

SPECIFIC AND ORIENTED IMMOBILIZATION OF PROTEINS ON GOLD NANOPARTICLES

Cristina Vaz Domínguez, José M. Abad, Stijn F.L. Mertens, Marcos Pita, Victor M. Fernández and David J. Schiffrin.

Instituto de Catálisis y Petroleoquímica, CSIC, C/ Marie Curie 2, 28049 Madrid, Spain
vaz@icp.csic.es

Over the past years, there has been a noticeable interest on the coverage of gold surfaces with monolayers of proteins based on the molecular recognition properties of biological systems¹. In this sense, the immobilization of proteins on surfaces retaining their full activity and stability constitutes a challenging goal. Most of the common methods are difficult to control and usually yield randomly bound proteins. On the contrary, an ideal immobilization would produce saturation coverage of specifically bound proteins. The formation of protein layers is induced by anchoring them to gold surfaces functionalized with active molecules, such as transition metal complexes with affinity to repetitive histidine sequences. A feasible method to uniformly cover gold surfaces consists on the self-assembly of thiols by oxidative-chemisorption over the gold². Reversible monolayers of histidine-tagged proteins have been produced using a gold layer covered with a chelator thioalkane monolayer³. In order to avoid a highly complex organic synthetic work, step-by-step construction of the functional monolayer over a template of thiocarboxylic acid chemisorbed onto gold has been developed⁴.

Because of its simplicity, both, from a conceptual as well as from a practical point of view, the step-by-step synthesis of a functional self assembled monolayer is accessible to most of the laboratories working on enzyme technology in spite of having limited facilities for organic synthesis. This synthetic strategy allows, by a judicious design of the synthetic route, the development of a multiplicity of architectures on SAMs. Different SAM strategies have been developed in our group for controlled and oriented immobilization of enzymes onto gold surfaces, using them as amperometric electrodes in the characterization of the enzymatic catalytic performance^{4,5,6}.

We present a next step of these SAM strategies towards functionalization of gold nanoparticles' surface for oriented immobilization of model proteins. The SAM provides the ability to discriminate between specific and non-specific proteins attachment⁷.

References:

¹ M. Lösche (1997) Protein monolayers at interfaces. *Current Opinion in Solid State & Materials Sciences*, 2:546-556.

² Ulman, A. (1996) Formation and structure of self-assembled monolayers. *Chem. Rev.* 96, 1533-1554.

³ P. Rigler, W.-P. Ulrich, P. Hoffmann, M. Mayer and H. Vogel (2003) Reversible immobilization of peptides: Surface modification and *in situ* detection by attenuated total reflection FTIR spectroscopy. *Chem. Phys. Chem.*, 4, 268-275.

⁴ Madoz, J., Kuznetsov, B.A.; Medrano, F.J.; García, J.L., Fernández, V.M. (1997) Functionalization of gold surfaces for specific and reversible attachment of a fused β -Galactosidase and choline-receptor protein. *J. Am. Chem. Soc.*, 119, 1043-1051.

⁵ Madoz-Gúrpide, J., Abad, J.M., Fernández-Recio, J., Vélez, M., Vázquez, L., Gómez-Moreno, C. and Fernández, V.M. (2000) Modulation of electroenzymatic NADPH oxidation through oriented immobilization of ferredoxin:NADP⁺ reductase onto modified gold electrodes. *J. Am. Chem. Soc.*, 122, 9808-9817.

⁶ Abad, J.M., Vélez, M., Santamaría, C., Guisán, J.M., Matheus, P.R., Vázquez, L., Gazaryan, I., Gorton, L., Gibson, T. and Fernández, V.M. (2002) Immobilization of peroxidasa glycoprotein on gold electrodes modified with mixed epoxy-boronic acid monolayers. *J. Am. Chem. Soc.*, 124, 12845-12853.

⁷ José M. Abad, Stijn F.L. Mertens, Marcos Pita, Víctor M. Fernández and David J. Schiffrin (2005) Specific and Oriented Immobilization Of Proteins On Gold Nanoparticles, *J. Am. Chem. Soc.*, 127, in press.

Figures:

