Supporting Information

Optical control over bioactive ligands at supramolecular surfaces

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General methods

Chemicals were purchased from Sigma Aldrich or from Acros Organics and used without further purification. Reactions were carried out using dried solvent and under an atmosphere of argon. Reactions were monitored by thin-layer chromatography (TLC), which was performed on 0.2 mm Merck precoated silica gel 60 F254 aluminum sheets. Spots were visualized by treatment with basic $KMnO_4$ solution. Column chromatography was carried out on silica gel 60 (0.063-0.2 mm, Merck). NMR spectra were recorded on Bruker spectrometers (AV400). Chemical shifts are given in units of parts per million (ppm) and expressed relative to the signals of deuterated solvents. Coupling constants (*J*) are reported in Hertz (Hz). Mass spectra were recorded with a MicroToF spectrometer (Bruker).

Synthesis of the azobenzene-carbohydrate conjugates

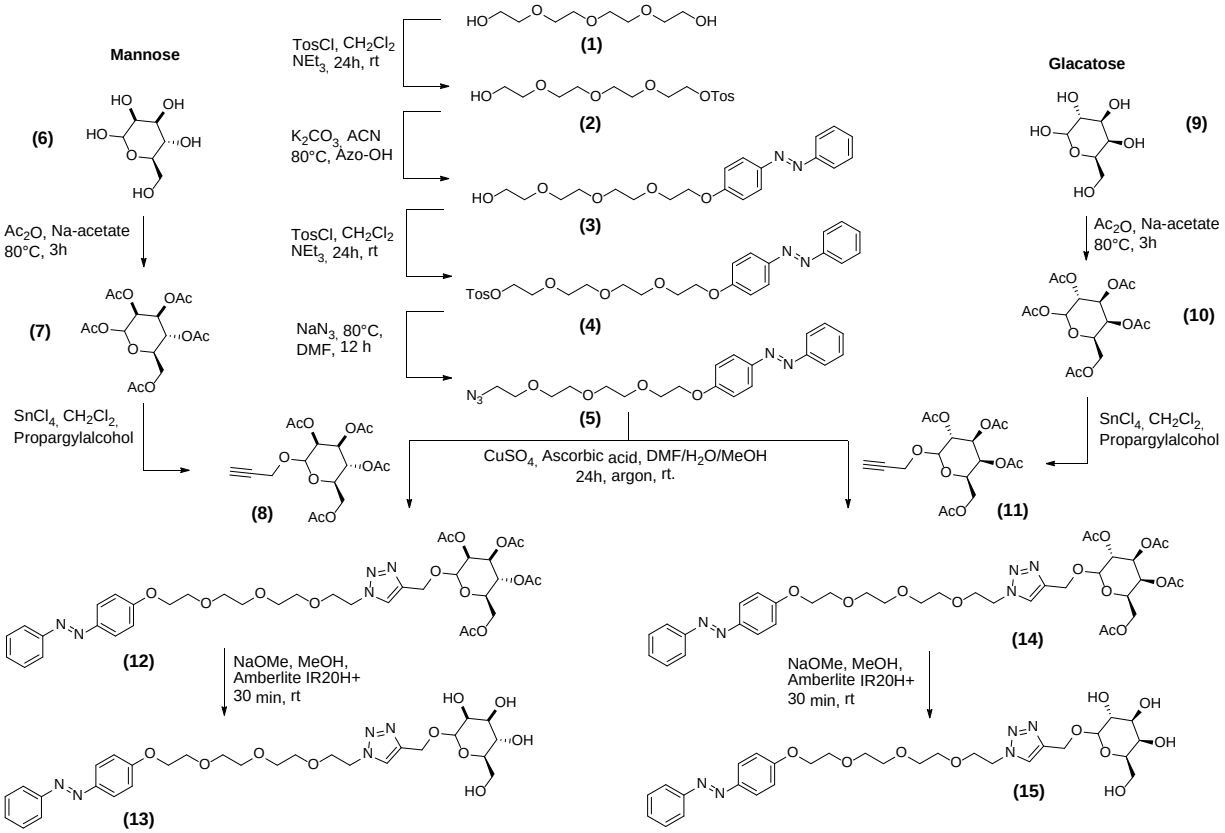
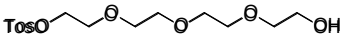


Fig. S1: Synthesis route of the azobenzene substituted glycosides.

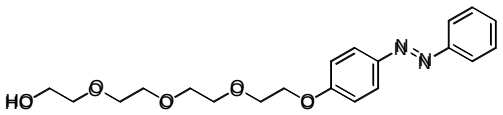
The synthesis of compound **2**,**3**,**7**,**8**,**10** and **11** were carried out according to a known literature procedure.[1, 2]

2-(2-(2-hydroxyethoxy)ethoxy)ethyl 4-methylbenzenesulfonate (2)[2]



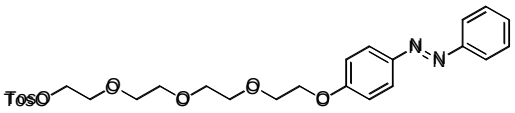
To a stirred solution of **(1)** (10.0 g, 5.1 mmol, 1 eq.) in DCM was added NEt_3 (0.4 mL, 3.3 mmol, 1 eq.) and a catalytic amount of DMAP (20 mg). The mixture was stirred for 30 minutes. After that time TosCl was added portionwise (2.41 g, 13 mmol, 1 eq.). The reaction mixture was stirred overnight followed by evaporation of the solvent. The residue was redissolved in chloroform and extracted three times with water. The organic layer was separated and dried over $MgSO_4$. The solvent was removed under reduced pressure. The title compound was obtained as colorless oil. **Yield:** 3.01 g, 63 %. **^{1}H NMR (300 MHz, $CDCl_3$, 298 K):** δ = 2.37 (s, 3H, CH_3), 3.44-3.51 (m, 10H, 5 CH_2), 3.61 (t, 2H, CH_2), 4.09 (t, 2H, CH_2), 7.27 (d, *J* = 6.0 Hz, 2H, 2CH), 7.73 (d, *J* = 6.0 Hz, 2H, 2CH). **ESI-MS (m/z)**: calculated for $[C_{13}H_{20}O_6SNa]^+$: 329.1814, found: 329.1917.The spectroscopic data are in agreement with those reported in literature.

(*E*)-2-(2-(2-(2-(4-(phenyldiazenyl)phenoxy)ethoxy)ethoxy)ethoxy)ethanol (3)[3]



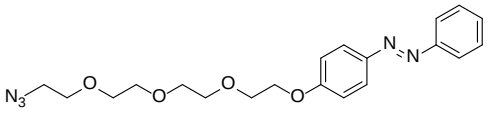
To a stirred solution of **(2)** (2.62 g, 75 mmol) in 100 ml of dry acetonitrile, containing of K_2CO_3 (5.03 g, 75 mmol) and a catalytic amount of LiBr, (50 mg) of 4-phenylazophenol dissolved in 25 ml of ACN was added and the reaction mixture was refluxed for 72 h under argon. After that all solvents were removed in vacuo. The residue was dissolved in 60 ml of DCM, washed once with 150 ml of water and three times with 150 ml of brine. The organic layer was dried over $MgSO_4$ and concentrated. The residue was subjected to silica gel column chromatography (DCM / methanol 90:10) to afford the title compound as an orange oil which solidified upon standing. **Yield:** 2.62 g, 92 %. **^{1}H NMR (300 MHz, $CDCl_3$, 298 K):** δ = 2.57 (s, 1H, OH), 3.58 – 3.50 (m, 2H, CH_2), 3.72 – 3.61 (m, 10H, $5CH_2$), 3.83 (m, 2H, CH_2), 4.16 (m, 2H, CH_2), 7.03 – 6.95 (m, 2H, 2CH), 7.50 – 7.35 (m, 3H, 3CH), 7.92 – 7.80 (m, 4H, 4CH). The spectroscopic data are in agreement with those reported in literature.

(*E*)-2-(2-(2-(4-(phenyldiazenyl)phenoxy)ethoxy)ethoxy)ethyl-4-methylbenzensulfonate (4)



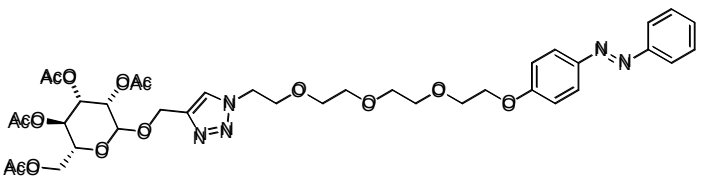
To a stirred solution of **(3)** (3.00 g, 10 mmol, 1 eq.) in CH_2Cl_2 was added NEt_3 (1,2 mL, 10 mmol, 1 eq.) and a catalytic amount of DMAP (50 mg). The mixture was stirred for 30 minutes. After that time p-toluenesulfonylchloride was added portionwise (2.07 g, 10 mmol, 1 eq.). The reaction mixture was stirred overnight followed by evaporation of the solvent. The residue was redissolved in chloroform and extracted 3 times with water. The organic layer was separated and dried over $MgSO_4$. The solvent was removed under reduced pressure. The residue was subjected to silica column chromatography ($CHCl_3$). The product was obtained as orange oil. **Yield:** 1,78 g, 33 %. **^{1}H-NMR (400 MHz, $CDCl_3$):** 7.89-7.84 (m, 4H, 4CH); 7.76-7.74 (d, 2H, 2CH); 7.48-7.45 (m, 2H, 2CH); 7.42-7.40 (m, 1H, CH); 7.29-7.27 (m, 2H, 2CH); 7.00-6.98 (m, 2H, 2CH); 4.18-4.16 (m, 2H, CH_2); 4.13-4.10 (m, 2H, CH_2); 3.86-3.83 (m, 2H, CH_2); 3.68-3.54 (m, 10H, $5CH_2$); 2.37 (s, 3H, CH_3). **ESI-MS (m/z):** calculated for $[C_{27}H_{32}N_2O_7SH]^+$: 529.1930, found: 529.1976.

***(E)*-2-(2-(2-(4-(phenyldiazenyl)phenoxy)ethoxy)ethoxy)ethyl-azide (5)**



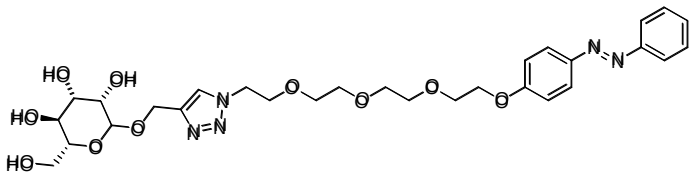
To a stirred solution of **(4)** (150 mg, 0.28 mmol, 1 eq) in 10 mL of DMF was added NaN_3 (100 mg, 1.51 mmol, 5.4 eq.) and the suspension was heated to 80°C and stirred overnight. After that, the solvent was removed in vacuo and the crude residue was dissolved in 20 mL of $CHCl_3$ and washed three times with 20 mL of distilled water. The organic layer was collected and dried over $MgSO_4$. The solvent was evaporated and the title compound was obtained as orange oil. **Yield:** 103 mg, 91 %. **^{1}H-NMR (400 MHz, $CDCl_3$):** 7.90-7.86 (m, 4H, 4CH); 7.46-7.43 (m, 2H, 2CH); 7.43-7.41 (m, 1H, CH); 7.02-7.00 (m, 2H, 2CH); 4.21-4.18 (t, 2H, *J* = 4.6 Hz CH_2); 3.89-3.86 (t, 2H, *J* = 4.6 Hz, CH_2); 3.72-3.63 (m, 10H, $5CH_2$); 3.43-3.34 (t, 2H, *J* = 4.6 Hz, CH_2). **ESI-MS (m/z):** calculated for $[C_{20}H_{25}N_5O_4H]^+$: 400,1907 found: 400.0972.

