Cellular Response of Therapeutic Nanoparticles

7

Avnesh Kumari, Rubbel Singla, Anika Guliani, Amitabha Acharya and Sudesh Kumar Yadav

Abstract

Nanoparticles (NPs) are being extensively used in the field of nanomedicines. Different types of NPs are administered into the body by various routes. NPs come in contact with cells inside the body. Cellular response of NPs is affected by size, shape, surface chemistry, and cellular uptake pathways of NPs. In addition to this, type of cells, various cell lines, and growth media are also found to affect the cellular response of NPs. NPs induce diverse cellular responses like apoptosis, necrosis, and reactive oxygen species (ROS) production. NPs also form a protein corona inside the biological media which may alter their identity and behaviour as compared to bare NPs. In this chapter, we have made an attempt to throw light on cellular uptake pathways of NPs, monitoring of endocytic pathways followed by NPs, factors affecting cellular responses of therapeutic NPs, and protein corona formation, characterisation and its implications on fate of NPs.

Keywords

Cellular uptake · Endocytic pathways · Protein corona · Hard corona · Soft corona

A. Kumari · R. Singla · A. Guliani · A. Acharya (✉) · S.K. Yadav
Department of Biotechnology, Council of Scientific and Industrial Research-Institute of Himalayan Bioresource Technology, Palampur 176061, Himachal Pradesh, India
e-mail: amitabhachem@gmail.com
amitabha@ihbt.res.in

A. Kumari · R. Singla · A. Guliani · A. Acharya · S.K. Yadav
Academy of Scientific and Innovative Research, New Delhi, India

S.K. Yadav (✉)
Department of Biotechnology, Center of Innovative and Applied Bioprocessing (CIAB), Mohali 160071, Punjab, India
e-mail: skyt@rediffmail.com; sudesh@ciab.res.in

S.K. Yadav (ed.), *Nanoscale Materials in Targeted Drug Delivery, Theragnosis and Tissue Regeneration*, DOI 10.1007/978-981-10-0818-4_7

Contents

7.1 Introduction

Recent developments in the biomedical sciences such as hyperthermia cancer therapy (Hainfeld et al. 2004; El-Sayed et al. 2005; El-sayed et al. 2006), targeted gene, and drug delivery (Sandhu et al. 2002; Koo et al. 2005) involve the interaction of nanoparticles (NPs) with living organisms, at the cellular level (Alivisatos et al. 2005; Parak et al. 2005; Luccardini et al. 2007). All these applications of NPs involve administration of NPs by different routes. After administration by various routes, NPs come in contact with cells. NPs may not only enhance the pharmacological effects of drugs, but also cause unwanted effects in the target cells (Nishikawa et al. 2009). Many factors affect the biological response of NPs with cells.

Cellular response of NPs can be varied by changing the type of NPs. Changing of even one variable can induce a very different outcome for NPs exposed to cells (Oh et al. 2011). The biological responses of NPs are dependent on size, charge, chemical composition, shape and surface chemistry (Verma and Stellacci 2010). In addition, targeting moieties such as tripeptide glutathione, cell penetrating peptides, nuclear localisation signal peptides, and proteins can also affect the cellular response (Oh et al. 2011). These parameters can also affect the cellular uptake, and the biological response of NPs (Oberdorster et al. 2005; Xia et al. 2008a, b). NPs in the blood stream are known to encounter with plasma proteins and immune cells. Processes such as adsorption of proteins create unfavourable condition for cellular uptake. Uptake of NPs by immune cells may occur by various pathways and can be enhanced by adsorption of plasma proteins. NPs coated with targeting moieties activate their receptor-mediated internalisation. The activation of membrane receptors strongly depends on the size of nanomaterials. NPs coated with targeting moieties are also found to be activating cell signalling processes essential for basic cell functions including cell death (Jiang et al. 2008).

Cellular uptake of NPs also has an influence on response of NPs towards cells. They are involved in manipulation of signal transductions leading to the expression of cell functions (Nishikawa et al. 2009).

Owing to currently arising opportunities and concerns associated with NPs in living systems, it is of immense importance to develop an understanding of the complex processes that govern their cellular response and intracellular

fate of nanomaterials (NMs). Interestingly, the interaction between NPs and molecules of the biological milieu results in the formation of a biological coating on the surface of NPs (Lynch and Dawson 2008). Therefore, biological entity which interacts with cells, tissues and organs, is completely different from the original surface of the NPs. Formation of biomolecule corona is a dynamic process and biomolecules like proteins and lipids present in the biological fluids compete for NPs surface to form biomolecule corona (Mahmoudi et al. 2011a, b). The biomolecule corona is usually enriched with about 10–50 proteins, that have the maximum affinity for the NPs surface, out of several thousand proteins of the biological milieu. New and advanced techniques are required for deep understanding of both the thermodynamics and kinetics of the biomolecule corona evolution and its subsequent biological impacts (Mahmoudi et al. 2011a, b).

The biological responses to NPs are greatly affected by the key forces at the bio-nano interface and also by the inherent characteristics of the NPs like size, shape, charge, coatings, surface modifications with targeting ligands, crystallinity, electronic states, surface wrapping in the biological medium, hydrophobicity, and wettability. Therefore, a thoughtful investigation of the NP biomolecule complex is essential for the development of therapeutically safe NPs (Nel et al. 2009). In this chapter, we have covered pathways for cellular uptake of NPs, monitoring of endocytic pathways, cellular response of therapeutic NPs, and formation, characterisation, and factors affecting biomolecule corona of NPs.

7.2 Pathways for Cellular Uptake of Nanoparticles

The invasion of NPs in human body occurs via inhalation, ingestion or through the skin. Once these tiny particles enter a biological milieu, they will inevitably come into contact with a huge variety of biomolecules including proteins, sugars and lipids which are dissolved in body fluids. Thus, the NPs have to be viewed as evolving systems which adapt to varying concentrations of the biomolecules present in the fluid. NPs enter the cells mainly through the passive diffusion or active transport, while nanomedicines got entry into cells via endocytosis which helps the drug to penetrate the specific cells and get accumulated (Fig. 7.1). The endocytosis pathway has been classified according to the proteins which play a major role in the process. Correspondingly, the mechanism of interaction of NPs with cytomembrane which governs the entrance and travel of NPs inside cells has also been extensively studied.

Various aspects such as pathways of entrance, factors affecting the pathways, functions of some proteins involved in endocytosis are still uncertain and are not absolutely proven. Such study is necessary for better understanding of the novel field of multifunction nanomedicines. NPs uptake into cells occurs through endocytosis (Jones et al. 2003), a process by which cells absorb NPs from outside by engulfing them within their cell membrane (Conner and Schmid 2003). This process of cellular uptake is further categorised into two phenomena, namely phagocytosis and pinocytosis (Fig. 7.1). Phagocytosis was originally discovered in macrophages. Phagocytes such as macrophages, neutrophils and monocytes destroy foreign particles such as NPs in blood through the phagocytosis process (Watson et al. 2005). Relatively, large NPs prefer this mechanism which initially involves recognition by opsonin viz., immunoglobulin (IgG and IgM), complement component (C3, C4, and C5) and blood serum proteins. Thereafter, the NPs bind to the cell surface receptors inducing the cup-shaped membrane extension formation. Such membrane extensions encircle the NPs and then internalise them, forming the phagosomes of diameter 0.5–10 μm which finally move to fuse with lysosomes (Aderem and Underhill 1999). Pinocytosis has been reported to occur by four different mechanisms: macropinocytosis, caveolin-dependent endocytosis, clathrin-dependent endocytosis and clathrin/caveolin-independent endocytosis (Fig. 7.1) (Swanson and Watts 1995; Patel et al. 2007; Xu et al. 2009). Macropinocytosis is a growth factor-induced, actin-driven endocytosis and a non-selective process for uptake of solute molecules or cargo. Macropinocytosis involves the formation

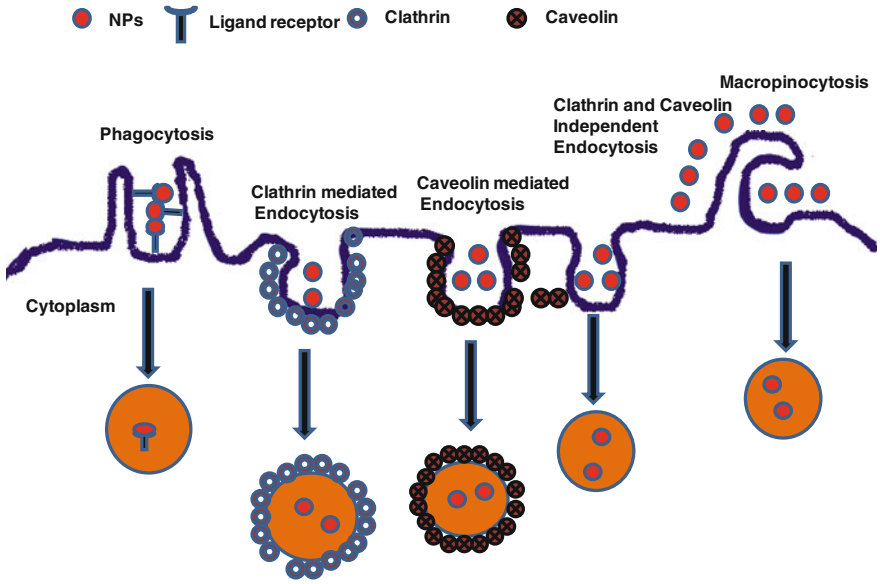

Fig. 7.1 Different uptake pathways followed by NPs for internalisation inside the cells. NPs follow phagocytosis, clathrin mediated endocytosis, caveolin mediated endocytosis, clathrin and caveolin-independent endocytosis and macropinocytosis pathways

