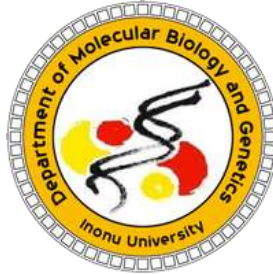


## GENERAL BIOLOGY LABORATORY II



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Week 5

### **Bioassays of major biomolecules: *Proteins***

Proteins are the most abundant biomolecules in cell or organism. The dry weight of a cell (or organism) is about 50% proteins and the rest is other molecules (such as carbohydrates, lipids, nucleic acids and other organic or inorganic small molecules). In other words, proteins are the workhorses of cells. They have a plethora of functions from their role in cell membrane integrity as structural or functional components to catalyzing reactions (such as enzymes) and acting as transcription factors. Given the prevalence and their diverse functions, proteins are may be the most studied components of cells.

As you have already seen in General Biology (and also Intro Mol Biol Gen) course, proteins are composed of amino acids. There are 20 of these building blocks and they are attached (dehydration reaction) to one another in a string by a covalent bond called peptide bond. Each of 20 amino acids has a distinct character. Given these unique properties of amino acids and the peptide bond, proteins in a biological source can be determined through a variety of methods.

Protein quantitation is an integral part of any laboratory workflow involving protein extraction, purification labeling or analysis. In this experiment we will learn how proteins are quantitatively and qualitatively assayed, using a protocol widely known and applied by the scientific community.

Spectrophotometric methods are the most applied ones. Since proteins and their building blocks (i.e., amino acids) behave differently under a range of different wavelengths, these kind of behavior can be utilized to determine the concentration of proteins in biological fluid. The simplest and most direct assay method for proteins in solution is to measure the absorbance at UV range. Amino acids containing aromatic side chains (i.e., tyrosine, tryptophan and phenylalanine) exhibit strong UV-light absorption. The most used UV range for proteins is 280 nm at which aromatic ring amino acids absorb light and 190 nm at which peptide bond character is responsible for absorption. However, proteins can also be treated with several of reagents that give a special color to the protein solution which can be measured at visible wavelengths (e.g. 600 nm). The most known protein assay methods are: Lowry method, Bradford method (dye-binding method), Biuret method, BCA (Bicinchoninic Acid) method, and etc.

The Lowry method of determining protein concentrations is one of the most widely used. The principle this method lies in the reactivity of the peptide nitrogen(s) with the copper ions under

alkaline conditions and the subsequent reduction of the Folin-Ciocaltey phosphomolybdicphosphotungstic acid to heteropolymolybdenum blue by the copper-catalyzed oxidation of aromatic amino acids. The Lowry method is sensitive to low concentrations of protein (0.01-0.2 mg protein ml<sup>-1</sup> solution).

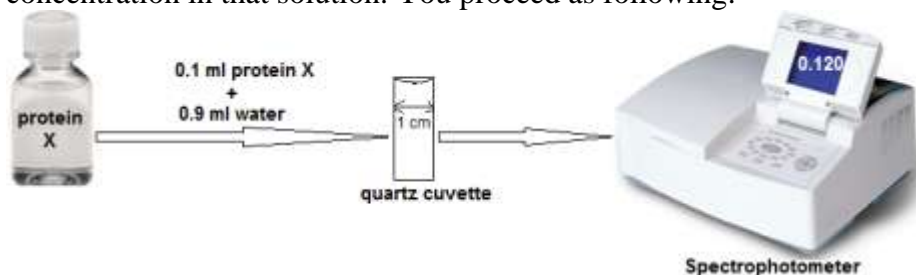
Since these assays are sensitive only to a low range of protein concentrations (mostly at 10-200 µg ml<sup>-1</sup>), any protein solution over 1 mg ml<sup>-1</sup> should be diluted to an appropriate dilution. However, how we will know the protein concentration in a solution at the first hand.

The most common way is to determine the protein concentration at 280 nm where aromatic ring amino acids absorb light. Use the following formula to roughly estimate protein concentration. (Light path length for most spectrometers is 1 cm).

Concentration (mg/ml) = Absorbance at 280 nm divided by path length (cm.)

This shows that the unit A<sub>280</sub> is roughly equal to unit protein concentration as mg ml<sup>-1</sup>. That is, if we read an absorbance 0.200 (in a 1 cm light path cuvette), the protein concentration in the solution is 0.2 mg ml<sup>-1</sup>. Let's work on another example!

Say you have an unknown protein solution in a bottle and you want to know the protein concentration in that solution. You proceed as following:



Since your spectrophotometer reading is 0.120, that means that you have 0.12 mg protein/ml solution in the cuvette. However, the question was how much protein in the bottle? Given the dilution of the original protein solution in the bottle [i.e., (0.1 + 0.9)/0.1 = 10]. This shows that protein solution in the bottle was 10 times diluted.

So, the protein solution in the bottle (i.e., protein X) has  $10 \times 0.12 = 1.2 \text{ mg ml}^{-1}$ .

Once we know the protein concentration of unknown by such a rough method, now we can determine its concentration in a more specific and sensitive assay such as the Lowry assay.

## Lowry protein assay

### Reagents

A. 500 ml 2% Na<sub>2</sub>CO<sub>3</sub> in 0.1 N NaOH

B. 50 ml 2% NaK Tartrate in H<sub>2</sub>O

C. 50 ml 1 % CuSO<sub>4</sub> · 5H<sub>2</sub>O in H<sub>2</sub>O

D. Alkaline copper reagent (98 ml A + 1 ml B + 1 ml C)

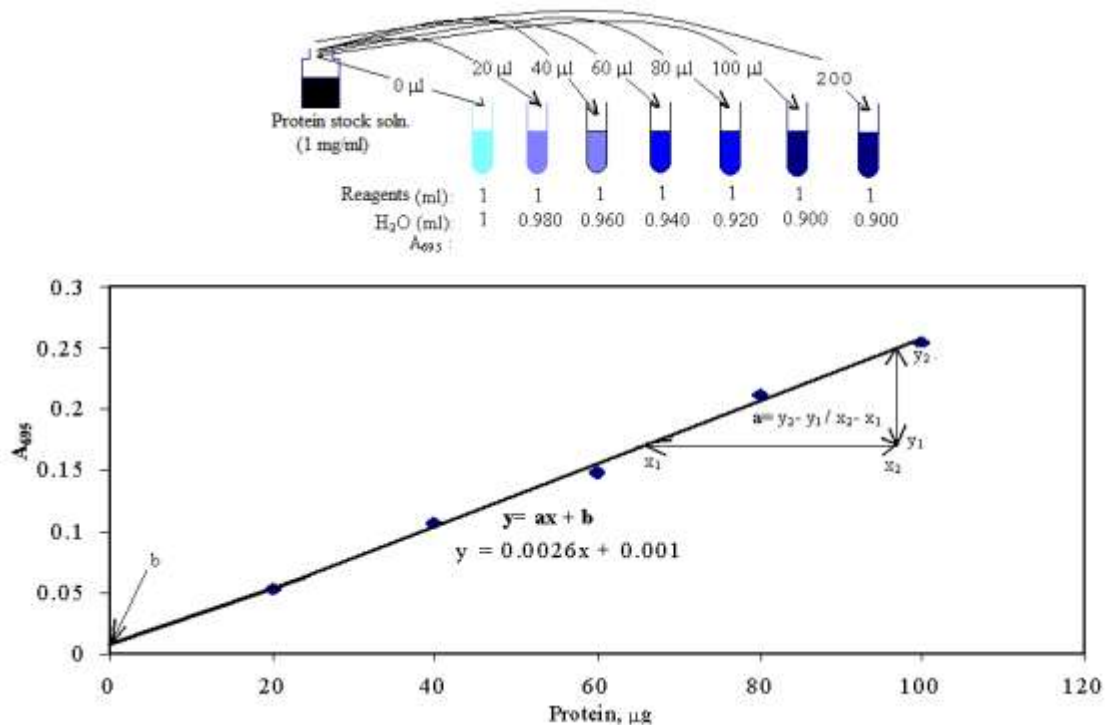
E. Folin-Ciocalteu reagent

- [Reagents A, B and C may be stored indefinitely]
- Stock BSA 10 mg/ml
- BSA Standard - 1 mg/ mL

### Procedure

Set up an identical parallel assay for all "unknowns" given to you.

1. Add 1 mg/ml stock BSA [0, 10, 20, 40, 80, 100, 200 μl] to test tubes.
2. Add appropriate volume of water so that the total volume in each tube is 1 ml.
3. Add 6 mL alkaline copper reagent (solution D) to each test tube.
4. Incubate for 10 minutes at room temperature.
5. Add 0.3 mL of Folin-Ciocalteu reagent to each tube.
6. Vortex each tube immediately and incubate at room temperature for 30 minutes.
7. Read and record the absorbance of each sample at 600 nm.
8. Plot absorbance vs μg protein to obtain a standard curve.



**State in your report**

1. What color you have observed and why after the protocol you have followed.
2. Differentiate between different protein assay methods by reading the paper given to you.
3. Why did we use the 280 nm wavelength before we start the Lowry method?
4. Calculate the concentrations of your unknowns from the standard curve you have constructed, taking into account your dilutions of original sample.