346
doi:10.1017/S1431927616002580

Microsc. Microanal. 22 (Suppl 3), 2016
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Characterization of Protein G B1 Immobilized Gold Nanoparticles using Time of Flight Secondary Ion Mass Spectrometry and X-ray Photoelectron Spectroscopy

Yung-Chen Wang[1] and David G. Castner[1,2]

[1.] National ESCA & Surface Analysis Center for Biomedical Problems, Department of Bioengineering, Box 351653, University of Washington, Seattle, WA 98195-1653 USA
[2.] National ESCA & Surface Analysis Center for Biomedical Problems, Department of Chemical Engineering, Box 351653, University of Washington, Seattle, WA 98195-1653 USA

Nanoparticles (NPs) have been widely used in many fields of science due to their unique physical properties. While many applications of NPs such as imaging probes or drug carriers often require the conjugation of proteins or biomolecules, the surface interactions between NPs and biomolecules remains underexplored [1]. For example, the immobilization of immunoglobulin G (IgG) onto nanoparticle surfaces is critical for the development of many immunosensors and drug delivery nanocarriers. Notably, the orientation of the immobilized IgG can have significant impact on the clinical outcomes of these carriers by impacting its biostability and efficacy [2].

In this work, Protein G B1, a protein that can selectively bind to the Fc tail of IgG, was immobilized onto gold NPs (AuNPs) functionalized with maleimide self-assembled monolayers (SAMs), oligo-(ethylene glycol) (OEG)-OH SAMs, and OEG-COOH SAMs. Depending on the SAM, Protein G B1 was immobilized on AuNPs using carbonyldiimidazole (CDI) chemistry, Ethyl-3-(3-dimethylaminopropyl)-carbodiimide)/ N-Hydroxysuccinimide (EDC/NHS) chemistry, or maleimide-cysteine interaction. Two different cysteine mutant (T11C and V21C) was introduced to Protein G B1 as the wild type (WT) protein does not contain a cysteine amino acid. We use the surface sensitive analysis techniques of X-ray photoelectron spectroscopy (XPS) and time of flight-secondary ion mass spectrometry (ToF-SIMS) to characterize the immobilization of protein G B1. XPS is a well-established and commonly used technique in the field of surface analysis and was previously used to investigate protein adsorption on flat surfaces. Utilizing x-ray excitation to generate element-specific photoelectrons, it is possible to quantify elemental composition of NPs with high sensitivity. Also, the combination with ToF-SIMS technique has been shown to be an effective method to characterize various types of SAMs on gold surfaces and determining the orientation of immobilized protein G.

The functionalization and protein immobilization chemistry was systematically characterized using XPS. XPS analysis confirmed the CDI and EDC/NHS activation of the OEG-SAMs AuNPs by detecting the nitrogen containing active intermediate and the attenuation of gold signal. After incubation with protein, the immobilization of the protein was demonstrated by the increased nitrogen signal on the surface for both chemistries. For AuNPs without the treatment of EDC/NHS chemistry, no nitrogen species was observed after protein incubation, suggesting the complete removal of non-specifically adsorbed protein. Successful protein immobilization was also observed using the maleimide-cysteine coupling strategy (Figure 1). As Protein G B1 WT does not naturally contain a cysteine for the maleimide-cysteine coupling, only limited adsorption might have taken place on the AuNPs which could have contributed to the attenuation of the gold signal. After incubation with the V21C mutant Protein G B1, an increase in the nitrogen signal was observed along with minor attenuation to the gold and sulfur signal. This increase in the nitrogen signal and attenuation of the gold and sulfur signal was also found with the Protein G B1 T11C mutant. Notably, the elemental composition of both the V21C and the

https://doi.org/10.1017/S1431927616002580 Published online by Cambridge University Press

T11C mutant were almost identical. This was expected as the only difference between the two Protein G B1mutants is the location of the cysteine.

