

Studying Antibodies and Fc Related Proteins by DSC

Current Trends in Microcalorimetry

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Forensic & Biophysical Analysis

Global Cellular & Analytical Resources

Amgen, Inc.

Outline

- Introduction
- Applications of DSC to the study of antibodies and Fc related proteins
 - Correlation between thermal stability and protein stability
 - Effect of pH on stability
 - Buffer screening for the best processing conditions
 - Candidate screening for manufacturability
 - Effect of carbohydrate on Stability
 - Thermal reversibility
- Conclusions

Introduction

DSC is a useful tool for:

Research

- pH and buffer conditions for purification/storage
- Candidate screening for manufacturability

Process development

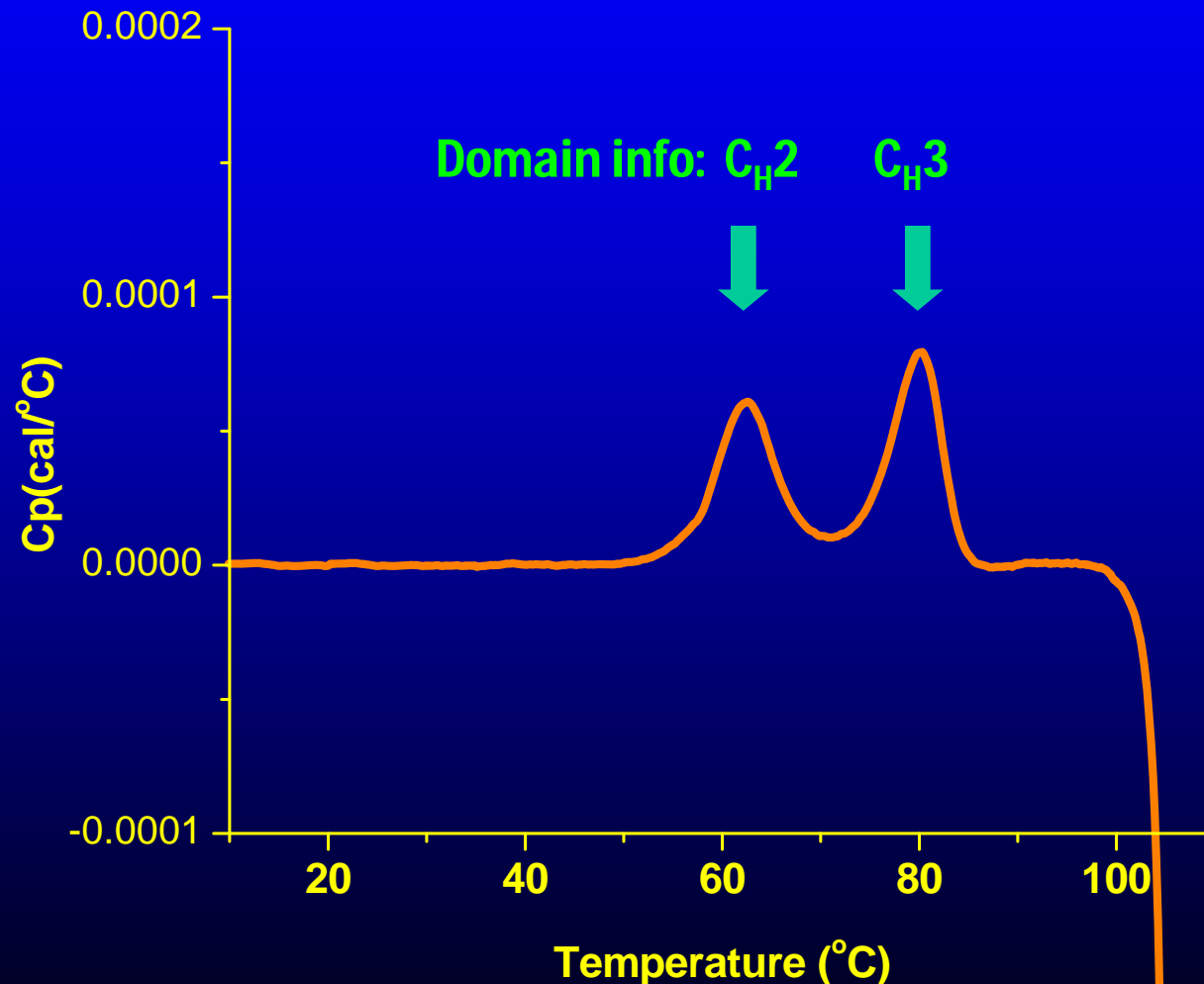
- pH and buffer screening for processing conditions
- Temperature optimization for the storage/processing conditions

Regulatory filing

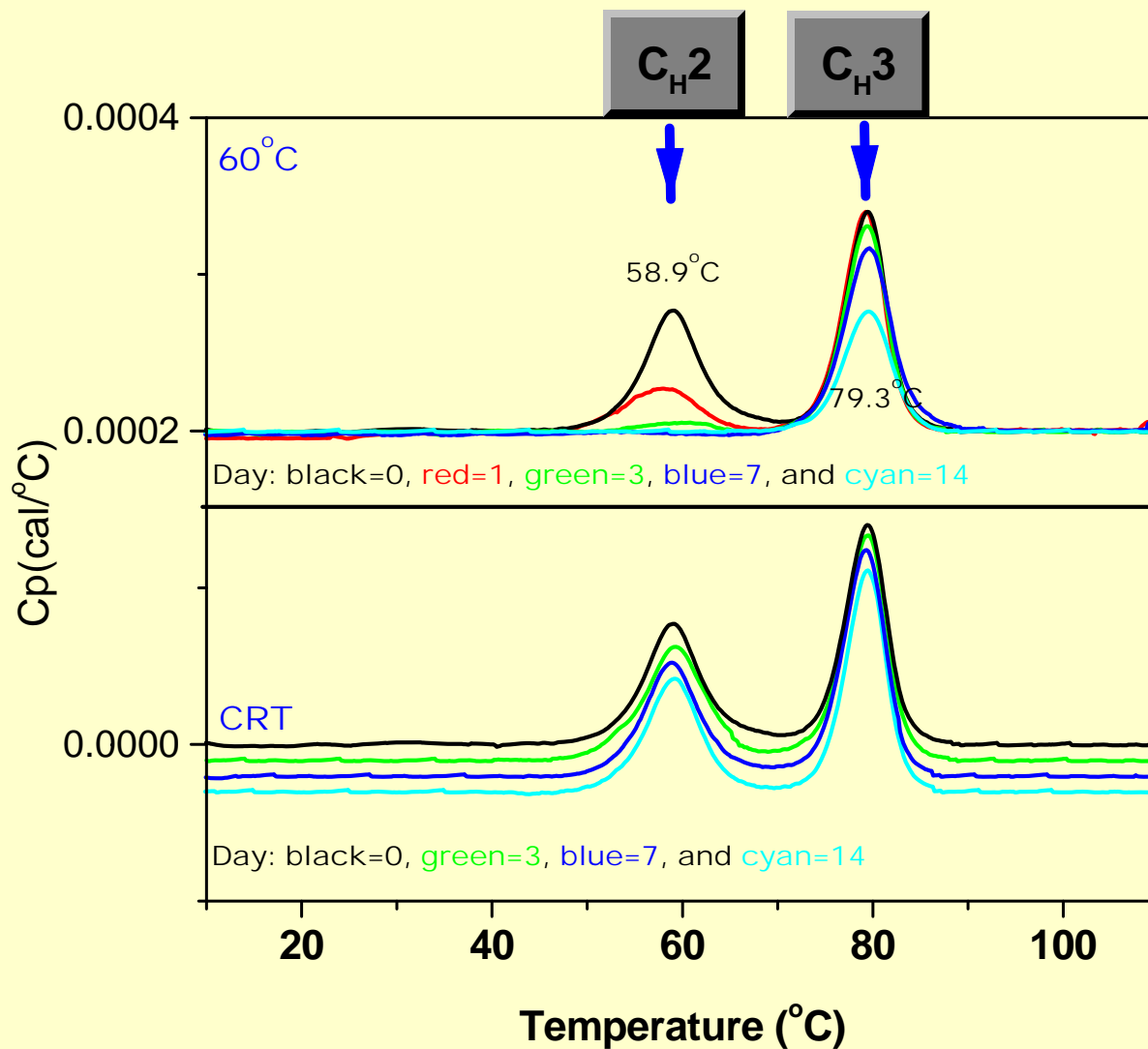
- Reference standard characterization
- Comparability study

Advantage for studying Fc-conjugated proteins:

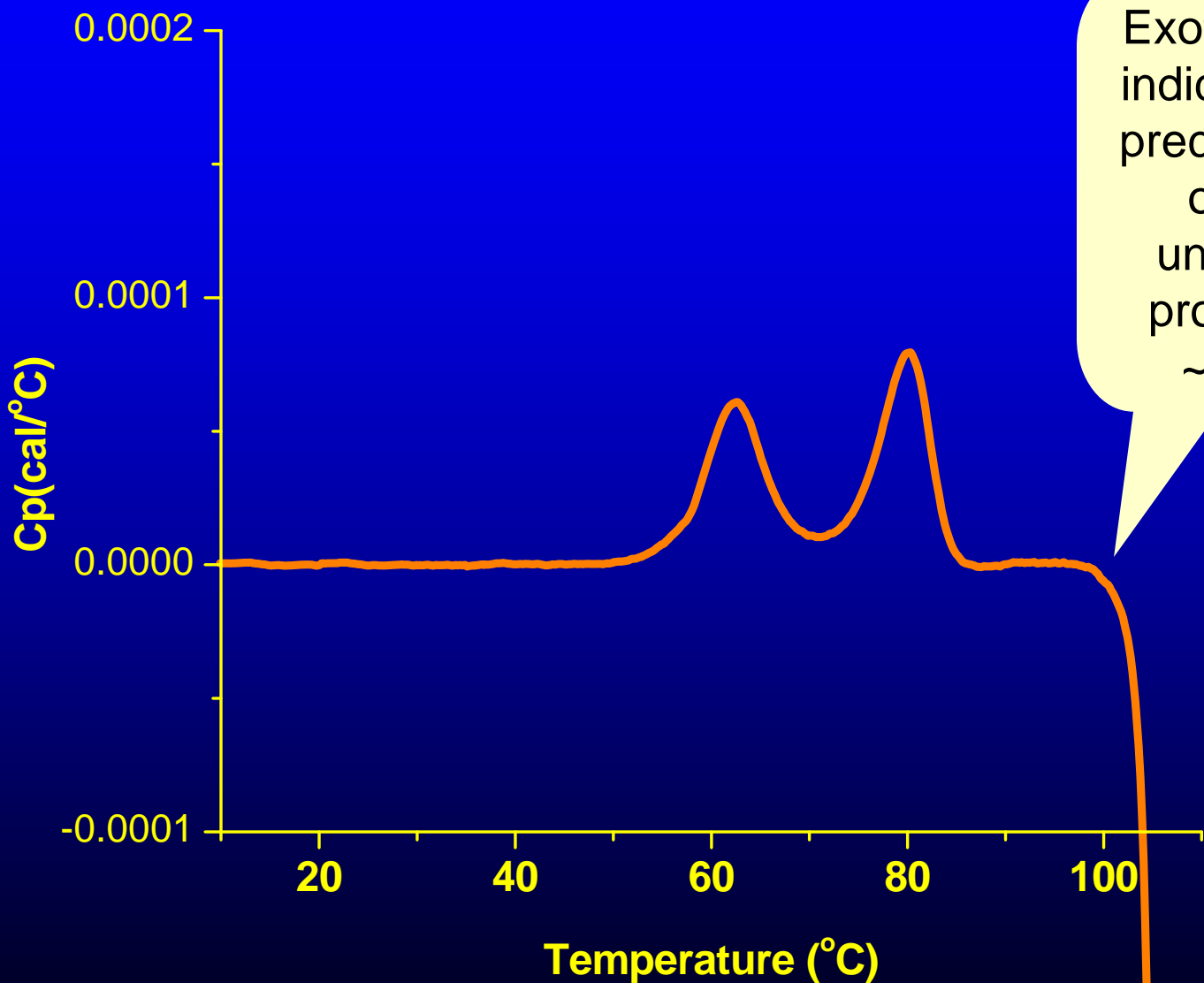
Identification of transitions of individual domains



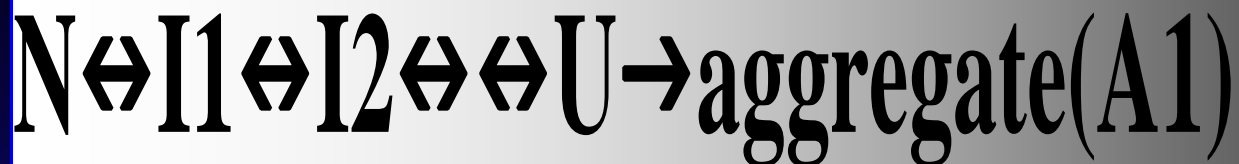
Tracking the changes of individual domains



Solubility of the unfolded protein



Schematic illustration of protein unfolding



A2 A3

I1 and I2 indicate intermediates;
A1, A2, etc. indicate aggregates, which could be different structurally;
N is the folded protein;
U is the unfolded protein.

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Correlation between thermal stability and protein stability

Sample Preparation:

Each sample was prepared at specified pH for DSC and HPLC analysis.

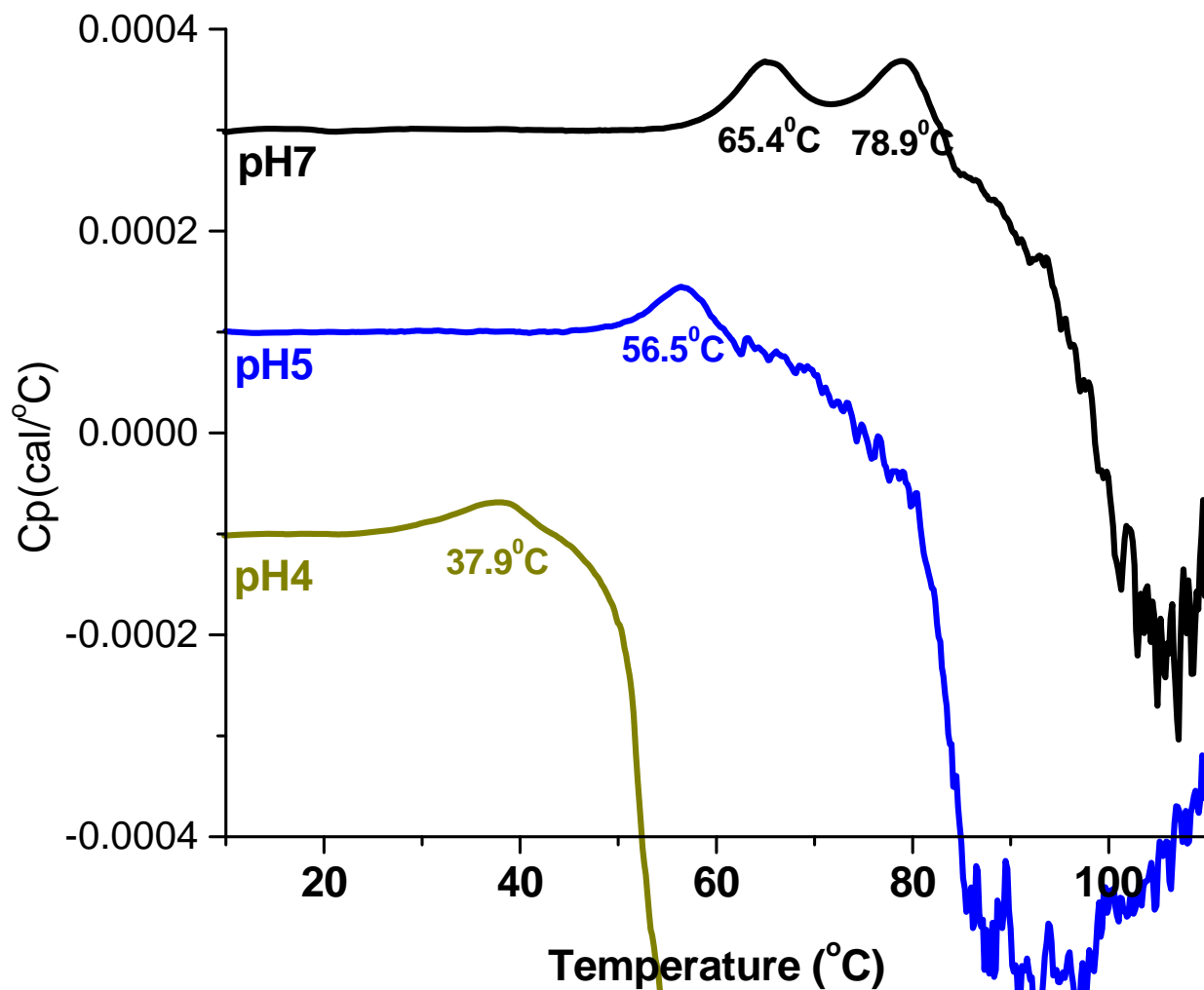
Storage Temperature:

- 4°C was the default storage temperature for all the samples.
- Room temperature and 37°C were also used for accelerated studies.

