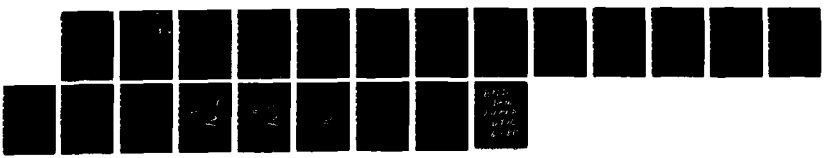
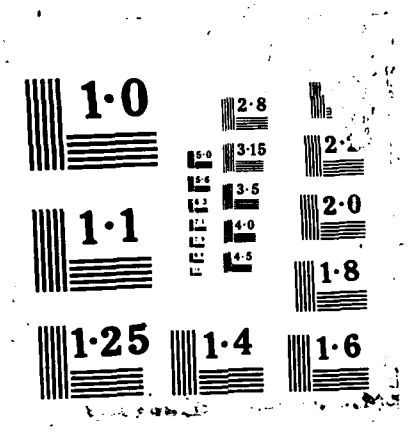
AD-A192 386 PRESSURE STUDIES OF PROTEIN DYNAMICS(U) ILLINOIS UNIV 1/1
AT URBANA DEPT OF PHYSICS H FRAUENFELDER ET AL
26 FEB 88 N00014-86-K-0270

UNCLASSIFIED F/G 6/1 NL




1·0
1·1
1·25
2·8
3·15
3·5
4·0
4·5
2·0
1·8
1·4
1·6

ONR Grant #N00014-86-K-0270

PRESSURE STUDIES OF PROTEIN DYNAMICS

Hans Frauenfelder and Robert D. Young

Department of Physics
University of Illinois at Urbana-Champaign

Annual Report to Office of Naval Research

1. Summary of Research Goals and Methods

The key goal of biophysical and biochemical research is a fundamental understanding of the relation among protein structure, motions, and function (1). We have developed a hierarchical model to describe protein conformations and motions (2,3). We study the binding of small ligands like carbon monoxide (CO) and dioxygen to myoglobin (Mb) and other heme proteins. Our main experimental tool is flash photolysis over wide ranges of time, temperature, and pressure as well as solvent viscosity and pH (4,5). Studies of ligand binding to myoglobin over wide ranges of pressure and temperature probe protein states and motions which are not accessible by varying only temperature or pressure. Although living systems function over wide ranges in pressure from high altitude to the ocean depths, pressure effects have been relatively unexplored in protein science because of their complexity and subtlety (6,7,8). We have discovered a superb marker, the infrared CO strectching bands in

SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

1a. REPORT SECURITY CLASSIFICATION (U)	1b. RESTRICTIVE MARKINGS NA
2a. SECURITY CLASSIFICATION AUTHORITY NA	3. DISTRIBUTION/AVAILABILITY OF REPORT Distribution Unlimited
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE NA	
4. PERFORMING ORGANIZATION REPORT NUMBER(S) NA	5. MONITORING ORGANIZATION REPORT NUMBER(S) NA
6a. NAME OF PERFORMING ORGANIZATION University of Illinois 6b. OFFICE SYMBOL (*If applicable*) NA	7a. NAME OF MONITORING ORGANIZATION Office of Naval Research
6c. ADDRESS (*City, State, and ZIP Code*) Department of Physics University of Illinois at Urbana-Champaign 1110 W. Green St., Urbana, IL 61801	7b. ADDRESS (*City, State, and ZIP Code*) 800 North Quincy Street Arlington, VA 22217-5000
8a. NAME OF FUNDING/SPONSORING ORGANIZATION Office of Naval Research 8b. OFFICE SYMBOL (*If applicable*) ONR	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER N00014-86-K-0270
8c. ADDRESS (*City, State, and ZIP Code*) 800 North Quincy Street Arlington, VA 22217-5000	10. SOURCE OF FUNDING NUMBERS PROGRAM ELEMENT NO. 61153N; PROJECT NO. RR04106; TASK NO. 4413015; WORK UNIT ACCESSION NO

11. TITLE (*Include Security Classification*)

(U) Pressure Studies of Protein Dynamics

12. PERSONAL AUTHOR(S)
Hans Frauenfelder and Robert D. Young

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (*Year, Month, Day*)	15. PAGE COUNT
Annual	FROM 3/1/87 TO 2/28/88	2-26-88	

16. SUPPLEMENTARY NOTATION

17. COSATI CODES			18. SUBJECT TERMS (*Continue on reverse if necessary and identify by block number*)
FIELD	GROUP	SUB-GROUP	Protein dynamics, pressure, myoglobin, [illegible] hierarchy of substates, flash photolysis, (CO) stretching bands, glass-like properties

19. ABSTRACT (*Continue on reverse if necessary and identify by block number*)

In this research we extend and deepen our studies of the relation between dynamic structure and function in proteins. We study protein dynamics using flash photolysis together with near ultraviolet, visible, and near and mid-infrared spectroscopies over wide ranges in time (50 ns-10 ks), temperature (60-320K), and pressure (0.1-100 MPa). Initially we study a simple biomolecular reaction -- carbon monoxide (CO) binding to myoglobin (Mb). We have greatly expanded knowledge of pressure effects on proteins by using the infrared CO stretching frequencies in MbCO as a probe. The combined pressure and temperature studies shed new light on various features of the hierarchical model of protein substates and motions. These studies also provide much information on the glass-like behavior of proteins, including the slaved glass transition and glass-like relaxation processes near the transition temperature.

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT ☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT. ☐ DTIC USERS	21. ABSTRACT SECURITY CLASSIFICATION (U)
22a. NAME OF RESPONSIBLE INDIVIDUAL Dr. M. Marron	22b. TELEPHONE (*Include Area Code*) (202) 696-4038 22c. OFFICE SYMBOL ONR

DD FORM 1473, 84 MAR 83 APR edition may be used until exhausted. All other editions are obsolete.

SECURITY CLASSIFICATION OF THIS PAGE

MbCO, for exploring the effects of pressure on proteins and protein reactions (9,10,11,12).

