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Review

Protein Corona of Nanoparticles: Isolation and Analysis

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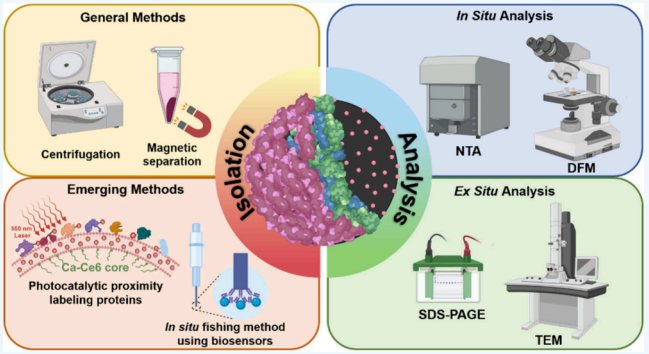
ABSTRACT: Nanoparticles entering biological systems or fluids inevitably adsorb biomolecules, such as protein, on their surfaces, forming a protein corona. Ensuing, the protein corona endows nanoparticles with a new biological identity and impacts the interaction between the nanoparticles and biological systems. Hence, the development of reliable techniques for protein corona isolation and analysis is key for understanding the biological behaviors of nanoparticles. First, this review systematically outlines the approach for isolating the protein corona, including centrifugation, magnetic separation, size exclusion chromatography, flow-field-flow fractionation, and other emerging methods. Next, we review the qualitative and quantitative characterization methods of the protein corona. Finally, we underscore the necessary steps to advance the efficiency and fidelity of protein corona isolation and characterization on nanoparticle surfaces. We anticipate that these insights into protein corona isolation and characterization methodologies will profoundly influence the development of technologies aimed at elucidating bionano interactions and the role of protein corona in various biomedical applications.



KEYWORDS: *Protein corona, Nanoparticles, Separation and analysis, Bionano interaction, Nanomedicine*

1. INTRODUCTION

Engineered nanoparticles (NPs) have a wide range of applications in the biomedical field, including imaging,[1,2] drug delivery,[3,4] diagnosis,[5,6] and treatment.[7−10] However, due to the high surface energy of NPs, once they encounter a biological fluid, their surface inevitably adsorbs specific biomolecules forming a biomolecular corona.[11] In cell culture medium and blood, the biomolecular corona is mainly composed of proteins, and most of the current research reports are also aimed at the protein components of the corona. Therefore, this review focuses on the latest developments in the protein corona (PC). The formation of the corona is a dynamic evolution process (Figure 1a). Initially, due to the Vroman effect, the high abundance proteins first bind to the surface of NPs and then dynamically exchange with low abundance and higher affinity proteins.[12,13] Thus, the enrichment of PC composition at the NPs surface does not correlate with the abundance of proteins in the biological fluid.[14,15] The formation of a corona is influenced by multiple factors, including (1) the physical and chemical properties of NPs: type,[16,17] size and shape,[18−23] rigidity,[24] surface morphology,[25] surface charge,[16,22] surface functionalization,[26−29] and so on; (2) formation condition: the medium type,[30−32] temperature,[33] pH,[34] concentration,[35−37] fluidity of

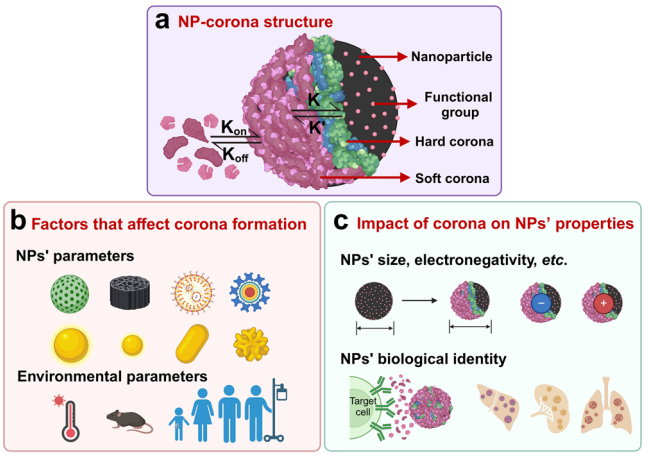


Figure 1. (a) Basic structure of NP-PC complexes. (b) Factors affecting the formation of PC. (c) The impact of PC formation on NPs. Created with BioRender.com.

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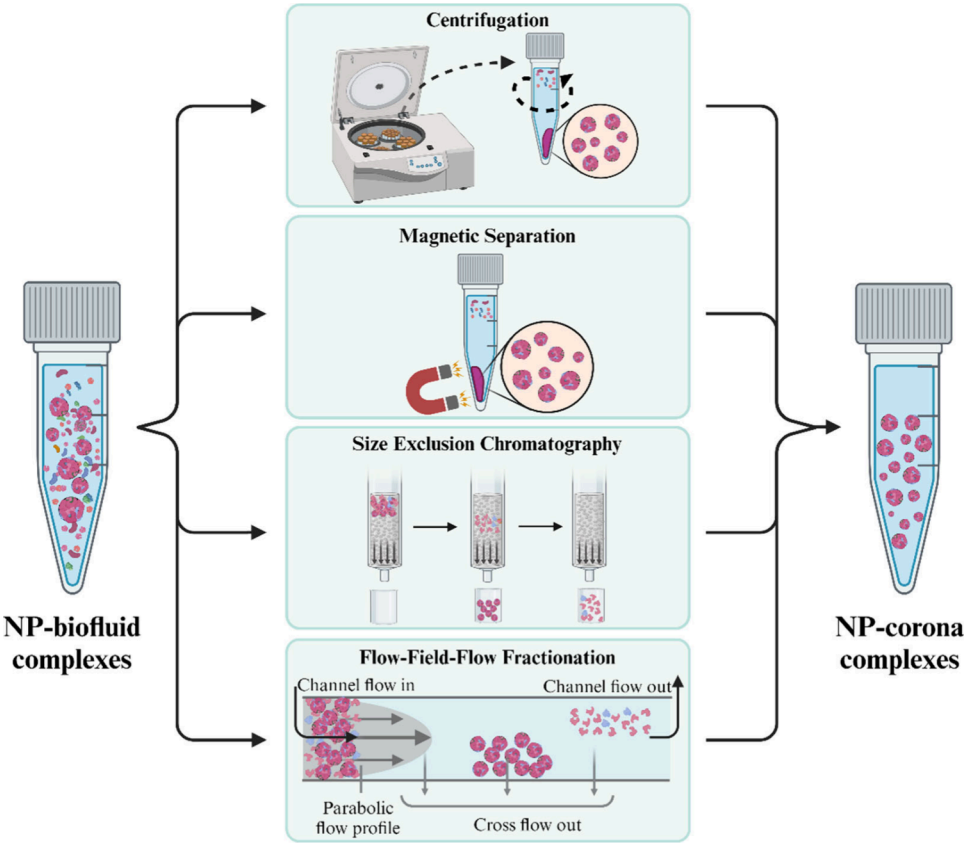

Figure 2. General methods for NP-PC complex isolation such as centrifugation, magnetic isolation, size exclusion chromatography and field-flow fractionation. Created with BioRender.com.

medium,[38] and incubation time;[39] and (3) the source of biological body fluids: the species,[40,41] age,[42] gender,[43] habits,[12] disease and medication taken by the subjects,[44−46] and administration route of NPs (Figure 1b).[47] When the NP-PC complexes are distributed to a new biological environment, the composition of the corona and the conformation of the protein molecules therein also change accordingly. Therefore, to accurately understand and evaluate the real biological effects of PC, it is essential to have the capability to detect and analyze NP-PC complexes from the media both *in situ* and *in vivo*. As shown in Figure 1a, according to the difference in relative affinity of proteins for NP, the PC can be divided into a "hard corona" (HC) and a "soft corona" (SC). The former carries the tightly associated proteins on the NP surface, while the latter majorly consists of loosely bound proteins.[48] Initially, high-abundance proteins preferentially come into contact with NPs, followed by gradual replacement of low-affinity proteins by high-affinity proteins.[49] When analyzing the interactions over time, it is evident that the relaxation time of the HC is much larger than the recognition time, which implies that the proteins in the HC are relatively stable during the recognition process and are not easily replaced or rearranged.[50] This stability makes the HC play a vital role in the cellular recognition process and will directly participate in the cellular recognition and signaling process, thus affecting the biological fate of the NPs.[51,52] In contrast, the relaxation time of the SC is shorter, usually in the range of milliseconds to seconds, which indicates that the molecules in the SC are more prone to dynamic changes, such as adsorption, desorption, rearrangement of molecules, *etc.*[50] The SC is not directly involved in the cellular recognition process, but the dynamic exchanges of molecules in the SC affect the formation of the HC, indirectly affecting the cellular response to NPs. For instance, specific molecules may transfer from the SC to the HC, or molecules in the HC may rearrange as a result of changes in the molecular structure of the SC.[50,51]

The PC endows NPs with a new biological identity and impacts the interaction between NPs and biological systems, such as toxicity, biodistribution, biodegradation, and targeting efficiency (Figure 1c).[53−56] Therefore, efficient extraction and isolation of NP-PC complexes from biological fluids is paramount in advancing the application of NPs by deciphering the biological information on PC. In this review, we initially summarized the isolation method of PC, including centrifugation, magnetic separation, size exclusion chromatography, field flow separation, and other merging methods. Next, we reviewed the approaches to analyze PC morphology, qualitative and quantitative biomolecular analyses, adsorption kinetics, and structural characterization. Then, we outlined the emerging PC purification methods and characterization techniques, emphasizing the challenges in developing and regulating specific biomolecular fingerprints. Finally, we summarized progress, challenges, and opportunities in PC research to understand the current state-of-the-art.

2. ISOLATION OF NP-PC COMPLEXES

Protein adsorption on the surface of NPs is a highly dynamic and complex process. Accurate interpretation and comparison of corona across various NPs and studies necessitate dependable methods for isolating the PC from protein-rich

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Table 1. Summary of the General NP-PC Isolation Methods and Their Advantages and Disadvantages

Methods	Advantages	disadvantages
Centrifugation	Based on the density difference	Cause NPs aggregation or damage
	Most frequently used technique	Lead to changes in PC composition
		Not suitable for separating NPs from high-viscosity or dense biological fluids
	High throughput	PC composition is affected by centrifugation force, washing duration, and washing solution volume
Magnetic Separation	Minimal impact on the structure of NP-PC composites	Limited to magnetic NPs
	High throughput	Separation efficiency decreases with the decrease of magnetism and is more time-consuming
Size Exclusion Chromatography	Separate low-density NPs	Low throughput and more time-consuming
	High resolution	Not suitable for larger or more dispersed NPs, as well as NPs that are easily adhered to column materials
Flow-Field-Flow Fractionation	Keep the structure of NP-PC composites	Sample loss due to adsorption on the membrane
	The separation process is flexible and controllable	Complex protocol: must be optimized for different particle types, and not widely available
		High cost and low throughput

Table 2. In Situ and Ex Situ Characterization Methods of PC

Characterization parameters	*In situ* **analysis method**	ref.	*Ex situ* **analysis method**	ref.
Morphology	Dynamic light scattering	112	Atomic force microscopy	145,146
	NP tracking analysis	113,114	Scanning electron microscopy	147,148
	Fluorescence correlation spectroscopy	115,116	Transmission electron microscopy	147−149
	Dark-field microscopy	117−119	Cryo-electron microscopy	63
			Differential centrifugation sedimentation	56,152,153
Qualification, Quantification, and Epitope analysis	Isotope labeling & NMR	124−127	Bicinchoninic acid assay	154
	Quartz crystal microbalance	129	Mass spectrometer	154
	Stochastic optical reconstruction microscopy	132,133	Bradford protein analysis	156,157
	3D single-molecule active Real-time Tracking	134	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis	158−161
	Stimulated emission depletion	135		
	Flow cytometry	136	Immuno-Au	56,165
	Microfluidic technology	137,138	Isobaric labeling	166
Adsorption kinetics	Real-time optical NP analysis scattering microscope	139	Isothermal titration calorimetry	167,168
	Fluorescence resonance energy transfer	140,141	Localized surface plasmon resonance	169,170
Structural characteristics	Synchrotron-Radiation Circular Dichroism	143	Fourier transform infrared spectroscopy	171
	Sum-frequency generation spectroscopy	144	Circular dichroism	172
			Surface-enhanced Raman spectroscopy	173

matrices. In this section, we systematically summarized the methods for isolation of PC (Figure 2 and Table 1), including density gradient centrifugation, differential centrifugation sedimentation, magnetic separation, size exclusion chromatography (SEC), and flow-field-flow fractionation (F4). Furthermore, we have also critically reviewed emerging methods for PC extraction.

