Supporting Information

Green Light Lithography: A general strategy to create active protein and cell micropatterns

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Materials: MiCy (Addgene plasmid # 54565), TurboRFP (Addgene plasmid # 54858), dKatushka (Addgene plasmid # 54775) and fibronectin (FN) (pET28a-ybbR-HIS-10FNIII(x4)-DocI, Addgene plasmid #58712) were acquired from Addgene. The CarH plasmid (Ampicillin resistance) was inserted into the vector pET-22b between the NdeI and BamHI cutting sites. BL21 (DE3) *E. coli* was purchased from New England Biolabs. The lipids 1,2-dioleoyl-sn-glycero-3-{[N-(5-amino-1-carboxypentyl) iminodiacetic acid) succinyl]} (DGS-NTA) and 2-{[(2,3-bis-(oleoyloxy)propyl]dimethylammonio}ethyl hydrogen phosphate (DOPC) were acquired from Avanti Polar Lipids. 4-arm PEG-Succinimidyl NHS ester (MW 10 kDa) was purchased from Creative PEG Works. All other chemicals were purchased from Sigma-Aldrich. Buffers and aqueous solutions were prepared with Milli-Q water.

Protein preparation: Each protein expression plasmid was transformed into BL21(DE3) *E. coli* and plated on an LB-Agar plate with the appropriate antibiotic (50 µg/ml) at 37 ºC overnight. A single colony was inoculated into 10 mL LB medium with the appropriate antibiotic (50 µg/mL) and incubated at 37 ºC, 250 rpm overnight. The overnight culture was transferred to 1 L LB medium with the appropriate antibiotic and incubated at 37 ºC, 250 rpm until the OD_{600} = 0.6-0.8. Then protein expression was induced with 1 mM IPTG for CarH and FN and with 1 g/L L-(+)-Arabinose for MiCy, TurboRFP and dKatushka, temperature was reduced to 18 ºC and the cultures were incubated overnight at 250 rpm. Next day, the

cultures were centrifuged at 6000 rpm, 4 ºC for 8 min (Beckman Coulter Avanti J-26S XP, JA-10 rotor), supernatant was discarded and the bacteria pellet collected. Then the bacteria pellet was resuspended in 20 mL buffer A (50 mM Tris-HCl, 300 mM NaCl, pH 7.4) supplemented with 1 mM protease inhibitor phenylmethane sulfonyl fluoride (PMSF) and 1 mM DL-dithiothreitol (DTT). The bacteria were lysed by sonication and the lysate was cleared by centrifugation at 12000 rpm (Beckman Coulter Avanti J-26S XP, JA-25.50 rotor) for 30 min, followed by filtration through a 0.45 μm filter (ROTH, KH 55.1) twice. The lysate was loaded onto a 5 mL Ni^{2+}-NTA agarose column. The column was washed with 50 mL buffer C (Buffer A with 25 mM imidazole and 1 mM DTT) and the protein was eluted with 10 mL buffer B (Buffer A with 250 mM imidazole and 1 mM DTT). The purified proteins were dialyzed against 2 L buffer A with 1 mM DTT twice for at least 6 h.

Preparation of CarH tetramer: The purified CarH monomer was incubated with fivefold excess of Vitamin B_{12} (Sigma-Aldrich, V2876) for 30 min in the dark to form the CarH tetramer. Unbound Vitamin B_{12} was removed from the resulting CarH tetramer by size exclusion chromatography using a HiLoad™ 16/600, Superdex™ 200 pg size exclusion column. If necessary CarH tetramer was concentrated using a centrifugal filtration devices (10 kDa molecular weight cutoff).

Preparation of 4-arm-PEG-NTA: N_{α},N_{α}-Bis(carboxymethyl)-L-lysine hydrate (NTA-Lys) (0.24 mmol, 65 mg) was first dissolved into 10 ml MeOH and then 4-arm-PEG N-hydroxysuccinimide ester (4-arm-PEG-NHS, MW 10 kDa) (0.06 mmol, 600 mg) was added and dissolved. Then 60 μl 4-methylmorpholine was added to the solution as catalyst. After reacting for 6 h at room temperature,[1] the product was precipitated by adding diethyl ether (100 mL) and then vacuum dried.

