Supporting Information

Reversible biomechano-responsive surface based on Green Fluorescent Protein genetically modified with unnatural amino acids

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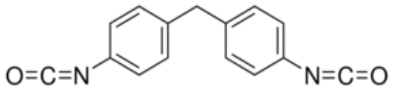
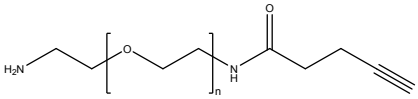
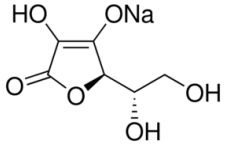
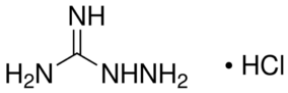
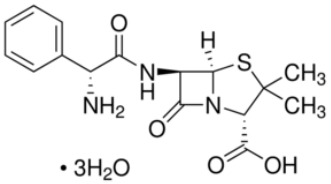
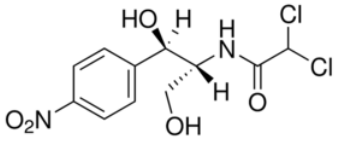
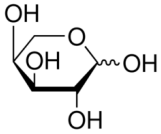
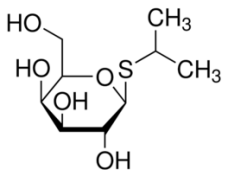
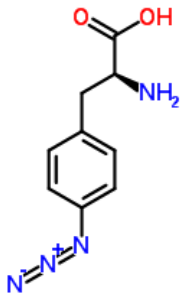
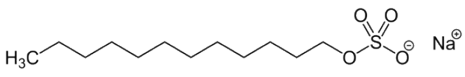
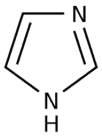
Material and methods

1. List of chemicals

All chemicals used in this work are gathered in the following table.

Name and Acronyme	Molecular structure	Mw (g.mol-1)	Supplier	CAS number
4,4'-Methylenebis(phenyl isocyanate) MDI		250.25	Sigma-Aldrich	101-68-8
alpha-Amino-omega-propargylacetamido poly(ethylene glycol)		3000	Iris-Biotech	--
Copper sulfate pentahydrate	$CuSO_4.5H_2O$	249.69	Sigma-Aldrich	7758-99-8
(+)-Sodium L-ascorbate		198.11	Sigma-Aldrich	134-03-2
Aminoguanidine hydrochloride		110.55	Sigma-Aldrich	1937-19-5
Ampicillin		403.45	AppliChem	7177-48-2
Chloramphenicol		323.13	Roth	56-75-7
L-(+)-Arabinose		150.13	Roth	5328-37-0

Isopropyl β-D-1-thiogalactopyranoside		238.30	Gerbu	367-93-1
p-azidophenylalanin (*p*AzF)		206.20	BACHEM	33173-53-4
1-dodecanthiol	$CH_3(CH_2)_{10}CH_2SH$	202.40	Sigma-Aldrich	112-55-0
Sodium dodecyl sulfate		288.37	Roth	151-21-3
Imidazole		68.08	AppliChem	288-32-4
Acrylamide-solution			AppliChem	

2. UVO set-up

Oxidation treatments of the PDMS surface was done by using a "UV-Ozone ProCleaner™ Plus" purchased from Bioforce-Nanosciences.

3. Contact angle measurements

Contact angle measurements were performed with a DIGIDROP-GBX® coupled with a camera by using 6 uL pure water droplets. Values result from the average of three independent measurements done at different area of the PDMS surface studied.

4. Infrared spectroscopy

All the experiments were performed by using an FTIR spectrometer (Vertex 70, Bruker, Billerca, MA, USA) equipped with a deuterated triglycine sulfate (DTGS) detector. Total reflectance ATR germanium crystal accessory was used for IR measurements. All spectra acquisitions were performed at 2 cm^{-1}resolution over 20 scans within a range of 4000-800 cm^{-}

[1]. This analytical method was performed to observe the hydroxyl group formation (silanol groups) on the PDMS surface at various UVO exposition times. Immediately after the UVO treatment, the oxidized PDMS was analyzed by IR: the surface was directly brought into contact with the ATR diamond crystal, and the spectra were recorded.

