On the Reversible Photoisomerization of Spiropyran-Modified Zeolite L Single Crystals


Photoisomerization of spiropyrans has been the subject of many interesting studies in a wide number of fields, such as sensors for cations,[1] sensors of pH,[2] switchable surfaces,[3] lasing effect,[4] among many others. This molecule can be photoisomerized upon UV irradiation, giving rise to the zwitterionic merocyanine (MC) form, which dramatically changes its hydrophilicity and photophysical properties (see Scheme 1). Irradiation in the visible region or heating brings back the spiropyran (SP) to the MC form. Solvent polarity and pH have been already shown to strongly influence the properties of spiropyrans.[5] SP does not absorb in the visible range, while the merocyanine, which absorbs at ca 570 nm (in DMSO), exhibits red fluorescence and its lifetime is typically about 260 ps.[6]

Recently the use of spiropyrans in the field of dual emission for bio-imaging has been reported, where silica nanoparticles containing spiropyran and a green-emitting perylene derivative were shown to exhibit green or red emission depending on the UV/Vis irradiation.[7] Such systems may become even more versatile porous materials, such as zeolite L, which are known to strongly influence the properties of spiropyrans.[8] Zeolite L is a porous aluminosilicate material consisting of thousands of one-dimensional nanochannels parallel to each other, which run throughout the long axis of the crystal.[9] Zeolite L can be used not only as luminescent devices,[10] in bio-imaging,[11] or in photodynamic treatments,[12] but also in many other applications.[13] This class of crystals can still be functionalized on glass, indium–tin oxide or other substrates and can be synthesized from 50 nm up to 20 µm,[13] which may be strategic, depending on the application. SP-functionalized zeolite L crystals with encapsulated dyes may be an interesting option to make new dual emitters, which can be used in bio-imaging experiments, even without taking advantage of energy transfer between encapsulated dyes and SP. Because cells may exhibit auto-fluorescence, a system having two different emissions at different times allows for distinguishing between the auto-fluorescence and the luminescence of the dual-emitters, simply by direct visualization of the sample in the microscope.

The advantages of using zeolite L instead of, for example, silica nanoparticles, are: 1) the size of the zeolite L crystals can be synthetically tuned from 50 nm to 20 µm, in order to be used in different experiments; 2) the luminescence of the encapsulated dyes, which can be highly anisotropic, is also strong because the inserted dyes are in the monomeric form and are completely isolated from the outer bio-environment; 3) zeolite L has been already shown to be easily functionalized with targeting groups, enabling them to attach to different living organisms.[11]

Herein we show for the first time the reversible photoisomerization of a spiropyran derivative (at the single crystal level) functionalized at the channel entrances of zeolite L and discuss the use of such single crystals as dual emitters in the field of bio-imaging. In a first step zeolite L can be loaded with a green-emitting dye (pyronine) and afterwards its outer surface can be functionalized with a spiropyran derivative. This system is also an interesting model for sensors based on energy transfer, because the spectral overlap necessary to make a Förster-type energy transfer from the encapsulated dye to the spiropyran (or vice versa, depending on the dye) strongly depends on the form of the spiropyran (SP or MC), which may be tuned by UV/Vis irradiation of the system.

The ninhydrin test used to quantify the number of amino groups functionalized at the outer surface of the 1 µm long zeolite L crystals indicates that there are ca 5.23 x 10⁻⁸ mol NH₂ groups in 1 mg of zeolite–NH₂. This value is 75 times larger than the expected one, which would have been obtained given 100% NH₂ coverage of zeolite L. The maximum number of amino groups expected to cover the zeolite L crystals was roughly estimated by considering a perfect cylinder with length/diameter of 1 µm/800 nm, whereas the size distribution...
of the crystals, as well as the existence of terraces, were not taken into account. Considering the approximations used in this estimate, one concludes that the zeolite L surface is covered with amino groups 100%, as indeed suggested by Figure 2c. However, an excess of FMOC–APMS used in the amino functionalization of the zeolite L, which would lead to some cross-linked material on the surface of the crystals, cannot be excluded, since it would give rise to a larger number of amino groups detected in the ninhydrin test, as indeed observed.