Preparation of LbL multiprotein films on PEG-Ni^{2+}-NTA functionalized glass Surfaces with protein: The glass surfaces were functionalized similarly as previously reported.[2] In short, glass slides (20 × 20 mm) were cleaned with freshly prepared Piranha solution (3:1

(v/v) concentrated H_2SO_4:H_2O_2 (30%)) for 1 h, rinsed 3 times with Milli-Q water and dried in an N_2 stream. For the PEGylation reaction, surfaces were immersed in a solution of PEG_{3000}-azide (10 mg PEG_{3000}-azide, MW = 3500 g/mol) and 200 µl dry triethylamine in dry toluene and kept at 80 ºC overnight under a N_2 atmosphere. The surfaces were first washed with ethyl acetate for 5 min by sonication, then with methanol for 5 min by sonication and dried in a N_2 stream. The PEG-coated surfaces were incubated with 100 µl of reaction solution containing 100 mM L-ascorbic acid, 100 mM Tris HCl (pH 9.0), 150 µM of NTA-alkyne and 1 mM $CuSO_4$ in a moisture chamber for 2 h. The surfaces were incubated with the following solutions to obtain PEG-Ni^{2+}-NTA functionalized surfaces: (1) 50 mM EDTA (pH 7.4) for 5 min; (2) Buffer A twice for 5 min; (3) 0.1 M $NiCl_2$ in water for 5 min; (4) Buffer A for 5 min. Afterward the surfaces were incubated with the following solutions to obtain the protein functionalized LbL substrates through NTA-Ni^{2+}-His tag interaction: (1) 1st layer: 5 µM purified protein for 30 min; (2) Buffer A for 10 min; (3) 25 µM 4-arm-PEG-NTA+100 µM $NiCl_2$ in Buffer A for 30 min; (4) Buffer A for 5 min; (5) 5 µM purified protein for 30 min (2nd protein layer); (6) Buffer A for 10 min. To generate subsequent protein layers step 3-6 were repeated to assemble the LbL protein film.

Protein patterning: Substrates with LbL films with CarH in the 1st and TurboRFP in the 2nd layer were used for all protein patterning experiments. The logo of the Max Planck Society, the Minerva, was patterned on an inverted fluorescence microscope (DMi8, Leica) equipped with a 10x objective and a digital micromirror device (Mosaic, Andor) and illuminating for 1 min with 10% intensity of the 525 nm LED line. Line patterns of proteins were produced on the confocal laser scanning microscopy (Leica TCS SP8) equipped with an argon laser and 20x objective. Lines were projected onto the LbL film using 20% intensity of the 552 nm laser for 10 s. To demonstrate the temporal control, substrates were patterned in buffer A solution and after each patterning step imaged using 1% intensity of the 552 nm laser and 0.5 s exposure time. All other substrates were first fixed with 4% paraformaldehyde for 20 min,

mounted in Mowiol-488 and then imaged using 1% intensity of the 552 nm laser and 1 s exposure time.

