

The Center for Research  
and Education in Bioluminescence  
and Biotechnology  
(CREBB)  
Presents:

# Protein Purification:

## Principles and Practice

### A Five and One-Half Day Hands-On Laboratory Course

<http://www.rci.rutgers.edu/~crebb/protein.html>



January 7-12, 2007

March 11-16, 2007

June 3-8, 2007

July 8-13, 2007

July 22-27, 2007

## Limited Enrollment

Participants will learn skills to effectively design separation and purification strategies for downstream processing. This course uses GFP (Green-Fluorescent Protein), a novel marker for gene expression, as the source material. While this is a general course applicable to all proteins, we use GFP as a highly effective visual teaching tool.

## Instructors

### Faculty Coordinator

*Dr. William W. Ward,  
Associate Professor of  
Biochemistry  
School of Environmental  
and Biological Sciences,  
Rutgers University  
and Director of C.R.E.B.B*



Dr. Ward is a noted teacher and seminar leader with more than 20 years of experience with adult professional audiences. He created "Protein Purification" in 1989 and the course has been running continuously ever since, reaching an estimated audience of over 1,200 scientists from across the globe. Dr. Ward specializes in the chemical and physical properties of the green-fluorescent protein and other proteins involved in marine bioluminescence. He is published in 112 books, book chapters, journal articles and abstracts. Dr. Ward has co-authored (with Catherine Thomson, Ph.D.) "A Guide to Green-Fluorescent Protein: Applications in Cell Biology and Drug Discovery" for D&MD Publications. This resource-oriented practical guide presents an overview of the most important features and technological applications of Green-Fluorescent Protein (GFP) and its variant forms and illustrates how GFP is currently being used in the biopharmaceutical industry. He has also authored "Biochemical and Physical Properties of Green-Fluorescent Proteins", a chapter in "Green-Fluorescent Protein: Properties, Applications, and Protocols, 2nd Edition", Chalfie and Kain (eds.), Wiley-Liss, Inc. 2006.

### Additional Instructors

*Dr. Gavin Swiatek*, Post Doctoral Associate, Microbiology,  
School of Environmental and Biological Sciences, Rutgers University

*Ms. Caren Villano*, Graduate Assistant, Biochemistry,  
School of Environmental and Biological Sciences, Rutgers University

*Dr. Richard Ludescher*, Professor of Food Science,  
School of Environmental and Biological Sciences, Rutgers University

*Mr. Robert Muldowney*, Associate Director, Structural Biology  
Computational Laboratory, School of Environmental and Biological  
Sciences, Rutgers University

*Dr. Olubayi Olubayi*, Asst. Professor, Middlesex Community College

## **The Center for Research and Education in Bioluminescence and Biotechnology (CREBB)**

*The Center offers a series of continuing education workshops each year featuring nationally renowned presenters. The Center for Research and Education in Bioluminescence and Biotechnology (CREBB) is a component of the School of Environmental and Biological Sciences, Rutgers University. The CREBB mission is to perform basic research on bioluminescence and to utilize bioluminescence (especially the Green Fluorescent Protein) as a tool to educate the scientific and industrial communities in the field of biotechnology.*

**M**ore than 1,200 scientists from around the world have strongly recommended this intensive course as an opportunity to develop protein research and analytical skills in a retreat setting. Participants work hard, identify and solve problems in the lab and enjoy camaraderie and good food and drink with colleagues.


**T**his five-day laboratory course covers a wide variety of conventional methods for protein isolation, purification, and characterization. The course format integrates hands-on laboratory exercises with classroom lectures, demonstrations, study breaks, and short take-home assignments.

**A** special feature of the course is that all laboratory work will be performed on the same starting sample (*Aequorea* GFP or recombinant GFP), which will be purified from an exceedingly crude form (starting with tissue or bacterial cell extraction) to near homogeneity as judged by high performance liquid chromatography (HPLC), SDS gel electrophoresis, isoelectric focusing, and Western blotting. This feature provides a continuity of purpose, integrating dozens of preparative and analytical protein techniques in a way that few competing courses can match.

**A** problem-solving approach will be used throughout the course. Under the guidance of experienced lab instructors, participants will work in groups of three to plan their own protocols, analyze data, and interpret results. A student-teacher ratio not greater than 8:1 will be maintained and the faculty coordinators will be present throughout the course.

### *Special Recognition*

We would like to recognize and thank Dr. Dennis Willows, former Director of University of Washington's Friday Harbor Laboratories, for allowing us to use FHL facilities for collection and processing of the *Aequorea* jellyfish used in this course.

 "I am sending you another note of thanks to tell you again how much I fondly remember the course at Rutgers. I recently attended a course given by another company which greatly illuminated the great job that you are doing with your courses, in terms of providing expert information in an environment that because of its enjoyable atmosphere is highly conducive to learning. Keep up the good work!"

