Film Self-Assembly of Oppositely Charged Macromolecules Triggered by Electrochemistry through a Morphogenic Approach

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Supporting Information

ABSTRACT: The development of new surface functionalization methods that are easy to use, versatile, and allow local deposition represents a real scientific challenge. Overcoming this challenge, we present here a one-pot process that consists in self-assembling, by electrochemistry on an electrode, films made of oppositely charged macromolecules. This method relies on a charge-shifting polyanion, dimethylmaleic-modified poly(allylamine) (PAHd), that undergoes hydrolysis at acidic pH, leading to an overall switching of its charge. When a mixture of the two polyanions, PAHd and poly(styrenesulfonate) (PSS), is placed in contact with an electrode, where the pH is decreased locally by electrochemistry, the transformation of PAHd into a polycation (PAH) leads to the continuous self-assembly of a nanometric PAH/PSS film by electrostatic interactions. The pH decrease is obtained by the electrochemical oxidation of hydroquinone, which produces protons locally over nanometric distances. Using a negatively charged enzyme, alkaline phosphatase (AP), instead of PSS, this one-pot process allows the creation of enzymatically active films. Under mild conditions, self-assembled PAH/AP films have an enzymatic activity which is adjustable simply by controlling the self-assembly time. The selective functionalization of microelectrode arrays by PAH/AP was achieved, opening the route toward miniaturized biosensors.

INTRODUCTION

Functional materials are predicted to have an enormous impact on many aspects of society, among them next-generation health care and energy-related technologies. Bottom-up approaches are considered to be the most appropriate routes for their synthesis.^{1,2} For almost 40 years, the scientific community has explored self-assembling systems, and numerous complex and hierarchical structures have been described.³ However, almost all of these structures obtained by mixing molecules were realized in solution or in a step-by-step manner on a surface. The layer-by-layer (LbL) assembly of polyanions and polycations, leading to so-called polyelectrolyte multilayer films, is certainly the strategy that has attracted the most attention.^{4,5} It has opened new avenues in fields such as surface treatment,^{6–8} biomaterials,^{9–11} energy storage,^{12,13} and optical properties.^{6,14,15} Enzyme-based LbL's are especially interesting in reactor sensing,¹⁶ glucose monitoring,¹⁷ drug delivery,¹⁸ and optical and electrochemical biosensors.¹⁹ On the basis of the

stepwise formation of polyanion/polycation complexes held together by electrostatic interactions, it allows the formation of organic films whose thicknesses range from nanometers to micrometers. However, even if LbL buildup is a powerful functionalization tool, it is a tedious one often requiring numerous steps. It was reported that one can also build up, in a step-by-step manner, films from a single solution containing polycation/polyanion complexes, PEDOT/PSS, respectively [PEDOT is poly(3,4-ethylenedioxythiophene); PSS is poly(styrenesulfonate)].²⁰ This film was obtained by a step-by-step adsorption of PEDOT/PSS realized by spin coating and followed by a drying step after each deposition. Recently, a polyelectrolyte complex made of PSS and poly(diallyldimethylammonium) (PDADMAC) in KBr solution

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Figure 1. (a) Principle of the charge-shifting polyelectrolytes: PAHd (negatively charged) hydrolyzes under acidic conditions into PAH (positively charged). (b) Schematic representation of the one-pot self-assembly of the PAH/PSS film triggered by the application of a constant current leading to a pH decrease, transforming PAHd into PAH that complexes with PSS present in the solution near the electrode. The experiments were performed in the presence of 60 mM hydroquinone in a 150 mM $NaNO_3$ aqueous solution adjusted to pH 7.7.

was spin coated to form thin and homogeneous films that can be released as free-standing membranes.²¹ Using a one-pot method triggered by an external stimulus seems to be the best way to induce film buildup. The electrochemically triggered formation of films can be divided into two categories: one encompassing the methods based on weak interactions such as precipitation^{22,23} or the continuous adsorption^{24–26} of polyelectrolytes and self-assemblies of physical gels²⁷ or proteins^{28–30} and another one where the film relies on the formation of covalent bonds between monomers (electropolymerization).³¹

Inspired from processes found in developmental biology,³² we introduced the concept of morphogen-driven film buildup. When extended to chemistry, a morphogen is defined as a molecule or an ion that is produced at an interface and diffuses into the solution, thus creating a concentration gradient, and that locally induces a chemical process. Electroclic chemistry was used to create the first morphogen-driven film buildup constituted of two interacting polymers.^{33–37} The role of the morphogen was played by Cu(I) generated at the electrode which catalyzed the reaction between polymers bearing either azide or alkyne moieties. On the basis of controlled polymerization, Caruso and collaborators introduced a similar concept of the continuous assembly of polymers to develop nanoscale films in a single step using macro-cross-linkers.^{38,39} The self-construction on surfaces of films whose cohesion is entirely based on electrostatic interactions represents a real challenge since polyelectrolytes with opposite charges spontaneously self-assemble to form polyanion/polycation complexes when mixed in solution. Merging the fields of polyelectrolyte multilayers and morphogen-driven film buildup, we present here a strategy which allows the self-assembly of oppositely charged macromolecules on a surface. Our strategy relies on a charge-shifting polymer:⁴⁰ a dimethylmaleic-modified poly(allylamine) (PAHd) that undergoes hydrolysis at acidic pH leading to a polycation, poly(allylamine hydrochloride) (PAH), was synthesized (Figure 1a).⁴¹ Protons can be electrogenerated by the oxidation of hydroquinone, leading to a pH gradient on the surface of the electrode. In the presence of another polyanion, poly(styrenesulfonate) (PSS), the rapid hydrolysis of PAHd into PAH initiates the formation of PAH/PSS complexes. Confined over nanometric distances near the electrode, the pH gradient leads to the continuous self-assembly of a nanometric PAH/PSS film (Figure 1b). We started by using a solution containing both PAHd and PSS, a polyanion fully charged over a large pH range. In a second trial, PSS was replaced by an enzyme.

