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Supporting Information for

Spatially Resolved Photochemical Coding of Reversibly Anchored Cysteine-Rich Domains

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1. Materials

Pentafluorophenol (PFP, Aldrich, ≥99 %), 4-(*N,N*-dimethylamino)pyridine (DMAP, Aldrich, >99%), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC, Carl Roth, >99%) hydrochloride, diisopropyl ethyl amine (DIPEA, Carl Roth, ≥ 99%), glutathione (GSH, AppliChem, ≥97%), glutathione oxidized (GSSG, TCI, >98%), L-arginine (Carl Roth, >98.5%), 2-bromo-2-methylpropionic acid (Aldrich, 98%), monoethylfumarate (Aldrich, 95%), sodium hydroxide (NaOH, Carl Roth, ≥98%), dipotassium hydrogenphosphate (Carl Roth, ≥99%), dichloromethane (DCM, Fischer, p.a.), *N*-dimethylformamide (DMF, Fischer, p.a.) and toluene (Fischer, p.a.) were used as received. The tetrazole silane was synthesized according to literature.[1] PEG-fumarate was prepared according to a literature procedure.[2] The CRD with the sequence $GPC_3GSYC_7PSVC_{11}APAC_{15}APVC_{19}C_{20}A$ was purchased from Biomatik (http://www.biomatik.com) in a purity of >95% (HPLC). Dialysis tubes used were from SpectrumLabs, employing Spectra/Por 6 with a MWCO of 1 kDa made of regenerated cellulose.

1.1. Folding of the CRD

Characteristic of the CRD peptide:

Sequence (one letter code):

GPCGSYCPSVCAPACAPVCCA[a]

Molecular weight: 1959.34 g/mol (reduced state)

In a round bottom flask, 100 mg of the CRD in reduced state (0.051 mmol,), 1.5 mg of GSH (0.005 mmol), 27.5 mg of GSSG (0.045 mmol) and 871 mg of L-arginine (5 mmol) were dissolved in 100 mL Millipore water. The solution was stirred at ambient temperature overnight. The next day, the reaction solution was transferred into a dialysis tube (MWCO 1 kDa) and dialyzed against deionized water for three days by exchanging the dialysis bath three times. Finally, the solution was freeze-dried to obtain a colorless white powder (50 mg, yield: 50%). ESI-MS: $[M+H]^+_{exp}$: 1952.7127, $[M+H]^+_{calc}$: 1952.6926.

For the ESI-MS spectrum of the CRD in reduced state please refer to Figure S1

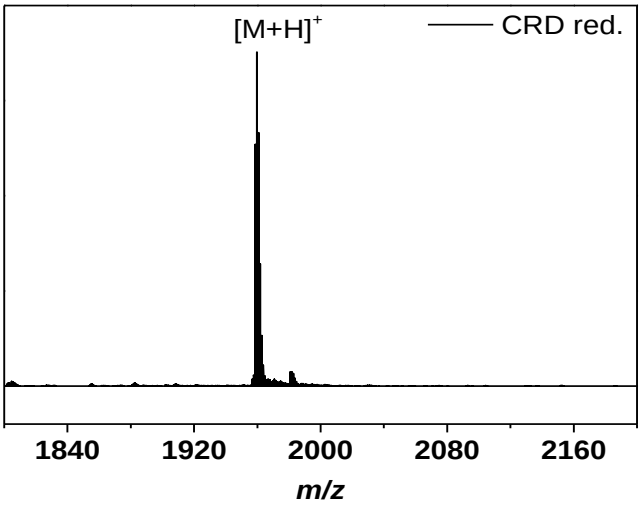

Figure S1. ESI-MS spectrum of the CRD in reduced state. Spectrum was recorded in MeCN/H_2O mixture with the addition of 1 % acetic acid. ESI-MS: $[M+H]^+_{exp}$: 1958.7538, $[M+H]^+_{calc}$: 1958.7396.