5. Fluorescence microscopy

The fluorescence measurements described in this work were performed by using an inverted light microscope (images were captured using Nikon Elipse TE200 with 63× PL APO (1.4 NA) objective equipped with Nikon Digital Camera (DS-Q11MC with NISElements softwares), and processed with ImageJ (http://rsb.info.nih.gov/ij/). The mercury lamp operates between 470 nm and 490 nm for excitation and above 500 nm for detection. The image analysis was performed by using ImageJ software (Raspband, W. S, ImageJ, U.S. National institutes of Health, Bethesda, USA). The measured fluorescence emission corresponds to means ± standard deviations from at least three independent experiments.

6. Elecstrospray mass spectroscopy

Mass spectrometric measurements were performed on an LTQ Orbitrap XL mass spectrometer (Thermo Fisher Scientific, Bremen, Germany) coupled to an Agilent 1200 nanoflow-HPLC (Agilent Technologies GmbH, Waldbronn, Germany). Samples were applied directly onto the column without pre-column. A gradient of A [0.5% acetic acid in water] and B [0.5% acetic acid in 80% ACN/water] with increasing organic proportion was used for peptide separation. The flow rate was 250 nl/min and for sample application 500 nl/min. The mass spectrometer was operated in the data-dependent mode and switched automatically between MS (max. of 1 x106 ions) and MS/MS. For MS/MS, wideband activation was enabled. The mass range for MS was m/z = 350 to 2,000 and signal threshold was 1,000. The resolution was set to 60,000. Data analysis was performed using Mascot.

7. Stretching devices

A homemade stretching device was designed and elaborated in our group (Figure S-1). It permits to stretch surface-modified PDMS substrates in a uniaxial direction up to 100% elongation.

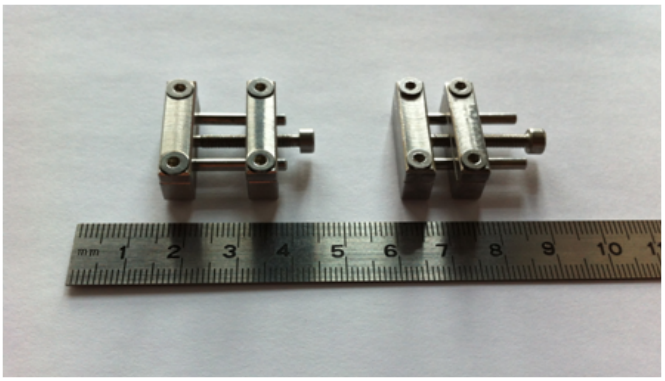


Figure S-1: Picture of the stretching devices. The silicone sheets are placed between the two clamps**.**

The stretching degree is defined as:

$$\alpha = \frac{(l - l_0)}{l_0} \cdot 100$$

With l_0 and l corresponding respectively to the initial and the stretched length of the silicone substrate. All the stretching experiments were performed at room temperature and with PDMS surface kept always wet (never dried).

8. SDS-PAGE

Gel preparation

Solutions for the preparations of gels were prepared according to Laemmli et *al.*[1] The separating gel was 15%, the stacking gel was 6%. The electrophoresis was performed in a Mini-Protean Tetra Cell (Bio-Rad Laboratories). The separating gel was firstly filled in, and carefully overlaid with isopropanol to allow a flat surface. After polymerization, the alcohol was removed. The stacking gel was prepared and poured until the top of the plate, the desired comb was inserted. After polymerization, the comb was removed and the wells were washed with running buffer.

