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Supplementary Information

Polyelectrolyte multilayer coatings that resist protein adsorption at rest and under stretching

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Materials and Methods

Materials

Milli-Q water was used for all the experiments. Poly(ethylene imine) (PEI, Sigma, MW 750,000), poly(allylamine hydrochloride) (PAH, Aldrich, MW 70,000), poly(sodium 4-styrene sulfonate) (PSS, Aldrich, MW 70,000), poly(acrylic acid) (PAA, Aldrich, 35 wt.% in water, MW 100,000) were used as received. Phosphorylcholine modified PAA (PAA-PC, Scheme 1 main text) was synthesized as described in [A. Reisch, J.C. Voegel, G. Decher, P. Schaaf, P.J. Mésini *Synthesis of polyelectrolytes bearing phosphorylcholine moieties*, Macromol. Rapid. Comm. **28**, 2217-2223 (2007)]. Fetal bovine serum (FBS, Gibco), bovine serum albumin (Sigma, ≥99%), chicken egg white lysozyme (Sigma, ref. L6876) and human plasma fibrinogen (Sigma, ref. F4883) were used in the adsorption tests. Albumin-fluorescein isothiocyanate conjugate (Alb^{FITC}, derived from bovine albumin) used for adsorption tests with fluorescence microscopy was purchased from Sigma.

The silicone sheets (254µm thick, Speciality Manufacturing Inc. SMI, Saginaw, Michigan, USA), measuring 18 x 18 mm, were cleaned with ethanol and extensively rinsed with water prior to use.

QCM measurements

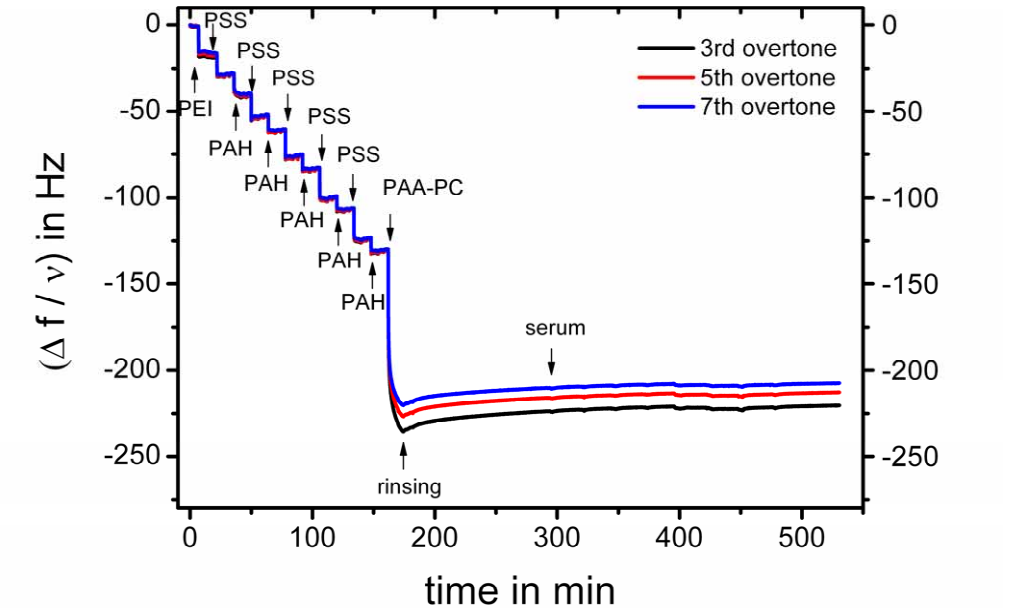
Quartz crystal microbalance (QCM) measurements were performed on a QCM-D D300 device (Q-sense, Sweden), using gold coated crystals at room temperature. The fundamental resonance frequency was of the order of 5 MHz. The change in frequency of the third, fifth and seventh harmonic were used for analysis. The adsorbed masses of polyelectrolytes, proteins and serum per surface area were calculated using the Sauerbrey equation for the third, fifth and seventh harmonic divided by the number of the harmonic and taking the average of these three values.

One can point out that the experiments performed by QCM are given to show that our surfaces indeed exhibit an anti-fouling character and to do so the treatment of the QCM data by using the Sauerbrey relation is entirely sufficient. Nevertheless, before using this relation we verified that the relative frequency changes $\Delta f_\nu / \nu$ where fairly independent of ν, ν being the overtone number. This is the case with 15% deviation for the worst situation (see Figure

S1). This thus shows that Sauerbrey's relation can be used and that the determined amounts are given within 15% or better.

