

## Electronic Supplementary Information (ESI)

# Morphogen-driven Self-Construction of Covalent Films Built from Polyelectrolytes and Homobifunctional Spacers : Film Buildup and pH Response

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## SECTION 1: Syntheses of PAA-*Alk* and homobifunctional spacers

### General Informations

All starting materials were obtained from commercial suppliers and were used without further purification.  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR spectra were recorded on Bruker Advance DPX400 (400 MHz) spectrometers. The NMR chemical shifts are reported in ppm relative to tetramethylsilane ( $\text{CDCl}_3$  or  $\text{MeO-d}_4$ ) or tert-butanol (1.24 ppm) in  $\text{D}_2\text{O}$  (s:singlet, t:triplet, q:quadruplet, dd:doublet of doublet, br:broad). Infrared spectra were obtained on a Thermo Electron Corporation Nicolet 380 FT-IR equipped with ATR. Merck RP-18 F254S plates were used for analytical thin layer chromatography. Silica gel 60 (particle: 40 – 60  $\mu\text{m}$ ) was used for flash chromatography.

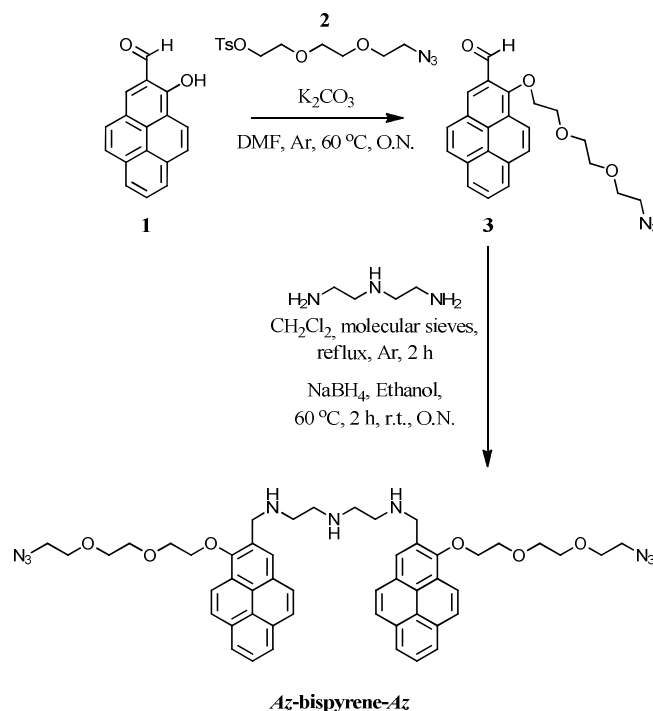
**Synthesis of PAA-*Alk* 5%.** PAA- *Alk* 5 % was synthesized according to Rydzek, G.; Thomann, J. S.; Ben Ameur, N.; Jerry, L.; Mésini, P.; Ponche, A.; Contal, C.; El Haitami, A. E.; Voegel, J.-C.; Senger, B.; Schaaf, P.; Frisch, B.; Boulmedais, F. *Langmuir* **2009**, *26*, 2816–2824.

**Synthesis of bispyrene** has been prepared according to Shiraishi, Y.; Tokitoh, Y.; Hirai, T. *Org. Lett.* **2006**, *17*, 3841-3844.

**Synthesis of Az-Bispyrene-Az.** 1-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)pyrene-2-carbaldehyde (**3**). To a solution of 100 mg (0.41 mmol) of compound **1** (Y. Zhou *et al.*, *Org. Lett.* 2009, **11**, 4442-4445) in 5 mL DMF was added 561 mg (4.06 mmol, 10 eq) of  $\text{K}_2\text{CO}_3$  under argon. To this bright red solution, 147 mg (0.45 mmol, 1.1 eq) of tosylate **2** in 1 mL DMF was added slowly and heated at 60 °C during overnight. Water was added and solvent evaporated under reduced pressure. The residue was extracted with  $\text{CH}_2\text{Cl}_2$  (3 times) and the combined organic phase was washed with water, dried over  $\text{MgSO}_4$ , solvent evaporated to give yellowish residue. This was flashed in a silica gel column eluting with 0-3 % ethyl acetate in  $\text{CH}_2\text{Cl}_2$  to get 110 mg (67 %) of product **3** as light brown semi solid (contains little tosylate starting material).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ) :  $\delta$  = 10.82 (s, 1H), 8.47 (s, 1H), 8.36 (d,  $^3\text{J}$  = 9.0 Hz, 1H), 8.08 (d,  $^3\text{J}$  = 7.5 Hz, 1H), 8.04 (d,  $^3\text{J}$  = 7.5 Hz, 1H), 8.03 (d,  $^3\text{J}$  = 9.0 Hz, 1H), 7.95 (t,  $^3\text{J}$  = 7.5 Hz, 1H), 7.90 (d,  $^3\text{J}$  = 9.0 Hz, 1H), 7.85 (d,  $^3\text{J}$  = 9.0 Hz, 1H), 4.40 (m, 2H), 3.93 (m, 2H), 3.75 (m, 2H), 3.72 (m, 2H), 3.68 (t,  $^3\text{J}$  = 5.0 Hz, 2H), 3.38 (t,  $^3\text{J}$  = 5.0 Hz, 2H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ) :  $\delta$  = 190.9, 155.7, 131.8, 131.6, 128.9, 127.9, 127.5, 127.5, 127.3, 127.1, 126.1, 125.3, 125.2, 124.5, 124.3, 123.5, 121.2, 70.8, 70.6, 70.2, 70.0, 50.7; MS (ESI)  $m/z$

calcd for  $C_{23}H_{21}N_3NaO_4$   $[M + Na]^+ = 426.14$ , found 426.00; IR (neat,  $cm^{-1}$ ): 2921, 2853, 2100, 1683, 1123.

