Imaging Molecules and Cells

R Sharma*
Center of Nanobiotechnology, TCC and Florida State University, Tallahassee, FL 32304 USA

ABSTRACT

Proteins provide the building blocks for multicomponent molecular units, or pathways, from which higher cellular functions emerge. These units consist of either assemblies of physically interacting proteins or dispersed biochemical activities connected by rapidly diffusing second messengers, metabolic intermediates, ions or other proteins. Genetics to identifies the proteins that constitute functional units and to establish the first-order connectivity. The dynamics of interactions within these protein machines can be assessed in living cells by the applications of MR microscopy, fluorescence spectroscopy on a microscopic level, using fluorescent proteins that are introduced within these functional units. Fluorescence is sensitive, specific and non-invasive, and the spectroscopic properties of a fluorescent probe can be analyzed to obtain information on its molecular environment. The development and use of biosensors based on the genetically encoded variants of green-fluorescent proteins has facilitated the observation of 'live' biochemistry on a microscopic level, with the advantage of preserving the cellular context of biochemical connectivity,compartmentalization and spatial organization. Active proteins such as myoglobin and troponin activities in the heart and protein-protein interactions can be imaged and localized within a single cell, allowing correlation with cell cycle, migration and morphogenesis.

Keywords: bioimaging, active molecule imaging, NMR, flurescence

1 INTRODUCTION

Remarkable optical, electrical and mechanical properties of nanostrctured materials provided a new set of materials for drug delivery and medical therapeutics. The development of nanoscale molecules in imaging began with ultra small sensing elements. Science grew up on nanomaterials (particles, rods, wires, tubes, cubes, tetrapods, or triangles), and the readiness for surface functionalization (physical, chemical, or biological). Integration of molecules (e.g. proteins, peptides, or DNA) with semiconductor quantum dots (QDs) and metal nanoparticles (NPs) expanded the impact of biophotonics and bioelectronics, particularly in optical imaging and biosensing, as well as therapeutic strategies.

For example, gold is considered to be a noble metal in bulk state, but the nanoparticles of gold are considered as good catalyst. Nanoparticles of gold, silver and semiconductors (quantum dots) such as CdSe, CdS etc can be used in diagnosis of DNA, proteins, and other biomolecules. Some nanostructured fluorescent materials such as LnF_3:Ln, $NaYF_4$:Ln, $LnPO_4$ and their functionalized doped materials have been utilized for protein labeling and quantitative DNA hybridization detection. Due to strong superparamagnetic behavior of iron oxide nanoparticles have been employed as a targeting material in biomedical sciences. Immunoglobulin G (IgG) and streptavidin was conjugated to CdSe QDs with different emission spectra to label the breast cancer marker Her2 on the surface of live cancer cells.

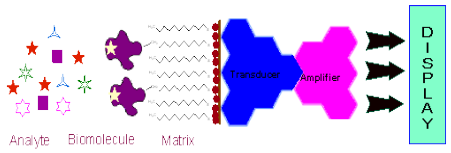

Figure 1. Schematic of nanomaterials based biosensor.

2 BIOSENSORS

Biosensor molecules detect signal using a transducer e.g. optical, electrochemical, piezoelectric and gravimetric transducers are used to get signals in these devices. So the central theme of detection is the signal transduction associated with the selective recognition of a biological or chemical species. There are two different types of biosensors: biocatalytic and bioaffinity-based biosensors. The biocatalytic biosensor uses mainly enzymes as the biological compound, catalyzing a signaling biochemical reaction. The bioaffinity-based biosensor, designed to monitor the binding event itself, uses specific binding proteins, lectins, receptors, nucleic acids, membranes, whole cells, antibodies or antibody-related substances for biomolecular recognition. Based on their unique physical, chemical and electrocatalytic properties, these nanoparticles play variety of roles in different biosensing systems.

