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Electronic Supporting Information

Ultrasensitive impedimetric glycan biosensor with controlled glycan density for detection of lectins and influenza hemagglutinins

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Chemicals

11-mercaptoundecanoic acid (MUA), 6-mercapto-1-hexanol, potassium hexacyanoferrate(III), potassium hexacyanoferrate(II) trihydrate, potassium chloride, potassium phosphate monobasic, potassium phosphate dibasic, sodium hydroxide, sulphuric acid, ethanolamine hydrochloride, *N*-hydroxysuccinimide (NHS), *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDC), sodium dodecyl sulfate (SDS), phosphate buffered saline tablet (PBS, one tablet dissolved in 200 mL of deionized water yields 0.01 M phosphate buffer, 0.0027 M potassium chloride and 0.137 M sodium chloride, pH 7.4, at 25 °C.), lectin from *Maackia amurensis* (MAA) and lectin from *Datura stramonium* (DSL) were purchased from Sigma Aldrich (USA). Recombinant hemagglutinin-influenza A virus H5N1 Indonesia 05/2005 and recombinant hemagglutinin-influenza A virus H1N1 New Caledonia 20/99 were obtained from MyBioSource, Inc. (USA), amino terminated glycan 3'Sialyllactose-β-Aminopropyl was purchased from Elicityl Oligotech (France). Ethanol for UV/VIS spectroscopy (ultra-pure) was purchased from Slavus (Slovakia). All buffer components were dissolved in deionised water (DW).

Electrode pre-treatment and SAM preparation

The cleaning of planar polycrystalline gold electrodes (1.6 mm diameter, Bioanalytical systems, USA) was done as previously described[1], with laboratory potentiostat/galvanostat

[1] Tkac, J.; Davis, J. J. *Journal of Electroanalytical Chemistry* **2008**, *621*, 117.

Autolab PGSTAT 128N (Ecochemie, Netherlands) in a three electrode cell with Ag/AgCl reference and a counter Pt electrode (Bioanalytical systems, USA). The first step is a reductive desorption of a previously bound thiols in 0.1 M solution of NaOH, by applying a cyclic potential scanning from a potential of -1,500 mV to -500 mV under N_2 atmosphere with a rate of 1 V s^{-1} for about 50 scans (until a stable scan was obtained). The second step is a mechanical polishing of the electrodes on a polishing pad using 1 μm particles for 5 min and 0.3 μm particles for 5 min (Buehler, USA). After mechanical polishing, electrodes were sonicated two times in DW for 5 min. The third step is the immersion of the electrodes into the solution of freshly prepared piranha (H_2O_2:H_2SO_4 – 1:3) for 20 min. After piranha treatment, the electrodes were again sonicated in DW for 5 min. The fourth and fifth steps are electrochemical polishing and electrochemical stripping of gold oxide in 0.1 M solution of H_2SO_4. Electrochemical polishing was performed by cyclic voltammetry, from -200 mV to 1,500 mV at a scan rate of 100 mV s^{-1} up to 25 scans, and gold oxide stripping was realized by 10 cycles starting from +750 mV to +200 mV at a scan rate of 100 mV s^{-1}. The electrodes were washed with DW and ultrapure ethanol. After the cleaning process, the electrodes were immersed into the 1 mM solution of thiols (mixtures of 11-mercaptoundecanoic acid (11-MUA) and 6-mercapto-1-hexanol (6-MH) in ratio 1:2, if not specified otherwise). The electrodes were incubated overnight in dark at room temperature. The 1 mM stock solution of 11-MUA and 6-MH was prepared in ultrapure ethanol and stored at -20 °C until use.

Preparation of the glycan biosensor

After incubation of the electrodes in a solution of thiols they were washed with ultrapure ethanol and DW. Carboxyl groups on SAM layer were activated with 40 μl solution of 200 mM EDC and 50 mM NHS in ratio 1:1 (solutions of EDC and NHS were previously prepared in DW and stored at -20°C in aliquots) for 15 min. After activation, the electrodes were washed with

DW and 100 μM amino terminated glycan was applied to the biosensor surface (glycan was previously dissolved in DW and stored at -20°C in aliquots). The glycan immobilisation was performed at room temperature for 1.5 h.

