Supporting information

Modular synthesis of supramolecular ureidopyrimidinone-peptide conjugates using an oxime ligation strategy

i. General materials

Rink Amide MBHA resin, FMOC-protected amino acids were purchased from Nova Biochem. Heterobifunctional OEG amines [O-(2-aminoethyl)heptaethylene glycol, O-(2-aminoethyl)pentadecaethylene glycol (**1a**) and O-(2-azidoethyl)nonadecaethylene glycol] (**1b**) were obtained from Polypure. Palladium on matrix activated carbon, Degussa type E101 was obtained from Sigma Aldrich. N′-Boc-aminooxyacetyl N-hydroxysuccinimide ester was kindly provided by Freek Hoeben (SyMO-Chem, Eindhoven, NL). All other commercial grade reagents and chemicals of the highest purity were obtained from Sigma Aldrich and used as received. Water was deionized prior to use.

ii. General methods

Automated solid phase peptide synthesis was performed on a Prelude synthesizer (Protein Technologies). Purification was carried out on a Biotage flash chromatography system equipped with a C18 column and using preparative RP-HPLC on a Varian Pro Star HPLC system with a Vydac protein & peptide C18 column. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on Varian Mercury 400 MHz NMR spectrometers at 298K (400 MHz for ^{1}HNMR and 100 MHz for ^{13}C NMR.) Chemical shifts are reported in ppm(δ) relative to trimethylsilane (TMS) as a standard. Matrix assisted laser desorption/ionization time of flight (MALDI-TOF) mass spectra were obtained on a PerSpective Biosystems Voyager DE-PRO spectrometer using acid α-cyanohydroxycinnamic acid matrix (CHCA) or neutral 2-[(2E)-3-(4-tert-butylphenyl)-2-methylprop-2enylidene] malonitrile (DCTB) matrices. LC-MS analyses were performed using a Shimidazu SCL-10 AD VP series HPLC coupled to a diode array detector (Finnigan Surveyor PDA Plus, Thermo Electron Corporation) and an Ion-Trap (LCQ Fleet Thermo Scientific). Atomic force micrographs were obtained on a Digital Multimode Nanoscope IV. Silicon cantilever tips (PPP-NCHR, 304-497 kHz, 10-130 N/m from Nanosensors) were used in tapping mode.

iii. Syntheses of Boc-protected UPy-OEG(6-14)-oxy-amine supramolecular units

Synthesis of 2a

A one-pot synthesis of **2a** commenced with Boc-protection of O-(2-aminoethyl)heptaethylene glycol (**1a**) (0.32 g, 0.81 mmol) using Boc-anhydride(0.18 g, 1.62 mmol) and N,N-diisopropylethylamine [DIPEA](0.56 mL, 3.24 mmol) in $CHCl_3$ (10 mL). Stirred at room temperature for 1 hour the intermediate was verified by LC-MS (t=5.07 min, *m/z*: 370.67 $[M+H\text{-}Boc]^+$, 470.33 $[M+H]^+$). 1,1'-Carbonydiimidazole (0.19 g, 1.17 mmol) was added to the reaction mixture, stirred for an additional 2 hours and evaluated by LC-MS (t= 5.30 min, *m/z*: 564.50 $[M+H]^+$). Cbz-protected dodecyl diamine (0.40 g, 1.21 mmol) was subsequently added, along with DIPEA (0.50 mL) and refluxed overnight. The product was purified using C18 column chromatography. A gradient from 5-100% CH_3CN/H_2O in 60 minutes was used. The purified product was rotavapped to dryness, and dried *in vacuo* at 40 ^oC overnight, yielding a white film.

Yield: 0.45 g, 67% ^{1}H-NMR (δ[ppm], $CDCl_3$, 400 MHz): 7.35-7.29 (m, 5H), 5.22 (br s, 1H), 5.08 (m, 3H), 4.20 (t, 2H), 3.82-3.65, (m, 28H), 3.30 (q, 2H), 3.15 (m, 4H), 2.63 (br s, 1H), 1.54-1.42(m, 10 H), 1.36-1.2 (m, 9H). ^{13}C-NMR (δ[ppm], $CDCl_3$, 100 MHz): 156.6, 156.2, 136.9, 128.6, 128.2, 128.1, 79.1, 70.7, 70.6, 70.6, 70.3, 69.8, 66.6, 63.8, 41.2, 41.1, 40.5, 30.0, 29.6, 29.4, 28.5, 26.8. MALDI-TOF-MS (positive mode): *m/z* calcd. for $[M+H]^+$ 830.53; found: 730.38$[M+H\text{-}Boc]^+$, 852.47$[M+Na]^+$, 868.45$[M+K]^+$. HPLC: 8.24 min, *m/z*: 731.00$[M+H\text{-}Boc]^+$, 848.00$[M+H+H_2O]^+$, 853.00$[M+Na]^+$.

Synthesis of 2b

A one-pot synthesis of **2b** commenced with Boc-protection of O-(2-aminoethyl)pentadecaethylene glycol (**2a**) (0.56 g, 0.78 mmol) using Boc-anhydride (0.37 g, 1.67 mmol) and N,N-diisopropylethylamine [DIPEA](0.17 g, 3.16 mmol) in $CHCl_3$ (15 mL). Stirred at room temperature for 1 hour the product was verified by LC-MS (t= 5.48 min, *m/z*: 362.00 $[M+2H\text{-}Boc]^{2+}$, 411.83 $[M+2H]^{2+}$, 722.92 $[M+H\text{-}Boc]^+$, 822.42 $[M+H]^+$). 1,1'-Carbonydiimidazole (0.17 g, 1.07 mmol) was added to the reaction mixture, stirred for an additional 2 hours and evaluated by LC-MS (t= 5.65 min, *m/z*: 458.75 $[M+2H]^{2+}$, 916.75 $[M+H]^+$). Cbz-protected dodecyl diamine (0.3774 g, 1.12 mmol) was subsequently added, along with DIPEA (0.50 mL) and refluxed overnight. The product was purified using C18 column chromatography. A gradient from 5-100% CH_3CN/H_2O in 60 minutes was used. The purified product was rotavapped to dryness, and dried *in vacuo* at 40 ^oC overnight yielding a white film.

