LECTURE-32

Nanotechnologies in Proteomics

Handout

PREAMBLE

Within very short period of time proteomics research has been established as one of the major focal point for interdisciplinary biological research. One hand proteomics has emerged in other related disciplines of life science; similarly other technologies are also incorporated in proteomics research to improve the capabilities of existing proteomics techniques and overcome their limitations. In this aspect, successful merging of proteomics with nanotechnology has generated an amalgamated platform know as "nanoproteomics". Improvement of sensitivity and multiplexing capability of regularly used proteomics techniques is the prime ambition of this inter-disciplinary approach (Ray et al., 2010; Ray et al., 2011a). While during the last decade quite a few nanostructured materials have been incorporated in proteomics research; quantum particles (QDs), carbon nanotubes (CNTs), Si nanowires (SiNWs), and gold nanoparticles (AuNPs) have attracted considerable interest of research communities, since those are very compatible with MS-based or array-based proteomics and offer multiple advantages, which are very promising for clinical research. In this lecture, we will provide a brief introduction and working principles of different nanotechnological approaches, which are commonly applied in proteomics. Diverse applications, advantages and limitations of those techniques will also be discussed.

OUTLINE OF LECTURE

I. Proteomics and Nanotechnology

II. Promising Nanotechniques and their applications in proteomics

(a) Carbon nanotubes and nanowires

(b) Silicon nanowire field effect transistor (SiNW-FET)

(c) Quantum dots

(d) Gold nanoparticles and nanocages

III. Nanoproteomics: technical advantages over conventional proteomic and immunoassay-based approaches

IV. Challenges

V. Conclusions

Box-1. Terminology

Nanoproteomics: The growing demands of proteomics have led to disciplines like nanoproteomics being evolved to unravel the complex proteome. Nanotechniques have found an increasing number of applications for proteomic studies due to their advantages over conventional approaches such as assay miniaturization, high sensitivity, real-time multiplexed analysis and low sample consumption.

Antibody-coated carbon nanotube: Hollow, cylindrical graphite sheets that exhibit high levels of chemical stability and mechanical strength can be functionalized with suitable antibodies that bind to the protein of interest. These carbon nanotubes have found an increasing number of applications for real-time label-free detection studies. Carbon nanowires, which are made up of multi-walled carbon nanotubes consisting of a long, linear carbon chain inserted into its innermost tube, are also being employed for various proteomic applications.

Conductance: It is a property that measures how easily electricity flows along a certain path through an electrical element.

Si nanowire: Semiconducting silicon nanowires hold great potential for fabrication of biosensors owing to their excellent electrical properties and ability to fine-tune according to the sensitivity requirements. These nanowires are suitable for immobilization with various biological species thereby facilitating their use as extremely sensitive detection devices.

Antibody-coated quantum dots: Inorganic fluorophores known as quantum dots are composed of a semiconductor core surrounded by the shell of another semiconductor having a larger spectral band gap. Excitons of higher energy than this band gap are formed when the quantum dots are excited with incident light. When these excitons return to their lower energy level, there is emission of a narrow energy band, which is monitored during detection of various analytes. These quantum dots can be conjugated to several biomolecules such as antibodies, proteins etc and have offered great potential for sensitive, label-based detection studies.

Gold nanoparticle surface: A resonant coherent oscillation of free electrons is induced at the surface of gold nanoparticles due to an electromagnetic frequency for which the absorption lies in the visible region. This phenomenon is known as surface plasmon resonance and allows the improved optical properties of the nanoparticle surface to be detected by spectroscopic techniques like surface enhanced Raman scattering. Adsorption of a target molecule onto a gold surface can provide improved signal output by augmenting the intensity of Raman-scattered light. Any change in spectra upon binding to the target analyte can be detected and forms the basis for use of gold nanoparticles in detection studies.

