# 3-D Printing and Arduino in the Chemical Engineering Classroom: Protein Structures, Heat Exchangers, and Flow Cells

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## Abstract

Unlike other engineering disciplines, chemical engineering (ChE) students rarely get the opportunity to design and build models, devices, and prototypes in the classroom. ChE students are also less likely to obtain valuable skills like computer-aided drafting (e.g., AutoCAD or SolidWorks), programming (e.g., Arduino), and circuitry. These are undeniably useful skills, but it is often challenging to find practical ways to incorporate them into ChE courses that are already dense with material. This paper describes 3 different modules that simultaneously teach core ChE & BioChE concepts while also introducing the concepts of 3D printing, drafting, and programing/circuitry with Arduino. The overall goal is to use these interdisciplinary techniques to enhance the students' understanding of ChE concepts.

## Module 1:  3D-Printed Amino Acid Building Blocks to Teach Protein Structure

This first module uses 3D-printed alpha carbon atoms ($C_\alpha$) and peptide bond groups (CONH) to show students how amino acids assemble into peptides and form complex structures simply by rotating the bonds around the alpha carbons. Students can use the models to prepare their own Ramachandran plots or build secondary structures (e.g., alpha helices and beta sheets). No drafting or coding experience is required for this module, but a 3D printer is needed to print the parts.

## Module 2:  3D-Printed Plate & Frame Heat Exchangers

This module allows students to design, build, and test their own plate & frame heat exchanger. The plates for the heat exchanger can be easily drafted in SolidWorks and modified to include corrugation or other geometries that increase turbulence and heat transfer. The 3D printed plates can then be assembled and used for heat transfer experiments in which the students estimate heat transfer coefficients. Coding can also be included by using an Arduino to monitor inlet & outlet temperatures with thermocouples.

## Module 3:  An Arduino-Controlled 3D-Printed Flow Cell to Measure Enzyme Kinetics

In this module, students design and build their own rudimentary spectrophotometer, which consists of an LED light source, photoresistor, cuvette, and an Arduino. The Arduino powers the LED and uses the photoresistor to measure how much light passes through the sample in the flow cell. This flow cell can be used to monitor chromogenic chemical reactions, including the reaction of beta galactosidase with ONPG, which produces a yellow product that absorbs light. Varying the concentration of ONPG allows students to collect data for Michaelis-Menten plots. **Note: Materials for each module are available from the primary author upon request.**

## Introduction

### *Motivation: Expanding the Skill Set of Chemical Engineering Students*

Traditional chemical engineering courses focus mainly on molecular phenomena (e.g., chemistry, molecular biology, reaction kinetics, etc.). These courses are excellent preparation for most chemical engineering jobs, but they typically lack some practical skills that are frequently taught in other engineering disciplines. For example, while mechanical, civil, electrical, and computer engineering students are taught how to use computer-aided drafting software (e.g., AutoCad or SolidWorks), most ChE students do not encounter these programs. Similarly, most ChE students will never have a chance to use a 3D printer, solder a circuit, or program a microcontroller (e.g., Arduino). One consequence of this knowledge gap is that many ChE's may not truly understand how a vital piece of equipment works and, when that instrument fails, they are not able to troubleshoot or repair it. In addition, ChE's are usually forced to rely on existing commercially available devices, which are expensive and may not even meet their specific needs. However, giving ChE students an opportunity to design, build, and test an electronic device could help them to better comprehend analytical equipment and may even enable them to design their own novel devices that are customized improve a chemical process.

All that being said, there is simply not enough time available in most ChE curricula to include rigorous training on circuits, programming, and fabrication. Instead, the goal of this work is to design small modules that can be incorporated into existing ChE courses and will simultaneously give students a brief introduction to practical skills like 3D printing while also introducing or reinforcing core ChE concepts. Specifically, this paper describes three different modules that expose students to 3D printing, computer-aided drafting (CAD), circuitry, and/or programming (see Table 1), which vary in complexity. In the simplest module, customized atomic building blocks are 3D printed and students use them to assemble peptides that they can play with during class to help them understand protein structure. The second module requires students to use drafting software (e.g., SolidWorks) to design and 3D print plates for a plate and frame heat exchanger that can then be tested. The third module challenges students to construct and program their own simple spectrophotometer, which is used to measure enzyme kinetics. If these modules pique the student's interest, they can then take more detailed mechanical, electrical, or computer engineering elective courses. However, at the very least, exposure to these concepts could help ChE students to better understand and collaborate with other engineers later in their careers.

| Modules | 3D Printing | Drafting/CAD | Circuitry | Programming | Description |
|---|---|---|---|---|---|
| 1: Protein Building Blocks | X | | | | Assemble custom atomic building blocks into proteins |
| 2: Heat Exchanger | X | X | O | O | Design plates, then build and test real heat exchanger |
| 3: DIY Spectrophotometer | X | X | X | X | Design, wire, and program a functional spectrophotometer |

**Table 1** – Overview of Modules

*Examples of Other 3D Printed Devices*

        Intuitive and disruptive technologies like Arduino, Raspberry Pi, and 3D printing (i.e., additive manufacturing) have made prototyping and device development easier than ever. Indeed, many scientists have already taken advantage of these technologies to design their own lab equipment (see Table 2 for examples), including small centrifuges, syringe pumps, pipettes, thermal cyclers, microscopes that can save images on a smartphone, and many more [1,2]. Specialized devices for chemical engineering applications have also been developed, including microfluidic devices (complete with valves, pumps, and mixers) [3–5], structured monolithic catalysts [6], reactors with complex geometries to enhance chemical synthesis [7,8], and chromatography columns. Aside from their lower cost, the main benefit of these devices is that they can be specifically designed for unique applications in the lab. 3D printing also allows the user to create complex structures and shapes that cannot be fabricated by other traditional methods.

| Labware | Reference(s) |
|---|---|
| Syringe pump | [1] |
| Centrifuge | |
| Pipettes | [2] |
| Thermal Cycler | |
| Microscope | |
| Microfluidic Devices | [3–5] |
| Catalysts | [6] |
| Chemical Reactors | [7,8] |
| Chromatography Columns | [9] |
| Turbidimeter | [10] |
| Spectrometer | [11] |

**Table 2** – Examples of 3D Printed Labware

*A Generic Protocol for 3D Printing and Programming your own Devices*

        In addition to being relatively inexpensive, the devices shown in Table 2 are also relatively easy to download/design, print, and assemble. A detailed description of the design, fabrication, and assembly of the DIY spectrophotometer used in Module 3 is available upon request from the instructor, but a general overview of the steps (along with a few important and helpful tips) required to 3D print a prototype are listed below for first time users:

1. **Generate or download STL (Standard Tessellation Language) files, which describe the dimensions of the object you would like to 3D print**
   a. Option 1: Find STL files on internet databases like STL Finder, GrabCAD, or Thingiverse (all of the parts described in this paper are archived on Thingiverse)
   b. Option 2: Generate your own models in SolidWorks, Autodesk Inventor, Google Sketchup, or another CAD program
   c. Note: When creating STL files, keep in mind that some printers tend to print objects slightly larger and holes slightly smaller than intended. Consequently, if you are printing pieces that fit together, you may need to do some test prints first.

