

Supporting Information

Synthesis and Photo-Postmodification of Zeolite L Based Polymer Brushes

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Table of Contents

1 General information	2
1.1 Techniques and materials	2
1.2 Analytical methods	2
2 GPC data	4
3 ¹H NMR spectra for determination of monomer incorporation ratios	5
4 IR spectra of 12 and photo-postmodified 20a-g	6
5 Zeta potentials	10
6 Control experiments	10
6.1 Control experiment 1: Photo-postmodification in absence of acyloin moieties	10
6.2 Control experiment 2: Postmodification of 12 in the dark	11
6.3 Control experiment 3: Fluorophore labeling without preceding photo-postmodification	11
7 Literature	11

1 General information

1.1 Techniques and materials

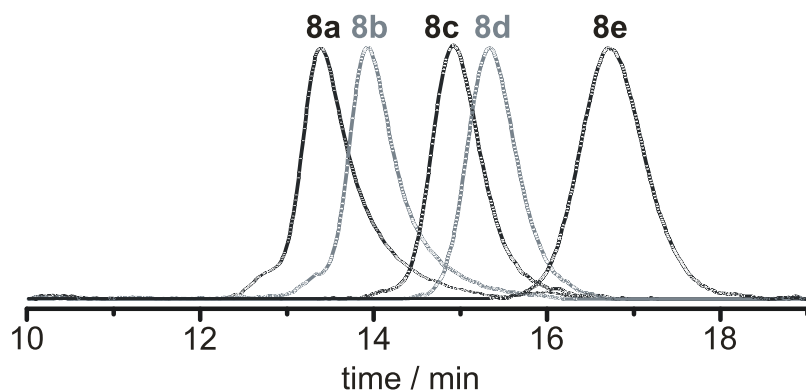
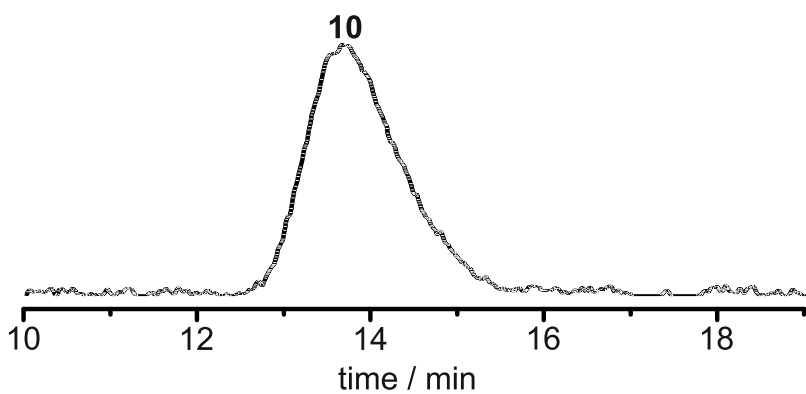
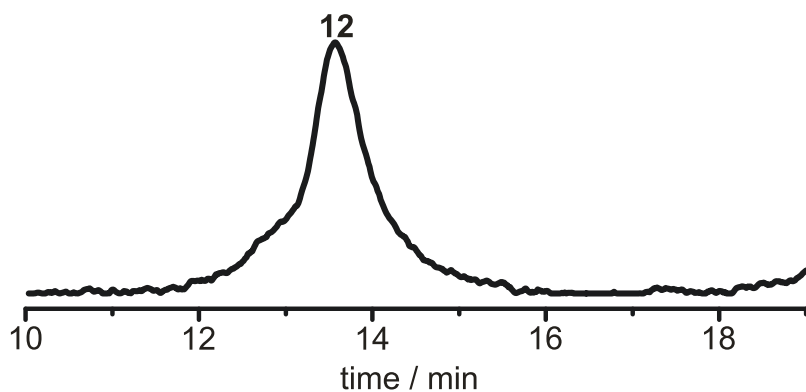
Zeolite L crystals were either purchased from Süd-Chemie (1.0-1.5 × 1.0 μm) or synthesized following reported procedures¹ (4.0 × 1.0 μm). **Reagents** were purchased from ABCR, Aldrich, Acros Organics, Alfa Aesar, Merck or TCI and used as received. Rhodamin-labelled ConA (5.0 mg/mL in HEPES buffer) was purchased from VECTOR Laboratories and Oyster[®]-488-tagged SAV (1.0 mg/mL in PBS buffer) from Luminartis. Styrene and MMA were destabilized by distillation over CaH₂. **Solvents** that were used for reactions with oxygen and/or moisture sensitive starting materials, intermediates or products were dried by standard procedures, stored under Ar and cooling or freshly distilled prior to use: Benzene (Na); CH₂Cl₂ (P₂O₁₀); Et₂O (1. KOH, 2. K/Na-alloy); THF (1. KOH, 2. Na, 3. K). DMF (99.8%, AcroSeal[®], Extra Dry over Molecular Sieve) and toluene (99.85%, AcroSeal[®], Extra Dry over Molecular Sieve) were purchased from Acros Organics. The solvents used for oxygen sensitive radical reactions were degassed by three freeze cycles or by bubbling of Ar through the reaction mixture for three minutes prior to the reaction. Reactions with oxygen and/or moisture sensitive starting materials, intermediates or products were conducted using Schlenk technique in glassware that was dried with a heat gun under high vacuum and flooded with argon prior to the reaction. Solvents and liquid reagents were measured with syringes and injected into the reaction tube through a septum plug. The reaction process was observed by TLC if possible. **Thin layer chromatography** (TLC) was conducted with glass plates from Merck (Silica 60, F₂₅₄). UV-active compounds were detected with an UV-lamp (λ = 254 nm). Additionally, the chromatograms of all compounds were made by dipping of the plates into a dip solution and subsequent warming with a heat gun. Potassium permanganate-dip solution: 5 g NaHCO₃, 1.5 g KMnO₄, 400 mL H₂O. **Flash chromatography** (FC) was conducted on Silica 60 (grit size 40–63 μm) from Merck with an argon pressure of ca. 0.4 bar. The solvents used for FC were technical grade and were distilled prior to use. Diethylether was distilled over FeSO₄ and KOH. EtOAc, acetone, dichloromethane, methanol and pentane were distilled without a drying agent. **Ultra-pure water** (18.2 mQ) was generated with an Elga Maxima. **Centrifugations** were performed with a microprocess guided centrifuge Labofuge 200 from Heraeus or mini G from IKA. **Sonication** was done in a Bandelin Sonorex RK 100.