I. PROTEOMICS AND NANOTECHNOLOGY

In recent years the applications of nanotechnology in proteomics have increased significantly (Ray et al., 2011a; Ray et al., 2011b). While the introduction of nanomaterials in MS-based techniques found to be beneficial for improving detection sensitivity as well as selective enrichment of target proteins and sample preparation; the major goal of applying nanomaterials in protein microarrays is to improve enhanced hybridization speeds and sensitivity. Diverse nanomaterials are useful for MS-based or array-based proteomics and incorporated as per the requirement of the researchers. Current progresses in nanotechnology leading to the development of novel nanomaterials with attractive features, makes it very promising for proteomics research. Proteomics aims for comprehensive study of entire set of proteins of an organism at a particular condition, and often experience poor coverage of complex proteome due to the inadequate dynamic range and sensitivity of the techniques. In this aspect, application of nano-sized devices or materials in proteomics is highly attractive since those can offer numerous advantages such as superior limit of detection, broader dynamic range, improved sample processing, better accuracy, real-time sensing and multiplexing capacity.

II. PROMISING NANOTECHNIQUES AND THEIR APPLICATIONS IN PROTEOMICS

(a) Carbon nanotubes and nanowires

Carbon nanotubes (CNTs) are composed of thin cylindrical graphite sheets; which exhibit fast electron transfer capabilities and high surface area-to-weight ratio (Hersam 2008). Electrical Conductance of the device is altered when the target proteins binds to the functionalized CNTs. Real-time detection of target molecules is conducted by measurement of the change in conductance (Fig 1).

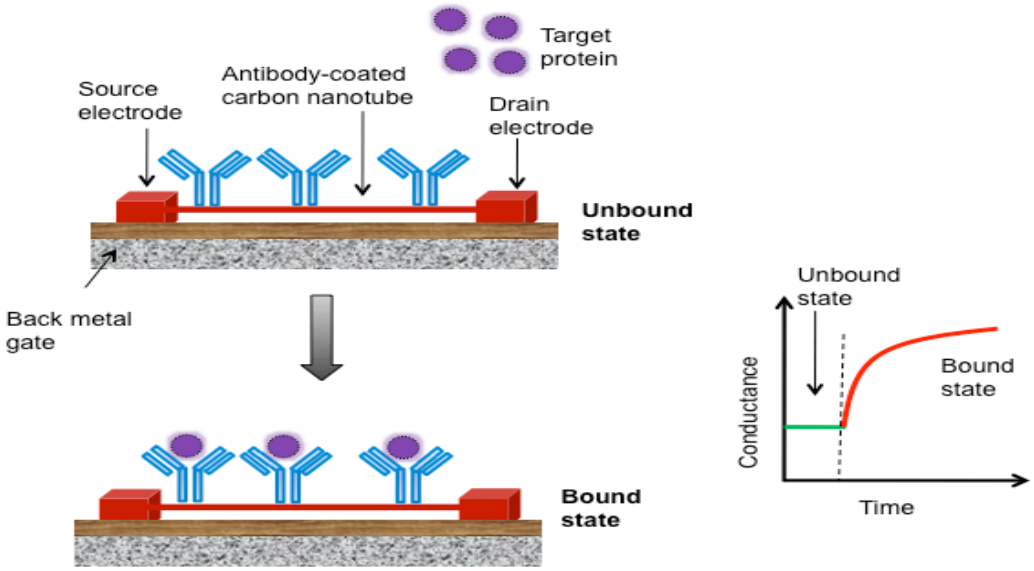

Fig 1. Working principle of carbon nanotubes (CNTs). CNTs contain thin cylindrical graphite sheets, show rapid electron-transfer abilities and high surface area-to-weight ratio. Binding of target proteins to the functionalized CNTs leads to alterations in electrical conductance of device, which is measured for real-time detection of target analytes.

Due to the following properties carbon nanoparticles are used for biosensing;

• Outstanding potential for signal amplification

• Fast electron-transfer capabilities and compatibility for multiplex analysis

• High surface area-to-weight ratio for making ultra-compact devices

• High level of selectivity for biomolecules after functionalization

• Superior chemical stability and mechanical potency

• Change conductance upon binding of charged macromolecules

Illustration: Carbon nanotubes

Carbon nanotubes are hollow cylindrical graphite sheets that exhibit very high levels of chemical and mechanical stability. These nanotubes can be suitably functionalized with antibodies or other agents like aptamers. Antibody-coated nanotubes show no variations in conductance with time when they are in the unbound state. Binding of target protein to the antibody is detected by a change in conductance of the carbon nanotube with time. These devices have been extremely useful for real-time, label-free detection of low abundance proteins and analyte and can achieve sensitivity in the nM-pM range.

