SIGNIFICANT IMPACT OF CONSUMABLE MATERIAL AND BUFFER COMPOSITION FOR LOW-CELL NUMBER PROTEOMIC SAMPLE PREPARATION

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ABSTRACT: Proteomics, essential for understanding gene and cell functions, faces challenges with peptide loss due to adsorption onto vial surfaces, especially in samples with low peptide quantities. Using HeLa tryptic digested standard solutions, we demonstrate preferential adsorption of peptides, particularly hydrophobic ones, onto polypropylene (PP) vials, leading to non-uniform signal loss. This phenomenon can alter protein quantification (e.g., Label-Free Quantification, LFQ) if no appropriated data processing is applied. Our study is based on understanding this adsorption phenomenon to establish recommendations for minimizing peptide loss. To address this issue, we evaluated the nature of surface material and buffer additives to reduce peptide-surface non-covalent binding. Here, we report that using vials made of polymer containing polar monomeric units such as polymethylmethacrylate (PMMA) or polyethylene terephthalate (PET) drastically reduces the hydrophobic peptide loss, increasing the global proteomics performances (fourfold increase in identified peptides for the single-cell equivalent peptide content range). Additionally, the incorporation of non-ionic detergents like polyethylene oxide (PEO) and n-Dodecyl-Beta-Maltoside (DDM) at optimized concentrations (0.0001% and 0.0075% respectively) improves overall proteomic performance and consistency, even when different vial materials are used. Implementing these recommendations on 0.2 ng/µL HeLa tryptic digest results in a tenfold increase in terms of peptide signal. Application to True Single Cell sample preparation without specialized instrumentation dramatically improves performance, allowing for the identification of approximately 650 proteins, compared to none with classical protocols.

INTRODUCTION

Proteomics is a scientific discipline dedicated to the comprehensive study of proteins, which are essential for understanding the functions of genes and cells. This interdisciplinary field encompasses a variety of techniques, such as imaging, array experiments, and genetic assays. Mass spectrometry (MS) has emerged as a powerful analytical tool for studying complex protein samples, with the current trend being the integration of liquid chromatography separation with electrospray ionization mass spectrometry[1,2]. Moreover, recent technological improvements in MS have significantly increased its sensitivity and the ability to downsize proteomic workflows. These advancements have allowed the analyses of samples containing a low amount of biological material (i.e., low amounts of proteins). Such capabilities are crucial for exploring specific areas like rare cell subpopulations[3,4], conducting spatial-omics on biological tissues[5,6], and investigating the proteome at the single-cell level[7–10].

Scaling down biological material quantities for proteomic analysis often results in a significant decrease in performance. This reduction is primarily attributed to limitations in the sensitivity of instruments (limit of detection and limit of quantification) and the loss of sample material during the preparation and purification processes[11]. The sensitivity of instruments being dependent on the equipment available on the market, the critical importance of sample preparation in enhancing the overall quality of the analysis necessitates more in-depth investigation. Optimized sample preparation approaches for samples containing low amounts of biological material, such as mPOP protocol, aim to eliminate sample cleaning, non-essential steps in the preparation process and sample transfer[12]. The mPOP protocol has been integrated in the Single Cell ProtEomics by Mass Spectrometry (SCoPE-MS) workflow developed by the Slavov's group for single-cell proteomics analysis. This workflow is based on the use of Tandem Mass Tag (TMT) and a carrier channel to boost peptide signals and counter the limit detection of current instruments[9,10], therefore allowing the identification and quantification of thousand proteins from a single cell.

Even when employing these approaches, material loss due to adsorption on surfaces can become critical when processing such very low amounts of starting biological material. Indeed, unspecific protein or peptide adsorption is already known to occur on solid surfaces (e.g., pipet tips, vials and instrumentation parts) through noncovalent interactions (e.g., electrostatic, hydrophobic) and depend on experimental conditions (e.g. peptide properties, physical state of the surface and sample environmental properties)[13]. When this adsorption phenomenon is not negligeable, peptide intensity signals in LC-MS analysis are not linearly correlated with the initial concentration in vials[14]. As

an example, Law and Shih reported that the calcitonin adsorption on soda lime silica glass is dependent on the concentration and that the adsorption isotherms are of the Langmuir and Freundlich type, depending on the solution pH[15]. The prediction of peptide adsorption on specific surfaces, based on their physicochemical properties is challenging since it results from a complex interplay of the properties of the surface (e.g., nature, shape, topology), the protein/peptide (e.g., hydrophobicity, charge, residue distribution, intramolecular and intermolecular interaction, conformation) and the buffer solvent (e.g., solvation force, composition, pH, temperature)[16–20]. Therefore, choosing the appropriate vial material and sample preparation buffer is crucial when processing samples with low protein concentrations[19]. Various strategies have been developed to minimize the adsorption of proteins and/or peptides onto surfaces. These strategies mainly involve addition of exogenous protein-rich sample (e.g., bovine serum albumin, BSA)[19,21], organic solvent (e.g., dimethyl sulfoxide or acetonitrile)[22], or surfactant agents agents[23–28]. The use of organic solvents can influence the binding of hydrophilic peptides on the LC column[22,29] while incorporating external proteins into samples with low protein content is suboptimal, as it may lead to ionization competition. Therefore, the use of adapted surfactant agents seems to be an adequate approach for improving low-cell number proteomic performance. With the objective of eliminating cleaning steps, surfactants agent must be LC-MS compatible without interfering with the peptide separation and ionization. Among the compatible surfactant agents, n-Dodecyl-Beta-Maltoside (DDM)[27,30] and polyethylene oxide (PEO)[25] have already reported as effective additives reducing peptide adsorption on the vial surface.

Nowadays, minimizing peptide adsorption on surface has emerged as a priority for manufacturers of laboratory consumables for low-cell number proteomics as supported with innovations such as the QuanRecovery vial from Waters (UK) and the proteoCHIP from Cellenion instrument (France)[7]. Another interesting approach, called the nanoPot workflow[31], has emerged from this need to reduce peptide loss due to surface adsorption. In that workflow, all sample preparation steps are carried out within a droplet to minimize molecular interactions with the surface and reduce the sample volume. However, this sample preparation procedure requires dedicated instruments for precise sub-microliter liquid dispensing.

In this study, we evaluate the effects of surface properties (i.e., the nature of the polymeric material) and the presence of surfactants on peptide loss due to surface adsorption. Some recommendations and strategies to minimize peptide loss when scaling down protein starting amounts from standard proteomic analysis to low cell numbers and single-cell samples are reported. These recommendations are based on (1) a ONE-pot strategy (aimed at reducing sample transfers and contact with laboratory consumables such as vials and tips), (2) the use of adapted surface materials, and (3) the use of surfactant agents to minimize peptide affinity for surfaces.

MATERIAL AND METHOD

Single cell type sample: HeLa Tryptic digest peptides

HeLa tryptic digest standard solution from ThermoFisher Scientific (Pierce™ HeLa Protein Digest Standard, 88328) has been considered as a model for monitoring peptide binding on surfaces. HeLa tryptic digest stock solutions of 500 ng/µl (equivalent protein content) have been prepared from the solubilization of standard HeLa tryptic digest in 0.1% trifluoroacetic acid (TFA) in MilliQ water, aliquoted by 2.44 µL and stored at -80°C in 0.6 mL Eppendorf vial (polypropylene). Two distinct peptide sample series were prepared by diluting the stock solution with 0.1% trifluoroacetic acid (TFA) in MilliQ water. These series are specifically designed to analyze peptide samples of either 10 ng or 0.2 ng (injected peptide quantities in LC-MS). (1) 10 ng corresponds to the expected quantity in SCoPE-MS workflows that involve a carrier channel comprising 50 to 100 cells. HeLa peptide solutions with concentration ranging from 1.1 to 20 ng/µL have been freshly prepared from the stock solutions to evaluate the concentration influence while keeping 10 ng of injected peptides. Injection volumes of this series were ranging from 9 to 0.5 µL, respectively, to reach the target of 10 ng injected in the LC-MS system (Trap-Elute mode). (2) For the second series, solutions of HeLa digest at 0.2 ng/µL were used, allowing precise injection of 0.2 ng of peptide which corresponds to the expected peptide amount for "True" single-cell proteomics. For both series, the target peptide quantities were selected based on protein quantification experiments of HeLa cells, revealing an approximate protein content of 0.2 ng/cell (see supporting information for protein quantification experiment results).

Stock solutions of polyethylene oxide (PEO) and n-Dodecyl-Beta-Maltoside (DDM) have been prepared in MilliQ water at 0.1% (w/w). The peptide solutions with PEO and DDM have been prepared by the dilution of HeLa stock solution spiked with PEO or DDM stock solutions and diluted with 0.1% TFA in MilliQ water to reach the desired peptide and PEO/DDM concentrations.

Cell sample preparation

HeLa cells (ATCC) were cultured in DMEM high glucose (Biowest, L0104-500), supplemented with 10% Fetal Bovine Serum (ThermoFisher, 10270-106) and 1% Penicillin/Streptomycin (Biowest, L0022-100). HeLa cells were prepared to a single cell solution (0.05 % Trypsin-EDTA, Gibco, 25300-54) on the day of Fluorescence-Activated Cell Sorting (FACS - SONY MA900). Ten or one HeLa cells were deposited into a 96-well PCR polypropylene plate from Eppendorf (Eppendorf twin.tec PCR Plate 96, Cat. No. 0030133366) pre-filled with 5 µL of sterile cold PBS (Biowest, L0615-500). The cells were sorted into the plate, allocating half of it with 10 cells and the other half with 1 cell per well (specifically, wells from columns 1 to 6 received single cells, while columns 7 to 12 received 10cells per well). The plates containing the sorted cells were centrifuged 300g for 3 minutes at 4°C in a swinging bucket rotor (ThermoScientific, Megafuge 40R) promptly frozen on dry ice and stored at -80°C. Prior to proteomic sample preparation, the buffer solutions were adjusted by adding 1 µL Tris-HCl (pH = 8) at 200 mM. Three conditions were tested: samples without additive (referred to as "NoAdd"), samples with 0.006% PEO in mass-to-mass ratio (referred to as "+PEO"), and samples with 0.045% DDM in mass-to-mass ratio (referred to as "+DDM"). The addition of PEO and DDM was performed to assess their advantages in sample preparation. The samples were centrifuged at 12,000 g for 1 min, followed by heating at +80°C for 5 min using a ThermoMixer C from Eppendorf. Subsequently, the samples were centrifuged again at 12,000 g for 2 min to recover condensed droplets. All subsequent reagent additions were made without direct contact with the sample to minimize material adsorption on the tips, followed by a 12,000 g centrifugation

for 2 minutes. A 1 µL aliquot of Benzonase (1 U/µL) was added to degrade DNA and RNA, with a 30-minute incubation. Then, 1 µL of 45 mM DDT was added to reduce disulfide bridges, incubating at 56°C for 30 minutes. Following this, 1 µL of 100 mM IAA in 100 mM Tris-HCl was added, and the sample was placed in the dark at room temperature for 30 minutes. Subsequently, 1 µL of 25 ng/µL Trypsin in 50 mM Tris-HCl was added, and protein digestion was carried out overnight at 37°C. Finally, 1 µL of TFA (1.2% v/v) was added to quench the digestion. The final volume in the vial was 12 µL, and the concentrations of PEO or DDM in "+PEO" and "+DDM" samples were, respectively, 0.0001% and 0.0075%.

The digestion of some samples prepared without PEO or DDM ("NoAdd" samples) were quenched by adding 1.2% TFA with 0.0012% PEO or 0.09% DDM to reach a final concentration of 0.0001% or 0.0075%, respectively. This experiment was designed to compare the influence of adding PEO or DDM at the beginning (i.e., cell level) or at the end (i.e., peptide level) of the sample preparation process.

Vial design and production

Vials have been molded by injection in different polymeric materials to investigate the impact of surface nature on proteomic performances. For this study, Polymethyl methacrylate (PMMA), polycarbonate (PC), polyethylene terephthalate (PET), Polyvinylidene fluoride (PVDF), cyclic olefin polymer & copolymer (COP and COC, respectively), and polypropylene (PP) were evaluated. These materials are all compatible with a 1% TFA solution and cover different polymer properties such as hydrophobicity and polarity. The compatibility with other commonly used solvents has also been investigated. It is important to note that some of these polymers, such as PMMA and PET, are not compatible with solvents containing acetonitrile (ACN) or methanol, which are primarily used in multiplex proteomic approaches. 0.3 mL PP vial from ThermoFisher Scientific (Cat. No. 11717597), 1.5 mL TotalRecovery Glass vial from Waters (Cat. No. 186005663CV), and 0.3 mL QuanRecovery PP vial from Waters (Cat. No. 186009186) have been evaluated as commercial vials.

Liquid Chromatography-Mass Spectrometry

Peptide solutions have been injected on an Acquity UPLC MClass liquid chromatography system from Waters (UK) connected to a timsTOF Pro2 or a timsTOF SCP instruments from Bruker (Bremen, Germany). Data dependent acquisition (DDA) analyses were performed on the timsTOF Pro2 for the analysis of 10 ng peptide samples while data-independent acquisition (DIA) analyses were performed on the timsTOF SCP for the analysis of 0.2 ng peptide samples. Detailed LC-MS instruments configuration and settings are reported in supporting information.

Data analysis

Identification and quantification of peptides and proteins have been computed on FragPipe 2.0.1 and DIA-NN 1.8.1 for DDA and DIA data, respectively, with match-between-run (MBR). Swiss-Prot reviewed human protein database (FASTA file generated from UniProt) has been used for the protein identification on DDA data. A spectral library has been generated for DIA analysis from the analysis of 200 ng HeLa tryptic digest by DDA. Python 3.11 scripts (involving Pandas, Scipy, Numpy, and Matplotlib libraries) have been developed to monitor peptide/protein intensities or counts in function of their properties (e.g., retention time, mass and charge) or experimental conditions (e.g., the nature of the vial, concentration in vial and buffer composition) and report 1D or 2D distribution plots (i.e., histogram or colored-based heatmaps). These scripts use the outputs generated by FragPipe 2.0.1 or DIAN-NN 1.8.1.

RESULTS AND DISCUSSIONS

Understanding peptide loss attributable to surface binding.

To evaluate the impact of non-specific adsorption on the inner surface of vials and LC system, we monitored the total intensity of identified peptides from a consistent injected peptide quantity while varying the peptide concentration. This approach was selected because peptide loss resulting from surface adsorption is concentration-dependent and should not be influenced by the injected quantity in LC instruments. Therefore, various solutions of standard HeLa tryptic digest with concentrations varying from 1.1 to 20 ng/µL were prepared in commercially available polypropylene vials from VWR, a commonly used consumable in LC-MS proteomics. To ensure constant surface exposure regardless of peptide concentration, a fixed final volume of 20 µL was employed. For each sample, the injection volume was set to correspond to an expected peptide quantity of 10 ng (i.e., injection volumes ranging from 9 to 0.5 µL for peptide concentrations from 1.1 to 20 ng/µL, respectively). Notably, 10 ng of HeLa tryptic digest is roughly equivalent to the protein content of 50 HeLa cells, taking into account an estimated protein content of approximately 0.2 ng per HeLa cell (as detailed in the supporting information). This quantity aligns with the SCoPE-MS workflow and is compatible with the sensitivity of the Bruker timsTOF Pro2 instrument.

In the absence of peptide adsorption, one would expect consistent peptide intensity regardless of the peptide concentration, as the same quantity of peptide is injected. However, as shown in Figure 1A, reducing the concentration of peptides while maintaining the same injected amount correlates with a decrease in peptide intensity. This finding indicates that the actual amount of peptide injected in the LC-MS system is less than the anticipated 10 ng, and this discrepancy relies on the effective concentration. It supports peptide loss due to adsorption phenomenon, since peptides adsorption on the surface diminishes their effective concentration, subsequently reducing the injected peptide quantity. This phenomenon is particularly pronounced at lower concentrations, where the proportion of adsorbed peptides becomes more significant. Interestingly, the observed decrease in total intensity with lower concentrations is also related to the peptide retention times, as shown in the color-coded heatmap in Figure 1B. This heatmap plots peptide intensities (log scale, represented by colors) in function of peptide concentration (X-axis) and retention time (ranging from 8 to 23 minutes on the Y-axis). In fact, the total peptide intensities significantly decline in the latter third of the LC gradient (particularly after 16 minutes), experiencing a fivefold drop when the concentration is lowered from 20 to 1.1 ng/µL. Conversely, this decrease is about threefold in the middle third and is minimal in the initial third. These observations suggest a potential correlation between peptide adsorption and peptide hydrophobicity. It appears that peptides with higher hydrophobicity are more susceptible to adsorption onto the surface, especially when using PP vials. This observation is consistent with the expected behavior for a PP surface, given the inherently hydrophobic nature of this material. Notably, polypropylene chains can engender

Van der Waals interactions with hydrophobic residues of peptides, reducing their exposure to water.

