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3.B Kinetic X-Ray Diffraction of Purple Membrane

Since the demonstration at LLE of good quality x-ray diffraction photographs from membrane specimens using a laser plasma x-ray source just over one year ago,[1] we have been preparing to perform kinetic protein structure experiments. This note will serve as a progress report on these experiments and show the first kinetic x-ray diffraction results obtained using the laser plasma source.

The protein system we are studying is the purple membrane of the *Halobacterium halobium*. Purple membrane contains a single protein, bacteriorhodopsin (BR), which serves as a light activated hydrogen ion pump.[2] The BR protein contains retinal, the same chromophore which activates the mammalian visual process. As a result, BR may serve as a model transmembrane ion pump, as well as a model for visual phototransduction. The goal in our experiment is to observe conformational changes in the BR protein during the photopumping cycle.

Our experimental arrangement is shown schematically in Figure 12. A pulse of light is used to stimulate photoactivity in our protein specimen. This light pulse is synchronized to the fire command signal of the GDL laser system, the output of which is used to produce an intense, point source pulse of monochromatic x-rays to interrogate the molecular structure of the protein. The purple membrane system is organized in the form of a perfect two-dimensional crystal; the naturally occurring membrane fragments are 50 Å thick and typically have a lateral extent of 1-2 μm. The static structure of the protein has been worked out to 7 Å resolution using a variety of biochemical and physical techniques including Fourier transform electron microscopy and x-ray diffraction.[3,4] Recently the complete amino acid sequence of BR has been deduced.[5,6] In addition, a model has been proposed for the positions of the amino acids in the BR structure.[7]

A specimen consisting of a large number of membrane fragments may be studied in a standard power diffraction camera. In our experiment a special focussing x-ray diffraction camera was constructed to enable a laser produced plasma to serve as the x-ray source,[1] providing nanosecond time resolution of the protein structure.

To stimulate our BR samples we have assembled the laser system shown in Figure 13. A CW pumped Nd^{+3}:YAG oscillator is Q-switched by an acousto optic modulator to produce pulses of 150 nsec duration at 1.064 μm. In our experiments the acousto optic Q-switch is external-

