# **Supporting Information**

1	Ge	neral		. 3
	1.1	Ger	eral Information	. 3
	1.2	Abł	previations	. 4
2	Sy	nthese	es	. 5
	2.1	Ger	eral Procedure for Zeolite L Modification: General Conditions C	. 5
	2.2	Ger	eral Procedure for the Synthesis of Alkoxyamines: General Conditions D	. 5
	2.3	All	coxyamine Syntheses	. 6
	2.3	.1	Synthesis of Alkoxyamine 19	. 6
	2.3	.2	Synthesis of Alkoxyamine 20	. 7
	2.3	.3	Synthesis of Nitroxide 21	. 7
	2.3	.4	Synthesis of Alkoxyamine 25	. 7
	2.4	Nitı	oxide Syntheses	11
	2.4	.1	Syntheses of Nitroxide Dyes 2 and 3	11
	2.4	.2	Syntheses of Bioconjugated Nitroxides	12
	2.4	.3	Synthesis of Succinimido Maleimide Nitroxide 15	14
3	Sit	e-Spe	cific Nitroxide Exchange Reactions at Zeolite L	15
	3.1	Ger	eral Procedure for the Nitroxide Exchange Reaction: General Conditions A	15
	3.2	Pro	of-of-principle: Nitroxide Exchange Reactions at 125 °C with 2 and 3	16
	3.2	.1	Reaction with Rhodamine Nitroxide 2	16
	3.2	2	Control Experiment 1: Adsorption of <b>2</b> onto the Surface	17
	3.2	.3	Reaction with NBD Nitroxide <b>3</b>	17
	3.3	Var	iation of Reaction Conditions	19
	3.3	.1	Variation of Reaction Temperature	19
	3.3	.2	Variation of Solvent	20
	3.3	.3	Variation of Solvent and Temperature	20
	3.4	Mo	nitoring Experiments: Studies towards Reversibility	21
4	Sit	e-Spe	cific Immobilization of Proteins	22
	4.1	Ger	eral Procedure for Protein Immobilization: General Conditions B	22
	4.2	Imn	nobilization of Concanavalin A (ConA)	22
	4.2	.1	Control Experiment 2: Adsorption of Concanavalin A onto the Surface	23
	4.2	.2	Control Experiment 3: Adsorption of Concanavalin A onto Alkoxyamine-Surfaces	24
	4.3	Imn	nobilization of Streptavidin	24

Supplementary Material (ESI) for Chemical Communications This journal is (c) The Royal Society of Chemistry 2011

6	Lite	ratu	re	35
	5.4	4-(1- acid	-[{4-( <i>tert</i> -Butyl)-2,2,6,6-tetraethyl-3-oxopiperazin-1-yl}oxy]-2-methylpropyl)-benzoid (25)	с 34
	5.3	Meth benz	hyl-4-(1-[{4-( <i>tert</i> -butyl)-2,2,6,6-tetraethyl-3-oxopiperazin-1-yl}oxy]-2-methylpropyl) coate ( <b>24</b> )	- 33
	5.2	Metl	hyl-4-(1-bromo-2-methylpropyl)-benzoate (23)	32
	5.1	4-(1-	-Bromo-2-methylpropyl)-benzoic acid (22)	31
5	<sup>1</sup> H-	and <sup>1</sup>	<sup>3</sup> C-NMR spectra of unknown compounds	31
	4.6.	3	Control Experiment 6: Adsorption of Bovine Serum Albumin (BSA) onto the Surface 29	•
	4.6.2	2	Immobilization of Bovine Serum Albumin (BSA)	28
	4.6.	1	Synthesis of Bovine Serum Albumin Nitroxide 31	28
	4.6	Imm	obilization of Bovine Serum Albumin (BSA)	28
	4.5.2	2	Immobilization of $\beta$ -Lactoglobulin A (LGA)	27
	4.5.	1	Synthesis of $\beta$ -Lactoglobulin A Nitroxide <b>30</b>	27
	4.5	Imm	obilization of β-Lactoglobulin A (LGA)	27
	4.4	Imm	obilization of eGFP	26
	4.3.2	2	Control Experiment 5: Adsorption of Streptavidin onto Alkoxyamine-Surfaces	26
	4.3.	1	Control Experiment 4: Adsorption of Streptavidin onto the Surface	25

#### 1 General

#### **1.1 General Information**

All reactions involving air- or moisture-sensitive reagents or intermediates were carried out in heat-gun-dried glassware under an argon atmosphere and were performed by using standard Schlenk techniques. All solvents for extraction and flash chromatography were distilled before use. TLC was carried out on *Merck* silica gel 60 F<sub>254</sub> plates; detection by UV or dipping into a solution of NaHCO<sub>3</sub> (5.0 g), KMnO<sub>4</sub> (1.5 g) and H<sub>2</sub>O (0.20 L) followed by heating. Flash chromatography was carried out on *Merck* or *Fluka* silica gel 60 (40 – 63  $\mu$ m) with an argon pressure of about 0.1 - 0.5 bar. Centrifugations were carried out on a Heraeus microprocessor controlled laboratory centrifuge Labofuge 200. <sup>1</sup>H-NMR and <sup>13</sup>C-NMR spectra were recorded on a Bruker DPX 300, a Bruker AV 400 or on a Bruker Varian 300 spectrometer. Chemical shifts  $\delta$  in ppm are referenced to the TMS peak as an internal standard. ESI-MS and HRMS were performed using a Bruker MicroTof and a Waters-Micromass Quattro LCZ (only ESI-MS). Melting points were determined on a Stuart Scientific SMP 10 apparatus and are uncorrected. Infrared spectra were recorded on a Digilab FTS 4000 equipped with a MKII Golden Gate Single Reflection ATR System, a Bruker IFS 28 or on a Shimadzu FTIR 8400S. Epifluorescence microscopy images were measured with an Olympus Reflected Fluorescence System CKX41 microscope equipped with excitation and emission filters and a color Kappa Opto-Electronics camera (images were edited as seen through the microscope). Confocal fluorescence microscopy was performed on a stage-scanning confocal microscope (Microtime 200 – Pico-Quant GmBH, Germany) with a 100x oil immersion objective (Planapochromat, NA 1.4). The samples were excited with a 532 laser (a) 40 MHz. Elementary analysis was performed using a Elementar-Analysensysteme GmbH Vario EL III. Zeta potential measurements were performed with a Beckman Coulter Delsa Nano C Particle Analyzer. THF for reactions was distilled from K/Na under argon, dichloromethane (CH<sub>2</sub>Cl<sub>2</sub>, DCM) and 1,2-dichloroethane (DCE, 99.8+%, extra pure from Acros Organics) were distilled from P<sub>2</sub>O<sub>5</sub>. Acetonitrile (Acros, 99.9 % over molecular sieve), methanol (Acros, 99.9 % over molecular sieve), toluene (Acros, 99.9 % over molecular sieve) and DMF (Acros, 99.9 % over molecular sieve) were used as dry solvents. All other chemicals were purchased and used as received from Acros, Sigma-Aldrich, Alfa-Aesar, Fluka, Degussa, Lancaster, Merck Darmstadt and abcr. All proteins used in this study are commercially available. Streptavidin was received from Luminartis, eGFP from BioCat, concanavalin A from Vector Laboratories, bovine serum albumin from *Invitrogen* and β-lactoglobulin A from *Sigma-Aldrich*.