1.2. Synthesis of PFP-fumarate

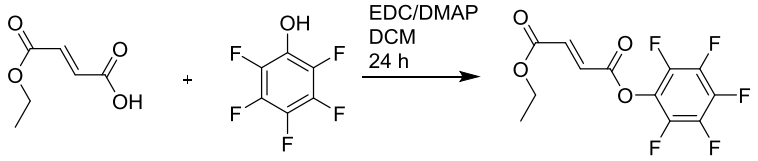

Figure S2. Esterification of monoethylfumarate yielding pentafluorophenol-fumarate.

In a round bottom flask, monoethylfumarate (2.00 g, 13.8 mmol, 1 eq.), PFP (3.19 g, 17.4 mmol, 1.25 eq.) and DMAP (0.34 g, 2.8 mmol, 0.2 eq.) were dissolved in 25 mL of a mixture of DCM/THF, using only as much THF as necessary to dissolve the monoethylfumarate. Next, the solution was cooled to 0 °C. The reaction was started by adding EDC (3.32 g, 17.4 mmol, 1.25 eq.) and the mixture was allowed to warm up to ambient temperature overnight. The next day, the solvent was removed under reduced pressure and the crude product was dissolved in DCM. The organic phase was washed with 5% hydrochloric acid, $NaHCO_3$ and brine solution. Subsequently, the united organic phase was dried over Na_2SO_4 and the solvent was removed under reduced pressure. The final product was obtained after gel flash chromatography, using cyclohexane/ethyl acetate (3:1 v/v) as eluent, obtaining a pale yellowish oil (2.8 g, 65%). ^{1}H- NMR (400 MHz, $CDCl_3$, 25 °C): δ (ppm): 7.14 - 7.04 (m, 2H, *H*C=C*H*), 4.32 (q, 2H, O-C*H*$_2$, ^3J = 7.4 Hz), 1.34 (t, 3H, C*H*$_3$-CH$_2$, ^3J = 7.4 Hz). ^{19}F-NMR (377 MHz, $CDCl_3$, 25 °C): δ (ppm): -152.4 (m, 2F, *ortho*), -157.2 (m, 1F, *para*), -161.9 (m, 2F, *meta*).

1.3. Synthesis of PFP-bromide

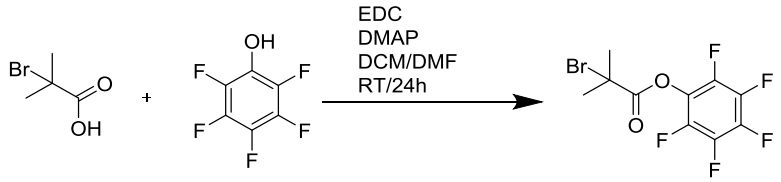

Figure S3. Esterification of 2-bromo-2-methyl propionic acid yielding pentafluorophenol-bromide.

In a round bottom flask, 2-bromo-2-methyl propionic acid (835 mg, 5 mmol, 2 eq.), PFP (460 mg, 2.5 mmol, 1 eq.) and DMAP (152 mg, 2.8 mmol, 1.25 eq.) were dissolved in 40 mL of DCM. Then, the solution was cooled to 0 °C. The reaction was started by adding the EDC (959 g, 5 mmol, 2 eq.) and the mixture was allowed to warm up to ambient temperature overnight. The crude mixture was washed with 5% hydrochloric acid, $NaHCO_3$ and brine solution. Subsequently, the united organic phase was dried over Na_2SO_4 and the solvent was removed under reduced pressure. The final product was obtained after gel flash chromatography, using cyclohexane/ethyl acetate (10:1 v/v) as eluent, obtaining a colorless oil (1.06 g, 63%). ^{1}H-NMR (400 MHz, DMSO, 25 °C): δ (ppm):

2.08 (s, 6H, *CH₃*). ^{19}F-NMR (377 MHz, DMSO, 25 °C): δ (ppm): -153.8 (m, 2F, *ortho*), -157.3 (m, 1F, *para*), -162.3 (m, 2F, *meta*).

