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Photoactivatable Reaction for Covalent Nanoscale Patterning of Multiple Proteins

Shengwang Zhou^{†,‡,||}, Kevin J. Metcalfe^{‡,||}, Pradeep Bugga[§], Jennifer Grant[§], and Milan Mrksich^{*,†,‡,§}

[†]Institute of Chemical Biology and Nanomedicine, State Key Laboratory of Chemo/Biosensing and Chemometrics, College of Chemistry and Chemical Engineering, Hunan University, Changsha 410082, People's Republic of China

[‡]Department of Biomedical Engineering, Northwestern University, 2145 Sheridan Road, Evanston, Illinois 60208, United States

[§]Department of Chemistry, Northwestern University, 2145 Sheridan Road, Evanston, Illinois 60208, United States

Abstract

This article describes a photochemical approach for independently patterning multiple proteins to an inert substrate, particularly for studies of cell adhesion. A photoactivatable chloropyrimidine ligand was employed for covalent immobilization of SnapTag fusion proteins on self-assembled monolayers of alkanethiolates on gold. A two-step procedure was used: first, patterned UV illumination of the surface activated protein capture ligands, and second, incubation with a SnapTag fusion protein bound to the surface in illuminated regions. Two different fluorescent proteins were patterned in registry with features of 400 nm in size over a 1 mm² area. An example is given wherein an anti-carcinoembryonic antigen (anti-CEA) scFv antibody was patterned to direct the selective attachment of a human cancer cell line that express the CEA antigen. This method enables the preparation of surfaces with control over the density and activity of independently patterned proteins.

Graphical Abstract

*Corresponding Author milan.mrksich@northwestern.edu.

Author Contributions

^{||}S.Z. and K.J.M. contributed equally to this work.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsami.8b16736.

Ligand synthesis and NMR and MS spectra, time-course study of deprotection in solution, LC/MS spectra of ligand—SnapTag coupling, calculation of expected molecular mass of species, time-dependent SAMDI-MS spectra of the SnapPPG ligand, mass spectrum of the mVenus-SnapTag protein, quantification of fluorescence micrographs, micrographs of cell lines cultured on patterned surfaces, tables of plasmid synthesis, and full DNA and protein sequences (PDF)

The authors declare no competing financial interest.

Keywords

immobilization; proteins; surface chemistry; photochemistry; monolayers

1. INTRODUCTION

Surfaces that are patterned with proteins have been important for studies of cell adhesion and signaling on extracellular matrices,^{1–4} for directing cellular differentiation,^{5–8} for creating protein arrays,^{9–12} and for many other applications.^{13–15} Recent work has aimed to reduce the feature sizes of patterned proteins—particularly at submicron length scales—and accommodate the co-patterning of multiple proteins in registry.^{8–11} However, current methods still face tradeoffs in feature size, interfeature distance, and preservation of activity of protein domains. Here, we describe a photochemical method that achieves diffraction-limited feature sizes of two different protein identities with homogeneous covalent attachment by combining active-site-directed protein immobilization^{16–22} with self-assembled monolayers.

Our strategy for immobilizing proteins uses a fusion protein that can selectively and covalently bind to an irreversible ligand presented on the monolayer.¹⁶ This strategy is significant because it gives excellent control over the density and surface orientation of the protein and it can be performed on self-assembled monolayers that are compatible with a broad range of analytical methods and prevent nonspecific protein adsorption. We first described this approach with the serine esterase cutinase,^{16–18} and then, we used SnapTag, the engineered alkyltransferase developed by Johnsson and co-workers,^{23,24} SnapTag binds to benzylguanine and benzyl chloropyrimidine moieties,^{23,25} and for the latter, the nucleophilic Cys145 displaces the chloropyrimidine group to form a covalent thioether bond with the ligand.^{23,26} Here, we prepare a self-assembled monolayer that presents a photocaged analogue of the benzyl chloropyrimidine ligand and we demonstrate that the monolayer can be activated with light to pattern the immobilization of a fusion protein into features of approximately 400 nm in size (Figure 1). Importantly, repeated cycles of deprotection and immobilization²⁷ were performed to independently immobilize multiple proteins through the same linkage by spatiotemporal activation of the photoprotected capture ligand.

