Electronic Supplementary Information

for

Precise Supramolecular Control of Surface Coverage Densities on Polymer Micro- and Nanoparticles

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1. Materials and Methods

Reagents for synthesis were from Fluka, Sigma-Aldrich, or Acros Organics NV, Belgium. Azobisisobutyronitrile (AIBN) was from Molekula GmbH, Germany. Analytical thin layer chromatography (TLC) was performed on SIL G/UV_{254} (Macherey-Nagel). Buffers and salts were of the highest purity available from Fluka, Sigma-Aldrich and used as received. Methyl methacrylate was destabilized with aluminum oxide (neutral for chromatography 50-200 μm, 60A) prior to use. Poly(styrene) nanoparticles (average diameter 110 nm) with surface carboxylic acid groups were from Kisker Biotech GmbH & Co. KG (Steinfurt, Germany). Cucurbit[7]uril (CB7) was synthesized according to established literature methods.[1] Functionalization of microspheres was carried out in standard Eppendorf plastic tubes. Concentrations of fluorescent dye stock solutions were determined using an extinction coefficient of 90800 $M^{-1}cm^{-1}$ in acetonitrile/phosphate buffer for Ada-Rho.[2]

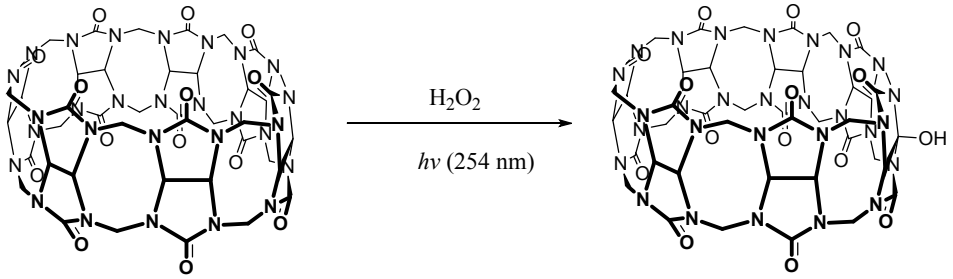
The photoreactions were carried out in a Luzchem LZC-4V photoreactor with 14 G8T5 lamps from SANKYO DENKI (six lamps from top and four lamps from each side) in a 250-mL quartz glass round bottom flask. IR spectra were recorded on a Bruker Equinox 55 equipped with an IRScope and ATR unit and are reported as wavenumbers in cm^{-1} with band intensities indicated as s (strong), m (medium), w (weak), and br (broad). ^1H spectra were recorded on a Jeol ECS400 MHz and chemical shifts (δ) are reported in ppm relative to TMS (δ = 0 ppm). ESI-MS was performed on a Bruker HCT ultra and mass spectra are reported as mass-per-charge ratio m/z (intensity in %, [assignment]). Absorbance measurements were performed with a Varian Cary 4000 spectrophotometer. Fluorescence was measured with a Varian Cary Eclipse spectrofluorometer equipped with a temperature controller. All spectroscopic measurements were performed in 3.5 mL polymethacrylate fluorimeter cuvettes (Sigma-Aldrich) or 3.5-mL quartz glass cuvettes (Hellma Analytics, Müllheim, Germany). Fluorescence microscopy images were captured by an Axiovert 200 (Zeiss) with a filter (BP 546/12, LP 590) through an Evolution QEi Media Cybernetics camera by using a 40× objective, and processed with the software ImageJ 1.48 V (https://imagej.nih.gov/ij/index.html).

2. Abbreviations

AIBN: azobisisobutyronitrile; AMADA: 1-aminomethyladamantane; ATA: 11-azido-3,6,9-trioxaundecan-1-amine; CB7: cucurbit[7]uril; EDC: 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride salt; DMSO: dimethyl sulfoxide; EDTA: ethylenediaminetetraacetic acid; ESI-MS: electrospray ionization mass spectrometry; FT-IR: Fourier transform infrared spectroscopy; HR-MS: high resolution mass spectrometry; MES: 2-(*N*-morpholino)ethanesulfonic acid; m.p.: melting point; NMR: nuclear magnetic resonance spectroscopy; IR: infrared; PAA: poly(acrylic acid); PMMA: poly(methyl methacrylate); rcf: relative centrifugal force; r.t.: room temperature; SDS: sodium dodecyl sulfate; TBTA: tris(benzyltriazolylmethyl)amine; TLC: thin layer chromatography.

3. Synthesis

3.1 Synthesis of CB-OH



1 g (0.86 mmol) CB7, synthesized as previously reported,[1] was dissolved in 125 mL of a mixture of Millipore water and 12 M HCl (3:2 v/v) and introduced in a 250-mL quartz glass round bottom flask under nitrogen. 65 µL (0.62 mmol) 30% hydrogen peroxide in H_2O was added and the solution was vigorously stirred during irradiation of UV light (254 nm) for 48 h. The reaction was monitored by ^{1}H NMR by taking aliquots of the reaction mixture. The solvent was then evaporated under reduced pressure affording a white solid. The crude product containing a mixture of CB7-$(OH)_n$ (with n = 0, 1, 2, 3),[3] was purified by column chromatography. Therefore, the mixture was dissolved in 950 µL H_2O/HCOOH 1:1 and loaded onto silica gel 60 (0.04-0.063 mm) and the column was eluted with H_2O/AcOH/HCOOH 10:10:1.5. The eluent was collected in fractions of 2 mL (>250 fractions) and the fractions containing pure CB7-OH (as confirmed by TLC, see Fig. S2) were combined. Evaporation of the solvent gave 150 mg CB7-OH as a white solid. The ^{1}H NMR was in accordance with the reported spectrum,[4] and the identity and purity of the obtained material was additionally confirmed by mass spectrometry (Fig. S1) and TLC (Fig. S2).

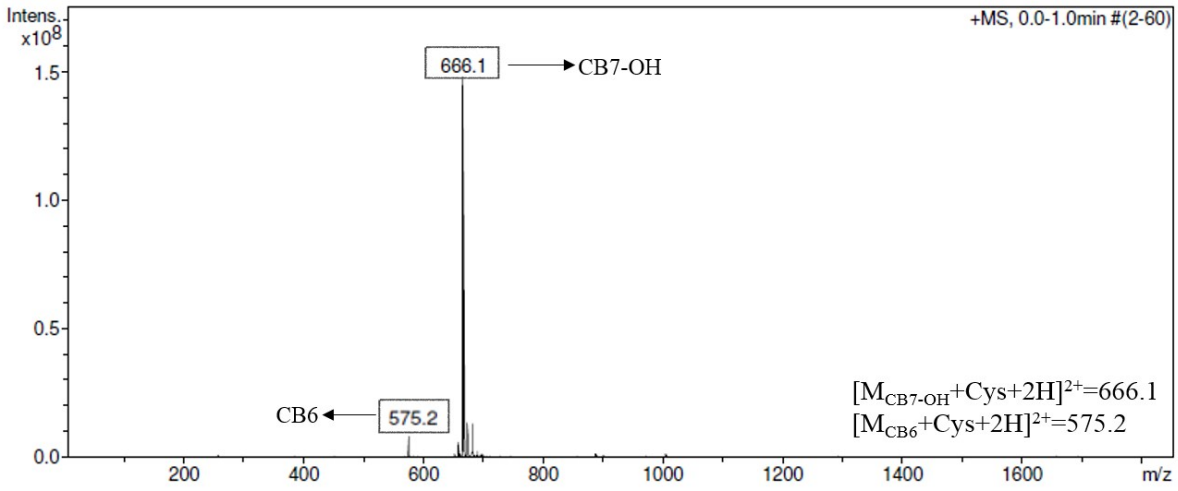


Fig. S1 Mass spectrum of CB7-OH with 1 mM cystamine in Millipore water. Traces of CB6 were presumably enriched during column chromatography (cf. Fig. S2).

