This protective mechanism has been demonstrated in the cyanophyte *Gloeocapsa* (*11*).

Our laboratory findings complement field observations made in Thompson Lake. As the bloom of *Anabaena spiroides* proceeds, accompanied by increased O_2 supersaturation, the ratio of carotenoid to chlorophyll a increases. The phycocyanin content tends to decrease, a phenomenon serving two purposes. (i) Phycocyanin has been shown to be a source of N_2 under conditions of nitrogen stress (*12*). (ii) The decrease in this pigment aids in minimizing photosynthetic O_2 evolution.

An additional mechanism by which *Anabaena* can maintain optimal CO_2 and N_2 fixation in O_2-supersaturated lake water is through a temporal separation of these two processes. In the field as in the laboratory, CO_2 fixation appears more sensitive to O_2 effects than N_2 fixation. If integrated under a square meter of lake surface, maximum CO_2 fixation rates consistently precede maximum N_2 fixation rates as well as maximum O_2 supersaturation (Fig. 3). The afternoon depression in CO_2 fixation could not be linked to a decrease in the inorganic carbon pool, since no significant changes in this pool occurred on a diurnal basis. Furthermore, an inorganic carbon limitation was not demonstrated by bioassays (*13*). The total inorganic carbon was 15 to 20 mg liter^{-1} in both lakes.

Sequential optimization of CO_2 and N_2 fixation, which can be maintained for several months in natural *Anabaena* populations, has a number of advantages. By separating the optimal rates of two light-driven processes in time, competition for photogenerated reductant between both processes is minimized. A higher proportion of photogenerated reductant is allocated to CO_2 fixation during morning than afternoon hours. As CO_2 fixation shows signs of afternoon depression, NA plays an increasingly important role as a recipient for photogenerated reductant. In effect, during the afternoons N_2 fixation acts as a reductant sink, accepting increasing shares of photoreductant as CO_2 fixation becomes less able to utilize photosynthetically active radiation. The afternoon decrease in CO_2 fixation reduces O_2 evolution, which aids in minimizing further O_2 inhibition of N_2 fixation. Finally, adequate supplies of newly fixed carbon skeletons are assured in order to accept fixed nitrogen.

Earlier work has shown that, per unit biomass, a diatom community that does not fix N_2 had patterns and efficiencies of CO_2 fixation similar to *Anabaena*, being similarly inhibited during the afternoon (*14*). The ability of *Anabaena* to divert photoreductant to N_2 fixation pathways during this inhibited period therefore represents much more efficient use of the total daily energy input. Furthermore, 20 to 30 percent of the daily *Anabaena* N_2 fixation occurs in the dark, relying on endogenous carbon pools accumulated during photosynthetic periods.

Under thermally stratified conditions, surface lake waters often become depleted of combined nitrogen, forcing phytoplankton that do not fix N_2 to greater depths where the supplies of combined nitrogen are ample but photosynthetically available radiation is not. The ability of *Anabaena* to successfully dominate surface waters stems from its capacity to utilize N_2, to deal with high light intensity and O_2 concentrations, and to maximize the efficiency of utilization of the total daily available radiant energy.

HANS W. PAERL*
PENELOPE E. KELLAR*
Canada Centre for Inland Waters,
Process Research Division,
Post Office Box 5050,
Burlington, Ontario, Canada

References and Notes

1. H. L. Golterman, *Physiological Limnology* (Elsevier, Amsterdam, 1975); J. N. Eloff, Y. Steinitz, M. Shilo, *Appl. Environ. Microbiol.* **31**, 119 (1976).
2. G. E. Fogg, W. D. P. Stewart, P. Fay, A. E. Walsby, *The Blue-Green Algae* (Academic Press, London, 1973); N. G. Carr and B. A. Whitton, Eds., *The Biology of Blue-Green Algae* (Blackwell, Oxford, 1973).
3. R. Y. Stanier and G. Cohen-Bazire, *Annu. Rev. Microbiol.* **31**, 225 (1977).
4. B. K. Burnison, in preparation.
5. R. J. Flett, R. D. Hamilton, N. E. R. Campbell, *Can. J. Microbiol.* **22**, 43 (1976).
6. E. Steeman-Nielsen, *J. Cons. Cons. Int. Explor. Mer.* **19**, 309 (1954); C. R. Goldman, *Verh. Int. Ver. Theor. Angew. Limnol.* **15**, 365 (1964).
7. J. W. G. Lund, C. Kipling, E. D. LeCren, *Hydrobiologia* **11**, 143 (1958).
8. M. M. Mullin, P. R. Sloan, R. W. Eppley, *Limmol. Oceanogr.* **11**, 307 (1966).
9. *Anabaena oscillarioides* was isolated from the Waikato River, New Zealand, and maintained in anexic cultures by Dr. C. Lam, Ministry of Works, Hamilton, New Zealand.
10. H. W. Paerl, *Oecologia (Berlin)* **32**, 135 (1978).
11. L. De Vasconcelos and P. Fay, *Arch. Microbiol.* **96**, 271 (1974).
12. J. R. Gallon and T. A. LaRue, *Can. J. Microbiol.* **26**, 1633 (1974).
13. H. W. Paerl and P. E. Kellar, *J. Phycol.* **14**, 254 (1978).
14. H. W. Paerl, unpublished data.
15. This work was completed with the aid of the Department of Scientific and Industrial Research, Ecology Division, Taupo, New Zealand, and the Canada Centre for Inland Waters, Burlington, Ontario. Special thanks to D. R. S. Lean for encouraging this research.
* Present address: Institute of Marine Sciences, University of North Carolina, Morehead City 28577.

