Interactomics: Protein Arrays and Label-Free Biosensors.

Professor Sanjeeva Srivastava.
Department of Biosciences and Bioengineering.
Indian Institute of Technology, Bombay.

Lecture 20.

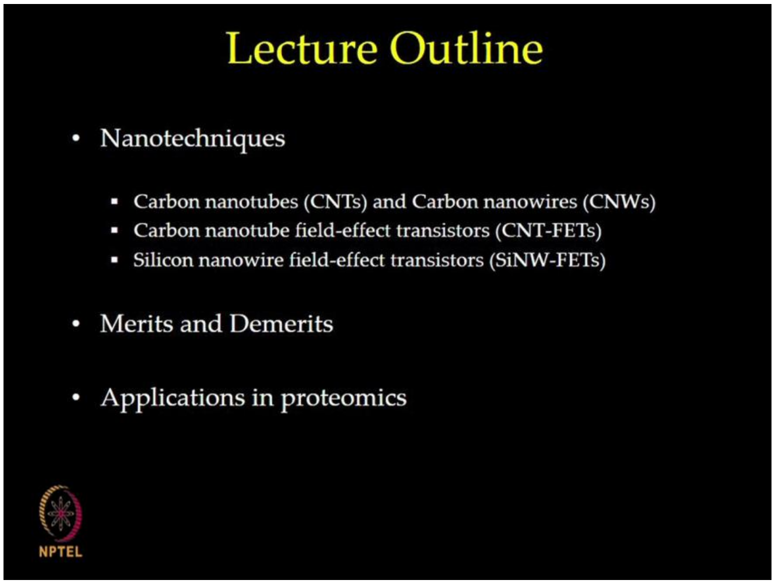
Nanotechniques in proteomics - I

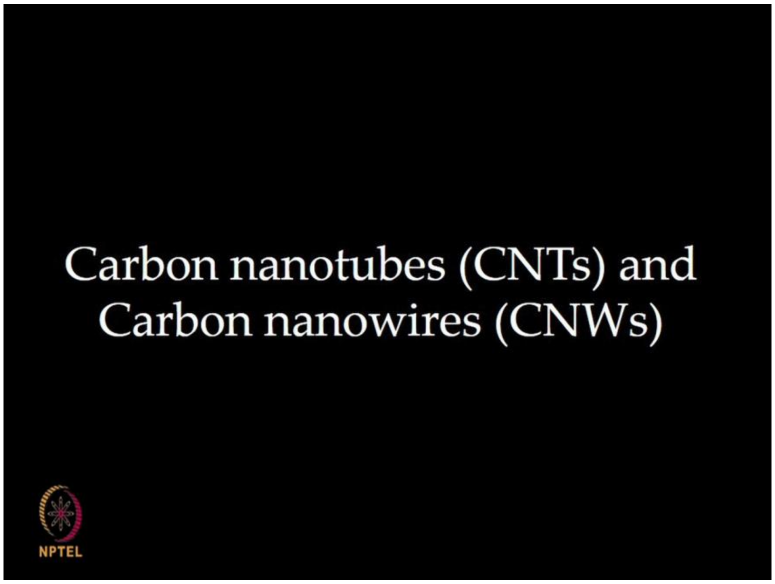
In today's lecture we will about Nanotechniques in proteomics. In the last few lectures we talked about label free technologies such as SPR, SPRI, Ellipsometry, Interferometry, and different kind of platforms. Today we will talk about different type of Nanotechniques. How they can be used for various types of Proteomics applications. We will discuss more on their applications, discuss about their advantages, and disadvantages and compare each of these methods with others and then we will touch upon how these Nanotechniques can be applied for proteomics based applications.

We will learn about some of the Nanotechniques which are applied for Proteomic applications. Nanotechniques offer several advantages over conventional Proteomic methodologies, such as miniaturization of assays, real time multiplexing capability, low sample and region consumption, very high sensitivity, and fastest assay time. There are several Nanotechniques such as carbon nanotubes, and nano wires, quantum dots, core nano particles, silicon nanowire field effect transistors which are now increasingly being used for various proteomic applications. These applications include biomarker discovery, amino acids, and different types of biomolecular interaction studies including protein protein interactions.

Successful integration of Nanotechnologies with Proteomics has now introduced a new field in the clinical research, known as Nano Proteomics. This is one of the very rapid emerging area for the Bio Medical Research which may ultimately have tremendous therapeutic potential. In today's lecture we will talk about different Nano techniques used for Proteomic Applications. We will talk about Carbon Nano Tubes, Nano Wires, Carbon Nano Tubes field defect transistors, CNT FETs, quantum dots, KODs, Gold Nanoparticles, and Nanocages and Microfluidics.

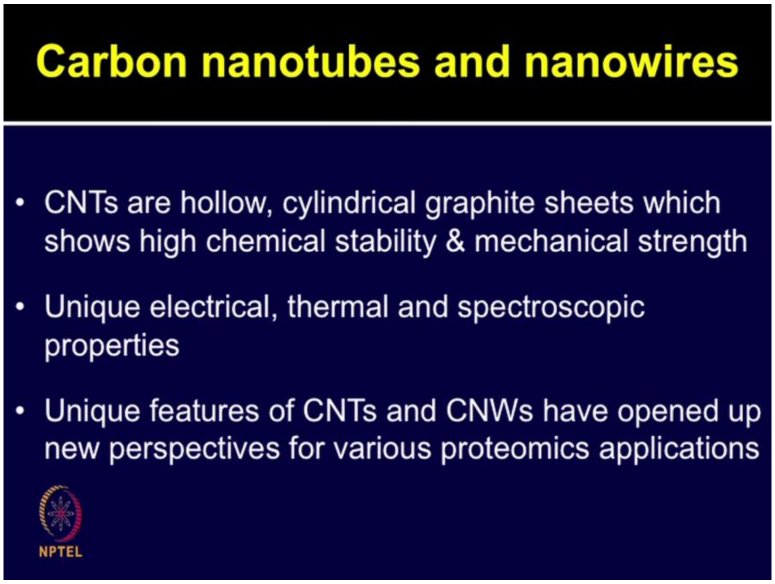
(Refer Slide Time: 2:53)





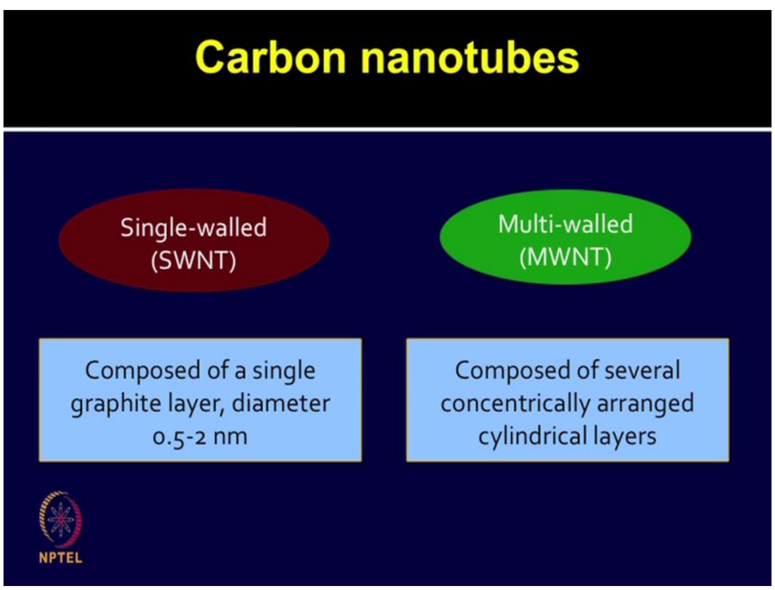
Let us first talk about Carbon Nanotubes, and Nanowires. There are various novel in organic nanomaterial which have been explored in Biological Research with an intention of developing new type of analytical tools. Due to rapid advances and synthesis and surface chemistry optimization, there are various classes of Nano structure including Nanowires, nanotubes, and nano crystals that have been used for the clinical Proteomic Research. CNTs or CNWs carbon nanotubes, or nanowires, they detect changes in the electrical conductance of a target binds, and they show sensitivity in Nanomolar to picomolar range.

