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Synthesis and photo-postmodification of zeolite L based polymer brushes

Zeolite L is a versatile material with unique properties that serves as a highly interesting building block for innovative systems. Within this work a novel type of zeolite L/polymer hybrid material is presented. Polymer brush particles are generated using surface-functionalized zeolite L crystals as macroinitiators in controlled radical polymerization processes. Copolymerization of a photo-cleavable monomer and subsequent spin trapping of functionalized nitroxides under UV irradiation leads to a variety of highly functionalized zeolite L-based core–shell particles in a modular approach.

As featured in:

Synthesis and photo-postmodification of zeolite L based polymer brushes†

Tim Buscher, Álvaro Barroso, Cornelia Denz* and Armido Studer*†

A novel type of zeolite L/polymer hybrid material is presented. Polymer brush particles are generated using surface-functionalyzed zeolite L crystals as macroinitiators in controlled polymerization processes. Copolymerization of a photo-cleavable monomer and subsequent spin trapping of functionalized nitroxides under UV irradiation lead to a variety of highly functionalized zeolite L based core–shell particles.

Introduction

The mineralogical group of zeolites comprises all hydrated aluminosilicates with over 200 natural and artificial modifications.1 Zeolites are industrially used in heterogeneous catalysis and are also well known as ion exchange materials.2 Synthetic zeolite Linde Type L (LTL, zeolite L) crystals are solid, optically transparent and physiologically stable nanocontainers with a size range from 30 nm up to 10 µm. The microporous structure of zeolite L is unique for its hexagonally arranged one-dimensional strictly parallel pores (0.76–1.26 nm) along the crystal c-axis and their channel entrances end only at the base of the crystal.3 Due to its versatile properties, zeolite L can be functionalized in various ways and has gained increasing interest as a base for innovative materials.4 For example, incorporation of organic molecules and metal complexes such as dyes or drugs into the channels leads to stable pigments or agent carriers.5 As one of the first groups recognizing the high potential of zeolite L, Calzaferri et al. developed methods for specific zeolite surface functionalization.5a,6 Furthermore, it was shown by De Cola et al. as well as by our group that modified zeolite L crystals can self-assemble by utilizing either complexation7 or reversible covalent bond formation between complementary functionalized crystals.8,9 De Cola et al. also documented that amino-functionalized and therefore positively charged zeolite L crystals can assemble with living cells.9a,9b Recently, we have shown that this electrostatic-type binding can be exploited for the controlled fabrication of such bio-hybrid systems with holographic optical tweezers (HOT),9b,9c by which multiple optical traps can be generated for the simultaneous manipulation of several zeolite L crystals or motile bacteria in a contactless and thus, minimally invasive way.10

Surprisingly, only a few papers dealing with zeolite L/polymer hybrid materials have appeared to date. Notably, this type of organic/inorganic hybrid system should combine the properties of the versatile inorganic zeolite L core material and also those of the functional soft polymers. After demonstrating that non-functionalized zeolite L crystals can be incorporated into polymer fibers via electrospinning,6h Botta et al. also showed that negatively charged zeolites can aggregate into well-defined fiber-like structures after complexation with cationic polymers.6i Calzaferri et al. reported covalent attachment of polymers to the zeolite L surface by copolymerizing methyl methacrylate (MMA) and methacrylate-functionalized crystals as macro cross-linkers.6k Taking advantage of the host–guest behavior then leads to various transparent organic/inorganic hybrid materials showing interesting properties.11

To the best of our knowledge, polymer coated zeolite L hybrid materials prepared in a grafting-from process by surface initiated radical polymerization are unknown. It is obvious that the polymer shell in these core–shell systems will readily allow for tuning surface properties.12

Herein, we present the synthesis of zeolite L based polymer brush particles. Alkoxysamine- and α-bromoisobutyrate-functionalized zeolite L crystals are utilized as macrorinitiators for surface initiated nitroxide mediated polymerization (siNMP)13 and surface initiated atom transfer radical polymerization (siATRP).12 To document the success of polymerization and as evidence for controllable polymer chain length and therefore surface concentration of functionalities, a fluorescent model polymer is used. We demonstrated recently, that polymers bearing 2-hydroxy-2-methyl-1-phenylpropan-1-one as backbone substituents can be cleaved in a Norrish-Type-I photoreaction to generate acyl radicals that can trap persistent radicals such
as nitroxides in excellent yields. This led to a novel efficient approach for postmodification of polymers and polymer brushes in a modular manner. Along these lines, we will discuss herein the synthesis of hybrid particles containing acylol moieties in their polymer layer that can be photochemically postmodified. The potential of this methodology is documented by the successful preparation of a small representative library of differently functionalized zeolite L crystals.

