ogy. A chemically layered mantle is a distinct possibility.

REFERENCES AND NOTES

1. E. Anders and M. Ebihara, *Geochim. Cosmochim. Acta* **46**, 2363 (1982).
2. D. L. Anderson and J. Bass, *Nature* **320**, 321 (1986).
3. H. H. Breneman and E. C. Stone, *Astrophys. J.* **299**, L57 (1985). These results are based on solar energetic particles (SEP) which generally agree with recent spectroscopic values, as reviewed by J.-P. Meyer, *Astrophys. J. Suppl. Ser.* **57**, 173 (1985).
4. D. L. Anderson, *Annu. Rev. Earth Sci.* **5**, 179 (1977).
5. E. Ohtani and A. E. Ringwood, *Earth Planet. Sci. Lett.* **71**, 85 (1984).
6. R. Jeanloz and T. Ahrens, *Geophys. J. R. Astron. Soc.* **62**, 505 (1980).
7. A. Dziewonski and D. L. Anderson, *Phys. Earth Planet. Inter.* **25**, 297 (1981).
8. The average composition of the terrestrial upper mantle is unknown. It is usually estimated from the compositions of peridotites or lherzolites (olivine- and orthopyroxene-rich rocks) that are brought to the surface as accidental inclusions in basalts and kimberlites or by tectonic and erosional processes. However, these are samples of only the shallow mantle (<200 km), and may not even be representative of this region. There is no reason to suppose that they are representative of the whole upper mantle (<650 km). In some scenarios of mantle differentiation and evolution, the shallow mantle represents the more buoyant products (FeO- and Al_2O_3-poor) of accretional melting and differentiation; for example, D. L. Anderson, *Science* **213**, 82 (1981).

   It is often asserted that mantle xenoliths must be representative of the entire mantle, or at least the entire upper mantle, because the mantle convects in some manner and this will tend to homogenize the upper mantle. Convection, however, is different from stirring. Light melts clearly separate from dense crystals, even in a convecting mantle, and a two- or three-layer mantle may also convect without homogenization. It is quite possible, and seems probable from seismic tomography, that the major basalt reservoir in the mantle is much deeper than the region from which lherzolite xenoliths have been plucked. It is also possible that the lower mantle is chemically distinct and isolated from the upper mantle reservoirs (*2*).
9. R. Butler and D. L. Anderson, *Phys. Earth Planet. Inter.* **17**, 147 (1978); R. Jeanloz and E. Knittle, *Adv. Phys. Geochim.* **6**, 275 (1986).
10. E. Ito, E. Takahashi, Y. Matsui, *Earth Planet. Sci. Lett.* **67**, 238 (1984).
11. The composition of the Martian and lunar mantles are estimated from their densities, which in turn are estimated from the observed radii, mean densities, and moments of inertia of these bodies. In the case of the moon, there is additional information available from the composition of lunar rocks. Pressures are low in these bodies, and unknown properties of high-pressure phases do not complicate the interpretation of chemistry from the density. Corrections for temperature are also small. There is also seismic data for the lunar interior, which has been interpreted in terms of a high FeO content; D. L. Anderson, *J. Geophys. Res.* **77**, 789 (1972); H. Wänke and G. Dreibus, in *Tidal Friction and the Earth's Rotation II*, P. Brosche and J. Sundermann, Eds. (Springer-Verlag, Berlin, 1982), pp. 322–334; M. J. Drake, in *Origin of the Moon*, W. K. Hartmann, R. J. Phillips, G. J. Taylor, Eds. (Lunar and Planetary Institute, Houston, 1986), pp. 105–124.
12. G. Dreibus and H. Wänke, *Meteoritics* **20**, 367 (1985).
13. D. L. Anderson, *Geophys. Res. Lett.* **7**, 101 (1980).
14. Piclogite is a gt- and cpx-rich rock with less than 50% olivine that can yield basalts by partial melting (*2, 15*).
15. D. L. Anderson, *J. Geophys. Res.* **93**, 4688 (1988); T. Duffy and D. L. Anderson, *ibid.*, in press.
16. A. E. Ringwood, *Composition and Petrology of the Earth's Interior* (McGraw-Hill, New York, 1975).
17. The high gradient of seismic velocities between 400 and 500 km depth may be a result of the formation of a garnet-structured solid solution of $(Mg,Fe)_3Al_2Si_3O_{12}$-garnet and diopside (*17, 18*). This transformation is complete by about 400 km if the diopside/gt ratio of the mantle is as low as that for MORB (*19*). Irifune and his colleagues (*19*) used the low transformation pressure of diopside to argue against a deeper transformation, but they did not account for the dependency on the diopside/gt ratio and the effect of orthopyroxene. Orthopyroxene-bearing rocks with high cpx/gt have cpx stable to depths in excess of 400 km.
18. D. L. Anderson and J. D. Bass, *Geophys. Res. Lett.* **11**, 637 (1984).
19. T. Irifune, T. Sekine, A. E. Ringwood, W. O. Hibberson, *Earth Planet. Sci. Lett.* **77**, 245 (1986); T. Irifune and A. E. Ringwood, in *High Pressure Research in Mineral Physics*, M. Manghnani and Y. Syono, Eds. (Terra Scientific, Tokyo, Japan, 1987), p. 231.
20. H. C. Nataf, I. Nakanishi, D. L. Anderson, *Geophys. Res. Lett.* **11**, 109 (1984); D. L. Anderson, in *Magmatic Processes*, B. O. Mysen, Ed., (Spec. Publ. No. 1, Geochemical Society, University Park, PA, 1987), pp. 3–12.
21. H. Palme and K. G. Nickel, *Geochim. Cosmochim. Acta* **49**, 2123 (1985).
22. D. L. Anderson, *Episodes* **1980**, 3 (1980); *Geophys. Res. Lett.* **6**, 433 (1979); *J. Geophys. Res.* **84**, 6297 (1979); *Science* **223**, 347 (1984); see also (*12, 17, 18*).
23. ———, *Earth Planet. Sci. Lett.* **57**, 1 (1982); *Adv. Earth Planet. Sci.* **12**, 301 (1982).
24. S. Maaloe and K. Aoki, *Contrib. Mineral. Petrol.* **63**, 161 (1977).
25. F. R. Boyd, J. J. Gurney, S. H. Richardson, *Nature* **315**, 387 (1985).
26. D. L. Anderson, *J. Geophys. Res.* **84**, 7555 (1979).
27. N. T. Arndt and E. G. Nisbet, Eds., *Komatiites* (Allen and Unwin, Boston, 1982).
28. K. Frederiksson and J. Kerridge, *Meteoritics* **23**, 35 (1988).
29. S. R. Taylor, *Planetary Science: A Lunar Perspective* (Lunar and Planetary Institute, Houston, 1982).
30. J. Morgan and E. Anders, *Geochim. Cosmochim. Acta* **43**, 1601 (1979).

