Modified Zeolites

Sequential Functionalization of the Channel Entrances of Zeolite L Crystals**

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Inorganic–organic host–guest systems are of great interest for designing supramolecular devices and machines.[1] We are especially interested in artificial antenna systems that mimic natural photosynthesis. A convenient host is zeolite L, a crystalline aluminosilicate in which corner-sharing SiO_4 and AlO_4^- tetrahedra give rise to one-dimensional channels arranged in a hexagonal structure. The channels have a smallest free opening diameter of 7.1 Å and a channel-to-channel distance of 18.4 Å.[2,3] They can be filled with suitable organic guest molecules, although only guests that can pass through the 7.1-Å opening are able to enter the channels. Pure zeolite L crystals with lengths between 30 and 7000 nm have been synthesized previously.[4].

Due to the channel entrances, the chemical and physical properties of the base and coat of the cylindrical crystals are different. Stopcocks are molecules that can only partly enter the channels. They typically consist of a head, a spacer, and a label, as shown in Figure 1. Only the label and the spacer can enter the channels due to size restrictions,[5] and, depending on the reactivity of the label, they can either be reversibly or irreversibly attached. While fluorescent stopcocks can be used to extract or inject electronic excitation energy from or

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[**] This work was supported by the Schweizerischer Nationalfonds zur Förderung der wissenschaftlichen Forschung NFP47 (4047-057481) and by the Schweizerische Bundesamt für Energiewirtschaft (project 10441). We thank Portlandzement AG for financial support. The large zeolite L crystals were synthesized by A. Zabala Ruiz and the small ones by R. Bühler.

  DOI: 10.1002/anie.200461114

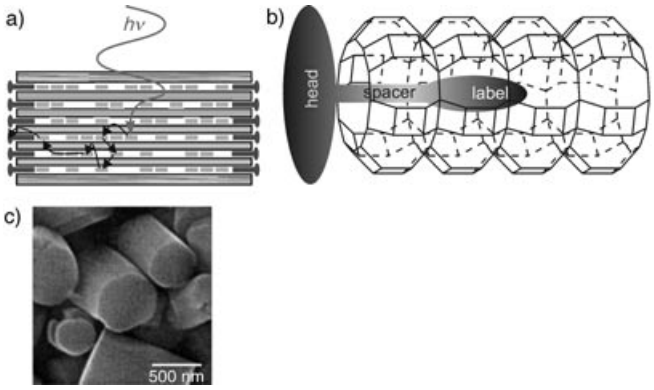


Figure 1. a) Schematic picture of a zeolite L crystal loaded with donor dye molecules and modified with acceptor stopcocks at the base. Excitation energy is channeled and focused from inside the zeolite crystal to the outside. b) Enlargement showing details of a channel, the smallest free opening of which is 7.1 Å, and the shape of a stopcock molecule. Only the spacer and label are small enough to enter the zeolite L channels; the head is too big. c) Scanning electron micrographic picture of zeolite L crystals.

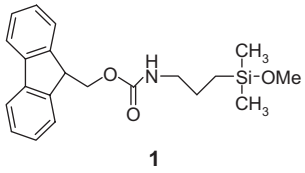
into the zeolite L crystals by radiationless Förster-type energy transfer,[6–8] others can be used to block the channel entrances against small molecules such as oxygen or water, or to hinder encapsulated dye molecules from leaving the channels.

Selective functionalization of only the channel entrances is an important topic in the development of highly organized materials, since these molecules are at the interface between the interior of a zeolite L crystal and its surroundings. We describe here a very versatile new five-step reaction principle (Figure 2), which can be summarized as follows:

1) Protection of the functionalizing group with a molecule that is too big to enter the channels of zeolite L. This step is necessary to ensure that the stopcock enters the channels in the desired direction.
2) Adsorption of the reactive stopcock at the channel entrances of the zeolite crystals.
3) Reaction between the adsorbed stopcock and the channel interior, which leads to irreversible fixation. The nature of the bond is not yet known, but water molecules and protons inside the channels seem to play an important role. Therefore, the bond is simply symbolized by a hook in Figure 2.
4) Removal of the protecting group, leaving the crystals with free functionalizing groups at the channel entrances.
5) Coupling of the head bearing the desired property; it can be a fluorescing dye or another molecule.