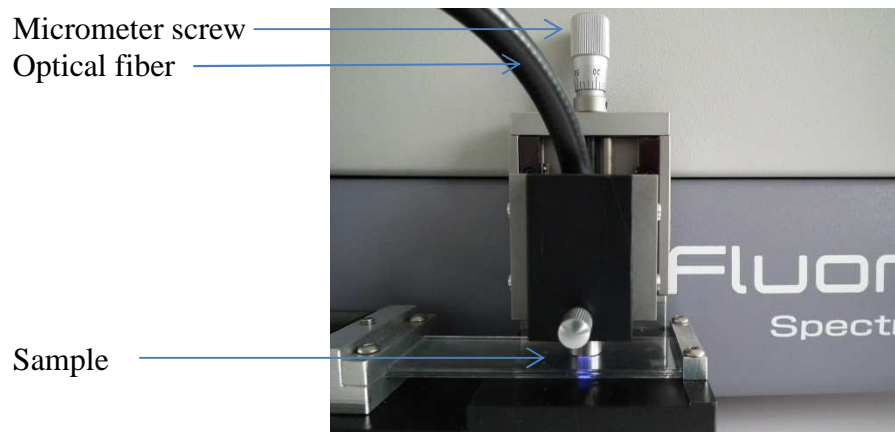
N1-((1-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)pyren-2-yl)methyl)-N2-((1-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)pyren-2-yl)methylamino)ethyl)ethane-1,2-diamine (**Az-bispyrene-Az**). A stirring solution of 110 mg (0.27 mmol, 2 eq) of Pyrene derivative (**3**) and 14 mg (15  $\mu$ L, 0.14 mmol) of diethylenetriamine in 12 mL  $CH_2Cl_2$  with few molecular sieves was refluxed for 2.5 h under argon. The reaction mixture was cooled down and filtered off the molecular sieves and the filtrate evaporated. To this semi oily yellow residue 13 mL ethanol and 31 mg (0.82 mmol, 6 eq) of  $NaBH_4$  were added and heated at 60  $^{\circ}C$  for 4 h and stirring continued for 16 h at room temperature. Solvent was evaporated under reduced pressure and the residue was taken into  $CH_2Cl_2$ , washed with 1 M NaOH solution (3 times), water and concentrated by evaporation to give impure compound **Az-bispyrene-Az** as light orange semisolid. This compound was used without further purifications.  $^1H$  NMR (400 MHz,  $CDCl_3$ ):  $\delta = 8.36$  (d,  $^3J = 9.0$  Hz, 2H), 8.11 (m, 4H), 8.03 (m, 4H), 7.92 (m, 6H), 4.22 (m, 6H), 4.00 – 3.30 (m, 22H), 2.81 (m, 8H);  $^{13}C$  NMR (100 MHz,  $CDCl_3$ ):  $\delta = 155.7$ , 144.7, 131.0, 130.9, 128.6, 127.4, 127.3, 127.0, 125.9, 124.0 – 125.5 (5C), 121.3, 70.7, 70.6, 70.0, 69.1, 68.7, 50.7, 50.6; MS (Maldi-TOF)  $m/z$  calcd for  $C_{50}H_{56}N_9O_6$   $[M + H]^+ = 878.435$ , found 878.669; IR (neat,  $cm^{-1}$ ): 2924, 2868, 2104, 1123.

