Electronic Supplementary Material (ESI) for RSC Advances
This journal is © The Royal Society of Chemistry 2013

Supporting Information

Biomolecular Patterning of Glass via Strain-Promoted Cycloaddition of Azides and Cyclooctynes

Marloes A. Wijdeven,[†] *Carlo Nicosia,*[§] *Annika Borrmann*[†] *Jurriaan Huskens*[§] *and Floris L. van Delft**[†]

[†]Institute for Molecules and Materials, Radboud University Nijmegen, Heyendaalseweg 135, 6525 AJ Nijmegen, The Netherlands [§]Molecular Nanofabrication group, MESA[+] Institute for Nanotechnology, University of Twente, P.O box 217, 7500 AE Enschede, The Netherlands

Content

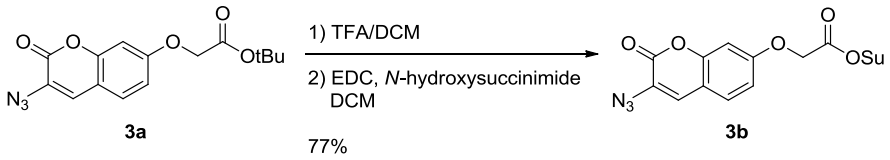
This journal is © The Royal Society of Chemistry 2013

General remarks

Solvents were distilled from appropriate drying agents prior to use and stored under nitrogen. Chemicals were purchased from Sigma-Aldrich and used as received, unless stated otherwise. Reactions were carried out under an inert atmosphere of dry nitrogen or argon. Standard syringe techniques were applied for the transfer of dry solvents and air- or moisture-sensitive reagents. Reactions were followed using thin layer chromatography (TLC) on silica gel–coated plates (Merck 60 F254) with the indicated solvent mixture. IR spectra were recorded on a Bruker Tensor 27 FTIR spectrometer. NMR spectra were recorded on a Bruker DMX 300 (300 MHz), and a Varian 400 (400 MHz) spectrometer in $CDCl_3$ solutions (unless otherwise reported). Chemical shifts are given in ppm with respect to tetramethylsilane (TMS) as internal standard. Coupling constants are reported as *J*–values in Hz. Column or flash chromatography was carried out using ACROS silica gel (0.035–0.070 mm, and ca 6 nm pore diameter). High resolution mass spectra were recorded on a JEOL AccuTOF (ESI). Fluorescence microscopy images were taken using an Olympus inverted research microscope IX71 with a mercure burner U-RFL-T as light source or a Leica TCS SP2 AOBS microscope with a 50W HBO fluorescence lamp.

This journal is © The Royal Society of Chemistry 2013

Synthetic procedures



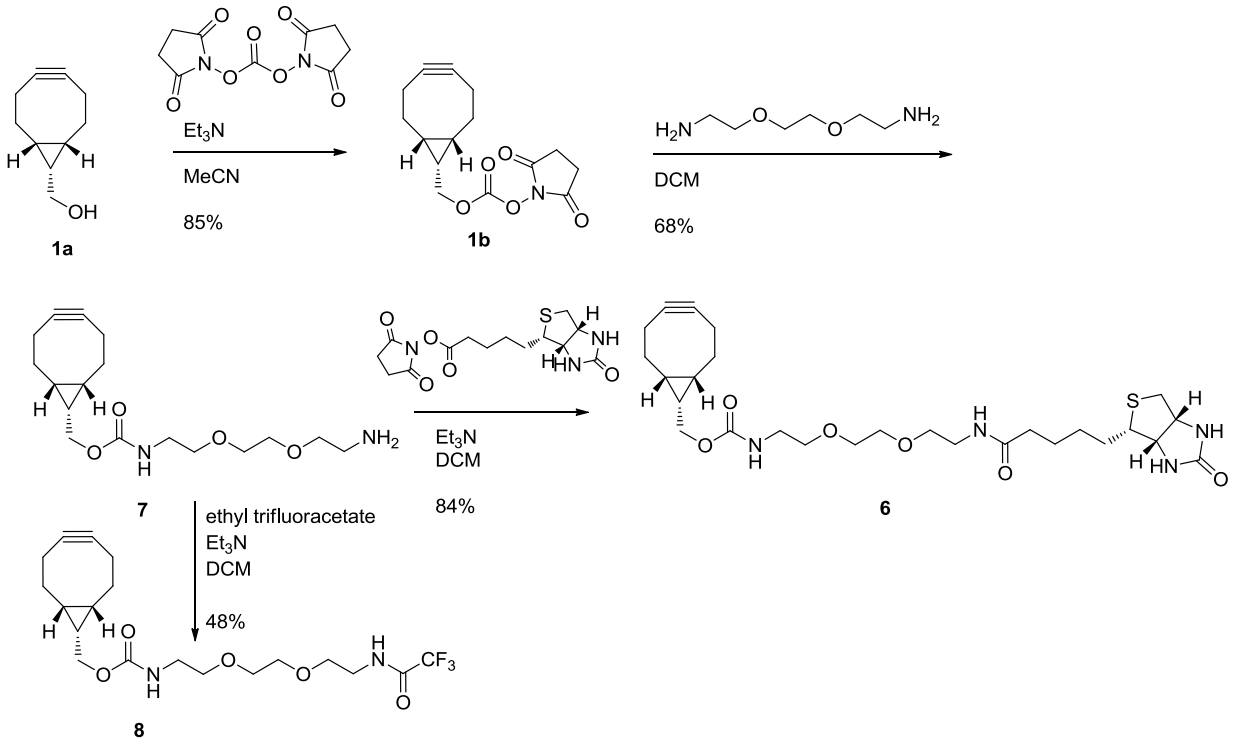
Scheme S1

Azidocoumarine **3a** was synthesized according to a literature procedure.[1]