2. EXPERIMENTAL SECTION

2.1. Materials.

All chemicals were purchased from Sigma, unless stated otherwise. Ultrapure water was prepared by a Millipore filtration unit and used for all experiments.

2.2. Organic Synthesis.

See the Supporting Information section for the detailed synthetic route of **1** (pp S3–S13).

Cyclic RGD (RGDfC) (f denotes a phenylalanine residue having the D-configuration at the α carbon) was synthesized as previously described.^{28,29}

2.3. ¹H NMR Spectroscopy.

¹H NMR spectra were recorded on an Agilent DD2 500 MHz system (HFX 5 mm probe w/Z-Gradient).

2.4. Electrospray Ionisation Mass Spectrometry (ESI-MS) Analysis of Small Molecules.

ESI-MS spectra were acquired on a Bruker AmaZon SL LC/MS mass spectrometer using electrospray ionization (ESI) with direct injection.

2.5. DNA Cloning.

All cloning was performed in the *Escherichia coli* strain DH5 α (NEB). Expression plasmids based on the pET-28b(+) backbone (Novagen) were constructed using the Golden Gate cloning strategy³⁰ by *Bsa*I restriction enzyme (NEB) and T4 ligase (NEB). A 10 μ L reaction was performed, with ~50 ng of the recipient vector and ~1:1 molar ratio of insert(s) to the recipient vector. A list of the plasmids used in this study is given in Table S1. Linear double-strand inserts were prepared by the polymerase chain reaction using Q5 polymerase according to the manufacturer's instructions (NEB). A list of the primers used to make these plasmids is given in Table S2. All primers were purchased from IDT. All DNA purification was performed using Qiagen kits according to the manufacturer's protocol. All purified proteins contain a C-terminal hexahistidine tag and a (EAAAK)₂ linker in between each domain in fusion proteins.

2.6. Protein Production and Purification.

Proteins were produced in the *E. coli* BL21 DE3 cell line (NEB). The culture was grown overnight in an orbital shaker at 30 °C and 240 rpm in lysogeny broth (Lennox) supplemented with 50 μ g/mL kanamycin. The overnight culture was diluted 1:100 into fresh 2xYT media in a baffled flask supplemented with 0.005% v/v antifoam A-204 and 50 μ g/mL kanamycin. The subculture was then grown at 30 °C for ~4 h at 240 rpm until the culture reached an OD₆₀₀ of 0.6–1.0. Isopropyl β -D-1-thiogalactopyranoside (IPTG) was added to a final concentration of 500 μ M, and the culture was cooled to 20 °C and grown at 240 rpm for ~16 h. Cultures were then spun down and frozen as cell pellets. Frozen cell pellets were thawed in 3 $\times$ phosphate buffered saline (PBS) and sonicated on ice for 5 min (5 s on and 5 s off) with a Fisherrand Model 550 Sonic Dismembrator. The lysate was pelleted, and the

supernatant was incubated with Ni-NTA resin (Qiagen) using a manual column (Kimble). The resin was washed thrice with wash buffer (3× PBS + 10 mM imidazole, pH 7.4) and eluted with 4 column equivalents of elution buffer (3× PBS + 250 mM imidazole, pH 7.4). Nickel column elution buffer was then precipitated with (NH₄)₂SO₄ (final concentration of (NH₄)₂SO₄ = 50% sat.), pelleted, and resuspended in 1× PBS. The concentrated protein was then separated on a size-exclusion column (GE) using an AKTA pure FPLC unit (GE). Fractions were collected, and the purity and identity of proteins were determined by sodium dodecyl sulfate (SDS)–polyacrylamide gel electrophoresis and liquid chromatography/mass spectrometry (LC/MS), respectively.

2.7. LC/MS Analysis.

To prepare samples for LC/MS analysis, protein samples were diluted in water to a final concentration of 1 μM. LC/MS analysis was performed using an Agilent 1200 series high-performance liquid chromatography system connected to an Agilent 6210A time-of-flight (TOF) mass spectrometer. A 10 μL injection of each sample was captured on a C18 trap column (Waters) and eluted using a gradient from 5 to 95% acetonitrile and 0.1% formic acid in water at a flow rate of 0.25 mL/min. Blank injections were run before each sample, and the extracted mass spectrum of the blank was subtracted from the mass spectrum of the sample. Each mass spectrum was deconvoluted using a maximum entropy routine. Data was analyzed using Agilent MassHunter Qualitative Analysis B.04.00.

For conjugation reactions, the SnapTag protein was coupled with compound **1** before and after UV irradiation of the sample. For the UV-irradiated sample, compound **1** (250 μM in dimethyl sulfoxide) was irradiated using a CL1000-L UV lamp (UVP) at a high power for 5 min (365 nm, 1500 mJ/cm²) and quantitative deprotection was assumed. Both ligand samples were incubated in a 50 μL reaction with 250 pmol of SnapTag and 2 equiv of ligand (5 μM SnapTag and 10 μM compound **1** in reaction) in PBS for 5 min at room temperature. The reaction was quenched with the addition of 10 μL of 0.6% v/v formic acid in water (the final concentration of formic acid was 0.1% v/v).

2.8. Preparation of Thin-Film Gold Substrates.

2.8.1. Steel Chips.—Steel chips were used in the self-assembled monolayer desorption/ionization (SAMDI) experiments of photoprotected SnapTag ligand functionalization, photodeprotection, and protein capture. For samples prepared on stainless steel surfaces, custom-fabricated stainless steel chips (18 × 18 mm) were first cleaned with hexanes, ethanol, and deionized (DI) water. An aluminum mask having an array of 2.8 mm circles in a standard 384-well format was placed on the chips, and an electron-beam evaporator (Thermionics Laboratory Inc., Hayward, CA) was used to first deposit a titanium layer (5 nm at 0.02 nm/s) onto the chips only on the exposed holes. Next, a gold layer (35 nm at 0.05 nm/s) was deposited on top of the titanium layer. The evaporator was operated at (1–5) × 10^{−6} mTorr. Steel chips were stored under vacuum until use.

2.8.2. Glass Slides.—Glass slides were used in the microscopy and surface plasmon resonance (SPR) experiments. For samples prepared on glass surfaces, the purchased glass slides (24 × 50 mm) were first rinsed by sonicating successively in the solutions of ethanol,

DI water, and ethanol for 20 min each. For microscopy experiments, first titanium (2 nm) and then gold (11 nm) layers were evaporated onto the glass slides using an electron-beam evaporator (Thermionics VE-100) at a rate of 0.02 nm/s for titanium and 0.05 nm/s for gold. For SPR experiments, the titanium (5 nm) and then gold (50 nm) layers were evaporated onto the glass slides using the electron-beam evaporator (Thermionics VE-100) at a rate of 0.02 nm/s for titanium and 0.05 nm/s for gold. The evaporator was operated at $(1-5) \times 10^{-6}$ mTorr. Glass slides were stored under vacuum until use.

2.9. Preparation of Self-Assembled Monolayers (SAMs).

The substrates were immersed for 24 h at 4 °C in an ethanolic solution containing the background symmetric 11-carbon alkyl disulfide terminated with tri(ethylene glycol) groups and asymmetric alkyl disulfide terminated with a maleimide group and a tri(ethylene glycol) group in a 3:2 ratio, with a 1 mM total disulfide concentration.