EXPERIMENTAL SECTION

Chemicals. Polyethylenimine (PEI, $M = 750\,000$ g/mol, CAS 9002-98-6), poly(sodium 4-styrenesulfonate) (PSS, $M = 70\,000$ g/mol, CAS 25704-18-1), poly(allylamine hydrochloride) (PAH, $M = 56\,000$ g/mol, CAS 71550-12-4), sodium nitrate ($NaNO_3$, 7631-99-4), potassium hexacyanoferrate(II) ($M = 422.41$ g/mol, CAS 14459-95-1), hydroquinone ($M = 110.11$ g/mol, CAS 123-31-9), para-nitrophenyl phosphate liquid substrate (pNPP, P7998, CAS 4264-83-9), deuterated sodium hydroxide ($NaOD$), and deuterated hydrochloric acid (DCl, 36% in D_2O) were purchased from Sigma-Aldrich. Alkaline phosphatase (AP, P0252, CAS 9001-78-9) was purchased from TCI Europe. D_2O (99%) was purchased from Eurisotop. All chemicals were used as received. AP labeled with rhodamine (APrho) was synthesized as described in a previous work.⁴² PEI, PSS, and PAH solutions were prepared at 1 mg/mL by the dissolution of an adequate amount in 150 mM $NaNO_3$ aqueous solution. PAHd and PSS mixture solutions were prepared at 1 mg/mL in a 150 mM $NaNO_3$ aqueous solution with 60 mM hydroquinone and were adjusted to pH 7.7 unless otherwise stated. PAHd and AP mixture solutions were prepared at 1 mg/mL in a 150 mM $NaNO_3$ aqueous solution with 60 mM hydroquinone and were adjusted to pH 7.

Synthesis of Dimethylmaleic-Modified Poly(allylamine) (PAHd). The procedure was inspired by Lynn and collaborators.⁴¹ PAH (0.5 g, 5.3 mmol, 1.0 equiv) was dissolved in a 1 M NaOH aqueous solution (15 mL) and stirred overnight. 2,3-Dimethylmaleic anhydride (2.7 g, 21.4 mmol, 4.0 equiv) was added by portion, maintaining the pH of the reaction solution above 10 by the addition of a 6 M NaOH aqueous solution. The reaction mixture was stirred overnight at room temperature and then dialyzed with a cellulose ester membrane (MWCO 14 000–16 000) against Milli-Q water (adjusted to pH >10 by using NaOH aqueous solution) for 3 days. The final solution was freeze-dried to give the desired PAHd as a white solid (0.938 g, 80%). FTIR (neat): 3264, 2914, 2852, 1630, 1554, 1392, 1305 cm^{-1} . 1H NMR (400 MHz, D_2O , δ) 3.25 (bs, 2H), 1.86 (m, 7H), 1.27 (bs, 2H). ^{13}C NMR (100 MHz, D_2O , δ) 179.1, 175.2, 138.3, 129.5, 43.3, 36.3, 32.7, 16.7, 16.2.

Electrochemical Quartz Crystal Microbalance (EC-QCM) with Dissipation Monitoring. The electrochemical quartz crystal microbalance (EC-QCM) experiments were performed on a Q-Sense E1 apparatus from Q-Sense AB (Gothenburg, Sweden) by monitoring the changes in the resonance frequency f_r and the dissipation factor D_r of an oscillating quartz crystal upon adsorption of a viscoelastic layer (ν represents the overtone number, equal to 1, 3, etc.). The quartz crystal is excited at its fundamental frequency (5 MHz), and the measurements are performed at the first, third, fifth, and seventh overtones, corresponding to 5, 15, 25, and 35 MHz. The QCM measurement is sensitive to the amount of water associated with the adsorbed molecules and senses the viscoelastic changes in the interfacial material. Only the normalized frequency shifts of the fundamental frequency (Δf_1) and the third overtone ($\Delta f_3/3$) are presented. The gold-coated QCM sensor acted as a working electrode. A platinum electrode on the top wall of the chamber and a no-leak Ag/

AgCl reference electrode fixed in the outlet flow channel were used as counter and reference electrodes, respectively. Electrochemical measurements were performed on a CHI660E apparatus from CH Instruments (Austin, TX) coupled to the QCM-D (named EC-QCM) apparatus. Before the buildup of the polymer film, in order to test the quality of the EC-QCM cell, a capacitive current and a faradic current of a 1 mM potassium hexacyanoferrate (II) aqueous solution were recorded. A 150 mM NaNO₃ aqueous solution was prepared to measure the capacitive current of the EC-QCM cell. Potassium hexacyanoferrate (II) (1 mM) was prepared in a 150 mM NaNO₃ aqueous solution and placed in contact with the crystal to monitor its cyclic voltammogram. The polymer film buildup was controlled by applying a fixed current (chronopotentiometry in galvanostatic mode) as described in the text.

