Selective functionalization of laser printout patterns on cellulose paper sheets coated by surface-specific peptides

Supporting Information

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1. Materials

1.1. Peptide Synthesis

N-α-Fmoc protected amino acids, Fmoc-Rink Amide resin (loading: 0.59 mmol/g), 2-(1H-benzotriazole-1-yl)-1,1,3,3-tertamethyluronium-hexafluorophosphate (HBTU) and *N*-methyl-2-pyrrolidone (NMP, 99.9 %, peptide synthesis grade) were used as received from IRIS Biotech GmbH (Marktredwitz, Germany). *N,N*-Diisopropyl-ethylamine (DIPEA, peptide grade), N,N-Diethyldithiocarbamate and Tetrakis(triphenylphosphine)palladium(0) were purchased from Acros Organics and used without further purification. Barbituric acid (Lancester), triethylsilane (TES, 98+ %, Alfa Aesar, Karlsruhe, Germany) and α-Cyano-4-hydroxy-cinnamic acid (α-CHCA, 99 %, Sigma Aldrich) have been applied as received. Trifluoracetic acid (TFA, peptide grade) as well as piperidine (peptide grade) were obtained from Acros Organics and distilled prior to use. Dichlormethane (DCM, peptide grade, IRIS Biotech) was distilled from CaH_2 prior to use.

1.1. Paper functionalization

Citric acid, ammonium peroxodisulfate (APS) and tris(bipyridine)ruthenium(II) chloride (Ru-bpy) were purchased from Sigma Aldrich and used as received.

2. Instrumentation

2.1. Peptide synthesis

Synthesis of peptides was performed automatically on an Applied Biosystems ABI 433a peptide synthesizer with ABI-Fastmoc protocols.

2.2. Semi-preparative HPLC

Peptides were purified via semi-preparative HPLC at 22 mL/min on a Shimadzu (Germany) Prominence LC 20-AP system equipped with a CBN-20A Communications Bus Module, a LC-20AP Preparative Liquid Chromatograph, a SIL-20A HT Auto Sampler and a FRC-10A Fraction Collector. UV-Vis detection was conducted on a SPD-10A UV-Vis Detector. Separation of the products was achieved using a Synergy 4n Fusion-RP 80 A column (Phenomenx Synergi 4µ

Fusion-RP 80A, Ax) and Solvent A/Solvent B-mixtures (Solvent A: 99.9 % MilliQ H_2O: 0.1 % formic acid; Solvent B: 99.9 % acetonitrile: 0.1 % formic acid) as solvents.

2.3. MALDI-TOF-MS

MALDI-TOF-MS measurements were performed on a Shimadzu Axima Confidence with matrix assisted laser desorption/ionization and time of flight detector using α-CHCA (dissolved in 50:50:0.1 (v/v/v) acetonitrile/water/TFA) as matrix.

2.4. UPLC-ESI-MS

UPLC analysis was performed on an Acquity-UPLC H-class CM Core system (Waters Corporation, Milford, USA) with Acquity-UPLC PDA and QDa detectors and a LCT Premier XE mass spectrometer for HRMS. An Acqutiy-UPLC HSS T3 column (Waters) was used at 40°C.

2.5. MALDI-imaging

MALDI-TOF-MS measurements for imaging experiments were performed on a MALDI LTQ Orbitrap XL from Thermo Fisher Scientific (Waltham, MA, USA) and evaluated with the ImageQuest 1.0.1 software (Thermo Fisher Scientific).

2.6. ToF-SIMS

ToF-SIMS was conducted with a TOF.SIMS5 instrument (ION-TOF GmbH, Münster, Germany), equipped with a Bi cluster liquid metal primary ion source and a non-linear time-of-flight analyzer. The Bi source was operated in the bunched mode providing 0.7 ns Bi_3^+ ion pulses at 25 keV energy and a lateral resolution of approx. 4 μm for all surfaces. The short pulse length allowed high mass resolution to analyze the complex mass spectra of the immobilized organic layers. Images larger than the maximum deflection range of the primary ion gun of 500×500 μm^2 were obtained using the manipulator stage scan mode. Primary ion doses were kept below 10^{11} ions m^{-2} (static SIMS limit). Spectra were calibrated on the C^-, CH^-, CH_2^-, CH_3^-, or on the C^+, CH^+, CH_2^+, and CH_2N^+.

2.7. Photoreactor

Light triggered reactions were performed in a custom built photo reactor as shown in Figure S1, equipped with a compact low-pressure lamp (Arimed B6, Cosmedico GmbH, Germany) emitting at λ_{max} = 320 nm (± 30 nm, 36 W). Cellulose sheets were placed into glass vials (Pyrex, diameter 20 mm), crimped airtight with a styrene/butadiene rubber seal before being immersed with a tetrazole solution. After degassing with Ar for 5 min, the vials were placed into the sample holder at a distance of 40–50 mm to the light source.

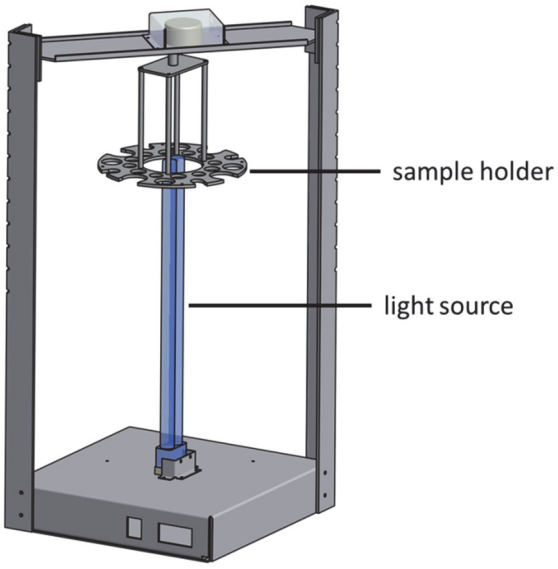


Figure S1. Depiction of the custom-built photo reactor applied for light triggered reactions.

