The Dual Role of Protein Corona in Nano-based Cancer Theranostics

Jiewen Zheng†

School of Pharmacy Queen's University Belfast Belfast Northern Ireland, UK

†jzheng10@qub.ac.uk

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Abstract: Based on the unique advantages of nanomaterials, they have been considered as attractive biomedical devices in cancer nanomedicine. When exposed to the biological milieu, nanoparticles would inevitably interact with biomolecules and generate a protein coating layer called the 'protein corona'. Protein corona has been proven to be decisive in nanoparticle biological fate and following cellular responses by enriching low abundant proteins in the biological fluid. However, the comprehension of nanoparticle-protein corona complex still remains insufficient, which would result in the limitation of nano-based therapeutics development or even induce cytotoxicity to normal cells. This paper mainly focuses on protein corona's dynamic properties and intracellular mechanisms in nano-bio interactions and its applications in cancer nanomedicine, including targeted drug delivery, biomarker discovery, and personalized precision medicine. Understanding the specific role of protein corona would advance the discovery of potential drug targets and the design of novel nano-based theranostics strategies. Thereafter, the modification and control of the nanoparticle surface and the components of the protein corona are supposed to provide high-precision detection and promising therapeutic effects to predict and enhance cancer treatment.

1. Introduction

The emergence of nano-biotechnology has greatly inspired the development of cancer nanomedicine to overcome the intrinsic limitations of conventional cancer therapeutics [1]. The versatile functions and tunable physicochemical properties of nanoparticles (NPs) have been developed as imaging and diagnostic devices and drug nanocarriers for further targeted drug development in cancer treatment [2]. Ideally, the NPs would circulate in the biological fluid and subsequently combine with the target receptors once injected into the human body. However, when the NPs enter into a biological environment, they will interact with the biological components and generate a biomolecular layer on the surface, namely the protein corona (PC), which would determine the biological fate of the NPs, including circulation half-life, tumor accumulation, and tumor internalization [3].

The composition of PC is in a dynamic change. It mainly consists of a relatively stable 'hard' corona and a rapidly altered 'soft' corona of protein molecules, as is shown in Figure 1 [3]. Typically, both of the physicochemical properties of NPs, including NP size, surface properties, and exposure time, and the biological environment, such as pH value, temperature, and plasma concentration, would result in the difference of PCs [4]. Consequently, the cellular uptake and targeting efficiency, control of drug release, and NP biocompatibility are all associated with the PC properties. Therefore, understanding the composition and characteristics of protein corona and the process of nano-bio interactions is of great importance to further investigate the NPs behaviours in bio-milieu and boost the design of novel nano-based cancer therapeutics and diagnosis (Figure 1).

Herein, PCs' dynamic properties, molecular mechanisms, and applications are elucidated to provide a systemic comprehension of nano-bio interactions that would benefit future cancer nanomedicine.

