Electronic Supplementary Material (ESI) for Chemical Communications

Quantification of Surface Functional Groups on Polymer Microspheres by Supramolecular Host-Guest Interactions

Andreas Hennig,*[a] Angelika Hoffmann,[a] Heike Borcherding,[b] Thomas Thiele,[b] Uwe Schedler[b] and Ute Resch-Genger*[a]

[a] *BAM – Federal Institute for Materials Research and Testing, Berlin, Germany*

[b] *PolyAn GmbH, Berlin, Germany*

1. Materials and Methods

Reagents for synthesis were from Fluka and Aldrich. All reactions were performed under N_2 atmosphere. Reversed-phase column chromatography was carried out on silica gel 90 C_{18}-reversed phase (Fluka, 40-63 µm). Analytical thin layer chromatography (TLC) was performed on aluminium sheets coated with silica gel (Fluka, 25 µm) and silica gel 60 RP-18 (Merck). Buffers and salts were of the highest purity available from Fluka, Sigma-Aldrich or AppliChem (Darmstadt, Germany) and used as received. Poly(methyl methacrylate) microspheres (diameter 6 µm) with varying amounts of grafted poly(acrylic acid) were individually prepared by PolyAn GmbH (Berlin, Germany) for this study. Cucurbit[7]uril was synthesized according to established literature methods[S1] or purchased from Aldrich, which both performed equally. Functionalization of microspheres was carried out in standard Eppendorf plastic tubes.

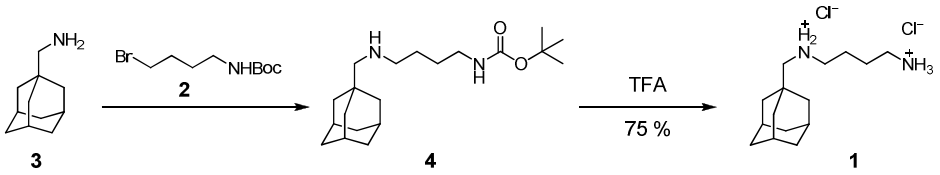
Melting points (m.p.) were determined on a heating table from VEB Kombinat Nagema (Germany). IR spectra were recorded on a Bruker Equinox 55 equipped with an IRScope and ATR unit. Spectra are reported as wavenumbers in cm^{-1} with band intensities indicated as s (strong), m (medium), w (weak), and br (broad). ^1H and ^{13}C NMR spectra were recorded on a Bruker AVANCE II 300 MHz or 500 MHz spectrometer and are reported as chemical shifts (δ) in ppm relative to TMS ($\delta = 0$). Spin multiplicities are reported as singlet (s), doublet (d), triplet (t), quartet (q) and quintet (quint) with coupling constants (J) given in Hz, or multiplet (m). ^1H resonances were assigned with the aid of H,H-COSY and ^{13}C resonances with additional information from DEPT 135 spectra. ESI-MS was performed on a Waters Micromass Q-Tof Ultima and are reported as mass-per-charge ratio *m/z* (intensity in %, [assignment]). Fluorescence measurements were performed in 3 mL polymethacrylate fluorimeter cuvettes (Sigma-Aldrich) with a Perkin Elmer LS-50B spectrofluorometer.

Absorption measurements were performed either with a Varian Cary 5000 equipped with a temperature controller or a Bruins Instruments OMEGA 10. Optical microscopy was carried out with an Olympus BX51 microscope equipped with a XC30 digital colour camera.

2. Abbreviations

Boc: *tert.*-butyloxycarbonyl; calc.: calculated; CB7: cucurbit[7]uril; EDC: 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride salt; 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; TFA: trifluoroacetic acid; TLC: thin layer chromatography; XPS: X-ray photoelectron spectroscopy.

3. Synthesis



N-adamantylmethylbutane-1,4-diamine (**1**): 152 mg (0.60 mmol) 4-(Boc-amino)-butylbromide **2** was dissolved in 8 mL abs. THF under inert atmosphere. Subsequently, 100 mg (0.60 mmol) adamantylmethylamine **3**, 245 mg K_2CO_3 (1.8 mmol), and 70 mg (0.42 mmol) KI were added and the reaction mixture was refluxed for 18 h. The solid residue was filtered, the solvent removed by rotary evaporation. The obtained intermediate **4** was deprotected without further purification. Therefore, the residue was taken up in 5 mL abs. CH_2Cl_2 and cooled to 0 °C. 1 mL TFA was slowly added and the reaction mixture was stirred at r.t. over night. Solvent removal and purification by preparative reversed-phase column chromatography with MeOH/HCl 1:1 gave **1** as a white product (141 mg, 75%). Mp: 233 °C; IR (neat): 2902 (s), 2848 (m), 1592 (m), 1514 (w), 1572 (s), 1447 (m); ^{1}H NMR (300 MHz, CD_3OD): 2.99-3.10 (m, 4H), 2.76 (s, 2H), 2.06 (br s, 3H), 1.68-1.91 (m, 16H); ^{13}C NMR(75 MHz, CD_3OD): 60.7 (t), 49.5 (t), 40.7 (t), 40.1 (t), 37.5 (t), 33.4 (s), 29.5 (d), 25.7 (t), 23.7 (t); MS (ESI, MeOH/HCOOH): 237 (100, $[M+H]^+$).

