SUPPORTING INFORMATION

A model study for chemoselective tethering of (bio)active molecules to biomaterial surfaces through arginine.

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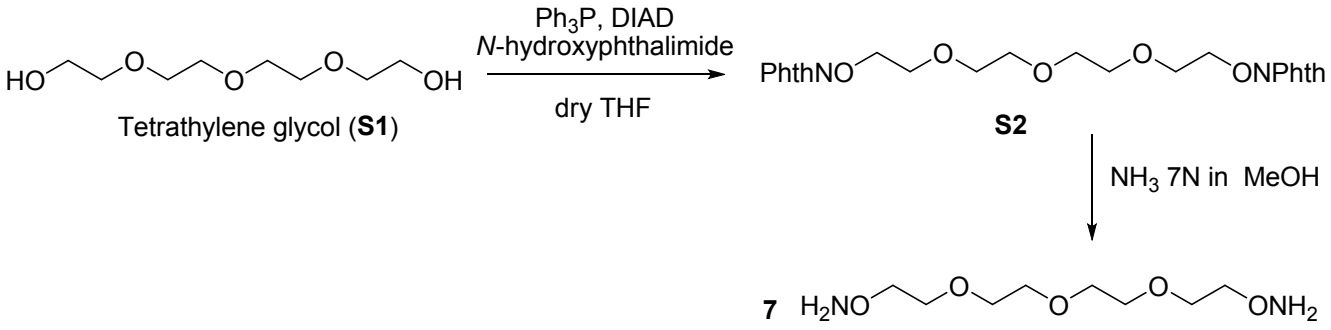
Chemistry.

General methods. All chemicals were purchased from Sigma-Aldrich except for Biotin-ONH_2 (**4**) that was purchased from Dojindo EU GmbH Germany (catalogue n° A305-10 – ARP). Solvents were dried over molecular sieves, for at least 24 h prior to use, when required. When dry conditions were required, the reaction was performed under Ar atmosphere. Thin-layer chromatography (TLC) was performed on silica gel $60F_{254}$ coated glass plates (Merck) with UV detection when possible, charring with Dragendorff reagent. Flash column chromatography was performed on silica gel 230-400 mesh (Merck). Routine ^1H NMR spectra were recorded at 400 MHz on a Varian Mercury instrument. Chemical shifts are reported in parts per million downfield from TMS as an internal standard; *J* values are given in Hz. Mass spectra were recorded on a System Applied Biosystems MDS SCIEX instrument (Q TRAP, LC/MS/MS, turbon ion spray).

Synthesis of compound 7.



*Compound **S2**.* To a solution of commercially available tetraethylene glycol (**S1**, 150 mg, 0.793 mmol) in dry THF (12 mL), Ph_3P (624 mg, 2.38 mmol) and *N*-hydroxyphthalimide (258 mg, 1.59 mmol) were

added. A solution of DIAD (469 μL, 2.38 mmol) in 3 mL of dry THF was finally added dropwise. After completion of the reaction, the solvent was evaporated to dryness, and the crude purified by flash chromatography (EP:AcOEt 0.5:9.5). 223 mg of hygroscopic white solid was obtained (58 % yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46-7.40 (m, 8 H, Ph-H), 4.30 (bt, 4H), 3.77 (bt, 4H), 3.54 (bt, 4H), 3.43 (bt, 4H). Elemental analysis calcd (%) for $C_{24}H_{24}N_2O_9$ (484.4): C 59.50, H 4.99, N 5.78; found C 59.47, H 5.01, N 5.75. MS: m/z = 485.3 $[M+H]^+$, 507.3 $[M+Na]^+$, 523.3 $[M+K]^+$.

