Journal of Materials Chemistry B

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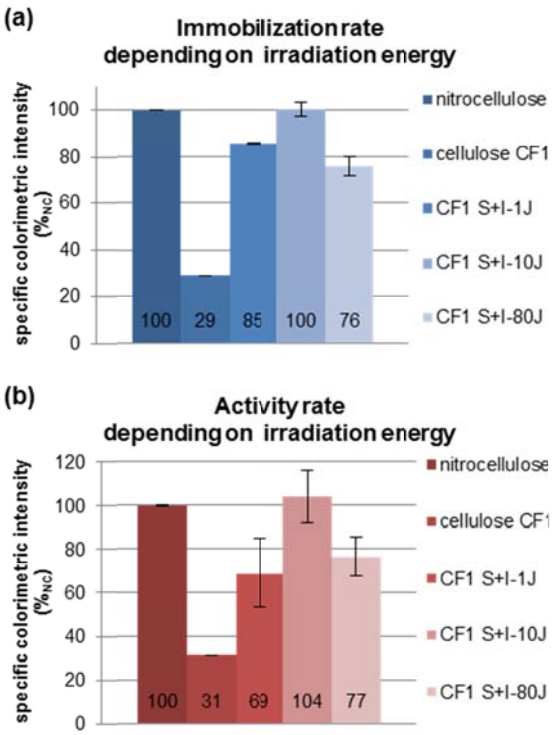
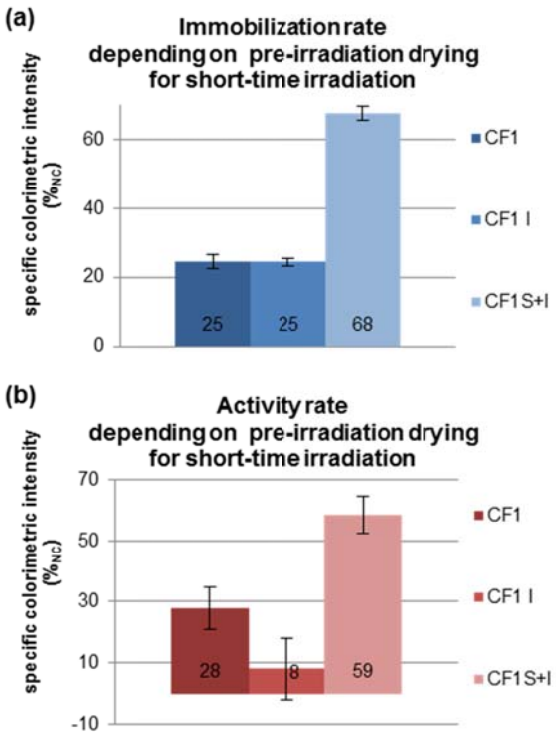
Supplementary Information

Photolinker-free photoimmobilization of antibodies onto cellulose for the preparation of immunoassay membranes

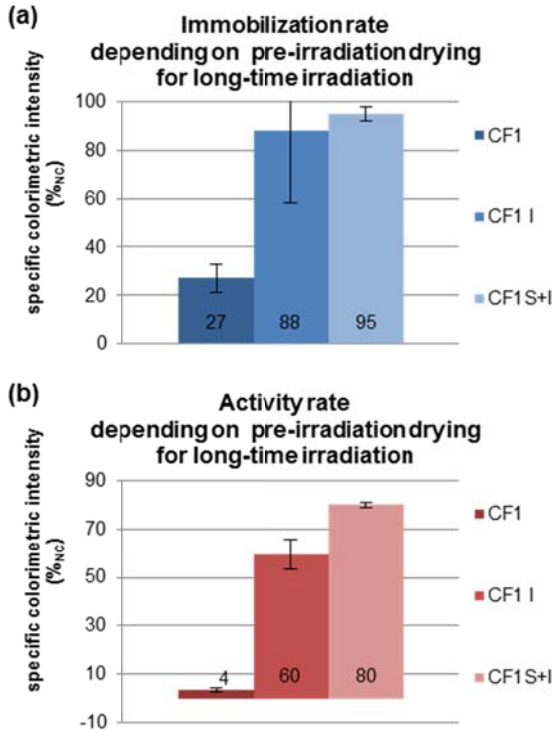
Julie Credou,[a] Hervé Volland[b] and Thomas Berthelot[a]* ,



