Use of ITC/DSC for the studies of biomolecular stability and interaction of excipients with proteins

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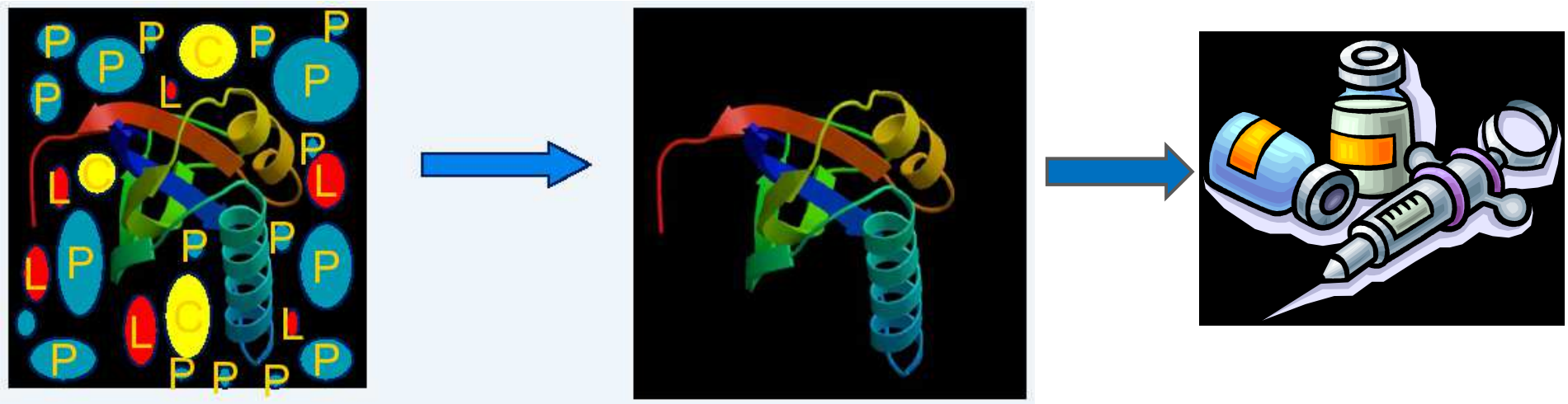
Molecular Interactions in Biopharmaceutical Formulations:
Can stability be rationalised and predicted?
Tuesday 30th October 2012 Trinity Centre, Cambridge, UK

MIBio 2012

Outline

- Brief intro to the specifics of protein stability profiling and optimization
- Key benifits of the application of lable-free biophysical technologies
- Introduction into microcalorimetric technology principles and the kind of information they can provide
- Examples on the use of DSC and ITC

Complex task of protein stabilization



Chemical stress (pH, ionic strength, etc.)

Physical stress (UF/DF, temperature)

Freeze-thaw-induced stress

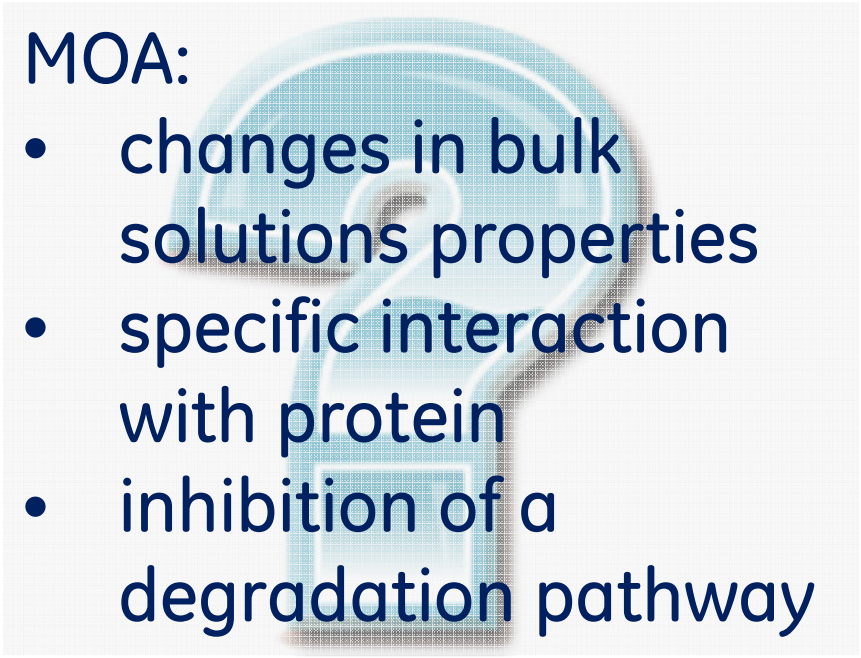
Numerous choices of excipeints available for empirical screening

