# Preview Slides for Introduction to Protein Formulation and Delivery

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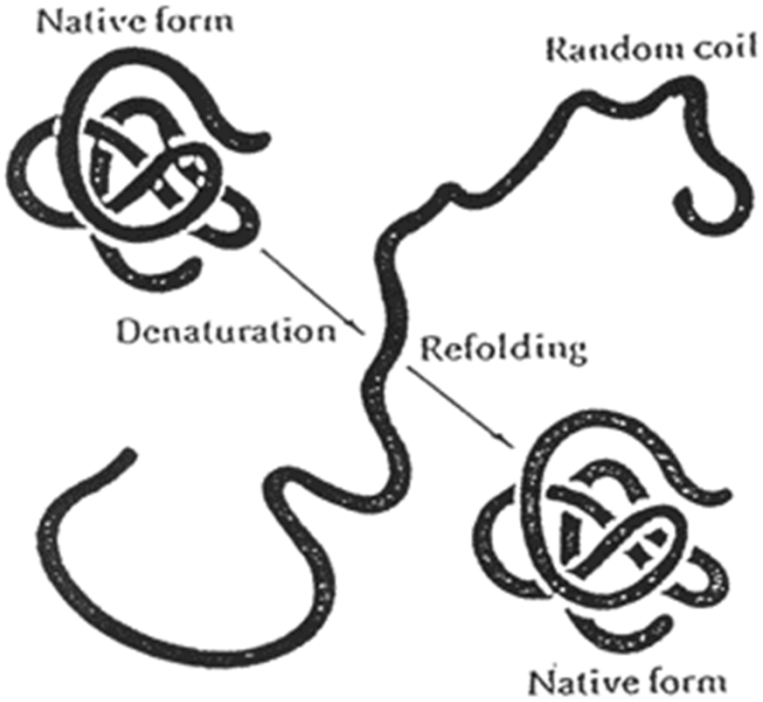
# Protein Folding: The Denatured State

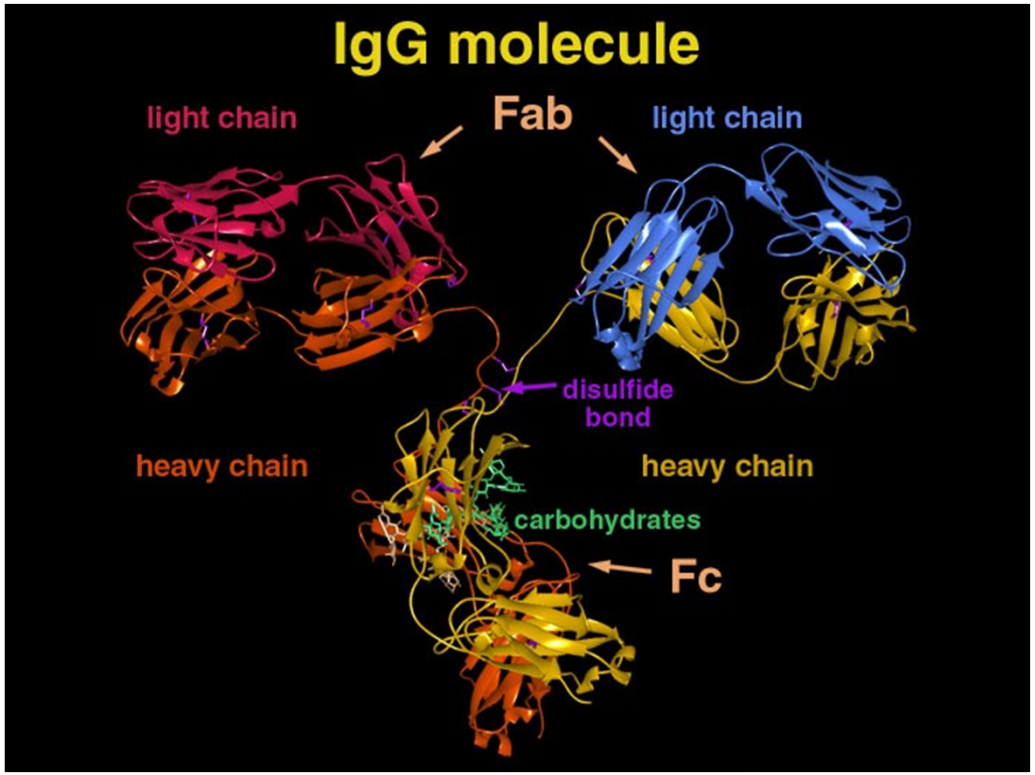
- Refers to the mostly unfolded state
- Usually D lacks any ordered structural elements, and is the higher-energy state under physiological conditions
  - Some D states have been experimentally shown to contain local, ordered structures
- D is stabilized by the high degree of conformational entropy due to the flexibility for attaining several conformations
- D is also stabilized by several H-bonds between the polypeptide groups and the solvent ($H_2O$) molecules