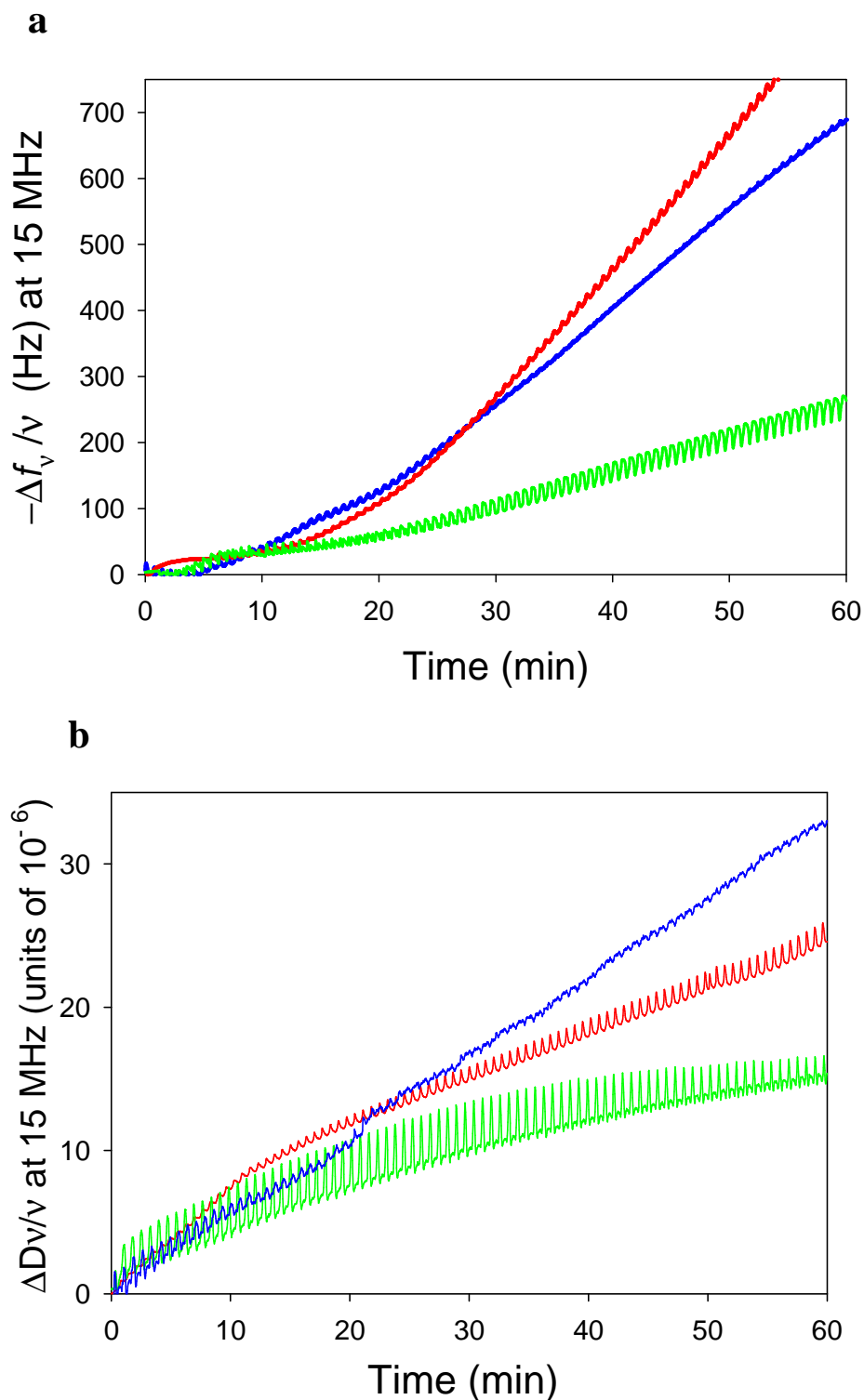


## SECTION 2 : Fluorescence measurements on the film PAA-*Alk*/Az-Bispyrene-Az.

All fluorescence intensities were measured by using the spectrofluorometer FluoroMax-4 from HORIBA JOBIN YVON at 432 nm of excitation wavelength. Emission fluorescence intensity of the film **PAA-*Alk*/Az-Bispyrene-Az** film was measured as follows:

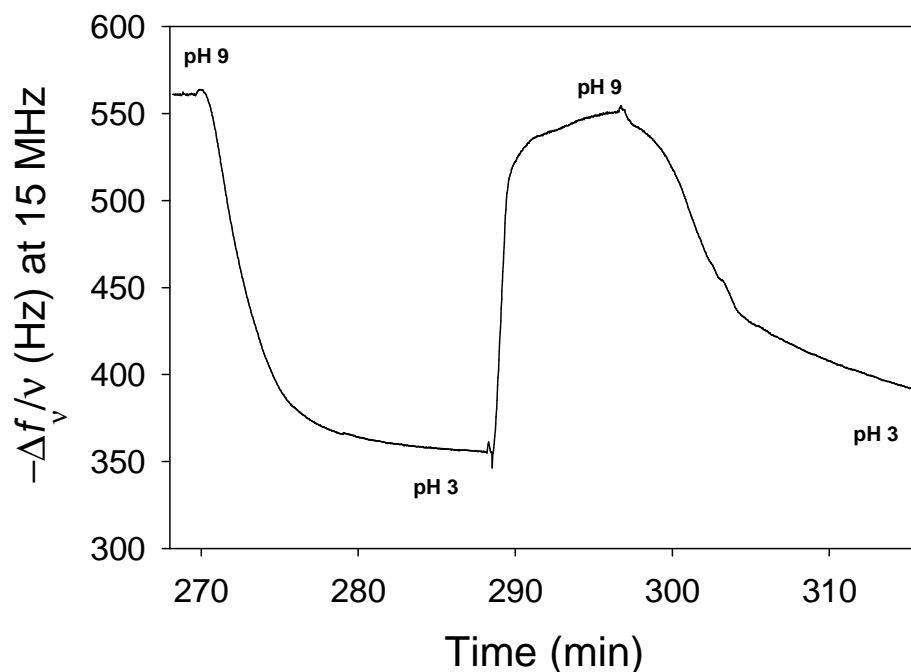
1. The self-constructed **PAA-*Alk*/Az-Bispyrene-Az** film was prepared on an ITO substrate by using the EC-QCM-D according to the description process given in the Materials and Methods section of the article. To measure fluorescence, the buildup of the film studied was stopped when  $\Delta f_v/v$  corresponding to the third overtones reached -950 Hz by the QCM.
2. The ITO substrate covered with the **PAA-*Alk*/Az-Bispyrene-Az** film was dipped in 15 mL of MilliQ solution at desired pH (this pH was obtained by using diluted solutions of HCl and NaOH).
3. The ITO substrate was deposited on a microscope glass slide with the film on the top. Then, the film was covered with another, thinner glass slide. This system was maintained in a home-made adaptor allowing fixing both the ITO substrate and an optical fiber. Thus, reproducible experiments have been performed.
4. The optical fiber F-3000, purchased from HORIBA JOBIN YVON, was fixed in our home-made adaptor and put in contact with the top glass slide on the ITO substrate by using a micrometer screw (see picture below).
5. Excitation of the film at 342 nm and fluorescence emission was done through the optical probe into a dark room.



