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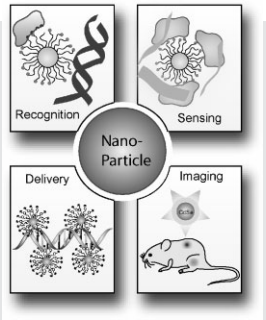
Applications of Nanoparticles in Biology**

By *Mrinmoy De, Partha S. Ghosh,* and *Vincent M. Rotello**

The wide variety of core materials available, coupled with tunable surface properties, make nanoparticles an excellent platform for a broad range of biological and biomedical applications. This Review provides an introduction to nanoparticle–biomolecular interactions as well as recent applications of nanoparticles in biological sensing, delivery, and imaging of live cells and tissues.



1. Introduction

The use of nanomaterials in biotechnology merges the fields of material science and biology. Nanoparticles provide a particularly useful platform, demonstrating unique properties with potentially wide-ranging therapeutic applications.[1] The field of nanoparticles in biology is certainly a burgeoning one, with the estimated number of papers in the area (based on Web of Science) rising from 11 in 1991 to nearly 10000 in 2007. Clearly, we cannot exhaustively cover the field, so this Review provides a brief overview of recent studies using spherical nanoparticles with metallic, metal oxide, semiconductor, and silica cores.

The unique properties and utility of nanoparticles arise from a variety of attributes, including the similar size of nanoparticles and biomolecules such as proteins and polynucleic acids. Additionally, nanoparticles can be fashioned with a wide range of metal and semiconductor core materials that impart useful properties such as fluorescence and magnetic behavior.[2] The applicable properties of some well-known core materials and corresponding possible ligands used for surface functionalization with their possible applications are summarized in Table 1.

[*] Prof. Vincent M. Rotello, M. De, P. S. Ghosh
Department of Chemistry
University of Massachusetts
710 North Pleasant Street
Amherst, MA 01003 (USA)
E-mail: rotello@chem.umass.edu

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In this Review we will discuss general approaches to the integration of nanoparticles with biomolecules. We will then discuss three major areas of nanoparticle application: (i) drug and gene delivery, (ii) biosensing, and (iii) bioimaging.

2. Nanoparticle–Biomolecule Interactions

Biomacromolecule surface recognition by nanoparticles as artificial receptors provides a potential tool for controlling cellular and extracellular processes for numerous biological applications such as transcription regulation, enzymatic inhibition, delivery and sensing. The size of nanoparticle cores can be tuned from 1.5 nm to more than 10 nm depending on the core material, providing a suitable platform for the interaction of nanoparticles with proteins and other biomolecules (Fig. 1).[3]

The conjugation of nanoparticles with biomolecules such as proteins and DNA can be done by using two different approaches, direct covalent linkage and non-covalent interactions between the particle and biomolecules.[4–9] The most direct approach to the creation of integrated biomolecule–nanoparticle conjugates is through covalent attachment.[10] This conjugation can be achieved either through chemisorption of the biomolecule to the particle surface or through the use of heterobifunctional linkers. Chemisorption of proteins onto the surface of nanoparticles (usually containing a core of Au, ZnS, CdS, and CdSe/ZnS) can be done through cysteine residues that are present in the protein surface (e.g., oligopeptide, serum albumin),[11] or chemically using 2-iminothiolane (Traut's reagent).[12] Bifunctional linkers provide a versatile

Table 1. Characteristics, ligands and representative applications for various metal and semiconductor materials.

Core material	Characteristics	Ligand(s)	Applications
Au	Optical absorption, fluorescence and fluorescence quenching, stability	Thiol, disulfide, phosphine, amine	Biomolecular recognition, delivery, sensing
Ag	Surface-enhanced fluorescence	Thiol	Sensing
Pt	Catalytic property	Thiol, phosphine, amine, isocyanide	Bio-catalyst, sensing
CdSe	Luminescence, photo-stability	Thiol, phosphine, pyridine	Imaging, sensing
Fe_2O_3	Magnetic property	Diol, dopamine derivative, amine	MR imaging and biomolecule purification
SiO_2	Biocompatibility	Alkoxysilane	Biocompatible by surface coating

means of bioconjugation. Biomolecules are often covalently linked to ligands on the nanoparticle surface via traditional coupling strategies such as carbodiimide-mediated amidation and esterification.[13] For biological applications oligoethylene glycol (OEG) or polyethylene glycol (PEG) is used in the linker to enhance the stability of the attached biomolecules and minimize non-specific adsorption of other materials.

Non-covalent assembly provides a highly modular approach to the biofunctionalization of nanoparticles. DNA–NP binding can be effected through electrostatic interactions, groove binding, intercalation, and complementary single-strand DNA binding.[14] Nanoparticles provide an attractive receptor for nucleic acids, providing a direct analogy to protein–DNA interactions.[15,16] One approach to particle–DNA assembly uses complementary electrostatic interactions to promote high affinity of nanoparticle–DNA binding. The use of cationic ligands on the nanoparticle surface provides a complementary surface for binding the negatively charged backbone of DNA, for example the use of **NP1** to recognize a 37-mer DNA duplex (Fig. 2a and b) by Rotello.[17] The binding of the DNA inhibited transcription by T7 RNA polymerase, indicating the high affinity of the NP–DNA complex, and pointing out a

Mrinmoy De received his B.Sc. in Chemistry from Vidyasagar University, India in 2000 and M.Sc. in Organic Chemistry from the Indian Institute of Technology-Bombay, India in 2002. He is currently pursuing a Ph.D. at the Department of Chemistry, University of Massachusetts at Amherst, USA under the guidance of Prof. Vincent M. Rotello. His current research is focused on the use of nanoparticles for biomolecular recognition, their applications in biology, and the development of biomaterials.

Partha S. Ghosh received his B.Sc. in Chemistry from Ramamkrishna Mission Vidyamandira at Belur, India in 2002. He received his M.Sc. from the Indian Institute of Technology-Kanpur, India in 2004. He is currently a graduate student at the Department of Chemistry, University of Massachusetts at Amherst, USA under the guidance of Professor Vincent M. Rotello. His current research is focused on the use of nanoparticles for delivery applications.

Vincent M. Rotello received his B.Sc. from the Illinois Institute of Technology, USA in 1985, his Ph.D. in 1990 from Yale University, USA and was an NSF postdoctoral fellow at M.I.T., USA from 1990–1993. Since 1993, he has been at the University of Massachusetts at Amherst,USA where he is currently the Charles A. Goessmann Professor of Chemistry. His research focuses on the application of synthetic chemistry to polymers, nanotechnology, and biological systems.

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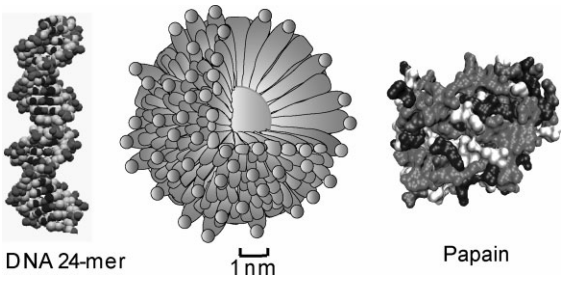

Figure 1. Schematic representation of a 2 nm gold nanoparticle with 11-mercaptoundecanoic acid monolayer and relative sizes of papain and a 24-mer DNA duplex.

potential use of these systems in gene regulation. Intercalation provides another mechanism for DNA binding, as demonstrated by Murray et al.[18] A third approach to DNA conjugation exploits the high affinity and specificity of DNA–DNA interactions (Fig. 2c).[19] This approach is discussed further in the biosensor portion of this Review.

Nanoparticle–protein interactions can regulate multiple biological processes such as protein–protein interactions, protein–nucleic acid interactions, and enzyme activity. As with DNA, electrostatic assembly provides a direct means of conjugation. One system that has been explored is the binding of α-chymotrypsin (ChT), exploiting the ring of cationic residues around active site of ChT (Fig. 3).[20] Time-dependent inhibition of ChT activity was observed upon incubation with negatively charged **NP 2**.[21] A two-step binding process with a fast reversible association followed by a slower irreversible denaturation was established.[22] This interaction could be reversed using cationic surfactants (Fig. 3b), restoring ChT activity.[23] Based on the dynamic light scattering (DLS) data two distinct mechanisms were postulated: alkyl surfactants **3** and **4** form a bilayer structure, whereas cationic thiol **5** and alcohol **6** directly modify the monolayer to liberate the bound proteins.

