Biomolecular Coronas Provide the Biological Identity of Nanomaterials

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The search for understanding interactions of nano-sized materials with living organisms is leading to the rapid development of key applications including improved drug delivery by targeting nanoparticles, and resolution of the potential threat of nanotechnological devices to organisms and the environment. Unless specifically designed to avoid it, nanoparticles in contact with biological fluids are rapidly covered by a selected group of biomolecules to form a corona that interacts with biological systems. Here we review the basic concept of the nanoparticle corona, its structure and composition, and highlight how the properties of the corona may be linked to its biological impacts. We conclude with a critical assessment of the key problems that need to be resolved in the near future.

The surfaces of all materials (including nano-sized materials) have higher free-energy than the bulk material itself. This means that unless the surfaces of nanoparticles have been designed to do otherwise, nanoparticles will progressively and selectively adsorb biomolecules when they contact complex biological fluids[1-7]. This 'corona' of biomolecules lowers the surface energy of the nanoparticle and promotes its dispersion. We hypothesize that in many cases it is the biomolecular corona that is interacting with biological systems, and thereby constitutes a major element of the biological identity of the nanoparticle (Fig. 1a)[1,4,8-11]. For several nanoparticles such as gold[12], polystyrene[8,13,14], silica[5,8,14,15], titania[15] and zinc oxide[15], a near-monolayer of biomolecules (called the 'hard' corona) bind tightly, but not completely irreversibly, to the nanoparticle surface. On top of this 'hard' corona is

a ‘soft’ corona consisting of a more loosely associated and rapidly exchanging layer of biomolecules[8,12-14,16] (Fig. 1b).

One expects those examples to be typical for nanoparticles with similarly high energy of the bare surface. Crucially, it is observed that only few of the biomolecules available in typical biological environments are found in the hard corona. In the purely illustrative examples in Table 1, out of roughly 3,700 human blood plasma proteins[17], only at most a few tens are ever abundant in the hard corona. Furthermore, these tens of proteins rarely correspond to the most abundant proteins in plasma, and are not necessarily those with the highest individual affinity to the nanoparticle surface[5,6,18-24].

Because the hard corona is distinctively stable[5,8,12-14], any subsequent exposure of the nanoparticle to a new environment with different biomolecules may lead to only partial displacement of the original hard corona by new molecules[25,26]. Biomolecules that are not replaced would serve as a corona ‘memory’ of the nanoparticle’s previous environment. Therefore the corona composition could potentially depend not only on the current environment of the nanoparticle, but on all environments it has moved through[26,27]. Figure 2 illustrates this concept for inhaled nanoparticles carrying typical lung surfactant proteins, and other membrane components, when they reach the blood circulation[28,29].

The fact that the biomolecular corona defines the biological interactions of nanoparticles has been considered surprising because we often classify substances according to the ‘intrinsic’ properties of the bare material, rather than ‘extrinsic’ properties derived from the environment. However, the importance of extrinsic properties for the biological activity of small molecule drugs, biomaterial implants and cell scaffolds is already well-known. For example, 99% of the anticoagulant drug

warfarin is typically bound to plasma proteins *in vivo,* and this protein-bound drug fraction is retained much longer in the body than the more biologically active unbound fraction, thereby improving the overall circulation half-life and effect of the drug[30]. Also, it is the biomolecules bound to the surface of biomaterial implants that are understood to interact with the surrounding tissue[31]; these biomolecules may mitigate material-induced tissue damage (including late-stage thrombosis[31,32]), or promote early inflammatory responses.

Despite the similarities, nanomaterials are different. Unlike macroscopic surfaces, nanoparticles can travel to almost every location within organisms[27-29] via endogenous transport pathways[33,34] and potentially retain biomolecules from their previous environments in their corona, including those relevant to the transport process itself. Examples include apolipoproteins, which are abundant in the corona of very different nanomaterials (including polystyrene and other polymeric nanoparticles[1,13,35], silica[5] and quantum dots[36]) and are important for crossing biological barriers.

It is worth emphasizing that the biomolecular corona is also relevant to nanoparticles with grafted antibodies, proteins and peptides that are specifically designed for targeting in nanomedicine[37,38]. While surface modifications (such as PEGylation[39]) reduce the binding of additional biomolecules, some association of biomolecules may still occur[35,40]. Their presence may lead to more subtle and poorly understood biological consequences, issues possibly related to current struggles in achieving efficient targeting of nanomedicines *in vivo*. Indeed, in future, instead of chemical grafting, the corona itself might be controlled (e.g. by surface templating approaches[41]) and used as a novel means to target diseases[21,36].

Nanoscale engagement with biological processes

Fundamentally all interactions between the nanoparticle-corona complex and the biological machinery are mediated by physical forces common to all molecular systems (e.g. van der Waals, electrostatic, and others[2]). A more useful classification is whether these interactions involve non-specific physical interactions[6,42], or specific biological recognition[10]. Such recognition can, for example, be driven by either peptide sequences (epitopes), or more subtle properties, such as curvature of the complex[43]. Recognition is expected to lead to well-regulated biological processing and functional impacts. In contrast, strong non-specific interactions between particles and cells could lead to a range of quite different, but equally complex, outcomes. For example, in several cases such as carbon nanotubes[6], silica[42] and graphene oxide[44], cell damage occurs in the absence of a protein corona (that is, cells exposed to particles in medium without proteins) but is mitigated in its presence. In such cases particles in the absence of protein may instead acquire a corona from biomolecules in the cell membrane, suggesting a mechanism for the damage[42]. Exceptional cases of direct entry into cytosol can also be related to a lack of corona[42,45]. In nanoparticle-cell interactions, though the intended purpose in supplying cells with serum protein is to feed them, an important accidental role of serum is to limit direct contact of the nanoparticle high energy surface with cells.

