

Covalent Layer-by-Layer Assemblies of Polyelectrolytes and Homobifunctional Spacers

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Synthesis of functionalized poly(acrylic acid) and bifunctionalized ethylene glycol spacer. All starting materials were obtained from commercial suppliers and were used without further purification. ^1H NMR and ^{13}C NMR spectra were recorded on a Bruker Advance DPX300 (300 MHz) or DPX400 (400 MHz) spectrometers. The NMR chemical shifts are reported in ppm relative to tetramethylsilane or tert-butanol (1.24 ppm). 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 μm , Merck, Darmstadt, Germany) was employed for flash chromatography. Low-resolution mass spectroscopy was performed on an Agilent MSD with Agilent 1200 SL HPLC equipped with DAD.

Synthesis of PAA-EG₁₃-Az 5%. PAA-EG₁₃-Az 5 % was synthesized according to Rydzek *et al.*¹

Synthesis of PAA-EG₄-Az 5%. Poly(acrylic acid) with Azide functionality (PAA-EG₄-Az) was synthesized by the following procedure: PAA (Mw: 250 KDa, 33 % in water) was freeze dried. Solid PAA (1 g; 13.8 mmol) was dissolved in 50 mL of DMF then, BOP (331 mg; 0.74 mmol) was added and the reaction mixture was stirred at room temperature for 10 minutes. 2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethanamine² (165 mg; 0.75 mmol) and DIEA (2.4 mL; 13.8 mmol) were added. After 1 hour under stirring, the solvent was evaporated under reduced pressure. The residue was dissolved into aqueous solution of NaOH (8 mL; 2 N) and evaporated under reduced pressure again. Solid product was washed with diethyl ether, dissolved in milli Q water (pH 7 with HCl 0.1 M), then dialyzed (Spectra/Por, cut off at MW

12000) for 12 h against milli Q water (pH 7 with NaOH 0.1 M). The final product was then dialyzed (Spectra/Por, cut off at MW 12000) for 24 h against milli Q water and freeze dried to give 1 g of product. By ^1H NMR, comparing the integration signal at 1.1-2.5 ppm (CH_2CHCO of polyacrylic acid) with that at 3.36 ppm (CH_2NHCO of PEG spacer), the effective degree of modification was found to be 5%.

^1H NMR (400 MHz, D_2O , δ): 3.72 (m, 0.6H, CH_2O), 3.64 (m, 0.12H, $\text{CH}_2\text{-CH}_2\text{-N}_3$), 3.52 (t, $J = 5.1$ Hz, 0.12H, $\text{CH}_2\text{-N}_3$), 3.38 (m, 0.12H, $\text{CH}_2\text{-NHCO}$), 2.1 (bm, 1H, CH-CH_2), 1.56 (bm, 2H, CH-CH_2)

IR: The peaks for Azido function can be identified at 2097 cm^{-1} .

Synthesis of PAA-EG₃-Alk 5%. PAA-EG₃-Alk 5 % was synthesized according to Rydzek et al.¹

Synthesis of NH₂-EG₁₃-Alk. Hexanoic acid (320 mg; 2.8 mmol) and BOP (1.3 g; 3.04 mmol) were dissolved in CH_2Cl_2 (15 mL). After 10 minutes, $\text{NH}_2\text{-EG}_{13}\text{-NH}_2$ (3.6 g; 6.11 mmol) and DIEA (0.53 mL; 3.04 mmol) in CH_2Cl_2 (5 mL) were added to the mixture. The reaction was stirred at room temperature for 3 h. Removal of the volatiles in vacuum provided a residue, which was purified by silica gel column chromatography with using 10 % MeOH in CH_2Cl_2 to afford 1.7 g of $\text{NH}_2\text{-EG}_{13}\text{-Alk}$ (Yield 82 %). R_f : 0.28 ($\text{CH}_2\text{Cl}_2/\text{MeOH}/\text{Et}_3\text{N}$ 9/1/0.1)

^1H NMR (400 MHz, CDCl_3 , δ): 6.3 (bs, 2H, NHCO), 3.69 (t, $J = 5.2$ Hz, 2H, $\text{OCH}_2\text{-CH}_2\text{-NH}_2$), 3.55 (m, 44H, $\text{OCH}_2\text{-CH}_2\text{O}$), 3.48 (t, $J = 5.2$ Hz, 2H, $\text{CH}_2\text{-CH}_2\text{-NHCO}$), 3.35 (q, 2H, $\text{CH}_2\text{-CH}_2\text{-NHCO}$), 3.03 (t, $J = 5.2$ Hz, 2H, $\text{CH}_2\text{-NH}_2$), 2.23 (t, $J = 6.9$ Hz, 2H, $\text{NHCO-CH}_2\text{-CH}_2$), 2.16 (dt, $J = 2.4$ Hz, $J = 6.9$ Hz, 2H, CH_2CCH), 1.91 (t, $J = 2.4$ Hz, 2H, CH_2CCH), 1.75 (quint, $J = 7.2$ Hz, 2H, $\text{CH}_2\text{-CH}_2\text{-CH}_2$)

^{13}C NMR (100 MHz, CDCl_3 , δ): 172.8 (NHCO), 84.2 (CH_2CCH), 71.0, 70.8, 70.7, 70.66, 70.4, 70.3, 70.2, 69.6, 68.5 (CH_2O , CH_2CCH), 40.9 ($\text{CH}_2\text{-NHCO}$), 39.6 ($\text{CH}_2\text{-NH}_2$), 35.5 ($\text{NHCO-CH}_2\text{-CH}_2$), 24.8 (CH_2CCH), 18.4 ($\text{CH}_2\text{-CH}_2\text{-CH}_2$)

Synthesis of PAA-EG₁₃-Alk 5%. Poly(acrylic acid) with alkyne functionality (PAA-EG₁₃-Alk) was synthesized by the following procedure: PAA (Mw: 250 KDa, 33 % in water) was freeze dried. Solid PAA (500 mg; 6.9 mmol) was dissolved in 50 mL of DMF then, BOP (220 mg; 0.5 mmol) was added and the reaction mixture was stirred at room temperature for 10 minutes. ($\text{NH}_2\text{-EG}_{13}\text{-Alk}$) (250 mg; 0.36 mmol) and DIEA (1.3 mL; 6.9 mmol) were added. After 1 hour under stirring, the solvent was evaporated under reduced pressure. The residue was dissolved into aqueous solution of NaOH (4 mL; 2 N) and evaporated under reduced pressure again. Solid product was washed with acetonitrile, dissolved in milli Q water (pH 7 with HCl 0.1 M) then dialyzed (Spectra/Por, cut off at MW 12000) for 12 h against milli Q water (pH 7 with NaOH 0.1 M). The final product was then dialyzed (Spectra/Por, cut off at MW 12000) for 24 h against milli Q water and freeze dried to give 0.7 g of PAA-EG₁₃-Alk. By ^1H NMR, comparing the integration signal at 3.2-3.85 ppm (NHCH_2 , CH_2NH , CH_2OCH_2 of PEG) with that at 1.1-2.5 ppm (CH_2CHCO of polyacrylic acid and $\text{CH}_2\text{CH}_2\text{CH}_2\text{CCH}$ of PEG), the effective degree of modification was found to be 5 %.

