska M, D'Alessandro A, Burke TA, Kelher MR, Moore EE, Banerjee A, Silliman CC, West BF, Hansen KC (2015a) Proteomics of apheresis platelet supernatants during routine storage: gender-related differences. J Proteomics 112:190–209

Dzieciatkowska M, D'Alessandro A, Hill RC, Hansen KC (2015b) Plasma QconCATs reveal a gender-specific proteomic signature in apheresis platelet plasma supernatants. J Proteomics 120:1–6

Eidelman O, Jozwik C, Huang W, Srivastava M, Rothwell SW, Jacobowitz DM, Ji X, Zhang X, Guggino W, Wright J, Kiefer J, Olsen C, Adimi N, Mueller GP, Pollard HB (2010) Gender dependence for a subset of the low-abundance signaling proteome in human platelets. Hum Genomics Proteomics 2010:164906

Finamore F, Pieroni L, Ronci M, Marzano V, Mortera SL, Romano M, Cortese C, Federici G, Urbani A (2010) Proteomics investigation of human platelets by shotgun nUPLC-MSE and 2DE experimental strategies: a comparative study. Blood Transfus 8:s140–s148

Floyd CN, Goodman T, Becker S, Chen N, Mustafa A, Schofield E, Campbell J, Ward M, Sharma P, Ferro A (2014) Increased platelet expression of glycoprotein IIIa following aspirin treatment in aspirin-resistant but not aspirin-sensitive subjects. Br J Clin Pharmacol 78:320–328

Freson K, Wijgaerts A, van Geet C (2014) Update on the causes of platelet disorders and functional consequences. Int J Lab Hematol 36:313–325

Fröbel J, Cadeddu RP, Hartwig S, Bruns I, Wilk CM, Kündgen A, Fischer JC, Schroeder T, Steidl UG, Germing U, Lehr S, Haas R, Czibere A (2013) Platelet proteome analysis reveals integrin-dependent aggregation defects in patients with myelodysplastic syndromes. Mol Cell Proteomics 12:1272–1280

Gambaryan S, Kobsar A, Rukoyatkina N, Herterich S, Geiger J, Smolenski A, Lohmann SM, Walter U (2010) Thrombin and collagen induce a feedback inhibitory signaling pathway in platelets involving dissociation of the catalytic subunit of protein kinase A from an NFkappaB-IkappaB complex. J Biol Chem 285:18352–18363

Garcia A, Prabhakar S, Hughan S, Anderson TW, Brock CJ, Pearce AC, Dwek RA, Watson SP, Hebestreit HF, Zitzmann N (2004a) Differential proteome analysis of TRAP-activated platelets: involvement of DOK-2 and phosphorylation of RGS proteins. Blood 103:2088–2095

Garcia A, Prabhakar S, Brock CJ, Pearce AC, Dwek RA, Watson SP, Hebestreit HF, Zitzmann N (2004b) Extensive analysis of the human platelet proteome by two-dimensional gel electrophoresis and mass spectrometry. Proteomics 4:656–668

Garcia BA, Smalley DM, Cho H, Shabanowitz J, Ley K, Hunt DF (2005) The platelet microparticle proteome. J Proteome Res 4:1516–1521

Gevaert K, Eggermont L, Demol H, Vandekerckhove J (2000) A fast and convenient MALDI-MS based proteomic approach: identification of components scaffolded by the actin cytoskeleton of activated human thrombocytes. J Biotechnol 78:259–269

Gevaert K, Van Damme J, Goethals M, Thomas GR, Hoorelbeke B, Demol H, Martens L, Puype M, Staes A, Vandekerckhove J (2002) Chromatographic isolation of methionine-containing peptides for gel-free proteome analysis: identification of more than 800 Escherichia coli proteins. Mol Cell Proteomics 1:896–903

Gevaert K, Goethals M, Martens L, Van Damme J, Staes A, Thomas GR, Vandekerckhove J (2003) Exploring proteomes and analyzing protein processing by mass spectrometric identification of sorted N-terminal peptides. Nat Biotechnol 21:566–569