1.2 Analytical methods

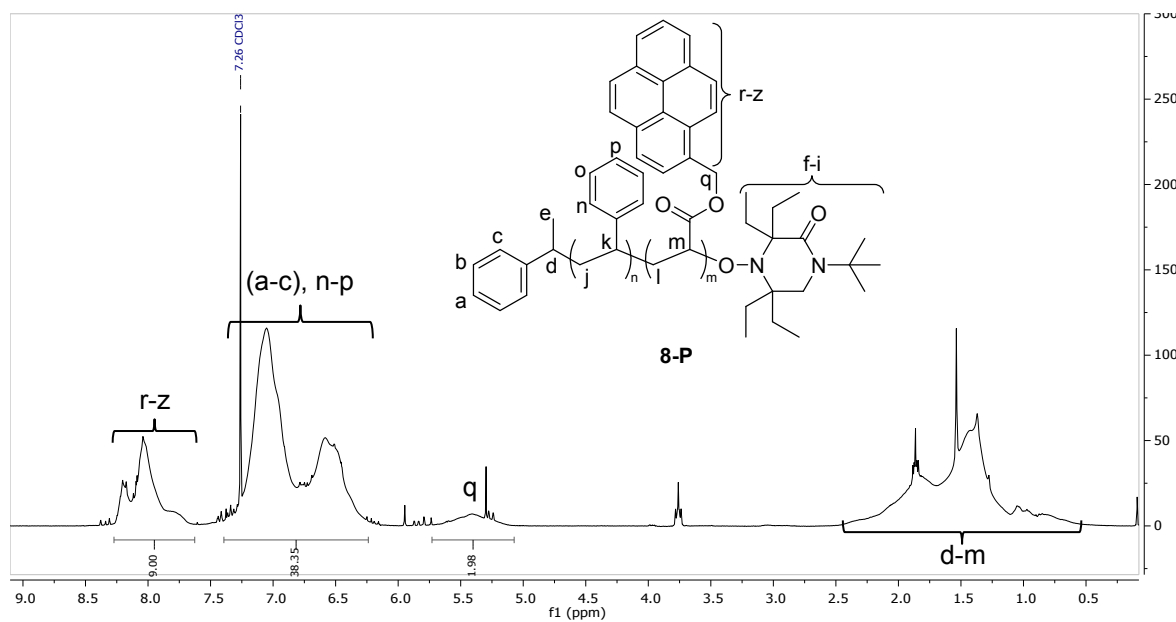
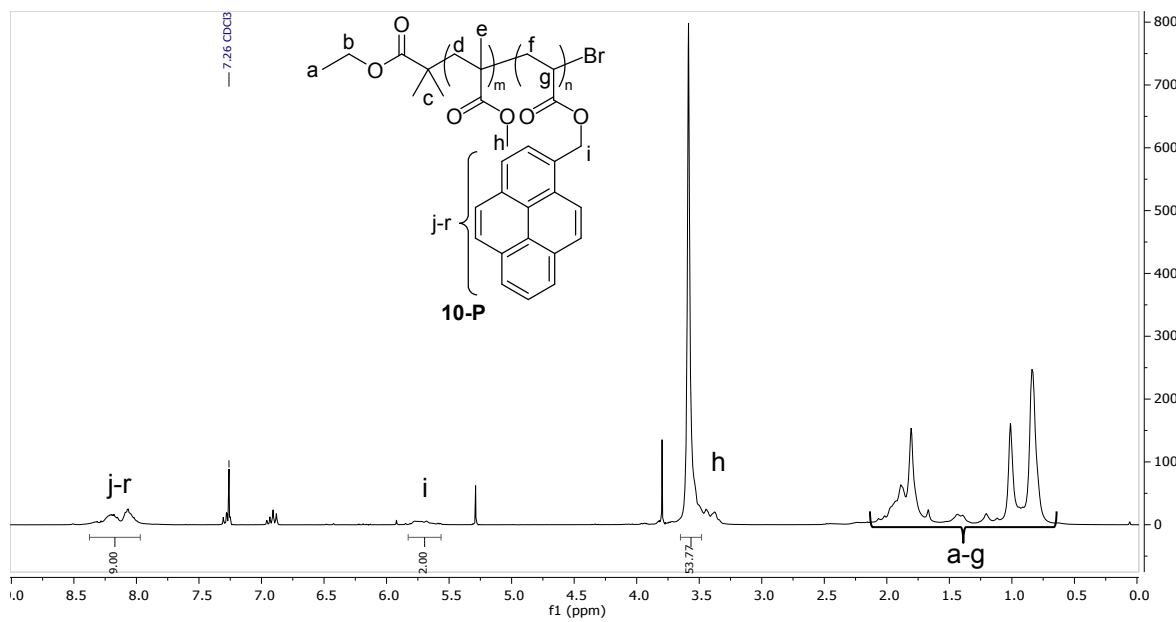
Melting points were determined with an SMP10 from Stuart Scientific and are uncorrected. **IR** spectra were recorded with a Digilab FTS 4000 equipped with a Specac MKII Golden Gate Single Reflection ATR System. The absorption bands are specified in wave numbers (cm⁻¹) with following abbreviations for their intensity: *br* = broad signal, *s* = strong, *m* = medium, *w* = weak. **¹H-NMR** spectra were recorded in the NMR spectroscopy lab of the Organic Institute of the Westfälische Wilhelms-Universität Münster (Münster University) in deuterated solvents with a DPX 300 (300 MHz; at 298 K) from Bruker with a preset pulse program. The chemical shifts δ in ppm are stated in relation to the resonance signal of the ¹H nuclei of tetramethylsilane (δ = 0.00 ppm). For a calibration of the spectra we correlated the resonance signal of the residual solvent protons with the value reported in the literature² (δ_{CHCl₃} = 7.26 ppm). The multiplicities of the resonance signals are abbreviated with *s* (singlet), *d* (doublet), *t* (triplet) or *m* (multiplet). Broad signals are abbreviated with *br s* (broad signal). Coupling constants *J* are specified in Hz. **¹³C-NMR** spectra were recorded in the NMR spectroscopy lab of the Organic Institute of the

Westfälische Wilhelms-Universität Münster (Münster University) in deuterated solvents with a DPX 300 (75 MHz; at 298 K) from Bruker with a preset pulse program. The chemical shifts δ in ppm are stated in relation to the residual resonance signal of the internal standard tetramethylsilane ($\delta = 0.00$ ppm). For a calibration of the spectra we used the deuterium coupled resonance signal of the particular solvent.² 90° DEPT-13C- and 135° DEPT-13C-spectra were recorded, as well as H,H-COSY (GCOSY) or C,H correlation experiments (GHSQC, GHMBC) were performed as a further tool for the interpretation of the ¹³C-NMR spectra, if necessary. **High resolution mass spectra (HRMS)** were recorded in the mass spectrometry lab of the Organic Institute of the Westfälische Wilhelms-Universität Münster (Münster University) as electrospray ionisation spectra (ESI spectra) with a Bruker Daltonics MicroTof (exact mass). The detected signals *m/z* are specified in u. **Gas chromatography (GC)** was performed with a Agilent Technologies 7890 GC System with a Hewlett Packard HP-5 column (30 m × 0.32 mm, film thickness 0.25 μ m) and FID by using H₂ (ca. 1 bar) as a carrier gas. **Fluorescence microscopy** images were recorded with an epi-fluorescence microscope Olympus reflected fluorescence system CKX41, which was equipped with a Lumen Dynamics X-Cite Series 120Q excitation source, excitation/emission filters and an Olympus U-LS30-3 camera. **Zeta potential** measurements were recorded with a DTS Zetasizer Nano ZS from Malvern. The measurements were performed in ammonium formate buffer (20 mM, pH = 7.4, 25 °C) at room temperature in a cell purged with dry methanol prior to the measurements. For interpretation the Smoluchowski equation was applied. **Gel permeation chromatography (GPC)** was carried out in degassed THF as eluent with a flow rate of 1.0 mL/min at rt on a system consisting of a Knauer HPLC Pump 64, a set of two PLgel 5 μ m MIXED-C columns (300 × 7.5 mm, Polymer Laboratories) and a Shodex RI differential refractometer detector. Calibration was done with Varian polystyrene (PS) calibration kit, PL2010-0100, S-M-10, Lot 103. Signals of the PS standards ranged from $M_n = 1530$ to 1319000 g/mol. Data analysis was done with Polymer Standards Service WinGPC Compact V.7.20 software. **Holographic optical tweezers (HOT)** experiments were performed on an inverted fluorescence microscope Nikon Eclipse Ti with a high numerical aperture microscope objective (Nikon Apo TIRF, 100×/1.49 Oil-immersion) and a Nd:YVO₄ laser ($\lambda = 1064$ nm, power at sample plane 400 mW approx.) as light source for optical trapping. The implemented HOT system is described in detail in literature.³

