VIP Self-Assembly

DOI: 10.1002/anie.200905827

Dynamers at the Solid–Liquid Interface: Controlling the Reversible Assembly/Reassembly Process between Two Highly Ordered Supramolecular Guanine Motifs**

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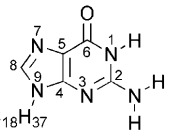
Complex systems and phenomena in nature are dominated by reversible noncovalent interactions. Nucleobases, which are essential components of the genetic material in all living organisms, encode sophisticated programs for self-assembly into highly ordered and complex architectures, such as the fascinating double helix of DNA.[1] Alongside Watson–Crick base pairing,[1] which directs the formation of the helical structure of DNA, nucleobases can interact through other hydrogen-bonded motifs to form various complex supramolecular architectures,[2] such as guanine (G) quadruplexes.[3] The G quartet G_4, an hydrogen-bonded macrocycle typically formed by cation-templated assembly, was first identified in 1962 as the basis for the aggregation of 5′-guanosine monophosphate (5′-GMP),[4] and fits particularly well with contemporary studies in molecular self-assembly and noncovalent synthesis.[5–10] Guanine is an extremely versatile building block: depending on its environment, it is able to self-assemble into various discrete architectures including dimers, ribbons, and macrocycles.[11,12] In the presence of certain cations, either G_4-based octamers or columnar aggregates (supramolecular polymers) can be formed, depending on the concentration of the cation and nucleobase. The guanine-based structures are interesting for applications in organic electronics[13] and synthesis of supramolecular hydrogels,[14] whereas G quartets are known to have potential in several biological processes[15] and in anticancer drug design, as they can act as enzyme telomerase inhibitors, and therefore are of importance for controlling tumor immortalization.[3,16,17]

While the self-assembly of guanines into G_4-based architectures (not templated by a metal center) on solid surfaces has been studied by STM under ultrahigh vacuum (UHV),[18,19] STM explorations at the solid–liquid interface have been primarily carried out on guanosine derivatives.[20–23] Although the structure of a guanine quadruplex templated by a metal center was introduced over 40 years ago,[4] its visualization by STM once assembled at the solid–liquid interface has not been reported to date.

We have studied the metal-templated reversible assembly/reassembly process of a N^9-alkylguanine into highly ordered quartets and ribbons. Herein, we present a submolecular-resolution STM visualization of such a process at the solid–liquid interface on highly oriented pyrolitic graphite (HOPG) surfaces. We focused our attention on the octadecyl guanine derivative **1** (see the Supporting Information for preparation). The presence of a long aliphatic side chain and the absence of the sugar with respect to previously studied guanosines were expected to promote the molecular physisorption on HOPG. The self-assembly of **1** alone on HOPG has been studied, and, upon subsequent addition of [2.2.2]cryptand, potassium picrate ($K^+(pic)^-$), and trifluoromethanesulfonic acid (HTf), the reversible interconversion between two different highly ordered supramolecular motifs is triggered (Scheme 1). This process was previously indirectly shown by ^{1}H NMR and circular dichroism spectroscopy to occur in solution.[24]



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In general, the generation of hydrogen-bond-stabilized ordered motifs at the solid–liquid interface requires fine tuning of the interplay between interactions that involve solvent molecules, solute molecules, and the substrate.[25] The STM observation of a conformational or assembly switching process that occurs at the solid–liquid interface cannot be obtained by operating when concentrated solutions are used, as dictated by the thermodynamics of physisorption at the solid–liquid interface. In a solution containing a large number of identical molecules that have two or more conformations, the component with a greater affinity for the substrate, that is, the component that offers a minimization of the free interface energy per unit area, will assemble on the surface, whereas the other components will remain in the supernatant solution.[26,27] To have full control over the switching process and immobilize all the components on the surface, and thus achieve a complete physisorption of all different components at the solid–liquid interface, it is mandatory to tune the stoichiometry of the molecules absorbed at surface.[28,29] At the solid–liquid interface, the number of molecules that are in