Importantly, as compared to zeolite L crystals that are functionalized with monolayers, brush-type zeolites as reported herein possess a much higher surface concentration of functionalities, a higher dispersibility in various solvents, and also softer surfaces. Potential applications of this novel type of zeolite L material fall within the scope of medicine and biology, optics and photonics or microelectronics for example. Utilizing the host-guest behavior of zeolite L in combination with highly functionalized polymer shells is interesting for drug delivery and release. Also incorporation of photo-active units into the strictly parallel channel structure results in Förster resonance energy transfer (FRET) within the crystal and tuning the surface properties by polymer chemistry can facilitate its utilization in OLEDs or organic solar cells for instance. In general these core-shell particles can be readily incorporated into different polymer materials by established macromolecular chemistry and due to the special advantages of zeolite L, micro- and macroscopic properties of these materials can be adjusted.

Results and discussion

Preparation of zeolite L based polymer brushes

Zeolite L crystals of about 1 µm diameter and 1 µm or 4 µm length were all-over functionalized with (3-aminopropyl)triethoxysilane (APTES) by dispersing the crystals in toluene and heating the mixture in the presence of an excess APTES to obtain amino-functionalized particles 1a (1 µm) and 1b (4 µm). Successful surface modification was supported by zeta potential (ζ) measurements in ammonium formate buffer (AFB, 20 mM, pH = 7.4, 25 °C), revealing a positive potential of ζ1a = +34.5 mV and ζ1b = +41.9 mV. Subsequent amidation of the surface-bound amines in 1a and 1b with active esters of NMP- and ATRP-initiators 2 and 3 resulted in macroinitiators 4a/b and 5, respectively (Scheme 1).

A fluorescent model polymer bearing pyrene moieties was used to study and document the success of the surface initiated polymerization via fluorescence microscopy. To this end, pyren-1-ylmethyl acrylate 6 was prepared from pyrene-1-carbaldehyde by LAH-reduction and subsequent acylation. Following standard protocols, 13 siNMP macroinitiator 4b was reacted with styrene/pyrene monomer 6 (12:1) and alkoxyamine 7  into the strictly parallel channel structure results in Förster resonance energy transfer (FRET) within the crystal and tuning the surface properties by polymer chemistry can facilitate its utilization in OLEDs or organic solar cells for instance. In general these core-shell particles can be readily incorporated into different polymer materials by established macromolecular chemistry and due to the special advantages of zeolite L, micro- and macroscopic properties of these materials can be adjusted.

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Table 1 Influence of the initiator concentration on the polymer brush synthesis via siNMP.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Zeolite</th>
<th>Initiator mol%</th>
<th>Conv. %</th>
<th>M$_n$exp./kg mol$^{-1}$</th>
<th>M$_n$thor./kg mol$^{-1}$</th>
<th>PDI</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>8a</td>
<td>0.05</td>
<td>50</td>
<td>120</td>
<td>67</td>
<td>1.23</td>
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<tr>
<td>2</td>
<td>8b</td>
<td>0.10</td>
<td>56</td>
<td>66</td>
<td>38</td>
<td>1.22</td>
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<tr>
<td>3</td>
<td>8c</td>
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<td>29</td>
<td>17</td>
<td>1.12</td>
</tr>
<tr>
<td>4</td>
<td>8d</td>
<td>0.50</td>
<td>60</td>
<td>14</td>
<td>11</td>
<td>1.13</td>
</tr>
<tr>
<td>5</td>
<td>8e</td>
<td>1.00</td>
<td>25</td>
<td>3.0</td>
<td>2.9</td>
<td>1.15</td>
</tr>
</tbody>
</table>

The incorporation ratio of MMA/pyrene acrylate (Scheme 3). Particles of type 4a/b lacking the PEG-linker, siNMP failed. We assume that shorter or no linkers result in macroinitiator deactivation by dimerization of the initiating transient radicals due to surface radical confinement.

