Kinetic Analysis of the Conformational Stability and Aggregation of mAbs Using Calorimetry
or
Two New Things To Do With Your Calorimeter

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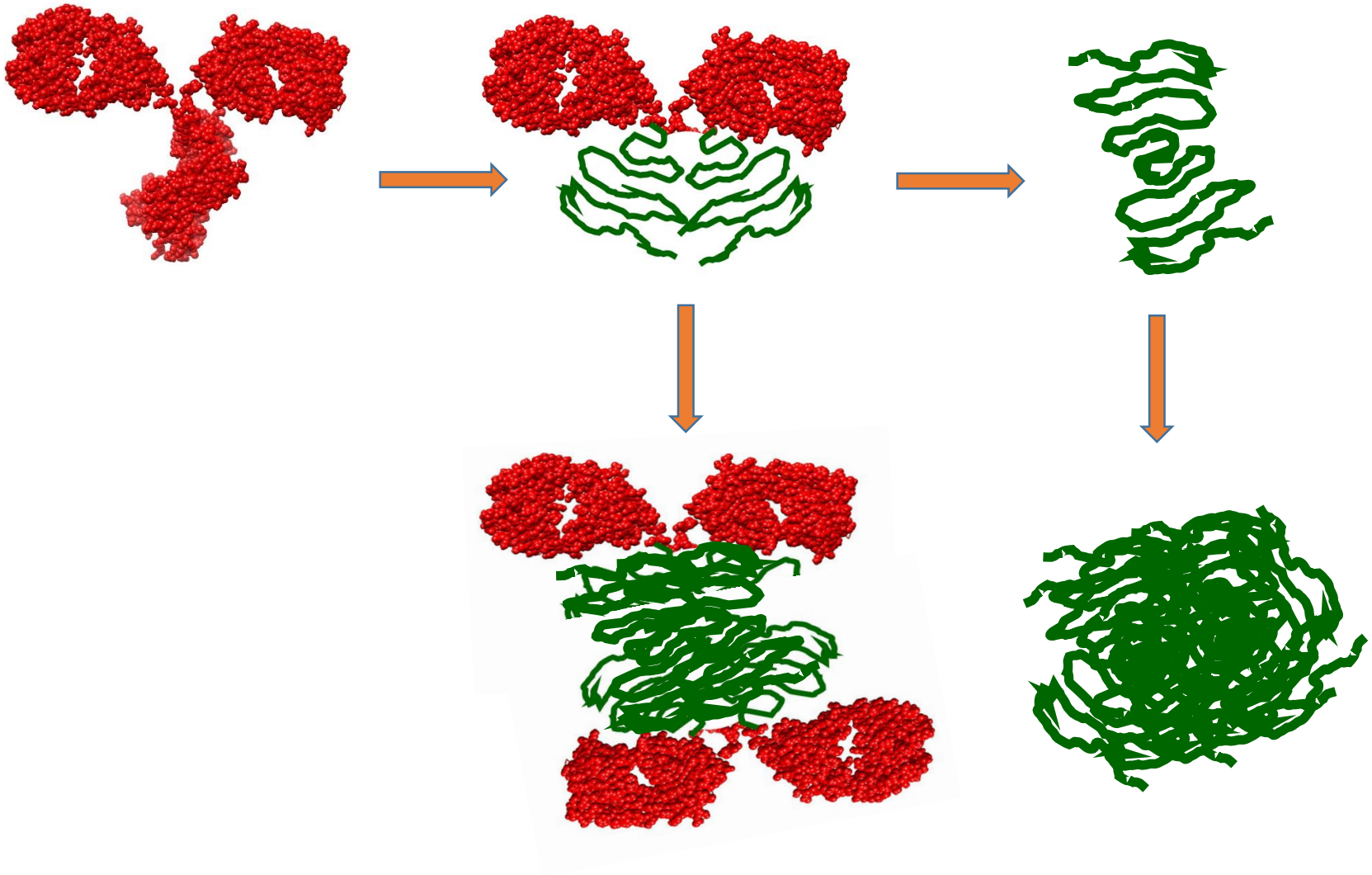
Freire, E.

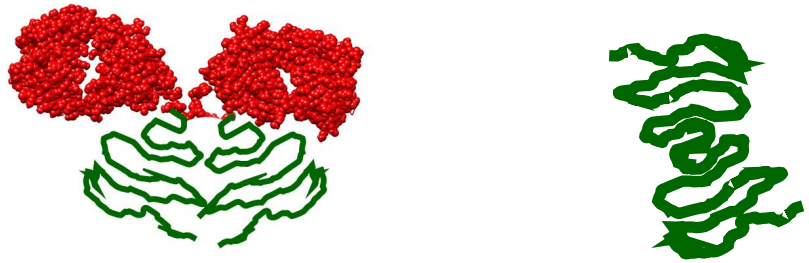
mAbs Denature and Aggregate



Partial Denaturation or Complete Denaturation Can Lead to Aggregation

Strategies to Slow Down Denaturation/Aggregation

- Lower Concentration of Aggregating Species



Solution: Increase Tm or ΔG

- Decrease Intrinsic Rate of Aggregation

Solution: Increase Activation Energy ΔH^*

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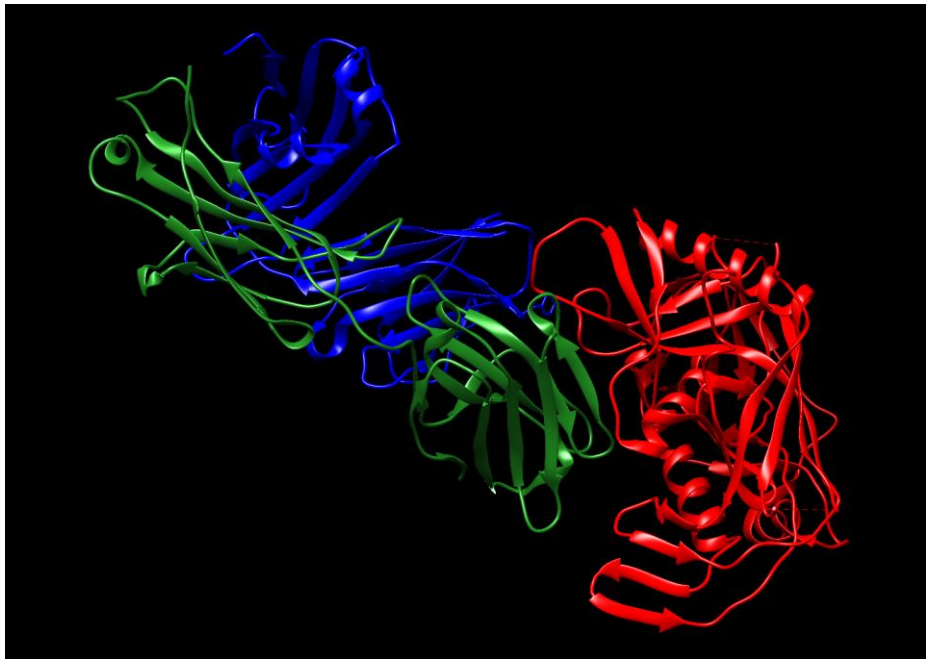
Case Study: VRC07-523LS, a Broadly Neutralizing HIV-1 Antibody Currently in Phase 1 Clinical Trials

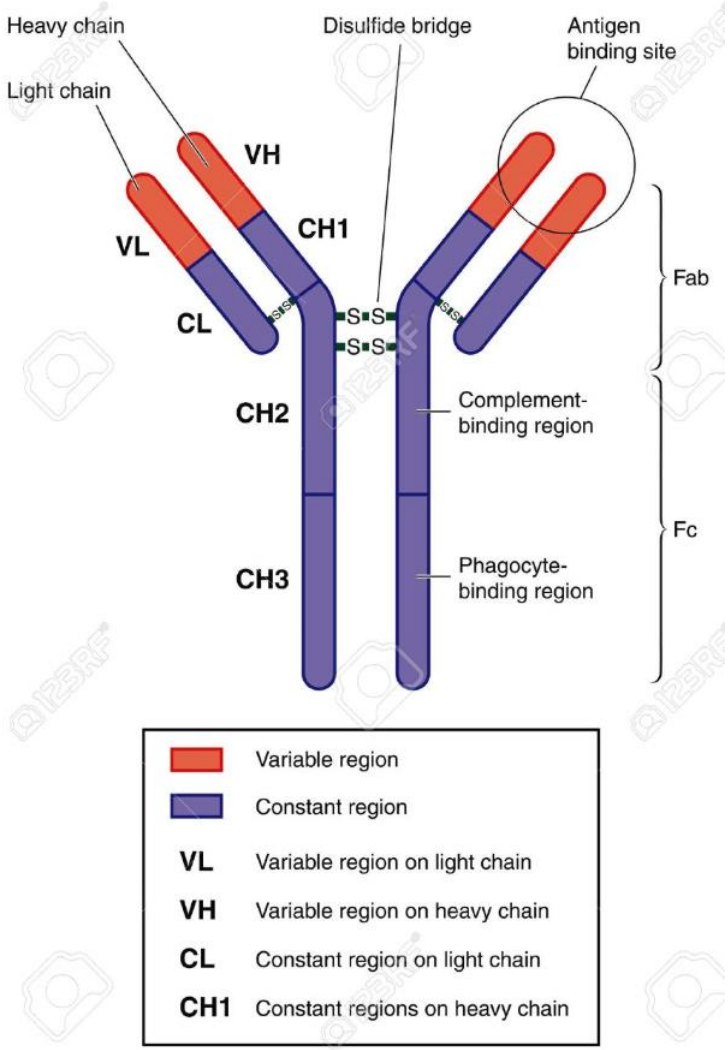
- VRC07-523LS is a VRC01 class IgG1 neutralizing antibody
- mAb VRC01 binds to CD4 binding site in envelope glycoprotein gp120. It neutralizes ~90% of HIV-1 strains with an IC50 of less than 50 µg/ml
- VRC07 was identified through sequencing antibody-gene transcripts of VRC01 donor B cells
  - Structure-based design was used to further optimize VRC07
  - VRC07-523LS is 5- to 8-fold more potent than VRC01
  - It neutralizes 96% of viruses tested



PDB: 4OLY, Kwon, Y.D., Kwong, P.D. 2014

Temperature Denaturation of mAbs is an Irreversible Process

DSC Usually Shows Peaks Corresponding to Fab, CH2 and CH3 Domains



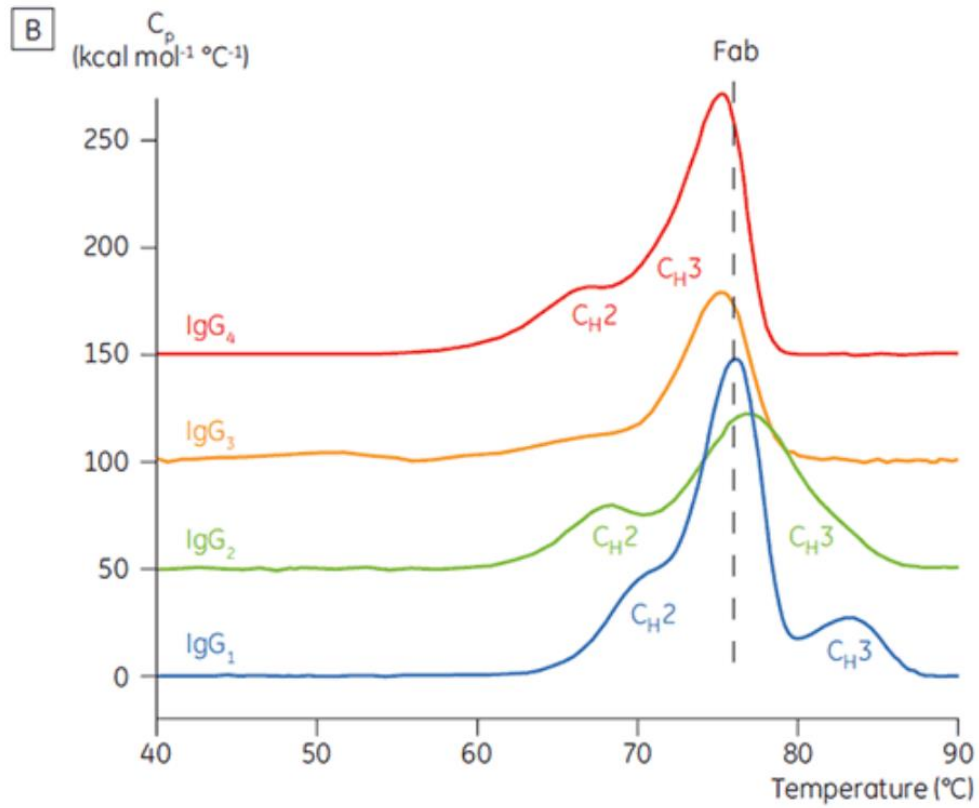
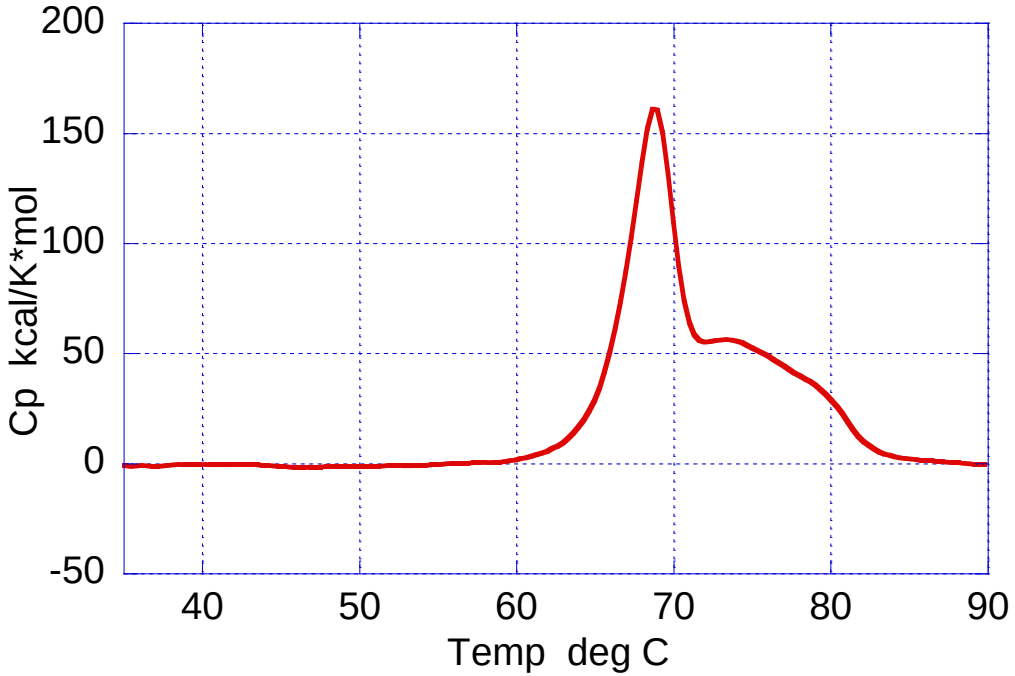
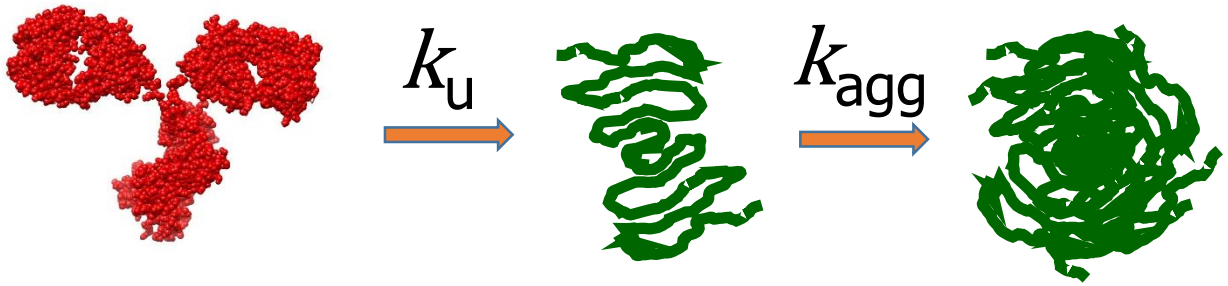


Fig 1. (A) DSC curve of the BIIB7 IgG_1. Above the curves are structures of a full-length human IgG_1 antibody (13). (B) DSC curves representing the four human IgG subclasses.

