GEORGIA INSTITUTE OF TECHNOLOGY
OFFICE OF CONTRACT ADMINISTRATION
SPONSORED PROJECT INITIATION

Date: 10/25/80

Project Title: Laser-Excited Raman Spectroscopy of Biopolymers

Project No: G-33-G05

Project Director: Dr. Nai-Teng Yu

Sponsor: DHEW/PHS/NIH - National Institute of General Medical Sciences; Bethesda, MD 20014

Agreement Period: From 9/1/80 Until 8/31/81 (10 year)

Type Agreement: Grant No. R01 GM18894-10

Amount: $78,384 PHS Funds (G-33-G05)
$ 4,200 GIT Contribution (G-33-330)
$82,584 TOTAL

NOTE: By verbal agreement with the sponsor, the initial budget was rearranged causing a reduction in indirect costs. Adjusted sponsored total to be $71,084.

Reports Required: Annual Progress Reports with Continuation Applications; Terminal Progress Report upon Grant expiration.

Sponsor Contact Person (s):

Technical Matters

Arthur E. Heming, PhD
Assoc. Director for Program Activities
National Institute of General Medical Sciences
Bethesda, MD 20014

Program Administrator
Dr. Marvin Cassman
(301) 496-7463

NOTE: FOLLOW-ON TO PROJECT G-33-G04
(09 YEAR)

Contractual Matters
(thru OCA)

Evelyn W. Carlin, Grants Mgt. Officer
Office of Assoc. Director for Program Activities
National Institute of General Medical Sci
Bethesda, MD 20014

Grants Management Specialists
K. McKnight/M.J. Carow
(301) 496-7166

Defense Priority Rating: None

Assigned to: Chemistry (School/~~Laboratory~~)

COPIES TO:

- Project Director
- Division Chief (EES)
- School/Laboratory Director
- Dean/Director–EES
- Accounting Office
- Procurement Office
- Security Coordinator (OCA)
- Reports Coordinator (OCA)
- Library, Technical Reports Section
- EES Information Office
- EES Reports & Procedures
- Project File (OCA)
- Project Code (GTRI)
- Other OCA Research Property Coordinator
- Project Code (OCA)

CA–3 (3/76)

GEORGIA INSTITUTE OF TECHNOLOGY OFFICE OF CONTRACT ADMINISTRATION

SPONSORED PROJECT TERMINATION SHEET

Date 7/12/83

Project Title: Laser-Excited Raman Spectroscopy of Biopolymers

Project No: G-33-G05

Project Director: Dr. Nai-Teng-Yu

Sponsor: DHEW/PHS/NIH - National Institute of General Medical Sciences; Bethesda, MD 20014

Effective Termination Date: 8/31/82

Clearance of Accounting Charges: 8/31/82

Grant/Contract Closeout Actions Remaining:

NONE

- ☐ **Final Invoice and Closing Documents**
- ☐ **Final Fiscal Report**
- ☐ Final Report of Inventions
- ☐ Govt. Property Inventory & Related Certificate
- ☐ Classified Material Certificate
- ☐ Other _____

NOTE: Follow-on project (1 Year) - G-33-G06

Assigned to: Chemistry (School/~~Laboratory~~)

COPIES TO:

~~Administrative Coordinator~~ RAN	Research Security Services	EES Public Relations (2)
Research Property Management	~~Reports Coordinator~~ (OCA)	Computer Input
Accounting	Legal Services (OCA)	Project File
Procurement/EES Supply Services	Library	Other GTRI

FORM OCA 10:781

Renal Report

APPLICANT: REPEAT GRANT NUMBER SHOWN ON PAGE	GRANT NUMBER
SECTION IV—SUMMARY PROGRESS REPORT	GM18894-11

PRINCIPAL INVESTIGATOR OR PROGRAM DIRECTOR (Last, First, Initial)	PERIOD COVERED BY THIS REPORT	
Yu, Nai-Teng	FROM	THROUGH
NAME OF ORGANIZATION	09/01/79	06/10/80
Georgia Institute of Technology		

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

Laser-excited Raman Spectroscopy of Biopolymers

G-33-G05/Yu/Chem

1 List all publications, not previously reported, resulting from work supported by this grant (author(s), title, page numbers, year, journal or book). List manuscripts separately as submitted for publication or accepted for publication.
2 Provide two reprints of publications not previously submitted to the awarding unit.
3 Progress Report (See instructions)

1(a). (i) Nai-Teng Yu and R. B. Srivastava "Resonance Raman Spectroscopy of Heme Proteins with Intensified Vidicon Detectors: Studies of Low Frequency Modes and Excitation Profiles in Cytochrome c and Hemoglobin" J. Raman Spectrosc. 9, 166 (1980).

(ii) Nai-Teng Yu and M. Tsubaki "Resonance Raman Spectra of Manganese Myoglobin and Its Azide Complex. Assignment of a New Charge-Transfer Band to Azide (π) → Porphyrin (π^*) Transition" Biochemistry, 19, 4647 (1980).

(iii) M. Tsubaki, R. B. Srivastava and Nai-Teng Yu "Temperature Dependence of Resonance Raman Spectra of Azide Metmyoglobin and Methemoglobin. Detection of Resonance enhanced Bound Azide Vibrations and Iron-Azide Stretch" Biochemistry, 20, 946 (1981).

(iv) R. B. Srivastava, C. Pace and Nai-Teng Yu "Comparative Raman Studies of Cytochrome b_{562} and Cytochrome c" J. Raman Spectrosc. 11, 20 (1981).