The concepts of conformational substates (13,14) and a slaved glass transition (10) in proteins are proving to be crucial in understanding the relation among protein structure, motions, and function. The concept of conformational substates implies that the ground state of a protein is highly degenerate and consists of many nearly isoenergetic minima in the conformational energy surface. A similar situation exists for spin glasses and other complex systems (15). The slaved glass transition (SGT) in myoglobin is similar to the "freezing" transition in a spin glass or glass (16,17). Our combined pressure and temperature experiments probe the glassy properties of proteins and, thus, connect two distant fields of research. This situation may lead to a more detailed understanding of both biomolecular relaxation and reactions and the physics of amorphous solids.

2. Summary of Accomplishments in Year Two

Significant progress has occurred during the second contract year. We have presented several papers on our results at meetings of the Biophysical Society (9,11,12). The work reported here has also resulted in one doctoral dissertation (18). We are preparing these results for a detailed publication. We only outline the main results in this report.

CO can bind to Mb in at least three positions which can be distinguished by their infrared CO stretching bands (19). In MbCO

the three main stretching bands, denoted by A_0(~1966 cm^{-1}), A_1(~1945 cm^{-1}), and A_3(~1930 cm^{-1}), are sensitive to external influences such as pH, solvent. composition, temperature, and, most importantly for this project, pressure (9,10,11,12,18). Observation of the infrared band is done with two systems: (i) quasi-static and slow kinetics experiments are performed on a Fourier transform infrared (FTIR) spectrometer (Mattson Sirius 100 FTIR); (ii) fast kinetics experiments are performed on a homebuilt, microsecond, mid-infrared flash photolysis system using a tunable infrared-diode to produce the monitoring light. We have also measured the heat capacity of the protein samples in a differential scanning calorimeter (11).

Two related classes of experiments were performed on MbCO: quasi-static and relaxation. In the quasi-static experiments we measure the characteristics of the CO stretching bands as function of temperature, pressure, pH, and solvent composition. We call the situation quasi-static because the glass-like behavior of the protein below a characteristic glass temperature Tg makes attainment of true equilibrium impossible on any reasonable timescale. In the relaxation experiments we observe the relaxation of the protein by monitoring the CO stretching bands after a fast pressure release at a temperature near to the glass temperature.

(a) Quasi-static Experiments. We have measured the IR spectrum of MbCO from 1910 to 1990 cm^{-1} in a 75% glycerol/water solvent (pH 7 with potassium phosphate buffer) from 140K to 340K in temperature and 0.1 MPa to 100 MPa in pressure. (1 MPa = 10

bar). The spectra are resolved into three bands denoted in order of decreasing wavenumber by A_0, A_1, and A_3 which correspond to conformational substates of tier 0 also denoted by A_0 to A_3. The main results of the quasi-static experiments can be seen in Fig. 1 where we plot the temperature dependence of the ratio $r_0 = A_0/A_1$ of the total areas of the two bands at two different pressures. The ratio r_0 shows six different regions denoted by i-vi starting at low temperature: (i) Glass region. The protein is in a glass-like, non-equilibrium state in which large scale motions are frozen, but motions of groups of atoms still occur. This situation is dramatically illustrated by the temperature independence of the ratio r_0 over a timescale of several hours. (ii) Slaved glass transition region. Depending on the observation time and temperature, large scale motions (relaxation) are observable or appear frozen. Region ii extends from about 170K to about 210K and is centered at about 185K in 75% glycerol/water solvent. We have found a strong dependence of the glass temperature on the composition of the external solvent in our FTIR measurements. This dependence has been confirmed by the heat capacity measurements with the DSC on several different solvents with and without MbCO. Thus region ii shows a very strong dependence on external solvent. (iii) Lower equilibrium region. Transitions among the substates A_0 to A_3 occur faster than the time of observation so that the ratio r_i follows a van't Hoff relation. The temperature and pressure dependence of the ratios $r_i = A_i/A_1$ allows the energies, entropies, and volumes of the substates A_i to be determined. We find that A_0 is more tightly bound than A_1 and A_3 and that A_0 has the smallest volume and

entropy. (iv) Lower transition region. This is also an equilibrium region in which the change in slope of r_0 suggest a new transition. A possible explanation is the breaking of a few hydrogen bonds in the protein. (v) Upper equilibrium region. The region again follows a simple van't Hoff relation but the change in slope indicates that a new substate A'_o is dominant which is less tightly bound than A_0 and which has a larger entropy and volume than substate A_1. This second substate A'_0 may indicate an intermediate protein state to unfolding. (vi) Unfolding. The protein begins to unfold irreversibly.

Figure 2 shows the heat capacity of MbCO in 75% glycerol/water solvent as measured with a DSC. The rapid increase in the rate of heat absorption dH/dt centered at 186K is characteristic of a glass transition. No pronounced anomoly occurs until 390K (the helix-coil transition).

(b) Relaxation Experiments. In a typical pressure release experiment, a pressure of 100 MPa is applied at 240K and the sample is cooled to a temperature between 210K and 170K. The temperature is held for about 100s and the pressure is released in 10sa or less to a lower pressure (7 MPa). Infrared spectra in the CO stretching region are then taken at exponentially increasing times from 10s to 100ks.

Our main results are summarized as follows: Between 170K and 190K the width narrows and the peak frequency shifts toward the low pressure values in the case of the A_0 absorption band while the total area of A_0 remains the same, indicating internal redistribution

in the A_0 substate. This internal redistribution corresponds to transitions within tier 0 of the hierarchy of substates. Slow interconversion of A_1 and A_3 also occurs in this temperature range resulting in a concommitant change in the areas of these two bands. Fig. 3 shows the time behavior of the A bands after the fast pressure release at 180K.

