Chapter 6
Biomolecules–Nanoparticles: Interaction in Nanoscale

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6.1 Introduction

Nanomaterials that measure 1–1,000 nm allow unique interaction with biological systems at the molecular level (Yezhelyev et al. 2006). As nanoparticles and biomolecules are of similar length scale, it seems logical that the combination of biomacromolecules to nanomaterials can provide interesting tool for mimicking the biomolecules which are present at cellular systems, probing the mechanisms of biological processes, as well as developing chemical means by handling and manipulating biological components (Katz and Willner 2004). The interactions of biomolecules and metal nanoparticles arise that determine the size of nanoparticles, modify the surface of nanoparticles to enhance solubility/biocompatibility/bio-recognition, and detoxification of toxic metals.

Nanoparticles can be synthesized in sizes ranging from a few nanometers to few hundreds of nanometers, which is comparable to those of a cell (5–100 μm), a virus (10–500 nm), a protein (1–50 nm), or a nucleic acid (2 nm wide and 5–100 nm long). This allows close interaction between the nanoparticles and the biological entity of interest thus leading to better integration of nanotechnology and biotechnology. In addition to the advantage in their size, the physicochemical properties of nanoparticles that are very different from those of bulk materials enhance their proposed applications.

With sophistication and advances in various fields, the macromolecule and nanoparticles interaction helped in ultra-trace detection, imaging, biomolecules detection, drug and DNA/RNA delivery, cancer therapy, and photodynamic therapy. The different groups of macromolecules that interact with nanoparticles are given in Fig. 6.1. The interaction of nanoparticles with nucleic acids and proteins are very high due to the presence of active functional groups on the surface; while that of carbohydrates and lipids is comparatively lower.

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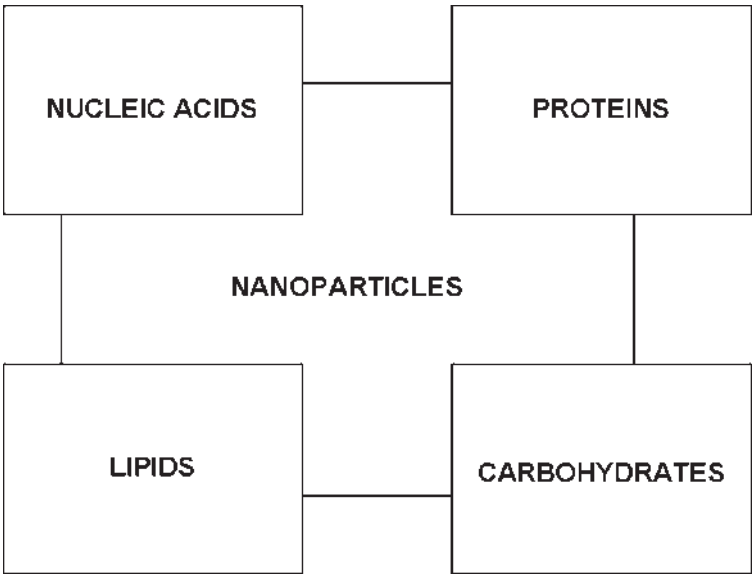

Fig. 6.1 Interaction of nanoparticles with various biomolecules

6.2 Nucleic Acid–Nanoparticles Interaction

Among the various nanoparticles, gold followed by silver and platinum are highly explored for use in various molecular level applications due to their high affinity with nucleic acids. A major breakthrough happened in 1996 when Mirkin, Alivisatos and co-workers coupled nanoparticles to DNA (Mirkin et al. 1996; Alivisatos et al. 1996) and proved that nanoparticles were highly sensitive spectroscopic reporters for the base-pairing of DNA. Mirkin showed that high-packing density of SiRNA on the surface of gold nanoparticles inhibits its degradation by various enzymes and facilitates it uptake by HeLa cells (Giljohann et al. 2009). A new colorimetric sensor for mercuric ions was developed by Lie (Xue et al. 2008) by exploiting the highly cooperative base-pairing of DNA bound gold nanoparticles that result in sharp melting transitions. The binding of mercuric ions between mismatched thymine base tips the balance in favor of hybridization and particle aggregation at room temperature. The folding state of DNA aptamers on gold nanoparticles surfaces modulates their electrostatic interaction and thus also affects the aggregation of particles. The oligonucleotides are usually coupled on the surface of gold nanoparticles by means of thiol adsorption.

As electrochemical intercalators, gold (Au) nanoparticles show high catalysis activity and compatibility for detection of biological molecules. An electrochemical approach for sequence-specific DNA-binding transcription factor detection by Au nanoparticle-catalyzed silver (Ag) enhancement at interface between electrodes and electrolyte solutions was reported by Pan et al. 2008 (Fig. 6.2). Here unimolecular hairpin oligonucleotides were self-assembled onto Au electrode surface and their elongation on Au electrode surface was carried out to form double-stranded oligonucleotides with transcription factor NF-κB (nuclear factor-kappa B) binding sites. Au nanoparticle-catalyzed Ag deposition was detected by anodic

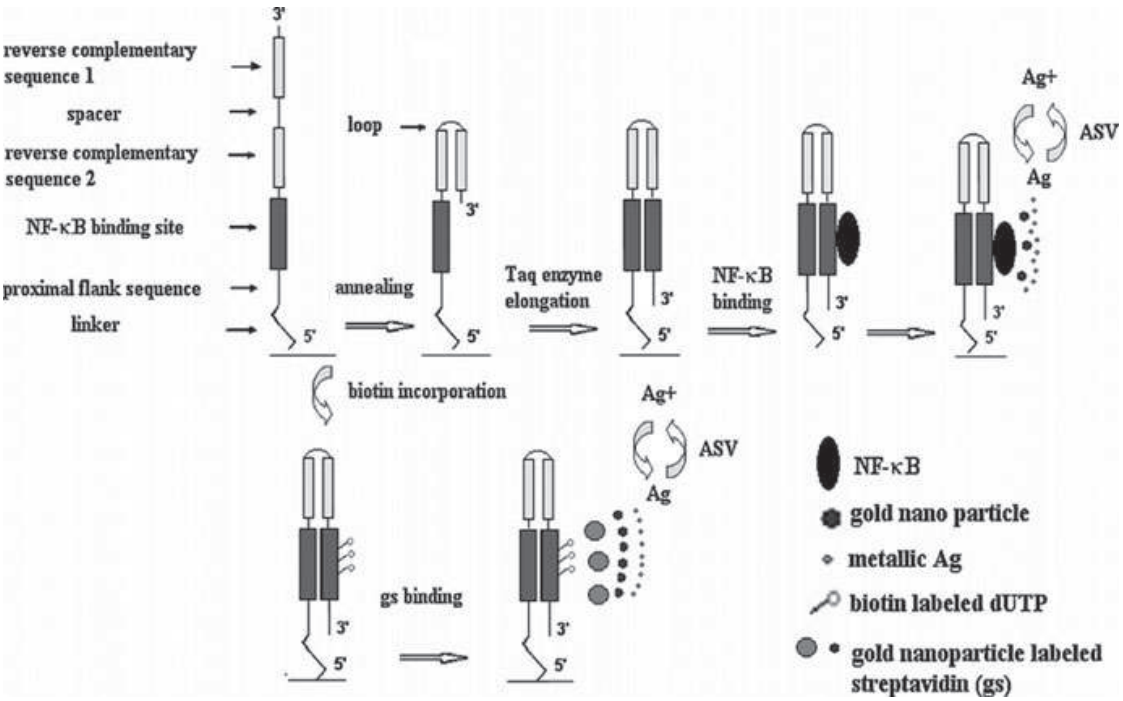

Fig. 6.2 Principle of Au nanoparticle-based electrochemical detection of NF-κB and DNA interaction. Unimolecular hairpin oligonucleotides were immobilized on Au electrodes with 5′ modified with thiol group. Then elongation of the unimolecular hairpin oligonucleotides to double-stranded oligonucleotides was carried out. Electrochemical approaches were applied to verify the elongation on Au electrodes. One was detection of the intrinsic guanine signals of double-stranded DNA without any indicators. The other was incorporation of biotin-labeled dUTP during the elongation followed by the addition of Au nanoparticle-labeled streptavidin and detection of ASV signals of Au nanoparticle-catalyzed Ag deposition. After the verification steps, NF-κB was bound to elongated double-stranded DNA and Au nanoparticles were reacted with the bound NF-κB. Subsequently, the ASV signals of Au nanoparticle-catalyzed Ag deposition were analyzed [with permission from Pan et al. (2008)]

stripping voltammetry (ASV) for NF-κB binding. It was found that this method for the detection of sequence-specific DNA-binding protein showed pronounced specificity and that the detection limit was as low as 0.1 pM.

6.3 Protein–Nanoparticles Interaction

Calvo exploits both the scattering and electrochemical properties of gold–glucose oxidase (core–shell) nanoparticles to develop a surface-enhanced Raman-based sensor for detection of glucose in the millimolar concentration (Scodeller et al. 2008). The optical spectroscopic study (Delfino and Cannistraro 2009) on the hybrid system obtained by conjugating the electron transfer blue copper protein Az to 20-nm sized gold nanoparticles provided interesting information on the interactions occurring at the interface between the protein and the metal surface