(Refer Slide Time: 3:58)



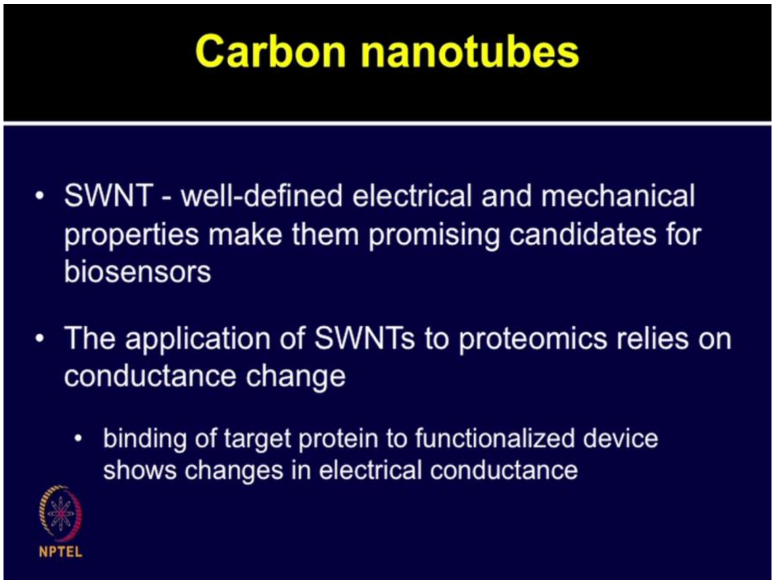
So what are CNTs, these are hollow, cylindrical graphite sheets which shows high chemical stability and mechanical strength. The Carbon Nanotubes, and Nanowires offer very unique electrical, thermal, as well as spectroscopic properties, and these unique features of Carbon Nanotubes, and Nanowires have opened up many new prospective in the field of nano proteomics.

(Refer Slide Time: 4:28)



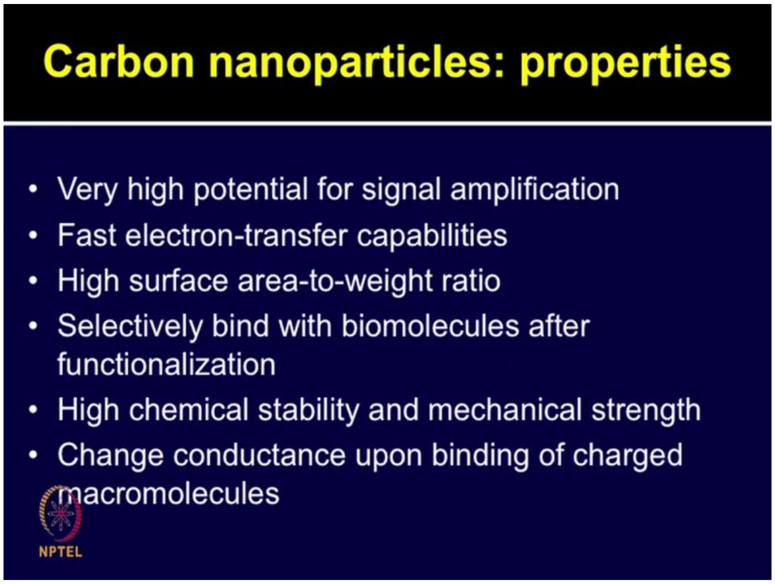
There are two different types of carbon Nanotubes, single walled Nanotubes SWNT, and multi walled Nanotubes MWNT. The single walled Nanotubes are composed of single graphite layer with a diameter in the range of point 5 to 2 nanometre. Whereas the multi walled Nanotubes are composed of seven concentrically arranged cylindrical layers. There are various properties associated with each of this group. Today we will focus more on how these Nanotubes can be applied for Proteomic applications.

(Refer Slide Time: 5:15)



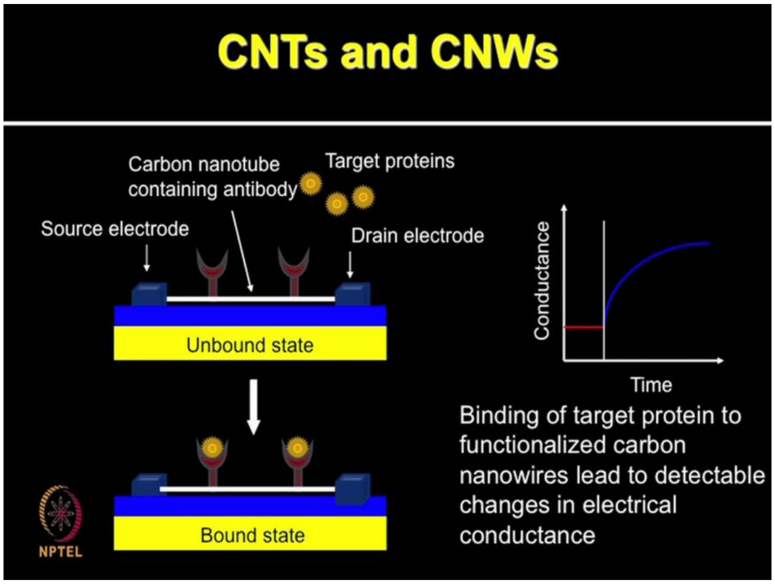
The single walled Nanotube possess well defined electrical and mechanical properties which makes them promising candidate as a biosensor. The application of single walled Nanotubes in Proteomics relies on the conductance change. Binding of the target protein to the functionalized device brings a change in the electrical conductance that is measured for binding interactions. These carbon nano particles have various properties, and some of these general properties are listed here.

(Refer Slide Time: 5:52)



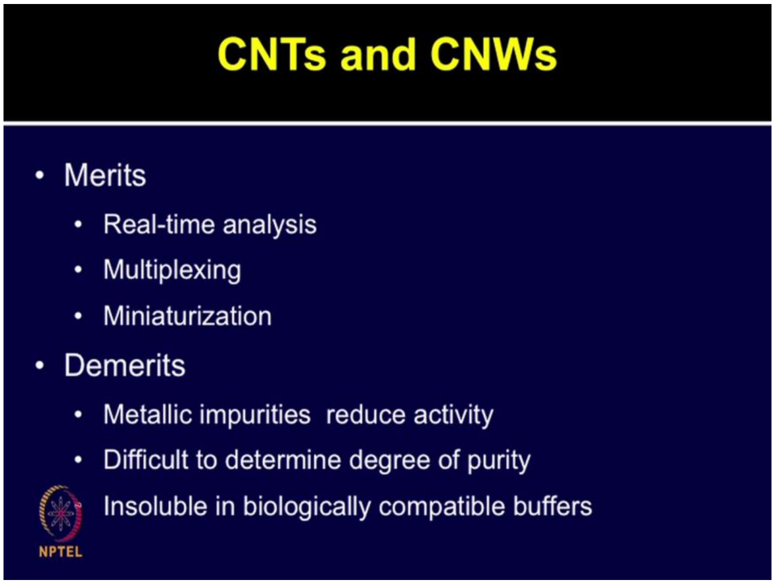
They have very high potential for signal amplification. They have a fast electron transfer capabilities exhibit very high surface area to weight ratio. They can selectively bind to biomolecules after functionalization and selective binding with high sensitivity is the key advantage of using these Nano Techniques. There is a bit high chemical stability, and mechanical strength as discussed earlier, and these changes in the conductance due to the binding of charged macromolecules can be measured for monitoring binding reactions.

(Refer Slide Time: 6:35)



This slide shows the image for carbon nanotubes with functionalized antibodies. The image on the top represents the unbound state where you have a sourced electrode and drain electrode. Some target proteins are observed to be binding in the bottom image showing change in conductance displayed in the graph on the right. The binding of the target protein to the functionalized carbon nanowires lead to detectable change in electrical conductance, and change in the conductance is measured for detecting different types of proteins as well as small particles including viruses. There are various applications showing the potential of latest technology.