Proteins

Lectins were dissolved in 10 mM PBS solution with appropriate pH (MAA solution with pH 7.4 and DSL with pH 6.8) at a concentration of 1 mg ml^{-1} and were stored at -20°C in aliquots. Hemagglutinins (0.1 mg ml^{-1} aliquots stored at -20°C until use) from H5N1 and from H1N1 were dissolved in PBS solution (10 mM PBS with 150 mM NaCl) having pH 7.2.

Electrochemical impedance spectroscopy (EIS) measurements

EIS was measured in an electrolyte containing 5 mM potassium hexacyanoferrate(III), 5 mM potassium hexacyanoferrate(II) and 0.1 M KCl. The analysis was run at 50 different frequencies (ranging from 0.1 Hz up to 100 kHz) under Nova Software 1.9 (Ecochemie, Netherlands). The results were shown in a Nyquist plot, with an equivalent circuit R(C[RW]). The charge transfer resistance (R_{CT}) parameter was used to calibrate the biosensor with each measurement performed at least in triplicate with an independent biosensor device and results are shown with a standard deviation (± SD) calculated in Excel. EIS values obtained in presence of proteins were subtracted from values obtained in a plain electrolyte.

Atomic force microscopy (AFM) measurement

For atomic force microscopy imaging Bioscope Catalyst instrument and Olympus IX71 microscope in conjunction with NanoScope 8.15 software at a scan rate 0.5 line s^{-1} with the tip

set of 200 pN (Scan Asyst, Bruker, USA) was used. Measurements were made with a peak force tapping mode in air. Bare gold, SPR sensor chip (XanTec bioanalytics GmbH, Germany) was cleaned three times with piranha (H_2O_2:H_2SO_4 – 1:3) for 2-3 s, afterwards with DW and ultrapure ethanol, and were modified with thiols and glycans as described above. SCANASYST-AIR silicon tip on nitride lever, sharpened to have a tip radius of 2 nm, was used (Bruker, USA, with f_0=50-90 kHz and k=0.4 N m^{-1}),

Surface plasmon resonance (SPR) measurements

Experiments were performed using a dual channel Reichert SR7000DC SPR system controlled by the SPR Autolink System software (AMETEK, Reichert Technologies, USA). For the SPR measurements bare gold, SPR sensor chip (XanTec bioanalytics GmbH, Germany) was used. The bare gold SPR sensor chip was cleaned three times with freshly prepared piranha (H_2O_2:H_2SO_4 – 1:3) for 2-3 s, afterwards with DW and ultrapure ethanol, and were modified with thiols as described above. A chip was preconditioned before the immobilisation by two consecutive pulses of 10 µL of 100 mM HCl, 50 mM NaOH, 0.5% SDS, 50 mM glycine, and water injected at the flow rate of 100 µL min^{-1}. Immobilization of glycans to the prepared sensor chip was performed at the flow rate of 5 µL min^{-1} employing standard amine coupling procedure. For glycan immobilisation, the sensor chip with SAM was activated for 10 min (both working and reference channels of the SPR flow cell using a fresh mixture of 0.2 M EDC and freshly prepared 0.05 M sulfo-NHS at the flow rate of 20 µL min^{-1}), which was followed by a glycan injection for 48 min (only working channel). The glycan concentration used for immobilisation was 100 µM in a 10 mM sodium acetate buffer (pH 4.5). The immobilisation procedure was completed by injection of 1 M ethanolamine hydrochloride solution to deactivate remaining activated carboxylic groups for 10 min at a flow rate of 20 µL min^{-1} (both channels).

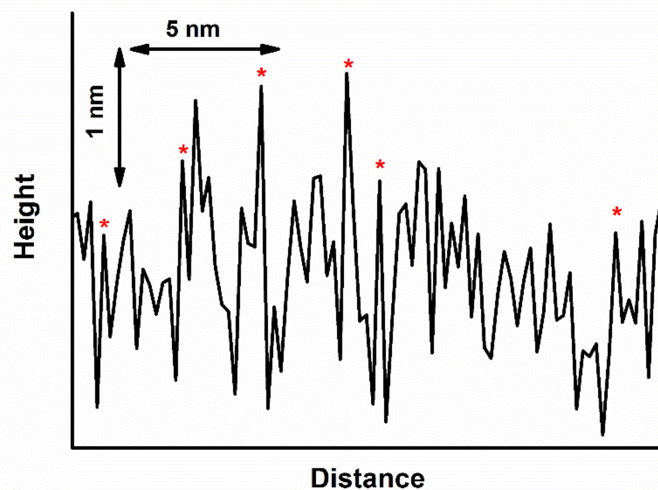

Fig. S1 A detailed topological profile of glycan patterned interface. For calculation of a glycan surface density, topological features with height difference larger than 1.5 nm were counted (marker by a red asterisk), since length of a sialyllactose glycan is 1.8 nm (see Fig. S3).

