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Direct Immobilization of Capture Antibodies on Injection Molded Thermoplastics

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This paper demonstrates for the first time a method for transferring functional antibodies to plastic substrates using injection molding. Our group has previously reported immobilization of other proteins using this method, such as horseradish peroxidase, fibronectin and avidin¹. In addition, Heyries et. al. has reported a similar immobilization method of antibodies^{2,3} and DNA⁴ on PDMS.

Polyclonal rabbit anti-mouse IgG were adsorbed from PBS onto a nickel mold insert. The molds were subsequently rinsed with MilliQ water and dried before a 250°C warm melt of cyclo-olefin copolymer (COC) were injected into the cavity, which was kept at 20°C. Although exposing the proteins to denaturing conditions, it is believed that the extremely fast cooling at the mold interface helps retain the protein functionality.

To evaluate the functionality, a sandwich ELISA was performed with the immobilized anti-mouse IgG as a capture antibody, and a peroxidase linked detection antibody for an enzymatic readout (Fig. 1). We achieved a standard curve with IgG from mouse serum as the antigen, which was comparable to “conventional” ELISA where the capture antibody is immobilized directly on the plastic by passive adsorption. The limit of detection (LoD), defined as the signal of zero concentration plus three standard deviations, was found to be 100 pg/mL.

The presented work is a new, innovative demonstration of functional antibody immobilization, compatible with high-volume production techniques of polymer devices, using materials that are superior to PDMS for cell culture applications or where long-term stability of the surface is required. The functional surfaces have the potential to be integrated into biochips, using bonding techniques with localized heating, such as laser or ultrasonic bonding, without any detrimental effect on the protein activity.

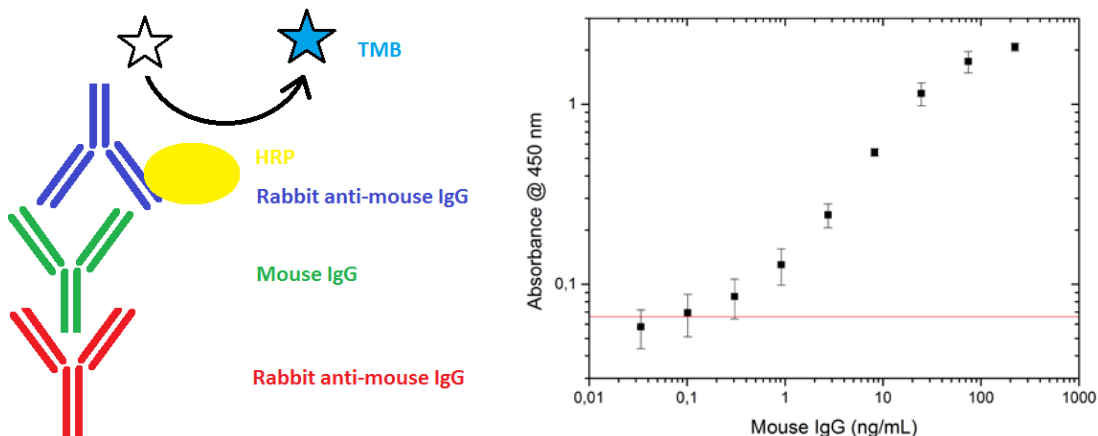


Figure 1: A standard curve for IgG from mouse serum generated by using the immobilized rabbit anti-mouse IgG as a capture antibody. The red curve marks the limit of detection. The errorbars are the standard deviation for 5 individual samples.

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