of lamellipodia-like plasma membrane extensions. Interestingly, macropinosomes can uptake NPs of >200 nm in size (Walsh et al. 2006). Cellular uptake by macropinocytosis has been reported for PEGylated-poly-L-lysine NPs (Walsh et al. 2006). Receptor-mediated endocytosis process is assisted by specific proteins, either clathrin or caveolae (Bao and Bao 2005). Caveolin, a protein exists in most cells plays a dominant role in caveolae-dependent endocytosis process. This pathway bypasses lysosomes (Benmerah and Lamaze 2007), thus many pathogens including viruses and bacteria select this pathway to avoid lysosomal degradation (Medina-Kauwe 2007). Transferrin-coated PLGA NPs are highly absorbed by brain endothelial cells via caveolae pathway (Chang et al. 2009). Similarly, clathrin-coated pits have the ability to accumulate NPs only up to 100 nm (Pelkmans and Helenius 2002) and targeted (receptor) NPs are internalised by clathrin-mediated endocytosis (Walsh et al. 2006). The internalisation is more efficient for NPs smaller than the caveolae. Clathrin/caveolae-independent endocytosis is a distinct pathway which relies on cholesterol and requires specific lipid compositions. NPs endocytosis by cells not only depends on the size, but also on surface properties of the NPs. Carboxy dextran-coated superparamagnetic iron oxide NPs (SPION) have been internalised by human mesenchymal stem cells and the efficiency of internalisation was correlated with the amount of carboxyl groups on the NPs surface (Mailänder et al. 2008). Cationic NPs enter HeLa cells in greater amounts than anionic NPs (Harush-Frenkel et al. 2007; Dausend et al. 2008). NPs uptake may also depends on the length of the molecules on the surface coating (Chang et al. 2009), or the type of cells (Xia et al. 2008a, b).

7.3 Monitoring Endocytic Pathways

Researchers have been interested in identifying the different pathways that NPs use during their internalisation to cells. Either endocytic markers or inhibitors have been used for long time to locate NPs and confirm whether the corresponding pathway plays an important role in the

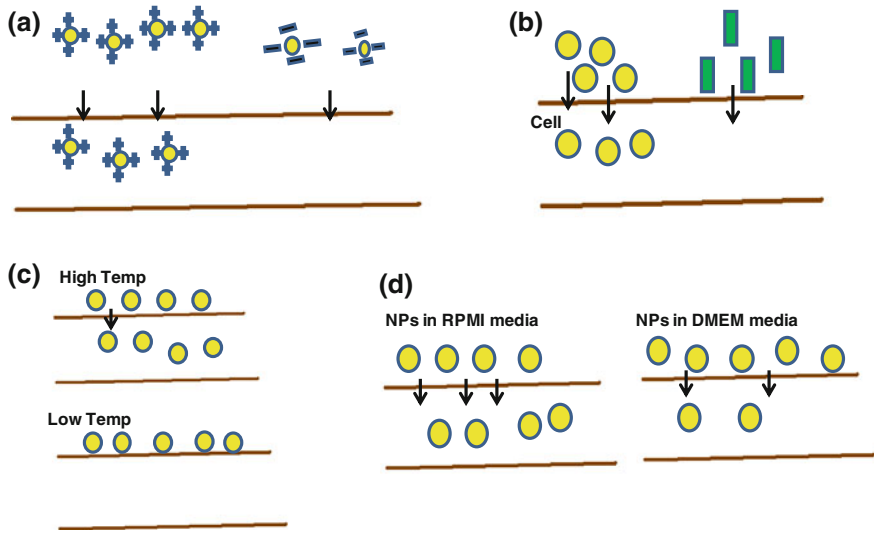

Fig. 7.2 Factors affecting cellular response of NPs **a** *Size* Positively charged NPs are well taken up by cells. **b** *Shape* Spherical NPs are efficiently taken up by cells **c** *Temperature* High temperature allows both cellular uptake and interaction, whereas at low temperature only interaction of NPs takes place. **d** *Cell culture media* NPs incubated in RPMI media showed more cell uptake than NPs incubated in DMEM media

uptake of the NPs. More recently, a combined approach has been postulated for better understanding of such mechanisms. Low-density lipoprotein (Duit et al. 2010) and transferrin (Liu et al. 2010a) enter into cells through clathrin-dependent endocytosis, and hence these are commonly used markers for clathrin-dependent endocytosis process. Similarly, caveolin-1 is mostly used as a marker of caveolae-dependent endocytosis, and dextran is the marker of macropinocytosis (Petrescu et al. 2009). Inhibitors can be used to block certain endocytic pathways to verify whether this route has been used by NPs to enter cells. It has been reported that amiloride, cytochalasin D and rottlerin can block macropinocytosis (Diken et al. 2011), whereas chlorpromazine (50–100 μm), hypertonic sucrose (0.4–0.5 m) and potassium depletion can be used to inhibit the clathrin-dependent endocytosis (Ivanov 2008). Similarly, methyl-β-cyclodextrin, filipin, nystatin and cholesterol oxidase can be used as the inhibitors for caveolae-dependent endocytosis (Diken et al. 2011).

7.4 Factors Affecting Cellular Response of Nanoparticles

NPs possess intricate structures and surface chemistry. In biological systems, they may play an undecided role (Xie et al. 2009). Response of NPs with cells depends on the type of cells, cell culture media, size, shape, surface charge, surface moieties, temperature and route of administration of NPs (Fig. 7.2). These are the key parameters for particle binding and wrapping by the surface membrane as well as defining the path of cellular uptake (Xia et al. 2008a, b). Type of cells and cell lines also affect the cellular response. Cell lines grown in different culture media showed different cellular response to NPs (Maiorano et al. 2010). In addition to this, type of NPs also has an effect on its fate inside the cells. NPs are synthesised from materials of different compositions. Biodegradable NPs are rapidly cleared from the cells (Chellat et al. 2005), while non-biodegradable particles are retained inside the body for longer duration (Niidome et al.

2006). NPs uptake by cells is also affected by their shape. Shape of NPs has not only affected the cellular uptake but also the internalisation. Spherical particles are internalised at a higher rate in endothelial cells (Jun et al. 2005).

Surface chemistry of NPs has immense role to play in the cellular response of NPs (Bartneck et al. 2010) Polyethylene oxide (PEO)-coated gold nanorods with amine end groups have exhibited anti-inflammatory properties, whereas carboxy terminated led to proinflammatory effects. NMs surface chemistry has influence on the expression of inflammatory genes and phenotype of macrophages (Bartneck et al. 2010). Surface charge of NPs is also reported to affect the response of NPs with cells. NPs with cationicsurface charge show positive response with negatively charged cell membrane. The internalisation of negatively charged NPs is believed to occur through non-specific binding and clustering of NPs on cationic sites of the plasma membrane and their subsequent endocytosis. Negatively charged NPs have displayed a less efficient rate of endocytosis. NPs with positive surface charge depolarize the plasma membrane leading to increased Ca^{2+} influx (Yue et al. 2011). Positively charged NPs normally escape from lysosome and reside in perinuclear region, whereas the negatively charged NPs prefer to co-localise with lysosome (Yue et al. 2011).

Cellular uptake of positively charged particles into lysosomal compartments could lead to cytotoxicity by acidifying proton pump (Boussif et al. 1995). In addition, the positively charged NPs (Fig. 7.2) have been shown to target cell membranes through strong binding to phospholipid components, which can lead to membrane disruption (Asokan and Cho 2002).

Charged NPs can also activate complement system, hemolysis and thrombogenicity. Charged NPs are more aggressively invoked complement system than neutral ones (Bartlett and Davis 2007; Nagayama et al. 2007). Gold NPs activate the immune system (Bastus et al. 2009). Bare lipid NMs show high cellular uptake due to non-specific internalisation through charge interaction of the positively charged NPs with the negatively charged cell surface. The PEGylation of the NPs limited the non-specific charge interaction and resulted in reduced cellular uptake of NPs (Wang et al. 2009).

Targeting moieties attached to the surface of NPs decide their fate inside the cells. NPs with specific recognition moieties bound to the surface have a good potential for site-selective uptake as well as improved specificity for drug targeting (Dinauer et al. 2005). This strategy is used to direct NPs to cell surface carbohydrates, receptors and antigens (Sinha et al. 2006). Moieties attached to the surface can include any molecule that selectively recognises and binds molecules on target cells (Sapra and Allen 2003). Antibodies, oligopeptides, carbohydrates, glycolipids and folic acid are the most widely used moieties for targeting different cells and tissues. Targeted NPs can bind more specifically to cancer cells than normal cells. NPs without specific moieties can attack normal cells as well. Peptide-conjugated gold NPs activate the macrophage system. Macrophage activation by gold NPs depend on the peptide pattern at the NPs surface (Bastus et al. 2009). Targeted NPs are internalised by cancer cells through receptor-mediated endocytosis enhancing cancer cell killing (Hu et al. 2010). NPs are internalised by different uptake pathways which also affect the cellular response (Xia et al. 2008a, b; Kumari and Yadav 2011). Multiwalled carbon nanotubes (MWCNTs) and quantum dots delivered by nano-channel electroporation undergo nonendocytic uptake in BEAS-2B cells and result in higher cell death (Zhao et al. 2015). Cellular uptake of NPs is also affected by the type of cells. Different types of cells internalise NPs to a different extent. Gold NPs with positive surface charge have shown more cell internalisation ability than those with negative surface charge in non-phagocytic cells. However, the internalised amount of negatively charged gold NPs was similar with that of the positively charged gold NPs in phagocytic cells (Liu et al. 2013).