1.4. Functionalization of the CRD-Peptide

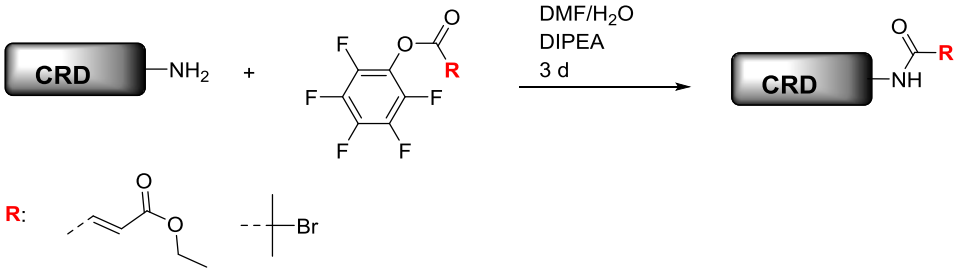

Figure S4. Introduction of functional groups to the CRD by active ester chemistry using PFP ester molecules bearing a fumarate and bromide group.

The functionalization of the CRD-peptide was conducted with the same procedure for both linker molecules: The folded CRD (30 mg, 15 mmol, 1 eq.) and the respective linker (linker 1 and linker 2, see below) was dissolved in 10 mL of a DMF/water mixture of 1:1 (v/v). After the CRD was dissolved, 5.5 µL of DIPEA (4 mg, 31 mmol, 2 eq.) were added to the solution. The reaction mixture was stirred at ambient temperature for three days. Afterwards, the solution was transferred into a dialysis tube (MWCO: 1000 kDa) and kept there for three days, changing the dialysis bath three times. Finally, the solution was freeze-dried to obtain a white colorless powder.

Linker 1 (PFP-fumarate): 6.0 µL, 9.5 mg, 31 mmol, 2 eq. Yield: 7.5 mg (25%)

Linker 2 (PFP-bromide): 5.5 µL, 10.2 mg, 31 mmol, 2 eq. Yield: 5 mg (16%)

CRD-fumarate: ESI-MS: $[M+Na]^+_{exp}$: 2101.7277, $[M+Na]^+_{calc}$: 2101.7096.

CRD-bromide: ESI-MS: $[M+Na]^+_{exp}$: 2124.6506, $[M+Na]^+_{calc}$: 2124.6249.

1.5. Preparation of Tetrazole-functionalized Silicon Surfaces[3]

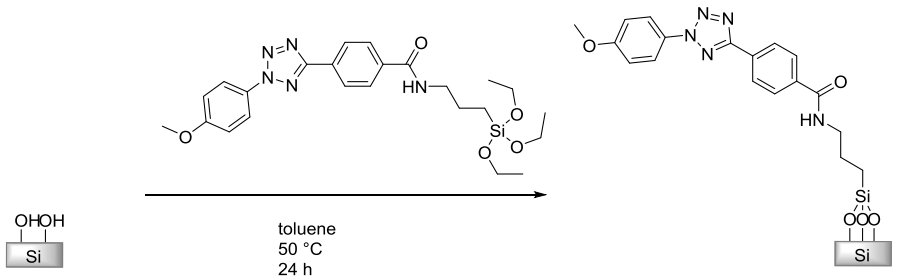

Figure S5. Functionalization with tetrazole groups of the silicon wafer by silanization.

A silicon wafer was cut into pieces of 1×1 cm² and cleaned by rinsing with organic solvents and water. In order to activate the silicon surface, the wafer was transferred into a plasma oven (air) and kept their at the maximum power level for at least 30 min. Meanwhile, a solution of the

tetrazole-silane in toluene at a concentration of 2 g·L^{-1} was prepared in a headspace vial. After the wafer have been exposed to the plasma, it was (polished side up) transferred immediately into the headspace vial and sealed. The headspace vial was then put into an incubator, shaking the vial for 24 h at 50 °C under light exclusion. The next day, the surface was rinsed with toluene, DCM and ethanol and finally dried under a nitrogen stream.