2.1. Centrifugation. Centrifugation (including ultracentrifugation) separates NP-PC complexes from the matrix based on the density difference between complexes and proteins[57] and has been widely used, especially in inorganic NPs research.[58,59] However, this method still faces some challenges. First, NPs with lower density, such as liposomes and lipid NPs (LNPs), typically necessitate high g-force, which may lead to NPs aggregation or disruption, inevitably altering the corona composition.[60] The ultrahigh centrifugal speed may also lead to the isolation of protein aggregates along with the complexes, deposition of proteins, and other biological molecules onto NP-PC complexes that do not naturally exist in the PC or the dissociation of loosely bound proteins of SC from the NP-PC complexes.[61,62] For example, Francia et al. found that high levels of protein contamination, originating from nonadsorbed proteins with similar density, such as lipoproteins, also segregated into the same layer as the NPs. This cosegregation may potentially lead to a misunderstanding of experimental results.[60] Additionally, Sheibani et al. discovered that the PC layer on polystyrene NPs after centrifugation could harbor a substantial amount of small-sized, agglomerated impurities (≤10 nm) that are unrelated to the corona composition.[63] More importantly, this method is not suitable for isolating NPs from high-viscosity or dense biological fluids.[64]

To improve this approach, differential centrifugation sedimentation (DCS) and density gradient centrifugation (DGC) have been proposed and utilized in numerous PC studies.[57,65−67] DCS, also termed the two-layer settling method, separates materials of varying mass by gradually increasing centrifugal speeds. DCS offers higher isolation efficiency than conventional centrifugation with no significant

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differences in the qualitative composition of the corona.[68] DGC separates the NP-PC complexes based on the difference in centrifugation coefficients of samples in different density gradients. The NP-PC complexes are loaded into a medium of graded density, and different density fractions are subsequently collected to identify the one containing NP-PC complexes. This approach is less disruptive compared to traditional centrifugation, as particles are more likely to remain suspended and presumably intact.[69]

Notably, centrifugation and washing conditions need to be tailored according to the types and properties of NPs and the culture media used to develop the PC. For example, Brückner et al. found that the choice of washing media significantly influences protein stability and, consequently, the interaction with the NPs.[70] In addition, the formation of the PC can increase the density of NP-PC complexes in some cases, potentially enhancing their collection by centrifugation or enabling precipitation at a lower centrifugation speed. Therefore, optimization of the centrifugation protocol for the number of washing steps, centrifugation time, and speed is crucial in effectively isolating NP-PC complexes from a protein-rich medium. By fine-tuning these parameters, it is possible to mitigate structural damage to soft NPs, loss of PC, or excessive protein sedimentation. However, such optimization is often overlooked or neglected, leading to failure in reproducing the reported separation efficiency or achieving a uniform yield on repetition. Thus, optimizing various parameters in the centrifugal separation process warrants greater attention from researchers to achieve the optimum separation of NP-PC complexes and, subsequently, obtain more reliable information about PC.

2.2. Magnetic Separation. Magnetic separation is another strong technique for the isolation of NP-PC complexes of magnetic NPs, such as superparamagnetic iron oxide NPs and magnetic multicore NPs.[71−74] The separation efficiency is related to the magnetic strength of the NPs.[74] Compared with the centrifugation, the magnetic separation has a minimal impact on the NP-PC complex structure, reducing the occurrence of false positive proteins and limited protein loss during washings.[75,76] For example, Francia et al. successfully realized the mild isolation of LNPs-PC complexes from biological fluids with magnetic separation method.[77] Encapsulation of magnetic NPs inside LNPs facilitated relatively moderate isolation under a magnetic field while maintaining the structural integrity of LNPs and the composition of PC. Similarly, Hoang et al. successfully collected the gold-coated iron oxide NP-PC complexes from biological systems.[78]

2.3. Size Exclusion Chromatography. SEC is a separation technique based on the fluid dynamic volume of the analyte.[79] During SEC, NP-PC complexes can be fractionated according to their size, facilitating the subsequent analysis.[80] Throughout SEC separation, smaller particles tend to interact more with the porous particles of the stationary phase, requiring more time to traverse the column, whereas larger particles lack such interaction and elute more rapidly. Kristensen et al. compared the efficiency of conventional centrifugation and SEC to separate the LNP-PC from serum. Although centrifugation did not completely precipitate LNP as a fine pellet, SEC was able to successfully separate LNP-PC complexes, despite some protein contamination.[81] The resolution of SEC can be improved by selecting the correct resin type, increasing the length and diameter of the chromatographic column, or combination with other methods.[60] For instance, Hadjidemetriou et al. integrated membrane ultrafiltration as a secondary purification step to ensure the removal of large proteins and protein aggregates, thereby isolating liposome-HC complexes from unbound and loosely bound plasma proteins.[82,83]

The application of SEC is limited by time-consuming procedures and relatively low sample throughput. In addition, this approach is not suitable for larger or polydisperse NPs, as well as those prone to adhere to column materials.[84] More importantly, the competitive interactions among NPs, PC, and stationary phases as well as the presence of shear forces can result in the desorption of the PC from the NPs surface.[75]

2.4. Flow-Field-Flow Fractionation. The Field-Flow Fractionation (FFF) method combines chromatography and field-driven techniques for the separation of macromolecular species using the nonuniform distribution between various external fields and cross-sectional flow in fine channels. Similar to chromatography, FFF operates as an elution technique based on the principles of differential flow displacement; and like field-driven techniques, it relies on the application of external fields or gradient-like force to separate components.[85,86] FFF can be classified into various types based on external fields such as gravitational/centrifugal, thermal, magnetic, and electric fields. Among them, F4 has been most widely studied and applied for the NP-PC complexes isolation.

F4 applies an additional lateral flow that moves perpendicular to the main flow channel and causes the analyte to be pushed toward the semipermeable wall of the channel, where it is isolated by a membrane that only allows current to pass through.[87] Particles with different diameters possess distinct diffusion coefficients in fluid dynamics and are located at different flow velocity levels. The application of an external lateral flow further segregates the analyte for collection at different times and locations.[88] There are three types of F4, including symmetrical F4 (SF4), asymmetrical F4 (AF4), and hollow-fiber F4 (HF5). Among them, AF4 is considered more acceptable due to the simple channel design and lower cost.[87,89−91]

The F4 method offers a significant advantage over the other FFF techniques. The absence of the stationary phase eliminates the mechanical and shear stress on PC, thereby preventing conformational changes or dissociation of proteins during the purification process.[88,92−94] Moreover, a cross-flow that acts as a perpendicular field in F4 makes the isolation process gentler, and the adjustable cross-flow intensity enhances the flexibility and controllability of the isolation process.[92,95] These features make F4 a preferred choice for isolating the NP-PC complexes with wide size range (1 nm to a few μm).[92,96,97] For example, Alberg et al. purified polymeric NP-PC complexes with a hydrodynamic radius of only 20−30 nm by AF4 that were difficult to separate by centrifugation.[94] The gentle nature of F4, especially AF4, allows for the capture and analysis of delicate molecular interactions. In a study by Marin et al., AF4 was applied to measure the binding isotherms and dissociation constants between poly[di(carboxylatophenoxy)phosphazene] (PCPP) and two model proteins, lysozyme (model antigen) and human serum albumin (HSA). By combining AF4 with isothermal calorimetry, they uncovered differences in the binding mechanisms between the polymer and the proteins, with antigen polymer complexes found to be more stable.[98]

The techniques discussed above are commonly used but inevitably result in the loss of SC components or alterations in the molecular composition of HC (Table 1). Their

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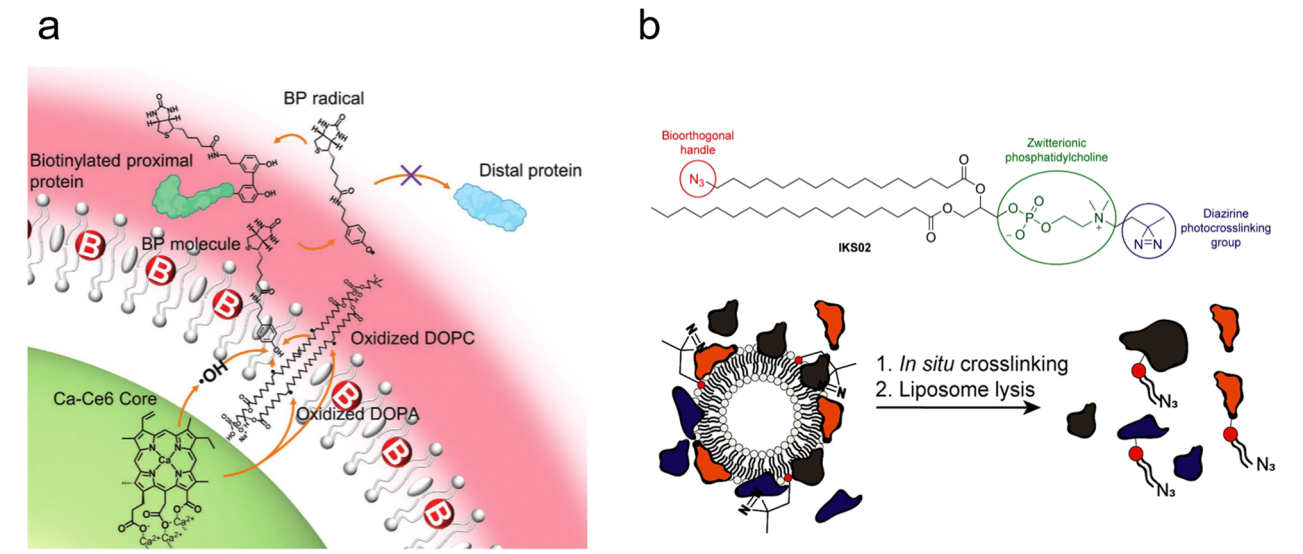

Figure 3. Separation method by labeling PC proteins. (a) Photocatalytic proximity labeling protein technology in NPs (nano-PPL). Reproduced with permission from ref 99. Copyright 2023, John Wiley and Sons. (b) Based on unbiased photoaffinity to capture proteins from PC. Reproduced with permission from ref 101. Copyright 2020, American Chemical Society.

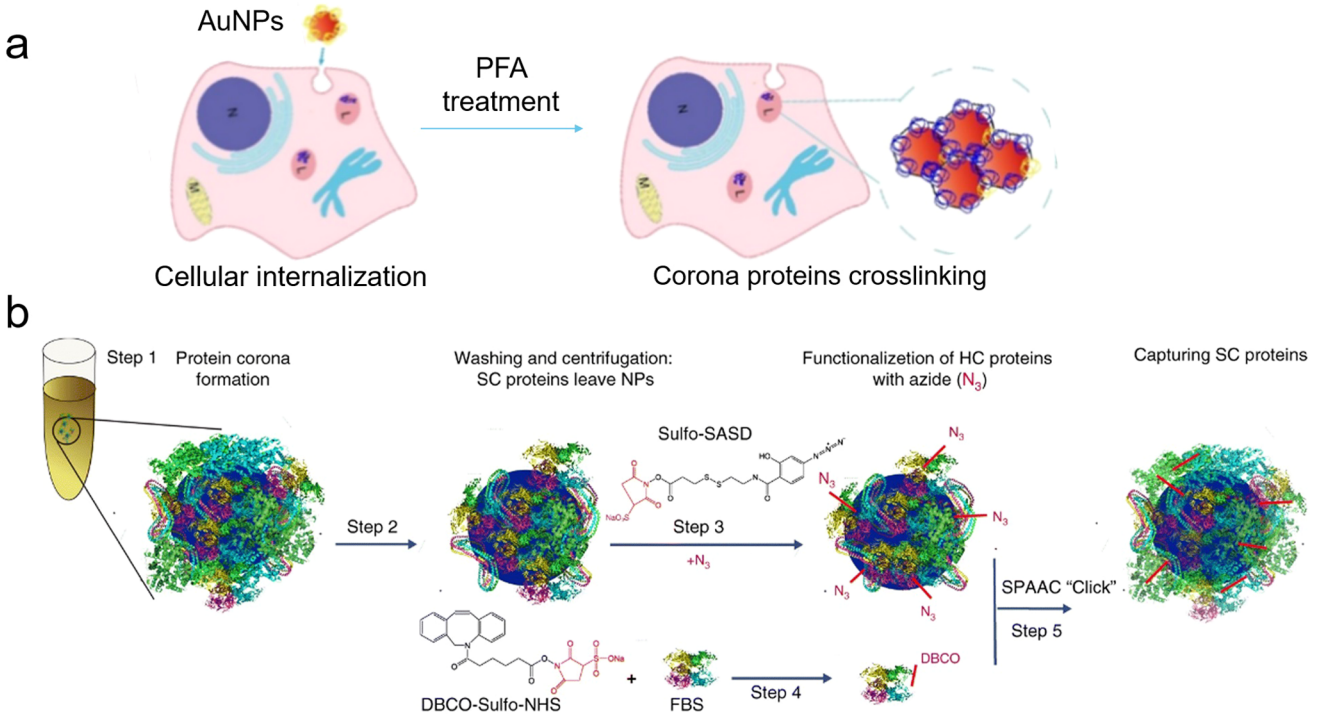

Figure 4. Engineering method for PC isolation. (a) Photocatalytic proximity labeling technology in NPs. Reproduced with permission from ref 102. Copyright 2021, American Chemical Society. (b) Unbiased photoaffinity to capture PC. Reproduced with permission from ref 105. Copyright 2020, Springer Nature.

applications are also limited, as they cannot accurately capture the delicate interaction between NPs and biological fluids during the brief contact time. These limitations hinder the rapid and high-resolution analysis of PC. Therefore, there is a demand for reliable, real-time, *in situ* monitoring methods to separate NP-PC complexes from the biological environment with the least contamination and without altering the corona's composition and spatial orientation.

2.5. Emerging Methods. Currently, many new approaches have been investigated for the NP-PC complexes analysis. Based on this principle, emerging methods can be divided into the following three categories, including labeling of PC, fixing the PC onto the NPs, and utilizing the biological-specific interaction between protein and ligand.

