SCIENCE CHINA
Chemistry

• REVIEWS •

April 2010 Vol.53 No.4: 704–719
doi: 10.1007/s11426-010-0110-3

Protein analysis based on molecular beacon probes and biofunctionalized nanoparticles

SHI Hui, HE XiaoXiao, YANG XiaoHai, WANG KeMin*, WANG Qing, GUO QiuPing & HUO XiQin

State Key Laboratory of Chemo/Biosensing and Chemometrics; College of Chemistry and Chemical Engineering, Biomedical Engineering Center, Hunan University; Key Laboratory for Bio-Nanotechnology and Molecule Engineering of Hunan Province, Changsha 410082, China

Received January 15, 2010; accepted February 7, 2010

With the completion of the human genome-sequencing project, there has been a resulting change in the focus of studies from genomics to proteomics. By utilizing the inherent advantages of molecular beacon probes and biofunctionalized nanoparticles, a series of novel principles, methods and techniques have been exploited for bioanalytical and biomedical studies. This review mainly discusses the applications of molecular beacon probes and biofunctionalized nanoparticles-based technologies for real-time, *in-situ*, highly sensitive and highly selective protein analysis, including the nonspecific or specific protein detection and separation, protein/DNA interaction studies, cell surface protein recognition, and antigen-antibody binding process-based bacteria assays. The introduction of molecular beacon probes and biofunctionalized nanoparticles into the protein analysis area would necessarily advance the proteomics research.

proteomics, protein analysis, molecular beacon, aptamer, silica nanoparticles, protein/DNA interaction

1 Introduction

Protein is one of the most significant parts of organisms, which is involved in virtually all cell functions [1–3]. Some proteins are involved in structural support, while others are involved in bodily movement, or in defense against germs. Upon the completion of the human genome-sequencing project, there has been a dramatic change in the focus of studies from genomics to proteomics [4, 5]. Proteomics includes not only the identification and quantification of proteins, but also the determination of their localization, modifications, interactions, activities, and functions [4]. Apparently, the development of proteomics primarily depends on the advances of protein analysis technologies. However, conventional methods for protein analysis, which usually include gel electrophoresis, enzyme-linked immunosorbent assay (ELISA), Western blot and immunochemistry, are often laborious, time-consuming, and are, in general, not applicable to real-time monitoring of proteins [4, 6–13]. It is very urgent to explore rapid, simple, highly efficient, real-time and *in-situ* protein analysis methods for proteomics studies.

The rapid development of molecular biology and nanotechnology paves a new way for analysts to perform protein analysis. A series of novel principles, methods and techniques based on molecular beacon probes and biofunctionalized nanoparticles have been developed for bioanalysis and biomedical research [14–20], by which the biological and chemical information of many significant life processes at the level of single cells, subcells, and even single molecules, could be acquired and monitored. The introduction of molecular beacon probes and biofunctionalized nanoparticles into protein analysis would necessarily advance the proteomics research. In this review, we will mainly discuss the molecular beacon probes and nanoparticles-based tech-

*Corresponding author (email: kmwang@hnu.cn)

nologies and their applications in protein analysis.

2 Protein analysis based on molecular beacon probes

Fluorescent probes for biomolecular analysis have been used for mechanism studies of biological functions and in ultrasensitive detection of biological species responsible for many diseases. In the post-genome era, the qualitative and quantitative studies of biomolecules for disease diagnosis and prevention and drug discovery would be the rapid growing areas of research and development. This has led to a continued demand for advanced biomolecular recognition probes with high sensitivity and specificity. The molecular beacon (MB) probe, which was first described in 1996 by Tyagi and Kramer [21], is an excellent example of biomolecular analysis probes and appears to be a very promising probe for genomic and proteomic studies. The thermodynamic stability of the hairpin structure, the highly efficient intrinsic signal switching, and the possibility of using a variety of fluorophores make MBs exceptional biomolecular analysis probes with excellent sensitivity, selectivity and real-time detection capability. MBs are therefore used for a variety of applications, such as DNA and RNA detection [21–30], monitoring of living systems [31–36], investigation of enzymatic processes [37–45], design of biosensors [46–50], study of protein/DNA interactions [51–57], and the fabrication of biochips [58, 59]. Here, the scope of this review is limited to the fundamental aspects of MB's principles, advantages and the recent applications of MBs in protein recognition and protein/DNA interaction studies.

2.1 Principles and advantages of MB probes

The hybridization of a nucleic acid strand to its complement target is one of the most specific molecular recognition events. MBs are a class of DNA probes which are single-stranded oligonucleotides that possess a stem-and-loop structure (Figure 1) [16], and their signal transduction mechanism for molecular recognition is based on fluorescence energy transfer. The loop portion of the MBs could report the presence of a specific complementary nucleic acid. The stem has five to seven base pairs which are complementary and contain a fluorophore and quencher linked to the two ends of the stem. The fluorescent dye serves as an energy donor, and the non-fluorescent quencher plays the role of an acceptor. The stem keeps these two moieties in close proximity to each other, causing the fluorescence of the fluorophore to be quenched by energy transfer. When the probe encounters a target molecule, the loop forms a hybrid that is longer and more stable than the stem, and the MB undergoes a spontaneous conformational reorganization that forces the stem apart, leading to the restoration of fluorescence. Unhybridized MBs do not fluoresce, thus it is not necessary to remove them to observe hybridized probes. Different MBs could be designed by selecting loop sequences and sizes [21]. The quencher and the fluorophores could be changed according to the application [23].

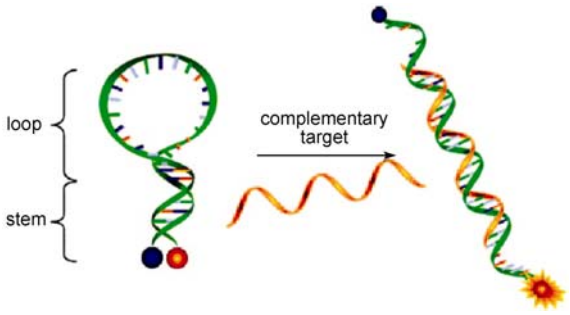

Figure 1 The working mechanism of a molecular beacon [16]. The MB adopts a stem-loop structure and thus holds the fluorophore (orange) and quencher (blue) in close proximity. As a result, the fluorescence emission of the fluorophore is strongly suppressed (in the absence of a target). The target sequence hybridizes with the loop domain of the MB and forces the stem helix to open, whereupon fluorescence is restored because of the spatial separation of the fluorophore from the quencher.

The inherent fluorescent signal transduction mechanism enables an MB to function as a sensitive probe with a high signal-to-background ratio for real-time monitoring. Its fluorescence intensity increases more than 200 folds when it meets the target under optimal conditions [21]. Therefore, MBs have a significant advantage over other fluorescent probes in ultrasensitive analysis. With this inherent sensitivity, individual MB DNA molecules have been imaged, and the hybridization process for a single molecule has been monitored [60]. MBs could be used in situations where it is not possible or desirable to isolate the probe-target hybrids from an excess of the unhybridized probes, such as in the real-time monitoring of polymerase chain reactions (PCRs) in sealed tubes or in the detection of mRNAs within living cells. This feature enables the synthesis of nucleic acids to be monitored as it is occurring, in sealed tubes or in living specimens, and without additional manipulation. Another major advantage of MBs is their molecular recognition specificity. They are extraordinarily target-specific, ignoring nucleic acid target sequences that differ by as little as a single nucleotide. This specificity of an MB comes from its loop-and-stem structure. The stem hybrid acts as a counterweight for the loop hybrid. Experiments have shown that the range of temperatures within which perfectly complementary DNA targets form hybrids but mismatched DNA targets do not is significantly wider for MBs than for the corresponding range of conventional linear probes [61]. Therefore, MBs could readily discriminate DNA targets that differ from one another by a single nucleotide.

2.2 Protein recognition and protein/DNA interaction study using MB probes

In the post-genome era, the quantitative analysis and real-

time monitoring of proteins are of significance. The application of MB probes for real-time protein detection demonstrates advantages in the understanding of many significant biological processes involving two key biomolecules: nucleic acid and protein.

2.2.1 *Nonspecific protein detection*

As the sensitive probes for monitoring interactions of proteins and enzymes with DNA, the protein-recognition ability of MBs was first demonstrated with a single-stranded DNA-binding protein (SSB) from *E. coli* [62]. The fluorescence enhancement caused by SSB and by complementary DNA was very comparable. Using MB-SSB binding, it was possible to detect SSB at a concentration as low as 2×10^{-10} mol/L by using a conventional spectrometer with a mercury lamp. Similarly, interactions between lactate dehydrogenase (LDH) and ssDNA have also been studied by using MBs [14]. Variant LDH isoenzymes were found to have different binding affinities for ssDNA, for example, a binding stoichiometry of 1:1 and a binding constant of 1.9×10^{-7} M^{-1} were measured for the complex LDH-5/MB. Interactions between enzymes and DNA, for example, the digestion of ssDNA by three different nucleases (S1 nuclease, DNase I, and mung bean nuclease) [37] can also be detected with MBs. The cleavage of the loop sequence destabilizes the stem duplex and restores fluorescence.

2.2.2 *Protein detection with specificity*

The nonspecific DNA binding protein studies open up the possibility for the further development of readily obtainable modified DNA molecules for real-time detection of specific proteins. Aptamers, which are a new type of designer molecules and could be used in protein recognition as antibodies [63, 64], are usually DNA or RNA molecules that have been selected from random pools based on their ability to bind to such ligands as small organic or inorganic compounds, peptides, proteins, and even the entire organisms. As antibodies, aptamers have high affinity and specificity for their targets, and thus have many potential uses with advantages over antibodies, such as facile synthesis, easier labeling, good reproducibility, easier storage, faster tissue penetration, and shorter blood residence. However, an aptamer itself cannot be used as a fluorescent probe since it lacks signal transduction capability to report the binding. The combination of MBs' sensitive signal transduction mechanism and aptamers' specific protein-binding capability results in a novel class of analytical probes termed molecular beacon aptamers (MBAs) [65], which have a great potential in the real-time analysis of proteins.

The MBAs developed to date could be described by three general classifications: FRET fluorophore/quencher pairs, FRET donor/acceptor pairs, and single labeled anisotropy probes. An example of a FRET-based quenching MBA is the one developed using the known and well-studied thrombin aptamer [65]. When bound to thrombin, the aptamer exists primarily in its quadruplex form containing two G-quartet structures, but in free solution, it could adopt either conformation, partially depending on the ionic strength and temperature. This conformation shift provides the basis for the MBA. By labeling the two ends of the aptamer with a fluorophore and quencher pair, aptamer binding of thrombin forces the quencher adjacent to the fluorophore, resulting in a substantial decrease in fluorescence.

Similarly, a donor and acceptor fluorophore could be positioned on either end to create a two fluorophore FRET probe [47]. Angiogenin (Ang), one of the most potent angiogenic factors, is related with the growth and metastasis of numerous tumors [66, 67]. Serum concentration of Ang is commonly detected with antibody-based enzyme-linked immunosorbant assay (ELISA). Here, a sensitive method for rapid Ang detection by using a dual-labeled FRET aptamer-based probe was discussed (Figure 2). As the donor and the acceptor, 6-carboxyfluorescein (FAM) and 6-carboxytetramethylrhodamine (TMR) were labeled at 5′- and 3′-termini of the aptamer probe, respectively. The dual- labeled probe showed apparent fluorescence changes due to the specific binding between aptamer and Ang. By monitoring the ratio of the fluorescence intensity of the donor and acceptor, quantitative Ang detection could be achieved. This assay is highly specific and sensitive, with a detection limit of 2.0×10^{-10} mol/L and a linear range of 5.0×10^{-10} to 4.0×10^{-8} mol/L Ang. The detection limit meets the requirement of clinical tests for Ang, and Ang in serum samples of health and lung cancer were also detected.

The third class of molecular aptamer probes is based on monitoring the change in fluorescence anisotropy upon protein binding [68, 69]. This signal transduction strategy, which takes advantage of the sensitive anisotropy signal change of fluorophore-labeled aptamer upon protein/ aptamer binding, has been introduced for quick Ang recognition and real-time quantitative analysis in homogeneous solutions [48]. When the labeled aptamer is bound with its target, the increased molecular weight causes the rotational

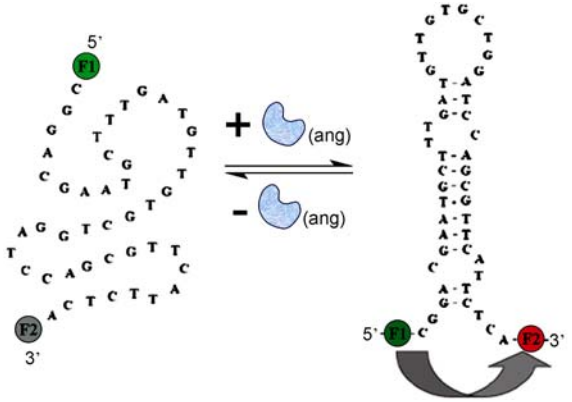

Figure 2 Schematic illumination of the FRET-based aptamer probe for binding with the target protein, Ang. (Left) The random coil structure and (right) the predicted secondary structure.

motion of the fluorophore attached to the complex to become much slower. Therefore, the increasing of the amount of Ang results in a raised anisotropy value of the Ang/ aptamer. By monitoring the anisotropy change, we are able to detect the binding events between the aptamer and Ang, and measure Ang concentration quantitatively in homogeneous solutions. This assay is highly selective, with a detection limit of 1 nM of Ang. The dissociation constant of the Ang/aptamer binding is determined in the nanomolar range and changes with increasing salt concentration. This assay could also be used to compare the binding affinities of different ligands for the target molecules.

Apart from MBAs, other aptamer-related protein analysis methods have been exploited [49, 50]. We have developed a simple, sensitive and specific proximity-dependent protein assay method with dual DNA aptamers [49]. As shown in Figure 3, thrombin is used as the model protein, and two aptamer probes with complementary sequence at 3′-end are designed for the two distinct epitopes of the protein. Association of the two aptamers with thrombin results in stable hybrids due to the proximity of 3′-end, and polymerase reaction is subsequently induced. The amount of obtained dsDNA is then indicated using the fluorescence dye Sybr Green I. The results show that the initial velocity of polymerase reaction has a positive correlation with the concentration of thrombin. The advantages of this dual-aptamer-based approach include simple and flexible design of aptamer probes, high selectivity and sensitivity. The detection limit is 6.9 pmol/L. Another example is a novel fluorescence-based method for protein detection, which is developed by using the aptamer probe as the recognized molecule and cationic conjugated polymers (CCP) as the reporter [50]. The quencher-labeled aptamer probe attaches to CCP by electrostatic interaction, leading to fluorescence quenching of CCP. When the target protein is added, it binds specifically to the aptamer probe, making the quencher detached from CCP and then the fluorescence of CCP is resumed. The results show that the fluorescence resuming ratio is proportional to the concentration of target protein, and the detection method for thrombin had a linear range of 1.7–40 nmol/L.

The selective recognition ability of aptamer probes for proteins has also been utilized to monitor the interaction of proteins and living cells in real time. In the post-genomic era, one of the most significant challenges bioscience faces is the ability to gain a detailed understanding of the function and interplay of proteins throughout human growth and development. The cellular behavior has been a critical focus for the development of imaging technologies [70, 71], involving the real-time observation of protein internalization and tracking the proteins inside living cells to determine subcellular localization. Angiogenin is a potent angiogenic factor that is significant in tumor angiogenesis. For angiogenin imaging, the conventional antibody-based immunofluorescence microscopy is accomplished with stopping cell culture, fixing with organic solvent and further immunofluorescence staining [72, 73]. The direct real-time imaging in living cells cannot be obtained. By using fluorophore-labeled aptamer and confocal laser scanning microscopy, we have developed a novel and simple method to visualize the real-time process of angiogenin internalization [36]. The results revealed that, when aptamer-angiogenin conjugates were added into cell cultures, conjugates could be selectively bound to HUVE cells (human umbilical vein endothelial cells) and MCF-7 cells (human breast cancer cells). Nuclear staining and *z*-axis scanning studies demonstrated that the aptamer-angiogenin conjugates were internalized to intracellular organelles, and dynamic confocal imaging studies indicated that the conjugates were rapidly internalized (Figure 4). The spatiotemporal process of angiogenin internalization was for the first time directly visualized with confocal laser scanning microscopy upon binding with a fluorophore-labeled aptamer. These results demonstrate the potential of (1) a fluorophore-labeled aptamer used as a recognition and labeling probe for real-time protein internalization and (2) an aptamer-ligand conjugate used for targeted cellular delivery in the implementation of novel diagnostic and therapeutic strategies.

2.2.3 *Protein/DNA interaction studies*

The replication, repair and recombination of nucleic acids are essential life processes, in which such biomolecules as enzymes, proteins and ribozymes participate. Along with increasing understanding of the characteristics and functions of MBs, MB-based assays have been designed to study more-specific and more-sophisticated processes involving DNA and proteins, such as DNA ligation and phosphorylation [38, 39, 55–57], DNA polymerization and dephosphorylation [40–42], and DNA methylation [43]. All of these developments would facilitate the obtaining of de-

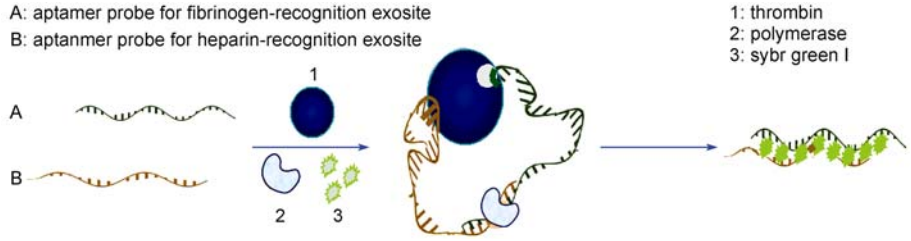

Figure 3 The schematic diagram of proximity-extension detection of thrombin.

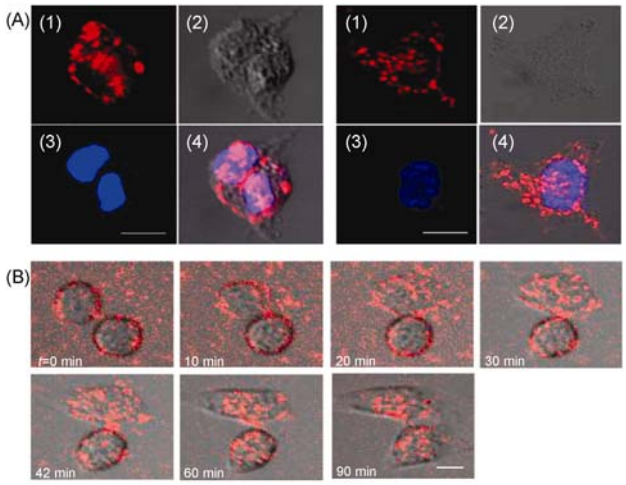

Figure 4 (A) Intracellular distribution of internalized angiogenin-aptamer conjugate in HUVE (left) and MCF-7 (right) cells revealed by confocal microscopy. (1) Fluorescence images of Cy5-labeled aptamer-angiogenin conjugate; (2) bright field images; (3) fluorescence images with Hoechst 33258 nuclear staining; (4) overlap of fluorescence images and the light bright field image. (B) Time-lapse imaging of MCF-7 cells cultured with angiogenin-aptamer conjugate. The overlaps of fluorescence images and bright field images are shown here. Scale bar, 10 μm.

signed DNA molecules for genomics and proteomics studies, molecular diagnosis of diseases and new drug development.

(1) Real-time monitoring of DNA ligation and phosphorylation

DNA ligase is indispensable in catalyzing the formation of phosphodiester bonds at single strand breaks between adjacent 3′-hydroxyl and 5′-phosphate ends in double strand DNA [74]. Nucleic acid ligation is a significant tool for editing DNA both *in vivo* and *in vitro*, and such nucleic acid assay methods as Ligase Chain Reaction (LCR) and Amplified Fragment Length Polymorphism (AFLP) have been developed on the basis of it [75, 76]. The ligation process is conventionally assayed by radial ^{32}P labeling, denaturing gel electrophoresis and autoradiography [74–76]. These methods are time-consuming, discontinuous and not sensitive, making real-time monitoring of nucleic acid ligation impossible. For the monitoring of DNA ligation, MBs are designed to bridge two ssDNA sequences to form a nick structure (Figure 5(a)). The two ssDNA sequences are complementary to two adjacent zones of the loop portion of the MB. Because the melting temperature of these two short sequences is much lower than that of the MB, the loop-stem structure of the MB remains intact. When the DNA ligase is introduced, it catalyzes the junction of the nick structure by using the loop of the MB as the template to form a longer DNA sequence that is complementary to the entire loop sequence. This process leads to the separation of the stem and the restoration of fluorescence, and the DNA-ligation process could thus be monitored in real time [38, 56, 57]. A series of DNA ligations catalyzed by different DNA ligases, including T4 DNA ligase and the DNA ligase from *E. coli*, have been explored by using this principle. The DNA ligase from *E. coli* operated much more precisely than T4 DNA ligase, but a T-G mismatch at the 5′-end of the nick led to the highest error rate for the ligation with both ligases. The effects of metal ions, small biomolecules, and drugs on the activity of DNA ligases have also been examined. This assay might be useful for the identification and development of ligase-targeted drugs [56]. However, as we still do not know exactly how the ligation process occurs, the results of the studies with molecular beacons are interpreted with caution.

A similar strategy with MBs was used to study DNA phosphorylation [39] (Figure 5(b)). The phosphorylation of the 5′-hydroxyl termini of nucleic acids is significant in nucleic acid metabolism and is indispensable to the repair of nucleic acids during strand interruption [77]. The polynucleotide kinase is found to be widespread in cells and considered to be a putative DNA repair enzyme that phosphorylates the 5′-hydroxyl of DNA and RNA [77, 78]. We have studied the process of phosphorylation using MB probes in real time and with high selectivity. The MB em-

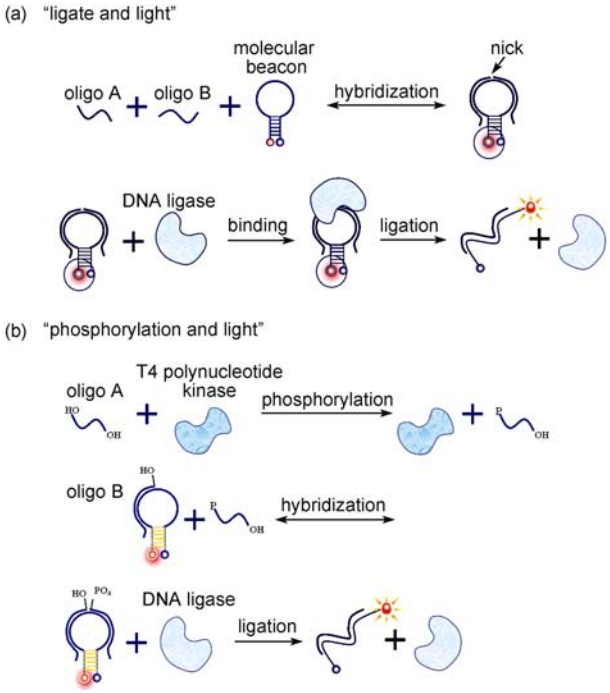

Figure 5 Application of MBs in DNA ligation and phosphorylation studies. a) Real-time monitoring of nucleic acid ligation. Two oligonucleotides that are complementary to opposite halves of the MB loop hybridize with the MB, whereby a nick is formed and the stem may be opened slightly. The DNA ligase binds to the nick and catalyzes the ligation of the two short oligonucleotides to form a longer oligonucleotide. The ligation product hybridizes with the MB to restore fluorescence. b) Monitoring of nucleic acid phosphorylation. Oligo A is first phosphorylated at the 5'-hydroxy group by the polynucleotide kinase. The nick formed upon the hybridization of oligo B and phosphorylated oligo A with MB can be sealed by the DNA ligase, whereupon the stem helix of the MB is opened, and fluorescence is restored.

ployed in this method is devised to sense the product of a "phosphorylation-ligation" coupled enzyme reaction. In detail, the DNA fragment located at the 5′-side of the nick was modified to mimic dephosphorylation damage at the 5′-end of DNA, i.e., one hydroxy group at the 5′-end of the DNA is not phosphorylated, so the nick cannot be sealed by a DNA ligase. Phosphorylation of the damaged DNA under the catalysis of a polynucleotide kinase (PNK) is required before the two DNA fragments are ligated to form the longer oligonucleotide sequence complementary to the MB loop. Compared with the conventional assays, this novel method is convenient, rapid, selective, highly sensitive and capable of real-time monitoring in a homogenous solution. Based on this principle, a rapid and accurate technique for assaying the kinase activity of T4 PNK has been developed with a wide linear detection range from 0.002 to 4.0 U/mL in 3 min. These novel approaches enable us to investigate the interactions between proteins and nucleic acids in a homogenous solution, such as those found in DNA repair or in drug development.

(2) Real-time monitoring of DNA polymerization and dephosphorylation

DNA polymerase is one of the responsible enzymes for replication and repair of DNA along the sequence of a template strand. DNA polymerases are the workhorses in numerous significant biological core technologies, such as PCR, cDNA cloning and genome sequencing. Because of the biological significance of DNA polymerases and their wide use, a novel method for real-time monitoring of the activity of DNA polymerase based on primer extension reaction using MB has been developed [40]. As illustrated in Figure 6(a), the primer is hybridized to half of the loop of the MB to form a primer-MB duplex. When DNA polymerase is introduced, it binds to the 3′-terminus of the primer-MB duplex and catalyze the extension of the primer to form a longer DNA strand that is complementary to the MB, resulting in the complete opening of MB and leading to a full fluorescence restoration. Under optimized conditions, Klenow analysis could be realized within 5 min with a detection limit down to 0.003 U/mL. There are no other methods having comparable sensitivity and speed. This assay appears to have general utility because it is also suitable for assessing the activity of other polymerases such as Klenow fragment (which has exonuclease/proofreading activity) and T4 DNA polymerase. The novel approach is potentially a useful tool in investigating the interactions between proteins and nucleic acids in homogeneous solution such as DNA replication, DNA repair, and drug research and development.

The similar principle has also been employed for real-time monitoring of *Rsa* I endonuclease activity [41]. Restriction endonucleases are one of the most significant enzymes in molecular biology. These enzymes are essential in recombinant technology, genotyping, mapping, and sequencing of large strands of DNA [79]. As illustrated in Figure 6(b), on hybridization of oligo A with oligo B, the *Rsa* I endonuclease recognition site is formed. After cleavage, the resulting oligo C, which is complementary with the 3′ half part of MB's loop, is expected to readily dissociate from the duplex. The oligo C could be hybridized to the MB and polymerized by DNA polymerase, resulting in the complete opening of MB and leading to a full fluorescence restoration. Thus, the activity of *Rsa* I endonuclease could be monitored in real time using this principle.

DNA dephosphorylation, which is involved in DNA replication, recombination, and repair of DNA damage induced by a variety of genotoxic agents, has been detected as well [42]. A novel method, which is simple, rapid, cost effective, and requires one oligonucleotide, has been developed for real-time monitoring of the dephosphorylation process by using MB DNA probes. Oligonucleotide A (Oligo A) is dephosphorylated and hybridized with the MB to form an oligonucleotide-MB duplex. In the presence of DNA polymerase, dephosphorylated Oligo A could be elongated by polymerization, which opens the MB and restores its fluorescence. In these procedures, the DNA polymerase plays a role as a converter that transforms the information of "oligonucleotide has been dephosphorylated" into restoration of fluorescence in the MB. Thus, dephosphorylation of an oligonucleotide could be monitored in real time by using this "dephosphorylation and polymerization" enzyme-coupled reaction.

(3) Real-time monitoring of DNA methylation

DNA methylation, a common gene protection approach, is significant in both prokaryotes and eukaryotes. This process is carried out by DNA methyltransferases (MTases),

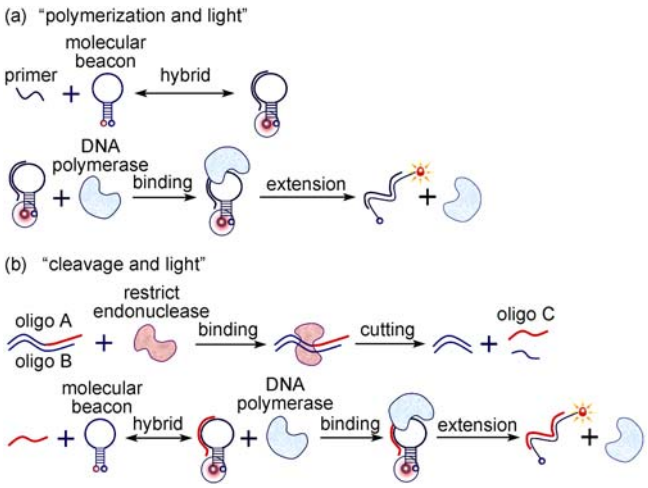

Figure 6 Schematic diagram of real-time monitoring of the activity of polymerase (a) and restriction endonuclease (b) based on molecular beacon probes. (a) A primer-MB duplex forms after primer hybridizing to MB, and the DNA polymerase binds to the 3'-terminus of the primer-MB duplex and catalyzes the extension of primer to form a longer oligo. Then the DNA polymerase leaves and the extension product restores the fluorescence of MB. (b) DNA is cleaved by restriction endonuclease, and the cleavage product hybridizes with the MB and can be polymerized by DNA polymerase, which restores the quenched fluorescence of the MB.

which specifically recognize the short palindromic sequences and catalyze the transfer of a methyl group from S-adenosyl-Lmethiolnine (SAM) to the target adenine or cytosine. We have constructed a new strategy to study methylase activity using fluorescent probes coupled with enzyme-linkage reactions [43]. A hairpin DNA probe is prepared with a fluorophore and a quencher linked at the 5′- and 3′-terminus of the probe. A disturbance of the stem sequence by DNA methylation would cause the separation of the fluorophore and the quencher, resulting in the restoration of the fluorescence. In our study, DNA adenine methylation (Dam) methyltransferase (MTase) and *Dpn* I endonuclease, both having a 5′-G-A-T-C-3′ recognition sequence, were used. Dam MTase catalyzed the methylation of the sequence of 5′-GATC-3′, and *Dpn* I cut the sequence of 5′-G-Am-T-C-3′, as shown in Figure 7. The fluorescence of the hairpin probe was restored when it was cleaved by *Dpn* I endonuclease during the course of methylation. Unlike conventional methods, this assay was done in real time and could be used to monitor the dynamic process of methylation. The method, which is simple and nonradioactive, yet as efficient as gel electrophoresis in detecting the activity of methylase, has the potential to screen suitable inhibitor drugs for Dam methylase.

In addition to MBs, other types of molecular probes have been applied for real-time monitoring of protein/DNA interaction. As a highly conserved damage repair protein, uracil-DNA glycosylase (UDG) mainly catalyzes the excision of uracil from DNA to sustain the genome integrity. A novel method for monitoring the uracil removal in real time is introduced [44]. Double-stranded DNA probes modified

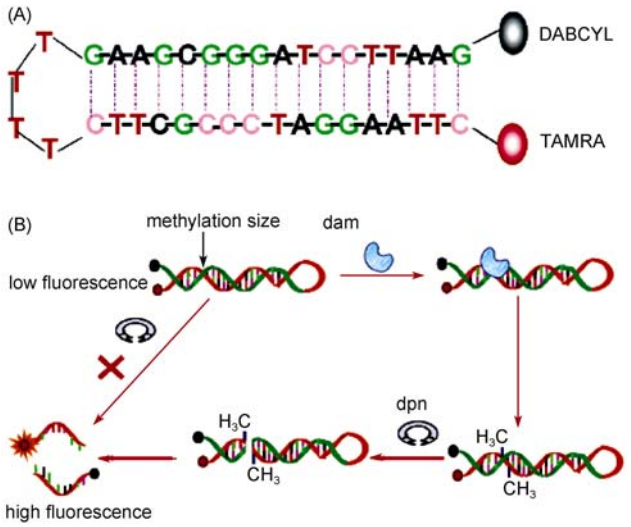

Figure 7 The schematic diagram of the strategy of real-time monitoring of the methylation process by a hairpin probe [43]. (A) The sequence of the designed hairpin probe labeled TAMRA and DABCYL; the probe had low fluoresce in the hairpin conformation. (B) The Dam MTase methylated the hairpin probe at the recognition site, yielding the methylated DNA probe. The methylated probe substrate was recognized and cleaved by Dpn I endonuclease, restoring the fluorescence of TAMRA.

with uracil residues that could induce FRET were used as substrates and detecting probes in a homogeneous solution. This method overcame the drawbacks of conventional radioactive assays, such as discontinuity and being time-consuming and complex, and was used to accurately determine the kinetic constant of UDG as well. The detection limit of UDG was 0.033 U/mL. The K_M and K_{cat} were 0.11 μM and 4 s^{-1}, respectively. The method was applied to investigate the influence of chemical drugs on UDG activity. The results showed that 10 mM fluorouracil (5-FU) and gentamicin are inhibitors to UDG. The *in vitro* detection of UDG in A549 cells showed that the activity of UDG was four times greater after the cells were treated with cisplatin. These results showed that this method could monitor uracil removal in real time and conveniently assay UDG activity with ultrasensitivity and excellent specificity in the homogeneous solution.

3 Protein analysis based on biofunctionalized nanoparticles

With the development of nanotechnology, new opportunities have appeared for resolving the key problems in obtaining biological and chemical information at levels of single cells, subcells, and even single molecules, e.g., nerve glia, and single base pairs of nucleotide acid [80, 81]. Particles with at least one dimension (d)⩽100 nm could universally be considered as nanoparticles (NPs). The fact that NPs are similar to many common biomolecules with respect to size magnitude permits them to have widespread use in biotechnological systems. Numerous studies have been published concerning the use of nanomaterials for biotechnological research and more are in progress, including the use of nanotechnology for gene delivery [82–86], drug delivery [87, 88], and immunoassays [89–91]. We have developed uniform biofunctionalized core/shell NPs, composed of a functionalized fluorescent or magnetic core, a modifiable coating shell, and the biomolecules modified on the surface of the nanoparticles, as shown in Figure 8. The three parts all prominently contribute to their application of biomedicine, such as cell staining [92–102], bacteria detection [103, 104], nanosensing [105, 106], biomolecule detection and separation [107–111], gene and drug delivery [112–116], and biotechnological application in DNA protection [117]. Here, a few applications for protein detection and separation using biofunctionalized nanoparticles are discussed.