1.6. Immobilization of Polyethylene Glycol onto the Surface

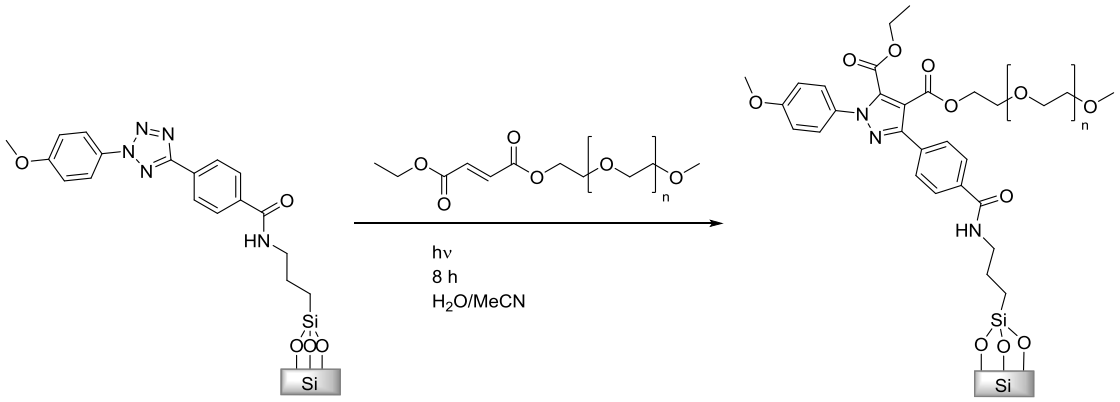

Figure S6. Immobilization of PEG-fumarate onto the tetrazole functionalized surface.

The immobilization of the PEG was performed using a dotted photomask. First, the silicon wafer was mounted to a special sample holder, employing on top of the wafer the photomask. A solution of PEG-fumarate was prepared with a concentration of 1 g·L^{-1}. The surface mounted in the sample holder was immersed in the solution and irradiated by UV light (λ_{max} = 310 nm) for 8 h. Subsequently, the surface was demounted from the sample holder, rinsed with DCM, ethanol and water and dried under a nitrogen stream.

1.7. Immobilization of CRD-Fumarate onto the Surface

In order to covalently link the CRD-fumarate to the surface, a solution of CRD-fumarate with a concentration of 1 g·L^{-1} in Millipore water/acetonitrile (1:1 v/v) was prepared. The surface was then immersed into the solution and irradiated with UV light for 24 h. After the irradiation, the surface was cleaned with Millipore water, 0.1 M NaOH solution and ethanol. Finally, the surface was dried under a nitrogen stream.

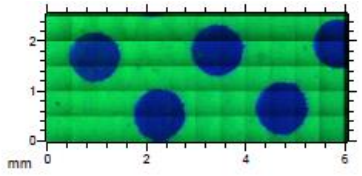

Figure S7. Surface after immobilization of the CRD-fumarate and the PEG-fumarate. ToF-SIMS overlay of the signals from S^-, HS^-, S_2^- (green), $C_2H_5O^-$ (blue).

1.8. Encoding the Surface via CRD-CRD Dimerization

The immobilization of the CRD-bromide onto the surface was carried out in two subsequent steps. First, a solution of the CRD-bromide was prepared at 0.25 $g \cdot L^{-1}$ in 10 mM PBS at pH = 7. Additionally, the surface was transferred into 1 mL of a 10 mM PBS solution at pH = 7. Both solutions were percolated with nitrogen and incubated for 2 h. After 2 h, the silicon wafer with the CRD-fumarate was transferred into the CRD-bromide solution and incubated for 6 h at ambient temperature. Afterwards, the surface was rinsed off with Millipore water, 0.1 M NaOH and ethanol. Finally, the surface was dried under a nitrogen stream.