Polystyrene NPs have been efficiently internalised by human ATI cell line (TT1) cells, while uptake of NPs by primary human ATII cells was negligible (Thorley et al. 2014). It has been

shown that extent of NPs uptake, kinetics of NPs uptake and NPs internalisation mechanisms differ between primary cells and phenotypically linked cells (Lunov et al. 2011). Pericellular matrix (PCM) of cell has been documented for a role in accumulation and enhancing uptake of NPs (Zhou et al. 2012). Cellular uptake of NPs is also modulated by cell surface area and membrane tension. It has been reported that cellular internalisation is enhanced linearly with cell surface area and decreased exponentially with increasing membrane tension (Huang et al. 2013). Cell culture media also has an influence on NPs uptake and internalisation. TiO_2 NPs incubated in three different media showed different extent of uptake and internalisation. Uptake of TiO_2 NPs was maximum in media with highest amount of protein (Strickland et al. 2013). Gold NPs incubated in RPMI media showed more cellular uptake than NPs incubated in DMEM media (Maiorano et al. 2010).

7.5 Cellular Response of Therapeutic Nanoparticles

NPs are increasingly used in the field of drug delivery. NPs show various responses with cells. These include interactions with cellular membrane and cellular uptake, signalling pathways, ROS production, cell cycle dysregulation and necrosis or apoptosis (Jones and Grainger 2009). Many in vitro models are used to study the response of NPs with cells (Kumar et al. 2012). Metallic and polymeric NPs are extensively used in the field of nanomedicine due to their ease of preparation and surface modification. Understanding the cellular response of metallic, polymeric and silica NPs has recently fascinated the attention of scientific community.

7.5.1 Metallic Nanoparticles

Toxicity of silver nanoparticles (Ag NPs) to human hepatoma cells is the result of oxidative stress and is independent of the toxicity of Ag^+ ions (Jun et al. 2005). Polysaccharide-coated and uncoated Ag NPs are distributed differently and cause different levels of DNA damage in mouse embryonic stem cells (MES) and mouse embryonic fibroblast cells (MEF). Both types of Ag NPs induce p53 protein expression, DNA double-strand breakage and apoptosis responses in MES and MEF cells (Ahamed et al. 2008). Upon exposure to 6.25 μg/mL Ag NPs, morphology of both HT-1080 and A431 types of cells remain unaltered. However, at higher concentrations (6.25–50 μg/mL) of Ag NPs, cells became less polyhedra, more fusiform and shrunken. Changes in the levels of catalase and glutathione peroxidase in A431 and A431 types of cells are statistically insignificant. Ag NP exposure caused the DNA fragmentation in cells (Arora et al. 2008). Citrate-stabilised NPs show significant cellular response, while cellular treatment with nucleic acid or BSA functionalised NPs caused no detectable changes in gene expression, cell cycle progression or apoptosis induction (Massich et al. 2010). Nucleic acid-modified gold NPs have been reported for less immune response (Massich et al. 2009). The L929 cells become round and even shrunken on exposure to TiO_2 NPs (Jin et al. 2008). Moreover, TiO_2 NPs-treated cells either show condensation of fragmented chromatin or directly necrosed. Cells cultured in a medium containing 300 μg/mL TiO_2 have increased the number of lysosomes and damaged some of the cytoplasmic organelles. Gold NPs with weakly bound ligands have shown significant cellular responses, while gold NPs with strongly bound ligands have shown weak cellular responses (Massich et al. 2010). Gold NPs have also been observed to induce cell death in human carcinoma lung cell line A549. In contrast, BHK21 (baby hamster kidney) and HepG2 (human hepatocellular liver carcinoma) cell lines remain unaltered by gold NPs treatment (Patra et al. 2007). ZnO NPs showed dose-dependent toxicity in RAW 264.7 cells, higher cellular uptake and elevated intracellular ROS level (Hong et al. 2013).

7.5.2 Silica Nanoparticles

SiO_2 NPs are extensively used in the field of cancer therapy (Hirsch et al. 2003), DNA delivery (Bharali et al. 2005), drug delivery (Venkatesan et al. 2005) and enzyme immobilisation (Qhobosheane et al. 2001; Chen and von Mikecz 2005). SiO_2 NMs have caused decrease in number of molecules released per mast cell granule (Maurer-Jones et al. 2010). Exposure to 25–500 μg/mL of mesoporus SiO_2 NPs have been reported to inhibit cellular respiration in a concentration and time-dependent manner (Jin et al. 2007). Dye-doped silica NPs have shown low level of genotoxicity and cytotoxicity against the tested A549 cells (Jin et al. 2007). Silica nanotubes have exhibited growth inhibition in epithelial breast cancer cell line (MDA-MB-231) and primary umbilical vein endothelial cell line (HUVEC) (Nan et al. 2008) in a concentration-dependent manner.

7.5.3 Polymeric Nanoparticles

PLA NPs have elicited a strong cytotoxic T lymphocyte (CTL) response and a strong T helper cells-biased cytokine release in mice (Ataman-Önal et al. 2006). HIV envelope glycoproteins 140 carrying carnuba wax NPs have been reported to induce strong cellular/humoral response without inflammation (Arias et al. 2011). Polysiloxane NPs were endocytosed via caveolae in human aortic endothelial cells and enhanced nitric oxide release (Nishikawa et al. 2009). The incorporation of a bombesin peptide or RGD peptide via a PEG spacer in polymeric NMs was resulted in receptor-mediated cellular uptake and high gene silencing efficiency in U87 cells (Wang et al. 2009). Ligand-receptor recognition between cRGD and $\alpha_v\beta_3$ integrin has mediated the surface binding of RGD-targeted NMs to HUVECs and probably induced cRGD- targeted NPs to enter cells through caveolae and localised in the perinuclear regions (Liu et al. 2010b). cRGD functionalization of PLGA NPs has appreciably improved NP accumulation in tumour cells in vitro and resulted in improved tumour accumulation of NPs in a mouse model (Toti et al. 2010). Cationic polystyrene NPs have caused necrotic and apoptotic cell deaths in BEAS-2B and RAW264.7 cells, respectively (Xia et al. 2008a, b).

7.5.4 Quantum Dots

Quantum dots (QDs) with unique photochemical properties such as high photoluminescence and photo stability have shown great potential as a bimodal imaging agent, cancer diagnostics and drug delivery (Lee et al. 2010). Cetuximab-coated quantum dots have shown enhanced uptake in EGFR overexpressing A549 cells (Lee et al. 2010). Quantum dots have also undergone transformation in biological systems (Mahendra et al. 2008; Pettibone et al. 2013). Speciation of four types of CdSe/ZnS QDs in HepG2 cells has been studied in a recent report and it was observed that two chemical forms, named as QD-1 and QD-2, have been detected in HepG2 cells. QD-1 and QD-2 has been confirmed as QD-like NPs and a kind of cadmium metallothioneins complex, respectively (Peng et al. 2015). Matrix metalloproteases (MMP-2 and MMP-7) decorated quantum dots also have shown enhanced uptake in HT-1080 cells (Zhang et al. 2006). Ligand-conjugated QDs were not accumulated in vesicles of the early sorting pathways and were also able to reach the lysosomes of dendritic cells (Cambi et al. 2007). Tat peptide-conjugated quantum dots (Tat-QDs) has been internalised by macropinocytosis. The internalised Tat-QDs have sticked to the inner vesicle surfaces and trapped in cytoplasmic organelles and actively transported by molecular machines such as dyneins along microtubule tracks (Ruan et al. 2007).

7.5.5 Liposomes

Liposomes have long been considered good candidates for drug delivery. Effect of surface charge on the binding and endocytosis of

liposomes has been investigated on human ovarian carcinoma cell line (HeLa) and a murine-derived mononuclear macrophage cell line J774. HeLa cells have been found to endocytose positively charged liposomes to a greater extent than either neutral or negatively charged liposomes (Miller et al. 1998). In contrast, the extent of liposome interaction with J774 cells was greater for both cationic and anionic liposomes than for neutral liposomes (Miller et al. 1998). Tumour penetrating peptides (TPP) targeting liposomes have exhibited remarkably increased cellular accumulation by PC-3 tumour cells than bare liposomes (Yan et al. 2014). Liposomes modified with octaarginine have enhanced the efficiency of cross-presentation of ovalbumin in mouse bone marrow-derived dendritic cells (Nakamura et al. 2014). Hyaluronan (HA) coated liposomes have shown cellular uptake via lipid raft-mediated endocytosis in A549 cells. Once within cells, HA-liposomes have localised primarily to endosomes and lysosomes (Qhattal and Liu, 2011). Aptamer targeted liposomes have shown enhanced binding specificity and selectivity to CD44 expressing A549 and MDA-MB-231 cells (Alshaer et al. 2015). Annexin A5 functionalised liposomes bind to phosphatidylserine exposing apoptotic K562 cells with high specificity (Garnier et al. 2009).