3. Binding Titrations[S2,S3]

Titrations were carried out in 3 mL polymethacrylate cuvettes (Sigma-Aldrich) at ambient temperature. The association constant K_a of acridine orange with CB7 was determined by successive addition of titrant to a cuvette containing acridine orange of known concentration. The fluorescence titration plot was obtained by plotting the fluorescence intensity FI (λ_{exc} = 450 nm, λ_{em} = 510 nm) against the total concentration of CB7 $[H]_0$ and was analyzed according to equation [S1]

$$FI = I_{gh} + (I_g - I_{gh})\frac{\frac{1}{2}\left([G]_0 - [H]_0 - 1/K_a\right) \pm \sqrt{\frac{1}{4}\left([H]_0 + [G]_0 + 1/K_a\right)^2 - [H]_0[G]_0}}{[G]_0} \qquad [S1]$$

where FI is fluorescence intensity, I_{gh} is fluorescence intensity of the host-guest complex, I_g is fluorescence intensity in absence of host, $[G]_0$ is total concentration of fluorescent guest (bound and unbound), $[H]_0$ is total concentration of CB7 (bound and unbound), and K_a is the association constant (dissociation constant $K_d = 1/K_a$). The absorption titration plot was obtained by plotting the absorption (λ_{obs} = 500 nm) against the total concentration of CB7 $[H]_0$. Equation [S1] can also be used for analysis of absorption titrations, in which FI is absorption, I_{gh} is absorption of the host-guest complex, I_g is absorption in absence of host, and I_h is absorption in absence of guest. Fluorescence (Fig. S1) and absorption (Fig. S2) titrations gave identical results of $K_a = 2 \times 10^6$ M^{-1}, which is slightly higher than previously reported by one of us.[3] We now noted, that the absorption of acridine orange slowly decreases with time in quartz glass cuvettes, which results in a more pronounced curvature of the fluorescence titration plot leading to an underestimate of the binding constant.

Competitive titrations were performed in 3 mL polymethacrylate cuvettes (Sigma-Aldrich) at ambient temperature by successive addition of known amounts of competitor **1** to solutions containing CB7 and acridine orange. Care was taken to prevent dilution effects during the titration. The binding titration plot was analyzed by a competitive binding model as previously described.[S2] In brief, the data was analyzed according to equation [S2]

$$FI = I_g + (I_{gh} - I_g)\frac{K_g[H]}{1 + K_g[H]} \qquad [S2]$$

where FI is fluorescence intensity, I_{gh} is fluorescence intensity of the host-guest complex, I_g is fluorescence intensity in absence of host, K_g is the association constant of guest and CB7 (as determined from a 1:1 titration), and $[H]$ is the concentration of free CB7, which is defined by equation [S3]

$$0 = a[H]^3 + b[H]^2 + c[H] - d \text{, where}$$
$$a = K_c K_g,\ b = K_c + K_g + K_c K_g([G]_0 + [C]_0 - [H]_0) \qquad \text{[S3]}$$
$$c = K_c([C]_0 - [H]_0) + K_g([G]_0 - [H]_0) + 1, \text{ and } d = -[H]_0$$

where K_c is the association constant of competitor and CB7, $[G]_0$ is total concentration of fluorescent guest, $[H]_0$ is total concentration of CB7, and $[C]_0$ is total concentration of competitor. The fitting was implemented into OriginPro 8G (OriginLab Corporation, Northampton, MA) by using a subroutine to solve the cubic equation [S3] with the Newton-Raphson method.

Strong evidence for a 1:1 binding stoichiometry between **1** and CB7 is indicated by the fact that first, full displacement of acridine orange occurs, when CB7 and **1** are in an equimolar ratio (see Fig. S3), and second, the NMR spectrum recorded for an equimolar ratio of **1** (Fig. S4) and CB7 remained unaffected when an excess of CB7 was added (spectrum not shown). Note that the binding titration was carried out under conditions, in which complexation is quantitative, i.e. the concentrations of the binding partners are much higher than the presumed dissociation constant.

4. Functionalization of Microspheres & Analysis

10 mg microspheres were washed* into 660 µL reaction buffer (0.1 M MES, pH 5.0) and 60 µL 40 mM **1** 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 V_{MS} was 800 µL, final conditions were 12.5 mg/mL microspheres, i.e. m_{MS}, (corresponding to 0.35-70 µmol COOH groups), 2.4 µmol **1**, and 42 µmol EDC. After 3 h reaction time, the microspheres were washed into 1 mL 10 mM $(NH_4)_2HPO_4$, pH 7.2. The reaction with microsphere preparations **MS-7** to **MS-9** were also carried out with 12 µmol **1**, which gave identical results for **MS-7** and **MS-8**, but a higher loading capacity for **MS-9**. Another reaction of **MS-9** with 24 µmol **1** gave the same result as with 12 µmol. Although EDC and **1** are not in excess relative to the total number of carboxy groups, this indicates that all concentrations of reactants were sufficient to react all accessible COOH groups. The values reported in Table 1 in the main text refer to the maximum loading capacity.

For analysis, aliquots V_{beads} of the functionalized microsphere suspension were diluted with 10 mM $(NH_4)_2HPO_4$, pH 7.2 and CB7 stock solution (volume V_{CB7} and concentration

* Washing steps were performed by repeated (≥ 5 x) centrifugation for 1 min at 16,000 rcf, removal of supernatant, and addition of fresh buffer. The microspheres were resuspended by vigorous vortexing and ultrasonication.

c_{CB7}) was added to a final volume of 300 µL (final concentration of CB7 was 16 µM). After shaking for 5 min, the suspension was centrifuged for 1 min at 16000 rcf. 200 µL of the supernatant was transferred into a 3 mL polymethacrylate cuvette and diluted to 2000 µL with 10 mM $(NH_4)_2HPO_4$, pH 7.2. Subsequently, 4 µL 1 mM acridine orange in 10 mM $(NH_4)_2HPO_4$, pH 7.2 was added and a fluorescence spectrum was recorded (λ_{exc} = 450 nm). The fluorescence intensities were normalized to the initial fluorescence intensity in absence of microspheres and plotted against the aliquot volume. Linear fitting of the initial linear decrease of the titration plot gave the slope of the fitted line *a* and the y-intercept *b* (Fig. 2 and Fig. S7 to S14). The loading capacity of the microsphere, *i.e.* the number of **1** per gram of microsphere is then obtained *via* equation [S4]

$$\text{loading capacity } (\mu\text{mol/g of particles}) = \frac{a \cdot c_{CB7} \cdot V_{CB7} \cdot V_{MS}}{m_{MS} \cdot (y_\infty - b)} \qquad [S4]$$

where *a* is slope of the fitted line, *b* is the y-intercept, c_{CB7} and V_{CB7} are the concentration and volume of CB7 in the stock solution added to the functionalized microspheres, V_{MS} is the total volume of the derivatization solution, m_{MS} is the mass of microspheres during the derivatization, and y_∞ is the relative fluorescence intensity in absence of CB7, *i.e.* when all CB7 has been extracted by the microspheres functionalized with **1**.

