Nucleic Acid Structure

Guanine Quartet Networks Stabilized by Cooperative Hydrogen Bonds**

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Hydrogen bonding between DNA or RNA bases is one of the main interactions that determines the conformation and biochemical activity of nucleic acids.[1,2] Apart from Watson–Crick base pairing,[1] which predominates in the double-helical structure of DNA, nucleobases can form other H-bonded aggregates that lead to different DNA structures such as guanine quadruplexes[3,4] and i-motifs.[5] Despite increasing evidence for the presence and function of these structures

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in vivo,[6] the exact physiochemical nature of the hydrogen bonds and the importance of charge-transfer contributions to the stabilization energy attributed to hydrogen bonding[7] in these structures is still under debate.[8] Semiclassical molecular dynamics (MD) simulations based on empirical potentials have generally been used to explain the observed nucleic acid structures and conformations.[9] Assessing the accuracy of these simulations requires a thorough study of charge-transfer effects on hydrogen bonds and their chemical nature. The MD calculations rely heavily on a number of assumptions, such as mutual cancellation of polarization effects between solute and solvent, whose validity can be determined only by comparison with experimental data.[9,10] Therefore, results of experiments carried out under controlled conditions may be used as benchmarks to test the predictions of MD simulations and the accuracy of the force fields in use. Herein we show, by high-resolution variable-temperature STM, that guanine deposited under ultraclean conditions onto an inert Au(111) substrate self-assembles into a H-bonded network of G quartets with the same structure as that found in quadruplex telomeric DNA.[3,4] Comparison with our DFT calculations shows that the strong preference of guanine to form quartets arises from a cooperative effect that strengthens the hydrogen bonds within the G-quartet network relative to those in isolated G dimers. These findings provide a framework to evaluate the accuracy of the different assumptions that are commonly used in MD simulations of nucleic acids.[9]

To study the H-bond interactions between nucleic acid bases in the absence of any other perturbing interactions, we deposited guanine onto inert Au(111) substrates under well-controlled ultrahigh-vacuum (UHV) conditions at room temperature. Ultraclean deposition ensures that no other chemical species affect the interactions between guanine molecules. The noble-metal close-packed Au(111) substrate was chosen to minimize surface interference with guanine; in this way, assembly was mainly determined by the intermolecular H-bond interactions of guanine, as discussed below.

Scanning probe microscopy is the technique of choice to investigate the assembly of organic and biological molecules at surfaces with atomic precision.[11] STM images recorded at 150–170 K show that guanine molecules, upon deposition onto Au(111) at room temperature, self-assemble into well-ordered islands with irregular shapes (Figure 1a). The adsorption of guanine does not reconstruct the gold surface, as the well-known herringbone reconstruction characteristic of clean Au(111) surfaces can still be observed as modulations of the corrugation of guanine islands (Figure 1b). Figure 1c shows a closer view of the self-assembled G-network structure, the lattice parameter of which is 1.5 ± 0.1 nm. Despite the hexagonal symmetry of the substrate, the G networks depict an almost square symmetry with a unit-cell composition of four molecules.

In high-resolution STM images, guanine molecules are imaged as triangular protrusions. The same signature is found if the Tersoff–Hamann[12] approach is used to calculate STM images of guanine molecules adsorbed flat against the Au surface (Figure 1d). This geometry, which has been generally found for purine molecules adsorbed onto metal surfaces,[13,14] arises from the interaction between the delocalized π-molecular orbitals and the metallic electronic density of the substrate.[15] First-principles DFT calculations of a single adsorbed guanine molecule on Au(111) demonstrate that the molecule–substrate interaction is rather weak. The calculated adsorption energy of a single guanine molecule is only 0.25 eV, with a rather large equilibrium distance of 0.375 nm between the molecule and the substrate. By placing the molecule at several different positions and orientations with respect to the surface, we found a very small corrugation of the potential energy surface in which energy differences do not exceed 0.05 eV from the minimum energy adsorption configuration (Figure 1d). In contrast, the calculated formation energy of the freestanding G-quartet network (in the absence of substrate) is 4.73 eV per unit cell which corresponds to a binding energy of 1.18 eV per guanine molecule.[16] This shows that the H-bonding interaction among guanine molecules completely determines the features of the observed G network, whereas the role of the Au(111) surface is minor; the surface serves primarily as a template to accommodate the molecules in a planar geometry.

