

## Articles

# Developing a New Interdisciplinary Lab Course for Undergraduate and Graduate Students: Plant Cells and Proteins\*

Received for publication, April 30, 2007, and in revised form, June 20, 2007

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Studies of protein function increasingly use multifaceted approaches that span disciplines including recombinant DNA technology, cell biology, and analytical biochemistry. These studies rely on sophisticated equipment and methodologies including confocal fluorescence microscopy, mass spectrometry, and X-ray crystallography that are beyond the scope of traditional laboratory courses. To equip the advanced undergraduate and beginning graduate students with an enabling base of knowledge and initial experience with advanced protein research methodologies, a laboratory course entitled *Plant Cells and Proteins* was developed in a partnership between Washington University and the Donald Danforth Plant Science Center in St. Louis. In this one semester course, 10–12 students obtain hands-on experience with plant tissue culture, gene transformation, subcellular localization of fluorescent recombinant proteins using confocal microscopy, purification of affinity-tagged recombinant proteins, isolation of total protein extracts, enzymatic assays, one- and two-dimensional gel electrophoresis, MALDI-TOF and ESI-Q-TOF mass spectrometry, protein crystallization, and X-ray diffraction. The course is taught as a series of modules, each led by an expert researcher. Students are evaluated based on a series of graded written reports and tests of their mastery of key concepts, interpretations, and the limitations of the experimental methods.

**Keywords:** Undergraduate, graduate, teaching laboratory, interdisciplinary, fundamental skills.

The conceptual and technical skills of upper-level undergraduate students and beginning graduate students are partly the product of traditional laboratory courses dedicated to specific topics and methods. Although teaching laboratories provide solid foundations in the principles and techniques of their respective fields, traditional classes are often limited by available facilities or faculty expertise. Classic biochemistry classes often do not reflect contemporary research practices that blend multiple disciplines, modern instrumentation, and a variety of experimental approaches. Likewise, traditional

plant biology laboratories tend to focus on whole plant physiology or genetics rather than molecular-based problems. In many curricula, implementation of research-oriented teaching leads to major revisions in existing courses [1–3]. With the goal of addressing this issue, a new laboratory class, which integrates current biochemistry, proteomics, microscopy, and plant biology approaches, was developed to provide students with the breadth of understanding and scientific skills to successfully apply such methods to advanced topics in plant biochemistry and molecular biology.

\* [http://www.biology.wustl.edu/pikaard/Plant\\_Cells\\_proteins\\_course\\_page.html](http://www.biology.wustl.edu/pikaard/Plant_Cells_proteins_course_page.html); Proteomics and Mass Spectrometry Facility: <http://www.danforthcenter.org/msb/>; Integrated Microscopy Facility: <http://www.danforthcenter.org/imf/>.

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### Course Development

As part of a curriculum update, a new combined lecture and laboratory class was designed to effectively introduce a range of methodologies that are defining current experimental practice to research-oriented senior undergraduates and first or second year graduate students. The new course serves students interested in biochemistry, but not necessarily plants, as well as those interested in plant biology and who also have the opportunity to enroll in lecture courses that cover plant genetics, physiology, and molecular biology. The course also capitalized on the facilities of the Donald Danforth Plant

Science Center (DDPSC)<sup>1</sup> ([www.danforthcenter.org](http://www.danforthcenter.org)), a nonprofit research institution with close ties to Washington University, particularly with the Biology Department ([www.wustl.edu/biology](http://www.wustl.edu/biology)). *Biology 4024: Plant Cells and Proteins* focuses on project-oriented approaches by offering research-based experiments that combine plant tissue culture and transformation, protein chemistry, X-ray crystallography, mass spectrometry, proteomics, and microscopy.

#### OBJECTIVES AND DESIGN

Development of Bio4024 aimed to serve the needs of undergraduate and graduate students majoring in biology or chemistry who are starting, or contemplating, a research career path at the interface of these disciplines. The goals of the course are as follows:

1. To introduce the advanced laboratory skills that reinforce basic principles and techniques learned in introductory biology, chemistry, and physics courses;
2. To enhance the breadth of general laboratory experiences needed for future independent research;
3. To provide research outcomes that develop critical thinking and analysis skills;
4. To improve communication skills through group participation, written reports, and oral presentations; and
5. To expose students to how technology is changing the types of biochemical and molecular questions asked in plant biology.