The use of simple alkyl-based monolayers generally results in protein denaturation; an unfavorable outcome for a number of applications in delivery and biotechnology. Relying on the resistance of OEG to nonspecific interactions with biomolecules,[24] tetra(ethylene glycol) spacers were introduced at the nanoparticle–protein interface.[25] Structural data obtained from fluorescence and circular dichroism (CD) studies revealed that the nanoparticle-bound ChT remained with native structure. Further studies demonstrated that nanoparticle–protein complexation can considerably stabilize the bound proteins against denaturation at the air/water interface.[26]

Specific biomacromolecular interactions such as streptavidin/biotin complementarity ($K_a \sim 10^{14}\ M^{-1}$)[27] have been used to provide specific protein–NP binding. Zheng and Huang introduced biotin and glutathione-functionalized gold nanoparticles functionalized with tri(ethylene glycol)-terminated thiols.[28] These particles bind specifically to streptavidin and glutathione-*S*-transferase, respectively, to give stable complexes with minimal nonspecific binding with other proteins (Fig. 3d). Biotin functionalized quantum dots (QDs) were also used for specific protein binding in a time-resolved fluoroimmunoassay.[29] Another way to specifically bind proteins is through the use of transition metal complexes that can bind with surface-exposed histidines of proteins.[30] Xu et al. fabricated FePt magnetic nanoparticles **NP 7**, with nickel-terminated nitrilotriacetic acid (NTA).[31] These NPs show high affinity and specificity towards histidine-tagged proteins (proteins with six consecutive histidine residues) (Fig. 3e). In comparison to commercial magnetic microbeads, these NPs have a great protein binding capacity owing to their high surface-to-volume ratio. This concept can be employed to manipulate the histidine-tagged recombinant proteins and bind other biological substrates at low concentrations.[32,33]

In analogy to proteins, nanoparticles can be used as a multivalent receptor to enhance low-affinity interactions such as carbohydrate–protein interactions.[34] As an example, Lin et al. prepared mannose-functionalized gold nanoparticles and investigated their interaction with the lectin, concanavalin A (Con A).[35] These nanoparticles showed a high affinity to Con A with a K_a of 10^7–$10^8\ dm^3\ mol^{-1}$; an affinity 10-100-fold higher than that of monovalent mannose ligands, an approach that has been used for the sensing of agglutinin proteins.[36–38]

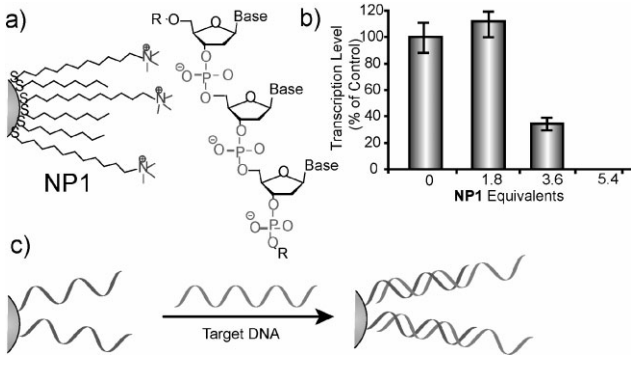

Figure 2. The DNA-nanoparticle interactions. a) Structure of **NP1** scaffold and the DNA backbone. b) Transcription level as a function of DNA–**NP1** stoichiometry. c) Binding of DNA through complementary oligonucleotide hybridization.

3. Nanoparticles in Biosensing

The sensing of biological agents, diseases, and toxic materials is an important goal for biomedical diagnosis, forensic analysis, and environmental monitoring.[39] A sensor generally consists of two components: a recognition element for target binding and a transduction element for signaling the binding event. The unique physicochemical properties of NPs[9] coupled with the inherent increase in signal-to-noise ratio provided by miniaturization[40] makes these systems promising candidates for sensing applications.[41] As an example, gold nanoparticles exhibits unique optical and electronic properties based on size and shape. Gold nanoparticles show an intense absorption peak from 500 to 550 nm[42] arising from surface plasmon resonance (SPR).[43–45] SPR occurs from the collective

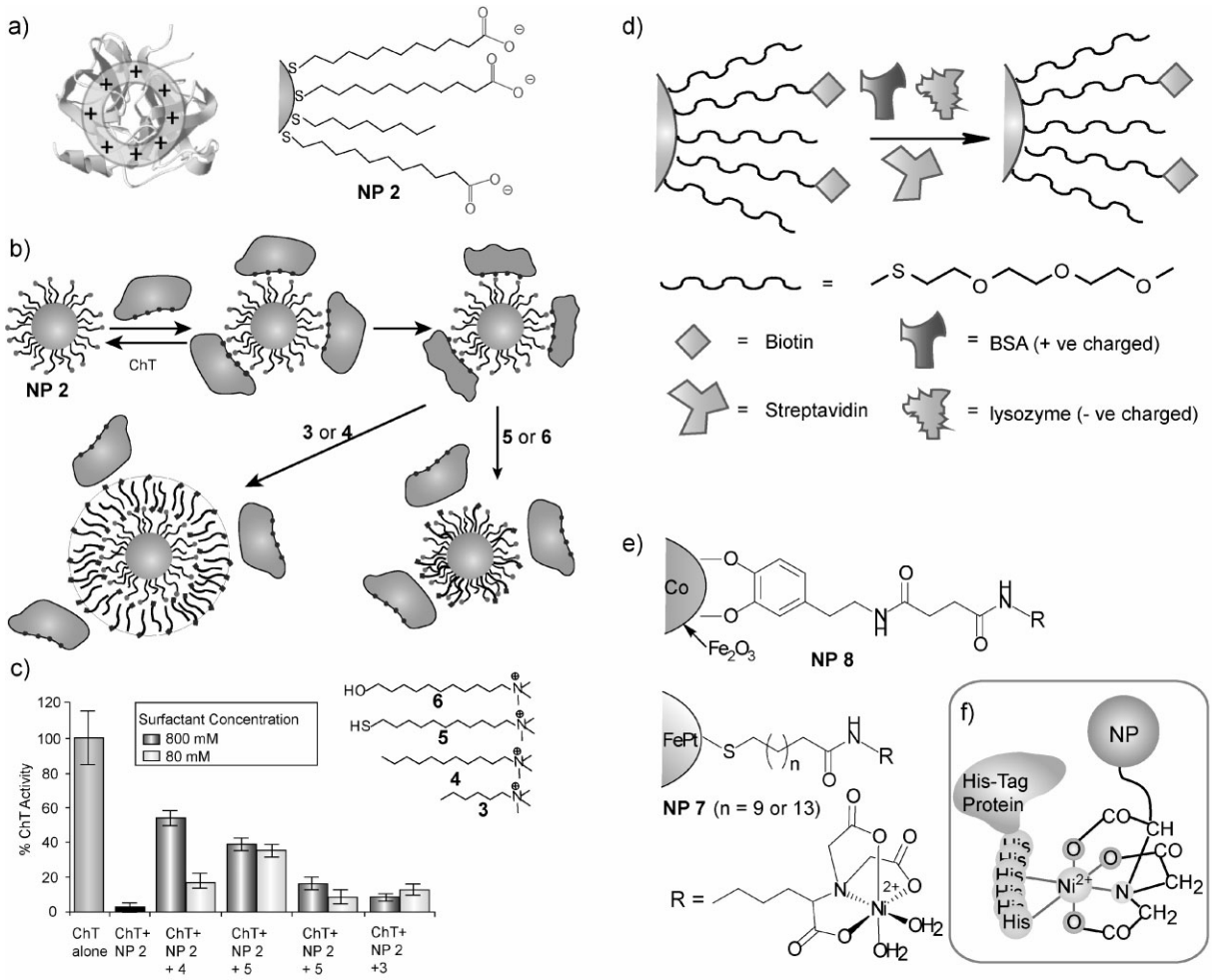

Figure 3. Protein–nanoparticle conjugation and its applications. a) Electrostatic targeting of ChT by anionic **NP 3** b) Complexation of ChT with anionic nanoparticles and its release mechanisms by addition of various surfactants. Addition of cationic alkyl surfactant (**3** and **4**) forms a bilayer structure, whereas addition of cationic thiol and alcohol (**5** and **6**) amends the monolayer. c) Different degree of restoration of enzymatic activity of nanoparticle-bound ChT by addition of various positively charged surfactants. d) Specific interaction of biotin-functionalized nanoparticles with streptavidin. e) Structure of NTA-modified magnetic nanoparticles. f) The NTA–Ni^{2+} functionalized magnetic nanoparticles selectively bind to histidine-tagged proteins.

oscillation of the conductive electrons owing to the resonant excitation by the incident photons, although the fundamental physical principles of SPR are very complex. The SPR band is sensitive to the surrounding environment, signaling changes in solvent and binding. A particularly useful output is the red-shift (to ca. 650 nm) and broadening of the plasmon band due to the interparticle plasmon coupling.[46] This phenomenon leads to the popular and widely applicable colorimetric sensing. Metallic nanoparticles also possess superb quenching ability[47,48] and photoluminescence[49–51] under certain conditions.

3.1. Colorimetric Sensing

The oligonucleotide-mediated nanoparticle aggregation process has been extensively used for the development of simple and highly sensitive colorimetric biosensors for oligonucleotides by Mirkin[52,53] and others.[54–56] The detection of specific oligonucleotide sequences is now very important in diagnosis of genetic and pathogenic diseases and quantifying the amount of product generated by polymerase chain reaction (PCR). The general procedure for detection of oligonucleotides is through the fabrication of nanoparticles, functionalized with single-stranded DNA. Upon addition of the target sequence the particles aggregate, changing the color of the solution (Fig. 4a). Using this method oligonuceotides were detected at sub-picomolar level without the assistance of PCR.[53] This methodology was also applied for the colorimetric screening of DNA binders[57] and triplex DNA binders (Fig. 4b).[58]

Based on the similar approach a highly selective and sensitive lead (Pb^{2+}) biosensor was reported by Lu et al.[59,60] In their sensor design, they used a Pb^{2+}-specific ''DNAzyme''

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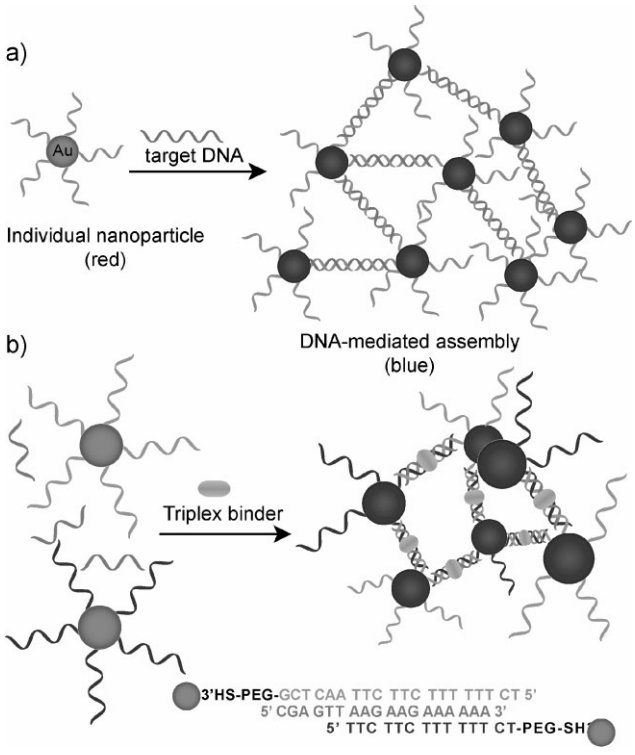

Figure 4. Schematic illustration of a) DNA-induced nanoparticle aggregation, and b) sensing of DNA triplex binder using DNA-directed AuNP assembly.

composed of a catalytic and a substrate strand. In the presence of Pb^{2+}, the substrate strand cleaves into two pieces (Fig. 5a), resulting in head-to-tail (Fig. 5b) or tail-to-tail (Fig. 5c) aggregation with a concomitant red to blue color shift with a sensing limit of 100 nM, which is unaffected by other divalent metal ions.

Another attractive sensor approach uses aptamers, single-stranded oligonucleic acid-based binding molecules that can bind a wide range of targets with high affinity and specificity.[61] An example is cocaine sensing, using a cocaine-specific aptamer (Fig. 6).[62] The sensor features a particle functionalized with two different sequences of single-stranded DNA, one for conjugation and another with the cocaine aptamer. In the presence of cocaine the nanoparticles are deaggregated, with a concomitant blue-to-red color change. This method has been extended to mixed-aptamer systems that respond to combinations of analytes,[63,64] as well as the detection of other biomolecular systems such as platelet-derived growth factors (PDGFs)[65] and thrombin.[66]

Nanoparticles featuring ligands targeted at specific biomolecules provide another avenue for the colorimetric detection of proteins. The bivalent lectin agglutinin specifically recognizes β-D-galactose, inducing the aggregation of galactose-functionalized nanoparticles at 1 ppm.[67] Other glyconanoparticles have been used for sensing various proteins such as Concanavalin A and cholera toxin.[68,69]

Dithiols such as C- and N-terminal cysteinyl peptides can serve as bridging agents to assemble nanoparticles, and have been used for the colorimetric detection of proteases. In a representative study, Stevens et al. reported a two-stage approach by using Fmoc-protected peptides (substrate of thermolysin) with a cysteine amino acid attached to gold nanoparticles.[70] In presence of thermolysin the peptides are fragmented and the assembly changes color from blue to red with a sensitivity of 90 zg mL^{-1} (i.e., less than 380 molecules of protease).[71] This approach has been extended to kinases[72] and phosphatases[73,74] at low concentration.

3.2. Fluorescence Sensing

The exceptional quenching ability of metallic nanoparticles makes them excellent materials for Förster resonance energy transfer (FRET)-based biosensors,[47] for example, for the fabrication of molecular beacons for sensing DNA.[75] In this approach, the dye molecule is close to the nanoparticle surface in the absence of the target DNA strand due to hairpin structure of the attached DNA, resulting in fluorescence quenching (Fig. 7a). Hybridization of the target DNA opens up the hairpin structure, resulting in a significant increase in

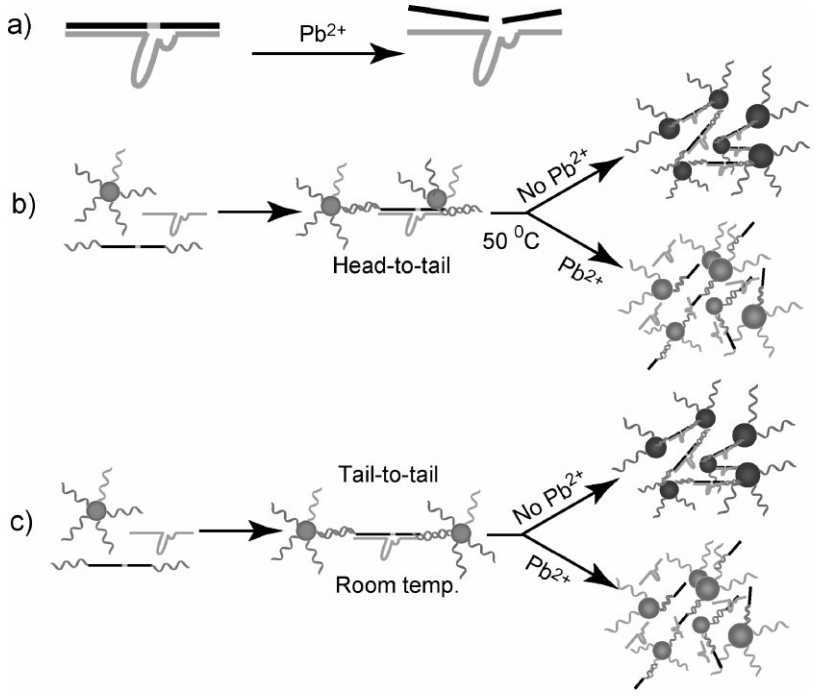

Figure 5. a) Cleavage of the substrate strand of DNAzyme in the presence of Pb^{2+}. DNAzyme mediated assembly of gold nanoparticles in b) a head-to-tail or c) a tail-to-tail manner.

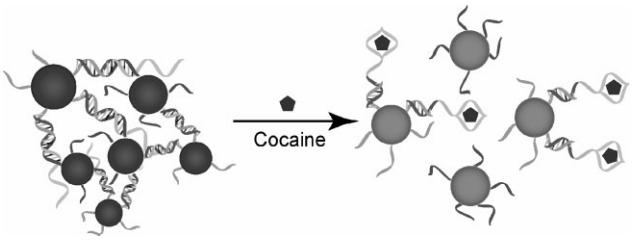

Figure 6. Schematic depiction of the aptamer-based colorimetric detection of cocaine by disassembling the nanoparticles.

fluorescence. A range of single-strand DNA and DNA cleavage processes have been monitored using this molecular beacon approach.[76,77]

Semiconductor QDs have also been used for the sensing of DNA and proteins. Melvin et al. have developed a fluorescence competition assay for DNA detection using QDs and gold nanoparticles as a FRET donor–acceptor couple.[78] In presence of complementary oligonucleotides, the gold particle is released from the QD, regenerating QD fluorescence

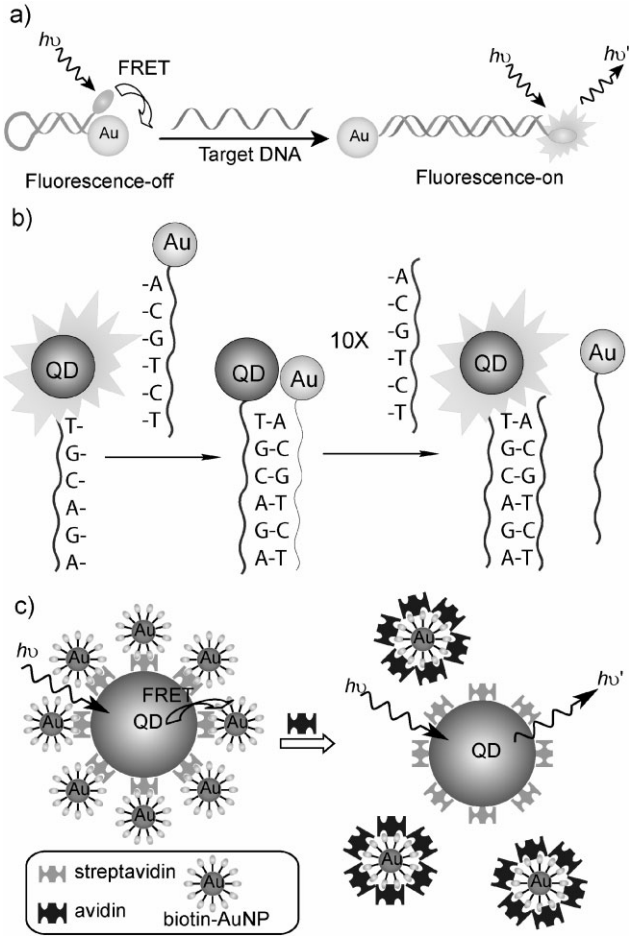

Figure 7. Schematic representation of a) molecular beacon for the detection of target DNA, b) quenching and reestablishment of emission from QDs by using gold nanoparticle and unlabelled complementary oligonucleotides and c) competitive inhibition assay for the detection of avidin by using QD-gold nanoparticle couple.

(Fig. 7b). Kim and co-workers have used the similar method for sensing the avidin (Fig. 7c),[79] and glycoproteins.[80]

Another paradigm for sensing relies on array-based sensing using selective recognition elements, that is, the ''chemical nose approach''. Rotello and collaborators have fabricated a sensor array by using cationic nanoparticles with various head groups and anionic poly(*p*-phenyleneethynylene) (PPE) fluorescent polymer.[81,82] In this sensor design, the cationic nanoparticles quench the fluorescence of the PPE polymer. Competitive binding of analyte proteins then release the PPE polymer, resulting in fluorescence restoration (Fig. 8a). Depending on the protein-nanoparticle interactions, different fluorescence response patterns were generated for individual proteins (Fig. 8b). Linear discrimination analysis (LDA) provided identification of unknown proteins which are identified with 94.2% accuracy on the basis of 52 samples. The application of the AuNP-conjugated polymer systems have also been expanded to the detection of bacteria.[83] Similar to the protein detection, in presence of various bacteria, the initially quenched PPE polymers regain their fluorescence depending on the relative affinity between the bacteria and nanoparticles. The sensor array successfully identifies 12 micro-organisms with more than 95% detection accuracy.

