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The Use of His-tagged EGFP- and mCherry-*Drosophila* Ultrabithorax (Ubx) Fusion Proteins for Student Designed Biomaterials Formation Projects

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The Ultrabithorax (Ubx) protein from *Drosophila melanogastor* is a Hox protein that serves as a transcription factor *in vivo*, regulating fly development. *In vitro*, the protein self-aggregates into remarkably stable, elastic, heat-resistant ordered materials making Ubx a promising candidate for development of a wide variety of biological applications. In this module, students purify either his-tagged EGFP- or his-tagged mCherry-Ubx fusion protein using affinity chromatography. Students design their own experiments to test the biomaterial forming characteristics of the purified fusion proteins. Students learn how to do polyacrylamide gel electrophoresis, Bradford assays, and protein purification through their participation in this project.

Keywords: biomaterials, ultrabithorax, protein purification, column chromatography, polyacrylamide gel electrophoresis (PAGE), fluorescent protein, molecular biology

Introduction

A curriculum module for the senior-level Biochemistry II Laboratory course at the University of Houston was developed which merges molecular biology techniques, protein purification, and biomaterials assembly and applications into a true research experience for undergraduate students. The Ultrabithorax (Ubx) protein from *Drosophila melanogastor* (fruit fly) is a Hox protein that serves as a transcription factor *in vivo*, regulating fly development. It is a homeotic selector gene that specifies the identity and developmental pathway of cells in the posterior thorax of the fly (Bondos et al., 2004). *In vitro*, the protein forms remarkably stable, elastic, heat-resistant ordered materials (Greer et al., 2009). Because these materials self-assemble rapidly in gentle buffer conditions, assembly of Ubx fusions with other proteins should maintain the activity of the fusion protein, making Ubx a promising candidate for development of a wide variety of binding, catalysis, and sensing applications.

This project provides students an opportunity to learn some of the basic biochemistry techniques used by researchers studying protein activity and function. In this module, students will learn how proteins are heterologously expressed in *E. coli* and purified using affinity chromatography. Students next learn the principles of affinity chromatography by purifying histidine-tagged Ubx fused to either enhanced green fluorescent protein (EGFP) or mCherry (a monomeric red fluorescent protein) using a nickel-NTA column. The use of an Ubx-fluorescent protein fusion allows easy visualization of the purification process. Students use SDS-PAGE to determine the purity of their protein samples and perform Bradford assays to calculate the concentration of their protein sample.

Students design their own experiments to test the characteristics of the fusion proteins. They either develop experiments to optimize materials formation (the development of ropes, films, or sheets) or they design novel ways to work with the materials such as developing novel structures. Past projects have included testing different buffers for materials formation, forming a cylindrical tube of Ubx protein, and devising a hand-held device capable of generating a twisted 3-strand-rope of protein. The experience provides students with a chance to formulate their own questions and hypotheses, develop a protocol to test their hypothesis, try their experiments, evaluate the results, redesign protocols based on the initial experimental trials, to report the data in a written, journal-style manuscript, and finally to share the results with the class in an informal seminar-type talk.

Students are provided with a number of checkpoints as they progress through the project. Students work in teams of three to four students to develop a project. Students are expected to review the current literature on Ubx and to use

research literature to guide the development of their research questions. Each group meets with the section teaching assistant and the course instructor to discuss their ideas. Meetings typically last for 15-20 minutes. Each student is expected to bring three ideas for possible projects to the meeting. The merits or potential issues of each idea are discussed. The group leaves the meeting with a list of ideas that they could reasonably develop and are allowed to choose which one(s) they will pursue. A week later, each group hands in a draft of their proposed protocol. Each team again meets with the course instructor to fine-tune the protocol. A final draft is handed in and one last round of suggestions is provided as written commentary on the protocol and returned to the students. This process helps ensure that students design projects that can reasonably be accomplished given the resources, space, and time available for the project.

One to two weeks of open lab time is allotted for the students to complete the project. Regular laboratory times are cancelled to allow the classroom space to be used throughout the day and to provide the space needed for project setup. At least one teaching assistant is on staff in the classroom anytime the lab is open. Undergraduate volunteers also staff the room to help students find equipment and supplies. Having the room staffed addresses safety concerns and provides students with immediate assistance and guidance as needed as they learn to manage a research project.

Some class time is utilized to teach scientific writing. The elements of a journal article are discussed along with the expectations for style and formality. Once the project is complete, students each write a journal-style article to report their work. Although this is a group project, each student is expected to write their own paper (figures, tables, and pictures may be shared). The first draft of the paper is peer edited by members of their own group. This helps ensure all the important elements of the experiment are included. Next, the papers are peer-edited by a classmate that is not a group member to ensure that the writing is clear to someone not intimately involved in the project. PowerPoint slides are prepared as a group and each member is expected to participate in the delivery to the class in their oral presentation.

Implementation of this curriculum module is labor intensive. At the University of Houston, this module is run with four class sections in a semester with a total enrollment of approximately 68 students with the assistance of four teaching assistants and two to three preparatory assistants. If teaching assistants are not available, using undergraduate students as teaching assistants to keep the workload manageable is recommended. Once the project has been run once, former students can be invited to serve as teaching assistants. These students are usually eager and willing to assist and already understand the project.

A suggested time line for implementation is provided in Table 1.

Table 1. Suggested timeline for project implementation.

Time	Activity	Student Assignment
Class 1	Introduction to the Ubx project	Read selected Ubx journal articles and answer questions on the reading
Class 1	Practice pulling rope	Develop a list of three or more ideas to test
Class 2	Transform Rosetta cells with Ubx-pET19 plasmid	
Class 3	Discuss ideas for projects; Select colonies for overnight culture; Instructor induces culture	Discuss the idea list with the instructor; select a project to pursue
Class 4	Purify protein	Write rough draft of methods
Class 5	Purify more protein	
Class 5	Quantitate protein and SDS-PAGE gels	Review methods with instructor
Class 6	Discuss protocols for projects	Final revision of methods
Class 7-8	Student experiments (1-2 weeks open lab)	Write journal-style manuscript
Class 9	Edit draft of manuscript	Develop PowerPoint presentation to present work
Class 10	Class presentations	Class presentations

Student Outline

Educational Objectives

Objective 1

Students will learn methods related to the study of proteins through this project-based activity. Students will use affinity chromatography to purify a his-tagged, Ubx-fusion protein, check the purity of the protein preparation using SDS-PAGE, and quantitate the yield (Bradford assay). Students will perform activity assays to verify proper function and folding of their his-tagged, Ubx-fusion protein.

Objective 2

Students will gain experience in materials manipulation as they experiment with different methods of pulling Ubx protein into ropes and sheets and forming bundles, lattices, and baskets with the protein.

Objective 3

Students will formulate their own questions and hypothesis, develop a protocol to test their hypothesis, try their experiments, evaluate the results, and redesign protocols to improve their results.

Objective 4

Students will present their findings in a journal-style paper to practice their technical writing skills. Students will read the primary literature on Ubx materials and will research potential applications of biomaterials with similar properties to Ubx (such as silk, elastin, and collagen) and propose future experiments for their fusion constructs within their journal-style paper.

Ultrabithorax Protein

The Ultrabithorax (Ubx) protein is a *Drosophila* Hox protein. During the development of bilaterally symmetric animals, Hox transcription factors specify and differentiate tissue fates along the anterior/posterior axis (Bondos et al., 2006; Hughes and Kaufman, 2002). Ubx specifies the development of the posterior thorax and anterior abdomen of *Drosophila*, including the halters, legs, midgut, and portions of the nervous system, ectoderm, and musculature of the fly (Bondos and Tan, 2001; Hughes and Kaufman, 2002).

In the course of purifying the Ubx protein to study its properties as a transcription factor, it was noted that the protein self-assembles under gentle buffer conditions to form ordered biomaterials (Greer et al., 2009). Ubx aggregates have not been found *in vivo*. Curiously, the materials formed *in vitro* are quite stable at room-temperature for years. These unusual properties have been studied more closely by the Matthews lab (Rice University) and the Bondos lab (Texas A & M Health Science Center) (Greer et al., 2009; Huang et al., 2010; Majithia et al., 2011).

