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Thiol-ene click chemistry towards easy microarraying of half-antibodies

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Supporting Information

1. Materials

Glass microscope slides were obtained from Labbox (Barcelona, Spain). Triethoxyvinylsilane, tris(2-carboxyethyl)phosphine (TCEP), bovine serum albumin (BSA), human C-reactive protein (CRP) and bovine serum albumin polyclonal antibody (IgG αBSA) were purchased from Sigma Aldrich. The human C-reactive protein monoclonal antibody (IgG αCRP) and Alexa Fluor 647 NHS ester, and NuPAGE™ Bis-Tris Welcome Pack, 4-12%, for SDS electrophoresis were purchased from ThermoFisher Scientific. Human cardiac troponin I (cTnI) and the human cardiac troponin I monoclonal capture antibody (IgG αcTnI) were ordered from Abcam. The human cardiac troponin I monoclonal detection antibody (IgG αcTnI) was obtained from Hytest and then labelled by the methodology described below. Toluene and 2-propanol were purchased from Scharlau. Ellman's reagent 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) was acquired from Acros Organics. Milli-Q water with a resistivity above 18 mΩ was used to prepare the aqueous solutions. The employed buffers were phosphate buffer saline (PBS 1x, 0.008 M sodium phosphate dibasic, 0.002 M sodium phosphate monobasic, 0.137 M sodium chloride, 0.003 M potassium chloride, pH 7.5), PBS-T (PBS 1x containing 0.05 % Tween 20), acetate buffer (0.15 M sodium acetate, 0.01 M EDTA, 0.1 M sodium chloride, pH 4.5) and bicarbonate buffer (0.1 M sodium bicarbonate, pH 8.3). All the buffer solutions were filtered through a 0.22 µm pore size nitrocellulose membrane from Whatman GmbH (Dassel, Germany) before use.

2. Instrumental methods

Surface activation was carried out with a UV−ozone cleaning system UVOH150 LAB (FHR, Ottendorf-Okrilla, Germany). Microarrays were printed with a low-volume non-contact dispensing system from Biodot (Irvine, CA, USA), model AD1500. Probe photoattachment was done with the same UV-ozone cleaning system described above. Contact angle measurements were taken with Attension Theta Lite (by Biolin Scientific) and images were processed with OneAttension (version 3.1; Biolin Scientific). Measurements were taken in triplicate at room temperature with a volume drop of 5 µL employing 18 mΩ water quality. The fluorescence signal of the spots in the microarrays was recorded with a homemade surface fluorescence reader (SFR),[1] with a high-sensitivity charge-coupled device camera Retiga EXi from Qimaging, Inc. (Burnaby, Canada), equipped with light-emitting diodes Toshiba TLOH157P as the light source or with a GenePix 4000B Microarray Scanner (Axon instruments). Microarray image treatment and quantification were done using the GenePix Pro 4.0 software from Molecular Devices, Inc. (Sunnyvale, CA, USA). The concentrations of proteins and antibodies were determined by measuring the optical density at 280 nm in a NanoDrop ND 1000 Spectrophotometer (ThermoFisher Scientific, Wilmington, Delaware, USA).

[1] D. Mira, R. Llorente, S. Morais, R. Puchades, A. Maquieira and J. Martí, *Proc. SPIE–Int. Soc. Opt. Eng.*, 2004, **5617**, 364.

3. Reduction of IgG to hIgG

IgG in acetate buffer (0.15 M sodium acetate, 0.01 M EDTA, 0.1 M sodium chloride, pH 4.5) at the 4 mg/mL concentration, in the presence of 25 mmol/L TCEP, were incubated for 90 minutes at 37°C. The corresponding hIgG were purified by employing a 50 kDa centrifugal filter unit. The concentrations of the solutions were determined by a NanoDrop spectrophotometer. hIgG were characterised by Ellman's assay and SDS-PAGE electrophoresis.

4. SDS-PAGE electrophoresis.

SDS-PAGE assays were performed using NuPAGE 4-12% Bis-Tris minigels under non-reducing conditions. Firstly, 5 µg of whole antibodies and antibody fragments were solved in 2.5 µL NuPAGE LDS sample buffer and water up to a volume of 20 µL. Then each sample was loaded to a well and gels were run at a constant voltage of 200 V for 35 minutes. After electrophoresis, gels were developed with Coomassie Brilliant Blue R solution for 1 h, washed with water milli-Q and destained 3 times with acetic acid/methanol (40%/10%) for 15 minutes until any excess staining was removed. Finally, gels were rehydrated in milli-Q water.