Sample preparation

Protein solution was prepared by adding 5 X SDS sample buffer (225 mM Tris-HCl pH6.8, 250 mM 1,4-Dithiothreitol, 50% Glycerin, 5% SDS, 0.05% Bromphenol blue) and heated at 99% for 3 min, centrifuged and stored at -20°C until analysis.

Running conditions

Electrophoresis was performed at room temperature at a voltage of 120 V for 1h until the tracking dye reached the bottom of the gel.

Staining and destaining

After ending electrophoresis, gel was removed from the plate and placed in a preheated staining solution containing 50% Ethanol, 10% acetic acid and 0.1% Coomassie Brillant Blue R250 for 30 min. Destaining of the gel was accomplished by replacing the gel in a preheated destaining solution containing 30% Ethanol and 10% acetic acid for 30 min. The destaining procedure was repeated for 2-3 times.

Gel scanning

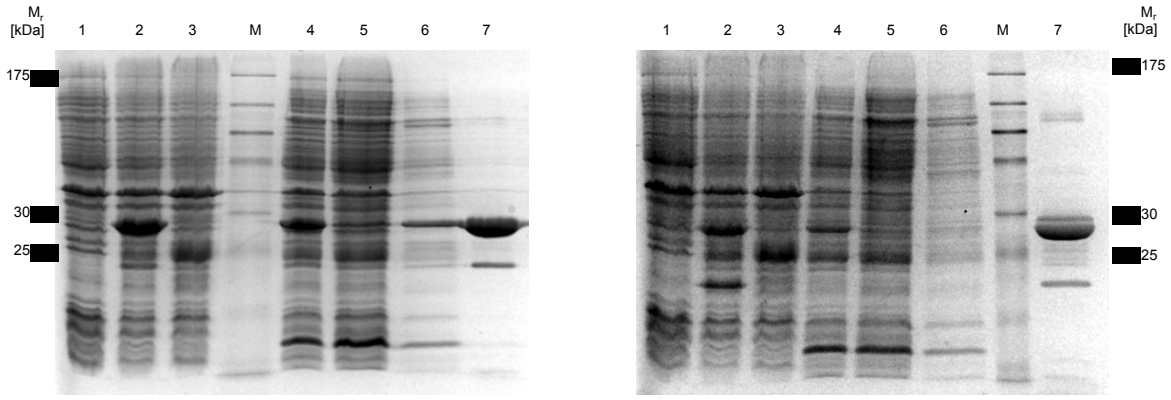
Scanning of the gels was performed by using Image System (Image Reader LAS-4000, Fujifilm) and saving the files in TIFF format.

[1] Laemmli. U. K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**, 680-685.

Part 1: Preparation and characterization of GFP wild type (wt) and N_3-GFP (39) and N_3-GFP-N_3 (39, 182)

Plasmid pET21a/GFPY39TAG or pET21a/GFP-Y39TAG-Y182TAG (plasmid pET21a/GFP was from Group Suzuki, Saitama University Japan; pET21a/GFPY39TAG and pET21a/GFP-Y39TAG-Y182TAG were made for this work), which contain the *gfp* gene (with amber mutation at codon 39 or, 39 and 182), and plasmid pEVOL/*p*AzF (Group Schultz, The Scripps Research Institute, USA) which contains the *M. jannaschii* tyrosyl-RS/ $tRNA^{r}_{CUA}$ gene were cotransformed into *E. coli* BL21 (DE3). Cells were amplified in 2xYT containing ampicillin (50 mg L^{-1}) and chloramphenicol (34 mg L^{-1}) at 37°C. Protein expression was induced at an optical density of 0.8-1.0 at 600 nm by addition of arabinose (0.02%) and isopropyl β-D-1-thiogalactopyranoside (1 mM), *p*-azidophenylalanin (*p*AzF) 2 mM (in case of one amber codon) or 4 mM (in case of two amber codons) were added. After induction, cells were grown at 30°C for overnight. Cells were harvested by centrifugation before lysis by sonication using PBS buffer (pH 8). GFP-Y39*p*AzF or GFP-Y39*p*AzF-Y182*p*AzF were purified via a C-terminal hexahistinine residue (His-tag) by using PerfectPro Ni-NTA Agarose (5 PRIME)

according to the manufacturers instruction. The purified proteins were determined with SDS-PAGE (Figure S-2).