One primary approach involves labeling proteins in the PC followed by their isolation, collection, and analysis. In one study, Zhang et al. employed photocatalytic proximity labeling in NPs (nano-PPL) to isolate NP-PC complexes (Figure 3a). The authors prepared LNP (NPs@CeBP) with a "core-shell" structure containing a photosensitive catalyst (chlorin e6, Ce6) and a probe biotinylphenol (BP). After exposure of NPs@CeBP to biological fluids, a photocatalytic reaction can be

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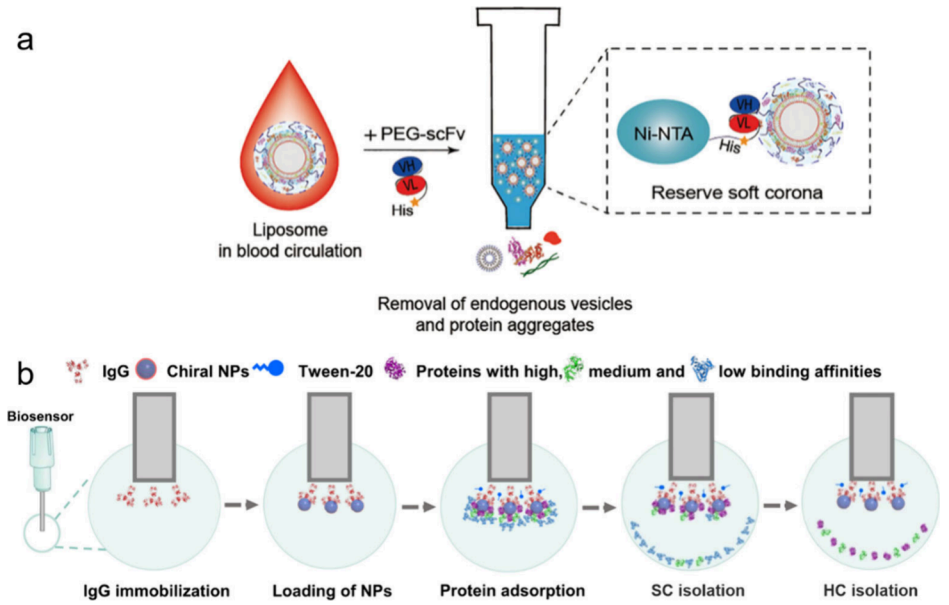

Figure 5. Separation is based on special interaction forces or high constraint forces. (a) Specific binding of poly(ethylene glycol) antibodies to poly(ethylene glycol) liposomes. Reproduced with permission from ref 106. Copyright 2021, American Chemical Society. (b) *In situ* fishing method using biosensors. Reproduced with permission from ref 107. Copyright 2022, Springer Nature.

initiated by irradiation, in which the photocatalyst activates BP and causes its release from the NPs. The BP radicals rapidly couple with the surrounding proteins with a biotin tag.[99] The half-life of BP radicals is extremely short, and the biotinylation of proteins was confined to a small range, demonstrating high spatial resolution.[99,100] This technology can accurately identify proximal interacting proteins within a range of 78 nm from the surface of NPs within 5 s of interaction time. Pattipeiluhu et al. proposed a technique based on unbiased photoaffinity to capture liposomes-PC complexes (Figure 3b).[101] They designed a probe (IKS02) with a structure similar to phospholipids containing long-chain fatty acids (>C12) with a diazirine functional group at one end and an azide functional group at the other end. Under irradiation, diazirine functionality generates highly active carbene intermediates that can spontaneously cross-link with proteins bound to the surface of liposomes. Then, liposomes are separated from the biological medium and solubilized to collect protein−phospholipid conjugates by coupling the fluorescent alkyne-Cy5 probe with another terminal azide functionality.[101]

To avoid the loss of PC components, some separation methods were developed by fixing PC onto the NPs. For example, Wang et al. developed a paraformaldehyde (PFA) cross-linking method to stabilize the corona compositions during PC extraction (Figure 4a).[102] Due to its excellent compatibility, PFA has the potential to work both *in situ* and *in vivo*. The extremely small molecular length limits the cross-linking distance between two functional groups, and it empowers PFA to preserve the original PC compositions formed in cells during the extraction process. In addition, the cross-linking induced by the PFA can be reversed by exposing it to high temperatures (>90 °C), which provides a possibility for the subsequent quantitative and qualitative analysis of NP-PC complexes.[102−104] The results demonstrated that PFA-induced cross-linking suppressed the dissociation or exchange of PC's proteins.[102] Similarly, Mohammad-Beigi et al. developed an experimental method based on a bioorthogonal reaction between dibenzocyclooctyne (DBCO) and azide to immobilize SC proteins on the NPs surface (Figure 4b).[105] In detail, NP-HC complexes were modified with an azide group and then incubated with fetal bovine serum (FBS) modified with DBCO. The SC is formed on the surface of the HC and immobilized by the bioorthogonal reaction between two functional groups. The NP-PC complexes containing both the HC and SC can be separated by centrifugation.

Meanwhile, a new method based on biospecific interaction between protein and ligand has been used to isolate PC. Chu et al. employed antibodies targeting poly(ethylene glycol) (PEG-scFv) with a strong affinity for PEG to isolate and characterize total PC on PEGylated liposomes (Figure 5a). His-tagged PEG-scFv efficiently captures PEGylated liposomes without altering the composition of PC, allowing for the isolation of PEGylated liposomes-PC complexes from plasma protein aggregates and endogenous vesicles through the Ni-NTA column.[106] This method exhibited the advantages of high sensitivity, accuracy, and nondestructive for isolating PEGylated liposomes from biological environments. In addition, Baimanov et al. developed the "*in situ* Fishing" method based on the high binding affinity between the immunoglobulin G (IgG) and NPs (Figure 5b).[107] The researchers immobilized IgG on the biosensor via an amine-coupling reaction and immersed it in Tween 20 buffer to exclude nonspecific protein adsorption, and then the NPs were captured into the IgG-coated biosensor. Subsequently, the sensor was immersed in serum to form a corona. The high binding affinity between IgG and NPs enables the NPs to remain on the sensor even after washing with washing buffer, which makes it possible to separate PC *in situ*. Interestingly, this technique allows for the isolation of SC and HC proteins under different elution conditions. It is worth noting that the combination of NPs and IgG may affect the type and quantity of corona proteins on the NPs.

The aforementioned methods possess distinct advantages and drawbacks, which restrict their application to specific conditions. Presently, a comprehensive approach that seamlessly integrates real-time, *in situ*, and nondestructive

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capabilities while accurately preserving both HC and SC components remains elusive. Nonetheless, we can select the most suitable method for the desired application and actively pursue the development of novel techniques.

3. ANALYSIS OF PC

Quantitatively and qualitatively characterizing PC through various techniques is core to understanding the mechanism behind the biological effect of PC.[67,108−111] In this section, we classify existing characterization techniques of PC into *in situ* and *ex situ* categories based on whether it is necessary to separate the NP-PC complexes from the culture medium. The former can directly detect the complex in the culture medium, while the latter needs to separate the complex from the culture medium before detection. *In situ* characterization makes it possible to monitor the dynamic evolution of NP-PC complexes in real time.

3.1. In Situ Analysis. The current *in situ* techniques for analyzing PC involve complex sample processing steps that disturb the adsorption equilibrium of the NP-PC complexes, leading to the desorption of some SC proteins. Therefore, understanding the mechanism of PC formation and structure is to design simple procedures with minimum handling steps. This is also necessary for real-time and dynamic observation of the evolution of PC over time in a physiological environment. In this section, *in situ* characterization techniques are broadly categorized into four distinct groups including morphology characterization, qualitative and quantitative characterization, adsorption kinetics characterization, and protein structure characterization.

3.1.1. Methods for Morphological Characterization. The characterization technologies for the thickness and morphology of NP-PC complexes are relatively highly developed due to advancements in analytic techniques used to study proteins and other biological macromolecules. The characterization technologies for the thickness of NP-PC complexes mainly include dynamic light scattering (DLS), NP tracking analysis (NTA), and fluorescence correlation spectroscopy (FCS). The principle of both DLS and NTA is based on Brownian motion and light scattering technology. They involve the determination of the sample's apparent diffusion coefficient and then calculate the particles' hydrodynamic radius based on the Stokes−Einstein equation.[112] Comparatively, DLS is suitable for analyzing monodisperse NPs and determining their size distribution. However, the results of DLS may be affected by the dispersion of unbound proteins. On the other hand, NTA is less susceptible to interference from numerous large NPs and provides the added advantage of measuring NPs concentration.[113,114]

Furthermore, FCS empowers us to track subtle alterations in the NP diffusivity resulting from protein adsorption from a solution with precisely defined protein concentrations. Leveraging the Stokes−Einstein relation, the diffusion coefficient can be translated into the hydrodynamic radius, affording us the capability to ascertain NP-PC sizes with subnanometer precision, thereby facilitating *in situ* analysis.[115,116] Compared to DLS, FCS only detects fluorescent molecules without interference from other nonfluorescent components, such as the unbound proteins within the culture medium. However, this technique depends on the fluorescent-labeled NPs and proteins, which may cause a change in PC composition and lead to distortion of the results.

Microscopy, such as Dark-field microscopy (DFM), has also been used to visualize the thickness and morphology of NP-PC complexes in biological fluids *in situ*.[117−119] When NPs are exposed to biological fluids, the adsorption of a large number of biomolecules causes changes in localized surface plasmon resonance (LSPR), resulting in changes in light intensity and color at the level of individual NPs, which can be observed through DFM.[120,121] DFM is especially suitable for metal NPs.[117,122,123] For example, Lin et al. used high-speed DFM to detect the size changes of optical anisotropic gold nanorods before and after PC formation.[117]

3.1.2. Methods for Qualification, Quantification, and Epitope Analysis. It is well-known that determining the composition of the PC is of great significance for studying the biocompatibility and potential toxicity of NPs. However, since only the biomolecules in the outer layer of the corona are involved in binding to other biomolecules, cell membranes, and cell receptors, it is not sufficiently capable of studying the types and quantities of proteins in the PC and elucidating key epitope information, including its location and orientation. *In situ* qualitative and quantitative techniques for PC analysis include combining isotope labeling with nuclear magnetic resonance (NMR), quartz crystal microbalance (QCM), 3D Single-Molecule Active Real-time Tracking (3D-SMART), stochastic optical reconstruction microscopy (STORM), and combining flow cytometry with microfluidic technology. At the same time, some of these techniques can also be used to determine the epitopes of corona proteins.

Isotopic labeling, an *in situ* analysis method, quantitatively detects PC adsorbed on NPs.[124,125] Isotopes, such as ^{2}H, ^{15}N, ^{13}C, and ^{19}F, have been used to label NPs for the detection by NMR and decode the structure information on surface PC based on the protein peak intensity, chemical shift, and line width of the NMR results.[126,127] Due to no interference from unlabeled substances, this method can be used in complex, opaque biological fluids such as intracellular compartments. In addition, NMR can be used to study the orientation, structure, and kinetic changes of PC proteins on the surfaces of NPs. Assfalg et al. discussed the application of solution NMR spectroscopy in the study of protein-NP interactions. They highlighted that analyzing the changes in chemical shifts can identify the binding sites and orientations of proteins on the surface of NPs. Additionally, the changes in the signal intensity of the proteins represent the proteins' coverage on the surface of NPs as a measure of the degree of adsorption. Finally, measuring the amide proton exchange rate and relaxation parameters of proteins can reveal the dynamic behavior and structural stability of proteins in PC.[126] Lin et al. also investigated the orientation of proteins on gold NPs with different modifications by NMR spectroscopy.[128]

In addition, QCM is also widely investigated to analyze NP-PC complexes in biological fluids. The adsorption of proteins will cause changes in the vibration frequency of quartz crystals which is used to calculate the mass of the proteins and the affinity and binding strength between proteins and specific ligands or other biological molecules. Therefore, QCM is mainly utilized to study protein binding and dissociation rates to reveal the kinetic properties between proteins and their interacting molecules.[129,130] Gianneli et al. quantified the accessible functional epitopes of NPs with transferrin (Tf) coronas through a QCM-based "sandwich" biochemical analysis method and correlated them with the size and surface functionalization of the NPs.[129] QCM is highly flexible and can

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be combined with other analytical techniques to expand its application. QCM combined with Dissipation Monitoring (QCM-D) technology utilizes resonance frequency with the dissipation of oscillations for higher resolution. For example, Wang et al. used QCM-D to study the effect of the chirality of NPs on the orientation of the corona protein and its interaction with receptors.[131]

Super-resolution microscopy has developed as a versatile and powerful imaging technique for biological study and has recently been used for the PC study. STORM is a super-resolution imaging technique that uses photoswitchable fluorophores to achieve subdiffraction limit resolution (20−30 nm). STORM has enabled direct visualization and quantification of the individual proteins formed on the surface of silica NPs.[132,133] For instance, Clemment et al. developed a quantitative method based on STORM to study the penetration of proteins within porous silica NPs.[132] Relying on the 3D-SMART microscope, Tan et al. developed real-time three-dimensional single-particle tracking (RT-3D-SPT) spectroscopy. Using RT-3D-SPT, they can continuously and simultaneously monitor single particles and measure the protein and particle interaction at a single protein level.[134] In addition, Wang et al. studied PC on individual silica NPs using stimulated emission depletion (STED) microscopy to analyze the structural characteristics of PC on individual particles.[135] They found that the distribution of PC on the surface of NPs was uneven and speculated that it was caused by uneven surface functionalization during protein adsorption.

A modified flow cytometry method was also developed to quantify levels of specific proteins by analyzing the interaction between fluorescently labeled immunoquantum dots (Immuno-QDs) and proteins by measuring the intensity and distribution of the fluorescent signal. The immuno-QDs (conjugates of quantum dots with specific antibodies) are incubated with NP-PC complexes to enable the antibodies to recognize and bind to specific epitopes on the surface of the NPs.[136] After the application of the laser, the scattered light and fluorescence signals of these NP-immuno-QD complexes are utilized to provide information about the size and shape of the NPs. The fluorescence signals reflect the binding of the immuno-QDs to infer the number, distribution, and availability of specific epitopes on the surface of the NPs. A higher concentration of immune-QDs can be used to simultaneously identify multiple protein epitopes.