Between 195K and 210K slow interconversion of A_0 with A_1 and A_3 is observed while A_1 and A_3 exchange rapidly. The transitions between different A bands correspond to transitions within tier 1 of the hierarchy. Fig. 4 shows the time behavior of the A bands after the fast pressure release at 195K. Fig. 4a gives absolute absorption spectra and Fig. 4b shows the spectra normalized to IR band A_1. Fig. 4b indicates that A_1 and A_3 are in equilibrium at 195K.

These results show similarities to the dynamics of spin glasses and glasses: The observed relaxations are highly nonexponential in time indicating a distribution of relaxation rates. Each A substate can thus consist of many sub-substates. The relaxations "freeze out" within a narrow temperature interval of about 20K with the rates having a Vogel-Fulcher like temperature behavior in which the relaxation rates have a singularity at a temperature T_o significantly below the glass temperature T_g. An important difference between the glass transition in myoglobin and the glass transition in amorphous solids is the "slaving" of the transition to the composition of the external solvent in the case of proteins.

(c) Preliminary Pressure Titration Experiments. The concept of the pressure titration experiment (7,20,21) was described in the Annual Report for Year One to the Office of Naval Research. We have been conducting preliminary experiments using the microsecond, mid-infrared flash system to monitor the A_1 infrared band after photodissociation by a laser pulse. The goal of these experiments is to probe relaxation of the A_1 substate in tier 1 of the hierarchy. Freezing out of motions within tier 1 of the hierarchy of conformational substates is responsible for the nonexponential rebinding kinetics of MbCO at low temperature. In 75% glycerol/water solvent the motions in tier 1 freeze out below about 180K (13,14). Preliminary measurements are promising

3. Plans for the Third Contract Year - 1988

Work on this contract is progressing well. We are in the process of improving the design of the pressure cell. One benefit from the improved design of the cell is a smaller thermal mass which will greatly improve the efficiency of the refrigeration system used in the pressure titration experiments. We are continuing to design and build the special equipment needed to do frequency domain studies of pressure effects. We are planning the following experiments during the third contract year.

(a) Frequency domain studies of pressure effects. The sensitivity of the CO stretching bands to pressure makes it possible to modulate the pressure with a piezoelectric crystal and to observe the corresponding pressure changes with a lock-in technique. In

principle we can extend our time (frequency) range by several orders of magnitude. We are building such a system and plan to apply it to studies of the slaved glass transition in myoglobin including observation of nonexponential relaxation at temperatures above the glass temperature T_g.

(b) Pressure titration studies of the CO stretching bands. We will continue the pressure titration studies on the A_1 band using the microsecond, mid-infrared flash photolysis system. The rather dramatic sensitivity of the intensity of the CO stretching bands makes these experiments much more feasible than in the past.

(c) Waiting time dependence of relaxation. Relaxation studies of the magnetization in metallic spin glasses below the glass transition temperature have shown that the relaxation of the magnetization depends on the waiting time before the external field is decreased to zero. These waiting time effects have been attributed to the underlying hierarchical free energy surface (22,23) but are not fully understood. In our pressure release experiments typically the waiting times were kept at about 100s before pressure was released. We will vary the waiting time from a few seconds to about 10ks in order to determine any waiting time effects on the relaxation of the A bands.

(d) Pressure studies on mutant myoglobins. Steve Sligar of the University of Illinois has recently succeeded in producing sperm whale myoglobin using genetic engineering (24). We have performed exploratory measurements on several mutant myoglobins

in which the distal histidine has been replaced by a different amino acid residue. We intend to extend our measurement of the infrared CO stretching bands to include the effects of pressure on the mutant myhoglobins.

(e) Ligand binding studies. Studies of the slaved glass transition and relaxation of the CO stretching bands after pressure release have proven to be unexpectedly rewarding but time-consuming. We have therefore delayed studies of the pressure dependence of ligand rebinding after flash photolysis but plan to return to them.

References

[Note: The references indicated by an asterisk(*) were supported in part by this contract and were published during Year Two.]

1. Structure, Dynamics, and Function of Biomolecules, Ed. Ehrenberg, R., Rigler, R., Graslund, A., and Nilsson, L., Springer-Verlag, (1987).

2. Frauenfelder, H. in Structure and Motion: Membranes, Nucleic Acids and Proteins, Eds. Clementi, E., Corongiu, G., Sarma, M. H., and Sarma, R. H., Adenine Press, new York, p. 205, (1985).

3. Ansari, A. et al, Proc. Natl. Acad. Sci. USA 82, 5000 (1985).

4. Beece, D. et al, Biochemistry 19, 5147 (1980).

5. Doster, W. et al, Biochemistry 21, 4831 (1982).

6. Hasinoff, B., Biochemistry, 13, 3111 (1974).

7. Eisenstein, L. and Frauenfelder, H., in Frontiers of Biological Energetics, Vol. 1, 680, Academic Press (1978).

8. Weber, G. and Drickamer, H., Q. Rev. Biophys. 16, 89 (1983).

9. Ansari, A., Biophys. J. 51, 289a (1987).

*10. Ansari, A. et al, Biophys. Chem. 26, 337 (1987).

*11. Iben, I. E. T. et al, Biophys. J. 53, 275a (1988).

*12. Iben, I. E. T. et al, Biophys. J. 53, 275a (1988).

13. Austin, R. H. et al, Biochemistry 14, 5355 (1975).

14. Frauenfelder, H., Parak, F. and Young, R. D., Ann. Rev. Biophys. Biophys. Chem. 17, 451 (1988).

15. Stein, D., Proc. Natl. Acad. Sci. USA 82, 0. 3670 (1985).

16. Binder, K. and Young, A. P., Rev. Mod. Phys. 58, 801 (1986).

17. Jaeckle, J., Rep. Prog. Phys. 49, 171 (1986).

*18. Iben, I. E. T., Ph.D. Dissertation, University of Illinois at Urbana-Champaign (1988).

19. Ormos, P. et al, Biophys. J. 53, 274a (1988).

20. Frauenfelder, H. and Young, R. D., Comments Mol. Cell. Biophys. 3, 347 (1986).

21. Young, R. D. in The Time Domain in Surface and Structural Dynamics, Eds. Grandjean, F. and Lang, G., Reidel Press, Dordrecht, p. 107 (1988).