2.8. UV-Vis measurements

UV-Vis measurements were performed by irradiation at Samples were then irradiated at 330 nm (Bandpassfilter 330 nm FWHM 10 nm, Optoprim GmbH) and spectra were recorded on a Cary 60 UV-Vis spectrophotometer (Agilent).

3. Synthesis of substances

3.1. Peptide Synthesis and functionalization

Synthesis of peptides was performed automatically on an Applied Biosystems ABI 433a peptide synthesizer by standard Fmoc-strategy. ABI-Fastmoc protocols (single coupling up to the 10th amino acid, double coupling from the 11th amino acid; additional capping) were applied and peptide synthesis was conducted in NMP using Polystyrene Rink Amide resin (loading 0.59 mmol/g, 0.1 mmol) as solid support. Fmoc-amino acid coupling was facilitated by HBTU/DIPEA. For *N*-terminal modification with maleimide, *N*-Maleoyl-β-alanine (synthesis reported elsewhere)[1] was attached to the deprotected peptide using 4 eq. of the maleimide, 3.6 eq. HBTU, 8 eq. DIPEA and 4 eq. HOBt in NMP (2 x 1 h). For *C*-terminal modification with tetrazole moiety, Fmoc-Lys(alloc)-OH allowed the removal of the allyloxycarbonyl protective group prior to Fmoc deprotection using 1 eq. Tetrakis(triphenylphosphine)palladium(0), (Pd(PPh3)4) and 10 eq. barbituric acid in dry DCM (2 x 30 min). Subequent removal of catalyst was performed with *N,N*-Diethyldithiocarbamate (5% (w/v) in NMP) for 30 min and subsequent NMP/DCM washes (5 x NMP, 5 x DCM, 5 x NMP). Functionalization of free ε-amine with triazole compound (4-(2-(4-methoxyphenyl)-*2H*-tetrazol-5-yl)benzoic acid, synthesis reported elsewhere)[2] was conducted using 1 eq. triazole, 0.9 eq. HBTU, 2 eq. DIPEA and 1 eq. HOBt in NMP (2 x 1 h). NMP/DCM washes were followed by *N*-terminal Fmoc deprotection in piperidine/NMP (1:1 [v/v]; 2 x 30 min). Subsequently, the resin was washed with NMP (5 times) and DCM (10 times). Liberation of all peptides was conducted with 95:3:2 (v/v/v) TFA/H_2O/TES for 2.5 h and resulted in fully deprotected peptides. Products were isolated by precipitation with diethyl ether and subsequent centrifugation. Purification was performed via semi-preparative HPLC.

3.2. Analysis of GGGSGVYKVAYDWQH (TBP)

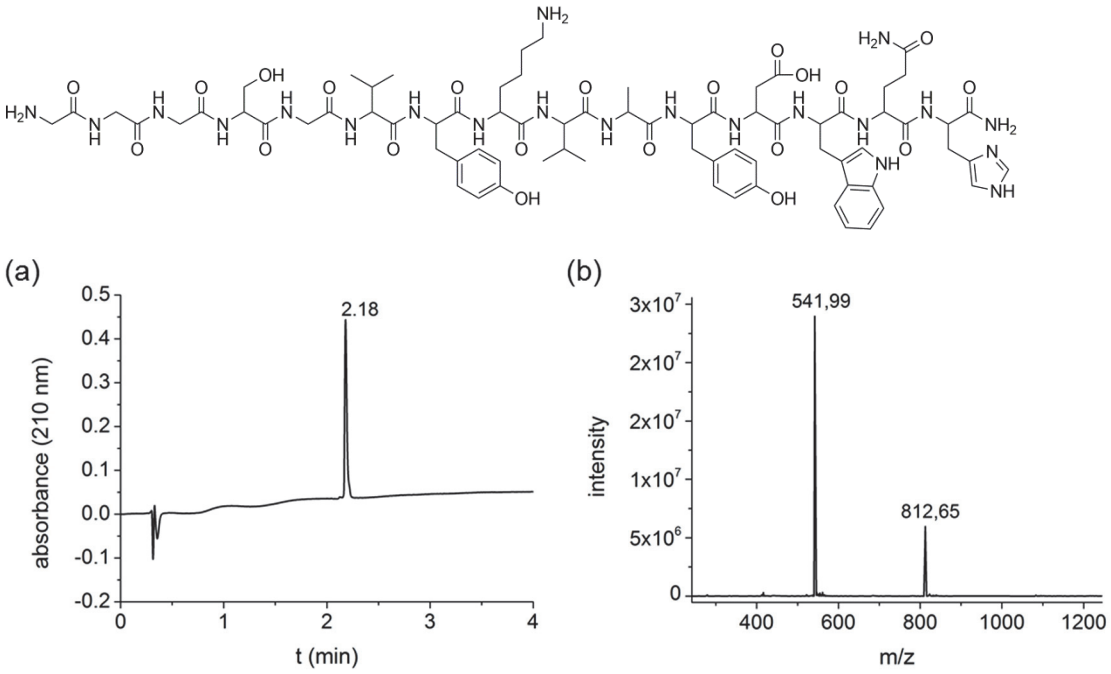


Figure S2. (a) HPLC of TBP_1 using a gradient of 5 % - 50 % Acetonitrile in ultrapure water within 4 minutes (rt = 2.18 min) and (b) ESI-MS of the product peak (542.0 z=3; 812.7 z=2).

