**DEVELOPMENT AND APPLICATION OF PHOTOSENSITIVE DEVICE
SYSTEMS TO STUDIES OF BIOLOGICAL AND ORGANIC MATERIALS**

Second Year Progress Report

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I) INTRODUCTION & SUMMARY

This report describes the progress of the second year of a 3-year DOE grant DE-FG-02-87ER60522 for the fiscal period 1/1/91 to 12/31/91 as of July 1991. The overall goals of the grant are to develop novel instrumentation and techniques for the performance of biological and materials research and to apply the new developments to basic biological and materials research problems. Since the last progress report, dated July, 1990, there has been significant progress on most of the originally proposed instrumentation and applications research. The overall research goals proposed for the next year have not changed from those originally listed in the 3-year proposal.

The research is well guided by the philosophy that useful instrumentation development and basic biological research are inseparably coupled and proceed most effectively when the instrumentation development is driven by specific needs of basic research problems. To insure this coupling, the Princeton Biophysics Group has avoided the division of the group into "instrumentation" and "applications" specialists; rather, each group member is simultaneously involved in both the development of the instrumentation and the application to basic research problems being worked on by that individual.

Specific accomplishments of the last year include:

- A) A prototype large area CCD x-ray detector was assembled and evaluated at the CHESS synchrotron facility;
- B) Fiber optic CCD coupling methods have been developed and are being applied to in-house detector needs;
- C) Novel detector control and calibration software was developed and refined;
- D) Novel high pressure x-ray diffraction and dilatometric apparatus were designed and built, and are being applied to the study of membrane-lipid phase behavior;
- E) A time-resolved x-ray diffraction study of lipid phase transitions was used to demonstrate that conventional calorimetry does not accurately determine the phase transition parameters;
- F) The elastic properties of amphiphilic layers were studied both theoretically and experimentally;
- G) A re-entrant hexagonal-lamellar-hexagonal lipid phase transition was discovered and studied.

Each of these accomplishments are detailed, below.

II) RESEARCH ACCOMPLISHMENTS

A) Large Format CCD X-ray Detectors for Synchrotron Applications

The most significant accomplishments of the last year pertain to success with the first tests of a large area CCD detector for synchrotron diffraction research. It is now generally recognized that progress in structural biology will likely involve crystallographic data collected at synchrotrons from large unit cell crystals (e.g., viruses), weakly diffracting specimens, and time-resolved Laue diffraction of proteins. It has also become clear that the bottleneck in the collection of such diffraction data is the lack of efficient x-ray detectors capable of collecting data at the prodigious rate at which data is produced at a storage ring source. The lack of suitable detectors on existing beamlines has led to a vast underutilization of expensive synchrotron resources; moreover, the bottleneck is projected to become more severe as newer and brighter beamlines, such as those expected at the

Advanced Photon Source (APS) become available. It is telling that a recent detailed poll of the crystallographic community (Biosync Report: Structural Biology and Synchrotron Radiation: Assessment of Resources and Needs. J.L. Smith & K.D. Watenpaugh, 1991) indicated that fast and efficient detectors were identified as *the most important* critical need of the biostructural community operating at synchrotron sources, ranking above needs for improved source characteristics and all other source facilities and resources.

Largely as the result of detector development research supported by this grant, CCD-based area x-ray detectors are recognized as the technology with the greatest potential to fulfill the desired detector needs. However, a major difficulty in adapting CCD technology to x-ray problems has been that the size of the required detective area, or detector aperture, has generally been larger than the size of CCDs which were readily available. In the absence of large aperture CCD detectors, many synchrotron groups were planning to install detectors based on storage phosphors (Miyahara et al, 1986), which are available in large aperture sizes. However, storage phosphors have severe limitations: Since they must be read off-line in a sophisticated, and generally slow, readout device, they lack the flexibility, speed, and convenience of electronic detectors. Moreover, as researchers begin to use imaging plates under different conditions, systematic problems have begun to emerge. In particular, Templer (1991; Who, incidentally, received his post-doctoral training in our laboratory) has critically examined the characteristics of image plates and has demonstrated fundamental difficulties associated with plate readout.