Synthesis of coumarine **3b**

To a solution of coumarine **3a** (225 mg, 0.71 mmol) in DCM (10 mL) was added trifluoroacetic acid (TFA) (2 mL) and the reaction was stirred overnight followed by the addition of citric acid (10% in H_2O, 15 mL) and DCM (10 mL). The organic layer was washed with citric acid (10% in H_2O, 2 × 15 mL), dried over Na_2SO_4, filtrated and concentrated under reduced pressure. The crude acid was dissolved in DCM (10 mL) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) (112 mg, 0.59 mmol) and *N*-hydroxysuccinimide (66 mg, 0.59 mmol) were added. After 4 h stirring, water (10 mL) was added and the organic layer was washed with water (3 × 10 mL), dried over Na_2SO_4, filtrated and concentrated *in vacuo* giving coumarine **3b** (195 mg, 0.55 mmol, 77%).
IR (film) 2133, 1727, 1606, 1204, 1067 cm^{-1}.^{1}H-NMR ($CDCl_3$, 300 MHz) δ 7.39-7.36 (m, 1H), 7.17-7.14 (m, 1H), 6.95-6.91 (m, 2H), 5.03 (s, 2H), 2.87 (m, 4H). ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 128.0, 125.5, 125.2, 113.7, 113.0, 112.4, 102.0, 101.2, 63.0, 25.1.

DiBAC **2** was synthesized according to a literature procedure.[2]



Scheme S2

This journal is © The Royal Society of Chemistry 2013

BCN **1a** was synthesized according to a literature procedure.[3]

Synthesis of BCN-OSu **1b**

To a solution of BCN **1a** (4.03 g, 26.8 mmol) in acetonitrile (300 mL) disuccimidylcarbonate (10.3 g, 40.2 mmol) and Et_3N (11.16 mL, 80.5 mmol) were added and the reaction was stirred for 3h. EtOAc (300 mL) and water/brine (9:1, 200 mL) were added and the organic layer was washed with water/brine (9:1, 2 × 200 mL), dried over Na_2SO_4, filtrated and concentrated in vacuo. Flash column chromatography (pentane:EtOAc 5:1-1:3) afforded product **1b** (6.60 g, 22.8, 85%).
^{1}H-NMR (300 MHz, $CDCl_3$) δ 4.44 (d, *J* = 8.4 Hz, 2H), 2.81 (s, 4H), 2.34-2.18 (m, 6H), 1.61-1.42 (m, 3H), 1.09-0.98 (m, 2H). ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 168.3, 151.1, 98.2, 68.8, 28.5, 25.0, 20.8, 20.2, 16.7.

Synthesis of BCN derivative **7**

2,2'-(Ethylenedioxy)bis(ethylamine) (11.78 mL, 80.5 mmol) was dissolved in DCM (200 mL) and BCN-OSu **1b** (7.82 g, 26.8 mmol) in DCM (100 mL) was added dropwise over 3 h. After complete addition the mixture was stirred for 10 min followed by washing with saturated aqueous NH_4Cl (3 × 200 mL). The organic layer was dried over Na_2SO_4, filtrated and concentrated *in vacuo*. Flash column chromatography (DCM:MeOH 99:1-93:7 + 1% Et_3N) gave product **7** (5.95 g, 54.7 mmol, 68%).
^{1}H-NMR (300 MHz, $CDCl_3$) δ 5.38 (s, 1H), 4.13 (d, *J* = 8.1 Hz, 2H), 3.59 (s, 4H), 3.56-3.50 (m, 4H), 3.35 (q, *J* = 5.1 Hz, 2H), 2.88 (t, *J* = 5.1 Hz, 2H), 2.32 (br s, 2H), 2.27-2.15 (m, 6H), 1.62-1.42 (m, 2H), 1.33 (qn, *J* = 8.7 Hz, 1H), 0.97-0.85 (m, 2H). ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 156.4, 98.3, 68.7 (2C), 62.2, 45.5, 40.3, 40.2, 28.6, 20.9, 19.6, 17.3. HRMS (ESI+) calcd for $C_{17}H_{28}N_2NaO_4$ (*M*+Na^+) 347.1947, found 347.1952.

Synthesis of BCN-biotin **4**

To a solution of BCN derivative **7** (0.80 g, 2.47 mmol) in DCM (25 mL) were added biotin-OSu (0.93 g, 2.71 mmol) and Et_3N (0.86 mL, 6.16 mmol). The reaction mixture was stirred for 5 h and subsequent saturated aqueous $NaHCO_3$ (20 mL) was added. The organic layer was washed with saturated aqueous $NaHCO_3$ (2 × 20 mL), dried over Na_2SO_4, filtrated and concentrated under reduced pressure. Flash column chromatography (DCM:MeOH 99:1-92:8) afforded BCN biotin **4** (1.14 g, 2.1 mmol, 84%).
^{1}H-NMR (400 MHz, $CDCl_3$): δ 6.57 (s, 1H), 6.44 (s, 1H), 5.48 (s, 1H), 5.37 (s, 1H), 4.52-4.48 (m, 1H), 4.33-4.30 (m,1H), 4.16 (d, *J* = 8 Hz, 2H), 3.62 (s, 4H), 3.57 (t, *J* = 5.2 Hz, 4H), 3.45 (q, *J* = 5.2 Hz, 2H), 3.40-3.36 (m, 2H), 3.17-3.12 (m, 1H), 2.92 (dd, *J* = 8, 4.8 Hz, 1H), 2.72 (d, *J* = 12.8 Hz, 1H), 2.33-2.18 (m, 8H), 1.88 (br s, 1H), 1.80-1.57 (m, 6H), 1.49-1.33 (m, 3H), 0.95 (t, *J* = 9.6 Hz, 2H). ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 172.9, 163.6, 156.4, 98.3, 69.6, 61.3, 59.7, 55.2, 40.3, 40.0, 38.7, 35.5, 28.6, 27.8, 27.6, 25.1, 21.0, 19.7, 17.3. HRMS (ESI+) calcd for $C_{27}H_{43}N_4O_6S$ (*M*+H^+) 551.2903, found 551.2911.

Synthesis of BCN derivative **8**

To a solution of BCN derivative **7** (100 mg, 0.31 mmol) in DCM (5 mL) were added ethyl trifluoracetate (44 µL, 0.38 mmol) and Et_3N (64 µL, 0.46 mmol). The reaction mixture was stirred overnight and subsequent saturated aqueous $NaHCO_3$ (10 mL) was added. The organic layer was washed with saturated aqueous $NaHCO_3$ (2 × 10 mL), dried over Na_2SO_4, filtrated and concentrated under reduced pressure. Flash column chromatography (EtOAc:heptane 1:1-6:1) afforded BCN derivative **8** (62 mg, 0.15 mmol, 48%).

This journal is © The Royal Society of Chemistry 2013

IR (film) 3317, 2932, 1709, 1532, 1221, 1173, 1152 cm^{-1}.^{1}H-NMR ($CDCl_3$, 300 MHz) δ 7.20 (bs, 1H), 5.12 (bs, 1H), 4.10 (m, 2H), 3.60-3.53 (m, 10H), 3.35-3.33 (m, 2H), 2.27-2.11 (m, 6H), 1.50-1.53 (m, 2H), 1.33-1.31 (m, 1H), 0.94-0.92 (m, 2H). ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 156.3 (2C), 115.7 (q, J = 286 Hz), 93.3, 70.0, 69.7, 68.2, 62.3, 40.2, 39.2, 28.6, 20.9, 17.3. HRMS (ESI+) calcd for $C_{19}H_{27}F_3N_2NaO_5$ (*M*+Na^+) 443.1770, found 443.1769.

This journal is © The Royal Society of Chemistry 2013

Surface modifications

Substrate and monolayer preparation

The microscope glass slides were oxidized with piranha solution for 45 min (concentrated H_2SO_4 and 33% aqueous H_2O_2 in a 3:1 ratio, **Warning:** piranha reacts violently with organic compounds and should be handled with extreme care) and rinsed with MiliQ water. After drying in a stream of nitrogen, the substrates were immersed immediately in toluene with 1% N-[3-(trimethoxysilyl)propyl] ethylenediamine (TPEDA) for 16 h.[4] Next, the substrates were rinsed with dichloromethane and ethanol and dried in a stream of nitrogen.

Microcontact printing

Stamps were prepared by casting a 10:1 (v/v) mixture of poly(dimethylsiloxane) and curing agent (Sylgard 184, Dow Corning) against a silicon master. After curing overnight at 60 ^{o}C, the stamps were used without oxidation. Inking was performed via incubation with 150 µL of solution of coumarin **3b** or BCN (**1b**) (5 mM in acetonitrile) for 5 min. The stamp was dried under a stream of nitrogen and brought into contact with the substrate for 10 min. After removal of the stamp the substrate was washed with acetonitrile, sonicated in acetonitrile for 1 min, rinsed with ethanol and dried under a stream of nitrogen.

Incubation with cyclooctyne or coumarine

After immobilization of BCN **1b** or coumarin **3b** substrates were further reacted via incubation with respectively coumarine **3a** (10 mM in methanol) or a cyclooctyne (BCN **1a** or DiBAC **2a**, 10 mM in methanol) for 20 min and subsequent rinsed with acetonitrile, sonicated in acetonitrile for 1 min, rinsed with ethanol and dried under a stream of nitrogen.

Incubation with BCN-biotin 4

Substrates functionalized with coumarine **3b** were first immerged in a solution of DCM (10 mL) with Ac_2O (40 µL) for 60 minutes. Then the plates were rinsed with acetonitrile and ethanol and dried under a stream of nitrogen followed by incubation with BCN-biotin **4** (5 mM in methanol) for 30 min. Afterwards the substrates were rinsed with acetonitrile, sonicated in acetonitrile for 1 min, rinsed with ethanol and dried under a stream of nitrogen.

Incubation with streptavidin

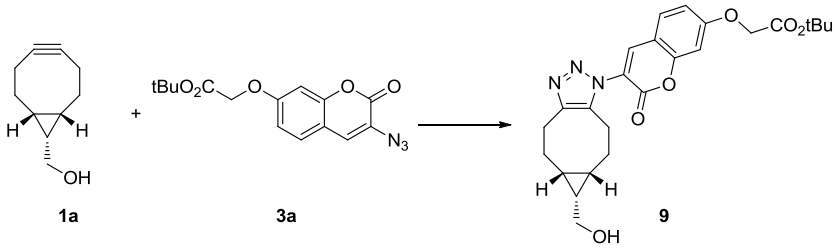
Substrates functionalized with BCN-biotin **4** or with coumarin **3b** (as blank) were incubated with streptavidin (0.01 mg/mL) in PBS buffer (pH = 7.4) for 30 min. Afterwards the samples were rinsed with PBS buffer, sonicated in PBS + tween (0.1%) for 5 min, rinsed with PBS buffer and MiliQ and dried under a stream of nitrogen.