2.10. Addition of the Photocaged SnapTag Ligand to the Surface.

The functionalized chips/slides were rinsed with water and ethanol, and then, a solution of 100 μ M compound **1** in a 50% v/v 100 mM Tris (pH 8.0) and 50% v/v ethanol solution at 20 °C was spotted onto the substrate and incubated at room temperature (rt) for 10 min in a humidified chamber. Samples were then rinsed with ethanol, DI water, and ethanol and dried under N₂.

2.11. Deprotection of the PPG-SnapTag Ligand on the Surface.

Surfaces with SAMs presenting compound **1** were then illuminated with UV light in one of the following two ways: (1) flood illumination using a UV crosslinker unit and (2) laser illumination using a confocal microscope.

2.11.1. Deprotection with the UV Cross-Linker Unit.—The photoprotected SnapTag ligand was deprotected using a UV cross-linker unit at the maximum power for 0.5–10 min (UVP CL1000-L, 365 nm, 5 mW/cm², 115 V/60 Hz/0.7 A). The Gilder mesh grids (TED Pella, Inc.) were sandwiched between two cover glasses with DI water and then laid on top of the surface of the slides wetted with DI water.

2.11.2. Deprotection with a Confocal Microscope.—The substrates functionalized with compound **1** were photopatterned using a Nikon Ti Eclipse confocal microscope. Using NIS-Elements software, nanoscale features were obtained by a sequential operation of region of interest (ROI) pattern drawing and photoactivation area designation. In this section, the substrate was illuminated with the stimulation laser beam (405 nm, 100 kW/cm², 200 μ s pixel dwell time, 0.22 μ m pixel size, 20 J/cm²) with regard to the ROI pattern. The pattern feature size was optimized by changing the photo-activation area specification and ROI editor.

2.12. SAMDI Mass Spectrometry of the PPG-SnapTag Ligand.

Steel chip substrates were prepared to have a SAM that presents compound **1**, as described in previous sections. For deprotected samples, the substrate was illuminated as described in the previous section. A 20 mg/mL solution of 2,4,6-trihydroxyacetophenone in acetone was

applied to the substrate, and the surface was analyzed using a 5800 MALDI-TOF/TOF mass spectrometer (AB Sciex, Framingham, MA) in a positive-ion reflector mode.

2.13. Protein Capture.

Steel chip substrates were prepared to have a SAM that presents compound **1**, as described in previous sections. For deprotected samples, the substrate was illuminated as described in a previous section. The purified fusion protein (50 μ M) was incubated on surfaces at rt for 1 h in a humidified chamber. The nonspecifically absorbed protein was removed by rinsing with the SDS buffer (0.5 mg/mL) and DI water and then dried under a stream of nitrogen.

2.14. MALDI-TOF and SAMDI of Protein.

Steel chip substrates were prepared to have a SAM that presents compound **1**, as described in previous sections. For deprotected samples, the substrate was illuminated as described in a previous section. The protein was incubated on the surface as described in a previous section. The matrix solution of sinapinic acid (10 mg/mL in 4:1 solution of acetonitrile and water with 1% trifluoroacetic acid) was applied to the substrate and dried. For MALDI-TOF samples, the protein was desalted, mixed with matrix, and applied to a MALDI plate. Mass spectra were acquired using the 5800 MALDI-TOF/TOF mass spectrometer (AB Sciex, Framingham, MA) in a linear high-mass positive reflector mode.

2.15. Surface Plasmon Resonance (SPR).

Glass slide substrates were prepared to have a SAM that presents compound **1**, as described in previous sections. For deprotected samples, the substrate was illuminated as described in a previous section. Substrates were taped to an SPR sensor chip holder and docked to a Biacore 2000 system. All experiments were performed at 25 °C with PBS + 0.01% v/v Triton X-100 (pH 7.4) as the running buffer at a flow rate of 10 μ L/min. Each run began with a 10 min application of the running buffer. The proteins were diluted to 10 μ M in a buffer of PBS + 0.01% v/v Triton X-100 (pH 7.4) and applied to the substrate for 20 min, followed by a 10 min wash with the running buffer. Proteins that are weakly physisorbed are removed during the wash step. Next, the PBS + 0.5 mg/mL sodium dodecyl sulfate (SDS) solution was made to flow over the substrate for 10 min, followed by a 10 min wash with the running buffer. Washing with SDS removes physisorbed species and leaves covalently bound species on the surface.^{18,31} Biacore software outputs data in the arbitrary unit (resonance units, RU), which represents the shift in the resonance angle, such that 1000 RU = 0.1° = 1 ng/mm².³²

2.16. Confocal Microscopy Imaging.

Surfaces were imaged using the Nikon Ti Eclipse confocal microscope in fluorescence mode.