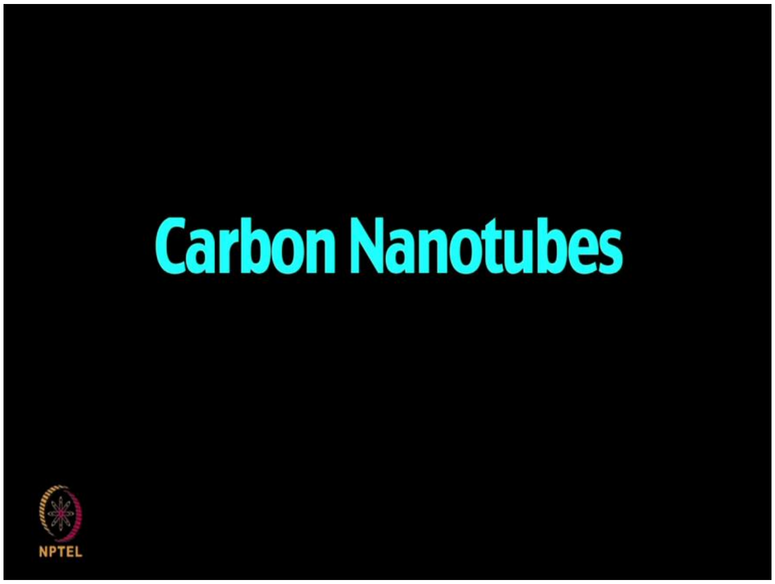
(Refer Slide Time: 7:33)

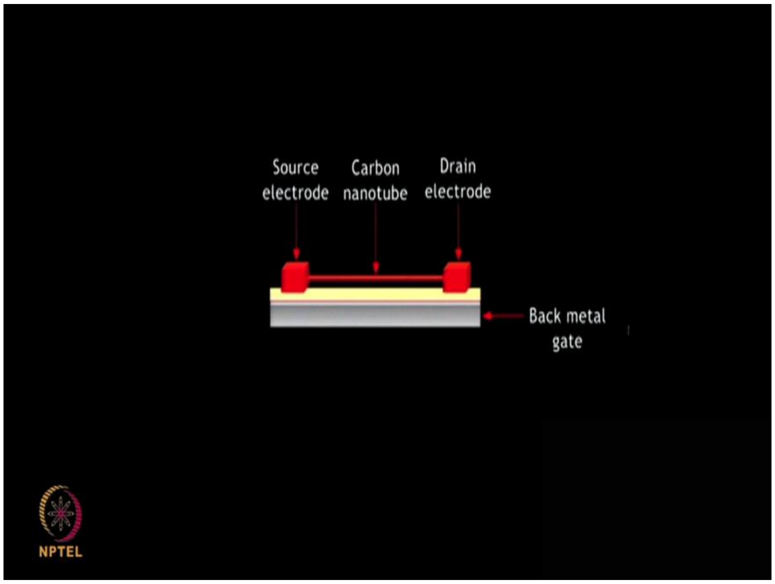


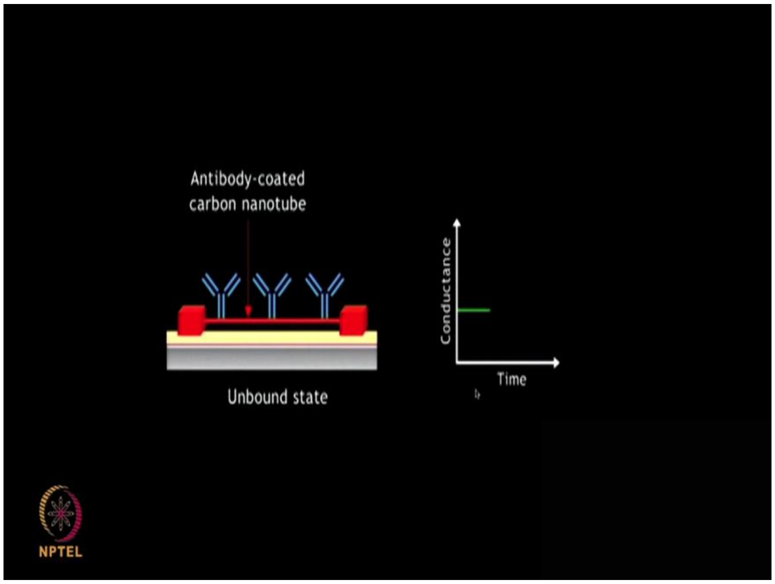
Let us discuss some merits, and demerits of using carbon nanotubes, and nanowires. They allow real time analysis, similar to some of the previously discussed label free techniques. In today (())(07:38) where we had opportunity to monitor the reaction in real time which is not the case with label based techniques which are mostly end point analysis. The approach also provides the multiplexing capability offers miniaturization. There are also various demerits associated with this approach. Firstly, the metallic impurities can reduce activity of these assays. It is not very easy to determine how pure these preparations are. So the degree of the purity is one of the limitations here.

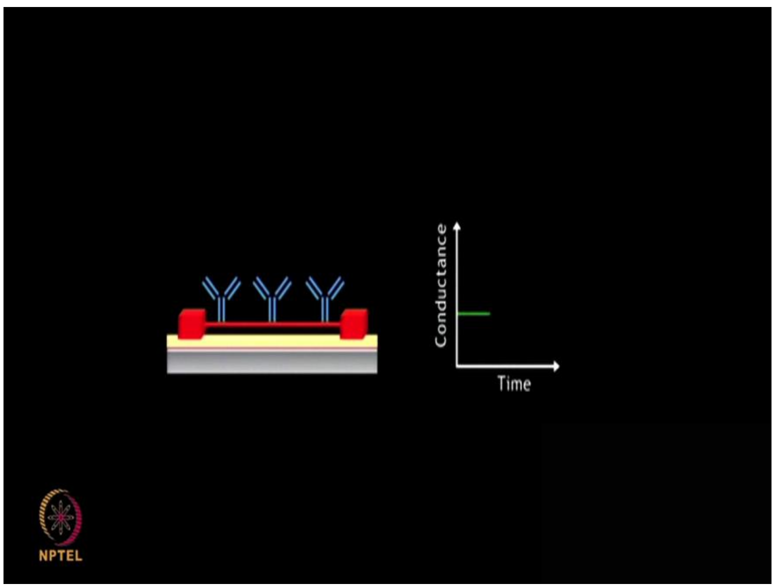
One of the major constrain is insolubility in biologically compatible buffers. Since they are not compatible in all biological buffers. To demonstrate the principle of carbon nanotube, and nanowires, let us look at this animation.

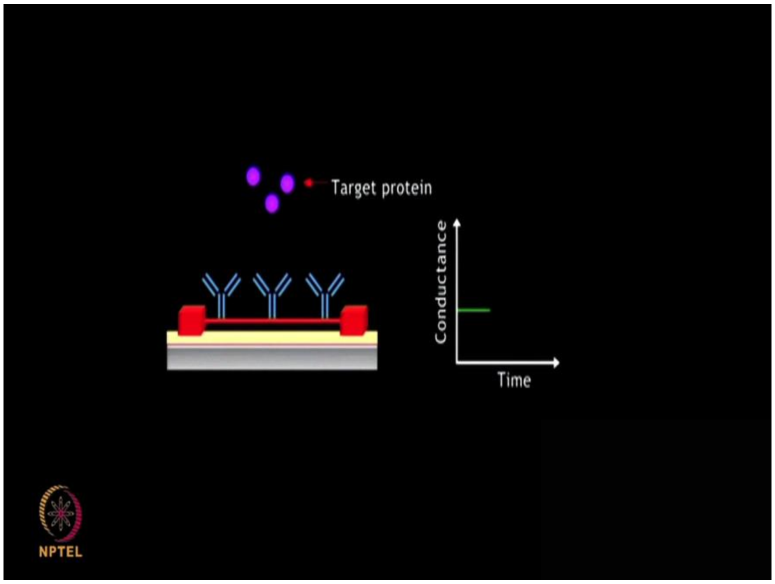
(Refer Slide Time: 8:39)

