Electronic Supplementary Material (ESI) for Analyst.

Supporting Information

Rapid and simultaneous detection of ricin, staphylococcal enterotoxin B and saxitoxin by chemiluminescence-based microarray immunoassay

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Content

SI – 1: Experimental details

Preparation of monoclonal anti-idiotypic antibody

To induce anti-idiotypic antibodies in mice, animals were immunized with anti-STX mAb 7H11 coupled to the highly immunogenic carrier protein keyhole limpet hemocyanine (KLH) by using glutaraldehyde. Each animal received an intraperitoneal injection of 60 µg of the conjugate emulsified in Freunds incomplete adjuvant. Booster injections using the same composition and amount of Immunogen were given 12 weeks after the primary injection. Finally, 3 days before fusion, mice exhibiting high serum titers of anti-idiotypic antibodies received a final booster injection of 80 µg mAb 7H11-KLH conjugate in PBS alone.

To detect anti-idiotypic antibodies in the sera of immunized mice as well as in culture supernatants, microtitre plates were coated with Fc-specific anti-mouse antibodies (5 mg/L) overnight at ambient temperature. After adding dilutions of the testing solutions (mice sera or culture supernatants) bound anti-idiotypic antibodies were detected by addition of Fab fragments of the mAb 7H11 conjugated to horseradish peroxidase. Differentiation between Ab2α and those Ab2β/γ antibodies reacting within the antigen-binding site of mAb 7H11 was done under competitive EIA-conditions in which saxitoxin and the anti-idiotypic antibodies under study competed for limited mAb 7H11 binding sites.

MCR-3 System

The buffers and reagents required for the measurement are located in the storage

containers, in the syringe units and in the sample syringe. The injection unit consists of a small glass syringe with a volume of 25 mL for biotinylated antibodies and a larger one with a volume of 50 mL for conjugates. The only manual component is the 1 mL sample syringe, which must be refilled after each measurement. The microarray chips are supplied with the required reagents, using tubing, valves and pumps. Each microarray chip has two flow cells and the shape of the flow cell is to ensure that the solutions are fed uniformly on the microarray chip. The operation can be understood as the MCR3 combination of modules that are located in the housing of the MCR3. The components in the system are controlled by a computer program with the LabVIEW 8.2.

Enzyme linked immunosorbent assay for HRP and PolyHRP40 comparison

The antibody mAb R109/3 for ricin was diluted to a concentration of 10 mg/L with PBS buffer (10 mM KH_2PO_4, 70 mM K_2HPO_4, 145 mM NaCl). Then, 50 µL of each diluted antibody solutions were added into the wells and incubated overnight at 4°C. Unbound antibodies were removed by washing three times with washing buffer(0.001mMKH_2PO_4, 0.007 mM K_2HPO_4, 0.015 mM NaCl, 0.05% Tween 20). Unspecific binding sites of the microtiter plate were blocked with 200 µL of 2% casein/well on a shaker for 2 hours at RT. After blocking, the wells were washed three times with PBST (10 mM KH_2PO_4, 70 mM K_2HPO_4, 145 mM NaCl with 0.05% (v/v) Tween20). The calibration standards were prepared by dilution with PBST at the concentration range of 0 to 1000 g/L of agglutinin and ricin. Subsequently, 50 µL of each calibration standards was added into the wells and incubated on a shaker for 2 h at RT. After a washing step (3 × 300 µL with PBST), an aliquot of 50 µL of R18/1-bio was added in PBST. The microtiter plate was again

incubated on a shaker for 1 h at RT and then washed (3 × 300 µL with PBST). Subsequently, 50 µL of SA-HRP or SA-PolyHRP40 was added to the respective wells in a dilution of 1:5000. After one hour of incubation, the plate was washed (3 × 300 µL with PBST) and 100 µLof a freshly prepared substrate solution of TMB was added into the wells. The microplate was conserved in a dark environment with aluminum foil for about 5 minutes at RT until sufficient blue color was visible. The reaction was ended after adding 100 µL of stopping solution (5% H_2SO_4), changing the color to yellow. The absorbance was measured at 450 nm. The washing steps were performed using an automatic washing system, ELx 405 Select, and the absorbance measurements were performed in a Synergy HT ELISA-reader from BioTek Instruments GmbH (Bad Friedrichshall, Germany). Each point was measured in quadruplicate using the software Gen 05.