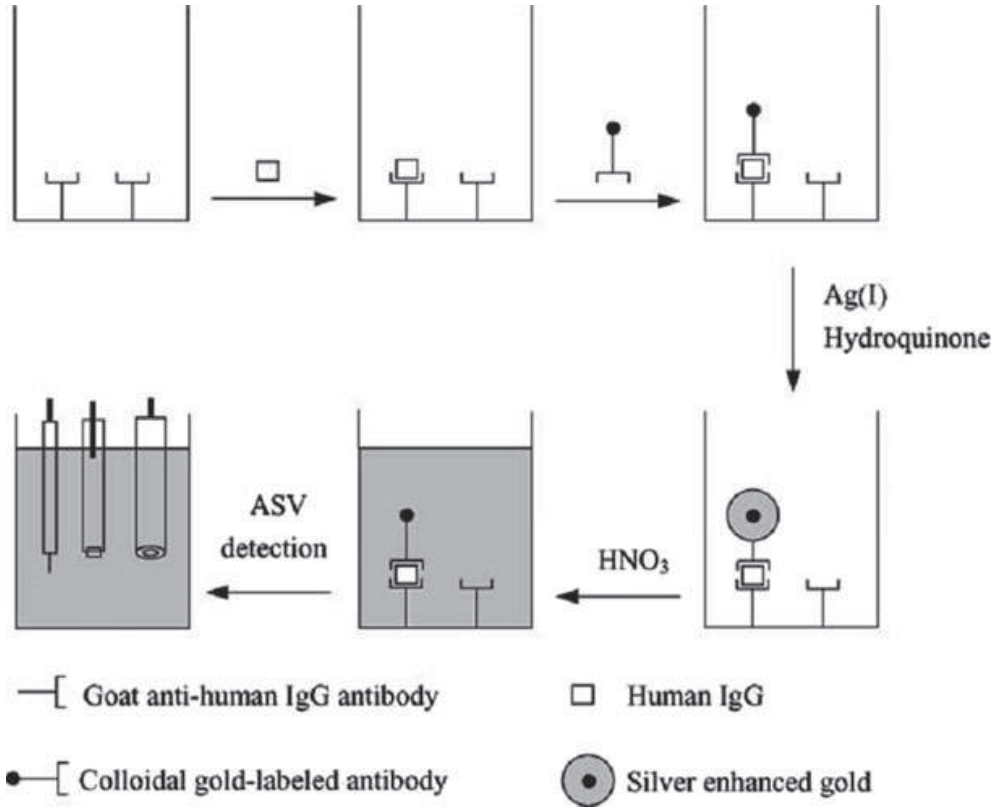

Fig. 6.3 Schematic representation of the analytical procedure of the heterogeneous electrochemical immunoassay based on silver-enhanced gold nanoparticle label [with permission from Chu et al. (2005)]

and on the resulting photophysical properties of the system. The analysis of the plasmon resonance band of the composite along with DLS results confirmed the interaction of Az with gold surface, probably involving a monolayer of protein molecules grafted on AuNPs. Each nanoparticle was estimated to bind at maximum 140 Az molecules which were thought to preserve their natural folding. The binding constant of proteins is proved to vary depending on the shape of nanoparticles; it was higher for BSA with spherical shaped gold nanoparticles than to that of rod shaped (Iosin et al. 2009). The principle of the heterogeneous electrochemical immunoassay (Chu et al. 2005) based on silver-enhanced colloidal gold is depicted in Fig. 6.3 and it was applied to human IgG analyte. Primary antibodies specific for human IgG are adsorbed passively on the walls of a polystyrene microwell. The human IgG analyte is first captured by the primary antibody and then sandwiched by a secondary colloidal gold-labeled antibody. After removal of the unbound labeled antibody, the silver-enhancer solution is added and incubated in the dark. As the silver ions in the silver-enhancer solution can only be catalytically reduced exclusively on the gold colloids, a large amount of specific silver deposition is produced at the walls of the polystyrene microwell through the catalytic reduction of the silver ions on the antibody–colloidal gold conjugate. The silver metal thus deposited is then dissolved in an acidic solution and the silver ions (AgI) released in solution are quantitatively determined at a glassy-carbon electrode by ASV.

The electrochemical signal is directly proportional to the amount of analyte (human IgG) in the standard solution or sample.

The paracrystalline proteinaceous surface layers (S-layers) are one of the most common surface structures present in all major phylogenetic groups of bacteria and in almost all archaea. They are composed of protein or glycoprotein monomers of a molecular weight between 40 and 200 kDa with the ability to self-assemble into two-dimensional paracrystalline arrays exhibiting oblique (p1, p2), square (p4), or hexagonal (p3, p6) symmetries or other structures. Due to the crystalline arrangement of the S-layer, functional groups such as carboxyl-, amino- or hydroxyl groups, are found in well-defined position and orientation on the protein meshwork. S-layers have been used as templates for the fabrication of different inorganic nanocrystal arrays (Pollmann et al. 2006).

6.4 Lipids–Nanoparticles Interaction

Pulmonary surfactant adsorption to form a surface film normally occurs through unique vesicular structures known as tubular myelin (Bakshi et al. 2008). Gold nanoparticles inhaled into the alveolar space during the breathing process could impact with such surface films, become wetted, and lined with phospholipids bilayers (Fig. 6.4).

6.5 Carbohydrates–Nanoparticles Interaction

Although several researchers worked on nanomaterials functionalized with proteins, peptides, DNA and RNA during the last decade, very few of them reported on nanoparticles covered with carbohydrates. The accurate detection of carbohydrates is of interest for improving long-term health care. Numerous efforts have been made using spectroscopic methods such as absorbance and luminescence to investigate carbohydrate binding to labeled organic compounds or superstructures. Tiopronin-coated silver nanoparticles [Fig. 6.5 (Zhang et al. 2004)] were prepared using a modified Brust reaction with a 1:1 mole ratio of tiopronin and silver nitrate in methanol. The boronic acid capped on the silver nanoparticle had a higher detective sensitivity for the polysaccharide. This method may help to facilitate the study of complex macromolecular structures such as glycoproteins, which have a substantial polysaccharide component.

Though at its nascent stage, a variety of applications (Fig. 6.6) of glyco-quantum dots, gold glyconanoparticles and magnetic glyconanoparticles will be developed in the near future (Fuente and Penades 2006). The interesting physical, chemical, and biological properties of the glyconanoclusters will allow them to extend their utility in Biomedicine, Biotechnology, and Material Science.

