



Preview Slides for Introduction to Protein Formulation and Delivery

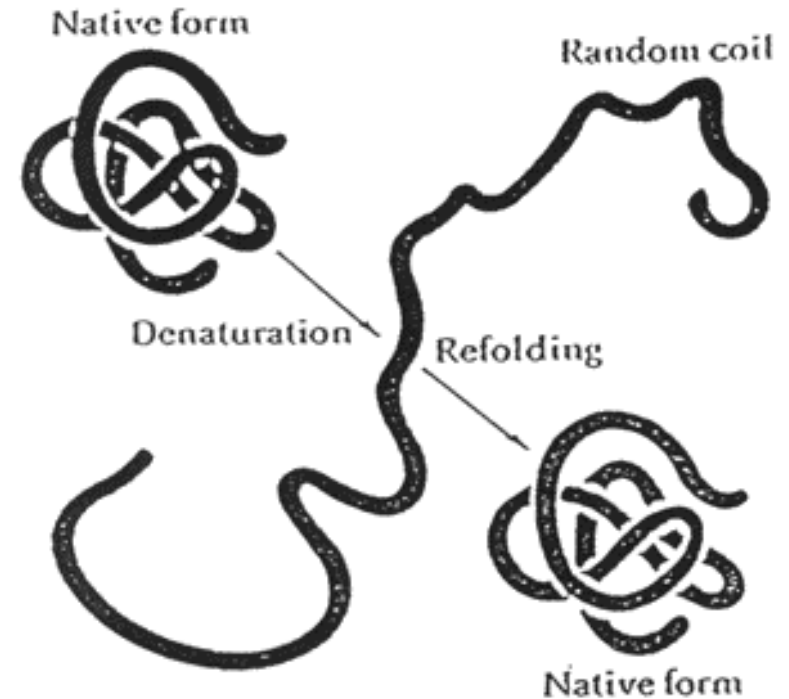
PepTalk 2014, Palm Springs CA

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Protein Folding: The Denatured State

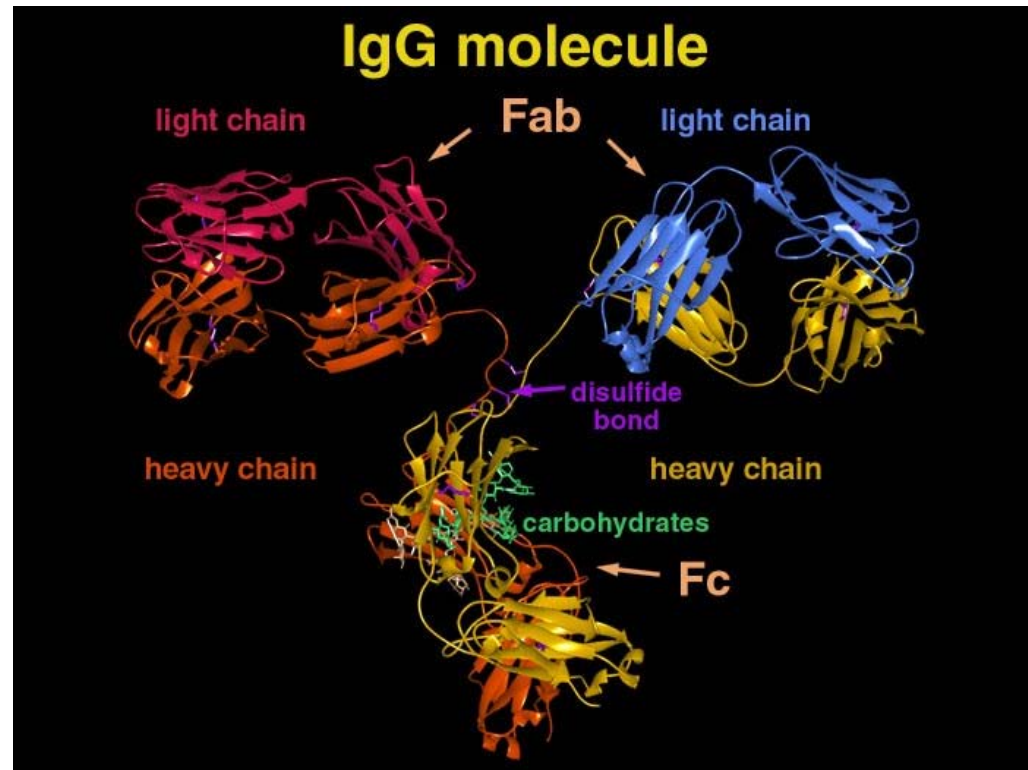
- Refers to the mostly unfolded state
- Usually D lacks any ordered structural elements, and is the higher-energy state under physiological conditions
 - Some D states have been experimentally shown to contain local, ordered structures
- D is stabilized by the high degree of conformational entropy due to the flexibility for attaining several conformations
- D is also stabilized by several H-bonds between the polypeptide groups and the solvent (H₂O) 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