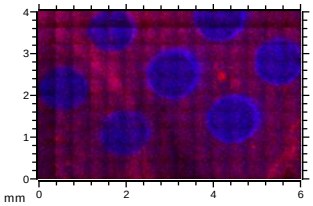

Figure S8. Surface after attaching the CRD-bromide to the surface. ToF-SIMS overlay of Sum of $^{81}Br^-$, Br^- (red), $C_2H_5O^-$ (blue).

1.9. Erasing of the CRD-Bromide from the Surface

In order to remove the immobilized CRD-bromide from the surface, the surface was transferred into a 10 mM PBS solution at pH = 7 containing 2.5 M GSH. The surface was shaken in an incubator for 10 min, rinsed with Millipore water and 0.1 M NaOH and transferred to a fresh 10 mM PBS solution, containing 2.5 M GSH. This step was repeated 5 times to finally rinse the surface with Millipore water, 0.1 M NaOH, and ethanol. Finally, the surface was stored in 10 mM PBS solution overnight in order to reoxidize the disulfide bonds of the CRD immobilized to the surface. Afterwards, the surface was rinsed with Millipore water and ethanol to finally dry the surface under a nitrogen stream.

2. Instruments

2.1. ESI-MS

High-resolution mass spectra (HRMS) were obtained using a Q Exactive (Orbitrap) mass spectrometer (Thermo Fisher Scientific, San Jose, CA, USA) equipped with a HESI II probe. The instrument calibration was carried out in the m/z range 74 - 1822 using calibration solutions from Thermo Scientific. A constant spray voltage of 4.7 kV and a dimensionless sheath gas of 5 were applied. The capillary temperature and the S-lens RF level were set to 320 °C and 62.0, respectively. The samples were dissolved in a H_2O/MeCN mixture (1:1 v/v) containing 1 vol. % of acetic acid and injected with a flow of 5 to 15 $\mu L \cdot min^{-1}$, respectively.

2.2. Nuclear Magnetic Resonance NMR

The NMR measurements were carried out on a Bruker Avance III 400 spectrometer (^{1}H: 400 MHz, $^{13}C\{^{1}H\}$: 100 MHz) . The δ scale was referenced to deuterated solvent, indicated in the respective spectrum. Evaluation was performed by TopSpin 7.1 PL7.

2.3. Photo Reactor Setup

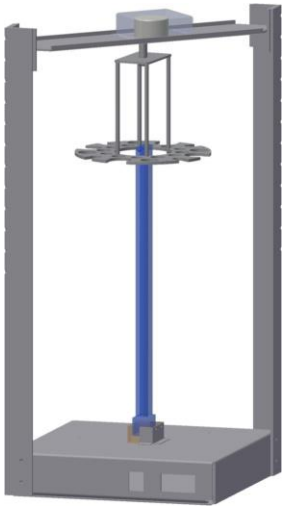

Figure S9. Schematic setup of the photoreactor used in this study. The samples are placed in the holder and rotated during the irradiation.

The photo-induced reactions were performed in a custom-built photoreactor (Figure S9), employing an ARIMED B6 UV lamp from Cosmedico with a spectral emission in the range of 290-400 nm (λ_{max} = 310 nm, Figure S10).

