Supporting Information I: Experimental section

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Supporting Information I: Experimental section

**Materials.** All aqueous solutions were prepared using ultrapure water (resistivity close to 18.2 MΩ.cm) and stored at 3°C.

Poly(L-lysine) bromide (PLL, 60 kDa), poly(diallyldiamine ammonium) chloride (PDADMA, 200-350 kDa) and sodium poly(styrene sulfonate) (PSS, 60 kDa) were supplied by Sigma-Aldrich (Saint Quentin Fallavier, France). Sodium hyaluronate (HA, 400 kDa) was supplied by Lifecore Biomedical (Chaska, USA). Alkaline Phopshatase (ALP, type XXIV from human placenta) was purchased from Sigma-Aldrich (Saint-Quentin Fallavier, France) and fluorescein diphosphate (FDP) from Invitrogen (Cergy Pontoise, France). Rhodamine Red®-X succinimidyl ester (Rho), which was used for the labeling of ALP, was purchased from Invitrogen (Cergy Pontoise, France).

1 mg/mL polyelectrolyte solutions were prepared by dissolution of the respective adequate polymer amounts in a 0.15 M NaCl aqueous solution buffered at pH 7.4 with 0.01 M tris(hydroxymethyl)aminomethane (Tris).

The labeling of ALP with Rho was performed as follow: ALP was dissolved at 5 mg/mL in a sodium bicarbonate solution (0.1 M, pH = 8.5) whereas Rho was extemporaneously dissolved in dimethylsulphoxide at 10 mg/mL. The two solutions were mixed, to have a ratio of 10 moles of Rho per mol of ALP, and gently agitated at 3°C during 1 hour. Then, ALPRho was purified by several dialyses with the bicarbonate buffer (Cut Off 10000 Da, Spectra Por 7 Dialysis Membrane, Spectrum Europe B.V., Breda, Netherlands), until the presence of Rho was no more detectable by UV-visible spectroscopy. The two final dialyses were performed with the previously described NaCl/Tris buffer solution.

ALPRho and FDP solutions were prepared at 0.5 mg/mL and 0.1 mg/mL respectively, with the same buffer solution as used to dissolve polyelectrolytes.

**Step-by-step build-up of films.** 18×18 mm² polydimethylsiloxane sheets (Specialty Manufacturing
Inc., Saginaw, Michigan, U.S.A.) with a thickness of 250 µm, were used as substrates. They were cleaned with absolute ethanol and ultra-pure water. PLL/HA films were constructed with an automatic dipping machine (Dipping Robot DR3, Riegler & Kirstein GmbH, Potsdam, Germany) by alternate dipping of the PDMS sheets in a PLL solution (5 min), two rinsing solutions (2 × 5 min), a HA solution (5 min), two rinsing solutions (2 × 5 min), and so on. PDADMA/PSS films were constructed with a micropipette by covering alternatively the substrates with a PDADMA solution (1 min), a rinsing solution (1 min), a PSS solution (1 min), again a rinsing solution (1 min) and so on.

After build up of (PLL/HA)30/PLL films, a 0.1 mg/mL FDP solution was brought in contact with it during 30 min, and the obtained system was rinsed three times with buffer solution (3 × 10 min). Then, the coated PDMS was fastened on a lab-made stretching device (see the “stretching device” part below). Finally, a (PDADMA/PSS)10 system was built in situ on the previous one and ALP Rho was deposited on top of it by putting a 0.5 mg/mL ALP Rho solution in contact with the film during 30 min and by rinsing three times with buffer solution (3 × 10 min). The final system can be described with the following formula: (PLL/HA)30/PLL/FDP/(PDADMA/PSS)10/ALP. It was maintained always hydrated – even in the stretched state – in contact with buffer solution.

**Stretching device.** The stretching device enabled elongation of the silicone substrates directly under the confocal microscope. The stretching degree is defined by the parameter $\alpha = (l - l_0) / l_0$, where $l_0$ and $l$ correspond respectively to the initial and to the stretched length of the silicone sheet. The stretching motion is achieved by a precision electric motor at a velocity of 0.74 mm/s. In all experiments the typical stretching time from one stretching degree to the next was of the order of 15 s. The film was then kept in a fixed stretched state for more than 1500 s. The time steps between two consecutive fluorescence measurements was 60 s. Thus stretching took place over a much shorter time than those over which the increase of fluorescence intensity was measured. No deconvolution between time and stretching rate has thus to be performed. This should be done if much slower stretching rate would be used.
All of the stretching experiments were carried out at ambient temperature in liquid conditions.