## 1.2 Abbreviations

aq.	aqueous	МеОН	methanol
d	day(s)	MS	mass spectrometry
DMAP	4-N,N-dimethylaminopyridine	MTBE	tert-butylmethylether
DMF	N,N-dimethylformamide	NMR	nuclear magnetic resonance
EDCI	1-ethyl-3-(3-dimethylaminopro-	NEt <sub>3</sub>	Triethylamine
	pyl)-carbodiimide	NHS	N-hydroxysuccinimide
equiv	equivalents	NMM	N-methyl-morpholine
ESI	MS: electrospray ionisation	ppm	parts per million
Et <sub>2</sub> O	diethylether	rt	room temperature
EtOAc	ethylacetate	sat.	saturated
EtOH	ethanol	mp	melting point
FC	flash chromatography	TEMPO	2,2,6,6-tetramethylpiperidin- <i>N</i> -oxyl
h	hour(s)		radical
HRMS	high resolution MS	TFA	trifluoroacetic acid
HOBt	1-hydroxybenzotriazole	THF	tetrahydrofurane
IR	infrared spectroscopy	DCM	dichloromethane
DCC	dicyclohexylcarbodiimide	PBS	phosphate buffered saline
DCE	1,2-dichloroethane	min	minute(s)

#### 2 Syntheses

#### 2.1 General Procedure for Zeolite L Modification: General Conditions C

The cylindrical synthetic zeolite L crystals used in this work were prepared according to a procedure described in the literature.<sup>[1]</sup> The crystals had a mean length of about 3.0 µm and a mean diameter of about 1.0 µm. Amino termination of the crystals was performed according to the reference.<sup>[2]</sup> Alkoxyamine coupling to the zeolite surface amino groups was performed by first activating the carboxy groups of 19, 20 and 25. In general, 75 µmol of 19, 20 or 25 dissolved in а solution of 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide were hydrochloride (EDCI·HCl) (50.0 µmol) and N-hydroxysuccinimide (NHS) (82.5 µmol) in 2.00 mL of dry DMF and the solution was stirred at room temperature (rt) for 24 h. Afterwards 40.0 mg of amino functionalized zeolites were added and the resulting suspension was ultrasonicated for 15 min followed by stirring at room temperature for 24 h. The samples were cleaned by centrifugation for 15 min at 5.300 rpm followed by removal of the supernatant. The crystals were then suspended in 10.0 mL Et<sub>2</sub>O followed by centrifugation. That purification procedure was repeated two times. Afterwards, the samples were used for the nitroxide exchange reactions.

#### 2.2 General Procedure for the Synthesis of Alkoxyamines: General Conditions D

Cu(OTf)<sub>2</sub> (1 mol-%) and 4,4'-di-*tert*-butyl-2,2'-bipyridyl (4 mol-%) were suspended under an argon atmosphere in benzene (1.25 mL/mmol nitroxide). Nitroxide (1.21 equiv), bromide (1.00 equiv) and Cu powder (1.05 equiv) were each dissolved in benzene and added. The reaction mixture was stirred in a sealed tube under an argon atmosphere at 55-75 °C for 17 h. The solids were removed by filtration over silica gel (washing with CH<sub>2</sub>Cl<sub>2</sub>). Purification by flash chromatography (MTBE/pentane) afforded the alkoxyamine.<sup>[3]</sup>

#### 2.3 Alkoxyamine Syntheses

## 2.3.1 Synthesis of Alkoxyamine 19

#### 4-(1-Bromo-2-methylpropyl)-benzaldehyde (17)

Bromide **17** was synthesized according to a literature procedure.<sup>[4]</sup>

#### 4-[2-Methyl-1-(2,2,6,6-tetramethylpiperidin-1-yloxy)-propyl]-benzaldehyde (18)



Alkoxyamine **18** was synthesized according to a literature procedure.<sup>[4]</sup>

#### 4-[2-Methyl-1-(2,2,6,6-tetramethylpiperidin-1-yloxy)-propyl]-benzoic acid (19)



Jones reagent (CrO<sub>3</sub> in H<sub>2</sub>SO<sub>4</sub>/H<sub>2</sub>O, 2.6 M, 900  $\mu$ L, 2.36 mmol, 1.50 equiv) was added to a solution of aldehyde **18** (500 mg, 1.58 mmol, 1.00 equiv) in acetone (11.0 mL) at 0 °C. The reaction mixture was stirred for 1 h at 0 °C, isopropanol (1.00 mL) was then

added during 30 min at rt to destroy surplus  $CrO_3$ . To stop the reaction,  $H_2O$  (20 mL) was added and the crude mixture was again stirred for 30 min. The aqueous layer was extracted with  $CH_2Cl_2$  (3×20 mL). The combined organic layers were dried over MgSO<sub>4</sub>. After filtration and removal of the solvents *in vacuo*, the crude product was purified by flash chromatography (pentane/Et<sub>2</sub>O 8:1). Alkoxyamine **19** was obtained as a colourless solid (431 mg, 1.29 mmol, 82%). The analytical data were in accordance to those described in the literature.<sup>[5]</sup>

<sup>1</sup>**H-NMR** (300 MHz, CDCl<sub>3</sub>, 298 K):  $\delta = 10.60$  (*br s*, 1H, CO<sub>2</sub>H), 8.04 (*d*, *J* = 8.2 Hz, 2H, Aryl-H), 7.33 (*d*, *J* = 8.2 Hz, 2H, Aryl-H), 4.68 (*br s*, 1H, CHCH(CH<sub>3</sub>)<sub>2</sub>), 2.67-2.53 (*m*, 1H, CHC*H*(CH<sub>3</sub>)<sub>2</sub>), 1.68-0.53 (*m*, 24H, 3×CH<sub>2</sub>, 2×C(CH<sub>3</sub>)<sub>2</sub>, CH(CH<sub>3</sub>)<sub>2</sub>). **HRMS (ESI)**: calculated for [M+H]<sup>+</sup>: 334.2377; found: 334.2389.

## 2.3.2 Synthesis of Alkoxyamine 20

# 1-[2-Methyl-1-{4-(13-[2,5,8,11-tetraoxa]-tridecanic acid)}-phenylpropoxy]-2,2,6,6tetramethylpiperidine (20)



## 2.3.3 Synthesis of Nitroxide 21

## 1-tert-Butyl-3,3,5,5-tetraethyl-2-piperazinone-4-oxyl radical (21)



Nitroxide 21 was synthesized according to a literature procedure.<sup>[6]</sup>

#### 2.3.4 Synthesis of Alkoxyamine 25

#### 4-(1-Bromo-2-methylpropyl)-benzoic acid (22)



(2.12 mL) and the solution was stirred for 30 min at rt. The reaction mixture was treated with  $H_2O$  (15 mL) and stirred for additional 30 min. The aqueous layer was extracted with  $CH_2Cl_2$  (3×20 mL) and the combined organic layers were dried over MgSO<sub>4</sub>. After filtration, the solvents were removed *in vacuo*, the acid **22** was isolated as a colourless solid (530 mg, 2.06 mmol, 99%) and used without further purification.

**Mp**.: 142 °C. **IR (neat)**: 2965*w*, 2872*w*, 2674*w*, 2551*w*, 1685*s*, 1610*m*, 1425*s*, 1315*m*, 1282*s*, 1182*m*, 867*m*, 703*s* cm<sup>-1</sup>. <sup>1</sup>**H-NMR** (300 MHz, CDCl<sub>3</sub>, 298 K):  $\delta = 11.63$  (*s*, 1H, CO<sub>2</sub>H), 8.07 (*d*, *J* = 8.4 Hz, 2H, Aryl-H), 7.46 (*d*, *J* = 8.4 Hz, 2H, Aryl-H), 4.72 (*d*, *J* = 8.4 Hz, 1H, CHCH(CH<sub>3</sub>)<sub>2</sub>), 2.41-2.21 (*m*, 1H, CHCH(CH<sub>3</sub>)<sub>2</sub>), 1.18 (*d*, *J* = 6.6 Hz, 3H, CH(CH<sub>3</sub>)), 0.86 (*d*, *J* = 6.7 Hz, 3H, CH(CH<sub>3</sub>)). <sup>13</sup>C-NMR (75 MHz, CDCl<sub>3</sub>, 298 K):  $\delta = 171.8$  (C), 147.5 (C), 130.4 (CH), 128.9 (C), 128.1 (CH), 62.5 (CH), 36.4 (CH), 21.3 (CH<sub>3</sub>), 20.4 (CH<sub>3</sub>). **MS (ESI)**: 258, 257, 256, 255 ([M-H]<sup>-</sup>). **HRMS (ESI)** calculated for [M-H]<sup>-</sup>: 257.0006. found: 257.0000. **Element. Anal.** calculated for C<sub>11</sub>H<sub>13</sub>BrO<sub>2</sub>: 51.58, H 5.10; found: C 51.59, H 5.08.

#### Methyl-4-(1-bromo-2-methylpropyl)-benzoate (23)



Thionylchloride (110  $\mu$ L, 1.56 mmol, 4.00 equiv) was added at 0 °C to a solution of acid **22** (100 mg, 390 mmol, 1.00 equiv) in methanol (4.00 mL) and the reaction mixture was stirred for 5 h at rt. H<sub>2</sub>O (10 mL) was added and the layers were separated after the addition of CH<sub>2</sub>Cl<sub>2</sub> (50 mL). The

organic layer was washed with  $H_2O$  (2×12 mL) and brine (aq. sat., 6 mL), dried over MgSO<sub>4</sub> and after filtration the solvent was removed *in vacuo*. The crude product was purified by flash chromatography (pentane/MTBE 40:1) and the ester **23** was isolated as a colourless solid (94.3 mg, 0.350 mmol, 89%).

**Mp**.: 25 °C. **IR** (**neat**): 2965*w*, 2873*w*, 1720*s*, 1610*m*, 1435*m*, 1416*w*, 1275*s*, 1179*m*, 1110*s*, 1019*m*, 966*w*, 865*w*, 708*s*, 659*w* cm<sup>-1</sup>. <sup>1</sup>**H-NMR** (300 MHz, CDCl<sub>3</sub>, 298 K):  $\delta$  = 7.98 (*d*, *J* = 8.4 Hz, 2H, Aryl-H), 7.42 (*d*, *J* = 8.4 Hz, 2H, Aryl-H), 4.70 (*d*, *J* = 8.4 Hz, 1H, CHCH(CH<sub>3</sub>)<sub>2</sub>), 3.89 (*s*, 3H, OCH<sub>3</sub>), 2.38-2.20 (*m*, 1H, CHCH(CH<sub>3</sub>)<sub>2</sub>), 1.16 (*d*, *J* = 6.5 Hz, 3H, CH(CH<sub>3</sub>)), 0.84 (*d*, *J* = 6.6 Hz, 3H, CH(CH<sub>3</sub>)). <sup>13</sup>**C-NMR** (75 MHz, CDCl<sub>3</sub>, 298 K):  $\delta$  = 166.6 (C), 146.5 (C), 129.8 (CH), 127.9 (CH), 77.2 (C), 62.7 (CH), 52.1 (CH<sub>3</sub>), 36.4 (CH), 21.3 (CH<sub>3</sub>), 20.4 (CH<sub>3</sub>). **MS (ESI)**: 293 ([M+H]<sup>+</sup>). **HRMS (ESI)** calculated for [M+H]<sup>+</sup>: 293.0148. found: 293.0144. **Element. Anal.** calculated for C<sub>12</sub>H<sub>15</sub>BrO<sub>2</sub>: C 53.15, H 5.58; found: C 53.26, H 5.66.

Methyl-4-(1-[{4-(*tert*-butyl)-2,2,6,6-tetraethyl-3-oxopiperazin-1-yl}oxy]-2-methylpropyl)benzoate (24)



According to general conditions D with  $Cu(OTf)_2$  (1.33 mg, 3.69 mmol, 0.0100 equiv) and 4,4'-di-*tert*-butyl-2,2'-bipyridine (4.16 mg, 20.0 µmol, 0.0400 equiv) in benzene (0.4 mL) and nitroxide **21** (115 mg, 410 µmol, 1.10 equiv) in benzene (0.3 mL). Bromide **23** (100 mg, 370 µmol, 1.00 equiv)

in benzene (0.3 mL) and Cu powder (24.6 mg, 390  $\mu$ mol, 1.05 equiv) were added. The reaction mixture was stirred for 17 h at 55 °C. After work up, the crude product was purified by flash chromatography (pentane/MTBE 60:1). A diastereomeric mixture (pyramidal N-atom) of alkoxyamine **24** was isolated as a colourless solid (0.14 mg, 0.29 mmol, 78%).