Ubx fibrils form at the air-surface interface of the buffered protein solution within 1-4 hours depending upon the experimental design. A nanoscale-thick film of Ubx forms across the liquid surface of a drop or pool of buffer containing purified protein. The film can continue to polymerize to form a thick, tough sheet or can be drawn into ropes (Figure 1) with diameters varying between 2 and 30 μm (Huang et al., 2010). The materials are heat-resistant, surviving in solution at 98°C for several hours. Ropes and sheets can be dried and stored at room temperature for years. The ropes are quite flexible, stretching up to an additional ~150% of their original length before rupturing, making these unique fibers more extensible than collagen or silkworm and spider dragline silk and as extensible as elastin (Huang et al., 2010). A number of interesting and potentially useful structures can be constructed with Ubx because the material is self-adhesive (Greer et al., 2009). Ropes can be aligned into fused parallel or twisted bundles. Lattices are formed using hanging drops or pulling rope from developed sheets.

Advantages of using Ubx for biomaterials formation over other elastomeric proteins include the fact Ubx self-assembles under very mild conditions eliminating the need for the use of high temperatures, non-physiological pH, or organic solvents and UBX assembles at lower concentrations and in hours rather than the days or weeks required for other proteins (Greer et al., 2009). These exceptional qualities uniquely enable functionalization of Ubx materials by gene fusion, since the heterologous protein is far less likely to be unfolded or inactivated by the assembly process (Huang et al., 2011).

Fluorescent Proteins

An array of fluorescent proteins are produced naturally by corals, jellyfish, and anemones. One of the earliest studied fluorescent proteins was green fluorescent protein, isolated from the jellyfish *Aequorea victoria*. Green fluorescent protein requires no cofactors to fluoresce. GFP can fluoresce as a monomer (Figure 2). This is useful because, unlike other naturally occurring fluorescent proteins, it does not need to form dimers or tetramers to fluoresce, which is helpful when fusing GFP to other proteins

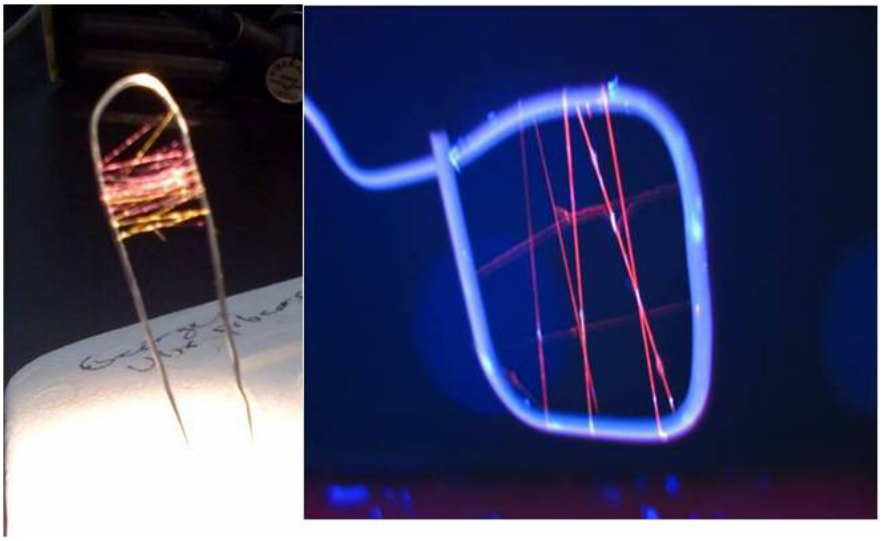

Figure 1. A rope of EGFP and mCherry-Ubx on the left and mCherry-Ubx on the right. The picture on the left is under white light. The picture on the right is under UV light.

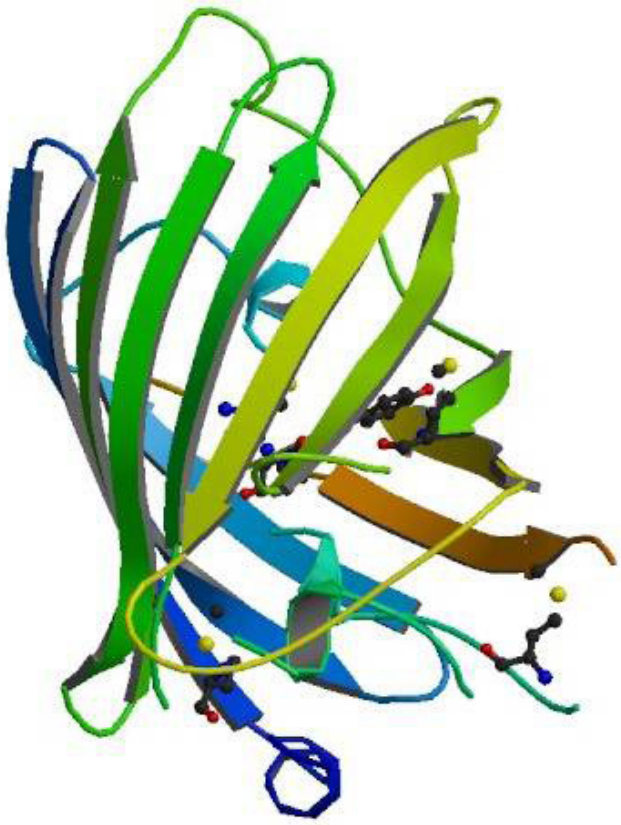

Figure 2. Crystal structure of green fluorescent protein. GFP is a monomeric protein comprised of a beta barrel. Image from PDB code: 1EMA.

because dimerization or tetramerization can interfere with the function of the protein to which the protein is fused. mOrange, mCherry, and mPlum were developed by Roger Tsien's lab from DsRed1. The DsRed1 protein fluoresces only after tetramerization (Figure 3) (Stepanenko et al., 2008). All of the Fruit Fluorescent proteins were developed by Roger Tsien's group and are marketed by Clontech. The Fruit Fluorescent proteins that carry the "m" designation are monomeric proteins engineered to fluoresce as monomers. mOrange, mCherry, and mPlum are all monomeric derivatives of DsRed1 (Shu et al., 2006).

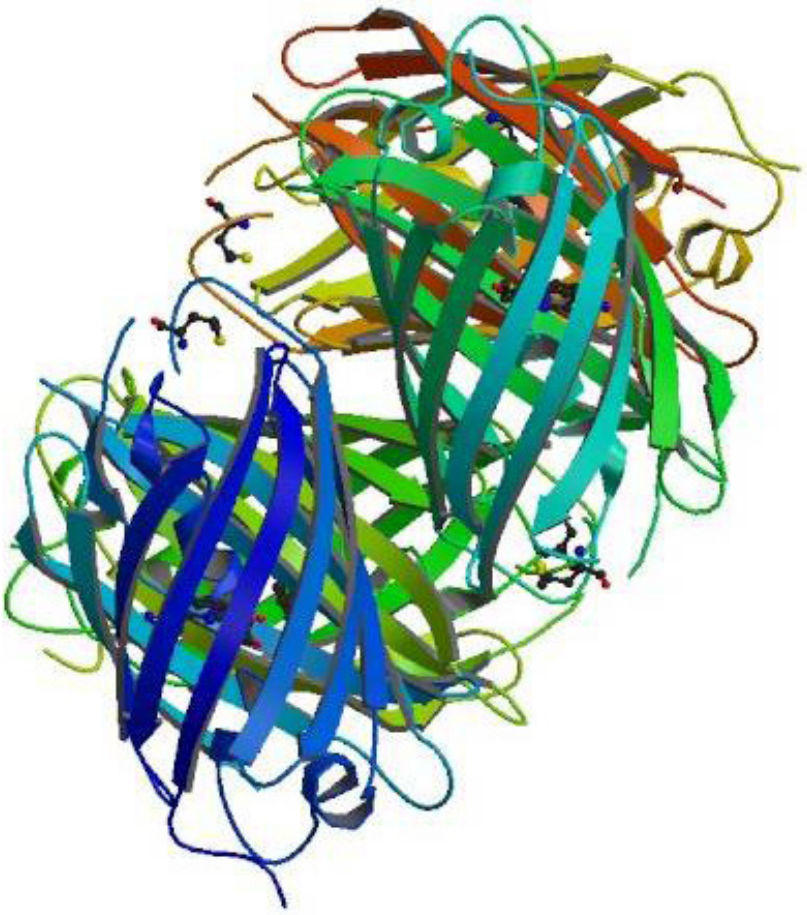

Figure 3. Crystal structure of DsRed1 from *Discosoma*. Image from PDB: Code 1G7K.

The excitation and emission spectrum for mCherry and several other fluorescent proteins are shown in Figure 4 and Table 2. The existence of proteins with overlapping emission and excitation spectra is useful in developing FRET-based assays which are used to detect macromolecular interactions and measure distances between two interacting molecules *in vivo*.

