Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

Supplementary Information

Controlled surface density of RGD ligands for cell adhesion: evidence for a ligand specificity using QCM-D

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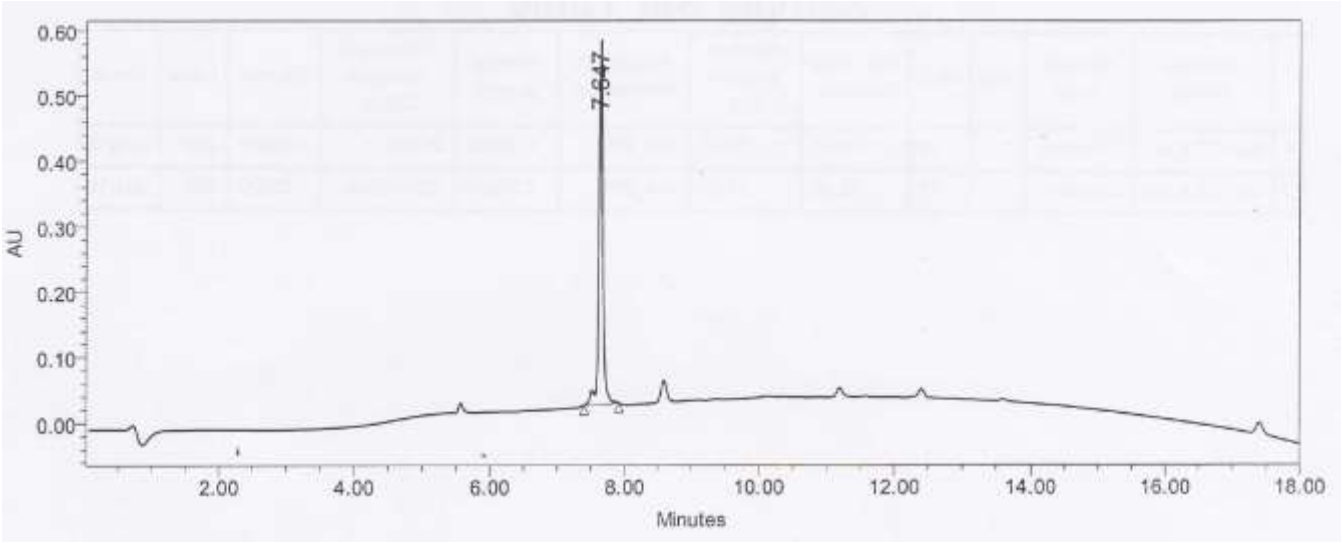
General procedures for analyses:

RP-HPLC analyses were performed on Waters equipment consisting of a Waters 600 controller, a Waters 2487 Dual Absorbance Detector and a Waters In-Line Degasser. The analytical column used was a Nucleosil 100 Å 5 µm C_{18} particles, 250 x 4.6 mm operated at 1 mL/min with linear gradient program in 30 min run time (routine program: 5% to 100 % B in 20 min). UV monitoring was performed at 214 nm. Solvent A consisted of H_2O containing 0.1% TFA and solvent B consisted of CH_3CN containing 9.9% H_2O and 0.1% TFA. Water was of Milli-Q quality. CH_3CN and TFA were of HPLC use quality.

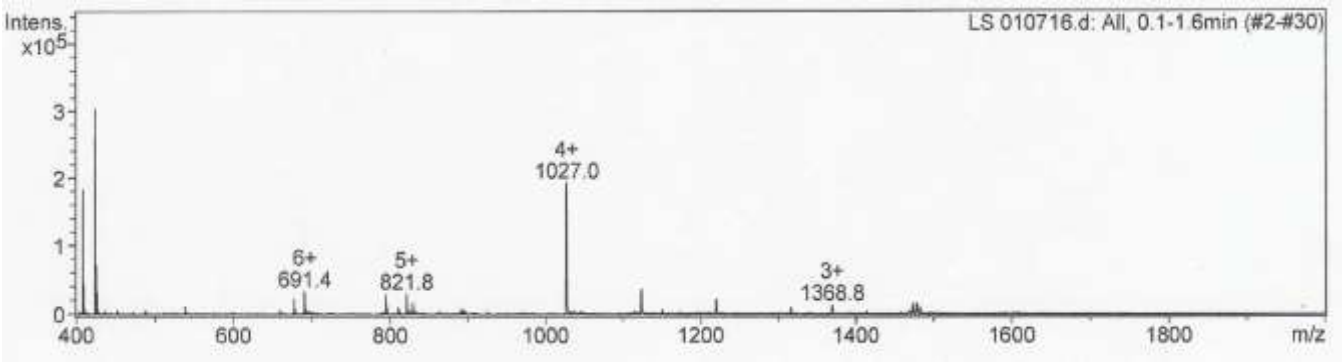
Electron spray ionization (ESI-MS) mass spectra were obtained on an Esquire 3000 (Bruker). The multiply charged data produced by the mass spectrometer on the m/z scale were converted to the molecular weight.