5. References

1 (*a*) W.-H. Huang, S. Liu, L. Isaacs, in *Modern Supramolecular Chemistry*, 2008, pp. 113-142; (*b*) J. Kim, I.-S. Jung, S.-Y. Kim, E. Lee, J.-K. Kang, S. Sakamoto, K. Yamaguchi, K. Kim, *J. Am. Chem. Soc.* 2000, **122**, 540; (*c*) A. I. Day, A. P. Arnold, R. J. Blanch, B. Snushall, *J. Org. Chem.* 2001, **66**, 8094; (*d*) C. Marquez, F. Huang, W. M. Nau, *IEEE Trans. Nanobiosci.* 2004, **3**, 39.

2 A. Hennig, H. Bakirci, W. M. Nau, *Nat. Methods* 2007, **4**, 629.

3 (*a*) W. M. Nau, G. Ghale, A. Hennig, H. Bakirci, D. M. Bailey, *J. Am. Chem. Soc.* 2009, **131**, 11558; (*b*) M. Shaikh, J. Mohanty, P. K. Singh, W. M. Nau, H. Pal, *Photochem. Photobiol. Sci.* 2008, **7**, 408.

6. Supporting Figures

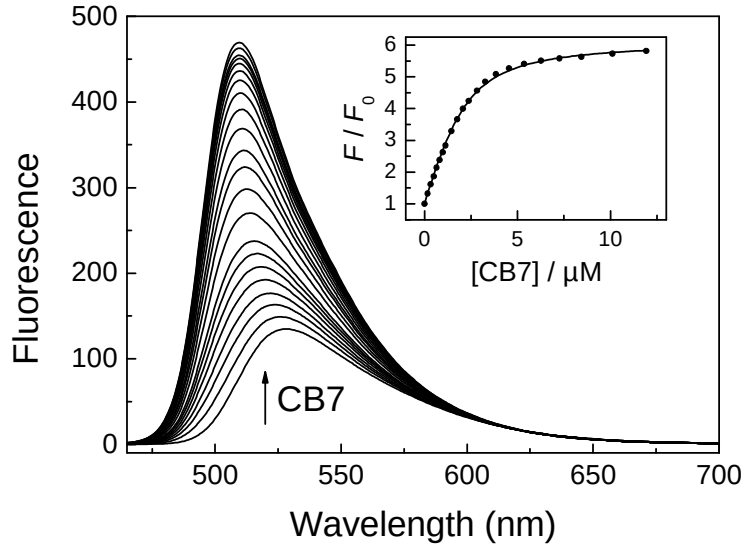


Fig. S1. Variation of fluorescence spectra (λ_{exc} = 450 nm) during titration of 2 µM acridine orange with CB7 in 10 mM $(NH_4)_2HPO_4$, pH 7.2. The inset shows the corresponding fluorescence titration (λ_{em} = 510 nm) plot normalized to the initial fluorescence intensity and fitted according to equation [S1].

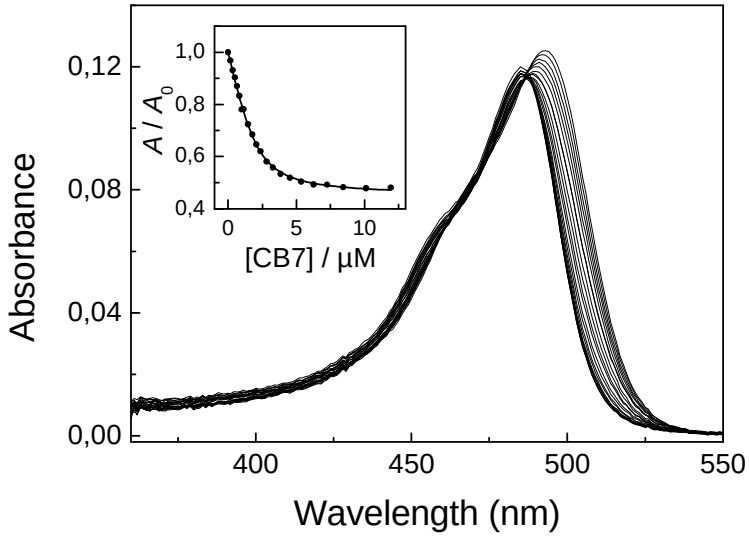


Fig. S2. Variation of absorption spectra during titration of 2 µM acridine orange with CB7 in 10 mM $(NH_4)_2HPO_4$, pH 7.2. The inset shows the corresponding normalized absorption titration plot (λ_{obs} = 500 nm) fitted according to equation [S1].

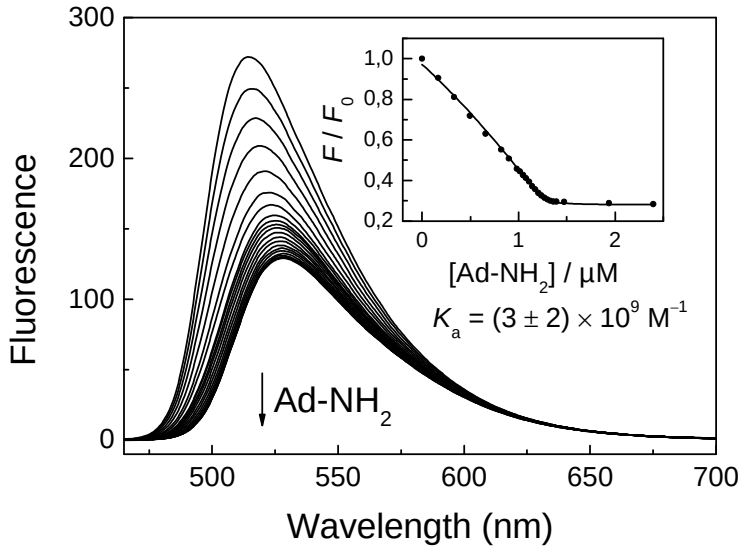


Fig. S3. Competitive fluorescence titration (λ_{exc} = 450 nm) of 2 µM acridine orange and 1.3 µM CB7 in 10 mM $(NH_4)_2HPO_4$, pH 7.2. The inset shows the corresponding fluorescence titration (λ_{em} = 510 nm) plot normalized to the initial fluorescence intensity and fitted according to equation [S2].