Table 2. Excitation and Emission Maxima for Clontech Fruit Fluorescent Proteins

Protein	Excitation Maxima	Emission Maxima
mBanana	540 nm	553 nm
mOrange	548 nm	562 nm
mCherry	587 nm	610 nm
mPlum	590 nm	649 nm

Overview

In this research project, fused fluorescent-UBX protein will be expressed and purified. The protein product will be used to create ropes, sheets, and films. The work flow for the project is depicted in Figure 5.

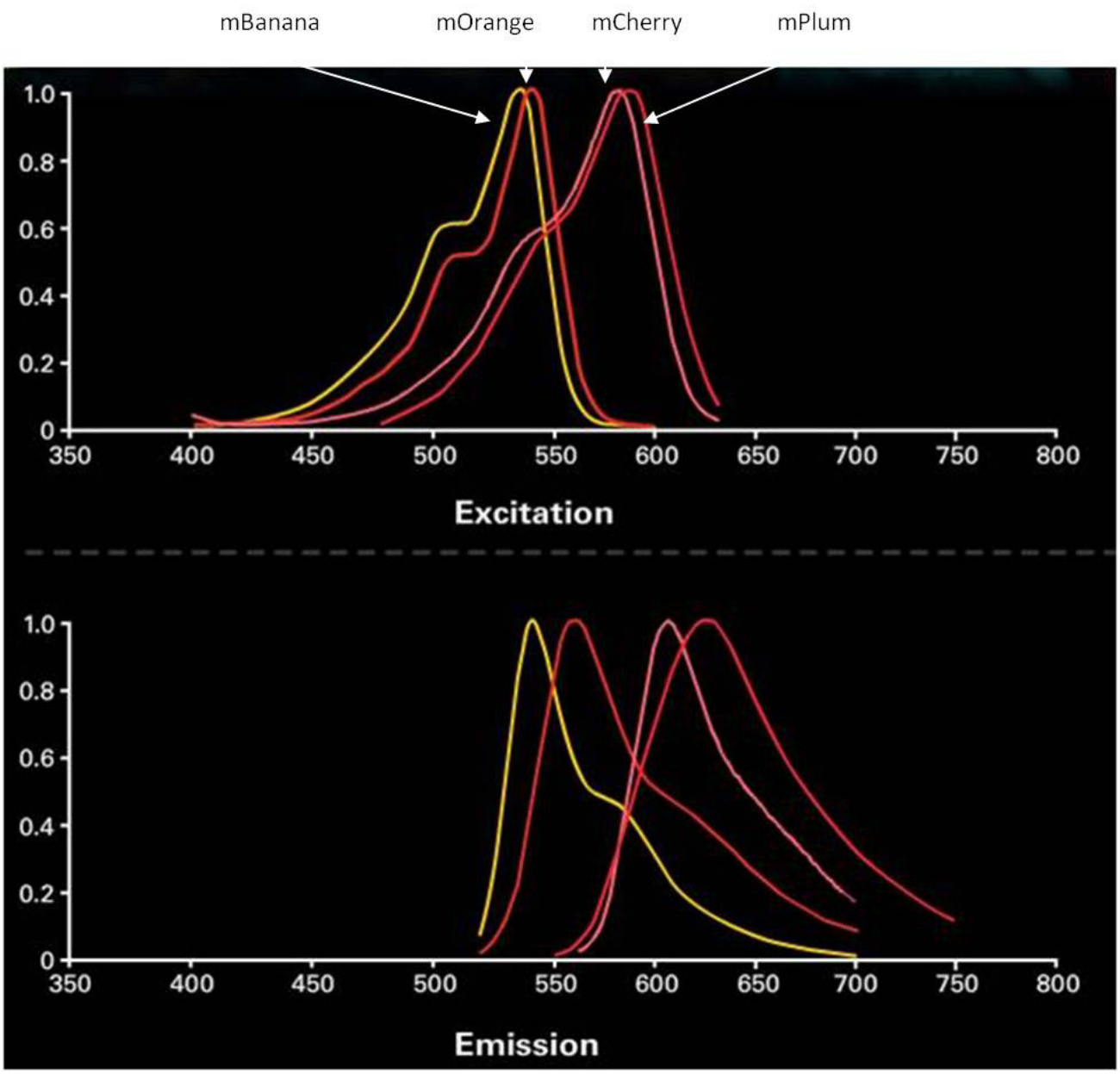

Figure 4. Emission and Excitation Spectra of Fruit Fluorescent Proteins. (retrieved on March 11, 2010 from **http://www.clontech.com/upload/images/WP9X2790_FP.html**).

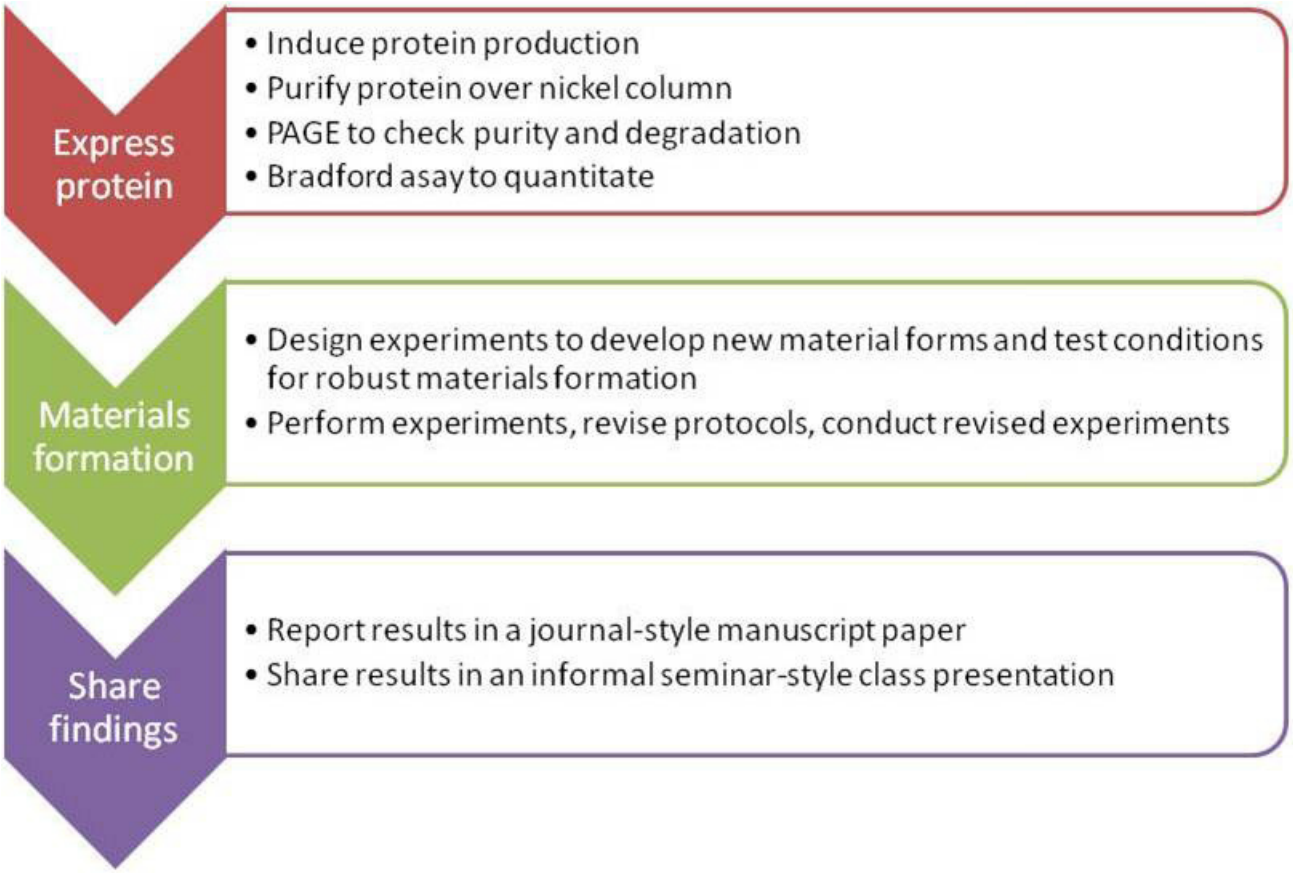

Figure 5. Project overview. The Ubx protein will be purified and checked for quality and quantity. Students will design and implement their experiments with the protein and results will be presented both orally and in writing.