Garber and Demarest BBRC 355 (2007) 751–757

Irreversible Denaturation is Kinetically Controlled

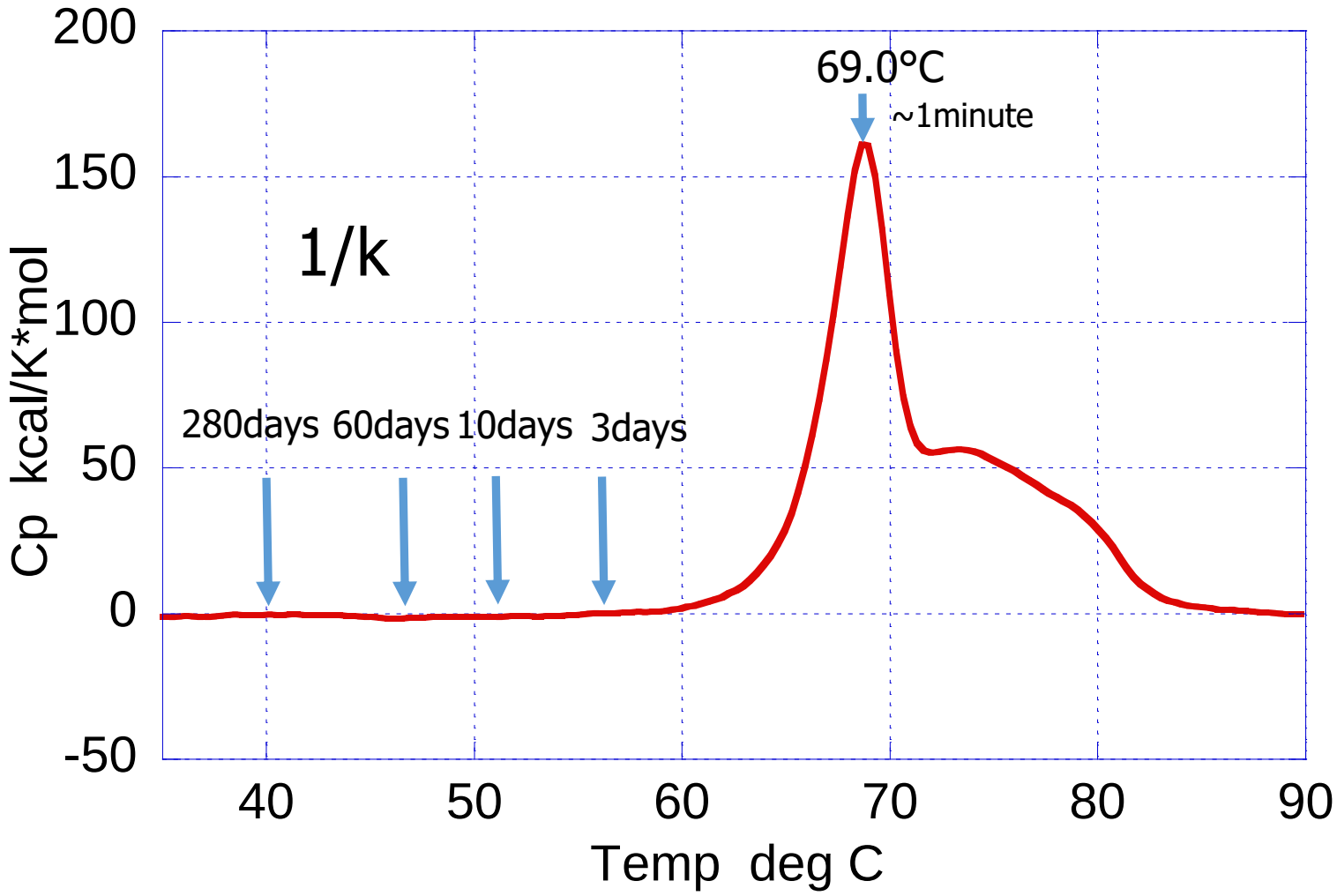




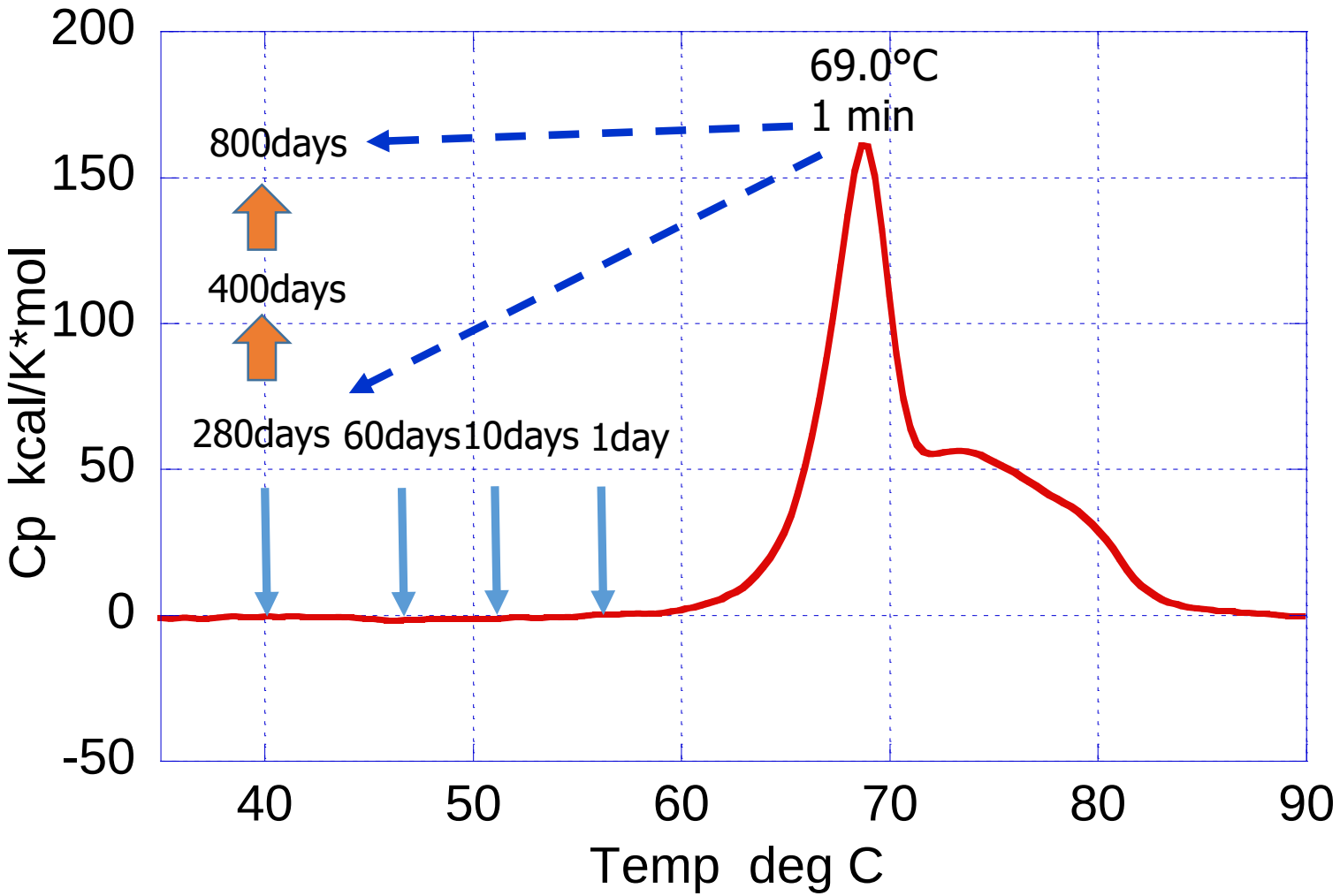
The kinetic control of irreversible protein denaturation in DSC was first discussed by Sanchez-Ruiz et al Biochemistry (1988) 27 1648.

The Rate of Denaturation Increases With Temperature

At Tm the Rate of Denaturation is ~ 1 minute



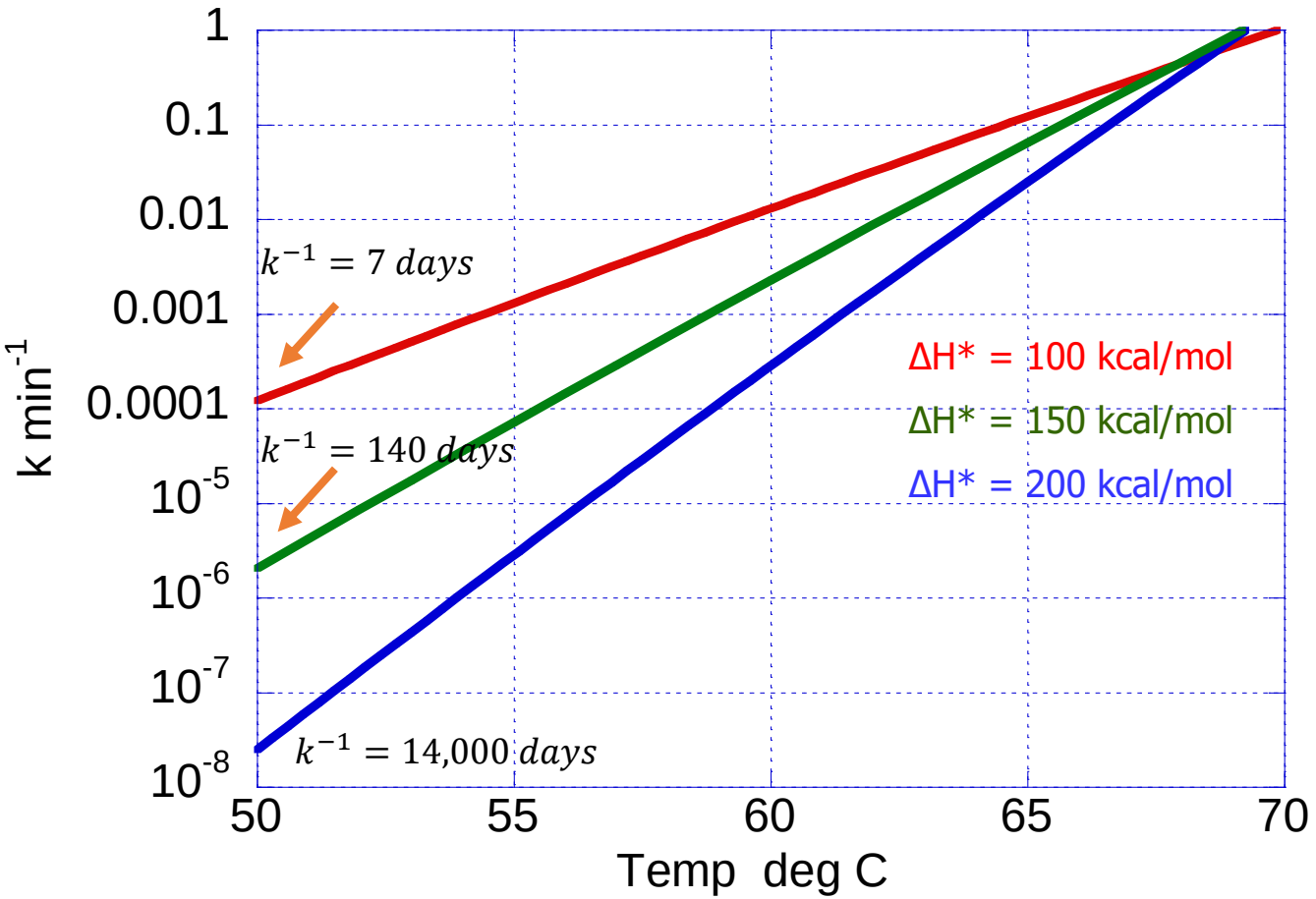
Temperature Denaturation is Kinetically Controlled



The Stability at Lower Temperatures Depends on the Activation Enthalpy ΔH^*.

- The Higher ΔH^*, the Higher the Stability.
- In Formulation or mAb Selection we want to Increase ΔH^* to Maximize Long Term Stability

The Temperature Dependence of the Rate of Denaturation is Determined by the Activation Energy ΔH*



The Higher ΔH* the Faster the Decrease of the Rate of Denaturation/Aggregation Upon Lowering the Temperature

Kinetic Deconvolution of DSC for Irreversible Denaturation

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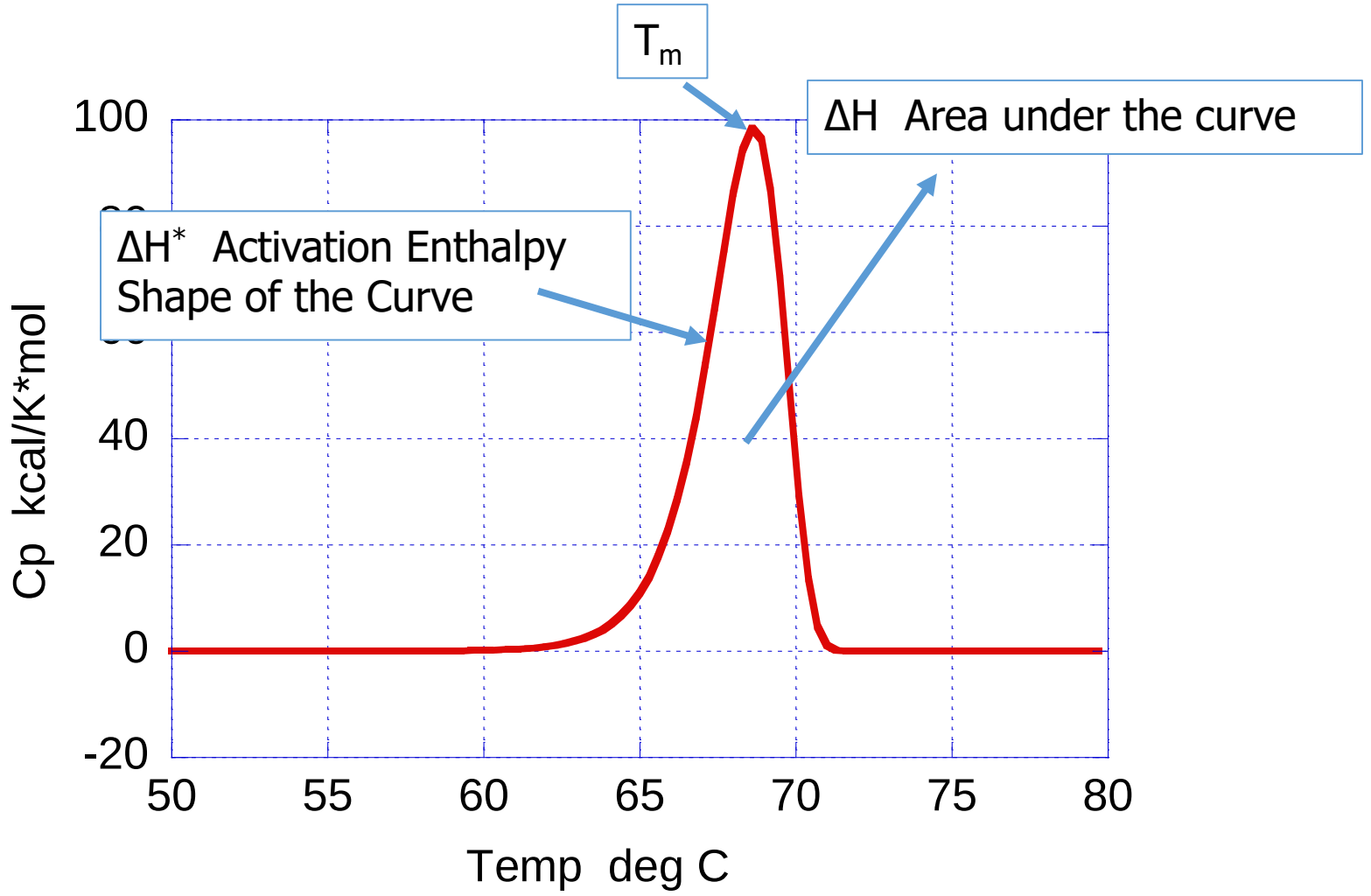

