Electronic Supporting Information

Cationically rendered biopolymer surfaces for high protein affinity support matrices

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Experimental

Materials

Trimethylsilyl cellulose (TMSC, Avicel) with a DS_{Si} value of 2.8 was purchased from TITK (Rudolstadt, Germany) and used as starting material for cellulose film preparation. Toluene (99.9%), sodium acetate (anhydrous), disodium phosphate heptahydrate ($Na_2HPO_4 \cdot 7\ H_2O$), sodium dihydrogen phosphate monohydrate **(**$NaH_2PO_4 \cdot H_2O$), fluorescein isothiocyanate-bovine serum albumin (FITC-BSA) and tetramethylrhodamine isothiocyanate-bovine serum albumin (TRITC-BSA) were purchased from Sigma-Aldrich Austria and used as received. Trimethylchitosan chlorides (M_w: 90 kDa, medical grade) were purchased from Kitozyme, Belgium, with two different degrees of substitutions (**TMC_L**: Degree of acetylation: 20%, $DS_{NMe3+Cl}$-: 27%; **TMC_H**: Degree of acetylation: 32%, $DS_{NMe3+Cl}$-: 66%). Cyclic olefin polymer slides (COP, Zeonor1060R slides) were gratefully provided from Sony DADC Austria and used as received. QCM-D sensors (QSX303) were purchased from LOT-Oriel (Germany). Milli-Q water from a Millipore water purification system (Millipore, USA) (resistivity = 18.2 Ω^{-1} cm^{-1}) was used for contact angle and QCM-D investigations.

Substrate cleaning and cellulose film preparation

Prior to spin coating, QCM-D Au-sensors were soaked into a mixture of H_2O/H_2O_2 (30 wt. %)/NH_4OH (5:1:1; v/v/v) for 10 min at 70 °C, then immersed in a "piranha" solution containing H_2O_2 (30 wt. %)/H_2SO_4 (1:3; v/v) for 40 s, and then rinsed with MQ-water and finally blow dried with N_2 gas. For spin coating of TMSC onto QCM sensors, 50 µl of TMSC solution (1 wt.%, dissolved in toluene by heating to 60 °C followed by cooling down to room temperature, filtered through 5 µm PTFE filter) was deposited onto the static substrate, rotated for 60 s at a spinning speed of 4000 rpm and an acceleration of 2500 rpm s^{-1}. For converting TMSC into pure cellulose, the sensors were placed in a polystyrene petri-dish (5 cm in diameter) containing 3 ml of 10 wt.% hydrochloric acid (HCl). The dish was covered with its cap and the TMSC films were exposed to the vapors of HCl for 15 min. The

regeneration of cellulose from TMSC coated films was verified by water contact angle, XPS and ATR-IR measurements as reported elsewhere.[1-3] Water contact angles of TMSC and regenerated cellulose films were determined to be 97 ± 1° and 33 ± 1°, respectively. The film thickness of regenerated cellulose was 21.0 ± 0.3 nm.

For the coating of COP slides (25 mm × 75 mm), 2 ml of a TMSC solution (1 wt.%) in toluene/cyclohexanone (1:9; v/v) were dropped on the static substrate and spin coated with the same spinning parameters as above.

Preparation of patterned cellulose films on COP slides and stability test

The TMSC coated COP slides were covered with an aluminum mask (75 mm × 25 mm, thickness: 1 mm) bearing 16 squared holes (50 mm × 50 mm). The slides were placed into a 1000 ml desiccator containing 300 ml of 10 wt.% HCl. The TMSC was regenerated for 15 min and the slides were taken out of the desiccator. The details of the spin coating and regeneration procedure are reported elsewhere.[4] After the regeneration, the slides were immersed into a buffer solution (100 ml) of different pH values (4, 5, 6, 7 and 8). Subsequently, the slides were rinsed with MQ-water for 10 min and blow dried in the stream of nitrogen gas.

Sample preparation for QCM-D measurements

TMC_L and **TMC_H** samples (0.1 wt.%) were dissolved in pure MQ-water. The ionic strength of the sample was adjusted to 150 mM with sodium chloride (NaCl) electrolyte at pH 7. The pH of the solution was adjusted by using either 0.1 M NaOH or 0.1 M HCl. All solutions were stirred overnight at room temperature and filtered through 5 µm PTFE syringe filters.