The entry of nanoparticles into cells and their translocation across biological barriers are important active processes[33,34]. Small hydrophobic molecules, loosely speaking, partition in biological compartments according to physicochemical equilibrium principles[46], while larger micron-sized objects engage significantly with macrophages and other elements of the front-line immune system[29]. In contrast, because of their size and surface, nanoparticle-corona complexes can engage with a

wide range of endogenous cellular uptake and other processes, and potentially reach all cellular and organ compartments[27-29,35], interact with them, and initiate signalling processes[10]. Endocytic mechanisms of cellular uptake are known from the extensive biomolecular transport literature[43,47]. Whether these or novel mechanisms are being utilized in nanoparticle uptake[33,48] will need to be clarified[49]. Almost certainly a variety of mechanisms is at work in the uptake of nanoparticles under different conditions. Several nanoparticles (e.g. polystyrene[33,46], silica[50] and others[49]) follow the endo-lysosomal pathway, leading finally to lysosomal accumulation.

However, there is now need to revise nanoparticle uptake questions in the context of the nanoparticle surface *in situ*. For example, multiple reports suggest that the rate of accumulation of nanoparticles in cells depends on the detailed nature of the biological milieu[4,22,51,52]. In several cases of cell types and nanoparticles, including silica[42], carbon[53], iron oxide[54], polystyrene[55] and others[11], formation of a serum-derived corona leads to reduced cell-uptake. Other differences in uptake levels can arise from rather subtle differences in nanoparticle dispersion preparation, such as serum heat inactivation[51], or even just different culture media, despite being supplemented with the same serum[22]. In order to rule out such changes as a source of differences, exact matching of the media will be required in future comparative experiments.

Similar implications apply for *in vivo* and *in vitro* comparative experiments, typically performed at different protein concentrations, and thus, resulting in different corona composition[14]. Furthermore, corona proteins from biological media derived from one species may not be recognized (or be recognized to a different degree) by receptors in different species, unless there is inter-species conservation. Thus, the role of

serum in studies of cells from different species may need to be questioned, possibly requiring matching also of the supplemented serum and organism in the future.

Lest it be forgotten, we emphasize that not only proteins may be recognised by the biological machinery. For example, the lipid component, especially relevant for inhalation scenarios, also plays a crucial role for biorecognition[56]; thus carbon nanotubes covered by a phospholipid uptake signal, phosphatidylserine, are recognised and cleared by macrophages both *in vitro* and following inhalation *in vivo*[57].

In cases where the corona biomolecules have the propensity of being recognized by the biological machinery there may be a combination of effects. Firstly, a non-specific lowering of the direct physical interactions between the bare particle and the cell surface. Secondly, a specific interaction between adsorbed biomolecules and the biological machinery, resulting in the activation of more specific regulated pathways. There have also been suggestions that surface biomolecules (including adsorbed endogenous proteins) could influence the crossing of biological barriers, such as the blood-brain barrier[35,58,59]. Mastering these phenomena will have deep implications for both nanosafety and nanotherapeutics[4].

Our hypothesis is that the biodistribution and biokinetics of many nanoparticles *in vivo* would largely be determined by the nanoparticle-corona complex rather than the bare material[2,4,11,28,29,59,60]. It is not yet known to what degree details of the preparation of nanoparticle dispersions and the biomolecules present in different animal species will affect the outcome. Individuals may express different molecules in their blood[17] and corona[61], and there have been propositions that the corona can identify cancer diagnostic markers[62]. These, and many others, are relevant questions that should be addressed.

A key question is whether it is the original corona at the point-of-entry (e.g. blood, lung or other), or a corona modified by subsequent translocation, that determines biodistribution and effects of nanoparticles *in vivo*. Currently the detailed fate of the original corona, as it passes membranes and barriers and interacts with the extracellular matrix is unknown. The original corona may be replaced by new biomolecules (as illustrated in Fig. 2), or remain intact. Early results suggest that (part of) the original corona may be carried into the cell, rather than being stripped off by contact with cell-membrane proteins and lipids[63,64]. This implies that the original corona, albeit somewhat modified by interactions at the cell membrane, may continue to hinder the direct contact between intracellular components and the bare nanomaterial surface. It is possible that the corona may be degraded entirely only if the particles end up in lysosomes or phagosomes[65]. For most of their early interactions with an organism, some variant of the corona would then constitute the biological identity of the nanoparticles, until final accumulation or clearance.

The role of the corona in signalling processes has also begun to emerge[66]. This highlights the fact that biological impacts might be driven by both the composition of the biomolecular corona and disturbances to the conformation of the proteins following adsorption on the nanoparticles[10,67-72]. One example where the mechanism has been worked out involves the nanoparticle-induced protein unfolding leading to initiation of the NF-Kβ pathway and inflammation[10]. Protein unfolding due to nanoparticles may also lead to nucleation of protein fibrillation[73]. Certainly interactions of nanoparticles and subsequent modulation of the immune system[40,61,74-77] requires a systematic study in the context of the *in situ* nanoparticle-surface in the relevant biological media.

Structure, Composition, Dynamical and Kinetic Aspects

Due to the complexity of the topic, we focus on two of the most immediate questions: Which aspects of the biomolecular corona are biologically relevant? And, what factors, besides the properties of the bare nanoparticle surface, determine these aspects of the corona?

Firstly, even if the nanoparticles remain within the same biological compartment, the corona biomolecules must stay long enough on the surface of the nanoparticle if they are to confer a biological identity[8]. Thus, besides the structure and composition of the corona, dynamical exchange processes will also determine what is biologically relevant (Fig. 1b). Biomolecules residing longer than the characteristic timescale of a given biological process may be relevant to that process[8], if appropriately oriented. For example, endocytosis of nanoparticles across the plasma membrane occurs within minutes[46,50], and, consequently, a biomolecule must stay on the nanoparticle surface at least this long in order to be 'seen' and processed by the endocytic machinery. For unmodified nanoparticles in biological media, several examples show that the tightly bound hard corona exchanges slowly, often over several hours[8,12,16]. In the case of the much more rapidly exchanging outer soft corona, we expect an exchange time of seconds or less[16]. This separation of timescales suggests that the key biological determinant for nanoparticles will often be the interface between the hard and soft corona. It may be that neither materials of the future nor some PEGylated particles will possess this simple separation of timescales, but the basic paradigm that the residence time determines the outcome will remain valid.

