LECTURE-38

NANOTECHNIQUES IN PROTEOMICS

TRANSCRIPT

Welcome to proteomics course. In today's lecture we will talk about nanotechniques in proteomics. In the last few lectures we talked about different type of proteomic technologies. We started with conventional get based techniques, we moved on to mass spectrometry, different type of quantitative proteomics using MS. We then discussed about microarrays, different type of microarray platforms including conventional protein microarrays and cell free expression based protein microarrays. Then we started discussing about different type of label-free techniques. In the last few lectures we focused more on new and different type of label-free methods such as SPR, SPRi, ellipsometry, interferometry and different type of platforms. In the same theme and same continuation today we will talk about different type of nanotechniques, how they can used for various type of proteomic application. We will discuss more on their principle, some of the advantages and disadvantages which each of these methods offer and very briefly we will touch upon how these nanotechniques can be applied for proteomics based applications?

So in the previous lecture just to refresh you, we discussed comparison of label-based and label-free techniques, we talked about advantages and disadvantages of each of these types of detection techniques. We then focused more on specific label-free techniques such as SPR, ellipsometry based label-free techniques and interferometry based label-free techniques. Now today we will talk about some of the nanotechniques which are applied for proteomic applications. The nanotechniques offer several advantages over the conventional proteomic techniques such as miniaturization of assay, real time multiplexing capability, low sample and reagent consumption very high sensitivity and quicker assaying time. There are several nanotechniques such as carbon nanotubes and nano-wires, quantum dots, gold nano particles, silicon nano-wire field effect transistors which are now increasingly being used for various proteomic

applications. These applications include biomarker discovery, immunoassays, label-free detection as well as different type of biomolecular interaction studies including protein-protein interactions. Successful integration of nanotechnologies with proteomics has now introduced a new field in this clinical research area known as nanoproteomics. This is one of the very rapidly emerging areas for the biomedical research, which may have ultimately tremendous therapeutic potential.

So in today's lecture we will talk about different nanotechniques which are applied for proteomics applications. We will talk about carbon nanotubes (CNTs) and nano-wires (CNWs), carbon nano tube field effect transistors (CNT-FETs), quantum dots (QDs), gold nanoparticles and nanocages, microfluidics. Then there are many new emerging techniques which are also being used for proteomics, very briefly we will touch those techniques as well. Then further we will discuss about challenges of this new field nanoproteomics. How integration of nanotechniques and proteomics has still various challenges and how one can overcome those challenges?

So let's first talk about carbon nanotubes and nanowires. There are various novel inorganic nanomaterials which have been explored in biological research with an intension of developing new types of analytical tools. Due to the rapid advances in synthesis and surface chemistry optimization process there are various classes of nanostructures including nanowires, nanotubes and nanocrystals have been used for the clinical proteomics research. CNTs or CNWs, they detect changes in the electrical conductance after the target binding and they show sensitivity in the nano molar to pico molar range.

So what are CNTs? These are hollow, cylindrical graphite sheets which show high chemical stability and mechanical strength. The CNTs and nanowires they offer very unique properties electrical, thermal as well as spectroscopic properties. These various unique features of CNTs and CNWs have opened up new perspectives for various proteomics applications.

These carbon nanotubes, there are two different types- single-walled nanotubes (SWNT) and multi-walled nanotubes (MWNT). SWNT are composed of a single graphite layer with the diameter in the range of 0.5-2 nm whereas the MWNT are composed of several concentrically arranged cylindrical layers. There are various properties for each of this group but we will more focus on how these nanotubes are applied for proteomic application.

The SWNT are well defined electrical and mechanical properties which make them promising candidates for biosensors. The applications of SWNTs to proteomics relies on conductance change. When a target protein is functionalized on SNWT device after that a binding partner binds then electrical conductance changes and that is measured for the monitoring the change in the binding.

These carbon nanoparticles have various properties. This is the broad field; these are general properties for nanoparticles-They have very high potential for signal amplification. They have fast electron-capability, they exhibit very high surface area to weight ratio, they can selectively bind with biomolecules after functionalization and this selective binding with very high sensitivity is the key advantage of using these nanotechniques. They exhibit high chemical stability and mechanical strength as we discussed earlier and then changes in the conductance upon binding of the charged macromolecules are monitored for these binding reactions.

