Creation of a Protein Standard Curve Using the SpecPhone

Materials:

- Bovine Serum Albumin (BSA)
- Bradford Reagent, kept cold
- PBS Buffer or deionized water
- Volumetric flask, 100 mL capacity or larger
- 5 mL pipette tips and pipette or graduated cylinder
- Test tubes (7)
- Test tube rack
- Cuvettes, 1 cm^3 (8)
- Lens Cloth (KimWipes)
- SpecPhone apparatus
- Light source (desk lamp)
- iPhone or another camera-containing mobile device

Introduction:

This protocol involves creating a protein concentration standard curve using BSA and Bradford Reagent. The results can be plotted and a line of best fit obtained. If a protein sample of unknown concentration is given, the absorbance value can be plugged into the equation of the line of best fit, to solve for the concentration. A stock solution is prepared, and then serially diluted to yield increasingly dilute samples. Bradford reagent binds to protein, and this binding causes a change in color from a grey-brown color to a brilliant blue. The intensity of the color change is proportional to the amount of protein present. Subsequently, the darker a solution, the more light it will absorb when in the spectrometer, and thus the lower the percent transmission (%T, or the amount of light that is allowed to pass through the sample). As the protein concentration decreases, the saturation of the blue color in the solution proportionally decreases, and thus more light will be allowed to pass through, leading to a higher %T value. Transmission values can be converted to absorbance values using the equation: A=-log(%T). The Bradford Reagent absorbs the most light at the wavelength of 595 nm. However, if this value was not know, it would be necessary to conduct trials using a wavelength-calibrated spectrometer to determine the wavelength of maximum absorbance.

Procedure:

Sample Preparation:

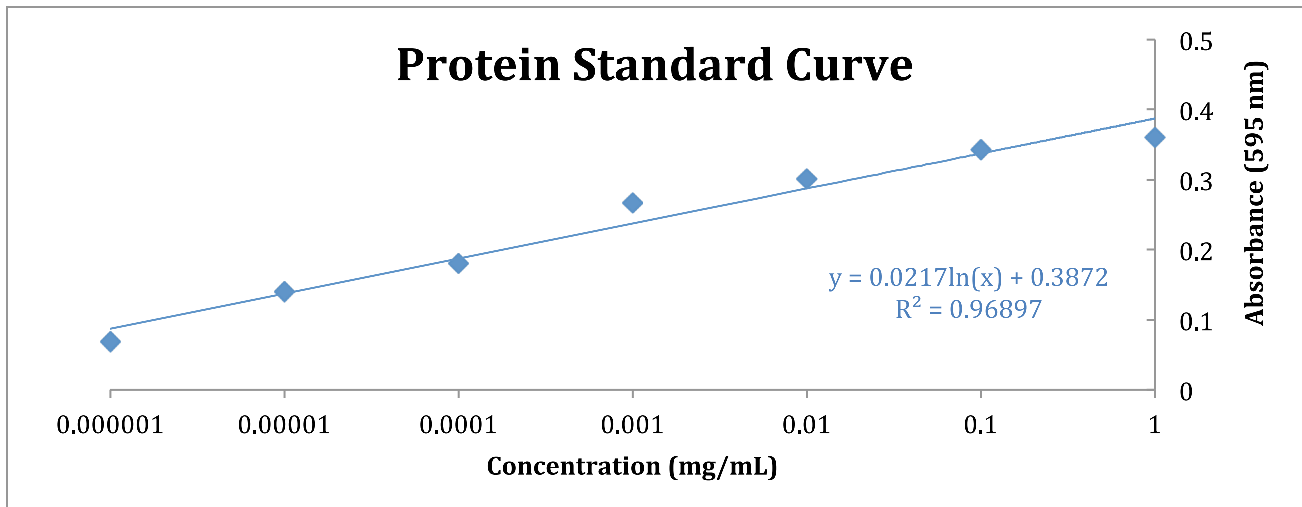
1. Create the stock solution, which will have a concentration of 1 mg/mL by dissolving 100 mg of BSA into 100 mL of buffer in the volumetric flask. Ensure there is adequate mixing and solvation of the protein.

2. Using a pipette or graduated cylinder, transfer 5 mL of the sock solution to a test tube. This should be labeled Test Tube 1.
3. Label six additional test tubes Test Tube 2-7, and fill each with 4.5 mL of the buffer using the pipette.
4. Using a pipette, transfer 0.5 mL of the solution in Test Tube 1 to Test Tube 2. Ensure there is adequate mixing, and then transfer 0.5 mL of the solution in Test Tube 2 to Test Tube 3, and continue this process down the line of tubes. This will create a serially diluted sample set of decreasing protein concentrations. Each dilution reduces the protein concentration by a factor of 10, for a range of 1 mg/mL to 0.000001 mg/mL.
5. Add 2 mL of the Bradford Reagent to each test tube, again ensuring there is adequate mixing. Make sure a fresh pipette tip is used. Allow the samples to sit for several minutes. Note the color gradation in accordance with the amount of protein in each sample.

Analysis Using SpecPhone:

1. Prepare the SpecPhone. Ensuring the surroundings are as free of outside light as possible, and that the light source to be used is bright enough. Place the mobile device on the SpecPhone apparatus and launch the camera app.
2. The first step is to find proper lighting. None of the camera pixels should be saturated by the light source, and the spectrum should have even intensity across all colors. Once adequate lighting is found, tape the apparatus in place and do not adjust it throughout the analysis, to maintain accuracy across all measurements.
3. Fill one cuvette with the buffer solution only, this will serve as the blank or reference cuvette. The blank should be used in between all sample measurements.
4. Insert the blank cuvette into the SpecPhone and take a photo of the resulting spectrum. Be sure to wipe all cuvettes with a KimWipe before using them.
5. Begin taking measurements, using a pipette to fill cuvettes with about 1 mL of each sample. Switch to a fresh tip between transfers.
6. Insert the first sample cuvette into the SpecPhone, and use the phone to take a photo of the resulting spectrum
7. Remove the first sample, and insert the blank cuvette. Take a photo of the blank spectrum, and then insert the second sample cuvette. Repeat this process, alternating between the blank and the sample solutions until a spectrum has been captured for all seven samples.

    Tip: It may be useful to photograph some object at the beginning and end of the series of photos to denote them in the camera roll. Notes should also be taken throughout the process, to recall the order of blank and sample.
8. Images can be imported to a computer containing ImageJ and a photo browser. Follow the instructions for image analysis provided in the *Image Processing and Calibration* protocol. The resulting data points can be used to construct a standard curve.



9. If an unknown sample is given, finding the absorbance value at 595 nm, and then using the equation of the line of best fit can solve for the concentration. Because serial dilutions were performed here, the trendline is logarithmic.