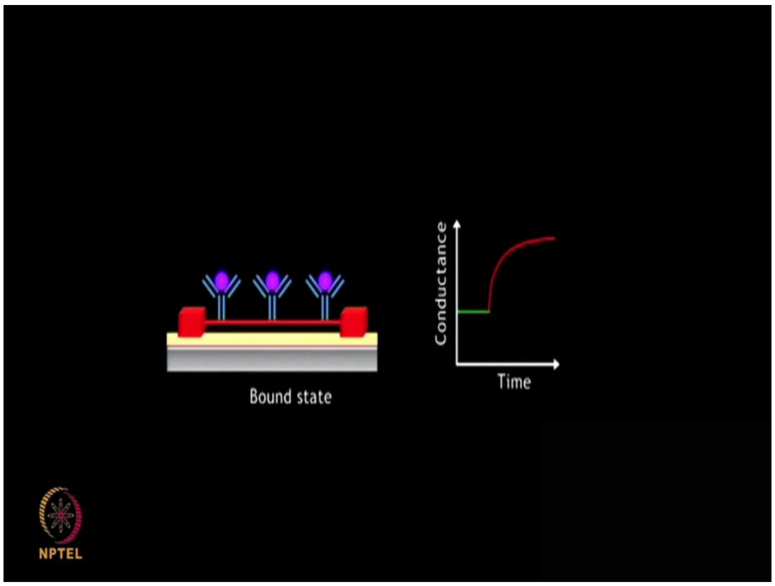





Antibody-coated
carbon nanotube
Conductance
Time
Unbound state
NPTEL


Conductance
Time
NPTEL



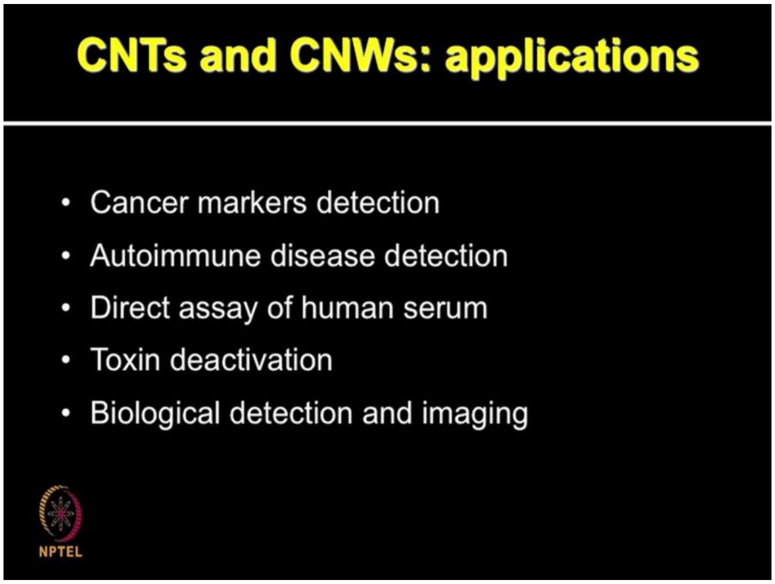


Carbon Nanotubes are hollow cylindrical graphite sheets that exhibit very high levels of chemical and mechanical stability. As you have seen here there is a drain electrode source electrode, and on top of carbon nanotube the antibodies are functionalized. These nanotubes will be suitably functionalized with the antibodies or other agents like aptamers. The antibody coated nanotubes show no variations in conductance when they are in the unbound state. Binding of 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 axis on the X axis conductance is spotted on the Y axis which is showing a change in the conductance due to the binding state of these target proteins. These Nanotube devices have been extremely useful for real time label free detection of low abundance protein, and sensitivity range is formed from Nanomolar to Picomolar range.

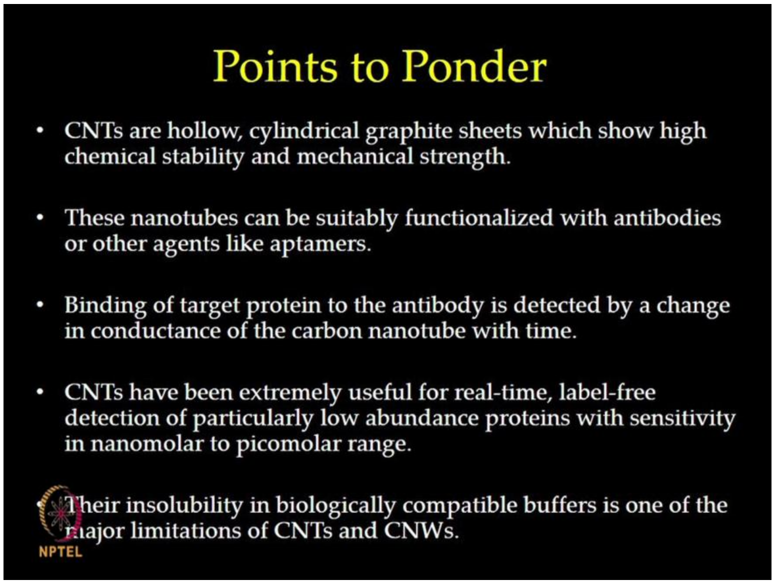
Now after looking at the principle of nanotubes, let us briefly discuss about its applications. The carbon nanotubes, and nanowires have shown unlimited potential for various applications in different fields. However here our discussion will be centred on applications in context of proteomics.

(Refer Slide Time: 10:36)

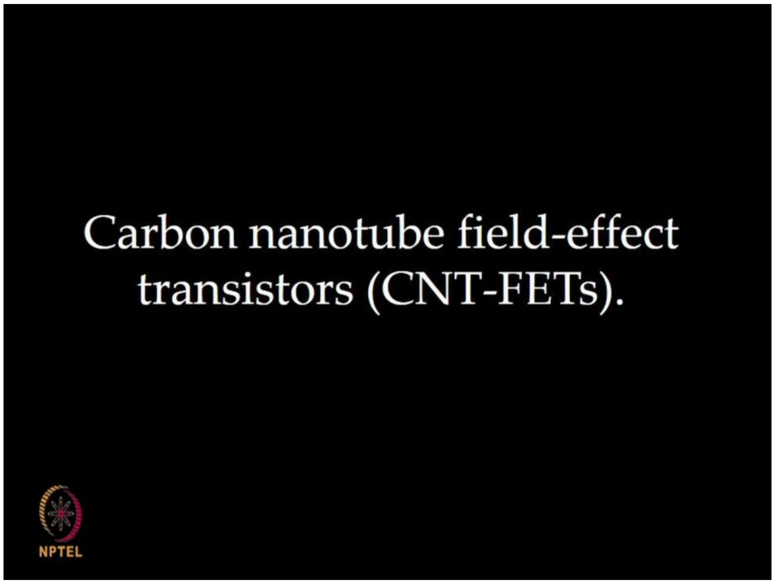


So various types of clinical studies have been performed using nanotubes, and nanowires to test the potential of these nanotechniques for cancer markers bio detection, autoimmune disease detection, direct assay of human serum, deactivations of toxins, and biological detection and imaging. This is very smallest of the unlimited applications which have been demonstrated by using carbon nanotubes, and nanowires.