In analogy, α-bromoisobutyrate-functionalized zeolite L crystals 5 were converted with MMA/pyrene acrylate 6 (12 : 1) using ethyl α-bromoisobutyrate (9, EBiB, 0.1 mol%) as an external initiator and CuBr$_2$/tris[2-(dimethylamino)ethyl]amine (Me$_6$TREN) in combination with ascorbic acid as a catalyst system at 50 °C for 6 h to hybrid particles of type 10 (Scheme 3). Particles 10 and unbound polymers were separated in three centrifugation–washing cycles in THF and GPC analysis of the unbound polymer revealed an estimated molecular weight $M_n$ for the brush polymers of 59 kg mol$^{-1}$ with a PDI of 1.31. The incorporation ratio of MMA/pyrene acrylate 6 into the unbound polymer is around 18 : 1 according to $^1$H NMR analysis of the corresponding signals. As expected for brush materials containing 6, strong fluorescence was observed for the organic/inorganic hybrid 10.

**Photo-postmodification of polyacyloin brush particles**

Previous studies in our group revealed that polymeric acyl radicals generated via a Norrish-Type-I reaction are quantitatively trapped by persistent nitroxide radicals. We therefore decided to run siNMP with a styrene derivative bearing an acyloin entity resulting in a polyacyloin brush material that should be readily activated by UV light ($\lambda = 365$ nm) and postmodified *via* spin trapping using various functionalized nitroxides. This approach will allow us to readily prepare a library of functionalized zeolite L/polymer hybrid particles in a modular approach starting with a single type of mother zeolite particle.

The photo-cleavable monomer 2-hydroxy-2-methyl-1-(4-vinylphenyl)propan-1-one (11) was prepared according to a literature procedure. SiNMP-active zeolite 4a was reacted with styrene/styrene derivative 11 (4 : 1) and alkoxyamine 7 (0.1 mol%) at 110 °C for 16 h to provide photo-postmodifiable zeolites 12 with an estimated $M_n$ of 74 kg mol$^{-1}$ and a PDI of 1.21, as determined by GPC analysis of the unbound polymer (Scheme 4).

To show the diversity of our modular zeolite L modification approach a variety of functionalized nitroxides 13–19 was used for the photo trapping reaction (Fig. 2). 2,2,6,6-Tetramethylpiperidine-1-oxyl (13, TEMPO) is commercially available. Nitroxides 14–19 were synthesized according to the cited literature.

Photo-postmodification was conducted with zeolites 12 (15 mg) and an excess of the respective nitroxides 13–19 (2.0 mg) in DMF (0.3 mL) using a LED ($\lambda = 365$ nm, 3 mW) at room temperature for 1 h (Scheme 5). Excess starting materials and byproducts were removed in several centrifugation–washing cycles (see the Experimental section for details). Success of the postmodification was...
proved by IR-analysis and zeta potential measurements before and after photochemical modification.

Based on the fact that conversion of the photo-active moiety in polymers to TEMPO esters in solution occurs quantitatively based on NMR analysis, we assume that the surface bound photoactive moieties are also activated and trapped in high yield. IR spectra of the resulting particles were recorded and new peaks at 1705 and 1748 cm\(^{-1}\) were assigned to the C=O stretching mode of the TEMPO ester moiety which was not present in the starting particles indicating successful ester formation (Fig. 3). The amide carbonyl stretching band expected in materials is hidden below the large band of the zeolite at around 1663 cm\(^{-1}\). Unfortunately, due to peak overlap we were not able to quantify the chemical postmodification. However, based on our previous reports we expect the modification to occur in high yields.

Zeta potentials \(\zeta\) of zeolite L crystals measured in AFB (20 mM, pH = 7.4, 25 °C) are summarized in Table 2. Polyaclayn brush particles show a negative potential of \(\zeta = -30.4\) mV (entry 1). Functionalization with neutral to partially negatively charged nitroxides leads to particles which feature only a slightly changed potential (entries 2, 5–8). Attachment of 4-amino-TEMPO provides particles that are protonated within the buffered medium and therefore positively charged (\(\zeta = +15.9\) mV, entry 3). Zeolites 20e show a strong positive charge of \(\zeta = +38.4\) mV which supports successful conjugation of 15 to the zeolite L crystal (entry 4).

