

Aipotou I & II: Genetics & Biochemistry

Objectives:

- To reinforce your understanding of Genetics, Biochemistry, and Molecular Biology
- To show the connections between these three disciplines
- To show how these three approaches can be combined to give a complete picture of a biological phenomenon
- To figure out a complete explanation of a biological phenomenon
- To experience how scientists develop and test hypotheses

Introduction:

Aipotou (pronounced “ā pō too” – the word is “utopia” spelled backwards) is a simulated world where users can explore a biological phenomenon in terms of genetics, biochemistry, molecular biology, and evolution.

The Biological Phenomenon Under Study

In this lab, you will explore the biological mechanisms behind the expression of flower color in a hypothetical plant. These flowers can be white, red, orange, yellow, green, blue, purple, or black.

Scenario:

You are the chief biologist for a breeder of fine flowers. Your company sells seeds to customers to plant in their gardens. Since most of your customers expect that the flowers will grow each year from seeds produced the previous year, you try to produce true-breeding plants whenever you can.

You’ve found a new species of flower with an attractive shape. You’ve collected four plants from the wild: two green, one red, and one white. Your customers would really like to have purple flowers from this plant. You set out to create a true-breeding purple flower.

Hypothesis Testing

In the Aipotou lab, you will use a process much like that used by practicing scientists as they conduct research. Although this process almost never follows a formula, it often proceeds as follows:

1. **Observe Patterns.** Observe the natural world and look for patterns, exceptional events, etc. For example, you might observe that red flowers sometimes have white offspring.
2. **Develop hypotheses.** From the observations, you define testable hypotheses – statements or questions that can be addressed experimentally. Continuing the example, you might reasonably hypothesize that red is dominant to white.
3. **Test hypotheses.** You then set up experiments or observations that will collect data that bear on your hypothesis. In the example, you might cross pure-breeding white with pure-breeding red. If your hypothesis is correct, all the offspring will be red. If you get another result, your hypothesis is incorrect.

4. **Revise hypotheses as necessary.** If your results do not match your prediction, you need to revise your hypothesis and go to Step (3) again until they do match. You have already been doing this informally in the VGL labs. You should note that this process never really ends – typically there is more to investigate once you’ve reached a satisfactory conclusion.

The “answer” vs the “point”

Although there is an answer – the complete model of color formation in these plants – and you can find it with the tools in Aipotu, *having* the answer is not as important as *finding* the answer. We could just tell you the answer, but that would leave out the major learning goals of this lab:

- Experiencing how scientific hypothesis testing works.
- Experiencing how you can use the tools of modern biology to completely understand a phenomenon.

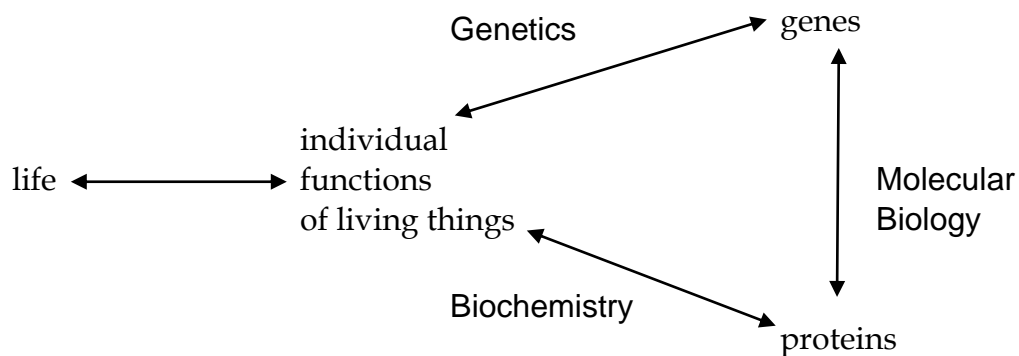
So, while we will work together to find ‘the answer’, the real *point* of the lab is the journey.

Unifying Three Parts of Modern Biology

The three major parts of this course are:

- **Genetics** = explaining biological phenomena in terms of genes. *How is flower color inherited?*
- **Biochemistry** = explaining biological phenomena in terms of proteins and other molecules. *How does protein sequence determine protein structure and color?*
- **Molecular Biology** = explaining the connection between genes (DNA) and protein. *How does the DNA sequence of the color gene lead to a particular color?*

The connections between these three fields of biology are shown below in a diagram credited to David Botstein (each of the arrows corresponds to “can be explained in terms of”, the words in this font are the different disciplines of biology):



Understanding any biological phenomenon requires information from all three of these disciplines. Today, time permitting you will use all three to investigate the model of color production in these flowers.