22. Hoogerbeets, R., Luo, W.-L., Orhbach, R., Phys. Rev. B34, 1719 (1986).

23. Springer, B. and Sligar, S. G., Proc. Natl. Acad. Sci. USA 84, 8961 (1987).

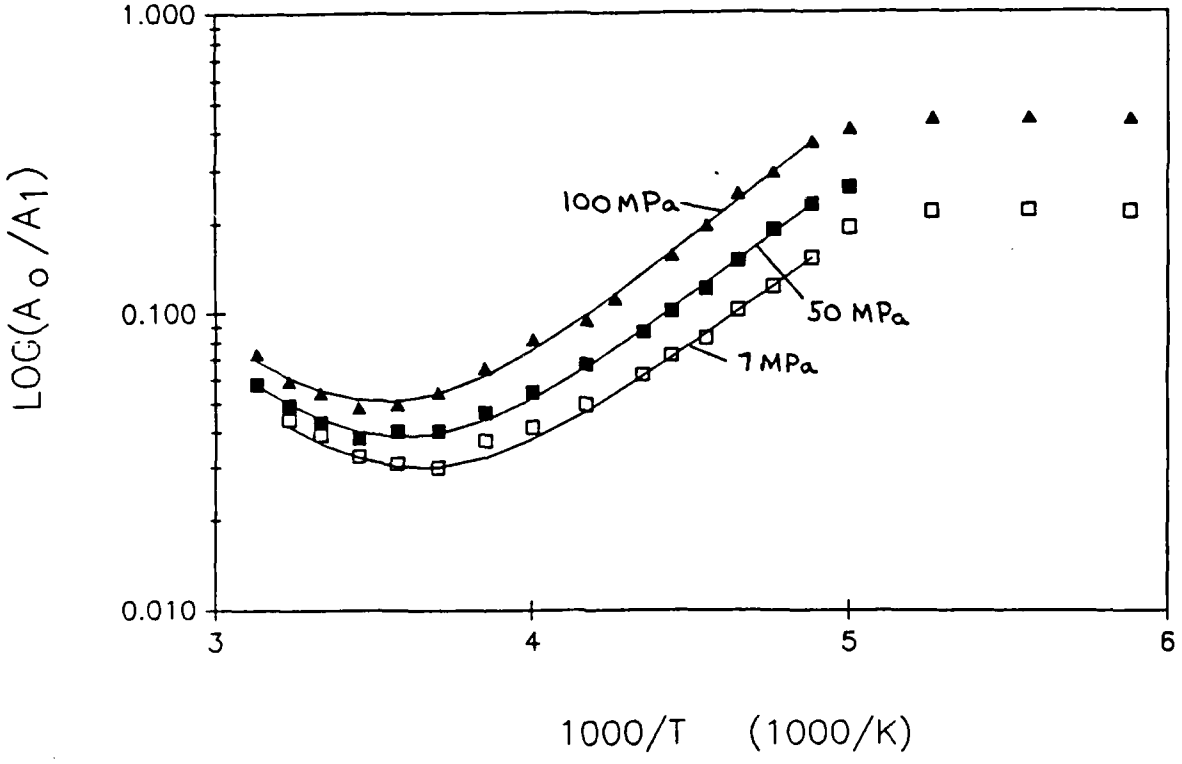
Figure Captions

1 (a) Logarithm of the ratio of areas for substates A_0 and A_1 versus inverse Kelvin temperature (1000/T) for three pressures: 7 MPa, 50 MPa, and 100 MPa. Solvent: 75% glycerol/water buffered to pH 7. (b) Definitions of temperature regions at 7 MPa: (i) Glass region, (ii) Glass transition region, (iii) Lower equilibrium region, iv) Upper transition region, (v) Upper equilibrium region, (vi) Denaturation region.

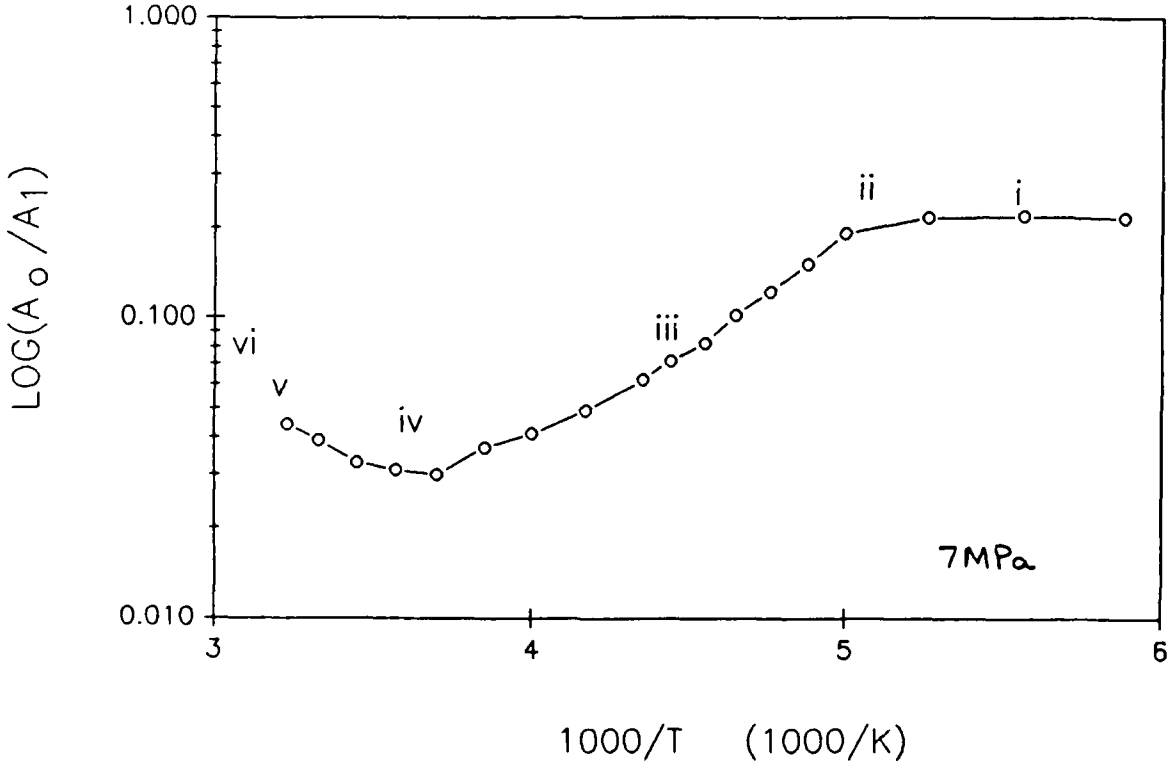
2 Thermal response of MbCO (75% glycerol/water buffered to pH 7 with potassium phosphate) using differential scanning calorimetry. Rate of heat absorption versus temperature (Celsius).