(Refer Slide Time: 11:11)

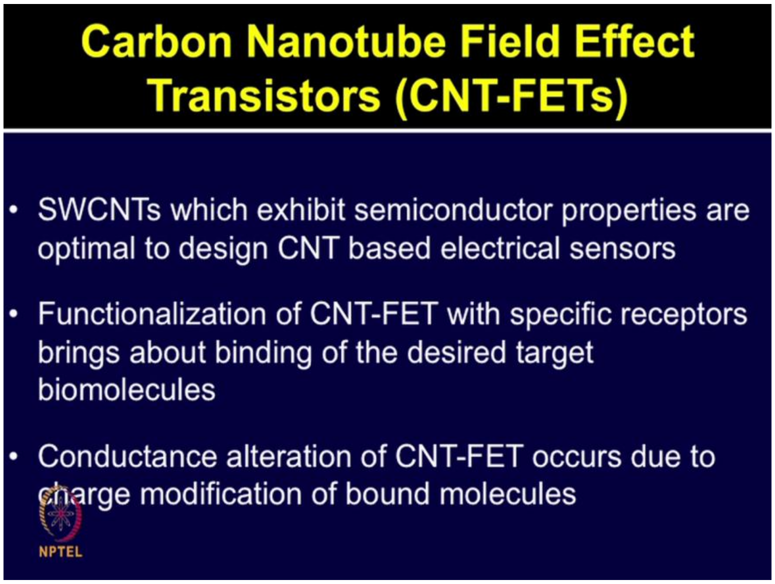


(Refer Slide Time: 11:34)



Let us 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 had led to the development of this novel (())(12:00) known as CNT FET. As we have discussed there are different type of carbon nanotubes SWNTs and MWNTs.

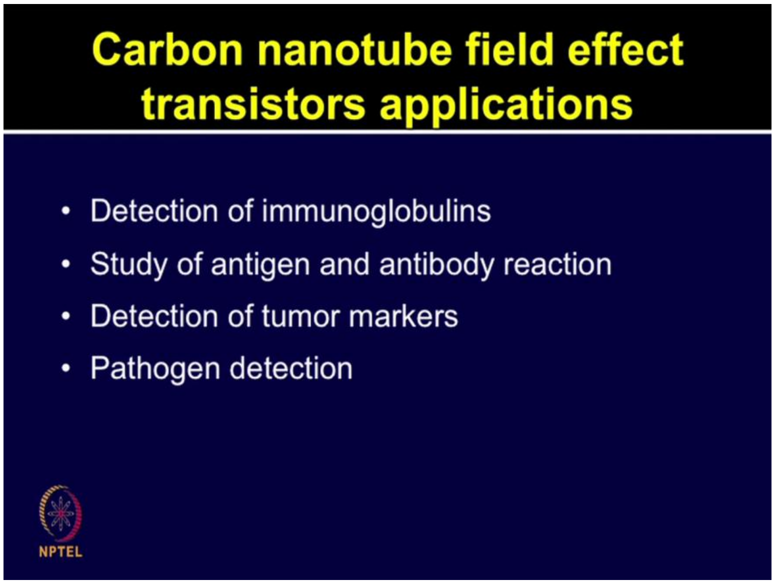
(Refer Slide Time: 12:14)



The single walled nanotubes exhibit semi conductance properties which are optimal to design the CNT based electrical sensors. The functionalization of Carbon Nanotube Field Effect Transistors 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.

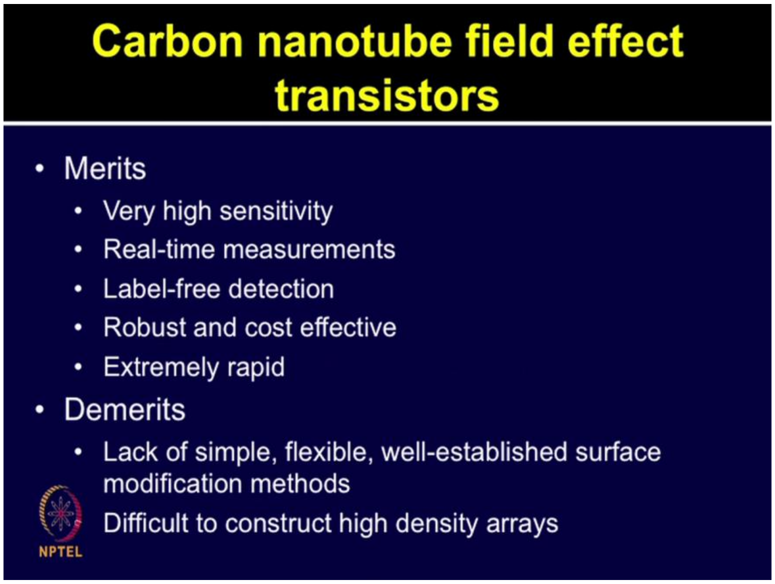
The biomolecular binding can be monitored by looking at the change in the conductance. This conductance alteration of CNT FET occurs due to charge modification of bound molecules. There are many applications of CNT FETs, here we will discuss it in context of Proteomics.

(Refer Slide Time: 13:10)



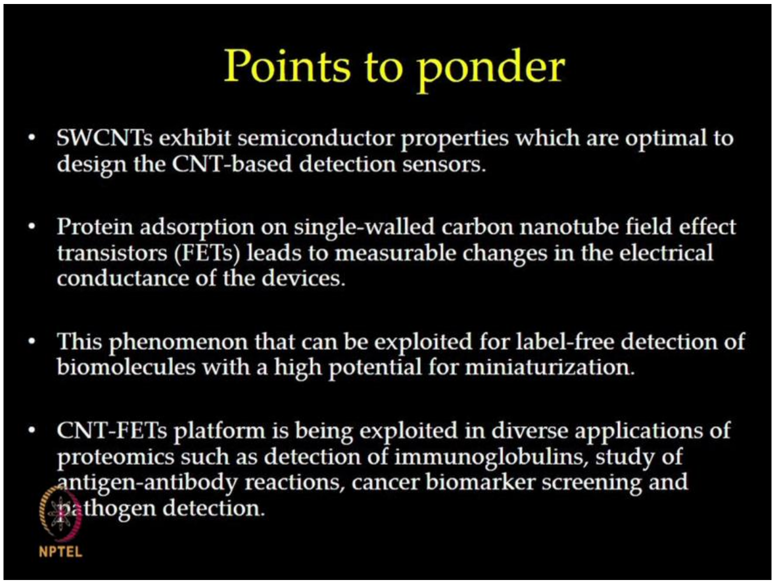
The platform has been applied for the detection of immunoglobulin, study of antigen, and antibody reactions, detection of various cancer biomarkers, and pathogen detection. One of the interesting topic to study these days is biodefense, and how one can detect various pathogens which has potential of bio hazard. Different type of bioterrorism attacks have caught attention especially with anthers, and different type of SAR based biological agents. So these nanotechniques have shown some various detection of various pathogens, and now apply for potential application in biodefense.

(Refer Slide Time: 14:02)

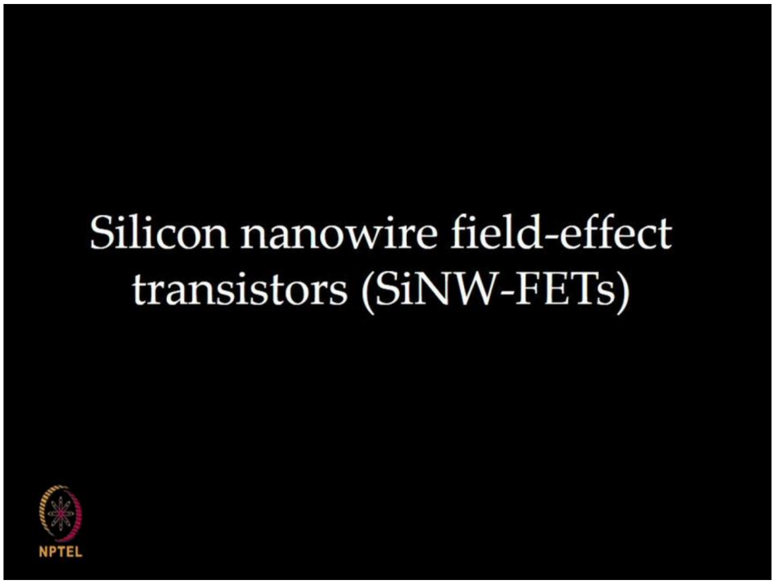


Let us now discuss merits and demerits of CNT FETs. The technology offers very high sensitivity with real time measurement capability. One can perform assay in a label free environment where there is no need of adding tag molecule. It is cost effective, and robust platform which is extremely rapid as well. At the same time there are many demerits which are associated with CNT FET platform including the lack of simple flexible, and well established surface modification protocols. When we talk about the application of Nanotechniques as an array platform then this particular platform is difficult to construct for the high density arrays.

