Supporting information

Self-sufficient Ultrasensitive Immunosensor Exploiting Supramolecular Construction for Diffusion-free Electrochemical Detection

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Reagents

All chemicals used were of analytical grade and used as received without any further purification. β-cyclodextrin was a generous gift from Roquette (France). Carboxymethyl cellulose sodium salt (CMC, M_W 90 kDa) potassium hexacyanoferrate, 1-ethyl-3-(3-dimethyl-aminopropyl) carbodiimide hydrochloride (EDC), phosphate-buffered saline (PBS) with 0.05% v/v Tween 20 (dry powder), hydroquinone (HQ), adamantane carboxylic acid, gliadin, rabbit anti-gliadin polyclonal-IgG-antibody, anti-rabbit IgG (whole molecule)–peroxidase antibody produced in goat (Ab-HRP), lactate oxidase (EC 1.13.12.4, from *Pediococcus sp*., 20 units/mg solid), choline oxidase (EC 1.1.3.17, from *Arthrobacter globiformis*, 8-20 units/mg solid) and glucose oxidase (EC 1.1.3.4, from *Aspergillus niger*, 200 units/mg) were purchased from Sigma-Aldrich. All solutions were prepared with Milli-Q water (Millipore Inc., Ω = 18 MΩ·cm).

The thiolated β-cyclodextrin polymer (CDPSH. Mw ~ 18 000 mol/L, degree of substitution: 13 mol SH per mol polymer)[1] was prepared as previously described.

Synthesis

Synthesis of Fc-CMC. 0.5 g of aminated CMC[2] (1.5 mmol of aminohexane groups) were dissolved in 25 mL of Milli-Q water. A solution of 0.32 g (1.5 mmol) of ferrocenecarboxaldehyde in 2 mL of DMSO was added dropwise with continuous magnetic stirring. After 3 hours an excess (60 mg, 15 mmol) of sodium borohydride was added and the solution was stirred overnight at room temperature. The mixture was concentrated to about half the initial volume by roto-evaporation and dialysed for 24 hours to remove impurities and was then dried *in vacuum* to give Fc-CMC (Yield: 0.31 g). ^{1}H-NMR (300 MHz, D_2O, 300 K) δ (ppm): 1.9-3.2 (m, Fc-CH_2-N, N(CH_2)$_6$N); 3.2-4.6 (m, overlapped Fc and glucose skeletal protons). 4.9-5.3 (m, anomeric protons). ^{13}C-NMR (75 MHz, D_2O, 300 K); 169 (bs, N*C*=O), 181 (bs, O*C*=O). UV-Vis: λ_{max} 430 nm (ε = 1700 cm·M^{-1}, Fc M→L charge transfer). The amount of Fc units (0.86 mol Fc/mol glucose) was estimated by UV-Vis spectroscopy at 400 nm by interpolation of absorbance values of a polymer solution in a calibration curve prepared using aminoferrocene.

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1 Fragoso, A.; Sanromà, B.; Ortiz, M.; O'Sullivan, C. K. *Soft Matt.* **2009**, *5*, 400
2 Ramirez, H. L.; Cao, R.; Fragoso, A.; Torres-Labandeira, J. J.; Dominguez, A.; Schacht, E. H.; Baños M.; Villalonga, R. *Macromol. Biosci*., **2006**, *6*, 555.

Conjugation of digested gliadin to Fc-CMC. 0.1 g of Fc-CMC were dissolved in 5 mL of 0.1 M acetate buffer at pH 5. To this solution, EDC (0.2 g) was added and the mixture was stirred for 1 hour at 4ºC. Digested gliadin (MW ~ 2 kDa)[3] was conjugated by adding 0.01 g (5 µmol) to the activated polymer and the solution was stirred overnight. The Fc-CMC-GLI polymer was purified using a Microcon® centrifugal filter device (Mw cut-off 10 kDa) and freeze-dried. IR (KBr), ν_{max} (cm^{-1}): 1641, 1529 (s, C=O amide), 1601 (s, COO^-), 1446 (m, C-N). UV-Vis λ_{max} (H_2O): 279 nm, 431 nm. The amount of gliadin in the polymer was estimated by UV spectroscopy at 279 nm using a digested gliadin calibration curve and the measured absorbances were subtracted to an Fc-CMC solution. This analysis gave a gliadin content of 0.09 mol/mol of polymer. The antigenic properties of gliadin bound to the polymer was tested by ELISA as described previously.[3]