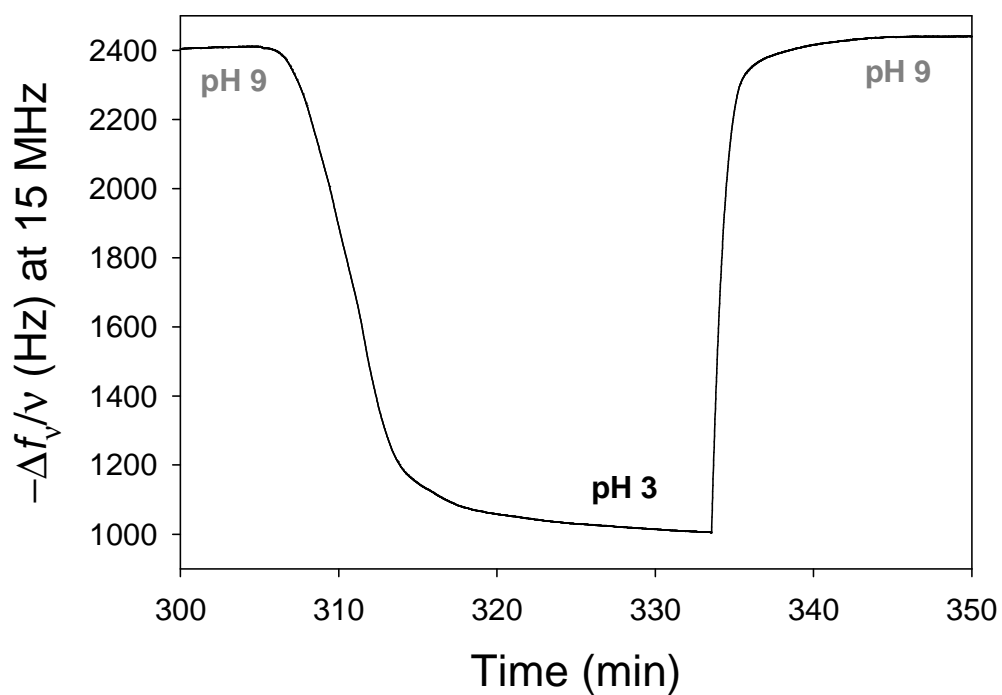


**Fig. S-1** Evolution of the opposite of the normalized frequency shift (**a**) and dissipations (**b**), measured at 15 MHz ( $\nu = 3$ ) by EC-QCM, during the self-construction of PAA-*Alk*/Az-EG<sub>2</sub>-Az (green line), PAA-*Alk*/Az-EG<sub>13</sub>-Az (red line) and PAA-*Alk*/Az-EG<sub>50</sub>-Az films (blue line). A mixture solution (PAA-*Alk*, Az-EG<sub>n</sub>-Az and CuSO<sub>4</sub>) is in contact with the gold substrate

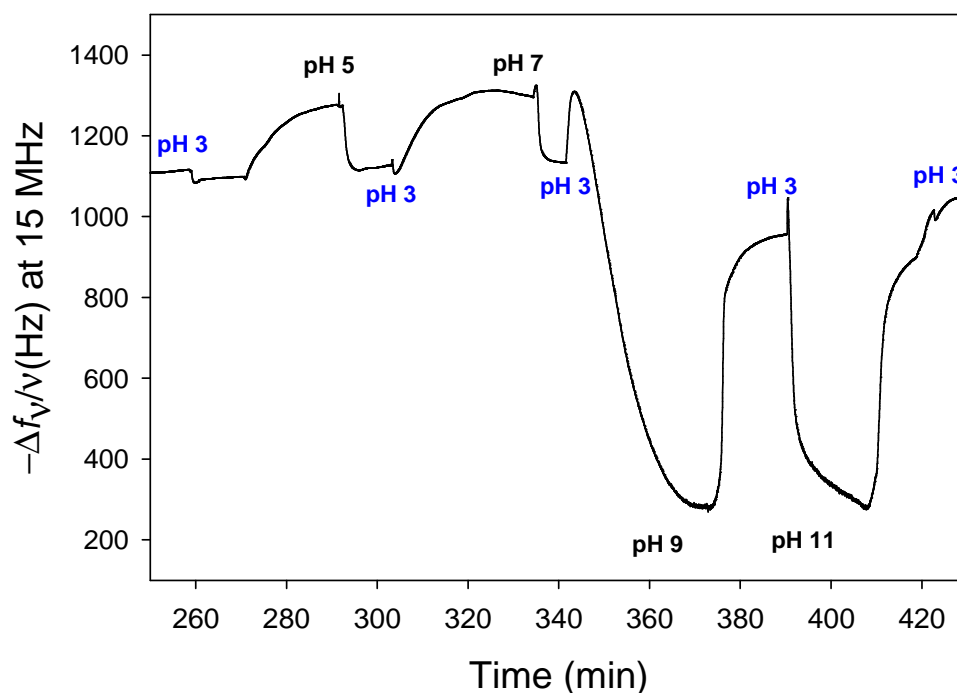
(QCM crystal) and a cyclic voltammetry (CV) between -350 mV and + 600 mV (vs Ag/AgCl, scan rate of 50 mV/s) is applied. The film is built on a PEI pre-coated surface.