The absorption spectrum of the synthesized SP in DMSO, before and after UV irradiation, as well as the red emission of the photo-generated MC, are shown in Figure 1. The solvent DMSO was chosen because of its basic pH, which avoids the protonation of the negatively charged oxygen of the MC form, giving rise to an absorption spectrum peaked at 570 nm. The MC species is subject to strong solvatochromism, a strong red shift being observed upon increasing the polarity of the solvent, which is due to the high hydrophilicity of that species. Interestingly, the SP shown in Scheme 1 has exhibited the rather rare negative photochromism[14] when in H2O/MeOH mixed in a 1:1 ratio (v/v), where the coloured MC species is the most stable form and is in fact spontaneously formed in the dark (Figure S1, Supporting Information). This may be due to a strong stabilization of the open form (MC) by hydrogen bonds and/or protonation. Protonation, however, seems to be a secondary effect to explain the negative photochromism of SP in the H2O/MeOH mixture, since solutions of SP in acidic solvents like CH3COOH, where protonation may occur, give rise to a positive photochromism, where the colourless SP is the most stable form in the dark. After UV irradiation at 366 nm, excitation of MC at 460 nm produces a red luminescence peaked at ca 650 nm, whose intensity slowly decreases as a result of the thermally induced back-isomerization MC → SP (Figure 1). Excitation at the main absorption band of MC (ca 570 nm) also induces the back-isomerization MC → SP.

The confocal picture of a SP-functionalized zeolite L crystal is shown in Figure 2a, which was obtained upon excitation at 535 nm, after short exposure of the crystals to the daylight. One can see from the distribution of the red emission of MC that the 4 µm long zeolite L crystals are predominantly functionalized at the channel entrances. The functionalized single crystals exhibit reversible photoisomerization, as shown in Figure 2b. Functionalization of the small zeolite L crystals (method 2, Experimental Section) gave rise to crystals completely covered by MC, whose photoisomerization was also shown to be reversible, as shown in Figure 2c. To the best of our knowledge, this is the first time that a reversible photoisomerization of a spiropyran at the single-crystal level is reported, as shown in Figure 2b. Figure 3 shows the colour changes of zeolite L crystals loaded with pyronine and functionalized with SP after irradiation with UV. The luminescence was generated by continuously exciting at 360 nm with the microscope excitation cube. Immediately after UV irradiation, when SP is photoisomerized to MC,
orange luminescence is produced, which is the sum of the green luminescence of pyronine and the red luminescence of MC. The green emission of pyronine radiatively isomerizes MC back to the non-emissive SP form, which results in the increasing green content in the emission of the samples upon going from panels 1 to 4 (Figure 3).

Figure 3. Fluorescence microscopy pictures showing dual emission coming from pyronine-loaded zeolite L crystals functionalized with SP (in air). The samples were continuously excited at 360 nm, and then pictures 1 to 4 were taken with time intervals of 1 min.

Förster resonant energy transfer (FRET) from pyronine to MC could not be observed by means of emission spectra, perhaps because the loading of the encapsulated dye was small (less than 1%), which means that the distance between the encapsulated pyronine and the spiropyran was considerably larger than the Förster radius $R_0$. The use of a higher loading of the encapsulated dye would in fact lead to smaller distances between the spiropyran and the pyronine, but on the other hand would not lead to a system showing dual-emission properties, since in this case only the extremely intense luminescence of the pyronine would be observed, and this is out of the scope of the present work.

The results shown in Figure 3 open new possibilities for using hybrid materials based on zeolite L as dual-emitters. It is worth saying that the whole hybrid supramolecular system can be further covered with a protective layer of silica, which may enable one to study dual emission using the functionalized zeolites not only in the external membrane surface, which may be in contact with the physiological medium, but additionally inside the cells, where the high hydrophobicity would make it difficult for SP to photoisomerize to the highly hydrophilic MC species. A sensor for cations is another interesting application for such hybrid systems. As an example, the closure time of the SP ring has shown to have a $\tau_{1/2}$ of 2.4 s in CH$_3$CN, while addition of a very small amount of Co$^{3+}$ has increased this time to 6.9 s (Figure S2).

We showed for the first time a reversible photoisomerization of a derivative of spiropyran at the level of a single crystal by using zeolite L. The synthesized spiropyran exhibited rare negative photochromism when dissolved in H$_2$O-EtOH 1:1. The spiropyran was indeed functionalized predominantly at the channel entrances in the case of 4 µm long crystals, as shown by the fluorescence microscopy pictures. The supramolecular hybrid system has shown to have a potential to be used as dual-emitters for bio-imaging, as nicely shown by the change from reddish to green fluorescence with the time. The development of SP-zeolite L systems for dual-emission and bio-sensors is currently being investigated in our group.