Excipient types

- ✓ Buffering agents
- ✓ Amino acids
- ✓ Osmolytes
- ✓ Sugars and carbohydrates
- ✓ Proteins and polymers
- ✓ Salts
- ✓ Surfactants
- ✓ Chelators and antioxidants
- ✓ Preservatives
- ✓ Specific ligands

MOA:

- changes in bulk solutions properties
- specific interaction with protein
- inhibition of a degradation pathway



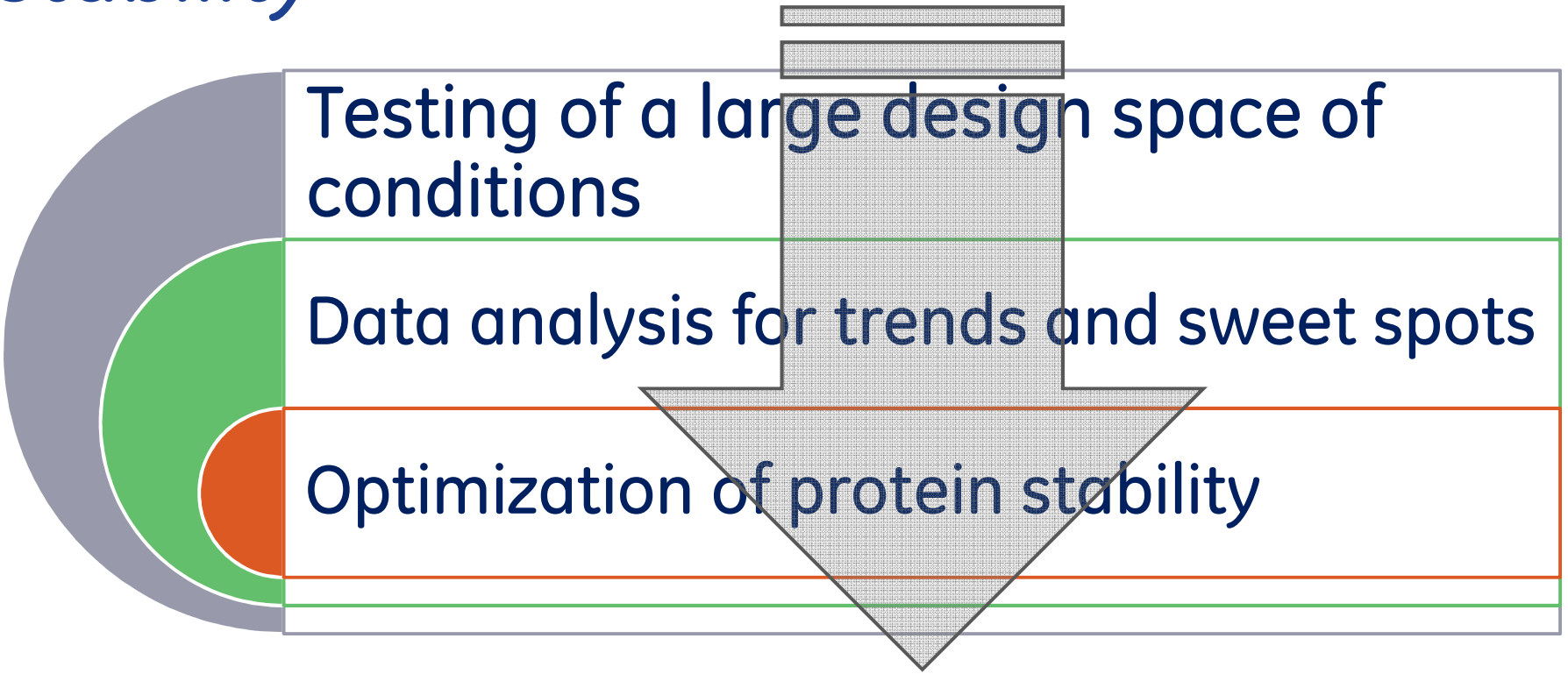
Empirical vs rational approaches

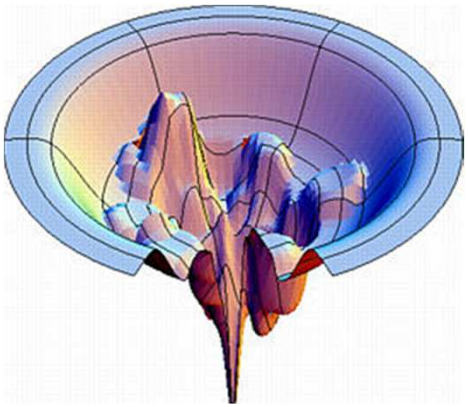
Proteins exhibit a wide range of degradation phenomena.