**Mp**.: 124 °C. **IR (neat)**: 2963*m*, 2933*w*, 2877*w*, 1726*s*, 1642*s*, 1418*m*, 1278*s*, 1115*m*, 1011*m*, 855*w*, 802*w*, 773*w*, 711*m* cm<sup>-1</sup>. <sup>1</sup>**H-NMR** (300 MHz, CDCl<sub>3</sub>, 298 K): diastereomeric mixture:  $\delta = 7.96 \ (d, J = 7.8 \text{ Hz}, 2\text{ H}, \text{ Aryl-H})$ , 7.30 (d, J = 7.7 Hz, 2 H, Aryl-H), 4.54 (*br s*, 1H, C*H*CH(CH<sub>3</sub>)<sub>2</sub>) one diastereoisomer, 4.38 (*d*, *J* = 6.3 Hz, 1H, C*H*CH(CH<sub>3</sub>)<sub>2</sub>) one diastereoisomer, 4.38 (*d*, *J* = 6.3 Hz, 1H, C*H*CH(CH<sub>3</sub>)<sub>2</sub>) one diastereoisomer, 3.90 (*s*, 3H, OCH<sub>3</sub>), 3.14-2.86 (*m*, 2H, NCH<sub>2</sub>), 2.03 (*m*, 9H, CHCH(CH<sub>3</sub>)<sub>2</sub>, 4×CH<sub>2</sub>CH<sub>3</sub>), 1.35 (*s*, 9H, C(CH<sub>3</sub>)<sub>3</sub>), 1.12-1.00 (*m*, 3H, CH(CH<sub>3</sub>)), 0.99-0.62 (*m*, 12H 4×CH<sub>2</sub>CH<sub>3</sub>), 0.61-0.35 (*m*, 3H, CH(CH<sub>3</sub>)). <sup>13</sup>C-NMR (75 MHz, CDCl<sub>3</sub>, 298 K): diastereomeric mixture (double set of resonance obtained for specific carbon atoms):  $\delta = 172.7$  (C), 172.6 (C), 167.1 (C), 128.8 (CH), 128.5 (CH), 92.0 (CH), 91.2 (CH), 73.8 (C), 73.3 (C), 62.8 (C), 62.3 (C), 57.0 (C), 52.0 (CH<sub>3</sub>), 46.0 (CH<sub>2</sub>), 26.6 (CH<sub>2</sub>), 24.7 (CH<sub>2</sub>), 24.5 (CH<sub>2</sub>), 20.4 (CH<sub>3</sub>), 20.0 (CH<sub>3</sub>), 17.4 (CH<sub>3</sub>), 16.1 (CH<sub>3</sub>), 11.8 (CH<sub>3</sub>), 9.4 (CH<sub>3</sub>), 9.1 (CH<sub>3</sub>), 8.2 (CH<sub>3</sub>). **MS (ESI)**: 971 ([2M+Na]<sup>+</sup>), 497 ([M+Na]<sup>+</sup>), 475 ([M+H]<sup>+</sup>). **HRMS (ESI)** calculated for [M+Na]<sup>+</sup>: 497.3350. found: 497.3348. **Element. Anal.** calculated for C<sub>28</sub>H<sub>46</sub>N<sub>2</sub>O<sub>4</sub>: C 70.85, H 9.77, N 5.90; found: C 70.61, H 9.94, N 5.88.

# 4-(1-[{4-(*tert*-Butyl)-2,2,6,6-tetraethyl-3-oxopiperazin-1-yl}oxy]-2-methylpropyl)-benzoic acid (25)



Alkoxyamine **24** (50.0 mg, 110  $\mu$ mol, 1.00 equiv) was dissolved in methanol (5.00 mL) and NaOH (aq., 0.25 M, 1.26 mL, 320  $\mu$ mol, 3.00 equiv) was added. The reaction mixture was stirred for 2 d at rt, CH<sub>2</sub>Cl<sub>2</sub> (2 mL) and HCl (aq.,

1 M, 2 mL) were added and the layers were separated. The aqueous layer was extracted with CH<sub>2</sub>Cl<sub>2</sub> (3×8 mL) and the combined organic layers were dried over MgSO<sub>4</sub>. After filtration, the solvents were removed in vacuo and a diasteromeric mixture of alkoxyamine 25 was isolated as a colourless solid (46.8 mg, 100 µmol, 97%) and used without further purification. Mp.: 148 °C. IR (neat): 2965w, 2926w, 2879w, 1714m, 1626m, 1419w, 1219m, 1174m, 1111w, 991m, 864w, 811w cm<sup>-1</sup>. <sup>1</sup>H-NMR (300 MHz, CDCl<sub>3</sub>, 298 K): diastereomeric mixture:  $\delta = 8.04$  (d, J = 7.9 Hz, 2H, Aryl-H), 7.39-7.25 (m, 2H, Aryl-H), 4.57 (br s, 1H, CHCH(CH<sub>3</sub>)<sub>2</sub>) one diastereoisomer, 4.41 (d, J = 6.3 Hz, 1H, CHCH(CH<sub>3</sub>)<sub>2</sub>) one diastereoisomer, 3.12-2.98 (m, 2H, NCH<sub>2</sub>), 2.57-1.51 (m, 8H, 4×CH<sub>2</sub>CH<sub>3</sub>), 2.12-2.01 (m, 1H, CHCH(CH<sub>3</sub>)<sub>2</sub>), 1.38 (s, 9H, C(CH<sub>3</sub>)<sub>3</sub>), 1.13-1.03 (m, 3H, CH(CH<sub>3</sub>)), 1.01-0.66 (m, 12H,  $4 \times CH_2CH_3$ ), 0.60-0.47 (*m*, 3H, CH(CH<sub>3</sub>)). <sup>13</sup>C-NMR (75 MHz, CDCl<sub>3</sub>, 298 K): diastereomeric mixture (double set of resonance obtained for specific carbon atoms):  $\delta =$ 171.7 (C), 171.5 (C), 170.4 (C), 130.5 (CH), 129.4 (CH), 128.5 (CH), 128.1 (CH), 127.5 (CH), 127.2 (CH), 91.0 (CH), 90.2 (CH), 76.4 (C), 72.4 (C), 61.9 (C), 61.4 (C), 56.1 (C), 45.0 (CH<sub>2</sub>), 44.7 (CH<sub>2</sub>), 34.7 (C), 32.8 (CH<sub>2</sub>), 31.3 (CH), 31.0 (CH), 28.7 (CH<sub>2</sub>), 28.3 (CH<sub>2</sub>), 27.2 (CH), 25.7 (CH<sub>2</sub>), 23.7 (CH<sub>2</sub>), 23.5 (CH<sub>2</sub>), 19.4 (CH<sub>3</sub>), 19.0 (CH<sub>3</sub>), 17.9 (CH<sub>3</sub>), 16.4 (CH<sub>3</sub>), 15.1 (CH<sub>3</sub>), 10.8 (CH<sub>3</sub>), 10.6 (CH<sub>3</sub>), 8.4 (CH<sub>3</sub>). **MS (ESI)**: 483 ([M+Na]<sup>+</sup>), 461 ([M+H]<sup>+</sup>). **HRMS (ESI)** calculated for [M+Na]<sup>+</sup>: 483.3193. found: 483.3185.

#### 2.4 Nitroxide Syntheses

#### 2.4.1 Syntheses of Nitroxide Dyes 2 and 3

#### 4-Amino-2,2,6,6-tetramethyl-piperidin-1-oxyl radical (26)



# 2-(6-(Diethylamino)-3-(diethyliminio)-3*H*-xanthen-9-yl)-5-(*N*-(1-oxyl-2,2,6,6-tetramethyl-piperidin-4-yl)-sulfamoyl)-benzenesulfonate radical (2)



Nitroxide **2** was synthesized according to a literature procedure.<sup>[5]</sup>

# 2,2,6,6-Tetramethyl-4-([7-nitrobenzo[*c*][1,2,5]oxadiazol-4-yl]-amino)-piperidin-1-oxyl radical (3)



Nitroxide **26** (0.20 g, 1.2 mmol, 1.0 equiv) was dissolved in methanol (4.0 mL) and cooled to  $0 \,^{\circ}$ C. 4-Chloro-7-nitrobenzo[*c*][1,2,5]-oxadiazole (0.23 g, 1.2 mmol, 1.0 equiv) was dissolved in methanol

(8 mL) and the solution was added dropwise to the nitroxide solution at 0 °C. The reaction mixture was allowed to warm up to rt and was stirred at rt for 17 h. The solvents were removed *in vacuo* and the crude product was purified by flash chromatography (CH<sub>2</sub>Cl<sub>2</sub>/methanol 300:1  $\rightarrow$  60:1). Nitroxide **3** was isolated as an orange solid (0.17 g, 0.51 mmol, 42%).