2.1 Colorimetric sensors

These devices are mainly based on the observation of color changes due to anion-cation interactions and for monitoring colorimetric reactions and for metal detection. Fundamentally, molecular recognition involves the interactions between molecules: i.e. bond formation, acid–base interactions, hydrogen-bonding, dipolar and multipolar interactions, vander Waals interaction and physical adsorption. The oligonucleotide-mediated nanoparticle aggregation gives highly sensitive colorimetric biosensors for oligonucleotides. The detection of specific oligonucleotide sequences is used in diagnosis of genetic and pathogenic diseases and quantifying the amount of product generated by polymerase chain reaction (PCR) to detect sub-picomolar level. This methodology was also applied for the colorimetric screening of DNA binders and triplex DNA binders. A new Pb^{2+} specific ''DNAzyme''works on principle, the substrate strand cleaves into two pieces, resulting in head-to-tail or tail-to-tail aggregation with a concomitant red to blue color shift with a sensing limit of 100 nM in presence of Pb^{2+}. Aptamers are single-stranded DNA or RNA molecules bind with target molecules with high affinity and specificity. The conformation of an aptamer changes upon binding to its target analyte, and

this property has been used in a wide variety of sensing applications of fluorescence intensity, polarization, energy transfer, electrochemistry or color change. Colorimetric sensors are particularly important because they minimize or eliminate the necessity of using expensive and complicated instruments. Gold nanoparticles for colorimetric sensing is a new hope.

2.2 Acoustic wave based piezoelectric sensors

Acoustic wave biosensors are based on the detection of mechanical acoustic waves and incorporate a biological component piezoelectric, magnetostrictive, optical, and thermal techniques. The piezoelectric transduction effect, acoustic biomedical sensor molecules act as oscillating crystal resonating at a fundamental frequency. If crystal coated with a an antibody can measure antigen quantity by change occurs in the resonant frequency of the crystal proportional to mass changes at the crystal surface. Quartz crystal, high-density particles (e.g. Au, Pt, CdS, Fe_3O_4, ZnO, TiO_2, polymers) may be also suitable.

2.3 Conductometric biosensors

Conductometric biosensors measure the changes in the conductance of the biological component arising between a pair of metal electrode as biosensors for estimation of glucose, urea neutral lipid/lipase and hemoglobin/pepsin by monitoring the change in the electronic conductivity arising as a change in redox potential and/or pH of the microenvironment in the polymer matrix. More applications of immunoassays based on magnetite nanoparticles for Escherichia coli (E. coli) detection using conductometric measurements, Staphylococcus epidermidis (S. epidermidis) are in progress as 1 CFU/ml of E. coli induces a conductivity variation of 35 μS. Other new conductivity biosensors based on double-codified nanogold particles are in progress for hepatitis B surface antigen (HBsAg) detection by using HRP-conjugated *anti*-HBs as secondary antibodies, while the assay sensitivity by using double-codified nanogold particles could be further increased to 0.01 ng/mL with the linear range from 0.1 to 600 ng/mL HBsAg.

2.4 Calorimetric biosensors

Enzyme-catalyzed reactions show an enthalpy change as thermal biosensors for a broad range of applications. A number of substrates, enzymes and antigens have been estimated using thermister biosensors. Differential scanning calorimetric studies and enzyme/protein stabilities which are bound to nanoporous inorganic materials indicate new hopes.

2.5 Electrochemical sensors

The attachment of nanoparticles onto electrodes drastically enhances the conductivity and electron transfer from the redox analytes to make them electroanalytical sensor. Based on this concept, enzyme glucose oxidase (apo-GOx) onto gold nanoparticles that was functionalized with N6-(2-aminoethyl) flavin adenine (FAD). This enzyme–nanoparticle hybrid system was linked to the electrode through dithiols, or alternatively the FAD-functionalized nanoparticles were assembled onto the electrode followed by the addition of apo-GOx. Similar electron transfer from protein to nanoparticles was used for monitoring hydrogen evolution from zinc-substituted cytochromec immobilized TiO_2 nanoparticles. Detection of DNA by selective deposition of oligonucleotide-functionalized nanoparticles between two electrodes is new hope. Biomolecules (glucose, cholesterol, urea, lactase, DNA, antigen and pesticides detection) from samples has opened a new window. Gold nanoparticles, platinum nanoparticles, silica, carbon nanotubes (SWCNT & MWCNT), niobium oxide (Nb_2O_5), manganese oxide (MnO_2), zinc oxide (ZnO), antimony oxide (Sb_2O_3), zirconium oxide (ZrO_2), cerium oxide (CeO_2), iron oxide (Fe_3O_4), tin oxide (SnO_2), titanium oxide (TiO_2) and composite of these nanomaterials are examples. Biomolecules such as enzyme, antigen or DNA have same dimensions of nanoparticles (NPs) and suggest possibility of biomolecule–NPs hybrid composite. Biosensors based on metal nanoparticles modified CNT electrodes lower the over potential of biochemical reaction and avoid the interference from other co-existing electroactive species. Various enzymatic, DNA and immunosensors have been reported using nanoparticles of metal, metal oxide and semiconductors.

