Fundamentals of Protein Chemistry
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Module - 11
Protein Macromolecule Interactions II
Lecture - 55
Protein Nanoparticle Interactions

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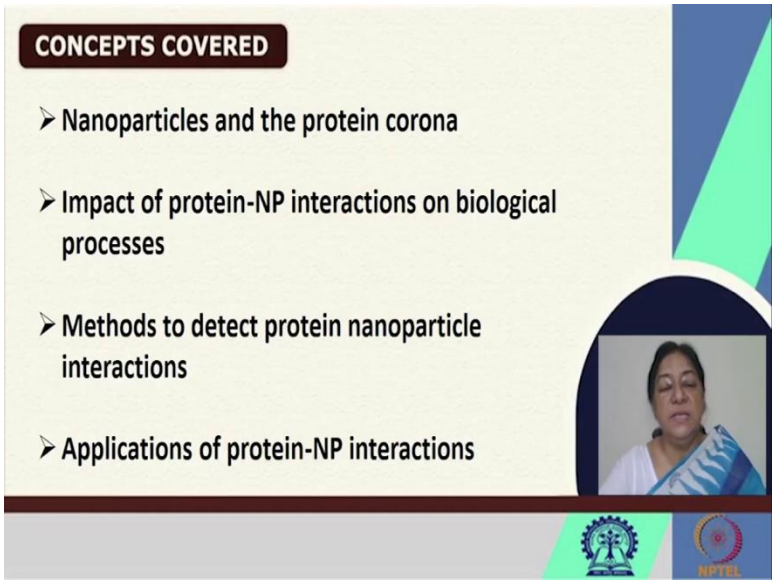

In our final discussion on protein macromolecular interactions, we will be looking at protein nanoparticle interactions. These are important interactions because they deal with a protein interaction in the term of a protein corona. We will look at the impact of protein nanoparticle interactions on biological processes, methods to detect them and how they are applied in nano medicine.

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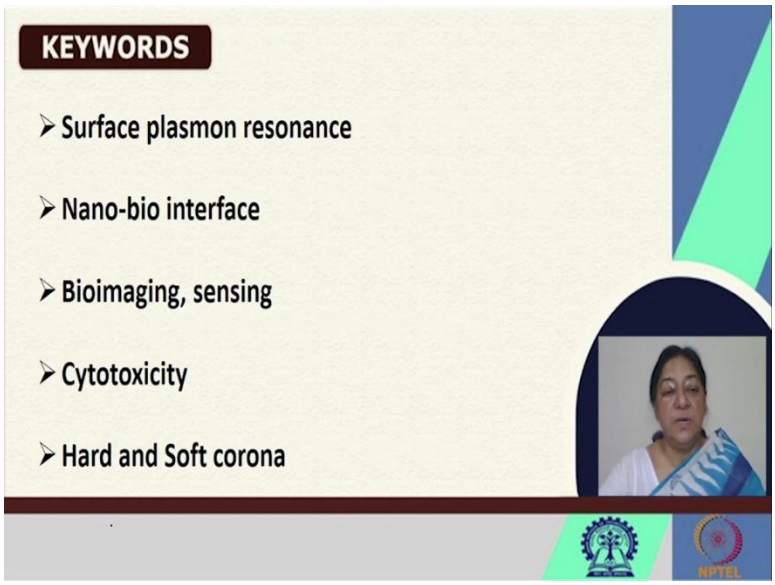

When we look at these specific terminologies, we will be looking at what we mean by surface plasmon resonance, the nano-bio interface, bioimaging, cytotoxicity, the hard and soft corona.

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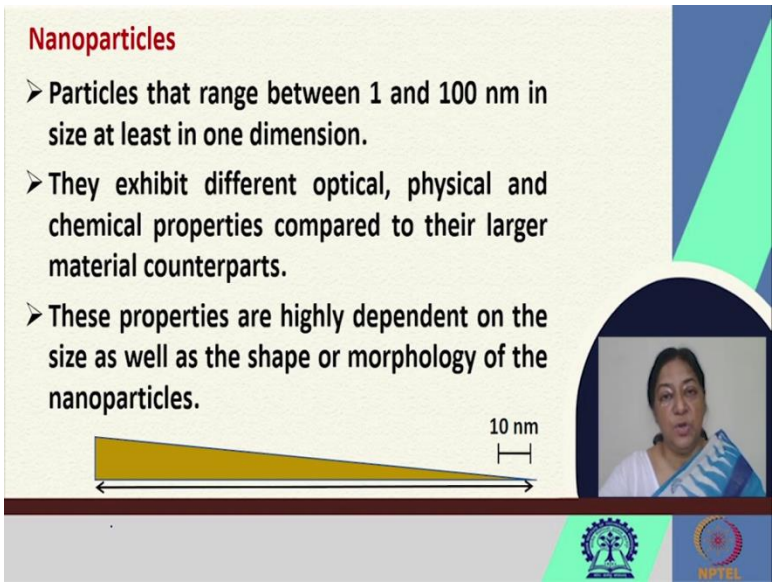

When we try and understand what nanoparticles are, these are particles that range between 1 and 100 nm in size at least in one dimension. They exhibit different optical, physical and chemical properties, compared to the larger material bulk counterpart. These properties are highly dependent on the size as well as the shape or the morphology of the nanoparticles.

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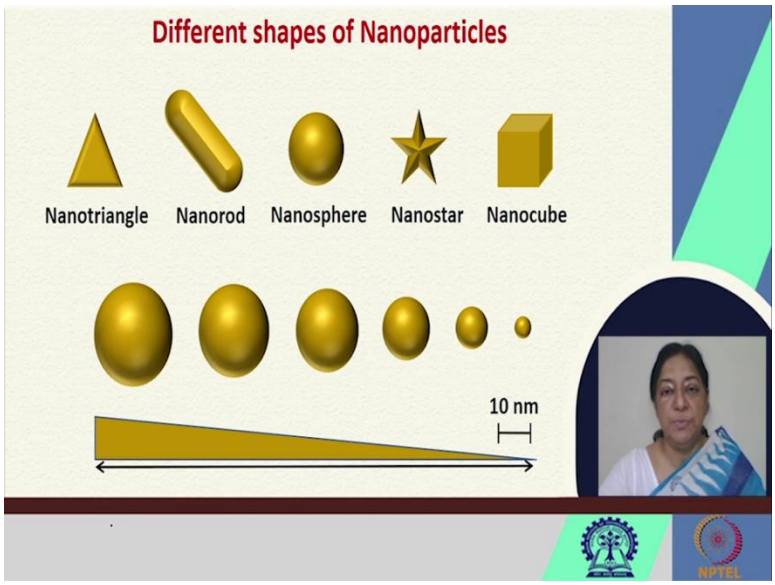

So if we look at the different shapes of nanoparticles that are possible, we could have nano triangles, rods, spheres, stars, even flowers, nano cubes and so on and so forth and depending upon the shapes and the sizes, they have different properties that can be exploited in various ways.

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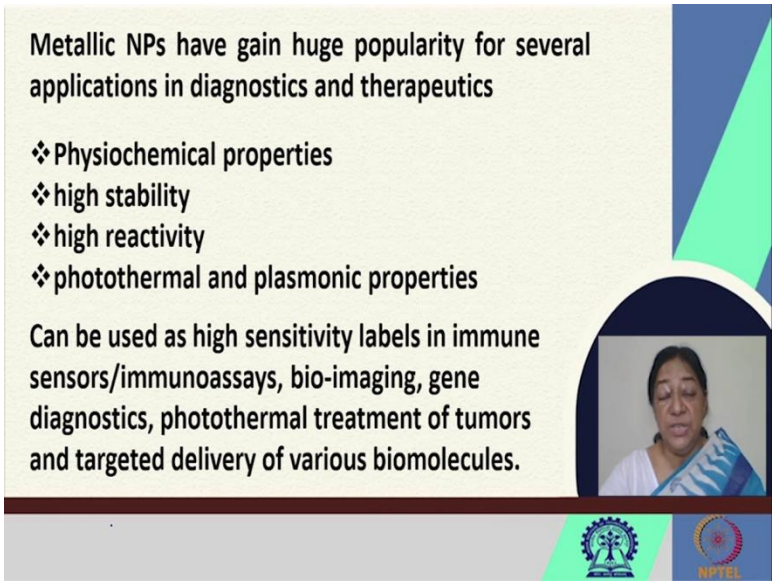

Metallic nanoparticles have gained a huge popularity for several applications in diagnostics and therapeutics, where the concept of a protein nanoparticle interaction or an understanding is important. The properties that are exploited are the physiochemical properties that has high stability, high reactivity and photothermal and plasmonic properties.

Thus, they can be used as high sensitivity labels in immune sensors or immunoassays, bio imaging, gene diagnostics, photothermal treatment of tumors and targeted delivery of various biomolecules and drugs.

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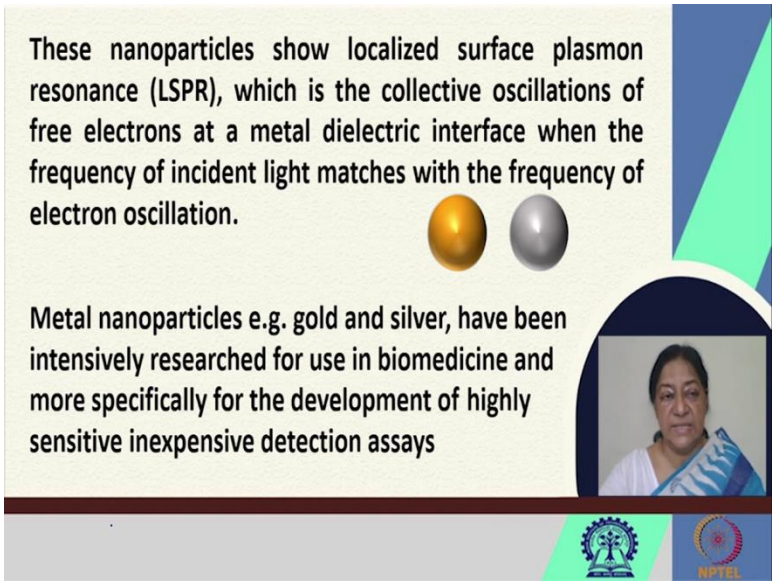

When we look at the nanoparticles, what they do is they show localized surface plasmon resonance, which is the collective oscillations of the free electrons at a metal dielectric interface, when the frequency of the incident light matches with the frequency of the electron oscillation thus being a resonance.