**Mp**.: decomposition above 220 °C. **IR (neat)**: 3230*w*, 2980*w*, 2922*w*, 2845*w*, 2361*m*, 2338*m*, 1581*s*, 1493*m*, 1319*s*, 1285*s*, 1240*s*, 1182*m*, 1028*w*, 999*w*, 904*w*, 814*w* cm<sup>-1</sup>. **MS (ESI)**: 357 ( $[M+Na]^+$ ). **HRMS (ESI)** calculated for  $[M+Na]^+$ : 357.1408. found: 357.1411. **Element. Anal.** calculated for  $C_{15}H_{20}N_5O_4$ : C 53.88, H 6.03, N 20.95; found: C 53.74, H 6.10, N 20.79.

## 2.4.2 Syntheses of Bioconjugated Nitroxides

4-(1-Oxo-2-(2,3,4,6-tetra-*O*-acetyl-mannopyranosyl)-ethylamino)-2,2,6,6-tetramethylpiperidin-1-oxyl radical (27)



Mannose nitroxide **27** was synthesized according to a literature procedure.<sup>[7]</sup>

# 4-(1-Oxo-2-(2,3,4,6-tetrahydroxy-mannopyranosyl)-ethylamino)-2,2,6,6-tetramethylpiperidin-1-oxyl radical (9)



Sugar nitroxide **27** (50.0 mg, 89.4  $\mu$ mol, 1.00 equiv) and K<sub>2</sub>CO<sub>3</sub> (61.8 mg, 447  $\mu$ mol, 5.00 equiv) were dissolved in methanol (0.70 mL) and H<sub>2</sub>O (0.70 mL). The reaction mixture was stirred at rt for 17 h and the solvents were removed *in* 

*vacuo*. The crude product was purified by flash chromatography (CH<sub>2</sub>Cl<sub>2</sub>/methanol 20:1  $\rightarrow$  2:1) and nitroxide **9** was obtained as a red solid (35.0 mg, 89.4 µmol, quant.).

**Mp**.: 90 °C. **IR (neat)**: 3298*w*, 2950*w*, 1654*m*, 1543*s*, 1411*m*, 1313*m*, 1246*m*, 1132*m*, 1062*s*, 972*s*, 815*w*, 666*m*, 603*m* cm<sup>-1</sup>. **MS (ESI)**: 414 ([M+Na]<sup>+</sup>). **HRMS (ESI)** calculated for [M+Na]<sup>+</sup>: 414.1973. found: 414.1968.

# *N*-(1-Oxyl-2,2,6,6-tetramethylpiperidin-4-yl)-5-((3*a*S,4S,6*a*R)-2-oxohexahydro-1Hthieno[3,4-d]imidazole-4-yl)-pentaneamide radical (11)



4-Amino-TEMPO **26** (85.6 mg, 500  $\mu$ mol, 1.00 equiv) was dissolved in DMF (10.0 mL) and D-(+)-biotin (122 mg, 500  $\mu$ mol, 1.00 equiv) was added. EDCI·HCl (115 mg, 600  $\mu$ mol, 1.20 equiv), HOBt·H<sub>2</sub>O (91.9 mg, 600  $\mu$ mol, 1.20 equiv) were

added. The reaction mixture was stirred for 72 h at rt and  $CH_2Cl_2$  (15 mL) and HCl (aq., 1 M, 10 mL) were added subsequently. The layers were separated and the aqueous layer was extracted with  $CH_2Cl_2$  (4×20 mL). The combined organic layers were washed with NaHCO<sub>3</sub>

(aq. sat., 20 mL) and NaCl (aq. sat., 20 mL). The aqueous layer was again extracted with  $CH_2Cl_2$  (3×20 mL) and the combined organic layers were dried over MgSO<sub>4</sub>. The solvents were removed *in vacuo*. The crude product was purified by flash chromatography (CH<sub>2</sub>Cl<sub>2</sub>/methanol 20:1) to give **11** as a red solid (139 mg, 350 µmol, 70%).

**Mp**.: 170 °C. **IR (neat)**: 3272*m*, 2974*w*, 2932*m*, 1694*s*, 1643*s*, 1544*m*, 1460*m*, 1363*w*, 1332*w*, 1242*m*, 1179*w*, 731*m* cm<sup>-1</sup>. **MS (ESI)**: 420 ([M+Na]<sup>+</sup>), 817 ([2M+Na]<sup>+</sup>). **HRMS** (**ESI)** calculated for [M+Na]<sup>+</sup>: 420.2166; found: 420.2153.

## 1-Oxyl-2,2,6,6-tetramethylpiperidin-4-carboxylic acid radical (28)

Nitroxide **28** was synthesized according to a literature procedure.<sup>[5]</sup>

## 2,5-Dioxopyrrolidin-1-yl 1-oxyl-2,2,6,6-tetramethylpiperidin-4-carboxylate (29)



Nitroxide **28** (50.0 mg, 250  $\mu$ mol, 1.00 equiv) was dissolved in DMF (1.00 mL) and cooled to 0 °C. At this temperature, NHS (28.8 mg, 250  $\mu$ mol, 1.00 equiv) and DCC (72.9 mg, 325  $\mu$ mol, 1.30 equiv) were added. After stirring for 1 h at 0 °C, the reaction mixture was stirred for

additional 48 h at rt. Afterwards, the precipitates were filtered off and washed with DMF (5 mL). The solvents were removed *in vacuo* and the crude product was directly used for further reactions without purification.

**MS (ESI)**: 320 ( $[M+Na]^+$ ). **HRMS (ESI)** calculated for  $[M+Na]^+$ : 320.1343; found: 320.1338.

# (S)-2,2'-([1-Carboxy-5-(1-Oxyl-2,2,6,6-tetramethylpiperidin-4-carboxamido)-pentyl]azanediyl)-diacetic acid radical (13)

 $(S)-2,2^{\circ}-([5-Amino-1-carboxypentyl]-azanediyl)-diacetic acid$ (78.6 mg, 300 mmol, 1.20 equiv) was dissolved in H<sub>2</sub>O(1.30 mL) and NaHCO<sub>3</sub> (83.9 mg, 999 mmol, 4.00 equiv) wasadded. The active ester**29**(74.2 mg, 250 µmol, 1.00 equiv) was dissolved in ethanol (2.5 mL)

added. The active ester 29 (74.2 mg, 250 µmol, 1.00 equiv) was dissolved in ethanol (2.5 mL) and acetone (0.25 mL). The resulting solution was added to the lysine solution. The reaction

mixture was stirred for 17 h at rt, the reaction mixture was treated with  $H_2O$  (1.3 mL) and was then washed with NaHCO<sub>3</sub> (0.25 M, 0.52 mL). The solvents were removed *in vacuo* and the crude product was purified by flash chromatography (CH<sub>2</sub>Cl<sub>2</sub>/methanol/H<sub>2</sub>O 3:1:0.2) and NTA-lysine nitroxide **13** was isolated as a red solid (73.6 mg, 177 µmol, 71%).

**Mp**: decomposition above 218 °C. **IR (neat)**: 3220*w*, 2925*m*, 2861*w*, 2162*w*, 1979*w*, 1586*s*, 1407*s*, 1328*m*, 1242*m*, 1176*m*, 1079*w*, 1004*w*, 923*w*, 721*m*, 649*m*, 606*m* cm<sup>-1</sup>. **MS (ESI)**: 467 ([M+Na]<sup>+</sup>). **HRMS (ESI)** calculated for [M+Na]<sup>+</sup>: 467.22381; found: 467.22356.