UPLC/ESI-MS M[calc.] = 541.9 $(M+3H)^{3+}$, 812.4 $(M+2H)^{2+}$

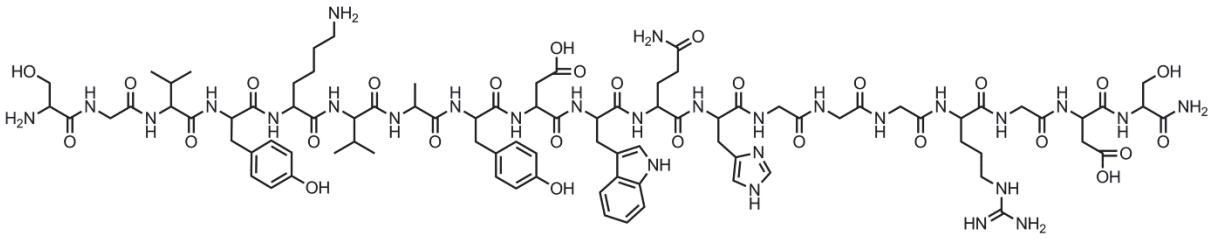
M[found] = 542.0 $(M+3H)^{3+}$, 812.7 $(M+2H)^{2+}$

MALDI-TOF-MS M[calc.] = 1622.8 $(M+H)^{+}$

M[found] = 1623.1 $(M+H)^{+}$

The signals can be assigned within ± 1 Da accuracy.

3.3. Analysis of SGVYKVAYDWQHGGGRGDS (TBP-RGDS)



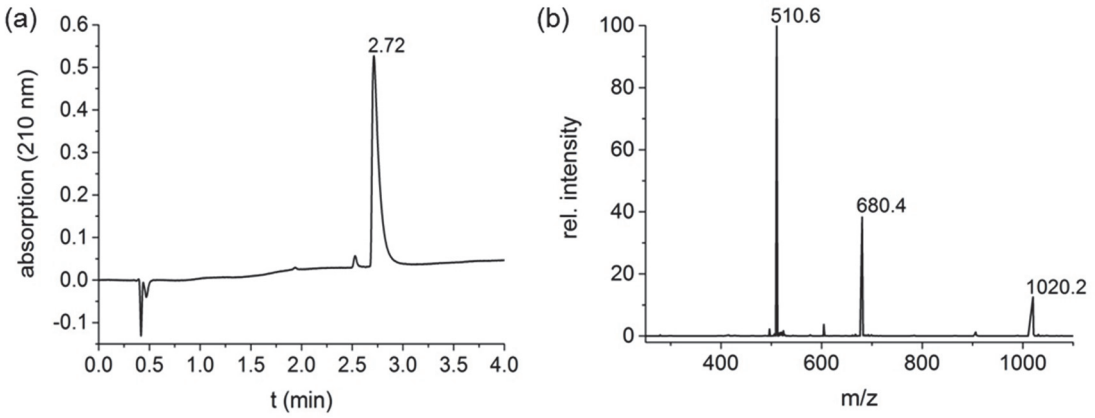


Figure S3. (a) UPLC of SGVYKVAYDWQHGGGRGDS (TBP-RGDS) using a gradient of 5 % - 30 % acetonitrile in ultrapure water within 4 minutes (r.t. = 2.72 min) and (b) ESI-MS of the product peak.

UPLC/ESI-MS M[calc.] = 1020.1 $(M+2H)^{2+}$, 680.4 $(M+3H)^{3+}$, 510.6 $(M+4H)^{4+}$

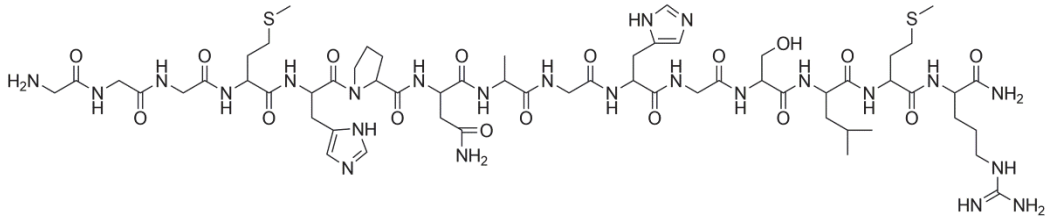
M[found] = 1020.2 $(M+2H)^{2+}$, 680.4 $(M+3H)^{3+}$, 510.6 $(M+4H)^{4+}$

MALDI-TOF-MS M[calc.] = 2039.2 $(M+H)^{+}$

M[found] = 2040.5 $(M+H)^{+}$

The signals can be assigned within ± 1.5 Da accuracy.

3.4. Analysis GGGMHPNAGHGSLMR (CBP)



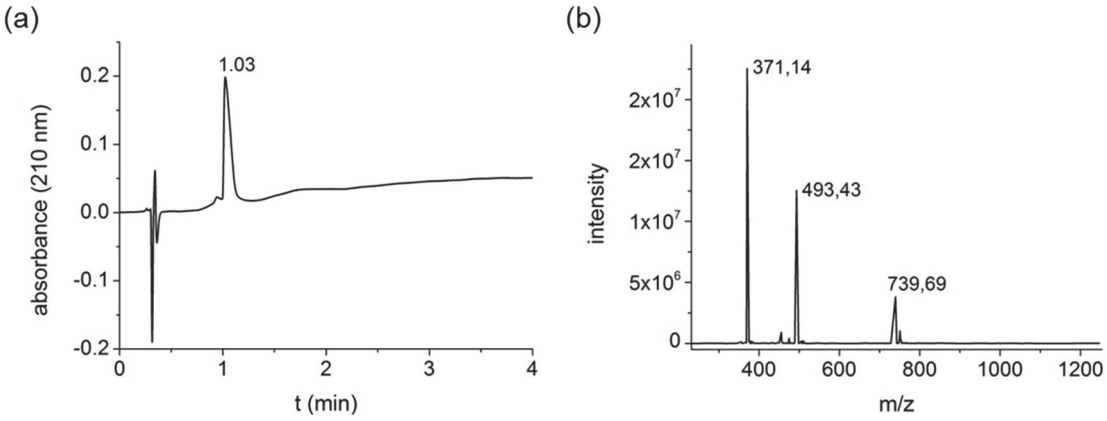


Figure S4. (a) HPLC of CBP_1 using a gradient of 5 % - 50 % Acetonitrile in ultrapure water within 4 minutes (rt = 1.03 min) and (b) ESI-MS of the product peak (371.14 z=4; 493.43 z=3; 739.69 z=2).