Protein stability depends on many parameters.

Profiling and optimization of protein stability remains a combination of trail-and-error and rational approaches.

Profiling and optimization of protein stability





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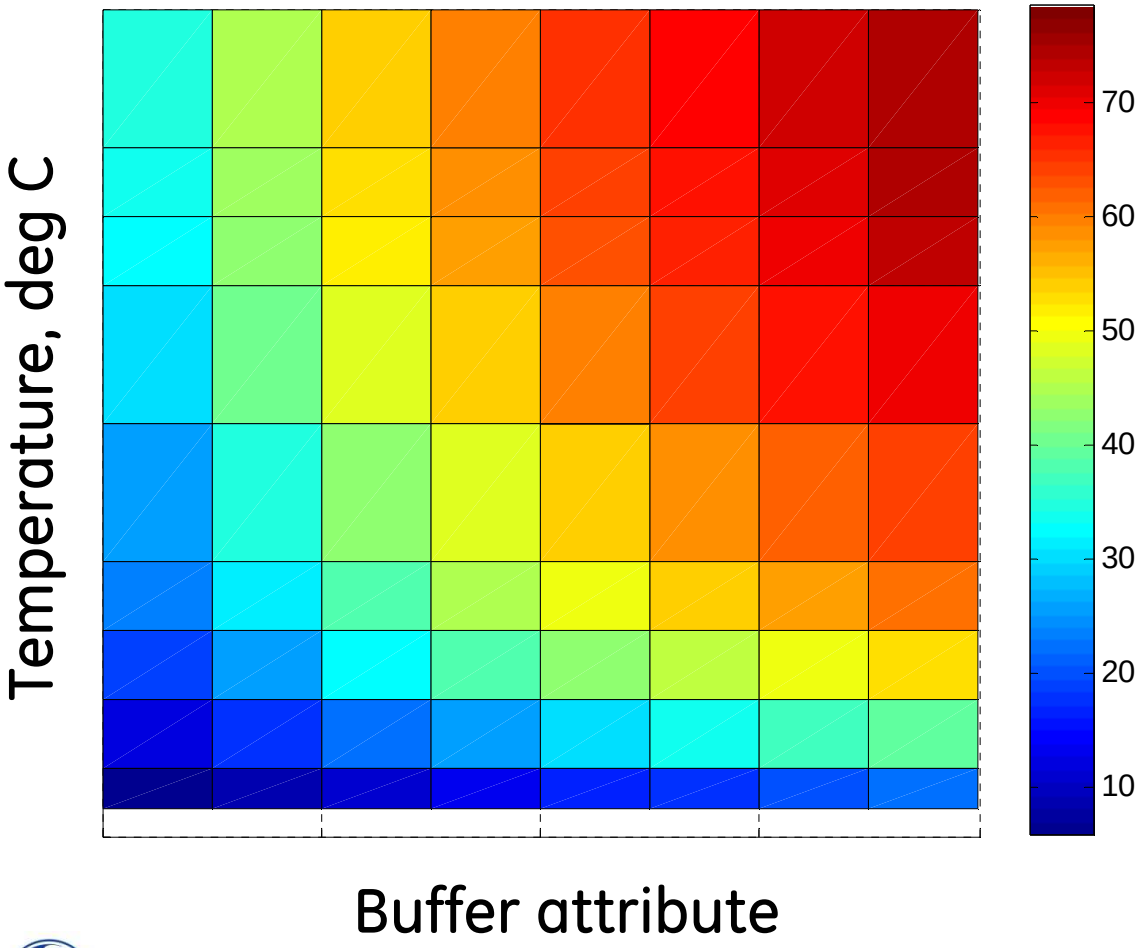
Looking for trends and sweet spots: Empirical phase diagram



Understanding of factors (intrinsic and extrisic) critical to a protein stability on the molecular level is needed for implementaion of the rational approach to optimization of protein stability

Need for orthogonal approach: No single technique works well in all cases

Every single technique has limitations associated with the underlying measuring principles. The results can be model and method dependent.