3 Time evolution of the infrared absorption spectra corresponding to the CO stretching bands in MbCO at 180K after a fast pressure release from 100 MPa to 7 MPa. Time range: 10s to 3.8ks (75% glycerol/water buffered to pH 7 by potassium phosphate).

4 Time evolution of the infrared absorption spectra corresponding to the CO stretching bands in MbCO at 195K after a fast pressure release from 100 MPa to 7 MPa. Time range: 23s to 2.4ks (75% glycerol/water buffered to pH 7 by potassium phosphate). a) Absolute spectra, b) Spectra normalized to A_1.


1.000
0.100
0.010
LOG(A o /A1)
100 MPa
50 MPa
7 MPa
3
4
5
6
1000/T (1000/K)

(a)


1.000
0.100
0.010
LOG(A o /A1)
vi
v
iv
iii
ii
i
7MPa
3
4
5
6
1000/T (1000/K)

(b)

FIG. 1

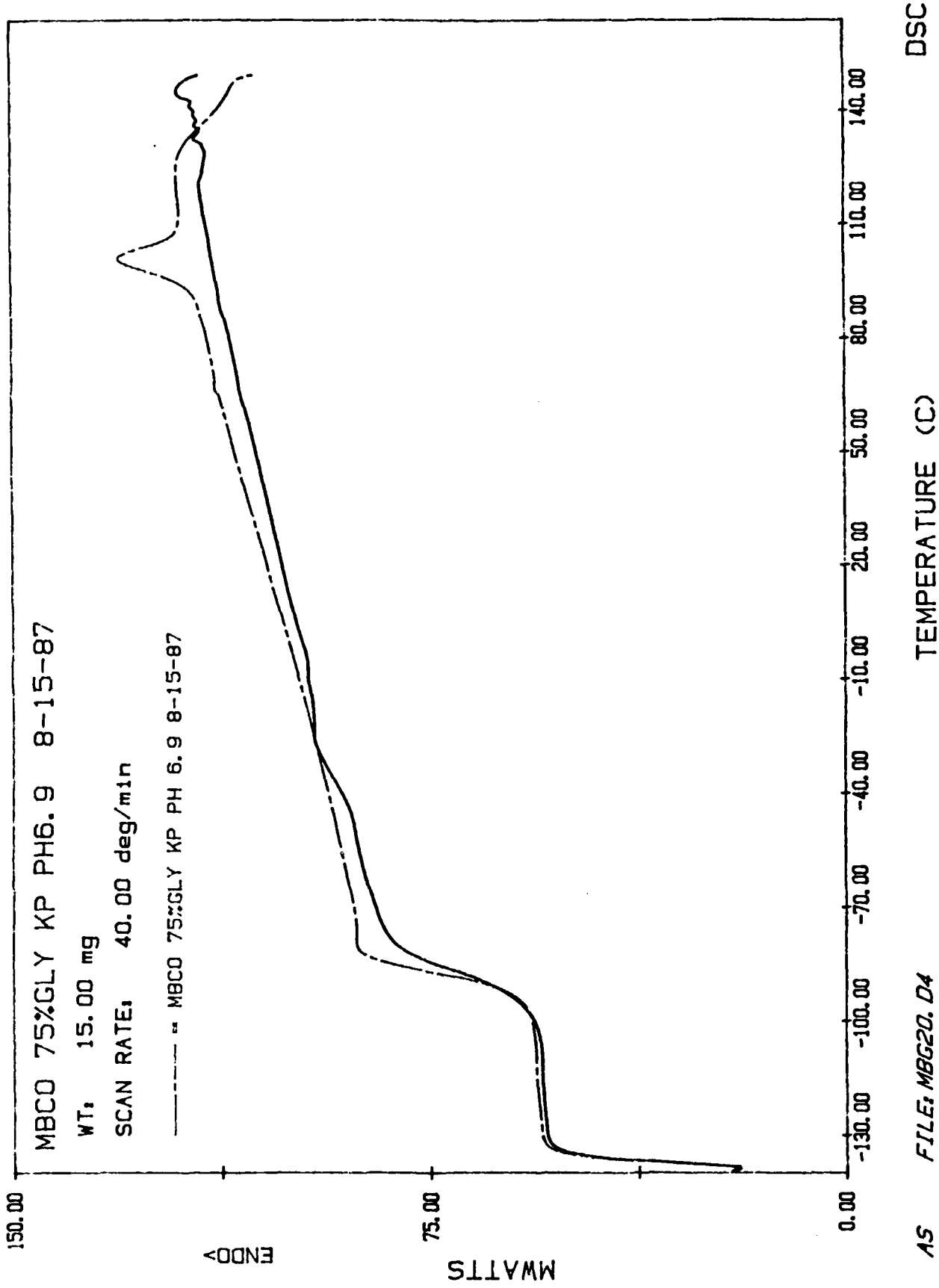

MBCO 75%GLY KP PH6.9 8-15-87
WT: 15.00 mg
SCAN RATE: 40.00 deg/min
MBCO 75%GLY KP PH 6.9 8-15-87
150.00
75.00
0.00
MWATTS
ENDO>
-130.00
-100.00
-70.00
-40.00
-10.00
20.00
50.00
80.00
110.00
140.00
TEMPERATURE (C)
DSC
AS
FILE: MBG20.D4
DATE: 87/12/29
TIME: 12:58