To prove necessity of the acyloin moiety together with irradiation and refute non-specific binding, the following control experiments were conducted. Polystyrene brush particles were synthesized in the absence of monomer and reacted with 4-amino-TEMPO following the general procedures. Polyaclayn brush particles were incubated with nitroxide in the absence of light. Both control experiments show no changes regarding IR absorption, zeta-potential or fluorescence activity after subsequent labeling (see the ESI† for details). Thus, specificity of the reported approach is proven.

In particular, positively charged zeolite L crystals are especially interesting for the realization of bacteria–zeolite L hybrid systems, where the positively charged zeolite L surface binds electrostatically to living bacteria, which feature a negatively charged cell envelope. Recently, we have demonstrated that such bio-hybrid assemblies can be finely fabricated using HOT. By means of this optical approach, we show the capability of zeolites to get attached to living bacteria using as a model of bacteria a wild type strain of self-propelling rod-shaped Bacillus subtilis in chemotaxis buffer and therefore demonstrate one possible application of this novel type of zeolite L material (Fig. 4).

Finally, photochemically postmodified non-fluorescent zeolites were reacted with various fluorophores to obtain...
zeolites 21b,d–f which were analyzed via fluorescence microscopy after several centrifugation–washing cycles to support successful modification (Scheme 6). Along these lines, amino-functionalized zeolites 20b were reacted with fluorescein isothiocyanate (FITC) to afford fluorescent particles 21b (Scheme 6a). The alkyne-functionalized crystals 20d were successfully conjugated via copper(i)-catalyzed azide–alkyne cyclo-addition (CuAAC) with N-(2-azidoethyl)-7-nitrobenzo[c][1,2,5]oxadiazol-4-amine (22) to give strongly fluorescent zeolite crystals 21d (Scheme 6b). Biotin-conjugated crystals 20e upon immersion with fluorophore-labeled streptavidin (SAV) resulted in intense green fluorescent protein decorated particles 21e (Scheme 6c). Similarly, glycoconjugated crystals 20f were recognized by rhodamine-labeled concanavalin A (ConA) to afford red fluorescent zeolite L crystals 21f containing ConA at the surface (Scheme 6d). Control experiments were conducted by immediate treatment of 12 with fluorophores under respective conditions without preceding photo-modification to disprove non-specific adsorption (see the ESI† for details). Reaction of 12 with FITC or 22 leads to particles showing barely any fluorescence proving that there is no non-specific binding of these fluorophores. Immobilization of labeled SAV or ConA onto 12 results in comparatively weak fluorescent particles. Remaining residual adsorption of the proteins might occur from non-specific adsorption due to interactions with the polystyrene part of the polymer shell, but clearly protein decoration is highly increased by preceding conjugation of the respective biomolecules.

### Conclusion

In summary, we have successfully prepared novel types of zeolite L/polymer hybrid materials by a straightforward modular approach comprising surface initiated radical polymerization and subsequent photochemical postmodifica-

Table 2  Zeta potentials of photo-functionalized zeolites 20a–g in AFB (20 mM, pH = 7.4, 25 °C)

<table>
<thead>
<tr>
<th>Entry</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitroxide</td>
<td>—</td>
<td>13</td>
<td>14</td>
<td>15</td>
<td>16</td>
<td>17</td>
<td>18</td>
<td>19</td>
</tr>
<tr>
<td>Zeolite</td>
<td>12</td>
<td>20a</td>
<td>20b</td>
<td>20c</td>
<td>20d</td>
<td>20e</td>
<td>20f</td>
<td>20g</td>
</tr>
<tr>
<td>ζ/mV</td>
<td>−30.4</td>
<td>−43.9</td>
<td>15.9</td>
<td>38.4</td>
<td>−27.6</td>
<td>−40.2</td>
<td>−38.8</td>
<td>−25.2</td>
</tr>
</tbody>
</table>

Fig. 4  Optical microscopy images that show the optical assembly of 20c and self-propelling B. subtilis with HOT (red circles). The dashed red circles indicate the position of the dynamic optical traps. After turning off the HOT laser, the bacterial cell carries the particle along the blue trajectory. Scale bars are 2 µm.