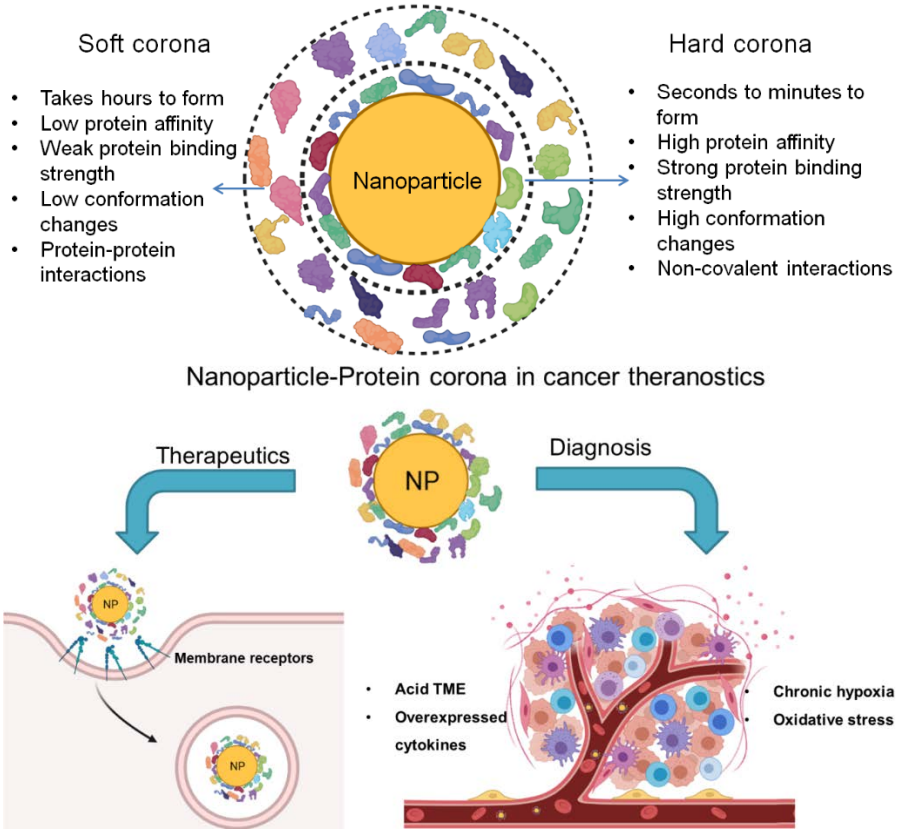

Figure 1. Protein coronas in cancer nanomedicine. TME: tumor microenvironment.

2. Mechanistic understanding of protein corona in nano-bio interactions

2.1. Dynamic formation and composition of the protein corona

The process of the plasma proteins' adsorption to the NP surface, according to the incubation time, protein abundance, and surface properties, is termed to be the 'Vroman effect' [5]. According to the 'Vroman effect', the NP-PC complex formation is a dynamic process. It may vary depending on the protein conformational change, affinity to the NP surface, and protein-protein interactions (PPI) [6]. In this process, the multi-layered PC is divided into two parts, the 'hard' corona and the 'soft' corona. The long-lived hard corona is likely to be consisted of proteins with higher affinity to form a tight, stable biomolecular coat on a short time scale. It would play an important role in further intracellular processing [7]. However, the loosely structured soft corona, which is demonstrated to constitute proteins with less affinity for NP surface, undergoes a constant exchange and would be mainly mediated via the PPI rather than the direct NP-PC interaction [8]. After the initial phase, the soft corona's absorbed protein shell would be replaced by proteins with higher affinity but low plasma abundance to achieve a longer residence time.

Except for time evolution and protein affinity, it has been reported that not only the physicochemical properties, such as the NP size, surface charge, morphology, and hydrophobicity but also the non-covalent interactions would influence the protein adsorption and subsequently result in the kinetic and thermodynamic differences of PCs [9].

Although PC composition is quite complicated and would vary according to the different initial biological environments, the 'adsorbome' would be unique for each type of NPs [10]. Typically, the PC composition, such as apolipoproteins, complement factors, immunoglobulins (IGs), and other assorted proteins, could be categorized into opsonins and dysopsonins [1]. For example, the opsonin, composed of IGs, complement factors, and coagulation proteins, would induce an immune response and trigger the presentation to the mononuclear phagocyte system (MPS) [11]. Besides, dysopsonin, which mainly consists of apolipoproteins and albumin, has been demonstrated to improve NP stealth and result in prolonged circulation time [12].

Furthermore, to qualify and quantify the PC composition, analytical techniques have been applied in many studies. For instance, spectroscopic methods, light scattering techniques, and separation techniques have been widely used for PC evaluation [13]. Also, to overcome the drawbacks of conventional cellular experiments, large-scale proteomics-based approaches and high-throughput screening models have been established for further nano-based drug development [4].

2.2. Protein coronas affect the nanoparticle biological fate

There has been no consensus on the specific role of the PC in regulating the biological fate of NPs, due to the complicated NP-PC composition, which would vary in different bio-environment. On the one hand, when treating lung cancer cells, the PC has been reported to 'screen' the transferrin-conjugated NPs to hinder the binding with the specific receptors, thus leading to the loss of the targeting capabilities [14]. On the other hand, in the study of bio-inspired liposomes for brain cancer treatment, the plasma protein apolipoprotein E (ApoE) in the PC layer could enable the NP to overcome the Blood-Brain Barrier (BBB) and promote the corona-mediated targeting efficiency for brain cancer therapy [15]. Therefore, PCs with various compositions would exhibit a dual character in regulating the functions of NPs, and more studies should concentrate on novel PC-based strategies development that would accumulate in the targeted cells or tissues in different diseases.