2. **Convert the STL file into G-Code, a numerical control programming language that tells the 3D printer how to print an object, using Cura, Slic3r, or another program**
   a. To prepare G-Code, you will need to input specific parameters for the type of filament (e.g., PLA, PMMA) and printer (e.g., Lulzbot, Object, etc.) that you are using. For example: filament diameter (ex: 2.85 mm), extruder head temperature (ex: 210°C for PLA) and nozzle diameter (ex: 0.5 mm), bed temp (ex: 60°C).
   b. You will also need to specify variables associated with the way that the filament is laid down, including print speed (ex: 50 mm/s), the fill density (ratio of void to filament inside object, e.g., 20%), and the thickness of each printed layer (ex: 1 mm for first layer and 0.25 mm for subsequent layers).
   c. Keep in mind that the printer will create your object from the ground up by laying down consecutive layers of material. Consequently, it is best to avoid any vertical gaps or voids. For example, it would be best to print a cup right-side-up instead of upside down. However, if voids are unavoidable, most programs include an option to print the object with a "filler" material that temporarily fills the void during the print and can be removed after the object is complete.
3. **Transfer the G-Code to your 3D printer and print!**
   a. Refer to the manufacturer's instructions for this step, but here are some tips:
   b. Make sure that the extruder head and bed are clean before starting the print.
   c. Check to see if you have enough filament to complete the print. Some printers use a balance to monitor the amount of filament, but this can be done manually.
   d. Note: 3D printing takes a long time! However, print times can be shortened by changing the orientation of the part or decreasing the fill density.
   e. Note: Use a backup power supply to prevent failures due to power outages.
4. **Construct a Circuit**
   a. Getting started: Some excellent introductory videos on circuits and electronic components can be found on YouTube or at Khan Academy:
      i. https://www.khanacademy.org/science/physics/circuits-topic
   b. In general, it is always best to design a printed circuit board (PCB, available from several different companies) and then solder components to it.
   c. A quick and easy (but less stable/reliable) alternative is a breadboard, which allows the user to plug wires into ports and remove them later, if necessary.
   d. Either way, the circuit can be powered by the Arduino via 3.3V/5.0V and GND ports. Sensors can be connected to the analog (A0-A5) or digital ports.
5. **Programming an Arduino**
   a. Start by writing your own code or downloading existing open source code from one of several websites (e.g., Arduino Playground) or online forums/threads
   b. Connect the Arduino to your computer, then select the board (e.g., Tools → Board → Arduino Uno) and port (e.g., Tools → Port → Com1) that you are using.
   c. Download the Arduino IDE, then copy and paste your code into the window. Verify the code (checkmark button), then upload (right arrow button) it to the Arduino. View the data generated by the Arduino by opening the Serial Monitor.

## Module 1:  3D Printed Models of Molecules, Polymers, and Proteins

*Preface*
      This first module describes the preparation of molecular building blocks that students can assemble in class to assemble proteins and play with them to form different secondary structures. It is relatively simple and only requires a 3D printer.  The instructor may choose to tailor the parts for their specific lessons in SolidWorks or another drafting program, but no circuitry or programming is required.  All the instructor needs to do is download the STL files (https://www.thingiverse.com/thing:2758246 or https://www.thingiverse.com/thing:2509708) and generate the G-Code for their specific 3D printer using Cura (as described above).  A word of warning, however - it may take several days and $100-200 of filament to print enough parts for a class of around 30 students.

*Overview*
      A classic challenge in chemistry and biology is that molecules are three dimensional, but the chalkboards and whiteboards in the classroom are only two dimensional.  Many different commercial kits with small balls (atoms) and sticks (bonds) have been designed to give students a 3D representation of molecules, but they lack important characteristics that are specific to proteins. The goal of this module was to design a kit of parts that represented the unique dimensions, bonds, and flexibility of a protein.  However, it is worth noting that this concept could also be applied to other polymers (e.g., DNA, PEG, etc.).

      While modeling a protein that contains hundreds of different amino acids may seem overwhelming, the truth of the matter is that most of the bond lengths (e.g., C-C = 1.51A, C-N = 1.33A, etc.) and bond angles (e.g., O=C-N = 123.5°, C-N-C = 122°, etc.) within a protein are fixed. In addition, the carbonyls (C=O) in the protein backbone also keep the peptide bond planar. Consequently, an entire peptide backbone can be built with only 4 unique parts (see Figure 1):  a tetrahedral amine ($NH_2$) for the N-terminus, tetrahedral alpha carbons ($C_\alpha H_2$) and planar peptide bonds (O=C-N-H) for the backbone, and a carboxyl group (COOH) for the C-terminus.  The 20 amino acid side chains can also be included, but they require additional parts (e.g., hydroxyls, primary amines, sulfhydryls, and unique cyclic groups for proline, histidine, phenylalanine, tyrosine, and tryptophan).  Nonetheless, the simplicity and modular nature of the peptide backbone allows it to be easily modeled using simple 3D printed parts.

      While most of the bond lengths and angles within a protein are fixed, there are two dihedral angles ($\phi$ and $\psi$) around the alpha carbon of each amino acid side chain that are free to rotate (Figure 2).  However, it is important to note that the range of feasible dihedral angle values is significantly limited by steric hindrance between atoms in the peptide backbone and the amino acid side chains.  For example, $\psi = \phi = 0°$ creates a steric clash between the carbonyl oxygen and the hydrogen attached to the secondary amine.  In contrast, some dihedral angles correspond to unique secondary structures like $\alpha$-helices (e.g., $\psi = \phi = -50°$ to $-60°$) that contain numerous hydrogen bonds.  This phenomenon is illustrated by the Ramachandran plot shown in Figure 2, in which each dot represents the dihedral angle combination of a specific residue in a protein.  Except