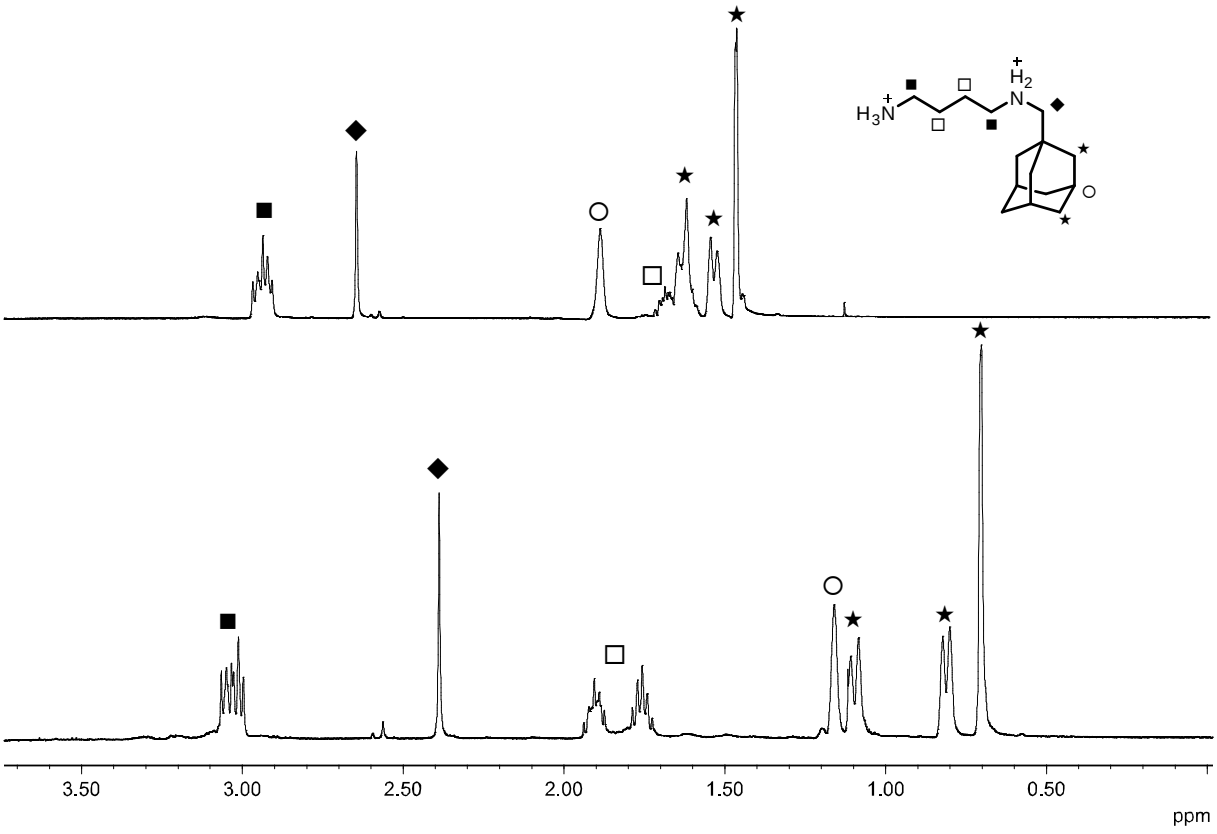


Fig. S4. ^{1}H NMR spectra of 5 mM **1** in D_2O/0.1% DCl in absence (top) and presence (bottom) of 5 mM CB7.

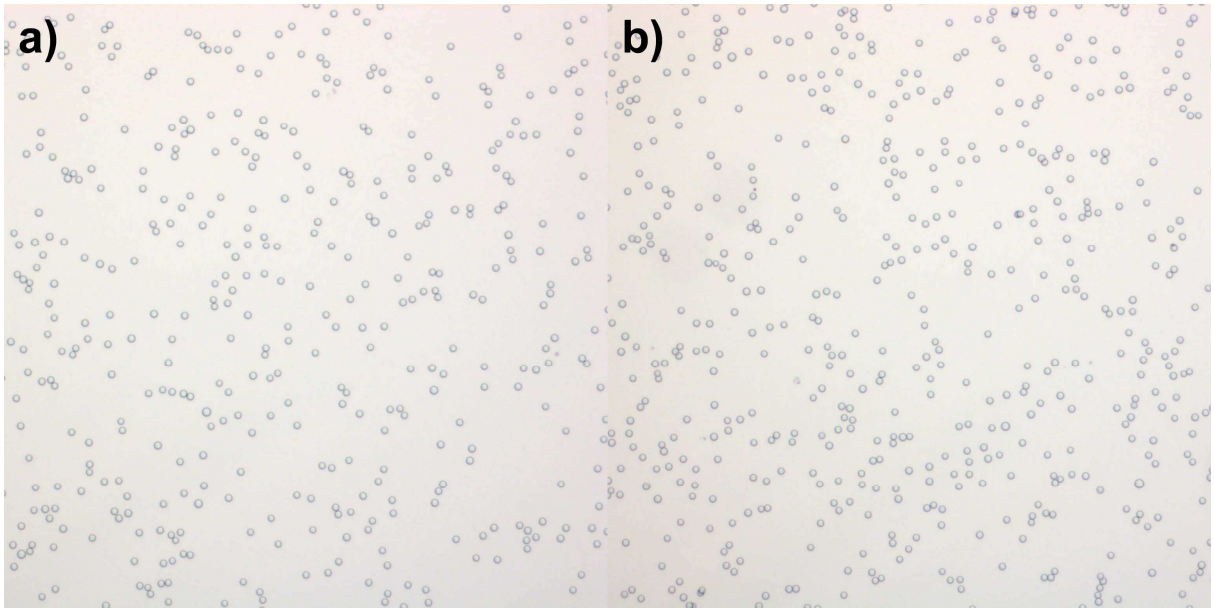


Fig. S5. Optical microscopy image of microsphere preparation **MS-3** a) before and b) after derivatization with adamantylmethylamine derivative **1**. Microsphere concentration was 10 mg/ml in 10 mM $(NH_4)_2HPO_4$, pH 7.2.