2.17. Mammalian Cell Culture.

Cell lines (CHO-K1, BT-474, and MDA-MB-231) were obtained from ATCC and cultured using standard techniques. Cells were cultured in Dulbecco's modified Eagle's medium (4.5 g/L D-glucose, 584 mg/L L-glutamine, and 110 mg/L sodium pyruvate) (Gibco #11995–

065) supplemented with 10% fetal bovine serum and grown at 37 °C in a 5% CO₂ atmosphere.

Patterned protein surfaces for cell culture were prepared using an aseptic technique. Glass slides were prepared as described above. Experimental samples presented **1**. Positive control surfaces presented the cRGD ligand and was coupled to the monolayer via the cysteine thiol. Slides with self-assembled monolayers that presented **1** were deprotected using a mask with parallel bars (RB90, Electron Microscopy Science). The purified anti-carcinoembryonic antigen (anti-CEA) scFv-SnapTag protein (50 μM) was incubated with slides for 1 h in a humidified chamber. The excess protein was washed with PBS buffer.

To grow cells on patterned protein surfaces, cells were washed with Dulbecco's phosphate buffered saline, trypsinized, and resuspended in culture media. Cell density was determined using Trypan blue exclusion. Cell dyes (Vybrant DiI cell-labeling solution (Invitrogen #V22885), Red CMTPX dye (Invitrogen #C34552), and Green CMFDA dye (Invitrogen #C7025)) were incubated with each cell line according to the manufacturer's instructions. The dye-labeled cells were diluted to a concentration of 10 000 cells/mL, and 1 mL of culture was added to a 24-well culture dish containing a patterned glass chip in each well. Cells were cultured for 6 h and imaged using a confocal microscope.

3. RESULTS AND DISCUSSION

3.1. Ligand Design, Synthesis, and Characterization.

We designed a photoprotected SnapTag ligand (**1**) where the amino group of the benzyl chloropyrimidine (Figure 1, pink) is blocked by a nitrophenyl photoprotecting group (Figure 1, blue).^{33,34} The molecule also includes a thiol for immobilization of the ligand to self-assembled monolayers that present maleimide groups against a background of tri(ethylene glycol) groups (Figure 1). The latter are effective at preventing unwanted protein adsorption, which is necessary to direct protein immobilization only to the photoactivated regions.³¹ The nitrophenyl protecting group was selected to undergo efficient photolysis after low-energy UV illumination³⁵ to obtain covalent and site-specific immobilization. High-energy UV irradiation of self-assembled monolayers causes photo-oxidation of the thiolate^{36,37} or photolysis of the tri(ethylene glycol) moiety,³⁸ a strategy for nonspecific protein patterning³⁹ that would compete with the desired immobilization strategy.

In solution-phase experiments, we first used ESI mass spectrometry to confirm that the photocaged ligand **1** underwent efficient deprotection when irradiated with UV light (365 nm, 300 mJ/cm²) for 1 min (Figures 2A and S12). We also used high-resolution LC/MS to show that the deprotected ligand forms a covalent adduct with SnapTag following UV irradiation, but not prior to photoactivation (Figures 2B and S13, see calculation on p S20). The photoprotected chloropyrimidine appears to have less back-ground reactivity with SnapTag when compared with recent reports of a photoprotected *O*6-benzylguanine ligand.^{22,40} These results show that **1** is activated for protein conjugation by irradiation with UV light (Figure 1).