3.1 Protein detection using biofunctionalized dye-doped silica NPs

Dye-doped silica NPs are used in many areas of protein analysis. With numerous dye molecules trapped inside a single nanoparticle, dye-doped silica NPs exhibit extraordi-

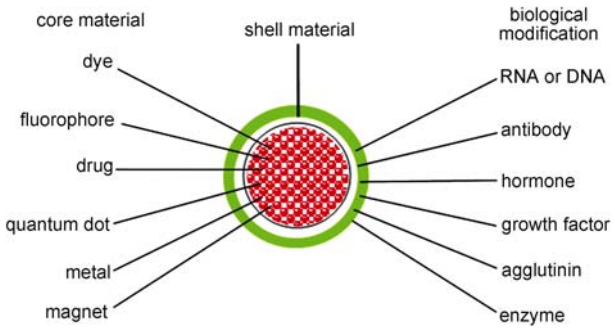

Figure 8 Schematic diagram of the core-shell biofunctionalized nanoparticles.

nary signaling strength and significantly improved the analytical sensitivity [118, 119]. As the dye is doped inside the silica matrix, which provides an effective barrier keeping the dyes from the surrounding environment, both photobleaching and photodegradation phenomena that often affect conventional dyes could be minimized [92, 97, 99, 100]. The excellent photostability makes these NPs suitable for applications where high intensity or prolonged excitations are required. The flexible silica chemistry provides versatile routes for surface modification, and different types of functional groups could be readily introduced onto the NPs for conjugation with biomolecules. All the aforementioned properties make dye-doped silica NPs excellent labeling reagents for protein analysis.

3.1.1 Synthesis, surface modification and bioconjugation of dye-doped silica NPs

There are two reported synthetic routes for the dye-doped silica nanoparticles, water-in-oil (W/O) microemulsion method [120–126] and the Stöber method [127, 128]. The W/O microemulsion is a thermodynamically stable system which is composed of water, surfactant and oil. The stabilized water nanodroplets formed in the oil solution act as small microreactors, where silane hydrolysis and the formation of NPs with dye trapped inside occur [122]. By studying the mechanism of stable core/shell silica NP preparation, we found that electrostatic interaction of the core materials with shell materials would determine whether the stable core/shell silica NPs formed or not [121]. Such conventional significant factors as molecular weight of core materials or the thickness of the shell have no apparent relationship with it. Based on this principle, Tris(2,2′-bipyridyl) dichlororuthenium (II) (RuBpy) was trapped into the silica matrix to form the stable fluorescent RuBpy-doped silica NPs. As for other dyes, which cannot be stably doped in the silica shell by the conventional method, a modified protocol has been employed to significantly improve this situation by forming fluorescent dye complexes [124]. It has been found that the FITC-IgG complexes could be encapsulated into silica NPs with much higher entrapment efficiency, compared to that of free FITC. Using this improved method, such fluorescent dyes as tetramethylrhodamine (TMR), Cy3, Cy5 and Alexa Fluor 647 have been doped into the silica NPs without leakage. In addition to single-dye doping, multiple-dye incorporation into the silica matrix has been reported [125, 126]. This provides more information upon detection. Figure 9 shows the photos and transmission electron microscope (TEM) image of several dye-doped silica NPs that we have prepared.

For the further application in bioanalysis, dye-doped silica NPs have been modified by a variety of trialkoxysilane molecules with carboxylic, vinyl, thiol, amino, or methacryloxy functionalities. Various functional groups could be readily introduced onto the particle surface by adding alkoxysilane reagents into the microemulsion along with tetraethyl orthosilicate (TEOS) to produce functionalized silica shell coated NPs [129, 130]. Once the silica surface has been modified, such biomolecules as proteins, enzymes, antibodies and oligonucleotides, could be directly linked to the NPs following standard conjugation protocols, which was discussed in detail in our previous reviews [17–19]. These finally obtained biofunctionalized NPs could be further applied in bioanalytical and biomedical research.

3.1.2 Bioeffect studies of silica NPs

With the development of silica NPs' application, the safety and overall bioeffects of silica NPs have emerged as a question that necessarily affects their use in most types of biomedical applications where compatibility with the biological milieu is required. For this reason, several groups, including ours, have explored the interaction and toxicity of silica NPs with biomolecules [117, 131], cells [132–134] and life organisms [135]. For instance, studies have demonstrated that the positively charged amino-modified silica NPs could enrich plasmid DNA and protect it from enzymatic cleavage [117], and as illustrated in Figure 10, the DNA strands have the same properties as free DNA strands when released from the nanoparticles, which are biologically active. Bioeffects of silica NPs as the carrier for enzyme immobilization have been investigated and compared with silica microparticles [131]. By selecting bovine liver catalases and horseradish peroxidases as the multimeric

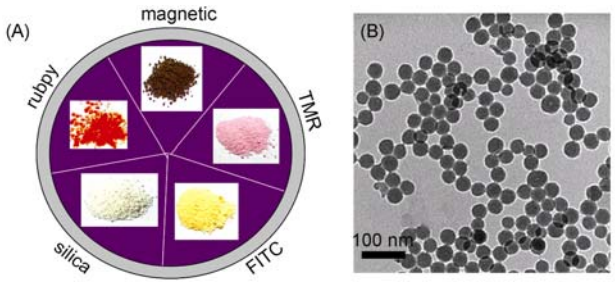

Figure 9 (A) Photos of core-shell silica nanoparticles, including pure silica NPs, magnetic silica NPs and dye-doped silica NPs, such as RuBpy-doped silica NPs, TMR-doped silica NPs and FITC-doped silica NPs; (B) the TEM image of Cy5-doped silica nanoparticles.

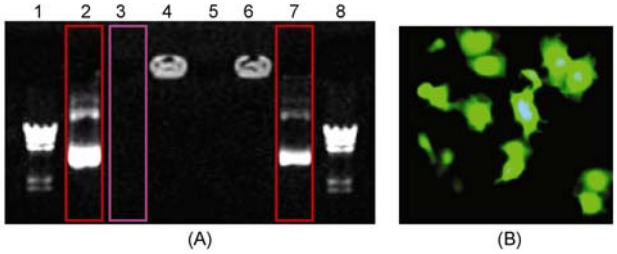

Figure 10 (A) Agarose gel electrophoresis of plasmid DNA and DNA-NP complexes. Lanes 1 and 8, the DNA marker; lane 2, the undigested free plasmid DNA; lane 3, the digested free plasmid DNA; lane 4, the plasmid DNA-NP complexes; lane 5, the pure silica NP after incubation with plasmid DNA; lane 6, the plasmid DNA-NP complexes digested with DNaseI; lane 7, the DNA released from the DNA-NP complexes that have been digested with DNaseI; (B) the fluorescence image of the expression of GFP plasmid DNA in COS-7 cells.

enzyme model and monomer enzyme model, respectively, four kinds of immobilized enzymes were obtained through the covalently binding method. After a series of characterization of the above four immobilized enzymes, silica NPs were demonstrated to exhibit non-selective excellent biocompatibility to both enzyme models.

Because of the wide applications of silica NPs in cellular research, their biocompatibility with target cells is inevitably a significant issue to evaluate whether silica NPs could be used safely in biomedical field right along. Studies have been conducted to investigate the toxicity of silica NPs to various cell lines, including normal tissue cells and tumor cells. The results indicated that the bioeffects of silica NPs on cells were concentration-dependent and silica NPs were nontoxic at low dosages [132–134]. Different surface modification could induce the diverse interactions of silica NPs with cells [134]. For example, the bioeffects of silica NPs, phosphorylate-terminated NPs (PO_4-NPs) and amino-terminated NPs (NH_2-NPs) on HaCaT cell line (skin epidermal cell line) were studied. The results indicated that while the cytotoxicities of the three kinds of functionalized NPs on HaCaT cells would increase with NPs' concentration increasing, the following order was observed: NH_2-NPs > silica NPs > PO_4-NPs. The quantity and rapidity of cellular uptake of NPs by HaCaT cells were diverse due to the different functional groups. Under the same conditions, NH_2-NPs was the most and most rapidly internalized by HaCaT cells, followed by silica NPs, and PO_4-NP was the least and slowest. These results provided theoretical foundation for the safe application and further modification of silica NPs, which may broaden the application of silica NPs in biomedicine.

Similarly, in view of potential *in vivo* applications, elucidating the *in vivo* pharmacokinetics of administered silica NPs, as an indication of the *in vivo* behavior, is considered significant in the context of the underlying medical debate regarding the safety of novel nanomaterials. In order to obtain the real-time information of silica NPs, we used an *in vivo* optical imaging system to study the bioeffects of intravenous injected silica NPs by using RuBpy dye doped in the silica matrix as an adoptable method to track silica NPs [135]. As shown in Figure 11, the *in vivo* biodistribution and urinary excretion of three types of surface-modified silica NPs (OH-NPs, COOH-NPs, and PEG-NPs) in animals have been investigated. The results show that the intravenous injected OH-NPs, COOH-NPs, and PEG-NPs, with a size of ~45 nm, could all be cleared from the circulation and presented inside organs. The PEG-NPs exhibit relatively long blood circulation time and low uptake by the liver than OH–NPs and COOH-NPs. The *in vivo* imaging reveals that all the intravenous injected types of silica NPs are partly excreted through the renal route. The *in vivo* imaging results were confirmed by *ex vivo* organ optical imaging, TEM imaging, and energy-dispersed X-ray spectrum analysis of urine samples. This work puts forward direct implications for the use of silica NPs as delivery systems and imaging tools in live animals.

3.1.3 Cell surface protein detection using dye-doped silica NPs

Dye-doped silica NPs possess four significant properties that make them highly useful in protein detection: extremely high optical intensity, high photostability, good biocompatibility and easy bioconjugation. Different types of bio-

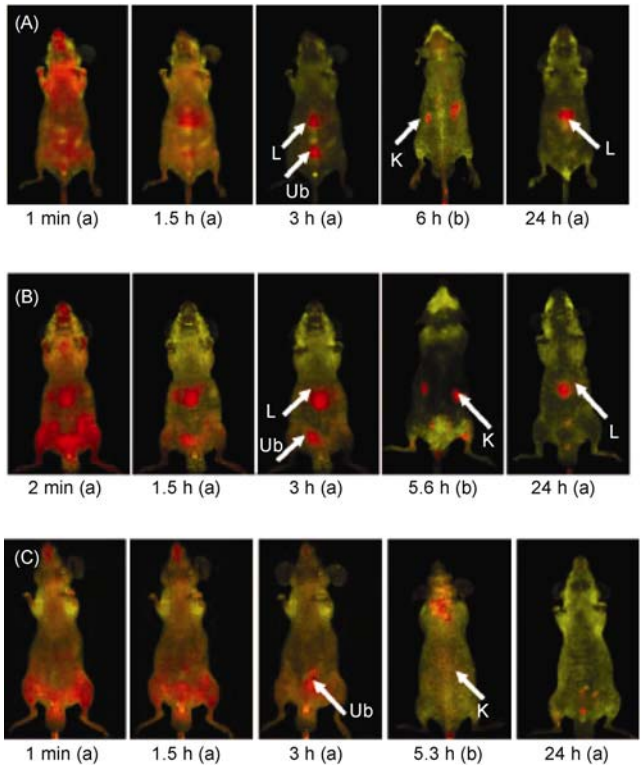

Figure 11 *In vivo* imaging biodistribution of different intravenous injected surface-modified silica NPs at different postinjection time points [135] (A–C: (a) abdomen imaging; (b) back imaging). (A) OH-NPs; (B) COOH-NPs; (C) PEG-NPs. Arrows mark the location of the kidney (K), liver (L), and urinary bladder (Ub).

functionalization have been explored for using dye-doped silica NPs to target cell surface proteins.