UPLC/ESI-MS M[calc.] = 370.4 $(M+4H)^{4+}$, 493.6 $(M+3H)^{3+}$, 739.9 $(M+2H)^{2+}$

M[found] = 371.1 $(M+4H)^{4+}$, 493.4 $(M+3H)^{3+}$, 739.7 $(M+2H)^{2+}$

MALDI-TOF-MS M[calc.] = 1477.7 $(M+H)^{+}$

M[found] = 1478.0 $(M+H)^{+}$

The signals can be assigned within ± 1 Da accuracy.

3.5. Analysis of Mal-GGGMHPNAGHGSLMR (Mal-CBP)

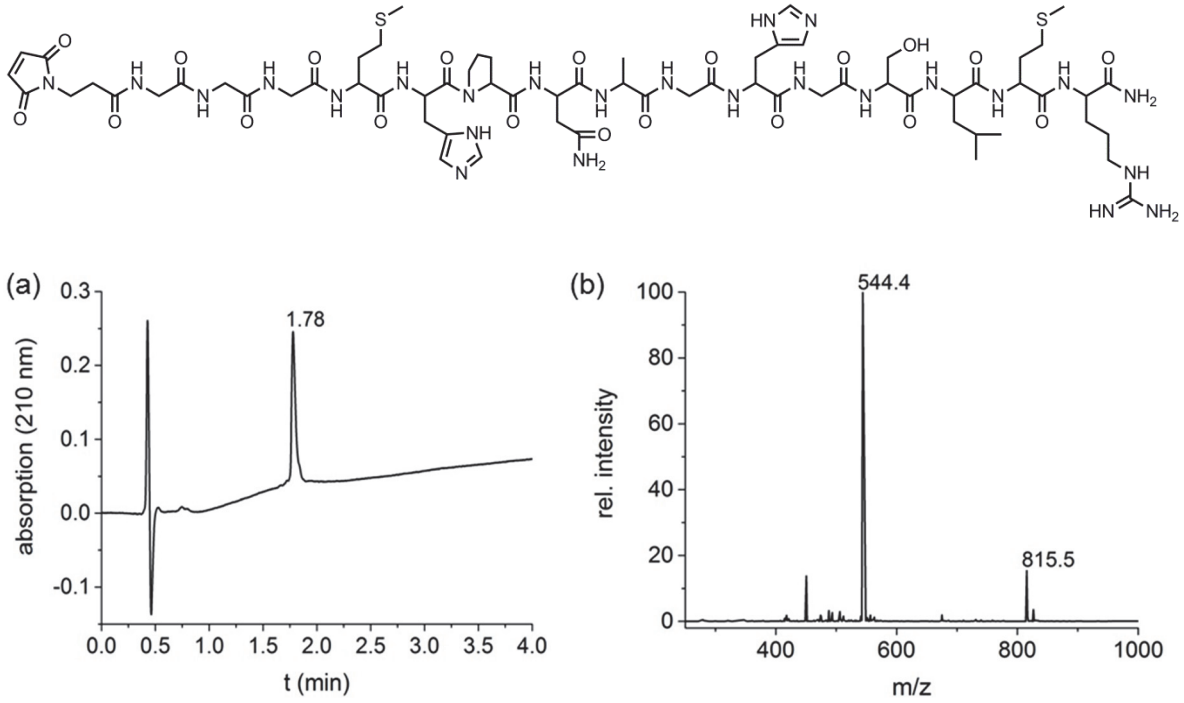


Figure S5. (a) UPLC of Mal-GGGMHPNAGHGSLMR (Mal-CBP) using a gradient of 05 % - 30 % acetonitrile in ultrapure water within 4 minutes (r.t. = 1.78 min) and (b) ESI-MS of the product peak.

UPLC/ESI-MS M[calc.] = 815.9 $(M+2H)^{2+}$, 544.2 $(M+3H)^{3+}$

M[found] = 815.5 $(M+2H)^{2+}$, 544.4 $(M+3H)^{3+}$

MALDI-TOF-MS M[calc.] = 1629.8 $(M+H)^{+}$

M[found] = 1630.4 $(M+H)^{+}$

The signals can be assigned within ± 1 Da accuracy.