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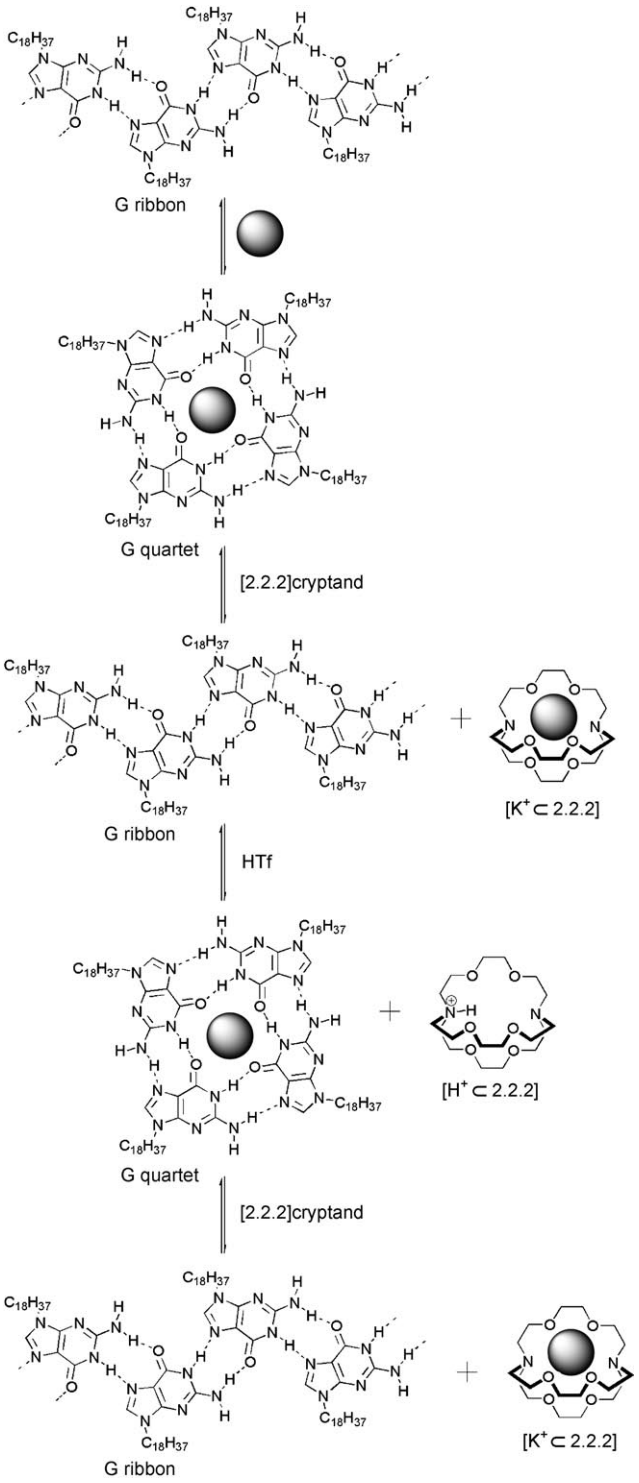
[**] This work was supported by the COST Network G4-NET (MPNS Action MP0802), the EC Marie Curie RTN PRAIRIES (MRTN-CT-2006-035810) and the EC Marie Curie ITN-SUPERIOR (PITN-GA-2009-238177), the EC FP7 ONE-P large-scale project no. 212311, and the International Center for Frontier Research in Chemistry (FRC, Strasbourg).

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.200905827.



Scheme 1. The stepwise reversible interconversion between the G ribbon ($\mathbf{1}_n$) and the G quartet K($\mathbf{1}$)$_4$. The gray sphere represents the K^+ ions.

solution and will be applied to the surface should be lower than the number required to form a monolayer of physisorbed molecules lying flat on the substrate.

We initially investigated the self-assembly of **1** by applying a drop of a (100 ± 2) μM solution of **1** in 1,2,4-trichlorobenzene (TCB) on the HOPG surface. The STM image of the obtained monolayer (Figure 1 a) showed a crystalline structure consisting of ribbonlike architectures.[30] In this 2D crystal, the octadecyl side chains are physisorbed flat on the