2.6 Amperometric sensors

Amperometric biosensors measure the current produced during the oxidation or reduction of a product or reactant usually at a constant applied potential. Nanostructured materials provide a direct redox mediator free electron communication surface between the analyte (enzymes or biomolecules) and electrode, various nanostructured materials have used for enzyme immobilization may affect significantly the response of biosensors sensitive to given species. Examples are amperometric H_2O_2 biosensor horse radish peroxidase (HRP) immobilized on Au NPs supported by the thiol tailed group of the cysteamine (cyst) monolayer; CNT affinity for hydrogen peroxide and NADH permitted effective low potential amperometric biosensing of glucose and ethanol (glucose oxidase and alcohol dehydrogenase/NAD^+) within the three-dimensional CNT/Teflon matrix; graphite/Teflon.

2.7 Potentiometric sensors

Potentiometry is the measurement of an electrical potential difference (voltage) between two electrodes when the cell current is zero. Nanostructured materials are useful in potentiometric sensors for ultratrace level detection limits on the order of nanomolar or lower concentrations ranges. Modified ion selective electrodes (ISEs) detect DNA hybridization detection for capturing a secondary oligonucleotide bearing CdS-nanocrystal tags.

2.8 Electrochemical impedance spectroscopy (EIS)

Impedance spectroscopy measures the behavior of the modified electrodes for analyte detection by impedance at specific frequencies depending on amplitude of the current and potential signals and the resulting phase difference between voltage and current, which depends on the nature of the system under study, dictates the system impedance.

Construction of impedimetric biosensors to monitor various biological reactions by immobilizing biomolecules such as enzymes, antibodies, nucleic acids, cell, and microorganisms is new approach. Nanoparticles enhance the signal in impedimetric biosensor for electronic, photonic, and catalytic

properties of biomolecules and nanoparticle-antibody conjugate s that induce the change of the electrical properties such as resistance and capacitanceproportional to impedance changed.

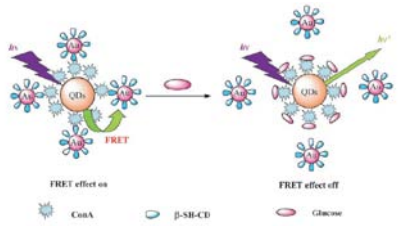

Figure 2. Chemical structure of the QDs-ConA-b-CDs-AuNPs nanobiosensor and schematic illustration of its FRET-based operating principles.

2.9 Surface plasmon resonance sensors

SPR biosensors measure changes in refractive index caused by structural alterations in the vicinity of a thin film metal surface. A glass plate covered by a gold thin film is irradiated from the backside by p-polarised light (from a laser) via a hemispherical prism, and the reflectivity is measured as a function of the angle of incidence, θ. The resulting plot is a curve showing a narrow dip. This peak is known as the SPR minimum. The trend is toward using nano-technology to develop sensing matrices and surfaces that are embedded the nano-particles.

2.10 Fluorescence sensors

Fluorescence occurs when a valence electron is excited from its ground state to an excited singlet state. The excitation is produced by the absorption of light of sufficient energy. When the electron returns to its original ground state it emits a photon at lower energy. Antibodies may be conjugated to fluorescent compounds; the most common of which is fluoresce in isothiocyanate (FITC). Fluorescence resonance energy transfer (FRET) biosensors are based on the transfer of energy from a donor fluorophore to an acceptor fluorophore shown in Figure 2. Semiconductors QDs sense DNA and proteins. Gold nanoparticles act as FRET donor–acceptor couple. In presence of complementary oligonucleotides, the gold particle is released from the QD, regenerating QD fluorescence. Examples are avidin and glycoproteins, anionic poly(p-phenyleneethynylene) (PPE) fluorescent polymer. Protein-nanoparticle interactions can be deduced from fluorescence response patterns for individual proteins by linear discrimination analysis (LDA).

3 OTHER SENSING METHODS

3.1 Surface-enhanced Raman scattering (SERS)

SERS is an extension and variation of standard Raman spectroscopy, vibrational spectroscopic technique gives molecular structural information and also provides ultrasensitive detection limits, including single molecule sensitivity; it has been used to detect pathogens that include bacteria and viruses biosensing, intrinsic or extrinsic, as shown in Figure 3. Raman reporter molecule Au nanoparticle is immobilized on SERS-active substrate with additional coating of SiO_2, TiO_2, or a polymer, makes a core-shell complex with antibodies outermost. Oligonucleotide, proteins and bar coded gold particles to target DNA, can be detected. The magnetic microparticle bound protein subsequently interacts with gold nanoparticles by antigen–antibody interaction.