Yield: 0.70 g, 77% ^{1}H-NMR (δ[ppm], $CDCl_3$, 400 MHz): 7.35-7.30 (m, 5H), 5.084 (m, 3H), 4.97 (m, 1H), 4.20 (t, 2H), 3.82-3.46 (m, 60 H), 3.31 (q, 2H), 3.15 (m, 4H), 2.53 (br s, 1H), 1.49-1.44 (m, 10H), 1.27-1.25 (m, 9H). ^{13}C-NMR (δ[ppm], $CDCl_3$, 100 MHz): 156.6, 156.1, 136.9, 128.6, 128.2, 128.2, 79.2, 70.7, 70.7, 70.7, 70.6, 70.4, 70.3,

69.8, 66.6, 63.9, 41.2, 41.2, 40.5, 30.1, 29.6, 29.4, 28.6, 26.9. MALDI-TOF-MS (positive mode): *m/z* calcd. for $[M+H]^+$ 1182.74; found: 1082.59 $[M+H\text{-}Boc]^+$, 1204.60 $[M+Na]^+$. HPLC: t=8.19 min, 542.17$[M+2H\text{-}Boc]^{2+}$, 592.08$[M+2H]^{2+}$, 1083.25$[M+H\text{-}Boc]^+$ 1205.17$[M+Na]^+$.

Synthesis of 3a

A one-pot synthesis of **3a** started with the deprotection of Cbz-protecting group on **2a** (0.26 g, 0.31 mmol) using triethylsilane (0.5 mL, 3.10 mmol) and Pd/C (0.033 g, 0.03 mmol) in MeOH (10 mL). Triethylsilane was added dropwise resulting in an effervescent solution. Once bubbling was stopped, the product was filtered over celite to remove all Pd catalyst. An LCMS spectrum (t= 5.45 min, *m/z*: 696.83 $[M+H]^+$, 718.83 $[M+Na]^+$) was taken to verify the amine intermediate, rotavapped to dryness and dried overnight at 30 °C *in vacuo*. Subsequently, the dried product was dissolved in $CHCl_3$ (10 mL) and UPy-urea-C6-isocyanate (0.091 g, 0.31 mmol) and N,N-diisopropylethylamine [DIPEA] (0.25 mL, 1.43 mmol) were added. The turbid solution was stirred for 4 hours until clear. The product was purified using C18 column chromatography. A gradient from 5-100% CH_3CN/H_2O in 60 minutes was used. The purified product was rotavapped to dryness, and dried *in vacuo* at 40 °C overnight yielding a white film.

Yield: 0.18 g, 59% ^{1}H-NMR (δ[ppm], $CDCl_3$, 400 MHz): 13.16 (br s 1H), 11.82 (br s, 1H), 10.07 (br s, 1H), 5.83 (s, 1H), 5.13 (m, 1H), 4.94 (m, 1H), 4.76 (m, 1H), 4.21 (m, 2H), 3.82-3.15 (m, 38H), 2.24 (s, 3H), 1.59-1.25 (m, 37H). ^{13}C-NMR (δ[ppm], $CDCl_3$, 100 MHz): 173.6, 159.0, 156.8, 155.8, 155.1, 148.8, 106.9, 79.5, 70.9, 70.6, 70.0, 64.1, 41.4, 40.8, 40.7, 40.4, 39.9, 30.7, 30.3, 30.2, 29.8, 29.7, 29.6, 28.8, 27.3, 27.1, 26.6, 26.5, 19.3. MALDI-TOF-MS (positive mode): *m/z* calcd. for $[M+H]^+$ 989.64; found: 989.49$[M+H]^+$, 1011.50$[M+Na]^+$, 1027.49$[M+K]^+$. HPLC: 7.09 min, 445.67 $[M+2H]^{2+}$, 495.33 $[M+2H\text{-}Boc]^{2+}$, 989.50 $[M+H]^+$, 1011.67$[M+Na]^+$.

Synthesis of 3b

A one-pot synthesis of **3b** started with the deprotection of Cbz-protecting group on **2b** (0.62 g, 0.52 mmol) using triethylsilane (0.5 mL, 5.43 mmol) and Pd/C (0.033 g, 0.05 mmol) in MeOH (15 mL). Triethylsilane was added dropwise resulting in an effervescent solution. Once bubbling was stopped, the product was filtered over celite to remove all Pd catalyst. An LCMS spectrum (HPLC: t=5.62 min, *m/z*: 475.33 $[M+2H\text{-}Boc]^{2+}$, 525.00 $[M+2H]^{2+}$, 1049.08 $[M+H]^+$, 1070.92 $[M+Na]^+$) was taken to verify the amine intermediate, rotavapped to dryness, and dried overnight at 30 °C *in vacuo*. Subsequently, the dried product was dissolved in $CHCl_3$ (10 mL) and UPy-urea-C6-isocyanate (0.16 g, 0.55 mmol) and N,N-diisopropylethylamine [DIPEA] (0.40 mL, 2.93 mmol) were added. The turbid solution was stirred for 4 hours until clear. The product was purified using C18 column chromatography. A gradient from 5-100% CH_3CN/H_2O in 60 minutes was used. The purified product was rotavapped to dryness, and dried *in vacuo* at 40 °C overnight yielding a white film.

Yield: 0.62 g, 89% ^{1}H-NMR (δ[ppm], $CDCl_3$, 400 MHz): 13.16 (br s, 1H), 11.82 (br s, 1H), 10.07 (br s, 1H), 5.83 (s, 1H), 5.10 (m, 1H), 4.95 (m, 1H), 4.81 (m, 1H), 4.24 (m, 2H), 3.82-3.47 (m, 56 H), 3.31- 3.15 (m, 10H), 2.24 (s, 3H), 1.59-1.25 (m, 37H). ^{13}C-NMR (δ[ppm], $CDCl_3$, 100 MHz): 173.5, 159.0, 156.7, 156.2, 155.0, 148.8, 106.8, 79.5, 70.8, 70.5, 69.9, 64.0, 41.3, 40.7, 40.3, 39.9, 30.7, 30.2, 30.1, 29.9, 29.8, 29.8, 29.6, 29.6, 29.5, 28.7, 27.2, 27.0, 26.5, 26.5, 19.2. . MALDI-TOF-MS (positive mode): *m/z* calcd. for $[M+H]^+$ 1341.85; found: 1363.77$[M+Na]^+$, 1379.74$[M+K]^+$ HPLC: t=7.14 min, 671.25$[M+2H]^{2+}$, 1341.50$[M+H]^+$, 1363.42$[M+Na]^+$.