ΔH_i^* Can be Determined from Kinetic Deconvolution

Kinetic Deconvolution of DSC for Irreversible Denaturation Using Exact Equations

$$C_p = \sum \Delta H_i \frac{dF_{d,i}}{dT}$$

$$\frac{dF_{d,i}}{dt} = k_i F_{d,i}$$

$$\frac{dF_{d,i}}{dT} = \frac{k_i F_{d,i}}{\alpha} \qquad \alpha = \frac{dT}{dt} = scan\ rate$$

$$\frac{d\ lnF_{d,i}}{dT} = \frac{k_i}{\alpha} = \frac{\exp(-\frac{\Delta G_i^*}{RT})}{\alpha}$$

$$k_i = \exp\left(-\frac{\Delta G_i^*}{RT}\right) = \exp(-\frac{\Delta H_i^* - T\Delta S_i^*}{RT})$$

ΔH_i^* Can be Determined from Kinetic Deconvolution

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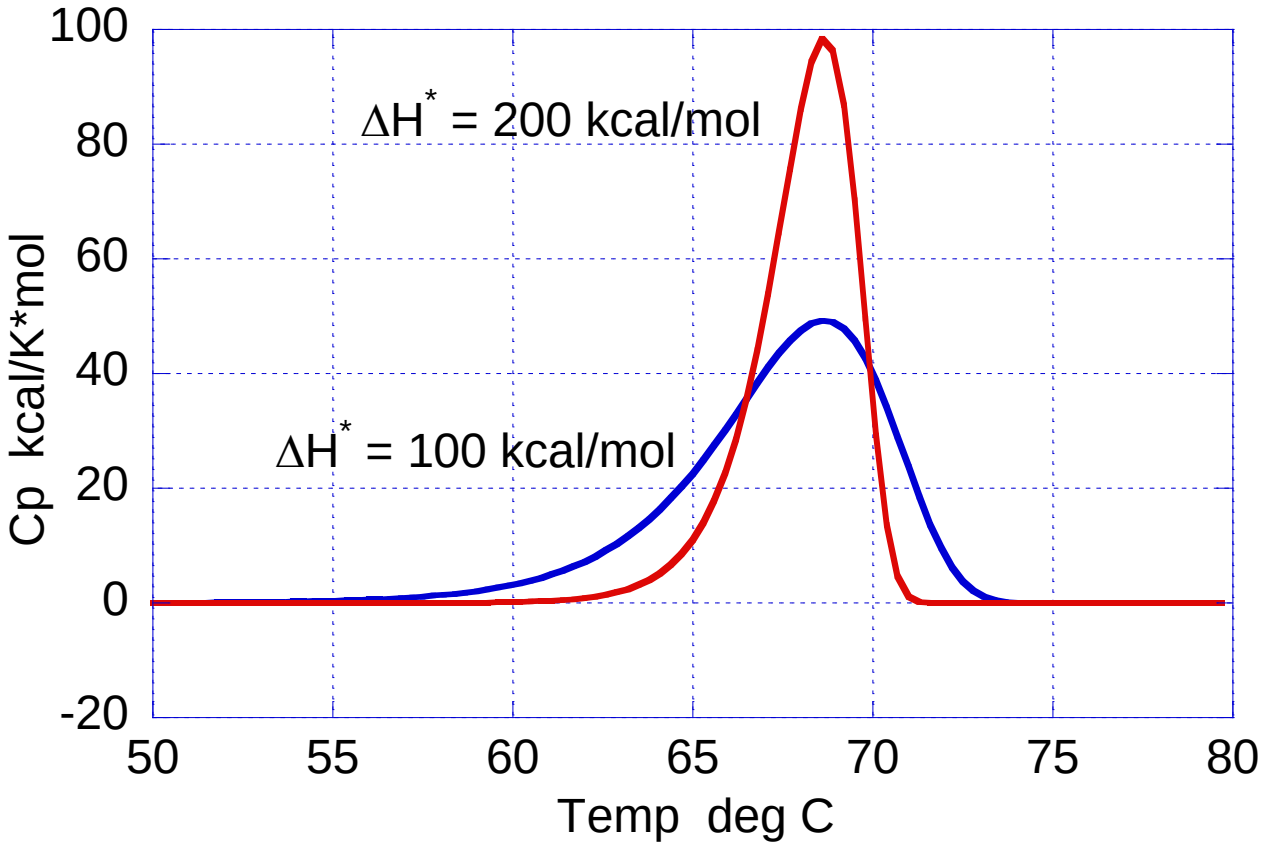
The Effect of the Activation Enthalpy (ΔH*) on the Shape of the DSC Curve



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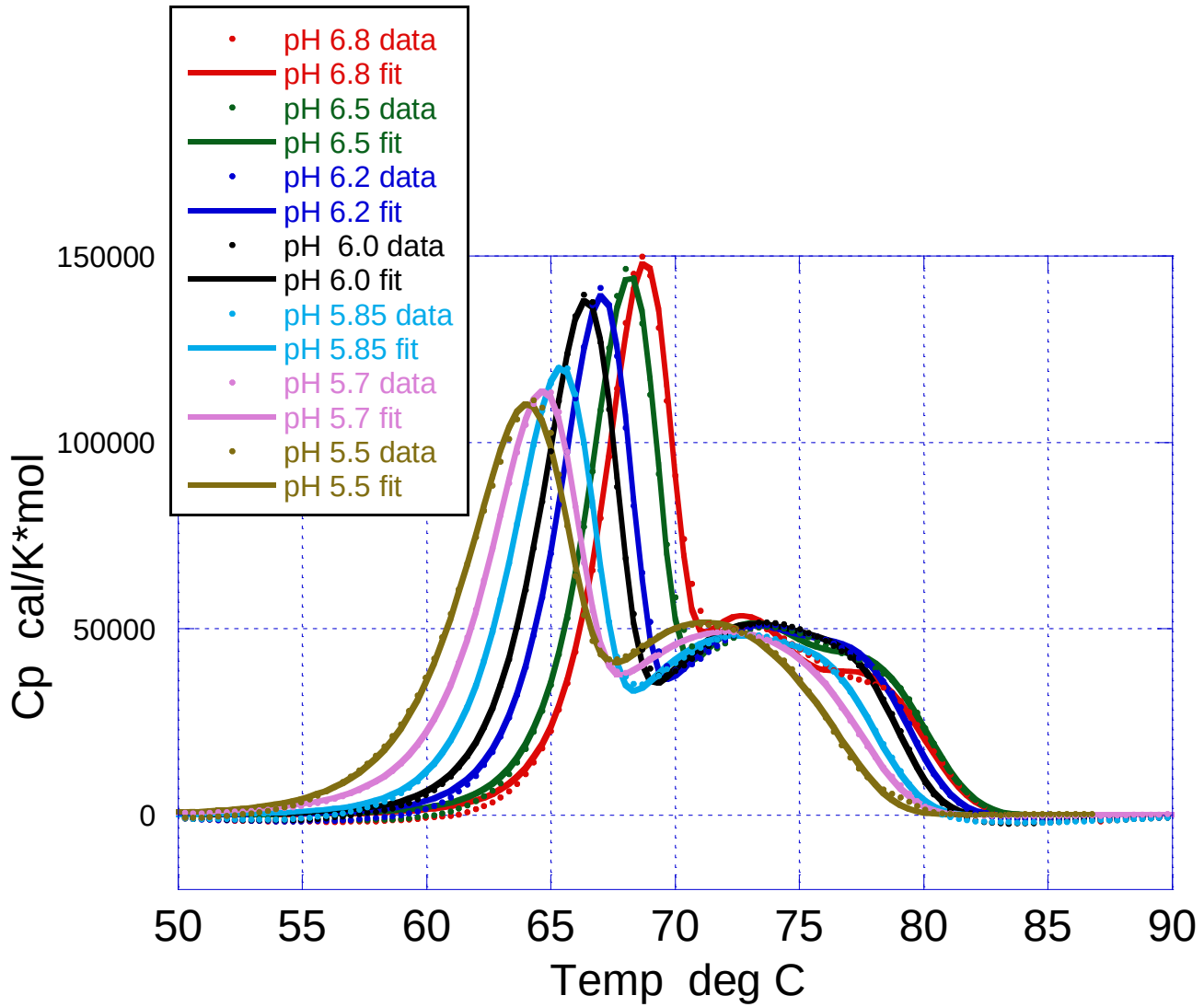
DSC Data and Kinetic Deconvolution as a Function of pH for mAb VRC07



50mM Histidine, 100mM NaCl

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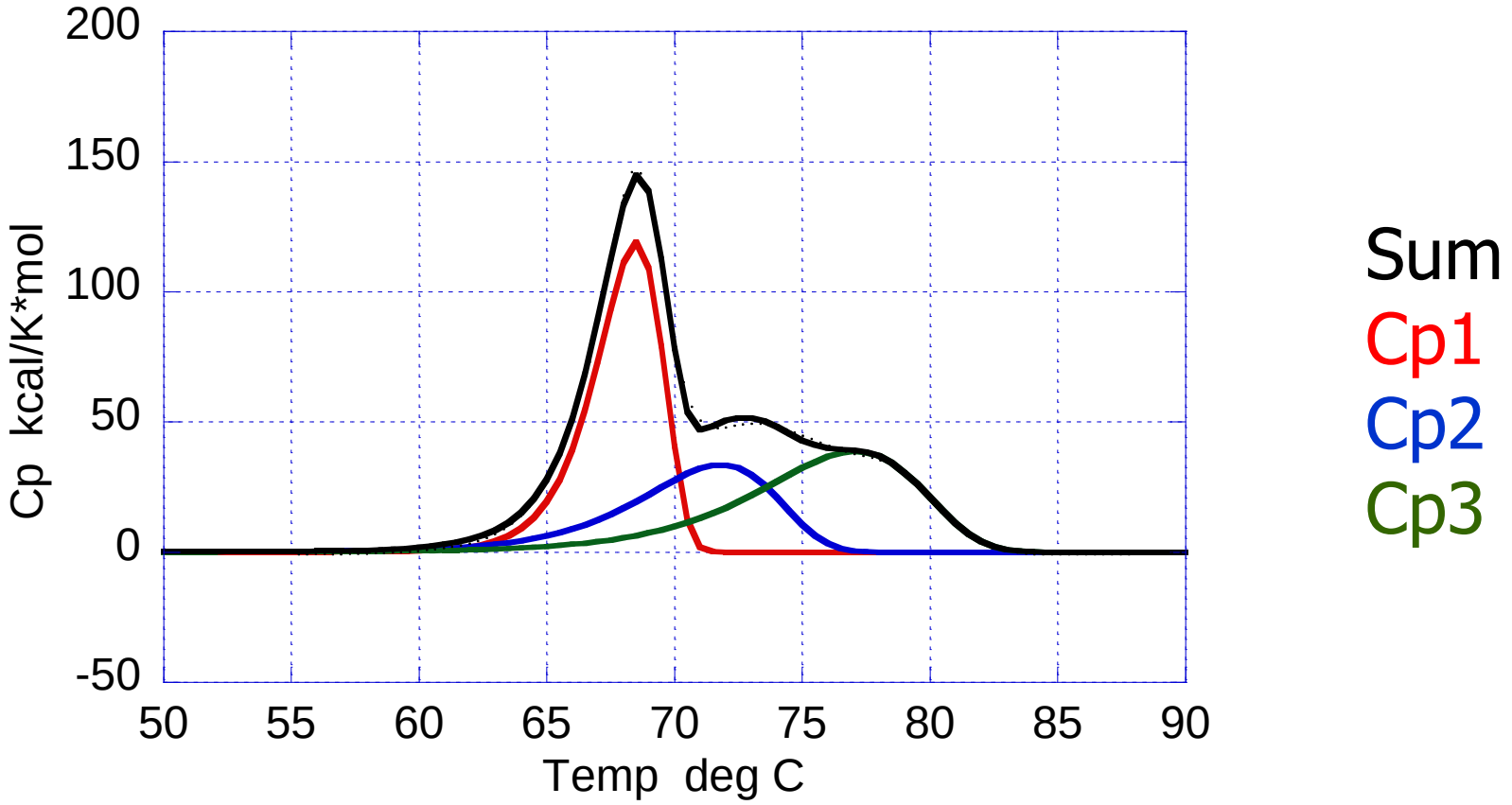
Kinetic Deconvolution of Cp Function of VRC07 pH 6.8