Further, by utilizing ToF-SIMS high surface sensitivity and sampling depth (2nm), it was possible to determine the orientation of immobilized protein G B1 of similar thickness (3nm) by comparing the ratio of secondary ion intensity originating from the opposite ends of the protein. The orientation control of the Protein G B1 was achieved through site-specific maleimide-cysteine based protein immobilization strategy.

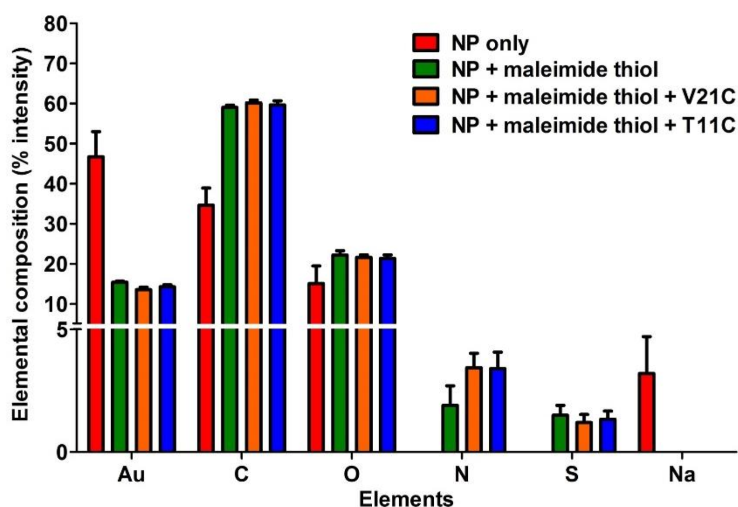

Figure 1. XPS elemental composition (atomic %) of Protein G B1 immobilization based on the maleimide-cysteine coupling.

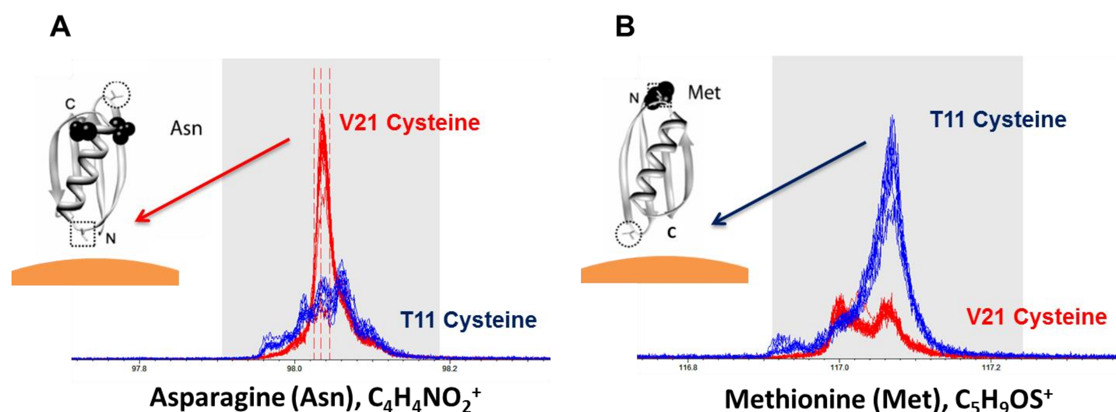

Figure 2. ToF-SIMS spectra of Protein G B1 immobilized AuNPs. V21C mutant is located on the loop near the N-terminus of the protein. T11C mutant is located on the loop close to the C-terminus of the protein. The orange surface below the protein indicates the surface of the AuNP. (A). Peak intensity comparison of Asparagine (88.03 m/z) for the V21C mutant (red) and T11C mutant (blue). (B). Peak intensity comparison of Methionine (117.03 m/z) for the V21C mutant (red) and T11C mutant (blue). Protein structure adopted from [3].

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[4] Y.-C. Wang and D.G. Castner gratefully acknowledge the support from National Institutes of Health grant EB-002027 to NESAC/BIO from the National Institute of Biomedical Imaging and Bioengineering.

https://doi.org/10.1017/S1431927616002580 Published online by Cambridge University Press