(a) (b)

Figure S-2: SDS-PAGE analysis of the purification-steps. (a) show purification of N_3-GFP (39) and (b) show purification of N_3-GFP-N_3 (39,182). Lane M: prestained protein marker, 7-175 kDa; lane 1: non-induced cell expression; lane 2: induced cell expression in the presence of *p*AzF; lane 3: induced cell expression in the absence of *p*AzF; lane 4: cytosolic proteins after sonication and centrifugation; lane 5: flow-through fraction; lane 6: wash fraction; lane 7: purified mutant GFP fraction.

The modified GFP's, N_3-GFP (39) and N_3-GFP-N_3 (39,182), were characterized with ESI-MS (Table S-1). First proteins were enzymatically digested into small peptide fragments. The peptide fragment (amino acids 27-41), which contains the first incorporated *p*AzF at position 39 was detected in both samples. The peptide fragment, which contains the second incorporated *p*AzF at position 182 could not be detected. Nevertheless, an expression test showed the evidences: 1) no full length GFP protein was produced in the absence of *p*AzF; 2) a truncated mutant N_3-GFP(39)-Y182STOP was obtained in the presence of *p*AzF. This suggests that the produced full length protein should be the mutant N_3-GFP-N_3 (39,182).

Protein	Sequence (26-42)	M (cal.)	M (found)
GFP	K.FSVSGEGEGDATYGK.L	1502,6489	1502,6525
GFP-Y39*p*AzF	K.FSVSGEGEGDATY*GK.L	1527,6616	1527,6590
GFP-Y39*p*AzF-Y182*p*AzF	K.FSVSGEGEGDATY*GK.L	1527,6616	1527,6590

Table S-1. Estimation of incorporation efficiency analyzed by ESI-MS. Peptide fragment (amino acid 27-41, FSVSGEGEGDATY*GK, Y* = incorporation of *p*AzF) was obtained.

Part 2: Preparation of the PDMS-Sylgard 184

Poly(dimethylsiloxane) PDMS Sylgard-184 is prepared by using a mixture composed of a "base" and a "curing agent" in a ratio of 10:1, both purchased from Dow Corning. After mixing the two components together, this mixture is degased for 1h under reduced pressure to remove all bubbles formed during the mixing step. Then, this bubble-free solution is melt into PMMA molds and cured overnight at 90°C at 254 mmHg. PDMS substrates are then unmold and washed, first with a solution of n-heptane and 1-dodecanethiol (0.01 %) for 1 h and then two times with n-heptane for 1h each time. These washing steps are performed in order to remove the remaining unreacted chains and the Pt catalyst from the material. The silicone sheets are then dried in two steps, first, under pressure at room temperature during 1h, and finally in the oven at 60°C overnight. The PDMS substrate obtained is transparent and displays elastic properties. Prior to the manipulations, PDMS substrates with a defined size of 2x1 cm are prepared and cleaned with water and intensively rinsed with milli-Q water to remove possible dust adsorption.

Part 3: Chemical modification and characterization of the PDMS surface

Part 3.1: UVO treatment and characterization

PDMS substrates are first activated under UV-Ozone for 60 min. Immediately after, the substrates are immersed in a diisocyanate solution for 1 h at room temperature under slow

agitation. After this reaction the surfaces are rinsed with acetone then with Milli-Q water and immersed in a 1 mg mL^{-1} PEG-amine solution in Milli-Q water at room temperature overnight. After rinsing with Milli-Q water the samples are kept in a PBS buffer until use.