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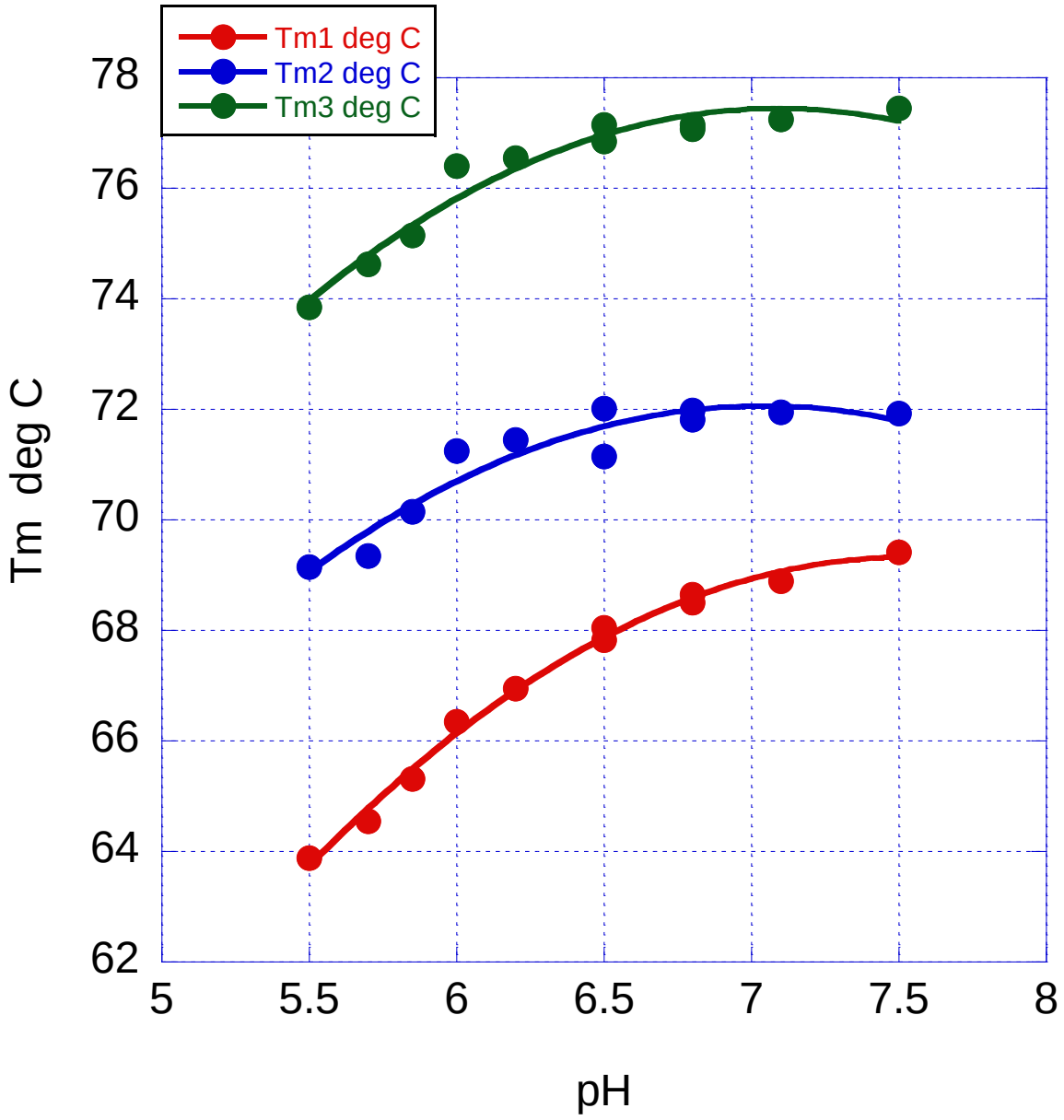
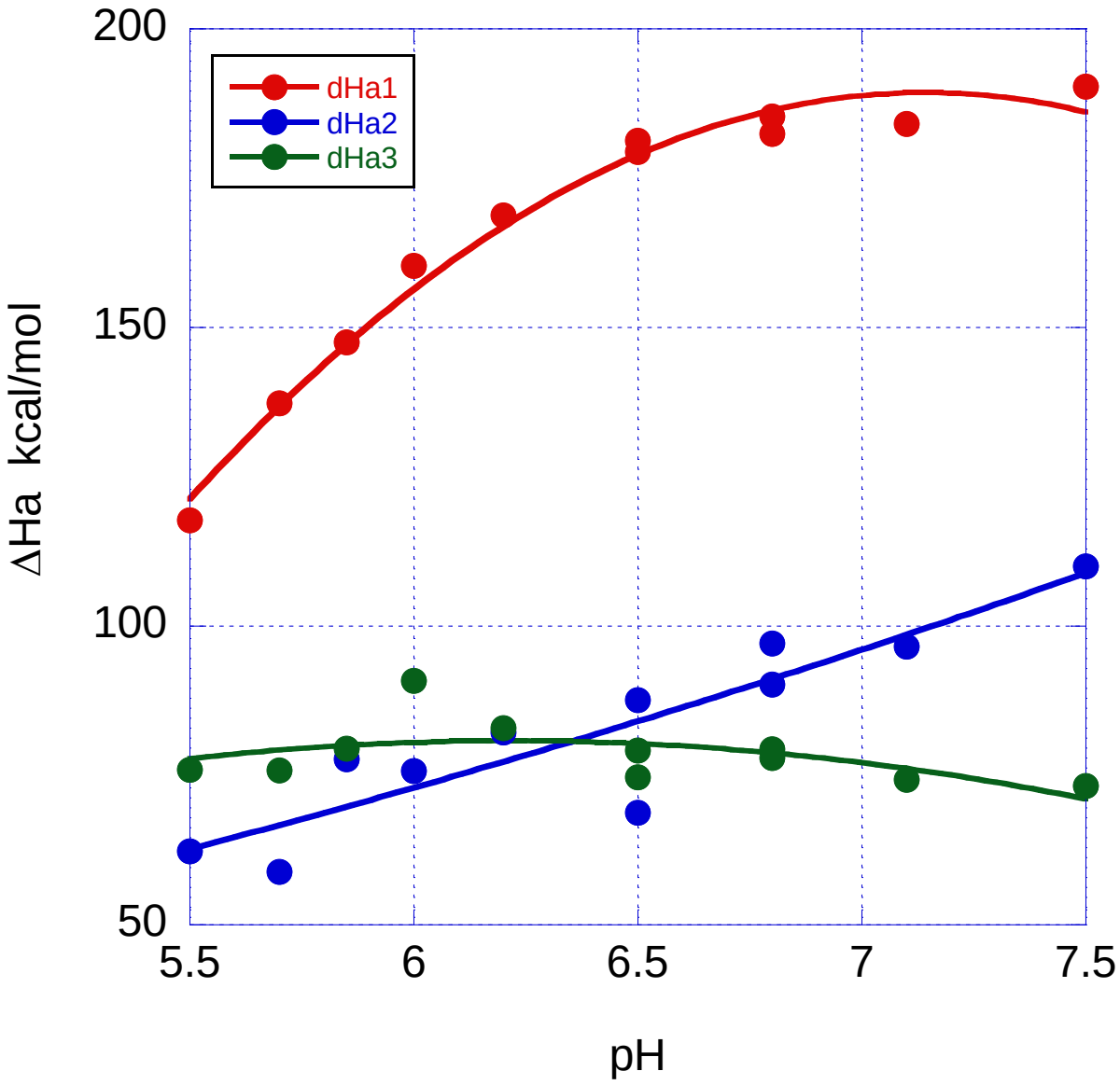
pH Dependence of Tm and Activation Enthalpy





Combination of Tm and Activation Enthalpy Indicate that pH 6.8 Offers Best Stability Profile

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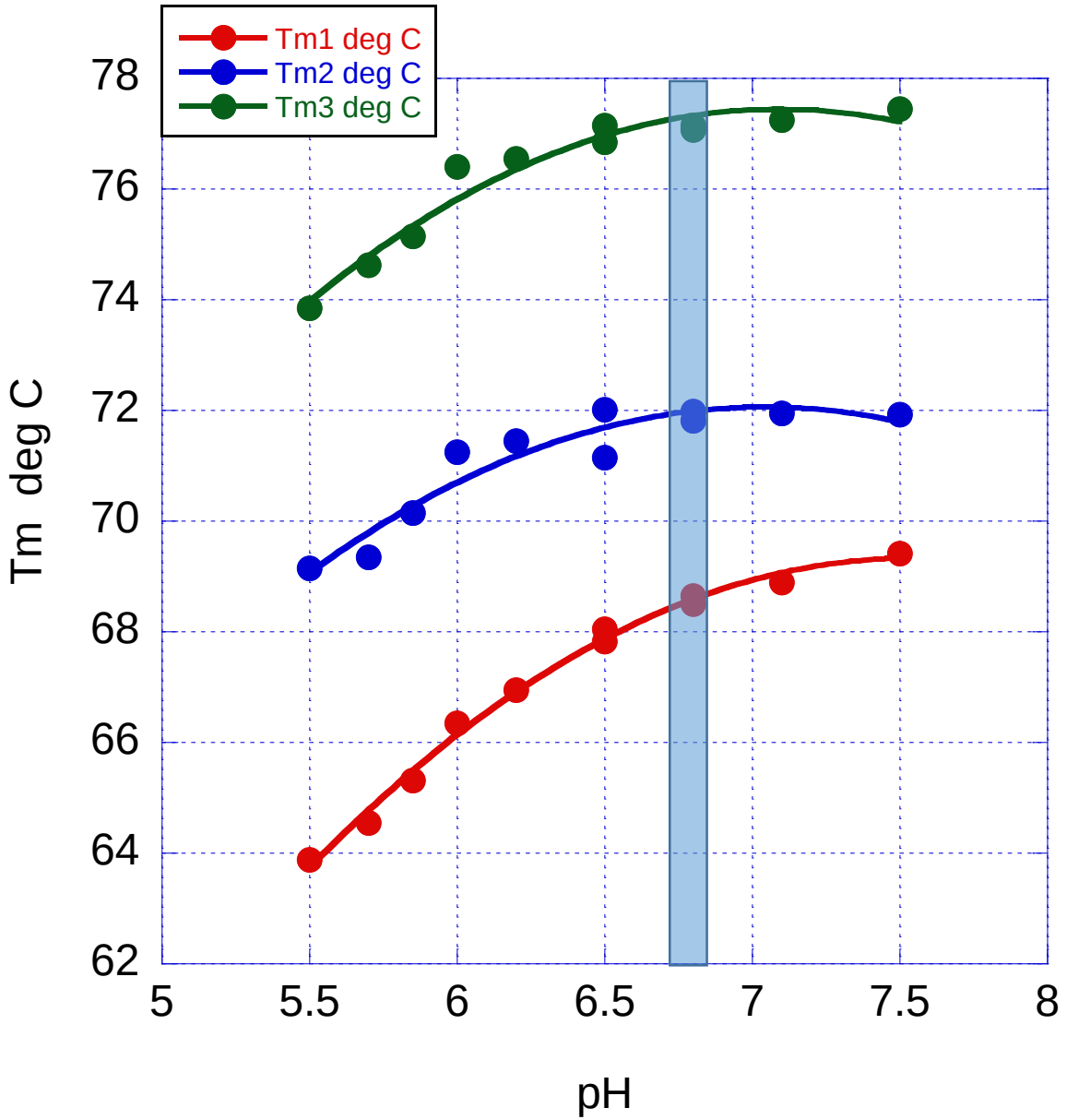

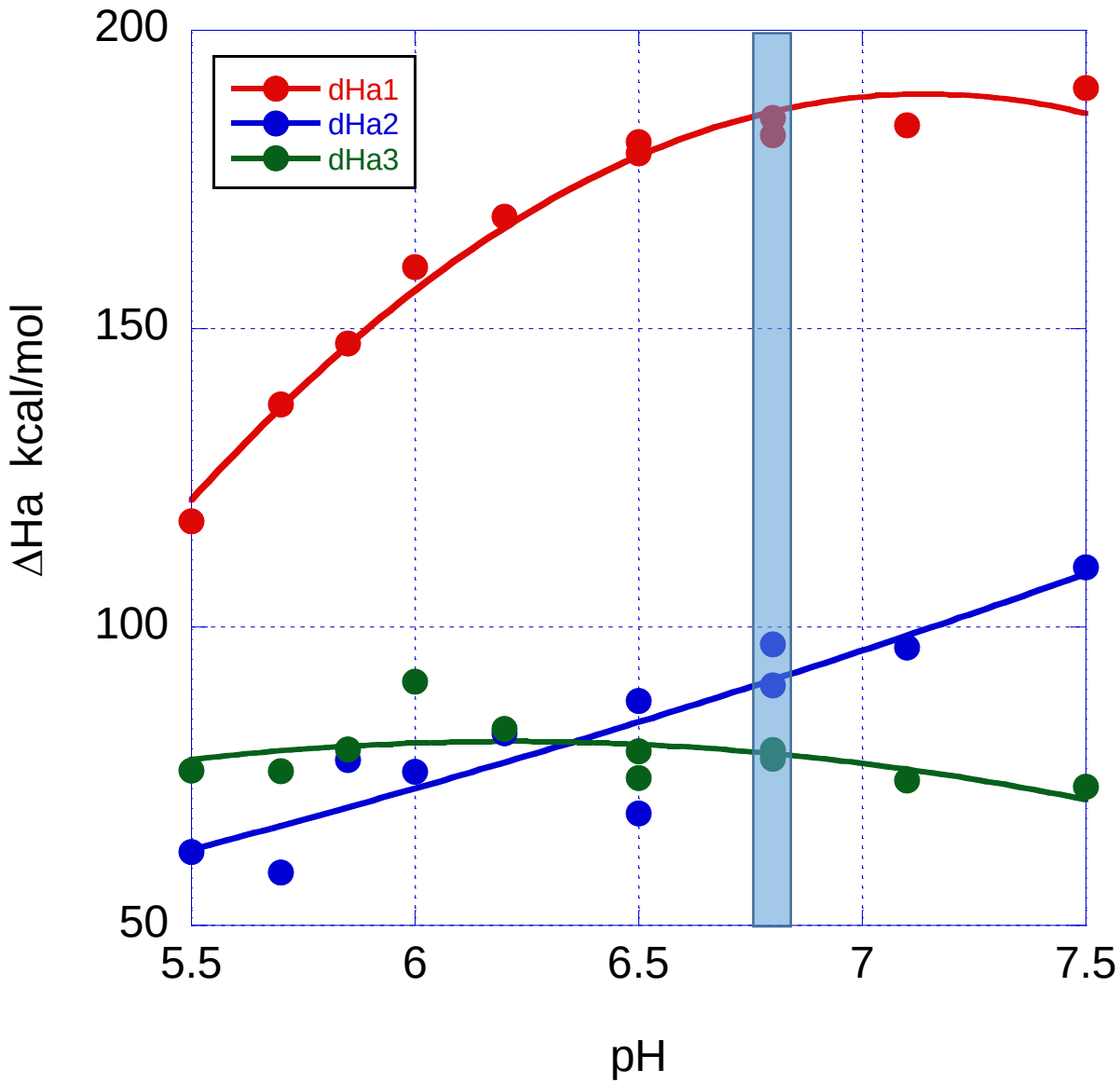

Low Temperature Region Provides Clues to Long Term Stability. Kinetic Deconvolution Allows Extrapolation of Cp Components

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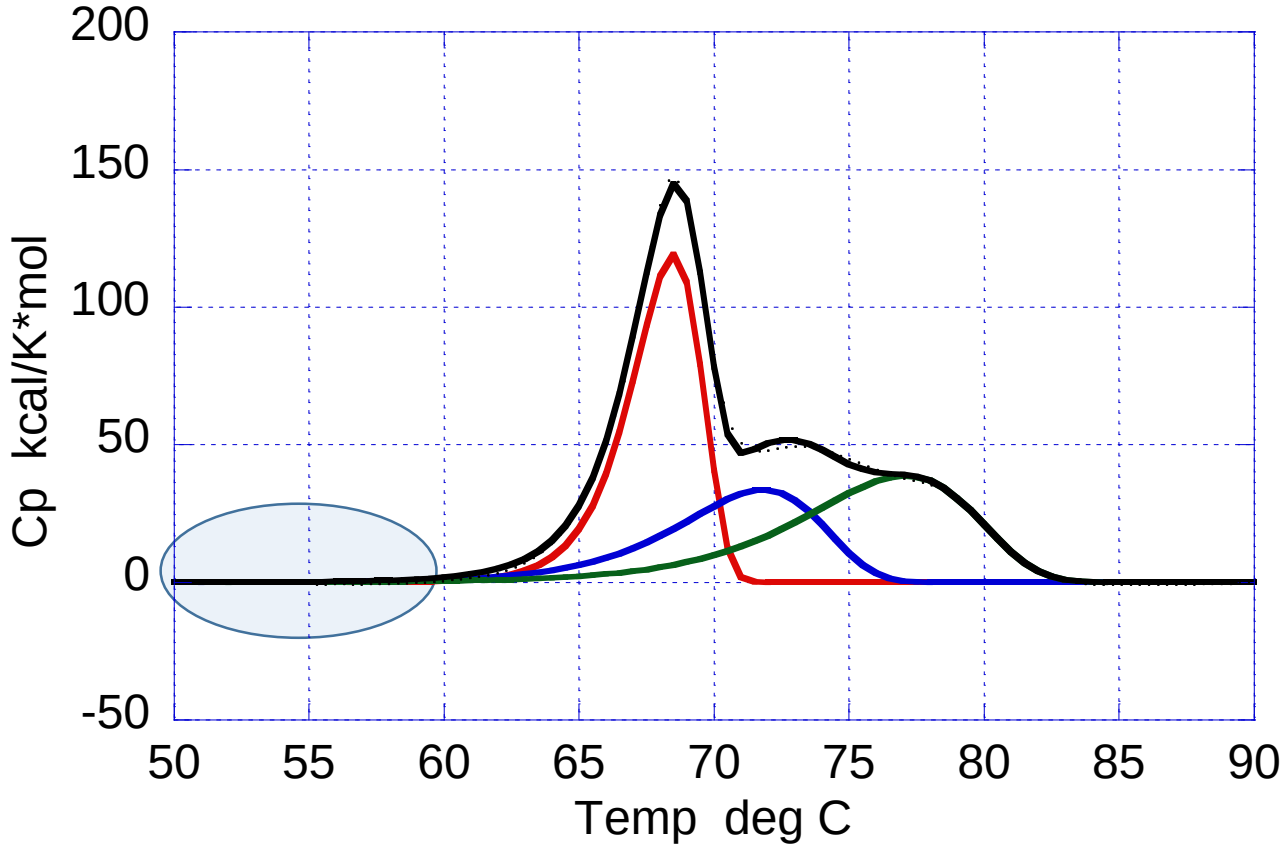