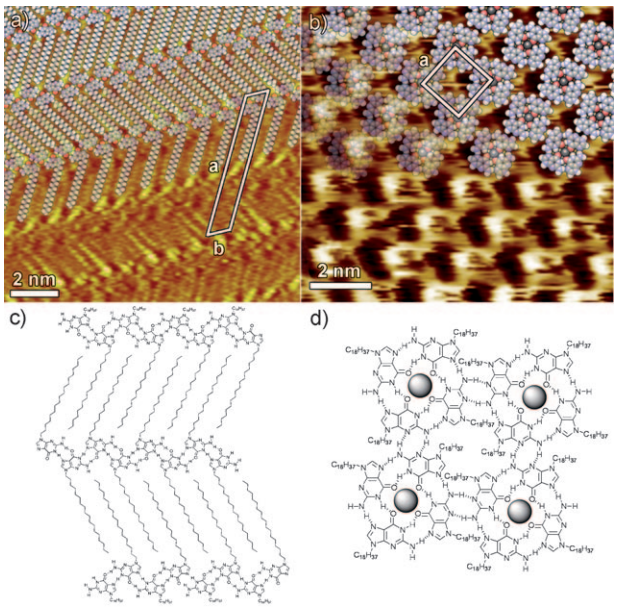


Figure 1. STM images of monolayers of supramolecular architectures of **1** at the solid-.liquid interface self-assembled from TCB solution; a) ribbonlike structure and b) G_4-based architecture. The packing motif models are shown in (c) and (d), respectively. Tunneling parameters: a) average tunneling current (I_t) = 15 pA, bias voltage (V_t) = 350 mV; b) I_t = 5 pA, V_t = 200 mV. The gray sphere represents the K^+ ion.

surface and are interdigitated between adjacent supramolecular ribbons. The unit cell parameters, $a = (5.8 \pm 0.2)$ nm, $b = (1.0 \pm 0.2)$ nm, and $\alpha = (64 \pm 3)°$, lead to an area $A = (5.21 \pm 1.35)$ nm^2, where each unit cell contains four molecules. Thus, the area occupied by a single molecule **1** corresponds to (1.3 ± 0.33) nm^2. The supramolecular motif can be well-described by the formation of a 1D hydrogen-bonded ribbon that involves the pairing NH(2)–O(6) and NH(1)–N(7) (see model in Figure 1 c and Figure S6 in the Supporting Information). This self-assembly behavior is in good agreement with previous observations on guanosine derivatives.[20] Although in general, STM investigation of sub-monolayer-thick films obtained from solutions with low concentrations may lead to polymorphism, such behavior was ruled out in our case: concentration-dependent experiments revealed that by applying a drop of (10 ± 2) μM or (500 ± 2) μM solutions of **1** in TCB onto the HOPG surface, only the ribbons shown in Figure 1 a were formed (see Figure S5 in the Supporting Information).

The STM study of a monolayer of guanine solution containing 100 equivalents of potassium picrate revealed the formation of a G_4-based architecture at the surface (Figure 1 b).[31,32] The unit-cell parameters, $a = (1.6 \pm 0.2)$ nm and $\alpha = (90 \pm 3)°$, lead to an area $A = (2.56 \pm 0.45)$ nm^2, where each unit cell contains four molecules of **1** and one potassium ion. The area occupied by a single molecule of **1** amounts to (0.64 ± 0.1) nm^2. Figure 1 d shows the proposed molecular packing motif, which can be well described by the formation

of a hydrogen-bonded macrocyclic structure that involves the pairing NH(1)–O(6) and NH(2)–N(7). The high-contrast circular feature in Figure 1b can probably be ascribed to the potassium ions located in the center of G_4 structure. It is most likely that the coordinated potassium ions are not in the same plane as the four molecules of **1** that form the G_4 quartet, as is also observed in solution.[33,34] To enable formation of NH(2)–N(3) hydrogen bonds between adjacent G_4 quartets (Figure 1d), thus stabilizing the entire supramolecular arrangement, the alkyl chains of **1** are back-folded into the supernatant solution. The G_4 packing motif of Figure 1d differs from that observed in UHV on quartets formed in absence of templating ions.[18] The presence of $C_{18}H_{37}$ alkyl side chains attached to the N(9) position of the guanine prevents the formation of the NH(9)–N(3) bond. Therefore, to stabilize the entire supramolecular structure into a G_4 quartet, another well-defined intraquartet hydrogen bond, that is, the NH(2)–N(7) bond, is formed.