31. Supported by NSF grant EAR-85-09350. Contribution No. 4705, Division of Geological and Planetary Sciences.

12 September 1988; accepted 31 October 1988

Direct Observation of Native DNA Structures with the Scanning Tunneling Microscope

THOMAS P. BEEBE, JR., TROY E. WILSON, D. FRANK OGLETREE, JOSEPH E. KATZ, ROD BALHORN, MIQUEL B. SALMERON, WIGBERT J. SIEKHAUS

Uncoated double-stranded DNA dissolved in a salt solution was deposited on graphite and imaged in air with the scanning tunneling microscope (STM). The resolution was such that the major and minor grooves could be distinguished. The pitch of the helix varied between 27 and 63 angstroms in the images obtained. Thus the STM can be useful for structural studies of a variety of uncoated and isolated biomolecules.

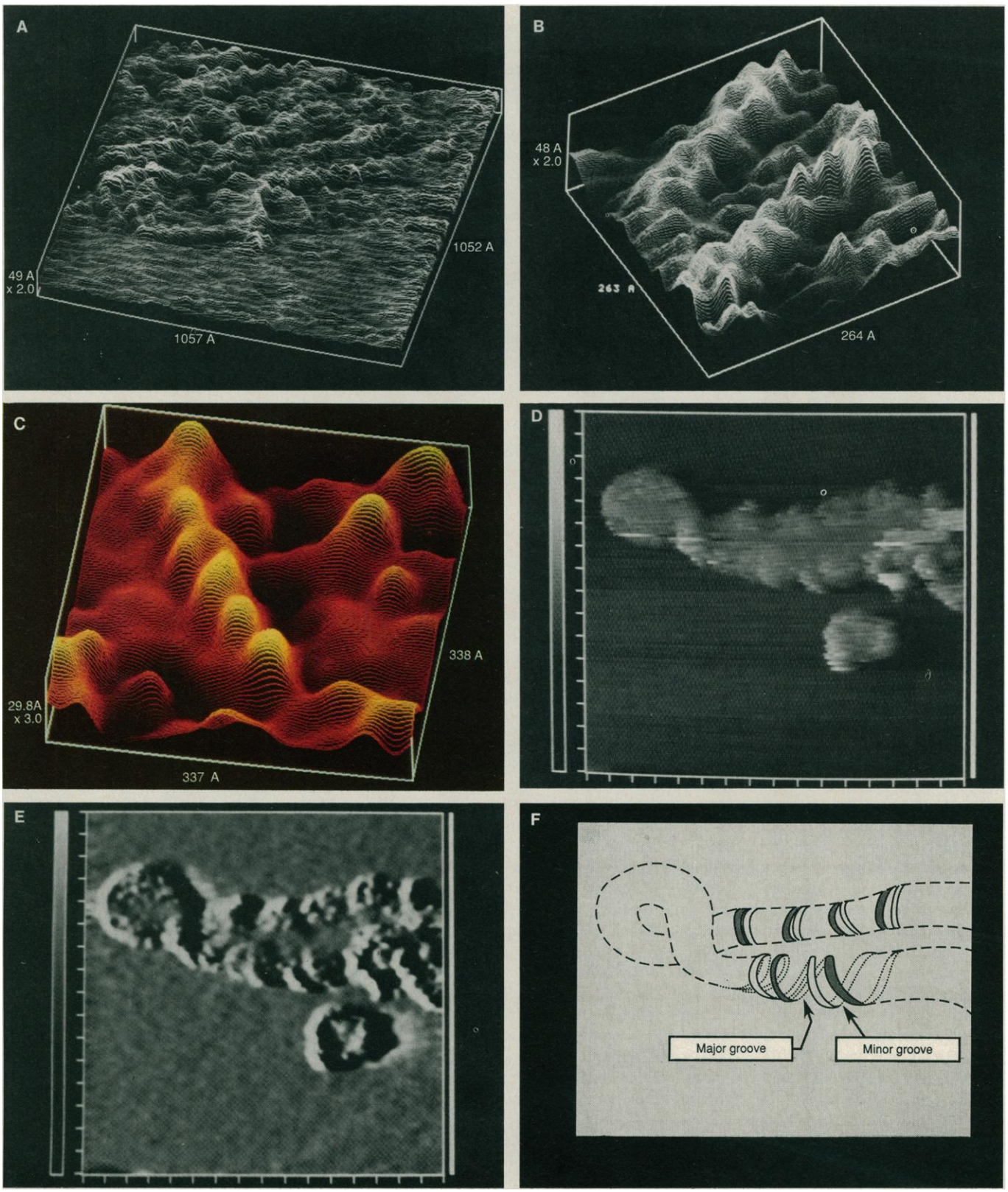
THE STM HAS BEEN USED TO STUDY the atomic structure of many surfaces other than metals and semiconductors (*1*). Organic, inorganic, and biological molecules have been imaged, both in air and under a variety of other media. These samples include, among others, bacteriophage virus particles (*2*), metal-shadowed recA-DNA complexes (*3, 4*), native closed circular DNA (*4*), and other smaller organic molecules (*5*). These early studies are promising because it was shown that the advantages of the STM [simplicity of operation, low cost, angstrom resolution, both laterally (x and y directions) and normal to the

T. P. Beebe, Jr., T. E. Wilson, W. J. Siekhaus, Lawrence Livermore National Laboratory, L-357, Department of Chemistry, Livermore, CA 94550.
D. F. Ogletree and M. Salmeron, Lawrence Berkeley Laboratory, MS 66-212, Center for Advanced Materials, Berkeley, CA 94720.
J. E. Katz, Lawrence Berkeley Laboratory, MS 70A-3363, Electronics Engineering Department, Berkeley, CA 94720.