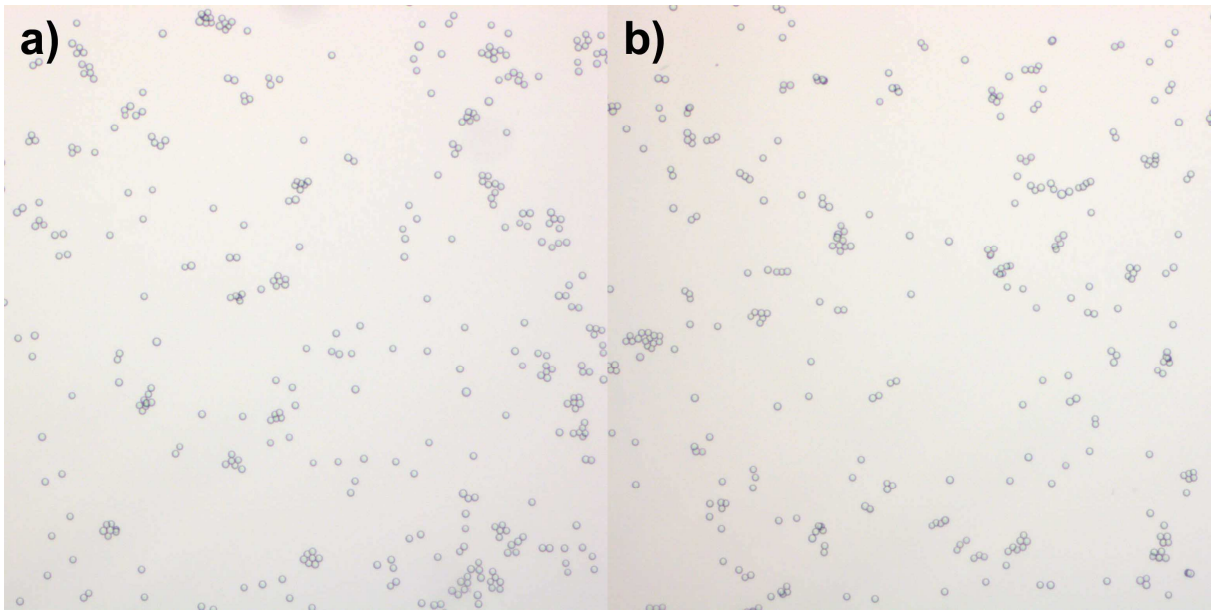


Fig. S6. Optical microscopy image of microsphere preparation **MS-6** a) before and b) after derivatization with adamantylmethylamine derivative **1**. Microsphere concentration was 10 mg/ml in 10 mM $(NH_4)_2HPO_4$, pH 7.2.

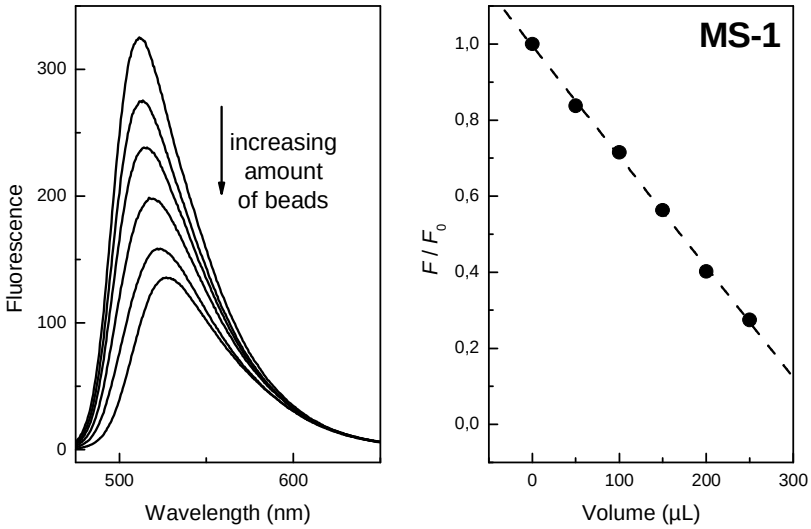


Fig. S-7. a) Variation of fluorescence intensity of a solution containing 2 µM acridine orange, 1.6 µM CB7 and varying amounts of previously derivatized polymer microspheres **MS-1** (10 mg/mL) in 10 mM $(NH_4)_2HPO_4$, pH 7.2 buffer.

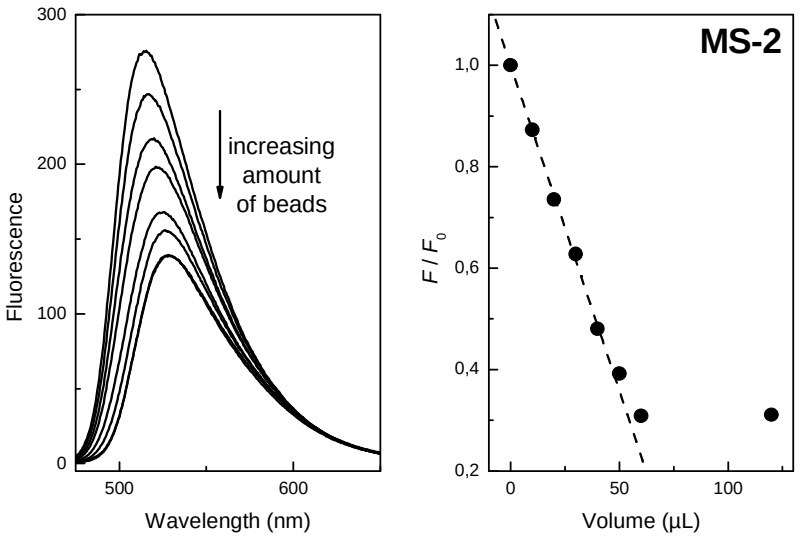


Fig. S-8. a) Variation of fluorescence intensity of a solution containing 2 µM acridine orange, 1.6 µM CB7 and varying amounts of previously derivatized polymer microspheres **MS-2** (10 mg/mL) in 10 mM $(NH_4)_2HPO_4$, pH 7.2 buffer.