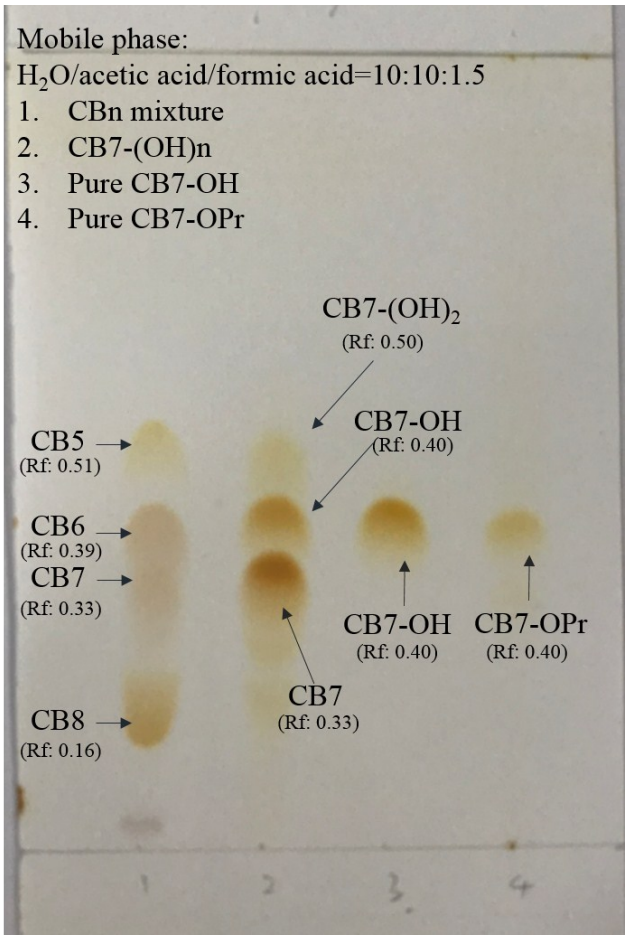
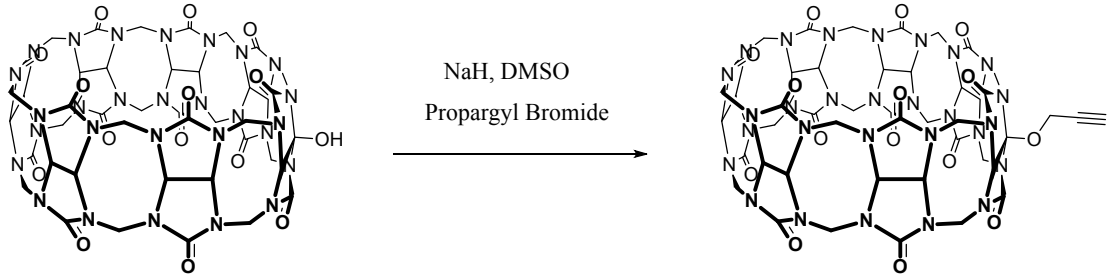


Fig. S2 TLC of CB*n* mixture and CB7 derivatives.

3.2 Synthesis of CB7-OPr



20 mg (17 µmol) CB7-OH was dissolved in 1.5 mL anhydrous DMSO. 10 mg (0.4 mmol) NaH (95% purity as solid) was added, and the mixture was stirred at room temperature for 3 h. Subsequently, the mixture was cooled to 0 °C, 0.5 mL (4.4 mmol) propargyl bromide was added, and the reaction mixture was stirred at room temperature for 12 h. 50 mL diethyl ether was added, and the resulting precipitate was three times triturated with 25 mL MeOH. Drying under high vacuum afforded a pale yellow solid, which was subjected a second time to the same reaction conditions. This gave the desired CB7-OPr quantitatively as confirmed by mass spectrometry (Fig. S3), ^{1}H NMR (Fig. S4), and IR spectroscopy: MS (ESI, +ve): 685.3 (100, [CB7-OPr+Cys+2H]$^{2+}$). IR (KBr) cm^{-1} 806 (s), 968 (s), 1234 (s), 1322 (s), 1376 (s), 1473 (s), 1733 (s), 2120 (w), 2933 (m), 2998 (w), 3432 (s).[5]

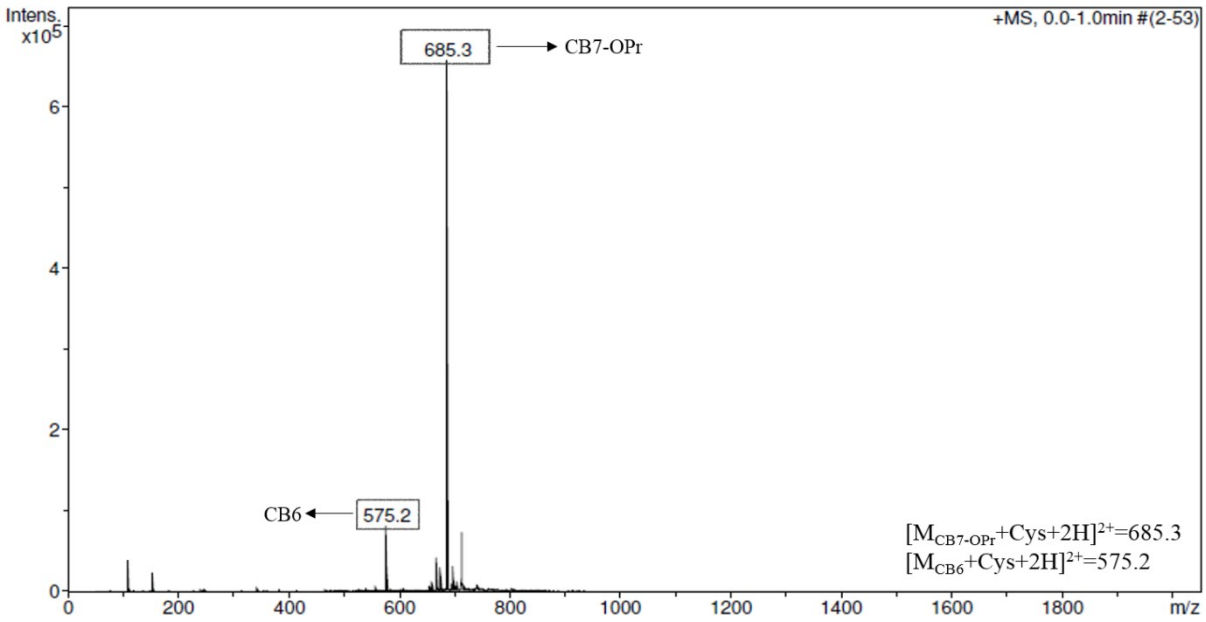


Fig. S3 Mass spectrum of CB7-OPr with 1 mM cystamine in Millipore water.

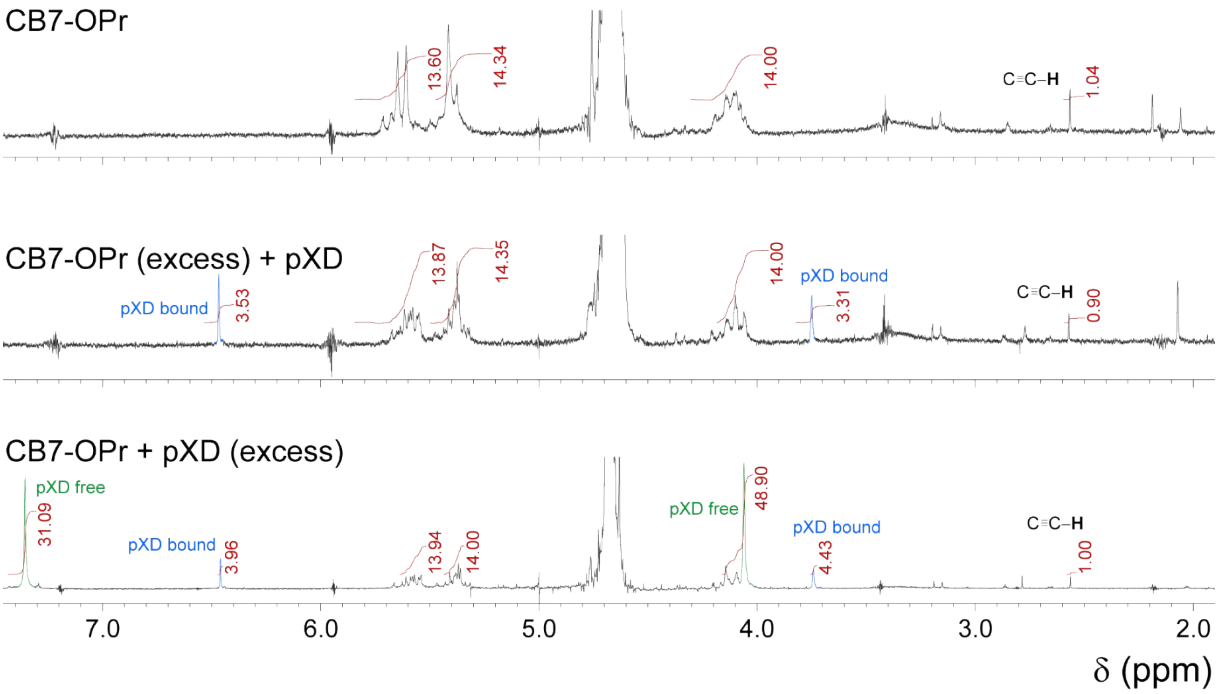
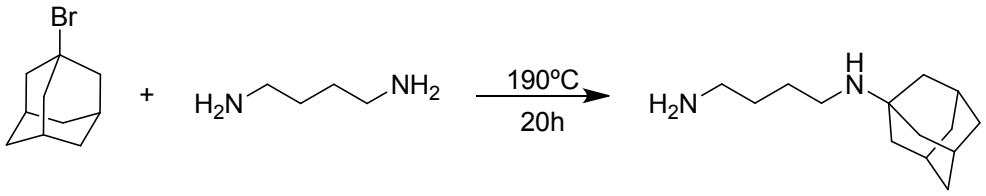
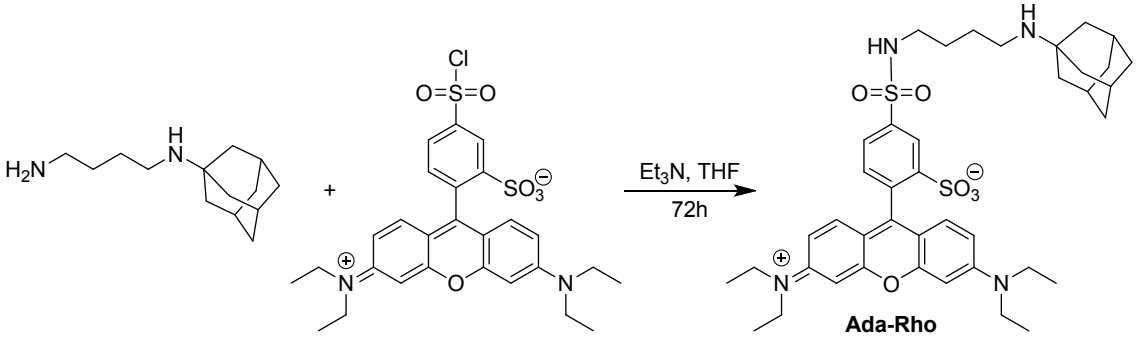