3.3. Electrochemical Sensing

The conductivity and catalytic properties of metallic and semiconductor nanoparticles have been applied to electroanalytical sensing.[84] The attachment of nanoparticles onto electrodes drastically enhances the conductivity and electron transfer from the redox analytes.[85] Based on this concept, Willner et al. reported several systems using nanoparticle–enzyme hybrids as electrochemical sensors. In one example, a bioelectrocatalytic system was constructed by connecting the redox enzyme glucose oxidase (apo-GOx) onto a gold nanoparticle that was functionalized with N^6-(2-aminoethyl) flavin adenine (FAD) (Fig. 9a).[86] This enzyme–nanoparticle hybrid system was linked to the electrode through dithiols, or alternatively the FAD-functionalized nanoparticle was assembled onto the electrode followed by the addition of apo-GOx. This system exhibited a highly efficient electrical communication with the enhanced turnover rates as compared to native Gox, and provided an effective sensor for glucose in the physiological concentration regime. An analogous electron transfer from protein to nanoparticles was used for monitoring hydrogen evolution from zinc-substituted cytochrome *c* immobilized TiO_2 nanoparticles, as reported by Yeni Astuti et al.[87]

Mirkin et al. have developed a method for the detection of DNA by selective deposition of oligonucleotide-functionalized nanoparticles between two electrodes.[88] In this approach short-chain oligonucleotides were deposited onto a SiO_2 surface between two electrodes. In the presence of target DNA, oligonucleotide-functionalized gold nanoparticles hybridize on the the surface. Deposition of silver by using

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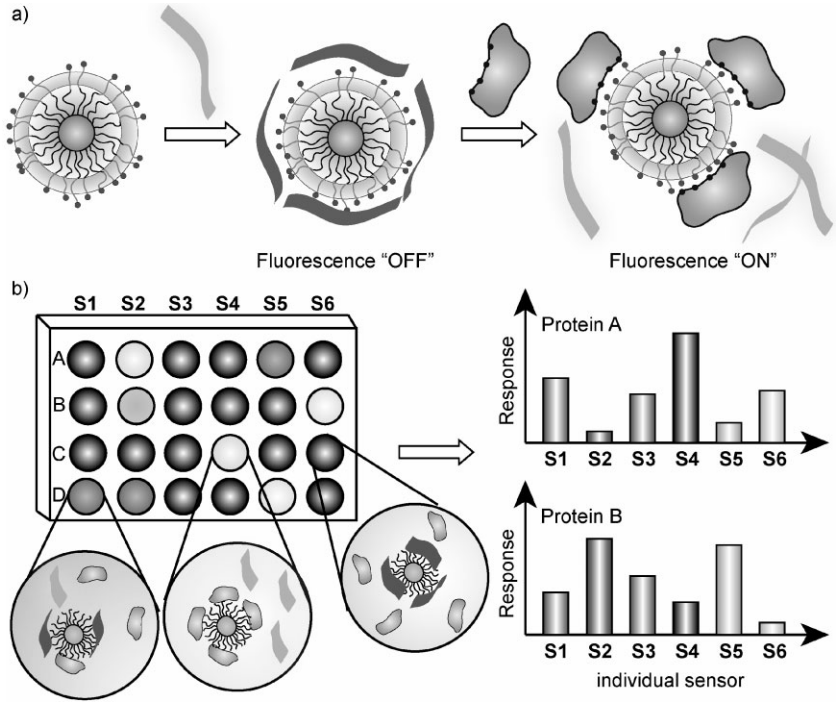

Figure 8. Schematic drawing of a ''chemical nose'' sensor array based on nanoparticle and fluorescence assay. a) The competitive binding between protein and quenched polymer leads to the fluorescence light-up. b) The combination of an array of sensors generates fingerprint response patterns for individual proteins.

Ag^+ salt and hydroquinone on the gold nanoparticle enhances the conductivity providing sensitivity down to 500 fM with a point mutation selectivity factor of about 100000:1 (Fig. 9c).[88] A similar antigen–antibody-based protein sensor was designed by Velev and Kaler, detecting human IgG at 0.2 pM.[89]

3.4. Other Sensing Methods

Surface enhanced Raman scattering (SERS) has been successfully exploited in biological sensing using nanoparticles.[90,91] Mirkin et al. used gold nanoparticles labeled with oligonucleotides and Raman-active dyes to achieve multiplexed detection of different DNA targets.[92] The SERS method was also employed for the detection of the protein–small molecule and protein–protein interactions by fabricating the nanoparticles with proteins and Raman dyes.[93]

Another ultrasensitive ''bio-barcode'' biosensor for proteins and nucleic acids has been developed by Mirkin's group, where the oligonucleotide is amplified and then detected either by surface hybridization or by PCR amplification, providing a means for

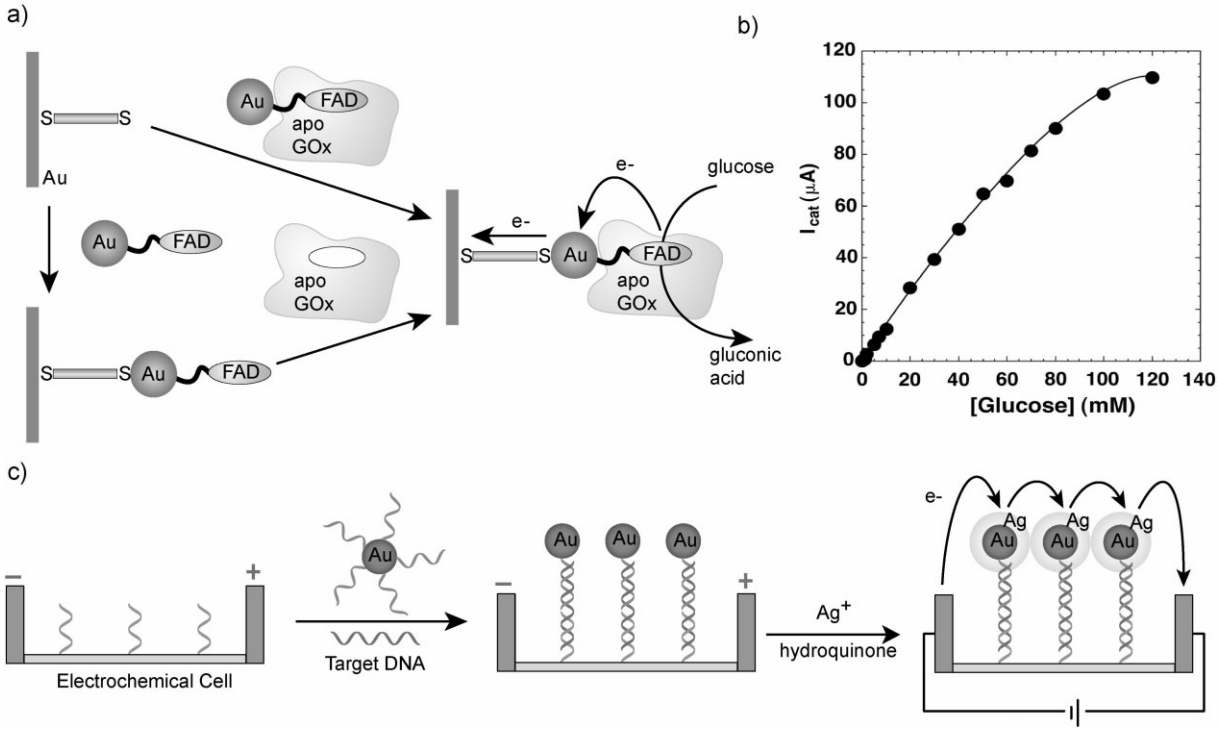

Figure 9. a) Fabrication of a GOx electrode by the reconstitution of apo-enzyme on a FAD-functionalized gold nanoparticle. b) Plot of the current developed by the reconstituted GOx electrode in the presence of different concentrations of glucose. c) Electrical detection of DNA based on the ''sandwich'' hybridization with DNA-functionalized AuNPs followed by silver deposition.

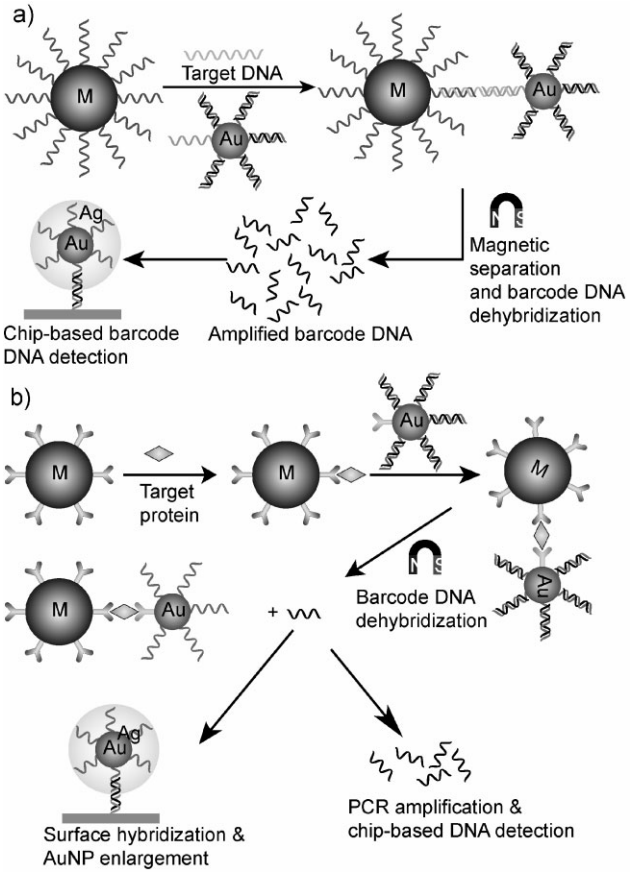

Figure 10. Schematic illustration of ''bio-barcode'' assays for a) DNA and b) proteins.