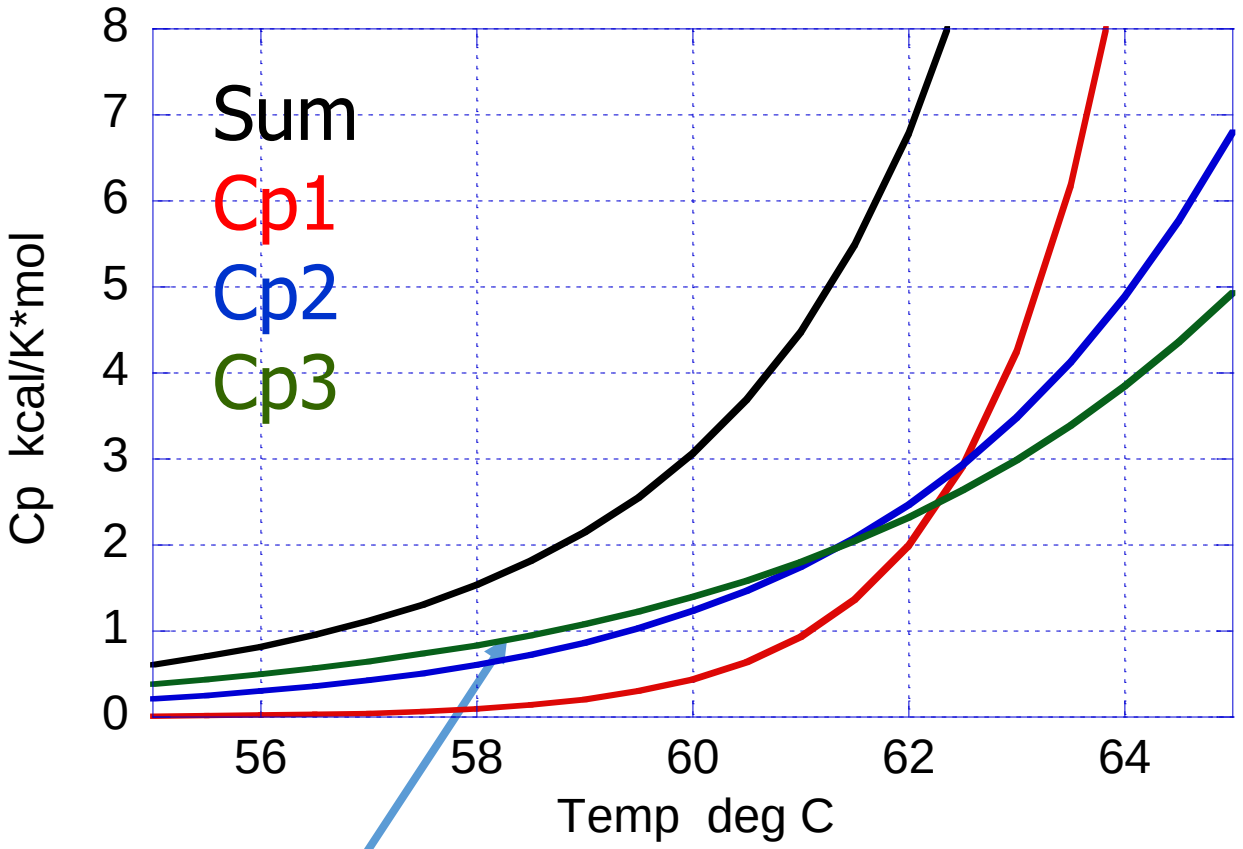

Cp2 and Cp3 are the first to start denaturation. Even though they have higher Tm's they have lower ΔH^*

At Low Temperatures Cp3 is the Least Stable Domain. pH 6.8 Appears to be the Most Appropriate pH

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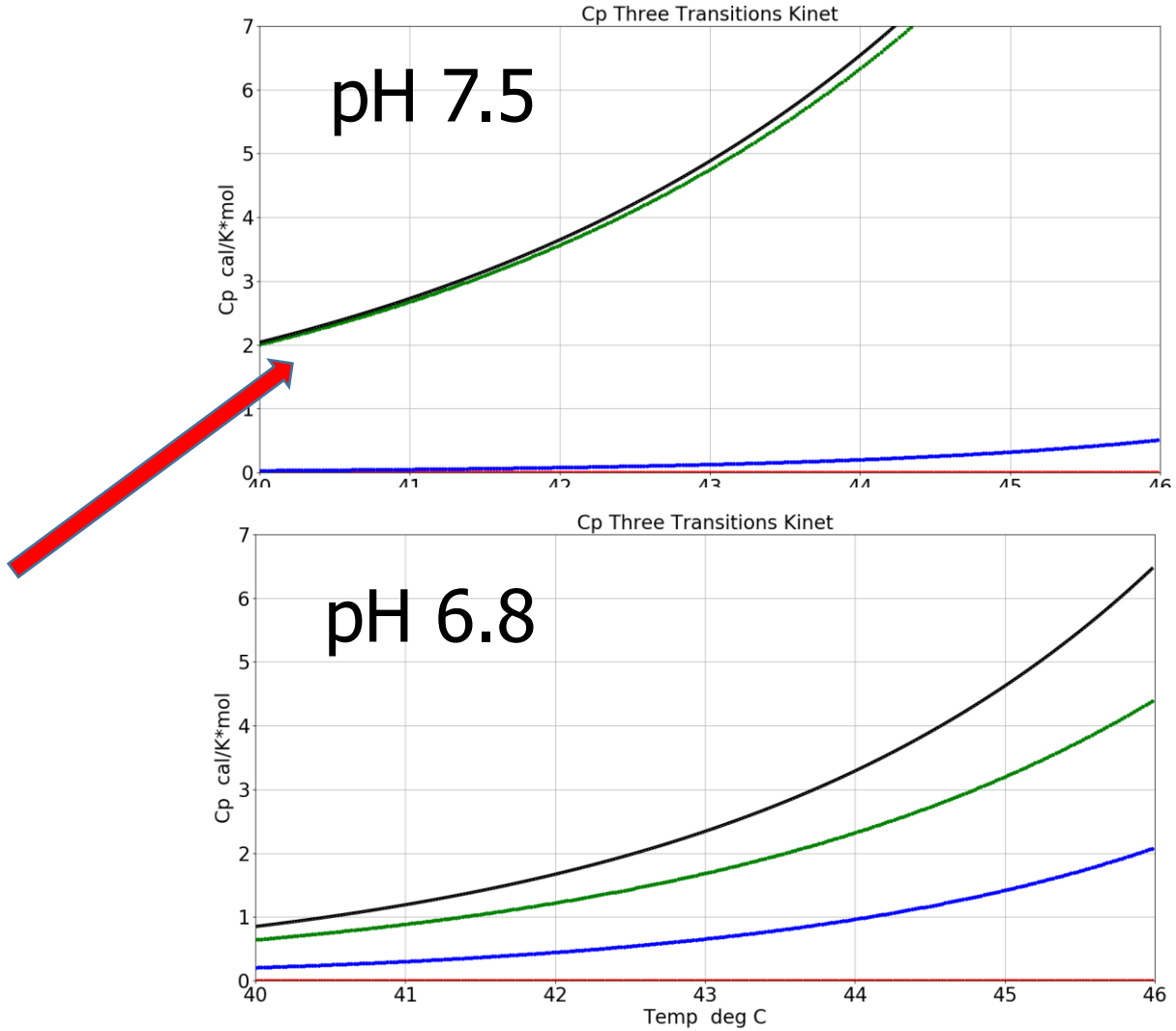

Sum
Cp1
Cp2
Cp3

Further Formulation Optimization at pH 6.8. Addition of Excipients: DSC of VRC07 in Buffers 1 - 4

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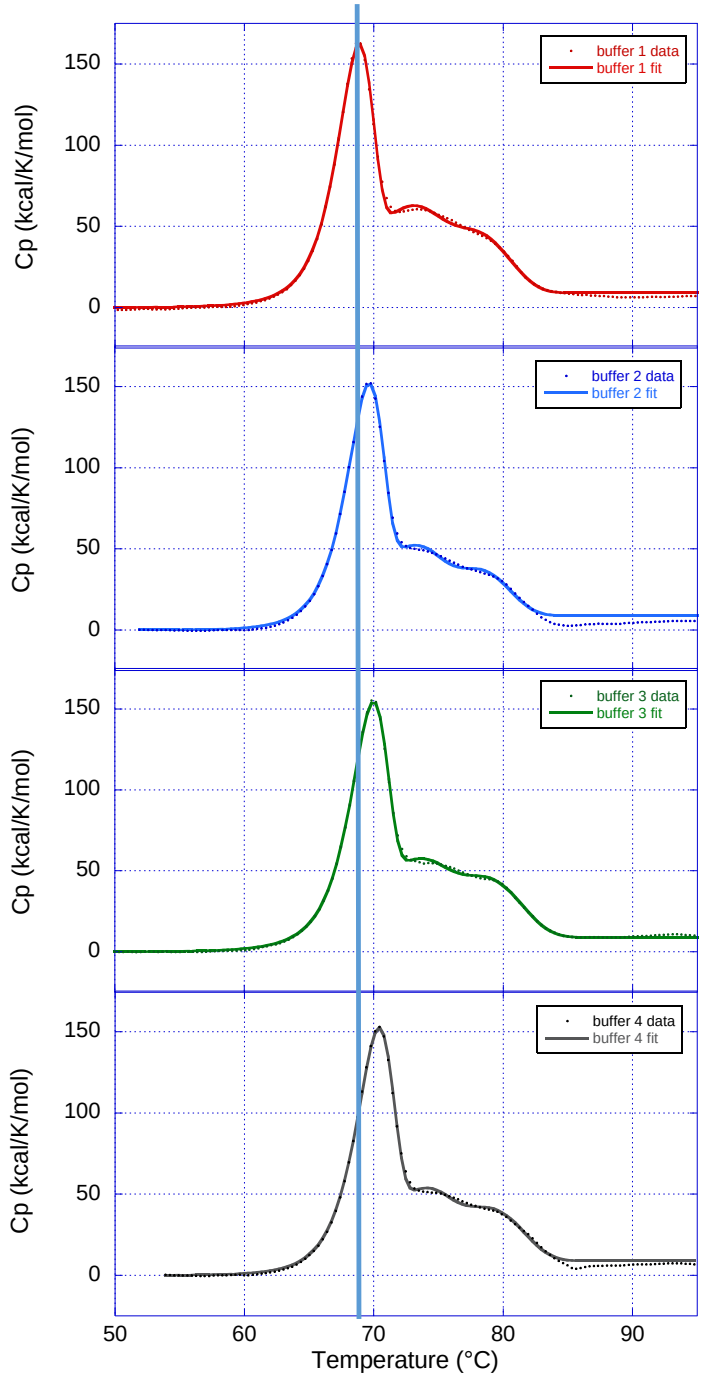