The availability of large aperture CCDs has changed markedly over the last year or so. Recognizing that large aperture CCDs would soon become available, last summer, in collaboration with John Lowrance of Princeton Scientific Instruments (PSI, a CCD controller manufacturer) and Don Bilderback of the CHESS staff, we assembled a test CCD detector based on a borrowed prototype TEK 2048 $\times$ 2048 pixel CCD. This detector had a 50 mm aperture and consisted of a rare-earth oxysulfide phosphor fabricated directly onto a fiber-optic plate which was, in turn, directly coupled to the CCD. The key elements of this detector, in addition to the CCD were the phosphor deposition and liquid-film fiber-optic to CCD coupling methods developed under this grant.

Our calculations indicated that quantum limited detection should be feasible without the need for image intensification (Gruner, 1989). Eliminating the image intensifier enormously simplifies the detector. Test results acquired at CHESS confirmed these expectations. Moreover, we were able to show unprecedented spatial resolution of Laue patterns, with feature sizes on the order of 50 μ m and with a dynamic range of at least 16 bits.

These results have been published (Eikenberry et al, 1991) and presented at a number of scientific meetings. The response from, especially, the crystallographic community has been dramatic, sufficiently so that CHESS has announced that it is reconsidering extensive investment in image plate detectors until more test data is available. Toward this end, Don Bilderback of the CHESS staff would like to collaborate with PSI and our group to install a 2048 $\times$ 2048 demonstration system within the 6-8 month time frame. The idea is to use the detector on virus diffraction patterns, which are amongst the most taxing of all crystallographic diffraction patterns. Then, based on the experiences and results, we would design and seek support for a dedicated 4096 $\times$ 4096 pixel system. We feel that this collaboration, and the 2048 $\times$ 2048 detector demonstration which would result, would be important steps toward solving many of the detector problems at synchrotron sources and have, therefore, agreed to proceed with the work. The assembly and installation of the 2048 $\times$ 2048 demonstration system within a 6-8 month time frame will tax the resources of all three parties in the collaborations. However, we are convinced that installation and use

of such a device would be the most efficacious route toward solving many of the detector needs of the biostructural community. It may be necessary to seek supplemental funding to carry this ambitious project through.

B) CCD-Fiber Optic coupling

One of the major difficulties involved in constructing CCD detectors is the need to couple fiber optic plates and tapers directly to CCDs. The methods which have been used tend to fail at the low temperatures at which the CCDs are operated because the CCD silicon and fiber optic glass have different thermal expansion coefficients. The liquid film coupling method described in Eikenberry et al (1991) is not suitable for long-term operation because the coupling agent eventually flows out. In consequence, we have been investigating compliant, silicone gel couplings. A number of silicone gel agents are being tested for low-temperature operation.

The silicone gel coupling agents are being tested on two in-house detector systems, both of which involve 512×512 CCDs. The first system uses a custom manufactured reducing intensifier bonded to a 2:1 fiber optic taper, which is in turn bonded to a Tektronix CCD. This is being used to replace a previous system (see Templer et al, 1988) which had a multi-stage image intensifier with a much smaller input area. The second system, which has been partially funded by a grant from the NSF, uses a proximity focussed image intensifier coupled to a large ratio (5:1) fiber-optic taper bonded to a Thomson CCD. It has been used as a test bed to evaluate the characteristics of large taper ratio fiberoptics and for the evaluation of red phosphors for increased CCD sensitivity (before the image tube was installed). This information is essential for the design of a large format CCD system because it is too risky to use expensive, large format CCDs to determine these parameters. Both of these detectors are now undergoing final testing. Our intention is to publish the results of the tests, including the new fiber optic bonding procedures and the phosphor evaluation and deposition procedures, as soon as possible.