2 GPC data

Figure SI1: GPC traces of unbound polymer from preparation of **8a-e**.Figure SI2: GPC trace of unbound polymer from preparation of **10**.Figure SI3: GPC trace of unbound polymer from preparation of **12**.Table SI1: GPC data of unbound polymer from preparation of **8a-e**, **10** and **12**.

Entry	Zeolite	7 / mol-%	Conv. / %	$M_{n, \text{theo.}}$ / kg/mol	$M_{n, \text{exp.}}$ / kg/mol	PDI
1	8a	0.05	50	120	67	1.23
2	8b	0.10	56	66	38	1.22
3	8c	0.25	61	29	17	1.12
4	8d	0.50	60	14	11	1.13
5	8e	1.00	25	3.0	2.9	1.15
6	10	0.10 EBiB (9)	72	82	59	1.31
7	12	0.10	60	73	74	1.21

3 ^1H NMR spectra for determination of monomer incorporation ratiosFigure S14: Representative ^1H NMR spectrum of unbound polymer from synthesis of **8**.Figure S15: ^1H NMR spectrum of unbound polymer from synthesis of **10**.

4 IR spectra of 12 and photo-postmodified 20a-g

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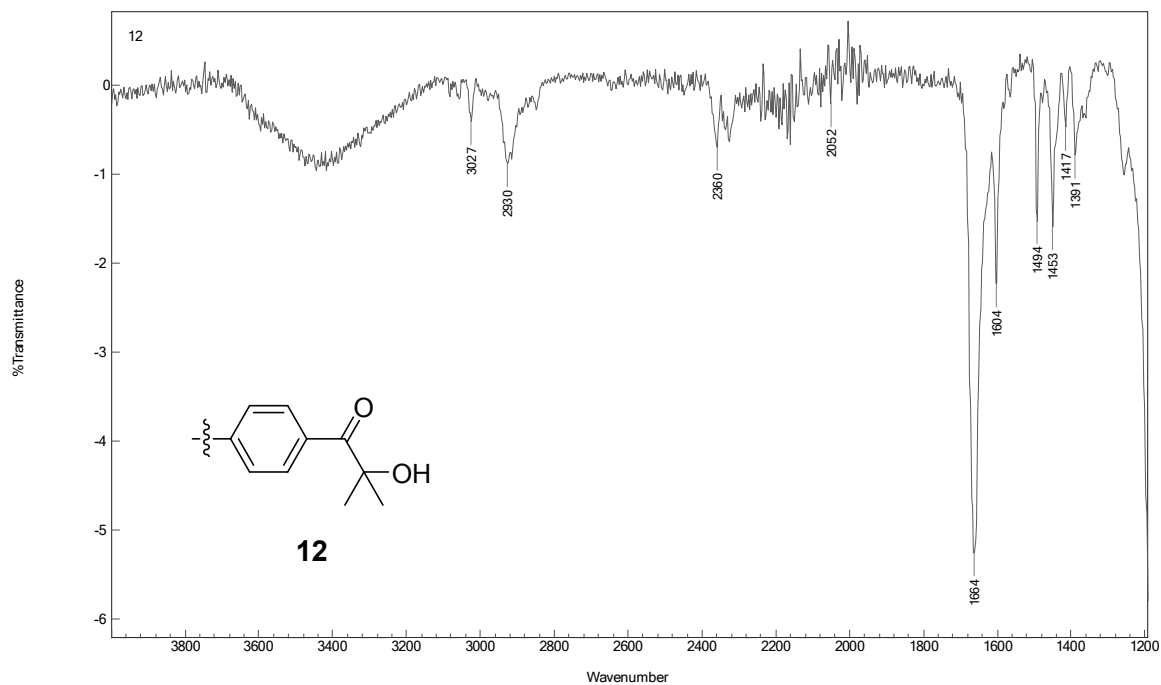


Figure SI6: IR spectrum of 12.

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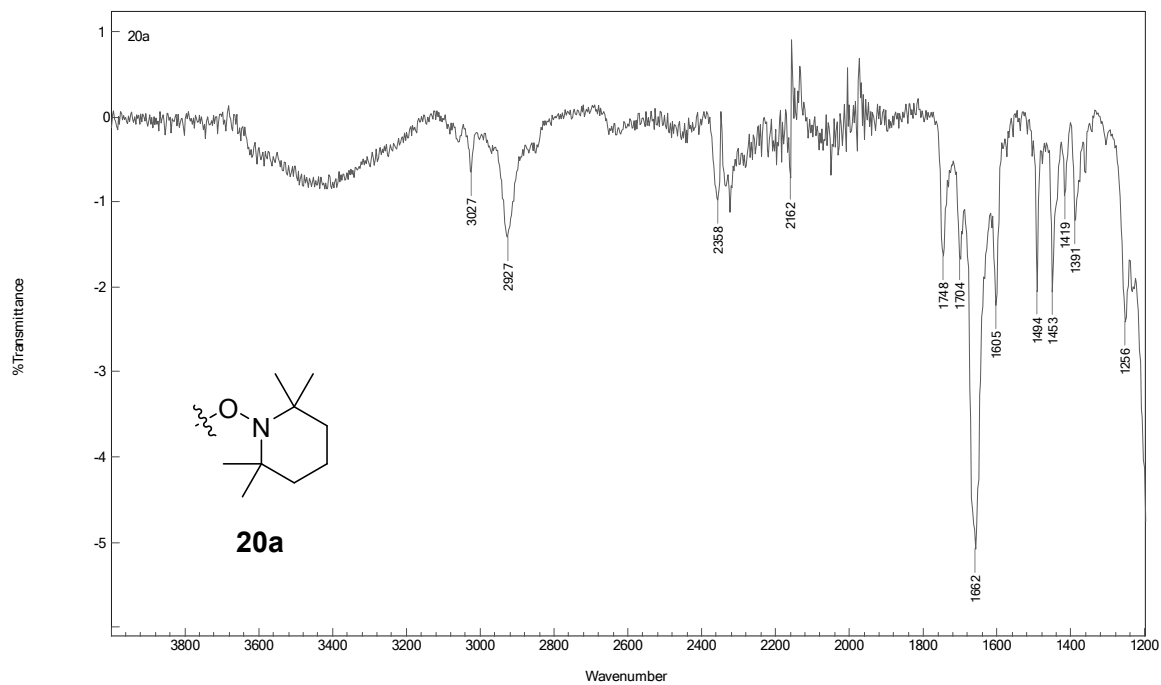


Figure SI7: IR spectrum of 20a.