Scheme 6  Photo-postmodification and fluorescence labeling of zeolite L based polymer brushes 12: (a) FITC conjugation; (b) CuAAC-click reaction; (c) immobilization of labeled SAV; (d) attachment of ConA. Scale bars are 2 µm.
Zeolite L based photo-active polymer brushes were generated in a grafting-from process using zeolite L crystals as macoinitiators for s>NoMP and s>ATRP. Copolymerization with a fluorescent monomer resulted in fluorescent brushes where the molecular weight of the surface attached polymers and hence the fluorescence intensity can be adjusted. Surface initiated copolymerization of styrene with a photo-cleavable monomer results in photoactive brushes that can be readily photochemically modified by UV irradiation in the presence of functionalized nitrooxides to afford the corresponding modified polymer brush particles. With the robust photochemistry, various functionalities can be introduced at the surface without the need of any protecting group strategies. For example, introduction of quaternized ammonium groups resulted in positively charged particles which were then used for the optical tweezer-assisted fabrication of bio-hybrid micro-robots. Also biotin or non-protected glucose was readily conjugated to the surface bound polymers. Moreover, alkynyl groups can be introduced which can be further chemically modified via established click chemistry. Importantly, our method features robustness in terms of late stage functionalization and due to the polymeric nature of the modifiable units, a high density of functionalities at the surface can be achieved in these novel organic/inorganic hybrid systems.

### Experimental

#### General information

Zeolite L crystals were either purchased from Süd-Chemie (1.0–1.5 × 1.0 µm) or synthesized following reported procedures\(^1\) (4.0 × 1.0 µm). Reagents were purchased from ABCR, Aldrich, Acros Organics, Alfa Aesar, Merck or TCI and used as received. Rhodamine-labeled ConA (5.0 mg mL\(^{-1}\) in HEPES buffer\(^\ast\)) was purchased from VECTOR Laboratories and Oyster®-488-tagged SAV (1.0 mg mL\(^{-1}\) in PBS buffer) from Luminartis. Styrene and MMA were destabilized by distillation over CaH\(_2\). Reactions were carried out in heat-gun-dried flasks equipped with a MKII Golden Gate single reflection ATR camera. IR spectra were recorded on a Digilab FTS 4000 equipped with an MKII Golden Gate single reflection ATR system. Fluorescence images were recorded on an epifluorescence microscope Olympus XLUV with a Lumen Dynamics X-Cite Series 120Q excitation source, excitation/emission filters and an Olympus U-LS30-3 camera. IR spectra were recorded on a Digilab FTS 4000 equipped with a Maclike Golden Gate single reflection ATR system. ζ-Potentials were measured on a Malvern DTS Zetasizer Nano ZS using the Smoluchowski equation. 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:YVO\(_4\) laser (λ = 1064 nm, power at sample plane 400 mW approx.) as the light source for optical trapping. The implemented HOT system is described in detail in the literature.\(^{40}\) H and \(^{13}\)C NMR spectra were recorded on a Bruker DPX 300 (300 MHz; at 298 K). Chemical shifts δ are noted in ppm and relatively to \(^1\)H or \(^{13}\)C signals of tetramethylsilane (0.0 ppm). Spectra are referenced to the solvent residual peak. High resolution mass spectra (HRMS) were recorded on a Bruker Daltonics MicroToF after electrospray ionization (ESI). Detected m/z signals are given in u. Melting points (MP) were determined on a Stuart SMP10 and are uncorrected.

Active esters 2\(^4\) and 3\(^2\) alkoxyamine 7,\(^5\) styrene derivative 11,\(^10\) nitrooxides 14,\(^16\) 15,\(^18\) 16,\(^16\) 17,\(^16\) 18\(^2\) and 19\(^16\) were prepared according to literature procedures.

#### General procedure for amino-functionalization of zeolite L

Unmodified zeolite L crystals (a: 1.0–1.5 × 1.0 µm; b: 4.0 × 1.0 µm) were dispersed in dry toluene (c = 100 mg mL\(^{-1}\)) and an excess of APTES (c = 100 µL mL\(^{-1}\)) was added. The suspension was sonicated for 3 min and stirred at 85 °C for 4 h. The mixture was centrifuged, the supernatant decanted and the residue washed three times with Et\(_2\)O in subsequent centrifugation–washing cycles. Functionalized crystals 1a/b were dried under reduced pressure.

