Critical Thinking Skills

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LA IUPI UPRRP

1. Preparing for an experiment

- Contextualize your experiment by reading the literature.

  - What methodology has been applied?
  - What instrument was used to collect data?
  - How were data analyzed?
  - What conclusions were made?

1. Preparing for an experiment

- Determine what instruments, chemicals, and tools are available

  - Plan ahead. Determine these things before the day of the experiment.

  - When possible, separate what you need into your own storage container.

  - Prepare buffers and stock solutions. Store solutions in appropriate glass or plastic ware and refrigerate, if necessary, to maximize stability and to accelerate the process of repeating experiments. I highly recommend protecting metal solutions from light as light can induce ligand degradation and/or metal redox changes.

  - Periodically check the quality of everything that you are using. Metal solutions have the tendency to become unstable due to compound dissociation, precipitation, and other factors.

1. Preparing for an experiment

- Working with chemicals

  - Be aware of the chemicals that you are working with and why you are using them.
  - It is important to know stability, solubility, and storage conditions.
  - Depending on the experiment, the choice of protonated or deuterated solvents is extremely important.
  - Be aware of characteristic solution absorbance signals especially if you are performing an experiment involving UV/Vis spectroscopy. We will return to this concept.
  - If working with proteins, make sure that you prepare protein solutions for immediate experimental use. In fact, it should be the last stock solution that you prepare. When not being used, the solution should be stored in the refrigerator. Avoid using solutions beyond a week old.

1. Preparing for an experiment

- Construct Workplans

  - These are meant to formalize your thoughts on how to perform an experiment. I highly recommend creating a workflow.

  Experiment → Data Collection → Analysis → Interpretation of Results → Next step

  - Help you to define expectations and to prepare alternative plans

  - Organize how you will analyze your results to measure success or to identify unexpected results

  - Cuts down on wasted lab time and inefficient use of instruments and chemicals

-Case Studies-

Focus on experiments using UV/Vis Spectroscopy

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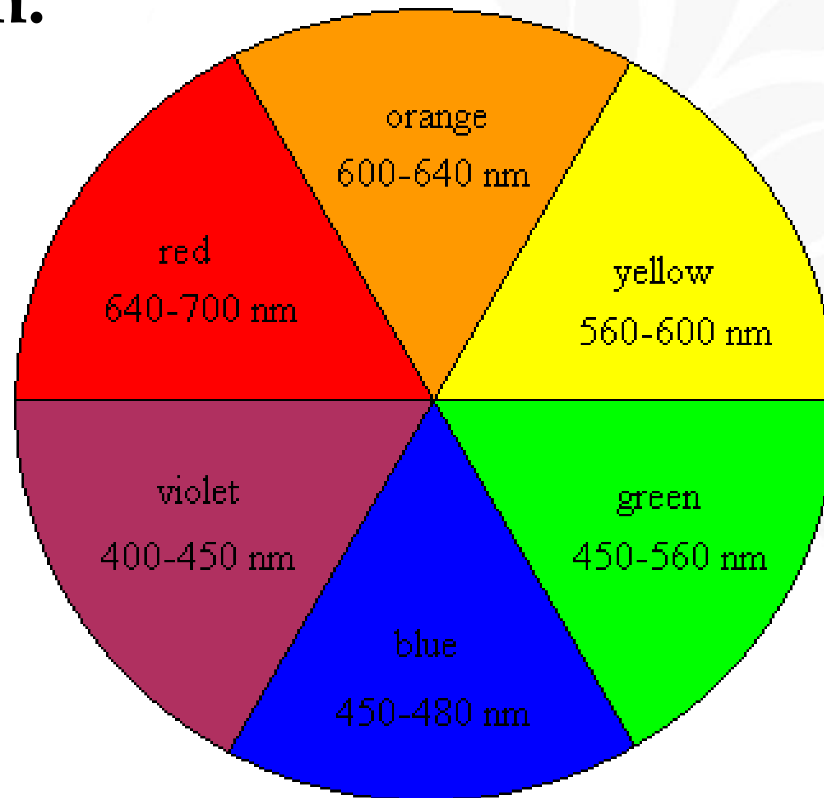
Key concept 1- The color wheel can help guide the interpretation of electronic transitions in the visible region.