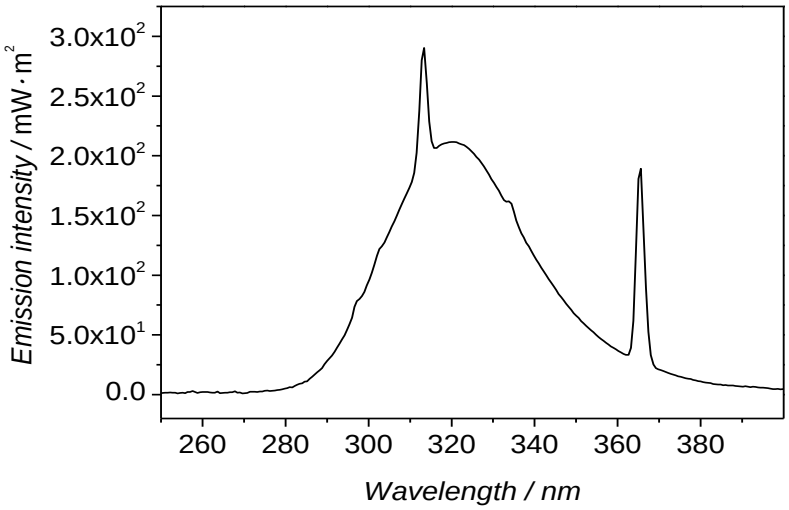

Figure S10. Emission spectrum of the Cosmedico ARIMED B6 lamp.

2.4. ToF-SIMS

Time-of-flight secondary ion mass spectrometry (ToF-SIMS) was conducted with a TOF.SIMS[5] 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 1 ns Bi_3^+ ion pulses at 25 keV energy and a lateral resolution of approx. 4 µm for all surfaces. Images larger than the maximum deflection range of the primary ion gun of 500×500 µm² were obtained using the manipulator stage scan mode. Primary ion doses were kept below 10^{11} ions·cm^{-2} (static SIMS limit).

2.4.1. ToF-SIMS Spectra

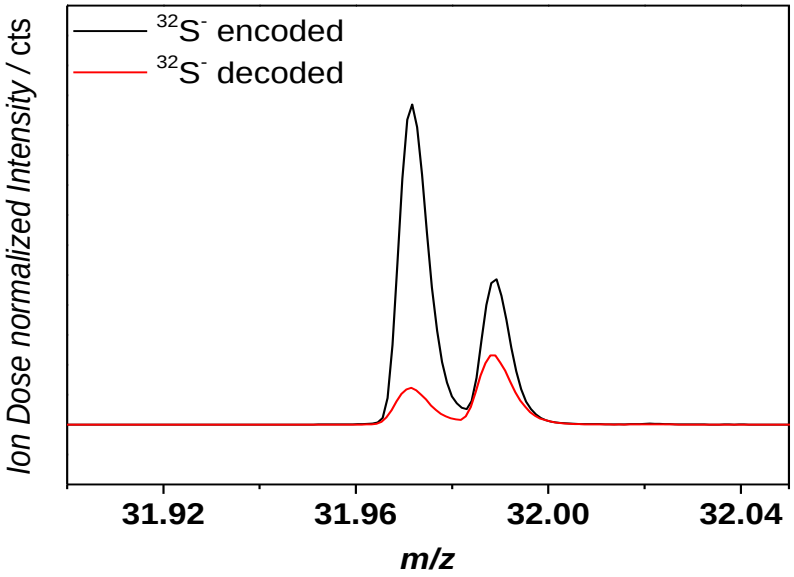

Figure S11. ToF-SIMS spectra of the main sulfur signal, m/z = 31.97, with primary ion dose normalization. (From Fig. 3 d) (black) and f (red))