Secondly, it is important to connect the structure and composition of the hard corona to the nature of the original nanoparticle surface. This is not a simple task because the corona is not at thermodynamic equilibrium. Indeed, the molecular

dynamical exchange times of the hard corona biomolecules can be many hours[8,12,16]. This means that the composition and structure of the corona are not (and cannot be) equilibrated on a timescale of typical biological experiments, and the nearly stationary composition and structure is actually a consequence of the very slow exchange. Current efforts to describe the hard corona by conventional methods of equilibrium physical sciences (e.g. affinity constants and Langmuir isotherms) may therefore be incomplete. In other words, one should not expect the hard corona structure and composition to reflect the basic interactions between the nanoparticle and biofluid in a simple manner.

For example, in the absence of rapid exchange, some biomolecules arriving early to the nanoparticle surface may subsequently either be displaced by those arriving later or, if they adhere strongly, may themselves play a role in determining what adsorbs next (see Fig. 3). These processes are all affected by the abundance and rate of diffusion of each biomolecule, as well as their affinity for the surface and/or other species already associated with the surface[72]. Consequently, nanoparticle-biomolecule and biomolecule-biomolecule interactions, along with the conditions in which the corona was formed, are all relevant considerations. Still, practical approaches have been sought to predict the relationship between nanoparticle properties and the nature of the corona by the measurement of binding affinities of a panel of small molecules to a nanoparticle surface[78]. It is too early to draw firm conclusions. Since the biomolecular corona may not always be simply related to individual nanoparticle-biomolecule affinities, it may in the end be necessary to unravel the details of the sequential kinetics of the formation of the corona. Still, practically speaking, the corona, being relatively stationary in time, can be studied and characterized, allowing efforts to relate it to biological interactions.

Some factors that control the corona are now clear, and it will be useful if future studies are carried out as a function of these parameters. For example, as with any surface coverage phenomenon, the ratio of nanoparticle surface area to protein concentration is an important factor[1,14]. Still, there are strong indications that radius of curvature of the nanoparticle is also a key parameter for several reasons[1,5,13,18,19]. Thus the surface energy of a bare nanoparticle depends on the radius of curvature because a highly curved surface causes local stresses on the microscopic organization of atoms at the surface. Indeed, the energy of a particle surface can be much larger than a flat surface of the same material[79].

The nanoparticle radius of curvature, if sufficiently high, will also affect the corona directly as large proteins seek to pack around small spaces[5,13,19]. For example, one expects radically different coronas in high aspect ratio materials (such as carbon nanotubes), where one of the lengths is typically smaller than that of the abundant proteins[80,81]. In this case, biomolecules may align along the long axis in order to fit on the nanoparticle surface. Larger globular biomolecules, on the other hand, may simply not fit and hence be excluded from the corona.

All these considerations bring into question the concept of nanomaterial classification for the purposes of regulation. For example, variations in fine details of size, shape, and physicochemical properties of the bare surface, beyond the capacity of current nanoparticle characterization methods to fully specify, can lead to different hard coronas. Therefore, classifying nanomaterials solely by their properties in the absence of their biological milieu may be insufficient. Instead, knowledge of what is strongly bound to nanoparticles in canonical biological and other environments, such as lung fluid, blood or river water, may be a better predictor of a range of biological interactions. In turn, such information may help categorize

nanomaterials according to their likely impacts, such as how they distribute and accumulate in different organs of humans or different parts of the environment.

One can make progress in characterizing the corona, and connecting its properties to biological impacts. Systematic and good quality information on the composition of the hard corona is now emerging, both in terms of identities[13,15,19,20,23,35,82-84] and, more recently, quantities[5,14,18,20], of the different species. For animal-derived biological media, those corona compositions currently known are largely composed of proteins[5,13,14,18-20,23,35,82-84] lipids[56,85] and sugars[86], but other components, derived from the overall composition of the environment, may be identified in future. Recent studies have gone further by application of tools from bioinformatics to correlate potential biological functions of the corona with nanoparticle properties[5].

It should be noted that most current methods to determine the corona composition first separate all of the strongly adsorbed biomolecules from the particles into a single biomolecule sample, and then use mass spectrometry to identify the recovered proteins or other components. However, because the corona is not at thermodynamic equilibrium, there could be statistical fluctuations in its composition and organization from particle to particle within the same sample[8,87]. Besides emphasizing that most of the hard corona is composed of few proteins, Table 1 suggests that there are many more proteins in the average corona than what would fit on one particle surface. If, as we believe, the sample of proteins extracted from the particle surface is not due to contamination by background proteins, this suggests that nanoparticles within the same sample may have slightly different hard coronas and, consequently, different biological actions. In future, different approaches are required to identify such corona subclasses, rather than averaging over them.

The hard corona structure can also be studied by isolating the particle-corona complexes[8], and using physical characterization methods well-known from colloid and interface science[88]. For example, dynamic light scattering[8] and fluorescent correlation spectroscopy[9,16] measure the hydrodynamic radius, and differential centrifugal sedimentation (DCS)[8,14] sheds light on the distributions of such nanoparticle-corona complexes. From DCS the corona thickness can be estimated, and information on dynamical exchanges assessed by time-resolved experiments. It is also becoming common practice to use average size and zeta potential measurements to monitor the corona formation when nanoparticles are added to biological media and characterise the quality of the resulting dispersion of these complexes. Several techniques are now available to study adsorption kinetics of blood plasma proteins or enzymes on nanoparticles, including infrared and Raman spectroscopies[68,69,80,89-91], fluorescence correlation spectroscopy[6,9,16,91], surface plasmon resonance[32,86,92], small angle X-ray scattering[67,91,93], quartz crystal microbalance[94], isothermal titration calorimetry[86,93,95], UV-vis and fluorescence spectroscopies[68,96]. Adsorption on the nanoparticle surface may result in protein conformational changes, and these have been studied by circular dichroism[6,67,68,72,80], infrared spectroscopy[69,80], NMR[85,97], and enzyme activity assays[67,68,98]. Morphological analysis of the corona-nanoparticle complexes has been carried out using transmission electron microscopy and atomic force microscopy[6,67] and emerging computational studies seek to correlate with experimental results[6,99-101].

