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Tunneling readout of hydrogen-bonding based recognition

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Abstract

Hydrogen bonding has a ubiquitous role in electron transport^{1,2} and in molecular recognition, with DNA base-pairing being the best known example.³ Scanning tunneling microscope (STM) images⁴ and measurements of the decay of tunnel-current as a molecular junction is pulled apart by the STM tip, ⁵ are sensitive to hydrogen-bonded interactions. Here we show that these tunnel-decay signals can be used to measure the strength of hydrogen bonding in DNA basepairs. Junctions that are held together by three hydrogen bonds per basepair (e.g., guanine-cytosine interactions) are stiffer than junctions held together by two hydrogen bonds per basepair (e.g., adenine-thymine interactions). Similar, but less-pronounced, effects are observed on the approach of the tunneling probe, implying that hydrogen-bond dependent attractive forces also have a role in determining the rise of current. These effects provide new mechanisms for making sensors that transduce a molecular recognition event into an electronic signal.

Electron tunneling *through an analyte molecule* can yield chemical information.^{6,7,8} Given that hydrogen bonds enhance electron tunneling rates over vacuum tunneling,⁹ we have proposed a new approach using self-assembled, hydrogen bonded tunnel junctions to give good contacts¹⁰ and chemical selectivity simultaneously.^{5,11} To demonstrate the feasibility of this approach, we functionalized a gold STM probe with a DNA base that was brought into contact with a monolayer of nucleosides on a gold surface under 1,2,4-trichlorobenzene (Figure 1 and see Methods). A steady tunnel current set-point (I_{SP}) was established under servo-control, the servo broken, and the current recorded as the probe was pulled away from the surface. Current-decay curves for a number of hydrogen-bond molecular junctions are

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Author contributions

SC and JH carried out tunneling measurements. AK carried CAFM measurements. ML and OS performed the DFT calculations. PZ synthesized the materials. SL managed the experimental design and analyzed the data.

Competing financial interests

SL, JH and PZ are inventors named on pending patent applications.

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Website summary: DNA base pairs are held together by hydrogen bonds. It has now been shown that a scanning tunneling microscope can be used to measure the strength of hydrogen bonding in such base pairs. The results provide a basis for new types of electronic biosensors and chemosensors.

shown in Figure 2. Clearly, the junctions held together by three hydrogen bonds (guanine-deoxycytidine, G-C and 2-aminoadenine-thymidine, 2AA-T) give signals that extend farther than those held together by two (adenine-thymidine, A-T and 2 aminoadenine-deoxycytidine, 2AA-C). Curves for other base-pair combinations are given in the supporting information. Current decays even more rapidly in solvent alone or with a non-hydrogen-bonding molecule (thiophenol) attached to the probe (supporting information). The curves shown in Figure 2 are raw, unselected data taken from several regions of the substrate. Remarkably, the identity of the hydrogen-bonded molecules can often be determined from a single curve.

Several factors indicate that the signals are not purely electronic in origin: Firstly, the decay distance is much too long to correspond to an electronic process⁵ where tunneling decay lengths are typically Å not nm.^{12,13} Secondly, the change in shape of the decay curves with I_{SP} is not consistent with a simple tunneling process. We illustrate this in Figure 4c with a set of averaged curves (red lines) for each I_{SP} for the G-C interaction. If the decay at lower set-point currents had the same shape as the $I_{SP} = 3\text{nA}$ curve, the curves would lie on the blue dashed lines. They clearly do not. Thirdly, there is considerable hysteresis in the data. On approach, the probe must be brought closer to the surface to restore a signal (supporting information) and its growth is more rapid than the decay was on withdrawal. Fourthly, a theoretical simulation (see below) indicates that the electronic conductance of a base-nucleoside pair is *not* particularly sensitive to hydrogen-bonding, being dominated by the low conductance across the sugar ring.