RP-HPLC profile and ESI analyses of 1

HPLC profile of **1**

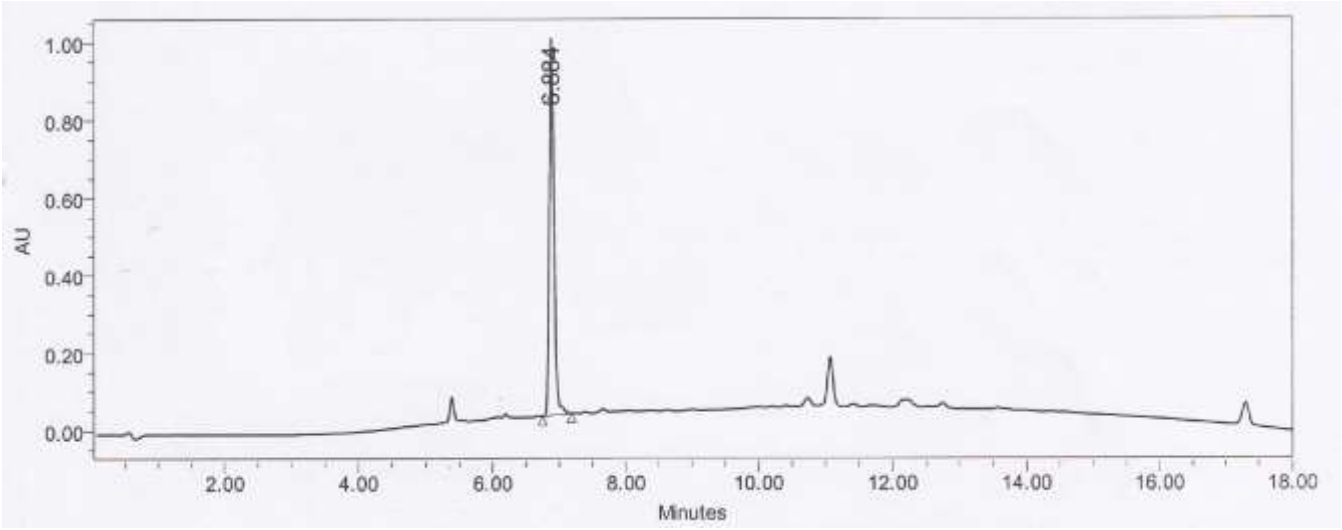


ESI analysis of **1**

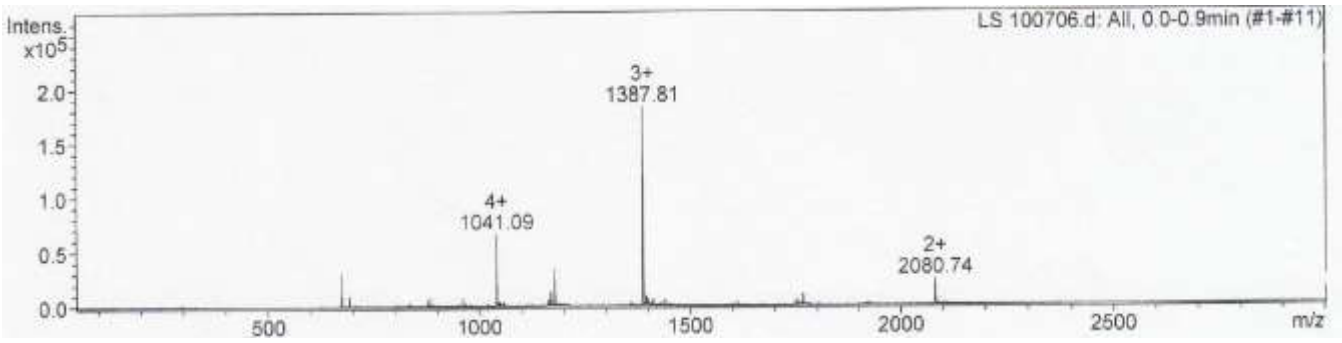


RP-HPLC profile and ESI analysis of 2

HPLC profile of **2**

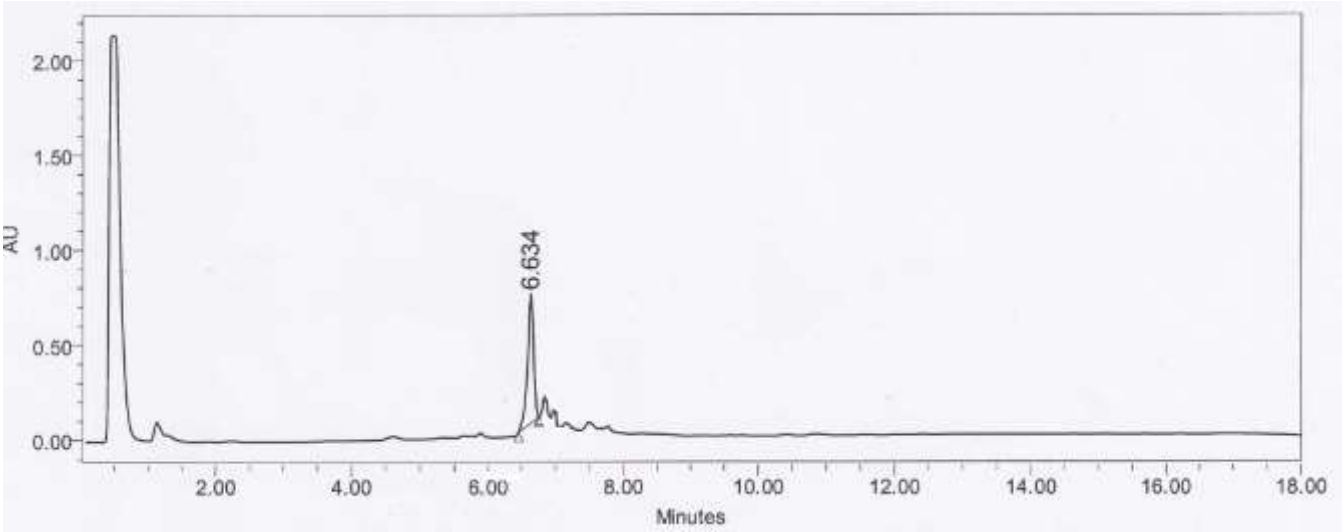


ESI analysis of **2**

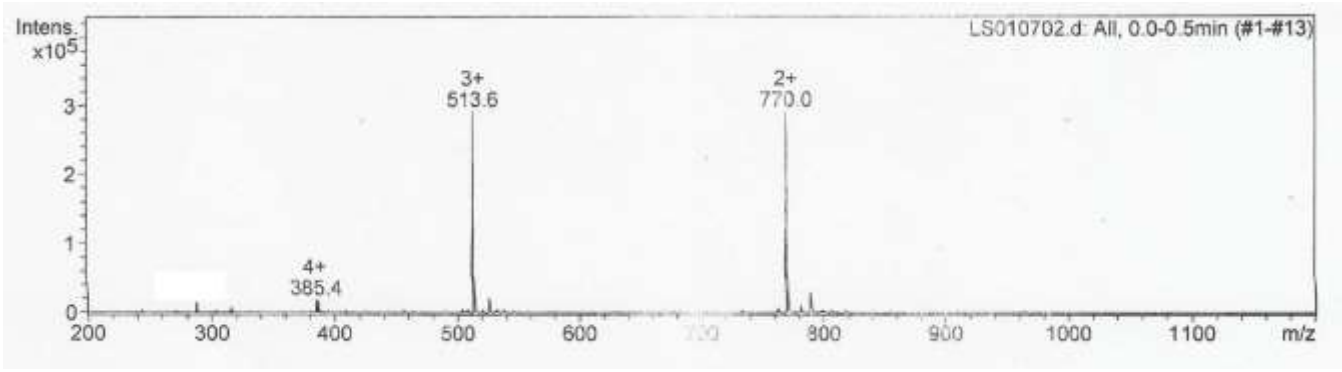


RP-HPLC profile and ESI analysis of 7

HPLC profile of **7**



ESI analysis of **7**



Supplementary data

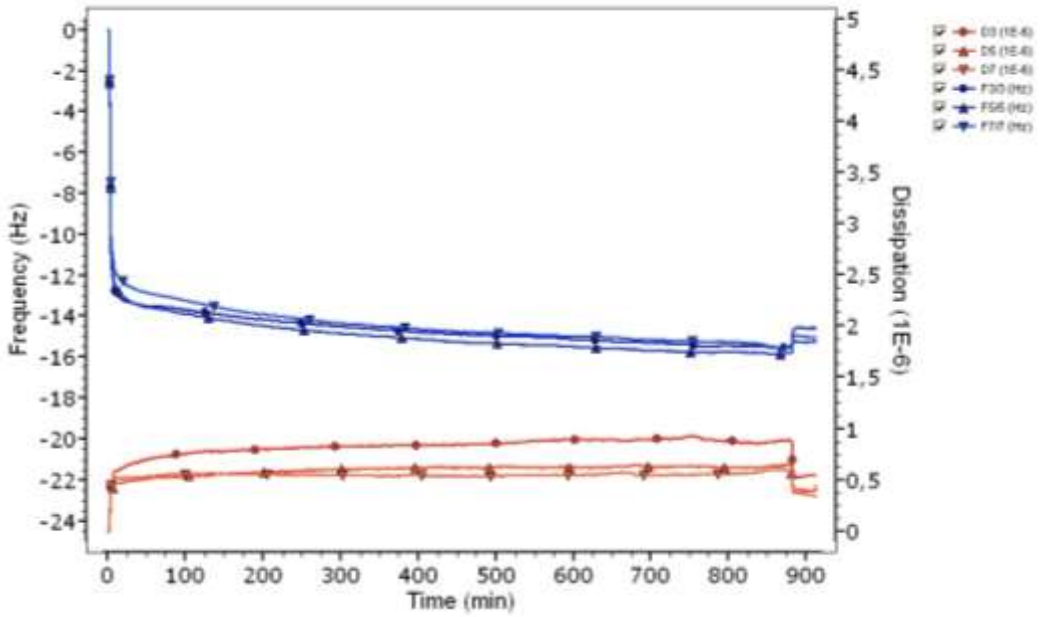


Figure S1: QCM-D profile characterizing the adsorption of SAM-EG_6Biot(50%) prepared from a 1 mM mixture of EG_4OH/EG_6Biot (50:50 molar ratio) in ethanol. (Frequency in blue and dissipation in red for the 3th, 5th and 7th overtones)

Figure S1 shows QCM-D profiles obtained during the adsorption of the thiols mixture (corresponding of 50:50 EG_4OH:EG_6OH) onto gold sensor using ethanol as solvent. After a fast negative shift in frequency, corresponding to the fixation of alkanethiolates onto the surface, the frequency decreases slowly during the following hours, the requisite time for the monolayer to rearrange into a highly ordered structure. Rinsing the measurement chamber with pure ethanol induces only a slight increase in frequency. As the change in dissipation is very low, Sauerbrey relation was applied to obtain an acoustical surface mass of 267 ng cm^{-2} for the SAM.

