GOLD NANOPARTICLES FUNCIONALIZED WITH ANTIBODIES: STUDYING THE STOICHIOMETRY BETWEEN ANTIBODIES AND PARTICLES.

E. Polo, V. Grazú, M.P. Pina, J.M. de la Fuente, J. Santamaría.

Instituto de Nanociencia de Aragón, University of Zaragoza, Edif. Inter II. C/ Pedro Cerbuna 12, 50009 Zaragoza Spain
esterpol@unizar.es

Gold nanoparticles (AuNPs) have been used for analytical and biomedical purposes for many years. Rapid and simple chemical synthesis, optical and electrochemical properties, a narrow size distribution, a large surface-to-volume ratio, and efficient coating by thiols and other bioligands has enabled gold nanoparticles to be used for several biorecognition binding applications.[1] These biomolecular recognition events occurring at the nanoparticles surface have an influence on the optical and/or electrical properties of the system allowing the development of more sensitive and flexible sensing systems.

The availability of versatile chemistry for functionalizing gold nanoparticles surface, allows preparation of various bioconjugates and these bioconjugates are generally stable in aqueous solution in a wide range of pH values, and ionic strength, providing a particularly useful platform for the application in biodetection. For example, signal enhancement of gold nanoparticles functionalized with capture antibodies has been used for immunoassay detections of analytes.[2]

In this work, water-soluble gold clusters protected by monolayers of tiopronin (N-2-mercaptopropionylglycine) were synthesized, using the method reported by *Murray et al. in 1999*,[3] with a core diameter size between 2 and 3 nm (**Au@Tiopronin**). These **Au@Tiopronin NPs** have been functionalized with antibodies by a covalent strategy binding directly to the nanoparticle surface.[4]

The stoichiometry between **Au@Tiopronin NPs** and antibodies has been studied by assays based on the aggregation of gold nanoparticle (AuNPs) with a bigger size and coated with the corresponding antigen.[5] 14 nm size-gold nanoparticles were synthesized by citrate method[6], and antigens were absorbed on the AuNP surface via electrostatic interactions. Antibody-labelled **Au@Tiopronin NPs** bind to the antigen adsorbed onto AuNPs to generate a sandwich system.

The antibody-antigen molecular event occurring at the surfaces of these nanoparticles, results in measurable changes and shifts of nanoparticle surface plasmon absorption band *(Figure 1)*. Gold nanoparticle exhibit a strong surface plasmon band in the visible region of the electromagnetic spectrum, at 520 nm, and this SP band depends on the shape, size and the surrounding medium of the particles.[7] The aggregation of AuNPs leads to the formation of a new absorption band at longer wavelengths, and causes a characteristic transition in solution colour from red to purple-violet.[8]

The rate of aggregation of antigen-**AuNPs** in the presence of antibodies-**Au@Tiopronin NPs** was measured by monitoring the absorption change of the AuNPs upon aggregation. The aggregation process was also monitored by transmission electron microscopy.

References:

[1] Rosi N.L.; Mirkin C.A.; *Chem.Rev*., (2005), 105, 1547-1562.
[2] Baptista, P.; Pereira, E.; Eaton, P.; Doria, G.; Miranda, A.; Gomes, I.; Quaresma, P.; Franco, R.; *Anal. Bioanal. Chem*. (2008), 391, 943-950.
[3] Templeton, A.C.; Chen, S.; Gross, S.M.; Murray, R.W.; *Langmuir* (1999), 15, 66-76.
[4] Bonroy, K.; Frederix, F.; Reekmans, G.; Dewolf, E.; de Palma, R.; Borghs, G.; Declerck, P.; Goddeeris, B.; *Journal of Immunological Methods* 2006, 312, 167-181.
[5] Thanh, K.; Rosenzweig, Z.; *Anal. Chem*. (2002), 74, 1624-1628.
[6] Enüstün, B.V.; Turkevich, J.; *J. Am. Chem. Soc*. (1963), 85, 3317.
[7] Zhong, Z.; Patskovskyy, S.; Bouvrette, P.; Luong, J.H.T.; Gedanken, A.; *J.Phys. Chem*.; (2004), 108, 4046-4052.
[8] Schneider, G.; Decher, G.; *Langmuir* (2008), 24, 1778-1789

Figures:

Figure 1