Golebiewska EM, Poole AW (2015) Platelet secretion: from haemostasis to wound healing and beyond. Blood Rev 29:153–162

Goubau C, Buyse GM, Di Michele M, Van Geet C, Freson K (2013) Regulated granule trafficking in platelets and neurons: a common molecular machinery. Eur J Paediatr Neurol 17:117–125

Gravel P, Sanchez JC, Walzer C, Golaz O, Hochstrasser DF, Balant LP, Hughes GJ, Garcia-Sevilla J, Guimon J (1995) Human blood platelet protein map established by two-dimensional polyacrylamide gel electrophoresis. Electrophoresis 16:1152–1159

Gygi SP, Rochon Y, Franza BR, Aebersold R (1999a) Correlation between protein and mRNA abundance in yeast. Mol Cell Biol 19:1720–1730

Gygi SP, Rist B, Gerber SA, Turecek F, Gelb MH, Aebersold R (1999b) Quantitative analysis of complex protein mixtures using isotope-coded affinity tags. Nat Biotechnol 17:994–999

Haudek VJ, Slany A, Gundacker NC, Wimmer H, Drach J, Gerner C (2009) Proteome maps of the main human peripheral blood constituents. J Proteome Res 8:3834–3843

Hernandez-Ruiz L, Valverde F, Jimenez-Nunez MD, Ocana E, Saez-Benito A, Rodriguez-Martorell J, Bohorquez JC, Serrano A, Ruiz FA (2007) Organellar proteomics of human platelet dense granules reveals that 14-3-3zeta is a granule protein related to atherosclerosis. J Proteome Res 6:4449–4457

Holly SP, Chang JW, Li W, Niessen S, Phillips RM, Piatt R, Black JL, Smith MC, Boulaftali Y, Weyrich AS, Bergmeier W, Cravatt BF, Parise LV (2013) Chemoproteomic discovery of AADACL1 as a regulator of human platelet activation. Chem Biol 20:1125–1134

Immler D, Gremm D, Kirsch D, Spengler B, Presek P, Meyer HE (1998) Identification of phosphorylated proteins from thrombin-activated human platelets isolated by two-dimensional gel electrophoresis by electrospray ionization-tandem mass spectrometry (ESI-MS/MS) and liquid chromatography-electrospray ionization-mass spectrometry (LC-ESI-MS). Electrophoresis 19:1015–1023

Ishihama Y, Oda Y, Tabata T, Sato T, Nagasu T, Rappsilber J, Mann M (2005) Exponentially modified protein abundance index (emPAI) for estimation of absolute protein amount in proteomics by the number of sequenced peptides per protein. Mol Cell Proteomics 4:1265–1272

Karmakar S, Saha S, Banerjee D, Chakrabarti A (2015) Differential proteomics study of platelets in asymptomatic constitutional macrothrombocytopenia: altered levels of cytoskeletal proteins. Eur J Haematol 94:43–50

Klockenbusch C, Walsh GM, Brown LM, Hoffman MD, Ignatchenko V, Kislinger T, Kast J (2014) Global proteome analysis identifies active immunoproteasome subunits in human platelets. Mol Cell Proteomics 13:3308–3319

Krishnan S, Gaspari M, Della Corte A, Bianchi P, Crescente M, Cerletti C, Torella D, Indolfi C, de Gaetano G, Donati MB, Rotilio D, Cuda G (2011) OFFgel-based multidimensional LC-MS/MS approach to the cataloguing of the human platelet proteome for an interactomic profile. Electrophoresis 32:686–695

Kruger M, Moser M, Ussar S, Thievessen I, Luber CA, Forner F, Schmidt S, Zanivan S, Fassler R, Mann M (2008) SILAC mouse for quantitative proteomics uncovers kindlin-3 as an essential factor for red blood cell function. Cell 134:353–364

Larance M, Lamond AI (2015) Multidimensional proteomics for cell biology. Nat Rev Mol Cell Biol 16:269–280