3 November 1978; revised 29 January 1979

Nanosecond X-ray Diffraction from Biological Samples with a Laser-Produced Plasma Source

Abstract. *By using 4.45-angstrom radiation generated by Cl^{+15} ions in a laser plasma and nanosecond exposures, low-angle x-ray diffraction patterns were obtained from dried rat spinal nerves and a powder of cholesterol. Three to four 400-picosecond, 45-joule pulses were required for the exposure. This new technique should have wide application in structural kinetic studies.*

X-ray diffraction has provided important and often unique information about the structure of biological macromolecules such as proteins and nucleic acids and of macromolecular assemblies such as membranes and muscles. In one area x-ray diffraction has unfulfilled potential of great significance: real-time kinetic studies of structures and macromolecules responding to well-defined stimuli. With conventional sources such as x-ray tubes exposure times are too long for kinetic studies. The use of powerful synchrotron x-ray sources has reduced the required exposure times significantly, and with the completion of the National Synchrotron Light Source at the Brookhaven National Laboratory exposure times as short as a few milliseconds may be possible in x-ray diffraction work (*1*).

In this report we present x-ray diffraction patterns of biological structures obtained with a laser plasma x-ray source. The exposure time for these patterns was of the order of 1 nsec. We believe that the laser plasma x-ray source can be developed into an effective tool for structural kinetic studies with high time resolution. The important characteristics of the source that make this application possible are its highly localized distribution in space and time combined with high spectral brightness (*2*).

If a high-power laser pulse is focused on a solid, an electron avalanche is driven by the optical field turning the solid into an ionized gas. If the laser pulse is of nanosecond duration or less, most of the energy absorbed by the plasma cannot be transported away from the focal region. As a result, very high particle temperatures are produced in a small region, and the plasma exhibits a high degree of ionization. At focused intensities of 10^{14} W/cm^2 or greater, there is significant pro-

0036-8075/79/0511-0622$00.50/0

duction of x-rays, an important component of which is line radiation from the plasma ions (*3*). With a proper choice of target materials and laser intensity, the x-ray line radiation can be concentrated in a few narrow lines. For many applications little filtering is needed to obtain adequate monochromaticity.

In our studies at the University of Rochester's Laboratory for Laser Energetics (LLE) we used a single-beam Nd^{+3}:glass development laser (GDL), which was constructed for engineering evaluation studies of systems and hardware to be employed on the 24-beam OMEGA laser under construction at LLE (*4*). The GDL system is being used to perform a variety of basic laser-matter interaction experiments. The laser is designed to deliver single pulses of radiation at a wavelength of 1.054 μm with a full width at half-maximum (FWHM) that can be varied between 50 and 600 psec. Peak pulse powers greater than 700 GW have been obtained with short pulses, and a maximum energy exceeding 160 J is available with long pulses. A full-power beam divergence of 100 μrad is measured from the 9-cm output aperture of the laser, indicating nearly diffraction-limited performance. The use of liquid-cooled rod amplifiers up to the full 9-cm output aperture permits a system repetition rate of two pulses per hour, the highest of any such glass laser system in operation or under construction. Our experiments were carried out in a vessel 24 inches (61 cm) in diameter, which was evacuated to 1×10^{-5} torr by a system of oil diffusion pumps and liquid nitrogen traps. A schematic diagram of the experimental chamber is given in Fig. 1.