The slow decay of current with withdrawal distance suggests that mechanical interactions play a role, a mechanism first suggested by Pethica.¹⁴ The stiffness of a molecular junction (directly measured using an atomic force microscope) is roughly the same as that of the bonds that hold the molecules to the surfaces (and on the order of a few N/m).¹⁵ Thus the extension of a molecular junction is accommodated both by distortion of the molecular system, and by distortion of the junction itself. A mechanical model for this is shown in Figure 3. If the stiffness of the probe-molecule bond (K_2) is smaller than that of the molecular junction (K_1) that spans the gap, then adjustments of the probe position (X_p) result in smaller changes in the tunnel gap (X_1). This results in tunnel signals that can appear to decay very slowly when plotted as a function of the external adjustment, X_p . We explored these mechanical interactions using conducting atomic force microscopy (CAFM) to measure interaction forces simultaneously with tunnel current. Adhesion forces are proportional to bond strengths, as opposed to the bond stiffness that appears in the model shown in Figure 3. However, if the shape of the interaction potential is similar for all the H-bond interactions, then bond stiffness is generally proportional to the bond strength, $K_{HB} \propto S_{HB}$. A typical current decay curve is shown by the red data points in Figure 4a, with the simultaneously acquired interaction force shown by the blue points. With this AFM cantilever, presumably much softer than an STM probe, the “tunneling” signal now extends out to 4 nm, illustrating the role of the probe stiffness in determining the extent of the signal. This effect is demonstrated more clearly by comparing current decay curves acquired with a soft probe ($K=0.35\text{ N/m}$, red traces in the inset in 4a) with those obtained with a stiffer probe ($K=2\text{ N/m}$, blue traces in the inset). The curves obtained with the stiffer probe begin to

resemble the STM results. The adhesion force between probe and surface can be read directly from these plots (F_{ad} in Figure 4a). Figure 4b plots F_{ad} vs. the set-point current for a 2AA probe (3 H-bonds, red points) and an A-probe (2 H-bonds, blue points). We used a thiophenol functionalized probe as a non-hydrogen bonding control (black points). Adhesion increases rapidly with set-point at low currents, but then more slowly as the set-point current increases. The electronic conductance through the molecular junctions containing base-nucleoside pairs is not strongly dependent on the number of the hydrogen bonds (right-hand column, Table 1), so the number of molecules probed in the junction at a given set-point does not depend on their hydrogen bonding, but rather on the area of the junction. This contact area increases linearly with the indentation of the probe and the current increases

exponentially with this indentation. Thus we expect that $F_{ad} \propto \sum_{N_{HB}} S_{HB} \ln(BI_{SP} + 1) + F_{NS}$ where N_{HB} is the number of hydrogen bonds per intermolecular interaction, S_{HB} is the strength of each hydrogen bond, B is a constant and F_{NS} is a non-specific adhesion force. This function fits the experimental data quite well (solid lines) with the assumption that the strength of the hydrogen bonds are all equal (bond strengths are similar for 2AA-T and A-T at 5 to 7 kcal/mol 17). Turning now to the case of the STM junction, and assuming $K_{HB} \propto S_{HB}$, the number of molecular contacts increases with the logarithm of I_{SP} , so that force equilibrium in the elastic-interaction model (Figure 3) requires that

$$X_1 = \frac{1}{1 + CN_{HB} \frac{K_1}{K_2} \ln(BI_{SP} + 1)} X_P \quad (1)$$

where K_1 represents the “spring constant” of a single hydrogen bonded interaction, K_2 is the stiffness of the probe (or probe-molecule bond) and $X_P = X_1 + X_2$ is the total displacement of the probe. For simplicity, we have assumed that all the hydrogen bonds are of equal

stiffness, though we shall see that this is not generally true. Two parameters, B and $\frac{CK_1}{K_2}$, determine changes in the tunnel gap as a function of I_{SP} and the measured probe displacement. This model is only valid for small departures from equilibrium and cannot describe the non-linear interactions that dominate the eventual breaking of the bonds. These non-linear interactions clearly contain additional useful information, as can be seen from the difference in shape of the G-C and 2AA-T curves (Figures 2a and b).