ζ-Potential in ammonium formate buffer (AFB, 20 mM, pH = 7.4, 25 °C): ζ\(_{1a}\) = +34.5 mV, ζ\(_{1b}\) = +41.9 mV.

#### General procedure for functionalization of zeolite L with active esters 2 and 3

Amino-functionalized zeolite L crystals 1a/b were dispersed in DMF (c = 50 mg mL\(^{-1}\)) and sonicated for 5 min. The respective active ester solution of 2 or 3 in DMF (50 mM) was added in excess and the suspension was stirred at rt for 24 h. The mixture was centrifuged, the supernatant decanted and the residue washed three times with Et\(_2\)O in subsequent centrifugation–washing cycles. Functionalized crystals 4a/b or 5 were dried under reduced pressure.

#### Synthesis of pyren-1-ylmethyl acrylate (6)

Pyren-1-ylmethanol. A solution of pyrene-1-carboxaldehyde (2.30 g, 10.0 mmol, 1.0 eq.) in THF (10 mL) was cooled to 0 °C and LiAlH\(_4\) (417 mg, 11.0 mmol, 1.1 eq.) was added portion-wise. The mixture was stirred at 0 °C for 10 min and at rt for 1 h. The reaction was quenched by addition of H\(_2\)O (0.5 mL), NaOH (aq., 4.0 M, 0.5 mL) and H\(_2\)O (1.0 mL). The suspension was filtered, the residue washed with THF (30 mL) and the filtrate evaporated in vacuo. Pyren-1-ylmethanol was obtained as a yellow solid (2.33 g, 10.0 mmol, quant.) and used without further purification.

Pyren-1-ylmethyl acrylate (6). A solution of pyren-1-ylmethanol (2.33 g, 10.0 mmol, 1.00 eq.) and acryloyl chloride (950 mg, 850 µL, 10.5 mmol, 1.05 eq.) in CH\(_2\)Cl\(_2\) (80 mL) was cooled to 0 °C and Et\(_3\)N (1.21 g, 1.66 mL, 12.0 mmol, 1.20 eq.) was added. The mixture was stirred at rt for 18 h. H\(_2\)O (80 mL)
was added and the aqueous phase was extracted with CH₂Cl₂ (3 × 50 mL). The combined organic layers were dried over MgSO₄ and the solvent was removed in vacuo. Purification by flash chromatography (pentane/EtOAc = 14:1 → 9:1) provided acrylate 6 as a pale yellow solid (2.47 g, 8.63 mmol, 86%).

\[^1\]H NMR (300 MHz, CDCl₃, 298 K): δ = 8.32–7.99 (m, 9H, CH₃(α), 6.48 (dd, J = 17.3 Hz, J = 1.5 Hz, 1H, C=CH/H)); 6.20 (dd, J = 17.3 Hz, J = 10.4 Hz, 1H, CH); 5.93 (s, 2H, CH₂); 5.85 (dd, J = 10.4 Hz, J = 1.5 Hz, 1H, CH, 13C NMR (75 MHz, CDCl₃, 298 K): δ = 166.2 (C₆H₅), 131.9 (C₆H₅), 131.3 (CH₃), 131.3 (C₆H₅), 129.7 (C₆H₅), 129.8 (C₆H₅), 128.5 (CH), 128.3 (CH), 127.9 (CH), 127.8 (CH), 127.4 (CH), 126.2 (CH), 125.6 (CH), 125.5 (CH), 125.0 (C₆H₅), 124.8 (C₆H₅), 124.7 (CH), 123.0 (CH), 64.91 (CH₃), 1H NMR (300 MHz, CDCl₃, 298 K): δ = 7.99 (m, 9H, CH₃(α)), 7.68 (dd, 3J = 10.4 Hz, 3J = 8.5 Hz, 1H, CH₃(α)).