- Complementary colors- The color "you" see is complementary to the color of absorbance.

Key concept 2- The extinction coefficient at a particular absorbance maximum is a unique identifier of a species in solution.

- Beer's Law

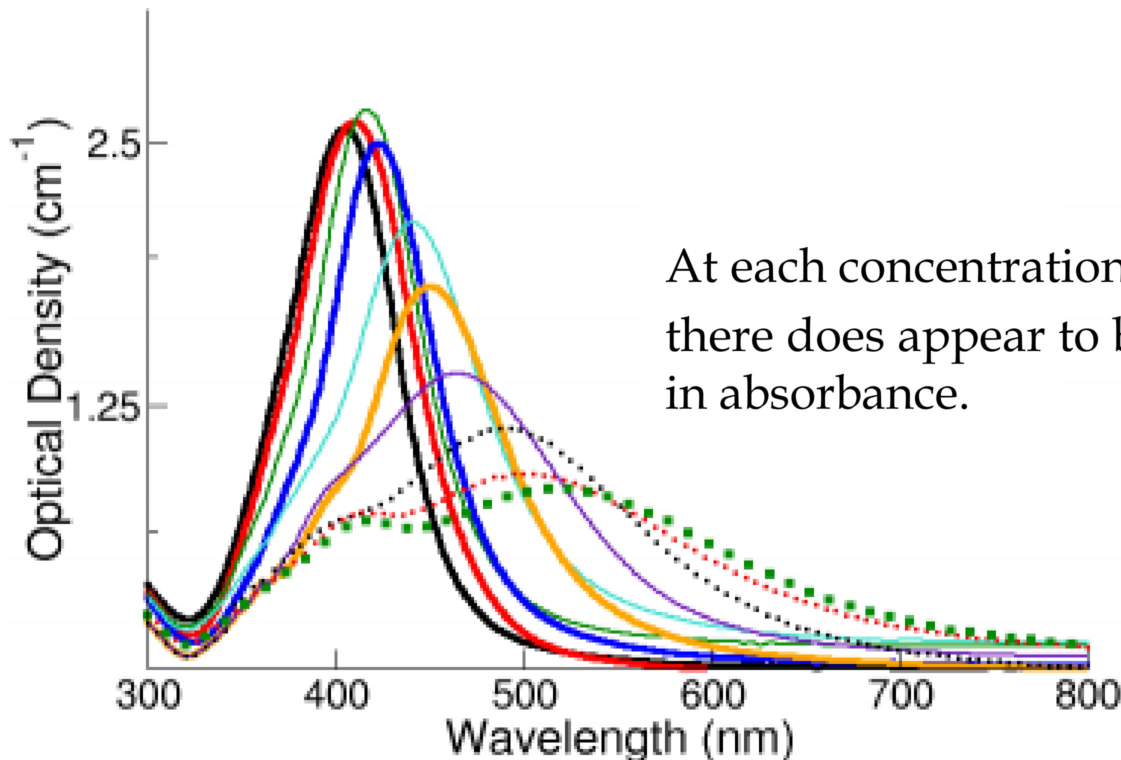
Absorbance = extinction coefficient x pathlength x concentration

$$A = \epsilon \times b \times c$$

For this relationship to hold, your pathlength and your absorbance maximum must be constant.

The extinction coefficient ($M^{-1}cm^{-1}$) is a numerical constant that defines your species. It can help you quantify how much of your species is present in solution.

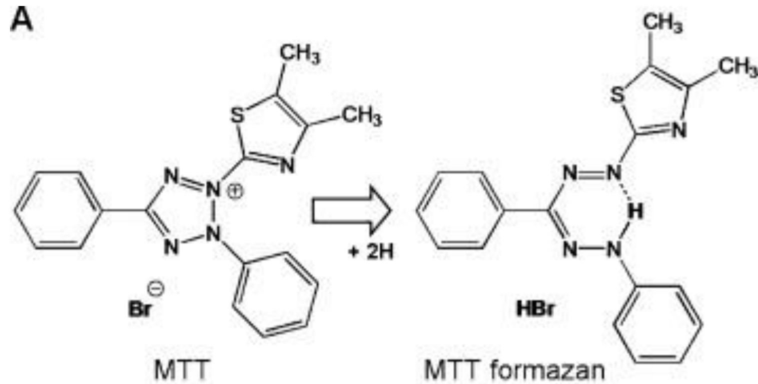
Case Study 1- You perform a colorimetric assay to quantify the amount of a species in solution. Its maximum absorbance is 450 nm. You prepare solutions of your standards.