Lewandrowski U, Moebius J, Walter U, Sickmann A (2006) Elucidation of N-glycosylation sites on human platelet proteins: a glycoproteomic approach. Mol Cell Proteomics 5:226–233

Lewandrowski U, Zahedi RP, Moebius J, Walter U, Sickmann A (2007) Enhanced N-glycosylation site analysis of sialoglycopeptides by strong cation exchange prefractionation applied to platelet plasma membranes. Mol Cell Proteomics 6:933–1941

Lewandrowski U, Lohrig K, Zahedi R, Wolters D, Sickmann A (2008) Glycosylation site analysis of human platelets by electrostatic repulsion hydrophilic interaction chromatography. Clin Proteomics 4:25–36

Lewandrowski U, Wortelkamp S, Lohrig K, Zahedi RP, Wolters DA, Walter U, Sickmann A (2009) Platelet membrane proteomics: a novel repository for functional research. Blood 114:e10–e19

Liu J, Li J, Deng X (2014) Proteomic analysis of differential protein expression in platelets of septic patients. Mol Biol Rep 41:3179–3185

Londin ER, Hatzimichael E, Loher P, Edelstein L, Shaw C, Delgrosso K, Fortina P, Bray PF, McKenzie SE, Rigoutsos I (2014) The human platelet: strong transcriptome correlations among individuals associate weakly with the platelet proteome. Biol Direct 9:3

Lopez-Farre AJ, Zamorano-Leon JJ, Azcona L, Modrego J, Mateos-Caceres PJ, Gonzalez-Armengol J, Villarroel P, Moreno-Herrero R, Rodriguez-Sierra P, Segura A, Tamargo J, Macaya C (2011) Proteomic changes related to "bewildered" circulating platelets in the acute coronary syndrome. Proteomics 11:3335–3348

Macaulay IC, Carr P, Gusnanto A, Ouwehand WH, Fitzgerald D, Watkins NA (2005) Platelet genomics and proteomics in human health and disease. J Clin Invest 115:3370–3377

Maguire PB, Wynne KJ, Harney DF, O'Donoghue NM, Stephens G, Fitzgerald DJ (2002) Identification of the phosphotyrosine proteome from thrombin activated platelets. Proteomics 2:642–648

Majek P, Reicheltova Z, Stikarova J, Suttnar J, Sobotkova A, Dyr JE (2010) Proteome changes in platelets activated by arachidonic acid, collagen, and thrombin. Proteome Sci 8:56

Marcus K, Immler D, Sternberger J, Meyer HE (2000) Identification of platelet proteins separated by two-dimensional gel electrophoresis and analyzed by matrix assisted laser desorption/ionization-time of flight-mass spectrometry and detection of tyrosine-phosphorylated proteins. Electrophoresis 21:2622–2636

Marcus K, Moebius J, Meyer HE (2003) Differential analysis of phosphorylated proteins in resting and thrombin-stimulated human platelets. Anal Bioanal Chem 376:973–993

Marques M, Sacristán D, Mateos-Cáceres PJ, Herrero J, Arribas MJ, González-Armengol JJ, Villegas A, Macaya C, Barrientos A, López-Farré AJ (2010) Different protein expression in normal and dysfunctional platelets from uremic patients. J Nephrol 23:90–101

Martens L, Van Damme P, Van Damme J, Staes A, Timmerman E, Ghesquiere B, Thomas GR, Vandekerckhove J, Gevaert K (2005) The human platelet proteome mapped by peptide-centric proteomics: a functional protein profile. Proteomics 5:3193–3204

Mateos-Caceres PJ, Macaya C, Azcona L, Modrego J, Mahillo E, Bernardo E, Fernandez-Ortiz A, Lopez-Farre AJ (2010) Different expression of proteins in platelets from aspirin-resistant and aspirin-sensitive patients. Thromb Haemost 103:160–170