for glycine, which is highly flexible due to its lack of a side chain, the Ramachandran plot shows that most dihedral angles occur within one of three regions that correspond to specific secondary structures: β-strands/sheets ($\psi$ = -60° to -180°, $\phi$ = 60-180°), right handed $\alpha_R$-helices ($\psi$ = -30° to -90°, $\phi$ = -30° to -90°), and another for left handed $\alpha_L$-helices ($\psi$ = 30-90°, $\phi$ = 30-90°). However, it is important to note that amino acids in unstructured loops also fall within these three regions, since these dihedral angle combinations represent conformations with relatively lower steric hindrance.
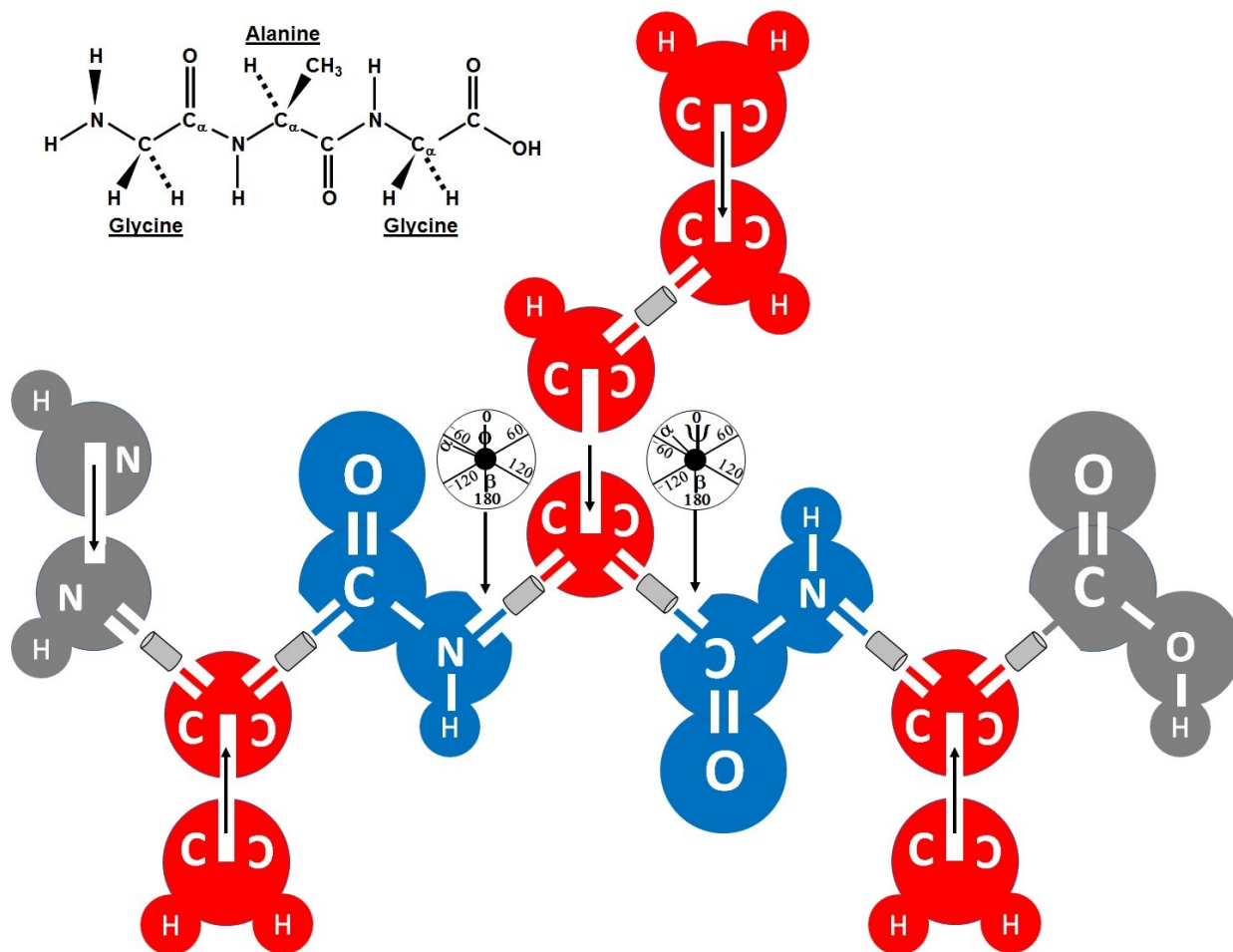


**Figure 1** – Assembly of a tripeptide (Gly-Ala-Gly) from 3D printed parts. Hollow rectangular slots in the tetrahedral atoms (e.g., $NH_2$, $C_\alpha$, & $C_\beta$) allow them to slide together to form a 3D structure, while the backbone pieces and the carbons of the alanine are connected with 1/8" ID 1/4" OD flexible plastic tubing that allows them to rotate. The $\phi$ and $\psi$ angle dials fit around the plastic tubing and have slots that allow them to be aligned/fixed to the carbon & nitrogen atoms.
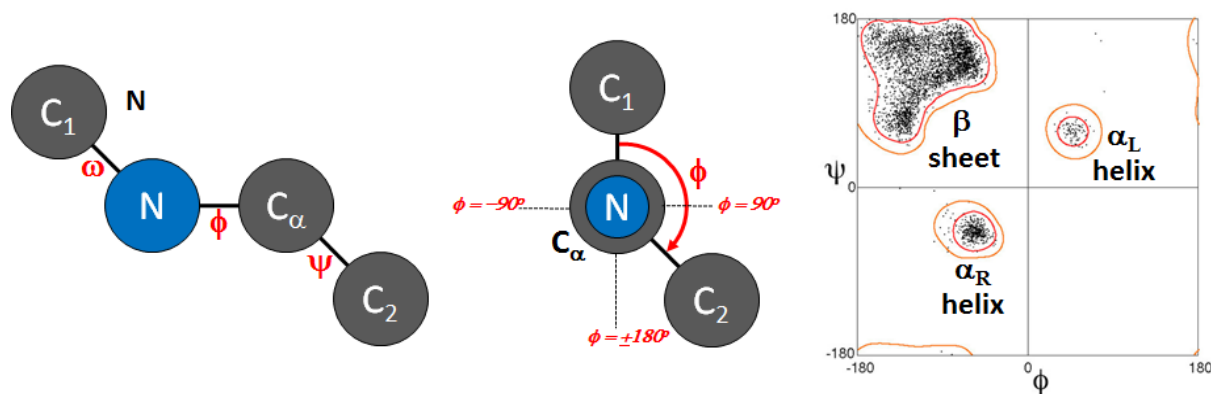
**Figure 2** – Dihedral angles in protein backbones. There are 3 types of dihedral angles in the peptide backbone ($\psi$, $\phi$, & $\omega$), but $\omega$ is fixed at 180° due to the planarity of the peptide bond (left pane). In contrast, $\phi$ and $\psi$ can rotate from 0 to +/- 180° (middle pane), but steric hindrance induced by amino acid side chains restricts most $\phi$ and $\psi$ angles to the regions shown in the Ramachandran plot, which also indicates the dihedral angles found in the common 2' structures of alpha helices and beta sheets (right plot).

### *Implementation in the Classroom*

If you are scratching your head at this point, you are not alone. Even with the description above and the 2D visual aids in Figure 2, dihedral angles are still a difficult concept to grasp for students and faculty alike. To help students develop an intuitive sense for the way that dihedral angles rotate and can be adjusted to create secondary structures, several sets containing pieces shown in Figure 1 were printed on a Lulzbot Taz6 3D printer using 2.85 mm PLA filament. This tripeptide (Glycine-Alanine-Glycine) consists of 27 different atoms ($C_7O_4N_3H_{13}$), but fusing the fixed, rigid, and planar atoms together into single pieces (e.g., the planar peptide) simplifies assembly and only requires 7 distinct pieces. Dials that indicate the dihedral angles can also be included in the model to allow students to easily adjust the protein structure and observe the effects of different dihedral angle values.