Fig. S4 ^{1}H NMR spectra of CB7-OPr in 1% DCl in D_2O in absence (top) and presence of substoichiometric amounts (middle) or excess (bottom) of the cavity binder *p*-xylene diamine (pXD).

3.3 Synthesis of Ada-Rho



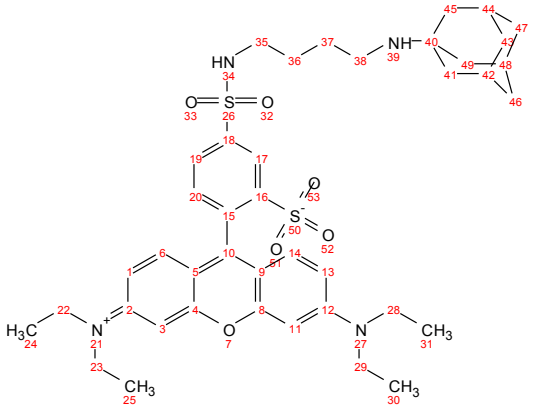
A mixture of 1-bromoadamantane (0.5 g, 2.32 mmol) and 1,4-diaminobutane (1.89 g, 11.62 mmol) was heated in a sealed tube at 190 °C for 20 h. Then, 2 M HCl (60 mL) and diethylether (60 mL) were added, and the aqueous layer was separated and made alkaline with 50% aq. NaOH (60 mL). The product was extracted with diethylether, the organic layer was dried over anhydrous $MgSO_4$, and after removal of the solvent, a pale yellow semi-solid was obtained (0.26 g, 1.17 mmol, 50% yield). The product was used without further purification in the next step. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 2.70 (t, J = 6.6 Hz, 2H), 2.58 (t, J = 6.6 Hz, 2H), 2.06 (s, 3H), 1.71-1.53 (m, 12H), 1.51-1.44 (m, 4H).



N-Adamantanyl-1,4-diaminobutane (15 mg, 0.067 mmol) and triethylamine (15 mg, 0.14 mmol) were dissolved in 1 mL dry tetrahydrofuran and the solution was stirred for 5 minutes. Afterwards, lissamine rhodamine B sulfonyl chloride (38.9 mg, 0.067 mmol), dissolved in 5 mL dry tetrahydrofuran, was added and the mixture was heated at 70 ºC in a sealed tube for 72 hours. After that time, the solvent was removed and the crude was subjected to purification by silica gel column chromatography, using dichloromethane/methanol (9/1) as eluent. This procedure yielded the final product Ada-Rho as purple solid (18 mg, 0.024 mmol, 35% yield). ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ (ppm): 8.52 (m, 1H, NH-adamantyl), 8.43 (d, J = 1.9 Hz, 1H, CH-phenylsulfonate), 8.05 (t, J = 6.0 Hz, 1H, SO_2NH), 7.96 (dd, J = 8.0 and 1.9 Hz, 1H, CH-phenylsulfonate), 7.49 (d, J = 8.0 Hz, 1H, CH-phenylsulfonate), 7.11-6.92 (m, 6H, 6 × CH-xanthylium), 3.64 (q, J = 7.0 Hz, 8H, 4 × $CH_3C\underline{H_2}N$), 2.92 (dt, J = 6.0 Hz, 2H, $SO_2NHC\underline{H_2}CH_2CH_2CH_2NH$), 2.88-2.78 (m, 2H, $SO_2NHCH_2CH_2CH_2C\underline{H_2}NH$), 2.09 (s, 3H, 3 × CH-adamantyl), 1.84 (s, 6H, 3 × CH_2-adamantyl), 1.71-1.48 (m, 10H, 3 × CH_2-adamantyl,

$SO_2NHCH_2C\underline{H_2}C\underline{H_2}CH_2NH$), 1.21 (t, J = 7.0 Hz, 12H, 4 × $C\underline{H_3}CH_2N$) ppm; ^{13}C NMR (101 MHz, $(CD_3)_2SO$) δ 157.3[a], 157.1[a], 155.0[a], 147.9[a], 141.6[a], 133.0[a], 132.6[b], 130.7[c], 126.6[c], 125.7[c], 113.7[a], 113.4[b], 95.4[b], 56.1 (quart. C-adamantyl), 45.3 (4 × $CH_3\underline{C}H_2N$), 42.0 ($SO_2NH\underline{C}H_2CH_2CH_2CH_2NH$), 38.5 ($SO_2NHCH_2CH_2CH_2\underline{C}H_2NH$), 37.5 (3 × CH_2-adamantyl), 35.2 (3 × CH_2-adamantyl), 28.4 (3 × CH-adamantyl), 26.3 ($SO_2NHCH_2\underline{C}H_2\underline{C}H_2CH_2NH$), 23.5 ($SO_2NHCH_2\underline{C}H_2\underline{C}H_2CH_2NH$), 12.5 (4 × $\underline{C}H_3CH_2N$) ppm.

[a] quaternary C corresponding to xanthylium or phenylsulfonate skeleton; [b] CH corresponding to xanthylium skeleton; [c] CH corresponding to phenylsulfonate skeleton



Position	^{1}H NMR (400 MHz, DMSO-d_6) δ	^{13}C NMR (101 MHz, DMSO-d_6) δ	Multiplicity, integration
39	8.52	-	(m, 1H)
17	8.43	126.6	(d, J = 1.9 Hz, 1H)
34	8.05	-	(t, J = 6.0 Hz, 1H)
19	7.96	125.7	(dd, J = 8.0, 1.9 Hz, 1H)
20	7.49	130.7	(d, J = 8.0 Hz, 1H)
1, 3, 6, 11, 13, 14	7.11-6.92	95.4, 113.4, 132.6	(m, 6H) xanthylium
22, 23, 28, 29	3.64	45.3	(d, J = 7.1 Hz, 8H)
35	2.92	42.0	(dt, J = 6.0 Hz, 2H)
38	2.88-2.78	38.5	(m, 2H)
42, 44, 48	2.09	28.4	(s, 3H)
41, 45, 49	1.84	37.5	(s, 6H)
36, 37	1.71-1.48	26.3, 23.5	(m, 4H)
43, 46, 47	1.71-1.48	35.2	(m, 6H)
24, 25, 30, 31	1.21	12.5	(t, J = 7.1 Hz, 12H)
2, 4, 5, 8, 9, 10, 12, 15, 16, 18	-	113.7, 133.0, 141.6, 147.9, 155.0, 157.1, 157.3	quart. carbons xanthylium and phenylsulfonate
40	-	56.1	quart. C adamantyl