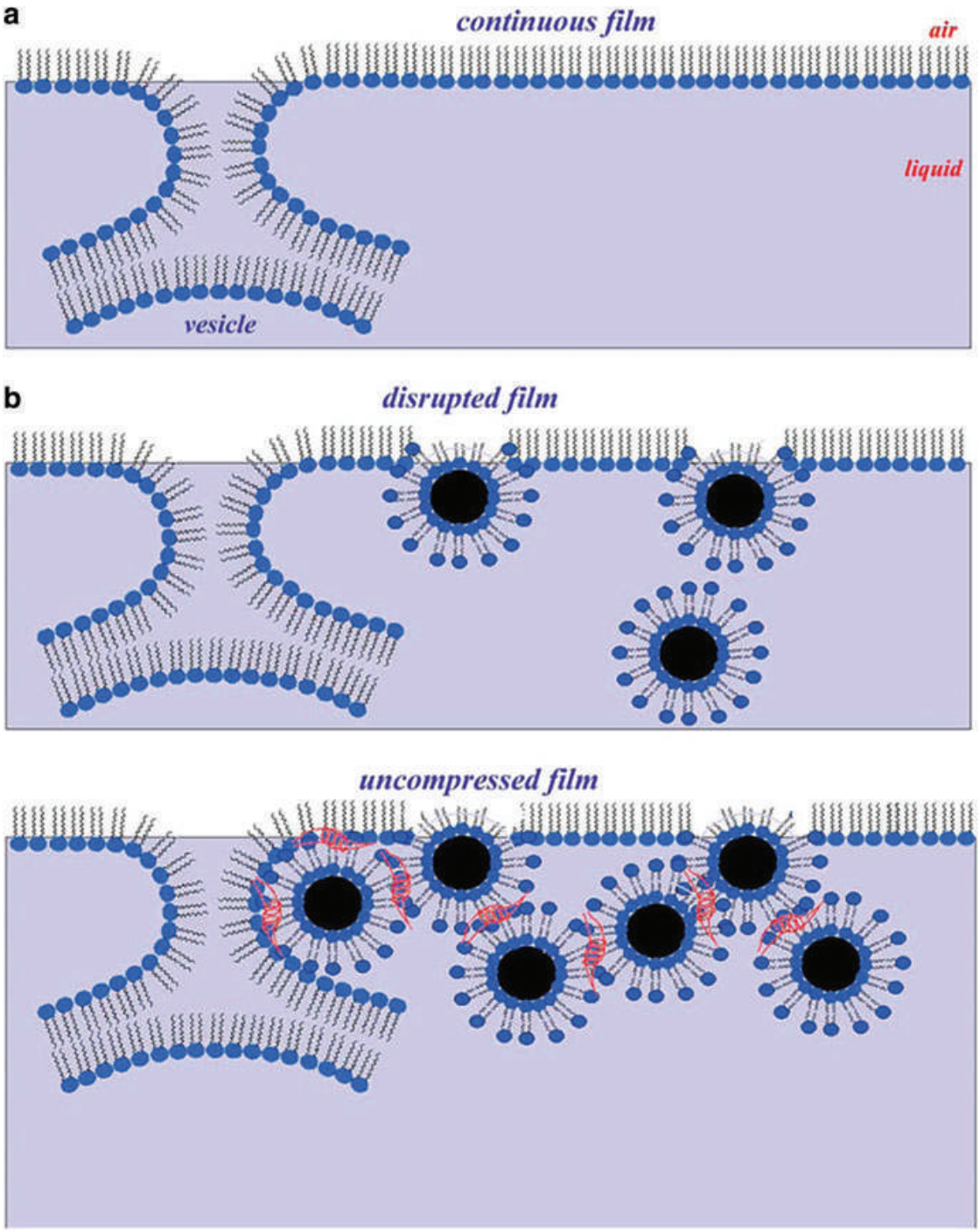

Fig. 6.4 Schematic representation of potential inhibitory effects of Au NP on pulmonary surfactant in the alveolar space (not to scale). (**a**) A continuous surfactant film (monolayer and underlying multilayer) formed by the rupture of pulmonary surfactant vesicles at the air–liquid interface as the hypothetical rate-limiting intermediate structure between bilayer vesicles and the interfacial monolayer (see the literature (3, 7, 67) for further details). (**b**) The disrupted interfacial surfactant film due to the entrapping of Au NP (from the air phase as pollutants) by pulmonary surfactant. (**c**) The self-aggregation of lipid capped Au NP in the presence of SP-B shown in ladder like lines. [with permission from Bakshi et al. (2008)]