The two model parameters (B and $\frac{CK_1}{K_2}$) are readily obtained from a subset of the tunneling decay data shown in Figure 2. In order to illustrate the fitting process, we have averaged 26 representative curves to show typical curves (red) for G-C interaction (Figure 4c) and A-T

interactions (Figure 4d). We used the G-C data to obtain B and $\frac{CK_1}{K_2}$ (supporting information). We then used equation 1 to scale the measured displacement to correspond to that in the $I_{SP} = 3\text{nA}$ curves, so that a polynomial fit to this curve could be used to predict the decays at lower set points (green lines in Figures 4c and d). The agreement with experiment is good, showing that the mechanical interactions revealed by the conducting AFM account for the STM data also. This simple model based on equal bond-stiffness works well for this case because the hydrogen bonds in A-T and G-C have an average

strength of 7 to 9 kcal/mol each¹⁷ and so are probably of similar stiffness. In addition, the underlying shape of the curve (i.e., $I(X_1)$) does not appear to change with set-point. This is not the case for 2AA-T and 2AA-C for which the underlying decay function changes noticeably (supporting information).

The data are highly reproducible from probe to probe, sample to sample and day to day, and examples are given in the supporting information. This is surprising, given that K_2 is determined by properties of the (variable) probe. One reason for this may be that the STM probes are much stiffer than the molecule-metal bond, so that variations in probe geometry do not affect the overall stiffness of the junction significantly.

Fits to the current decay data are complicated by factors that affect $I(X_1)$ and that are not incorporated into this simple mechanical model. The model is better-tested by integrating the current decay curves to obtain the charge transferred in each interaction (when the x axis is converted to time using the known retraction speed of the probe). This removes the explicit dependence on $I(X_1)$. The mean charge transferred, Q , as a function of initial set-point is plotted for a number of types molecular junctions in Figure 5. The error bars are ± 1 sd of the distributions for each point. The mechanical model of gap distortion yields the following result for Q :

$$Q = \frac{I_{SP}}{\beta v} \left(1 + \frac{CK_1}{K_2} N_{HB} \ln(BI_{SP} + 1) \right). \quad (2)$$

Here $v = \frac{dX_p}{ds}$ is the retraction speed of the STM probe (100 nm/s) and β is the intrinsic decay constant (taken to be the 6 nm^{-1} measured in solvent - supporting information) and B

and $\frac{CK_1}{K_2}$ are the parameters introduced in Equation 1. Calculated values of Q are shown by the solid lines in Figure 5 for $N_{HB} = 0, 1, 2$ and 3. The experimental data fall close to the corresponding lines, with the exception of the data for 2AA-C which falls on the $N_{HB} = 1$ line, presumably because the two H-bonds in this complex are significantly less stiff. It is interesting to note that the data for G-C (9 kcal/mol for each H-bond¹⁷) lies above the line while the data for 2AA-T (5 kcal/mole for each H-bond¹⁷) are closer to the prediction.

Theoretical calculations help us understand how the set point current can be unaffected by the hydrogen bonding. We investigated the conductance of both paired-bases and base-nucleoside pairs using a density functional theory (DFT) Green's function scattering method^{18,19} based on the Landauer approach.^{20,21} Semi-infinite gold electrodes were connected to sulfur atoms (yellow in the illustration of guanine-cytosine and guanine-deoxycytidine pairs in Table 1). Further details are given in the supporting information. Calculated conductances (Table 1) are much larger for bases joined by three hydrogen bonds than for those joined by two. However, when one of the bases in the pair is replaced with a nucleoside (right column) the conductance is reduced substantially and no longer varies systematically with the number of hydrogen bonds. More importantly, a comparison of calculations and measurements for a number of molecular junctions suggests that the calculated conductance is often a factor 10 too high.¹² Thus one molecular junction probably contributes only a little to the overall current.