^1H NMR (400 MHz, D_2O , δ): 3.72 (m, 1.8H, CH_2O), 3.63 (m, 0.18H, $\text{CH}_2\text{-CH}_2\text{-NHCO}$), 3.4 (m, 0.18H, $\text{CH}_2\text{-NHCO}$), 2.39 (m, 0.18H, $\text{NHCO-CH}_2\text{-CH}_2$), 2.26 (m, 0.09H, $\text{CH}_2\text{-CH}_2\text{CCH}$), 2.08 (bm, 1H, CH-CH_2), 1.81 (m, 0.09H, $\text{CH}_2\text{-CH}_2\text{CCH}$), 1.55 (bm, 2H, CH-CH_2)

Synthesis of Az-EG₄-Az. 1,2-bis(2-azidoethoxy)ethane was synthesized according to Bonger et al.³

Synthesis of Az-EG₁₃-Az. HO-EG₁₃-OH (25 g; 41 mmol) and triethylamine (14 mL; 100 mmol) were dissolved in 500 mL of CH₂Cl₂. The mixture was cool down at 0°C, then methanesulfonyl chloride (7.75 mL; 100 mmol), was added drop by drop. After 1 night under stirring, cold water (10 mL) was added, solvent was removed and MsO-EG₁₃-OMs was partially purified by silica column chromatography with using 10 % MeOH in CH₂Cl₂ to afford 25g of MsO-EG₁₃-OMs as an sticky yellow oil, slightly contaminated by triethylamine salt. MsO-EG₁₃-OMs (25 g; 33.4 mmol) was dissolved in acetonitrile (100 mL). Sodium Azide (10.6 g; 164 mmol) was added and the mixture has been heated to reflux during 2 days. The solvent was removed under vacuum. The oil residue was purified by silica gel column chromatography using (CH₂Cl₂/MeOH 9.5/0.5), to afford 16 g of Az-EG₁₃-Az (Yield 74 %). R_f: 0.35 (CH₂Cl₂/MeOH 20/1)

¹HNMR (300 MHz, CDCl₃, δ): 3.53 (m, 48H, OCH₂), 3.25 (t, *J* = 5.9 Hz, 4 H, CH₂-N₃)

¹³CNMR (75 MHz, CDCl₃, δ): 70.9, 70.8, 70.3 (24 CH₂O), 50.9 (CH₂-N₃)

Synthesis of NH₂-EG₁₃-NH₂. Az-EG₁₃-Az (4.5 g; 7.02 mmol) and PPhe₃ (7.36 g; 28 mmol) were dissolved in 30 mL of THF. After five minutes under stirring, 1.5 mL of milli Q water was added to the mixture. The reaction was stirred under reflux over the night. The solvent was removed by evaporation under vacuum. The crude product was purified by silica gel column chromatography with CH₂Cl₂/MeOH/Triethylamine (9/1/0.1) then (7/3/0.2) as eluent to afford 3.6 g of NH₂-EG₁₃-NH₂ (yield 87 %) as a yellow oil. R_f: 0.05 (CH₂Cl₂/MeOH/Et₃N 9/1/0.1)

¹HNMR (300 MHz, CDCl₃, δ): 3.7 (m, 48H, OCH₂-CH₂O), 3.52 (t, *J* = 5.2 Hz, 4H, CH₂-CH₂-NH₂), 2.87 (t, *J* = 5.2 Hz, 4H, CH₂-CH₂-NH₂) ; 2.73 (bs, 4H, 2NH₂)

¹³CNMR (75 MHz, CDCl₃, δ): 73.1 (CH₂-CH₂-NH₂), 71.9, 71.1, 70.8, 70.4 (22CH₂O), 42.1 (CH₂-NH₂)

Synthesis of *Alk*-EG₁₃-*Alk*. Hexinoic acid (311 mg; 2.77 mmol) and BOP (1.3 g; 3.04 mmol) were dissolved in CH₂Cl₂ (15 mL). After 10 minutes, NH₂-EG₁₃-NH₂ (800 mg; 1.3 mmol) and DIEA (0.53 mL; 3.04 mmol) in CH₂Cl₂ (5 mL) were added to the mixture. The reaction was stirred at room temperature for 3 h. Removal of the volatiles in vacuum provided a residue, which was purified by silica gel column chromatography with using 10 % MeOH in CH₂Cl₂ to afford 900 mg of *Alk*-EG₁₃-*Alk* (Yield 79.5 %) as a yellow oil. R_f: 0.42 (CH₂Cl₂/MeOH 9/1)

¹HNMR (400 MHz, CDCl₃, δ): 6.55 (bs, 2H, NHCO), 3.56 (m, 44H, OCH₂-CH₂O), 3.48 (t, *J* = 5.2 Hz, 4H, CH₂-CH₂-NHCO), 3.37 (m, 4H, CH₂-CH₂-NHCO), 2.25 (t, *J* = 7.2 Hz, 4H, NHCO-CH₂-CH₂), 2.17 (dt, *J* = 2.4 Hz, *J* = 7.2 Hz, 4H, CH₂CCH), 1.93 (t, *J* = 2.4 Hz, 2H, CH₂CCH), 1.78 (quint, *J* = 7.2 Hz, 4H, CH₂-CH₂-CH₂)

¹³CNMR (100 MHz, CDCl₃, δ): 172.9 (NHCO), 84.2 (CH₂CCH), 71.0, 70.7, 70.4, 69.6 (CH₂O, CH₂CCH), 39.7 (CH₂NHCO), 35.5 (NHCO-CH₂-CH₂), 24.8 (CH₂CCH), 18.4 (CH₂-CH₂-CH₂)

Synthesis of *Alk*-EG₃-*Alk*. Hexinoic acid (665 mg; 5.93 mmol) and BOP (2.6 g; 5.95 mmol) were dissolved in CH₂Cl₂ (15 mL). After 10 minutes NH₂-EG₃-NH₂ (400 mg; 2.69 mmol) and DIEA (1.04 mL; 6 mmol) in CH₂Cl₂ (5 mL) were added to the mixture. The reaction was stirred at room temperature for 3 h. Removal of the volatiles in vacuum provided a residue, which was purified by silica gel column chromatography with using 10 % MeOH in CH₂Cl₂ to afford 600 mg of *Alk*-EG₃-*Alk* (Yield 67 %) as a white solid. R_f: 0.52 (CH₂Cl₂/MeOH 9/1)

¹HNMR (400 MHz, CDCl₃, δ): 6.26 (bs, 2H, NHCO), 3.59 (m, 4H, OCH₂-CH₂O), 3.53 (t, *J* = 5.2 Hz, 4H, CH₂-CH₂-NHCO), 3.43 (m, 4H, CH₂-CH₂-NHCO), 2.31 (t, *J* = 7.2 Hz, 4H,

NHCO-CH₂-CH₂), 2.21 (dt, $J = 2.4$ Hz, $J = 6.8$ Hz, 4H, CH₂CCH), 1.94 (t, $J = 2.4$ Hz, 2H, CH₂CCH), 1.82 (quint, $J = 6.8$ Hz, 4H, CH₂-CH₂-CH₂)
¹³CNMR (100 MHz, CDCl₃, δ): 173.2 (NHCO), 84.2 (CH₂CCH), 70.8, 70.5, 69.8 (CH₂O, CH₂CCH), 39.8 (CH₂NHCO), 35.6 (NHCO-CH₂-CH₂), 24.8 (CH₂CCH), 18.5 (CH₂-CH₂-CH₂), LRMS (ESI): ([M + Na]⁺ calcd 359.19, found 359.2

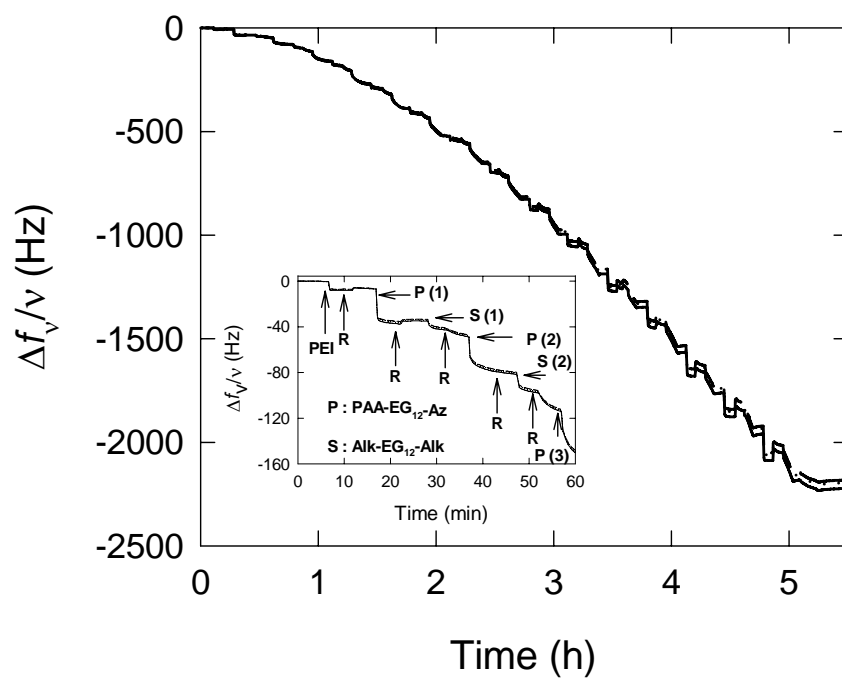


Figure S-1: Evolution of the normalized frequency shift, measured by QCM-D at 15, 25 and 35 MHz, during the buildup of PAA-EG₁₃-Az/Alk-EG₁₃-Alk film. Inset: P is the injection of PAA-EG₁₃-Az and S is the injection of Alk-EG₁₃-Alk; R means rinsing.

Table S-1: Median, first and third quartiles, mean and standard deviation (identical with Root Mean Square (RMS)) obtained from the distribution of the height of the film surface analyzed on the AFM images of PEI-(PAA-EG₁₃-Az/Alk-EG₁₃-Alk)_n-PAA-EG₁₃-Az film with $n = 4$, $n = 9$ and $n = 14$. Each AFM image is composed by 512×512 pixels, i.e. each distribution is derived from 262144 values of height.

<i>n</i>	Median (1st q., 3rd q.) (nm)	Mean (SD) (nm)
4	22.1 (18.2, 28.6)	26.7 (16.3)
9	49.3 (35.9, 73.0)	60.1 (36.7)
14	90.0 (64.0, 125.4)	102.5 (57.3)

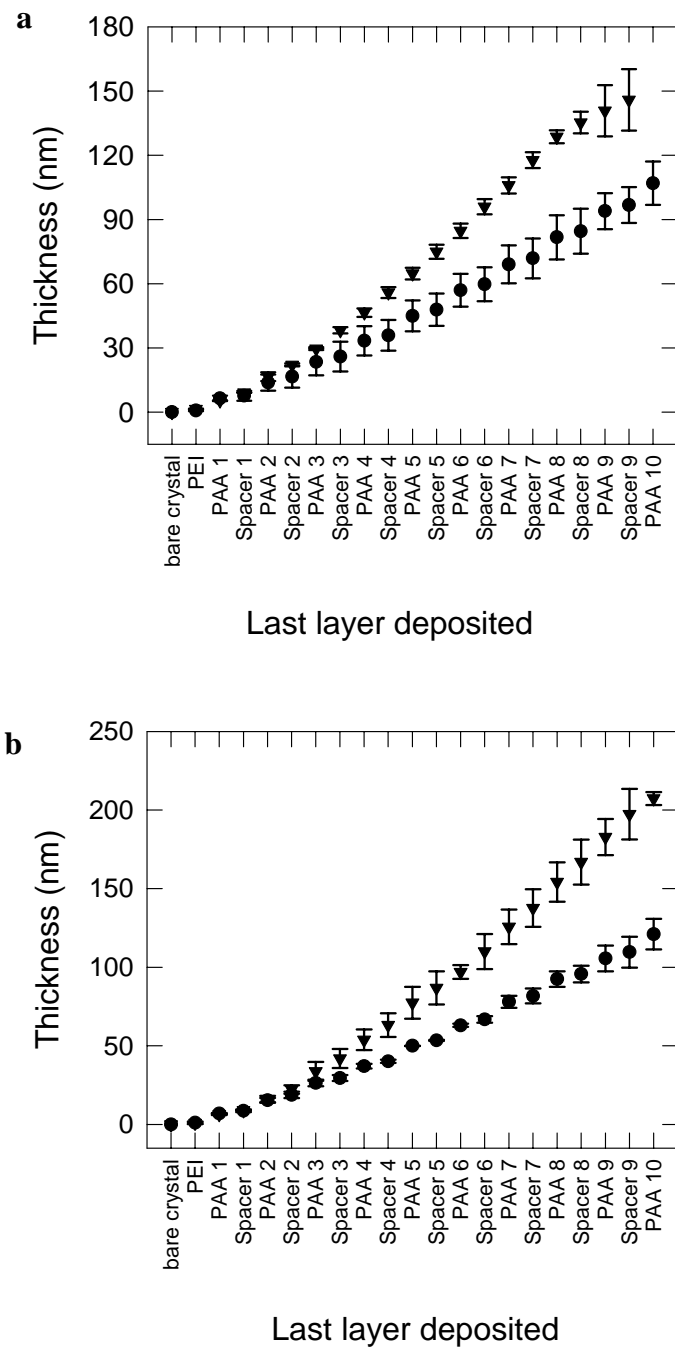


Figure S-2. Evolution of the thickness of PAA-EG_p-Az/Alk-EG_q-Alk films with (a) PAA-EG₃-Az/Alk-EG₃-Alk (circles) or with Alk-EG₁₃-Alk (triangles). (b): PAA-EG₁₃-Az with Alk-EG₃-Alk (circles) or with Alk-EG₁₃-Alk (triangles). The error bars represent the standard deviation of three independent experiments. The thickness is derived from the frequency shift for $\nu = 3$ using Sauerbrey's relation.

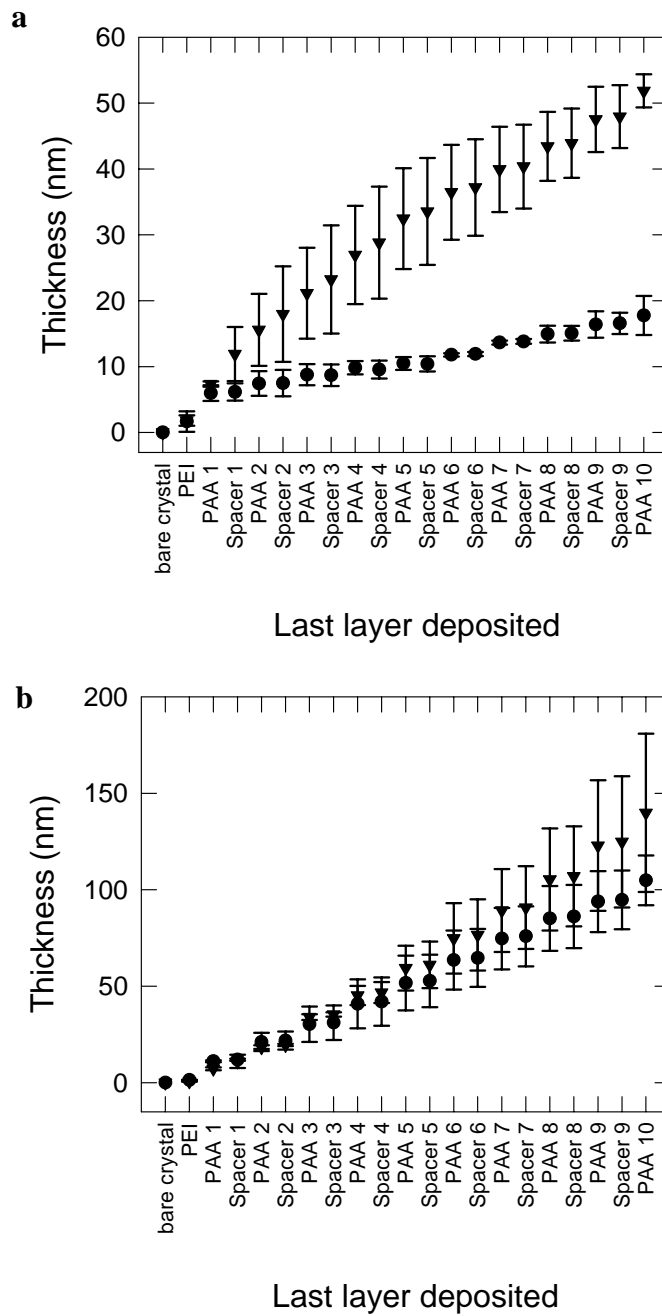


Figure S-3. Evolution of the film thickness built with PAA-EG_p-Alk and Az-EG_q-Az according to the length of the ethylene glycol chain. (A): PAA-EG₃-Alk with Az-EG₃-Az (circles) or with Az-EG₁₃-Az (triangles). (B): PAA-EG₁₃-Alk with Az-EG₃-Az (circles) or with Az-EG₁₃-Az (triangles). The error bars represent the standard deviation of three independent experiments. The thickness is derived from the frequency shift for $\nu = 3$ using Sauerbrey's relation.