Moreover, during the recruitment, the PC has been demonstrated to regulate the cellular uptake, cell adhesion, cytotoxicity, and immunogenicity of NP, which would bring novel properties to the NP-PC complex and subsequently influence the NP fate [16]. It has been highlighted that the PC's varied physical and biological impacts on the polymer-based NPs and demonstrated that the nanogels bearing the corona layer would significantly improve the macrophage cytokine release.

2.3. Molecular mechanism of nanoparticle-protein interactions

Generally, the NP would interact with the cellular interface and get entered into the cells through several pathways, including phagocytosis, macropinocytosis, caveolae-dependent endocytosis, clathrin-dependent endocytosis, non-specific endocytosis, and multiple pathways, based on different NP physicochemical properties and cellular receptors (**Figure 2**) [17]. The endocytosis pathways always involve the initial adsorption and the following internalization steps, and the NP-PC complex is supposed to play an important role in these steps. For example, a cellular uptake alteration mechanism of polystyrene NPs in both serum-free and serum-containing solutions has been demonstrated [18]. Without the serum proteins, the two types of NPs would get inside the cells through endocytosis or macropinocytosis pathway. In contrast, the NPs internalization pathway would change into phagocytosis in the serum-containing environment. Also, the grafted molecules on the NP surface, such as the polyethylene-glycol (PEG) molecule and the modified chemical groups, would synergistically affect the recognition and subsequent uptake by various cells [19].

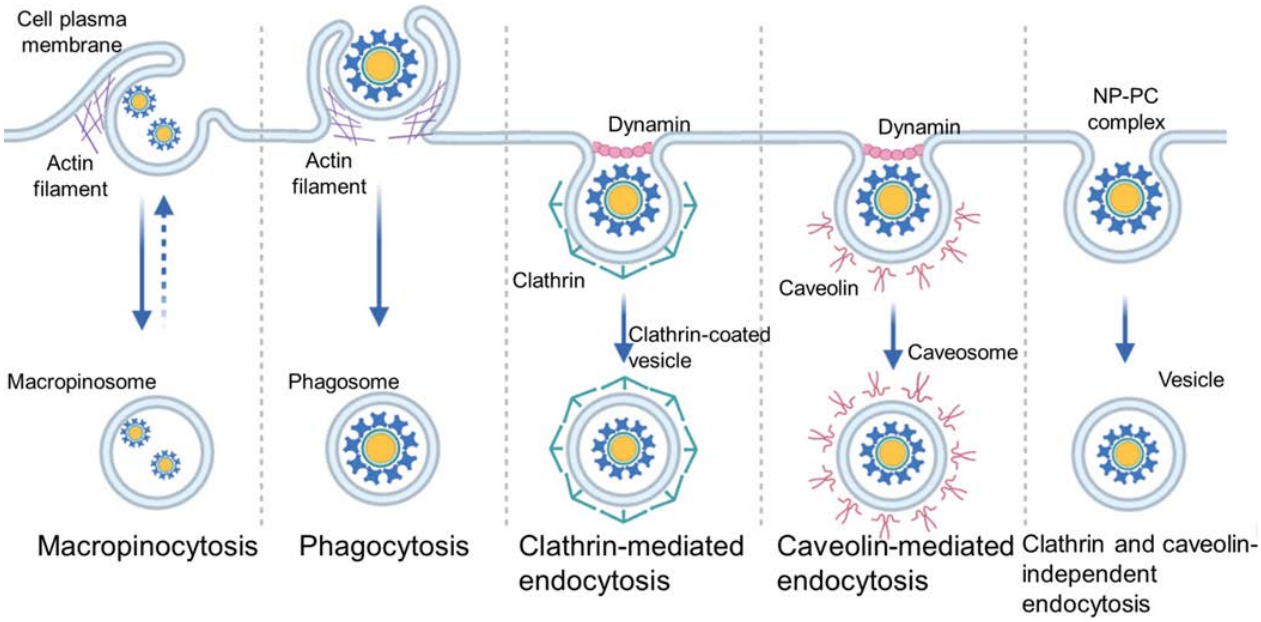

Figure 2. Schematic of the mechanisms of the NP-PC recognition and cell internalization

