

## On the way to supramolecular photochemistry at the single-molecule level\*

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**Abstract:** Two examples of artificial supramolecular host–guest systems derived from resorc[4]arenes (calix[n]arenes based on resorcinol) and ammonium ions as guests have been studied by atomic force microscopy (AFM). For the first time, real single-molecule events have been determined for this type of supramolecular complexes and off-rates as well as molecular parameters of single-molecule aggregates such as the depths of the binding pocket (molecular length parameter) could be measured by applying the methods of dynamic force spectroscopy. In addition, this technique was also applied to differentiate between the two states (open and closed) of a photoswitchable resorc[4]arene-anthracene tweezer. An investigation of the exchange rates of various complexes in the gas phase by means of Fourier transform ion cyclotron resonance (FTICR) mass spectrometry confirmed the results of the AFM study.

**Keywords:** resorc[4]arenes; photoswitches; atomic force microscopy; single-molecule processes; mass spectrometry.

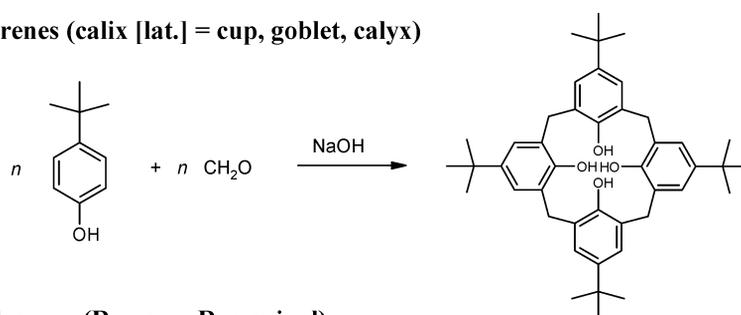
### INTRODUCTION TO CALIXARENE CHEMISTRY

Calixarenes are synthetic macrocycles generally derived from *tert*-butylphenols and formaldehyde or from resorcinol and aldehydes [1]. They have goblet-like structures (Scheme 1), and therefore they provide a receptor cavity for charged and neutral guest molecules [2]. Besides phenolcalix[n]arenes (with generally  $n = 4, 6, 8$ ) and resorc[4]arenes, there are other macrocycles accessible derived, for example, from pyrrole [3], 2,6-dihydropyridine [4], and even higher benzoid arenes [5], to mention only a few.

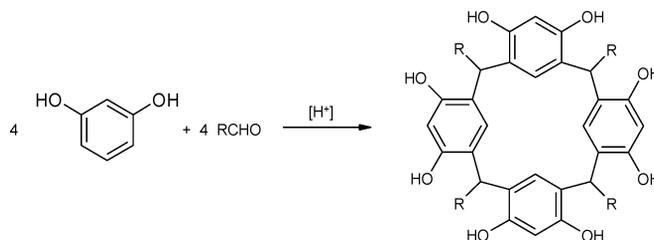
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**Calix[n]arenes (calix [lat.] = cup, goblet, calyx)**



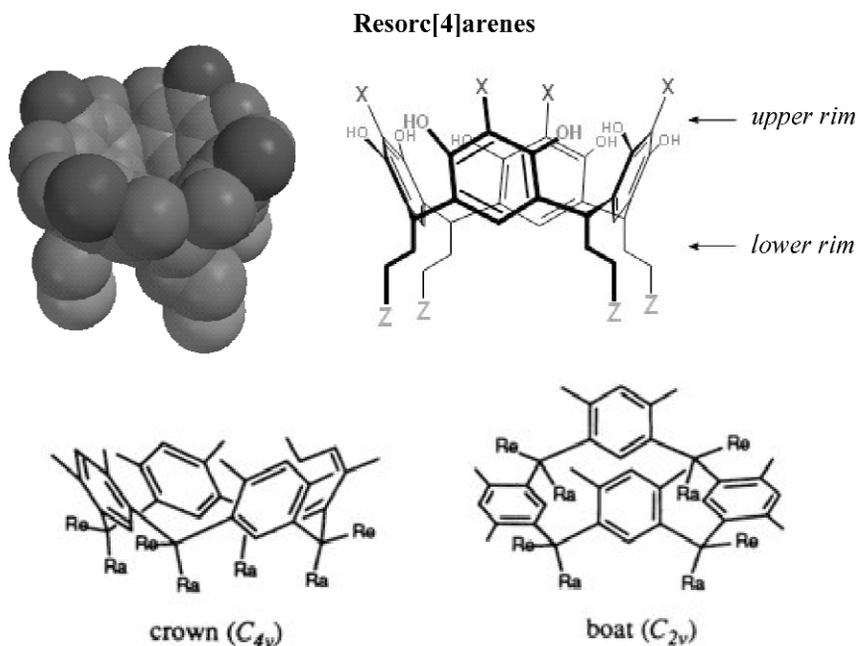
**Resorc[4]arenes (Resorc = Resorcinol)**



