Bovine Serum Albumin binding to mercapto-capped gold nanoparticles: study of different capping agents on bioconjugation reaction

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Surface-enhanced Raman scattering (SERS) is a powerful spectroscopic technique combining nanotechnology and Raman spectroscopy, being able to detect traces of closely adsorbed molecules on plasmonic nanostructures (often Au and Ag). SERS-active substrates are expected to meet some critical design conditions at nanoscale for creating surface plasmons at the appropriate wavelengths. Therefore, one of the major ongoing challenges for widening the use of SERS as a characterization and analytical tool is to obtain a reproducible substrate, which can be structurally tuned at the nanoscale. One of the fundamental requirement in the development of bio-conjugated materials for imaging, diagnosis and therapeutics is the understanding of how the various elements present in the conjugate interact with each other. Nanoparticle (NP) – protein conjugates are becoming extremely relevant for a wide variety of bio-applications.

The most common method of protein-nanoparticle conjugation is through covalent bond formation via a linker molecule. The use of a linker creates a space between the protein and the nanoparticle surface, thus avoiding biomolecule denaturation or undesirable biomolecule-nanoparticle interactions. It is reasonable to assume that the linker’s physiochemical properties must be carefully considered before performing the chemical reactions. The lack of attention paid to this issue is surprising, especially when the nanoparticle’s size and its surface properties are considered to be the two most important parameters for the mediation of biocompatibility of new materials used in nanomedicine.

In this work, in order to gain insight into nanoparticle-capping agent-protein interaction, the coating of gold nanoparticles (AuNPs) was carried out with different molecules bearing the mercapto group: 3-mercaptopropionic acid (3MPA), 4-mercaptobenzoic acid (4MBA) and 11-mercaptoundecanoic acid (11Mu) (Figure 1A). The characteristic SEM image of the AuNPs is shown in Figure 1B, where spherical particles in the range of 30 nm are observed. We systematically investigated different ratios between mols of gold and mols of the capping agent in order to establish the best condition, which would be the smallest concentration of the capping agent to completely modify the AuNP. The ratios of 1, 5, 10 and 20 % were investigated. The covalent conjugation of AuNPs to the protein Bovine Serum Albumin (BSA), via the crosslinker reaction EDC/NHS, was systematically investigated on different reaction conditions. All of the products were analyzed via SERS, electronic spectroscopy and electrophoresis gel technique.

Figure 1 (A) Capping agents used as linkers between nanoparticles’ surface and Bovine Serum Albumin. (B) Representative SEM image of AuNPs.
Figure 2 shows extinction spectra related to the coated gold nanoparticles by different mercapto ligands in the presence with small amounts of salt, to probe each of the coated-nanoparticles’ stability towards aggregation. The appearance of a SPR band located at higher wavelengths induced by the increase of ionic force, and the concomitant decrease in the intensity of the 530 nm SPR band indicates that anisotropic colloidal assemblies are formed; two plasmons bands related to individual and aggregated AuNPs are noticeable in the presence of the smaller capping agents (4MBA and 3MPA), but this is no so evident for 11MUA.

![Figure 2](image)

**Figure 2** Extinction spectra of (1%) mercapto-capped AuNPs before (black lines) and after (red lines) addition of salt: (A) 4MBA, (B) 3MPA and (C) 11MUA.

Studies of the efficiency of the coating process of AuNPs with different ratios of capping agents were conducted by SERS using 785 nm laser. Figure 3 shows that for 4MBA, a high signal of the capping molecule is observed, restricting further analysis; for 3MPA, specific bands vanish with increasing concentration of the ligand, which is related to the replacement of citrate (which initially coats the AuNPs), and for 11MUA, difficulties related to the reproducibility of the signal are observed, and further studies will be conducted. The SERS results show that the ratio of 5% mols of gold/mols of the capping agent is the optimized relative concentration to further chemical modifications of the AuNP with biomolecules.

![Figure 3](image)

**Figure 3** SERS of AuNPs coated with (A) 4MBA, (B) 3MPA and (C) 11MUA in concentrations in the range between 1 % and 10 %. $\lambda_{\text{exc.}} = 785$ nm.

**ACKNOWLEDGMENTS**

The authors thankfully acknowledge the support of Capes, Fapesp and CNPq.

**REFERENCES**