The plan is to use the fiber optic taper, phosphor and fiber optic bonding information gathered on the small format CCD detectors for the CHESS project. Much work remains to be done, especially on long-term evaluation of the fiber optic-CCD bonding, before we will feel sufficiently confident to attempt to bond a 2048×2048 CCD. Problems which still have to be addressed include minimization of moire effects arising from the beating of CCD and fiber optic rasters and reduction of optical interference fringes coming from index mismatches in the gel bonding layer.

CCD-fiber optic bonding is difficult. We have been contacted by researchers at the MIT-Lincoln Labs who are attempting to perform rigid, epoxy bonding to large format CCDs. So far, their attempts have failed temperature cycling. They were keenly interested in our compliant bonding methods and requested to be kept informed of our progress.

C) Software Developments

Considerable effort has been invested in the development of software to control CCD detectors and integrate the data acquisition process with the operation of the detectors. The result is an operating system which allows movement and manipulation of large data images as though they were numbers. Considerable attention has also been given to improving the codes used to determine the distortions introduced via image intensifier and fiber optic taper components and to removing these distortions. These codes were developed for the 512×512 pixel data sets available from the in-house detectors. However,

these codes will have to be modified for the much more demanding specifications of the 2048×2048 detectors. A major task for the coming year is the extension of the detector control and correction codes to larger format images. It is already clear that the 386-PC platforms on which our current codes are based will be too slow for the larger data sets. For this reason, we anticipate having to move to more powerful work station computers. There will also be more emphasis on code portability to decrease the load involved in upgrading software in the future.

A description of the software correction procedures is being prepared for the literature. We had hoped to have already submitted an article on corrections by this time, but the writeup of the software has fallen behind because of a shortage of personnel to both write up past developments and develop new codes. In the meantime, the codes have been made freely available to the community. Most recently, a copy of the control and correction codes were provided to Dr. B. Chu, of the SUNY-Stonybrook PRT at the NSLS. Scientists from Dr. Chu's laboratory were also trained in the use of the codes.

D) High Pressure X-ray and Dilatometric Apparatus

Pressures in the range of 0 to 1 kbar are known to affect many of the processes of living cells, including such bizarre effects as the reversal of anesthesia and alcohol intoxication (Janoff & Miller, 1982). However, there is no known, accepted mechanism whereby such moderate pressures affect cellular function. We have suggested that pressure affects cellular function by modulation of the anisotropic compressibility of biomembrane lipids and a concomitant coupling to membrane proteins (Gruner & Shyamsunder, 1991). Toward this end, apparatus has been constructed for the performance of membrane and lipid diffraction at pressures ranging up to 3 kbar.

The results have indicated that the pressure dependent lipid-water phase diagram is a very sensitive function of pressure; indeed, the mesomorphic phases of lipid-water dispersions are amongst the most pressure dependent non-gaseous phase transitions known (Shyamsunder, et al, 1989). A detailed study of two lipid-water systems was carried out with the aim of understanding the structural changes which occur as pressure is applied. However, it became clear that a full understanding of the thermodynamics required either knowledge of the entropy changes as a function of pressure or, equivalently (via the Clausius-Clapeyron relation), of the volumetric changes. It was determined that high pressure dilatometry would be simpler and more accurate to perform on small samples than high pressure calorimetry because the heavy walled vessels needed to contain pressure make it difficult to thermally isolate the specimen. Unfortunately, we were unable to find a description in the literature of a dilatometer suitable for measuring the very small volume changes (a part in 10^6) on very small samples (< 100 microliters) over the necessary pressure range (to 3 kbar).

In consequence, a high pressure dilatometer was designed and constructed and is now being used to acquire the dilatometric data to complement the x-ray diffraction studies. Our hope is to have completed the dilatometric studies early by this Fall. Descriptions of the high pressure x-ray and dilatometric apparatus and the results of the lipid studies will appear in the Ph.D. thesis of Peter So and in subsequent publications.

