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Original Research

Synthesis and Surface Modification of Nanomaterials for Tumor Targeting: Gold nanoparticle conjugation, Direct Adsorption of Proteins and Role of Free Thiol on Protein Adsorption to Gold Nanoparticles

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Abstract:

Nanomaterials' ability to modulate drug release rate and dosage in drug transport makes them useful in tumor-targeted therapy. Before the medicine reaches its intended target, they prevent it from degrading and allow for its regulated release in that setting. This literature review aims to analyse the efficacy of drug delivery systems based on nanoparticles for cancer treatment. Anticancer agents may be better delivered, systemic toxicity reduced, and therapy outcomes improved with the use of nanoparticles. Extensive research has demonstrated encouraging outcomes in both preclinical and clinical trials. To improve clinical translation, however, problems including unstable drug loading capacities, possible side effects, and other obstacles must be overcome. Improving drug loading capacity is a hot topic in the research community. Possible solutions include creating new drug encapsulating techniques or tweaking the surfaces of nanoparticles. The therapeutic effectiveness of these systems can be greatly improved by increasing drug loading. Another obstacle in clinical translation is stability concerns. Scientists are looking for ways to make nanoparticles more stable, like applying protective coatings or tweaking the composition, in order to solve stability problems. To further reduce the likelihood of adverse effects, researchers are taking great care to synthesise nanoparticles from biocompatible materials and are performing extensive toxicity tests prior to beginning clinical trials.

Keywords: Nanomaterials, Tumor Targeting, Synthesis, Surface Modification.

Introduction:

Nanoparticles have the potential to treat a wide range of disorders, including cancer, and there has been a dramatic increase in research into this area throughout the last decade. The first step in making a therapeutic nanoparticle is making a nanoparticle with the exact characteristics (such as magnetic properties, optical absorption, etc.) that will provide the desired therapeutic effect. Successful delivery of the particle to the tumour site is the next step. In order to make them more suitable for intravenous distribution to tumour locations, the surfaces of most nanoparticle materials must be modified in some way. With the ever-growing field of nanoparticle-based therapeutics, it would be extremely challenging to provide a complete inventory of all the different combinations of nanoparticle production processes, nanoparticle materials, geometries, and features [1, 2]. We will go over how to make some of the most common nanoparticle systems, and then how to tweak them so that they may be delivered to tumours.

Surface and Synthesis Elevated Metal Particle Functionalization Biologically active gold and silver nanoparticles have a long history of usage due to their low reactivity, low toxicity profiles[3,4], and relative ease of functionalization. These nanoparticles have also found utility in a number of therapeutic contexts. Particles of gold and silver can exhibit size-dependent surface plasmon resonances, which give their suspensions their distinctively bright hues. Because of their powerful optical resonances, they have attracted attention as potential agents for surface-enhanced Raman scattering and fluorescence tests, as well as for use in thermal ablation [5-7]. They have also been utilised as contrast agents for electron microscopy [8, 9] and to improve the effectiveness of radiation therapy [10] due to their electron density. Synthetic methods for gold and silver nanoparticles have proliferated in recent years, resulting in a diverse range of sizes and forms, including spherical, rod-shaped, prismatic, and cube-shaped [11–13]. Silver particles have been used in wound dressings due to their intriguing antibacterial characteristics.

Gold nanoparticles are the most used noble metal particle for many in vivo applications due to their low toxicity and enhanced durability. Particle sizes ranging from around 12 to 200 nm are ideal for therapeutic uses that aim to accumulate tumours. Particles smaller than 10 nanometers can be swiftly eliminated from the bloodstream by means of the kidneys. The interstitial region surrounding tumours may be inaccessible to large-diameter particles (>250 nm) due to the tumours' leaky vasculature. In this section, we will concentrate on the manufacture of gold particles, which are commonly utilised in therapeutic applications, because there is insufficient room to discuss manufacturing procedures for all forms of metal nanoparticles.