Enrollment of the course was limited to 12 students in a single section to ensure a close interaction with the instructors. All students were required to have completed their introductory biology (three semesters) and chemistry (four semesters with labs) courses as prerequisites. The course schedule involved a weekly 1-hour lecture held at Washington University and two 4-hour laboratory sessions at the DDPSC. The lecture component of the class provided the background information and theory on the techniques and experimental systems used in the laboratory. The small class size is very conducive for the presentation and discussion of lecture and laboratory material. An important feature of the laboratory section is that the students use the same facilities and state-of-the-art equipment used by DDPSC researchers (Table I). For example, during the mass spectrometry sections of the protein chemistry and proteomics modules, students learn the basic operation of matrix-assisted laser desorp-

TABLE I  
Summary of facilities at the Donald Danforth Plant Science Center

Core facility	Expertise
Tissue culture and transformation	Plant cell suspension cultures, <i>Agrobacterium</i> -mediated transformation, biolistics, and electroporation
Mass spectrometry and proteomics	2D gel electrophoresis, 2D gel image analysis, protein spot excision, in-gel protein digestion, MALDI-TOF MS analysis, ESI-Q-TOF MS/MS analysis, HPLC, surface plasmon resonance (BIAcore), MS data analysis, and database searching
Microscopy and imaging	Living cell microscopy (confocal/multiphoton), digital image reconstruction, high pressure freezing, transmission electron microscopy

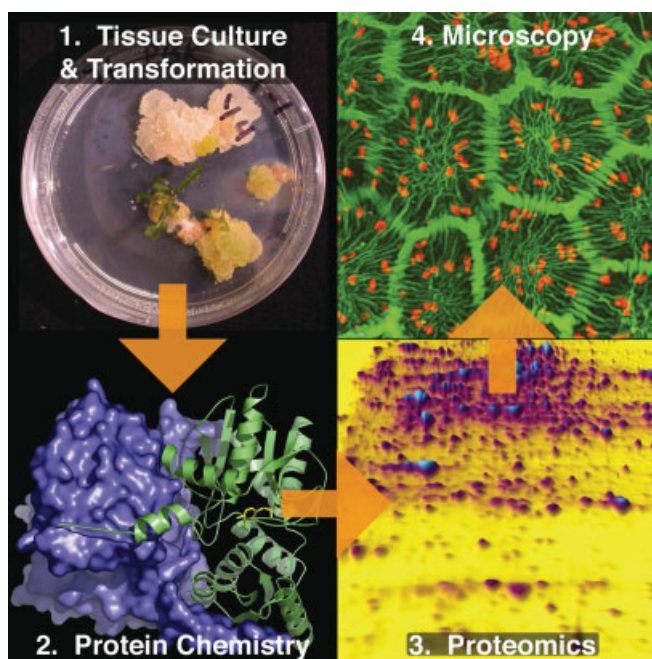


FIG. 1. **Progress of course modules.** The four units of Bio4024 cover a range of topics to highlight the current methods for studying plant proteins and cells both *in vitro* and *in vivo*.

tion ionization (MALDI) and electrospray ionization (ESI) time-of-flight (TOF) instruments and compare the results obtained from each instrument.

#### OVERVIEW OF COURSE MODULES

The course material is presented through a series of laboratory modules, each designed to teach basic experimental principles and methods through research-oriented projects (Fig. 1). The modular aspect of the course allows flexibility for the progression of experiments, instrument use, and topics within each unit [4]. The first unit on plant tissue culture and genetic transformation involves the transformation of plants with genes encod-

<sup>1</sup>The abbreviations used are: MALDI-TOF, matrix-assisted laser desorption ionization-time-of-flight; ESI-Q-TOF, electrospray ionization-quadrupole-time-of-flight; DDPSC, Donald Danforth Plant Science Center; MALDI, matrix-assisted laser desorption ionization; ESI, electrospray ionization; TOF, time-of-flight; GFP, green fluorescent protein; RNAi, RNA interference; SDS-PAGE, SDS-polyacrylamide gel electrophoresis; OASS, O-acetylserine sulfhydrylase; 2,4-D, 2,4-dichlorophenoxyacetic acid; 2DE, two-dimensional gel electrophoresis; 2D, two-dimensional; YFP, yellow fluorescent protein.