Compound 7. Compund **S2** (390 mg, 0.805 mmol) was dissolved in dry MeOH (3 mL) and a 7N NH_3 solution in MeOH (0.5 mL) was added.[1] The reaction was allowed to stir for 24 h, then the solvent evaporated. Since the alkoxyamines are better isolated as their hydrochloride salts,[2] the residue was suspended in dichloromethane, 0.2 mL of 4N HCl in dioxane was added. The white precipitate was collected, and thoroughly washed with diethyl ether (80% yield). ^{1}H NMR (400 MHz, D_2O) δ 4.07 (bt, 4H), 3.48 (bt, 4H), 3.55 (bs, 8H). Elemental analysis calcd (%) for $C_8H_{20}N_2O_5$ (224.2): C 42.85, H 8.98, N 12.49; found C 42.88, H 8.95, N 12.51. MS: m/z = 225.2 $[M+H]^+$, 247.2 $[M+Na]^+$, 263.2 $[M+K]^+$.

Collagen Film preparation. Type I collagen from equine tendon (1% in acetic acid) was diluted 1:6 w/v in ultrapure water, homogenized at 4°C with a mixer for 2 minutes at maximum speed. After removal of the aggregates by filtration, 80 mL of collagen solution was poured into a 35-mm diameter culture dish and the solvent evaporated in the fume hood for two days.

*Collagen-MK (**1**)*. Collagen patches were immersed in a 0.24 M methylglyoxal solution in 83 mM $NaHCO_3$ buffer solution (pH 11) and stirred for 1h, then thoroughly washed with aq. 0.1 M Na_3PO_4.

*General procedure for derivatization of collagen-MK with compounds **4**, **5**, **7**.* Collagen-MK (**1**) was immersed in a 1M solution of the tag in acetate buffer (pH 4.5) and reacted overnight. The samples were then thoroughly washed with acetate buffer, then with water.

*Collagen-PEG-Lac (**9**).* Collagen-PEG-ONH_2 was immersed in a 1M lactose solution in acetate buffer (pH 4.5) and reacted overnight. After this time the collagen film was thoroughly washed first with acetate buffer, then with water.

X-ray photoelectron spectroscopy (XPS).

XPS analysis was performed in an instrument of our own design and construction**,** consisting of a preparation and an analysis UHV chamber, equipped with a 150 mm mean radius hemispherical electron analyser with a four-elements lens system with a 16-channel detector giving a total instrumental resolution of 1.0 eV as measured at the Ag $3d_{5/2}$ core level. MgKα non-monochromatised X-ray radiation (hν = 1253.6 eV) was used for acquiring core level spectra of all samples (C1s, F1s, N1s, S2p and O1s). The spectra were energy referenced to the C1s signal of aliphatic C atoms having a binding energy BE = 285.00 eV. Atomic ratios were calculated from peak intensities by using Scofield's cross section values and calculated λ factors[3]. Curve-fitting analysis of the C1s, F1s, N1s, S2p and O1s spectra was performed using Voigt profiles as fitting functions, after subtraction of a Shirley-type background[4].

Table 1S. XPS data collected on samples Collagen-MK (1), Collagen-biotin (2) and Collagen-TFEH (3), as well as on the pristine collagen used as reference for spectra interpretation. Core level binding energy (BE), full width at half-maxima (FWHM) and atomic ratio $N_{i(tot)}/N_{N(tot)}$ values are reported, together with the proposed signal assignments.