**Scheme 1** Calix[n]arenes derived from *tert*-butylphenol and resorcinol.

All these macrocycles exist in various geometries, however, the crown ( $C_{4v}$ ) and the boat ( $C_{2v}$ ) conformation are shown to be the most abundant ones at least for resorc[4]arenes (Fig. 1). Both conformers are in a dynamic equilibrium which can be influenced by substitution pattern and medium effects such as solvents [6]. In addition, the *crown*-conformation can be fixed by forming cavitands following Cram's procedure [7]. Due to their easy access and their broad functionalization, calixarenes have widely been used in many areas of supramolecular chemistry such as host–guest complexes, sensors, self-assemblies, and mesoscopic systems, etc. [8,9].

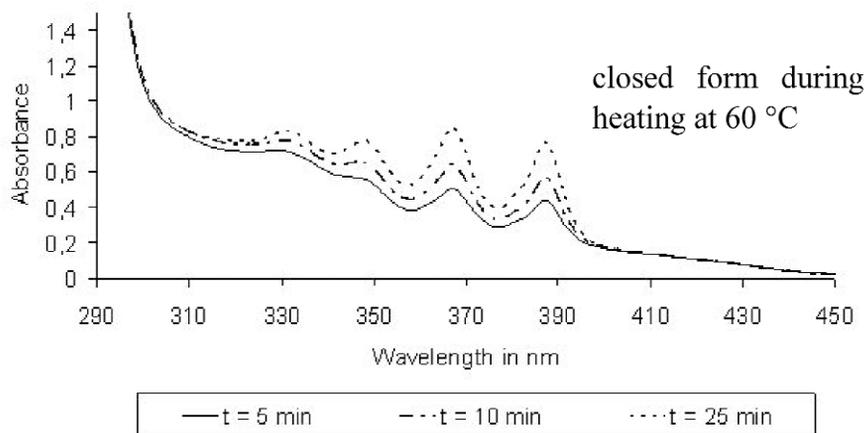
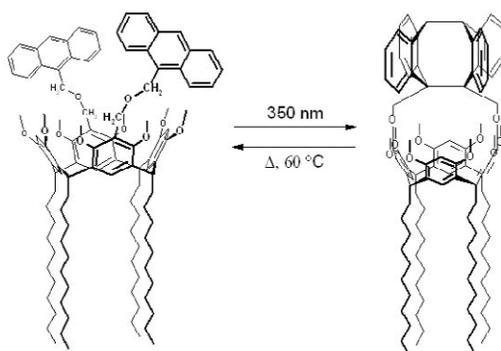
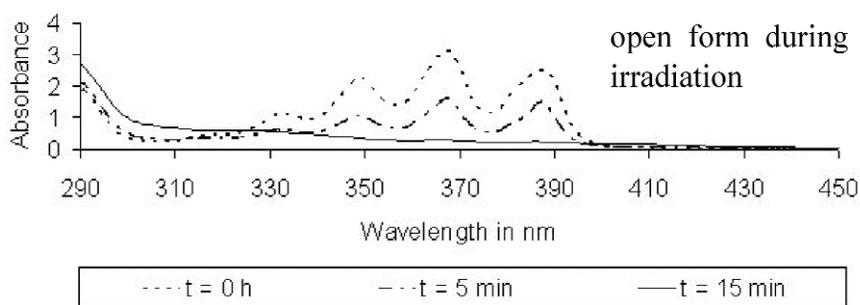
In this report, we will focus on selected aspects of host–guest features of resorc[4]arenes which we have investigated earlier in solution, in the solid state, and in the gas phase, i.e., molecular recognition of these resorc[4]arenes with ammonium cations and amines studied at the single-molecule level by atomic force microscopy (AFM). Thereby, we will discuss in detail two examples, a cavitand and a photoswitchable resorc[4]arene derivative. Finally, we will briefly present our results of a gas-phase study by means of mass spectroscopy.