3.1. Bioimaging, Bio-Labeling, Biomarker

Molecular imaging techniques such as optical/fluorescence imaging, positron emission tomography (PET), magnetic resonance imaging (MRI), X-ray computed tomography (CT), single photon emission computed tomography (SPECT), ultrasound and microwave have been employed for imaging the structure and function of systems of *in vitro* and *in vivo* biological specimens using luminescent and magnetic nanoparticles advances bioimaging technologies for optical imaging (OI) and MRI. There are also dual-mode nanoparticles for simultaneous imaging by OI and MRI. **3.2**

3.2 Optical imaging

Optical imaging is an imaging technique (also known as molecular imaging) that involves inference from the deflection of light emitted from a laser or infrared source to anatomic or chemical properties of material (e.g. cell tissue).

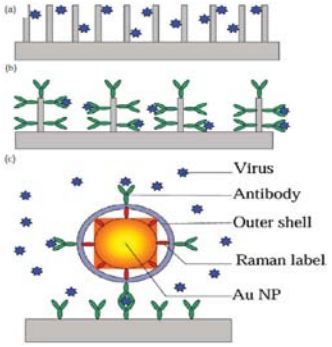

Figure 3. Different SERS detection configurations:

(a) Direct intrinsic detection;
(b) indirect intrinsic detection; and
(c) extrinsic detection.

Quantum dots and fluorescent nanostructured materials can detect bio-molecules or cells *in vivo* and *in vitro*. Most nanoparticle-based optical imaging agents can be subdivided into three categories: latex nanospheres, luminescent quantum dots (QDs) & dye-doped nanoparticle QDs and optically active luminescent nanostructure materials (such as gold, silver and luminescent lanthanide nanoparticles). Self illuminating QD luminesces without external excitation by coupling QDs to a modified luciferase. The energy released by luciferase catalysis is transferred to QDs via resonance energy transfer resulting in emission from QDsand can be applied to bioimaging systems. QD functionalized nanoparticles' surfaces with the enzymes glutamate dehydrogenase and lactate dehydrogenase makes use of them for cell membrane staining. Other fluorescent biomarkers are lanthanides as sources of fluorescence in luminescent assays with several important advantages (good stability, low background luminescence under normal light conditions and large Stoke's shift) better than fluorophores. These nanostructured materials can be useful for biotagging application as NIR light strongly reduces the autofluorescence background of the biomaterials in the visible range. Compared with the traditionally used down-conversion fluorescent organic dyes and quantum dots, conceivable advantages of NIR-to-visible upconversion fluorescent bioprobes include an improved signal-to-noise ratio due to the absence of autofluorescence and reduction of

light scattering and the noninvasive excitation of NIR light that falls within the "water window" (the gap in the absorption spectrum of tissue between chromophores(< 800 nm) and water (> 1200 nm)). In vivo imaging can be easily achieved because of the ability of NIR radiation to strongly penetrate tissue. Photobleaching can be greatly reduced because of the resistance to photobleaching of these inorganic particles. Multiple labeling can also be achieved by fluorescent particles with different emissions under the same excitation. In recent years, a large number of reports have been published on lanthanide (III) doped nanostructured materials because of their potential applications in proteins labeling.