So far, the focus has been on blood plasma-induced corona on nanoparticles. Studies of the corona of nanoparticles recovered from many other biologic fluids, such as urine, synovial fluid, cerebrospinal fluid, and pleural effusion, are also

emerging[20]. Very different coronas can form also when nanoparticles are dispersed in river water or similar aqueous media used for environmental studies[102,103]. We should be aware that the environmental fluid first in contact with nanoparticles may also affect later interactions of the nanoparticles with organisms. As noted earlier, nanoparticles could derive their initial biomolecular corona from different sources — for example, lung entry leads to contact with lung fluids (see Fig. 2) and early studies have focussed on protein surfactants, such as Sp-A[60,104] and related biomolecules. In a recent study, the selective adsorption of surfactant was demonstrated on single-walled carbon nanotubes recovered from broncheo-alveolar lavage of mice and the presence of this surfactant lipid-protein corona was shown to influence the degree of macrophage uptake[56]. There is ample evidence showing that small nanoparticles pass the alveolar-capillary barrier in the lungs and enter the bloodstream, but it is not yet clear to what degree this is assisted by the early binding of lung-borne biomolecules[28,29,105].

Future Challenges

Although much progress has been made, there are several key problems of the biomolecular corona that still need to be addressed[106]. The macroscopic composition of biomolecules that form the hard corona is valuable but to fully link the properties of the corona to the biology, more detailed information on the composition, organization and dynamics of these biomolecules is needed. Techniques developed for protein characterization are likely valuable for identifying biologically functional peptide sequences (known as epitopes) at the interface of the hard and soft coronas. See Fig. 4 for an illustration. Screening the biomolecules using antibody and protein microarrays will give information on the different types of binding targets and

ultimately the epitopes exposed on corona complexes. Such methods also have the possibility to identify subclasses of particles with different bound biomolecules in different arrangements.

To determine the structure of the hard-soft corona interface in detail requires other methods than those currently used in the field. Several techniques from related areas, such as X-ray and neutron scattering, previously successful in studies of complex interfaces, have now started to be used for the present purposes[7], and both spectroscopic and NMR approaches will be of help. It is clear that the combination of physicochemical and biological methodologies will continue, requiring scientists studying these problems to work across the traditional disciplines.

It is worth stressing that all of these approaches could support efforts to correlate and predict aspects of the biological interactions of new materials. Thus, quite different nanomaterials that share similar corona properties (such as size, shape, exchange dynamics, and surface expressed epitopes) will likely have similar early biological interactions, implying potentially similar biodistributions. Methods to fingerprint these (even without understanding them) will be of practical importance. Such approaches are considered important for nanosafety because the range and complexity of relevant nanomaterials makes their individual study very challenging.

All these questions are relevant also to nanoparticles made for medical applications. Although grafting targeting ligands onto nanoparticles can reduce non-specific binding of molecules from the environment, when studied in the presence of biological media, it will likely emerge that the interface they form with biological targets is much more complex than currently envisaged, possibly even partly explaining the sometimes puzzling lack of success of some targeting strategies when applied *in vivo*[107].

It will be important to understand how the passage of nanoparticles inside cells, through biological barriers, and between compartments and organs *in vivo* is determined by and controls the evolving corona (Fig. 2). A key scientific question is the characterization, and understanding, of the *in situ* biomolecular corona inside cells and organisms; particularly interesting is the detailed relationship it bears to trafficking to specific cellular and barrier targets[108]. Almost certainly this question has to be resolved for predicting biodistribution and biokinetics from nanomaterial design. It also impinges on broader questions, such as how nanoparticles that have passed through a variety of natural (say river) or production environments are changed from their pristine state[109,110], *via* dissolution mechanisms as well as adsorption of molecules, and subsequently interact with organisms.

The general conceptual framework for interactions between nanoparticles and living systems is now falling into place, accompanied by an interdisciplinary community working on the question. We may thus hope that a rational basis for biological identity at the nanoscale is a genuine possibility.

References

1. Cedervall, T. *et al.* Understanding the nanoparticle-protein corona using methods to quantify exchange rates and affinities of proteins for nanoparticles. *Proc. Natl. Acad. Sci. USA* **104**, 2050-2055 (2007). **Introduction of the concept of "nanoparticle-corona" and linkage of biomolecular exchange time-scale to biological identity.**
2. Nel, A.E. *et al.* Understanding biophysicochemical interactions at the nano-bio interface. *Nature Mater.* **8**, 543-557 (2009).
3. Lynch, I. & Dawson, K.A. Protein-nanoparticle interactions. *Nano Today* **3**, 40-47 (2008).
4. Aggarwal, P., Hall, J.B., McLeland, C.B., Dobrovolskaia, M.A. & McNeil, S.E. Nanoparticle interaction with plasma proteins as it relates to particle biodistribution, biocompatibility and therapeutic efficacy. *Adv. Drug Deliv. Rev.* **61**, 428-437 (2009).

5. Tenzer, S. *et al.* Nanoparticle size is a critical physicochemical determinant of the human blood plasma corona: a comprehensive quantitative proteomic analysis. *ACS Nano* **5**, 7155-7167 (2011).
Extensive determination of protein corona composition, and bioinformatics analysis of protein function.
6. Ge, C. *et al.* Binding of blood proteins to carbon nanotubes reduces cytotoxicity. *Proc. Natl. Acad. Sci. USA* **108**, 16968-16973 (2011).
7. Ang, J.C., Lin, J.-M., Yaron, P.N. & White, J.W. Protein trapping of silica nanoparticles. *Soft Matter* **6**, 383-390 (2010).
8. Walczyk, D., Baldelli Bombelli, F., Monopoli, M.P., Lynch, I. & Dawson, K.A. What the cell "sees" in bionanoscience. *J. Am. Chem. Soc.* **132**, 5761-5768 (2010).
9. Röcker, C., Pötzl, M., Zhang, F., Parak, W.J. & Nienhaus, G.U. A quantitative fluorescence study of protein monolayer formation on colloidal nanoparticles. *Nature Nanotech.* **4**, 577-580 (2009).
This is one of the first examples in which the corona lifetime has been determined.
10. Deng, Z.J., Liang, M., Monteiro, M., Toth, I. & Minchin, R.F. Nanoparticle-induced unfolding of fibrinogen promotes Mac-1 receptor activation and inflammation. *Nature Nanotech.* **6**, 39-44 (2011).
Here it is demonstrated that the corona itself can include new (cryptic) epitopes due to unfolding upon adsorption on nanoparticle surface, leading to activation of NFkβ pathway.
11. Walkey, C.D. & Chan, W.C.W. Understanding and controlling the interaction of nanomaterials with proteins in a physiological environment. *Chem. Soc. Rev.* **41**, 2780-2799 (2012).
12. Casals, E., Pfaller, T., Duschl, A., Oostingh, G.J. & Puntes, V. Time evolution of the nanoparticle protein corona. *ACS Nano* **4**, 3623-3632 (2010).
An early paper in which the kinetics of maturation of the protein hard corona is explored.
13. Lundqvist, M. *et al.* Nanoparticle size and surface properties determine the protein corona with possible implications for biological impacts. *Proc. Natl. Acad. Sci. USA* **105**, 14265-14270 (2008).
14. Monopoli, M.P. *et al.* Physical-chemical aspects of protein corona: relevance to in vitro and in vivo biological impacts of nanoparticles. *J. Am. Chem. Soc.* **133**, 2525–2534 (2011).
Semi-quantitative determination of hard coronas. Also shows how the corona composition changes as the protein concentration is varied, for instance corresponding to typical *in vitro* and *in vivo* protein contents.
15. Deng, Z.J. *et al.* Differential plasma protein binding to metal oxide nanoparticles. *Nanotech.* **20**, 455101 (2009).
16. Milani, S., Baldelli Bombelli, F., Pitek, A.S., Dawson, K.A. & Rädler, J. Reversible versus irreversible binding of transferrin to polystyrene nanoparticles: soft and hard corona. *ACS Nano* **6**, 2532-2541 (2012).
17. Anderson, N.L. & Anderson, N.G. The human plasma proteome. *Mol. Cell. Proteomics* **1**, 845-867 (2002).
18. Zhang, H. *et al.* Quantitative proteomics analysis of adsorbed plasma proteins classifies nanoparticles with different surface properties and size. *Proteomics* **11**, 4569-4577 (2011).

