Summer Scholar Report
By Kathleen Fleming and Joanne Stubbe

ACS Senior Chemists Task Force
By Morton Z. Hoffman and Michaeline Chen

National Chemistry Week Events
2011 James Flack Norris Award
To Peter Mahaffy of King’s University College
The Original Part-Time Chemistry Evening Graduate Program in New England

All courses meet for a two-and-a-half hour period one evening per week and carry three semester-hours of graduate credit toward the 30 semester-hour requirement for a coursework M.S. degree.

Classes start September 7, 2011

Principles of Chemical Biology-5621-Monday
Drug Discovery and Development-5645-Monday

Principles of Mass Spectrometry-5612-Tuesday
Thermodynamics-5636-Tuesday
Radiochemistry (new)–5668 - Tuesday

Principles and Analysis of Carbohydrates-5644-Wednesday
Organic Synthesis 1-5626-Wednesday
Optical Methods-5613-Wednesday

Analytical Separations-5611-Thursday
Mechanistic and Physical Organic Chemistry-5627-Thursday

Students new to the program must have completed an application for admission.

For additional information including admission requirements please contact:

Jean Harris
Department of Chemistry & Chemical Biology
Tel: (617) 373-2824
H.Harris@neu.edu
www.chem.neu.edu
Contents

ACS Senior Chemists Task Force 4
By Morton Z. Hoffman and Micheline Chen

August Historical Events in Chemistry 5
By Leopold May, Catholic University of America

Announcements 6
2011 James Flack Norris Award to Peter Mahaffy, NCW 2011:
Design a T-shirt Contest, RE-SEED – Retirees Enhancing Science
Education through Experiments and Demonstration

Announcements 7
Save the Date-10th Annual Undergraduate Symposium on Sustainability and the
Environment, National Chemistry Week Events

Summer Scholar Report 8
In Vitro Study of Human Ribonucleotide Reductase Enzymatic Activity and
Assembly of Diferric-tyrosyl Radical Cofactor
By Kathleen Fleming and Joanne Stubbe

Cover: Participants in the Northeast Student Chemistry Research Conference
(NSCRC): (l-r) Michael Lacy (Tufts University), winner of the Excellent Poster
Presentation by an Undergraduate Student Award (sponsored by Strem Che-
micals); April Jewell (Tufts University), Chair, NESACS Younger Chemists
Committee and organizer of NSCRC; Jiazuo "Henry" Feng (Boston University),
winner of the Brauner Memorial Book Award for his oral presentation; Jennifer
Bento (Simmons College), recipient of a NESACS Undergraduate Grant-in-Aid,
which provided funds for her to attend and present her poster (in background) at
the ACS National Meeting in Anaheim. Photographe by at the May Education
Night Meeting. (Photo by Morton Z. Hoffman).

Deadlines: October 2011 Issue: August 11, 2011
November 2011 Issue: September 15, 2011

The Nucleus Summer 2011
The Senior Chemists Task Force (SCTF) was established in 2009 and is currently composed of 21 members. Its purpose is to serve as the focal point of programming and representation for senior chemists over the age of 50 within the ACS and the chemistry enterprise at large. Its mission, broadly stated, is to encourage and serve as a conduit for senior members to volunteer and contribute their energy and talent to the ACS, including governance, education, mentoring, and community projects; to provide useful services and information to seniors, such as retirement and estate planning, consulting and part-time opportunities, and travels/tours; to foster networking opportunities among seniors, both nationally and locally; and to represent senior chemists in their interaction with other elements of ACS governance, bringing awareness of their needs, fostering collaborations, and creating synergies.

The age demographics of the ACS demonstrate the need for institutional interest in senior chemists; of its approximately 160,000 members, at least 50% are 50 years of age or older, and about 30% are over 60. SCTF is needed in order to provide services to this continually growing segment of the membership, to encourage seniors to stay involved with ACS, to coordinate local section activities that involve seniors, and to make the rest of the Society aware of the needs of seniors.

From a programming standpoint, SCTF is in a position to organize, sponsor, and co-sponsor symposia and events at ACS national meetings, and provide guidance for communications with seniors at regional meetings and within local sections. It can also provide information at its link on the ACS website, through the SCTF connections on the ACS Network, and with articles in local section newsletters, the Councilor Bulletin, and Committee News.

With regard to SCTF programming at ACS national meetings, the most enduring has been the Senior Chemists Breakfasts, which have attracted sell-out crowds. Since 2009, the speakers have included Peter Stang, University of Utah (Salt Lake City, 2009); Luis Echegoyen, NSF.
August Historical Events In Chemistry

by Leopold May, The Catholic University of America, Washington, DC 20064

August 1, 1885
One hundred and twenty-six years ago, on this date Georg von Hevesy was born. He was a researcher in radioisotopes and discovered hafnium (Hf, 72) in 1923 with Dirk Coster. In 1943, he was awarded the Nobel Prize in Chemistry for his work on the use of isotopes as tracers in the study of chemical processes.

August 5, 1936
Robert R. Williams and J. K. Cline synthesized vitamin B₁ on this date.