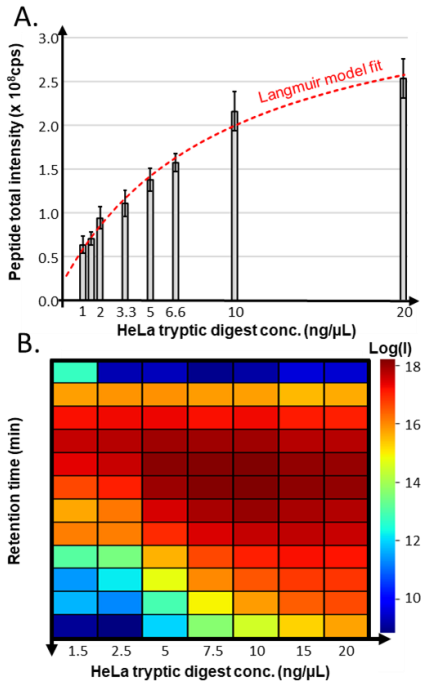

Figure 1: A) Peptide total intensity (sum of intensities of identified peptide) from 10ng of HeLa tryptic digest solutions analyses with concentration ranging from 1 to 20ng/µL. Peptide were separated with a 25 minutes LC gradient and MS/MS spectra were acquired in DDA-PASEF mode (see Materials and Methods for more information). Peptides are identified and quantified by FragPipe with an FDR < 1% without any normalization on 3 replicates. Dash red line corresponds to a fit with a Langmuir model. B) Distribution of peptide total intensity (expressed in Log10) in function of the retention time (Y-axis) and the HeLa tryptic digest concentration in PP vial.

Consequently, hydrophobic peptides tend to be more readily adsorbed onto a hydrophobic surface. In contrast, hydrophilic peptides, being well solubilized, exhibit lower propensity for surface adsorption. This discrepancy on adsorption behaviors leads to a non-uniform loss of peptides, which can have a significant impact on protein identification but also on protein quantification. Indeed, when aggregating peptides for protein quantification, such as in Label-Free Quantification (LFQ), the intensities of hydrophobic peptides are considerably affected, resulting in greater signal variability. This could lead to misinterpretation in differential studies, even with the use of normalization algorithms, especially for low-abundant proteins containing hydrophobic part (e.g., membrane proteins). This adsorption phenomenon seems to be especially critical in samples with low peptide concentrations (below 20 ng/µL). The evolution of peptide total intensity as a function of peptide concentration aligns well with a simple implementation of the Langmuir adsorption model, modified to establish a relationship between intensity and concentration (illustrated in Equation 2).The Langmuir model equation used to establish this theoretical correlation is reported in Eq. 1, where $\%_{ads}$ is the equilibrium fractional occupancy, denoting the fraction of active sites occupied at equilibrium relative to the total number of available active sites, c is the molecule concentration, and K stands for the equilibrium constant governing the adsorption reaction. The adaptation of this equation to relate the measured total intensity of peptides (denoted $\boldsymbol{Intensity}$) with the concentration leads to Eq. 2 (explanation and demonstration of this equation are reported in supporting information); where α is the correlation factor between the infused quantity and the resulting measured intensity which is related to a weighted-average ionization efficiency for the different peptides; $\boldsymbol{n_{expect\ peptide}}$ is the expected quantity of injected peptide (i.e., considering as the peptide concentration multiplicated by the injection volume) expressed in moles; $\boldsymbol{n_{site}}$ is the number of available adsorption site on the surface of the vial; and $\boldsymbol{V_{vial}}$ is the solution volume in the vial. It is important to note that $\boldsymbol{V_{vial}}$ was constant in this experiment. The Eq. 2 can be parametrized to obtain the Eq. 4 that relates the measured intensities as a function of concentration while considering adsorption. This parametrization involves three parameters: A, B, and K. The definition of A and B are reported in Eq. 3.

$$\%_{ads} = \frac{K.c}{1 + K.c} \qquad \text{Eq. 1}$$

$$Intensity = \alpha \,.\, n_{expect\ peptide} \left(1 - \frac{\frac{K}{1+K.c} \,.\, n_{site}}{V_{vial}}\right) \qquad \text{Eq. 2}$$

$$\mathrm{A} = \alpha \,.\, n_{expect\ peptide} \qquad B = \frac{\mathrm{n_{site}}}{V_{vial}} \qquad \text{Eq. 3}$$

$$\mathrm{I} = \mathrm{A}.\left(1 - \mathrm{B}\left(\frac{\mathrm{K}}{1 + \mathrm{K.C}}\right)\right) \qquad \text{Eq. 4}$$

The parameter A is related to the expected intensity if no peptide adsorption, i.e., the expected total intensity of peptides from a 10ng HeLa tryptic digest injection in the case of this experiment. This parameter was constant for the HeLa solution series since the expected quantity of injected peptide was fixed to 10 ng. By definition, the parameter A is independent of the vial nature but depends on LC-MS instrumentation and methods (e.g., LC gradient, ionization source, ion optics in MS device) due to the α factor. The parameter B is directly related to the number of available adsorption sites and is therefore strongly depend on the surface nature and surface contact area. Here, B parameter is considered as constant for a given vial type and a given solution volume ($\boldsymbol{V_{vial}}$). Conversely to A parameter, B should be independent of the LC-MS instrument and method. Finally, K parameter represents a weighted-average equilibrium constant for all peptides since each peptide is defined with its own affinity for a given surface, depending for example on its hydrophobicity (among other factors) as discussed above. These three parameters were considered as constant for each concentration range tested since the same LC-MS settings were used and all vials were filled with the same volume of the peptide solution (i.e., constant $\boldsymbol{V_{vial}}$). The strong correlation observed between experimental intensity and the Langmuir-type model (reported in Figure 1A) provides strong evidence that the signal loss is closely linked to peptide adsorption onto the surface.

Based on these observations, a key strategy to reduce peptide loss and increase the portion of injected peptide is to decrease the peptide's affinity for the vial surface and LC system, specifically by lowering the adsorption equilibrium constant (K). Indeed, K is a fundamental parameter that characterizes the extent of adsorption of molecules on a solid surface. K is influenced by several factors, including the characteristics of the surface, the properties of the buffer, and the specific type of interactions involved (especially hydrophobic interaction in the case of PP

vials). The nature of the surface where adsorption takes place is a key determinant of K. A surface with a strong affinity for the molecules will result in a higher K value, indicating a bigger negative impact. Additionally, the composition of the buffer can substantially influence K. When the interactions between the molecules and buffer are highly favorable, it can lead to a situation where the liquid phase competes with the solid surface for interactions with the molecules. This competition may reduce adsorption on the solid surface because the buffer can effectively displace the molecules from the surface. There are therefore two strategies for reducing peptide affinity and improving the overall performance of the analyses by: (1) adapting the nature of the vial surface to reduce its affinity for peptides; (2) changing the solvent affinity for the peptide. These two strategies will be introduced and discussed in the following sections.

Evaluation of the surface nature influence on peptide adsorption

Different vials with identical geometries but made from various raw materials were designed and manufactured to assess the influence of surface properties on peptide adsorption. In this experiment, a range of polymeric materials with varying degrees of hydrophobicity were examined to modulate hydrophobic interactions. These materials included Polymethyl methacrylate (PMMA), Polycarbonate (PC), polyethylene terephthalate (PET), Polyvinylidene fluoride (PVDF), cyclic olefin polymers & copolymers (COP and COC, respectively), and polypropylene (PP).

As for the PP commercial vial, the peptide losses have been evaluated on these polymeric materials by the evolution of the total peptide intensities in function of the concentration for an expected injection of 10 ng HeLa tryptic digest. Peptides were separated during a 20-minutes LC gradient and identified by DDA. The results of this study are reported in Figure 2. The total peptide intensity evolution when using the PP vial from VWR is also reported in these results in white as reference. Since the number of available sites of peptide adsorption could be influenced by the nature of the polymeric material, the peptide intensities reported in Figure 2A were normalized to the total peptide intensity of the 20 ng/µl samples for each polymeric material respectively (i.e., total peptide intensities for the 20 ng/µL samples were set to 100% for each polymeric material, as shown for the 20 ng/µL results in Figure 2A). This allows evaluating the peptide resulting from the adsorption equilibrium constant (K) for the different polymeric material removing the contribution of the other material properties. As an initial observation from these results, the more hydrophobic polymers, such as COC, COP, and PP, result in a significant decrease in total peptide intensity with concentration compared to the more hydrophilic polymeric materials such as polycarbonate (PC) and polymethyl methacrylate (PMMA). This suggests that using hydrophilic polymeric material allows reducing hydrophobic interaction between hydrophobic peptide and the surface. The Figure 2B reported the counts of peptide identification with FDR < 1%, with the concentration for the different polymeric material nature. As expected, the peptides counts are strongly correlated to the peptide total intensities.

