# Summary Abstract: Ferritin deposition on tungsten and its desorption in a high electric field [a]

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The interaction of an organic molecule with other molecules or a metallic substrate can influence the course of many physical and biological reactions. The high resolution electron microscope can probe details of molecular structure, but is generally restricted to an examination of two dimensional molecular crystals, or individual molecules stained with heavy metals and placed on nonmetallic substrates. X-ray crystallography can examine molecular structure within an ordered crystal of molecules, but cannot examine a metal-molecule interface.

The Field-Electron Emission Microscope and Field-Ion Microscope have the potential to image molecules at a metal-molecule interface, but imaging attempts have not been particularly successful. Organic and biological molecules deposited on clean metal surfaces produced images that were either highly structured, but unrelated to a molecule's known conformation; or were transient, or nonreproducible.[1] For several years we have attempted to use controlled field-desorption to probe the structure of molecules deposited on a metal surface. Recently, this method has yielded reproducible images of unstained ferritin clusters[2] and individual ferritin molecules placed on a tungsten substrate.[3]

The imaging method is straightforward: ferritin (a nearly spherical protein molecule that contains iron) is deposited onto the surface of a large radius field-emitter tip.[4] The tip radius is chosen to be much larger than the diameter of the ferritin molecule (11 nm). This insures that surface tension forces during the deposition processes will be minimized, and the molecule, once deposited, will not act as an appreciable protrusion on the nearly spherical tip surface. The tip is placed in UHV, and cooled to below 30 K. A thick layer of ultra pure benzene is condensed onto the tip surface from the gas phase. The immobile benzene layer completely surrounds and covers the deposited molecules. By establishing a moderate electric field at the tip surface (of $\approx 2$ V/nm), the benzene layer can be controllably field-desorbed. The benzene ions produced during the desorption event are radially projected onto a chevron CEMA detector where they form a highly magnified image of their relative positions on the surface of the condensed layer. As long as the layer covers the deposited ferritin molecules the image on the CEMA screen appears bright, but without significant contrast variations. However, as soon as a molecule or cluster is uncovered, a dark region appears in the image. The contour of the dark region reflects the contour of the deposited molecule at a height above the surface equal to the thickness of the remaining benzene layer. By field-desorbing the benzene layer, contours successively closer to the tip surface are revealed in the image. If a series of contour images are superimposed, a three-dimensional picture of molecular morphology is obtained.

We use a digital processing technique developed at Sandia to enhance the three-dimensional detail inherent in a series of contour images. All contour images in a series are digitized, and then digitally summed. The resulting composite image is shifted by several video lines, digitally (low-pass) filtered, and then subtracted from the unshifted composite image.[5] The result is an image with highlights and shadows which displays a three-dimensional quality similar in appearance to that seen in a scanning electron micrograph.

We have used this process to reconstruct images of ferritin cluster morphology on tungsten following the application of a field sufficient to desorb the most weakly bound clusters.[6] These desorb at field strengths of $\approx 4$ V/nm, well above the field required to image them and far below the field (of 7 V/nm) required to desorb isolated ferritin molecules. These
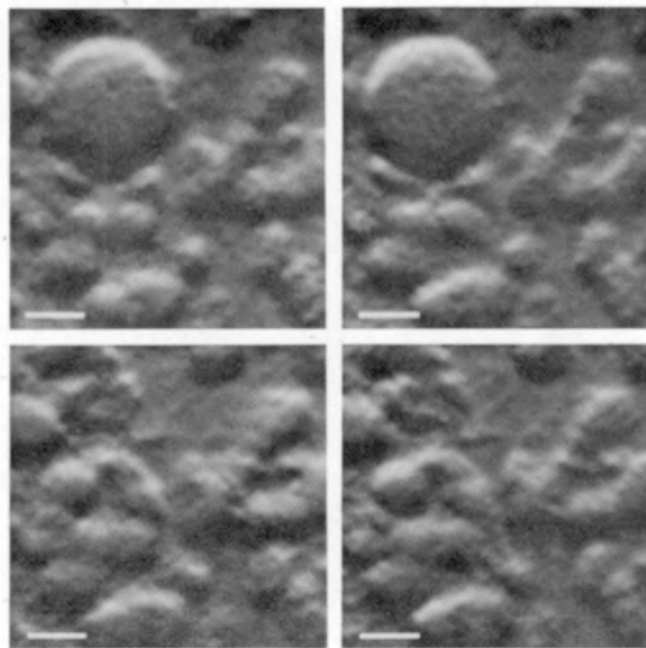


FIG. 1. Two digitally generated stereo pair images of unstained ferritin clusters on tungsten before applying a field of 4 V/nm (upper), and after applying this field strength (lower). These are the first images ever taken of unstained protein molecules on a metal surface. Scale marker = 20 nm.

studies indicate that our imaging process is nondestructive.