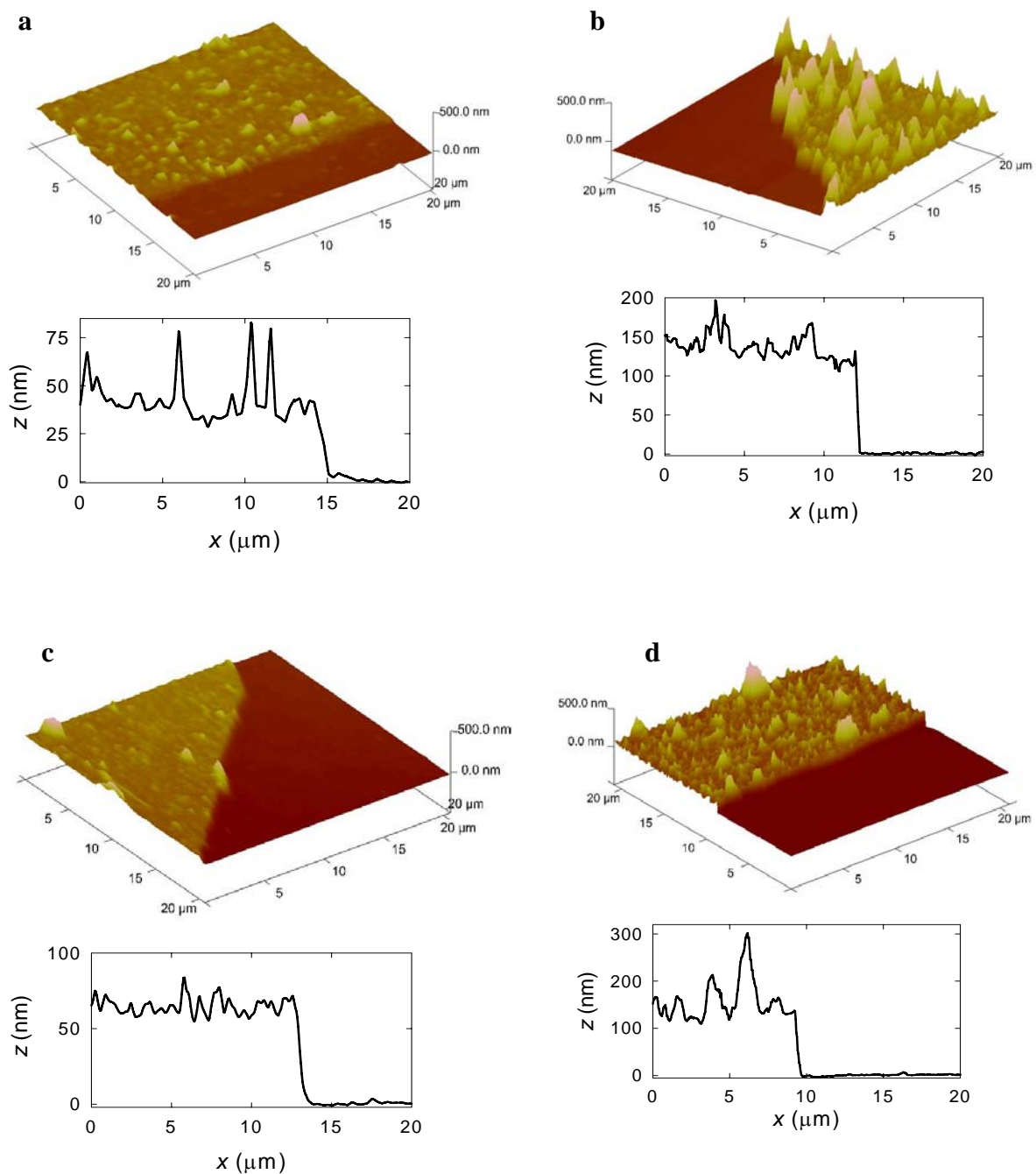


Figure S-4. AFM height mode scratched images in liquid state of various films accompanied with their profilometric sections for film thickness measurements: (a). PEI-(PAA-EG₃-Az/Alk-EG₃-Alk)₉-PAA-EG₃-Az, (b). PEI-(PAA-EG₃-Az/Alk-EG₁₃-Alk)₉-PAA-EG₃-Az, (c). PEI-(PAA-EG₁₃-Az/Alk-EG₃-Alk)₉-PAA-EG₁₃-Az, (d). PEI-(PAA-EG₁₃-Az/Alk-EG₁₃-Alk)₉-PAA-EG₁₃-Az.

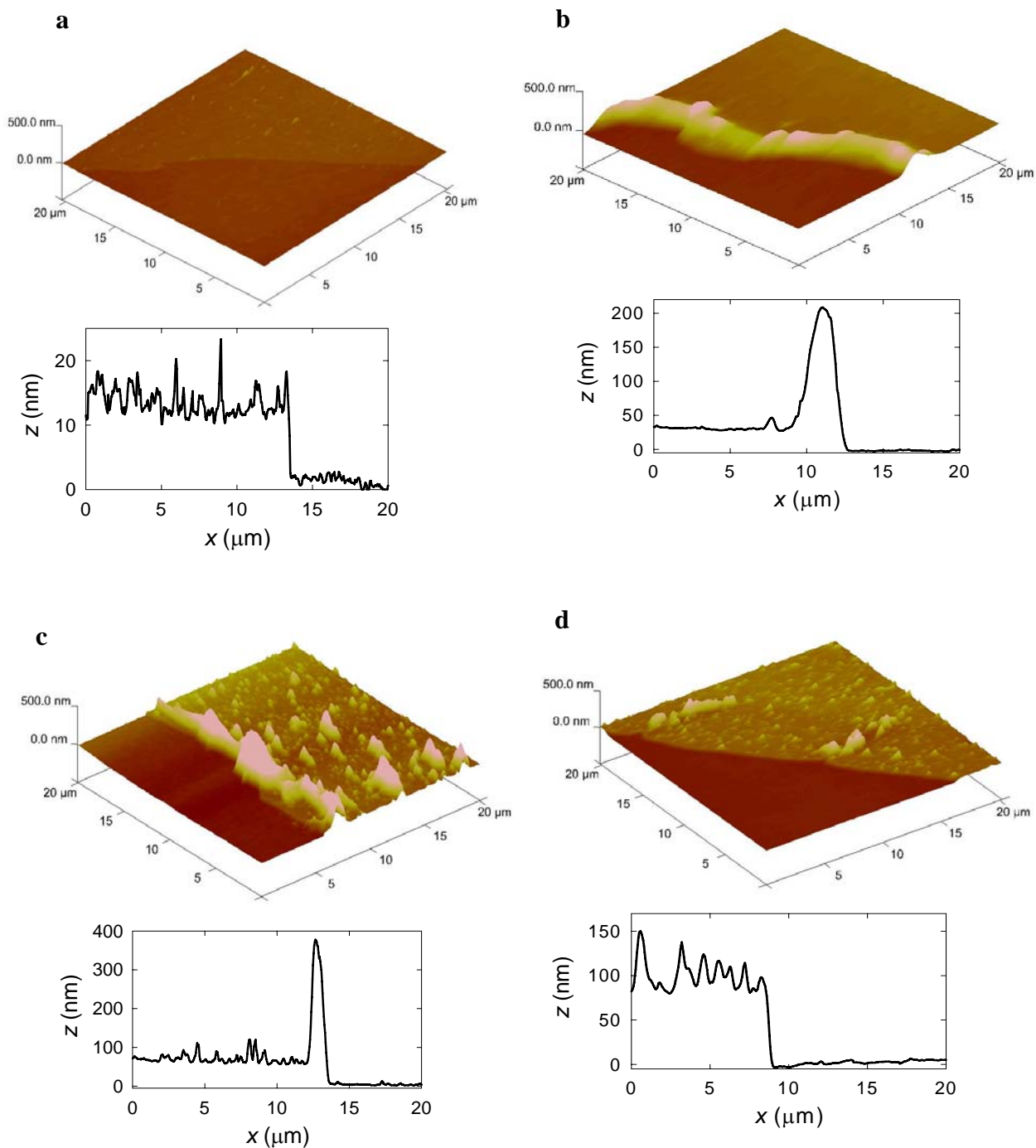


Figure S-5. AFM height mode scratched images in liquid state of various films accompanied with their profilometric sections for film thickness measurements: (a). PEI-(PAA-EG₃-*Alk*/Az-EG₃-Az)₉-PAA-EG₃-*Alk*, (b). PEI-(PAA-EG₃-*Alk*/Az-EG₁₃-Az)₉-PAA-EG₃-*Alk*, (c). PEI-(PAA-EG₁₃-*Alk*/Az-EG₃-Az)₉-PAA-EG₁₃-*Alk*, (d). PEI-(PAA-EG₁₃-*Alk*/Az-EG₁₃-Az)₉-PAA-EG₁₃-*Alk*

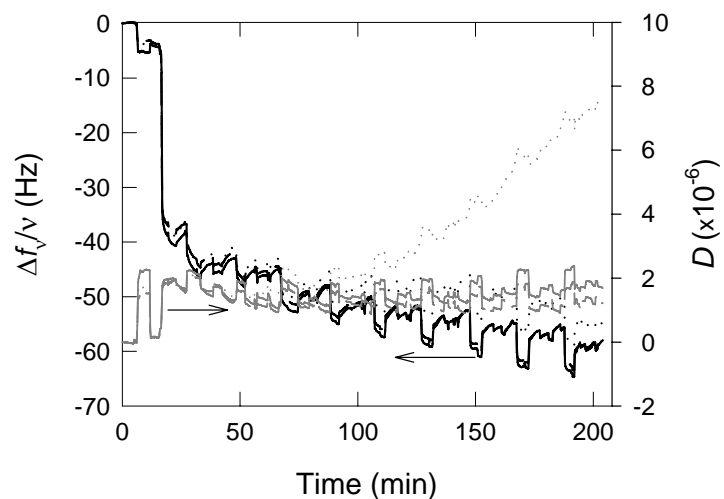


Figure S-6: Evolution of the normalized frequency shift, measured by QCM-D at 15, 25 and 35 MHz, during the buildup of PAA-EG₁₃-Az/PAA-EG₁₃-Alk film.