Proteins on the cell surface are usually related with the development of a number of diseases and life processes, in which cell surface proteins act as either the biomarkers for diseases or the indicator of cell physiological changes. As illustrated in Figure 12, by employing the affinity and specificity associated with the antigen-antibody recognition process, dye-doped silica NPs-based immunoassay techniques have been widely developed for targeting cell surface proteins and diagnosing such diseases as tumors. As the excellent signal element, NPs were covalently conjugated with antibodies to recognize $SmIgG^{+}B$ lymphocyte for the immune-diagnosis of Systematical Erythema Lupus [94], HepG liver cancer cells [95], MDA-MB-231 breast cancer cells [98] and MCF-7 cancer cells [97] selectively and efficiently. A representative example is that, lactobionic acid (LA, a ligand of asialoglycoprotein receptor on a hepatocyte membrane) has been attached to dye-doped silica NPs through EDAC linkage, enabling the specific identification of target cells from the background cells by a laser confocal scanning microscope [96] (Figure 13). The precise identification of a few liver cancer cells in the blood has been demonstrated, which confirms the excellent capability of galactose-conjugated fluorescent nanoparticles (GCFNPs) in identifying specific cells in a large host cell background.

In order to further improve the reliability of target cell detection, a dye-doped silica NPs mediated improved double immunofluorescence labeling method has been applied to analyze two proteins in gastric cancer cells simultaneously [99]. The MGC-803 gastric cancer cells were incubated with the mixture of anti-CEA antibody-conjugated FITC-doped FSiNPs and anti-CK19 antibody-conjugated RuBpy-doped FSiNPs, and then imaged using laser scanning confocal microscopy (CLSM). As shown in Figure 14, only the MGC-803 gastric cancer cells could be labeled with two dye-doped silica NPs simultaneously, which further confirms the precise detection of target cells. With this

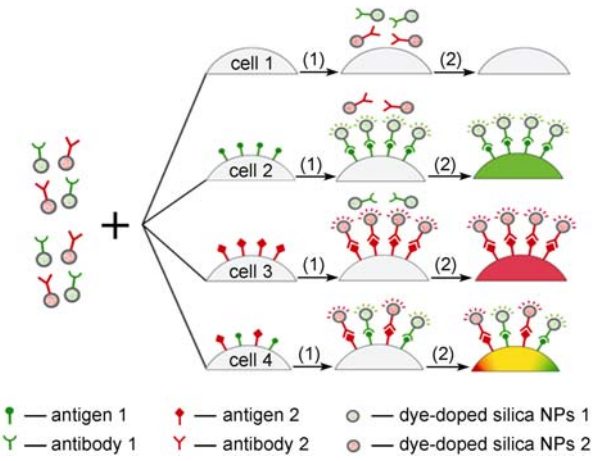

Figure 12 The schematic diagram of the dye-doped silica NPs-based immunoassays for cell surface proteins. (1) Cells were incubated with antibody-conjugated dye-doped silica NPs for 30 min at 37 °C; (2) cells were washed with PBS three times after incubation and then imaged by a laser scanning confocal microscope.

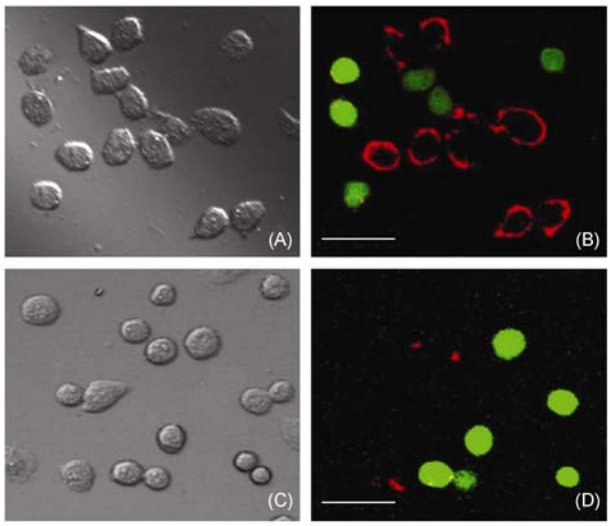

Figure 13 Recognition of liver cancer cells from mixed heterogeneous cells with GCFNPs by a laser confocal microscope [96]. (A) and (B) bright-field and fluorescent-field images of cells incubated with GCFNPs. GCFNPs located on the surface of liver cancer cells and very few nanoparticles adsorbed on the surface of MCF-7-GFP. (C) and (D) bright-field and fluorescent-field images of cells incubated with bare fluorescent nanoparticles. No signal was apparent. Scale bar, 50 μm.

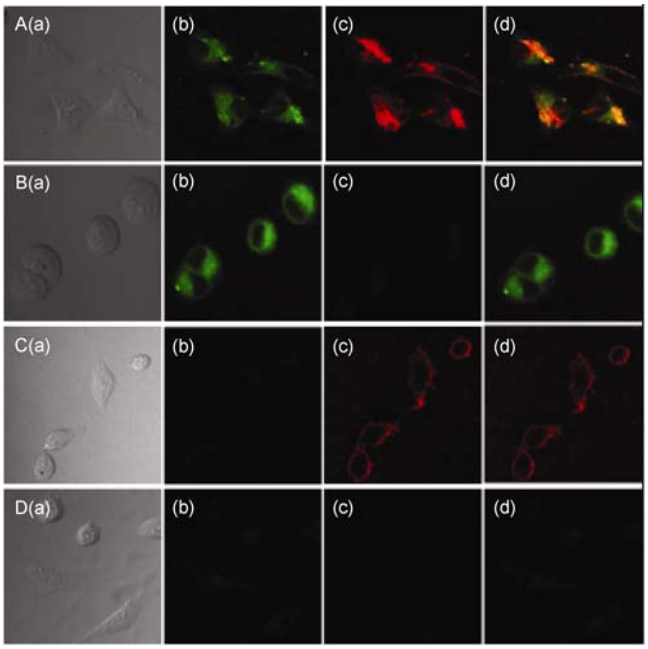

Figure 14 Bioconjugated FSiNPs mediated double immunofluorescence images by CLSM of the cells that were incubated with the mixture of anti-CEA antibody-conjugated FITC-doped FSiNPs and anti-CK19 antibody-conjugated RuBpy-doped FSiNPs [99]. A, B, C and D, MGC-803 cells, Hela cells, 1E8 cells and COS-7 cells, respectively. a, b, c, and d, the images acquired in the bright-field, channel 1 (green signal), channel 2 (red signal) and merged images of the green channel and the red channel.

method, both *in vitro* cultured MGC-803 cells in blood and the *ex vivo* primary MGC-803 cells that came from the tumor tissues of mice bearing MGC-803 gastric cancer tumor xenografts, were double labeled and distinguished through antigen-antibody recognition. These results demonstrate this new bioconjugated FSiNPs mediated double immunofluorescence staining was reliable to effectively image interested cells. The biofunctionalized dye-doped silica NPs mediated immunofluorescence staining may have potentials for bioimaging of early diagnosis of disease *in vitro* and even *in vivo*.

Similar to cell recognition principles, some life processes could be monitored by targeting cell surface proteins based on dye-doped silica NPs [100]. Apoptosis, or programmed cell death, is a universal and significant process in the development of multicellular organisms, regulation of the immune system, and clearance of abnormal cells [136]. As a complex biological phenomenon, apoptosis involves many subprocesses and changes that could be divided into three phases: initiation, decision, and execution. The externalization of phosphatidylserine (PS) from the inner to the outer membrane is an early and major event in the apoptotic process, which has been used to detect and stain early-stage apoptosis by binding PS sitting on the outer membrane of apoptotic cells [137–138]. In our study, a novel bioprobe based on Rhodamine B isothiocyanate-doped silica NPs (RBITC-DSNPs) has been developed through modifying Annexin V with NPs for early-stage apoptosis detection and imaging. It was demonstrated that the bioprobe could specifically recognize early-stage apoptotic cells, and the RBITC-DSNPs labeling method was applied to monitor the physiological change of apoptotic cells along with the extended induction time. As illustrated in Figure 15, with the extension of culture time with paclitaxel, the number of the apoptotic cells stained by Annexin V-functionalized RBITC-DSNPs increased ever more. At higher magnification, it was observed that the red fluorescence on the outer membrane of the recognized apoptotic cells changed as the treatment time was prolonged, from weak to strong, from partially to completely surrounding the cell membrane. It is indicated that dye-doped silica NPs are potential biomarkers that would play significant roles in bioanalysis and bioimaging, especially in monitoring the changes of biochemical information over a long time.

In addition to targeting cell surface proteins, the antigen-antibody recognition process could be introduced into pathogenic bacteria assays. Detection of pathogenic bacteria is vital in food and environment safety, clinical diagnosis and anti-bioterrorism. Conventional methods for the detection of pathogenic bacteria either lack sensitivity or take a long time for analysis. By utilizing the advantages of nanomaterials, a method of dye doped NPs-based indirect immunofluorescence microscopy (FNP-IIFM) was developed for the rapid detection of *Mycobacterium tuberculosis* [103]. An anti-*Mycobacterium tuberculosis* antibody was used as a primary antibody to recognize *Mycobacterium tuberculosis*, and then an antibody binding protein (Protein A) labeled with RuBpy-doped silica NPs was used to generate fluorescent signals for microscopic examination (Figure 16). With this method, *Mycobacterium tuberculosis* in bacterial mixture as well as in spiked sputum was detected. The use of the fluorescent nanoparticles reveals amplified signal intensity and higher photostability than the direct use of conventional fluorescent dye as the label. In order to further decrease false positives caused by aggregates of nanoparticle-bioconjugates and nonspecific binding of nanoparticle-bioconjugates to background debris, another method using an improved two-color flow cytometric analysis by a

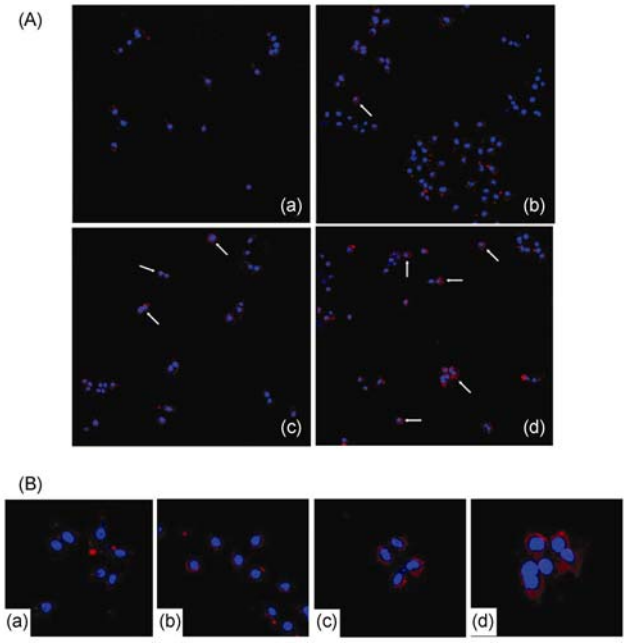

Figure 15 Confocal microscopy fluorescence images of early-stage apoptotic MCF-7 cells that were treated with the same concentration of paclitaxel for different time periods [100]: (a) 0 min, (b) 100 min, (c) 210 min, and (d) 400 min, incubated with RBITC-DSNPs conjugated to Annexin V. Images of groups A and B represent the photographs taken at the lower and higher magnifications, respectively. White arrows point to the cells detected by RBITC-DSNPs conjugated to Annexin V.