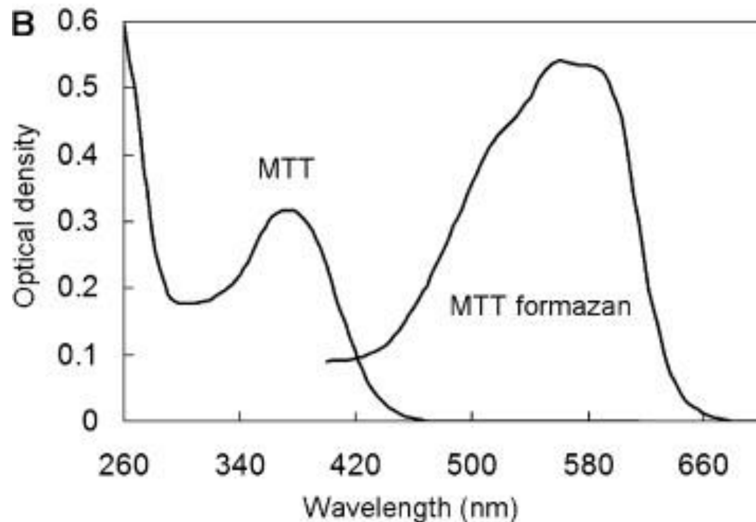


At each concentration of standard, there does appear to be an increase in absorbance.

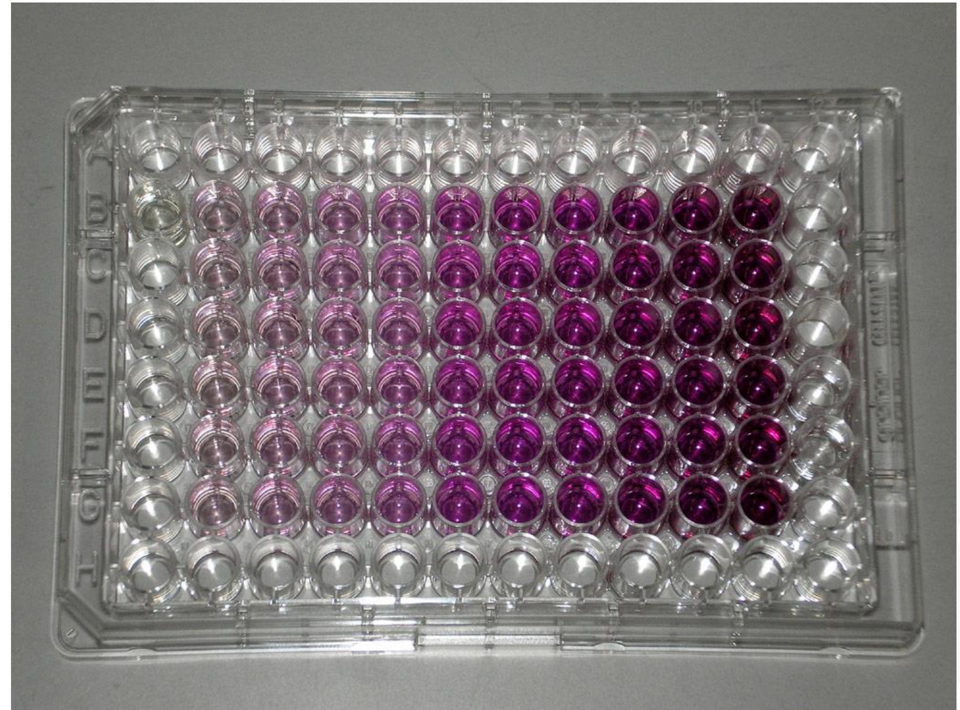
- Can you use these data? Explain.