TABLE II  
Description of lab sections and expected outcomes

Experiment	Sessions <sup>a</sup>	Tasks	Content
1. Tissue culture and transformation	4	Examine the effect of nutrients and plant growth regulators using plant tissue culture methods; transformation of plant tissues with GFP constructs	Contamination testing, seed and leaf sterilization, effect of macronutrients on shoot and root growth, plant growth regulation by hormones, soybean hairy root transformation, tobacco leaf disc transformation, agrobacterium methods
2. Protein Chemistry	10	Purification and biochemical characterization of a heterologously expressed plant protein; introduction to protein mass spectrometry; kinetic analysis of wild-type and mutant proteins; crystallize and evaluate X-ray diffraction of protein	Bacterial expression, affinity purification, SDS-PAGE, protein determination assays, UV/Vis spectroscopy, gel extraction and protease digestion, enzyme assays, steady-state kinetics, enzyme, protein crystallization, X-ray diffraction
3. Proteomics	6	Analyze the effect of environmental or hormone-dependent changes on plant protein abundance; use of mass spectrometry to identify proteins whose abundance changed due to treatment	Plant protein extraction, 2D gel electrophoresis, image analysis, spot picking, trypsin digestion, sample preparation, MADLI-TOF & ESI-Q-TOF mass spectrometry, data analysis
4. Microscopy	6	Protein visualization within cells; confocal microscopy and digital image reconstruction of transformed cells	GFP visualization of soybean hairy roots and transgenic <i>Arabidopsis</i> seedlings by confocal microscopy, image processing, analysis of organelle localization

<sup>a</sup> Each lab session is up to 4 h in length.

ing proteins that have been engineered such that they are translationally fused to green fluorescent protein (GFP). The last section of the course, which covers microscopy and imaging, examines the intracellular localization of these proteins in the transformed plants. The second and third modules cover *in vitro* and *in vivo* aspects of protein chemistry. The second unit involves a comprehensive biochemical characterization of a plant enzyme, including protein expression and purification, spectroscopic and kinetic analysis, protein identification by mass spectrometry, protein crystallization, and an introduction to three-dimensional structural studies by X-ray crystallography. The third module expands on the introduction to mass spectrometry using this technique for proteomic analysis of proteins isolated from plants that have been subjected to different treatments and resolved by two-dimensional gel electrophoresis (2DE).

The overall coordination of the course is the responsibility of a Washington University professor and a DDPSC investigator. A DDPSC principal investigator with expertise in the unit's topic teaches an individual module. This investigator delivers lectures and runs the laboratory sessions. For the three modules performed in the DDPSC core facilities, a staff scientist from the facility assists the module instructor during the laboratory periods. In addition, the course has a graduate student-teaching assistant assigned throughout the semester.

The activities of the first week of the course provide students with the typical information, including a course overview, description of the syllabus, grading expectations, and formal laboratory safety training by an institutional safety officer. Moreover, if students are involved in research projects, they are encouraged to incorporate their own work, if possible, into the course. This informa-

tion is also provided to the students before the semester begins to allow for material preparation. The transformation/microscopy and proteomics modules are well suited for this option. For example, two graduate students from a laboratory, studying plant pathogen interactions, examined the differences in the proteomes of untreated and pathogen-treated *Arabidopsis thaliana* (thale cress). The detailed features of each module and the experimental tasks and content are summarized in Table II.

#### MODULE ONE: PLANT TISSUE CULTURE AND TRANSFORMATION

The plant tissue culture and transformation module is split into two sessions (Weeks 2 and 6). In the first half of this unit, students learn sterile technique and plant tissue-culture methods to test how the effects of nutrient supply and the interaction of growth regulators affect plant development. To examine the role of macronutrients on growth and development, seeds of the model plant *A. thaliana* are germinated on media lacking nitrogen, phosphorus, calcium, magnesium, sulfur, or potassium. Similarly, the interplay of plant growth regulators, including auxins (1-naphthaleneacetic acid), cytokinins (6-benzylaminopurine), and gibberellins, is tested using different combinations of these hormones for the growth of explants from tobacco leaves and stems.

The second part of this unit introduces students to *Agrobacterium*-mediated plant transformation methods. In the laboratory, students generate transgenic plants using two transformation systems for subsequent analysis in the microscopy module of the course. Generation of transgenic hairy roots on soybean cotyledons uses *Agrobacterium rhizogenes* to express a series of GFP-fusion proteins tagged with organelle-specific localization

signals [5]. Culturing of the hairy roots depends on the tissue-culture methods learned in the first week. In parallel, students use a disarmed *Agrobacterium tumefaciens* strain to make transgenic tobacco leaf discs expressing scorable and selectable markers [6]. For this experiment, an RNA interference (RNAi) construct targeting magnesium chelatase, an enzyme required for chlorophyll biosynthesis, is used to demonstrate how the expression of a specific protein can be downregulated by targeting its mRNA for RNAi-mediated degradation. Collectively, the parts of this module emphasize the relative ease and versatility of using plant tissue culture and transformation methods to manipulate plant development and study plant gene and protein functions.