(b) Silicon nanowire field effect transistor (SiNW-FET)

Silicon nanowires are semiconducting particles, very apt for the immobilization of biological or chemical molecules. SiNWs-based sensing approaches detect selective binding of the target analytes to the functionalized NWs by measuring variations in conductance (Fig 2) (Cui et al., 2001; Patolsky et al., 2006).

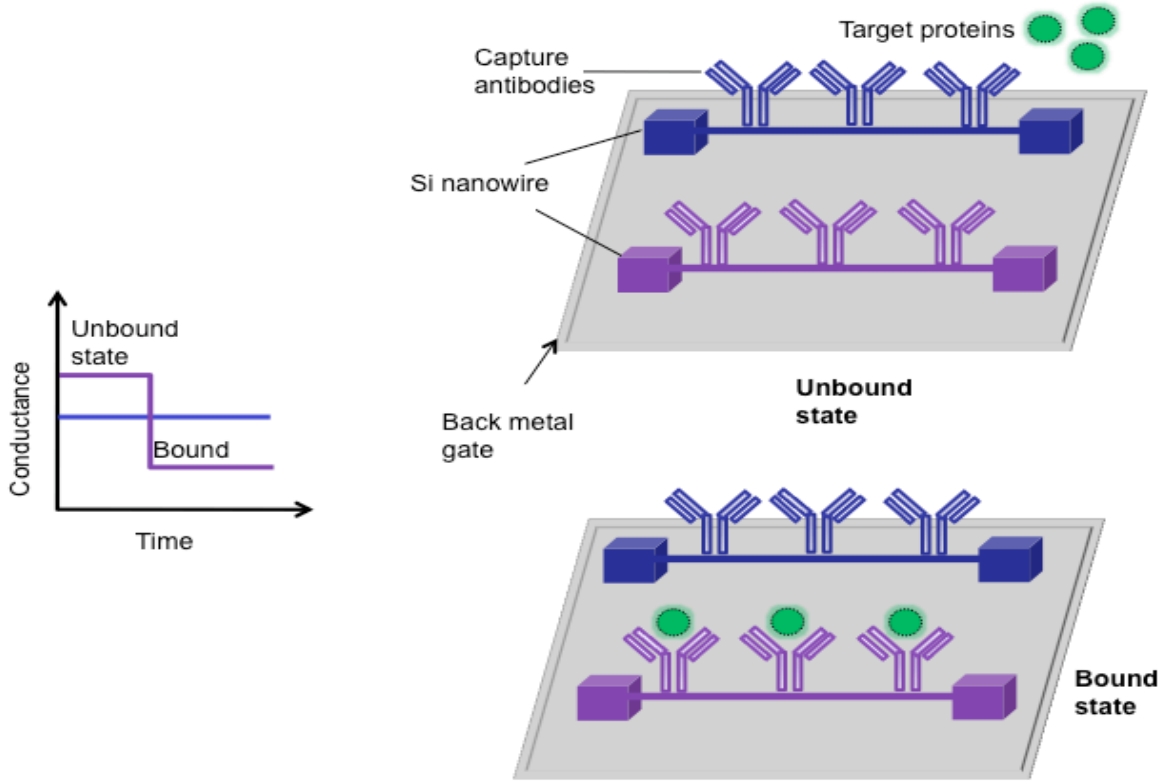

Fig 2. Working principle of silicon nanowire (SiNW). Selective binding of target analytes to the functionalized NWs leads to the alterations in the conductance of the device, which is measured for detection of biomolecules.