FIG. 2

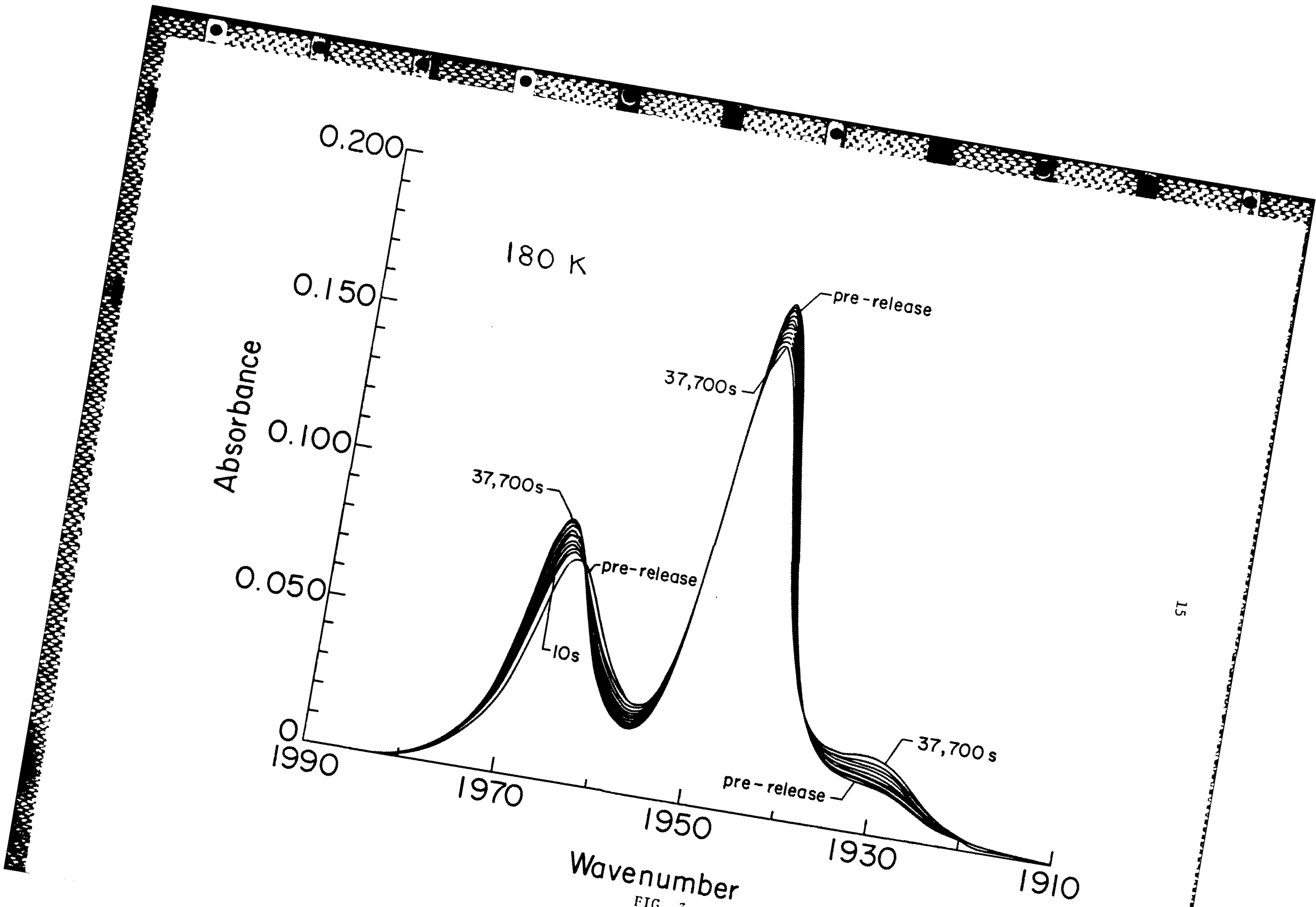

0.200
0.150
0.100
0.050
0
Absorbance
180 K
pre-release
37,700s
37,700s
pre-release
10s
37,700 s
pre-release
1990
1970
1950
1930
1910
Wavenumber