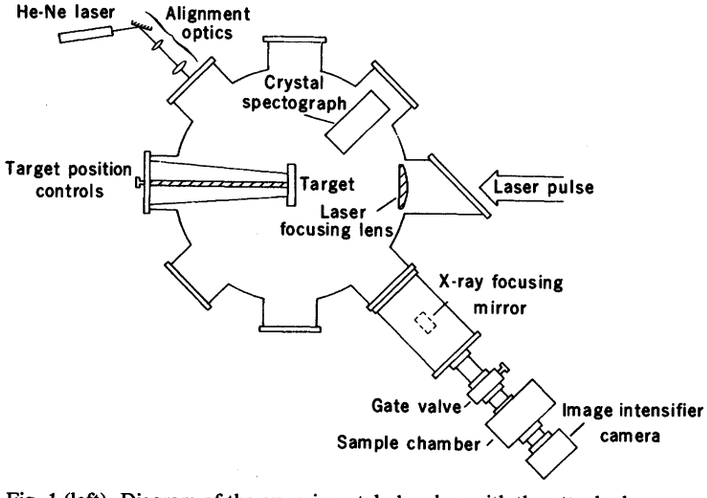
Laser pulses enter the chamber through an optical window and are brought to focus near the tank center by an $f/3.5$ single-element aspheric lens designed and fabricated at the University of Rochester. The laser focal spot diameter was approximately 100 μm. A mechanical assembly mounted on a flange opposite the entrance window allows various targets to be micropositioned in or near the focus while the tank is evacuated. Up to eight different targets may be selected on a target wheel without breaking vacuum.

For our experiments we constructed an x-ray diffraction camera that attaches directly to the high-vacuum chamber described above. The grazing reflection toroidal mirror is similar to one employed by Henke and DuMond (*5*) but has a mean angle of incidence of 0.92°, allowing a useful level of reflectivity to be obtained to wavelengths as short as 2.6 Å. A controlled-environment sample chamber and a scintillator-image intensifier system are included in the camera. The 40-mm ZnS(Ag) scintillator is located 125 cm from the laser plasma source and is coupled to the Amperex model XX1360 25-mm channel plate image intensifier through a fiber optic reducer. The intensifier has an adjustable gain of up to 7×10^4 and is housed in a mount permitting direct attachment of various 35-mm and Polaroid cameras. A lead disk 1.2 mm in diameter is mounted just in front of the scintillator plane to block the direct beam from the x-ray mirror.

Our laser pulses were focused on saran targets. Strong resonance line radiation was obtained from Cl^{+15} ions in the plasma. Most of this line radiation was produced at ~ 4.45 Å. A weaker background continuum was also produced; the softer components were filtered by a beryllium foil, while the very hard components were not reflected by the mirror.

The GDL system was not configured for maximum output energy during our experiments. Laser pulse energies of approximately 45 J with a FWHM of 400 psec were delivered to our targets. Approximately 8×10^{13} x-rays at 4.45 Å were produced on each shot. To simulate the full-energy single-shot capability of our x-ray source, our diffraction patterns were obtained by summing three or four laser shots.

Our first results with this system are shown in Fig. 2. We worked with two different samples, a powder of cholesterol (*6*) and an array of dried rat spinal