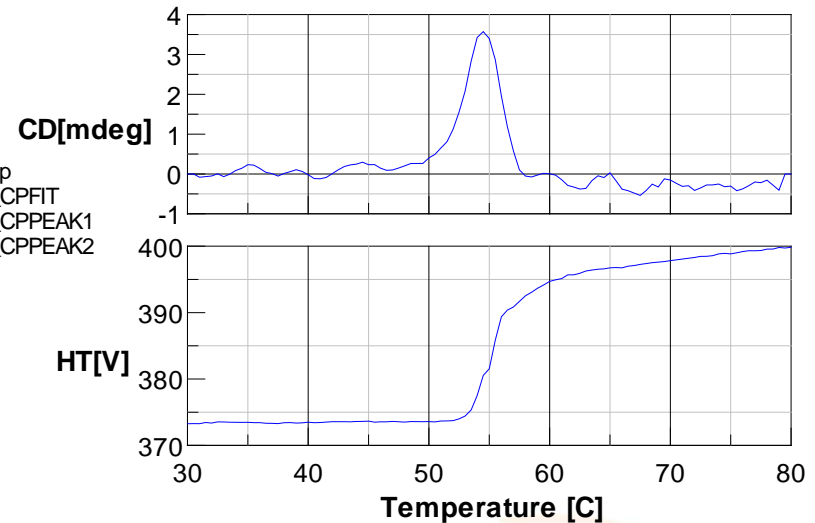
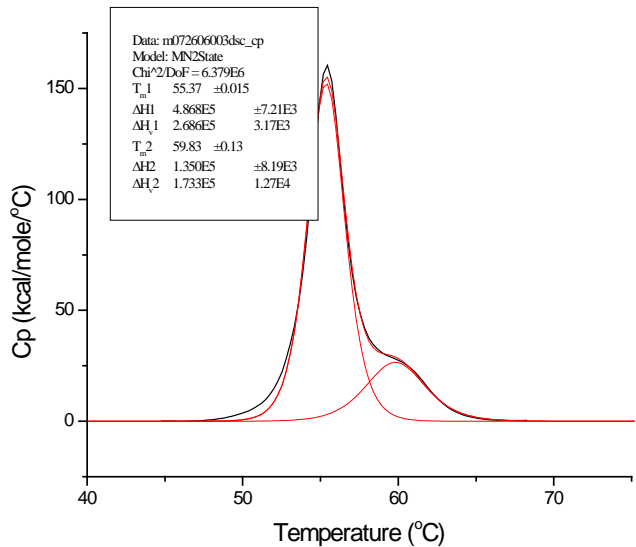
http://www.bmb.psu.edu/faculty/tan/lab/gallery_proteins.html

