# Circular dichroism CD Binding assay (two different topics)

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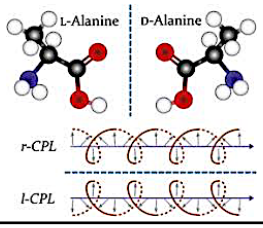
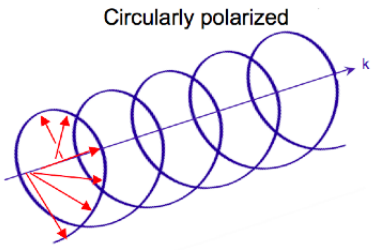
## What is a Circular dichroism ?

- Circular dichroism is a spectrophotometer observes differences in absorption of right and left circularly light by chiral molecules (of protein).
- $\Delta E = E_R - E_{L.}$
- It the protein molecules are chiral? How?

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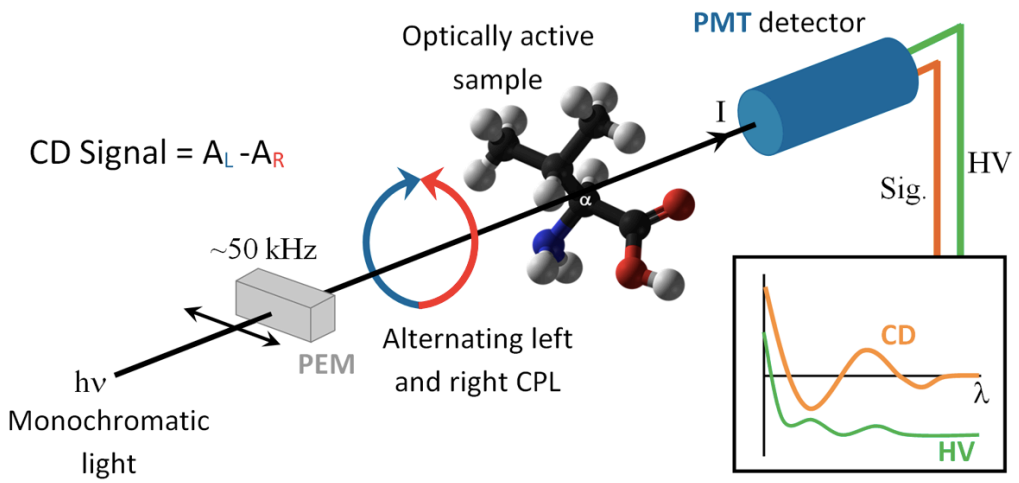


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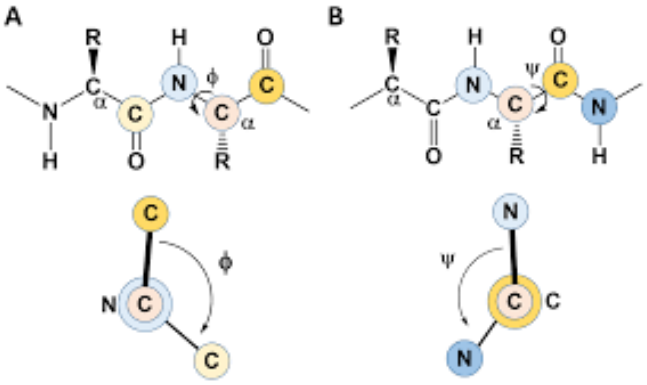
**How many two types of dihedral angles for peptides that can rotate the polarized light?:**

- There are two types of dihedral angles for peptides that can rotate the polarized light:
- Ψ Psi is between C-Cα
- Θ Phi is between Cα-N

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# What is the Circular dichroism used for?

CD spectroscopy is mainly used to:

1. To determine whether an expressed, purified protein is folded or not.
2. To determine the secondary structure of proteins.
3. To determine if a mutation affects its conformation or stability.
4. It can be used to study protein interactions.

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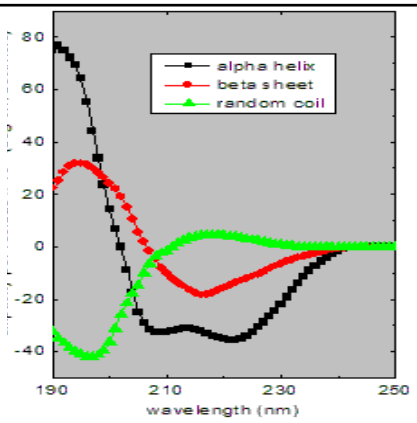
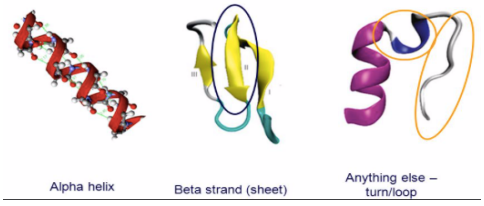
# How does CD spec. show the spectra? Explain?

- CD determine the type of the secondary structure of the protein as shown:
- Also to to estimate the percentage of the helixes, beta sheet and random coil.




Secondary structure is usually divided into three categories:

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**Usually we compare the CD spectra of the WT and the mutants.**

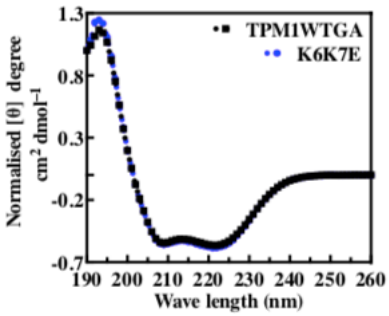


**Table 4.1 α-helical calculation and beta strands in the TPM secondary structure (195-260 nm).**

| TPM | α Helix % | β Sheet (Parallel and anti-parallel) % | β bends % | Random coil % |
|---|---|---|---|---|
| TPM1WTGA | 99.45 ± 0.1 | 0.65 ± 0.1 | 1.7 ± 0 | 1.8 ± 0.1 |
| K6K7E | 98.7 ± 1.6 | 0.75 ± 0.1 | 1.7 ± 0 | 1.65 ± 0.1 |

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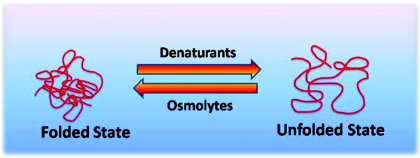
# How does CD signal change with Temperature?

- With Temperature increases, the protein begins to unfold till becomes totally unfolded.
- So, at this point we can collect the points of the spectra and fir it as an exponential curve to calculate Tm˚C.
- What is Tm.˚C?
- It the mid-point between folded and unfolded state.

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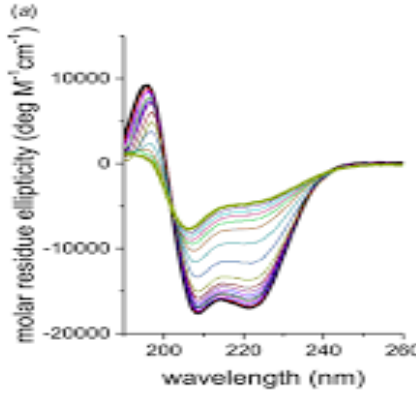
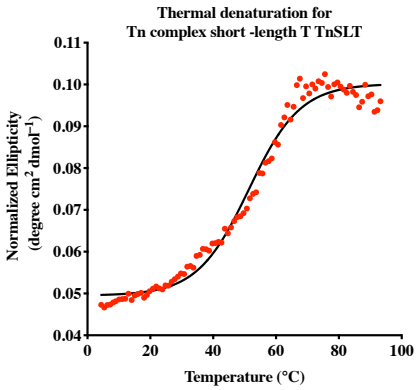
## Describe the change in the CD spectra with T ˚C increases

1. The point are collected at 222 nm and then fitted as an exponential curve.
2. The mid-point is calculated.
3. Then we compare the mid point of the WT with mutants.




