FOUNDED 1898
NORTHEASTERN SECTION • AMERICAN CHEMICAL SOCIETY
NESACS

THE NUCLEUS

April 2007 *Vol. LXXXV, No. 8*

Monthly Meeting

Esselen Award Meeting at Harvard, Prof. Michael Marletta, UCAL Berkeley, speaks on "Nitric Oxide in Biology: From Discovery to Therapeutics"

Summer Research Scholar

"Engineering Monovalent Avidin" by Jessica A. Lynch, Mark Howarth and Alice Y. Ting

Book Review

"Nontraditional Careers for Chemists: New Formulas in Chemistry," reviewed by Lynne A. O'Connell

Symposium

"Advances in Chemical Sciences" to be held March 30, 2007 at the Royal Sonesta Hotel in Cambridge, MA

SUMMER RESEARCH SCHOLAR

Engineering Monovalent Avidin

Jessica A. Lynch, Mark Howarth, Alice Y. Ting
Department of Chemistry, Massachusetts Institute of Technology, Cambridge MA, 02139

Introduction

The binding of the vitamin biotin by avidin or streptavidin is one of the tightest interactions known in nature. These two proteins are widely used by biologists for biomolecule labeling, immobilization and purification. Streptavidin and avidin have also shown promise in medical applications, for example, delivering radioisotopes to tumors.

Despite the existence of numerous applications for streptavidin and avidin, many limitations still exist. Because both proteins are tetramers, they can tetramerize the biotin conjugates to which they bind. This can lead to aggregation, interfering with the use of avidin/streptavidin for tracking cell surface proteins and promoting complement activation when used *in vivo*. Also, immunogenicity is a problem, particularly with streptavidin. As the therapy is repeated, antibodies are generated against the foreign protein and efficacy decreases. Another challenge is to engineer the avidin/streptavidin to bind biotin analogs that are not present endogenously in the body, to give an orthogonal labeling system. The Ting lab is working to address all of these limitations, by rational engineering and selection of random mutants.

The lab recently solved the valency problem by developing a monovalent streptavidin with a single femtomolar biotin binding site, which was utilized in cross-linking-free labeling of biotinylated neuroligin-1 on the surface of living neurons. It is essential to keep streptavidin tetrameric or biotin binding affinity decreases ten thousand fold. The goal was to produce a streptavidin tetramer consisting of three subunits unable to bind biotin (termed "Dead") and one subunit that binds biotin as effectively as wild-type streptavidin (termed "Alive"). To generate the desired mutants, three alterations were made in the binding site. A 6His tag was then added to the Alive subunit and then combined with Dead and Alive subunits at a 3:1 molar ratio in the presence of guanidinium hydrochloride. The subunits were then rapidly refolded and monovalent streptavidin was then purified on a nickel-nitrilotriacetic acid column[1].

The Ting lab has also developed a method of site-specific labeling of cell surface proteins utilizing biotin ligase. *E. coli* biotin ligase (BirA) sequence-specifically ligates biotin to a 15-amino-acid acceptor peptide (AP)[2]. Cell surface proteins are then labeled by detecting the biotin with monovalent streptavidin.

The goal of my project was to engineer a monovalent avidin, for use in drug targeting and cellular labeling. This is important because avidin behaves differently from streptavidin *in vivo*; notably, it is much less immunogenic than its bacterial analog.

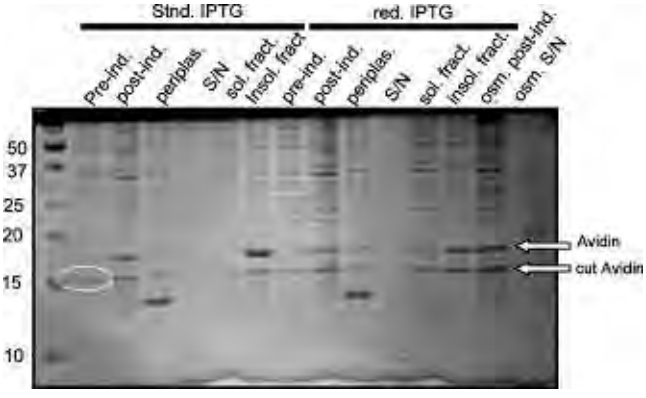

Figure 1

Results

The first task was to determine an appropriate expression and purification method for avidin, which, when expressed cytoplasmically, forms inclusion bodies which are time-consuming to purify. We decided to express the avidin periplasmically, and the avidin was thus engineered to have a bacterial leader sequence directing the protein to the periplasmic space. Because the yield was low and the leader was not cleaved properly, we decided on several methods of improvement, including using 3-fold less IPTG (70 μM), and expressing the protein under stress conditions: 0.5M Sorbitol, 4% NaCl and 10mM glycine betaine[3] (**Fig. 1**). We also experimented with GET buffer[4] (0.5M glucose, 1mM EDTA and 200 mM Tris, pH 7.4) with 0.15 mg/mL lysozyme and sonication at 40% duty cycle 30 seconds on, 30 seconds off, two times to decrease the viscosity of the lysate and increase our yield. To aid with leader cleavage, we added a 4-amino acid spacer to the N-terminus of the protein. Despite these improvements, subsequent yields of f of the Ni-NTA column remained low (**Fig. 2**).