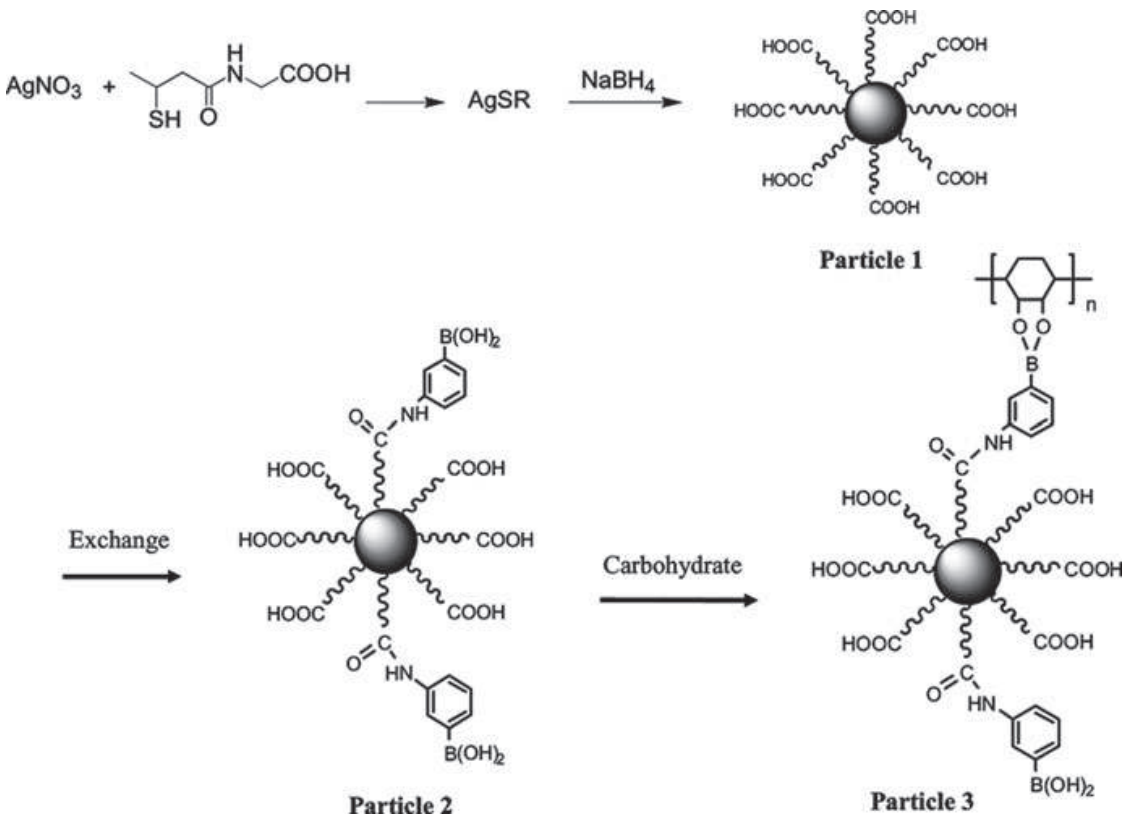

Fig. 6.5 Preparation of tiopronin-monolayer-protected silver nanoparticle, ligand exchange by compound 1, and coupling with saccharide [with permission from Zhang et al. (2004)]

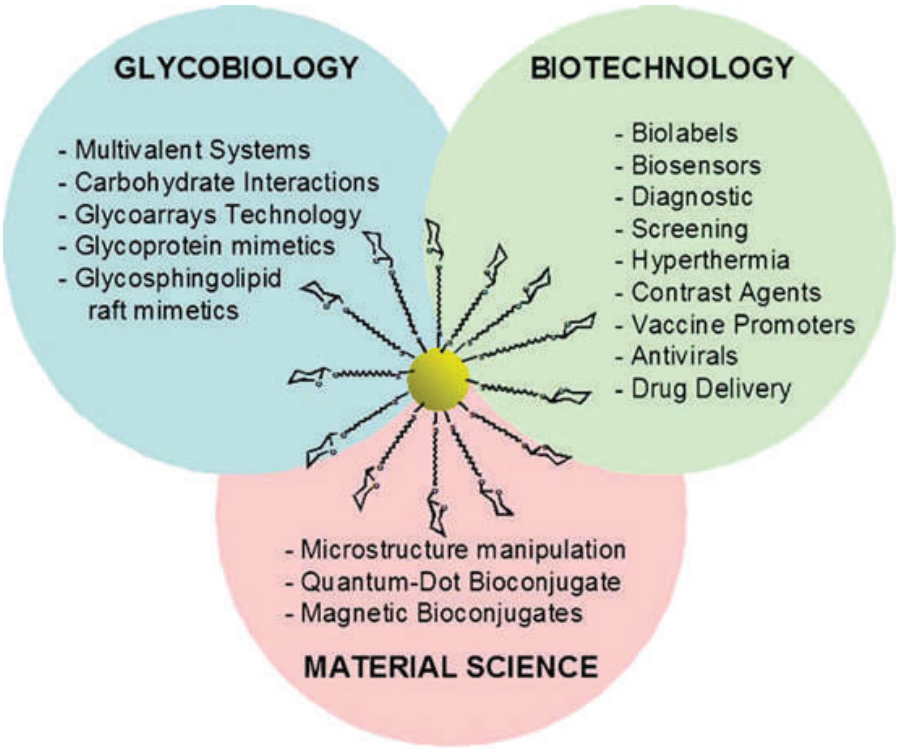

Fig. 6.6 The glyconanoparticle concept and its potential applications [with permission from Fuente and Penades (2006)]

Although the use of colloidal particles of metals and semiconductors as pigments dates back many centuries, and although the recipe for stable 6 nm diameter particles of gold ("Ruby gold") was famously devised by Faraday in 1857 (Zhu et al. 2008) the unique properties of nanomaterials and their promise for applications in biochemistry, cell biology, and medicine have only recently been appreciated.