HPLC Injection Time Points:

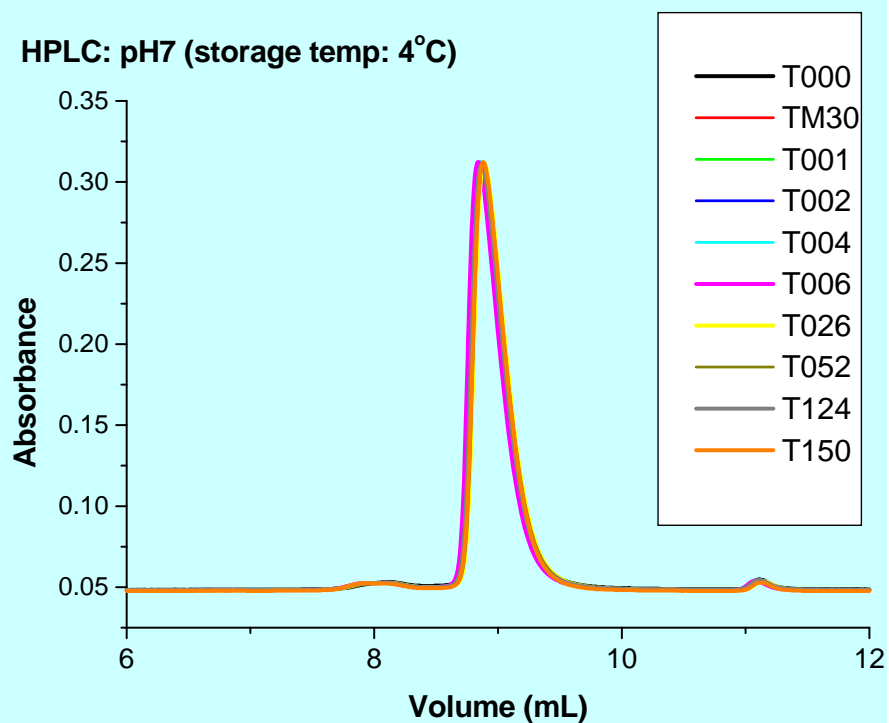
0, 0.5, 1, 2, 4, 6, 24, 48 hours, ..., (up to weeks)

DSC Profiles of Fc-conjugated Protein X

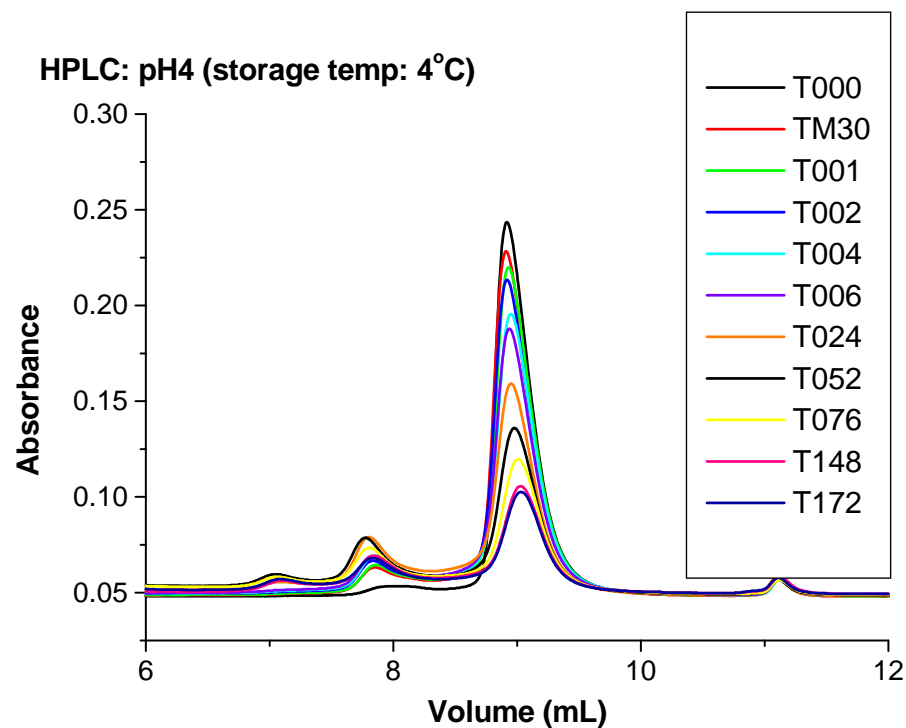


SEC Data of Fc-conjugated protein X

pH 7 (storage temp: 4°C)

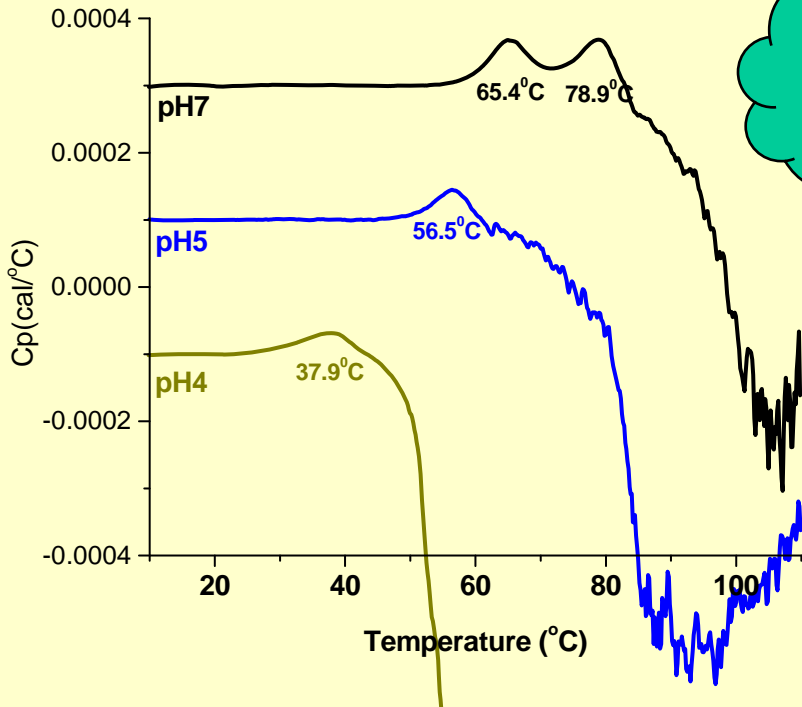


pH 4 (storage temp: 4°C)



DSC Data vs. HPLC Data

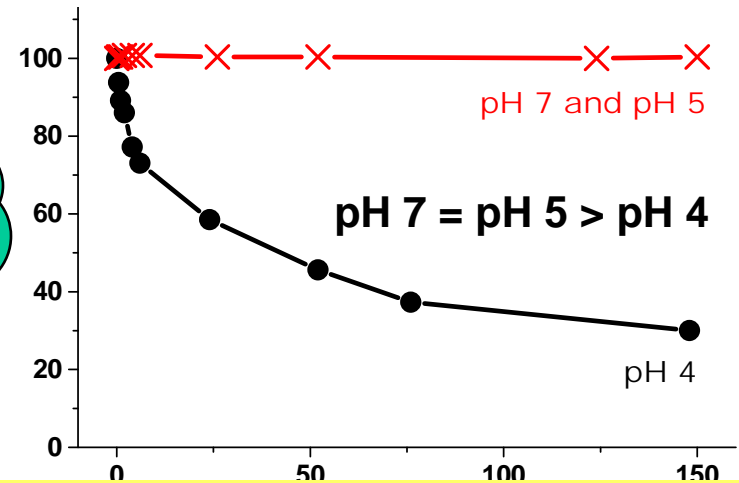
DSC Results



Is DSC able to predict?