3.6. Analysis of KRWRIRVRVIRKGGK-tetrazole (AMP-Tet)

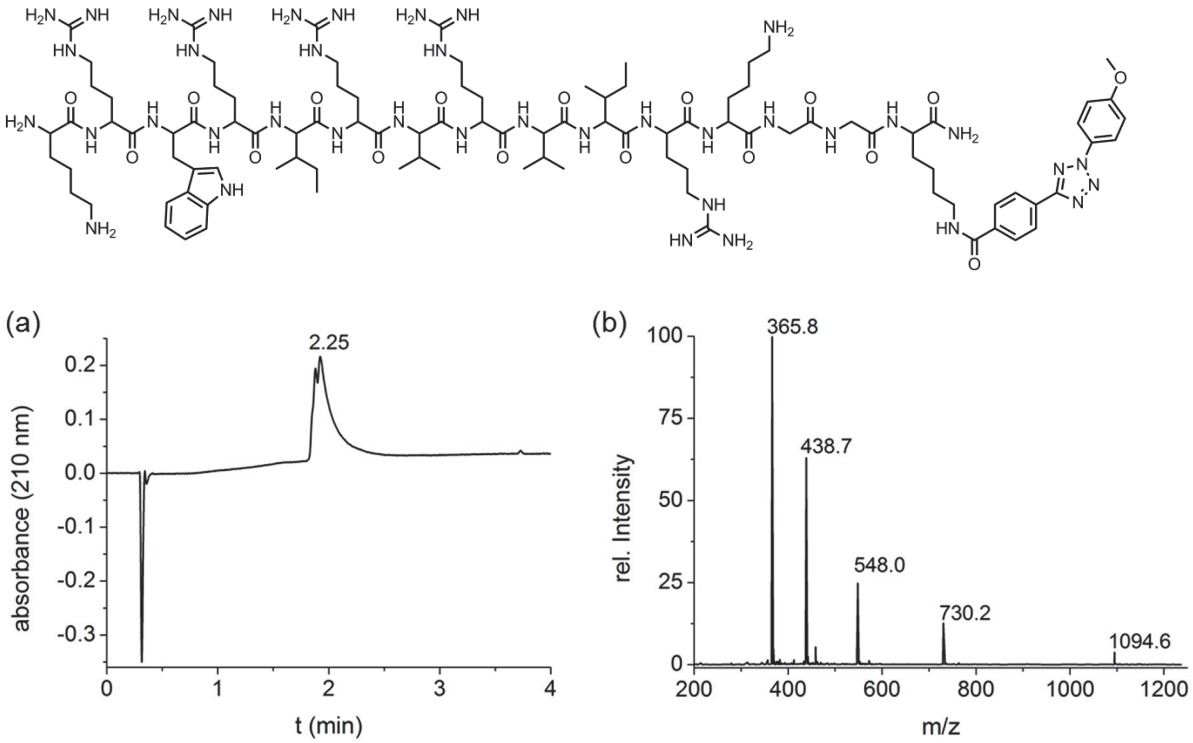


Figure S6. (a) UPLC of KRWRIRVRVIRKGGK-tetrazole (AMP-Tet) using a gradient of 05 % - 30 % acetonitrile in ultrapure water within 4 minutes (rt = 2.25 min) and (b) ESI-MS of the product peak.

UPLC/ESI-MS M[calc.] = 1094.30 $(M+2H)^{2+}$, 729.83 $(M+3H)^{3+}$, 547.62 $(M+4H)^{4+}$, 438.30 $(M+5H)^{5+}$, 365.42 $(M+6H)^{6+}$

M[found]= 1094.6 $(M+2H)^{2+}$, 730.2 $(M+3H)^{3+}$, 548.0 $(M+4H)^{4+}$, 438.7 $(M+5H)^{5+}$, 365.8 $(M+6H)^{6+}$

MALDI-TOF-MS M[calc.] = 2187.49 $(M+H)^{+}$

M[found] = 2188.2 $(M+H)^{+}$

The signals can be assigned within ± 1 Da accuracy.

4. MALDI-imaging

Coated samples were fixed to a glass slide with double-faced adhesive tape and coated with matrix solution (10 mg/mL α-CHCA in H_20/MeOH 50:50 v/v) by airbrush. The slide was then fixed to a MALDI carrier. A spectrum was taken each 100 µm with 2 shots per spectrum over an area of 13.3 mm x 14.0 mm.

5. ToF-SIMS

5.1. Analysis of TBP-RGDS coated printouts

Filter paper was printed with a HP LaserJet Pro CM1415fn color MFP (Böblingen, Germany). Printed patterns were cut out and separately washed carefully in citrate buffer (citric acid 50 mM, pH 6.5) for 30 min, then incubated with TBP/TBP-RGDS (0.2 mg/mL in citrate buffer) for 15 minutes. After washing with citrate buffer two times for 2 min at pH 6.5 and 4.0 each, coated printouts were dried under air. Measurements were performed both with non-coated and coated samples. For TBP-RGDS coated samples, specific signals for amino acids valine $[C_5H_7O]^+$, histidine $[C_4H_5N_2]^+$ and a fragment of m/z = 720.50 (assignable to $[SGVYKVA^{CONH}]^+$) were observed (cf. Figure S7), confirming the presence of TBP-RGDS on toner printed sites. For plain toner-printed structures, no image for the specific fragments could be constructed, as masses were absent.

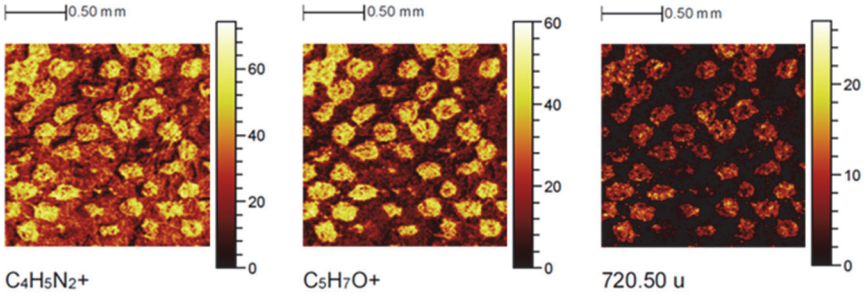


Figure S7. ToF-SIMS analysis of printed structures after incubation with TBP-RGDS in positive mode showing 2D images for $[C_4H_5N_2]^+$ and $[C_5H_7O]^+$ ions as well as a fragment with m/z = 720.5.

5.2. Analysis of Mal-CBP coated printouts

Filter paper was printed with a HP LaserJet Pro CM1415fn color MFP (Böblingen, Germany). Printed patterns were cut out and separately washed carefully in citrate buffer (citric acid 50 mM, pH 6.5) for 30 min, then incubated with TBP/TBP-RGDS (0.2 mg/mL in citrate buffer) for 15 minutes. After washing with citrate buffer two times for 2 min at pH 6.5 and 4.0 each, the peptides were cross-linked on the surface via PICUP. Therefor they were placed each separately on object slide, covered with 25 µL of 1 mM ammonium peroxodisulfate (APS) and 25 µL 1mM tris(bipyridine)ruthenium(II) chloride (Ru-bpy) and illuminated for 10 s with the light source of an Axio Observer.A1 microscope (Carl Zeiss Microscopy, Thornwood, USA) on maximum light intensity. Washing two times for 2 min with citrate buffer was followed by incubation with CBP/Mal-CBP (0.2 mg/mL in diluted TBST buffer [25 µM tris-HCl [pH 7.5], 75

µM NaCl, 0.25 ‰ v/v Tween 20]) for 10 min. Finally the samples were washed 3 times for 2 min with diluted TBST buffer and dried under air.