Supporting Information

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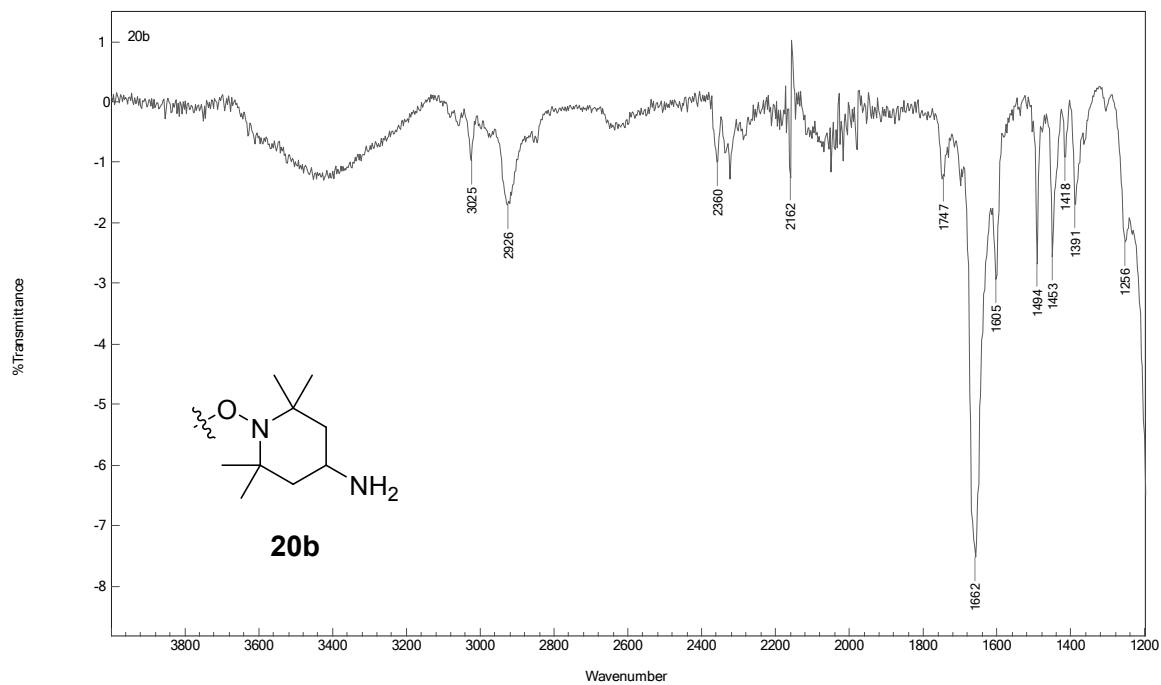


Figure SI8: IR spectrum of **20b**.

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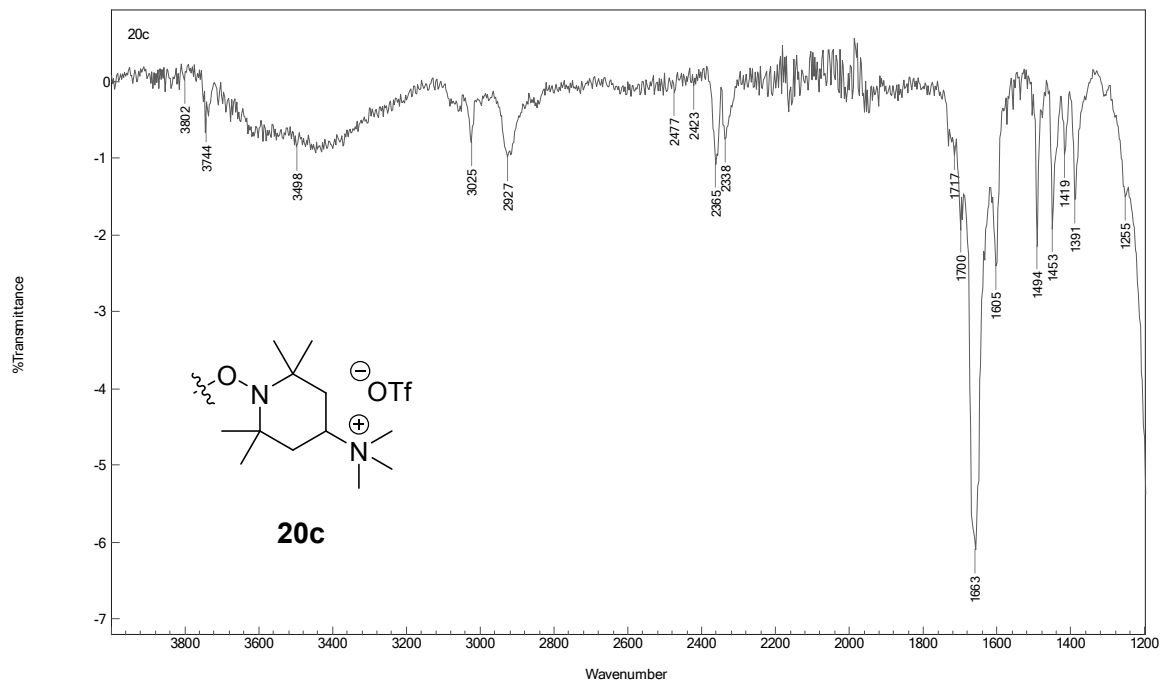
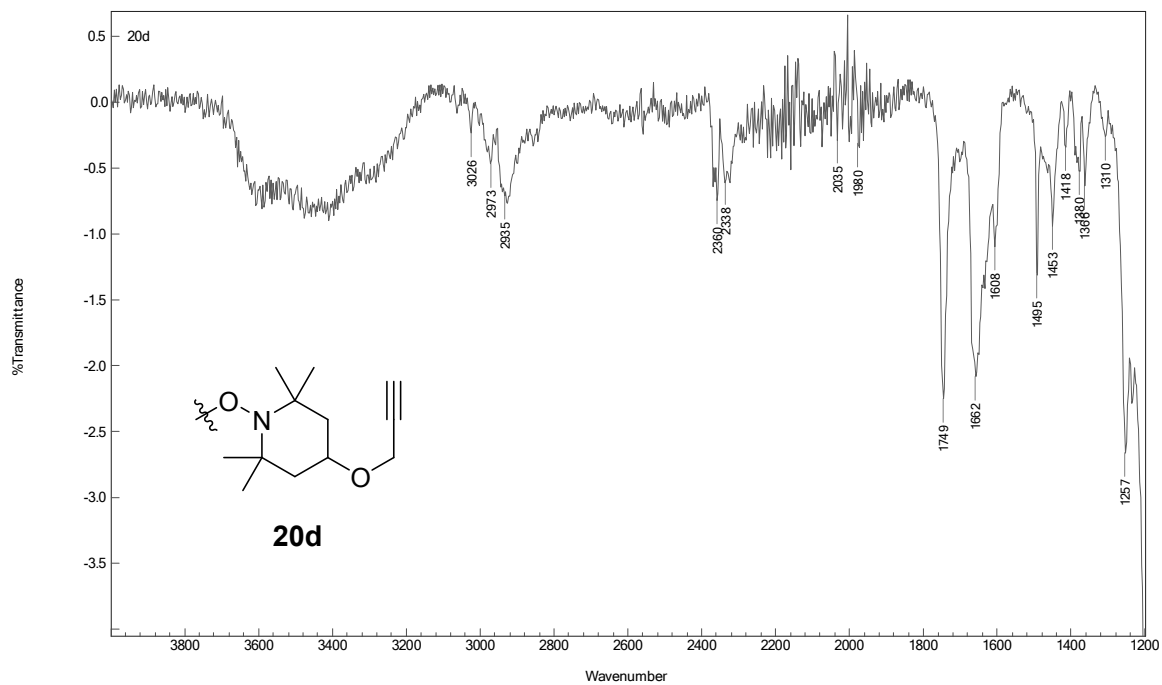