All the experiments were carried out at least two times, adsorption of the polyelectrolytes was measured at least four times. For the latter ones the standard deviation is given as error bars (see Table 1). Protein and serum adsorption were measured at least two times for each value given. In Tables 2 and 3 we report the mean value and the error bar corresponds to the difference between the measured values and the mean value. The detection limit of our QCM device is of the order of 5 ng cm^{-2}. This limit is given as the error bar for the experiences where the measured values are close to zero and the difference to the mean value is $\leq$ 5 ng cm^{-2}.

Figure S1: Construction of a PEI(PSS/PAH)$_5$PAA-PC multilayer (PAA-PC with a grafting degree of 25%) and serum adsorption on this multilayer followed by QCM. The third, fifth and seventh harmonic are shown.



FTIR experiments

Fourier transform infrared (FTIR) spectra were recorded on a Bruker Vertex 70 spectrometer. Construction of multilayers was realized using a compartment ATR (attenuated total reflectance) cell (GRASEBY-SPECAC, U.K.) equipped with a top plate (110 µL cell volume) and fitted with a 45° trapezoidal ZnSe crystal (internal reflection element, 6 reflections,

dimensions of 72x10x6 mm) and a liquid-nitrogen-cooled MCT detector. All experiments were conducted using tris buffer solutions prepared with D_2O.

Fluorescence Microscopy

Images were obtained on an inverted light microscope (Nikon Microphot-FXA, Japan) equipped with a mercury lamp and operating between 470 and 490 nm for excitation and above 520 nm for detection. A 40x dry objective and a digital camera were used. Image analysis was performed using ImageJ (U.S. National Institutes of Health, Bethesda, Maryland, USA, http://rsb.info.nih.gov/ij/).

The grey scale (0 – 255) was used for quantification of intensities. Background values were determined with the same imaging conditions after removal through scratching of the multilayers previously in contact with an Alb^{FITC} solution. Several factors may contribute to the background signal: i) the filters are not 100% effective, ii) rest light may be captured by the camera, iii) fluorescence of dust particles in air, iv) weak fluorescence of the silicone sheets.

Mechanical stretching of functionalized films

A homemade stretching device allowed the elongation of the silicone substrates directly under the microscope. The stretching motion is achieved by a precision electric motor at a velocity of 0.24 mm s^{-1}. All the stretching experiments were performed at ambient temperature with the modified side of the film being in contact with buffer solution. The film was left to stabilize at each stretching step for 4 min prior to adsorption experiments.

The mechanical resistance of the multilayers was monitored by fluorescence microscopy of PAH/PSS $(PAH^{FITC}/PAA\text{-}PC)_2$ modified silicone sheets under stretching. No formation of cracks was observed for stretching up to $l/l_0 = 1.6$ (Figure S5).

Multilayer assembly

In the QCM experiments, adsorption of proteins and serum was evaluated on a $PEI(PSS/PAH)_5$ multilayer on which the polyanion to be tested was adsorbed. Furthermore $PEI(PSS/PAH)_5$-PAA-PC/PAH/PAA-PC multilayers (PAA-PC: DS 25%) were constructed using the QCM device and the adsorption of proteins and serum thereon was measured. The adsorption of $PAH(PSS/PAH)_3$PAA-PC/PAH/PAA-PC multilayers was followed by IR spectroscopy. PAH/PSS$(PAH/PAA\text{-}PC)_n$ multilayers were constructed on the silicone sheets prior to $albumin^{FITC}$ adsorption.

Filtered solutions of 1 mg/mL of PEI, PSS, PAH and of 0.5 mg/mL of PAA or modified PAA in tris buffer (20 mM, NaCl 150 mM) at pH 7.4 were used for the construction of the multilayers. For the IR experiments D_2O was used for the preparation of the buffer solution. The multilayers were assembled by bringing the substrate (QCM crystal, ZnSe crystal or silicone sheet) in contact with the polyelectrolyte solutions for 6 min in the case of PEI, PSS and PAH and for 12 min in the case of PAA and modified PAA followed by rinsing twice with the buffer solution during 4 min each. After deposition of the last layer and rinsing, the multilayer was left to stabilize in contact with the buffer solution for at least 30 min prior to further adsorption experiments.

Adsorption of serum

Serum adsorption, that is the adsorption from solutions containing serum, was determined by QCM-D experiments: Solutions of fetal bovine serum (FBS) were obtained by dilution of pure FBS with tris buffer (20 mM, NaCl 150 mM) at pH 7.4, followed by filtration, yielding concentrations of 1 to 20 volume% of FBS, corresponding to protein concentrations of 0.44 to 8.78 mg/mL. FBS solutions were used within 3 hours after preparation. The adsorption of serum was studied by successive injections of six FBS solutions, going from low to high concentration, without intermediate rinsing, into the QCM cell after construction of the multilayer in situ. Each solution was left in contact with the multilayer for 26 min.

