# Lecture 9
# Biomolecule Characterization

## Question 1: Electromagnetic radiation and matter

a) UV/Vis spectrometry relies on electromagnetic radiation with energy that corresponds to:
- Transitions between vibrational levels of chemical bonds
- Interference with nuclear energy levels
- Transitions between electron energy levels
- Molecular diffusion states

b) Select a statement that is **false**:
- Differential scanning calorimetry is a spectroscopic method
- Visible light has higher energy compared to microwave radiation
- Radio-frequent radiation is used in resonance experiments
- UV absorbance increases with the content of conjugated double bonds

c) Circular dichroism is based on:
- Differential scattering of left- and right- polarized UV radiation
- Differential absorption of non-polarized UV radiation
- Differential absorption of left- and right- polarized UV radiation
- Dynamic motion (vibrations) of secondary structure element

d) Select a statement that is **true**:
- Chromophores display stronger UV/Vis absorbance compared to fluorophores
- Fluorophores have larger optically-active groups compared to chromophores
- Fluorophores emit radiation of lower energy compared to the absorbed radiation.
- Fluorophores emit radiation of lower wavelength compared to the absorbed radiation.

e) Stokes-Einstein equation is used in which method:
- Circular dichroism
- Differential Scanning Fluorimetry
- X-ray crystallography
- Dynamic light scattering

f) Select a statement that is **true**:
- The same chemical groups in proteins are used to measure UV absorbance and DSF.
- Unlike proteins, nucleic acids do not have an extinction coefficient at 280nm.
- The presence of double bonds in carbohydrates makes them detectable by UV.
- All amino-acids are detectable by circular dichroism.

## Question 2: Select a biophysical method for your experiments

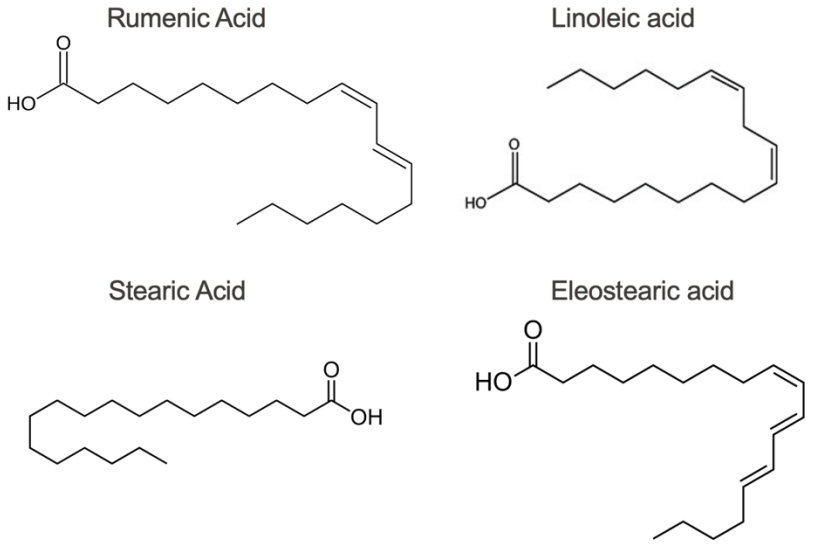
You produced a bacterial protein that has not been characterized previously. After performing extensive chromatographic purification step you wish to learn more about this protein. Select the biophysical methods from lectures 8 and 9 that you can use to measure the below-mentioned properties:

| | |
|---|---|
| Inspect protein purity | |
| Determination of molecular radius in native state | |
| Determine the content of secondary structure elements | |
| Determination of molecular weight in denatured state | |
| Measure concentration | |
| Measure protein stability to thermal denaturation | |
| Determine atomic structures | |
| Study protein aggregation | |
| Protein localization inside the cell | |

The method options are:
- Dynamic Light Scattering (DLS)
- Sodium-dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE)
- Circular dichroism (CD)
- UV spectrometry/spectroscopy
- Differential Scanning Fluorimetry (DSF)
- Fluorescent microscopy
- Cryo-electron microscopy (cryoEM)
- Nuclear Magnetic Resonance (NMR)
- X-ray crystallography (XRC)
- Fourier-Transform Infrared Spectroscopy (FT-IR)

## Question 3: UV absorbance

a) Below are structures of several fatty acids containing different content of double-bonds, resulting in different minimal energy difference between π and π* levels. Order the molecules based on the location of their maximum UV absorbance wavelength ($\lambda_{max}$), from highest to lowest.




b) Below are the sequences of 3 different short proteins (peptides). Based on the content of UV-active amino acids in each peptide, can you rank them based on the expected extinction coefficient at 280nm (from highest to lowest)?