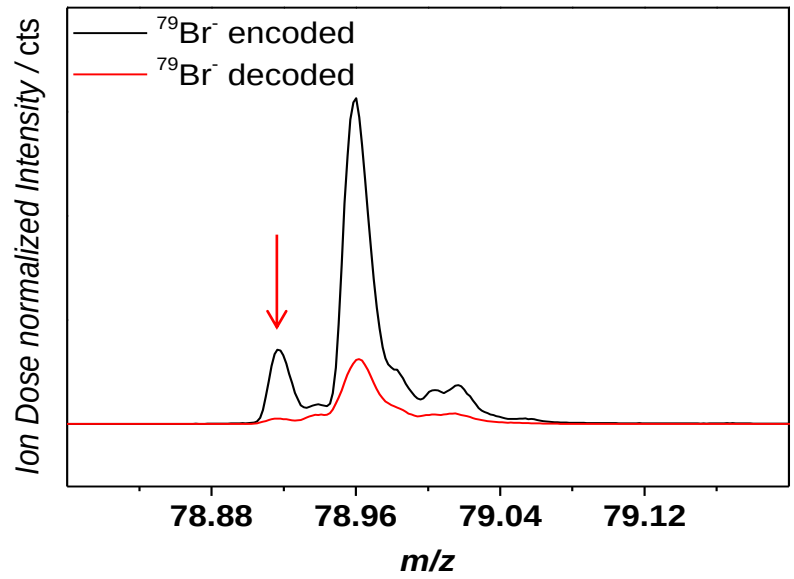

Figure S12. ToF-SIMS spectra of the $^{79}Br^-$ fragment, m/z = 78.92 Th (peak indicated with red arrow) with primary ion dose normalization. (From Figure 3 e) (black) and g (red))

2.5. Sample Holder

The parts of the sample holder and the photomask consist of stainless steel. The setup is assembled with two screws (Figure S13). The photomask is placed between the silicon wafer and the lid of the sample holder.

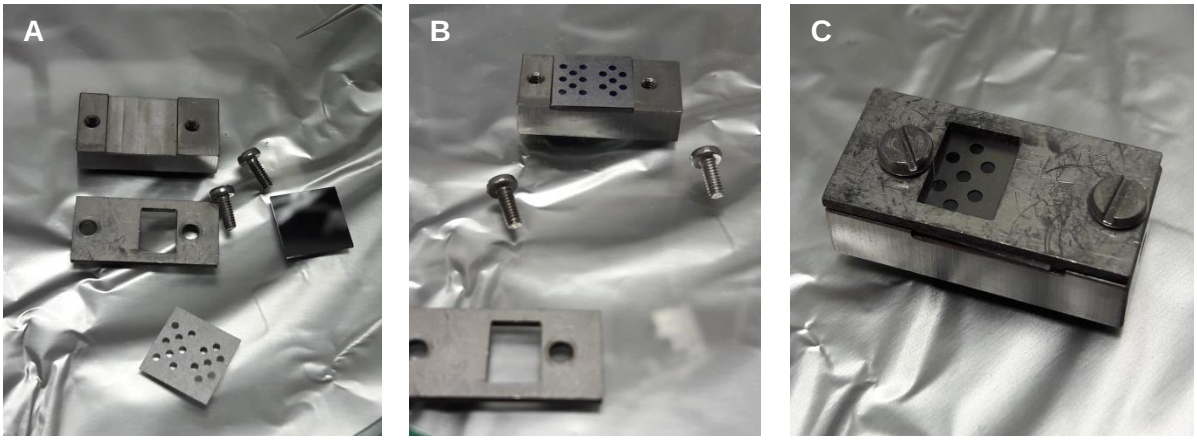

Figure S13. Assembly of the sample holder for the spatially resolved surface immobilization. A: Individual parts disassembled, B: Surface and photomask placed onto the sample holder. C: Sample holder fully assembled with the photomask mounted onto the silicon wafer.

3. References

(1) Peled, A.; Naddaka, M.; Lellouche, J.-P. *J. Mater. Chem.* **2011**, *21*, 11511–11517.

(2) Nebhani, L.; Sinnwell, S.; Lin, C. Y.; Coote, M. L.; Stenzel, M. H.; Barner-Kowollik, C. *J. Polym. Sci. Part A Polym. Chem.* **2009**, *47*, 6053–6071.

(3) Blasco, E.; Piñol, M.; Oriol, L.; Schmidt, B. V. K. J.; Welle, A.; Trouillet, V.; Bruns, M.; Barner-Kowollik, C. *Adv. Funct. Mater.* **2013**, *23*, 4011–4019.