Buffer 1 - 50mM Histidine, 100mM NaCl, pH 6.8

Buffer 2 - 50mM Histidine, 100mM NaCl, 5% w/v sucrose, pH 6.8

Buffer 3 - 50mM Histidine, 100mM NaCl, 5% w/v sorbitol, pH 6.8

Buffer 4 - 50mM Histidine, 50mM NaCl, 5% w/v sucrose, 2.5% w/v sorbitol, pH 6.8

Clarkson et al (2018) Analytical Biochemistry

Effect of Excipients in Tm's and ΔH*'s of VRC07 at pH 6.8

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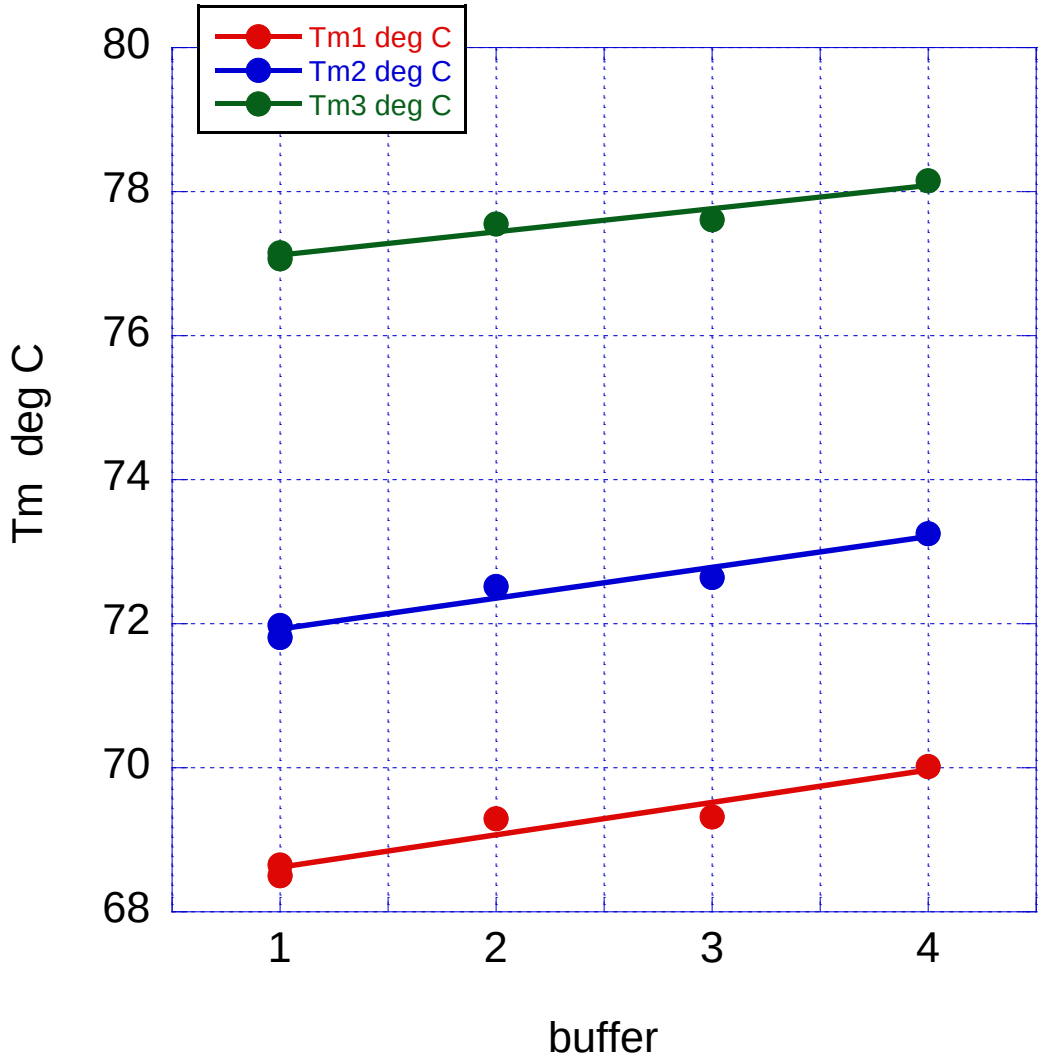
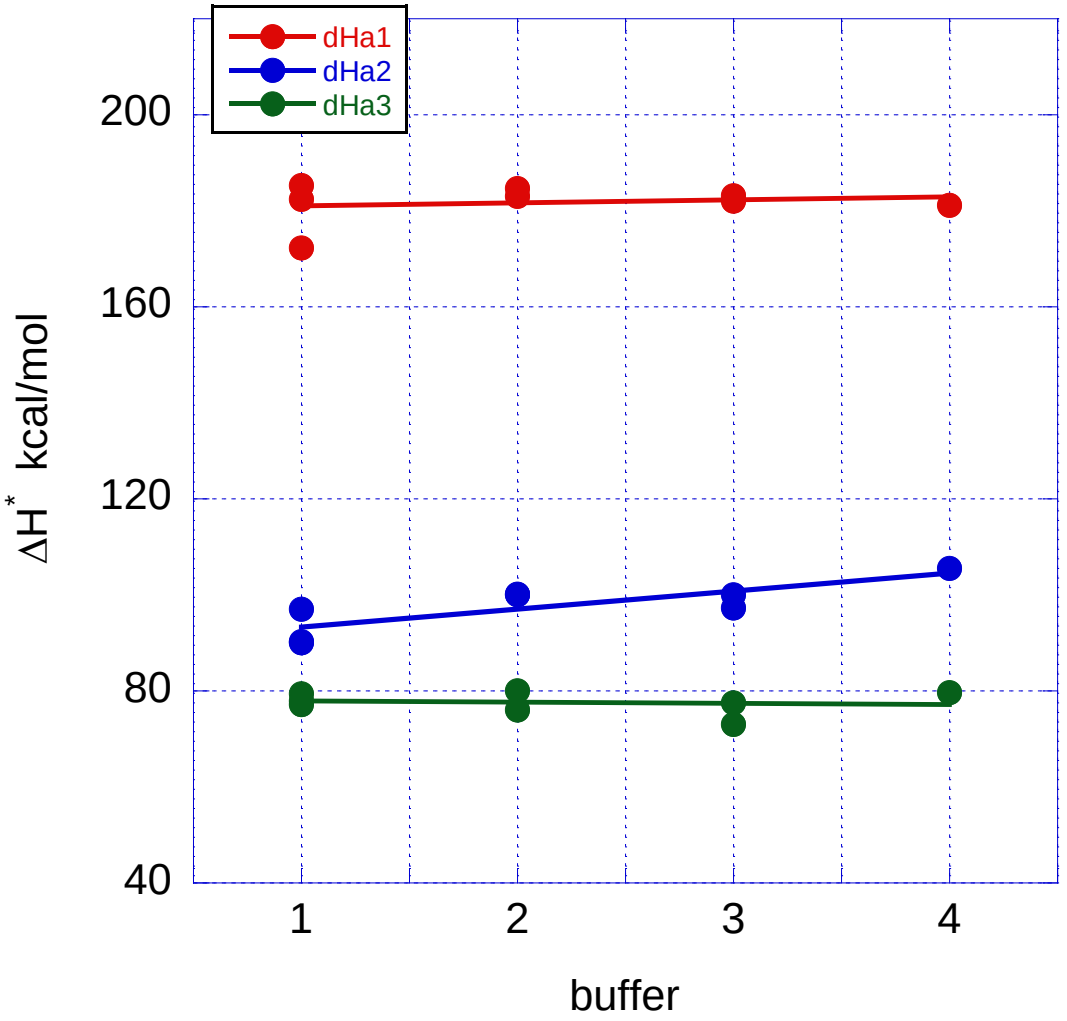
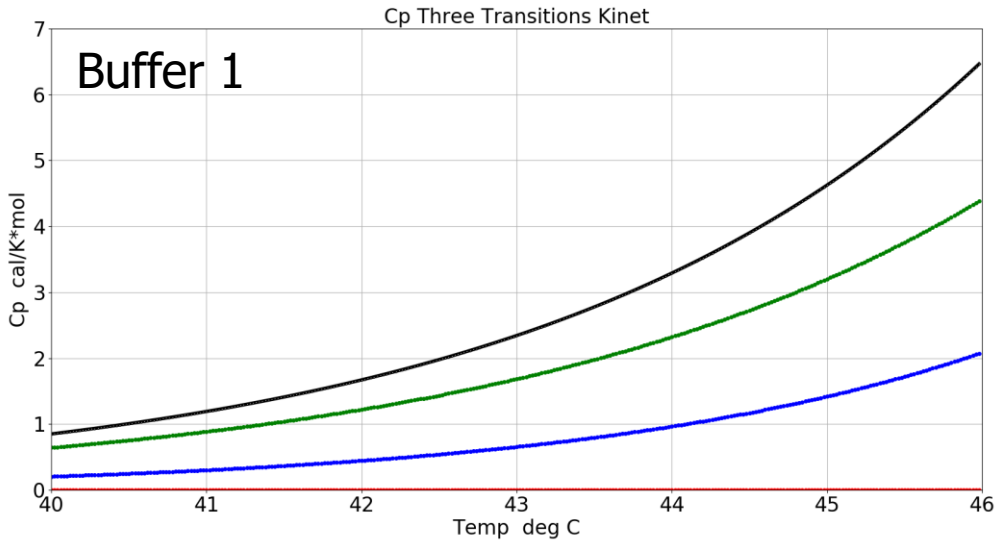
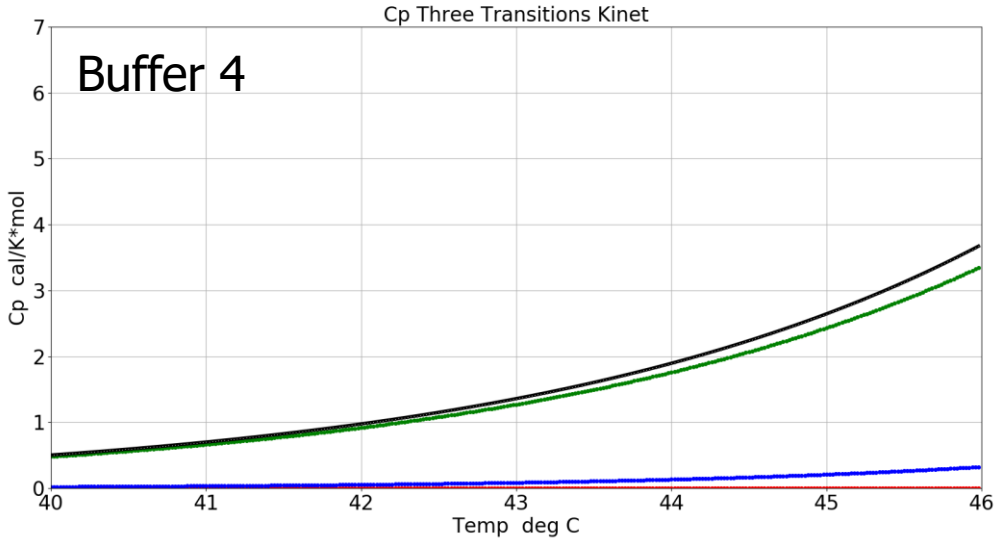




Tm's show an increase for all transitions. Activation enthalpy is fairly constant for transitions 1 and 3. Buffer 4 is the best overall.

Improvement of Low Temperature Stability by Excipients





Sum
Cp1
Cp2
Cp3

Notice that Formulation Optimization is a Combination of Tm and Activation Enthalpy

Isothermal Calorimetry

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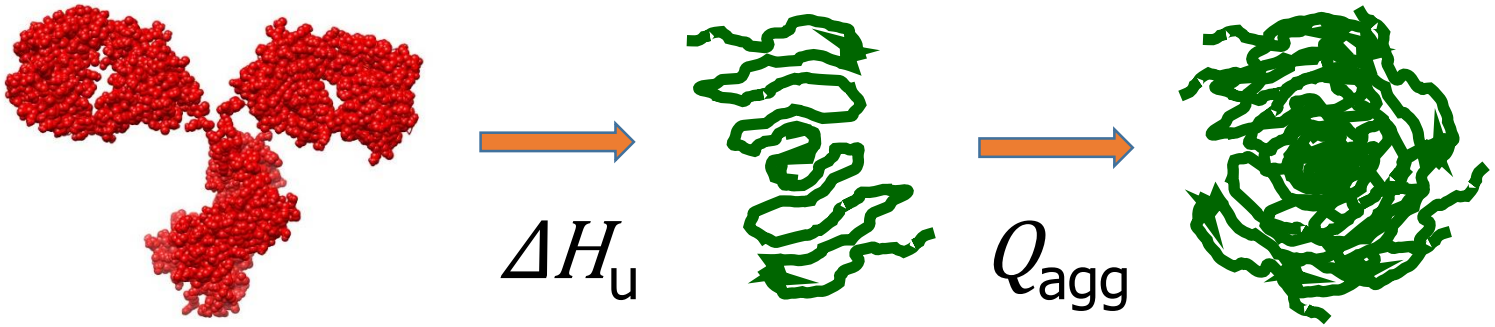

Denaturation and Aggregation Involve the Absorption or Release of Heat and Therefore Can Be Measured Calorimetrically at Constant Temperature

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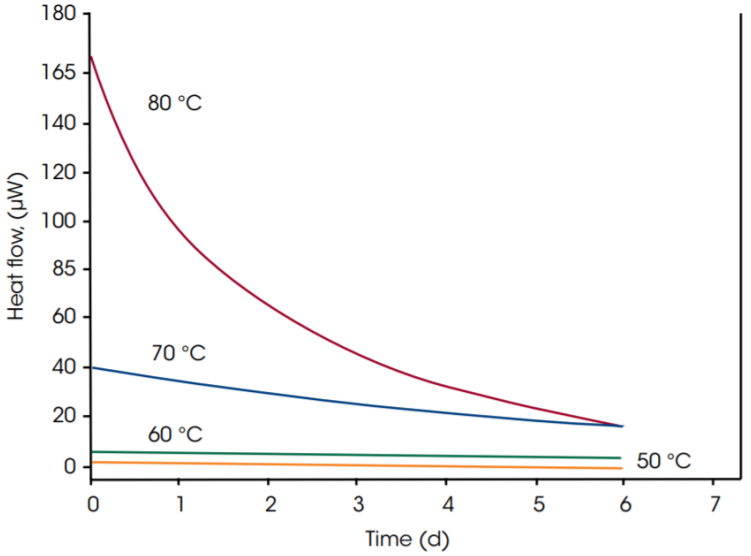
TAM Isothermal Calorimeter

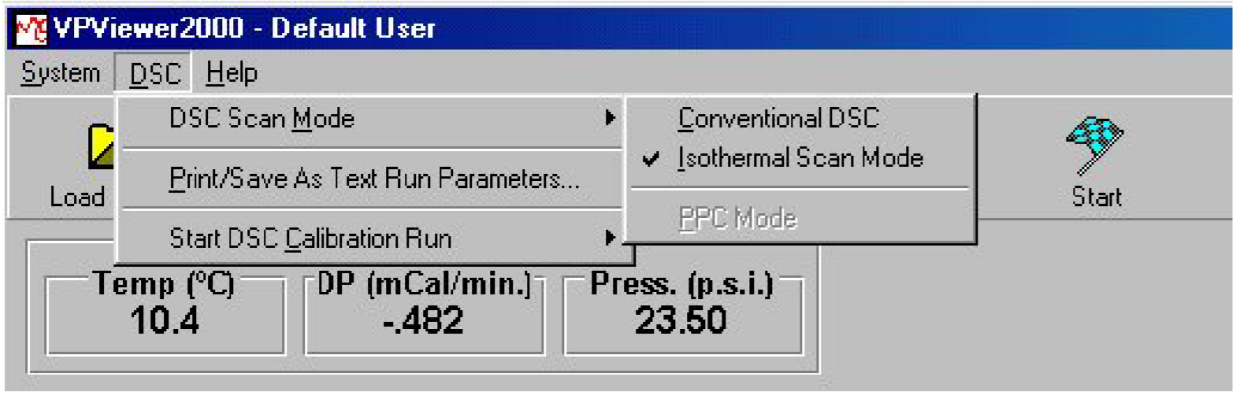


VP DSC. Little Known Isothermal Mode

Isothermal Operation

The VP-DSC has two basic modes of operation; scanning (Conventional DSC) and isothermal (Isothermal Scan Mode). Scanning mode uses a linearly increasing or decreasing temperature ramp function, while measuring the differential power between the reference and sample cells. This mode is referred to as 'Conventional DSC' from within VPViewer.

A second mode of operation is also available, namely Isothermal mode. In Isothermal mode, a constant temperature is maintained for relatively long periods of time while measuring differential power between the reference and sample cells. Isothermal mode is commonly used in evaluating the stability of drug formulations, as in shelf life studies. To carry out an Isothermal mode scan (IsoScan), the user must first select the Isothermal mode of operation from within the Experimental Control window. From the Experimental Control window, select the menu Cell 1|DSC Scan Mode|Isothermal Scan Mode, as shown below.



Isothermal Calorimetry

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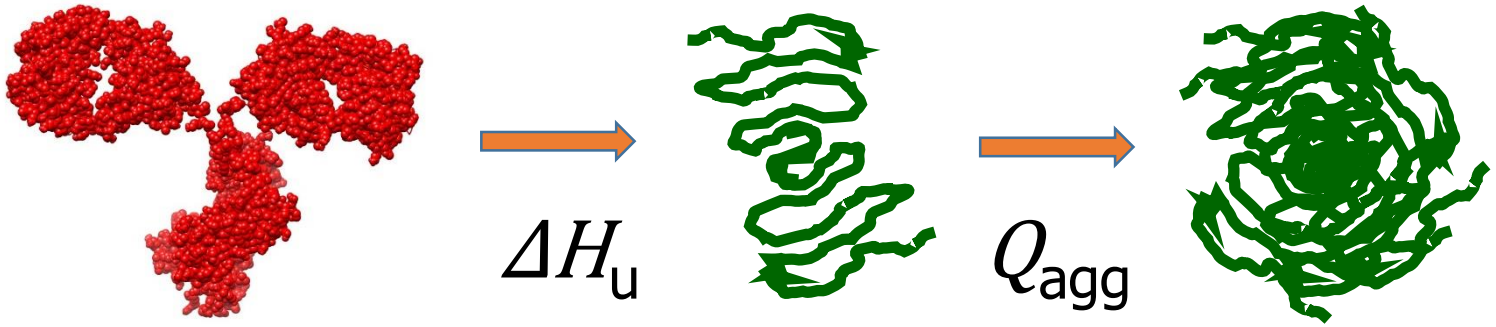