FIG. 3

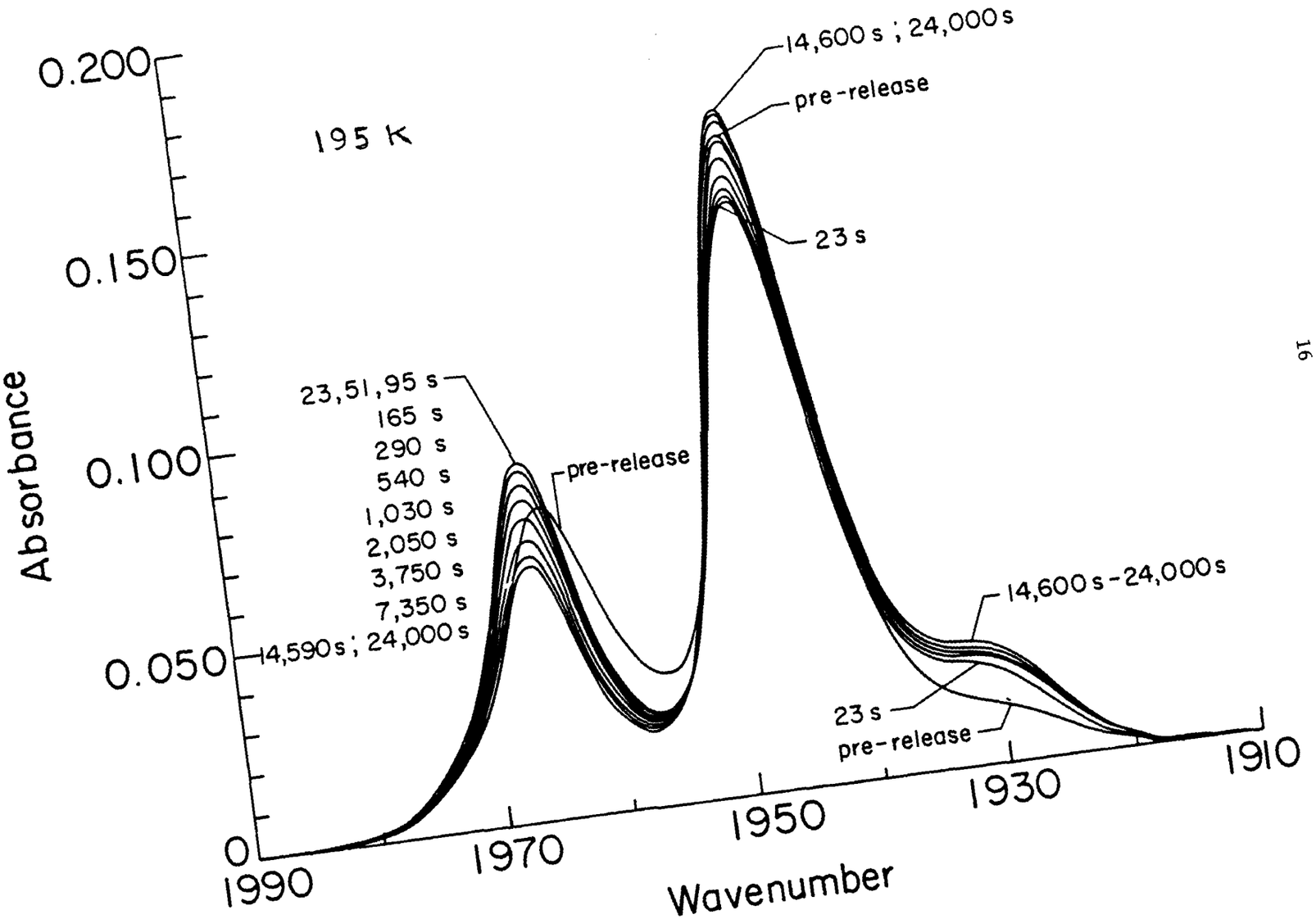

0.200
0.150
0.100
0.050
0
Absorbance
195 K
14,600 s ; 24,000 s
pre-release
23 s
23,51,95 s
165 s
290 s
540 s
1,030 s
2,050 s
3,750 s
7,350 s
14,590 s ; 24,000 s
pre-release
14,600 s - 24,000 s
23 s
pre-release
1990
1970
1950
1930
1910
Wavenumber

FIG. 4a

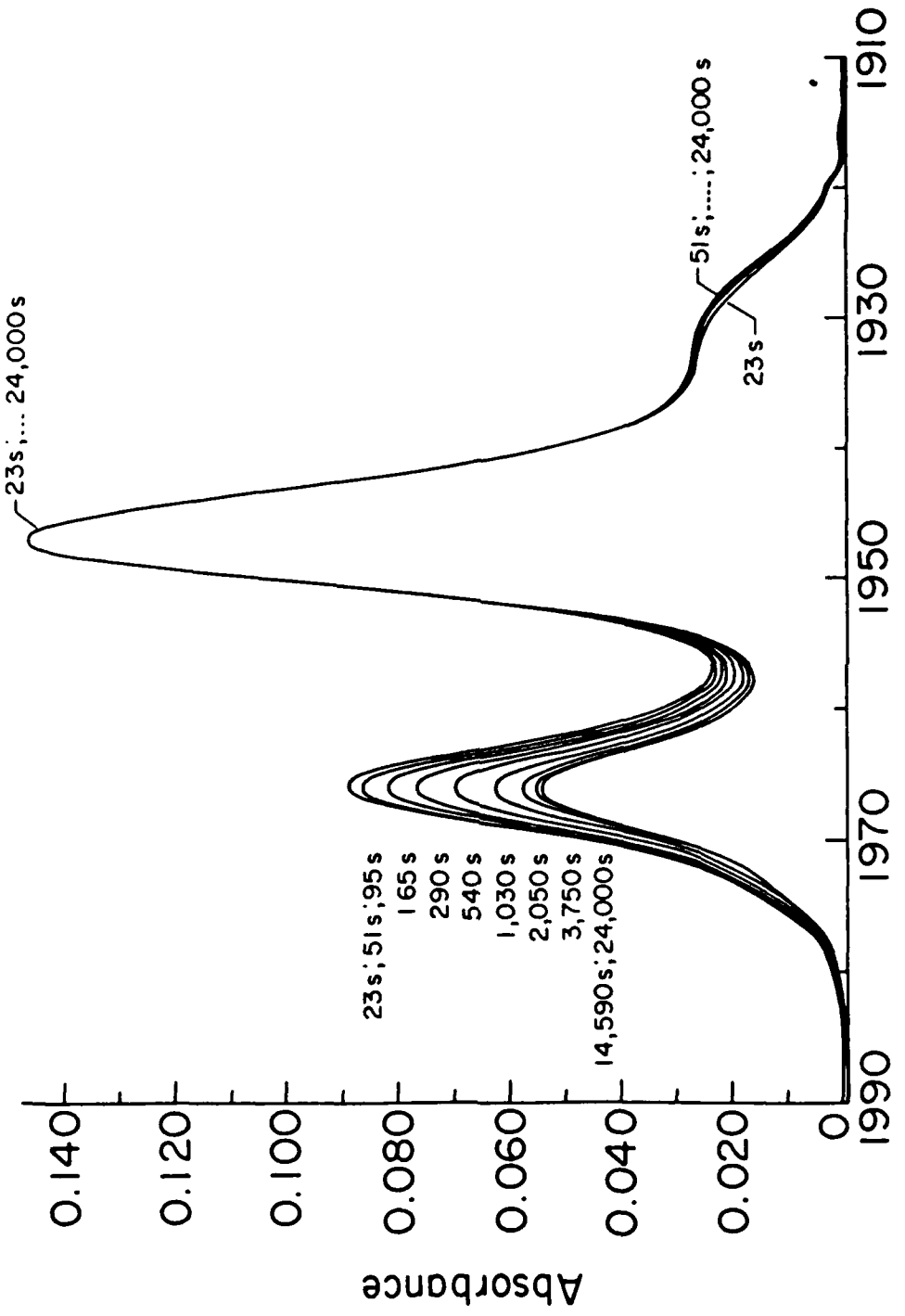

Absorbance
0.140
0.120
0.100
0.080
0.060
0.040
0.020
0
1990
1970
1950
1930
1910
Wavenumber
23s;... 24,000s
23s;51s;95s
165s
290s
540s
1,030s
2,050s
3,750s
14,590s;24,000s
51s;....;24,000s
23s