7.6 Protein Corona Formation on Therapeutic Nanoparticles

Upon entering in biofluids, NPs surfaces were rapidly covered by selective sets of blood plasma proteins forming the protein corona (Cedervall et al. 2007a, b; Lindman et al. 2007; Mahmoudi et al. 2009; Mahmoudi et al. 2010; Walczyk et al. 2010). Upon entry of NPs into biological millieu, they were initially surrounded by high concentrations of free protein. Proteins moved towards the NP surface either by diffusion, or by travelling down a potential energy gradient. Protein adsorption in the neighbourhood of the NPs surface has occurred spontaneously only if it is thermodynamically favourable (Walkey and Chan 2012). In other words, if:

$$\Delta G_{ads} = \Delta H_{ads} - T\Delta S_{ads} < 0$$

where ΔG_{ads}, ΔH_{ads} and ΔS_{ads} are the changes in Gibbs free energy, enthalpy and entropy, respectively, during adsorption, and T is the temperature.

The formation of covalent and non-covalent bonds between NPs and protein, rearrangement of interfacial water molecules or conformational changes in either the protein or the NPs surface contribute to favourable changes in enthalpy ($\Delta H_{ads} < 0$), or entropy ($\Delta S_{ads} > 0$) (Walkey and Chan 2012). Type of protein (Lindman et al. 2007) and physiochemical properties of NPs determine the mechanism involved during adsorption and protein corona formation (De et al. 2007). Proteins interact with NPs surface through a portion known as domain during adsorption and protein corona formation. Adsorption of high-molecular weight protein kininogen to iron oxide NPs has occurred through its domain 5 (Simberg et al. 2009). Adsorption of proteins to NPs surface may involve interactions through many domains of the proteins (Walkey and Chan 2012). ΔG_{ads} determines the stability of the protein NPs corona. Proteins with large ΔG_{ads} stay with NPs surface, while proteins with small ΔG_{ads} desorb and return to solution (Norde 1994). Hydrophobic NPs interact strongly with proteins than those of hydrophilic (Walkey and Chan 2012). Hydrophobic or charged NPs cause more conformational changes in protein than their hydrophilic counterparts. Conformational changes of proteins are either reversible or irreversible and depend on the structure and chemistry of protein and NPs (Walkey and Chan 2012).

Protein corona is further divided into two types, viz. hard corona and soft corona (Fig. 7.3). Hard corona consists of an inner layer of selected proteins with a lifetime of several hours in slow exchange with the environment (Fig. 7.3). Soft corona

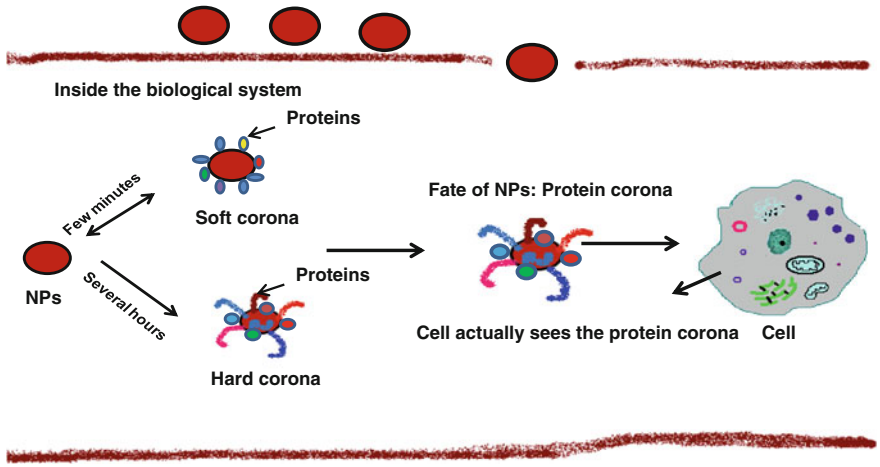

Fig. 7.3 Nanoparticles inside the biological systems. NPs form soft and hard corona after interaction with plasma proteins. Fate of NPs is decided by the protein corona of NPs

comprises of an outer layer of weakly bound proteins, which are characterised by a faster exchange rate with the free proteins (Mahmoudi et al. 2010). It is now believed that the hard corona interacts with cellular receptors and defines the fate of NPs in a biofluid due to the long lifetime of the hard protein corona (Mahmoudi et al. 2011a). It has been documented in a study that negatively charged NPs did not show the formation of hard protein corona while positively charged NPs show the formation of hard protein corona (Casals et al. 2010). First monolayer (hard corona) of transferrin binds irreversibly to polystyrene NPs and second monolayer (soft corona) is capable of exchanging proteins with solution (Milani et al. 2012).

7.7 Characterisation of Protein Corona on Nanoparticles

Upon contact with biological fluids, NPs interact strongly with proteins and other biomolecules, which drastically alter their surface characteristics. NPs with improved and changed biological activity will have the influence on NPs bio-distribution and their in vivo fate. Therefore, it is essential to apply and develop analytical tools and techniques to investigate the interactions of NPs with proteins in order to understand the protein corona composition and their possible biological activity. When NPs are meant for biological applications, it is necessary to investigate their physicochemical characteristics in the biological milieu (Mahmoudi et al. 2011a, b). As reported, many studies have been performed to shed light on the protein NPs association/dissociation processes in serum and plasma. As more than 3700 proteins coexist and compete for binding to the NPs surface, the determination of binding rates, affinities, and stoichiometries of protein association, and dissociation with NPs in biological fluids is particularly tedious and complicated process (Mahmoudi et al. 2011a, b). Protein corona is characterised by five parameters like thickness and density, identity and quantity, arrangement and orientation, conformation, and affinity of proteins on NPs. Together, these parameters describe the interaction of NPs with a biological environment (Walkey and Chan 2012). The composition and structure of the protein corona has been studied using either in situ or ex situ techniques. In situ techniques measure the protein corona, while the NPs is dispersed in a biological milieu. These techniques are limited in number and typically provide the least amount of information. Ex situ measurements require isolation of the NPs with its bound protein from the biological environment (Walkey and Chan 2012). Methods used for the characterisation of protein corona are Fourier transform infrared spectroscopy (FTIR), Raman spectroscopy, fluorescence correlation

spectroscopy, differential centrifugal sedimentation (DCS), isothermal titration calorimeter (ITC), LC-MS, electrophoresis, size exclusion chromatography, and dynamic light scattering.

7.7.1 Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectroscopy is known as an important technique for the examination of protein conformation in H_2O-based solution, resulting in a greatly expanded use in studies of protein secondary structure and protein dynamics in the past decade (Kong and Yu 2007). FTIR spectroscopy has been used for characterising the interactions of NPs with proteins (Wang et al. 2012).

7.7.2 Raman Spectroscopy

Raman scattering technique is a vibrational molecular spectroscopy in which a laser photon is scattered by a sample molecule and during the process energy is either lost or gained (Kengne-Momo et al. 2012). The amount of energy lost is seen as a change in energy (wavelength) of the irradiating photon which is characteristic for a particular bond in the molecule. The Raman signal produces a precise spectral sample fingerprint, unique to each atom, group of atoms or individual molecule (Kengne-Momo et al. 2012). A recent study has used surface enhanced Raman scattering spectroscopy to study changes in the proteins secondary structure as well as the effect on integrity and conformations of disulfide bonds immediately on the NP surface (Grass and Treuel 2014).

7.7.3 Fluorescence Correlation Spectroscopy

Fluorescence correlation spectroscopy (FCS) is a technique used to study the molecular movements and interactions. This technique monitors the fluctuation in the fluorescence signal from fluorescently labelled molecules inside the small confocal volume (Röcker et al. 2009; Maffre et al. 2011). In FCS, one usually considers the dynamics of number fluctuations in an open sampling volume of a macroscopic system that fluctuates about average equilibrium concentrations which are determined by the surrounding medium and its thermodynamics (Röcker et al. 2009; Maffre et al. 2011). The concentration fluctuations of each species can occur by in situ chemical reactions and by diffusion of each species in and out of the sampling volume. FCS is a highly accurate method to simultaneously determine binding affinities and the thickness of the protein corona on NPs (Röcker et al. 2009; Maffre et al. 2011). This method permits the quantitative observation of protein adsorption in situ on NPs (Treuel et al. 2014). Recent study has used FCS to quantitatively monitor HSA adsorption onto dihydrolipoic acid quantum dots (Treuel et al. 2014).

7.7.4 Differential Centrifugal Sedimentation

Differential centrifugal sedimentation (DCS) is based on the ability to separate NPs of the same density by mass, i.e. size (Cölfen 2004). DCS is a fast, accurate and relatively inexpensive, resolves multimodal size distributions, and uses relatively small sample volumes (Krpetic et al. 2012). DCS have been extensively used to measure size distribution, and hydrodynamic radii of NPs (Machtle 1999; Müller 2004, 2006). In addition to particle size, DCS has been used to determine changes in surface structure, study of binding isotherms of NPs and showed the attachment of DNA binding protein to NPs (Salvati et al. 2013). DCS can also be used for measuring thickness of protein corona.

7.7.5 Isothermal Titration Calorimetry

Isothermal titration calorimetry (ITC) is the only technique that can simultaneously determine all binding parameters in a single experiment. ITC measures heat transfer during binding which

enables accurate determination of binding constants (K_D), reaction stoichiometry (n), enthalpy (ΔH) and entropy (ΔS). This provides a complete thermodynamic profile of the molecular interaction. ITC also helps in elucidating the mechanisms underlying molecular interactions. The strength of protein interactions can be assessed using ITC and it also provides additional information on the thermodynamics of protein adsorption (Lindman et al. 2007).