19. Dobrovolskaia, M.A. *et al.* Interaction of colloidal gold nanoparticles with human blood: effects on particle size and analysis of plasma protein binding profiles. *Nanomed. Nanotech. Biol. Med.* **5**, 106-117 (2009).
Here it is shown how particles of different sizes form different coronas in human blood and their effects on complement activation and coagulation.
20. Martel, J. *et al.* Comprehensive proteomic analysis of mineral nanoparticles derived from human body fluids and analyzed by liquid chromatography-tandem mass spectrometry. *Anal. Biochem.* **418**, 111-125 (2011).
This is one of the first studies which includes protein corona composition in several human body fluids other than blood serum or plasma.
21. Dufort, S., Sancey, L. & Coll, J.-L. Physico-chemical parameters that govern nanoparticles fate also dictate rules for their molecular evolution. *Adv. Drug Deliv. Rev.* **64**, 179-189 (2012).
22. Maiorano, G. *et al.* Effects of cell culture media on the dynamic formation of protein-nanoparticle complexes and influence on the cellular response. *ACS Nano* **4**, 7481-7491 (2010).
23. Simberg, D. *et al.* Differential proteomics analysis of the surface heterogeneity of dextran iron oxide nanoparticles and the implications for their in vivo clearance. *Biomater.* **30**, 3926-3933 (2009).
24. Mahmoudi, M. *et al.* Protein−nanoparticle interactions: opportunities and challenges. *Chem. Rev.* **111**, 5610-5637 (2011).
25. Gasser, M. *et al.* The adsorption of biomolecules to multi-walled carbon nanotubes is influenced by both pulmonary surfactant lipids and surface chemistry. *J. Nanobiotechnology* **8**, 31 (2010).
26. Lundqvist, M. *et al.* The evolution of the protein corona around nanoparticles: a test study. *ACS Nano* **5**, 7503-7509 (2011).
27. Schleh, C. *et al.* Size and surface charge of gold nanoparticles determine absorption across intestinal barriers and accumulation in secondary target organs after oral administration. *Nanotoxicology* **6**, 36-46 (2012).
28. Choi, H.S. *et al.* Rapid translocation of nanoparticles from the lung airspaces to the body. *Nature Biotech.* **28**, 1300-1303 (2010).
29. Oberdörster, G., Elder, A. & Rinderknecht, A. Nanoparticles and the brain: cause for concern? *J. Nanoscience Nanotech.* **9**, 4996-5007 (2009).
Early discussion of how secondary surface coating by molecules at portal of entry and during translocation may affect nanoparticle biodistribution in organisms, including to the brain. Results relevant to those reported in ref. 28.
30. Gaddum, J. *Gaddum's Pharmacology*, Edn. 9. (Oxford University Press, Oxford, 1985).
31. Ekdahl, K.N. *et al.* Innate immunity activation on biomaterial surfaces: a mechanistic model and coping strategies. *Adv. Drug Deliv. Rev.* **63**, 1042–1050 (2011).
32. Gorbet, M.B. & Sefton, M.V. Biomaterial-associated thrombosis: roles of coagulation factors, complement, platelets and leukocytes. *Biomater.* **25**, 5681-5703 (2004).

