# Stability issues for protein pharmaceuticals

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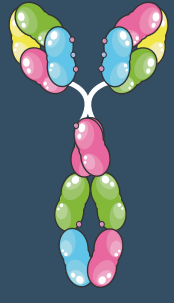
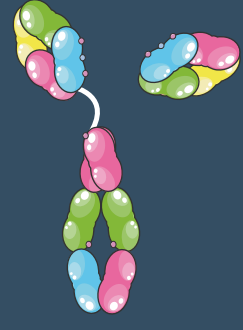
# Stability is key for function





Chemical

Physical

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# Chemical stability

**Key degradation mechanisms**

- Deamidation and Isomerization
- Oxidation
- Hydrolytic fragmentation
- Racemization and β-Elimination
- Maillard reactions

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# General issues effecting degradation

- Temperature
- Protein structure
  - Primary sequence
  - Tertiary and quaternary structure
- pH
- Choice of buffer and ionic strength
- Presence of contaminants from excipients and production - for example, catalytic metals and ROS
- Light and air (oxygen)

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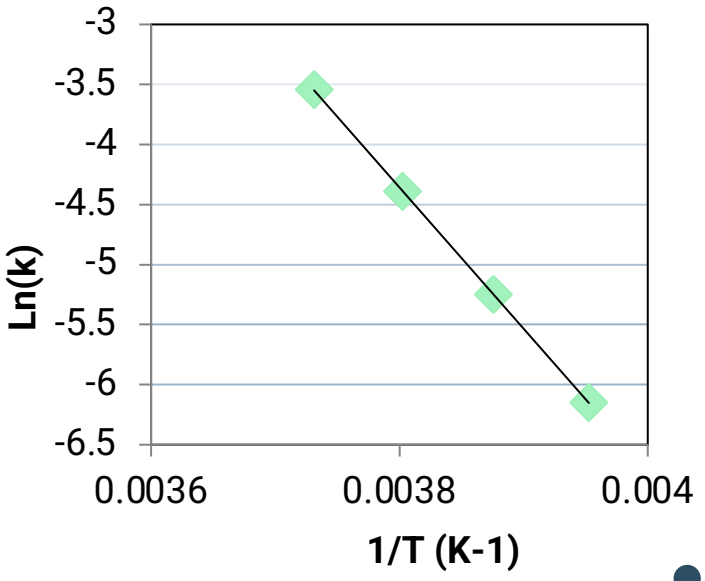
# Arrhenius or not Arrhenius

- Chemical reactions often follows the Arrhenius equation
- This is to some extent true for proteins
- Exceptions due to
  - Limiting by reactive impurities
  - Non-Arrhenius conformational changes
- Challenge- many parallel reactions that can affect each other

The Arrhenius equation

$$k = k_0 e^{-E_a/RT}$$

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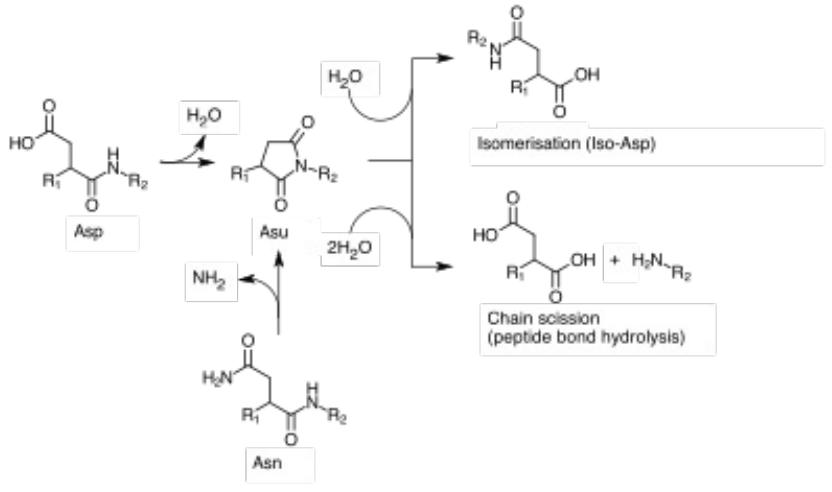
# Deamidation and isomerization