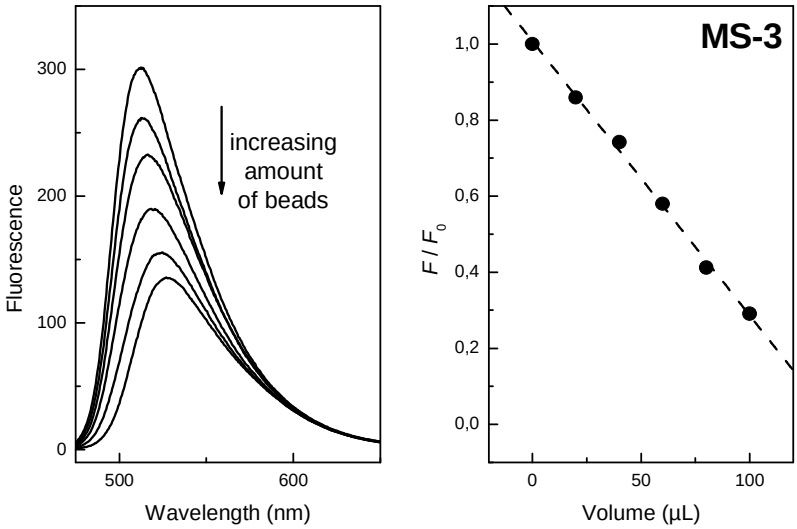


Fig. S-9. a) Variation of fluorescence intensity of a solution containing 2 µM acridine orange, 1.6 µM CB7 and varying amounts of previously derivatized polymer microspheres **MS-3** (10 mg/mL) in 10 mM $(NH_4)_2HPO_4$, pH 7.2 buffer.

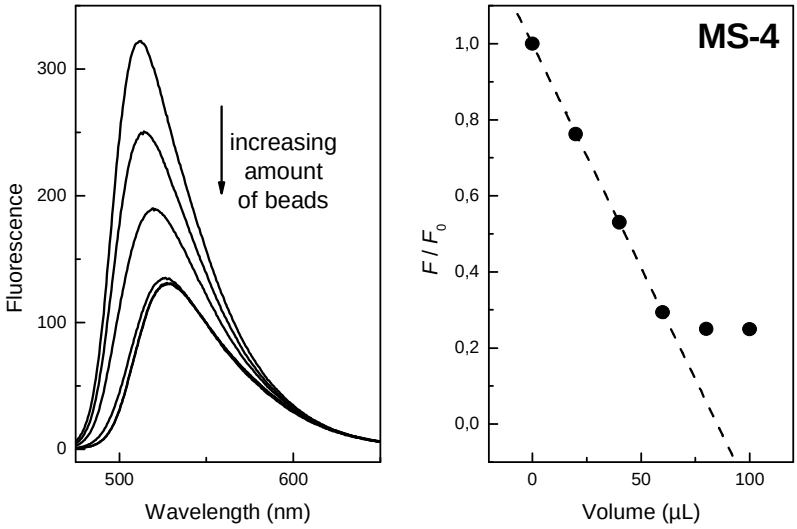


Fig. S-10. a) Variation of fluorescence intensity of a solution containing 2 µM acridine orange, 1.6 µM CB7 and varying amounts of previously derivatized polymer microspheres **MS-4** (10 mg/mL) in 10 mM $(NH_4)_2HPO_4$, pH 7.2 buffer.

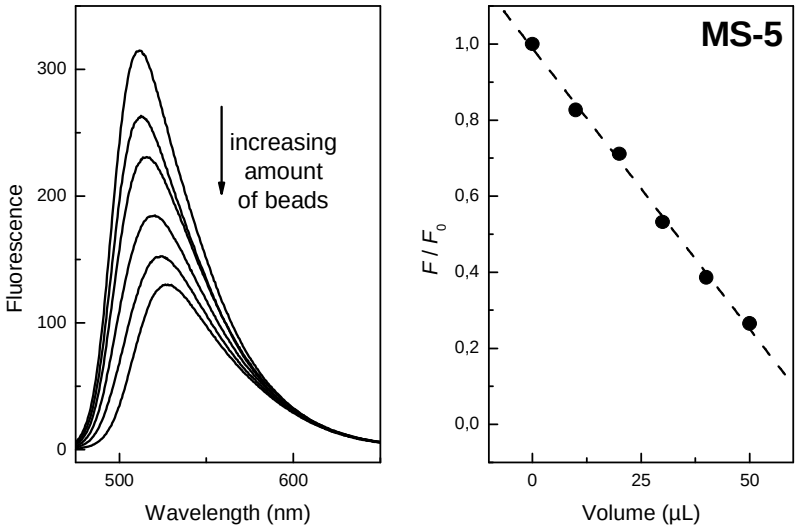


Fig. S-11. a) Variation of fluorescence intensity of a solution containing 2 µM acridine orange, 1.6 µM CB7 and varying amounts of previously derivatized polymer microspheres **MS-5** (10 mg/mL) in 10 mM $(NH_4)_2HPO_4$, pH 7.2 buffer.

