Protein concentration measurement by UV

Concentration of a purified protein is best measured spectrophotometrically using absorbance at 280 nm and calculated molar absorption coefficient (ε_{280nm}).

There are two main variations to the method: one can measure the absorbance either under native conditions using folded protein, or by denaturing the protein with guanidine hydrochloride. The latter will expose all aromatic residues to equivalent environment and minimize the effect of the folded protein on their absorbance, but at the same time it will also hide any problems with aggregation of the material as all protein will be denatured and solubilised. Depending on the method, the absorption coefficient should be calculated using either values from Pace et al (1995, native conditions) or Gill and von Hippel (1989, denatured samples).

This method is relatively accurate for most proteins, assuming there is sufficient number of aromatic residues that absorb at 280 nm (Trp and Tyr). Disulfides absorb as well, and for proteins rich in disulfide bonds this needs to be take into account when calculating the absorption coefficient.

Any contaminants, co-factors and buffer components that absorb at 280 nm will naturally distort the readings. Appropriate buffer for measuring the baseline will minimize these problems, assuming these components in exactly the same concentration in the sample as well. A very common issue is with imidazole which has UV absorbing contaminants and eluted samples from His-tag purification will have significant baseline shift due to this.

One should *always* measure complete UV spectrum from 340nm to 220nm in order to see the shape of the spectrum and not obtain single reading at 280nm. Protein spectrum should have maximum at around 278-280 nm (depending on how many aromatics and what type there are) and a minimum around 250 nm. Contamination by nucleic acids for example will cause significant absorption at 260 nm, and this can be easily detected when a complete spectrum is measured. For a quick estimate a "Nanodrop" kind of instrument is sufficient, but for final samples that are to be used for quantitative measurements or assays, the spectrum is best measured using a quartz cuvette with 10 mm path length and a "traditional" spectrophotometer. A bit more work, but very little compared to the time it took you to prepare that protein and the time it will take you to do the important quantitative measurements.

Protocol for native measurement:
- Record a baseline using buffer in which the protein is dissolved, including all components from 340nm – 220 nm.
    - Make sure there are no bubbles in the sample and that you are using a clean UV transparent cuvette, ideally a quartz one. Pay attention to the absorption readings while recording the baseline, they should not be excessive, typically < 0.2 AU with non-absorbing buffer and quartz cuvette.
- Dilute the protein (if need be) to be in linear range of the spectrophotometer, typically below 1.5 AU at 280 nm (far UV readings will be way off, but we are not using these).
- Record the spectrum and read the absorbance at 280 nm.
    - Repeat the measurement at least once (ideally twice) and use the average reading. Substract 2 x the absorbance at 333 nm from the reading at 280 nm to correct for scattering in the sample, which is wavelength dependent. Assuming you have measured

your baseline correctly, the only absorbance at 333 nm is from aggregates/dust/particles.

- Calculate protein concentration using the following formula:

$$\frac{(Abs\ 280nm - 2 * Abs\ 333\ nm) * dilution\ factor}{e280nm/1000} = [protein]\ (in\ mM)$$

Multiply the [protein] by molecular weight in kDa to obtain concentration in mg/ml. I like doing the calculation with mM extinction coefficient and MW in kDa as these are the kind of concentrations/values we use in our daily work in the lab. Molar solutions of proteins are hardly possible!

Protocol for denatured measurement:

- Use 6 M guandinine chloride, 50 mM Na Phosphate, pH 7.5 to dilute all the samples (at least 10x), including the baseline sample.
  - Measure the same sample at least twice, doing the dilution every time to account for pipetting errors. Make sure ton and get drawn into the tip after it has been used on use fresh, un-wetted tip every time as proteins will have significant surface tensio ce.
  - Calculate the concentration as above, but use the Gill and von Hippel values for ε_{280}
  - You are unlikely to see scattering by aggregates as they will be dissolved by GndHCl. In native method scattering at longer wavelength can be a useful information and indicative of protein's state at the time of measurement, and you might want to do do the measurement in both conditions, for the peace of mind if nothing else.

References:

Pace CN, Vajdos F, Fee L, Grimsley G, Gray T. (1995) How to measure and predict the molar absorption coefficient of a protein. Protein Sci. 4(11):2411-23

Gill SC, von Hippel PH. (1989) Calculation of protein extinction coefficients from amino acid sequence data. Anal Biochem. 182(2):319-26

Example absorption curves:
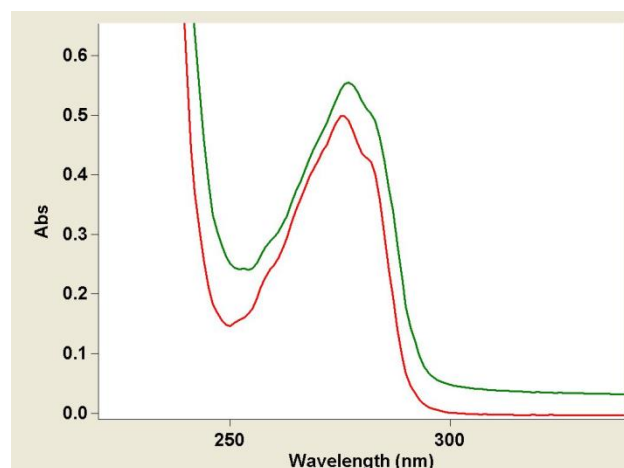
UV absorption curve for native (green) and denatured sample (red) of a same protein sample, at same dilution. Note increasing absorbance at longer wavelength for the native sample, indicating aggregation or precipitation, or other insoluble contaminants that scatter light. Note also differences in the details of the curves and slightly shifted maximum of absorption between the samples.

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