(Refer Slide Time: 14:02)



(Refer Slide Time: 15:07)

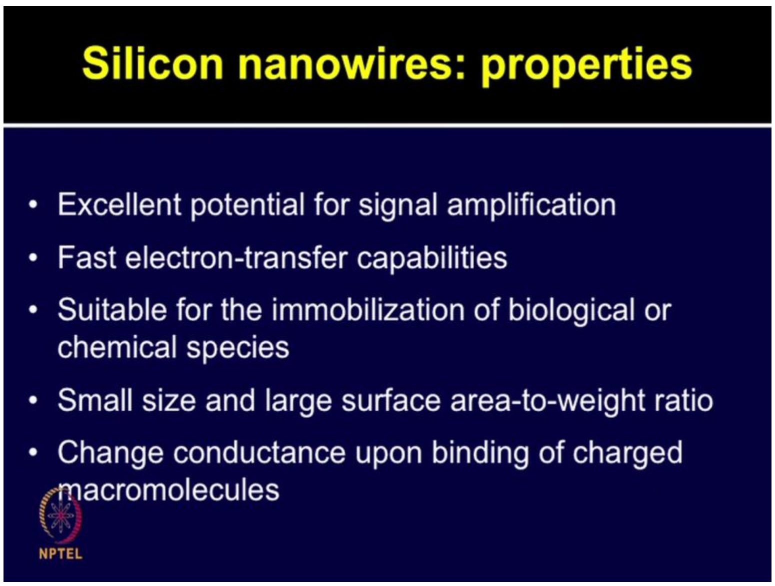


Let us now discuss silicon nanowire field effect transistors. The semi-conductor channel is placed between the source and drain electrodes with the a gate electrode of the bottom to control the conductivity of semiconductor channel. The biological reacceptance are attached to the surface of semiconductor channel. By chemical modification to buying to the target analyte in buffer.

The target receptor interaction changes the surface potential of semiconductor channel modulates the channel conductance, and the signal is eventually measured by a detection system. The physical parameters sometimes post constrain for sensor fabrication nonetheless local FETs have overcome these limitations to a large extent, but still they have their own drawback. For example, the presence of metallic nanotube, and lack of well-established surface modification protocols.

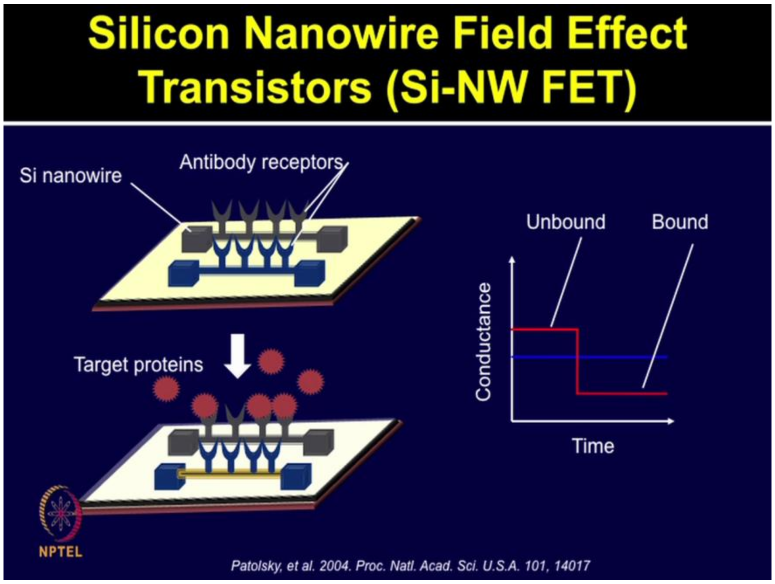
The silicon nanowires are the building blocks of this approach. They allow tuning of the sensitivity as per the requirement by controlling the type and amount of dopant in the semi-conductance. Let us now discuss some of the properties of the silicon nanowires.

(Refer Slide Time: 16:41)



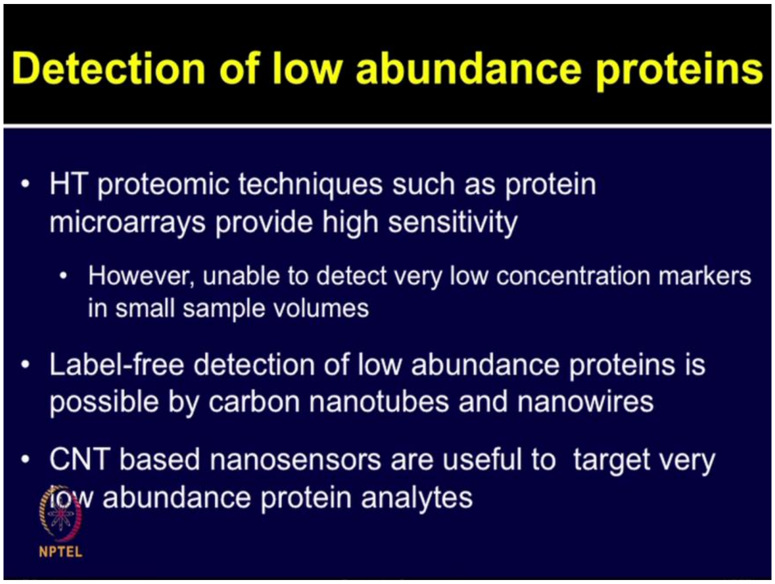
They have very high potential for signal amplification with fast electron transfer capabilities. Silicon nanowires are suitable for immobilization of various biological or chemical species. They are small in size with large surface area to weight ratio. Again the changes in conductance are measured here upon binding of charged biomolecules to their interacting partner.

(Refer Slide Time: 17:22)



Let us now discussed how these silicon nanowire field effect transistors are applied for some of the proteomics based applications. As you can see in this slide, the image on the top shows silicon nanowires coated antibody receptors. When you want to study some target proteins, and there is any interactions of target protein with antibodies as shown in the bottom image. These target proteins will bind to antibody receptors and the silicon nanowire will show the change in conductance. As you can see in the graph on the right hand side, conductance is plotted on the Y axis with time on the X axis. From the unbound to bound state, there is change in conductance which is measured to study biomolecular interactions.

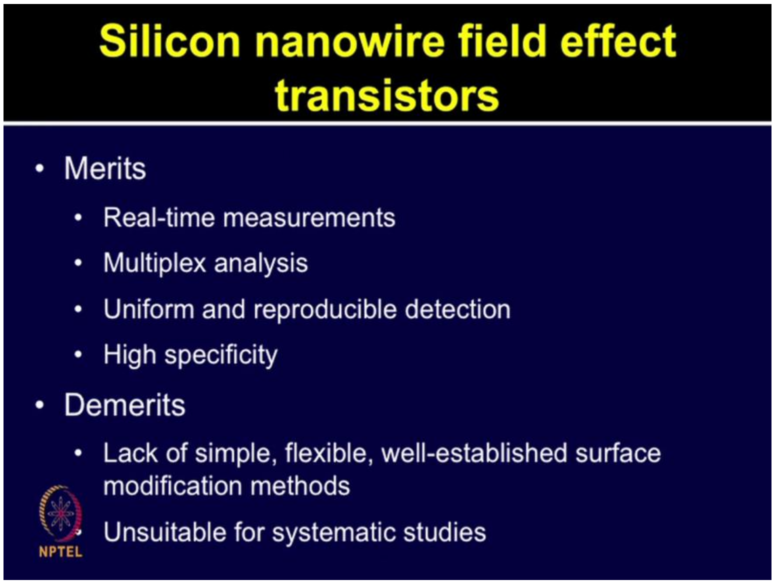
(Refer Slide Time: 18:10)



In proteomics deduction of low abundance proteins remains one of the major challenge. The high throughput proteomic techniques such as protein microarrays, and various label free platform provide good sensitivity. But when we are screening biological fluids for detection of very low concentration of biomarkers in very small sample volume, it becomes extremely challenging. Therefore various label free detection techniques are aiming towards detection of the low abundance proteins by using carbon nanotubes, and nanowires.