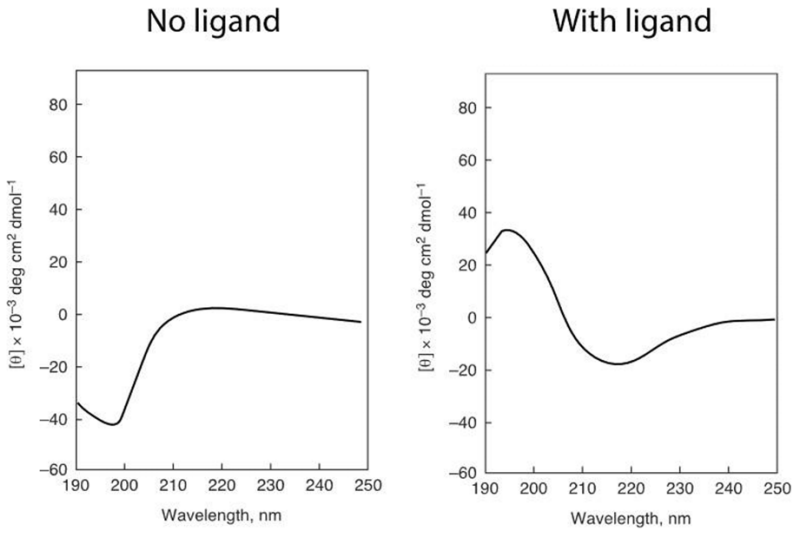
Peptide M: MSTRKRVVNTSPLGNEDPKNYQMILAPRESNQGNKILSSGQEDPRTN
Peptide N: MTGSTTVNGTWADTIEGKQGRSDGDSYILSSASGSDRTYESNMPSL
Peptide O: MNGRGAGALMYRSDKDAKNGYLANVDAKDLVKKENGAASKLMIPNT

c) You wish to determine the concentration of Protein X in aqueous buffer. Its' molecular weight is 67kDa and the molar extinction coefficient at 280nm wavelength ($\varepsilon_{280}$) is 43'824 L/(mol*cm). Using a cuvette with a light path (l) of 1cm, you measured the absorbance at 280nm to be 3.5. Based on these values can you determine the molar concentration of this sample (mol/L). What is the mass concentration (g/L)?

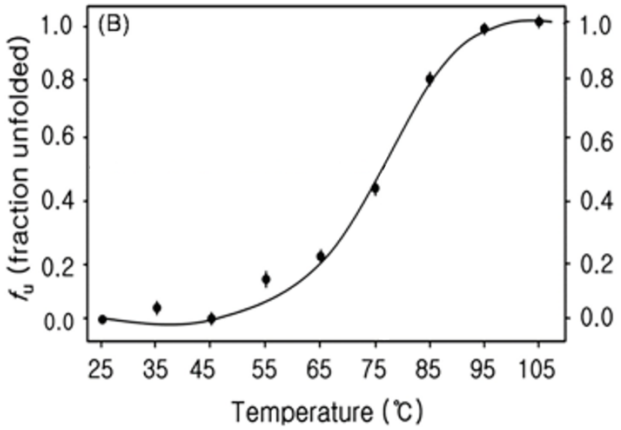
## Question 4: Circular dichroism

You expressed and purified a bacterial carbohydrate-binding protein Z. This protein can detect galactose and switch on downstream signaling pathways to metabolize this sugar. You are interested in uncovering the molecular processes that regulate this signaling and discover a way to exploit it for therapeutic purposes.

a) You performed circular dichroism (CD) on protein Z in the presence and absence of the ligand (at 25˚C). Please describe what type of an effect the ligand has on protein? Based on comparison to the reference CD spectra shown on lecture slides can you elaborate on the specific details of this change?




b) You wanted to measure the thermal stability of protein Z in the presence of the ligand using CD and you obtain the graph shown below. Can you estimate the Tm value from this plot? What would the raw CD curve (ellipticity vs wavelength) look like at temperatures >95˚C?

