Supporting Information

Functionalizing the glycocalyx of living cells with supramolecular guest ligands for cucurbit[8]uril-mediated assembly

Emanuela Cavatorta,† Mark L. Verheijden,† Wies van Roosmalen, Jens Voskuhl, Jurriaan Huskens* and Pascal Jonkheijm*

Molecular Nanofabrication Group, MESA+ Institute for Nanotechnology, Department of Science and Technology, University of Twente, P. O. Box 217, 7500 AE, Enschede, The Netherlands. *Corresponding authors: E-mail: j.huskens@utwente.nl and p.jonkheijm@utwente.nl

General methods

Chemicals were purchased from Sigma Aldrich or from Acros Organics unless differently specified and used without further purification. The purity of each batch of cucurbit[8]uril (Sigma Aldrich) was assessed by UV-Vis titration with cobaltocenium according to a literature procedure.[S1] Dulbecco's Phosphate Buffered Saline (PBS, Sigma Aldrich) with pH 7.4 at 25 °C containing 0.01 M phosphate buffer, 0.0027 M potassium chloride and 0.137 M sodium chloride was used. TR was purchased from Invitrogen and all other lipids as well as polycarbonate membranes were purchased from Avanti Polar Lipids. Cell staining reagents were purchased from Invitrogen. FluoBCN was purchased from Synaffix. Reactions were monitored using thin-layer chromatography (TLC), which was performed on 0.2 mm Merck precoated silica gel 60 F254 aluminum sheets. Spots were visualized using a basic $KMnO_4$ solution unless otherwise specified. Column chromatography was carried out on silica gel 60 (0.063-0.2 mm, Merck). NMR spectra were recorded on a Bruker spectrometer (Ascend 400). 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). High-resolution mass spectrometry (HRMS) was performed using a Waters ESI(+)-ToF spectrometer (Micromass LCT) calibrated on the molecular ion of reserpine. The elemental analysis was performed on an Interscience analyzer (Flash 2000 CHN). Zeta potentials of lipid vesicles (0.5 mg/ml in 0.5x PBS) were measured in folded capillary cells at 25 ºC on a Malvern Instrument system (Zetasizer NanoZS) using a laser with a wavelength of 633 nm and backscatter detection at an angle of 173°. Fluorescence microscopy was performed using an Olympus (1X71) microscope. For confocal microscopy, a Nikon confocal (A1) microscope was used equipped with a 488 nm laser and a 500-550 nm emission filter. Flow cytometry was carried out on BD bioscience flow cytometer (FACS Aria II) equipped with a 488 nm laser. For each measurement, the flow cytometer was calibrated using a Molecular Probe (L14821, 2.5 µm) LinearFlow™ Green Flow Cytometry Intensity Calibration Kit. Fluorescence spectroscopy was performed using a Perkin Elmer fluorimeter (LS 55).

Synthesis of NphBCN

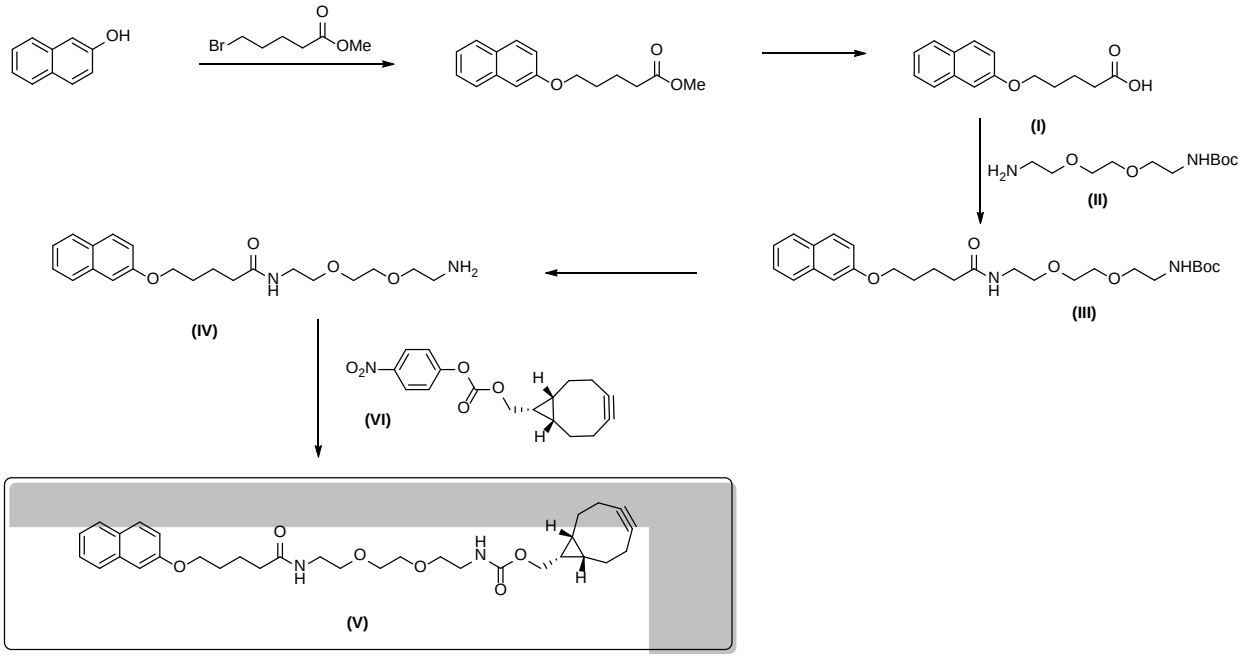
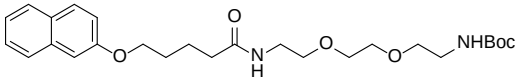


Fig. S1. Synthesis route of NphBCN (V) starting from 2-naphthol.

Compounds (I)[S2], (II)[S3] and (VI)[S4] were synthesized according to known literature procedures.

Nph-C_5-EG_3-NHBoc (III)



Compound (I) (98 mg, 0.25 mmol, 1eq.) was dissolved in dry CH_2Cl_2 (20 mL) followed by the addition under argon of EDC (0.2 mL, 1.3 mmol, 3.1 eq.), HOBt (61 mg, 0.25 mmol, 1 eq.) and a catalytic amount of DMAP (20 mg). The solution was stirred for 30 minutes followed by the addition of compound (II) (100 mg, 0.4 mmol, 1eq.) and DIPEA (0.1 mL). Stirring was continued for another 18 h at room temperature. The solution was then diluted with 50 mL of CH_2Cl_2 and extracted three times with water (20 mL). The organic layer was dried over $MgSO_4$ and the solvent was evaporated *in vacuo*. The residue was subjected to silica column chromatography (CH_2Cl_2/MeOH: 9/1). The product was obtained as brownish oil. Yield: 68 mg (58 %); ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.76-7.70 (m, 3H, 3CH); 7.42 (t, J = 7.9 Hz, 1H, CH); 7.32 (t, J = 7.9 Hz, 1H, CH); 7.14-7.12 (m, 2H, 2CH); 6.16 (s, 1H, NH); 5.00 (s, 1H, NH); 4.10 (m, 2H, CH2); 3.60-3.47 (m, 10H, 5 CH2); 3.31 (bs, 2H, CH2); 2.30 (t, J = 6.8 Hz, 2H, CH_2); 1.89 (m, 4H, $2CH_2$); 1.44 (s, 9H, $3CH_3$). ^{13}C-NMR (101 MHz, $CDCl_3$): δ = 157.10, 134.72, 129.48, 129.10, 129.02, 127.76, 126.85, 126.47, 123.64, 119.07, 109.43, 106.70, 100.98, 77.36, 70.43, 70.36, 70.31, 70.05, 67.67, 38.87, 36.07, 28.88, 28.55, 22.60.