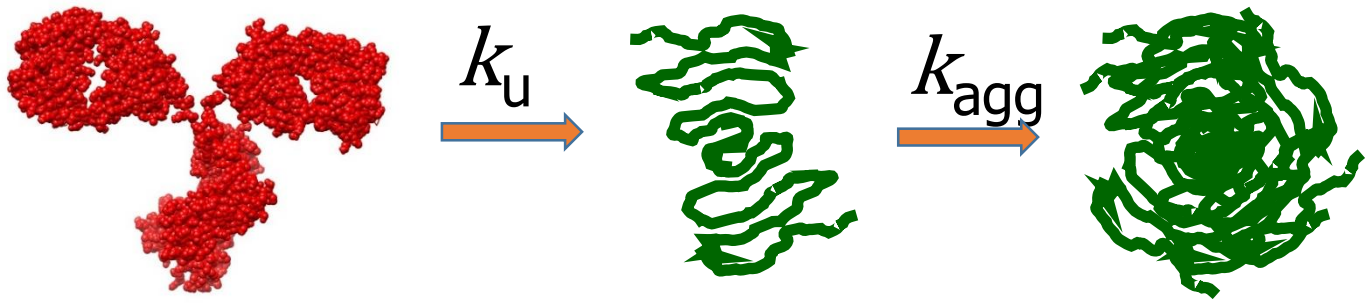
$$Q = \frac{Heat}{v[P_T]} = \Delta H_u F_u + Q_{agg} F_u F_{agg}$$

$$Q = \Delta H_u\left(1 - e^{-k_u t}\right) + Q_{agg}\left(1 - e^{-k_u t}\right)\left(1 - e^{-k_{agg} t}\right)$$

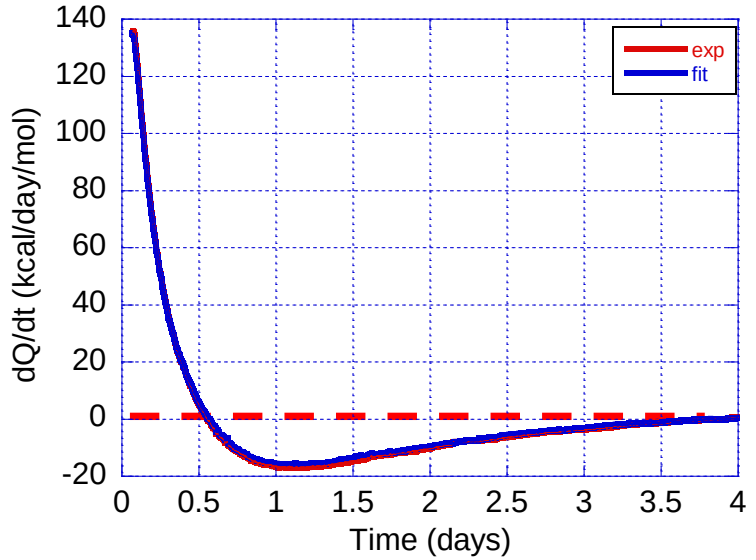
Quantity Measured by Calorimeter is Heat Flow: dQ/dt

$$dQ/dt = k_u \Delta H_u e^{-k_u t} + k_{agg} Q_{agg} e^{-k_{agg} t} + k_u Q_{agg} e^{-k_u t} - (k_u + k_{agg}) Q_{agg} e^{-(k_u + k_{agg})t}$$

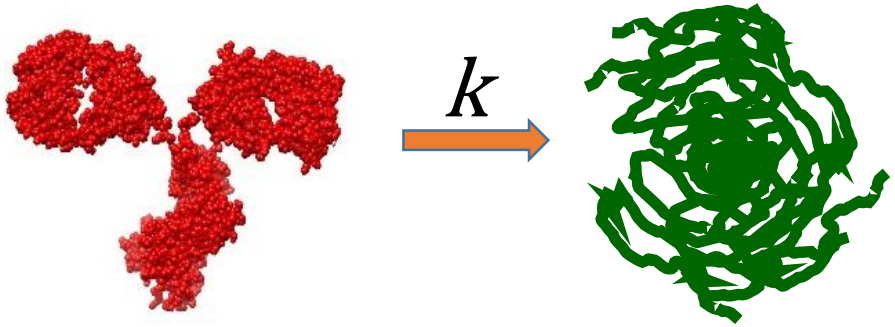
Two Different Situations for Protein Denaturation/Aggregation



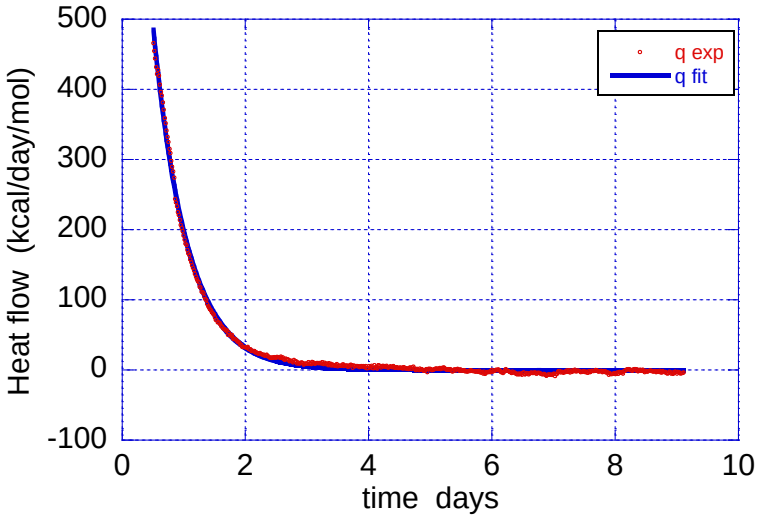
$$k_u >> k_{agg}$$



Denaturation Precedes Aggregation



$$k_u << k_{agg}$$



Denaturation, Aggregation Occur Simultaneously

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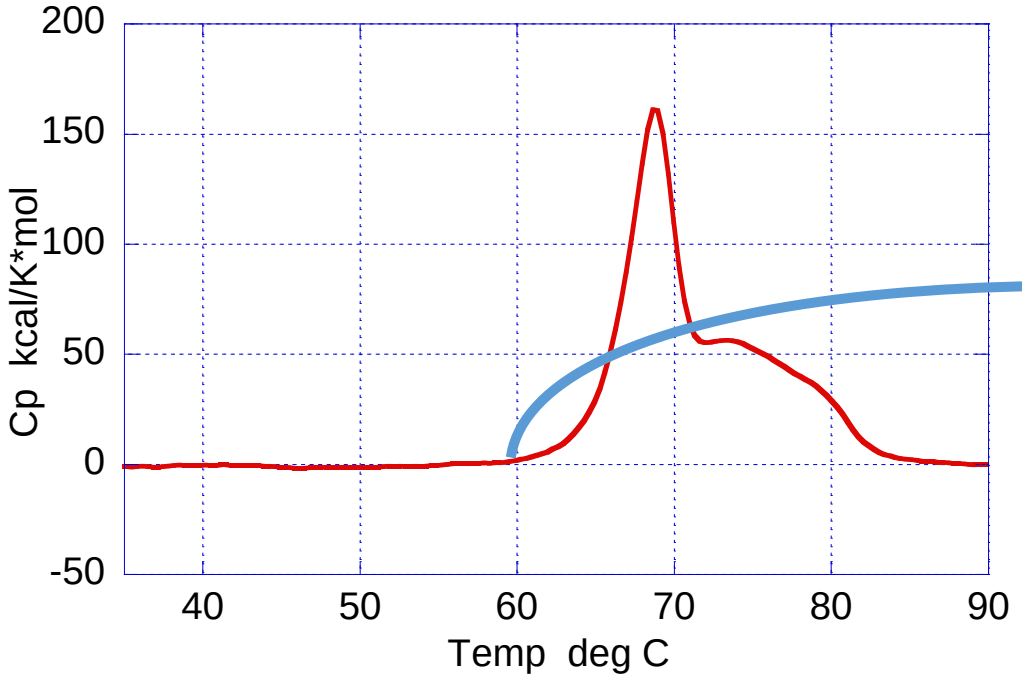
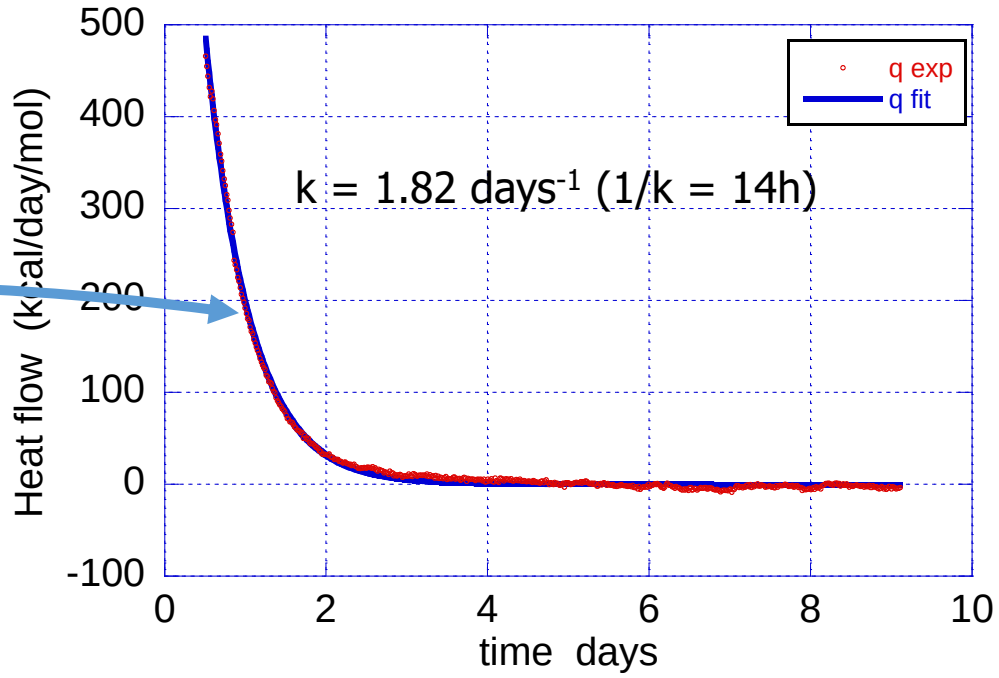
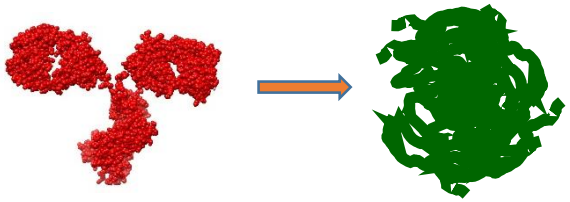
Temperature Denaturation and Aggregation of mAb VRC07



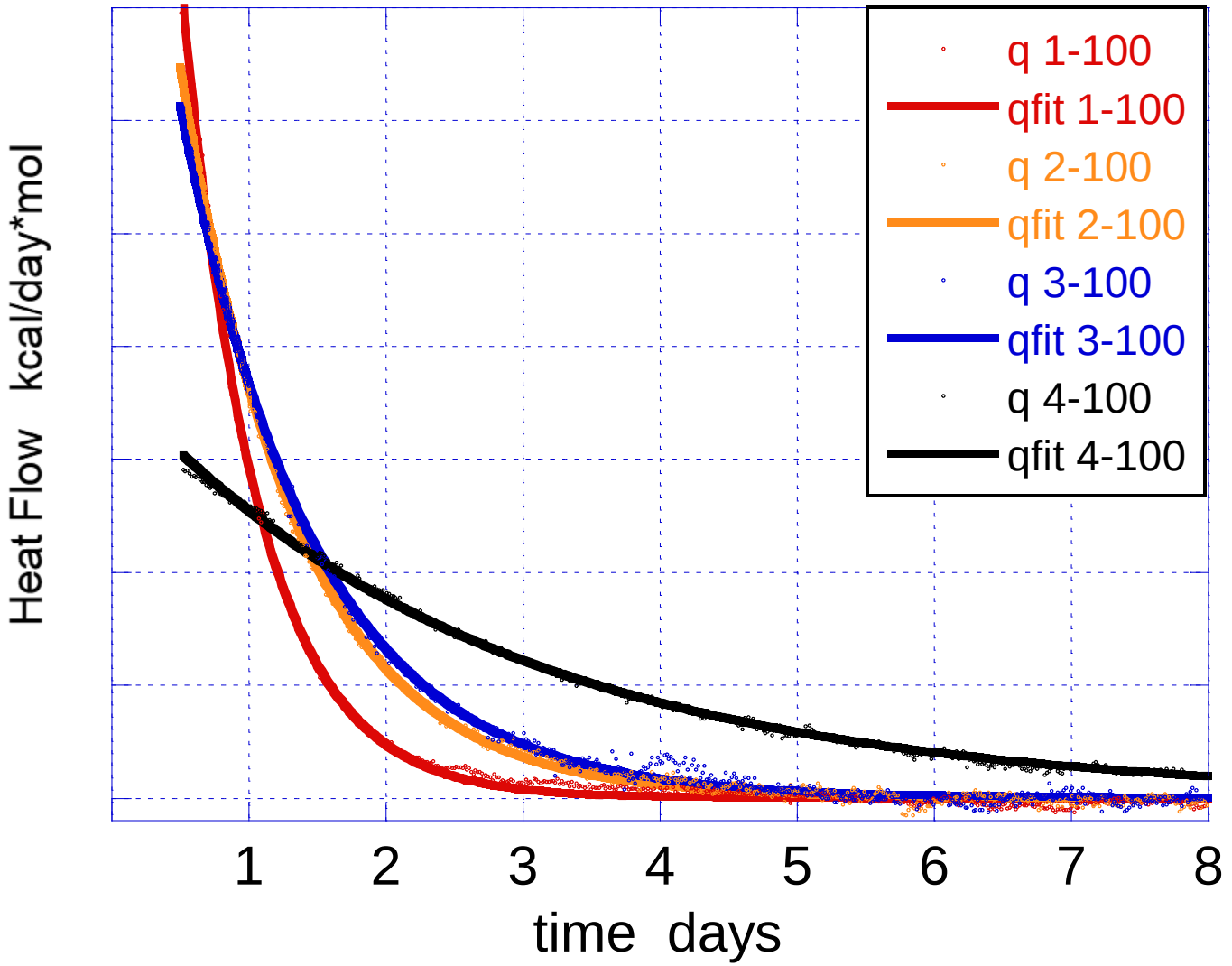
DSC of VRC07



Isothermal Calorimetry at 60°C, 100 mg/mL

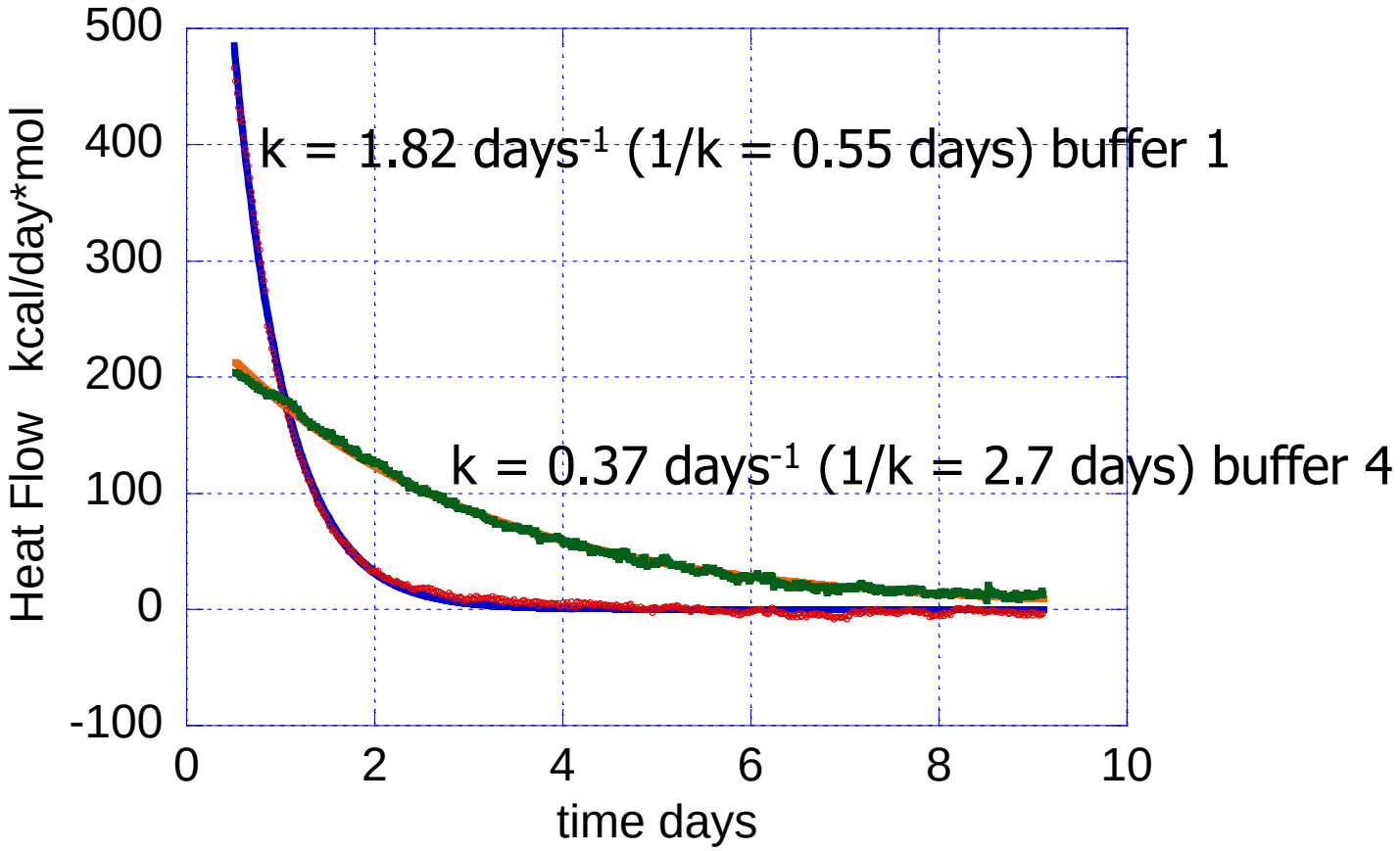


Denaturation/Aggregation Measured by Isothermal Calorimetry (100mg/mL)