Convergence of evidence from several techniques is often needed to gain insights into conditions critical to protein stability and to deduce the mechanism of protein-excipient interaction

Biophysical techniques

Upsides of biophysical techniques:

- first principle data produced with fewer assumptions and simplifications
- possibility to cross-correlate experimental findings to assure their reliability
- better control over experimental parameters and the reacting species ▸ better possibility to eliminate artifacts

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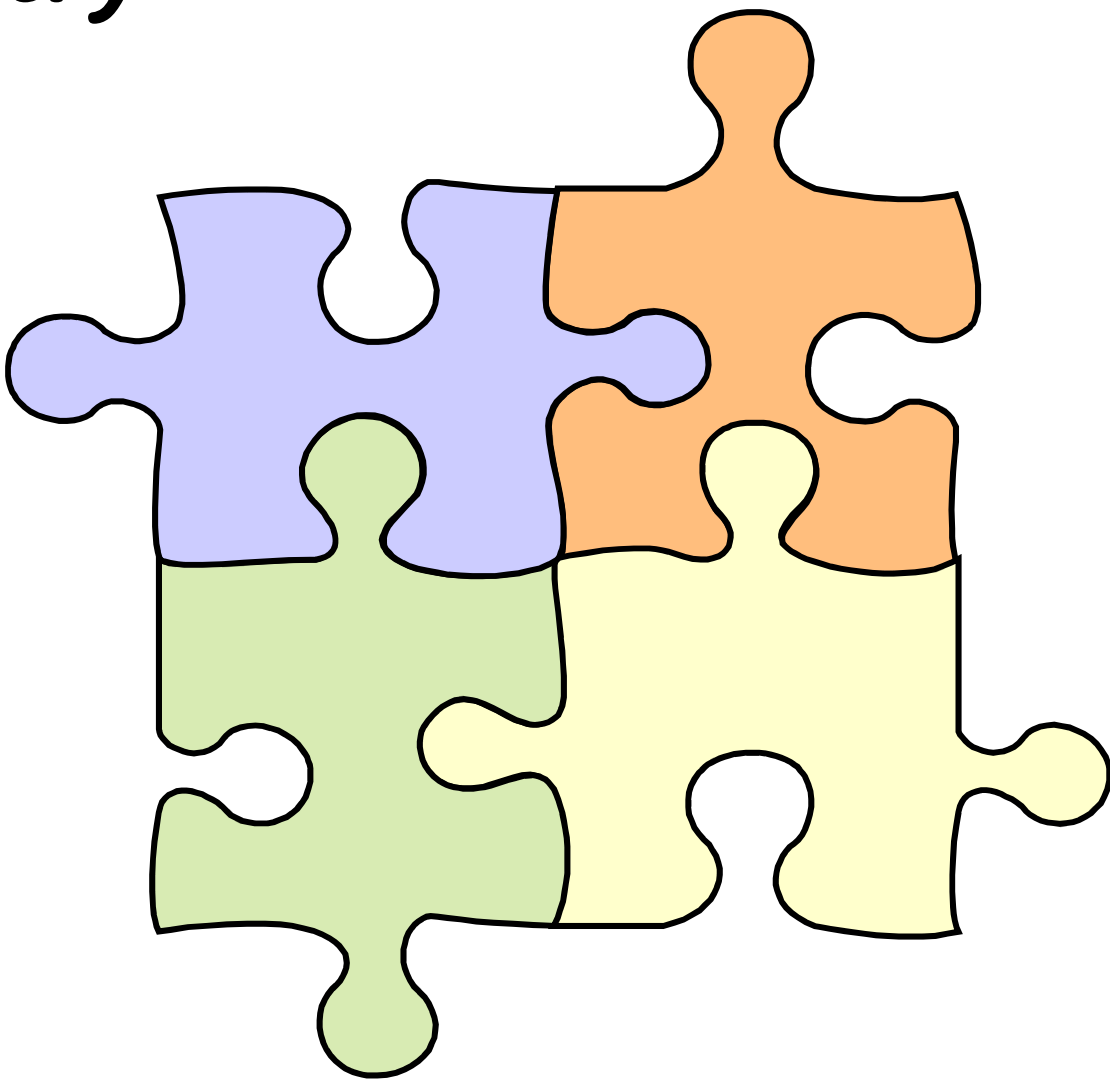
Microcalorimetry



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Microcalorimetry

ITC, Isothermal Titration Calorimetry

- Heat of interaction is measured
- Binding reaction of two components
- One temperature

DSC, Differential Scanning Calorimetry

- Thermal denaturation of protein is monitored
- Thermal stability of proteins at different conditions (buffers, excipients, adjuvants)
- Temperature is ramped