Getting Aipotu

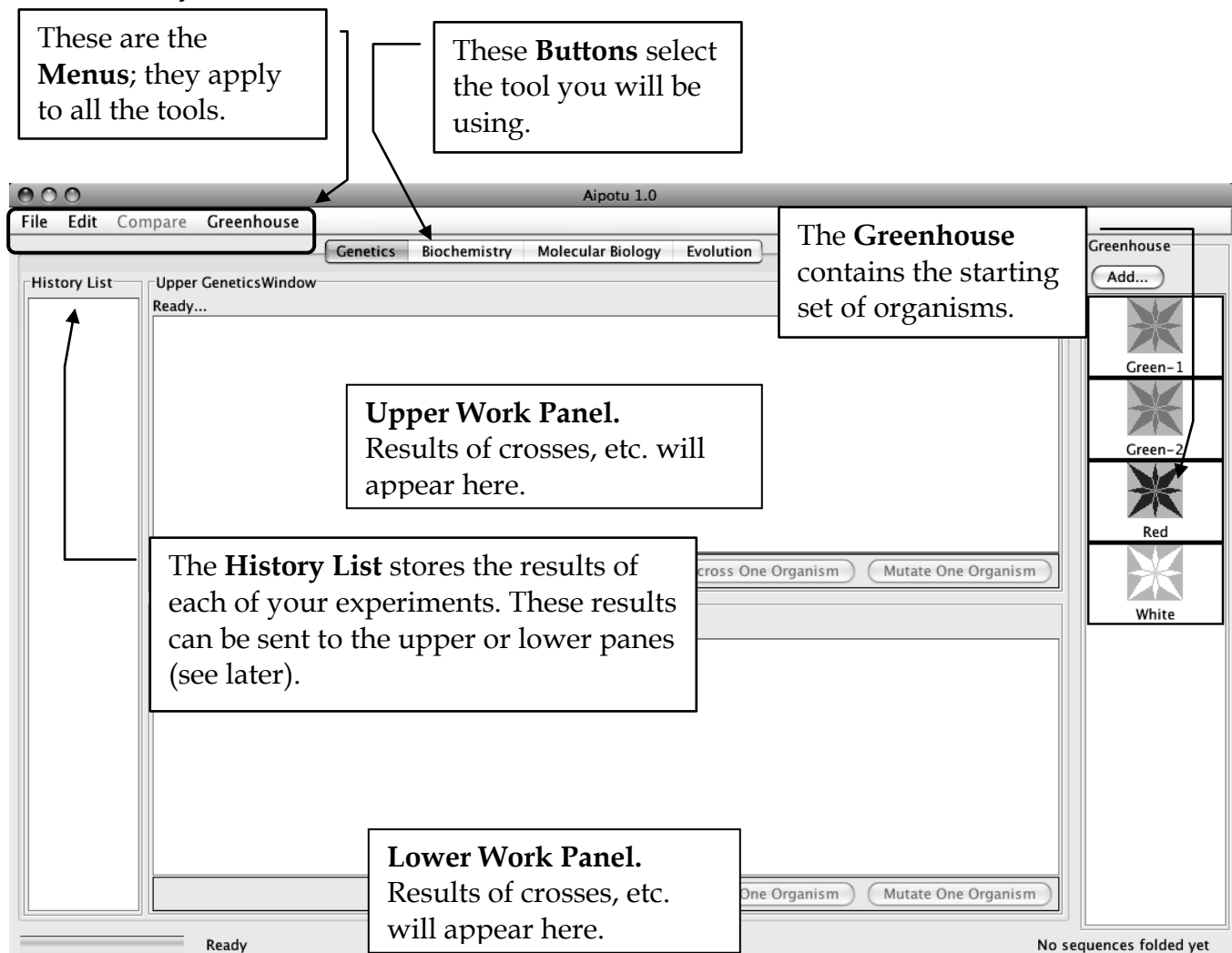
You can download and install this free software which Pr. Brian White created here at UMass Boston and have it on your own computer. You should download it from <http://intro.bio.umb.edu/aipotu/> or the link on the course online component. It is already installed on the desktops of the computers in your course laboratory.

Tools Available in Aipotu

For each of the three disciplines, there is a different tool in Aipotu that will allow you to explore the same set of creatures using a different set of techniques:

- **Genetics.** The flowers in this simulation are diploids. As with most flowers, they are all hermaphrodites (both male and female). With this tool, you can perform the following experiments:
 - *Cross any two organisms.* A new window will appear with the offspring of this cross.
 - *Self-cross any organism.* In this case, the single selected organism is both mother and father to the resulting offspring.
 - *Mutate any organism.* A new window will appear with a set of flowers that are mutant versions of the selected organism.
- **Biochemistry.** The color in these flowers results from the form(s) of pigment proteins present in an individual plant. With this tool, you can perform the following experiments:
 - *Examine the pigment proteins present in a plant.* The tool shows you the amino acid sequence and two-dimensional structure of the pigment proteins present in a given plant.
 - *Design your own proteins.* You can edit an existing protein sequence or type in an entirely new sequence. The program will then predict the two-dimensional structure of the resulting protein as well as its color. It will also predict the color resulting from the combination of any two proteins.
- **Molecular Biology.** The pigment proteins in these plants are produced by pigment protein genes. With this tool, you can perform the following experiments:
 - *Examine the pigment protein genes present in a plant.* The tool shows the DNA, pre-mRNA, mature mRNA, and protein sequences present in a given plant. You can explore the introns, exons, etc. of these genes.
 - *Design your own genes.* You can edit an existing DNA sequence or type in an entirely new sequence. The program will then predict the mRNA, protein sequence, two-dimensional structure of the resulting protein as well as its color. It will also predict the color resulting from the combination of any two proteins.
 - *Design your own plants.* You can save edited DNA sequences as new organisms for further study.

When you start Aipotou, the program will load the four starting flower types into the Greenhouse, you will then see a screen like this:



Each organism is shown as a flower:



The color of the picture shows the color of the flower. These flowers can be white, red, orange, yellow, green, blue, purple, or black. When any organism is selected, the black border turns green to show that it has been selected.

The next sections of this manual will show you the various tools and the tasks that you will need to carry out for Aipotou I Genetics and Aipotou II Biochemistry, you will continue with Aipotou III Molecular Biology at a later point in the semester. Therefore, take good notes now as you work through parts I & II.