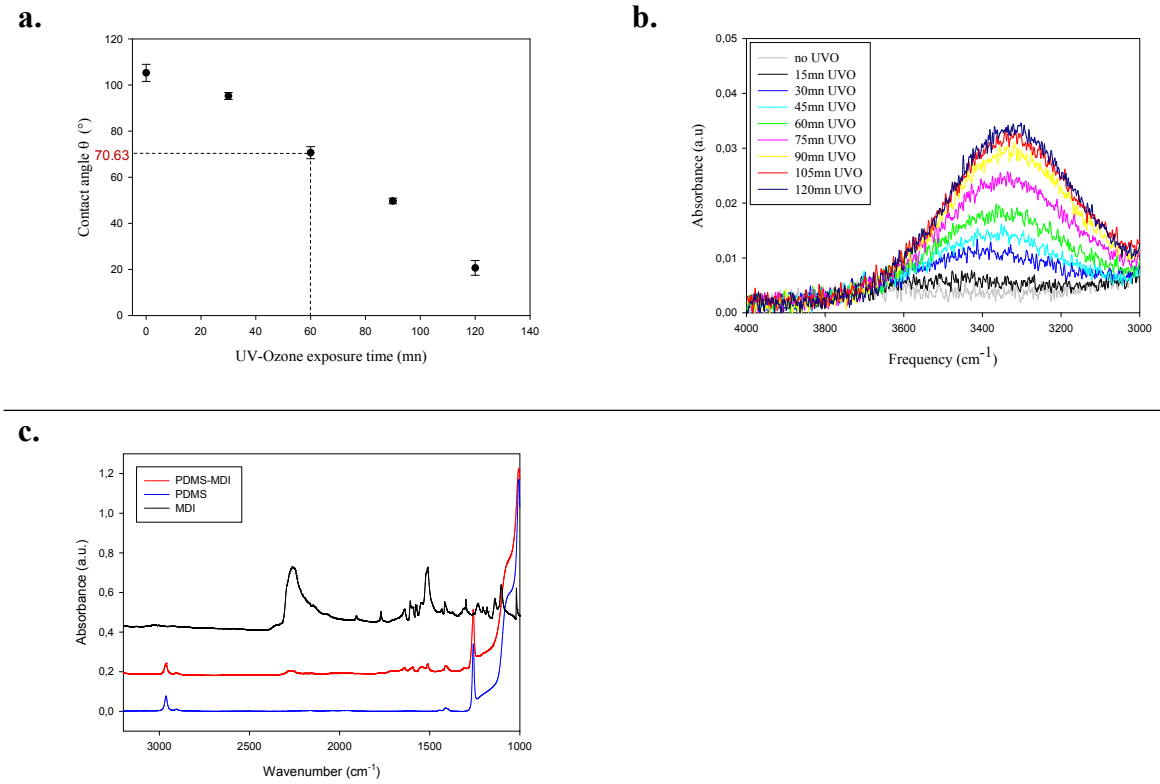


Figure S-3: (**a**) Contact angle measurements on PDMS substrates exposed for different times to UVO. These measurements were performed with a DIGIDROP-GBX® coupled with a camera by using 6 uL pure water droplets. Series of three measures were performed and averaged for each treatment; (**b**) Evolution of the O-H stretching vibration band observed at 3370 cm^{-1}, due to silanol group's formation when exposed to increasing UVO exposition times; (**c**) ATR-IR spectra of unmodified PDMS (blue), MDI (black) and PDMS modified with MDI, PDMS-MDI (red).

Part 3.2: Introduction of MDI

In order to modify our PDMS substrate, different bifunctional linkers were used. PDIT and MDI were solved at 2 mg ml^{-1} in anhydrous acetone solution while DIDS is solved at 1 mg mL^{-1} in Milli-Q water.