Film Buildup Procedure. An anchoring bilayer of PEI/PSS was deposited onto the gold working electrode by the LbL technique prior to film buildup. The aqueous PEI solution (1 mg/mL in 150 mM NaNO₃) was injected into the electrochemical cell (600 μ L) at a flow rate of 600 μ L/min with a peristaltic pump. The adsorption of the polycation was monitored by EC-QCM. Once the signal stabilized, i.e., after ca. 5 min, 600 μ L of a 150 mM NaNO₃ aqueous solution was injected into the cell to rinse it for 2 min. The aqueous polyanion solution (PSS at 1 mg/mL in 150 mM NaNO₃) was then injected into the cell (600 μ L) with the peristaltic pump. The adsorption of the polyanion on the previous polycation layer was monitored by EC-QCM. Once the signal stabilized, i.e., after ca. 5 min, an aqueous solution of 150 mM NaNO₃ was injected into the cell to rinse it (600 μ L) for 2 min. An aqueous solution of 150 mM NaNO₃ containing PAHd (1 mg/mL), PSS (1 mg/mL), and hydroquinone (60 mM) adjusted to pH 7.7 was then injected into the electrochemical cell with a continuous flow rate of 50 μ L/min. When using AP instead of PSS, an aqueous solution of 150 mM NaNO₃ containing PAHd (1 mg/mL), AP (1 mg/mL), and hydroquinone (60 mM) adjusted to pH 8.4 was used. Once the EC-QCM signal stabilized, a galvanostatic current was then applied for 1 h to trigger the hydroquinone oxidation and start the self-assembly of the film. After the self-assembly, an aqueous solution of 150 mM NaNO₃ was injected into the cell (600 μ L). The gold working electrode was then unmounted from the EC-QCM and dried with a flow of dry air for 5 min, and the samples were stored in the dry state.

Atomic Force Microscopy. Polyelectrolyte film samples, to be studied by atomic force microscopy (AFM) in the liquid state, were built on the quartz crystal inside the EC-QCM apparatus. AFM images were obtained in contact mode under liquid conditions with the Nanoscope IV from Veeco (Santa Barbara, CA). Cantilevers with a spring constant of 0.03 N/m and silicon nitride tips (model MSCATAUHW, Veeco) were used. We always performed several scans over a given surface area. These scans had to produce comparable images to ascertain that there was no sample damage induced by the tip. Deflection and height images (10 μ m $\times$ 10 μ m) were scanned at a fixed scan rate (2 Hz) with a resolution of 512 $\times$ 512 pixels.

X-ray Photoelectron Spectroscopy. The chemical composition of the films was determined by X-ray photoelectron spectroscopy (XPS) analysis. This analysis was performed with a Thermo VG Scientific spectrometer equipped with an Al K α X-ray source (1486.6 eV). It operated at 225 W under ultrahigh vacuum (pressure lower than 5.0×10^{-8} mbar). The incident angle and the source-to-analyzer angle were set to 45 and 90°, respectively. The probing depth of the technique is estimated to range from 5 to 8 nm. The survey scans were collected from 0 to 1100 eV with a pass energy of 50 eV, and the high-resolution scans were performed with the pass energy adjusted to 20 eV. The spectra were recorded with Advantage V.2.26 software and analyzed with Casa XPS 2.3.16 software. For calibration purposes, the carbon C 1s electron bond energy corresponding to aliphatic carbon was referenced to 285.0 eV. Raw areas determined after background subtraction were corrected according to Scofield sensitivity factors (C 1s, 1.00; N 1s, 1.80; S 2p, 1.68). The curve fitting was performed using a convolution of Gaussian and Lorentzian line shapes with a typical ratio of 70:30. This peak-fitting procedure was repeated until an

acceptable fit was obtained with a consideration of peak position and full width at half-maximum.

Enzymatic Activity of PAH/AP Films. A multidetector spectroscopy UV (Xenius XC, SAFAS, Monaco) equipped with a microplate reader was used to monitor the catalytic activity of the enzymes within PAH/AP films in contact with their substrate, the paranitrophenyl phosphate. The solution of this compound was prepared at 10% w/w with 10 mM Tris buffer at pH 8.0. The enzymatic reaction yields *para*-nitrophenol, which becomes an intensely yellow soluble product monitored at the wavelength of 405 nm every 2 min for 80 min.