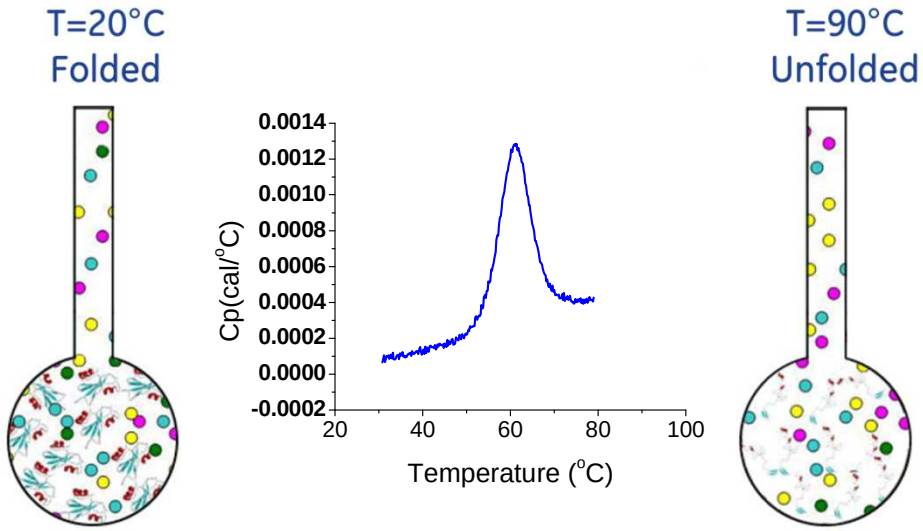
MicroCal™ systems

Differential scanning calorimetry (DSC) delivers information on protein stability

Isothermal titration calorimetry (ITC) allows to assess binding affinity and stoichiometry in one simple assay

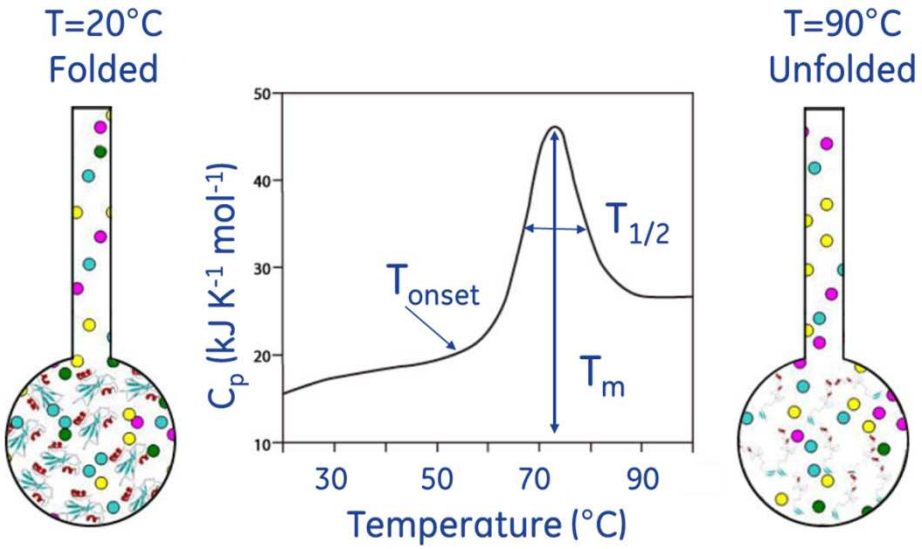
What does DSC actually measure?

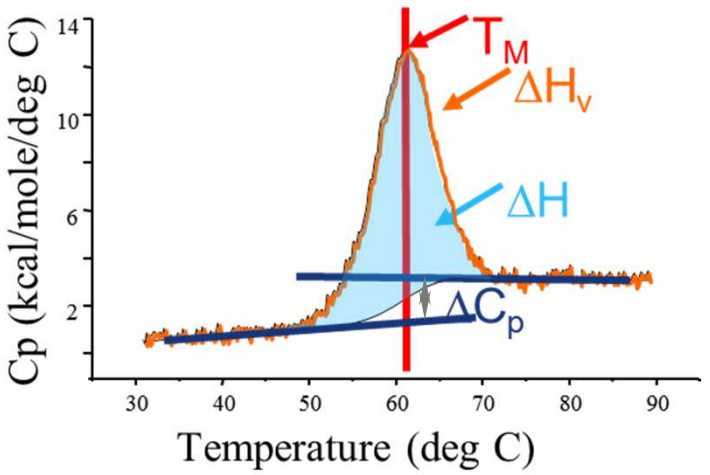
- Measures heat capacity change associated with thermal unfolding
- Provides a thermogram which is a qualitative and quantitative fingerprint of protein unfolding profile