**Bruce Baldwin**, Eli Lilly & Company

## Course Location

The course will be held on the School of Environmental and Biological Sciences Campus, New Brunswick, NJ. A map with written directions to the course site will be included with your confirmation of registration. If you do not receive your confirmation ten days before the course starts, please call our Registration Desk at (732) 932-9763 ext. 225 or 216.

**For information on travel and lodging**, please contact: Gloria Kierniesky at (732) 932-9763 ext. 225 or e-mail her at [kierniesky@aesop.rutgers.edu](mailto:kierniesky@aesop.rutgers.edu), or phone the Rutgers Information Service at (732) 932-INFO. The following Rutgers website contains information on discounted travel and lodging:  
<http://www.ruttravel.rutgers.edu/discountprograms.html>

## Refunds

You may withdraw from this course with a full refund (less a \$100 processing fee) provided our office is notified at least five (5) full business days prior to the start of the course. Beyond that time, you may be responsible for the full tuition fee if you register but do not attend. Substitutions are encouraged.

CREBB reserves the right to cancel this course due to insufficient enrollment.

## Registration Information

The tuition fee for this five and one-half day course is \$2,695; register three weeks prior to the start of the course and pay only \$2,195. A discounted fee of \$1,995 per person is available if two or more registrants from the same company register. There is also a discounted fee of \$1,395 for attendees from academia. Fees **MUST BE PAID** three (3) weeks prior to the start of the course to be eligible for the discounted rates. The tuition fee includes continental breakfasts, lunches, coffee breaks and three dinners, along with all course materials and hand-outs.

## Ways to Register



### By Phone

9:00 a.m. – 4:00 p.m., Monday – Friday

Registration Desk: (732) 932-9763 ext. 225 or 216



### By Mail

Send check or money order (payable to Rutgers, the State University) to:

**CREBB Registration Desk**

**Rutgers University**

**School of Environmental and Biological Sciences**

**Department of Biochemistry and Microbiology**

**76 Lipman Drive**

**New Brunswick, NJ 08901-8525**



### By Credit Card

Visa or MasterCard only

Call Registration Desk (732) 932-9763 ext. 225 or 216



### By Fax

(732) 932-3633, 24 hours

Please fax a copy of check or money order with fax registration.



### Via the Internet

<http://www.rci.rutgers.edu/~crebb/registerprotein.html>

## Course Format

Course participants will extract an easily visualized chromo-protein, the green-fluorescent protein, (Science vol. 263 pp. 802-805, 1994) from a frozen tissue sample or bacterial cell pellets, clarify the extract, and then concentrate and purify the protein by “salting out.” Gel filtration, ion exchange, hydrophobic interaction, and size exclusion HPLC chromatography will then be employed to extensively purify the desired protein (GFP) from the crude extract. The unique nature of this brilliantly fluorescent protein allows you to follow all phases of the purification with a simple hand-held mineral light, enhancing your understanding of each process.

The purified protein will be characterized by SDS and native gel electrophoresis, isoelectric focusing, ion exchange FPLC, size exclusion HPLC, and Western blotting. Each group will prepare a detailed purification table and graphs (homework assignments), and will characterize the protein with respect to purity, charge, molecular weight, isoelectric point, unique spectral features, subunit composition, isoprotein composition, and the chemical nature of the chromogenic peptide.

This course integrates lecture and laboratory sessions to provide a comprehensive learning experience. The course begins with an introductory lecture on Sunday afternoon. Everyone is strongly encouraged to attend this session, but participants who cannot arrive for the Sunday lecture may begin the course on Monday morning (at the laboratory location).

The course concludes Friday afternoon with an interactive problem-solving workshop and a molecular modeling workshop at the Rutgers Structural Biology Computational Laboratory (<http://cesario.rutgers.edu>) facilities in Lipman Hall, Rutgers. Out-of-state registrants should try to schedule flight times out of Newark International Airport no earlier than 6:00 p.m. so they do not miss this session.

*“Dr. Ward was very available and open to questions. He sincerely tried to answer every question asked and was very patient. The TAs were awesome, very well prepared and eager to help.”*

**Bridget Ferdinand**, Manufacturing Associate, Antigenics, L.L.C.

*“I got far more out of the course than I had even anticipated, and it was certainly money VERY WELL spent. In that one week, the training was far more intense and the knowledge I gained more directly applicable to what I am working on than I could have attained otherwise. I still refer to the manual that you provided. I also appreciated the assistance and knowledge of your collaborators.”*

**Robert A. Bollinger**, Dept. of Radiology, UT Southwestern Medical Center

# Course Outline

## **Sunday**

**3:00 p.m. - 8:00 p.m.**

Registration and Reception	3:00 p.m. - 4:00 p.m.
Course Introduction and Overview	4:00 p.m. - 7:00 p.m.
Dinner	7:00 p.m. - 8:00 p.m.