Case Study 2- The MTT cell proliferation/viability assay. Focus on the pH dependence of results.

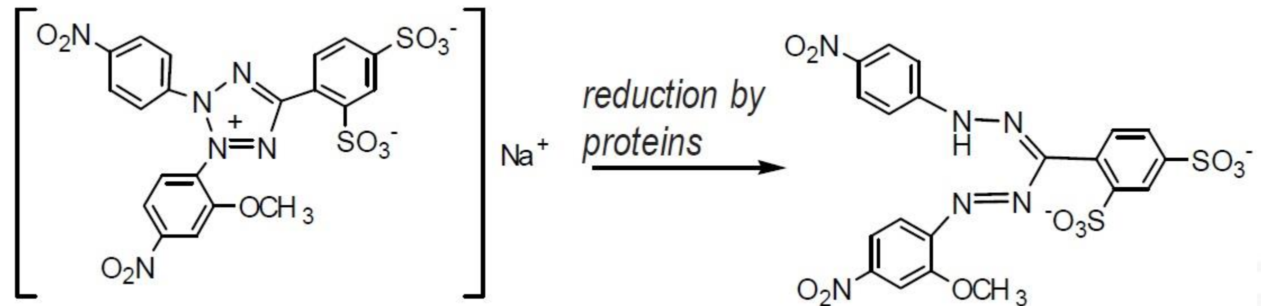




Higher cell proliferation from left to right.

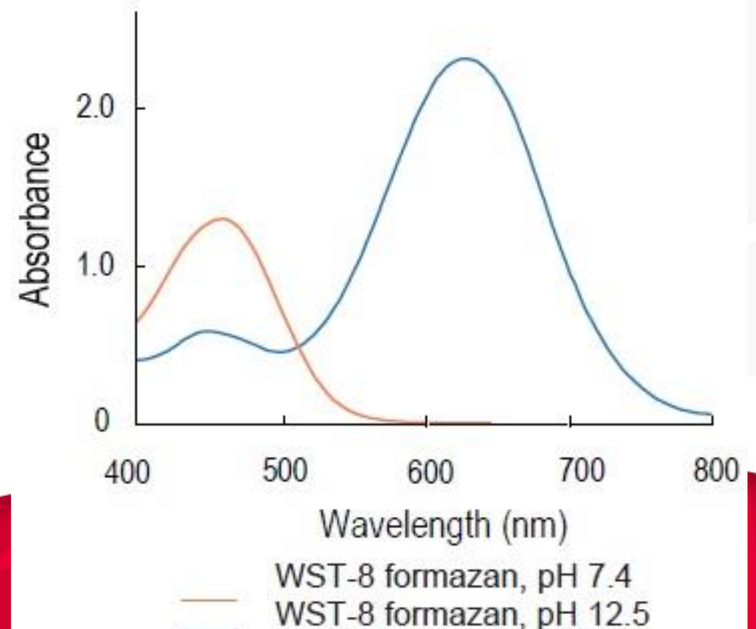


Case Study 2- The MTT cell proliferation/viability assay. Focus on the pH dependence of results.