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

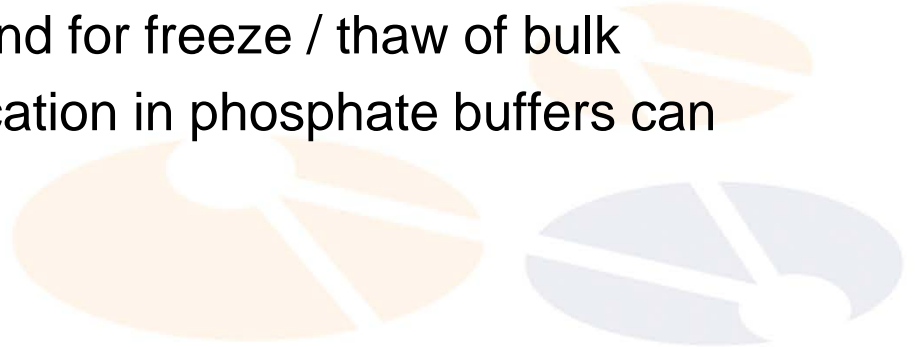


T_{m1} = 55°C
Onset of unfolding = 47°C
T_{m2} = 60°C

T_m = 55°C
Onset of unfolding = 49°C

Buffer Selection Considerations

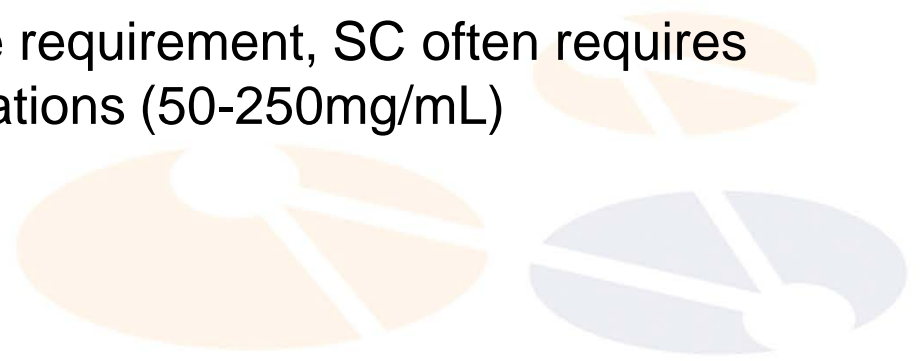
- Freeze / thaw and Lyophilization Concerns
 - Changes in pH can occur as a result of buffer salt crystallization and precipitation during freezing
 - Inorganic salts – freezing point of mono-ionized salt can be different than that the non-ionized free acid or base
- Phosphate buffer (mono- and dibasic 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 lyophilization and for freeze / thaw of bulk
 - Replace Na cation with K cation in phosphate buffers can reduce this effect



Routes of Administration

- Subcutaneous (SC)

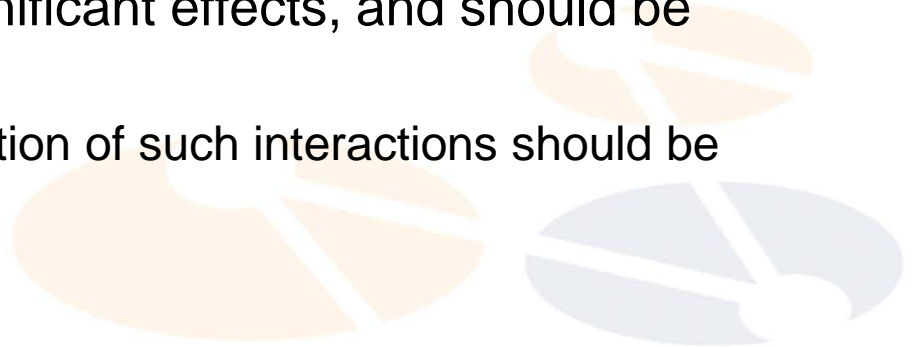
- More convenient for frequent / chronic administration
- Can be self-administered outside medical facility
- Couple with delivery devices such as pre-filled syringes and autoinjectors
- Significant marketing advantages
- Requires small volumes (<1.5mL)
- Many proteins, such as antibodies, require high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