Adsorption of proteins

Protein adsorption on PAA and PAA-PC was tested by QCM-D. Solutions of 20 mg / 100 mL of albumin, lysozyme and fibrinogen in tris buffer (20 mM, NaCl 150 mM) at pH 7.4 were used. Albumin and lysozyme solutions were used as obtained, the fibrinogen solutions were centrifuged and only the supernatant was used within 15 min after preparation. Protein concentration was controlled by UV-Vis spectroscopy at 280 nm.

Adsorption of the different proteins on PAA was tested by bringing the protein solutions for 1 h in contact with the multilayer followed by rinsing and leaving in contact with the buffer solution for 1 h. In the case of PAA-PC (DS 25%) the same multilayer was brought in contact successively with albumin, lysozyme and fibrinogen solutions with intermediate rinsing.

Albumin adsorption on the multilayer coated silicone sheets was tested by bringing them in contact with a 20 mg / 100 mL solution of Alb^{FITC} in the tris buffer during 10 min. (After stretching the multilayer was left for 4 min before beginning the Alb^{FITC} adsorption.) The surfaces were rinsed twice during 4 min before taking fluorescence micrograph images. The

amount of irreversible adsorbed Alb^{FITC} was determined by measuring the grey values for fixed imaging conditions. Each experiment was performed at least twice. The standard deviations of the values measured at different regions of the sample are given as error bars in Table 4. Images of Alb^{FITC} on PAH were recorded at the same time in order to compensate for intensity changes of the mercury lamp.

For the adsorption experiments under stretching, adsorption of protein was performed first at rest and then repeated at every given degree of stretching.

Zeta potential measurements

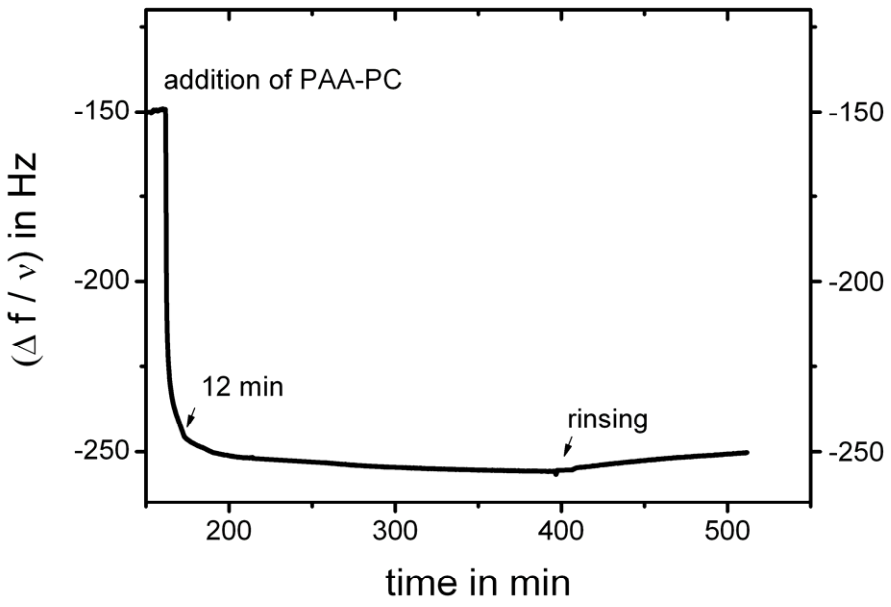
For the determination of the zeta potential colloidal particles (polystyrene latexes, diameter 1 μm) were coated with a $PEI(PSS/PAH)_2$ multilayer capped with the polyanion under consideration. Multilayer assembly was performed by bringing a diluted dispersion of particles in contact with the polyelectrolyte solution followed by centrifugation. The particles were redispersed in buffer solution and the buffer removed by centrifugation twice. After redispersion the next layer was applied. A low level of aggregate formation was controlled by optical microscopy.

The zeta potential of the particles was then determined with a Zetasizer 3000 HS in buffer solution.

Adsorption of PAA-PC

Adsorption of a single layer

Figure S2: Long time adsorption of PAA-PC with a grafting degree of 25% on a $(PSS/PAH)_5$ multilayer followed by quartz crystal microbalance (5 MHz, the first harmonic is shown). The point at which rinsing is started in the other experiments is marked (12 min).



Adsorption of PAA-PC/PAH/PAA-PC

Figure S3 : Adsorption of PAA-PC/PAH/PAA-PC on top of a $(PSS/PAH)_5$ multilayer measured by quartz crystal microbalance (5 MHz, the first harmonic is shown). PAA-PC with a grafting degree of 25% is used.