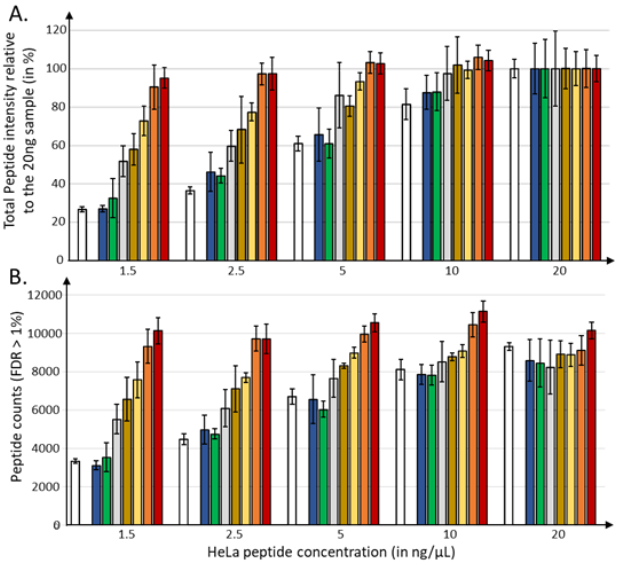

Figure 2: A) Total peptide intensities expressed as percentages relative to the 20 ng samples and (B) the peptide counts as a function of both the polymeric material nature of the vial and its concentration in LC-MS analysis, from a 10ng injection. A range of polymeric materials, including COP (blue), COC (green), PP (grey), PVDF (brown), PC (yellow), PET (orange), and PMMA (red), was examined. Additionally, a PP commercial vial from VWR was included, represented in white. Peptides were identified and quantified using FragPipe 2.0 software, with a false discovery rate (FDR) less than 1% and match-between-run (MBR). Relative total peptide intensities were calculated using MaxLFQ intensities provided by the FragPipe software. The reported values and error bars represent the averages and uncertainties derived from triplicate experiments.

The Figure 3 reported the peptide intensity heatmap for COC, PP, PC and PMMA in function of the retention time (in Y-Axis) and the concentration (in X-axis). These graphs show that the total intensity of hydrophobic peptide (i.e., higher retention times) are gradually increased from the two most hydrophobic evaluated polymeric materials (i.e., COC and PP) to the two least hydrophobic (i.e., PC and PMMA) at low peptide concentration. This supports that the use of low hydrophobic polymeric materials (i.e., more "polar" monomeric units) should enable the recovery of hydrophobic peptide signals when decreasing peptide concentration, thereby reducing the hydrophobic interactions with the surface. From literature, it is well established that predicting peptide adsorption on specific surfaces based solely on their biochemical characteristics is barely reliable[19,20]. However, in the context of bottom-up proteomic samples, it becomes evident that the hydrophobicity of the peptide and the exposed surface are critical parameters explaining peptide loss. One might speculate that the primary challenge in predicting peptide affinity for a given polymeric surface lies in accurately determining its hydrophobicity. Indeed, the prediction of the hydrophobicity of a polymeric material solely based on its chemical composition could not be sufficient, as the polymeric chains, structure, and geometry of the material can also influence its hydrophobic properties. Unfortunately, such detailed information is not always readily available from polymer or vial suppliers. To illustrate this point, even though the chemical composition of the monomeric units in PC suggests that this material should be more hydrophilic than PMMA, PC polymeric material exhibits slightly more pronounced hydrophobic interactions than PMMA, resulting in a slight decrease in total

peptide intensities. As a recommendation, the chemical nature can be considered as a main criterion for a large selection of potential candidates but experimental screening of the candidates must be performed to identify the best polymeric material for proteomic analysis on low-concentrate samples.

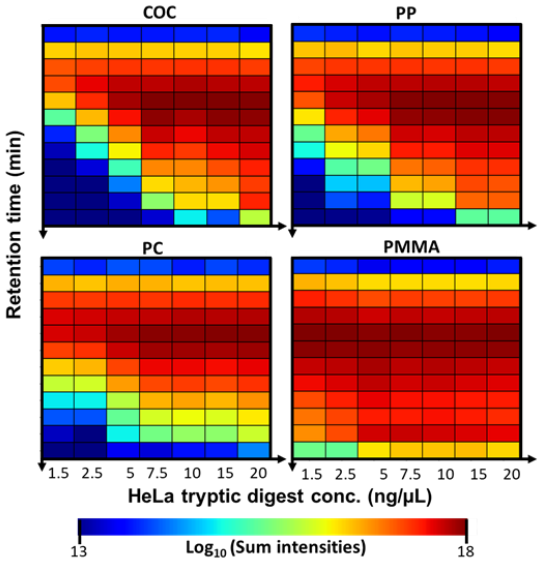

Figure 3: Peptide intensity distribution as a function of the retention time (Y-axis, in minutes) and the HeLa tryptic digest peptide solution concentration (X-axis, in ng/µL) for vials molded in COC (1), PP (2), PC (3), PMMA (4). Peptide intensity values are logarithmically scaled (log_{10}) and visualized using a color gradient, ranging from blue to red for, respectively, log_{10}(intensity) of 13 to 18.

For practical applications, vial manufacturers typically limit their choices to some versatile polymer materials, such as PP (polypropylene) and glass. However, in situations where the resources for crafting custom vials are unavailable, researchers are compelled to work with the materials that are readily accessible. It is essential, however, to be able to compare these commercial vials to select the most suitable option based on the proteomics applications. As an illustration, an experimental screening of three commercially available vials (a PP vial from VWR, a PP QuanRecovery vial from Waters, and a glass Total Recovery vial from Waters) were conducted for "True" single-cell proteomics purpose. Injection of 0.2 ng of HeLa tryptic digest peptide standard solution (concentration: 0.2 ng/µL) has been considered as a model of True single-cell proteomics. The outcomes of this study are depicted in Figure 4 and are compared to the PMMA vials. The proteomics performances were monitoring based on the total peptide intensity expressed in counts. The values presented in these figures are averaged from triplicate analyses. Regarding total ion intensity, the PP vials from VWR yielded the lowest total peptide intensity, followed by the glass vial from Waters. Remarkably, the QuanRecovery vial demonstrated impressive results, surpassing even the performance of the custom PMMA vial, despite being categorized as a PP vial. An undisclosed specific surface treatment has been applied to these latter to reduced peptide adsorption (based on the manufacturer saying), which could explain this gain in peptide total intensity compared to the other PP vial. As discussed above, the peptide counts is strongly correlated with the total peptide intensities.

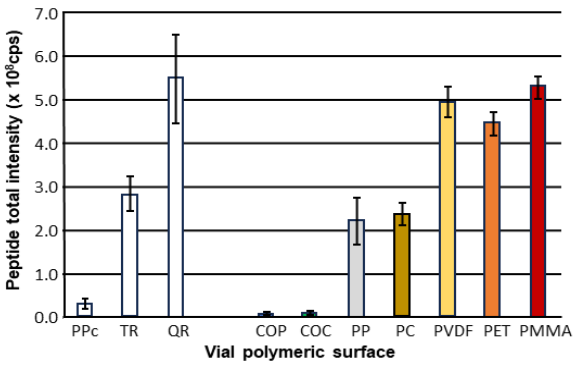

Figure 4: Absolute total peptide intensities expressed as counts in function of the vial nature including commercial vial PPc: PP commercial vial from VWR, WTR: Glass vial Total Recovery from Waters, QR: QuanRecovery vial from Waters and our custom vials, COP (blue), COC (green), PP (grey), PVDF (brown), PC (yellow), PET (orange), and PMMA (red) for 0.2ng HeLa tryptic digest standard solution (0.2ng/µL in 0.1% TFA). Data were acquired on the timsTOF SCP instrument in PASEF-DIA mode. Peptides were identified and quantified using DIA-NN 1.8.1 software, using an in-house spectral library with a false discovery rate (FDR) less than 1% and match-between-run (MBR). The reported values and error bars are the average values and uncertainties derived from triplicate experiments.