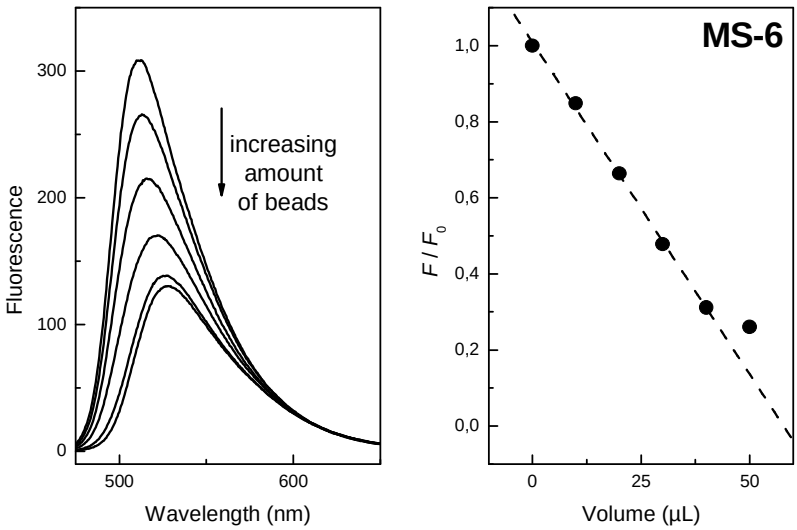


Fig. S-12. a) Variation of fluorescence intensity of a solution containing 2 µM acridine orange, 1.6 µM CB7 and varying amounts of previously derivatized polymer microspheres **MS-6** (10 mg/mL) in 10 mM $(NH_4)_2HPO_4$, pH 7.2 buffer.

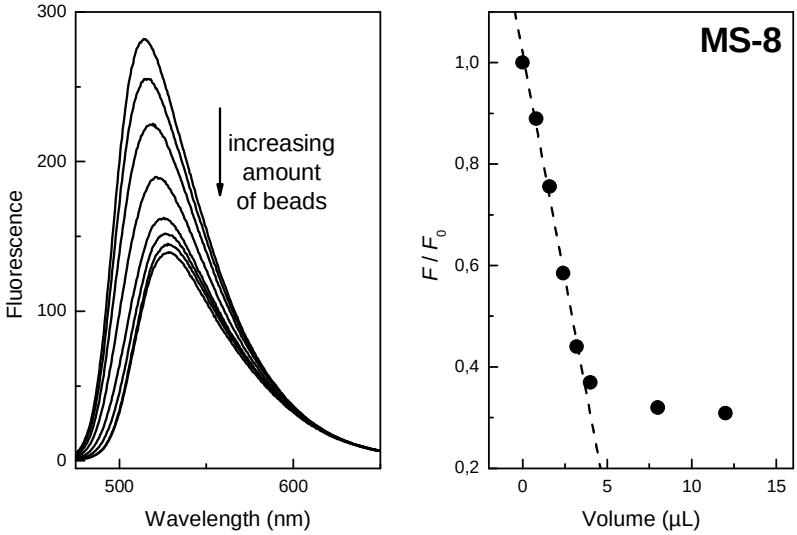


Fig. S-13. a) Variation of fluorescence intensity of a solution containing 2 µM acridine orange, 1.6 µM CB7 and varying amounts of previously derivatized polymer microspheres **MS-8** (10 mg/mL) in 10 mM $(NH_4)_2HPO_4$, pH 7.2 buffer.

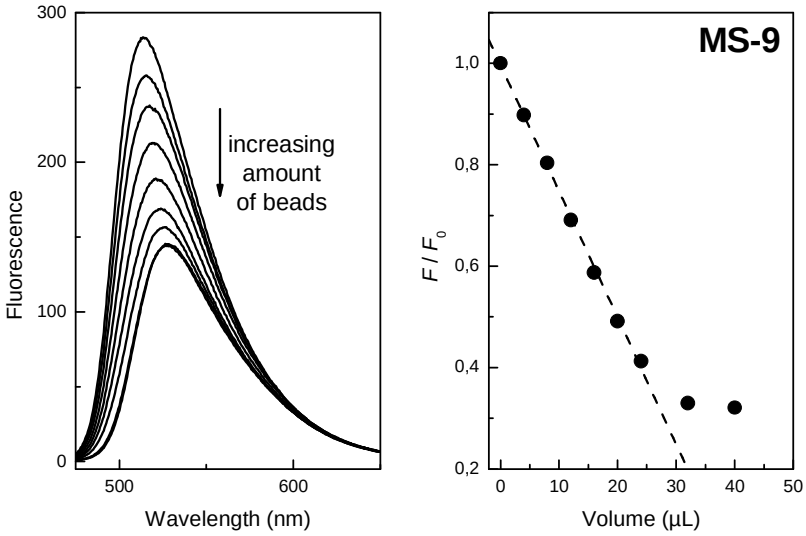


Fig. S-14. a) Variation of fluorescence intensity of a solution containing 2 µM acridine orange, 1.6 µM CB7 and varying amounts of previously derivatized polymer microspheres **MS-9** (10 mg/mL) in 10 mM $(NH_4)_2HPO_4$, pH 7.2 buffer.

