# TEACHING ANALYTICAL INSTRUMENTAL ANALYSIS TO UNDERGRADUATES IN SPECIALIZED DEGREE PROGRAMS WITH HETEROGENEOUS PRIOR KNOWLEDGE: REFLECTIONS ON INQUIRY-BASED LECTURE ACTIVITIES

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## ABSTRACT

*An adjunct faculty member and graduate instructional assistant (GIA) introduced inquiry-based activities into a 20-student undergraduate analytical instrumental analysis (AIA) lecture course, and reflect on their teaching assumptions, practices and experiences. The increased need for interdisciplinary scientific programs now has an AIA course serving multiple Bachelor of Science degrees in environmental sciences, forensic sciences, molecular biology, and secondary science education. However, we learned degree specialization also introduces into a course, student populations possessing heterogeneous prior knowledge, making an instructor's rendering of student prerequisite skills a greater challenge. Guided by a pretest assessment, instructional activities were modified or developed by the authors and aimed at enhancing student engagement and motivation to mitigate prior knowledge gaps, improve analytical problem-solving skills, and facilitate a deeper understanding of modern instrumentation design and function. Detailed activity rationale and descriptions are presented. Activities included using readily available Internet bioinformatics and database tools for analytical problem-solving; demonstrating principles of electronic hardware and software design and integration; and creating interdisciplinary scientific narratives using biological, environmental, and industrial molecular exemplars. Our teaching reflections reference weekly post-lecture instructor/GIA discussions, strategic student questioning, collaborative classroom activity observations, and formative assessments. We propose continual instructional reflection is essential for a course serving multiple specialized degrees programs in a scientific field and facilitates preparation for students entering the workforce or graduate school. Further, our observations suggest inquiry-based, real-world activities relevant to modern instrumentation and its applications, assisted students in resolving heterogeneous prior knowledge gaps.*

## STATEMENT OF PURPOSE

Using a range of technologies adaptable to a lecture classroom setting, our purpose was to develop, implement, and reflect upon active learning activities utilized in an Analytical Instrumental Analysis (AIA) course characterized by students with heterogeneous prior knowledge. The reflective lens utilized in our teaching practices involved four areas—technical, descriptive, dialogic, and critical—as previously described by Hatton and Smith (1995).

While teaching AIA for the first time as an adjunct faculty member, it was noted that students' prior knowledge varied markedly. Hence, when teaching the course subsequently and in collaboration with a GIA, it was decided by these authors to assess—using pretesting—student prior knowledge in biology, chemistry, and physics. Our pretesting revealed considerable differences within our student population in skills and knowledge in the areas of computation, algebra manipulation, knowledge in biology, chemistry and important concepts in general physics. Literature indicates that prior knowledge is foundational for learning new concepts (Wright, 2004), and we believed inadequate scientific prior knowledge would challenge a student's ability to learn the conceptual underpinnings for a curriculum in AIA, which comprehensively introduces analytical and instrumental methodologies (Schafer & Bruck, 2013).

During week one of instruction, a 20-minute (pretest) assessment was administered. The pretest questions included knowledge of facts and processes, application of concepts and principles, and manipulation of variables as suggested by Lazarowitz and Lieb (2006). The student knowledge gaps identified were then incorporated into active learning exercises as we taught the established course curriculum.

This assessment aided by classroom experiences were combined to make critical adjustments to instructional practices and assessments in our AIA course. For example, when introducing spectrophotometry and the Beer-Lambert Law, calculations and methods to express analyte concentration were reviewed and practiced as a group activity. Understanding the Beer-Lambert Law, which provides a direct proportional relationship between absorbance of a substance at a specific wavelength and its concentration, is a central pillar in AIA (Swinehart, 1962). Similarly, basic electronics' concepts were introduced as demonstrations with active involvement by students prior to describing how energy interacting with a sample is first recorded as an analog (electrical) signal and subsequently converted to a digital value. In part, these examples illustrate our challenge in teaching topics in AIA to students with heterogeneous prior knowledge in chemistry and physics.

The framework for our reflective practices involved four areas: technical, descriptive, dialogic, and critical as described by Hatton and Smith (1995). Implementation of modified or newly developed active learning activities and demonstrations were categorized under technical reflections. Within the technical reflective lens, we focused on the immediate needs of the students to comprehend and learn key concepts in AIA.

Descriptive reflections were based on classroom relationships with our students, encouraging them to present rationale for why they acted or decided the way they did. Problem-solving skills were categorized under dialogic reflections. Using strategic questioning aimed at developing critical thinking skills, we asked students to utilize their self-examination and consider why a result occurred the way it did; for example, critiquing empirical data. We then queried students to explain their rationale to the class, creating and expanding classroom dialog and debate.

Our final lens of reflection was critical reflections that relied on considering historical, ideological and social forces as we taught. Using these reflections, we intended to improve our teaching practices by implementing lecture activities and demonstrations that would support better student comprehension and problem-solving skills.

## STATEMENT OF PROBLEM

Rapid technological innovations are demanding that university faculty continually revise student learning objectives and curriculum for advanced undergraduate courses (Ben Kei, 2018). As a result, consideration of a student proficiency in a particular subject area may not completely align with the skills needed for optimal course learning outcomes. When students enroll in an upper-level undergraduate course such as AIA, they have taken a series of prerequisite courses. Instructors viewing these prerequisites courses, make assumptions on what students should know. However, we unexpectedly discovered with our AIA student population, gaps in biology, chemistry, mathematics, and physics prior knowledge. These revelations prompted us to reevaluate the approach of using a traditional presentation style of curriculum. Given the degree of student heterogeneous prior knowledge observed, reliance on a standard PowerPoint lecture approach, we believed, would result in decreased student engagement and motivation.

Today, with more specialized majors being offered, a course once designed to serve a single Bachelor of Science program (such as chemistry), is now tasked to instruct students in specialized degrees including biochemistry, bioinformatics, environmental, and forensic sciences, molecular biology, neuroscience and secondary science education (Fahey & Tyson, 2006). In this report, we define this course as a Multiple Program Interdisciplinary Course (MPIC), and we suggest MPIC curriculum content and instructional activities require continual updating and reflection for relevant and effective inquiry-based student engagement.

As Glaze (2018) pointed out, faculties in the sciences bring assumptions into their classroom including expectations of what students should know before entering given courses. Importantly, as Rose (2009) suggested, instructors should not automatically assign student knowledge gaps to learning deficiencies, particularly in a classroom that bundles students from multiple specialized degree programs. We note that multiple factors influence undergraduate scientific literacy, but rather than question who is responsible for rigor-wise or lack of content knowledge, our focus was to develop active-learning exercises and demonstrations, which encouraged higher levels of scientific literacy. Hence, understanding a student's prior knowledge is a key consideration when teaching advanced concepts in analytical chemistry and modern instrumental analysis, mandating instructors adjust instruction to account for these knowledge differences.

The authors believe opportunity exists to optimize student learning outcomes in an AIA-MPIC through inquiry-based content delivery when guided by a pretest assessment, which explicitly identifies key student knowledge gaps. Moreover, given rapid changes in instrumental informatics and data analysis, greater attention regarding emphasis of what instrumental methods are taught in lecture and in corresponding co-requisite laboratory courses is needed (Fahey & Tyson, 2006). We anticipate our reflective practices and activities for this AIA-MPIC provide a partial roadmap for generating and modifying lecture-based critical-thinking activities in heterogeneous prior knowledge student populations, assist in serving multidisciplinary scientific degree programs, and better prepare students for graduate school or industrial positions.

## ACTIVITY DESCRIPTIONS

As Glaze (2018) stated, "There is no one-size-fits-all approach to improving scientific literacy and active engagement in the undergraduate classroom" (p. 6). Given the heterogeneous prior knowledge we observed, the instructor and GIA collaborated to modify and develop activities to enhance skills in dimensional analysis, critical thinking, and expand content knowledge. In-lecture activities and demonstrations presented were conducted at the beginning of lecture periods and completed within 15 to 25 minutes. Lectures were 70 minutes, meeting three times a week.

Our in-lecture activities were intended to be authentic, inquiry-based, real-world scenarios confronting a workforce scientist or graduate student developing practical approaches to a problem. Criteria for activity development included focusing on creating student engagement, allowing students opportunities to fill knowledge gaps, applying mathematical skills, and introducing new instrumentation concepts prior to formal lectures. Importantly, our activities and demonstrations were not meant to supplant AIA laboratory procedural based learning which typically relies on a fixed result.

Our AIA in-lecture activities were categorized into seven areas, which were complementary to established AIA-MIPC curriculum and facilitated alleviation of prior knowledge heterogeneity we gleaned from pretesting assessment and classroom observations. The following activities described include: (1) Formative Assessment: Prior Content

Knowledge Skills (Pretesting); (2) Practical Critical Thinking Exercise: Bioinformatic Explorations; (3) Basic Instrumental Analysis Interpretation Skills, Spectral Analysis; (4) Concept Enhancement: Instrumental Design and Data Domain Transduction; (5) Measurement: Evaluating Component Accuracy, Precision and Tolerance; (6) Multidisciplinary Student Engagement: Variations on Molecular Themes; and (7) Critiquing Experimental Data: Gel Electrophoresis. Each activity included use of strategic questioning to assess comprehension.

## FORMATIVE ASSESSMENT: PRIOR CONTENT KNOWLEDGE SKILLS (PRETESTING)

Formative assessment is quintessential when instructing students with heterogeneous prior knowledge. We continually used formative assessment, questioning, quizzes, and real-time checks for understanding to make instructional adjustments and to gain information about students' progress, and we routinely incorporated prior knowledge gaps into quizzes and examinations to assess comprehension, and as needed, we further amplified a topic or skill while teaching the established analytical instrumental analysis curriculum.

Our pretest assessment included the following topics: drawing structures of organic molecules, defining Avogadro's number, defining and using units of measure such as pressure and temperature, ranking organic molecules based on hydrophobicity, calculating molarity and density, utilizing dimensional analysis skills, describing the basic structure of DNA, distinguishing classes of biological molecules, defining Ohm's law, and listing an eminent scientist they admired.

The majority of students correctly ranked the hydrophobicity of ethane, ethene, and ethanol, and about 70% of students were able to name and draw simple organic molecules. But, students had significant difficulty performing molarity and density calculations and distinguishing classes of biomolecules. With regards to listing an eminent scientific figure they admired, overwhelmingly the response was either Einstein or no response was given. Upon discussing this latter response with a few full-time faculty members, we learned only brief mentions of historically significant scientists are included in prerequisite chemistry courses. This lack of emphasis on history of science in prerequisite curricula was quite surprising to us as much of the processes and theories in science routinely used today are expressed through the nature, philosophy and chronicles of scientific discovery. This assessment also revealed that most students had limited or no background in basic physics and electronics.

## PRACTICAL CRITICAL THINKING EXERCISE: BIOINFORMATIC EXPLORATIONS

Choosing a chromatographic separation method involves multiple considerations, including: composition of the chemical mixture to be separated into its constituent molecules, time required for the separation, and the chromatography system's mobile and stationary phases properties. Our first quiz assessment revealed students had difficulty understanding how molecules separated based on hydrophobicity and net molecular charge. We noted that this was in contrast to results obtained from our basic pretest assessment—we surmised, students were not connecting a molecule's characteristics, such as hydrophobicity (understanding confirmed on our pretest) and applying this knowledge when selecting an appropriate separation technique. Hence, we developed an inquiry-based activity using easily accessible bioinformatics Internet tools. Our aims were two-fold: first, encourage student learning through collaboratively developing an authentic real-world scenario, and second, make informed judgments and decisions about which separation method(s) approach is best based on their scenario, an activity having practical workflow applications in both graduate training and commercial scientific applications.

The activity begins with students creating a hypothetical mixture of six to 10 proteins of various unknown concentrations, assumed to be present over several orders of magnitude, micro-molar to millimolar. After developing this hypothetical mixture and using bioinformatics tools, theoretical data about each protein is generated, compiled, and reviewed by the class. The class is queried to think critically about a methodological approach with questions from the instructor, including: what chromatographic separation method should be applied based on the physicochemical properties of individual proteins in the mixture? Which method(s) would be best for detection; for example, absorption or fluorescence? What are limitations of the methods chosen?

Just prior to beginning the activity, students are instructed how to use the bioinformatics Internet resources. First, students utilize bioinformatics, obtaining a protein's amino acid sequence that is subsequently submitted to another algorithm which calculates a protein's theoretically molecular weight, isoelectric point, hydrophobicity, and extinction coefficient value. Results are then tabulated on the whiteboard (Table 1), and the class considers how to represent this data to make decisions regarding the appropriate chromatographic approach. With instructor facilitation, students graph hydrophobicity, grand average of hydropathicity (GRAVY), versus isoelectric point (pI). Based on considerations such as data clustering, students collaborate as a team determining which chromatographic method(s) is best suited based on their hypothetical protein mixture; for example, ionic exchange, size-exclusion and reverse phase HPLC. Shown in Table 1 is an example of actual student generated data in our classroom as well as a graphical representation (Figure 1).

Table 1: Theoretical protein physical and chemical properties using the ExPASy ProtParam tool. All data are from human amino acid sequences.

| Protein | Accession Number | Molecular Weight (g mol$^{-1}$) | pI | GRAVY | Extinction Coefficient (M$^{-1}$ cm$^{-1}$) |
|---|---|---|---|---|---|
| Calmodulin | P0DP23 | 16,706.39 | 4.09 | - 0.671 | 2,980 |
| Collagen | P02452 | 94,796.09 | 9.29 | - 0.881 | 5,960 |
| Elastin | E7ENM0 | 58,506.24 | 10.37 | 0.691 | 22,475 |
| Hemoglobin α subunit | P69905 | 15,126.36 | 8.73 | 0.035 | 9,970 |
| Histone H1 | Q02539 | 21,710.90 | 10.99 | -0.758 | 1,490 |
| Keratin | Q16195 | 22,898.90 | 4.68 | -0.671 | 13,075 |
| Myosin | P12882 | 223,145.43 | 5.58 | -0.777 | 111,590 |

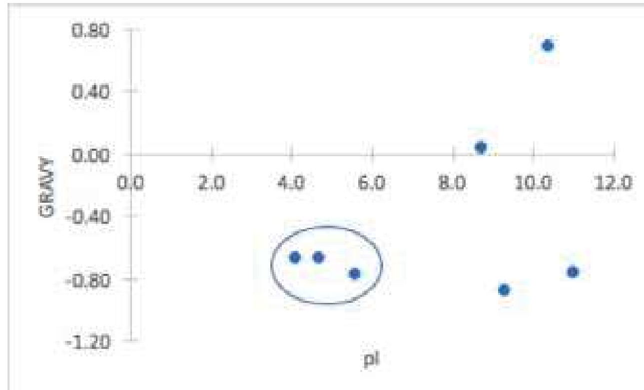


Figure 1: Plot of Hydrophobicity (GRAVY value) versus Isoelectric Point (pI). Values plotted are from Table 1. The bioinformatics tools employed in this activity included the UniProt Knowledgebase (The UniProt Consortium, 2017), and the ExPASy ProtParam tool (Gasteiger et al., 2005). Students can be questioned why clustering of data points (circled) may indicate multiple chromatographic methods are needed for separation.

## PROTEIN BIOMARKERS AND MASS SPECTROMETER

A logical segue from chromatography methods discussions and activities directs attention to the next step in analytical workflow, identification of the separated molecules. Integrated and sequenced next into most instrumental analysis curricula is mass spectrometry as it occurs downstream from gas or liquid chromatography. During lecture, students learn the key components of a mass spectrometer including the ionization method, general principles of mass analyzer design, and ion detection. But, in general, less emphasis is placed on spectrum interpretation or the use of identification software in introductory analytical instrumental analysis textbooks (Harris, 2016).

In forensic sciences, identification of organic combustion products and illicit drugs are unusual exemplars for undergraduate lectures; for example, cocaine, methamphetamine or anabolic steroids. And, while an undergraduate teaching institution laboratory may be equipped with gas chromatography mass spectrometer (GC-MS), licensing costs, policy for usage of controlled substances, and software training may result in limited student exposure to informatics software for compound identification.

An additional consideration is, students completing general and organic chemistry prerequisites have either not encountered or retained nominal mass, accurate (experimental) mass and exact mass terminology, and its relevance to accurate and precise high-resolution mass spectrometry data for compound identification (Brenton & Godfrey, 2010).

To overcome the aforementioned issues and address prior knowledge gaps, an activity was developed using data sets collected on a Matrix Assisted Laser Desorption Ionization—Time-of-Flight Mass Spectrometer (MALDI-TOF MS) of known (Table 2 & Appendix A) and unknown (Appendix A) trypsin-digested proteins. Students gained experience querying the peptide fingerprinting database MASCOT (Koenig et al., 2008). And, while high-resolution MALDI-TOF MS instrumentation is not readily available at smaller undergraduate teaching institutions, using these data, introduced students to compound identification informatics and reinforced the importance of accuracy and precision in mass spectrometry measurements.

Using MASCOT, students investigate search parameter set-up using known standards such as bovine serum albumin (Appendix A) or calmodulin (Table 2), simulating decision-making processes occurring in graduate training programs and academic or commercial research. For example, using the mass accuracy parameter, decisions about tolerance values for each data set are established. The activity concludes in a collaborative effort where students are presented unknown data sets and search for probable protein matches. Data sets are included, which do not give definitive results, and students collaborate as to other methods useful to identify the unknown protein, defending their decision-making via classroom discourse or online student journal entry.

Table 2. An appended peptide peak list with mass-to-charge ratio (m/z), peak intensity and signal-to-noise values for trypsin-digested purified human calmodulin. This data is used to establish search parameters in order to confirm reliability and avoid false positives. Appendix A lists data sets, available from authors, for bovine serum albumin (BSA), known proteins used as controls and unknown proteins. Data were obtained on a Bruker Ultraflex II ToF/ToF equipped with a N2 laser (337 nm) operating in linear mode.

Masses reported are MH+ masses.

| m/z | Intensity | S/N |
|---|---|---|
| 662.12 | 7328.33 | 23.81 |
| 805.26 | 23434.82 | 81.06 |
| 821.25 | 2169.49 | 7.50 |
| 832.20 | 10473.58 | 35.94 |
| 1265.47 | 3605.36 | 13.96 |
| 1352.45 | 3950.95 | 14.84 |
| 1563.49 | 12353.03 | 46.91 |
| 1596.49 | 2436.65 | 9.58 |
| 1754.63 | 18379.51 | 88.19 |
| 1842.73 | 1433.87 | 6.05 |
| 1844.59 | 12265.92 | 54.01 |
| 2400.89 | 8531.25 | 39.21 |

## BASIC INSTRUMENTAL ANALYSIS INTERPRETATION SKILLS: SPECTRAL ANALYSIS

After completing a lecture course in AIA, students are expected to know underlying principles of how instruments in well-equipped commercial or academic laboratory in principle operate. However, given the breath of instrumentation that must be covered in an AIA course, spectral interpretation is typically not emphasized in AIA course curriculums, and taught in either advanced undergraduate organic chemistry courses or at the graduate level.

Our experience indicated that students enrolled in our AIA-MPIC had limited or no prior knowledge in interpreting basic mass spectrometry, infrared or NMR spectral data. Students did indicate having some exposure interpreting spectral data in first and second semester organic chemistry. We believe covering basic spectral interpretation skills as an activity needs to be included as part of describing instrument operation and thus identified resources useful to both instructors and students.

Using the National Institute of Standards and Technology (NIST) Chemistry WebBook (Linstrom & Mallard, 2001), students were shown how to retrieve spectral data, providing students with examples for spectral interpretation and analysis. The NIST Chemistry WebBook resource was used throughout our course to facilitate class discussions and to enhance skills in the interpretation of known molecules. We found that practicing spectral interpretation regularly throughout the course and creating supplemental take-home worksheets improved assessment scores in subsequent quizzes and exams, suggesting enhanced student problem-solving skills. An example of a NIST dataset used in our classroom is shown in Figure 2.

Given the heterogeneous backgrounds of the students, spectral interpretation assessments were designed with a primary goal of identifying major fragmentation patterns and functional groups within the spectrum using data tables that were also provided during a quiz or an exam. This activity also demonstrated the importance of continually reflecting upon the level of assessment rigor in our AIA-MPIC classroom, uncovering potential knowledge gaps within a specialized degree program. An environmental science major may not include multiple organic chemistry courses, yet all AIA-MPIC students should to be familiar with at least the basic methods of interpreting spectral data important for their academic and career development.