Using PEO or DDM to reduce peptide loss

A second option to reduction the surface affinity for peptide in solution is to add non-ionic detergent directly to the solvent. The use of non-ionic detergents in peptide solutions could reduce adsorption by forming micelles, decreasing adhesion forces, and/or changing vial surface properties by passivation. One can suspect that the adsorption of detergent on "apolar" surface such as COC, COP, and PP could functionalize these surfaces reducing their hydrophobicity. However, it is important to note that the inverse phenomena can occur on more "polar" surface leading to an increase of hydrophobicity by masking polar moieties. As for estimating surface hydrophobicity, predicting the efficiency of detergents in reducing peptide adsorption is challenging. Therefore, experimental screenings appear to be the best approach for evaluating their influence. In this study, we explored the use of polyethylene oxide (PEO) and n-Dodecyl-Beta-Maltoside (DDM) as non-ionic detergents compatible with the ONE-pot strategy requiring minimal preparation steps for low cell number sample. Their advantage lies in their compatibility with MS analysis, eliminating the need for a cleaning procedure before injection into LC-MS. The minimum concentration required to observe the beneficial effects of DDM and PEO was estimated by downscaling their concentration from 0.1% to 0.001% and 0.001% to 0.00001% (weight-to-weight percentages), respectively, on 0.2 ng HeLa tryptic digest (see supporting information, SX). The minimum concentrations of DDM and PEO required for 0.2 ng HeLa were determined to be 0.0075% and 0.00001%, respectively for PP vials, in weight-to-weight ratio. We therefore used these concentrations to evaluate the gain in proteomic performances using DDM and PEO on the different vial discussed in the preceding section. For PEO, we also assessed a tenfold increase in the minimal concentration, as the latter appears to be insufficient for certain vial types such as COC and COP. Note that with 0.001%, the signal of PEO was detected in the total ion chromatogram, especially at high retention times due to the polymer chain distribution

(MW: 20,000). However, these signals seemed not to downgrade the MS/MS analysis of peptides. These additives have been evaluated on the injection of 0.2 ng HeLa tryptic solution (0.2 ng/µL, injected volume = 1µL) as a single-cell model. The labels 'noAdd,' 'DDM,' 'PEO,' and 'PEOx10' corresponds to 0.2 ng HeLa tryptic digestion without additives, with 0.0075% DDM, with 0.0001% PEO, and with 0.001% PEO, respectively. The total peptide intensity gains (expressed in counts and represented in gray) using PEO, PEOx10 and DDM additives are reported in Figure 5.a, 5.b, and 5.c, respectively. The peptide identification gains (represented in gray) are also reported for PEO, PEOx10 and DDM in Figure 5.d, 5.e, and 5.f, respectively. These gains are represented in regard to the results obtained for the same solution without additive ("noAdd", represented in white).

As for the modification of the vial material, the peptide distributions based on retention times have been monitored (box plots in Supporting information, Figure S3. As already discussed, less hydrophobic peptides are detected for PP, COP, and COC vials without additives. Indeed, the mean retention time for peptides identified in COC, COP, PP are approximately 21 min, 21 min and 23 min, respectively, while the mean retention time when using PMMA vial is close to 26 min. These results are in perfect agreement with the previous observations. In presence of 0.0001% of PEO, the peptide distribution observed for COC and COP vial is right drifted to 24 min, while all the other vial exhibits similar distributions. This support that hydrophobic peptides are recovered in presence of PEO and suggest that PEO reduce the surface affinity difference between the different materials investigated in this study. At higher PEO concentration, the difference between all polymeric materials is reduced, suggesting that more PEO was required for the COP and COC vial to reduce peptide affinity for the surface. It is interesting to note that with 0.001% PEO, all peptide distributions are similar regardless of the polymeric material. This leveling effect on surface peptide adsorption becomes more pronounced when employing DDM, as it ensures uniform distribution of all peptides no matter the vial material used. In presence of these additives, the COC and COP vial seem to be as much adapted for single cell proteomics than PMMA supporting that all investigated polymeric material can be considered for low-cell number sample if PEO or DDM are added. Moreover, UPSET plot representation of these data (see supporting information Figure S4) show that the majority of peptides is commonly detected in all the investigated vials supporting that addition of PEO or DDM allows minimizing the vial nature influence on peptide adsorption and thus proteomic performances.

It is important to consider that PEO or DDM are detected in MS instrument and could contaminate the LC-MS system as they are detected in their corresponding retention time. Nevertheless, PEO and DDM contamination are detected after the peptide elution (i.e., higher retention time) without overlapping peptide signal. High-chain PEO have been selected to ensure this behavior. Interestingly, DDM ion and cluster ion are detected with ammonium adduct ($[C_{24}H_{46}O_{11} + NH_4]^+$, $[(C_{24}H_{46}O_{11})_2 + NH_4]^+$, $[(C_{24}H_{46}O_{11})_3 + NH_4]^+$ = 528.3384, 1038.6424, 1548.9464) at the same retention time suggesting the even if the concentration in solution is maintained below the micellar concentration threshold, some cluster can be form during the LC separation. This can explain it elution at higher retention time. These contaminations are therefore not critical for bottom-up proteomic analyses. However, we suggest to adapt the LC method to ensure a sufficient cleaning step at higher acetonitrile content solvent after the elution of PEO or DDM to avoid their accumulation in the LC system (e.g., columns and tubing). It is important to note that the DDM contamination is dependent on the injected quantity and should be different in function of the injected volume in LC system.

Recommendation for single-cell proteomic experiments

All the previous results have been obtained from protein digest samples equivalent to low-cell number, i.e., diluted standard solution of HeLa tryptic digest. The compatibility of PEO or DDM with proteomic sample preparation was assessed using 10 HeLa cells isolated thanks to a FACS, representing approximately 2 ng of proteins. These cells were collected in a polypropylene (PP) 96-well PCR plate and were prepared for proteomic analysis directly within the wells, employing an on-pot strategy. The final volume of the sample preparation was 12µL leading to a protein concentration of +/- 0.17 ng/µL. 9 µL of the sample was finally injected for LC-MS analysis, corresponding to an expected injected quantity of 1.5 ng.

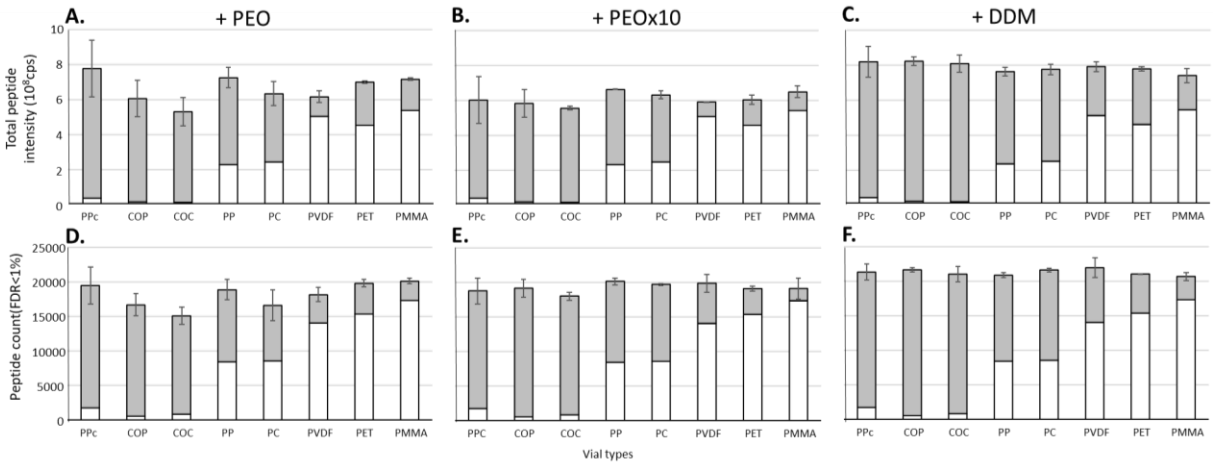

Figure 5: (A-C) Total peptide intensities (expressed in 10^8 counts) as a function of the polymeric material nature of the vial for different buffer compositions: (A) PEO 0.0001%, (B) PEO 0.001%, and (C) DDM 0.0075%. (D-F). Number of identified peptides with FDR < 1% as a function of the polymeric material nature of the vial for different buffer compositions: (D) PEO 0.0001%, (E) PEO 0.001%, and (F) DDM 0.0075%. White bars correspond to the results in absence of detergent (PEO or DDM) while grey bars are the observed increase using the corresponding buffer composition. All three buffer compositions contained 0.1% of TFA.

Sample preparation with PEO or DDM addition during the cell lysis (i.e., at the beginning of the proteomic sample preparation) has been compared with the addition of PEO or DDM after the protein digestion (i.e., at the end of the proteomic sample preparation). In both cases, the final concentration (w/w) of PEO or DDM was 0.001% and 0.0075%, respectively. The proteomic performances are reported in Figure 6 in terms of total peptide intensities (Figure 6.A, in log scale), identified peptide counts with 1% FDR (Figure 6.B) and identified protein counts (Figure 6.C) for 5 replicates. A first observation is that the total peptide intensities are by far lower than the peptide intensity expected from a standard solution of HeLa tryptic digests at equivalent quantity (i.e., 2ng). This can be explained by (1) important loss of material during the sample preparation, (2) low sample preparation efficiency (ex. cell lysis efficiency, trypsin digestion yield). One can also observe that error bars reported for the 10-HeLa cells sample are higher than with equivalent standard solution of HeLa, but this can be explained by the heterogeneity of HeLa cells. From this comparison study, it clearly appears that there is an advantage to perform the entire sample preparation in the presence of non-ionic detergent as the proteomic performances are better when PEO or DDM are added during the cell lysis. Moreover, DDM seems to lead to better performance that PEO for this precise case involving 10 HeLa cells and PP 96-well plate.