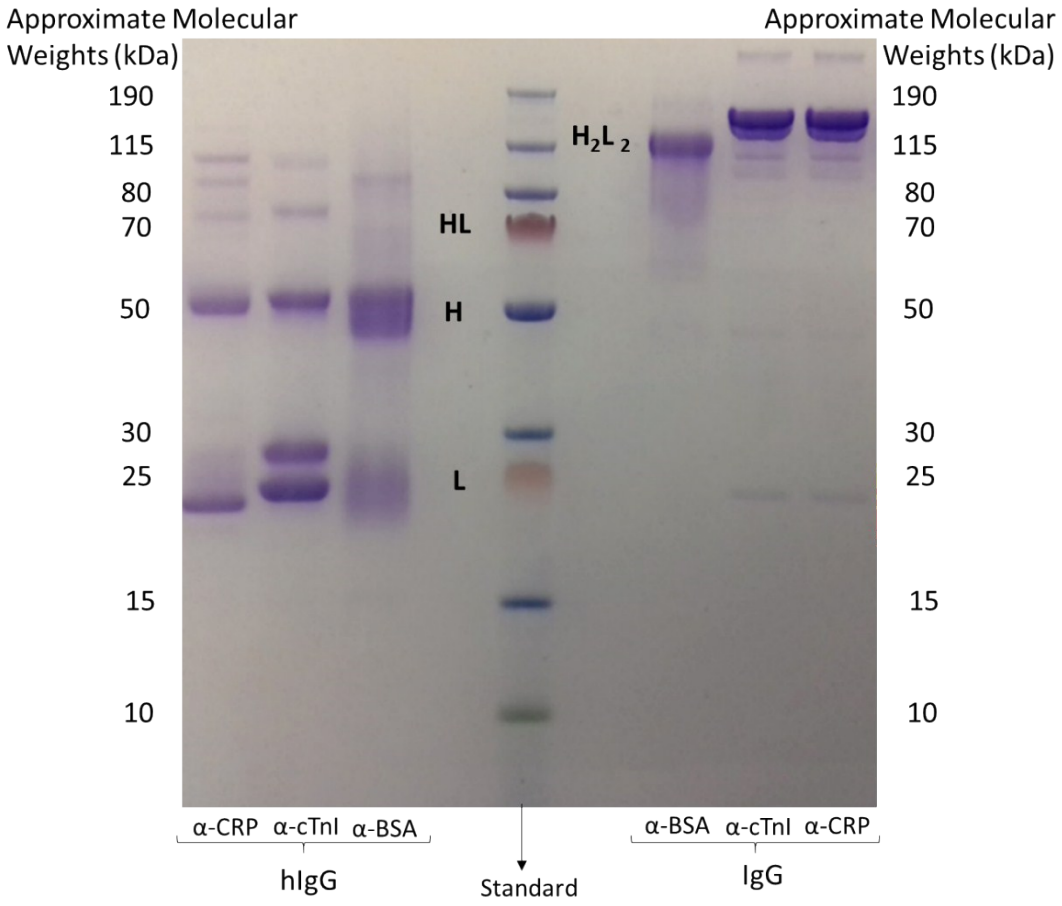

Fig. S1 SDS-PAGE analysis of α-BSA, α-CRP and α-cTnI in the reduced and non-reduced forms. IgG antibodies show mainly the expected H_2L_2 bands, while hIgG antibodies display the corresponding HL, H and L fragments.

5. Ellman's assay

Ellman's reagent (10-fold molar excess) was added to a solution of 3.5 mg/mL in phosphate buffer and the resulting solution was stirred at room temperature for 15 minutes. The formation of 2-nitro-5-thiobenzoic acid was quantitatively determined at 412 nm with an extinction coefficient of 14,150 $M^{-1}cm^{-1}$.

6. Surface chemical modification

Glass microscope slides were cut into pieces of ≈ 2 × 1 cm^2, cleaned with water and 2-propanol, and then air-dried. Afterwards they were placed in the UV−ozone cleaner and irradiated for 10 min at 254 nm. Subsequently, chips were immersed in a solution of triethoxyvinylsilane 2% in toluene for 2 h at room temperature. Finally chips were washed with 2-propanol and air-dried before being baked for 20 min at 100°C.