Incubation with GFP-BCN (5)

Substrates functionalized with coumarin **3b** were incubated with GFP-BCN (**5**)[5] (1 µM) in PBS buffer (pH = 7.4) for 1h. Afterwards the samples were rinsed with PBS buffer, sonicated in PBS + tween (0.1%) for 5 min, rinsed with PBS buffer and MiliQ and dried under a stream of nitrogen.

This journal is © The Royal Society of Chemistry 2013

Fluorescence in solution



Scheme S4

Azidocoumarine **3a** (2 mg, mmol) and BCN **1a** (1.2 mg, mmol) were dissolved in methanol (3 mL) and diluted to a 2 µM solution (4 µL in 5 mL methanol). After 20 min the emission (Figure S2) and excitation (Figure S3) were measured and after 1h the emission was determined (Figure S4). As a blank the same concentration of azidocoumarine **3a** was measured.

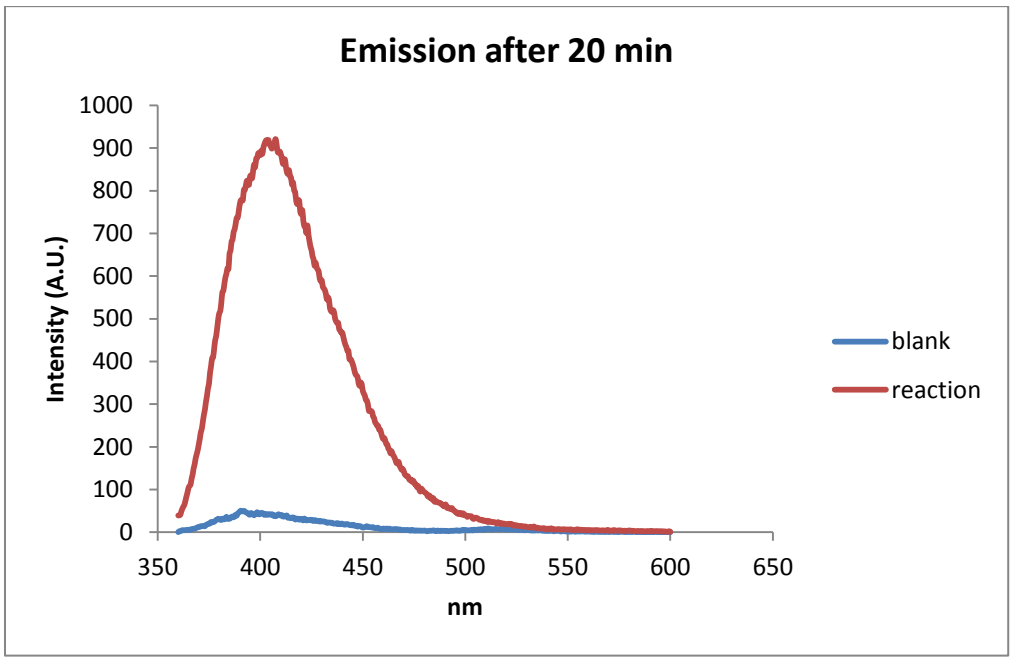


