SIGMAPLOT EXERCISE: HOW TO GRAPH DATA
CELL MOL BIO LAB (B. STITH)

“The advent of online grant and manuscript submission, publishing, and presentation has created a need for tools for creating, analyzing, and presenting data electronically. SigmaPlot 2000 from SPSS Science provides a versatile environment for plotting and analyzing data. The latest version includes several enhancements targeted at electronic document creation.” Graphing Wizardry; Carol A. Bertrand. From: Science 15 September 2000;Vol. 289. no. 5486, p. 1894. See the very end of this document for a list of what SigmaPlot can do.

This exercise mimics the research experience (I recommend that instead of asking me what to do next, that YOU discover how to overcome obstacles). Thus, THIS EXERCISE TESTS YOUR DRIVE, INGENUITY, INITIATIVE AND PERSEVERANCE! BE CLEVER TO OVERCOME CHALLENGES! Many of experiments in this Cell Bio/Mol Bio lab are like real research- there may be a variety of correct answers or methods. Like real research, you must think of ways to overcome problems, develop methods and come up with the answers independently (e.g., how to record the distance proteins travel in the gel, find help on the web, in SigmaPlot's help manual, etc.).

Recommended steps for lab experiments: (1) define problems (what you need to do), (2) how to address problems; brainstorm solutions (3) evaluate collected data (you have to answer the question of “how to do this?”).

Fight procrastination (do you continue to ask me questions that you should answer?); jump right in, avoid excuses. Independent thinkers will proceed faster.

Examples of graphs and information follow…
Begin your use of SigmaPlot by following the directions below. Open the program on your computer and remember to save your data as you go along. Put your data on a floppy or flash drive, or email it yourself.

The SigmaPlot help manual can be accessed from within the program, or go to this web site: http://www.cof.orst.edu/net/software/sigplot/workshop.php

Information for graphing, scanning, presentations:
http://bama.ua.edu/~hsmithso/class/bse_695/links/index.shtml

TIP: click on something in the graph or highlight data that you want to change.

Plot the data shown in Fig. 2-9 instead of Fig. 2-8 (later is not shown).
Continue entering the rest of the data shown in Figure 2–8. When you are finished with one column, move to the top of the next column by clicking the first cell of the next column.

Σ

To correct a cell entry, click the cell and type the new value.

Titling Columns

To enter the column titles shown in Figure 2–8, select the entire column by moving the cursor over the column number and clicking the column. Type Area 1 into the edit box at top of the worksheet, and press Enter. Then highlight column 2 and enter Density 1. Press Enter.

Plotting Graph Data

When you are finished entering the data, you are ready to create a graph. Drag the mouse over the two column titles to select both columns of data, as shown in Figure 2–9.
To specify a graph type and style, select the Line/Scatter Plot button icon from the 2D graph bar, then select the Line and Symbol button from the palette.

The Graph Wizard appears. Select XY Pair from the list as a data format, then select Next.

The next window of the Graph Wizard prompts you to pick data from your worksheet to assign to the graph. Because you highlighted the columns before opening the Graph Wizard, the Area 1 and Density 1 columns are pre-assigned as your X and Y data in the Selected Columns list.

If you did not pre-select the columns in the worksheet, either highlight the row in the Selected Columns list and reselect the correct column from the worksheet or the Data for Y list, or clear the row by double-clicking it.

Select Finish. You have created the 2D graph shown in Figure 2–12.

Next, learn how to manipulate data in the table: switch the Y value for the two X values of 6 and 8. Then, add a Y value for 12: 14.
Print off the figure you generate.

Next, change the Y axis to log (not linear); try double clicking on the axis and then clicking on SCALING, and then on the new page pick SCALE TYPE and change to LOG.
Note that you have transformed the curved line to a straight line. Next, find the regression line (equation of the line defined by the numbers). Click on STATISTICS, LINEAR REGRESSION. Click on “All data in plot,” and “Extend to axes.” Click on APPLY. Then click on RESULTS, and look for the following:

All curves:
Coefficients:
\[ b[0] = 0 \]
\[ b[1] = 0.0927183211 \]

\[ b[0] \] stands for the y intercept of the equation \( Y = mx + b \)

\[ b[1] \] stands for the slope m

(if you have an \( X^2 \) term, you would find a number listed as \( b[2] \) for the number associated with \( X^2 \)).

What does the r value mean? (r or R ranges from 0 to 1, 0.9 to 1 means good data; .4 or below means redo the experiment)

Print off the linear form of the graph that shows the regression line.

Thus, if you have a new X value, you can calculate the Y value from the equation (do this- pick a number such as 15). Double check your answer by using the graphs that you printed out. This may be a process upon which I will test on upcoming exams.

Don’t forget to save your data. In addition to printing off graphs, you should bring floppies or flash drives to future labs to copy your data; or send you files to yourself by email.

---

**Exercise on the use of SigmaPlot:**

Gel electrophoresis is a technique that separates your protein of interest from other proteins and tells you how big your protein is (surprisingly, size is one of the most important characteristics of a protein as we often identify proteins based on their size).

Read these pages in World of the Cell (6th ed): 176-177 (see copies at the end of this document).

View the gel electrophoresis video on the course web site (test questions from video).

Be able to understand the technique (you might look it up on the web); briefly,

1. Cells are homogenized (proteins are now in a “soup”)
2. Proteins are denatured (what does this mean? Look it up on the web if you don’t know) and bound by detergent (often, sodium dodecyl sulfate or SDS).
3. Proteins are added to the top of a jello-like material (polyacrylamide typically)
4. Negatively-charged proteins move down the gel as they are attracted to the positive electrode in the bottom of the gel
5. As the proteins make their way through the channels (or “caves”) in the gel, the smaller proteins move with less resistance—thus move faster than the bigger proteins. (Longer migration for smaller proteins)

6. This separates the proteins by size. Standard proteins (proteins of known weight) are added to other lanes, so we can find out how big our unknown protein of interest is by comparison.

Read your text (see below) & the directions given below, but first look at this web site: http://www5.amershambiosciences.com/aptrix/upp00919.nsf/(FileDownload)?OpenAgent&docid=2343097446FC389FC1256EB400417D11&file=11B06413.pdf
Using the SigmaPlot program, graph the following (X, Y) data from Fig. 7-22 of the text:
Spectrin: 280,000 Daltons (Dalton is a measure of size; it is the weight of a proton)
Anion Exchange Protein: 96,000 Daltons; Actin is 43,000 Daltons. Use semi-log plot.

How big are GPD and Ankyrin? Record all details of how you got the answer and the calculations themselves in your lab notebook.
You need some information to find the answers; first, Read the manual for a protein standard kit:
http://www.piercenet.com/Products/Browse.cfm?fldID=02051027

Follow the instructions for the standard protein marker kit:

In summary, what you want to do; measure how far all proteins migrated from the top of the gel. With the standard protein sizes noted above, plot out with SigmaPlot (you have to use semi-log), print off for your lab notebook. Find the numerical values for the standard line (y=mx + b but SigmaPlot uses the format of y= (2)X + (1)) using SigmaPlot’s regression line program. Calculate the size of the unknown proteins by plugging their migration value into the equation and record this in your lab notebook.

Transfer graphs that you generate in SigmaPlot to a Word file: on the graph page, go to EDIT and SELECT ALL, then hit control-S to save. Then open a Word document and hit control-V to paste the SigmaPlot graph into the document. Now you can add text around the figure. From within Word: if you want to move the figure to the right side of the page, right click on the figure and change its properties.