R. Balhorn, Lawrence Livermore National Laboratory, L-452, Biomedical Sciences Division, Livermore, CA 94550.

Fig. 1. (Facing page) DNA deposited on graphite and imaged in air with the STM. All of the images were obtained at constant tunnel current (topographic images). (**A**) Sample area, 1050 Å by 1050 Å; total height, 49 Å; sample bias, −155 mV; current setpoint, 0.9 nA; gap resistance, 172 MΩ; acquisition time, 210 s, (tip velocity of 1300 Å/s). Postacquisition image processing consisted of digital bandpass filtering with the removal of all Fourier components $>9.72 \times 10^{-2}$ Å^{-1} and $<2.43 \times 10^{-3}$ Å^{-1}. Variation of these values within reasonable ranges caused no significant changes in the images. The image is presented in a projected three-dimensional format with gray scale, as viewed from a perspective 45° above the plane, and 20° clockwise in the plane. (**B**) Magnified view of center of (A), under the same conditions, area 260 Å by 260 Å; total height range 48 Å; viewing perspective is 45° above the plane and 30° counterclockwise in the plane. (**C**) Area 340 Å by 340 Å; total height range, 29 Å; sample bias, −155 mV; current setpoint, 0.8 nA; gap resistance, 194 MΩ; viewing perspective is 45° above the plane and 20° clockwise in the plane. (**D** and **E**) Area 400 Å; total height range, 132 Å; sample bias, −97 mV; current setpoint, 3.3 nA; gap resistance, 29 MΩ; acquisition time 42 s (tip velocity of 2500 Å/s). (D) Raw image. (E) Postacquisition image processing consisted of nine point two-dimensional smoothing applied once, followed by simulated light source shading from a point 15° above the plane. (**F**) Schematic of the DNA structure in (D) and (E).