3.3 Magnetic resonance imaging

MR imaging is one of the most powerful non-invasive imaging modalities utilized in clinical medicine today [600-607]. MR imaging is based on the property that hydrogen protons will align and process around an applied magnetic field, B_0, which monitored by the nuclear magnetic resonance (NMR) spectrometer. Upon application of a transverse radiofrequency (rf) pulse, these protons are perturbed from B_0. The subsequent process through which these protons return to their original state is referred to as the relaxation phenomenon. Two independent processes, longitudinal relaxation (T_1-recovery) and transverse relaxation (T_2-decay), can be monitored to generate an MR image. Local variation in relaxation, corresponding to image contrast, arises from proton density as well as the chemical and physical nature of the tissues within the specimen. Exogenous contrast agents are generally introduced to enhance the tissue contrast, including complexes of Gd^{III} and magnetic nanoparticles. Complexes of Gd^{III} in liposomes or micelles are widely used as a MRI contrast agents; however, these systems suffer from drawbacks such as Gd^{III} ion exchange with endogenous metals (e.g., Zn, Cu), and uptake of complexes in extra vascular space. Magnetic and fluorescent silica microspheres fabricated by incorporating magnetic (g-Fe_2O_3) nanoparticles and CdSe/CdZnS core/shell QDs into a silica shell around preformed silica microspheres. The monodisperse, cross-linked iron oxide nanoparticles provide non-toxic MRI contrast agents. These iron oxide nanoparticles are superparamagnetic (SPION) in nature. The cross-linked iron oxide nanoparticles are highly stable, and convenient ''clickable'' nanoparticles have been used for targeted imaging with high cellular uptake. This superparamagnetic behavior of iron oxide nanoparticles play an important role as MRI contrasts agents, to better differentiate healthy and pathological tissues. Recent developments in MR imaging have enabled *in vivo* imaging at near microscopic resolution. In order to visualize and track stem and progenitor cells by MR imaging, it is necessary to tag cells magnetically. Tat protein-derived peptide sequences are an efficient way of internalizing a number of marked proteins into cells. Biocompatible magnetic particles could be derivatized with similar sequences and that entire particles could be efficiently ferried into haematopoietic and neural progenitor cells in quantities upto 10–30 pg of superparamagnetic iron per cells. Iron incorporation did not affect cell viability, differentiation, or proliferation of CD34+ cells. Following intravenous injection into immunodeficient mice, 4% of magnetically CD34+ cells homed to bone marrow per gram of tissue, and single cells could be detected by MRI in tissue samples. In addition, magnetically labelled cells that had homed to the bone marrow could be recovered by magneticsep aration columns. Trans gene expression can be visualized noninvasively by MRI *in vivo*. Conjugated human holo-transferrin to iron oxide nanoparticles increases in receptor levels at the cell surface and cause considerable changes in MRI signals. These superparamagneticiron oxide nanoparticles are relatively non-toxic while iron oxide core is biodegradable. Iron oxide nanoparticle degradation theoretically allows multiple imaging of transgene expression over time.

The availability of a universal MR marker gene to image gene expression could be particularly important in monitoring gene therapy, in which exogeneous genes are introduced to ameliorate a genetic defect or to add an additional gene function to cells, and construction and testing of such vectors is currently under way. The desired strategy can also be used to image endogenous gene expression during development and pathogenesis of disease. With advances in establishing transgenic mouse models, an animal line might be developed with an imaging marker gene under the control of a given promoter under study, so that the promoter activity can be directly visualized. The work opens an exciting avenue for developing additional and complementary strategies to image gene expression in deep organs by MRI. Magnetic nanoparticles have been used to detect apoptosis by MRI. Apoptosis is an active process of cellular self-destruction that plays an important role in number of disorders including neurodegenerative diseases, cerebral and myocardial ischaemia and organ rejection following transplant. Therapeutic treatment of tumour cells in vivo results in changes in MR image contrast that are thought to reflect the morphological features of apoptosis, such as cell shrinkage and membrane blebbing. The C2 domain of synaptotagmin I, which binds to anionic phospholipids in cell membranes binds to the plasma membrane of apoptotic cells by both flow cytometry and confocal microscopy. Administration of C2-SPION can lead to significant increases in image contrast in those regions of a tumour containing relatively large number of apoptotic cells. Conjugation of the protein to SPION is detectable by MRI. Apoptotic cells in vitro, with isolated apoptotic tumour cells and in vivo in a tumour can be MRI visible. The MRI technique can detect apoptosis at an early stage in the process and has the advantages over other methods such as magnetic resonance spectroscopy and radionuclide techniques, that it can detect apoptoticregions with relatively high spatial resolution. The SPION label is highly sensitive to MR detection and is also relatively non-toxic. SPION has been approved for clinical use as a blood pool agent for MRI.

4. CONCLUSION AND FUTURE PROSPECTS

Different imaging molecules with potentials of imaging are explored as nanowires, nanobelts, nanorods, nanotube and nanodisks in broad range applications in biomedical sciences including biosensors (enzymatic biosensors, DNA biosensor, pH sensor and immunosensors), drug delivery and bioimaging engineered for possible biomolecular recognition, therapeutic delivery, biosensing, and bioimaging. Nanoparticles have already been used for a wide range of applications both *in vitro* and *in vivo*.