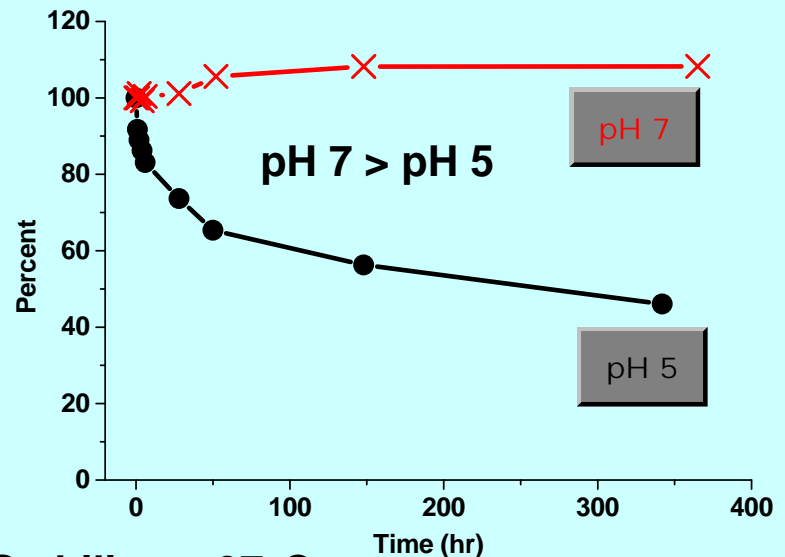
Stability at 4°C



SEC Results

pH 7 = pH 5 > pH 4

Combining both 4 and 37°C HPLC data, we get: pH 7 > pH 5 > pH 4



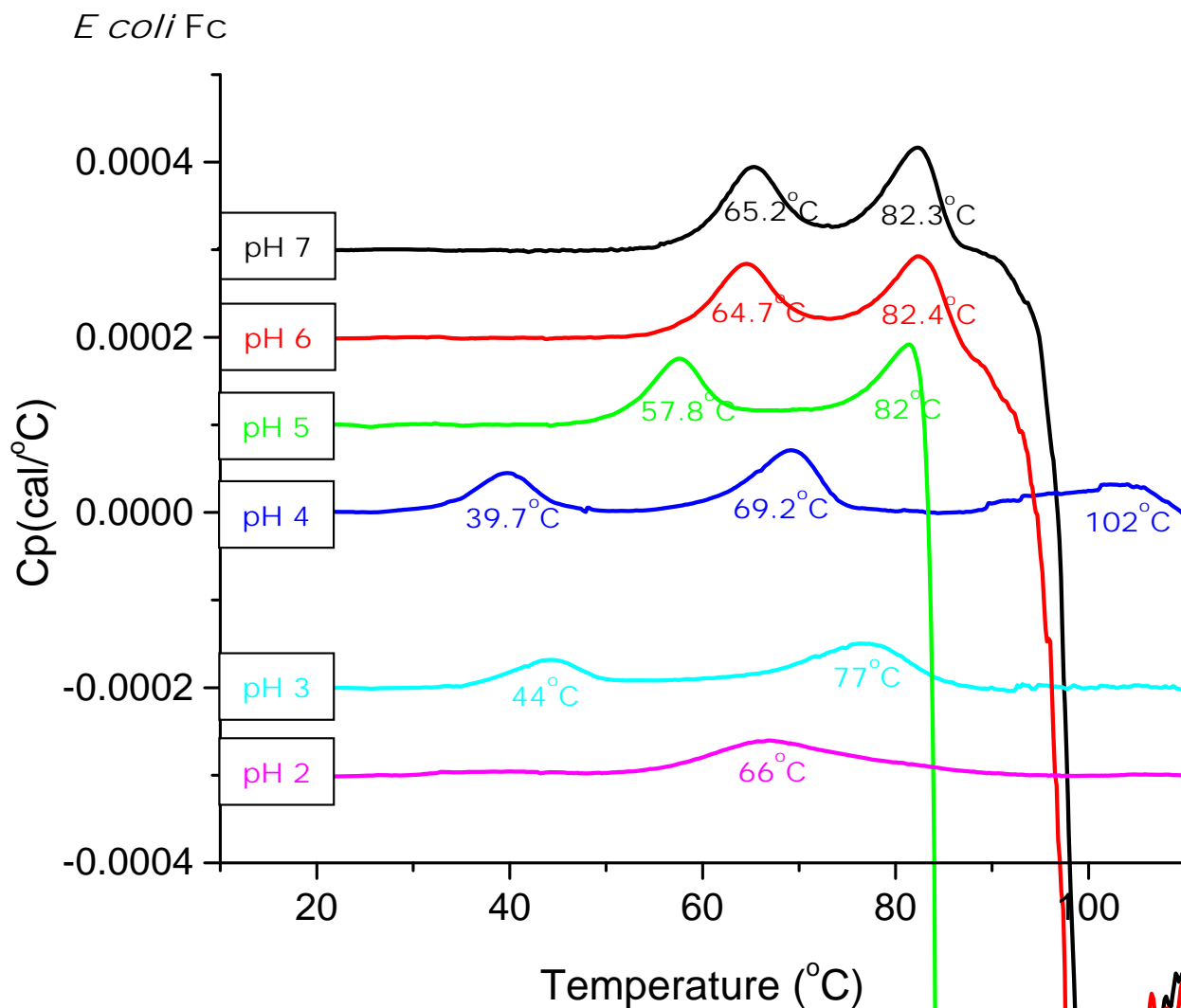
Stability at 37°C

The data suggest that DSC is able to predict the long-term storage stability.

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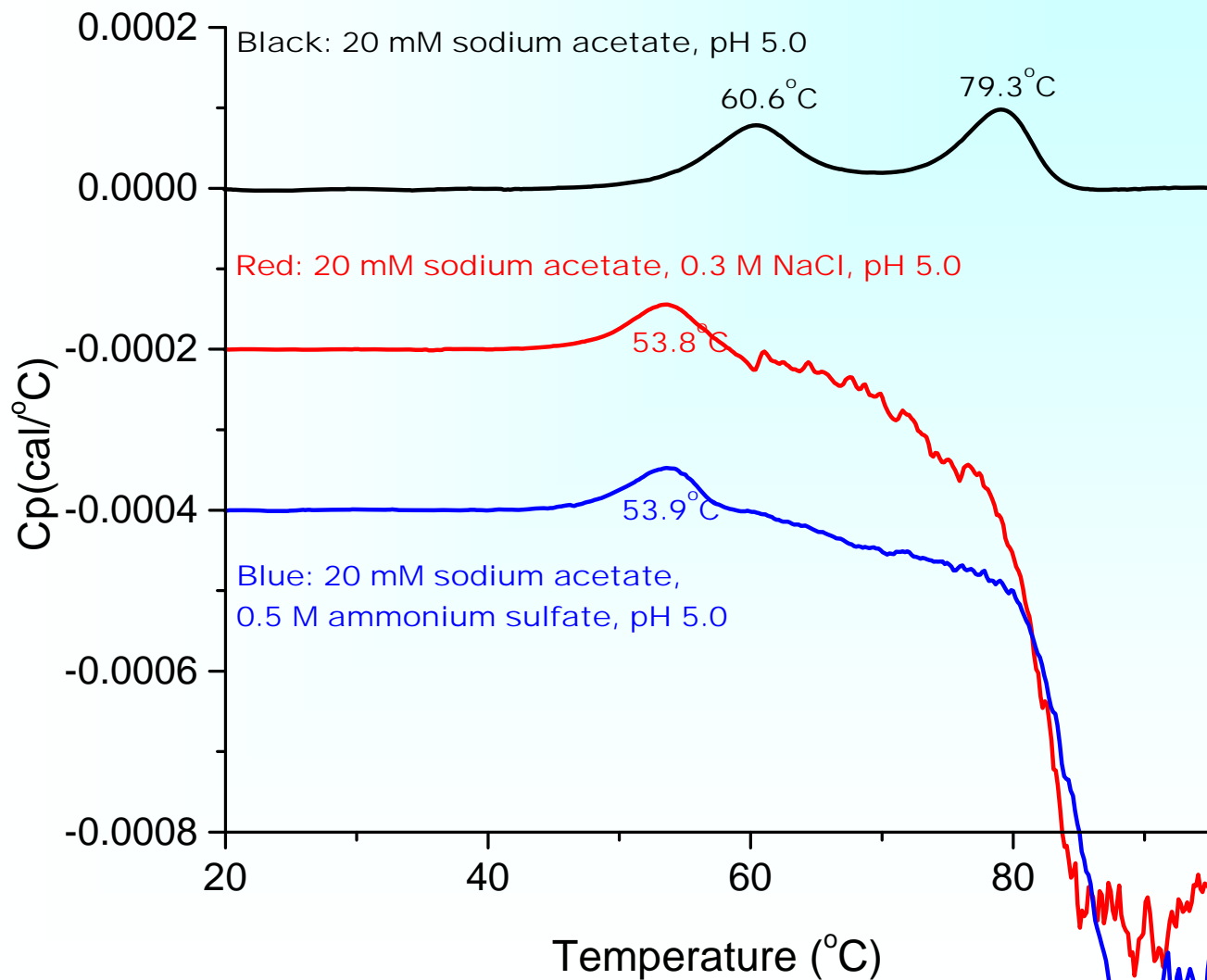
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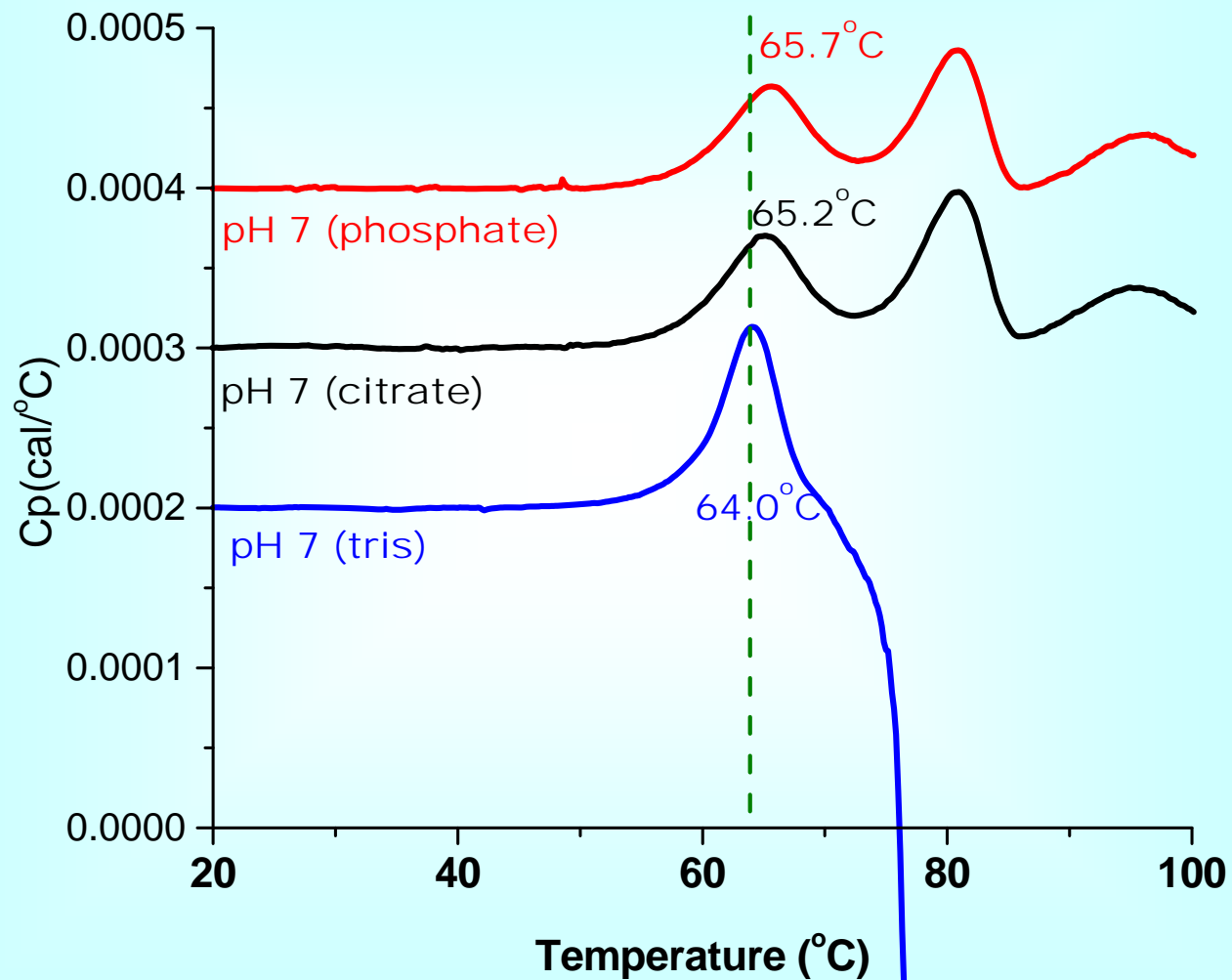
Effect of pH on stability