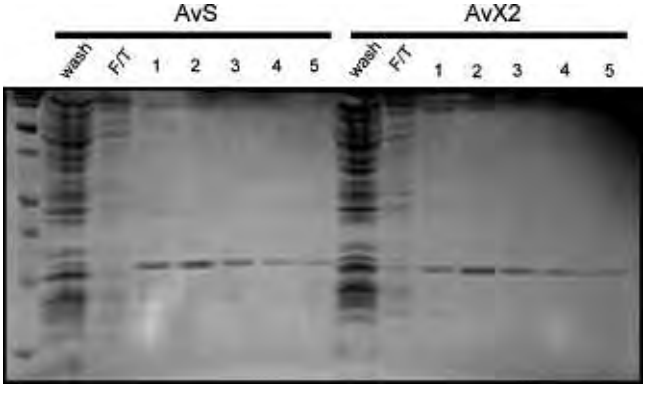

Figure 2

continued on page 10

Contents

Cover: *April Speaker, Professor Michael A. Marletta, (Photo Courtesy of Prof. Marletta)*

Deadlines: *Summer 2007 Issue: June 15, 2007*
September 2007 Issue: July 13, 2007

THE NUCLEUS

The Nucleus is distributed to the members of the Northeastern Section of the American Chemical Society, to the secretaries of the Local Sections, and to editors of all local A.C.S. Section publications. Forms close for advertising on the 1st of the month of the preceding issue. Text must be received by the editor six weeks before the date of issue.

Editor:	Michael P. Filosa, Ph.D., editor, ZINK Imaging LLC, 1265 Main Street, Waltham, MA 02451 Email: Michael.Filosa(at)zink.com; Tel: 781-386-8479.
Associate Editors:	Myron S. Simon, 20 Somerset Rd., W. Newton, MA 02465, Tel: 617-332-5273 Nancy Simons, Analytical Chemist, Corporate R&D, Boston Scientific Corp., 1 Boston Scientific Place A4, Natick, MA 01360-1537. Email: Nancy.Simons(at)bsci.com; Tel. 508-650-8603; Fax 508-647-2329 Sheila E Rodman, Konarka Techologies Inc., 116 John St. Suite 12, Lowell, MA 01852 email:srodman(at)konarka.com tel 978-569-1414
Board of Publications:	Vivian K. Walworth (Chair), Mary Mahaney, David Cunningham
Business Manager:	Karen Piper, 19 Mill Rd., Harvard, MA 01451, Tel: 978-456-8622
Advertising Manager:	Vincent J. Gale, P.O. Box 1150, Marshfield, MA 02050, Tel: 781-837-0424; FAX: 781-837-1453
Contributing Editors:	Morton Hoffman, Feature Editor; Dennis Sardella, Book Reviews
Calendar Coordinator:	Sheila Rodman, email: srodman(at)konarka.com
Photographers:	Morton Z. Hoffman and James Phillips
Proofreaders:	Donald O. Rickter, Myron S. Simon, Vivian K. Walworth, E. Joseph Billo
Webpage:	Webmaster: Sathish Rangarajan, sathish.rangan2(at)gmail.com

Call for Papers

Northeast Student Chemistry Research Conference 2007

The 9th Annual Conference is open to undergraduates, graduates, and postdoctoral fellows in all areas of chemical research.

Saturday, April 21, 2007

MIT

The Ray and Maria Stata Center

9 am - 4 pm

Visit the NESACS YCC for more information: http://www.nsycc.org/

Abstracts will be accepted on this site. There is no registration fee.

Students are invited to present a poster or a 15-minute oral presentation.

Deadlines:
Oral presentation: April 6, 2007
Poster presentations: April 13, 2007 ◇

Call for Volunteers

Boston National ACS Meeting

The National ACS meeting will be held in Boston from August 19-23, 2007. The Northeastern Section will host the meeting. The Hospitality Center, General Interest Program and Student Personnel along with volunteer workers are being organized for the meeting. The Hospitality Center will need volunteers to provide information on points of interest, transportation, restaurants, etc. The center will be open from approximately 9AM to 5PM daily. Hours for volunteers are flexible. More information on the scheduling will be forthcoming. For more information, please contact the following:

Hospitality—Mary Burgess
617-522-9165 mbrburgess(at)aol.com

General Chairperson-
(Planning Committee) - Robert Lichter
Rlichter(at)MerrimakLLC.com ◇

Corporate Patrons and Golf Iridium
Astra Zeneca R&D Boston
Genzyme Drug Discovery & Dev
Hovione
IBM
IRIX Pharmaceuticals
Johnson-Matthey Pharma Solutions
Lyophilization Service of New England
Merck
Phasex Corporation
Sepracor
Strem Chemicals, Inc.
Zone Enterprises

Corporate Sponsors and Golf Rhodium
Aerodyne Research, Inc.
Cambridge Isotope Laboratories
Cambridge Major Laboratories
New England BioLabs, Inc.
Pfizer
Sigma-RBI
Shasun Pharma Solutions

Donors and Golf Tungsten
Consulting Resources Corp.
Dishman Pharmaceuticals and Chemicals Limited
Houghton Chemical Company
Organix, Inc.
Vertex

BRIC Meeting

Boston Regional Inorganic Chemistry Meeting (BRIC) to be held at UNH

The 13th Boston Regional Inorganic Chemistry (BRIC) Meeting, is scheduled for Saturday, May 5, 2007, at the University of New Hampshire in Durham, NH from 9:30 am to 3:30 pm. The program will include three to four lecturers and presentation of posters.

Speakers will include Rachel N. Austin, Bates College; Howard H. Patterson, University of Maine at Orono; and Dean E. Wilcox, Dartmouth College.

Informal lunch will be provided. Questions contact Roy Planalp, roy.planalp(at)unh.edu. ◇

Monthly Meeting

The 878th Meeting of the Northeastern Section of the American Chemical Society

Esselen Award Meeting

Thursday, April 12, 2007

Harvard University, Cambridge, MA
Harvard Faculty Club, 20 Quincy Street

2:30 pm Career Services Presentations and Resume Reviews, Mukund Chorghade, Chair NESACS; Megan Driscoll and Jennifer Sass of PharmaLogics Recruiting.