Functionalization of Microelectrodes. To deposit the PEI–PSS anchoring bilayer, the interdigitated array electrode (IDA ref: A-01212S, Biologic) was immersed in a PEI solution (1 mg/mL in 150 mM NaNO₃) followed by a rinsing step in a 150 mM NaNO₃ solution and a PSS solution (1 mg/mL in 150 mM NaNO₃) followed by a rinsing step in the 150 mM NaNO₃ solution. An aqueous solution of 150 mM NaNO₃ containing PAHd (1 mg/mL), AP (0.9 mg/mL), APrho (0.1 mg/mL), and hydroquinone (60 mM) adjusted to 8.4 was brought into contact with the microelectrodes. The microelectrode was addressed for 15 min through the application of a current (10 μ A) with the CHI 660e potentiostat/galvanostat in a three-electrode cell where the working electrode was one of the IDA electrodes, the counter electrode was part of the IDA, and the reference electrode was a no-leak Ag/AgCl reference electrode. A SARFUS IMAGING HR station (Nanolane, Le Mans) was used in bright field and fluorescence mode to image the IDA electrodes.

RESULTS AND DISCUSSION

Although amide bond hydrolysis is slow in aqueous solution, maleic amide bonds are cleaved easily at low pH, providing free amino groups. Using this property, Lynn introduced charge-shifting polyelectrolytes to release, under a pH trigger, a fluorescent polycation in a controlled manner from polyelectrolyte multilayers.⁴¹ We first investigated the hydrolysis of PAHd into PAH in D₂O solution as a function of pD (pD = pH + 0.41)⁴³ by ¹H NMR spectroscopy at room temperature. The degree of hydrolysis of PAHd was followed as a function of time for different pD values (Figure S1a in [Supporting Information](#), SI). The hydrolysis reaction takes place rapidly in less than 10 min, reaching a plateau value after 20 min. The final proportion of hydrolyzed PAHd increases with the decrease in pD (Figure S1b in SI). For pD values below 3, almost 100% of the dimethylmaleic groups are hydrolyzed. PAHd thus appears to be the charge-shifting polyelectrolyte of choice to self-assemble films of oppositely charged macromolecules rapidly by using a pH (or pD) gradient. The most common way to establish a pH gradient in electrochemistry consists of the electrolysis of water, which generates protons or hydroxide. To generate protons, the oxidation of hydroquinone is preferred because of the lower oxidative potential required (0.3–0.4 V compared to 1.5–1.7 V for water electrolysis), which is well suited to the gold-coated working electrode. By the electrochemical oxidation of hydroquinone into 1,4-benzoquinone, two electrons are transferred to the electrode concomitantly with the production of two protons released in the media. It has been calculated that this oxidation leads to a pH of 3.6 in close proximity to the surface that increases gradually to neutral away from it.²⁷

Electrochemical-quartz crystal microbalance (EC-QCM) was used to monitor the self-assembly in situ during the application of the current with the gold QCM crystal acting as the working electrode. We used two frequencies for measurements depending on the thickness of the deposition. The acoustic wave with the fundamental frequency (near 5 MHz) has a

Figure 2. (a) Evolution of the opposite of the frequency shift as a function of time at an applied current of 90 μA for a PAHd/PSS mixture in contact with a PEI–PSS precoated electrode in the presence of 60 mM hydroquinone. (b) Typical 3D AFM image, in contact mode and the liquid state, and a section profile of a scratched PAH/PSS film obtained after 60 min of self-assembly.

higher penetration depth and allows us to follow the buildup of thick films. Less sensitive to the boundary effects of the crystal, the third harmonic at 15 MHz ($-\Delta f_3/3$) is more stable for thin depositions. The electrode was first covered with a layer of branched poly(ethylenimine) (PEI) to ensure a strong anchoring on the surface, followed by the deposition of a PSS layer rendering the surface negatively charged. This latter deposition was intended to prevent the nonspecific adsorption of PSS or PAHd. When the PEI–PSS precoated electrode was brought into contact with the negatively charged PAHd in the presence of 60 mM hydroquinone and 150 mM NaNO_3 at pH 7.7, a small frequency shift was observed as expected. As soon as a current of 90 μA was applied, the opposite of the normalized frequency shift increased rapidly and stabilized at about 18 Hz (Figure S2 in SI), corresponding typically to the deposition of one PAH layer on the previously adsorbed PSS layer. Now, when a mixture of PAHd/PSS (instead of only PAHd or PSS) in the presence of 60 mM hydroquinone and 150 mM NaNO_3 at pH 7.7 is injected onto a PEI–PSS precoated electrode, no signal is observed in the absence of current. A flow rate of 0.05 mL/min was imposed to ensure a constant concentration of reactants in the electrochemical cell. When a current of 90 μA was applied, the normalized frequency shift increased, in a first approximation, linearly with time, showing the self-assembly of a PAH/PSS film (Figure 2a). In the sole presence of PSS and hydroquinone in solution, a small frequency shift (10 Hz) was observed (Figure S2b in SI). The presence of both partners is thus required, which strongly indicates the self-assembly of a PAH/PSS film.

The morphology of the deposited film was characterized by atomic force microscopy (AFM) in contact mode and the liquid state (Figure S3 in SI). After scratching, the thickness of the film was also determined. Figure 2b shows a typical morphology corresponding to a PAH/PSS film self-assembled for 60 min at pH 7.7 with an applied current of 90 μA . The film uniformly covers the whole substrate with a thickness of 388 ± 22 nm (determined from three measurements of the z section) and a roughness of 47 nm (determined on $10 \times 10 \mu\text{m}^2$ images). The film thickness increases as a function of the application time of the current whereas the film roughness stays constant at about 50 nm (Figure 3). The constant roughness is

due to the self-assembly of PAH/PSS complexes on the substrate along the process.

