GREAT, YOU HAVE PROTEIN, NOW WHAT?

- What is your yield?
- How pure is it?
- Is it active?
- Does it have other spectroscopic properties?
- Does it interact with other proteins?
- What does the structure look like?
- Does it have a substrate range?
- What cellular processes does it play in?
LET'S ADDRESS THE FIRST QUESTION - WHAT WAS OUR YIELD?

- Proteins that have aromatic residues (Tyr, Phe, Trp) absorb light at 280 nm.

- Some proteins have other spectroscopic handles such as PTM chromophores (mCherry), cofactors (FMN, FAD, etc) and metal centers (iron-sulfur clusters, Cu, etc).

- No matter which absorbance handle you use, you can determine your yield by using Beer's law and the molar extinction coefficient for your chromophore.

Beer's (not beers) Law

- Not related to the delicious drink...sorry

\[ A = \epsilon c l \]

- \( A \) = absorbance
- \( \epsilon \) = extinction coefficient
- \( c \) = concentration
- \( l \) = pathlength (most often in cm)
Beer's (not beers) Law

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\[ A = \varepsilon cl \]

- A = absorbance
- \( \varepsilon \) = extinction coefficient
- c = concentration
- l = pathlength (most often in cm)
Example

A protein absorbance is 1.2 A.U. It also has an extinction coefficient of 60300 cm\(^{-1}\)M\(^{-1}\). What is the concentration of protein? The cuvette pathlength is 1 cm.

\[
c = \frac{A}{(E \cdot 1)}
\]

\[
c = \frac{1.2}{(60.2)(1)}
\]

\[
= \, 0.000019 \text{ M or 0.019 mM or 19 uM}
\]
So how do you find your yield if you don't know your extinction coefficient?

- You can use various assays (Bradford, Lowry, BCA) which are all based on standard curves using known proteins
Bradford Assay
Standard curves

Concentration Curve

\[ y = 0.2431x + 0.0379 \]

\[ R^2 = 0.9434 \]
BSA Drawback

- BSA exhibits strong dye response
  - That means using it will underestimate the actual protein concentration!!!!

- Good alternatives are lysozyme and Immunoglobulin G
Questions

Given an absorbance, extinction coefficient, and pathlength, can you find concentration?

Given a concentration, pathlength, and absorbance, can you find $E$?

Given an equation for a standard curve, and an absorbance, can you find concentration?

What are the drawbacks of using BSA?
After the lecture extras!!!

There are lots of resources out there to look at protein information. Google protparam, enter a sequence, hit enter and BAM! You will get your protein MW, isoelectric point, and a theoretical molar extinction coefficient. Very useful tool!!!
Try out Uniprot!

Uniprot is another database full of surprises. Got to uniprot.org, type in mCherry and click on X5DSL3. That's your protein!!!! Click around, see what information you can dig up.