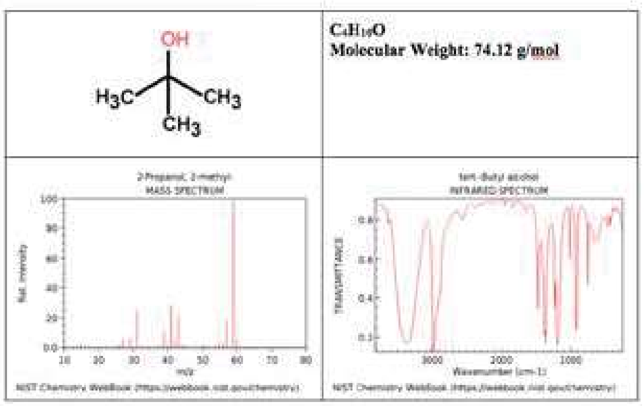


Figure 2: NIST Spectral Data, t-butyl alcohol

## CONCEPT ENHANCEMENT: INSTRUMENTAL DESIGN AND DATA DOMAIN TRANSDUCTION

Students enrolled in an AIA-MIPC perceive, based on our questioning, an instrument as a "BLACK BOX"—a sample is inserted into an instrument (the input), and a spectrum or a numerical reading is obtained (the output). However, this conceptualization, having little or no knowledge of the instrument's internal functions, is incomplete. And, while providing detailed subject coverage of electronic signal amplification and modification requires some background physics and electronics, the key concepts of data transformation are important to all AIA—MPIC curriculum. Our challenge, given most students did not have some electronics' or even a minimal physics or engineering background, made explaining data transformations, hardware, and software complexities of modern instrumentation a problematic teaching task. Hence, we utilized a simple, yet elegant demonstration for our students to partially crack open the black box's lid on modern instrumentation.

We employed the use of an open source computer hardware and software development tool (Arduino, 2018). The Arduino Uno setup shown in Figure 4 was used in conjunction with the breadboard resistor/thermistor voltage divider circuit (Figure 3) to make a simple digital thermometer. The Arduino Uno board is interfaced via USB to a laptop computer for uploading a short software sketch and digital readout using Arduino IDE software. Software sketches are available via the Internet and easy to understand with minimal programming knowledge, allowing students to appreciate the transfer of information from the physical world to an analog signal and ultimately a digital output. The software sketch code used for our demonstration is available online.

Students began to conceptualize instrument design using this simple circuit example and real-time observation of data sampling and signal transformation as the thermistor resistance decreases in response to a student pinching the thermistor. Students were asked what would happen if the 10 kΩ fixed resistor was replaced with a 100 kΩ resistor? How does tolerance of the resistor affect the accuracy of the measurement? This demonstration afforded opportunities to review Ohm's law and series circuits, allowing students to think critically about each component and its function in this simple instrument.

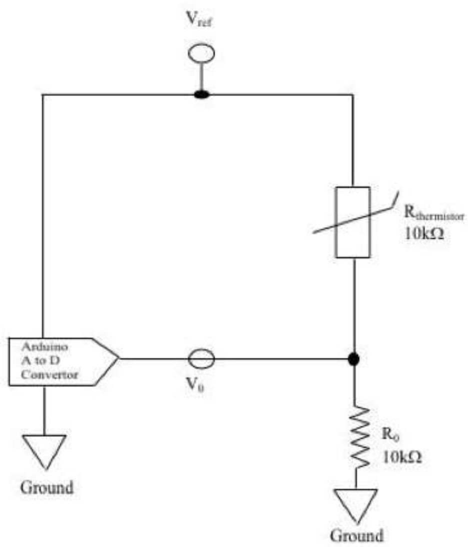


Figure 3: Voltage Divider Circuit and Arduino Uno

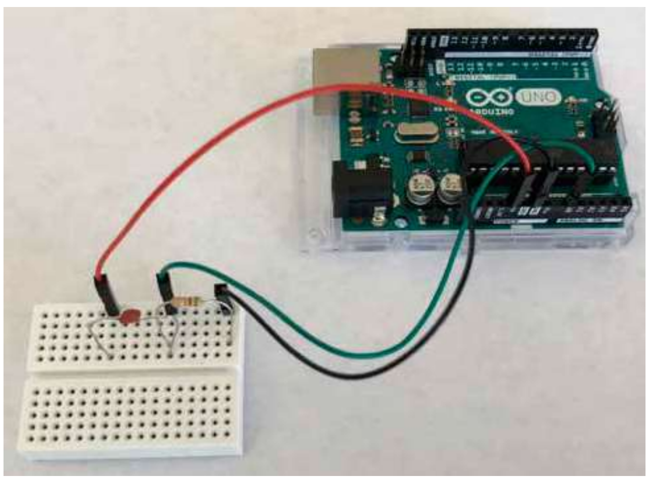

Figure 4: Arduino Uno Connected to Breadboard Circuit

## MEASUREMENT: EVALUATING COMPONENT ACCURACY, PRECISION AND TOLERANCE

We noted over 50% of our students had an incomplete understanding of the terms accuracy, precision and tolerance. And, while students were generally familiar with accuracy and precision explanatory "target" models as presented in Figure 5, when tasked to differentiate these terms using a set of experimental data, assessment revealed gaps in understanding.

Hence, we employed a simple activity demonstrating the application of these terms. The class generated an original data set using thermistors, selected randomly from a "grab bag," all rated at the same temperature-dependent resistance and tolerance value specified by the manufacturer. Upon compiling their group data in a tabular format (Table 3), the class was asked to describe how accuracy (conformity to a known standard or value), precision (repeatability of a measurement without generating random errors) and tolerance (predictable or allowable deviation from a standard) were reflected in their data set. Students were prompted to consider the importance of the measurements in terms of instrumentation design and cost. Would they use this thermistor to design and manufacture a digital thermometer? How would the thermistor be calibrated to actual temperature readings? How might components having lower or higher tolerance values affect accuracy and precision of an instrument's measured value?

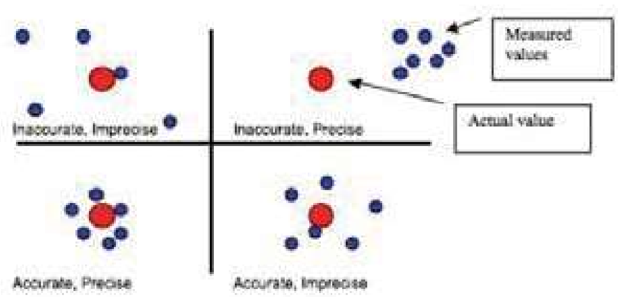


Figure 5: Graphical Representation of Accuracy and Precision

Students used a digital voltmeter to measure thermistor resistance at both room temperature and a simulated body temperature reading by pinching the thermistor between their thumb and forefinger. Were thermistor resistance values similar at room temperature? If an outlier value was recorded, students were asked to suggest probable explanations. Using descriptive statistics, a tolerance value was estimated from the data set and compared to the manufacturer's value, and fixed resistors used as control measurements (Table 3). Measurements of body temperature were used to discuss accuracy and precision experimental variations in measurements.

We noted given varied student coursework, some students were working with a digital voltmeter for the first time. While this is a relatively simple activity in measurement, it illustrates important concepts in measurement and considerations for instrument design that were incorporated into our lecture discussions. Additional activities might include sensitivity and response of various thermistors over a temperature range.

| Student Measurement | Resistance, Room Temperature (kΩ) | Resistance, Body Temperature (kΩ) |
|---|---|---|
| 1 | 9.800 | 7.315 |
| 2 | 9.329 | 7.451 |
| 3 | 8.864 | 7.313 |
| 4 | 8.837 | 7.173 |
| 5 | 9.037 | 7.274 |
| 5 (control) 10 kΩ | 9.902 | 9.887 |
| 6 (control) 10 kΩ | 9.918 | 9.886 |

Table 3: Resistance measurements using 10 kΩ thermistors with 10% tolerance and two 10 kΩ non-thermistor resistors (control) with 10% tolerance ratings.

## MULTIDISCIPLINARY STUDENT ENGAGEMENT: VARIATIONS ON MOLECULAR THEMES

Given the heterogeneity of student knowledge and a program's course specificity, we envisioned an activity to leverage these circumstances, creating an engaging multidisciplinary experience, empowering program majors to become classroom subject matter experts. Periodically, lectures began with an activity we entitled, Variations on Molecular Themes. Molecules employed in a significant analytical method, environmentally newsworthy or used in an industrial application, were purposefully selected, appealing to environmental science, forensic science, and medical field program majors. Discussions included methodological approaches to identify and characterize the molecule, and at times, revealed biological and societal implications. We share several examples.

When lecturing on High Performance Liquid Chromatography (HPLC)—a method used to separate mixtures of molecules—the relative molecular polarities, ultraviolet absorbance characteristics, and column elution order of the amino acids phenylalanine, tryptophan, and tyrosine were discussed. Instructor questioning began by sharing biologically relevant information that tryptophan and tyrosine are precursors for the neurotransmitters serotonin and dopamine, respectively. This led to a brief discussion about the compound L-dopa (structurally similar to dopamine), used in the treatment of Parkinson's disease, and methods of chiral drug separation, creating multidisciplinary engagement between biochemistry, biology, and chemistry majors. One student reflected about their relative using L-dopa for the treatment of Parkinson's disease. Phenylalanine prompted another student to mention the disease phenylketonuria, which causes elevated levels of phenylalanine in the blood. Each discussion assembled memorable connections to course content and provided context to the importance of specific analytical methods to identify and quantitate the molecules being discussed.

Examples of other molecules included SYBR green, a nucleic acid dye used in electrophoresis and Real-Time Quantitative Polymerase Chain Reaction (PCR) in the field of molecular biology (Dragan et al., 2012), and bisphenol A, an organic synthetic compound used in the making of plastics in items such as water bottles and studied extensively by environmental scientists for toxicity (Vaughn, 2010).

## CRITIQUING EXPERIMENTAL DATA: GEL ELECTROPHORESIS

Entering our classroom, analytical instrumental-analysis students possessed varying levels of practical laboratory knowledge but had had little or no experience in critically critiquing the quality of experimental empirical data. The instructor, a retired industrial science practitioner, suggests this is a significant knowledge gap or deficiency, and believes greater attention to the quality control and critique of datasets are needed when developing university lecture and laboratory course curriculums.

Beginning in introductory biology and through an advanced course in biochemistry, students are exposed to gel electrophoresis. Typically, most attention is directed to the final gel readout. However, an AIA course requires a more thorough understanding of electrophoretic mobility principles of molecules and the factors influencing quality of separation. Using traditional gel electrophoresis (agarose or polyacrylamide), lends itself to discuss technique limitations and parameters affecting optimal molecular separation. This activity provides a logical transition to discuss methodological advancements and applications in electrophoresis; for example, capillary electrophoresis (Fahey & Tyson, 2006).

In this activity, an agarose gel was used for qualitative analysis (Figure 6). Analyzing empirical data differs from analyzing numerical data as it is observation-based. Students were questioned on the use of appropriate molecular weight standards, explanations for the distortions in gel bands, use of positive and negative controls, detection method (SYBR green I dye), and quality of separation based on the concentration of the gel matrix. Students were asked to critically evaluate parameters for improving this experiment. Forensic science majors were engaged by this activity as it involved genetic profiling experiment conducted by instructor at a recent workshop.

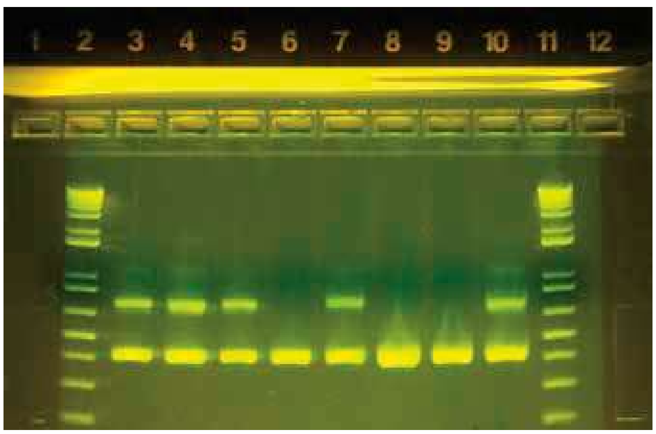


Figure 6: Electrophoresis Gel: screen for the presence of the PV92 Alu insertion (Batzer et al., 1994).

## REASONS FOR THE PROBLEM

An AIA course at an institution in the southwestern United States serves multiple degree programs including biochemistry, chemistry, chemistry for secondary education, environmental science, forensic science, and molecular biology. While these degree programs initially have similar prerequisite coursework, requirements begin to diverge as each program progresses. For example, an environmental science program's requirements include environmental law and human health risk assessment, while chemistry requires additional analytical, organic and physical chemistry lecture and laboratory coursework. How did academic institutional factors contribute to our pretest findings of AIA student prior knowledge heterogeneity?

A multiple degree program AIA course enrolls junior and senior undergraduates, each bringing prior knowledge and skills from their respective program. We began our reflection by assuming basic scientific knowledge was taught consistently to students in prerequisite chemistry and biology courses, but we considered what happened as students progressed in their degree program. Did divergent upper-division coursework contribute to the heterogeneity in "basic" student prior knowledge scientific capabilities? Were specialized instructional faculty reintroducing and reinforcing previously taught scientific concepts and skills, maintaining consistency in scientific literacy? We inferred inadequate reinforcement of previously taught scientific concepts and skills within specialized program coursework contributed to lower levels of scientific prior knowledge and problem-solving skills.