detection of both proteins and DNA (Fig. 10). In case of DNA detection, a magnetic microparticle carrying partially complementary DNA to target DNA was hybridized with bio-barcoded gold nanoparticles in presence of target DNA. After the magnetic separation of the sandwich assemblies and thermal dehybridization, the barcode was released for analysis. The detection limit of this method was found at 500 zM, comparable to many PCR-based approaches.[94] For protein detection the magnetic microparticles carry antibodies that specifically bind the target protein. The magnetic microparticle-bound protein subsequently interacts with gold nanoparticles by antigen–antibody interaction. The detection limit for the chip-based sandwich hybridization process was observed at 30 aM, which was reduced using PCR amplification to 3 aM.[95] This approach is also used for multiplexed detection of protein and DNAs by using mixture of different bio-barcoded gold nanoparticle probes.[96]

4. Nanoparticles as Drug Delivery Systems

Drug delivery systems (DDSs) can improve several crucial properties of ''free'' drugs, such as solubility, in vivo stability, pharmacokinetics, and biodistribution, enhancing their efficacy.[97] In this aspect nanoparticles can be used as a potential DDS owing to their advantageous characteristics, as mentioned previously. As an example of cellular delivery, mixed monolayer protected gold clusters were exploited for in vitro delivery of a hydrophobic fluorophore (BODIPY); an analog of hydrophobic drugs.[98] The cationic surface of the nanoparticles facilitated the penetration through cell membrane, and the payload release was triggered by intracellular glutathione (GSH), relying on the ca. 1000-fold higher intracellular concentration of GSH relative to the extracellular environment (Fig. 11a). Release of the dye was established by fluorogenesis upon release of the dye from the quenching nanoparticle (Fig. 11b and c). The controlled release of the fluorophore was observed in mouse embryonic fibroblast (MEF) cells; containing ca. 50% lower GSH levels than Hep G2, through incubation with glutathione monoethyl ester (GSH-OEt) which is processed to GSH by esterases transiently increasing intracellular GSH concentrations (Fig. 11d).

Lin et al. have demonstrated that thiols, such as dihydrolipoic acid (DHLA) and dithiothreitol (DTT), can likewise act as stimuli to remove caps of the pores in mesoporous silica nanoparticles and hence release of trapped molecules inside the pores.[99,100] The pores were capped with removable CdS or Fe_3O_4 nanoparticles through disulphide linkers that cleave in a reducing environment. Release of encapsulated fluorescein isothiocyanate (FITC) from magnetic nanoparticle-capped MCM-41 was observed in cancer cells owing to the presence of significant amounts of intracellular DHLA.

pH-responsive nanomateials provide an alternate mechanism for release, relying on the acidic condition inside tumor and inflamed tissues (pH ~ 6.8) and cellular compartments including endosomes (pH ~ 5.5–6) and lysosomes (pH ~ 4.5–5.0).[101] Toward this end, magnetic nanoparticles (Fe_3O_4) were covalently functionalized with doxorubicin (DOX), an anticancer drug, through an acid-labile hydrazone linker.[102] The carrier was then encapsulated with thermosensitive polymer for temperature-controlled release of the drug. The hybrid system released DOX efficiently in mild acidic buffer solution of pH 5.3. Schoenfisch et al. have likewise shown that nitric oxide (NO) can be efficiently released at acidic pH from gold nanoparticles.[103]

Besides the surface chemistry of nanoparticles, the unique physical properties of nanoparticles have been utilized in the design of DDSs. Ford et al. have designed a water-soluble nanocontainer for NO storage based on electrostatic assembly of DHLA-coated quantum dots and cationic dinitro complexes that uses energy transfer from the core to release NO.[104] In another approach, doping of Ag/Au nanoparticles serves as an antenna to absorb the energy from a laser beam of ''biologically friendly'' near-infrared (NIR) region, causing local heating and disruption of microcapsules. More recently, Bhatia et al. designed multifunctional superparamagnetic nanoparticles for remote release of bound drugs (Fig. 12).[105] The particles transduce external electromagnetic force (EMF) at 350–400 kHz to local heating for breaking hydrogen bonds between DNA chains.

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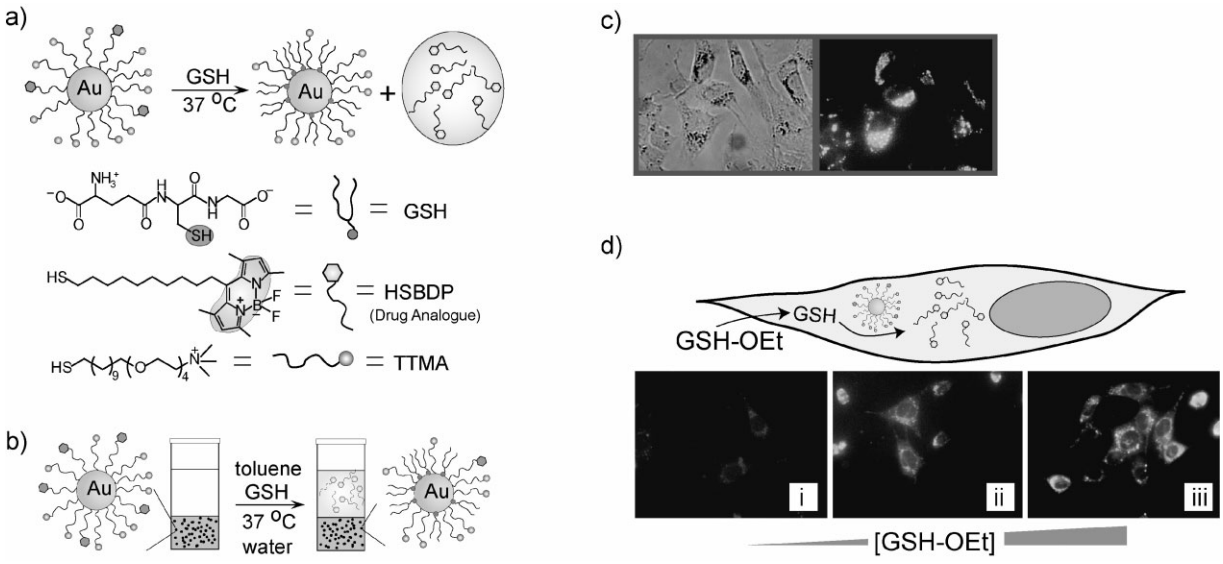

Figure 11. a) Schematic illustration of GSH-mediated surface monolayer exchange reaction/payload release. b) In-cuvette release of BODIPY ligand mediated by GSH. c) Bright field and fluorescence images of Human Hep G2 cells incubated with AuNP for 96 h. d) Fluorescence images of MEF cells displaying GSH-controlled release of the fluorophore after incubation with of 0, 5, and 20 mM GSH-OEt.

4.1. Nanoparticles as Delivery Vehicles for Biomolecules

Nanoparticles can provide effective carriers for biomolecules such as DNA, RNA, or proteins, protecting these materials from degradation and transporting them across the cell-membrane barrier. ''Safe'' delivery of these biomolecules provides access to gene therapy as well as protein-based therapeutic approaches. For successful delivery, carriers must: (i) form condensed complexes with biomolecules, (ii) facilitate penetration of the cell membrane after complexation, and (iii) unload their payloads inside of cells. An example of gene delivery was provided by Rotello et al. using a series of cationic nanoparticles with different positive charge coverage and hydrophobicity.[106] In their experiment NPs were incubated with DNA plasmid encoding for β-galactosidase and then used to transfect embryonic kidney cells. Maximizing hydrophobicity without precipitation was found to play a key role in enhancing efficiency (Fig. 13a–c). Building upon these studies, Klibanov et al. covalently attached 2 kDa polyethylenimine (PEI2) onto the surface of gold nanoparticle **NP 12** (Fig.13d).[107] The transfection efficiency of these systems varied with the PEI to gold molar ratio in the conjugates, with the best conjugate being 12 times more potent than the unmodified polycation. The efficiency of the delivery could be doubled by the addition of the *N*-dodecyl-PEI2 to the conjugate during complex formation (Fig. 13e), a further demonstration of the role of hydrophobicity

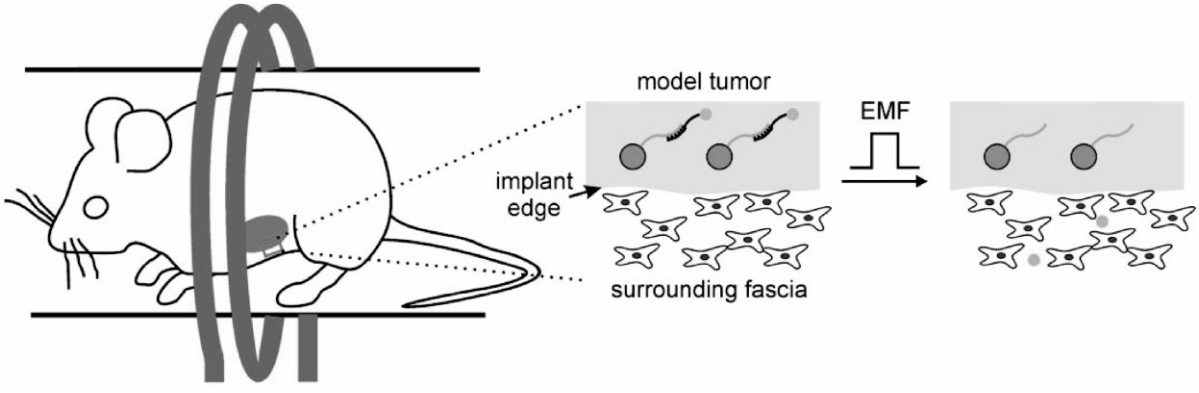

Figure 12. Controlled release of payloads using oligonucleotide-modified iron oxide nanoparticles for drug delivery at a remote location. Adapted with permission from [105].

