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Quantitation of Non-Specific Protein Adsorption at Solid-Liquid Interfaces for Single-Cell Proteomics

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ABSTRACT:

Protein non-specific adsorption that occurred at the solid-liquid interface has been subjected to intense physical and chemical characterizations due to its crucial role in a wide range of applications including food and pharmaceutical industries, medical implants, biosensing, and so on. Protein-adsorption caused sample loss has largely hindered the studies of single-cell proteomics; the prevention of such loss requires the understanding of protein-surface adsorption at the proteome level, in which the competitive adsorption of thousands and millions of proteins with vast dynamic range occurs. To this end, we feel the necessity to review current methodologies on their potentials to characterize—more specifically to quantify—the proteome wide adsorption. We hope this effort can help advancing single-cell proteomics and trace proteomics.

INTRODUCTION

Proteins are complex amphiphilic biopolymers with flexible structures that can denature at solid surfaces due to adsorption, which is caused by complex interactions including but not limited to hydrophobic, hydrophilic, van der Waals, and electrostatic interactions^{1,2}. The process occurs spontaneously and rapidly³. The adsorbed molecules are commonly referred to as “adsorbate” and the surface to which they attach are “adsorbent”⁴. The adsorption process is extremely complex and involves at least the diffusion of proteins to the solid-liquid interface, the replacement of existing adsorbed molecules, and the conformation and orientation changes of the adsorbed proteins on surfaces^{2,3} before the subsequent desorption. Therefore, not only the properties of proteins, but also those from the solvent and the surface, affect the adsorption process and have been detailed reviewed⁵⁻⁷.

In complex protein mixture, however, the additional Vroman effect, i.e. the competitive adsorption and displacement of different protein species over time at the surface, further complicates the kinetics and thermodynamics of the adsorption and desorption⁸. Even though physical, chemical, and mathematical efforts have been applied to investigate the factors contributed to these events, it remains challenging to understand and predict how different proteins behave at surface in a mixture.

Because proteins are widely used in research and in our daily life, the adsorption events have been concerned in numerous fields^{2,9}, including but not limited to dairy industry¹⁰, biomaterials¹¹, biomedicine¹², biotechnology¹³, biosensing¹⁴ as well as protein-related analyses¹⁵⁻¹⁷. For example, protein adsorption is one of the major

challenges in the use of medical implants and in packaging biologics for therapies. For the former, inflammation and thrombogenicity raised from biofouling of implant surfaces have hindered the use of many materials ¹⁸. For the latter, the packaging and storage condition of biologics in the pharmaceutical industry can greatly impact the availability and efficacy of biologics ^{12,19-21}. In the field of biosensing, non-specific protein adsorption poses challenges to the selectivity and sensitivity of measurements ¹⁴, and passivation of the device surface has become a default step. Numerous detergents ^{22,23} as well as different surface chemistries ²⁴ have also been explored for eliminating non-specific protein adsorption.

Recent interests on single-cell proteomics ^{25,26}, in which thousands of protein species need to be accurately identified and quantified for structural as well as functional properties, have demanded an in-depth understanding of protein-surface nonspecific interaction at proteome level. The nearly complete prevention of sample loss is required by single-cell proteomics owing to the minute amount of proteins available in the samples ²⁷. Within such trace samples, there are not only thousands to millions of different protein species but also presented in a dynamic range of 5-6 orders of magnitude. Unlike nucleic acids, these proteins cannot be amplified. Therefore, even though single cell genomics and transcriptomics have received substantial advancements in recent ^{25,28} years, the direct studies of single-cell proteomics are limited ²⁹. Recently, matrix assisted laser desorption/ionization (MALDI) and secondary ion mass spectrometry (SIMS) based *in situ* single-cell mass spectrometry imaging techniques ³⁰⁻³² have started to emerge to tackle the spatial distribution of biomolecules in single cells.