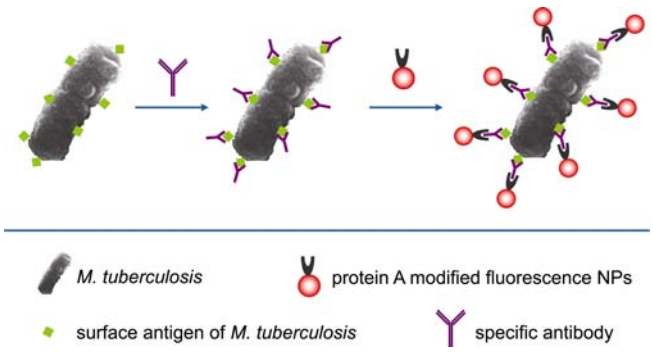

Figure 16 Schematic representation of the principle of *M. tuberculosis* detection with the dye-doped fluorescent silica nanoparticle-based indirect immunofluorescence assay [103].

combination of biofunctionalized dye-doped silica NPs and SYBR Green I (FSiNP@SG-FCM) has been developed for detection of pathogenic *Mycobacterium tuberculosis* [104]. This assay allowed for detection of as low as 3.5×10^3 and 3.0×10^4 cells mL^{-1} *M. tuberculosis* in buffer and spiked urine respectively, with higher sensitivities than the FITC-based conventional flow cytometry. The total assay time including sample pretreatment was within 2 h. This proposed FSiNP@SG-FCM method has potentials for rapid detection of *M. tuberculosis* or other pathogenic bacteria in clinical samples.

3.2 Protein separation using biofunctionalized magnetic silica NPs

The efficient separation and purification of proteins is a significant prerequisite for the ultrasensitive protein analysis. There are various procedures to isolate proteins and peptides, such as chromatography, electrophoresis, ultrafiltration, and precipitation. These classical protein separation systems are usually very complex, and involve different techniques with many time-consuming steps, which have in most cases led to a very low overall process yield and thus a high product cost. Magnetic separation is a powerful separation method for biomolecules [107–111]. In our research, silica coated magnetic nanoparticles, employing Fe_3O_4 as the core and silica as the shell, have been prepared using a water-in-oil microemulsion technique. The procedure was first completed using synthesis of aqueous magnetic ferrofluid with the precipitation of the chloride mixture with the base. Then the silica coating was completed through a water-in-oil microemulsion technique. The obtained magnetic nanoparticles are also superparamagnetic.

For selective separation of proteins, two kinds of unique pH-dependent magnetic nanoadsorbents based on silica coated magnetic nanoparticles (SMNPs) and amino-silica coated magnetic nanoparticles (ASMNPs) have been exploited [110]. With different isoelectric points, SMNAs and ASMNAs could respectively adsorb proteins with different charges, and the interactions between proteins and magnetic nanoadsorbents changed with the solution pH. Thus, the adsorption or desorption between proteins and magnetic nanoadsorbents could be controlled by changing the solution pH according to the charge of the proteins. The magnetic nanoadsorbents could be separated and recycled simply with a magnet. As model adsorbates, Cyt-c and BSA are selectively separated from simple protein mixtures with SMNAs and ASMNAs, respectively. Cyt-c was adsorbed on SMNAs at pH 8.0 at 0.160 mg mg^{-1}. BSA was adsorbed on ASMNAs at pH 5.0 at 0.142 mg mg^{-1}.

Biofunctionalized silica coated magnetic nanoparticles were developed as affinity protein adsorbents, which were activated with CNBr and modified by trypsin inhibitor. Due to the recognition between trypsin and its inhibitor, trypsin in simple model protein mixture or pig pancreas was objectively separated with the biofunctionalized silica coated magnetic nanoparticles. This study provided a new affinity adsorbent for protein separation with silica coated magnetic nanoparticles, which has magnetic cores and biomodified surfaces.

4 Perspectives

With the completion of human genome-sequencing project, key issues in this post-genome era focus on research across many disciplinary interfaces and the development and use of new quantitative tools for proteomics. The MB probe technology and bionanotechnology have been extensively explored for bioanalysis and biomedical applications, and demonstrated to be promising tools for protein analysis. The following areas are of interest for protein analysis based on molecular beacon probes and bionanoparticles.

First, since the introduction of MBs in 1996, the advantages of MBs have led to the development of a broad spectrum of applications in biology, chemistry, biomedicine, and biotechnology. Efforts to improve MBs would continue to focus on the optimization of signaling transduction and the use of modified bases to endue MBs additional useful properties for applications in intracellular gene/protein interaction monitoring, such as the resistance to nuclease digestion, a higher hybridization affinity, and minimized nonspecific protein binding. MB aptamers, which are protein probes that combine the advantages of the signal transduction mechanism of MBs and the specificity of molecular recognition by aptamers, are expected to be further explored and widely applied for the highly sensitive and selective detection of proteins in solution, live cells, tissues and even *in vivo*.

Second, with respect to biofunctionalized nanoparticles, although the inherent features, including flexible conjugation, excellent photostability, superparamagnetism and ultrasensitivity, have made them a useful tool in protein detection and separation, more studies are required to develop strategies for manipulating the properties of the NP matrix and surface to prevent aggregation and inhibit nonspecific binding. Aggregation and nonspecific binding are the major issues blocking or slowing the realization of the power and ultrasensitivity of NPs in bioanalysis, which demands new strategies to reduce NP background signals for detection of ultratrace amounts of analytes. The technological advances in cell surface protein labeling and aqueous protein separation allow the intracellular proteins and the related organelles to be analyzed in real time and *in situ* with higher sensitivity and selectivity.

Third, MBs could be linked to nanoparticles to generate molecular sensors for studying molecular interactions, which has expanded the application scope of protein analysis techniques. With the combination of NPs and MBs, exciting results may be obtained by utilizing the signal transduction mechanism of MBs and the particular properties of

NPs, such as the DNA protection from cleavage, the fluorescence quenching effect and the unique color alteration phenomenon.

The authors express their great thanks for the support from the Program for Changjiang Scholars and Innovative Research Team in University, the Natioual High Technology Research and development Program of China (Grant No. 2007AA022007), Program for New Century Excellent Talents in University (Grant No. NCET-06-0697), the National Natural Science Foundation of China (Grant Nos. 90606003 & 20775021), and Outstanding Youth Foundation of Hunan Province (Grant No. 08JJ10002).

1 Dive C, Hickman JA. Drug-target interactions: only the first step in the commitment to a programmed cell death? *British J cancer*, 1991, 64(1): 192–196
2 Lennon SV, Martin SJ, Cotter TG. Dose-dependent induction of apoptosis in human tumour cell lines by widely diverging stimuli. *Cell proliferation*, 1991, 24(2): 203–214
3 Mathews DJ, Kopczynski J. Using model-system genetics for drug-based target discovery. *Drug Discovery Today*, 2001, 6(3): 141–149
4 Fields S. PROTEOMICS: proteomics in genomeland. *Science*, 2001, 291(5507): 1221–1224
5 Abbott A. And now for the proteome…. Nature, 2001, 409(6822): 747–748
6 Voller A, Bartlett A, Bidwell DE. Enzyme immunoassays with special reference to ELISA techniques. *J Clin Path*, 1978, 31(6): 507–520
7 Dalmau J, Furneaux HM, Gralla RJ, Kris MG, Posner JB. Detection of anti-Hu antibody in the serum of patients with small cell drug cancer—a quantitative western blot analysis. *Ann Neurol*, 1990, 27(5): 544–552
8 Matkowskyj KA, Cox R, Jensen RT, Benya RV. Quantitative immunohistochemistry by measuring cumulative signal strength accurately measures receptor number. *J Histochem Cytochem*, 2003, 51(2): 205–214
9 Alban A, David SO, Bjorkesten L, Andersson C, Sloge E, Lewis S, Currie I. A novel experimental design for comparative two-dimensional gel analysis: two-dimensional difference gel electrophoresis incorporating a pooled internal standard. *Proteomics*, 2003, 3(1): 36–44
10 Fenn JB, Mann M, Meng CK, Wong SF, Whitehouse CM. Electrospray ionization for mass spectrometry of large biomolecules. *Science*, 1989, 246(4926): 64–71
11 Oda Y, Huang K, Cross FR, Cowburn D, Chait BT. Accurate quantitation of protein expression and site-specific phosphorylation. *Proc Natl Acad Sci USA*, 1999, 96: 6591–6596
12 Gygi SP, Rist B, Gerber SA, Turecek F, Gelb MH, Aebersold R. Quantitative analysis of complex protein mixtures using isotope-coded affinity tags. *Nature Biotechnol*, 1999, 17(10): 994–999
13 Abbott A. A post-genomic challenge: learning to read patterns of protein synthesis. *Nature*, 1999, 402(6763): 715–720
14 Fang X, Li JJ, Perlette J, Tan W, Wang K. Molecular beacons: novel fluorescent probes. *Anal Chem*, 2000, 72(23): 747A–753A
15 Li J, Tang Z, Wang K, Tan W. Real-time protein monitoring based on molecular beacons. *Current Proteomics*, 2004, 1: 315–324
16 Wang K, Tang Z, Yang CJ, Kim Y, Fang X, Li W, Wu Y, Medley CD, Cao Z, Li J, Colon P, Lin H, Tan W. Molecular engineering of DNA: molecular beacons. *Angew Chem Int Ed*, 2009, 48(5): 856–870
17 Tan W, Wang K, He X, Zhao X, Drake T, Wang L, Bagwe RP. Bionanotechnology based on silica nanoparticles. *Med Res Rev*, 2004, 24(5): 621–638
18 Wang L, Wang K, Santra S, Zhao X, Hilliard LR, Smith JE, Wu Y, Tan W. Watching silica nanoparticles glow in the biological world. *Anal Chem*, 2006, 78(3): 646–654
19 Yan J, Estévez MC, Smith JE, Wang K, He X, Wang L, Tan W. Dye-doped nanoparticles for bioanalysis. *Nanotoday*, 2007, 2(3): 44–50
20 Liu JB, Yang XH, Wang KM, Wang D, Zhang PF. Chemical etching with tetrafluoroborate: a facile method for resizing of CdTe nanocrystals under mild conditions. *Chem Commun*, 2009, 40: 6080–6082
21 Tyagi S, Kramer FR. Molecular beacons: probes that fluoresce upon hybridization. Nat Biotechnol, 1996, 14(3): 303–308
22 Tyagi S, Marras SAE, Kramer FR. Wavelength-shifting molecular beacons. *Nat Biotechnol*, 2000, 18(11): 1191–1196
23 Tyagi S, Bratu DP, Kramer FR. Multicolor molecular beacons for allele discrimination. *Nat Biotechnol*, 1998, 16(1):49–53
24 Zhang P, Beck T, Tan W. Design of a molecular beacon DNA probe with two fluorophores. *Angew Chem Int Ed*, 2001, 40(2): 402–405
25 Guo Q, Yang X, Wang K, Tan W, Li W, Tang H, Li H. Sensitive fluorescence detection of nucleic acids based on isothermal circular strand-displacement polymerization reaction. *Nucleic Acids Res*, 2009, 37(3): e20
26 Yang X, Zhao X, Zuo X, Wang K, Wen J, Zhang H. Nucleic acids detection using cationic fluorescent polymer based on one-dimensional microfluidic beads array. *Talanta*, 2009, 77(3): 1027–1031
27 Zuo X, Yang X, Wang K, Tan W, Li H, Zhou L, Wen J, Zhang H. Improving the performance of immobilized molecular beacons through cleavage. *Anal Chim Acta*, 2006, 567(2): 173–178
28 Tang H, Yang X, Wang K, Tan W, Li W. mRNA detection in living cell using phosphorothioate-modified molecular beacon. *Chin Sci Bull*, 2009, 54(9): 1507–1514
29 Tang H, Yang X, Wang K, Tan W, Liu B, He L, Wang W. Monitoring p21 mRNA expression in living cell based on molecular beacon fluorescence increasing rate. *Chin Sci Bull*, 2007, 53(3): 357–361
30 Liu B, Wang K, Xiao Z, Wang W, Tan W, Sun Y, Tang H, Yang X. Quantitative detection of ING1 mRNA under different gene regulation based on molecular beacon. *Chin Sci Bull*, 2006, 51(17): 2059–2064
31 Bratu DP, Cha BJ, Mhlanga MM, Kramer FR, Tyagi S. Visualizing the distribution and transport of mRNAs in living cells. *Proc Natl Acad Sci USA*, 2003, 100(23): 13308–13313
32 Perlette J, Tan W. Real-time monitoring of intracellular mRNA hybridization inside single living cells. *Anal Chem*, 2001, 73(22): 5544–5550
33 Medley CD, Drake TJ, Tomasini JM, Rogers RJ, Tan W. Simultaneous monitoring of the expression of multiple genes inside of single breast carcinoma cells. *Anal Chem*, 2005, 77(15): 4713–4718
34 Santangelo PJ, Nix B, Tsourkas A, Bao G. Dual FRET molecular beacons for mRNA detection in living cells. *Nucleic Acids Res*, 2004, 32(6): e57
35 Meng X, Wang K, Tan W, Li J, Tang Z, Guo Q, Huang S, Li D. Ribozyme probe based on molecular beacon for real time monitoring of enzymatic cleavage process. *Chin Sci Bull*, 2003, 48(23): 2581–2584
36 Li W, Yang X, Wang K, Tan W, He Y, Guo Q, Tang H, Liu J. Real-time imaging of protein internalization using aptamer conjugates. *Anal Chem*, 2008, 80(13): 5002–5008
37 Li JJ, Geyer R, Tan W. Using molecular beacons as a sensitive fluorescence assay for enzymatic cleavage of single-stranded DNA. *Nucleic Acids Res*, 2008, 28(11): e52
38 Tang Z, Wang K, Tan W, Li J, Liu L, Guo Q, Meng X, Ma C, Huang S. Real-time monitoring of nucleic acid ligation in homogenous solutions using molecular beacons. *Nucleic Acids Res*, 2003, 31(23): e148
39 Tang Z, Wang K, Tan W, Ma C, Li J, Liu L, Guo Q, Meng X. Real-time investigation of nucleic acide phosphorylation process using molecular beacons. *Nucleic Acids Res*, 2005, 33(11): e97
40 Ma C, Tang Z, Wang K, Tan W, Li J, Li W, Li Z, Yang X, Li H, Liu L. Real-time monitoring of DNA polymerase activity using molecular beacon. *Anal Biochem*, 2006, 353(1): 141–143
41 Ma C, Tang Z, Wang K, Tan W, Yang X, Li W, Li Z, Lv X. Real-time monitoring of restriction endonuclease activity using molecular beacon. *Anal Biochem*, 2007, 363(2): 294–296
42 Ma C, Tang Z, Wang K, Tan W, Yang X, Li W, Li Z, Li H, Lv X. Real-time monitoring of nucleic acid dephosphorylation by using

