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Final Report

GRANT #: N00014-90-J-1455

PRINCIPAL INVESTIGATOR: S.M. Lindsay

INSTITUTION: Arizona State University

<u>GRANT TITLE:</u> Biological Applications of STM and AFM in Water /High Resolution Microscopy of Nucleoprotein Complexes in Water

AWARD PERIOD: (Jan. 1, 1990 - September 30, 1996)

<u>OBJECTIVES:</u> The work carried out under this grant was aimed at: (1) Developing reliable methods for imaging samples on surfaces under liquids and under potential control and (2) Applying these imaging techniques to problems in biomolecular structure.

ACCOMPLISHMENTS: The first goal was achieved, and, along the way, many contributions were made to the understanding of and technology of electrochemical scanning probe microscopy. The second goal was delayed as failures taught us much about the limitations of (and possibilities for) scanning tunneling microscopy applied to biological samples. The last year of the grant was spent applying methods developed for improving atomic force microscopy in 1992. The new technique (MA/C mode AFM) shows great promise for gentler imaging at higher resolution than heretofore possible.

<u>CONCLUSIONS</u>: (1) STM Images: A simple interpretation of the images is that they reflect only the points where the molecule touches the metal substrate. This information is adequate for, say following the path of a DNA molecule, but useless in most other cases. The limits of the technique are illustrated in some recent work in which we used covalent chemistry to bond DNA to gold substrates covalently. The oligomers used in that work gave understandable images and, subject to some assumptions, we even determined the bending angle in bulged DNA. In an (unpublished) study of ribozymes carried out in collaboration with F. Eckstein and T. Jovin of the Max Planck Institutes we showed that although the images were reproducible, 3D structural interpretation was not possible.

(2) AFM Imaging: Using MA/C mode, we have studied DNA microcircles spontaneously adsorbed onto mica in an aqueous solution [37]. The gentleness of the new mode is illustrated by our ability to obtain images in the presence of Mg alone. With tapping mode, Mg will not bind DNA spontaneously strongly enough to be imaged. We found a remarkable result: Axially strained microcircles had a small tendency to form kinks (localized sharp bends) in the presence of Mg. However, when the cation was changed to Zn, a four-fold increase in kinking occurred. Static bending was replaced by straight DNA connected by kinks (illustrated below). The same behavior is found in physiological solutions containing both Mg and Zn.

<u>SIGNIFICANCE</u>: The program has contributed significantly to what is now becoming routine use of the AFM for structural and micromechanical studeis of biomolecules. The understanding of electron transfer in the STM has opened a new program aimed at electrochemical identification of single molecules.

PATENT INFORMATION:

"Method of Electrochemical Detection/Identification of Single Organic Molecules Using Scanning Tunneling Microscopy" Nongjian Tao and S.M. Lindsay US Patent 5,497,000, March 5, 1996. "Controlled Force Microscope for Operation in Liquids" S.M. Lindsay US Patent 5,515,719, May 14, 1996. AWARD INFORMATION: H. Willard Davis Lectureship in Chemistry, University of South Carolina (1994, Humbolt Senior Scientist Research Award (1993, Faculty Mentoring Award, ASU (1993), Vice-Chair Division of Biological Physics of the American Physical Society, 1994-5, Chair, Division of Biological Physics of the American Physical Society, 1995-6, Elected Fellow of the American Physical Society (1990) for: "Pioneering studies in the application of scanning tunneling microscopy to imaging biomolecules, especially images of the DNA double helix in water".

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