Microarray preparation

For production of the antibody microarrays, the capture antibodies were covalently immobilized on the activated slides. Up to nine micro-arrays were manufactured in a spotting operation. The application of the analyte solutions was performed with a transference volume of approximately 3 nL. To prevent drying of the deposited analytes (spots), the humidity was set to 50% at a temperature of 15 °C. The capture antibodies were spotted at 1g/L with spotting buffer containing 0.005% Pluronic with 10% trehalose. The anti-idiotypic antibodies were spotted at 0.5 g/L in PBS with 0.09% NaN_3.The positive control consisted of 1.0 g/L anti-HRP in PBS with 0.005% Pluronic and 10% trehalose. After spotting, the spotted solutions were kept overnight (15 h) at 4

°C. In order to prevent non-specific binding, all microarrays were blocked for 15 min in an ultrasonic bath with TRIS-HCl buffer (1M TRIS with addition of HCl). Then, the microarrays were washed in distilled water for 5 minutes and methanol for 5 min. Subsequently, they were placed in an ultrasonic bath and dried under nitrogen. A finished microarray chip had two separate flow cells with a distance of 11.75 mm. In each flow cell, the antibodies were in a series with a distance of 1.1 mm (y-direction) to each other, repeated 5 times, and 1.3 mm of spacing in the rows (x-direction). The final microarray was then combined with a black PMMA plastic carrier (needed for the inlet and outlet of the solutions) by using a double-sided adhesive film having cut-outs for the two flow cells. The cell has 43.3 µL of volume and it possesses an active field of detectable spotted microarray area of 12 mm × 8 mm. Subsequently, the finished microarray chips were used for measurements in the MCR3 system. More details are described by Kloth (2009).

Measurement of the antibody microarray with MCR3

The microarray was connected to the fluidic system of the automated microarray chip read-out platform MCR (GWK Präzisionstechnik, Munich, Germany) by placing it in the drawer of chip loading unit. The CCD camera (Linos MX 916 from Starlight Xpress, Holyport, UK) was located below the flow cell with the microarray situated on its upper surface. The chemicals were pumped through the flow cell by using a fluidic system. The software LabView 8.2 enabled setting of individual assay programs, whereas fluid volumes, flow rates, and flow intervals could be defined individually. The assay program was performed automatically.

In the detection unit, the CCD camera is placed above the microarray chip. This camera has a quantum efficiency of 65% at 420 nm .The obtained image files have a resolution of 696 pixels × 520 pixels with a maximum signal of 65,535 a.u. measured per pixel. For the evaluation of the measurements, the background images of the CCD camera were subtracted from the measured images using the LabVIEW 8.2 software. By using the program version 0.3.2.1 MCR Image Analyzer, CL signal values were obtained for each of the spots background-corrected measurement images. The calculation of the mean and the standard deviation of the five spot replicates were carried out on the ten brightest pixels per spot. The graphical analysis was performed using Origin 7.5. The mean values of the background-corrected signals were CL semi-logarithmically plotted against the toxin concentration. The standard deviations of the CL signals were displayed in the graph as error bars.

SI – 2: Comparison between horseradish peroxidase-streptavidin (SA-HRP) and poly(horseradish peroxidase)-streptavidin (SA-PolyHRP40)