## **Monday**

**7:30 a.m. - 8:00 p.m.**

- *Lecture:* Protein Structure
- Laboratory Introduction and Overview
- Morning Laboratory Exercises: Filtration, Precipitation with Ammonium Sulfate, Centrifugation, Fluorimetric and Spectrophotometric Assays, and Gel Filtration Chromatography
- *Lecture:* General Preparative Methods of Protein Purification
- Evening Laboratory Exercises: Do Biochemical Assays, Plot Gel Filtration Data, Select Ion-Exchange Conditions, Start Dialysis, and Begin Purification Table

## **Tuesday**

**7:30 a.m. - 5:30 p.m.**

- *Lecture:* Open Column Purification
- Demonstration: Analytical Gel Filtration
- Morning Laboratory Exercises: Centrifuge Dialyzed Sample, Begin Ion-Exchange Chromatography
- Afternoon Laboratory Exercises: Choose HIC Matrix and Eluting Solvent, Assay Ion-Exchange Fractions, Plot Data

## **Wednesday**

**7:30 a.m. - 8:30 p.m.**

- Morning Laboratory Exercises: Run HIC on Peak Ion-Exchange Fractions
- *Lecture:* HPLC Theory
- Morning Laboratory Exercises: Assay HIC Fractions
- Afternoon Laboratory Exercises: Concentrate and Desalt Samples, Purify by SEC-HPLC and Calculate Molecular Weight, Analyze Pure GFP Spectrally
- *Lecture:* Electrophoresis
- Evening Laboratory Exercises:
  - Demonstration: Tangential Flow Ultrafiltration (Pall Minimate TFF System)
  - Demonstration: Three Phase Purification of Proteins using t-Butanol
- Interactive Workshops
  1. Affinity Chromatography of His-tagged Protein on Qiagen Nickel Column
  2. Western Blotting on the Novex System
  3. Titration Curve on GE Healthcare "Phast" System

## **Thursday**

**7:30 a.m. - 5:30 p.m.**

- Demonstration: Preparative PAGE on BioRad Prep Cell
- Morning Laboratory Exercises: Pour SDS Running Gel, Load Isoelectric Focusing Gel
- Demonstration: "Phast" System, Native Gel with Western Blot
- *Lecture:* Analysis of Protein Structure
- Laboratory Exercises (continued): Pour SDS Stacking Gel, Stain IEF Gel
- Afternoon Laboratory Exercises: Run SDS Gel, Stain and Destain SDS Gels

## **Friday**

**7:30 a.m. - 4:00 p.m.**

- *Lecture:* Molecular Biology of GFP or HPLC of Proteins and Peptides
- Morning Laboratory Exercises: Analyze SDS Gels, Plot SDS Molecular Weight Data, Analyze IEF Gel, Determine Isoelectric Point, Analyze "Phast" IEF, Complete Purification Table
- Interactive Workshop: Determination of Chromopeptide Structure: Analyze Chromopeptide Purification Data. Discuss and Analyze: (1) Amino Acid Composition Data, (2) Edman Sequencing Data, (3) Pronase/Carboxypeptidase Data (4) DNA Sequence Data and (5) Model Compound Analysis.
- Molecular modeling workshop: Rutgers Structural Biology Computational Laboratory

**Note:** Breakfast and Lunch included Monday - Friday. Dinners provided Sunday, Monday and Wednesday.

### *Biochemical Separations* Introduction to Laboratory Techniques (2 days, lecture/demonstration)

This course provides an introduction to techniques used to isolate, purify and characterize biological macromolecules. Emphasis is placed on techniques used in protein isolation and purification – both from native and recombinant sources. Techniques include extraction, filtration, centrifugation, column chromatography (including HPLC), electrophoresis, isoelectric focusing, western blotting and capillary zone electrophoresis. This is an introductory level course designed for scientists having limited experience with laboratory separations.

**Course Cost:** \$995

February 15-16, 2007 (Thursday & Friday)

April 26-27, 2007 (Thursday & Friday)

August 2-3, 2007 (Thursday & Friday)

Website: <http://www.rci.rutgers.edu/~crebb/biosep.html>

  *New for 2007*  

### *Tutorials in Protein Purification*

(3 days, hands-on instruction in Downstream Processing)

In an intimate, small group laboratory setting, learn the details of protein purification from an expert. Join Professor William Ward as he leads you through the step-by-step purification of a model protein, hands-on from start to finish. Under his constant supervision, you will prepare a crude extract, administer several batch purification steps, and then achieve purity in a series of high resolution chromatography steps. You will then judge purity of the final product in comparison with your crude extract by calculating specific activity and by analyzing your product by size exclusion HPLC and/or SDS gel electrophoresis.