DSC thermogram provides multiple descriptors of protein thermal stability

- T_m is the thermal transition midpoint
- ΔH, ΔH_{vH}, ΔC_p
- T_{onset} - unfolding start
- $\Delta T_{1/2}$ - homogenity of population
- Domain resolution
- Assessment of oligomerization and aggregation propensity
- Percent reversibility of the transition
- Kinetics of irreversible process





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Advantages

- Generic: almost all transitions have an enthalpy change
- Domain resolution
- Broad dynamic range of attainable temperatures, scan rates (10÷240 ^{o}C/h) and possibility for re-scans
- Temperature resolution to 0.2 ^{o}C
- No optical probes
- No labelling, allows to study sample as it is
- Can run on turbid solutions

DSC applications

DSC can be used to assess:

- Thermodynamic and kinetic stability
- Shelf life/stability
- Contribution of different groups to the stability
- Effect of buffer and storage condition
- Protein-ligand interaction

MicroCal VP-Capillary DSC System

Automated system
medium throughput
screening

Up to 50 samples/
day; down to ~50 µg
of a protein per scan

96-well plate
format

Auto VP-Capillary
DSC
130 µl cell

✓ Measures the temperature (Tm) associated with a thermal unfolding of a protein

✓Tm is an indicator of stability

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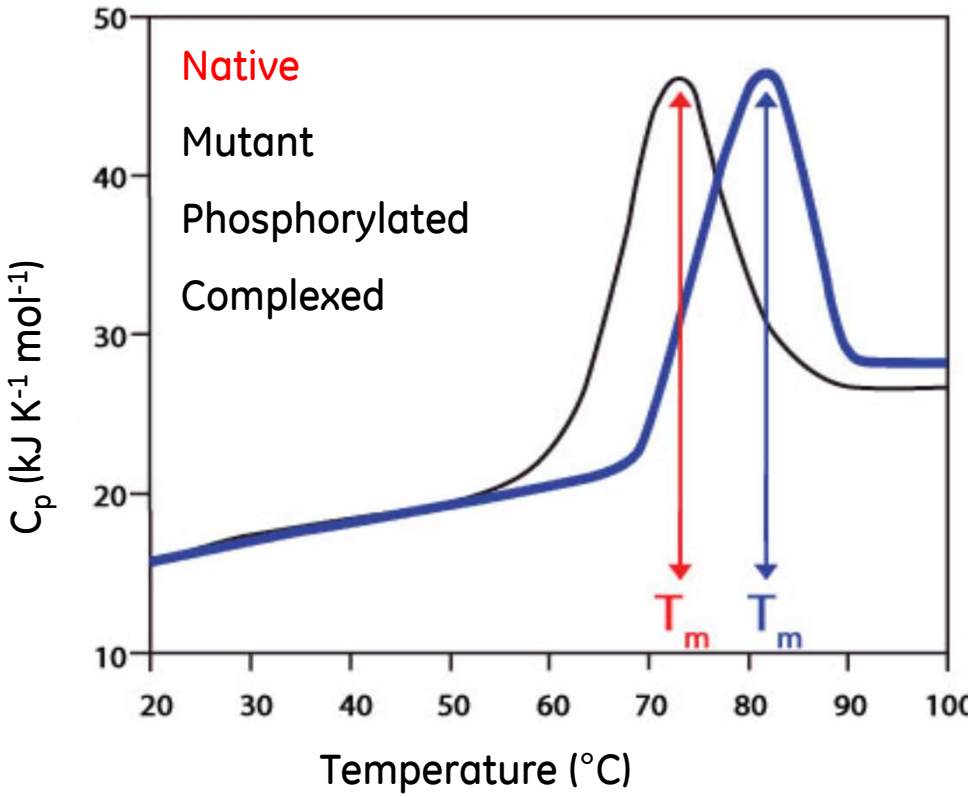
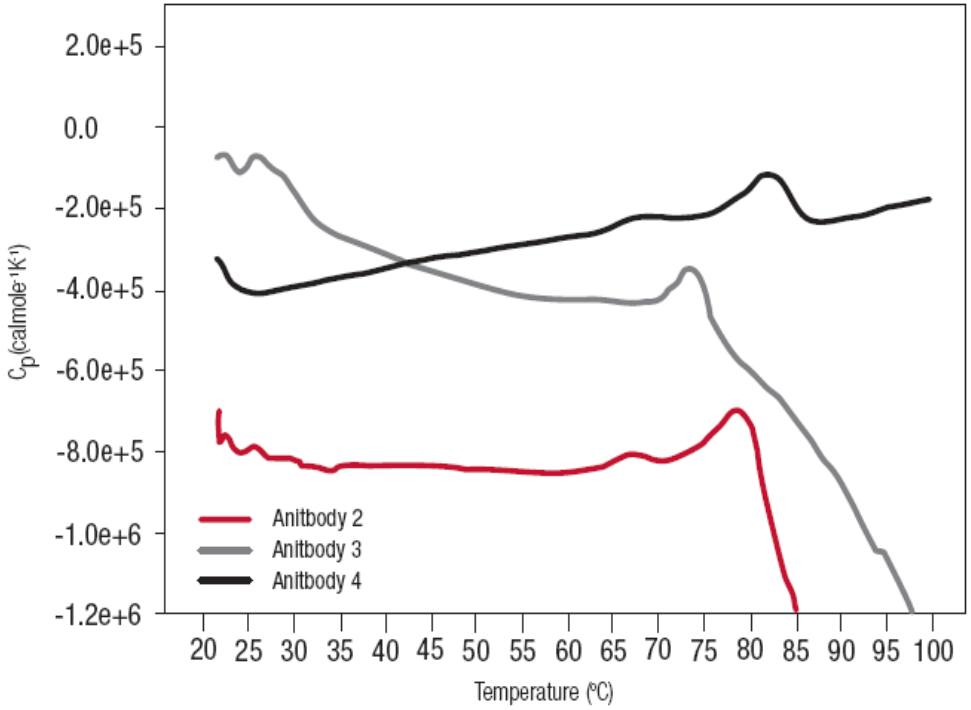
Concentration Requirements

- ✓ Minimum concentration 0.02 mg/ml (~10 μg)
- ✓ As starting point min 0.1-0.2 mg/ml
- ✓ Maximum concentration 5 - 10 mg/ml

Comparison of native, altered and mutant forms



Construct selection: IgG1 Thermal Stability



Antibody 3 would be removed from the drug development pipeline

MicroCal Application Note, F. Ollila, 2004

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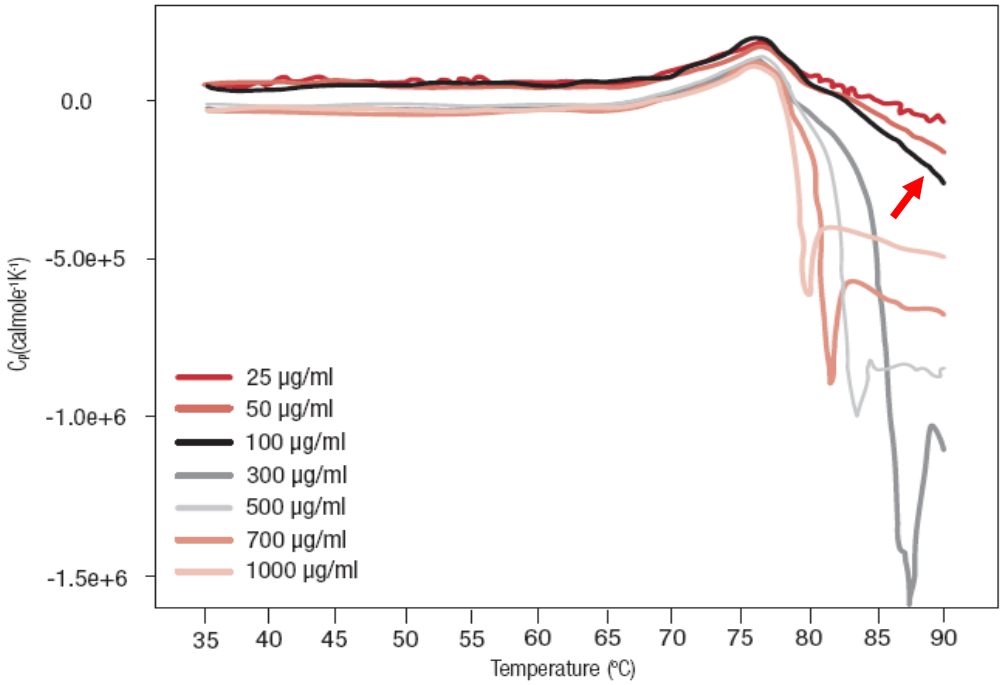
Colloidal stability at different concentrations: IgG1 Thermal Stability



