Reports File

GEORGIA INSTITUTE OF TECHNOLOGY
OFFICE OF RESEARCH ADMINISTRATION

RESEARCH PROJECT INITIATION

Date: August 17, 1972

Project Title: Laser-Excited Raman Spectroscopy of Biopolymers

Project No: G-33-654

Principal Investigator: Dr. Nai-Teng Yu

Sponsor: Public Health Service

Agreement Period: From September 1, 1972 Until August 31, 1973

Type Agreement: Grant No. 5 R01 GM 18894-02

Amount:

$27,045	PHS
6,645	Cost Sharing Ga. Tech Contribution, Account G-33-343
$33,690	Total Project Budget

Reports Required:

Progress report accompanying application for continuation support due May 31, 1973. (Final report due 12/29/73 only if project is not continued.)

Sponsor Contact Person (s):

Dr. Walter L. Newton, Deputy Chief
Research Grants Branch
National Institute of General Medical Sciences
Bethesda, Maryland 20014
(or his designated representative)

Assigned to: School of Chemistry

COPIES TO:

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Patent Coordinator	Other

RA-3 (6-71)

Reports File

GEORGIA INSTITUTE OF TECHNOLOGY

OFFICE OF RESEARCH ADMINISTRATION

RESEARCH PROJECT TERMINATION

Date: October 31, 1973

Project Title: "Laser-Excited Raman Spectroscopy of Biopolymers"

Project No: G-33-654

Principal Investigator: Dr. Nai-Teng Yu

Sponsor: National Institute of General Medical Sciences

Effective Termination Date: August 31, 1973

Clearance of Accounting Charges: Charges should clear by Nov. 31, 1973

Research effort G-33-654 supported by PHS, NIH grant 5 R01 GM18894-02 continues as project G-33-672 supported by grant 5 R01 GM18894-03.

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Photographic Laboratory
Terminated Project File No. _____
Other _____

A-4 (5/70)

APPLICANT: REPEAT GRANT NUMBER SHOWN ON PAGE 1 ⟶	GRANT NUMBER
SECTION IV—SUMMARY PROGRESS REPORT	GM 18894-03

PRINCIPAL INVESTIGATOR OR PROGRAM DIRECTOR (Last, First, Initial)	PERIOD COVERED BY THIS REPORT	
Yu, Nai-Teng	FROM	THROUGH
NAME OF ORGANIZATION		
Georgia Institute of Technology	09/01/72	05/20/73

TITLE (Repeat title shown in Item 1 on first page)

Laser-excited Raman Spectroscopy of Biopolymers

1. List publications: (a) published and not previously reported; (b) in press. Provide five reprints if not previously submitted.
2. List all additions and deletions in professional personnel and any changes in effort.
3. Progress Report. (See Instructions)

1. (a) 1. Nai-Teng Yu, C. S. Liu and D. C. O'Shea, "Laser Raman Spectroscopy and the Conformation of Insulin and Proinsulin", J. Mol. Biol. 70,117 (1972).
   2. Nai-Teng Yu and C. S. Liu, "Laser Raman Spectra of Crystalline and Aqueous Glucagon", J. Amer. Chem. Soc., 94, 5127 (1972).
   3. Nai-Teng Yu, B. H. Jo, and C. S. Liu, "A Laser Raman Spectroscopic Study of the Effect of Solvation on the Conformation of Ribonuclease A", J. Amer. Chem. Soc., 94, 7572 (1972).
   4. Nai-Teng Yu, B. H. Jo and D. C. O'Shea, "Laser Raman Scattering of Cobramin B, a Basic Protein from Cobra Venom", Arch. Biochem. Biophys. 156, 71(1973)

   (b) 1. Nai-Teng Yu and B. H. Jo, "Comparison of Protein Structure in Crystals and in Solution by Laser Raman Scattering: I Lysozyme", Arch. Biochem. Biophys (1973) in press.
   2. Nai-Teng Yu and B. H. Jo, "Comparison of Protein Structure in Crystals and in Solution by Laser Raman Scattering: II Ribonuclease A and Carboxypeptidase A", J. Amer. Chem. Soc. (1973) in Press.
   3. Nai-Teng Yu and Y. Kyogoku, "Specific H-Bonding of Glutarimides and Hydantoins to Derivatives of RNA Bases in Chloroform Solution", submitted to Biochim. Biophys Acta.

