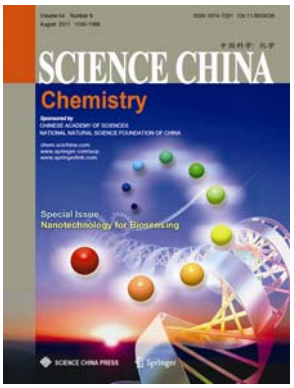
SCIENCE CHINA
Chemistry

Contents

Vol.54 No.8 August 2011



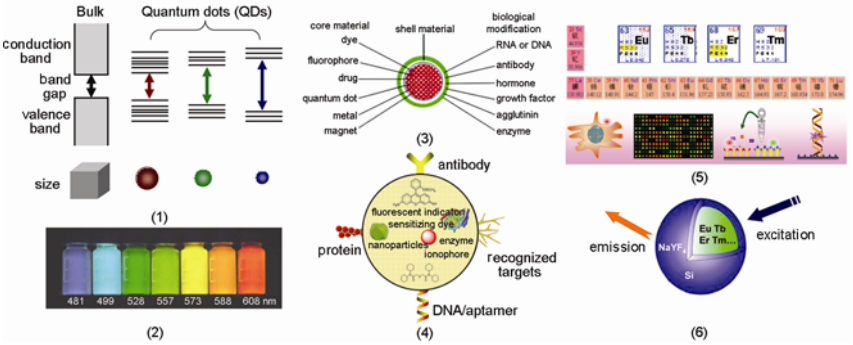
COVER Fluorescence-based sensing is currently the dominant analytical tool and is widely used in the field of chemical analysis, environmental monitoring and biological imaging. However, the traditional organic dyes show low absorption coefficients and weak signal, which leads to their reduced sensitivity and response of the targets. Fluorescent nanoparticles have attracted wide interest due to their unique optical properties. NPs-based detection platforms can provide advantages in terms of the signal sensitivity, stability, and capability for multiplexing. Their small size scale can provide an opportunity for high signal-to-noise ratio response and signal amplification. Meanwhile, their molecular size minimizes physical perturbations to such delicate systems as living cells. We focus on the developments and analytical applications of fluorescent NPs in the chemical and biological sensing of pH, ions, organic compounds, small biological molecules, nucleic acids, proteins, virus and bacteria. Fluorescent imaging at the cell and body levels, and the advantages of NPs brought to the field of sensing are also discussed. (see the review by LIU JianBo, WANG KeMin, *et al*. on page 1157–1176)

Special Issue: Nanotechnology for Biosensing

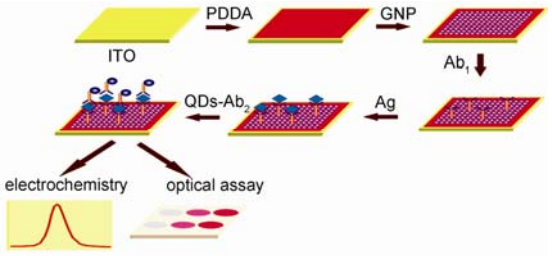
Preface

REVIEWS

Fluorescent nanoparticles for chemical and biological sensing



Optical applications of quantum dots in biological system



Advances of multiplex and high throughput biomolecular detection technologies based on encoding microparticles

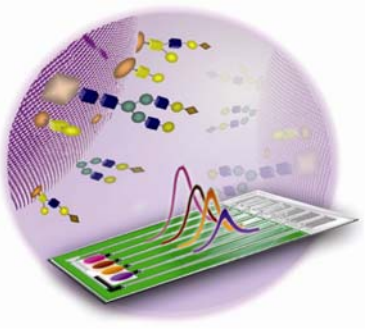
ZHAO XiangWei, ZHAO YuanJin & GU ZhongZe

Sci China Chem, 2011, 54(8): 1185–1201

Sensitive biosensing strategy based on functional nanomaterials

JU HuangXian

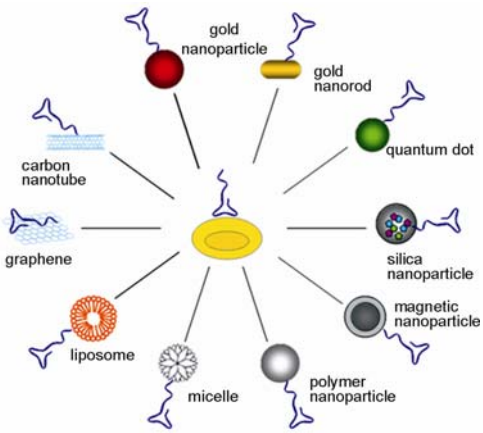
Sci China Chem, 2011, 54(8): 1202–1217



Cell-SELEX-based aptamer-conjugated nanomaterials for enhanced targeting of cancer cells

KONG RongMei, CHEN Zhuo, YE Mao, ZHANG XiaoBing & TAN WeiHong

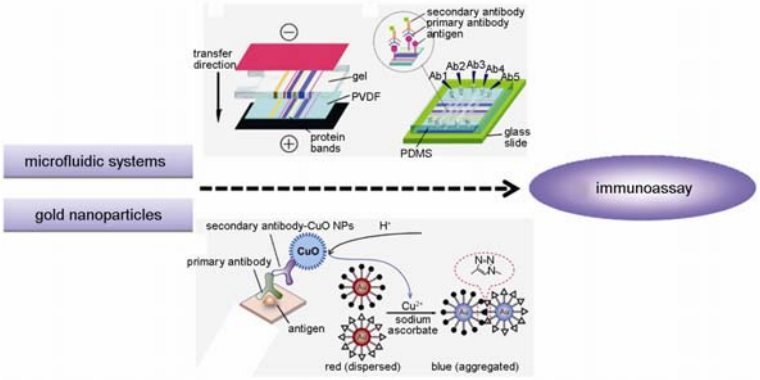
Sci China Chem, 2011, 54(8): 1218–1226

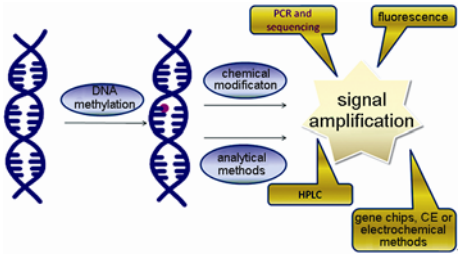


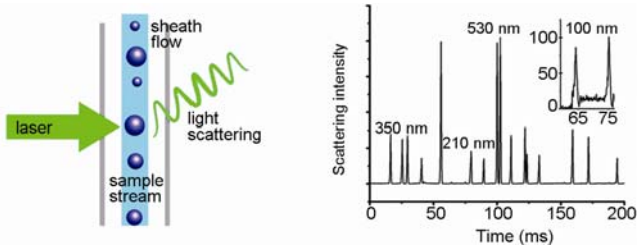
Recent progress in the application of microfluidic systems and gold nanoparticles in immunoassays

CHEN WenWen, LI TangSong, HE Sha, LIU DingBin, WANG Zhuo, ZHANG Wei & JIANG XingYu

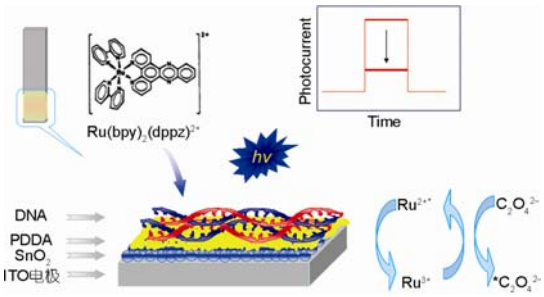
Sci China Chem, 2011, 54(8): 1227–1232

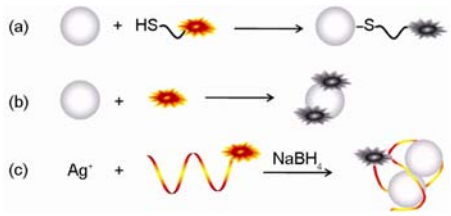


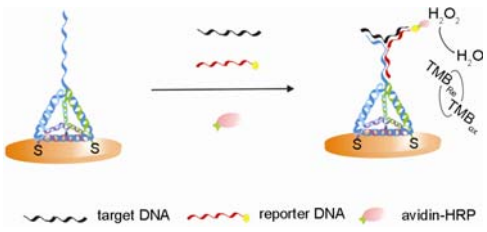




ARTICLES



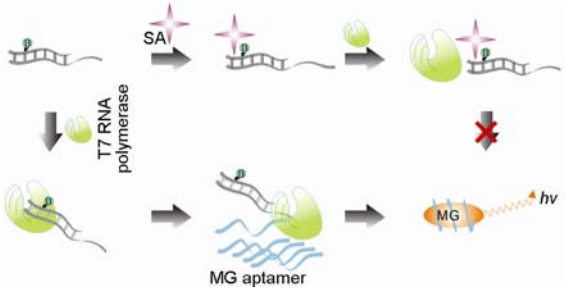




Homogeneous label-free fluorescent assay of small molecule-protein interactions using protein binding-inhibited transcription nanomachine

ZHOU DianMing, WU YiDan, LIU Pei, BAI HaoTian, TANG LiJuan, YU RuQin & JIANG JianHui

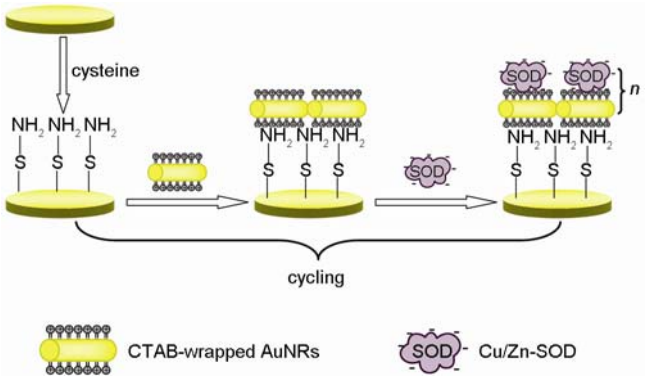
Sci China Chem, 2011, 54(8): 1277–1283



Assembly of layer-by-layer films of superoxide dismutase and gold nanorods: A third generation biosensor for superoxide anion

WANG MengDong, HAN YiTao, LIU XingXing, NIE Zhou, DENG ChunYan, GUO ManLi & YAO ShouZhuo

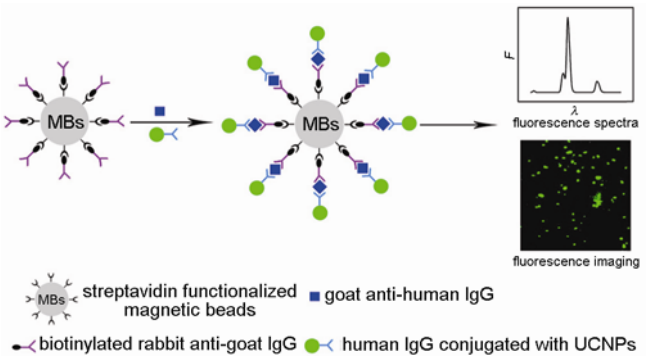
Sci China Chem, 2011, 54(8): 1284–1291



Surface modification of hydrophobic $NaYF_4$:Yb,Er upconversion nanophosphors and their applications for immunoassay

LIU ChengHui, WANG Zhe, WANG XiaoKe & LI ZhengPing

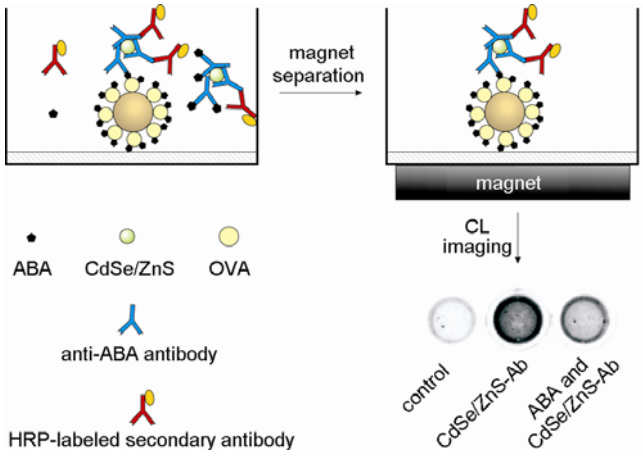
Sci China Chem, 2011, 54(8): 1292–1297



Immunomagnetic assay combined with CdSe/ZnS amplification of chemiluminescence for the detection of abscisic acid

ZHOU GuoHua, WANG Ping, YUAN Ju, QIU Ting & HE ZhiKe

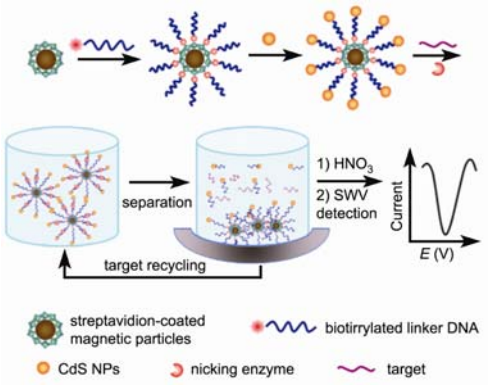
Sci China Chem, 2011, 54(8): 1298–1303



Magnetic particles and cadmium sulfide nanoparticles tagging for signal-amplifying detection of nucleic acids

DONG XiaoYa, ZHAO WeiWei, XU JingJuan & CHEN HongYuan

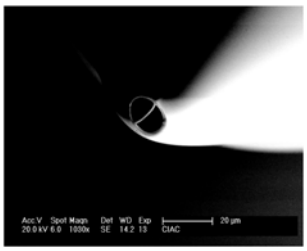
Sci China Chem, 2011, 54(8): 1304–1310



Simulation and experimental verification of the dependence of collection efficiency on the shape of a dual micropipette

GAO Zhao, LI Bo, ZHAO WenBo, CHEN Yong, HU Mo, LIANG ZhongWei, ZHOU ShuLin & SHAO YuanHua

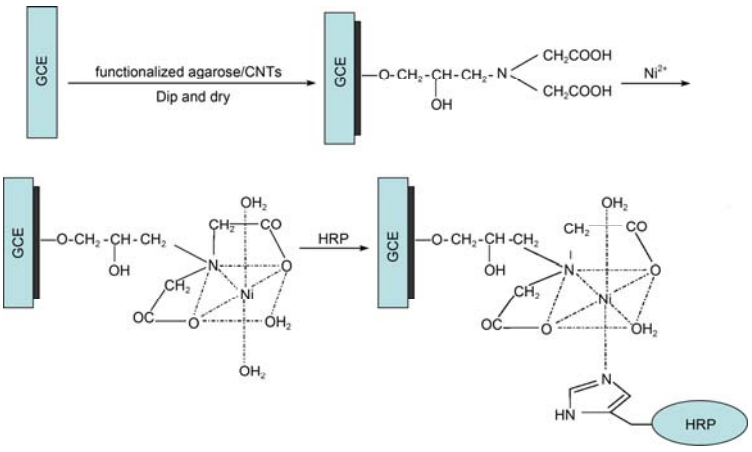
Sci China Chem, 2011, 54(8): 1311–1318



Metal chelate affinity to immobilize horseradish peroxidase on functionalized agarose/CNTs composites for the detection of catechol

TU XinMan, LUO ShengLian, LUO XuBiao, ZHAO YingJie, FENG Li & LI JingHong

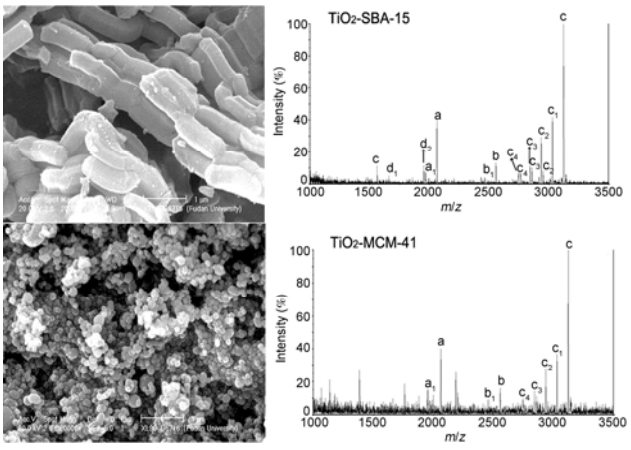
Sci China Chem, 2011, 54(8): 1319–1326

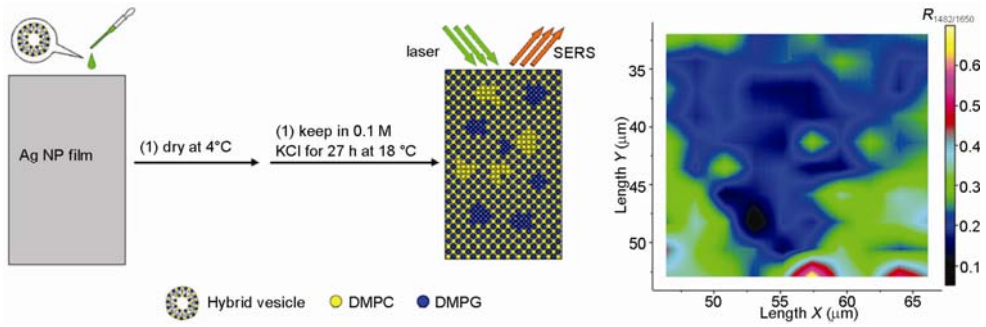


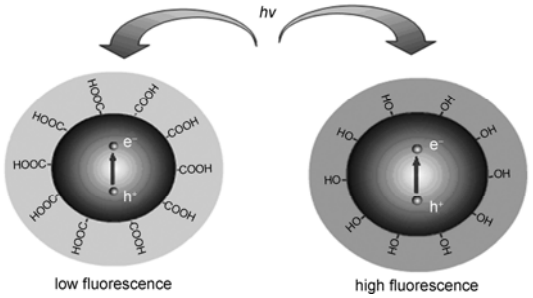
TiO_2-functionalized mesoporous materials for sensitive analysis of multi-phosphopeptides

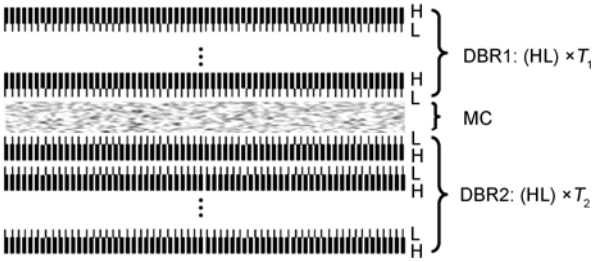
GUO WeiChao, WAN JingJing, QIAN Kun, YU ChengZhong, KONG JiLie, YANG PengYuan & LIU BaoHong

Sci China Chem, 2011, 54(8): 1327–1333









NEWS & COMMENTS

SCIENCE CHINA
Chemistry

• REVIEWS •

August 2011 Vol.54 No.8: 1202–1217
doi: 10.1007/s11426-011-4339-2

Sensitive biosensing strategy based on functional nanomaterials

JU HuangXian*

State Key Laboratory of Analytical Chemistry for Life Science; Department of Chemistry, Nanjing University, Nanjing 210093, China

Received May 3, 2011; accepted May 22, 2011

The first decade of the 21st century has been labeled as "the sensing decade". The functional nanomaterials offer excellent platforms for fabrication of sensitive biosensing devices, including optical and electronic biosensors. A lot of works have focused on the biofunctionalization of different nanomaterials, such as metal nanoparticles, semiconductor nanoparticles and carbon nanostructures, by physical adsorption, electrostatic binding, specific recognition or covalent coupling. These biofunctionalized nanomaterials can be used as catalysts, electronic conductors, optical emitters, carriers or tracers to obtain the amplified detection signal and the stabilized recognition probes or biosensing interface. The designed signal amplification strategies have greatly promoted the development of stable, specific, selective and sensitive biosensors in different fields. This review introduces some novel principles and detection strategies in the area of biosensing, based on functional nanomaterials. The general methods for biofunctionalization of nanomaterials with biomolecules and their biosensing application in immunoassay of protein, DNA detection, carbohydrate analysis and cytosensing are also described.

nanobiosensing, biosensors, nanomaterials, biofunctionalization, signal amplification, bioanalysis, biological recognition

1 Introduction

The first decade of the 21st century has been labeled as "the sensing decade". Among sensing, biosensing, based on nanomaterials, is one of the hottest topics in bioanalysis and nanoscience. The unique properties of nanomaterials offer excellent platforms as electronic and optical signal transduction to design a new generation of biosensing devices and produce the novel concepts for sensitive biosensing in clinical, environmental, and industrial applications [1]. A wide variety of nanoscale materials with different sizes (1–100 nm), shapes, and compositions have been introduced into biosensing area. The small sizes of nanoparticles break through the limitation of structure miniaturization, leading to lower detection limits, even reaching zeptomolar concentrations. Furthermore, the biofunctional nanoparticles can produce a synergic effect among catalytic activity, conductivity, and biocompatibility to accelerate the signal transduction, leading to the quick development of stable, specific, selective and sensitive biosensors in different fields.

The biofunctionalization of nanomaterials is the most important procedure in the preparation of nanomaterial-based biosensors. Over the past decade, different nanomaterials such as metal nanoparticles, semiconductor nanoparticles and carbon nanostructures have been functionalized with biomolecules by physical adsorption, electrostatic binding, specific recognition or covalent coupling. These biofunctionalized nanomaterials can be used as catalysts, electronic conductors, optical emitters, carriers or tracers to obtain the amplified detection signal and the stabilized recognition probes or biosensing interface. As catalysts they can induce the tracer or target to produce the detectable signal relative to the target content. As electronic conductors they can accelerate the electron transfer between the tracer or target and electrode to enhance the amperometic response or produce reagentless biosensing. As optical emitters they can be labeled onto recognition probes to produce detectable optical or electrogenerated chemilumi-

*Corresponding author (email: hxju@nju.edu.cn)

nescent signal for the detection of the targets. As carriers they can load more tracer molecules to amplify the recognition signal or provide biocompatible environments to stabilize the immobilized biomolecules. As tracers they lead to novel biosensing principles and detection strategies and some interestingly bioanalytical application, including biomimetic and single-molecular detection.

In this review, the general methods for biofunctionalization of nanomaterials with biomolecules and their biosensing application in immunoassay of protein, DNA detection, carbohydrate analysis and cytosensing are summarized. The novel principles and detection strategies in the area of biosensing based on functional nanomaterials are also introduced. More consideration will focus on the signal amplification using the biofunctionalized nanomaterials.

2 Biofunctionalization of nanomaterials for biosensing

Although the unique properties of nanomaterials have attracted considerable attention in designing new biosensing devices, the drawbacks of nanoparticles in biocompatibility and biological recognition ability limit their application in bioanalysis. Biofunctionalization of nanomaterials can endow them with good biocompatibility for immobilization of biomolecules, tissue or cells and high specificity for biological recognition [2], which leads to stable biosensing systems with good selectivity and reproducibility. Generally the biofunctionalization of nanomaterials can be performed with two approaches: noncovalent interaction including physical adsorption and entrapment of biomolecules around the nanomaterials, and covalent linkage of biomolecules to the functional groups on nanomaterial surface [3, 4]. The noncovalent approach can avoid destruction of conjugated skeleton and loss of electronic properties of the nanomaterials, while covalent binding can lead to better stability and reproducibility.

2.1 Biofunctionalization by noncovalent assembly

The simple adsorption of biomolecules on nanomaterials is a convenient method for biofunctionalization of nanomaterials. For example, gold nanoparticles (AuNPs) produced by citrate reduction can be functionalized with carcinoembryonic antibody molecules at pH values that lie slightly above the isoelectric point of the citrate ligand [5]. Other example of protein coating through electrostatic interactions includes the direct adsorption of oxidases at thioglycolic acid (TGA)-capped CdSe quantum dots (QDs) particles [6]. Single strand DNA (ssDNA) can wrap around single-walled carbon nanotubes (SWNTs) through aromatic interaction to form a soluble DNA-SWNTs complex, which has been used for construction of a highly sensitive sensor for target protein [7]. For the carbon-based nanomaterials, aromatic molecules, such as pyrene, porphyrin, and their derivatives, can interact with the sidewalls of carbon nanomaterials by means of π-π stacking interaction. The direct π-π interaction between porphyrins and SWNTs plays an important role to achieve ordered assembly of protonated porphyrin on SWNTs surface [8].

Another method for noncovalently immobilizing biomolecules on nanomaterials is to entrap them in biocompatible polymer such as poly(ethylene glycol), Nafion, chitosan and copolymer. The coating polymers not only prevent the aggregation of nanomaterials but also provide the abundant positions for functionalization with second biomolecules. For example, polytyrosine has been used as an electroactive label for the detection of prostate-specific antigen (PSA) with the limit of detection of about 1 nM [9]. Layer-by-layer assembly can be used for the preparation of functional nanomaterial films with a high density of enzyme molecules. For example, a uniform and stable multilayer membrane of multi-walled carbon nanotubes (MWNTs) and glucose oxidase (GO*x*) can be prepared through this technique after modification of MWNTs with polyelectrolytes poly(dimethyldiallylammo- nium chloride) (PDDA) (Figure 1) [10]. A simple electrostatic method for polyelectrolytes coating submicrometer-size latex spheres has been assembled with AuNPs for the ultrasensitive detection of DNA [11].

2.2 Covalent biofunctionalization of nanomaterials

Controlled chemisorption via covalent binding is preferable to unspecific physisorption in terms of stability and reproducibility of the surface functionalization. The functional groups on the nanomaterial surfaces can be directly bonded to reactive ligands by a linkage reaction facilitated with the aid of catalysts. For example, nanomaterials decorated with carboxylic acid groups can be covalently bound to biomolecules bearing primary amines through 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide/N-hydroxysuccinimide linkers [12]. This approach has been used in the attachment of DNA, aptamers and antibody-antigen to nanomaterials. Similarly, amino-decorated nanomaterials can conjugate with biomolecules bearing carboxylated groups for the attachment of peptides, proteins, antibodies, and enzymes to nanomaterials.

Biomolecules, especially for protein, in direct contact

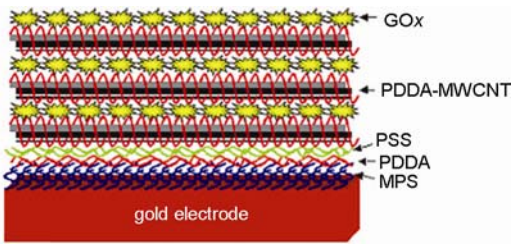


Figure 1 Schematic structure of PDDA-MWNTs/GO*x* films on gold electrodes.

with an unprotected solid substrate is subject to more or less severe denaturing and the ensuing loss of their specific biochemical functionality. Low-molecular bifunctional linkers, which have anchor groups for their attachment to nanomaterial surfaces and functional groups for their further covalent coupling to the target biomolecules, have been extensively used in the generation of covalent-tethered conjugates of biomolecules with various nanomaterials. Anchor groups such as thiols, disulfides, or phosphine ligands are often used for the binding of the bifunctional linkers to Au, Ag, CdS, and CdSe nanoparticles (NPs). The primary binding of thiolated molecules, such as oligopeptides, to AuNPs can provide a means for the covalent tethering of biomolecules to nanoparticles [13]. Elsewhere, CdTe NPs capped with dimercaptosuccinic acid through the strong binding of its thiol groups to Cd^{2+} ionic sites on the surface of the nanoparticles can be functionalized with biomolecules by direct chemical reaction [14]. When no thiolated residue is available in the native proteins, thiol groups can be produced with 2-iminothiolane by chemical means [2]. Metal oxide nanoparticles can be modified with silanes, carboxylate and phosphonates to produce functional groups including amino, cyano, carboxylic acid, epoxy groups, etc. for subsequent functionalization [4].

2.3 Biofunctionalization by click chemistry

"Click" chemistry, a Cu-catalyzed azide-alkyne cycloaddition, is a relatively new approach of direct conjugation. The NP surfaces can be decorated with either alkyne or azide functionalities for conjugation to the complementarily functionalized bioactive molecules. Via one-step click reaction, the drug-loaded polymer nanoparticles can be functionalized with folate, biotin, and gold nanoparticles (Figure 2) [15]. A gradient of a glycine-arginine-glycine-aspartate-serine linear peptide has been fabricated on a versatile substrate by using click chemistry technology as a tool for screening surface-directed cell function [16].

3 Signal amplification for biosensing with functional nanomaterials

One of the major goals to develop novel biological assay methods for the detection of biomolecules and DNA hybridization is to achieve high sensitivity. By combining with molecular biological amplification such as polymerase chain reaction (PCR) and rolling circle amplification (RCA), many high sensitive methods have been developed for the detection of DNA and proteins. However, the sensitive monitoring of proteins is particularly challenging owing to the absence of PCR-like amplification protocols. The unique properties of nanomaterials and the problems existing in the molecular biological amplification techniques have led to the quick development of nanomaterial-based signal amplification strategy. A series of nanoparticle probes have been designed to amplify recognition events of targets and significantly lower the detection limit of biosensing techniques.

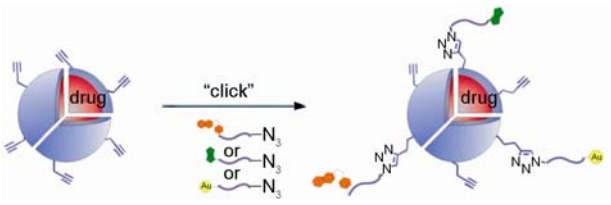


Figure 2 Schematic illustration of modification of alkyne-functionalized NPs with azide-functionalized biomolecules via click chemistry.

3.1 Nanoparticle-amplified optical assay

Among all detection methods used in biosensors, optical-based technique is the most popular one because of its high sensitivity and the ability to remotely interrogate the information on biosensor using light or laser. Metal nanoparticles, such as gold and silver nanoparticles, exhibit plasmon absorbance bands in the visible spectral region that are controlled by the size of the respective particles. The unique plasmon absorbance features of Au NPs and specifically the interparticle-coupled plasmon absorbance of conjugated particles have been widely used for DNA [17] and antibody-antigen [18] analyses. By using a carboxylated dextran film to eliminate nonspecific adsorption of ODN-capped Au NPs, a signal amplification method has been designed to show a remarkable detection limit of DNA as low as 2.1×10^{-20} mol, corresponding to 1.38 fM [19]. By combining the specific recognition with signal amplification of adenosine aptamer (Apt) coupled with Au NPs via G-quartetinduced nanoparticle assembly, a sensitive resonance light scattering (RLS) detection method for adenosine in human urine has been reported [20].

The use of gold development results in greater signal enhancement than the typical silver development, and multiple rounds of metal development have been found to increase the resulting signal compared to one development [21]. The gold or silver NPs can be catalytically deposited on a chip using electroless deposition techniques to increase the light scattering signal of the immobilized Au NP probes. The use of gold deposition may have significant utility in scanometric detection schemes and broader clinical and research applications.

Another approach to increase the signal intensity of dye-based assays is metal-enhanced fluorescence [22]. The fluorescent signal is the result of interaction between fluorophore and surface plasmon in metallic nanostructure, most typically in Ag and Au. Enhanced emission can be obtained as a result of an amplification of the incident electric field, which effectively increases the fluorophore's absorption cross-section, or by an acceleration of the radiative decay rate [23]. Using hybrid nanomaterials as the signal amplifi-

ers, a new way to improve the performance of fluorescence technologies for biological imaging through a fluorescence resonance energy transfer (FRET) approach has been designed [24]. From the materials viewpoint, the emission wavelength, charge nature, and diameter of polyhedral oligomeric silsesquioxane (POSS)-based fluorescent nanoparticles can be easily adjusted through chemical modification of fluorescent, which arms so as to fulfill the different requirements of specific applications.

Similarly, the functionalized nanoparticals have been used to enhance the chemiluminescence (CL) or electrogenerated chemiluminescence (ECL) intensity. By the DNA polymerase I (Klenow fragment)-induced coupling of the nucleotide-modified nanoparticle probe to the mutant sites of duplex DNA under the Watson-Crick base-pairing rule, an ultrasensitive chemiluminescence method based on the Au nanoparticles amplification for the quantitative detection of SNPs in genomic DNA has been accomplished [25]. A FI-CL detection platform of DNA hybridization based on bio-bar-code functionalized magnetic nanoparticle labels has also been developed [26].

The ECL of QDs can be greatly enhanced by carbon nanotubes (CNTs) [27]. Based on this phenomenon, a sensitive ECL immunosensor for the detection of human IgG down to 0.6 pg/mL has been proposed. The principle of ECL detection for target is based on the increment of steric hindrance after immunoreaction, which results in the decrease of ECL intensity.

3.2 Nanoparticle-amplified electrochemical detection

The formation of conductive domains provides an attractive route for electrochemical transduction of biorecognition events. For example, based on nanoparticle-induced changes in the conductivity across a microelectrode gap, a conductivity immunoassay method for proteins has been proposed [28]. Sandwich immunoassay leads to the binding of a secondary gold-labeled antibody, followed by the catalytic deposition of a silver layer to "bridging" two microelectrodes to produce conductive paths across interdigitated electrodes, which leads to a measurable conductivity signal and the ultrasensitive detection of human IgG down to the 2×10^{-13} M level.

Powerful nanoparticle-based electrochemical DNA hybridization assays have been developed using Au and Ag metal tracers [29]. Such protocols rely on capturing the gold or silver nanoparticles to the hybridized target and use anodic-stripping voltammetry for electrochemical measuring the metal tracers. For example, an electrochemical method is employed for the Au-nanoparticle-based quantitative detection of the 406-base human cytomegalovirus DNA sequence (HCMV DNA) [30]; and a novel ultrasensitive multiplexed immunoassay method has been developed by combining alkaline phosphatase-labeled antibody functionalized gold nanoparticles and enzyme-Au NP catalyzed deposition of silver nanoparticles at disposable immunosensor array (Figure 3) [31]. The deposited silver measured by anodic stripping analysis in KCl solution shows the amplified signal for multiplexed immunoassay in wide linear ranges over four orders of magnitude with the detection limits down to 4.8 pg/mL.

Recent activities have demonstrated that inorganic nanocrystals offer an electrodiverse population of electrical tags for multiplexed bioanalysis. For example, Wang's group used encoding nanoparticles (cadmium sulfide, zinc sulfide, copper sulfide, and lead sulfide) to multiplexed detection of DNA targets [32], SNPs [33], and antigens [34]. The multi-target electrical detection capability was coupled to the

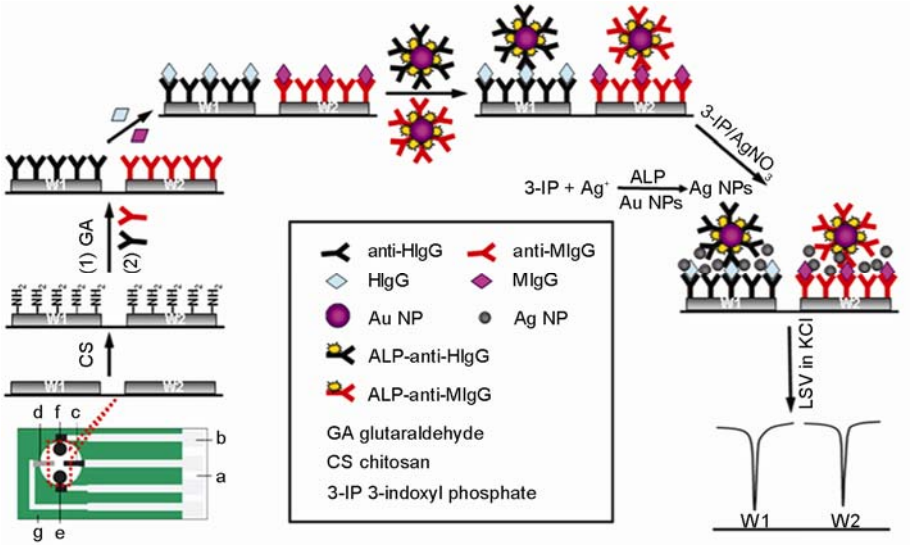


Figure 3 Schematic representation of the preparation of immunosensor array and the detection strategy by sandwich-type immunoassay and linear sweep voltammetric stripping analysis of enzymatically deposited Ag NPs. (a) Nylon sheet, (b) silver ink, (c) graphite auxiliary electrode, (d) Ag/AgCl reference electrode, (e) W1, (f) W2, and (g) insulating dielectric.

amplification feature of electrochemical stripping transduction (to yield fmol detection limits) and an efficient magnetic separation (to minimize nonspecific adsorption effects). Each biorecognition event yielded a distinct voltammetric peak, whose position and size reflected the identity and level of the corresponding target. Recently, Wang's group designed a QD/aptamer-based ultrasensitive electrochemical biosensor to detect multiple protein targets (Figure 4) [35]. Such electronic transduction obtained a detection limit of 20 ng/L (0.5 pM). With the use of RCA technique, a cascade signal amplification strategy was proposed for the detection of protein target at ultralow concentration [36]. As shown in Figure 5, the RCA product containing tandem-repeat sequences could serve as excellent template for periodic assembly of QDs, which presented per protein recognition event to numerous quantum dot tags for electrochemical readout. Both the RCA and the multiplex binding system showed remarkable amplification efficiency. The designed strategy could quantitatively detect protein down to 16 molecules in a 100 μL sample with a linear calibration range from 1 aM to 1 pM. The proposed cascade signal amplification strategy seems to be a powerful tool for proteomics research and clinical diagnostics.

Metal nanoparticles can amplify the electrochemical impedance and capacitance signals for the detection of biomole- cules. This allows construction of highly sensitive

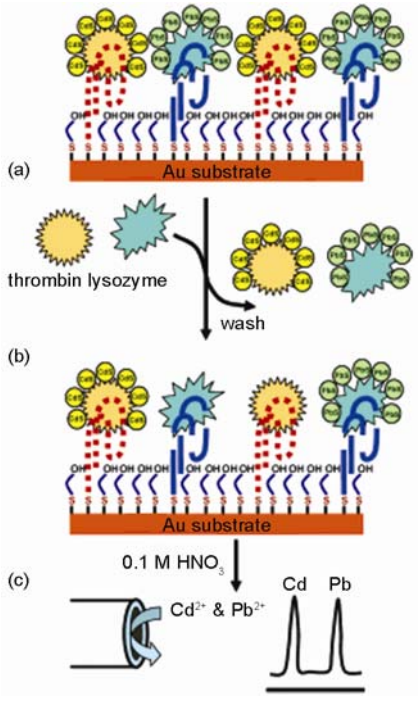


Figure 4 Operation of the aptamer/quantum-dot-based dual-analyte biosensor, involving displacement of the tagged proteins by the target analytes: (a) mixed monolayer of thiolated aptamers on the gold substrate with the bound protein-QD conjugates; (b) sample addition and displacement of the tagged proteins; (c) dissolution of the remaining captured nanocrystals followed by their electrochemical-stripping detection at a coated glassy carbon electrode.

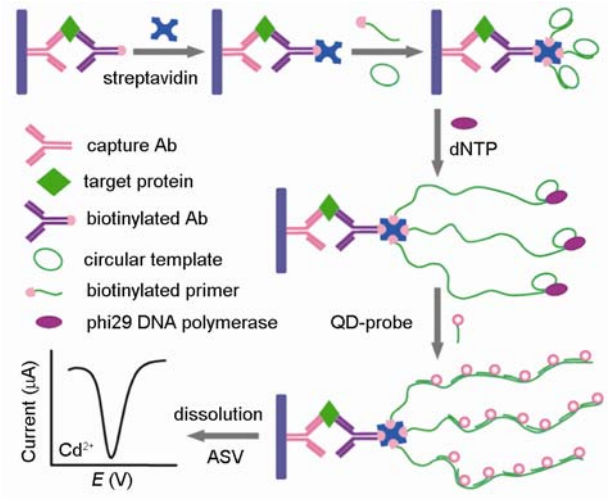


Figure 5 Schematic representation of the cascade signal amplification strategy for protein detection.

electrochemical impedance biosensors [37]. Au NPs-based amplification of voltammetric signal has also been suggested for analyzing sequence-specific DNA using ferrocene-capped gold nanoparticles/streptavidin conjugates [38]. After hybridization with target DNA, the functional gold nanoparticles are introduced to enhance the electrochemical signal of ferrocene.