Measurements were performed both with non-coated and coated samples. For non-coated printed structures, images for methionine $[C_2H_5S]^+$, proline $[C_4H_8N]^+$ and leucine $[C_5H_{12}N]^+$ (cf. Figure S8) show no or unspecific appearance of these fragments.

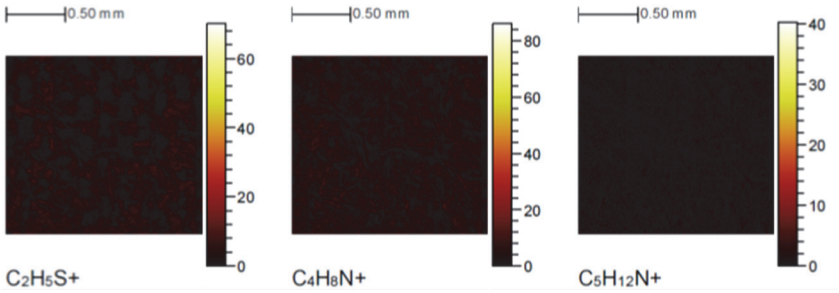


Figure S8. ToF-SIMS analysis of printed structures prior to coating in positive mode showing 2D images for $[C_4H_8N]^+$ and $[C_5H_{12}N]^+$ ions.

In the negative mode, specific signals for sulfur ions $[S, HS]^-$ resulting from methionine (cf. Figure S9) are nearly absent.

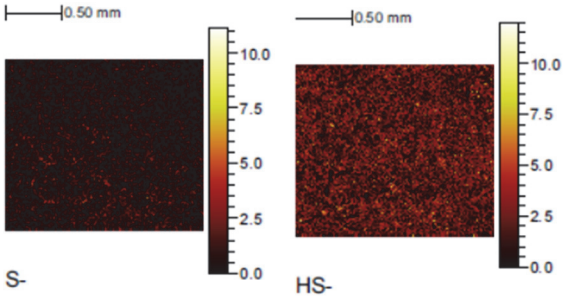


Figure S9. ToF-SIMS analysis of printed structures prior to coating in negative mode showing 2D images for $[S]^-$ and $[HS]^-$ ions.

For Mal-CBP coated samples, specific signals for amino acids methionine $[C_2H_5S]^+$, proline $[C_4H_8N]^+$ and leucine $[C_5H_{12}N]^+$ were observed (cf. Figure S10) in the positive mode, confirming the presence of Mal-CBP on cellulose. In addition, sulfur ions $[S, HS]^-$ resulting from methionine were observed in the negative mode (cf. Figure S11), further proving the surface-specific functionalization of cellulose with Mal-CBP.

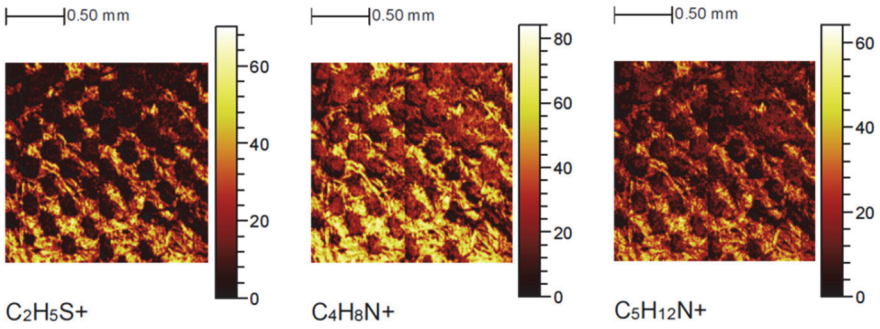


Figure S10. ToF-SIMS analysis of printed structures after incubation with TBP-RGDS, PICUP and Mal-CBP coating in negative mode showing 2D images for $[C_2H_5S]^+$, $[C_4H_8N]^+$ and $[C_5H_{12}N]^+$ ions.

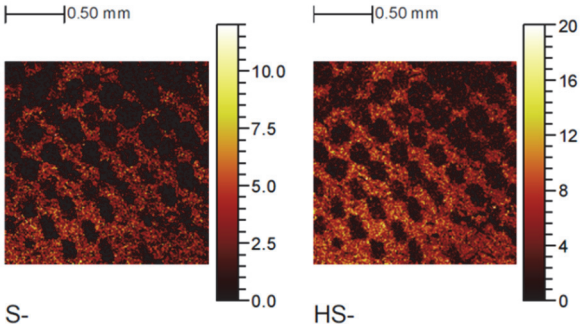


Figure S11. ToF-SIMS analysis of printed structures after incubation with TBP-RGDS, PICUP and Mal-CBP coating in negative mode showing 2D images for $[S]^-$ and $[HS]^-$ ions.

5.3. Functionalization of coated printouts

For identification of AMP-Tet on functionalized surfaces, a dropcast of AMP-Tet was recorded and compared with signals arising for the irradiated coated surface. A series of 13 masses were singled out due to revealing spatial resolution on sections capable to undergo pyrazoline formation with AMP-Tet (see Table S1).

Table S1. Specific fragments for AMP-Tet.

mass number	1	2	3	4	5	6	7
m/z	27.02	39.01	42.03	43.02	56.04	58.04	68.02

mass number	8	9	10	11	12	13
m/z	69.03	71.00	72.06	84.06	98.03	99.02

Reference experiment

In order to exclude physisorption of the AMP-Tet on cellulose sections, a non-coated printout was placed in a vial and crimped air tight before being covered with a solution of AMP-Tet (1 mg in 1 mL acetonitrile, 5 Vol.% DMSO) and purged with argon for 5 min. The sample was kept in the dark for 1 h before 1 mL of MQ water was added dropwise. After being kept in the dark for another hour, the cellulose sheet was rinsed extensively with acetonitrile and MQ water before being dried with N_2. Subsequent ToF-SIMS measurements show no accumulation of specific fragments for AMP-Tet (cf. Figure S12).