(*E*)-2-(2-(2-(4-(phenyldiazenyl)phenoxy)ethoxy)ethoxy)ethyl-triazoyl-peracetylmannopyranoside (12)



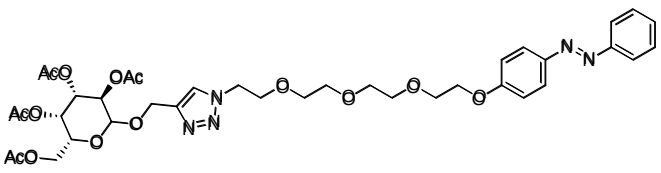
To a mixture of **(5)** (50 mg) and **(8)** (50 mg) in a degassed solvent mixture of MeOH, DMF and water (1:2:1) was added sodium ascorbate (20 mg) and $CuSO_4$ (10 mg). The suspension was stirred for 24 h under an atmosphere of argon followed by evaporation of all solvents. The residue was dissolved in $CHCl_3$ (1 mL) and subjected to silica column chromatography ($CHCl_3$/MeOH, 9:1). The product was obtained as brown-orange oil. **Yield:** 91 mg, 92%. **^{1}H-NMR (400 MHz, $CDCl_3$):** 7.87-7.81 (m, 4H, 4CH); 7.77 (s, 1H, $CH_{triazole}$); 7.47-7.43 (m, 2H, 2CH); 7.41-7.37 (m, 1H, CH); 6.99-6.96 (m, 2H, 2CH); 5.27-5.25 (m, 2H, 2CH); 5.20-5.19 (m, 1H, CH); 4.92 (d, 1H, *J* = 1,6 Hz, CH); 4.71 (dd, 2H, *J* = 12,2 Hz, 53.1 Hz); 4.51–4.48 (2H, *J* = 5.2 Hz, CH_2); 4.29-4.23 (m, 1H, CH); 4.18-4.16 (2H, *J* = 4.9Hz, CH_2); 4.08 (d, 1H, *J* = 2.4Hz, CH); 4.05-4.02 (m, 1H, CH); 3.86-3.82 (m, 4H, $2CH_2$); 3.71-3.69 (m, 2H, CH_2); 3.64-3.61 (m, 2H, CH_2); 3.60-3.57 (m, 4H, $2CH_2$); 2.09 (s, 3H, CH_3); 2.07 (3H, CH_3); 1.98 (s, 3H, CH_3); 1.93 (s, 3H, CH_3). **^{13}C-NMR: (100 MHz, $CDCl_3$):** 170.90, 170.86, 170.17, 170.01, 169.86, 161.33, 152.80, 147.18, 143.33, 130.55, 129.16, 124.84, 124.51, 122.67, 114.92, 96.88, 92.29, 77.43, 70.96, 70.67, 70.63, 70.62, 69.72, 68.78, 67.82, 66.43, 66.15, 66.15, 62.47, 61.71, 60.97, 50.45, 20.99, 20.91, 20.82, 20.79. **ESI-MS (m/z):** calculated for $[C_{37}H_{47}N_5O_{14}H]^+$: 786.3198 found: 786.0652.

(*E*)-2-(2-(2-(4-(phenyldiazenyl)phenoxy)ethoxy)ethoxy)ethyl-triazoyl-mannopyranoside (13)



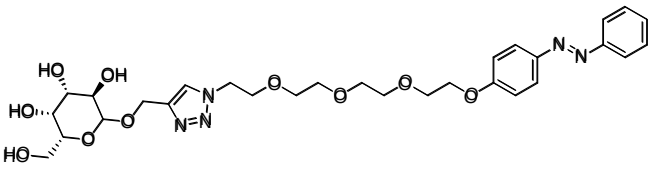
Compound **(12)** (90 mg, 0.11 mmol) was dissolved in 10 mL of dry methanol followed by the addition of a catalytical amount of NaOMe (20 mg). After 30 minutes Amberlite® IR 120 (H^+) ion exchange resin was added until a pH of 3 was reached. The resin was filtered off and the solution was evaporated to dryness. The title compound was obtained as an orange-brown hygroscopic solid. **Yield:** 61 mg, 87%.**^{1}H-NMR (400 MHz, CD_3OD):** 8.22 (s, 1H, $CH_{triazole}$);7.82-7.79 (m, 4H, 4CH); 7.46-7.44 (m, 2H, 2CH); 7.42-7.40 (m, 1H, CH); 7.02-7.00 (d, 2H, *J* = 8.8 Hz, 2CH); 4.82 (s, 1H, CH); 4.71 (dd, 2H, *J* = 46,2 Hz, 12,7 Hz, CH_2); 4.56 (t, 2H, *J* = 5Hz, CH_2); 4.15 (t, 2H, *J* = 4.8 Hz; CH_2); 3.84 (t, 2H, *J* = 4.7 Hz, CH_2); 3.81-3.79 (m, 2H, CH_2); 3.77 (bs, 1H, CH); 3.67-3.62 (m, 4H, $1CH_2$, 2CH); 3.59-3.55 (m, 8H, $4CH_2$); 3.50-3.47 (m, 1H, CH). **^{13}C-NMR: (100 MHz, CD_3OD):** 168.90, 163.00, 154.10, 148.32, 131.73, 130.86, 130.27, 127.43, 127.16, 125.83, 123.58, 116.04, 101.26, 96.38, 75.20, 71.95, 71.80, 71.56, 71.53, 71.46, 70.74, 69.96, 69.07, 68.79, 68.58, 62.98, 60.12, 52.49. **ESI-MS (m/z):** calculated for $[C_{29}H_{39}N_5O_{10}H]^+$: 617.27 found 617.97

(*E*)-2-(2-(2-(4-(phenyldiazenyl)phenoxy)ethoxy)ethoxy)ethyl-triazoyl-peracetylgalactopyranoside (14)



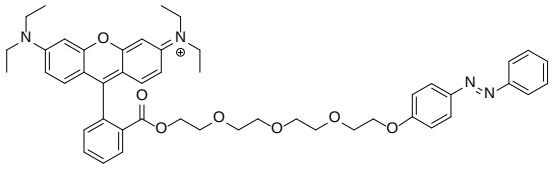
To a mixture of **(11)** (50 mg) and **(5)** (60 mg) in a degassed solvent mixture of MeOH, DMF and water (1:2:1) was added sodium ascorbate (20 mg) and $CuSO_4$ (10 mg). The suspension was stirred for 24 h under an atmosphere of argon followed by evaporation of all solvents. The residue was dissolved in $CHCl_3$ (1 mL) and subjected to silica column chromatography ($CHCl_3$/MeOH, 9:1). The product was obtained as an orange oil. **Yield:** 88 mg, 89%.**^{1}H-NMR (400 MHz, $CDCl_3$):** 7.88-7.82 (m, 4H, 4CH); 7.72 (bs, 1H, $CH_{triazole}$); 7.46 (t, J = 7.2 Hz, 2H, 2CH); 7.40 (t, J = 7.2 Hz, 1H, CH); 6.98 (d, J = 8.7 Hz, 2H, 2CH); 5.36 (d, J= 3.3 Hz, 1H, CH); 5.18-5.16 (m, 1H, CH); 4.99-4.97 (m, 1H, CH); 4.85 (dd, J= 12Hz, 43 Hz, 2H, CH_2); 4.63 (d, J= 6Hz, 1H, CH); 4.51-4.48 (m, 2H, CH_2); 4.18 (t, J = 4.3 Hz, 2H, CH_2); 4.13-4.11 (m, 2H, CH_2); 3.91 (t, J = 6.6 Hz, 1H, CH); 3.86-3.83 (m, 4H, $2CH_2$); 3.70 (t, J = 4 Hz, 2H, CH_2); 3.63-3.59 (m, 6H, 3 CH_2); 2.10 (s, 3H, CH_3); 2.02 (s, 3H, CH_3); 1.94 (s, 3H, CH_3); 1.93 (s, 3H, CH_3). **^{13}C-NMR: (100 MHz, $CDCl_3$):** 170.27, 170.10, 170.08, 169.92, 169.36, 161.05, 152.53, 146.91, 130.27, 128.88, 124.56, 122.39, 114.65, 100.14, 95.35, 90.44, 77.12, 70.69, 70.62, 70.42, 70.38, 70.33, 69.46, 69.20, 68.59, 67.56, 66.90, 62.56, 61.10, 50.21, 20.57, 20.54, 20.50, 20.41. **ESI-MS (m/z):** calculated for $[C_{37}H_{47}N_5O_{14}H]^+$: 786.3198 found: 786.0656.