*http://albumen.stanford.edu/library/c20/messier1991a.html*

# Protein Folding: The Native State

- Final product in a protein folding pathway
- N brings amino acids, which are distant in the primary sequence, in spatial proximity
- The 3d structure of N is a delicate balance of several counteracting forces
  - Reduced entropy: limited number of conformations available to N
  - Favorable interactions: non-covalent and covalent
- The structure is determined by the collective influence of many individually weak interactions
- The goal of preformulation development studies is to identify conditions that maximize the stability of N relative to D under both stressed and non-stressed conditions



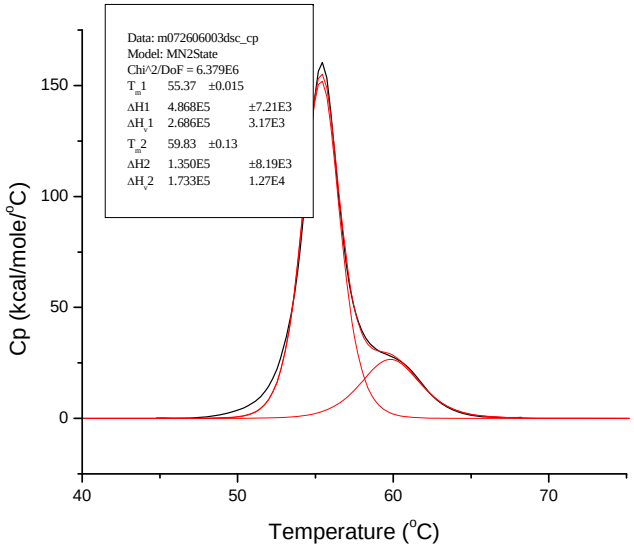

*http://www.bmb.psu.edu/faculty/tan/lab/gallery_proteins.html*

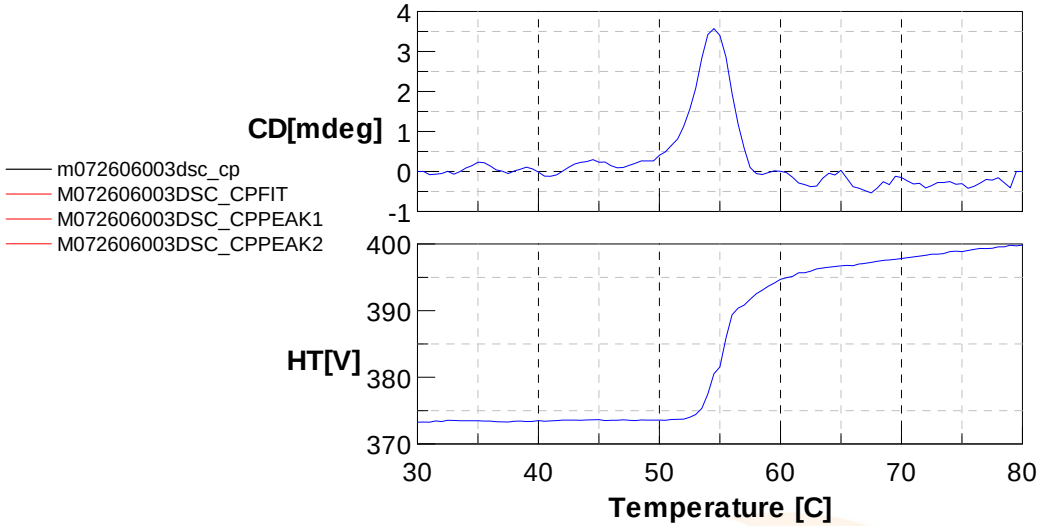
# Protein Aggregation

- Generally caused by heat denaturation or agitation
  - Agitation stress is related to increased interaction of protein with interfaces
- Proteins tend to denature and aggregate at interfaces
  - Air / Liquid
  - Liquid / Liquid
  - Liquid / Solid (including ice)
- Due to mixture of polar and non-polar side chains, proteins tend to concentrate at interfaces
- Aggregation generally increases with protein concentration
  - Increase the interactions between protein molecules, cause hydrophobic regions to interact

# Comparison of DSC and CD for Thermal Analysis

## Enzyme B




Tm1 = 55° C
Onset of unfolding = 47° C
Tm2 = 60° C

Tm = 55° C
Onset of unfolding = 49° C

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# Buffer Selection Considerations