#### MODULE TWO: PROTEIN CHEMISTRY

The second 5-week module (Weeks 3–5 and 7–8) covers protein purification and characterization methods and includes bacterial expression of His-tagged proteins, affinity chromatography, protein quantitation, SDS-polyacrylamide gel electrophoresis (SDS-PAGE), analysis of enzyme function, mass spectrometry, and protein crystallography. The techniques introduced on this module were chosen to demonstrate the range of analytical tools used by practicing biochemists. During the 2005 and 2006 spring semesters, these experiments used *O*-acetylserine sulfhydrylase (OASS), a cysteine biosynthesis enzyme, from *A. thaliana* [7]. Pairs of students were assigned either wild-type enzyme or one of several OASS mutant enzymes with impaired function. For a class of 12 students, this allowed the examination and comparison of wild-type OASS and five mutant enzymes. The site-directed mutants were previously generated in the laboratory of an author (J.M.J.); however, many of the mutants had not been functionally tested before the class began.

The experimental core of the module involves the expression, purification, and biochemical characterization of each group's OASS variant. This set of exercises guides students through a set of techniques routinely used in protein chemistry labs, including bacterial expression, purification, SDS-PAGE analysis, protein concentration determination, and enzyme kinetics. As a prelude to the next module, students learn methods for mass spectrometric determination of molecular weight and protein identification through peptide mass fingerprinting and tandem mass spectrometry sequencing of a trypsin-digested protein sample. The final exercise in this module introduces students to the methods of structural biology, including the principles and practice of protein crystallization and how to collect X-ray diffraction data.

A key feature to the protein chemistry module is an emphasis on teamwork. Given the mix of undergraduate and graduate students and the range of their laboratory backgrounds, some students have previously had experience with one or more methods used in this section. These students are encouraged to share the benefit of their experience by teaming up with less experienced students. Although each pair of students works independently to characterize their OASS protein, the whole

class functions as a team to characterize and evaluate the set of mutant proteins relative to the wild-type enzyme. The written report for this section emphasizes the organization and interpretation of the data obtained collectively by the entire class.

#### MODULE THREE: PROTEOMICS AND MASS SPECTROMETRY

The proteomics module spans a 3-week period (Weeks 9–11). This module aims to introduce students to the limitations and capabilities of proteomic studies using plants as the experimental material. This module of the course is tightly linked to the earlier protein chemistry section in the use of gel electrophoresis to separate and resolve individual proteins and the use of mass spectrometry to analyze proteins. The proteomics section differs from the protein chemistry section in that the scale of the analysis is larger, such that changes in the abundance of many proteins are investigated as opposed to the previous focus on an individual protein.

Our experimental material is the model plant *A. thaliana*. Because the genome of this species is fully sequenced, the protein databases used for searching the mass spectra are complete. *Arabidopsis* can also be easily grown on Petri plates or in soil. Plants are treated in various ways and the changes in the proteome are analyzed using 2DE. In the 2005 semester, proteins expressed in plants subjected to cold temperatures were compared to plants grown under normal (control) conditions to evaluate changes in the observed protein profiles. During the 2006 semester, plants were treated with 2,4-dichlorophenoxyacetic acid (2,4-D), a synthetic auxin that mimics many effects of the natural plant hormone indole acetic acid (an auxin). At high concentrations, 2,4-D is a potent herbicide commonly used on lawns to control dicotyledonous weeds (nongrasses). Alternatively, students compared proteins of wild-type *Arabidopsis* plants to mutants that they were studying in their own independent projects, such as their graduate thesis work. The corresponding lectures for this module provide background on the physiology associated with the stress treatment used and cover the technical details of the section. One lecture provided the background material on how plants adapt to the temperature changes or on the biological significance and action of the hormone auxin. Other lectures focused on the principles of 2DE, the utility of proteomics, and the analysis of two-dimensional gel (2D) images. The basics of mass spectrometry were covered previously in the protein chemistry module, but high-throughput technologies including robotic spot picking and sample handling are discussed as part of the proteomics module. In addition, to understand how mass spectrometry can identify protein sequences using bioinformatic tools, the students are also given a manual *de novo* sequencing exercise.