Many solution based single-cell proteomic efforts including single-cell mass cytometry^{33,34}, sensitive capillary electrophoresis – mass spectrometry/mass spectrometry (CE-MS/MS) single-cell proteomics^{35,36}, as well as bioreactor based integrated liquid chromatography – mass spectrometry/mass spectrometry (LC-MS/MS) platforms³⁷⁻³⁹ are also actively pursued.

Without a complete understanding and quantification on how proteins are lost in sample preparation due to the adsorption to surfaces, the results from these solution-based single-cell proteomics will not provide accurate and desired biological insights^{40,41}. Even though various detergents²² and surface chemistry⁴²⁻⁴⁴ have been explored for lowering nonspecific protein binding, none of these strategies were evaluated on their efficiency to prevent the adsorption of the cellular proteome with high complexity and dynamic range. The challenge is largely due to the availability of methodologies that are suitable to characterize protein adsorption at such complexity on diverse surfaces encountered in proteomics.

To facilitate the advancement of single-cell represented trace proteomics, we reviewed here current technologies capable of quantifying surface adsorption caused sample loss, with a particular focus of the proteomic environment and, if possible, trace proteomics, i.e. from attomole to a few protein molecules in a mixture of thousands of these protein species. Specifically, as the current sample preparation workflow in proteomics typically requires a diverse collection of surfaces ranging from Eppendorf vials, pipette tips, filters, magnetic nanoparticles, to micrometer-sized resins and tubings to name a few, we will focus on the flexibility of current adsorption characterization

techniques to analyze surfaces with irregular shapes, topology, optical and electrical properties, and to analyze complex mixtures of thousands of protein species. Due to the vast available technologies and the long history of research on protein-surface interaction, we selected over 14 methods to represent the current field. We hope the information could leverage the existing methodologies and encourage future technical innovations to better fulfilling the demand raised from single-cell proteomics.

OVERVIEW OF THE METHODS

In decades of protein-adsorption studies, many methodologies have been developed, and their sensitivity and limitations have been reviewed^{8,45,46}. However, no review, to our knowledge, has focused on the multiplexity of these methods for thousands of proteins in the proteome. Moreover, no reviews have been focusing on the flexibility of these methods in characterizing the irregular surfaces of vials, tips, and capillaries. We include here more than 14 methods (Figure 1), such as labeling and label free spectroscopic, optical, and separation based methods. These methods can be catalogued into direct and indirect methods based on the site where proteins are obtained, i.e. immobilized at the surface (direct) or free in solution (indirect, or “solution depletion” method). In solution depletion analysis, the change of protein concentration in solution before and after contacting the target surface is measured to deduce the amount of adsorbed proteins^{2,4,41,45}.

Table 1 lists these methods. Many of them, such as surface plasmon resonance (SPR), ellipsometry (ELM), total internal reflection fluorescence (TIRF), atomic force microscopy (AFM), neutron reflection (NR), and quartz crystal microbalance (QCM), can perform real time *in situ* measurement ⁴⁷, whereas others only measure *ex situ*, such as x-ray photoelectron spectroscopy (XPS), sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and mass spectrometry (MS). Due to instrumental constraints, most spectroscopic analyses such as XPS ⁴⁸ and SPR ⁴⁹ can characterize curved surfaces only if nanoparticles or nanorods are employed as the adsorbent; whereas, labeling approaches and separation based *ex situ* analyses, such as SDS-PAGE and MS, can handle any surfaces, including sample vials, channels, beads, and fibers that are irrelevant to their optical or plasmonic properties. Below, we discuss the capacity of these methods on their multiplexity and surface flexibility, with additional information on their analysis sensitivity.

LABELING TECHNIQUES

Radiolabeling. Radioisotope-labeling technique is an early developed and commonly used approach that has been widely applied to investigate protein nonspecific adsorption. Proteins labeled by radioisotopes, such as ¹³¹I ⁵⁰ or ¹²⁵I ⁵¹, emit gamma ray that can be sensitively detected due to the low background. The quantitation is achieved by measuring the radioactivity using a liquid scintillation counter on either the adsorbed proteins stripped off the target surface ⁵² (direct analysis), or the protein solution before

and after incubation with the surface (indirect analysis). Therefore, the method is flexible on the types of surfaces ⁴⁵.