Furthermore, the secondary conformational alteration of corona protein has been indicated to be an essential factor in regulating the NP-PC and cell-surface interaction.

Fleischer and coworkers reported a novel NP internalization mechanism according to the opposite-charged NPs, leading to the difference in cellular receptors recognition [20]. In the bovine serum albumin (BSA) solution, the cationic NPs were more likely to be redirected to scavenger receptors, while the anionic NPs preferred the albumin receptors. This might be due to the misfolded and structural-changed proteins in the corona layer of the NP-BSA complex. Then, as a single complex with the corona layer, the NP has been transported through the cell membrane to reach the endosomes or lysosomes in a microtubule-dependent manner. The NPs would be subsequently secreted out of the cell via the exocytosis pathway or induce cell death.

During the interaction of the proteins in the PC and the cells, the composition remains stable and would not be easily displaced. Therefore, the PC could inspire the bio-mimetic strategy development to design advanced bio-inspired NPs and avoid immune recognition [10].

3. Nanoparticle-protein interactions in cancer nanomedicine

Nanomaterials have been widely used in the field of cancer nanomedicine according to the unique advantages, such as enhancing circulation time, increasing drug bioavailability, and reducing cytotoxicity to normal cells [1]. However, the protein layer would form around the NPs in vivo and thus influence the NP efficiency and following biological responses. Therefore, how the PC would affect the behavior of NPs and the application of PC manipulating the design of cancer nanomedicine should be depicted more properly.

3.1. Nano-based drug delivery for cancer therapeutics

The nano-based drug carriers are supposed to deliver therapeutic agents to the target cells or tissues according to the bio-functionalization of the NPs with bioactive molecules, including membrane receptor ligands, aptamers, and monoclonal antibodies (mAbs) [21]. In the in vivo recruitment, the identity of the PC has been reported to determine the ultimate localization of the nano-system. However, the specific role of PC in NP targeting still remains controversial.

The PC has been reported to display a dual role in the nano-based drug delivery system (DDS) [10]. For example, a novel nano-based DDS, utilizing MUC1 aptamers to functionalize the chitosan NPs, has been developed to treat human colon adenocarcinoma [22]. However, compared with the bare NPs, the results showed that protein adsorption at the NPs has significantly hampered the

targeting potentials of NPs, and thus reduced cytotoxicity to cancer cells. Additionally, another study also examined the influences of PC on NP targeting capabilities [23]. With the presence of the PC layer, the polymer-capsuled and core-shell NPs, functionalized with the humanized A33 mAb targeting the colon cancer cells, showed retained antibody-impelled cell antigen-binding efficiency, regardless of different human serum concentrations and PC compositions. These findings imply that the PC, as the novel identity of the NP, would restrict the behavior of the functional molecules and lead to the reduction of targeting efficiency and therefore affect the payload capacity of nano-based DDS.

On the contrary, the components in the PC would act as the potential receptors for further NP targeting delivery. Therefore, a thorough understanding of the role of the PC in the nano-bio interactions could provide promising information to overcome the limitations through redesigning and controlling the NPs to increase the binding specificity [24]. For instance, Ju et al. focused on developing an engineered hyaluronic acid (HA)-based capsule with metal-phenolic coating via the PC [25]. The results revealed that this particle showed a molecular weight (MW)-dependent manner in targeting delivery. The existence of the PC would significantly enhance the specific interaction between the HA capsules with high MW and the CD44 over-expressed cancer cells. Thus, the PC layer formed around the metal-phenolic coating could be potentially applied as a surface engineered tool for efficient drug delivery in the future.

