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Techniques for the experimental investigation of the protein corona

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Due to its enormous relevance the corona formation of adsorbed proteins around nanoparticles is widely investigated. A comparison of different experimental techniques is given. Direct measurements of proteins, such as typically performed with mass spectrometry, will be compared with indirect analysis, in which instead information about the protein corona is gathered from changes in the properties of the nanoparticles. The type of measurement determines also whether before analysis purification from unbound excess proteins is necessary, which may change the equilibrium, or if measurements can be performed *in situ* without required purification. Pros and contras of the different methods will be discussed.

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Introduction

Frequently colloidal nanoparticles (NPs) adsorb proteins (and other (bio-)molecules) spontaneously on their surface when they are placed in biological fluids, forming the so-called protein corona (Figure 1) [1,2]. This is a dynamic process [3,4] governed by the binding affinities and equilibrium constants of each type of protein to the respective NP surface, and variations in the protein composition of biological fluids [5••]. The nature and composition of the corona can affect the fate, uptake, and performance of NPs in a biological context [6••,7,8]. Indeed, it may provide NPs with additional colloidal stability and reduced toxicity [9,10], influence the cellular uptake of NPs [11] or their *in vivo* circulating times [12], as it may interfere with receptor–ligand interactions [13].

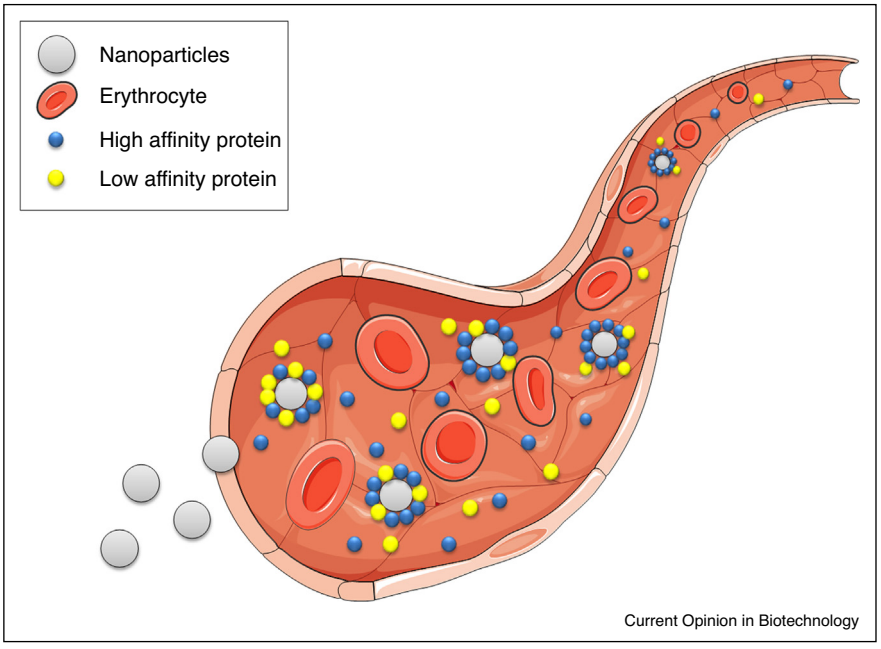
Hence, the protein corona is a subject of interest [14••,15,16,17•]. Concerning experimental analysis, the adsorbed proteins can be directly analyzed with mass spectrometry, circular dichroism, *etc*, or indirectly via measuring modifications in the NPs properties, such as changes in size. In both cases, some techniques allow for detection of the protein corona *in situ*, while others require the removal of unbound proteins before measurements, which may however change equilibrium properties [15].

Direct methods

Direct methods directly analyze the proteins that are adsorbed on the NP surface (Figure 2). Besides the proteins bound to the NP also free proteins in solution will be present, which may interfere in the read-out. Thus unbound proteins have to be removed before measurements, which however leaves the purified NPs in non-equilibrium [15]. Loosely bound proteins, sometimes referred to as 'soft corona' may be lost in the purification step, which would not be detected.