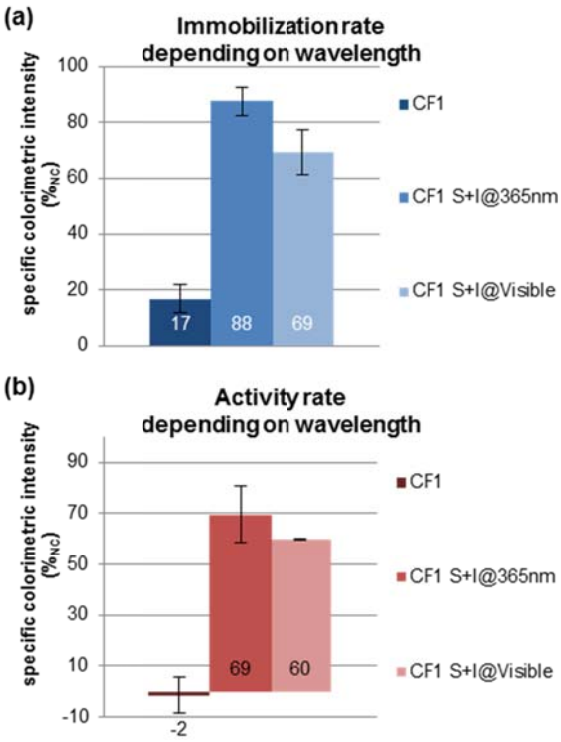
SI-Figure 1: Influence of irradiation energy on antibody immobilization (a) and biological activity (b).
(a) Antibodies immobilized on nitrocellulose or cellulose, after optional drying (S) of the membrane, and irradiation (I) at 1 J cm^{-2}, 10 J cm^{-2} or 80 J cm^{-2}, are revealed by gold-labeled goat anti-mouse tracer antibodies. On ungrafted papers, no signal is detected. On antibody-grafted papers, performances of nitrocellulose are reached for an irradiation energy of 10 J cm^{-2}. The results corresponding to 2 different immobilizations are shown for each condition.
(b) Antibodies immobilized on nitrocellulose or cellulose, after optional drying (S) of the membrane, and irradiation (I) at 1 J cm^{-2}, 10 J cm^{-2} or 80 J cm^{-2}, are exposed to OVA antigen. The capture of the latter by the immobilized antibodies is highlighted by gold-labeled murine anti-OVA tracer antibodies. In absence of OVA antigen, no signal is detected. In presence of OVA antigen, performances of nitrocellulose are reached for an irradiation energy of 10 J cm^{-2}. The results corresponding to 2 different immobilizations are shown for each condition.



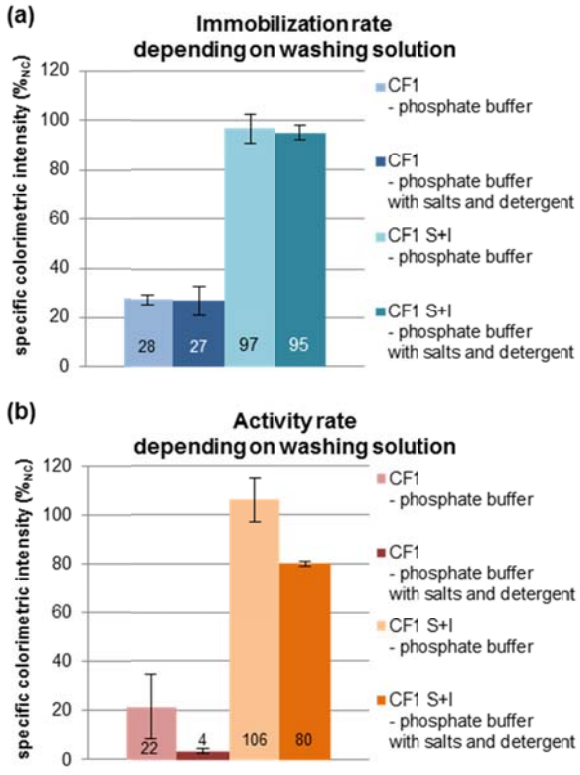
SI-Figure 2: Histograms showing the immobilization (a) and activity (b) rates of antibodies immobilized onto cellulose, after irradiation (I) or drying and irradiation (S+I), for short irradiation time. The results from 2 different immobilizations are presented for each condition.