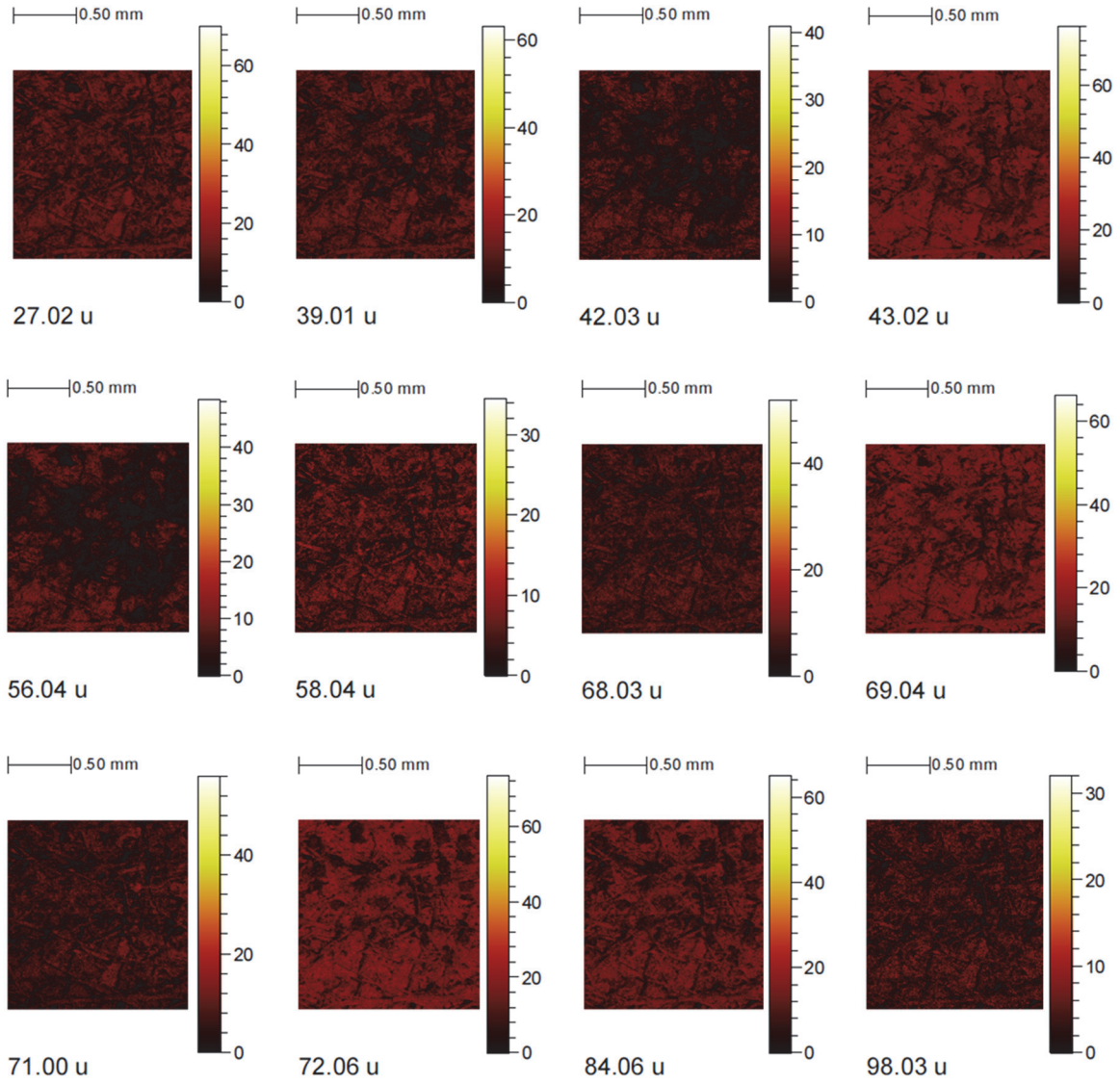


Figure S12a. ToF-SIMS analysis of non-coated printed structures after incubation with APM-Tet in positive mode showing 2D images for specific fragments according to AMP-Tet fingerprint.

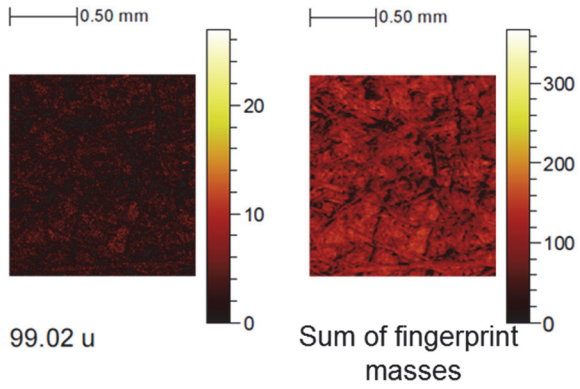


Figure S12b. ToF-SIMS analysis of non-coated printed structures after incubation with APM-Tet in positive mode showing 2D images for specific fragments according to AMP-Tet fingerprint.

Functionalization with AMP-Tet

Printed structures were coated according to chapter 5.2. A coated printout was placed in a vial and crimped air tight before being covered with a solution of AMP-Tet (1 mg in 1 mL acetonitrile, 5 Vol.% DMSO) and purged with argon for 5 min. The vial was placed in a custom built photo reactor and irradiated with λ_{max} = 320 nm (Arimed B6) for 1 h before 1 mL of MQ water was added dropwise followed by an additional hour of irradiation. Subsequently, the cellulose sheet was rinsed extensively with acetonitrile and MQ water before being dried with N_2. Subsequent ToF-SIMS measurements show the accumulation of specific fragments for AMP-Tet on the cellulose surface (cf. Figure S13).