The kits were implemented in two separate courses: a ChE elective (BioEngineering Lab Techniques) and a course in the Chemistry department (Biochemistry I). In both cases, the models were used in an introductory lesson that focused on dihedral angles and their relationship to secondary structures ($\alpha$-helices and $\beta$-strands). Students were given the individual pieces and asked to construct the tripeptide using the diagram shown in Figure 1. The instructor then guided the students through a few combinations of dihedral angles, some of which created steric clashes and others which created hydrogen bonds. Those observations were then used to create a Ramachandran plot from scratch, which highly resembled the experimental plot shown in Figure 2. A short video demonstrating the use of the model to investigate different dihedral angles is also available online (https://www.youtube.com/watch?v=1usemtIYe_s). It is worth mentioning that this video was viewed over 1,300 times in the first six months after it was posted.

*Student Feedback*

The results of a survey administered at the end of the class in which the models were used are shown in Figure 3. Overall, most students enjoyed using the models and agreed that they helped them to understand dihedral angles and the Ramachandran plot. Student comments on the surveys that scored the exercise relatively low (in the 2-4 range) indicated that the models were somewhat difficult to assemble and that the experience would be more rewarding if the students had more time to play with the models. Indeed, as an instructor, I observed that students did take a long time (~5-10 minutes) to properly assemble the models. While we had hoped that the assembly process would help students to see and remember the different bonds in the peptide backbone, I concede that more time could be spent on more advanced topics (e.g., secondary structure) or simply playing with the models if they are pre-assembled before class.
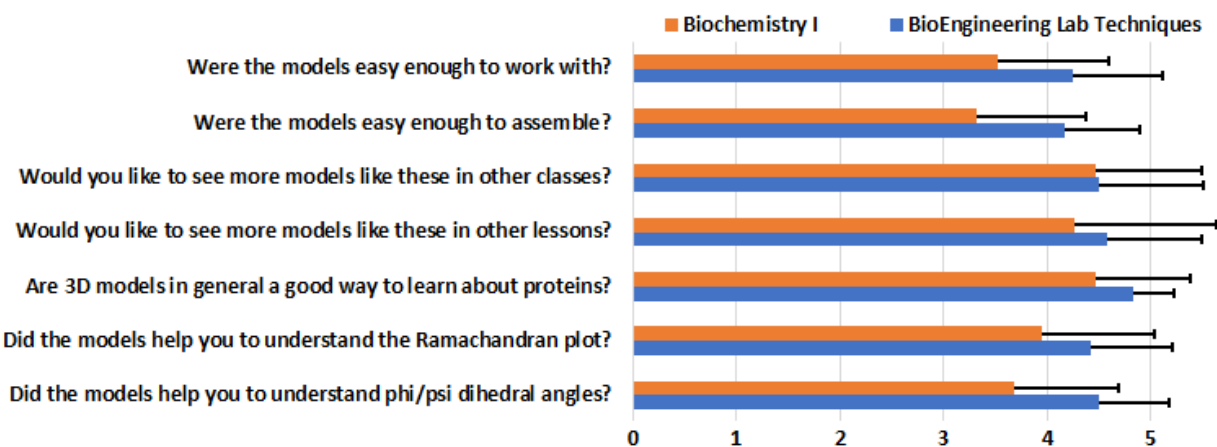


**Figure 3** – Results of student surveys administered after a lesson with the models. (Likert scale, 1 = Strongly Disagree/No, 2 = Disagree/No, 3 = Neutral, 4 = Somewhat Agree/Yes 5 = Strongly Agree/Yes)

## Module 2:  Plate and Frame Heat Exchanger

*Preface*

Like the molecular building blocks described in Module 1, the parts needed for this module can simply be downloaded (https://www.thingiverse.com/thing:2452341) and printed. However, the students can also be challenged to modify the plate designs (e.g., add corrugations to induce turbulence and increase heat transfer) in a drafting program like SolidWorks. Either way, the printed plates can be assembled and used to transfer heat between streams of hot ($\leq 40^{\circ}C$) and cold (~$4^{\circ}C$) water, but additional equipment is required (pumps, tubing, and thermocouples). In the most advanced iteration of this project, thermocouples can be attached to an Arduino that automatically records the stream temperatures.

*Overview*

While there are many different modes of heat transfer, including conduction, convection, and radiation, most chemical engineers are primarily concerned with the transfer between two

liquid streams via convection and conduction in some sort of heat exchanger. Many different types of heat exchangers exist, but the most common designs are double pipe, shell and tube (S&T), and plate and frame (P&F, see Figure 4) [12]. Double pipe heat exchangers are the simplest design, consisting of two concentric pipes, but they are also the least efficient. Shell and tube heat exchangers utilize several smaller tubes within a large shell to achieve higher heat transfer coefficients, but they can be expensive, difficult to clean, and relatively large. In contrast, P&F heat exchangers can provide similar heat transfer coefficients in a much smaller package due to their high surface area to volume ratio. Generally speaking, P&F heat exchangers consist of several plates which can be stacked together to form channels for fluid flow between the plates. Flat plates can be used to provide flat rectangular channels, but corrugating the plates simultaneously increases their total surface area and the overall rate of heat transfer between the streams. The plates are held together with a frame, which can be easily assembled or disassembled for cleaning. The simplicity of the plate design and ease of assembly makes P&F heat exchangers uniquely suited for 3D printing and testing in the classroom, as described in this module.
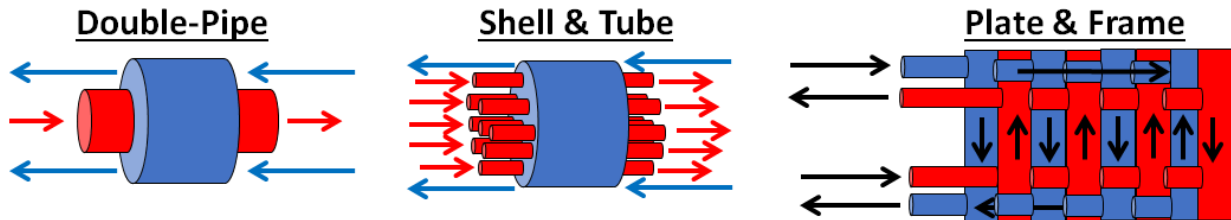


**Figure 4** – Common types of heat exchangers being operated in counter-current flow.

***Implementation in the Classroom***

Thus far, this project has only been implemented as an independent study/honors project for one student. Consequently, student feedback and learning outcome data are not available for this module. However, the sections below describe how the module was carried out and how it could potentially be introduced to an entire heat transfer course.