In order to provide a more accurate three-dimensional reconstruction of ferritin morphology on tungsten, we have also used a digital processing technique to create stereo pairs from a series of contour images.[5] Two images are created. One, digitally shifted to the right, corresponds to a left eye view; and one, digitally shifted to the left, corresponds to a right eye view. Two examples are shown in Fig. 1. The upper stereo pair shows ferritin cluster morphology on a tungsten surface prior to applying a field of 4 V/nm. The lower pair shows the same region of the surface after a field strength of 4 V/nm was applied. Such images provide a direct way to examine macromolecular interactions with a surface, and the effect of denaturing on protein morphology. Experiments

are planned which will examine polymer interaction with metallic substrates, and adhesion phenomena on a nanometer scale.

[1]J. A. Panitz, in *Experimental Methods of Surface Physics*, edited by R. L. Park and M. G. Lagally (Wiley, New York) (in press).
[2]J. A. Panitz, J. Microsc.12511, 1 (1982).
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[4]J. A. Panitz and I. Giaever, Ultramicroscopy 6, 3 (1981).
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# Summary Abstract: Low energy electron diffraction study of crystalline graphite and potassium overlayers on graphite

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Graphite is well known as a favorable substrate for studying phase transitions of physisorbed gases as well as an ideal host lattice for studying the quasi-two dimensional behavior in intercalation compounds. It is attractive, therefore, to study the surface structural properties of graphite itself and of chemisorbed overlayers on it.

LEED and AES were used to study both clean graphite (0001) surface and the graphite (0001) surface with adsorbed and absorbed potassium atoms.

A standard four-grid LEED-Auger system mounted in a UHV chamber (pressure $1 \times 10^{-10}$ Torr) was used for the experiment. Natural graphite crystal platelets 2–4 mm in diameter were initially cleaved in air with a dry nitrogen gas stream. The sample was then mounted on a manipulator, placed in the vacuum chamber and a clean large grain was defined by AES and LEED prior to baking the vacuum chamber. A potassium molecular beam source shielded from the graphite by a shutter was also installed in the vacuum chamber.

After baking the chamber, the graphite surface was aligned normal to the incident electron beam under the criterion of equivalence (to within a few percent) of degenerate beams. First and second order diffracted beam intensities of graphite were measured from the fluorescent screen via spot photometer. The experimental intensity profiles were then normalized to the incident beam current via computer.

Potassium was evaporated from the molecular beam source onto the graphite substrate at different source and substrate temperatures. After each potassium deposition the

coverage was determined by AES using low (600-eV) and high (3-KeV) primary energy electrons. Order of the potassium-graphite surface was examined by LEED.

The perfect graphite crystal is characterized by a layered structure with an hcp lattice (ABAB...stacking). The single layer has a honeycomb structure with two carbon atoms per unit cell. Normal termination of this structure for the (0001) face and the limited penetration depth of low energy electrons will result in a three-fold LEED pattern for the (0001) surface if only one surface termination (A or B) is present and a sixfold LEED pattern if both A and B terminations are present on the surface due to the existance of monoatomic steps. In fact, both three-fold and six-fold diffraction patterns were observed with the three-fold pattern present for virgin samples and those samples not much perturbed in the chamber (e.g., by heating or ion bombardment). LEED *I–V* spectra for four normal incidence beams of a three-fold (single termination) clean graphite surface have been compared to dynamical calculations for two separate surface structure models. In the dynamical LEED calculations, the multiple scattering within each layer was treated with the reverse scattering perturbation (RSP) method and for the interlayer scattering the renormalized forward scattering (RFS) was used. The two structural models were the normal stacking (ABAB...) terminated surface and a stacking fault (AA-BAB...) terminated surface.

The comparison between the calculated LEED *I–V* curves and the experimentally determined ones indicates that bulk ABAB... stacking exists at the clean surface of