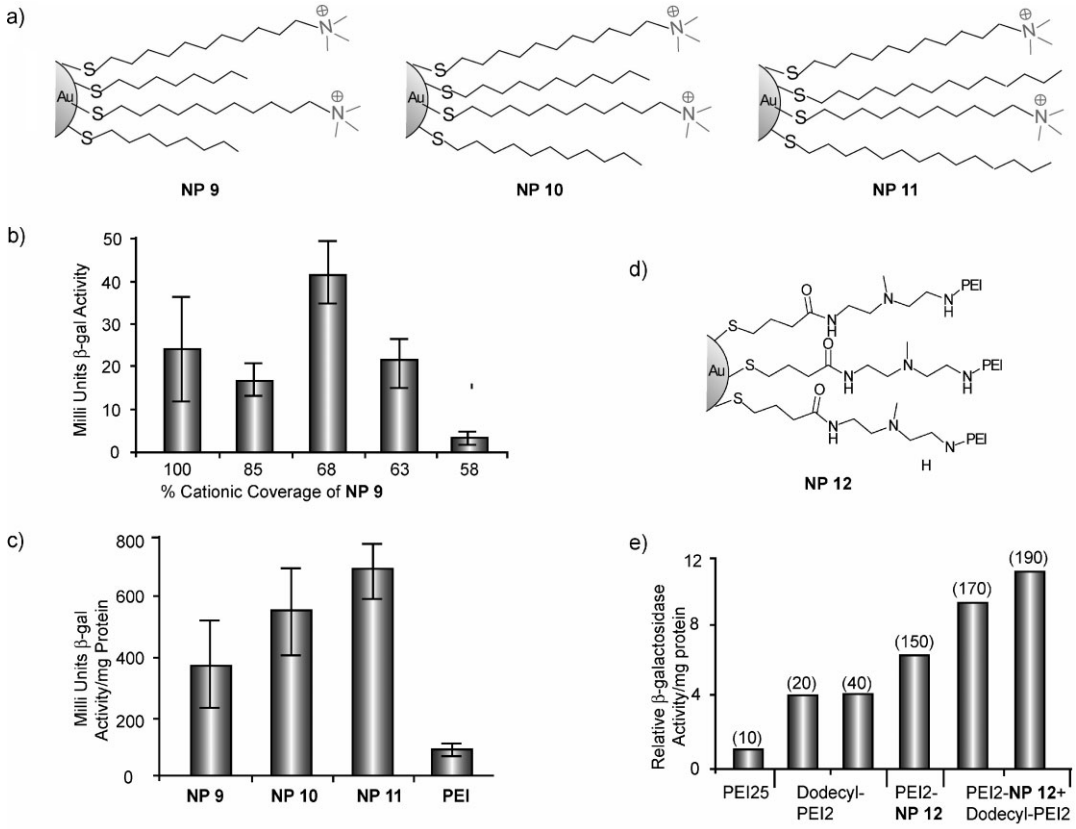

Figure 13. a) Structures of nanoparticles used for transfection by Rotello. b) β-Galactosidase transfection using various nanoparticle-DNA complexes at 2200:1. c) Transfection efficiency of nanoparticles **9**, **10**, **11** (2200:1 nanoparticle/DNA ratio) and PEI (60 kDa). All transfections were performed in the presence of 100 μM chloroquine and 10% serum. d) **NP 12** fabricated by Klibanov with branched 2 kDa polyethylenimine (PEI2) conjugated to a gold core. e) Incorporation of PEI2 onto nanoparticles increases the transfection efficiency. Further addition of dodecyl-PEI2 to **NP 12** increases the transfection efficiency. The numbers in the parentheses indicate the ratio of PEI nitrogen to DNA phosphate.

in facilitating transfection. Lin et al. have recently reported mesoporus silica nanoparticles (MSNs)-mediated delivery of DNA into plants.[108]

In contrast to polycation-mediated transfection, cellular internalization of oligonucleotide-functionalized gold nanoparticles carrying high negative surface potential has emerged as a new approach to gene therapy.[109] Antisense nanoparticles (ASNP) were effective in low concentration to suppress EGFP signal in C166 cells. Investigation on mechanism of cellular uptake of ASNP revealed that the endocytosis is initiated by adsorption of a large number of serum proteins onto the particle surface.[110]

Rotello et al. have functionalized the monolayer of gold nanoparticle with a photo-cleavable *o*-nitrobenzyl ester moiety that dissociates upon light irradiation to alter the surface potential from positive to negative, thereby releasing adsorbed DNA (Fig. 14a),[111] as established in-cuvette by a T7 RNA polymerase assay (Fig. 14b). A FITC-labeled DNA was successfully delivered into mammalian cells with nuclear localization of the released DNA observed (Fig. 14c and d), an important requirement for genetic therapy.

RNA technology has emerged as a potential tool for curing disease at an early stage. A small interfering RNA (siRNA), generally consisting of 19–21 base pairs, can efficiently slice the gene of interest. For in vitro delivery, siRNA has been conjugated by a thiol linker with variety of nanoparticles, such as gold,[112] quantum dots,[113] or iron oxide.[114] Moore et al. have designed a multifunctional superparamagnetic nanoparticle that can: (i) carry the siRNA, (ii) deliver it in a site-specific manner, and (iii) probe the delivery by magnetic resonance imaging as well as optical imaging.[114] The multifunctional nanoparticles were effective for in vitro and in vivo gene silencing via a specific pathway.

Protein delivery is complementary to nucleic acid therapies in the field of biomedicine. Nanoparticles can efficiently bind protein, and hence be used as protein delivery systems. Lin et al. have fabricated MCM-41 type mesoporous silica nanoparticles (MSNs) as protein carriers (Fig. 15).[115] These MSNs can incorporate cytochrome *c*, a membrane-impermeable protein, into their large pores (diameter = 5.4 nm), and slowly release the proteins in active form under physiological conditions.

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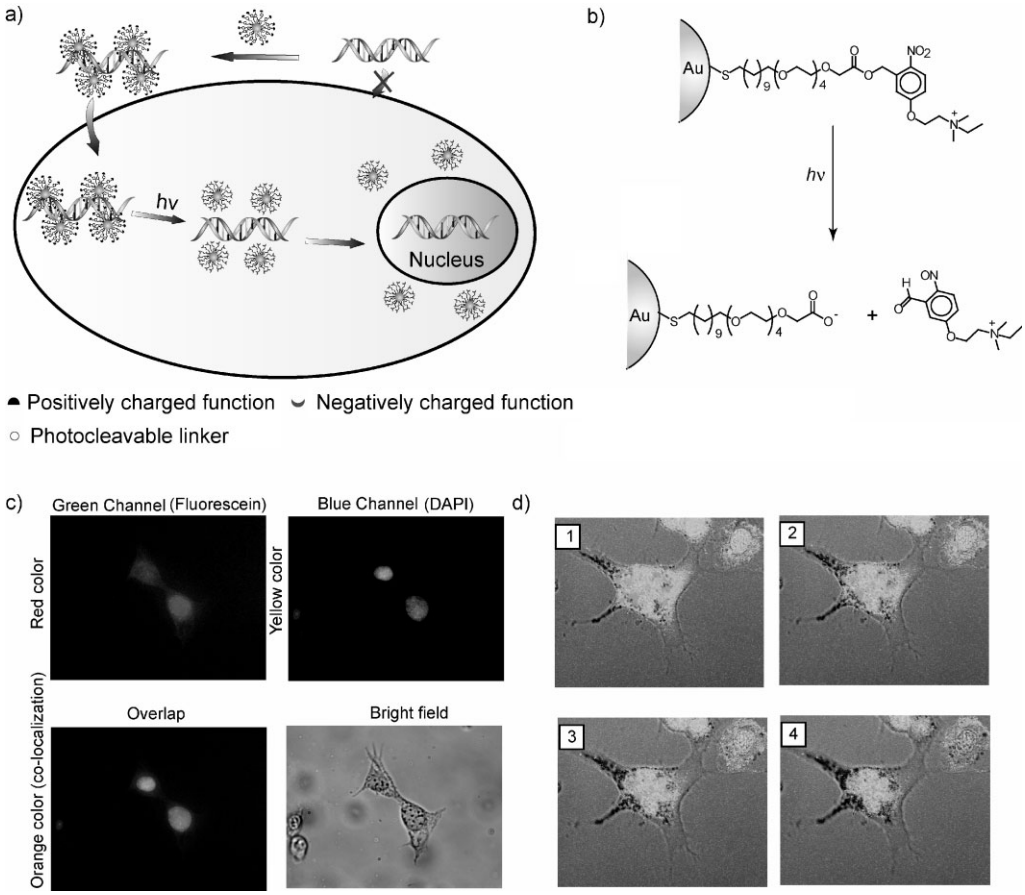

Figure 14. a) Depiction of photo-triggered release of DNA inside living cells. b) Illustration of light-induced surface transformation of nanoparticles. c) Fluorescence and bright field micrographs of cells after photo irradiation. To clarify the overlap of F-DNA and nuclei stain DAPI; green (Fluorescein) and blue (DAPI) channel are depicted with red and yellow color, respectively. d) Confocal microscopy images illustrating that the photo-released DNA accumulates inside the nucleus. Panels 1, 2, 3, and 4 show four consecutive slices of middle sections of z-series confocal images (interval = 1.0 μm).