Use the SigmaPlot Program to analyze the data below with a t Test:
Control Experimental group
55.2000 70.0000
62.4000 66.0000
58.0000 71.0000
61.0000 69.0000
66.0000 67.0000

Enter the data into a new file (name it and save it), then click on Statistics and (Pooled) t Test or Paired t Test. Analyze the data both ways and tell me if there is a significant difference between the two groups (you will get two different P values; record them with the appropriate t Test).
Read the following section on SDS Polyacrylamide Gel Electrophoresis from “World of the Cell (6th ed):” (tip: a test may ask you to define what SDS is…)
group. After attachment, the farnesyl or geranylgeranyl group is inserted into the lipid bilayer of the membrane.

Many lipid-anchored proteins attached to the external surface of the plasma membrane are covalently linked to glycosylphosphatidylinositol (GPI), a glycolipid found in the outer monolayer of the plasma membrane (Figure 7-19g). These GPI-anchored membrane proteins are made in the endoplasmic reticulum as singlepass transmembrane proteins, which subsequently have their transmembrane segments cleaved off and replaced by GPI anchors. The proteins are then transported from the ER to the exterior of the plasma membrane by a pathway we will encounter in Chapter 12. Once at the cell surface, GPI-anchored proteins can be released from the membrane by the enzyme phospholipase C, which is specific for phosphatidylinositol linkages.

Proteins Can Be Separated by SDS–Polyacrylamide Gel Electrophoresis

Before continuing our discussion of membrane proteins, it is useful to consider briefly how membrane proteins are isolated and studied. We will look first at the general problem of solubilizing and extracting proteins from membranes, and we will then learn about an electrophoretic technique that is very useful in the fractionation and characterization of proteins.

Isolation of Membrane Proteins. A major challenge to protein chemists has been the difficulty of isolating and studying membrane proteins, many of which are hydrophobic. Peripheral membrane proteins are in general quite amenable to isolation. As noted earlier, they are bound to the membrane by weak electrostatic interactions and hydrogen bonding with either the hydrophilic portions of integral membrane proteins or the polar head groups of membrane lipids. Peripheral proteins can therefore be extracted from the membrane by changes in pH or ionic strength; in fact, peripheral membrane proteins were originally defined as those that could be extracted from membranes with an alkaline carbonate solution of a specific pH and ionic strength. Peripheral membrane proteins can also be solubilized by the use of a chelating (cation-binding) agent to remove calcium or by addition of urea, which breaks hydrogen bonds. Lipid-anchored proteins are similarly amenable to isolation, though with the requirement that the covalent bond to the lipid must first be cleaved. Once extracted from the membrane, most peripheral and lipid-anchored proteins are sufficiently hydrophilic to be purified and studied with techniques commonly used by protein chemists.

Integral membrane proteins, on the other hand, are difficult to isolate from membranes, especially in a manner that preserves their biological activity. In most cases, they can be solubilized only by the use of detergents that disrupt hydrophobic interactions and dissolve the lipid bilayer. As we shall now see, the use of strong ionic detergents such as sodium dodecyl sulfate (SDS) allows integral membrane proteins not just to be isolated, but also to be fractionated and analyzed by the technique of electrophoresis.

SDS–Polyacrylamide Gel Electrophoresis. Cells contain thousands of different macromolecules that must be separated from one another before the properties of individual components can be investigated. One of the most common approaches for separating molecules from each other is electrophoresis, a group of related techniques that utilize an electrical field to separate electrically charged molecules. The rate at which any given molecule moves during electrophoresis depends upon its charge as well as its size. Electrophoresis can be carried out using a variety of support media, such as paper, cellulose acetate, starch, polyacrylamide, or agarose (a polysaccharide obtained from seaweed). Of these media, gels made of polyacrylamide or agarose provide the best resolution and are most commonly employed for the electrophoresis of nucleic acids and proteins.