We can estimate the number of molecules in the junction (and thus their conductance) if we assume that each hydrogen bond contributes about 200 pN to the excess adhesion force over the control sample at the loading rate used here.²² Thus rupturing 2AA-thymidine requires 600 pN while A-thymidine pairs require 400 pN. Subtracting the background signal (1.7 nN – Figure 4b) from the measured adhesion and dividing the remainder by 600 (2AA-T) or 400 pN (A-T) yields a range of 2 to 10 molecules in these junctions over the range of set points (0.1 to 3 nA). Dividing the current at zero force ($I(F = 0)$, Figure 4a) by the estimated number of molecules in each junction yields an estimate of the conductance of each molecular pair as 100 to 300 pS. This is indeed about a factor ten lower than calculated. In consequence, most of the current at the initial set point must come from through-space and not through-bond tunneling. The CAFM data (Figure 4a) offer further evidence that the molecular junctions have a low conductance, because the current signal generally falls to a negligible value when the H-bonds are fully stretched at the point of maximum adhesion. These simple calculations do not take account of fluctuations. Doing so would probably result in a smaller predicted conductance (supporting information).

In summary, we have demonstrated a method whereby the STM can be used to characterize the stiffness of hydrogen bonds in few-molecule interactions, and have accounted for the extent of the withdrawal signals with an electro-mechanical model. When H-bonds are of similar stiffness, the measurements can identify targets by counting H-bonds. Weaker attractive interactions, presumably owing to transient hydrogen-bonding, result in significant chemical sensitivity in the approach curves also. The sensitivity of the approach curves to H-bonding suggests that it is the H-bonds and not the Au-S bonds that break. The curves are highly repeatable, indicating that the H-bonds can be broken and reformed apparently indefinitely. These mechanisms provide a basis for new types of electronic biosensors and chemosensors based on hydrogen bonding, transducing molecular recognition directly into an electrical signal with a high degree of chemical specificity.

Methods

Preparation of reagents and surfaces is described in the supporting information. Tunneling measurements were carried out on a PicoSTM (Agilent, Chandler) with the sample and probe submerged in 1,2,4-trichlorobenzene. Approach and retraction curves were recorded using custom Labview software. Conducting AFM (PicoAFM, Agilent, Chandler) measurements used probes with nominal spring constants of 0.35 and 2 N/m (Mikromasch) sputter coated with alternating layers of chrome and gold (25 Å Cr, 50 Å Au, 25 Å Cr, 50 Å Au, 25 Å Cr, 100 Å Au, 25 Å Cr and 300 Å Au). The final probe radii, determined by SEM lay between 100 and 200 nm. Probes were immersed in 1 mM solutions of 8-mercaptoadenine or 2-amino-8-mercaptoadenine in DMF or 1 mM thiophenol in methanol (control experiments) for 2 to 12 h immediately after removal from the sputter-coater. Simultaneous force and conductance measurements were taken at a series of nominal current setpoints between 0.5 and 9 nA with a bias of 0.4V in 1,2,4-trichlorobenzene using thymidine monolayers and a probe retraction speed of 2000 nm/s. After taking measurements, each probe was calibrated using the thermal-noise method.²³

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

Illustration of the STM measurements. A sharp gold probe, functionalized with a thiolated base, is approached to a gold (111) surface functionalized with a monolayer of thiolated nucleosides until the desired set-point current is obtained, and then retracted while the tunnel current is recorded. The current-distance curves can be used to characterize the strength of the hydrogen-bonded interactions.

Figure 2.

Tunnel-current decay curves. The decay curves were obtained at a bias (V in Fig. 1) of 0.4V as the probe was retracted from a monolayer of nucleosides at a rate of 100 nm/s. a, an 8-mercaptoguanine probe interacting with a deoxycytidine monolayer (G-C); b, 2-amino-8-mercaptoadenine functionalized probe interacting with a thymidine monolayer (2AA-T); c, an 8-mercaptoadenine probe interacting with a thymidine monolayer (A-T); d, a 2-amino-8-mercaptoadenine functionalized probe interacting with a deoxycytidine monolayer (2AA-C). All nucleosides were 5'-thiolated. The initial setpoint currents were 3 nA (green), 2.4 nA (brown), 1.2 nA (purple), 0.8 nA (blue), 0.4 nA (khaki) and 0.1 nA (black). The curves are a combination of repeated measurements on the same point and measurements made at different points on the substrate. The signals from non-hydrogen-bonded controls decay much more rapidly (supporting information). Logarithmic plots are available in the supporting information.

Figure 3.

A mechanical model of the tunnel gap. A displacement of the probe by an amount X_p is distributed between distortions of the molecule-probe bonds (X_2) and the molecule-molecule bonds (X_1) according to their relative stiffness (K_1/K_2).

Figure 4.

Determination of elastic-interaction parameters and prediction of the tunnel decay curves. a, Conducting AFM measurements of interaction force (blue points) and simultaneously acquired tunnel current (red points) for A-thymidine with a cantilever of spring constant 0.35 N/m. The inset shows how the extent of the current-distance curves is controlled by the stiffness of the cantilever (black curves, $K=2$ N/m, red curves, $K=0.35$ N/m). b, Adhesion force plotted vs. set-point current for a 2-amino-8-mercaptoadenine functionalized probe interacting with a thymidine monolayer (red points) and a 8-mercaptoadenine probe interacting with a thymidine monolayer (blue points). The black dots are control data obtained with thio-phenol functionalized probes. The solid lines are fits to $F_{ad} \propto N_{HB} \ln(BI_{SP} + 1) + F_{NS}$ (assuming all bonds are of equal strength) with B set to 2.83 nA^{-1} . The coefficient of the log terms are 1.97 ± 0.3 (AA-T) and 1.0 ± 0.16 (A-T), so their ratio is $1.96 (+0.7, -0.5)$ a range that spans the expected value of 1.5 ($N_{HB} (2AA - T) / N_{HB} (A - T) = 3/2$). Representative STM decay curves (red) for an 8-mercaptoguanine probe interacting with a deoxycytidine monolayer (c) and an 8-mercaptoadenine probe interacting with a

thymidine monolayer (d) for several set-points (each curve is the average of 26 raw data curves). The blue dashed-lines show how the current should decay from each set point if it follows the form of the 3nA data. The green lines are predicted by the elastic distortion model described in the text where the same two parameters are used to fit both the G-C and A-T data.

Figure 5.

Charge transfer, obtained from integration of the tunnel current decay curves, plotted as a function of set-point current for different molecular junctions. The junctions plotted are non-hydrogen bonding controls (triangles), 2AA-deoxycytidine (black dots), A-thymidine (green dots), 2AA-Thymidine (red dots) and G-deoxycytidine (blue dots). The two sets of controls correspond to a bare probe and a thio-phenol functionalized probe interacting with a thymidine monolayer. The withdraw speed was 100 nm/s and the error bars are ± 1 sd. The solid lines are calculated according to equation 2 for $N_{HB} = 0, 1, 2$ and 3 using the values of B and CK_1/K_2 obtained from fits to the G-C data in Figure 4c.

Table 1

Conductances calculated with density-functional theory for hydrogen-bonded bases spanning a pair of gold electrodes (middle column) and for nucleosides hydrogen bonded to bases (the sulfur atoms that attach to the gold are shown in yellow for the C:G examples illustrated).

Base-pair (N_{HB}) [Base-nucleoside]	Conductance Base:Base (nS)	Conductance Nucleoside:base (nS)

C:G (3) [Cytidine:G]	83.8	0.96
T:2AA (3) [Deoxythymidine:2AA]	119	1.43
T:A (2) [Deoxythymidine:A]	22.2	1.62
T:G (2) [Deoxythymidine:G]	23.7	0.54