**Fig. 1** General features of resorc[4]arenes (a: axial, e: equatorial).

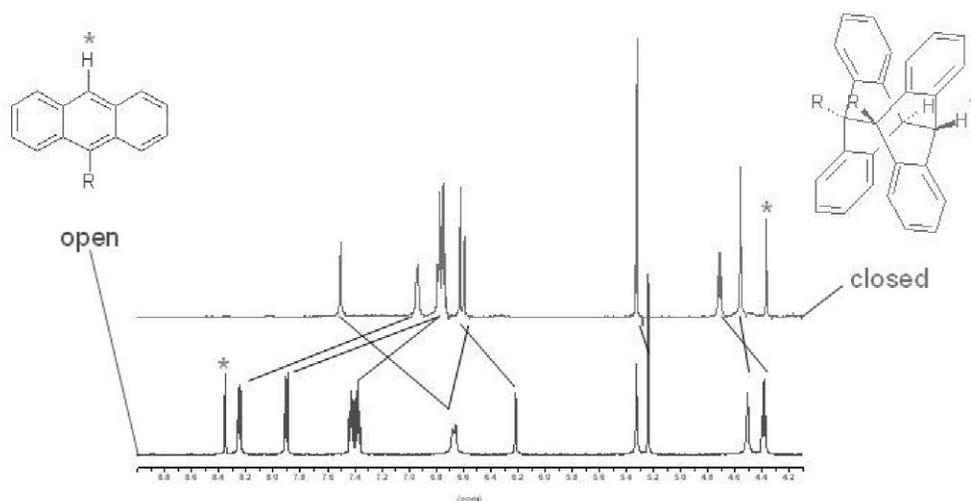
### PHOTOSWITCHABLE CALIXARENES: THE RESORC[4]ARENE-ANTHRACENE TWEEZER

Anthracene was used to realize a molecular tweezer on the basis of resorc[4]arenes [10]. This photo-switchable tweezer can be deactivated upon irradiation of the open form at 350 nm (Scheme 2) leading to the closed form by a [4+4] cycloaddition of the anthracene units. The latter one can be photochemically or thermally reactivated again by reopening the cavity. All the details of synthesis, photochemical, and thermal behavior have already been reported elsewhere [11]. In Scheme 2, both the photo-induced closure and the thermal reopening of the resorc[4]arene-anthracene tweezer is shown, which can be easily followed by UV/vis absorption spectroscopy.



**Scheme 2** UV/vis absorption spectra of the open and closed form of the resor[4]arene-anthracene tweezer.

Regarding the structure of the closed form, NMR studies and quantum chemical calculations strongly support a head-to-head dimerization of the anthracene chromophors (Fig. 2) [12].



**Fig. 2** Changes of selected  $^1\text{H}$  NMR signals of the open and closed resorc[4]arene-anthracene tweezer.

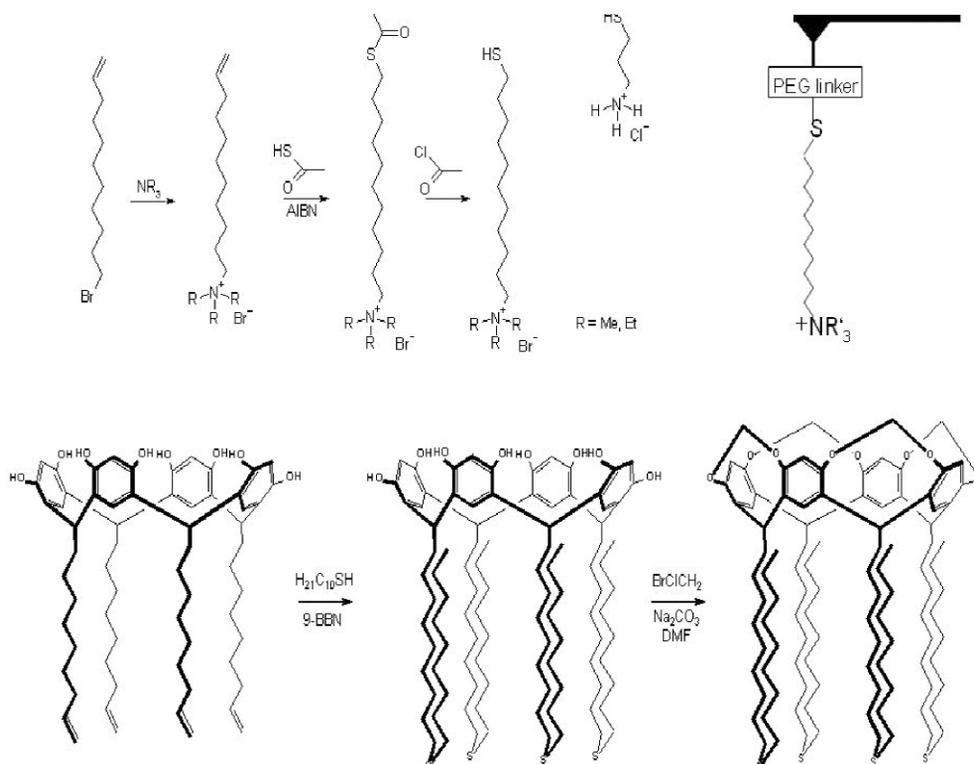
### SINGLE-MOLECULE PROCESSES STUDIED BY AFM: STATE OF THE ART—THE CAVITAND-AMMONIUM COMPLEX

To measure forces of the interaction between single-molecule AFM has become one of the major tools [13]. By this method, a single molecule (e.g., a guest molecule) attached to the tip of the AFM microscope (cantilever) interacts with another molecule (e.g., a host molecule) immobilized at a surface. There are several questions arising from this approach:

- i. Is it possible to measure forces of complex disruption within the general cantilever spring constants ranging from  $10 \text{ pN nm}^{-1}$  up to  $100 \text{ pN nm}^{-1}$ ?
- ii. How can real single-molecule processes be realized?
- iii. Is it possible to extract molecular parameters from these experiments such as off-rates and molecular length scales?