General procedure for preparation of polymer brush particles via sINMP
Alkoxyamine-modified zeolite L crystals 4a/b (c = 10 mg mmol⁻¹ monomer mixture⁻¹), functional monomer 6 or 11 (n = 0.08–0.2 eq.), styrene (1.0–n eq.) and alkoxyamine 7 (0.05–1.0 mol%) were added to benzene (c = 50 µL mmol⁻¹ monomer mixture⁻¹). The suspension was degassed in three freeze–thaw cycles, sonicated for 3 min and stirred at rt for 2 h. Polymer brush particles and unbound polymers were separated in three centrifugation–washing cycles with THF (3 × 10 mL) and solvents were evaporated in vacuo. The unbound polymer was dissolved in a minimum amount of CH₂Cl₂, precipitated from methanol and dried in vacuo.

General procedure for preparation of polymer brush particles via sIA TRP
α-Bromoisobutyrate-functionalized zeolite L crystals 5 (2.0 mg), pyrene acrylate 6 (23 mg, 80 µmol, 0.08 eq.) and MMA (98 µL, 0.92 mmol, 0.92 eq.) were added to EBiB (stock solution in anisole, 20 mM, 50 µL, 1.0 µmol, 0.1 mol%) and CuBr₂/Me₆TREN (stock solution in anisole, CuBr₂ = 2.0 mM, Cu₆TREN = 20 mM, 200 µL, nCuBr₂ = 0.4 µmol, nCu₆TREN = 4.0 µmol, 0.04 mol%/0.4 mol%). The suspension was degassed in three freeze–thaw cycles and sonicated for 3 min. Ascorbic acid (0.2 mg, 1 µmol, 0.1 mol%) was added and the suspension was stirred at 50 °C for 6 h. Polymer brush particles 10 and unbound polymers were separated in three centrifugation–washing cycles with THF (3 × 10 mL) and solvents were evaporated in vacuo. The unbound polymer was dissolved in a minimum amount of THF, filtered through a short plug of silica, precipitated from methanol and dried in vacuo.

General procedure for photo-postmodification of zeolite L based polyacrylon brushes with nitroxides 13–19
Polyacrylon brush particles 12 (15 mg) were dispersed in DMF (0.3 mL) and the respective nitroxides 13–19 (2.0 mg, excess) were added to the suspension. The mixture was sonicated for 3 min and stirred under LED irradiation (λ = 365 nm, 3 mW) at rt for 1 h. Polymer brush particles 20a–g 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-Potentials in AFB (20 mM, pH = 7.4, 25 °C) are summarized in Table 2.

General procedure for preparation of HOT experimental samples
Zeolite L crystals 20c (c = 0.1 mg mL⁻¹) were dispersed in a chemotaxis buffer (10 mM K₂HPO₄/KH₂PO₄ buffer (pH = 7.0), 0.14 mM CaCl₂, 0.30 mM (NH₄)₂SO₄, 0.10 mM EDTA (pH = 5.0), 5.0 mM sodium lactate, 0.05% glycerol) by sonication for 5 min. B. subtilis were washed with chemotaxis buffer in three centrifugation–washing cycles, dispersed in chemotaxis buffer and added to the zeolite suspension. One droplet of the mixture was applied on a microscope slide.

General procedure for FITC conjugation
Amino-functionalized zeolite L 20b (2.0 mg) was dispersed in DMF (2.0 mL) and FITC (1.0 mg, 3.6 µmol, excess) and EtN (10 µL, 72 µmol, excess) were added. The suspension was sonicated for 3 min and stirred at rt for 2 h. Polymer brush particles 21b were washed in five centrifugation–washing cycles with DMF (2.0 mL), MeOH (2 × 2.0 mL) and DCM (2 × 2.0 mL) and dried in vacuo.

Synthesis of N-(2-azidoethyl)-7-nitrobenzo[c][1,2,5]oxadiazol-4-amine (22)
N-(2-Bromoethyl)-7-nitrobenzo[c][1,2,5]oxadiazol-4-amine. 4-Chloro-7-nitrobenzo[c][1,2,5]oxadiazole (100 mg, 500 µmol, 1.0 eq.) was dissolved in EtOAc (3.0 mL). 2-Bromoethylammonium chloride (102 mg, 500 µmol, 1.0 eq.) and NaHCO₃ (126 mg, 1.50 mmol, 3.0 eq.) were added and the suspension was stirred at 40 °C for 23 h. The mixture was filtered and the residue was washed with CH₂Cl₂ (20 mL). HCl (aq, 1 M, 15 mL) was added to the filtrate and the aqueous phase was extracted with CH₂Cl₂ (3 × 20 mL). The combined organic layers were dried over MgSO₄ and solvents were removed in vacuo. Purification by flash chromatography (pentane/EtOAc = 1:1) provided N-(2-bromoethyl)-7-nitrobenzo[c][1,2,5]oxadiazol-4-amine as a red solid (105 mg, 366 µmol, 73%).