Since the delicate part of the reaction are steps 1–3, this principle allows us to synthesize a large variety of materials that would otherwise be very difficult to obtain.

This principle was demonstrated to work by functionalizing the channel ends of zeolite L crystals as follows: As a reactive stopcock, 9-fluorenylmethyl carbamate *N*-hydroxysuccinimidyl ester[9–11] (FMOC-NHS) was reacted with (3-aminopropyl)methoxydimethylsilane (APMS) to give **1**



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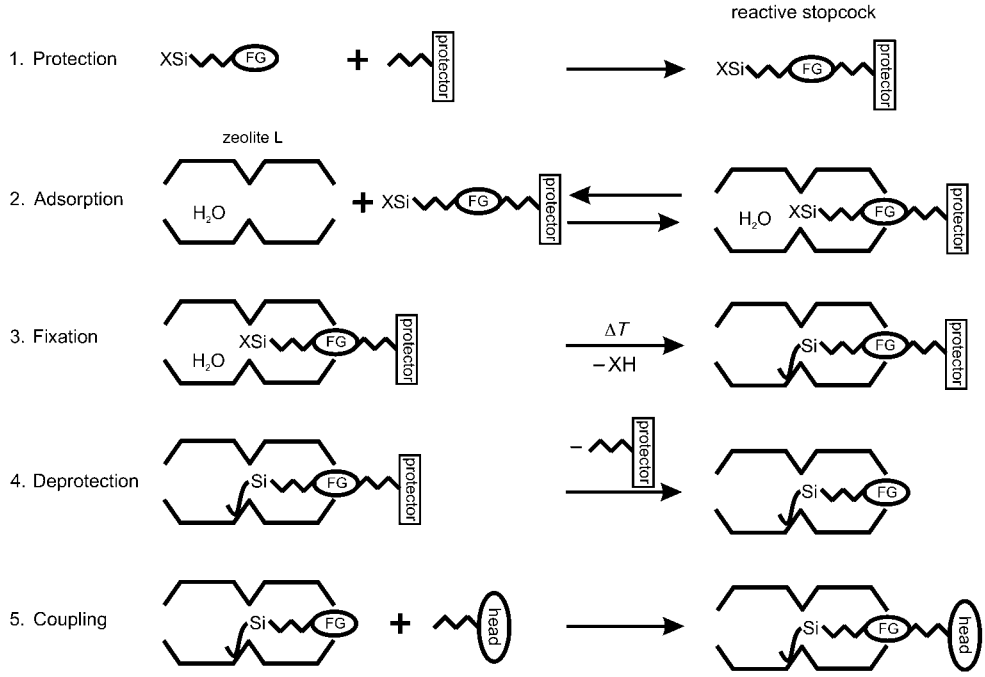


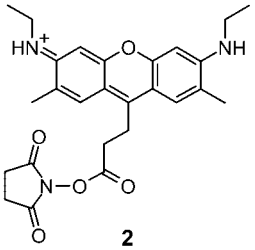
Figure 2. Reaction principle for the irreversible functionalization of the channel entrances of zeolite L crystals. FG: desired group to functionalize the channel ends; protector: the part of the molecule that is too big to enter the channels and that can be removed afterwards; XSi: the group that can penetrate the channels and bind to the zeolite L framework; head: the FG can be further reacted with a head if desired.

(FMOC-APMS). A weighed amount of zeolite L was suspended in *n*-hexane, the number of channel entrances was calculated, and the same amount of **1** was added to the suspension. Knowing the number of channel entrances per milligram of zeolite is very important, since an excess of **1** to channel entrances leads to crystals modified on the whole external surface. The stopcock molecule **1** has a hydrophobic head (FMOC) and a hydrophilic tail (methoxysilane); therefore the tail prefers to penetrate the zeolite L channels, which are hydrophilic, while the hydrophobic head prevents the molecule from entering the channels completely due to the size restriction imposed by the channel openings. The type of reaction between the methoxysilane and the interior of the zeolite channel must be investigated further. Since there are no free silanol groups inside the channels, reactions described for flat surfaces,[12] nanoparticles,[13] and the surface of zeolites[14] are not very probable. However, pentacoordinate silicon oxide compounds are well known,[15a] and pentacoordinate Al^{3+} in zeolite L has been proposed recently.[15b]

The selectivity of the shape dependence of the adsorption was shown by performing the same reaction with FMOC-protected (3-aminopropyl)triethoxysilane (FMOC-APTES). The triethoxysilane group is too bulky to enter the channels of zeolite L under these conditions. Moreover, APTES is known to form clusters in solution, which would also hinder a selective adsorption only at the channel entrances. Therefore, the molecule is expected to adsorb all over the outer surface of the crystals, whereas the smaller stopcock **1** can adsorb selectively at the channel entrances. The same amount of zeolite was used for both modifications and therefore exactly the same amount of FMOC-APMS and FMOC-APTES. Both samples were processed as described in the Experimental Procedure giving NH_2-zeolite L.

The number of amino groups linked to the zeolite L was determined by a quantitative ninhydrin colorimetric reaction, also known as the Kaiser test,[16] and compared to the calculated number of channel entrances. The results showed that essentially 100% of both FMOC-APMS and FMOC-APTES were attached to the zeolite, which means that every zeolite has as many amino groups on the surface as channel entrances for both reactants.

To show the spatial distribution of the amino groups on the zeolite L surface for both samples, the NH_2 groups can be marked with fluorescing dyes. Amino-reactive fluorescing dyes are used by biochemists for protein analysis and are readily available.[17] *N*-Hydroxysuccinimidyl ester (NHS) derivatives, such as dyes **2** (ATTO520-NHS) and **3** (ATTO610-NHS), are well known to react selectively with primary amino groups in high yield, giving an amide bond.



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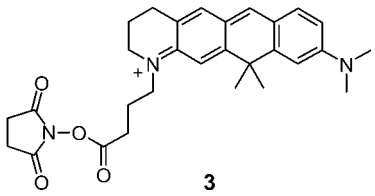

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Dye **3** was therefore coupled to the free amino group of APMS- and APTES-modified crystals. Confocal fluorescence microscopy pictures were taken of both, showing the spatial distribution of the fluorescing dye and therefore of the amino groups (Figure 3). The difference between the results

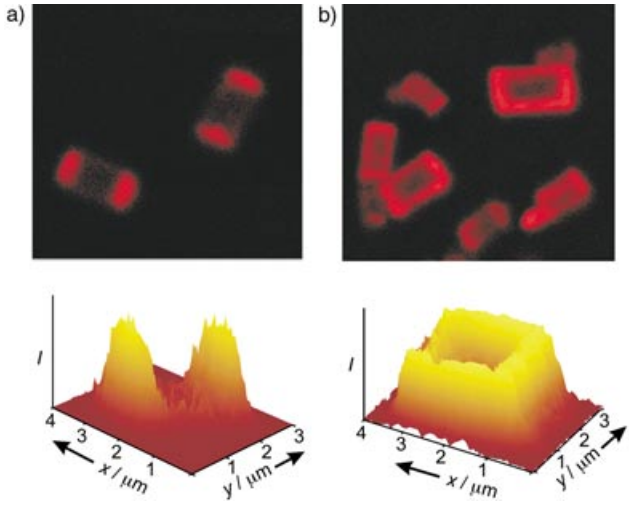

Figure 3. Confocal fluorescence microscopy pictures of zeolite L single crystals. a) Two crystals functionalized with APMS and coupled with the strongly fluorescing dye **3** (top). We also show the relative intensity distribution of one of these crystals (bottom). b) Several crystals functionalized with **3** coupled to APTES (top) and the corresponding relative intensity distribution of one of these crystals (bottom). Both samples are modified with the same amount of dye **3**, therefore the overall fluorescence intensity of the single crystals is similar.

obtained with the monomethoxysilane APMS and the triethoxysilane APTES is striking. The overall fluorescence intensity of single crystals of both samples is about the same, since the same number of amino groups is available, but the distribution of the amino groups on the zeolite surface is completely different: while FMOC-APMS is observed only at the base of the crystals, FMOC-APTES is observed to be present over the whole surface. The only way to explain this is by assuming that the smaller methoxydimethylsilyl group can enter the channels, while the more bulky triethoxysilyl cannot. Control experiments were performed by treating unmodified zeolite L crystals with dye **3**. Since there are no amino groups on the surface, one would expect the dye to be washed away completely. This is indeed the case, and no fluorescence was observed from these samples.