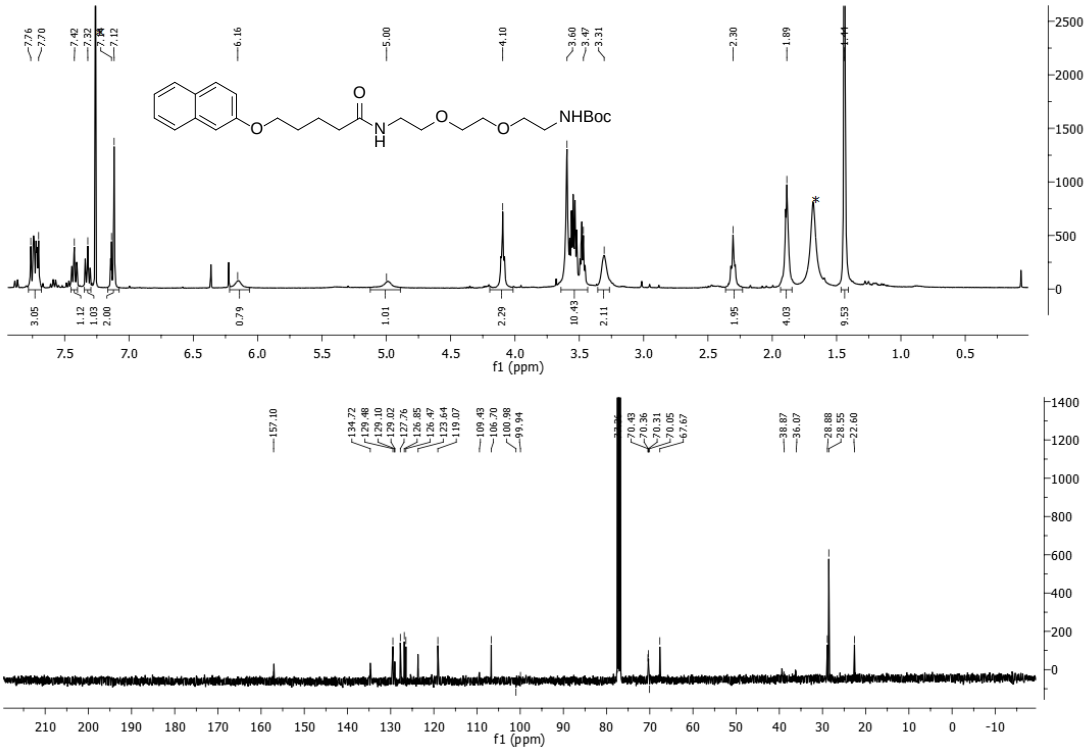
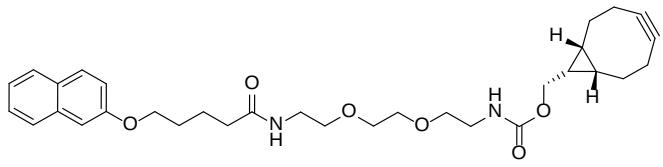


Fig. S2. ^{1}H-NMR and ^{13}C-NMR spectra of Nph-C_5-EG_3-NHBoc (III).

Nph-C_5-EG_3-NH-Cyclooctyne (NphBCN, V)



Compound (III) (100 mg, 0.19 mmol, 1 eq.) was dissolved in 5 mL of dry MeOH. The solution was cooled to 0 ºC followed by the dropwise addition under argon of acetylchloride (0.05 mL). Stirring was continued at room temperature for another 3 h. Completion of the reaction was monitored using TLC. After that time all solvent was evaporated and the product was obtained as brown oil. The product (IV) was used without further purification in the next step. Compound (IV) (50 mg, 0.13 mmol, 1.3 eq.) was dissolved in 5 mL of DMF followed by the addition of NEt_3 (0.1 mL). After stirring for 5 minutes, (VI) (30 mg, 0.10 mmol, 1 eq.) dissolved in 2 mL of DMF was added dropwise. The solution was stirred at room temperature was continued for 24 h followed by evaporation of all solvent *in vacuo*. The residue was purified by silica column chromatography (CH_2Cl_2/MeOH: 9/1). The desired product was obtained as pale yellow oil. Yield: 33 mg, (60%); ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.70-7.76 (m, 3H, 3CH); 7.42 (t, J = 7.8 Hz, 1H, CH); 7.32 (t, J = 7.8 Hz, 1H, CH); 7.11-7.14 (m, 2H, 2CH); 6.12 (s, 1H, NH); 5.18 (s, 1H, NH); 4.14 (d, J = 7.9 Hz, 2H, CH_2); 4.09 (bs, 2H, CH_2); 3.48-3.59 (m, 10H, 5 CH_2); 3.36 (bs, 2H, CH2); 2.21-2.30 (m, 8H, 4CH_2); 1.88 (s, 4H, CH_2); 1.52-1.58 (m, 2H, CH_2); 1.33 (q, J = 8.5 Hz, 1H, CH); 0.87-0.83 (m, 2H, CH_2). ^{13}C-NMR (101 MHz, $CDCl_3$): δ = 172.87; 156.99; 156.92; 134.68; 129.47; 129.02; 127.73; 126.82; 126.46; 123.67; 119.01; 106.67; 98.91; 70.39; 69.67; 67.66; 67.39; 65.97; 63.72; 62.97; 61.78; 40.87; 40.13; 39.35; 38.80; 38.27; 37.15; 36.36; 30.37; 29.14; 28.86; 26.77; 22.59; 21.52; 20.22; 17.86; 15.39. Traces of ethyl acetate are present in the ^{1}H-NMR and ^{13}C-NMR. HRMS $[M+Na]^+$ = 573.2936 (calc. 573.2941), $[M+K]^+$ = 589.2888 (calc. 589.2680), $[M+2ACN+H]^+$ = 655.2214 (calc. 655.3471).

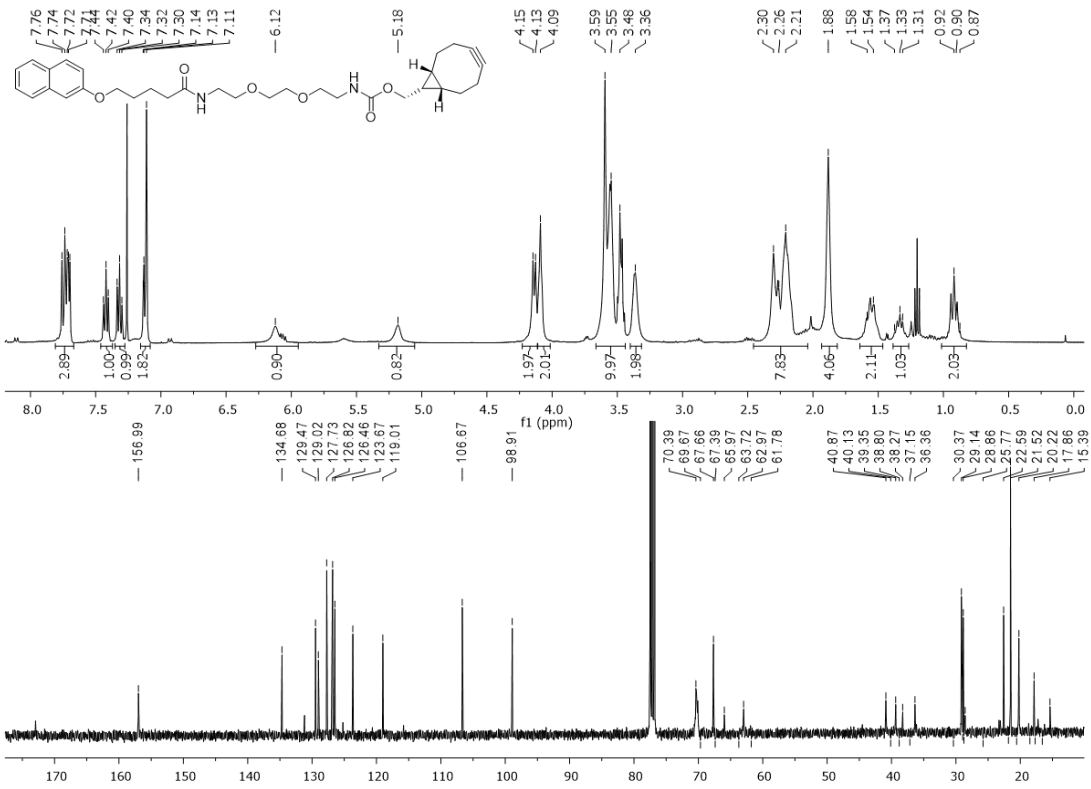


Fig. S3. ^{1}H-NMR and ^{13}C-NMR spectra of NphBCN (V). Traces of ethyl acetate are present.