sample	signal	BE (eV)	FWHM (eV)	*Iratio $_{exp.}$	assignment
Collagen (reference)					
	C1s	285.00 286.40 288.20 289.95	1.73 1.73 1.73 1.73	56.3% 24.6% 16.6% 2.5%	C-C C-N, C-O C=O, N-C=O (amide-like) O-C=O
	N1s	399.80 401.42	1.71 1.71	89.2% 10.8%	N Histidine-like + free -NH_2 N^+ + amide-like N
	O1s	530.24 531.81 533.42	2.00 2.00 2.00	18.4% 65.7% 15.9%	C=O C-O-C; C-OH physisorbed H_2O
Collagen-MK (1)					
	C1s	285.00 286.42 288.00 289.73	1.69 1.69 1.69 1.69	23.8% 46.5% 18.8% 10.9%	C-C C-N, C-O C=O, N-C=O (amide-like) O-C=O (collagen)
	N1s	399.86 401.28	1.77 1.77	86.5% 13.5%	N Histidine-like + free -NH_2 N^+ + amide-like N
	O1s	530.28 531.92 533.50	1.95 1.95 1.95	55.7% 37.7% 6.6%	C=O C-O-C; C-OH physisorbed H_2O
Collagen-biotin (2)					
	C1s	285.00 286.36 288.10 289.92	1.73 1.73 1.73 1.73	25.3% 54.7% 12.9% 7.1%	C-C C-N, C-O, C-S C=O, N-C=O (amide-like) O-C=O (collagen)
	N1s	399.81 401.52	1.75 1.75	43.6% 56.4%	N Histidine-like + free -NH_2 N^+ + amide-like
	O1s	530.40 531.90 533.40	1.98 1.98 1.98	40.7% 44.3% 15.0%	C=O C-O-C; C-OH physisorbed H_2O

	$S2p_{3/2}$	164.69	1.84	N/S = 6.7/1	S (biotin) ($Iratio_{theor.}$: N/S = 7/1)
Collagen-TFEH (3)					
	C1s	285.00 286.52 288.26 289.88 293.00	1.71 1.71 1.71 1.71 1.71	26.9% 46.9% 16.2% 8.2% 1.8%	C-C C-N, C-O C=O, N-C=O (amide-like) O-C=O (collagen) C-F (TFEH)
	N1s	399.78 401.50	2.19 2.19	16.7% 83.3%	N Histidine-like + free -NH2 N^+ + amide-like N
	O1s	530.25 531.82 533.51	1.90 1.90 1.90	58.0% 32.3% 9.7%	C=O C-O-C; C-OH physisorbed H_2O
	F1s	689.92	2.17	F/C_{C-F} = 2.8/1	F TFEH ($Iratio_{theor.}$: F/C_{C-F} = 3/1)

Fluorescence microscopy. All images were acquired using an inverted microscope (Axiovert; Zeiss, Oberkochen, Germany). Collagen-PEG-Lac (**9**) and pristine collagen are suspended in a PBS solution of Lectin from *Arachis hypogaea* (peanut) FITC conjugated (PNA-FITC, Sigma-Aldrich catalogue n° L7381, 40 µg/ml) and stirred for 2 h in dark conditions. After this time the collagen films were thoroughly washed with PBS buffer (63 times x 15 min). The samples were air-dried and analysed for their fluorescence.

ELLA assay.[5] Pristine collagen and collagen-PEG-Lac (**9**) samples were treated with a solution of 2% BSA in PBS (100 µL) and shaken (14 h, 5°C), according to manufacturer protocol. The films were then removed and incubated at room temperature with a solution of the lectin from peanut (*Arachis hypogaea*) peroxidase labelled (Sigma-Aldrich, catalogue n° L7759) (0.01 mg/mL, 200 µL) in PBS for 2 h with shaking. The films were then thoroughly washed with PBS to remove unbound lectin and then treated with a solution of OPD (SIGMAFAST^{TM} OPD, Sigma-Aldrich, catalogue n°P9187) (500 µL, 1 h). The absorbance of an aliquot of this solution (200 µL) was measured at 450 nm.

Additional analysis

In order to exclude the possibility of trifluoroethyl hydrazine chemisorbtion on collagen substrates, FT-IR were performed (Figure S1):