We found that the yield of the reaction between attached amino groups and the reactive dye is mainly determined by the size of the dye and the shape of the crystals. For dyes **2** and **3** we obtained an almost quantitative reaction with the amino groups, and hence full coverage of the channel entrances, when using crystals with a flat base and well-defined shape without any intergrowth, as shown in the SEM picture in Figure 1. The yield decreases to about 10% when zeolite L crystals with a very rough surface are used, indicating the importance of the morphology.

The excitation and emission spectra of **2**, **3**, and the near-IR-emitting dye ATTO680 (**4**) attached to the base of the

zeolite L crystals are shown in Figure 4a. (Note that the structure of **4** has not yet been revealed by the supplier). The excitation and emission spectra of **2** and **3** bound to zeolite L crystals are similar to those recorded for a dilute solution,

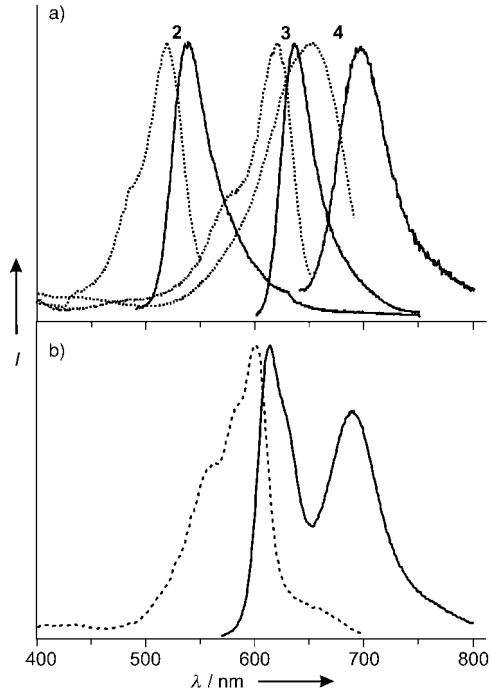

Figure 4. a) Excitation (dotted) and emission (solid) spectra of dyes **2** (ATTO520), **3** (ATTO610), and **4** (ATTO680) attached to zeolite L channel entrances. b) Excitation (dotted) and emission (solid) spectra of ATTO680,Ox^+-zeolite L. The emission spectrum was recorded when Ox^+ was excited selectively at 540 nm. The excitation spectrum was recorded at 720 nm, where essentially only ATTO680 emits.

showing that the dyes are present as monomers. However, the excitation spectrum of **4** is broadened and blue-shifted. Although the structure of **4** is not yet available, we can assume that its dimensions are substantially larger than those of **2** or **3**, allowing electronic interaction between dye molecules located at different channel entrances. This effect is even more pronounced when excitation and emission spectra are measured for a thick layer, in which the chances of intercrystalline electronic interactions between dye molecules bound to different crystals increase.

An additional proof to show that the amino-reactive dye is fixed on the zeolite L crystal can be obtained from energy-transfer experiments. Oxonine-loaded zeolite L (Ox^+-zeolite L) is a strongly emitting and stable material that has its emission maximum at 615 nm. The excitation and emission spectra of Ox^+-zeolite L were published in ref. [18]. The emission spectrum of Ox^+-zeolite L shows a pronounced overlap with the excitation spectrum of ATTO680-zeolite L. This allows Förster-type energy transfer from Ox^+ located inside the channels to ATTO680 at the channel entrances in an ATTO680,Ox^+-zeolite L sample, if the distance between Ox^+ and ATTO680 is in the range of the Förster radius. Therefore, the excitation energy absorbed inside the zeolite L is transported spatially and focused at the channel entrances. The excitation and emission spectra of an ATTO680,Ox^+-zeolite L sample are shown in Figure 4b. Selective excitation of Ox^+ causes an important fluorescence of ATTO680 with a maximum at 700 nm; this is obviously due to electronic excitation energy transfer. The ATTO680 is more strongly excited due to energy transfer from Ox^+ than by direct excitation at its absorption maximum at 660 nm, as can be seen from the excitation spectrum.