Transformation of Ubx-pET19b into Rosetta2(DE3) cells for protein expression

Heterologous protein expression (expression of protein from one organism in a different organism, most often *E. coli*) is a commonly used technique when large amounts of protein are needed for study. This allows the addition of tags that make purification of the specific protein of interest easier. The *Drosophila* Ubx gene was cloned into the pET19b vector from Novagen. Because *Drosophila* is a eukaryotic organism, the Ubx gene contains introns. Bacteria lack the machinery to splice out introns. This lack of splicing machinery prevents bacteria from making functional proteins from eukaryotic genes. To get around this problem, *Drosophila* mRNA was used to create a type of DNA known as copy DNA or cDNA. The cDNA made from the mRNA template lacks introns because mature mRNA lacks introns.

Ultimately, it is the Ubx cDNA that is cloned into the pET19b vector (Novagen). mCherry and EGFP were then cloned in front of the Ubx gene. The stop codon of the fluorescent protein was removed to allow the creation of a fusion protein with Ubx. In addition, a histidine-tag is added to the N-terminal end of the fusion protein construct (Figure 6) to allow for easy purification using a metal affinity column.

Figure 6. Fusion protein expressed from the modified pET19 vector.

The modified pET19 vector must be transformed into an expression line of bacteria in order to make the desired fusion protein. The Ubx protein makes the bacteria a bit sick so it is important to do a new transformation with the plasmid each time protein is expressed. Expression cell lines tend to rearrange the plasmid DNA if the protein being expressed negatively impacts cell fitness. The Rosetta (Novagen) cell line used in this project is a BL21 derivative. BL21 lines are used for expression of heterologous proteins in *E. coli*. BL21 cells are derived from B strain *E. coli* carrying a lambda lysogen that prevents infection and lysing by bacteriophage 21 (hence the name BL21). The Rosetta line enhances expression of eukaryotic proteins by providing tRNAs for 7 codons rarely used by *E. coli* (AGA, AGG, AUA, CUA, GGA, CCC, and CGG) on a chloramphenicol-resistant plasmid. By providing the tRNAs for these rare codons, higher levels of protein expression are possible. Strains designated DE3 are lysogens of lambda phage DE3 (λDE3). This means that lambda DNA has been integrated into the *E. coli* host chromosome and is stably maintained there, even when the *E. coli* replicates, without inducing phage replication and cell lysing. The Rosetta2(DE3) strain, therefore, has a copy of the T7 RNA polymerase since the gene for this protein is carried on the lambda genome. This polymerase is needed for expression of genes cloned behind a T7 promoter, such as in the pET vectors used in this work. The full genotype of the Rosetta2(DE3) strain is described in Table 3 below.

Table 3. Genotype: Rosetta2(DE3): F- *ompT hsdS*$_B$ (r_B-m_B-) *gal dcm* (DE3) pRARE2 (Cam^R)

Definition	Genotype
F-	Lacking an F plasmid needed for mating.
ompT	Lacks an outer membrane protease to allow increased yields of heterologously expressed proteins.
hsdSB	Strain is both methylase and restriction deficient.
gal	A mutation in one of the genes of the galactose operon.
dcm	Cytosine methylation in the sequence CCWGG is abolished allowing for digestion of DNA with dcm sensitive restriction enzymes in downstream applications.
(DE3)	Lambda DE3 is an engineered version of the lambda virus. The coding sequence for T7 virus RNA polymerase has been added. Expression of the RNA polymerase is under the control of the lacUV5 promoter. The lacUV5 promoter is a mutated version of the lac operon promoter. The mutation helps reduce the leaky basal level expression of the gene typical of the lac promoter.
pRARE2	Encodes tRNAs for 7 codons rarely used by E. coli (AGA, AGG, AUA, CUA, GGA, CCC, and CGG) on a chloramphenicol-resistant plasmid.
Cam^R	Chloramphenicol-resistant.

Transformation Protocol

1. Thaw three 50 µL aliquots of chemically competent Rosetta2(DE3) cells on ice. Label one tube (U) for UBX, one tube (+) for the positive control, and one (-) for the negative control.
2. Add 3 µL of the selected UBX plasmid to the (U) tube. Mix gently by pipetting.
3. Add 3 µL sterile deionized water to the (-) tube. Mix gently by pipetting.
4. Add 3 µL pUC18/19 to the (+) tube. Mix gently by pipetting.
5. Place tubes on ice for 30 minutes.
6. Heat shock the cells in a 42°C water bath for 1.5 minutes.
7. Immediately place the tubes back on ice.
8. Chill the tubes on ice for 5 minutes.
9. Add 200 µL 2YT media.
10. Incubate cells at 37°C for one hour.
11. Make dilutions for each cell sample as indicated in Table 4.

Table 4. Cell Sample Dilutions

Dilution	2YT	Cells
1:1	0 µL	200 µL
1:10	180 µL	20 µL
1:100	198 µL	2 µL

12. Plate all 200 µL of each dilution out onto a labeled LB-ampicillin/chloramphenicol (100 µg/mL / 34 µg/mL of antibiotics respectively) agar plate. Be sure to label the bottom and not the lid. Spread evenly with a cell spreader. Be sure to flame the spreader between cell samples and allow the spreader to cool adequately before spreading the cells. Alternately, use an autoclavable plastic spreader and use a fresh spreader for each plate (do not throw out the plastic spreaders. They are autoclavable and reusable).
13. Place the plates lid-side down in a 37°C incubator overnight.
14. Check colonies the next day.
15. Add 10 mL of 2YT media supplemented with 100 µg/mL ampicillin and 34 µg/mL chloramphenicol to three sterile culture tubes. Label the tubes.
16. Select three colonies from the transformation plates to culture overnight to use as starter culture for the induction of protein expression. You chose three colonies instead of just one colony just in case one of the colonies is not truly transformed with the plasmid and has "escaped" selection on the plate. Use a wooden applicator stick to gently touch a selected colony. Swirl the stick around in the 2YT culture tube. Dispose of the applicator stick in the biohazard waste container. Repeat for the remaining colonies and tubes.
17. Incubate cultures overnight at 37°C on a shaker.

Expression of the Ubx fusion protein from the transformed Rosetta2 (DE3) cells

Expression of the his-tagged EGFP-, mCherry-Ubx fusion protein is under the control of the lambda phage T7 viral promoter. The Ubx fusion protein cannot be produced until T7 polymerase is produced by the host cell line (Rosetta2(DE3) in this case). The T7 polymerase transcribes the DNA coding for the Ubx fusion protein into mRNA which is then translated by the cell into protein. The gene for the T7 polymerase is carried on the bacterial chromosome in the Rosetta2(DE3) line. It is important that the T7 polymerase is not expressed before the cell density reaches an OD600 of at least 0.6. Once expression of any protein under control of the T7 polymerase begins, most of the resources of the cell are directed toward production of the heterologously expressed protein. If the cell density is too low before the cells' resources for division are diverted to protein production, the yield of the recombinant protein will be low. Additionally, if the heterologously expressed protein is toxic to the cell, cell death will occur before the density of the cell population is high enough to result in a high protein yield. Thus, it is important that the T7 polymerase not be transcribed until the OD600 of the cell culture reaches at least 0.6.

The expression of the T7 polymerase is under the control of the lacUV5 promoter. The lacUV5 promoter is a mutated version of the bacterial lac promoter. The lac promoter tends to be "leaky", thus allowing some basal level expression of the proteins of the lac operon β-galactosidase (*lacZ*), lactose permease (*lacY*), and a transacetylase (*lacA*). Bacteria are not able to completely shut down transcription of the operon even when a transcriptional inhibitor, such as lacI, is present. The mutation in the lacUV5 promoter prevents "leaky" expression of the T7 polymerase gene. The pET19b vector carries the coding sequence for lacI, an inhibitor of the lac operon. When the lacI protein is bound to the operator sequence in the regulatory region preceding the T7 polymerase gene in the host bacterial chromosome, the T7 polymerase cannot be transcribed due to fact lacI sterically hinders the RNA polymerase from binding to the promoter sequence. In order for transcription to occur, lacI must bind lactose. Once lactose is bound, lacI falls off the operator sequence and the polymerase can bind to the lacUV5 promoter and transcribe the T7 polymerase. In this system, isopropyl β-D-1-thiogalactopyranoside (IPTG) is often used instead of lactose to remove lacI from the operator. Lactose can be cleaved to glucose and galactose by β-galactosidase in the cell. Once lactose is cleaved, it is no longer available to bind to lacI. IPTG serves as a molecular analog of lactose. The lacI protein binds IPTG and falls off the promoter but the IPTG is not degraded so levels of IPTG remain constant throughout the experiment. Once the T7 polymerase is translated into protein, it begins transcribing the Ubx-fusion protein on the pET19b plasmid.