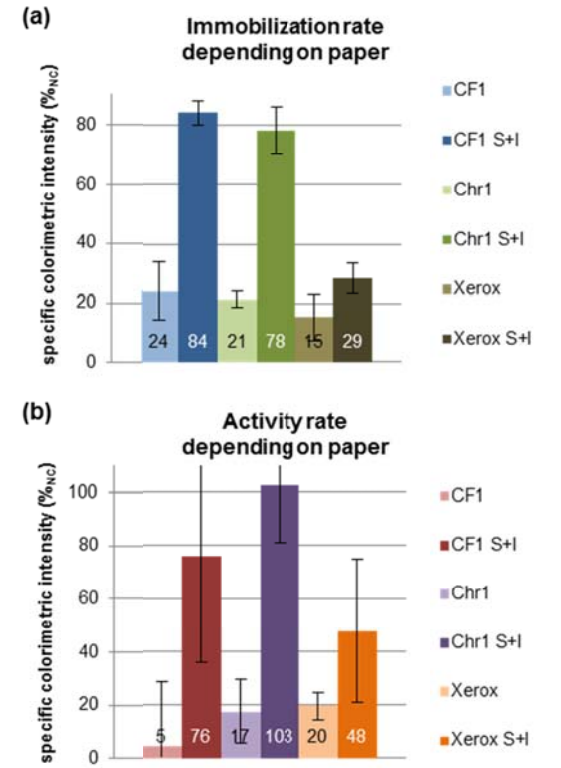
SI-Figure 3: Histograms showing the immobilization (a) and activity (b) rates of antibodies immobilized onto cellulose, after irradiation (I) or drying and irradiation (S+I), for long irradiation time. The results from 2 different immobilizations are presented for each condition.



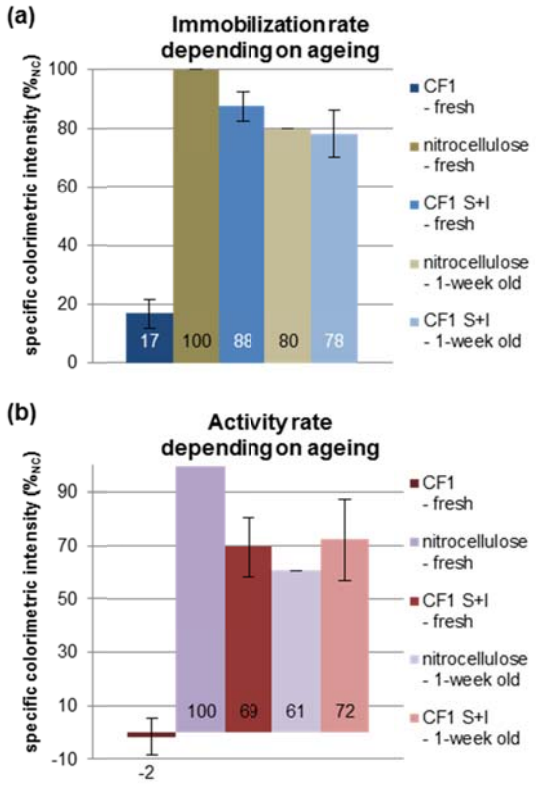
SI-Figure 5: Histograms showing the immobilization (a) and activity (b) rates of antibodies immobilized onto cellulose, after drying (S) and irradiation for 2h40 at either 365 nm (I@365) or under visible light (I@visible). The results from 3 different immobilizations are presented.