Due to the following features SiNWs are considered as potential candidates for biosensing;

• Tremendous potential for signal amplification and compatible for multiplex analysis

• Rapid electron-transfer capabilities and allows real-time detection

• Apt for the immobilization of various types of biological or chemical molecules

• Minute size and huge surface area-to weight ratio

• Alterations in conductance upon binding of charged macromolecules

Illustration: Silicon nanowire-field effect transistors

Silicon nanowires can be functionalized with several chemical and biological species and used as sensitive detection devices. Antibodies are commonly immobilized on silicon nanowires for detection of protein antigens. No change in conductance of the nanowire is observed in the unbound state. Binding of target protein to the antibody is detected by a change in conductance of the Si nanowire over time while the conductance of the unbound antibody-functionalized nanowire remains unaltered. These devices offer excellent sensitivity in the pM-fM range and are capable of detecting molecules even at a single particle level.

(c) Quantum dots

Quantum dots (QDs) are inorganic flourophores, whose excitons are confined in all three spatial dimensions. QDs are semiconductor in nature and attractive labeling mediator for the sensitive detection of target proteins in HT format. Detection of target proteins are performed by monitoring the emission spectra of bound QDs (Fig 3) (Azzazy et al., 2007; Resch-Genger et al., 2008).

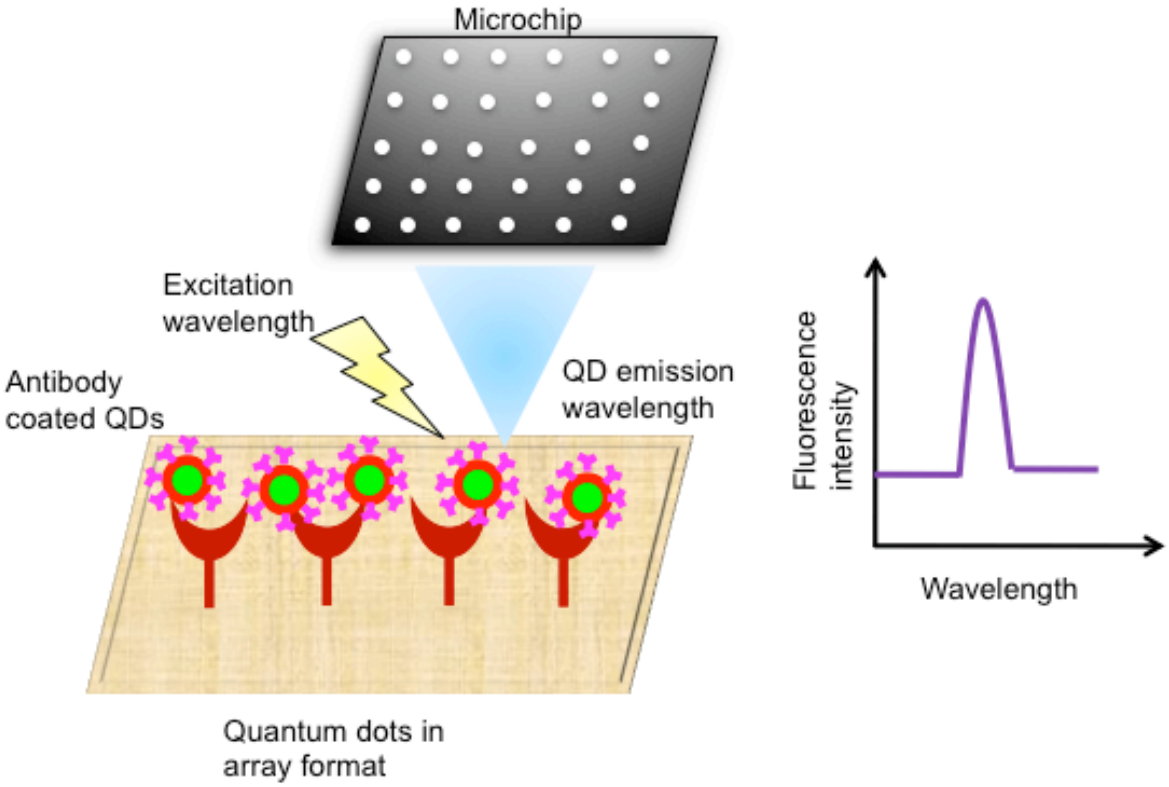

Fig 3. Working principle of quantum dots (QDs). Biomolecules can be labeled using quantum dots (QDs). Monitoring of emission spectra of bound QDs allows real-time

detection of the targets analytes. Fluorescence Resonance Energy Transfer (FRET) between fluorescence acceptors and a QD donor generates fluorescence emission.