Expression Protocol

1. Use the 10 mL overnight culture from the transformation protocol to inoculate 1 L LB supplemented with 50 µg/mL carbenicillin (or ampicillin) / 100 µg/mL chloramphenicol). Incubate on a shaker at 37°C until the culture reaches an OD600 of 0.6-0.8
2. Induce Ubx expression by adding 2 mL 500 mM IPTG (for a final concentration of 1 mM IPTG in LB). Grow at room temperature on a platform shaker for a minimum of 3 hours (incubate at 28°C if a shaker capable of maintaining 28°C is available because 28°C provides higher yields). Cultures may be allowed to grow overnight.
3. Harvest cells by centrifugation at 7,000 x g for 15 minutes. Pour off supernatant. Add 0.1 g lysozyme. Mix with a little LB broth as possible to resuspend. Transfer to a 50 mL disposable centrifuge tube for storage. Store pellets at -80°C until ready for use.

Purify the UBX fusion Protein

The his-tagged EGFP-Ubx and his-tagged mCherry-Ubx protein will be purified using a nickel-nitrilotriacetic acid (Ni-NTA) resin from Qiagen. The nitrilotriacetic acid binds nickel ions. The histidines in the 6x-his tag appended to the EGFP-Ubx and mCherry-Ubx bind the nickel. Any proteins that happen to have a lot of histidines exposed on their surface may also bind to the resin. If really pure protein is needed, researchers will often use more than one type of column to isolate the desired protein from the cell lysate. In this protocol, the desired protein will be isolated using a gravity column. Immidazole will be used to elute the bound protein off the column. Histidine contains an imidazole ring. When the column is washed with a buffer containing successively higher concentrations of imidazole, the free imidazole displaces the imidazole ring of the histidine in the 6x-his tag and the protein is eluted off the column. Lower concentrations of imidazole in the initial washes help remove proteins that are not bound as tightly to the column as proteins with the 6x-his tag.

To check the purity of eluted protein, an SDS-PAGE gel will be run. The EGFP-Ubx and mCherry-Ubx protein will run at about 84 kD. The approximate concentration of the protein can be obtained using a Bradford assay.

Protein Purification Protocol

1. Add 10 mL lysis buffer (Buffer G) to the pellet. Add half of a protease inhibitor tablet (Roche). Add 20 µL 2-mercapto-ethanol. Thaw pellet in a beaker of room temperature water. Resuspend the pellet with a serological pipette or disposable transfer pipette. Do not make bubbles! As the cells lyse, the solution will become viscous. This is caused by the release of DNA from the cells.
2. Freeze the pellet at -80°C (5-10 minutes or until completely frozen). Repeat twice (more if needed) until the cells have clearly lysed. The solution will be very "snotty". If the sample has been freeze/thawed several times and is still not "snotty", add sodium phosphate dibasic (100 mM in water) to bring the pH up over 7.6 (to about 8).
3. Add 160 µL DNase I solution. Rock at 4°C until the "snottiness" dissolves. There should be no DNA strings left. This usually takes less than 30 minutes. Check at 20 minutes.
4. Centrifuge the sample at 18,000 rpm in a JA-20 rotor for 30 minutes at 4°C. Be sure to balance the centrifuge tubes. Pour off the supernatant into a fresh tube. Add a quarter tablet of protease inhibitor to the supernatant. Dissolve by gently inverting the tube. Set aside 20 µL of sample at 4°C to run on an SDS-PAGE gel later.
5. While the sample is in the centrifuge (step 4), add 6 ml Ni-NTA resin (Qiagen) to a glass column (be sure to resuspend the resin in the storage solution to create a slurry before transfer to the column) and allow the buffer to drain off. To equilibrate the column, add 30 mL Buffer G. Pipet the solution into a 50 mL disposable centrifuge tube. Place at 4°C until ready for use.
6. Add the supernatant from step 4 to the resin. Be sure the stopcock is closed. Use a serological pipette to thoroughly mix the resin with the protein. Allow the resin to settle. The color of the fluorescent protein will develop and become more intense over time. The color of mCherry will develop more slowly than EGFP. The resin will begin to look rather bluish-purple as the mCherry matures and then eventually will turn magenta (Figure 7).
7. Once the resin has settled and there is clear buffer (may be the color of the fluorescent protein) above the column resin, begin draining the liquid out of the column. Collect the flow-through as one fraction in a disposable 50 mL centrifuge tube. Save a 20 µL sample of the fraction for SDS-PAGE analysis. It is advisable to save the complete fraction until the experiment is complete in case there is significant UBX-fusion protein in the flow-through that can be collected later.

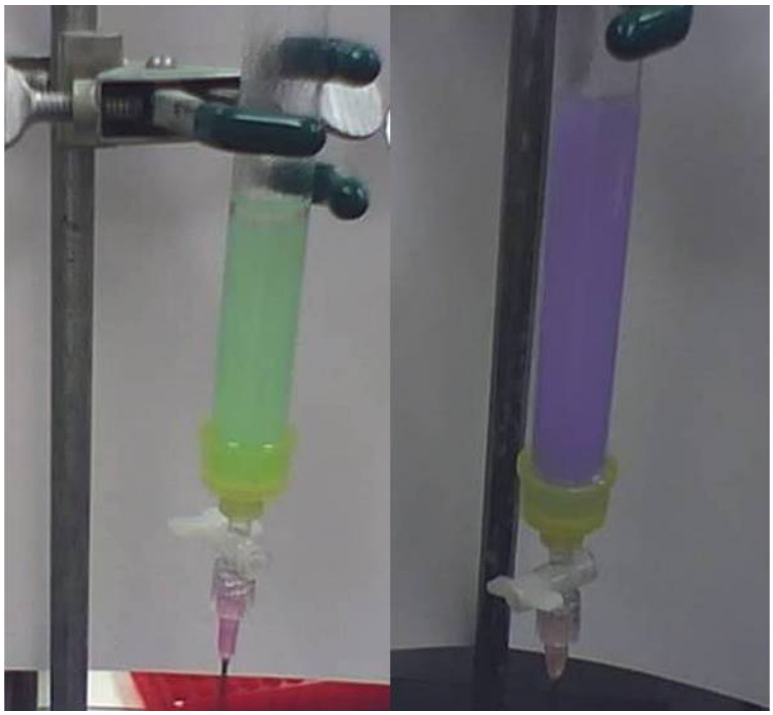

Figure 7. EGFP-Ubx and mCherry-Ubx protein on a Ni-NTA resin column.

8. When the buffer level is even with the column resin, close the stopcock. Be careful not to let the column run dry as it damages the resin. Add the wash buffer. Use the serological pipette to mix the resin with the new buffer. It helps to "fluff" the resin each time a new wash is added, because this is a very sticky protein. This step is not typical for most protein purifications but for this particular protein, it will help keep the columns flowing at a reasonable rate. Allow the resin to settle (at least until a thick layer is formed; it is not necessary to wait for all of the resin to settle). Collect the wash buffer in a 50 mL disposable centrifuge tube .

   Wash 1: 50 mL Buffer G + quarter tablet protease inhibitor (optional step)

   Wash 2: 50 mL Buffer G + 20 mM imidazole, pH 8.0 + quarter tablet protease inhibitor

   Wash 3: 50 mL Buffer G + 40 mM imidazole, pH 8.0 + quarter tablet protease inhibitor

   Wash 4: 50 mL Buffer G + 80 mM imidazole, pH 8.0 + quarter tablet protease inhibitor

9. Set aside a 20 µL sample from each wash for SDS-PAGE analysis later. Store wash fractions at 4°C until the fractions have been analyzed in case there is a lot of UBX-fusion protein that can be collected later.

10. Elute the UBX-fusion protein from the column:

    a. Add 2 mL elution buffer to the column and collect the fraction in a 15 mL disposable centrifuge tube.

    b. Add 2 mL elution buffer to the column, mix it into the resin. Let the resin sit for 10 minutes. Collect the fraction. Repeat two more times.

    c. Add 6 mL elution buffer to the column and elute in 2 mL fractions.

    d. Add 4 mg DTT (or 21 µL of a 1.25 M DTT solution) to each 2 mL fraction. Mix by inverting and store at 4°C.

ALTERNATELY: Add 10 mL elution buffer, mix with the resin, wait 10 minutes and collect the eluent as one 10 mL fraction. This procedure works well if class time is limited. It usually yields a protein concentration high enough for use in student designed experiments.