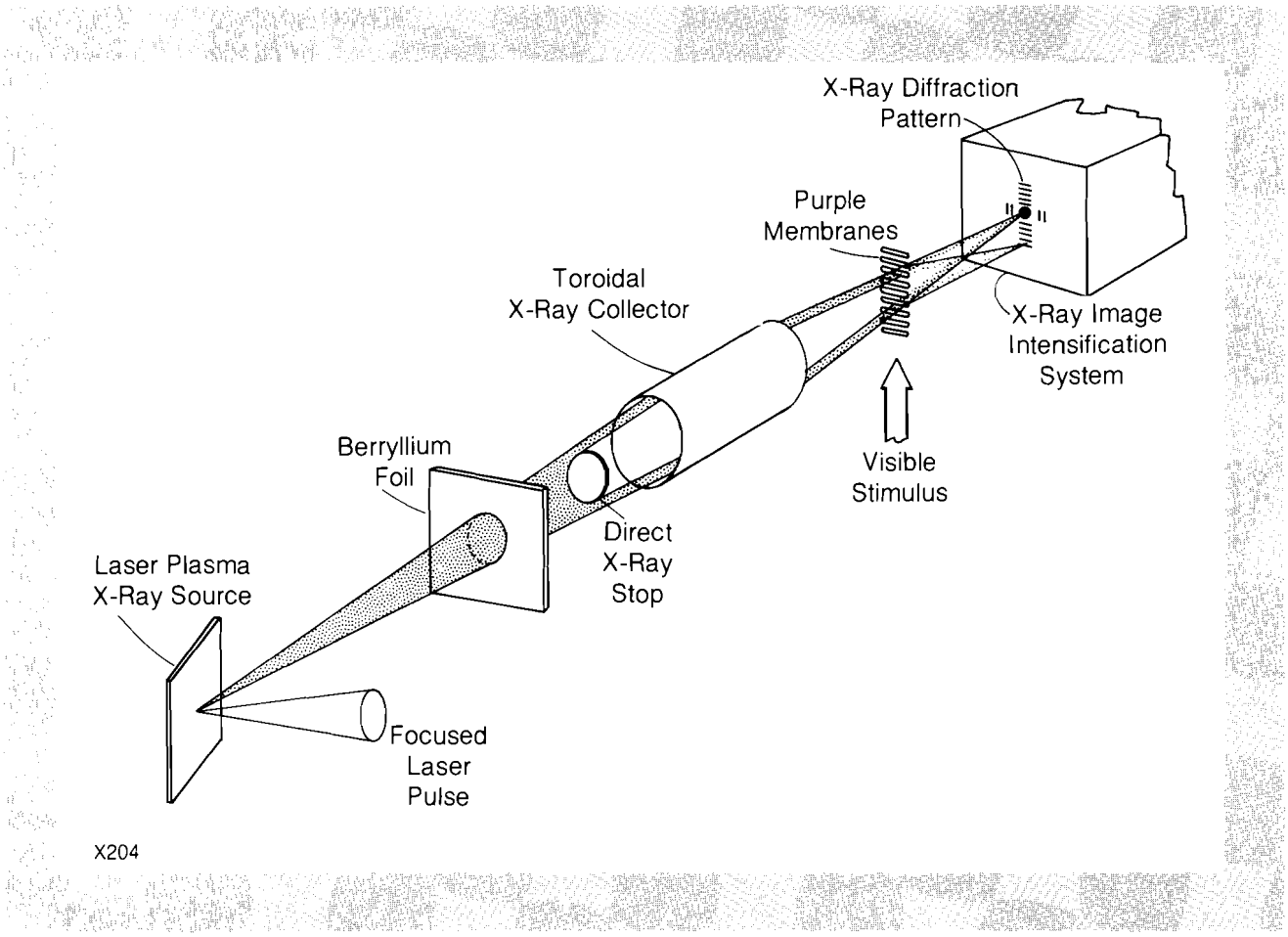

Fig. 12
Conceptual layout of pulsed x-ray diffraction experiment.

ly triggered by a burst generator, allowing us to produce a pulsetrain of arbitrary length, with interpulse spacings down to 40-50 μsec. The train of pulses is then amplified by a double pass, pulsed pumped, Nd^{+3}:glass amplifier and focussed into a temperature tuned CDA crystal to produce a train of 0.53 μm pulses. This pulsetrain is transported to our BR specimen in our x-ray diffraction camera by a series of dielectric coated mirrors which attenuate the unconverted 1.064 μm radiation from the crystal.

The optical stimulus pulsetrain is supplied to our BR specimen by placing in the x-ray diffraction camera a dichroic mirror consisting of a thin (600 Å) aluminum coating on a 1.5 μm thick mylar foil. This thin mirror is transparent to the focussed soft x-ray radiation from the laser plasma and highly reflecting to the green light pulses.

Since our demonstration of single shot recordings of protein x-ray diffraction patterns we have constructed a lens coupled, two-dimensional, digital vidicon recording system for our camera. The diffraction pattern is formed on a ZnS:Ag scintillator deposited on a fiberoptic plate which is directly coupled to a 25 mm Amperex microchannelplate image intensifier. The image intensifier output is lens coupled to the PAR OMAII vidicon and 1216 controller which are operated by interfacing to a DEC 11/23 computer equipped with 128 K of 16 bit memory,

