

Lecture 12: AFM measurement of chemical bonding forces (*and ... molecule weight*)

- **Chemical bonding (Atomic force)** *measured by* **atomic force** microscope;
- What are the challenges?
- Direct measurement of single-covalent bonding;
- Evaluation of inter-chain interaction (H-bonding) of DNA.

😊 Measuring molecule weight?

AFM vs. atomic resolution imaging

- Although originally invented based on atomic force, but not commonly used for atomic resolution imaging because of the additional forces brought in between the tip and sample surface including the adhesion, friction, etc..
 - The major factors limiting the high resolution are “fat-tip” effect, thermal agitation at room temperature, and surface contamination.
 - Some representative literatures for atomic imaging:
 2. F. Ohnesorge, G. Binnig, *Science* **260**, 1451 (1993).
 3. F. J. Giessibl, *Science* **267**, 68 (1995).
 4. S. Kitamura, M. Iwatsuki, *Jpn. J. Appl. Phys.* **34**, 145 (1995).
-

Chemical bonding: *strong, short-range force between two atoms*

- Chemical bonding --- *attraction between two atoms when they are in proximity (bond formation), leading to formation of chemical compounds, which contain two or more atoms. For the chemical bonding in molecules, its strength of bonds varies considerably, and can be classified as "strong bonds" such as covalent bonds and "weak bonds" such as hydrogen bonding (e.g. the interaction holding water molecules together in water, and the base-pairing holding the DNA double strands together).*
- When AFM tip is in proximity with the sample surface --- attraction occurs --- *that is covalent bonding between a single-pair of atoms! --- one atom is the outmost atom on tip, and the other is from the sample surface. Draw two schemes on board: atomic force vs. distance, tip over the sample.*
- Stiffness of a cantilever can be as small as 10^{-3} N/m --- considering an oscillation of 1 nm, the force acted to the tip is around 10^{-3} nN, or 1 pico-Newton, sensitive enough to measure the chemical bonding, which is normally around a few nN.
- AFM is just perfect for measuring the chemical bonding (short-range force) due to the highly controlled tip-sample (i.e., the inter-atomic) distance.
- However, it turns out to be quite challenging: *see the later slide for reasons.*

Long range atomic force: *van der Waals force and electrostatic interaction*

- The **van der Waals force** (or van der Waals interaction), named after Dutch scientist Johannes Diderik van der Waals, is the sum of the attractive or repulsive forces between atoms or molecules (or between parts of the same molecule) other than those due to covalent bonds or to the electrostatic interaction of ions with one another or with neutral molecules.
 - The **electrostatic interaction** can be repulsion or attraction between two charged species, which, in the AFM imaging, could be the tip and the sample surface. It is typical long range force.
-

Comparison between **short** and **long** range atomic force:

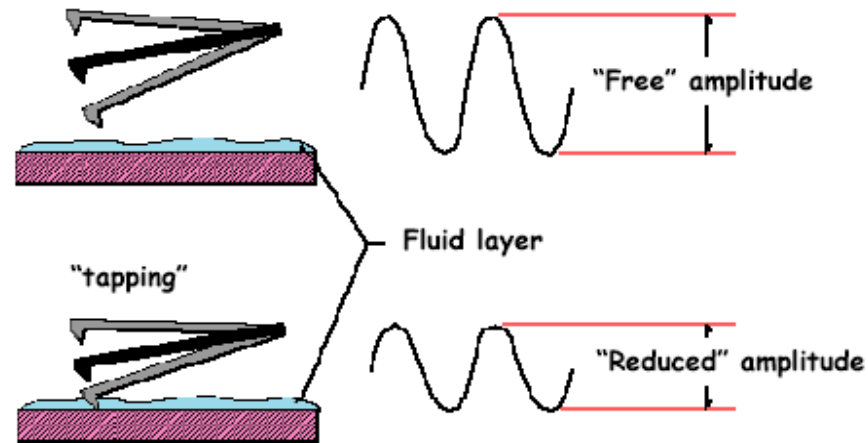
Force type	strength	distance	Dissociation energy
Covalent bond	Strong, a few nN	~ 0.1 nm	~ 100 kcal/mol
Hydrogen bond	Weak, ~ 10% of above	In between	1-10 kcal/mol
Van der Waals force	Even weaker, ~ 10% of above	~> 0.3 nm	< 1 kcal/mol

The measurement of short-range bonding forces with the AFM has been difficult to achieve for several reasons:

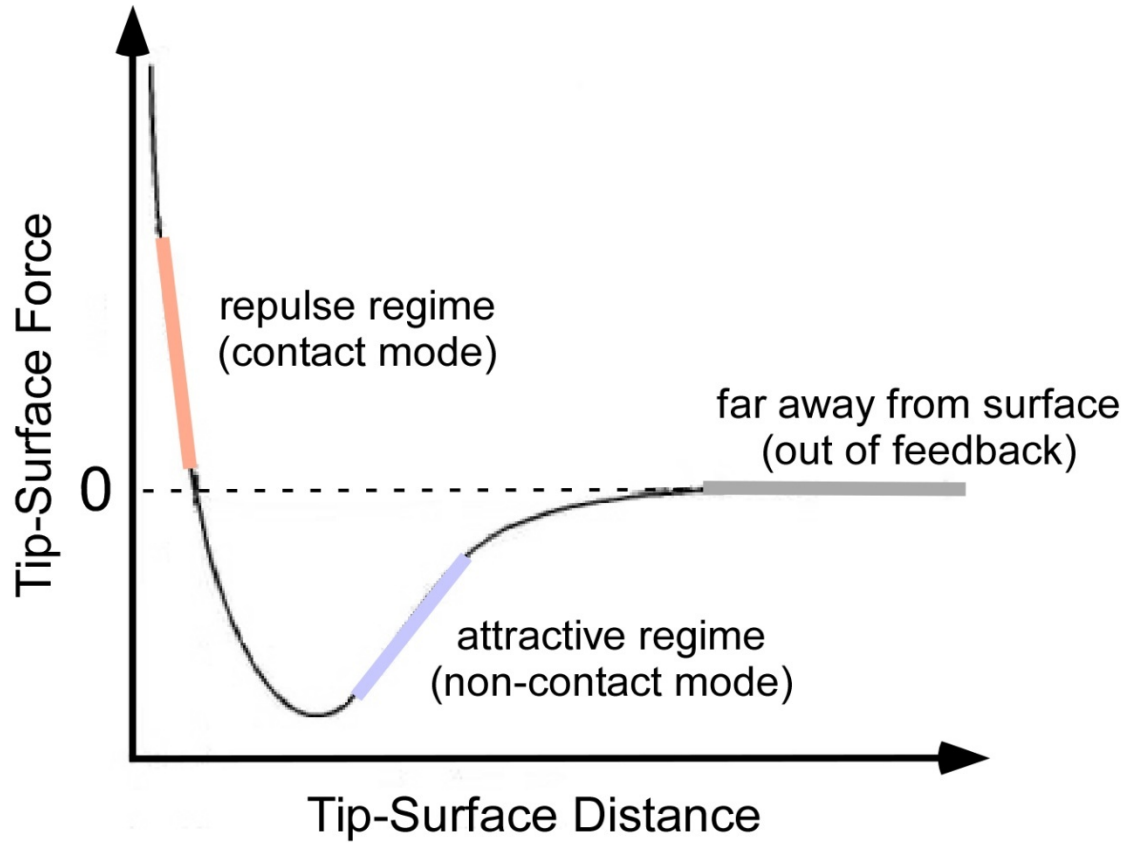
1. At room temperature, **thermal drift** and piezoelectric **scanner creep** make it difficult to reliably position the tip above a specific lattice position.
 2. Most atomic-resolution AFM images have been obtained using a dynamic technique in which the tip-bearing cantilever is driven on its fundamental resonant frequency with a typical amplitude of several nanometers. When the cantilever tip comes close to the sample surface, the force acting on the tip weakly perturbs the cantilever oscillation, giving rise to a small **shift Δf** in the resonance frequency. The **frequency shift** is used as a **feedback** parameter to control the tip-sample spacing, and images therefore correspond to **contours of constant frequency shift**. Because of the large tip excursion, the **relation between the measured frequency shift and the force acting on the tip is not straightforward**. Recently, however, progress has been made in quantitatively understanding and inverting this relation.
 3. In general, both **short-range forces** (such as **covalent bonding** forces) and **long-range forces** [such as **van der Waals (vdW)** and **electrostatic forces**] act on the tip. Separating these contributions in order to isolate the **short-range chemical bonding** force is a nontrivial problem.
 4. It is difficult to determine whether the measured chemical force involves more than just **a single pair of atoms**.
- *(Keep this list on board till the slide of how to solve these problems)*