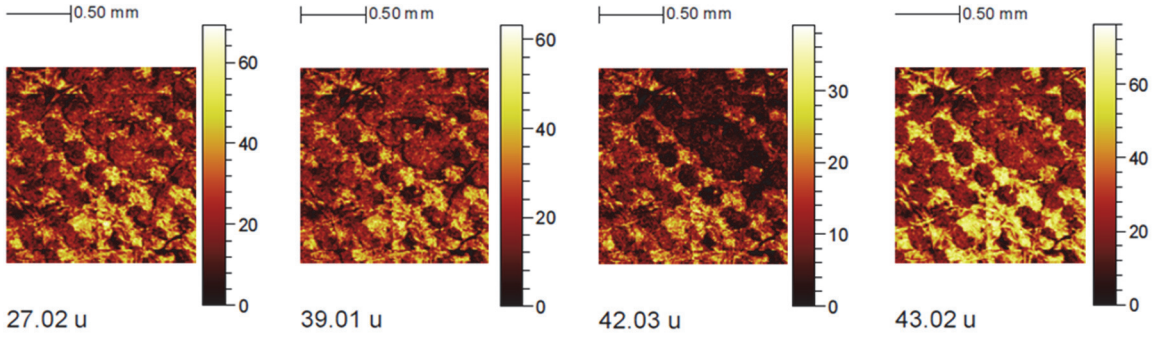


Figure S13a. ToF-SIMS analysis of functionalized printed structures after incubation with TBP-RGDS, PICUP, Mal-CBP coating and NITEC modification in positive mode showing 2D images for specific fragments according to AMP-Tet fingerprint.

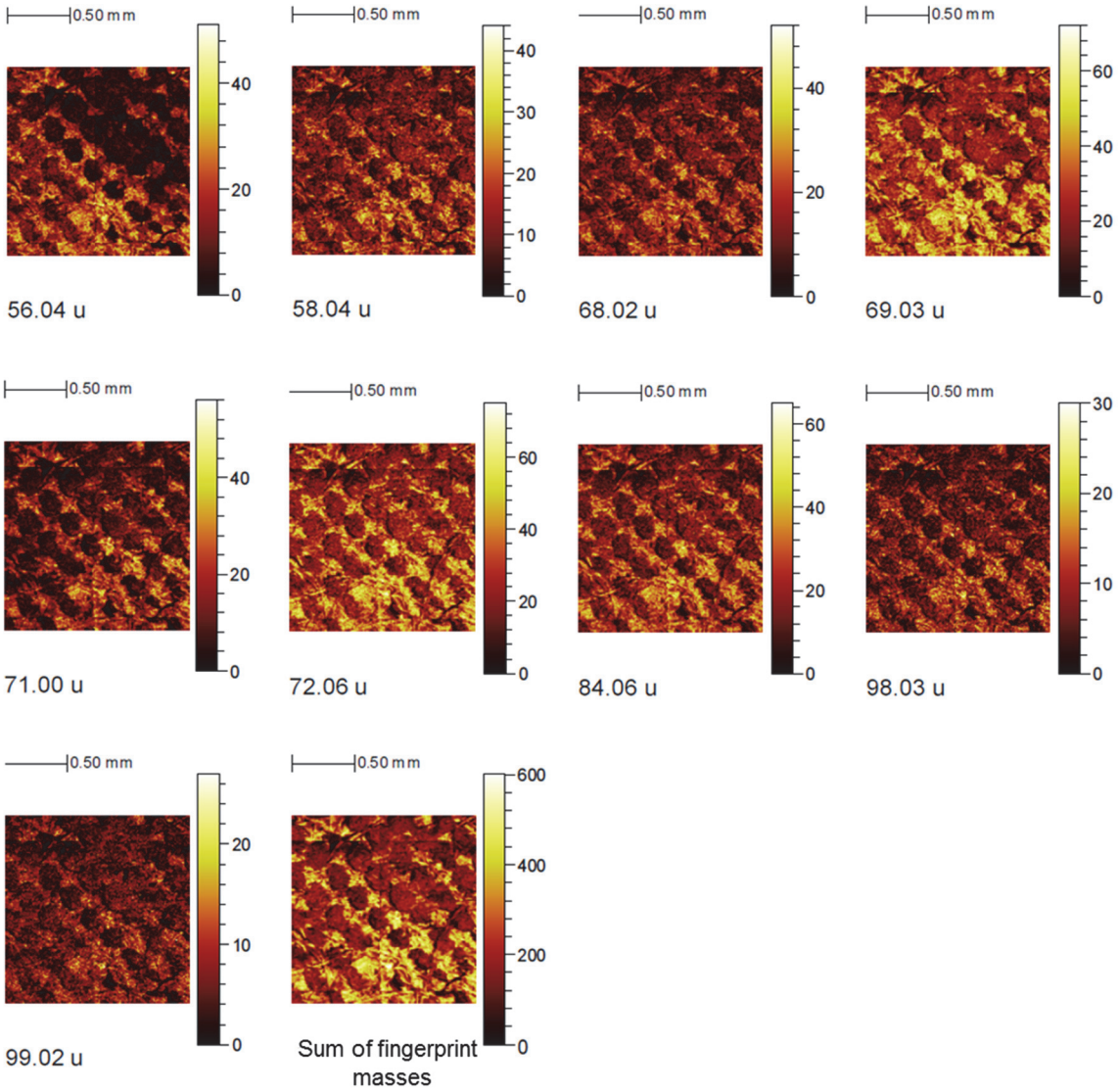


Figure S13b. ToF-SIMS analysis of functionalized printed structures after incubation with TBP-RGDS, PICUP, Mal-CBP coating and NITEC modification in positive mode showing 2D images for specific fragments according to AMP-Tet fingerprint.

6. UV-Vis measurements

Under red light, a solution of 4-(2-(4-methoxyphenyl)-*2H*-tetrazol-5-yl)benzoic acid (tetrazole) and Mal-CBP (each 3 µmol in 1 mL acetonitrile, 5 Vol.% DMSO) was prepared and purged with argon for 5 min. Subsequently, the sample was irradiated at λ_{max} = 330 nm for ten minutes and spectra were recorded for every minute (cf. Fig 3a manuscript).

7. References

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