Quartz crystal microbalance with dissipation (QCM-D)

A QCM-D instrument (model E4) from Q-Sense, Gothenburg, Sweden was used. The instrument simultaneously measures changes in the resonance frequency (Δf) and energy dissipation (ΔD) when the mass of an oscillating piezoelectric crystal changes upon

increase/decrease in the mass of the crystal surface due to the added/deduced mass. Dissipation refers to the frictional losses that lead to damping of the oscillation depending on the viscoelastic properties of the material. For a rigid adsorbed layer that is fully coupled to the oscillation of the crystal, Δf_n is given by the Sauerbrey equation[5] (1)

$$\Delta m = C \frac{\Delta f_n}{n} \qquad (1)$$

where Δf_n is the observed frequency shift, C is the Sauerbrey constant (-17.7 ng Hz^{-1} cm^{-2} for a 5 MHz crystal), n is the overtone number (n = 1, 3, 5, etc.), and Δm is the change in mass of the crystal due to the adsorbed layer. The mass of a soft (*i.e.* viscoelastic) film is not fully coupled to the oscillation and the Sauerbrey relation is not valid since energy is dissipated in the film during the oscillation. The damping (or dissipation) (D) is defined as

$$D = \frac{E_{diss}}{2\pi E_{stor}} \qquad (2)$$

where E_{diss} is the energy dissipated and E_{stor} is the total energy stored in the oscillator during one oscillation cycle.

Adsorption of TMC and protein on unstructured cellulose films using QCM-D

Cellulose coated QCM sensors were mounted in the QCM chambers and were firstly equilibrated to water and then to a 100 mM NaCl solution. After equilibration of the films (ca. 60 minutes), aqueous solutions of TMC (c = 1 mg ml^{-1}) at an ionic strength of 100 mM NaCl was pumped over the sensors at a flow rate of 0.1 ml min^{-1} for 60 min. After rinsing with NaCl (30 min) and water (45 min), Δf and ΔD values were used to determine the adsorbed masses via viscoelastic modelling (Voigt model)[6]. All experiments have been performed in three parallels. In the Voigt model, the adsorbed layer was treated as a viscoelastic layer between the quartz crystal and a semi-infinite Newtonian liquid layer. For calculating the adsorbed mass (Γ_{QCM}), film thickness (h_f),viscosity (η_f) and elastic shear modulus (μ_f) of the

TMC coated layer, the different overtones (n = 3, 5, 7, 9 and 11) were used. All calculations were carried out using the software package QTools 3.0.12 (Q-Sense). The fitting parameters used the modelling are: viscosity, from 1×10^{-4} to 0.1 N·s·m^{-2}; elastic shear modulus, from 1×10^{4} to 1×10^{8} N·m^{-2}; and thickness, from 1×10^{-10} to 1×10^{-6} m. It is worth noting that the values of h_f and ρ_f were not independent variables. In order to calculate the effective thickness and adsorbed mass (equation 3), the density ρ_f values was varied between 1000 and 1400 kg m^{-3}. It turned out that no mass change was occurred by changing the density value and therefore the density (ρ_f) of 1000 kg m^{-3} was used for all calculation (eq. 3).

$$\Gamma_{QCM} = h_f \rho_f \quad (3)$$

Table S1. Viscoeleastic properties of TMC adsorbed on cellulose thin films.

Sample	Γ_{QCM} (mg m^{-2})	h_f (nm)	η_f x 10^{-3} (N s m^{-2})	μ_f x 10^{4} (N m^{-2})
TMC$_L$	24.6 ± 0.7	24.6 ± 0.7	1.3 ± 0.01	5.6 ± 0.3
TMC$_H$	10.0 ± 0.1	10. 1 ± 0.3	1.6 ± 0.02	10.8 ± 1.5

Adsorption of FITC-BSA on cationically rendered unstructured cellulose films by QCM-D

FITC-BSA was dissolved at different concentrations (0.001-1000 µg ml^{-1}) in a 10 mM buffer at pH values of 5 (acetate buffer), 6 (PBS) and 7 (PBS). The ionic strength of the buffer solutions was adjusted to 100 mM NaCl electrolyte. After equilibration of the cationically rendered cellulose films in water and buffer (60 minutes) at the corresponding pH value, the different FITC-BSA solutions were pumped through the QCM-D chambers at a flow rate of 0.1 ml min^{-1} for 30 minutes. After rinsing with corresponding buffer solution for 30 minutes, Δf and ΔD values were used to determine the adsorbed masses via viscoelastic modelling (Voigt model). In this case, the adsorbed mass of F-BSA was calculated by keeping all the

fitting parameters constant except the density (ρ_f = 1100 kg m^{-3}). Langmuir parameters have been calculated according to equation (4). All experiments have been performed in three parallels.