5:30 pm Social Hour,

6:30 pm Dinner

8:15 pm Award Meeting, Mallinckrodt Building, 12 Oxford Street
Pfizer Lecture Hall (MB23), Ground Floor

Dr. Mukund Chorghade, NESACS Chair, presiding

Welcome - Dr. Robert Langer, Chair, Esselen Award Committee

The Esselen Award – Dr. Myron Simon, Founding Member of the Esselen Award Committee

Introduction of the Award Recipient – Dr. Robert Langer, Massachusetts Institute of Technology

Presentation of the Award – Gustavus J. Esselen, III

Nitric Oxide in Biology: From Discovery to Therapeutics. Dr. Michael A. Marletta, Aldo DeBenedictus Distinguished Professor of Chemistry and Chair of the Department of Chemistry, University of California, Berkeley

Dinner reservations should be made no later than noon, Friday, April 6. Please call or fax Marilou Cashman at (800) 872-2054 or e-mail at MCash0953(at)aol.com. Reservations not cancelled at least 24 hours in advance must be paid. Members, $30.00; Non-members, $35; Retirees, $20; Students, $10.

THE PUBLIC IS INVITED

Any one who needs special services or transportation, please call Marilou Cashman a few days in advance so that suitable arrangements can be made.

Free Parking in the Broadway Street Garage (3rd level or higher), enter from Cambridge Street via Felton Street. Directions to the Harvard Faculty Club can be found at http://www.hfc.harvard.edu/ .

Next Meeting: The May meeting is Education Night. It will be on May 10, 2007 at Northeastern University (Raytheon Amphitheater). Speaker TBA. Please note that the board meeting will begin a half hour early (4pm to ensure that it is over in time for the reception, etc. (The reception will be at 5:15pm and dinner will be at 6pm.)

Abstract

Nitric Oxide in Biology: From Discovery to Therapeutics

Nitric oxide (NO) is now firmly established as a vital mediator in mammalian biochemistry. Parallel lines of research focused on mechanisms of immune system killing and cardiovascular function converged in the middle 1980's on NO as the common component. NO is highly toxic, consistent with a killing function in the immune system but inconsistent with a role in the cell-to-cell signaling. The subsequent discovery of nitric oxide synthase revealed a complex redox protein that carries out the conversion of L-arginine to NO and citrulline. Important mechanistic questions still remain; however, much progress has been made on this complicated reaction. Given the toxicity of NO, signaling mechanisms must respond to low concentrations. The primary NO receptor is guanylate cyclase, an enzyme that converts GTP to cyclicGMP once activated by NO. Biological responses such as vasodilation are mediated by cyclicGMP. The mechanism of guanylate cyclase activation by NO has been extensively studied and molecular details continue to emerge. The fundamental studies on nitric oxide synthase, guanylate cyclase and other enzymes in NO pathway have found application in a number of diseases such as septic shock and erectile dysfunction. ◇

Biography

Michael A. Marletta was born in Rochester New York on February 12, 1951. After an A.B. degree in biology and chemistry from SUNY College at Fredonia, in 1973, he received a Ph.D. in 1978 from the University of California, San Francisco, working under Prof. George L. Kenyon. He then joined Prof. Christopher Walsh at M.I.T. for a 2-year postdoctoral appointment.

Marletta then joined the faculty at M.I.T. as an Assistant Professor of Toxicology in the Department of Applied

Continued on page 8

Book Review

Nontraditional Careers for Chemists: New Formulas in Chemistry,

by Lisa M. Balbes (Oxford University Press, 2006, 320 pp., ISBN 0195183673; $27.95 paperback)

Reviewed by Lynne A. O'Connell
Department of Chemistry, Merkert Chemistry Center
Boston College, Chestnut Hill, MA 02467

Chemists are passionate about research. Investigating the unknown, asking questions about chemical reactions and processes, designing and performing experiments to solve problems—these are the activities that get a chemist's creative juices flowing. But for some chemists, a moment arrives when they realize their passion for laboratory research is no longer there; at this point, the question inevitably arises, "What do I do with my chemistry degree now?" The book *Non-Traditional Careers for Chemists: New Formulas in Chemistry*, by Lisa M. Balbes, provides a comprehensive answer to this question.

The title of each chapter in the book begins with the words "Chemistry and … ." A sampling of the subjects that replace the ellipsis shows the variety of fields that are described: communication, information science, sales and marketing, business development, regulatory affairs, public policy, computers, and education. The beginning of each chapter introduces the reader to the profession and lists the various positions that would be appropriate for someone in this field with a background in chemistry. For instance, the chapter entitled "Chemistry and Patents" describes the jobs of patent examiner, patent searcher, technical specialist and patent agent, in addition to patent attorney. Profiles of actual people who hold these positions follow the job descriptions. These profiles are the heart of the book and give the reader real insights into the professions that are discussed.

Each person who is profiled starts by describing the position that he or she currently holds, including details of daily tasks and an overview of all the responsibilities encompassed by the job. Some indication is usually given concerning the amount of travel required for the position, as well as the salary, which is simply compared to that of a chemist working at the bench in an industrial setting. In the lengthiest section of each profile, the individual explains how he or she arrived at the current position. These sections help the reader to see what factors prompt people to leave the laboratory setting, what advanced degree options should be considered if one is going to enter the line of work described, and what personality traits are important for success in the field. Since many of the speakers have been employed in a variety of environments, the advantages and disadvantages of working in small versus large companies are discussed, as are the pros and cons of owning one's own business. In an "Advice" section, the speaker is asked to provide specific suggestions on how to enter and succeed in the profession. The profiles conclude with predictions about the future direction of the field, as well as a list of references where the reader can find more information.

There are a number of themes that recur throughout the book. Virtually everyone who is profiled indicates that, although they are no longer working in a laboratory, the experience they gained while studying chemistry prepared them well for their current career. The skills learned in lecture and lab courses that are repeatedly cited as being invaluable include the ability to break down complex issues

Continued on page 7

Book Review

Continued from page 6

into simpler parts in order to solve problems, as well as the ability to acquire data, analyze it and assess its accuracy. In addition, having a strong background in science facilitates communication with practicing scientists, which is a necessary part of all the professions covered. The excitement associated with talking to scientists who are working on cutting edge research was mentioned by many as being a highly rewarding aspect of their job. Some generalizations can also be drawn from the advice given by those profiled—an ability to adapt to new situations is essential; one must always be ready to take advantage of new opportunities; and networking at conferences and through professional societies is important for advancement.

