Carbohydrate binding modules as a generic tool to anchor biomolecules and metal nanoparticles on the surface of paper-based biosensors

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Abstract

There is a global demand for affordable, sensitive, selective and rapid analytical platforms usable in low-tech contexts to perform health diagnostics, environmental monitoring and food quality testing. Paper-based analytical devices have emerged as one of such platforms, with the additional advantages of being biodegradable, easy-to-use and portable [1]. Paper can be modified and adapted to perform biological assays by adding appropriate biorecognition and reporting agents (e.g. antibodies, enzymes, oligonucleotides and DNA aptamers) to the test areas [2]. Additionally, paper surfaces can be modified with metal nanoparticles (e.g. Au, Ag, and Cu, among others) to introduce optical and electronic properties better suited for biosensing applications [3,4]. One of the keys for the success of these paper surfaces is the ability to master the immobilization of biomolecules and metal nanoparticles, while adequately preserving functionality and stability.

Covalent attachment to the cellulose fibers is not a strict requirement for the incorporation of either biomolecules or metal nanoparticles into paper. In fact, dry paper itself is able to sorb aqueous solutions in such a way that the non-volatile components of the solutions are left in the paper structure after drying. However, impregnation without attachment may not be a robust strategy to immobilize biomolecules or metal nanoparticles because subsequent exposure to aqueous solutions (e.g. washing buffers or biological samples) is likely to leach these components. Furthermore, it is difficult to control the orientation of biomolecules in the paper structure, especially in the case of antibodies, with recognition sites taking different positions in space after random immobilization, resulting in hindered interactions with their binding target [5].

We have developed an immobilization platform that uses specialized proteins named Carbohydrate Binding Modules (CBMs) that have a natural affinity to cellulose, to anchor biomolecules and metal nanoparticles on paper surfaces as an alternative to conventional methods like covalent binding and physical adsorption. The strategy relies on the fusion of biosensing molecules (e.g. affinity handles, enzymes, oligonucleotides) with CBMs and on their subsequent immobilization on paper via affinity interactions.

In this communication, specific applications are presented that rely on CBM3-ZZ, a fusion protein that combines the cellulose-binding properties of CBM3a from *Clostridium thermocellum* with the antibody-binding properties of a double Z-domain from the staphylococcal protein A [5]. Using these fusion proteins, properly oriented antibodies could be anchored on paper surfaces (Fig. 1a). By further exploring the recognition ability of these antibodies, we were able to immobilize 40 nm gold nanoparticles (AuNPs, Fig. 1b) and capture DNA hybrids labeled with AuNPs (Fig. 1c) on the surface of chromatographic paper (Whatman N. 1). Our results have shown that colorimetric signals could be generated that differed substantially from the ones presented when AuNPs or DNA hybrids labeled with AuNPs were simply deposited on paper, without the assistance of CBM3–ZZ fusions (Fig. 2). A SEM analysis revealed that the difference in the colorimetric signals could be attributed to the fact that AuNP homogenously distribute in the paper matrix when immobilized via CBM-3-ZZ fusions, whereas they tend to aggregate when they are simply deposited over paper. By plasmon resonance effect these differences in AuNP aggregation then generated the observed color differences (Fig. 2).

As a proof-of-concept, a strategy for the detection of nucleic acids from *Trypanosoma brucei*, the causative agent of sleeping sickness was developed. In order to confine fluids to specific regions of paper, a wax printing methodology was used to print hydrophobic barriers that delineate circular reaction areas. We then combined the CBM3-ZZ–based anchoring of antibodies with DNA probes specific for *T. brucei* conjugated with gold nanoparticles. The methodology involved i) the pre-conjugation of CBM3-ZZ with an anti-biotin antibody, ii) the deposition of the CBM3-ZZ:antibiotin antibody conjugate on paper, iii) the pre-hybridization of biotin labeled target with AuNP labeled probes off-paper and application on the bioactive circular region of paper and iv) the visual detection of colored

signals. Our results showed that colorimetric readouts in the form of red spots were generated only when DNA strands complementary to the probe were tested.

In summary, we have developed a bioaffinity based platform for the immobilization of biomolecules and metal nanoparticles on paper that is compatible with biosensing applications. Furthermore, we demonstrated that the methodology enhances plasmon resonance effects induced by AuNPs on paper surfaces, making it possible to perform simple molecular and immunological diagnostics tests.

References

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Figures

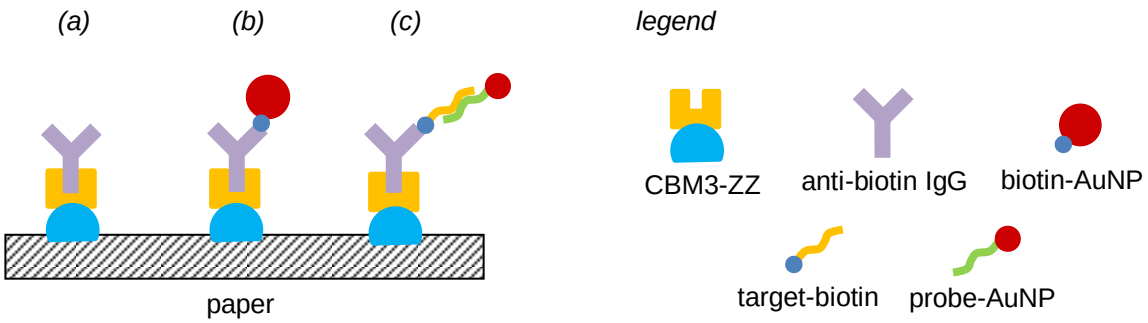

Figure 1. Schematic representation of the use of CBM3-ZZ fusions to anchor biomolecules and metal nanoparticles on the surface of paper-based biosensors. (a) Anchoring of antibodies, (b) immobilization of AuNPs and (c) capture DNA hybrids labeled with AuNPs.

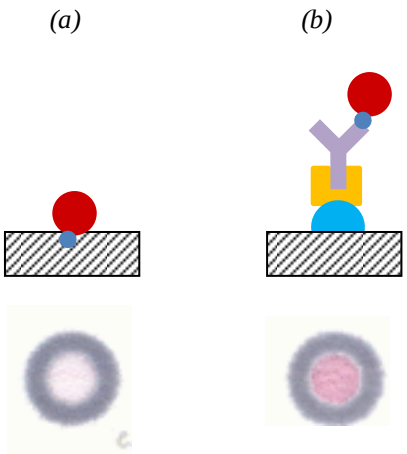

Figure 2. Effect of CBM-based anchoring on the colorimetric signals generated by AuNPs on paper. Circular regions (4 mm) were defined by wax printing on Whatman N. 1 chromatographic paper and 2.3 fmol of biotin labeled AuNPs (40 nm) were deposited. (a) Plain adsorption of AuNPs. (b) Affinity anchoring of AuNPs with CBM3-ZZ:antibotin antibody conjugates.