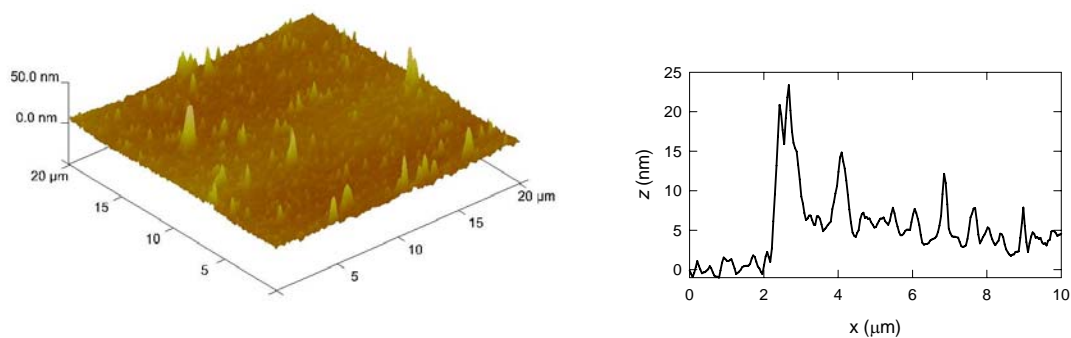
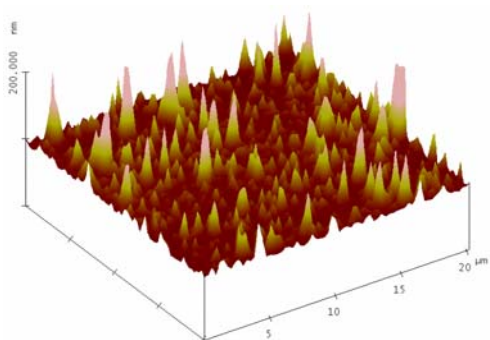
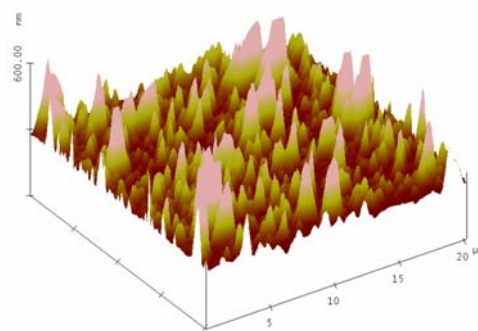


Figure S-7: AFM height mode images in liquid state of PEI-(PAA-EG₁₃-Az/PAA-EG₁₃-Alk)₉-PAA-EG₁₃-Alk film with the profilometric section of the scratch.

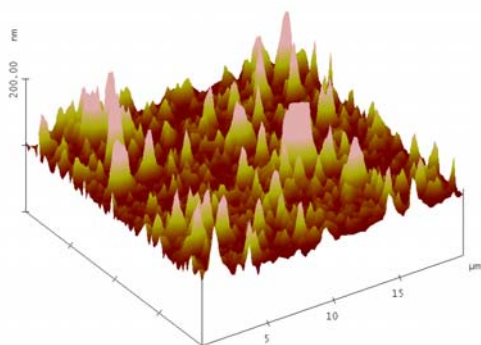
a PAA-EG₃-Az / Alk-EG₃-Alk



b PAA-EG₃-Az / Alk-EG₁₃-Alk



c PAA-EG₁₃-Az / Alk-EG₃-Alk



d PAA-EG₁₃-Az / Alk-EG₁₃-Alk

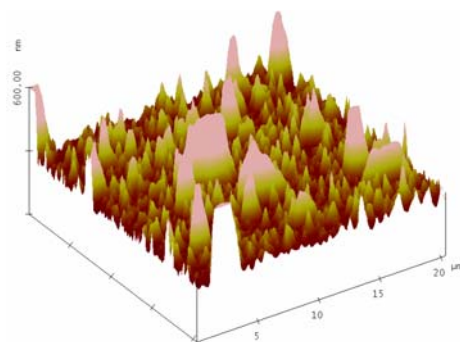


Figure S-8. AFM height mode images in liquid state of various films (a) PEI-(PAA-EG₃-Alk/Az-EG₃-Az)₉-PAA-EG₃-Alk, (b). PEI-(PAA-EG₃-Alk/Az-EG₁₃-Az)₉-PAA-EG₃-Alk, (c). PEI-(PAA-EG₁₃-Alk/Az-EG₃-Az)₉-PAA-EG₁₃-Alk, (d). PEI-(PAA-EG₁₃-Alk/Az-EG₁₃-Az)₉-PAA-EG₁₃-Alk.