While not explicitly stated, it appears that the book is intended for students at both the graduate and undergraduate levels. However, there are few profiles of people who are just embarking on their careers. Most have advanced degrees or have been in their fields a long time and risen to a high-level positions. While the absence of recent chemistry graduates might initially appear to be an oversight, it becomes clear that the experiences of these more seasoned individuals have given them big picture views of their respective fields, which allows them to speak about opportunities at every level, from entry to executive. Their knowledge of and enthusiasm for their fields is inspiring. In fact, the vast array of opportunities presented was so enticing that it made me want to go back and major in chemistry all over again. ◇

Symposium

Inaugural Symposium: Advances In Chemical Sciences

Symposium sponsored by NESACS, IUPAC and RSC-US

We wish to announce that the inaugural "Advances in Chemical Sciences Symposium" conference in the Boston area will be held on March 30, 2007; the symposium will begin at 9.00 a.m. The venue will be the Royal Sonesta Hotel in Cambridge, MA. Under the auspices of these conferences, eminent scientists from the strategic triad of government and industry and academia will deliver plenary lectures. The conference will bring together 200+ scientists for a scholarly event focusing on medicinal chemistry, organic synthesis and methodology. Robert Grubbs (Caltech), William Greenlee (Schering Plough), Eric Jacobsen (Harvard) and Steven Ley (Cambridge). The proposed topical focus areas – medicinal chemistry, pharmaceutical and organic synthesis – are areas where the chemical enterprise in our area has a well-deserved reputation for excellence. We invite all of you to join us for this event, share ideas, and build networks.

The symposium registration fee will be $35.00. Please send your contact information and checks payable to NESACS to Dr. James Piper, Treasurer NESACS, 19 Mill Road, Harvard, MA 01451

Please contact the following for additional details:

Chorghade(at)comcast.net,

Michael.P.Pollastri(at)pfizer.com,

Leslie.Mcquire(at)novartis.com,

ralphscannell(at)comcast.net,

jamedio(at)ziopharm.com ◇

Summerthing 2007

Lowell Spinners Baseball
Saturday, July 14, 2007
5:00 pm

Join your NESACS friends and their families as we travel to LaLecheur Park in Lowell, MA to watch the Lowell Spinners (BOS) take on the Auburn Doubledays (TOR) in a Class A baseball game. The game will be on Saturday July 14th, 2007 at 5:00 PM.

Following the game, stick around to play catch on the field and watch a feature presentation on the state of the art video board. Tickets are limited and will be available on a first come, first ticketed basis. Cost will be $10.00 per ticket.

For directions to LaLecheur Park, see the Lowell Spinners Website at www.Lowellspinners.com.

Some Pregame Festivities at LaLecheur Field in 2005: Peter Filosa prepares to throw a ceremonial first pitch. Rene Rancourt of Boston Bruins fame followed with his famous rendition of the National Anthem and O Canada.

(Photo by Michael Filosa)

For reservations, please call Marilou Cashman at the NESACS office at 1-800-**872-2054** or **(508) 653-6329** or e-mail to **mcash0953(at)aol.com.** ◇

Historical Notes

Daniel F. Lord

Daniel F. Lord died May 25, 2006 in Marblehead, MA. He was 82. He was born in Cambridge, MA in 1923, graduated from Marblehead High School in 1941, and began college at MIT. He served in the U.S. Army in World War II, then returned to MIT, graduating with the Class of 1947. Subsequently, he studied chemical engineering at Lehigh University.

Lord worked as a chemical engineer at J.S.Barnet and Sons in Lynn, at Gillette in North Andover, and other firms. He also ran the Cliff Family Tree Farm in Salem, NH. He was a member of the American Chemical Society, the American Institute of Chemical Engineers and MENSA.

He is survived by his wife of 56 years, Norma MacMullen Lord, a daughter, Karen Cunningham, two sons, Jonathan K. Lord and Alan Lord, a brother, Robert S. Lord, six grandchildren, and one great-grandchild.

Kenneth A. Moon

Kenneth A. Moon, a long time member of the American Chemical Society, died on March 16, 2006. He held B.S. and M.S. degrees from Queens University in Kingston, Ontario and a Ph.D. in Physical Chemistry from M.I.T. His chemical career was at the Army laboratories at the Watertown Arsenal.

He was the recipient of many awards and honors for his dedication to conservation and environmental issues. He was a founding member of the Sudbury Valley Trustees and the Wayland Conservation Commission. He is survived by his wife,Elizabeth. sons David and Peter, daughters-in-law Krista and Kate and four grandchildren.

MSS

We present here short biographies of chemists and chemical engineers whose deaths have been reported to us during the past year. We thank members of the Northeastern Section who have sent us obituary notices appearing in newspapers we do not see. ◇

Biography

Continued from page 5

Biological Sciences. He was promoted to Associate Professor in 1986. In 1987 he joined the faculty at the University of Michigan as Associate Professor of Medicinal Chemistry in the College of Pharmacy and, in 1989, Associate Professor of Biological Chemistry in the Medical School. In 1991 he was promoted to Professor in both departments and appointed the John G. Searle Professor of Medicinal Chemistry. In 1997 he became an Investigator in the Howard Hughes Medical Institute. Marletta moved to the University of California, Berkeley in 2001, where he assumed the positions of Professor of Chemistry, Department of Chemistry, and Professor of Biochemistry and Molecular Biology, Department of Molecular and Cell Biology. He also holds an appointment as Professor of Cellular and Molecular Pharmacology at UCSF and Faculty Scientist at the Lawrence Berkeley National Lab. He was appointed the Aldo DeBenedictis Distinguished Professor of Chemistry in 2002. On 1 July 2005, he became Chair of the Department of Chemistry at Berkeley.