**Confocal laser scanning microscopy (CLSM).** Films were images with a Zeiss LSM 510 confocal microscope equipped with an Argon laser (488 nm) and a HeNe laser (543 nm). A 40× objective lens with a numerical aperture of 0.3 (Zeiss Achromplan) was used. Fluorescein and fluorescein monophosphate (FMP) (enzymatic dephosphorylation of FDP by ALP yields to FMP and fluorescein) fluorescence was detected after excitation at $\lambda = 488$ nm with a cut-off dichroic mirror of 488 nm and an emission band-pass filter of 505-530 nm (green emission). Rhodamine fluorescence was detected after excitation at $\lambda = 543$ nm, with a dichroic mirror of 543 nm and an emission band-pass filter of 505-530 nm (red emission). A z-section of the films and their supernatant can be obtained after deconvolution and reconstruction of (x, y) stacked images. This allow to localize fluorescent molecules in depth, and to measure distances in the z-direction, e.g. to determine the film thickness. Moreover, catalytic reactions were followed in situ by measuring supernatant intensities with time. Intensities were deduced from image analyses of CLSM images (x,z section images of 77×49 µm$^2$). Error bars corresponding to standard deviations were determined by analyzing intensities on 10 areas of 10×5 µm$^2$ selected in each section image.

**Quartz Crystal Microbalance.** The build-up process of multilayer films was monitored in situ by quartz crystal microbalance-dissipation using the Q-Sense 401 flow module (QCM-D, E1, Q-Sense, Västra Frölunda, Sweden). The QCM technique consists in measuring the resonance frequency $f$ of a quartz crystal induced by molecule adsorption on the crystal, in comparison with the crystal in contact with buffer solution. The crystal used here is a QSX 303 (Q-Sense), it is coated with a 100 nm thick SiO$_2$ film. The quartz crystal is excited at its fundamental frequency, 5 MHz, and its harmonic frequencies, 15, 25, 35, 45, 55 and 65 MHz. Changes in the resonance frequency, $\Delta f$ during each adsorption step are measured for each excitation frequency. A shift in $\Delta f$ can be associated, in first approximation, with a variation of the mass adsorbed on the crystal. To characterize the film at a given step, only the frequency at the end of the rinsing steps following the exposure to either polycation or
polyanion were taken into consideration.

**Fluorimetry.** The presence of fluorescein and FMP in solutions was monitored by fluorescence intensity measurements with a Bio-Rad Versafluor™ fluorometer (Bio-Rad, Marnes-la-Coquette, France). It is equipped with a UV lamp and wavelength filters: excitation at 490 nm (10 nm band width) and emission at 520 nm (10 nm band width).

**Atomic force microscopy.** Atomic force imaging was performed using a Multimode scanning probe microscope (Veeco, Santa Barbara, CA). The apparatus operated in the contact mode in dry condition. Cantilevers with a spring constant of 0.03 N.m\(^{-1}\) ending with a silicon tip were used (MSCT model, Veeco, Santa Barbara, CA). Surfaces of PDMS sheets coated with films were imaged after the stretching procedure, if necessary, and after drying by an air flow.

**Zeta potential measurements.** The streaming potentials of films deposited on PDMS sheets were measured with a ZetaCAD device (CAD Instrumentation, Les Essarts le Roi, France). Two PDMS sheets were mounted parallel to each other in the plexiglass sample holder and were separated by a 500-μm-thick poly(tetrafluorethylene) (PTFE) spacer. The PEM film was deposited on the substrates ex situ using the usual deposition times. The streaming potential was measured five times on the same sample and the obtained values were averaged. All of these experiments were performed under the same conditions as the other experiments, i.e. in a 0.15 M NaCl aqueous solution buffered at pH 7.4 with 0.01 M TRIS. The ζ potential was calculated from the measured streaming potential ΔE/ΔP which is namely the slope of the potential difference versus pressure difference curve. The potential difference ΔE was measured between two Ag/AgCl reference electrodes located on both sides of the measurement cell. The pressure difference ΔP between the two electrolyte compartments was varied with compressed air in increments of 5 kPa between −30 and +30 kPa.
Supporting Information II

Supplementary figure S1.