Infrared spectra of modified PDMS-SMI substrate is given in Figure 3c: the asymmetric vibration band of the NCO group at 2200 cm^{-1} is observed onto the PDMS-MDI.

Part 3.3: Preparation of PEG-brushes silicone surface

1 Once the PDMS surface functionalized with alkyne groups, the reaction between the modified GFP and the alkyne-terminated PEG was performed following an optimized Copper(I)-catalyzed Azide-Alkyne Cycloaddition (CuAAC) protocol for bioconjugation developed by Finn and coworkers (V. Hong, S. I. Presolski, C. Ma, M. G. Finn, *Angew. Chem. Int. Ed.*, 2009, **48**, 9879). For this reaction, all the reagents were mixed in a specific order to prevent degradation of the proteins. It must be noted that all the compounds except the GFP are solubilized in Milli-Q water.

The reagents are added in a 2 mL Eppendorf tube according to the following order:

- 432 µl of a 3.35 µg mL^{-1} GFP solution in phosphate buffer (PBS, 0.1 M, pH 7.4)
- 7.5 µL of a premixed solution of $CuSO_4$ / THPTA. 2.5 µL of a 20 mM $CuSO_4$ solution is mixed to 5 µL of a 50 mM THPTA solution.
- 25 µL of 100 mM aminoguanidine solution.
- 25 µL of 100 mM sodium ascorbate solution.

The solution is then quickly homogenized and put in contact with the functionalized PDMS substrate for one hour. Once the reaction occurred, the surfaces were washed and stored in a phosphate buffer solution (PBS, 0.1M, pH 7.4) until analyzed.

Part 3.4: XPS analysis

The chemical composition of the modified PDMS surface 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 1000 eV with pass energy of 50 eV and the high resolution scans were performed with the pass energy adjusted to 20 eV. The spectra were recorded by Avantage V.2.26 software and analyzed by CasaXPS 2.3.16 software. For calibration purposes, the silicon Si 2p electron bond energy was referenced to 101.78 eV (P. Louette, F. Bodino, J.-J. Pireaux, *Surface Science Spectra* **2005**, *12*, 38-43). Raw areas determined after Shirley background subtraction

were corrected according to Scofield sensitivity factors (C(1s): 1.00; N(1s): 1.80; O(1s): 2.93; Si(2p): 0.817). 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 consideration of peak position, full width at half-maximum.

The atomic ratio Si(2p)/C(1s) measured on PDMS surface (unmodified), PDMS surface modified by MDI (PDMS-MDI) and PDMS-MDI surface modified by PEG chains (PDMS-SMI-PEG) prepared as described above in this ESI section is 0.634, 0.586 and 0.446 respectively (Figure S-4). The decrease of this ratio going from PDMS, to PDMS-MDI and finally PDMS-MDI-PEG is due to an increase proportion of carbon atom on surface: PEG chains bringing more carbon atom than MDI, these values and trend are in agreement with the effective modification of PDMS at each step.

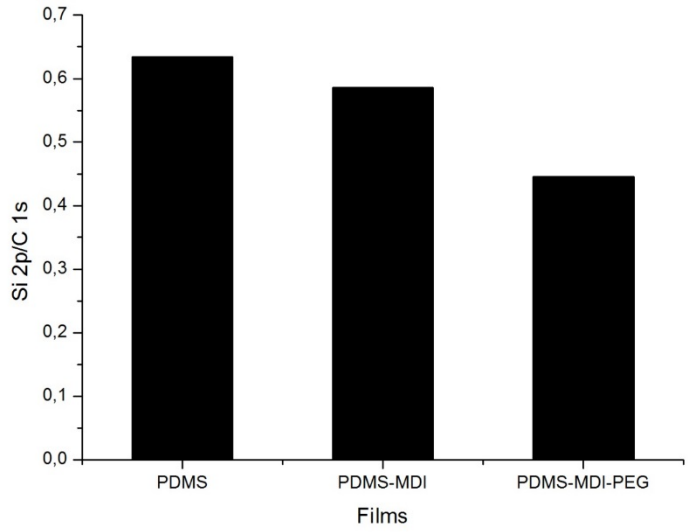


Figure S-4. Comparison of the atomic ratio Si(2p)/C(1s) measured by XPS onto the following surfaces: unmodified PDMS (PDMS) , PDMS modified by MDI (PDMS-MDI) and PDMS-MDI-PEG films.