Microfluidic technology allows real-time and *in situ* monitoring of the dynamic PC formation process. Srivastava et al. combined integrated microfluidic platforms with electrical resistance measurement devices to achieve *in situ* monitoring of PC formation.[137] This method can accurately control fluid flow in dynamic environments and can be used to simulate natural biological environments. Further, by combining microfluidics with confocal laser scanning microscopy, Weiss et al. in situ captured the adsorption process of proteins onto silica NPs and revealed three phases of PC formation, including proteins irreversibly and directly bound, irreversibly bound proteins interacting with preadsorbed proteins, and reversibly bound "soft" protein corona proteins.[138]

3.1.3. Methods for Characterizing Adsorption Kinetics. For a deeper understanding of the dynamic evolution of PC, a real-time, all-optical analysis of the adsorption kinetics between NPs and proteins at the single-particle level can be achieved with scattering microscopy (RONAS).[139] This method leverages the sensitivity of a particle's scattering cross-section to its local surroundings, allowing for the simultaneous monitoring of PC formation in serum across multiple particles. A study by Dolci et al. utilized RONAS to study protein adsorption kinetics and PC evolution in various NP morphologies. They found that the surface chemistry is the primary factor influencing protein adsorption. The chemical nature of the core or the size of the NPs may also play a role depending on the specific type of NPs.

Fluorescence resonance energy transfer (FRET) can be used for *in situ* analysis of the affinity and adsorption orientation of the surface PC on NPs. For example, a study by Qu et al. found that the different chirality of InP@ZnS QDs affected the affinity and adsorption orientation of proteins.[140] Similarly, Gao et al. detected the binding affinity and thermodynamic parameters of HSA on GSH-CdSe/ZnS core/shell quantum dots (GSH-QDs) using FRET and FCS. FRET measured the distance between the fluorophore and GSH-QDs to reveal the orientation of HSA on the QDs surface. At the same time, FCS revealed the monolayer structure and thickness of HSA-PC around 5.5 nm.[141]

3.1.4. Methods for Characterizing Protein Conformation. The physical binding and conformation of corona protein are the basis for the transmission of complex nanostructured information across membranes, which is crucial for the biorecognition and subsequent biological effects of NPs.[142] The conformation of corona protein often directly affects its function and its interaction with NPs, which in turn affects the stability and composition of the corona and the transport, metabolism, and toxicity of NPs *in vivo*. The *in situ* examination of corona protein conformation poses a challenge with many existing technologies overlooking the intricacies of protein conformational change. The PC on the surface of NPs is always in a dynamic state; therefore, real-time *in situ* characterization of the PC is crucial for understanding the interaction between NPs and proteins as well as between NPs and cell membranes. However, in addition to the PC bound to NPs, there is also free protein in the medium, which may interfere with the accuracy of the analysis and is regarded as the main challenge in characterizing PC *in situ*.

A few methods can do *in situ* analysis of the protein conformation in PCs. For example, Sanchez-Guzman et al. analyzed the secondary structural changes of proteins adsorbed on NPs using the Synchrotron-Radiation Circular Dichroism (SRCD) technique. The researchers measured the circular dichroism changes of proteins at different wavelengths to identify secondary structural changes in proteins, such as α-helix, β-folding, *etc.* after interaction with NPs.[143] Zhang et al. analyzed the transition of the PC from soft to hard utilizing molecular dynamics (MD) simulations and sum-frequency generation (SFG) spectroscopy, including the deflection and distortion of the protein's tertiary structure and the change of the PC's ring structure from vertical to horizontal.[144] However, this method revealed the structural changes of the PC as a whole during interaction with the lipid bilayer rather than on the detailed conformation of the individual protein molecules in the PC. The current methods do not accurately analyze the conformational changes of individual proteins in the PC, which also indicates that the development of real-time dynamic corona protein conformation analysis methods will be an important direction.

3.2. Ex Situ Analysis. *3.2.1. Methods for Morphological Characterization.* Visualization techniques such as atomic force microscopy (AFM), scanning electron microscopy

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(SEM), transmission electron microscopy (TEM), and cryo-electron microscopy (cryo-EM) have been widely used to study the morphology of PC on the surface of NPs.[63,145−150] AFM's high lateral and vertical resolution allows for detailed imaging of PC morphology, providing information about NP-PC interactions, such as changes in adhesion; the sample preparation of AFM is relatively simple, only requiring the sample to be fixed on the substrate but with slow imaging speed. SEM can provide high-resolution imaging of PC morphology and NPs surface morphology, but it requires coating the sample with conductive materials, which may affect the morphology of PC. TEM can provide detailed structural information about NP-PC interactions, including the internalization process, but its sample preparation involves cutting the specimen into thin sections, and imaging necessitates invasive staining procedures, which may destroy the PC structure. Cryo-EM images samples that have been rapidly frozen in vitreous ice, preserving their native structure without artifacts and allowing for high-resolution imaging of biological specimens. In certain studies, a combination of multiple characterization techniques is employed to gain more comprehensive insights. For instance, Zhou et al. utilized both AFM and TEM for the characterization of PC. TEM was used to identify the presence of PC followed by AFM analysis, which further elucidated the surface adhesion force between NPs and the incubation medium.[151]

Recently, researchers have been repurposing conventional characterization techniques like ultracentrifugation and DCS to analyze the particle size distribution of NPs.[56,152,153] For example, Perez-Potti et al. used DCS to characterize different types of functionalized NPs. This method measures the adsorption of polymers and biomolecules on the surface of NPs by detecting changes in the effective density of the NP. The density of the core and surface coatings of NPs, such as polymer coatings and PC, is the main factors responsible for the density change depicted as sedimentation shift and altered sedimentation time.[153]

3.2.2. Methods for Qualification, Quantification, and Epitope Analysis. Bicinchoninic acid (BCA) assay, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and proteomics are the main strategies for qualitative and quantitative detection of proteins.[154,67,155] The BCA assay is a colorimetric method to quantify the protein concentration in a sample by detecting peptide bonds. It not only quantifies protein concentration but also provides qualitative information about the presence or absence of proteins in a sample by comparing the color intensity to known protein standards. Bradford assay is also commonly used as an indirect quantitative method to determine the concentration of proteins adsorbed on the surface of NPs.[156,157] SDS-PAGE is widely used for the qualitative analysis of proteins by visualizing their separation patterns on the gel. It can also provide semiquantitative information by comparing band intensities between samples.[158−161] SDS-PAGE has advantages such as wide use, low cost, and simple operation, but it also has disadvantages such as low flux, low sensitivity, and artifacts.[47]

Liquid chromatography-tandem mass spectrometry (LC-MS/MS) based proteomics is a comprehensive qualitative and quantitative method to reveal the composition of PC on NP surface.[162] After isolation of the NP-PC complexes, the corona proteins are digested into peptides with a proteolytic enzyme such as trypsin. Then, the peptides are separated by LC, ionized by ion, and separated under an electromagnetic field based on the mass-to-charge ratio. The proteins are identified by comparing the mass spectrum with that of the existing protein database. To accurately identify and quantify corona protein, quantitative proteomics, such as tandem mass tagging (TMT) or stable isotope labeling of amino acids in cell culture (SILAC), also can be used for PC analysis.[163,164] For example, Cai et al. cultured HeLa cells with SILAC medium and extracted intracellular proteins through cell lysis. They coincubated extracted proteins with NPs, separated the NP-PC complexes, and analyzed the proteins therein using LC-MS/MS.[164] Comparison of the signal intensities of different SILAC-labeled proteins was performed to quantify the binding efficiency of proteins to NPs. Compared with the direct use of LC-MS/MS, SILAC-LC-MS/MS can compare multiple samples in the same mass spectrometry run. The isotopic labeling provides distinct signals for quantification, which can improve the accuracy and reproducibility of the quantification. However, this method does not produce a clear model of protein orientation.[56,165]

To map the protein orientation on NPs surfaces with MS, Liessi et al. developed a new strategy using the isobaric labeling method.[166] This method first uses an isotopically labeled tag (e.g., TMT) covalently bound to the amino acid residues (usually lysine or N-terminal amino acids) on the outer surface of the PC. The PC is then detached from the surface of the NPs by denaturation treatment, exposing the inner surface, and labeled with another tag of equal weight. The labeled PC was digested by trypsin, and the generated peptides were analyzed by LC-MS/MS. Due to the equal-weight properties of the two tags, different labeled forms of the same peptide will have the same mass-to-charge ratio in the mass spectrometry profile but will produce different reporter ion signals in the MS/MS spectrum. The relative orientation of the peptide in the PC can be inferred by comparing the relative intensities of the two reporter ions in the MS/MS spectra; if the reporter ion signal of the outer surface label is stronger than the signal of the inner surface label, then it indicates that the peptide is more outwardly oriented in the corona and vice versa. Similarly, the reporter ion signals of all of the peptides were counted and analyzed to determine the overall orientation trend of the corona protein.

Meanwhile, Kelly et al. have proposed an immuno-Au labeling technique to identify and locate specific protein epitopes on the surface of NPs.[56] The immuno-Au labeling uses the ability of antibodies to recognize and bind to tiny regions of specific antigens (i.e., epitopes) to determine the spatial distribution. In this method, antibody-Au NP complexes (immuno-Au labeling) combine the antibody's specificity and the Au NPs' high electron density. The immuno-Au complexes are incubated with the NP-PC complexes to bind the target epitope (such as Tf), and the unbound immuno-Au complex is removed by washing. Then, the number of immuno-Au markers can be determined by TEM to quantify the number of epitopes, and their spatial distribution on the surface of the NPs can be reconstructed, which in turn determines the number and spatial location of epitopes.[165]

3.2.3. Methods for Characterizing Adsorption Kinetics. Isothermal titration calorimetry (ITC) has been applied to characterize the adsorption kinetics of proteins on the surface of NPs. ITC measures the process of heat exchange between biomolecules with a calorimeter and evaluates the thermodynamic parameters of PC formation, such as affinity constant

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and binding stoichiometry. In this way, ITC reflects the strength and adsorption kinetics of the interaction between NPs and proteins, which can deepen our understanding of the NP-PC formation mechanism.[167,168] In addition, the LSPR characteristics in the UV−vis-NIR spectral range of metal NPs can also be used to characterize the adsorption kinetics of proteins on the surface of NPs. The principle of this method is based on the interaction between free electrons on the surface of metal NPs and the incident light field. When the size of NPs approaches the wavelength of light, free electrons will resonate and oscillate, producing a surface plasmon resonance. This resonance amplifies the electromagnetic field on the surface of metal NPs, forming a locally enhanced electromagnetic field. When biomolecules (such as proteins) adsorb onto the surface of metal NPs, they alter the local electromagnetic field on the surface of the NPs, thereby affecting the properties of the LSPR signals. However, research has shown that LSPR frequency is susceptible to small changes in the surrounding environment of NPs.[169,170]

3.2.4. Methods for Characterizing Protein Conformation. Fourier transform infrared spectroscopy (FT-IR) and circular dichroism (CD) are classical methods to detect protein conformation. FT-IR is highly sensitive to the displacement and shape changes of the vibrational bands corresponding to amide bonds and can observe changes in the secondary structure of proteins and measure the trend of binding between PC and NPs.[171] CD is also employed to determine the changes in the secondary structure of proteins as discussed above.[172] In addition, surface-enhanced Raman spectroscopy (SERS) can provide information on the structure, composition, and interaction of molecules near the surface of metal NPs.[173]

Overall, many analysis techniques are currently available to characterize NP-PC complexes comprehensively. To compensate for the limitations of a single method, researchers often combine multiple techniques and make adjustments based on the scientific problem being studied and specific needs. A summary of current *in situ* and *ex situ* characterization methods is provided in Table 2 to aid readers in making well-informed decisions.

4. CONCLUSION AND OUTLOOK

NPs offer immense potential in biomedical science, yet a thorough understanding of their behavior and fate within biological systems is essential for their safe application. Nevertheless, the complex dynamics of bionano interactions present significant challenges. The formation of PC upon NPs in biological fluids confers a new biological identity and masks their original biofunctions. Therefore, a comprehensive understanding of the complex interplay between NP-PC complexes and biological entities is key for nanomedicine research. Accurate and nondestructive isolation and robust characterization of PC will guide the development of safer and more effective NPs for human health and the environment. Consequently, this review delineates strategies for PC isolation and analysis using *in situ* and *ex situ* methods to study its morphology, composition, structure, and orientation.

The big challenge for PC analysis is composition loss and protein contamination from the biological samples. The currently available techniques still have several limitations for PC isolation and characterization, but it is rapidly evolving. The future of corona analysis technology relies on integrating multiple omics methodologies, advancing single-particle analysis techniques, and establishing high-throughput screening platforms. Moreover, the formulation of standardized protocols and reference materials for PC analysis will promote comparability and reproducibility across diverse research endeavors and applications. Discrepancies in PC composition are due to differences in the experimental environments used in various laboratories, such as the types and concentrations of biological fluids, and the methods and instrument models used in isolation and analysis methods.[111,174,175] Furthermore, as stated by Boselli et al., MS is a reference technique for protein analysis, but only a few studies report proteomic data in available formats; the availability of proteomic data in accessible formats will speed up the PC research also.[176] Meanwhile, we need to continue to expand the application of computational biology and bioinformatics tools in the field of PC analysis to model and predict PC formation and function at the molecular level. These efforts will ultimately contribute to the development of a safer and more effective nanomedicine.