2. Mr. C.S. Liu - received his M.S. degree in July of 1972 and is now a Ph.D. candidate at M.I.T.
   Dr. Emily J. East - received her Ph.D. degree in 1972 from Emory University. She will join our research group in September, 1973.

3. SUMMARY PROGRESS REPORT:

   1. OBJECTIVES:
   The overall objectives of the total project are: (a) to develop the techniques and procedures necessary to obtain and interpret the Raman spectra of biopolymers; (b) to derive significant structural information of proteins not obtainable by other research techniques; and (c) to correlate the structure-function relationship of these biomolecules.

   2. MAIN SCIENTIFIC FINDINGS AND THEIR SIGNIFICANCE:
   (a) During the past year and one-half, we have developed Raman techniques to a point where extremely detailed Raman spectra of proteins can be readily obtained. We have obtained the first detailed Raman spectrum of a single crystal of protein, ribonuclease A. It was found that in the 500-700 cm^{-1} region, where the S-S and C-S stretching and the tyrosyl ring vibrations appear the spectral feature of RNase A single crystal is somewhat similar to that of lyophilized powder but different from that of solution. The spectral differences between crystal and solution phases may be interpreted as due to changes

in the geometry of the disulfide linkages and the local environment of the "buried" tyrosines upon crystallization. However, in the amide III backbone region (1220-1300 cm^{-1}), a good agreement exists in both frequencies and line-widths between the crystal and solution spectra, indicating that the backbone conformation of RNase A is the same between the two phases. In addition, we compared the Raman spectrum of carboxypeptidase A (Anson) crystals to that of solution and showed that there existed a small difference in the line-shape of the amide III region. This may be a reflection of the subtle backbone conformational changes when CPDase A is crystallized.

(b) We have made a successful attempt at obtaining extremely detailed Raman spectra of native intact lens from calf (shown in Figures 1 (a) and 1 (b)). These two spectra were obtained with very low laser power (about 30 mW at the sample), high resolution (2 cm^{-1} spectral slit-width) and fast scanning speed (25 cm^{-1}/min.). The sulfhydryl (-SH) groups in lens proteins were clearly seen at 2580 cm^{-1} (see Figure 1(b)). From the amide I (1630-1700cm^{-1}) and amide III (1220-1300 cm^{-1}) regions, we concluded that the average backbone conformation of lens proteins (α-, β-,and γcrystallins) were of β-pleated sheet type. At present, we are trying to obtain the Raman spectra of cataractous lenses from rats, calves, and humans to determine the exact nature of cataract lens formation. We believe that the laser Raman technique may be used to investigate some biological processes <u>in vivo</u>.

(c) Cobramine B, a small basic protein from cobra venom, has been selected as a model for studying the scattering intensity of tyrosyl ring vibrations in the Raman spectra of proteins. All three tyrosines in this protein appear to be "buried" in the interior of the molecule and probably involved in interactions which are similar to those of the three "buried" tyrosines in RNase A when it is dissolved in water. The Raman spectra in the 300-1800 cm^{-1} region of cobramine B in the solid and solution are compared quantitatively. Several differences exist between the two spectra and may be interpreted in terms of the difference in conformation. In the amide I region, a strong single line was observed at 1672 cm^{-1} both in the solid and solution spectra, suggesting that this protein may contain a large fraction of antiparallel β structure. This is supported by the presence of a line at 1235 cm^{-1} in the amide III region. In addition to cobramine B, we also examined the Raman spectra of cobramine A and neurotoxin α. A large fraction of antiparallel β structure was also found in these proteins.

(d) Raman spectra of C-peptide from proinsulin and S-peptide from ribonuclease were obtained. Based on the criteria established by our laboratory previously (J. Mol. Biol., <u>70</u>, 117 (1972); J. Amer. Soc., <u>94</u>, 5127 (1972), we concluded that these two peptides existed in random-coiled form both in the solid state and in solution.

(e) We have examined the Raman spectra of oxytoxin and neurophysin II in the lyophilized powder form. Spectral interpretations are in progress.

3. RESEARCH GOALS FOR THE COMING YEAR:

(a) To continue our Raman spectral studies of the interaction of barbiturates with adenine derivatives in various solvents.

(b) To investigate the structure and conformation of cataractous lens and its purified fractions.