Another important nanoparticle-amplified electrochemical detection is based on the catalytic behavior of nanoparticles. For example, MWNT-cobalt porphyrin compositions display excellent catalytic performance for oxygen reduction in acidic media at room temperature [39]. With low catalyst loading, the oxygen reduction rates are more than 1 order of magnitude, higher than previously reported values for free cobalt porphyrin catalysts. A sandwich nanohybrid of single-walled carbon nanohorn-TiO_2-porphyrin has been prepared to show an excellent electrocatalytic activity toward the reduction of chloramphenicol in neutral media,

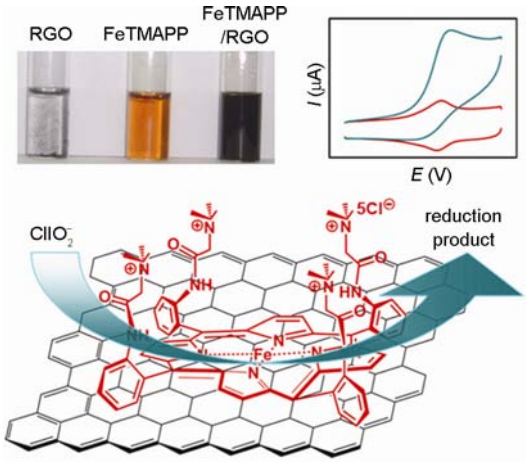


Figure 6 Schematic representation of the noncovalent assembly and electrocatalysis of FeTMAPP on RGO.

which leads to a rapid response to chloramphenicol concentration in a linear range from 0.9 nM to 135.7 mM [40]. Reduced graphene oxide functionalized with picket-fence porphyrin shows good dispersion and highly efficient electrocatalytic activity for reduction of chlorite (Figure 6) [41]. The resulting electrochemical biosensor displays a linear increase with increasing chlorite concentration from 5.0×10^{-8} to 1.2×10^{-4} mol/L.

3.3 Nanoparticles as carriers for signal amplification

Various nanomaterials including Au NPs, carbon nanotubes, bionanospheres and magnetic beads have been used as excellent carriers for preparation of labels by loading numerous signal tags such as enzymes, quantum dots, oligonucleotide and dyes on these carriers. The prepared labels can greatly amplify the transduction signal of recognition event in bioassay. Au NPs are extensively used as carriers of the signaling molecules for the amplification detection of DNA [42] and protein target [42]. Using a nanoporous gold electrode and HRP labeled secondary antibody-Au NPs bioconjugates, a highly sensitive protein detection method has been described (Figure 7) [44]. The sensitivity of immunoassay for target protein is about 100 times higher than ELISA. Using Au NPs-based signal amplification, an electrochemiluminescence aptasensor [45] and an electrochemical sensor [46] have been proposed for sensitive and cost effective detections of target thrombin and Hg^{2+} ions, respectively.

CNTs are a good carrier for biomolecules and signal molecules [47]. Using CNT-based labels, Lee and coworkers developed an amplified nucleic acid detection [48]. The CNT labels significantly enhanced the nucleic acid assay sensitivity by at least 1000 times compared to that of conventional labels used in enzyme-linked oligosorbent assay. Recently, a novel biosensing strategy for selective electrochemical detection of DNA down to attomolar level with a linear range of 5 orders of magnitude has been developed by the specific recognitions of target DNA and streptavidin to biotin labeled molecular beacon and signal amplification of streptavidin-horseradish peroxidase functionalized carbon nanotubes (Figure 7) [49]. This biosensor can differentiate single-base mismatched and three-base mismatched sequences of DNA, which makes it promising for genetic target analysis in biomedical and bioanalytical application.

Due to the small size, high surface-to-volume ratio and good biocompatibility, silica nanoparticles have become another normally used carrier for signal-amplified bioanalysis. For example, a fluoroimmunoassay for antigen detection has been proposed by immobilizing the prime antibody on the silica NP surface to capture antigen and Cy3 labeled secondary antibody in a sandwich assay format [50]. The assay allowed a detection limit of 50 ng/mL IgG by a naked eye detection.

A nanoparticle label capable of amplifying the electrochemical signal of DNA hybridization has been fabricated

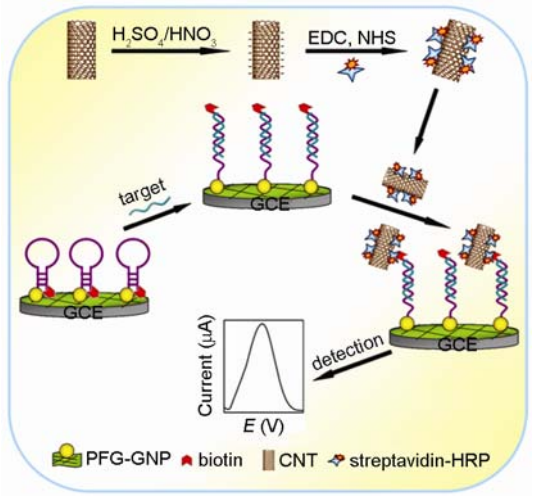


Figure 7 Schematic representation of functionalization of CNTs with streptavidin-HRP and detection of DNA hybridization with MB as switch.

by functionalizing poly(styrene-co-acrylic acid) microbeads with CdTe quantum dots [51]. As shown in Figure 8, the CdTe-tagged polybeads are attached to DNA probes by streptavidin-biotin binding to construct a DNA biosensor. The detection of the DNA hybridization process is achieved by the square-wave voltammetric detection of Cd^{2+} after the dissolution of the CdTe tags with HNO_3. The efficient carrier-bead amplification platform, coupled with the highly sensitive stripping voltammetric measurement, gives rise to a detection limit of 0.52 fmol/L and a dynamic range spanning 5 orders of magnitude.

4 Sensitive biosensing applications of functional nanomaterials

Due to the signal amplification behaviors of functional nanomaterials, they have been extensively used for the design of sensitive biosensing methods. Particularly, some biofunctional nanoparticles can produce a synergic effect among catalytic activity, conductivity, and biocompatibility to accelerate the signal transduction, leading to the quick development of stable, specific, selective and sensitive biosensors for immunoassay of protein, DNA detection, carbohydrate analysis and cytosensing.

4.1 Nanomaterials for immunosensing of proteins

As promising approaches for selective and sensitive analysis, immunoassays have gained increasing attention in environmental monitoring, clinical diagnosis, food safety, pharmaceutical analysis and bacterial identification. Rapid development of nanotechnology offers unique opportunities for designing ultrasensitive immunosensing methods. The remarkable sensitivity of the novel nanoparticles-based immunosensing protocols opens up a new possibility for

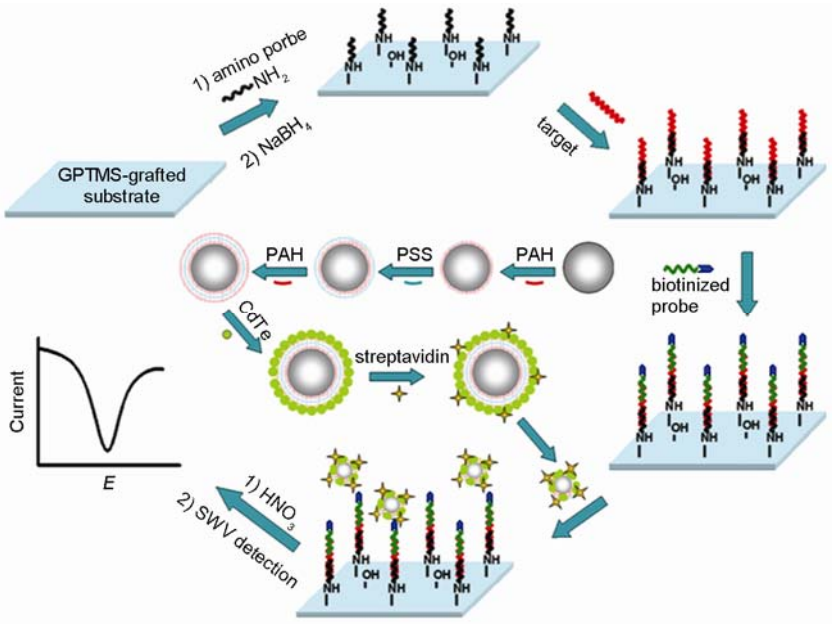


Figure 8 Schematic diagram of the assembly process for preparation of streptavidin/CdTe-tagged polybeads and highly sensitive DNA detection.

analyzing very low levels of disease markers, biothreat agents, or environmental pollutions that cannot be detected by other conventional methods. Such highly sensitive biodetection schemes provide useful techniques for early diagnosis of disease or warning of terrorist attack. The sensitivity of immunoassays depends on several factors including the affinity of antibodies, amount of immobilized immunological recognition elements, choice of transducer and signal probe. Therefore, the improvement of immunoassay performance mainly relies on the development of antibody preparation techniques, the improvement of immobilization and tagging methods, and the adoption of high performance transduction manner. This review mainly discusses the immobilization of immunoreagents on various biocompatible nanomaterials (e.g., noble metals, magnetic oxides and carbon nanoparticles) and the use of nanoparticles as signal tags to provide a great probability for ultrasensitive immunoassays.

In immunosensors and heterogeneous immunoassays, immunological recognition elements (either antigens or antibodies) are usually presented as immobilized forms on solid substrates. The surface characteristics of solid substrates are crucial factor for obtaining good assay performance. The continuous progress of nanotechnology in material science has led to the development of nano-size materials fit for acting as biomolecule immobilization substrate. AuNPs are a kind of nanomaterial with surprising electrostatic adsorption ability for proteins, and have been extensively used as an immobilized matrix for retaining the bioactivity of antigens and antibodies, and promoting the direct electron transfer of the immobilized proteins. These characters make AuNPs to be widely used for fabrication of various types of electrochemical immunosensors especially amperometric immunosensors. For example, AuNPs doped chitosan composite has been used to immobilize immunological recognition elements on screen-printed carbon electrode for improving the reversibility of the electrochemical reaction of substrate and increasing the detection sensitivity [52]. In the presence of AuNPs the labeled horseradish peroxidase can show direct electrochemical signal for the design of reagentless immunosensors. With this advantage several disposable reagentless immunosensor arrays for simple immunoassay of panels of tumor markers by individually embedding different kinds of HRP-labeled antibody-modified AuNPs in a designed biopolymer/sol-gel matrix formed on SPCEs have been prepared (Figure 9) [53]. Such kind of immunosensor arrays provide a simple multi-analyte immunoassay with no need for substrate and no between-electrode cross-talk, offering the capability of point-of-care testing. Ag NPs have also been reported as immobilization substrates to fabricate various electrochemical immunosensors. A label-free capacitive immunosensor has been developed for the detection of microcystin-LR

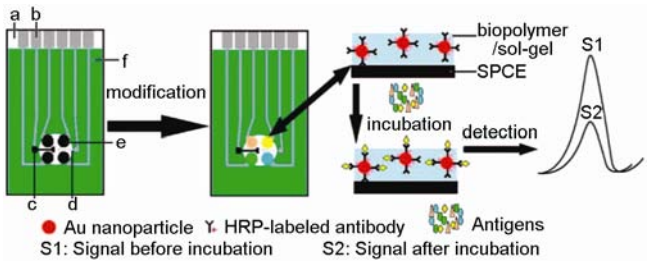


Figure 9 Schematic diagrams of immunosensors array and multianalyte electrochemical immunoassay system. (a) Nylon sheet; (b) silver ink; (c) graphite auxiliary electrode; (d) Ag/AgCl reference electrode; (e) graphite working electrode; (f) insulating dielectric.

using a gold electrode modified with self-assembled thiourea monolayer incorporated with Ag NPs. Comparing to the modified electrode without Ag NPs, its signal could be obtained up to 43 times [54].

Besides noble metal nanoparticles, some carbon nanomaterials including nanotubes, nanofibers and nanohorns and silica nanoparticles (Si NPs) show good immobilization abilities. Wohlstadter's group [55] used CNTs as an immobilization phase to fabricate an electrochemiluminescence (ECL)-based sensing device for the immunoassay of α-fetoprotein. Ju's group [56] used soluble carbon nanofiber to construct an immunosensor for rapid separation-free immunoassay for carcinoma antigen 125. Latterly, this group used single-walled carbon nanohorns as an immobilization scaffold to develop a novel immunosensor for microcystin-LR (MC-LR) [12]. The unique properties of CNTs have made them be used as both the carriers of conventional electrochemical labels [5, 47] and the labels to facilitate electron transfer between the electrochemical probe and the electrodes for immunoassay [57]. Jiang *et al*. [57] used phospholipids coated CNTs as the electrochemical labels to develop an electrochemical immunoassay strategy, in which the CNT-based labels were attached on an insulating self-assembled monolayer (SAM) modified electrode to mediate the electrochemistry of redox indicator, creating a very sensitive and specific tactic for signal transduction. This group [58] further combined the signal transduction process with SWNT wrapping ssDNA terminally tethered to small molecule for protein recognition, which prevented digestion of ssDNA by exonuclease I and precluded adsorption of DNA-wrapped SWNTs on SAM modified electrode, thus leading to sensitive electrochemical detection of protein recognition.

Si NPs have no obvious electrochemical activity as noble metal and carbon nanoparticles. However, they still can be considered for immobilization of antigens/antibodies to fabricate immunosensors due to their high surface-to-volume ratio and good biocompatibility [59]. Recently, Ju's group [60] synthesized a biofunctionalized three-dimensional ordered nanoporous silica film to construct a CL immunosensing device. As shown in Figure 10, the three-dimensional ordered nanopores have high capacity for loading of streptavidin and antibodies and for improving the mass transport of immunoreagents for immunoreaction, thus the resulting CL immunosensor shows high sensitivity and wide dynamic range for fast immunoassay, and good reproducibility and stability.

For immuno-recognition events, the tags with high sensitivity are obviously advantageous to improve the signal intensity and lower the detection limits of immunosensing. Compared with the conventional signal tags such as enzyme and organic fluorescent dye, nanomaterial tags are showing greater promise for developing ultrasensitive immunoassay methods by improving the signal transduction and increasing the signal molecules contained in a single nano-size tag.

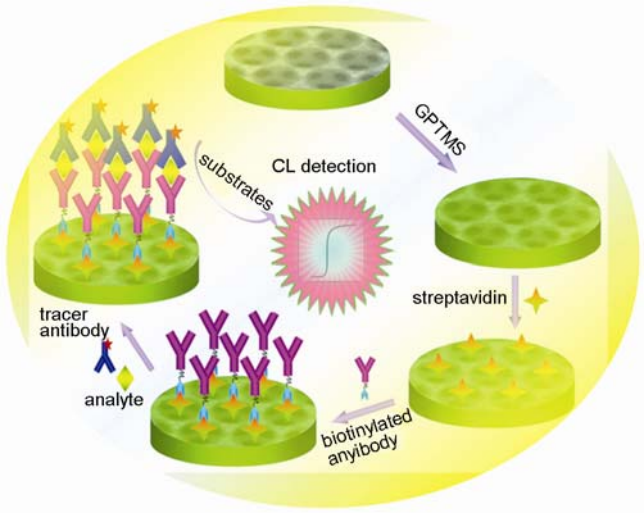


Figure 10 Schematic diagram for biofunctionalization of 3D nanoporous SiO_2 film and one-step sandwich immunoassay procedure for CA 125.

The single nano-size tag containing large number of signal molecules produces much higher signal than the single tag containing only one or several signal molecules (traditional tag). Usually nano-size tags for immunoassay include metal-based tags and nanocrystals. AuNPs as the representative of metal-based tags show unique optical and electronic properties when used as signal probe. They have been used for preparation of immunochromatographic strips by absorption measurement and development of colorimetric immunoassays. By dissolving the AuNPs tag to form $AuCl_4^-$, which can react with luminol to generate strong CL emission, some highly sensitive CL immunoassay methods have been developed [61]. These methods can be further improved since the stripping procedure results in high background. AuNPs have been utilized as signal probes for electrochemical immunoassays. For example, Ju's group [62] proposed a simple, sensitive and low-cost inherently crosstalk-free multiplexed immunoassay by combining a disposable chip with AuNP as an electrochemical label. The anaytes could be detected by electrooxidization of AuNPs in 0.1 M HCl. A highly sensitive piezoelectric immunosensor has been proposed using AuNPs tag and applied to detect aflatoxin B_1 in contaminated milk [63], in which the indirect competitive immunoassay strategy is applied for the detection of the target using AuNPs as a "weight tag" to the secondary antibodies for amplifying the response. A highly sensitive immunoassay is proposed based on time-resolved inductively coupled plasma mass spectrometry with AuNPs (15 nm) as tags to antibodies [64]. The similar mass spectrometric immunoassay strategy has also been proposed to detect human IgG in immunomicroarray mode with a detection limit of 0.012 ng/mL [65].

Nanocrystals (QDs) exhibit some important differences as compared to traditional fluorophores such as organic fluorescent dyes and naturally fluorescent proteins. Vinayaka

et al. [66] developed a reliable and rapid fluoroimmunoassay method for analysis of 2,4-dichlorophenoxyacetic acid by using CdTe QDs to tag 2,4-dichlorophenoxyacetic acid. The intrinsic redox properties and the sensitive electrochemical stripping analysis of the metal components of QDs make the tags in the electrochemical biosensors to be very sensitive. This concept has been extended to immunoassay [67]. Recently, by combining RCA technique with QDs and multiplex binding of biotin-strepavidin system, Ju's group [36] proposed an ultrasensitive immunosensor for the detection of protein target at ultra-low concentration. QDs have also been used as signal tags for potentiometric immunoassay. Thurer *et al*. [68] reported a potentiometric immunoassay of IgG in a microtiter plate format using CdSe as tags. After sandwich immunoreaction on the microtiter plate, the captured QDs were dissolved to release Cd^{2+} ions that were sensitively detected by Cd^{2+} selective micropipette electrodes. Using the enhanced ECL of CdSe quantum dots composited with CNTs, Zhu's group [27] proposed a sensitive immunoassay method based on the decrease of ECL intensity upon the formation of immunocomplex due to the increasing impedance. By coupling with an enzymatic amplification with the ECL of QDs at a relatively low emission potential with a self-produced coreactant, H_2O_2 (Figure 11), Ju's group [69] proposed a novel ECL immunosensor for HIgG with wide calibration range of 0.05 ng/mL –5 μg/mL. This facile immunosensing strategy opened a new avenue for detection of proteins and application of QDs in ECL biosensing.

4.2 Nanomaterials for sensitive DNA detection

DNA analysis has a particular interest in genetics, molecular diagnosis, pathology, food safety, biothreat detection, criminology, environment protection, and so on. The main principle of DNA biosensors is immobilizing one single-stranded DNA (or PNA) on transducer surface to precisely recognize its partner of complementary base sequence, probe DNA, consequently, the recognition event is detected with a useful readable signal. Over past two decades, due to the huge surface area and excellent optical/electrochemical/magnetic properties of nanomaterials, many important nanomaterials and technologies have been used to design novel DNA sensing methods and devices, which have led to enormous improvements in sensitivity, selectivity, multiplexing capacity and simplicity. For example, using CNTs as support to increase the surface loading of DNA probe a method for label-free and sequence specific DNA detection with a low detection limit has been reported [70]. A CNT-based amplified bioelectronic protocol by enzymatic amplification and chronopotentiometric stripping detection has also been prepared at a CNT-modified electrode [71]. Recent studies have demonstrated that metal nanoparticles such as silver and Au NPs can be readily assembled on DNA. The proposed methods dramatically increase DNA attachment quantity and complementary ssDNA detection sensitivity for its large surface area and good charge transport characteristics [72]. An electrochemical DNA biosensor based on ZnO nanoparticles and MWNTs for DNA immobilization and enhanced hybridization detection has been proposed by DPV measurement of methylene blue as an indicator [73]. The sensor could effectively discriminate different DNA sequences related to PAT gene in the transgenic corn, with a detection limit of 2.8×10^{-12} mol/L of target sequence.

QDs are another most frequently used semiconductor nanoparticles for biological detection of DNA. For example, based on the fluorescence resonance energy transfer (FRET) between QDs and graphene oxide, Ju's group [74] designed an effective sensing platform for DNA target (Figure 12). The strong interaction between molecular beacon and graphene oxide led to the fluorescent quenching of QDs. Upon the recognition of the target, the distance between the QDs and graphene oxide increased, and the interaction between target-bound molecular beacon and graphene oxide became

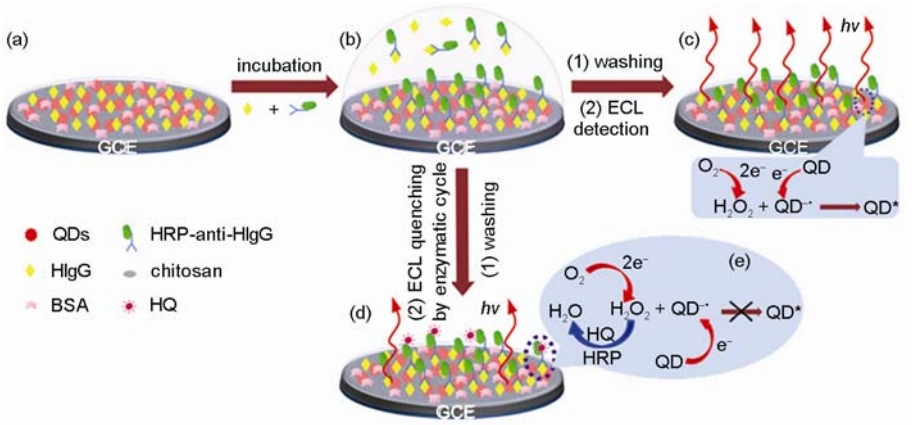


Figure 11 Construction (a) and incubation (b) of the immunosensor, and ECL detection without (c) and with (d, e) the enzymatic amplification by consumption of H_2O_2 as coreactant.

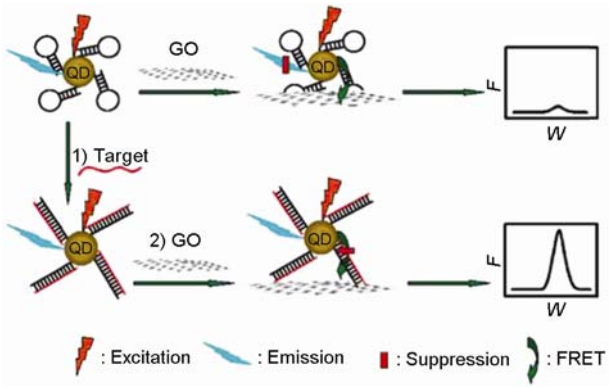


Figure 12 Schematic representation of graphene oxide-induced fluorescence quenching of molecular beacon-QDs and biosensing mechanism.

weaker, which significantly hindered the FRET, leading to the increase of the fluorescence of QDs. The change in fluorescent intensity could be used for the detection of the target with a detection limit down to 12 nM. QDs are also widely used as tags in ultrasensitive electrochemical DNA assays due to their intrinsic redox properties and the sensitive electrochemical stripping analysis of the metal components of QDs [51]. The efficient carrier-bead amplification platform can provide a hybridization detection of DNA down to 0.52 fmol/L. The ECL emission of QDs will be another way to sensitively detect target DNA concentration and sequence by using QDs as the tags.

Advances in biochip/microarray areas are giving scientists and researchers new methods for sequence detection of DNA by immobilizing DNA probe or oligonucleotide on a solid support. Although much effort is contributed to the development of label-free detection methods for monitoring hybridization processes, the use of labels still dominates microarray technology. Fluorescent QDs have emerged as a promising new type of label due to their narrow emission wavelength and controllable size and chemical composition [75]. Metal colloids, especially gold [76] and silver-enhanced gold nanoparticles [77] have also been proposed as the tags for the optical and electrical detection of DNA biochip/microarray. The advantage of fully electrical chips is the intrinsic high spatial resolution and direct signal coupling of the biosensing element and the transducer. Chen's team [76] used self-assembly gold nanoparticle multilayer to conduct and amplify electrical signals and developed an array-based CMOS biochip to replace the conventional DNA microarray for DNA identification by electrical detection. They demonstrated an electrical detection method on a DNA biochip that employed an approach for ultra sensitive detection of DNA using self-assembled gold nanoparticles and bio-bar-code-based amplification (BCA) DNA [78]. The detective concentration of target DNA with electrical DNA biosensor was as low as 1 fM for the analysis of current-voltage curves.

4.3 Nanomaterials for carbohydrate detection

Carbohydrates are involved in many biological processes and metabolism, and appear to play critical roles in determining biological functions. Glycosylation also affects the biological activity, lifetime, cellular uptake, and specificity of proteins. Therefore, the study and characterization of carbohydrates become increasingly important and has emerged as the "new frontier" for elucidating fundamental biochemical processes and for identifying new pharmaceutical substances. For most carbohydrate, the secondary and higher-order structures in solution are not readily defined, due to their inherent flexibility, which makes it difficult to define a universal protocol for their analysis. Since generally carbohydrates do not contain intrinsic chromophores (neither fluorescent nor emitting in the UV-visible range), electrochemical approaches for carbohydrate determination have become increasingly important in a variety of biological and pharmaceutical samples [79], benefited from the electrocatalytic and signal-amplified properties of nanomaterial. In combination with adsorptive stripping voltammetry, hesperidin down to 7 nM can be detected using multi-walled carbon nanotube modified electrodes [80]. Several transition metals nanoparticles/nanostructures have been synthesized to construct electrodes with high electrocatalytic capability toward oxidation of carbohydrates. For example, various Ni based nanostructures have been applied to the detection of sugars [81].

Lectins are a broad family of proteins involved in diverse biological processes, and can reversibly bind to mono- and oligosaccharides, and glycans with high specificity [82]. Based on such properties, they have been extensively exploited as recognizing components for carbohydrate sensing. The protocol of electrochemical recognition of sugars can be established by competitions of target sugars and the quantum dots-labeled sugars. As shown in Figure 13, the bioassay process involves the immobilization of the lectin onto the gold surface, competition between a QD-labeled sugar and the target sugar for the carbohydrate binding sites on lectins, and monitoring the extent of competition through highly sensitive electrochemical stripping detection of the captured nanocrystal. The protocol relies on an one-step competitive lectin-sugar recognition to yield a distinct cadmium stripping voltammetric current peak [83].

Glycans have great potential as disease biomarkers and therapeutic targets. By integrating the specific carbohydrate recognition and enzymatic signal amplification of proteins on Au nanoparticles, a dual-functionalized nanoprobe has been designed for highly sensitive and selective *in situ* evaluation of glycans on living cells [84]. This method uses a nano-scaffold of nanohorns functionalized with arginine-glycine-aspartic acid-serine tetrapeptide to capture cell and enhance electrical connectivity, which allows a detection limit down to 15 cells and monitoring of dynamic variation of carbohydrate expression on cancer cells in response

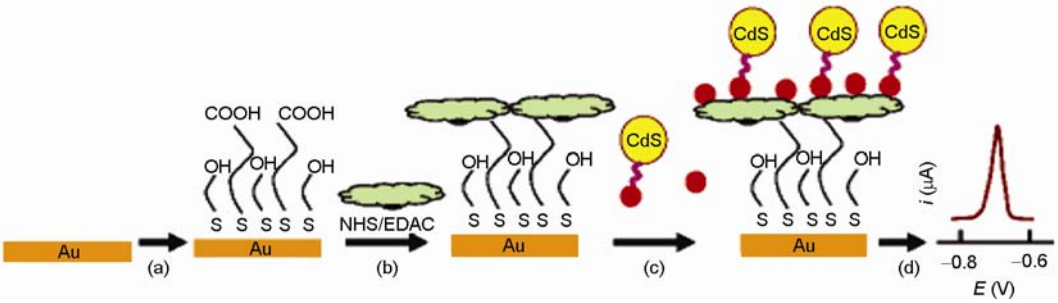


Figure 13 (a) Mixed SAM on the gold substrate; (b) covalent immobilization of the lectin; (c) addition of the tagged and untagged sugars; (d) dissolution of the captured nanocrystals, followed by their stripping voltammetric detection at a mercury-coated glassy carbon electrode.

to drugs. A strategy for profiling cell surface carbonhydrate expression patterns by evaluating cell-binding patterns on lectin arrays has been presented using an inverted optical microscope [85]. This strategy provides a proof-of-principle demonstration of the direct qualitative profiling of cell surface carbohydrate expression patterns by simple microscopic observation of cell binding to lectin arrays, and may readily be expanded to encompass a wider variety of carbohydrate structural motifs by increasing the number of lectins or other specific ligands present in the array.

4.4 Nanomaterials for cytosensing

In the context of cytosensing, researchers require the ability to track molecules within their native environments. Thus the efficiency of sensing systems is critically related to the outcome of the detection process in terms of the response time, signal-to-noise (S/N) characteristics, sensitivity, and selectivity of the system. The combination of nanotechnology with chemistry, biology, physics, engineering and medicine has emerged as a cornerstone solution. It may revolutionize the research on cell adhesion, glycobiology, cell trafficking and signal transduction along with structural biology, transcriptional regulation, disease diagnosis and treatment. The nanomaterial-based techniques, combined with nano-fluidics, single-molecule detection, and multiplexing methodologies excitingly provide new possibilities for ultrasensitive cytosensing.

Cytosensing modalities include optical fluorescence, molecular magnetic resonance imaging, surface-enhanced Raman scattering (SERS), colorimetry, scanometric detection and electrochemistry. Fluorescence imaging is an important approach to extract information on processes and functions in living cells. The present fluorophores are increasingly being replaced with NPs since their several advantages. NPs tend to have superior optical properties, substantially greater chemical stability, and stability against photobleaching. The most widely studied NPs for optical imaging applications are QDs. As a popularly used recognition pair, biotin-avidin (or streptavidin) has constituted an alternative option to specificly bind QDs to living cells. Cell labeling with ferro/paramagnetic substances is an increasingly common method for *in vivo* cell study as the labeled cells can be detected by magnetic resonance imaging (MRI) [86]. The iron oxide NPs targeted to a cell surface molecule can remain bound to their target, thus being useful as molecular MRI probes [87]. SERS is a promising analytical technique that allows intracellular sensing and diagnosis and treatment of cancer. A class of biocompatible and nontoxic pegylated AuNPs has been developed for *in vivo* tumor targeting and SERS detection with large optical enhancements [88]. Gambhir's group [89] demonstrated the ability of Raman spectroscopy to separate the spectral fingerprints of up to 10 different types of SERS NPs in a living mouse. Colorimetric methods are extremely attractive for rapidly identifying rare and low-abundance disease markers on cell surface because they can be easily read out with the naked eye, in some cases at the point of use. The unique physical properties of Au NPs make them attractive building blocks for nanoscale signal transducers and/or signal amplifiers in a variety of colorimetric platforms. A direct colorimetric assay for direct detection of diseased cells has been developed by Tan's group [90], which can detect 1000 target cells by the naked eye. Recently, scanometric detection has attracted considerable interest in cytosensing field because it combines the advantage of colorimetric detection with the array format and the information-recording and acquirement procedure can be performed with a flatbed scanner. This assay utilizes the catalytic properties of the NPs in a subsequent amplification event to affect the reduction of Ag^+ in the presence of hydroquinone, which simply needs to measure the grayscale value of the resulting silver spots to quantify the amount of target present [91]. For example, a convenient and label-free scanometric approach for *in situ* cell surface carbohydrate assay has been designed by integrating the bioconjugation and aggregation of glyconanoparticles, silver signal amplification, and spot test (Figure 14) [92]. The same group has also proposed another information transfer strategy for visualization of carbohydrate expression and scanometric detection of cell concentration in a wide range down to 5×10^4 cells/mL (Figure 15) [93]. This technology can be employed for studying other active components on cell surface and expanded to achieve information transfer between different substrates to better fit

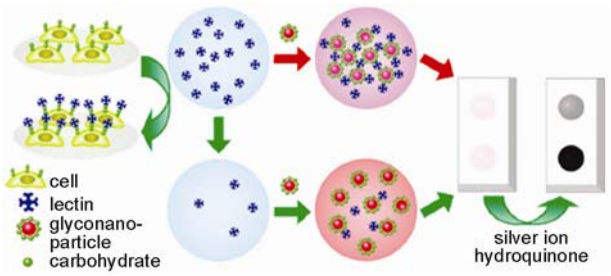


Figure 14 Scheme of the scanometric strategy for *in situ* detection of mannose groups on living cells.

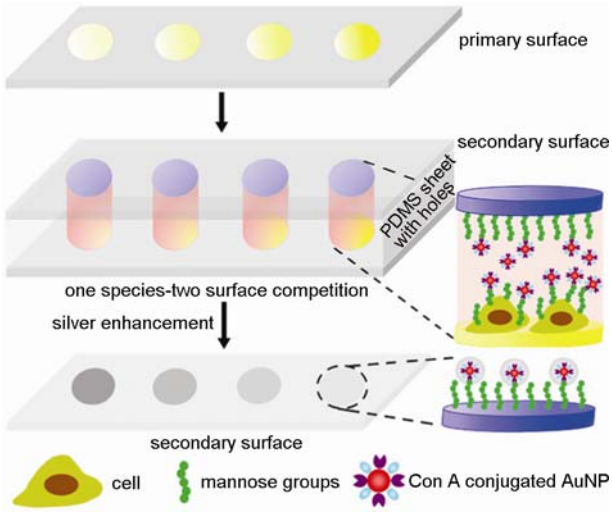


Figure 15 Schematic representation of mannose information transfer from a cell-adhered primary surface to a mannose-presenting secondary surface and visualization of cell surface carbohydrate expression.

the detection requirement.

An explosion of interest has occurred in the use of various kinds of zero-, one-, two-, and three-dimensional nanomaterials for electrochemical cytosensing. Examples of such materials include semiconductor QDs, metallic NPs, CNTs, nanostructured conductive polymers or nanocomposites thereof, and various other nanomaterials. The significant roles of nanomaterials in cytosensing lie in the ability to address some of the key issues, including design of the cell-compatible interface, facilitation of the electron transfer in electrochemical reactions, achievement of efficient transduction of the biorecognition event, increases in the sensitivity and selectivity, and improvement of response times [94]. The voltammetric responses of the redox centers in living cells usually show irreversible electron transfer related to the oxidation of guanine, however, the presence of nanomaterials on electrode for cell immobilization can both significantly reinforce the electrochemical response and maintain cell viability [93, 94]. Ju's group has developed a series of nanomaterials, such as AuNPs, MWNTs and nanocomposites to modify electrodes for electrochemical investigation of exogenous effect [95] and electrochemical anti-tumor drug sensitivity test [96]. Their first work is to use colloidal AuNPs to construct a non-toxic biomimetic interface for immobilization of AsPC-1 cells on a carbon paste electrode surface, which efficiently retains the activity of living tumor cells and prevents cell leakage from the electrode interface [95]. The voltammetric response can then be used to investigate the influence of exogenous factors on physiological function of living cells, evaluate anti-tumor drug effect and differentiate between normal and disease cells. Using a MWNTs-modified glassy carbon electrode, a simple, *in vitro*, electrochemical antitumor drug sensitivity test has been developed [94]. Comparing with conventional methods, the developed drug sensitivity test exhibits good performance, such as high sensitivity, desirable accuracy, low cost and simplified procedures.

Owing to the unique properties of nanomaterials, they have been widely used for the fabrication of impedance-based cytosensor to improve cell immobilization, retain cell viability, increase electrode surface area and decrease the background impedance, thus enhancing detection sensitivity [97, 98]. By combining the biocompatibility of chitosan and excellent conductivity of carbon nanofiber, this group prepared a nanocomposite film for the immobilization and cytosensing of K562 cells over the range of 10^4–10^8 cells/mL with a detection limit of 8.7×10^2 cells/mL [97]. This cytosensing method can be used for monitoring adhesion, proliferation, and apoptosis of cells on electrodes. Magnetic NP-antibody conjugates (MNAC) could be utilized to concentrate separated cells into a small volume, and further in the active layer of interdigitated array microelectrode (IDAM), at which cells were then detected [99].

Recently, cell surface carbohydrate assay by assembly of NPs has been quickly developed owing to their capability of tagging, signal amplification, integration of multiple functions and effective cell capture. Functional nanomaterials are mainly used in two roles as nanoscaffolds for immobilization of lectins and cell under investigation and as nanoprobes to combine the carbohydrate recognition, signal transduction and signal amplification abilities. Based on the specific recognition of integrin receptors on cell surface to arginine-glycine-aspartic acid-serine (RGDS)-functionalized SWNTs, the living cells can be captured on electrode surface, the cell surface carbohydrate then can be assayed using enzyme-linked lectin [100]. Combined with screen-printed carbon electrodes, this method can be further expanded for preparation of cytosensor array and effective monitoring of the dynamic variation of glycans on cancer cell surfaces during both drug inducement and erythroid differentiation of K562 cells (Figure 16) [101]. By functionalizing CdSe QDs with lectin on electrode surface, an ECL-based biosensor for cells and cell surface carbohydrate has been developed by the decrease of ECL intensity upon the specific capture of cells by the lectin-carbohydrate recognition [102].

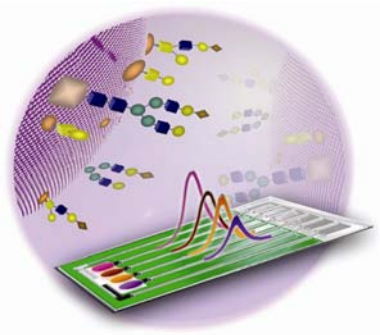


Figure 16 Schematic representation of the electrochemical cytosensor array for cell surface glycan analysis.

Nanoprobes suitable for carbohydrate sensing have emerged in electrochemical, fluorescent, flow cytometric, scanometric, and ECL detection. An earlier work for fabrication of nanostructure to recognize cell surface glycan was performed by Bertozzi's group [103]. In order to increase the detection sensitivity, a mannan carbohydrate monolayer was latterly developed to compete with cell surface carbohydrate to recognize lectin-functionalized QD probes [104]. The nanoprobes captured by mannan monolayer then could be detected by anodic stripping voltammetric technique. This first-reported "one molecule-two surfaces competition format" allowed *in situ* analysis of cell surface mannose moieties and cell concentration without need of cell labeling. A type of trifunctional nanospheres of QDs with excellent fluorescence, magnetism, and recognition of cancer cells surface-expressed with sialic acid and N-acetylglucosamine has been developed and functionalized with different types of lectins for qualitative and quantitative analysis of the glycoconjugates on A549 cell surface [105]. To solve the drawbacks in this method, CdTe QDs have been linked to Con A for recognizing cell surface mannosyl groups [106]. The decrease in fluorescence intensity of the nanoprobe solution is related to the cell amount and the expression extent of the mannosyl groups on the K562 cells. A facile ECL strategy for label-free monitoring of carbohydrate expression on living cells has been designed based on carbohydrate-functionalized CdS QDs/CNTs nanocomposites, which act as ECL emitting species on sensor surface [107]. Upon competitive binding of Con A to the carbohydrate groups on both cell surface and electrode surface, the ECL changed, which depends on both the cell amount and the expression level of cell surface carbohydrate. Water-soluble COOH-functionalized CdSe QDs have also been conjugated with plant-derived lectins to develop a fluorescent probe for flow cytometric identification of leukemia cells from normal lymphocytes [108]. More nanoprobes will be designed as optical and electrical probes for flow cytometric, optical and electrochemical analysis of cells and membrane acceptors such as carbohydrates, proteins and glycoproteins on cell surface.

5 Conclusions and perspectives

Biosensing technologies provide reliable, rapid, quantitative, cheap and high throughout detection of biomolecules and have been extensively used in different fields. Highly sensitive biosensing methods are urgently needed for measuring different markers present at ultra-low levels. Small, fast and high-throughput devices are also highly desired for replacing time-consuming laboratory-analyses to realize the screening of large population. The emergence of nanotechnology opens new horizons for the development of highly efficient biosensing strategies, nanoprobes, nanosensors and nanosystems suitable for fast, selective, sensitive and convenient detection of biomolecules. Functional nanomaterials offer improved biocompatibility, additional binding sites and high signal intensity. Hence, they are being gradually applied to biosensors for markedly enhancing the sensitivity and specificity of detection [109]. Plenty of nanomaterials such as carbon, metal, semiconductor and oxide-based nanoparticles, nanotubes, nanowires, nanoflakes and nanoribbons as well as dendrimers, fullerences, atomic clusters and molecular sieves are being functionalized to achieve highly efficient biological detection. Moreover, nanomaterials-based biosensors and nanofabrication can allow for miniaturization of the biosensors, which improves the sensitivity and reduces the sample and reagent volumes, making the detection process more efficient. Further work will focus on the integration of nanomaterial-based biosensors with fluidics, optics and electronics on a chip for automatic bioanalysis and high-throughput multi-analyte detection.

Recently, Wang's group discovered an unusual increase in the speed of catalytic nanowire motors in the presence of silver ions [110], which led to a fundamentally new nanomotor-based bio-detection platform for specific DNA and bacterial ribosomal RNA detection. This new nanomotor concept may be readily expanded to the detection of a broad range of target biomolecules in connection with different biomolecular interactions and motion transduction principles. Similarly, new concepts, new materials and new phenomena in nanoscience will inspire new ideals of bioanalysis, produce new research ways of biosensing and promote the quick development of biosensors. New nanostructured materials will constitute new platforms for biomolecular sensing that may provide increased sensitivity and amenability to miniaturization.

The demand in life science and clinic diagnosis for specific detection of biomolecules and discovery of new biomarkers will continue to be the motivity to develop new nanoprobes, nanocarriers and biosensing methods. Although current biosensing methods have been used for the detection of glucose, disease biomarkers, gene, viruses, microorganisms, cancer cells and even microRNA in single cell [111], the diagnosis at single cell and molecule level and the demand for point-of-care diagnosis and personalized medicine

will lead to new nanomaterial-based amplification strategies, nanomaterial-based molecular electronics and nanoscale biosensors and bioarrays. These progresses will also result in commercial advances of biosensing technology in different fields.

The author wish to thank the National Basic Research Program of China (2010CB732400), the National Natural Science Foundation of China (20821063 & 20875044), and the Natural Science Foundation of Jiangsu (BK2008014).

1 Ju HX, Zhang XJ, Wang J. *Nanobiosensing: Principles, Development and Application*. New York: Springer, 2011
2 Katz E, Willner I. Integrated nanoparticle-biomolecule hybrid systems: Synthesis, properties, and applications. *Angew Chem Int Ed*, 2004, 43: 6042–6108
3 Daniel MC, Astruc D. Gold nanoparticles: Assembly, dupramolecular chemistry, quantum-size-related properties, and applications toward biology, catalysis, and nanotechnology. *Chem Rev*, 2004, 104: 293–346
4 Neouze MA, Schubert U. Surface modification and functionalization of metal and metal oxide nanoparticles by organic ligands. *Monatsh Chem*, 2008, 139: 183–195
5 Lai GS, Yan F, Ju HX. Dual signal amplification of glucose oxidase–functionalized nanocomposites as a trace label for ultrasensitive simultaneous multiplexed electrochemical detection of tumor markers. *Anal Chem*, 2009, 81: 9730–9736
6 Jiang H, Ju HX. Enzyme-quantum dots architecture for highly sensitive electrochemiluminescence biosensing of oxidase substrates. *Chem Commun*, 2007, 404–406
7 Zheng M, Jagota A, Semke ED, Diner BA, Mclean RS, Lustig SR, Richardson RE, Tassi NG. DNA-assisted dispersion and separation of carbon nanotubes. *Nat Mater*, 2003, 2: 338–342
8 Hasobe T, Fukuzumi S, Kamat PV. Ordered assembly of protonated porphyrin driven by single-wall carbon nanotubes: J- and H-aggregates to nanorods. *J Am Chem Soc*, 2005, 127: 11884–11885
9 Gao Y, Cranston R. Polytyrosine as an electroactive label for signal amplification in electrochemical immunosensors. *Anal Chim Acta,* 2010, 659: 109–114
10 Zhao HT, Ju HX, Multilayer membranes for glucose biosensing via layer-by-layer assembly of multiwall carbon nanotubes and glucose oxidase. *Anal Biochem*, 2006, 350: 138–144
11 Pinijsuwan S, Rijiravanich P, Somasundrum M, Surareungchai W. Sub-femtomolar electrochemical detection of DNA hybridization based on latex/gold nanoparticle-assisted signal amplification. *Anal Chem*, 2008, 80: 6779–6784
12 Zhang J, Lei JP, Xu CL, Ding L, Ju HX. Carbon nanohorn sensitized electrochemical immunosensor for rapid detection of microcystin-LR. *Anal Chem*, 2010, 82: 1117–1122
13 Hu KC, Lan DX, Li XM, Zhang SS. Electrochemical DNA biosensor based on nanoporous gold electrode and multifunctional encoded DNA-Au bio bar codes. *Anal Chem*, 2008, 80: 9124–9130
14 Cheng LX, Liu X, Lei JP, Ju HX. Low-potential electrochemiluminescent sensing based on surface unpassivation of CdTe quantum dots and competition of analyte cation to stabilizer. *Anal Chem*, 2010, 82: 3359–3364
15 Krovi SA, Smith D, Nguyen ST. "Clickable" polymer nanoparticles: a modular scaffold for surface functionalization. *Chem Commun*, 2010, 46: 5277–5279
16 Gallant ND, Lavery KA, Amis EJ, Becker ML. Universal gradient substrates for "click" biofunctionalization. *Adv Mater*, 2007, 19: 965–969
17 Moon S, Kim DJ, Kim K, Kim D, Lee H, Lee K, Haam S. Surface-enhanced plasmon resonance detection of nanoparticle-conjugated DNA hybridization. *Appl Optics*, 2010, 49: 484–491
18 Maier I, Morgan MRA, Lindner W, Pittner F. Optical resonance-enhanced absorption-based near-field immunochip biosensor for allergen detection. *Anal Chem*, 2008, 80: 2694–2703
19 Yao X, Li X, Toledo F, Zurita-Lopez C, Gutova M, Momand J, Zhou FM. Sub-attomole oligonucleotide and p53 cDNA determinations via a high-resolution surface plasmon resonance combined with oligonucleotide-capped gold nanoparticle signal amplification. A*nal Biochem*, 2006, 354: 220–228
20 Zhang JQ, Wang YS, He Y, Jiang T, Yang HM, Tan X, Kang RH, Yuan YK, Shi LF. Determination of urinary adenosine using resonance light scattering of gold nanoparticles modified structure-switching aptamer. *Anal Biochem*, 2010, 397: 212–217
21 Kim D, Daniel WL, Mirkin CA. Microarray-based multiplexed scanometric immunoassay for protein cancer markers using gold nanoparticle probes. *Anal Chem*, 2009, 81: 9183–9187
22 Stewart ME, Anderton CR, Thompson LB. Nanostructured plasmonic sensors. *Chem Rev*, 2008, 108: 494–521
23 Fu Y, Zhang J, Lakowicz JR. Silver-enhanced fluorescence emission of single quantum dot nanocomposites. *Chem Commun*, 2009, 3: 313–315
24 Pu KY, Li K, Liu B. Cationic oligofluorene-substituted polyhedral oligomeric silsesquioxane as light-harvesting unimolecular nanoparticle for fluorescence amplification in cellular imaging. *Adv Mater*, 2010, 22: 643–646
25 Ding CF, Wang ZF, Zhong H, Zhang SS. Ultrasensitive chemiluminescence quantification of single-nucleotide polymorphisms by using monobase–modified Au and CuS nanoparticles. *Biosens Bioelectron*, 2010, 25: 1082–1087
26 Bi S, Zhou H, Zhang SS. Bio-bar-code functionalized magnetic nanoparticle label for ultrasensitive flow injection chemiluminescence detection of DNA hybridization. *Chem Commun*, 2009, 37: 5567–5569
27 Jie GF, Li LL, Chen C, Xuan J, Zhu JJ. Enhanced electrochemiluminescence of CdSe quantum dots composited with CNTs and PDDA for sensitive immunoassay. *Biosens Bioelectron*, 2009, 24: 3352–3358
28 Velev OD, Kaler EW. In situ assembly of colloidal particles into miniaturized biosensors. *Langmuir*, 1999, 15: 3693–3698
29 Wang J, Xu D, Kawde AN, Polsky R. Metal nanoparticle-based electrochemical stripping potentiometric detection of DNA hybridization. *Anal Chem*, 2001, 73: 5576–5581
30 Authier L, Grossirod C, Brossier P, Limoges B. Gold nanoparticle-based quantitative electrochemical detection of amplified human cytomegalovirus DNA using disposable microband electrodes. *Anal Chem*, 2001, 73: 4450–4456
31 Lai GS, Yan F, Wu J, Leng C, Ju HX. Ultrasensitive multiplexed immunoassay with electrochemical stripping analysis of silver nanoparticles catalytically deposited by gold nanoparticles and enzymatic reactio. *Anal Chem*, 2011, 83: 2726–2732
32 Wang J, Liu G, Merkocüi A. Electrochemical coding technology for simultaneous detection of multiple DNA targets. *J Am Chem Soc*, 2003, 125: 3214–3215
33 Liu G, Lee TMH, Wang J. Nanocrystal-based bioelectronic coding of single nucleotide polymorphisms. *J Am Chem Soc*, 2005, 127: 38–39
34 Liu G, Wang J, Kim J, Jan MR, Collins GE. Electrochemical coding for multiplexed immunoassays of proteins. *Anal Chem*, 2004, 76: 7126–7130
35 Hansen JA, Wang J, Kawde A, Xiang Y, Gothelf KV, Collins G. Quantum-dot/aptamer-based ultrasensitive multi-analyte electrochemical biosensor. *J Am Chem Soc*, 2006, 126: 2228–2229
36 Cheng W, Yan F, Ding L, Ju HX, Yin YB. Cascade signal amplification strategy for subattomolar protein detection by rolling circle amplification and quantum dots tagging. *Anal Chem*, 2010, 82: 3337–3342
37 Bonanni A, Pividori MI, Campoy S, Barbe J, del Valle M. Impedimetric detection of double-tagged PCR products using novel amplification procedures based on gold nanoparticles and protein G. *Analyst*, 2009, 134: 602–608
38 Li JH, Hu JB. Functional gold nanoparticle-enhanced electrochemical

determination of DNA hybridization and sequence-speciric analysis. *Acta Chim Sinica*, 2004, 62: 2081–2088
39 Zhang W, Shaikh AU, Tsui EY, Swager TM. Cobalt porphyrin functionalized carbon nanotubes for oxygen reduction. *Chem Mater*, 2009, 21: 3234–3241
40 Tu WW, Lei JP, Ding L, Ju HX. Sandwich nanohybrid of single-walled carbon nanohorns-TiO_2-porphyrin for electrocatalysis and amperometric biosensing towards chloramphenicol. *Chem Commun*, 2009, 28: 4227–4229
41 Tu WW, Lei JP, Zhang SY, Ju HX. Characterization, direct electrochemistry and amperometric biosensing of graphene by noncovalent functionalization with picket-fence porphyrin. *Chem Eur J*, 2010, 16: 10771–10777
42 Du P, Li HX, Cao W. Construction of DNA sandwich electrochemical biosensor with nanoPbS and nanoAu tags on magnetic microbeads. *Biosens Bioelectron*, 2009, 24: 3223–3228
43 Liu XP, Wu HW, Zheng Y, Wu ZS, Jiang JH, Shen GL, Yu RQ. A sensitive electrochemical immunosensor for α-Fetoprotein detection with colloidal gold-based dentritical enzyme complex amplification. *Electroanalysis*, 2010, 22: 244–250
44 Ding CF, Li H, Hu KC, Lin JM. Electrochemical immunoassay of hepatitis B surface antigen by the amplification of gold nanoparticles based on the nanoporous gold electrode. *Talanta*, 2010, 80: 1385–1391
45 Fang LY, Lü ZZ, Wei H, Wang E. A electrochemiluminescence aptasensor for detection of thrombin incorporating the capture aptamer labeled with gold nanoparticles immobilized onto the thio-silanized ITO electrode. *Anal Chim Acta*, 2008, 628:80–86
46 Zhu ZQ, Su YY, Li J, Li D, Zhang J, Song SP, Zhao Y, Li GX , Fan CH. Highly sensitive electrochemical sensor for mercury(II) ions by using a mercury-specific oligonucleotide probe and gold nanoparticle-based amplification. *Anal Chem*, 2009, 81: 7660–7666
47 Carrillo-Carrión1 C, Simonet1 BM, Valcárcel M. Carbon nanotube-quantum dot nanocomposites as new fluorescence nanoparticles for the determination of trace levels of PAHs in water. *Anal Chim Acta*, 2009, 652: 278–284
48 Lee AC, Ye JS, Tan SN, Poenar DP, Sheu FS, Heng CK, Lim TM. Carbon nanotube-based labels for highly sensitive colorimetric and aggregation–based visual detection ofnucleic acids. *Nanotechnology,* 2007, 18: 455102
49 Gao WC, Dong HF, Lei JP, Ji HX, Ju HX. Signal amplification of streptavidin-horseradish peroxidase functionalized carbon nanotubes for amperometric detection of attomolar DNA. *Chem Commun*, 2011, 47: 5220–5222
50 Wang YY, Liu B. Conjugated polymer as a signal amplifier for novel silica nanoparticle-based fluoroimmunoassay. *Biosens Bioelectron*, 2009, 24: 3293–3298
51 Dong HF, Yan F, Ji HX, Wong DKY, Ju HX. Quantum-dot-functionalized poly(styrene-co-acrylic acid) microbeads: step-wise self-assembly, characterization, and applications for sub-femtomolar electrochemical detection of DNA hybridization. *Adv Funct Mater*, 2010, 20: 1173–1179
52 Wu J, Tang JH, Dai Z, Yan F, Ju HX, EL Murr N. A disposable electrochemical immunosensor for flow injection immunoassay of carcinoembryonic antigen. *Biosens Bioelectron*, 2006, 22: 102–108
53 Wu J, Yan F, Zhang XQ, Yan YT, Tang JH, Ju HX. Disposable reagentless electrochemical immunosensor array based on a biopolymer/sol-gel membrane for simultaneous measurement of several tumor markers. *Clin Chem*, 2008, 54: 1481–1488
54 Loyprasert S, Thavarungkul P, Asawatreratanakul P, Wongkittisuksa B, Limsakul C, Kanatharana P. Label-free capacitive immunosensor for microcystin-LR using self-assembled thiourea monolayer incorporated with Ag nanoparticles on gold electrode. *Biosens Bioelectron*, 2008, 24: 78–86
55 Wohlstadter JN, Wilbur JL, Sigal GB, Biebuyck HA, Billadeau MA, Dong LW, Fischer AB, Gudibande SR, Jamieson SH, Kenten JH, Leginus J, Leland JK, Massey RJ, Wohlstadter SJ. Carbon nanotube-based biosensor. *Adv Mater*, 2003, 15: 1184–1187
56 Wu LN, Yan F, Ju HX. An amperometric immunosensor for separation-free immunoassay of CA125 based on its covalent immobilization coupled with thionine on carbon nanofiber. *J Immunol Methods*, 2007, 322: 12–19
57 Nie HG, Liu SJ, Yu RQ, Jiang JH. Phospholipid-coated carbon nanotubes as sensitive electrochemical labels with controlled-assembly-mediated signal transduction for magnetic separation immunoassay. *Angew Chem Int Ed*, 2009, 48: 9862–9866
58 Wu Z, Zhen Z, Jiang JH, Shen GL, Yu RQ. Terminal protection of small-molecule-linked DNA for sensitive electrochemical detection of protein binding via selective carbon nanotube assembly. *J Am Chem Soc*, 2009, 131: 12325–12332
59 Liu Y, Jiang H. Electroanalytical determination of carcinoembryonic antigen at a silica nanoparticles/titania sol-gel composite membrane-modified gold electrode. *Electroanalysis*, 2006, 18: 1007–1013
60 Yang ZJ, Xie ZY, Liu H, Yan F, Ju HX. Streptavidin-functionalized three-dimensional ordered nanoporous silica film for highly efficient chemiluminescent immunosensing. *Adv Funct Mater*, 2008, 18: 3991–3998
61 Hu DH, Han HY, Zhou R, Dong F, Bei WC, Jia F, Chen HC. Gold(III) enhanced hemiluminescence immunoassay for detection of antibody against ApxIV of Actinobacillus pleuropneumoniae. *Analyst*, 2008, 133: 768–773
62 Leng C, Lai GS, Yan F Ju HX. Gold nanoparticle as an electrochemical label for inherently crosstalk-free multiplexed immunoassay on a disposable chip. *Anal Chim Acta*, 2010, 666: 97–101
63 Jin XY, Jin XF, Chen LG, Jiang JH, Shen GL, Yu RQ. Piezoelectric immunosensor with gold nanoparticles enhanced competitive immunoreaction technique for quantification of aflatoxin B_1. *Biosens Bioelectron*, 2009, 24: 2580–2585
64 Hu SH, Liu R, Zhang SC, Huang Z, Xing Z, Zhang XR. A new strategy for highly sensitive immunoassay based on single–particle mode detection by inductively coupled plasma mass spectrometry. *J Am Soc Mass Spectr*, 2009, 20: 1096–1103
65 Hu SH, Zhang SC, Hu ZC, Xing Z, Zhang XR. Detection of multiple proteins on one spot by laser ablation inductively coupled plasma mass spectrometry and application to immunomicroarray with element-tagged antibodies. *Anal Chem*, 2007, 79: 923–929
66 Vinayaka AC, Basheer S, Thakur MS. Bioconjugation of CdTe quantum dot for the detection of 2,4-dichlorophenoxyacetic acid by competitive fluoroimmunoassay based biosensor. *Biosens Bioelectron*, 2009, 24: 1615–1620
67 Liu GD, Wang J, Kim J, Jan MR, Collins GE. Electrochemical coding for multiplexed immunoassays of proteins. *Anal Chem*, 2004, 76: 7126–7130
68 Thurer R, Vigassy T, Hirayama M, Wang J, Bakker E, Pretsch E. Potentiometric immunoassay with quantum dot labels. *Anal Chem,* 2007, 79: 5107–5110
69 Liu X, Zhang YY, Lei JP, Xue YD, Cheng LX, Ju HX. Quantum dots based electrochemiluminescent immunosensor by coupling enzymatic amplification with self-produced coreactant from oxygen reduction. *Anal Chem*, 2010, 82: 7351–7356
70 Zhu NN, Lin YQ, Yu P, Su L, Mao LQ. Label-free and sequence-specific DNA detection down to a picomolar level with carbon nanotubes as support for probe DNA. *Anal Chim Acta*, 2009, 650: 44–48
71 Wang J, Liu GD, Jan MR. Ultrasensitive electrical biosensing of proteins and DNA: carbon-nanotube derived amplification of the recognition and transduction events. *J Am Chem Soc*, 2004, 126: 3010–3011
72 Niu SY, Han B, Cao W, Zhang SS. Sensitive DNA biosensor improved by Luteolin copper(II) as indicator based on silver nanoparticles and carbon nanotubes modified electrode. *Anal Chim Acta*, 2009, 651: 42–47
73 Zhang W, Yang T, Huang DM, Jiao K. Electrochemical sensing of DNA immobilization and hybridization based on carbon nanotubes/nano zinc oxide/chitosan composite film. *Chin Chem Lett*, 2008, 19: 589–591
74 Dong HF, Gao WC, Yan F, Ji HX, Ju HX. Fluorescence resonance

energy transfer between quantum dots and graphene oxide for sensing biomolecules. *Anal Chem*, 2010, 82: 5511–5517
75 Bally M, Halter M, Voros J, Grandin HM. Optical microarray biosensing techniques. *Surf Interf Anal*, 2006, 38: 1442–1458
76 Cheng YT, Pun CC, Tsai CY, Chen PH. An array-based CMOS biochip for electrical detection of DNA with multilayer self-assembly gold nanoparticles. *Sensor Actuat B-Chem*, 2005, 109: 249–255
77 Lytton-Jean AKR, Han MS, Mirkin CA. Microarray detection of duplex and triplex DNA binders with DNA-modified gold nanoparticles. *Anal Chem*, 2007, 79: 6037–6041
78 Chang TL, Tsai CY, Sun CC, Uppala R, Chen CC, Lin CH, Chen PH. Electrical detection of DNA using gold and magnetic nanoparticles and bio bar-code DNA between nanogap electrodes. *Microelectron Engineering*, 2006, 83: 1630–1633
79 Baldwin RP.Electrochemical determination of carbohydrates: Enzyme electrodes and amperometric detection in liquid chromatography and capillary electrophoresis. *J Pharm Biomed Anal*, 1999, 19: 69–81
80 Sims MJ, Li Q, Kachoosangi RT, Wildgoose GG, Compton RG. Using multiwalled carbon nanotube modified electrodes for the adsorptive striping voltammetric determination of hesperidin. *Electrochim Acta*, 2009, 54: 5030–5034
81 Elahi MY, Mousavi MF, Ghasemi S. Nano-structured Ni(II)-curcumin modified glassy carbon electrode for electrocatalytic oxidation of fructose. *Electrochim Acta*, 2008, 54: 490–498
82 Sharon N. Lectins: Carbohydrate-specific proteins that mediate cellular recognition. *Chem Rev*, 1998, 98: 637–674
83 Babu P, Sinha S, Surolia A. Sugar quantum dot conjugates for a selective and sensitive detection of lectins. *Bioconjugate Chem*, 2006, 18: 146–151
84 Ding L, Ji QJ, Qian RC Cheng W, Ju HX. Lectin-based nanoprobes functionalized with enzyme for highly sensitive electrochemical monitoring of dynamic carbohydrate expression on living cells. *Anal Chem*, 2010, 82: 1292–1298
85 Zheng T, Peelen D, Smith LM. Lectin arrays for profiling cell surface carbohydrate expression. *J Am Chem Soc*, 2005, 127: 9982–9983
86 Gupta AK, Gupta M. Synthesis and surface engineering of iron oxide NPs for biomedical applications. *Biomaterials*, 2005, 26: 3995–4021
87 Laurent S, Boutry S, Mahieu I, Vander Elst L, Muller RN. Iron oxide based MR contrast agents: from chemistry to cell labeling. *Curr Med Chem*, 2009, 16: 4712–4727
88 Qian XM, Peng XH, Ansari DO, Yin-Goen Q, Chen GZ, Shin DM, Yang L, Young AN, Wang MD, Nie SM. In vivo tumor targeting and spectroscopic detection with surface-enhanced Raman NP tags. *Nat Biotechnol*, 2008, 26: 83–90
89 Zavaleta CL, Smith BR, Walton I, Doering W, Davis G, Shojaei B, Natan MJ, Gambhir SS. Multiplexed imaging of surface enhanced Raman scattering nanotags in living mice using noninvasive Raman spectroscopy. *Proc Natl Acad Sci*, 2009, 106: 13511–13516
90 Medley CD, Smith JE, Tang ZW, Wu YR, Bamrungsap S, Tan WH. Gold NP-based colorimetric assay for the direct detection of cancerous cells. *Anal Chem*, 2008, 80: 1067–1072
91 Lee JS, Mirkin CA. Chip-based scanometric detection of mercuric ion using DNA-functionalized gold NPs. *Anal Chem*, 2008, 80: 6805–6808
92 Ding L, Qian RC, Xue YD, Cheng W, Ju HX. *In situ* scanometric assay of cell surface carbohydrate by glycoNP-aggregation-regulated silver enhancement. *Anal Chem*, 2010, 82: 5804–5809
93 Ding L, Xiao XR, Chen YL, Qian RC, Bao L, Ju HX. Transfer of carbohydrate expression information on cell-adhered surface to a secondary surface by competition of two surfaces for one species. *Chem Commun*, 2011, 47: 3742–3744
94 Yang WR, Ratinac KR, Ringer SP, Thordarson P, Gooding JJ, Braet F. Carbon nanomaterials in biosensors: Should you use nanotubes or graphene? *Angew Chem Int Ed*, 2010, 49: 2114–2138
95 Du D, Liu SL, Chen J, Ju HX, Lian HZ, Li JX. Colloidal gold NP modified carbon paste interface for studies of tumor cell adhesion and viability. *Biomaterials*, 2005, 26: 6487–6495
96 Chen J, Du D, Yan F, Ju HX, Lian HZ. Electrochemical anti-tumor drug sensitivity test for leukemia K562 cells at a carbon nanotubes modified electrode. *Chem Eur J*, 2005, 11: 1467–1472
97 Ding L, Hao C, Xue YD, Ju HX. A bio-inspired support of gold NPs-chitosan nanocomposites gel for immobilization and electrochemical study of K562 Leukemia cells. *Biomacromolecules*, 2007, 8: 1341–1346
98 Ding L, Hao C, Zhang XJ, Ju HX. Carbon nanofiber doped polypyrrole nanoscaffold for electrochemical monitoring of cell adhesion and proliferation. *Electrochem Commun*, 2009, 11: 760–763
99 Varshney M, Li Y. Interdigitated array microelectrode based impedance biosensor coupled with magnetic nanoparticle-antibody conjugates for detection of Escherichia coli O157:H7 in food samples. *Biosens Bioelectron*, 2007, 22: 2408–2414
100 Cheng W, Ding L, Lei JP, Ding SJ, Ju HX. Effective cell capture with tetrapeptide functionalized carbon nanotubes and dual signal amplification for cytosensing and evaluation of cell surface carbohydrate. *Anal Chem*, 2008, 80: 3867–3872
101 Cheng W, Ding L, Ding SJ, Ying YB, Ju HX. A simple electrochemical cytosensor array for dynamic analysis of carcinoma cell surface glycans. *Angew Chem Int Ed*, 2009, 48: 6465–6468
102 Han E, Ding L, Lian HZ, Ju HX. Cytosensing and dynamic monitoring of cell surface carbohydrate expression by electrochemiluminescence of quantum dots. *Chem Commun*, 2010, 5446–5448
103 Ohtsubo K, Marth JD. Glycosylation in cellular mechanisms of health and disease. *Cell*, 2006, 126: 855–867
104 Ding L, Cheng W, Wang XJ. Ding SJ, Ju HX. Carbohydrate monolayer strategy for electrochemical assay of cell surface carbohydrates. *J Am Chem Soc*, 2008, 130: 7224–7225
105 Xie M, Hu J, Long YM, Zhang ZL, Xie HY, Pang DW. Lectin-modified trifunctional nanobiosensors for mapping cell surface glycoconjugates. *Biosens Bioelectron*, 2008, 24: 1311–1317
106 Xu X, Ding L, Xue YD, Ju HX. A simple fluorescent method for in situ evaluation of cell surface carbohydrate with a novel lectin-nanoprobe. *Analyst*, 2010, 135: 1906–1908
107 Han E, Ding L, Jin S, Ju HX. Electrochemiluminescent biosensing of carbohydrate- functionalized CdS nanocomposites for in situ label-free analysis of cell surface carbohydrate. *Biosens Bioelectron,* 2011, 26: 2500–2505
108 Zhelev Z, Ohba H, Bakalova R, Jose R, Fukuoka S, Nagase T, Ishikawa M, Baba Y. Fabrication of quantum dot-lectin conjugates as novel fluorescent probes for microscopic and flow cytometric identification of leukemia cells from normal lymphocytes. *Chem Commun*, 2005, 15: 1980–1982
109 Rosi NL, Mirkin CA. Nanostructures in biodiagnostics. *Chem Rev*, 2005, 105: 1547–1562
110 Wu J, Balasubramanian S, Kagan D, Manesh KM, Campuzano S, Wang J. Motion-based DNA detection using catalytic nanomotors. *Nat Commun*, 2010, doi:10.1038/ncomms1035
111 Dong HF, Ding L, Yan F, Ji HX, Ju HX. The use of polyethylenimine-grafted graphene nanoribbon for cellular delivery of locked nucleic acid modified molecular beacon for recognition of microRNA. *Biomaterials*, 2011, 32: 3875–3882