Kako proteinska korona određuje sudbinu nanočestica u biološkom okolišu

Capjak, Ivona; Šupraha Goreta, Sandra; Domazet Jurašin, Darija; Vinković Vrček, Ivana

Capjak I, et al. How protein coronas determine the fate of engineered nanoparticles in biological environment
Arh Hig Rada Toksikol 2017;68:245-253

245

How protein coronas determine the fate of engineered nanoparticles in biological environment

Ivona Capjak[1], Sandra Šupraha Goreta[2], Darija Domazet Jurašin[3], and Ivana Vinković Vrček[4]

Croatian Institute of Transfusion Medicine[1], University of Zagreb, Faculty of Pharmacy and Biochemistry[2], Ruđer Bošković Institute[3], Institute for Medical Research and Occupational Health[4], Zagreb, Croatia

Nanomedicine is a booming medical field that utilises nanoparticles (NPs) for the development of medicines, medical devices, and diagnostic tools. The behaviour of NPs *in vivo* may be quite complex due to their interactions with biological molecules. These interactions in biological fluids result in NPs being enveloped by dynamic protein coronas, which serve as an interface between NPs and their environment (blood, cell, tissue). How will the corona interact with this environment will depend on the biological, chemical, and physical properties of NPs, the properties of the proteins that make the corona, as well as the biological environment. This review summarises the main characteristics of protein corona and describes its dynamic nature. It also presents the most common analytical methods to study the corona, including examples of protein corona composition for the most common NPs used in biomedicine. This knowledge is necessary to design NPs that will create a corona with a desired efficiency and safety in clinical use.

KEY WORDS: *hard corona; nano-bio interface; nanomedicine; soft corona*

Nanomedicine is a growing medical field that utilises nanomaterials for new applications in medicine, including their clinical use in disease diagnosis and treatment (1-3). According to the European Commission (4), *'Nanomaterial' means a natural, incidental or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50 % or more of the particles in the number size distribution, one or more external dimensions is in the size range 1-100 nm*. However, in medicine the term *nanoparticle* includes particles with dimensions of up to 1000 nm.

Due to a large surface-area-to-volume ratio, nanoparticles (NPs) have exceptional functional and structural properties that make them suitable to carry many diagnostic and therapeutic agents (5). Recent advances in nanomedicine have resulted in the development of biodegradable nanodrug delivery systems, nanocrystals for magnetic resonance imaging (MRI), and luminescent NPs for multiplexed molecular diagnostics (1, 3, 5, 6).

Because of the small size, NPs can enter almost every part of the body, including tissues, organs, and organelles (mitochondria, lysosomes, and endosomes) by different routes (e.g., inhalation, ingestion, injection, or physical contact with cuts or wounds) (2, 3, 7-9).

There are many types of NPs, including polymeric NPs, liposomes, carbon nanotubes, quantum dots, or metal-based NPs (gold, silver iron oxide, silica, titanium dioxide, etc.). Owing to the exceptional properties, these NPs may be used for targeted drug and contrast delivery, photothermal therapy, optical sensing, biochromatography, bioanalytical electrochemistry, biocidal agents and coatings, or a variety of bioassays (1-3, 5).

Their distribution, excretion, metabolism, and pharmacokinetics may be quite complex and pose a challenge for developing safe and effective nano-based biomedical agents. One of the key issues to resolve is rapid NP uptake and clearance by the reticuloendothelial system (RES), active *vs.* passive targeting, and penetration into tumour tissues (5).

Even though thousands of research papers have already been published on the interaction between NPs and biological systems, little is still known about the mechanistic details of these interactions (5). What are the biological interfaces that facilitate the interaction between NPs and cell components? This question should be addressed from the perspective of colloidal chemistry (10).

Blood as a biological medium contains more than a thousand biomolecules like proteins, lipids, and nucleic acids (1, 10). As soon as NPs enter the medium, ions, small molecules, proteins, and cells compete to adsorb on the NP surface due to its high reactivity (11). Plasma proteins have a critical role in creating nano-bio interfaces, as they opsonise NPs and form coronas (5, 12-16).

What kind of a protein corona forms around an NP's surface will depend on the NP's properties (size, shape, composition, surface functional groups, and surface charges), biophysical properties of the biological medium (blood, interstitial fluid, or cell cytoplasm), and the time of interaction. In other words, how will proteins adsorb on

Correspondence to: Ivana Vinković Vrček, Institute for Medical Research and Occupational Health, Ksaverska cesta 2, 10000 Zagreb
E-mail: *ivinkovic@imi.hr*

246

Capjak I, et al. How protein coronas determine the fate of engineered nanoparticles in biological environment
Arh Hig Rada Toksikol 2017;68:245-253

NPs will depend not only on protein-NP interaction but also on protein-protein interactions.

Once formed, a protein corona will determine the physicochemical behaviour of an NP. Its properties are more important in determining the biological response (agglomeration, cellular uptake, circulation lifetime, signalling, kinetics, transport, accumulation, and toxicity) than NP's properties. In other words, to find out what will be the distribution, metabolism, and elimination of NPs in the body before it is applied in clinical practice, one needs to determine how protein corona affects them.

Knowing how to control the formation of the protein corona is crucial for most clinical uses of NPs (9, 10, 17, 18). This review summarises the current knowledge on the nano-bio interface between NPs and proteins.