surface (z direction), and imaging in a wide variety of environments, including air or liquids] can be brought to bear on structural questions in the biological field, even if the nature of the electron energy levels of the organic molecule that participate in the tunneling process is not known or understood. Also, the STM does not require sample preparation conditions as harsh as those needed for most conventional electron microscopy techniques (vacuum conditions and conducting coatings).

We present high-resolution observations of DNA structures with the STM in native (non–metal-shadowed) specimens. In these



images both the major and minor grooves of double-helix DNA were resolved. In addition, the distances between these grooves have been measured.

We have constructed an STM based on an earlier design (*6*). Our STM used a micrometer-driven differential spring mechanism for sample movement and a tubular piezo rather than a tripod to scan the tip. We used mechanically cut tips consisting of a 60% Pt, 40% Rh alloy. The particular conditions of tunneling are typically 150-mV sample bias (either polarity) and few nanoamperes tunneling current. No difference in the images was observed as a function of these parameters.

Solutions of calf thymus DNA were prepared as described in (*7*). A droplet of the aqueous solution (1 mg of DNA per milliliter in 10 m*M* KCl) was allowed to evaporate in air on a freshly cleaved, highly ordered pyrolytic graphite (HOPG) substrate, which provides a conductive surface with atomically flat crystal planes over thousands of angstroms. In the images we present, tunneling was initiated immediately after the last amounts of water were observed to evaporate. Thus the DNA was not subjected to particularly harsh or intricate sample preparation procedures. Sample blanks consisting of 10 m*M* KCl solutions subjected to identical treatments, but that contained no DNA, showed no topographic structure in the STM images other than the expected atomic features and occasional steps that are characteristic of cleaved graphite surfaces.

Some of the problems encountered with DNA prepared in this way included clumping of the DNA into large unrecognizable aggregates that did not give stable tunneling conditions, and the other extreme, that of not being able to find any DNA molecules within the scanning range of our microscope (our largest scans were 0.5 μm by 0.5 μm). Movement or displacement of the DNA, although occasionally observed, was not found to be a problem in these images. The complete evaporation of the DNA solution commonly left a 4-mm area on the graphite surface consisting of a series of concentric rings due to the evaporation process and the salts that the shrinking droplet left behind. In the center of this area there was usually a 1-mm spot in which the last amounts of salt precipitated out of solution. This region was avoided because of the unstable tunneling conditions caused by the thick layer of nonconducting salt.

An image constructed from the topographic contours followed by the STM tip as it traversed the DNA adsorbed on the graphite, while the feedback control electronics maintained a constant tunneling current, is shown in Fig. 1A. This image is typical of our large-area images, in that it shows a double-stranded DNA molecule (DNA duplex) that makes many convolutions on the surface, and consists both of segments that are isolated from the rest of the duplex, as well as segments in which there are overlapping and apparently tangled DNA duplexes. The periodic bumps along the duplex occur with a range of spatial periodicities from 27 to 50 Å and correspond to the helix pitch. The observed DNA structures were stable and reproducible from scan to scan. Magnified views of different regions on the sample are shown in Fig. 1, B and C. In Fig. 1B two DNA duplexes lie nearly parallel on the substrate; the pitch periodicities range from 28 to 50 Å, with an average over nine measurements along the duplex corresponding to approximately 36 Å. The appearance of a twisted ladder is seen here, although no alternation in groove size is apparent, as would be expected if the major and minor grooves were resolved. In Fig. 1C, which is taken from another sample area, a right-handed DNA duplex is shown in greater detail. Four measurements of the DNA pitch taken along this particular length range from 46 to 52 Å, with an average of 49 Å. The apparent width of the DNA, which seems large in this image (approximately 60 Å), is actually a result of the finite size of the tip. This problem becomes more pronounced for structures with a large relief from the substrate, as tunneling from the side of the tip is possible. The apparent width is thus a convolution of the actual DNA structure with that of the tip. The height above the substrate (that is, change in the z coordinate) is not affected in the same way, and this value is typically 20 to 30 Å, which is near the expected value.