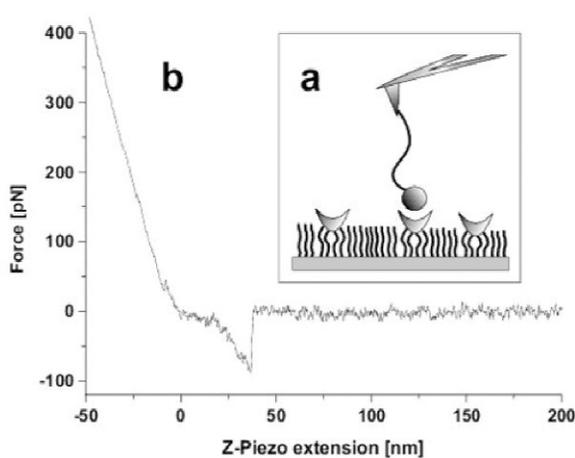
In the following section, we will discuss in some detail a system which we selected in order to answer all these questions which have not been solved at that time. More details are reported elsewhere [14].

The synthesis of a suitable host (cavitand) and suitable guests (ammonium cations) is shown in Scheme 3. In both cases, the key steps are the introduction of sulfur substituents at the  $\omega$ -position of an alkyl chain in order to immobilize them at a gold surface and the AFM tip, respectively. For the cavitand, long-chain sulfides are used following a procedure developed by Reinhoudt et al. [15]. In addition, we diluted this layer by adding didecylsulfide in a 1:40 mixture, allowing real single-molecule events [16]. The ammonium residue is attached to an  $\text{Si}_3\text{N}_4$  AFM tip by a flexible polymer linker via a thiol unit.



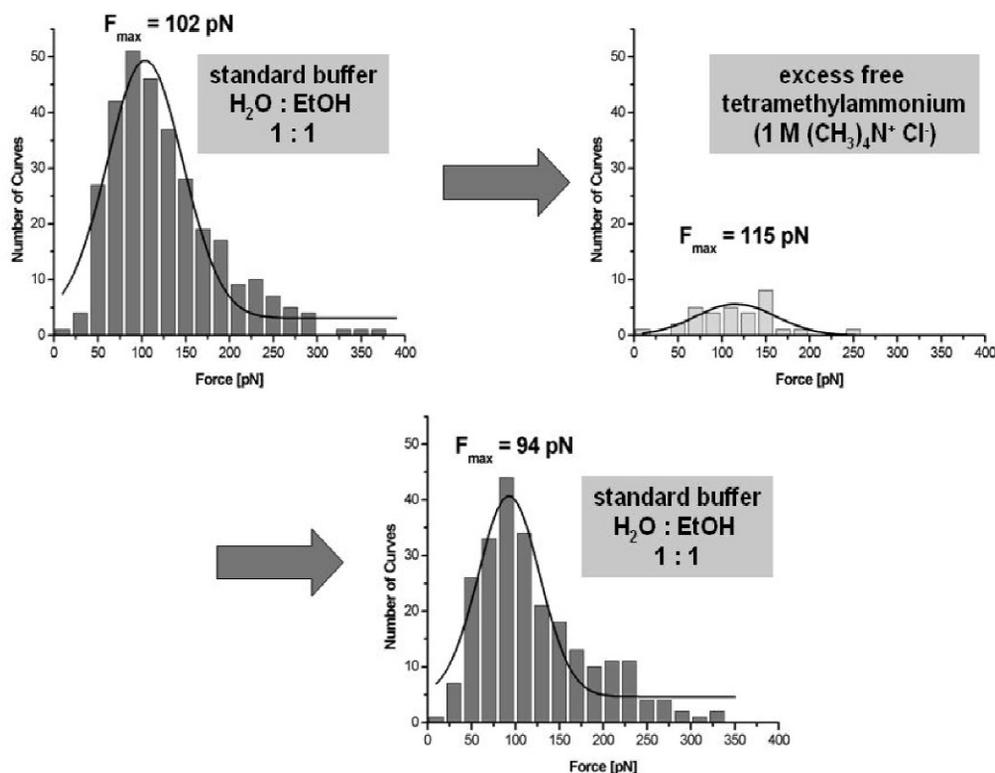
**Scheme 3** Synthesis of a *lower-rim* functionalized cavitand and of an ammonium cation for immobilizing on gold and the AFM tip.

Molecular unbinding processes could be identified by plotting the force response of the AFM cantilever against the z-position of the piezo actuator (of the cavitand surface, Fig. 3). The elastic stretching of the poly(ethylene glycol) (PEG) spacer before the point of detachment, which shows an elasticity curve in accordance with the worm-like polymer-elasticity model, served as the criterion to discriminate real single-binding events from unspecific adhesion.



