Self-Assembly of Bacteria

DOI: 10.1002/anie.200701019

# Self-Assembling Living Systems with Functional Nanomaterials**

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*Dedicated to Professor Vincenzo Balzani*

Assembling molecules in large architectures,[1] or in functional supramolecular systems,[2–12] together with the understanding of the type of interactions between molecules and/or molecule and substrate is an interesting and growing field for the realization of molecular devices.[13,14] Inspired by nature, scientists have designed and created simple systems that could mimic natural functions by connecting biological components to abiotic materials[15–17] to understand the workings of the biological system[18–20] or to take advantage of the unique properties of the "nonbiological" components in a natural setting (in vivo and in vitro). For this purpose, recently, nano- and microscale objects such as nanoparticles,[21] micrometer plates,[22] and nanorods[23] have been assembled with the aim to bridge the gap between the nano- and the macroscopic worlds or to reproduce structures with dimensions similar to biomacromolecules. However, so far no attempts have been published on self-assembling bacteria by using artificial functional nano- and micromaterials to enable, eventually, communication between the cells.

With this goal in mind and with the ambition to realize the first step toward the exchange of specific information between the synthetic systems and/or bacteria, we have functionalized biocompatible artificial nanocontainers (zeolite L) and attached them to nonpathogenic bacteria (*Escherichia coli*; *E. coli*). We demonstrate herein that the living system attached to the zeolite can be easily visualized by using fluorescence spectroscopy and, owing to the particularly defined geometrical arrangement of the zeolite and bacteria, we are also able to self-organize two bacteria by using the nanocontainer as a junction.

Zeolites are framework silicates consisting of interlocking tetrahedrons of $SiO_4$ and $AlO_4$. Each Al atom in the framework contributes a negative charge that is compensated by the exchange of cations such as sodium, calcium, and others that reside in the large vacant spaces or cages in the structure.[24,25] Zeolite L contains one-dimensional channels running through the whole crystal with an opening of 0.71 nm, a large free diameter of 1.26 nm, and a unit-cell length of 0.75 nm (Figure 1). The center-to-center distance between two channels is 1.84 nm.[24,25]

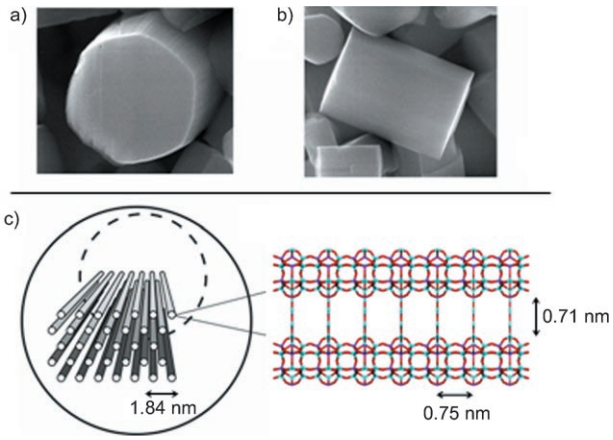


***Figure 1.*** Framework and morphology of zeolite L. SEM images of the base (a) and coat (b) of a crystal are shown. c) The image in the circle shows how these materials consist of a large number of strictly parallel channels going through the whole crystal. The image on the right shows the side-on view of a modeled single channel.

As an example, a crystal with a diameter of 550 nm consists of about 80000 parallel channels. Important properties of these crystals are their versatility to host molecules that possess desired emission properties,[26] for example, dyes. Furthermore, there is the possibility to prepare them in different aspect ratios and sizes ranging from 30 nm up to several thousand nm,[27] and the possibility to chemically modify the channel entrances in a specific way with stopcock molecules.[26,28–30] It has also been demonstrated that ions can be exchanged in and out of the channels.[31]

Finally, owing to their biocompatibility and unidimensional porous character, crystals of zeolite L can be used to realize artificial assemblies in which new properties and functions, not present in the more common nanoparticles, can be implemented. To prove that it is indeed possible to expand the assembly concept to living systems and not only to molecules and nanoobjects, we have realized a hybrid

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[**] Supported by Ministry of Science and Research (MWF), Northrhine Westfalia, Germany, SFB 656, MoBil Muenster, Germany, and by the Swiss National Science Foundation, project NF 200020-105140. We thank Dr. Kathrin Bissantz and Ramona Wesselmann for providing *E. coli* samples. We appreciate the efforts of Dr. Dingyong Zhong in helping with the SEM measurements and Dr. Arantzazu Zabala Ruiz for the zeolite preparation.

Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.

construction based on cells and zeolites L. The organism we have selected for the assembly is the nonpathogenic *E. coli* (strain JM109). This cell belongs to the family of Gram-negative bacteria. The outer cell membrane of *E. coli* is blistered with lipopolysaccharides that are phosphorylated at multiple locations.

Such a property was used for the construction of the assembly as we decided to explore an electrostatic-type binding between the negatively charged outer cell membrane and a positively charged zeolite L crystal. Interestingly we are able to position the charge only at the entrance of the channels so that the entire crystal retains its own character on the surface. To realize the construction, we first loaded 1-μm-long zeolite L with the green luminescent organic dye pyronine through an ion-exchange procedure[26] (Figure 2).

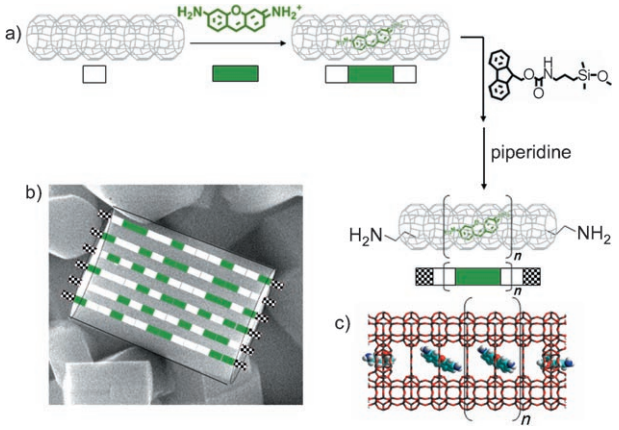


***Figure 2.*** Simplified stepwise synthetic procedure for the amino functionalization of zeolites L. a) A single channel is depicted (the white boxes represent the unit cell) and after insertion of the pyronine (fluorescent dye, green rectangular) the entrance is functionalized with a silane derivative. Hydrolysis of the amido group results in a primary amine group (black and white checkerboard). b) A superimposition of the cartoon-type representation and the real SEM picture are shown. c) A view of the functionalized crystal and the molecule inserted is sketched in a simplified molecular-modeling structure.

We then functionalized the channel entrances[28] of the zeolite with thousands of amino derivatives, which under our conditions are protonated, leading therefore to the desired positively charged systems. Amino-functionalized zeolite crystals and bacteria in an estimated 1:1 ratio were then incubated together for 1 hour at 37 °C in phosphate-buffered saline (PBS) solution.

We observe a stable formation of the hybrid assembly most probably owing to electrostatic interactions even though we cannot exclude hydrogen-bond formation. The nonfunctionalized zeolite L crystals do not form, as expected, stable assemblies with the bacteria. To characterize the assembly, fluorescence microscopy was employed in solution. In the aggregate, the zeolite can be easily detected because upon excitation, in the range 420–490 nm, the excited state of the encapsulated pyronine dye is generated and green emission is observed (Figure 3a).

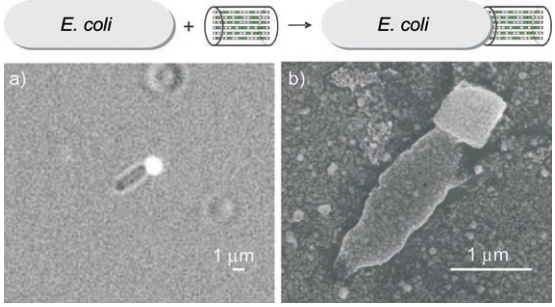


***Figure 3.*** Assembly of 1:1 zeolite L/bacterium in PBS buffer solution. Image (a) was taken upon white- and blue-light illumination in an optical microscope. b) SEM image of the assembly after evaporation of the solvent and subsequent coating with silver.

Further analysis performed on dry samples by using scanning electron microscopy (SEM) confirmed the solution results (Figure 3b). It is interesting to note that the zeolite-bacterium assembly is able to live under the physiological conditions and that the movement of the living system is not prevented. The organism is able to "swim" in the solution even with a heavy load such as the 1-μm zeolite! (see movies in the Supporting Information). The movement of the bacterium can be easily tracked under a fluorescent microscope by using the light emitted upon excitation of the entrapped dyes (pyronine). As can be noticed after investigation of several samples (visible in Figure 3 and movies in the Supporting Information), the zeolite is predominantly attached to the pole of the bacterium. This observation is corroborated by literature data that show that micrometer particles are also attached mainly at the end of the *E. coli* bacteria,[32] and even though it is not fully understood, such behavior can be due to the different domains of the cell surface.[33] We cannot exclude that in the first milliseconds after mixing the components, the zeolites and the bacteria are more randomly assembled and a migration of the zeolite along the cell occurs to maximize the zeolite–bacteria interaction. At this point, we would like to stress that the assembly is not a casual event but occurs for most of the bacteria (>70%) present in solution. This can also be observed in the movies present in the Supporting Information in which several bacteria are visible (out of focus) that possess an attached zeolite.