The STM results show that each unit cell is composed of four molecules.[17] From the arrangement of the triangles the chirality of the structure can be observed.[18] (Figure 1d) Thus, the unit cell is reminiscent of the G-quartet Hoogsteen-

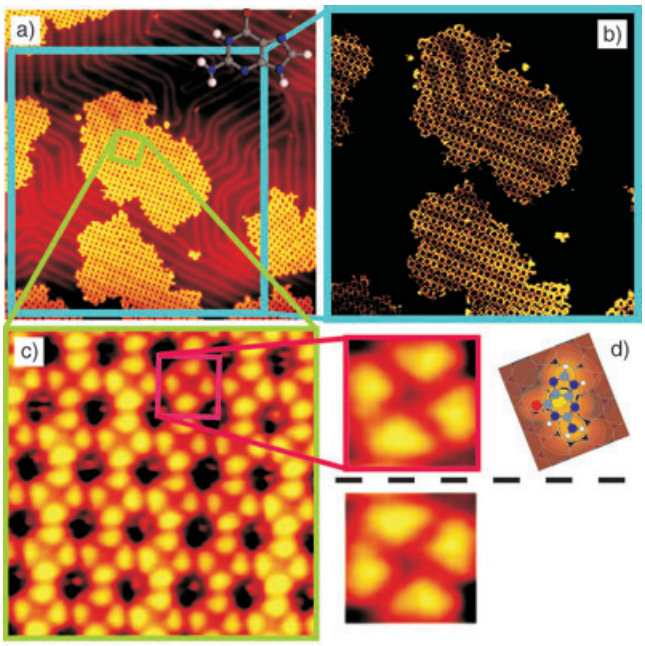

Figure 1. a) STM image (100×100 nm^2) of several self-assembled G islands on Au(111); b) higher magnification (80×80 nm^2) of the previous image, in which the color scale has been adjusted so that the herringbone reconstruction can be recognized on top of the G islands; c) STM image (8×8 nm^2) showing that the G network has an almost square geometry in which the lattice parameter is ≈ 1.5 nm, and the unit cell is composed of four guanine molecules; d) higher magnification (1.5×1.5 nm^2) of image c) showing a high-resolution image of the unit cell. guanine molecules appear as triangular protrusions, in good agreement with theoretical calculations for guanine adsorbed flat onto Au(111) terraces. The unit cell can be recognized as a chiral structure from the geometry of the guanine molecules within; a mirror image is shown below the dashed line.

bonded structures known to hold up to four G-rich DNA strands together under suitable solution conditions.[2,3] Indeed, by superimposing the G-quartet structure determined by X-ray crystallography on G-quadruplex DNA crystals[19] and the STM images reported herein (Figure 2a), a good correspondence is observed between the former and the unit cell of our G network. Furthermore, the lateral interaction between different unit cells consisting of G quartets can be explained by the formation of eight new hydrogen bonds between the N3 and N9 atoms of adjacent guanine molecules (Figure 2b). We therefore conclude that guanine, upon deposition onto Au(111) under ultraclean conditions, spontaneously self-assembles into a H-bonded network of G quartets with structures similar to those found in telomeric DNA, even in the absence of the DNA backbone and metal ions in solution. Other examples of guanosine derivatives that can self-assemble into G-quartet structures in the absence of templating metal ions have been previously reported.[20]