XPS analysis of pure powder of MDI exhibits a peak at 399.1 eV corresponding to the presence of the nitrogen atom N(1s) involved in both isocyanate group (NCO). When MDI does react onto oxidized PDMS surface, XPS analysis (decomposition peaks) shows the presence of two peaks localized at 399.1 and 399.9 eV (Figure S-5). The first one proves the

presence of remaining NCO groups and the second is assigned to the N(1s) atom of carbamate groups (NHCOO): these last one are formed from the reaction between isocyanate of MDI and silanol group present onto oxidized PDMS surface. The area of each peak localized at 399.1 and 399.9 eV are equivalent. XPS analysis of pure powder of MDI exhibits also a peak at 287.4 eV corresponding to the O(1s) atom involved in both isocyanate group (NCO). When oxidized PDMS is brought in contact with MDI, two peaks at 287.4 and 288.7 eV are detected (Figure S-6). The new second peak at 288.7 eV is assigned to O(1s) atom of carbamate groups (NHCOO, oxygen atom of the carbonyl group). Area of both peaks are equivalent. This XPS analysis provides evidence that MDI is covalently linked to PDMS surface and is still reactive to allow the further nucleophilic addition of amino ended PEG chains.

When PDMS-MDI surface is treated with NH_2-PEG-Alkyne chains, the XPS analysis of the resulting surface shows that the peak at 399.1 eV has disappeared due to a full conversion of free isocyanate groups from MDI and a new peak at 399.4 eV is detected. This last one is assigned to the nitrogen atom N(1s) of the urea bond (NHCONH). This observation proves the covalent coupling between PDMS-MDI surface and amino-terminated PEG chains.