One of the most common chemical reactions in formulated proteins

Linked to increased immunogenicity

**Key amino acids**

hydrolysis of Asn and Gln

isomerization Asp

**Key conditions**

Sensitive to the mobility of polypeptide chain

pH-dependent with a minimum at pH 3-6

**Key strategies to mitigate**

pH control

Excipients such as sugars and polyols

Choice of buffer (TRIS not Phosphate) and ionic strength (low)

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# Oxidation

## Key amino acids

methionine, tryptophane and occasionally in histidine, cysteine, phenylalanine, or tyrosine residues.

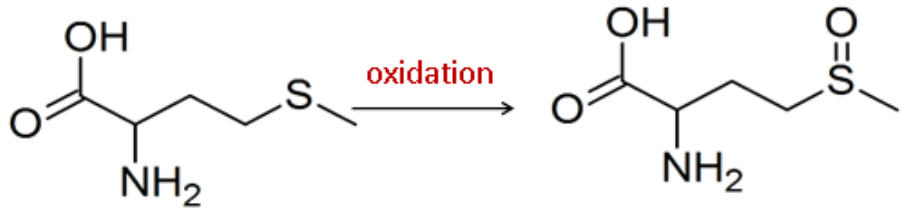
## Key triggers

- Metal-catalyzed oxidation
- Photosensitivity
- ROS (peroxides, free radicals)

## Key strategies to mitigate

- Antioxidants
- Chelating agents
- Protection against light
- Control of excipients
- Oxygen free packing and production
- pH

Methionine one of the most sensitive amino acids




Methionine

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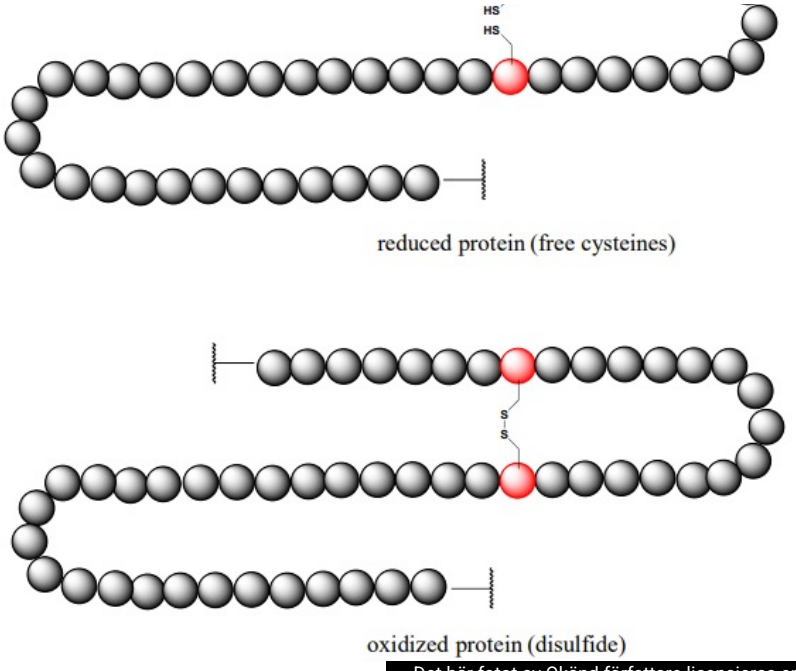
# Photostability

- When a protein is exposed to light, species are generated that will cause chemical oxidation
- amino acids that are sensitive to light-induced oxidation, are Trp, Tyr and Phe
- Trp is especially sensitive
- Excipients can be photosensitive, especially polysorbates