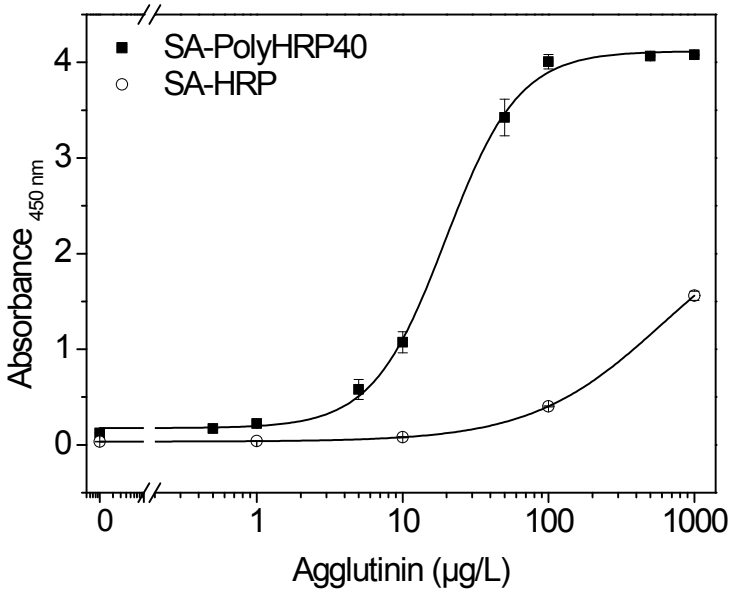
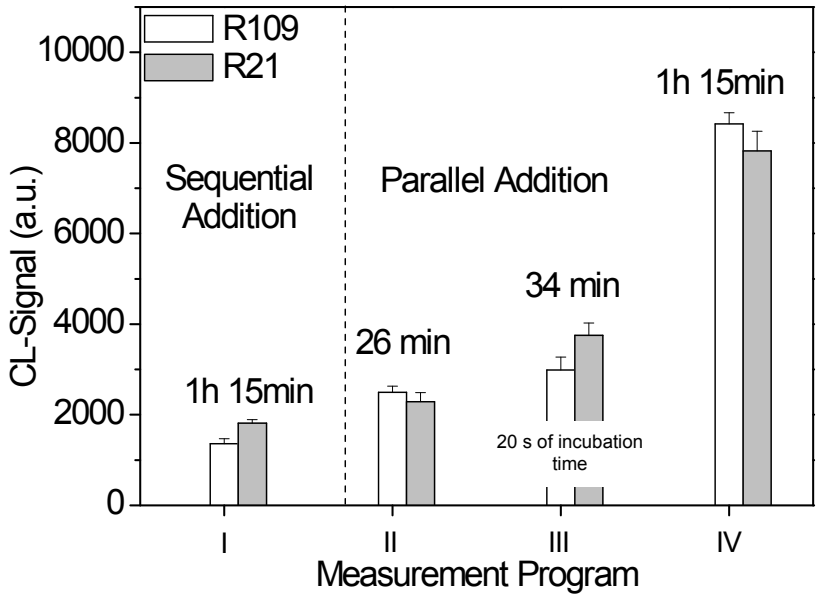


Figure SI – 2. Calibration curve for SA-HRP and SA-PolyHRP40 detected with R18.

SI – 3: Influence of the sequential and parallel addition of reactants



(I) Sequential addition of reactants (1 µL/s) with 10 s of interaction time (duration: 1h 15 min);

(II) Parallel addition in continuous flow at 1 µL/s (duration: 26 min);

(III) Parallel addition with pre-incubation step of the detection antibody and the analyte for 1 min in the MCR3 loop, injecting 50 µL of sample at 1 µL/s with 20 s of incubation time (duration: 34 min);

(IV) Parallel addition with pre-incubation step, injecting 5 µL of sample at 1 µL/s with 10 s of incubation time (duration: 1h 15 min).

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SI – 4: Influence of the flow rate and interaction time on the CL signal

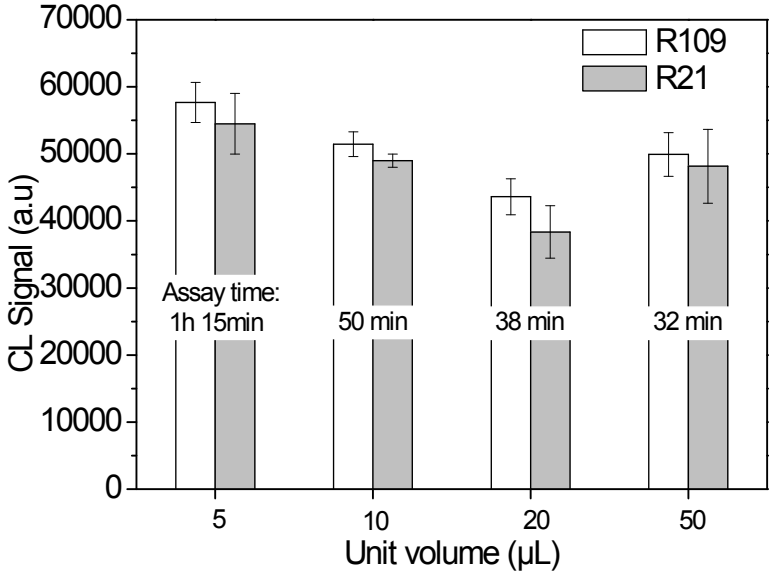

Figure SI – 4. Variation of the unit volume. Measurements were performed using the stopped-flow principle at 1 µL/s with 10 s of incubation time. Ricin concentration was 500 µg/L.