33. Rejman, J., Oberle, V., Zuhorn, I.S. & Hoekstra, D. Size-dependent internalization of particles via the pathways of clathrin- and caveolae-mediated endocytosis. *Biochem. J.* **377**, 159-169 (2004).
34. Chithrani, B.D., Ghazani, A.A. & Chan, W.C.W. Determining the size and shape dependence of gold nanoparticle uptake into mammalian cells. *Nano Lett.* **6**, 662-668 (2006).
35. Kim, H.R. *et al.* Analysis of plasma protein adsorption onto PEGylated nanoparticles by complementary methods: 2-DE, CE and protein lab-on-chip system. *Electrophoresis* **28**, 2252-2261 (2007).
36. Prapainop, K., Witter, D.P. & Wentworth, P. A chemical approach for cell-specific targeting of nanomaterials: small-molecule-initiated misfolding of nanoparticle corona proteins. *J. Am. Chem. Soc.* **134**, 4100-4103 (2012).
37. Ferrari, M. Cancer nanotechnology: opportunities and challenges. *Nature Rev. Cancer* **5**, 161-171 (2005).
38. Stephan, M.T., Moon, J.J., Um, S.H., Bershteyn, A. & Irvine, D.J. Therapeutic cell engineering with surface-conjugated synthetic nanoparticles. *Nature Med.* **16**, 1035-1041 (2010).
39. Otsuka, H., Nagasaki, Y. & Kataoka, K. PEGylated nanoparticles for biological and pharmaceutical applications. *Adv. Drug Deliv. Rev.* **55**, 403-419 (2003).
40. Hamad, I. *et al.* Distinct polymer architecture mediates switching of complement activation pathways at the nanosphere-serum interface: implications for stealth nanoparticle engineering. *ACS Nano* **4**, 6629-6638 (2010).
41. Hoshino, Y. *et al.* Recognition, neutralization, and clearance of target peptides in the bloodstream of living mice by molecularly imprinted polymer nanoparticles: a plastic antibody. *J. Am. Chem. Soc.* **132**, 6644-6645 (2010). **One of the first efforts to use molecular imprinting to control the protein corona on the nanoparticle surface.**
42. Lesniak, A. *et al.* Effects of the presence or absence of a protein corona on silica nanoparticle uptake and impact on cells. *ACS Nano* **6**, 5845-5857 (2012).
43. Mercer, J., Schelhaas, M. & Helenius, A. Virus entry by endocytosis. *Annu. Rev. Biochem.* **79**, 803-833 (2010).
44. Hu, W. *et al.* Protein corona-mediated mitigation of cytotoxicity of graphene oxide. *ACS Nano* **5**, 3693-3700 (2011).
45. Verma, A. *et al.* Surface-structure-regulated cell-membrane penetration by monolayer-protected nanoparticles. *Nature Mater.* **7**, 588-595 (2008).
46. Salvati, A. *et al.* Experimental and theoretical comparison of intracellular import of polymeric nanoparticles and small molecules: towards models of uptake kinetics. *Nanomed. Nanotech. Biol. Med.* **7**, 816-826 (2011).
47. Doherty, G.J. & McMahon, H.T. Mechanisms of endocytosis. *Annu. Rev. Biochem.* **78**, 857-902 (2009).
48. Dausend, J. *et al.* Uptake mechanism of oppositely charged fluorescent nanoparticles in HeLa cells. *Macromol. Biosci.* **8**, 1135-1143 (2008).
49. Iversen, T.G., Skotland, T. & Sandvig, K. Endocytosis and intracellular transport of nanoparticles: present knowledge and need for future studies. *Nano Today* **6**, 176-185 (2011).
50. Shapero, K. *et al.* Time and space resolved uptake study of silica nanoparticles by human cells. *Mol. Biosyst.* **7**, 371-378 (2011).

51. Lesniak, A. *et al.* Serum heat inactivation affects protein corona composition and nanoparticle uptake. *Biomater.* **31**, 9511-9518 (2010).
52. Ehrenberg, M.S., Friedman, A.E., Finkelstein, J.N., Oberdörster, G. & McGrath, J.L. The influence of protein adsorption on nanoparticle association with cultured endothelial cells. *Biomater.* **30**, 603-610 (2009).
53. Zhu, Y. *et al.* Effects of serum proteins on intracellular uptake and cytotoxicity of carbon nanoparticles. *Carbon* **47**, 1351-1358 (2009).
54. Bajaj, A., Samanta, B., Yan, H., Jerry, D.J. & Rotello, V.M. Stability, toxicity and differential cellular uptake of protein passivated-Fe3O4 nanoparticles. *J. Mater. Chem.* **19**, 6328-6331 (2009).
55. Lunov, O. *et al.* Differential uptake of functionalized polystyrene nanoparticles by human macrophages and a monocytic cell line. *ACS Nano* **5**, 1657-1669 (2011).
56. Kapralov, A.A. *et al.* Adsorption of surfactant lipids by single-walled carbon nanotubes in mouse lung upon pharyngeal aspiration. *ACS Nano* **6**, 4147-4156 (2012).
57. Konduru, N.V. *et al.* Phosphatidylserine Targets Single-Walled Carbon Nanotubes to Professional Phagocytes In Vitro and In Vivo. *PLoS ONE* **4**, e4398 (2009).
58. Georgieva, J.V. *et al.* Surface characteristics of nanoparticles determine their intracellular fate in and processing by human blood-brain barrier endothelial cells in vitro. *Mol. Ther.* **19**, 318-325 (2011).
59. Kreuter, J. *et al.* Apolipoprotein-mediated transport of nanoparticle-bound drugs across the blood-brain barrier. *J. Drug Target.* **10**, 317-325 (2002).
60. Schleh, C., Rothen-Rutishauser, B. & Kreyling, W.G. The influence of pulmonary surfactant on nanoparticulate drug delivery systems. *Eur. J. Pharm. Biopharm.* **77**, 350-352 (2011).
61. Boraschi, D., Costantino, L. & Italiani, P. Interaction of nanoparticles with immunocompetent cells: nanosafety considerations. *Nanomedicine (Lond.)* **7**, 121-131 (2011).
62. Huo, Q. *et al.* A facile nanoparticle immunoassay for cancer biomarker discovery. *J. Nanobiotechnology* **9**, 20 (2011).
63. Ghosh, P. *et al.* Intracellular delivery of a membrane-impermeable enzyme in active form using functionalized gold nanoparticles. *J. Am. Chem. Soc.* **132**, 2642-2645 (2010).
64. Doorley, G.W. & Payne, C.K. Cellular binding of nanoparticles in the presence of serum proteins. *Chem. Commun.* **47**, 466-468 (2011).
65. Sée, V. *et al.* Cathepsin L digestion of nanobioconjugates upon endocytosis. *ACS Nano* **3**, 2461-2468 (2009).
66. Marano, F., Hussain, S., Rodrigues-Lima, F., Baeza-Squiban, A. & Boland, S. Nanoparticles: molecular targets and cell signalling. *Arch. Toxicol.* **85**, 733-741 (2011).
67. Wang, J. *et al.* Soft interactions at nanoparticles alter protein function and conformation in a size dependent manner. *Nano Lett.* **11**, 4985-4991 (2011).
68. Gagner, J.E., Lopez, M.D., Dordick, J.S. & Siegel, R.W. Effect of gold nanoparticle morphology on adsorbed protein structure and function. *Biomater.* **32**, 7241-7252 (2011).