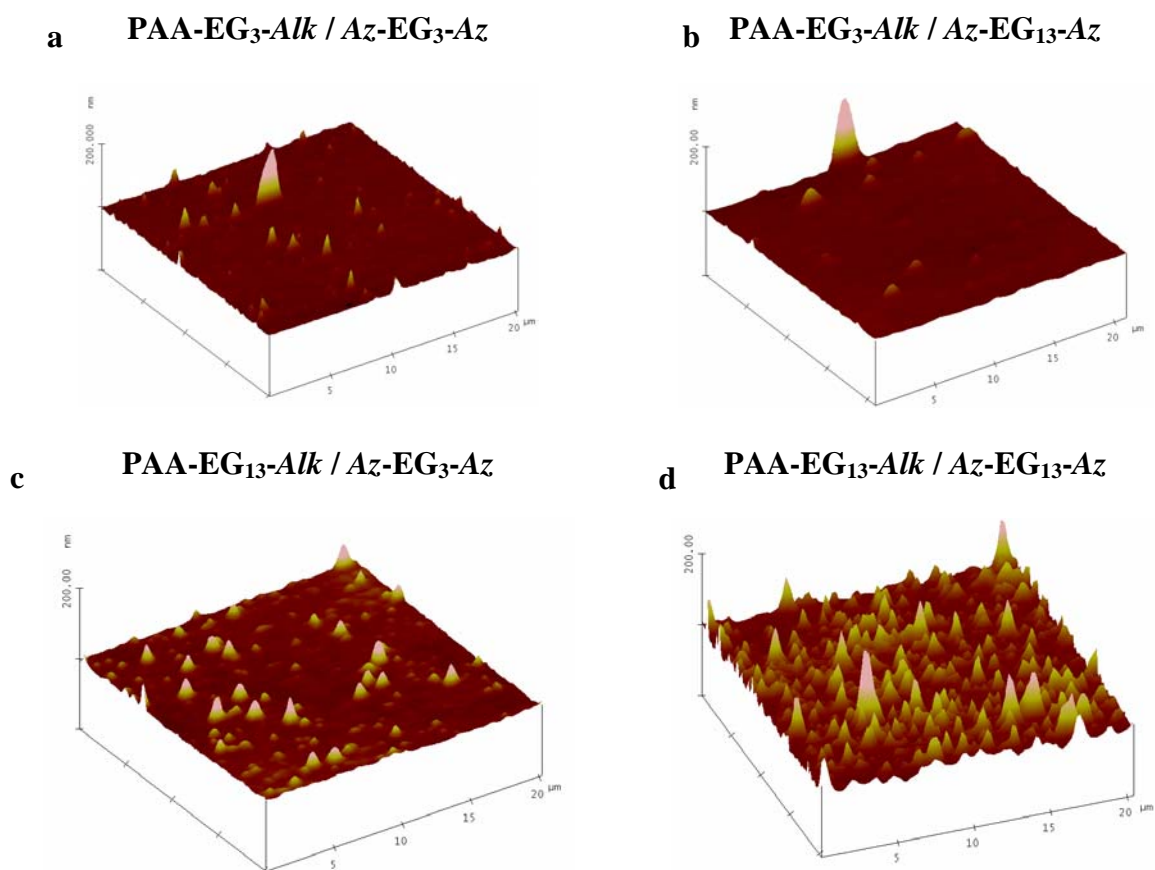


Figure S-9. AFM height mode images in liquid state of various films (a). PEI-(PAA-EG₃-Alk/Az-EG₃-Az)₉-PAA-EG₃-Alk, (b). PEI-(PAA-EG₃-Alk/Az-EG₁₃-Az)₉-PAA-EG₃-Alk, (c). PEI-(PAA-EG₁₃-Alk/Az-EG₃-Az)₉-PAA-EG₁₃-Alk, (d). PEI-(PAA-EG₁₃-Alk/Az-EG₁₃-Az)₉-PAA-EG₁₃-Alk.

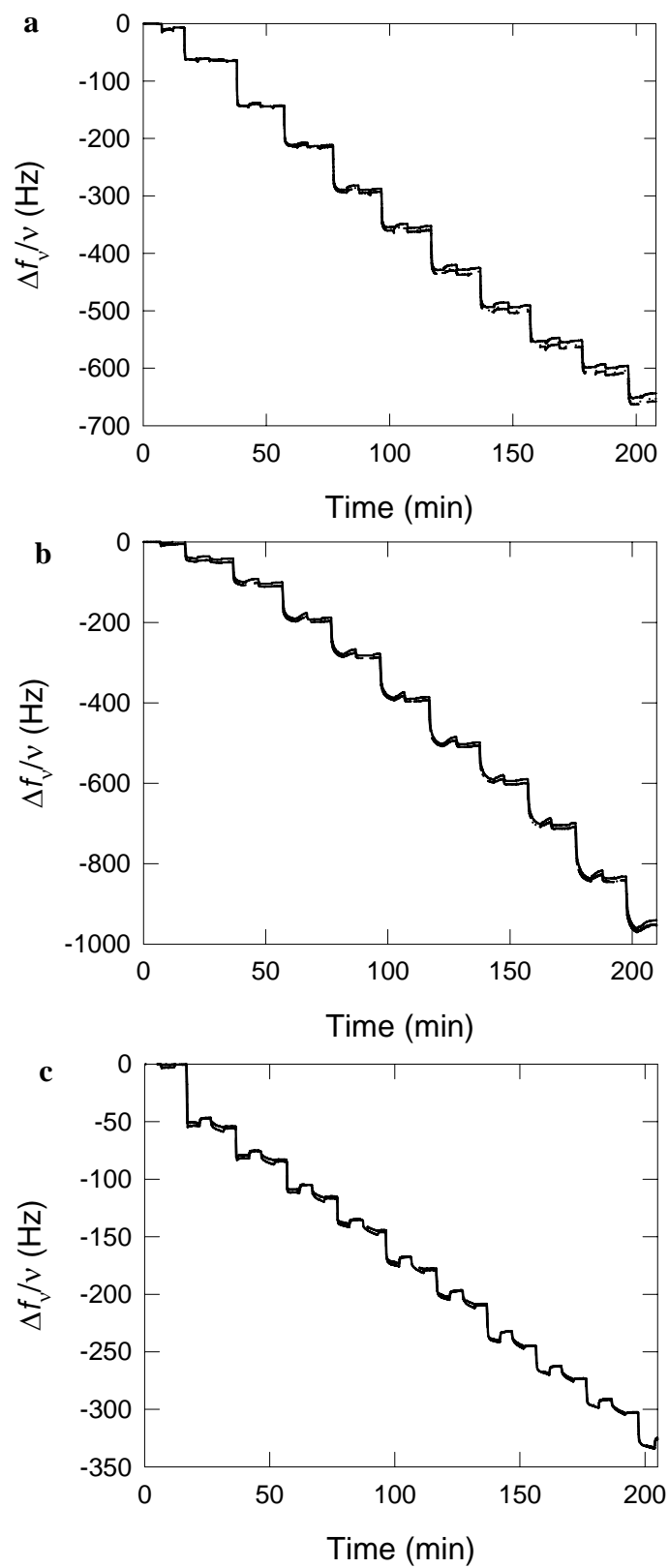


Figure S-10: Buildup of (a) PAA-EG₁₃-Alk/Az-EG₃-Az (b) PAA-EG₁₃-Alk/Az-EG₁₃-Az and (c) PAA-EG₁₃-Alk/Az-EG₅₀-Az at pH 3.5 followed by QCM-D.