3.2. Surface Characterization.

To pattern the immobilization of proteins to surfaces, we immobilized **I** to a self-assembled monolayer of alkanethiolates on gold presenting maleimide groups. We used the self-assembled monolayers for MALDI-TOF mass spectrometry (SAMDI-MS) technique⁴¹ to confirm immobilization and to show the efficient removal of the photoprotecting group upon UV illumination to reveal the active SnapTag ligand, as indicated by the loss of 281 *m/z* units after illumination (365 nm, 300 mJ/cm²) (Figures 3A and S14). Following the irradiation, we applied the fluorescent fusion protein mVenus-SnapTag to the surface for 60 min, rinsed the protein, and again analyzed the surface using SAMDI-MS. The peak corresponding to the immobilized protein increased by 726 Da, which is consistent with the formation of a covalent adduct with the ligand-functionalized alkanethiolate. In contrast, incubation of monolayers that were not irradiated gave essentially no protein immobilization (Figure 3B). These results confirm that the protein site-specifically and covalently attaches to the surface.^{42–44} We also used surface plasmon resonance spectroscopy to confirm that the fusion protein did not adsorb nonspecifically to the surface that was not irradiated and that attachment to the photoactivated surface was specific and covalent (Figure 3C,D).

3.3. Fluorescent Protein Patterning.

We next used photomasks to activate regions of the monolayer for microscale patterning of SnapTag fusion proteins. We separately irradiated monolayers with masks having either an array of square features 37 μm in size or circular features 6.5 μm in diameter (365 nm, 300 mJ/cm²). We then applied a solution of the mVenus-SnapTag fusion protein to the substrate over an area greater than 1 mm². After 60 min, we rinsed the monolayers and acquired fluorescence images that revealed immobilization of the protein into the patterned features, demonstrating the selectivity of the protein–ligand reaction (Figure 4A,B). Fluorescence images from mVenus confirmed that the protein preserved its fold after immobilization.⁴⁵ We observed a similar patterning fidelity using a different photomask with 6.5 μm circular features (Figure 4C).

We created nanoscale patterns of multiple proteins using a focused laser on a confocal microscope (405 nm, 20 J/cm²) to activate regions of the monolayer. We patterned “SAMDI” on the surface using the microscope laser. Incubation of the mVenus-SnapTag protein resulted in a pattern of ~420 nm line width, near the diffraction limit (Figure 4D). As for the surfaces generated with a photomask, we observed high contrast between illuminated and nonilluminated regions.

In a second example, we first patterned the word NANO using a microscope laser with a translating stage and incubated the surface with the mCerulean-SnapTag protein. Second, we aligned the field to the first pattern, illuminated a box around this patterned word, and incubated the surface with the mCherry-SnapTag protein. This resulted in co-localized features of different proteins with feature widths of ~420 nm (Figures 4E and S16).

3.4. Antibody-Directed Cell Patterning.

In a final example, we patterned antibodies on a surface to direct the attachment of mammalian cell lines. We used the single-chain variable fragment of an antibody that

specifically binds a carcinoembryonic antigen (anti-CEA scFv),⁴⁶ a surface protein that is expressed on cancer cell lines.⁴⁷ To demonstrate the ligand-directed cell attachment using our photopatterning strategy, we used the human breast cancer cell lines MDA-MB-231 and BT-474, which express the CEA antigen.^{47,48} Microscale features (92 μm columns, 276 μm pitch) were patterned using a photomask (365 nm, 300 mJ/cm²), and then, the anti-CEA scFv-SnapTag fusion protein was captured on the surface in illuminated regions. The MDA-MB-231 cells attached to the surfaces that present both cyclic RGD (cRGD) and the patterned anti-CEA scFv-SnapTag (Figure 5). Cells attached in a columnar pattern on the photopatterned surface, as they were directed by the patterned substrate. The unpatterned regions were inert to cell attachment, as they presented the tri(ethylene glycol)-terminated alkanethiolate.⁴⁹ This columnar pattern was also observed for BT-474 cells (Figure S17). In contrast, the cell line CHO-K1, which does not express the CEA antigen,⁵⁰ did not attach to the patterned surface (Figure 5). Thus, photopatterning of an scFv antibody-directed mammalian cell attachment in distinct regions is based on cell type.