The metal nanoparticles for example, gold and silver have been intensively researched for use in biomedicine and more specifically for the development of high sensitive inexpensive detection assays.

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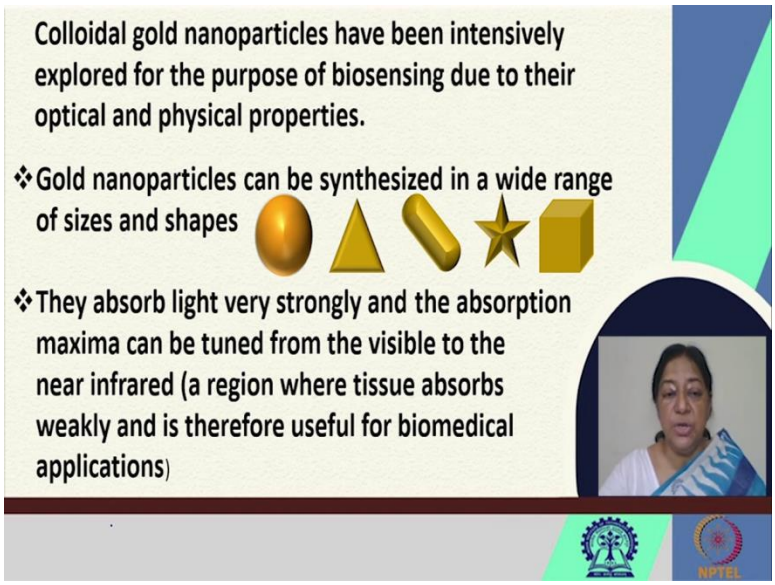

The colloidal gold nanoparticles have been intensively explored for the purpose of biosensing, due to their optical and physical properties. So the gold nanoparticles as we saw, can be synthesized in various sizes and shapes. They absorb light very strongly and the absorption maxima, can be tuned from the visible to the near infrared. This is the region where the tissue absorbs weakly and thus they can be used for biomedical applications.

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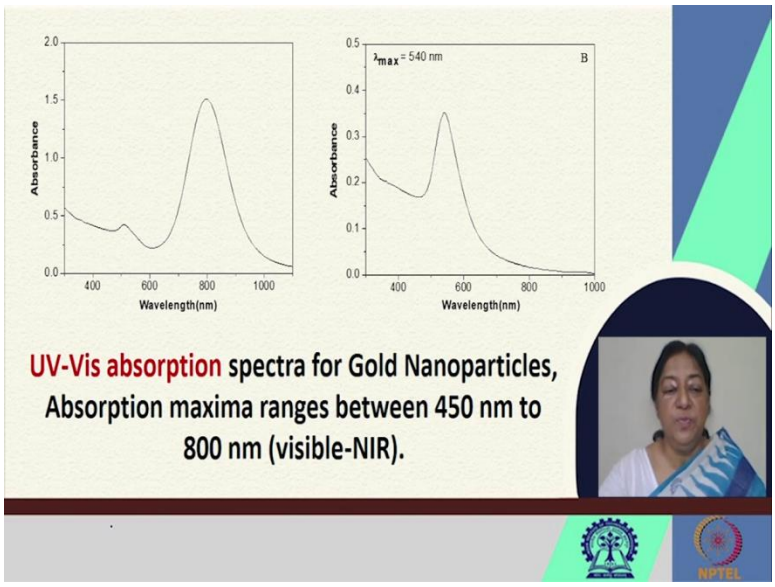

This [refer to slide] is a typical spectra for the UV-Vis absorption spectra for gold nanoparticles, where the absorption maxima ranges between 450 and 800 nm.

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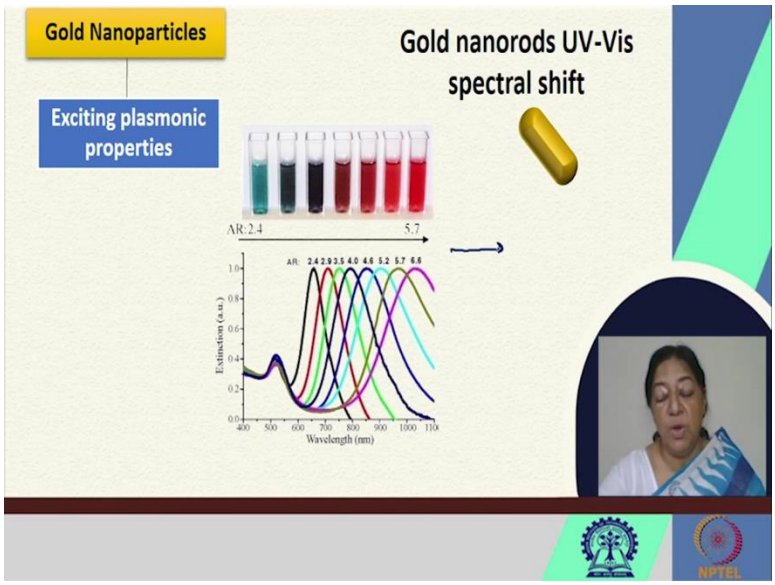

There are exciting plasmonic properties of these gold nanoparticles and what happens for these gold nano rods, there is a spectral associated with the size and the shape of the nanoparticle.

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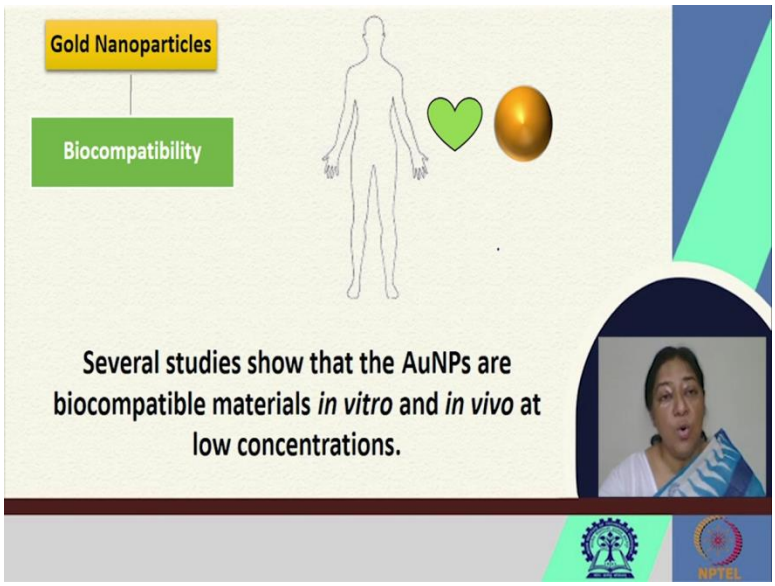

Their bio compatibility is also extremely important and several studies with gold nanoparticles, show that they are biocompatible in vitro and in vivo at low concentrations.

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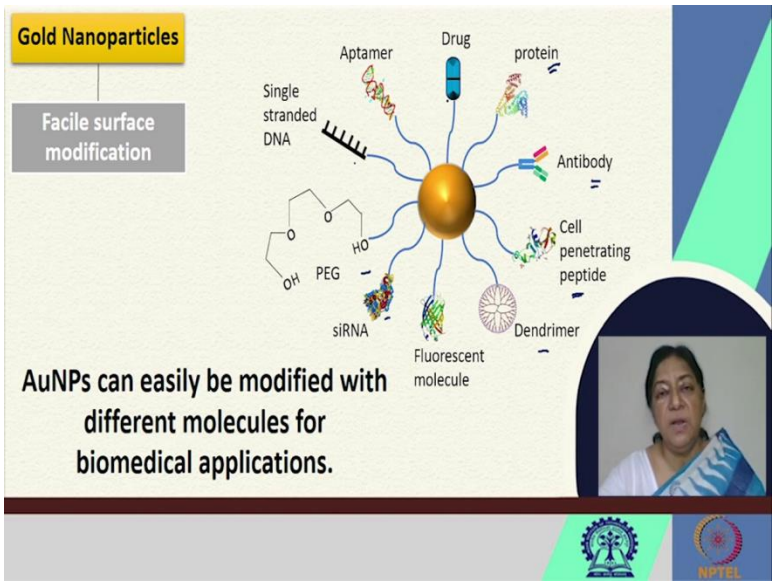

The other advantage of gold nanoparticles is, it is possible for surface functionalization or surface modification. This surface modification as we can see [refer to slide] in the figure, can be accomplished by many other smaller biomolecules that can be used for various properties. So whether we are attaching a protein, an antibody, a cell penetrating peptide, a dendrimer, a fluorescent molecule, siRNA, PEG, single stranded DNA, an aptamer or a drug, would depend upon the specific application that we would be interested in.

So, these gold nanoparticles can easily be modified with different molecules for several biomedical applications.