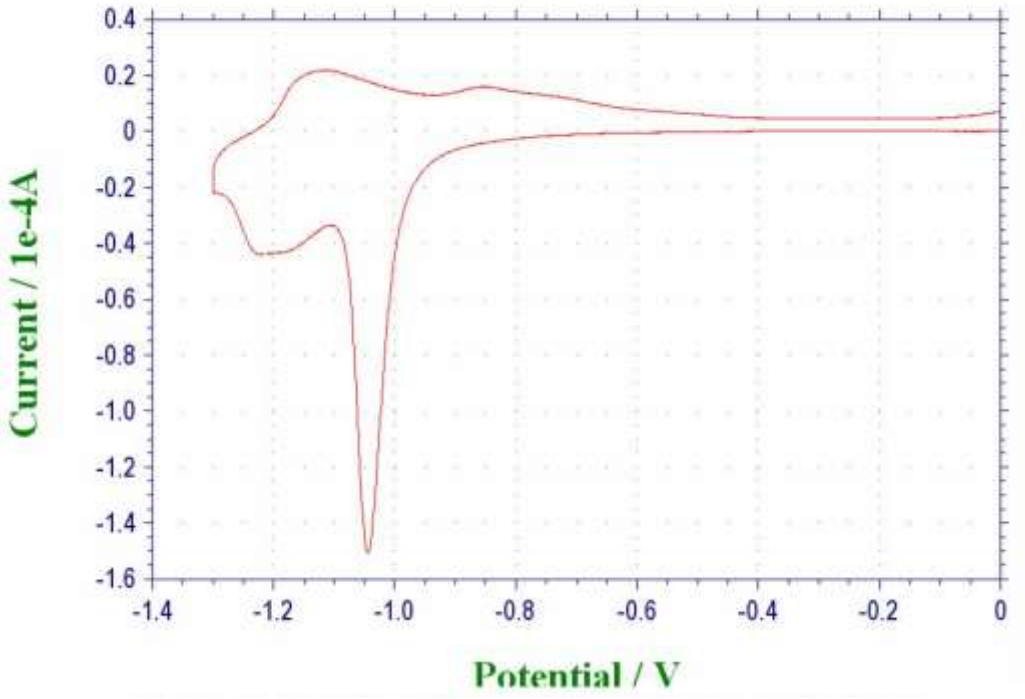


Figure S2: Cyclic voltammogram recorded during the reductive desorption of alkanethiolates EG_4OH on a gold quartz crystal in deaerated NaOH 0.5 M solution (scan rate: 20 mV s^{-1}).

Figure S2 shows a typical voltammogram recorded on the modified gold of quartz crystals during the reductive desorption of alkanethiolates EG_4OH in NaOH 0.5 M. A marked cathodic peak appears at -1.04 V. In addition, the voltammogram exhibits a small wave at more negative potential, the wave being centered at -1.17 V. Such additional wave has often been observed and its origin has been associated to the desorption from different binding sites on stepped surface sites of the polycrystalline Au and/or tightly packed domains of the adsorbed thiol[1,2]. In addition, we have observed that this wave is more marked in the case of scratched gold surfaces.

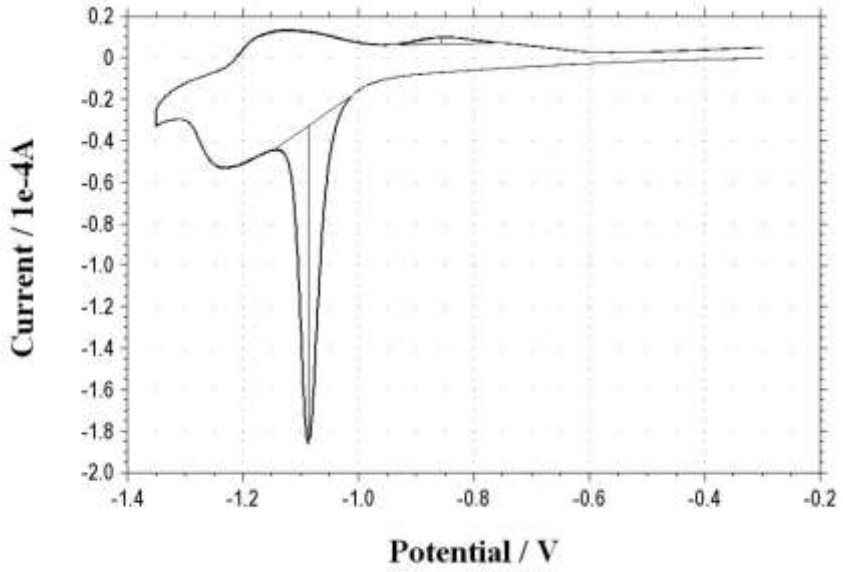


Figure S3: Cyclic voltammogram of reductive desorption of EG_6Biot 10 % SAM in deaerated 0.5 M NaOH aqueous solution at a sweep rate of 20 mV/s.