Figure 3. Evolution of PAH/PSS film thickness and roughness, measured by AFM in contact mode in the liquid state, obtained after 15, 30, 45, and 60 min of self-assembly for an applied current of 90 μA . The film thickness was evaluated on three different profilometric sections of the scratched film (Figure S-3 in SI). The roughness of the films was evaluated on $10 \times 10 \mu\text{m}^2$ AFM images. The data represent the mean and the standard deviation of three measurements.

The simultaneous presence of both partners in the film was checked through X-ray photoelectron spectroscopy (XPS). The atomic composition of films obtained at 40, 60, 80, and 90 μA has been analyzed after 60 min of self-assembly. For all of these films, the presence of PAH and PSS is confirmed by the presence of sulfur atoms, S(2p), from PSS and nitrogen atoms, N(1s), from PAH. Data are gathered in Tables S1 and S2 with typical XPS spectra in the SI. The measured atomic ratio of S(2p)/N(1s) lies between 1.12 and 1.34, showing a small excess of PSS in self-assembled PAH/PSS films whatever the applied current (Figure S4a in SI). The measurement of the ratio of nitrogen involved in the amide bonds (NHCO) allows access to the degree of hydrolysis of PAHd. This hydrolysis is complete for current intensities above 60 μA (Figure S4b in SI).

To gain more insight in the mechanism, several parameters that might influence the buildup process were varied. The current intensity was varied from 30 to 90 μA while keeping the pH of the mixture solution at 7.7. The increase in the normalized frequency shift, related to the mass adsorbed, varies

with the applied current intensity (Figure S5 in SI). Thus, the PAH/PSS film self-assembly kinetics strongly depend on the applied current: higher current intensities lead to faster buildup kinetics (Figure 4a). For currents such as 30 and 40 μA , almost

Figure 4. (a) Opposite of the frequency shift measured after 60 min of self-assembly with a PAHd/PSS mixture at pH 7.7 at different current intensities (from 30 to 90 μA). (b) Evolution of the opposite of the frequency shift as a function of time during PAH/PSS self-assembly at an applied current of 80 μA for 40 min in two steps of 20 min separated by 60 min without any current.

no buildup is observed. Higher current intensity leads to more acidic conditions in the vicinity of the working electrode, leading to a faster transformation of PAHd in PAH. The increase in the frequency shift stops as soon as the current is switched off and starts again when the current is switch on (Figure 4b). These experiments prove the continuous buildup of a film in the presence of PAHd and PSS under electrochemical control.

The influence of the pH of the mixture solution was also investigated for an applied current of 90 μA (Figure S6a in SI). At pH 6.5, the buildup process cannot be realized because the PAHd/PSS solution becomes rapidly turbid, indicating the rapid hydrolysis of PAHd into PAH and the formation of PAH/PSS complexes in solution. At pH 7.5 and 7, the buildup kinetics are decreased compared to those at pH 7.7. Above pH 7.7, the self-assembly kinetics are also decreased until no film builds up at pH 8.7. It thus comes out that the optimal pH window allowing self-assembly is fairly narrow, lying between 7.7 and 8.5. When the experiments were realized at pH 7.7 in 0.75 mM HEPES buffer solution, the buildup kinetics

dramatically decreased (Figure S6b in SI). This is expected since the production of protons at the interface is neutralized in a buffer solution. The pH gradient near the electrode is thus strongly reduced, hindering the hydrolysis of PAHd. These experiments highlight the crucial role of the pH gradient from the surface on the self-assembly of the film.

To generalize this concept, PSS was replaced by an enzyme, alkaline phosphatase (AP), whose isoelectric point is close to 4. By changing the pH and the current applied, the self-assembly of PAH/AP films was optimized (Figure S7 in SI). The best buildup was obtained with a mixture solution at pH 8.4 and with a current application in two steps, i.e., 100 μA for 10 min followed by 90 μA . A small increase in the opposite of the normalized frequency shift (30 Hz) is observed at the injection of the PAHd/enzyme mixture solution due to electrostatic adsorption. As soon as the current is applied, an increase in the signal takes place (Figure S8 in SI).

The self-assembly kinetics of the PAH/AP film is much slower than that of the PAH/PSS film (Figure 5a). After 15 min of self-assembly, the substrate is totally covered with a film (Figure S9 in SI) whose thickness is about 24 ± 1 nm with a roughness of 10 nm. For longer times, a linear increase in the thickness is observed up to 33 ± 2 nm for 60 min (Figure 5b, and the roughness seems to stabilize at about 30 nm (Figure S10 in SI).

Using paranitrophenyl phosphate (a chromogenic substrate of AP), we monitored the enzymatic activity of self-assembled PAH/AP films built at an applied current of 100 μA for 10 min, followed by 90 μA for 15, 30, and 45 min. In the presence of AP, paranitrophenyl phosphate is transformed into paranitrophenol that can be measured by UV spectroscopy at 405 nm (Figure S11 in SI). The enzymatic activity of the film increases with the self-assembly time, i.e., the quantity of enzyme adsorbed (Figure 6a), until reaching a plateau. This plateau can be explained by the substrate diffusion limitations and the reduced enzyme accessibility that is well established for multilayers of enzymes as the thickness increases.^{44–46} This result is extremely interesting for future biosensor applications since it should allow us to control the sensor sensitivity simply by tuning the film deposition time.