**Fig. S-2** Evolution of the opposite of the normalized frequency shift, measured at 15 MHz ( $v = 3$ ) by EC-QCM-D, of PAA-*Alk/Az*-EG<sub>2</sub>-*Az* film during pH changes back and forth from 9 to 3. After the film buildup on a PEI precoated surface, aqueous solutions at pH 9 and pH 3 were put into contact alternately with the film under a flow rate of 1 mL/min. The swelling and shrinking recorded are attributed to reversible deprotonation/protonation of the carboxylic functions of PAA.

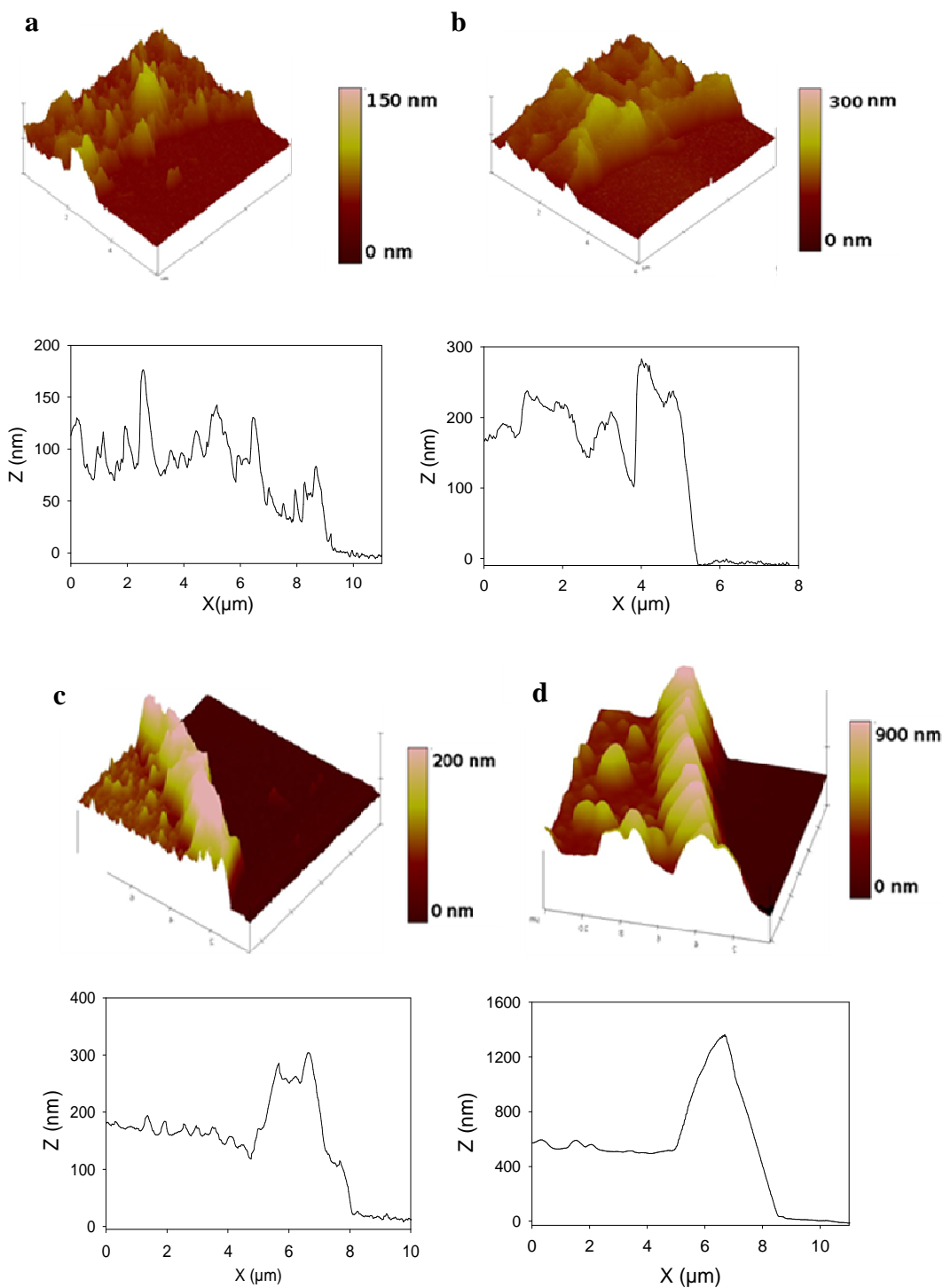


**Fig. S-3** Evolution of the opposite of the normalized frequency shift, measured at 15 MHz ( $\nu = 3$ ) by EC-QCM-D, of PAA-Alk/Az-EG<sub>13</sub>-Az film during pH changes back and forth from 9 to 3. After the film buildup on a PEI precoated surface, aqueous solutions at pH 9 and at pH 3 were put into contact alternately with the film under a flow rate of 1 mL/min. The swelling and shrinking recorded are attributed to reversible deprotonation/protonation of the carboxylic functions of PAA.

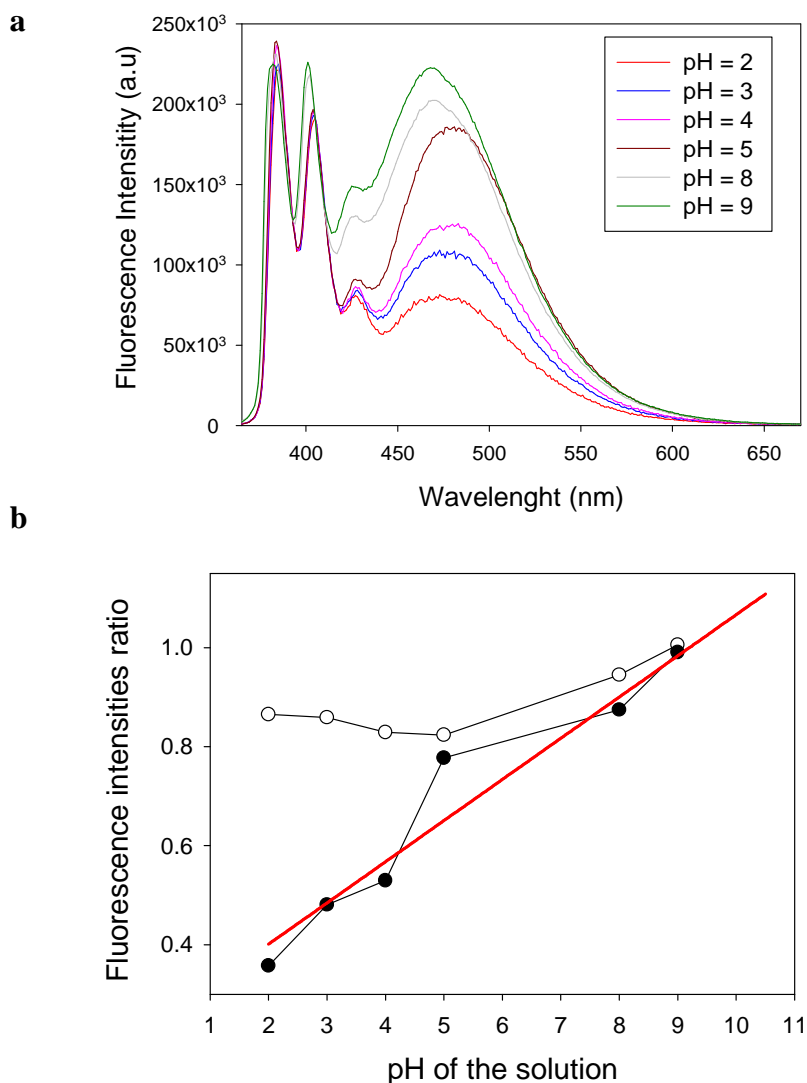


**Fig. S-4** Evolution of the opposite of the normalized frequency shift, measured at 15 MHz ( $\nu = 3$ ) by EC-QCM-D, of a PAA-AIk/Az-EG<sub>50</sub>-Az film during gradual pH increase from pH 3 to pH 11. After the film buildup on a PEI precoated surface, aqueous solutions at different pH were put into contact alternately with the film under a flow rate of 1 mL/min. After an expected increase of the frequency shift with the increase in pH, one observes a strong decrease of the frequency shift when the pH increases at pH 9 and 11. Correlatively the frequency of higher harmonics is no longer measured by the apparatus. Such a decrease of the frequency shift is fully compatible with a film that becomes extremely thick (Xu, F. Yao, G. D. Fu, E. T. Kang, *Biomacromolecules*, **2010**, *11*, 1810).