1(b). (i) M. Tsubaki and Nai-Teng Yu "Resonance Raman Investigation of Dioxygen Bonding in Oxycobaltmyoglobin and Oxycobalthemoglobin: Structural Implication of Splittings of Bound O-O Stretching Vibration" Proc. Natl. Acad. Sci. USA (in press).

(ii) Nai-Teng Yu, A. Lanir and M. M. Werber "Laser Raman Scattering and Preresonance in Co(III)-ATP Complexes" J. Raman Spectrosc., (in press).

(iii) M. Tsubaki, R. B. Srivastava and Nai-Teng Yu "Resonance Raman Investigation of Carbon Monoxide Bonding in Carbonmonoxy Hemoglobin and Influence of the Quaternary Structure Change" Biochemistry, (submitted).

(iv) M. Tsubaki and Nai-Teng Yu "Resonance Raman Investigation of Nitric Oxide Bonding in Nitrosyl Hemoglobin A and Myoglobin: Detection of Bound N-O Stretch and Fe-NO Stretch Vibrations from Hexacoordinated NO-Heme Complex" Biochemistry, (subbmitted).

2. Two copies each of the above papers are provided with this progress report.

3. (1) Brief statement of the general scientific goals of the project: no change.

(2) Concise description of the studies conducted during the budget year, the results obtained and their significance.

(i) We have solved a great puzzle in biochemical journals concerning the ν(O-O) stretching vibration in hemoproteins. This vibrational frequency has been provided so far by infrared spectroscopy. For the first time we observed this vibrational modes by resonance Raman spectroscopy. To our surprising, we detected a total of three (3) isotope-sensitive Raman lines at 1103 (1107),

1137 (1137) and 1153 (1152) cm^{-1} in oxy CoMb (or oxy CoHbA). The first two frequencies arise from resonance interaction between a ν(O-O) mode at ~1122 cm^{-1} and an accidentally degenerate porphyrin ring mode at 1123 (1121) cm^{-1}, whereas the third one represents an "unperturbed" ν(O-O) vibration from a different conformer. The ν(Co-O) stretch was detected at ~538 cm^{-1} which is considerably lower than the ν(Fe-O) frequency at ~570 cm^{-1} in oxy FeMb and oxy FeHbA. The Co-O bond is longer and weaker than the Fe-O bond. Enhancement of both ν(O-O) and ν(Co-O) indicates the existence of a charge-transfer transition underlying the Soret band, which may be assigned as $\pi^*(\pi_g^*O_2/d_{xz}) \rightarrow \sigma^*(d_{z^2}Co/\pi_g^*)$. The presence of two ν(O-O) vibrations at (~1122 and ~1152 cm^{-1}) but only one ν(Co-O) mode at (~538 cm^{-1}) means that the two species in oxy CoMb or oxy CoHbA have the same Co-O bond lengths but different O-O bond lengths. The bound dioxygen in a bent, end-on configuration may have two allowed orientations which differ in the extent of $SP^2(N_\varepsilon) \rightarrow \pi^*(O_2)$ donation from distal histidine.

This study enhanced our understanding on the exact nature of dioxygen bonding and the factors controlling the binding of molecular oxygen to hemoproteins.

(ii) We have made the first identification of the iron-carbon bond in carbonmonoxy hemeproteins by resonance Raman spectroscopy. The Fe-CO stretching, Fe-C-O bending and bound C-O stretching vibrations have been observed at 508 (512), 578 (577) and 1951 (1944) cm^{-1}, respectively, in the resonance Raman spectrum of carbonmonoxy human HbA (or sperm whale Mb) upon excitation at 406.7 nm within the Soret band. These assignments were made on the basis of frequency shifts with the isotopes $^{13}C^{16}O$, $^{12}C^{18}O$ and $^{13}C^{18}O$. Calculated isotope shifts according to the model Im-Fe-C-O (but not Im-Fe-O-C) agree well with the observed data. The possible mechanisms of resonance Raman enhancement of these vibrations are discussed in terms of $d\pi(Fe) - \pi^*(CO)$ interaction.

Careful examination of the Fe-CO stretching mode at 507 cm^{-1} in carbonmonoxy HbA and Hb Kansas both with and without inositol hexaphosphate (IHP) reveals no changes in either frequency nor intensity. However, resonance Raman spectrum of carbonmonoxy carp Hb exhibits a broadening of the Fe-CO stretching line on the lower energy side upon switching the quaternary structure from R- to T-form, suggesting the presence of a new conformer with a weaker Fe-CO bond or a somewhat different tilt angle between the Fe-C-O group and the heme normal.

3. Specific objectives for the coming year:

(a) To replace the present SIT (silicon intensified target) detector with a more sensitive "intensified SPD (silicon photodiode) array detector" (PAR model 1420). We ordered this detector in October 1980 but not yet received due to difficulties in meeting the specifications by the manufacturer. However, we have been assured for a speedy delivery by the end of July 1981. This detector is far more superior in the UV region, reaching ~25% quantum efficiency between 250-400 nm; compared to ~2% in SIT.

(b) To study the ligand binding properties of Co-, Fe-, Mn- and Cr- "picket fence" porphyrin and compare the results with those from hemeproteins.

(c) With the installation of our new detector (PAR model 1420), we will collect data for UV resonance Raman spectra of nucleic acid components.

(d) In collaboration with Prof. Dave Lambeth of Emory University (Dept. of Biochemistry) we will be studying cytochrome P-450_{SCC} from adrenal cortex.

(e) In collaboration with Prof. C. A. Yu of Oklahoma State University (Dept. of Biochemistry) we will be studying cytochrome C_1 and cytochrome oxidase by resonance Raman spectroscopy.

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.

June 18, 1981
Date

Nai-Teng Yu
Principal Investigator