E) Time-Resolved Lipid Phase Transitions

A combined synchrotron radiation and rotating anode generator study of lipid mesomorphic phase transitions was completed and submitted for publication. The mesomorphic

transitions which were examined are of the bilayer to nonlamellar variety, which involve topologically discontinuous rearrangements of the lipid and water volumes within a lipid-water dispersion. These phase transitions are of interest because it is thought that the energetics and kinetic pathways involved in local bilayer destabilization during mesomorphic transitions are related to the local disruptions which occur during important cell membrane fusion events (Siegel, 1986).

The synchrotron radiation part of the study involved application of ohmic temperature jumps to lipid-water dispersions to induce the phase transitions. The use of 2-dimensional detectors allowed snapshot acquisition of the diffraction patterns on the 10 msec timescale. It was shown that the bilayer-nonlamellar (H_{II}) transition was kinetically hindered and proceeded on the roughly 100 msec time scale. Analysis of the lattice size showed that these lyotropic transitions are at least partially hindered by the time required for water to equilibrate between the two phases.

The rotating anode part of the study was based on the observation that, although the phase transition is thought to be largely first order, a small fraction of the mass of the sample appears to lag behind the bulk of the specimen after the heating event. It was shown that the transition kinetics can be modeled as a combination of exponentially and algebraically decaying processes. An important consequence of the algebraic component is that for small temperature jumps a nonnegligible portion of the specimen is still undergoing the phase transition weeks after the temperature jump! This calls into question the classic procedures used for performing calorimetry on membrane and lipid systems, by which heating rates of a degree/hr is considered to be sufficiently slow for complete equilibrium. We believe that these results also demonstrate the existence of a new class of kinetics active in phase transitions of interfacial surfaces, namely, topological hindrance. Study of such a class of phase transitions may ultimately prove to be very important for understanding mesomorphism in a variety of specimens consisting of bulk interfaces, including diblock copolymers, lyotropic liquid crystals and cell membranes.

F) Elastic Properties of Amphiphilic Layers

Amphiphilic layered systems include biological membranes, lyotropic liquid crystals and diblock copolymers. Over the last ten years there has been enormous progress, both theoretically and experimentally, in understanding the physical properties of such systems. In particular, the x-ray structural and theoretical work of our group has demonstrated the importance of curvature elasticity in lipid and biomembrane systems (for reviews see Gruner, 1989b; Tate et al, 1991). This work has been based on a theoretical model of lipid-water mesomorphism first presented by Kirk et al in 1984 which held that curvature elasticity drives mesomorphic transitions. These are, in turn, controlled by competing free energies associated with the packing of hydrophobic lipid chains in the hydrocarbon cores of the mesomorphs. Over the last year, we have completed a series of studies aimed at directly verifying central aspects of the theory.

Three studies were performed. The first involved the development of a novel method for solving the structure of the hexagonal (H_{II}) phase of lipid water dispersions directly from the x-ray diffraction derived structure factors. Former methods involved assumptions about circular symmetry in the mesomorphs and so were inadequate for investigation of the hydrocarbon chain packing effects which arise directly from noncircular symmetry of the lipid-water tubes which the H_{II} lattice. Armed with this method, we demonstrated that entropically expensive density variations in the lipid hydrocarbon domain diverge as the

luminescence does result from this process, but it is not yet clear what the chemiluminescence is due to. So far, both photomultiplier and spectral records have been obtained. The phenomenon is a possible analytic tool for the polymerization process and we plan to conduct a thorough study during the coming year. (3) A feasibility study demonstrating color capability for image intensification has been published (Reynolds, 1991) and some interest has been shown by participants in the ONR Ocean Optics Program. (4) We continue to act as consultants to various biologists in the detection of low light level phenomena such as bioluminescence fluorimetry.

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