Electronic Supplementary Material (ESI) for RSC Advances.

Supporting Information for RSC Advances

A simple two-step silane-based (bio-) receptor molecule immobilization without additional binding site passivation

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Figure S1. False colored green fluorescence image of parts of channel 1 and 3 further away from the middle cross (see Fig. 2D) and a schematic drawing of the mask layout that was used during photoresist exposure for creating the stripe pattern. The diagonal lines explicitly allow distinguishing between exposed and unexposed areas. Since we used a positive resist, the diagonal lines were exposed and later on covered with TESPSA, which is proven by the fluorescent signal. The unexposed stripes exhibited protein repellent properties so that we conclude that they were still covered with PEG silane after the preparation procedure. The dark spots that overlap with the fluorescent lines are due to support columns of the PDMS channels preventing the channel collapse.[1]

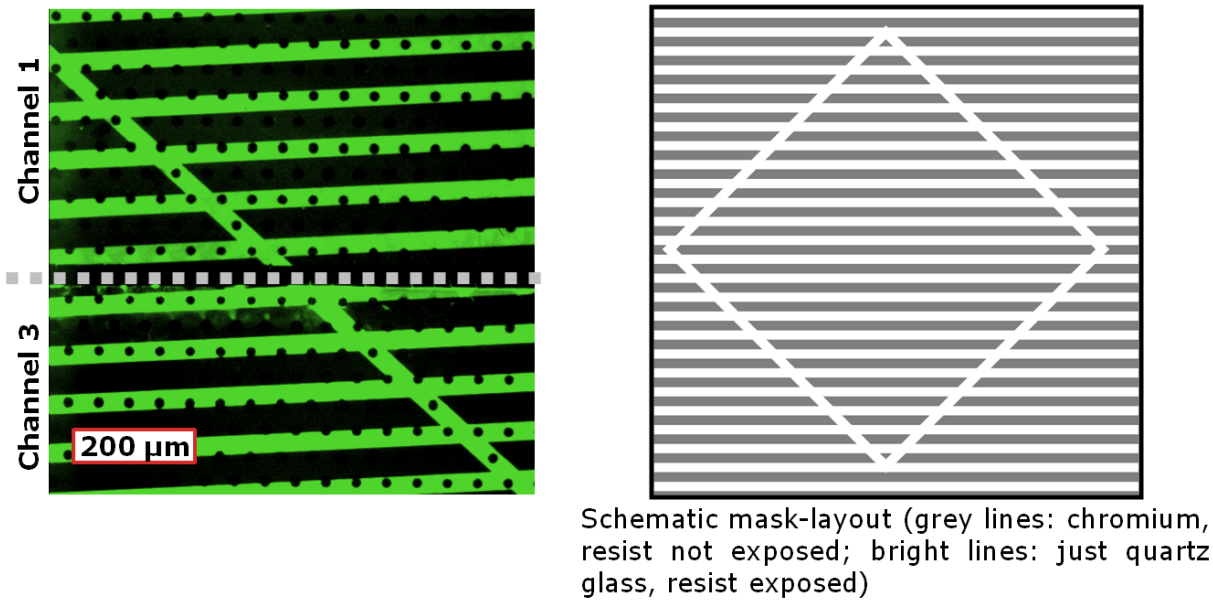

Figure S1

Figure S2. Analysis of the fluorescence intensity. Merged fluorescence microscopy images of green and red filter for the three different receptor / analyte incubation stages – in four microfluidic channels (indicated by grey dotted lines). Receptor and analyte molecules were incubated with the surface in different orders as schematically depicted next to the channels (Figure 2). Fluorescence intensities of red and green filter were measured along the white dashed lines across the images at each stage, respectively. The corresponding grey values were plotted below the respective images. At stage **(1)** an intense red fluorescence peak was detected in channel 4. The two lower red peaks in channel 4 are attributed to decreased fluorescence intensities close to the channel walls. In channel 3, two intense green fluorescence peaks were detected, and one peak with lower intensity at a position very close to the channel wall. The peaks at positions close to the channel walls (always indicated with ▼) also appeared in the subsequent two stages, however, with changing intensities, because the lines along which the grey values were measured did not perfectly match in the different stages.