Awards he has received include the George H. Hitchings Award for Innovative Methods in Drug Discovery and Design (1991) sponsored by the Burroughs Wellcome Fund and a Faculty Recognition Award from the University of Michigan (1992). He was awarded the Outstanding Alumni Achievement Award from SUNY Fredonia in 1993. In 1995 he received a MacArthur Fellowship awarded by the John D. and Catherine T. MacArthur Foundation. He was elected Senior Fellow in the Michigan Society of Fellows and elected to the SUNY Honor Role in 1996. He was elected to the Institute of Medicine in 1999. He was awarded the Distinguished Faculty Lectureship Award in Biomedical Research by the University of Michigan Medical School for 2000 and honored as the Michigan Scientist of the Year (2000) by the Impression 5 Science Museum. Also in 2000, he was a

Continued on page 9

Biography

Continued from page 8

Lecture Platform Speaker at the Chautauqua Institution and selected for the Distinguished Faculty Achievement Award at the University of Michigan. In 2001 he was elected to the American Academy of Arts and Sciences and became a fellow of the American Association for the Advancement of Science. In 2004 he was the recipient the Harrison Howe Award of the American Chemical Society. He was elected to the National Academy of Sciences in 2006. In 2007 he will receive the Repligen Award of the ACS Division of Biological Chemistry.

He is a member of the American Chemical Society and the American Society for Biochemistry and Molecular Biology. He currently serves on the Board of Editors of ACS Chemical Biology and on the editorial boards of a number of other journals. He is a consultant for a number of pharmaceutical companies and has served on the scientific advisory boards of NitroMed. Inc. and Oxon Medica Inc. He is a member of the Fredonia College Foundation Board of Directors.

He lives with his wife, Margaret Gutowski, and son in Berkeley, California. ◇

UNH Faculty

Margaret E. Greenslade Joins Faculty at UNH

B.A., 1998, Bryn Mawr College

Ph.D., 2005, University of Pennsylvania

Research Scientist, 2005-present, Cooperative Institute for Research in Environmental Science (CIRES) at University of Colorado, Boulder and an affiliate of the National Oceanic and Atmospheric Administration (NOAA)

In the fall of 2007, Mar garet E. Greenslade will begin as an Assistant Professor in the Department of Chemistry at the University of New Hampshire. Her research will revolve around physical and chemical transformations of aerosols observed from an optical and morphological point of view. Experimental work, both in the laboratory and the field, will be complemented with theory and model studies. ◇

Forgotten Genius

Please join us to view the NOVA documentary on Dr. Percy Julian and discuss his life and achievements

Friday, April 13, 2007

1:30 pm

Bridgewater State College
Moakley Center

Co-sponsored by the BSC Chemistry Department, Center for the Advancement of Science Education, and the American Chemical Society

A discussion on the life of Dr. Julian, and the opportunities and challenges for minority scientists will be led by Dr. James Shoffner from the American Chemical Society Department of Diversity Programs.

Please RSVP to Dr. Steve Haefner
shaefner(at)bridgew.edu
508-531-2984 ◇

Summer Scholar

Continued from page 2

We therefore decided to purify avidin from inclusion bodies as we had done previously with streptavidin.[1] In order to do this, the bacterial leader originally engineered onto the avidin had to be removed through site-directed mutagenesis. The wild-type avidin without the bacterial leader was smaller by 78 base pairs and there was a visible shift in product size when visualized on an agarose gel alongside the wild-type construct. Once the leader was removed and the sequence confirmed, we began expressing the protein in inclusion bodies.

Charge mutant avidin

In the process of testing the wild-type avidin purified from the inclusion bodies, we observed that the tetramer did not enter the separating gel during SDS-PAGE analysis, but instead became immobilized in the stacking gel. It is reported[6] that making a reduced charge mutant of avidin allows for better tetramer visualization on SDS-PAGE and so we made a series of 4 mutations to change surface charge residues and reduce the pI. Additionally, lowering the pI may reduce stickiness when using avidin to label cell surface proteins. The following mutations were made: R122A, R124A, R2A, K3E in two rounds of mutagenesis. Both the double mutant (R122A, R124A) and the quadruple mutant (R122A, R124A, R2A, K3E) were functional, as determined by testing with a biotin-4-fluorescein assay (**Fig. 3**).

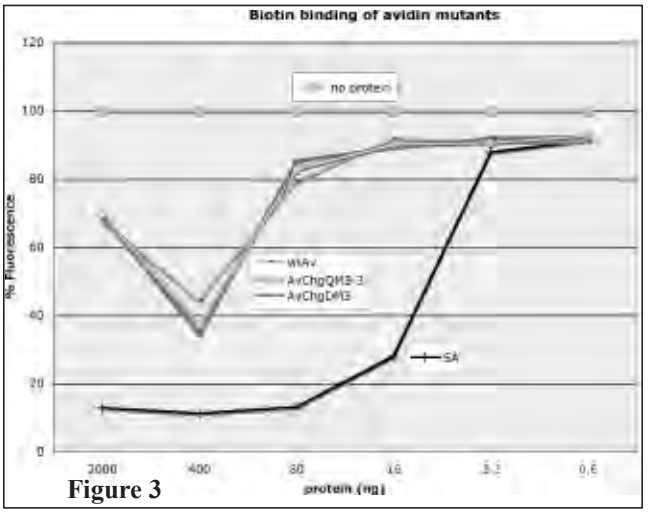

Figure 3

When run on a gel, we expected to visualize tetramer, however, this was not the case. It appears as though much of the protein is monomeric due to misfolding and work is still being done to optimize the re-fold conditions and maximize tetramer yield. We utilized a new loading buffer containing no DTT to maintain disulfide bonds and supplemented the refold buffer with 0.5M Arginine (0.2M NaHCO3, 0.5mM GSSG, 1 mM GSH)[7] and this has made a small impact on tetramer formation (**Fig. 5**). 5mM DTT was added fresh to the guanidinium hydrochloride to encourage correct disulfide bond formation. Work is still in progress to recover more tetramer and more charge mutations may be made at a later date.

Engineering "dead" avidin

Based on the mutations made in streptavidin (N23A, S27D, S45A) which all lie within the binding site (**Fig. 4**)[8], analogous mutations were made in avidin (N12A, S16D, T35A).

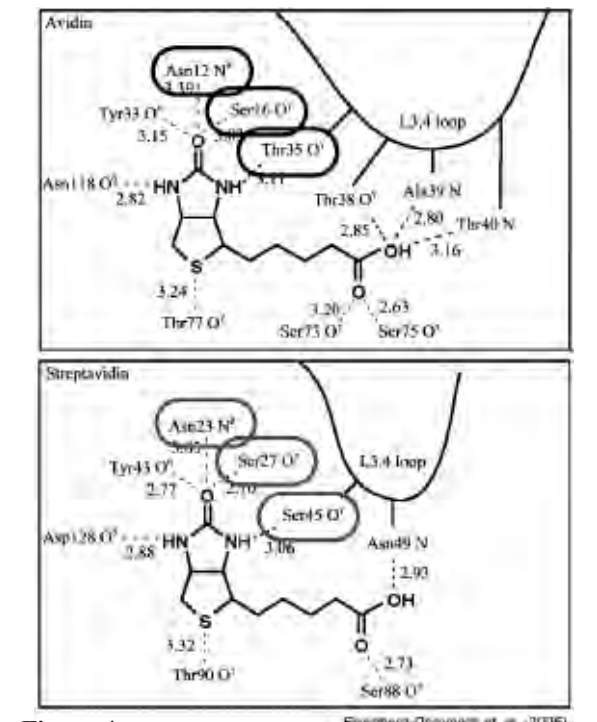

Figure 4

Site-directed mutagenesis was performed with primers designed to introduce the desired point mutations. The mutagenesis was performed in two rounds, the first round introducing N12A and S16D and the second introducing T35A. The protein was purified using the modified renaturation buffer containing 0.5M Arginine and the preliminary results show the presence of a small amount of tetramer (**Fig. 5**). Using the biotin-4 fluorescein assay to determine activity, the double mutant (N12A, S16D) shows little to no biotin binding (**Fig. 6**). The triple mutant (N12A, S16D, T35A) was also cloned and sequenced, but was unable to fold properly.

The extinction coefficient of avidin was based on the Edelhoch method, is described by Pace.[9] The extinction coefficient at 280 nm for the non-glycosylated form of avidin was calculated as 22,125 $M^{-1}cm^{-1}$ per subunit in water, and as 22,665 $M^{-1}cm^{-1}$ in guanidinium hydrochloride. These values agree with Kulomaa's published value[4] of 24,280 $M^{-1}cm^{-1}$.

During the course of the project, "dead" avidin and the charge mutant avidin were cloned, purified and tested for their abilities to bind biotin. For the future, refolding conditions for both wild type and mutant avidin will be explored, which we hope will maximize tetramer yield. Eventually, we hope to produce a monovalent avidin by combining the "alive" and "dead" avidin in different molar ratios followed

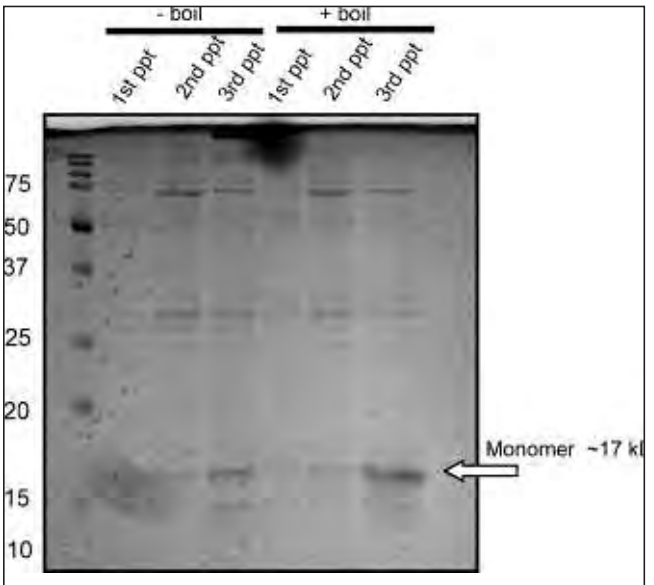

Figure 5

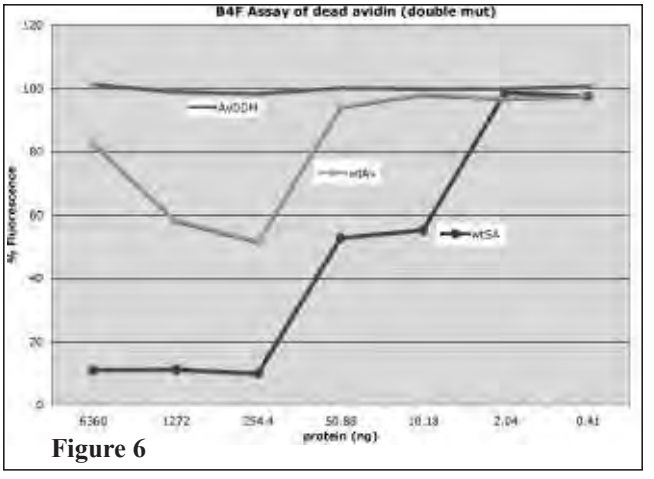

Figure 6

by purification on a Ni-NTA column in much the same way that monovalent streptavidin is produced.

Acknowledgements

I would like to thank the Northeastern Section of the American Chemical Society for awarding me the James Flack Norris and Theodore William Richards Undergraduate Summer Scholarship. The scholarship was instrumental in allowing me to pursue a valuable research experience. I would also like to thank Dr. Mark Howarth, with whom I worked very closely on this project, for his guidance, Professor Alice Ting for allowing me the opportunity to spend the summer as a member of her group, and the members of the Ting lab for all their help and support.

References

[1] Howarth, M., Chinnapen, D., Gerrow, K., Dorrrestein, P.C., Grandy, M.R., Kelleher, N.L, El-Husseini, A.& Ting, A.Y. A monovalent streptavidin with a single femtomolar biotin binding site. *Nat. Methods*. **3**, 267-273 (2006).