7.7.6 Liquid Chromatography-Mass Spectrometry

Liquid chromatography-mass spectrometry (LC-MS) is a technique applied to a wide range of biological molecules. Mass spectrometers operate by converting the analyte molecules to a charged state, with subsequent analysis of fragment ions that are produced during the ionisation process, on the basis of their mass to charge ratio (*m*/*z*) (Pitt 2009). LC-MS plays an important role in several areas of clinical biochemistry and competes with conventional liquid chromatography and other techniques such as immunoassay. LC-MS has been used recently in a study to quantify the proteins in protein corona formed on SiO_2 and polystyrene NPs. The composition of protein corona has been investigated by LC-MS on polystyrene NPs with average diameters slightly above 100 nm and resulted in identification of approximately 170 different adsorbed proteins (Ritz et al. 2015).

7.7.7 Matrix-Assisted Laser Desorption/Ionisation Time of Flight Mass Spectrometer (MALDI-TOF MS)

The sample for matrix-assisted laser desorption/ionisation (MALDI) is uniformly mixed in a large quantity of UV-absorbing matrix and then followed by time of flight mass spectrometry (Marvina et al. 2003).The matrix absorbs the UV light and converts it into heat energy. A small part of the matrix is heated and rapidly vaporised, together with the sample. Different matrices are used for different kind of samples. Derivatives of benzoic acid, cinnamic acid and other related aromatic compounds are usually approved as good quality MALDI matrices for protein analysis (Hillenkamp et al. 1991). Charged ions of various sizes are generated on the sample slide, a potential difference between the sample slide and ground attracts the ions in a direction. As the potential difference is constant with respect to all ions, ions with smaller m/z value and more highly charged ions move faster through the drift space until they reach the detector. Consequently, the time of ion flight differs according to m/z value of the ion (Marvina et al. 2003). MALDI-TOF-MS has recently been used in a study to identify the proteins of NPs protein corona of nanosized welding particles formed in vitro (Ali et al. 2015).

7.7.8 Electrophoresis

Electrophoresis is used for the identification of proteins after separation of the NPs protein complex that has been separated from excess plasma proteins. Most commonly used method for this purpose is two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) (Blunk et al. 1993; Gessner et al. 2002; Seehof et al. 2000). To identify individual proteins, it is common practice to compare the 2-D protein gels to a 2-D master map of human plasma proteins. However, differences in donor plasma and anticoagulants used during the blood collection process (EDTA, sodium citrate, lithium heparin), can result in variations in plasma protein maps, and hence may contribute to a misinterpretation of the 2D data when compared to a specific protein master map. The sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) is used for identifying proteins in hard protein corona (Winzen et al. 2015).

7.7.9 Size Exclusion Chromatography

Size exclusion chromatography (SEC) separates the NPs according to their size in dilute solutions (Sun et al. 2004). SEC is the second method investigated for its potential to reveal quantitative information on protein–NPs interactions. NPs and protein solution was passed through the chromatographic resin which allows protein and NPs to be resolved, but not different proteins. There has been a clear difference in the elution profile of HSA mixed with NPs, compared with free albumin, which implies an interaction between the protein and the NPs. Different proteins show different elution profiles when in protein NPs mixture than in free form. The rates of association/dissociation are very different from protein to protein, depending on the overall protein NP composition (Cedervall et al. 2007a, b). The strength of interactions of proteins with NPs can also be studied using SEC (Cedervall et al. 2007a, b).

7.7.10 Dynamic Light Scattering

Dynamic light scattering (DLS) measures the hydrodynamic diameter of NPs. DLS measures the size of NPs typically in the sub-micron region, also referred to as photon correlation spectroscopy or quasi-elastic light scattering. NPs suspended within a liquid undergo Brownian motion. DLS monitors the Brownian motion with light scattering. DLS measurements have been used to determine changes in the NP diameter before and after incubation with proteins. Proteins bind strongly to the NPs surface when the incubation time is increased, and they are stable against desorption in the serum free media. NPs hydrodynamic diameter is also increased as a result of the interactions of proteins with NPs. Adsorption of proteins onto the NPs surface has increased the overall size of NPs and not due to NPs aggregation (Casals et al. 2010). The thickness of the protein corona can be measured in situ using DLS (Walczyk et al. 2010).

7.7.11 Bioinformatic Tools

In silico simulation studies of protein NPs interactions is attracting attention as an alternative to experimental techniques (Walkey and Chan 2012). Simulation has been successfully adapted to study the adsorption of proteins on NPs. Simulation results have been used to study the interaction of a nanomaterial with individual amino acids to be observed over femto second timescales (Walkey and Chan 2012). Three strategies commonly used to simulate protein adsorption are quantum mechanical (QM), all atom empirical force field (AA) and coarse grained (CG) (Makarucha et al. 2011). Experimentally measured reference values are required to ensure the validity of simulation results. In silico simulations have been used to study the protein adsorption onto NPs as a function of surface ligand structure (Makarucha et al. 2011), surface curvature (Hung et al. 2011), and protein identity (Ge et al. 2011). However, computational power is presently unable to handle the complexity of competitive protein adsorption in a biological milieu, and there is a scarcity of availability of relevant force fields and descriptions of salvation (Walkey and Chan 2012).

7.8 Properties of NPs Affecting Protein Corona Formation

Interaction of NPs with biomolecules and composition of the resulting protein corona is affected by many factors (Foroozandeh and Aziz 2015). The physicochemical properties of NPs and the biological environment are vital parameters governing protein corona formation. Therefore, studying and understanding each of these parameters are essential for safe and smart designing of NPs for targeted drug delivery. The affinities and identities of proteins that bind to NPs are affected by the composition and surface chemistry of NPs. It has been reported that different proteins bind to different NPs with the same surface charge (Deng et al. 2009). Similar proteins can be adsorbed onto the TiO_2 and SiO_2

NPs, whereas significantly different proteins can compose the hard corona of ZnO NPs. The corona of TiO_2 and SiO_2 NPs were comprised of clusterin, apolipoprotein D and alpha-2-acid glycoprotein, while these were not observed in the corona of ZnO. Fascinatingly, transferrin, Ig heavy chain alpha and haptoglobin have been reported in the corona of ZnO NPs.

The size of NPs plays a vital role in the adsorption of proteins, conformational changes and composition of protein corona (Lundqvist et al. 2008; Lynch and Dawson 2008). It has been reported that the thickness of protein corona progressively increases as the size of NP increases. In addition, conformational change upon adsorption on the NPs surface showed enhancement with the size of NPs. Moreover, more proteins adsorbed on smaller sized NPs than on larger sized NPs (Dobrovolskaia et al. 2009). Interestingly, even 10 nm variations in NPs size have remarkably affected the protein corona composition (Tenzer et al. 2011). Shape of NPs has also affected the formation of protein corona on NPs surface (Deng et al. 2009). Surface charge of NPs also play vital role in the composition and formation of protein corona on NPs. Negatively charged NPs have been shown for enhancement in plasma protein absorption with an increase in the surface charge density of NPs (Gessner et al. 2002). In addition, proteins with isoelectric points (PI) of less than 5.5 adsorbed on positively charged NPs whereas proteins with isoelectric points of higher than 5.5 bound to negatively charged NPs (Foroozandeh and Aziz 2015). More proteins can adsorb onto the surface of hydrophobic NPs than their hydrophilic counterparts and lose their native structure (Roach et al. 2005). It has been observed that protein corona can drastically affect the cellular uptake and internalisations of NPs. Distinct corona signatures are indeed able to predict the cellular uptake of NPs. Hence, covering NPs surface with physiological proteins can indeed enhance or inhibit their cellular uptake, whereas the surface charge of the uncoated NPs appear to be less important (Monopoli et al. 2012; Lesniak et al. 2012; Zhang et al. 2014). Protein corona fingerprints can be modulated for increasing the cellular uptake of NPs across many biological barriers. It has been shown that apolipoproteins promote the transport of NPs across the blood brain barrier (Zhu et al. 2013) and different immunoglobulins and opsonins enable their uptake into monocytes (Riehemann et al. 2009), while dysopsonins inhibit the uptake of NPs (Cedervall et al. 2007a, b). NPs targeted with transferrin have been studied in foetal bovine serum albumin for their targeting efficiency (Salvati et al. 2013). It has been observed that serum decreased the overall uptake of NPs through transferrin receptors. These results have demonstrated that targeted NPs may lose their targeting abilities in biological media. This behaviour is attributed to the proteins in the serum forming a protein corona around the NPs, which masks the transferrin and stops it from binding to the targeted receptors on the cells. Hence, experiments carried out in vitro cannot be used to provide a conclusive analysis of targeting efficiency. Therefore, targeted NPs formulations should be smartly designed by taking into account the protein corona effect, and the problems faced due to the route of administration, organs, tissues and cellular uptake. This will help in developing targeted NPs with clinical applications and achieving the concept of personalised medicine (Gaspar 2013).

In addition to the properties of NPs, the composition of protein corona is also affected by the biological media in which NPs are incubated. It has been reported that formation of protein corona by utilising Dulbecco's Modified Eagle's Medium (DMEM) media is significantly time dependent, while using Roswell Park Memorial Institute (RPMI) media leads to different dynamics and reduction of protein corona (Maiorano et al. 2010). Besides NPs properties and biological milieu, other factors which affect protein corona at bio-nano interfaces are gradient plasma, plasma concentration, cell observer, temperature and cell membrane composition. Detailed investigations must be carried out to understand these ignored factors as to enable the development of better and effective nanomedicine (Foroozandeh and Aziz 2015).