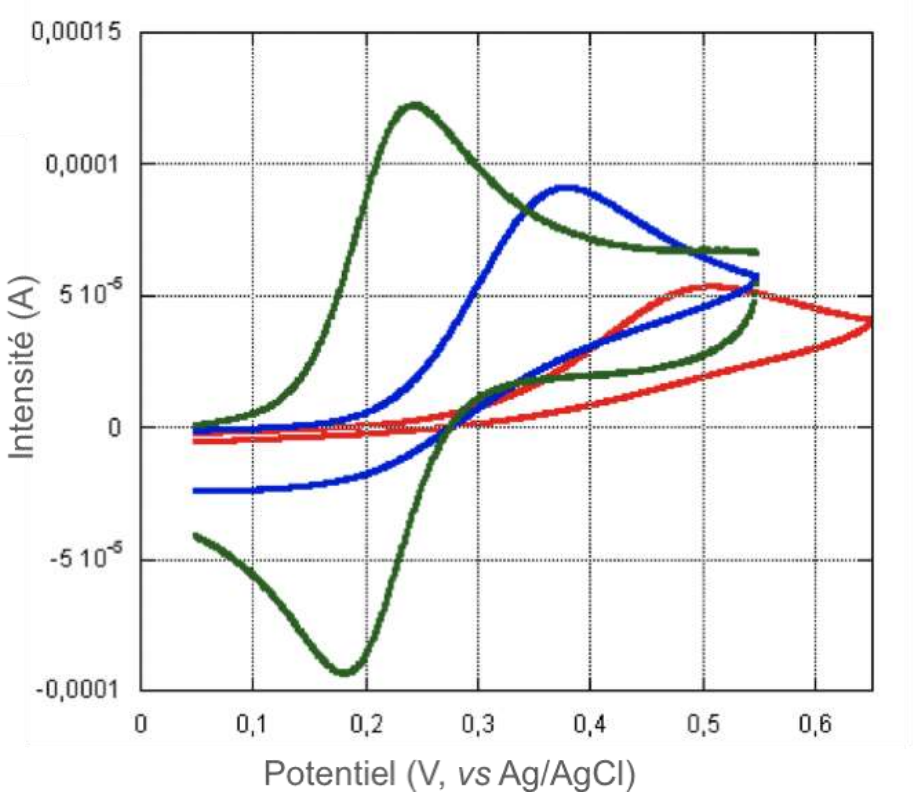
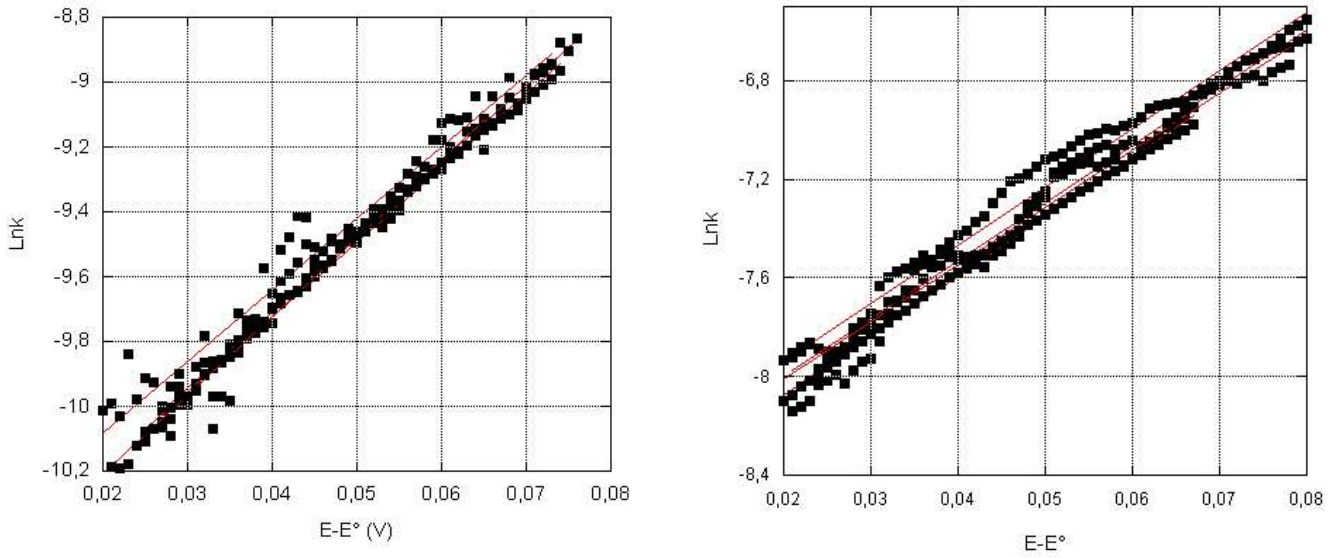


Figure S4: Comparison of the voltammetric responses of 5×10^{-4} M ferrocenemethanol at bare Au surface (green curve) and gold coated with a monolayer of pure EG_4OH (red curve) or monolayer of pure EG_6Biot (blue curve) in 0.1 M Na_2SO_4 (scan rate: 100 mV s^{-1}).

Electrochemical characterization of EG_4OH and EG_6Biot SAMs: Figure S6 shows Tafel plots of ln(k) as a function of E-E° for 5 different potential scan rates with $k = \frac{I}{nFAC}$ and $\ln k = \ln k° + \alpha_0 \frac{nF}{RT}(E - E°)$ (with E°= 0.212V). The faradaic current was obtained by subtracting the capacitive current recorded in pure electrolyte to the current measured in the presence of ferrocenemethanol (FcMeOH).



A B

Figure S5: Tafel plots for 0.5 mM FcMeOH oxidation recorded in 0.1 M Na_2SO_4 on EG_4OH (A) and EG_6Biot (B) SAM surfaces at potential scan rates of 0.2 V/s; 0.4 V/s; 0.8 V/s; 1V/s and 2V/s. The solid lines (red lines) represent the linear fitting.