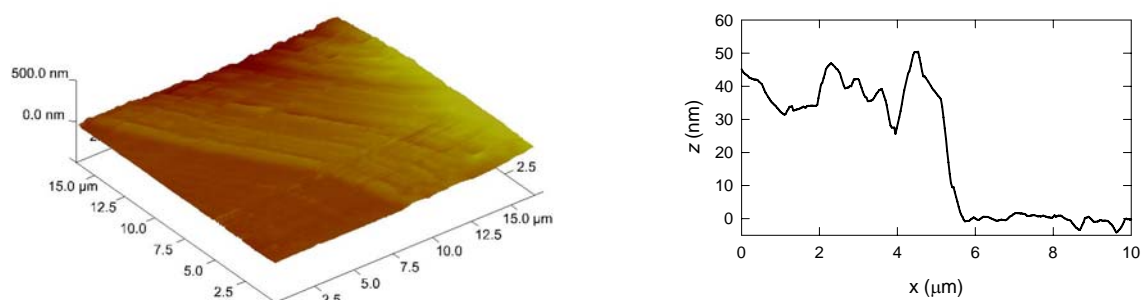


Figure S-11: AFM height mode scratched images in liquid state of PEI-(PAA-EG₁₃-*Alk*/A_z-EG₅₀-A_z)₉-PAA-EG₁₃-*Alk*

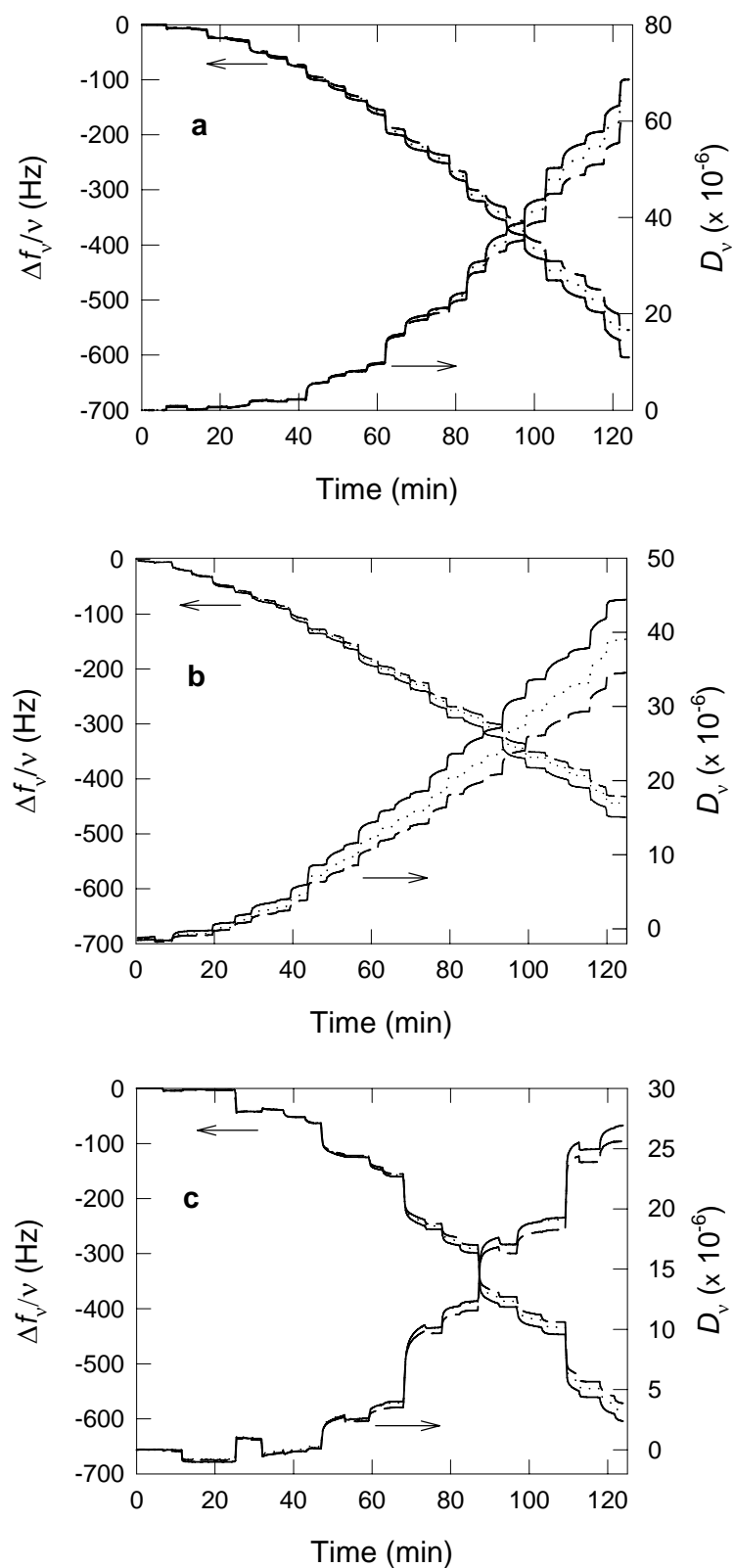


Figure S-12: Buildup of PEI-(PAA-EG₃-Az/Alk-EG₃-Alk)₅-PAA-EG₃-Az films with (a) 5%, (b) 9% and (c) 31% of functionality, monitored by QCM-D. In the three cases, the pH of the polymer buildup solutions was 4.4.

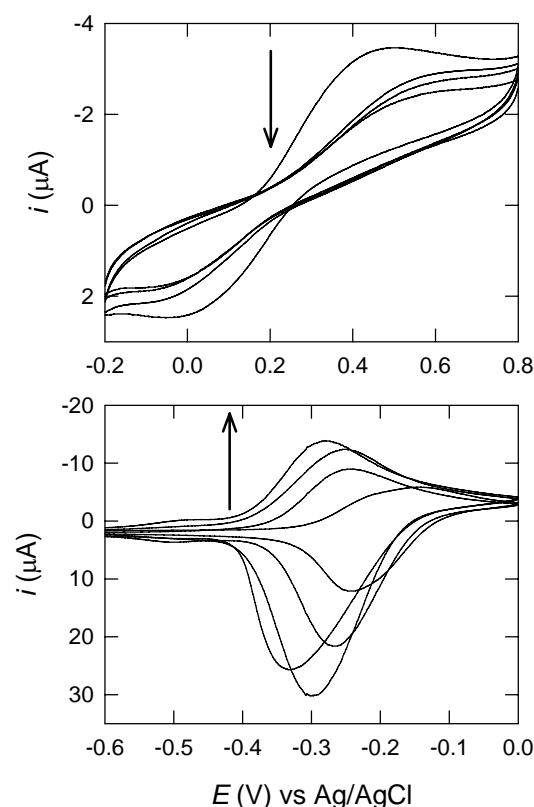


Figure S-13: Cyclic voltammograms of PEI-(PAA-EG₁₃-Alk/Az-EG₃-Az)₅-PAA-EG₁₃-Alk film built by click-chemistry on a GC electrode in contact with NaNO₃ 0.15 M supplemented with 1 mM of either Fe(CN)₆⁴⁻ ions (upper panel) or Ru(NH₃)₆²⁺ ions (lower panel) at pH increasing from ≈ 3.5 up to ≈ 10 . Sweep rate $\nu = 50$ mV/s. The arrows indicate the direction of increasing pH.

References

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