Multiplexing can be achieved by labeling with distinct radioisotopes, such as $^{131}\text{I}/^{125}\text{I}$ ⁵³, or $^3\text{H}/^{14}\text{C}$ ⁵², but the degree of multiplexing is limited due to the available isotopes ⁵⁴. The method is regarded as one of the most sensitive techniques for protein quantitation ⁵⁵; nevertheless, it is hampered by its health hazard ^{54,56,57}. The labeling step also increases the analysis time and risks of altering protein conformation, thereby the adsorption properties ⁵⁸⁻⁶⁰.

Fluorescence labeling. As an alternative to radioisotopes, fluorophores are frequently used to study protein adsorption because of its safe of use, simplicity of detection, and high sensitivity ⁶¹. The fluorescence intensity can be used for quantitative analysis *in situ* or *ex situ* ⁶². Yet the detection limit and dynamic range vary largely based on the stability and brightness (i.e. the quantum yield) of the fluorophores ⁶³, as well as the environmental factors such as the ionic strength, pH, solvent polarity, and the presence of metal or metal ions ⁶⁴. The development of quantum dots has overcome many of these limitations ⁶³. Similar to radiolabeling, fluorescence labeling is flexible for both direct and indirect analyses on various surfaces.

The multiplexed fluorescence detection has been widely explored in bioimaging and Fluorescence Aid Cell Sorting (FACS), with known limitations on the number of different fluorophores that can be distinguished simultaneously ^{64,65}. Labeling of proteins with external fluorophores also risks the perturbation to protein structure, and the intrinsic fluorescence emanated from residues of tyrosine, tryptophan, and phenylalanine

⁴⁵ has been explored. Yet, these signals are often too weak for sensitive measurement ⁶⁶, and contribute greatly to the background noise.

Total Internal Reflection Fluorescence (TIRF) A common challenge in quantification of protein surface adsorption is to distinct the adsorbed proteins from those free in solution. It can be overcome by confining the detection at the interface, and TIRF is one of the techniques that are developed for such purposes. Using TIRF, only molecules within ~100–200 nm from surface can be excited by the evanescent wave for fluorescence monitoring ^{2,67}. The details of the technique and its applications in surface characterization can be found in the recent review ⁶⁸, including the adsorption isotherms, kinetics, the conformation and orientation of the proteins at the surface. The sensitivity of the technique can detect single molecules ⁶⁹. Again, the measurement requires labeling of different fluorophores to study competitive adsorption of a few proteins ⁶⁷. Owing to the optical constraints, the technique is suitable only for planar and optically transparent surfaces that are more constrained than those in regular fluorescence measurements.

LABEL-FREE TECHNIQUES

Fourier transform infrared spectroscopy (FTIR) The structure of proteins at the solid interface can be directly characterized by a number of spectroscopic methods without any labeling. With calibrated molar absorptivity, the obtained spectra can be used for quantifications of the absorbate. Among these methods, FTIR and circular dichroism (CD) are two popular approaches. In FTIR, the vibrational modes of the amide bands and

the secondary structures of proteins, such as α -helices, β -sheets, and turns, can be monitored spectroscopically. Similar to TIRF, the attenuated total internal reflection (ATR) ^{70,71} technique is often coupled to FTIR (i.e. ATR-FTIR) to directly analyze proteins at the surface submerged in solution. In such *in situ* analyses, the evanescent wave developed through a waveguide is used to interrogate the proteins at the solid interface. The method is difficult for multiprotein studies even though attempts on the binary protein mixture have been demonstrated ⁷². The sensitivity of the method is relatively poor and reported in micromolar protein concentration ⁷³. It remains difficult to use the method for studying curvature effect as the analyses are mostly conducted on flat surface of the waveguide.