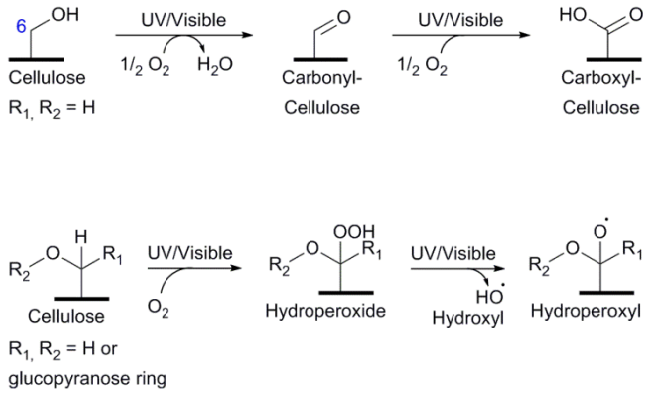
SI-Figure 4: Histograms showing the immobilization (a) and activity (b) rates of antibodies immobilized onto cellulose, after drying, irradiation (S+I) and washing with phosphate buffer or phosphate buffer with salts and detergent. The results from 3 different immobilizations are presented.



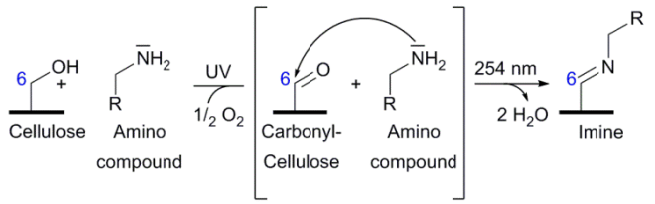
SI-Figure 6: Histograms showing the immobilization (a) and activity (b) rates of antibodies immobilized onto various papers according to the optimal procedure. The results from 3 different immobilizations are presented.



SI-Figure 7: Histograms showing the immobilization (a) and activity (b) rates of antibodies immobilized onto cellulose according to the optimal procedure. Fresh and aged strips were compared. The results from 3 different samples are presented.



SI-Figure 8: Photooxidation processes occurring in cellulose during photo-ageing according to ref [56].



SI-Figure 9: Photooxidation of cellulose treated with amino compounds according to ref [57].

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Additional experiment

Photolinker-free photoimmobilization of antibodies onto cellulose for the preparation of immunoassay membranes

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1. Experimental

1.1. Reagents and materials

Papers used for performing the immunoassay membranes comprise celluloses CF1 and AE 98 Fast nitrocellulose from Whatman (Maidstone, Kent, UK). Immunochromatographic strips were prepared using No. 470 absorbent pad from Schleicher and Schuell BioScience GmBH (Dassel, Germany) and MIBA-020 backing card from Diagnostic Consulting Network (Carlsbad, CA, USA). Materials were cut using an automatic programmable cutter Guillotine Cutting CM4000 Batch cutting system from BioDot (Irvine, CA, USA). Proteins (ovalbumin (OVA), Bovine Serum Albumin (BSA) and porcine skin gelatin), as well as chemical products for preparing buffers and colloidal gold solution were obtained from Sigma-Aldrich (St Louis, MO, USA). Water used in all experiments was purified by the Milli-Q system (Millipore, Brussels, Belgium). Monoclonal murine antibodies (murine mAbs) were produced at LERI (CEA, Saclay, France) as previously described [1]. Goat anti-mouse antibodies (IgG + IgM (H+L)) were purchased from Jackson ImmunoResearch (West Grove, PA, USA). Irradiations were conducted at room temperature in a UV chamber CN-15.LV UV viewing cabinet (Vilber Lourmat, Marne-la-Vallée, France). 96-Well polystyrene microplates (flat-bottom, crystal-clear, from Greiner Bio-One S.A.S. Division Bioscience, Les Ulis, France) were used as container for migrations on immunochromatographic strips. Colorimetric intensity resulting from colloidal gold was quantified with a Molecular Imager VersaDoc^{TM} MP4000, in association with the software Quantity One 1-D Analysis (Bio-Rad, Hercules, CA, USA).