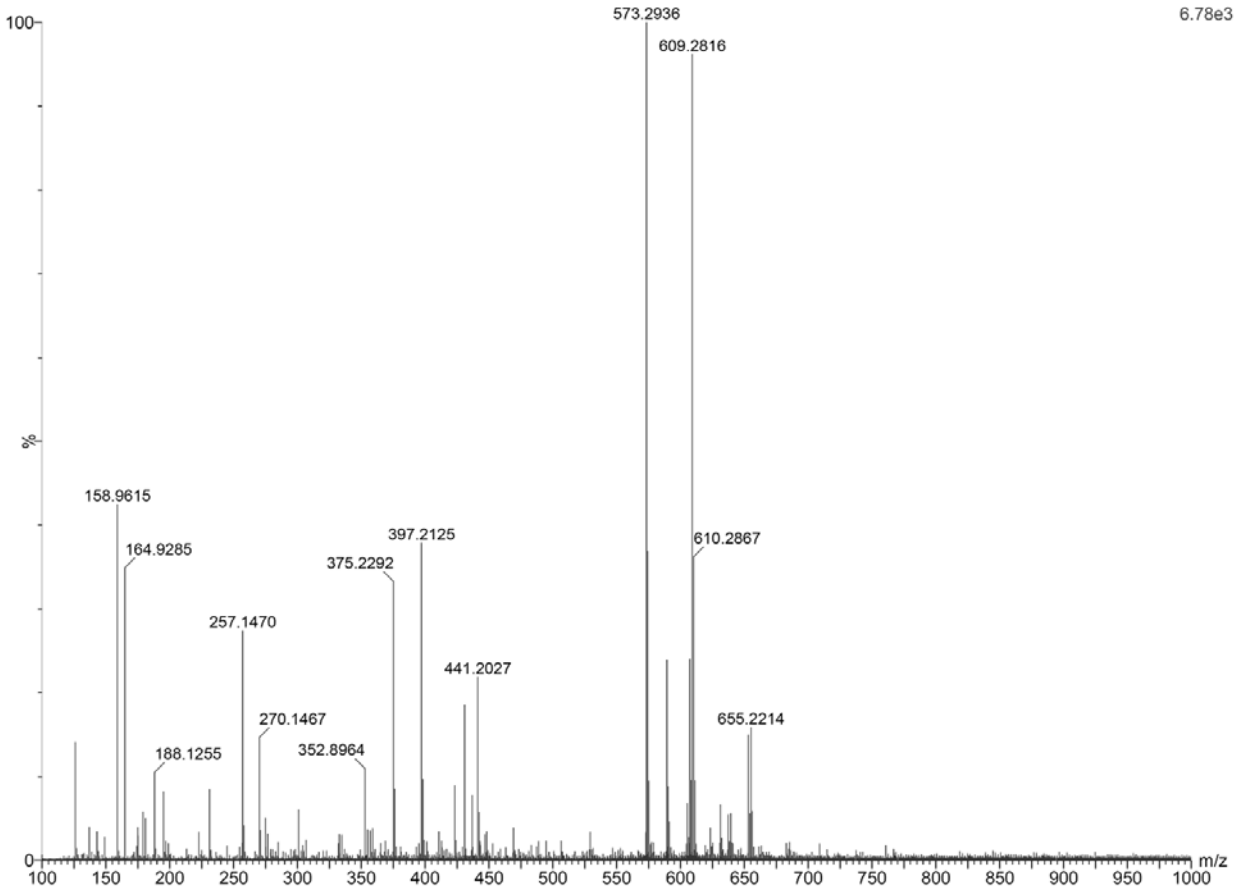
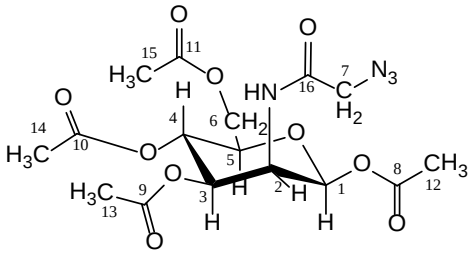


Fig. S4. ToF-ESI(+) HRMS spectra of NphBCN (V). $[M+Na]^+$ = 573.2936 (calc. 573.2941), $[M+K]^+$ = 589.2888 (calc. 589.2680), $[M+2ACN+H]^+$ = 655.2214 (calc. 655.3471). Some minor peaks in the HRMS were assigned to fragments of V formed by rupture of the bonds of the carbamate group: $[M-C_{10}H_{12}O+Na]^+$ = 441.20 (calc. 441.20), $[M-C_{11}H_{12}O_2+H]^+$ = 375.23 (calc. 375.23), $[M-C_{11}H_{12}O_2+Na]^+$ = 397.21 (calc. 397.21), $[M-C_{11}H_{12}O_2+2H]^{2+}$ = 188.13 (calc. 188.12). Other minor peaks were due to the solvents: [sodium trifluoroacetate+Na]$^+$ = 158.96 (calc. 158.96), [3xDMSO+Na]$^+$ = 257.15 (calc. 257.02). Reserpine molecular ion $[M+H]^+$ was used as a standard and set at 609.2816 m/z.

Synthesis of MAN



The procedure from Laughlin and Bertozzi[4] for the synthesis of peracetylated N-azidoacetylmannosamine was used. For TLC, spots were visualized by treatment with 0.05 M solution of triphenylphosphine in toluene and subsequent staining with a 0.08 M ninhydrin solution in 1-butanol containing 0.5 M acetic acid. Yield: 91 mg (6.6%), white wax. ^{1}H-NMR: (400MHz, $CDCl_3$) δ = 2.00 (s, 3H), 2.06 (s, 3H), 2.11 (s, 3H), 2.12 (s, 3H), 3.83-3.87 (ddd, 1H, $J_{5,6a}$ = 2.4, $J_{5,6b}$ = 4.5, $J_{5,4}$ = 9.4), 4.12-4.16 (dd, 1H, $J_{6a,5}$ = 2.4, $J_{6a,6b}$ =12.0), 4.16 (s, 2H), 4.23-4.27 (dd, 1H, $J_{6b,5}$ = 4.5, $J_{6b,6a}$ = 12.0), 4.72-4.75 (ddd, 1H, $J_{2,3}$ = 1.4, $J_{2,NH}$ = 3.6, $J_{2,1}$ = 9.0), 5.14-5.19 (app t, 1H, $J_{4,5}$ = 9.4), 5.91 (d, 1H, $J_{3,2}$ = 1.4), 6.85-6.88 (d, 1H, $J_{1,2}$= 9.0). ^{13}C-NMR: (101MHz, $CDCl_3$): δ = 20.6, 20.6, 20.6, 20.7, 50.1, 52.3, 61.7, 64.9, 71.4, 73.3, 90.1, 168.4, 168.7, 169.8, 170.3, 170.8. Minor peaks in the ^{1}H-NMR and ^{13}C-NMR are attributed to the anomeric isomer. HRMS $[M+Na]^+$ = 453.1248 (calc. 453.1234), $[M+K]^+$ = 469.0982 (calc. 469.0973), $[2M+Na]^+$ = 883.2366 (calc. 883.2570), $[2M+K]^+$ = 899.2091 (calc. 899.2309). IR: 2100 cm^{-1} ν_{as} N_3 (strong). Anal. Calcd. for $C_{16}H_{22}N_4O_{10}$: C 44.65, H 5.15, N 13.02; found: C 43.34, H 4.88, N 12.33.