Research in the current decade has led to a much more sophisticated set of tools for controlling the size, shape, dispersity, and surface chemistry of nanoparticles. The complexity of nanoparticle structures as nanoshells, prisms, Janus particles, derivatized nanotubes, core–shell particles, and striped nanowires, to name a few, offers new tools to tailor particles to specific problems in ultra-trace detection, imaging, drug delivery, DNA/RNA delivery, and therapy. Sophisticated schemes for signal amplification, enhanced bioaffinity through multivalency, and cooperative binding highlight the synergy of nanoparticles as platforms for biological molecular recognition. The deliberate design of nanoparticles for biological applications, for example for drug delivery or for dual functions (such as imaging and magnetic manipulation of cells), has been enabled by these new advances in nanoparticle synthesis. Nanoparticles can have several functionalities that enhance their effectiveness for drug delivery, including transport and targeting within cells and controlled release.

Both biotechnology and materials science meet at the same length scale (nanometer). To exploit and to utilize the concepts administered in natural nanometer-scale systems, the development of nanochemistry is crucial (Philp and Stoddart 1996). On the other hand, commercial requirements to produce increasingly miniaturized microelectronic devices strongly motivate the elaboration of nanoscale systems. The structural dimensions of computer microprocessors are currently in the range of about 200 nm. They are only just available by conventional top down processes (miniaturization processes) such as photolithography, but for the foreseeable future, such technologies hardly allow the large-scale production of parts that are significantly smaller than 100 nm. "There is plenty of room at the bottom", as Nobel physicist Richard Feynman pointed out more than 50 years ago (Feynman 1961).

6.6 Interactions of Macromolecules and Nanoparticles in Various Applications

6.6.1 Biotemplates and Biomimetics

The study of biosynthesis of nanomaterials offers a valuable contribution into materials chemistry. Yan et al. (2003) have reported DNA templated synthesis of highly conductive, uniform-width, silver nanowires. The ability of some microorganisms such as bacteria and fungi to control the synthesis of metallic nanoparticles offers a viable approach as an alternative to chemical and physical ones. Recently, Sadowski et al. (2008) have reported the biosynthesis of silver nanoparticles using *Penicillium*

fungi. Various organic molecules and polymers such as amino acids, citric acid, vitamins, cyclodextrin, chitosan, starch, etc. can be employed as biotemplates for the synthesis of metal nanoparticles. Recently Xia et al. reported a facile synthetic approach for preparing water-soluble Fe_3O_4 NPs using cyclodextrin in aqueous medium.

Biomimetic processes have attracted huge attention in recent years due to their significant applications in biomedical areas such as bone tissue engineering. Titanium is a well-known bone repairing material widely used in orthopedics and dentistry. It has a high fracture resistance, ductility, and weight to strength ratio. Unfortunately, it suffers from the lack of bioactivity, as it does not support cell adhesion and growth well. Apatite coatings are known to be bioactive and to bond to the bone. Ma et al. (2003) have employed a biomimetic process, to form a nanocrystallite apatite coating on metal. A thin bone-like apatite layer was coated onto titanium (Ti) metals via an alkali pretreatment. Their work has shown that the apatite layer grown in this way exhibits nanostructure and has similar stoichiometry to that of natural bone. It was also observed that the thickness of the apatite layer increases as the immersion period increases. Their studies have shown that a uniform coating of carbonate-containing apatite (hydroxyapatite) is firmly adhered on the Ti metal. The adhesion of the apatite layer on the Ti substrate was confirmed by a shear test, which showed an average value of 9.5 MPa indicating good mechanical properties. The bioactivity of the coating was examined by cell culturing experiments and was found to be satisfactory.

6.6.2 Drug Delivery

Nanoparticle-based drug delivery systems are increasingly being used for treatment of certain types of cancer, as opposed to chemotherapy or radiation therapy. The conventional treatments are administered resulting in deleterious side-effects as the drug/therapy attacks normal, healthy cells in addition to the target tumor cells. The application of magnetic nanoparticles (MNPs) as carriers for drug delivery overcomes this major disadvantage of nonspecificity. The objectives are twofold: (1) to reduce the amount of systemic distribution of the cytotoxic drug, thus reducing the associated side-effects; and (2) to reduce the dosage required by more efficient, localized targeting of the drug. In magnetically targeted therapy, a cytotoxic drug is attached to a biocompatible magnetic nanoparticle carrier such as superparamagnetic iron oxide nanoparticles (SPIONs) of γ-Fe_2O_3 or Fe_3O_4. These particles are well dispersed in water, or form composites with organic or inorganic matrices in the form of beads. Superparamagnetic magnetization can reach nearly the magnetization saturation of ferromagnetic iron oxide by application of an external magnetic field. This behavior allows the tracking of such particles in a magnetic field gradient without losing the advantage of a stable colloidal suspension. These drug/carrier complexes are injected into the patient via the circulatory system usually in the form of a biocompatible ferrofluid. When the particles have entered the

bloodstream, external, high-gradient magnetic fields are used to concentrate the complex at a specific target site within the body. Once the drug/carrier is concentrated at the target, the drug can be released either via enzymatic activity or changes in physiological conditions such as pH, osmolality, or temperature, and be taken up by the tumor cells.

Depending on the synthesis procedure, nanocapsules are obtained where the MNPs are coated with a biocompatible molecule attached through a linker. Coating with a neutral and hydrophilic compound (i.e., polyethylene glycol, polysaccharides, dysopsonins (HSA), biotin, etc.) increases the circulatory half-life from minutes to hours or days. The key parameters in the behavior of MNPs are related to surface chemistry, size (magnetic core, hydrodynamic volume, and size distribution), and magnetic properties (magnetic moment, remanence, coercivity).