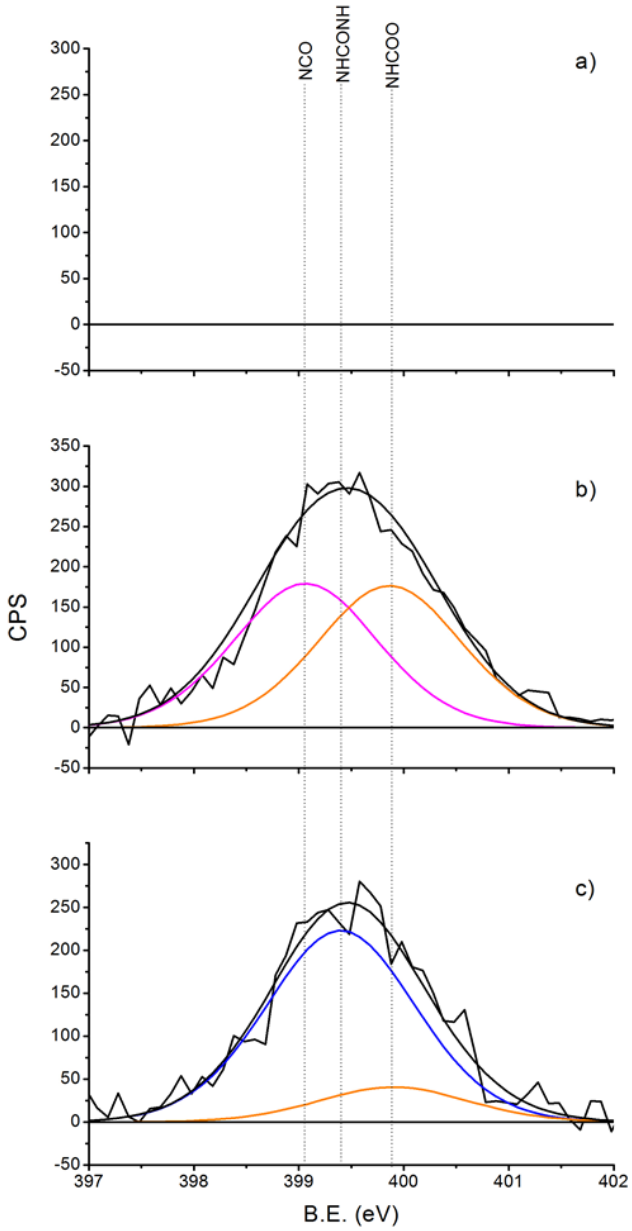


Figure S-5. High resolution spectrum obtained by XPS and focused in the N(1s) region between 398 and 402 eV of the following surfaces and compounds analyzed: (a) unmodified PDMS (PDMS), (b) PDMS surface modified by MDI (PDMS-MDI) and (c) PDMS-MDI surface modified with amino and alkyne PEG chains (PDMS-MDI-PEG) . CPS=Count per second.

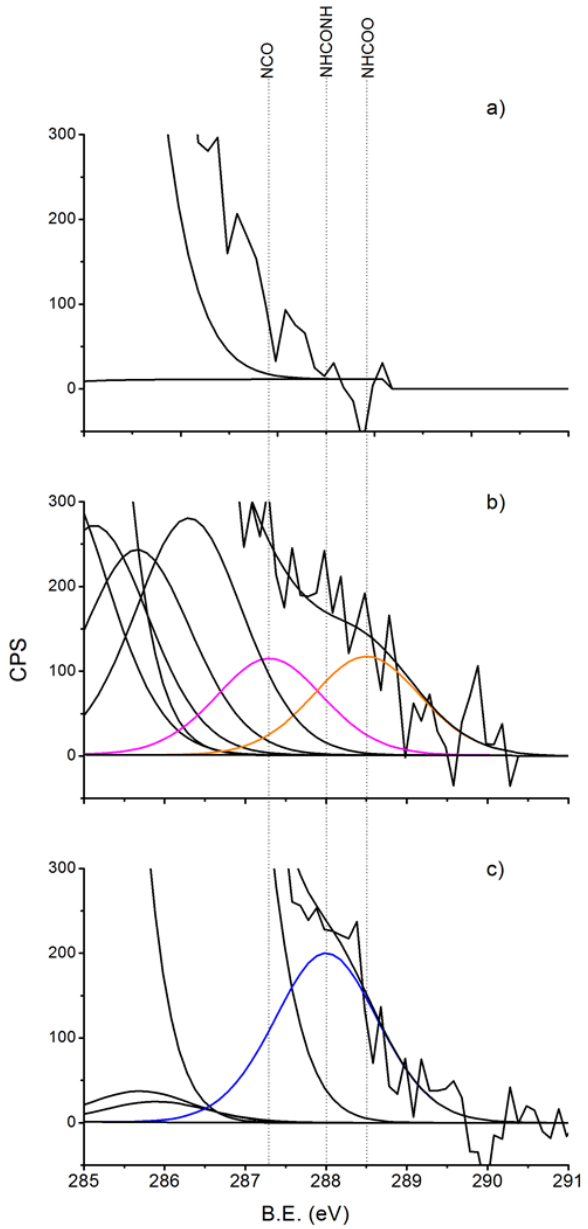


Figure S-6. High resolution spectrum obtained by XPS and focused in the O(1s) region between 285 and 295 eV of the following surfaces and compounds analyzed: (a) unmodified PDMS (PDMS), (b) PDMS surface modified by MDI (PDMS-MDI) and (c) PDMS-MDI surface modified with amino and alkyne ended PEG chains (PDMS-MDI-PEG). CPS = count per second.