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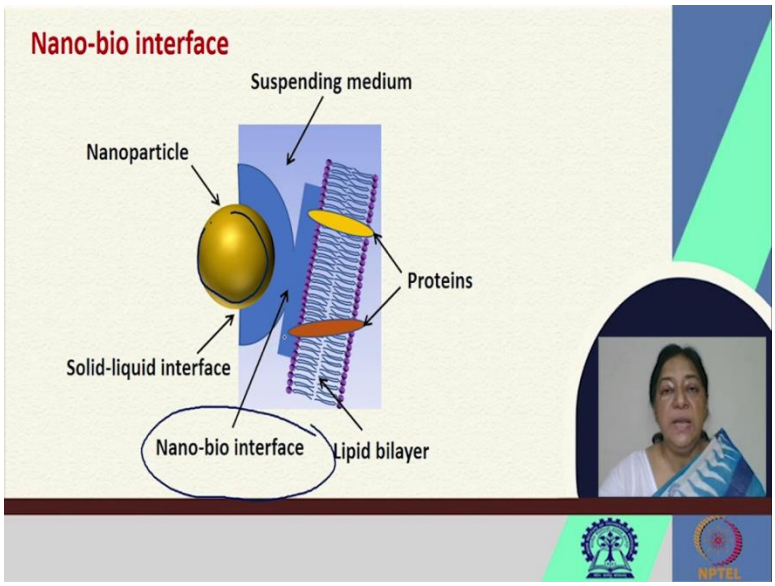

The nano-bio interface is an important aspect because we consider their attachment to the cell or their interaction with body fluids. We have a solid liquid interface and there are the special

properties of the lipid bilayer where we have the embedded proteins, where there could be specific interactions depending upon the type of process that is being looked at.

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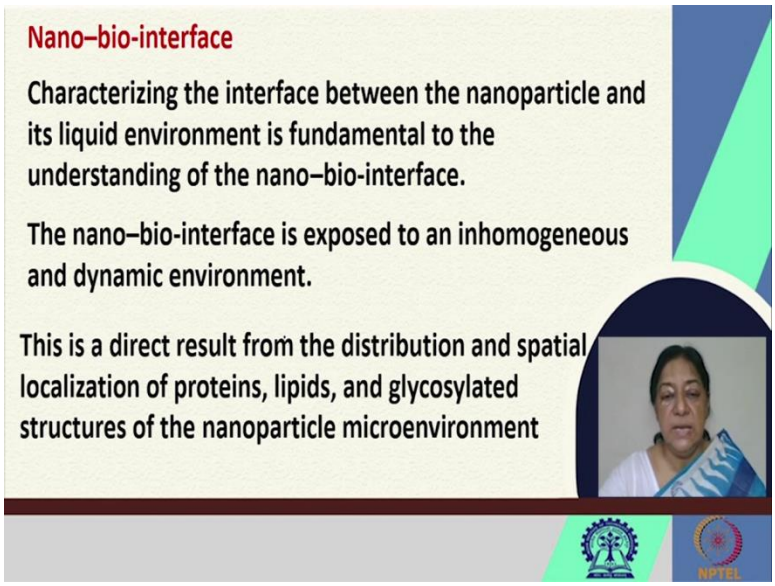

So characterizing this nano-bio interface between the nanoparticle and its liquid environment, is fundamental to the understanding of this specific interface. This is because it is exposed to an inhomogeneous and dynamic environment, given that it is interacting with the cellular environment; the extracellular and intracellular environment, if it is allowed to pass the cell membrane.

This is a direct result from the distribution and spatial localization of the proteins, the lipids and glycosylated structures of the nanoparticle micro environment and each of these are going to have a specific feature or a specific characteristic that is going to affect the overall property of the nanoparticle.

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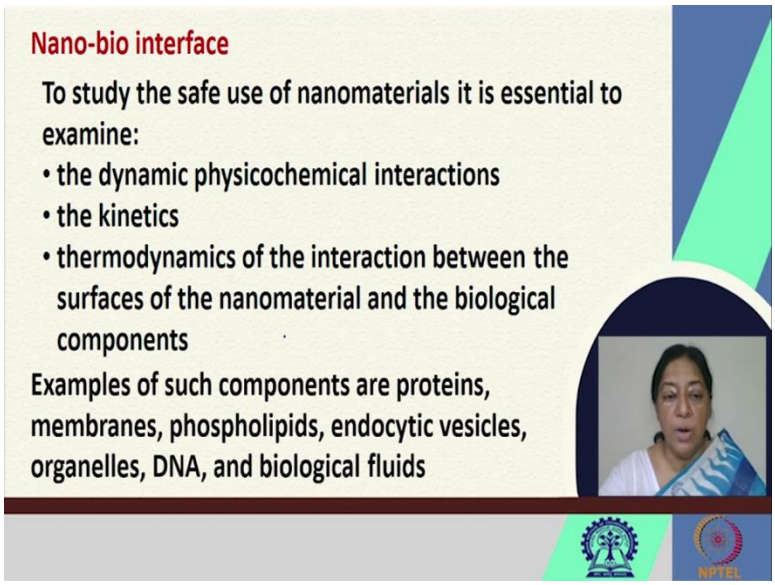

To study the safe use of nanomaterials, it is essential to examine the dynamic physiochemical interactions, the specific kinetics and the thermodynamics of the interactions between the surfaces of the nanomaterial and the biological components. Examples of the components that the interaction can occur with are proteins, membranes, phospholipids, endocytic vesicles, several organelles DNA and biological fluids.

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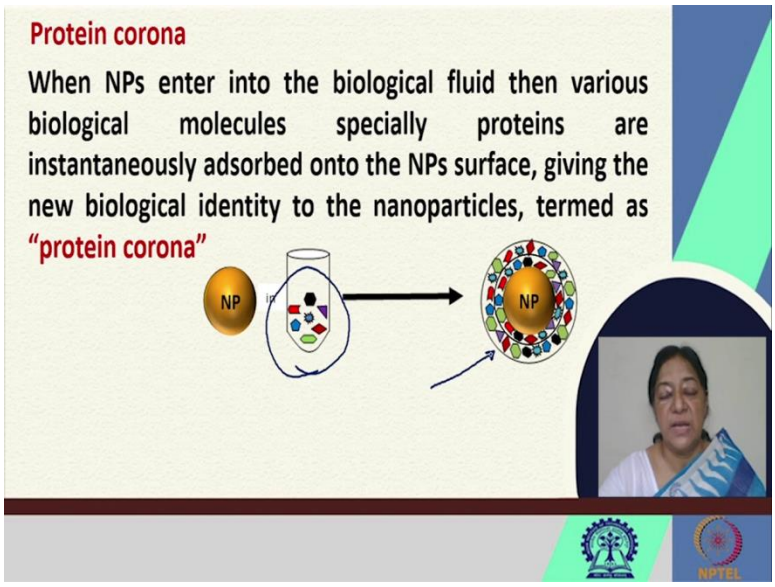

In this case we will be looking at the interaction with proteins in the formation of what is called a protein corona. When nanoparticles enter into the biological fluid, then there are various molecules particularly proteins, that are instantaneously adsorbed onto the nanoparticle surface. The interaction with biofluids, on their first interaction when a nanoparticle enters a biological fluid, these proteins adsorb on the surface of the nanoparticle and this is termed as the protein corona.

So we have a protein corona that has the nanoparticle, several proteins that can interact with the nanoparticles. If we have a mixture of proteins they can interact with the nanoparticles and their interaction would depend upon the properties of the proteins, their charge and their specific characteristics, depending upon the surface interactions that are going to interact with the nanoparticles.

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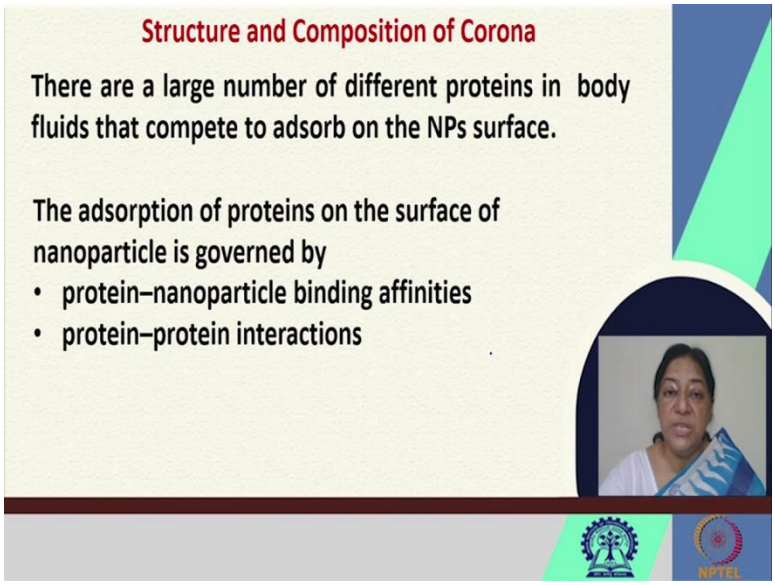

The structure and the composition of the corona based on the proteins involved in this case, there are a large number of different proteins in the body fluids that can compete to adsorb on the nanoparticle surface. In this case the affinity with the nanoparticle is going to be important. So, the absorption of the proteins on the surface of the nanoparticle is governed by the protein nanoparticle binding affinities and protein-protein interactions.

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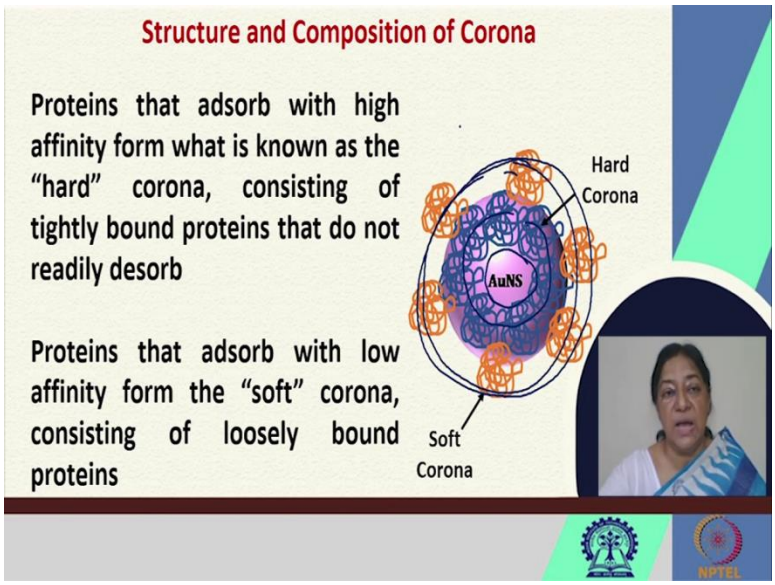

The proteins that adsorb with high affinity from what is called the hard corona, consisting of tightly bound proteins that do not readily desorb from the surface and the proteins that adsorb with low affinity, from what is called the soft corona that consists of loosely bound proteins. We have a hard corona if we look at a gold nanosphere, where we have the proteins that are tightly bound to the surface because of their high affinity.