Synthesis of 4a

A synthesis of **4a** started with the deprotection of Boc-protecting group on **3a** (0.18 g, 0.18 mmol) using trifluoroacetic acid (3 mL) and $CHCl_3$ (1 mL) for 30 minutes. The intermediate was confirmed by LC-MS analysis (t= 5.62 min, *m/z*: 445.33$[M+2H]^{2+}$, 889.50$[M+H]^+$) and evapourated using a gentle stream of N_2. The intermediate was redissolved in $CHCl_3$ (10 mL) with N,N-diisopropylethylamine (1.0 mL) and N′-Boc-aminooxyacetyl N-hydroxysuccinimide ester (0.085 g, 0.30 mmol). The reaction mixture was stirred for two hours. The product was purified using C18 column chromatography. A gradient from 5-100% CH_3CN/H_2O in 60 minutes was used.

Yield: 0.12 g, 61% ^{1}H-NMR (δ[ppm], $CDCl_3$, 400 MHz): 13.16 (br s, 1H), 11.81 (br s, 1H), 10.06 (br s, 1H), 8.54 (br s, 1H), 7.97 (br s, 1H), 5.82 (s, 1H), 5.10-4.96 (m, 3H), 4.33-4.20 (m, 4H), 3.67-3.15 (m, 38H), 2.24 (s, 3H), 1.58-1.25 (m, 37H). ^{13}C-NMR (δ[ppm], $CDCl_3$, 100 MHz): 173.5, 169.5, 159.0, 157.7, 156.7, 155.0, 148.8, 106.8, 82.5, 75.9, 70.8, 70.6, 69.9, 69.8, 64.0, 41.3, 40.6, 40.3, 39.9, 39.1, 30.6, 30.4, 30.2, 30.1, 29.8, 29.8, 29.6, 29.6, 29.5, 28.4, 27.2, 27.0, 27.0, 26.6, 26.5, 19.2. MALDI-TOF-MS (positive mode): *m/z* calcd. for $[M+H]^+$ 1062.66; found: 1084.53$[M+Na]^+$. HPLC: t=6.86 min, *m/z*: 531.75$[M+2H]^{2+}$, 1062.50$[M+H]^+$.

Synthesis of 4b

A one-pot synthesis of **4b** started with the deprotection of Boc-protecting group on **3b** (0.62 g, 0.46 mmol) using trifluoroacetic acid (3 mL) and $CHCl_3$ (1 mL) for 1 hour. The intermediate was confirmed by LC-MS analysis (t= 5.78 min, *m/z*: 414.58 $[M+3H]^{3+}$, 621.50 $[M+2H]^{2+}$, 1241.67 $[M+H]^+$) and evapourated using a gentle stream of N_2. The intermediate was redissolved in $CHCl_3$ (10 mL) with N,N-diisopropylethylamine (1.0 mL) and N′-Boc-aminooxyacetyl N-hydroxysuccinimide ester (0.1610 g, 0.56 mmol). The reaction mixture was stirred for two hours. The product was purified using C18 column chromatography. A gradient from 5-100% CH_3CN/H_2O in 60 minutes was used.

Yield: 0.37 g, 56% ^{1}H-NMR (δ[ppm], $CDCl_3$, 400 MHz): 13.16 (br s, 1H), 11.82 (br s, 1H), 10.07 (br s, 1H), 8.34 (br s, 1H), 7.90 (br s, 1H), 5.84 (s, 1H), 4.96 (m, 1H), 4.83 (m, 1H), 4.64 (m, 1H), 4.33 (s, 2H), 4.20 (t, 2H), 3.81-3.46 (m, 60H), 3.24-3.14 (m, 10H), 2.24 (s, 3H), 1.60-1.22 (m, 37H). ^{13}C-NMR (δ[ppm], $CDCl_3$, 100 MHz): 173.6, 169.4, 158.9, 157.7, 156.8, 155.1, 148.8, 106.9, 82.6, 76.1, 70.9, 70.6, 70.0, 69.9, 64.1,

41.4, 40.8, 40.4, 39.9, 39.2, 30.7, 30.3, 30.1, 29.9, 29.8, 29.7, 29.6, 28.5, 27.3, 27.0, 26.6, 26.5, 19.3. MALDI-TOF-MS (positive mode): *m/z* calcd. for $[M+H]^+$ 1414.87; found: 1436.81$[M+Na]^+$, 1452.77$[M+K]^+$. HPLC: t=6.97 min, *m/z*: 707.92$[M+2H]^{2+}$, 718.92$[M+H+Na]^{2+}$, 1414.58$[M+H]^+$, 1436.67[M+Na]+ .

iv. Syntheses of peptides

Standard FMOC-based protocols were used to synthesize peptides (**6**-**11**) on a 200 µmol scale using Rink Amide MBHA resin. Prior to synthesis the resin was swollen for 20 minutes in N-methylpyrrolidone (NMP). Every synthetic cycle started with removal of the FMOC-protecting group with 20% (v/v) piperidine in NMP (2 mL, 2 x 5 min) proceeded by an NMP wash (6 x 30 s). HBTU (1 mL, 400 mM) activation of the FMOC amino acid (2 mL, 200 mM) in the presence of N, N'-diisopropylethylamine (DIPEA, 1 mL, 600 mM) ensued with a coupling time of 2 x 20 minutes. Several NMP washes (6 x 30s) were employed to remove any uncoupled amino acids. A final FMOC-deprotection step was used to remove the protecting group prior to resin cleavage. A standard cleavage cocktail consisting of 5 mL of TFA 95%: Triisopropylsilane (TIS) 2.5%: H_2O (2.5%) was used for peptides **6**, **7**, **8** and **9**. For peptides **10** and **11**, containing the levulinic acid (LA) moiety, a deprotection of mixture of TFA 90%: DCM 10% was used. Deprotected peptides were then precipitated using cold diethyl ether (45 mL), stored in the freezer for ~30 min. The peptides were then centrifuged (2500 rpm, 10 min) and the ethereal layer was decanted. The solid pellet was then redissolved in 50% CH_3CN/50% H_2O/0.5% TFA and put on the lyophilizer. Peptides containing serine at the N-terminus, **6**, **7**, **8**, and **9**, were then oxidized using sodium m-periodate ($NaIO_4$). From a 1 M solution of sodium periodate in phosphate buffer (20 mM NaH_2PO_4/ Na_2HPO_4), two equivalents of $NaIO_4$ were added to the crude peptide in a 5 mL volume for 30 minutes. Oxidization of the N-terminal serine was verified using LC-MS and purified using C18 flash column chromatography using a CH_3CN/H_2O gradient from 5-40% CH_3CN over 14 minutes, then a gradient from 40-100% CH_3CN over 3 minutes (**6**, **7**: ESI-MS (positive mode) *m/z* calcd. for $[M+H]^+$ 717.28, found: 717.42 $[M+H]^+$, 735.50$[M+H+H_2O]^+$; **8**, **9**: ESI-MS (positive mode) *m/z* calcd. for $[M+H]^+$ 837.35, found: 427.83$[M+2H]^{2+}$, 837.35$[M+H]^+$, 854.50$[M+H+H_2O]^+$). Collagen I binding peptides (**10**, **11**) were used without further purification, ESI-MS (positive mode) *m/z* calcd. for $[M+H]^+$ 1264.62, found: 633.08 $[M+2H]^{2+}$, 1264.58 $[M+H]^+$.