$$\Gamma_{eq} = \Gamma_{max} \frac{K_L C}{1 + K_L C} \quad (4)$$

with

Γ_{eq} – equilibrium surface excess concentration of adsorbate at concentration C

Γ_{max} – maximum surface concentration at infinite bulk concentration

C – bulk concentration of adsorbate

K_L – Langmuir constant

Table S2. Calculated Langmuir adsorption isotherm parameters for FITC-BSA adsorption onto cellulose and N-trimethyl chitosan chloride (TMC) coated cellulose substrates at pH 5 to 7.

	Cellulose		**TMC_L@cellulose**		**TMC_H@cellulose**	
	Γ_{max} [mg m^{-2}]	K_L [L mg^{-1}]	Γ_{max} [mg m^{-2}]	K_L [L mg^{-1}]	Γ_{max} [mg m^{-2}]	K_L [L mg^{-1}]
pH 5	20.9 ± 0.1	0.9 ± 0.1	41.4 ± 0.1	3.1 ± 0.6	62.4 ± 4.8	0.83 ± 0.02
pH 6	1.3 ± 0.8	0.24 ± 0.02	1.8± 0.7	0.22 ± 0.04	2.3 ± 0.7	0.31 ± 0.04
pH 7	0.3 ± 0.01	0.15 ± 0.03	1.4 ± 0.1	0.13 ± 0.09	1.8 ± 0.1	0.22 ± 0.01

The QCM-data for FITC-BSA adsorption on uncoated cellulose is depicted in Figure S1.

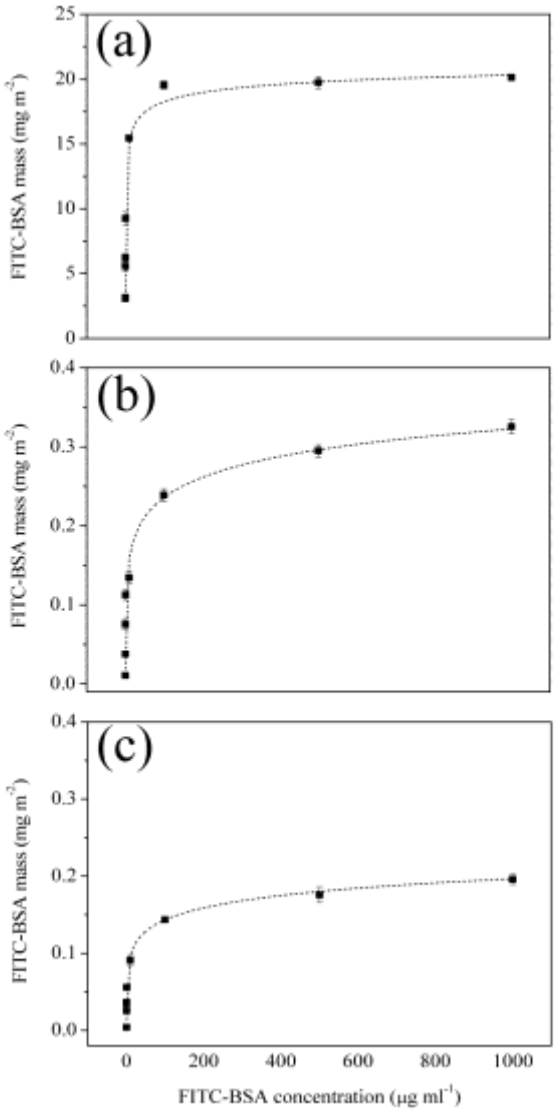

Figure S1. Comparison of FITC-BSA adsorption at different concentrations onto pure cellulose surfaces at pH values of 5 (a), 6 (b), and 7 (c).

Atomic force microscopy of the cationically rendered surfaces

Surface morphology of cellulose films rendered with TMC were characterized by AFM in the intermittent contact mode using an Agilent 5500 AFM multimode scanning probe microscope (Digital Instruments, Santa Barbara, CA). Silicon cantilevers (ATEC-NC-20, Nanosensors, Germany) with a resonance frequency of 210-490 kHz and a force constant of 12-110 N m^{-1} were used. The scanned image size was 5 × 5 μm^2. All measurements were performed at room temperature in ambient atmosphere. The images are depicted in Figure S2.