Continued on page 12

The Gustavus John Esselen Award for Chemistry in the Public Interest

In 1985 an inquiry was made as to whether the Section would wish to honor a former leader of the Northeastern Section. The Esselen family proposed to donate a sum of money to provide for an award in the memory of Gustavus John Esselen II, Chairman in 1922 and 1923, and a member of the ACS Board of Directors for many years. In 1948, Dr. Esselen received a special award, the James Flack Norris Honor Scroll, "as the person who has done most to advance the interests of the Northeastern Section." A committee consisting of William O. Foye, Truman S. Light, Arthur S. Obermayer, and Myron S. Simon, Section Chairman, met with Esselen's son, Gustavus J. Esselen III, and recommended to the Board of Directors that the Section accept the offer. The committee and Mr. Esselen agreed that the award should not be in a specific field of chemistry, but instead should have the special purpose of emphasizing the positive values of chemistry to mankind. In light of the climate of the day, with the disaster of Bhopal on every chemist's mind and the public receiving nothing but negative stories about chemistry, this was to be a small step toward establishing a balance.

Mr. Esselen proposed to add a bronze medal to the monetary award. A prominent sculptor from Newton, Massachusetts, Lloyd Lillie, was selected to prepare the design which was then approved by members of the Esselen family. The fact that Dr. Esselen had done much work in plastics during his career led his son to propose that the bronze medal be imbedded in a block of clear plastic. This was done, giving a very distinctive addition to the ceremonial presentation.

The first presentation of the Gustavus John Esselen Award for Chemistry in the Public Interest was made in 1987 to F. Sherwood Rowland and Mario J. Molina for their work on the chemical processes which were destroying the stratospheric ozone layer, explaining the formation of the Antarctic Ozone Hole. Since then, the award has been given to chemists in several fields of chemistry and has become a much coveted prize.

This is an edited version of a more detailed history of the origins of the Esselen Award. A comprehensive history written by Myron S. Simon was most recently published on page 10 of the May 2006 issue of The Nucleus. ◇

NESACS Candidates for 2007

Chair-Elect
Dr. E. Joseph Billo

Secretary
Dr. Michael Singer

Trustee
Dr. Esther A. H. Hopkins

Councilor/Alt. Councilor
Dr. Mukund Chorghade
Dr. David Cunningham
Dr. Timothy B. Frigo
Dr. Mark Froimowitz
Dr. Thomas Gilbert
Dr. Robert Lichter
Dr. Pamela Nagafuji
Dr. Eriks Rozners
Dr. Michael Singer

Director-at-Large
Ms. Laila Dafik
Mr. Steve Lantos

Nominating Committee
Dr. David Cunningham
Dr. Jerry Jasinski
Dr. Pamela Nagafuji
Dr. Dorothy J. Phillips

Esselen Award Committee
Dr. Sean Lu
Dr. Alfred Viola

Richards Medal Committee
Dr. Peter Jacobi
Dr. Barry Karger
Dr. Daniel Nocera
Dr. Dagmar Ringe ◇

Summer Scholar

Continued from page 11

[2] Chen, I., Howarth, M., Lin, W., & Ting, A.Y. Site-specific labeling of cell surface proteins with biophysical probes using biotin ligase. *Nat. Methods.* **2**, 99-104 (2005).

[3] Barth, S., Huhn, M., Matthey, B., Lkimka, A., Galinski, E.A., and Engert, (2000) A. Compatible-Solute-Supported Periplasmic Expression of Functional Recombinant Proteins under Stress Conditions. *Applied and Environmental Microbiology.*. **66**, 1572-1579.

[4] Hytönen, V.P., Laitinen, O.H.,, Airenne, T. T., Kidron, H., Meltola, N.J., Prokka, E.J., Hörha, J. Paldanius, T., Määttä, J.A.E., Nordlund, H.R., Johnson, M.S., Salminen, T.A., Airenne, K.J., Ylä-Herttuala, S., and Kulomaa, M.S. (2004). Efficient production of active chicken avidin using a bacterial signal peptide in *Escherichia coli*. *Biochem J.* **384**, 385-390.

[6] Marttila, A.T., Airenne, K.J., Laitinen, O.H., Kulik, T., Bayer, E.A., Wilchek, M., and Kulomaa, M.S. (1998). Engineering of chicken avidin: a progressive series of reduced charge mutants. *FEBS Letters* **441**, 313-317.

[7] Nardone, E., Rosano, C., Santambrogio, P., Curnis, F., Corti, A., Magni, F., Siccardi, A. G., Paganelli, G., Losso, R., Apreda B., Bolognesi, M., Sidoli, A., and Arosio, P. (1998) Biochemical characterization and crystal structure of a recombinant hen avidin and its acidic mutant expressed in *Escherichia coli*. Eur. J. Biochem. **256,** 453-460.

[8] Eisenberg-Domovich, Y., Hytönen, V.P., Wilchek, M., Bayer, E.A., Kulomaa, M.S. and Livnah, O. (2005) High resolution crystal structure of an avidin-related protein: insight into high-affinity biotin binding and protein stability. *Acta Cryst.* **61**, 528-538.

[9] Pace, C.N., Vajdos, F., Fee, L., Grimsley, G., and Gray, T. (1995) How to measure and predict the molar absorption coefficient of a protein. *Protein Science*. **4**, 2411-2423. ◇

BUSINESS DIRECTORY

SERVICES

VOLUNTEERS

Reporters needed to cover ACSNES monthly meeting lectures

Phone for more details
Myke S. Simon
Nucleus Associate Editor
Tel: 617-332-5273

WANT MORE ARTICLES

When you tell our advertisers that you saw their ads here they have more confidence in our newsletter's viability as an advertising medium. They advertise more. This supports our many activities.