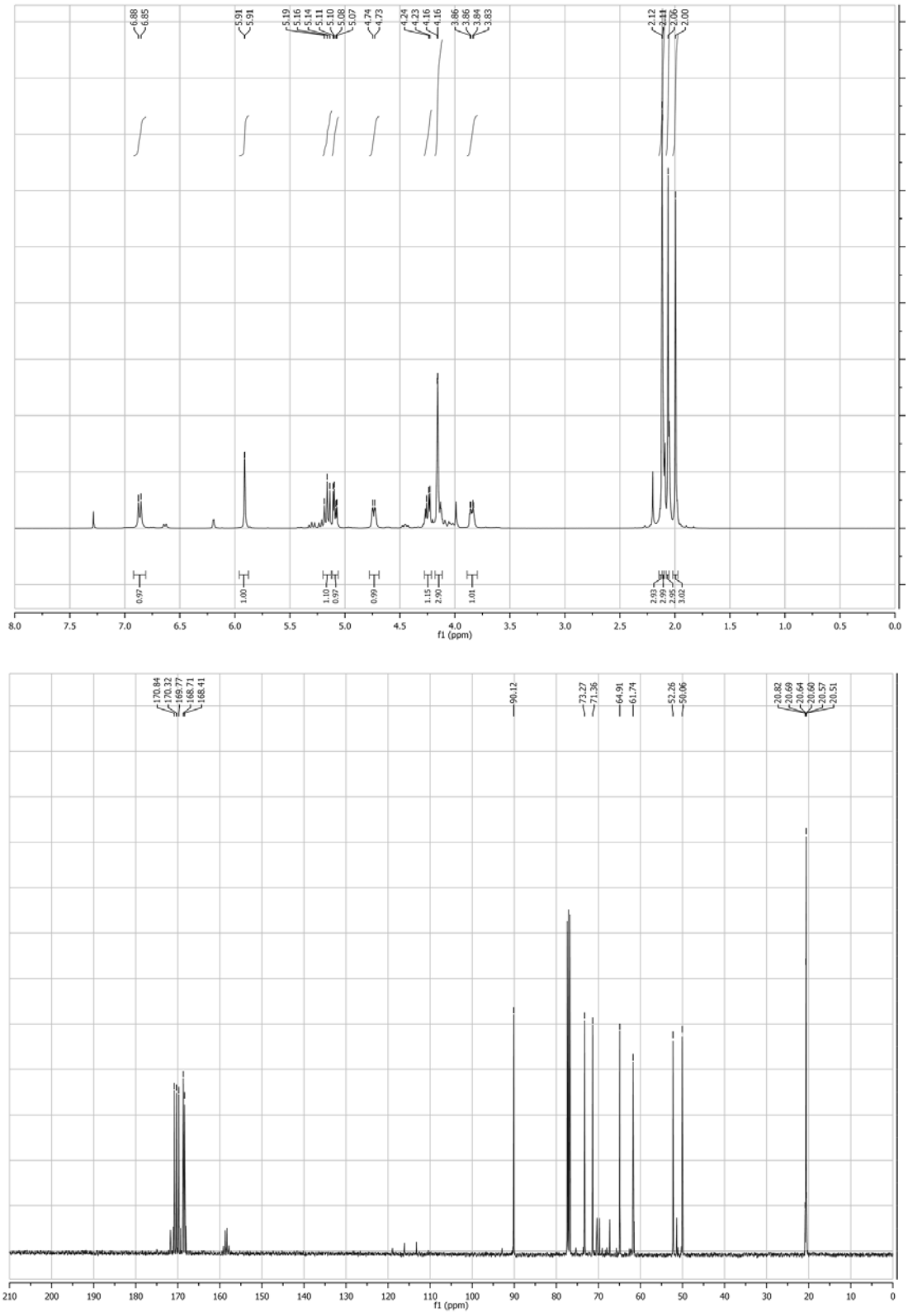


Fig. S5. ^{1}H-NMR and ^{13}C-NMR spectra of MAN. Minor peaks are attributed to the anomeric isomer.

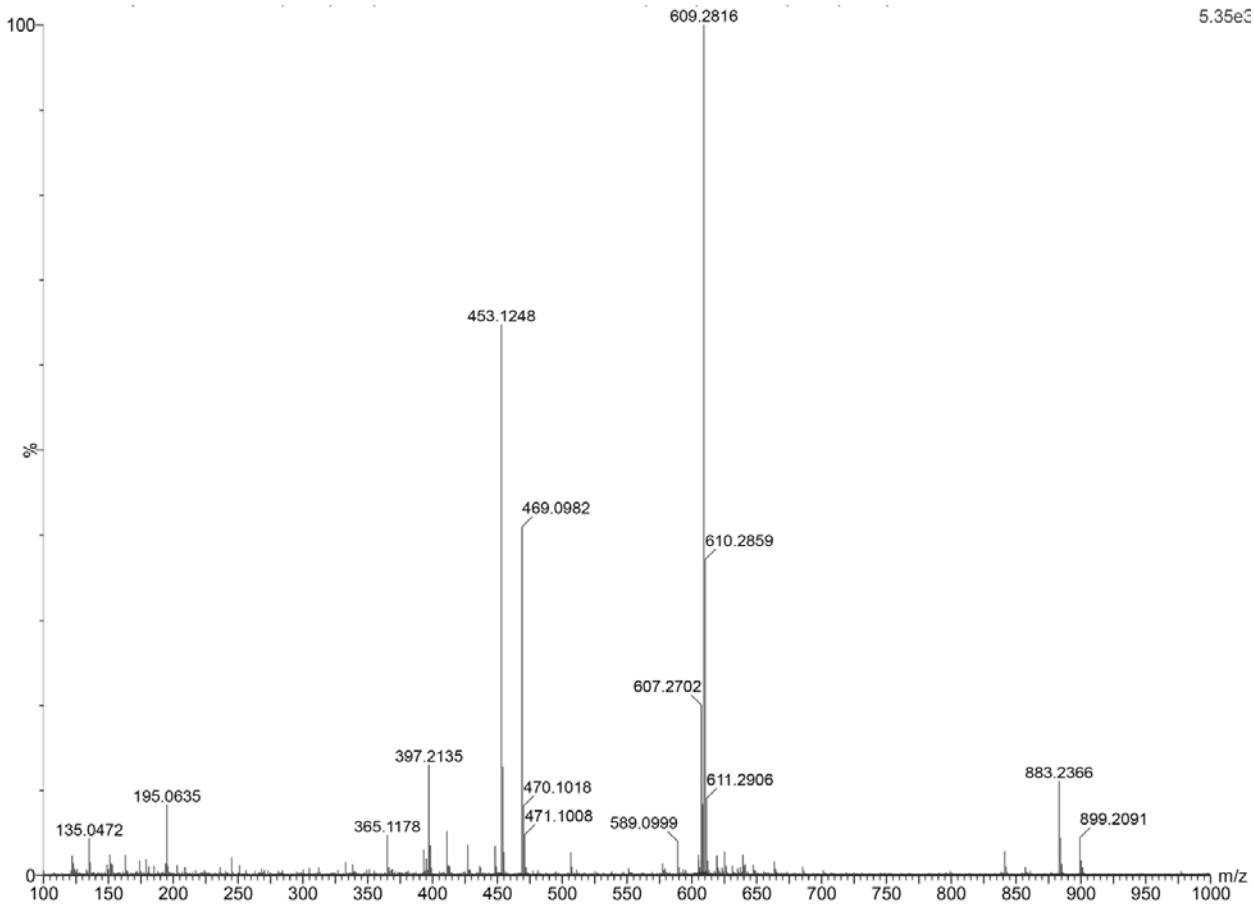


Fig. S6. ToF-ESI(+) HRMS spectra of MAN. Reserpine molecular ion $[M+H]^+$ was used as a standard and set at 609.2816 m/z.

Synthesis MV

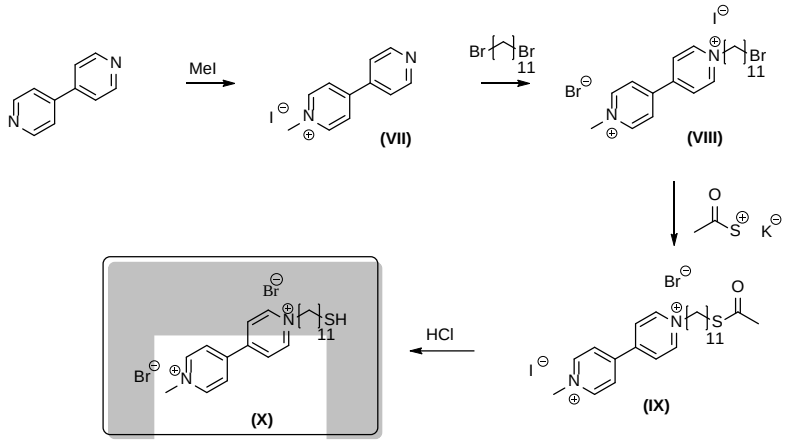


Fig. S7. Synthesis route of viologen derivative MV (X).

Synthesis of compounds (VII), (VIII) and (IX) was adapted from Rauwald, et al.[S5] and González-Campo, et al.[S2]

1-Methyl-4,4'-bipyridinium iodide (VII)

4,4'-bipyridine (5.0 g, 32 mmol) was dissolved in DCM (75 ml). Methyl iodide (2.5 ml, 40.5 mmol) was dissolved in DCM (25 ml) and this solution was added dropwise to the refluxing solution while stirring. Upon addition, the colorless solution turned into an orange suspension. The suspension was refluxed for 1 hour and allowed to cool down overnight while stirring. The product was filtered and washed with DCM. The residue was recrystallized from methanol. The product was filtered and washed with diethyl ether, yielding a yellow, crystalline solid. Compound (VII) was used for the following synthetic step without further characterization.

12-Bromo-1-(methyl-4,4'-bipyridinium)-undecane (VIII)

1,11-Dibromoundecane (2.07 g, 6.6 mmol) was dissolved in acetonitrile (15 ml) and DMF (3 ml). Compound VII (500 mg, 1.68 mmol) was dissolved separately in a warm mixture of acetonitrile (10 ml) and DMF (2 ml) and added dropwise to the refluxing solution while stirring. Stirring and refluxing conditions were continued for 48 h. The lukewarm solution was filtrated and the residue was washed with acetonitrile. Ether was slowly added to the clear filtrate while stirring. The product was collected by centrifugation (10 minutes at 7000 rpm) and decantation, and dried. Recrystallization from methanol and acetonitrile and drying *in vacuo* yielded an orange crystalline solid. Compound (VIII) was used for the following synthetic step without further characterization.

