

# 1. Molecule Microscopy

## Academic and Research Staff

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## 1.1 Desorption Studies

We are continuing the experiments begun by D. Lysy<sup>1</sup> and B. Silver<sup>2</sup> on the thermal and electron stimulated desorption of water and other small molecules from biological materials for four reasons. (1) To understand the contrast mechanism in molecule microscopy; (2) to measure the total water content of various samples; (3) to measure the binding energy of this water; and (4) to determine the order of the kinetics of the desorption process. The importance of these experiments comes from the central role of water in biological processes at the molecular level.

### Progress

During 1983 we adapted the laser desorption apparatus of J. Yorker<sup>3</sup> for thermal desorption studies carried out in collaboration with Dr. D. Atkinson of the Biophysics Institute at Boston University. An essential part of the work was the development of sample holders that can be made cheaply in quantity so that many samples can be prepared at one time at Boston University for later study at M.I.T. The current design satisfies almost all requirements. It is cheap, has an inert, wettable surface, and can be installed easily in the apparatus along with the miniature thermocouple wires required for temperature monitoring. Improvements were made in the temperature control system, which includes LN<sub>2</sub> cooling and heating by current passing through a graphite rod.

Over 100 desorptions were made from a variety of samples, mostly cholesterol applied to the sample holder by micropipette from solutions with ethanol, methanol, and hexane. Reproducible results were obtained showing a dominant, anomalously narrow desorption peak at moderate temperature (60 C). We interpret this as associated with a phase change in the sample. Similar data were obtained with thymine, cytosine, and uracyl monohydrates. We believe that the sample is in the form of crystallites which undergo a phase change with the emission of water.

Rather than continue work with these imperfectly characterized samples, we decided to study monolayers of cholesterol and DPPC. Accordingly, a Langmuir trough with appropriate dipping mechanism was used, but two problems have appeared. First we find the cleaning procedure of the sample holder to be very critical and secondly its shape, essentially a cylinder, makes it difficult to coat only the end. We are planning further development of the sample holder, at the same time that we are analyzing the data that we have in terms of new theoretical models.

### References

1. D.G. Lysy, "Measurement of the Activation Energy for Desorption and other Relevant Parameters by Thermal Desorption Techniques for Water Adsorbed on Biological Macromolecules," Ph.D. Thesis, Department of Physics, M.I.T., 1976.
2. B.R. Silver, "Scanning Desorption of Small Molecules from Model Biological Surfaces," Ph.D. Thesis, Department of Physics, M.I.T., 1976.
3. J.G. Yorker, "Thermal Desorption of Small Molecules from Biological Surfaces with Spatial Resolution," Ph.D. Thesis, Department of Physics, M.I.T., 1982.

## 1.2 Nanometer Resolution Scanning Desorption Molecule Microscopy

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We are designing an SDMM capable of resolution between 1 and 10 nm. The pivotal invention is the use of a tungsten needle with a 1  $\mu\text{m}$  radius tip maintained at low temperature which oscillates from 1  $\mu\text{m}$  above the sample to the entrance of a time-of-flight mass spectrometer. When the needle is above the sample, molecules desorbed from the sample freeze on the tip — later when it is at the entrance of the mass spectrometer these molecules are field desorbed and field ionized by a short high voltage pulse. The molecules are desorbed from the sample by electron stimulated desorption using focused pulses of electron that can be scanned across the sample.

In this way we achieve good solid angle and high efficiency in the detection of neutral molecules whereas high spatial resolution is achieved by exploiting advanced scanning electron microscopy techniques. With this instrument we expect to determine the position and amounts of water and other desorbable molecules on biological samples, thus complementing data attainable in a limited number of cases by x-diffraction.

### Progress

During 1983, we have set up experiments to confirm and extend our earlier measurements of ESD cross sections, to desorb molecules from tips with 200 – 400 kv pulses and to investigate ways of controlling and producing these pulses. The design of the instrument will thus be based on well-tested elements.

## 1.3 Scanning Micropipette Molecule Microscopy (SMMM)

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The major goal of this research continues to be the study of water transport at the cellular level across hormonally responsive tissues that model the distal tubules of the mammalian kidney, specifically amphibian urinary bladder. The elucidation of these processes is basic to an understanding of the cellular and molecular mechanisms by which the kidney maintains salt and water balance in man.