Golden Balls:

Among metal nanoparticles, spherical gold is among the most common and ubiquitous. Since Faraday's seminal work in 1857 (Faraday 1857), spherical gold nanoparticles have been extensively studied and exploited. There are a lot of businesses offering gold particles of different sizes due to the growing interest in using gold nanoparticles in various biological applications. A few of the most common ways that gold colloid is prepared are going to be covered here. There may be a lot of synthetic methods for spherical particles out there, but the fundamentals are the same for the vast majority of them. A gold-containing salt, often HAuCl4, is dissolved in water in these procedures [12]. A reducing substance is introduced to start the particle production process. You can regulate the size of the nanoparticles that come out by adjusting the concentration of salt in the solution and the strength of the reducing agent.

An aqueous phase is used exclusively or primarily for the production of these particles. Nanoparticle preparation in a two-phase systems is a well-established area, with studies by Goulet et al. (2010) and Li et al. 2011b describing how particles stabilised by monolayers of alkanethiols dissolved in the organic solvent make up the bulk

of the field. The utilisation of organic solvents may be off-putting to certain labs, despite the potential biological utility of these monolayer protected clusters. To avoid any potential toxicity, it is necessary to eliminate any leftover organic solvents before conducting in vivo experiments. Therefore, the preparations based on water will be the primary focus of this chapter.

Citric acid, sodium:

A widely used method for creating spherical gold colloid involves reducing the gold salt with sodium citrate. It is simple to adjust this recipe so that particles with a diameter between 12 and 50 nm are produced. Most of the time, people follow the procedures laid out by Turkevich and Frens (1973) when they prepare. Usually, when working with particles smaller than 12 nm, a solution of HAuCl4 is heated to a boil and then vigorously stirred. Add sodium citrate quickly to start particle growth. Starting with the yellow HAuCl4 solution, the colour will transition via a black intermediate and settle on a reddish-burgundy hue. To get particles with a narrow size distribution, keep mixing well when adding the reducing agent [13–16]. This kind of preparation usually results in a diameter size distribution of around 10%. By modifying the ratio of reducing agents to gold salt, the total particle diameter can be customised.

Hyperthermia-Inducing Near-Infrared Absorbing Particles:

While it has been demonstrated in certain studies that tumor-resident metal nanoparticles can improve radiation therapy, the majority of current efforts are devoted to direct thermal ablation of tumours and the generation of localised hyperthermia to boost radiation efficacy [17, 18]. Gold nanoshells and gold nanorods are the two most popular particles that absorb near-infrared light. In the near-infrared (NIR), both particle types exhibit robust, geometry-dependent optical resonances that can be tuned.

Thin films of gold:

It was Oldenburg et al. (1998, 1999) who first created the silica-gold nanoshell, which is composed of a core of silica and a thin layer of gold. The interaction between the outer and inner surface plasmons of the gold shell is responsible for the tunability of the optical resonance for these particles. By adjusting the thickness of the gold layer around the particle, one can adjust the optical resonance of the particle by plasmon hybridization [19–21]. A 120 nm silica core is suitable for near-infrared optical resonances, the most popular choice for hyperthermia applications. In the Stöber technique, tetraethyl orthosilicate is hydrolyzed by a base and subsequently condensed into SiO2 to form this core. Aminating the silica cores involves adding amino propyltriethoxysilane to a silica particle dispersion in order to enable the deposition of a gold layer on the silica surface. Centrifugation and resuspension in fresh ethanol are used to remove excess silane [22].

Following the procedure outlined by Duff and Baiker (1993), small gold colloid particles (1-3 nm in diameter) are added to the surface of the silica particles. This is done by mixing approximately 3.5 mL of silanized silica particles with 1 L of a gold colloid solution. Afterwards, the nucleating sites on the surface of the silica core are occupied by the gold colloid, which can be transformed into a full-fledged gold shell by adding a gold plating solution (~400 Mm HAuCl4, 2 mM K2CO3 in water) while formaldehyde is present. You can regulate the ultimate thickness of the gold shell and the optical resonance that results by adjusting the concentration of the silica seed particles in the plating solution.