Maurer-Spurej E, Kahr WH, Carter CJ, Pittendreigh C, Cameron M, Cyr TD (2008) The value of proteomics for the diagnosis of a platelet-related bleeding disorder. Platelets 19:342–351

Maynard DM, Heijnen HF, Horne MK, White JG, Gahl WA (2007) Proteomic analysis of platelet alpha-granules using mass spectrometry. J Thromb Haemost 5:1945–1955

McRedmond JP, Park SD, Reilly DF, Coppinger JA, Maguire PB, Shields DC, Fitzgerald DJ (2004) Integration of proteomics and genomics in platelets: a profile of platelet proteins and platelet-specific genes. Mol Cell Proteomics 3:133–144

Moebius J, Zahedi RP, Lewandrowski U, Berger C, Walter U, Sickmann A (2005) The human platelet membrane proteome

reveals several new potential membrane proteins. Mol Cell Proteomics 4:1754–1761

O'Neill EE, Brock CJ, von Kriegsheim AF, Pearce AC, Dwek RA, Watson SP, Hebestreit HF (2002) Towards complete analysis of the platelet proteome. Proteomics 2:288–305

Ong SE, Blagoev B, Kratchmarova I, Kristensen DB, Steen H, Pandey A, Mann M (2002) Stable isotope labeling by amino acids in cell culture, SILAC, as a simple and accurate approach to expression proteomics. Mol Cell Proteomics 1:376–386

Parguiña AF, Grigorian-Shamajian L, Agra RM, Teijeira-Fernández E, Rosa I, Alonso J, Viñuela-Roldán JE, Seoane A, González-Juanatey JR, García A (2010) Proteins involved in platelet signaling are differentially regulated in acute coronary syndrome: a proteomic study. PLoS One 5:e13404

Parguina AF, Grigorian-Shamagian L, Agra RM, Lopez-Otero D, Rosa I, Alonso J, Teijeira-Fernandez E, Gonzalez-Juanatey JR, Garcia A (2011) Variations in platelet proteins associated with ST-elevation myocardial infarction: novel clues on pathways underlying platelet activation in acute coronary syndromes. Arterioscler Thromb Vasc Biol 31:2957–2964

Pieroni L, Finamore F, Ronci M, Mattoscio D, Marzano V, Mortera SL, Quattrucci S, Federici G, Romano M, Urbani A (2011) Proteomics investigation of human platelets in healthy donors and cystic fibrosis patients by shotgun nUPLC-MSE and 2DE: a comparative study. Mol Biosyst 7:630–639

Piersma SR, Broxterman HJ, Kapci M, de Haas RR, Hoekman K, Verheul HM, Jimenez CR (2009) Proteomics of the TRAP-induced platelet releasate. J Proteomics 72:91–109

Premsler T, Lewandrowski U, Sickmann A, Zahedi RP (2011) Phosphoproteome analysis of the platelet plasma membrane. Methods Mol Biol 728:279–290

Qureshi AH, Chaoji V, Maiguel D, Faridi MH, Barth CJ, Salem SM, Singhal M, Stoub D, Krastins B, Ogihara M, Zaki MJ, Gupta V (2009) Proteomic and phospho-proteomic profile of human platelets in basal, resting state: insights into integrin signaling. PLoS One 4: e7627

Ramacciotti E, Hawley AE, Wrobleski SK, Myers DD, Strahler JR, Andrews PC, Guire KE, Henke PK, Wakefield TW (2010) Proteomics of microparticles after deep venous thrombosis. Thromb Res 125:e269–e274

Ramaiola I, Padró T, Peña E, Juan-Babot O, Cubedo J, Martin-Yuste V, Sabate M, Badimon L (2015) Changes in thrombus composition and profilin-1 release in acute myocardial infarction. Eur Heart J 36:965–975

Randriamboavonjy V, Isaak J, Elgheznawy A, Pistrosch F, Fromel T, Yin X, Badenhoop K, Heide H, Mayr M, Fleming I (2012) Calpain inhibition stabilizes the platelet proteome and reactivity in diabetes. Blood 120:415–423