The apparent rate-limiting electron transfer constants k° for FcMeOH oxidation onto EG_4OH and EG_6Biot SAM surfaces were determined from the averages of intercepts: (A) $k^0_{EG4OH} = 4.1\ 10^{-5} cm/s$ and (B) $k^0_{EG6Biot} = 3.3 10^{-4}\,cm/s$.

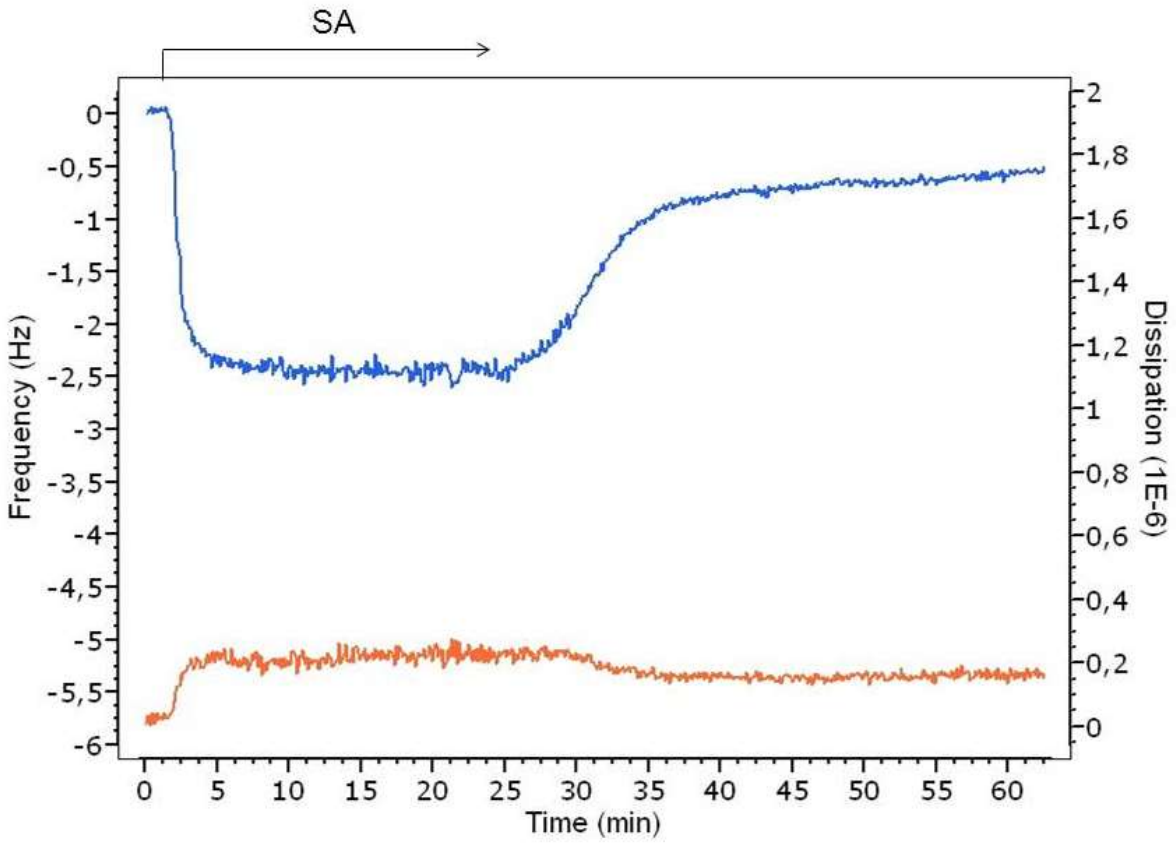


Figure S6: QCM-D profile illustrating the non-specific adsorption of SA (50 µg/mL) on single-component EG_4OH SAM.

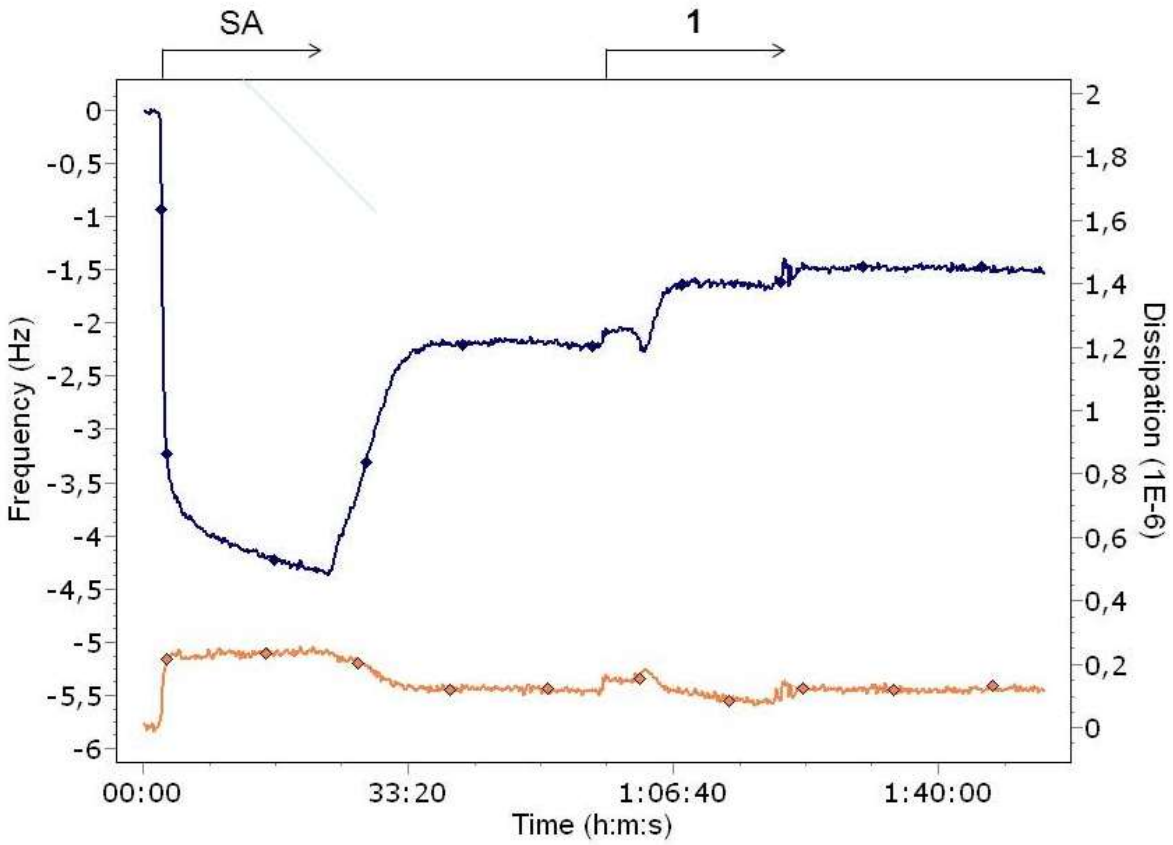


Figure S7: QCM-D profile characterizing the building of the biomolecular architecture on a SAM platform prepared with a thiol mixture containing 0.05 %. EG_6Biot, SA 50 µg/mL and compound **1** 0.1 µg/mL.