Figure 5. (a) Evolution of the opposite of the normalized frequency shift of the third overtone, measured by EC-QCM, as a function of time during the self-assembly of PAH/AP film on a PEI–PSS precoated electrode at an applied current of 100 μA for 10 min, followed by 90 μA for 1 h. Mean and standard deviation of the obtained films from three independent measurements after the subtraction of the contribution of the PEI–PSS precursor film. PAHd and AP were each at 1 mg/mL in the presence of hydroquinone (60 mM) at pH 8.4. (b) Typical 3D AFM image and section profile, obtained in contact mode and the liquid state, of a scratched PAH/AP film obtained after 60 min of self-assembly.

Figure 6. (a) Enzymatic reaction rate of PAH/AP films obtained by self-assembly in two steps, i.e., 100 μA for 10 min followed by 90 μA for different self-assembly times. The reaction rate corresponds to the hydrolysis of paranitrophenyl phosphate in contact with a PAH/AP film measured by the slope of the optical density (OD) at 405 nm versus time. Optical microscope images of interdigitated array (IDA) electrodes (b) in bright field and (c) in fluorescence mode where three electrodes within IDA were addressed to self-assemble PAH/APrho (rhodamine-labeled AP) films at an applied current of 10 μA for 15 min. The scale bar represents 25 μm .

Since the pH shift is localized near the electrode, it should be adequate to functionalize microelectrodes. Using rhodamine-labeled AP (APrho), a PAH/APrho film was built up on interdigitated arrays (IDA) of electrodes (Figure S-12 in SI) by the application of 10 μA on one of the two arrays for 15 min. The microelectrodes were imaged by optical microscopy in bright field and in fluorescence to check for the presence of APrho and to verify its spatial localization (Figure 6b,c). The PAH/APrho film exhibits an excellent spatioselectivity since high fluorescence is observed only on the addressed microelectrodes.

CONCLUSIONS

On the basis of the concept of the morphogen-driven buildup of films, the self-assembly of oppositely charged macromolecules was addressed by using a mixture of polyanion (or enzyme) and a charge-shifting polyanion, PAHd, which hydrolyzes into PAH under acidic conditions. By generating protons locally through the oxidation of hydroquinone under a constant current, a pH decrease is obtained near the electrode and leads to the continuous self-assembly of a PAH/PSS nanometric film. When using an enzyme instead of PSS, i.e., PAHd/enzyme solutions, an enzymatically active film whose activity increases with deposition time is self-assembled. This process is well suited to the functionalization of microelectrodes by enzymes, opening the route toward miniaturized biosensors.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.langmuir.5b02749.

¹H NMR study of PAHd hydrolysis in solution, EC-QCM study of PAHd and PSS solutions, AFM study of PAH/PSS self-assembled film, XPS analysis of PAH/PSS self-assembled film, influence of the current and the pH on the self-assembly of PAH/PSS film, and physico-chemical study of PAH/AP self-assembled films (PDF)

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Author Contributions

The manuscript was written through the contributions of all authors. All authors have given approval to the final version of the manuscript. A.D. and T.G. contributed equally.

Notes

The authors declare no competing financial interest.

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REFERENCES

- (1) Whitesides, G. M.; Grzybowski, B. Self-assembly at all scales. *Science* **2002**, *295*, 2418–2421.
- (2) Lehn, J. M. *Supramolecular Chemistry Concepts and Perspectives*; VCH: Weinheim, 1995.
- (3) Whitesides, G. M.; Boncheva, M. Beyond molecules: Self-assembly of mesoscopic and macroscopic components. *Proc. Natl. Acad. Sci. U. S. A.* **2002**, *99*, 4769.
- (4) Decher, G. Fuzzy nanoassemblies: Toward layered polymeric multicomposites. *Science* **1997**, *277*, 1232–1237.
- (5) Ariga, K.; Yamauchi, Y.; Rydzek, G.; Ji, Q. M.; Yonamine, Y.; Wu, K. C. W.; Hill, J. P. Layer-by-layer nanoarchitectonics: Invention, innovation, and evolution. *Chem. Lett.* **2014**, *43*, 36–68.
- (6) Bravo, J.; Zhai, L.; Wu, Z. Z.; Cohen, R. E.; Rubner, M. F. Transparent superhydrophobic films based on silica nanoparticles. *Langmuir* **2007**, *23*, 7293–7298.
- (7) Farhat, T. R.; Schlenoff, J. B. Corrosion control using polyelectrolyte multilayers. *Electrochem. Solid-State Lett.* **2002**, *5*, B13–B15.
- (8) Shchukin, D. G.; Zheludkevich, M.; Yasakau, K.; Lamaka, S.; Ferreira, M. G. S.; Möhwald, H. Layer-by-layer assembled nanocontainers for self-healing corrosion protection. *Adv. Mater.* **2006**, *18*, 1672–1678.
- (9) Kerdjoudj, H.; Berthelemy, N.; Rinckenbach, S.; Kearney-Schwartz, A.; Montagne, K.; Schaaf, P.; Lacolley, P.; Stoltz, J.-F.; Voegel, J.-C.; Menu, P. Small vessel replacement by human umbilical arteries with polyelectrolyte film-treated arteries in vivo behavior. *J. Am. Coll. Cardiol.* **2008**, *52*, 1589–1597.
- (10) Thompson, M. T.; Berg, M. C.; Tobias, I. S.; Rubner, M. F.; Van Vliet, K. J. Tuning compliance of nanoscale polyelectrolyte multilayers to modulate cell adhesion. *Biomaterials* **2005**, *26*, 6836–6845.
- (11) Zheng, H. P.; Berg, M. C.; Rubner, M. F.; Hammond, P. T. Controlling cell attachment selectively onto biological polymer-colloid templates using polymer-on-polymer stamping. *Langmuir* **2004**, *20*, 7215–7222.
- (12) Xiang, Y.; Lu, S. F.; Jiang, S. P. Layer-by-layer self-assembly in the development of electrochemical energy conversion and storage devices from fuel cells to supercapacitors. *Chem. Soc. Rev.* **2012**, *41*, 7291–7321.