Rendu F, Brohard-Bohn B (2001) The platelet release reaction: granules' constituents, secretion and functions. Platelets 12:261–273

Rogowska-Wrzesinska A, Le Bihan MC, Thaysen-Andersen M, Roepstorff P (2013) 2D gels still have a niche in proteomics. J Proteomics 88:4–13

Rotilio D, Della Corte A, D'Imperio M, Coletta W, Marcone S, Silvestri C, Giordano L, Di Michele M, Donati MB (2012) Proteomics: bases for protein complexity understanding. Thromb Res 129:257–262

Schubert P, Culibrk B, Coupland D, Scammell K, Gyongyossy-Issa M, Devine DV (2012a) Riboflavin and ultraviolet light treatment potentiates vasodilator-stimulated phosphoprotein Ser-239 phosphorylation in platelet concentrates during storage. Transfusion 52:397–408

Schubert P, Culibrk B, Karwal S, Slichter SJ, Devine DV (2012b) Optimization of platelet concentrate quality: application of proteomic technologies to donor management. J Proteomics 76:329–336

Schweigel H, Geiger J, Beck F, Buhs S, Gerull H, Walter U, Sickmann A, Nollau P (2013) Deciphering of ADP-induced, phosphotyrosine-dependent signaling networks in human platelets by Src-homology 2 region (SH2)-profiling. Proteomics 13:1016–1027

Senis YA, Tomlinson MG, Garcia A, Dumon S, Heath VL, Herbert J, Cobbold SP, Spalton JC, Ayman S, Antrobus R, Zitzmann N, Bicknell R, Frampton J, Authi KS, Martin A, Wakelam MJ, Watson SP (2007) A comprehensive proteomics and genomics analysis reveals novel transmembrane proteins in human platelets and mouse megakaryocytes including G6b-B, a novel immunoreceptor tyrosine-based inhibitory motif protein. Mol Cell Proteomics 6:548–564

Shai E, Rosa I, Parguina AF, Motahedeh S, Varon D, Garcia A (2012) Comparative analysis of platelet-derived microparticles reveals differences in their amount and proteome depending on the platelet stimulus. J Proteomics 76:287–296

Shevchenko A, Tomas H, Havlis J, Olsen JV, Mann M (2006) In-gel digestion for mass spectrometric characterization of proteins and proteomes. Nat Protoc 1:2856–2860

Sim DS, Dilks JR, Flaumenhaft R (2007) Platelets possess and require an active protein palmitoylation pathway for agonist-mediated activation and in vivo thrombus formation. Arterioscler Thromb Vasc Biol 27:1478–1485

Snyder EL, Dunn BE, Giometti CS, Napychank PA, Tandon NN, Ferri PM, Hofmann JP (1987) Protein changes occurring during storage of platelet concentrates. A two-dimensional gel electrophoretic analysis. Transfusion 27:335–341

Staes A, Demol H, Van Damme J, Martens L, Vandekerckhove J, Gevaert K (2004) Global differential non-gel proteomics by quantitative and stable labeling of tryptic peptides with oxygen-18. J Proteome Res 3:786–791

Staes A, Impens F, Van Damme P, Ruttens B, Goethals M, Demol H, Timmerman E, Vandekerckhove J, Gevaert K (2011) Selecting protein N-terminal peptides by combined fractional diagonal chromatography. Nat Protocols 6:1130–1141

Stes E, Laga M, Walton A, Samyn N, Timmerman E, De Smet I, Goormachtig S, Gevaert K (2014) A COFRADIC protocol to study protein ubiquitination. J Proteome Res 13:3107–3113

Thomas A, Rahmanian S, Bordbar A, Palsson BØ, Jamshidi N (2014) Network reconstruction of platelet metabolism identifies metabolic signature for aspirin resistance. Sci Rep 4:3925