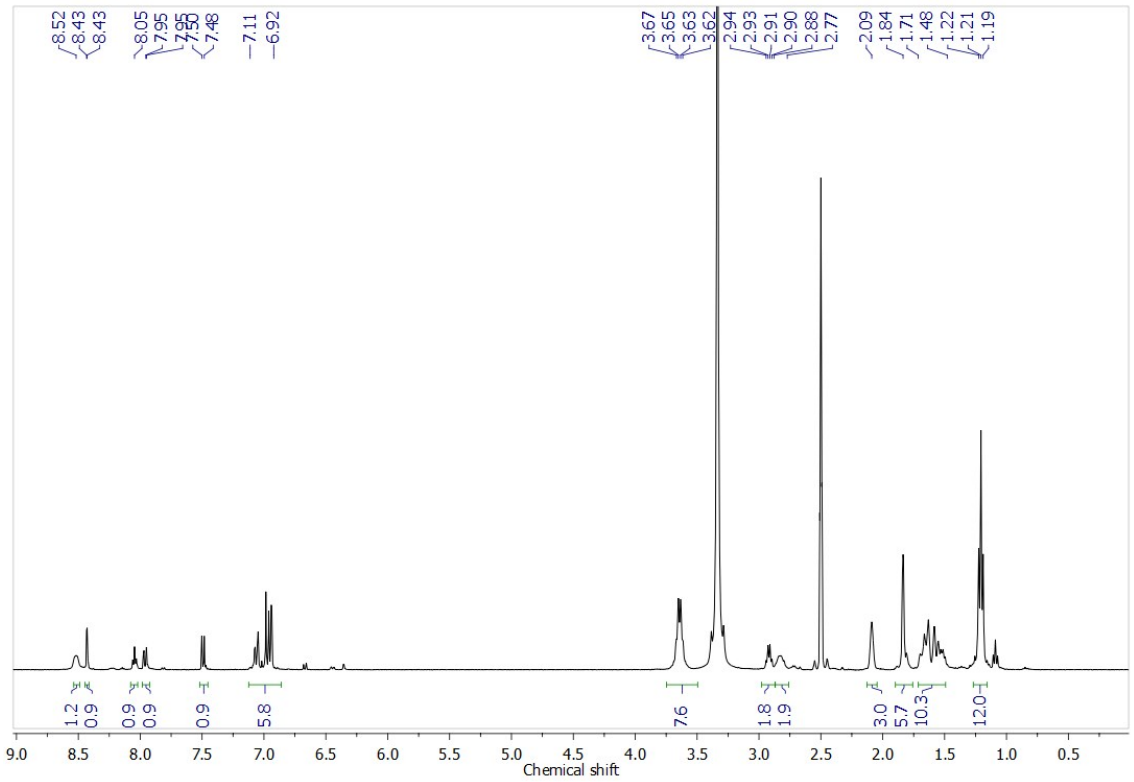


Fig. S5 ^{1}H NMR spectrum of Ada-Rho in $(CD_3)_2SO$.

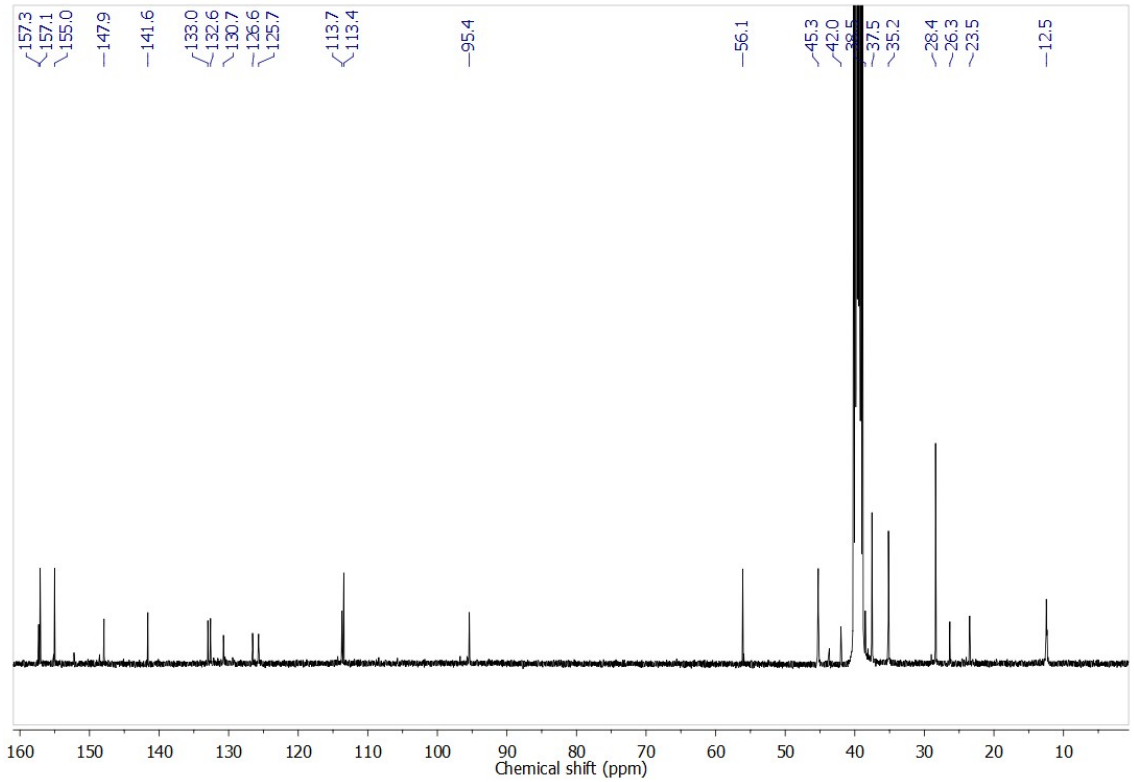


Fig. S6 ^{13}C NMR spectrum of Ada-Rho in $(CD_3)_2SO$.

4. Preparation and Characterization of CB7-Functionalized Particles

4.1 Synthesis of PMMA/PAA Microparticles

PMMA microparticles: Poly(methylmethacrylate) (PMMA) polymer microbeads were prepared by dispersion polymerization.[6,7] In order to obtain a narrow size distribution, a custom-made modified reaction chamber was employed to control the polymerization temperature and the stirring speed of the sealed polymerization flasks. The bead size can be controlled by parameters like monomer, stabilizer, and radical initiator concentration as well as reaction temperature. A typical procedure for bead preparation is given in the following: 7 g poly(vinylpyrrolidine) K90 (average molecular weight 1.300.000) and 600 mg (1.35 mmol) sodium bis(2-ethylhexyl) sulfosuccinate (aerosol-OT) were dissolved in 170 ml methanol. The mixture was transferred to the reaction flask containing 15 ml (14.1 g, 141 mmol), destabilized methyl methacrylate, and 200 mg (1.2 mmol) azobisisobutyronitrile (AIBN). The sealed flask was placed in the reaction chamber, where the polymerization was performed at a stirring speed of 20 rpm at a temperature of 55 °C for 21 hours. After cooling down to room temperature, the resulting bead suspension was poured into 600 ml water to precipitate the PMMA beads. After decantation of the water, the beads were washed several times with water and removed from the solution by centrifugation in 50 ml Falcon tubes. These washing-centrifugation cycles were repeated until no more methyl methacrylate could be detected in the supernatant.

Bead functionalization:[8] 300 ml of a 0.17 % (w/v) suspension of 2.55 µm PMMA particles were mixed with 9 g (125 mmol) acrylic acid, 150 mg (0.52 mmol) sodium dodecylsulfate (SDS), and 1.50 ml of 0.15 M benzophenone as photoinitiator in methanol. After 5 min equilibration time, the suspension was exposed for 8 min to UV light with 20 mW/cm^2 intensity, while the suspension was vigorously stirred. After irradiation, the suspension was centrifuged and washed several times with distilled water to remove unreacted compounds, additives, and homopolymer. Absence of PAA in solution was confirmed by conductometry (conductance <10 µS/cm).

4.2 Azide-Functionalized Particles

ATA-functionalized PMMA microparticles: 10 mg PMMA microparticles (111 µmol/g COOH) were washed into 660 µL reaction buffer (0.1 M MES, pH 5.0 or 10 mM $(NH_4)_2HPO_4$, pH 7.2) by repeated centrifugation, supernatant removal and resuspension cycles. Subsequently, 60 µL of 40 mM 11-azido-3,6,9-trioxaundecan-1-amine (ATA) in reaction buffer were added. The reaction was started by adding 80 µL of 100 mg/mL (0.52 M) EDC hydrochloride freshly dissolved in 4 °C cold water. Total reaction volume was 800 µL, final

conditions were 12.5 mg/mL microparticles (corresponding to 1.11 µmol COOH groups), 2.4 µmol ATA, and 42 µmol EDC. After 3 h or 6 h reaction time, the particles were washed into 1 mL 10 mM $(NH_4)_2HPO_4$, pH 7.2 to afford a 10 mg/mL stock solution of ATA-functionalized particles.

ATA-functionalized PS nanoparticles: 10 mg commercially available PS nanoparticles were washed into 660 µL reaction buffer (0.1 M MES, pH 5.0) and 18 µL of 400 mM 11-azido-3,6,9-trioxaundecan-1-amine (ATA) in reaction buffer were added. The reaction was started by adding 74 µL of 1 g/mL (5.2 M) EDC hydrochloride freshly dissolved in 4 °C cold water. Total reaction volume was 800 µL, final conditions were 12.5 mg/mL nanoparticles, 7.2 µmol ATA, and 389 µmol EDC. After 3 h reaction time, the particles were washed into 1 mL 10 mM $(NH_4)_2HPO_4$, pH 7.2 (by 10x centrifugation at 28600 rcf for 40 min) to afford a 10 mg/mL stock solution of ATA-functionalized particles.