**Fig. 3** Force-distance curve upon disruption of the cavitand-ammonium complex.

Since the molecular unbinding process is of stochastic nature, rupture forces from many rupture events (typically 200) were compiled in a force histogram (Fig. 4). The mean value resulting from a single-modal Gaussian fit to the histogram distribution is the most probable unbinding force (for the trimethyl ammonium guest  $F_{\max} = 102$  pN in 1:1  $\text{H}_2\text{O}=\text{EtOH}$  mixture). In Fig. 4, the control experiment for validating the specificity of the host–guest interaction is also shown: an excess of free ammonium ions was added to the solvent as competing guest molecules resulting in a significantly reduction of binding events. After washing tip and sample again with the pure solvent, the system could be reactivated to its full former unbinding functionality.



**Fig. 4** Single-molecule competition experiments.

Similar experiments were performed using guests of different sizes: ammonium and triethyl ammonium. Whereas the former cation shows comparable behavior, the latter one shows a much lower unbinding probability. Furthermore, we utilized dynamic force spectroscopy (force-loading-rate plots) to reveal details about the kinetics of the binding and information concerning the molecular length scale of the interaction [17]. The results are presented in Fig. 5, yielding a higher off-rate for ammonium compared to trimethyl ammonium ( $0.99 \text{ s}^{-1}$  and  $0.0187 \text{ s}^{-1}$ ). Accordingly, the lifetime changes from  $\tau = 1.01 \text{ s}$  (ammonium) to  $\tau = 53.5 \text{ s}$  (trimethyl ammonium). In addition, from the inverse slope of the loading-rate dependency, the molecular lengths can be extracted, yielding  $\chi_\beta = 0.22 \text{ nm}$  for ammonium and  $\chi_\beta = 0.38 \text{ nm}$  for the trimethyl ammonium ions. These results correspond with the calculated van der Waals diameters of  $0.3 \text{ nm}$  for ammonium and  $0.6 \text{ nm}$  for trimethyl ammonium [18].