(*E*)-2-(2-(2-(4-(phenyldiazenyl)phenoxy)ethoxy)ethoxy)ethyl-triazoyl-galactopyranoside (15)



Compound **(14)** (90 mg, 0.11 mmol) was dissolved in 10 mL of dry methanol followed by the addition of a catalytic amount of NaOMe (20 mg). After 30 minutes Amberlite® IR 120 (H^+) ion exchange resin was added until a pH of 3 was reached. The resin was filtered off and the solution was evaporated to dryness. The title compound was obtained as an orange-brown hygroscopic solid. **Yield:** 66 mg, 97%.**^{1}H-NMR (400 MHz, CD_3OD):** 8.07 (s, 1H, $CH_{triazole}$); 7.91-7.85 (m, 4H, 4CH); 7.54-7.52 (m, 2H, 2CH); 7.48-7.46 (m, 1H, CH); 7.09 (d, *J* = 9.1 Hz, 2H, 2CH); 4.96 (d, *J* = 12.4 Hz, 1H, CH_2); 4.76 (d, *J* = 12.4 Hz, 2H CH_2); 4.55 (t, *J* = 4.9 Hz, 2H, CH_2); 4.33 (d, *J* = 7.6 Hz, 1H, CH); 4.24-4.22 (m, 2H, CH_2); 3.89-3.86 (m, 4H, $2CH_2$); 3.82-3.70 (m, 4H, $2CH_2$); 3.62-3.57 (m, 6H, $3CH_2$); 3.55-3.52 (m, 2H, CH_2); 3.48-3.44 (m, 1H, CH). **^{13}C-NMR: (100 MHz, CD_3OD):** 163.43, 154.13, 148.60, 131.66, 130.23, 128.41, 126.15, 125.78, 123.54, 116.05, 104.27, 76.84, 74.94, 71.82, 71.59, 71.50, 70.76, 70.38, 70.35, 69.07, 63.01, 62.62, 51.47. **ESI-MS (m/z):** calculated for $[C_{29}H_{39}N_5O_{10}H]^+$: 617.27 found: 617.93.

Rhodamine-azobenzene conjugate (16)



(2) (300 mg, 0,8 mmol) was dissolved in 20 mL of dichloromethane followed by the addition of HOBt (110 mg, 0,8 mmol) and EDCI (0,13 mL, 0,8 mmol). After 20 minutes of stirring rhodamine B (380 mg, 0.8 mmol) was added and stirring was continued for 18 h. After that time the solvent was removed in vacuo and the residue was subjected to silica gel column chromatography. (DCM/EtOH – 9:1, Rf = 0,88), **Yield:** 58 mg, 9%. **^{1}H-NMR (400 MHz, $CDCl_3$):** 8.30 (dd, J = 7.0 Hz, 0.7 Hz, 1H, CH); 7.91-7.83 (m, 4H, 4CH); 7.78 (dt, J = 6.5 Hz, 0.8 Hz, 1H, CH); 7.69 (dt, J = 7.0 Hz, 0.8 Hz, 1H, CH); 7.50-7.39 (m, 3H, 3CH); 7.29 (d, J = 7.5 Hz, 1H, CH); 7.04-6.96 (m, 4H, 4CH); 6.82-6.80 (m, 4H, 4CH);4.22-4.16 (m, 2H, CH_2); 3.89-3.84 (m, 2H, CH_2); 3.75-3.52 (m, 20H, 10 CH_2); 1.27 (t, J = 7.0 Hz, 12H, $4CH_3$). **^{13}C-NMR: (100 MHz, $CDCl_3$)**: 165.66, 161.91, 159.52, 158.47, 156.19, 153.43, 153.38, 147.79, 147.74, 134.39, 133.86, 132.17, 131.98, 131.16, 131.08, 131.04, 130.99, 130.40, 129.75, 129.72, 127.50, 125.41, 123.25, 116.86, 115.57, 115.53, 115.49, 114.79, 114.26, 97.11, 77.83, 73.23, 71.53, 71.52, 71.34, 71.26, 71.22, 71.17, 70.98, 70.31, 69.39, 68.42, 68.38, 46.77, 30.39, 13.32. **ESI-MS**: calculated for $[C_{48}H_{55}N_4O_7]^+$: 799.4065, found 799.5651.