We have a soft corona that is loosely bound, where there is an exchange with the fluid and with the surface of the soft corona, whereas the hard corona proteins are tightly bound.

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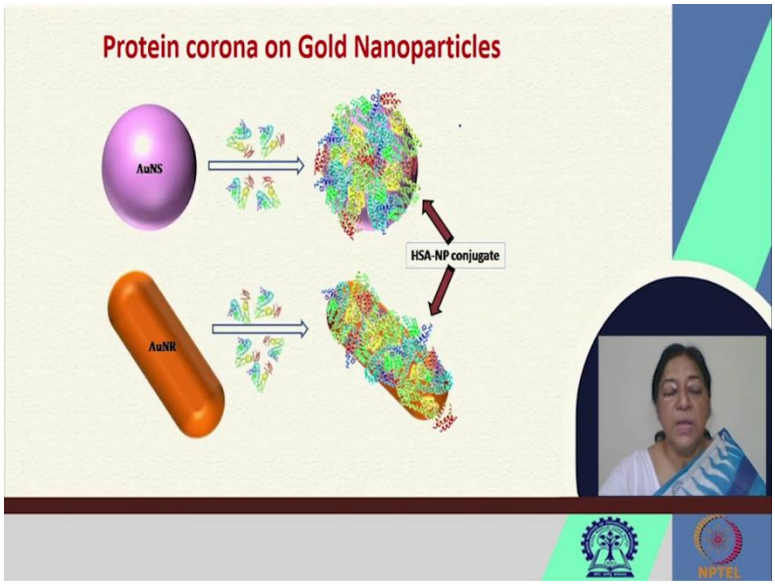

If we look at the protein corona on the gold nanoparticles, depending upon the shape, the protein nanoparticle conjugate can have different notations, different ways in which they could interact and different ways in which the affinity for the gold nanoparticle may be affected.

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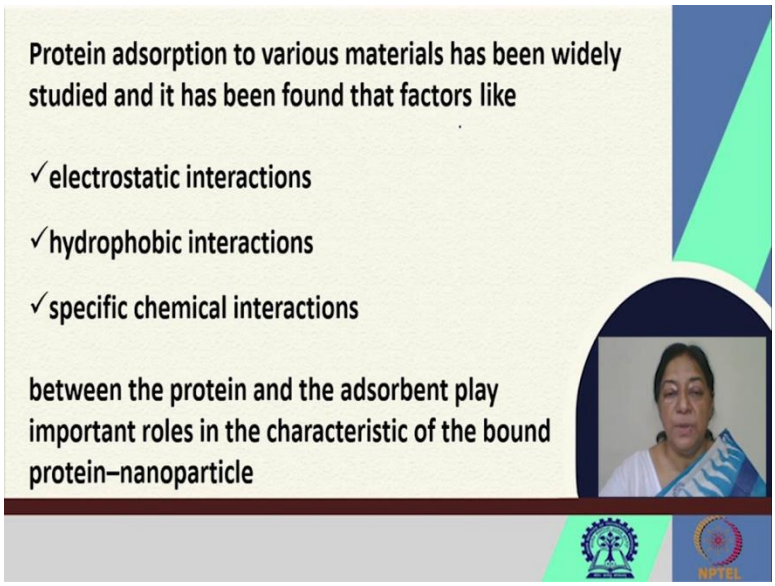

The protein adsorption on the materials has been widely studied and it has been found that factors like the electrostatic interactions, the hydrophobic interactions, the specific chemical interactions are important between the protein and the adsorbent, that play important roles in the characteristic of the bound protein nanoparticle.

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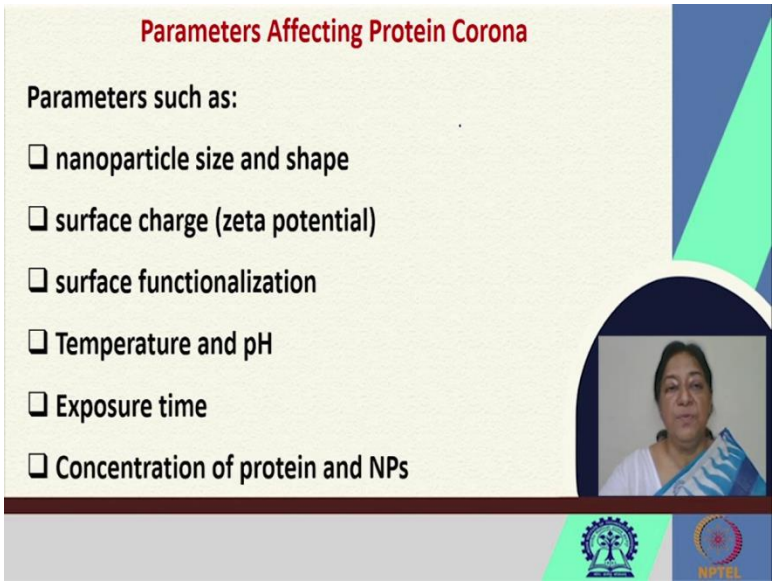

The parameters that can affect this protein corona are the nanoparticle size and shape; the surface charge; the surface functionalization; the temperature and the pH because that in turn will affect the structure of the protein and also the charge on the protein would be affected by changes in pH; then the exposure time meaning how long the protein has been allowed to interact with the specific nanoparticle and of course, the concentration of the protein and the nanoparticles.

All of these factors are going to affect the adsorption of the formation of the protein corona that is the proteins on the nanoparticle surface.

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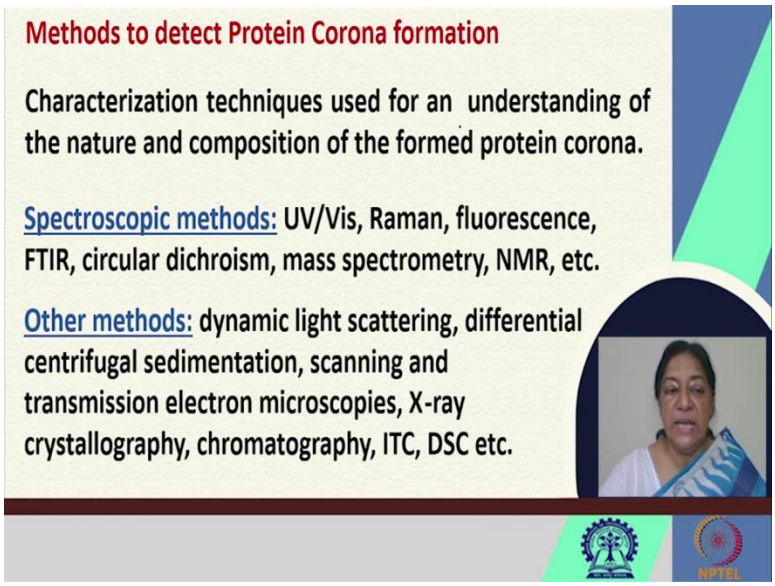

If we want to find methods that are going to detect the protein corona formation to understand whether the protein has interacted with the specific nanoparticle, then there are specific characterization techniques that are used for an understanding of the nature and composition of the formed protein corona.

Spectroscopic methods such as UV visible, Raman, fluorescence, FTIR, CD that is circular dichroism, mass spectrometry, NMR. These techniques can be used in addition to optical methods or other methods that could be dynamic light scattering, differential centrifugal sedimentation depending upon the size, then scanning and transmission electron microscopy, X-ray crystallography, chromatography, ITC and DSC.

So, all of these are going to look at the presence of the protein on the nanoparticle and specific interactions that may be possible, the property that may be affected of the nanoparticle and of the protein, due to the interaction that has occurred.

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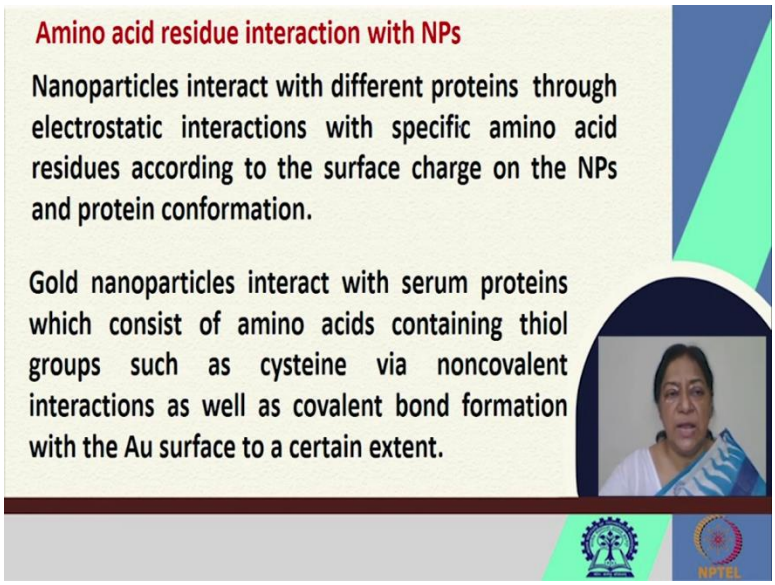

The amino acid residue interactions with the nanoparticles, they interact with different proteins through electrostatic interactions with specific amino acid residues, according to the surface charge on the nanoparticle as well as the protein. Gold nanoparticles are known to interact with serum proteins which consists of amino acids, that contain thiol groups such as cysteine via non-covalent interactions as well as covalent bond formation, with the gold surface to some extent.