1-methyl-4,4'-bipyridinium-undecanethioacetate (IX)

Compound VIII (226 mg, 0.4 mmol) was dissolved in a mixture of Milli-Q (12.5 ml) and ethanol (5 ml). potassium thioacetate (114 mg, 1.0 mmol) was dissolved in Milli-Q (12.5 ml) and ethanol (5 ml) and added to the refluxing solution while stirring. The reaction mixture was kept under N_2 and refluxed for 48 h resulting in a light green-blue solution. NH_4PF_6 (0.26 g, 1.6 mmol) was added to the reaction mixture. The crème-white precipitate was filtered under vacuum and redissolved in acetonitrile. Any brown, solids were removed by filtration. The product was again precipitated by adding tetrabutylammonium chloride (0.44 g, 1.6 mmol) resulting in a white precipitate. The product was filtered under vacuum and further dried *in vacuo*. The product was obtained as a pale yellow powder. Compound (IX) was used for the following synthetic step without further characterization.

1-Methyl-4,4'-bipyridinium-undecanethiol (MV, X)

Compound IX (44 mg, 72 µmol) was dissolved in the 1.25 M HCl solution in methanol (0.58 ml) while stirring. The mixture was refluxed overnight. The product was dried *in vacuo* and then dissolved in Milli-Q water (25 ml). The product was precipitated by adding NH_4PF_6 (0.53 g, 3.25 mmol). After vacuum filtration, the residue was dissolved in acetonitrile (10ml) and again precipitated by adding tetrabutylammonium bromide (1.08 g, 3.25 mmol). Vacuum filtration and further drying *in vacuo* yielded the final product as a yellow-green solid. Yield: 19 mg (51%). ^{1}H-NMR (400 MHz, D_2O): δ = 9.12-9.05 (dd, 4H, CH); 8.56-8.52 (m, 4H, CH); 4.72 (t, 2H, CH_2), 4.50 (s, 3H, CH_3); 2.71 (t, 2H, CH_2), 2.08 (m, 2H, CH_2), 1.63 (m, 2H, CH_2), 1.36-1.26 (m, 14H, CH_2). ^{13}C-NMR: (150 MHz, D_2O): δ = 23.74-38.45, 48.34, 62.21, 126.60, 126.93, 145.41, 146.31, 149.93 HRMS $[M^{2+}\text{-}H]^+$ = 357.2354 (calc. 357.2354), $[M^{2+}\text{-}C_{11}H_{22}SH]^+$ = 171.0896 (calc.171.0917).

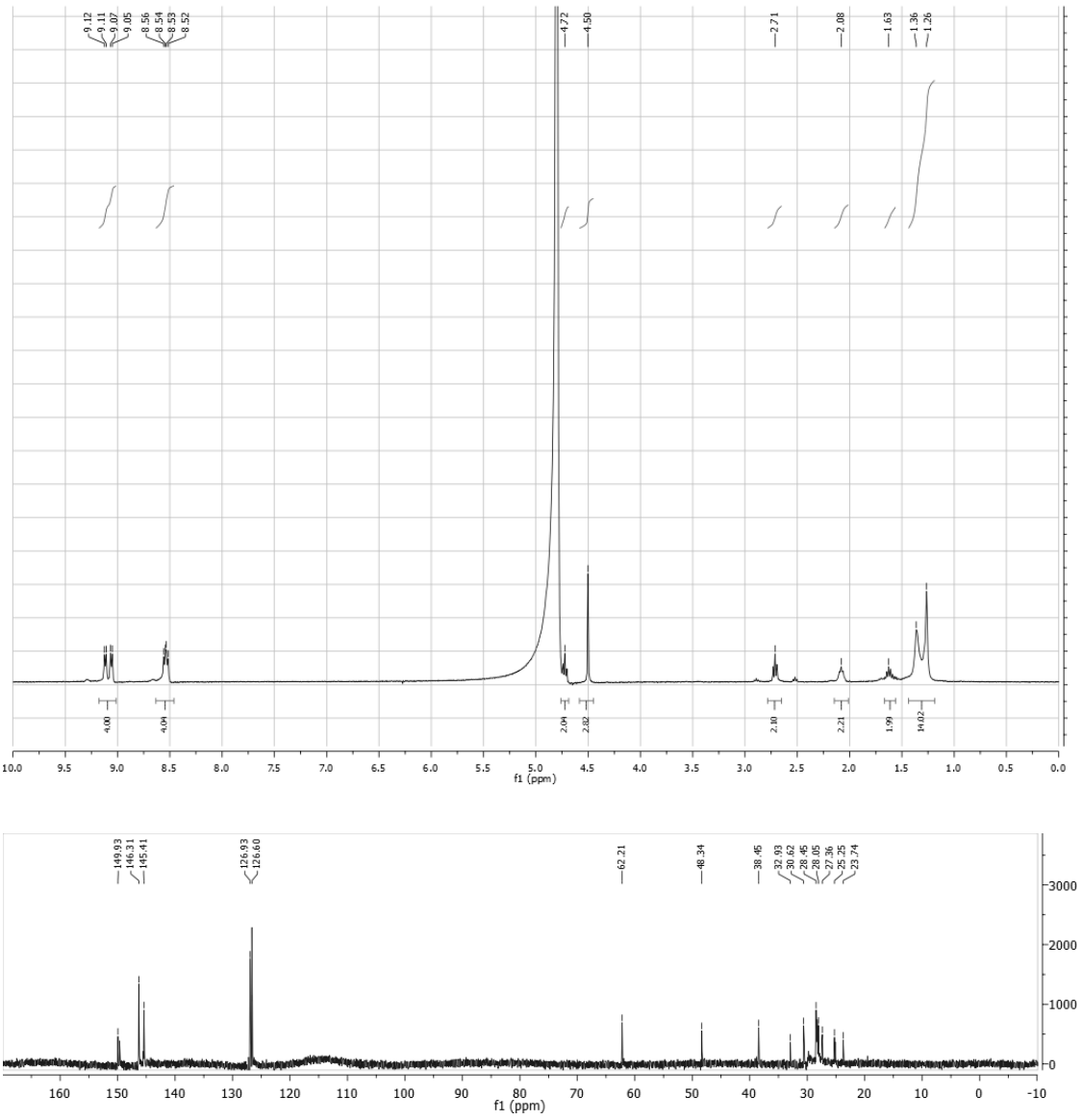


Fig. S8. ^{1}H-NMR and ^{13}C-NMR spectra of MV (X).