#### 2.4.3 Synthesis of Succinimido Maleimide Nitroxide 15

4-(2,5-Dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)-*N*-(1-oxyl-2,2,6,6-tetraethylpiperidin-4-yl)butanamide radical (15)



4-Amino-TEMPO **26** (30.5 mg, 350  $\mu$ mol, 1.00 equiv) and 2,5dioxopyrrolidin-1-yl 4-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)butanoate (50.0 mg, 350  $\mu$ mol, 1.00 equiv) were dissolved in THF

(4.00 mL). The solution was stirred for 17 h at rt. The solvents were removed *in vacuo* and the crude product was purified by flash chromatography (pentane/acetone  $10:1 \rightarrow 2:1$ ). The maleimide nitroxide **15** was isolated as a red oil (51.0 mg, 152 µmol, 43%). The analytical data are in accordance with those reported in the literature.<sup>[8]</sup>

**MS (ESI)**: 359 ( $[M+Na]^+$ ). **HRMS (ESI)** calculated for  $[M+Na]^+$ : 359.1816; found: 359.1819.

#### **3** Site-Specific Nitroxide Exchange Reactions at Zeolite L

#### 3.1 General Procedure for the Nitroxide Exchange Reaction: General Conditions A



Alkoxyamine functionalized zeolite L crystals were suspended in DCE or  $H_2O$  in a sealed tube (2.0 cm length and 0.5 cm diameter). A nitroxide was added and the tube was purged with argon for 3 min. The tube was sealed and the reaction mixture was ultrasonicated for additional 3 min. The reaction was carried out at 125 °C, 90 °C, 70 °C or at rt for 1-4 h. After cooling to rt, the reaction mixture was suspended in THF or  $H_2O$ , the suspension was then transferred to a centrifuge tube and centrifuged for 15 min (5300 rpm, 1-5 times). The supernatant solution was decanted and the resulting zeolites were dried *in vacuo*.

Preparation of the microscope slide: The zeolites were suspended in toluene or  $H_2O$  (~0.2 mg/mL), ultrasonicated for 3 min and a droplet of the suspension was put on a microscope slide. After evaporation of the solvent at rt, the zeolites were analyzed by fluorescence and confocal microscopy.

For analysis of the reaction success by zeta potential measurements, the zeolites ( $\sim 0.1 \text{ mg}$ ) were suspended in NH<sub>4</sub>HCO<sub>2</sub> buffer (pH 7.4) or in H<sub>2</sub>O (mQ), ultrasonicated for 1 min and the solution was converted to the measuring cell. The measurements were performed at rt.

#### 3.2 Proof-of-principle: Nitroxide Exchange Reactions at 125 °C with 2 and 3



## 3.2.1 Reaction with Rhodamine Nitroxide 2

According to general conditions A, with alkoxyamine 19, 20 or 25 site-selectively functionalized zeolite L crystals (2.0 mg each) and nitroxide 2 (10 mg each) were suspended in DCE (0.4 mL each) and stirred for 4 h at 125 °C. The reaction success was verified by fluorescence microscopy (Figure S1 a and b) and by confocal microscopy (Figure S1 c and d) for zeolite conjugates 1. Conjugates obtained from 7 (conjugates with alkoxyamine 25) are shown in Figure S1 e and f. Conjugates obtained from 8 (conjugates with alkoxyamine 20) are shown in Figure S1 g and h.

*Figure S1*: Functionalization of: **a**, **c**, **e**, **g**: pore entrances; **b**, **d**, **f**, **h**: of the whole surface.



#### 3.2.2 Control Experiment 1: Adsorption of 2 onto the Surface



To exclude non-specific adsorption onto the crystal surface, unfunctionalized zeolite L crystals (2.0 mg) were reacted with nitroxide **2** (1.2 mg) in DCE (0.4 mL) for 4 h at 125 °C (general conditions A). Fluorescence microscopy showed barely any fluorescence after washing with THF,  $CH_2Cl_2$  and methanol (Figure S2 **a**).

*Figure S2*: **a**: Fluorescence image of control experiment 1: adsorption of nitroxide **2**; **b**: site-specific fluorescence under general conditions A with zeolite conjugates of type **1**.



#### 3.2.3 Reaction with NBD Nitroxide 3

According to general conditions A, with alkoxyamine **19** site-selectively functionalized zeolite L crystals (2.0 mg each) and nitroxide **3** (5.0 mg each) were suspended in DCE (0.4 mL each) and stirred for 4 h at 125 °C. The reaction success was verified by fluorescence microscopy (Figure S3, **a**, **b**).

Supplementary Material (ESI) for Chemical Communications This journal is (c) The Royal Society of Chemistry 2011

*Figure S3*: Functionalization of **a**: pore entrances; **b**: of the whole surface.





#### 3.3 Variation of Reaction Conditions

#### 3.3.1 Variation of Reaction Temperature

According to general conditions A, the nitroxide exchange reaction was performed with zeolite conjugates 1 or 7 (2.0 mg each) and rhodamine derivative 2 (1.00-10.0 mg each) at 125 °C, 90 °C, 70 °C and at rt in DCE (0.4 mL each) for 4 h. The reaction success was verified by fluorescence microscopy (Figure S4).

*Figure S4*: Fluorescence images from variation of temperature for the nitroxide exchange reaction with nitroxide **2**: **a**, **b**: entrance functionalization (**a**: conjugation with **19**; **b**: conjugation with **25**), **c**: whole surface modification with **25**. **1**: exchange at 125 °C; **2**: exchange at 90 °C; **3**: exchange at 70 °C; **4**: exchange at rt.



#### 3.3.2 Variation of Solvent

According to general conditions A, the nitroxide exchange reaction was performed with zeolite conjugates 1 or 7 (2.0 mg each) and rhodamine derivative 2 (1.0 mg each) for 4 h at 125 °C in H<sub>2</sub>O (0.4 mL each). The reaction success was verified by fluorescence microscopy (Figure S5).

*Figure S5*: i) Exchange with zeolites 7. ii) Fluorescence images from nitroxide exchange reactions with nitroxide 2 in  $H_2O$ : a: entrance functionalization, b: whole surface modification. 1a, 1b: conjugation with 19; 2a, 2b: conjugation with 25.



#### 3.3.3 Variation of Solvent and Temperature

According to general conditions A, the nitroxide exchange reaction was performed with zeolite conjugates 7 (2.0 mg each) and rhodamine derivative 2 (1.0 mg each) for 4 h at 125 °C in DCE (0.4 mL) or for 4 h at rt in H<sub>2</sub>O (0.4 mL). The reaction success was verified by fluorescence microscopy (Figure S6).

*Figure S6*: i) Exchange with zeolites 7. ii) Fluorescence images from nitroxide exchange reactions with nitroxide 2 in different solvents at different temperatures: **a**: entrance functionalization, **b**: whole surface modification. **1a**, **1b**: reaction for 4 h at 125 °C in DCE; **2a**, **2b**: reaction for 4 h at rt in  $H_2O$ .