Part I A: Genetics - Crosses

Tasks:

- Determine how color is inherited in these flowers. **NOTE:** the color is controlled by one gene only.
 - Determine the colors of the alleles present in the original set of organisms.
 - Which alleles are dominant?
 - Which alleles are recessive?
 - How do the alleles combine to produce the overall color of the plant?
- Construct a purple organism to demonstrate your understanding of this process.

Using the tool:

You can switch to this tool by clicking the “Genetics” tab near the top of the window.

There are three kinds of experiments you can perform with this tool. The following sections use examples to show you how to do each; you will need to devise your own experiments to carry out the tasks above.

1) *Cross Two Organisms.* Suppose that you wanted to cross Red and White:

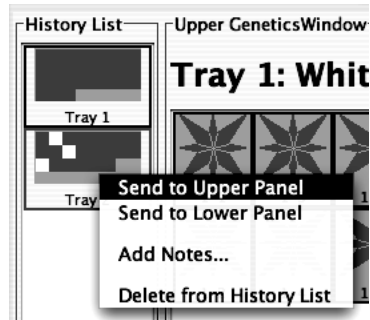
1) Click on Green-1 and then on White in the **Greenhouse**. The rectangular borders of both should turn green to show that they have been selected. The “Cross Two Organisms” buttons in the **Upper** and **Lower Work Panels** should be activated.

2) Click the “Cross Two Organisms” button in the **Upper Work Panel**. You should see something like this:

These are the offspring of the cross. You can select any of these for crossing, etc.

This represents the **Tray** of plants containing the offspring you just produced. The colored squares represent the different colors of the offspring produced.

If you double-click on the **Tray** in the **History List**, you get a pop-up menu with a list of useful options:



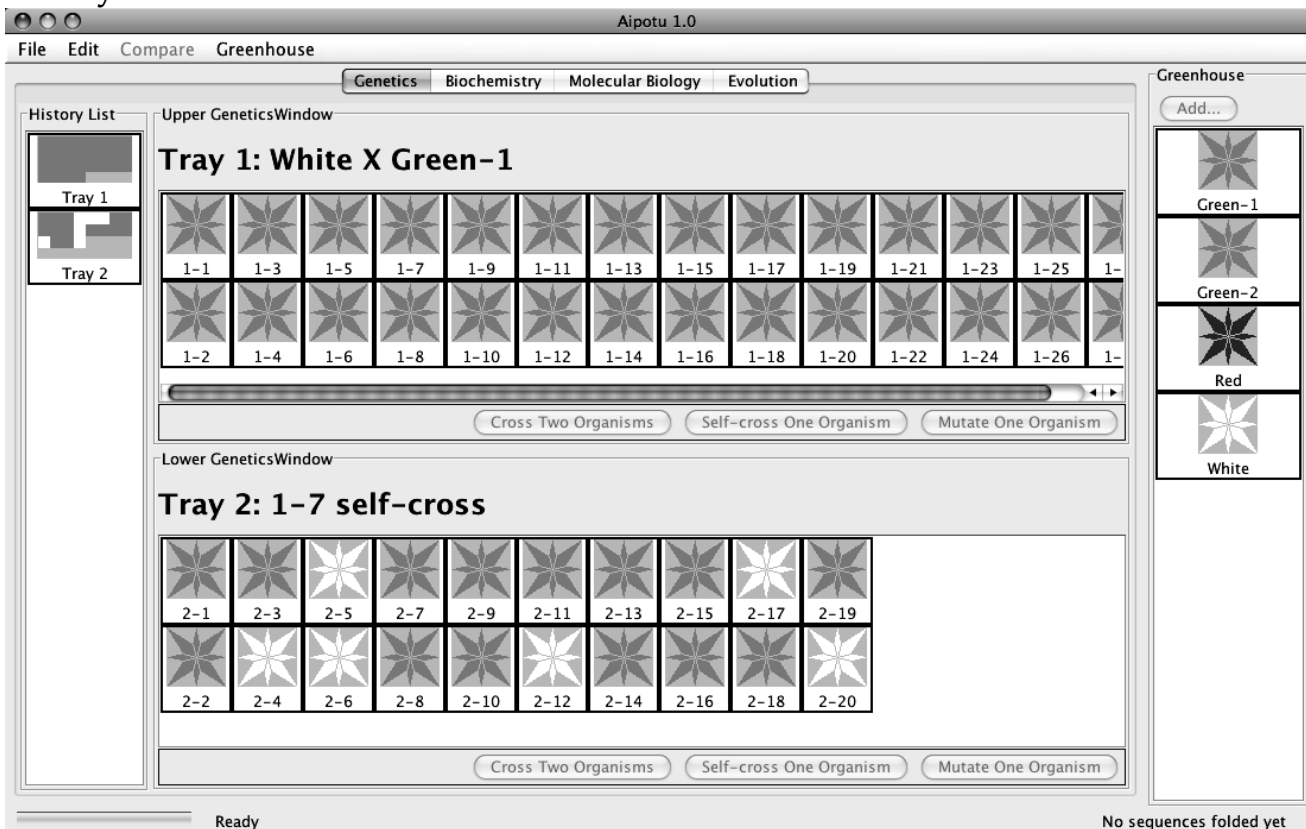
- **Send to Upper Panel:** Sends this **Tray** to the **Upper Panel** so you can cross those organisms.
- **Send to Lower Panel:** Sends this **Tray** to the **Lower Panel** so you can cross those organisms.
- **Add Notes...:** Allows you to add notes to the **Tray** in the **History List**. These notes will appear if you leave the cursor over the **Tray** for a few seconds.
- **Delete from History List:** deletes the **Tray** from the **History List**; this is cannot be undone.