1.2. Photoimmobilization of antibodies

Murine monoclonal antibodies directed against OVA epitopes (1 mg mL^{-1} in 0.1 M potassium phosphate buffer, pH 7.4, 40 µL cm^{-2} deposit) were photoimmobilized onto pristine CF1 cellulose paper. They also were adsorbed onto nitrocellulose (positive control) and onto pristine CF1 cellulose paper (negative control) by regular 1-hour incubation at room temperature. Results obtained after photoimmobilization were compared to positive and negative controls.

According to optimization results [2,3], the photoimmobilization process for antibody immobilization onto cellulose can be described as follows: (i) an antibody solution was dispensed onto a cellulose sheet (40 µL cm^{-2} on a 0.5 cm x 0.5 cm cellulose sheet); (ii) antibodies were concentrated by drying of the impregnated paper at 37°C, in a ventilated oven, for 15 minutes; (iii) the system was irradiated at 365 nm (1050 µW cm^{-2}) for 2h40 (about 10 J cm^{-2}) for inducing photoimmobilization; and (iv) papers were intensively rinsed with a washing buffer (0.1M potassium phosphate buffer, pH 7.4, containing 0.5 M NaCl and 0.5% (v/v) Tween 20) for removing non-immobilized antibodies.

In order to discuss the mechanism involved in the photoimmobilization process, an experiment in which the substrate was irradiated prior to antibody deposit was performed. Thus, the steps previously described then occurred in the following order: (i) papers were dried at 37°C, in a ventilated oven, for 15 minutes; (ii) papers were irradiated at 365 nm (1050 µW cm^{-2}) for 2h40 (about 10 J cm^{-2}); (iii) an antibody solution was dispensed onto the irradiated cellulose sheets (40 µL cm^{-2} on a 0.5 cm x 0.5 cm cellulose sheet) and left to incubate for 1 hour; and (iv) papers were intensively rinsed with a washing buffer (0.1M potassium phosphate buffer, pH 7.4, containing 0.5 M NaCl and 0.5% (v/v) Tween 20) for removing non-immobilized antibodies.

All membranes were then saturated with a gelatin solution (0.1 M potassium phosphate buffer, pH 7.4, containing 0.5% (w/v) porcine gelatin and 0.15 M NaCl) for preventing nonspecific protein adsorption on membranes during immunoassays. Saturation was performed by impregnating and incubating the membranes with the gelatin solution overnight at 4°C, and then drying them at 37°C in a ventilated oven for 30 minutes.

1.3. Immunochromatographic assays (LFIA)

Immobilization rate and biological activity rate of the immobilized antibodies were evaluated by colloidal-gold-based lateral flow immunoassays (LFIA) [4]. The signal intensity was quantitatively estimated by colorimetric measurement. All results were compared with adsorption on pristine cellulose (negative control) and nitrocellulose (positive control).

Considering that adsorption on nitrocellulose is the most frequently used method for immunochromatographic assays, it is herein considered as the reference and has been assimilated to 100% for both the immobilization rate and the activity rate.

All the reagents were diluted in the analysis buffer (0.1 M potassium phosphate buffer, pH 7.4, containing 0.1% (w/v) BSA, 0.15 M NaCl, and 0.5% (v/v) Tween 20), at room temperature, 30 minutes prior to migration in order to reduce nonspecific binding. Each assay was performed at room temperature by inserting a strip into a well of a 96-well microtiter plate containing 100 µL of the test solution. The mixture was successively absorbed by the various pads and the capillary migration process lasted for about 15 minutes. Colorimetric intensity was further measured using the molecular imager. Since this intensity depended on parameters such as temperature and moisture content of paper at the time of measurement, all strips were dried for 30 minutes at 37°C in a ventilated oven and then rehydrated with the analysis buffer just before measurement [5].

1.3.1. Preparation of colloidal-gold-labeled antibodies

Tracer antibodies were labeled with colloidal gold according to a known method previously described [6]. Two types of tracer were prepared: a goat polyclonal antibody anti-mouse tracer to reveal the immobilized murine antibodies, and a murine monoclonal antibody anti-OVA tracer to highlight the capture of OVA by the immobilized antibodies.

