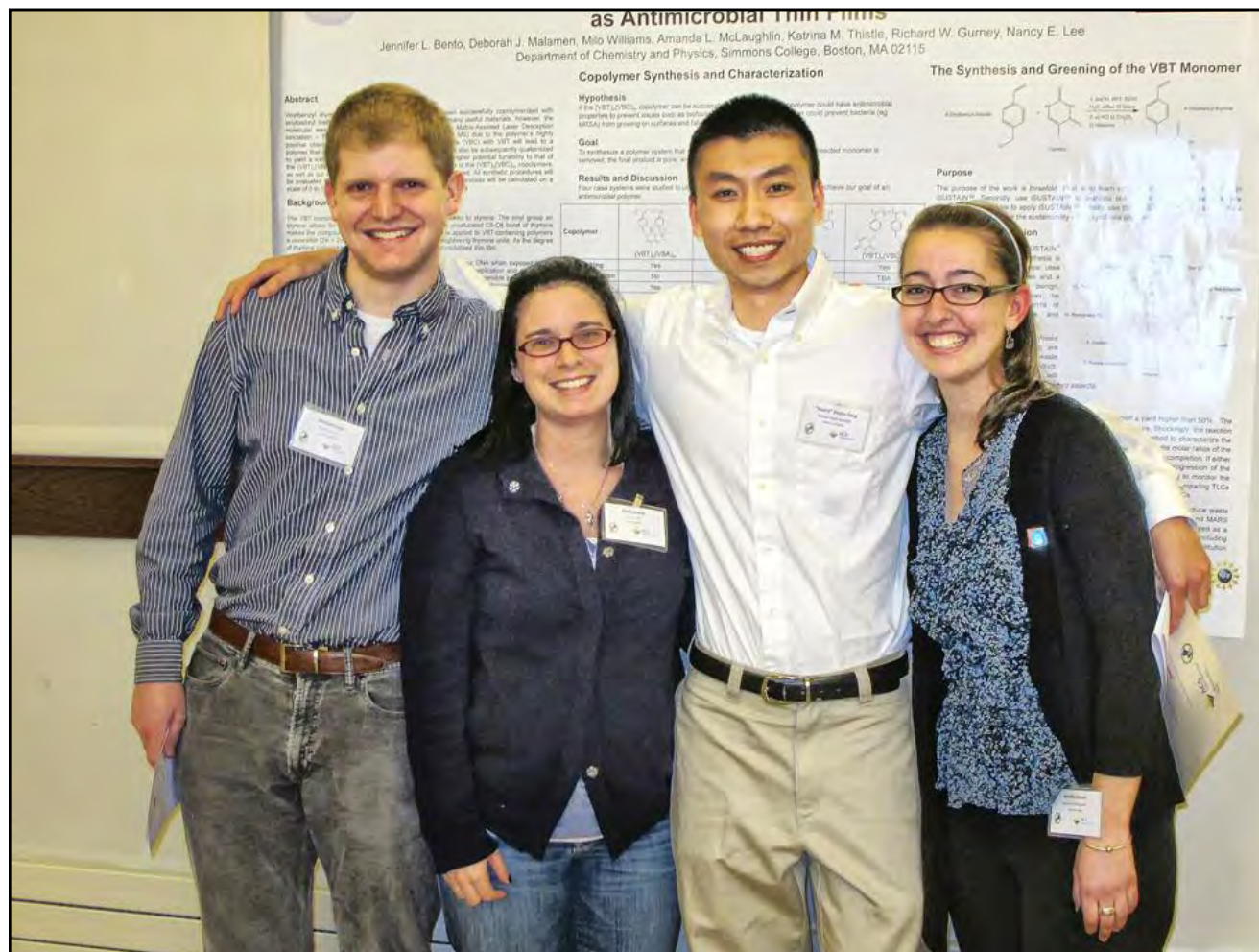


# THE NUCLEUS

Summer 2011

Vol. LXXXIX, No. 10



## 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



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**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 Chemicals); 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. Photographed at the May Education Night Meeting. (Photo by Morton Z. Hoffman).