AFM images:

As a comparison with the QCM-D, the density of binding protein on the surface was evaluated by using AFM imaging. The study is executed on a flat planar template stripped gold surface as described in the literature.[3] The substrate was functionalized by using the same technique as for gold quartz crystal and imaged by AFM by under tapping mode. The probe used has a very low spring constant to protect the protein and consequently obtain more precise results on the determination of protein density on the surface.

In order to determine the density of SA on the surfaces prepared from 0.05% EG_6Biot solution the AFM images were investigated by using AxioVision software (Zeiss) considering the protein spot as discret objects.

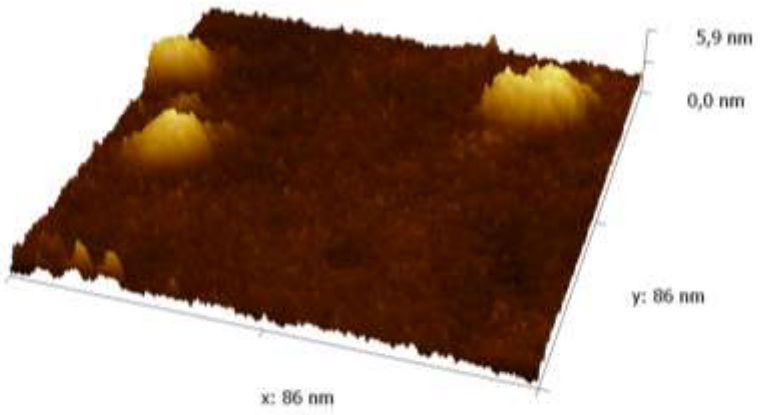


Figure S8: Tapping AFM images of SA grafted on biotinylated SAMs (formed from thiol mixture containing 0.05 % of EG_6Biot). Images display a surface area of 86 nm x 86 nm. The height of the biomolecular layer is 5.9 nm.

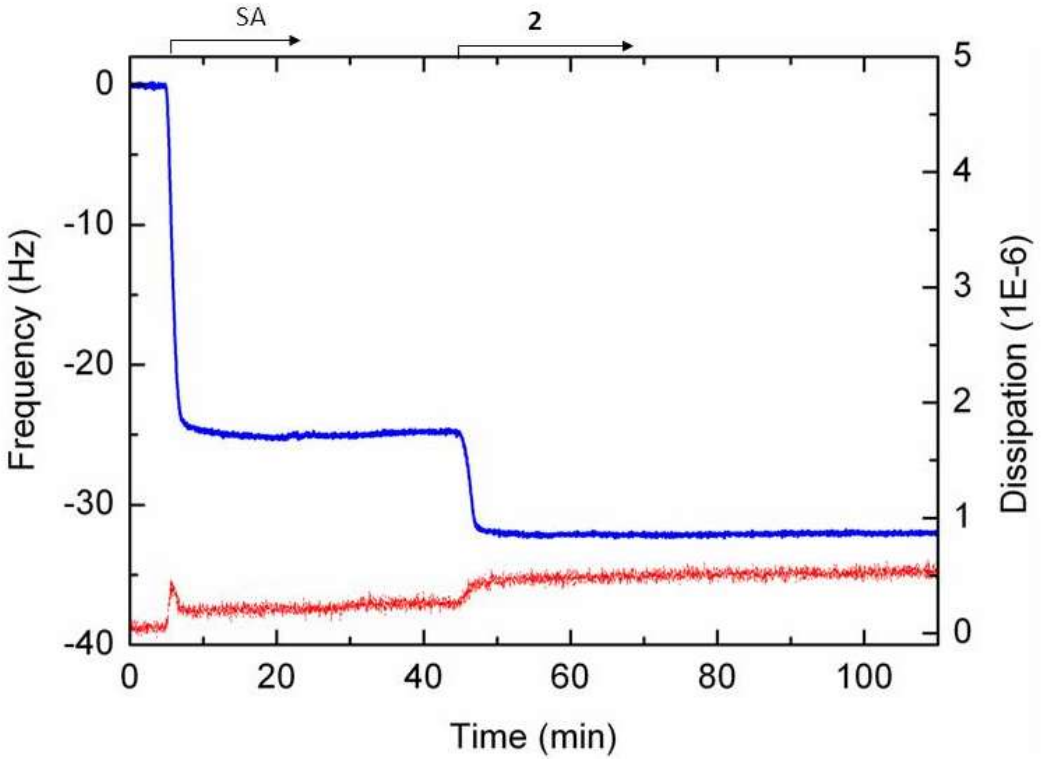


Figure S9: QCM-D profile (shifts in resonant frequency (blue curve) and in dissipation (red curve) *vs.* time) illustrating the building of the biomolecular assembly SA/**2** on SAM-Biot prepared with 10 % of EG_6Biot, SA: 50µg/mL and compound 1: 1 µg/mL (7th overtone data)