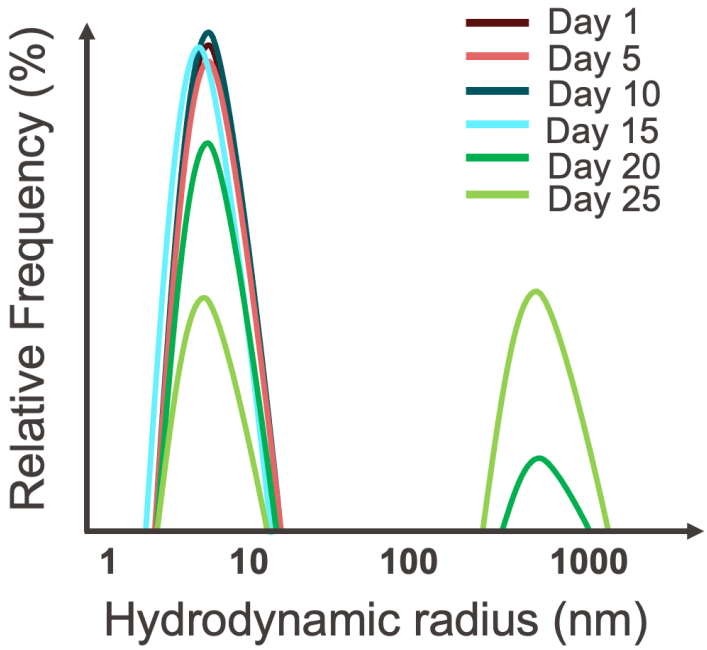



c) You are interested in developing a new antibiotic candidate that will inhibit this critical galactose detection mechanism and block downstream metabolism. You have a library of potential compounds to screen from. How would you setup the CD assay to determine which of these candidate compounds can block the activity of this protein?

## Question 5: Light Scattering

Dynamic and multi-angle light scattering methods are particularly useful in pharmaceutical industry for monitoring the quality and homogeneity of different therapeutic formulations.

a) Below you will see a DLS size-distribution curve of a monoclonal antibody cocktail after storage at room temperature for different periods of time. Can you infer what could be happening to the sample over time? What would be the max storage time under these conditions to assure the sample is still effective for therapeutic use?

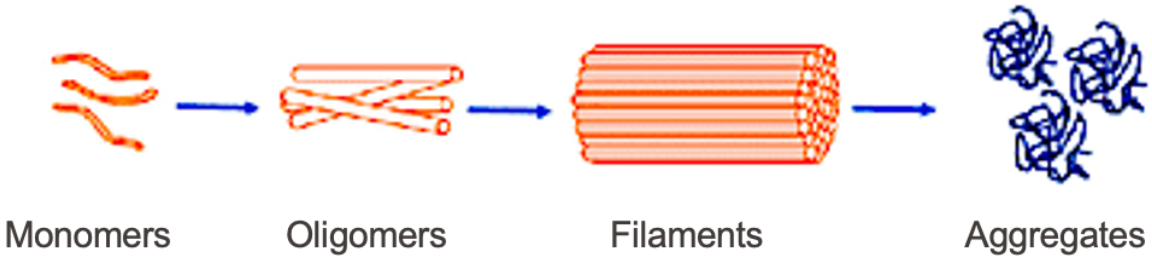



b) You propose that one way to preserve the sample is to store it at lower temperatures (e.g., 4˚C). The viscosity of aqueous solutions is 2x higher under these conditions. How will this affect the diffusion coefficient of the monoclonal antibody? What about the hydrodynamic radius? Qualitative descriptions are sufficient.

c) Calculate the $r_h$ for this monoclonal antibody in storage solution at 4 ˚C (T = 277 K). The diffusion coefficient (D) is $2.1*10^{-11}$ $m^2/s$ and the viscosity (η) of the storage solution at this temperature is $1.6*10^{-3}$ Pa*s (= $J*s/m^3$). Assume that the Boltzmann constant ($k_b$) is $1.38*10^{-23}$ J/K, and π=3.14

## Question 6: Protein aggregation

Aggregation of certain cellular proteins (e.g., alpha-synuclein) is believed to be the primary causative factor of neurodegenerative disorders. You discovered one protein of only 80 amino-acids (MW = 9.5kDa) that resembles alpha-synuclein, and you suspect that it may undergo similar aggregation process upon triggering with chemical stimuli (e.g., ligand or low pH).




a) Which methods would you use to determine if the protein is structured or disordered before chemical induction?