Due to the following properties QDs can act as an effective signal amplifier:

• Wide range of excitation and stable optical properties

• Size-tunable narrow and symmetric emission spectra

• High photostability and fluorescence quantum yields, fluorescence lifetimes: 10-100 ns

• Surface chemistry controls stability and bigger surface area

• Suitable for labeling biomolecules and single molecule analysis

• Semiconducting nature and resistant to chemical and thermal effects

Illustration: Quantum dots

Changes in emission wavelength upon binding to the antibody-conjugated QDs are recorded by the microchip and used for detection of various biomoleules. QDs are capable of detecting molecules down to fM levels and provide significant advantages over conventionally used organic fluorophores.

(d) Gold nanoparticles and nanocages

Gold nanoparticles (AuNPs) possess unique optical, electronic and catalytic properties and can efficiently immobilize biomolecules, which makes them suitable for biosensing (Fig 4) (Boisselier and Astruc, 2009). They are also well suited for HT applications in array design.

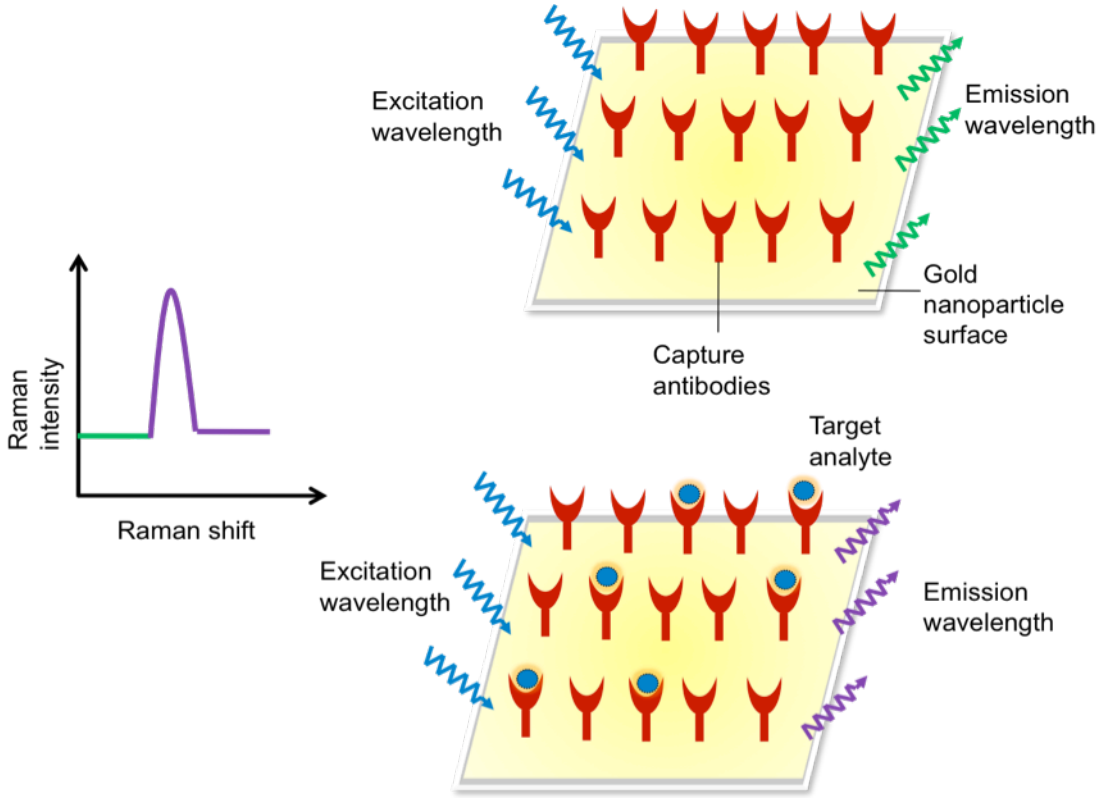

Fig 4. Working principle of gold nanoparticles (AuNPs). AuNPs are compatible for selective and ultrasensitive detection of protein biomarkers due to their unique optical, electronic and catalytic properties and signal enhancement capability. AuNPs can amplify signal effectively if introduced in electronic biosensing process.

Due to the following properties AuNPs can act as an efficient signal amplifier:

• Much smaller than the wavelength of light

• Strong scattering and absorption peaks in the near-infrared region

• Narrow spectral bandwidth

• Excellent prospective for signal amplification and compatibility for multiplex HT analysis

• Potential optical probes for reflectance-based optical imaging

• Binding of biomolecules leads alterations in spectra of scattered light

• Ability to conjugate with antibodies or peptides and homogeny in target binding

Illustration: Gold nanoparticles

The gold nanoparticle surface is functionalized suitably by antibodies, which binds to the analyte of interest. Any target binding is depicted by a change in the emission spectra. The analyte of interest is detected by the antibody-functionalized gold nanoparticle surface. Changes in the emission spectra get recorded indicating binding. Gold nanoparticles thus form a very useful and sensitive label-free detection system that can detect particles down to pM levels.