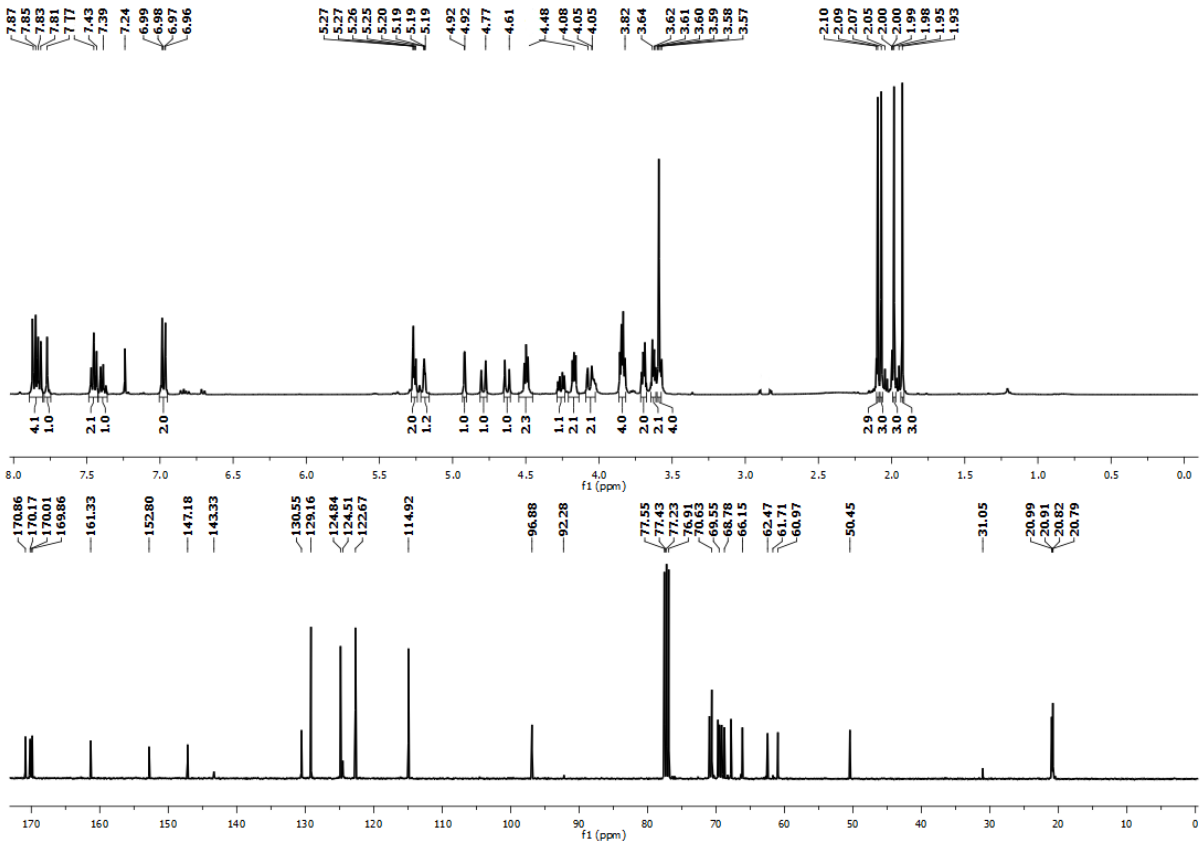


Fig. S2: ^{1}H-NMR and ^{13}C-NMR of compound **(12)** in $CDCl_3$

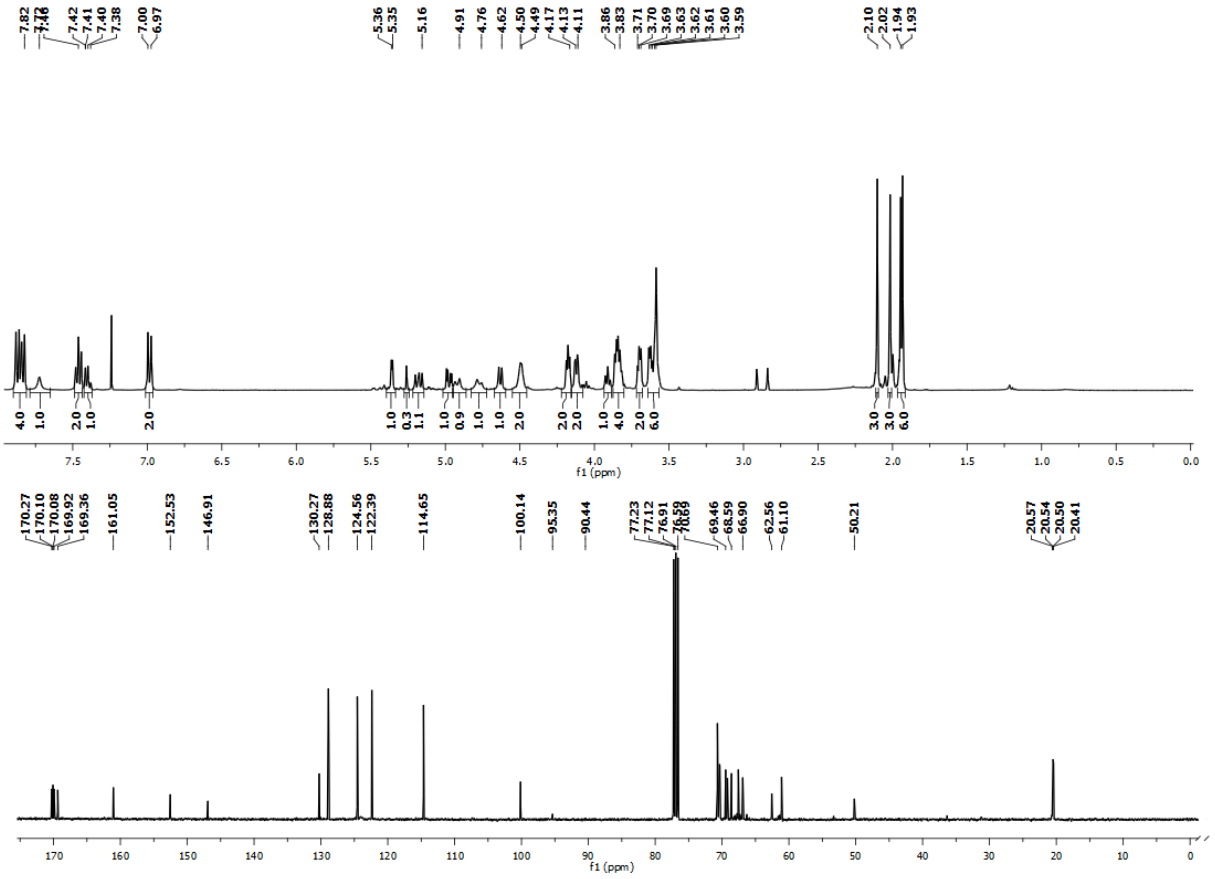


Fig. S3: ^{1}H-NMR and ^{13}C-NMR of compound **(14)** in $CDCl_3$.