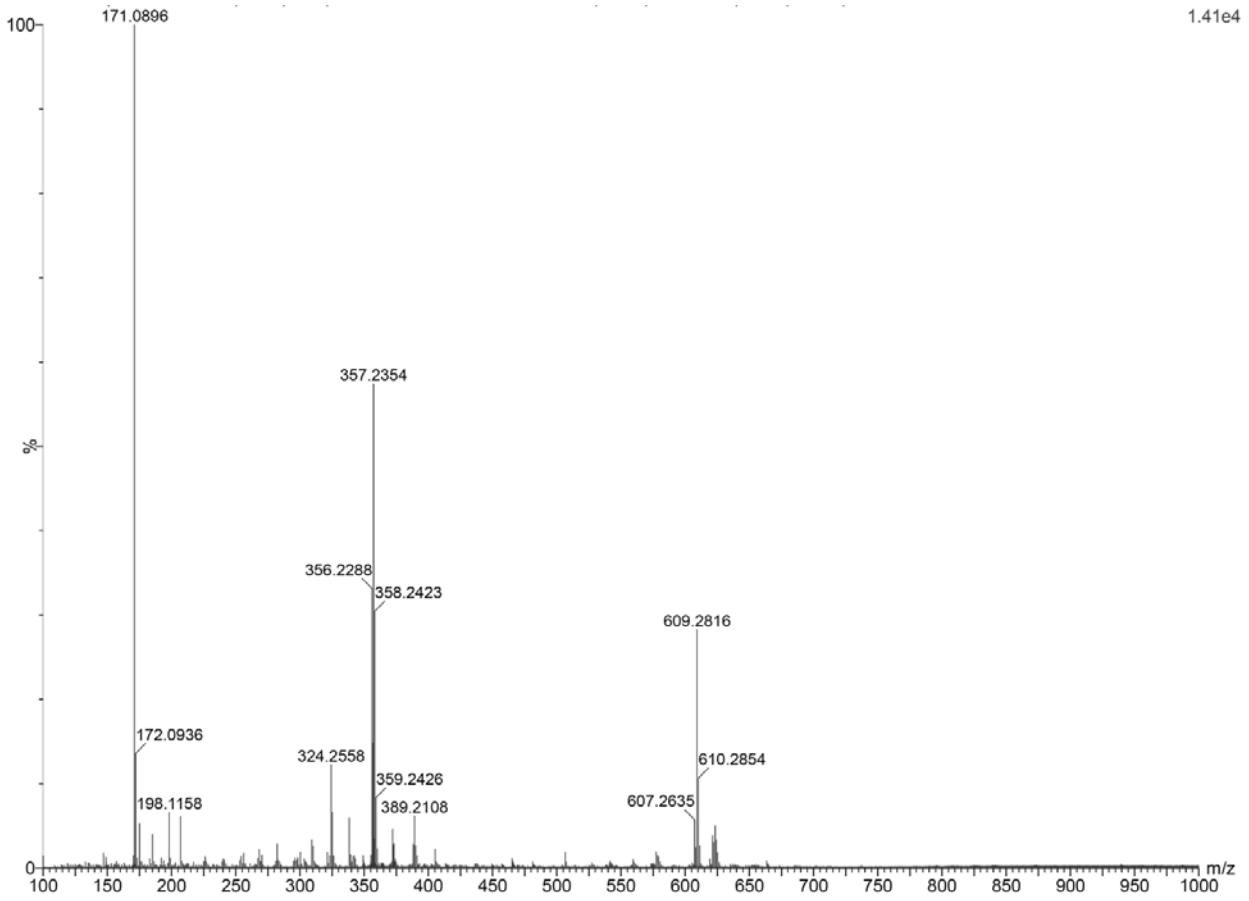


Fig. S9. ToF-ESI(+) HRMS spectra of MV (X). Reserpine molecular ion $[M+H]^+$ was used as a standard and set at 609.2816 m/z.

Cell culture

Jurkat cells were cultured in RPMI medium with 10% fetal bovine serum at a density of 10^5 - 10^6 cells/ml at standard cell culture conditions (37 ºC with 5% CO_2). Cells between passages 11 and 25 were used. For metabolic labeling of cells, 2.5 10^6 cells in 10 ml of the same culture medium were incubated with MAN in the incubator for 3 days and then washed with PBS three times. After that 10^6 cells per condition were allowed to react for 1 h in the incubator with NphBCN (5% DMSO in PBS) or fluoBCN (PBS) depending on the experiments. Finally cells were washed with PBS three times. Cells for negative controls were treated according to the same procedures but replacing the addition of only solvents as described in the main text.

The viability of cells was tested for a range of concentrations of DMSO and CB[8]. The concentrations of 5% DMSO and 20 µM CB[8] were found to be optimal. The staining experiments were conducted according to standard procedures of the LIVE/DEAD Cell Viability assay from Invitrogen using fluorescence microscopy. Green color (λ_{ex} = 460-490 nm and λ_{em} = 525 nm) corresponds to live cells and red color (λ_{ex} = 510-550 nm and λ_{em} = 590-LP) corresponds dead cells.

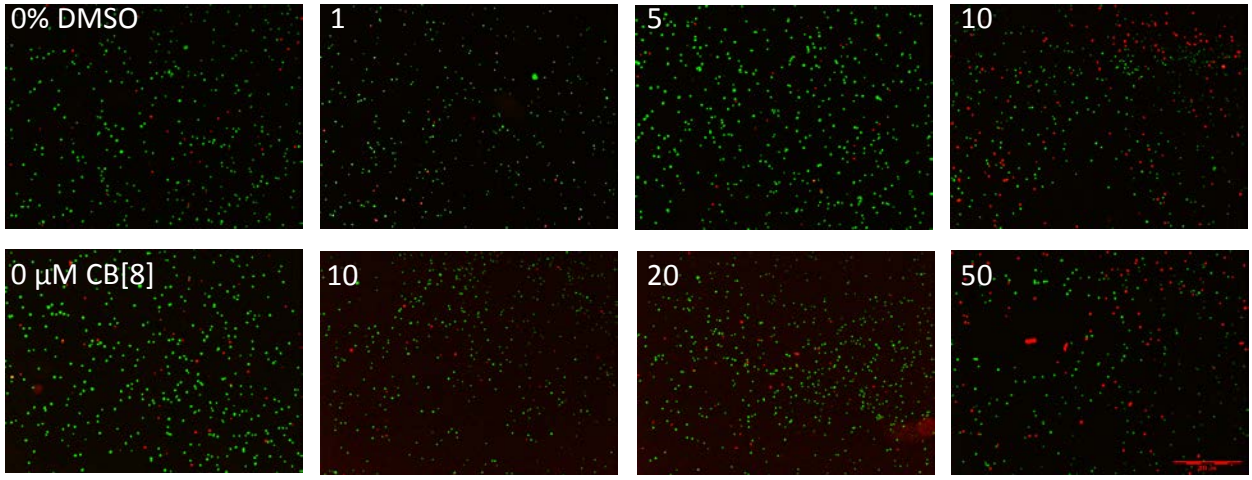


Fig. S10. Live-dead assay of Jurkat cells. Cells were exposed for 1 h in the incubator to percentages of DMSO between 0 to 10% (top panel) and of CB[8] between 0 and 50 µM in PBS (bottom panel). Scale bar 200 µm.

Analysis of Jurkat cell surface functionalization

Localization of reacted BCN-fluo by confocal microscopy

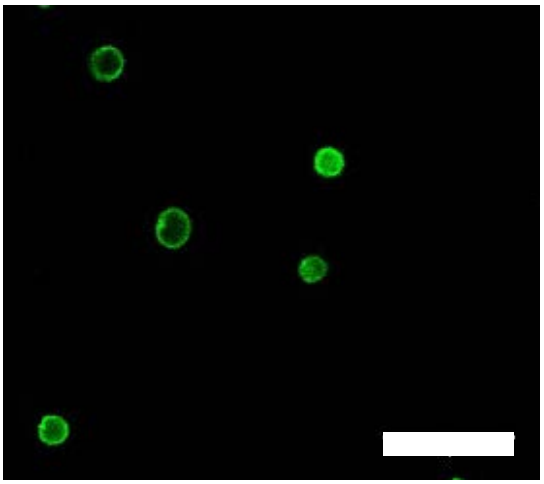


Fig. S11. Representative confocal image of fluoBCN labeled Jurkat cells Jurkat cells were imaged after 3 day incubation with 50 µM MAN and subsequent SPAAC with fluoBCN (scale bar 50 µm).

Flow cytometry

Samples of cells (10^6 cells/ml, 1 ml) were loaded in the flow cytometer after brief vortexing and each measurements was set to count a total of 10^5 cells. For all experiments the fluorescence intensity was calibrated with commercial beads. The flow cytometer was operated with a 488 nm laser and emission was detected at 530 nm.

Turnover of functional groups at the cell surface

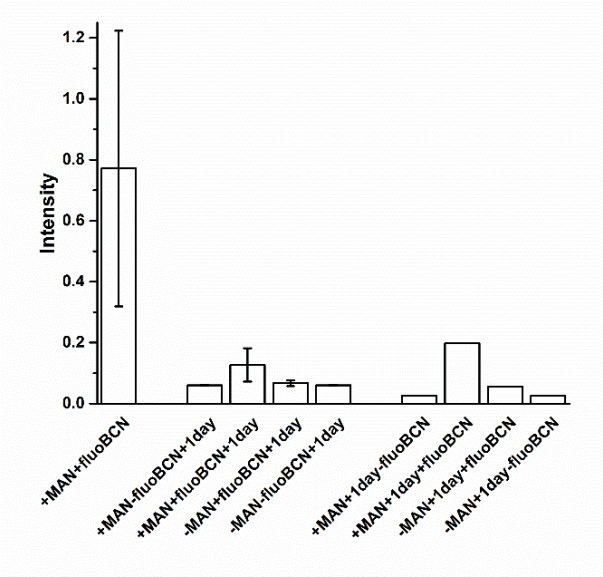


Fig. S12. Turnover study of azide (functional group of MAN) and of fluoBCN groups at the cell surface by flow cytometry. Turnover of azide (+MAN+1day+fluoBCN, n=1) was tested by introducing 1 day of cell incubation in medium between the metabolic labeling and the click reaction. Turnover of fluoBCN (+MAN+fluoBCN+1day, n=2) was tested by introducing 1 day of cell incubation in medium after the click reaction. For both turnover experiments the corresponding negative controls are presented as well.