Table 1. Different applications of nanoproteomics approaches

Technique	Applications
(a) Carbon nanotubes and nanowires	• Diagnosis of cancer (Peng et al., 2008) • Autoimmune diseases detection (Chen et al., 2008) • Human body fluids analysis (Drouvalakis et al., 2008) • Microbial toxins deactivation of (Joshi et al., 2008) • Biological detection and imaging (Chen et al., 2004) • Analysis of drug effect (Goyal et al., 2011)
(b) Silicon nanowire field effect transistor (SiNW-FET)	• Detection of cancer biomarkers (Zheng et al., 2005; Lee et al., 2009) • Detection of cardio-vascular biomarkers (Lin et al., 2010) • Detection of bacterial toxin (Mishra et al., 2008) • Detection of virus particles (Patolsky et al., 2004) • Therapeutics (Brammer et al., 2009) • Study of small molecule interactions (Wang et al., 2005)
(c) Quantum dots	• Diagnosis of cancer (Wu et al., 2003) • Cancer theranostics (Singh 2011) • Diagnostic imaging (Gao et al., 2004) • Tissue specimens profiling (Gao et al., 2003) • Immunohistochemistry (Xing et al., 2007) • Virus detection (Zhang et al., 2010) • Biosensing (Zhang et al., 2005)

(d) Gold nanoparticles and nanocages	• Cancer markers detection (Kim et al., 2010) • Photothermal destruction of cancer cells (Cobley et al., 2010) • Immunosensing (Nam et al., 2003; Ahirwal et al., 2010) • Drug effect analysis (Goyal et al., 2011) • Drug delivery (Cobley et al., 2010)

Some of the interesting applications of nanoproteomics are illustrated here.

Illustration: Application - Immunological studies

Ref: Lee et al. Biosens Bioelectron. 2009; 24(6):1801-5.

Lee et al. (2009) innovatively made use of aptamers as recognition elements for real-time, label-free detection of cancer markers. These RNA aptamers showed specificity for binding to VEGF, a useful cancer marker. Binding of VEGF to the anti-VEGF RNA aptamer on the silicon nanowire surface brought about localized charge transfers, which in turn caused a change in current. This localized current change was detected and was useful for monitoring such binding events.

Illustration: Application – Protein interaction studies

Ref: Chen et al., 2005. Chembiochem. 6, 1169-1173.