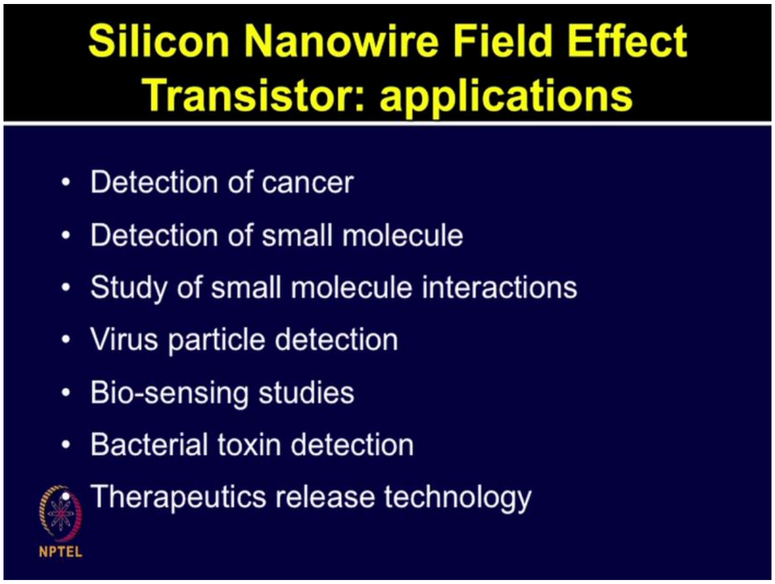
These studies have shown the potential of using nanotechniques for such applications where low abundance protein can be detected with high sensitivity, and this is one of the major advantages of using these platforms.

(Refer Slide Time: 19:08)



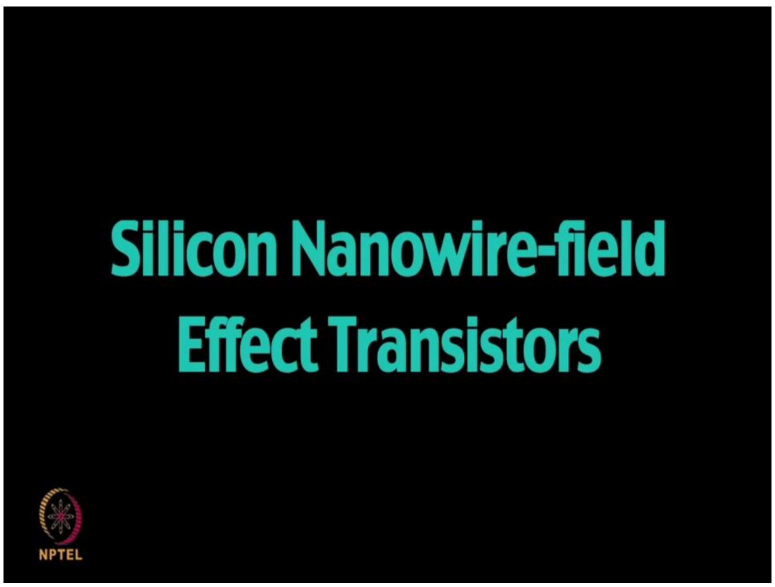
Continuing our discussion on silicon nanowires field effect transistors we will just go through some of the merits and demerits. This platform provides real time measurement capability with multiplexing analysis ability. It is uniform and delivers reproducible results for various biomolecule detection. It also provides high specificity. Now discussing about demerits it lacks simple, flexible, and well established surface modification protocol, and that is one of its major limitation. Additionally it is not very suitable for systematic studies.

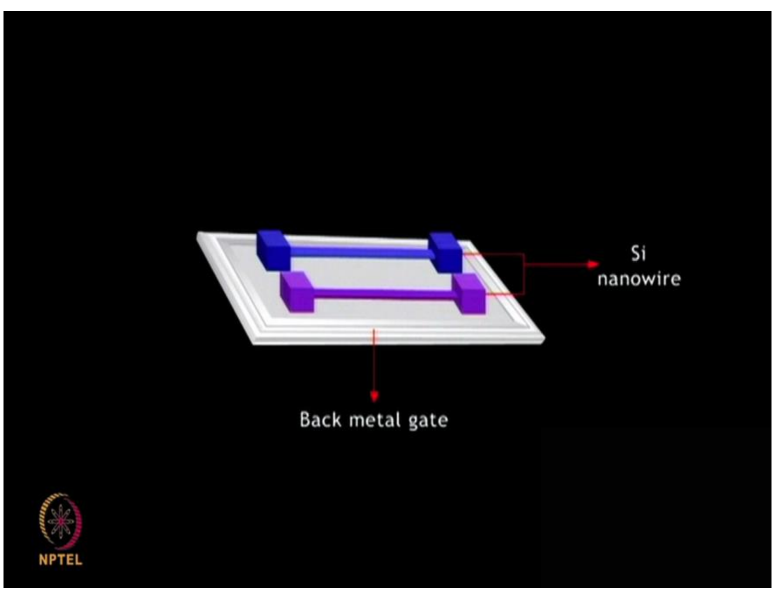
(Refer Slide Time: 20:00)

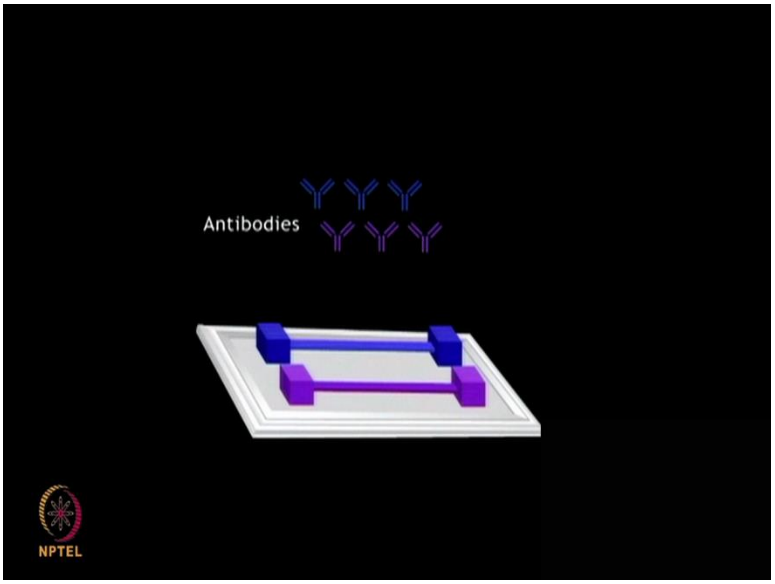


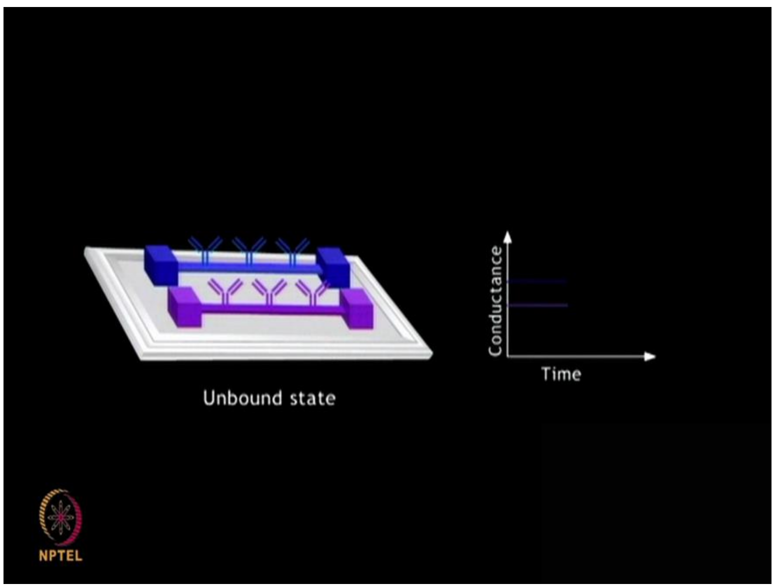
Despite some of the challenges, and demerits of silicon nanowire field effect transistors, they have been applied for various studies in proteomics including detection of cancer, detection of small molecule, study of small molecule interactions, detection of virus particles, different type of bio-sensing studies, and again similar to viral particle detection, different type of bacterial toxin detection studies have also been performed by using this technique. So overall many therapeutics based studies have shown the potential of silicon nanowires field effect transistors which can be applied for cancer biomarkers, and other diseases.