Now in this slide, I am showing you the image for CNT which has a functionalized antibody the top one is showing the unbound state where you have source electrode, drain electrode and there are some target proteins after binding which is shown in the bottom panel there is a change in the conductance which is shown in the right panel. The binding of the target protein to the functionalized carbon nanowires lead to detectable changes in electrical conductance, this is very sensitive. And this change in conductance is measured for measuring different type of proteins as well as some small particles including viruses. There are various applications have shown the potential of this technology.

Now let's discuss merits and demerits of using CNTs and CNWs-

They allow real time analysis similar to what we have talked previously about some of the label-free techniques including SPR where we have opportunity to monitor the reaction in the real time which is not the case with the label-based techniques which are mostly end point assays. Now here we can monitor how reactions are changing and one can actually change the experimental conditions in the real time to modify the binding. This also provides the multiplexing capability and gives the miniaturization. There are various demerits as well- metallic impurities can reduce activity of these assays. It is not very easy to determine how pure these preparations are so degree of purity is one of the limitations here. One of the other major limitation is their insolubility in biologically compatible buffer they are not are compatible in the various biological buffers.

To demonstrate the principle of using CNTs and CNWs now let's look at this animation-

Carbon nanotubes-The carbon nanotubes are hollow, cylindrical graphite sheets which exhibit high chemical stability and mechanical strength. As I have shown here there is a drain electrode, source electrode and on top of carbon nanotube antibodies are functionalized so these nanotubes can be suitably functionalized by nanotubes or other agents like aptamers. The antibody coated nanotubes show no variation in the conductance when they are in the unbound state, the binding of the target protein to the antibody is detected by a change in conductance of the carbon nanotubes with time. As you can see in the right panel the time on the X-axis and conductance spotted on the Y-axis which is showing a change in the conductance due to the binding state of these target proteins. These nanotubes devices have been extremely useful for real time label-free detection of low abundance protein and analytes and it can achieve sensitivity from nano molar to pico molar range.

So now after looking at the animation you are clear with the principle of using these nanotubes. Now let's discuss briefly about various applications. So CNTs and CNWs have shown unlimited potential for various applications in different field. Here we are just talking in the context of proteomics so for the proteomic applications various type of

clinical studies people have performed and used nanotubes and nanowires to test the potential of these nanotechniques for cancer biomarker detection, it has also been used for autoimmune disease detection, direct assaying of human serum, deactivation of toxins, biological detection and imaging. This is very small list of unlimited applications which have emerged out of these potential techniques.

Let's now move on to another platform which is carbon nanotubes field effect transistors (CNT-FETs).

The successful combination of carbon nanotubes with field effect transistors has led to the development of this novel sensing device known as CNT-FET.

As we have discussed different type of carbon nanotubes SWCNT and MWCNT. The SWCNTs exhibit semiconductor properties are optimal to design the CNT based electrical sensors. The functionalization of CNT-FET with specific receptors brings about binding of the desired target biomolecules. For example we can immobilize surface with specific receptor antibody and then target proteins can be used to detect the binding. If the biomolecules are showing binding then that binding can be monitored by looking at the change in the conductance. So the conductance alteration of CNT-FET occurs due to the charge modification of bound molecules.

Again there are many applications of CNT-FETs, here we looking at few based on proteomics applications. This platform has been applied for detection of immunoglobulins, study of antigen and antibody reaction, detection of various cancer markers and pathogen detection. So one of the interesting topics is bio-defense and how one can detect various types of pathogen which have potential for biohazards. So different type of bioterrorism attacks usually have taken attention specially on the anthrax and different type of SARS based biological agents so these nanotechniques have shown very specific detection of various pathogens and now those also have been applied for very potential application for bio-defense field.

Let's now discuss about merits and demerits of CNT-FETs.

This technology offers very high sensitivity. It provides real time measurement capability. One can monitor the label-free environment where there is no need of adding a label. This is very robust platform and also cost effective and it provides very high speed, extremely rapid. There are many demerits of CNT-FETs which include the lack of simple, flexible, well-established surface modification methods. When we talk about application of these nanotechniques with microarray platform then this particular platform is difficult to construct for the high density arrays. Again in the proteomics field there is a increasing trend, increasing interest to apply various type of label-free techniques as well as nanotechniques and couple them with the high density, high throughput array platforms so that one can not only monitor the reactions in the very low volumes, in the real time, in the label-free environment but also identify and study thousands of biomolecules at the same time on the same chip platform.