Quantification of cell adhesion: PEG-Ni^{2+}-NTA coated glass surfaces were either functionalized with the FN or a CarH (1st layer)/ FN (2nd layer) LbL film. While one set of surfaces was illuminated with green light for 5 min (15 W), the other one was kept in the dark. A PEG-Ni^{2+}-NTA functionalized surface without protein functionalization was used as a negative control. The surfaces were placed into the 6-well cell culture plates and were washed with phosphate buffered saline 1x (PBS 1x). Subsequently, 5×10^4 MDA-MB-231 cells were seeded per well in 2 ml Dulbecco's modified Eagle's medium (DMEM) (Gibco BRL) supplemented with heat inactivated fetal bovine serum (FBS, 10%) (Sigma Aldrich) and penicillin/streptomycin (P/S, 1%, Gibco BRL) and incubated at 37 ºC, 5% CO_2 for 4 h in the dark. After carefully washing the surfaces twice with PBS, the cells were fixed with 2% paraformaldehyde. The cells were mounted with Moviol-488 containg 1 μg/ml DAPI (Sigma Aldrich) for nucleus staining. Fluorescent images were acquired with an inverted fluorescence microscope (DMi8, Leica) in the DAPI channel with a 10x objective for an area of 5 mm^2 per sample. The number of cells on the surface were counted using the analyze particle tool in ImageJ.

Cell patterning: A PEG-Ni^{2+}-NTA modified glass surface with the CarH (1st layer)/FN (2nd layer) film was illuminated with green light through TRITC channel filter for 2 second by an inverted fluorescence microscope (DMi8, Leica) through a 20x objective with an adjustable field diaphragm. Then surfaces were incubated with 1×10^6 MCF-7 cells were seeded on the surface in the 2 ml DMEM supplemented with 10% FBS and 1% P/S at 37 ºC, 5% CO_2 overnight. After cell incubation, surfaces were washed with PBS 1x and bright filed images of the samples were acquired.

QCM-D measurement: All QCM-D measurements were performed on a Q-Sense E4 system (Q-Sense) with SiO_2 crystals (Q-sense) at room temperature with a flow rate of 300 μl/min.

The SiO_2 crystals were cleaned with a 2% SDS solution in water over night, rinsed 3 times with Milli-Q water and dried in a N_2 stream. After the QCM-D crystals were cleaned with oxygen plasma (TePla 200-G, 0.2 mbar, 150 W, 10 min), the crystals were functionalized with PEG-Ni^{2+}-NTA as described above. These crystals were placed into the QCM-D chamber and the following solutions were passed over the crystal to form the LbL protein films: (1) Buffer A, 5 min; (2) 5 μM of 1st protein, 30 min; (3) Buffer A, 10 min; (4) 100 μM $NiCl_2$+25 μM 4-arm-PEG-NTA, 30 min; (5) Buffer A, 5 min; (6) 5 μM of 2nd protein, 30 min; (7) Buffer A, 10 min. Steps 4-7 were repeated to form additional proteins layers. To remove the protein layers a solution of 250 mM imidazole in Buffer A was passed over the QCM-D crystals for 5 min followed by a washing step with Buffer A for 5 min. Experiments which included green light illumination were performed with a window QCM-D module and a 15 W green LED lamp (Osram) was used for illumination. The 7th overtone of the QCM-D measurements was represented in all graphs. The QCM-D data was fitted to the Sauerbrey equation using a density of 1056 g/L to determine the film thicknesses after each step.

Table S1. Film thicknesses obtained from QCM-D measurement in Fig. 2a by fitting the 7th overtone to the Sauerbrey equation.

Layer	Thickness (nm)
MiCy	4.2
TurboRFP	1.6
dKatushka	1.4

Table S2. Film thicknesses obtained from QCM-D measurement in Fig. 2b by fitting the 7th overtone to the Sauerbrey equation.

Layer	Thickness (nm)
CarH	3.35
TurboRFP	2.5
After green light	1.5

Table S3. Film thicknesses obtained from QCM-D measurement in Fig. 4a by fitting the 7th overtone to the Sauerbrey equation.