**Deadlines:** October 2011 Issue: August 11, 2011  
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# ACS SENIOR CHEMISTS TASK FORCE

Morton Hoffman and Michaelene Chen, SCTF Members



Tom Beattie (San Diego Local Section) and SCTF member, at left, with Harry Gray (Cal-Tech), who spoke at the Senior Chemists Breakfast at the ACS meeting in Anaheim, March 2011. Photo by M.Z. Hoffman

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

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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

continued on page 12

# 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 B<sub>1</sub> 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, Stimulated 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*



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# Announcements

## *James Flack Norris Award 2011 Award to Peter Mahaffy*



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](http://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 and learned from 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

## **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](http://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!!! ◇

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to Professor Mahaffy at the November 10 meeting of the Northeastern Section. ◇

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## **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](http://www.reseed.neu.edu) or by calling Paul Conroy at 617-373-8388 or by email to [pa.conroy@neu.edu](mailto:pa.conroy@neu.edu).

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



# 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 10<sup>th</sup> 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. ◇

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## Summer Scholar

*Continued from page 11*

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# National Chemistry Week Events



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**Chemistry—Our Health, Our Future!**

## October 23, 2011 – Museum of Science Boston

• *Phyllis A. Brauner Memorial Lecture by Dr. Bassam Shkhashiri*

Dr. Bassam Shkhashiri is a Professor of Chemistry at the University of Wisconsin-Madison and is the William T. Evjue Distinguished Chair for the Wisconsin Idea. Professor Shkhashiri 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 (2<sup>nd</sup> floor, Blue Wing) at 1:00 pm and 4:00 pm.

\* Admission to the museum is required. Free tickets to Dr.

Shkhashiri'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](mailto: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 year's theme.

## September 1 – October 21, 2011

K-12 students participate in the NCW **poetry contest**. Visit [www.nesacs.org](http://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-a4d5-76c2e0eb9d5ff](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-a4d5-76c2e0eb9d5ff) 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](http://www.nesacs.org) for more information, or see page 6 of this issue of the NUCLEUS. ◇

# Summer Scholar Report

## *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.<sup>[1]</sup> 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 Fe<sup>III</sup>Fe<sup>III</sup>-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.<sup>[1,2]</sup> Mechanism-based inhibitors (MBIs), such as Gemcitabine (Gemzar<sup>®</sup>, F<sub>2</sub>C, Figure 1a) have been utilized to successfully probe RNR catalytic activity.<sup>[3]</sup> Clofarabine (Clolar<sup>®</sup>, ClF, Figure 1b), a prodrug indicated for treatment of leukemia, is a nucleoside analog proposed to target RNR.<sup>[4]</sup> Elucidating the chemical inactivation mechanism of human RNR by Clolar<sup>®</sup> is of critical interest to further understand both the chemistry of RNR and the clinical efficacy of Clolar<sup>®</sup>. Prior to studying Clolar<sup>®</sup>, 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 diferric-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 Fe<sup>III</sup>Fe<sup>III</sup>-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 Clolar 5'-monophosphate from Clolar<sup>®</sup>.

### Results and Discussion

**Purification of H1 and H2 subunits of Human RNR:** Recombinant human (His)<sub>6</sub>-H1 and (His)<sub>6</sub>-H2 were expressed in *E. coli*, yielding 1.2 mg/L culture and 3.8 mg/L culture respectively.<sup>[5]</sup> Poor protein yield and purity prompted purification optimization efforts, which resulted in improved yield, specific activity, and purity.<sup>[6]</sup> Use of Talon column allowed the removal of Arna, a 74 kDa *E. coli* protein, that previously co-purified with H1.<sup>[6]</sup> Talon was thus used for the purification of (His)<sub>6</sub>-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 vitro* following purification. H1 was purified by a similar procedure. The specific activity (S.A.) of H1 (590–700