Dynamic nature of protein corona

When NPs come in contact with biological components, a nano-bio interface is formed. What makes it dynamic is a number of physicochemical interactions and thermodynamic exchanges between NP and biomolecular surfaces (7, 19-21). The dynamic nature of the nano-bio interface between NPs and proteins is best described by *soft* and *hard* coronas (Figure 1). Proteins with higher affinity for NP surface will exchange easily and quickly forming the *hard* corona, while proteins with low affinity exchange slowly forming the *soft* corona (19). The *hard* corona proteins interact directly with NP surface, while the *soft corona* proteins interact with the hard corona proteins via weak protein-protein interactions. The time needed for corona formation differs between the *hard* and *soft* corona.

The *hard* corona is formed very quickly, within seconds or a minute, while the formation of the *soft* corona may take hours or even days, as proteins with higher affinity replace those with lower affinity (21). This process depends on protein concentrations and the composition of the biological environment.

Some suggest that even at low plasma concentrations, corona proteins will completely envelope the surface of an NP and modify its nature and physicochemical properties (19). Soft corona proteins can also interact with the hard corona proteins, as they desorb from NP surface and free the slot for other biomolecules to interact. All these exchanges are based on competitive adsorption and desorption of proteins, which depends on interaction time, protein concentrations, and their adsorption affinity for the NP. These exchanges, known as the *Vroman effect* (22, 23) have two stages. In the early stage, proteins adsorb rapidly with the highest association rates, and in the late stage proteins with short residence times are being replaced by proteins with slower association rates but longer residence times (24).

This dynamic nano-bio system is determined by hydrodynamic, electrostatic, electrodynamic, solvent, and steric interactions (Table 1) (25, 26).

This is why the nano-bio interface changes continuously in a biological environment (Figure 2), especially in the living cells, where different cell products are being secreted. When NPs move from one biological compartment to another, protein corona will change its profile. Some proteins form only transient complexes with NPs, while
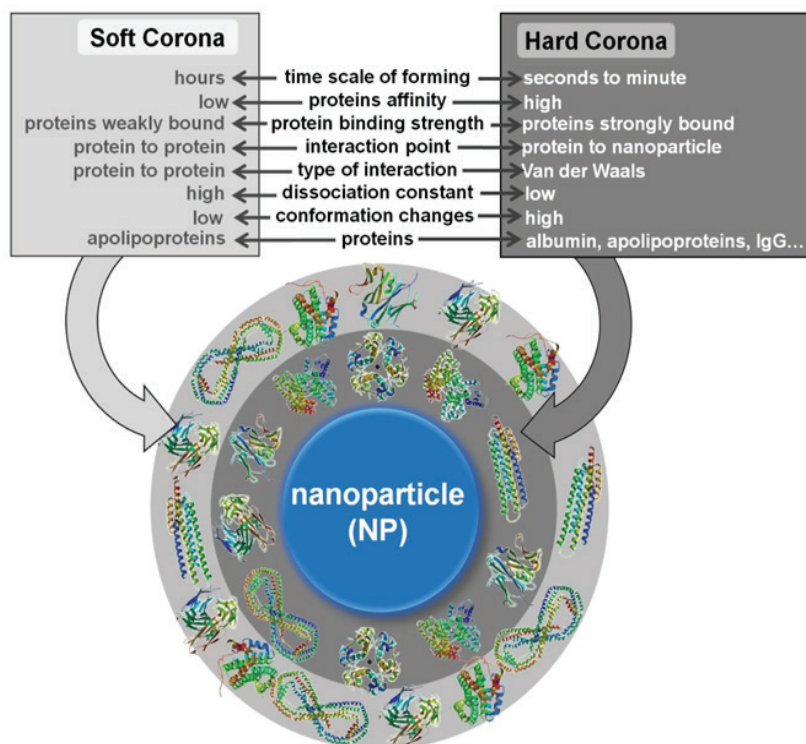

Soft Corona		Hard Corona
hours	time scale of forming	seconds to minute
low	proteins affinity	high
proteins weakly bound	protein binding strength	proteins strongly bound
protein to protein	interaction point	protein to nanoparticle
protein to protein	type of interaction	Van der Waals
high	dissociation constant	low
low	conformation changes	high
apolipoproteins	proteins	albumin, apolipoproteins, IgG...

Figure 1 *Nano-bio interface: properties of protein corona on NP surface*

Capjak I, et al. How protein coronas determine the fate of engineered nanoparticles in biological environment
Arh Hig Rada Toksikol 2017;68:245-253

247

Table 1 *Main forces at the nano-bio interface (26)*

Force	Range (nm)	Origin and properties
Hydrodynamic interactions	10^2-10^6	Long-range interactions; induced by particles moving in a viscous fluid, bulk transport, shear, lift, and Brownian diffusion; Increases the collision between NPs and other surfaces in the system
Electrostatic interactions	1-100	Coulomb interactions; induced by attraction or collision between charged interfaces and counter- or repel co-ions; characterized by the formation of an electrostatic double layer
Electrodynamic interactions	1-100	Van der Waals interactions that describe interactions between randomly oriented dipoles, between dipole and induced dipole, and fluctuating dipole and induced dipole
Solvent interactions	1-10	Interactions between lyophobic or lyophilic materials and solvent molecules
Steric interactions	1-100	Repulsive interactions with other interfaces; induced by adsorbed polymer layers on NPs surface; increase stability of individual NPs, but can interfere in cellular uptake

others will be tightly bound, depending on the specific NP type and the biological fluids in which NPs are suspended.

Protein adsorption on and interaction with NPs may induce conformational changes or crowding of proteins on the NPs surface or the formation of reactive oxygen species (ROS) that will cause oxidative damage to the adsorbed proteins (26). If a hydrophobic or charged protein sequence interacts with a hydrophobic or charged part of NP surface, this will induce thermodynamically favourable changes. Conformational changes of proteins induced by their interaction with NPs are typically irreversible (21) and may affect the downstream protein-protein interaction, cellular signalling, and DNA transcription, which directly affect enzyme activity (24). At the same time, protein binding that changes the shape, size, and surface charge of NPs will directly affect the agglomeration, cellular uptake, circulation lifetime, signalling, kinetics, transport, accumulation, and toxicity of NPs in a biological environment (10, 27).

A complex consisting of an NP and its protein corona is a new entity that cells can *see* (10, 28). For easier understanding, hard and soft coronas are usually presented as layers (Figure 1). The outer layer (soft corona) does not allow the inner layer (hard corona) to interact with the cell
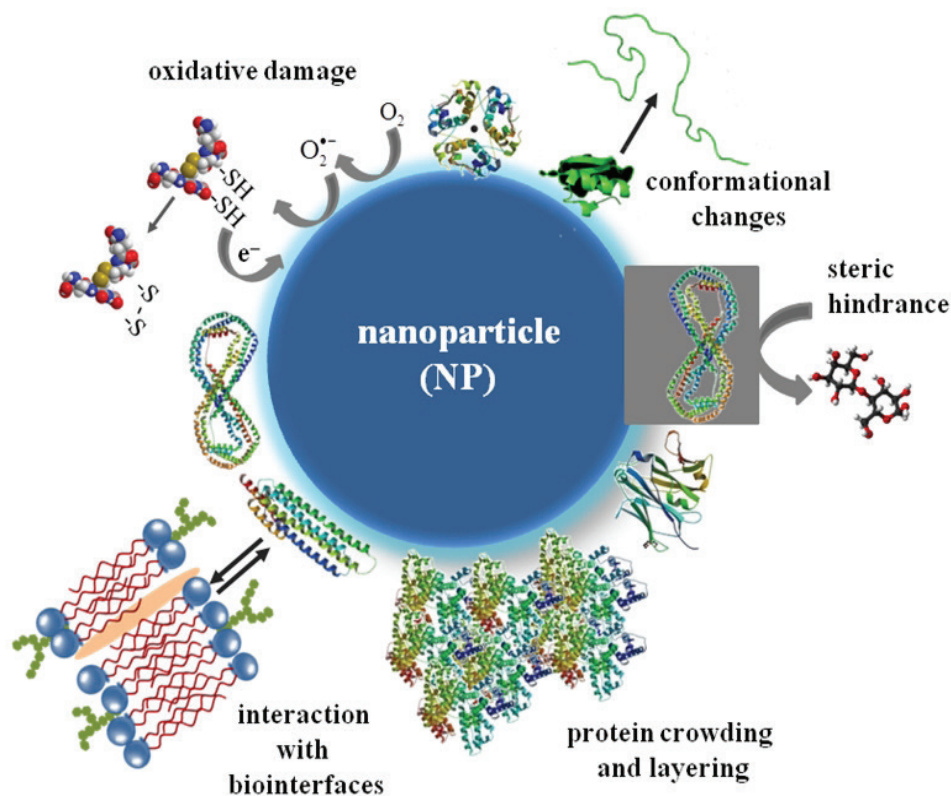

Figure 2 *Changes in the structure and function of NPs and proteins caused by events occurring at the nano-bio interface [inspired by (10)]*

medium (6, 7, 11, 14, 18, 29). Of course, this will depend on the thickness of the outer layer.

Figure 3 shows the types of interactions between NPs and the biological medium. These interactions promote or inhibit: (a) the adsorption of ions, detergents, and other molecules from the medium, (b) attachment/detachment of proteins, (c) competitive binding, (d) steric hindrance on the NPs surface, (e) formation of two or more layers on NP surface, (e) NP dissolution and/or degradation, (f) surface reconstruction, and (g) accumulation and/or agglomeration of NPs (12, 27, 30, 31).

The most important physicochemical properties of an NP that define protein corona formation and fate are chemical composition, shape, curvature, surface functionalisation and structure, porosity, crystallinity, heterogeneity, roughness, and hydrophobicity/hydrophilicity (2-4). Furthermore, effective surface charge, aggregation state, stability, biodegradability, and dissolution properties of the NP surface layer are also important parameters that need to be considered for the investigation of the nano-bio interface (4, 32). For example, surface curvature of an NP affects protein-binding affinities. Greater curvature makes the corona thicker but decreases protein-protein interactions and conformational changes of the adsorbed proteins. Higher surface charge increases corona thickness as well as conformational changes of proteins (13, 27, 30, 31, 33). It may also trigger protein denaturation (27). Higher hydrophobicity increases corona thickness and conformational changes of proteins, as well as the opsonisation rate (14, 34).

All these properties and interactions (Figures 2-3) determine the long-range and short-range forces governing the nano-bio interface (Table 1) (10, 35). Long-range forces originate from attractive van der Waals and repulsive electrostatic double-layer interactions, while short-range forces arise from charge, steric interactions, depletion, and solvent interactions (Table 2) (10, 35).