Another plausible contributing factor to student prior knowledge heterogeneity includes the challenge facility and curriculum development encounter while harmonizing prerequisite rigor to serve multiple specialty scientific degree programs, and its impact on AIA-MPIC lecture and corequisite laboratory content. Clearly, mitigating prior knowledge heterogeneity by requiring additional prerequisites offers partial resolution, but this may not be attainable in lieu of specialized degree program coursework.

Significant content overlaps in AIA methodologies within specialized scientific fields exists, making it impractical and cost-ineffective for institutions to breakout AIA courses into multiple courses to accommodate each specialized degree program. As a result, when an AIA course serves multiple degree programs, optimizing curriculum and learning activities should remain a central consideration. Under this AIA-MPIC model, differences in chemistry, math, and physics prior knowledge skills persist in its student population, necessitating teaching strategies and learning activities for students possessing heterogenous prior knowledge.

## EVALUATION OF REASONS FOR THE PROBLEM

Over the past decade, universities have added specialized scientific degree programs to their colleges, responding to industry's heightened interest in job applicant specialization (Fahey & Tyson, 2006). Since most specialized programs require AIA training, science departments have had to reorganize instructional and curriculum content to accommodate. But, program design should also carefully consider course prerequisites and sequencing prior to AIA student enrollment. As mentioned, an AIA course traditionally designed to serve a chemistry Bachelor of Science degree, is now tasked to instruct students in multiple specialized degrees, a contributing factor to student heterogeneous prior knowledge.

Adding further complexity to AIA coursework is instrumental technologies with biological applications (Fahey & Tyson, 2006). For example, soft ionization method advancements (electrospray ionization (ESI) & MALDI), have expanded applications for mass spectrometry in life sciences and should be introduced into AIA undergraduate curriculums. Given technological dynamics, continual AIA-MPIC content review adjusts for new analytical applications within a scientific discipline. This becomes another source for generation of student heterogeneous prior knowledge.

Evaluating sources of prior knowledge heterogeneity becomes a "course" resource for future content revisions and prerequisite realignment considerations, essential for optimizing student learning outcomes. As Fitzgerald and Lentmaier (2016) stated, "Prior knowledge is critical to students' success" (9:e Proceedings, p. 1). New concepts are more easily learned when students are equipped with a strong prior knowledge foundation, and students lacking knowledge necessary to understand new concepts may lose motivation (Oliver, 2006).

Have full-time instructional faculty had adequate teaching reflection opportunity to address knowledge gaps? Given heavier institutional teaching requirements and/or research commitments, are resources to mitigate heterogenous prior knowledge of a modified AIA classroom practical? Our reflections suggest practical steps for facility to enhance learning outcomes in an AIA-MPIC classroom.

Interdepartmental collaboration between faculty should facilitate basic scientific competencies are being achieved. Glaze (2018) suggested faculty at all levels should support achievement of higher levels of scientific literacy, and "achieving those higher levels should be our goal for instruction" (p. 2). An initial step includes periodic interdepartmental coordinated pretesting to support achieving higher levels of scientific literacy. Additionally, AIA-MIPC curriculum and its teaching activities within a heterogenous prior knowledge classroom are served by applying a developmental approach (Bettinger & Long, 2005), which requires understanding student areas of strength and weakness.

For example, an area of weakness observed was math-based problem-solving skills, lending support to our inference of inadequate teaching reinforcement of previously learned skills. Our course curriculum for AIA-MPIC included: (1) applying unit conversions; (2) dimensional analysis in chemical measurements; and (3) calculations of solution concentration. We added the skill of algebraic equation rearrangements. Through assessment and problem-solving activities, we identified areas where math knowledge gaps, and possibly deficiencies, were present. We found 50% of our students had some difficulty combining and rearranging the equations $v=c/\lambda$ and $E=h\upsilon$ so that $\lambda$ was the subject of the combined equations. Freeman, et al. (2014), reported that utilizing active learning measurably improves math skills, and we routinely had students demonstrate the utility of math applications by facilitating group discussions.

## DECISION

The unexpected finding from our AIA–MPIC was the degree of heterogeneous prior knowledge we observed in students. We suggest two key factors have manifested this situation. First is the desire of teaching universities to increase the breadth of specialized degree programs, supporting student future graduate school goals as well as marketplace needs. As a result, the traditional AIA course was recruited to provide instruction in modern instrumentation analysis to an increasing number of students pursuing specialized scientific degrees. In the past, an AIA course predominantly enrolling chemistry majors. Now, an AIA course multitasks serving multiple degree programs, incorporating increasing amount of content and students with heterogeneous scientific proficiencies.

Second, this has created challenges for faculty and program directors to incorporate new technologies into AIA lecture and laboratory curriculums. Science faculties must establish a balance between appropriate prerequisites to enroll a student in an AIA course, and the level of technical sophistication to adequately understand its concepts. Students may not possess or have limited prior knowledge skills in physics and chemistry to ensure optimal learning outcomes. Moreover, instructional faculty with heavy teaching assignments and resource limitations are challenged to adequately develop active learning activities, addressing the needs of a heterogenous prior knowledge classroom.

## REFLECTIVE CRITIQUE

We used the general framework of reflection outlined by Hatton and Smith (1995), identified as technical, descriptive, dialogic, and critical reflections. While this critique is organized into four categories, overlap likely exists given the complexity of teaching practices, the type of learning activity and subsequently reflecting upon effectiveness.

## TECHNICAL REFLECTIONS

Our instructional skills and effectiveness of introducing new activities and demonstrations are presented as technical reflections. The main goal of our technical reflections was to focus on the immediate needs of the students to understand the concepts presented. We routinely conducted instructor/instructional assistant debriefings after each lecture, assessing our impressions by comparing the level of student engagement from previous lectures. Did more students participate and ask questions beyond our usually high engagement students? How could each activity or exercise be improved to address heterogenous prior knowledge issues?

Our group learning enabled students with heterogeneous prior knowledge to more fully engage in discussion. Fitzgerald and Lentmaier (2016) suggested, “One method to mitigate the problem of heterogeneous prior knowledge among students is to use group-based teaching and learning activities, with a format aimed at helping students to complement their prior knowledge” (9:e Proceedings, p. 2). And, while direct instruction by lecture continued to occupy a role in our AIA-MPIC, activities presented here were introduced throughout the entire course, and during most lecture periods. Current research supports active learning as a way to engage students (Talbot, 2014; Yarnall, Toyama, Gong, Ayers, & Ostrander, 2007). Throughout the course activities, we constantly reviewed areas of strengths and weakness in learning and worked to structure these areas into our discussions with confirmation of learning through quizzes and examination.

Several exercises purposefully involved students selecting parameters that were gathered during classroom lectures; for example, lists of proteins studied in a previous course or obtaining resistance measurements using a digital voltmeter in order to create original data sets to be used to launch an exercise. Each exercise was authentic with outcomes which were designed not to be a fixed result. Tables 1 and 3 were generated in this fashion, allowing the class to participate various levels of inquiry. For example, we problem-solved collectively if database queries yielded unexpected results, discussing possibilities for graphically representing data sets.