- Freeze / thaw and Lyophilization Concerns
  - Changes in pH can occur as a result of buffer salt crystallization and precipitation during freezing
  - Inorganic salts – freezing point of mono-ionized salt can be different than that the non-ionized free acid or base
- Phosphate buffer (mono- and dibasic Na Phosphate)
  - Dibasic form can precipitate during freezing, resulting in dramatic pH shifts in the liquid medium containing the protein
  - Can cause protein denaturation
  - Problem for lyophilzation and for freeze / thaw of bulk
  - Replace Na cation with K cation in phosphate buffers can reduce this effect

# Routes of Administration

- Subcutaneous (SC)
  - More convenient for frequent / chronic administration
  - Can be self-administered outside medical facility
  - Couple with delivery devices such as pre-filled syringes and autoinjectors
  - Significant marketing advantages
  - Requires small volumes (<1.5mL)
  - Many proteins, such as antibodies, require high doses (>1mg/kg or >100mg/dose)
  - Coupled with small volume requirement, SC often requires very high protein concentrations (50-250mg/mL)

# Design of Experiement (DOE)
# 2-Level Factorial Design

- Allows evaluation of main effects and interaction effects
- 3 factor 2-level factorial design
  - Main effects: A, B, C
  - Interactions: AB, AC, BC, ABC
  - Total of 7 effects, the most you can estimate from an 8 run factorial design
    - » *One degree of freedom is required to estimate the overall mean*
- 3 factor interactions (ABC) are rare
- Interactions between >3 factors are not only extremely rare, but are almost by definition not significant effects, and should be disregarded statistically
  - Designs which include evaluation of such interactions should be regarded as inefficient

# Effect of Buffer Type on MAb Stability

- DOE Summary
  - Two fractional factorial designs
  - Selected to span a wide range of pH conditions
  - MAb concentrated to >200 mg/mL; 40°C for 4 weeks
- Full panel of analytics performed:
  - SEC showed effect of buffer/pH on HMW species
  - Phosphate buffer: HMW increases with increasing pH
  - Histidine buffer: HMW stable from pH 6 - 7