11. Run an SDS-PAGE gel to check fractions. Use 10 µL of each fraction + 20 µL loading dye per lane. Heat samples (except for the marker) at 98-100°C for 1 minute. Load gel.

    - Lane 1: 10 µL Kaleidoscope Plus Pre-stained Molecular Weight Marker
    - Lane 2: Wash 1
    - Lane 3: Wash 2
    - Lane 4: Wash 3
    - Lane 5: Wash 4
    - Lane 6: Elution Fraction 1
    - Lane 7: Elution Fraction 2
    - Lane 8: Elution Fraction 3
    - Lane 9: Elution Fraction 4
    - Lane 10: Elution Fraction 5
    - Lane 11: Elution Fraction 6
    - Lane 12: Elution Fraction 7

12. Run the gels at 200 volts for 35 minutes or until the bromphenol blue dye front has reached the bottom edge of the gel.
13. Stain one gel with BioSafe Coomasie to visualize all proteins present on the gel.
    a. Place the gel in a small tray with 200 ml of deionized water (gel should be covered). Place on rocking platform shaker for 5 minutes. Repeat twice.
    b. Replace the water with BioSafe Coomasie and stain the gel for 30-60 minutes. The gels can be left in the refrigerator until the next lab period if necessary.
    c. Destain the gel with three changes of deionoized water as in step over the course of 1-2 hours as needed to reduce background.
14. Combine the fractions containing reasonably pure UBX.
15. Dialyse the fractions containing UBX into freezing buffer (300 mM NaCl, 50 mM sodium phosphate buffer, 5% glucose, 1 mM DTT, pH 7.5).
    a. Pour the fractions into the open end of a clamped (or knotted) dialysis bag (MWCO10,000).
    b. Clamp the open end.
    c. Immerse in the freezing buffer in a beaker with a stir bar.
    d. Dialyze overnight at 4°C on a stir plate.
    e. Freeze in 1 mL aliquots in cryovials at -80°C.
16. Check the protein concentration using the BioRad protein assay.
    a. Label a microcentrifuge tube for each BSA standard (1 µg/mL, 2 µg/mL, 3 µg/mL, 4 µg/mL, 5 µg/mL, 6 µg/mL, 7 µg/mL, and 8 µg/mL) and each UBX protein sample (1:10,1:20, 1:50, and 1:100 dilutions) to be quantified.
    b. Set up dilutions as indicated in Table 5 (BSA Standard Curve) and Table 6 (Ubx quantification) as shown below.
    c. Mix by gently pipetting.
    d. Incubate at room temperature for 5 minutes.
    e. Measure absorbance at 595 nm.
    f. Plot the standards. Use this standard curve to determine the UBX protein concentration.

Table 5. BSA Standard Curve (BSA standard should be prepared in freezing buffer)

	Protein Dilutions							
	1 μg/mL	**2 μg/mL**	**3 μg/mL**	**4 μg/mL**	**5 μg/mL**	**6 μg/mL**	**7 μg/mL**	**8 μg/mL**
BSA (0.1 mg/mL)	100 μL	200μL	300μL	400μL	500μL	600μL	700μL	800μL
Freezing buffer	700 μL	600 μL	500 μL	400 μL	300 μL	200 μL	100 μL	0 μL
BioRad dye	200 μL	200 μL	200 μL	200 μL	200 μL	200 μL	200 μL	200 μL

Table 6. Ubx Dilutions for quantification of protein concentration

	Protein Dilutions			
	1:10	**1:20**	**1:50**	**1:100**
Ubx	100 μL	50 μL	20 μL	10 μL
Dialysis buffer	700 μL	750 μL	780 μL	790 μL
BioRad dye	200 μL	200 μL	200 μL	200 μL

Making Materials

Ubx materials can be made either from drops on siliconized slides or from pools of buffer. Siliconization of the glass slides creates a very hydrophobic surface and helps prevent protein binding to the glass surface. If the slide is properly siliconized, the protein drop will form a distinct bead. If the siliconization is inadequate, the protein will spread out over the slide. Teflon coated baking sheets and bars prevent the protein from sticking. The protein is very sticky and will stick to glassware and plasticware. This greatly reduces the amount of protein available to form materials.

When attempting to pull up sheets or rope, look for an oily sheen on the surface of the buffer. These oily-like patches are where your protein is most concentrated. Gently drag the paperclip or needle-tip through the patch while pulling up from the surface slightly to "catch" the protein. When pulling rope, use a slow, steady pace. If you pull too quickly, the rope will snap. You know you have a rope forming if you see a "Hershey kiss" shape on the surface of the buffer from the tension of the rope pulling away from the buffer surface as you drag the paperclip or needle tip upwards.

Ubx film

1. Dilute protein to a concentration of 0.6 mg/mL (or the desired concentration) using freezing buffer.
2. EDTA to a final concentration of 10 mM to prevent amorphous aggregates from forming instead of the desired ordered film.
3. Place 100 μL of the Ubx solution on the surface of a siliconized slide.
4. Cover the drop with a test tube or box lid to limit evaporation.
5. Incubate for 60-90 minutes.
6. Examine the drop for film formation.

Ubx sheets

1. Obtain a sample of Ubx protein at a concentration of 1 mg/mL.
2. Add EDTA to a final concentration of 10 mM.
3. Place 100 μL of the Ubx solution on the surface of a siliconized slide at room temperature.
4. Cover with a test tube cap or box lid to prevent excessive evaporation.
5. Leave the samples on the bench at room temperature overnight. Sheets should be present in the morning.

Figure 8. Pulling an mCherry-Ubx rope from a slide using a plastic-coated paperclip. Notice the "Hershey kiss" shape at the base that develops from the tension of the rope as it is pulled from the drop.

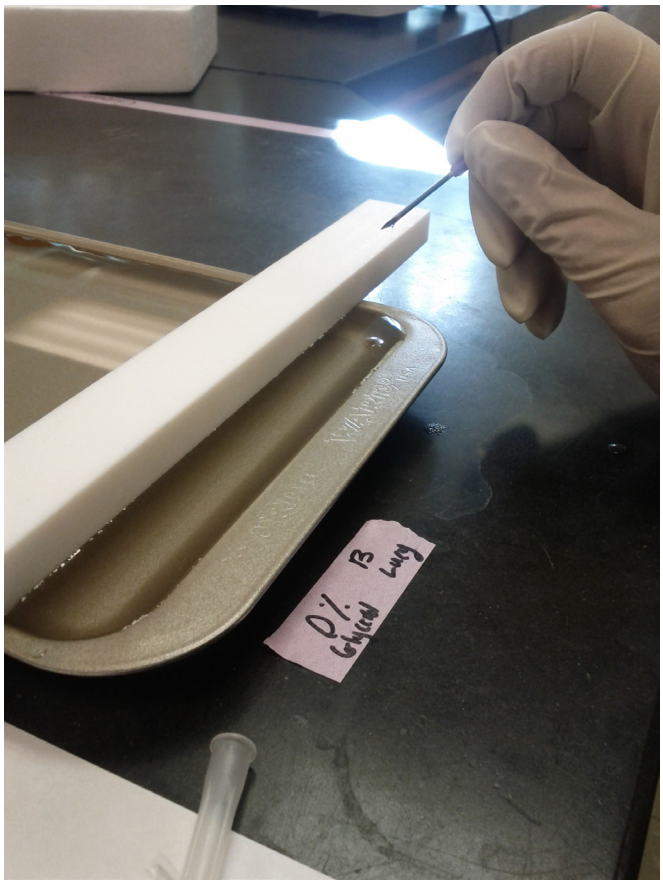

Figure 9. Setup for pulling rope from a Teflon coated pan. The pan is filled with Buffer G. Protein is pipetted along the surface of the buffer. The solution is allowed to stand undisturbed for 4 hours to overnight. A Teflon bar (or microcentrifuge rack) is gently placed at the back edge of the pan and dragged forward to within about 2.5 cm of the front edge of the pan. A syringe needle is used to pull the rope.

Ubx ropes

1. Place 100 μL of the Ubx solution on the surface of a siliconized slide.
2. Cover with a test tube cap or box lid to prevent excessive evaporation.
3. Incubate for 90 minutes at room temperature.
4. Use a needle or pipette tip to carefully draw out ropes from the sample (Figure 8).

****Alternative (AND RECOMMENDED) method for sheet and rope formation*

1. Place ~600 mL Buffer G in Teflon coated toaster oven pan or small cookie sheet.
2. Add ~1 mL of protein solution at 0.6 mg/mL or higher on the surface of the buffer. Lower concentrations work but product formation is reduced.
3. Wait 4 hours to overnight. Cover the pan with a cake pan or box lid to prevent dust from settling in the samples.
4. Carefully drag the side of a Teflon bar across the surface of the buffer to "crowd" the protein (Figure 9). The buffer level must be high enough that the bar is touching the surface of the liquid but low enough that it does not overflow from the pan. If evaporation has occurred or the level of buffer is too low, gently add Buffer G to the back side of the tray with a serological pipette disturbing the solution as little as possible. Once the bar has been dragged along the protein surface do not lift it and drag it across again. This will disturb the surface and the Ubx will need time to return to the surface to reform material.
5. Pull ropes using a pipette tip, needle, or paperclip. Pull sheets using a looped paper clip.

Materials

Equipment

Microcentrifuge
P10, P20, P200, P1000 pipettes
Water bath or heat block
Ice bucket
Power supplies
Horizontal gel electrophoresis boxes
Transilluminator box
Camera system for gel documentation (optional)
-80°C Freezer
100 mL graduated cylinders
Alcohol lamp
Cell spreaders
Balance
Chromatography column and ring stand
Pipette pump for serological pipettes
Oakridge centrifuge tubes for Beckman JA20 rotor
Nalgene centrifuge bottles (500 mL)
Beckman centrifuge with JA20 and JA10 rotor or similar
Flasks (100-250 mL, 4 L)
37°C shaker
Room temperature shaker
Spectrophotometer
Teflon coated cookie sheet or toaster oven pan (Nordic Ware cat. #: 43010; **http://www.nordicware.com/contact**)
Teflon bar (0.5" thick x 1" wide x 12" long) (K-mac Plastics cat. #: KBS-1311 **http://www.k-mac-plastics.com/**)
Dialysis tubing clamps
Large beakers (1 L or larger)
Stir bars
Stir plates
Test tube racks (able to hold 15 and 50 mL disposable centrifuge tubes)
Microcentrifuge tube racks

Supplies

Tips for P10, P20, P200, P1000 pipettes
Beaker of microcentrifuge tubes
Matches
Sharpie marker
Cryovials
Serological pipets
pH paper
15 and 50 mL disposable centrifuge tubes
Transfer pipets (disposable)
Dialysis tubing (MWCO 10,000) (VWR cat. #: PI168100; **www.vwrsp.com**)
Wooden applicator sticks
Syringe needles or paperclips
Siliconized slides

Reagents

Beaker of 70% ethanol for cell spreaders
10% Bleach bottle
80% glycerol

Transformation

0.5 mL sterile deionized water
4 µL pUC18/19 control DNA
1 mL aliquots of 2YT (16 g bacto-tryptone, 10 g bacto-yeast extract, 5 g NaCl and water to 1 L)
45 LB-ampicillin/chloramphenicol plates per section (if going into Rosetta2(DE3) cells
100 µg/mL ampicillin plates and 12.5 µg/mL chloramphenicol)
Competent Rosetta2(DE3) cells (50 µL aliquots) (Novagen cat. #:71397; **www.emdchemicals.com**)

Protein Expression

LB broth
500 mM IPTG (Sigma cat. #: I-5502; **www.sigmaaldrich.com**)
Lysozyme (Sigma cat. #: L-6876)

Protein Purification

Lysis buffer (Buffer G) (50 mM sodium phosphate (monobasic), 500 mM NaCl, 5% glucose)
Buffer G + 20 mM imidazole (imidazole from Sigma cat. #: I5513)
Buffer G + 40 mM imidazole
Buffer G + 80 mM imidazole
Freezing Buffer (300 mM NaCl, 50 mM sodium phosphate, 5% glucose, 1 mM DTT, pH 7.5)
2-mercaptoethanol (beta-mercaptoethanol)
Sodium phosphate dibasic
Protease inhibitor tablets (Roche: Complete Mini Protease Inhibitor, cat. #: 11-836-153-001; **https://www.roche-applied-science.com/servlet/new/solutions.jsp**)
Ni-NTA resin (Qiagen: cat. #: 30210, 25 ml; **www.Qiagen.com**)
DnaseI (2,000 Kunitz units/mL in 0.15 M NaCl; use 80 µL per L cells) (Roche: cat. #: 10-104-159-001, 100 mg; **https://www.roche-applied-science.com/servlet/new/solutions.jsp**)
DTT (dithiothreitol)

PAGE

SDS-PAGE gel running buffer (25 mM Tris/192 mM glycine/0.1% SDS)
SDS-loading buffer (60 mM Tris-Cl, 20% glycerol, 0.2% bromophenol blue, 4% (w/v) SDS (electrophoresis grade), 200 mM beta-mercaptoethanol)

NATIVE-PAGE gel running buffer (optional): (25 mM Tris/192 mM glycine) NO SDS
Native Gel Loading Dye (optional): 20% glycerol, 0.13 M Tris, 0.1% bromophenol blue, pH 6.8
Bio-Safe Coomasie (Bio-Rad: cat. #: 161-0787EDU)
Polyacrylamide gels
10 µL aliquots Kaleidoscope marker (Bio-Rad: cat.# 161-0375EDU)

Protein Quantification

BioRad Protein Assay Kit II (cat. #: 500-0002)

Notes for the Instructor

The preparatory work required for this laboratory exercise is extensive. If teaching assistants are not available, I recommend you use undergraduate students as teaching assistants to keep the workload manageable. Once the project has been run once, former students can be invited to serve as teaching assistants and are usually eager and willing to do so and best of all, you have already trained them and they understand the project.

Before students begin planning their experiments, it is critical that they have a firm understanding of the nature of the protein material with which they will be working. After the topic is introduced, it is important to set aside class time to allow students to attempt to pull ropes and sheets from samples that have been prepared ahead of time. The protein is fragile and students need to understand how difficult it is to pull and manipulate ropes before they begin planning experiments. Without this hands-on training before the planning stage, a high percentage of projects suggested will not be feasible within the constraints of materials and equipment available to students.

The Ubx-pET19 plasmid can be maintained in DH5α. There is a tendency for the plasmid to rearrange in the expression lines due to the fact the Ubx protein makes the cells a bit sick. For this reason, you must do a fresh transformation each time you wish to express protein. One overnight incubation is needed to allow colonies to develop on the transformation plates. A second overnight step is needed to grow up 10 mL of overnight culture from a selected colony. After induction with IPTG, cells will grow for 3 hours to overnight depending on the level of expression you desire or your time schedule. The best expression from mCherry-Ubx lines is obtained with an overnight incubation. The best expression from EGFP-Ubx lines is with a 3 hour incubation (although yields are still reasonable if the culture grows overnight after induction).

Following the protein purification steps from lysis of the pellet to elution takes approximately 6 hours. To condense the time frame into a 3 hour class period, the lysis step can be done before class so that students begin at the point the protein is loaded onto the column. If two class days are dedicated to protein purification, students can complete the lysis step one day and the column chromatography the second day. Teaching assistants or student volunteers will be needed to complete the purification on day one and to complete the lysis steps on day two. The advantage of this approach is that students get to go through the entire protocol and a great deal of protein is generated for downstream use in the student projects.

Really pure protein is not necessary for materials formation. In fact, ropes can be pulled from cell lysate without purifying the protein at all. However, the more pure the protein, the better the materials formation. For the purpose of classroom projects, simply pooling all the elutes from all the washes that have a reasonably deep color is sufficient.

The Ubx protein is sticky. It will stick to plastic and glass. It will also clog up the fret at the bottom of the chromatography column. Should this occur, simply clean the column with a 10% HCl solution and flush the column thoroughly. Because the protein sticks to plastic and glass, it is important to set up the experiments to pull materials in Teflon coated cookie sheets (or anything Teflon coated). Teflon bars can be purchased to crowd the materials. Microcentrifuge racks also work for this but some protein will be lost due to sticking to the rack.

While the Ubx protein is a remarkably stable biomaterial, it is not as stable in solution prior to material formation. Materials cannot be made from Ubx solutions that have been at 4°C for more than about 4 days. The quality of material that can be obtained declines with time. To circumvent this problem, protein can be dialyzed into freezing buffer and stored at -80°C. Aliquots of the protein can be thawed as needed for student projects. Be sure to carefully record the concentrations of each batch of protein aliquoted. The students will need that information to set up their experiments. Also, it is difficult to pull ropes when the ambient humidity is low, particularly in the winter months when the building heat is running. Room humidifiers can be used to improve rope formation.