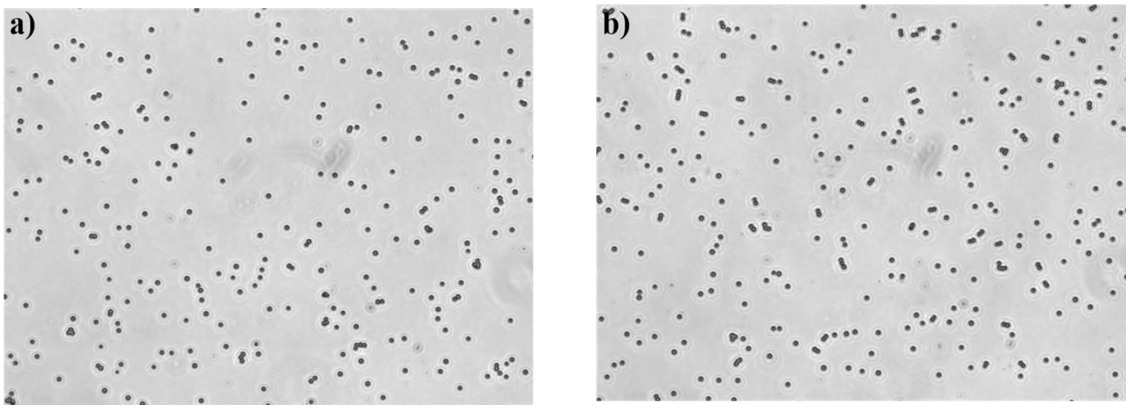


Fig. S7 Optical microscopy images (at 40fold magnification) of a) PMMA-PAA and b) ATA-functionalized microparticles.

4.3 CB7-Functionalized Particles

32 µL of a 3 mM CB7-OPr stock solution in DMSO was added to 400 µL ATA-functionalized particles (10 mg/mL) in 10 mM $(NH_4)_2HPO_4$, pH 7.2 buffer, and 10 µL of a freshly prepared 20 mM sodium ascorbate solution in 10 mM $(NH_4)_2HPO_4$, pH 7.2 was mixed with 20 µL of a 10 mM Cu^{2+}/tris(benzyltriazolylmethyl)amine (TBTA) in 55% DMSO stock solution. Both solutions were combined and the resulting reaction mixture was shaken for 24 h. The solution was then centrifuged (3.5 min, 16000 rcf) and the supernatant was discarded after centrifugation. The particles were thoroughly washed (20 times) with 10 mM $(NH_4)_2HPO_4$, pH 7.2 or 10 mM $(NH_4)_2HPO_4$, 1 mM EDTA, pH 7.2 (which gave identical results), and the total buffer volume was finally adjusted to afford a particle concentration of 10 mg/mL. Nanoparticles were prepared in the same way except for centrifugation at 28600 rcf for 40 min.

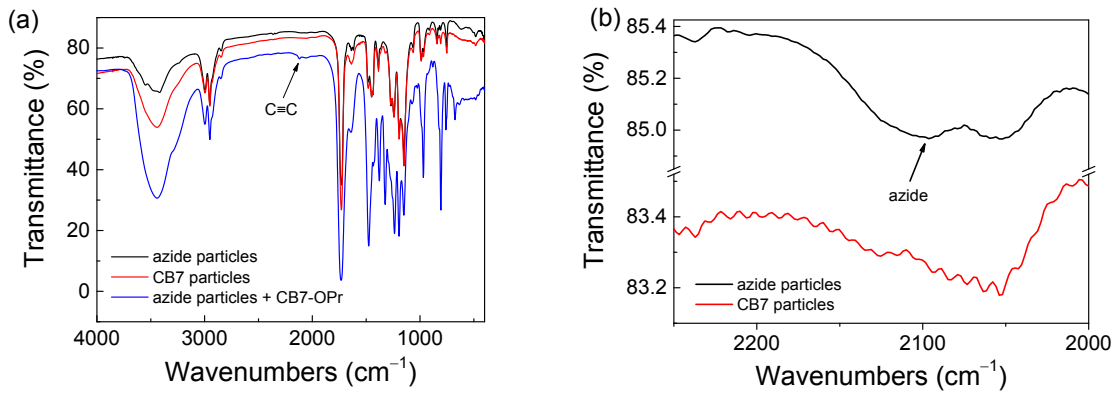


Fig. S8 (a) IR spectra (KBr pellet) of ATA-functionalized particles before (black) and after (red) click reaction with CB7-OPr, and a mixture of ATA-functionalized particles and CB7-OPr (blue). (b) IR spectra of ATA-functionalized particles before (black) and after (red) click reaction with CB7-OPr in a narrow range.

5. Surface Quantification Methods

5.1 Quantification of Surface-Bound CB7 with AMADA

For quantification of surface-bound CB7, aliquots from the as prepared CB7-functionalized particle stock solution (10 mg/mL) were diluted with 10 mM $(NH_4)_2HPO_4$, pH 7.2 to afford a final volume of 475 µL, to which 25 µL 60 µM AMADA was added (final AMADA concentration 3 µM). The mixture was briefly vortexed, sonicated, and then shaken for 5 min. After centrifugation for 10 min at 16000 rcf, 400 µL of the supernatant was transferred to a new Eppendorf tube and centrifugation was repeated. 350 µL of the final supernatant were transferred into a 3-mL poly(methylmethacrylate) cuvette containing 1290 µL 10 mM $(NH_4)_2HPO_4$, pH 7.2, and 160 µL 10 µM CB7 and 200 µL 10 µM acridine orange were added. The final volume was 2000 µL and final concentrations were 0.8 µM CB7, 1 µM AO, and 0-0.525 µM AMADA (depending on the amount of AMADA extracted).

Then, a fluorescence spectrum was recorded (λ_{exc} = 450 nm) and the fluorescence intensities at λ_{em} = 520 nm were plotted against the volume of particle stock solution and normalized to the fluorescence intensity in absence of particles (V = 0 µL). Linear fitting of the initial linear increase of the titration plot gave the slope of the fitted line, a, and the y-intercept, b (see, for example, inset of Fig. 2b in the main manuscript).

Assuming quantitative binding between AMADA and CB7 on the particle surface, the loading capacity of the particles, *i.e.* the amount of CB7 per particle mass, can be obtained from the intersection of the fitted line and the final plateau value in the titration plot, y_∞, indicating that all extraction of the molecule by the CB7-functionalized particles is complete. The volume of particle stock solution needed to completely extract the molecule, x, is thus:

$$x = \frac{y_\infty - b}{a} \qquad \text{[EQ1]}$$

The mass of particles needed to completely extract the molecule, m, is then obtained by the mass concentration of particle stock solution, $\rho_{Particle}$:

$$m = \frac{\rho_{Particle}\,(y_\infty - b)}{a} \qquad \text{[EQ2]}$$

This gives the loading capacity of the particles as the mass of particles needed to extract a specific amount of molecules, $n = c \cdot V$, as

$$Loading\ capacity\left(in\ \frac{\mu mol}{g} of\ particles\right) = \frac{a \cdot c \cdot V}{\rho_{Particle} \cdot (y_\infty - b)} \qquad \text{[EQ3]}$$

where a is slope and b the y-intercept of the fitted line, y_∞ is the final plateau value in the titration plot, $c \cdot V$ is the amount of the molecule to be extracted (e.g., 25 µL of 60 µM AMADA

stock solution or 500 µL of 3 µM during incubation), and $\rho_{Particle}$ is the mass concentration of particle stock solution (here: 10 mg/mL). The reproducibility of the method was evaluated by repeated measurements with a randomly selected batch of CB7-functionalized particles (Table S1).

5.2 Quantification of Surface-Bound Ada-Rho

Varying volumes of the CB7-functionalized particles (10 mg/mL) were transferred into 1.5 mL Eppendorf tubes and 10 mM $(NH_4)_2HPO_4$, pH 7.2 was added to achieve a total volume of 80 µL. Then, 100 µL of 20 µM Ada-Rho was added and the mixture was incubated for 17 min. After addition of 20 µL Triton X-100 to prevent unspecific absorption of Ada-Rho (sodium dodecylsulfate performed equally well), the mixture was briefly vortexed and then centrifuged for 27 min at 16000 rcf. Afterwards, 150 µL of the supernatant was diluted into 1850 µL 10 mM $(NH_4)_2HPO_4$, pH 7.2 and absorption and fluorescence spectra were recorded.

The fluorescence intensities at λ_{em} = 580 nm and the absorbance values at λ = 570 nm were plotted against the volume of particle stock solution (Fig. S10), and linear fitting of the initial linear decrease of the titration plot gave the slope of the fitted line, a, and the y-intercept, b.