Circular Dichroism (CD) Analogues to FTIR, CD is also a powerful *in situ* technique to reveal the secondary and tertiary structure of the adsorbed proteins that undergo conformational changes at the solid surfaces. CD is a spectropolarimeter that measures the differential absorption of two circularly polarized lights once they pass through proteins. The sensitivity of the method to small surfaces and low protein concentration can be improved by stacking multiple surfaces together ⁷⁴, or using the more intense synchrotron radiation ⁷⁵. The optically active molecules in the buffer other than those attached to the surface can complicate the target signals ⁷⁴. The method measures transmitted light therefore is flexible to surface shape and topology, but materials must be optically clear. The lack of ability to identify individual protein components renders the method incapable of decoupling complex adsorption events in a protein mixture.

Atomic Force Microscopy (AFM). AFM is a surface scanning method that directly characterizes adsorbed proteins on the surface without labeling. AFM consists of a cantilever, a tapered tip at the free end of cantilever, which probes the surface topology as well as molecular interactions. The distance between the cantilever tip and the adsorbed protein layer can be monitored for protein conformational and orientational changes⁷⁶. The thickness of the protein adlayer can be used for quantifying the adsorbed proteins^{2,76,77}. AFM can be carried out *ex situ* on dry surfaces and *in situ* in solution. Commonly used contact mode can cause large disruption of the adsorbed protein layer⁵. Tapping mode as well as force modulation and phase sensitive detection mode allows brief contact with the soft substrate and are frequently used for protein and biomaterial surface characterizations^{77,78}. The thickness of the protein adlayer and the viscoelasticity can be monitored^{5,79}. The high resolution of the cantilever probe also allows for single-protein analysis⁷⁸. Nevertheless, the interference of AFM tip to the experimental system has been observed, including the blocking of surface access of proteins in solution and the changed tip shape and dimension owing to the adsorption of proteins to the tip end⁷⁸ which require careful parameter design and calibration of the measurement for better interpretation of the results⁷⁹. In addition, the use of AFM to characterize individual behavior of multiprotein systems is elusive.

Ellipsometry (ELM). Different from CD, ELM uses reflected light for real time direct adsorption studies⁴⁵. The measurement is based on the changes in reflectance and phase of the polarized light from the planar surface after interrogating the protein coated surface. The dynamics of the adsorption can be quantified through the changes of the

density, the refractive index, and the thickness of the adlayer which also infers to the bulk change of protein conformation, and side-on or end-on orientation at the surface^{80,81}. The sensitivity of quantitation can reach $\sim \text{ng}/\text{cm}^2$ ⁸². To enhance the optical contrast, a thin layer of indium tin oxide (ITO) has been used⁸³. The method is highly sensitive for *in situ* protein overall structural changes, but cannot resolve structural details offered by FTIR and CD. It also suffers from the limited distinguishing power to individual protein species in mixture. The general applications of ELM are on the planner surface, and studies of nanoparticles and nanotubes on planner surfaces have also been reported⁸⁴.

Neutron Reflectometry (NR) Similar to ELM, neutron beams can be used for reflection analysis on protein surface adsorption as reviewed recently^{5,85,86}. Because the wavelength of these beams is orders of magnitude shorter than the visible light, NR offers atomic and molecular resolution in z-direction normal to the target interface⁸⁶. Using this method, angstrom resolution in the adlayer thickness can be achieved⁸⁷, which greatly facilitated mechanistic studies of protein surface adsorption, such as the side-on and head-on orientation, and the two-layer model of protein surface adsorption in the cases of bovine serum albumin (BSA) and caseins. The method is sensitive to surface topology and can be used to measure roughness of the surface. Using isotopic labeling, the method is capable of distinguishing different molecules as mixtures on the surface, but its ability to characterize complex systems such as proteomes is limited.

X-ray photoelectron spectroscopy (XPS) XPS is a sensitive and quantitative, but *ex situ* spectroscopic analysis conducted directly at the surface in ultra-high vacuum ($< 10^{-9}$ mbar). The analysis can directly obtain both chemical and elemental information except

for H and He elements⁸⁸ in the sample. It uses X-ray to irradiate the surface and to excite the core and valence electrons of samples (< 10 nm depth)⁴⁶. The energy of released photoelectron from the surface can be used to distinguish the element type as well as their chemical states⁸⁸, and has also been used to characterize the thickness, orientation, and coverage of the adsorbed protein layer^{89,90}. Both the planar⁸⁹ and highly curved bead surfaces⁴⁸ were probed by XPS. The sensitivity of XPS detection can reach ng/cm^2 ^{90,91}. Recent reviews have detailed its use in characterizing the protein adlayer^{46,88}. Similar to many other methods, XPS is limited to identify different proteins in mixture at the surface.