Tunable coverage of Jurkat cells

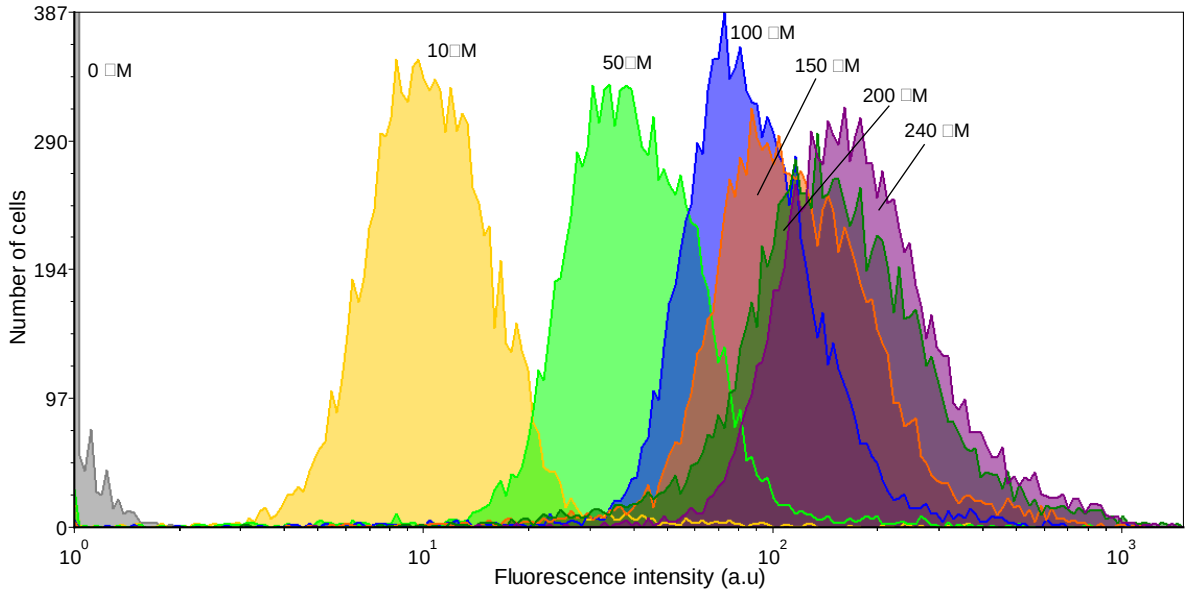


Fig. S13. Flow cytometry results for Jurkat cells labeled with fluoBCN. Concentrations indicated in the figure refer to concentration of fluoBCN used in the strain promoted click reaction. Concentration of MAN was 50µM in all conditions.

Determining cell coverage

Cells were labeled with fluoBCN. Their mean fluorescence was measured by flow cytometry. For each measurement, standard beads with various coverages of fluorescein were also measured. The average number of fluorescein molecules per cell can be calculated by quantifying the amount of fluorescein molecules per bead via a calibration obtained by fluorescence spectroscopy. Here, an example calculation is given for cells labeled with 50 µM of MAN and 48 µM of fluoBCN.

First, a calibration curve of known concentrations of fluorescein in Milli-Q water was made using a fluorescence spectrophotometer, see Fig. S14a.

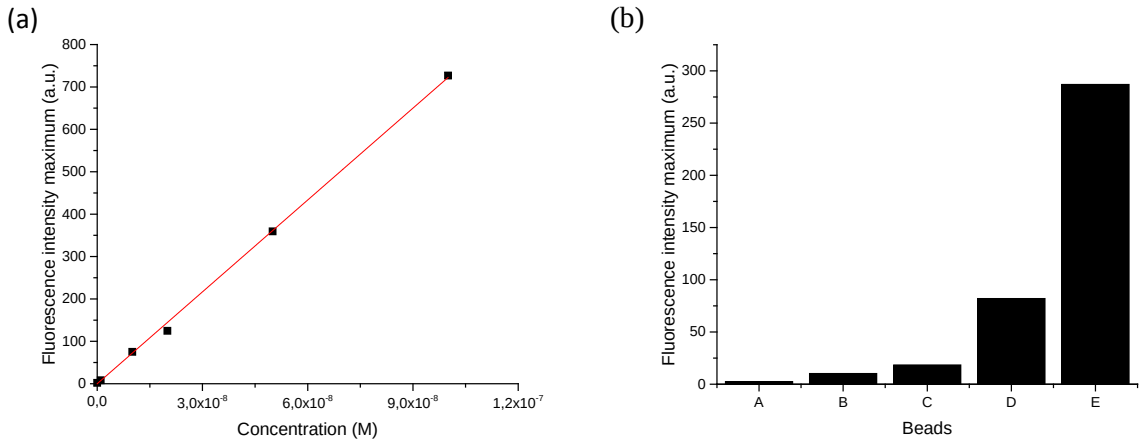


Fig. S14. (a) Fluorescence spectroscopy calibration curve of fluorescein solutions at concentrations between 0 and 0.1 µM constructed with the maximum intensity of their emission spectra. (b) Maximum intensities of the emission spectra of samples of commercial beads provided with a range of coverages of fluorescein. For beads A to F, the coverage of fluorescein at the surface of the beads increases. Beads F were not included for this calibration because the intensity was too high to measure with our fluorescence spectrophotometer.

Linear fitting presented in Fig. S14a results in:

$$C_{fluorescein}(M) = 1.38 * 10^{-10} * I_{max,fluorescence} \quad (1.1)$$

Similarly, fluorescence spectra were measured for a series of commercial beads with a range of coverages of fluorescein, see Fig. S14b**.** The intensity values obtained from the bead series were converted to fluorescein concentration using equation (1.1). This resulted in a known concentration of fluorescein in each sample of beads. The possible influences of scattering of the beads or fluorescence quenching due to proximity of dye molecules at the beads surface were assumed to be negligible.

The same set of beads was measured by flow cytometry as well. This resulted in Fig. S15, giving a mean intensity for each type of beads. The values of mean intensities from flow cytometry were correlated to the corresponding intensity measured by fluorescence spectroscopy as follows.

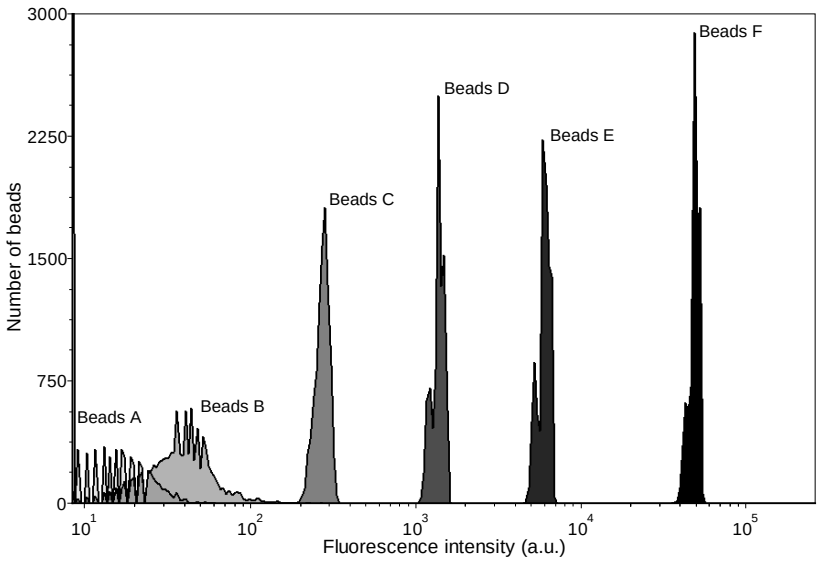


Fig. S15. Fluorescence intensity obtained with flow cytometry for a commercially available set of fluorescein labelled beads.

Each stock solution of beads had a given density of $6 \cdot 10^7$ beads/ml. For fluorescence spectroscopy, samples were prepared by adding one droplet of the sonicated bead solution in 2 ml of PBS. The volume of the droplet was determined to be 38 ± 1 µl (standard deviation, n=3) resulting in a final concentration of $1.1 \cdot 10^6$ beads/ml. With the concentration of fluorescein as well as the density of beads known, the coverages (number of fluorescein molecules/beads) of the different beads were determined.

Combining these calculated coverages of the beads, with their mean intensities from representative flow cytometry experiments, resulted in Fig. S16.