molecular beacons. *Chem Bio Chem*, 2007, 8(13): 1487–1490
43 Li J, Yan H, Wang K, Tan W, Zhou X. Hairpin fluorescence DNA probe for real-time monitoring of DNA methylation. *Anal Chem*, 2007, 79(26): 1050–1056
44 Liu B, Yang X, Wang K, Tan W, Li H, Tang H. Real-time monitoring of uracil removal by uracil-DNA glycosylase using fluorescent resonance energy transfer probes. *Anal Biochem*, 2007, 366(2): 237–243
45 Biggins JB, Prudent JR, Marshall DJ, Ruppen M, Thorson JS. A continuous assay for DNA cleavage: The application of "break lights" to enediynes, iron-dependent agents, and nucleases. *Proc Natl Acad Sci USA*, 2000, 97(25): 13537–13542
46 Fang X, Liu X, Schuster S, Tan W. Designing a novel molecular beacon for surface-immobilized DNA hybridization studies. *J Am Chem Soc*, 1999, 121(12): 2921–2922
47 Li W, Yang X, Wang K, Tan W, Li H, Ma C. FRET-based aptamer probe for rapid angiogenin detection. *Talanta*, 2008, 75(3): 770–774
48 Li W, Wang K, Tan W, Ma C, Yang X. Aptamer-based analysis of angiogenin by fluorescence anisotropy. *Analyst*, 2007, 132(2): 107–113
49 Yang X, Wang L, Wang K, Tan W, Tang H, Meng X, Guo Q. Novel protein detection method based on proximity-dependent polymerase reaction and aptamers. *Chin Sci Bull*, 2007, 53(2): 204–208
50 Yang X, Wang S, Wang K, Luo X, Tan W, Cui L. Novel method of protein detection based on cationic conjugated polymer and aptamer probe. *Chem J Chin Univ*, 2009, 30(5): 899–902
51 Fang X, Li JJ, Tan W. Using molecular beacons to probe molecular interactions between lactate dehydrogenase and single-stranded DNA. *Anal Chem*, 2000, 72(14): 3280–3285
52 Li JJ, Fang X, Schuster SM, Tan W. Molecular beacons: a novel approach to detect protein-DNA interactions. *Angew Chem Int Ed*, 2000, 39(6): 1049–1052
53 Tan W, Fang X, Li J, Liu X. Molecular beacons: a novel DNA probe for nucleic acid and protein studies. *Chem Eur J*, 2000, 6(7): 1107–1111
54 He X, Jin R, Yang L, Wang K, Li W, Tan W, Li H. Study on the specific interaction between angiogenin and aptamer by atomic force microscopy (AFM). *Chin Sci Bull*, 2008, 53(2): 198–203
55 Ma C, Tang Z, Huo X, Yang X, Li W, Tan W. Real-time monitoring of double-stranded DNA cleavage using molecular beacons. *Talanta*, 2008, 76(2): 458–461
56 Liu L, Tang Z, Wang K, Tan W, Li J, Guo Q, Meng X, Ma C. Using molecular beacon to monitor activity of *E. coli* DNA ligase. *Analyst*, 2005, 130(3): 350–357
57 Tang Z, Wang K ,Tan W, Li J, Liu L, Guo Q, Meng X, Huang S, Li D, Luo W. Real time monitoring of nucleic acids ligation based on molecular beacon. *Chin Sci Bull*, 2003, 48(3): 1215–1218
58 Yao G, Tan W. Molecular-beacon-based array for sensitive DNA analysis. *Anal Biochem*, 2004, 331(2): 216–223
59 Wang H, Li J, Liu H, Liu Q, Mei Q, Wang Y, Zhu J, He N, Lu Z. Label-free hybridization detection of a single nucleotide mismatch by immobilization of molecular beacons on an agarose film. *Nucleic Acids Res*, 2002, 30(12): e61
60 Fang X, Tan W. Imaging single fluorescent molecules at the interface of an optical fiber probe by evanescent wave excitation. *Anal Chem*, 1999, 71(15): 3101–3105
61 Bonnet G, Libchaber A, Kramer FR, Tyagi S. Thermodynamic basis of the enhanced specificity of structured DNA probes. *Proc Natl Acad Sci USA*, 1999, 96(11): 6171–6176
62 Li JJ, Fang X, Schuster SM, Tan W. Molecular beacons: a novel approach to detect protein-DNA interactions. *Angew Chem Int Ed*, 2000, 112(6), 1091–1094
63 Tuerk C, Gold L. Systematic evolution of ligands by exponential enrichment: RNA ligands to bacteriophage T4 DNA polymerase. *Science*, 1990, 249(4968): 505–510
64 Jayasena SD. Aptamers: An emerging class of molecules that rival antibodies in diagnostics. *Clin Chem*, 1999, 45(9): 1628–1650
65 Li JJ, Fang X, Tan W. Molecular aptamer beacons for real-time protein recognition. *Biochem Bioph Res Co*, 2002, 292(1): 31–40
66 Harper JW, Fox EA, Shapiro R, Vallee BL. Mutagenesis of residues flanking Lys-40 enhances the enzymatic activity and reduces the angiogenic potency of angiogenin. *Biochemistry*, 1990, 29(31): 7297–7302
67 Yoshioka N, Wang L, Kishimoto K, Tsuji T, Hu GF. A therapeutic target for prostate cancer based on angiogenin-stimulated angiogenesis and cancer cell proliferation. *Proc Natl Acad Sci USA*, 2006, 103: 14519–14524
68 Fang X, Cao Z, Beck T, Tan W. Molecular aptamer for real-time oncoprotein platelet-derived growth factor monitoring by fluorescence anisotropy. *Anal Chem*, 2001, 73(23): 5752–5757
69 Lou H, Brister JR, Li JJ, Chen W, Muzyczka N, Tan W. Adeno-associated virus Rep78/Rep68 promotes localized melting of the Rep binding element in the absence of ATP. *ChemBioChem*, 2004, 5(3): 324–332
70 Lang P, Yeow K, Nichols A, Scheer A. Cellular imaging in drug discovery. *Nat Rev Drug Discovery*, 2006, 5: 343–356
71 Ruan G, Agrawal A, Marcus AI, Nie S. Imaging and tracking tat-peptide conjugated quantum dots in living cells: new insights into nanoparticle uptake, intracellular transport, and vesicle shedding. *J Am Chem Soc*, 2007, 129: 14759–14766
72 Hu G, Xu C, Riordan JF. Human angiogenin is rapidly translocated to the nucleus of human umbilical vein endothelial cells and binds to DNA. *J Cell Biochem*, 2000, 76: 452–462
73 Li R, Riordan JF, Hu G. Nuclear translocation of human angiogenin in cultured human umbilical artery endothelial cells is microtubule and lysosome independent. *Biochem Biophys Res Commun*, 1997, 238(2), 305–312
74 Doherty AJ, Dafforn TR. Nick recognition by DNA ligases. *J Mol Biol*, 2000, 296: 43–56
75 Vos P, Hogers R, Bleeker M, Reijans M, Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M, Zabeau M. AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Res*, 1995, 23: 4407–4414
76 Kinoshita Y, Nishigaki K, Husimi Y. Fluorescence-, isotope- or biotin-labeling of the 5'-end of single-stranded DNA/RNA using T4 RNA ligase. *Nucleic Acids Res*, 1997, 25: 47–48
77 Wang LK, Lima CD, Shuman S. Structure and mechanism of T4 polynucleotide kinase: an RNA repair enzyme. *EMBO J*, 2002, 21: 3873–3880
78 Whitehouse J, Taylor RM, Thistlethwaite A, Zhang H, Karimi-Busheri F, Lasko DD, Weinfeld M, Caldecott KW. XRCC1 stimulates human polynucleotide kinase activity at damaged DNA termini and accelerates DNA single-strand break repair. *Cell*, 2001, 104: 107–117
79 Pingoud A, Jeltsch A. Structure and function of type II restriction endonucleases. Nucleic Acids Res, 2001, 29(18): 3705–3727
80 Tan W, Shi Z, Smith S, Birnbaum D, Kopelman R. Submicrometer intracellular chemical optical fiber sensors. *Science*, 1992, 258(5083): 778–781
81 Bratten CDT, Cobbold PH, Cooper JM. Single-cell measurements of purine release using a micromachined electroanalytical sensor. *Anal Chem*, 1998, 70(6): 1164–1170
82 Kneuer C, Sameti M, Bakowsky U, Schiestel T, Schirra H, Schmidt H, Lehr CM. A nonviral DNA delivery system based on surface modified silica-nanoparticles can efficiently transfect cells *in vitro*. *Bioconjug Chem*, 2000, 11(6): 926–932
83 Kneuer C, Sameti M, Haltner EG, Schiestel T, Schirra H, Schmidt H, Lehr CM. Silica nanoparticles modified with aminosilanes as carriers for plasmid DNA. *Int J Pharm*, 2000, 196(2): 257–261
84 Mao H, Roy K, Troung-Le VL, Janes KA, Lin KY, Wang Y, August JT, Leong KW. Chitosan-DNA nanoparticles as gene carriers: synthesis, characterization, and transfection efficiency. *J Control Release*, 2001, 70(3): 399–421
85 Benns JM, Kim SW. Tailoring new gene delivery designs for specific targets. *J Drug Target*, 2000, 8(1): 1–12
86 Truong-Le VL, Walsh SM, Schweibert E, Mao H, Guggino WB, August JT, Leong KM. Gene transfer by DNA-gelatin nanospheres. *Arch Biochem Biophys*, 1999, 361(1): 47–56
87 Soppimath KS, Aminabhavi TM, Kulkarni AR, Rudzinski WE.