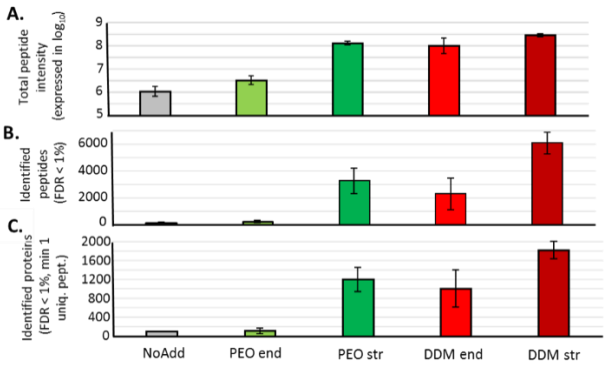

Figure 6: Proteomics performances obtained from 10 HeLa cells sample collected by FACS in commercial PP 96-well plate from ThermoFisher in function of the addition of detergent. "NoAdd" (gray bars) corresponds to the sample preparation without detergent. "PEO end" and "DDM end" (light green and light red, respectively) correspond to the sample preparation protocol involving the addition of PEO and DDM, respectively, before the LC-MS injection. "PEO str" and "DDM str" (dark green and dark red, respectively) correspond to the sample preparation protocol involving the addition of PEO and DDM, respectively, as the first step after cell collection. The proteomic performances are evaluated in terms of (A.) total peptide intensity (expressed in $\log_{10}$), (B.) count of identified peptides with FDR < 1% and (C.) count of identified protein with an FDR < 1% and with at least one unique peptide.

From these observations, we recommended adding DDM directly after the cell collection or to collect cells directly in a solution containing DDM. The interest to add PEO or DDM for the sample preparation has also been evaluated on True single cell experiments (See Supporting information, Figure S4). These single cells have been collected in PP 96-well plate using FACS sorter. No signal was detected in the absence of non-ionic detergent. Conversely, an average of approximately 300 proteins and 630 proteins were observed with 0.0001% PEO and 0.0075% DDM, respectively, across five replicates.

CONCLUSION

This study highlights that a proportion of peptides can be adsorbed onto the surface of vials, thereby reducing proteomics performance, especially for sample containing low amounts of proteins. Standard solution of HeLa tryptic digest peptide at different concentrations have been used as a model to evaluate this effect. The relationship between concentration and peptide intensities fits a Langmuir-type adsorption model, supporting peptide adsorption on surfaces. On commonly used vials made of polypropylene (PP), signal loss is predominant for high retention time eluted peptides, suggesting that hydrophobic peptides are more impacted than hydrophilic peptides. This could lead to important misinterpretations in differential studies based on label-free quantification (LFQ), even with the use of normalization algorithms. Based on the established equation relating intensity to peptide concentration, some recommendations have been suggested: (1) Reducing the exposition to the surface by prioritizing on-pot strategies and minimizing sample preparation. (2) Reducing peptide affinity for the surface by adapting the vial surface or the peptide buffer. (3) Reducing the working volume to increase concentration and reduce exposition to the surface. We demonstrate that the surface nature of vial directly influences peptide adsorption. The use of custom vials molded with more polar polymers, such as polymethylmethacrylate (PMMA), allows a 15-fold increase of total peptide intensity for very low-concentrate peptide solution (i.e. > 2 ng/µL) compared to commercial polypropylene vials by reducing the adsorption of peptides, especially hydrophobic ones. In other hand, the used of more hydrophobic polymeric material drastically increase the peptide loss supporting the influence of the surface nature on the proteomics performances. The use of non-ionic detergents such as polyethylene oxide polymer (PEO) and n-Dodecyl-Beta-Maltoside (DDM) at adapted concentrations allows the reduction of performance heterogeneity between the different investigated vial materials. For the investigated vial, the recommended concentration of PEO or DDM was estimated at 0.0001% or 0.0075%, respectively, which is below the currently reported values in the literature. However, refining these concentrations is recommended when using different vials. Using these compounds allows achieving similar results with commercial PP vials as those observed with PMMA vials. Advantages of PEO and DDM for the sample preparation of 10 HeLa cells or a single HeLa cell sorted in a PP 96-well plate has also been evaluated. From these results, we demonstrate that the use of PEO and DDM strongly increase the detection of peptides and proteins, especially when added at the beginning of the sample preparation, i.e., during cell lysis. For HeLa cell samples, DDM leads to better results than PEO. In the case of single-cell sample preparation with classical workflow in PP 96-well plate (i.e., without adapted lab consumables and equipment), we report that no protein was identified without the addition of PEO or DDM. However, when DDM is added during the cell lysis, we identified more than 600 proteins with an FDR < 1% without using match-between runs (MBR) algorithm. Based on these results, using a one-pot protocol with an appropriate additive appears to be crucial for enhancing proteomic analysis of samples with a low protein content, such as single-cell proteomics, especially when using standard sample preparation equipment. Furthermore, employing MBR for matching protein identification across acquisition data, along with the addition of isobaric or isotopic cell carriers, are two strategies that should be considered to further improve proteome coverage.

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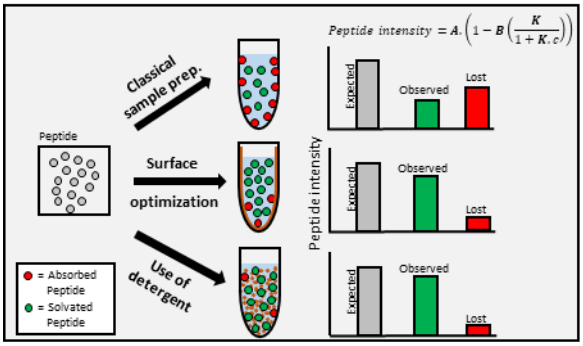
TOC (Graphical abstract)



Supporting information

SIGNIFICANT IMPACT OF CONSUMABLE MATERIAL AND BUFFER COMPOSITION FOR LOW-CELL NUMBER PROTEOMIC SAMPLE PREPARATION

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LC-MS instruments configuration and settings

Peptide solutions have been injected on an Acquity UPLC MClass liquid chromatography system from Waters (UK) coupled with timsTOF Pro2 or a timsTOF SCP instruments from Bruker (Bremen, Germany). Data dependent acquisition (DDA) were performed on the timsTOF Pro2 for the analysis of 10 ng peptide samples while). Data independent acquisition (DIA) analyses were performed on the timsTOF SCP for the analysis of 0.2 ng peptide samples. Instrument configurations and settings are reported in supporting information. The LC instrument was in Trap Elute configuration with a 10µL loop, a Symmetry C18 trap column from Waters (ACQUITY UPLC M-Class Symmetry C18 Trap Column, pore size: 100 Å, particle size: 5 µm, ID: 180 µm, length: 20 mm, Cat. No.: 186007496) followed by an analytic C18 columns AuroraGen3 Elite from IonOpticks (pore size: 120 Å, particle size: 1.7 µm, ID: 75 µm, length: 15 cm, Cat. No.: 186007496) equipped with a CaptiveSpray emitter tip. The LC method involved a 2-minute trapping step at 2% ACN followed by an analytical gradient ramping from 2% to 40% ACN over 25 minutes. MClass system was connected to a timsTOF Pro2 or a timsTOF SCP instruments from Bruker (Bremen, Germany) using a CaptiveSpray interface for nanoESI ionization. In both instruments, ion mobility and m/z values have been calibrated using Agilent TuneMix. Ion mobility separation was performed from 1/K0 from 0.7 to 1.3 with a ramp of 150 ms and an accumulation time of 150 ms (duty cycle ≈ 100%). Samples were analyzed in positive mode in data dependent acquisition (DDA) or in data-independent acquisition (DIA) using Parallel Accumulation–Serial Fragmentation (PASEF). For the DDA method, 6 cycles PASEF were performed. A polygonal selection has been applied based on the mass-to-charge (m/z) and inverted mobility (1/Ko) to remove monocharged peptide from the precursor selection. The DIA method has been first optimized using the py-diAID software (open-source Python package for dia-PASEF methods with Automated Isolation Design) released by Mann's laboratory using DDA analysis results and then slightly adjusted. The cycle time of a DIA analysis was 0.6 seconds. In the case of this study, DDA analyses were performed on the timsTOF Pro2 for the analysis of 10 ng peptide samples while DIA analyses were performed on the timsTOF SCP for the analysis of 0.2 ng peptide samples.