Surface Plasmon Resonance (SPR) spectroscopy. SPR is a frequently used optical technique for analysis of *in situ* direct adsorption in solution occurred at planar surfaces. The method monitors the changes of surface refractive index upon the adsorption of protein^{5,51}. The adsorbed protein adlayer can be quantified at a sensitivity < pg/mm^2 ^{51,92,93}. Reviews of the technique and its applications are available^{94,95}. Coupled to MS, SPR can resolve individual protein behaviors in mixtures^{96,97}. The newly developed NanoSPR can study protein adsorption on highly curved surfaces such as those of gold nanorods⁹⁸, but to our knowledge there is no report on using SPR for polypropylene vial and pipette-tip surfaces. Other similar optical techniques such as optical waveguide lightmode spectroscopy (OWLS)^{99,100} have also been developed for quantification of adsorbed proteins.

Quartz Crystal Microbalance (QCM) QCM represents a field of research that employs acoustic waves formed from oscillating piezoelectric, or single crystal quartz

plate¹⁰¹⁻¹⁰³, to sense direct protein adsorption *in situ*. Under this umbrella, similar techniques also include QCM with dissipation (QCM-D) and surface acoustic wave (SAW), generated and detected by interdigital transducers (IDT) on the surface of piezoelectric crystal^{104,105}. Quantification of these methods is achieved through the change of resonance frequency (f_n) of the quartz crystal resonator with respect to the mass load (QCM), or the energy dissipation rate (QCM-D). The method is capable of characterizing the viscoelastic layer of the adsorbed proteins¹⁰⁶, and can be used to study the conformational changes of proteins on surfaces, such as the side chain and torsional angle of proteins with nanogram sensitivity^{5,51}. The adsorbed mass detected by QCM includes not only the adsorbed protein but also the trapped water¹⁰⁷. As a result, QCM tends to over-estimate the quantity of protein compared with other techniques such as radiolabeling, ELM, OWLS, and SPR^{51,80,108}, and has been used more frequently in conjunction with other techniques such as SPR, and reflectometry (e.g. NR and ELM)^{109,110}.

Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

Different from spectroscopic and optical methods introduced above, quantification of protein adsorbates can also be carried out by *ex situ* separation approaches, so individual adsorbate in mixtures can be resolved after they are removed from the surface. Below, we summarize a few representative separation approaches in protein adsorption analyses, including SDS-PAGE, high pressure liquid chromatography (HPLC), CE, and MS. The key advantage of these analyses is their capacity to resolving protein mixtures. We will

focus on SDS-PAGE technique in this section and discuss other methods in the following sections.

SDS-PAGE is a commonly used electrophoretic technique for separating protein mixtures based on their molecular weight ¹¹¹⁻¹¹³. Together with colorimetric staining, such as coomassie and silver staining, the method can quantify proteins in the gel ^{114,115}. SDS-PAGE has been frequently employed for indirect “solution depletion” analysis of protein adsorption ^{114,116}, in which the adsorbates are quantified by measuring the changes of protein concentration in solution before and after the interaction with surfaces. Its quantitation (in sub $\mu\text{g}/\text{cm}^2$ range) is not as precise as that of aforementioned spectroscopic techniques. Nevertheless, because the method does not directly measure proteins on surfaces, it is flexible to tackle any types and shapes of surfaces.