II) *Self-cross a single Organism.* Suppose that you wanted to self-cross one of the offspring in **Tray 1**:

- 1) Select any one organism from **Tray 1** in the **Upper Work Panel**. You can de-select an organism by clicking on it. When you have only one organism selected, the “Cross Two Organisms” buttons will be grayed out and the “Self-Cross One Organism” and “Mutate One Organism” buttons will be activated.
- 2) Click the “Self-Cross One Organism” button in the **Lower Work Panel**. You should see something like this (since offspring are generated by random choice of parental alleles, you will likely see slightly different numbers of red and white offspring):

You should see something like this:

The offspring of this self-cross are in the **Lower Work Panel**. Note the addition of Tray 2 to the **History List**.



At this point, there are several other things you can do:

A) If you find an interesting organism, you can save it to the **Greenhouse**:

- 1) Click the "Add..." button at the top of the **Greenhouse**.
- 2) You will be prompted to give the organism a name. Give it a descriptive name and click "OK". You will see your new organism appear in the **Greenhouse**. You can now access it using the other tools in this program.
- 3) At this point, the organism is saved in the program, but not on the disk. To save the contents of the **Greenhouse** to disk, click on the **Greenhouse Menu** and select "Save Greenhouse".

B) You can cross or mutate any of the organisms visible in the **Greenhouse**, **Upper Work Panel**, or **Lower Work Panel**.

C) You can bring any **Tray** from the **History List** to a **Work Panel** by double-clicking the **Tray** and selecting the appropriate item in the pop-up menu.

D) You can add notes to any **Tray** in the **History List** by double-clicking the **Tray** and choosing the "Add Notes..." item from the resulting pop-up menu. These notes will appear if you leave the cursor over the **Tray** for a few seconds.

IMPORTANT NOTE:

This software is under development. Please treat it gently and be patient. Please report any bugs to your TA. **You should save your Greenhouse regularly, especially if you save a large number of organisms.**

Specific Tasks to do with this tool:

- a) How many different alleles are there? Which colors do they produce? It will be useful to use multiple-allele notation like this: C^R = red; C^G = green, etc.
- b) Which are dominant and which are recessive?
- c) How do the colors combine to produce an overall color? For this, it may help to make a genotype-phenotype table and then try to abstract rules from that. You should be sure that you have tried all the possible combinations of the alleles you found in part (a).
- d) Using the symbols you have developed, give the genotypes of the four starting organisms.
- e) Using this knowledge, construct a purple flower.
- f) Can you construct a pure-breeding purple flower with the starting set of organisms?

How to go about accomplishing these tasks

Follow the procedure from page (1) - look for patterns, make hypotheses, test hypotheses, revise hypotheses. You should be thinking in these terms and move on to using the other two tools (Aipotu II and III).

Put your data in the tables below:

(a) and (b) allele color

(c) Genotype Phenotype

What rules can you find to explain the data above?

(d) Using the allele symbols above, what are the genotypes of the starting strains?

Green-1:

Green-2:

Red:

White:

(e) Have your TA check off that you have constructed a purple flower. For full credit, you must be able to explain to your TA why the flower is purple.

(f) Can you construct a pure-breeding purple flower using only the four starting strains? Why or why not?

Part I B: Genetics - Mutation

Another very important way scientists study a process is to alter it by mutation and see what happens. You will make a mutant organism now and then explore it in detail.

Tasks:

- Make a mutant version of one of the starting alleles that has a different color.
- Note the change to its color, protein sequence, and DNA sequence.
- Save it for later analysis.

Using the Tool

Mutate any one organism. This simulates treating the cells of one organism with a strong dose of a mutagen and then growing each of those mutant cells into a separate plant. The flowers of the resulting mutant plants are displayed in a new **Tray**. This process can take a few minutes. Suppose that you wanted to mutate one of the white organisms in Tray 2:

- 1) Select a white organism from Tray 2. When you have only one organism selected, the “Cross Two Organisms” buttons will be grayed out and the “Self-Cross One Organism” and “Mutate One Organism” buttons will be activated.
- 2) Click the “Mutate One Organism” button in the **Upper Work Panel**. A pop-up will appear telling you that the program is busy making mutants. You can cancel this process if you like.

You will then see a new **Tray** with the mutants and a new entry in the **History List**.

Procedure

- (1) Make a pure-breeding strain of the color of your choice. Note the color here_____.
- (2) Select one of the pure-breeding organisms and click the **Mutate One Organism** button. Wait while it makes the mutants.
- (3) With luck, your mutant will have a different color. If not, try mutating the pure-breeding strain again until you see another color. Do not mutate an organism that was already mutated.
- (4) Save your mutant to the **Greenhouse** with a descriptive name like “Green from Red”.
- (5) From the **Greenhouse** menu, choose **Save Greenhouse**.
- (6) Find out how the mutation has changed the *Protein Sequence*.
 - a. Click on the **Biochemistry** tab at the top of **Aipotv**.
 - b. Double-click on the organism you just saved in the **Greenhouse**.