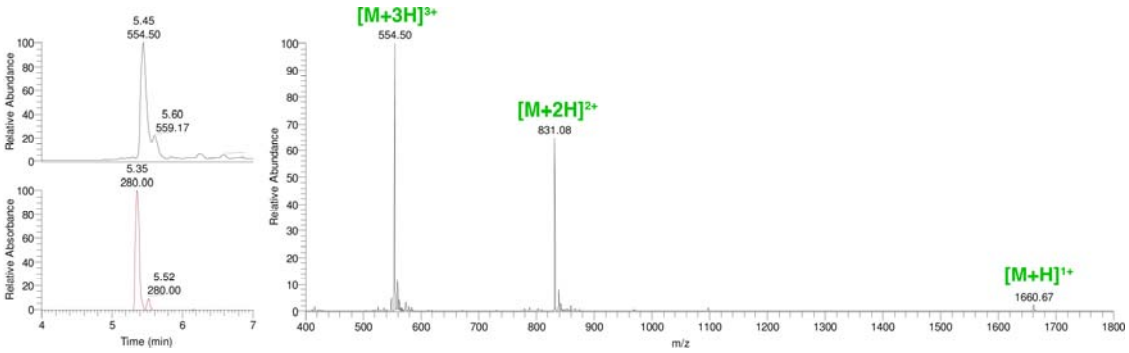
v. Synthesis and characterization of UPy-peptides: oxime ligations

Compounds **5a** and **5b** were Boc-deprotected using pure trifluoroacetic acid (TFA) (2 mL) for 1 hour at 0 ºC. LC-MS was used to verify the intermediate before TFA was evapourated using a gentle stream of N_2 (compound **5a**: t=6.22 min, 482.25 $[M+2H]^{2+}$, 963.00 $[M+H]^+$; compound **5b**: t=6.42 min, m/z= 658.42 $[M+2H]^{2+}$, 1315.33 $[M+H]^+$). The product was resuspended in deionized water and lyophilized. One equivalent of compound **5a** and two equivalents of the oxidized peptide sequences **6**, **7**, **8**, **9** or **10** were dissolved in 1.5 mL of DMF and 1.5 mL of 10 mM

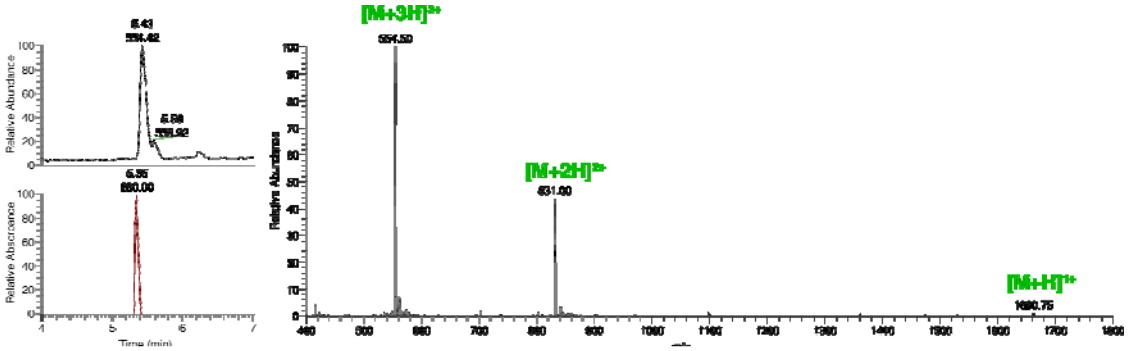
anilinium acetate buffer at pH 4.5. The reaction was stirred for overnight at 37 °C. Compound **5b** and peptides **6**, **10** and **11** were dissolved in 1.5 mL of DMF and 1.5 mL of 10 mM anilinium acetate buffer at pH 4.5. The reaction was stirred overnight at 37 °C. Products were purified using C18 column chromatography using a gradient of CH_3CN/H_2O from 0-60% CH_3CN over 16 minutes, then 60-100% CH_3CN over 2 minutes, and 100% CH_3CN for 3 minutes.