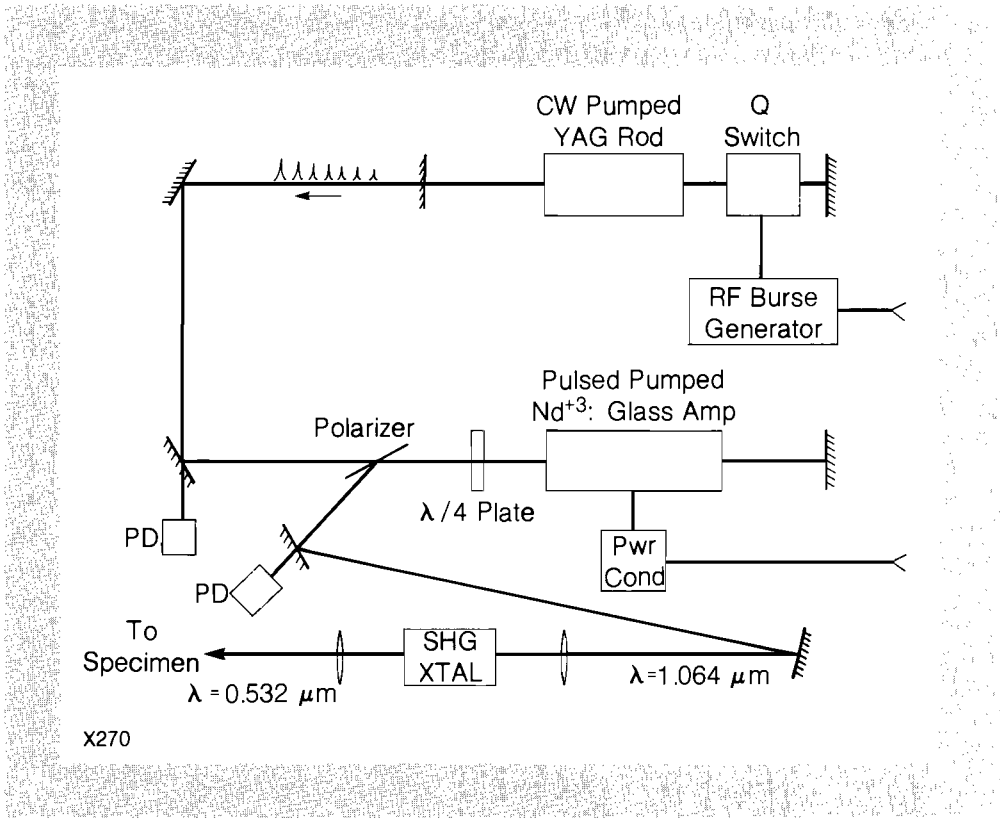

Fig. 13
Photostimulus system for purple membrane.

an RL-01 disc drive, and a Lear-Sigler graphics display terminal. Software has been written to permit fully two-dimensional scanning of a 256 x 512 point image field by the OMA head. To enable us to record the low light level events from our x-ray scintillator the OMA head is mounted in a refrigeration unit which operates at −40 °C. The vidicon unit is slow scanned, requiring approximately 10 seconds to read an x-ray diffraction pattern into the computer.

At this point our data reduction techniques are still under development and several components in our x-ray camera are awaiting final installation. However, we have been able to observe stimulated changes in purple membrane diffraction patterns during preliminary tests of our system.

In our recent experiments we have converted our laser target irradiation facility to the ultraviolet, third harmonic output of the GDL facility.[8] This change was dictated by the measurement of greatly enhanced conversion of high intensity UV laser light into x-ray radiation in previous GDL experiments.[9] In the results presented here approximately 40 Joule, 490 psec FWHM pulses of third harmonic laser energy were focussed by a 14 cm diameter f/12 lens to a 120 μm diameter focal spot onto flat targets made of saran foil. The strongest emission lines are produced by the helium-like and hydrogen-like ions of chlorine in the wavelength range of 3-4.5 Å. By foil filtration in the x-ray camera a single group of lines with overall spectral width of less than 1% may be isolated at 4.45 Å with high efficiency.[1] The use of this radiation wavelength dictates a protein specimen thickness of 50-60 μm for optimum x-ray diffraction efficiency. With purple membrane,

such specimen dimensions result in an optical density in the range of 2-3 at the wavelength of our stimulus system. (The use of shorter wavelength x-ray radiation would dictate the use of thicker specimens which would prove much more difficult to photostimulate. The use of longer wavelength radiation would reduce the potential structural resolution which could be obtained and prevent simple foil filtration for monochromatic radiation.)

Our specimens were prepared by air drying a centrifuge concentrated suspension of membranes obtained from a purified preparation supplied to us by Dr. Janos Lanyi.[10] The samples were air dried onto saran foil supports which also served to monochromatize the plasma x-ray radiation. For the experiments reported here the samples were placed directly in the x-ray diffraction camera which was then evacuated.

Representative results are shown in Fig. 14. In this figure we plot the average diffracted x-ray intensity observed as a function of linear distance on the scintillator plate away from the axis of our camera. To obtain these plots the digitized patterns are computed to assign each point a radius value from the coordinates corresponding to the camera axis. (The latter coordinates are determined initially by trial and error.) The average intensity for each radius value is then computed. Apart from this processing no other corrections to the raw data have been made in Fig. 14.