Protein corona has been recently exploited for evading mononuclear phagocytic system. Albumins form tight binding to SiO_2 NPs and may undergo

rapid unfolding to form hard corona. Albumin has been documented to promote NPs uptake into cells that are expressing class A scavenger receptors and resulted in internalisation of the protein NP complex via receptor-mediated endocytosis (Mortimer et al. 2014).

7.9 Conclusions

NPs may not only promote the pharmacological effects of delivered therapeutic molecules, but also cause undesirable effects in the target cells. Factors like size, shape and surface chemistry of NPs can influence their cellular responses. NPs have been observed to induce cellular responses like ROS production, apoptosis, necrosis and expression of genes. Most of the studies involving the cellular responses of NPs have been carried out on in vitro cell lines, and therefore final validation is required with primary cells. Effect of various surface ligands and the use of targeting moieties should be investigated to better understand the cellular responses of NPs. NPs should no longer be viewed as simple carriers for biomedical applications, but can also play dynamic role in mediating biological responses. NPs-biomolecule corona should be more carefully characterised and quantified for safe and effective use of NPs in nanomedicine. The molecular design of basic molecules for NPs and the response between NPs and cells should be considered more carefully in terms of the activation of cell functions.

Acknowledgments We are grateful to Director, IHBT for critical and valuable suggestions. Financial assistance from Council of Scientific and Industrial Research, Government of India is genuinely acknowledged.

References

Aderem A, Underhill DM (1999) Mechanisms of phagocytosis in macrophages. Annu Rev Immunol 17:593–623

Ahamed M, Karns M, Goodson M et al (2008) DNA damage response to different surface chemistry of silver nanoparticles in mammalian cells. Toxicol Appl Pharmacol 233:404–410

Ali N, Mattsson K, Rissler J et al (2015) Analysis of nanoparticle—protein coronas formed in vitro between nanosized welding particles and nasal lavage proteins. Nanotoxicology 1–9

Alivisatos AP, Gu W, Larabell C (2005) Quantum dots as cellular probes. Annu Rev Biomed Eng 7:55–76

Alshaer W, Hillaireau H, Vergnaud J et al (2015) Functionalizing liposomes with anti-CD44 aptamer for selective targeting of cancer cells. Bioconjugate Chem 26:1307–1313

Arias MA, Loxley A, Eatmon C et al (2011) Carnauba wax nanoparticles enhance strong systemic and mucosal cellular and humoral immune responses to HIV-gp140 antigen. Vaccine 29:1258–1269

Arora S, Jain J, Rajwade JM et al (2008) Cellular responses induced by silver nanoparticles: In vitro studies. Toxicol Lett 179:93–100

Asokan A, Cho MJ (2002) Exploitation of intracellular pH gradients in the cellular delivery of macromolecules. J Pharm Sci 91:903–913

Ataman-Önal Y, Munier S, Ganée A et al (2006) Surfactant-free anionic PLA nanoparticles coated with HIV-1 p24 protein induced enhanced cellular and humoral immune responses in various animal models. J Control Rel 112:175–185

Bao G, Bao XR (2005) Shedding light on the dynamics of endocytosis and viral budding. Proc Natl Acad Sci USA 102:9997–9998

Bartlett DW, Davis ME (2007) Physicochemical and biological characterization of targeted nucleic acid-containing nanoparticles. Bioconjugate Chem 18:456–468

Bartneck M, Keul HA, Singh S et al (2010) Rapid uptake of gold nanorods by primary human blood phagocytes and immunomodulatory effects of surface chemistry. ACS Nano 4:3073–3086

Bastus NG, Sanchez-Tillo E, Pujals S et al (2009) Homogeneous conjugation of peptides onto gold nanoparticles enhances macrophage response. ACS Nano 3:1335–1344

Benmerah A, Lamaze C (2007) Clathrin-coated Pits: Vive la Difference? Traffic 8:970–982

Bharali DJ, Klejbor I, Stachowiak EK et al (2005) Organically modified silica nanoparticles: a nonviral vector for in vivo gene delivery and expression in the brain. Proc Natl Acad Sci USA 102:11539–11544

Blunk T, Hochstrasser DF, Sanchez JC et al (1993) Colloidal carriers for intravenous drug targeting: plasma protein adsorption patterns on surface-modified latex particles evaluated by two-dimensional polyacrylamide gel electrophoresis. Electrophoresis 14:1382–1387

Boussif O, Lezoualc'h F, Zanta MA et al (1995) A versatile vector for gene and oligonucleotide transfer into cells in culture and in vivo: polyethylenimine. Proc Natl Acad Sci 92:7297–7301

Cambi A, Lidke DS, Arndt-Jovin DJ et al (2007) Ligand-conjugated quantum dots monitor antigen uptake and processing by dendritic cells. Nanolett 7:970–977

Casals E, Pfaller T, Duschl A et al (2010) Time evolution of the nanoparticle protein corona. ACS Nano 4:3623–3632

Cedervall T, Lynch I, Foy M et al (2007a) Detailed identification of plasma proteins adsorbed on copolymer nanoparticles. Angew Chem Int Ed 46:5754–5756

Cedervall T, Lynch I, Lindman S et al (2007b) 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

Chang J, Jallouli Y, Kroubi M et al (2009) Characterization of endocytosis of transferrin-coated PLGA nanoparticles by the blood-brain barrier. Int J Pharm 379:285–292

Chellat F, Grandjean-Laquerriere A, Le Naour R et al (2005) Metalloproteinase and cytokine production by THP-1 macrophages following exposure to chitosan-DNA nanoparticles. Biomaterials 26:961–970

Chen M, von Mikecz A (2005) Formation of nucleoplasmic protein aggregates impairs nuclear function in response to SiO2 nanoparticles. Exp Cell Res 305:51–62

Cölfen H (2004) Analytical ultracentrifugation of nanoparticles. In: Nalwa HS (ed) Encyclopedia of nanoscience and nanotechnology, vol. 1. American Scientific Publishers, Stevenson Ranch, pp 67–88

Conner SD, Schmid SL (2003) Regulated portals of entry into the cell. Nature 422:37–44

Dausend J, Musyanovych A, Dass M et al (2008) Uptake mechanism of oppositely charged fluorescent nanoparticles in HeLa cells. Macromol Biosci 8:1135–1143

De M, You CC, Srivastava S et al (2007) Biomimetic interactions of proteins with functionalized nanoparticles: A thermodynamic study. J Am Chem Soc 129:10747–10753

Deng ZJ, Mortimer G, Schiller T et al (2009) Differential plasma protein binding to metal oxide nanoparticles. Nanotechnology 20:455101

Diken M, Kreiter S, Selmi A et al (2011) Selective uptake of naked vaccine RNA by dendritic cells is driven by macropinocytosis and abrogated upon DC maturation. Gene Ther 18:702–708

Dinauer N, Balthasar S, Weber C et al (2005) Selective targeting of antibody-conjugated nanoparticles to leukemic cells and primary T-lymphocytes. Biomaterials 26:5898–5906

Dobrovolskaia MA, Patri AK, Zheng J et al (2009) Interaction of colloidal gold nanoparticles with human blood: effects on particle size and analysis of plasma protein binding profiles. Nanomed Nanotechnol Biol Med 5:106–117

Duit S, Mayer H, Blake SM et al (2010) Differential functions of ApoER2 and very low density lipoprotein receptor in Reelin signaling depend on differential sorting of the receptors. J Biol Chem 285:4896–4908

El-Sayed IH, Huang X, El-Sayed MA (2005) Surface plasmon resonance scattering and absorption of anti-EGFR antibody conjugated gold nanoparticles in cancer Diagnostics: applications in oral cancer. Nano Lett 5:829–834

El-Sayed IH, Huang XH, El-Sayed MA (2006) Photo-Thermal therapy of epithelial carcinoma using anti-EGFR antibody conjugated gold nanoparticles. Cancer Lett 239:129–135

Foroozandeh P, Aziz AA (2015) Merging worlds of nanomaterials and biological environment: factors governing protein corona formation on nanoparticles and its biological consequences. Nanoscale Res Lett 10:221

Garnier B, Bouter A, Gounou C et al (2009) Annexin A5-Functionalized liposomes for targeting phosphatidylserine-exposing membranes. Bioconjugate Chem 20:2114–2122

Gaspar R (2013) Nanoparticles pushed off target with proteins. Nature Nanotech 8:79–80

Ge C, Du J, Zhao L et al (2011) Binding of blood proteins to carbon nanotubes reduces cytotoxicity. Proc Natl Acad Sci 108:16968–16973

Gessner A, Lieske A, Paulke B et al (2002) Influence of surface charge density on protein adsorption on polymeric nanoparticles: analysis by two-dimensional electrophoresis. Eur J Pharm Biopharm 54:165–170

Grass S, Treuel L (2014) Mechanistic aspects of protein corona formation: insulin adsorption onto gold nanoparticle surfaces. J Nanopart Res 16:2254–2265

Hainfeld JF, Slatkin DN, Smilowitz HM (2004) The use of gold nanoparticles to enhance radiotherapy in mice. Phys Med Biol 49:N309–N315

Harush-Frenkel O, Debotton N, Benita S et al (2007) Targeting of nanoparticles to the clathrin-mediated endocytic pathway. Biochem Biophys Res Commun 353:26–32