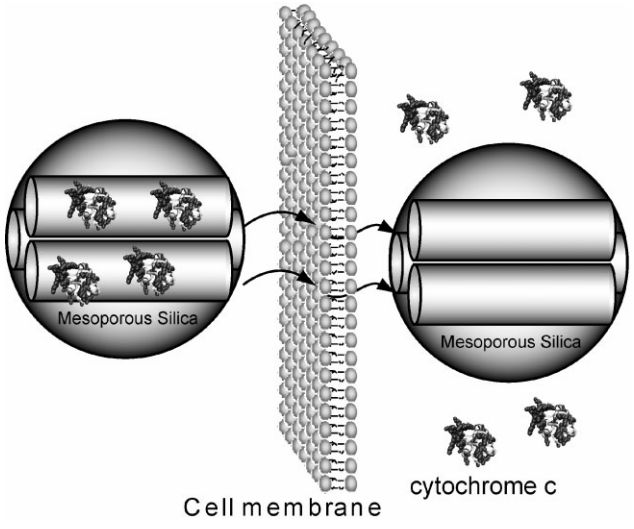

Figure 15. Schematic illustration of cellular delivery of cytochrome *c* using mesoporous silica nanoparticles.

4.2. In vivo Targeting of Nanoparticles

A key goal of delivery systems is to discharge their payloads specifically at the diseased tissue. Two approaches to serve this purpose are "passive" and "active" targeting.[116] Passive targeting relies on the homing of the carriers to infected tissues. In tumor tissues, the blood vessels are frequently leaky, facilitating accumulation of nanosized carriers. On the other hand, active targeting relies on specific recognition of the ligands that are displayed on delivery vehicles by cell surface receptors.

The ligand used for active targeting can be a small molecule, or a peptide or protein. Weissleder et al. have reported cell-specific targeting of fluorescent magnetic nanoparticles through multivalent attachment of small molecules.[117] For cancer therapy, folic acid (FA) or methotrexate (MTX) can be anchored onto nanoparticles for recognition by folate receptors, which are overexpressed on surfaces of many cancer cells. FA-grafted gold nanoparticles[118] or iron oxide[119] nanoparticles are specifically uptake by folate receptor-positive KB cells. Methotrexate provides an additional advantage as it can serve as a targeting ligand as well as a chemotherapeutic drug.

Zhang et al. have shown that cells expressing human folate receptor (MCF-7 and HeLa) internalize MTX-conjugated superparamagnetic nanoparticles, killing the cells.[120]

Many cancer cells overexpress receptors for the protein transferrin on their surface. He et al. have reported transferrin-mediated uptake of gold nanoparticles by tumor cells.[121] Nanoparticles have also been tagged with peptides or antibodies for targeting tumors in vivo.[122] Ruoslahti et al. have fabricated quantum dots coupled to different peptides, targeting nanocrystals to specific organs.[123] Paciotti et al. created a potential for cancer therapy based on colloidal gold (cAu-PEG-TNF) was decorated with PEG and protein tumor necrosis factor (TNFα).[124] Following intravenous injection into mice, cAu-PEG-TNF accumulated preferentially in MC-38 colon carcinoma tumors compared to other healthy organs, diminishing tumor mass more effectively than free TNF. The efficacy of this approach was further improved by grafting an anticancer drug, paclitaxel, onto colloidal gold.[125]

5. Nanoparticles for Bioimaging

A number of molecular imaging techniques, such as optical imaging (OI), magnetic resonance imaging (MRI), ultrasound imaging (USI), positron emission tomography (PET), and others have been reported for imaging of in vitro and in vivo biological specimens.[126,127] The current development of luminescent and magnetic nanoparticles advances bioimaging technologies.[128,129] Two different type of nanoparticles have been widely used for imaging: luminescent nanoprobes for OI and magnetic nanoparticles for MRI. There are also dual-mode nanoparticles for simultaneous imaging by OI and MRI.[130,131]

5.1. Optical Imaging

Most nanoparticle-based optical imaging agents can be subdivided into two categories: quantum dots (QDs) [132] and dye-doped nanoparticle QDs. The use of QDs for cell imaging was first reported by Nie's[133] (Fig. 16c) and Alivisatos' groups in 1998.[134] Compared to conventional fluorophores, QDs are photochemically stable,[135] brighter, have a narrow, tunable[136] and symmetric emission spectrum (Fig. 16a and b), and are metabolically stable.[137,138] There are, however, issues of toxicity, photo-oxidation, and water solubility associated with these materials.[139,140] The problem of acute toxicity and photo-oxidation can be overcome by capping with a protective shell of insulating material or semiconductor, for example, ZnS-coated CdSe core/shell QDs.[141] As water solubility is key to their applications in imaging, there are a range of methods reported to make the QDs water soluble and biocompatible for biological imaging, such as fabricating the surface with suitable thiolated ligand,[142] over-coating with silica,[143,144] and encapsulating with amine-modified polymer.[145] Likewise, there are a number of strategies for their functionalization.[146,147]

Taking the advantage of the size dependent emission of QDs Choi et al. determined the size- and charge-dependent renal clearance of QDs, a very important issue for the design of biologically targeted nanoparticles for medical applications.[148] Their study revealed that zwitterionic or neutral QDs with hydrodynamic diameter >15 nm prevent renal extraction. whereas rapid and efficient elimination was observed for QDs <5.5 nm in diameter.

The requirement of external excitation for QDs sometimes limits their in vivo applications due to tissue opacity. An attractive example of a self-illuminating QD conjugate was reported by So et al. for in vivo imaging.[149] They overcome

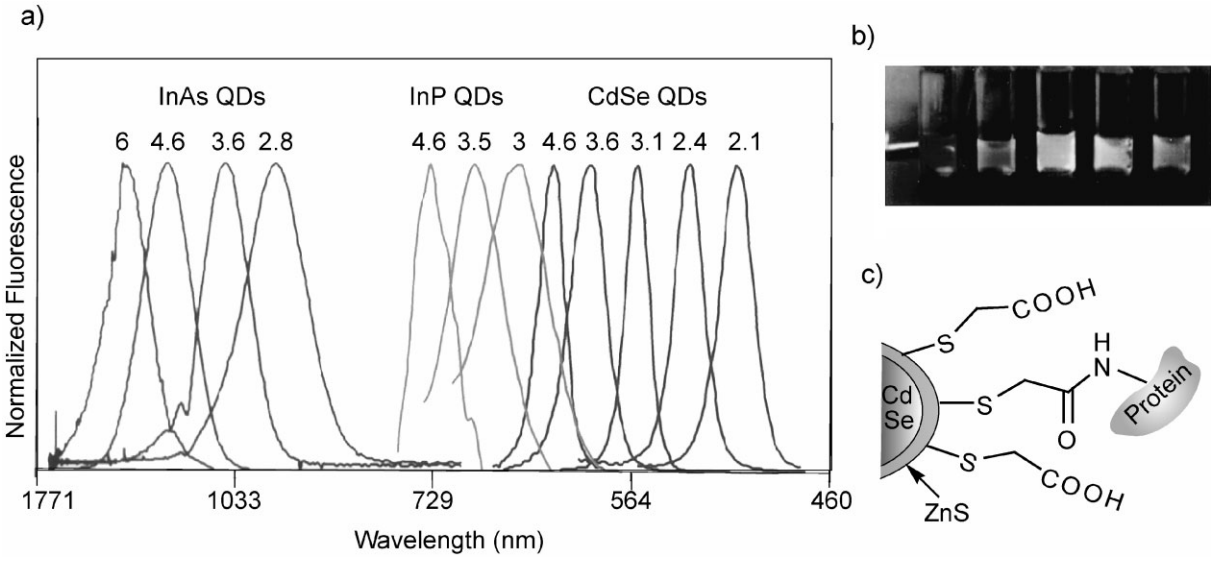

Figure 16. a) Size- and material-dependent emission spectra of several surfactant-coated QDs. b) A true-color image of a series of silica-coated core/shell CdSe/ZnS or CdS QDs. Adapted with permission from [134]. Copyright 1998 American Association for the Advancement of Science. c) Schematic of a ZnS-capped CdSe QD that is covalently coupled to a protein by mercaptoacetic acid.

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the issue of opacity in terms of excitation by designing a self-illuminating QD that luminesces without external excitation by coupling QDs to a modified luciferase. The energy released by luciferase catalysis was transferred to QDs via resonance energy transfer resulting in emission from QDs (Fig. 17).

QD–peptide conjugates have been applied to bioimaging systems.[150] A QD-peptide luminescent probe with inherent signal amplification upon interaction with a proteolytic enzyme was reported by Chang et al.[151] In this system, QDs are bound to gold nanoparticles via a proteolytically degradable peptide sequence. Upon proteolysis of the peptide linker, the QD becomes highly luminescent owing to the separation from the quenching gold NP (Fig. 18). Other examples of bioimaging using QDs have been summarized in many recent reviews,[152,153] demonstrating their utility in imaging lymph nodes and blood vessels.