Briefly, 4 mL of gold chloride and 1 mL of 1% (w/v) sodium citrate solution were added to 40 mL of boiling water under constant stirring. Once the mixture had turned purple, this colloidal gold solution was allowed to cool to room temperature and stored at 4°C in the dark. 25 µg of antibody and 100 µL of 20 mM borax buffer, pH 9.3, were added to 1 mL of this colloidal gold solution. This mixture was left to incubate for one hour on a rotary shaker at room temperature, therefore enabling the ionic adsorption of the antibodies onto the surface of the colloidal gold particles. Afterwards, 100 µL of 20 mM borax buffer, pH 9.3, containing 1% (w/v) BSA, was added and the mixture was centrifuged at 15 000 g for 50 minutes at 4°C. After discarding the supernatant, the pellet was suspended in 250 µL of 2 mM borax buffer, pH 9.3, containing 1% (w/v) BSA and stored at 4°C in the dark.

1.3.2. Preparation of immunochromatographic strips

An immunochromatographic strip is usually composed of a loading area (or sample pad), a detection area and an absorbent pad, the whole being affixed onto a plastic support. The detection area was therefore formed by an antibody-bearing membrane. Migration was supported by two surrounding sample wicking pads made of the same kind of paper than the detection area, free of antibodies and saturated with gelatin (see Figure 1).

1.3.3. Evaluation of the immobilization rate

The test solution was composed of a goat anti-mouse tracer diluted 10 times in the analysis buffer. Papers without antibody in the photoimmobilization solution (ungrafted paper) assessed the unspecific signal due to unspecific adsorption of the tracer onto the detection pad. The immobilization rate of the cellulose papers following the various procedures was measured by the difference between the antibody-grafted paper signal and the ungrafted corresponding one.

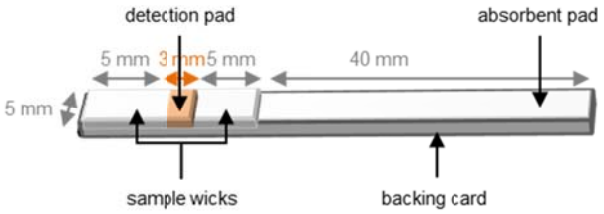

Figure 1: Schematic representation of an immunochromatographic strip.

1.3.4. Evaluation of the activity rate

Two test solutions were prepared and pre-incubated for 10 minutes. The first one was a solution of OVA and murine anti-OVA mAb tracer (1 µg mL^{-1} and 10-fold dilution, respectively) in the analysis buffer. The second one only contained murine anti-OVA mAb tracer diluted 10 times in the analysis buffer. This immunoassay without antigen (OVA) assessed the unspecific signal due to unspecific adsorption of the tracer onto the antibody–gelatin matrix during immunoassays. The biological activity rate of the grafted antibodies was measured by the difference between the antibody-grafted paper signal in the presence of OVA and the corresponding one in the absence of it.

2. Results and discussion

2.1. Proposed mechanism

Several studies may raise suggestions about the possible mechanism. Particularly, accelerated photo-ageing experiments demonstrated that cellulose exposure to long-scale UV and visible light ($\lambda \geq 340$ nm) induced extensive oxidative degradation of cellulose, along with formation of hydroxyl radicals and carbonyl groups. Photooxidative reactions resulted in an increase of carbonyl, carboxyl and hydroperoxide content. The species described in that study are depicted in Supplementary Information (SI-Figure 8) [7]. Furthermore, another study showed that carbonyl groups resulting from cellulose exposure to 254-nm UV light condensed with primary amino groups from species previously poured onto cellulose to form imines (see SI-Figure 9 in Supplementary Information). This phenomenon would be responsible for the yellowing of cellulose papers treated with amino compounds [8], which yellowing also occurs under natural light exposure ($\lambda \geq 280$ nm). To the best of our knowledge, no study has proved this imine formation under 365-nm UV light thus far. However, this is well conceivable given that carbonyl groups are produced during accelerated photo-ageing of cellulose at this same wavelength [7]. In addition these carbonyl groups are easily condensed with primary amines from biomolecules under mild conditions. This is actually a broadly used method for chemically immobilize biomolecules onto cellulose [9].