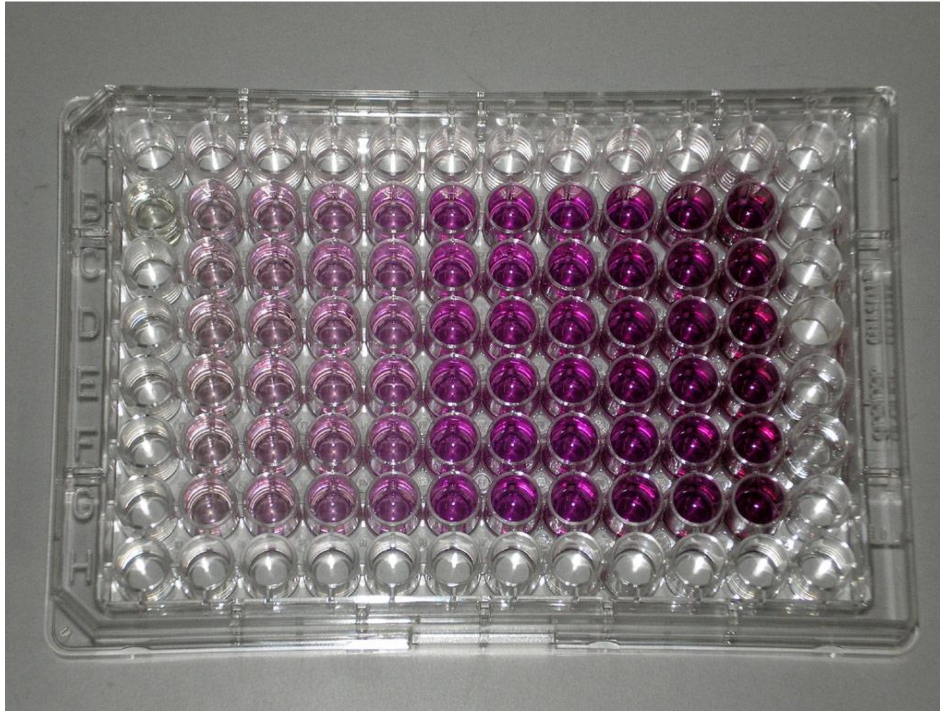


Buffering conditions are extremely important in order to not erroneously interpret data. Take the example of a water soluble form of MTT.

- Drifts in pH can dramatically change the intensity of the color being measured.
- Must have exact background matches.



Case Study 3- The MTT cell proliferation/viability assay. Nonsense results.

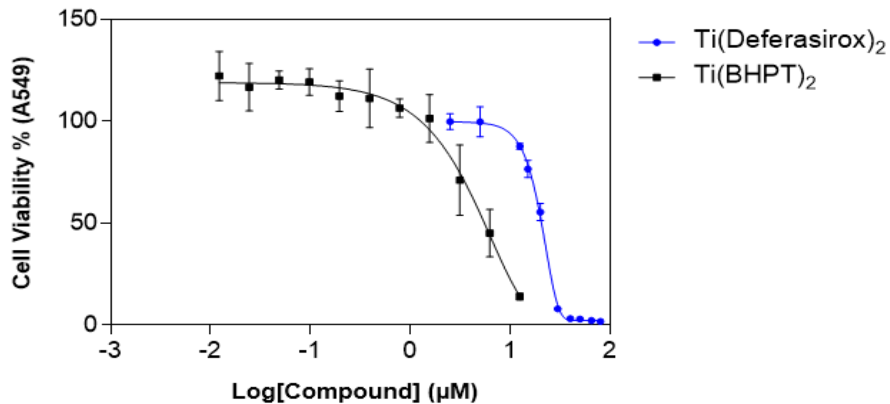


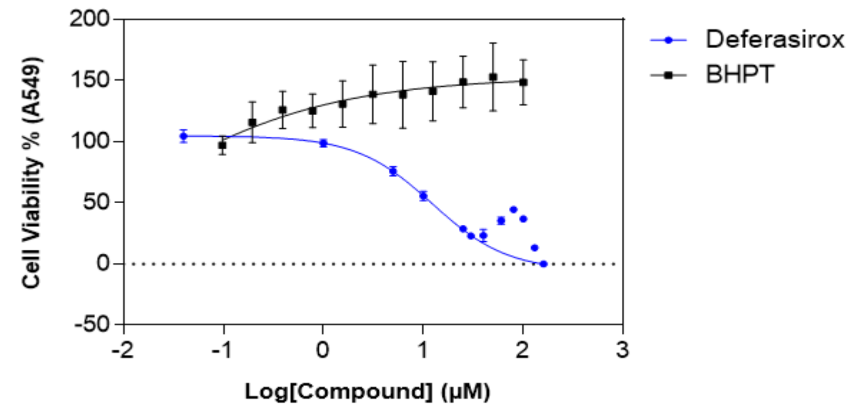
Avoid mindlessly collecting data. It will just waste your time and frustrate you.