To gain insight into the interconversion between the two structures, in situ successive assembly/reassembly cycles from ribbons to G_4-based architectures was accomplished. Upon in situ addition of 10 mM potassium picrate solution in TCB to the initial ribbonlike motif in Figure 2a, the G_4 supramolecular motif was obtained (Figure 2b). To sequester the ions from the G_4 quartet, we opted to use [2.2.2]cryptand since it offers an efficient complexation of K^+ to yield the cryptate $[K^+\subset 2.2.2]$.[35]

Upon subsequent in situ addition of a 10 mM solution of [2.2.2]cryptand in TCB to the G_4 supramolecular architectures on HOPG, the guanine reassembled into the original ribbon (Figure 2c). By adding a 10 mM solution of trifluoromethanesulfonic acid (HTf) in TCB, the potassium ions were released from the cryptate and the G_4 assembly was regenerated (Figure 2d). In order to complete the switching process, that is, to deprotonate the nitrogen atoms of the cryptand and to reform the ribbon structures, an alkaline solution was applied to the surface. Unfortunately, even in presence of a large excess of triethylamine it was not possible to trigger the formation of ribbons at surfaces. However, upon further addition of a [2.2.2]cryptand solution, the ribbon structure was regenerated (Figure 2d).

The similar area occupied by single molecules of **1** in the ribbon structure and in the G_4-based architecture on a surface (see Figure S7 in the Supporting Information) indicate that the switching process may have occurred on the HOPG surface, that is, without subsequent desorption and readsorption, although conclusive direct evidence could not be obtained.[36] The reproducibility of the submolecular-resolution STM imaging of the interconversion of **1** from ribbons to G_4-based architectures requires stringent experimental conditions characterized by an extremely low thermal gradient (below 3 °C) and a high control over the concentration of the employed solutions (the concentration of HTf could not exceed that needed to protonate only the nitrogen atoms of [2.2.2]cryptand, otherwise only disordered monolayers were obtained).

In summary, by using STM, we have provided direct evidence on the sub-nanometer scale of a dynamer operating