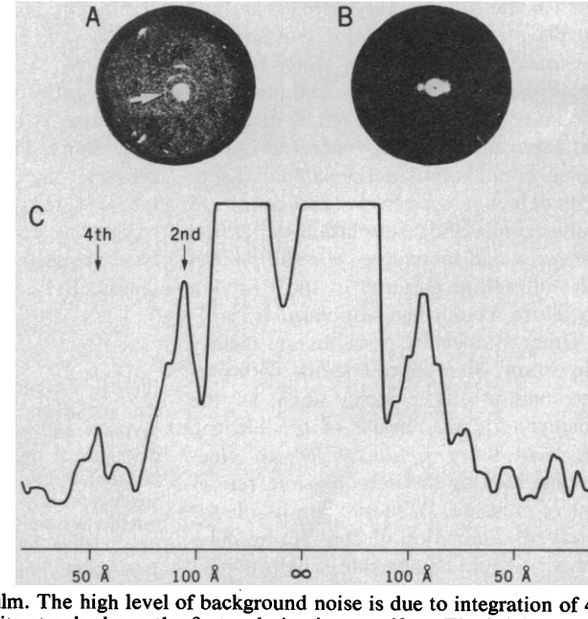


Fig. 1 (left). Diagram of the experimental chamber with the attached x-ray camera. The He-Ne laser was used to align the x-ray camera. Fig. 2 (right). Diffraction patterns obtained with a laser plasma x-ray source. (A) The arrow points to the first-order (34.9 Å) diffraction ring from a 30- to 50-μm-thick powder of cholesterol (*6*). Three 400-psec laser pulses with a total energy of 118 J were used. The pattern was recorded on Polaroid 410 film. The high level of background noise is due to integration of 45 seconds of dark current because the intensifier was manually gated. The white streak above the first-ordering is an artifact. The bright region around the central beam stop is due to x-rays scattered by the toroidal x-ray collector and not completely baffled. (B) Polaroid 410 photograph of second-order diffraction from dried rat spinal roots (*7*). The diffraction is due to the 170-Å repeat period in the myelin membranes around the axons of the nerve. Four 400-psec laser shots with a total energy of 190 J were used to obtain the exposure. (C) Densitometric scan of another diffraction pattern of dried rat spinal roots. The original exposure was obtained on Kodak 2475 film. Four 400-psec pulses with a total energy of 191 J were used to obtain the exposure. The second and fourth orders from the myelin membranes are labeled. The dip at ∞ is due to the central beam stop.

roots (*7*). The powder was dried from solution on a 50-μm beryllium foil to a thickness of 30 to 50 μm. The nerve fibers were removed from freshly killed albino rats and air-dried under slight tension. Twenty fibers were mounted in parallel to cover the full aperture of the x-ray beam. Fiber thicknesses ranged from 30 to 100 μm.

The samples were placed in vacuum approximately 5 cm from the scintillator plane. A stop was placed in front of the samples to limit scattered x-rays in the camera system (*5*). Although this was adequate to permit observation of the diffraction in this case, a smaller stop will be used in the future to more fully eliminate the bright feature in the center of the patterns.

The sharpness of the first-order diffraction ring from cholesterol (34.9 Å) indicates that our camera was in good focus. The diffraction from the spinal roots is due to the 170-Å repeat period in the myelin membranes. The more diffuse second- and fourth-order diffraction spots from the nerve are probably due to structural imperfections produced during drying and mounting. We believe that the use of wet samples with improved ordering, along with straightforward improvements in our x-ray diffraction camera, will enable us to obtain patterns with much improved detail on a single laser shot.

Many kinetic experiments in organic and inorganic systems are possible. In particular, photoresponsive membranes such as the rod outer segment (*8*) and the purple membrane of *Halobacterium halobium* (*9*) seem ideal for study by low-angle x-ray diffraction techniques with the laser plasma x-ray source. In a typical experiment a laser-generated optical pulse would stimulate the sample. Then, with delays ranging from nanoseconds to milliseconds, the laser plasma-generated x-rays would interrogate the sample. X-ray diffraction patterns of the evolving structure would thus be obtained.

Other studies are possible and clearly important. Membrane systems with predominantly one protein, such as the postsynaptic membrane of the electric organ of the *Torpedo californica* electroplax (*10*) or the sarcoplasmic reticulum of muscle (*11*), should be suitable for study. Examination of contracting muscle (*12*) would be possible. The prospect of low-angle x-ray scattering by macromolecules in solution (*13*) with the added dimension of time resolution opens many new experimental paths. The temporal evolution of the binding of an enzyme to its substrate, the assembly of macromolecular structures such as ribosomes and nucleosomes, and the folding of proteins might be studied.

The power and energy of large laser systems continues to increase. At the same time the mechanisms of the laser light-matter interaction are becoming better understood. We are confident that in the near future the laser plasma will produce a sufficient flux of x-rays to give single-shot subnanosecond x-ray diffraction patterns of high quality for structural kinetic studies.

ROBERT D. FRANKEL
Laboratory for Laser Energetics, University of Rochester, Rochester, New York 14623
JAMES M. FORSYTH
Institute of Optics and Laboratory for Laser Energetics, University of Rochester