Buffer screening for the best processing conditions

AMGEN





Outline

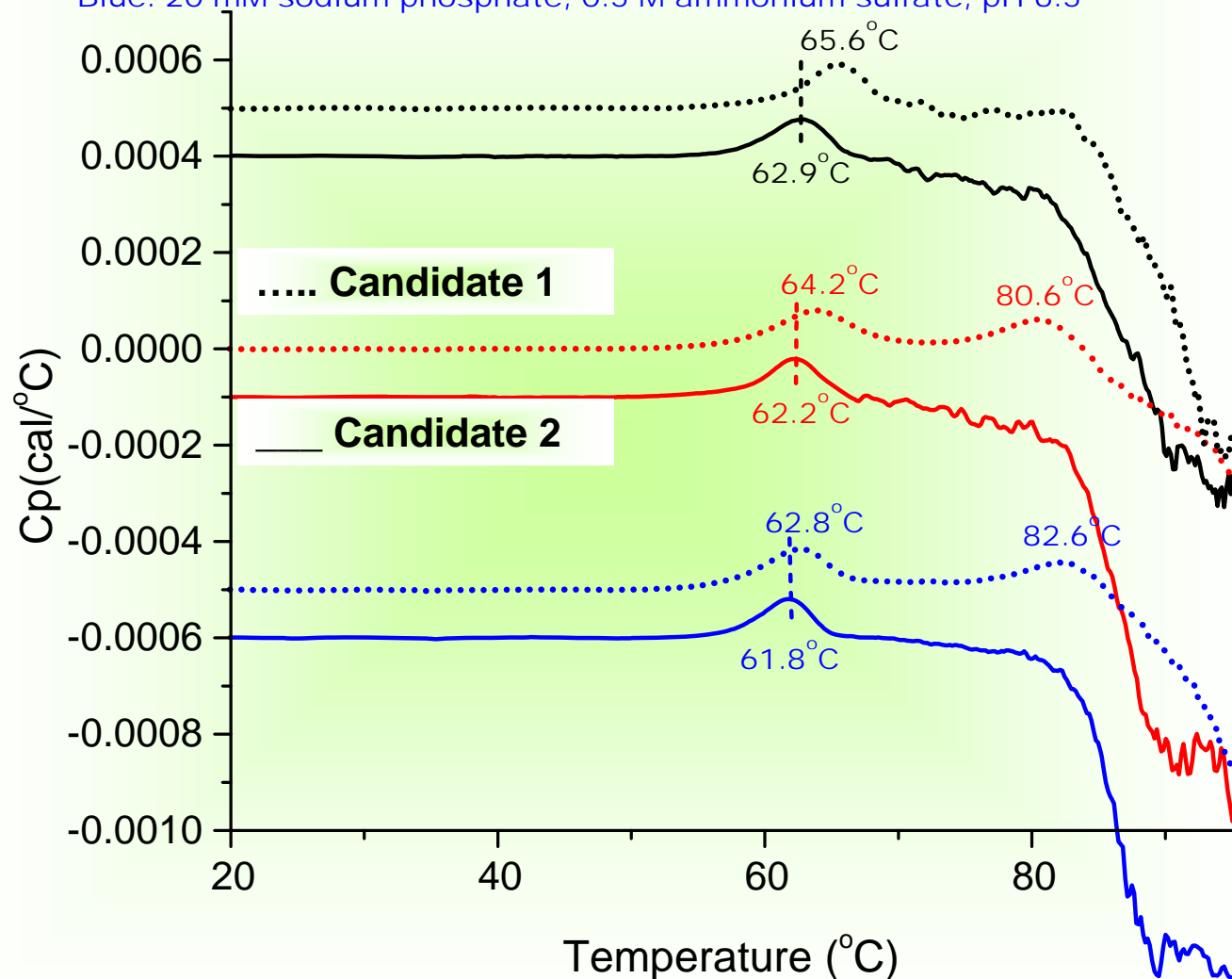
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Candidate screening for manufacturability

Black: 20 mM sodium phosphate, pH 6.5

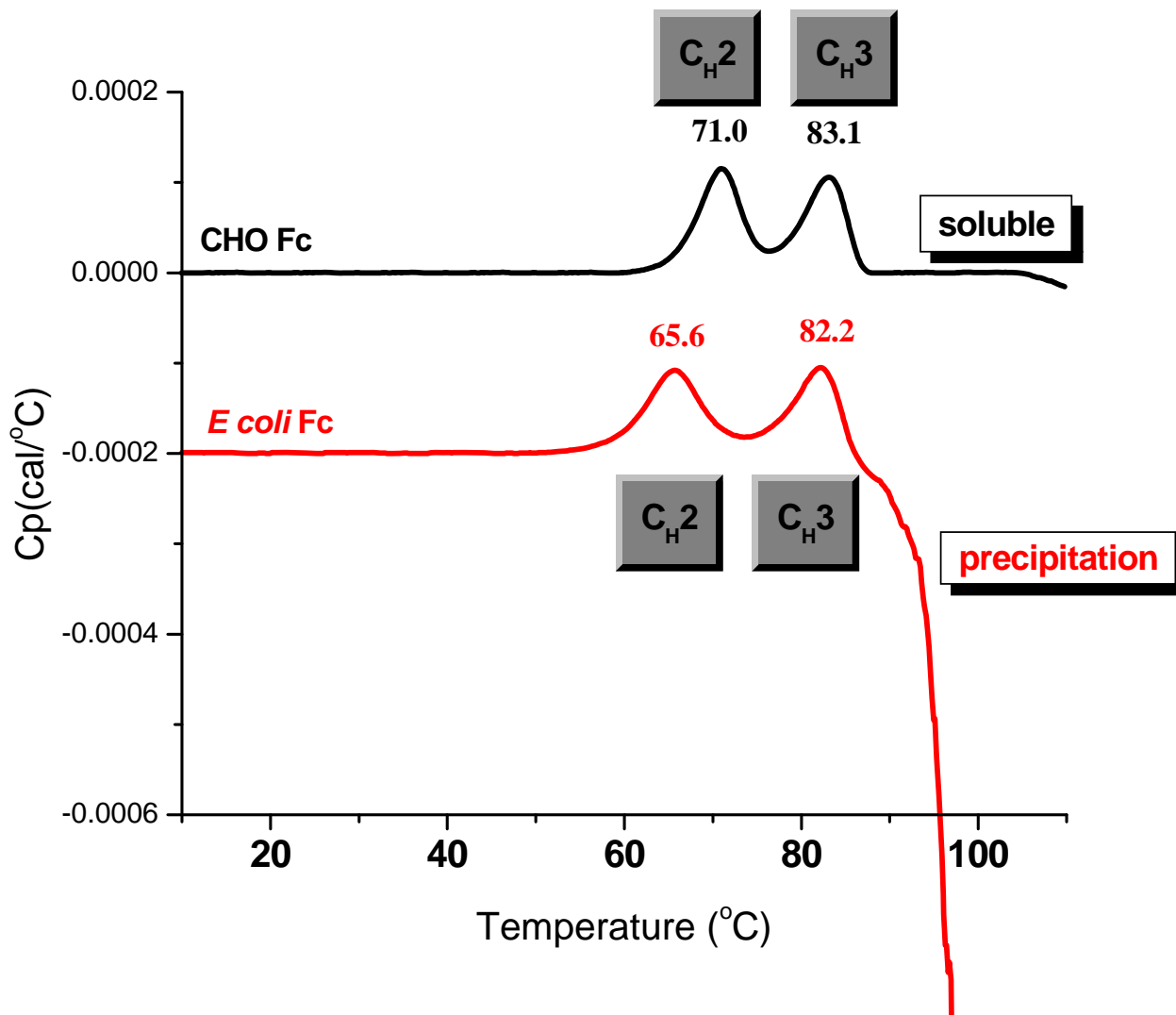
Red: 20 mM sodium phosphate, 0.3 M NaCl, pH 6.5

