# Reports

## Images of the DNA Double Helix in Water

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**The scanning tunneling microscope can image uncoated DNA submerged in water. The grooves of the double helix were clearly resolved in images of the 146–base pair fragment extracted from calf thymus nucleosome. In contrast to images obtained with dry DNA, the helix pitch varied only a small amount (36 ± 5 angstroms). The path of the helix shows considerable variation. It is quite straight when the molecules are densely packed, but it curves and bends in isolated molecules.**

THE SCANNING TUNNELING MICROscope (STM) has been used by a number of workers to obtain images of uncoated, dry DNA in air (*1, 2*). However, the hydration layer is an important part of the structure of the double helix (*3*), and we have imaged DNA with an STM operated in water in order to maintain the hydration of the molecule (*4*). We have achieved resolution adequate to observe the pitch of the double helix (*5*). There are two new points we wish to make: (i) in contrast to the results of Beebe *et al.* (*2*), we find that the helix pitch varies relatively little, and (ii) we observe remarkable variations (bending and curving) of the overall path of the backbone, which depend strongly on the packing density of the fragments. By operating the experiment in water, we can use electrochemistry to control the packing of the sample (*5*). We illustrate these points here with images of the 146-bp fragment obtained from the nucleosome (*6*).

We embedded the samples at the interface between a crystalline gold surface and an electrolyte by using Faradaic deposition of the anion component of a buffer salt, carried out so as to avoid known reactions with DNA (*4, 5*). Electrochemical control of the desposition gave a fairly uniform deposit at the interface, so that the experiments were quite reproducible (*7*).

The source of the nucleosomes used in this work is calf thymus (*6*), so the material is identical to that used in our earlier studies (*4, 5*), with the important difference that it is much shorter and very uniform in length [~500 Å (*6*)]. The present data were obtained by the methods we have described elsewhere (*5*), except for improvements in the tips and substrates. The platinum tips are insulated with Apeizon wax (*8*), and the substrates are gold that was epitaxially deposited onto mica. In control experiments, the substrates were found to be flat to within a few atomic steps over micrometer distances. The microscope is a prototype of a commercial product designed for electrochemistry studies (*8*). It was calibrated and linearized with a gold-coated optical diffraction grating. We operated with a tip bias of −60 mV and a tunnel current of 100 to 200 pA (*9*).

At high concentrations of DNA (≥100 μg/ml), dense aggregates predominate. An example of such an aggregate is shown in Fig. 1A. It consists of bundles of a characteristic 1000 Å size, which are composed of sheets of the 500 Å fragments stacked side by side. The individual molecules can be discerned in the sheet protruding into the center of the picture from the large aggregate on the left. High-resolution images of such tight aggregates show that the individual molecules pack as straight rods with a very regular helix pitch [a high-resolution

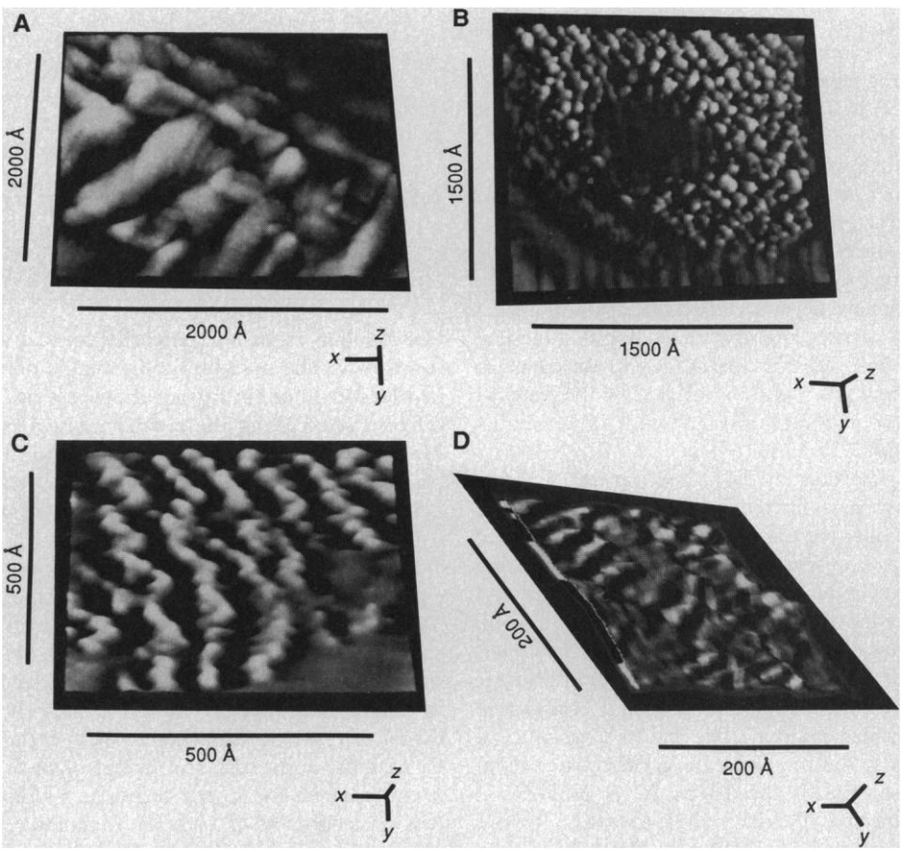


**Fig. 1.** Images of 146-bp DNA embedded on a gold surface under water; height information is encoded in the gray scale [white = +30 Å, but see (*9*)] and in the perspective projection (the viewing angle is illustrated by a projection of the *xyz* coordinate system at the bottom right of each image—the substrate is the *xy* plane). (**A**) A 2280 Å by 2280 Å region of dense aggregate. (**B**) A 1830 Å by 1830 Å area of more loosely packed DNA, surrounding a ~600 Å diameter "mesa" on the gold substrate. (**C**) A 620 Å by 620 Å area showing three complete fragments in the left side of the image. Images A, B, and C have been smoothed to remove features finer than ~15 Å. In (**D**), fine structure has been retained in a 246 Å by 246 Å region, aligned to give a nearly side-on view of a molecule that crosses from the upper-left to lower-middle regions. The image has been Fourier-filtered with a passband of $5 \times 10^{-2}$ Å$^{-1}$ to $1 \times 10^{-3}$ Å. Local variations have been enhanced by setting the gray scale with a Laplacian-filtered version of the image.

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image of a dense aggregate is given in (*5*)]. At somewhat lower concentrations (~10 μg/ml), a number of low-density patches form (Fig. 1B). The "popcorn" texture comes from the modulation due to the helix pitch (Fig. 1B is oriented so as to emphasize this). The helix pitch in most of the samples of random-sequence DNA that we have measured is 36 Å. The uncertainty in this number varies with the nature of the adsorbate being imaged. Densely packed aggregates with two-dimensional (crystal-like) order allow very accurate measurements to be made (particularly from the Fourier-transformed image) so that the uncertainty is ~0.1 Å. In loose aggregates, the uncertainty is far greater because of fluctuations in the helix direction (~0.5 Å). The helix is far less regular when the samples are dried. A larger range of pitches is seen in synthetic nucleic acids. For example, RNA (in what is probably A form) has a pitch of ~30 Å, whereas poly(dGC) · poly(dCG) (which may be Z form) has a pitch greater than 40 Å. Important exceptions to these results may occur near gross structural features (such as kinks). It becomes very difficult to define a pitch near regions of large structural fluctuation.

A close-up image showing three complete 146-bp fragments is shown in Fig. 1C (lower left to middle—the middle two fragments are in contact at their top end). Each shows structure compatible with 15 roughly full turns of the double helix along their length, but the helix meanders considerably. Since we have never seen such variation in images of dense aggregates of high molecular weight calf thymus DNA (*5*), we conclude that the structure is affected by the packing, a conclusion consistent with other studies (*10*).

The data in Fig. 1, A through C, were low pass–filtered to smooth out features smaller than about 15 Å. We have retained this fine structure in Fig. 1D (emphasizing it with a curvature-keyed gray scale and choice of perspective). It is almost a side-on view of a strand that crosses from the upper-left to lower-middle regions of the image (two other partially obscured strands are visible behind it). The regular striations that cross the molecule are approximately consistent with the 15 to 20 Å modulation expected for the minor to major groove variation in B-DNA (*3*). However, we do not consider the data reliable at this level of resolution because of the strong interaction between the tip and molecule, and we expect that the features are distorted in a complex way that depends on the angle of approach between tip and molecule, as well as the "local" deformability of the molecule (*5*).

Better control of the interaction between the tip and molecule is obtained with the atomic force microscope (AFM). Recent AFM experiments demonstrate what we believe to be the most important advantage of operation in water, which is the ability to monitor bimolecular reactions as they proceed (*11*). Finally, we note that DNA extracted from nucleosomes is probably not random sequence (*12*). We have observed recurring chain configurations that may in fact be sequence directed.


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7. The DNA was dissolved to a concentration of a few tens of micrograms per milliliter in a 20 m*M* tris buffer containing 10 m*M* sodium acetate, adjusted to *p*H 7.5 with HCl. The plating electrode was held at −2 V for 2 to 3 min.
8. Available from Angstrom Technology, 1815 West First Avenue, Mesa, AZ.
9. These tunnel currents are somewhat less than those used in (*5*). The contrast in the images presented here appears to be positive, but careful inspection of a trace over a DNA molecule shows both positive and negative contributions (the negative contribution appearing as the black shadow beside each "molecule," most obvious in Fig. 1C). The contrast arises from deformation of the tip, substrate and molecule, as well as from hysteretic effects associated with the servo response, as explained in (*5*). The apparent height (of about 30 Å) should not be interpreted literally.
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# Mammal-Like Dentition in a Mesozoic Crocodylian


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**Crocodylian teeth are generally conical with little differentiation in shape along the tooth row. The mandible is incapable of any fore-aft movement, and feeding typically involves little or no intraoral processing. Complex, multi-cusped, mammal-like teeth differentiated along the tooth row have been found in a Cretaceous crocodylian from Malawi. The morphology of the teeth and mandible indicates that food items were processed by back-to-front (proal) movement of the mandible, unlike living crocodylians but as in some mammals and *Sphenodon* (the tuatara).**


ALTHOUGH LIVING CROCODYLIANS display a variety of skull shapes ranging from the long, tubular snout of the Indian gharial to the broad, flat snout of the alligator, there is little diversity in dental morphology. Indeed, nearly all dental diversity so far observed within the Crocodylia, both fossil and living, involves lateral compression or the degree of bluntness or slenderness of teeth (*1*). Mandibular movement during feeding is also quite uniform among living crocodylians, consisting mainly of prolonged closure of the mouth on the prey (*2*). Unlike that of most other diapsid amniotes (for example, lizards and birds), the crocodylian skull is akinetic, and the mandible does not move backward or forward when it is adducted. The discovery of a fossil crocodylian with an extremely heterodont dentition including multicusped teeth and a mandible that moved fore and aft is therefore worthy of note.

The specimens of this new crocodylian were collected in 1984 and 1987 in northern Malawi, southeast Central Africa, from beds mapped as Lower Cretaceous (*3*). They include five partial skulls with lower jaws and several isolated teeth (*4*). All the skulls


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