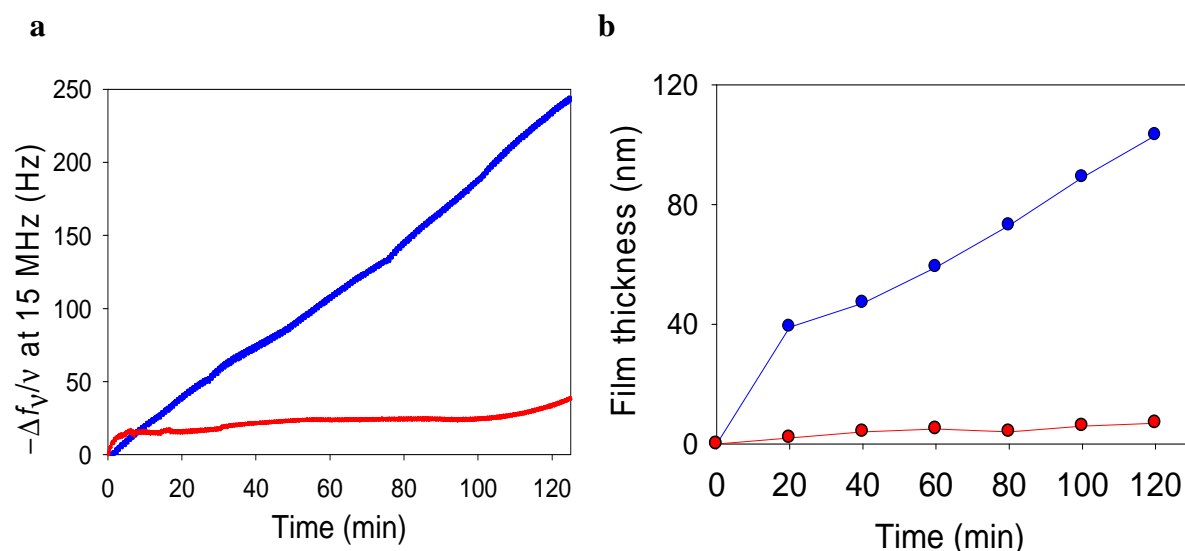




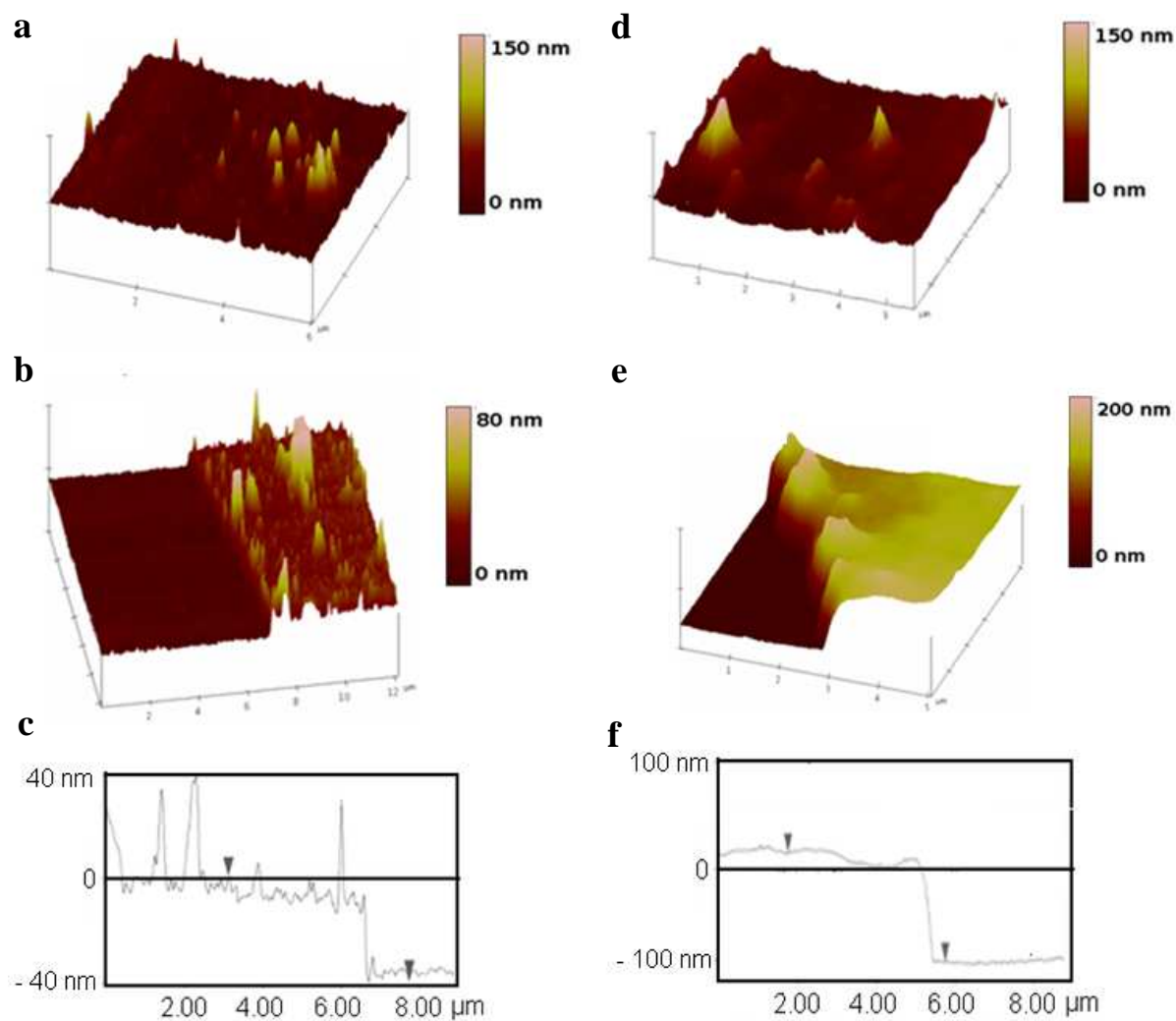
**Fig. S-5** Typical AFM height 3D images ( $12\ \mu\text{m} \times 12\ \mu\text{m}$ ) and their corresponding profilometric sections profiles of scratched (**a, b**) (PAA-*Alk*/*Az*-EG<sub>2</sub>-*Az*) and (**c, d**) (PAA-*Alk*/*Az*-EG<sub>13</sub>-*Az*) films obtained, in contact-mode, observed in liquid state at pH 3 (**a, c**) and pH 9 (**b, d**).



