Characterization of Functionalized Gold Nanoparticle and their Interactions with Model Peptides and Protein G

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Introduction: The purpose of this research is to study the interactions of biomolecules with nanoparticles using model systems. This model system is used to simplify the complex biological environment to develop a fundamental understanding of biomolecule/nanoparticle surface interactions. Gold nanoparticles (AuNPs) were used as the model nanoparticles surface. They are significantly less toxic than quantum dots (semiconductor nanoparticles), hence desirable for use in nanomedicine applications. Because of the higher percentage of surface atoms, AuNP properties depend highly on their size, shape, and surface chemistries. This opens up a great opportunity for scientists to manipulate these variables to obtain desirable outcomes. Here, the size and functionalizing ligands of the AuNPs were systematically varied to provide a range of AuNP surfaces. For the biomolecules, LKα–14 was used as a model peptide and the B1 domain of protein G was used as a model protein. LKα–14 is a synthetic peptide with alternating leucine and lysine residues (Ac-LKKLL KLLKK LLKL-OH). When adsorbed onto a surface, it is expected to retain its α–helical secondary structure with all the hydrophobic leucine side chains on one side and all the hydrophilic lysine side chains on the opposite side.[1,2] The wild type B1-protein G has a negatively charged surface. Through site-directed mutagenesis of specific charged residues, the global charge dipole along the long axis of the protein was changed to positively charged (D6) and neutral (D4). These variations in hydrophobicity and surface charge effect the biomolecule interactions with the AuNPs.

Methods: Three different diameters of AuNPs (14, 25 and 40 nm) were synthesized using the citrate reduction method. The AuNP surfaces were functionalized with self-assembled monolayers (SAMs) of carboxylic acid terminated thiols (C6, C8, C11 and C16 alkyl chain lengths). The size, shape and size distribution of the AuNPs were characterized with TEM. Surface chemistries of the SAMs were characterized with X-ray photoelectron spectroscopy (XPS) and time-of-flight secondary ion mass spectrometry (ToF-SIMS). XPS analysis was also used to optimize the synthesis and functionalization procedures so contaminants (e.g., Na) and unbound thiols were not detected. The SAMs crystallinity was studied with ATR-FTIR (attenuated total reflectance FTIR) and AuNPs stability and aggregation was studied with UV/VIS spectroscopy. Adsorption isotherms for the B1-protein G and LK onto the AuNP-SAM surfaces were studied using a colorimetric technique. Solid-state NMR was also used to study the orientation of the adsorbed peptide.

Results: The size distribution increased with increasing particle size, with 3σ values of 2.5 nm and 20nm for the 14 nm and 40nm AuNPs, respectively. As the AuNPs diameter decreased and SAMs chain length increased, the XPS C/Au ratio on the surface increased and the ToF-SIMS intensity ratio of $C_{1-4}H_xO_y$ ions/Au-containing-ions increased. In the ATR-FTIR study, as SAMs length increased, the CH_2 stretching vibration frequencies (υCH_2) decreased on both AuNPs and flat-Au surfaces. For a given chain length, the υCH_2 also decreased as the AuNPs particle size decreased. Colorimetric results showed that the positively charged protein G mutant adsorbed in the highest concentrations while the wild-type protein adsorbed in the lowest concentrations on the negatively charged AuNPs. Figure 1 shows an adsorption isotherm for the positively charged (D6) B1-protein G on the 14nm-C16COOH AuNPs.

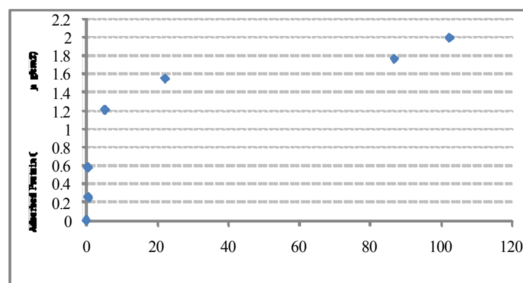

Figure 1: D6 protein G adsorption onto C16COOH covered 14nm AuNPs.

For the LKα–14 peptide the best adsorption isotherm results were obtained under basic pH conditions. NMR studies of a LKα–14 with deuterium labeled leucine side chains adsorbed onto the 14nm-C16COOH AuNPs surface suggested that the peptide was oriented with the leucine side chains facing away from the AuNPs surfaces.

Conclusions: Detailed characterization of the SAM covered AuNPs indicated that the longer carboxylic SAMs on the smaller AuNPs, 14nm with C16-COOH SAMs, produced the most ordered SAMs and well-defined samples. Thus, the C16-COOH AuNPs were used for investigating the adsorption of the LKα–14 peptide and B1-protein G. The amount of Protein G adsorbed onto the C16-COOH AuNPs depended on the protein surface charge. Further studies to improve AuNP surface area measurements, XPS characterization of the biomolecule covered AuNPs samples, and synthesis of AuNPs with other surface chemistries are currently underway.

References: 1) W. F. DeGrado, Science, 1989; 243:622-628; 2) A. M. Gronenborn, Science 1991; 253:657-661