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Notes

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REFERENCES

(1) Zhang, H. Molecularly Imprinted Nanoparticles for Biomedical Applications. *Adv. mater.* **2020**, *32* (3), 1806328.
(2) Wei, Q.; Chen, Y.; Ma, X.; Ji, J.; Qiao, Y.; Zhou, B.; Ma, F.; Ling, D.; Zhang, H.; Tian, M.; Tian, J.; Zhou, M. High-Efficient Clearable Nanoparticles for Multi-Modal Imaging and Image-Guided Cancer Therapy. *Adv. Funct. Mater.* **2018**, *28* (2), 1704634.
(3) Mitchell, M. J.; Billingsley, M. M.; Haley, R. M.; Wechsler, M. E.; Peppas, N. A.; Langer, R. Engineering precision nanoparticles for drug delivery. *Nat. Rev. Drug Discovery* **2021**, *20* (2), 101−124.
(4) Yang, J.; Yang, Y.-W. Metal-Organic Frameworks for Biomedical Applications. *Small* **2020**, *16* (10), 1906846.
(5) Krishnan, K. M. Biomedical Nanomagnetics: A Spin Through Possibilities in Imaging, Diagnostics, and Therapy. *IEEE Trans. Magn.* **2010**, *46* (7), 2523−2558.
(6) Lee, J.; Liao, H.; Wang, Q.; Han, J.; Han, J. H.; Shin, H. E.; Ge, M.; Park, W.; Li, F. Exploration of nanozymes in viral diagnosis and therapy. *Exploration* **2022**, *2* (1), 20210086.
(7) Soetaert, F.; Korangath, P.; Serantes, D.; Fiering, S.; Ivkov, R. Cancer therapy with iron oxide nanoparticles: Agents of thermal and immune therapies. *Adv. Drug Delivery Rev.* **2020**, *163*−*164*, 65−83.
(8) Lei, H.; Pei, Z.; Jiang, C.; Cheng, L. Recent progress of metal-based nanomaterials with anti-tumor biological effects for enhanced cancer therapy. *Exploration* **2023**, *3* (5), 20220001.
(9) Theivendran, S.; Lazarev, S.; Yu, C. Mesoporous silica/organosilica nanoparticles for cancer immunotherapy. *Exploration* **2023**, *3* (3), 20220086.
(10) Geng, Z.; Cao, Z.; Liu, J. Recent advances in targeted antibacterial therapy basing on nanomaterials. *Exploration* **2023**, *3* (1), 20210117.
(11) Trinh, D. N.; Gardner, R. A.; Franciosi, A. N.; McCarthy, C.; Keane, M. P.; Soliman, M. G.; O'Donnell, J. S.; Meleady, P.; Spencer, D. I. R.; Monopoli, M. P. Nanoparticle Biomolecular Corona-Based Enrichment of Plasma Glycoproteins for N-Glycan Profiling and Application in Biomarker Discovery. *ACS Nano* **2022**, *16* (4), 5463−5475.
(12) Kamaly, N.; Farokhzad, O. C.; Corbo, C. Nanoparticle protein corona evolution: from biological impact to biomarker discovery. *Nanoscale* **2022**, *14* (5), 1606−1620.
(13) Cedervall, T.; Lynch, I.; Foy, M.; Berggård, T.; Donnelly, S. C.; Cagney, G.; Linse, S.; Dawson, K. A. Detailed Identification of Plasma Proteins Adsorbed on Copolymer Nanoparticles. *Angew. Chem., Int. Ed.* **2007**, *46* (30), 5754−5756.
(14) Caracciolo, G.; Callipo, L.; De Sanctis, S. C.; Cavaliere, C.; Pozzi, D.; Laganà, A. Surface adsorption of protein corona controls the cell internalization mechanism of DC-Chol-DOPE/DNA lipoplexes in serum. *Biochimica et Biophysica Acta (BBA) - Biomembranes* **2010**, *1798* (3), 536−543.
(15) Wang, Y.-F.; Zhou, Y.; Sun, J.; Wang, X.; Jia, Y.; Ge, K.; Yan, Y.; Dawson, K. A.; Guo, S.; Zhang, J.; Liang, X.-J. The Yin and Yang of the protein corona on the delivery journey of nanoparticles. *Nano Res.* **2023**, *16* (1), 715−734.
(16) Lai, W.; Wang, Q.; Li, L.; Hu, Z.; Chen, J.; Fang, Q. Interaction of gold and silver nanoparticles with human plasma: Analysis of protein corona reveals specific binding patterns. *Colloids Surf., B* **2017**, *152*, 317−325.
(17) Li, X.; Yu, L.; He, M.; Chen, C.; Yu, Z.; Jiang, S.; Wang, Y.; Li, L.; Li, B.; Wang, G.; Shen, A.; Fan, J. Review on carbon dots: Synthesis and application in biology field. *BMEMat* **2023**, *1* (4), No. e12045.
(18) Lima, T.; Bernfur, K.; Vilanova, M.; Cedervall, T. Understanding the Lipid and Protein Corona Formation on Different Sized Polymeric Nanoparticles. *Sci. Rep.* **2020**, *10* (1), 1129.
(19) García-Álvarez, R.; Hadjidemetriou, M.; Sánchez-Iglesias, A.; Liz-Marzán, L. M.; Kostarelos, K. *In vivo* formation of protein corona on gold nanoparticles. The effect of their size and shape. *Nanoscale* **2018**, *10* (3), 1256−1264.
(20) Piloni, A.; Wong, C. K.; Chen, F.; Lord, M.; Walther, A.; Stenzel, M. H. Surface roughness influences the protein corona formation of glycosylated nanoparticles and alter their cellular uptake. *Nanoscale* **2019**, *11* (48), 23259−23267.
(21) Cheng, X.; Tian, X.; Wu, A.; Li, J.; Tian, J.; Chong, Y.; Chai, Z.; Zhao, Y.; Chen, C.; Ge, C. Protein Corona Influences Cellular Uptake of Gold Nanoparticles by Phagocytic and Nonphagocytic Cells in a Size-Dependent Manner. *ACS Appl. Mater. Interfaces* **2015**, *7* (37), 20568−20575.
(22) Xiao, B.; Liu, Y.; Chandrasiri, I.; Overby, C.; Benoit, D. S. W. Impact of Nanoparticle Physicochemical Properties on Protein Corona and Macrophage Polarization. *ACS Appl. Mater. Interfaces* **2023**, *15*, 13993−14004.
(23) Subramaniam, S.; Joyce, P.; Donnellan, L.; Young, C.; Wignall, A.; Hoffmann, P.; Prestidge, C. A. Protein adsorption determines pulmonary cell uptake of lipid-based nanoparticles. *J. Colloid Interface Sci.* **2023**, *641*, 36−47.
(24) Francia, V.; Montizaan, D.; Salvati, A. Interactions at the cell membrane and pathways of internalization of nano-sized materials for nanomedicine. *Beilstein J. Nanotechnol.* **2020**, *11* (1), 338−353.
(25) Chaudhary, A.; Khan, S.; Gupta, A.; Nandi, C. K. Effect of surface chemistry and morphology of gold nanoparticle on the structure and activity of common blood proteins. *New J. Chem.* **2016**, *40* (6), 4879−4883.
(26) Chetwynd, A. J.; Zhang, W.; Thorn, J. A.; Lynch, I.; Ramautar, R. The Nanomaterial Metabolite Corona Determined Using a Quantitative Metabolomics Approach: A Pilot Study. *Small* **2020**, *16* (21), 2000295.
(27) Ashby, J.; Pan, S.; Zhong, W. Size and surface functionalization of iron oxide nanoparticles influence the composition and dynamic nature of their protein corona. *ACS Appl. Mater. Interfaces* **2014**, *6* (17), 15412−15419.
(28) Chakraborty, D.; Chauhan, P.; Kumar, S.; Chaudhary, S.; Chandrasekaran, N.; Mukherjee, A.; Ethiraj, K. R. Utilizing corona on functionalized selenium nanoparticles for loading and release of doxorubicin payload. *J. Mol. Liq.* **2019**, *296*, 111864.
(29) Bertrand, N.; Grenier, P.; Mahmoudi, M.; Lima, E. M.; Appel, E. A.; Dormont, F.; Lim, J.-M.; Karnik, R.; Langer, R.; Farokhzad, O. C. Mechanistic understanding of in vivo protein corona formation on polymeric nanoparticles and impact on pharmacokinetics. *Nat. Commun.* **2017**, *8* (1), 777.
(30) Lesniak, A.; Fenaroli, F.; Monopoli, M. P.; Åberg, C.; Dawson, K. A.; Salvati, A. Effects of the Presence or Absence of a Protein Corona on Silica Nanoparticle Uptake and Impact on Cells. *ACS Nano* **2012**, *6* (7), 5845−5857.
(31) Mirshafiee, V.; Kim, R.; Mahmoudi, M.; Kraft, M. L. The importance of selecting a proper biological milieu for protein corona analysis in vitro: Human plasma versus human serum. *Int. J. Biochem. Cell Biol.* **2016**, *75*, 188−195.
(32) Castagnola, V.; Tomati, V.; Boselli, L.; Braccia, C.; Decherchi, S.; Pompa, P. P.; Pedemonte, N.; Benfenati, F.; Armirotti, A. Sources of biases in the in vitro testing of nanomaterials: the role of the biomolecular corona. *Nanoscale Horiz* **2024**, *9* (5), 799−816.
(33) Mahmoudi, M.; Shokrgozar, M. A.; Behzadi, S. Slight temperature changes affect protein affinity and cellular uptake/toxicity of nanoparticles. *Nanoscale* **2013**, *5* (8), 3240−3244.
(34) Lee, J. G.; Lannigan, K.; Shelton, W. A.; Meissner, J.; Bharti, B. Adsorption of Myoglobin and Corona Formation on Silica Nanoparticles. *Langmuir* **2020**, *36* (47), 14157−14165.
(35) Blundell, E. L. C. J.; Healey, M. J.; Holton, E.; Sivakumaran, M.; Manstana, S.; Platt, M. Characterisation of the protein corona using tunable resistive pulse sensing: determining the change and distribution of a particle's surface charge. *Anal. Bioanal. Chem.* **2016**, *408* (21), 5757−5768.
(36) Strojan, K.; Leonardi, A.; Bregar, V. B.; Križaj, I.; Svete, J.; Pavlin, M. Dispersion of Nanoparticles in Different Media Importantly Determines the Composition of Their Protein Corona. *PLoS One* **2017**, *12* (1), No. e0169552.
(37) Gräfe, C.; Weidner, A.; Lühe, M. V. D.; Bergemann, C.; Schacher, F. H.; Clement, J. H.; Dutz, S. Intentional formation of a protein corona on nanoparticles: Serum concentration affects protein

