EDITORIAL

Conjugation of nanoparticles

A nanoparticle–biomolecule conjugate is a nanoparticle with biomolecules attached to its surface. Nanoparticles (NPs) are minuscule particles, typically measured in nanometres (nm) that are used in nanobiotechnology to explore the functions of biomolecules. Properties of the ultrafine particles are characterized by the components on their surfaces more so than larger structures, such as cells, due to large surface area-to-volume ratios. Large surface area-to-volume-ratios of nanoparticles optimize the potential for interactions with biomolecules. Major characteristics of nanoparticles include volume, structure, and visual properties that make them valuable in nanobiotechnology. Depending on specific properties of size, structure, and luminescence, nanoparticles can be used for different applications.

Nanoparticles are valuable tools in identification of biomolecules, through the use of bio-tagging or labeling. Attachments of ligands or molecular coatings to the surface of a nanoparticle facilitate nanoparticle-molecule interaction, and make them biocompatible. Conjugation can be achieved through intermolecular attractions between the nanoparticle and biomolecule such as covalent bonding, chemisorption, and noncovalent interactions.

To enhance visualization, nanoparticles can also be made to fluoresce by controlling the size and shape of a nanoparticle probe. Fluorescence increases luminescence by increasing the range of wavelengths the emitted light can reach, allowing for biomarkers with a variety of colours. This technique is used to track the efficacy of protein transfer both *in vivo* and *in vitro* in terms of genetic alternations (1).

Nanoparticles provide a particularly useful platform, demonstrating unique properties with potentially wide-ranging therapeutic applications. The unique properties and utility of nanoparticles arise from a variety of attributes, including the similar size of nanoparticles and biomolecules such as proteins and polynucleic acids. Additionally, nanoparticles can be fashioned with a wide range of metal and semiconductor core materials that impart useful properties such as fluorescence and magnetic behaviour.

Biomacromolecule surface recognition by nanoparticles as artificial receptors provides a potential tool for controlling cellular and extracellular processes for numerous biological applications such as transcription regulation, enzymatic inhibition, delivery and sensing. The size of nanoparticle cores can be tuned from 1.5 nm to more than 10 nm depending on the core material, providing a suitable platform for the interaction of nanoparticles with proteins and other biomolecules.

The conjugation of nanoparticles with biomolecules such as proteins and DNA can be done by using two different approaches, direct covalent linkage and non-covalent interactions between the particle and biomolecules (2-7). The most direct approach to the creation of integrated biomolecule nanoparticle conjugates is through covalent attachment (8).

This conjugation can be achieved either through chemisorption of the biomolecule to the particle surface or through the use of hetero bifunctional linkers. Chemisorption of proteins onto the surface of nanoparticles (usually containing a core of Au, ZnS, CdS, and CdSe/ZnS) can be done through cysteine residues that are present in the protein surface (e.g., oligopeptide, serum albumin), or chemically using 2-iminothiolane (Traut's reagent). Bifunctional linkers provide a versatile means of bioconjugation. Biomolecules are often covalently linked to ligands on the nanoparticle surface via traditional coupling strategies such as carbodiimide-mediated amidation and esterification. For biological applications oligoethylene glycol (OEG) or polyethylene glycol (PEG) is used in the linker to enhance the stability of the attached biomolecules and minimize non-specific adsorption of other materials. Non-covalent assembly provides a modular approach to the biofunctionalization of nanoparticles. DNA–NP binding can be effected through electrostatic interactions, groove binding, intercalation, and complementary single-strand DNA binding. Nanoparticles provide an attractive receptor for nucleic acids, providing a direct analogy to protein–DNA interactions (9).

The conjugation of NPs to proteins is of increasing interest in biomedicine. NP–protein conjugates hold great promise in sensing/diagnostics, in targeted delivery, in the control of protein activity and in imaging. To this end, efforts have been made to synthesize biocompatible NPs that are nontoxic and stable at physiological pH. Several strategies have been developed to assemble NP-protein conjugates. A commonly used approach is to attach the NP on the protein via non-covalent interactions, mostly through electrostatic adsorption. In the 1970s, negatively charged gold NPs

were already linked non-covalently to various proteins, such as antibodies, horseradish peroxidase, and bovine serum albumin, to serve as electron dense labels for electron microscopy (EM) of tissue sections. Although this approach is relatively straightforward and does not require cloning or chemical cross-linkage, the NP can dissociate from the protein, especially at pH values above the protein isoelectric point (pI) or at high salt concentrations. In contrast, the formation of a covalent bond with either the NP core atoms or the NP ligand molecules creates a more stable NP–protein complex and allows controlling the site of attachment on the protein more easily. Such site-specific labelling is especially advantageous in imaging and sensing applications. In EM imaging applications, for instance, site-specific NP attachment on the protein enables one to go beyond the traditional histology applications and to localize specific structures within large proteins (10).

DNA, which carries genetic information, is potentially useful for high-density memory. DNA's specific hybridization may allow molecules or clusters to be arranged on a nanometre scale exceeding the limitations of photolithography, the patterning size of which is limited up to the wavelength of light ~i.e., several hundred nanometres. DNA–nanoparticle conjugates have attracted great interest, as there are numerous opportunities to utilize the properties of the nanomaterial and the DNA synergistically on small length scales.

Gold nanoparticles (AuNPs) in particular, are widely used in biomedical applications such as delivery, sensing, and imaging due to their exceptional biocompatibility. AuNPs have a relatively inert surface but can be easily conjugated to biomolecules by covalent linkage via the sulphur atom in a thiol. Therefore, thiol-capped DNA oligos have been popular for constructing AuNP– DNA conjugates. Thiol-aided conjugation is considered feasible for the laboratory; however, some important issues are often ignored in practice. The first is the stability of the nanoparticles during the conjugation process. Conjugation via a thiol group is typically performed in concentrated ionic buffers which screen the surface charge of the particles and DNA, so that electrostatic repulsion is diminished, and therefore increasing the collision rate between the two species. Using concentrated samples also raises this collision rate. Lyophilization is a method that can elevate both the ionic strength of solution and the concentration of each species extremely high as the water content of the solution dries out. Citrate-stabilized AuNPs are commonly used in laboratories but they irreversibly aggregate during lyophilization due to their low stability. A chemical ligand bis (p-sulfonatophenyl) phenylphosphine dihydrate dipotassium salt is thus used to replace the citrate molecules on the AuNP surface, and allows AuNPs to sustain stability under more severe ionic conditions (11).

Conjugation of antibodies to nanoparticles is important for the development of vehicles with targeting specificity, which can be used for diagnosis, and treatment of cancer and other diseases. The antibody association with nanoliposomes and other nanoparticles is achieved mostly by generation of covalent bonds based on their chemical and structural properties (12).

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