- (13) Taylor, A. D.; Michel, M.; Sekol, R. C.; Kizuka, J. M.; Kotov, N. A.; Thompson, L. T. Fuel cell membrane electrode assemblies fabricated by layer-by-layer electrostatic self-assembly techniques. *Adv. Funct. Mater.* **2008**, *18*, 3003–3009.
- (14) DeLongchamp, D. M.; Hammond, P. T. High-contrast electrochromism and controllable dissolution of assembled prussian blue/polymer nanocomposites. *Adv. Funct. Mater.* **2004**, *14*, 224–232.
- (15) DeLongchamp, D. M.; Hammond, P. T. Multiple-color electrochromism from layer-by-layer-assembled polyaniline/prussian blue nanocomposite thin films. *Chem. Mater.* **2004**, *16*, 4799–4805.
- (16) Ariga, K.; Ji, Q.; Hill, J. P. Enzyme-encapsulated layer-by-layer assemblies: Current status and challenges toward ultimate nano-devices. In *Modern Techniques for Nano- and Microreactors/Reactions*; Caruso, F., Ed.; Springer: Berlin, 2010; Vol. 229, pp 51–87.
- (17) McShane, M. J. Potential for glucose monitoring with nanoengineered fluorescent biosensors. *Diabetes Technol. Ther.* **2002**, *4*, 533–538.
- (18) Karamitros, C. S.; Yashchenok, A. M.; Möhwald, H.; Skirtach, A. G.; Konrad, M. Preserving catalytic activity and enhancing biochemical stability of the therapeutic enzyme asparaginase by biocompatible multilayered polyelectrolyte microcapsules. *Biomacromolecules* **2013**, *14*, 4398–4406.
- (19) Takahashi, S.; Sato, K.; Anzai, J. I. Layer-by-layer construction of protein architectures through avidin–biotin and lectin–sugar interactions for biosensor applications. *Anal. Bioanal. Chem.* **2012**, *402*, 1749–1758.
- (20) de Saint-Aubin, C.; Hemmerlé, J.; Boulmedais, F.; Vallat, M. F.; Nardin, M.; Schaaf, P. New 2-in-1 polyelectrolyte step-by-step film buildup without solution alternation: From pedot-pss to polyelectrolyte complexes. *Langmuir* **2012**, *28*, 8681–8691.
- (21) Kelly, K. D.; Schlenoff, J. B. Spin-coated polyelectrolyte coacervate films. *ACS Appl. Mater. Interfaces* **2015**, *7*, 13980–13986.
- (22) Krylova, I. Painting by electrodeposition on the eve of the 21st century. *Prog. Org. Coat.* **2001**, *42*, 119–131.
- (23) Payne, G. F.; Kim, E.; Cheng, Y.; Wu, H. C.; Ghodssi, R.; Rubloff, G. W.; Raghavan, S. R.; Culver, J. N.; Bentley, W. E. Accessing biology's toolbox for the mesoscale biofabrication of soft matter. *Soft Matter* **2013**, *9*, 6019–6032.
- (24) Ngankam, A. P.; Van Tassel, P. R. Continuous polyelectrolyte adsorption under an applied electric potential. *Proc. Natl. Acad. Sci. U. S. A.* **2007**, *104*, 1140–1145.
- (25) Ngankam, A. P.; Van Tassel, P. R. In situ layer-by-layer film formation kinetics under an applied voltage measured by optical waveguide lightmode spectroscopy. *Langmuir* **2005**, *21*, 5865–5871.
- (26) Olsen, C.; Van Tassel, P. R. Polyelectrolyte adsorption kinetics under an applied electric potential: Strongly versus weakly charged polymers. *J. Colloid Interface Sci.* **2009**, *329*, 222–227.
- (27) Johnson, E. K.; Adams, D. J.; Cameron, P. J. Directed self-assembly of dipeptides to form ultrathin hydrogel membranes. *J. Am. Chem. Soc.* **2010**, *132*, 5130–5136.
- (28) Wong, I. Y.; Footer, M. J.; Melosh, N. A. Electronically activated protein polymerization and alignment. *J. Am. Chem. Soc.* **2008**, *130*, 7908–7915.
- (29) Brusatori, M. A.; Tie, Y.; Van Tassel, P. R. Protein adsorption kinetics under an applied electric field: An optical waveguide lightmode spectroscopy study. *Langmuir* **2003**, *19*, 5089–5097.
- (30) Brusatori, M. A.; Van Tassel, P. R. Biosensing under an applied voltage using optical waveguide lightmode spectroscopy. *Biosens. Bioelectron.* **2003**, *18*, 1269–1277.
- (31) Waltman, R. J.; Bargon, J. Electrically conducting polymers - a review of the electropolymerization reaction, of the effects of chemical-structure on polymer film properties, and of applications towards technology. *Can. J. Chem.* **1986**, *64*, 76–95.
- (32) Potter, J. D. Morphogens, morphostats, microarchitecture and malignancy. *Nat. Rev. Cancer* **2007**, *7*, 464–474.
- (33) Rydzek, G.; Jier, L.; Parat, A.; Thomann, J. S.; Voegel, J.-C.; Senger, B.; Hemmerlé, J.; Ponche, A.; Frisch, B.; Schaaf, P.; Boulmedais, F. Electrochemically triggered assembly of films: A one-pot morphogen-driven buildup. *Angew. Chem., Int. Ed.* **2011**, *50*, 4374–4377.
- (34) Rydzek, G.; Garnier, T.; Schaaf, P.; Voegel, J.-C.; Senger, B.; Frisch, B.; Haikel, Y.; Petit, C.; Schlatter, G.; Jier, L.; Boulmedais, F. Self-construction of supramolecular polyrotaxane films by an electro-triggered morphogen-driven process. *Langmuir* **2013**, *29*, 10776–10784.
- (35) Rydzek, G.; Parat, A.; Polavarapu, P.; Baehr, C.; Voegel, J.-C.; Hemmerlé, J.; Senger, B.; Frisch, B.; Schaaf, P.; Jier, L.; Boulmedais, F. One-pot morphogen driven self-constructing films based on non-covalent host-guest interactions. *Soft Matter* **2012**, *8*, 446–453.
- (36) Rydzek, G.; Polavarapu, P.; Rios, C.; Tisserant, J. N.; Voegel, J.-C.; Senger, B.; Laval, P.; Frisch, B.; Schaaf, P.; Boulmedais, F.; Jier, L. Morphogen-driven self-construction of covalent films built from polyelectrolytes and homobifunctional spacers: Buildup and ph response. *Soft Matter* **2012**, *8*, 10336–10343.
- (37) Rydzek, G.; Terentyeva, T. G.; Pakdel, A.; Golberg, D.; Hill, J. P.; Ariga, K. Simultaneous electropolymerization and electro-click functionalization for highly versatile surface platforms. *ACS Nano* **2014**, *8*, 5240–5248.
- (38) Mertz, D.; Ochs, C. J.; Zhu, Z. Y.; Lee, L.; Guntari, S. N.; Such, G. K.; Goh, T. K.; Connal, L. A.; Blencowe, A.; Qiao, G. G.; Caruso, F. Atp-mediated continuous assembly of polymers for the preparation of nanoscale films. *Chem. Commun.* **2011**, *47*, 12601–12603.
- (39) Goh, T. K.; Guntari, S. N.; Ochs, C. J.; Blencowe, A.; Mertz, D.; Connal, L. A.; Such, G. K.; Qiao, G. G.; Caruso, F. Nanoengineered films via surface-confined continuous assembly of polymers. *Small* **2011**, *7*, 2863–2867.
- (40) Funhoff, A. M.; van Nostrum, C. F.; Janssen, A.; Fens, M.; Crommelin, D.; Hennink, W. E. Polymer side chain degradation as a tool to control the destabilization of polyplexes. *Pharm. Res.* **2004**, *21*, 170–S57176.
- (41) Liu, X. H.; Zhang, J. T.; Lynn, D. M. Polyelectrolyte multilayers fabricated from 'charge-shifting' anionic polymers: A new approach to controlled film disruption and the release of cationic agents from surfaces. *Soft Matter* **2008**, *4*, 1688–1695.
- (42) Mertz, D.; Vogt, C.; Hemmerlé, J.; Mutterer, J.; Ball, V.; Voegel, J.-C.; Schaaf, P.; Laval, P. Mechanotransductive surfaces for reversible biocatalysis activation. *Nat. Mater.* **2009**, *8*, 731–735.
- (43) Glasoe, P. K.; Long, F. A. Use of glass electrodes to measure acidities in deuterium oxide. *J. Phys. Chem.* **1960**, *64*, 188–190.
- (44) Jin, W.; Shi, X. Y.; Caruso, F. High activity enzyme microcrystal multilayer films. *J. Am. Chem. Soc.* **2001**, *123*, 8121–8122.
- (45) Caruso, F.; Schuler, C. Enzyme multilayers on colloid particles: Assembly, stability, and enzymatic activity. *Langmuir* **2000**, *16*, 9595–9603.
- (46) Onda, M.; Ariga, K.; Kunitake, T. Activity and stability of glucose oxidase in molecular films assembled alternately with polyions. *J. Biosci. Bioeng.* **1999**, *87*, 69–75.