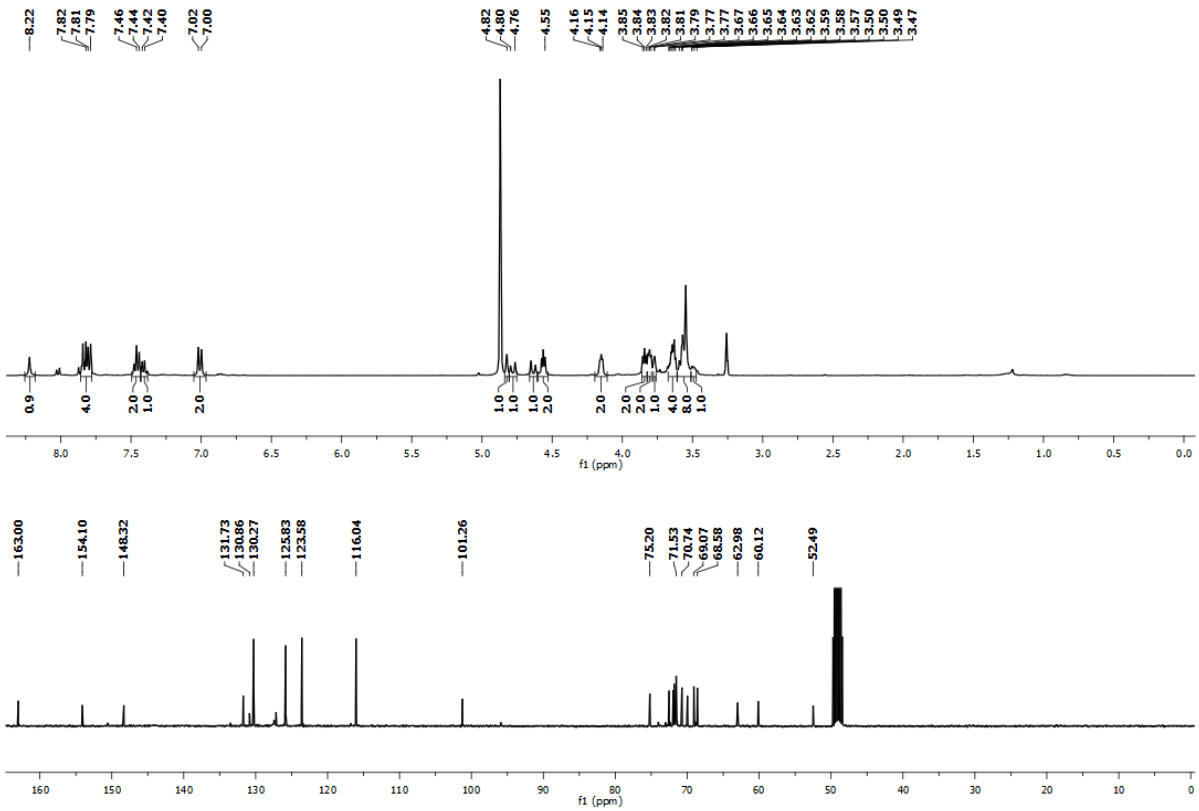


Fig. S4: ^{1}H-NMR and ^{13}C-NMR of compound **(13)** in $CDCl_3$

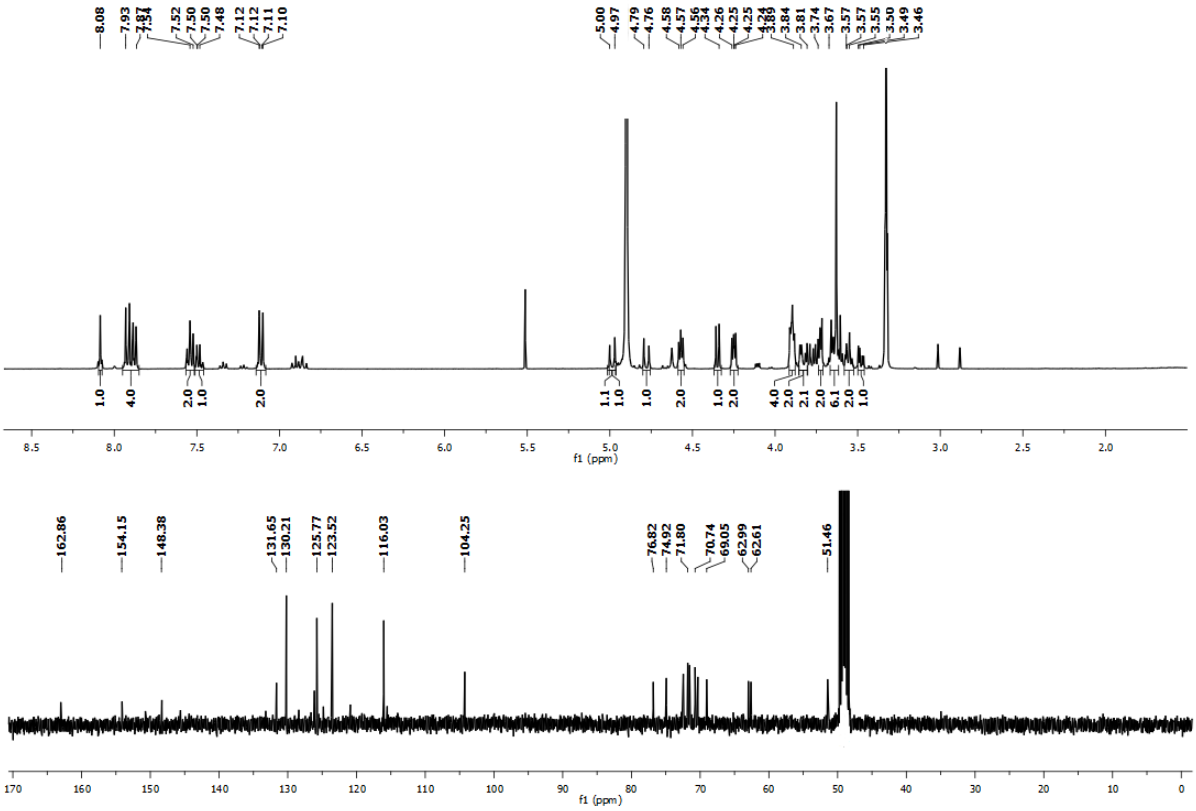


Fig S5: ^{1}H-NMR and ^{13}C-NMR of compound **(15)** in $CDCl_3$.