Figure S1

This journal is © The Royal Society of Chemistry 2013

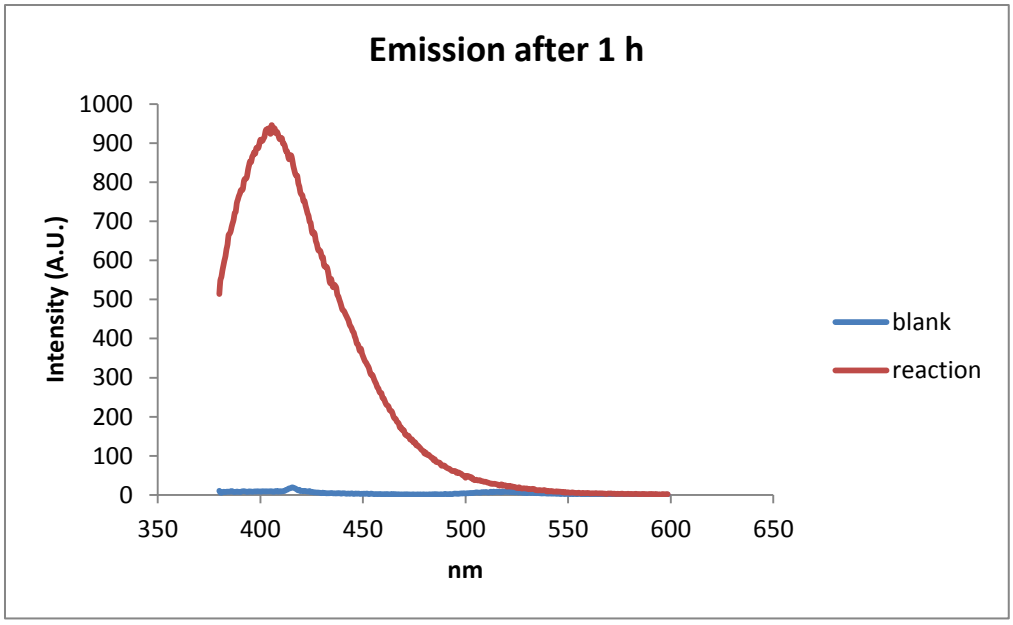


Figure S2

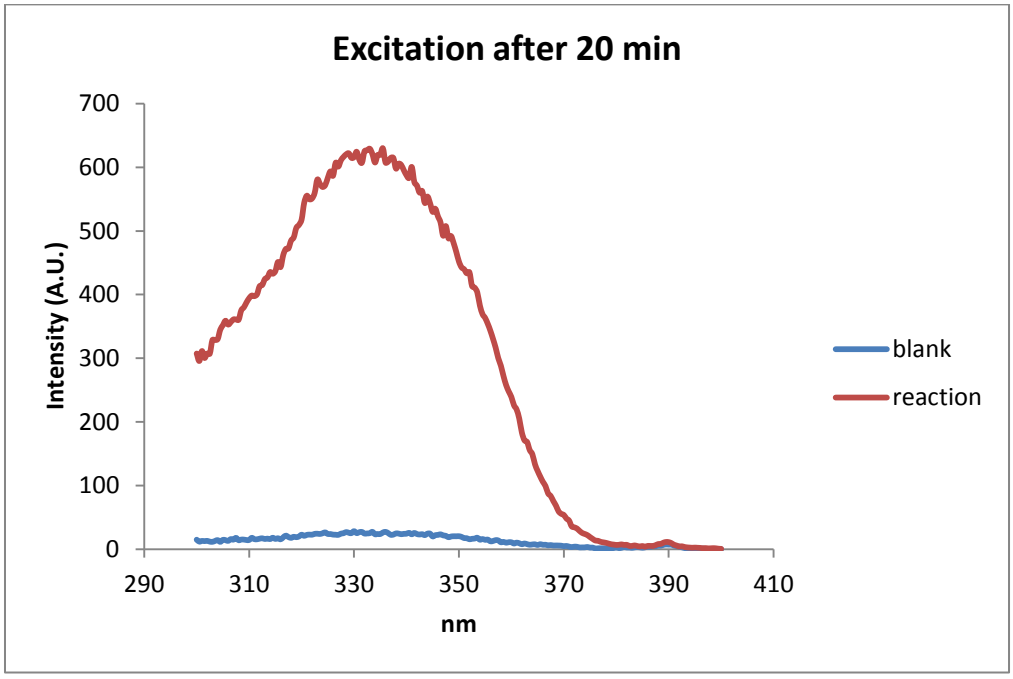


Figure S3

This journal is © The Royal Society of Chemistry 2013

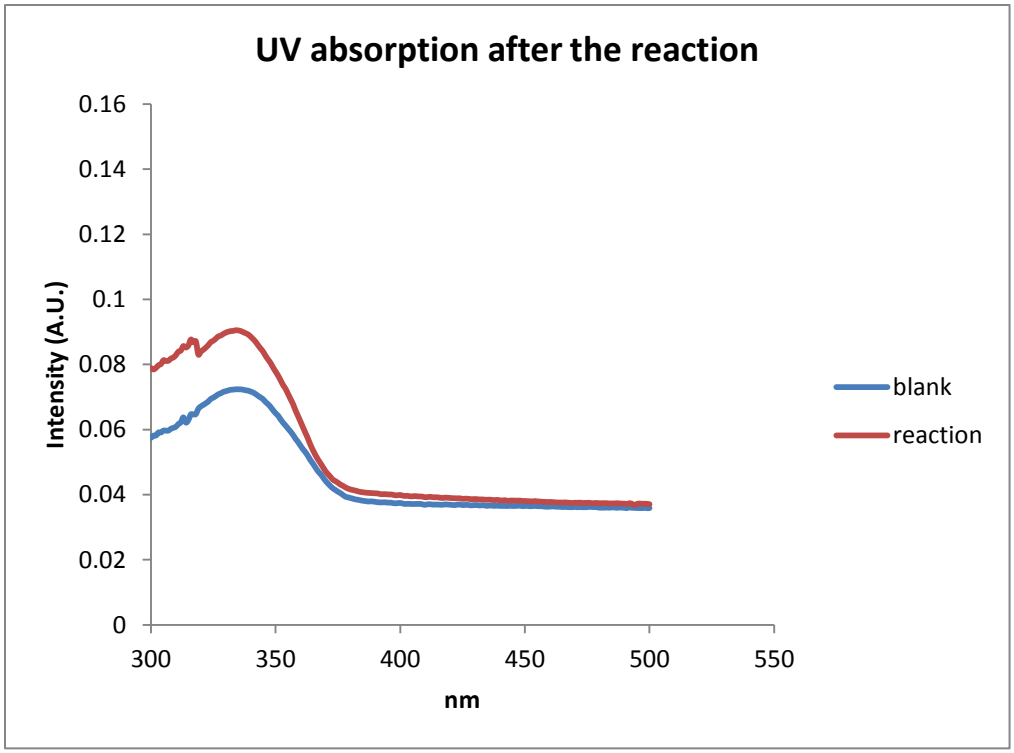


Figure S4

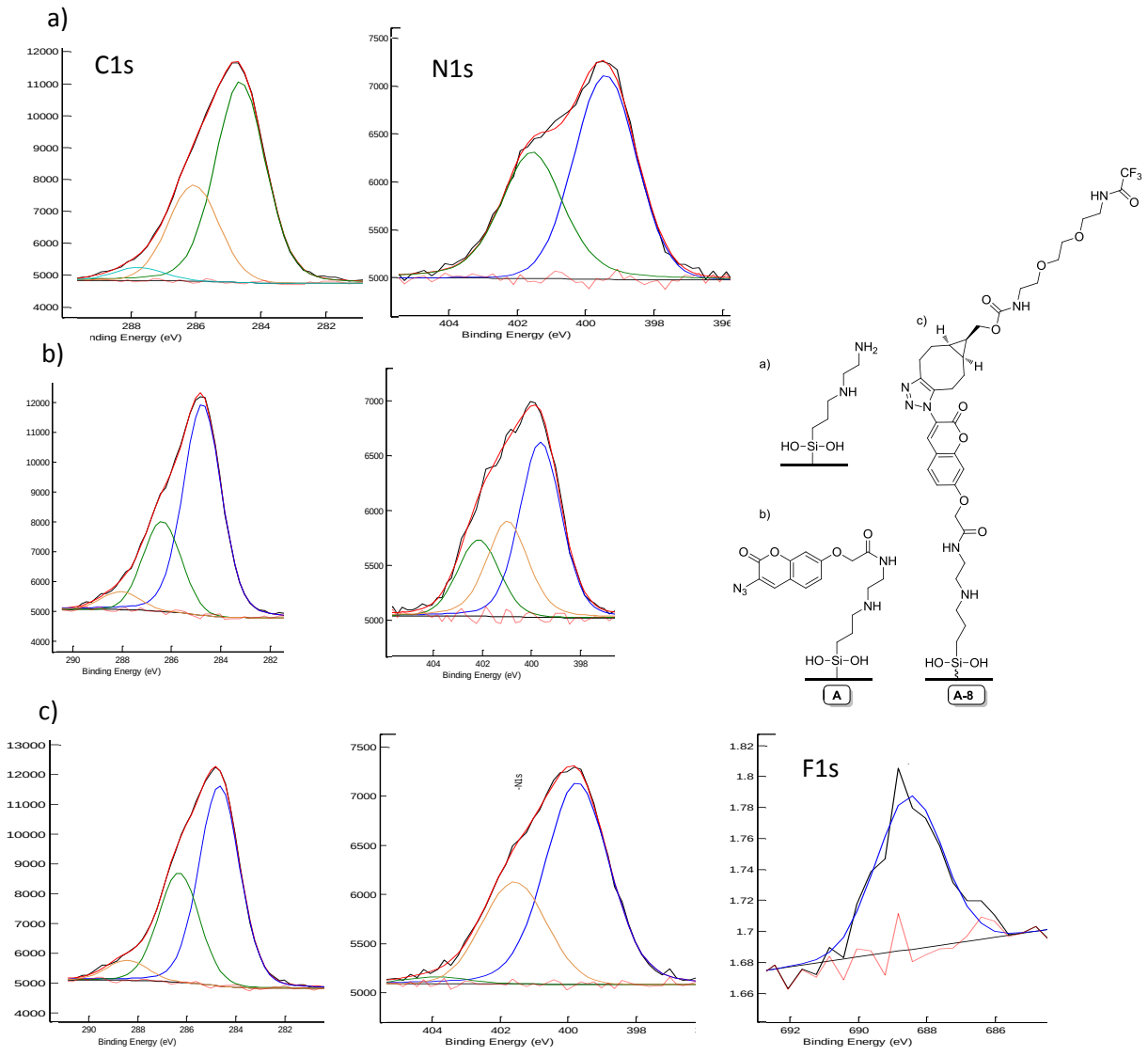
Surface characterization

XPS studies

X-ray photoelectron spectroscopy (XPS) was completed in order to further confirm the monolayer formation. The XPS of the amine monolayer showed peaks for N1s at 399 eV (1N) and 402 eV (1N) indicating the presence of two different nitrogens (primary and secondary amine). The peak located at 284 eV in the C1s region is attributed to the C-C components and C-N components are observed at 286 eV. The observed C/N ratio (2.5) is in reasonable agreement with the predicted value (3.6).

Upon immobilization of the azidocoumarine, a clear changing of the N1s was observed, indicative of the formation of the amide (401 eV) and the introduction of the nitogens of the azide group. A more pronounced peak at 288 eV in C1s region is typical of the presence of carbonyl groups. The observed C/N ratio (3.2) is higher in comparison with the amine monolayer and in reasonable agreement with the predicted value (4.5).

Finally, strain-promoted alkyne–azide cycloaddition (SPAAC) is confirmed via immobilization of a fluorine-modified BCN unit. The change of the XP spectra of the N1s region is typical for the formation of a triazol unit and the F1s peak recorded at 689 eV unequivocally confirms the immobilization of the fluorine-modified BCN group.