Design-Expert® Software

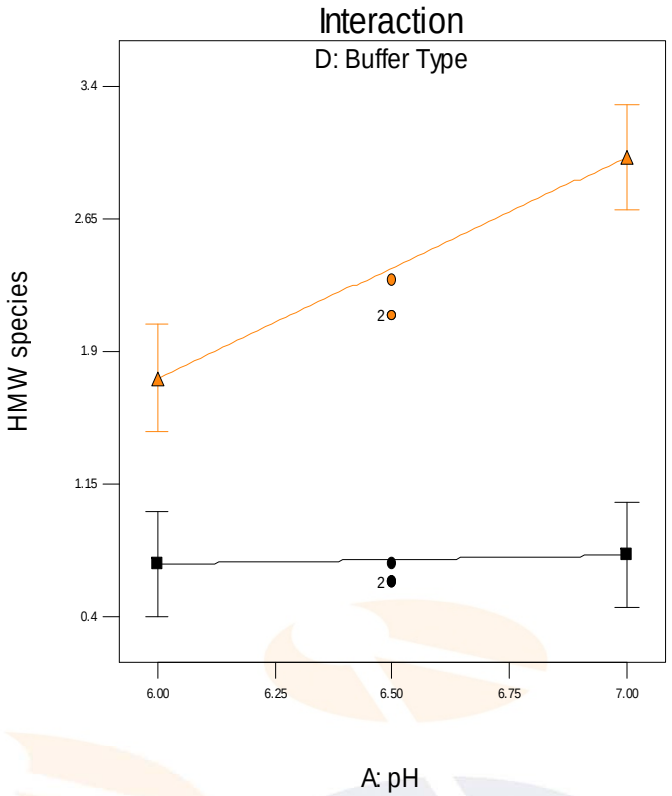
HMW species

● Design Points

■ D1 Histidine
△ D2 Phosphate

X1 = A: pH
X2 = D: Buffer Type

Actual Factors
B: Buffer Conc = 35.00
C: NaCl Conc = 75.00

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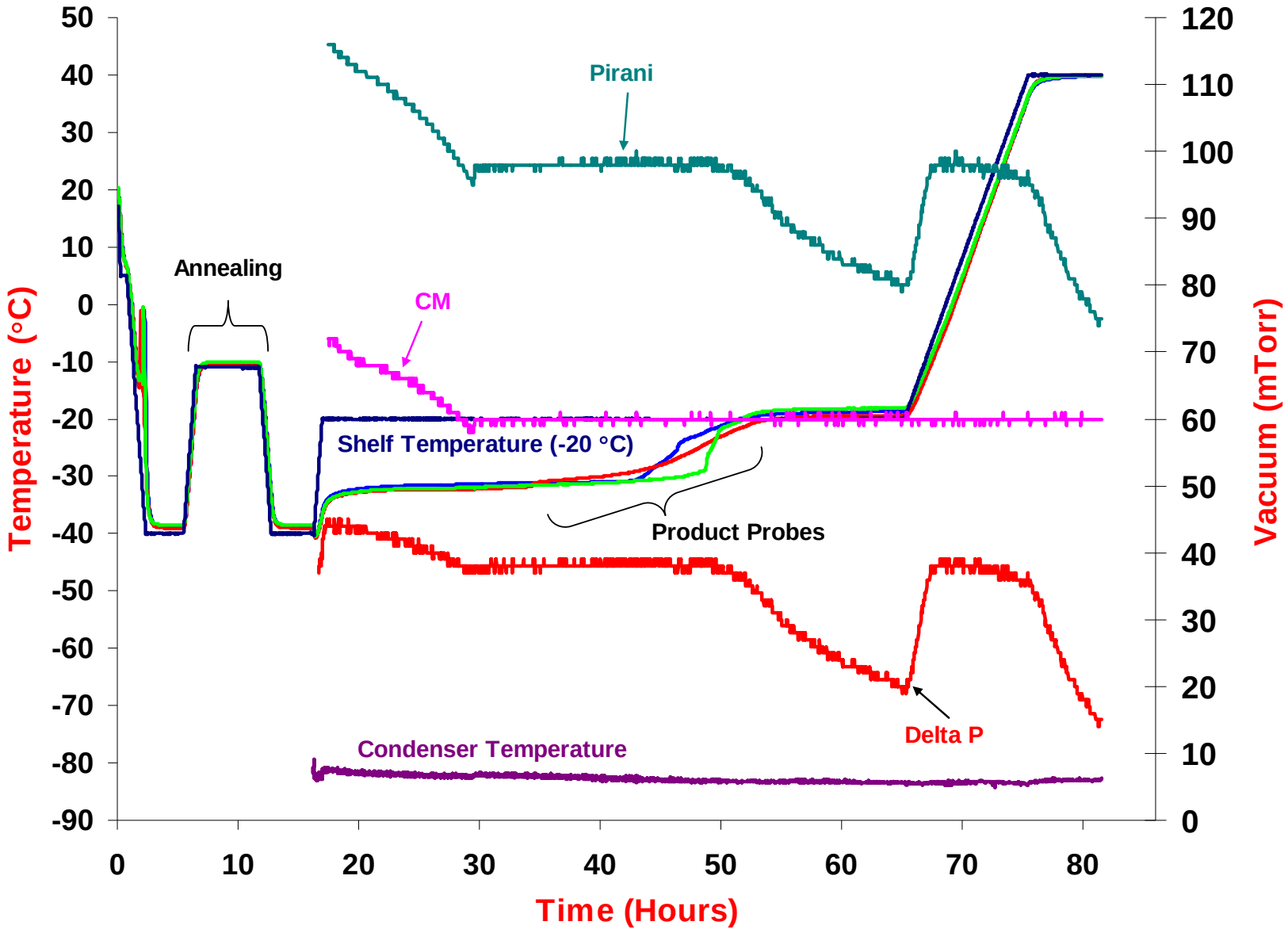
# Why Lyophilize Proteins?

- Protein is unstable in liquid formulation
- Freezing alone is not practical
  - Maintenance of -80° C cold chain
- Remove water
  - Reduce rate of hydrolytic reaction
- Reduce molecular mobility
  - Slow degradation processes
- Lyophilized products can be stored under refrigeration or perhaps at room temperature
- Provides dosing flexibility
  - Different reconstitution volumes will yield different protein concentrations

# Final Lyophilization Cycle



# Excipient Considerations – Crystalline Agents

- Must fully crystallize during freezing or collapse may result (annealing step)
- Must remove mannitol hydrate during secondary drying or stability may be compromised due to increase in moisture (high secondary drying temperature >40° C)
- May also require another stabilizing agent to protect protein during the lyophilization process and after reconstitution
  - Sucrose or Trehalose in combination with Mannitol
  - Ratio of at least 3:1 Mannitol:Sucrose (w/w) to get desired lyophilization properties
  - Consider protein concentration and final desired osmolality of reconstituted solution