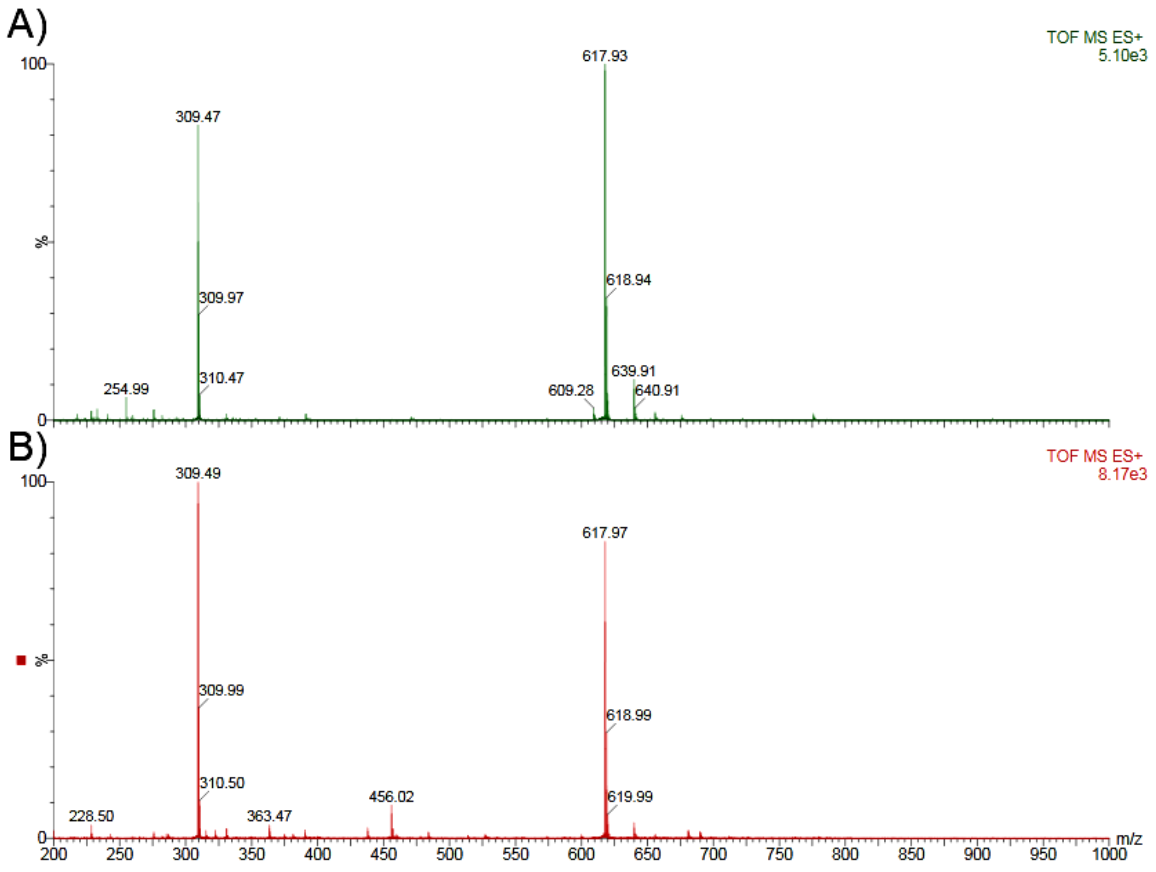


Fig. S6. ESI-TOF-MS of A) compound **(15)** and B) compound **(13)**

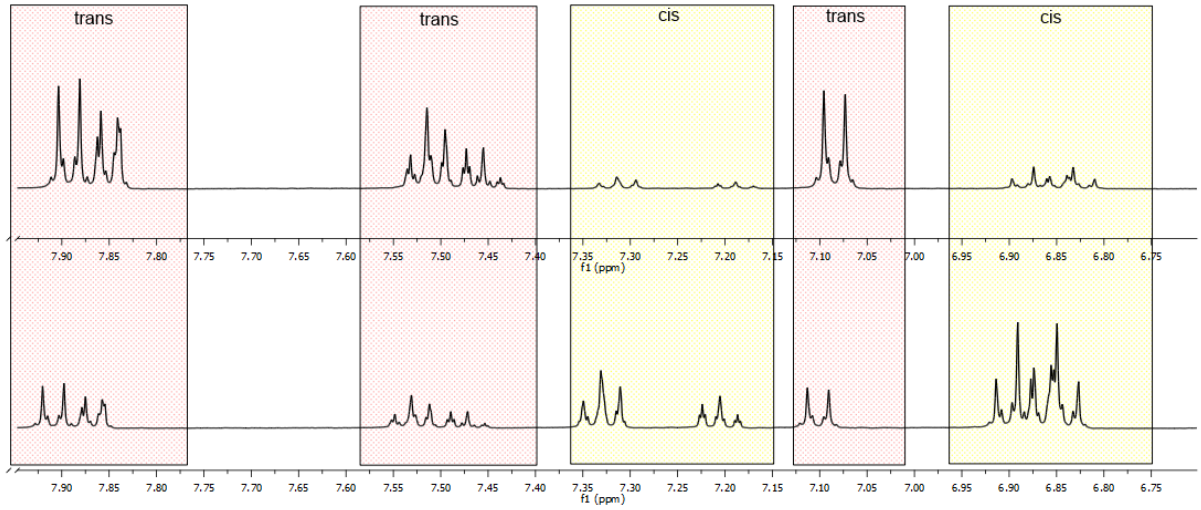


Fig. S7: ^{1}H-NMR of the aromatic region of compound **(15)** before and after irradiation for 10 minutes with a common UV-lamp (365 nm).

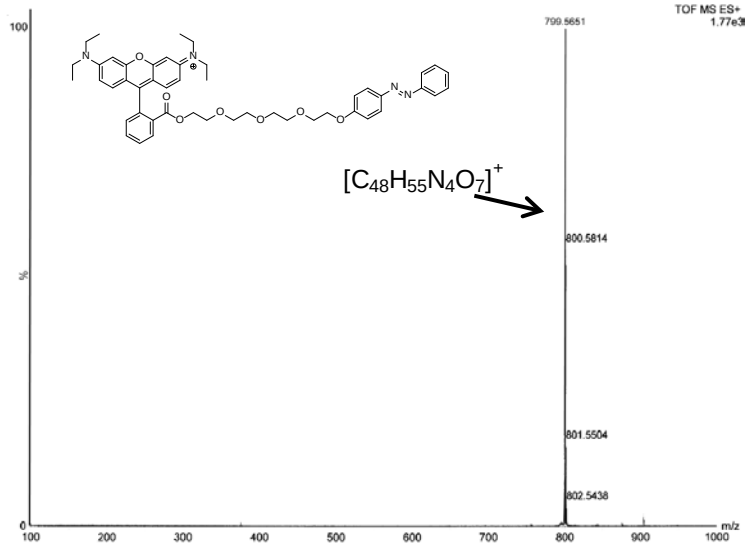


Fig. S8: TOF MS-ES+ spectrum of compound **(16)**

Monolayer preparation

Gold substrates for QCM

Standard gold coated QCM sensors from Q-sense (QSX301) were cleaned by immersing them in piranha (1:3 mixture of concentrated H_2SO_4 and 30% H_2O_2) for 15 s. (Warning: piranha should be handled with caution; it can detonate unexpectedly). After thorough rinsing with MilliQ water, they were placed in absolute ethanol for 10 min. The substrates were subsequently placed in a freshly prepared 0.1 mM solution of β-CD heptathioether in EtOH and $CHCl_3$ (1:2 v/v) for 16 h at 60 ° C. The substrates were then rinsed with $CHCl_3$, EtOH and MilliQ water.