Understanding how each physicochemical parameter of an NP affects corona formation is a key to designing new, efficient, and secure nanomaterials. Then these properties can be optimised, NPs pre-coated, and their surfaces functionalised to obtain the nature of the protein corona that would render an NP biocompatible (23). One should also take into account environmental factors, such as temperature, pH, protein concentrations, and time of interaction. NPs may also change adsorption, accumulation, degradation, agglomeration, dissolution, distribution, and clearance patterns after the protein corona has been formed, while the proteins forming the corona may pass through conformational changes, free energy release, restructuration, or change their binding profile and kinetics (10, 36, 37).

Mechanistic investigation of protein corona

The properties of metallic NPs can be investigated with a range of spectroscopic, electrophoretic, and microscopic methods (Table 2). The same methods can also be used to study protein corona formation and composition. The most common methods for determining NP properties are the transmission electron microscopy (TEM), light scattering techniques, and UV-visible and fluorescence spectroscopy.
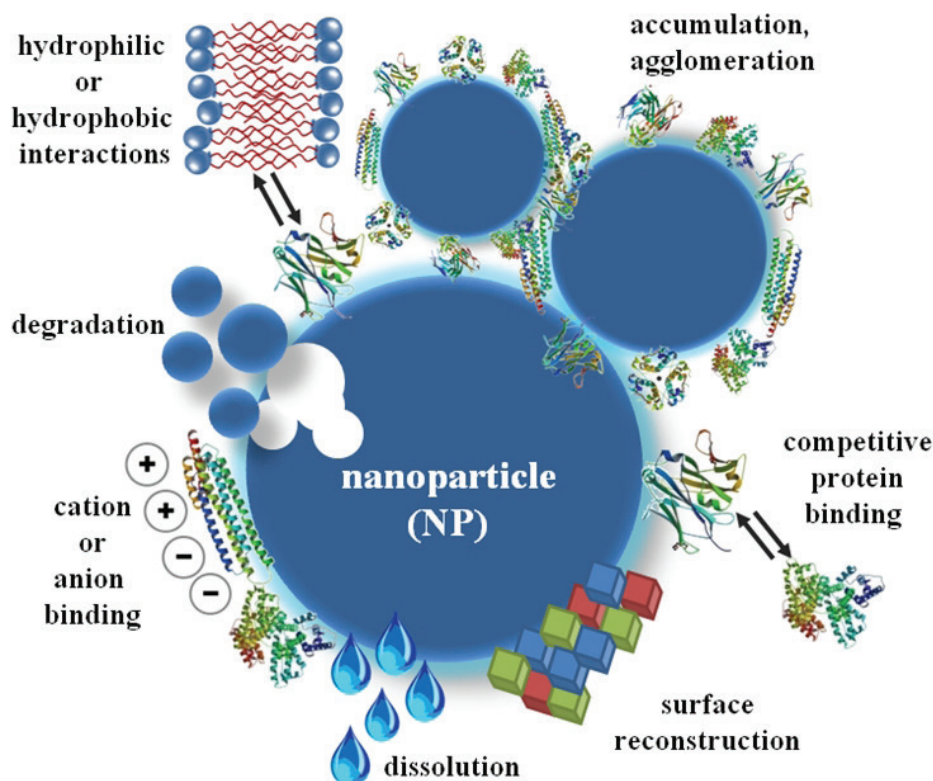

Figure 3 *Active interactions at the nano-bio interface [inspired by (10)]*

Capjak I, et al. How protein coronas determine the fate of engineered nanoparticles in biological environment
Arh Hig Rada Toksikol 2017;68:245-253

249

Table 2 *Analytical methods used to assess the of properties of NPs and their interactions at the nano-bio interface*

NP properties	Analytical technique	Brief analytical description
Size and charge	light scattering (dynamic and electrophoretic)	changes in the hydrodynamic diameter of NP upon binding to proteins
	analytical ultracentrifugation	changes in the hydrodynamic diameter of NP
Size, shape and structure	transmission electron microscopy (TEM)	visualisation of NPs
	atomic force microscopy (AFM)	visualisation of NPs
Structure	X-ray diffraction	determination of crystalline and chemical structure
Size, dissolution	UV-Vis spectroscopy	evaluation for surface plasmon resonance (SPR) peak
Dissolution, structure	inductively coupled plasma mass spectrometry (ICPMS)	determination of elemental composition
Surface area	Braunauer-Emmet-Teller method	measurement of specific surface area using adsorption of gas on the surface
Protein binding affinity	fluorescence spectroscopy	evaluation of changes in fluorescence spectra of proteins due to the NPs-protein interaction
	UV-Vis spectroscopy	evaluation of changes in absorption spectra due to the NPs-protein interaction
	isothermal calorimetry	determination of binding constant, thermodynamic parameters of NP-protein interactions
	quartz crystal balance	determination of changes in mass at the oscillating quartz surface in the NPs-protein complexes
	surface plasmon resonance	detection of change in oscillation of electrons on a metal surface in the NPs-protein complexes
	AFM	determination of adhesion forces and surface free energy during the protein corona formation
	fluorescence correlation spectroscopy	determination of binding characteristics depending on the fluctuation in florescence
Protein structural changes	circular dichroism spectroscopy	measurement of changes in secondary structure of proteins depending on chiral properties of proteins
	Fourier transformed infrared spectroscopy	measurement of adsorption of amide bonds in the proteins to derive structural change
	Raman spectroscopy	evaluation of molecular vibrations to predict structure
	nuclear magnetic resonance	determination of magnetic properties of atomic nuclei to predict structure
Composition of protein corona	capillary electrophoresis	separation of proteins and NPs-protein complexes using very small sample volumes
	LC-MS/MS	separation and identification of protein, accurate analysis of molecular weight (M_w) distribution
	polyacrylamide gel electrophoresis (PAGE)	identification of proteins

Newly synthesised metallic NPs are usually characterised in the medium used for their synthesis, such as water. Although quite demanding, NP evaluation in pure water is much less complicated than in any biological matrix.