Full demonstration of peptide intensity to concentration based on Langmuir model

The Langmuir model equation use for establish this theoretical correlation between total peptide intensity and concentration is reported in Eq. S1, where %ads is the equilibrium fractional occupancy, denoting the fraction of active sites occupied at equilibrium relative to the total number of available active sites, c is the molecule concentration, and K stands for the equilibrium constant governing the adsorption reaction.

$$\%_{ads} = \frac{K.c}{1 + K.c} \qquad \text{Eq. S1}$$

The quantity of adsorbed peptide ($n_{ads\ peptide}$, expressed in mole) can be expressed as a function of the number of adsorption site on the surface ($.n_{site}$, expressed in mole) and $\%_{ads}$ as reported in Eq. S2

$$n_{ads\ peptide} = \%_{ads} . n_{site} \qquad \text{Eq. S2}$$

The effective quantity of peptide in solution ($n_{sol\ peptide}$, expressed in mole) correspond to the total quantity of peptides ($n_{tot\ peptide}$) minus the quantity of adsorbed on the surface ($n_{ads\ peptide}$) as reported in Eq. S3. The effective concentration (C_{eff}, in mole/L) can be calculated with the Eq. S4 where V_{vial} is the volume solution in the vial (in L)

$$n_{\text{sol } peptide} = n_{tot\ peptide} - n_{ads\ peptide} \qquad \text{Eq. S3}$$

$$C_{eff} = \frac{n_{\text{sol } peptide}}{V_{vial}} = \frac{n_{tot\ peptide} - n_{ads\ peptide}}{V_{vial}} \qquad \text{Eq. S4}$$

The expected intensity ($Intensity$, in arbitrary unit) can be estimated from the C_{eff} and the volume of injection (V_{inj}, in L) with a scaling factor (α, in arbitrary unit) corresponding to a weighted-average ionization efficiency for the different peptides as shown in Eq. S5.

$$Intensity = \alpha . C_{eff} . V_{inj} \qquad \text{Eq. S5}$$

Developing the Eq. S5 with the C_{eff} expression from the Eq. S4, we obtain the Eq. S6

$$Intensity = \alpha . \frac{n_{tot\ peptide} - n_{ads\ peptide}}{V_{vial}} . V_{inj} \qquad \text{Eq. S6}$$

The $n_{ads\ peptide}$ parameter can also be developed using Eq. S2 and Eq. S1 to obtain, respectively Eq. S7 and Eq. S8.

$$Intensity = \alpha . \frac{n_{tot\ peptide} - \%_{ads} . n_{site}}{V_{vial}} . V_{inj} \quad \text{Eq. S7}$$

$$Intensity = \alpha . \frac{n_{tot\ peptide} - \frac{K.c}{1+K.c} . n_{site}}{V_{vial}} . V_{inj} \quad \text{Eq. S8}$$

$n_{tot\ peptide}$ can also be expressed as the total peptide concentration, c and V_{vial} to obtain Eq. S9

$$Intensity = \alpha . \frac{c . V_{vial} - \frac{K.c}{1+K.c} . n_{site}}{V_{vial}} . V_{inj} \quad \text{Eq. S9}$$

Eq. S9 can be transformed to obtain Eq. S10, Eq. S11, and Eq. S12

$$Intensity = \alpha . \left(\frac{c . V_{vial}}{V_{vial}} - \frac{\frac{K.c}{1+K.c} . n_{site}}{V_{vial}} \right) . V_{inj} \quad \text{Eq. S10}$$

$$Intensity = \alpha . \left(c - \frac{\frac{K.c}{1+K.c} . n_{site}}{V_{vial}} \right) . V_{inj} \quad \text{Eq. S11}$$

$$Intensity = \alpha . c \left(1 - \frac{\frac{K}{1+K.c} . n_{site}}{V_{vial}} \right) . V_{inj} \quad \text{Eq. S12}$$

Since $c . V_{inj}$ correspond to the expected quantity injected in the instrument (i.e., considering no adsorption in surfaces), $n_{expect\ peptide}$, Eq. S12 can be modified to obtain Eq. S13

$$Intensity = \alpha . n_{expect\ peptide} \left(1 - \frac{\frac{K}{1+K.c} . n_{site}}{V_{vial}} \right) \quad \text{Eq. S13}$$

Finally, Eq. S13 can be parametrize using 2 parameters, A and B expressed in Eq. S14 and Eq. S15 considering that (1) $n_{expect\ peptide}$ was constant in the experiments (10ng), (2) V_{vial} was fixed for all experiment and (3) and n_{site} is constant for a given vial. The resulting correlation between Intensity and concentration of peptides is the Eq. S16

$$A = \alpha . n_{expect\ peptide} \quad \text{Eq. S14}$$

$$B = \frac{n_{site}}{V_{vial}} \quad \text{Eq. S15}$$

$$Intensity = \mathrm{A}\left(1 - B\,.\,\frac{K}{1 + K.c}\right) \qquad \text{Eq. S16}$$

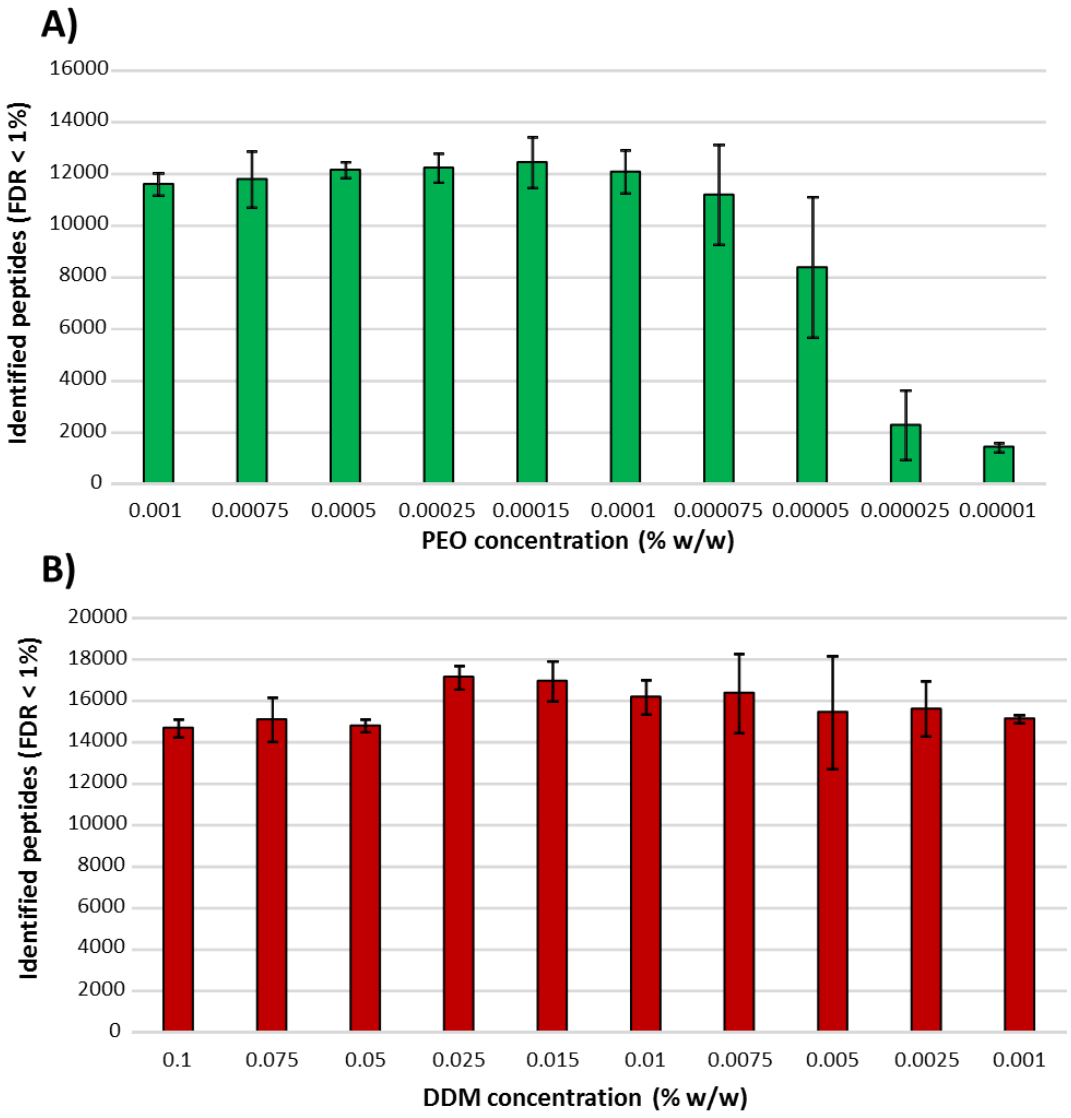


Figure S1: Proteomics performance (in terms of identified peptides with FDR < 1 %, no MBR) on 0.2ng HeLa tryptic digest in function of the PEO (A) or DDM (B) concentration in % (w/w). Concentration of PEO and DDM ranges from 0.00001% to 0.001% and 0.001 to 0.1%, respectively.