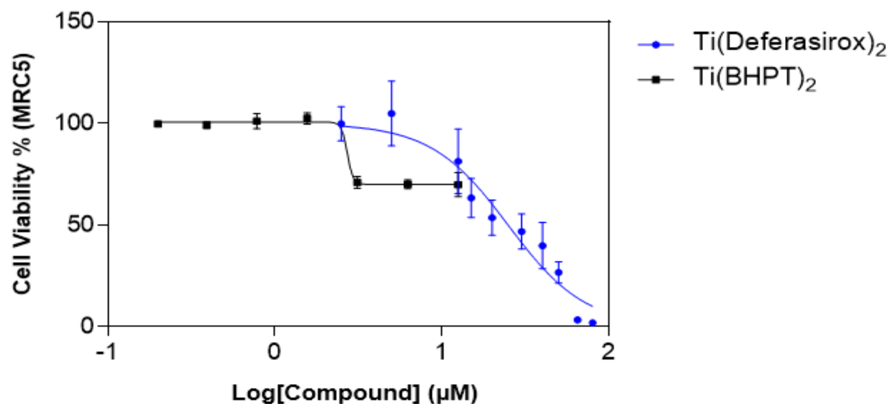
Also don't say an experiment did not work. Tell me what you observed. Chances are the real results are more promising or tell you about something you are doing to help troubleshoot.

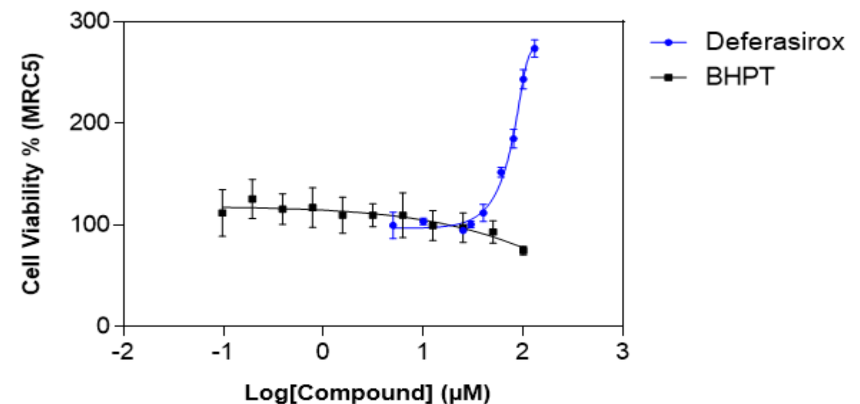
0.045	0.045	0.045	0.045	0.045	0.045	0.045	0.045	0.045	0.045	0.045	0.045
0.047	0.047	0.047	0.047	0.047	0.047	0.047	0.047	0.047	0.047	0.047	0.047
0.045	0.045	0.045	0.045	0.045	0.045	0.045	0.045	0.045	0.045	0.045	0.045
0.045	0.045	0.045	0.045	0.045	0.045	0.045	0.045	0.045	0.045	0.045	0.045
0.046	0.046	0.046	0.046	0.046	0.046	0.046	0.046	0.046	0.046	0.046	0.046
0.045	0.045	0.045	0.045	0.045	0.045	0.045	0.045	0.045	0.045	0.045	0.045
0.048	0.048	0.048	0.048	0.048	0.048	0.048	0.048	0.048	0.048	0.048	0.048
0.046	0.046	0.046	0.046	0.046	0.046	0.046	0.046	0.046	0.046	0.046	0.046

Case Study 3- The MTT cell proliferation/viability assay. Correct analysis of results.









Case Study 3- The MTT cell proliferation/viability assay. Correct analysis of results.

1. The MTT viability assay is a measure of how a chemical species affects the proliferation of cells. A lower proliferation in the presence of a chemical species does not imply that it is cytotoxic. It would be classified as antiproliferative. You will need to do additional analyses to determine if something is cytotoxic.

2. Always measure the absorbances at the wavelength of maximal absorbance and at a wavelength that is representative of baseline. I typically will use 750 nm or 800 nm as representative of baseline.
   - The baseline measurements will correct for any light scattering due to solution conditions.
   - Will correct for an instrumental drifting baseline.