Complexes that form between NPs and proteins are most often analysed with mass spectrometry (MS)-based proteomics. Spectroscopic methods like ultraviolet-visible (UV-Vis) and fluorescence spectroscopy, and circular dichroism (CD) are used to investigate nano-bio interface binding interactions due to their robustness and high sensitivity. UV-Vis spectroscopy can be used to measure the rate of protein binding as a function of change in plasmon λ_{max} over time. Fluorescence spectroscopy acquires the intrinsic fluorescence of the protein and can therefore measure binding to NPs. CD spectroscopy uses changes in the chiral properties of a protein to predict changes in its secondary structure. Measured interactions between plasma proteins and NPs can be quantified using several kinetic models and equations (38-40). All the methods described in Table 2 are quite accessible and straightforward to evaluate nano-bio interface in pure water or a simple buffer system. In complex media like blood plasma or cellular matrix, however, analytical performance and interpretation of results may become very complex.

250

Capjak I, et al. How protein coronas determine the fate of engineered nanoparticles in biological environment
Arh Hig Rada Toksikol 2017;68:245-253

Protein corona composition

The most extensively studied biological environment for protein corona is human blood plasma. Protein layer(s) that adsorb on NPs in blood can affect their uptake and distribution in the cells. For example, fibrinogen, immunoglobulin G (IgG), or complement factors are believed to promote phagocytosis and removal of NPs from the bloodstream, while human serum albumin (HSA) and apolipoproteins prolong their circulation time in blood (19).

In the early stage of corona formation, albumin, IgG, fibrinogen, and apolipoproteins seem to adsorb rapidly on metallic NPs in plasma (13, 35, 41). These proteins are found in the hard coronas of all studied NPs and are replaced by apolipoproteins and coagulation factors in the slow phase of corona formation (35). Changes in biological environment also reflect on a corona composition. Walkey and Chan (21) use the term *adsorbome* to denote a group of 125 most common plasma proteins in the corona. Table 3 lists some of them by the type of NP.

Only two to six of them strongly adsorb on metallic NPs. For most metallic NPs, the corona is dominated by albumin (42), which is at the same time the most abundant protein in plasma. Although it has a negative net charge at pH 7.4, albumin contains 60 positively charged lysine residues, which enable its interaction with both positively and negatively charged NPs. Albumin will form anionic corona complexes with NPs regardless of their net charge (36). These coronas are similar in size and effective surface charge, but their behaviour in contact with the cell will differ. Fleischert and Payne (36) believe that cationic NPs alter the structure of albumin proteins in the corona, while anionic NPs do not. These structural changes affect the behaviour of the albumin-NP complex at the cellular level, so that cell receptors bind coronas formed around anionic NPs and redirect those formed around cationic NPs to scavenger receptors. Another difference is that albumin adsorbs much more on the surface of anionic than cationic NPs (23% *vs.* 8% of surface coverage, respectively) (36).

Similar to albumin, fibrinogen was also identified in the corona of many NPs. Apolipoproteins will mainly adsorb on liposomes and polymeric NPs, as they have low affinity for metallic NPs. The main force involved in their interactions with NPs is hydrophobicity (29). Polymeric and hydrophobic NPs also attract proteins like transferrin, haptoglobin, fetuin A, kininogen, histidine-rich glycoprotein, and intrinsic clotting pathway factors. Most of these proteins will adsorb on metallic NPs (29).

Table 3 *Composition of protein corona by NP type*

NP type	Proteins detected in corona	Reference
Polystyrene NPs	coagulation factors, immunoglobulins, lipoproteins, acute phase proteins, complement proteins, plasminogen, anti-CD4, c4a, albumin	1, 23,42
Latex NPs	albumin, apolipoproteins, immunoglobulins, hemoglobin, haptoglobins	1, 23
Copolymer NPs	albumin, apolipoprotiens, fibrinogen, immunoglobulins, C4BP-α-chain	1
Supraparamagnetic iron oxide NPs	albumin, α-1-antitrypsin, fibrinogen chains, immunoglobulin chains, transferrin, transthyretin	1, 41, 42
Gold NPs	albumin, fibrinogen chains, apolipoprotein A1, transport proteins, coagulation factors, tissue development proteins	1, 23, 42
Carbon nanotubes	fibrinogen chains, immunoglobulin light chains, fibrin, albumin, ApoA1, complement, component proteins, fibronectin	1, 42
SiO$_2$ NPs	immunoglobulins, lipoproteins, complement proteins, coagulation proteins, acute phase proteins, cell proteins, serum proteins	1, 23, 19
TiO$_2$NPs, ZnONPs, SiO$_2$ NPs	albumin, immunoglobulins, fibrinogen, transferrin, apolipoprotein A1, complement proteins, immunoglobulin light chains, fibrin, albumin, fibronectin	1, 23
Magnetic NPs	albumin, apolipoprotein A1, complement factors, vitronectin, hemoglobin	1
Citrate-coated AgNPs	albumin, α-1-antiproteinase, α-2-HS-glycoprotein, apolipoprotein A1, serotransferrin, α-2-macroglobulin, α-fetoprotein, apolipoprotein B100, α-2-antiplasmin, complement C3, β-2-glycoprotein 1, fetuin-B, inter-α-trypsin inhibitor heavy chain H1, hemoglobin foetal subunit β, inter-α-trypsin inhibitor heavy chain H3, inter-α-trypsin inhibitor heavy chain H2, hemoglobin subunit α, complement factor B, hemopexin, serpin A3-6	46
AgNPs coated with polyvinylpyrrolidone	albumin, α-2-HS-glycoprotein, α-1-antiproteinase, apolipoprotein A1, serotransferrin, α-2-macroglobulin, α-fetoprotein, apolipoprotein B100, complement C3, α-2-antiplasmin, inter-α-trypsin inhibitor heavy chain H1, fetuin-B, β-2-glycoprotein 1, hemoglobin foetal subunit beta, inter-α-trypsin inhibitor heavy chain H3, inter-α-trypsin inhibitor heavy chain H2, vitamin D-binding protein, transthyretin, hemoglobin subunit α, complement factor B	46