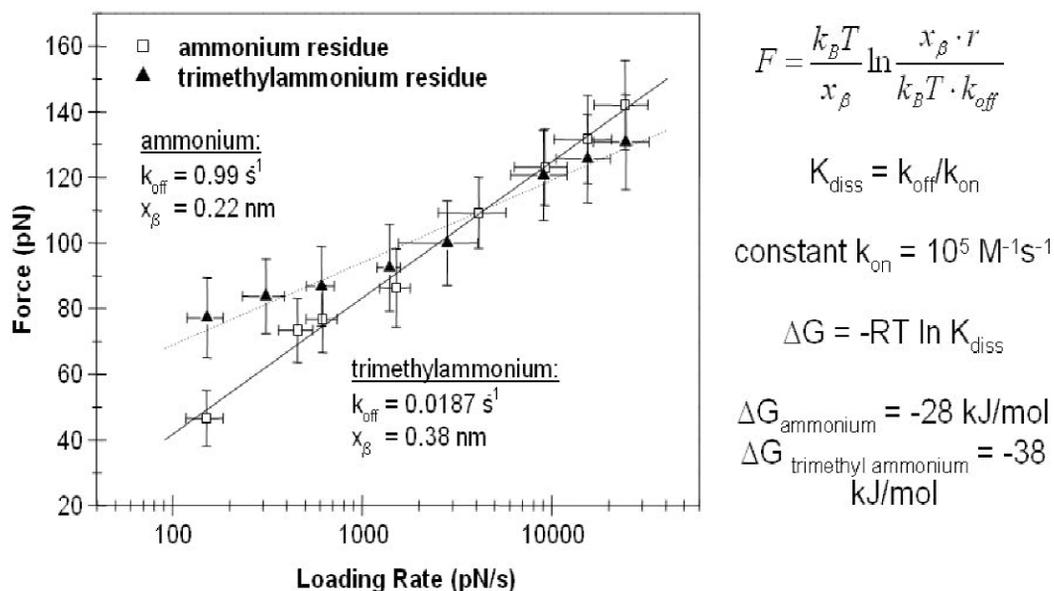


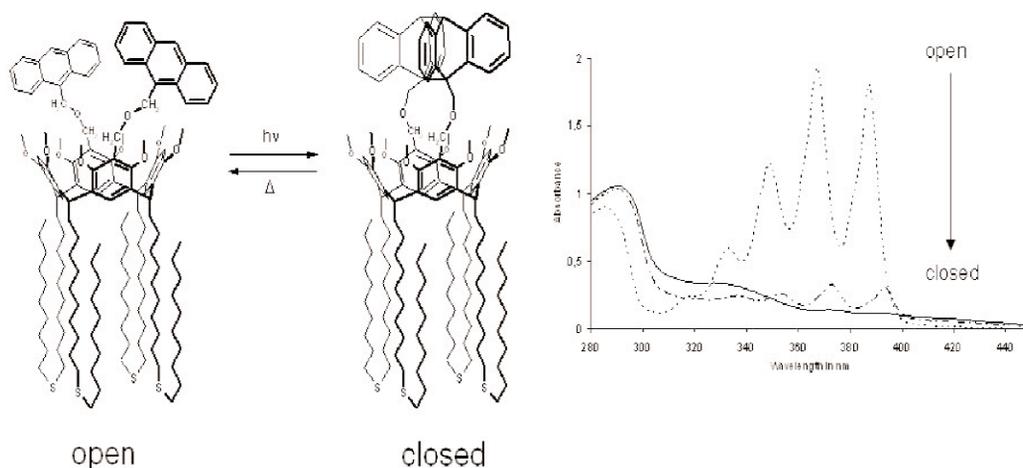
Fig. 5 Dynamic force spectroscopy experiments.

Summarizing this section, we could show for an artificial host–guest couple that the specific interaction and dissociation are measurable at a single-molecule level for forces in the 100 pN range. In addition, off-rates as well as molecular parameters such as the reaction lengths (depth of the binding pocket) can be determined. For the first time, various affinities for a series of host–guest pairs have been determined at a single-molecule level by means of AFM.

## PHOTOCHEMISTRY MEETS AFM: SINGLE-MOLECULE STUDIES OF A PHOTOSWITCHABLE MOLECULAR TWEEZER

As far as we know there is only one report on an AFM experiment of a photoswitchable compound: In 2002, Gaub et al. reported on a single-molecule optomechanical cycle [19]. An azobenzene polymer attached to a cantilever was exposed to 420 nm, putting the polymer in its lengthened trans state. Irradiation at 365-nm light causes the backbone to contract, pulling on the cantilever. Photoswitchable host–guest systems have not been investigated to date.

Therefore, we modified our own AFM experiments first by immobilizing a *lower-rim* sulfide-functionalized resorc[4]arene-anthracene phototweezer on gold surface (with ammonium as guest fixed to the cantilever as described above) and secondly by allowing the samples to be irradiated with light of 350-nm wavelength (Scheme 4) [12,20].



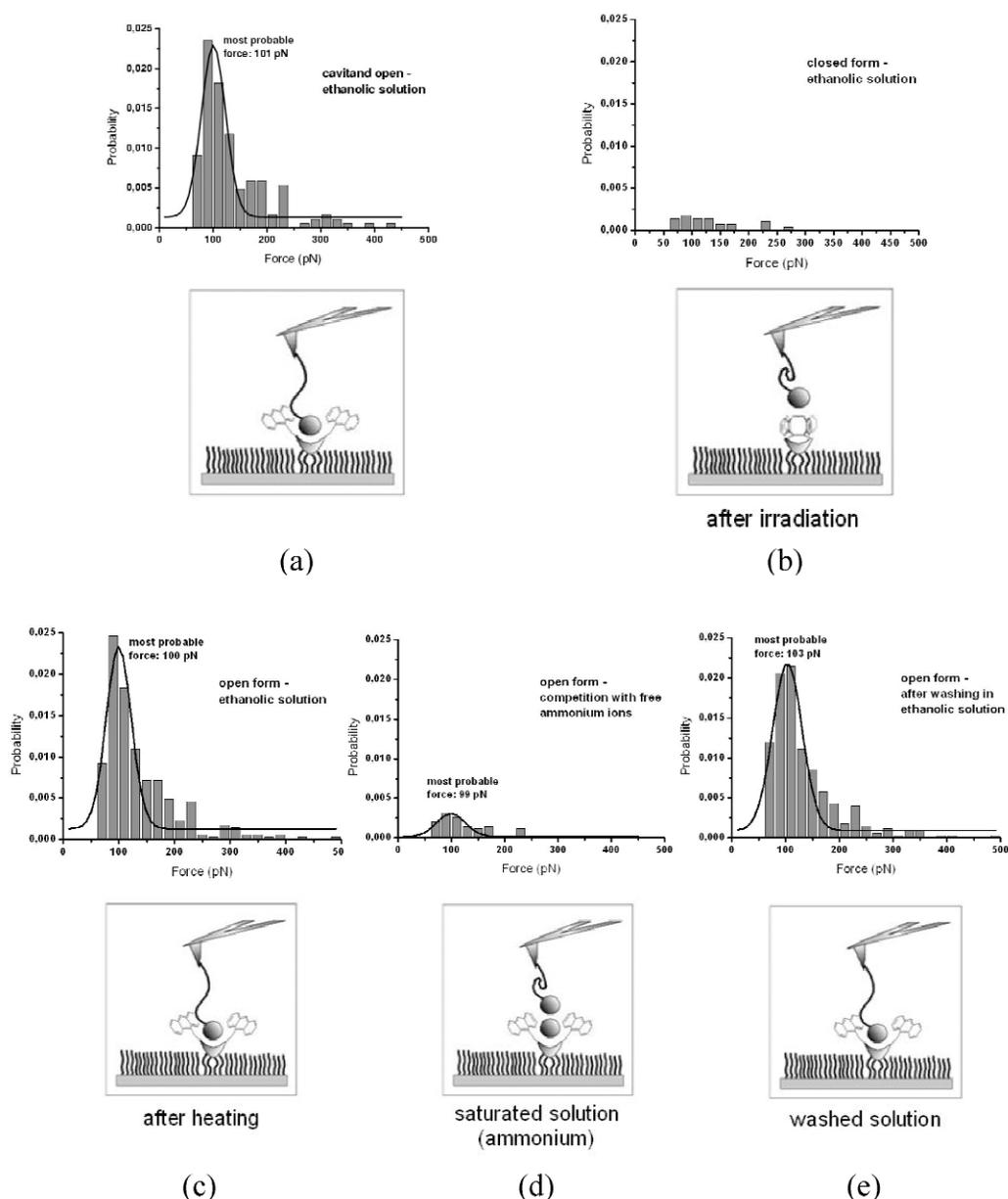
**Scheme 4** UV/vis absorption spectra of the *lower-rim* functionalized resoc[4]arene-anthracene phototweezer.