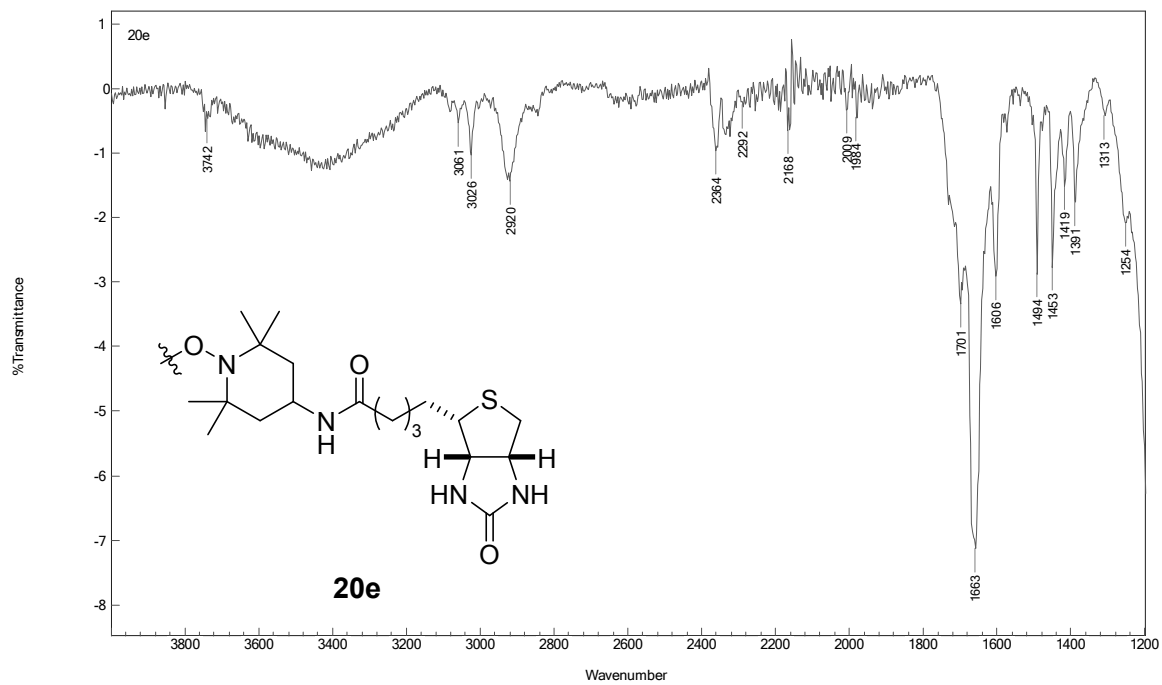
Figure SI9: IR spectrum of **20c**.

Supporting Information

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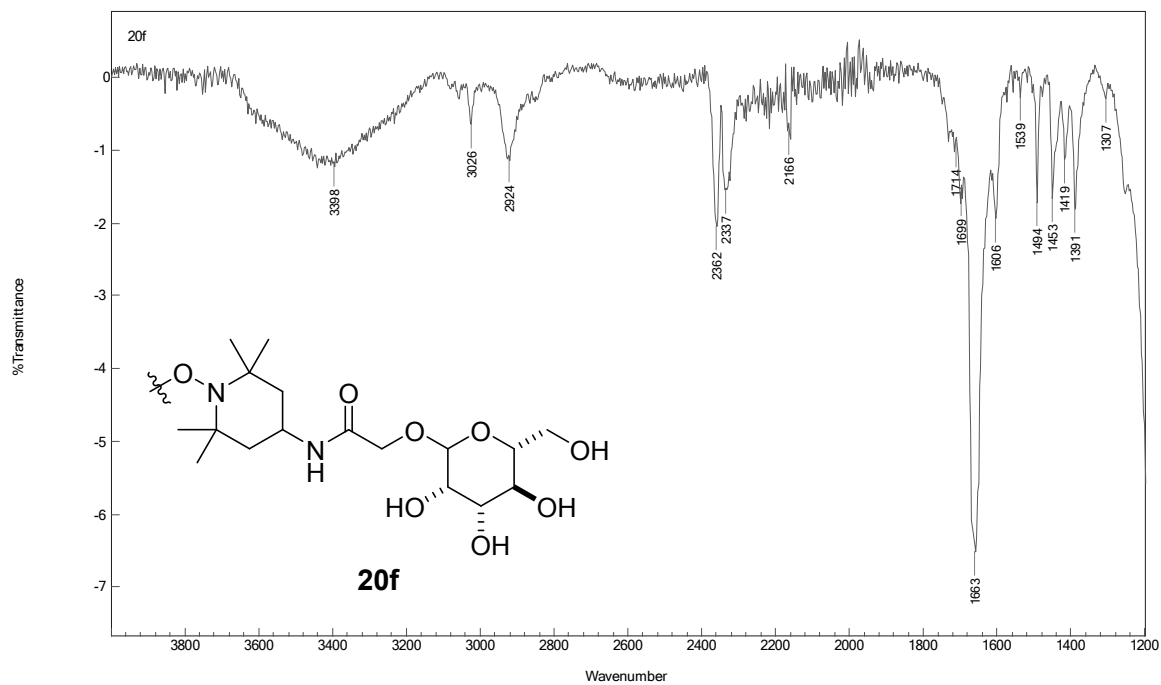


Figure S112: IR spectrum of **20f**.