This module requires the design and/or fabrication of 3 types of plates (Figure 5) – a top plate with inlet/outlet ports for the hot/cold streams, middle plates with rectangular channels for alternating hot and cold streams, and a bottom plate that caps the flow. Each plate also has holes for bolts that fasten the plates together (with a gasket or glue between each plate to prevent leaks). We observed that 5 total plates (providing 2 channels for the hot stream and 2 channels for the cold stream) is sufficient to obtain significant heat transfer (see Figure 5), but heat transfer can be easily increased by printing additional pairs of middle plates. It is worth mentioning that the plastics used for 3D printing have much lower thermal conductivities than the metals typically used for heat exchangers (e.g., k = 0.13 W/mK for PLA, k = 0.24 W/mK for ABS and PET, k = 400 W/mK for copper), but the large amount of surface area within the exchanger adequately compensates for the relatively low rates of conduction heat transfer. Custom 3D printed metallic parts can also be ordered online from a variety of sources, if desired.

Once the heat exchanger is assembled, peristaltic pumps can be used to pump ice water through the cold side and warm water ($\leq 40°C$ for plates printed with PMMA) through the hot side. The inlet and outlet temperatures can then be monitored in real time with a commercial thermocouple (e.g., Amprobe TMD-56 multi-logging digital thermometer). Alternatively, if the instructor wishes to challenge the students to do some programming and complete a simple circuit, several examples of simple thermocouple and thermistor circuits controlled by an Arduino are available online [13,14].
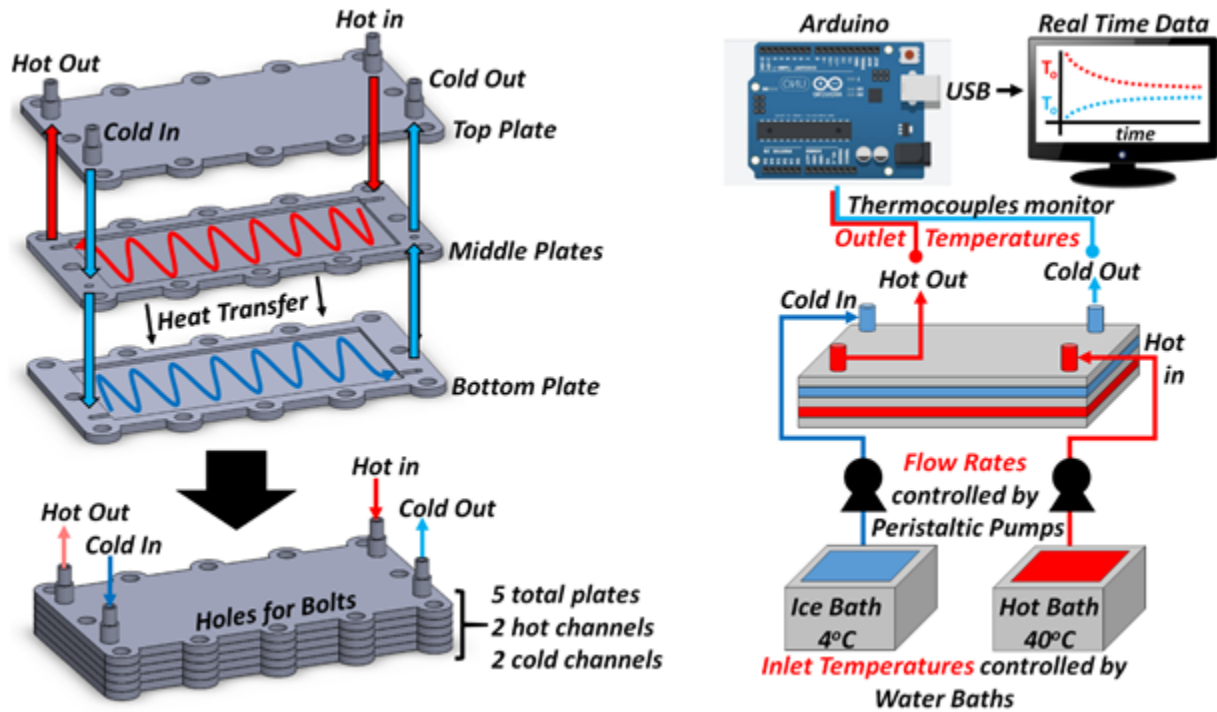


**Figure 4 – 3D Printed Plate and Frame Heat Exchanger.** **Left:** Plates are designed in SolidWorks, 3D printed, and fastened into a stack with bolts. In the example shown above, plate dimensions were approximately 3x6x1/8" (WxLxT). **Right:** The heat exchanger is tested by pumping cold (4°C) and hot (40°C) water through alternating layers while the outlet temperatures of each stream are monitored in real time by a thermocouple or an Arduino.
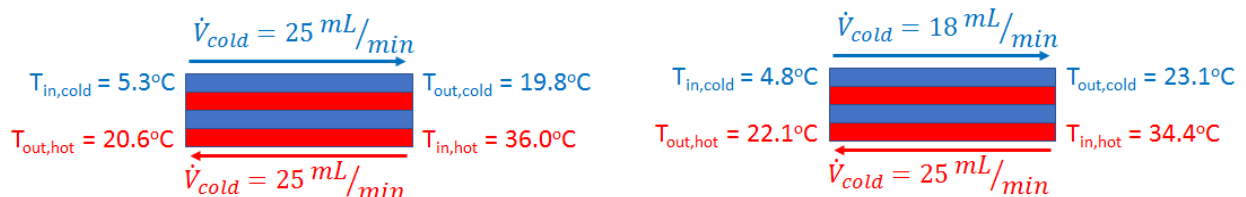


**Figure 5 – Results of 2 Experiments with the 3D Printed Plate and Frame Heat Exchanger.** **Left:** Temperatures achieved with hot and cold stream flow rates of 25 mL/min. **Right:** Temperatures achieved with hot stream flow rate = 25 mL/min & cold stream flow rate = 18 mL/min. Each plate had a 75 cm$^2$ of surface area for heat transfer (~2"x6").

Once the heat exchanger is assembled and tested to ensure it does not leak (internally or externally), several variables can be varied to investigate their effects on heat transfer. First of all, if the instructor wishes to include drafting in the project, the dimensions of the plate (e.g., length & width) can be easily varied in a CAD program. Ambitious students can also mimic real heat exchanger plates by adding chevron-shaped corrugations to the plates that increase turbulence and heat transfer. Once the heat exchanger is printed and assembled, the students can start by determining how long it takes the system to get to steady state by tracking the outlet temperatures over time. Once steady state is achieved, the parameters associated with each stream (e.g., inlet temperatures or flow rates) can be varied to determine their effects on the overall heat transfer coefficient, U. The flow patterns can also be easily switched between co-current and counter-current flow by switching the positions of the inlet and outlet tubing. The effects of additional plates or alternative fluids (besides water) can also be investigated, but some fluids may dissolve the materials used for 3D printing (e.g., ethanol dissolves PMMA).

Finally, it is important to emphasize that this is just one possible example of a 3D printed heat exchanger. The versatility of 3D printing allows the user to print a wide variety of different shapes and geometries, some of which cannot be fabricated with traditional methods. Indeed, several examples of 3D printed heat exchangers (plastic and metal) with novel geometries are available in the literature and on commercial websites [15–17].