Non-contact vs. tapping mode

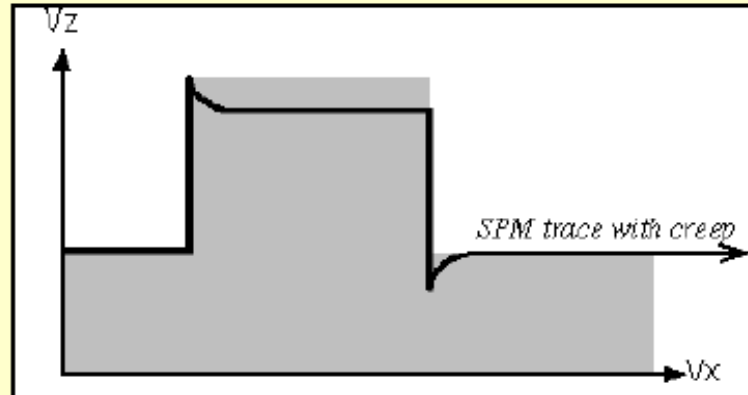
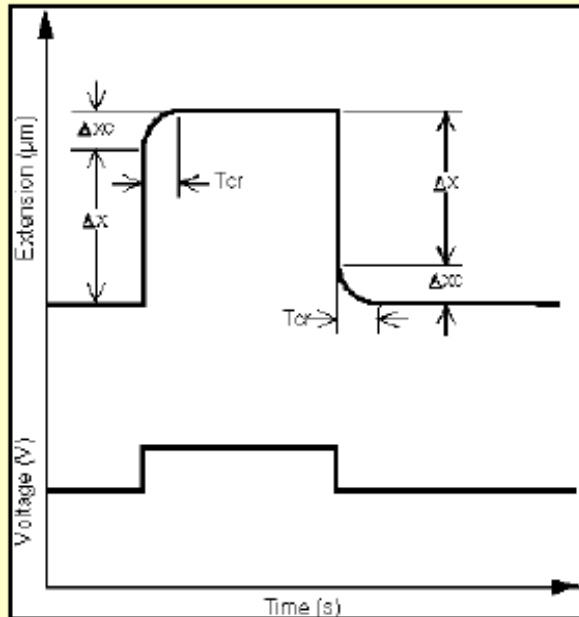
- Both are based on a Feedback Mechanism of constant oscillation amplitude.
- Contact mode: amplitude set as $\sim 100\%$ of “Free” amplitude;
- Tapping mode: amplitude set as $\sim 50 -60\%$ of “Free” amplitude.
- Tapping mode provides higher resolution with minimum sample damage.
- **Most of times, non-contact mode is operated as tapping mode.**



Atomic interaction



Scanner Creep



- ◆ When an abrupt change in voltage is applied, the piezoelectric reacts in two steps: the first step takes place in less than a millisecond, the second on a much longer time scale. The second step, Δx_c , is known as **creep**.
- ◆ Creep is the ratio of the second dimensional change to the first: $\Delta x_c / \Delta x$. It ranges from 1% to 20%, over times of 10 to 100 sec.

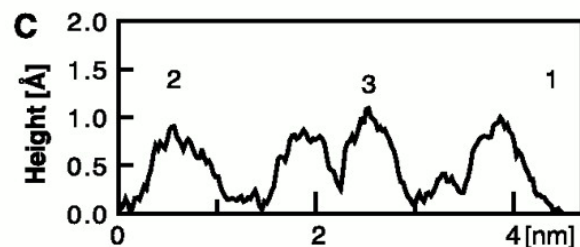
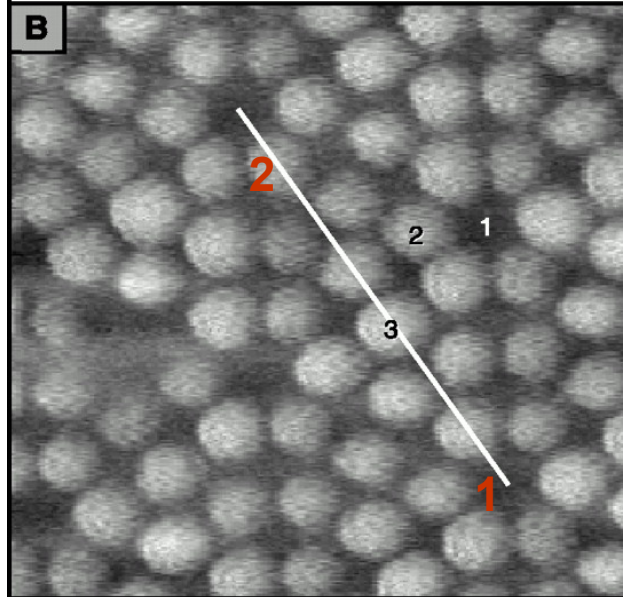
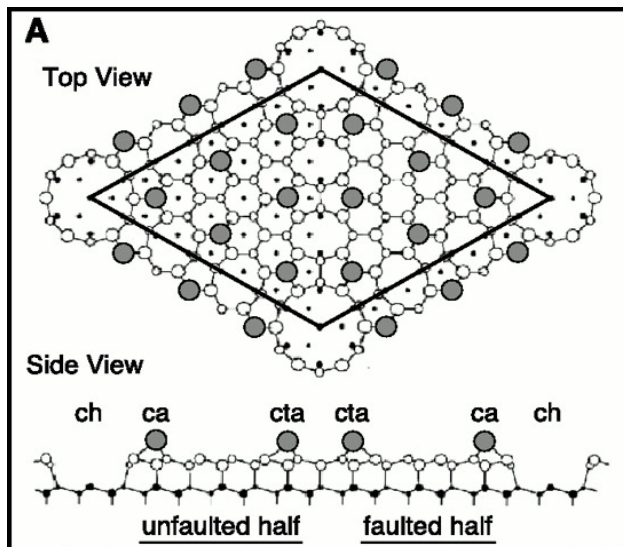
Quantitative Measurement of Short-Range Chemical Bonding Forces

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The measurement of short-range bonding forces with the AFM has been approached through some technical improvements:

1. Measured at low temperature (like 7.2 K) and UHV, to minimize or eliminate **thermal drift** and piezoelectric **scanner creep**, and remove the tip-sample interaction caused by the surface contaminations.
2. Using a well developed procedure (ref. 7 cited therein) to convert the **frequency-distance** data to **force-distance results** --- *frequency shift (Δf) is now quantitatively converted to the force acting on the tip, i.e., the interatomic force between the atom on tip and the atom on the sample.*
3. Measuring the force-distance over a non-specific site (like a **defect-hole** on a crystal surface of silicon, *draw on board*) as a control base-line to correct (subtract) the **van der Waals (vdW)**; by applying bias to the sample (here +1.16 V) to correct the **electrostatic forces**.
4. How to confirm --- the measured chemical force involves only **a single pair of atoms**?
 - Repeated measurements over the same site (atom) --- if it is due to multiple atoms, there should be no good reproducibility due to the damage to the tip by the scanning;
 - Fitting the data --- good agreement to the first principle calculations designed to model the same situation;
 - Evidenced by the atomic topographic image scanned by the same tip --- only after the real atomic image is obtained, is the force-distance measurement started.