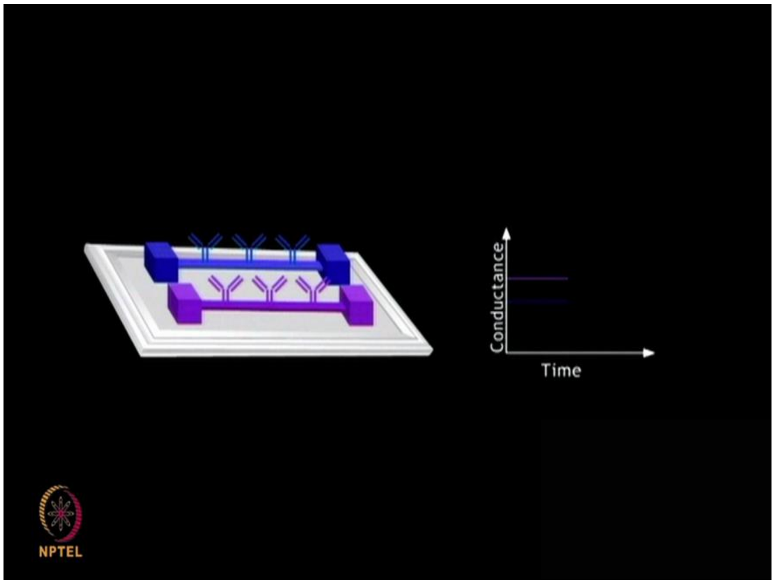
(Refer Slide Time: 20:44)



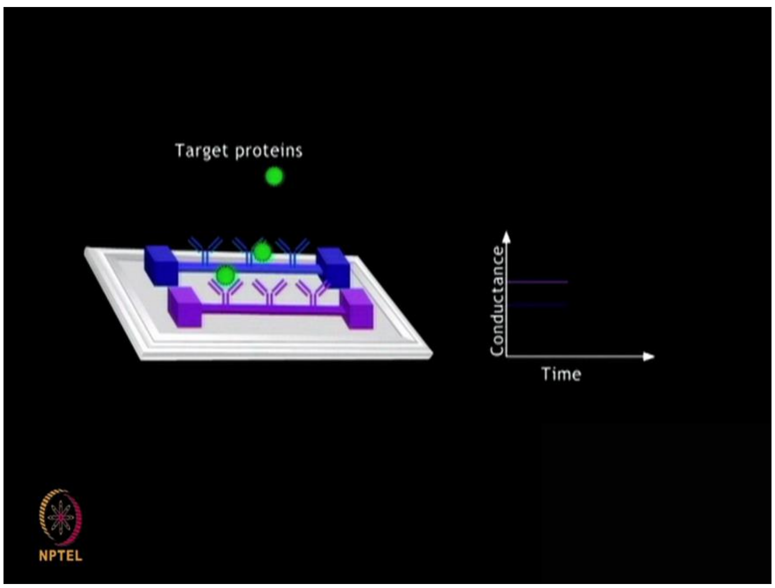



Antibodies
NPTEL

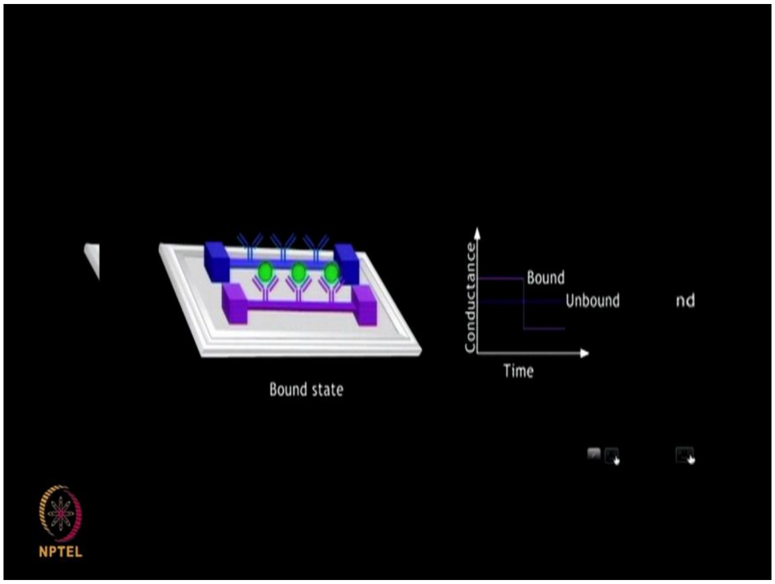

Conductance
Time
Unbound state
NPTEL



Conductance
Time
NPTEL



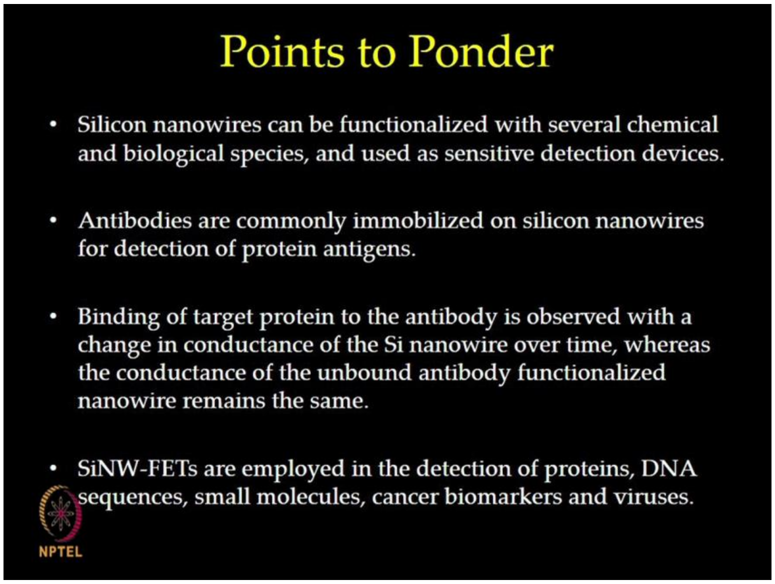
Target proteins
Conductance
Time
NPTEL



Let us now look at the animation to understand the principle of silicon nanowire field effect transistors more clearly. Silicon Nanowire field effect transistors, silicon nanowires can be functionalized with several chemical and biological molecule and used as sensitive detection devices. Antibodies are commonly mobilized on silicon nanowires for detection of protein antigens. No change in the conductance of nanowire is observed in the unbound state as you can see on the right hand side panel of the graph which is plotted between the time and conductance.

Bidning of target protein to the antibody is detected by change in the conductance of silicon nanowires overtime while the conductance of the unbound antibody functionalized nanowires remains unaltered. These devices offer excellent sensitivity in the picomolar to femtomolar range that are incapable of detecting molecules even at single particle level.

(Refer Slide Time: 21:56)



In sary, today we discussed about some of the Nanutechniques including carbon Nanotubes and Nanowires. Carbon Nanotubes field effect transistors and silicon field effect transistors has promising analytical tools for various applications. We will continue our discussion on few more nanotechniques, such as Quantum Dots, Gold Nanoparticles. Nano cages, and Microfludics in our next lecture. Thank You.