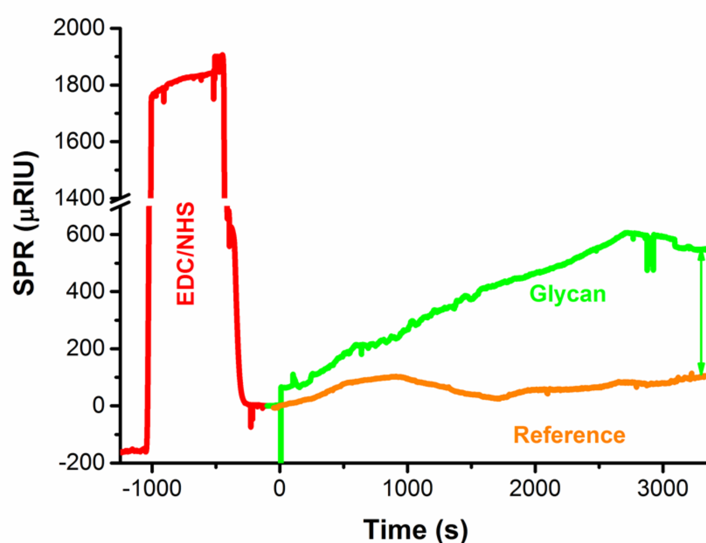

Fig. S2 SPR sensorgram obtained during covalent immobilisation of glycan on a mixed SAM layer showing activation of a –COOH group of 11-MUA by EDC/NHS (red) and covalent immobilisation of sialyllactose glycan (green). Change of SPR signal in a reference channel (brown) being activated by EDC/NHS, but without injection of glycan is shown, as well. Glycan immobilisation was carried out at a flow rate of 5 µL min^{-1} for 40 min.