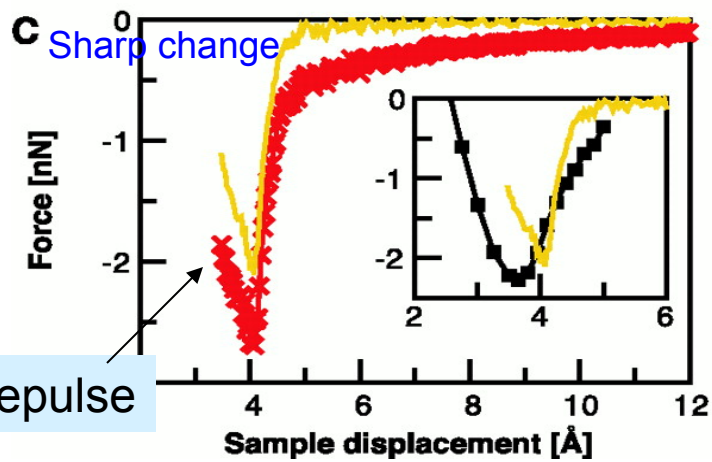
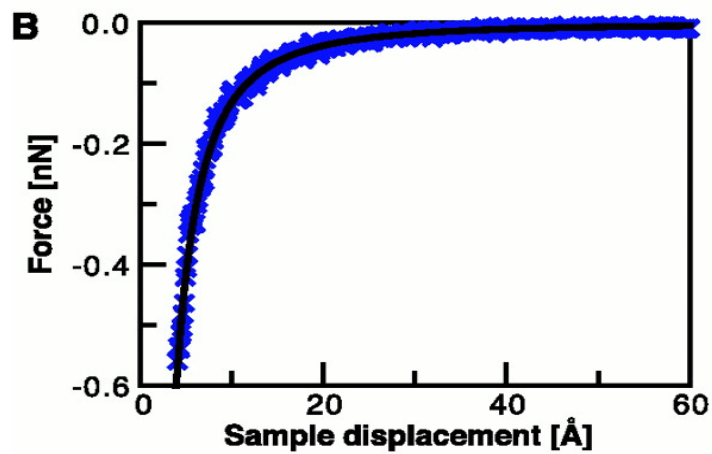
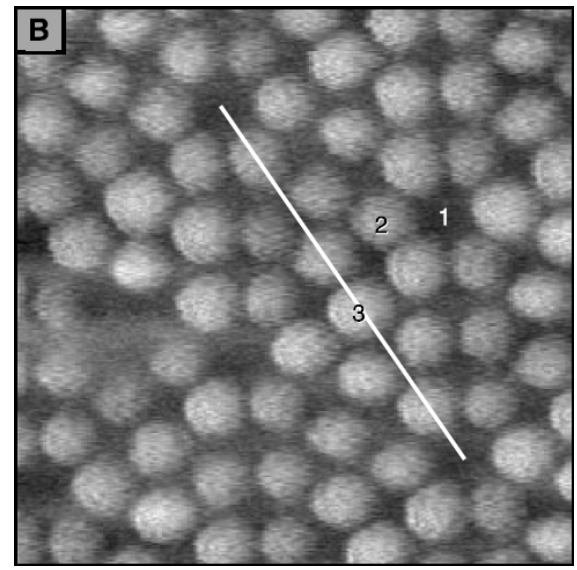
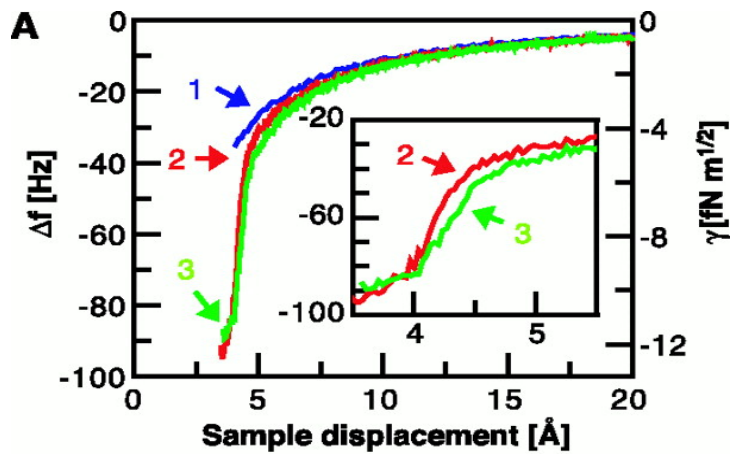
(A) Dimer adatom stacking-fault model of the Si(111) 7×7 surface. The unit cell is outlined by a black diamond. The adatoms are shown as gray circles; the side view shows the positions of the corner holes (**ch**), corner adatoms (**ca**), and center adatoms (**cta**).

(B) Constant frequency shift image ($\Delta f = -38$ Hz, root mean square error 1.15 Hz, scan speed 2 nm/s, image size 6 nm by 6 nm). The labels 1, 2, and 3 indicate the position of frequency distance measurements (see text).

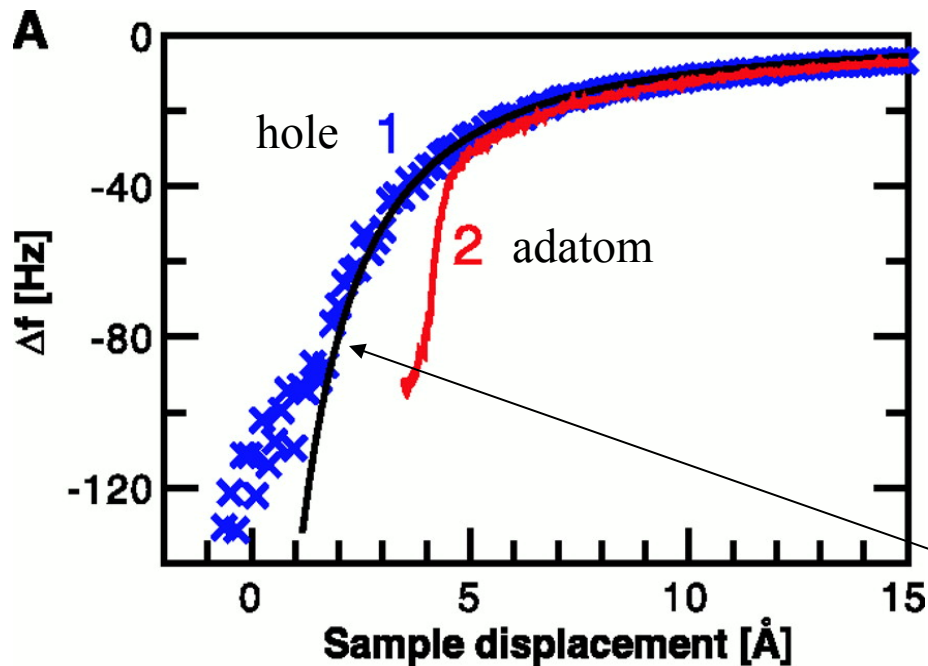
(C) The white line indicates the position of the line section. The corner hole position labeled 1 and the corner adatom labeled 2 in the line section are equivalent by trigonal symmetry to sites 1 and 2 in (A).

Some experimental conditions:

1. UHV.
 2. Tip cantilever was heated at 150 °C for 2 hours to remove contaminants.
 3. Tip was covered with native SiO₂.
 4. Temperature of measurement system, 7.2 K.
 5. AFM scanning at constant Δf (frequency) dynamic mode.
- The high resolution image was only obtained after couple times of preliminary scanning (low resolution) --- this might be due to the polishing of the tip or transfer of silicon atoms from sample surface to the tip.
 - A general measuring procedure:
 1. Getting a set-point from the high-resolution scanning;
 2. From the set-point (in feedback), retract the tip from the sample surface, say 63.07 Å, then slowly pull back the tip even further, say 64.33 Å, at a rate 6 Å/s. now, the tip is 1.26 Å further to the sample --- falling into the regime of long range force (van der Waals).
 3. From there, retract the tip again by 15.77 Å, then **very slowly** pull back the tip by 17.03 Å, at a rate of 1.7 Å/s. Now the tip is 1.26 Å closer to the sample --- falling into the short range force (covalent bonding).

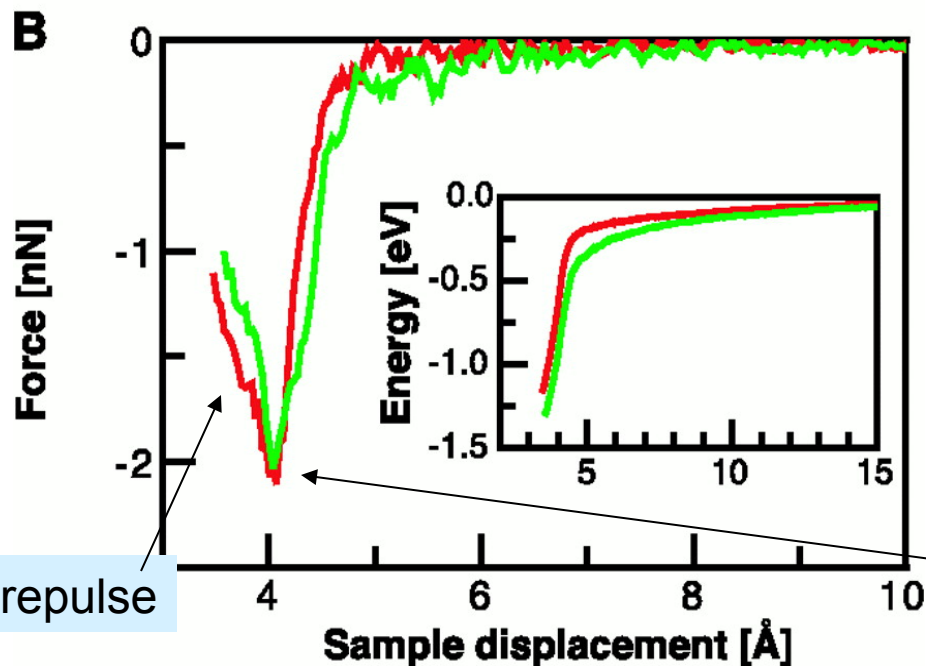


- (A) Frequency shift Δf and normalized frequency shift versus distance, as measured above the positions labeled 1, 2, and 3 in the last figure. The inset adjusts the scales for Δf and distance to give a better picture of the data acquired above the two inequivalent adatoms.
- (B) Force-distance relation determined above the **corner hole** (blue symbols) and a fit to the data using a sphere-plane model for the vdW force (black line).
- (C) Total force (**red line** with symbols) and short-range force (**yellow line**) determined above the **adatom** site labeled 2 in the last figure. In the inset, the measured short-range force is compared with a first-principles calculation (black line with symbols).



(A) Frequency shift measured above the corner hole (symbols) and extrapolated from the model fit to the data of last figure (blue line). For comparison, the data acquired above adatom site **2** from last figure are also plotted (red line).

For the hole atom (lower than the adatom #2), larger displacement is needed to reach the same amount of force.

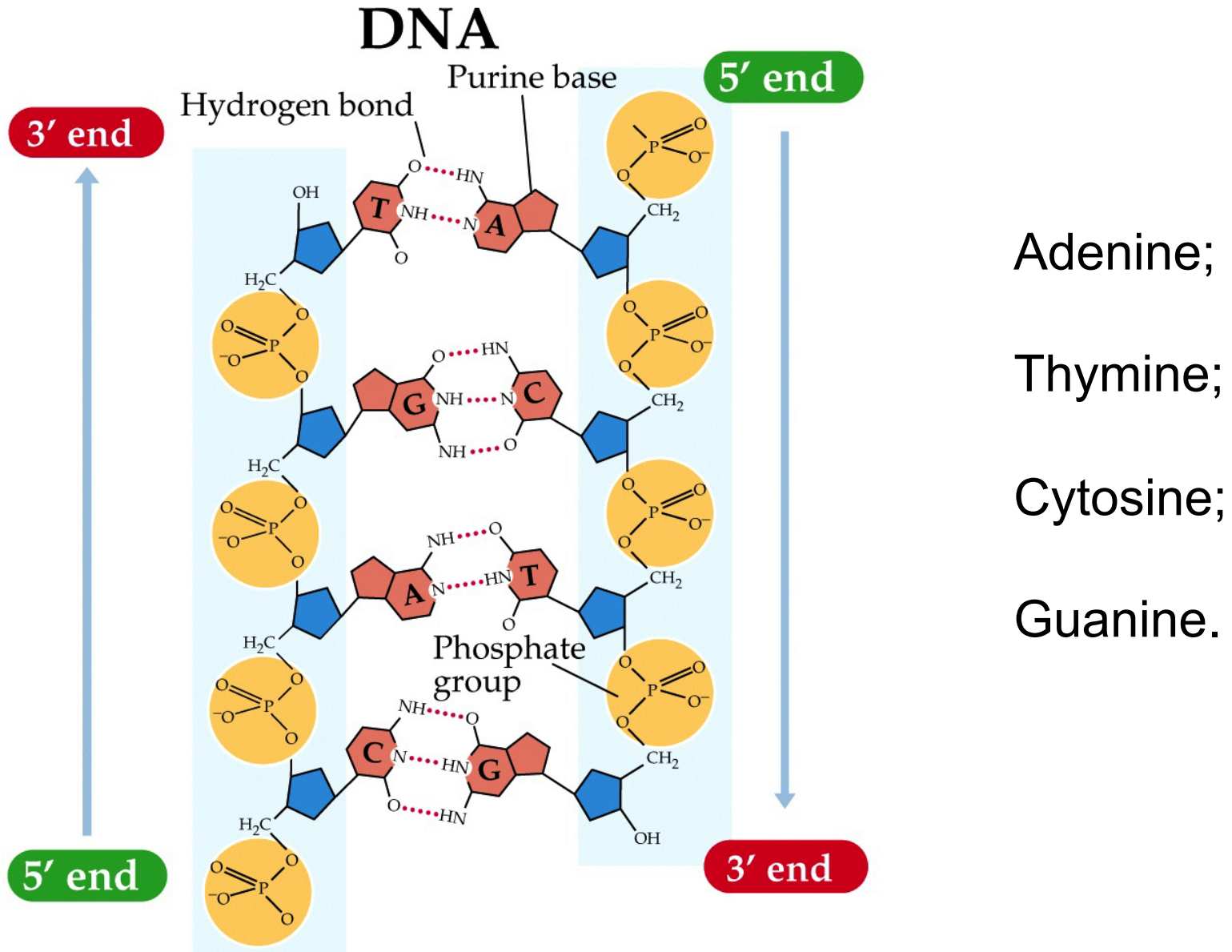


(B) Short-range force and interaction energy (inset) measured above the sites labeled 2 and 3 in Fig. 1.

2.1 nano-Newtons

repulse

Watson-Crick base pairing: H-bonding



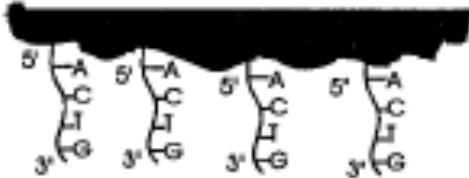
Direct Measurement of the Forces Between Complementary Strands of DNA

Gil U. Lee; Linda A. Chrisey; Richard J. Colton

Science, New Series, Vol. 266, No. 5186 (Nov. 4, 1994), 771-773.

The intra- and intermolecular forces of the DNA double helix are central to understanding its structure and rich functional behavior (1). Until recently, our knowledge of these molecular forces was based on indirect physical and thermodynamic measurements such as x-ray crystallography, light scattering, and nuclear magnetic resonance spectroscopy (2). Direct measurement of interaction forces requires that the state of a system be monitored while an independent force is applied.

20 base DNA single strand immobilized on tip and substrate surface.



ACTG-ACTG-ACTG-ACTG-ACTG



TGAC-TGAC-TGAC-TGAC-TGAC

The surface immobilization is much stronger than the H-bonding force, so, the measurement will not break up the surface binding.

Measurement procedure (see next figure):

- a. As the tip approaches the sample surface, **non-specific attraction** (due to the inter-chain interaction and the like) appears when the tip-sample distance falls below **5 nm**.
- b. Further approaching reaches the repulse force region.
- c. A **hysteresis** is observed when retracting the tip out-of contact with surface --- this is a result of adhesion force, ca. 1.56 nN.

Measurement statistics:

- a. Repeated measurements showed that the magnitudes of the adhesive forces fall into **4 distinct populations** centered at **1.52, 1.11, 0.83, 0.48 nN**.
- b. The 0.48 nN force is due to the non-H-bonding force, as evidenced by the measurement for the non-complimentary DNA strands, where a similar force of **0.38 nN** was obtained .
- c. The three distinct forces are due to the H-bonding between **20, 16, and 12** base pairs within a single-pair of DNA strands --- approximately linear dependence.

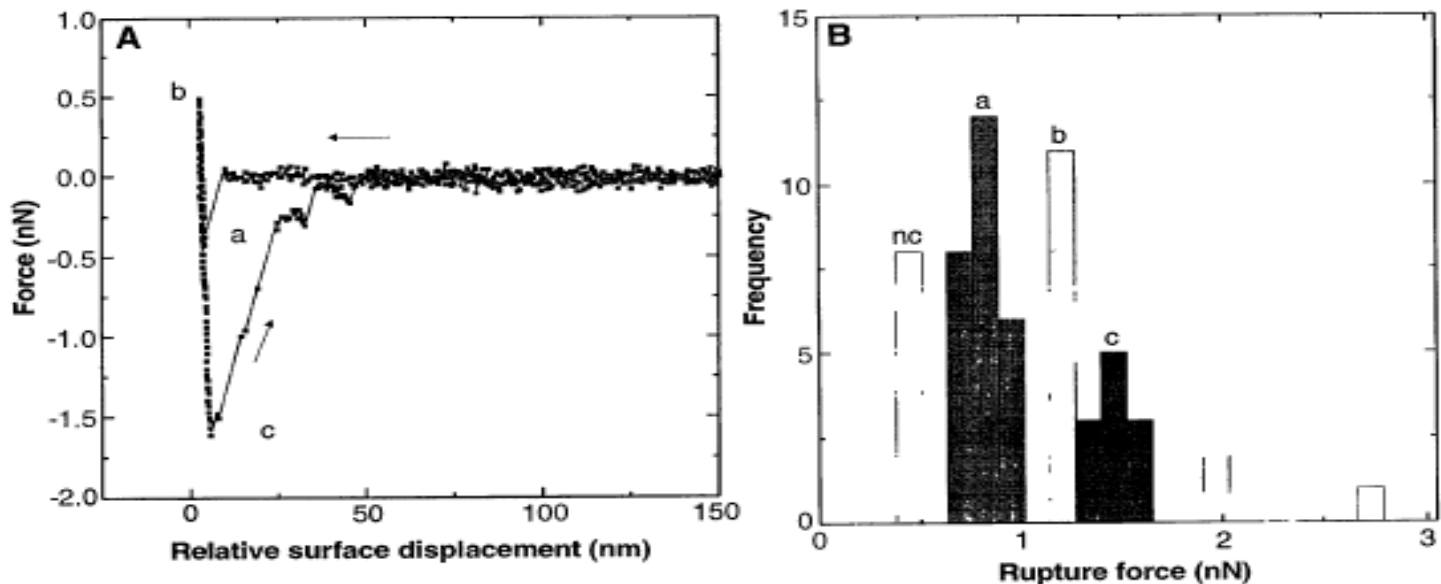


Fig. 2. (A) Force versus relative surface displacement measured between (ACTG)₅- and (CAGT)₅-functionalized surfaces in 0.1 N NaCl, pH 7.0, at 25°C (standard conditions). Points a, b, and c indicate the jump into contact, a region of repulsive force, and the jump away from contact, respectively. The rupture force, period, and length are 1.56 nN, 1.4 s, and 3 nm, respectively. Each data point (filled squares) on the curve represents 12 measurements taken for a \approx 10-ms period. The AFM used in this study was designed specifically for force measurements in liquid; an optical detection scheme (9) was used to measure both the deflection of the cantilever and the position of the surface. Although the theoretical limits of force detection imposed by the optical detector (24) and by thermal noise (25) are ± 0.002 nN and ± 0.001 nN, respectively, variations in the optical properties limit the precision of force measurements to ± 0.01 nN in practice. The instrument was operated in a variable-force mode in which the surface was ramped toward the probe at velocities of 10 to 0.1 nm/s until a repulsive force of ≈ 1 nN was sensed. Silica spheres, 60 to 120 μ m in diameter, were attached to silicone oxynitride-microfabricated cantilevers (8) with a chemically inert epoxy, and the spring constant of each cantilever was measured at the point of probe contact (6). **(B)** Histogram of the rupture forces measured for a single pair of surfaces over a 2-hour period. The distribution of rupture forces labeled nc is attributed to nonspecific surface forces. The distributions of rupture forces labeled a, b, and c are attributed to a single pair of oligonucleotides involving 12, 16, and 20 base pairs, respectively. The observation of adhesive forces greater than 2 nN is attributed to multiple molecular interruptions.

Further readings:

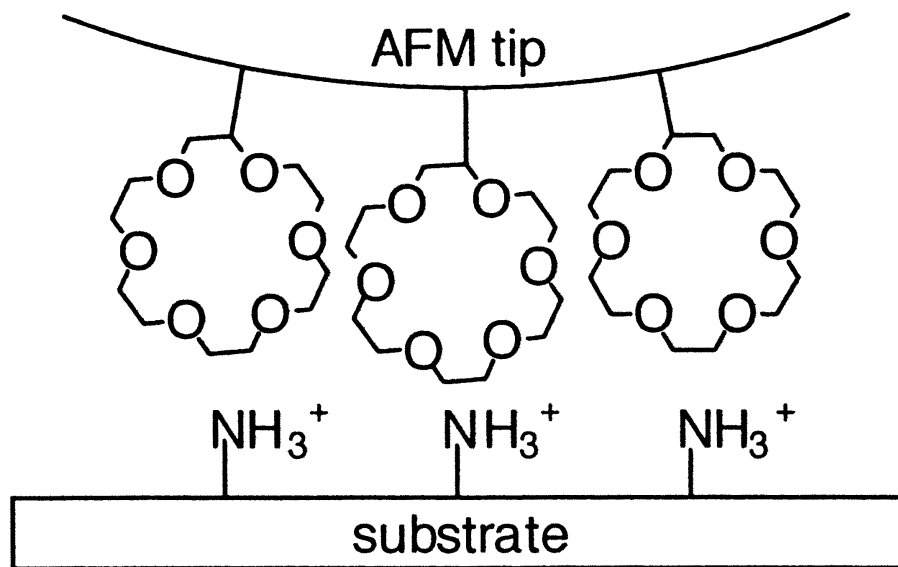
Protein interaction force measured by AFM

6. G. U Lee, D. A. Kidwell, R. J. Colton, *Langmuir* **10**, 354 (1994).
7. E.-L. Florin, V. T. Moy, H. E. Gaub, *Science* **264**, 415 (1994).

Single Complexation Force of 18-Crown-6 with Ammonium Ion Evaluated by Atomic Force Microscopy

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Direct Measurement of Interaction Forces between **Colloidal Particles** Using the Scanning Force Microscope

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Atomic force microscopy: A forceful way with **single molecules**

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Measuring Molecular Weight by Atomic Force Microscopy

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- Absolute-molecular-weight of cylindrical brush molecules were determined using a combination of the Langmuir Blodgett (LB) technique and Atomic Force Microscopy (AFM).
 - The LB technique gives mass density of a monolayer, i.e., mass per unit area, whereas visualization of individual molecules by AFM enables accurate measurements of the molecular density, i.e., number of molecules per unit area.
 - From the ratio of the mass density to the molecular density, one can determine the absolute value for the number **average molecular weight**.
 - The **length** distribution can be virtually identical to the **molecular weight** distribution.
 - The polymers used are four kinds of PBA brushes with different lengths.

Measurement procedure:

1. Prepare a solution of PBA (precise weighing of total mass);
2. Certain amount of solution poured into LB trough to form monolayer over water --- mass per unit area (m_{LB}) is known, where c is the concentration, V is the volume transferred, and S_{LB} is the area,

$$m_{LB} = c \cdot V / S_{LB} \quad (1)$$

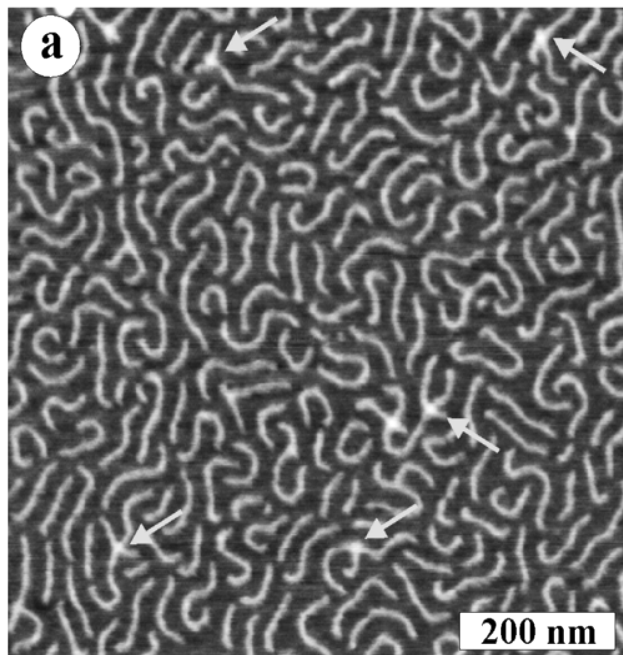
3. Transfer the monolayer onto a substrate for AFM measurement,
4. The area size changes after transfer, $S_{AFM} = S_{LB} / T$, T is the transfer ratio,
5. Molecule per unit area measured by AFM,

$$n_{AFM} = N / S_{AFM} \quad (2)$$

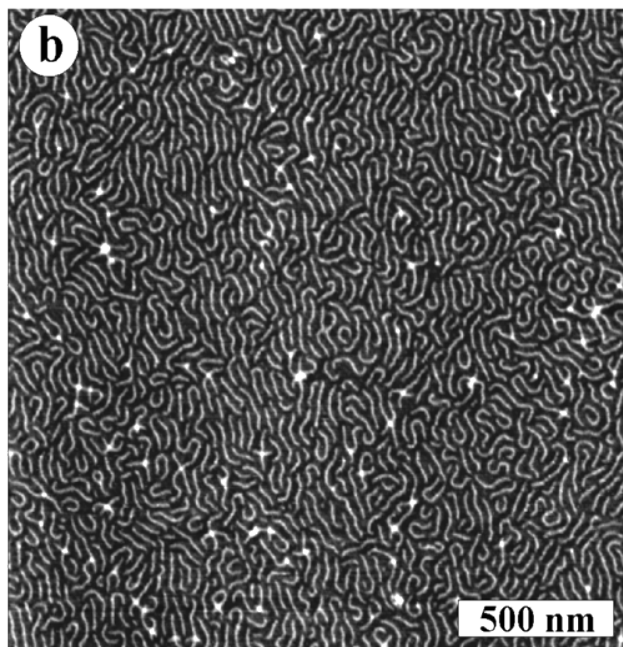
6. So, the averaged molecular weight M_n , where m_{am} is the atomic mass unit, 1.6605×10^{-24} g

$$M_n = \frac{m_{LB}}{n_{AFM}} \frac{T}{m_{am}} \quad (3)$$

Individual molecules of polymer B were clearly resolved by tapping mode AFM.



(a) The higher resolution AFM image demonstrates details of the molecular conformation including **crossing molecules** indicated by arrows. The larger scale image.



(b) demonstrates the **uniform coverage** of the substrate.

Table 2. Molecular Weights of PBA Cylindrical Brushes Determined by SLS, MALLS-GPC and the AFM-LB Methods

polymer	SLS		MALLS-GPC		AFM	
	$M_n^a 10^6$	$M_n^b 10^6$	M_w/M_n^c	$M_n^d 10^6$	L_n^e nm	L_w/L_n^f
A	1.1	0.8	1.39	0.8 ± 0.11	110 ± 8	1.24
B	1.4	1.6	1.54	1.5 ± 0.15	108 ± 6	1.33
C	2.5	2.4	1.39	2.5 ± 0.22	115 ± 5	1.24
D	3.9	4.7	1.46	4.0 ± 0.35	113 ± 5	1.20



Good agreement

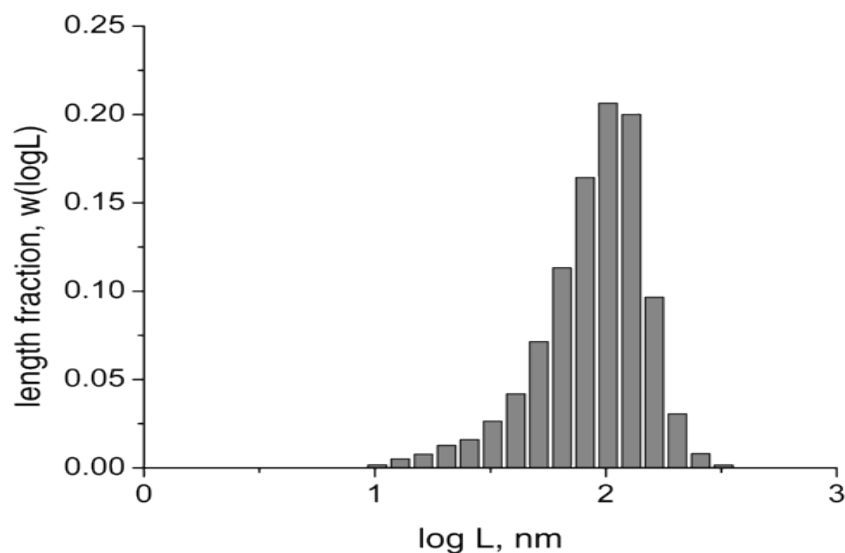
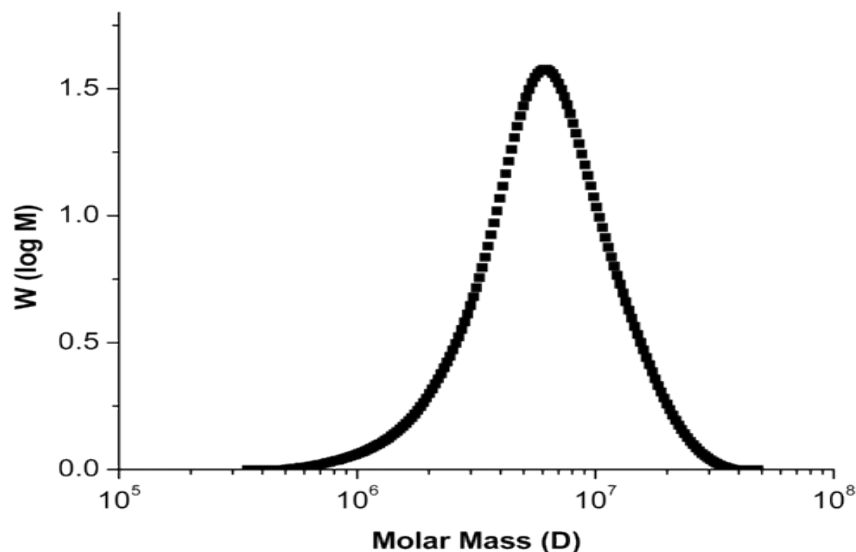
Static light scattering (SLS)

multi-angle laser light-scattering (MALLS)

gel permeation chromatography (GPC)

^e Number average length measured for an ensemble of 300 molecules with a statistical deviation of 5 nm.

^f Polydispersity index of the molecular length obtained from AFM images.



Comparison between molecular weight distribution and molecular length distribution measured by AFM for an ensemble of 3060 molecules.

- Statistics offers evaluation of the **homogeneity** of molecular weight;
- Also evaluates the linear chain structure of polymers --- **uniform vs. branched?**

Latest News

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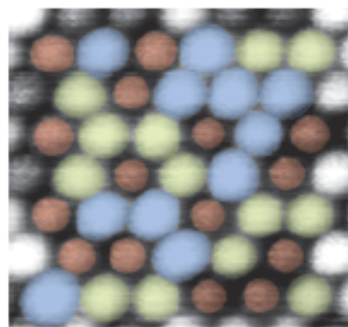
Atomic Force Microscopy

Individual Surface Atoms Identified

Technique fingerprints atoms at room temperature

Ron Dagani

YOU ARE BLINDFOLDED and presented with a tray filled with "marbles" of three different materials—glass, styrofoam, and gelat. Could you identify which marble is which simply by touch? Most people could do this quite easily.



© Nature 2007

Color coded Atomic force microscopy can identify the surface atoms of an alloy: silicon (red), tin (blue), or lead (pale green).

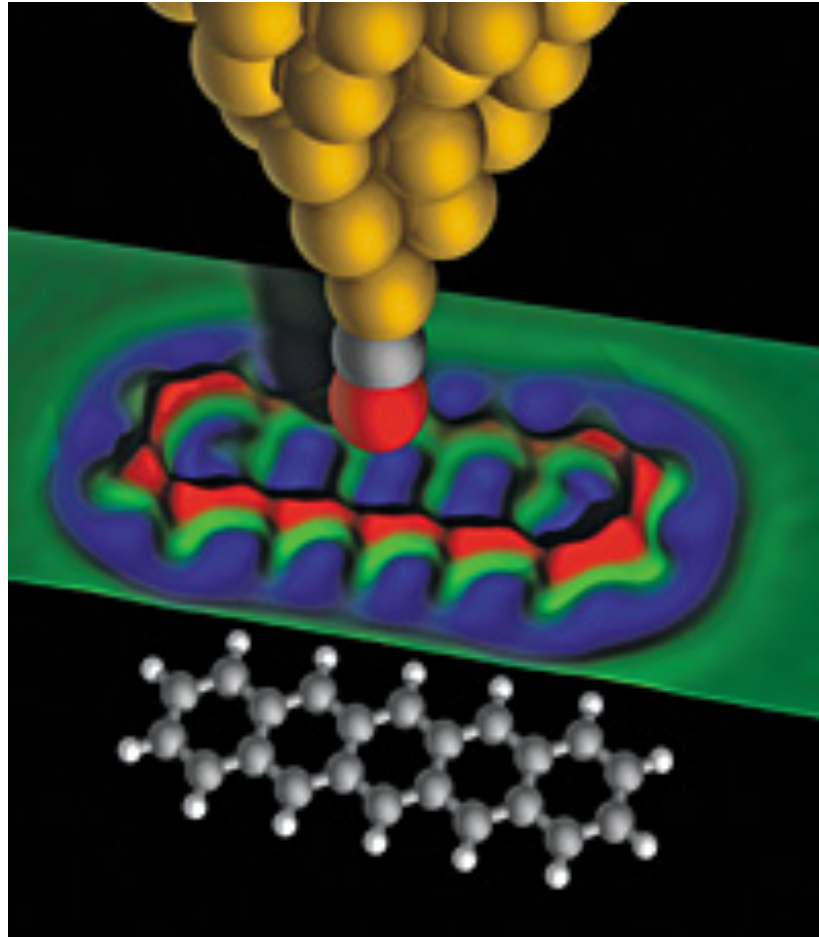
The nanoscale analog of this task—identifying different atoms on a surface—is much more difficult, but it now has been accomplished for the first time at room temperature, thanks to the exquisite touch of the atomic force microscope (AFM).

Scientists use the AFM to image and manipulate atoms and structures on a variety of surfaces. For imaging, the surface is scanned with the microscope's sharp, vibrating tip (a microscopic inverted pyramid), which is attached to a flexible cantilever. The atom at the apex of the tip "senses" individual atoms on the underlying surface when it forms incipient chemical bonds with them. Because these chemical interactions subtly alter the tip's vibration frequency, they can be detected and mapped.

Physicist Óscar Custance at Osaka University's Graduate School of Engineering, in Japan, and his colleagues now have exploited the short-range chemical forces acting between the AFM tip and surface atoms to distinguish between silicon, tin, and lead atoms on an alloy surface (*Nature* 2007, 446, 64).

