**Project Administration Data**

**OCA contact:** Kathleen R. Ehlinger  
894-4820

**Sponsor technical contact**  
DR. HELEN SUNSHINE  
(301)496-7309

**Sponsor issuing office**  
JANE UNSWORTH  
(301)496-7166

**NATL INST OF GENERAL MEDICAL SCIENCE**  
NATIONAL INSTITUTES OF HEALTH  
BETHESDA, MD 20892

**Security class (U,C,S,TS):**  

**Defense priority rating:**

**Equipment title vests with:**  
Sponsor  
GIT X

**Administrative comments**  
ISSUED TO INCORPORATE BUDGET REVISION DATED 6-12-89 SHIFTING FUNDS BETWEEN BUDGET CATEGORIES.
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:

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<tr>
<td>Final Invoice or Copy of Final Invoice</td>
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<tr>
<td>Final Report of Inventions and/or Subcontracts</td>
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<td>Government-Property Inventory &amp; Related Certificate</td>
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Comments CONTINUED BY G-33-G14

Subproject Under Main Project No. _____________

Continues Project No. G-33-G12___________

Distribution Required:

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<tr>
<td>Project Director</td>
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<td>Administrative Network Representative</td>
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<td>GTRI Accounting/Grants and Contracts</td>
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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.
<table>
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<tr>
<th>Name</th>
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<th>Notes</th>
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<tbody>
<tr>
<td>Yu, Nai-Teng (Ph.D.)</td>
<td>Professor, Principal Investigator</td>
<td>Chemistry, Georgia Tech</td>
<td>-5</td>
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<tr>
<td>Cai, Ming-Zhi</td>
<td>Res. Technician, instrument operator</td>
<td>Chemistry, Georgia Tech</td>
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<tr>
<td>Zhang, Fuli</td>
<td>Res. Assoc., Special techniques developer</td>
<td></td>
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<tr>
<td>Lee, Bao-Shiang (Ph.D.)</td>
<td>Postdoctor, Oxy Co hemes studies</td>
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<tr>
<td>Lin, S.-H. (Ph.D.)</td>
<td>Postdoctor (replaced by Dr. Lee)</td>
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<tr>
<td>Nie, Shuming (Ph.D.)</td>
<td>Postdoctor, FT-Raman studies</td>
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<td>+10</td>
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<tr>
<td>Gersonde, K. (Ph.D.)</td>
<td>Professor, Collaborator, West Germany</td>
<td></td>
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</table>

**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 Stiria, Czechoslovakia; (iii) Bell Lab. (visit Dr. Denis Rousseau) 6/11-6/18/89.

<table>
<thead>
<tr>
<th>Name</th>
<th>Position/Role</th>
<th>Institution</th>
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<tr>
<td>Lee, B. S.</td>
<td>Res. Assoc., Oxy Co hemes</td>
<td>Chemistry, Georgia Tech</td>
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<tr>
<td>Lipscomb, L.</td>
<td>Grad. Res. Assist., NO binding</td>
<td>Chemistry, Georgia Tech</td>
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<tr>
<td>Castillo, C.</td>
<td>Grad. Res Assist., Surface-enhanced Raman development</td>
<td>Chemistry, Georgia Tech</td>
</tr>
<tr>
<td>Tsubaki, M.</td>
<td>Res. Assoc. (summer)</td>
<td>visitor from Himeji Inst. of Technology</td>
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</table>
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.
The specific aims for the next year of support are: (1) To
continue our investigation on the $\nu(F^{II}-N_e)$ stretching mode in
deoxy Fe$^{II}$ adamantane porphyrin-6,6-cyclophane with N-
methyl imidazole as an axial base; (2) To investigate if the
gometry 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 $\nu$(Fe-CO) at 507 cm$^{-1}$ and low $\nu$(C-O) at
1951 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 $\nu$(Fe-CO) in $\beta$-subunit to 492 cm$^{-1}$ and an increase of $\nu$(C-O) to 1968 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 N$_e$ 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 N$_e$ 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 $\nu$(Fe-CO) and $\nu$(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 $\nu$(Fe-CO) and $\nu$(C-O).

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

Near infrared Fourier-transform (FT) Raman spectroscopy has been applied for the first time to investigate photolabile organocobalt 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 B$_{12}$ complexes of the type, RCo(DH)$_2$CH$_3$ (where DH = monoanion of dimethylglyoxime, R = pyridine, H$_2$O, triphenylphosphine, trimethylphosphine and tricyclohexylphosphine). The Raman-active Co-C stretching mode is unambiguously identified at 522 cm$^{-1}$ (or 504 cm$^{-1}$) in solid PyCo(DH)$_2$-CH$_3$ (or 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 σ-bound phenyl-Fe^III^ heme complex. Various phenyl ring vibrational modes of the phenyl-Fe^III^ myoglobin were enhanced upon excitation in the intense Soret absorption region. By 13C-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 ν(Fe-CO) and ν(C-O) stretching frequencies were observed at 508 and 1951 cm⁻¹, respectively. At low pH (5.5), however, the ν(Fe-CO) stretching modes split into two peaks at 508 and 494 cm⁻¹, and the ν(C-O) stretching frequencies were observed at 1951 and 1964 cm⁻¹, respectively. The results may indicate a differential effect of pH on the α- and β-like chains.

3. No change
4. Not Applicable
5. Publications:


