FUNCTIONALIZATION OF MAGNETIC NANOPARTICLES WITH ANTIBODIES: DOES THE ANTIBODY ORIENTATION MATTER?

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For the last 30 years, there is a growing interest in the use of magnetic nanoparticles (Nps) for applications in drug delivery, MRI contrast agents and quantitative and highly-sensitive biosensors. There has been a great effort for the development of strategies to provide nanoparticles with excellent physical properties, high magnetization values, perfect size distribution and high stability [1]. However, it is also very important the development of strategies for the adequate bio-functionalization of these nanoparticles to provide them with the appropriate features for biotechnological and biomedical applications.

Different biomolecules have been used to provide specificity and bioactivity to magnetic nanoparticles, going from aptamers, peptides to carbohydrates. However, the star of these biomolecules due to their extremely high specificity and high recognition efficiency, are antibodies [2, 3]. Antibodies are proteins used by the immune system to identify and neutralize foreign objects, such as bacteria and viruses. They are typically made of two large heavy chains and two small light chains. Although the general structure of all antibodies is very similar (Fc zone), a small region at the tip of the protein is extremely variable (Fab zone), providing specificity to the protein. Each of these Fab zones can recognize to a specific antigen. This huge diversity of antibodies plus the high efficiency in the antigen-antibody interaction make antibodies an excellent biomolecule to be incorporated to magnetic nanoparticles and explode them for biotechnological applications.

Different strategies have been reported for the incorporation of antibodies on surfaces and supports [4]. Among the reported protocols, adsorption of the antibody through immobilized protein A or protein G, site directed biotinilation, or covalent immobilization through the sugar chains present in the Fc region of the antibody, are the preferred options to immobilize the antibody by the Fc region leaving free the site where the antigen molecular recognition take place (Fab regions) [5,6,7,8,9]. But all these protocols are more or less sophisticated and in many cases involve the antibody modification. Moreover, few examples have been reported for nanoparticles, probably due to the difficulties of this kind of research [9,10]. There is a need, therefore, to develop very easy methodologies for the immobilization of non modified antibodies onto magnetic Nps without involving the IgG Fab regions during the immobilization process. We report a simple way to functionalize magnetic Nps via a two step strategy that involves a first ionically exchange (anionic or cationic) of the antibody followed by its further covalent attachment using epoxy or NHS/carbodiimide chemistry. Antiperoxidase from horseradish antibody has been used as model. Nps functionalized with anti-peroxidase immobilized through its more reactive amine groups or through its sugar chains were also prepared as control for ***random*** and ***oriented immobilization*** respectively. The capacity of the immobilized antibodies to capture peroxidase was evaluated, calculating the enzyme/antibody molar ratio. We have confirmed that Nps functionalized with antibodies anchored through their carbohydrate moieties are the only that retained 100% functionality. However, the biological activity of antibodies anchored to Nps through positive or negative charged riches zone was not strongly affected (> 80% of retained functionality in both cases).

The obtained results imply that the previous ionic adsorption of the antibody to carboxilated or aminated Nps followed by its further covalent attachment, did not involve the Fab regions of the antibody. These easy functionalization techniques are applicable to almost all

antibodies, and they will be very useful for the development of more bioactive nanoparticles conjugated with antibodies, improving selectivity and sensitivity of new nanodevices.

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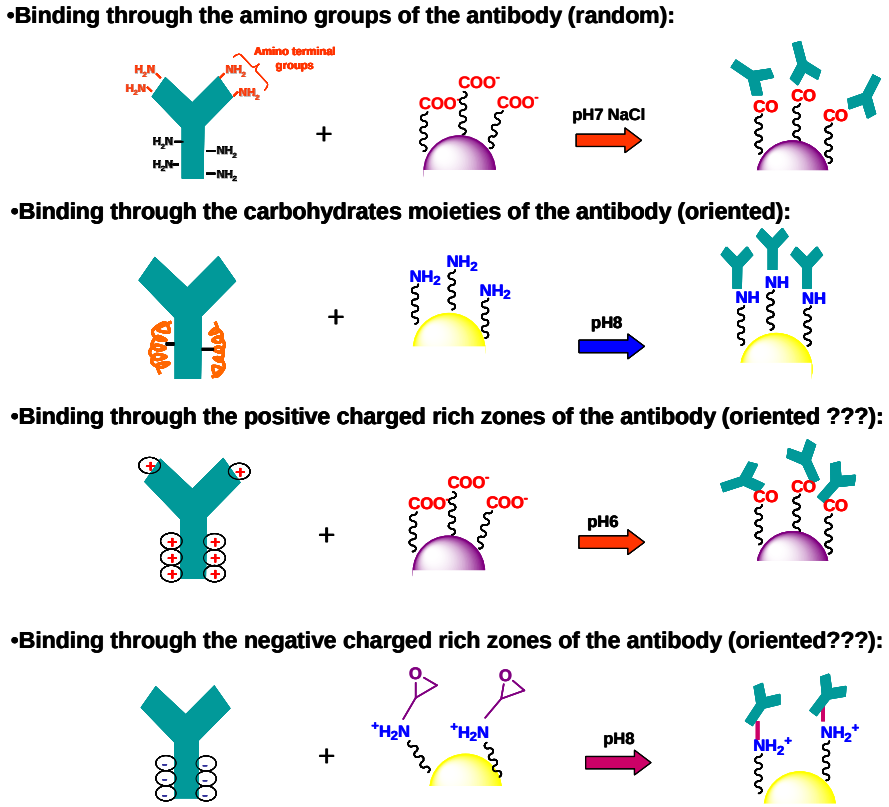

Figure 1. Different immobilization strategies used.

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