## Module 3: DIY Spectrophotometer

### *Preface*
This final module is the most advanced, since it requires the students to used CAD software to design components (https://www.thingiverse.com/thing:2760937), 3D print them, wire a simple circuit, and program an Arduino that controls the DIY spectrophotometer. Consequently, this module is currently implemented as the sole focus of a 6-week long freshman design course. However, it is also possible to construct one or more of these units before class and simply demonstrate its assembly and use during class. This project does require several additional materials (e.g., LEDs, resistors, Arduino board, cuvettes), but the total cost of each completed prototype is still only about $50. Finally, since the cost of the device is so low, an instructor for a lab course could build several devices to allow each lab group to have their own device (instead of sharing a single commercial instrument that is more expensive).

### *Overview*
Spectrophotometers allow a user to measure the concentration of a colorful and/or light scattering solute (e.g., proteins, dyes, or cells) by illuminating a sample with a specific wavelength of light and then measuring how much of that light is transmitted through the sample to a detector. The percent of light transmitted (%T) can then be used to calculate the absorbance (A) of the sample, which is then used to estimate solute concentration with the Beer-Lambert Law, $A = \varepsilon C l$.

In this equation, A = sample absorbance, C = solute concentration, e = extinction coefficient, and l = pathlength (distance that light travels through the sample). Note: This equation is only accurate when the absorbance of the sample is between 0.1-1.0.

The first spectrophotometer that could effectively measure transmittance (and absorbance) was invented by Arnold Beckman of National Technical Laboratories in 1940. Beckman's initial design used a glass prism to split light into various wavelengths and a vacuum tube photocell to measure transmittance, but later models used more reliable quartz prisms.

Modern spectrophotometers use essentially the same design, but with a few key changes (see Figure 6). The light source is typically a halogen light bulb, which emits wavelength in the visible range ($\lambda$ = 300-700 nm) and near-infrared, but a deuterium lamp may also be included to emit ultraviolet (UV, $\lambda$ = 200-300 nm) wavelengths. The emitted light must then be aligned into parallel rays with a collimating lens before hitting the prism, which separates the separate wavelengths of light. Alternatively, some instruments use a diffraction grating, which uses a reflection phenomenon to separate wavelengths of light. Either way, the desired wavelength of light is selected mechanically with a slitted wavelength selector, such that one or a few wavelengths of light ($\lambda$) passes through the slit to illuminate the sample (all other wavelengths are blocked by the selector). The monochromatic (containing only one wavelength) light then passes through the sample and the amount of light that passes through it is measured by some sort of photodetector.
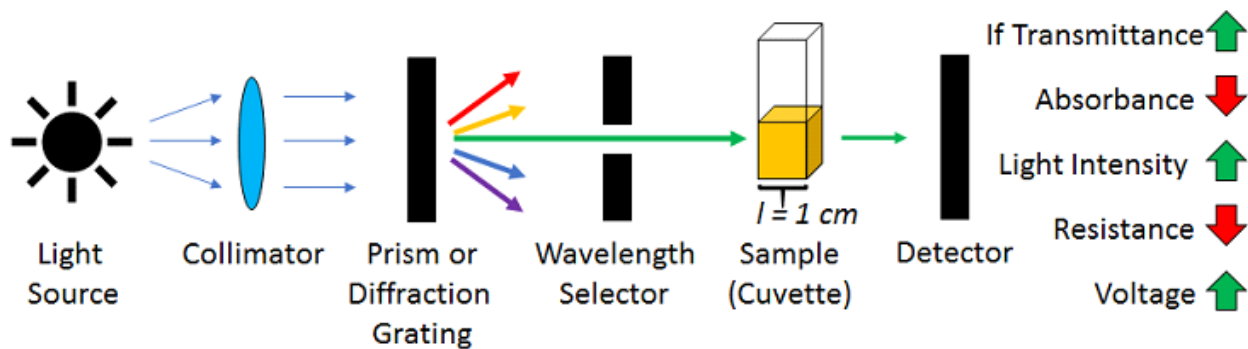


**Figure 6** –Spectrophotometer Design. The rays emitted from the light source are aligned with a collimating lens or mirror, then different wavelengths are separated by a prism or diffraction grating. Only one wavelength is selected to illuminate the sample and the amount of light that passes through the sample is measured by a detector that provides some sort of voltage signal.

Several different types of photodetector may be used (e.g., photoresistor, photocell, photosensor, photodiode, etc.), but most photodetectors are some sort of resistor. For example, the resistance of a photoresistor is inversely proportional to the light intensity on its surface. In other words, as more light hits a photoresistor, its resistance decreases. This change in resistance can easily be detected as a change in voltage across the photoresistor (e.g., voltage increases as resistance decreases) by a programmed electrical circuit. The measured voltage can then be

converted to a transmittance and absorbance. In addition, most modern spectrophotometers also boast a wide array of convenient capabilities, including automated injection, mixing, and measurement of sample absorbances in 96 well plates at programmable temperatures. This added convenience does come at a cost though, as most spectrophotometers cost thousands of dollars.

*Implementation in the Classroom*

The goal of this module is to challenge students to use concepts from mechanical, electrical, computer, and chemical engineering to design their own functional "DIY" spectrophotometer (Figure 7) at a much lower cost (~$50) than commercially available spectrophotometers ($1,000-$50,000). Since the scale of this module is relatively large, it is the focus of a (required) freshman design course that lasts 6 weeks (13 total meetings, 2 meetings/week, 75 minutes/class). The weekly schedule for this design course is shown in Table 3. A TA is also hired to assist the instructor in teaching the students how to draft their parts, 3D print them, and assemble/program their circuit. The course begins with an overview of spectrophotometry, then a tutorial on SolidWorks that guides the students through the process of designing components for the spectrophotometer (e.g., a cuvette holder with an LED on one side and a photoresistor on the other), which are then 3D printed. The instructor then guides the students through the process of wiring the spectrophotometer circuit shown in Figure 7, which consists of a few simple parts (LED, photoresistor, 330W and 10kW resistors) that can be easily connected (without soldering) using a breadboard. The resolution of the device can also be enhanced by including an optional 16-bit analog/digital converter (ADC), as shown in Figure 7. Specifically, a 16-bit ADC allows the device to detect changes in the analog photoresistor voltage drop signal (i.e. sample absorbance) as small as 76 $\mu$V ($5V/2^{16 \text{ bits}}$), while the 10-bit ADC on the Arduino can only detect changes in voltage of 4.8 mV ($5V/2^{10 \text{ bits}}$). Nonetheless, the device is still able to detect changes in ONP concentration if the ADC is omitted by directly connecting the negative lead of the photoresistor to the analog A0 port on the Arduino.
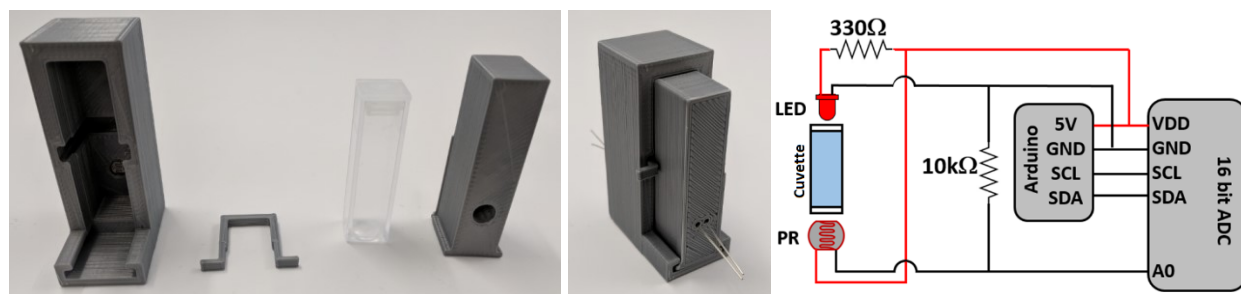


**Figure 7** –3D printed spectrophotometer. **Left/Middle:** Photographs of one example of a 3D printed cuvette holder. **Right:** Circuit diagram for spectrophotometer, which includes an Arduino Uno, 16 bit analog-digital converter (ADC), a violet (400 nm) LED, photoresistor, 330 Ω resistor for the LED, and a 10 kΩ shunt resistor for the photoresistor. All these components can be easily connected on a breadboard.

Once the circuit is complete, it is connected to the Arduino and the instructor shows the students how to program the board to monitor the photoresistor voltage. This step completes the device, which is then able to measure the absorbance of a sample by illuminating it with an LED of a specific wavelength, then measuring the amount of transmitted light by detecting the voltage across the photoresistor. Finally, it is important to mention that the parts and circuit shown in Figure 7 are only one possible design – students can be given the ability to tinker with completely different parts and order alternative materials.

In the final two weeks of the course, the students use the DIY spectrophotometer to measure the reaction rates ($V_o$) and kinetic parameters ($K_m$ and $V_{max}$) of the classic enzymatic reaction between β-galactosidase and a substrate (O-nitrophenyl galactoside, ONPG), which produces a product (O-nitrophenol, ONP) with a yellow color.

$$\text{β-galactosidase} + \text{ONPG} \rightarrow \text{β-galactosidase} + \text{ONP}$$
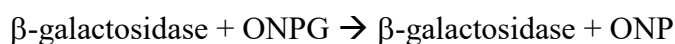
The students begin by preparing a standard curve the correlates the readings from the device (i.e., voltage across the photoresistor, which is displayed as a number of bits) to different increasing concentrations of the yellow ONP product. Once that relationship is established, the students can then perform several reactions in which the concentration of the ONPG substrate is increased from 0 to 10 mM. In each of these reactions, the device readings are recorded immediately after the enzyme is added to the substrate for a total of 2 minutes (see Figure 8). Using this raw data, the initial reaction rate ($V_o$, Δbits/min) at each substrate concentration [S] can then be calculated by dividing the total change in bits by the time elapsed (e.g., 2 minutes).

| Week | Day 1 (75 min) | Day 2 (75 min) |
|---|---|---|
| 1 | No Class | Intro to Biochemical/mechanical Engineering and Design Project |
| 2 | Introduction to Light and Spectrophotometry | Design Session: *Use SolidWorks to design device* |
| 3 | Introduction to 3D Printing *Receive device, redesign if necessary* | Introduction to Circuits *Assemble circuit & design enclosure* |
| 4 | Introduction to Arduino Programming *Assemble prototype, redesign if necessary* | Programming Session: *Compile & test code for Arduino* |
| 5 | Protein Structure and Enzyme Kinetics | Experiment Session I: *Begin collecting β-gal reaction data* |
| 6 | Excel Workshop *Analyze preliminary data in Excel* | Experiment Session II: *Continue collecting data* |

**Table 2** – Schedule for DIY Spectrophotometer Project

After the initial reaction rates are calculated, they can be used to estimate kinetic parameters for the enzyme, including the maximum reaction rate ($V_{max}$) and the relative affinity

of the enzyme for the substrate ($K_m$, also known as the Michaelis-Menten constant). Specifically, if it is assumed that the reaction between β-galactosidase (E) and ONPG (S) proceeds like so:

$$E + S \leftrightarrows ES \to E + P$$

…then it is possible to derive the following relationship between the initial reaction rate and substrate concentration, which is known as the Michaelis-Menten equation:

$$V_o = \frac{V_{max}[S]}{K_m + [S]}$$

According to this equation, if $V_o$ is plotted against [S], the resulting curve should be in the shape of a square hyperbola. Indeed, plotting initial reaction rates obtained by the device against their corresponding substrate concentrations yields the expected trend, as shown in Figure 8. This analysis can then be taken one step further by linearizing the data with the Hanes-Woolf model:

$$\frac{[S]}{V_o} = \left(\frac{1}{V_{max}}\right)[S] + \frac{K_m}{V_{max}}$$

Once the data is linearized (Figure 8), $V_{max}$ can be calculated as the inverse slope of the line, while $K_m$ can be calculated from the y-intercept. Using data obtained from the device, this analysis reveals a maximum reaction rate of $V_{max}$ = 140 ± 30 µmol ONP/min/mg β-gal and a Michaelis-Menten constant of $K_m$ = 0.9 ± 0.1 mM. These values are in excellent agreement with previously published values ($V_{max}$ = 134 µmol/min/mg β-gal, $K_m$ = 0.95 mM), thereby demonstrating the reliability of this DIY spectrophotometer [18,19].
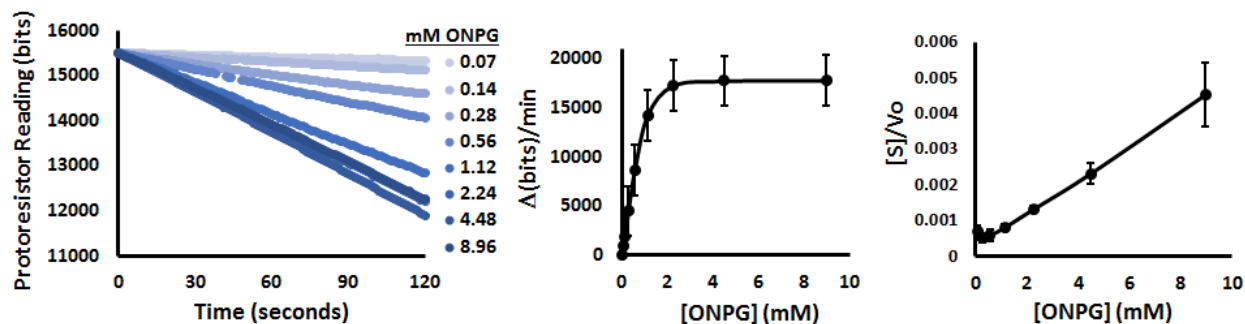


**Figure 8** – Analysis of kinetic data obtained with the 3D printed spectrophotometer. **Left:** The reaction of β-galactosidase with ONPG forms a yellow dye (ONP) that increases the absorbance of the sample, which leads to a subsequent decrease in the photoresistor reading (bits). **Middle:** Calculating the slopes of the raw data provides reaction rates (Δbits/min) that can be plotted against the corresponding substrate concentrations (mM ONPG) to obtain a Michaelis-Menten plot. **Right:** Linearizing the data to fit the Hanes-Woolf model reveals a linear trend that can be used to estimate $K_m$ and $V_{max}$. Error bars represent standard deviations from 3 separate experiments.