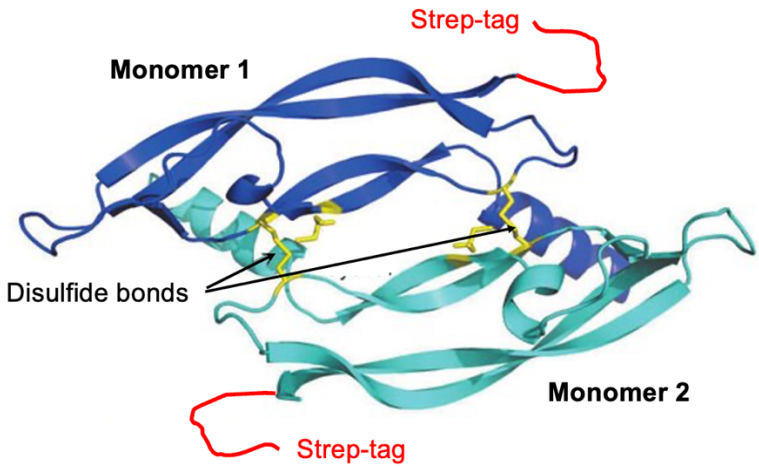
b) After getting a better understanding of the protein in its monomeric form, you now wish to study the aggregation process. You can trigger aggregating by lowering the pH of the buffer which allows to control the start of the process. Which method can you apply to study the size of the oligomers and aggregates at different time points?

c) In alpha-synuclein the transition to fully aggregated state proceeds through several intermediates (e.g., oligomers, filaments) which form regular b-sheet-enriched assembly. The transition usually takes a few hours. Which method could you use to monitor this process over time?

d) You are suspicious that the monomers undergo peptide bond cleavage and fragmentation into smaller segments, in order for the aggregation to occur. Which approach would you use to check if the fragmentation is indeed occurring?

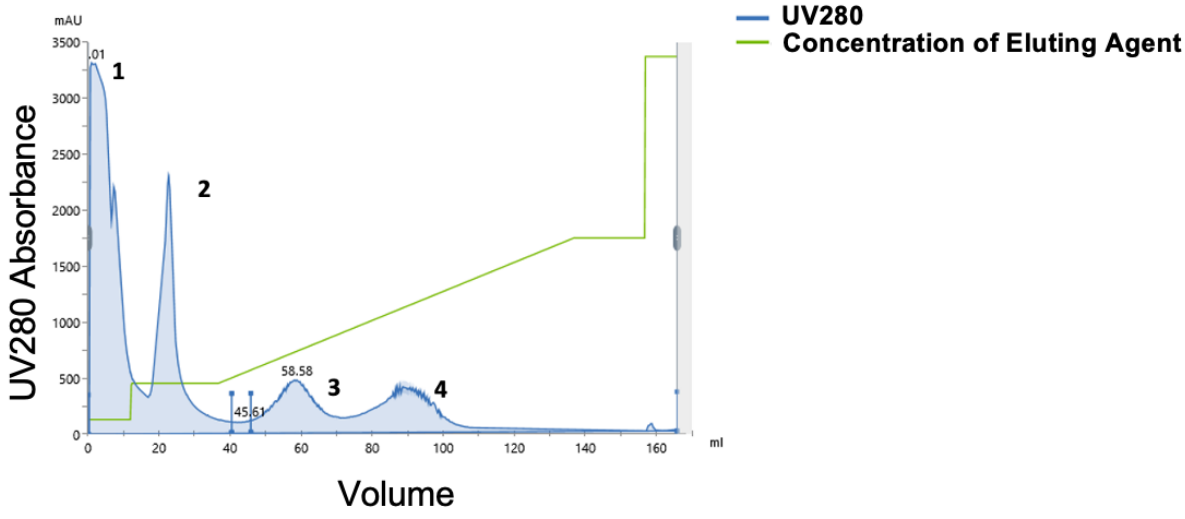
## Question 7: Gel electrophoresis coupled with chromatography