Capjak I, et al. How protein coronas determine the fate of engineered nanoparticles in biological environment
Arh Hig Rada Toksikol 2017;68:245-253

251

Importance of protein corona for biomedical application of nanoparticles

As the NP-corona complex is actually "what the cell sees" (28), it is more important to determine the biological response (i.e., immunogenicity) to the complex than the properties of an NP alone (43). For most biomedical purposes, hard corona will likely improve the interaction between NPs and proteins, membranes, phospholipids, endocytic vesicles, organelles, and DNA (44-46).

Corona is what controls which type of biomolecule will it bind and how, how will the NP-corona complex interact with cells receptors, and what will its distribution and elimination be (45-46). For a nano-drug delivery system it is important to define the affinity, stoichiometry, kinetics, and the concentrations of NPs for their interaction with specific proteins. At the moment, however, we still have a lot to learn. The biggest challenge for researches is to find out how protein corona could contribute to nanodrug distribution *in vivo*.

Conflicts of interest

None to declare.

REFERENCES

1. Saptarshi SR, Duschl A, Lopata AL. Interaction of nanoparticles with proteins: relation to bio-reactivity of the nanoparticle. J Nanobiotechnol 2013;11:26. doi: 10.1186/1477-3155-11-26

2. Huang R, Carney RP, Stellacci F, Lau BL. Protein-nanoparticle interactions: the effects of surface compositional and structural heterogeneity are scale dependent. Nanoscale 2013;5:6928-35. doi: 10.1039/c3nr02117c

3. Jurašin DD, Ćurlin M, Capjak I, Crnković T, Lovrić M, Babič M, Horák D, Vinković Vrček I, Gajović S. Sufrace coating affects behavior of metallic nanoparticle in a bilogical environment. Beilstein J Nanotechnol 2016;7:246-62. doi: 10.3762/bjnano.7.23.

4. European Commission. Commission recommendation of 18 October 2011 on the definition of nanomaterial (Text with EEA relevance) (2011/696/EU) [displayed 3 November 2017]. Available at https://ec.europa.eu/research/industrial_technologies/pdf/policy/commission-recommendation-on-the-definition-of-nanomater-18102011_en.pdf

5. Lane LA, Qian X, Smith AM, Nie S. Physical chemistry of nanomedicine: understanding the complex behaviours of nanoparticles *in vivo*. Annu Rev Phys Chem 2015;66:521-47. doi: 10.1146/annurev-physchem-040513-103718

6. Ding F, Radic S, Chen R, Chen P, Geitner NK, Brown JM, Ke PC. Direct observation of a single nanoparticle-ubiquitin corona formation. Nanoscale 2013;5:9162-9. doi: 10.1039/c3nr02147e

7. Duran N, Silveira CP, Duran M, Martinez DST. Silver nanoparticle protein corona and toxicity: a mini-review. J Nanobiotechnology 2015;13:55. doi: 10.1186/s12951-015-0114-4

8. Docter D, Westmeier D, Markiewicz M, Stolte S, Knauer SK, Stauber RH. The nanoparticle biomolecule corona: lessons learned - challenge accepted? Chem Soc Rev 2015;44:6094-121. doi: 10.1039/C5CS00217F

9. Kononenko V, Narat M, Drobne D. Nanoparticle interaction with the immune system. Arh Hig Rada Toksikol 2015;66:97-108. doi:10.1515/aiht-2015-66-2582

10. Nel AE, Mädler L, Velegol D, Xia T, Hoek EMV, Somasundaran P, Klaessig F, Castranova V, Thompson M. Understanding biophysicochemical interactions at the nano-bio interface. Nat Mater 2009;8:543-57. doi: 10.1038/nmat2442

11. Tavanti F, Pedone A, Menziani MC. Competitive binding of proteins to gold nanoparticles disclosed by molecular dynamics simulations. J Phys Chem C 2015;119:22172-80. doi: 10.1021/acs.jpcc.5b05796

12. Cedervall T, Lynch I, Foy M, Berggad T, Donnelly S, Cagney G, Linse S, Dawson K. Detailed identification of plasma proteins adsorbed on copolymer nanoparticles. Angew Chem Int Ed 2007;46:5754-6. doi: 10.1002/anie.200700465

13. Lundqvist M, Stigler J, Elia G, Lynch I, Cedervall T, Dawson KA. Nanoparticle size and surface properties determine the protein corona with possible implications for biological impacts. Proc Natl Acad Sci 2008;105:14265-70. doi: 10.1073/pnas.0805135105