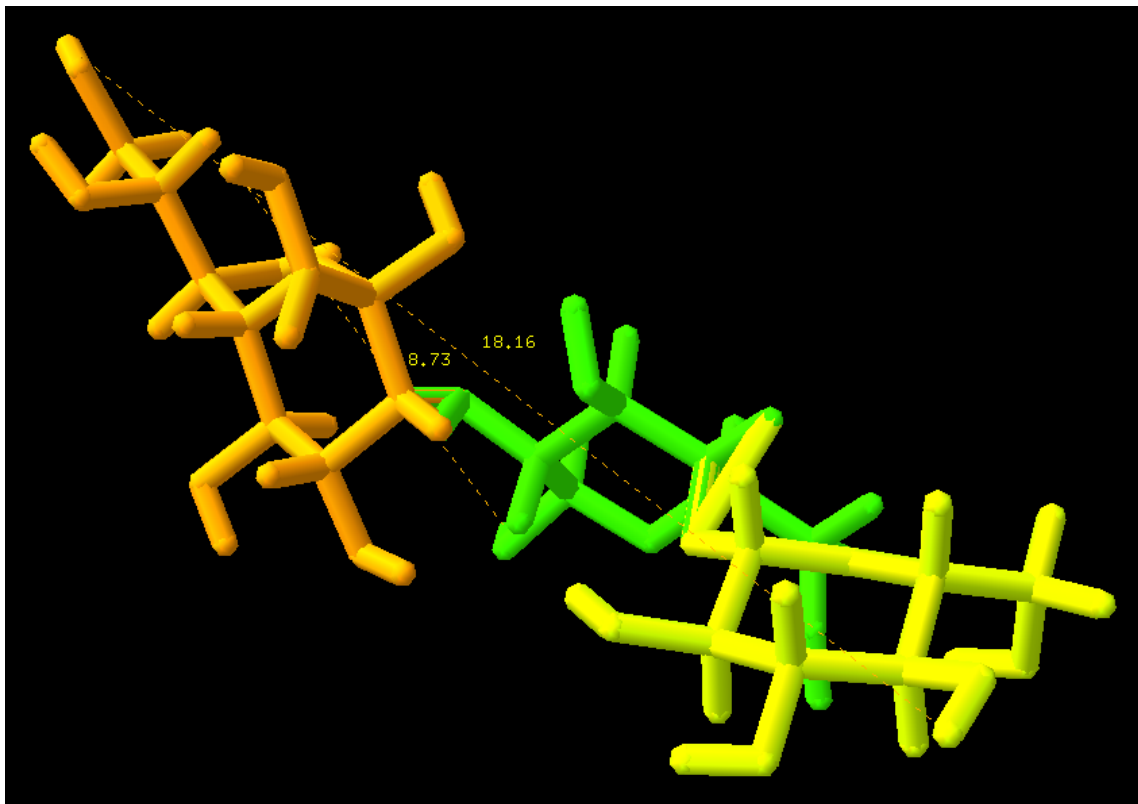

Fig. S3 Structure of sialyllactose glycan used in the study showing glycan chain length of ~18 Å and glycan chain width of ~9 Å.

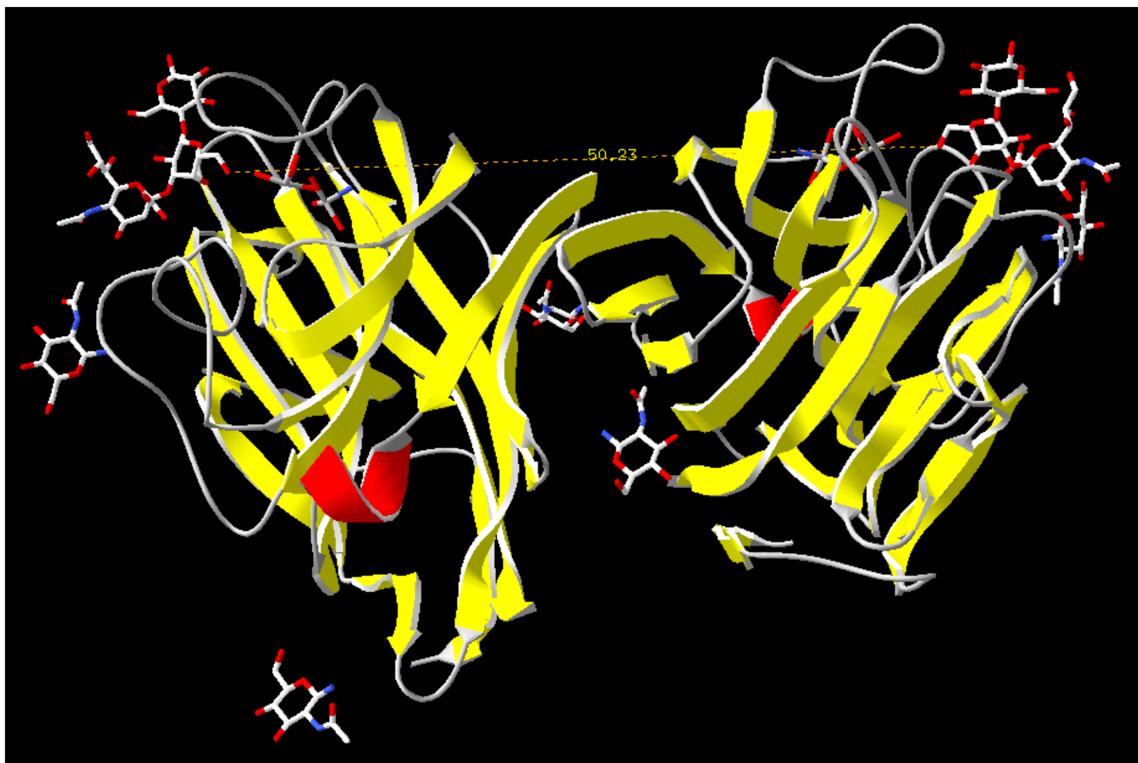

Fig. S4 Structure of MAA lectin having two binding sites docked with sialyllactose ligands showing distance between two binding sites of ~50 Å. Length (x-axis) of MAA in this image is ~69 Å and

diameter (y-axis) is ~53 Å. Image was prepared using DeepView/Swiss-PdbViewer using a pdb file 1DBN.

Fig. S5 Structure of H1N1 hemagglutinin with only one binding site docked with a sialyllactose ligand. Length (x-axis) of H1N1 is ~118 Å and diameter (y-axis) is ~35 Å. Image was prepared using DeepView/Swiss-PdbViewer using a pdb file 3HTT.