4. CONCLUSIONS

This article introduces a general method for independently patterning multiple proteins at submicron length scales. The ability to direct covalent protein immobilization with light will allow for subdiffraction-limited patterning,^{51,52} potentially realizing patterning at the single protein length scale. Light-activated covalent patterning affords precise spatiotemporal control⁵³ and gives greater immobilization stability, relative to noncovalent attachment.⁵⁴ The protein resistance of self-assembled monolayers presenting oligo(ethylene glycol) groups is critical to the sequential patterning of different proteins and will enable studies of cell signaling, coupled enzyme reactions, and energy transfer systems. Our method specifically addresses the challenge of site-specific covalent patterning of multiple proteins in registry, whereas previous methods were limited by irreversible surface passivation. Finally, patterned protein surfaces require maintenance of activity through homogeneous covalent attachment to standardize the surface orientation of active protein domains, which is not achieved with nonspecific protein attachment strategies.^{55,56}

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Schematic of photopatterning of proteins. Protein coupling to **1** is blocked by a nitrophenyl photoprotecting group (PPG), which yields a functional SnapTag ligand upon photolysis. The surface was prepared by self-assembly of a maleimide-presenting alkanethiolate monolayer. Then, **1** was immobilized to the surface. Next, the photoprotecting group was removed by UV illumination. The SnapTag fusion protein was covalently captured in illuminated regions.

Figure 2.

Solution-phase photodeprotection of the SnapTag ligand (**1**). (A) ESI-MS of **1** before (red) and after (blue) photodeprotection. UV irradiation yields the active ligand and photoprotecting group (PPG). (B) LC/MS deconvoluted mass spectra of the SnapTag protein incubated with **1** before (red) and after (blue) photodeprotection.

Figure 3.

Surface characterization of the photoprotected SnapTag ligand monolayer. (A) SAMDI-MS spectra to identify small-molecule surface species before (red) and after UV illumination for 60 s (blue). Disulfide and alkanethiolate ions are observed. (B) MALDI-TOF spectrum of mVenus-SnapTag (black) and SAMDI-MS spectra of mVenus-SnapTag captured on the surface before (red) and after UV illumination (blue). (C) Surface plasmon resonance sensorgrams of mVenus-SnapTag captured with (blue) and without (red) UV illumination. SDS, sodium dodecyl sulfate. (D) Schematic of protein chemisorption on surfaces with (top) and without (bottom) UV illumination. The photoprotecting group (star) is removed by UV illumination.

Figure 4.

Fluorescence micrographs of patterned surfaces. (A, B) Illumination through a photomask with square holes and capture of mVenus-SnapTag. (C) Illumination through a photomask with circular holes and capture of mVenus-SnapTag. (D) Illumination using a focused laser beam and capture of mVenus-SnapTag. (E) Sequential illumination and capture of mCerulean-SnapTag and mCherry-SnapTag using a focused laser on a microscope. (Left) mCerulean (blue) channel showing a pattern of the word "NANO". (Middle) mCherry (red)

channel showing a rectangular pattern. (Right) Merged image of the mCerulean and mCherry channels showing colocalization of the two patterned fluorescent proteins.

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Figure 5.

Bright-field micrographs of cell lines cultured on patterned surfaces. Two different cell lines, CHO-K1 (CEA⁻) and MDA-MB-231 (CEA⁺), were grown for 6 h on surfaces that present cyclic RGD (cRGD), the photoprotected ligand (**1**) incubated with anti-CEA scFv-SnapTag (no UV), and the photopatterned ligand (**1**) incubated with anti-CEA scFv-SnapTag (patterned).