14. Cedervall T, Lynch I, Lindman S, Berggård T, Thulin E, Nilsson H, Dawson KA, Linse S. Understanding the nanoparticle-protein corona using methods to quantify exchange rates and affinities of proteins for nanoparticles. Proc Natl Acad Sci USA 2007;104:2050-5. doi: 10.1073/pnas.0608582104

15. Lynch I, Dawson KA, Linse S. Detecting cryptic epitopes created by nanoparticles. Sci STKE 2006;2006(327):pe14. doi: 10.1126/stke.3272006pe14

16. Gref R, Lück M, Quellec P, Marchand M, Dellacherie E, Harnisch S, Blunk T, Müller RH. 'Stealth' corona-core nanoparticles surface modified by polyethylene glycol (PEG): influences of the corona (PEG chain length and surface density) and of the core composition on phagocytic uptake and plasma protein adsorption. Colloids Surf B Biointerfaces 2000;18:301-13. doi: 10.1016/S0927-7765(99)00156-3

17. Kittler S, Greulich C, Gebauer JS, Diendorf J, Treuel L, Ruiz L, Gonzalez-Calbet JM, Vallet-Regi M, Zellner R, Köller M, Epple M. The influence of proteins on the dispersability and cell-biological activity of silver NP. J Mater Chem 2010;20:512-8. doi: 10.1039/B914875B

18. Koshkina O, Lang T, Thiermann R, Docter D, Stauber RH, Secker C, Schlaad H, Weidner S, Mohr B, Maskos M, Bertin A. Temperature-triggered protein adsorption on polymer-coated nanoparticles in serum. Langmuir 2015;31:8873-81. doi: 10.1021/acs.langmuir.5b00537

19. Monopoli MP, Walczyk D, Campbell A, Elia G, Lynch I, Bombelli FB, Dawson KA. Physical-chemical aspects of protein corona: relevance to *in vitro* and *in vivo* biological impacts of nanoparticles. J Am Chem Soc 2011;133:2525-34. doi: 10.1021/ja107583h

20. Miclăuş T, Bochenkov VE, Ogaki R, Howard KA, Sutherland DS. Spatial mapping and quantification of soft and hard protein coronas at silver nanocubes. Nano Lett 2014;14:2086-93. doi: 10.1021/nl500277c

21. Walkey CD, Chan WC. Understanding and controlling the interaction of nanomaterials with proteins in a physiological environment. Chem Soc Rev 2012;41:2780-99. doi: 10.1039/c1cs15233e

252

Capjak I, et al. How protein coronas determine the fate of engineered nanoparticles in biological environment
Arh Hig Rada Toksikol 2017;68:245-253

22. Vroman L, Adams AL, Fischer GC, Munoz PC. Interaction of high molecular-weight kininogen, factor-XII, and fibrinogen in plasma at interfaces. Blood 1980;55:156-9. PMID: 7350935

23. Aggarwal P, Hall JB, McLeland CB, Dobrovolskaia MA, McNeil SE. Nanoparticle interaction with plasma proteins as it relates to particle biodistribution, biocompatibility and therapeutic efficacy. Adv Drug Deliv Rev 2009;61:428-37. doi: 10.1016/j.addr.2009.03.009

24. Göppert TM, Müller RH. Polysorbate-stabilized solid lipid nanoparticles as colloidal carriers for of drugs to the brain: comparison of plasma protein adsorption patterns. J Drug Target 2005;13:179-87. doi: 10.1080/10611860500071292

25. Gebauer JS, Malissek M, Simon S, Knauer SK, Maskos M, Stauber RH, Peukert W, Treuel L. Impact of the nanoparticle-protein corona on colloidal stability and protein structure. Langmuir 2012;28:9673-9. doi: 10.1021/la301104a

26. Treuel L, Nienhaus GU. Toward a molecular understanding of nanoparticle-protein interactions. Biophys Rev 2012;4:137-47. doi 10.1007/s12551-012-0072-0

27. Lynch I, Dawson KA, Linse S. Detecting cryptic epitopes created by nanoparticles. Sci STKE 2006;2006(327):pe14. doi: 10.1126/stke.3272006pe14

28. Walczyk D, Bombelli FB, Monopoli MP, Lynch I, Dawson KA. What the cell "sees" in bionanoscience. J Am Chem Soc 2010;132:5761-8. doi: 10.1021/ja910675v

29. Gessner A, Waicz R, Lieske A, Paulke B-R, Mäder K, Müller RH. Nanoparticles with decreasing surface hydrophobicities: influence on plasma protein adsorption. Int J Pharm 2000;196:245-9. doi: 10.1016/S0378-5173(99)00432-9

30. Lundqvist M, Stigler J, Cedervall T, Berggard T, Flanagan MB, Lynch I, Elia G, Dawson K. The evolution of the protein corona around nanoparticles: a test study. ACS Nano 2011;5:7503-9. doi: 10.1021/nn202458g

31. Mahmoudi M, Lynch I, Ejtehadi MR, Monopoli MP, Bombelli FB, Laurent S. Protein-nanoparticle interactions: opportunities and challenges. Chem Rev 2011;111:5610-37. doi: 10.1021/cr100440g

32. Klein J. Probing the interaction of proteins and nanoparticles. Proc Natl Acad Sci USA 2007;104:2029-30. doi: 10.1073/pnas.0611610104