References and Notes

1. G. Rosenbaum, K. C. Holmes, J. Witz, *Nature (London)* **230**, 434 (1971); J. P. Blewett, Ed., *Brookhaven Natl. Lab. Rep. 50595* (1977), pp. 3–15.
2. J. M. Forsyth, *Trans. Am. Crystallogr. Assoc.* **12**, 11 (1976).
3. N. G. Basov, V. A. Boiko, Yu. P. Voinov, E. Ya. Kononov, S. L. Mandel'shstam, G. V. Sklizokov, *Zh. Eksp. Teor. Fiz. Pisma Red.* **5**, 177 (1967), translated in *JETP Lett.* **5**, 141 (1967); P. J. Mollozzi, H. M. Epstein, R. G. Jung, D. C. Applebaum, B. P. Fairand, W. J. Gallagher, in *Fundamental and Applied Laser Physics*, M. S. Feld, A. Javan, N. A. Kurnit, Eds. (Wiley, New York, 1973), p. 165; V. A. Boiko, O. N. Krokhin, S. A. Pikuz, A. Ya. Faenov, *Sov. J. Quantum Electron.* **4**, 1212 (1975); D. J. Nagel, *Adv. X-Ray Anal.* **18**, 1 (1975).
4. J. M. Eastman, J. A. Boles, J. M. Soures, *Laser Focus* **14**, 48 (December 1978).
5. B. L. Henke and J. W. M. DuMond, *J. Appl. Phys.* **26**, 903 (1955).
6. R. S. Bear, J. J. Palmer, F. O. Schmitt, *J. Cell. Comp. Physiol.* **17**, 355 (1941).
7. F. O. Schmitt, R. S. Bear, G. L. Clark, *Radiology* **25**, 131 (1935).
8. C. R. Worthington, *Annu. Rev. Biophys. Bioeng.* **3**, 53 (1974).
9. R. Henderson, *J. Mol. Biol.* **93**, 123 (1975); A. E. Blaurock, *ibid.*, p. 139.
10. M. J. Ross, M. W. Klymkosky, D. A. Agard, R. M. Stroud, *ibid.* **116**, 635 (1977).
11. L. Herbette, J. Marquardt, A. Scarpa, J. K. Blaise, *Biophys. J.* **20**, 245 (1977).
12. H. E. Huxley and W. Brown, *J. Mol. Biol.* **30**, 383 (1967).
13. O. Kratsky and I. Pilz, *Q. Rev. Biophys.* **8**, 129 (1972).
14. Partially supported by the Laser Fusion Feasibility Project, which is sponsored by Exxon Research and Engineering Company, General Electric Company, Northeast Utilities, Empire State Electric Energy Research Company, and the New York State Energy and Research Development Administration. We thank W. Dickenson, H. Graf, and R. Taylor for technical assistance; S. Gruner, J. Milch, H. Sobell, and C. Worthington for helpful discussions; E. Koenig for use of facilities for the preparation of nerve fibers; and J. Puskin for the cholesterol.

13 February 1979

Hydroxylase Cofactor Activity in Cerebrospinal Fluid of Normal Subjects and Patients with Parkinson's Disease

Abstract. *A method for measuring hydroxylase cofactor activity in human cerebrospinal fluid is described. The hydroxylase cofactor content of cerebrospinal fluid from Parkinsonian patients is approximately 50 percent that of normal subjects. A significant correlation between hydroxylase cofactor and the concentration of homovanillic acid in the cerebrospinal fluid was observed.*

The initial step in the biosynthesis of dopamine is the enzymic hydroxylation of tyrosine. This reaction is catalyzed by tyrosine hydroxylase, which utilizes tyrosine, tetrahydrobiopterin (BH_4), and oxygen as cosubstrates. Recent studies have suggested that the hydroxylase cofactor, BH_4, plays a regulatory role in the synthesis of catecholamine neurotransmitters (*1*, *2*). Since the presence of hydroxylase cofactor in cerebrospinal fluid might reflect the in situ functional capability of central catecholamine neurons in man, we attempted to devise a sufficiently sensitive assay for this compound in cerebrospinal fluid (CSF). The

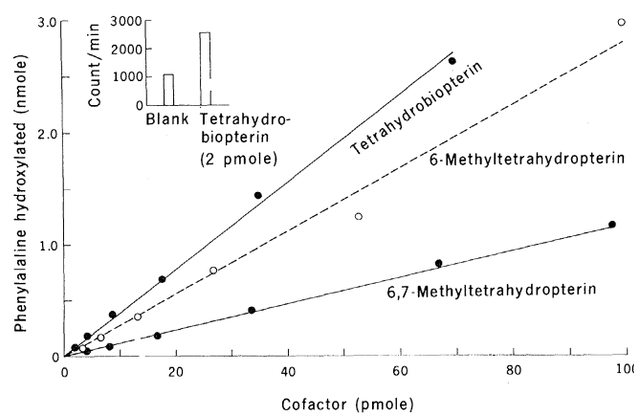


Fig. 1. Response of the cofactor assay system to various amounts of hydroxylase cofactors. In the absence of any cofactor the system gave a blank value of about 1000 count/min (upper left), whereas the addition of as little as 2 pmole of BH_4 generates more than twice this number of counts.

0036-8075/79/0511-0624$00.50/0