69. Roach, P., Farrar, D. & Perry, C.C. Surface tailoring for controlled protein adsorption: effect of topography at the nanometer scale and chemistry. *J. Am. Chem. Soc.* **128**, 3939-3945 (2006).
70. Mandal, H.S. & Kraatz, H.B. Effect of the surface curvature on the secondary structure of peptides adsorbed on nanoparticles. *J. Am. Chem. Soc.* **129**, 6356-6357 (2007).
71. Brown, D.M., Dickson, C., Duncan, P., Al-Attili, F. & Stone, V. Interaction between nanoparticles and cytokine proteins: impact on protein and particle functionality. *Nanotech.* **21**, 215104 (2010).
72. Lacerda, S.H. *et al.* Interaction of gold nanoparticles with common human blood proteins. *ACS Nano* **4**, 365-379 (2010).
73. Linse, S. *et al.* Nucleation of protein fibrillation by nanoparticles. *Proc. Natl. Acad. Sci. USA* **104**, 8691-8696 (2007).
74. Hubbell, J.A., Thomas, S.N. & Swartz, M.A. Materials engineering for immunomodulation. *Nature* **462**, 449-460 (2009).
75. Fadeel, B. & Garcia-Bennett, A.E. Better safe than sorry: understanding the toxicological properties of inorganic nanoparticles manufactured for biomedical applications. *Adv. Drug Deliv. Rev.* **62**, 362-374 (2010).
76. Karmali, P.P. & Simberg, D. Interactions of nanoparticles with plasma proteins: implication on clearance and toxicity of drug delivery systems. *Expert Opin. Drug Deliv.* **8**, 343-357 (2011).
77. Salvador-Morales, C. *et al.* Complement activation and protein adsorption by carbon nanotubes. *Mol. Immunol.* **43**, 193-201 (2006).
78. Xia, X.R., Monteiro-Riviere, N.A. & Riviere, J.E. An index for characterization of nanomaterials in biological systems. *Nature Nanotech.* **5**, 671-675 (2010).
79. Nanda, K.K., Maisels, A., Kruis, F.E., Fissan, H. & Stappert, S. Higher surface energy of free nanoparticles. *Phys. Rev. Lett.* **91**, 106102 (2003).
80. Chakraborty, S. *et al.* Contrasting effect of gold nanoparticles and nanorods with different surface modifications on the structure and activity of bovine serum albumin. *Langmuir* **27**, 7722-7731 (2011).
81. Dutta, D. *et al.* Adsorbed proteins influence the biological activity and molecular targeting of nanomaterials. *Toxicol. Sci.* **100**, 303-315 (2007).
82. Caracciolo, G. *et al.* Evolution of the protein corona of lipid gene vectors as a function of plasma concentration. *Langmuir* **27**, 15048-15053 (2011).
83. Gessner, A., Paulke, B.R. & Müller, R.H. Analysis of plasma protein adsorption onto polystyrene particles by two-dimensional electrophoresis: comparison of sample application and isoelectric focusing techniques. *Electrophoresis* **21**, 2438-2442 (2000).
84. Sund, J., Alenius, H., Vippola, M., Savolainen, K. & Puustinen, A. Proteomic characterization of engineered nanomaterial-protein interactions in relation to surface reactivity. *ACS Nano* **5**, 4300-4309 (2011).
85. Hellstrand, E. *et al.* Complete high-density lipoproteins in nanoparticle corona. *FEBS J.* **276**, 3372-3381 (2009).
86. Zeng, Z. *et al.* Synthetic polymer nanoparticle-polysaccharide interaction: a systematic study. *J. Am. Chem. Soc.* **134**, 2681-2690 (2012).
87. Lartigue, L. *et al.* Nanomagnetic sensing of blood plasma protein interactions with iron oxide nanoparticles: impact on macrophage uptake. *ACS Nano* **6**, 2665-2678 (2012).

88. Hunter, R.J. *Foundations of Colloid Science*, Edn. 2. (Oxford University Press, Oxford, 2001).
89. Tsai, D.H. *et al.* Adsorption and conformation of serum albumin protein on gold nanoparticles investigated using dimensional measurements and in situ spectroscopic methods. *Langmuir* **27**, 2464–2477 (2011).
90. Turci, F. *et al.* An integrated approach to the study of the interaction between proteins and nanoparticles. *Langmuir* **26**, 8336-8346 (2010).
91. Sapsford, K.E., Tyner, K.M., Dair, B.J., Deschamps, J.R. & Medintz, I.L. Analyzing nanomaterial bioconjugates: a review of current and emerging purification and characterization techniques. *Anal. Chem.* **83**, 4453-4488 (2011).
92. Li, L., Mu, Q., Zhang, B. & Yan, B. Analytical strategies for detecting nanoparticle-protein interactions. *Analyst* **135**, 1519-1530 (2010).
93. Henzler, K. *et al.* Interaction strength between proteins and polyelectrolyte brushes: a small angle X-ray scattering study. *Phys. Chem. Chem. Phys.* **13**, 17599-17605 (2011).
94. Brewer, S.H., Glomm, W.R., Johnson, M.C., Knag, M.K. & Franzen, S. Probing BSA binding to citrate-coated gold nanoparticles and surfaces. *Langmuir* **21**, 9303-9307 (2005).
95. Liu, S. *et al.* Investigations on the interactions between plasma proteins and magnetic iron oxide nanoparticles with different surface modifications. *J. Phys. Chem. C* **114**, 21270-21276 (2010).
96. de Puig, H., Federici, S., Baxamusa, S.H., Bergese, P. & Hamad-Schifferli, K. Quantifying the nanomachinery of the nanoparticle-biomolecule interface. *Small* **7**, 2477–2484 (2011).
97. Giri, J. *et al.* Interactions of poly(amidoamine) dendrimers with human serum albumin: binding constants and mechanisms. *ACS Nano* **5**, 3456-3468 (2011).
98. Wu, Z.C., Zhang, B. & Yan, B. Regulation of enzyme activity through interactions with nanoparticles. *Int. J. Mol. Sci.* **10**, 4198-4209 (2009).
99. Obata, S. & Honda, K. Dynamic behavior of carbon nanotube and bio-/artificial surfactants complexes in an aqueous environment. *J. Phys. Chem. C* **115**, 19659-19667 (2011).
100. Hung, A. *et al.* Ordering surfaces on the nanoscale: implications for protein adsorption. *J. Am. Chem. Soc.* **133**, 1438-1450 (2011).
101. Dell'Orco, D., Lundqvist, M., Oslakovic, C., Cedervall, T. & Linse, S. Modeling the time evolution of the nanoparticle-protein corona in a body fluid. *PLoS ONE* **5**, e10949 (2010).
102. Gao, J. *et al.* Dispersion and toxicity of selected manufactured nanomaterials in natural river water samples: effects of water chemical composition. *Environ. Sci. Technol.* **43**, 3322-3328 (2009).
103. Keller, A.A. *et al.* Stability and aggregation of metal oxide nanoparticles in natural aqueous matrices. *Environ. Sci. Technol.* **44**, 1962-1967 (2010).
104. Schulze, C., Schaefer, U.F., Ruge, C.A., Wohlleben, W. & Lehr, C.M. Interaction of metal oxide nanoparticles with lung surfactant protein A. *Eur. J. Pharm. Biopharm.* **77**, 376-383 (2011).
105. Kreyling, W.G. *et al.* Size dependence of the translocation of inhaled iridium and carbon nanoparticle aggregates from the lung of rats to the blood and secondary target organs. *Inhal. Toxicol.* **21**, 55-60 (2009).