Although the images in Fig. 1, A through C, were obtained from different regions of the sample and were acquired only minutes apart in time, the pitch periodicity differed by 37%. The hydration state of the DNA is only one factor that determines its molecular conformation. Since the conformation varies greatly even for samples that have had nearly identical dehydration times, other forces, perhaps surface-molecule interactions, are probably responsible for these observed structures.

In Fig. 1, D and E, an image is shown in which higher resolution was achieved that is typical of other images we have obtained (D is the raw image and E is processed). The DNA duplex as imaged enters the figure in the upper right corner of the image, makes a loop and crosses over itself in the upper left corner of the image, and leaves the image in the upper right corner (there is an unresolved structure below this, possibly a DNA fragment). A schematic image is shown in Fig. 1F. A long-short alternation occurs in the spacing of the ridges in the DNA duplex. These ridges are due to the phosphodiester backbone of DNA, which is composed of alternating deoxyribose sugar and phosphate groups. In Fig. 1C only the major groove could be observed, and each bump is due to the periodic double-stranded helix separated by the major grooves. In Fig. 1, D and E, where the resolution is higher, the additional structure due to the major-minor groove alternation is seen. The distance of one major-minor pair is approximately 63 Å across the bottom duplex of the image, and 49 Å across the top duplex of the image. On average, this corresponds approximately to a 55% length expansion compared with the crystalline state. DNA may undergo length expansions by 25 to 50% when intercalated with ionic species in solution (*8*). The DNA molecules have been subjected to the combined forces of the surface, dehydration, and possibly intercalation with ionic species, so that we are not surprised that the pitch periodicities vary as they do here.

REFERENCES AND NOTES

1. P. K. Hansma and J. Tersoff, *J. Appl. Phys.* **61**, R1 (1987).
2. A. M. Baro *et al.*, *Nature* **315**, 253 (1985).
3. M. Amrein *et al.*, *Science* **240**, 514 (1988).
4. G. Travaglini *et al.*, *Surf. Sci.* **181**, 380 (1987).
5. H. Ohtani, R. J. Wilson, S. Chiang, C. M. Mate, *Phys. Rev. Lett.* **60**, 2398 (1988); J. K. Gimzewski, E. Stoll, R. R. Schlitter, *Surf. Sci.* **181**, 267 (1987); P. H. Lippel and R. J. Wilson, *Proceedings of the Third International Conference on STM* (Oxford Univ. Press, Oxford, in press); T. Sleator and R. Tycko, *Phys. Rev. Lett.* **60**, 1418 (1988); J. E. Frommer and J. S. Foster, *Nature* **333**, 542 (1988); L. Eng *et al.*, *J. Vac. Sci. Technol.* **A6**, 358 (1988); C. A. Lang, J. K. H. Horber, T. W. Hansch, W. M. Heckl, H. Mohwald, *ibid.* p. 368; J. A. N. Zasadzinski, J. Schneir, J. Gurley, V. Elings, P. K. Hansma, *Science* **239**, 1013 (1988).
6. M. Salmeron, D. S. Kaufman, B. Marchon, S. Ferrer, *Appl. Surf. Sci.* **28**, 279 (1978). A modified version of this microscope is commercially available from McAllister Technical Services, Berkeley, CA.
7. Highly polymerized calf thymus DNA (Worthington Biochemical Corp., Freehold, NJ) was dissolved overnight in aqueous 10 m*M* KCl at a concentration of 4.5 mg/ml. Aliquots of this stock solution were subsequently diluted with 10 m*M* KCl to a working concentration of 1 mg/ml.
8. C. R. Cantor and P. R. Schimmel, in *Biophysical Chemistry. Part III: The Behaviour of Biological Macromolecules* (Freeman, New York, 1980), p. 1252; W. K. Olson, in *Nucleic Acids: Geometry and Dynamics*, R. Sarma, Ed. (Pergamon, New York, 1980), p. 383.

9. Supported by the Lawrence Livermore National Laboratory under contract number W-7405-ENG-48, and by an LLNL Institutional Research & Development grant. Also supported by the Lawrence Berkeley Laboratory through the Director, Office of Energy Research, Office of Basic Energy Sciences, Materials Division of the U.S. Department of Energy under contract number DE-AC03-76SF00098. We thank J. Holzrichter for support and insistent encouragement and A. W. Moore for providing HOPG.

28 October 1988; accepted 29 November 1988