Biodegradable polymeric nanoparticles as drug delivery devices. *J Control Release*, 2001, 70: 1–20
88 Sershen SR, Westcott SL, Halas NJ, West JL. Temperature-sensitive polymer-nanoshell composites for photothermally modulated drug delivery. *J Biomed Mater Res*, 2000, 51(3): 293–298
89 Kubitschko S, Spinke J, Bruckner T, Pohl S, Oranth N. Sensitivity enhancement of optical immunosensors with nanoparticles. *Anal Biochem*, 1997, 253(1): 112–122
90 Lyon LA, Musick MD, Smith PC, Reiss BD, Pena DJ, Natan MJ. Surface plasmon resonance of colloidal Au-modified gold films. *Sensor Actuat B-Chem*, 1999, 54:118–124
91 Shaertl S, Meyer-Almes FJ, Lopez-Calle E, Siemers A, Kramer J. A novel and robust homogeneous fluorescence-based assay using nanoparticles for pharmaceutical screening and diagnostics. *J Biomol Screen*, 2000, 5(4): 227–237
92 Santra S, Zhang P, Wang K, Tapec R, Tan W. Conjugation of biomolecules with luminophore-doped silica nanoparticles for photostable biomarkers. *Anal Chem*, 2001, 73(20): 4988–4993
93 He X, Wang K, Tan W, Xiao D, Li J, Yang X. A novel fluorescent label based on biological fluorescent nanoparticles and its application in cell recognition. *Chin Sci Bull*, 2001, 46(23): 1962–1965
94 He X, Wang K, Tan W, Li J, Yang X, Huang S, Li D, Xiao D. Photostable luminescent nanoparticles as biological label for cell recognition of system lupus erythematosus patients. *J Nanosci Nanotech*, 2002, 2: 317–320
95 He X, Duan J, Wang K, Tan W, Lin X, He C. A novel fluorescent label based on organic dye-doped silica nanoparticles for HepG liver cancer cell recognition. *J Nanosci Nanotech*, 2004, 4(6): 585–589
96 Peng J, Wang K, Tan W, He X, He C, Wu P, Liu F. Identification of liver cancer cells in a mixed cell system using galactose-conjugated fluorescent nanoparticles. *Talanta*, 2007, 71(2): 833–840
97 He X, Chen J, Wang K, Qin D, Tan W. Preparation of luminescent Cy5 doped core-shell SFNPs and its application as a near-infrared fluorescent marker. *Talanta*, 2007, 72(4): 1519–1526
98 Wu P, He X, Wang K, Tan W, Ma D, Yang W, He C. Imaging breast cancer cells and tissues using peptide-labeled fluorescent silica nanoparticles. *J Nanosci Nanotech*, 2008, 8(5): 2483–2487
99 He X, Ge J, Wang K, Tan W, Shi H, He C. FSiNPs mediated improved double immunofluorescence staining for gastric cancer cells imaging. *Talanta*, 2008, 76(5): 1199–1206
100 Shi H, He X, Wang K, Yuan Y, Deng K, Chen J, Tan W. Rhodamine B isothiocyanate doped silica-coated fluorescent nanoparticles (RBITC-DSFNPs)-based bioprobes conjugated to Annexin V for apoptosis detection and imaging. *Nanomedicine: Nanotechnology, Biology, and Medicine*, 2007, 3(4): 266–272
101 Li Z, Wang K, Tan W, Li J, Fu Z, Ma C, Li H, He X, Liu J. Immunofluorescent labeling of cancer cells with quantum dots synthesized in aqueous solution. *Anal Biochem*, 2006, 354(2): 169–174
102 Li Z, Wang K, Tan W, Li J, Fu Z, Wang Y, Liu J, Yang X. Preparation of luminescent CdTe quantum dots doped core-shell nanoparticles and their application in cell recognition. *Chin Sci Bull*, 2005, 50(16): 1703–1707
103 Qin D, He X, Wang K, Zhao X, Tan W, Chen J. Fluorescent nanoparticle-based indirect immunofluorescence microscopy for detection of Mycobacterium tuberculosis. *J Biomed Biotechnol*, 2007, ID: 89364
104 Qin D, He X, Wang K, Tan W. Using fluorescent nanoparticles and SYBR Green I based two-color flow cytometry to determine Mycobacterium tuberculosis avoiding false positives. *Biosens Bioelectron*, 2008, 24(4): 626–631
105 He X, Wang Y, Wang K, Peng J, Liu F, Tan W. Research of the relationship of intracellular acidification and apoptosis in Hela cells based on pH nanosensors. *Sci China Ser B-Chem*, 2007, 5(2): 258–265
106 Peng J, He X, Wang K, Tan W, Wang Y, Liu Y. Noninvasive monitoring of intracellular pH change induced by drug stimulation using silica nanoparticle sensors. *Anal Bioanal Chem*, 2007, 388(3): 645–654
107 Zhao X, Tapec-Dytioco R, Wang K, Tan W. Collection of trace amounts of DNA/mRNA molecules using genomagnetic nanocapturers. *Anal Chem*, 2003, 75(14): 3476–3483
108 Ashtari P, He X, Wang K, Gong P. An efficient method for recovery of target ssDNA based on amino-modified silica-coated magnetic nanoparticles. *Talanta*, 2005, 67(3): 548–554
109 He X, Huo H, Wang K, Tan W, Gong P, Ge J. Plasmind DNA isolation using amino-silica coated magnetc nanoparticles (ASMNPs). *Talanta*, 2007, 73(4): 764–769
110 He X, Chen Y, Wang K, Wu P, Gong P, Huo H. Selective separation of proteins with pH-dependent magnetic nanoadsorbents. *Nanotechnology*, 2007, 18(36): 365604 (6pp)
111 Gong P, He X, Wang K, Tan W, Xie W, Wu P, Li H. Combination of functionalized nanoparticles and polymerase chain reaction-based method for SARS-CoV gene detection. *J Nanosci Nanotech*, 2008, 8(1): 293–300
112 Peng J, Xing X, Wang K, Tan W, He X, Huang S. Influence of anions on the formation and properties of chitosan-DNA nanoparticles. *J Nanosci Nanotechnol*, 5(5): 713–717
113 Peng J, He X, Wang K, Tan W, Li H, Xing X, Wang Y. An antisense oligonucleotide carrier based on amino silica nanoparticles for antisense inhibition of cancer cells. *Nanomedicine: Nanotechnology, Biology and Medicine*, 2006, 2(2): 113–120
114 Gong P, Li H, He X, Wang K, Hu J, Tan W, Zhang S, Yang X. Preparation and antibacterial activity of Fe_3O_4@Ag nanoparticles. *Nanotechnology*, 2007, 18: 285604 (7pp)
115 He X, Wu X, Wang K, Shi B, Hai L. Methylene blue-encapsulated phosphonate-terminated silica nanoparticles for simultaneous *in vivo* imaging and photodynamic therapy. *Biomaterials*, 2009, 30(29): 5601–5609
116 Wu P, He X, Wang K, Tan W, He C, Zheng M. A novel methotrexate delivery system based on chitosan-methotrexate covalently conjugated nanoparticles. *J Biomed Nanotechnol*, 2009, 5(5): 557–564
117 He X, Wang K, Tan W, Liu B, Lin X, He C, Li D, Huang S, Li J. Bioconjugated nanoparticles for DNA protection from cleavage. *J Am Chem Soc*, 2003, 125(24): 7168–7169
118 Zhao X, Tapec-Dytioco R, Tan W. Ultrasensitive DNA detection using highly fluorescent bioconjugated nanoparticles. *J Am Chem Soc*, 2003, 125(38): 11474–11475
119 Zhao X, Hilliard LR, Mechery SJ, Wang Y, Bagwe RP, Jin S, Tan W. A rapid bioassay for single bacterial cell quantitation using bioconjugated nanoparticles. *Proc Natl Acad Sci USA*, 2004, 101(42): 15027–15032
120 Santra S, Wang K, Tapec R, Tan W. Development of novel dye-doped silica nanoparticles for biomarker application. *J Biomed Opt*, 2001, 6: 160–166
121 He X, Wang K, Tan W, Chen J, Duan J, Yuan Y, Lin X. Study on the effect of electrostatic interaction on core-shell nanoparticles preparation with microemulsion technique. *Chin Sci Bull*, 2005, 50(24): 2821–2826
122 Yamauchi H, Ishikawa T, Kondo S. Surface characterization of ultramicro spherical particles of silica prepared by w/o microemulsion method. *Colloids Surf*, 1989, 37: 71–80
123 Bagwe RP, Yang CY, Hilliard LR, Tan WH. Optimization of dye-doped silica nanoparticles prepared using a reverse microemulsion method. *Langmuir*, 2004, 20(19): 8336–8342
124 Zhao X, Bagwe RP, Tan W. Development of organic-dye-doped silica nanoparticles in reverse microemulsion. *Adv Mater*, 2004, 16: 173–176
125 Wang L, Yang C, Tan W. Dual-luminophore-doped silica nanoparticles for multiplexed signaling. *Nano Lett*, 2005, 5(1): 37–43
126 Wang L, Tan W. Multicolor FRET silica nanoparticles by single wavelength excitation. *Nano Lett*, 2006, 6(1): 84–88
127 Qhobosheane M, Santra S, Zhang P, Tan W. Biochemically functionalized silica nanoparticles. *Analyst*, 2001, 126: 1274–1278
128 Tapec R, Zhao J, Tan W. Development of organic dye-doped silica nanoparticle for bioanalysis and biosensors. *J Nanosci Nanotechnol*, 2002, 2: 405
129 Hilliard LR, Zhao X, Tan W. Immobilization of oligonucleotides

onto silica nanoparticles for DNA hybridization studies. *Anal Chim Acta*, 470(1): 51–56
130 Bagwe RP, Milliard LR, Tan W. Surface modification of silica nanoparticles to reduce aggregation and nonspecific binding. *Langmuir*, 2006, 22(9): 4357–4382
131 Shi H, He X, Wang K, Yuan Y, Tan W. Bioeffects of silica nanoparticles and silica microparticles as carriers for enzyme immobilization. *Chem J Chin Univ*, 2007, 28(9): 1690–1695
132 Chen M, Von Mikecz A. Formation of nucleoplasmic protein aggregates impairs nuclear function in response to SiO_2 nanoparticles. *Exp Cell Res*, 2005, 305: 51–62
133 Xing X, He X, Peng J, Wang K, Tan W. Uptake of silica-coated nanoparticles by HeLa cells. J Nanosci Nanotechnol, 2005, 5(10): 1688–1693
134 He X, Liu F, Wang K, Ge J, Qin D, Gong P, Tan W. Bioeffects of different functionalized silica nanoparticles on HaCaT cell line. *Chin Sci Bull*, 2006, 51(16): 1939–1946
135 He X, Nie H, Wang K, Tan W, Wu X, Zhang P. *In vivo* study of biodistribution and urinary excretion of surface-modified silica nanoparticles. *Anal Chem*, 2008, 80 (24): 9597–9603
136 Heemels MT. Apoptosis. *Nature*, 2000, 407:769
137 Fadok VA, de Cathelineau A, Daleke DL, Henson PM, Bratton DL. Loss of phospholipid asymmetry and surface exposure of phosphatidylserine is required for phagocytosis of apoptotic cells by macrophages and fibroblasts. *J Biol Chem*, 2001, 276: 1071–1077
138 Gac SL, Vermes I, Berg A. Quantum dots based probes conjugated to Annexin V for photostable apoptosis detection and imaging. *Nano Lett*, 2006, 6: 1863–1869