The chromatograms and corresponding mass spectra of all conjugates **12**-**19** are shown below (for every compound: *top left* = total ion current, *bottom left* = UV/Vis detection, *right* = ESI-MS spectrum)

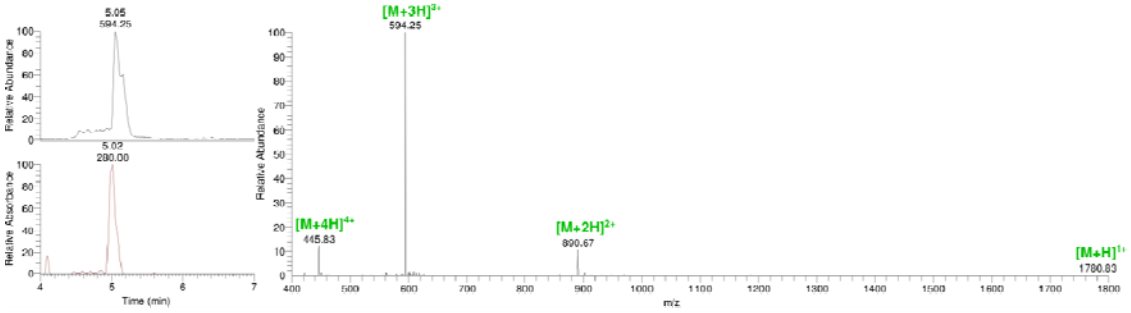
UPy-peptide (GGG-GRGDS) **12**, One equivalent of compound **5a** (22.5 mg, 0.023 mmol) and two equivalents of the oxidized peptide **6** (33.5 mg, 0.047 mmol) were reacted according to the above-mentioned conditions. Yield = 12.7 mg, 33 %



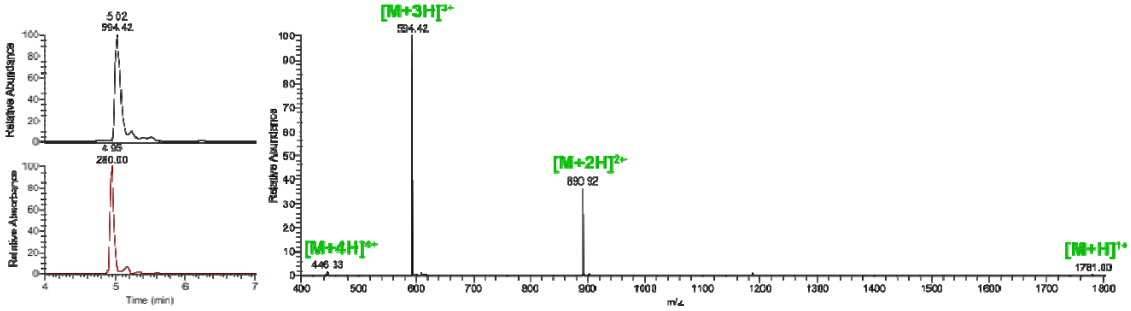
UPy-peptide (GGG-SGDRG) **13**, One equivalent of compound **5a** (17.0 mg, 0.018 mmol) and two equivalents of the oxidized peptide **7** (26.4 mg, 0.037 mmol) were reacted according to the above-mentioned conditions. Yield = 11.7 mg, 40 %



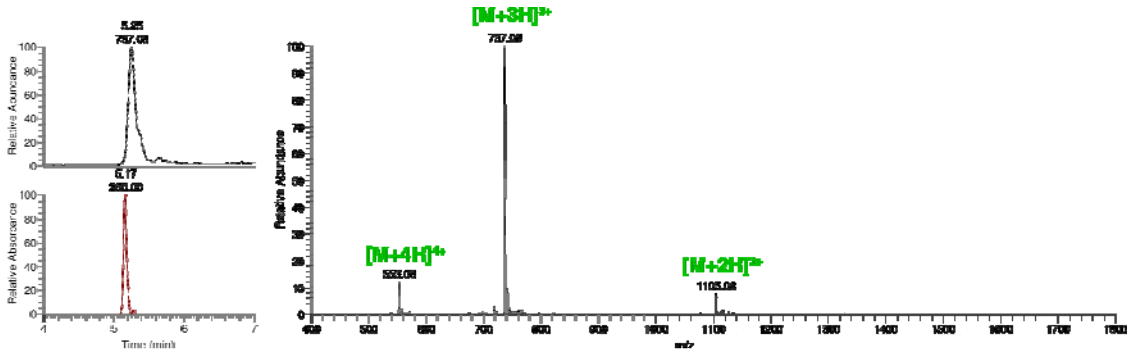
UPy-peptide (GGG-PHSRN) **14**



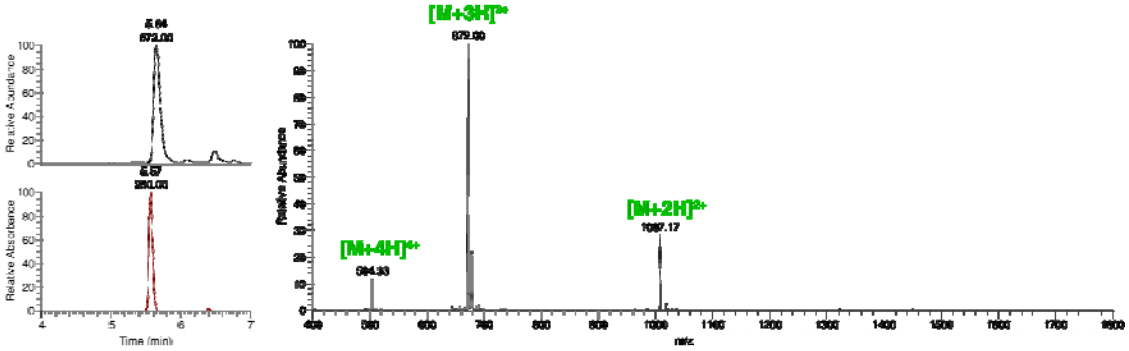
UPy-peptide (GGG-PNRHS) **15**



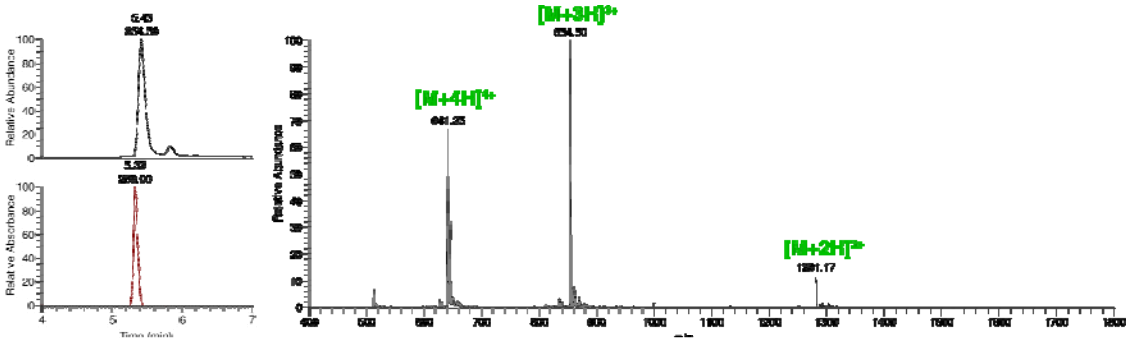
UPy-peptide (KGGG-HVWMQAP) **16**, One equivalent of compound **5a** (11.8 mg, 0.012 mmol) and two equivalents of the oxidized peptide **10** (30.0 mg, 0.024 mmol) were reacted according to the above-mentioned conditions. Yield = 19.7 mg, 73 %