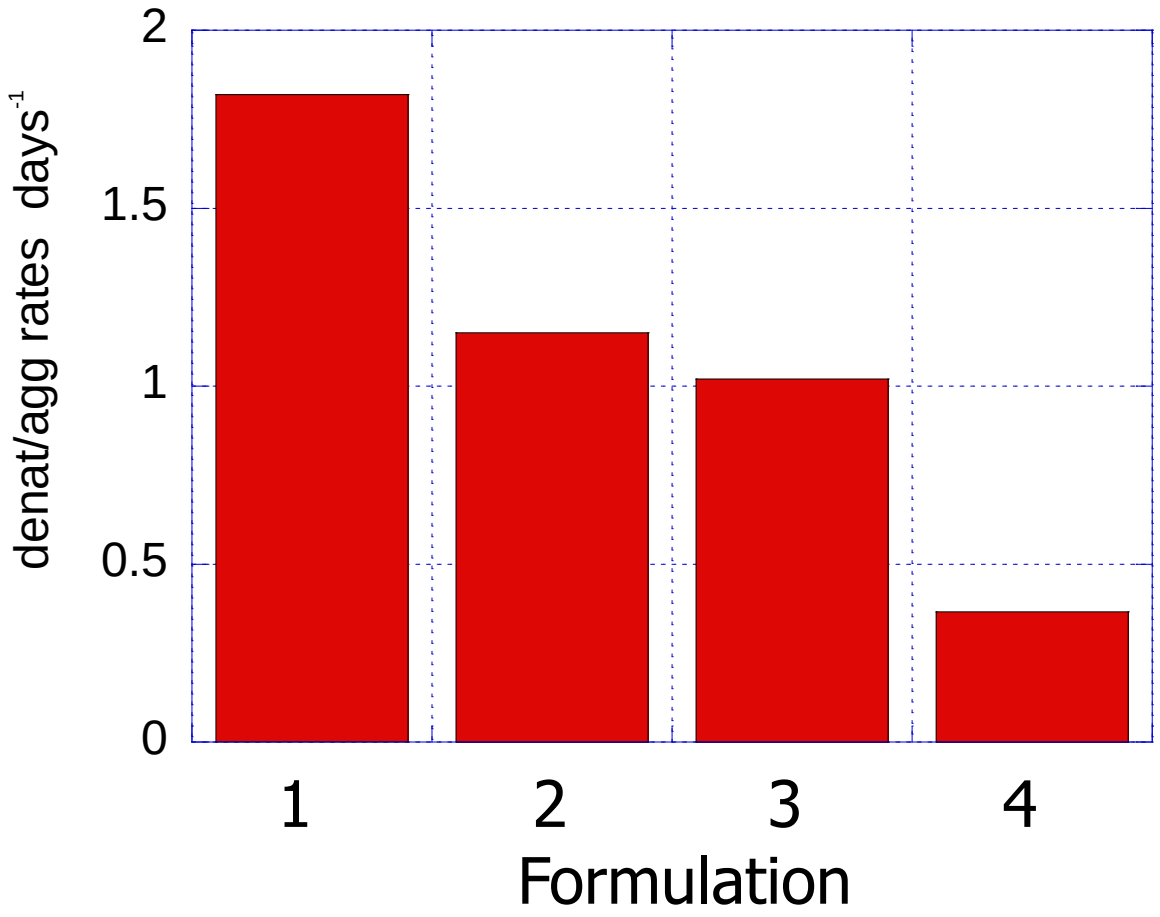
Clarkson et al (2018) Analytical Biochemistry, In Press

Kinetics of Denaturation/Aggregation Buffers 1 and 4 at 60°C (Isothermal Calorimetry Data)



Clarkson et al (2018) Analytical Biochemistry, In Press

VRC07 Denaturation/Aggregation Rates in Different Formulations at 60°C



Discrimination Between Different Formulations

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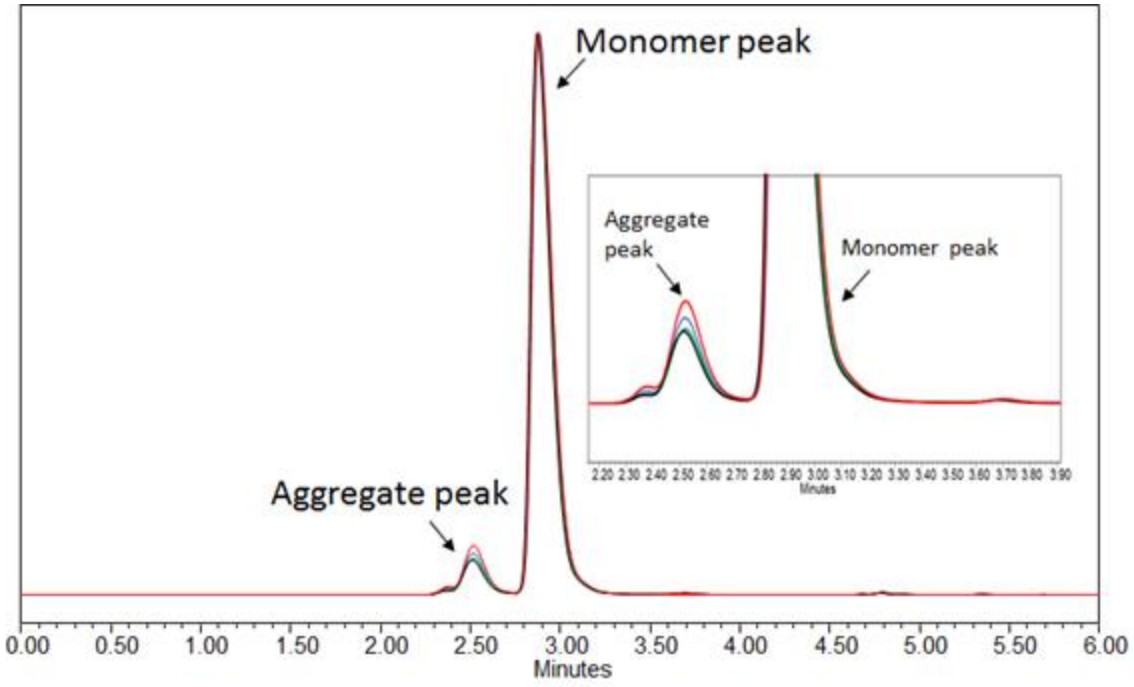
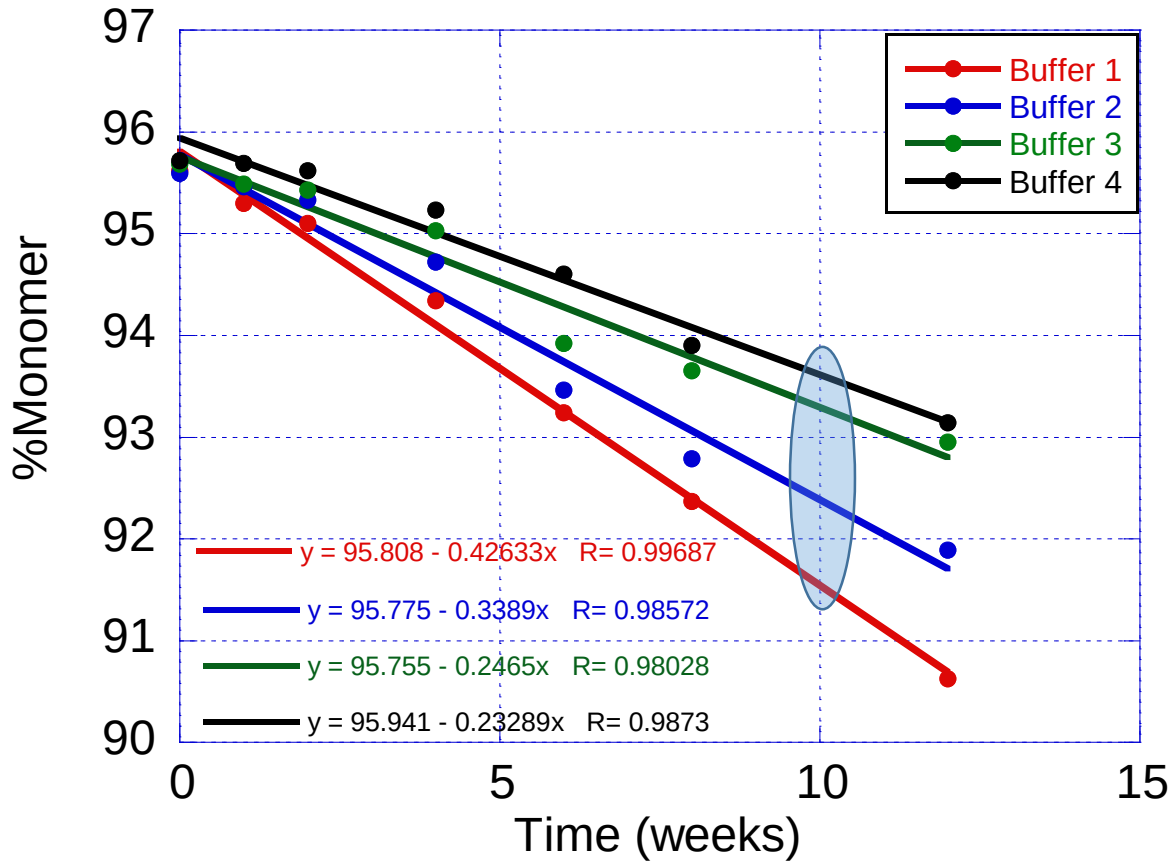
SEC chromatograms of VRC07 in the four formulation buffers after 12 weeks storage at 25°C:

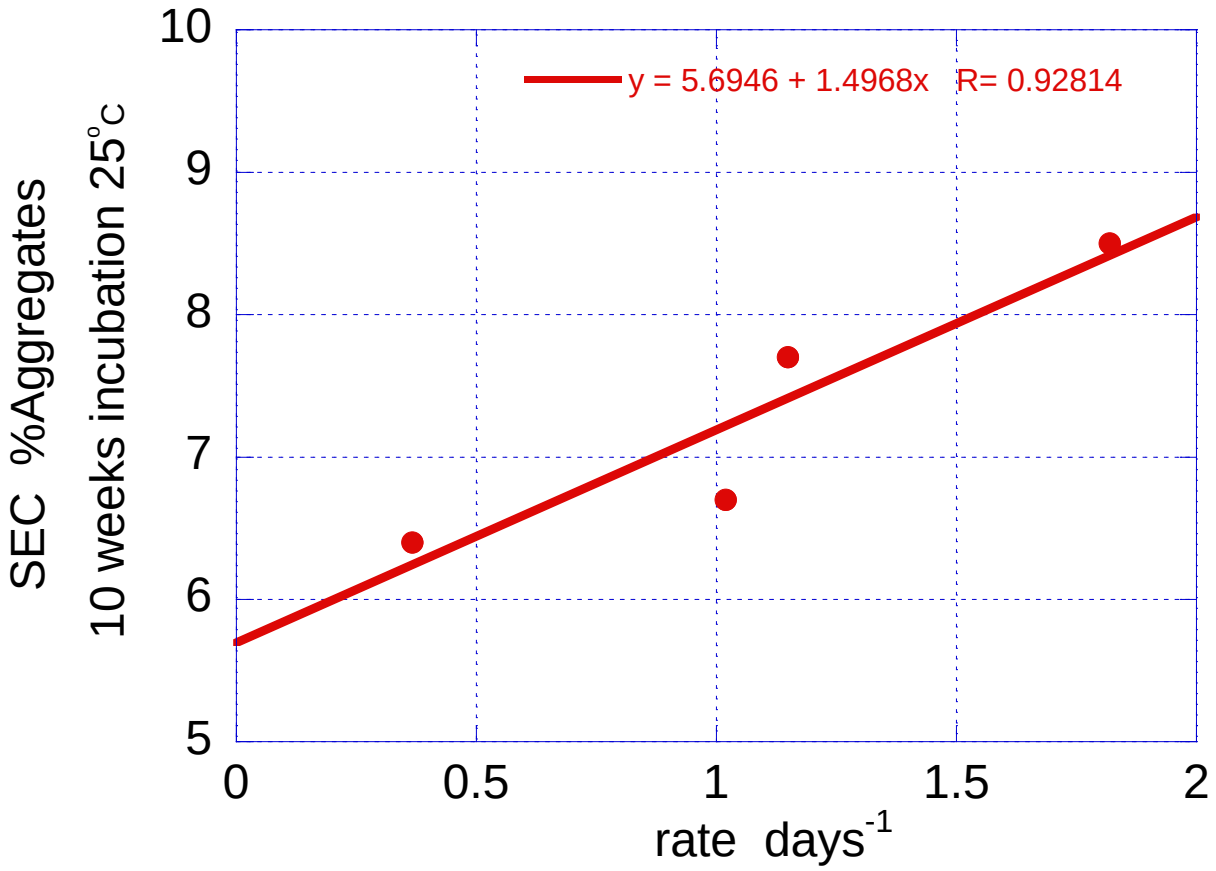


red: buffer 1
blue: buffer 2
green: buffer 3
black: buffer 4

SEC Data for Four Different Formulations at 25°C



Correlation Between SEC and Isothermal Calorimetry Rates



Conclusions

- Kinetic Deconvolution of DSC: Activation enthalpies for each denaturation peak. Temperature dependence of denaturation rates. Combined analysis of ΔH^* and Tm allows selection of best formulation.

- Isothermal Calorimetry: Rates of denaturation/aggregation at constant temperature can be determined in one week. Correlates well with SEC data and can be used to assess quality of antibody or formulation.

For additional information or to discuss a collaboration please contact E. Freire at ef@jhu.edu