Blue: 20 mM sodium phosphate, 0.5 M ammonium sulfate, pH 6.5



Effect of Carbohydrate on Stability

CHO Fc (with carbohydrate) vs. *E. coli* Fc (without carbohydrate)

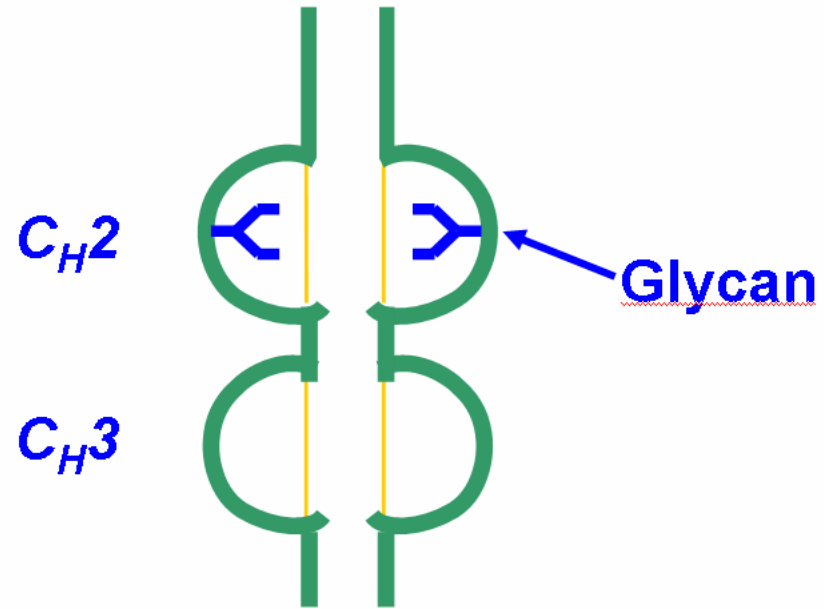
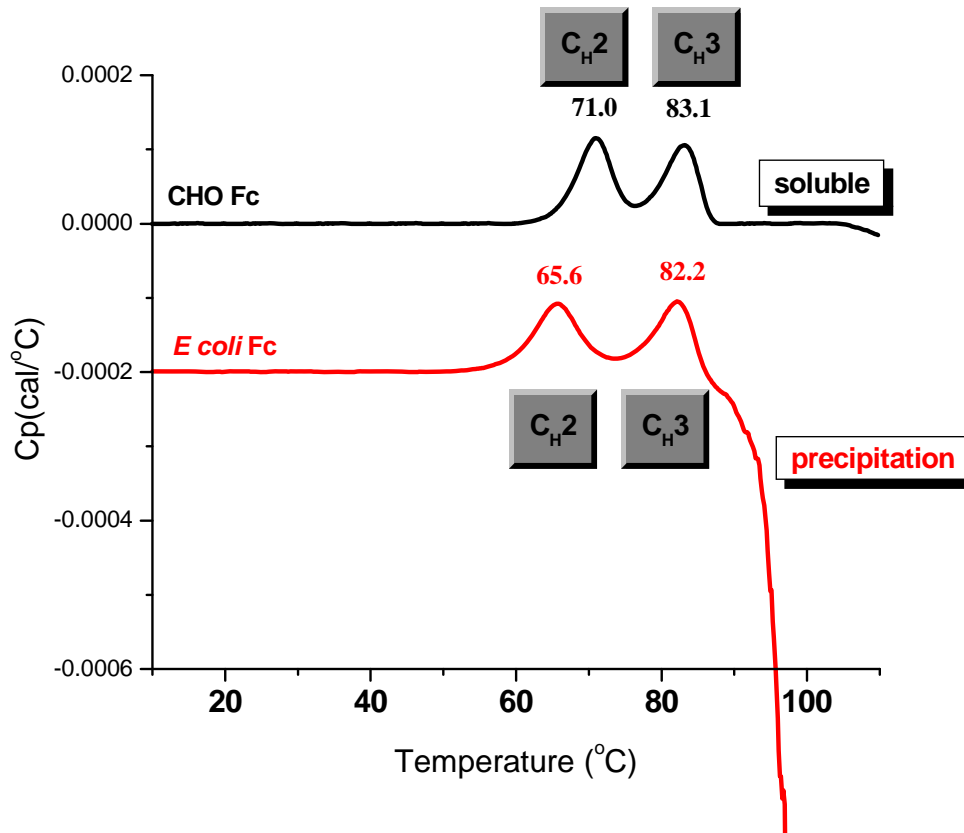


The DSC data suggest

1. The carbohydrates stabilize the domains (or protein)
2. The carbohydrates make the unfolded protein more soluble
3. The carbohydrates stabilize the C_{H2} domain more than the C_{H3} domain

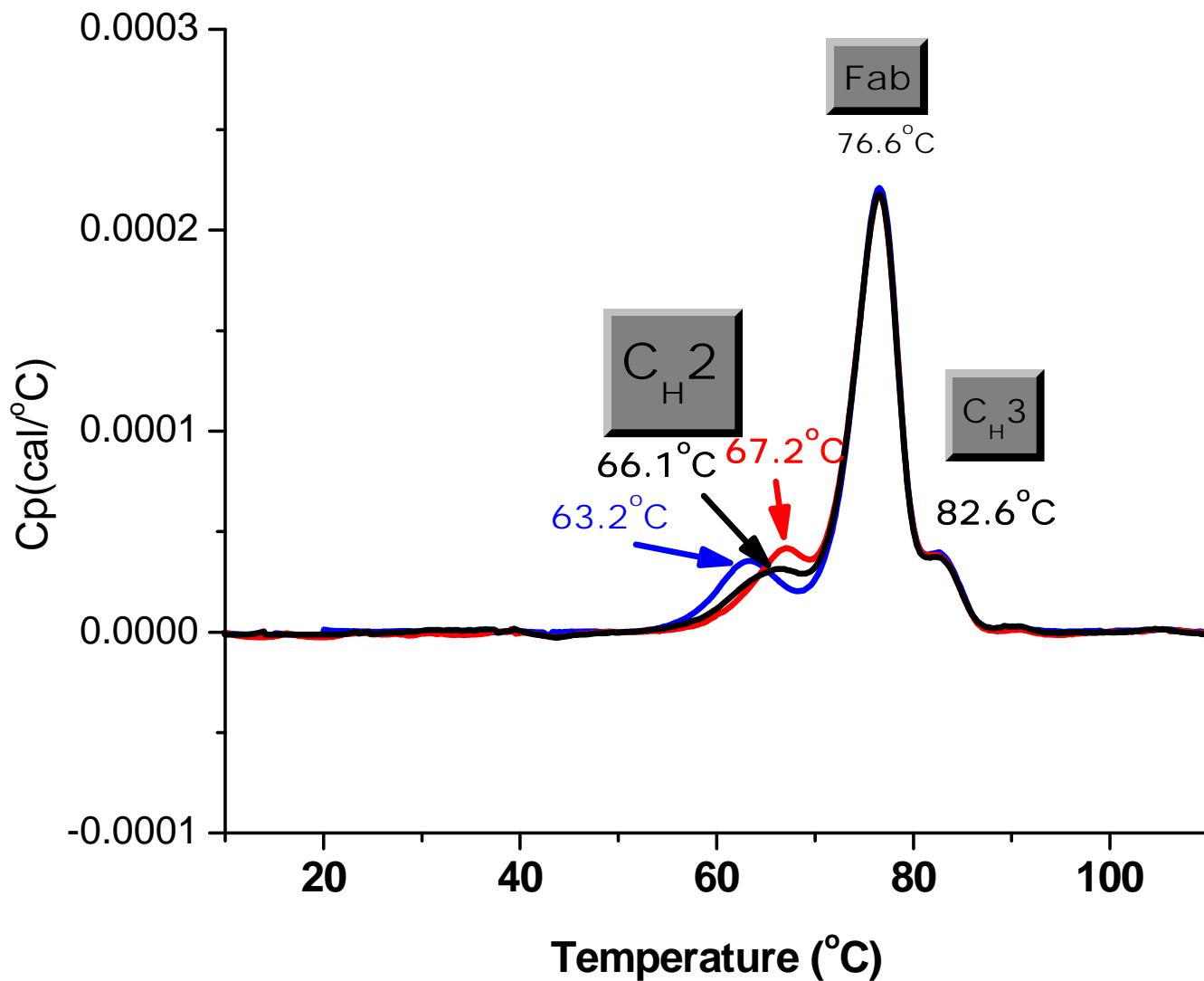
Carbohydrates vs. Stability (cont'd)

CHO Fc (with carbohydrate) vs. E. coli Fc (without carbohydrate)



This structure suggests that the carbohydrates can stabilize the C_H2 domain more than the C_H3 domain

Effect of sugars on stability



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Thermal reversibility

At least several factors need to be considered:

- Final temperature (upper limit temperature) of the scan
- pH and buffer components
- Holding time at final temperature
- Protein concentration
- Domains
- Scan rate

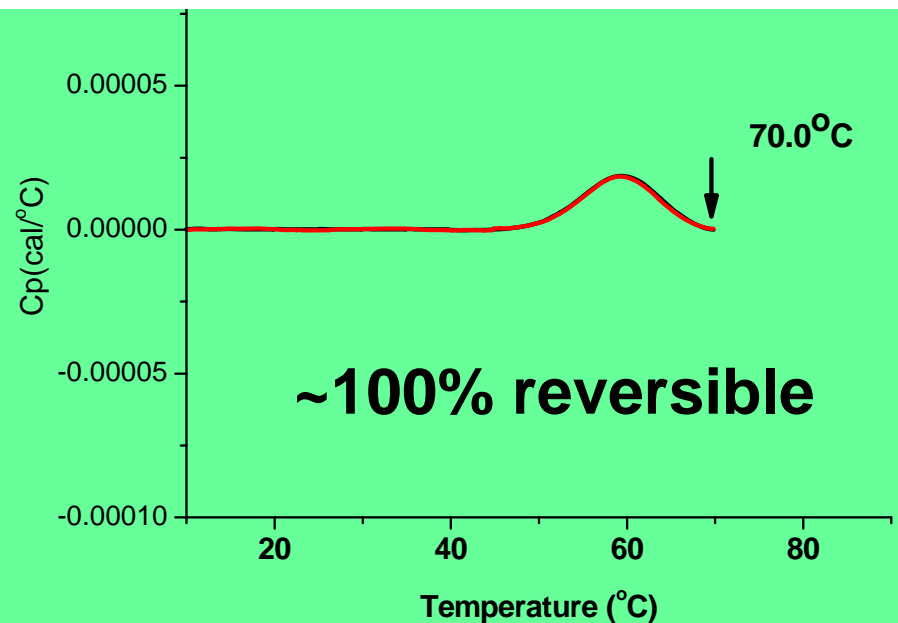
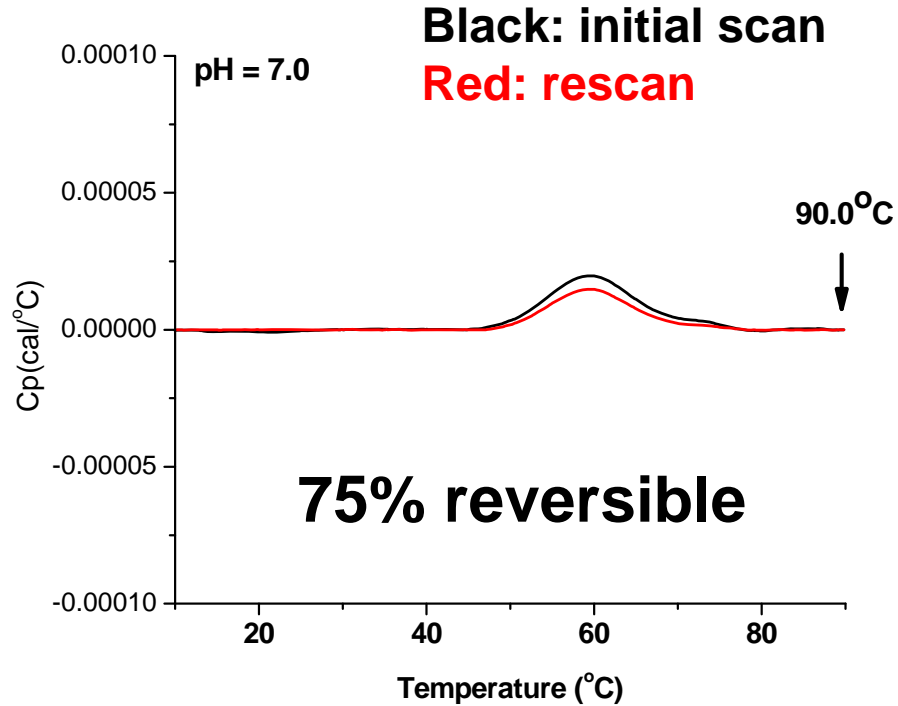
Thermal Reversibility

Dependence on end-point temperature

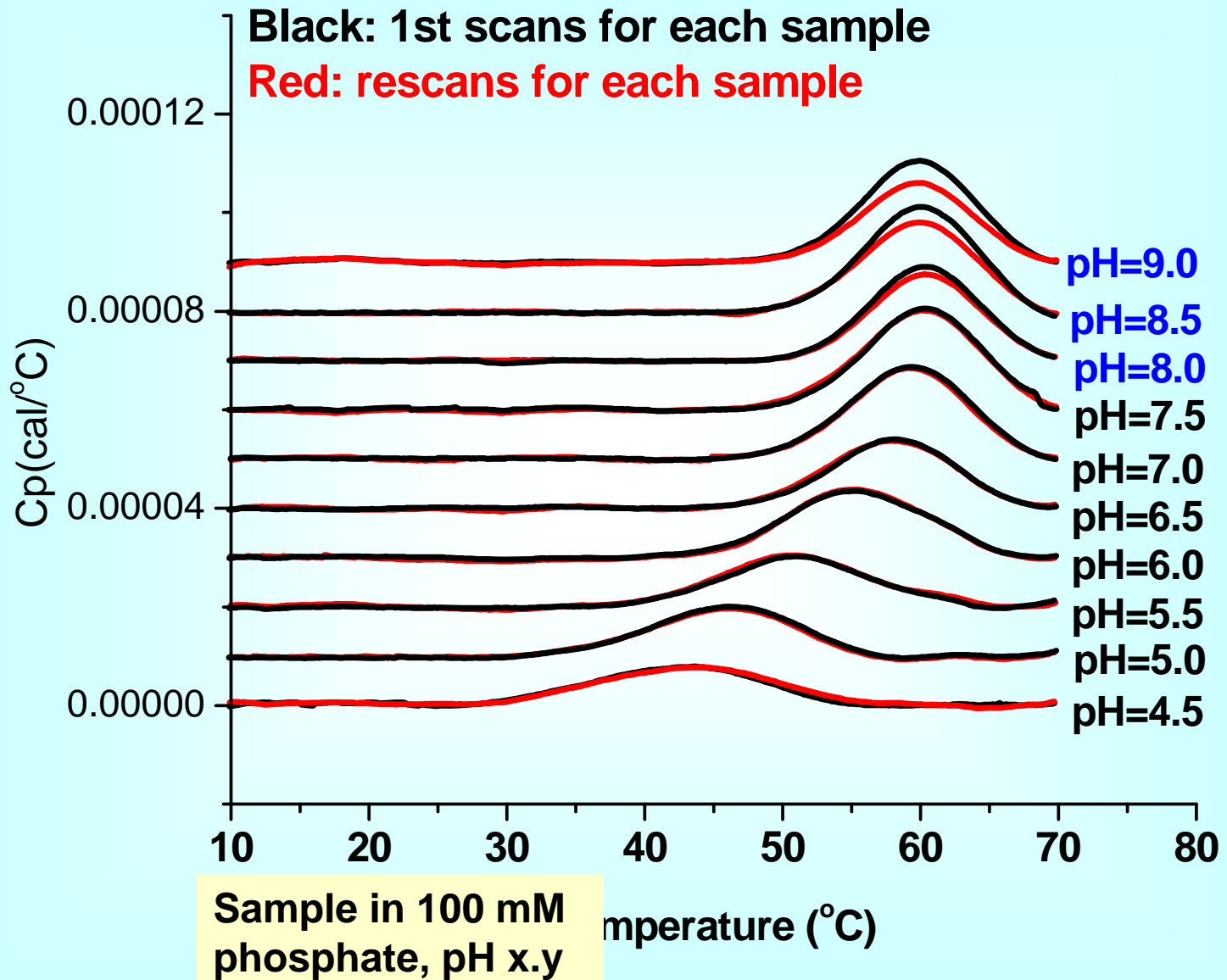
90°C →

vs.