The method has been employed to study Vroman effect in protein mixture. Vogler et al. have summarized such applications ^{117,118}. When using 2-dimensional (2D)-gel coupled with MS, the technique can tackle proteome-wide protein adsorption ¹¹⁹. The drawback of the method is its *ex situ* analysis that has limited its application from conducting fast kinetic studies. In addition, the relatively poor detection of intensity difference requires sufficiently large surfaces for distinguishable adsorbate quantity variation in solution before and after the interaction with surfaces. The suitable surfaces are typically achieved by using large number of small particles, resins, and beads.

To broaden the flexibility of suitable surfaces in this method, we have recently developed a directly protein analysis (DPA) approach, i.e. SDS-PAGE/DPA ⁴¹. This direct method quantifies stripped proteins from the surfaces instead of using the indirect

solution-depletion approach, as shown in Figure 2. In this method, because the protein adsorbates are quantified against the blank, the detection sensitivity can be a couple of nanograms of proteins that is orders more sensitive than the solution depletion method. The reason is analogous to the sensitivity improvement gained by fluorescence over UV-Vis detection, in which fluorescence appears from a dark background, whereas UV-Vis relies on the intensity difference of the incident beam after interrogating the target solution. As a result, our SDS-PAGE/DPA not only adapted to the advantages of SDS-PAGE for complex samples, but also is sensitive to small surfaces, such as those of Eppendorf vials. Using the method, we have successfully analyzed the BSA adsorption at an extremely low concentration of 4 pg/ μ L in 0.67 mL Eppendorf vials with less than 2 cm² surface area ⁴¹.

Other separation based quantification approaches. Besides SDS-PAGE, other separation based approaches used to study protein-surface adsorption include HPLC and CE. The elution peak intensity and retention time detected by UV-Vis ¹²⁰ or fluorescence ^{121,122} detectors were used for both qualitative and quantitative analysis of protein adsorption in the mixture. These methods were applied to both *ex situ* protein adsorption for stripped proteins ¹²³, or to *in situ* adsorption in the separation system itself ¹²⁴. In addition, MS has been increasingly coupled to HPLC and CE as the detector owing to its additional separation capacity in m/z and its high detection sensitivity ^{125,126} that will be further elaborated below.

Mass Spectrometry (MS) MS has been increasingly used in studying protein *ex situ* non-specific adsorption due to its high sensitivity and versatility of the acquired

information. Besides coupling to separation schemes such as SDS-PAGE, HPLC, and CE, MS can be directly used to image proteins on the surface. Particularly, the time-of-flight secondary ion mass spectrometry (ToF-SIMS) has been employed to replace previous quadrupole mass analyzer based analysis to directly detect proteins adsorbed to solid surfaces^{127,128}. The sensitivity of the measurement can be 10^7 - 10^{11} atoms/cm²¹²⁸. With the aid of multivariate analysis such as principal component analysis and partial least square, the method can distinguish different protein species by analyzing the small amino acid fragment at low molecular weight with extremely high mass accuracy and pure protein standards¹²⁸.

In addition to SIMS, MALDI has also been used to couple to the ToF mass analyzer for monitoring the adsorption by molecular ions¹²⁹. MALDI matrix was also used in SIMS sample preparation for enhanced sample ionization^{130,131}. Other than molecular MS, inductively coupled plasma (ICP)-MS¹²⁶ represented elemental MS as well as particle MS¹³² were also emerged for protein adsorption analysis. For the latter, the protein adsorbed nanoparticles that were introduced as a whole to MS for analysis. Therefore, MS analysis has been increasingly used in protein adsorption analysis in recent years.

Besides using MS for direct analysis at the surface, by stripping the proteins off the surface and using shotgun proteomics¹³³ (detection and quantification of unknown proteins based on sequencing their derived peptides), hundreds and thousands of types of proteins can be quantified simultaneously, which provided unprecedented information for the biofouling on medical implants¹³⁴⁻¹³⁷. Examples of these applications include the 2D-

gel MS as mentioned in the SDS-PAGE section and gel-free liquid chromatography separation and MS analyses.