Thompson A, Schafer J, Kuhn K, Kienle S, Schwarz J, Schmidt G, Neumann T, Johnstone R, Mohammed AK, Hamon C (2003) Tandem mass tags: a novel quantification strategy for comparative analysis of complex protein mixtures by MS/MS. Anal Chem 75:1895–1904

Thon JN, Schubert P, Duguay M, Serrano K, Lin S, Kast J, Devine DV (2008) Comprehensive proteomic analysis of protein changes during platelet storage requires complementary proteomic approaches. Transfusion 48:425–435

Tucker KL, Kaiser WJ, Bergeron AL, Hu H, Dong JF, Tan TH, Gibbins JM (2009) Proteomic analysis of resting and thrombin-stimulated platelets reveals the translocation and functional relevance of HIP-55 in platelets. Proteomics 9:4340–4354

Unlu M, Morgan ME, Minden JS (1997) Difference gel electrophoresis: a single gel method for detecting changes in protein extracts. Electrophoresis 18:2071–2077

van den Bosch MT, Poole AW, Hers I (2014) Cytohesin-2-phosphorylation by protein kinase C relieves the constitutive suppression of platelet dense granule secretion by ADP-ribosylation factor 6. J Thromb Haemost 12:726–735

van Holten TC, Bleijerveld OB, Wijten P, de Groot PG, Heck AJ, Barendrecht AD, Merkx TH, Scholten A, Roest M (2014) Quantitative proteomics analysis reveals similar release profiles following specific PAR-1 or PAR-4 stimulation of platelets. Cardiovasc Res 103:140–146

Vaudel M, Burkhart JM, Radau S, Zahedi RP, Martens L, Sickmann A (2012) Integral quantification accuracy estimation for reporter ion-based quantitative proteomics (iQuARI). J Proteome Res 11:5072–5080

Veitinger M, Umlauf E, Baumgartner R, Badrnya S, Porter J, Lamont J, Gerner C, Gruber CW, Oehler R, Zellner M (2012) A combined proteomic and genetic analysis of the highly variable platelet proteome: from plasmatic proteins and SNPs. J Proteomics 75:5848–5860

Vélez P, Parguiña AF, Ocaranza-Sánchez R, Grigorian-Shamagian L, Rosa I, Alonso-Orgaz S, de la Cuesta F, Guitián E, Moreu J, Barderas MG, González-Juanatey JR, García Á (2014) Identification of a circulating microvesicle protein network involved in ST-elevation myocardial infarction. Thromb Haemost 112:716–726

Vélez P, Izquierdo I, Rosa I, García Á (2015) A 2D-DIGE-based proteomic analysis reveals differences in the platelet releasate composition when comparing thrombin and collagen stimulations. Sci Rep 5:8198

Volpi E, Giusti L, Ciregia F, Da Valle Y, Giannaccini G, Berti S, Clerico A, Lucacchini A (2012) Platelet proteome and clopidogrel response in patients with stable angina undergoing percutaneous coronary intervention. Clin Biochem 45:758–765

Wandall HH, Rumjantseva V, Sørensen ALT, Patel-Hett S, Josefsson EC, Bennett EP, Italiano JE, Clausen H, Hartwig JH, Hoffmeister KM (2012) The origin and function of platelet glycosyltransferases. Blood 120:626–635

Washburn MP, Wolters D, Yates JR (2001) Large-scale analysis of the yeast proteome by multidimensional protein identification technology. Nat Biotechnol 19:242–247

Werner T, Sweetman G, Savitski MF, Mathieson T, Bantscheff M, Savitski MM (2014) Ion coalescence of neutron encoded TMT 10-plex reporter ions. Anal Chem 86:3594–3601