This journal is © The Royal Society of Chemistry 2013

Figure S5: X-ray photoelectron spectra of the C1s, N1s and F1s regions for a) amine monolayer, b) Surface **A**: coumarin monolayer and c) surface **A-8**: BCN-CF_3 monolayer

Table S1 Atomic ratios of elements C,N from XPS

Monolayer	**C/N calc**	**C/N XPS**
Amine monolayer (**a**)	2.5	3.6
Surface **A** (**b**)	3.2	4.5
Surface **A-8** (**c**)	5.0	5.6

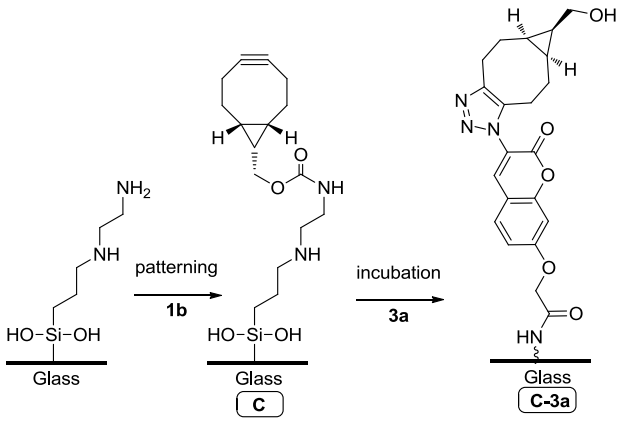
Table S2 Contactangles

Monolayer	**Advancing**	**Receding**
Amine monolayer (**a**)	83.2 ± 1.8	71.5 ± 0.2
Surface **A** (**b**)	78.4 ± 2.2	63.8 ± 3.2
Surface **A-8** (**c**)	76.9 ± 1.4	54.2 ± 0.5

This journal is © The Royal Society of Chemistry 2013

Fluorescence microscopy

As described in the article the TPEDA monolayer was functionalized via stamping with BCN derivative **1b** followed by incubation with coumarin **3a** (Scheme S5 and Figure S6). However, the BCN-containing surface turned out to be of low stability and reproducibility despite varying different parameters (stamping time, concentration, addition of radical inhibitor (dihydroquinone)), possibly as the result of polymerization of the strained alkynes after attachment to the surface.



Scheme S5

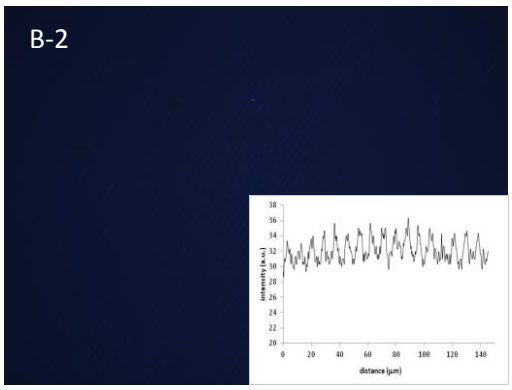


Figure S6 BCN slide (C) incubated with coumarine (C-3a) (mag. = 20x, 5 x 15 µm lines) Fluorescence microscope pictures after 20 min incubation (inset is the intensity profile, exposure time = 500 ms, λ_{ex} = 340 nm, λ_{em} = 420 nm).

In addition to Figure 2 of the article the blanc, thus the sole coumarine monolayer (surface A) the comparison of incubation with DIBAC (**2a**) and incubation with DIBAC (**2a**) and afterwards with BCN (**1a**) (Figure S7).

This journal is © The Royal Society of Chemistry 2013

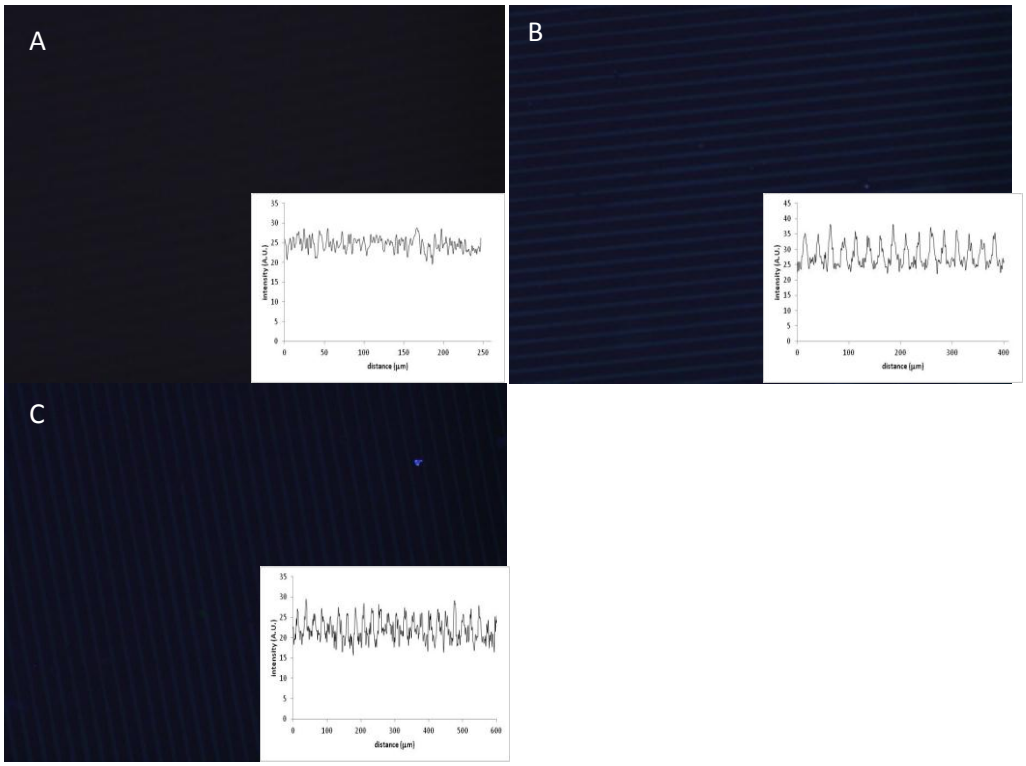


Figure S7 A) platform A, blank (magnification = 20x, 20 x 5 µm lines) B) coumarin slides incubated with DIBAC (**2a**) (mag. = 10x, 5 x 15 µm lines) C) coumarin slides incubated with DIBAC (**2a**) and subsequent with BCN (**1a**) (mag. = 10x, 5 x 15 µm lines) Fluorescence microscope pictures after 20 min incubation (inset is the intensity profile, exposure time = 500 ms, λ_{ex} = 340 nm, λ_{em} = 420 nm).