When electrophoresis is used to study membrane proteins, membrane fragments are first solubilized with the detergent SDS, which disrupts most protein-protein and protein-lipid associations. The proteins denature, unfolding into stiff polypeptide rods that cannot refold because their surfaces are coated with negatively charged detergent molecules. The solubilized, SDS-coated polypeptides are then layered on the top of a polyacrylamide gel, and an electrical potential is applied across the gel, such that the bottom of the gel is the positively charged anode (Figure 7-22). Because the polypeptides are coated with negatively charged SDS molecules, they migrate down the gel toward the anode. The polyacrylamide gel can be thought of as a fine meshwork that impedes the movement of large molecules more than that of small molecules. As a result, polypeptides move down the gel at a rate that is inversely related to their size.

When the smallest polypeptides approach the bottom of the gel, the process is terminated and the gel is stained with a dye that binds to polypeptides and makes them visible. (Coomassie brilliant blue is commonly used for this purpose.) The particular polypeptide profile shown in Figure 7-22 is for the membrane proteins of human erythrocytes, most of which we have already encountered (recall Figure 7-20b).

Determining the Three-Dimensional Structure of Membrane Proteins Is Proving to Be Increasingly Feasible

Determining the three-dimensional structure of integral membrane proteins has been hampered for many years, primarily because these proteins are generally difficult to isolate and purify. However, they are proving increasingly amenable to study by X-ray crystallography, which determines the structure of proteins that can be isolated in crystalline form. For the many membrane proteins that have not yet yielded to crystallographic study, an
alternative approach called hydrophobic analysis is available, provided that the protein can at least be isolated and sequenced. We will look briefly at each of these techniques.

X-Ray Crystallography. X-ray crystallography is widely used to determine the three-dimensional structure of proteins. A description of this technique is included in the Appendix (see pp. A1-A30). The difficulty of isolating integral membrane proteins in crystalline form virtually excluded these proteins from crystallographic analysis for many years. The first success was reported by Hartmut Michel, Johann Deisenhofer, and Robert Huber, who crystallized the photosynthetic reaction center from the purple bacterium, *Rhodopseudomonas viridis*, and determined its molecular structure by X-ray crystallography. Based on their detailed three-dimensional structure of the protein, these investigators also provided the first detailed look at how pigment molecules are arranged to capture light energy, a topic to which we will return in Chapter 11 (see Figure 11-11 on p. 303). In recognition of this work, Michel, Deisenhofer, and Huber shared the Nobel Prize for Chemistry in 1988.

Despite this breakthrough, the application of X-ray crystallography to the study of integral membrane proteins progressed very slowly until the late 1990s, particularly at the level of resolution required to identify
SigmaPlot new version 9 capabilities:

**Large, Scientific Worksheets**

- Over 32,000 columns by millions of rows
- Handles numeric, text (categorical), and date & time data
- Automatically generate column statistics
- Data sorting
- Rename column and row titles
- Insert color, symbols, line styles and bar patterns
- Insert and delete rows and columns
- Row and column cell clipping and in-place editing
- Independent graphically adjustable row height and column widths
- Missing data handling
- Data point sampling
- Multiple worksheets per session
- Graphical feedback of current curve and datapoint
- Text support of up to 256 characters
- Change the font type and grid colors
- Zoom in and zoom out
- Change font for worksheet
- Multiple Undo
- Format empty cells - formatted selected columns even if they do not contain data
- More flexible column titles allow for duplicates and numeric only titles
- Enhanced data/time recognition and more formats
- Arrow-key functionality is similar to that of Microsoft Excel
- Freeze Panes
- Multi-line editing - text wraps to fit the column while the row height automatically adjusts
- Print preview
- Find and replace data from among millions of rows and columns
- Format text based cells with independent fonts
- Multiple sorting

**Microsoft Office Integration**

- You can open Excel spreadsheets directly inside SigmaPlot, allowing you to use the many features Excel offers. Use in-cell formulas and other Excel data analysis tools on your data.
- One-click access to directly launch SigmaPlot from Microsoft Excel