https://doi.org/10.1021/cbe.4c00105
Chem Bio Eng. 2024, 1, 757−772

corona mass, surface charge, and nanoparticle-cell interaction. *Int. J. Biochem. Cell Biol.* **2016**, *75*, 196−202.
(38) Yu, K.; Andruschak, P.; Yeh, H. H.; Grecov, D.; Kizhakkedathu, J. N. Influence of dynamic flow conditions on adsorbed plasma protein corona and surface-induced thrombus generation on antifouling brushes. *Biomaterials* **2018**, *166*, 79−95.
(39) Hadjidemetriou, M.; Al-Ahmady, Z.; Kostarelos, K. Time-evolution of in vivo protein corona onto blood-circulating PEGylated liposomal doxorubicin (DOXIL) nanoparticles. *Nanoscale* **2016**, *8*, 6948−6957.
(40) Müller, J.; Prozeller, D.; Ghazaryan, A.; Kokkinopoulou, M.; Mailänder, V.; Morsbach, S.; Landfester, K. Beyond the protein corona - lipids matter for biological response of nanocarriers. *Acta Biomater.* **2018**, *71*, 420−431.
(41) Giulimondi, F.; Digiacomo, L.; Pozzi, D.; Palchetti, S.; Vulpis, E.; Capriotti, A. L.; Chiozzi, R. Z.; Laganà, A.; Amenitsch, H.; Masuelli, L.; Peruzzi, G.; Mahmoudi, M.; Screpanti, I.; Zingoni, A.; Caracciolo, G. Interplay of protein corona and immune cells controls blood residency of liposomes. *Nat. Commun.* **2019**, *10* (1), 3686.
(42) Ju, Y.; Lee, W. S.; Pilkington, E. H.; Kelly, H. G.; Li, S.; Selva, K. J.; Wragg, K. M.; Subbarao, K.; Nguyen, T. H. O.; Rowntree, L. C.; Allen, L. F.; Bond, K.; Williamson, D. A.; Truong, N. P.; Plebanski, M.; Kedzierska, K.; Mahanty, S.; Chung, A. W.; Caruso, F.; Wheatley, A. K.; Juno, J. A.; Kent, S. J. Anti-PEG Antibodies Boosted in Humans by SARS-CoV-2 Lipid Nanoparticle mRNA Vaccine. *ACS Nano* **2022**, *16* (8), 11769−11780.
(43) Ashkarran, A. A.; Gharibi, H.; Grunberger, J. W.; Saei, A. A.; Khurana, N.; Mohammadpour, R.; Ghandehari, H.; Mahmoudi, M. Sex-Specific Silica Nanoparticle Protein Corona Compositions Exposed to Male and Female BALB/c Mice Plasmas. *ACS bio & med chem Au* **2023**, *3* (1), 62−73.
(44) Hajipour, M. J.; Laurent, S.; Aghaie, A.; Rezaee, F.; Mahmoudi, M. Personalized protein coronas: a "key" factor at the nanobiointerface. *Biomater. Sci.* **2014**, *2* (9), 1210−1221.
(45) Caputo, D.; Papi, M.; Coppola, R.; Palchetti, S.; Digiacomo, L.; Caracciolo, G.; Pozzi, D. A protein corona-enabled blood test for early cancer detection. *Nanoscale* **2017**, *9* (1), 349−354.
(46) Hajipour, M. J.; Raheb, J.; Akhavan, O.; Arjmand, S.; Mashinchian, O.; Rahman, M.; Abdolahad, M.; Serpooshan, V.; Laurent, S.; Mahmoudi, M. Personalized disease-specific protein corona influences the therapeutic impact of graphene oxide. *Nanoscale* **2015**, *7* (19), 8978−8994.
(47) Docter, D.; Westmeier, D.; Markiewicz, M.; Stolte, S.; Knauer, S. K.; Stauber, R. H. The nanoparticle biomolecule corona: lessons learned - challenge accepted? *Chem. Soc. Rev.* **2015**, *44* (17), 6094−6121.
(48) Kihara, S.; van der Heijden, N. J.; Seal, C. K.; Mata, J. P.; Whitten, A. E.; Koper, I.; McGillivray, D. J. Soft and Hard Interactions between Polystyrene Nanoplastics and Human Serum Albumin Protein Corona. *Bioconjugate Chem.* **2019**, *30*, 1067−1076.
(49) Fleischer, C. C.; Payne, C. K. Nanoparticle-Cell Interactions: Molecular Structure of the Protein Corona and Cellular Outcomes. *Acc. Chem. Res.* **2014**, *47* (8), 2651−2659.
(50) Dawson, K. A.; Yan, Y. Current understanding of biological identity at the nanoscale and future prospects. *Nat. Nanotechnol* **2021**, *16* (3), 229−242.
(51) Villacorta, A. M.; Mielcarek, A.; Martinez, M. G.; Jorge, H.; Henschke, A.; Coy, E.; Gomez-Vallejo, V.; Llop, J.; Moya, S. E. The In Vivo Biological Fate of Protein Corona: A Comparative PET Study of the Fate of Soft and Hard Protein Corona in Healthy Animal Models. *Small* **2024**, 2309616.
(52) Onal Acet, B.; Gul, D.; Stauber, R. H.; Odabasi, M.; Acet, O. A Review for Uncovering the "Protein-Nanoparticle Alliance": Implications of the Protein Corona for Biomedical Applications. *Nanomaterials* **2024**, *14*, 823.
(53) Nierenberg, D.; Khaled, A. R.; Flores, O. Formation of a protein corona influences the biological identity of nanomaterials. *Rep. Pract. Oncol. Radiother.* **2018**, *23* (4), 300−308.
(54) Caracciolo, G.; Farokhzad, O. C.; Mahmoudi, M. Biological Identity of Nanoparticles In Vivo: Clinical Implications of the Protein Corona. *Trends Biotechnol.* **2017**, *35* (3), 257−264.
(55) Shi, J.; Kantoff, P. W.; Wooster, R.; Farokhzad, O. C. Cancer nanomedicine: progress, challenges and opportunities. *Nat. Rev. Cancer* **2017**, *17* (1), 20−37.
(56) Kelly, P. M.; Aberg, C.; Polo, E.; O'Connell, A.; Cookman, J.; Fallon, J.; Krpetic, Z.; Dawson, K. A. Mapping protein binding sites on the biomolecular corona of nanoparticles. *Nat. Nanotechnol* **2015**, *10* (5), 472−479.
(57) Hajipour, M. J.; Safavi-Sohi, R.; Sharifi, S.; Mahmoud, N.; Ashkarran, A. A.; Voke, E.; Serpooshan, V.; Ramezankhani, M.; Milani, A. S.; Landry, M. P.; Mahmoudi, M. An Overview of Nanoparticle Protein Corona Literature. *Small* **2023**, *19*, 2301838.
(58) Robertson, J. D.; Rizzello, L.; Avila-Olias, M.; Gaitzsch, J.; Contini, C.; Magoń, M. S.; Renshaw, S. A.; Battaglia, G. Purification of Nanoparticles by Size and Shape. *Sci. Rep.* **2016**, *6* (1), 27494.
(59) Di Silvio, D.; Rigby, N.; Bajka, B.; Mayes, A.; Mackie, A.; Baldelli Bombelli, F. Technical tip: high-resolution isolation of nanoparticle-protein corona complexes from physiological fluids. *Nanoscale* **2015**, *7* (28), 11980−11990.
(60) Francia, V.; Schiffelers, R. M.; Cullis, P. R.; Witzigmann, D. The Biomolecular Corona of Lipid Nanoparticles for Gene Therapy. *Bioconjugate Chem.* **2020**, *31* (9), 2046−2059.
(61) García-Álvarez, R.; Vallet-Regí, M. Hard and Soft Protein Corona of Nanomaterials: Analysis and Relevance. *Nanomaterials* **2021**, *11* (4), 888.
(62) Weber, C.; Morsbach, S.; Landfester, K. Possibilities and Limitations of Different Separation Techniques for the Analysis of the Protein Corona. *Angew. Chem., Int. Ed.* **2019**, *58* (37), 12787−12794.
(63) Sheibani, S.; Basu, K.; Farnudi, A.; Ashkarran, A.; Ichikawa, M.; Presley, J. F.; Bui, K. H.; Ejtehadi, M. R.; Vali, H.; Mahmoudi, M. Nanoscale characterization of the biomolecular corona by cryo-electron microscopy, cryo-electron tomography, and image simulation. *Nat. Commun.* **2021**, *12* (1), 573.
(64) Soliman, M. G.; Trinh, D. N.; Ravagli, C.; Meleady, P.; Henry, M.; Movia, D.; Doumett, S.; Cappiello, L.; Prina-Mello, A.; Baldi, G.; Monopoli, M. P. Development of a fast and simple method for the isolation of superparamagnetic iron oxide nanoparticles protein corona from protein-rich matrices. *J. Colloid Interface Sci.* **2024**, *659*, 503.
(65) Davidson, A. M.; Brust, M.; Cooper, D. L.; Volk, M. Sensitive Analysis of Protein Adsorption to Colloidal Gold by Differential Centrifugal Sedimentation. *Anal. Chem.* **2017**, *89* (12), 6807−6814.
(66) Vilanova, O.; Mittag, J. J.; Kelly, P. M.; Milani, S.; Dawson, K. A.; Rädler, J. O.; Franzese, G. Understanding the Kinetics of Protein-Nanoparticle Corona Formation. *ACS Nano* **2016**, *10* (12), 10842−10850.
(67) Tenzer, S.; Docter, D.; Kuharev, J.; Musyanovych, A.; Fetz, V.; Hecht, R.; Schlenk, F.; Fischer, D.; Kiouptsi, K.; Reinhardt, C.; Landfester, K.; Schild, H.; Maskos, M.; Knauer, S. K.; Stauber, R. H. Rapid formation of plasma protein corona critically affects nanoparticle pathophysiology. *Nat. Nanotechnol.* **2013**, *8* (10), 772−781.
(68) Fernández-Iglesias, N.; Bettmer, J. Complementary mass spectrometric techniques for the quantification of the protein corona: a case study on gold nanoparticles and human serum proteins. *Nanoscale* **2015**, *7* (34), 14324−14331.
(69) Chen, D.; Ganesh, S.; Wang, W.; Amiji, M. The role of surface chemistry in serum protein corona-mediated cellular delivery and gene silencing with lipid nanoparticles. *Nanoscale* **2019**, *11* (18), 8760−8775.
(70) Brückner, M.; Simon, J.; Jiang, S.; Landfester, K.; Mailänder, V. Preparation of the protein corona: How washing shapes the proteome and influences cellular uptake of nanocarriers. *Acta Biomater.* **2020**, *114*, 333−342.
(71) Sakulkhu, U.; Maurizi, L.; Mahmoudi, M.; Motazacker, M.; Vries, M.; Gramoun, A.; Ollivier Beuzelin, M.-G.; Vallée, J.-P.; Rezaee, F.; Hofmann, H. Ex situ evaluation of the composition of protein

corona of intravenously injected superparamagnetic nanoparticles in rats. *Nanoscale* **2014**, *6* (19), 11439−11450.
(72) Dutz, S. Are Magnetic Multicore Nanoparticles Promising Candidates for Biomedical Applications? *IEEE Trans. Magn.* **2016**, *52* (9), 1−3.
(73) Wu, W.; Wu, Z.; Yu, T.; Jiang, C.; Kim, W.-S. Recent progress on magnetic iron oxide nanoparticles: synthesis, surface functional strategies and biomedical applications. *Sci. Technol. Adv. Mater.* **2015**, *16* (2), 023501.
(74) Bonvin, D.; Chiappe, D.; Moniatte, M.; Hofmann, H.; Mionic Ebersold, M. Methods of protein corona isolation for magnetic nanoparticles. *Analyst* **2017**, *142*, 3805−3815.
(75) Böhmert, L.; Voß, L.; Stock, V.; Braeuning, A.; Lampen, A.; Sieg, H. Isolation methods for particle protein corona complexes from protein-rich matrices. *Nanoscale Advances* **2020**, *2* (2), 563−582.
(76) Blume, J. E.; Manning, W. C.; Troiano, G.; Hornburg, D.; Figa, M.; Hesterberg, L.; Platt, T. L.; Zhao, X.; Cuaresma, R. A.; Everley, P. A.; Ko, M.; Liou, H.; Mahoney, M.; Ferdosi, S.; Elgierari, E. M.; Stolarczyk, C.; Tangeysh, B.; Xia, H.; Benz, R.; Siddiqui, A.; Carr, S. A.; Ma, P.; Langer, R.; Farias, V.; Farokhzad, O. C. Rapid, deep and precise profiling of the plasma proteome with multi-nanoparticle protein corona. *Nat. Commun.* **2020**, *11* (1), 3662.
(77) Francia, V.; Zhang, Y.; Cheng, M. H. Y.; Schiffelers, R. M.; Witzigmann, D.; Cullis, P. R. A magnetic separation method for isolating and characterizing the biomolecular corona of lipid nanoparticles. *Proc. Natl. Acad. Sci. U. S. A.* **2024**, *121* (11), No. e2307803120.
(78) Hoang, K. N. L.; Wheeler, K. E.; Murphy, C. J. Isolation Methods Influence the Protein Corona Composition on Gold-Coated Iron Oxide Nanoparticles. *Anal. Chem.* **2022**, *94* (11), 4737−4746.
(79) Sun, T.; Chance, R. R.; Graessley, W. W.; Lohse, D. J. A Study of the Separation Principle in Size Exclusion Chromatography. *Macromolecules* **2004**, *37* (11), 4304−4312.
(80) Capriotti, A. L.; Caracciolo, G.; Cavaliere, C.; Colapicchioni, V.; Piovesana, S.; Pozzi, D.; Laganà, A. Analytical Methods for Characterizing the Nanoparticle-Protein Corona. *Chromatographia* **2014**, *77* (11), 755−769.
(81) Kristensen, K.; Münter, R.; Kempen, P. J.; Thomsen, M. E.; Stensballe, A.; Andresen, T. L. Isolation methods commonly used to study the liposomal protein corona suffer from contamination issues. *Acta Biomater.* **2021**, *130*, 460−472.
(82) Hadjidemetriou, M.; McAdam, S.; Garner, G.; Thackeray, C.; Knight, D.; Smith, D.; Al-Ahmady, Z.; Mazza, M.; Rogan, J.; Clamp, A.; Kostarelos, K. The Human In Vivo Biomolecule Corona onto PEGylated Liposomes: A Proof-of-Concept Clinical Study. *Adv. mater.* **2019**, *31* (4), 1803335.
(83) Hadjidemetriou, M.; Al-ahmady, Z.; Buggio, M.; Swift, J.; Kostarelos, K. A novel scavenging tool for cancer biomarker discovery based on the blood-circulating nanoparticle protein corona. *Biomaterials* **2019**, *188*, 118−129.
(84) Cedervall, T.; Lynch, I.; Lindman, S.; Berggård, T.; Thulin, E.; Nilsson, H.; Dawson, K. A.; Linse, S. Understanding the nanoparticle-protein corona using methods to quantify exchange rates and affinities of proteins for nanoparticles. *Proc. Natl. Acad. Sci. U. S. A.* **2007**, *104* (7), 2050−2055.
(85) Giddings, J. C. The conceptual basis of field-flow fractionation. *J. Chem. Educ.* **1973**, *50* (10), 667.
(86) Caldwell, K. D.; Kesner, L. F.; Myers, M. N.; Giddings, J. C. Electrical Field-Flow Fractionation of Proteins. *Science* **1972**, *176*, 296−298.
(87) Giddings, J. C.; Yang, F. J. F.; Myers, M. N. Flow-Field-Flow Fractionation: A Versatile New Separation Method. *Science* **1976**, *193* (4259), 1244−1245.
(88) Böhmert, L.; Girod, M.; Hansen, U.; Maul, R.; Knappe, P.; Niemann, B.; Weidner, S. M.; Thünemann, A. F.; Lampen, A. Analytically monitored digestion of silver nanoparticles and their toxicity on human intestinal cells. *Nanotoxicology* **2014**, *8* (6), 631−642.
(89) Zattoni, A.; Casolari, S.; Rambaldi, D.; Reschiglian, P. Hollow-Fiber Flow Field-Flow Fractionation. *Curr. Anal. Chem.* **2007**, *3* (4), 310−323.
(90) Wahlund, K. G.; Giddings, J. C. Properties of an asymmetrical flow field-flow fractionation channel having one permeable wall. *Anal. Chem.* **1987**, *59* (9), 1332−1339.
(91) Joensson, J. A.; Carlshaf, A. Flow field flow fractionation in hollow cylindrical fibers. *Anal. Chem.* **1989**, *61* (1), 11−18.
(92) Rambaldi, D. C.; Reschiglian, P.; Zattoni, A. Flow field-flow fractionation: recent trends in protein analysis. *Anal. Bioanal.Chem.* **2011**, *399* (4), 1439−1447.
(93) Yohannes, G.; Wiedmer, S. K.; Tuominen, E. K. J.; Kinnunen, P. K. J.; Riekkola, M.-L. Cytochrome c-dimyristoylphosphatidylglycerol interactions studied by asymmetrical flow field-flow fractionation. *Anal. Bioanal.Chem.* **2004**, *380* (5−6), 757−766.
(94) Alberg, I.; Kramer, S.; Schinnerer, M.; Hu, Q.; Seidl, C.; Leps, C.; Drude, N.; Möckel, D.; Rijcken, C.; Lammers, T.; Diken, M.; Maskos, M.; Morsbach, S.; Landfester, K.; Tenzer, S.; Barz, M.; Zentel, R. Polymeric Nanoparticles with Neglectable Protein Corona. *Small* **2020**, *16* (18), 1907574.
(95) Li, J.; Ge, J.; Yin, Y.; Zhong, W. Multiplexed Affinity-Based Protein Complex Purification. *Anal. Chem.* **2008**, *80* (18), 7068−7074.
(96) Pollastrini, J.; Dillon, T. M.; Bondarenko, P.; Chou, R. Y.-T. Field flow fractionation for assessing neonatal Fc receptor and Fcγ receptor binding to monoclonal antibodies in solution. *Anal. Biochem.* **2011**, *414* (1), 88−98.
(97) Onyema, H. N.; Berger, M.; Musyanovych, A.; Bantz, C.; Maskos, M.; Freese, C. Uptake of polymeric nanoparticles in a human induced pluripotent stem cell-based blood-brain barrier model: Impact of size, material, and protein corona. *Biointerphases* **2021**, *16* (2), 021004.
(98) Marin, A.; Kethanapalli, S. H.; Andrianov, A. K. Immunopotentiating Polyphosphazene Delivery Systems: Supramolecular Self-Assembly and Stability in the Presence of Plasma Proteins. *Mol. Pharmaceutics* **2024**, *21* (2), 791−800.
(99) Zhang, Z.; Ren, J.; Dai, W.; Zhang, H.; Wang, X.; He, B.; Zhang, Q. Fast and Dynamic Mapping of the Protein Corona on Nanoparticle Surfaces by Photocatalytic Proximity Labeling. *Adv. mater.* **2023**, *35* (9), 2206636.
(100) Rhee, H.-W.; Zou, P.; Udeshi, N. D.; Martell, J. D.; Mootha, V. K.; Carr, S. A.; Ting, A. Y. Proteomic Mapping of Mitochondria in Living Cells via Spatially Restricted Enzymatic Tagging. *Science* **2013**, *339* (6125), 1328−1331.
(101) Pattipeiluhu, R.; Crielaard, S.; Klein-Schiphorst, I.; Florea, B. I.; Kros, A.; Campbell, F. Unbiased Identification of the Liposome Protein Corona using Photoaffinity-based Chemoproteomics. *ACS Central Science* **2020**, *6* (4), 535−545.
(102) Wang, C.; Chen, B.; He, M.; Hu, B. Composition of Intracellular Protein Corona around Nanoparticles during Internalization. *ACS Nano* **2021**, *15* (2), 3108−3122.
(103) Vasilescu, J.; Guo, X.; Kast, J. Identification of protein-protein interactions using in vivo cross-linking and mass spectrometry. *Proteomics* **2004**, *4* (12), 3845−3854.
(104) Neupert, S.; Rubakhin, S. S.; Sweedler, J. V. Targeted Single-Cell Microchemical Analysis: MS-Based Peptidomics of Individual Paraformaldehyde-Fixed and Immunolabeled Neurons. *Chemistry & Biology* **2012**, *19* (8), 1010−1019.
(105) Mohammad-Beigi, H.; Hayashi, Y.; Zeuthen, C. M.; Eskandari, H.; Scavenius, C.; Juul-Madsen, K.; Vorup-Jensen, T.; Enghild, J. J.; Sutherland, D. S. Mapping and identification of soft corona proteins at nanoparticles and their impact on cellular association. *Nat. Commun.* **2020**, *11* (1), 4535.
(106) Chu, Y.; Tang, W.; Zhang, Z.; Li, C.; Qian, J.; Wei, X.; Ying, T.; Lu, W.; Zhan, C. Deciphering Protein Corona by scFv-Based Affinity Chromatography. *Nano Lett.* **2021**, *21* (5), 2124−2131.
(107) Baimanov, D.; Wang, J.; Zhang, J.; Liu, K.; Cong, Y.; Shi, X.; Zhang, X.; Li, Y.; Li, X.; Qiao, R.; Zhao, Y.; Zhou, Y.; Wang, L.; Chen,