August 6, 1960
Fifty years ago on this date, the first publication on the first working laser was published in the paper, Simulated optical radiation in ruby by Theodore H. Maiman in Nature, 197, 494 (1960).

August 8, 1779
Benjamin Silliman, who was born on this date, was a noted teacher at Yale University. He founded the oldest continuing journal of natural science in the United States, the American Journal of Science, familiarly called “Silliman’s Journal.” In 1807, a meteorite fell with spectacular sound and light effects in Weston, Connecticut. This was the first documented fall of a meteorite in the New World—only 25 miles from New Haven. He published an analysis of the meteorite.

August 9, 1896
Erich Armand Arthur Joseph Hückel developed the Hückel method of approximate molecular orbital (MO) calculations on pi-electron systems and with Peter Debye developed the Debye-Hückel theory of electrolytic solutions. He was born on this date.

August 12, 1793
James Muspratt, who was born on this date, improved the methods of manufacture of acids and other chemicals.

August 13, 1918
Frederick Sanger, a researcher on the structure of proteins and insulin and the base sequences of nucleic acids, was born on this date. He received the Nobel Prize in Chemistry in 1958 for his work on the structure of proteins, especially that of insulin, and in 1980 shared the Prize with W. Gilbert for their contributions concerning the determination of base sequences in nucleic acids and Paul Berg for his fundamental studies of the biochemistry of nucleic acids, with particular regard to recombinant-DNA.

August 17, 1893
Walter K. F. Noddack co-discovered rhenium in 1925, with his wife, Ida E. Noddack and O. Berg. He was born on this date.

August 18, 1916
Walter J. Kauzmann, who was born on this date, did research on the hydrophobic effect in the three-dimensional structure of proteins and the nature of supercooled liquids (Kauzmann’s paradox).

Continued on page 12
The Northeastern Section of the American Chemical Society is pleased to announce that Professor Peter Mahaffy is the recipient of the 2011 James Flack Norris Award for Outstanding Achievement in the Teaching of Chemistry. Within the classroom, Dr. Mahaffy is known for his highly effective and innovative teaching methods, including his commitment to help students, educators, scientists and the general public observe the intricate connections between science and their everyday lives. Dr. Mahaffy was instrumental in establishing and co-directing the King’s Centre for Visualization in Science which has allowed him to continue his development of digital learning resources that help learners see and understand scientific concepts that would otherwise be difficult to visualize. Each month, over 10,000 learners from over 70 countries advance their chemical understanding by visiting www.keys.ca where they access information on topics ranging from elementary science to chemistry, physics and climate change science. During the International Year of Chemistry, Dr. Mahaffy has interacted with many chemists and educators from around the world, observing the imaginative solutions they bring to the many challenges faced by scientists. He aspires to build on the IYC themes and to serve as a catalyst for education and understanding that enables the tools of imagination and science to make a positive difference. The Award will be formally presented to Professor Mahaffy at the November 10 meeting of the Northeastern Section.

National Chemistry Week 2011: Design a t-shirt contest
Would you like to design the NCW 2011 t-shirt worn by all NESACS NCW volunteers? The winning design will be on the front of the t-shirt. The Northeastern Section of the American Chemical Society Logo and NCW 2011 will be on the back of the t-shirt. This contest is open to all students K-12 in the Northeastern Section.

Contest rules:
1. Your design must either capture the NCW 2011 theme of Chemistry – Our Health, Our Future or the International Year of Chemistry Theme of Water. Please visit www.acs.org for more information.
2. You may use up to 4 colors in your design and your design must be on an unlined 8.5” x 11” sheet of paper.
3. The deadline for submission is September 20, 2011. The winner will be announced by October 1, 2011.
4. Please mail your original design to: Christine Jaworek-Lopes 400 The Fenway Emmanuel College Boston, MA 02115
5. All entries must have the following information included with the entry: student’s name, grade, home address, telephone number, school name, school address, teacher’s name, email, and school telephone number. Both addresses are used for sending prizes.
6. Have fun!!!

RE-SEED
Retirees Enhancing Science Education through Experiments and Demonstrations
Since 1991, the RE-SEED program at Northeastern University has trained retired scientists and engineers and others with backgrounds in science or mathematics to provide classroom assistance to K-12 science teachers. There are over eighty volunteers assisting science teachers in the greater Boston area through the Boston RE-SEED Center. After taking part in a comprehensive training program, participants typically assist in school classrooms one day a week for one academic year. The RE-SEED Program is part of the Center for STEM (science, technology, engineering and mathematics) Education at Northeastern University. Other programs focus on science teacher professional development and student assistance in science learning.

The Boston RE-SEED Center is currently recruiting volunteer retired scientists and engineers for the 2011-2012 school year. The recruiting campaign is focused on the Boston Public School, but volunteers may elect to serve closer to their homes. The 32-hour training is being held at the Boston Public Schools Science Center and will be conducted by the Northeastern University Center for STEM Education personnel with assistance from BPS staff. An information meeting is being held at Northeastern University on August 24, 2011, and the training will begin September 12, 2011. Call Paul Conroy at 617-737-8388 for more information and to register for the information session.

You can learn more about RE-SEED by visiting their website, www.reseed.neu.edu or by calling Paul Conroy at 617-373-8388 or by email to pa.conroy@neu.edu.

The Center for STEM Education, INV520, Northeastern University, 360 Huntington Avenue, Boston, MA 02115

Your one-stop source to career-related links in the Chemical Sciences
WWW.NESACS.ORG/CAREERS
Save the Date!

10th Anniversary!

10th Annual Undergraduate Symposium on Sustainability and the Environment
Saturday, November 19, 2011
Bridgewater State University

Please join us as we celebrate our 10th anniversary of the only symposium dedicated to undergraduate environmental research and projects that address sustainability issues from a campus, regional, national, or global perspective. The event will also include Phase I tours of our new science and math center.

Please email Ed Brush (ebrush@bridgew.edu) to add your name to our distribution list. A formal “Call for Abstracts” will be sent electronically in September.

National Chemistry Week Events

Celebrating Chemistry—Our Health, Our Future!

October 23, 2011 – Museum of Science Boston

• Phyllis A. Brauner Memorial Lecture by Dr. Bassam Shakhashiri
Dr. Bassam Shakhashiri is a Professor of Chemistry at the University of Wisconsin-Madison and is the William T. Evjue Distinguished Chair for the Wisconsin Idea. Professor Shakhashiri has captivated audiences with his scientific demonstrations at a variety of locations, including Boston’s Museum of Science, the National Academy of Sciences and the Smithsonian’s National Air and Space Museum in Washington.

Taking place in Cahners Theatre (2nd floor, Blue Wing) at 1:00 pm and 4:00 pm.

* Admission to the museum is required. Free tickets to Dr. Shakhashiri’s show will be available on a first come, first serve basis. Tickets are available via advance reservation. To reserve tickets, please contact the NESACS secretary either via email secretary@nesacs.org (preferred) or by phone 1-781-272-1966 before October 20, 2011. Tickets will be available for pick-up in the lobby of the museum at the ACS table.

• Kicking off National Chemistry Week 2011 festivities
Join us in a variety of hands-on activities related to the yearly theme. Taking place from 1:00 pm - 5:00 pm on October 23, 2011 throughout the Museum.

October 29, 2011 – Boston Children’s Museum
From 11 am – 4 pm, NCW volunteers will be on-hand throughout the museum to perform demonstrations and assist in hands-on activities related to this years theme.

September 1 – October 21, 2011
K-12 students participate in the NCW poetry contest. Visit www.nesacs.org and http://portal.acs.org/portal/acs/corg/content?nfpb=true&_pageLabel=PP_TRANSITIONMAIN&node_id=1033&use_sec=false&sec_url_var=region1&_uuid=c2ba266d-bd00-4469-a4d5-76c2e0eb9d5f for more information (after July 15, 2011).

June 1 – September 20, 2011
K-12 students participate in the Local Section design a t-shirt competition. Visit www.nesacs.org for more information, or see page 6 of this issue of the NUCLEUS.
**In vitro Study of Human Ribonucleotide Reductase Enzymatic Activity and Assembly of Diferric-Tyrosyl Radical Cofactor**

Kathleen Fleming and JoAnne Stubbe
Department of Chemistry, Massachusetts Institute of Technology, Cambridge, MA 02139

**Introduction**

Ribonucleotide reductases (RNRs) catalyze the conversion of nucleotides to deoxynucleotides in all organisms and play an essential role in DNA replication and DNA repair.[1] Because of their central role, RNRS are also successful targets of several drugs used clinically in the treatment of a number of malignancies. Structurally, human RNR (hRNR) consists of two subunits. The H1 subunit binds nucleoside diphosphates (NDPs) and the dNTP/ATP allosteric effectors. The H2 subunit houses the FeIIIFeIII-tyrosyl radical cofactor required to initiate inter-subunit radical propagation (>35Å) that leads to thyl radical generation at the active site on H1 to catalyze NDP reduction.[1,2] Mechanism-based inhibitors (MBIs), such as Gemcitabine (Gemzar®, F2C, Figure 1a) have been utilized to successfully probe RNR catalytic activity.[3] Clofarabine (Clolar®, ClF, Figure 1b), a prodrug indicated for treatment of leukemia, is a nucleoside analog proposed to target RNR.[4] Elucidating the chemical inactivation mechanism of human RNR by Clolar® is of critical interest to further understand both the chemistry of RNR and the clinical efficacy of Clolar®. Prior to studying Clolar®, it was first necessary to purify and characterize active hRNR H1 and H2 subunits. Unlike *E. coli* RNR, much remains unknown about hRNR; specifically the stability of the diferrie-tyrosyl radical cofactor of human H2 versus *E. coli* β is not well understood and presents a challenge to conducting *in vitro* studies. Efforts were made to improve protocols for both the purification of hRNR and the *in vitro* reassembly of H2 active cofactor. Reported here are (1) improvements to the purification of H2, (2) a reproducible method for *in vitro* assembly of the FeIIIFeIII-tyrosyl radical (Y•) cofactor, (3) preliminary stability studies of the Y• under physiological conditions (pH 7.6, 37°C), and (4) an alternative synthesis of Clofar 5’-monophosphate from Clolar®.

**Results and Discussion**

**Purification of H1 and H2 subunits of Human RNR:** Recombinant human (His)₆–H1 and (His)₆–H2 were expressed in *E. coli*, yielding 1.2 mg/L culture and 3.8 mg/L culture respectively.[5] Poor protein yield and purity prompted purification optimization efforts, which resulted in improved yield, specific activity, and purity.[6] Use of Talon column allowed the removal of Arna, a 74 kDa *E. coli* protein, that previously co-purified with H1.[6] Talon was thus used for the purification of (His)₆–H2 to >90% homogeneity, as judged by 10% SDS–PAGE (Figure 2). As–isolated H2 lacks fully active diferric-Y• cofactor required for nucleotide reduction. This cofactor must be assembled in *in vitro* following purification. H1 was purified by a similar procedure. The specific activity (S.A.) of H1 (590–700 nmol/min/mg) was measured using [5–³H] CDP for the formation of 2’–deoxycytidine 5’–diphosphate (dCDP) over time in the presence of the ATP allosteric effector and thioredoxin/thioredoxin reductase/NADPH reducing system. *In vitro Assembly (Reconstitution) of H2 Diferric-Y• Cofactor:* The inherent instability of as–isolated mammalian subunits (hRNR H2 and mouse RNR M2) compared to *E. coli* β present a significant challenge to all *in vitro* experiments. *In vitro Assembly (Reconstitution) of H2 Diferric-Y• Cofactor:* The inherent instability of as–isolated mammalian subunits (hRNR H2 and mouse RNR M2) compared to *E. coli* β present a significant challenge to all *in vitro* experiments. The *in vitro* Y• half–lives for *E. coli* RNR β subunit and mouse RNR M2 have previously reported to be on the order of several days and 10 min, respectively.[7] A purification and reconstitution protocol has previously been developed for *E. coli* RNR β subunit that allows study of the stoichiometry and time–scale of the tyrosine oxidation reaction.[8] Using this protocol, addition of Fe²⁺ in the presence of O₂ to the purified apo *E. coli* RNR β subunit spontaneously leads to assembly of the diferric center and oxidation of Y122 (Equation 1). A modified protocol has recently deve-
oped for the reconstitution of the H2 active cofactor. However, the difficulties in reproducibly generating active cofactor were noted in initial studies. Prior to studying putative mechanism based inhibitors (MBI) of hRNR, it is critical to quantify the assembly and stability of the H2 subunit. Here characterizations of the as–isolated and reconstituted hRNR H2 subunit are reported.

Previous reports of the activity of the reconstituted diferric-Y• cofactor of H2 in vitro yielded 0.8 Y•/H2 dimer and S.A. of 1089 nmol/min/mg and 75 nmol/min/mg, respectively. [5,9] I sought to provide a reproducible method for regenerating (His)6–H2 cofactor to the theoretical 1.0 Y•/H2 dimer by first characterizing the as–isolated H2’s iron loading, tyrosyl radical content, and specific activity. The iron content of the hRNR H2 subunit[5] as–isolated was measured using a standard ferrozine–based colorimetric assay. Ferrozine binds ferrous iron, but not ferric iron, and forms a complex that absorbs strongly at 562 nm (ε562=27870 M⁻¹cm⁻¹). [10] The specific activity of H2 was measured by radioactive assay and Y• content per H2 dimer was measured using EPR spin–quantitation and gave 1.4–1.6 iron equivalents (equiv)/dimer, 0.2 Y•/H2 dimer, and S.A. of 900–1250 nmol/min/mg. With this knowledge in hand, in vitro reconstitution was systematically investigated. One Y•/H2 dimer (Figure 3) following in vitro reconstitution was achieved reproducibly by reducing the amount of iron equivalents incubated with the H2 in the glove–box at 4°C and alteration of the addition rate of Fe2+ to adjust for the as–isolated protein not being in apo form, and to account for potential obligatory conformation changes that regulate iron binding. The reconstituted (His)6–H2 subunit had 3.4 iron equiv/dimer, 1.0 Y•/H2 dimer, and S.A. of 2100–2400 nmol/min/mg.

While working towards developing a reproducible in vitro reconstitution method, a publication came out reporting the isolation of H2 (3.1 iron equiv/dimer, 1.23 Y•/H2 dimer, and S.A. of 6000 nmol/mg/mg) without the in vitro assembly of cofactor. [11] These results were obtained by overexpression of (His)6–H2 subunit in E. coli, harvesting the cell pellet, cell lysate preparation with Bug-buster and Benonase incubation, purification with Ni-NTA resin with elution of protein from the resin by gravity, dialysis of eluate overnight, followed by concentration and activity measurements. Since the half life of the Y• is 25 min, dialysis overnight should leave little radical. In my hands, the report from this group was irreproducible; the (His)6–H2 subunit with 0.6 iron equiv/dimer, no detectable Y• and S.A. of 158 nmol/min/mg. Thus, we used our optimized protocol.

I conducted a preliminary study of the in vitro half-life of the H2 Y• 1.0 Y•/dimer, SA 2100 nmol/min/mg) by monitoring its time–dependent decay at 37°C, and pH 7.6. The half–life was 25 minutes (Figure 4). The instability of the human H2 subunit in vitro requires that the decay of Y• and the specific activity be monitored during all in vitro inhibition experiments to correct for the spontaneous enzyme decay. This in vitro half–life of human H2 contrasts with reported in vitro half–lives of E. coli and mouse β.

Alternative Preparation of CIFMP from Clolar®:
Recent work noted difficulty purifying CIFMP which was generated enzymatically from Clolar® and ATP with HdCK. [6] This procedure yielded an equilibrium mixture of starting material and products: CIFMP and ADP. A multi–step DEAE anion exchange chromatography was utilized to isolate CIFMP. I investigated an alternative two–step method for the purification of CIFMP from ADP, which, as found previously, coeluted on anion exchange chromatography at 350 mM triethylammonium bicarbonate (TEAB). A periodate cleavage step was introduced to destroy ADP, using a protocol previously reported. [12] Sodium periodate selec-

---

**Equation 1:**

\[ Y-OH + 2Fe^{2+} + 3/2O_2 + e^- + 3H^+ \rightarrow \bullet-O-Y + Fe^{3+}-O-Fe^{3+} + 2H_2O \]

Previous reports of the activity of the reconstituted diferric-Y• cofactor of H2 in vitro yielded 0.8 Y•/H2 dimer and S.A. of 1089 nmol/min/mg and 75 nmol/min/mg, respectively. [5,9] I sought to provide a reproducible method for regenerating (His)6–H2 cofactor to the theoretical 1.0 Y•/H2 dimer.
Summer Scholar
Continued from page 9

tively reacts with the cis–diol of the sugar of ADP to cleave the 2′–C-3′–C bond, generating a dialdehyde. The CIFMP is unreactive. After removal of excess periodate, excess methyl amine (pH 7.5) is added to form iminium ions, leading to the elimination of pyrophosphate. Inorganic pyrophosphatase irreversibly converts the inorganic pyrophosphate to inorganic phosphate. Anion exchange chromatography using a linear gradient from 0–600 mM TEAB (pH 6.8) allowed recovery of homogenous CIFMP (eluted at 350 mM TEAB).

Study of Inhibition of E. coli α Subunit by Clofarabine 5′–diphosphate in Presence of 10 Fold Molar Excess β: Preliminary progress curves for dCDP formation showed possible biphasic time-dependent inhibition. These studies suggested that CIFDP may be a slow-binding, reversible inhibitor of E. coli α RNR subunit (Figure 5).

Materials and Methods: General: Clofarabine was purchased from AK Scientific. The pET–9d expression vector for human deoxycytidine kinase (His6–HdCK) was obtained in E. coli BL21 (DE3) pLySS strain as a gift from Dr. Staffan Eriksson. The purification of E. coli thioredoxin (TR, 40 units/mg) [13] and E. coli thioredoxin reductase (TRR, 1400 units/mg). [14] HdCK (S.A. 150 nmol/min/mg measured by spectrophotometric assay using pyruvate kinase and lactate dehydrogenase) and UMP–CMP kinase (4.8 μmol/mg/ min by the γ–32 P]ATP phosphate transfer assay) have previously been described. [15] UV–vis absorption spectra were obtained and spectrophotometric assays were carried out using a Cary 3 UV–vis spectrophotometer (Varian, Walnut Creek, CA). X–band EPR spectra were acquired using a Bruker EMX spectrometer (Bruker, Madison, WI).

Isolation and Characterization of E. coli RNR α and β Subunits: Wild type E. coli RNR (His)α–α subunit (S.A. 1600-2000 nmol/min/mg) was purified and pre-reduced as previously described; [16] protein concentration was determined using E280mm = 189 mM-1 cm-1 (6). Wild type E. coli (His)β–β subunit was pre-incubated at 25°C using recombinant technology and purified from cellular extracts by affinity chromatography, Ni2+–NTA resin, as previously reported. [17,18] The diferic-tyrosyl-Y• cofactor was assembled in vitro as previously described. [17] Protein concentration was determined using E280nm = 131 mM-1 cm-1. [19] Specific activity (6000–7000 nmol/min/mg) was measured by radioactive and NADPH coupled spectrophotometric assay. Y• content (1–1.2 radicals per dimer) was measured both by the drop-line correction spectroscopy method and by EPR spectroscopy, as previously reported. [20] EPR spectra were acquired using a Bruker EMX X–band spectrometer at 77 K equipped with a quartz finger dewar and at 20 K using an Oxford Instruments liquid helium cryostat (9.38 GHz Microwave Frequency, 1 mW Microwave Power, 1 Gauss Modulation Amplitude). Radical content was quantified against a standard solution of 1 mM CuSO4 in 50 mM EDTA by double integration of spectra registered at non-saturating microwave levels by standard Bruker software.

Figure 5. NADPH Coupled Spectrophotometric Inhibition Assay, Biphasic Time-Dependent Inhibition of E. coli a RNR subunit by CIFDP at 25°C (A: 0 μM CIFDP, B: 20 μM, C: 40 μM, D: 80 μM). The reaction mixture (300 μL: 200 μM NADPH, 1 mM CDP, 3 mM ATP, 30 μM TR, 0.5 μM TRR, 50 mM Hepes (pH 7.6), 15 mM MgCl2, 1 mM EDTA, 0.2μM α, and 2μM β) without CIFDP (1 mM final concentration, saturating substrate conditions) or CIFDP (0-80 μM final concentration) was pre-incubated at 25°C for 1 min. CDP/CLDP was added and reduction of absorbance at 340 nm was continuously monitored for 1.5 minutes after addition; 1 nmol of NADPH oxidized per minute corresponds to 1 nmol of dCDP formed per minute.

Isolation and Characterization of Human RNR H1 and H2 Subunits: The (His)6–H1 and (His)6–H2 subunits were purified using a modified protocol reported previously. [21] Talon (Clontech) resin was used instead of Ni-NTA, and a dATP affinity column was used as a second step to achieve higher purity, higher specific activities and reduced purification time. In vitro Assembly of Human RNR H2 Active Cofactor: Diferic Y•– Human (His)6–H2 subunit (50 μM) in 500 μL of 50 mM Hepes (pH 7.6), 100 mM KCl, 10% glycerol was deoxygenated by six cycles of evaluation (for 3X10 s) followed by argon flushing using standard Schlenk line technique. The deoxygenated (His)6–H2 solution was brought into the glove–box (M. Braun, Stratham, NH) and stored at 4°C. Incrementally over a 15 min period 3 equivalents of Fe (II) (deoxygenated ferrous ammonium sulfate in 50 mM Tris (pH 7.6) and 100 mM KCl were added; the concentration of Fe(II) was determined by ferrozine assay. [10] The resulting mixture was incubated at 4°C for an additional 15 min. The protein was then removed from the glove–box and 170 μL (8-fold excess of 3.5 equiv/dimer required) of O2(g) saturated buffer was added and O2(g) was blown over the surface of the protein solution. Excess iron was removed by Sephadex G25 chromatography (40 mL, 2.5 X 30 cm). An activity assay in the presence of seven–fold molar excess human (His)6–H1 subunit was carried out, and 250 μL of the protein solution was transferred to an EPR tube and frozen in liquid N2 for EPR spin–quantiﬁcation of Y•/dimer.

Radioactive and Spectrophotometric Assays: Measurement of E. coli and Human RNR SA: The reduction of CDP by E. coli and human RNR was assayed by measuring the oxidation of NADPH coupled to dCDP formation and the forma-
tion of radioactive dCDP from [5–3H] CDP. In the NADPH oxidation method, the disappearance of A340 nm was followed continuously using a Cary 3 spectrophotometer (Varian). The following were incubated in a final volume of 300 μL: 200 μM NADPH, 1 mM CDP, 3 mM ATP, 30 μM TR, 0.5 μM TRR, 50 mM Heps (pH 7.6), 15 mM MgCl2, 1 mM EDTA, 2 μM (or 0.2 μM) α, and 0.2 μM (or 2 μM) β. The reaction mixture was pre-incubated at 25°C for 1 min. The subunit being assayed in 10-fold molar excess of the other subunit was added to initiate the reaction. Initial velocities were measured and used to calculate nmol of NADPH oxidized per min; 1 nmol of NADPH oxidized per min corresponds to 1 nmol of dCDP formed per min.[20] For the radioactive assay method, a reaction mixture contained in a final volume of 210 μL: 50 mM Heps (pH 7.6), 15 mM MgCl2, 1 mM EDTA, 0.3 μM (or 3 μM) α, 3 μM (or 0.3 μM) β, 3 mM ATP, 1 mM [5–3H] CDP, and 0.2 μM ClFDP in Presence of 10 Fold Molar Excess β. The reaction mixture contained in a final volume of 300 μL: 200 μM NADPH, 1 mM CDP, 3 mM ATP, 30 μM TR, 0.5 μM TRR, 50 mM Heps (pH 7.6), 15 mM MgCl2, 1 mM EDTA, 2 μM (or 0.2 μM) α, and 0.2 μM (or 2 μM) β. The reaction mixture without CDP (1 mM final concentration) or ClFDP (0-80 μM final concentration) was pre-incubated at 25°C for 1 min. CDP/ClDP was added and the reduction of absorbance at 340 nm was continuously monitored for 1.5 minutes after addition; 1 nmol of NADPH oxidized per minute corresponds to 1 nmol of dCDP formed per minute.[20]

Acknowledgements: Thank you to the ACS for supporting me as a recipient of the 2010 James Flack Norris/Theodore William Richards Summer Research Scholarship. Thank you to Dr. Stubbe and members of the Stubbe Laboratory for rigorous laboratory training and for sharing their love for biochemistry research.

References:
ACS Senior Chemists

Continued from page 4

Members of SCTF at the Senior Chemists Breakfast at the ACS meeting in Anaheim, March 2011, left-right, George Heinze (New Jersey Local Section), SCTF Chair, Morton Hoffman (NESACS), Ronald Archer (Connecticut Valley Local Section).

Photo by Linda Wang, C&EN

(Washington, 2009); Robert Grubbs, CalTech (San Francisco, 2010); Roald Hoffmann, Cornell University (Boston, 2010); Harry Gray, CalTech (Anaheim, 2011). A Senior Chemists Breakfast to be held in Denver on Tuesday, August 30, will feature Dr. Bassam Shakhashiri, current ACS President-Elect, as the guest speaker; he will speak on “Chemistry and Society: Looking Back, Looking Around, Looking Ahead.”

SCTF has organized, co-sponsored, or co-listed the following symposia on topics important to seniors and other attendees at the national meetings: being a consultant, volunteerism (Washington, 2009); the consulting business (San Francisco, 2010); governmental interface, connections to Germany and Europe, Medicare supplement workshop (Boston, 2010); aging and the ACS, diverse workforces in small businesses (Anaheim, 2011). In Denver (Fall 2011), SCTF will co-sponsor a symposium on interactions between the Younger Chemists Committee of ACS and the European Young Chemists Network of EuCheMS (European Association for Chemical and Molecular Sciences), as well as symposia on entrepreneurialism, health care reform and its impact on seniors, and the globalization of the chemistry profession.

SCTF is in the process of planning future activities for seniors, including assistance with consultancies, employment, income tax issues, and retirement and estate planning. It anticipates organizing trips for seniors to universities for educational visits, and to local governmental bodies for legislative visits. Seniors with academic or industrial backgrounds will become part of the “Chemistry Ambassadors” to interact with students and teachers at the K-12, undergraduate, and graduate levels. SCTF plans to work with local sections toward the establishment of their own senior chemists committees for the promotion of relevant activities of interest to their members in the areas of education, governmental affairs, and environmental improvement.

Later in 2011, the ACS Committee on Committees (ConC) will evaluate the programs and activities of SCTF with an eye toward the establishment of a national Senior Chemists Committee (SCC) that would be analogous to the current Younger Chemists Committee (YCC) and Women Chemists Committee (WCC).

Events in Chemistry

Continued from page 5

August 23, 1887
Bradley Dewey was the “Czar” of synthetic rubber production in World War II and served as President of ACS in 1946. He was born on this date.

August 25, 1812
Nicolai N. Zinin, who was born on this date, discovered the reduction of aromatic nitro compounds to amines, 1842, and the benzidine rearrangement. He founded and was the first president of the Russian Chemical Society, 1868-77.

August 31, 1786
Michel E. Chevreul was a researcher on dyes and physics of color and discovered stearin and margarine. He was born on this date and lived to 100.

Additional historical events can be found at Dr. May’s website, http://faculty.cua.edu/may/Chemistrycalendar.htm

ACS Senior Chemists

Continued from page 4

Members of SCTF at the Senior Chemists Breakfast at the ACS meeting in Anaheim, March 2011, left-right, George Heinze (New Jersey Local Section), SCTF Chair, Morton Hoffman (NESACS), Ronald Archer (Connecticut Valley Local Section).

Photo by Linda Wang, C&EN

(Washington, 2009); Robert Grubbs, CalTech (San Francisco, 2010); Roald Hoffmann, Cornell University (Boston, 2010); Harry Gray, CalTech (Anaheim, 2011). A Senior Chemists Breakfast to be held in Denver on Tuesday, August 30, will feature Dr. Bassam Shakhashiri, current ACS President-Elect, as the guest speaker; he will speak on “Chemistry and Society: Looking Back, Looking Around, Looking Ahead.”

SCTF has organized, co-sponsored, or co-listed the following symposia on topics important to seniors and other attendees at the national meetings: being a consultant, volunteerism (Washington, 2009); the consulting business (San Francisco, 2010); governmental interface, connections to Germany and Europe, Medicare supplement workshop (Boston, 2010); aging and the ACS, diverse workforces in small businesses (Anaheim, 2011). In Denver (Fall 2011), SCTF will co-sponsor a symposium on interactions between the Younger Chemists Committee of ACS and the European Young Chemists Network of EuCheMS (European Association for Chemical and Molecular Sciences), as well as symposia on entrepreneurialism, health care reform and its impact on seniors, and the globalization of the chemistry profession.

SCTF is in the process of planning future activities for seniors, including assistance with consultancies, employment, income tax issues, and retirement and estate planning. It anticipates organizing trips for seniors to universities for educational visits, and to local governmental bodies for legislative visits. Seniors with academic or industrial backgrounds will become part of the “Chemistry Ambassadors” to interact with students and teachers at the K-12, undergraduate, and graduate levels. SCTF plans to work with local sections toward the establishment of their own senior chemists committees for the promotion of relevant activities of interest to their members in the areas of education, governmental affairs, and environmental improvement.

Later in 2011, the ACS Committee on Committees (ConC) will evaluate the programs and activities of SCTF with an eye toward the establishment of a national Senior Chemists Committee (SCC) that would be analogous to the current Younger Chemists Committee (YCC) and Women Chemists Committee (WCC).
Rilas Technologies is your partner for all your chiral separations needs, from analysis to purification. Our services are fast, flexible and highly affordable. We offer:

- Chiral Analysis, enantiomer excess determination within 1-3 days
- Purifications of enantiomers from milligram to gram scale within 3-6 days
- Free sample pick up and delivery within Boston Metro area

The Advantage of Working with Rilas

- We offer over 25 years of experience
- There is no need to disclose structural information
- Simple pricing with no lengthy quoting and negotiating process

For more information:
www.rilastech.com
info@rilastech.com
857-231-2078

---

 Elemental Analysis
 CHNOS ash, ICP-AES, ICP-MS, TOC, TOX, BTU
 Problem Solving

 Elements By SFC

 NMR Service 500MHz

 NMR-IR/IR/UV/VIS/FL

 CAGE Code: 44ME9
 DUNS: 556785657

 ---

 Micron Analytical Services

 COMPLETE MATERIALS CHARACTERIZATION
 MORPHOLOGY   CHEMISTRY   STRUCTURE

 SEM/EDXA • EPM/WDXA • XRD • XRF • ESCA • AUGER • FTIR • DSC/TGA

 Registered with FDA • DEA • GMP/GLP Compliant

 3815 Lancaster Pike Wilmington DE 19805
 Voice 302-998-1184, Fax 302-998-1836
 E-Mail micronanalytical@compuserve.com
 Web Page: www.micronanalytical.com

 ---

 Robertson Microlit Laboratories

 Where speed and accuracy are elemental

 Elemental CHN, S, X. Analysis (same day service)
 Metals by ICP-OES, ICP-MS, A/A
 FTIR, UV/VIS Spectroscopy
 Ion Chromatography

 Bioavailability
 Polarimetry
 DSC, TGA, melting point
 KF Aquametry, Titrimetry

 1705 U.S. Highway 46 • Suite 1D • Ledgebrook, NJ 07852 • 973.966.6668 • F 973.966.0136
 www.robertson-microlit.com • email: results@robertson-microlit.com

 Rapid Results • Quality • Accuracy • Competitive Pricing
COSMOSIL HPLC Columns
Since 1979

New Phases Now Available!

HILIC (Triazole bonded)
- Unique stationary phase for highly polar compounds
- piNAP (Naphthylenylethyl group bonded)
- Enhanced interactions for unsaturated compounds

Cholesterol (Cholesteryl group bonded)
- New stationary phase for structural isomers

Nacalai USA, Inc. 1840 La Jolla Blvd. Suite A206 San Diego, CA 92121
Tel: 858-494-0493 Email: info@nacalaiusa.com
www.nacalaiusa.com

PolyOrg, Inc.
Chemical Solutions for the Life Science Industry

- Custom Organic Synthesis
- Process Development
- Contract R & D
- Pharmaceutical Intermediates
- Medicinal Chemistry Support
- Biotechnology Specialty Reagents
- Solid Support Reactions
- Process Validation
- Gram to Multi-Kilogram Synthesis

PolyOrg Inc.
10 Powers Street, Leominster, MA 01453
Phone: 978-465-7976 1-866-Poly-002
Fax: 978-465-0884 info@polyorginc.com
www.polyorginc.com

Index of Advertisers
BUCHI Corporation.........16
Boston College...............14
CreaGen Biosciences .......14
Eastern Scientific Co.........16
EMD Chemicals, Inc.........5
Front Run OrganX, Inc.......13
Huffman Laboratories, Inc...13
Mass-Vac, Inc.................4
Micron Inc........................13
Nacalai USA, Inc.............14
New Era Enterprises, Inc....13
Northeastern University ...2,15
NuMega Resonance Labs ...13
Organix, Inc...................13
PCI Synthesis .................12
PolyOrg, Inc...................14
Rilas Technologies, Inc.....13
Robertson Microlit Labs....13
Vacuubrand, Inc...............12
Waters Corporation..........14
The professional science master's (PSM) is an innovative degree designed to allow students to pursue advanced training and excel in science, while simultaneously developing highly-valued business skills necessary to adapt to a changing workplace.

The rapid growth of biopharmaceuticals has created a critical need for regulatory science professionals. The shift in the pharmaceutical industry from small molecules to biologics coupled with many blockbuster drugs coming off patent will revolutionize the industry and further increase the demand for regulatory science.

Enroll today for the career of tomorrow!
Learn more at www.northeastern.edu/biotech/regscism.html
It is now possible to sign up for electronic delivery of the Nucleus at www.nesacs.org. You can choose an electronic-only option, a paper-only option, or receive both an electronic copy and a paper copy. The electronic copy, in general, will be available two to three weeks before paper copies delivered by third class mail. Improved timeliness should greatly enhance the value of the Nucleus for our readers.

If you have any questions, contact the editor by email at michael.filosa(at)zink.com.