# Cystein

- Oxidation - formation of inter- or intra-molecular disulphide bridges
- Loss of stabilisation
  - Disulphide Scrambling
  - Reduction of disulphide bridges

Consequence conformational changes and aggregation



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# Proteolysis of the peptide backbone

**Key reactions**

- Aspargine hydrolysies associated backbone clevage
- Hinge region hydrolysis for Antibodies
- Protease related fragmentation

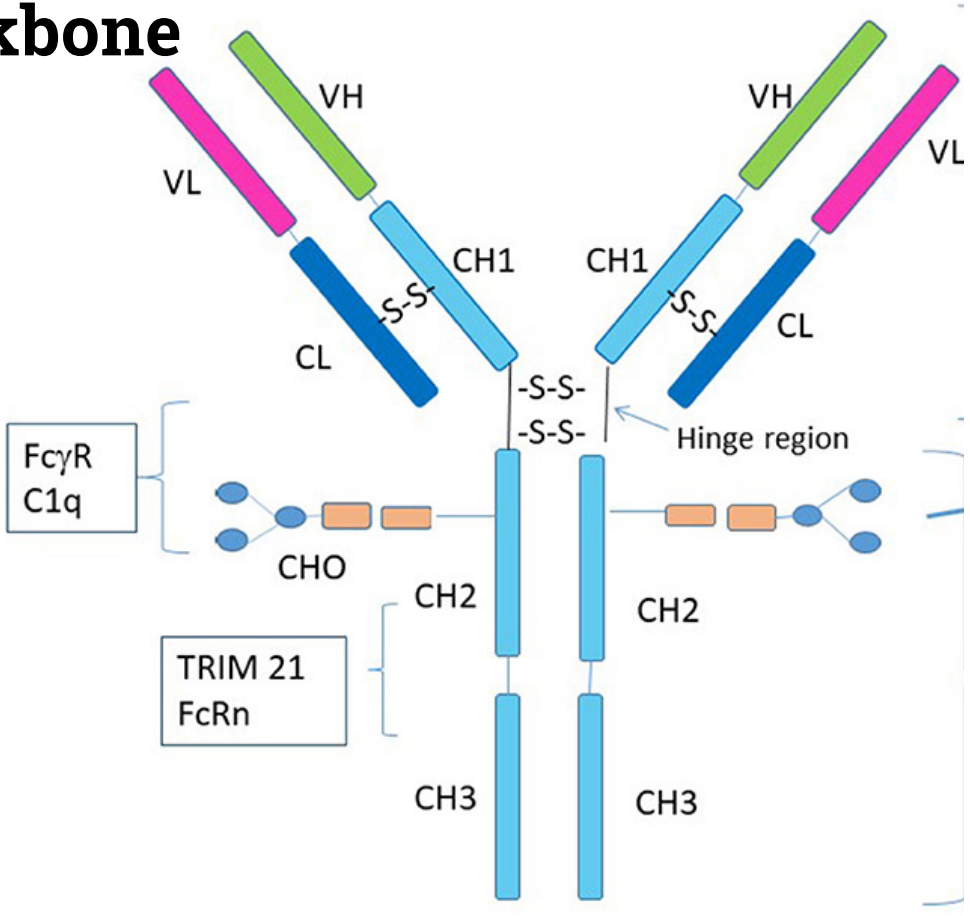
**Key conditions**

pH below 6

Asp-Pro especially sensitive

Hing region both acid and base driven

