Assignment for Modules 4/5

• Analyze the differentiation of 3T3-L1s into adipocytes.
  – Quantify changes in shape (ImageJ)
  – Quantify lipid storage (ImageJ)

• Details on the course website.

• Please review technical writing guidelines.
Lysing Cells-Review

• We collected a set of lysates stimulated +/- calf serum to compare in Module 7.

• Before analyzing changes in individual proteins in these samples (Module 7) we first need to quantify the amount of protein in our samples.
RIPA Buffer-Review

Radioimmunoprecipitation assay (RIPA) buffer

- Tris-Buffer prevents protein degradation
- NaCl-Prevents nonspecific protein interactions
- NP-40, SDS, sodium deoxycholate-detergents to extract proteins
- Protease and phosphatase inhibitor cocktail
Lysing Cells with RIPA Buffer-Review

- Wash 1X with ice-cold PBS
- Add lysis buffer
- Scrape cells off dish into lysis buffer
- Centrifuge for 10 minutes
- Collect supernatant
  - Insoluble fraction pellets
BCA Assay

- Bicinchoninic acid assay (Smith assay)
- Colorimetric detection and quantification of total protein
- Bovine serum albumin (BSA) used as a standard
BCA Assay-BSA standard

• BSA universal standard for protein quantification assays
  – Low cost
  – Stable
  – Little to no effect in biochemical assays

• Serial dilutions of BSA are used to form a reference standard

• We will use BSA again in Module 7...
BCA Assay-Chemistry

- Peptide bonds in proteins reduce Cu$^{2+}$ to Cu$^{1+}$ (temp dependent, alkaline conditions)
- Formation of Cu$^{1+}$ is proportional to amount of protein

http://www.chem.ucla.edu/harding/IGOC/P/peptide_bond.html
BCA Assay-Chemistry

• Colorimetric detection of Cu^{1+} ion using reagent containing BCA
• Purple color formed by chelation of two molecules of BCA with Cu^{1+}
• Read absorbance on the plate reader at 540 nm
Controls

• **Blank**: used to measure background in an assay
  – When have you used blanks this semester?

• **Positive Control**: sample known to give a positive test result, used to determine validity of measurement technique
  – When have you used positive controls this semester?

• **Negative Control**: sample known to give a negative result, identify **confounding variables**
  – When have you used negative controls this semester?
Experimental vs. Technical Replicates

• Experimental/biological replicates-
same organism/cells are treated in the
same conditions
  – Establish the variability in the
    organism/cell
  – Example: Doubling time of 6 flasks of
    3T3-L1 cells (same seeding density,
    media, passage number, etc)

• Technical replicates-Same sample
  analyzed multiple times
  – Establish the variability of a technique
  – Example: OD of a culture measured 3
times on the plate reader
Experimental vs. Technical Replicates

- What would be an experimental replicate in the BCA assay?

- What would be a technical replicate in the BCA assay?
“Error bars”: Standard deviation vs. standard error

Commonly misused!
• Standard deviation (SD)-Variation of the data (property of the data)
  – Not affected by sample size, nothing to do with error!
  – When to use: For experimental/biological replicates, demonstrate variation of samples, ex. Student height in BIOE202, performance on an assignment
• Standard error of the mean (SEM=SD/sqrt(N))-Uncertainty of the mean (property of the experiment)
  – Decreases with sample size (greater certainty with more measurements)
  – SEM is always smaller than SD
  – When to use: For technical replicates (usually)-demonstrate the the uncertainty of the measurement
• Both SD and SEM have the same units, report as mean+/sd or mean+/-sem

Useful article discussing sd, sem, and CI in relation to significance: http://www.nature.com/nmeth/journal/v10/n10/pdf/nmeth.2659.pdf
Standard Curves

• Standard curves are made with samples with known properties to map unknown samples to properties
• We will be using a standard curve to map protein amount to measured absorbance values
Technical point: using multichannel micropipettes

- Firmly press micropipette into tips-straight down, not at an angle!
- Plastic reservoirs (reusable!) allow for easier use
- Verify by eye that the fluid is pulling up evenly in all tips—if not one or more tips is loose
Abstract

• Should contain the following:
  – General statement about why you are doing this experiment (global picture)
  – Brief summary of materials and methods
  – Summary of results (include numbers). Give a reason for the reader to want to read the rest of the article.
  – Discussion/conclusions. Include specifics as well as global. (i.e., these experiments indicate....which means that....)
Methods Section

• The Methods section should include all the information such that another researcher could duplicate your results.

• In scientific literature, scientists must report where reagents were obtained from, for example “Cell viability was measured using Trypan blue (Sigma)”. Similarly, the source of cell lines or DNA constructs must also be acknowledged (including those not commercially available), for example, “3T3-L1 cells expressing GFP were obtained from the Jensen Lab”. This is not required for BIOE202 reports and assignments unless otherwise specified.

• Details that do not affect the experiment need not be included (ie “The dilution series was performed in column 2 of a 96-well plate.”) In this example, someone could obtain the same data if they performed the dilutions in column 3, so the details are not relevant.
Introduction Section

• Motivate the study and provide any background information that will help the reader understand the purpose of the study and the questions being addressed.
Results Section

- Every table or figure needs to have supporting text in the main body of the results section. Supporting text can be used to describe the figure or use the figure to help clarify your text.
- Observations should be included in the results section.
Discussion Section

• Think critically about your results and explain any discrepancies between what you saw and what was expected.

• Interpret your results. What does the data mean? Yes, it is significantly different, but is that good, bad, or does it really matter?

• Support your arguments with literature when possible (and include in your References section. If something went wrong with your experiments, explain what you believe the problem to be and why (don’t just guess or give a laundry list of reasons) and provide possible solutions.

• If the data are inconclusive, discuss the next steps in understanding/solving the problem.
Module 6 Lab Overview

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<thead>
<tr>
<th>Standards</th>
<th>Samples</th>
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<tr>
<td>A</td>
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<td>B</td>
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<td>C</td>
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- Standards A, B, C, D, E, F, G, H
- Samples 1, 2, 3, 4
- Dilutions: undiluted, 1:5, 1:10
- Symbols: +, -
Schedule and logistics

• No Return lab for Module 6
• Main Lab after spring break: Module 6 Protein Quantification (quiz during Main Lab).
  – Please study! This traditionally has been a challenging topic/quiz.
• Module 4/5 assignment due 4/1 at 5pm