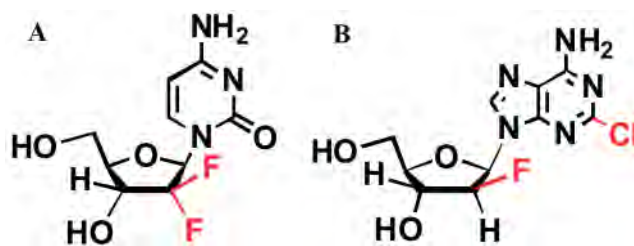


Figure 1. (a) Gemzar<sup>®</sup> (F<sub>2</sub>C) (b) Clolar<sup>®</sup> (ClF).

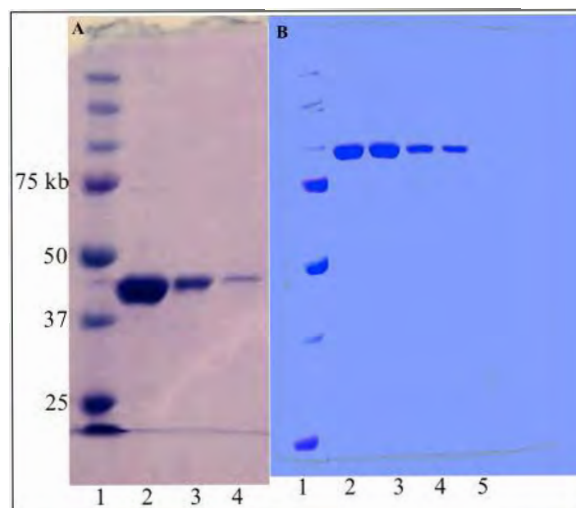
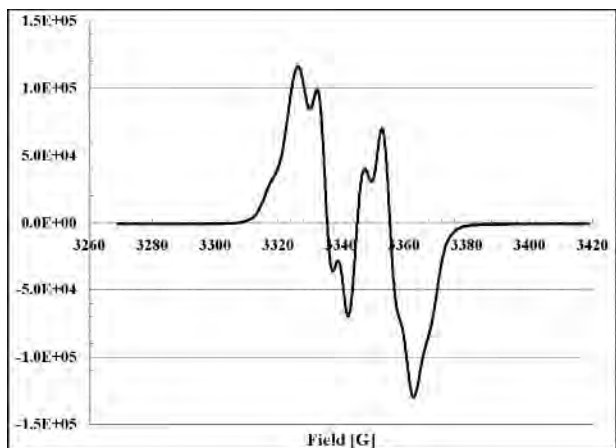


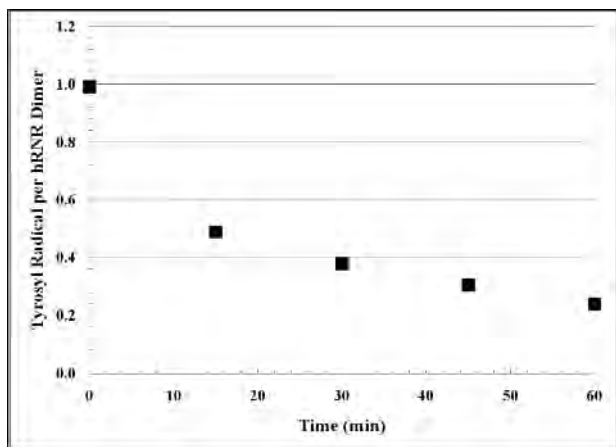
Figure 2. 10% SDS PAGE Analysis of Purified H2 (A) and H1 (B) Following Talon Affinity Chromatography Purification. The numbered lanes correspond to (1) Molecular Weight Markers, (2) 2.0 μg purified protein, (3) 1.0 μg purified protein, (4) 0.5 μg purified protein.