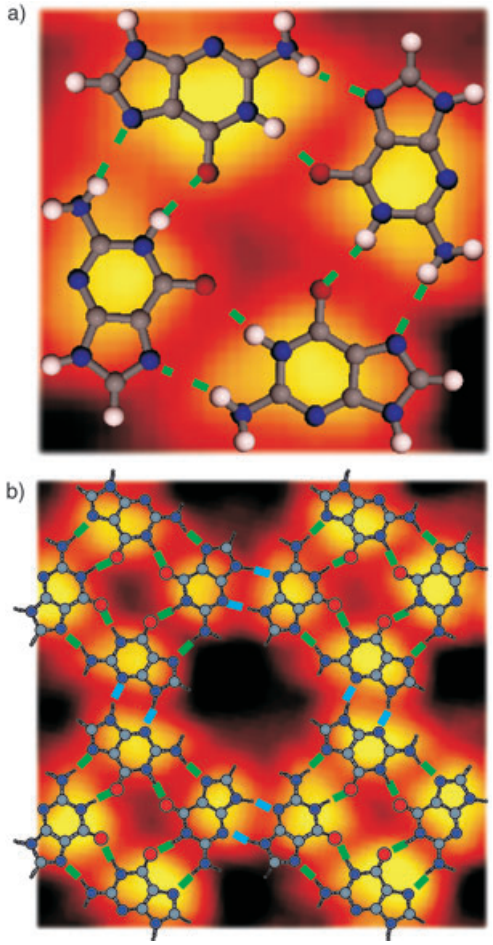

Figure 2. a) Comparison of a high-resolution STM image of the G-quartet unit cell with the Hoogsteen-bonded G-quartet structure determined by X-ray crystallography; b) comparison of an STM image of several G-quartet unit cells with the relaxed structure obtained by DFT calculations. The lateral interaction between G quartets occurs by eight new hydrogen bonds between the peripheral N3 and N9 atoms of neighboring guanine molecules. Intraquartet hydrogen bonds are shown in green; interquartet hydrogen bonds, in blue.

The G-quartet network is not the only H-bonded network that guanine adopts when deposited onto Au(111). By annealing the sample at 400 K, the G network changes irreversibly to another structure, depicted in Figure 3, and no trace of the G-quartet structure is found after annealing.

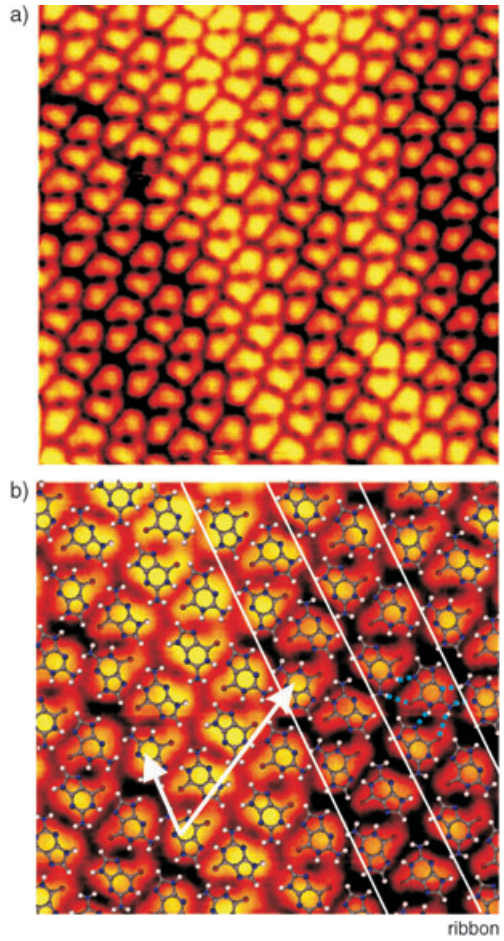

Figure 3. a) STM image (10×10 nm^2) of the high-temperature phase of guanine on Au(111); b) model for the H-bonded network that corresponds to the high-temperature phase of guanine on Au(111) with unit vectors displayed. The local environment of each guanine molecule within this network is similar to that within the G-quartet network, in that each molecule is coordinated by six hydrogen bonds to three nearest neighbors. Ribbon patterns are indicated.

The STM images (recorded at 150–170 K) of the high-temperature structure correspond perfectly to earlier results of self-assembled monolayers of guanine molecules on highly oriented pyrolytic graphite (HOPG) or MoS_2 formed from solution.[14] The high-temperature network is composed of antiparallel molecular "ribbons" (Figure 3b), which are also well-known motifs for self-assemblies of guanosine derivatives in solution.[4,21] The local environment of a guanine molecule in a ribbon network is similar to that in a G-quartet network; each molecule is doubly hydrogen bonded to three nearest neighbors. The entropy penalty for assembly formation is similar in both networks, but the molecular density is about 30% higher in the ribbon network (1.6 and 2.1 mol nm^{-2} for the G-quartet and ribbon networks, respec-

tively), which probably explains its increased stability. Therefore, although the G-quartet network is stable at room temperature, it does not correspond to the most stable arrangement of guanine molecules on the Au(111) surface. This suggests that the preference for the G-quartet network for depositions carried out at room temperature is a phenomenon governed by kinetics rather than thermodynamics. Our findings therefore raise the question: why do guanine molecules assemble *exclusively* into the G-quartet structure when deposited at room temperature, and what is the reason behind the stability of the metastable G-quartet network?