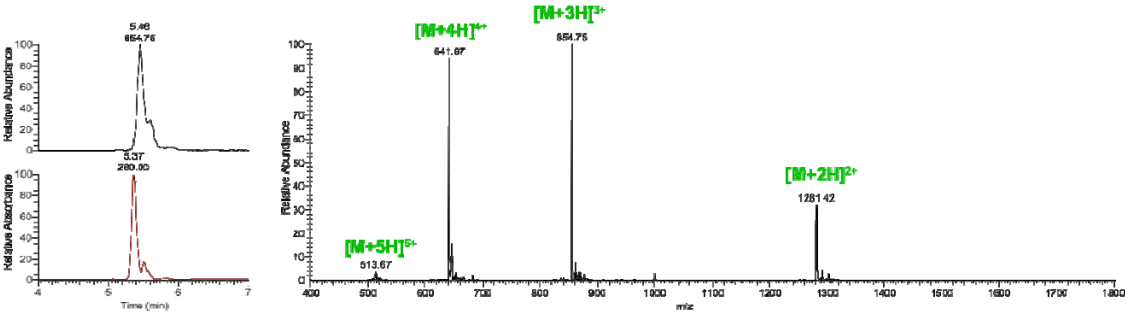
UPy-peptide (GGG-GRGDS) **17**, One equivalent of compound **5b** (24.2 mg, 0.018 mmol) and two equivalents of the oxidized peptide **6** (26.4 mg, 0.037 mmol) were reacted according to the above-mentioned conditions. Yield = 18.0 mg, 49 %



UPy-peptide (KGGG-HVWMQAP) **18**, One equivalent of compound **5b** (22.7 mg, 0.017 mmol) and two equivalents of the oxidized peptide sequence **10** (46.6 mg, 0.037 mmol) were reacted according to the above-mentioned conditions. Yield = 20.7 mg, 47 %



UPy-peptide (KGGG-AMPVQWH) **19**



vi. Sample preparation for atomic force microscopy (AFM) imaging

Solutions of **12**, **13**, **16**, **17**, **18** and **19** (100 μM) prepared in THF/deionized water (60:40) were drop cast on mica (10 μL), after several hours. The samples were allowed to dry overnight prior to imaging.

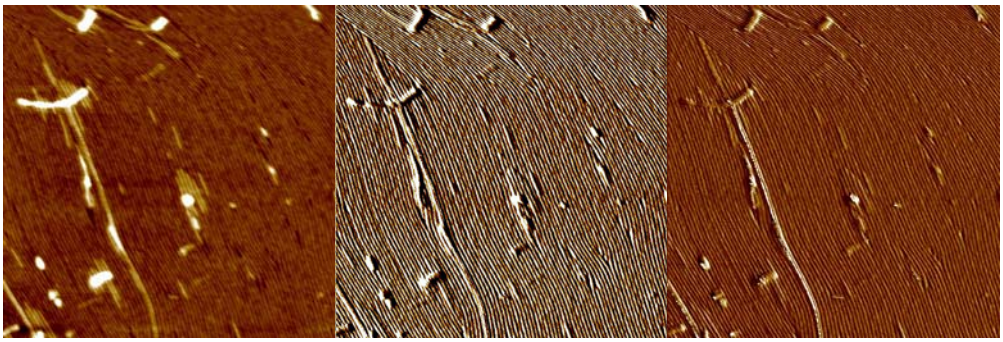


Figure 1: *AFM height (left), amplitude (center), and phase (right) images of* ***12****, 13 hours after dissolution (1 x 1 μm scale)*

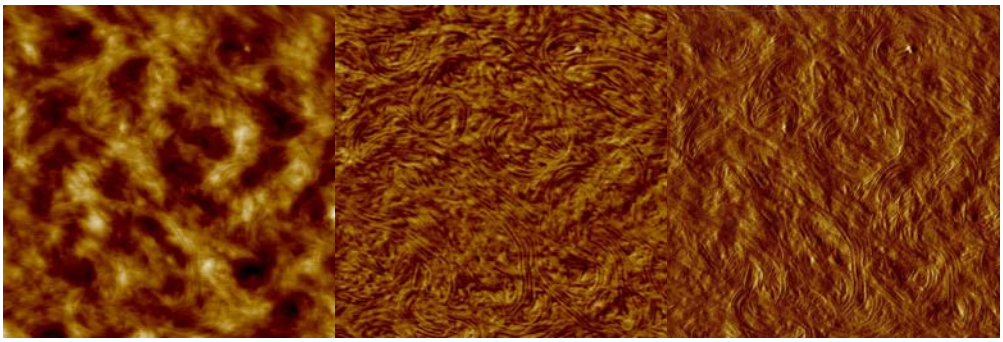


Figure 2: *AFM height (left), amplitude (center), and phase (right) images of* ***13****, 6 hours after dissolution (1 x 1 μm scale)*

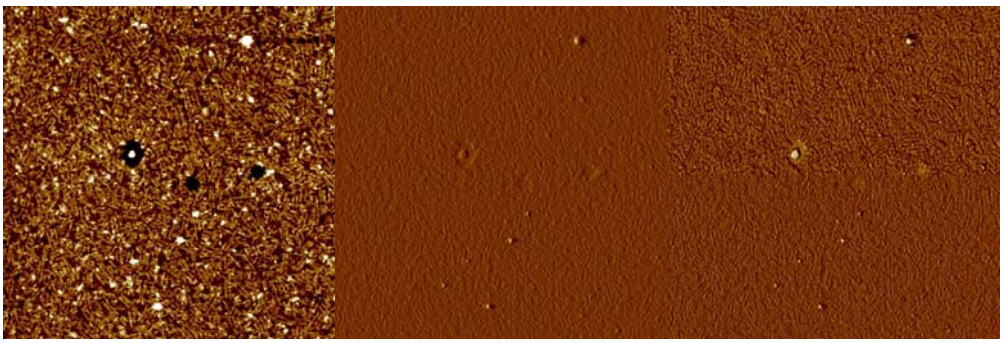


Figure 3: *AFM height (left), amplitude (center), and phase (right) images of* ***16****, 6 hours after dissolution (1 x 1 μm scale)*

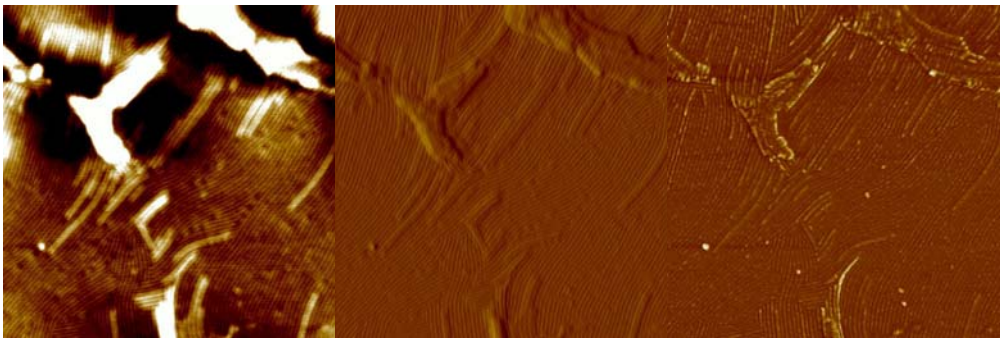


Figure 4: *AFM height (left), amplitude (center), and phase (right) images of* ***17****, 6 hours after dissolution (1 x 1 μm scale)*

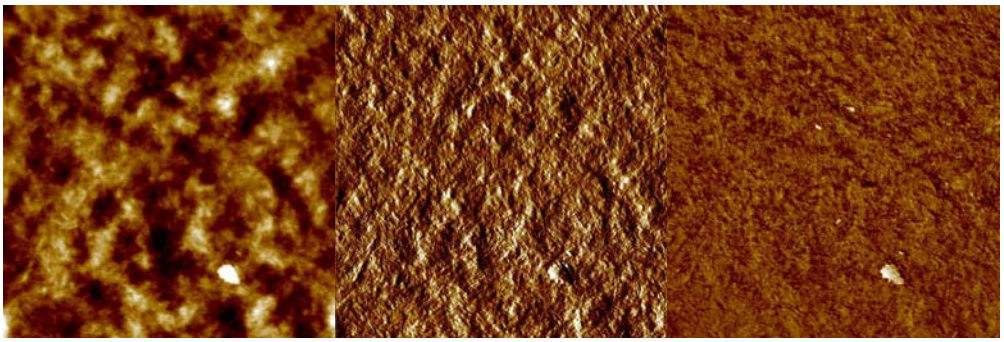


Figure 5: *AFM height (left), amplitude (center), and phase (right) images of* ***18****, 6 hours after dissolution (1 x 1 μm scale)*

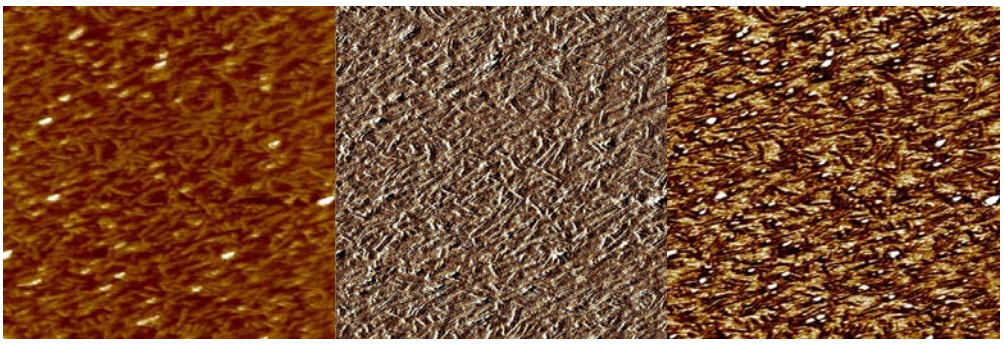


Figure 6: *AFM height (left), amplitude (center), and phase (right) images of* ***19****, 13 hours after dissolution (1 x 1 μm scale)*

Also, a solution of **12** in deionized water (100 μM) was placed in a sonication bath for 30 min until the solution turned clear. This solution was drop cast on mica (10 μL) several hours after sonication, and allowed to dry overnight. Fibre formation is visible, however, later it was determined that sonication decomposed **12** into the UPy-moiety and the peptide sequence. It is proposed that the oxime bond is broken.