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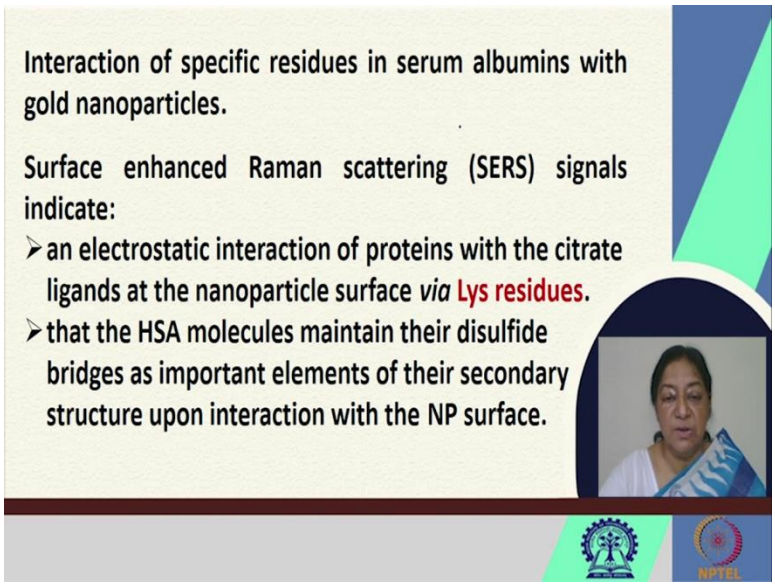

So the interaction of specific residues in the serum albumins with the gold nanoparticles, give us surface enhanced Raman scattering signals. These indicate an electrostatic interaction of the proteins with the citrate ligands at the nanoparticle surface via lysine residues, also that the HSL molecules maintain their disulfide bridges as important elements of their secondary structure, upon interaction with the nanoparticle surface.

The interaction that we can see occurs through the gold may occur through a thiol, may also occur through specific electrostatic interactions, depending upon the size and the charge of the specific protein and the nanoparticle of interest.

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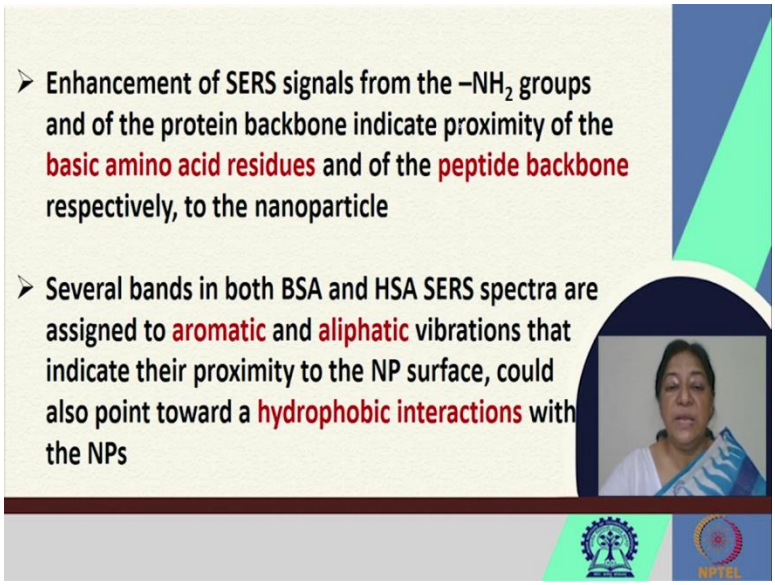

If we look at the enhancement of the SERS signals from the -NH_2 groups and of the protein backbone, these indicate proximity of basic amino acids and of the peptide backbone to the nanoparticle. There are several bands in both BSA and HSA, that is bovine serum albumin and human serum albumin spectra, that can also be assigned to aromatic and aliphatic vibrations that indicate their proximity to the nanoparticle surface, that could also point toward a hydrophobic interaction with the specific nanoparticles.

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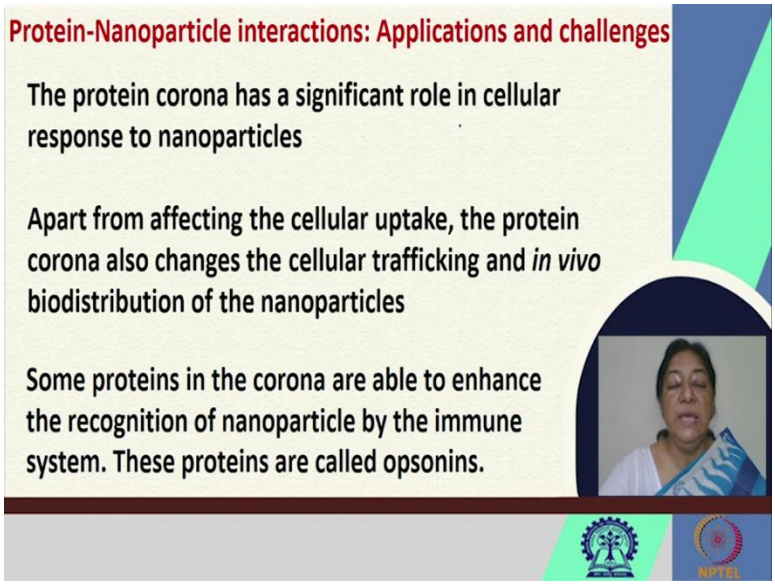

If we look at this protein nanoparticle interactions and the specific applications and challenges that come by, the protein corona has a significant role in cellular response to nanoparticles. Apart from affecting the cellular uptake, the protein corona also changes the cellular trafficking and in vivo biodistribution of the nanoparticles, which is dependent on the interactions.

So, some proteins in the corona are able to enhance the recognition of nanoparticles by the immune system. These specific proteins are called opsonins.

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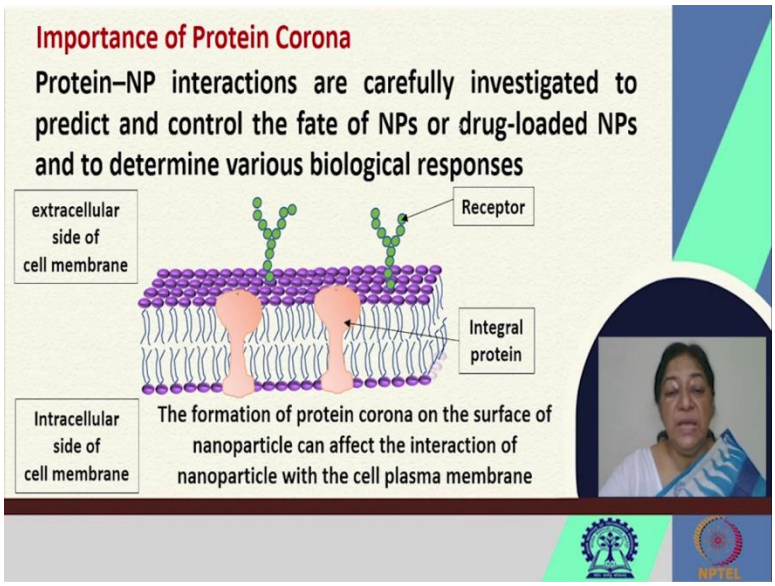

We can look at the importance of the protein corona in terms of the protein nanoparticle interactions, that can be carefully investigated to predict and control the fate of the nanoparticles or drug loaded nanoparticles and to determine various biological responses. So, we look at the interactions with the lipid bilayer. We have the integral protein, we have a specific receptor on the surface, the extracellular side of the cell membrane and the intracellular side of the cell membrane.

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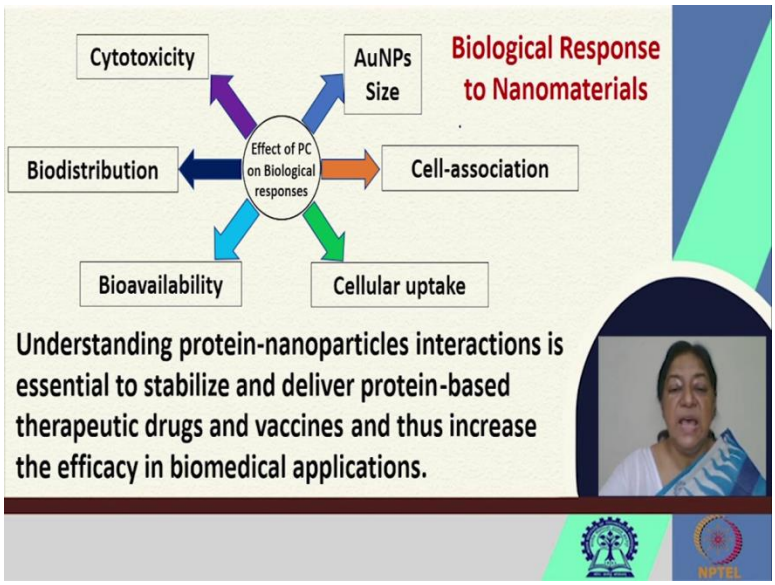

Now the formation of the protein corona on the surface of the nanoparticle can definitely affect the interaction of the nanoparticle with the cell plasma membrane, depending upon the protein that is present in the membrane or depending on the specific receptors of the surface, on the extracellular side of the cell membrane.

If we look at the specific biological responses to nanomaterials, the effect of a protein corona on these biological responses can result in cytotoxicity, biodistribution changes, bioavailability, cellular uptake, cell association and the variation due to the nanoparticle size. So understanding these protein nanoparticle interactions is essential, to stabilize and deliver protein based therapeutic drugs and vaccines and thus increase the efficacy in biomedical applications.

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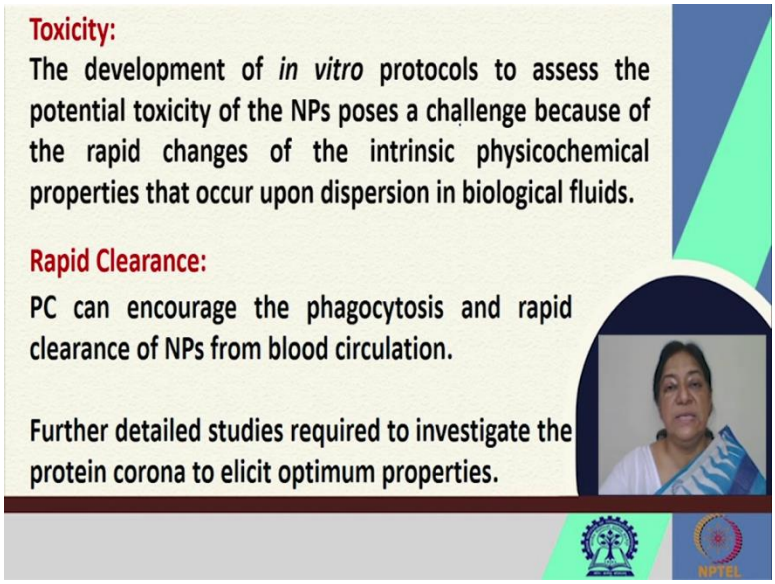

The development of in vitro protocols to assess the potential toxicity of the nanoparticles, poses a challenge because of the rapid changes in the intrinsic physiochemical properties that can occur upon dispersion in biological fluids because we have to understand that the content or the extent of the biological fluids is a dynamic process and is constantly changing.

So, there are going to be constant variations and this could give specific differences to the toxicity that is observed. A rapid clearance is also necessary where the protein corona can encourage phagocytosis and rapid clearance of the nanoparticles from blood circulation and so further detailed studies are required to investigate the protein corona to elicit optimum properties.

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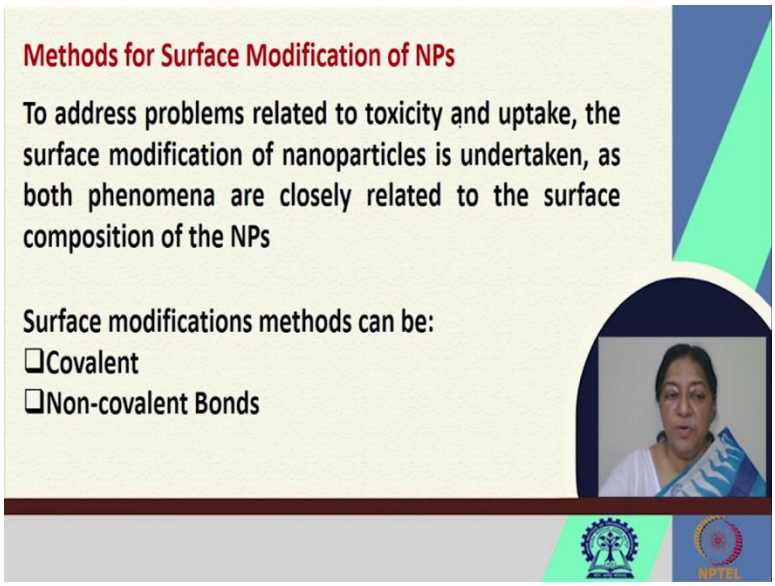

There are methods for surface modification of nanoparticles, so that they can be optimally utilized and to address problems related to the toxicity and the uptake, surface modification of nanoparticles is undertaken, as both phenomena are closely related to the surface composition of the nanoparticles. The surface modifications can be either covalent bond formation or non covalent interactions.

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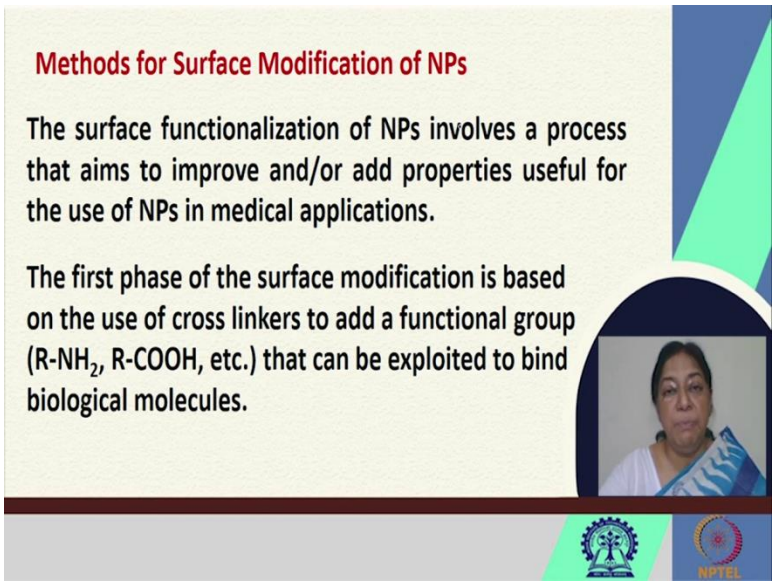

The methods for surface modification include surface functionalization, involving a process that aims to improve and/or add properties useful for the use of nanoparticles in medical applications. The first phase of the surface modification is based on the use of cross linkers. These add specific functional groups to the surface that can be exploited, to further bind biological molecules.

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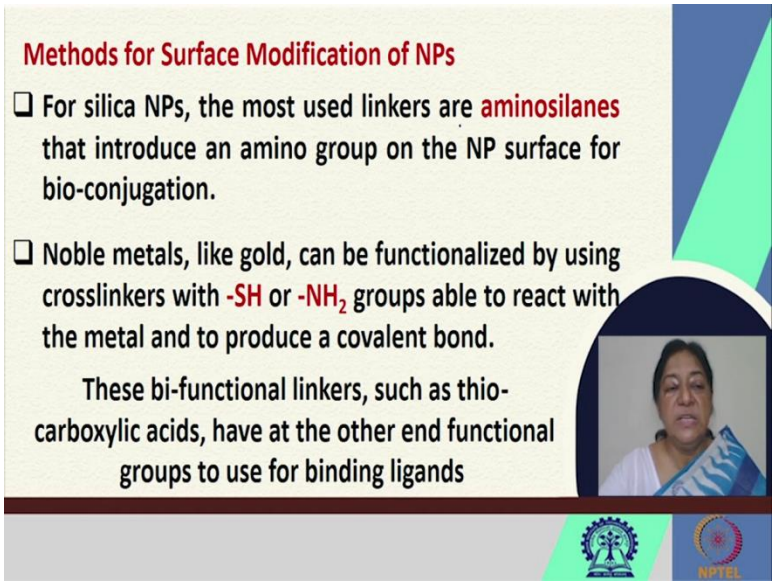

For example in silicon nanoparticles the most used linkers are aminosilanes, that introduce an amino group on the nanoparticle surface for possible bio-conjugation. Similarly for noble metals like gold, they can be functionalized by using cross linkers with -SH or -NH_2 groups attached to them, that can react to produce a covalent bond. So, such bifunctional linkers such as the thio-carboxylic acids, have at the other end functional groups that can be used for binding specific ligands.

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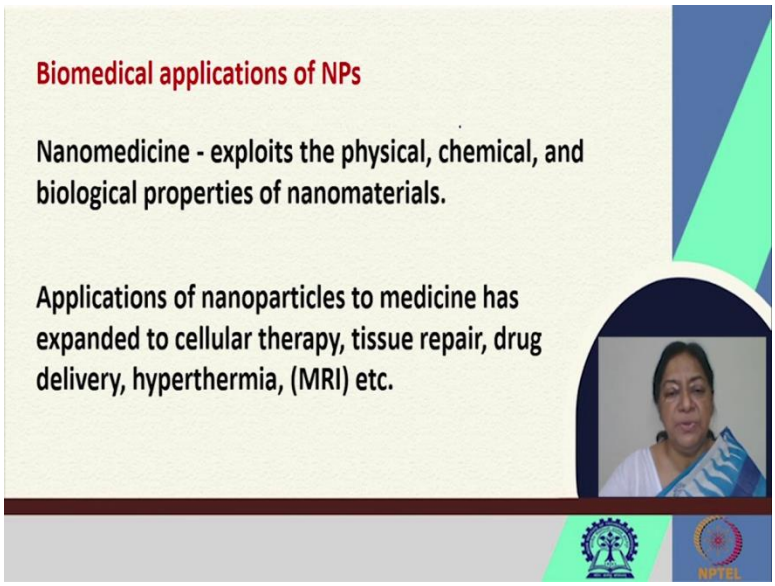

The biomedical applications of nanoparticles are very diverse. In nanomedicine, this exploits the physical, chemical and biological properties of the nanomaterials. So the applications of nanoparticles to medicine has expanded from cellular therapy, to tissue repair, to drug delivery, MRI, hyperthermia and many other methodologies.

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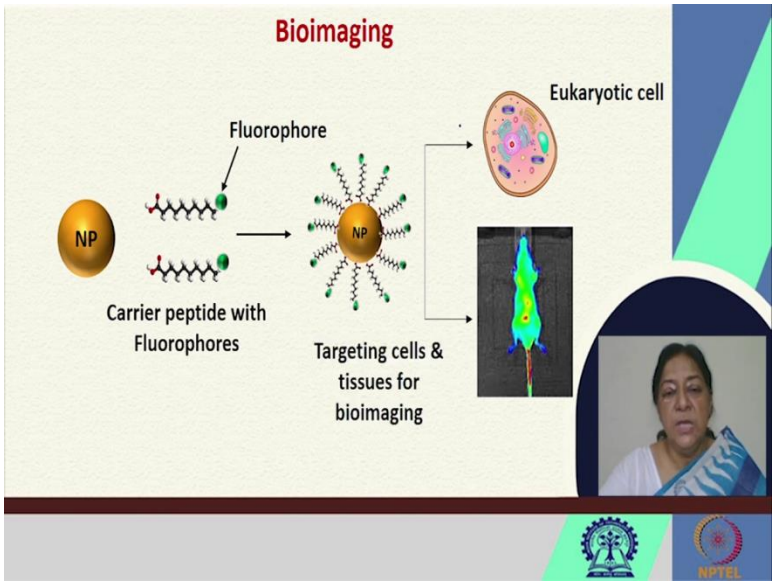

These can be summarized in the following slides [refer to the slides] where they can be used in bioimaging techniques, where the nanoparticle has a fluorophore and a carrier peptide with the fluorophore, that is then bound to the nanoparticle and then trapped in the eukaryotic cell in a bioimaging process, looking at targeting cells and tissues for bioimaging.