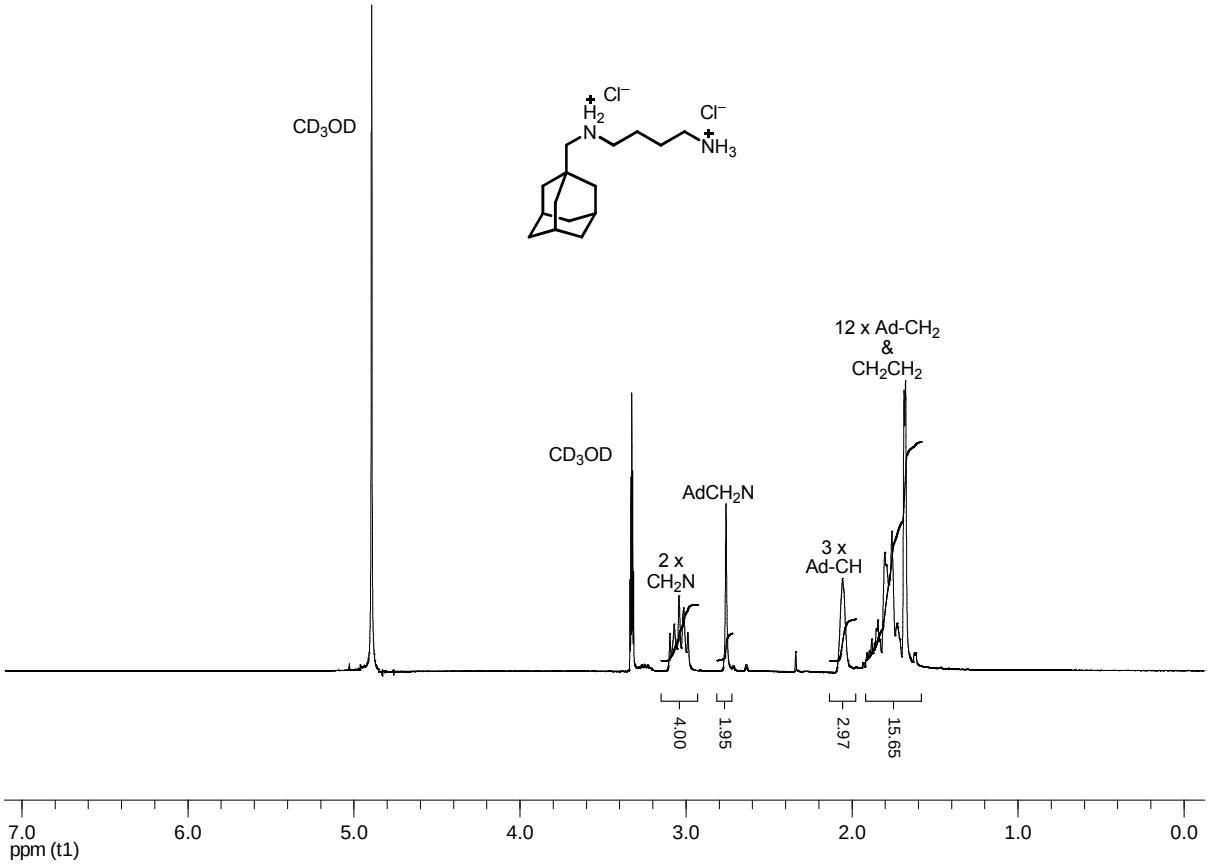


Fig. S-15. ^{1}H NMR of reaction product **1** in CD_3OD.

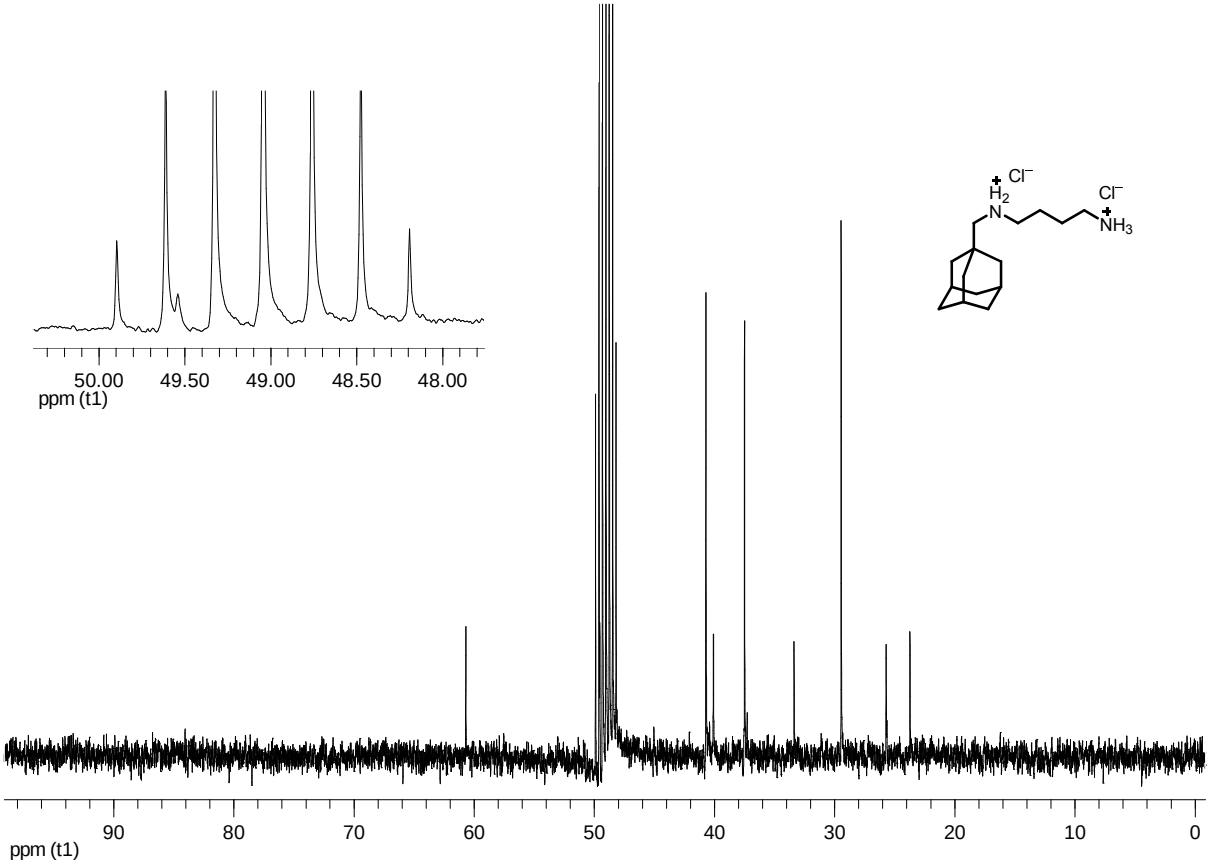


Fig. S-16. ^{13}C NMR of reaction product **1** in CD_3OD.