The loading capacity of the particles, i.e. the mass of particles needed to extract a specific amount of Ada-Rho, was calculated similarly as above (see EQ3) by additionally considering the amount of unspecifically absorbed Ada-Rho, $n_{unspecific}$:

$$Loading\ capacity\left(in\ \frac{\mu mol}{g} of\ particles\right) = \frac{a \cdot (c \cdot V - n_{unspecific})}{\rho_{Particle} \cdot (y_\infty - b)} \qquad \text{[EQ4]}$$

As controls, AMADA-blocked CB7-functionalized particles were prepared by incubation of 100 µL 10 mg/mL CB7-functionalized particles and 1 mL 10 µM AMADA in 10 mM $(NH_4)_2HPO_4$, pH 7.2 for 17 min and subsequent centrifugation. The pellet was resuspended in 10 mM $(NH_4)_2HPO_4$, pH 7.2 and then subjected to the procedure above to test whether Ada-Rho could still bind to the particles (Fig. S12).

Unoccupied binding sites on the Ada-Rho and CB7-functionalized were quantified by the AMADA-based method (Fig. S13, see Section 5.1 for experimental procedure).

6. Supramolecular Control of Surface Coverage Densities

In order to prepare particles with different surface coverage densities of Ada-Rho, varying volumes of CB7 functionalized particles (7 mg/mL) were transferred into 1.5 mL Eppendorf tubes and 10 mM $(NH_4)_2HPO_4$, pH 7.2 was added to achieve a total volume of 140 µL. Then, 200 µL of a solution containing Ada-Rho and AMADA (10 µM total concentration) was added and the mixture was incubated for 17 min. After addition of 100 µL 1% SDS, the mixture was briefly vortexed and then centrifuged for 27 min at 16000 rcf. Afterwards, 370 µL of the supernatant was diluted into 1630 µL 10 mM $(NH_4)_2HPO_4$, pH 7.2 and absorption spectra were recorded.

The absorbance values at λ = 570 nm were plotted against the volume of particle stock solution (Fig. S15), and linear fitting of the initial linear decrease of the titration plot gave the slope of the fitted line, *a*, and the y-intercept, *b*. The surface coverage density with Ada-Rho was then calculated using EQ4, in which *c* is the concentration of Ada-Rho only. The dependence of the resulting surface coverage density on the molar fraction of AMADA is shown in Fig. 4 in the main text.

Particles for fluorescence microscopy were then prepared by incubating 40 µL of 10 mg/mL CB7 functionalized particles with 200 µL of a solution containing Ada-Rho and AMADA (10 µM total concentration) for 17 min, addition of 100 µL 1% SDS and centrifugation for 27 min. The particles were finally washed with 10 mM $(NH_4)_2HPO_4$, pH 7.2 and the particle concentration was adjusted to 5 mg/mL. The fluorescence of the particle suspensions was determined by fluorescence spectroscopy (Fig. 5a in the main text) and by fluorescence microscopy (Fig. 5b and Fig. S16). For the latter, 5 µL of the particle suspension were deposited in the center of a Thermo SCIENTIFIC 76×26 mm clear-white glass slide and a 22×22 mm ROTH #1 cover glass was placed on the particle suspension to ensure that particles distribute homogeneously between glass slide and cover glass. The images were captured with a Zeiss Axiovert 200 and an Evolution QEi Media Cybernetics imaging camera through a 40× objective using a BP 546/12, LP 590 filter set. The dependence of the fluorescence intensities within the field of view on the surface coverage densities with Ada-Rho (Fig. 5b) was obtained by automatic assignment of the regions of interest (ROIs) and averaging the intensity within all ROIs with the software ImageJ 1.48 V (Fig. S16). The brightness and contrast of the image in the main manuscript (Fig. 3b) was enhanced. The images in the SI (Fig. S16) are unedited.

7. Supporting Figures and Tables

7.1 Supporting Tables S1 and S2

Table S1. Reproducibility measurements for the quantification of surface-bound CB7.

Experiment	loading capacity (µmol/g)	coupling yield (%)	CB7 surface density (nmol/cm^2)
1	5.66	5.10	0.286
2	5.81	5.23	0.294
3	5.52	4.97	0.279
4	5.88	5.30	0.297
5	5.63	5.07	0.285
6	5.74	5.17	0.290
7	5.80	5.23	0.293
average	5.72	5.15	0.289
standard deviation	0.11	0.10	0.006
coefficient of variation		ca. 2%	

Table S2. Reaction results for CB7 surface functionalization.[a]

entry #	reaction conditions[b]	loading capacity (µmol/g)	coupling yield (%)[c]	CB7 surface density (nmol/cm^2)
1	1) 3 h at pH 5.0, 2) 24 h (with EDTA)[d]	5.69 ± 0.04	5.13 ± 0.03	0.288 ± 0.002
2	1) 3 h at pH 5.0, 2) 24 h (without EDTA)	5.67	5.11	0.287
3	1) 3 h at pH 5.0, 2) 48 h	6.2	5.6	0.32
4	1) 3 h at pH 7.2, 2) 24 h	4.4	4.0	0.22
5	1) 6 h at pH 7.2, 2) 24 h	4.8	4.3	0.24

[a] Values were determined with the AMADA assay (Section 5.1). [b] Functionalization of COOH surface groups with 1) azide groups was performed in pH 5.0 or pH 7.2 buffer for 3 h or 6 h and then with 2) CB7-OPr in a click reaction for 24 h or 48 h (see Section 4.1 and 4.2 for details). [c] With respect to 111 µmol/g surface COOH. [d] From three replicates.

7.2 Supporting Figures S9 to S19

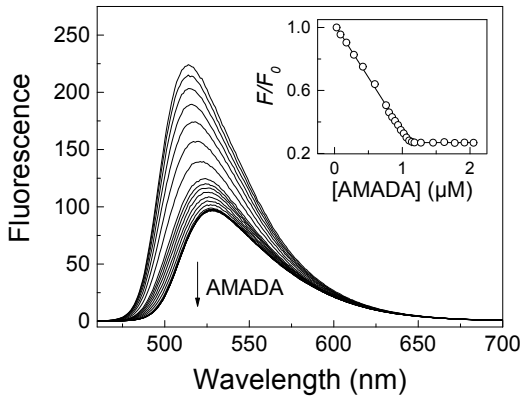


Fig. S9 Competitive fluorescence titration (λ_{exc} = 450 nm) of 2 μM AO and 1.1 μM CB7 in 10 mM $(NH_4)_2HPO_4$, pH 7.2. The inset shows the corresponding fluorescence titration (λ_{em} =510 nm) plot normalized to the initial fluorescence intensity and demonstrates quantitative 1:1 binding between AMADA and CB7 in solution.

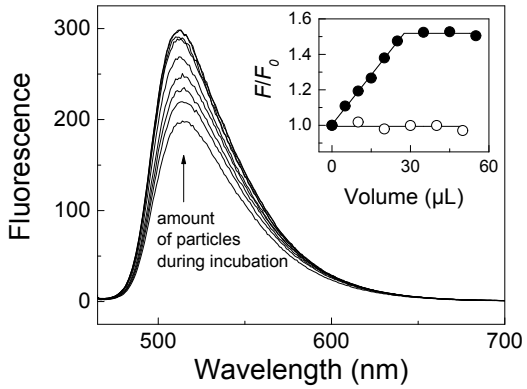


Fig. S10 Dependence of fluorescence spectral changes (λ_{exc} = 450 nm, λ_{obs} = 510 nm) of the supernatant on the volume of added CB7-functionalized polymer particles stock solution (10 mg/mL) during incubation with (dimethylaminomethyl)ferrocene (surface coverage density = 5.5 μmol/g). The inset compares CB7-functionalized (filled circles) and ATA-functionalized particles as control (open circles).

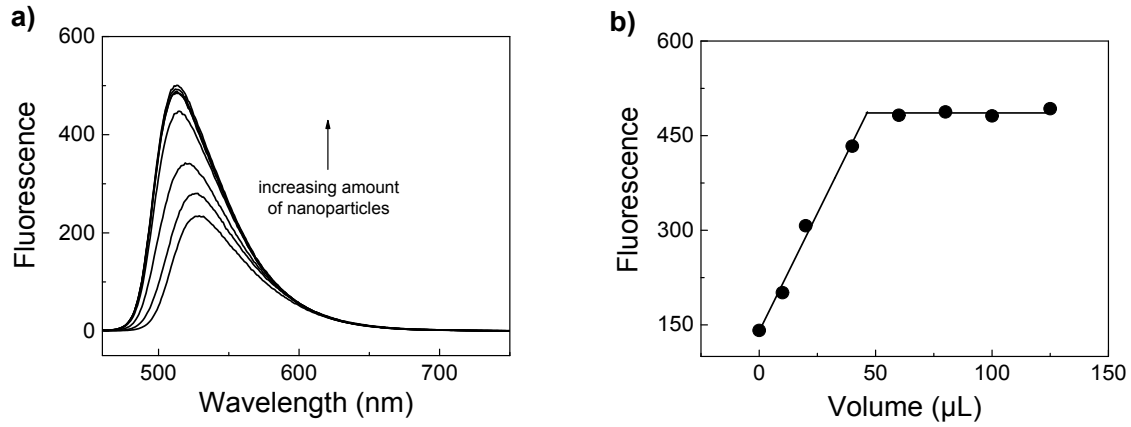


Fig. S11 a) Dependence of fluorescence spectral changes (λ_{exc} = 450 nm, λ_{obs} = 510 nm) of the supernatant on the volume of added CB7-functionalized (poly)styrene nanoparticles stock solution (10 mg/mL) during incubation with 4.56 µM AMADA. b) Respective titration plot.