Furthermore, a new PC precoating strategy has been established to improve the drug delivery efficiency of the nucleic acid drug-bearing chitosan-based nanocarriers [26]. The modified BSA with the cRGD peptide was used as the protein layer to coat on the redox-responsive chitosan-based NPs (TsR NPs), and the results demonstrated that the BSA-cRGD generated a stable corona on the nanocarrier to promote the stability, biocompatibility, intracellular uptake efficiency, and lysosomal escape, which would significantly enhance the antitumor activity of siRNA-loaded NPs (**Figure 3**). Also, the BSA-cRGD precoating strategy offered a possible way to avoid the influence of other plasma proteins on this nanocarrier.

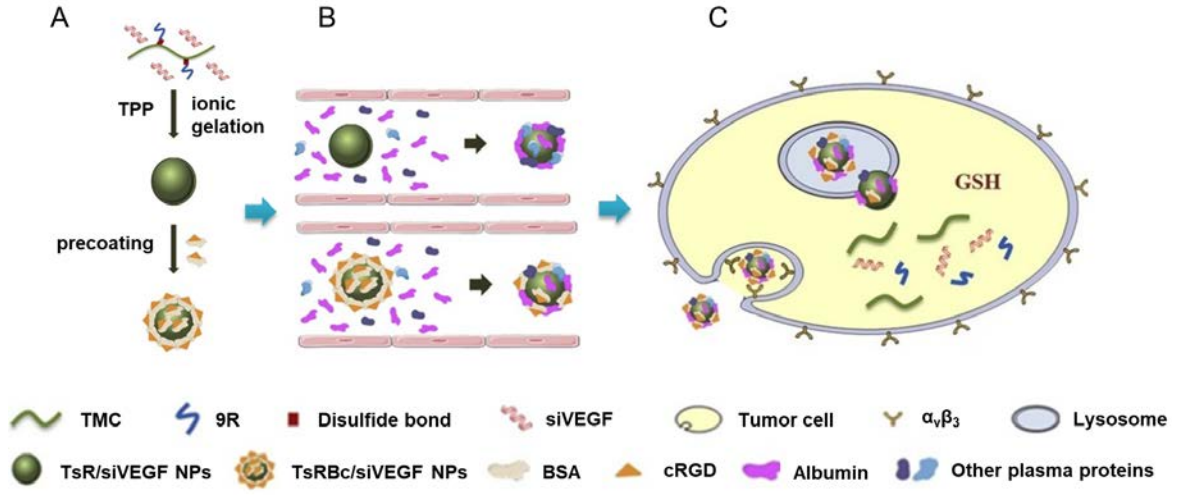

Figure 3. The PC-based nanocarrier design to improve the therapeutic effect and targeting ability [26].

A: NP synthesis and precoating; B: NP in the blood circulation will maintain targeting ability and reduce plasma protein adsorption; C: NPs improve target delivery efficiency and enhance the therapeutic effect. TPP: sodium tripolyphosphate, TMC: trimethyl chitosan, siVEGF: siRNA targeting vascular endothelial growth factor, TsRBc/siVEGF NPs: Bc-precoated TsR/siVEGF NPs, GSH: glutathione.

3.2. Biomarker discovery and cancer diagnosis

Nowadays, various nano-platforms, such as metal NPs, carbon nanotubes, and polymeric NPs, have been developed as the carrier for the detection of cancer biomarkers [27]. As has been discussed

previously, the PC would determine the biological fate of the NPs in vivo and enable them to become 'visible' for downstream analysis. Therefore, the characteristics of the NP-PC complex make it a potent platform to enrich low abundant proteins in the plasma and serum for downstream biomarker discovery and cancer diagnosis [28].

Based on the exploitation of the nano-bio interaction, researchers have further investigated cancer diagnosis strategies. Recently, it has been confirmed that the patients with various health conditions would show differences in the PC composition and function, even treated with the same NPs. Therefore, the new concept 'personalized protein corona' exhibited increasing importance in the field of PC-based cancer nanomedicine [29]. For instance, a useful diagnostic tool, combined with the haemoglobin level analysis, has been proposed for pancreatic ductal adenocarcinoma (PDAC) early detection upon the analysis of the PC formed around graphene oxide (GO) sheets in human plasma [30]. The PC compositions of the PDAC patients in this study were significantly discriminated in the proteins with the molecular weight from 20 to 30 kDa, and the apolipoprotein A1 (APOA1) was likely to be the most enriched component within this range. Also, APOA1 displayed a high affinity to the GO surface and thus could be recognized as a potential biomarker for PDAC.