#### 3.4 Monitoring Experiments: Studies towards Reversibility



According to general conditions A, the nitroxide exchange reaction was performed with zeolite conjugates (n = 0, 1) obtained from a functionalization with nitroxide 2 (1.00 mg) and an excess of TEMPO (40.0 mg) for 4 h or 24 h at 125 °C in DCE (0.4 mL). The reaction success was verified by fluorescence microscopy (Figure S7).

*Figure S7*: Fluorescence images before and after the reaction with excess TEMPO: **a**: zeolite conjugates with dye **2**; **b**: zeolite after the reaction for 24 h at 125 °C; **c**: zeolite after the reaction for 4 h at 125 °C.



#### 4 Site-Specific Immobilization of Proteins

#### 4.1 General Procedure for Protein Immobilization: General Conditions B



Large excess of protein (2-6  $\mu$ L solution in buffer) which was diluted with H<sub>2</sub>O, NH<sub>4</sub>HCO<sub>2</sub> or PBS buffer (0.1-0.2 mL, pH = 7.4) was added to the modified zeolite-bioconjugates obtained under general conditions A. The crystals were suspended in the reaction mixture for 3 min by ultrasonication and then reacted at rt between 1 h and 4 h. Work up was performed by centrifugation in H<sub>2</sub>O (up to 3 times) and analysis was conducted according to general conditions A.

#### 4.2 Immobilization of Concanavalin A (ConA)

According to general conditions A, the nitroxide exchange reaction was performed with zeolite conjugates 1 (2.0 mg) and sugar nitroxide 9 (10.0 mg) in DCE (0.3 mL) for 4 h at 125 °C or with zeolite conjugates 1 (2.5 mg) and sugar nitroxide 9 (1.0 mg) in H<sub>2</sub>O (0.5 mL) for 4 h at rt. Subsequent complexation with rhodamine tagged concanavalin A (2.0  $\mu$ L or 6.0  $\mu$ L) in H<sub>2</sub>O (mQ, 0.1 mL or 0.3 mL) for 4 h or 1 h at rt was performed (general conditions B). The reaction success was verified by fluorescence microscopy (Figure S8).

*Figure S8*: Fluorescence images after complexation of rhodamine labelled concanavalin A to mannose-modified zeolite L surfaces. **a**, **b**: entrance functionalization; **c**: whole surface modification.



## 4.2.1 Control Experiment 2: Adsorption of Concanavalin A onto the Surface



To exclude non-specific adsorption of the protein onto the crystal surface, unfunctionalized zeolite L crystals (0.5 mg) were reacted with concanavalin A (2.0  $\mu$ L) in H<sub>2</sub>O (0.1 mL) for 4 h at rt (general conditions B). Fluorescence microscopy showed barely any fluorescence after washing either with H<sub>2</sub>O or THF (Figure S9 **a**).

*Figure S9*: Fluorescence images after complexation of rhodamine labelled concanavalin A. **a**: adsorption onto a non-modified zeolite L crystal; **b**: complexation after entrance modification with mannose nitroxide **9**.



## 4.2.2 Control Experiment 3: Adsorption of Concanavalin A onto Alkoxyamine-Surfaces



To exclude non-specific adsorption of the protein onto the zeolite surface modified with alkoxyamines, zeolite L conjugates **1a** and **1b** (1.2 mg) were reacted with concanavalin A (6.0  $\mu$ L) in H<sub>2</sub>O (0.3 mL) for 1 h at rt (general conditions B). Fluorescence microscopy showed barely any fluorescence after washing with H<sub>2</sub>O (Figure S10 **a**, **c**).

*Figure S10*: Fluorescence images after complexation of rhodamine labelled concanavalin A. **a**: adsorption onto entrance-specific alkoxyamine-modified zeolite L crystals; **b**: complexation after entrance modification with mannose nitroxide **9**; **c**: adsorption onto all over alkoxyamine-modified zeolite L crystals; **d**: complexation after all over modification with mannose nitroxide **9**.



#### 4.3 Immobilization of Streptavidin

According to general conditions A, the nitroxide exchange reaction was performed with zeolite conjugates 1 (2.0 mg) and biotin nitroxide 11 (1.0 mg) in DCE (0.4 mL) for 4 h at 125 °C or with zeolite conjugates 1 (2.0 mg) and biotin nitroxide 11 (1.0 mg) in H<sub>2</sub>O (0.4 mL) for 4 h at 125 °C. Subsequent complexation with Oyster®-488 tagged streptavidin (1.0  $\mu$ L) in H<sub>2</sub>O (mQ, 0.1 mL) for 4 h at rt was performed (general conditions B). The reaction success was verified by fluorescence microscopy (Figure S11).

*Figure S11*: Fluorescence images after complexation of Oyster®-488 labelled streptavidin to biotin-modified zeolite L surfaces. **a**: entrance functionalization; **b**: whole surface modification.



# 4.3.1 Control Experiment 4: Adsorption of Streptavidin onto the Surface



To exclude non-specific adsorption of the protein onto the crystal surface, unfunctionalized zeolite L crystals (2.0 mg) were reacted with streptavidin (1.0  $\mu$ L) for 4 h at rt (general conditions B). Fluorescence microscopy showed barely any fluorescence after washing either with H<sub>2</sub>O or THF (Figure S12 **a**).

*Figure S12*: Fluorescence images after complexation of Oyster®-488 labelled streptavidin. **a**: adsorption onto a non-modified zeolite L crystal; **b**: complexation after entrance modification with biotin nitroxide **11**.



#### 4.3.2 Control Experiment 5: Adsorption of Streptavidin onto Alkoxyamine-Surfaces



To exclude non-specific adsorption of the protein onto the zeolite surface modified with alkoxyamines, zeolite L conjugates **1a** and **1b** (1.2 mg) were reacted with streptavidin (6.0  $\mu$ L) in H<sub>2</sub>O (0.3 mL) for 1 h at rt (general conditions B). Fluorescence microscopy showed barely any fluorescence after washing with H<sub>2</sub>O (Figure S13 **a**, **c**).

*Figure S13*: Fluorescence images after complexation of Oyster®-488 labelled streptavidin. **a**: adsorption onto zeolite alkoxyamine conjugates **1a**; **b**: complexation after entrance modification with biotin nitroxide **11**; **c**: adsorption onto all over alkoxyamine-modified zeolite L crystals **1b**; **d**: complexation after all over modification with biotin nitroxide **11**.



#### 4.4 Immobilization of eGFP

According to general conditions A, the nitroxide exchange reaction was performed with zeolite conjugates 1 (3.4 mg) and NTA lysine nitroxide 13 (4.0 mg) in H<sub>2</sub>O (0.6 mL) for 3 h at rt. After centrifugation in H<sub>2</sub>O (up to three times) and removal of the solvents *in vacuo*, NiSO<sub>4</sub> (aq., 1.0 mL, 100 mM) was added, the sample was ultrasonicated for 3 min and then reacted for 1 h at rt. Again the sample was centrifugated with H<sub>2</sub>O (up to three times) and dried *in vacuo*. Subsequently, his-tagged eGFP (10.0  $\mu$ L; 1 mg/mL in PBS buffer) in PBS buffer (1.0 mL, pH = 7.42) was complexated for 24 h at 4 °C. Work up was performed according to general conditions B. The reaction success was verified by fluorescence microscopy (Figure S14).