In 1996, the first Phase I clinical trial was carried out by Lubbe et al. (1996) in patients with advanced and unsuccessfully pretreated cancers using MNPs loaded with epirubicin. FeRx, Inc. (founded in 1997) produced doxorubicin-loaded MNPs (Goodwin et al. 1999) consisting of metallic Fe ground together with activated carbon. A Phase II clinical study in patients with primary liver cancer was conducted using these MNPs, although the trial was not successful. Chemicell GmbH has commercialized TargetMAG-doxorubicin NPs (Steinfeld and Pauli 2006) involving a multidomain magnetite core and a cross-linked starch matrix with terminal cations that can be reversibly exchanged by the positively charged doxorubicin. The particles have a hydrodynamic diameter of 50 nm and are coated with 3 mg/ml doxorubicin. These NPs loaded with mitoxantrone (Wiekhorst et al. 2006) have already been used in animal models with successful results. Chemicell has also commercialized FluidMAG® for drug delivery applications. MNP hydro-gel (MagNaGel®) from Alnis Biosciences, Inc. is a material comprising chemotherapeutic agents, Fe oxide colloids, and targeting ligands.

A wide variety of molecules has been loaded onto organic and inorganic shells, e.g., by chemical functionalization or physical absorption. The list includes tumor-recognition moieties such as antibodies in "smart" contrast agents, and enzymes, toxins, genes(transfection) (Schillinger et al. 2005), growth factors, radionucleotides, folic acid, and drugs (mitoxantrone, tamoxifen, cefradine, doxorubicin, ammonium glycyrrhizinate, fludarabine, danorubicin, cisplatin and gemcitabine, pingyangmycin, nonsteroidal anti-inflammatory drugs (Taepaiboon et al. 2006), amethopterin, mitomycin, paclitaxel, diclofenac sodium, and adriamycin) for drug delivery applications.

Another approach to cancer treatment is hyperthermia where the tumor region is heated locally to the intended temperature without damaging normal tissue. The procedure involves dispersing magnetic particles throughout the target tissue and applying an AC magnetic field with sufficient strength and frequency to cause the particles to heat. The generated heat conducts into the immediately surrounding diseased tissue, and the cancer is destroyed. Magnetic liposomes composed of Mn-ferrite have been prepared through thin film hydration (Pradhan et al. 2007), loaded with 2:1 ratio of egg-phosphatidyl choline (egg-PC) and cholesterol, which have been found suitable for treating hyperthermia.

In addition to cancer treatment, MNPs can also be used in anemic chronic kidney disease and disorders associated with the musculoskeletal system (i.e., local inflammatory processes, side effects). For those disorders, superparamagnetic Fe oxide NPs (SPION), in conjunction with external magnetic fields, seem a suitable alternative for drug delivery to inflammatory sites (Neuberger et al. 2005).

6.6.3 Bioimaging and Magnetic Resonance Imaging

The rapid development of bio-medical sciences demands new advanced techniques and instruments to investigate cells and cellular processes. In the last years, luminescent nanoparticles (NPs) have attracted growing attention as a versatile and promising tool for bio-imaging. Bio-imaging involves developing multifunctional nanoparticles with tailored optical and/or magnetic properties for visualizing complex cellular structures (in tissues and organs), receptors, tumor cells, and masses.

The II–VI semiconductor nanocrystals were prepared for use as fluorescent probes in biological staining and diagnostics. The advantages of the broad, continuous excitation spectrum were demonstrated in a dual-emission, single-excitation labeling experiment on mouse fibroblasts (Bruchez et al. 1998).

The II–VI quantum dots (QDs) have demonstrated usefulness for several applications ranging from cell labeling to tracking cell migration, from flow cytometry to genomic and proteomic detection, high throughput screening of biology, etc., however, their application in biology and medicine is hampered by their inherent chemical toxicity. Unlike bulk Si that is not a good light emitter, nanostructured Si can emit photons in the visible- near IR range with a reasonable efficiency. It follows that silicon nanoparticles (Si-NPs) have the potential to overcome the inherent limitations in the biomedical use of QDs since silicon is inert, nontoxic, abundant, and economical. Moreover, the silicon surface is apt to chemical functionalization, thus allowing for numerous stabilization and bioconjugation steps. For *in vitro* and *in vivo* applications, NPs were coated with a biocompatible polymer such as functional silanes terminated with amine or epoxy groups, and then were conjugated with poly (ethyleneglycol) to prevent the formation of large aggregates in order to improve biodistribution. It was also found that the use of HF/HNO_3 mixture as etching agent can make the Si-NPs photoluminescent with various emission colors depending on the etching time. Preliminary *in vivo* experiments were performed by detection of the photoluminescence signal in continuous mode after intravenous injection of a colloidal solution of Si-NPs in tail vein of a mouse.

Another class of materials of great interest for bio-imaging is the metallic NPs of Ag and Au. When compared with organic chromophores, the absorption and scattering cross-section of Au and Ag NPs are several orders of magnitude higher. As a result, these NPs have recently been explored as contrast agents (CA) for optical imaging of tumors. In addition, it is possible to tailor the spectral dependence of absorption and scattering coefficients of Au and Ag spherical NPs by engineering their geometrical parameters. The optical resonant behavior is due to the collective

electronic or plasmonic resonance which depends not only from the metal but also from the particle shape. Tuning the plasmon resonance, combined with the easy bioconjugation of Au nanostructures, results in a combination of features which are ideal for biomedicine.