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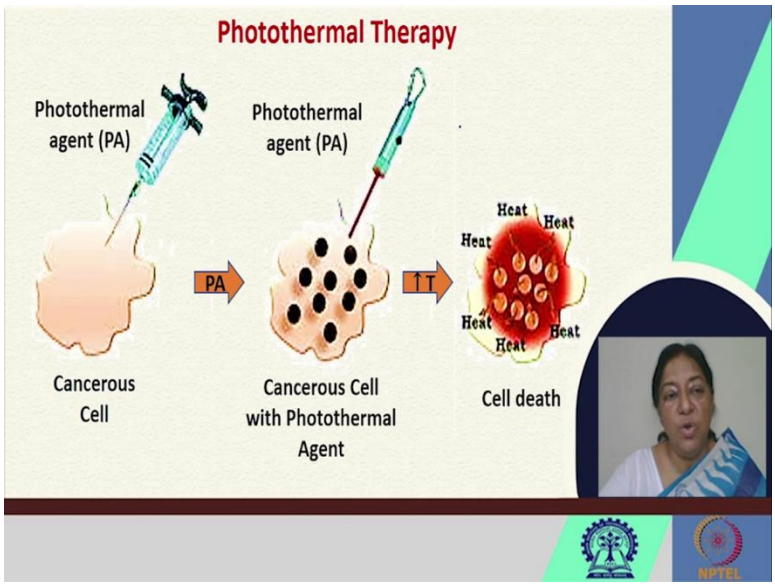

In photothermal therapy as the name implies, if there is a cancerous cell, a photothermal agent then can be targeted with a nanoparticle and a photothermal agent created and the cancerous cell, with the photothermal agent then get destructed with the application of heat.

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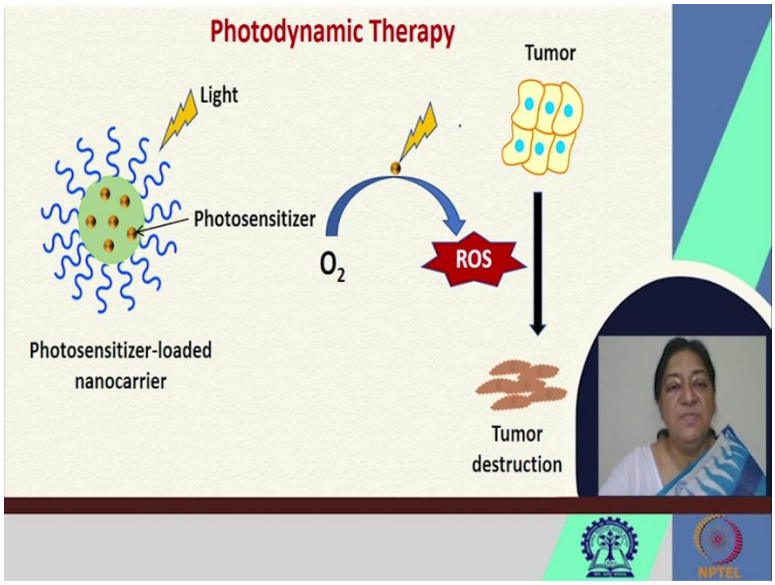

In a photodynamic therapy, we have photosensitizers loaded nanocarriers. In this [refer to slide] case there is a photosensitizer and specific light is going to then create specific reactive oxygen species, ROS and result in tumor destruction.

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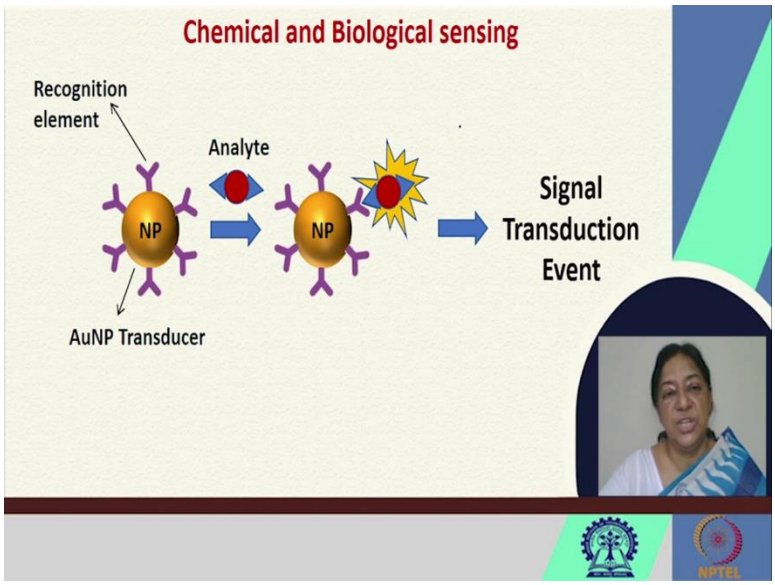

In chemical and biological sensing, we have the nanoparticles that have specific transducers attached to them, a recognition element such as an antibody that can attach specific molecules to it and can be used for biological sensing. So, this could generate a signal that then can be studied with a signal transduction event.

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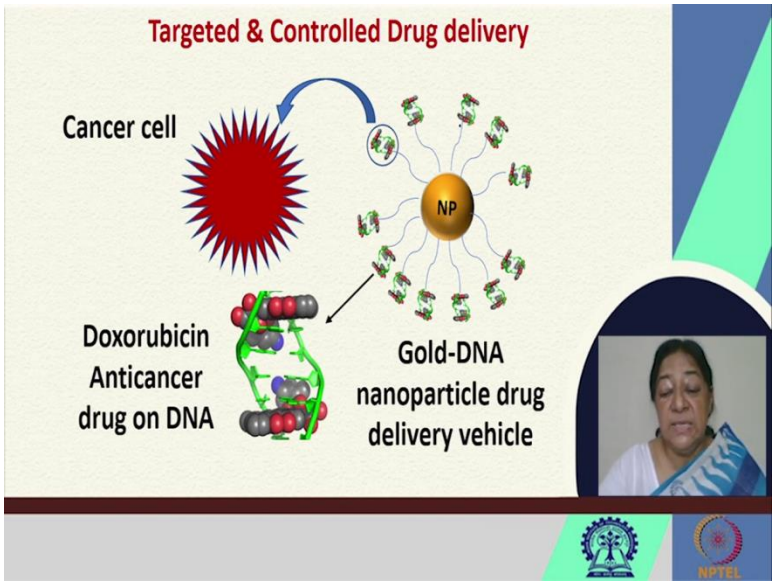

Targeted and controlled drug delivery is what is extremely important. So in this [refer to slide] case for example, doxorubicin anticancer drug on DNA and a cancer cell, the gold DNA nanoparticle drug delivery vehicle was able to deliver doxorubicin. But again this is extremely important in where it is going and how it is being delivered and of course, the clearance of the nanoparticle as well.

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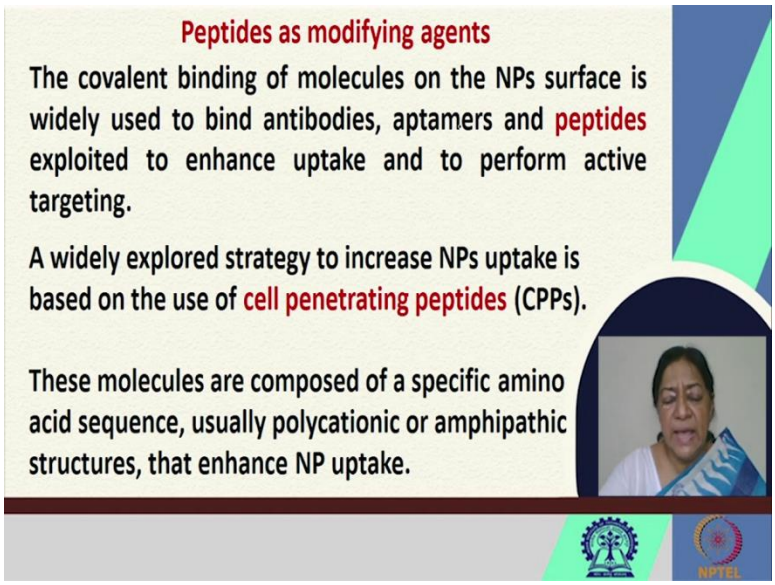

Here peptides can be used as modifying agents. The covalent binding of molecules on the nanoparticle surface is widely used to bind antibodies, aptamers and peptides, that are exploited to enhance uptake and to perform active targeting. A widely used explode strategy to do this, is using cell penetrating peptides, the topic that we discussed in protein peptide interactions. So, these molecules are composed of specific amino acid sequences that can enhance nanoparticle optic.