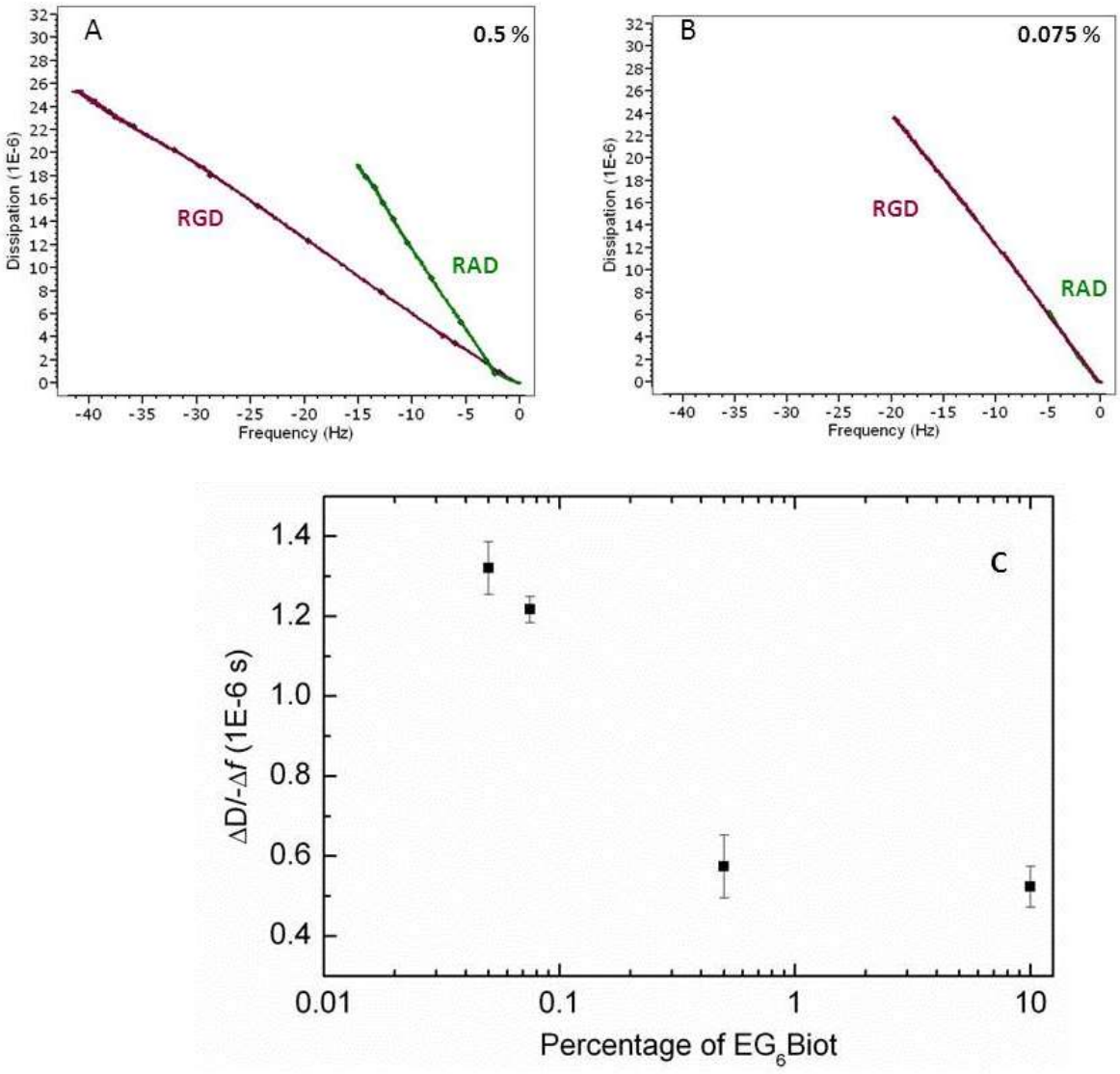


Figure S10: A-B) Δ*D* vs. Δ*f* plots corresponding to the first 40 min of HEK 293 (β3) cells flowing onto two different functionalized surfaces (SAM-EG_6Biot/SA/**1** in violet) and (SAM-EG_6Biot/SA/**2** (in green). C) Graph of Δ*D*/-Δ*f* ratio measured on the QCM-D profile for HEK β3 adhesion on SAM-EG_6Biot/SA/**1** as a function of the percentage of EG_6Biot in the thiol mixture used to prepare the SAM.

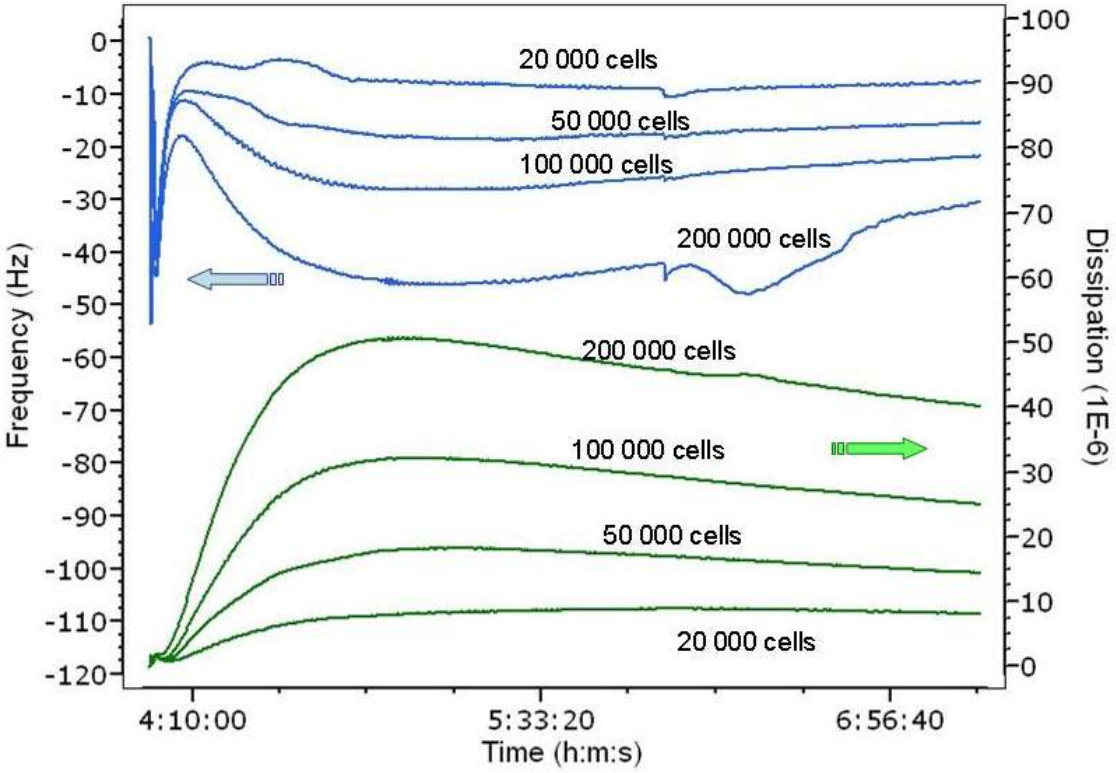


Figure S11: QCM-D profiles characterizing the seeding of a known amount of cells on RGD substrates prepared from 0.05 % of EG_6Biot in the SAM. These experiments were performed in open module (using E4 platform). The peak in frequency observed at time zero corresponds to the perturbation generated by the injection of the cells (20 to 200 µL of 10^6 cells/mL in 1 mL volume).