Westbury SK, Turro E, Greene D, Lentaigne C, Kelly AM, Bariana TK, Simeoni I, Pillois X, Attwood A, Austin S, Jansen SB, Bakchoul T, Crisp-Hihn A, Erber WN, Favier R, Foad N, Gattens M, Jolley JD, Liesner R, Meacham S, Millar CM, Nurden AT, Peerlinck K, Perry DJ, Poudel P, Schulman S, Schulze H, Stephens JC, Furie B, Robinson PN, van Geet C, Rendon A, Gomez K, Laffan MA, Lambert MP, Nurden P, Ouwehand WH, Richardson S, Mumford AD, Freson K, BRIDGE-BPD Consortium (2015) Human phenotype ontology annotation and cluster analysis to unravel genetic defects in 707 cases with unexplained bleeding and platelet disorders. Genome Med 7:36

Wijten P, van Holten T, Woo LL, Bleijerveld OB, Roest M, Heck AJ, Scholten A (2013) High precision platelet releasate definition by quantitative reversed protein profiling. Arterioscler Thromb Vasc Biol 33:1635–1638

Wilkins MR, Sanchez JC, Gooley AA, Appel RD, Humphery-Smith I, Hochstrasser DF, Williams KL (1996) Progress with proteome projects: why all proteins expressed by a genome should be identified and how to do it. Biotechnol Genet Eng Rev 13:19–50

Winkler W, Zellner M, Diestinger M, Babeluk R, Marchetti M, Goll A, Zehetmayer S, Bauer P, Rappold E, Miller I, Roth E, Allmaier G, Oehler R (2008) Biological variation of the platelet proteome in the elderly population and its implication for biomarker research. Mol Cell Proteomics 7:193–203

Wright B, Stanley RG, Kaiser WJ, Mills DJ, Gibbins JM (2011) Analysis of protein networks in resting and collagen receptor (GPVI)-stimulated platelet sub-proteomes. Proteomics 11:4588–4592

Xu X, Gnatenko DV, Ju J, Hitchcock IS, Martin DW, Zhu W, Bahou WF (2012) Systematic analysis of microRNA fingerprints in thrombocythemic platelets using integrated platforms. Blood 120:3575–3585

Yao X, Freas A, Ramirez J, Demirev PA, Fenselau C (2001) Proteolytic 18O labeling for comparative proteomics: model studies with two serotypes of adenovirus. Anal Chem 73:2836–2842

Zahedi RP, Begonja AJ, Gambaryan S, Sckmann A (2006) Phosphoproteomics of human platelets: a quest for novel activation pathways. Biochim Biophys Acta 1764:1963–1976

Zahedi RP, Lewandrowski U, Wiesner J, Wortelkamp S, Moebius J, Schutz C, Walter U, Gambaryan S, Sickmann A (2008) Phosphoproteome of resting human platelets. J Proteome Res 7:526–534

Zeiler M, Moser M, Mann M (2014) Copy number analysis of the murine platelet proteome spanning the complete abundance range. Mol Cell Proteomics 13:3435–3445

Zellner M, Winkler W, Hayden H, Diestinger M, Eliasen M, Gesslbauer B, Miller I, Chang M, Kungl A, Roth E, Oehler R (2005) Quantitative validation of different protein precipitation methods in proteome analysis of blood platelets. Electrophoresis 26:2481–2489

Zellner M, Baureder M, Rappold E, Bugert P, Kotzailias N, Babeluk R, Baumgartner R, Attems J, Gerner C, Jellinger K, Roth E, Oehler R, Umlauf E (2012) Comparative platelet proteome analysis reveals an increase of monoamine oxidase-B protein expression in Alzheimer's disease but not in non-demented Parkinson's disease patients. J Proteomics 75:2080–2092

Zimman A, Titz B, Komisopoulou E, Biswas S, Graeber TG, Podrez EA (2014) Phosphoproteomic analysis of platelets activated by pro-thrombotic oxidized phospholipids and thrombin. PLoS One 9:e84488

Zufferey A, Fontana P, Reny JL, Nolli S, Sanchez JC (2012) Platelet proteomics. Mass Spectrom Rev 31:331–351

Zufferey A, Schvartz D, Nolli S, Reny JL, Sanchez JC, Fontana P (2014) Characterization of the platelet granule proteome: evidence of the presence of MHC1 in alpha-granules. J Proteomics 101:130–140