Layer	Thickness (nm)
CarH	2
Fibronectin	1.25
After green light	0.25

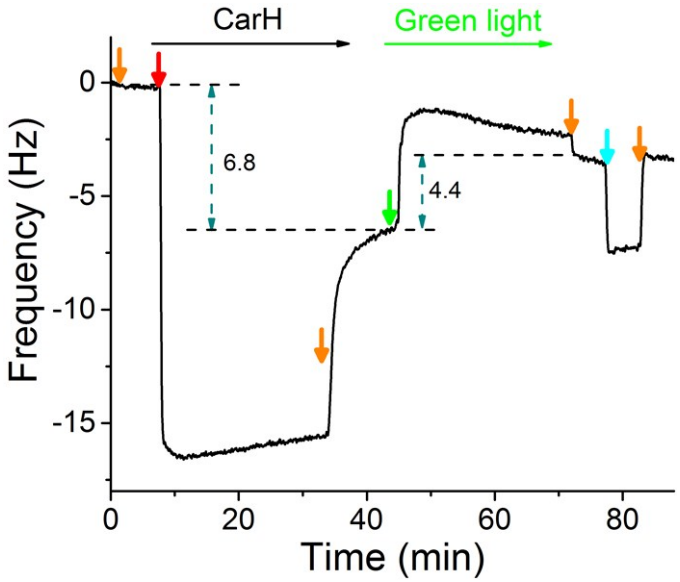

Fig. S1 QCM-D measurement of CarH tetramer, the CarH protein is dissociated from the surface in the green light step. (Arrow with different color means different solutions run on the silica crystal, orange arrows: buffer A; red arrow: 5 μM CarH; blue arrow: 25 μM 4-arm-PEG-Ni^{2+}-NTA; green arrow: green light illumination; cyan arrow: buffer B). The 7th overtone is presented. This step is used to test if the CarH is still light sensitive when immobilized and if the photocleavage of the CarH tetramer leads to any dissociation from the surface. In the QCM-D measurement, we observe that CarH tetramer dissociates from the surface when illuminate with green light (green arrow) and it indicates that CarH can bind efficiently to the surface as a tetramer through multiple His6-tags but not as a monomer with a single His6-tag.

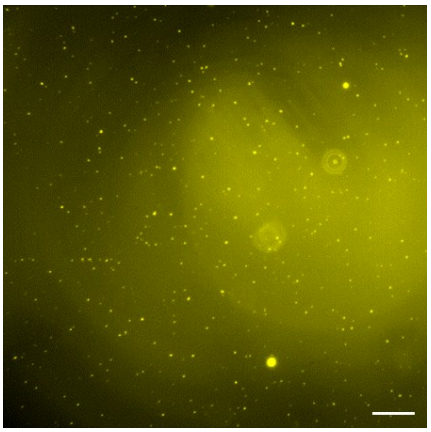

Fig. S2 Protein were locally patterned by projecting the logo of the Max Planck Society, the Minerva, onto the pure TurboRFP film in 10% intensity using a digital micromirror device, wavelength 525 nm, exposure time 1 min, scale bar: 100 µm.

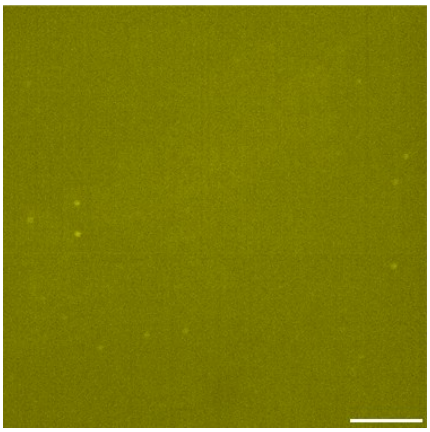

Fig. S3 Patterned protein lines with thickness values from 100 µm to 1 µm onto a just TurboRFP film in confocal microscopy, 20% intensity, wavelength 552 nm, exposure time 10 s, scale bar: 100 µm.

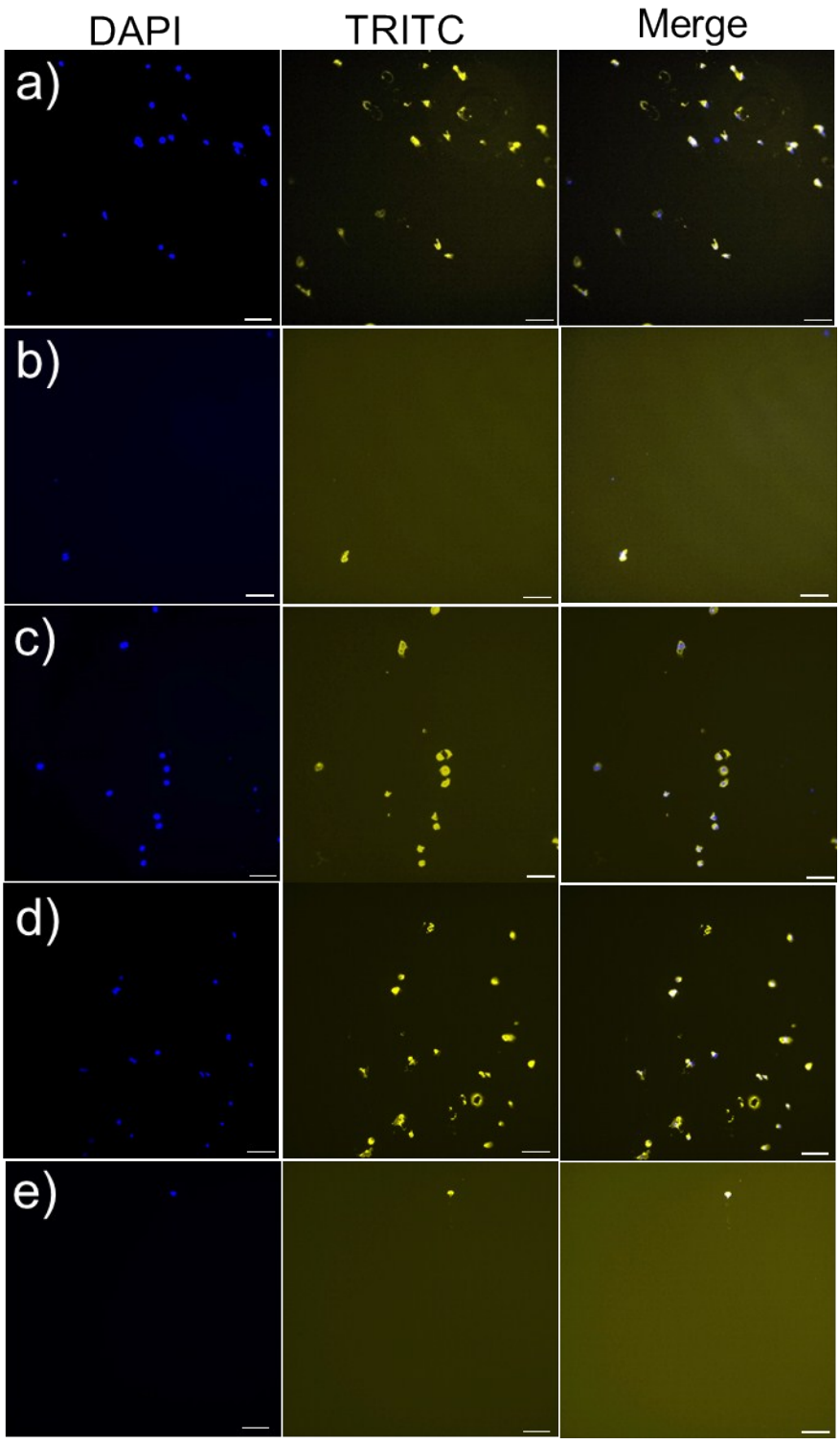

Fig. S4 The microscopy image of cell incubated on the a) CarH/FN modified glass surface under dark environment; b) CarH/FN modified glass surface under light environment; c) FN modified glass surface under dark environment; d) FN modified glass surface under light environment; e) PEG-NTA modified glass surface. Cell is stained by DAPI (DAPI channel) and Phalloidin / Actin (TRITC channel), scale bar is 100 μm.

References

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