If students need a higher concentration of protein than what is available, the protein can be concentrated using Vivaspin Concentrators (10 kD MWCO) from Vivaproducts (**http://www.vivaproducts.com/contact-vivaproducts.html**).

Adequate laboratory bench space is needed to implement the student-designed experiments portion of this curriculum. Space can be managed by marking out pre-set areas with lab tape. Each group is expected to stay in their own space. Premarking spaces eliminates territorial arguments that might otherwise arise. If space is limited, the student-designed experiment portion of the lab can be spread over two weeks with half the groups doing their work the first week and the other half working in the lab the second week.

Research is expensive. Clearly define limits on product use. For example, students in the BCHS4311 course at the University of Houston, are told from the beginning of the project that each group will only receive 6 mLs of purified protein. Supervise any reagent mixing and check calculations to avoid wasting supplies. Limit projects to those that can be

done with items students supply or common supplies stocked routinely in the lab. Be sure supplies are well-stocked ahead of time.

There are an endless number of ways this lab can be expanded to meet the needs of a molecular biology-rich course. One suggestion to add depth to this lab is to have students perform a Western blot on their collected fractions from the purification using antibodies to the histidine tag. Qiagen offers an Anti-His Antibody Selector kit (cat. #: 34698) that provides chromogenic detection which is often the method of choice for a teaching laboratory classroom.

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Literature Cited

Bondos, S. E., Catanese, D.J., Tan, X., Bicknell, A., Li, Likun, and Matthews, K. 2004. Hox transcription factor Ultrabithorax Ib physically and genetically interacts with Disconnected Interacting Protein 1, a double-stranded RNA-binding protein. *Journal of Biochemistry*, 279: 26433-26444.

Bondos, S. E., and Tan, X. 2001. Combinatorial transcription regulation: the interaction of transcription factors and cell signaling molecules with homeodomain proteins in *Drosophila* development. *Critical Review in.Eukaryotic Gene Expression*, 11: 145-171.

Bondos, S. E., Tan, X., and Matthews, K. S. 2006. Physical and genetic interactions link Hox function with diverse transcription factors and cell signaling proteins. *Molecular & Cellular Proteomics*, 5: 824-834.

Greer, A. M., Huang, Z., Oriakhi, A. Lu, Y., Lou, J., Matthews, L. S., and Bondos, S. E. 2009. The Drosophila transcription factor Ultrabithorax self-assembles into protein-based biomaterials with multiple morphologies. *Biomacromolecules*, 10: 829-37.

Huang, Z., Lu, Y., Majithia, R., Shah, J., Meissner, K., Matthews, K. S., Bondos, S. E., and Lou, J. 2010. Size dictates mechanical properties for protein fibers self-assembled by the *Drosophila* Hox transcription factor Ultrabithorax. *Biomacromolecules*, 11: 3644-3651.

Huang, Z., Salim, T., Brawley, A., Patterson, J., Matthews, K. S., and Bondos, S. E. 2011. Functionalization and patterning of protein-based materials using active ultrabithorax chimeras. *Advanced Functional Materials,* 21: 2633-2640.

Hughes, C., and Kaufman, T. 2002. Hox genes and the evolution of the arthropod body plan. *Evolution & Development*, 4: 459-499.

Majithia, R., Patterson, J. L., Bondos, S. E., and Meissner, K. E. 2011. On the design of composite protein-quantum dot biomaterials via self-assembly. *Biomacromolecules*, in press.

Shu, X., Shaner, N., Yarbrough, C., Tsien, R., Remington, S. 2006. Novel chromophores and buried charges control color in mFruits. *Biochemistry*, 45: 9639-47.

Stepanenko, O., Verkhusha, V., Kuznetsova, I., Uversky, V., and Turoverov, K. 2008. Fluorescent proteins as biomarker and biosensors: Throwing color lights on molecular and cellular processes. *Current Protein and Peptide Science*, 9: 338-69.

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Appendices

APPENDIX A: Siliconization Protocol

In order to pull materials using the slide method, the slides or the cover slips must be siliconized. Siliconized slides can be purchased or you can treat them yourself using the protocol below. SIGMACOTE (Sigma, cat. #SL2) is composed of silicone suspended in heptane. It forms a thin film on the glass that acts as a water repellent. Siliconizing the cover slips is less expensive. After the cover slip has been siliconized, it can be attached to a slide with clear nail polish for easier handling. Work in a fume hood!

1. Obtain a clean, dry cover slip.
2. Holding the slip with tweezers, dip the slip in SIGMACOTE (undiluted).

3. Allow the cover slip to air dry in a fume hood. Leaning them along the sides of an empty pipette box container rather laying them flat allows the coating on both sides to remain intact.
4. Rinse the cover slips with water. Allow to dry. Stack cover slips back in their original container. Be sure to label the container "siliconized".

APPENDIX B: Buffer and Solution Recipes

Buffer G (50 mM sodium phosphate, 500 mM sodium chloride, 5% glucose)

For 1 L:

1. Add the following to a beaker:

   29.22 g sodium chloride
   50 g glucose

2. Add 100 mM sodium phosphate buffer (pH 8) up to a final volume of 1 L. Stir.

Buffer G + 200 mM imidazole (1 L) (Stock)

1. Add the following to a beaker:

   29.22 g sodium chloride
   50 g glucose
   13.62 g imidazole

2. Add 100 mM sodium phosphate buffer (pH 8) up to 800 mL. Stir.
3. pH the solution to 8.
4. Bring the volume up to 1 L with 100 mM sodium phosphate buffer (pH 8).

Buffer G + 20 mM imidazole (1 L)

1. Add 100 mL 200 mM Buffer G + imidazole stock (see protocol for imidazole stock).
2. Add 900 mL Buffer G.
3. Stir.

Buffer G + 40 mM imidazole (1 L)

1. Add 200 mL 200 mM Buffer G + imidazole stock (see protocol for imidazole stock).
2. Add 800 mL Buffer G.
3. Stir.

Buffer G + 80 mM imidazole (1 L)

1. Add 400 mL 200 mM Buffer G + imidazole stock (see protocol for imidazole stock).
2. Add 600 mL Buffer G.
3. Stir.

Sodium phosphate buffer

To make sodium phosphate buffer, stock solutions of the mono and dibasic salts need to be made first.

Make 1 L of 1 M Na_2HPO_4:

1. Add 268.07 g sodium phosphate dibasic heptahydrate to a beaker.
2. Add deionized water up to 1 L.
3. Stir until dissolved.

Make 1 L of 1 M NaH_2PO_4:

1. Add 120 g sodium phosphate monobasic (M.W. 120).
2. Add deionized water up to 1 L.
3. Stir until dissolved.

Make 100 mM sodium phosphate buffer, pH 8:

1. Add 93.2 mL 1 M Na_2HPO_4 to a beaker.
2. Add 6.8 mL 1 M NaH_2PO_4
3. Add deionoized water to 1 L.

1x SDS PAGE Loading Buffer

(50 mM Tris-HCl (pH 6.8), 2% (w/v) SDS (electrophoresis grade), 0.1% bromophenol blue, 10% (w/v) glycerol, beta-mercaptoethanol (2-mercaptoethanol)

1. Add the following to a beaker:

   5 ml 50 mM Tris-HCl (pH 6.8)
   2 ml 10% SDS (electrophoresis grade)
   0.1 g bromophenol blue
   12.5 ml 80% glycerol

2. Mix on stir plate.
3. Add deionized water up to 100 mL.
4. Store at room temperature. Add 69 µL 14.3 M beta-mecaptoethanol just before use.

Freezing Buffer (300 mM NaCl, 50 mM sodium phosphate buffer, 5% glucose, 1 mM DTT)

1. Add the following to a beaker:

   17.5 g NaCl
   500 mL 100 mM sodium phosphate buffer
   (see protocol for phosphate buffer)
   50 g glucose
   0.15 g DTT

2. Mix well. Store at 4° C.

DNase I (20 mg/mL)

Dissolve 100 mgDNase I (Roche: cat. #: 10-104-159-001) in 5 mL cold 0.15 M NaCl to yield 2,000 Kunitz units/mL). Make 160 µL aliquots and store at -80°C.

IPTG (500 mM)

Add 1.2 g IPTG (238.31 g/mol) + sterile deionized water up to 10 mL.

2 x YT Medium

1. Add 900 mL deionized water to a beaker.
2. Add the following:

   16 g bacto-tryptone
   10 g bacto-yeast extract
   5 g NaCl
   Stir until dissolved.

3. Adjust pH to 7.0 with 5 N NaOH.
4. Bring volume up to 1 L with deionized water. Autoclave.

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