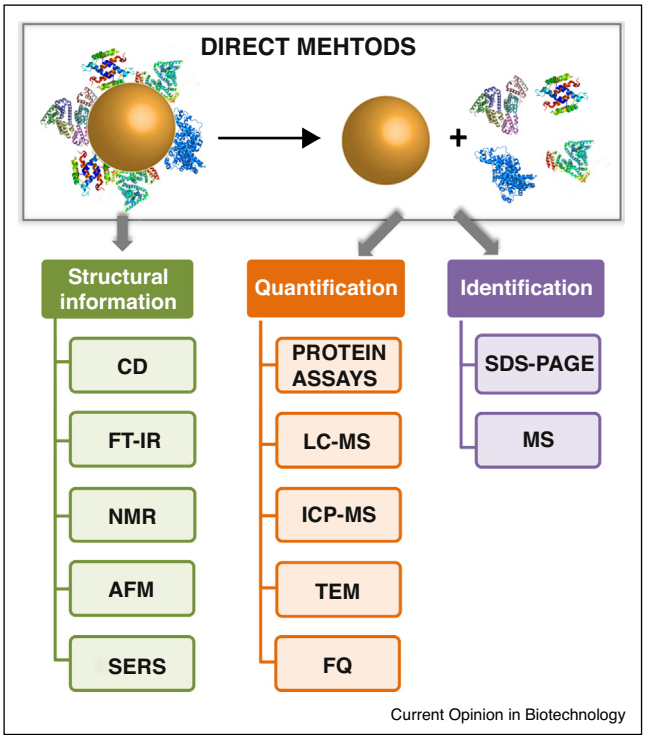
Adsorbed proteins can be directly visualized after negative staining with transmission electron microscopy (TEM) [14••]. While in this way adsorbed proteins can be directly detected, and thus the presence of proteins on the NP surface can be verified, this method so far could not be applied for quantitative analysis. For improved analysis, in order to study the amount of adsorbed proteins and the composition of the protein corona, the proteins typically need to be desorbed from the purified NPs. For a mere quantification of the amount of adsorbed proteins standard biochemical protein quantification assays, such as Bradford or bicinchoninic acid assay [9,18] can be applied. In the case of inorganic NPs, these studies can be further completed by inductively coupled plasma mass spectrometry (ICP-MS) which allows for the calculation of the total content of sulfur coming from cysteine residues of the proteins, as well as for the total content of metal coming from the NPs [19]. In this way the ICP-MS data provide the amount of NPs, which

Figure 1



Representation of protein corona formation. Modified from Servier Medical Art.

Figure 2



Summary of direct methods.

together with the results from the protein quantification assays yield the number of proteins *per* NP [20•].

Gel electrophoresis (GE) is a straightforward method to investigate the protein corona composition by using a protein molecular weight (M_w) standard marker to identify the different proteins by their apparent M_w. Frequently sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) systems are used, where proteins are denatured and disulfide bonds are partially digested prior to GE [21,22•,23,24]. In case the protein corona has been obtained from a complex mixture of proteins, such as those present in serum or plasma, GE can only provide qualitative information regarding the corona composition. Hence, this technique is often complemented with MS based proteomics to determine the identity of proteins [25,26]. Selected bands from SDS-PAGE are recovered from the gel and analyzed by tandem mass spectrometry (MS/MS), matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), or electrospray ionisation mass spectrometry (ESI-MS). By comparing the mass spectra of the recorded protein fragments with existing databases the composition of the protein corona can be obtained [27,28•,29•]. However, for a quantitative analysis yielding the absolute composition of proteins a chromatographic technique coupled with MS is required, such as liquid chromatography–mass spectrometry (LC–MS) [30,31]. Among these direct methods, TEM, GE, MS, and ICP–MS require purification from excess proteins and they