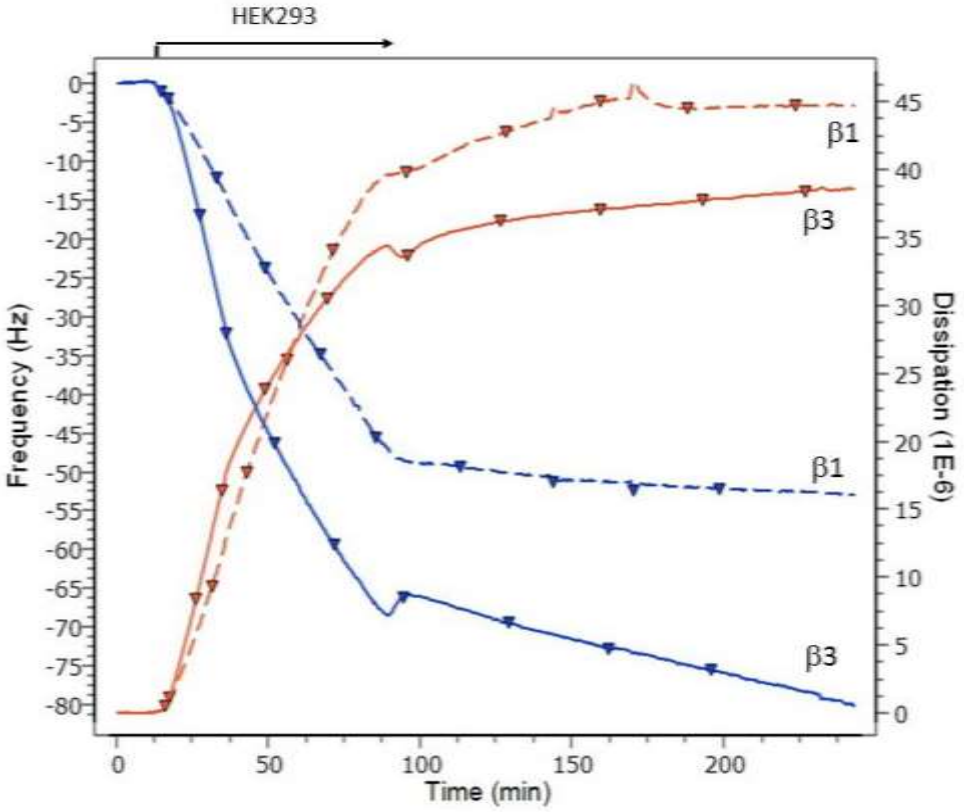


Figure S12: QCM-D profiles illustrating the cell adhesion for HEK293 (β_3) (solid line) cells and HEK293 (β_1) (dotted line) cells (100 000 cells/mL) flowing on SAM-EG$_6$Biot(10%)/SA/**1**. Frequency changes were presented as blue lines and dissipation as orange lines. The cellular medium is DMEM without serum. For clarity, only the 7th overtone is presented. The arrows indicate the time of injections (full: cells and empty: rinsing with DMEM).

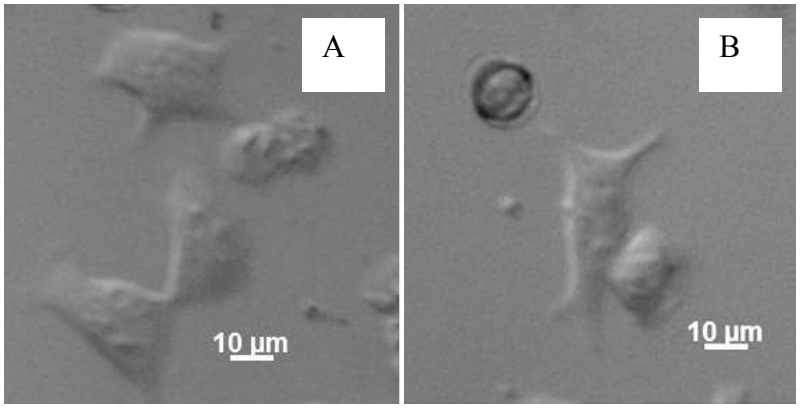


Figure S13: Optical microscopy images (DIC measurement) of HEK293 (β_3) (A) and HEK293 (β1) (B) on SAM-EG_6Biot (10%)/SA/**1** substrates (quartz crystal resonator) being captured during QCM-D experiment (Figure S12). Scale bar = 10 µm. Cell suspension (100 000 cell/mL) was injected at 100 µL/min

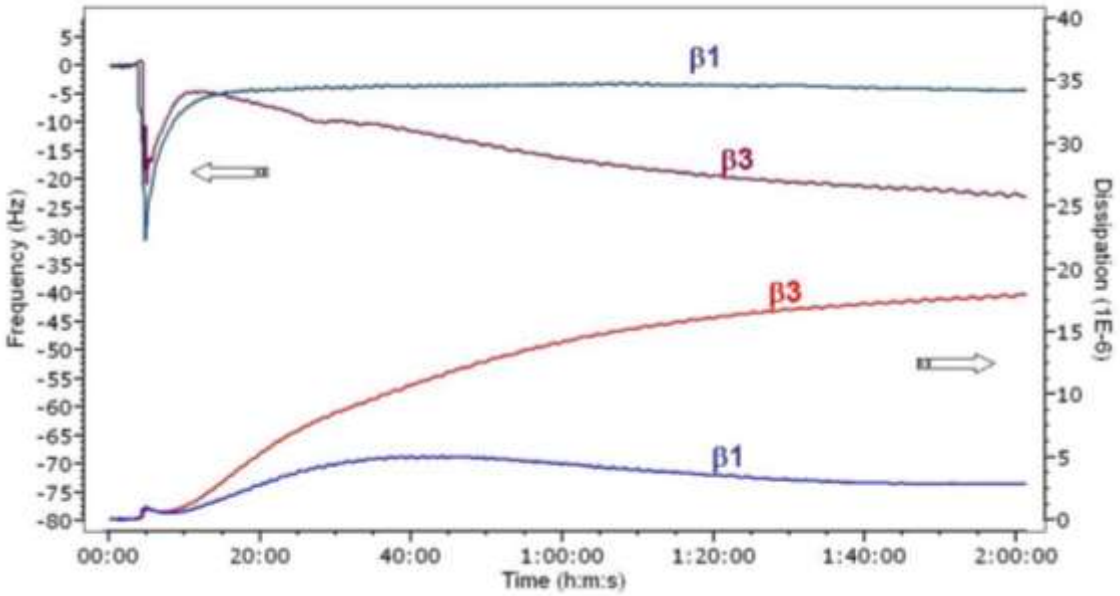


Figure S14: QCM-D profiles characterizing the seeding of 100 000 cells in static mode on RGD substrates prepared from 0.05 % of EG_6Biot.

Additional data: movies presenting cell adhesion

Movie 1: HEK293 (β3) cells adhesion and spreading on SAM-EG_6Biot(0.05%)/SA/**1** (scale bar: 50 µm)

Movie 2: HEK293 (β3) cell spreading on SAM-EG_6Biot(0.05%)/SA/**1** (scale bar: 20μA)

References:

[1] Kawaguchi, T.; Yassida, H.; Shimadzu, K.; Porter, M.D. *Langmuir* **2000**, 16, 9830-9840.

[2] Mokrani, C.; Fatisson, J.; Guérente, L.; Labbé, P. *Langmuir* **2005**, 21, 4400-4409.

[3] a) Hegner, M.; Wagner, P.; Semenza, G. Surf. Sci. 1993, 291, 39-46. (b) Wagner, P.; Hegner, M.; Güntherodt, H.-J.; Semenza, G. Langmuir 1995, 11, 3867-3875.