Glass substrates for microcontact printing

The glass substrates were cleaned and activated by immersing in piranha for 30 mins followed by washing with water, ethanol and thorough drying. The surface was then functionalized with N-[3-(trimethoxysilyl)propyl]ethylenediamine (TPEDA) by overnight chemical vapor deposition using a vacuum desiccator. The substrates were then thoroughly cleaned using ethanol and toluene after which it was incubated in a 1mM solution of 1,4-phenylene di-isothiocyanate (ITC) in dry toluene for 2 hr at 50^0C. The substrates were then washed thoroughly with toluene, ethanol and water and finally incubated with a 1mM solution of per-6-amino-β-cyclodextrin in MilliQ water for 2 hr at 50^0C. The substrates were finally washed thoroughly with water, dried and stored in N_2 atmosphere until use.

QCM experiments

The QCM experiments were conducted using the Q-Sense E1 instrument with the standard Flow Module. All binding studies were conducted with a flow rate of 100 μL/min at a temperature of 22 ^{0}C. Resonance frequency and dissipation values were determined automatically by the instrument and only the changes in these values were presented as the response. Binding affinity experiments of Azo-Man onto β-CD monolayers were done using water as the solvent. Binding experiments involving ConA and bacteria were done in a 10 mM HEPES, 137 mM NaCl, pH 7 buffer containing 1mM $MnCl_2$ and 1mM $CaCl_2$.

Microcontact printing

Poly(dimethylsiloxane) (PDMS) stamps were prepared by casting a 10:1 (v/v) mixture of Sylgard 184 elastomer and curing agent (Dow Corning) against a patterned silicon master. After curing the stamps at 60°C overnight, they were peeled off from the master before using. The individually cut out stamps were inked with the solution containing the azobenzene compounds for 10 mins after which they were dried with a stream of N_2 gas. These were then stamped on the substrate for 10 mins with 15 g weight on top. The substrate was then briefly washed with water and dried. Backfilling was done by incubating with a 1 mM Azo-PEG5000 solutions for 5 mins. Finally, the protein or bacteria were immobilized by incubating them on the substrate for 10 mins.

Bacterial immobilization

The bacterial strains ORN178 and ORN208 (kind gift from Prof. Luc Brunsveld, TU/e) were grown overnight in LB media using tetracycline as the selective antibiotic. These were then spun down at 5000g for 10 mins and the supernatant was discarded. The bacteria were

washed twice with 10 mM HEPES, 137 mM NaCl, pH 7 buffer by centrifugation and resuspension. Finally the bacteria were reconstituted in this buffer and their optical density at 600 nm was measured. For QCM and microcontact printing, the bacterial solutions were then diluted down to an O.D. of 0.1 and $MnCl_2$, $CaCl_2$ were added to final concentration of 1 mM each.

Fluorescence studies

An Olympus microscope IX71 with appropriate filters were used for recording fluorescent images.

ConA was conjugated with fluorescein using 5-carboxyfluorescein, succinimidyl ester (5-FAM) from Molecular Probes Europe. 5-FAM was added to a 1 mg/mL solution of ConA in pH 9 carbonate buffer to obtain a final dye concentration of 200 μM. This was incubated at room temperature for 60 mins with occasional shaking. The dye-conjugated protein was then separated from unreacted dye and rebuffered into the above mentioned buffer using Zeba desalting spin column with a cutoff of 7 kDa. ConA was conjugated with Cy3 dye from GE Healthcare using a similar protocol.

Surface immobilized bacteria were stained with a 1 μg/mL Hoechst (H33342) solution for 5 mins after which they were washed, dried and viewed under the microscope.

UV irradiation

UV irradiation was performed using a Spectroline miniature ultraviolet pencil lamp (model 36-380) having an average intensity of 1000 μW/cm^2 of 365 nm radiation at 1in. The lamp was always placed at approximately 3 cm away from the sample in a dark box. Subsequent washing and drying of the substrates was always carried out in the dark. UV irradiation of all dry patterned substrates, irrespective of what dye was used, did not diminish the fluorescence intensity, confirming that photo-bleaching of the dye did not occur.

QCM study of bacterial surface immobilization

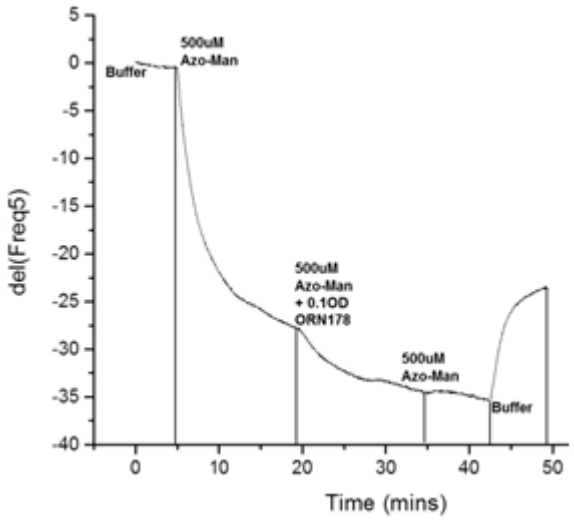


Fig. S10: (a) QCM response curve (ΔFrequency of 5th harmonic) for bacterial surface immobilization. β-CD SAM on gold was initially exposed to a solution of Azo-Man (500 μM) followed by flowing of ORN178 (in the presence of Azo-Man), which produced a clear change in frequency, which remained

unchanged on switching to Azo-Man only. Changing to a flow of buffer showed a change in frequency that is indicative of stable binding of bacteria to the supramolecular surface.

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