In a typical experiment, a self-assembled monolayer was generated consisting of the sulfide-functionalized photoswitch and free sulfide in a ratio of 1:40 on gold. Then a series of experiments were performed (Fig. 6):

- SMFS (single-molecule force spectroscopy) experiments of the open form of the photoswitch, i.e., after heating. The number of rupture events was high enough to analyze them by fitting a Gaussian to the histogram, yielding an overall binding probability of 9.1 % and most probably a force of 101 pN at a loading rate of 5260 pN s<sup>-1</sup>.
- After irradiation using UV light (340–400 nm), the new histogram clearly shows a dramatic reduction of the binding probability up to 0.9 %, indicating the formation of the closed form which gives no access to the ammonium guest.
- Heating to 60 °C for 2 h results in a force histogram very similar to the first one (a) clearly indicating reopening of the resoc[4]arene cavity.
- Control experiments similar to those described above, i.e., saturation with excess ammonium chloride and washing with pure solvents in order to recover the free receptor units.
- See (d).

Note that a similar control experiment with the closed photoswitch (cf. b) does not show a comparable effect which is also in line with a reduced access of free ammonium ions to the closed cavity of the host molecule.

In summary, we have clearly demonstrated that the reversible switching between two states of a photoswitchable resoc[4]arene can be followed by AFM at a single-molecule level.

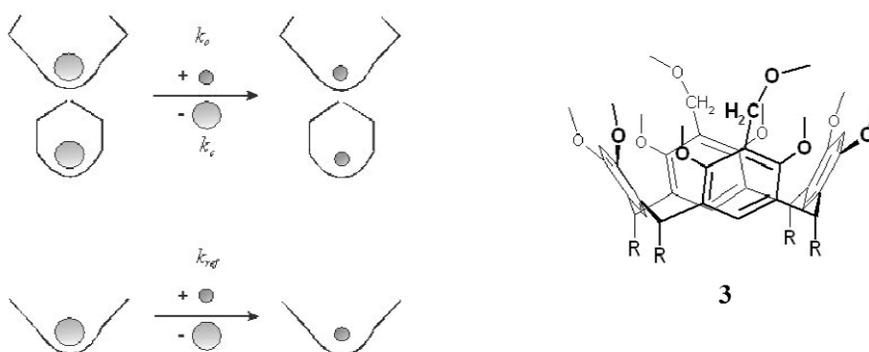


**Fig. 6** Single-molecule experiments of the open and closed photoswitch including competition experiments.

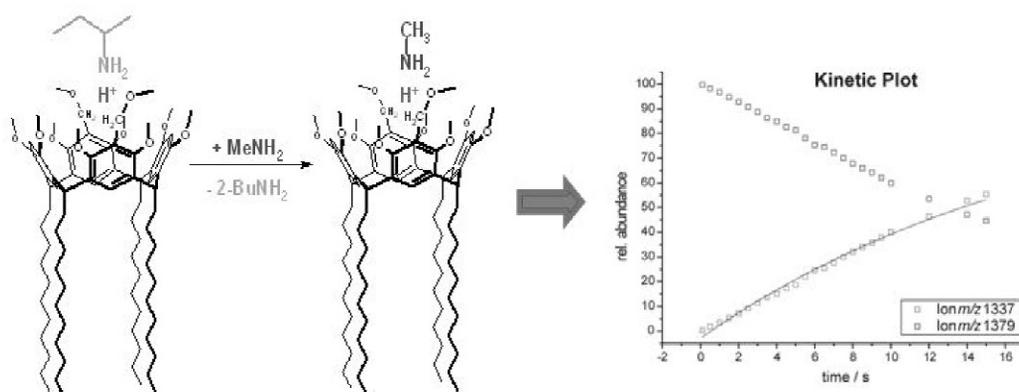
## STUDIES IN THE GAS PHASE

Recently, we have already studied molecular recognition phenomena such as host-guest complex formation between resor[4]arenes and metal cations as well as ammonium ions, even for chiral hosts and guests in the gas phase [8a,8b]. In addition, similar experiments by means of mass spectrometry had been performed for molecular capsules [8c]. All these experiments were additionally evaluated by quantum chemical calculations [18,21]. Therefore, we thought that the different affinities of the open and closed photoswitch might also be studied by means of Fourier transform ion cyclotron resonance (FTICR) mass spectrometry [22].