FINAL REMARK

The concerns of protein surface adsorption in sample preparation to the accuracy and sensitivity of shotgun proteomics have been addressed¹³⁸⁻¹⁴¹. The studies of prevention using various strategies have been actively pursued as mentioned at the beginning. The cumulative knowledge towards protein and peptide specific adsorptions as a function of surface chemistry and solvent property has received increased attention. The information on the intermolecular effects derived from the Vroman effect in proteome-wide protein adsorption will advance the comprehensive understanding protein-surface interaction. Breakthroughs in understanding, prediction, and prevention of adsorption caused sample loss will greatly facilitate the single-cell proteomics represented trace proteomics analysis.

We summarized here the techniques commonly used in characterization and quantification of protein-solid surface adsorption. The labeling techniques are sensitive and easy to implement that are flexible to the surfaces; yet the labeling process can alter native protein structure and interfere with protein adsorption properties. Various label-free spectroscopic and optical characterizations can directly measure protein structure and quantity at the intersurfaces, but majority of them are not suitable for proteome-wide analyses. The separation based methods such as SDS-PAGE, HPLC, CE, and MS are

suitable to resolve protein mixtures, and the hybrid techniques including 2D-gel-MS/MS, SPR-MS, CE-MS/MS, LC-MS/MS etc. can interrogate complex adsorption events with rich information.

In the perspective of single-cell proteomics, from attomole and zeptomole down to single-molecule quantity of thousands and millions of different protein species are required for precise and direct measurement without amplification. Techniques such as fluorescence measurement can reach single-molecule detection limit, while others such as PAGE has been demonstrated recently to resolve proteins from single cells^{142,143}. With the increasing accessibility of strong beams such as the synchrotrons, it will be possible to analyze minute amount of samples in many spectroscopic based methods. We hope this review can raise some of the critical challenges in characterization of proteome-wide protein adsorption for trace proteomics and stimulate our understanding of protein-surface interactions to a brand new level in the near future.

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Table 1. Summary of different quantification techniques of protein adsorption

Technique	Surface requirement	Sensitivity	Throughput	Kinetics	Method	Ref.
Radiolabeling	-	ng/cm ²	multiplex (limited)	yes	direct/indirect labeling	60
Fluorescence	-	ng/mL	multiplex (limited)	yes	direct/indirect labeling	12,64
TIRF	planar	ng/cm ²	multiplex (limited)	yes	direct/indirect labeling	68
FTIR	planar	μM	-	yes	direct label-free	73
CD	Planar/beads	ng/cm ²	-	yes	direct label-free, in-situ	74
AFM	planar	-	-	-	direct label-free	144
ELM	planar	sub-ng/cm ²	-	yes	direct label-free, in-situ	82,83
SPR	planar	sub-ng/cm ²	-	yes	direct label-free, in-situ	93
XPS	planar	ng/cm ²	-	-	direct label-free	90,91
OWLS	planar	sub-ng/cm ²	-	yes	direct label-free, in-situ	80
QCM	planar	sub-ng/cm ²	-	yes	direct label-free, in-situ	145,146
SAW	planar	pg/cm ²	-	yes	direct	104

					label-free, in-situ	
NR	planar	sub-ng/cm ²	-	-	direct label-free	85
SDS-PAGE	Extremely large	sub ug/cm ²	multiplex	limited	indirect label-free	147
SDS-PAGE/DPA	-	sub ng/cm ²	multiplex	limited	direct label-free	41
CE	-	ng/μL	multiplex	yes	direct labeling	126
MALDI/SIMS	-	10 ⁷ -10 ¹¹ atoms/cm ²	multiplex	-	direct label-free	128

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Figure 1. Illustration of methods for protein surface adsorption in the perspective of sample vials if possible. The analyses are divided by labeling and label-free techniques.

EOF: electroosmotic flow.

Figure 2. Schematic of quantitative analysis of the adsorbed proteins by SDS-PAGE/DPA, in which the adsorbed BSA prepared in various volumes in Eppendorf vials are stripped off the surface. The collected BSA were separated by SDS-PAGE and quantified by silver staining and BSA standards applied to the same gel. The adsorption amount increased proportionally to the contact area of the solution in the vial. The gel results and the image were adapted from ⁴¹.

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