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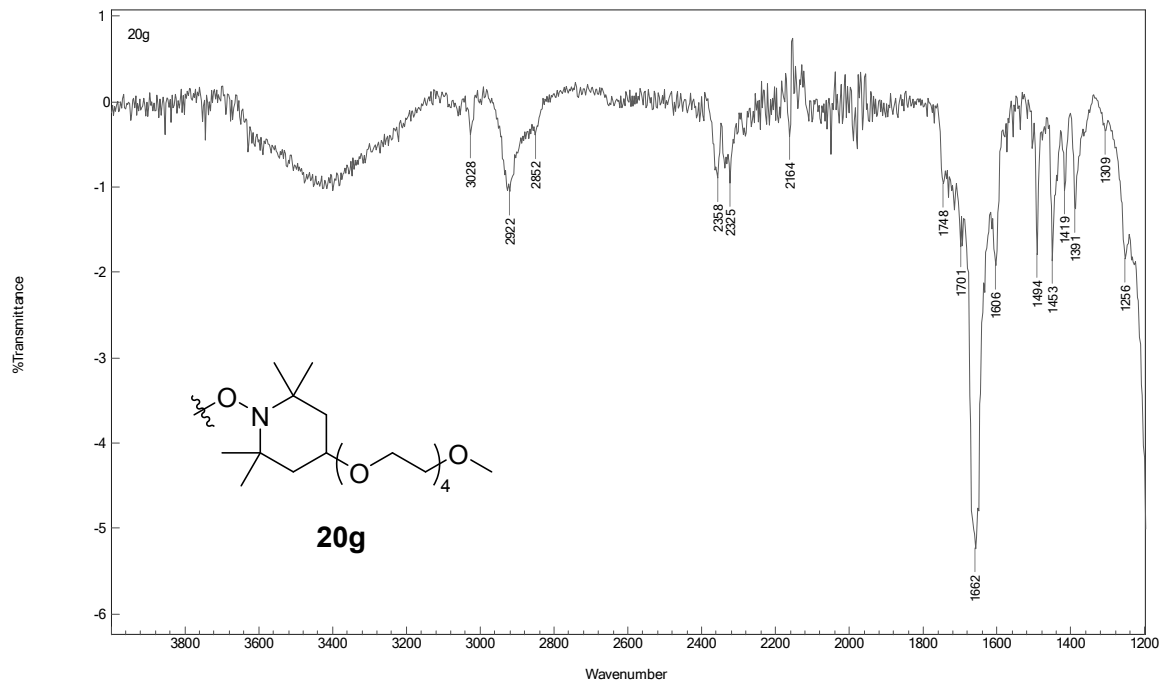


Figure S113: IR spectrum of **20g**.

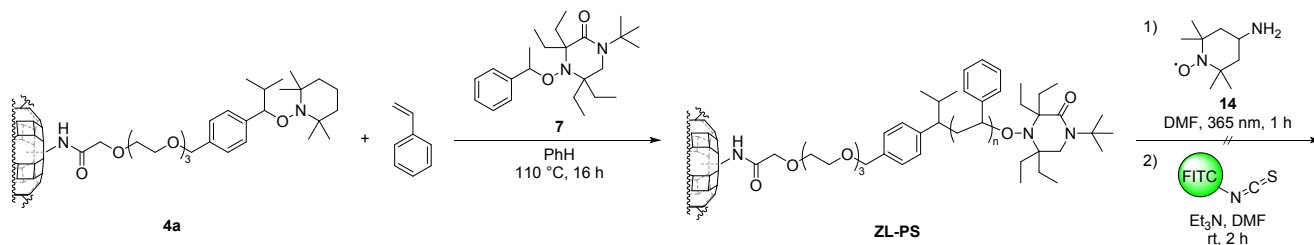
5 Zeta potentials

Table SI2: Zeta potentials of unfunctionalized zeolite L, **1a/b**, **12** and **20a-g** in ammonium formate (20 mM, pH = 7.4).

Entry	1	2	3	4	5	6	7	8	9	10	11	12
Zeolite	1×1 μm unfunctionalized	1×4 μm unfunctionalized	1a	1b	12	20a	20b	20c	20d	20e	20f	20g
ζ / mV	-57.9	-59.8	34.5	41.9	-30.4	-43.9	15.9	38.4	-27.6	-40.2	-38.8	-25.2

6 Control experiments

6.1 Control experiment 1: Photo-postmodification in absence of acyloin moieties

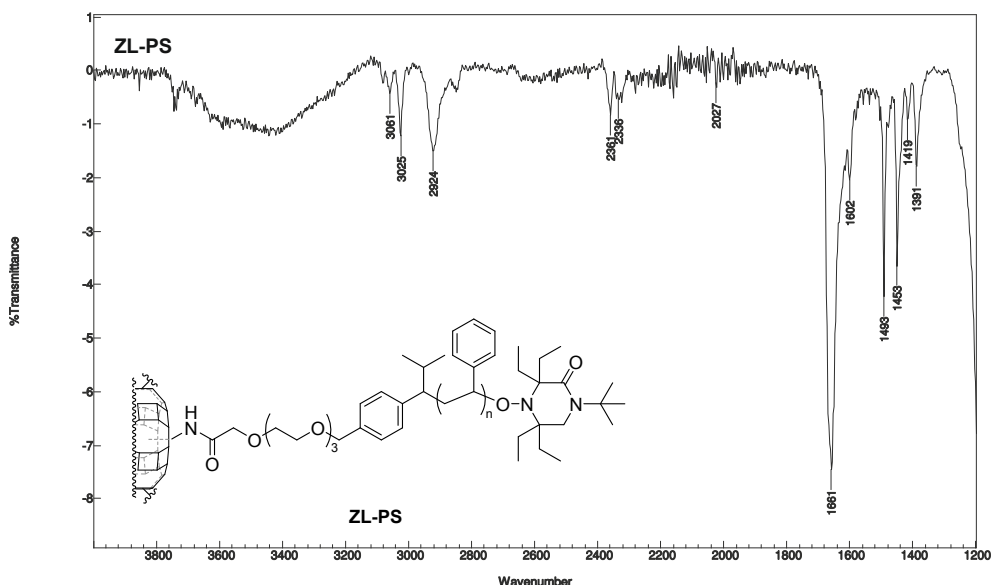


Scheme SI1: Synthesis and photo-postmodification of zeolite L based polystyrene brushes **ZL-PS** in absence of acyloin moieties.

To exclude non-specific reaction of nitroxides with polymer brush crystals, polystyrene brush particles were synthesized in absence of monomer **11** and used for the photo-postmodification experiment subsequently (Scheme SI1).

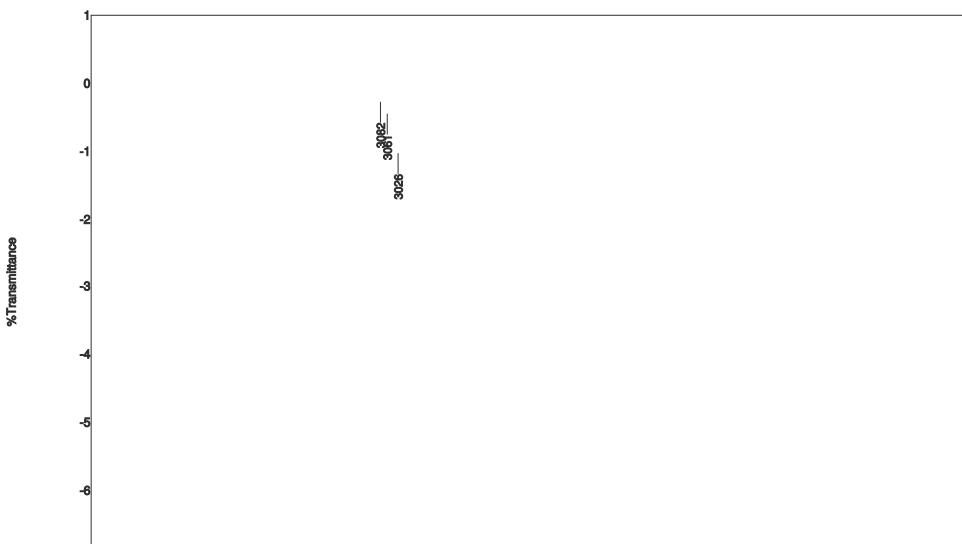
Alkoxyamine-modified zeolite L crystals **4a** (20 mg), styrene (2.0 mmol) and alkoxyamine **7** (0.1 mol-%) were added to benzene (100 μL). The suspension was degassed in three freeze-thaw-cycles, sonicated for 3 min and stirred at 110 °C for 16 h. Polymer brush particles **ZL-PS** and unbound polymer were separated in three centrifugation-washing-cycles with THF (3 × 10 mL) and solvents were evaporated *in vacuo*. Unbound polymer was dissolved in a minimum amount of CH₂Cl₂, precipitated from methanol and dried *in vacuo*. GPC: $M_n = 41$ kg/mol; PDI = 1.1. ζ-potential in ammonium formate buffer (AFB, 20 mM, pH = 7.4, 25 °C): ζ = -42.3 mV.