Experimental Section

Spiropyran: Spiropyran (SP) 1-[(carbosuccinimidyl-oxy)ethyl]-3,3-dimethyl-6-nitrospiro[2H–1]benzopyran-2,2-indoline (Scheme 1) has been synthesized according to a procedure previously described. The compound was purified by usual chromatography techniques and stored at 4 °C in the dark.

Zeoite L: Zeolite L was synthesized following a hydrothermal procedure described elsewhere. Zeolite L crystals featuring 4 µm/1 µm and 1 µm/800 nm length/diameter aspect ratios were used. All the crystals were cation exchanged with K$^+$ before any reaction. Some of the zeolite L crystals were loaded with the green emitting dye pyronine, by means of cation exchange from the solution.

Amino-Functionalized Zeolite L: The 1 µm long zeolite L crystals were completely amino-functionalized by following a well-known procedure: 9-fluorenyl methyl carbamate N-hydroxysuccinimidyl ester (FMOC-NHS) reacts with (3-aminopropyl)methoxydimethylsilane (APMS) to give FMOC-APMS, which then covalently binds to the free OH groups present in the external surface of the zeolite L crystals. The ninhydrin test was carried out to quantify the number of amino groups present in the functionalized zeolites.

SP-Functionalized Zeolite L: This was carried out following two different methods.

**Method 1:** Inside a teflon tube SP still activated with the succinimid group reacted with a stoichiometric amount of aminopropylmethoxydimethylsilane (APMS) to give FMOC-APMS, which then covalently binds to the free OH groups present in the external surface of the zeolite L crystals. The ninhydrin test was carried out to quantify the number of amino groups present in the functionalized zeolites.

**Method 2:** 1 µm long zeolite L crystals were first amino-functionalized as described above. A solution of 10$^{-4}$ M SP in freshly distilled CH$_2$Cl$_2$ was slowly added to a stirring suspension of amino-modified K-exchanged zeolite L (1 mg mL$^{-1}$) in the same solvent and left reacting for 1 h at room temperature. The crystals were then washed with CH$_2$Cl$_2$ and centrifuged several times, and afterwards stored in the dark at 4 °C.

**Method 3:** 1 µm long zeolite L crystals were first amino-functionalized as described above. A solution of 10$^{-4}$ M SP in freshly distilled CH$_2$Cl$_2$ was slowly added to a stirring suspension of amino-modified K-exchanged zeolite L (1 mg mL$^{-1}$) in the same solvent and left reacting for 1 h at room temperature. The crystals were then washed with CH$_2$Cl$_2$ and centrifuged several times, and afterwards stored in the dark at 4 °C.

Measurements: Absorption spectra were measured on a Varian Cary 5000 double-beam UV/Vis–NIR spectrometer and baseline corrected. Steady-state emission spectra were recorded on a HORIBA Jobin–Yvon IBH FL-322 Fluorolog 3 spectrometer equipped with a 450 W xenon arc lamp. The fluorescence pictures of the zeolites showing the reversible photoisomerization of SP were made with the confocal microscope DM IRB (Leica), the samples being irradia-
ed with a portable UV-lamp (366 nm). The fluorescence pictures showing dual-emission were taken with a confocal microscope MicroTime 200 (PicoQuant) in the normal mode. The confocal fluorescent picture of the single crystal was recorded on a confocal microscope TCS SPE (Leica).

Acknowledgements

RQA would like to acknowledge the Alexander von Humboldt Foundation and SFB TR161 for financial support.

Keywords: photochemistry · photoisomerization · single crystals · spiropyran · zeolites


On the Reversible Photoisomerization of Spiropyran-Modified Zeolite L Single Crystals

Having it both ways: The reversible photoisomerization of a derivative of spiropyran at the level of a single crystal by using zeolite L is demonstrated. The synthesized spiropyran exhibits rare negative photochromism when dissolved in a 1:1 mixture of H₂O and EtOH. The spiropyran is functionalized predominantly at the channel entrances in the case of 4 μm long crystals (see picture).