(c) To study the conformation of nucleic acid in viruses such as Qβ, T2, T4, and ϕX174.

(d) To study the effect of relative humidity on the spectra of DNA-histone and DNA-polylysine complexes.

(e) To continue the investigation on the structure of carboxypeptidase A and papain.

The undersigned agrees to accept responsibility for the scientific and technical conduct of the project and for provision of required progress reports if a grant is awarded as the result of this application

4/28/73
Date

Principal Investigator

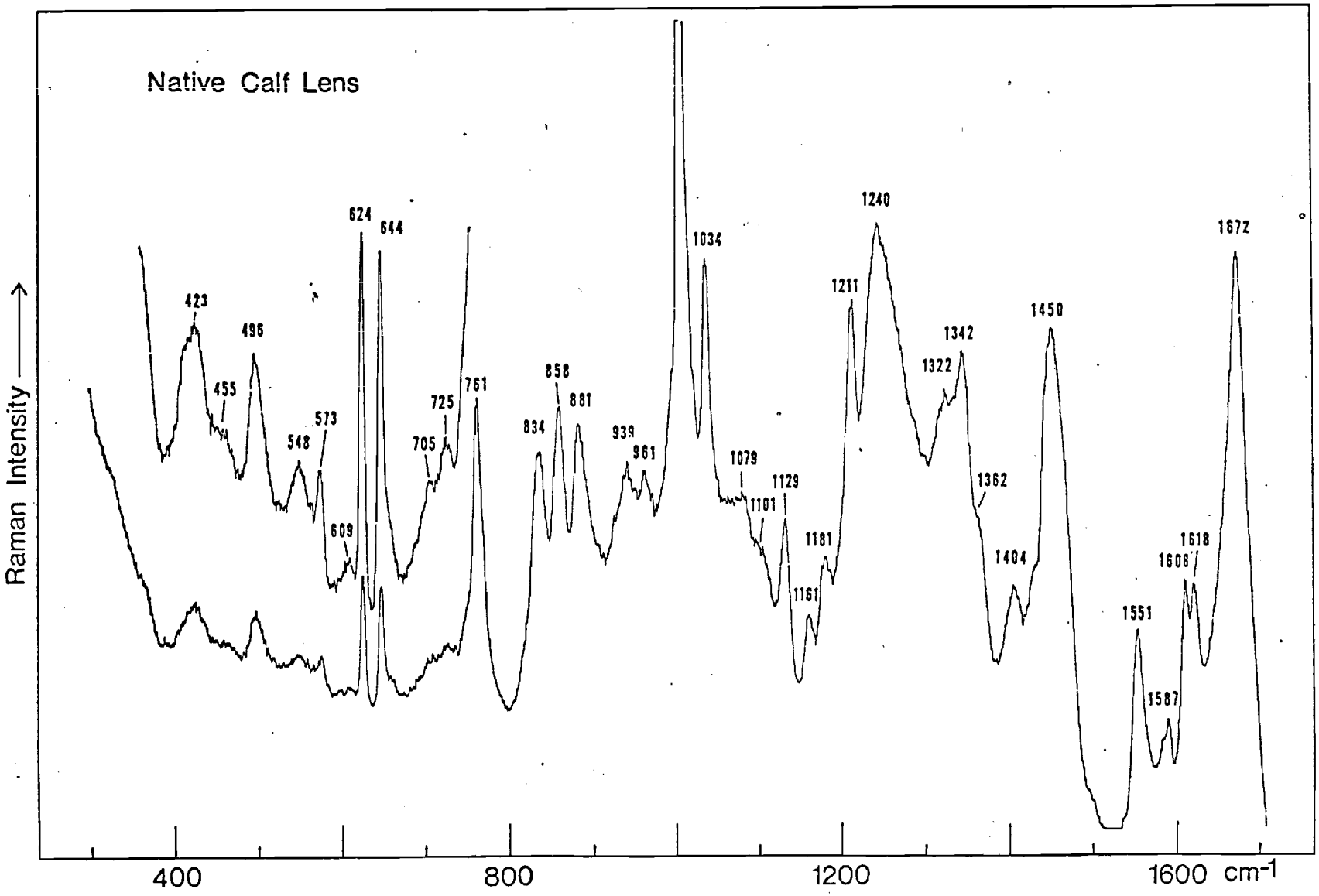
Native Calf Lens
Raman Intensity
423
455
496
548
573
609
624
644
705
725
761
834
858
881
939
961
1034
1079
1101
1129
1161
1181
1211
1240
1322
1342
1362
1404
1450
1551
1587
1608
1618
1672
400
800
1200
1600
cm-1