**Curve fitting**

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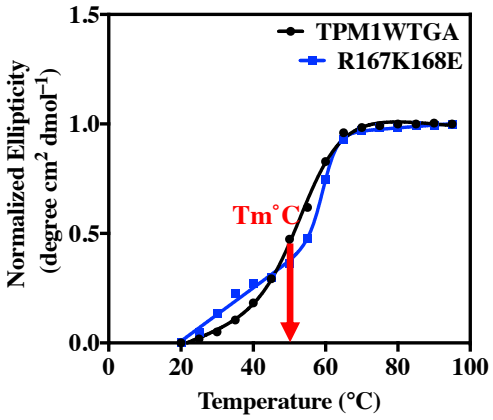
## Protein folding and unfolding

- Protein **Misfolding** could cause Disease.
- It can be studies by measuring Tm ˚C (mid temperature degree )using CD (thermal unfolding) technique.
- It shows a sigmodal curve.

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# Sedimentation assays ‘’Binding assay”

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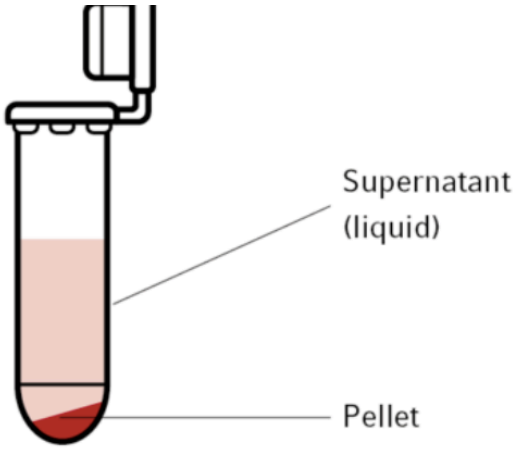
## What is the Co-sedimentation assay?

- Co-sedimentation assay is an in vitro assay
- It is done to assess the binding of the 2 proteins or more by sedimentation (centrifugation at high speed about 80,000 rpm).
- The aim of this assay is to examine the ability of filamentous protein such as acin to bind to non- filamentous protein.

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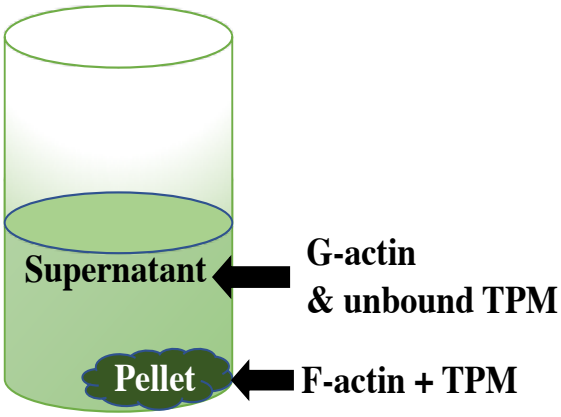
# Which protein does sedimented ?

- From history,
- We know that filamentous proteins only sedimented at high speed such as actin.
- However, non- filamentous protein never sedimented such as Tropomyosin Tpm and G-actin (non-filamentous)

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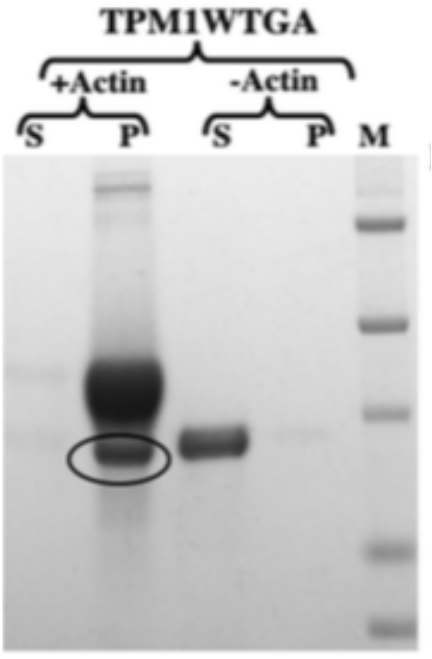


# How to check the binding of the Co- sedimentation?

- **The samples aare taken from the supernatant and the pellet and then checked by by SDS-page of gel electrophoresis:**
- **If the actin binds to Tropomyosin TPM, it will sedimented in the pellet.**
- **If not, it will remain in the supernatant.**

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