C. In situ analysis of nanoparticle soft corona and dynamic evolution. *Nat. Commun.* **2022**, *13* (1), 5389.
(108) Gebauer, J. S.; Malissek, M.; Simon, S.; Knauer, S. K.; Maskos, M.; Stauber, R. H.; Peukert, W.; Treuel, L. Impact of the Nanoparticle-Protein Corona on Colloidal Stability and Protein Structure. *Langmuir* **2012**, *28* (25), 9673−9679.
(109) Walczyk, D.; Bombelli, F. B.; Monopoli, M. P.; Lynch, I.; Dawson, K. A. What the Cell "Sees" in Bionanoscience. *J. Am. Chem. Soc.* **2010**, *132* (16), 5761−5768.
(110) Walkey, C. D.; Olsen, J. B.; Song, F.; Liu, R.; Guo, H.; Olsen, D. W. H.; Cohen, Y.; Emili, A.; Chan, W. C. W. Protein Corona Fingerprinting Predicts the Cellular Interaction of Gold and Silver Nanoparticles. *ACS Nano* **2014**, *8* (3), 2439−2455.
(111) Mahmoudi, M. The need for robust characterization of nanomaterials for nanomedicine applications. *Nat. Commun.* **2021**, *12* (1), 5246.
(112) Nguyen, V. S.; Rouxel, D.; Vincent, B. Dispersion of nanoparticles: From organic solvents to polymer solutions. *Ultrason. Sonochem.* **2014**, *21* (1), 149−153.
(113) Nienhaus, K.; Nienhaus, G. U. Brownian motion-based nanoparticle sizing—A powerful approach for in situ analysis of nanoparticle-protein interactions. *Biointerphases* **2020**, *15* (6), 061201.
(114) Ortiz-Orruño, U.; Quidant, R.; van Hulst, N. F.; Liebel, M.; Ortega Arroyo, J. Simultaneous Sizing and Refractive Index Analysis of Heterogeneous Nanoparticle Suspensions. *ACS Nano* **2023**, *17* (1), 221−229.
(115) Nienhaus, G. U.; Maffre, P.; Nienhaus, K. Studying the protein corona on nanoparticles by FCS. *Methods Enzymol.* **2013**, *519*, 115−137.
(116) Shang, L.; Nienhaus, G. U. In Situ Characterization of Protein Adsorption onto Nanoparticles by Fluorescence Correlation Spectroscopy. *Acc. Chem. Res.* **2017**, *50* (2), 387−395.
(117) Lin, X.; Pan, Q.; He, Y. *In situ* detection of protein corona on single particle by rotational diffusivity. *Nanoscale* **2019**, *11* (39), 18367−18374.
(118) Markhali, B. P.; Sriram, M.; Bennett, D. T.; Khiabani, P. S.; Hoque, S.; Tilley, R. D.; Bakthavathsalam, P.; Gooding, J. J. Single particle detection of protein molecules using dark-field microscopy to avoid signals from nonspecific adsorption. *Biosens. Bioelectron.* **2020**, *169*, 112612.
(119) Tang, Q. Application of dark-field microscopy for detection of DNA and protein. *Highlights Sci. Eng. Technol.* **2022**, *3*, 123−129.
(120) Li, Q.; Peng, M.; Wang, C.; Li, C.; Li, K.; Lin, Y. Sensitive Sulfide Monitoring in Live Cells by Dark-Field Microscopy Based on the Formation of Ag_2S on Au@AgI Core-Shell Nanoparticles. *ACS Sustainable Chem. Eng.* **2019**, *7* (24), 19338−19343.
(121) Ciobanu, V.; Roncari, F.; Ceccone, G.; Braniste, T.; Ponti, J.; Bogni, A.; Guerrini, G.; Cassano, D.; Colpo, P.; Tiginyanu, I. Protein-corona formation on aluminum doped zinc oxide and gallium nitride nanoparticles. *J. Appl. Biomater. Funct. Mater.* **2022**, *20*, 228080002211318.
(122) Murray, R. A.; Escobar, A.; Bastús, N. G.; Andreozzi, P.; Puntes, V.; Moya, S. E. Fluorescently labelled nanomaterials in nanosafety research: Practical advice to avoid artefacts and trace unbound dye. *NanoImpact* **2018**, *9*, 102−113.
(123) Xu, M.; Soliman, M. G.; Sun, X.; Pelaz, B.; Feliu, N.; Parak, W. J.; Liu, S. How Entanglement of Different Physicochemical Properties Complicates the Prediction of in Vitro and in Vivo Interactions of Gold Nanoparticles. *ACS Nano* **2018**, *12* (10), 10104−10113.
(124) Carril, M.; Padro, D.; Del Pino, P.; Carrillo-Carrion, C.; Gallego, M.; Parak, W. J. In situ detection of the protein corona in complex environments. *Nat. Commun.* **2017**, *8* (1), 1542.
(125) Padro, D.; Cienskowski, P.; Lopez-Fernandez, S.; Chakraborty, I.; Carrillo-Carrion, C.; Feliu, N.; Parak, W. J.; Carril, M. Toward Diffusion Measurements of Colloidal Nanoparticles in Biological Environments by Nuclear Magnetic Resonance. *Small* **2020**, *16* (36), 2001160.
(126) Assfalg, M.; Ragona, L.; Pagano, K.; D'Onofrio, M.; Zanzoni, S.; Tomaselli, S.; Molinari, H. The study of transient protein-nanoparticle interactions by solution NMR spectroscopy. *Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics* **2016**, *1864* (1), 102−114.
(127) Randika Perera, Y.; Hill, R. A.; Fitzkee, N. C. Protein Interactions with Nanoparticle Surfaces: Highlighting Solution NMR Techniques. *Isr. J. Chem.* **2019**, *59* (11−12), 962−979.
(128) Lin, W.; Insley, T.; Tuttle, M. D.; Zhu, L.; Berthold, D. A.; Kral, P.; Rienstra, C. M.; Murphy, C. J. Control of protein orientation on gold nanoparticles. *journal of physical chemistry. C, Nanomaterials and interfaces* **2015**, *119* (36), 21035−21043.
(129) Gianneli, M.; Polo, E.; Lopez, H.; Castagnola, V.; Aastrup, T.; Dawson, K. A. Label-free in-flow detection of receptor recognition motifs on the biomolecular corona of nanoparticles. *Nanoscale* **2018**, *10* (12), 5474−5481.
(130) Aramesh, M.; Shimoni, O.; Ostrikov, K.; Prawer, S.; Cervenka, J. Surface charge effects in protein adsorption on nanodiamonds. *Nanoscale* **2015**, *7* (13), 5726−5736.
(131) Wang, X.; Wang, M.; Lei, R.; Zhu, S. F.; Zhao, Y.; Chen, C. Chiral Surface of Nanoparticles Determines the Orientation of Adsorbed Transferrin and Its Interaction with Receptors. *ACS Nano* **2017**, *11* (5), 4606−4616.
(132) Clemments, A. M.; Botella, P.; Landry, C. C. Spatial Mapping of Protein Adsorption on Mesoporous Silica Nanoparticles by Stochastic Optical Reconstruction Microscopy. *J. Am. Chem. Soc.* **2017**, *139* (11), 3978−3981.
(133) Feiner-Gracia, N.; Beck, M.; Pujals, S.; Tosi, S.; Mandal, T.; Buske, C.; Linden, M.; Albertazzi, L. Super-Resolution Microscopy Unveils Dynamic Heterogeneities in Nanoparticle Protein Corona. *Small* **2017**, *13* (41), 1701631.
(134) Tan, X.; Welsher, K. Particle-by-Particle In Situ Characterization of the Protein Corona via Real-Time 3D Single-Particle-Tracking Spectroscopy**. *Angew. Chem., Int. Ed.* **2021**, *60* (41), 22359−22367.
(135) Wang, Y.; Soto Rodriguez, P. E. D.; Woythe, L.; Sánchez, S.; Samitier, J.; Zijlstra, P.; Albertazzi, L. Multicolor Super-Resolution Microscopy of Protein Corona on Single Nanoparticles. *ACS Appl. Mater. Interfaces* **2022**, *14* (33), 37345−37355.
(136) Lo Giudice, M. C.; Herda, L. M.; Polo, E.; Dawson, K. A. In situ characterization of nanoparticle biomolecular interactions in complex biological media by flow cytometry. *Nat. Commun.* **2016**, *7* (1), 13475.
(137) Srivastava, I.; Khan, M. S.; Dighe, K.; Alafeef, M.; Wang, Z.; Banerjee, T.; Ghonge, T.; Grove, L. M.; Bashir, R.; Pan, D. On-Chip Electrical Monitoring of Real-Time "Soft" and "Hard" Protein Corona Formation on Carbon Nanoparticles. *Small Methods* **2020**, *4* (7), 2000099.
(138) Weiss, A. C. G.; Krüger, K.; Besford, Q. A.; Schlenk, M.; Kempe, K.; Förster, S.; Caruso, F. In Situ Characterization of Protein Corona Formation on Silica Microparticles Using Confocal Laser Scanning Microscopy Combined with Microfluidics. *ACS Appl. Mater. Interfaces* **2019**, *11* (2), 2459−2469.
(139) Dolci, M.; Wang, Y.; Nooteboom, S. W.; Soto Rodriguez, P. E. D.; Sánchez, S.; Albertazzi, L.; Zijlstra, P. Real-Time Optical Tracking of Protein Corona Formation on Single Nanoparticles in Serum. *ACS Nano* **2023**, *17* (20), 20167−20178.
(140) Qu, S.; Sun, F.; Qiao, Z.; Li, J.; Shang, L. In Situ Investigation on the Protein Corona Formation of Quantum Dots by Using Fluorescence Resonance Energy Transfer. *Small* **2020**, *16* (21), 1907633.
(141) Gao, L.-X.; Hao, H.; Yu, Y.-Q.; Chen, J.-L.; Chen, W.-Q.; Gong, Z.-D.; Liu, Y.; Jiang, F.-L. Protein Labeling Facilitates the Understanding of Protein Corona Formation via Fluorescence Resonance Energy Transfer and Fluorescence Correlation Spectroscopy. *Langmuir* **2023**, *39* (43), 15275−15284.
(142) Chen, D.; Parayath, N.; Ganesh, S.; Wang, W.; Amiji, M. The role of apolipoprotein- and vitronectin-enriched protein corona on lipid nanoparticles for in vivo targeted delivery and transfection of