The lowest signal was observed for 20 µL and the CL signal of 50 µL was comparable to the CL signal level of 10 µL. A comparison between 5 and 50 µL showed a decrease in the CL signal intensity of 13.5%. Nevertheless, the assay time is much faster for 50 µL than for 5 µL. Therefore, the volume of 50 µL was chosen.It is important to say that a new antibody batch was used for this experiment in comparison to the SI-3. This explains the difference of the CL-signals between 5 µL and 50 µL for the two graphs. In addition, the higher CL signals were obtained with the detection antibody R109.

Flow rate

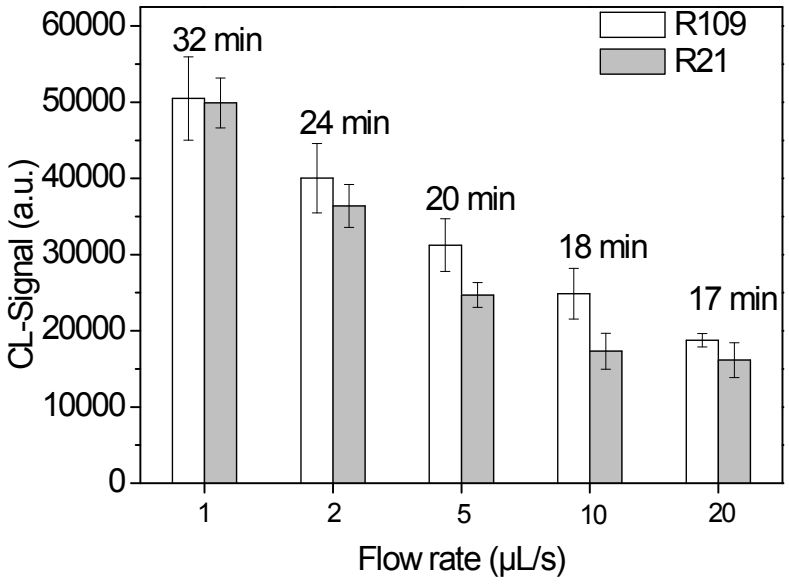

Figure SI – 4. Variation of flow rate from 1 to 20 µL/s. Measurements were performed with an interaction time of 10 s, unit volume of 50 µL and ricin concentration of 500 µg/L.

Interaction time

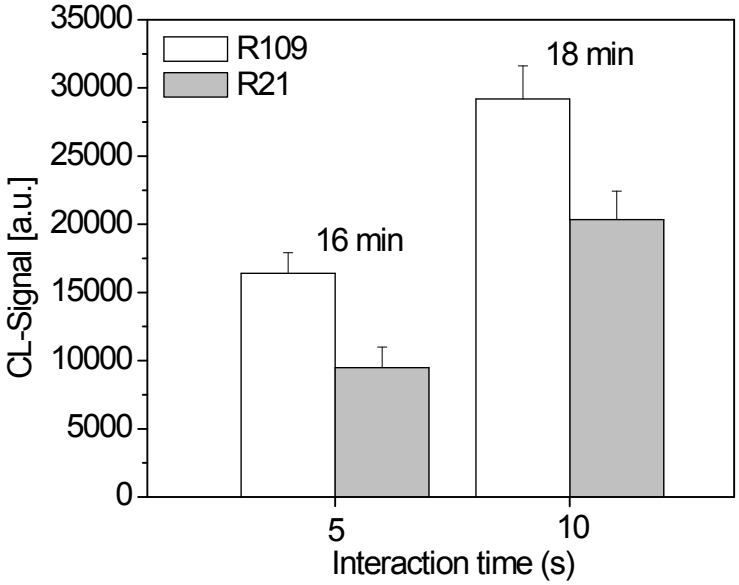

Figure SI – 4. Variation of the interaction time: 5 and 10 s. Measurements were performed with a unit volume of 50 µL, flow rate of 10 µL/s and ricin concentration of 500 µg/L.