Oriented antibody immobilization on carboxylate-modified COC

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A key challenge for many applications is to control the organization of antibodies on surfaces whilst retaining their biological activity.

SITUATION

The bioconjugation procedures employ commonly methods such as nonspecific adsorption, which are difficult to control and usually yield randomly bound biomolecules. An ideal bioconjugation system should clearly be based on a "biointerface" with interactions between the biological target molecule and tailored modifications of the surface, producing biomolecule attachment in a favourable orientation for catalysis (enzymes) or binding (antibodies, receptors), while avoiding nonspecific protein adsorption.

We address different strategies for immobilization of antibody molecules on COC surfaces modified with carboxylate ended groups.

APPROACH

Two methodologies were assayed and compared versus direct antibody physisorption onto COCs non-modified with carboxylic groups:

- **Covalent oriented immobilization:** Ab molecules were covalently oriented immobilized on carboxylate-modified COC by formation of stable amide bonds with primary amines of Ab.
- **Oriented immobilization of antibodies through metal-chelation:** COC surfaces were functionalized with a metal-chelate and Ab immobilization was carried out through the metal-chelation to histidine-rich metal binding site present in the Ab heavy chain (Fc).

RESULTS

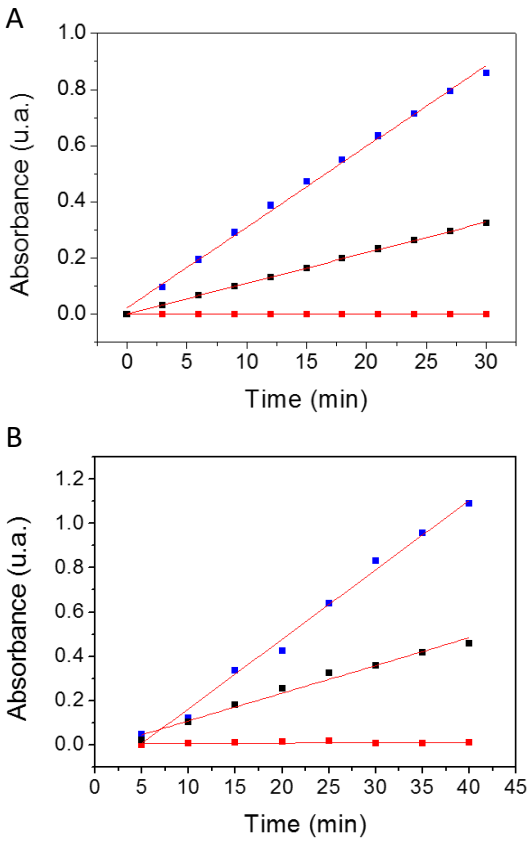

Figure 1_ Comparison between covalent oriented immobilization versus direct physiadsorption by measuring of activity of HRP enzyme molecules bound to anti-Horseradish peroxidase immobilized on carboxylate-modified COCs (blue symbol) and physisorbed directly on unmodified COCs (black symbol). As control of nonspecific protein adsorption, the measurement of activity of HRP enzyme molecules bound to antibodies Anti-Human CD3 (red symbol) is also showed. Conditions: 1 mM ABTS as electron donor and 1 mM H2O2 as electron acceptor in 50 mM sodium phosphate buffer, pH 6.0 at 25ºC. Wavelength 414 nm. **A.** Covalent oriented immobilization. **B.** Oriented immobilization of antibodies through metal-chelation

Both methods show approximately 2.5 times higher enzymatic activity to that obtained for physisorption, having the advantage of obtaining an antibody attachment in a favourable orientation for binding, while avoiding nonspecific protein adsorption

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