Let's now discuss silicon nanowire field effect transistors (SiNW-FETs). The planer field effect transistors modified with molecular receptors for the analyte and monitor binding of charged species through the accumulation of carriers on the surface of transistors although this transistor directly into readily measurable changes in conductance or related electrical properties. The physical parameters pose constrains for sensor fabrication nonetheless local FETs have overcome those limitations to large extent but they have their own drawbacks. For example the presence of the metallic nanotubes and lack of well established surface modification techniques. The silicon nanowires which are always semiconducting are potential building blocks. To fabricate functional nanosensors that overcomes some of these limitations which we just discussed, In addition they allow sensitivity to be fine tuned as per the requirement by controlling the type and amount of dopent in the semiconductors. Let's discuss some of the properties of the silicon nanowires.

They have very high potential for signal amplification and have fast electron-transfer capability. Silicon nanowires are suitable for the immobilization of various biological or chemical species. They are small in size and have large surface area to weight ratio.

Again the change in the conductance is measured here upon binding of charged macromolecules.

Now let's discuss how this silicon nanowire field effect transistors (SiNW-FET) are applied for some of the proteomics based applications. As you can see in this slide the top panel is showing silicon nanowires which are coated with the antibody receptors. Now when you want to study some target proteins and if there is any interaction of target proteins with the antibodies so as shown in the bottom panel these target proteins will bind to antibody receptors and now these silicon nanowires will show the change in the conductance. As you can see in the right panel where conductance is plotted on the Y-axis and time is on the X-axis

In proteomics detection of low abundance proteins remains one of the major challenges; so many high throughput proteomic techniques such as protein microarrays provide high sensitivity. They provide very high sensitivity but when we are screening with the biological samples, biological fluids detection of very concentration biomarker in a very small sample volume remains very challenging. If you remember the previous discussion we have talked about how from the serum the various types of high abundant proteins which are present one need to remove those proteins, deplete those serum to actually look for the low abundant proteins but there are many high abundant proteins which are present which mask the overall low abundant proteins. Similarly these are present in so low amount the technique has to be very sensitive. Therefore various label-free detection techniques are aiming towards detection of low abundance proteins and by using CNTs and CNWs various studies have shown the potential of using these nanotechniques for such applications where low abundance proteins can be detected with high sensitivity. So CNT based nanosensors have shown their use and applications to target very low abundance protein analytes and this is one of the major advantages of using these platforms.

So continuing on SiNW-FET, their different merits and demerits-

This platform provides real time measurement capability also it offers multiplexing analysis. It is uniform and provides reproducible results for various biomolecular detections and it shows very high specificity. Now discussing about demerits of this platform, the lack of simple, flexible, well-established surface modification methods is one of the limitation and it also not vey suitable for systematic studies.

Despite some of the challenges and some of the demerits of SiNW-FETs they have been applied for various types of studies in proteomics area including detection of cancer, detection of small molecules, studying small molecular interactions, detection of virus particles, different types of bio-sensing studies also have been performed and again similar to viral particle detection different type of bacterial toxin detection have been tested by using this technique. So overall many therapeutic based studies have shown the potential of SiNW-FETs which can be applied for cancer biomarkers and as well as other diseases.

So let's now look at this animation to understand the principle of SiNW-FETs more clearly.

SiNW-FETs-

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 the conductance of nanowires is observed in the unbound state as you can see in the right hand panel of the graph which is plotted between time and conductance. Binding of target protein to the antibody is detected by change in the conductance of silicon nanowires over time while the conductance of the unbound antibody functionalized nanowire remains unaltered. These devices offer excellent sensitivity in the pico molar to femto molar range and are capable of detecting molecules even at single particle level.

After discussing the principle of SiNW-FETs, let's now move on to quantum dots (QDs).

The QDs have shown wide variety of promising applications in different areas of biological sciences such as immune assays, nucleic acid detection, analysis of genetic polymorphism, diagnostic imaging, single molecule detection, cellular localization studies, drug delivery as well as discovery and proteomic and genomic studies. So you can realize it offers very wide range of applications in different field of biosciences. The superior optical properties and ability to conjugate with several biomolecules like peptides and nucleic acids and antibodies have established QDs as sensitive detection tool in clinical proteomics research.

So what are these QDs? These are semiconductors whose excitons are confined in three spatial dimensions. These are inorganic fluorophores that exhibit size-tunable emission, strong light absorbance, bright fluorescence, narrow symmetric emission bands and high photo stability. The long life, high chemical and thermal stability, resistance to photo-bleaching, ability to provide better fluorescence quantum yields of QDs have overcome several basic limitations of traditionally used organic fluorophores and that's why they have become a fluorophore of choice and applied for unlimited applications in different biological fields.

What are different properties of QDs? They have broad-range excitation, size-tunable narrow emission spectra, and symmetric emission spectra. The fluorescence life time ranges between 10-100 nano seconds. The surface chemistry controls the stability. It is suitable for labeling of variety of biomolecules. It is applied as semiconductors.

In this slide one of the applications of QDs is shown?

The application of semiconductor QDs as labeling agent sensitive detection of target proteins in an array format is shown here. The protein analytes are detected by monitoring the emission spectra of QDs. The excitation of QDs with incident light leads to formation of excitons due to the absorbance of a photon with higher energy than that of a band gap of component semiconductor and this fluorescence intensity is measured as shown on the right hand panel of the slide.

There are various merits and demerits of using quantum dots? Merits include high fluorescence quantum yield, long life-time, high chemical and thermal stability, resistance to photobleaching and it suitability for single molecular analysis. Its demerits include toxicity for cells, mechanism is not completely understood, and reproducibility of the labels is also limited. The cellular toxicity and biocompatibility there are the major limitations of QDs as there is a limited knowledge on their clearance in the living system. However there is ongoing research to make these QDs more biocompatible by surface modifications including hydrophobicity and including them within the phospholipid micelles. So more advanced research will definitely make them more widely applicable for different applications.

Let's now look at this animation of QDs. The changes in the emission wavelength upon binding to the antibody conjugated QDs are recorded by the microchip and used for detection of various biomolecules. QDs are capable of detecting molecules down to femto molar level and provide significant advantages over conventionally used organic fluorophores. As you can see on the right hand side the wavelength versus fluorescence intensity is plotted and change in the fluorescence intensity is observed when the molecule is binding on the antibody surface which is labeled with the QDs.

So as you have seen its principle is very easy and the same basic principle is applied for variety of applications such as diagnostic imaging, biomarker detection in cancer, studying of DNA-protein interactions, various studies to monitor the protein motion, detection of antigens, different type of tumor biopsy analysis has also been performed. Now, MS based studies have also started exploring QDs. It has been used for carbohydrate and protein interaction studies. These are some of the published studies but there are many studies which have shown the potential of using QDs for various proteomic applications.

Let's now move on to another platform gold nanoparticles and nanocages. The gold nanomaterials have shown versatile biomedical applications due to their attractive structural and physicochemical properties. The size of the gold nanoparticles

determines the proportion of absorption to scattering with the biological imaging requiring a very high cross-section which is achieved by increasing the size of the nanoparticles.

So these gold nanoparticles and nanocages are considered as promising nanomaterials for cancer diagnosis due to their high signal enhancement capability. Gold nanocages which have been developed more recently are nanostructures with porous walls and hollow interiors. They exhibit strong scattering and absorption peaks in near-infrared region.

Various type of surface modifications have been tried for gold nanoparticles by using different type of antibodies as well as molecular labels which can lead to the generation of surface-enhance Raman scattering (SERS) response. These modifications have made Au-NPs or gold nanoparticles suitable for development of immunosensors for selective and ultrasensitive detection of protein biomarkers.

Let's look at different properties of gold nanoparticles and nanocages. These are much smaller than the wavelength of light, they offer strong scattering and adsorption peaks in near-infrared region, they have very narrow spectral bandwidth, high potential for signal amplification and potential for optical probes for reflectance-based optical imaging. The change in spectra of scattered light on conjugation with biomolecules occurs by using these gold nanoparticles and nanocages. They can be easily conjugated to antibodies or peptides.

So these gold nanoparticles exhibit unique optical, electronic and catalytic properties and signal enhancement capabilities which make them suitable for selective and ultrasensitive detection of various biomarker. The implication of gold nanoparticles in electronic biosensing process leads to signal amplification. As shown in this slide, there are monoclonal antibodies which are immobilized on these gold nanoparticles surface which enables specific detection of target proteins.

Now let's discuss surface-enhanced Raman scattering (SERS), the nanotubes functionalized with Raman labeled antibodies.

The surface-enhanced Raman scattering or Raman spectroscopy which lags the sensitivity but silver and gold nanoparticles can be used to enhance the substrate. Signal intensity of Raman active molecules is enhanced due to increased local electric field at the nanoparticle surface and by electronic coupling between the absorbed molecule and the gold nanoparticle surface. As shown here in this slide these are gold nanoparticles which are functionalized with the Raman labeled antibodies now on the right hand side the graph shows change in the Raman intensity due to the binding effect.

There are various merits and demerits of using gold nanoparticles and nanocages. Various merits include its narrow spectral band width, resistance to phobleaching and quenching, simple detection systems, high throughput and multiplexing capability. In vivo molecular imaging is possible by using these nanomaterials. Its demerits include response is highly dependent on shape and size of nanoparticles and detection of molecules in complex solutions is difficult as well as toxicity associated with these nanoparticles.

The gold nanoparticles and nanocages have shown applications in variety of fields specifically in proteomics. They have been used in immunoassay studies, detection of cancer biomarkers, and detection of various biomolecular interactions and photothermal destruction of breast cancer cells. These are some of the published studies which have shown the potential of using gold nanoparticles and nanocages which can be used for proteomic studies.

So let's now look at principle of gold nanoparticles and nanocages in this interactive animation. The gold nanoparticle surface is functionalized suitably by antibodies which will bind to the analyte of interest. Any target binding is depicted by a change in emissions spectra. As you can see on the right hand side Raman intensity versus

Raman intensity is plotted after these gold nanoparticles are binding to the antibodies, a change in the Raman intensity is observed.

Now let's move on to next technology-microfluidics.

So processing of ultra-small fluid volumes with the help of channels of 10-100 μm which gives the sensitivity in the range of pico molar to femto molar,

In microfluidics, they manipulate fluids at nano levels with help of channels having dimensions of tens to hundred of micrometers. So in addition to primary advantages of improved heat and mass transport, microfluidics offers other benefits like low reagent and sample consumption, multiplexed analysis, process automation and enhanced reproducibility of assays.

Microfluidic devices now increasingly applied in the proteomics field and they are coupled with different type of proteomics platforms such as mass spectrometry as well as protein microarrays. There is lot of research going on in this field there is an increasing interest of applying microfluidic devices and coupling them with mass spectrometry. It can provide an excellent platform for highly sensitive and simultaneous analysis of complex proteome.   Another unique application for proteomics is by applying microfluidics is in the field of printing microarrays. Printing DNA, RNA and protein on the chip surface is always challenging and now different type of solutions are offered by microfluidics based systems to enhance the spot to spot reproducibility and lower sample consumptions for printing.

So microfluidics platform offers many merits such as improved heat and mass transport, less reagents and sample consumption, the process is very automated, it also offers higher reproducibility as well as provides capability for multiplexing analysis. The demirits of microfluidics includes the higher cost of the chips, sometimes no specific interactions occur due to the high surface to volume ratio and highly sensitive detection is still needed.

Now briefly look at some of the major applications of using microfluidics in proteomics field. The protein identification is performed by MS, now there is increasing trend of applying microfluidics based systems with MS and microfluidics is applied for sample preparation for MALDI based MS as well as it has been applied for other platforms such as SPR. Even SPR analysis now microfluidics based systems are increasingly used. It has been used for immunological studies and protein interactions, for different type of biomarker discovery including cancer biomarker detection and enzymatic reactors. These are just few microfluidic based applications.

Other than these nanotechniques which we discussed in some detail there are many promising nanotechniques which are also being used for proteomic applications. So there are various nanotechniques at various stages of development they have received increased attention due to their higher potential for proteomic based investigation although the success of these nanotechniques is still very limited but it has potential and significant importance for the future proteomic applications.

These other nanotechniques involve nanomechanical mass spectrometry, nanofluidics, microcantilivers, photonic microring resonators, various types of detection methods which employ inorganic and metal oxide nanoparticles, self assembled cationic peptide nanoparticles and polymeric nanoparticles.

So the study of proteomics which promises to provide solutions to several pathological conditions is still in need for possible new techniques to study the complex proteome. This has got together diverse field of proteomics and nanotechnology and offering a new field known as nanoproteomics. Over the last few years nanotechniques have made significant progress starting from proof of the concept designs to well established and reliable technological platform for handling the complex proteome. So these nanotechniques have been used for several diverse applications such as biomarker discovery, label-free protein detection studying various types of molecular interactions as well as coupling them with MS and protein microarrays. Nanoproteomics field hold great promise to become a technically robust and user friendly platform for clinical and

diagnostic studies. The advantages offered by various nanotechniques have allowed them to be successfully coupled them with rapidly expanding field of proteomics. In summary today we discussed about different type of nanotechniques such as CNTs and CNWs, CNT-FETs, QDs, gold nanoparticles and nanocages and microfluidics. We discussed merits and demerits each of these techniques as well as some of the potential applications. Now there still remain many challenges associated with this field. However by looking at some of these applications it can be concluded that nanotechniques can offer significant advancement in the proteomics research.

Thank you!