To understand the basis of the stability of the G-quartet network, we have calculated the formation energy of isolated G quartets and their constituent dimers and trimers as shown in Figure 4a. Our calculations show that the formation energy of the dimer, trimer, and quartet structures does not scale with the number of hydrogen bonds between guanine molecules. The average energy per hydrogen bond increases from 0.22 eV in the dimer to 0.31 eV in the trimer and 0.42 eV in the quartet. Previous MD calculations gave a value of $\approx$2.21 eV for the H-bonding energy of the G quartet[22] which corresponds to $\approx$0.27 eV per hydrogen bond. This value is much closer to our result for the dimer than for the quartet. This means that this cooperative effect, as expected, is not well-described by MD simulations. On the other hand, quantum mechanical (QM) approaches (particularly Hartree–Fock and DFT) have been used to estimate the binding energy of the G quartets, and these results (2.86 eV for the quartet, 0.36 eV per hydrogen bond) are in better agreement with our values.[8,23] Figures 4c and d depict the charge-density-perturbation plots for a G dimer and a G quartet, and illustrate the basis of these cooperative hydrogen bonds. The formation of a dimer leads to a rearrangement of the electron density of the guanine molecules to allow some electron charge to be transferred to the oxygen atom that is not involved in hydrogen bonding, thus helping the formation of a new hydrogen bond at this position (arrow in Figure 4c). The intramolecular charge perturbation ascribed to hydrogen bonding is about one order of magnitude greater than that associated with the adsorption of a single guanine molecule on the Au(111) substrate, as shown in Figure 4b. As a consequence of the charge transfer associated with hydrogen bonding, the perturbation of the charge density of guanine involved in a quartet (Figure 4d) is more pronounced (the maximum contour line is 30% larger) than the perturbation in the charge density of guanine in a dimer. This explains why the hydrogen bonds within a quartet are calculated to be about twice as strong as they are in a dimer. This phenomenon, referred to as resonance-assisted hydrogen bonding (RAHB), has been suggested to contribute to the stabilization energy of Watson–Crick base pairs in DNA[24] and G quartets.[8]

Our calculated energy values in combination with an assumed attempt frequency of 10^{13}–10^{14} s^{-1} (consistent with previous studies on the diffusion of organic molecules onto noble metal surfaces,[25]) gives an estimate of $\approx 10^{14}$–10^{15} s for the average lifetime of a guanine molecule within a G quartet at room temperature before its four hydrogen bonds are broken. For comparison, the average lifetimes of a guanine molecule within a dimer or a trimer before the hydrogen bonds are broken are as short as $\approx 5\times10^{-7}$ and 5×10^{-4} s, respectively. We thus conclude that G quartets are the smallest stable structures at room temperature. Once four guanine molecules meet to form a quartet, the structure they form will not break apart before the arrival of another