FIGURE 1 (a)

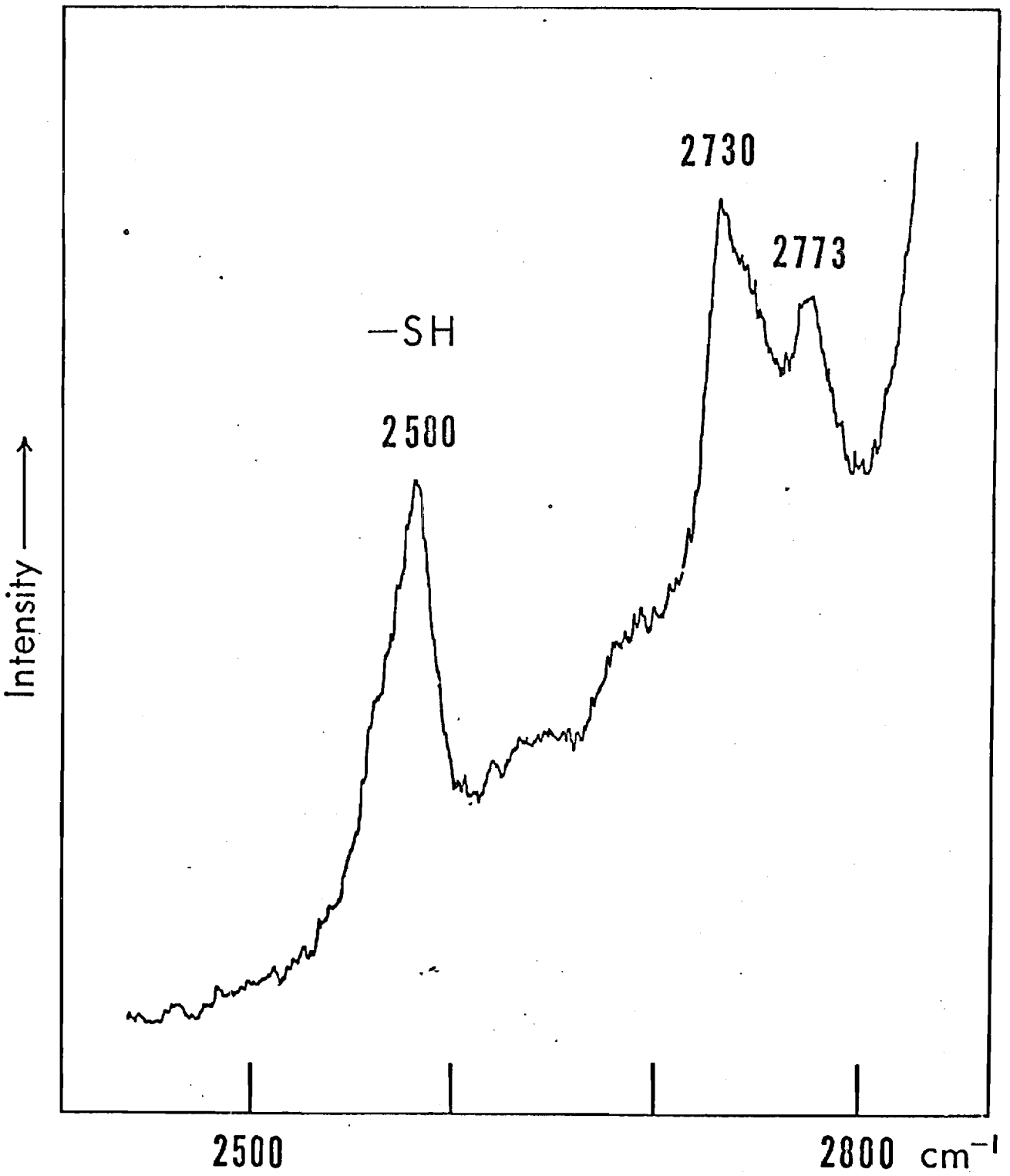

FIGURE 1 (b)