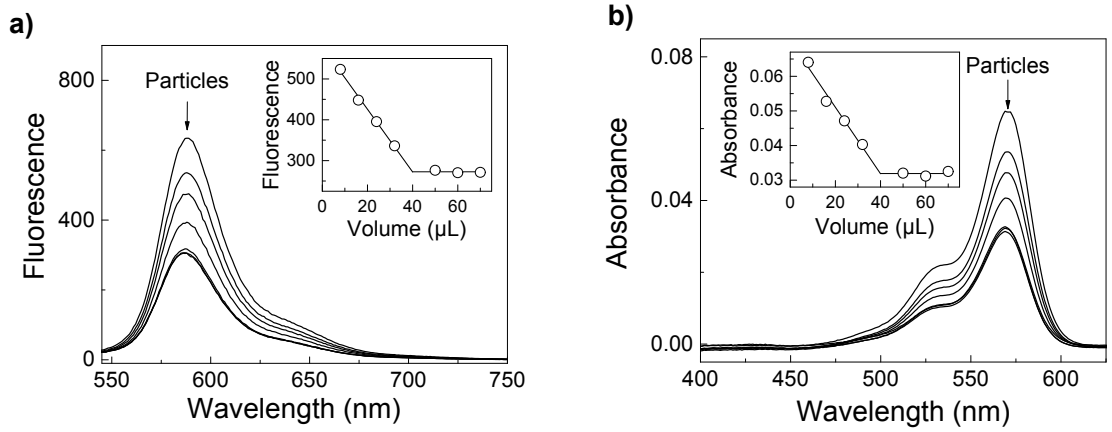


Fig. S12 Variation of a) fluorescence intensity (λ_{exc} = 520 nm, λ_{obs} = 580 nm) and b) absorbance (λ_{obs} = 570 nm) of the supernatant (150 µL) of a mixture of 10 µM Ada-Rho and varying amounts of CB7 particles (10 mg/mL) after centrifugation and dilution to 2000 µL in 10 mM $(NH_4)_2HPO_4$, pH 7.2.

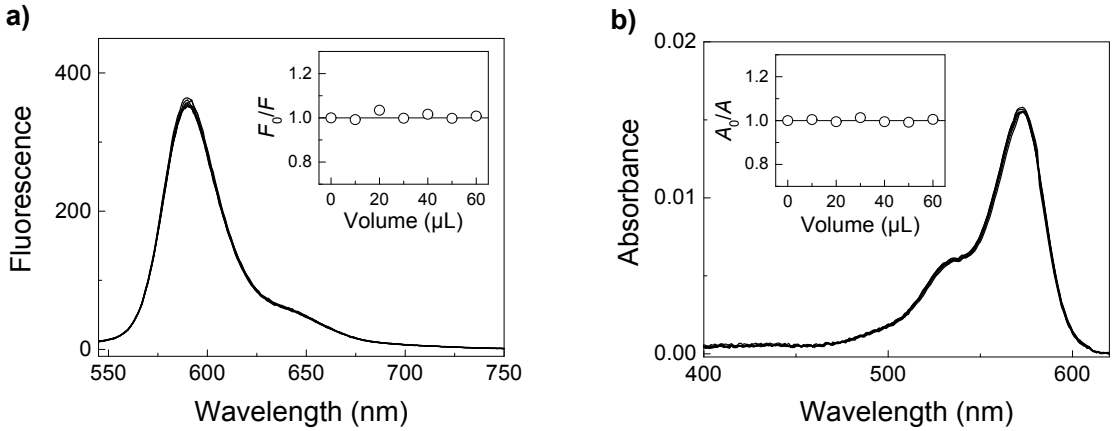


Fig. S13 Variation of a) fluorescence intensity (λ_{exc} = 520 nm, λ_{obs} = 580 nm) and b) absorbance (λ_{obs} = 570 nm) of the supernatant (220 µL) of a mixture containing 1.7 µM Ada-Rho and varying amounts of ATA-functionalized particles (10 mg/mL) after centrifugation and dilution to 2000 µL in 10 mM $(NH_4)_2HPO_4$, pH 7.2.

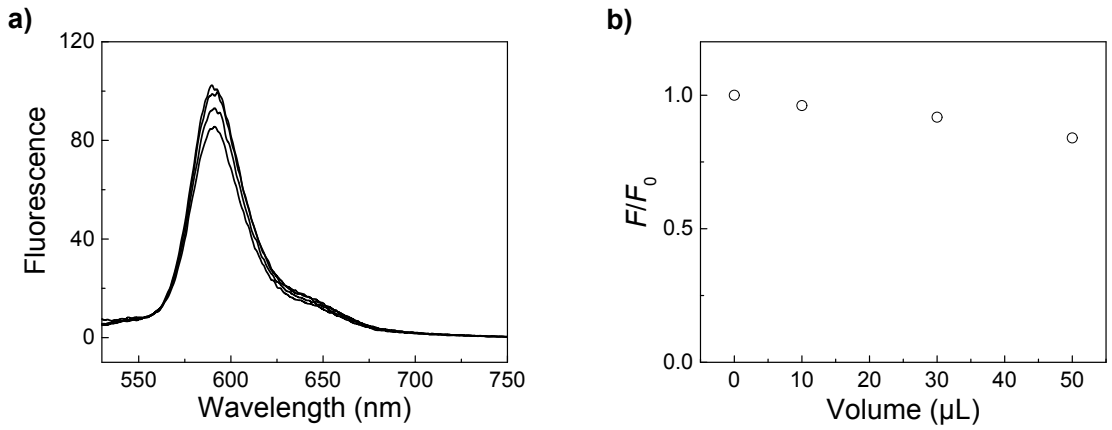


Fig. S14 Incubation of Ada-Rho with AMADA-blocked CB7-functionalized particles. a) Fluorescence spectra of different samples. b) Dependence of fluorescence spectral changes (λ_{exc} = 520 nm, λ_{obs} = 570 nm) of the supernatant on the volume of added AMADA-blocked CB7 functionalized polymer particles stock solution (10 mg/mL) during incubation with 2.5 µM Ada-Rho in 10 mM $(NH_4)_2HPO_4$, pH 7.2.

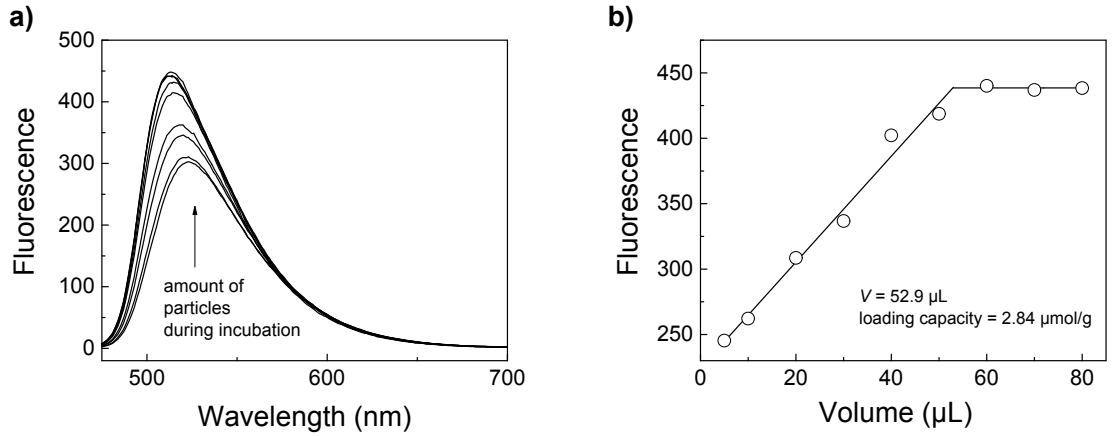


Fig. S15 Quantification of remaining CB7 binding sites on Ada-Rho-functionalized particle surfaces. a) Fluorescence spectra of different samples. b) Dependence of fluorescence spectral changes (λ_{exc} = 450 nm, λ_{obs} = 510 nm) of the supernatant on the volume of added functionalized polymer particles stock solution (10 mg/mL) during incubation with 25 µM AMADA in 10 mM $(NH_4)_2HPO_4$, pH 7.2.