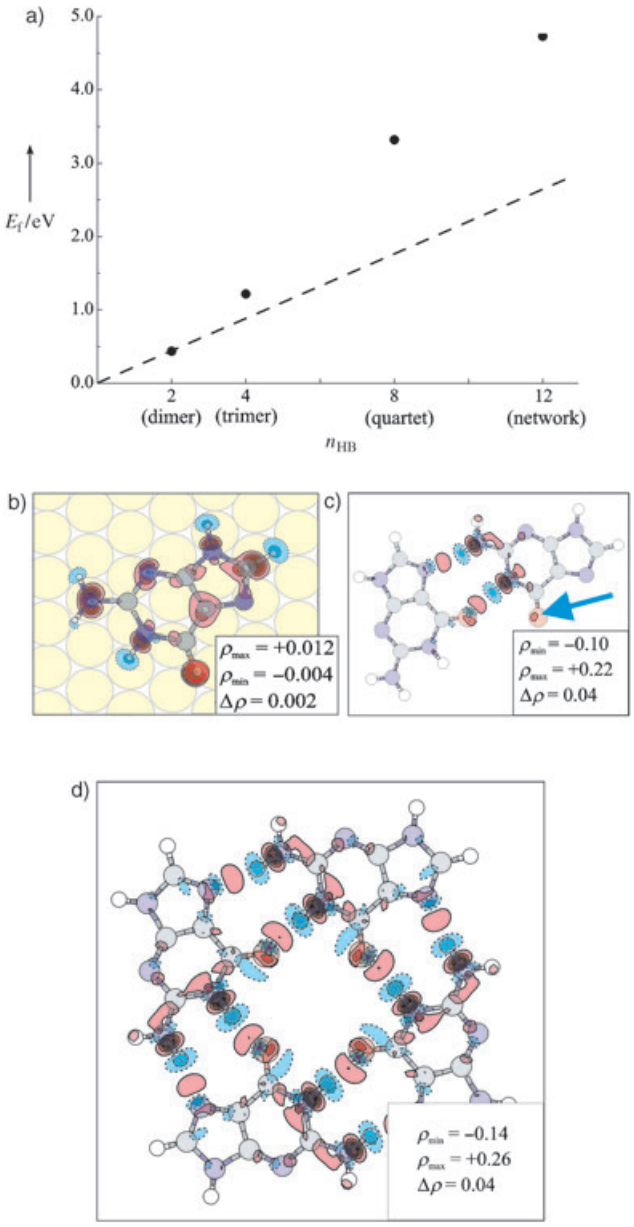

Figure 4. a) Formation energy (E_f) as a function of the number of hydrogen bonds (n_{HB}) in dimer, trimer, quartet, and network structures (as indicated). The dashed line represents the expected result if the hydrogen bonds were mutually independent and thus, if E_f scaled with n_{HB}. Charge-density perturbations determined by DFT calculations for b) adsorption of a molecule onto Au(111); c) the formation of a H-bonded dimer; and d) the formation of a H-bonded quartet. Insets show the values for the maximum and minimum contours and the interval between consecutive contours in $e\text{Å}^{-3}$. Results shown in parts c) and d) are from calculations performed in the absence of the Au substrate.

guanine molecule, thus directing the assembly of a G-quartet network, which precludes the formation of a more stable ribbon structure (Figure 3). In the absence of the RAHB effect, the energy required to break up a G quartet would be only $0.22\ \text{eV} \times 4 = 0.88\ \text{eV}$, which corresponds to an average lifetime of about 5–50 s at room temperature. With the deposition rate in our experiments (1.5×10^{-6} molecules per unit cell s^{-1}), only one to ten molecules would be deposited in an area of $100 \times 100\ \text{nm}^2$ during this time interval. In this case, the quartet structure would not be sufficiently stable at room temperature to direct the growth of the G-quartet network, and there would be nothing to prevent the molecules from assembly into the minimum-energy configuration. The fact that our STM results show formation of the G-quartet network after room-temperature deposition constitutes the first experimental evidence that charge-transfer effects, like the RAHB effect, decisively affect the strength of the hydrogen bonds among nucleobases.

According to the results of previous theoretical work, hydrogen bonding contributes between 30% (for MD simulations)[22] and 60% (for QM calculations)[23,26] of the total binding energy of G-quadruplex DNA in the gas phase, that is, in the absence of solvent. In solution, the remaining binding energy of G quadruplexes arises from complex formation with metal ions (mainly Na^+ and K^+).[27] New experiments are underway to explore the possibility of complex formation with these ions for adsorbed G-quartet networks under UHV conditions. In any case, we have demonstrated that a significant contribution of the H-bonding energy of the G quartets within G quadruplexes can be attributed to the RAHB effect, as previous DFT calculations have also shown.[28] The RAHB strengthening of the hydrogen bonds must therefore be included in any calculation of the structure of G quadruplexes.

In summary, we have demonstrated that by a careful choice of substrate it is possible to observe the geometry of molecular assembly governed by intermolecular interactions while minimizing surface-derived perturbations in such a way that structures known from biology or solution studies can be reproduced on solid surfaces. Exploitation of the RAHB-mediated increase of intermolecular H-bond energies can lead to a more efficient design of the molecular building blocks for 2D self-assembled architectures. The stabilizing effect of RAHB may therefore play an important role in the rational design of drugs, as G quartets have been proposed as targets for drug design in cancer therapeutics.[29]

Experimental Section

All experiments were performed in a UHV chamber (base pressure 10^{-10} Torr) equipped with a variable-temperature, fast-scanning STM (Aarhus type),[30] a homemade molecular evaporator, and facilities for preparing single-crystal surfaces. Au(111) substrates were prepared by repeated cycles of Ar^+-ion sputtering followed by annealing at 800 K. Guanine in powder form was purchased from Sigma-Aldrich (2-Amino-6-hydroxypurine, MFCD 00071533), and loaded into glass crucibles in the molecular evaporator. After thorough degassing, guanine was deposited onto the Au(111) surface by thermal evaporation (crucible temperature 450–470 K, substrate held at room temperature) at a rate of 1.5×10^{-6} molecules per unit cell s^{-1}. The STM experiments were carried out at 150–170 K to prevent tip interaction with the adsorbed guanine molecules. Typical tunneling parameters in our STM images were 0.5–2 V for the bias voltage and 0.2–0.7 nA for the tunneling current. Attempts to bring the tip in closer proximity to the surface usually resulted in an increased interaction between the tip and adsorbed molecules which led to manipulation events.

The first-principles DFT simulations were performed with a plane-wave basis set (expanded up to 35 Rydbergs) together with ultrasoft pseudopotentials,[31] with the exchange-correlation effects treated in the GGA-PW91 approximation.[32]

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[1] J. D. Watson, F. H. C. Crick, *Nature* **1953**, *171*, 737–738.
[2] R. R. Sinden, *DNA Structure and Function*, 1st ed., Academic Press, San Diego, **1994**.
[3] W. I. Sundquist, A. Klug, *Nature* **1989**, *342*, 825–829.
[4] J. T. Davis, *Angew. Chem.* **2004**, *116*, 684–716; *Angew. Chem. Int. Ed.* **2004**, *43*, 668–698.
[5] K. Gehring, J.-L. Leroy, M. Guéron, *Nature* **1993**, *363*, 561–565.
[6] a) D. Kipling, *The Telomere*, 1st ed., Oxford University Press, Oxford, **2002**; b) J. C. Darnell, K. B. Jensen, P. Jin, V. Brown, S. T. Warren, R. B. Darnell, *Cell* **2001**, *107*, 489–499.
[7] T. Steiner, *Angew. Chem.* **2002**, *114*, 50–80; *Angew. Chem. Int. Ed.* **2002**, *41*, 48–76.
[8] G. Louit, A. Hocquet, M. Ghomi, M. Meyer, J. Sühnel, *PhysChemComm* **2002**, *5*, 94–98.
[9] T. E. Cheatham III, P. A. Kollman, *Annu. Rev. Phys. Chem.* **2000**, *51*, 435–471.
[10] C. F. Guerra, F. M. Bickelhaupt, *Angew. Chem.* **2002**, *114*, 2194–2197; *Angew. Chem. Int. Ed.* **2002**, *41*, 2092–2095.
[11] a) J. V. Barth, J. Weckesser, C. Cai, P. Günter, L. Bürgi, O. Jeandupeux, K. Kern, *Angew. Chem.* **2000**, *112*, 1285–1288; *Angew. Chem. Int. Ed.* **2000**, *39*, 1230–1234; b) F. Oesterhelt, D. Oesterhelt, M. Pfeiffer, A. Engel, H. E. Gaub, D. J. Müller, *Science* **2000**, *288*, 143–146; c) A. Kühnle, T. R. Linderoth, B. Hammer, F. Besenbacher, *Nature* **2002**, *415*, 891–893.
[12] a) J. Tersoff, D. R. Hamann, *Phys. Rev. Lett.* **1983**, *50*, 1998–2001; b) J. Tersoff, D. R. Hamann, *Phys. Rev. B* **1985**, *31*, 805–813.
[13] a) Q. Chen, D. J. Frankel, N. V. Richardson, *Langmuir* **2002**, *18*, 3219–3225; b) M. Furukawa, H. Tanaka, T. Kawai, *J. Chem. Phys.* **2001**, *115*, 3419–3423.
[14] a) S. J. Sowerby, M. Edelwirth, W. M. Heckl, *J. Phys. Chem. B* **1998**, *102*, 5914–5922; b) W. M. Heckl, D. P. E. Smith, G. Binnig, H. Klagges, T. W. Hänsch, J. Maddocks, *Proc. Natl. Acad. Sci. USA* **1991**, *88*, 8003–8005.
[15] This π interaction contains an important contribution from van der Waals attraction between the surface and the molecule; it therefore scales with the number of atoms in the molecule, and can be very important for large molecules: F. Rosei, M. Schunack, P. Jiang, A. Gourdon, E. Lægsgaard, I. Stensgaard, C. Joachim, F. Besenbacher, *Science* **2002**, *296*, 328–331; R. Otero, F. Rosei, Y. Naitoh, P. Jiang, P. Thostrup, A. Gourdon, E. Lægsgaard, I. Stensgaard, C. Joachim, F. Besenbacher, *Nano Lett.* **2004**, *4*, 75–78; N. Katsonis, A. Marchenko, D. Fichou, *Adv. Mater.* **2004**, *16*, 309–312.
[16] This number is a lower bound, as DFT largely ignores van der Waals interactions. However, some van der Waals interaction has been reported with the same exchange-correlation