Figure S1. Confocal microscope images of a (x,z) section of a silicone sheet coated with a (PLL/HA)$_{30}$/PLL/FDP film and covered with a (PDADMA/PSS)$_{10}$ barrier. Green (a) and red (a’) channels of the film without enzyme and green (b) and red (b’) channels with enzymes ALP$^{\text{Rho}}$ (red labeling) adsorbed on top of (PDADMA/PSS)$_{10}$. This (PDADMA/PSS)$_{10}$ acts as a barrier and prevents diffusion of ALP$^{\text{Rho}}$ inside the underlying PLL/HA reservoir. No evolution of green fluorescence intensity was monitored with time. The dashed lines indicate the interface between the silicone sheet and the polyelectrolyte multilayer film. All images are acquired with the same parameters (detector gain settings, laser power and optical parameters). The white scale bars correspond to 8 µm in all images.
Supplementary figure S2.

Figure S2. Quartz crystal microbalance measurements monitoring the build-up of PDADMA/PSS multilayer films and the adsorption of alkaline phosphatase (ALP) on PSS (a) or on PDADMA (b) terminating film. The surface mass densities of ALP deposited on the PSS or on the PDADMA terminating films, estimated by the Sauerbrey relation, correspond to 450 and 2130 ng.cm\(^{-2}\) respectively.
Supplementary figure S3.

Figure S3. Green (a and a') and red (b and b') channels of confocal microscope section (x,z) images of a silicone sheet coated with a reservoir containing enzyme substrates [(PLL/HA)30/PLL/FDP] and covered by a barrier with enzymes adsorbed on top [PDADMA/PSS)10/PDADMA/ALP\textsuperscript{Rho}]. If ALP\textsuperscript{Rho} is deposited on a similar film but with a PDADMA terminating layer instead of PSS, the whole film section is entirely labeled in red, indicating that this PDADMA ending barrier is not permeable towards ALP\textsuperscript{Rho}. Images a and b were acquired at the ending of the build-up and images a' and b' 10 minutes later. The dashed lines indicate interface between the silicone sheet and the film. All images are obtained with the same microscope adjustments. The yellow dotted line corresponds to the upper surface of the silicone sheet. The white line is an 8 µm-scale.
Figure S4. Evolution with time of mean fluorescence intensity of buffer solution in contact with a reservoir containing the enzymatic substrate and without enzymes on the top [(PLL/HA)_{30}/PLL/FDP/(PDADMA/PSS)_{10} film]. Intensities were deduced from image analyses of CLSM images. Several stretching rates $\alpha$ were applied on the film: $\alpha = 0\%$ (black discs), $\alpha = 100\%$ (red discs). The film was stretched and maintained at stretching degree of 100% from $t = 1600$ s to 3100 s. The slope in both cases is $\approx 0.13$ a.u./s. For sake of clarity, scaling for y axis is similar to Figure 3.
Supplementary figure S5.

Figure S5. Evolution with time of mean fluorescence intensity of buffer solution in contact with a reservoir containing the enzymatic substrate and capped with a barrier layer having enzymes on the top [(PLL/HA)$_{30}$/PLL/FDP/(PDADMA/PSS)$_{10}$/ALP film]. Intensities were deduced from image analyses of CLSM images. Several stretching rates $\alpha$ were applied on the film: first at rest ($\alpha = 0\%$, black discs), then a stretching is applied above the critical stretching degree ($\alpha = 100\%$, red discs), and finally the film is brought back at rest. The error bars correspond to standard deviations calculated from image analyses.
Supplementary figure S6.

**Figure S6.** AFM images of a reservoir functionalized with FDP and capped with a barrier layer having enzymes on the top [(PLL/HA)$_{30}$/PLL/FDP/(PDADMA/PSS)$_{10}$/ALP film]. The film is observed a) in the non-stretched state ($\alpha = 0\%$) and b) for a stretching rate of $\alpha = 100\%$. Images size are 2.5 $\mu$m $\times$ 2.5 $\mu$m and Z scale is 150 nm. The mean roughness was 11 nm for both images.
Supplementary figure S7.

**Figure S7.** Evolution with time of mean fluorescence intensity of buffer solution in contact with a reservoir containing the enzymatic substrate and capped with a barrier layer having enzymes on the top. Two films composed of a thin (10 bilayers) or a thick (20 bilayers) barrier are tested: 

- (PLL/HA)$_{30}$/PLL/FDP/(PDADMA/PSS)$_{10}$/ALP (blue)
- (PLL/HA)$_{30}$/PLL/FDP/(PDADMA/PSS)$_{20}$/ALP (pink)

These films are in the non-stretched state $\alpha = 0 \%$ (discs) and then at $\alpha = 140 \%$ (circles). Intensities were deduced from image analyses of CLSM images.
References