Attachment of gliadin and oxidases to Fc-CMC. Fc-CMC (0.1 g) was activated as described above and treated with a mixture containing 5 µmol of digested gliadin and 0.5 µmol of the corresponding oxidase (lactate oxidase, glucose oxidase or chloline oxidase). The Fc-CMC-GLI/Ox polymer was purified using a Microcon® centrifugal filter device (Mw cut-off 100 kDa) and stored at 4ºC. The specific enzymatic activity of the polymers (~6 U/mg for Fc-CMC-GLI/LOx; 20 kU/mg for Fc-CMC-GLI/GOx; 9 U/mg for Fc-CMC-GLI/ChOx) was measured colorimetrically following Sigma protocols.[4]

Surface enhanced Raman spectroscopy (SERS)

SERS were obtained on a Renishaw Raman microscope operating at 785 nm and 10 mW laser power. Klarite® KLA-312 gold coated glass substrates were overnight exposed to a 1 mg/mL solution of CDPSH under nitrogen and the Raman spectra were recorded before and after overnight incubation with 1 mg/mL of Fc-CMC-GLI. The substrate was washed with water before recording the spectra to remove non-immobilized material.

To record a control spectrum of Fc-CMC-GLI (in the absence of CDPSH, a drop of 1 mg/mL of Fc-CMC-GLI was allowed to dry over the Klarite substrate.

Surface plasmon resonance (SPR) studies

SPR studies were carried out using a Biacore® 3000 instrument operating at 25°C. Gold chips from a Biacore SIA kit were cleaned with Piranha's solution (*Warning: Piranha's solution is very corrosive)* for 3 minutes, washing with water, followed by thorough washing with water and finally treated with ozone using a PSD-UVT cleaning instrument (from Novascan, USA) for 9 min, rinsed with ethanol and dried under a filtered Ar stream. The chip was modified with thiolated cyclodextrin polymer (CDPSH) by overnight immersion in a 10 mg/mL solution followed by extensive rinsing with water, after which the chip was mounted in the Biacore support and a 5 µL/min flow of running buffer (10 mM PBS pH 7.4) was established. After baseline stabilization (~ 3 hours) a layer of Fc-CMC-GLI polymer was created by injecting 50

3 Ortiz, M.; Fragoso, A.; O'Sullivan, C. K. *Anal. Chem.* **2011**, *83*, 2931.

4 For LOx assay see: http://www.sigmaaldrich.com/etc/medialib/docs/Sigma/General_Information/2/lactate_oxidase.Par.0001.File.tmp/lactate_oxidase.pdf. For ChOx see: http://www.sigmaaldrich.com/etc/medialib/docs/Sigma/General_Information/choline_oxidase.Par.0001.File.tmp/choline_oxidase.pdf; for GOx see: http://www.sigmaaldrich.com/etc/medialib/docs/Sigma/General_Information/glucose_oxidase.Par.0001.File.tmp/glucose_oxidase.pdf

μL of 1 μg/mL of antigliadin antibody in PBS. Surface regeneration was carried out using 10 mM glycine buffer pH 2.

Electrochemical measurements

Electrochemical measurements were performed on a PC controlled PGSTAT12 Autolab potentiostat (EcoChemie, The Netherlands) with a built-in frequency response analyzer FRA2 module. Electrode arrays were fabricated as previously described,[5] consisting of 16 gold working electrodes (dimensions: 1×1 mm^2) in a 4×4 arrangement. Each working electrode is positioned between a silver pseudo reference 0.2×1 mm^2 and a gold counter electrode of the same size. The working electrodes were electrocleaned by applying a series of 25 potential cycles in 1 M H_2SO_4 in the range 0-1.6 V vs. Ag/AgCl. The quality of the cleaning step was checked using cyclic voltammetry with 1 mM $K_3[Fe(CN)_6]$ in 0.1 M KCl.

Modification of gold electrodes and antibody detection.

The support layer was formed by spotting the electrodes with 5 μL of a 10 mg/mL solution of CDPSH in water and incubated overnight to form a SAM containing cyclodextrin hosts. After rinsing with water, 5 μL of 1 mg/mL of Fc-CMC-GLI or Fc-CMC-GLI/Ox in PBS were spotted onto the functionalized electrodes and allowed to incubate overnight. The next incubation steps were carried out immediately prior to the amperometric measurements. For this purpose, 5 μL of polyclonal anti-gliadin IgG antibody of the appropriate concentration in PBS was incubated for 30 minutes. After rinsing with water, a solution of anti-IgG peroxidase conjugate (IgG-HRP) conjugate was then added and incubated for 10 minutes.

The amperometric measurements were carried out by first recording the background response at 0.2 V in 10 mM PBS + 0.15 M NaCl pH 6, followed by the addition of 10 mM sodium L-lactate, choline or D-glucose.

5 Henry, O. Y. F.; Fragoso, A.; Beni, V.; Laboria, N.; Acero Sánchez, J. L.; Latta, D.; Von Germar, F.; Drese, K.; Katakis, I.; O'Sullivan, C. K. *Electrophoresis* **2009**, *30*, 3398.