33. Gessner A, Lieske A, Paulke BR, Müller RH. Influence of surface charge density on protein adsorption on polymeric nanoparticles: analysis by two-dimensional electrophoresis. Eur J Pharm Biopharm 2002;54:165-70. doi: 10.1016/S0939-6411(02)00081-4

34. Lindman S, Lynch I, Thulin E, Nilsson H, Dawson KA, Linse S. Systematic investigation of the thermodynamics of HSA adsorption to N-iso-propylacrylamide/N-tert-butylacrylamide copolymer nanoparticles. Effects of particle size and hydrophobicity. Nano Lett 2007;7:914-20. doi: 10.1021/nl062743+

35. Rahman M. Nanoparticle and protein corona. In: Rahman M, Laurent S, Tawil N, Yahia L, Mahmoudi M, editors. Springer Series in Biophysics. Vol. 15. Protein-nanoparticle interactions. Chapter 2. Berlin Heidelberg: Springer-Verlag; 2013. p. 21-44.

36. Fleischert CC, Payne CK. Nanoparticle-cell interactions: molecular structure of the protein corona and cellular outcomes. Acc Chem Res 2014;47:2651-9. doi: 10.1021/ar500190q

37. Laera S, Ceccone G, Rossi F, Gilliland D, Hussain R, Siligardi G, Calzolai L. Measuring protein structure and stability of protein-nanoparticle systems. Nano Lett 2011;11:4480-4. doi: 10.1021/nl202909s

38. Eskandari K, Kamali M, Ramezani M, Safiri Z, Keihan AH, Rashidiani J, Kooshki H, Zarei H. The effect of hydrophobicity and hydrophilicity of gold nanoparticle on proteins structure and function. Int J Bio-Inorg Hybrid Nanomat 2013;2:465-70.

39. Cui M, Liu R, Deng Z, Ge G, Liu Y, Xie L. Quantitative study of protein coronas on gold nanoparticles with different surface modifications. Nano Res 2013;7:345. doi: 10.1007/s12274-013-0400-0

40. Boulos SP, Davis TA, Yang JA, Lohse SE, Alkilany AM, Holland LA, Murphy CJ. Nanoparticle-protein interactions: a thermodynamic and kinetic study of the adsorption of bovine serum albumin to gold nanoparticle surfaces. Langmuir 2013;29:14984-96. doi: 10.1021/la402920f

41. Sakulkhu U, Mahmoudi M, Maurizi L, Salaklang J, Hofmann H. Protein corona composition of superparamagnetic iron oxide nanoparticles with various physico-chemical properties and coatings. Scientific Rep 2014;4:5020. doi: 10.1038/srep05020

42. Karmali PP, Simberg D. Interactions of nanoparticles with plasma proteins: implication on clearance and toxicity of drug delivery systems. Expert Opin Drug Deliv 2011;8:343-57. doi: 10.1517/17425247.2011.554818

43. Lee YK, Choi E-J, Webster TJ, Kim S-H, Khang D. Effect of the protein corona on nanoparticles for modulating cytotoxicity and immunotoxicity. Int J Nanomedicine 2015;10:97-113. doi: 10.2147/IJN.S72998

44. Yallapu MM, Ebeling MC, Jaggi M, Chauhan SC. Plasma proteins interaction with curcumin nanoparticles: implications in cancer therapeutics. Curr Drug Metab 2013;14:504-15. PMCID: PMC4030727

45. Zook JM, Halter MD, Cleveland D, Long SE. Disentangling the effects of polymer coatings on silver nanoparticle agglomeration, dissolution and toxicity to determine mechanisms of nanotoxicity. J Nanopart Res 2012;14:1165. doi: 10.1007/s11051-012-1165-1

46. Shannahan JH, Lai X, Ke PC, Podila R, Brown JM, Witzmann FA. Silver nanoparticle protein corona composition in cell culture media. PLoS One 2013;8:e74001. doi: 10.1371/journal.pone.0074001

Capjak I, et al. How protein coronas determine the fate of engineered nanoparticles in biological environment
Arh Hig Rada Toksikol 2017;68:245-253

253

Kako proteinska korona određuje sudbinu nanočestica u biološkom okolišu

Nanomedicina je iznimno napredno medicinsko područje u kojem se iskorištavaju nanočestice za razvoj inovativnih lijekova, medicinskih pomagala i dijagnostičkih postupaka. U *in vivo* uvjetima ponašanje nanočestica može biti vrlo kompleksno zbog bliskih interakcija s biološkim molekulama. Zbog međudjelovanja nanočestica i proteina u biološkim tekućinama nastaje dinamička proteinska korona koja obavija nanočestice i tvori novo sučelje između nanočestica i okoliša u kojem se one nalaze (krv, stanice, tkiva). Ta međudjelovanja ovise o biološkim, kemijskim i fizikalnim svojstvima samih nanočestica i proteina, ali i samog biološkog okoliša. U ovom preglednom radu dan je prikaz glavnih karakteristika koji određuju proteinsku koronu te opis njezine dinamičke prirode. Prikazane su najvažnije analitičke metode za istraživanje proteinske korone te primjeri sastava proteinske korone za najčešće korištene vrste nanočestica u biomedicini. Takvo je znanje nužno za dizajn i razvoj učinkovitih i sigurnih nanomedicinskih proizvoda.

KLJUČNE RIJEČI: *nano-bio interakcije; nanomedicina; meka korona; tvrda korona*