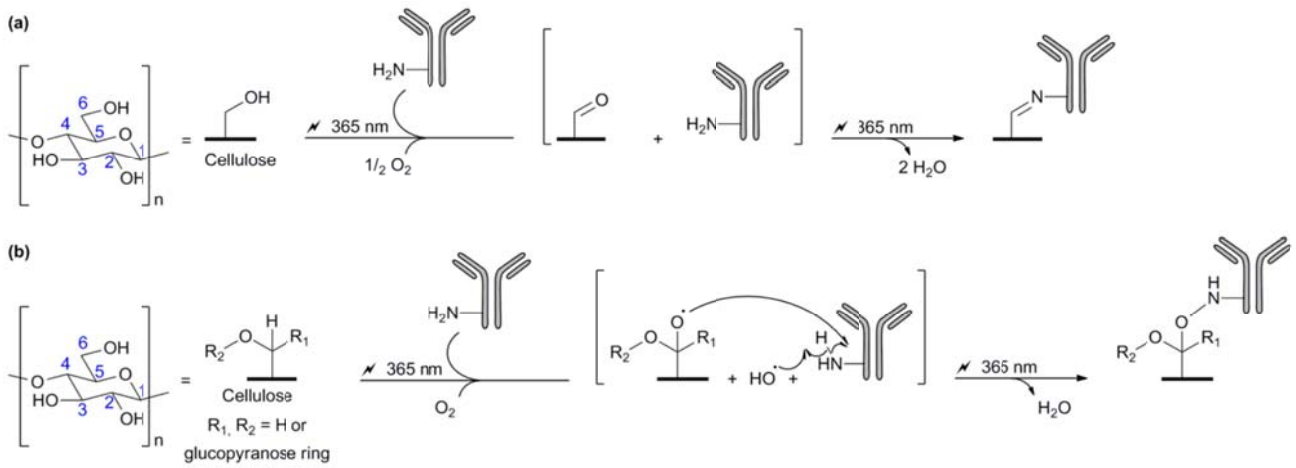

Figure 2: Proposed mechanisms for photoimmobilization of antibodies onto cellulose. The oxidative mechanism (a) is based on references [7–9], while the radical mechanism (b) is only based on reference [7].

Table 1: Antibody immobilization and activity rates depending on washing solution.

	Specific colorimetric intensity ($\%_{NC}$)	
	Immobilization rate (anti-mouse tracer)	Activity rate (anti-OVA tracer)
CF1+Ab	32 ± 5	18 ± 6
$[CF1+Ab]_{S+I}$	121 ± 10	81 ± 13
$[CF1]_{S+I}$ +Ab phosphate buffer with salts & detergent	32 ± 4	-1 ± 0.4

As a negative control, antibodies were adsorbed onto cellulose (CF1+Ab). Antibodies were photoimmobilized onto cellulose following the optimized procedure: an Ab deposit followed by drying and irradiation ($[CF1+Ab]_{S+I}$). As a comparison, cellulose substrate was irradiated prior to antibody deposit ($[CF1]_{S+I}$ +Ab). The results from 2 different series are presented.

In light of those readings, two mechanisms could be proposed for the photolinker-free photoimmobilization process presented herein: an oxidative mechanism involving carbonyl moieties (Figure 2a) and a radical mechanism (Figure 2b). If the carbonyl mechanism (Figure 2a) actually occurred, nearly no difference should be observed by irradiating the substrate prior to antibody deposit. This verification experiment had been conducted (results are shown in Table 1) and led to both immobilization rate and activity rate similar to the negative control (pristine unirradiated cellulose paper) values. The carbonyl mechanism was therefore excluded and the radical mechanism (Figure 2b) seemed to be the most likely. Besides, the latter would be consistent with both the need for antibody concentration observed during optimization experiments and the results observed with cellulose pre-irradiation aforementioned. Indeed, radicals have a short lifetime related to a high reactivity and therefore react in short range. Hence, the radicals created by pre-irradiation would have been degraded before the antibody deposit and would lead to results similar to unirradiated papers (above result). In addition, radicals would only react with the closest antibodies which are many more after a concentration step (optimization result). More experiments such as ESR are in progress in order to confirm this hypothesis.

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