Thin Films of Gold:

Nonspherical metal particles have seen a surge in usage throughout the last ten years. The capacity to more easily alter the optical properties of nonspherical particles by manipulating their geometry is one of the key advantages of these particles. The gold nanorod is one of the most widely utilised non-spherical particles in modern times. The particles in question are solid gold rods. Modulating the aspect ratio, which is the ratio of the nanorod's diameter to its length, allows one to alter the nanorod's optical resonance [28-

31]. By making some little adjustments to the synthetic procedures, these particles can be adjusted to absorb light in both the visible and near-infrared (NIR) sections of the spectrum. A seeded growth process is used to prepare most gold nanorods (Gole and Murphy 2004; Smith and Korgel 2008; Jana et al. 2001). The method begins with small, spherical gold particles. Particles typically need the surfactant cetyltrimethylammonium bromide (CTAB) to be grown into rod forms. If this surfactant is not present, increasing the concentration of the gold and reductant in the solution will result in spherical particles with bigger diameters. Particles become rod-shaped when the surfactant is present because they develop along certain crystal facets. The surfactant is essential for rod-shaped particle production, but also poses a problem for future applications, especially in laboratory settings and living organisms. There is strong evidence that CTAB is cytotoxic. It is also challenging to functionalize the gold nanorods further because they are coated with a bilayer of surfactant molecules [32]. It is necessary to extract the bulk of the CTAB solution before using the gold nanorods in biological contexts.

Numerous synthetic variants have been published, just like spherical gold particles. Jana et al. (2001) created the first protocol for making nano-rods. This recipe calls for the unaltered, unprocessed use of HAuCl4•3H2O, CTAB, NaBH4, AgNO3, and ascorbic acid. The initial step in making the gold "seed" particles is to combine 100 mM of CTAB with HAuCl4•3H2O, stirring the mixture for a short time. After that, 600 µL of a recently made, ice-cold solution of 10 mM NaBH4 is added, and then the mixture is mixed for 2 minutes. Add 40 mL of 100 mM CTAB, 1.7 mL of 10 mM HAuCl4•3H2O, 250 µL of 10 mM AgNO3, and then 270 µL of 100 mM ascorbic acid to make the nanorod growth solution. The seed solution was added to a solution that already included CTAB, HAuCl4•3H2O, AgNO3, and ascorbic acid in order to start the formation of the nanorods. After the solution has been carefully mixed, it is let to sit for 40 minutes. To stop the particles from "ageing," in which their sizes fluctuate over time, and the cytotoxicity caused by the CTAB, it is necessary to remove the excess reagents from the solution [33]. To remove any surplus of reagents, simply pellet the nanorods and resuspend them in fresh buffer. Additional methods for decreasing the CTAB concentration in the rod solution include dialysis, tangential flow filtration, and two-phase organic aqueous separations. It is important to avoid washing the nanorods too vigorously, as this could remove all of the CTAB [34]. The particles' surfactant coating stabilises the colloids and stops them from clumping together.

Protein Adsorption on Gold Nanoparticles Directly:

Proteins can be directly attached to gold nanoparticles using methods that have been available for quite some time. Immunohistochemistry has made considerable use of protein-labeled gold colloids as labels. Several vendors offer commercially available conjugates with common proteins, including avidin/streptavidin, albumin, protein A/G, and other antibodies [35, 36]. The effectiveness of protein attachment to the gold surface is pH-dependent because it occurs as a result of charge interaction between the protein and the gold particle.

When the solution pH is close to the protein's isoelectric point, protein attachment is most effective. When manipulating the pH of colloidal solutions, it is important to avoid adding too many ions, as this could lead to aggregation. A flocculation experiment can be run to find the best solution conditions for a protein's binding [37, 38]. This test involves changing the pH of a colloid solution and then adding the same quantity of protein to each of the solutions. After a predetermined amount of time has passed, often 30 to 60 minutes, the particles are centrifuged and resuspended in new buffer to eliminate any excess protein. Particles in the colloid solution that have weak surface-to-protein adhesion will clump together and precipitate out of the solution when sodium chloride is added.

This approach can be appealing for in vitro research when clearance by the immune system is not a concern, due to the relative ease of protein direct adsorption to the particles. In vivo circulation half-lives of particles generated using this technology are typically low because the particles are not "shielded" from the immune system. Verifying that the protein retains its biological activity following direct adsorption onto the surface of gold particles is essential [39–41]. It is possible that some proteins are inactive due to the lack of control over surface protein orientation. Additionally, denaturation of certain more fragile proteins could occur as a result of limitations on solution ionic strength and pH in relation to colloid stability.