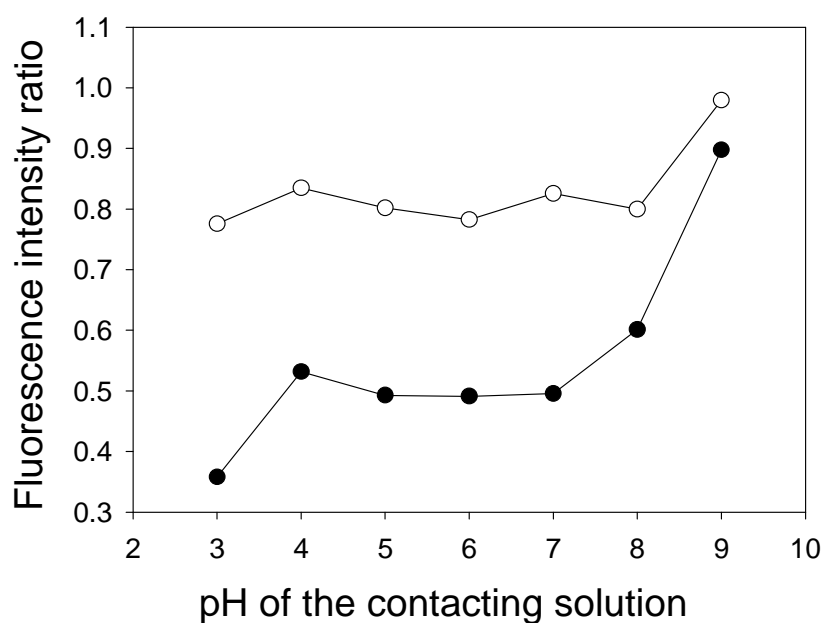
**Fig. S-6** (a) Fluorescence emission spectra of Az-bispyrene-Az molecule in solution measured at different pH with an excitation wavelength of 342 nm. The concentration of Az-bispyrene-Az was 0.2 mg/mL prepared in a 80:20 (v/v) water/ethanol solution. (b) Evolution of fluorescence intensities ratios of Az-bispyrene-Az in solution (●) between the excimer (480 nm) and a monomer (383 nm) peaks and (○) between the two monomer peaks (404 and 385 nm) as a function of pH. A calibration curve (red curve) can be plotted with the following equation: *Fluorescence Intensity Ratio of excimer* =  $0.0832 \times pH + 0.2347$  ( $R^2 = 0.977$ ).



**Fig. S-7 (a)** Evolution of the opposite of the normalized frequency shift, measured at 15 MHz ( $v = 3$ ) by EC-QCM-D, as a function of time in the presence of (PAA-*Alk*/*Az*-bispirene-*Az*) mixture (blue line) and of (PAA-*Alk*/azide-free bispirene) mixture (red line) during the CV application between -350 mV and +600 mV (vs Ag/AgCl, scan rate of 50 mV/s). **(b)** Evolution of the calculated thicknesses of PAA-*Alk*/*Az*-bispirene-*Az* film (blue line) and PAA-*Alk*/azide-free bispirene mixture (red line). The film thickness was calculated using the Voigt model (M. V. Voinova, M. Rodahl, M. Jonson, B. Kasemo, *Physica Scripta* **1999**, 59, 391) from EC-QCM-D data. The mixture solutions were prepared at pH 4 in water/ethanol 50/50 (v/v) solution with 0.5 g/L of polymer and of *Az*-bispirene-*Az* and 0.6 mM of CuSO<sub>4</sub>. The film is built on a PEI precoated surface.



**Fig. S-8** AFM 3D images of (a, d) non-scratched ( $6 \mu\text{m} \times 6 \mu\text{m}$ ) and (b, e) scratched ( $12 \mu\text{m} \times 12 \mu\text{m}$ ) PAA-*Alk*/*Az*-bispyrene-*Az* film with (c, f) their respective profilometric sections obtained in AFM height mode in liquid state at pH 3 (a, b, c) and pH 9 (d, e, f).



**Fig. S-9** Evolution of fluorescence intensity ratios of self-constructed PAA-*Alk*/*Az*-bispirene-*Az* film (●) between the excimer (435 nm) and a monomer (385 nm) peaks and (○) between the two monomer peaks (405 and 385 nm) as a function of pH. PAA-*Alk*/*Az*-bispirene-*Az* film was built on an ITO coated QCM crystal. The film was dipped into 15 mL Milli Q water at the different pH values during at least 2 h before measurement of the fluorescence intensity at an excitation wavelength of 432 nm.