

13:31:55

OCA PAD AMENDMENT - PROJECT HEADER INFORMATION

06/13/89

Active

Project #: G-33-G13  
Center # : Q5169-3A0

Cost share #:  
Center shr #:

Rev #: 3  
OCA file #:  
Work type : RES  
Document : GRANT  
Contract entity: GIT

Contract#: 5 R01 GM18894-18  
Prime #:

Mod #: BR DTD 6-12-89

Subprojects ? : N  
Main project #:

Project unit:  
Project director(s):  
YU N-T

CHEM  
CHEM

Unit code: 02.010.136  
(404)894-4007

Sponsor/division names: DHHS/PHS/NIH  
Sponsor/division codes: 108

/ NATL INSTITUTES OF HEALTH  
/ 001

Award period: 880901 to 890831 (performance) 891130 (reports)

Sponsor amount	New this change	Total to date
Contract value	0.00	172,107.00
Funded	0.00	172,107.00
Cost sharing amount		0.00

Does subcontracting plan apply ? : N

Title: LASER-EXCITED RAMAN SPECTROSCOPY OF BIOPOLYMERS

PROJECT ADMINISTRATION DATA

OCA contact: Kathleen R. Ehlinger 894-4820

Sponsor technical contact

Sponsor issuing office

DR. HELEN SUNSHINE  
(301)496-7309  
NATL INST OF GENERAL MEDICAL SCIENCE  
NATIONAL INSTITUTES OF HEALTH  
BETHESDA, MD 20892

JANE UNSWORTH  
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Security class (U,C,S,TS) :  
Defense priority rating :  
Equipment title vests with: Sponsor

ONR resident rep. is ACO (Y/N): N  
supplemental sheet  
GIT X

Administrative comments -

ISSUED TO INCORPORATE BUDGET REVISION DATED 6-12-89 SHIFTING FUNDS  
BUDGET CATEGORIES.



GEORGIA INSTITUTE OF TECHNOLOGY  
OFFICE OF CONTRACT ADMINISTRATION

NOTICE OF PROJECT CLOSEOUT

Closeout Notice Date 04/02/90

Project No. G-33-G13

Center No. Q5169-3A0

Project Director YU N-T

School/Lab CHEM

Sponsor DHHS/PHS/NIH/NATL INSTITUTES OF HEALTH

Contract/Grant No. 5 R01 GM18894-18

Contract Entity GIT

Prime Contract No.

Title LASER-EXCITED RAMAN SPECTROSCOPY OF BIOPOLYMERS

Effective Completion Date 890831 (Performance) 891130 (Reports)

Closeout Actions Required:	Y/N	Date Submitted
Final Invoice or Copy of Final Invoice	Y	
Final Report of Inventions and/or Subcontracts	N	
Government Property Inventory & Related Certificate	N	
Classified Material Certificate	N	
Release and Assignment	N	
Other	N	

Comments CONTINUED BY G-33-G14

Subproject Under Main Project No.

Continues Project No. G-33-G12

Distribution Required:

Project Director	Y
Administrative Network Representative	Y
GTRI Accounting/Grants and Contracts	Y
Procurement/Supply Services	Y
Research Property Management	Y
Research Security Services	N
Reports Coordinator (OCA)	N
GTRC	Y
Project File	Y
Other	N



Equipment : Recently we traded in our Spectra-Physics model 171 krypton-ion laser for the purchase of a more advanced Spectra-Physics model 3800 Nd:YAG laser. This laser delivers 80 ps pulses at 80 MHz with wavelength at 1064 nm. It can be easily frequency doubled to 532 nm. The requested pulse compressor is for further shortening the pulses to 5 ps, allowing a greater enhancement of the surface-enhanced hyperRaman effects, important for obtaining more information about the ligand vibrational modes.

Earlier, we have also traded in our obsolete CR-500K krypton-ion laser for a more powerful CR 100-K2 krypton laser, which is even more superior than the Spectra-Physics 171 krypton-ion laser.

The costs for these laser tubes replacement are shared between this grant and EY 01746. The third grant EY 07006 (involving the development of a clinical instrument) does not use these lasers.

112,681	0
59,426	0
172,107	0

Yu, Nai-Teng (Ph.D.) 585-28-9345	Professor, Principal Investigator Chemistry, Georgia Tech	-5
Cai, Ming-Zhi	Res. Technician, instrument operator Chemistry, Georgia Tech	+15
Zhang, Fuli	Res. Assoc., Special techniques developer	+10
Lee, Bao-Shiang (Ph.D.)	Postdoctor, Oxy Co hemes studies	+25
Lin, S.-H. (Ph.D.)	Postdoctor (replaced by Dr. Lee)	-25
Nie, Shuming (Ph.D.)	Postdoctor, FT-Raman studies	+10
Gersonde, K. (Ph.D.)	Professor, Collaborator, West Germany	-5

- C. (i) Water-to-Water Heat Exchanger (Neslab) for cooling CR-100K2 laser \$ 6,220  
(ii) Spectra-Physics 3800 Nd:YAG laser with a Mode-Locker (\$ 12,715 from this grant;  
\$29,815 from other sources)
- D. (i) Symposium on Oxygen Binding Heme Proteins (10/09-10/14/88) in Asilomar, CA.  
(ii) The 5th International Symposium on Biological Macromolecules(4/3-4/8/89) in Stiri  
Czechoslovakia; (iii) Bell Lab. (visit Dr. Denis Rousseau) 6/11-6/18/89.

Lee, B. S.	Res. Assoc., Oxy Co hemes	Chemistry, Georgia Tech
Lipscomb, L.	Grad. Res. Assist., NO binding	Chemistry, Georgia Tech
Castillo, C.	Grad. Res Assist., Surface- enhanced Raman development	Chemistry, Georgia Tech
Tsubaki, M.	Res. Assoc. (summer)	visitor from Himeji Inst. of Technology



NIH Grant : EY 01746-14

Title : Comparative Raman Studies of Human and Animal Lenses.

Entire Project Period : 5 years, ending 04/30/93.

Amount of Direct Costs for Period 05/01/89-04/30/90 : \$ 134,805

Percentage of Appointment : 0.25

(b) NIH Grant : EY 07006-03

Title : Clinical Monitor of Diabetic Lenses by Fluorescence.

Entire Project Period : 4 years, ending 03/31/91.

Amount of *requested* Direct Costs for Period 04/01/89-03/31/90 : \$ 108,003 (including \$ 64,725 subcontract to Joslin Diabetes Center in Boston).

Percentage of Appointment : 0.15

(2) APPLICATIONS PENDING REVIEW OR FUNDING : None.

Yu, Nai-Teng

09/01/88

06/26/89

Georgia Institute of Technology

Laser-excited Raman Spectroscopy of Biopolymers

## 1. The Plans for the Next Year of Support :

The specific aims for the next year of support are : (1) To continue our investigation on the  $\nu(\text{F}^{\text{II}}-\text{N}_\text{E})$  stretching mode in deoxy  $\text{Fe}^{\text{II}}$  adamantane porphyrin-6,6-cyclophane with N-methyl imidazole as an axial base; (2) To investigate if the geometry of the bound NO in nitrosyl myoglobin can really undergo a dramatic change when temperature decreases from 20 °C to 77 °K, a result claimed by EPR spectroscopists; (3) To initiate the studies of the mechanism of the allosteric control of ligand binding in dimeric CTT hemoglobins; (4) To continue our studies on ligand binding to liver fluke Dd hemoglobin. (5) To develop the FT-Raman techniques for the studies of metalloporphyrins and related derivatives.

## 2. Concise Description of the Studies Conducted during the Current Budget Year :

### (i) *The origin of the distal steric effect in carbonmonoxy hemoglobin*

The site-directed mutagenesis has produced human mutant hemoglobins in *E. coli* having : (1) His( $\alpha$ E7) replaced by Gln or Gly, (2) His( $\beta$ E7) by Gln, Val, Gly or Phe, (3) Val( $\alpha$ E11) by Ala, Leu or Ile, (4) Val( $\beta$ E11) by Ala, Met, Leu or Ile, and (5) Phe(CD1) by Gly or Tyr. The effects of these mutations on the vibrational properties of the Fe-C and C-O bonds in carbonmonoxy Hb A have been studied by Soret-excited resonance Raman spectroscopy. The relatively high  $\nu(\text{Fe-CO})$  at 507  $\text{cm}^{-1}$  and low  $\nu(\text{C-O})$  at 1951  $\text{cm}^{-1}$ , compared with those corresponding frequencies in free porphyrin model compounds, are indicative of a protein-induced CO distortion (off-axis CO bonding). The replacement of His( $\beta$ E7) by a bulkier but



nonpolar Phe causes a decrease of  $\nu(\text{Fe-CO})$  in  $\beta$ -subunit to  $492\text{ cm}^{-1}$  and an increase of  $\nu(\text{C-O})$  to  $1968\text{ cm}^{-1}$ , indicating a near absence of steric hindrance. In contrast, the substitution of His(E7) by a smaller but polar Gln in either  $\alpha$  or  $\beta$  subunits produces no effects. The ability of both His and Gln in causing the CO distortion may be attributed to their  $\text{N}_\epsilon$  atoms which can interact with the bound CO in a similar manner. The origin of the distal steric effect that causes the off-axis CO bonding is apparently **not** the steric bulk of the E7 residue; it may be the repulsive polar interactions between the lone-pair electrons of the  $\text{N}_\epsilon$  and those of the carbonyl oxygen. The Val(E11) in native Hb plays no significant role in causing the CO distortion, as evidenced by the finding that there are no detectable changes in  $\nu(\text{Fe-CO})$  and  $\nu(\text{C-O})$  for all the E11 mutants. Furthermore, we show that the substitution of Phe(CD1) by Gly or Tyr also produces no effects on  $\nu(\text{Fe-CO})$  and  $\nu(\text{C-O})$ .

(ii) *Near infrared FT-Raman spectroscopy of photolabile organocobalt  $\text{B}_{12}$  compounds*

Near infrared Fourier-transform (FT) Raman spectroscopy has been applied for the first time to investigate photolabile organocobalt  $\text{B}_{12}$  compounds. The use of near infrared laser excitation at 1064 nm prevents electronic absorptions, and thus leads to a complete elimination of fluorescence interference and Co-C bond photolysis. Fluorescence-free, high-quality FT-Raman spectra have been obtained for  $\text{B}_{12}$  complexes of the type,  $\text{RCo}(\text{DH})_2\text{CH}_3$  (where DH = monoanion of dimethylglyoxime, R = pyridine,  $\text{H}_2\text{O}$ , triphenylphosphine, trimethylphosphine and tricyclohexylphosphine). The Raman-active Co-C stretching mode is unambiguously identified at  $522\text{ cm}^{-1}$  (or  $504\text{ cm}^{-1}$ ) in solid  $\text{PyCo}(\text{DH})_2\text{-CH}_3$  (or  $\text{CD}_3$ ), and is found to exhibit an unusually large solvation effect in chloroform.

(iii) *Resonance Raman studies of phenyl iron complex of horse heart myoglobin*

The reaction of horse heart myoglobin with phenylhydrazine provides a stable  $\sigma$ -bound phenyl-Fe<sup>III</sup> heme complex. Various phenyl ring vibrational modes of the phenyl-Fe<sup>III</sup> myoglobin were enhanced upon excitation in the intense Soret absorption region. By <sup>13</sup>C-phenyl-isotope labelling and excitation profile studies, possible assignments of the phenyl ring modes were suggested. The resonance enhancement of the phenyl ring modes may be caused by a charge-transfer transition.

(iv) *Resonance Raman studies of carbonmonoxy spot hemoglobins*

The ligand binding affinity of Spot hemoglobin is extremely pH dependent (the Root effect). At high pH (8.5), the  $\nu(\text{Fe-CO})$  and  $\nu(\text{C-O})$  stretching frequencies were observed at 508 and 1951 cm<sup>-1</sup>, respectively. At low pH (5.5), however, the  $\nu(\text{Fe-CO})$  stretching modes split into two peaks at 508 and 494 cm<sup>-1</sup>, and the  $\nu(\text{C-O})$  stretching frequencies were observed at 1951 and 1964 cm<sup>-1</sup>, respectively. The results may indicate a differential effect of pH on the  $\alpha$ - and  $\beta$ -like chains.

3. No change

4. Not Applicable

5. **Publications :**

- (a) Yu, N.-T., Lin, S.-H., Chang, C. K., and Gersonde, K. (1989) "Resonance Raman enhancement of the Mn-N-O bending mode in nitrosyl manganese 'strapped' and 'open' heme complexes" *Biophys. J.* **55**, 1137-44.
- (b) Gersonde, K., Yu, N.-T., Lin, S.-H., Smith, K. M. and Parish, D. W. (1989) "Resonance Raman assignment and evidence for noncoupling of individual 2- and 4-vinyl vibrational modes in a monomeric cyanomethemoglobin" *Biochemistry*, **28**, 3960-66.



Yu, Nai-Teng

585-28-9345

- (c) Lin, S.-H., Yu, N.-T., Tame, J., Renaud, J.-P., Shih, D. T.-b. and Nagai, K. (1989) "The origin of the distal steric effect in carbonmonoxy hemoglobin: evidence from resonance Raman study of E. coli synthesized mutants" Proc. Natl. Acad. Sci., USA (to be submitted).
- (d) Nie, Shuming, Yu, N.-T., and Marzilli, L. (1989) "Near infrared FT-Raman spectroscopy of photolabile organocobalt B12 compounds. I Detection of the cobalt-carbon stretching mode in both solid state and solution" J. Am. Chem. Soc. (to be submitted).
- (e) Liu, Hsiao-Hua, Lin, S.-H. and Yu, N.-T. (1989) "Resonance Raman studies of phenyl iron complex of horse heart myoglobin" Biochemistry (in preparation).