nmol/min/mg) was measured using [5-<sup>3</sup>H] 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. The *in vitro* Y• half-lives for *E. coli* RNR β subunit and mouse RNR M2 subunit have previously reported to be on the order of several days and 10 min, respectively.<sup>[7]</sup> 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.<sup>[8]</sup> Using this protocol, addition of Fe<sup>2+</sup> in the presence of O<sub>2</sub> to the purified apo *E. coli* β subunit spontaneously leads to assembly of the diferric center and oxidation of Y122 (Equation 1). A modified protocol has recently devel-



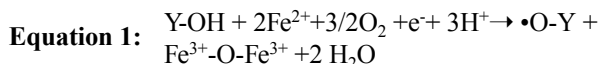


**Figure 3.** EPR Spectrum at 20 K of Reconstituted H2 (23.6  $\mu\text{M}$ ). The diferric-tyrosyl radical cofactor of H2 was assembled *in vitro*. EPR spin-quantitation was used to quantify the tyrosyl radical content per H2 dimer. EPR parameters: 9.37 GHz microwave frequency, 0.5 mW modulation amplitude, 100 kHz modulation frequency, 15 scans,  $DI/N$  79.5,  $DI/Nc=1.85$ ,  $Y\bullet/H2=[Y\bullet]_{std}^*/(DI/Nc)_{sample}/(DI/Nc)_{std}/[H2, dimer]=1.0 Y\bullet/H2 dimer$ .



**Figure 4.** Time-Dependent *in vitro* H2  $Y\bullet$  decay. Reconstituted H2 (1.0  $Y\bullet/H2$  dimer) was incubated at 37°C. At 0, 15, 30, 45, and 60 minute time points 200  $\mu\text{L}$  aliquots were transferred to EPR tubes and immediately flash frozen in liquid  $N_2$  for EPR spin-quantitation of  $Y\bullet$ /dimer.

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- $Y\bullet$  cofactor of H2 *in vitro* yielded 0.8  $Y\bullet/H2$  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)<sub>6</sub>-H2 cofactor to the theoretical 1.0  $Y\bullet/H2$

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 ( $\epsilon_{562}=27870 \text{ M}^{-1}\text{cm}^{-1}$ ). [10] The specific activity of H2 was measured by radioactive assay and  $Y\bullet$  content per H2 dimer was measured using EPR spin-quantitation and gave 1.4-1.6 iron equivalents (equiv)/dimer, 0.2  $Y\bullet/H2$  dimer, and S.A. of 900-1250 nmol/min/mg. With this knowledge in hand, *in vitro* reconstitution was systematically investigated. One  $Y\bullet/H2$  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  $\text{Fe}^{2+}$  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)<sub>6</sub>-H2 subunit had 3.4 iron equiv/dimer, 1.0  $Y\bullet/H2$  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  $Y\bullet/H2$  dimer, and S.A. of 6000 nmol/min/mg) without the *in vitro* assembly of cofactor. [11] These results were obtained by overexpression of (His)<sub>6</sub>-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  $Y\bullet$  is 25 min, dialysis overnight should leave little radical. In my hands, the report from this group was irreproducible; the (His)<sub>6</sub>-H2 subunit with 0.6 iron equiv/dimer, no detectable  $Y\bullet$  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  $Y\bullet$  1.0  $Y\bullet$ /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  $Y\bullet$  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  $\beta$ .

**Alternative Preparation of ClFMP from Clolar®:** Recent work noted difficulty purifying ClFMP which was generated enzymatically from Clolar® and ATP with HdCK. [6] This procedure yielded an equilibrium mixture of starting material and products: ClFMP and ADP. A multi-step DEAE anion exchange chromatography was utilized to isolate ClFMP. I investigated an alternative two-step method for the purification of ClFMP 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-

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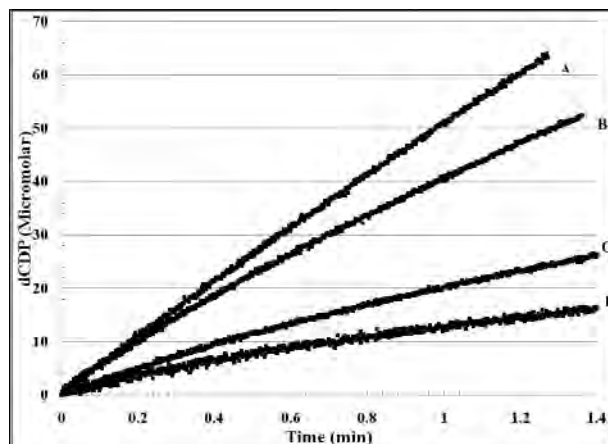
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tively reacts with the cis-diol of the sugar of ADP to cleave the 2'-C-3'-C bond, generating a dialdehyde. The CIFMP is unreactive. After removal of excess periodate, excess methyl amine (pH 7.5) is added to form iminium ions, leading to the elimination 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  $\alpha$  Subunit by Clofarabine 5'-diphosphate in Presence of 10 Fold Molar Excess  $\beta$* : 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*  $\alpha$  RNR subunit (Figure 5).

**Materials and Methods: General:** Clofarabine was purchased from AK Scientific. The pET-9d expression vector for human deoxycytidine kinase (His<sub>6</sub>-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)<sup>[13]</sup> and *E. coli* thioredoxin reductase (TRR, 1400 units/mg),<sup>[14]</sup> HdCK (S.A. 150 nmol/min/mg measured by spectrophotometric assay using pyruvate kinase and lactate dehydrogenase) and UMP-CMP kinase (4.8  $\mu$ mol/mg/min by the [ $\gamma$ -<sup>32</sup>P]ATP phosphate transfer assay) have previously been described.<sup>[15]</sup> 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  $\alpha$  and  $\beta$  Subunits:** Wild type *E. coli* RNR (His)<sub>6</sub>- $\alpha$  subunit (S.A. 1600–2000 nmol/min/mg) was purified and pre-reduced as previously described;<sup>[16]</sup> protein concentration was determined using  $\epsilon_{280\text{nm}} = 189 \text{ mM}^{-1}\text{cm}^{-1}$ (6). Wild type *E. coli* (His)<sub>6</sub>- $\beta$  subunit was over-expressed in *E. coli* using recombinant technology and purified from cellular extracts by affinity chromatography, Ni<sup>2+</sup>-NTA resin, as previously reported.<sup>[17,18]</sup> The diferric-tyrosyl-Y• cofactor was assembled *in vitro* as previously described.<sup>[17]</sup> Protein concentration was determined using  $\epsilon_{280\text{nm}} = 131 \text{ mM}^{-1}\text{cm}^{-1}$ .<sup>[19]</sup> 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.<sup>[8]</sup> 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 CuSO<sub>4</sub> 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*  $\alpha$  RNR subunit by CIFDP at 25°C (A: 0  $\mu$ M CIFDP, B: 20  $\mu$ M, C: 40  $\mu$ M, D: 80  $\mu$ M). The reaction mixture (300  $\mu$ L: 200  $\mu$ M NADPH, 1 mM CDP, 3 mM ATP, 30  $\mu$ M TR, 0.5  $\mu$ M TRR, 50 mM Hepes (pH 7.6), 15 mM MgCl<sub>2</sub>, 1 mM EDTA, 0.2  $\mu$ M  $\alpha$ , and 2  $\mu$ M  $\beta$ ) without CDP (1 mM final concentration, saturating substrate conditions) or CIFDP (0–80  $\mu$ M 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)<sub>6</sub>-H1 and (His)<sub>6</sub>-H2 subunits were purified using a modified protocol reported previously.<sup>[5]</sup> 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, Diferric Y•:** Human (His)<sub>6</sub>-H2 subunit (50  $\mu$ M) in 500  $\mu$ L 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)<sub>6</sub>-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.<sup>[10]</sup> The resulting mixture was incubated at 4°C for an additional 15 min. The protein was then removed from the glove-box and 170  $\mu$ L (8-fold excess of 3.5 equiv/dimer required) of O<sub>2</sub>(g) saturated buffer was added and O<sub>2</sub>(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)<sub>6</sub>-H1 subunit was carried out, and 250  $\mu$ L of the protein solution was transferred to an EPR tube and frozen in liquid N<sub>2</sub> for EPR spin-quantitation 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-