Using adsorption to conjugate gold nanoparticles:

The initial approach reported by Frens [42] for creating citrate stabilised gold particles in "water" is the basis for our in-house developed procedures that are utilised to manufacture AURION Conventional Gold Nanoparticles, sizes 6-10-15 and 25nm. A negative surface charge is carried by these gold nanoparticles in a Frens goldsol because of surface plasmon resonance and the presence of citrate ions on their surface. Particles of colloidal gold also exhibit hydrophobic characteristics. When a macromolecule approaches its isoelectric point, its hydrophobic interactions become most apparent. The ideal pH for conjugation is typically around half a pH unit more than the molecule's IEP, which is the pH at which the molecule is expected to be adsorbed on the surface of the particle. This pH prevents clumping because the protein has a little negative overall charge. While the gold particles are negatively charged, the molecule will have enough positive charges (lysine and tryptophan rich regions) to encourage electrostatic contact. Gold particles bind most strongly and steadily to proteins through the chemisorption of thiol groups [43–46] from surface-exposed cysteines. In 1981, Goodman et al. proved that the most stable gold particles had just one layer of protein on their surface. Conjugates become less stable and more multi-layered when there is an excess of added protein. Modern extremely sensitive analytical methods like Differential Centrifugal Sedimentation and Dynamic Light Scattering have confirmed the existence of a hard corona and a soft-corona. Because of this, there are two important steps in the "classic" technique for conjugating gold nanoparticles: first, finding the minimum protective protein concentration that forms the hard corona; and second, purifying the soft corona to remove any immunoreactive molecules. To provide the best possible stability and activity of the conjugate, a combination of thiol group chemisorption, hydrophobic interaction, and electrostatic binding of a single layer of proteins on the surface of the gold nanoparticles is used. Both research and diagnostics can benefit from the use of such conjugates. Many of the commercially available tests rely on immunogold conjugates that are generated via adsorption; for example, pregnancy tests. 100 nm Prior to being subjected to 8M urea for 60 minutes, the particles of gold that were conjugated to F(ab')2 fragments of goat anti-rabbit IgG were treated. Urea has been utilised in affinity chromatography since the mid-sixties due to its ability to breakdown antibody clusters and dissociate high affinity antigen-antibody interactions. Not only does the absence of colour change or flocculation in the presence of 8M urea prove conjugate stability, but it also reveals that incubation in this solution has no effect on the reactivity of the gold reagent. A review by Bendayan in 1989 and a publication by Esbach et al. describe the binding and intracellular processing of Ultra Small gold nanoparticle (MD ~ 0.8nm) conjugated acetylated low density lipoproteins (Ac-LDL-Au) in rat liver endothelial cells, which are two other instances of enzyme-gold nanoparticle conjugates that retain bioactivity after conjugation using the adsorption method.

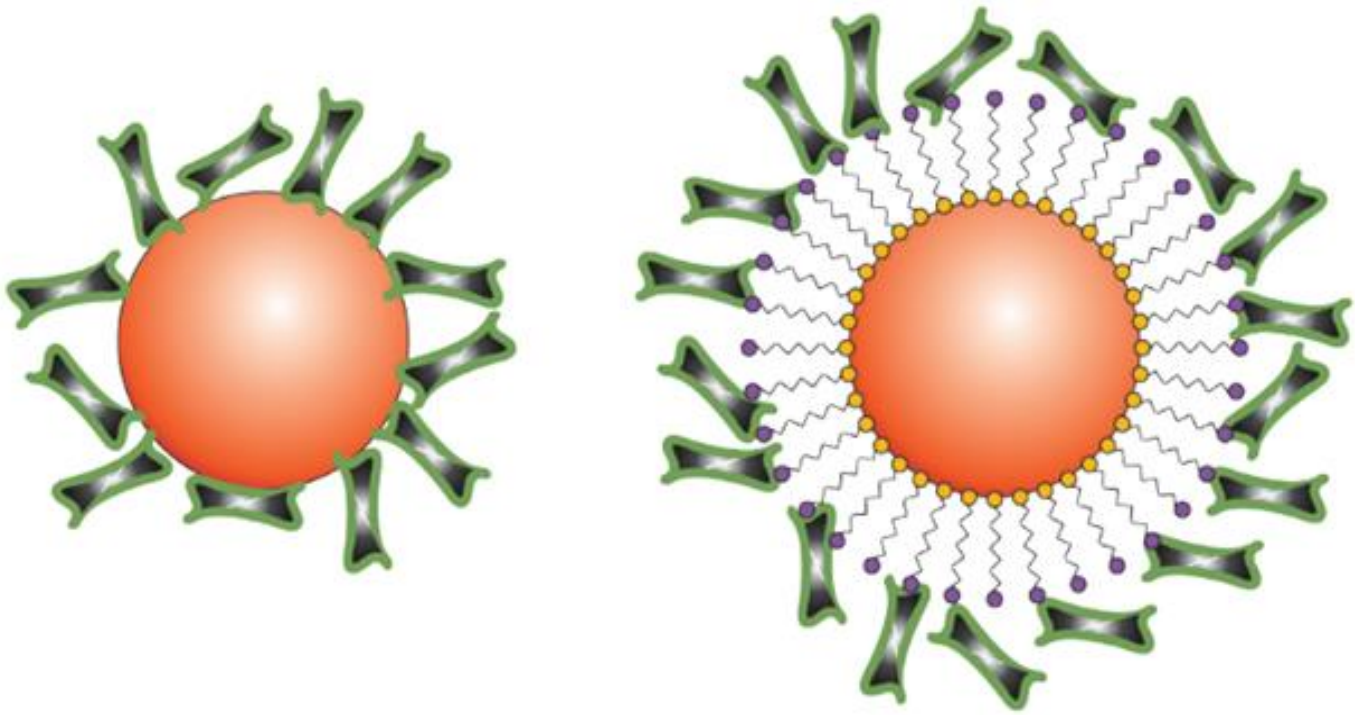

Figure 1: A Protein is shown schematically A conjugate of gold nanoparticles made by "classic" adsorption and covalent bonding. In the "classic" conjugate, the ligand-to-gold nanoparticle distance is the lowest. When it comes to resolution, this is helpful. Covalent conjugates with PEG chains of less length than the particles provide the ligand greater steric freedom, which might improve labelling density.

Intratumorally Administered Gold Nanoparticles: Impact of Surface Protein Adsorption on Distribution and Retention:

Innovative methods for non-invasively detecting and diagnosing the illness at its earliest premalignant stage and for providing targeted therapy against its progression and recurrence are available in theranostic nanomedicine for cancer management [48]. But the interaction between nanomaterial and tumour microenvironment is one of the biggest obstacles to bringing theranostic nanomedicine to the clinic. Specifically, protein corona adsorbed on nanoparticle surfaces can change their stability, dispersibility, biodistribution, pharmacokinetics [49], and toxicity profile as a result of interactions between nanoparticles and proteins in biological systems. Gold nanoparticles (GNPs) have shown promise in nanotheranostic cancer applications thanks to their distinctive optical features, excellent biocompatibility, and absence of toxicity. Various solid gold particles have been rationally created for use in cancer therapy, including nanobelts, nanowires, nanostars, and core-shell gold-coated particles [50]. The protein corona layer's interactions with the surrounding cells are crucial for the GNPs to have successful in vivo results [51]. In order to design, manufacture, and translate GNP-based nanotheranostics, it is essential to understand GNP-protein interactions. Extensive research has demonstrated that in order to govern opsonization on GNPs, the protein corona is affected by the surface chemistry and size of spherical GNPs. When given to living organisms, particles form a complex network of adsorbed proteins called a protein corona. This network includes albumin, immunoglobulin, glycoproteins, and apolipoproteins, which are abundant but have lower affinity proteins that bind at first. Later on, higher affinity proteins like fibrinogen or lysozyme replace apolipoproteins [52–55]. An inner layer of proteins known as the hard corona interacts directly with the surface of the GNP, and an outer layer of proteins known as the soft corona are connected through weak protein-protein interactions. These two layers of proteins are the main components of the GNP. A complicated, dynamic, and competitive mechanism stabilises

the GNPs in a physiological environment by dislodging the hydration layer, which forms the overall particle corona. Because of this arrangement, GNPs can be recognised by phagocytes, leading to their quick clearance from plasma and accumulation in the liver and spleen, because epitopes that are typically buried in the interior sites of proteins can be exposed outwards from the soft corona layer of the particle [56]. The ability to regulate the physiological characteristics of GNPs is crucial for mediating a variety of functions, including cellular absorption [57], immunological response, toxicity, circulation time, transit between organs, and clearance. The protein content of blood and the enhanced permeability and retention effect (EPR) have been the subject of numerous studies regarding particle behaviour in relation to tumours and systemic administration. However, it is worth noting that other biological compartments, like tumour interstitial fluid, also contain a high protein content, which could influence particle behaviour when injected intratumorally. Thus, the processes investigated, which are associated with the hematic system's protein corona creation, are generalizable to other fluids. Cells from the stroma, including endothelial cells and fibroblasts, are present in solid tumours like lung malignancies. In addition, LLC tumours have subpopulations of cells with wildly varying metastatic potentials, which contributes to their very diverse nature [58–61]. Because drugs do not distribute uniformly throughout tumours, tumour heterogeneity is a major contributor to treatment resistance. We postulate that the tumor's particle zonal distribution can be controlled by regulating protein adsorption on the GNP surface. We have already shown that our spherical GNPs have great qualities as CT contrast agents in living organisms and a strong radio-sensitization property in laboratory settings, where they induce DNA damage in Lewis lung cancer (LLC) cells.

Despite the fact that nanomaterial can be directly injected into the interstitium of malignant tissue through intratumoral injections, the interstitial tumoral pressure is significantly higher compared to healthy tissue. The injected formulation is typically pushed out of the tumour and more medication leaks into the surrounding tissue by this raised interstitial fluid pressure gradient. Due to its potential role as a physical mechanism of drug resistance and its clinical relevance in determining the efficacy of nanomedicine-based therapy, determining the intra-tumoral distribution of nanoparticles is an important goal in clinical research. Given the current understanding that physical processes are not the only ones involved in radiosensitization, but also chemical and biological ones [62], it stands to reason that the various distribution patterns found depending on particle functionalization would have an effect on radiosensitization. Possible factors contributing to these effects include the number and proximity of particles in the area, the percentage of uptake, and the precise placement of particles within or close to distinct cell types that are going through different stages of replication. Cells that are metabolically active and dividing rapidly, for instance, are known to be more radiosensitive. Hence, improving radiosensitization effects through protein surface modification that leads to greater particle distribution in high-turnover cells should be beneficial for future clinical applications. This study highlights the importance of surface passivation in the intratumoral biodistribution of GNPs, which can lead to substantial heterogeneity in the tumour microenvironment, as seen in vivo [63]. The majority of BSA-GNP perfusion happens around the tumor's periphery, with very little deposition occurring across the entire tumour. We believe that the aberrant and heterogeneous vascular structure of the LLC tumour, which indicates that perfusion, not the permeability of the GNPs, is the limiting factor for tumour accumulation, although off-center injection or tumour growth could also play a role. Even though BSA surface passivation causes perivascular accumulation, it can nevertheless influence the retention and distribution of GNPs within tumours.

The Effect of Free Thiol on the Adsorption of Proteins on Gold Nanodevices:

There are numerous possible uses for protein-gold nanoparticle (AuNP) bioconjugates in the field of

nanomedicine. In order to construct these functional bioconjugates with desirable features, it is crucial to have a comprehensive understanding of the protein-AuNP interaction. Here, we study how the protein's free thiols affect the protein-AuNP conjugate's stability. Three different forms of human serum albumin (HSA) were produced by varying the molar excess of the chemical modifier 2-iminothiolane (Traut's reagent), resulting in 1, 5, and 20 free thiols on the protein surface. Using this HSA species and an IgG antibody that displayed 10 free thiols, protein exchange assays on AuNPs were carried out. We prepared, purified, and dispersed antibody-AuNP conjugates in solutions containing all of the HSA species. Extensively thiolated HSA displaying 20 free thiols displaced 85% of the antibody on the AuNP surface, but neither the HSA nor the modified HSA containing 5 thiols showed any evidence of protein exchange. In addition, we dispersed HSA species in an antibody solution and preadsorbed them onto AuNP to examine the effect of the protein adsorption [64] sequence. The antibody removed all traces of the HSA containing one thiol from the AuNP in three hours, but it took twenty-four hours to remove all traces of the modified HSA having five thiols, and it failed miserably when faced with an HSA containing twenty thiols. Based on these findings, the binding relationship between the protein and the AuNP is controlled by the number of Au-S contacts [65-67]. The binding mechanism between proteins and AuNPs is better understood thanks to this work, which also identifies key concepts for designing altered proteins to improve bioconjugates.

Gold Surfaces Modified with Thiols:

When it comes to attaching molecules to the surface of gold particles, thiol moieties are perhaps the most employed approach. Extensive research on planar gold surfaces and nanoparticles has established the robust connection between the sulphur atom in the thiol moiety and the gold surface. On crystalline gold surfaces, thiols self-assemble form ordered monolayers. It is the characteristics of the thiol that determines the monolayer's order and the extent to which it covers the surface. A highly organised mono-layer of relatively tiny alkane thiols (e.g., mercaptoundecanol) [68] will develop on the surface of the gold. Monolayers formed by larger molecules, like thiol-modified polyethylene glycols, tend to be less organised than those formed by smaller, less rigid molecules.

Thiols self-assemble onto gold surfaces, making them a potent tool for surface modification. The use of thiol attachment pro- vides a number of benefits over electrostatic interactions: (1) orientation control of the attached molecule, (2) capacity to build well-defined mixed monolayers, and (3) ability to attach a wide variety of molecules independent of charge, hydrophobicity, etc. Thiols will reassert themselves during self-assembly, replacing other molecules like electrostatically adsorbed ions, due to the fact that the thiol-gold interaction is one of the strongest interactions that a gold surface can participate in [72-73]. One thiol-containing molecule can displace another on the surface of gold, despite the strength of the thiol-gold connection. Mixed monolayers for multifunctional surfaces (e.g., targeting and immune response protection) can be created by taking advantage of this lability.

Gold nanoparticles treated with pegrylation:

For biological applications requiring some type of protection from the immune system, coating gold surfaces with polyethylene glycol is quite prevalent. Past research on drug delivery systems based on liposomes has demonstrated that adding a PEG layer significantly increases the circulating half-life of nanoparticles in the bloodstream. The increase in circulation half-life is related to the capacity of the PEG layer to suppress opsonization by the body, which acts to hide nanoparticles from the reticuloendothelial system (RES) [73]. The inclusion of a PEG layer also considerably boosts colloid stability in high ionic strength conditions by providing steric stabilization of the particles to prevent aggregation. Because of the inherent “stickiness” of bare gold surfaces with respect to biomolecules, which was discussed in the section on attaching proteins directly to the surface of gold, opsonizing

proteins in the bloodstream will readily attach to the surface of a bare particle, and the circulating half-life of bare particles is measured in minutes.

PEGylation of gold surfaces can be carried out using a thiol-modified PEG molecule of the type CH3O(CH2CH2O)n CH2CH2SH such as the commercially available mPEG-SH MW 5000 (Laysan Bio). This molecule will self-assemble onto the bare gold surface through the thiol–gold interaction discussed earlier. After assembly, the molecule offers a nonreactive methoxy (CH3O–) group to the solvent. Particle size has a small but significant impact on the length of the PEG chain needed for (1) particle stabilisation and (2) RES activation prevention. For example, gold nanoshells (particle diameter ~150 nm) require a PEG molecule with an average MW of around 5000 Da to provide stability and to hide the particles from the RES. The stability and circulating half-life of gold nanoshells synthesised with a 2000-MW mPEG-thiol coating are much lower than particles prepared with a 5000-MW mPEG-thiol [74-75]. Much smaller PEG chain lengths can be utilised to stabilize tiny colloid preparations, although care must be made to not use very small PEG molecules (<1500 MW), which have been demonstrated to have some level of toxicity in vivo.

PEGylation of the naked gold particles can be carried out by adding an excess of mPEG-SH to the solution containing the bare gold particles [76-80]]. Generally, a substantial excess of mPEG-SH is employed to stimulate the assembly of molecules on the surface. The amount of PEG excess required can be determined by the total surface area of the gold to be coated. Because of the flexibility in the PEG chain, the footprint—or the effective surface area—taken up by a 5000-MW PEG molecule is relatively big. Brush (extended) or mushroom (compacted) configurations are possible for the PEG molecules. The anticipated footprint of 5000 MW PEG-SH, according to previous research, is around 20 nm2. This figure can be used to estimate the amount of PEG molecules required to cover one particle. In just two or three hours, you can get adequate PEG monolayer cover-age by using a 5- to 6-fold excess of mPEG-SH [81-83]. Longer periods of time in solution can cause mPEG-SH molecules to reorganise on the surface, leading to better packing and improved surface coverage.

Gold surface PEGylation protects against the RES system and increases blood circulation half-life, but it does not target cancer cells specifically. The increased permeability and retention (EPR) effect causes PEG-coated particles to concentrate in tumours [84-86]. Gold particles can escape the bloodstream and collect around tumours due to their leaky vasculature. Drug delivery and photothermal therapy have both made use of this "passive" targeting of tumours to deliver particles. It might be useful to make the particles able to target cancer cells in particular, depending on the use case. Adding a targeting molecule (antibody, peptide, etc.) does not significantly change the amount of particles that collect in the tumour, but it significantly changes their distribution and localization inside the tumour, according to recent research. For some uses, like gene therapy and medication delivery, this enhanced absorption by cancer cells is crucial. Additionally, it may improve the effectiveness of photothermal treatments and radiation dose enhancement by dispersing the effects more uniformly across the tumour [87].

To make PEGylated particles more selective for cancer cells while still providing some protection from RES and making the particles more stable, there are a number of ways to attach a targeting agent to them [89]. Two of the most common methods for functionalizing surfaces in this way are(1) functionalizing the PEG molecules and(2) mixed surface preparation. As mentioned previously in this chapter, the targeting moiety—typically a protein—is first physically bonded to the surface of the gold particles during mixed surface preparation. To finish filling the gaps between the proteins, mPEG-SH is applied to the surface of the particles. There are a number of major problems with this approach. The same orientation problems that were discussed before are still relevant here, and the PEG layer can act as a steric "shield" to reduce the protein's

functionality in living organisms. One potential cost consideration while making the conjugated particles is the amount of protein needed for functionalization; this method's main benefit is the low amount of protein needed. The second approach, which involves incorporating targeting moieties into the PEG molecules [90–92], is a little more reliable. It makes use of a polyethylene glycol (PEG) molecule that is bifunctional, meaning it has a thiol group as well as another chemically reactive group. The molecules are attached to the gold surface via the thiol group, and the targeting moiety can be connected to the other reactive group.

Conclusion:

Through quantitative CT and ICP-OES analysis, we were able to demonstrate that regulating protein absorption on the GNP surface resulted in a considerable variation in the intratumoral distribution and retention of the particles. In addition, our research showed that the process of cellular uptake in vitro is controlled by the surface passivation of GNPs. Additional research will help us understand how to regulate the surface passivation of GNPs, which is important for translating nanoparticle-based treatments into the clinic. Using high-resolution preclinical CT imaging, protein surface adsorption can impact GNP biodistribution in an animal model of NSCLC. Through quantitative CT and ICP-OES analysis, we were able to demonstrate that regulating protein absorption on the GNP surface resulted in a considerable variation in the intratumoral distribution and retention of the particles. In addition, our research showed that the process of cellular uptake in vitro is controlled by the surface passivation of GNPs. Additional research will help us understand how to regulate the surface passivation of GNPs, which is important for translating nanoparticle-based treatments into the clinic.

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