FIG. 4b

Dr. Margo G. Haygood
Office of Naval Research
800 North Quincy Street
Arlington, VA 22217-5000

Dr. M. Marron
Code 1141MB
Office of Naval Research
800 North Quincy Street
Arlington VA 22217-5000

Adminstrator (2 copies, Enclose DTIC Form 50)
Defense Technical Information Center
Building 5, Cameron Station
Alexandria, VA 22314

ANNUAL AND FINAL REPORTS ONLY (One copy each)
Commander
Chemical and Biological Sciences Division
Army Research Office
P. O. Box 12211
Research Triangle Park, NC 27709

Directorate of Life Sciences
Air Force Office of Scientific Research
Bolling Air Force Base
Washington, DC 20332

Chemistry and Atmospheric Sciences Directorate
Air Force Office of Scientific Research
Bolling Air Force Base
Washington, DC 20332

Director
Biotechnology Division
CRDEC
Aberdeen Proving Grounds, MD 21010-5423

Administrative Contracting Officer
ONR Resident Representative
(Address varies - obtain from your business office)

Director, Code 12
Applied Research and Technology Directorate
Office of Naval Research
800 North Quincy Street
Arlington, VA 22217-5000

Director, Code 22
Support Technology Directorate
Office of Naval Technology
800 North Quincy Street
Arlington, VA 22217-5000

Director, Code 112
Environmental Sciences Directorate
Office of Naval Research
800 North Quincy Street
Arlington, VA 22217-5000

Director, Code 113
Chemistry Division
Office of Naval Research
800 North Quincy Street
Arlington, VA 22217-5000

FINAL AND TECHNICAL REPORTS ONLY
Director (6 copies)
Naval Research Laboratory
Attn: Technical Information Division, Code 2627
Washington, DC 20375

AMZEL, L. Mario
Department of Biophysics
Johns Hopkins School of Medicine
725 North Wolfe Street
Baltimore, MD 21205

BEVERIDGE, David L
Department of Chemistry
Wesleyan University
Hall-Altwater Laboratories
Middletown, CT 06457

FICHT, Allison R.
Department of Medical
Biochemistry & Genetics
Texas A&M University
College Station, TX 77843

FRAUENFELDER, Hans
Department of Physics
University of Illinois
Urbana, IL 61801

GABER, Bruce P.
Code 6190
Bio/Molecular Engineering Branch
Naval Research Laboratory
Washington, DC 20375

HONIG, Barry
Columbia University
Dept of Biochemistry and
Molecular Biophysics
630 West 168th St.
New York, NY 10032

LAURSEN, Richard
Chemistry Department
Boston University
590 Commonwealth Avenue
Boston, MA 02215

LENZ, Robert W.
Chemical Engineering Department
University of Massachusetts
Amherst, MA 01003

LEWIS, Randolf V.
Biochemistry Department
University of Wyoming
University Station Box 3944
Laramie, WY 82071

LINDSAY, Stuart M.
Department of Physics
Arizona State University
Temp, AZ 85278

McCONNELL, Harden M.
Stanford University
Department of Chemistry
Stanford, CA 94305

MEYER, Edgard F.
Texas A&M University
Dept of Biochemistry and
Biophysics, Box 3578
College Station, TX 77843

NAGUMO, Mark
Bio/Molecular Engineering Branch
Code 6190
Naval Research Laboratory
Washington, DC 20375-5000

NOVOTNY, Jiri
Laboratory of Cellular and
Molecular Research
Massachusetts General Hospital
Boston, MA 02114

PABO, Carl O.
Department of Biophysics
Johns Hopkins University
School of Medicine
Baltimore, MD 21205

PRENDERGAST, Franklyn
Mayo foundation
200 first st. SW
Rochester, MN 55905

RICH, Alexander
Department of Biology
Massachusetts Institute of
Technology
Cambridge, MA 02139

RICHARDS, J. H.
California Institute of
Technology
Division of Chemistry and
Chemical Engineering
Pasadena, CA 91125

SHULER, Michael
School of Chemical Engineering
Cornell University
Ithaca, New York 14853

SINSKEY, Anthony J.
Laboratory of Appl Microbiology
Dept of Appl Biological Sciences
MIT
Cambridge, MA 02139

URRY, Dan W.
Laboratory of Molec Biophysics
University of Alabama
P. O. Box 311
Birmingham, AL 35294

WAITE, J. Herbert
College of Marine Studies
University of Deleware
Lewes, DE 19958

WARSHEL, Arieh
Department of Chemistry
University of Southern California
University Park
Los Angeles, CA 90089-0482

WILLIAMS, Jon I.
Allied Corporation
Columbia Rd and Park Ave.
Morristown, NJ 07960

CHANG, Eddie L.
Bio/Molecular Engineering
Code 6190
Naval Research Laboratory
Washington, D.C. 20375-5000

CORDINGLEY, John S.
Department of Molecular Biology
University of Wyoming
Box 3944 University Station
Laramie, WY 82071

CASE, Steven T.
Department of Biochemistry
University of Mississippi Medical
Center
2500 North State Street
Jackson, MS 39216-4505

END

DATE

FILMED

DTIC

6-88