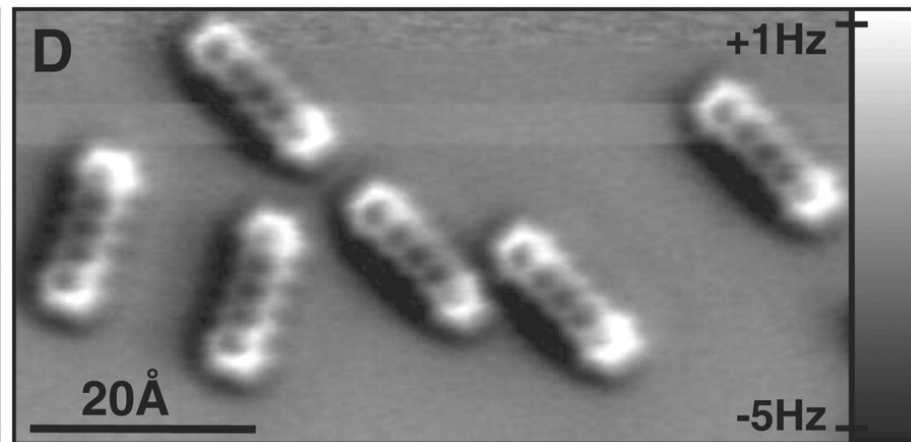
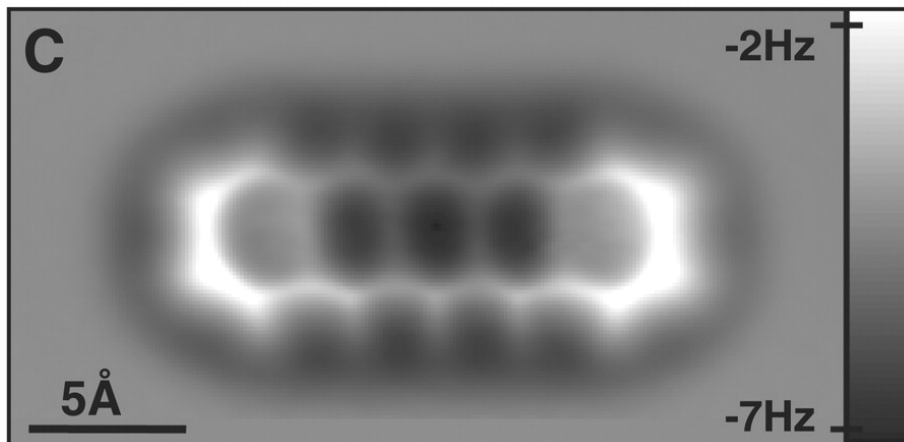
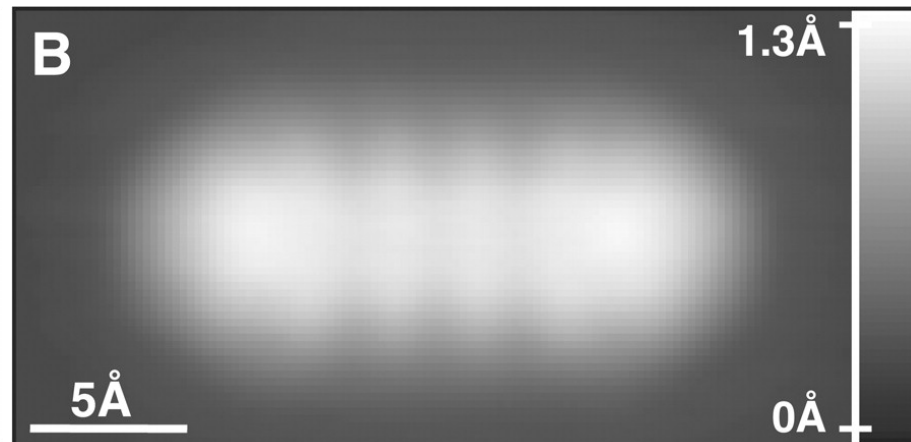
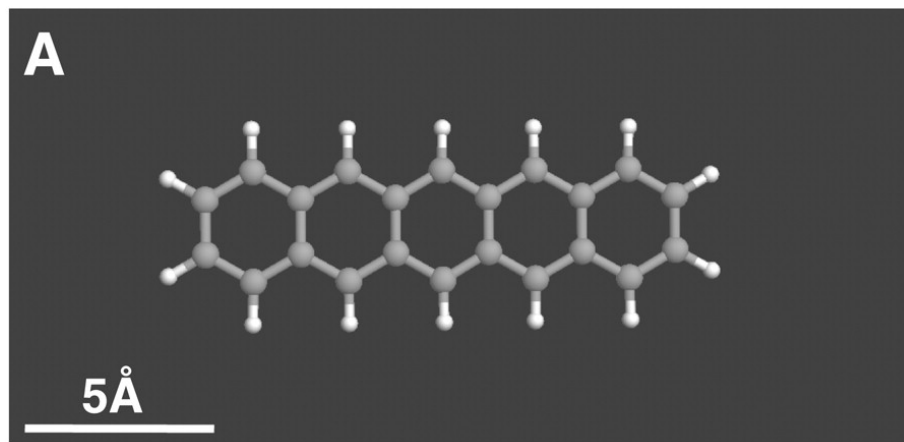


Molecule's Atoms, Bonds Visualized by AFM:
Enhanced tip resolution by attaching a CO molecule



Leo Gross, et al. *Science*, 28 August 2009: Vol. 325. no. 5944, pp. 1110 - 1114

CO-tip AFM image (C, D) reveals atoms and bonds of pentacene (A) on Cu(111), whereas conventional STM image (B) cannot. Scale bars are 5 Å.



Real-Space Identification of Intermolecular Bonding with Atomic Force Microscopy

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Also using CO-functionalized tip

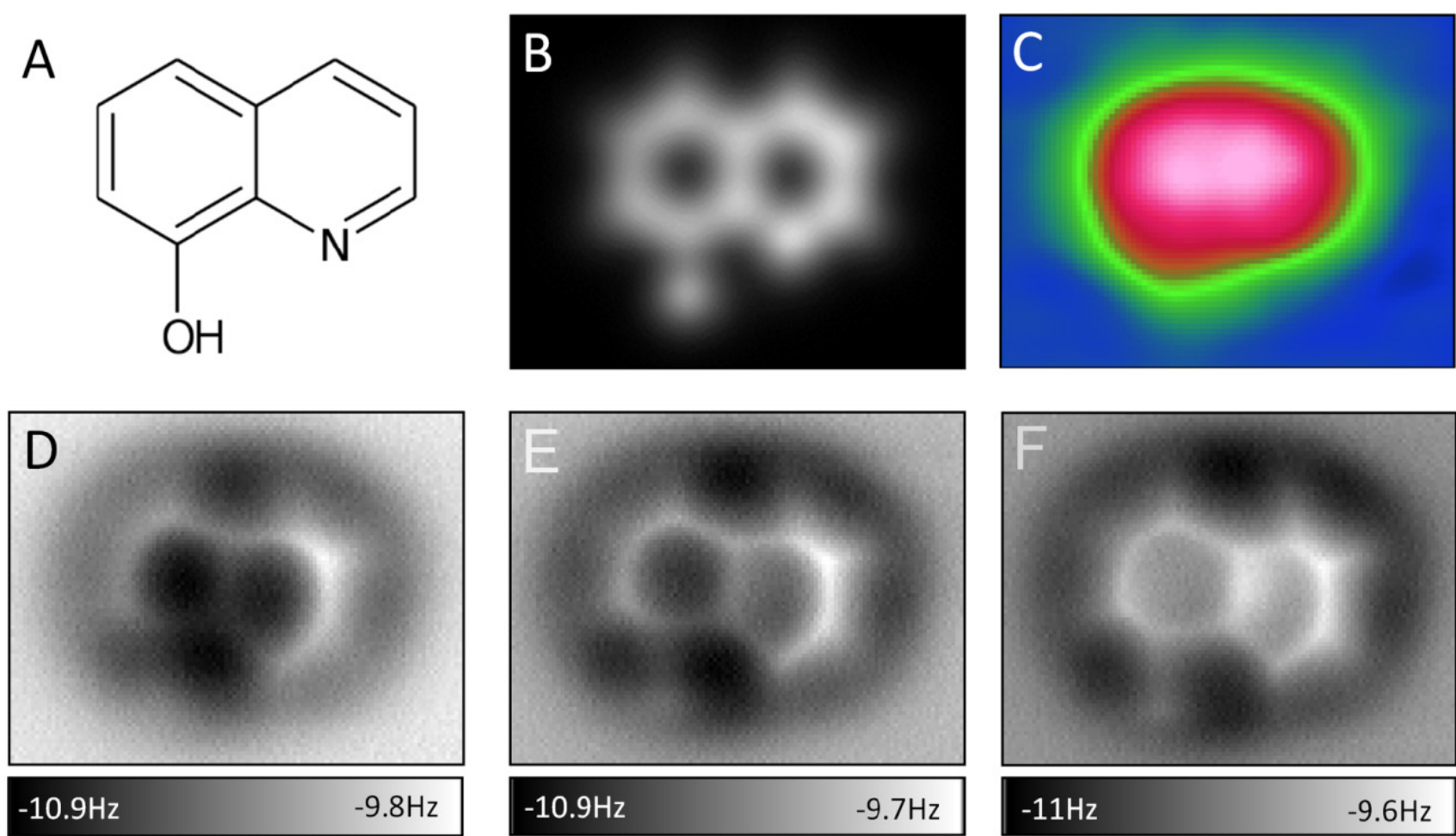


Fig. 1. SPM measurements and DFT calculations of single 8-hydroxyquinoline (8-hq) on Cu(111). (A) Chemical structure of 8-hq. (B) DFT-calculated molecular electron density maps at a distance of 150 pm above the molecule. (C) Constant-current STM topography image ($V = -100$ mV, $I = 100$ pA) with a CO-functionalized tip. (D to F) Constant-height AFM frequency shift images ($V = 0$ V, $A = 100$ p.m.) at different tip heights. The tip height Δz was set with respect to a reference height given by the STM set point above (-100 mV, 100 pA) the bare Cu(111) substrate in the vicinity of the molecule. The plus (minus) sign means the increase (decrease) of tip height. (D): $\Delta z = +30$ p.m.; (E): $\Delta z = +10$ p.m.; (F): $\Delta z = 0$ pm. The size of all images is 1.3 nm \times 1.0 nm.