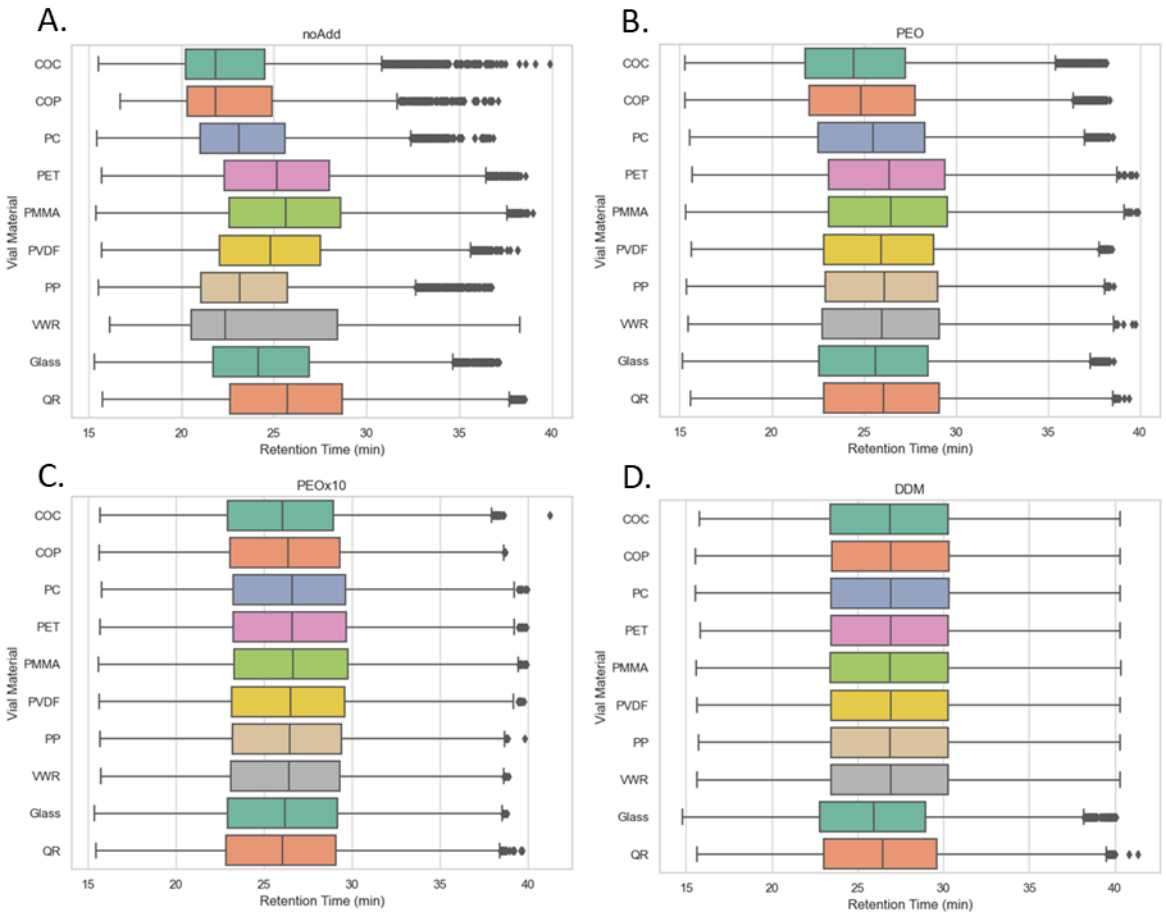
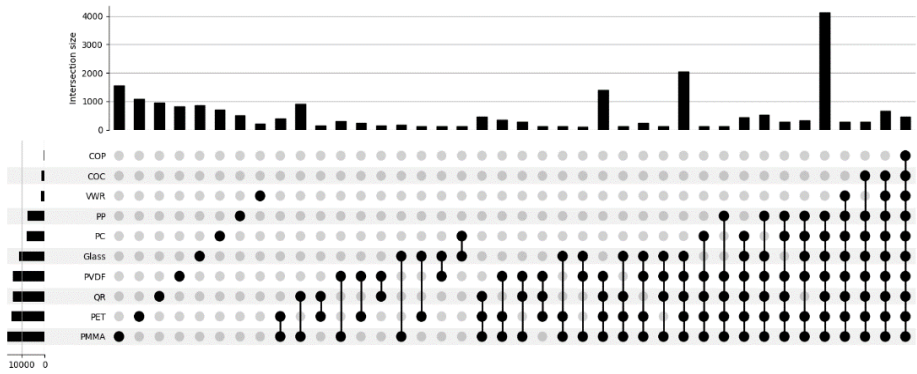
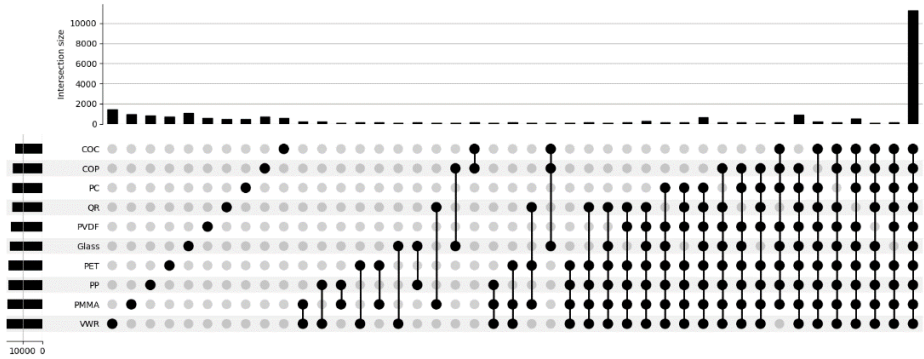


Figure S2: Box plots representing the peptide distributions in function of the retention time using different vials. (A.) without addition of detergent, (B.) with a buffer solution containing 0.0001% of PEO (mean mass weight = 20,000), (C.) with a buffer solution containing 0.001% of PEO (mean mass weight = 20,000), and (D.) with a buffer solution containing 0.0075% of DDM.

A)



B)



C)

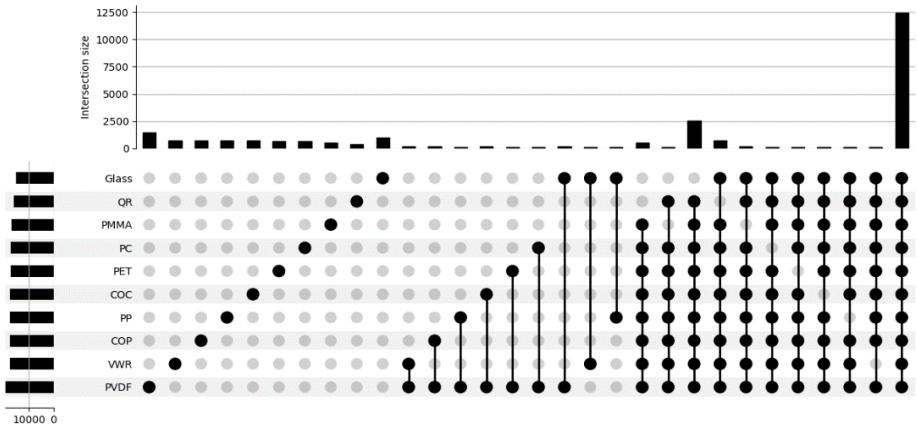


Figure S3: UPSET plot of the differential peptide analysis as a function of the polymeric material nature and the surfactant illustrated that PEO and DDM reduce the surface affinity difference between the vials investigated in this study. Only the 100 first groups are represented. A) No additive, B) addition of 0.0001% of PEO, and C) addition of 0.0075% of DDM.

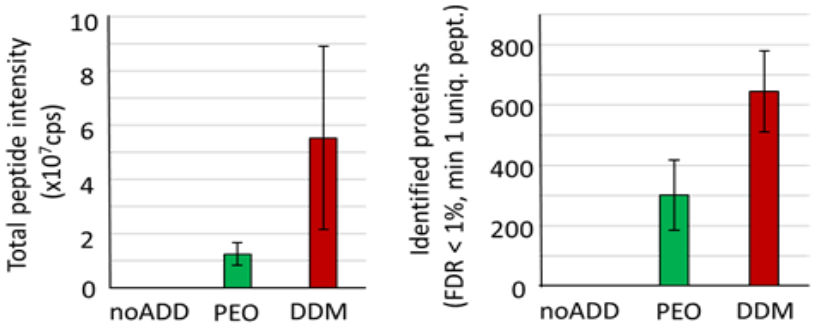


Figure S4: Proteomics performances obtained from single HeLa cells sample collected by FACS in commercial PP 96-well plate from ThermoFisher in function of the addition of detergent. "NoAdd" (grey bars) corresponds to the sample preparation without additive "PEO" and "DDM" (dark green and dark red, respectively) correspond to the sample preparation protocol involving the addition of, respectively, PEO and DDM, in the first step of the sample preparation (i.e., cell lysis). The proteomic performances are evaluated in terms of (A.) total peptide intensity (expressed in 107 counts) and (C.) count of identified protein with an FDR < 1% and with at least one unique peptide.