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Figure SI14: IR spectrum of **ZL-PS**.

Polystyrene brush particles **ZL-PS** (10 mg) were dispersed in DMF (0.3 mL) and nitroxide **14** (2.0 mg, excess) was added to the suspension. The mixture was sonicated for 3 min and stirred under LED irradiation ($\lambda = 365$ nm, 3 mW) at rt for 1 h. Polymer brush particles were washed in four centrifugation-washing-cycles with DMF (2.0 mL), MeOH (2×2.0 mL) and DCM (2.0 mL) and dried *in vacuo*. ζ -potential in ammonium formate buffer (AFB, 20 mM, pH = 7.4, 25 °C): $\zeta = -42.7$ mV.

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Figure SI15: IR spectrum of **ZL-PS** after incubation with **14**.

Resulting polymer brush particles (2.0 mg) were dispersed in DMF (2.0 mL) and FITC (1.0 mg, 3.6 μ mol, excess) and Et₃N (10 μ L, 72 μ mol, excess) were added. The suspension was sonicated for 3 min and stirred at rt for 2 h. Polymer brush particles were washed in five centrifugation-washing-cycles

with DMF (2.0 mL), MeOH (2×2.0 mL) and DCM (2×2.0 mL), dried *in vacuo* and fluorescence samples were prepared.

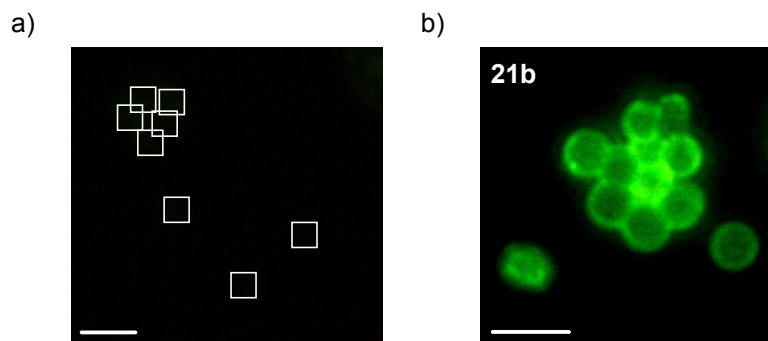
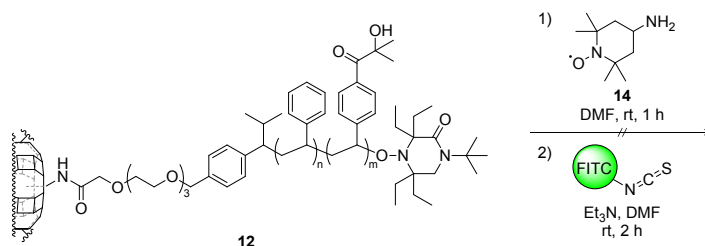


Figure SI16: Fluorescence images of: a) **ZL-PS** after incubation with **14** and FITC labeling (white squares indicate position of zeolite L crystals); b) FITC labeled **21b**. Scale bars are 2 μm .

Particles resulting from photo-postmodification of acyloin-free **ZL-PS** with **14** show no peaks at 1705 and 1748 cm^{-1} in the FTIR (Figure SI15), which were assigned to the C=O stretching mode of the TEMPO ester moiety. Furthermore there is no detectable change of the ζ -potential ($\zeta = -42.3$ mV to $\zeta = -42.7$ mV) and labeling with FITC leads to particles that show barely any fluorescence (Figure SI16a), which proves the necessity of incorporation of **11** for the postmodification.

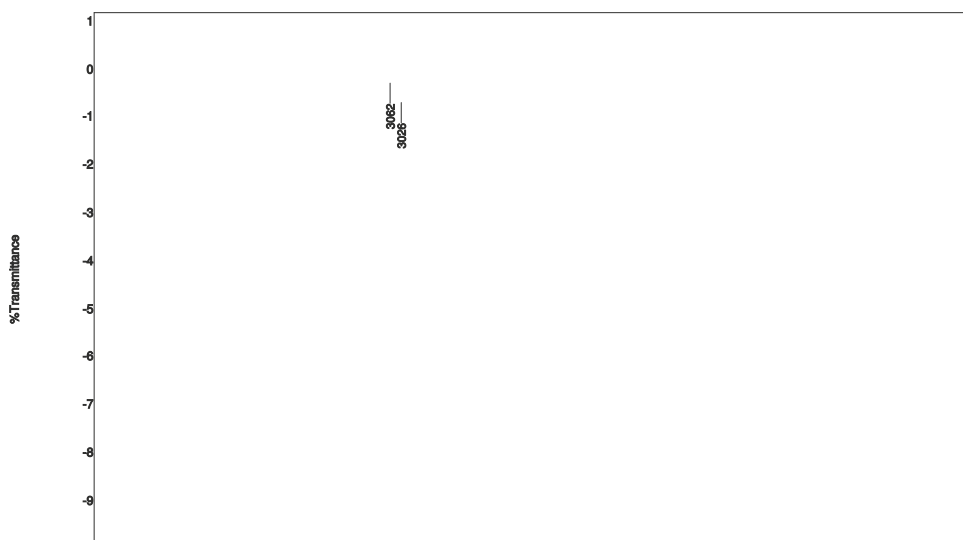
6.2 Control experiment 2: Postmodification of **12** in the dark



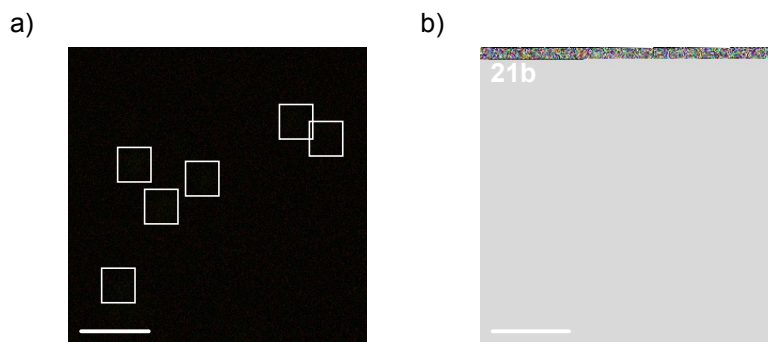
Scheme SI2: Photo-postmodification of **12** with **14** in the dark.

To prove that irradiation is needed for the postmodification, polyacyloin brush **12** was incubated with **14** under exclusion of light (Scheme SI2).