We assessed conceptual understanding through quizzes and exams, using multiple-choice, essay, calculation-based questions, and problems relating to our activities. We asked ourselves: were students able to perform critical-thinking and problem-solving skills reinforced during our activities, demonstrations, and formal lectures? In general, we continually assessed student ability to perform mathematical operations and apply dimensional analysis skills.

## DESCRIPTIVE REFLECTIONS

Descriptive reflections critiqued our subject knowledge and classroom interpersonal relationships. Kane, Sandretto, and Heath (2004) described the importance of a research-teaching nexus in tertiary (post-secondary) in their reflective practice model, where teaching reflection is the hub with spokes including subject knowledge, skills, interpersonal relationships, and personality.

Given the instructor’s 25 years as an industrial biotechnology practitioner, aspects of the AIA course curricula were linked to industrial method and product development with applications in mass spectrometry (Pingerelli et al., 2009). The GIA also brought her expertise as a recent graduate in chemistry and undergraduate research work, contributing to classroom discussions As Brownell and Tanner (2012) propose, introducing the concept of a scientist’s professional identity is important to our teaching responsibilities, engaging students, and reflecting on work in the context of our discipline and status among professional colleagues. Results from the end-of-course survey, using a Likert scale, strongly agreed (4.9/5.0) that the instructor and GIA effectively related expertise in the subject matter area.

Kane et al. (2004), pointed out that, "Tertiary teaching does not take place in a vacuum but occurs within a relationship between the teacher and the students" (p. 295). The interpersonal classroom relationship between the instructor and GIA was enhanced through strategic questioning of the class. When students did not respond to instructor questions, the instructor sought input from his GIA. For instance, the instructor asked students to describe the chromatography Van Deemter equation. Lacking student volunteers, the GIA shared experiences to stimulate student engagement, continuing the class discussion. When asked on the end-of-course survey if the instructor was engaged in discussion in a helpful and meaningful way, the Likert score was 5.0/5.0 (strongly agreed).

As detailed in our Multidisciplinary Student Engagement activity, our reflective practice included continual review of nomenclature during group activities such as, Variations on a Molecular Theme. These problem-solving sessions created a "brainstorming" atmosphere, engaging the entire classroom community to build relationships with each other as we worked through a problem. Too often in higher education passive learning is utilized during lecture, limiting faculty's ability to uncover heterogeneous knowledge gaps and build a stronger connection with students. We advocate undergraduate faculty increasingly engage in active learning approaches that have been long advocated in K-12 education (Faust & Paulson, 1998).

## DIALOGIC REFLECTIONS

As described by Hatton and Smith (1995), dialogic reflection is hearing one's voice exploring alternative ways to solve problems in a professional situation. A key consideration and challenge of this AIA-MPIC was having students with different levels of mathematical skills; for example, statistics, algebraic manipulations, scientific notation, and proper use of significant figures. When discussing instrumental noise, we discovered many students had difficulty in expressing $n/\sqrt{n}$ using fractional powers. Hence, we took opportunities to conduct impromptu math reviews and included these reviewed skills in future quiz assessments as well as practice exercises in take-home handouts.

Supplemental handouts were created and routinely used to help students with heterogenous prior knowledge and knowledge gaps. For mass spectrometry, we derived the kinetic energy equation using a non-calculus-based method, but also covered the calculus-based derivation for more advanced students. We stipulated students only needed to know the results and how kinetic energy was important for discrimination of charged ions in a mass analyzer. We discovered students utilized our supplemental handouts as study guides for both quizzes and exams.

## CRITICAL REFLECTIONS

Hatton and Smith (1995) defined critical reflections as, "Thinking about the effects upon others of one's actions, taking account of social, political, and/or cultural forces" (p. 45).

Throughout the course, theories and equations developed by prominent scientists were discussed, including those of Boltzmann, De Broglie, Einstein, Heisenberg, Newton, and Planck, and how their ideas were important to the development of modern instrumentation. As mentioned earlier, we discovered students had limited historical background when asked to name an important scientist on their pretest formative assessment. Early in the semester, the instructor asked students to stand and remain standing if they could answer questions about a modern cultural icon. Typically, 100% percent of the class remained standing. When asked who Dr. Jonas Salk was, the entire class seated itself. Reflecting on these experiences, we routinely incorporated short biographies of prominent scientists into the course curriculum.

We purposefully including brief vignettes on the history of science in areas which were relevant to our course content. When introducing a prominent scientist, the instructor invited the scientist to metaphorically join the class for the remainder of the course. Asking rhetorical questions such as: how would Newton approach this question?

The GIA worked with an instructor who

is quadriplegic. The GIA facilitated slide presentations, hand-out of materials, and assisting the instructor with illustration using the whiteboard. Both the GIA and instructor did not know each other prior to the course. Effective communication was key to good classroom management, and when the instructor asked for a graphic or a representation to be drawn on the whiteboard, students witnessed in real-time the importance of effective communication. Initially, this process did not go as smoothly, but over time, we believe, students recognized how collegial working relationships are developed. One of the participants from the Kane et al. (2004), study explained that good tertiary teaching "exhibit much of their own personality" (p. 298). Both the instructor and GIA reflected their enthusiasm and shared their professional and academic experiences throughout the course. The instructor learned through years of instruction that students, particularly scientists, often are besieged with curiosity regarding one's particular circumstances. This creates engagement and the instructor shared his educational experiences through a short story entitled, "A different place to learn" (Pingerelli, 2016).

Future Directions and Activities for Analytical Instrumental Analysis

A more detailed approach should be given to our pretesting assessment to reveal additional areas of heterogeneous personal knowledge and knowledge gaps and organizing its findings by mapping them to the appropriate active learning activities.

Future activities include equipping an Arduino with a simple circuitry interface which includes an infrared Light Emitting Diode (LED) to measure heart rate, demonstrating data domain transfer of a physiological response into an analog signal and conversion into an interpretable digital value (Arduino, 2018).

An interesting post-lecturer presentation activity might include having students use their online classroom forum to briefly report on their independent survey of Arduino Internet resources, and describing an instrument they would be interested in prototyping using the Arduino platform.

During lecture presentations of chromatographic methodologies such as High Performance Liquid Chromatography (HPLC), a list of molecules could be presented to students to complete an outside assignment. Students would need to research how best to separate a mixture of molecules contained in the list, determining column type (normal or reverse phase), a preferred mobile phase and other method parameters, requiring undergraduates to develop systematic literature search criteria in order to identify useful research reports. A demonstration of online HPLC simulators to develop separation method would be presented (Run HPLC Simulator, 2018).

Beyond the use of strategic questioning, other opportunities to encourage engagement and make informal real-time formative assessments of student prior knowledge would be beneficial. We used the activity Variations on Molecular Themes for the first third of the semester to introduce molecules of interest, different classes of biological molecules (steroids, neurotransmitters, toxins), molecules used for analytical applications, molecules which impact the environment and human health. In the future, students would select molecules and give a two- to three- minute presentation on its chemical properties, applications, social controversies or environmental considerations associated with the molecule and detection using analytical instrumental analysis.