Finally, it is important to mention that the data shown in Figure 8 were obtained by the instructor with the instructor's own device. The devices built by the students are somewhat error-

prone and tend to require some troubleshooting.  Some of the most frequent problems observed by the instructor have been:

1. Components plugged into the breadboard are loose or disconnected.
2. Some photoresistors are faulty and will provide relatively higher resistances under all circumstances.  Try replacing the photoresistor if the blank readings seem low.
3. The incorrect shunt resistor is used for the photoresistor (should be 10 k$\Omega$).
4. The LED or photoresistor are not firmly connected to the circuit.
5. The LED or photoresistor are not fixed in place – moving the photoresistor only a short distance towards or away from the LED can significantly affect its reading.
6. The cuvette must be covered during the reaction to prevent background light contamination, which causes readings to be higher than expected.

Students also appear to struggle most with the following specific concepts, so make sure that you emphasize them during lecture:

1. LEDs:  LEDs will only light up if the longer lead is connected to a positive source!
2. Pipetting:  Students that have never pipetted before have difficulty learning the "two-stop" system and will often transfer too much substrate or enzyme solution to the cuvette.
3. Data Analysis:  Some freshmen have never used Excel to perform calculations. Consequently, an entire class session is set aside to explain Excel functions in our class.

## Conclusion

The modules described in this paper give ChE students the opportunity to learn about 3D printing and other valuable interdisciplinary skills.  The students can substantially benefit from these experiences, since their expanded skill set allows them to design, build, and/or test prototypes for novel devices that could be used to optimize or analyze a process.

Since the numerous steps and nuances involved in each of these modules cannot be completely encapsulated within this brief paper, the primary author is willing to assist anyone who wishes to implement these modules.  Finally, it is also worth mentioning that several other 3D printed and/or Arduino-controlled devices and learning tools can be found online at Thingiverse and other websites.  Even if you do not have a 3D printer, many of these websites will also print and ship 3D printed parts to you.

## Transfer to Other Institutions & Scalability

The author is happy to help any other interested instructors adopt the modules described in this paper.  A short list of the steps needed to implement the modules is given below:

1. Obtain a 3D printer
   a. A Lulzbot Taz6 was used for the Amino Acids & DIY Spectrophotometer
   b. A Stratasys Objet was used for the heat exchanger, but the Lulzbot might work
2. Download STL files from Thingiverse
   a. Amino acids connected by set screws:https://www.thingiverse.com/thing:2758246
   b. Amino acids connected with tubing:  https://www.thingiverse.com/thing:2509708
   c. Plate & Frame Heat Exchanger:  https://www.thingiverse.com/thing:2452341
   d. Spectrophotometer:  https://www.thingiverse.com/thing:2760937
   e. Solidworks files are also available if you wish to modify the parts
3. Generate G-Code
   a. Install **Cura** and input parameters for your printer and filament
   b. Load the STL files and generate the G-Code
4. Print and assemble!

The time required to print parts for each module will vary significantly depending on the printer used and the number of students in the class.  For example, it took several days of printing and $75 to print 15 of the amino acid kits.  However, all of the parts for the spectrophotometer module (with 13 groups of students) were printed in a single overnight print for less than $10.  All that being said, any of these modules can be scaled up to accommodate larger class sizes, but it may require much longer lead times or several printers working simultaneously to generate the parts.

## Complete Bill of Materials for DIY Spectrophotometer

The table below shows all of the electronic components required to construct ten photometers.
Note: The 16-bit ADC is optional. The device is $15 cheaper (and still works) if it is not included.
Note: Most of these components can be reused for subsequent classes, but some components will become damaged (LEDs, resistors, and photoresistors) and need to be replaced over time.

| Item | Supplier | Catalog # | Price $ | Quantity | Net |
|------|----------|-----------|---------|----------|-----|
| Arduino Uno R3 | Adafruit | 50 | 24.95 | 10 | 249.50 |
| ADS1115 16-bit ADC w/4 channels | | 1085 | 14.95 | 10 | 149.50 |
| Half-Size Breadboard | | 64 | 5.00 | 10 | 50.00 |
| Purple LED (400 nm) (10 pack) | | 1793 | 4.95 | 1 | 4.95 |
| Photoresistor | | 161 | 0.95 | 10 | 9.50 |
| 330 Ω Resistors (25 pack) | | 2780 | 0.75 | 1 | 0.75 |
| 10 kΩ Resistors (25 pack) | | 2784 | 0.75 | 1 | 0.75 |
| Male/Male Jumper Wires (20) | | 1956 | 1.95 | 5 | 9.75 |
| Male/Female Jumper Wires (40) | | 826 | 3.95 | 1 | 3.95 |
| Cuvette Holder | 3D printer | 1 kg spools of PLA are ~$15 each | | ~2 | 30.00 |
| Arduino/Breadboard Enclosure | | | | | |
| **Estimated Total for 10 Devices** | | | | | **$508.65** |
| **Approximate Cost of a Single Device** | | | | | **$50.87** |

**In addition to the electronics shown in the table above, the following reagents are also required:**

| Item | Supplier | Catalog # | Price $ | Quantity | Net |
|------|----------|-----------|---------|----------|-----|
| β-galactosidase (enzyme) | Sigma Aldrich | G5635-5KU | 291.00 | 1 | 291.00 |
| ONPG (substrate for reaction) | | N1127-1G | 38.25 | 1 | 38.25 |
| O-nitrophenol (ONP, to prepare standard curves) | | N19702-5G | 25.40 | 1 | 25.40 |
| Monobasic sodium phosphate | | 71500-250G | 26.00 | 1 | 26.00 |
| Dibasic sodium phosphate | | S9390-100G | 35.25 | 1 | 35.25 |
| Potassium chloride | | P5405-250G | 26.25 | 1 | 26.25 |
| Magnesium chloride | | 793612-500G | 74.00 | 1 | 74.00 |
| Dithiothreitol (DTT) | | D0632-1G | 47.00 | 1 | 47.00 |
| Ultrapure water | | --- | --- | --- | --- |
| **Total Cost of Chemicals** | | | | | **$563.15** |

Note: The estimated cost of chemicals may have to be adjusted depending upon how many students are in the class and how many experiments the instructor wishes to perform. The total shown above is more than enough for 13 groups to each perform 32 experiments (4 replicates of 8 reactions with varying ONPG concentrations of 8, 4, 2, 1, 0.5, 0.25, 0.125, & 0.0625 mM).

Note: The β-galactosidase and ONPG solutions can be aliquoted and frozen at -72°C until needed.

Note: General consumables (tubes, cuvettes, pipettes, pipette tips, etc.) will also be needed.

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