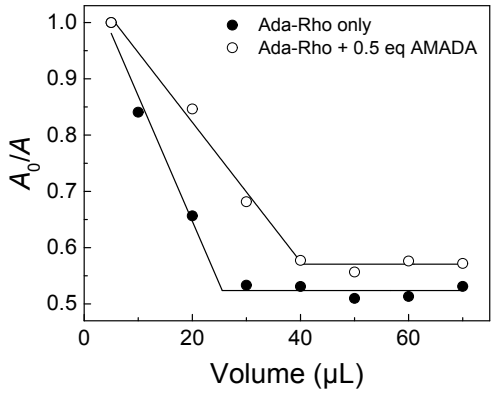


Fig. S16 Extraction with CB7-functionalized beads using solutions of 0.44 µM Ada-Rho (solid circles) or a mixture of 0.44 µM Ada-Rho and 0.22 µM AMADA (open circles). The intersections refer to final Ada-Rho surface loadings of 3.0 µmol/g and 1.8 µmol/g, respectively.

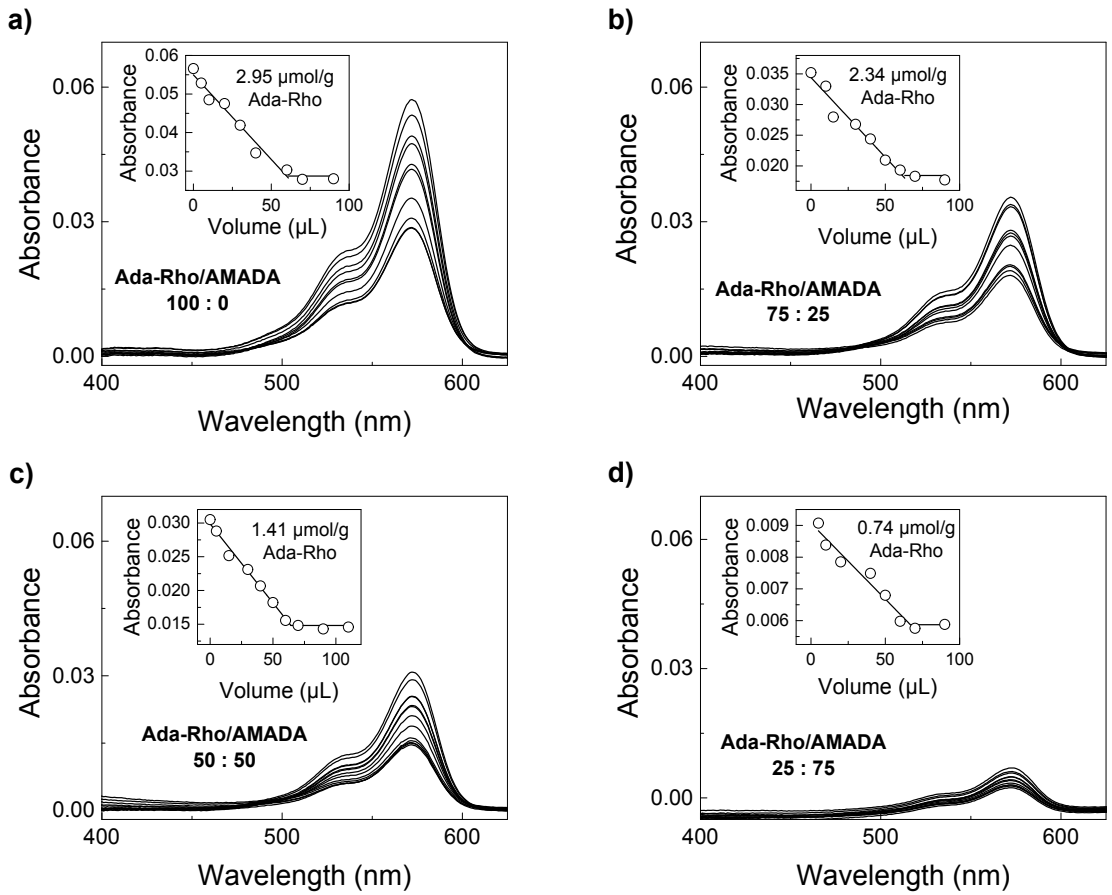


Fig. S17 Variation of absorbance (λ_{obs} = 570 nm) of the supernatant (370 µL) containing a mixture of 2 nmol AMADA and Ada-Rho (molar fraction of Ada-Rho: a) 1.0, b) 0.75, c) 0.5, and d) 0.25) incubated varying amounts of CB7 functionalized particles after centrifugation and dilution to 2000 µL in 10 mM $(NH_4)_2HPO_4$, pH 7.2. The insets show the dependence of absorbance at λ_{obs} = 570 nm on the volume of added particle stock solution (7 mg/mL).

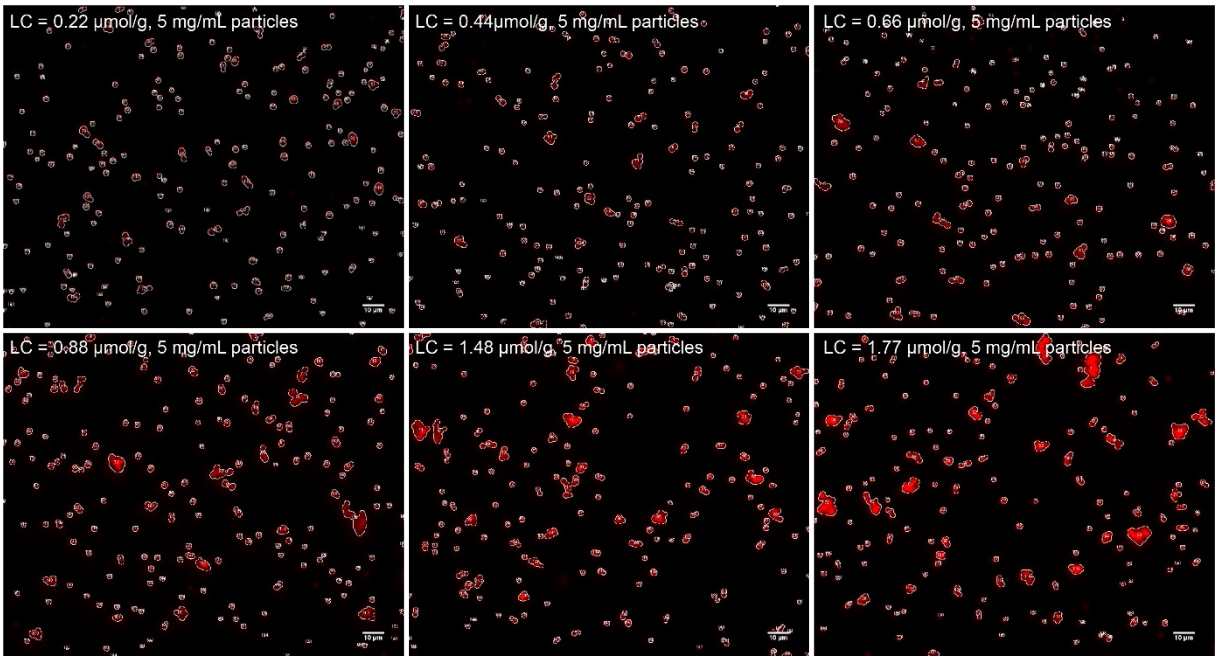


Fig. S18 Fluorescence microscopy images (546 nm bandpass filter) of Ada-Rho-labeled particles (5 mg/mL) with increasing surface coverage densities of Ada-Rho (expressed as loading capacities, LC). The white circles represent the automatically assigned ROIs by the software ImageJ.

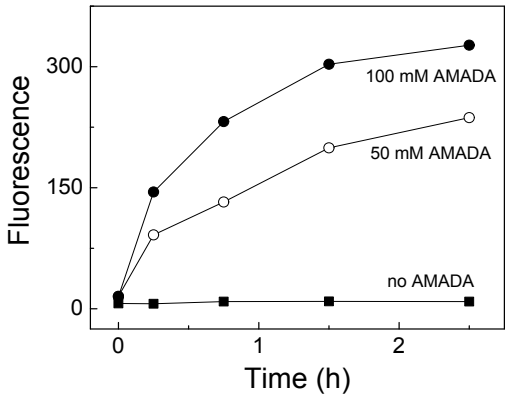


Fig. S19 Time-dependent dissociation of Ada-Rho from CB7-functionalized particles in presence of the competitor AMADA. Ada-Rho functionalized particles (2 mg/mL) were incubated with 50 mM (open circles) or 100 mM (filled circles) AMADA in 10 mM $(NH_4)_2HPO_4$, pH 7.2 and the fluorescence of the supernatant (λ_{exc} = 520 nm, λ_{obs} = 585 nm) was measured after certain time intervals. As control, the fluorescence of the supernatant did not show any release of surface-bound Ada-Rho in absence of the competitor AMADA (filled squares).

8. References

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