An interesting and novel application of nanoparticles in biology is their use as intracellular magnetic labels in nuclear magnetic resonance imaging (MRI). The presence of MNPs near a cell results in a much faster rate of magnetic relaxation of protons in the cell. Since NMR imaging is based on the rate of magnetic relaxation, there is distinct contrast introduced. Iron oxide nanoparticles are the most commonly used superparamagnetic contrast agents. Dextran-coated iron oxides are biocompatible and are excreted via the liver after the treatment. They are selectively taken up by the reticuloendothelial system, a network of cells lining blood vessels whose function is to remove foreign substances from the bloodstream. MRI contrast relies on the differential uptake of different tissues; thus by modifying the size and the nature of coating, it is possible to modify the organ distribution and the pharmacokinetics and consequently the clinical application. Magnetic iron oxide NPs can be used as negative MRI contrast agents (Laurent et al. 2008). They have marked T2 relaxivity due to their high magnetic moment, which generates microscopic field inhomogeneities. Consequently they produce a strong decrease in signal intensity in the organs in which they accumulate. There are currently two distinct classes in this family of products depending on the size of the particles: SuperParamagnetic Iron Oxide (SPIO), with mean particle diameter greater than 50 nm, and Ultra-small SPIO particles (USPIO) smaller than 50 nm.

Cengelli et al. (2006) have developed SPIONs coated with dextran or polyvinyl alcohol, to enhance detection of neurodegenerative diseases. They monitored the uptake by isolated brain-derived endothelial and microglial cells and found no cytotoxicity or inflammatory activation even at a much higher levels.

In another study concerning atherosclerosis by Hildebrandt et al. (2007), SPIONs were coated with dextran having negatively charged functionalities, in order to connect specific peptide labels by electrostatic interactions. Peptides containing a specific recognition sequence and amino acids that are positively charged under physiological conditions were bound to the negatively charged dextran. These peptides can then selectively bind to the receptors on the damaged artery walls and can be imaged. Similar work using PEG-gallol has been carried out (Amstad et al. 2007) but with Neutravidin, a peptide binding protein, as the linker between PEG and peptide.

6.6.4 Sensors and Biosensors

Localized surface plasmon resonance of metal nanoparticles has been exploited in several ways for sensing applications, because this optical characteristic is the basis of various new and highly promising set ups to transduce biorecognitive interactions into visible signals. Semiconductor nanoparticles are viable for sensors due to

three main reasons – (1) the sensitivity of surface plasmon band to its immediate environment offers an opportunity to detect attached molecules and environmental changes, (2) the reversible aggregation of plasmon resonant particles through specific linkers provides an excellent means for colorimetric assays, and (3) the ultra-bright light scattering from each plasmon resonant particle makes the optical detection of a single molecular target possible.

Nam et al. (2003) have developed an ultra-sensitive nanoparticle-based bar-coding method for detecting protein analytes. The system relies on magnetic microparticle probes with antibodies that specifically bind a target of interest [prostate-specific antigen (PSA) in this case] and nanoparticle probes that are encoded with DNA that is unique to the protein target of interest and antibodies that can sandwich the target captured by the microparticle probes. Magnetic separation of the complexed probes and target followed by dehybridization of the oligonucleotides on the nanoparticle probe surface allows the determination of the presence of the target protein by identifying the oligonucleotide sequence released from the nanoparticle probe. Because the nanoparticle probe carries with it a large number of oligonucleotides per protein binding event, there is substantial amplification and PSA can be detected at 30 aM concentration.

Edelstein et al. (2000) have developed the BARC sensor for the detection of biological warfare agents. The Bead ARray Counter (BARC) is a multi-analyte biosensor that uses DNA hybridization, magnetic microbeads, and giant magneto-resistive (GMR) sensors to detect and identify biological warfare agents. The current prototype is a table-top instrument consisting of a microfabricated chip (solid substrate) with an array of GMR sensors, a chip carrier board with electronics for lock-in detection, a fluidics cell and cartridge, and an electromagnet. DNA probes are patterned onto the solid substrate chip directly above the GMR sensors, and sample analyte containing complementary DNA hybridizes with the probes on the surface. Labeled, micron-sized magnetic beads are then injected that specifically bind to the sample DNA. A magnetic field is applied, removing any beads that are not specifically bound to the surface. The beads remaining on the surface are detected by the GMR sensors, and the intensity and location of the signal indicate the concentration and identity of pathogens present in the sample. The current BARC chip contains a 64-element sensor array; however, with advancement in technology, chips with millions of these GMR sensors will soon be commercially available, allowing simultaneous detection of thousands of analytes. Because each GMR sensor is capable of detecting a single magnetic bead, in theory, the BARC biosensor should be able to detect the presence of a single analyte molecule.

Enzymes are also commonly used in biosensors because of their high specificity. Biosensor applications require a highly active immobilized enzyme system that allows the maintenance of an efficient connection between the sensing molecule and the transduction component of the biosensor. Immobilization strategies include covalent bonding, physical adsorption, cross-linking, encapsulation, or entrapment. Betancor et al. (2006) have reported a strategy for chemically associating silica nanospheres containing entrapped β-galactosidase to a silicon support for the detection of lactose. The immobilization strategy resulted in a three-dimensional