106. Faunce, T.A., White, J. & Matthaei, K.I. Integrated research into the nanoparticle–protein corona: a new focus for safe, sustainable and equitable development of nanomedicines. *Nanomedicine (Lond.)* **3**, 859-866 (2008).
107. Salvati, A. *et al.* Nanoparticle targeting efficiency is determined by the biological milieu; protein corona effects at the bio-nano interface. *Nature Nanotech.*, Under review (2012).
108. Sandin, P., Fitzpatrick, L.W., Simpson, J.C. & Dawson, K.A. High-speed imaging of Rab family small GTPases reveals rare events in nanoparticle trafficking in living cells. *ACS Nano* **6**, 1513-1521 (2012).
109. Lowry, G.V., Gregory, K.B., Apte, S.C. & Lead, J.R. Transformations of nanomaterials in the environment. *Environ. Sci. Technol.* **46**, 6893-6899 (2012).
110. Quik, J.T. *et al.* Effect of natural organic matter on cerium dioxide nanoparticles settling in model fresh water. *Chemosphere* **81**, 711-715 (2010).

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Additional information

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Figure legends

Figure 1. The nanoparticle-corona complex in a biological environment. **a,** It is the nanoparticle corona complex, rather than the bare nanoparticle, that interacts with biological machinery, here with a cell membrane receptor. **b,** Relevant processes

(arrows), in both directions (on/off), for a nanoparticle interacting with a receptor. Biomolecules in the environment adsorb strongly to the bare nanoparticle surface (k_1), forming a tightly bound layer of biomolecules, the 'hard' corona, in immediate contact with the nanoparticle. Other biomolecules, the 'soft' corona, have a residual affinity to the nanoparticle-hard corona complex (primarily to the hard corona itself), but this is much lower, so those molecules are in rapid exchange with the environment (k_2). If sufficiently long-lived in the corona, a biomolecule may lead to recognition by a cell membrane receptor of the nanoparticle-corona complex as a whole (k_3). The same biomolecule alone can also be recognised by the receptor (k_4). If present, the bare surface of the nanoparticle may also interact with cell surface receptors (k_5) or other constituents of the cell membrane.

Figure 2. The evolution of the nanoparticle corona through the body. **a,** An example for inhaled nanoparticles in the alveoli of the lungs. **b,** An original corona (blue) forms when the nanoparticle comes in contact with the lung fluids in the alveoli. Upon subsequent translocation across the lung barrier (composed of a layer of epithelial cells), nanoparticles reach the blood stream. In blood, some biomolecules from the original corona may be displaced by different biomolecules (purple) in this new compartment, forming a new corona. **c,** Nanoparticle crossing the epithelial cells of the lung barrier, via regulated import, further transport through different membrane compartments and final export out from the cell. Partial displacement of the original corona (blue) by intracellular biomolecules (green) could potentially occur at any point along the pathway, or only once the nanoparticle is exposed to biomolecules in the blood (purple).

Figure 3. Formation of the biomolecular corona. From left to right: An initial corona forms from those biomolecules (in green) that arrive first to the surface (typically highly abundant proteins). An initially adsorbed molecule with low affinity (in green) is subsequently displaced by a different molecule with higher affinity (in blue) arriving later. A third molecule (in yellow), that initially had a low affinity for the bare surface, now adsorbs on the nanoparticle surface, due to favourable interactions with the adsorbed (green and blue) biomolecules. A different biomolecule (in red) does not adsorb at all.

Figure 4. Epitope exposure on the nanoparticle-corona interface. **a**, If the epitopes of proteins in the corona are not exposed, they are not recognised by the corresponding receptor (or other ligand). **b,** The epitope on the corona is exposed and can be recognised. **c,** Multiple (similar) epitopes are exposed, leading to a higher biological recognition. **d,** Several different epitopes of different proteins (shown in different colours) are exposed, leading potentially to anomalous or unusual combinations, which could be recognised by a different receptor (in green).

Nanoparticle and protein sample	Relative abundance of top 20 proteins (%)	Total number of proteins identified
PS unmodified 200nm in 10% plasma[14]	85.4	52
PS unmodified 200nm in 55% plasma[14]	92.9	49
Silica 200nm in 10% plasma[14]	94.9	47
Silica 200nm in 55% plasma[14]	90.2	51
Carbon apatite in human serum[20]	73.5	100
Silica 55 nm (si125) in plasma[5]	71.2	125
Silica 16 nm (si20) in plasma[5]	72.1	125
Silica 10 nm (si8) in plasma[5]	70.4	125

Table 1. Quantitative abundance of proteins in the biomolecular corona of some example nanoparticles exposed to blood proteins (from previous literature). The 20 most abundant proteins identified constitute the majority of the proteins in the 'average corona'. The abundance of the remaining proteins are, however, not negligible, and the number of such proteins is large. This gives an idea of the variance of protein abundance; specifically, it suggests that nanoparticles do not have identical coronas (e.g. it seems clear that not all 125 proteins can be found on the 10 nm particle of the last sample, even if packed in a bilayer).