Electrospray ionization (ESI) is used to generate a host–guest complex of the photoswitch (open and closed) and a suitable guest such as ammonium or an amine in the gas phase. Then the kinetics of exchange reactions by addition of another guest is measured for all combinations of small and larger guest molecules. A schematic depiction of the exchange experiments is shown in Scheme 5. The detailed kinetic analysis is summarized in Scheme 6. The detailed kinetic analysis is summarized in Scheme 6.



**Scheme 5** Schematic presentation of exchange experiments in the gas phase (resor[4]arene **3** is used as reference host compound).



Host@Guest	Methylamine	Ethylamine	sec-Butylamine
<b>1</b> @ NH <sub>4</sub> <sup>+</sup>		1.40 ± 0.13 (11.1%)	1.97 ± 0.12 (18.5%)
<b>2</b> @ NH <sub>4</sub> <sup>+</sup>		1.21 ± 0.12 (9.6%)	1.73 ± 0.07 (16.2%)
<b>3</b> @ NH <sub>4</sub> <sup>+</sup>		2.80 ± 0.3 (22.2%)	7.13 ± 0.18 (66.4%)
<b>1</b> @ secBuNH <sub>3</sub> <sup>+</sup>	0.142 ± 0.006 (1.1%)	0.168 ± 0.006 (1.3%)	
<b>2</b> @ secBuNH <sub>3</sub> <sup>+</sup>	0.155 ± 0.008 (1.2%)	0.205 ± 0.016 (1.6%)	
<b>3</b> @ secBuNH <sub>3</sub> <sup>+</sup>	0.267 ± 0.005 (2.1%)	0.46 ± 0.02 (3.7%)	

Kinetic constants are given in 10<sup>-10</sup> cm<sup>3</sup> / (s molecule) units. The calculated efficiencies are reported in brackets. Errors result from curve fitting and the scattering of different measurement series.

**Scheme 6** Kinetic analysis of guest exchange processes in the gas phase.

In brief, the results of these exchange experiments can be summarized as follows:

- The fastest reaction is always observed with reference **3** indicating the less hindered *upper rim* in this resorc[4]arene.
- In case of small guests (e.g.,  $\text{NH}_4^+$ ), the open photoswitchable tweezer exchanges faster than the closed one, indicating an easier access of the added guest to the captured guest in the cavity.
- In the case of big guest molecules (e.g., *sec*- $\text{BuNH}_3^+$ ), the closed photoswitch reacts faster than the open one, indicating the big guest not to be incorporated in the relatively small cavity of the phototweezer.
- The reaction rates increase with proton affinity of the collision gas.

Summarizing this section, the host–guest displacement processes in the gas phase provide information about the intrinsic dynamics of these reactions. In particular, these experiments allow us to discriminate between the two states of the photoswitch. It is assumed that only the smaller guests can enter the resorc[4]arene cavity, leading to a lower rate in the case of the closed host.

## SUMMARY AND PERSPECTIVES

After the establishment of single-molecule spectroscopy (see, e.g., contributions of W. E. Moerner and Frans DeSchryver to this issue) the study of chemical processes between single molecules is a great challenge for the future. In this context, molecular recognition (e.g., host–guest interaction) of single molecules is most important in the area of supramolecular chemistry. We have studied two examples of artificial host–guest systems, i.e., calixarene-ammonium ions, and we have shown for the first time that real single-molecule events can be evaluated in the range of ca. 100 pN by means of AFM. In addition, applying the methods of dynamic force spectroscopy we were able to determine off-rates of decomposition of single supramolecular complexes as well as molecular parameters such as molecular lengths (corresponding to the depth of the binding pocket). Furthermore, the AFM technique was also applied to differentiate between the two states of a photoswitchable host–guest system for the first time. An investigation of the exchange rates of various complexes in the gas phase by means of FTICR mass spectrometry confirms the results of the AFM study.

These and also other results from our and other groups (cf. ref. [19]) open a new horizon for the study of various types of supramolecular systems, artificial as well as natural ones. The information about single molecules and aggregates extracted from these investigations will give a deeper insight into the fundamentals and will have a great impact on the development of bio-nano-science and technology.

## ACKNOWLEDGMENTS

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