This work is being carried out using the scanning micropipette molecule microscope.<sup>1</sup> This instrument uses a micropipette of about 1  $\mu\text{m}$  tip radius sealed with a permeable plug and connected to a mass spectrometer to make concentration measurements of tracers such as labelled water. Thus spatial and temporal variations in the permeation of these tracers across epithelia can be monitored in vitro.

### Progress

During the past year, we have continued our studies using microiontophoretic injection of the fluorescent dye, Lucifer Yellow. We have expanded this effort to address the long-standing issue of the role of the basal cells in cell-cell coupling in this epithelium. Our preliminary data suggest, contrary to the currently-held hypothesis, that the basal cells do not provide a major coupling pathway.

We have also initiated a collaboration with Dr. K. Zaner at the Massachusetts General Hospital to measure the diffusional water permeability of various cytoplasmic gel extracts from lung macrophages. The scientific objective is to determine to what extent cytoplasmic gels may restrict intracellular diffusional transport. Our initial measurements on water indicate that there is no significant long-range ordering of intracellular water as has been suggested by others.

Lastly, we have embarked on an effort to determine the feasibility of cryo-preserving amphibian urinary bladders. If this proves possible, it will allow us to use the technique of radiation inactivation to determine, in situ, the molecular weight of several important putative transport proteins.

### References

1. J.A. Jarrell, J.G. King, and J.W. Mills, "A Scanning Micropipette Molecule Microscope," *Science* **211**, 277 (1981).

### Publications

- Jarrell, J.A., "Reversible  $\text{CO}_2$ -induced Inhibition of Dye-Coupling in Necturus Gallbladder," *Am. J. Physiol.* **244**, C419 (1983).
- Jarrell, J.A., C.A. Rabito, and J.G. King, "Applications of Intracellular Dye Injection and Mass Spectrometry to the Study of Epithelial Transport," in M.A. Dinno, T. Rozzell, and A. Callahan

(Eds.), Physical Methods in the Study of Cellular Biophysics (A.R. Liss, New York, 1983).  
Jarrell, J.A., "Dye Coupling in Amphibian Urinary Bladder," Abstract for the 1983 meeting of the American Society of Cell Biology.

## 1.4 Electrical Neutrality of Molecules

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The present experimental upper limit on any departure from neutrality of molecules is approximately  $10^{-21}$  of one elementary charge. This can be interpreted as a limit on the proton–electron charge difference, or on the charge on the neutron, or on some combination of these. The occurrence of innumerable transformations involving both leptons and baryons, and our long–standing belief in the conservation of electric charge as a fundamental law of nature, certainly support the assumption of exact equality of charges of either sign on all elementary particles. Nevertheless, the possibility that there might be some minute difference between electron and proton charges is something that cannot be ruled out on the basis of any existing experimental evidence or theoretical scheme, and its existence would have profound implications.

The fundamental interest of this question has led to a number of experiments by various investigators. These experiments have involved three different approaches: (1) direct tests for any net charge in a volume of gas released from a container; (2) tests for a charge on an isolated body (analogous to the Millikan oil–drop experiment); (3) molecular–beam deflection experiments.

In 1973 King and Dylla<sup>1</sup> pioneered a new technique based on the fact that an electric field, periodic in time, applied to a homogeneous medium will excite acoustic waves at the same frequency if the molecules of the medium are not completely neutral. (This is separable from any induced polarization effects, which will occur at doubled frequency).

### Progress

We have designed and built a cylindrical resonant cavity in which an approximately uniform axial oscillating electric field can be established. One end of the cavity is a solid plug and the other end, adjustable in position, is a resonant diaphragm condenser microphone. We use the 5 MHz bridge technique developed by Linsay and Shoemaker<sup>2</sup> to measure vibration of the diaphragm near the thermal limit. The first runs will be in  $\text{CCl}_4$  followed by  $\text{LHe}^4$  and  $\text{LH}_2$  and eventually  $\text{LHe}^3$  and  $\text{LD}_2$ .

### References

1. H. Dylla and J. King, Phys. Rev. A 7, 1224 (1973).
2. P.S. Sinsay and D.H. Shoemaker, Rev. Sci. Inst. 53, 1014 (1983).