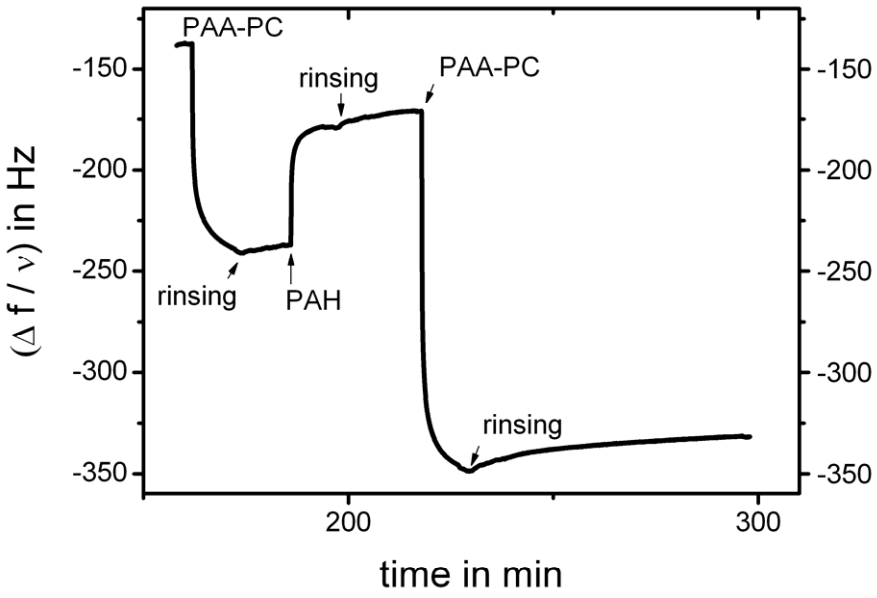
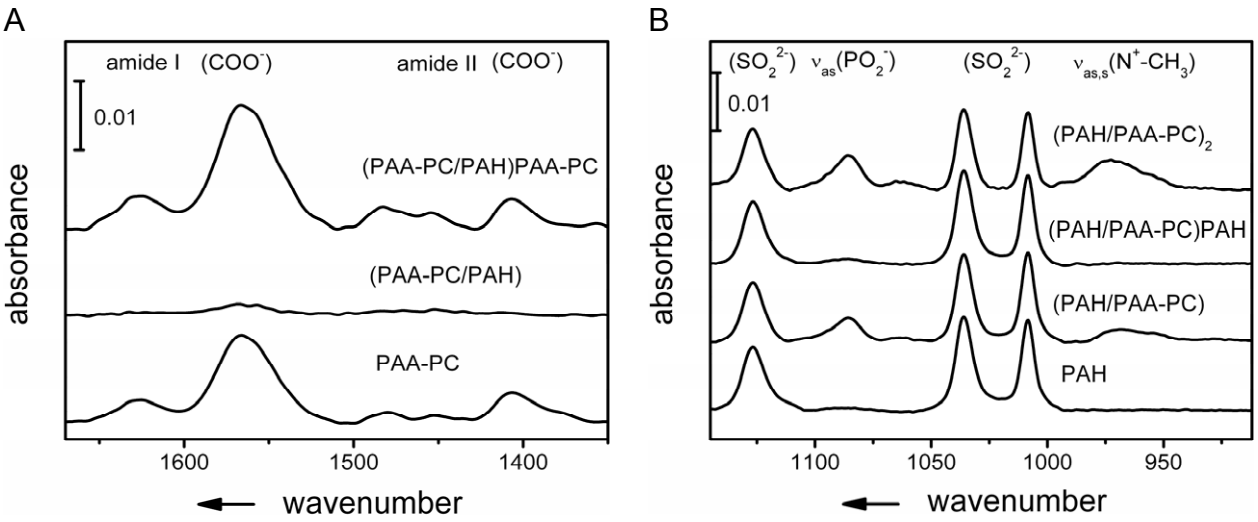


Figure S4 : Adsorption of PAA-PC/PAH/PAA-PC on top of a $(PSS/PAH)_3$ multilayer followed by infrared spectroscopy. PAA-PC with a grafting degree of 25% is used. A) Spectra after the adsorption of PAA-PC, (PAA-PC/PAH) and (PAA-PC/PAH)PAA-PC after the substraction of the spectrum of the underlying $(PSS/PAH)_3$ multilayer. B) Spectra of the entire multilayer after adsorption of PAH, (PAH/PAA-PC), (PAH/PAA-PC)PAH and $(PAH/PAA\text{-}PC)_2$ on top of $(PSS/PAH)_2PSS$.



Stability of PAH/PSS (PAH/PAA-PC)$_2$ multilayers under stretching

Figure S5: Fluorescence images of PAH/PSS (PAH^{FITC}/PAA-PC)$_2$ multilayers at rest (1) and at an elongation of 1.6. The white bar corresponds to 20µm.