*Figure S14*: Fluorescence images after complexation of eGFP to NTA-Ni<sup>2+</sup>-modified zeolite L surfaces. **a**: entrance functionalization; **b**: whole surface modification.



# 4.5 Immobilization of β-Lactoglobulin A (LGA)

## 4.5.1 Synthesis of β-Lactoglobulin A Nitroxide 30



LGA (2.00 mg, 109 nm, 1.00 equiv) was dissolved in NH<sub>4</sub>HCO<sub>2</sub> buffer (1.09 mL, 20.0 mM, pH = 7.4). Succinimido maleimide nitroxide **15** (73.0  $\mu$ g, 218 nmol, 2.00 equiv), dissolved in acetonitrile (20.0  $\mu$ L), was added and the reaction mixture was stirred for 24 h at rt. Purification was performed by dialysis (620 mg NH<sub>4</sub>HCO<sub>2</sub> in 500 mL H<sub>2</sub>O using *Spectra/Por Dialysis Membranes*, MWCO: 6000-8000) for 24 h at 0 °C. The reaction success was verified by mass spectrometry.

**MS (ESI)**: 18699 ([M+H]<sup>+</sup>). **HRMS (ESI)** found: [M+H]<sup>+</sup>: 18699.61660.

#### 4.5.2 Immobilization of β-Lactoglobulin A (LGA)



According to general conditions A, the nitroxide exchange reaction was performed with zeolite conjugates **1** (2.0 mg) and  $\beta$ -lactoglobulin A nitroxide **30** (2.0  $\mu$ L of a 100  $\mu$ M solution in NH<sub>4</sub>HCO<sub>2</sub> buffer (pH = 7.4)) in H<sub>2</sub>O (mQ, 0.4 mL) for 4 h at rt. Work up was performed

according to general conditions B. The reaction success was verified by zeta potential measurements (Table S15).

*Table S15*: Zeta potential measurements of different surface modifications **a**: entrance modified zeolite conjugates and **b**: all over modified zeolite conjugates. Measurements were performed at rt in NH<sub>4</sub>HCO<sub>2</sub> buffer (pH = 7.4).

type of functionalization	a: entrance functionalization ζ (mV)	b: whole surface functionalization ζ (mV)
no functionalization	-60.89	
NH <sub>2</sub>	-46.81	-16.99
alkoxyamine <b>19</b>	-49.06	-9.56
LGA	-23.71	-5.42

#### 4.6 Immobilization of Bovine Serum Albumin (BSA)

#### 4.6.1 Synthesis of Bovine Serum Albumin Nitroxide 31



Rhodamine labelled BSA (2.00 mg, 30.3 nm, 1.00 equiv) was dissolved in NH<sub>4</sub>HCO<sub>2</sub> buffer (0.303 mL, 20.0 mM, pH = 7.4). Succinimido maleimide nitroxide **15** (163  $\mu$ g, 484 nmol, 16.0 equiv), dissolved in acetonitrile (10.0  $\mu$ L), was added and the reaction mixture was stirred for 24 h at rt. Purification was performed by dialysis (620 mg NH<sub>4</sub>HCO<sub>2</sub> in 500 mL H<sub>2</sub>O using *Spectra/Por Dialysis Membranes*, MWCO: 6000-8000) for 24 h at 0 °C.

#### 4.6.2 Immobilization of Bovine Serum Albumin (BSA)



According to general conditions A, the nitroxide exchange reaction was performed with zeolite conjugates 1 (2.5 mg) and rhodamine labelled bovine serum albumin nitroxide 31 (10.0  $\mu$ L of a 100  $\mu$ M solution in NH<sub>4</sub>HCO<sub>2</sub> buffer (pH = 7.4)) in H<sub>2</sub>O (mQ, 0.5 mL) for 4 h at rt. Work up was performed according to general conditions B. The reaction success was verified by fluorescence microscopy and by zeta potential measurements (Figures S16 and Table S17).

*Figure S16*: Fluorescence microscopy images of **a**: entrance modified BSA-zeolite conjugates and **b**: all over modified BSA-zeolite conjugates.



*Table S17*: Zeta potential measurements of different surface modifications **a**: entrance modified zeolite conjugates and **b**: all over modified zeolite conjugates. Measurements were performed at rt in  $NH_4HCO_2$  buffer (pH = 7.4).

type of functionalization	a: entrance functionalization ζ (mV)	b: whole surface functionalization ζ (mV)	
no functionalization	-60.89		
NH <sub>2</sub>	-46.81	-16.99	
alkoxyamine <b>19</b>	-49.06	-9.56	
BSA	-31.32	-2.89	

4.6.3 Control Experiment 6: Adsorption of Bovine Serum Albumin (BSA) onto the Surface



To exclude non-specific adsorption of the protein onto the crystal surface, unfunctionalized zeolite L crystals (2.3 mg) were reacted with rhodamine labelled bovine serum albumin (10.0  $\mu$ L of a 100  $\mu$ M solution in NH<sub>4</sub>HCO<sub>2</sub> buffer (pH = 7.4)) in H<sub>2</sub>O (mQ, 0.5 mL) for 4 h at rt (general conditions B). Fluorescence microscopy showed barely any fluorescence after washing either with H<sub>2</sub>O or THF (Figure S18 **a**).

*Figure S18*: Fluorescence images after complexation of rhodamine labelled bovine serum albumin. **a**: adsorption onto a non-modified zeolite L crystal; **b**: complexation after entrance modification with nitroxide **28**.



# 5 <sup>1</sup>H- and <sup>13</sup>C-NMR spectra of unknown compounds

# 5.1 4-(1-Bromo-2-methylpropyl)-benzoic acid (22)





5.2 Methyl-4-(1-bromo-2-methylpropyl)-benzoate (23)

# 5.3 Methyl-4-(1-[{4-(*tert*-butyl)-2,2,6,6-tetraethyl-3-oxopiperazin-1-yl}oxy]-2methylpropyl)-benzoate (24)



5.4 4-(1-[{4-(*tert*-Butyl)-2,2,6,6-tetraethyl-3-oxopiperazin-1-yl}oxy]-2-methylpropyl)benzoic acid (25)



## 6 Literature

- [1] A. Z. Ruíz, D. Brühwiler, T. Ban and G. Calzaferri, Monatsh. Chem., 2005, 136, 77.
- [2] S. Huber and G. Calzaferri, Angew. Chem. Int. Ed., 2004, 43, 6738.
- [3] K. Matyjaszewski, B. E. Woodward, X. Zhang, S. G. Gaynor and Z. Metzner, *Macromolecules*, 1998, 31, 5955.
- [4] M. K. Brinks, M. Hirtz, L. Chi, H. Fuchs and A. Studer, *Angew. Chem. Int. Ed.*, 2007, 119, 5324.
- [5] B. Schulte, M. Tsotsalas, M. Becker, L. De Cola and A. Studer, Angew. Chem. Int. Ed., 2010, 49, 6881.
- [6] S. Miele, P. Nesvadba and A. Studer, *Macromolecules*, 2009, 42, 2419.
- [7] C. B. Wagner and A. Studer, *Eur. J. Org. Chem.*, 2010, **30**, 5782.
- [8] G. Wu and W. L. Hubbell, *Biochemistry*, 1993, **32**, 879.