Remove protease during down stream processes

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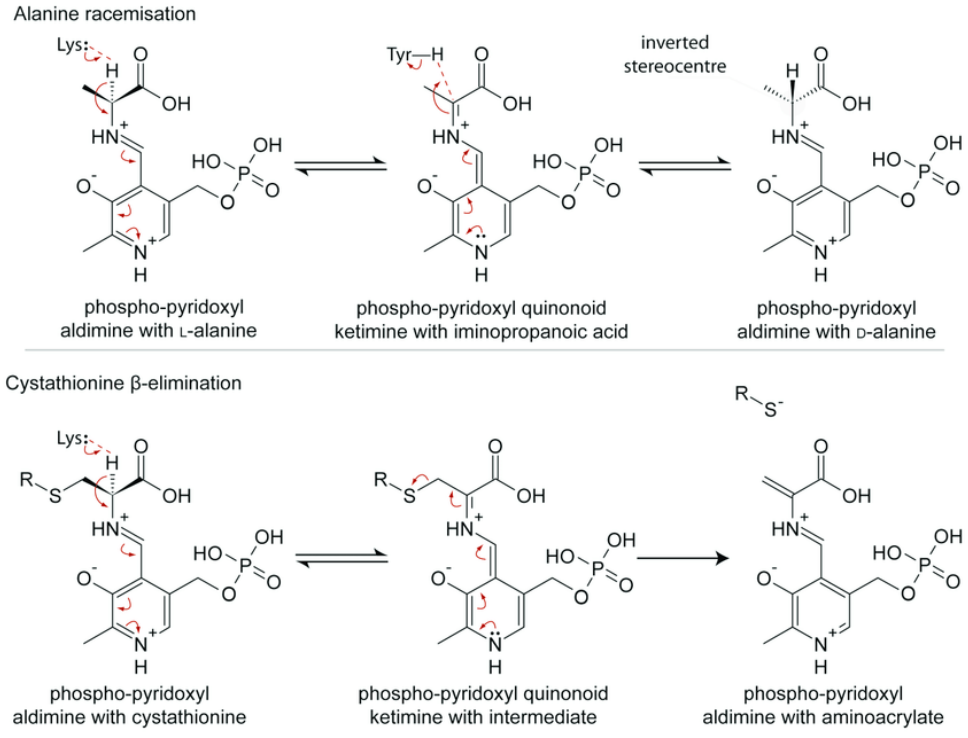
# Racemization and β-Elimination




Similar start a deprotonation of the hydrogen on the α-carbon

**Key amino acids**

Ser, Asp and Asn but normally slow

Cys important for β-Elimination

**Key conditions**

Racemisation; avoid basic pH

pH; β-Elimination in IgG minimum at pH 6

metal ions; catalyse β-Elimination

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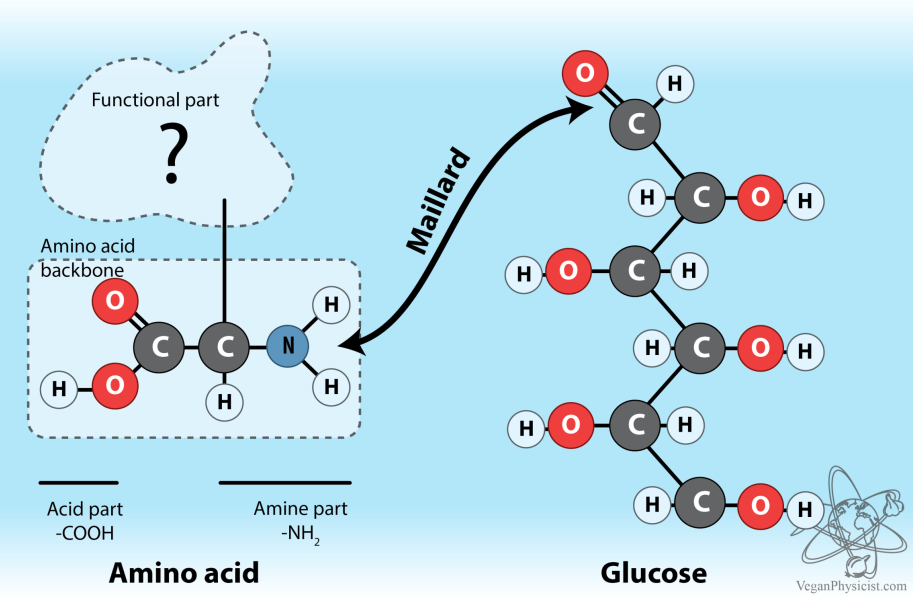
# Maillard reactions

Complex reactions between *primary amines* and *reducing sugars* (sugars carrying an aldehyde function)

- Amino acids: Lysine
- Monosaccharides: glucose, galactose
- Disaccharides: lactose, maltose

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# Strategies to increase chemical stability

**Avoid**

- Peroxides
  - PEG and PEG-based surfactants
  - Excipients treated by $H_2O_2$
- Protease impurities
  - Check manufacturing process
- Metal ions (particularly transition metal ions)
  - Add EDTA, or other chelating compound

**Add**

- Antioxidants
  - Ascorbic acid
- Osmolytes
  - Carbohydrates
  - Amino acids
  - Polyols- Glycerol, PEG, Manitol

Use

- Oxygen free packaging
- Light protection
- pH control

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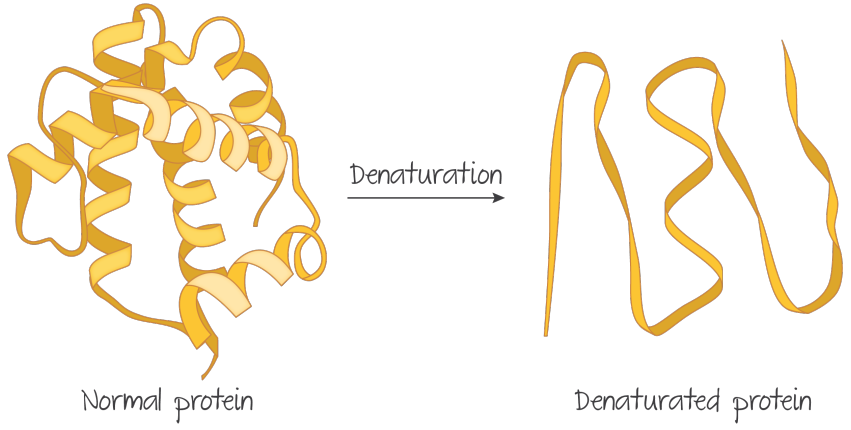
# Stability– conformational changes

## Effect

- Loss of activity
- Aggregation – Fibrils or particles - Risk of adverse effects such as immunogenicity

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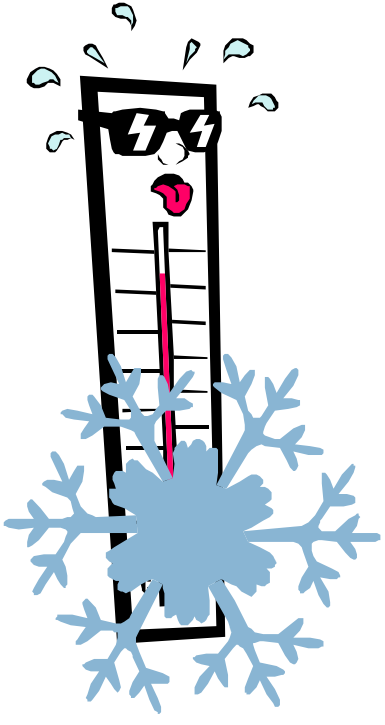
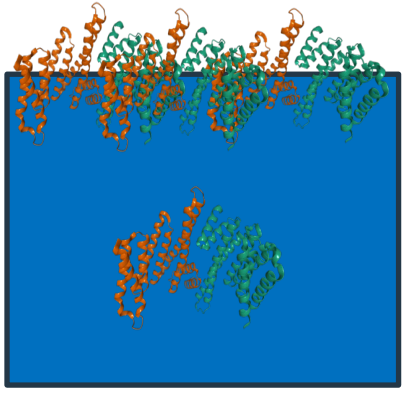
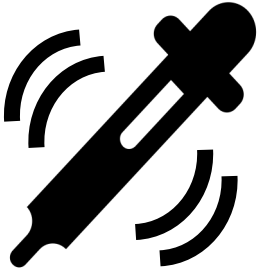
# What triggers aggregation



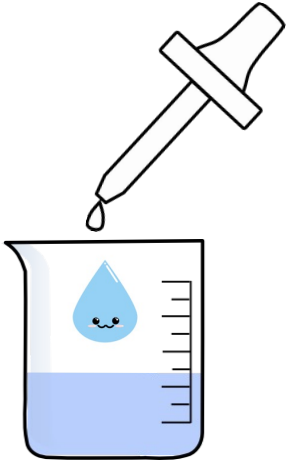
Heat, cold and freezing



Interfaces



Shaking and dropping



Dilute



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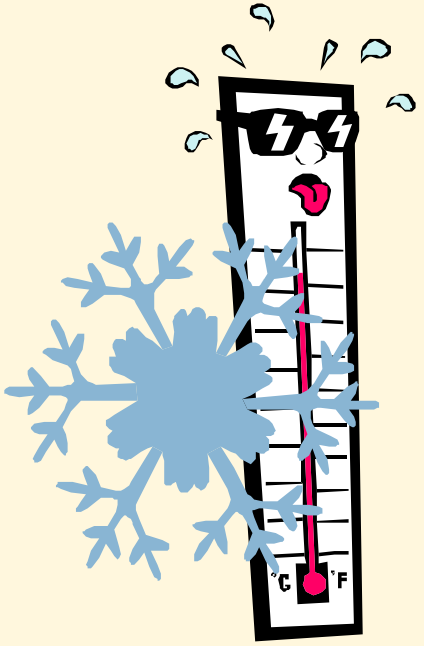
# Heat and cold




- Proteins can denature/change structure both at high and low temperatures
- Dropping a product can cause cavitation and local high-temperature
- Ice formation can change the composition of the drug product
- Proteins can adsorb to ice crystals

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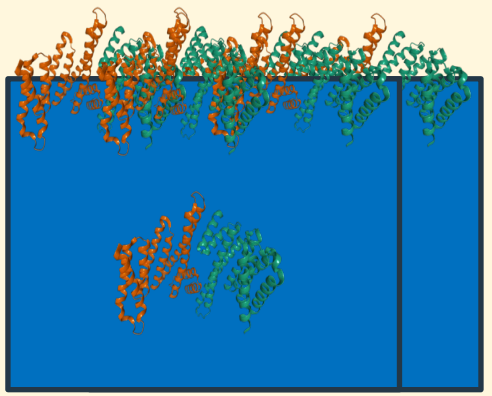
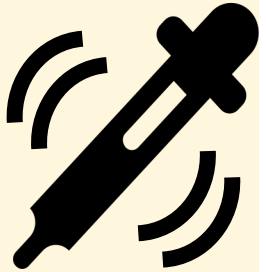
# Interfaces, Shake and flow





- Induction of aggregation through contact with interfaces
  - Air bubbles
  - Silicon oil
- Extensional flow aggregation

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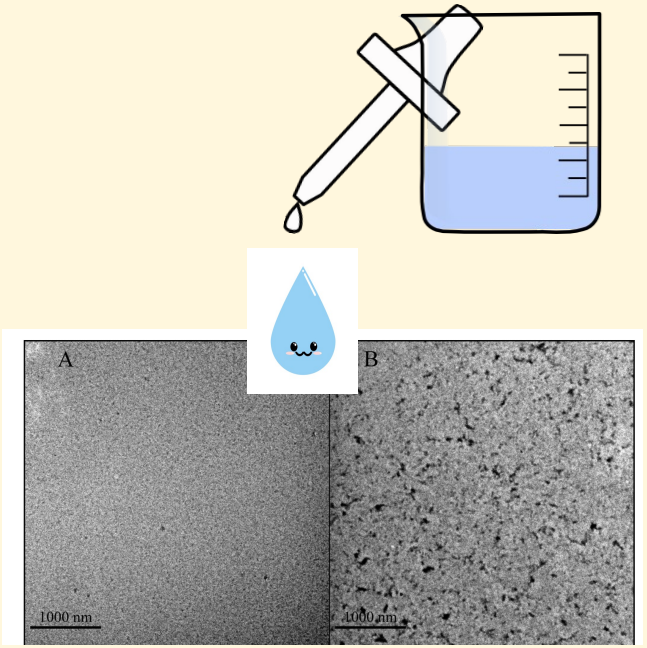
# Dilution




- Change in composition of formulation
  - pH
  - Ionic strength
  - Concentration of surfactant
- Example Trastuzumab
  - OK to dilute in 0,9% NaCl
  - Not ok 5% Dextrose

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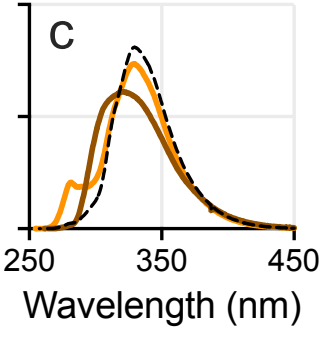
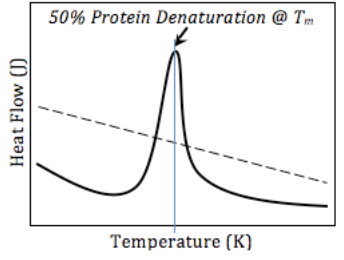
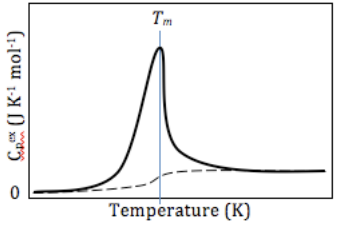
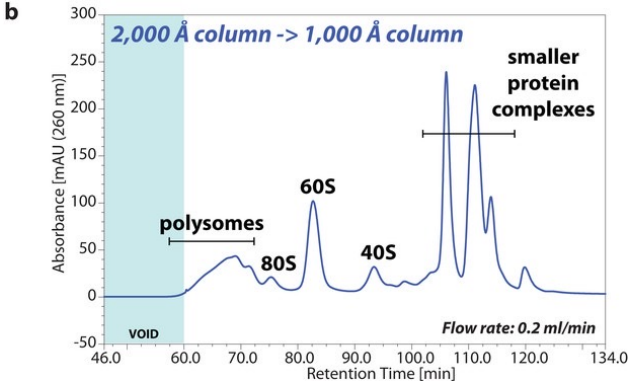
# How to evaluate structural stability

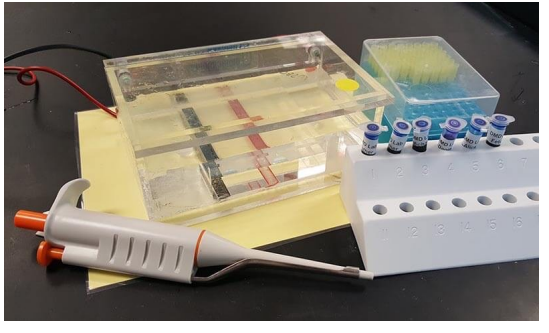
- UV spectra,fluorimetry and Circular dichroism spectra
- Size
  - Rheology
  - Light scatter, DLS
  - AUC
  - SEC and FFF
- Changes in the electrophoretic pattern
- Immunological tests
- Affinity binding
- DSC and DSF
- NMR





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## Real World Handling of Protein Drugs – Exploration, Evaluation & Education

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