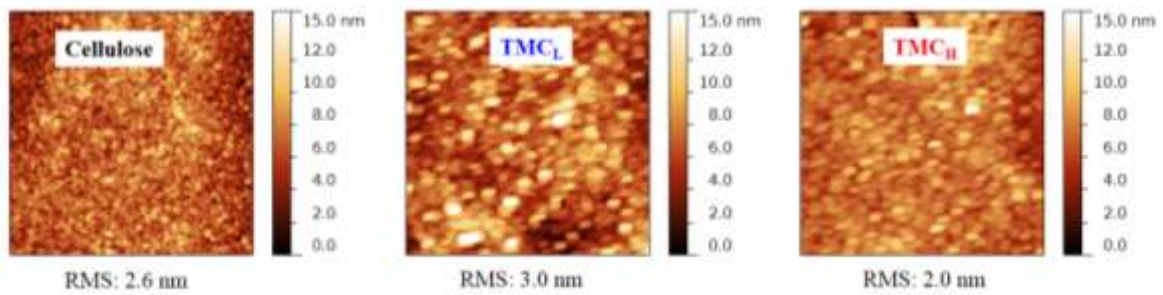

Figure S2. AFM images of neat cellulose surfaces and those rendered with **TMC$_L$** and **TMC$_H$**.

Adsorption of TMC and TRITC-BSA on structured cellulose films

The immobilization of TMC and TRITC-BSA on structured cellulose surfaces was carried out using a similar protocol as it was done for QCM-D measurements but under static conditions. **TMC$_L$** and **TMC$_H$** (30 µl, c = 1 mg ml^{-1} in 150 mM NaCl, pH 7) were dropped onto the structured cellulose pads (eight per column with **TMC$_L$** and eight in the other column with **TMC$_H$**) deposited on COP slides and incubated for 60 minutes. Afterwards, the solutions on the pads were exchanged by electrolyte solution (30 µl, 150 mM NaCl, pH 7) and incubated for another 30 minutes. Then, each pad was incubated with pure water (30 µl) for 45 minutes. For loading experiments, solutions of TRITC-BSA with a concentration of 1000, 500, 100, 10, 1, 0.1, 0.01, and 0.001 µg ml^{-1} at pH values of 5, 6 and 7 (100 mM NaCl) have been prepared. Accordingly, 30 µl of each of these TRITC-BSA solutions were dropped on the **TMC$_L$** or **TMC$_H$** coated cellulose pads and incubated for 30 minutes. Then, the TRITC-BSA solution was exchanged against 30 µl washing buffer at the corresponding pH value for 30 minutes followed by a final rinsing step using MQ water (30 minutes). Finally, the slides were blow dried in a stream of nitrogen. All the experiments were performed at room temperature and under the exclusion of light.

Microarray Scanning

Microarray fluorescence scanning was performed on a microarray scanner DNAscope LM +from GeneFocus, USA with green excitation (523 nm) and red emission. The images (Figure S3) were colored red by the software.