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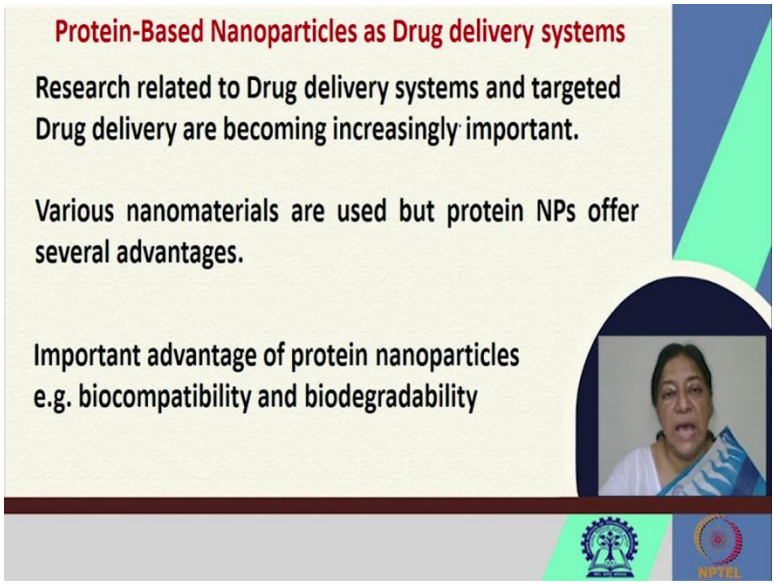

The protein-based nanoparticles are thus used as drug delivery systems. The research related to drug delivery systems and targeted drug delivery are becoming increasingly important. Various nanoparticles have been used, but protein nanoparticles offer several advantages. First, the important advantage of the use of protein nanoparticles, is their biocompatibility and their biodegradability.

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The preparation of protein nanoparticles and the corresponding encapsulation process of drugs or specific compounds, involve relatively mild conditions with no toxic chemicals or organic solvents that are primarily used. The stability, activity and half-life, can be improved by protecting the drug from enzymatic degradation and the protein nanoparticles can be used in a variety of targeted therapies including cancer therapy.

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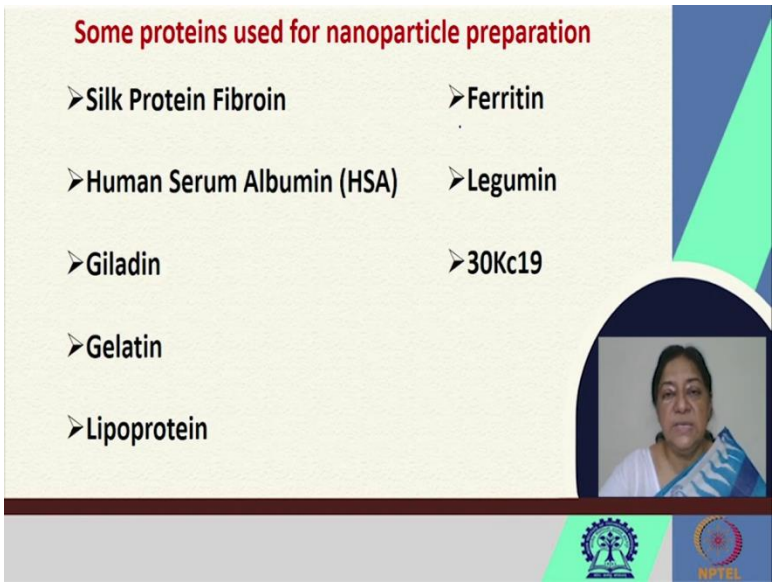

These [refer to slide] are some proteins that are used for nanoparticle preparation.

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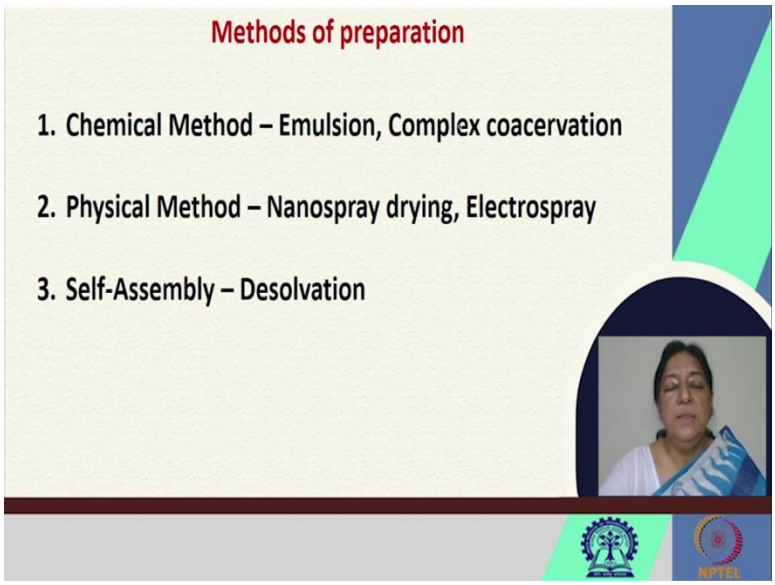

The methods of preparation include chemical methods, physical methods and even self - assembly where we have the protein nanoparticle being prepared.

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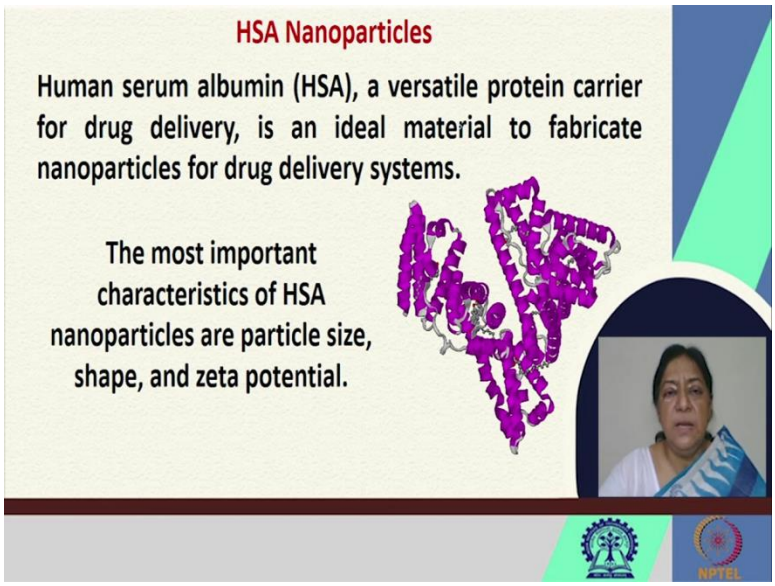

For example human serum albumin nanoparticles HSA, is a versatile protein carrier for drug delivery and is an ideal material to fabricate nanoparticles for drug delivery systems, for the preparation of such systems, to not only see the protein as a protein corona on a nanoparticle, but also create the nanoparticle from HSA itself and the most important characteristics of HSA nanoparticles are their particle size, their shape and the charges that can be determined by the zeta potential.

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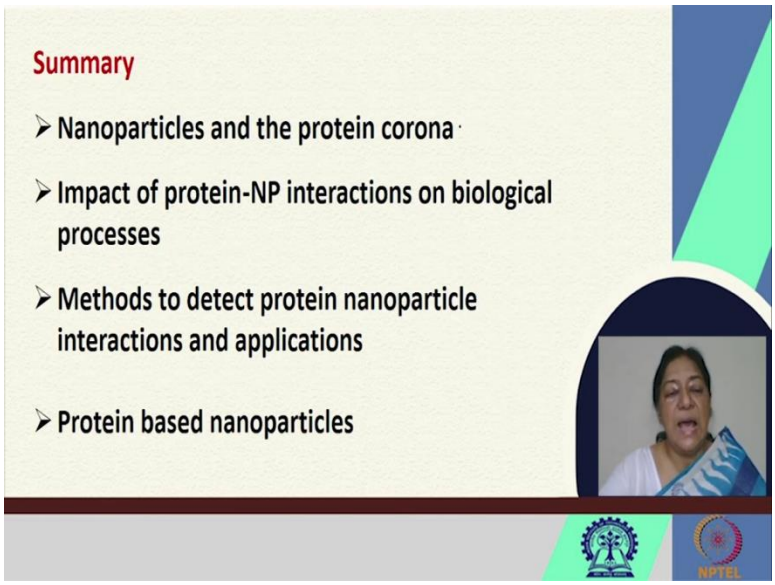

So in summary, we looked at nanoparticles and the protein corona, the importance of the protein corona and trying to understand how interactions can occur, the impact of protein - nanoparticle interactions on biological processes, how we can develop methods to detect protein nanoparticle interactions and their specific applications particularly in biomedicine, where we can look at bioimaging, phototherapy, dynamic or photochemical therapy and several methodologies that can be used to develop this for a nanomedicine. We also looked at protein-based nanoparticles, the advantage of being their biocompatibility and their biodegradability.

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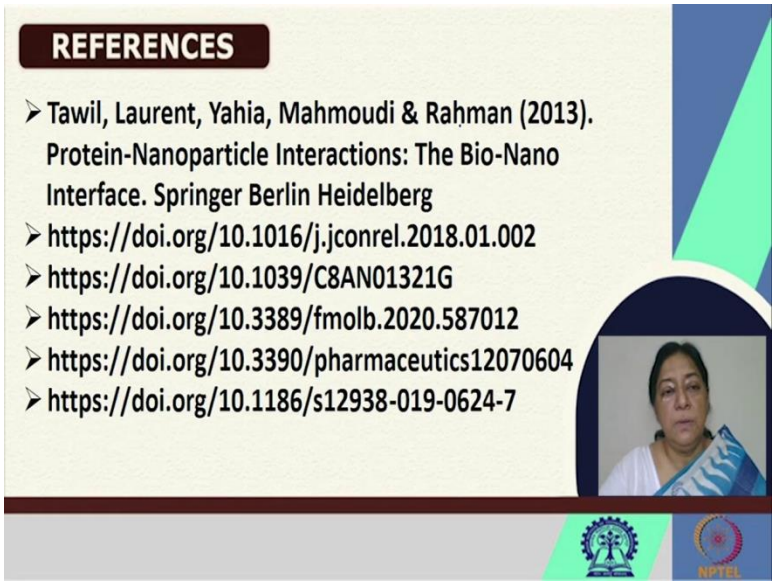

These [refer to slide] are the references.