At stage **(2)**, weak red fluorescence signals were detected in channel 2 and channel 3, at positions where previously other antibodies had been immobilized (indicated with *). This signal is attributed to minor unspecific interactions between $IgG_{Control\_red}$ and immobilized $IgG_{Receptor}$ (channel 2) as well as IgG_{Target_green} (channel 3). However, no similar effect could be observed in channel 4 in the green fluorescence signal. We assume the $IgG_{Control\_red}$ has a tendency to interact unspecifically with the antibody covered surfaces. In channel 4, the IgG_{Target_green}, however, proves that any unspecific interactions are not due to unsaturated binding sites of the TESPSA layer. A surface blocking, for example with bovine serum albumin,[2] might prevent undesired non-specific attachment entirely.

At stage **(3)**, a high green fluorescence signal was detected in channel 2 (indicated with ●). This signal is attributed to a specific interaction between the immobilized $IgG_{Receptor}$ and the IgG_{Target_green}. The fluorescence intensity in channel 2 was not as high as in the reference channel 3. This result implies that during the initial binding of IgG to the TESPSA surface more molecules attach than during the specific receptor-analyte-interaction. The dark lines in-between the TESPSA stripes showed a similar low brightness as the areas outside the channels. Therefore, the PEG silane stripes can be considered to be efficient in protein repulsion.

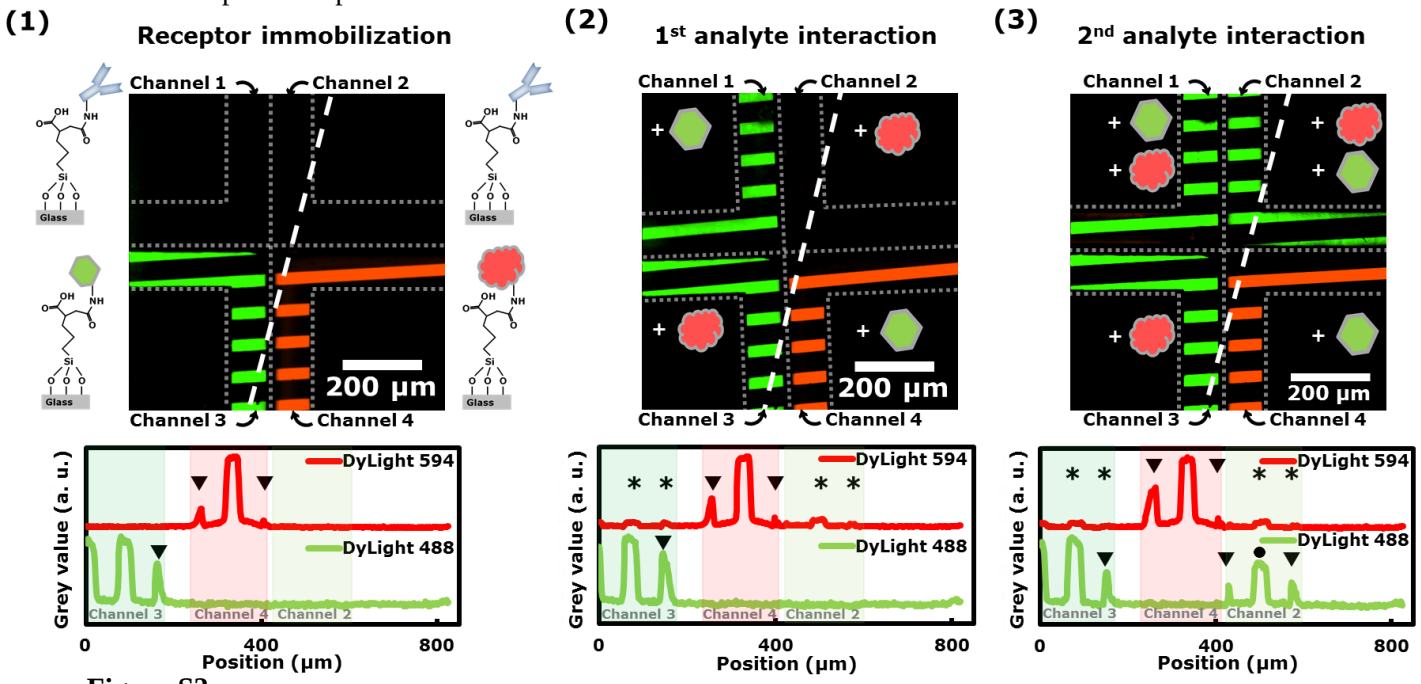

Figure S2

References

1 A. Gang, N. Haustein, L. Baraban and G. Cuniberti, *RSC Adv.*, 2015, **5**, 11806.

2 M. Steinitz, *Anal. Biochem.*, 2000, **282**, 232.