# References

Arduino. (2018). What is Arduino? Retrieved from https://www.arduino.cc/

Batzer, M., Stoneking, M., Alegria-Hartman, M., Bazan, H., Kass, D., Shaikh, T., . . . & Herrera, R. (1994). African origin of human-specific polymorphic Alu insertions. Proceedings of the National Academy of Sciences, 91(25), 12288–12292.

Ben Kei, D. (2018). Reimaging research methodology as data science. Big Data and Cognitive Computing, 2(1), 4. doi:10.3390/bdcc2010004

Bettinger, E. P., & Long, B. T. (2005). Addressing the needs of under-prepared students in higher education: Does college remediation work? [PDF] Retrieved from http://www.nber.org/papers/w11325.pdf

Boeckmann, B., Bairoch, A., Apweiler, R., Blatter, M. C., Estreicher, A., Gasteiger, E., . . . Schneider, M. (2003). The SWISS-PROT protein knowledgebase and its supplement TrEMBL in 2003. Nucleic Acids Research, 31, 365–370.

Brenton, A. G., & Godfrey, A. R. (2010). Accurate mass measurement: Terminology and treatment of data. Journal of the American Society Mass Spectrometry, 21, 1821–1835.

Brownell, S. E., & Tanner, K. D. (2012). Barriers to faculty pedagogical change: Lack of training, time, incentives and . . .tensions with professional identity. CBE Life Sciences Education, 11, 339–346.

Dragan, A. I., Pavlovic, R., McGivney, J. B., Casas-Finet, J. R., Bishop, E. S., Strouse, R., . . . & Geddes, C. D. (2012). SYBR Green I: Fluorescence properties and interaction with DNA. Journal of Fluorescence, 22(4), 1189–1199. doi:10.1007/s10895-012-1059-8

Fahey, A., & Tyson, J. (2006). Instrumental analysis in the undergraduate curriculum. Analytical Chemistry, 78(13), 4249–4254. doi:10.1021/ac069421a

Faust, J. L., & Paulson, D. R. (1998). Active learning in the college classroom. Journal on Excellence in College Teaching, 9(2), 3–24.

Freeman, S., Eddy, S. L., McDonough, M., Smith, M. K., Okoroafor, N., Jordt, H., & Wenderoth, M. P. (2014). Active learning increases student performance in science, engineering, and mathematics. Proceedings of the National Academy of Sciences USA, 111, 8410–8415.

Fitzgerald, E., & Lentmaier, M. (2016, December 15). Strategies for teaching students with heterogeneous prior knowledge. LTHs 9:e Pedagogiska Inspirationskonferens. Retrieved from https://www.lth.se/genombrottet/lths-pedagogiska-inspirationskonferens/

Gasteiger, E., Hoogland, C., Gattiker, A., Duvaud, S., Wilkins, M. R., Appel R. D., & Bairoch, A. (2005). Protein identification and analysis tools on the ExPASy server. In J. M. Walker (Ed.), The proteomics protocols handbook (pp. 571–607), Totowa, NJ: Humana Press.

Glaze, A. L. (2018). Teaching and learning science in the 21st century: Challenging critical assumptions in post-secondary science. Education Sciences, 8(1), 12. Retrieved from https://www.mdpi.com/2227-7102/8/1/12

Harris, D. C. (2016). Quantitative chemical analysis (9th ed.). New York, NY: W.H. Freeman and Co.

Hatton, N., & Smith, D. (1995). Reflection in teacher education: Towards definition and implementation. Teaching & Teacher Education, 11(1), 33–49.

Kane, R., Sandretto, S., & Heath, C. (2004). An investigation into excellent tertiary teaching: Emphasising reflective practice. Higher Education, 47, 283–310.

Koenig, T., Menze, B. H., Kirchner, M., Monigatti, F., Parker, K. C., Patterson, T., . . . & Steen, H. (2008). Robust prediction of the MASCOT score for an improved quality assessment in mass spectrometric proteomics. Journal of Proteome Research, 7(9), 3708–3717. doi:10.1021/pr700859x

Linstrom, P. J., & Mallard, W. G. (2001). The NIST chemistry webbook: A chemical data resource on the Internet. Journal of Chemical & Engineering Data, 46(5), 1059–1063. doi:10.1021/je000236i.

Lazarowitz, R., & Lieb, C. (2006). Formative assessment pre-test to identify college student prior knowledge, misconceptions and learning difficulties in biology. International Journal of Science and Mathematical Education, 4, 741–762.

Oliver, R. (2006). Exploring a technology-facilitated solution to cater for advanced students in large undergraduate classes. Journal of Computer Assisted Learning, 22(1), 1–12.

Pingerelli, P. L. (2016). A different place to learn. Pentimento, 6, 13–15.

Pingerelli, P. L., Ozols, V. V., Saleem, H., Anderson, C., & Burns, R. S. (2009). The calcium-modulated structures of calmodulin and S100b are useful to monitor hydrogen/deuterium exchange efficiency using matrix-assisted laser desorption ionization time-of-flight mass spectrometry. European Journal Mass Spectrometry, 15, 739–746.

Rose, M. (2009). Why school? Reclaiming education for all of us. New York, NY: The New Press.

Run HPLC Simulator. (2018). HPLC Simulator. Retrieved from http://www.hplcsimulator.org/simulator.php

Schafer, A., & Bruck, R. (2013). Teaching strategies for undergraduate laboratories with students having heterogeneous prior knowledge. IEEE Global Engineering Education Conference (EDUCON), pp. 112–117. doi:10.1109/EduCon.2013.6530094

Swinehart, D. F. (1962). The Beer-Lambert Law. Journal of Chemical Education, 39(7), 333. doi:10.1021/ed039p333

Talbot, B. (2014). Beyond active learning in undergraduate science. Retrieved from https://medium.com/@Bud_T/beyond-active-learning-in-rule science-60e41ef7a32b

The UniProt Consortium. (2017). UniProt: The universal protein knowledgebase. Nucleic Acids Research, 45, D158–D169.

Vaughn, B. C. (2010). Bisphenol A and phthalates: Uses, health effects and environmental risks. Hauppauge, NY: Nova Science Publishers.

Wright, L. E. (2004). Reform in undergraduate science teaching: university and federal perspective, pp. 12Y17. International Conference on Learning and Assessment in Science, Engineering & Management in Higher Education. In O. Herscovitz (Ed.) Conference proceedings, Technion City, Haifa, Israel, December 2004.

Yarnall, L., Toyama, Y., Gong, B., Ayers, C., & Ostrander, J. (2007). Adapting scenario-based curriculum materials to community college technical courses. Community College Journal of Research and Practice, 31, 583–601.

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# Appendix A (via request to authors).

1. Bovine Serum Albumin Excel Data File
2. Calmodulin Excel Data File
3. Unknown Protein Excel Data Files
4. Arduino Thermistor Software Sketch