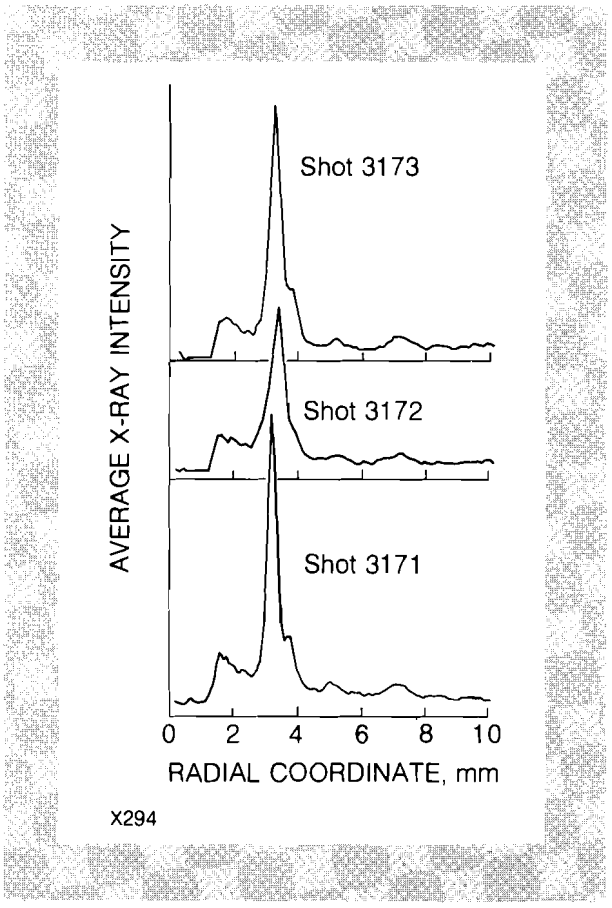

Fig. 14
Time-resolved x-ray diffraction from dried purple membranes of the halobacterium halobium.

The diffraction patterns for three shots are shown, all taken using the same purple membrane specimen. Shot 3171 was taken with the specimen unstimulated. On shot 3172 approximately 300 microjoules of 0.53 μm energy was delivered to a 1 mm² area of the specimen in a train of 5 pulses. A train of pulses was used to stimulate this system because during any photoactivation event only 30% of the BR can be "launched" on the photopath, the remainder of the system being constrained to the ground state. However, 2 μsec after an impulse excitation, 30% of the remaining ground state population may be photoactivated. By repeating this process (i.e. using a pulsetrain) we can reversibly photoactivate the entire system, as our measurements of the time dependent absorption have shown. The stimulated diffraction pattern was recorded 50 msec after the stimulus pulsetrain was delivered. It shows a distinct shift in the position of the strongest diffraction peak and an overall reduction in the diffraction efficiency of the sample. (The x-ray beam samples an annular area of the specimen approximately 0.6 mm in diameter, well inside the overall dimensions of the stimulated region.) On the next shot, number 3173, we recorded an unstimulated diffraction pattern again, and observe a return to the original pattern features, indicating that the system is recycling. However, we observed that repeated stimulation of the sample on subsequent shots caused the diffraction pattern to return to that of shot 3172 and to remain in that form for the rest of the day even when left in the dark. We observed that if the specimen were then exposed to normal laboratory atmosphere overnight, the diffraction pattern would be restored to that of shot 3171 the next morning. It appears that in the dried state a certain portion of the molecules cannot complete the photocycle (proton release followed by proton uptake) due to diffusion and loss of some of the hydrogen ions. Rehydration in normally humid air appears to correct this problem and suggests that we may observe a different time course of structural evolution when we repeat the experiment with fully hydrated specimens.

We have recently completed tests with a special specimen chamber which supports a humidified atmosphere for the specimens inside the camera. Stimulated experiments with this chamber and with an improved scintillator detector system should begin soon. Exciting possibilities for a great many kinds of structural kinetic experiments are suggested by the present results, particularly as developments in laser and materials technology continue to make higher x-ray intensities available.

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