Gold nanoparticles functionalized with suitable carbohydrate ligands were used by Chen et al. (2008) for targeting specific proteins from a mixture. They carried out separation of galactophilic lectins from Pseudomonas aeruginosa by binding galactose and another carbohydrate (P^k antigen) to the gold nanoparticle surface. The target lectin proteins bound selectively to the carbohydrate ligands on the surface of the gold nanoparticles. Proteolytic enzymes were then added for digestion of the bound target proteins. The enzymes cleaved the bound proteins into peptide fragments, some of which remained bound to the NP while others went into solution. The authors carried out direct on-probe MS analysis using MALDI-TOF-TOF following separation of the nanoparticles by centrifugation. A clean MS profile was observed with no significant peaks being observed due to the NPs, indicating the tremendous potential of nanoprobe-based affinity mass spectrometry.

Illustration: Application – Biomarker detection

Ref: Chen et al., 2008. Nat Biotechnol. 26, 1285-1292.

The proteinase 3 antigens were captured on a gold-coated microarray surface. Chen et al. (2008) successfully detected autoantibodies in human serum against proteinase 3, a clinically important biomarker for diagnosis of the autoimmune disorder Wegener's granulomatosis. SWNT conjugated to GaH-IgG were used for detection of antigen-antibody binding. Multiplexed detection of more than one antigen was successfully achieved by using multicolored Raman labels. Anti-human and anti-mouse IgGs were bound to ^{13}C and ^{12}C SWNTs respectively. Rapid, protein detection was possible through excitation with 785 nm laser followed by comparing the Raman scattering intensity at their respective maxima, which showed very less cross-reactivity.

III. NANOPROTEOMICS: TECHNICAL ADVANTAGES OVER CONVENTIONAL PROTEOMIC AND IMMUNOASSAY-BASED APPROACHES

The detection limits of different nanoproteomics, conventional proteomics and immunoassays-based approaches for detection of two cancer markers; prostate-specific antigen (PSA) and carcinoembryonic antigen (CEA) was compared from different published articles, which suggested that use of nanotechniques in proteomics improve the sensitivity multiple orders of magnitude compared to the proteomics and immunoassays-based methods (Ray et al., 2011a). Increase in sensitivity is particularly attractive from diagnostic point of view; since most of the disease biomarkers are very low abundance proteins and their detection are frequently limited by the sensitivity of techniques.

Besides sensitivity and multiplexing ability, application of nanotechnology in proteomics allows label-free, real-time detection and helps to get rid of the need for any secondary-labeling agents. Additionally, the applications of diverse nanoparticles in regular immunoassays and proteomics can efficiently reduce the assay time.

IV. CHALLENGES

Although incorporation of nanomaterials in different proteomics technologies are found to be useful for improving hybridization speeds and sensitivity, and selective enrichment of low-abundance analytes; however, there are multiple concerns and basic limitations associated with this emerging field (Ray et al., 2010; Ray et al., 2011a);

• Toxicity, biosafety and biocompatibility are major concerns regarding use of nanostructured materials (Fan and Alexeeff 2010)

• Possible adverse effect of nanomaterials on environment (Oberdörster 2010)

• Inadequate knowledge on precise mechanism of action

• Insolubility of nanomaterials in biologically compatible buffers

• Short lifetime of nanomaterials

• Presence of metallic impurities in synthesized nanomaterial, which reduce their efficacy

• Difficulties in determination of purity of synthesized nanotubes and nanowires

• Lack of easy, robust, fixed low-cost surface modification techniques

V. CONCLUSIONS

Incorporation of different approaches from other disciplines in proteomics are found to be advantageous and useful to resolve many existing limitations of this field, particularly when detection of extremely low abundance analytes or very weak biomolecular interactions are concerned. To this end, coupling of nanotechnology with proteomics have successfully enhanced the dynamic range and improved sensitivity to allow selective detection of low-abundance analytes down to aM concentrations, which is highly promising from diagnostics point of view. Nevertheless, use of nanotechniques in proteomics is not used in routine proteomic analyses, primarily due to the possible adverse effect of the nanomaterials on human health and environment. Since the nanoproteomics have shown immense potential in proof-of-principle experiments, now the focus of coming decades should be to strengthen the promising amalgamated discipline by overcoming the existing limitations.

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