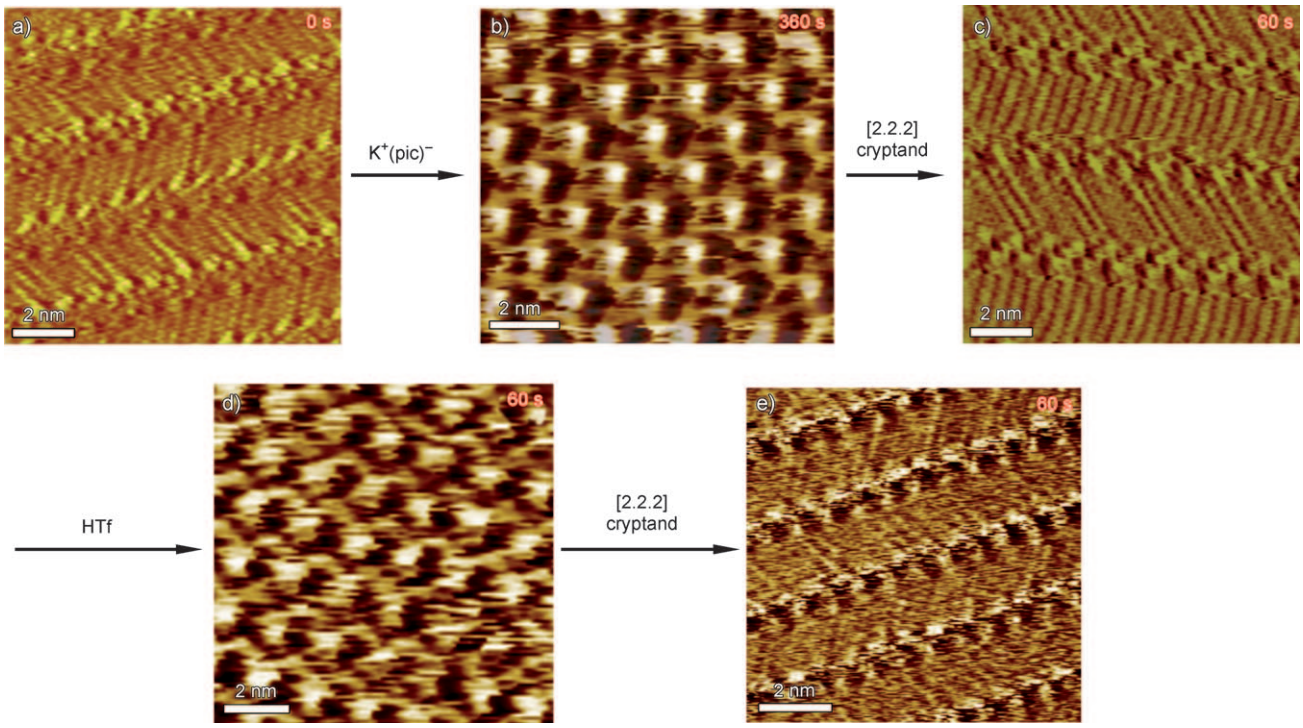


Figure 2. Consecutive STM images showing the structural evolution of a monolayer of **1** over a 9 min time scale (time range displays in the upper right part of the images correspond to the time that was needed to reach the equilibrium after addition of reacting agents). (a), (c), and (e) show ribbonlike structure, whereas (b) and (d) exhibit G_4-based architectures. Tunneling parameters: a), c), and e) $U_t = 350$ mV and $I_t = 15$ pA; b) and d) $U_t = 200$ mV and $I_t = 5$ pA.

at surfaces. The versatile guanine molecule **1** was reversibly interconverted at the solid–liquid interface between two highly ordered supramolecular motifs, that is, hydrogen-bonded ribbons and G_4-based architectures, upon subsequent addition of [2.2.2]cryptand, potassium picrate, and trifluoromethanesulfonic acid. The visualization of such supramolecular interconversion at the solid–liquid interface opens new avenues towards understanding the mechanism of formation and function of complex nucleobase architectures such as DNA or RNA. Furthermore, the in situ reversible assembly and reassembly between two highly ordered supramolecular structures at the surfaces represents the first step towards the generation of nanopatterned responsive architectures.

Received: October 16, 2009
Revised: January 7, 2010
Published online: February 4, 2010