The geometrically linear assembly shown in Figure 3 stimulated a perhaps obvious question: can we assemble living systems by using the nanocontainers as a junction?

To achieve this goal, we have changed the estimated ratio between the cells and the zeolite L (by using a large excess of bacteria). After mixing, we observe, under the microscope, a linear structure that does not correspond to a single cell. An accurate analysis of the assemblies proves that now the ratio between the zeolite and the bacteria is 1:2. Figure 4 shows the hybrid assembly, which was characterized by optical microscopy by using white- and blue-light irradiation. At this stage, we do not know if the zeolite can play an active role in the communication of the two linked systems. In fact, we wish to stress that our zeolite, used as a connector, is not only biocompatible and stable but also functional and modular.

  

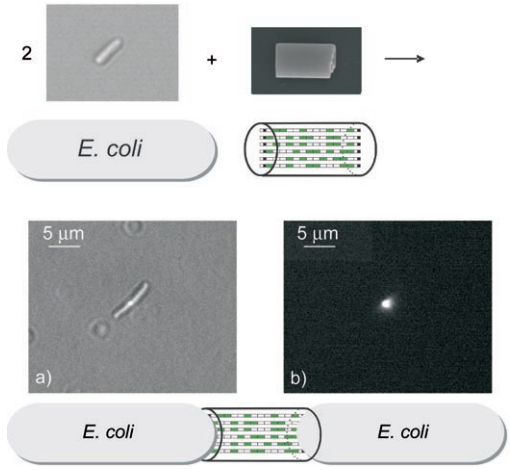


***Figure 4.*** Self-assembly of two bacteria with functionalized 1-μm zeolite L as the junction. a) Optical image in white- and blue-light illumination showing the entire assembly. b) In the same sample, fluorescence of the pyronine-filled zeolite, the junction, under blue-light excitation is observed.

The channels that span from one edge to the other can be empty or filled with many different chemicals.

The length of the zeolite as well as the width can be varied by up to three orders of magnitude, which suggests the possibility to explore topological effects in living systems. Furthermore, the selective functionalization of the zeolite in space and with different chemical groups opens infinite possibilities for connections and combination of living systems and materials.

In conclusion, we have shown that self-assembly of functional materials and living systems is possible through a chemically programmed construction. Self-assembly of bacteria can be achieved and we believe that an exchange of specific information between the zeolite and/or the bacteria is possible and that it will be fascinating to explore the consequences of this.

## Experimental Section

Zeolite modification: The cylindrical synthetic zeolite L crystals used in this work were prepared according to the procedure described in reference [27]. The crystals had a mean length and mean diameter of $1.0 \times 1.0\ \mu m^2$. Pyronine-loaded crystals were prepared by an ion-exchange procedure from water as described previously.[26] Amino termination of the crystals was performed as described previously.[28]

Experiments with bacteria *E. coli*: The bacteria sample, *E. coli* (strain JM109), was freshly prepared from an incubated stock solution in Lysogeny broth (LB) medium (LB medium is made from tryptone (10 g), yeast extract (5 g), and NaCl (10 g) in water (1 L); pH 7, adjusted by NaOH) and suspended in PBS solution. The concentration is estimated (from optical density) to be in the order of $10^9$ cells per mL. Zeolite (ca. 1 mg) was suspended in doubly distilled water (1 mL) and sonicated for 15 min. Bacteria and zeolite solutions were mixed in a 1:1 ratio or with the excess of the bacteria (with respect to the number of bacteria cells and zeolite L crystals; aliquots of 100 μL) and shaken at 37 °C for 1 h. After the incubation period, an aliquot was taken (10 μL), diluted 10 times, and deposited (10 μL) on a glass plate. The droplet was covered with a cover microscope glass and sealed. The optical microscope measurements were performed immediately.

The Camera used for the optical microscope recordings was a black and white Leica DFC350FXR2 digital camera mounted to the inverted Leica microscope. The sample was observed through an oil immersion 63X/1.4 objective with white and/or with fluorescence (with filter cube H3, Leica) illumination. SEM imaging was performed by using a filed-emission scanning electron microscope from LEO, type 1530 VP. SEM samples were prepared by depositing the zeolite/*E. coli* mixtures on a mica plate followed by deposition of 4 nm of silver after solvent evaporation. The imaging was performed at an acceleration voltage of 15 kV.

Received: March 7, 2007
Published online: July 3, 2007

**Keywords:** bacteria · fluorescence · nanochannels · self-assembly · zeolites

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