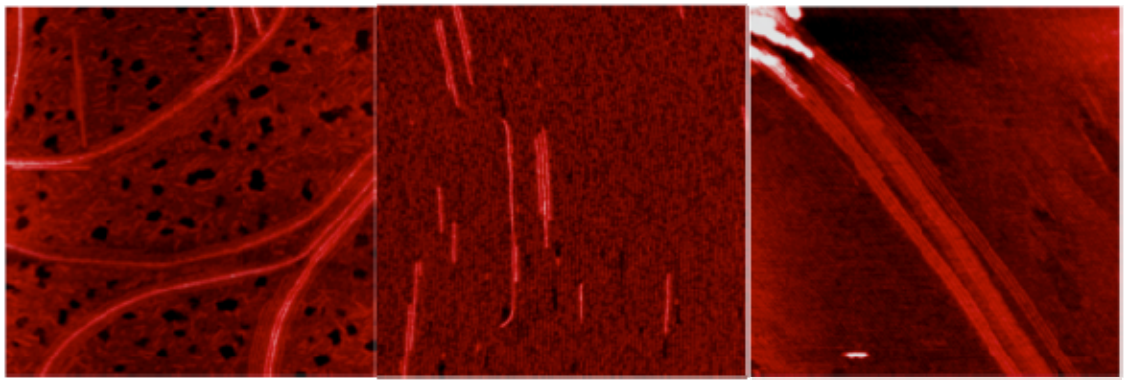


Figure 7: *AFM height images of* ***12*** *after sonication (1 x 1 μm scale)*

vi. Cell experiments

50 μL solutions of **12**, **13**, **17** (100 μM in 60 THF: 40 H_2O) were drop cast into a BD Falcon 8-chamber culture slide (BD BioSciences) and allowed to dry overnight at room temperature. In order to study the bioactivity of the UPy-peptides NIH/3T3 fibroblasts were seeded at a density of 7.5 x 10^4 cells/cm^2 and incubated at 37 °C and 5% CO_2 for 4 h under serum-free conditions in Dulbecco's Modified Eagle Medium (DMEM) (Invitrogen Corporation) supplemented with 1% Penicillin and Streptomycin (Invitrogen Corporation). Fibroblast adhesion to surfaces with **12**, **13**, and **17** was compared to fibronectin coated wells (50 μg/mL; BD BioSciences). Optical microscopy was performed after 4 h using a Zeiss Axiovert 40C microscope (Carl Zeiss MicroImaging) equipped with a Canon PowerShot A650 IS camera (Canon).

To assess cytoskeletal organization after 4 h, the fibroblasts were washed with pre-warmed 37 °C phosphate buffered saline (PBS; Sigma Aldrich) and fixed with 4% formaldehyde/PBS solution for 20 minutes at room temperature. Actin filaments were stained with phalloidin-TRITC (Sigma Aldrich; dilution 1:1000 in PBS) for 45 minutes at room temperature. Cell nuclei were counterstained simultaneously with 1 μM DAPI (Invitrogen Corpopration). Next, cells were washed with PBS and immersed in Citufluor Glycerol/PBS solution AF1 mounting medium (Agar Scientific). Fluorescence microscopy was performed with a Zeiss Axio Observer.D1 microscope equipped with a Zeiss Axio Cam MR3 camera and Zeiss Axio Vision SE64 Rel.4.8 software (all Carl Zeiss MicroImaging).

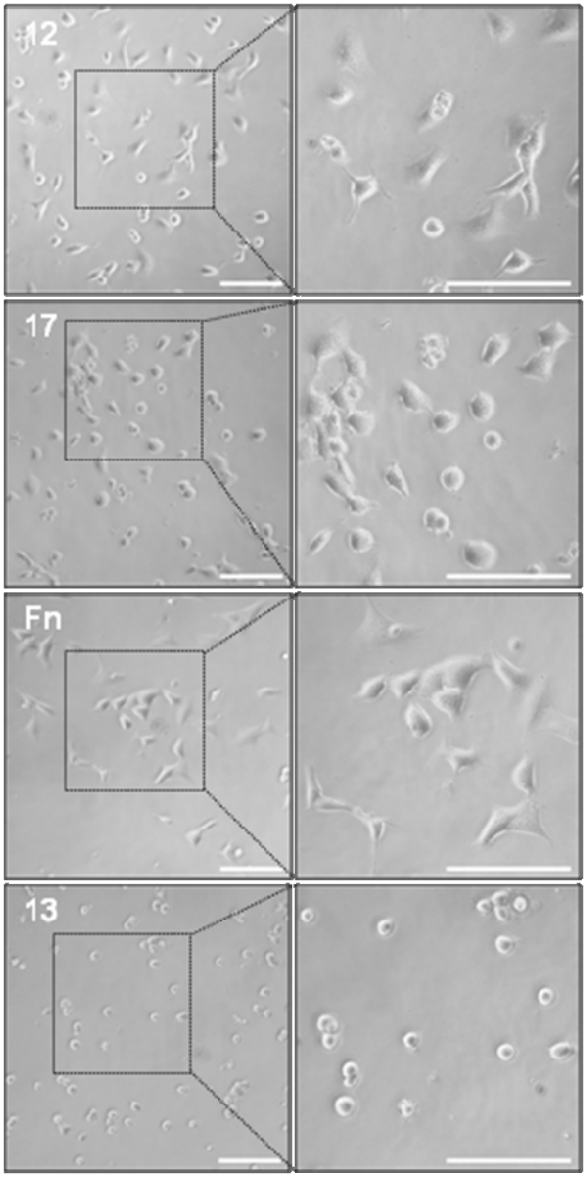


Figure 8: *Optical microscopy images of NIH/3T3 fibroblasts on peptide-coated surfaces. Fibroblasts were seeded at a density of 7.5 x 10^4 cells/cm^2 and incubated under serum-free conditions. Four hours after cell seeding fibroblasts adhered to the fibronectin (Fn) coated wells and the surfaces with the UPy-modified RGD peptides* ***12*** *and* ***17****, indicating bioactivity. No cell adherence was observed on the scrambled peptide* ***13****. Scale bars represent 100 µm.*

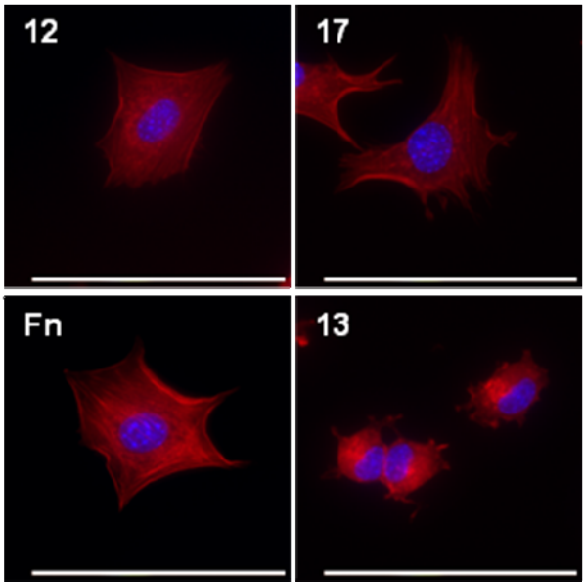


Figure 9: *Immunofluorescent images of actin filament organisation in NIH/3T3 fibroblasts on peptide-coated surfaces. Fibroblasts were fixated 4 hours after seeding and actin filaments and cell nuclei were stained with phalloidin-TRITC (red) and DAPI (blue), respectively. Actin filament organisation in fibroblasts adhered to UPy-modified RGD surfaces* ***12*** *and* ***17*** *was compared to cell-adhesion on fibronectin (Fn) coated wells. Scrambled peptide* ***13*** *served as negative control. Scale bars represent 100 µm.*