Hillenkamp F, Karas M, Beavis RC et al (1991) Matrix-assisted laser desorption/ionization MS of biopolymers. Anal Chem 63:1193–1202

Hirsch LR, Stafford RJ, Bankson JA et al (2003) Nanoshell-mediated near-infrared thermal therapy of tumors under magnetic resonance guidance. Proc Natl Acad Sci 100:13549–13554

Hong T, Tripathy N, Son H et al (2013) A comprehensive in vitro and in vivo study of ZnO nanoparticles toxicity. J Mater Chem B 1:2985–2992

Hu CM, Kaushal S, Tran Cao HS et al (2010) Half-antibody functionalized lipid-polymer hybrid nanoparticles for targeted drug delivery to carcinoembryonic antigen presenting pancreatic cancer cells. Mol Pharm 7:914–920

Huang C, Butler PJ, Tong S et al (2013) Substrate stiffness regulates cellular uptake of nanoparticles. Nano Lett 13:1611–1615

Hung A, Mwenifumbo S, Mager M et al (2011) Ordering surfaces on the nanoscale: implications for protein adsorption. J Am Chem Soc 133:1438–1450

Ivanov AI (2008) Pharmacological inhibition of endocytic pathways: is it specific enough to be useful? Methods Mol Biol 440:15–33

Jiang W, Kim BY, Rutka JT et al (2008) Nanoparticle-mediated cellular response is size-dependent. Nat Nanotechnol 3:145–150

Jin Y, Kannan S, Wu M et al (2007) Toxicity of luminescent silica nanoparticles to living cells. Chem Res Toxicol 20:1126–1133

Jin CY, Zhu BS, Wang XF et al (2008) Cytotoxicity of titanium dioxide nanoparticles in mouse fibroblast cells. Chem Res Toxicol 21:1871–1877

Jones CF, Grainger DW (2009) In vitro assessments of nanomaterial toxicity. Adv Drug Deliv Rev 61:438–456

Jones AT, Gumbleton M, Duncan R (2003) Understanding endocytic pathways and intracellular trafficking. A prerequisite for effective design of advanced drug delivery systems. Adv Drug Deliv Rev 55:1353–1357

Jun YW, Huh YM, Choi JS et al (2005) Nanoscale size effect of magnetic nanocrystals and their utilization for cancer diagnosis via magnetic resonance imaging. J Am Chem Soc 127:5732–5733

Kengne-Momo RP, Daniel P, Lagarde F et al (2012) Protein interactions investigated by the raman spectroscopy for biosensor applications. Int J Spectroscopy 2012: Article ID 462901, 7 pages. doi:10.1155/2012/462901

Kong J, Yu S (2007) Fourier transform infrared spectroscopic analysis of protein secondary structures. Acta Biochim Biophys Sin 39:549–559

Koo OM, Rubinstein I, Onyuksel H (2005) Role of nanotechnology in targeted drug delivery and imaging: A concise review. Nanomed Nanotechnol Biol Med 1:193–212

Krpetic Z, Singh I, Su W et al (2012) Directed assembly of DNA-functionalized gold nanoparticles using pyrrole-imidazole polyamides. J Am Chem Soc 134:8356–8359

Kumar V, Kumari A, Guleria P et al (2012) Evaluating the toxicity of selected types of nanochemicals. Rev Environ Contamin Toxicol 215:40–112

Kumari A, Yadav SK (2011) Cellular interactions of therapeutically delivered nanoparticles. Expert Opin Drug Deliv 8:141–151

Lee J, Choi Y, Kim K et al (2010) Characterization and cancer cell specific binding properties of anti-EGFR antibody conjugated quantum dots. Bioconj Chem 21:940–946

Lesniak A, Fenaroli F, Monopoli MP et al (2012) Effects of the presence or absence of a protein corona on silica nanoparticle uptake and impact on cells. ACS Nano 6:5845–5857

Lindman S, Lynch I, Thulin E et al (2007) 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 7:914–920

Liu AP, Aguet F, Danuser G et al (2010a) Local clustering of transferrin receptors promotes clathrin-coated pit initiation. J Cell Biol 191:1381–1393

Liu XQ, Song WJ, Sun TM et al (2010b) Targeted delivery of antisense inhibitor of miRNA for antiangiogenesis therapy using cRGD-functionalized nanoparticles. Mol Pharm 8:250–259

Liu X, Huang N, Li H et al (2013) Surface and size effects on cell interaction of gold nanoparticles with both phagocytic and nonphagocytic cells. Langmuir 29:9138–9148

Luccardini C, Yakovlev A, Gaillard S et al (2007) Intracellular uses of colloidal semiconductor nanocrystals. J Biomed Biotechnol 2007:1–9

Lundqvist M, Stigler J, Elia G et al (2008) Nanoparticle size and surface properties determine the protein corona with possible implications for biological impacts. Proc Natl Acad Sci 105:14265–14270

Lunov O, Syrovets T, Loos C et al (2011) Differential uptake of functionalized polystyrene nanoparticles by human macrophages and a monocytic cell line. ACS Nano 5:1657–1669

Lynch I, Dawson KA (2008) Protein-nanoparticle interactions. Nano Today 3:40–47

Machtle W (1999) High-Resolution submicron particle size distribution analysis using gravitational-sweep sedimentation. Biophys J 76:1080–1091

Maffre P, Nienhaus K, Amin F et al (2011) Characterization of protein adsorption onto Fept nanoparticles using dual-focus fluorescence correlation spectroscopy. Beilstein J Nanotechnol 2:374–383

Mahendra S, Zhu HG, Colvin VL et al (2008) Quantum dot weathering results in microbial toxicity. Environ Sci Technol 42:9424–9430

Mahmoudi M, Simchi A, Imani M (2009) Cytotoxicity of uncoated and polyvinyl alcohol coated superparamagnetic iron oxide nanoparticles. J Phys Chem C 113:9573–9580

Mahmoudi M, Simchi A, Imani M et al (2010) A new approach for the in vitro identification of the cytotoxicity of superparamagnetic iron oxide nanoparticles. Colloid Surf B 75:300–309

Mahmoudi M, Lynch I, Ejtehadi MR et al (2011a) Protein nanoparticle interactions: opportunities and challenges. Chem Rev 111:5610–5637

Mahmoudi M, Sant S, Wang B et al (2011b) Superparamagnetic iron oxide nanoparticles (SPIONs): development, surface modification and applications in chemotherapy. Adv Drug Delivery Rev 63:24–46

Mailänder V, Lorenz MR, Holzapfel V et al (2008) Carboxylated superparamagnetic iron oxide particles label cells intracellularly without transfection agents. Mol Imaging Biol 10:138–146

Maiorano G, Sabella S, Sorce B et al (2010) Effects of cell culture media on the dynamic formation of protein-nanoparticle complexes and influence on the cellular response. ACS Nano 4:7481–7491

Makarucha J, Todorova N, Yarovsky I (2011) Nanomaterials in biological environment: a review of computer modelling studies. Eur Biophys J 40:103–115

Marvina LF, Robertsb MA, Faya LB (2003) Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry in clinical chemistry. Clin Chim Acta 337:11–21

Massich MD, Giljohann DA, Seferos DS et al (2009) Regulating immune response using polyvalent nucleic