- c. Aipotou then displays the shapes of the proteins produced by the two alleles that this organism is carrying. One of these will be unchanged – it will have the color that you started with; the other will be mutated, resulting in an altered color.
 - d. From the **Compare** menu, choose **Upper vs Lower**. A window will pop up showing the two amino acid sequences with differences highlighted in red. Make a note of the difference in the space below.
- (7) Find out how the mutation has changed the *DNA Sequence*.
- a. Click on the **Molecular Biology** tab at the top of **Aipotou**.
 - b. Double-click on the organism you just saved in the **Greenhouse**.
 - c. Aipotou then displays the genes that encode the two alleles that this organism is carrying. One of these will be unchanged – it will have the color that you started with; the other will be mutated, resulting in an altered color.
 - d. From the **Compare** menu, choose **Upper vs Lower**. A window will pop up showing the two DNA sequences with differences highlighted in red. Make a note of the difference in the space below.

Aipotou II Biochemistry

You now need to expand your understanding to *how* these colors are produced and how they interact at a *Biochemical level*.

In this lab session, you will use a version of the Protein Investigator that computes the color of the proteins you fold. You should know that no real protein works this simply; however, the *way you figure out* how structure leads to color is the same as a biochemist would use in the lab.

Fine Tuning Your Hypothesis Testing

With all three of the Aipotou tools (genetics, biochemistry, molecular biology) you will use a process much like that used by practicing scientists as they conduct research. Although this process almost never follows a formula, it often proceeds as follows:

Observe Patterns. Observe the natural world and look for patterns, exceptional events, etc. For example, you might observe that red proteins tend to have long thin shapes.

Develop hypotheses. From the observations, you define testable hypotheses – statements or questions that can be addressed experimentally. Continuing the example, you might reasonably hypothesize that long thin proteins will be red.

Test hypotheses. You then set up experiments or observations that will collect data that bear on your hypothesis. In the example, you might type in a sequence of amino acids that would be expected to fold into a long thin shape, fold the protein, and

observe its color. If your hypothesis is correct, it will be red. If you get another result, your hypothesis is incorrect.

Revise hypotheses as necessary. If your results do not match your prediction, you need to revise your hypothesis and go to Step (3) again until they do match.

Important Note: It is always important to keep in mind, the ‘scientist’s mantra’: always be asking yourself “How could I be being fooled by this?” To continue the example from the previous page, consider the following:

Suppose that the long thin protein were red, you might congratulate yourself that you had found the connection between shape and color. However, what if the real mechanism is that proteins containing arginine are red and your long thin protein just happened to be made with arginine. The red color would be fooling you into thinking you had it right.

How do you avoid this trap? Even the best scientists sometimes fall into traps like this. The answer is to *always be thinking of alternative explanations for your results*. In the case above, one long thin red protein does not mean that “long & thin = red”. You have to collect more data: proteins that aren’t long and thin; long and thin proteins with different amino acids; etc.

Remember, it is the *process of science* rather than the *answer* that is most important. Don’t forget to collaborate as a class to solve this scientific problem.

Tasks:

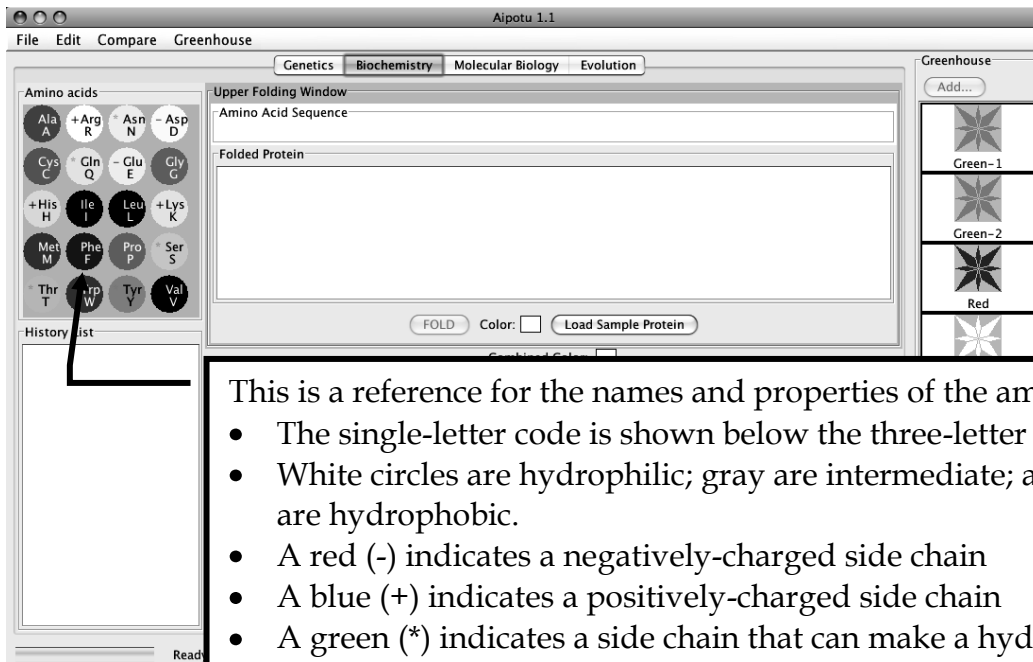
Work together as a class to:

- Determine the differences in amino acid sequence between the proteins produced by the alleles you found in Part I: Genetics.
- Determine how the amino acid sequence of a pigment protein determines its color.
- Explain, in terms of the proteins present, the interactions between the alleles you found in part I.
 - Why is the color phenotype of some pigment proteins dominant while others are recessive?
 - How do the pigment proteins combine to produce the overall color of the plant?
- Construct a purple protein to demonstrate your understanding of this process.

As in real science, these tasks are too big to be solved by one group alone. If you think of real research as solving an enormous jigsaw puzzle, each researcher works on only one little corner of the puzzle. Scientists publish papers and present findings at conferences in order to connect the corners of the puzzle that each is working on. Discuss your findings as a class.

