Biophysical Techniques to Probe Nanoparticle-Protein Interactions

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Nanoparticles (NP) have been used in the advancement of medicine and biotechnology, with applications ranging fromclinical diagnosis, drug delivery, tobiosensing. Nanoparticlecomposition canvary significantly from metals to inorganic semiconductors. Inaddition, there are a variety of surface modifications available depending on the desired physiochemical and biological activities. Identifying nanoparticle interfaces that facilitate both specific and non-specific interactions in biological media is a daunting task. Nano-bio assemblies require the use of various analytical approaches to gain fundamental insight on these novel materials. Furthermore, when NPs are introduced into biological media composed of nucleic acids, lipids, and proteins, theywill spontaneouslyinteract forming a biomolecular corona.Due to these phenomena, and given the broad medical applications of NPs, understanding NP-protein interactions isof paramount importance.It ishypothesized that thetranslocation of a protein fromaqueous medium to NP corona can affect the protein's conformation and dynamics, which in turn can interfere with thesurface properties of the NPs. Nanoparticle curvature is prone to induce conformational changesin both the secondary and tertiary structures of many proteins.

Different analytical tools have been employedto characterizebiocoronaformation on NP surfaces. These techniques include surface plasmon resonance (SPR), transmission electron microscopy (TEM),mass spectrometry(MS) based proteomics, chromatography, fluorescence spectroscopy,CDspectroscopy,electrophoresis,dynamic light scattering (DLS), isothermal titration calorimetry (ITC),and nuclear magnetic resonance spectroscopy (NMR). These techniques have been used to identify

the structural and conformational changes of various proteins onto the NP surface.

Under physiological conditions, biological molecules spontaneouslyadsorbonto NP surfaces.This in turn changes the surface properties of the NP by the formation ofa biocorona. Understanding the biophysical characteristics of the NP biocoronacan leadto new applicationsin thebiological and medical fields. The biocorona consists of proteins, carbohydrates, lipids, and nucleic acids that either loosely (soft corona) or tightly (hard corona) associate with the NP surface.Determinationof binding affinities, binding capacities, association and dissociation rates, and stoichiometries of these biomolecules are essentialfor a thoroughunderstanding of the corona's properties. Different analytical techniques have been employed to study NP-protein interactions, specifically UV-Vis, MS, DLS, ITC, and CD. Each techniquehas unique advantages and disadvantages when investigating NP-protein interactions.

In UV-Visible(UV-Vis) spectroscopy, the localized surface plasmon resonance (LSPR) phenomenon is used to characterizethe metallic NPs, such as gold nanoparticles(AuNPs) and silver nanoparticles (AgNPs), and its conjugates. LSPR is only present in plasmonic NPs and cannot be used in non-metallic systems, such as liposomes and SiNPs. The LSPR peak shape and size is caused by a collective oscillation of free electrons of the metallic particle. A shift and broadening of the absorption spectra for the NP-protein complex will depend on the bioconjugate size, aggregation state, and the local dielectric environment. Due to this phenomenon, UV-Vis is widely used to quantify metallicNPs and qualitatively measure conjugate binding. UV-Vis is a non-invasive method, where the integrity of the sample is not compromised,and is aninexpensivetechnique that requires little sample preparation. The major disadvantage of UV-Vis is that the adsorption spectrum ishighly influenced by the solvent, pH, temperature, and high electrolyte concentration. Moreover, very little can be learned about biomolecular structures using UV-Vis spectroscopy alone.

Fluorescence, Raman, and CD spectroscopy can be effectively used to detect NP-protein binding. Theadvantage of employing these techniques isthat they do not require plasmonic NP systems. However, there are significant factorswhich can complicate each of these methods. For example, fluorescence spectroscopy typically requires natural fluorophores, such as tyrosine or tryptophan in proteins.If these are not present, cysteine or aminescan be labeled with fluorescent probes, such as fluorescein, to study their structural and dynamic properties.Even though fluorescence is a highly sensitive technique,its main disadvantage is that the addition offluorescent probes can alter the NP-proteininteraction. Moreover,the inner filter effect (IFE) and light scatteringfrom the proteins or NPs may complicate the interpretation of fluorescence experiments.

Some of the drawbacks faced by fluorescence spectroscopy can be avoided using Raman spectroscopy, a non-destructive, highly specific technique thathas a distinct fingerprint for solid and liquid solutions. Ramanspectroscopy measures the NP bioconjugatein aqueous solutions with great spectral resolution.Surface enhanced Ramanspectroscopy (SERS) has improved the measurements of Raman spectroscopy with higher sensitivity and higher selectivity of chemical groups. SERS has been used todetermine the structural and conformational changes of protein on metal NPsurfaces. Apart from protein conformation data, the morphological changes in AuNPs when interacting with proteins, are also detected through SERS spectra. One drawback is the intense laser heating the NP-protein conjugate, which can alter its structure and conformation, giving rise to misleading results.

Neither fluorescence norRaman spectroscopy can detect secondary or tertiary structuralchanges in proteins; however,CD is used extensively to determine the secondary structure of proteins and how these structures change upon binding to NP surfaces. To exhibit a CD signal, a molecule must be chiral.However, NP surfaces are not typically chiral and will not generally interfere with signal and interpretation of data.As a drawback, UV-CD provides only a rough estimation of conformational changes, since the unbound (native) protein is typically left in the cuvette when the NP-bound protein is measured, and the unbound protein often dominates the observed signal. Separating the NP-bound protein by centrifugation, or performing a difference measurement are viable alternatives, althoughthe signal originating solely from NP-bound proteins is often very weak.

Other, non-spectroscopic approaches are also useful, and include dynamic light scattering, chromatography, mass spectrometry, and isothermal titration calorimetry. Dynamic light scattering (DLS) is a popular technique used to determine the hydrodynamic size of NPs in suspension.DLS measures the scattering intensity fluctuations caused by the Brownian motion of NPs in solution and uses the Stokes-Einstein equation to relate the diffusion coefficient to the NP size. The measured hydrodynamic diameter reflects the dimensions of the NP as well as thecorona layer bound to the NP surface in solution. The hydrodynamic radius measured by DLS can also be used to determine the binding ratio of protein to NP. Although DLS is a non-perturbative, fast, and accurate method,it requires adust free and dilute sample having a monodisperse population. Furthermore, this method suffers from low sensitivity toward small particles and possible interferences from light-absorbing species.

Chromatographic methods,like size exclusion chromatography (SEC) or gel filtration chromatography,are frequently used to separatecomplex mixtures of biological compoundsbased on their size. Gel filtration chromatography has been used to detect proteins bound to NPs and determine the exchange rate by comparing the bound versus free protein elution profiles.There are inherent drawbacks owing to the sensitivity of the technique, including lower precision, accuracy, and longer acquisition times. Electrophoresis is another useful technique to separate complex NP-protein mixtures thatprovidesqualitative and quantitative analysis. Polyacrylamide gel electrophoresis (PAGE) is one of the most widely used methods to separate NP-protein complexes. Macromolecules are differentiated according to their electrophoretic mobility, which is a function of the molecule'slength, conformation, and charge. For proteins, sodium dodecyl sulfate (SDS) is used to denature proteins and give them a uniform charge/size ratio.Even though SDS-PAGE is an effective tool to identify the composition of the protein corona, it suffers from poor protein separation if the protein mixtureis too complex, resulting in comigration of several proteinswith similar size.

Mass spectrometry (MS) is a high throughput, sensitive analytical technique used to monitor larger proteins (up to ~100 kDa) interacting with NPs. The two main ionization methods used to investigate biomolecules are matrix-assisted laser desorption ionization (MALDI) and electrospray ionization (ESI). Protein samples are often digested using proteolytic enzymes, into smaller fragments that are more suitable for the mass range of instruments. MS can provide both qualitative and quantitative information regarding the protein mixtures present on NP surfaces andcan be used in parallel with chromatographic and gel-based methods to identify the composition of the protein corona.Primarily, the use of protein fragments enables one to investigate structure on the NP surface. Even though MS is a destructive method, it provides qualitative and quantitative values that reflect the protein abundance in the protein corona, and it often uses comparatively small amounts of sample.

Isothermal titration calorimetry (ITC) can be used to directly measure the enthalpy change when proteins interact with NPs. In general, to measure the enthalpy change, the protein of interest is gradually added to a solution containing NPs and the evolved heat of binding is measured. These heats are calculated using the power required to maintain isothermal conditions.Fitting a thermodynamic model to the heats produces parameters like stoichiometry and the enthalpy of binding. Several drawbacks exist when using ITC to study protein-NP binding. For one, the method requires high sample concentrations (0.1-1 mM) and volumes (~1 mL). Secondly, adsorption may not produce a measurable heat, even when NPs are quite concentrated. Finally, if multiple steps occur (e.g. binding and unfolding), ITC thermograms may be challenging/difficult to interpret. Despite these challenges, ITC remains a useful tool for understanding the strength of biomolecule-NP interactions.

In summary, each of the analytical methods mentioned abovehas their own advantages and disadvantages that coincides with the physical properties being measured.

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