Keywords: dynamers · guanine · hydrogen bonding · interfaces · self-assembly

[1] J. D. Watson, F. H. C. Crick, *Nature* **1953**, *171*, 737.
[2] S. Sivakova, S. J. Rowan, *Chem. Soc. Rev.* **2005**, *34*, 9.
[3] J. T. Davis, *Angew. Chem.* **2004**, *116*, 684; *Angew. Chem. Int. Ed.* **2004**, *43*, 668.
[4] M. Gellert, M. N. Lipsett, D. R. Davies, *Proc. Natl. Acad. Sci. USA* **1962**, *48*, 2013.
[5] J. F. Stoddart, H. R. Tseng, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 4797.
[6] D. N. Reinhoudt, M. Crego-Calama, *Science* **2002**, *295*, 2403.
[7] G. M. Whitesides, E. E. Simanek, J. P. Mathias, C. T. Seto, D. N. Chin, M. Mammen, D. M. Gordon, *Acc. Chem. Res.* **1995**, *28*, 37.
[8] D. Philp, J. F. Stoddart, *Angew. Chem.* **1996**, *108*, 1242; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 1154.
[9] J. S. Lindsey, *New J. Chem.* **1991**, *15*, 153.
[10] J.-M. Lehn, *Supramolecular chemistry: concepts and perspectives*, VCH, New York, **1995**.
[11] J. T. Davis, G. P. Spada, *Chem. Soc. Rev.* **2007**, *36*, 296.
[12] S. Lena, S. Masiero, S. Pieraccini, G. P. Spada, *Chem. Eur. J.* **2009**, *15*, 7792.
[13] R. Rinaldi, G. Maruccio, A. Biasco, V. Arima, R. Cingolani, T. Giorgi, S. Masiero, G. P. Spada, G. Gottarelli, *Nanotechnology* **2002**, *13*, 398.
[14] N. Sreenivasachary, J. M. Lehn, *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 5938.
[15] S. Neidle, S. Balasubramanian, *Quadruplex nucleic acids*, Royal Society of Chemistry, Cambridge, **2006**.
[16] E. S. Baker, J. T. Lee, J. L. Sessler, M. T. Bowers, *J. Am. Chem. Soc.* **2006**, *128*, 2641.
[17] T. de Lange, *Science* **1998**, *279*, 334.
[18] R. Otero, M. Schock, L. M. Molina, E. Laegsgaard, I. Stensgaard, B. Hammer, F. Besenbacher, *Angew. Chem.* **2005**, *117*, 2310; *Angew. Chem. Int. Ed.* **2005**, *44*, 2270.
[19] R. Otero, W. Xu, M. Lukas, R. E. A. Kelly, E. Laegsgaard, I. Stensgaard, J. Kjems, L. N. Kantorovich, F. Besenbacher, *Angew. Chem.* **2008**, *120*, 9819; *Angew. Chem. Int. Ed.* **2008**, *47*, 9673.
[20] G. Gottarelli, S. Masiero, E. Mezzina, S. Pieraccini, J. P. Rabe, P. Samorì, G. P. Spada, *Chem. Eur. J.* **2000**, *6*, 3242.
[21] T. Giorgi, S. Lena, P. Mariani, M. A. Cremonini, S. Masiero, S. Pieraccini, J. P. Rabe, P. Samorì, G. P. Spada, G. Gottarelli, *J. Am. Chem. Soc.* **2003**, *125*, 14741.
[22] S. Lena, G. Brancolini, G. Gottarelli, P. Mariani, S. Masiero, A. Venturini, V. Palermo, O. Pandoli, S. Pieraccini, P. Samorì, G. P. Spada, *Chem. Eur. J.* **2007**, *13*, 3757.
[23] G. P. Spada, S. Lena, S. Masiero, S. Pieraccini, M. Surin, P. Samorì, *Adv. Mater.* **2008**, *20*, 2433.
[24] S. Pieraccini, S. Masiero, O. Pandoli, P. Samorì, G. P. Spada, *Org. Lett.* **2006**, *8*, 3125.
[25] S. De Feyter, F. C. De Schryver, *Chem. Soc. Rev.* **2003**, *32*, 139.
[26] C. A. Palma, M. Bonini, T. Breiner, P. Samorì, *Adv. Mater.* **2009**, *21*, 1383.
[27] C.-A. Palma, J. Bjork, M. Bonini, M. S. Dyer, A. Llanes-Pallas, D. Bonifazi, M. Persson, P. Samorì, *J. Am. Chem. Soc.* **2009**, *131*, 13062.
[28] C. A. Palma, M. Bonini, A. Llanes-Pallas, T. Breiner, M. Prato, D. Bonifazi, P. Samorì, *Chem. Commun.* **2008**, 5289.
[29] A. Ciesielski, G. Schaeffer, A. Petitjean, J. M. Lehn, P. Samorì, *Angew. Chem.* **2009**, *121*, 2073; *Angew. Chem. Int. Ed.* **2009**, *48*, 2039.
[30] ^{1}H NMR experiments (see Figure S3 in the Supporting Information,) also support the existence of hydrogen-bonded ribbon-like structures in solution, as previously reported for guanosine derivatives.[20]
[31] Solution ^{1}H NMR experiments (see Figure S4 in the Supporting Information) indicate that **1** self-assembles into G4 motifs in the presence of more than 0.25 equivalents of potassium picrate, as previously reported for lipophilic guanosine derivatives.[32]
[32] E. Mezzina, P. Mariani, R. Itri, S. Masiero, S. Pieraccini, G. P. Spada, F. Spinozzi, J. T. Davis, G. Gottarelli, *Chem. Eur. J.* **2001**, *7*, 388.
[33] A. L. Marlow, E. Mezzina, G. P. Spada, S. Masiero, J. T. Davis, G. Gottarelli, *J. Org. Chem.* **1999**, *64*, 5116.
[34] It is known that an isolated tetramer of guanine complexed with the metal center is not thermodynamically stable in solution, thus octameric or larger G_4-based stacks intercalated by cations are typically formed. In STM imaging at the solid–liquid interface, it is very difficult to perform a "tomography" of a molecular assembly in order to unambiguously determine, in our case, if the observed packing in Figure 1b corresponds to a single layer or double-decker (i.e., octamer).
[35] J. M. Lehn, J. P. Sauvage, *J. Am. Chem. Soc.* **1975**, *97*, 6700.
[36] If different areas are occupied by single molecules of the reactant and product that self-assemble at the solid–liquid interface in a reaction or switching process, the total number of molecules at the surface will changed, thus requiring the occurrence of a desorption and/or re-adsorption process. However, STM imaging at the solid–liquid interface cannot offer real-time monitoring of the reaction process in its intermediate states, thus the occurrence of the reaction at the surface or through a desorption/re-adsorption process cannot be unambiguously determined.