The surface was characterised by the WCA (water contact angle), with evidence for the presence of alkene groups on the surface shown in all cases.

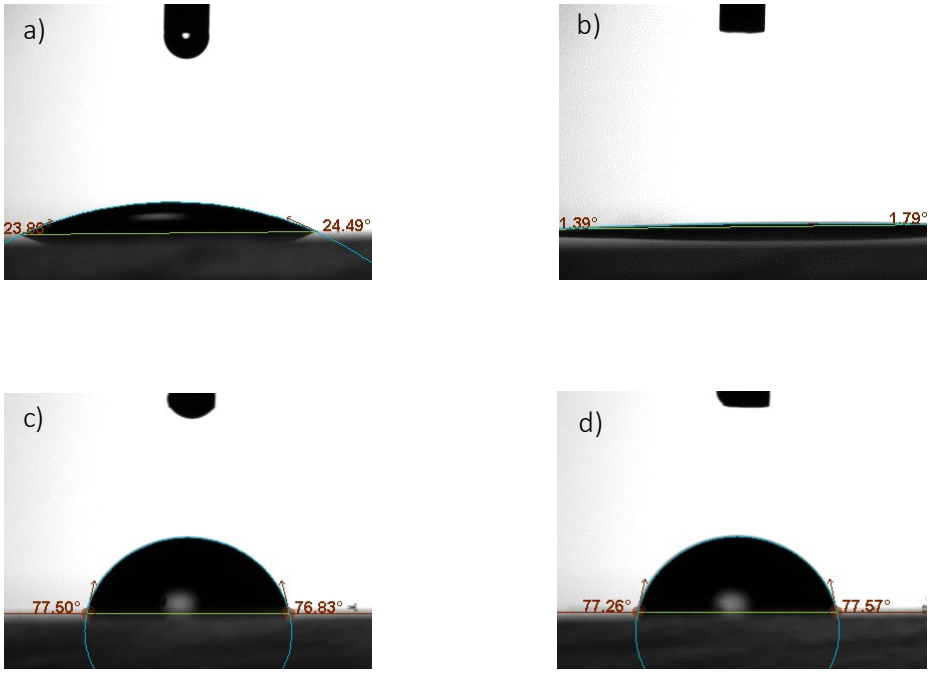

Fig. S2 The water contact angle for the surface a) before and b) after activation using UV light, c) for the surface functionalised with triethoxyvinylsilane and d) for the vinylated surface after the baking step.

7. Labelling probes with Alexa Fluor 647 (BSA, αBSA, CRP, αcTnI)

Firstly, 1 mg of protein was dissolved in 0.1 mL of bicarbonate buffer. Amine-reactive Alexa Fluor 647 (0.1 mg) was dissolved in 0.01 mL of DMSO and the resulting solution was immediately added to the solution of protein while stirring. The resulting mixture was protected from ambient light and stirred at room temperature for 1 h. The reaction mixture was purified by employing 30 kDa centrifugal filter units. The concentration and the label to probe ratio were determined by spectrophotometry.

8. Photoimmobilisation procedure

hIgG microarrays were printed over the previously functionalized glass chips with the low-volume non-contact dispensing system from Biodot. The buffer employed was acetate buffer (0.15 M sodium acetate, 0.01 M EDTA, 0.1 M sodium chloride, pH 4.5), and 25 nL/spot were employed for the microarrays read with the microarray scanner, while 50 nL/spot were dispensed for the microarrays read with the SFR. The microarray had 4 spots per row and in both cases only one drop was printed on a single spot.
Five minutes after printing, chips were irradiated for 5 seconds with UV light (λ = 254 nm) with the UV-ozone cleaner. Afterwards, chips were stored in the dark for 10 minutes and then washed with PBS-T, rinsed with water and dried. They were subsequently incubated in the dark with the labelled probe dissolved in 10% human serum for 30 min at ambient temperature. After washing with PBS-T and water, the fluorescence of the dried chips was measured by either the SFR or the microarray scanner. With the sandwich immunoassays, an additional 30-minute incubation step was run with the corresponding labelled detection antibody solution.