Rapid photo-bleaching of organic dyes is one of the biggest problems in their use as imaging agents. The optical transparent property of silica nanoparticles[154] was used to solve this problem through encapsulation, greatly stabilizing the dyes. Silica nanoparticles can be synthesized either by a sol–gel process[155] (Stober's method) or by a microemulsion technique.[156] These dye-doped silica particles provide biocompatibility,[157] signal amplification, and low toxicity[158] along with facile synthesis, encapsulation procedures, and easy surface modification for attaching biomolecules such as proteins and peptides.[158,159] Tan's group reported several procedures of covalent conjugation of biomolecules with dye-doped silica nanoparticles.[160,161] In one example, they have functionalized

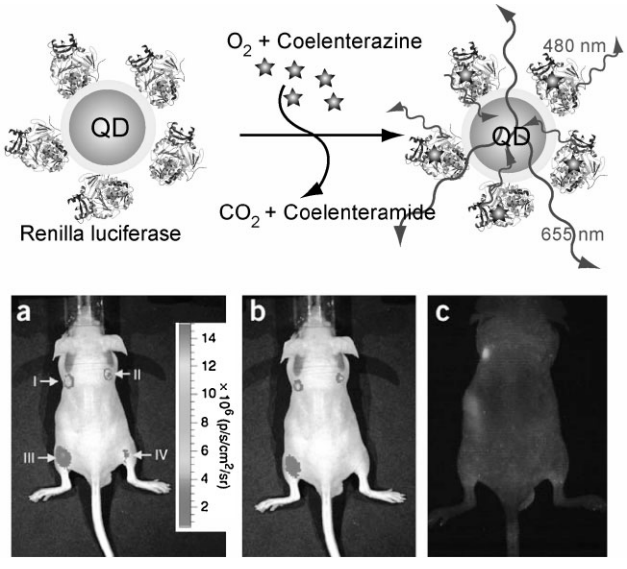

Figure 17. Conjugation of QDs with luciferase proteins. The luminescence released from QD during the luciferase catalyzed oxidation of coelenterazine is tranfered to the QD. Bioluminescence and fluorescence imaging of QD and luciferase protein injected at indicated sites (I and III QD, II and IV luciferase protein). (a) Without filters. (b) With 575- to 650-nm filter. (c) Fluorescence imaging with filter, 503–555 nm. Adapted with permission from [149]. Copyright 2006 Nature Publishing Group.

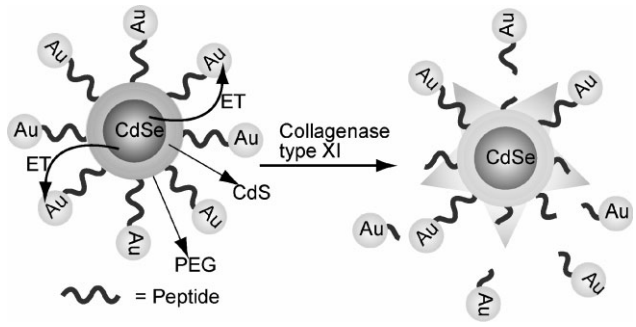

Figure 18. Illustration of suppression and activation of QD photoluminescence by protease cleavage of the peptide linker.

nanoparticles' surfaces with the enzymes glutamate dehydrogenase and lactate dehydrogenase, and used them for cell membrane staining. In another example they used aptamer-conjugated magnetic and dye-doped silica nanoparticles for selective collection and detection of cancer cells.[162]

5.2. Magnetic Resonance Imaging

MRI is another important non-invasive imaging technique. The MRI technique is based on the nuclear magnetic resonance of the various interacting nuclei, with most imaging applications focusing on proton resonance. The factors influencing MRI signal strengths are T_1 (spin-lattice/longitudinal relaxation time), T_2 (transverse relaxation time), and ρ (spin energy). Exogenous contrast agents are generally introduced to enhance the tissue contrast, including complexes of Gd^{III} and magnetic nanoparticles. Complexes of Gd^{III} in liposomes or micelles are widely used as a MRI contrast agents;[163] however, these sytems suffer from drawbacks such as Gd^{III} ion exchange with endogenous metals (e.g., Zn, Cu), and uptake of complexes in extravascular space. The monodisperse, cross-linked iron oxide (CLIO) nanoparticles reported by Weissleder's group provide non-toxic MRI contrast agents.[164,165] The CLIO nanoparticles are highly stable, and convenient ''clickable'' nanoparticles have been used for targeted imaging with high cellular uptake.[166–168]

In a recent study, antibody-conjugated magnetic Poly-(D,L-lactide-*co*-glycolide) (PLGA) nanoparticles with doxorubicin (DOX) were synthesized for the simultaneous targeted detection and treatment of breast cancer.[169] DOX and magnetic nanoparticles were incorporated into PLGA nanoparticles, with DOX serving as an anticancer drug and Fe_2O_3 nanoparticles used as an imaging agent (Fig. 19). They also used antibody Herceptin1 for targeting the breast cancer.

Taking advantage of both OI and MRI, multimodal imaging agents such as magnetofluorescent nanoparticles have been developed.[170–173] Targeted magnetofluorescent nanoparticles

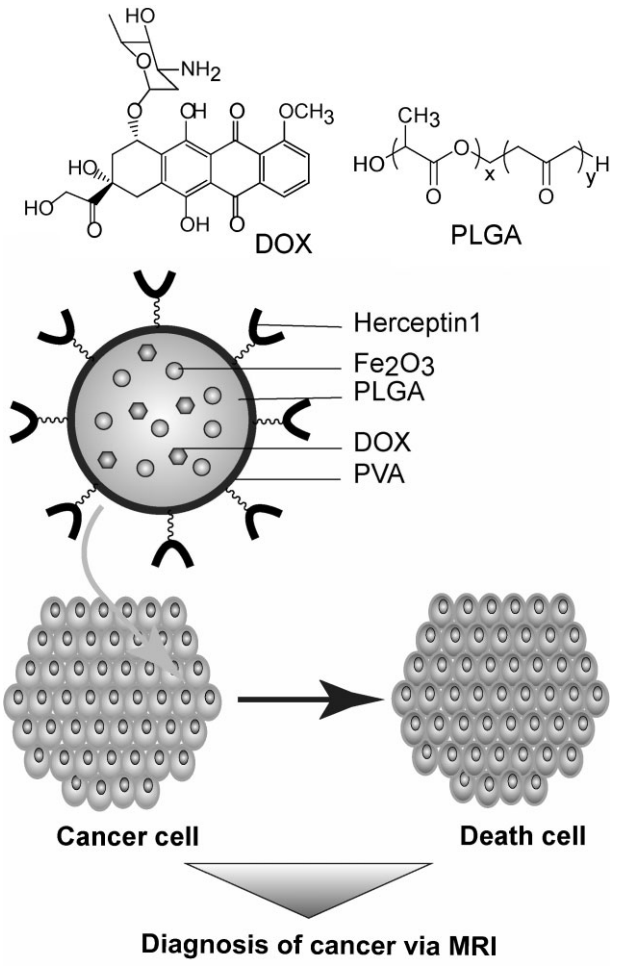

Figure 19. The use of magnetic nanoparticles embedded into PLGA nanoparticles for diagnosis and treatment of cancer.

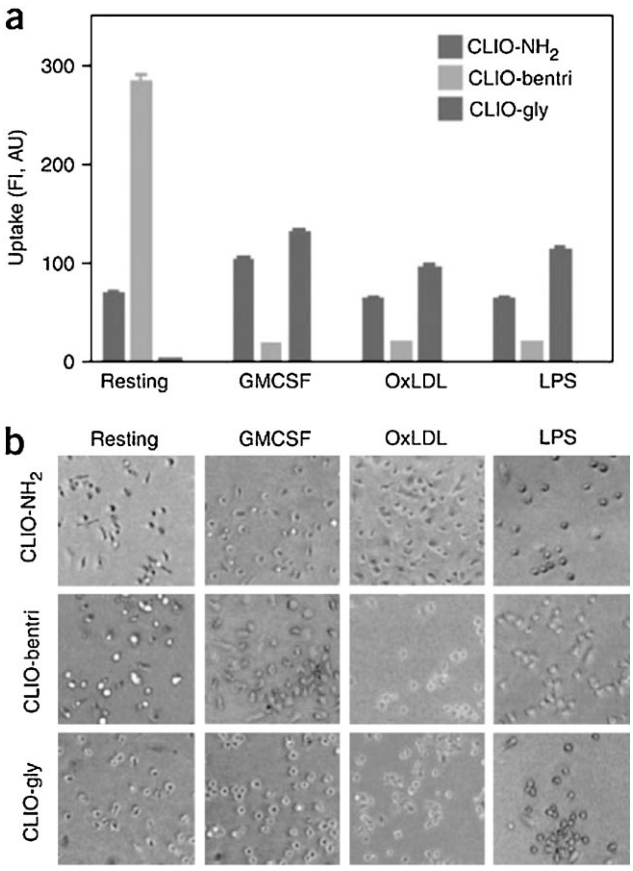

Figure 20. Nanoparticle uptake measured by quantitative fluorescence-activated cell sorting, were probed against resting and different activating macrophages. Adapted with permission from [117]. Copyright 2005, Nature Publishing Group.

have been developed by Weissleder's group using high-throughput screening techniques. For example, glycine-functionalized CLIO-Cy5.5 (CLIO-Gly) had a high affinity towards activated microphages, whereas the 3,3′,4,4′-benzophenontetracarboxylic dianhydride attached CLIO-Cy5.5 (CLIO-bentri) preferentially interacted with resting microphages (Fig. 20). Recently, Bawendi's group also described an efficient synthetic method for magnetic and fluorescent silica microspheres fabricated by incorporating magnetic (γ-Fe_2O_3) nanoparticles and CdSe/CdZnS core/shell QDs into a silica shell around preformed silica microspheres.[174]

6. Conclusions

Nanoparticles present a highly attractive platform for a diverse array of biological applications. The surface and core properties of these systems can be engineered for individual and multimodal applications, including biomolecular recognition, therapeutic delivery, biosensing, and bioimaging. Nanoparticles have already been used for a wide range of applications both in vitro and in vivo. Full realization of their potential, however, requires addressing a number of open issues, including acute and long-term health effects of nanomaterials as well as scalable, reproducible manufacturing methods and reliable metrics for characterization of these materials.

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