acid-gold nanoparticle conjugates. Mol Pharm 6:1934–1940
Massich MD, Giljohann DA, Schmucker AL et al (2010) Cellular response of polyvalent oligonucleotide-gold nanoparticle conjugates. ACS Nano 4:5641–5646
Maurer-Jones MA, Lin YS, Haynes CL (2010) Functional assessment of metal oxide nanoparticle toxicity in immune cells. ACS Nano 4:3363–3373
Medina-Kauwe LK (2007) "Alternative" endocytic mechanisms exploited by pathogens: new avenues for therapeutic delivery? Adv Drug Del Rev 59:798–809
Milani S, Bombelli FB, Pitek AS et al (2012) Reversible versus irreversible binding of transferrin to polystyrene nanoparticles: Soft and hard corona. ACS Nano 6:2532–2541
Miller CR, Bondurant B, McLean SD et al (1998) Liposome-cell interactions in vitro: effect of liposome surface charge on the binding and endocytosis of conventional and sterically stabilized liposomes. Biochemistry 37:12875–12883
Monopoli MP, Aberg C, Salvati A et al (2012) Biomolecular coronas provide the biological identity of nanosized materials. Nat Nanotechnol 7:779–786
Mortimer GM, Butcher NJ, Musumeci AW et al (2014) Cryptic epitopes of albumin determine mononuclear phagocyte system clearance of nanomaterials. ACS Nano 8:3357–3366
Müller HG (2004) Determination of very broad particle size distributions via interferences optics in the analytical ultracentrifuge. Prog Colloid Polym Sci 127:9–13
Müller HG (2006) Determination of particle size distributions of swollen (Hydrated) particles by analytical ultracentrifugation. Prog Colloid Polym Sci 131:121–125
Nagayama S, Ogawara K, Fukuoka Y et al (2007) Time-dependent changes in opsonin amount associated on nanoparticles alter their hepatic uptake characteristics. Int J Pharm 342:215–221
Nakamura T, Ono K, Suzuki Y et al (2014) Octaarginine-modified liposomes enhance cross-presentation by promoting the C-terminal trimming of antigen peptide. Mol Pharm 11:2787–2795
Nan A, Bai X, Son SJ et al (2008) Cellular uptake and cytotoxicity of silica nanotubes. Nano Lett 8:2150–2154
Nel AE, Madler L, Velegol D et al (2009) Understanding biophysicochemical interactions at the nano-bio interface. Nat Mater 8:543–547
Niidome T, Yamagata M, Okamoto Y et al (2006) PEG-modified gold nanorods with a stealth character for in vivo applications. J Control Rel 114:343–347
Nishikawa T, Iwakiri N, Kaneko Y et al (2009) Nitric oxide release in human aortic endothelial cells mediated by delivery of amphiphilic polysiloxane nanoparticles to caveolae. Biomacromolecules 10:2074–2085
Norde W (1994) Protein adsorption at solid surfaces: A thermodynamic approach. Pure Appl Chem 66:491–496
Oberdorster G, Oberdorster E, Oberdorster J (2005) Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles. Environ Health Perspect 113:823–839
Oh E, Delehanty JB, Sapsford KE et al (2011) Cellular uptake and fate of pegylated gold nanoparticles is dependent on both cell-penetration peptides and particle size. ACS Nano 5:6434–6448
Parak WJ, Pellegrino T, Plank C (2005) Labelling of Cells with Quantum Dots. Nanotechnol 16:R9–R25
Patel LN, Zaro JL, Shen WC (2007) Cell penetrating peptides: intracellular pathways and pharmaceutical perspectives. Pharm Res 24:1977–1992
Patra HK, Banerjee S, Chaudhuri U et al (2007) Cell selective response to gold nanoparticles. Medicine 3:111–119
Pelkmans L, Helenius A (2002) Endocytosis via caveolae. Traffic 3:311–320
Peng L, He M, Chen B et al (2015) Metallomics study of CdSe/ZnS quantum dots in HepG2 Cells. ACS Nano 9:10324–10334
Petrescu AD, Vespa A, Huang H et al (2009) Fluorescent sterols monitor cell penetrating peptide Pep-1 mediated uptake and intracellular targeting of cargo protein in living cells. Biochim Biophys Acta Biomembr 1788:425–441
Pettibone JM, Gigault J, Hackley VA (2013) Discriminating the states of matter in metallic nanoparticle transformations: what are we missing? ACS Nano 7:2491–2499
Pitt JJ (2009) Principles and applications of liquid chromatography mass spectrometry in clinical biochemistry. Clin Biochem Rev 30:19–34
Qhattal HSS, Liu X (2011) Characterization of CD44-mediated cancer cell uptake and intracellular distribution of hyaluronan-grafted liposomes. Mol Pharmaceutics 8:1233–1246
Qhobosheane M, Santra S, Zhang P et al (2001) Biochemically functionalized silica nanoparticles. Analyst 126:1274–1278
Riehemann K, Schneider SW, Luger TA et al (2009) Nanomedicine challenge and perspectives. Angew Chem Int Ed 48:872–897
Ritz S, Schottler S, Kotman N, Baier G et al (2015) Protein corona of nanoparticles: distinct proteins regulate the cellular uptake. Biomacromolecules 16:1311–1321
Roach P, Farrar D, Perry CC (2005) Interpretation of protein adsorption: Surface induced conformational changes. J Am Chem Soc 127:8168–8173
Röcker C, Pötzl M, Zhang F et al (2009) A quantitative fluorescence study of protein monolayer formation on colloidal nanoparticles. Nat Nanotechnol 4:577–580

Ruan G, Agrawal A, Marcus AI et al (2007) Imaging and tracking of tat peptide-conjugated quantum dots in living cells: new insights into nanoparticle uptake, intracellular transport, and vesicle shedding. J Am Chem Soc 129:14759–14766

Salvati A, Pitek AS, Monopoli MP et al (2013) Transferrin-functionalized nanoparticles lose their targeting capabilities when a biomolecule corona adsorbs on the surface. Nat Nanotech 8:137–143

Sandhu KK, McIntosh CM, Simard JM et al (2002) Gold nanoparticle-mediated transfection of mammalian cells. Bioconjugate Chem 13:3–6

Sapra P, Allen TM (2003) Ligand-targeted liposomal anticancer drugs. Prog Lipid Res 42:439–462

Seehof K, Kresse M, Mader K et al (2000) Interactions of nanoparticles with body proteins-improvement of 2D-PAGE-analysis by internal standard. Int J Pharm 196:231–234

Simberg D, Park JH, Karmali PP et al (2009) Differential proteomics analysis of the surface heterogeneity of dextran iron oxide nanoparticles and the implications for their in vivo clearance. Biomaterials 30:3926–3933

Sinha R, Kim GJ, Nie S et al (2006) Nanotechnology in cancer therapeutics: Bioconjugated nanoparticles for drug delivery. Mol Cancer Ther 5:1909–1917

Strickland J, Dreher K, Kligerman AD et al (2013) Effect of treatment media on the agglomeration of titanium dioxide nanoparticles: impact on genotoxicity, cellular interaction, and cell cycle. ACS Nano 7:1929–1942

Sun T, Chance RR, Graessley WW et al (2004) A study of the separation principle in size exclusion chromatography. Macromolecules 37:4304–4312

Swanson JA, Watts C (1995) Macropinocytosis. Trends Cell Biol 5:424–428

Tenzer S, Docter D, Rosfa S et al (2011) Nanoparticle size is a critical physicochemical determinant of the human blood plasma corona: a comprehensive quantitative proteomic analysis. ACS Nano 5:7155–7167

Thorley AJ, Ruenraroengsak P, Potter TE et al (2014) Critical determinants of uptake and translocation of nanoparticles by the human pulmonary alveolar epithelium. ACS Nano 8:11778–11789

Toti US, Guru BR, Grill AE et al (2010) Interfacial activity assisted surface functionalization: a novel approach to incorporate maleimide functional groups and cRGD peptide on polymeric nanoparticles for targeted drug delivery. Mol Pharm 7:1108–1117

Treuel L, Brandholt S, Maffre P et al (2014) Impact of protein modification on the protein corona on nanoparticles and nanoparticles cell interactions. ACS Nano 8:503–513

Venkatesan N, Yoshimitsu J, Ito Y et al (2005) Liquid filled nanoparticles as a drug delivery tool for protein therapeutics. Biomaterials 26:7154–7163

Verma A, Stellacci F (2010) Effect of surface properties on nanoparticle-cell interactions. Small 6:12–21

Walczyk D, Bombelli FB, Monopoli MP et al (2010) What the cell "sees" in bionanoscience. J Am Chem Soc 132:5761–5768

Walkey CD, Chan WCW (2012) Understanding and controlling the interaction of nanomaterials with proteins in a physiological environment. Chem Soc Rev 41:2780–2799

Walsh M, Tangney M, O'Neill MJ et al (2006) Evaluation of cellular uptake and gene transfer efficiency of pegylated poly-L-lysine compacted DNA: implications for cancer gene therapy. Mol Pharm 3:644–653

Wang XL, Xu R, Wu X et al (2009) Targeted systemic delivery of a therapeutic siRNA with a multifunctional carrier controls tumor proliferation in mice. Mol Pharm 6:738–746

Wang T, Bai J, Jiang X et al (2012) Cellular uptake of nanoparticles by membrane penetration: a study combining confocal microscopy with FTIR spectroelectrochemistry. ACS Nano 6:1251–1259

Watson P, Jones AT, Stephens DJ (2005) Intracellular trafficking pathways and drug delivery: fluorescence imaging of living and fixed cells. Adv Drug Deliv Rev 57:43–61

Winzen S, Schoettler S, Baier G et al (2015) Complementary analysis of the hard and soft protein corona: sample preparation critically effects corona composition. Nanoscale 7:2992–3001

Xia T, Kovochich M, Liong M et al (2008a) Cationic polystyrene nanosphere toxicity depends on cell-specific endocytic and mitochondrial injury pathways. ACS Nano 2:85–96

Xia T, Kovochich M, Liong M et al (2008b) Comparison of the mechanism of toxicity of zinc oxide and cerium oxide nanoparticles based on dissolution and oxidative stress properties. ACS Nano 2:2121–2134

Xie J, Huang J, Li X et al (2009) Iron oxide nanoparticle platform for biomedical applications. Curr Med Chem 16:1278–1294

Xu P, Gullotti E, Tong L et al (2009) Intracellular drug delivery by poly (lactic-co-glycolic acid) nanoparticles, revisited. Mol Pharm 6:190–201

Yan Z, Yang Y, Wei X et al (2014) Tumor-penetrating peptide mediation: An effective strategy for improving the transport of liposomes in tumor tissue. Mol Pharm 11:218–225

Yue ZG, Wei W, Lv PP et al (2011) Surface charge affects cellular uptake and intracellular trafficking of chitosan-based nanoparticles. Biomacromolecules 12:2440–2446

Zhang Y, So MK, Rao J (2006) Protease-modulated cellular uptake of quantum dots. Nanolett 6:1988–1992

Zhang Y, Bai Y, Jia J et al (2014) Perturbation of physiological systems by nanoparticles. Chem Soc Rev 43:3762–3809

Zhao X, Wu Y, Gallego-Perez D et al (2015) Effect of nonendocytic uptake of nanoparticles on human bronchial epithelial cells. Anal Chem 87:3208–3215

Zhou R, Zhou H, Xiong B et al (2012) Pericellular matrix enhances retention and cellular uptake of nanoparticles. J Am Chem Soc 134:13404–13409

Zhu M, Nie G, Meng H et al (2013) Physicochemical properties determine nanomaterial cellular uptake, transport, and fate. Acc Chem Res 46:622–631