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tion of radioactive dCDP from [5-<sup>3</sup>H] CDP. In the NADPH oxidation method, the disappearance of A<sub>340 nm</sub> 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 Hepes (pH 7.6), 15 mM MgCl<sub>2</sub>, 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.<sup>[20]</sup> For the radioactive assay method, a reaction mixture contained in a final volume of 210 μL: 50 mM Hepes (pH 7.6), 15 mM MgCl<sub>2</sub>, 1 mM EDTA, 0.3 μM (or 3 μM) α, 3 μM (or 0.3 μM) β, 3 mM ATP, 1 mM [5-<sup>3</sup>H] CDP (S.A. 5926 cpm/nmol, ViTrax, Placentia, CA) 30 μM *E. coli* TR, 0.5 μM TRR, and 1 mM NADPH. The assay mixture was pre-incubated at 37°C for 2 min, and the reaction was initiated by the addition of [5-<sup>3</sup>H] CDP. Aliquots, 30 μL each, were removed over a 10 min time period and quenched in a boiling water bath for 2 min. dC production was analyzed, subsequent to dephosphorylation with alkaline phosphatase as previously described,<sup>[21]</sup> and analyzed by the method of Steeper and Stuart.<sup>[10]</sup> The reduction of CDP by hRNR was measured only by the formation of [5-<sup>3</sup>H] dCDP. The NADPH coupled spectrophotometric assay could not be used to measure the specific activity of hRNR subunits or the holoenzyme, since the reduction of absorbance at 340 nm versus time is not appreciable compared to the background to allow for specific activity quantitation.

*Time-Dependent Decay of Human H2 Y• at 37°C and pH 7.6:* Reconstituted human H2 (1.0 Y•/dimer, SA 2100 nmol/min/mg) was incubated at 37°C. Aliquots (200 μL) were transferred to EPR tubes at 0, 15, 30, 45, and 60 min and samples were immediately flash frozen in liquid N<sub>2</sub> for EPR spin-quantitation of the Y•/dimer.

*Clofarabine to CIFMP:* The reaction mixture contained in a final volume of 9 mL: 1 mM Clofarabine, 5 mM ATP, 2 mM DTT, 0.5 mg/mL BSA, 0.1 mg/mL HdCK, 50 mM Tris (pH 7.6), 100 mM KCl, and 10 mM MgCl<sub>2</sub>. The reaction was initiated by the addition of HdCK and the mixture incubated at 37°C for 45 min. The reaction mixture was loaded on a DEAE-Sephadex A-25 column (20 mL X 20 cm X 1 cm) equilibrated with 5 mM TEAB (pH 6.8) and the column washed with 50 mL of 5 mM TEAB. The product was eluted using a 150 mL X 150 mL linear gradient from 5 to 400 mM TEAB. Fractions (5 mL) were assayed for A<sub>260nm</sub> and A<sub>280nm</sub>; the nucleotide containing fractions were combined and the solvent was removed *in vacuo*. CIFMP eluted at 350 mM TEAB. <sup>31</sup>P NMR and <sup>1</sup>H NMR revealed contaminating ADP in addition to the CIFMP product. CIFMP was purified by oxidative cleavage of ADP with periodate fol-

lowed by pyrophosphatase treatment to convert liberated pyrophosphate into inorganic phosphate as previously reported.<sup>[12]</sup> Following this step, a second DEAE anion exchange chromatography step with the same aforementioned gradient was used to purify CIFMP.