In summary, a new reaction principle for sequential functionalization of only the channel entrances of zeolite L has been developed. The reaction was demonstrated by attaching an aminomethoxysilyl group to the channel openings, which can be further used to selectively bind, for example, fluorescing dye molecules to the base of the crystals. Since amino-reactive fluorescing dyes are readily available, this allows the modification of the base of zeolite L crystals with dyes covering the whole visible and near-IR region. This has important consequences for the preparation of highly efficient antenna materials funneling the excitation energy from inside the zeolite L crystals to the channel ends, or vice versa.[7,8] The amino groups can also be transformed into thiol groups.[19] Such HS-zeolite L crystals are currently under investigation in our laboratory. The reaction principle is not restricted solely to zeolite L or to covalent modification with amino groups, thus opening up options for obtaining a wide range of new, functionalized zeolite-based materials, which otherwise would be very difficult, if not impossible, to obtain.

Experimental Section

Pure zeolite L crystals were synthesized and characterized as described in ref. [4]. The potassium-exchanged form was used for all experiments. The dyes **2**–**4** were obtained from Fluka and used as received. Confocal microscopy images were taken from zeolite L crystals having a length of about 3 μm; crystals of about 30 nm were used for the energy-transfer experiment. Fluorescence spectra were recorded on an LS 50B Perkin-Elmer luminescence spectrophotometer, and absorption spectra were measured with a Lambda 900 Perkin-Elmer UV/Vis/NIR equipment. Confocal microscopy was performed with a fluoview FV 300 (Olympus) accessory equipped with an argon-ion laser operating at 488 nm and an He/Ne laser operating at 543.5 nm.

Synthesis of FMOC-APMS (**1**): (3-Aminopropyl)methoxydimethylsilane (APMS, Acros, 97%; 10 μL, 8.7 mg, 0.06 mmol) and was added to 1 mL of CH_2Cl_2 (dried over CaH_2) in a Teflon tube. FMOC *N*-hydroxysuccinimide ester (FMOC-NHS, Fluka, >98%, HPLC; 30 mg, 0.089 mmol) was dissolved in 1 mL of dry CH_2Cl_2 and added dropwise to the APMS solution. The reaction mixture was stirred at room temperature and monitored by thin-layer chromatography. After 1 h the reaction was complete, and no more free amine could be detected by a ninhydrin test.

Synthesis of FMOC-APTES: This synthesis was carried out in analogy to that of FMOC-APMS with (3-aminopropyl)triethoxysilane (APTES, Fluka, ≥96%, GC; 10 μL, 8.5 mg, 0.053 mmol) instead of APMS.

Synthesis of APMS-zeolite L and APTES-zeolite L: Typically, zeolite L crystals (10 mg) were suspended in 2 mL of *n*-hexane (Merck, p.a.) in a Teflon tube. The number of channel entrances was calculated and 1 equiv of either FMOC-APMS or FMOC-APTES was added to the suspension. After the suspension was sonicated for 20 min to allow the stopcock to adsorb on the zeolite L crystals, it was heated at reflux for 3 h. The suspension was then centrifuged to give

FMOC-APMS-zeolite L or FMOC-APTES-zeolite L. The crystals were suspended in 1 mL of dry DMF containing 0.2 mL of piperidine and stirred for 1 h to remove the FMOC group. After the crystals were centrifuged and washed twice with 2 mL of MeOH, APMS-zeolite L or APTES-zeolite L (NH_2-zeolite L) was obtained.

Quantification of NH_2 groups bonded to zeolite L: The amine test solution consisted of ninhydrin (Fluka, ~99%, UV; 0.5 g, 2.81 mmol) dissolved in 40 mL of *n*-butanol and 10 mL of doubly distilled water. A 2-mL aliquot of the test solution was added to 0.05 μL, 0.025 μL, 0.0125 μL, and 0.005 μL (0.3 μmol, 0.15 μmol, 0.075 μmol, and 0.03 μmol, respectively) of APMS, and the solutions were stirred at 100°C for 35 min. The absorption at 570 nm was measured for all samples against a blank containing ninhydrin solution without APMS, giving a regression line. A weighed amount of typically 1–2 mg of NH_2-zeolite L was suspended in 2 mL of the ninhydrin test solution and stirred at 100°C for 35 min. After centrifuging, the absorption of the solution was measured at 570 nm, and the number of NH_2 groups per mg of zeolite L was calculated from the regression line.