**SigmaStat 3.1 Integration**

- SigmaStat's statistical capabilities are directly accessible in SigmaPlot through the statistics menu.
- Over 30 of the most frequently used statistical tests to analyze scientific research
- Advisor Wizard guides you through the process of choosing the appropriate statistical test
- Report generation that translates the statistics into plain and simple English
- Descriptive statistics
- Non-parametric tests: t-tests, ANOVA
• One-way, two-way, three-way ANOVA
• Repeated measures
• Rates and proportions
• Correlation
• Survival analysis (Kaplan-Meir)
• Power and sample size analysis

Symbol Types

• Over 80 symbol types
• 30 new symbol types that include half-filled and BMW styles
• Edit font when using text as symbol
• Access new symbols directly from graph properties dialog, toolbar, legend page, and the symbol dialog box
• More line types such as dash and gap patterns
• More fill patterns, for bar charts and area plots, that can be independently set from the line color

"Picking from Column" Option

• Enter colors, patterns, symbols, line styles, tick mark intervals, tick labels and more directly into your worksheet to customize your graph the way you want. Transforms and "picking from column" allow you to create data dependent color gradients, symbols and sizes.

SigmaPlot Notebook

• Can hold SigmaPlot worksheets, Excel worksheets, reports, documents, regression wizard equations, graph pages, and macros.
• New dialog-bar-based notebook that has several states: docks, resizable, hide-able, summary information mode, etc.
• Browser-like notebook functionality that supports drag-n-drop capabilities
• Direct-editing of notebook summary information

Import

• Axon Binary, Axon Text, ASCII Plain, Comma and general import filter, 1-2-3T, Symphony T, Quattro T, Excel, dBASE E, DIF, SigmaPlot for DOS 4.0, 4.1, 5.0, SigmaPlot 1.0, 2.0, and 3.0, 4.0, 5.0 for Windows, SigmaPlot 4.1 and 5.0 for Macintosh data worksheets, SYSTAT, SigmaScan Pro, SigmaScan, SigmaScan Image, Mocha
• Import and ODBC compliant database
• Run SQL queries on tables and selectively import information

Export

• ASCII Text, Tabbed, Comma, 1-2-3T, Excel, DIF, SigmaPlot 1.0, 2.0, and 3.0 for Windows, SigmaPlot 5.0 for Macintosh data worksheets, SigmaScan Pro
• PDF and HTML export of graphs and reports

Export Graphs Options
• Export an individual graph, a group of graphs and objects, or an entire page
• Different levels of resolution and color depths: EPS, TIFF, JPEG, WMF, BMP
• True color EPS vector
• Compressed CMYK TIFF
• Publication Help: guides user through the complexities of selecting the correct DPI, image size, file export format, etc.
• True CMYK EPS export

Publish as Web Page

• Export graphs as high-resolution Web objects
• **WebViewer:** free browser plug-in to view data used to create graph or print, pan and zoom in on graph without losing resolution
• The WebViewer supports IE 4.01 or higher. A screen-resolution JPEG file is automatically displayed for other browser applications and operating systems.

Automate Routine and Complex Tasks

• Visual Basic compatible programming using built-in macro language interface
• Macro recorder to save and play-back operations
• Full automation object support - use Visual Basic to create your own SigmaPlot-based applications
• Run built-in macros or create and add your own scripts
• Add menu commands and create dialog boxes
• Toolbox menu: helpful macros appear as a separate menu item
• Export graph to PowerPoint Slide (macro)
• New 'Insert Graph to Microsoft Word' Toolbox macro
• New keyboard shortcuts in the Graph Properties and most Microsoft Excel keyboard shortcuts in the worksheet

Windows Application

• Excel, Word and PowerPoint for Office 2000 and Windows 2000 support ToolTips
• Tips and Tricks at startup
• Full 32-bit implementation
• OLE 2 container and server
• Use Excel worksheets inside SigmaPlot
• Uninstaller
• Controls have bitmaps to give feedback about selections
• Right mouse button property editing
• Selection of objects on graph page
• Full precision and date/time Microsoft Excel copy and paste

★ New Features added in SigmaPlot 9