This journal is © The Royal Society of Chemistry 2013

In addition to Figure 3 of the article the fluorescence pictures before incubation with streptavidin AF488 at the green channel of the microscope (A) and the blanc (platform **A**) after incubation with streptavidin AF488 and biotin (B+C) (Figure S8).

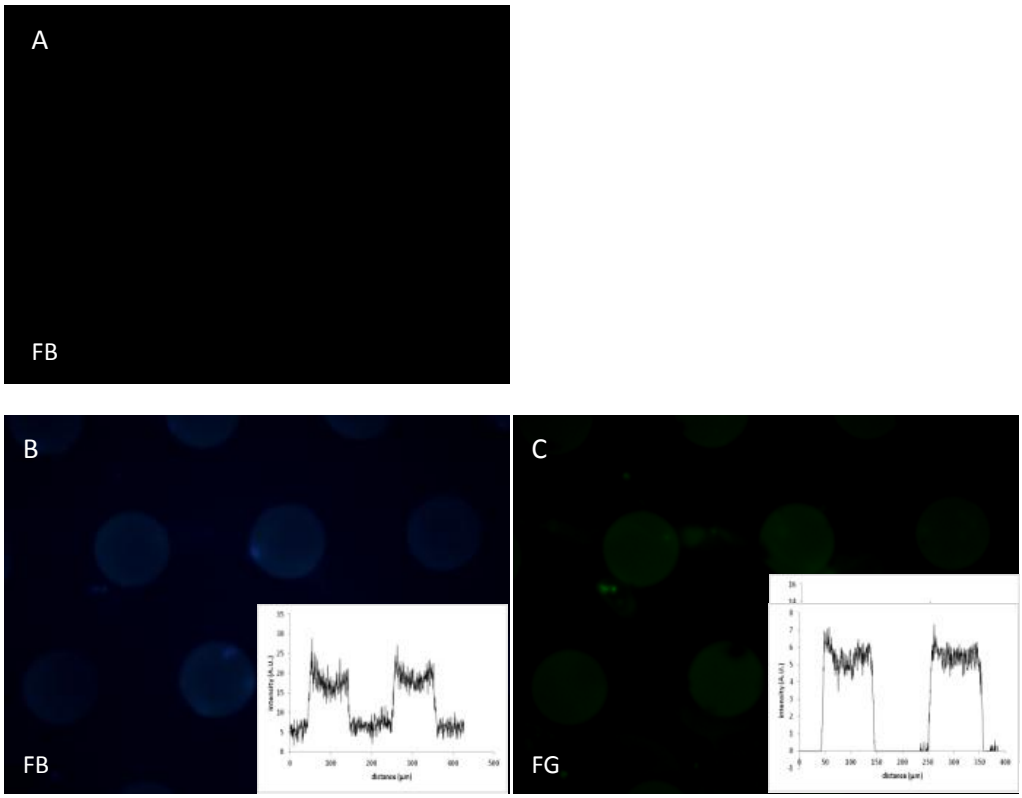


Figure S8 A) before incubation with streptavidin AF488 at the green channel of the microscope B) and C) images after incubation platform **A** with streptavidin AF488. Magnification 20x, 100 µm dots, exposure time = 500 ms, gain 2.3. Inset is intensity profile. Filter sets: FB: λ_{ex} = 340-380 nm, λ_{em} = 425 nm, FG: λ_{ex} = 450-490 nm, λ_{em} = 515 nm.

References

1) Sivakumar, K.; Xie, F.; Cash, B. M.; Long, S.; Barnhill, H. N.; Wang, Q. *Org. Lett.* **2004**, *6*, 4603.
2) Debets, M. F.; van Berkel, S. S.; Schoffelen, S.; Rutjes, F. P. J. T.; van Hest, J. C. M.; van Delft, F. L. *Chem Commun* **2010**, *46*, 97. Kuzmin, A.; Poloukhtine, A.; Wolfert, M. A.; Popik, V. V. *Bioconjugate Chem.* **2010**, *21*, 2076.
3) Dommerholt, J.; Schmidt, S.; Temming, R.; Hendriks, L. J. A.; Rutjes, F. P. J. T.; van Hest, J. C. M.; Lefeber, D. J.; Friedl, P.; van Delft, F. L. *Angew. Chem., Int. Ed.* **2010**, *49*, 9422
4) Hsu, S.-H.; Reinhoudt, D. N.; Huskens, J.; Velders, A. H. *J. Mater. Chem.* **2008**, *18*, 4959.
5) Borrmann, A.; Milles, J.; Plass, T.; Dommerholt, J.; Verkade, J. M. M.; Wießler, M.; Schultz, C.; van Hest, J. C. M.; van Delft, F. L.; Lemke, E. A. *ChemBioChem* **2012**, 2094-2099.