Using the tool:

Once you start Aipotu, you can switch to the tool for this section by clicking the “Biochemistry” tab near the top of the window. You will see something like this:



This part of the program uses the one-letter code for the 20 amino acids:

<u>Amino Acid</u>	<u>3-letter code</u>	<u>1-letter code</u>	<u>Mnemonic</u>
Alanine	Ala	A	<u>A</u> lanine
Arginine	Arg	R	a <u>R</u> ginine
Asparagine	Asn	N	asparagi <u>N</u> e
Aspartic acid	Asp	D	aspar <u>D</u> ic acid
Cystine	Cys	C	<u>C</u> ystine
Glutamine	Gln	Q	<u>Q</u> -tamine
Glutamic Acid	Glu	E	glu-t <u>E</u> -amic acid
Glycine	Gly	G	<u>G</u> lycine
Histidine	His	H	<u>H</u> istidine
Isoleucine	Ile	I	<u>I</u> soleucine
Leucine	Leu	L	<u>L</u> eucine
Lysine	Lys	K	lysi <u>nK</u>
Methionine	Met	M	<u>M</u> ethionine
Phenylalanine	Phe	F	<u>F</u> enylalanine
Proline	Pro	P	<u>P</u> roline
Serine	Ser	S	<u>S</u> erine
Threonine	Thr	T	<u>T</u> hreonine
Tryptophan	Trp	W	t <u>W</u> ptophan
Tyrosine	Tyr	Y	t <u>Y</u> rosine
Valine	Val	V	<u>V</u> aline

- Click in the **Amino Acid Sequence Box** at the top of the **Upper Folding Window**. Type a short sequence of letters and you will see a short amino acid sequence appear in the window. This tool converts the single-letter code to the three-letter code automatically.

- Click the “FOLD” button and a two-dimensional version of your amino acid sequence will appear in the **Folded Protein** window.

There are several important things to note about this folding process:

It is the same as you used in the Protein Investigator. This is a highly-simplified model of protein folding. It is not intended to predict the correct structures of any proteins; it is designed to illustrate the major principles involved in that process. The important features of proteins that this software retains are as follows:

- Amino acids have side-chains of varying hydrophobicity, charge, and hydrogen bonding capacity.
- The amino acids are connected in an un-branched chain that can bend.
- Hydrophobic amino acids will tend to avoid the water that surrounds the protein; hydrophilic amino acids will bind to the water.
- Amino acids that can form hydrogen bonds will tend to form hydrogen bonds if they can.
- Positively-charged amino acids will tend to form ionic bonds with negatively-charged amino acids if they can.
- Like-charged amino acids will repel each other if they can.
- Ionic interactions are stronger than hydrogen bonds, which are stronger than hydrophobic interactions.

Even though this software provides some important insights into protein folding, you should always keep in mind that this is an approximation. The most important "gotcha's" to be aware of are:

- This program folds proteins in 2-dimensions only.
- This program treats all amino acids as equal-sized circles.
- This program models an environment where disulfide bonds do not form.
- This program folds the protein based on the interactions between the side chains only.
- This program does not model secondary or quaternary structure.
- This program assumes that all side chains with hydrogen bonding capability can bond with each other.

These simplifications are necessary for two reasons. The first is technical: it turns out to be extremely difficult to predict the full 3-d folded structure of a protein given only its amino acid sequence. As of the writing of this lab manual, it takes a super-computer several days to predict the fully-folded shape of even a small protein like lysozyme. Even then, the predictions don't always match known structures.

The second reason is educational. Proteins are complex 3-dimensional molecules; thus, it can be hard to find your way around when inside one. Likewise, it would be very difficult to visually compare two protein molecules to observe the effects of changes to their amino acid sequence. It would be easy to miss the forest (the forces that control protein structure) for the trees (the tiny details of the structures).

For these reasons, we will use this simplification. It retains the properties of amino acids that are important for this lab while being simple and fast.

There are several kinds of experiments you can perform with this tool. The following sections use examples to show you how to do each; you will need to devise your own experiments to carry out the tasks from the previous page.

I) *Examine the Pigment Proteins Present in an Organism from the Greenhouse.* This simulates extracting the pigment protein(s) produced by the two alleles of the pigment protein gene that an organism possesses, displaying their two-dimensional structures, and displaying their colors.

1) Double-click on the **Green-2** organism in the **Greenhouse**. You should see this:



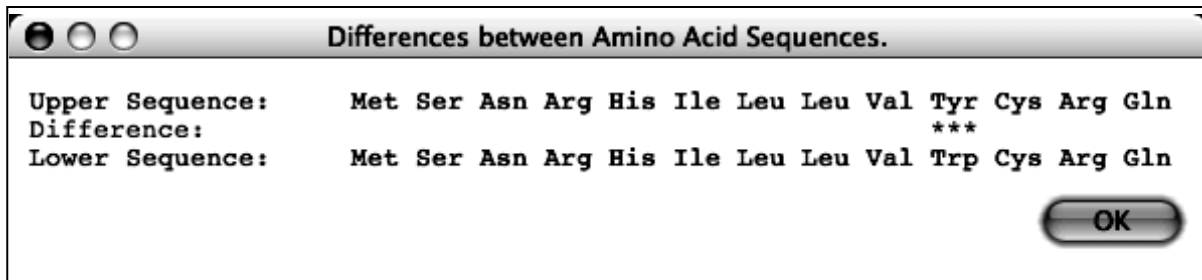
The **Green** organism contains two alleles of the pigment protein gene. Each of these alleles produces a different protein. One of these proteins is shown in the **Upper Folding Window**; it is a blue-colored protein as shown by the blue square next to the "Color:" label. The other protein is shown in the **Lower Folding Window**; this is yellow-colored protein. The combined color of the two proteins is green as shown by the **Combined Color** in between the two **Folding Windows**.