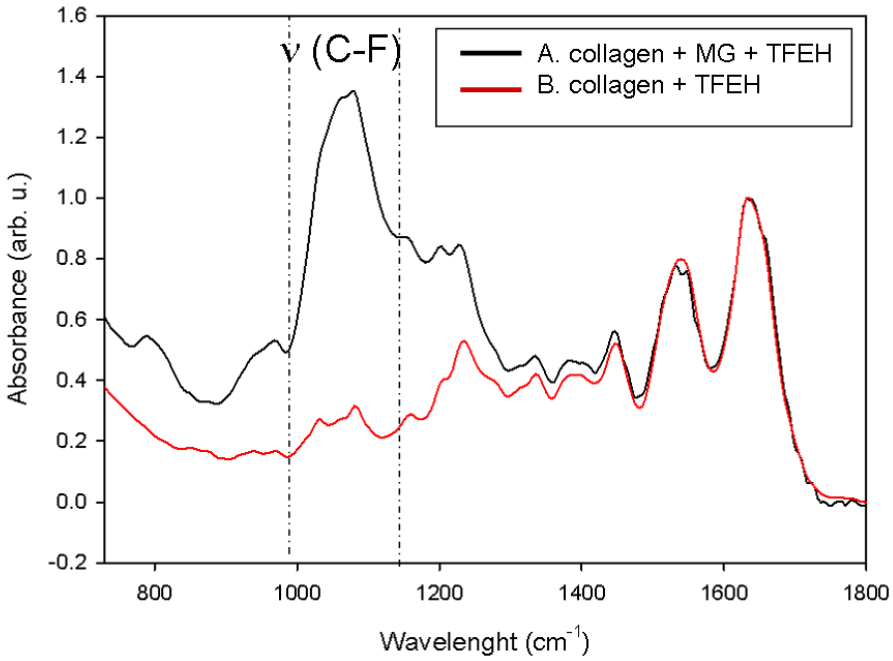


Figure S1. FT-IR spectra of linked and adsorbed trifluoroethyl hydrazine (TFEH). MG: methylglyoxal.

Fluorinated compounds display strong bands around 1000-1200 cm^{-1}; the presence of the typical signals in sample A (black line, collagen treated with methylglyoxal), and absence in sample B (red line, collagen without methylketone functionality) demonstrate that trifluoroethyl hydrazine is linked to collagen with covalent bonds (the only expected one, that is oxime bonds).

In the case of biotin and lactose, fluorescence analysis was used to exclude chemisorbtion (Fig. S2):

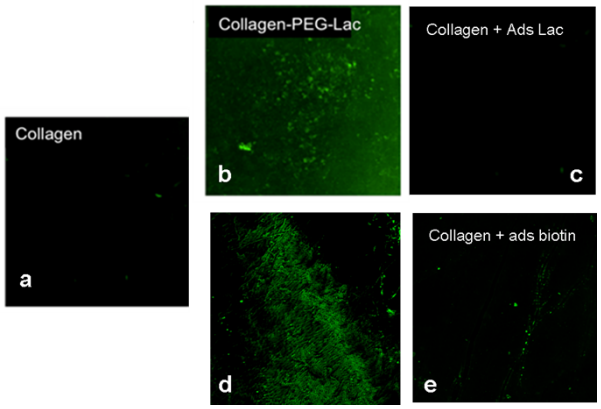


Figure S2. Fluorescence images after incubation with fluorescent probe of a) untreated collagen; b) collagen treated with i) MG, ii) compound **7**, iii) Lactose; c) collagen treated with lactose; d) collagen treated with i) MG, ii) biotin-ONH_2; e) collagen treated with biotin.

Fluorescence images show that biotin and lactose can be detected only if collagen is pre-treated with methylglyoxal (Fig. S2 b and d), while non fluorescence is detectable on sample c and e, treated directly respectively with biotin and lactose.

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[3] Swift, P.; Shuttleworth, D.; Seah, M.P. *Practical Surface Analysis by Auger and X-ray Photoelectron Spectroscopy*, D. Briggs and M. P. Seah (Eds.), J. Wiley & Sons, Chichester, 1983, chapter 5 and appendix 3

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[5] Slaney, A.M.; Wright, V.A.; Meloncelli, P.J.; Harris, K.D.; West, L.R.; Lowary, T.L.; Buriak, J.M. *ACS Appl. Mater. Interfaces* **2011**, *3*, 1601–1612.