Reaction of NH_2-zeolite L with an amino-reactive dye: NH_2-zeolite L (ca. 5 mg) was suspended in 1 mL of dry acetonitrile (Merck, DNA grade). Triethanalamine (Fluka, >99%, GC; 5 μL, 5.62 mg, 0.038 mmol) was added and the suspension was sonicated for 10 min. The amino-reactive dye (3–4 equiv relative to the NH_2 groups) was also dissolved in 1 mL of acetonitrile and added dropwise to the zeolite suspension. The dye-zeolite L was stirred for 1 h then washed twice with 2 mL of MeOH to remove excess dye. The amount of dye bound to the NH_2 groups was determined by destroying a known amount of dye-zeolite L with hydrofluoric acid and measuring the concentration of the dye by UV/Vis spectroscopy.

Synthesis of Ox^+-zeolite L: Ox^+ was synthesized as described in ref. [20] and inserted into zeolite L crystals by ion exchange.[7] Further reaction with ATTO680-NHS ester was performed as described previously for empty zeolite L.

Received: June 29, 2004

Keywords: fluorescence · organic–inorganic hybrid composites · silanes · surface chemistry · zeolites

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Sequential functionalization of the channel entrances of zeolite L crystals

In a teflon tube, 10 µl of (3–aminopropyl)dimethylmethoxysilane (APMS, 0.059 mmol) were diluted with 1 ml of CH_2Cl_2, and 30 mg of FMOC–N–hydroxysuccin-imidylester (FMOC–NHS, Fluka, > 98 % HPLC; 0.089 mmol, 1.5 eq) disolved in 1 ml CH_2Cl_2 were added dropwise. The reaction mixture was stirred at room temperature and followed by TLC. After stirring for 30 min, no more free amino groups could be detected with a ninhydrin test (see 8.3.4 for details) showing that FMOC–NHS had reacted with all free NH_2 groups to build FMOC–APMS. A weighted amount of zeolite L, typically 10–20 mg was suspended in a puffer solution of pH 5 (composition reported in section 8.2) and stirred for 1 h. After washing the crystals once with bidest. water, they were blown dry with N_2 and kept at 22% rel. humidity for some hours to rehydrate. The zeolite L crystals were then transferred to a teflon tube and suspended in 2 ml n–hexane. The amount of channel entrances was calculated usind Eq. ***3.5*** *and exactly the corresponding amount of APMS–FMOC solved in 10 µl CH_2Cl_2 was added. The suspension was sonicated for 15 min to allow adsorption of the FMOC–APMS stopcock at the channel entrances. Afterwards, the suspension was refluxed at 65°C for 3 h to covalently bind the stopcock molecules. After centrifuging, the FMOC–APMS–zeolite L sample was suspended in 2 ml of DMF and 0.2 ml of piperidine was added. The deprotection was complete after stirring the suspension for 30 min at room temperature, giving H_2N–zeolite L. The modified zeolite L sample was washed two times with 2 ml acetonitrile to get rid of the remaining piperidine and dried in a oven for 2 h at 80 °C. The zeolite L crystals modified covalently with amino groups at the channel entrances can be used as a precursor and any amino reactive substance can be bound to the free amino groups. The amount of free amino groups bound to the channel entrances of zeolite L was determined by a quantitative ninhydrin test (see 8.3.4).*

The strongly fluorescent, amino–reactive dye ATTO610–N–hydroxysuccinimidylester (ATTO610–NHS) was coupled to the precursor material H_2N–zeolite L as follows: About 10 mg of H_2N-zeolite L were suspended in 2 ml dry acetonitrile, 10 µl triethanolamine (TEA, Fluka, >99% GC; 0.076 mmol) was added to deprotonate the NH_2 groups and the suspension was heated to 70 °C. An excess of ATTO610–NHS was disolved in 1 ml of dry acetonitrile and half of the solution was slowly added to

the H_2N–zeolite L suspension. After stirring for 15 min at 70 °C, the second half of the dye solution was added slowly and the mixture was stirred for 1.5 h at 70 °C. After cooling, the suspension was centrifuged and the ATTO610–zeolite L was washed two times with methanol to get the final product.

Buffer solution pH 5.0:
Fluka 0.095 M citric acid, 0.2 M sodium hydoxide