*CIFMP to CIFDP:* This synthesis is reported in a publication under review.<sup>[6]</sup>

*Spectrophotometric Assay to Study Inhibition of E. coli α by CIFDP 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 Hepes (pH 7.6), 15 mM MgCl<sub>2</sub>, 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 CIFDP (0-80 μM final concentration) was pre-incubated at 25°C for 1 min. CDP/CIFDP 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.<sup>[20]</sup>

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**ACS Senior Chemists Events in Chemistry**

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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 Shkhashiri, 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, employ-

*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> ◇

ment, 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). ◇

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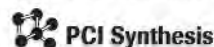
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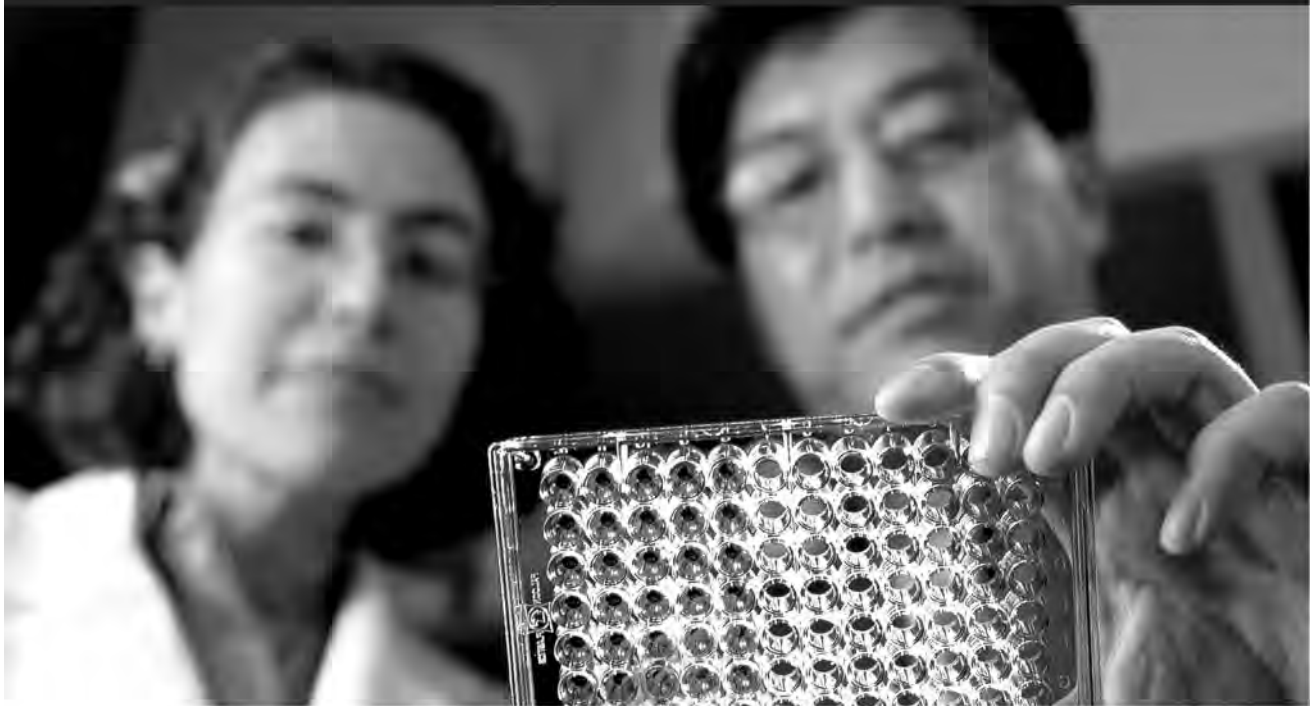
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