MP: 129 °C. \[^1\]H NMR (300 MHz, CDCl₃, 298 K): δ = 8.50 (d, J = 8.5 Hz, 1H, CH₃(α)), 6.39 (s, 1H, NH); 6.26 (d, J = 8.5 Hz, 1H, CH₃(α)), 4.04–3.88 (m, 2H, CH₂N₂), 3.70 (t, J = 6.0 Hz, 2H, CH₂Br). IR (film): 3360, 3094, 2463, 1618m, 1562s, 1527m, 1495s, 1460w, 1342m, 1385w, 1351m, 1275s, 1247s, 1174m, 1141m, 1061w, 1003m, 892m, 825m, 779m, 738m, 675w cm⁻¹. HRMS (ESI) calculated for C₈H₇BrN₂O₂Na⁺ ([M + Na⁺]): 308.9594. Found: 308.9621.

N-(2-Azidoethyl)-7-nitrobenzo[c][1,2,5]oxadiazol-4-amine (22). N-(2-Bromoethyl)-7-nitrobenzo[c][1,2,5]oxadiazol-4-amine (40 mg, 0.14 µmol, 1.0 eq.) and NaN₃ (27 mg, 0.42 µmol, 3.0 eq.) were dissolved in DMF (2.0 mL). The mixture was stirred at 80 °C for 4 h and at rt for 15h. H₂O (10 mL) was added, the aqueous phase was extracted with Et₂O (5 × 20 mL), the combined
organic layers were dried over MgSO₄ and the solvents were removed in vacuo. Purification by flash chromatography (pentane/Et₂O = 1:2 → 1:3) provided azide 22 as an orange solid (33 mg, 0.13 mmol, 95%).

MP: 136 °C. ¹H NMR (300 MHz, DMSO-d₆, 298 K): δ = 9.49 (bs, 1H, CHarom.); 8.52 (d, 3J = 8.8 Hz, 1H, CHarom.); 6.51 (d, 3J = 8.9 Hz, 1H, CHarom.); 3.69 (bs, 4H, 2 × CH₂). IR (film): 3226m, 3152w, 3098w, 3070w, 3002w, 2957w, 2119s, 2056w, 1616m, 1280s, 1250s, 1213s, 1192m, 1127s, 1091m, 1064m, 1035m, 998m, 985m, 972w, 900m, 838m, 812m, 778m, 740m, 679w, 640m cm⁻¹. HRMS [ESI] calculated for C₈H₇N₇O₃Na⁺ [M + Na⁺]: 272.0503. Found: 272.0503.

General procedure for CuAAC
Alkyne-functionalized zeolite L 20d (2.0 mg) was dispersed in THF (0.2 mL) and H₂O (0.1 mL). Azide 22 (1.0 mg, 4.0 µmol, excess), sodium ascorbate (1.0 mg, 3.6 µmol, excess) and CuSO₄ (~0.1 mg) were added. After degasification with an Ar stream the suspension was sonicated for 3 min and stirred at rt for 4 h. Polymer brush particles 21d were washed in five centrifugation steps and dried in vacuo.

General procedure for immobilization of fluorophore labeled proteins
Modified zeolite L crystals 20e or 20f (1.0 mg) were dispersed in H₂O (0.2 mL) by sonication for 1 min and an excess of the respective protein buffer solution (2.0 µL) was added. The suspension was sonicated for 3 min and stirred at rt for 4 h. Polymer brush particles 21e or 21f were washed in five centrifugation–washing cycles with MeOH (5 × 2.0 mL) and dried in vacuo.

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Notes and references
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19 The reaction is performed in the absence of water and O₂, but initial studies showed that DMF/H₂O mixtures as solvents are tolerated as well as the presence of air.