Your one-stop source to career-related links in the Chemical Sciences

WWW.NESACS.ORG/CAREERS

BUSINESS DIRECTORY

SERVICES

BUSINESS DIRECTORY

SERVICES

SERVICES

CAREER SERVICES

Index of Advertisers

THE NUCLEUS
19 Mill Road
Harvard, MA 01451

NONPROFIT ORG.
U.S. POSTAGE PAID
NORTHEASTERN
SECTION
AMERICAN CHEMICAL
SOCIETY

Calendar

Check the NESACS Homepage for late additions: http://www.NESACS.org

Note also the Chemistry Department web pages for travel directions and updates.
These include:
http://chemserv.bc.edu/seminar.html
http://www.bu.edu/chemistry/events/
http://www.chem.brandeis.edu/colloquium.shtml
http://www-chem.harvard.edu/events/
http://web.mit.edu/chemistry/
www.chem.neu.edu/web/calendar/index.html
http://chem.tufts.edu/seminars.html [CHEM.]
http://ase.tufts.edu/chemical/seminar.htm [CHEM. ENGG.]
http://www.chem.umb.edu/
www.umassd.edu/cas/chemistry/seminars.cfm
www.uml.edu/Dept/Chemistry/speakers.html
http://www.unh.edu/chemistry/seminars.html

Apr 3
Kim R. Dunbar(Texas A & M University)
TBA
UNH, Room L103
11:10 am

Prof. Antonio M. Echavarren, Institut Català d'Investigació Químa, Spain
"The Mechanistic Puzzle of Gold-Catalyzed Cyclizations of Enynes and Beyond"
Boston College, Merkert 130
4:00 pm

Apr 4
Dr. D. Venkataraman (U. Mass Amherst)
TBA
U. Mass Dartmouth, Building Group II, Rm. 115
4:00 pm

Apr 5
Prof. Chang Y. Rhu (Rensselaer Polytechnic)
"Polymer Adsorption into Nanopores and Its Applications for Polymer HPLC"
U. Mass. Lowell, Olney Hall Rm 218
3:30 pm

Apr 9
Chad Rienstra (UIUC)
"New Dimensions in Magic-Angle Spinning NMR of Membrane Proteins: Methods and Applications for Physical Chemistry and Structural Biology"
MIT, 56-114
4:30 pm

Eiichi Nakamura, (Univ. of Tokyo
TBA
Harvard, Pfizer Lecture Hall 4:15 pm

Apr 10
Professor Rob Doyle (Syracuse Univ.)
"Vitamin Bioconjugates for Drug Delivery"
UNH, Room L103
11:10 am

Professor Chuan He, (University of Chicago)
TBA
Boston College, Merkert 130
4:00 pm

Prof. Alanna Schepartz, (Yale University)
TBA
Tufts, Pearson Rm P-106
4:30 pm

Apr 11
Dr. Chuan He, (Univ. of Chicago)
"Regulations of Virulence, Antibiotic Resistance, and Metal Ion Concentrations in Bacteria"
Brandeis Univ., Gerstenzang 122
3:45 pm

Apr 12
Michelle Wang (Cornell)
TBA
Harvard, Pfizer Lecture Hall 4:00 pm

Apr 13
Dr. James Shoffner (ACS Department of Diversity Programs)
"A discussion on the life of Dr. Percy Julian, Forgotten Genius, and the opportunities and challenges for minority scientist."
Bridgewater State College, Moakley Center
1:30 pm
RSVP to Dr. Steve Haefner
shaefner@bridgew.edu
508-531-2984

Apr 16
Dr. Ken Caulton (Indiana Univ.)
"Spin States, Unsaturation, and Pi-Donation: Impact on Reactivity"
Brandeis Univ., Gerstenzang 122 3:45 pm

Apr 17
Dr. Maureen Fagan (Smith College)
"Synthesis and Reactivity of Palladium Enolate Complexes"
UNH, Room L103 11:10 am

Prof. Samuel J. Danishefsky, (Sloan-Kettering Institute & Columbia Univ.)
TBA
Boston College, Merkert 130 4:00 pm

Prof. Rick van Duyne (Northwestern)
"Molecular Plasmonics for Surface Enhanced Sensing and Raman Spectroscopy"
Tufts, Pearson Rm P-106 4:30 pm

Expose Yourself This Summer

Did you know 17,000 copies of the Summer Nucleus will be printed and 10,000 will be handed out to attendees at the Boston National ACS Meeting to be held in August 2007.

If you would like to submit an article or book review for the Summer Issue contact the Editor of the Nucleus, Michael Filosa at michael.filosa(at)zink.com.

The editorial deadline for the Summer Issue is June 15, 2007.

To advertise in this issue contact the Nucleus Advertising Manager, Vince Gale at www.mboservices.net.

Apr 19
Richard Mathies (UC Berkeley)
Title: Femtosecond Stimulated Raman Spectroscopy
Harvard / MIT Seminar Series
MIT 56-114
5:00 pm

Apr 23
Dr. Shannon Stahl, (Univ. of Wisconsin)
TBA
Brandeis Univ., Gerstenzang 122
3:45 pm

Tamar Seideman (Northwestern)
TBA
MIT, 56-114 4:30 pm

Apr 24
Professor Gregory Sotzing (U. Conn)
"Nanolithography, Nanofibers and Conducting Polymers"
UNH, Room L103
11:10 am

Professor Anna K. Mapp, (U. Michigan)
TBA
Boston College, Merkert 130 4:00 pm

Apr 26
Prof. Afred Crosby (U. Mass Amherst)
"Natures's Instabilities: Inspiring Materials Design and Characterization"
U. Mass. Lowell, Olney Hall Rm 218
3:30 pm

Apr 30
Dr. J. M. Brown (Oxford Univ.)
"Racemic Ligands in Catalytic Asymmetric Synthesis"
Brandeis Univ., Gerstenzang 122 3:45 pm

Notices for the Nucleus Calendar should be sent to:
Sheila E Rodman
250 Kennedy Drive
Unit #403, Malden, MA 02148.
E-MAIL: serodman(at)hotmail.com