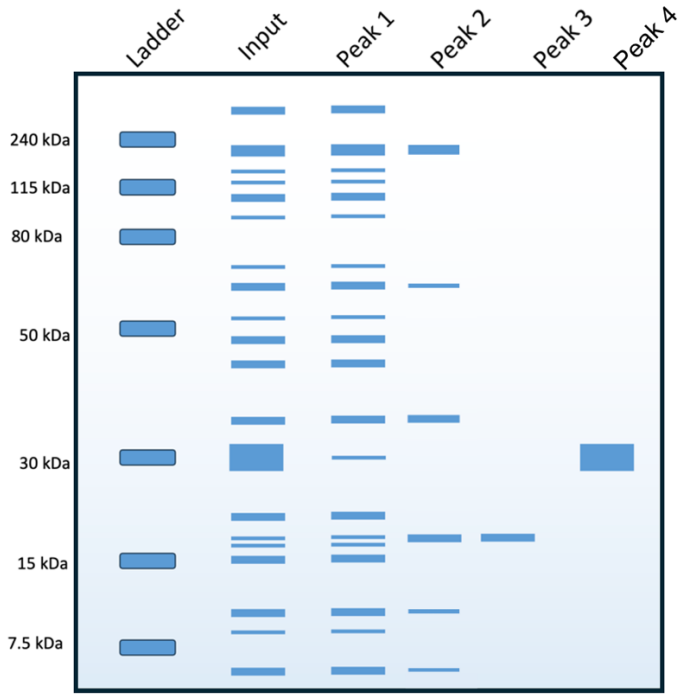
You are working with an enzyme with the predicted molecular weight of 30 kDa (based on amino-acid sequence). In nature this protein needs to dimerize in order to be in its' functional state. The dimeric state is further stabilized by 2 disulfide bonds formed between the monomers. The protein has been engineered with a C-terminal Strep-Tag and recombinantly expressed in *E. Coli.*



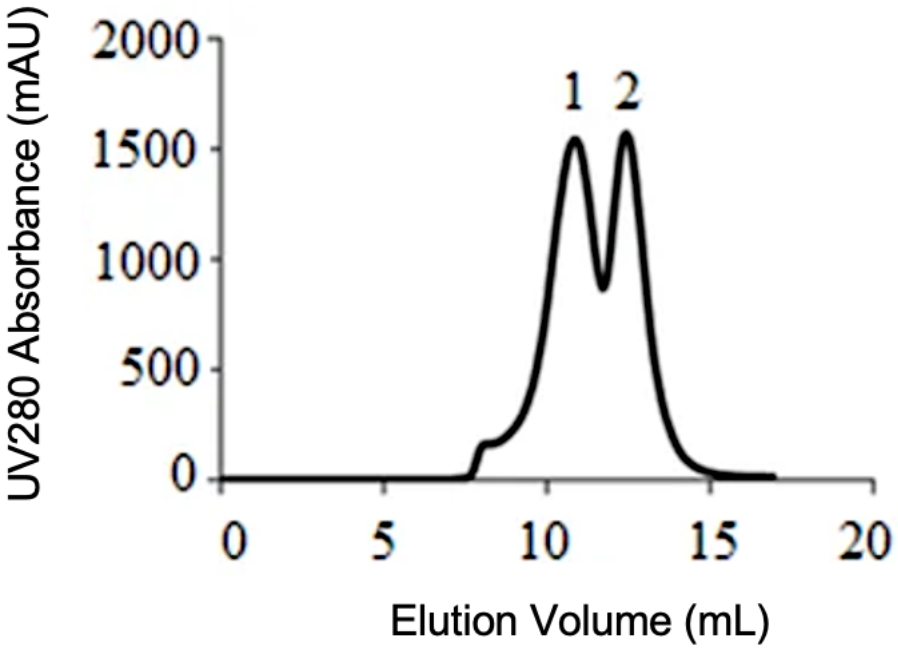

a) What type of affinity column would you use to purify this protein?

b) After purification you obtain the following chromatogram which shows more peaks than you anticipated. To determine which peak contains the pure protein you run small amounts on an SDS PAGE gel under reducing conditions (+ DTT). Which peak contains the pure protein of interest? Please explain.






c) To separate different oligomeric states of the protein (monomer vs dimer) you perform size-exclusion chromatography. In the chromatogram you see two peaks and you get very excited because you assume that one of the peaks belongs to the dimeric active form of the enzyme. Which peak corresponds to the monomer and which peak to the dimer based on the elution volume?



d) You take a small amount of each peak and denature it under reducing (+DTT) and non-reducing (-DTT) conditions, followed by SDS PAGE analysis. The results are shown below. What can you say about the status of inter-monomeric disulfide bonds in Peak 1 and Peak 2? How do these results correlate to size-exclusion chromatography in terms of the oligomeric state?