II) *Examine Pigment Proteins From the Mutant Organism(s) You Made in the first part of the Aipotu Lab, Genetics.* You can go back to the greenhouse as long as you saved mutants of interest they can be used again.

III) Compare the amino acid sequences of two pigment proteins. This aligns the two amino acid sequences so that the highest number of matching amino acids is obtained and then finds the remaining differences.

1) Double-click on the **Green** organism in the **Greenhouse**. You should see that the **Upper Folding Window** shows a blue protein and the **Lower Folding Window** shows a yellow protein.

2) You can compare the amino acid sequence of these two proteins by clicking on the "Compare" menu and choosing "Upper vs. Lower". A window will appear showing the differences between the two sequences. This is shown below:



This shows that the only difference is that, in the upper (blue) protein, amino acid 10 is tyrosine, while in the lower (yellow) protein, amino acid 10 is tryptophan.

⇒ You can also copy the sequence of a particular protein to the clipboard using the options in the **Edit** menu. You can then **Compare** a sequence to the one in the clipboard.

IV) Edit a Protein Sequence or Create a New Protein Sequence and Determine its Two-Dimensional Structure and Color. You can edit the sequence in either of the **Amino Acid Sequence** boxes and click the "Fold" button to predict the two-dimensional structure and color of the protein. The tool will also give the color that results from the combination of the colors in the Upper and Lower windows.

For example, click anywhere in the "Tyr" corresponding to amino acid 10 in the **Upper Amino Acid Sequence** box. Click the "delete" key and that amino acid will disappear. Type an "L" (the one letter code for leucine) and the amino acid sequence should be:

Met Ser Asn Arg His Ile Leu Leu Val Val Cys Arg Gln

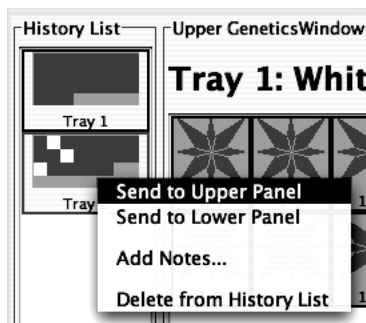
Click the "FOLD" button in the **Upper Folding Window** (or click the return key). You will see that the color of the new protein is white as shown by the "Color:" in the **Upper Folding Window**. You should also notice that:

- the "Combined Color" at the center of the window is now yellow.
- there is now an entry in the **History List** with your new protein. The background of **History List** entry is white to show the color of this protein.

You can also click the “Load Sample Protein” button. This will load a sample amino acid sequence that folds to a white-colored protein with a shape that is similar to many colored proteins.

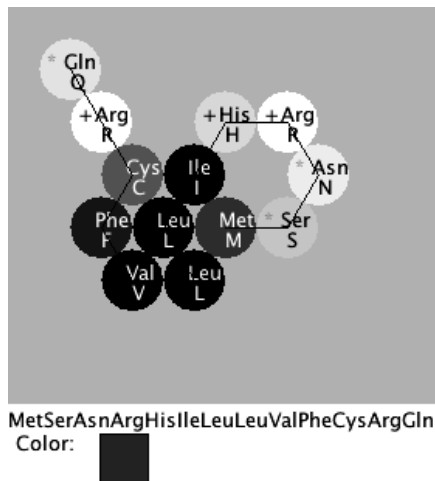
Please note that while Aipotu is an excellent teaching tool for exploring the connections between genes, proteins and phenotypes, in order for the software and exercises to be workable, Aipotu imposes certain simplifications on biological reality. It models proteins in two dimensions instead of three, as noted above. A second simplification is that within the “Aipotu world” flower color is determined directly by proteins that are themselves pigments. In the real world this is not the case. Genes that determine flower color code for enzymes that catalyze the synthesis of non-protein pigment molecules such as carotenoids, or for proteins that regulate the expression of these enzymes.

If you double-click an entry in the **History List**, you will get a pop-up menu with a list of useful options:



- **Send to Upper Panel:** Sends this **Tray** to the **Upper Panel** so you can cross those organisms.
- **Send to Lower Panel:** Sends this **Tray** to the **Lower Panel** so you can cross those organisms.
- **Add Notes...:** Allows you to add notes to the **Tray** in the **History List**. These notes will appear if you leave the cursor over the **Tray** for a few seconds.
- **Delete from History List:** deletes the **Tray** from the **History List**; this is cannot be undone.

⇒ You can also *take a Snapshot of either Work Panel* If you wish to save an image and email it to yourself you can take snapshots of particular proteins. A typical snapshot looks something like this; it shows the protein’s shape, amino acid sequence, and color:



You can take a snapshot in either of two ways:

- *Save a snapshot as a picture.* In the **File** menu, choose either **Save Image of Upper Panel...** or **Save Image of Lower Panel...**. You will then be asked to give the file a name and it will be (typically) saved to the desktop as *name.png*; you can then email it to yourself for your notes.
- *Take a snapshot to the clipboard.* In the **Edit** menu, choose either **Copy Image of Upper Panel to Clipboard** or **Copy Image of Lower Panel to Clipboard**. You can then paste the resulting image into another program, like Microsoft Word and email that to yourself.