The students are guided through a simple procedure for protein extraction, and then 2D gels are run; the first dimension being isoelectric focusing and the second being SDS-PAGE. To reduce the time and costs associated with students casting their own 2D gels, we use small precast gels with a group of two students analyzing

a pair of gels containing protein from both treated and untreated plants. We found that the 2,4-D treatment yielded more pronounced differences in protein profiles than did the cold treatment. After the gels are run, they are stained with Coomassie Blue and either scanned or photographed. Although students are given a lecture on gel image analysis, their analysis is done by visual inspection of gel images, where they look for differentially expressed proteins between gel images. The protein spots that are found to be differentially abundant between gels are manually picked, subjected to in-gel digestions with trypsin, and the resulting peptides are isolated from the gel. Finally, the peptides are analyzed using both MALDI-TOF and ESI quadrupole-TOF (ESI-Q-TOF) instruments, and the results obtained from each analysis are compared. The students must then use the resulting data for a computer-based search of relevant sequence databases using a web version of the Protein Prospector software (<http://www.prospector.ucsf.edu>). At the conclusion of this module, the students are responsible for a written report that incorporates a description of their methods, presentation of their results, and an interpretation of their findings. Students also give a short presentation of their results, so that the different groups share their experiences and results with the whole group.

#### MODULE FOUR: MICROSCOPY AND IMAGING

The availability of GFP (and related proteins) has revolutionized cellular imaging, subcellular localization studies, and the *in vivo* visualization of protein dynamics [8]. The last module of the course (Weeks 12–14) uses the transgenic plant material that was generated earlier in the semester in the plant tissue culture and transformation module. The students' goal is to determine the subcellular localization of different GFP-fusion proteins in soybean hairy roots and *Arabidopsis* seedlings. The constructs used to generate soybean hairy roots result in the expression of GFP-fusion proteins targeted to the cytosol, nucleus, endoplasmic reticulum, or mitochondria. In addition, students are provided with *A. thaliana* seedlings transformed with GFP- or yellow fluorescent protein (YFP)-fusion proteins that are known to be expressed in the nucleus, vacuole, or endosome. The differences in targeting are also useful for the students to identify different organelles in plants. The lecture component of this module provides the students with background material on fluorescence, confocal, and multiphoton microscopy for protein visualization, and how to use the confocal instrument and imaging software they will use for their analyses. The actual microscopy component is scheduled over the final 2 weeks of the class to provide sufficient time for students to use the instrumentation in the DDPSC Microscopy facility. Initially, the selection of transgenic root or *Arabidopsis* tissue for further analysis is conducted using a dissecting microscope and epifluorescence imaging. Next, the fluorescent tissues are dissected from the plant and placed on a coverslip for high-resolution imaging using an inverted confocal microscope. Students obtain multiple optical sections that must then be processed to generate confocal projections

revealing the localization of the target proteins. The written report for this section combines material from the plant tissue culture and transformation module and the microscopy module.

#### ASSESSMENT

Students in the class are evaluated primarily on laboratory performance, which accounts for half of the grade contribution. This includes teamwork, discussion participation, and maintaining a neat, orderly, and detailed laboratory notebook. The unit's instructor grades the laboratory notebooks and evaluates the students' performance after each module. The remaining half of the student's grade is based on three written reports covering 1) tissue culture, transformation, and microscopy, 2) protein chemistry, and 3) proteomics. Each report is a combination of responses to questions posed by the instructors concerning key concepts, classroom presentations of data, and evaluation and discussion of experimental findings. The respective instructor of each module grades these reports. The course coordinators oversee this process to provide students with consistent feedback between units.

University evaluations and individual student feedback have proved valuable information for evaluating the effectiveness of the course and improving its content. Very favorable responses from the students were received in both the years that the course was offered. Specifically, many of the students enjoyed the range of topics covered, the depth of information provided, the usefulness of the experimental techniques learned by them, and the ability to obtain hands-on experience with sophisticated state-of-the-art equipment. Moreover, Washington University graduate students, who have taken the class later, commented that the topics covered in Bio4024 enabled them to rapidly apply the skills learned and the course material in their own thesis research.

#### SUMMARY

The described course aimed to provide the students with hands-on experience in the range of methods used to study proteins and their functions, which is a rapidly evolving and important area of science. Modern research problems transcend classic biochemistry and/or molecular biology and can involve structural biology, analyzing global changes related to protein function using proteomics, and plant transformation and microscopy to provide another level for evaluating the "function" of individual proteins as they fit into the context of cell biology. With an understanding of how to use these varied methods, students are empowered to consider studies at levels of organization that range from analyses in the whole organism to the atomic scale.

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