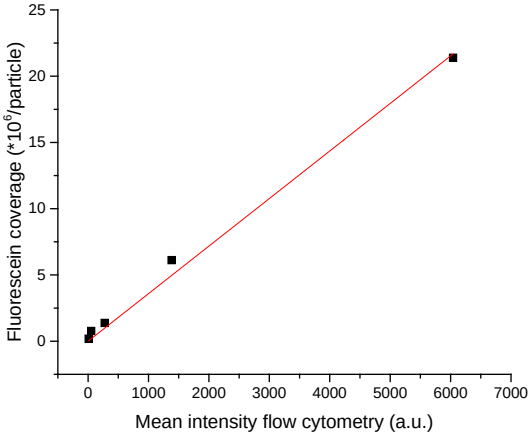


Fig. S16. Fluorescein coverage of fluorescent beads A-E as a function of their flow cytometry mean intensity. Coverages were calculated as described in the text.

Linear fitting presented in Fig. S16 results in:

$$\theta\ (\ 10^6 molecules/particle) = 3.59 * 10^{-3} * I_{FlowCytometry} \qquad (1.2)$$

Equation (1.2) provides the average fluorescein coverage of the cells. For the cell experiment corresponding to the measurement reported in Fig. S16, the +MAN+fluoBCN sample had a mean intensity

of 317.5, giving a coverage of $1.1*10^6$ (fluorescein molecules/cell). This is in the same order of magnitude as values obtained in similar studies using metabolic labeling.[S6]

Vesicle preparation

1,2-Dioleoyl-sn-glycero-3-phosphocholine (DOPC) was stored as a 10 mg/mL stock solution in chloroform at -20°C and used as the main constituent in SLBs. 1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine-N-[4-(p-maleimidomethyl)cyclohexane-carboxamide] (MCC) was stored as a 2 mg/mL stock in chloroform at -20 ºC. The lipid-dye conjugate, Texas Red-1,2-dihexadecanoyl-sn-glycero-3-phosphoethanolamine (TR) was stored as a 1 mg/mL stock solution in methanol at -20 ºC. The dissolved lipids were mixed in a molar ratio DOPC/MCC/TR of 99.3/0.5/0.2 and dried under a flow of nitrogen in a glass vial in order to create a film of lipid material at the glass wall. This film was further dried under vacuum for at least 1 h and subsequently hydrated by vortexing in MilliQ water to form multilamellar vesicles (MLVs) at 1 mg/mL. In the case of methylviologen functionalized vesicles, 100 µM of thiol-functionalized methylviologen in PBS was used for the hydration step and the reaction was carried out for 2 h. The lipid suspension was extruded 11 times through a polycarbonate membrane with 100 nm pore size resulting in large unilamellar vesicles (LUVs) as confirmed by DLS measurements (Fig. S17). The resulting LUVs were kept at 4 ºC and used within two weeks or within one day in case of the methylviologen functionalized vesicles.

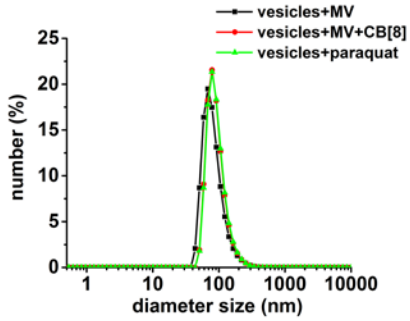


Fig. S17. Diameter size distribution as measured by DLS for vesicles of DOPC:MCC:TR (respectively 99.3, 0.5, 0.2 mol%) after reaction with MV (vesicles+MV) and addition of CB[8] (vesicles+MV+CB[8]) or after addition of paraquat (vesicles+paraquat).

Supported lipid bilayer (SLB) fabrication

SLBs were fabricated in a flow channel setup which was built as previously described.[S7] In short, the flow channel consisted of a standard glass microscope slide on which a polydimethylsiloxane (PDMS) flow channel was attached with a width of 1.5mm and a height of 50µm. A bright field microscopy overview is presented in Fig. S18. For repeated use of flow channels, the channels were rinsed with 1 wt% sodium dodecyl sulfate in MilliQ and the glass was activated for 1 h with a 1 M solution of sodium hydroxide in MilliQ. The channels were subsequently thoroughly rinsed with MilliQ. Supported lipid bilayer formation was achieved by dilution of the LUV solution to 0.1 mg/mL in PBS. Prior to LUV incubation, the channels were flushed briefly with PBS. The channels were then incubated with the vesicle suspension for at least 30 min to allow for vesicle adsorption and rupture to occur. Subsequently, the channels were washed with an excess of MilliQ water. From this point forth, care was taken to ensure no air bubbles entered the device.

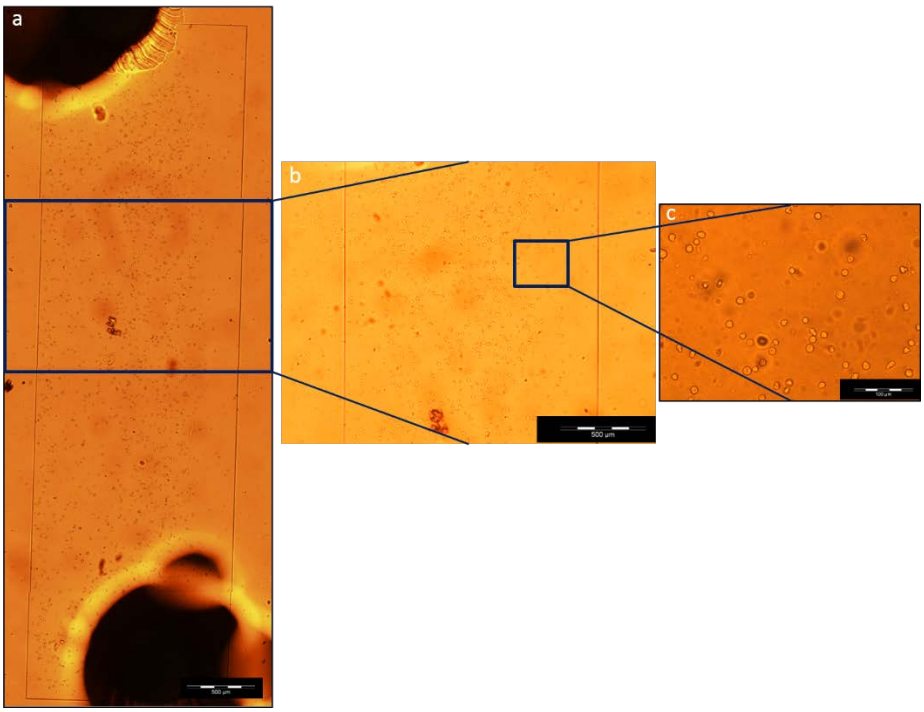


Fig. S18 (a) Stack view of the flow channel. Inlet and outlet are visible at the top and at the bottom. Cells are imaged at 4x magnification in a representative frame (b). Cells morphology is checked at 20x magnification (c). Scale bars 500 µm (a-b) and 100 µm (c).

The quality of the SLBs was checked always before and after each cell experiment via fluorescence microscopy by having a uniform red intensity from the TR present in the composition of the bilayer (Fig. S19). Moreover a negative control of untreated Jurkat cells (-MAN-fluoBCN-CB[8] in the main text) was flown on the SLB prior to using the same SLB for further experiments to further verify the consistent performance of different individual SLBs.

Fig. S19. Fluorescence images of the same SLB before and after an experiment with cells. Red colour from 0.2% TR in the SLB composition. Scale bar 100 µm.

References

S1. S. Yi and A. E. Kaifer, *J. Org. Chem.*, 2011, **76**, 10275-10278.

S2. A. González-Campo, M. Brasch, D. A. Uhlenheuer, A. Gómez-Casado, L. Yang, L. Brunsveld, J. Huskens and P. Jonkheijm, *Langmuir*, 2012, **28**, 16364-16371.

S3. R. J. Mart, K. P. Liem, X. Wang and S. J. Webb, *J. Am. Chem. Soc.*, 2006, **128**, 14462-14463.

S4. J. Dommerholt, S. Schmidt, R. Temming, L. J. A. Hendriks, F. P. J. T. Rutjes, J. C. M. Van Hest, D. J. Lefeber, P. Friedl and F. L. Van Delft, *Angew. Chem. Int. Ed.*, 2010, **49**, 9422-9425.

S5. U. Rauwald and O. A. Scherman, *Angew. Chem. Int. Ed.*, 2008, **47**, 3950-3953.

S6. Z. J. Gartner and C. R. Bertozzi, *Proc. Natl. Acad. Sci. U. S. A.*, 2009, **106**, 4606-4610.

S7. S. Sankaran, J. Van Weerd, J. Voskuhl, M. Karperien and P. Jonkheijm, *Small*, 2015, **11**, 6187-6196.