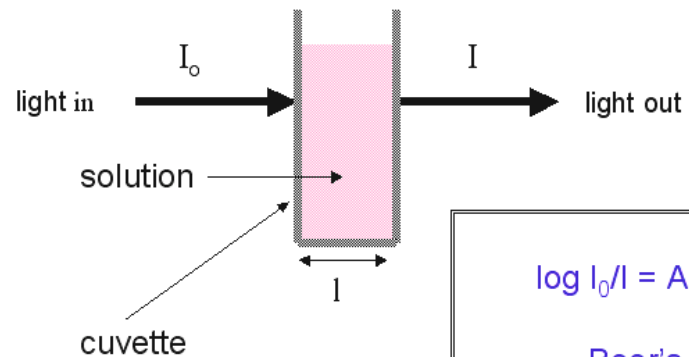


Spectrophotometric Analysis



$$\log I_0/I = \text{Absorbance}$$

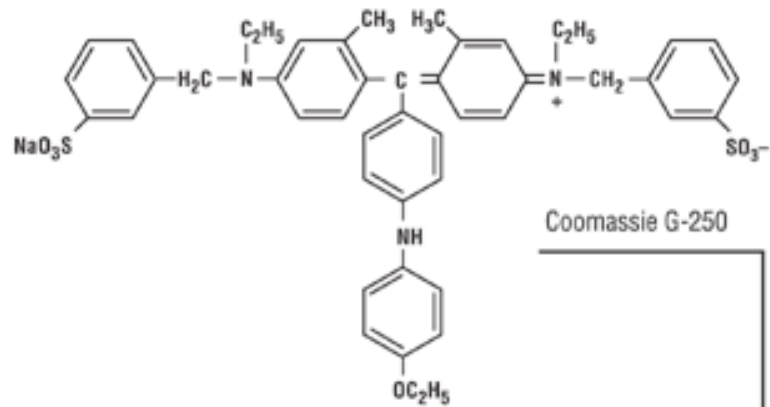
Beer's Law

$$\text{Absorbance} = \epsilon \times \text{conc} \times l$$

PROTEIN

Basic and Aromatic
Side Chains

+



Coomassie G-250

465 nm



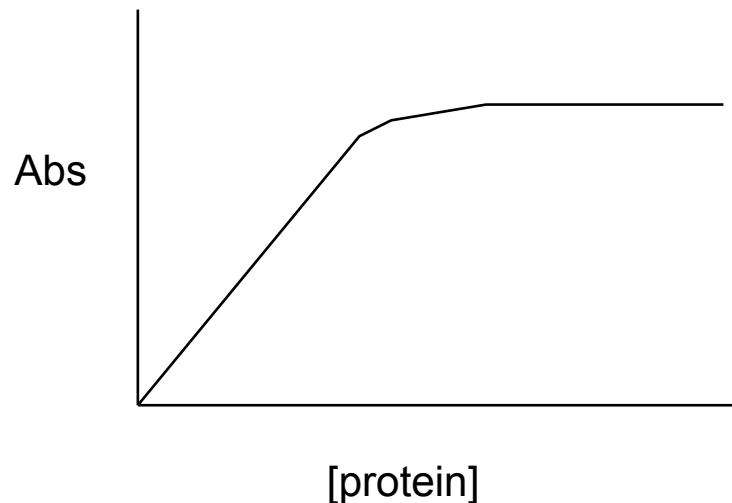
$A_{\max} = 595 \text{ nm}$

Protein-Dye Complex

Bradford Assay

- accurate over a concentration range from 0 – 1 $\mu\text{g}/\mu\text{l}$
- often, your sample will have a protein concentration far greater than 1 $\mu\text{g}/\mu\text{l}$!!

What's a Biologist to do?



The solution is dilution!

e.g. dilute sample 1/10 – then test it

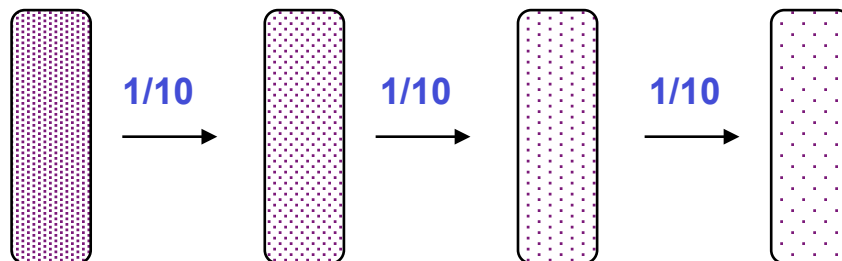
Dilution = 1/10

This gives you the [protein] of the diluted sample!

Multiple the interpolated concentration by the Dilution Factor (DF)

Serial Dilutions:

DF = 1/dilution



Measured [protein] = .25 $\mu\text{g}/\mu\text{l}$

Concentration of original
(undiluted) sample?