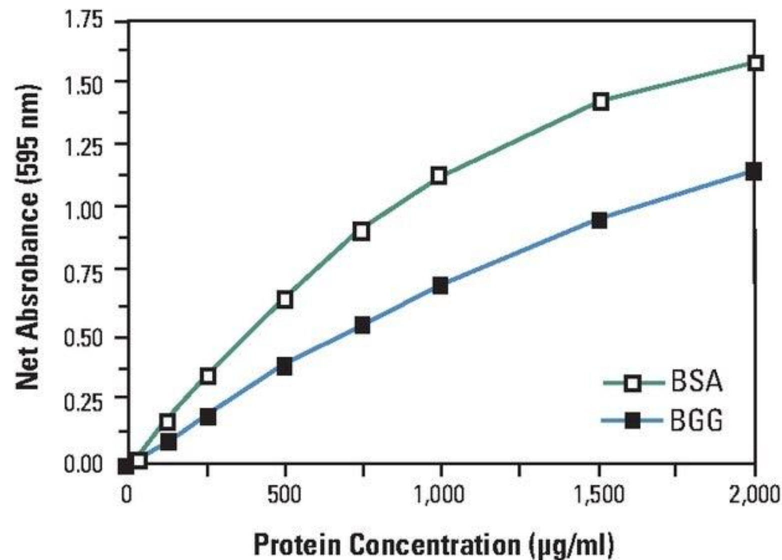
3. Set up proper controls to measure the maximal viability and also conditions for no viability. Compare your results to these high and low markers in order to determine dose dependent % viability.

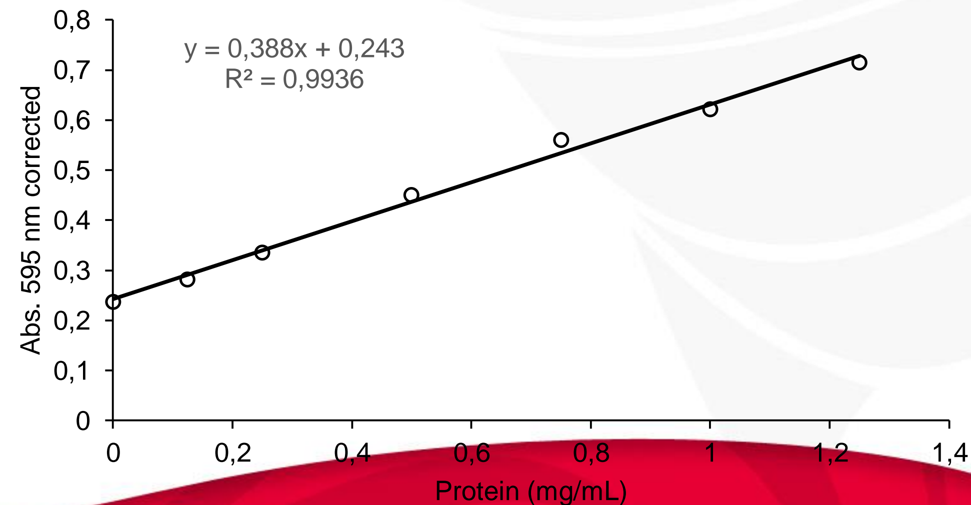
Case Study 5- The Bradford assay. An example of how to analyze differences in data output.

Protein + Bradford Reagent → **Blue Protein Complex** (A_{max} = 595 nm)

1. The technique is based on the reagent binding to the basic amino acids of the protein. As such, it is a relative quantitative approach for measuring protein content.

2. Absorbance response to protein content is not always linear.





Polynomial Fit

Linear Fit

Case Study 6- Buffer compatibility with temperature dependent studies.

The buffering capacity of a buffer depends on its pKa values. Some buffers can control pH at different pH ranges because they have more than one ionizable proton.

- Beware of the temperature dependence of the pKa of buffers as that can severely affect the buffering capacity of a buffer for temperature based studies.

pH of Tris Buffer (0.05 M)		
5°C	25°C	37°C
7.76	7.20	6.91
7.89	7.30	7.02
7.97	7.40	7.12
8.07	7.50	7.22
8.18	7.60	7.30
8.26	7.70	7.40

Questions?

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