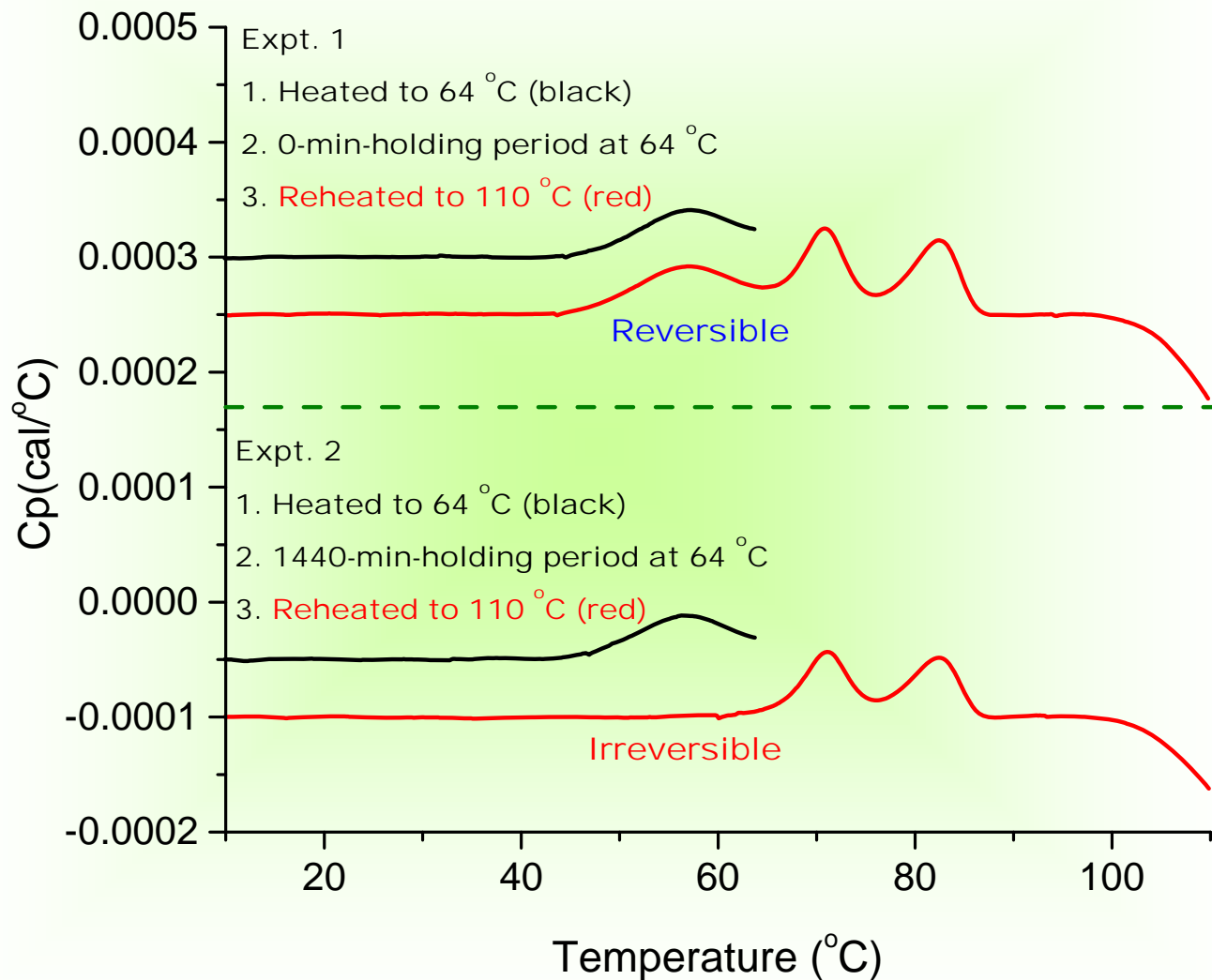
70°C →



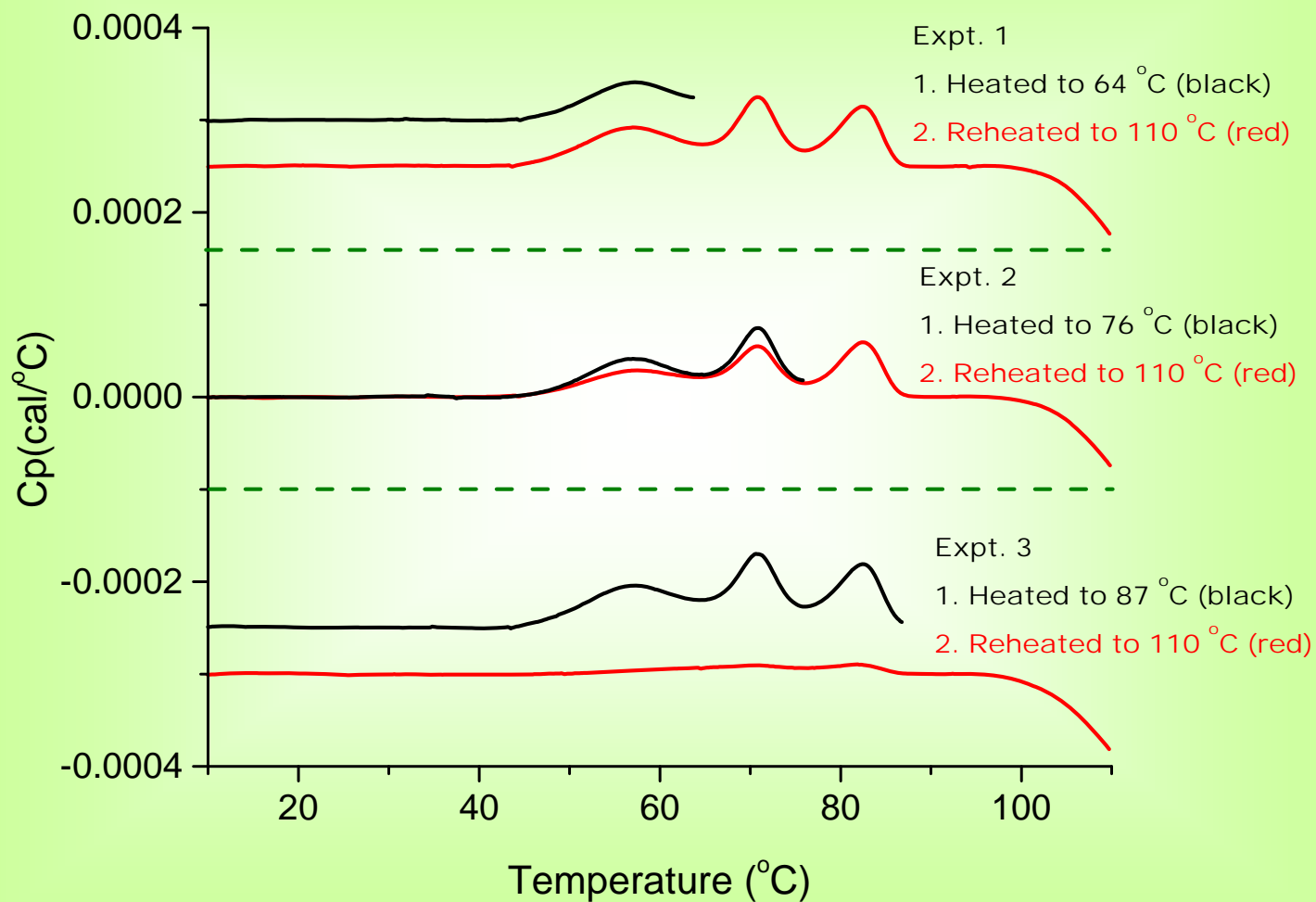
Effect of pH and buffer



Holding time at final temperature



Domain reversibility of an Fc-conjugated protein



Thermal Reversibility – Summary

The thermal reversibility of a protein can be a **relative term**. It depends on AT LEAST the following factors

- Final temperature (upper limit temperature) of the scan
- pH and buffer components
- Holding time at final temperature
- Protein concentration
- Scan rate

Different domains in a Fc conjugated protein may have different thermal reversibility.

Conclusions

- The identification of transitions of individual domains
- **Good correlation between thermal stability and protein stability**
- Able to identify the relative stability at different pHs
- **Able to screen buffers for the best processing conditions**
- Able to screen candidates for manufacturability
- **Able to evaluate the stability changes caused by carbohydrates (amount and/or different type)**
- **Thermal reversibility can a relative term (depending on final temperature, pH and buffer components, holding time at final temperature, protein concentration, scan rate, etc.); Different domains in a Fc conjugated protein may have different thermal reversibility**

Acknowledgements

- **Yijia Jiang**
- **Linda Narhi**
- **Summer interns: Kathryn Hymes and Ke Gong**
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