Moreover, the fingerprint study of the PC from the breast and prostate cancer patients' plasma has been fully investigated to establish a cross-reactive sensor array with various lipid-based NP formulations [31]. The authors identified that the most discriminated protein components, including complement factors, tumor suppressors, and immune regulators, were associated with breast and prostate cancer's molecular and cellular patterns. Therefore, tumor-associated proteins, such as the PHLD and C1S, in the PC of cationic liposome could be treated as biomarkers for breast and prostate cancer diagnosis, respectively. The advancement of this novel NP-PC profiling strategy showed high potentials for clinical translation and enabled the cancer early detection through a simple blood test.

However, the PC would also impede the efficiency of the NPs and decrease their circulation time on specific occasions. Therefore, to avoid the generation of the corona layer, further design and modification of the NPs are of great importance. Within this situation, the novel modification method on ultra-small super-paramagnetic iron oxide NPs (USPIONs) has been discovered [32]. The NPs were modified with sulfobetaine or N, N-diethylaminopropylamine to form zwitterionic NPs (ZW-NPs), which showed low affinity to proteins in the plasma, and no cytotoxicity to normal cells. Overall, the ZW-NPs achieved the 'stealth' property and avoided rapid scavenging by the MPS, indicating that they would become excellent candidates for cancer imaging and diagnosis.

3.3. Mass spectrometry-based proteomic analysis in nano-bio interactions

To fully investigate the components and functions of the PCs, the analytical workflows, especially the mass-spectrometry (MS)-based proteomics PC fingerprint studies, have been widely conducted. Generally, after the protein components have been extracted from the NP-PC complex, they will be digested into peptide fragments, which would be subsequently characterized and identified through the liquid chromatography (LC) MS/MS analysis (**Figure 4**). Several labeling methods would be used during the separation process, including metabolic labeling and chemical labeling (isobaric tag for relative and absolute quantitation and isotope-coded protein label), for comparative quantitative proteomics analysis [33].

For example, a potent therapeutic target for ovarian cancer has been recently discovered by modulating PC formation around the gold NPs (AuNPs) [34]. Based on the MS-based proteomics analysis, the hepatoma-derived growth factor (HDGF), which has been detected in PC, has been confirmed to be overexpressed on the malignant ovarian cells and thus become a significant biomarker for ovarian cancer. Moreover, the proteomic fingerprinting of the PC on the PEGylated multi-walled carbon nanotubes (MWCNT) has been conducted based on MS analysis, which identified the role of both hard corona and soft corona in immuno-regulation as well as the maintenance of cellular homeostasis [35]. Accordingly, this PEGylated MWCNT could be developed as a promising candidate for future drug delivery.

Based on the widely-applied MS-based proteomics strategy, researchers have recently developed a novel strategy and try to overcome the limitations caused by the complicated plasma proteome and

workflows. A highly parallel protein quantitation platform has been designed to conduct efficient proteomic profiling on the PCs on superparamagnetic iron oxide NPs (SPIONs) for non-small cell lung cancer biomarker discovery [36]. This novel approach improved the depth of coverage and throughput of proteome quantification and enabled a more precise way to understand the unique interaction between proteins and engineered NPs. Based on this newly developed method, it is anticipated that more precise, convenient, and high-throughput strategies would emerge for PC-based cancer biomarker detection in the future.

Additionally, the usage of the MS-based strategies could be applied to further explore intracellular signaling pathways and downstream cellular responses, which would provide a deeper understanding of the NP cellular uptake and potential extra- /intracellular targets for novel therapeutic development according to the nano-bio interactions [33].