Polyacyloin brush particles **12** (15 mg, $\zeta = -30.4$ mV) were dispersed in DMF (0.3 mL) and nitroxide **14** (2.0 mg, excess) was added to the suspension. The mixture was sonicated for 3 min. The flask was wrapped in aluminum foil and the mixture stirred at rt for 1 h. Polymer brush particles were washed in four centrifugation-washing-cycles with DMF (2.0 mL), MeOH (2×2.0 mL) and DCM (2.0 mL) and dried *in vacuo*. ζ -potential in ammonium formate buffer (AFB, 20 mM, pH = 7.4, 25 $^{\circ}\text{C}$): $\zeta = -25.4$ mV.

Varian Resolutions ProFigure SI17: IR spectrum of **12** after incubation with **14** in the dark.

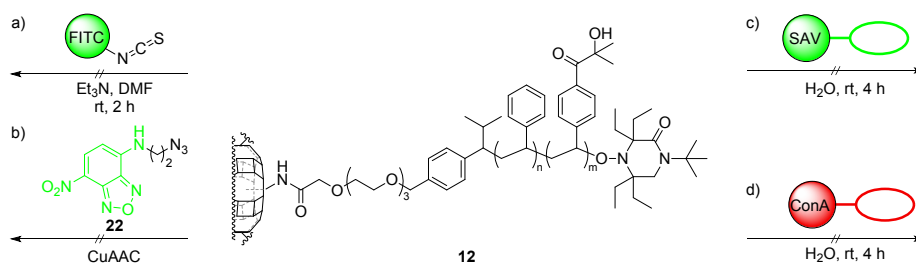
Resulting polymer brush particles (2.0 mg) were dispersed in DMF (2.0 mL) and FITC (1.0 mg, 3.6 μmol , excess) and Et_3N (10 μL , 72 μmol , excess) were added. The suspension was sonicated for 3 min and stirred at rt for 2 h. Polymer brush particles were washed in five centrifugation-washing-cycles with DMF (2.0 mL), MeOH (2×2.0 mL) and DCM (2×2.0 mL), dried *in vacuo* and fluorescence samples were prepared.

Figure SI18: Fluorescence images of: a) **12** after incubation with **14** in the dark and FITC labeling (white squares indicate position of zeolite L crystals); b) FITC labeled **21b**. Scale bars are 2 μm .

Particles resulting from postmodification of **12** with **14** in absence of light show no peaks at 1705 and 1748 cm^{-1} in the FTIR (Figure SI17), which were assigned to the C=O stretching mode of the TEMPO ester moiety. Furthermore there is only a slight change of the ζ -potential within the margin of error ($\zeta = -30.4$ mV to $\zeta = -25.4$ mV) and labeling with FITC leads to particles that show barely any fluorescence (Figure SI18a), which proves the necessity of UV-light for the postmodification.

6.3 Control experiment 3: Fluorophore labeling without preceding photo-postmodification

Supporting Information



Scheme SI3: Immediate fluorophore labeling of **12** without preceding photo-postmodification: a) FITC conjugation; b) CuAAC-click reaction with **22**; c) Immobilization of labeled SAV; d) Attachment of labeled ConA.

To prove specificity of the fluorophore labeling, polyacryloin brush particles **12** (2.0 mg) were converted with fluorophores under respective conditions and without preceding photo-postmodification (Scheme SI3).

Polymer brush particles **12** (2.0 mg each) were reacted with FITC, **22**, Oyster[®]-488 labeled SAV or rhodamine labeled ConA according to the general procedures (see Experimental Section). Fluorescence microscopy revealed barely any fluorescence after labeling with FITC or **22** (Figure SI19a,b). Immobilization of SAV or ConA onto **12** results in comparatively weak fluorescent particles (Figure SI19c,d). Remaining residual adsorption of the proteins might occur from non-specific adsorption due to the polystyrene part of the polymer shell, but protein decoration is highly increased by preceding conjugation of the respective biomolecules.

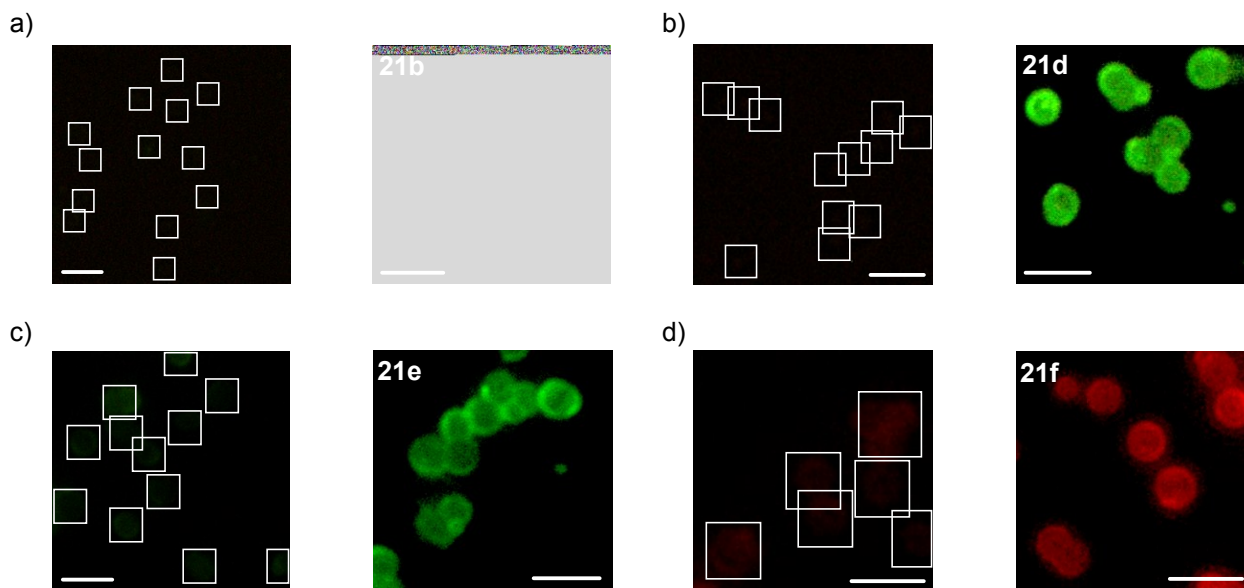


Figure SI19: Fluorescence images of: a) FITC conjugation; b) CuAAC-click reaction with **22**; c) Immobilization of labeled SAV; d) Attachment of ConA. Left: Control experiments with **12** (no preceding photo-postmodification). Right: Particles labeled according to the general procedures. White squares indicate position of zeolite L crystals. Scale bars are 2 μ m.

7 Literature

- 1 A. Z. Ruiz, D. Brühwiler, T. Ban and G. Calzaferri, *Monatsh. Chem.*, 2005, **136**, 77-89.

Supporting Information

- 2 H. E. Gottlieb, V. Kotlyar and A. Nudelman, *J. Org. Chem.*, 1997, **62**, 7512-7515.
- 3 Á. Barroso, S. Landwerth, M. Woerdemann, C. Alpmann, T. Buscher, M. Becker, A. Studer and C. Denz, *Biomed. Microdevices*, 2015, **17**, 1-8.