Design of Experiment (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

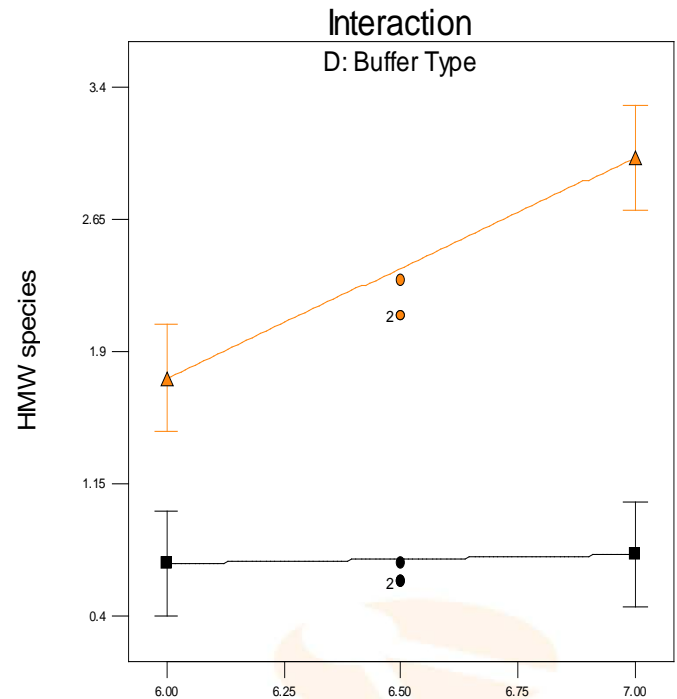
X1 = A: pH

X2 = D: Buffer Type

Actual Factors

B: Buffer Conc = 35.00

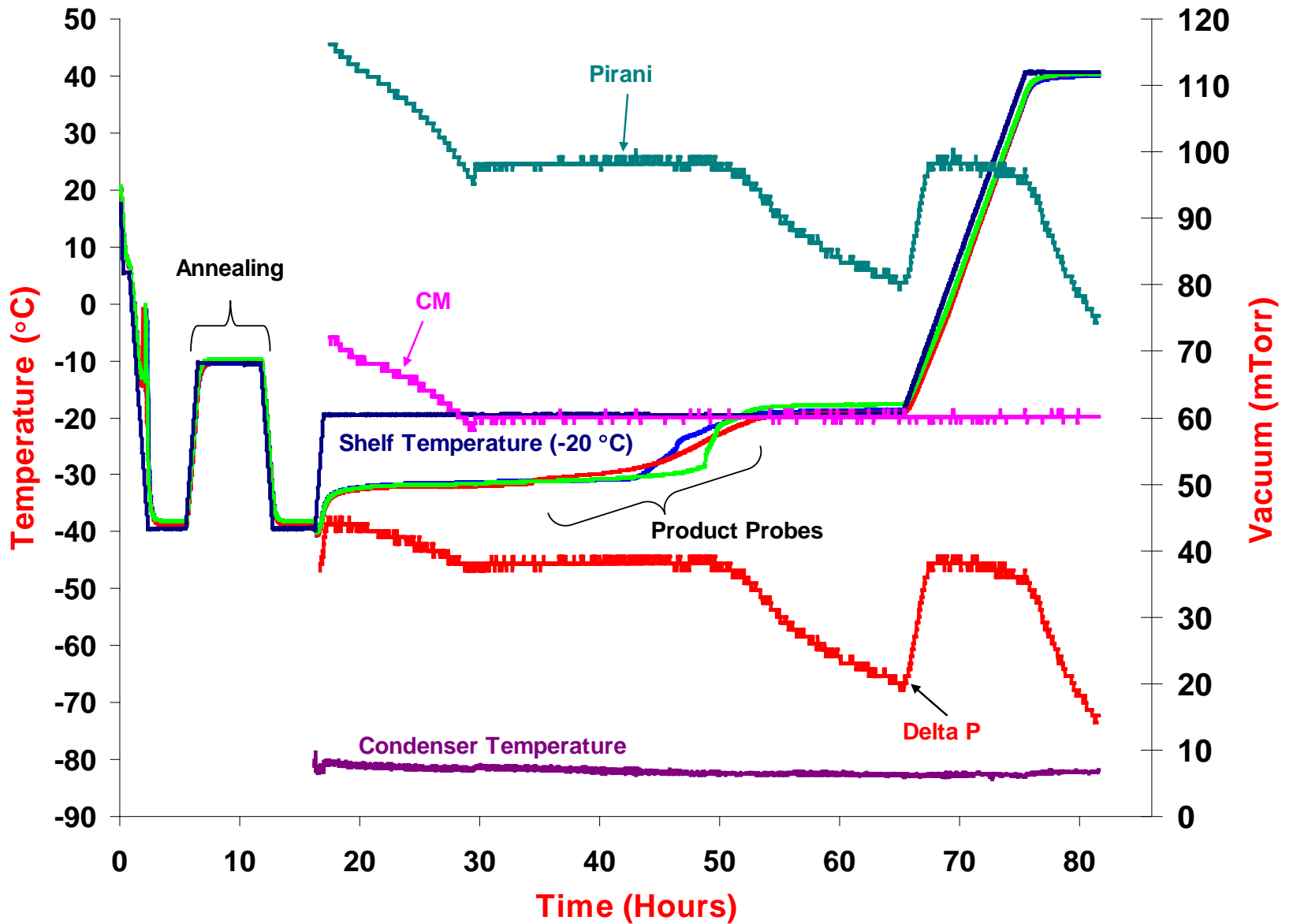
C: NaCl Conc = 75.00



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 $>40^{\circ}\text{C}$)
- 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