function that we employed (S. Grimme, *J. Comput. Chem.* **2004**, *25*, 1463 – 1473).
[17] We are confident that under our current conditions, most of the quartets are indeed empty. At the base pressure at which we work, the flux of molecules from the residual gas on the surface is estimated to be $< 2.4 \times 10^{14}$ molecules $m^{-2} s^{-1}$. To fill in all quartets in a full monolayer of G-quartet structures with molecules from the residual gas, a period longer than 1 day would be needed.
[18] Each guanine island is enantiomerically pure, but different islands can have different configurations, giving rise to an overall racemic mixture.
[19] G. Laughlan, A. I. H. Murchie, D. G. Norman, M. H. Moore, P. C. E. Moody, D. M. J. Lilley, B. Luisi, *Science* **1994**, *265*, 520 – 524.
[20] J. L. Sessler, M. Sathiosatham, K. Doerr, V. Lynch, K. A. Abboud, *Angew. Chem.* **2000**, *112*, 1356 – 1359; *Angew. Chem. Int. Ed.* **2000**, *39*, 1300 – 1303.
[21] T. Giorgi, F. Grepioni, I. Manet, P. Mariani, S. Masiero, E. Mezzina, S. Pieraccini, L. Saturni, G. P. Spada, G. Gottarelli, *Chem. Eur. J.* **2002**, *8*, 2143 – 2152.
[22] N. Spackova, I. Berger, J. Sponer, *J. Am. Chem. Soc.* **2001**, *123*, 3295 – 3307.
[23] J. Gu, J. Leszczynski, M. Bansal, *Chem. Phys. Lett.* **1999**, *311*, 209 – 214.
[24] G. Gilli, F. Bellucci, V. Ferretti, V. Bertolasi, *J. Am. Chem. Soc.* **1989**, *111*, 1023 – 1028. Evidence is mounting against a significant role for cooperative effects on the hydrogen bonds of Watson–Crick base pairs; see: J. Sponer, P. Jurecka, P. Hobza, *J. Am. Chem. Soc.* **2004**, *126*, 10142 – 10151 and reference [10]).
[25] M. Schunack, T. R. Linderoth, F. Rosei, E. Lægsgaard, I. Stensgaard, F. Besenbacher, *Phys. Rev. Lett.* **2002**, *88*, 156102.
[26] J. Gu, J. Leszczynski, *J. Phys. Chem. A* **2000**, *104*, 6308 – 6313.
[27] C. C. Hardin, A. G. Perry, K. White, *Biopolymers* **2000**, *56*, 147 – 194.
[28] M. Meyer, T. Steinke, M. Brandl, J. Sühnel, *J. Comput. Chem.* **2001**, *22*, 109 – 124.
[29] J.-L. Mergny, C. Hélène, *Nat. Med.* **1998**, *4*, 1366 – 1367.
[30] E. Lægsgaard, L. Österlund, P. Thostrup, P. B. Rasmussen, I. Stensgaard, F. Besenbacher, *Rev. Sci. Instrum.* **2001**, *72*, 3537 – 3542.
[31] D. Vanderbilt, *Phys. Rev. B* **1990**, *41*, 7892 – 7895.
[32] J. P. Perdew, J. A. Chevary, S. H. Vosko, K. A. Jackson, M. R. Pederson, D. J. Singh, *Phys. Rev. B* **1992**, *46*, 6671 – 6687.