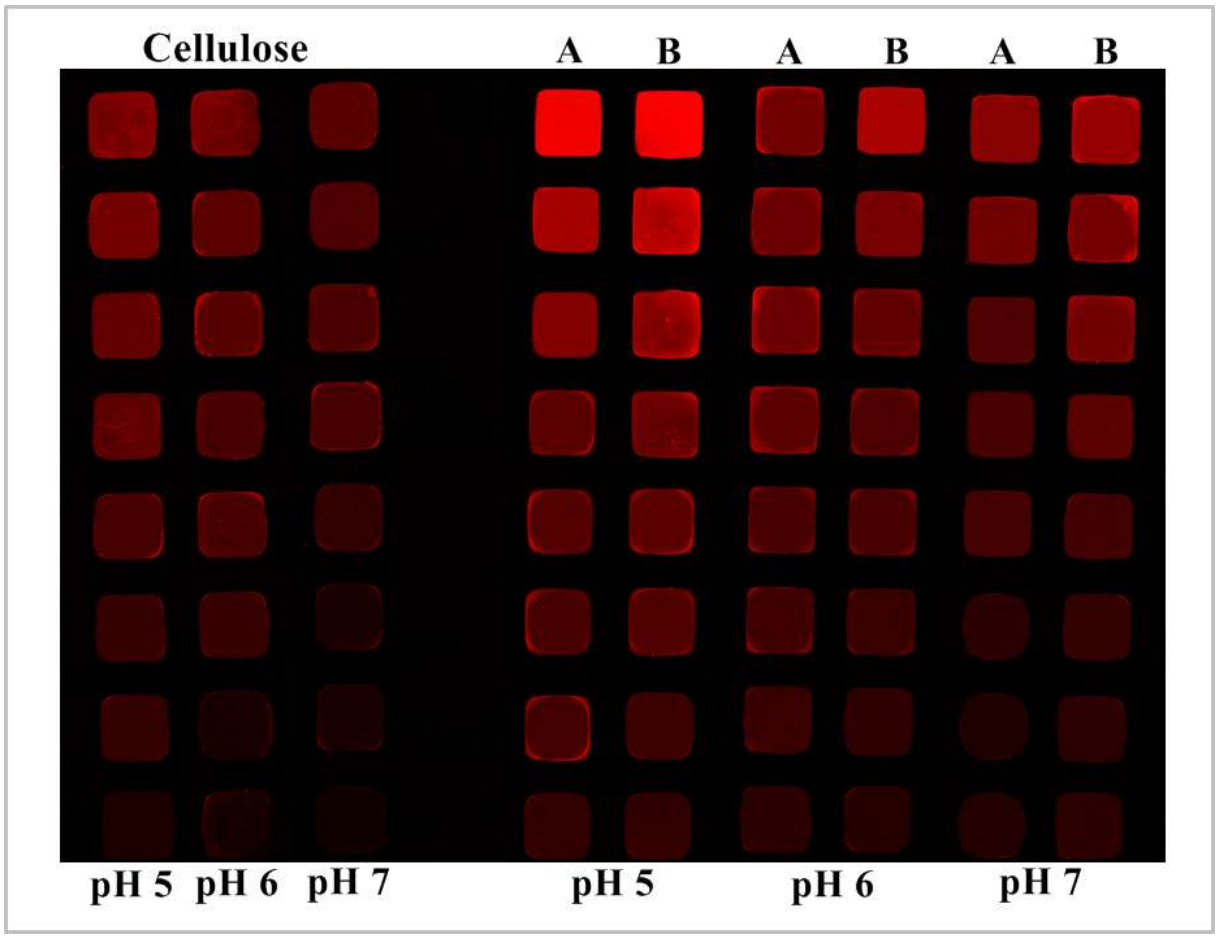

Figure S3. Sensor slides for TRITC-BSA detection at different pH values. Left: neat cellulose films after incubation and rinsing with TRITC-BSA. Right: Cellulose films that have been treated with **TMC_L** (A) and **TMC_H** (B) before TRITC-BSA incubation and rinsing.

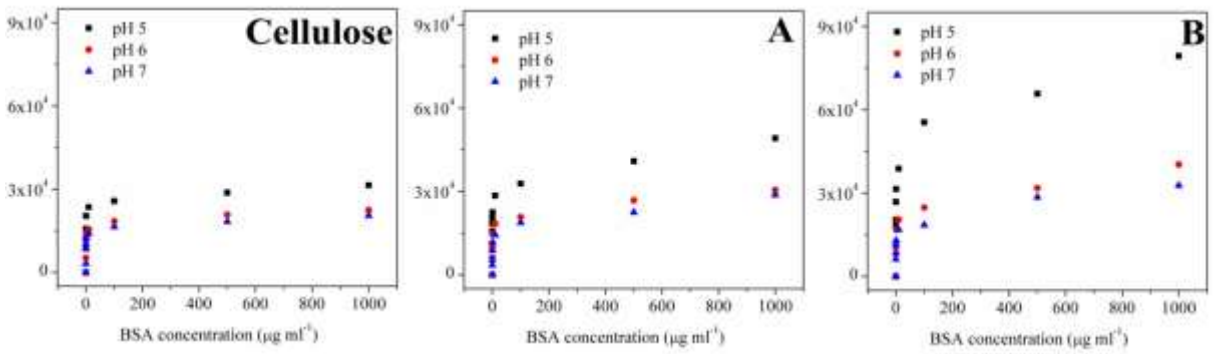

Figure S4. Fluorescent intensities of the sensor slides depicted in Figure S3.

References

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