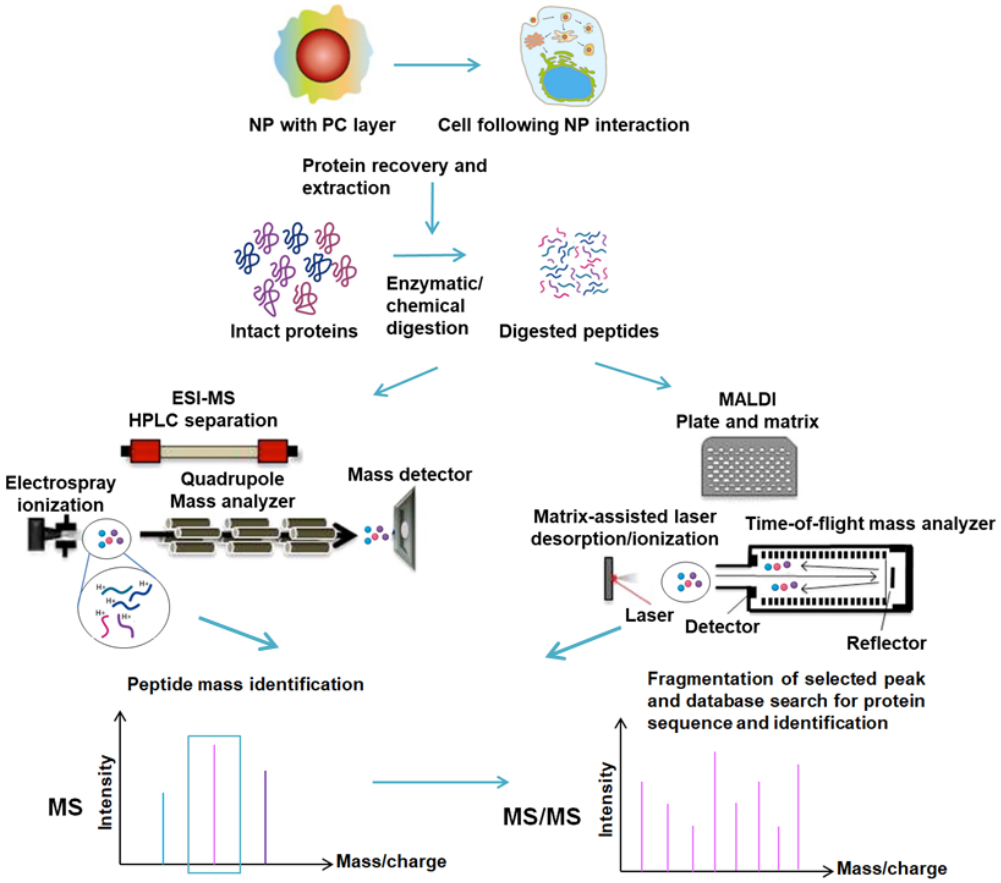

Figure 4. Schematic of the workflow showing the MS-based proteomics analysis on the protein corona in the nano-bio interactions [33]

4. Conclusion

In conclusion, this study summarized PC's dynamics properties and molecular mechanisms in nano-bio interactions and the theranostic applications of PC in cancer nanomedicine. It has been confirmed that the PC layer would exhibit a dual role in regulating the biological fate of nano-platforms and downstream biological responses. The structure and function of PC would vary according to the different physicochemical properties of NPs, natural parameters, and time evolution. Moreover, the heterogeneous biological environment, including different patients' health status and tumor types, has been reported to play an essential role in PC composition, which leads to the exploitation of personalized precision medicine based on nano-bio interactions. Thus, MS-based proteomics studies on the PC fingerprint have been widely applied in biomarker discovery to predict and improve cancer therapeutics.

The understanding of nano-bio interactions would significantly benefit the design of nano-based antiproliferative DDS and cancer diagnosis strategies. In the future, novel high-throughput technologies should get further developed to achieve an in-depth comprehension of the formation and composition of PC. Also, more investigations on PC-based novel nanomedicine for cancer treatment and diagnosis should be conducted by redesigning and modifying NPs to obtain more precise, faster, and cheaper approaches for targeting drug delivery and biomarker exploration.

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