Note taking as you work: A summary of your useful work should contain the following elements:

- **Title.** Enter this in the **Title** bar. It should contain your group name and a short phrase indicating the subject of your post. For example, “Brian, Tina, & Ling: This long protein is red!”
- **An Image of the protein.**
 - *First, save the image to the desktop.* Using Aipotú’s “**Save Image of ... Panel...**” from the **File** menu, save an image of the interesting protein to the Desktop. Give it a distinctive name so you can find it easily. Aipotú will add “.png” to the end of the file name to correctly identify it as an image. Now you can email it to yourself or transfer it to a USB drive.
- **A Hypothesis.** Using the format buttons, select bold type and type “Hypothesis:”. Go to the next line and, using regular type, write the hypothesis you were exploring. For example, “Long thin proteins will be red”.
- **The Experiment.** “Experiment:” should be bold and on it’s own line. Follow with a brief description of the experiment; for example, “We designed a long thin protein”.

- **The Result.** “Result:” should be bold and on it’s own line. Follow with a brief statement describing the result; for example, “The protein was colorless”.
- **The Conclusion.** “Conclusion:” should be bold and on it’s own line. Follow with a brief conclusion; in this case, “The hypothesis is incorrect; long thin proteins are not necessarily red.”

IMPORTANT NOTES:

(1) This software is under development. Please treat it gently and be patient. Please report any bugs to your TA. **You should save your Greenhouse regularly, especially if you save a large number of organisms.**

(2) Don’t shut down or restart the computer or you will lose all the pictures you’ve saved on the Desktop and all the organisms you’ve saved in the Greenhouse.

Specific Tasks for this section

- What are the differences in the amino acid sequences of the proteins produced by the alleles you define in Part I? Hint: use the **Compare** menu to find the difference(s) between the amino acid sequences.
- What features of the amino acid sequence make a protein pigmented?
- What features of the amino acid sequence make a protein a particular color?
- How do the colors combine to produce an overall color? How does this explain the genotype-phenotype rules you found in part (I)?
- Which proteins are found in each of the four starting organisms?
- Using this knowledge, construct a purple protein.

Hints:

- It may be useful, before formulating any hypotheses, to look for *patterns* in the data. Which features do colored proteins have in common that uncolored proteins lack?
 - Try comparing the amino acid sequences of proteins with different colors.
 - Here are some additional interesting sequences to try:
 - FFFFFFFFRRRRRR
 - RRRFFFFFFFRRR
 - KKKKKLLLLLLLF
 - KKKKKLLLLLLL
 - SLQLNITMEVDFW
 - EEEWWWWWWEEE
- Scientists, including yourselves, often find it useful to use mutation to study phenomena like this. Go to **Genetics** and make some mutants. Save any ones with interesting colors to the **Greenhouse**. Switch back to **Biochemistry** and look at the proteins they have.

Procedure:

1. Compare the proteins found in the starting strains to answer questions (a) and (b) on the following pages.
2. Your TA will assign your group one particular colored protein to study. Compare its sequence and shape to the “sample protein” that you get by clicking the **Load Sample Protein** button on one of the **Folding Windows** and then choosing from the **Compare** menu.
3. A representative from each group will come to the board to describe the sequence and shape difference(s) between their protein and the sample. Note that each subsequent group should relate their findings to the previously-presented data.
4. Based on these data, as a class, make several *specific* hypotheses that can be tested.
5. Each group should work on one or more of their hypotheses.
6. Your TA may stop for a mini-symposium to share data and design new hypotheses.
7. You will then be able to complete parts (d) through (f).

Put your data in the tables below:

(a) Which proteins are found in each of the four starting organisms?

Green-1

Green-2

Red

White

(b) allele color amino acid sequence (highlight differences)

(c) What features of a protein make it colored?

(d) What features of the amino acid sequence make a protein a particular color?

(e) How do the colors combine to produce an overall color? How does this explain the genotype-phenotype rules you found in part (I)?

(f) Show your TA that you have made a purple protein. For full credit, you need to explain to your TA *why* it is purple.

Follow-Up Assignment

Although you will perform these experiments as a group, each member of the group must turn in an individual assignment written in their own words for Aipotu I & II. You will also need to use your summarized information for Aipotu III Molecular Biology in two weeks, so be sure to make clear notes in your notebook and to keep a copy this assignment for yourself as you continue to work on part III.

You will describe one place where one hypothesis and some data interacted to lead to a firm conclusion. You should **not** describe all the experiments you did; just one place where you were able to draw a firm conclusion. The conclusion can be positive (“My hypothesis was supported because...”) or negative (“My hypothesis was not supported because...”). This need not be an experiment you did, but it must have happened during your lab session.

You should include:

- 1) Hypothesis - the hypothesis being tested. You must pick a clear and specific hypothesis that can be clearly and decisively tested by the experiment(s) you describe below. Your hypothesis need not be correct.
- 2) Experiments - a description of the experiment(s) you tried that addressed the hypothesis. Do not include all your experiments; only those relevant to the hypothesis from part (1). These must clearly and decisively test your hypothesis.
- 3) Results - pictures of the results of the experiments, save them during the lab and email to yourself. You may need to note the color(s) in text since most printers print in black and white.
- 4) Conclusions - do the data support the hypothesis or not. Explain your reasoning.

You will be graded on the quality of your argument: how clearly you described your hypothesis, experiment(s), and result(s) as well as how clearly and completely your conclusions are explained.