**Course Cost:** \$995

February 2-4, 2007 (Friday - Sunday)

May 4-6, 2007 (Friday - Sunday)

August 10-12, 2007 (Friday - Sunday)

Website: <http://www.rci.rutgers.edu/~crebb/Tutorials.html>

**Early Registration:** If you register three weeks prior to the start of the course you pay only \$895.

**Discounted Fee:** A discounted fee of \$850 per person is available if two or more registrants from the same company register.

The tuition fee includes continental breakfasts, lunches, and coffee breaks along with all course materials and hand-outs.

## Techniques and Instruments You Will Use

- ✓ Tissue Homogenizers (Omni)
- ✓ Filtration Devices including Tangential Flow Ultrafiltration (Millipore, Sartorius, Vivascience, Pall)
- ✓ Refrigerated (Sorvall) Centrifuges
- ✓ Recording UV-Vis Spectrophotometers (Spectronic, Cary)
- ✓ Filter Fluorometers (Turner Designs, Hoefer)
- ✓ Chromatography: Gel Filtration, Ion-Exchange, & Hydrophobic Interaction (GE Healthcare, BioRad)
- ✓ Immobilized Metal Ion Affinity Chromatography (Qiagen, BD Biosciences/Clontech)
- ✓ Fraction Collectors (Gilson, Isco)
- ✓ Column Monitors
- ✓ Electrophoresis (SDS, native and isoelectric focusing) (BioRad, Invitrogen/Novex, GE Healthcare)
- ✓ Western Blotting (Invitrogen/Novex)
- ✓ Coomassie Blue and Silver Staining (Sigma)
- ✓ Ion Exchange HPLC (Applied Biosystems)
- ✓ Size Exclusion HPLC (Thermo Electron Corporation, Phenomenex)
- ✓ GE Healthcare Phast Electrophoresis System
- ✓ Molecular Modeling
- ✓ Amino Acid Analysis\*
- ✓ Automated Edman Sequencing\*
- ✓ DNA Sequence Analysis\*
- ✓ Peptide Mapping\*
- ✓ Mass Spectral Analysis\*

\*Introduced in problem-solving session on Friday

*✍️ “I was extremely impressed with the quality of the course and caliber of all instructors ... best course like this I’ve been to ... Thank you for a wonderful learning opportunity and environment ...”*

**Margo Zanotto**, Manufacturing Associate, Biotech, Baxter Biotech

**Corporate Support:** Equipment, materials and laboratory supplies from the following companies are used in this course. We sincerely appreciate their support.



**Rutgers University, School of Environmental and  
Biological Sciences (CREBB)  
Department of Biochemistry and Microbiology  
76 Lipman Drive  
New Brunswick, NJ 08901-8525**

Name \_\_\_\_\_

First Name For Nametag \_\_\_\_\_

Employer \_\_\_\_\_

Job Title \_\_\_\_\_

Mailing Address \_\_\_\_\_

\_\_\_\_\_

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Work Phone \_\_\_\_\_

Home Phone \_\_\_\_\_

Fax No. \_\_\_\_\_

E-mail Address \_\_\_\_\_

### ***Protein Purification***

- January 7-12, 2007
- March 11-16, 2007
- June 3-8, 2007
- July 8-13, 2007
- July 22-27, 2007

### ***GFP Guide***

D&MD Publications is happy to extend a 10% discount to course registrants for “A Guide to Green-Fluorescent Protein: Applications in Cell Biology and Drug Discovery.”

To order the Guide, please contact D&MD's Customer Service Department at [cust.serv@drugandmarket.com](mailto:cust.serv@drugandmarket.com) or call +1 (508) 616-5566 and mention source code “9133-10%PP”.

**D&MD**  
PUBLICATIONS

### ***Tuition Fee:***

- \$2,195** (Early Registration)
- \$1,995** (Per Person for Multiple Early Registration of 2 or more attendees from the same company)
- \$1,395** (Academic Early Registration)
- \$2,695** (Regular Registration)

**NOTE: Fees MUST BE PAID** 3 weeks prior to the start of the course for all discounted rates.

- Check or Money Order enclosed in the amount of \$ \_\_\_\_\_
  - Visa or MasterCard # \_\_\_\_\_
- Exp. Date: \_\_\_\_\_

Rutgers University, School of Environmental and  
Biological Sciences (CREBB)  
Department of Biochemistry and Microbiology  
76 Lipman Drive  
New Brunswick, NJ 08901-8525

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# Protein Purification:

## Principles and Practice