https://doi.org/10.1021/cbe.4c00105
Chem Bio Eng. 2024, 1, 757−772

oligonucleotides in murine tumor models. *Nanoscale* **2019**, *11* (40), 18806−18824.
(143) Sanchez-Guzman, D.; Giraudon-Colas, G.; Marichal, L.; Boulard, Y.; Wien, F.; Degrouard, J.; Baeza-Squiban, A.; Pin, S.; Renault, J. P.; Devineau, S. *In Situ* Analysis of Weakly Bound Proteins Reveals Molecular Basis of Soft Corona Formation. *ACS Nano* **2020**, *14* (7), 9073−9088.
(144) Zhang, Y.; Zhang, L.; Cai, C.; Zhang, J.; Lu, P.; Shi, N.; Zhu, W.; He, N.; Pan, X.; Wang, T.; Feng, Z. In situ study of structural changes: Exploring the mechanism of protein corona transition from soft to hard. *J. Colloid Interface Sci.* **2024**, *654* (B), 935−944.
(145) Ge, C.; Du, J.; Zhao, L.; Wang, L.; Liu, Y.; Li, D.; Yang, Y.; Zhou, R.; Zhao, Y.; Chai, Z.; Chen, C. Binding of blood proteins to carbon nanotubes reduces cytotoxicity. *Proc. Natl. Acad. Sci. U. S. A.* **2011**, *108* (41), 16968−16973.
(146) Lu, X.; Xu, P.; Ding, H.-M.; Yu, Y.-S.; Huo, D.; Ma, Y.-Q. Tailoring the component of protein corona via simple chemistry. *Nat. Commun.* **2019**, *10* (1), 4520.
(147) Baimanov, D.; Wu, J.; Chu, R.; Cai, R.; Wang, B.; Cao, M.; Tao, Y.; Liu, J.; Guo, M.; Wang, J.; Yuan, X.; Ji, C.; Zhao, Y.; Feng, W.; Wang, L.; Chen, C. Immunological Responses Induced by Blood Protein Coronas on Two-Dimensional MoS2 Nanosheets. *ACS Nano* **2020**, *14* (5), 5529−5542.
(148) Theodorou, A.; Liarou, E.; Haddleton, D. M.; Stavrakaki, I. G.; Skordalidis, P.; Whitfield, R.; Anastasaki, A.; Velonia, K. Protein-polymer bioconjugates via a versatile oxygen tolerant photoinduced controlled radical polymerization approach. *Nat. Commun.* **2020**, *11* (1), 1486.
(149) Cai, R.; Ren, J.; Ji, Y.; Wang, Y.; Liu, Y.; Chen, Z.; Farhadi Sabet, Z.; Wu, X.; Lynch, I.; Chen, C. Corona of Thorns: The Surface Chemistry-Mediated Protein Corona Perturbs the Recognition and Immune Response of Macrophages. *ACS Appl. Mater. Interfaces* **2020**, *12* (2), 1997−2008.
(150) Caracciolo, G.; Palchetti, S.; Digiacomo, L.; Chiozzi, R. Z.; Capriotti, A. L.; Amenitsch, H.; Tentori, P. M.; Palmieri, V.; Papi, M.; Cardarelli, F.; Pozzi, D.; Laganà, A. Human Biomolecular Corona of Liposomal Doxorubicin: The Overlooked Factor in Anticancer Drug Delivery. *ACS Appl. Mater. Interfaces* **2018**, *10* (27), 22951−22962.
(151) Zhou, P.; Guo, M.; Cui, X. Effect of food on orally-ingested titanium dioxide and zinc oxide nanoparticle behaviors in simulated digestive tract. *Chemosphere* **2021**, *268*, 128843.
(152) Bekdemir, A.; Stellacci, F. A centrifugation-based physico-chemical characterization method for the interaction between proteins and nanoparticles. *Nat. Commun.* **2016**, *7*, 13121.
(153) Perez-Potti, A.; Lopez, H.; Pelaz, B.; Abdelmonem, A.; Soliman, M. G.; Schoen, I.; Kelly, P. M.; Dawson, K. A.; Parak, W. J.; Krpetic, Z.; Monopoli, M. P. In depth characterisation of the biomolecular coronas of polymer coated inorganic nanoparticles with differential centrifugal sedimentation. *Sci. Rep* **2021**, *11* (1), 6443.
(154) Ren, J.; Cai, R.; Wang, J.; Daniyal, M.; Baimanov, D.; Liu, Y.; Yin, D.; Liu, Y.; Miao, Q.; Zhao, Y.; Chen, C. Precision Nanomedicine Development Based on Specific Opsonization of Human Cancer Patient-Personalized Protein Coronas. *Nano Lett.* **2019**, *19* (7), 4692−4701.
(155) Qin, M.; Zhang, J.; Li, M.; Yang, D.; Liu, D.; Song, S.; Fu, J.; Zhang, H.; Dai, W.; Wang, X.; Wang, Y.; He, B.; Zhang, Q. Proteomic analysis of intracellular protein corona of nanoparticles elucidates nano-trafficking network and nano-bio interactions. *Theranostics* **2020**, *10* (3), 1213−1229.
(156) Bradford, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **1976**, *72* (1−2), 248−254.
(157) Spreen, H.; Behrens, M.; Mulac, D.; Humpf, H.-U.; Langer, K. Identification of main influencing factors on the protein corona composition of PLGA and PLA nanoparticles. *Eur. J. Pharm. Biopharm.* **2021**, *163*, 212−222.
(158) Zhao, Z.; Li, G.; Liu, Q. S.; Liu, W.; Qu, G.; Hu, L.; Long, Y.; Cai, Z.; Zhao, X.; Jiang, G. Identification and interaction mechanism of protein corona on silver nanoparticles with different sizes and the cellular responses. *J. Hazard. Mater.* **2021**, *414*, 125582.
(159) Ashkarran, A. A.; Ghavami, M.; Aghaverdi, H.; Stroeve, P.; Mahmoudi, M. Bacterial Effects and Protein Corona Evaluations: Crucial Ignored Factors in the Prediction of Bio-Efficacy of Various Forms of Silver Nanoparticles. *Chem. Res. Toxicol.* **2012**, *25* (6), 1231−1242.
(160) Walkey, C. D.; Olsen, J. B.; Guo, H.; Emili, A.; Chan, W. C. W. Nanoparticle Size and Surface Chemistry Determine Serum Protein Adsorption and Macrophage Uptake. *J. Am. Chem. Soc.* **2012**, *134* (4), 2139−2147.
(161) Pinals, R. L.; Yang, D.; Lui, A.; Cao, W.; Landry, M. P. Corona Exchange Dynamics on Carbon Nanotubes by Multiplexed Fluorescence Monitoring. *J. Am. Chem. Soc.* **2020**, *142* (3), 1254−1264.
(162) Ferdosi, S.; Tangeysh, B.; Brown, T. R.; Everley, P. A.; Figa, M.; McLean, M.; Elgierari, E. M.; Zhao, X.; Garcia, V. J.; Wang, T.; Chang, M. E. K.; Riedesel, K.; Chu, J.; Mahoney, M.; Xia, H.; O'Brien, E. S.; Stolarczyk, C.; Harris, D.; Platt, T. L.; Ma, P.; Goldberg, M.; Langer, R.; Flory, M. R.; Benz, R.; Tao, W.; Cuevas, J. C.; Batzoglou, S.; Blume, J. E.; Siddiqui, A.; Hornburg, D.; Farokhzad, O. C. Engineered nanoparticles enable deep proteomics studies at scale by leveraging tunable nano-bio interactions. *Proc. Natl. Acad. Sci. U. S. A.* **2022**, *119* (11), No. e2106053119.
(163) Wolf, M.; Poupardin, R. W.; Ebner-Peking, P.; Andrade, A. C.; Blochl, C.; Obermayer, A.; Gomes, F. G.; Vari, B.; Maeding, N.; Eminger, E.; Binder, H. M.; Raninger, A. M.; Hochmann, S.; Brachtl, G.; Spittler, A.; Heuser, T.; Ofir, R.; Huber, C. G.; Aberman, Z.; Schallmoser, K.; Volk, H. D.; Strunk, D. A functional corona around extracellular vesicles enhances angiogenesis, skin regeneration and immunomodulation. *J. Extracell Vesicles* **2022**, *11* (4), No. e12207.
(164) Cai, X.; Ramalingam, R.; Wong, H. S.; Cheng, J.; Ajuh, P.; Cheng, S. H.; Lam, Y. W. Characterization of carbon nanotube protein corona by using quantitative proteomics. *Nanomedicine: nanotechnology, biology, and medicine* **2013**, *9* (5), 583−593.
(165) Herda, L. M.; Hristov, D. R.; Lo Giudice, M. C.; Polo, E.; Dawson, K. A. Mapping of Molecular Structure of the Nanoscale Surface in Bionanoparticles. *J. Am. Chem. Soc.* **2017**, *139* (1), 111−114.
(166) Liessi, N.; Maragliano, L.; Castagnola, V.; Bramini, M.; Benfenati, F.; Armirotti, A. Isobaric Labeling Proteomics Allows a High-Throughput Investigation of Protein Corona Orientation. *Anal. Chem.* **2021**, *93* (2), 784−791.
(167) Prozeller, D.; Morsbach, S.; Landfester, K. Isothermal titration calorimetry as a complementary method for investigating nanoparticle-protein interactions. *Nanoscale* **2019**, *11* (41), 19265−19273.
(168) Fleischer, C. C.; Payne, C. K. Secondary Structure of Corona Proteins Determines the Cell Surface Receptors Used by Nanoparticles. *J. Phys. Chem. B* **2014**, *118* (49), 14017−14026.
(169) Ren, X.; Li, M.; Chen, M.; Dai, Y.; Shi, T.; Zhao, Y.-L. Characterization of protein-conjugating kinetics based on localized surface plasmon resonance of the gold nanoparticle. *Spectrosc. Lett.* **2016**, *49* (6), 434−443.
(170) Barbir, R.; Pem, B.; Kalčec, N.; Kastner, S.; Podlesnaia, K.; Csáki, A.; Fritzsche, W.; VinkovićVrček, I. Application of Localized Surface Plasmon Resonance Spectroscopy to Investigate a Nano-Bio Interface. *Langmuir* **2021**, *37* (5), 1991−2000.
(171) Wang, M.; Fu, C.; Liu, X.; Lin, Z.; Yang, N.; Yu, S. Probing the mechanism of plasma protein adsorption on Au and Ag nanoparticles with FT-IR spectroscopy. *Nanoscale* **2015**, *7* (37), 15191−15196.
(172) Capomaccio, R.; Ojea Jimenez, I.; Colpo, P.; Gilliland, D.; Ceccone, G.; Rossi, F.; Calzolai, L. Determination of the structure and morphology of gold nanoparticle-HSA protein complexes. *Nanoscale* **2015**, *7* (42), 17653−17657.
(173) Szekeres, G. P.; Montes-Bayón, M.; Bettmer, J.; Kneipp, J. Fragmentation of Proteins in the Corona of Gold Nanoparticles As Observed in Live Cell Surface-Enhanced Raman Scattering. *Anal. Chem.* **2020**, *92* (12), 8553−8560.

https://doi.org/10.1021/cbe.4c00105
Chem Bio Eng. 2024, 1, 757−772

(174) Ashkarran, A. A.; Gharibi, H.; Voke, E.; Landry, M. P.; Saei, A. A.; Mahmoudi, M. Measurements of heterogeneity in proteomics analysis of the nanoparticle protein corona across core facilities. *Nat. Commun.* **2022**, *13* (1), 6610.
(175) Mahmoudi, M. The need for improved methodology in protein corona analysis. *Nat. Commun.* **2022**, *13* (1), 49.
(176) Boselli, L.; Castagnola, V.; Armirotti, A.; Benfenati, F.; Pompa, P. P. Biomolecular Corona of Gold Nanoparticles: The Urgent Need for Strong Roots to Grow Strong Branches. *Small* **2024**, *20* (15), 2306474.

https://doi.org/10.1021/cbe.4c00105
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