MicroCal Application Note, F. Ollila, 2004

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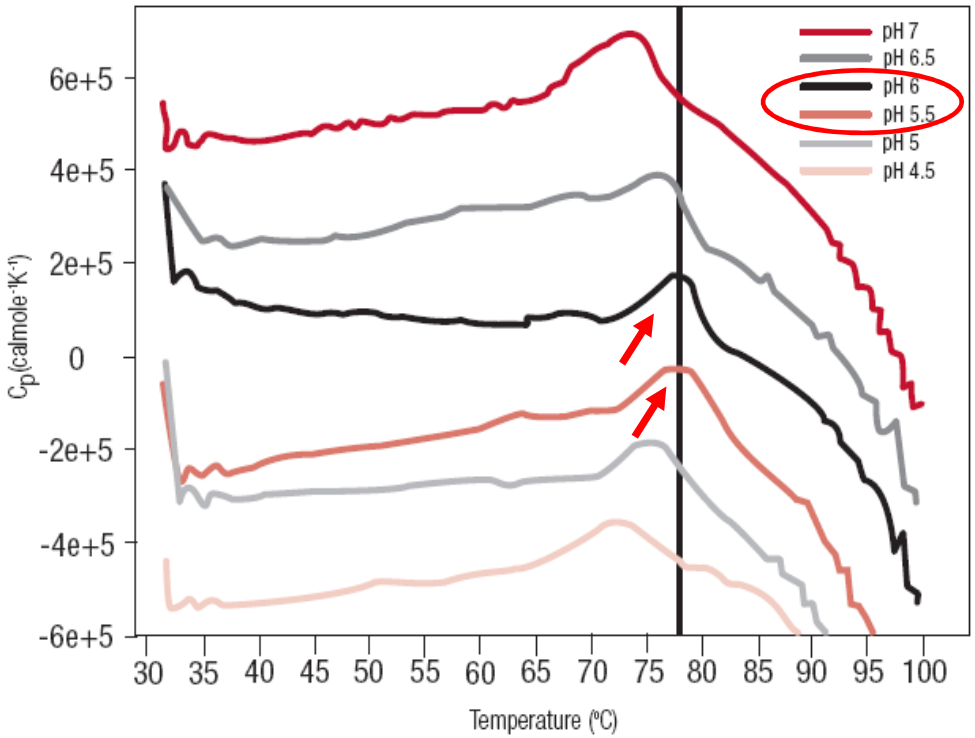
Screening different pHs:
IgG1 Thermal Stability



Best pH 6 or 6.5

MicroCal Application Note, F. Ollila, 2004

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How Does DSC Compare?

Results from a pH primary screen of a therapeutic antibody

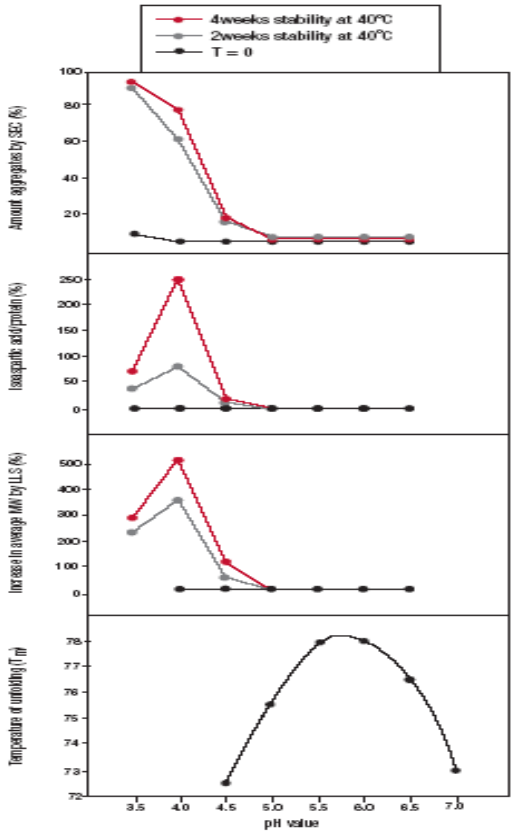
DSC was the most accurate and fastest predictor of suitable formulations

Size Exclusion Chromatography

Isoaspartate Formation CE

Laser Light Scattering

DSC T_M



Red=4 weeks at 40 ^{0}C
Grey=2 weeks at 40 ^{0}C
Black=T=0

MicroCal Application Note, F. Ollila, 2004

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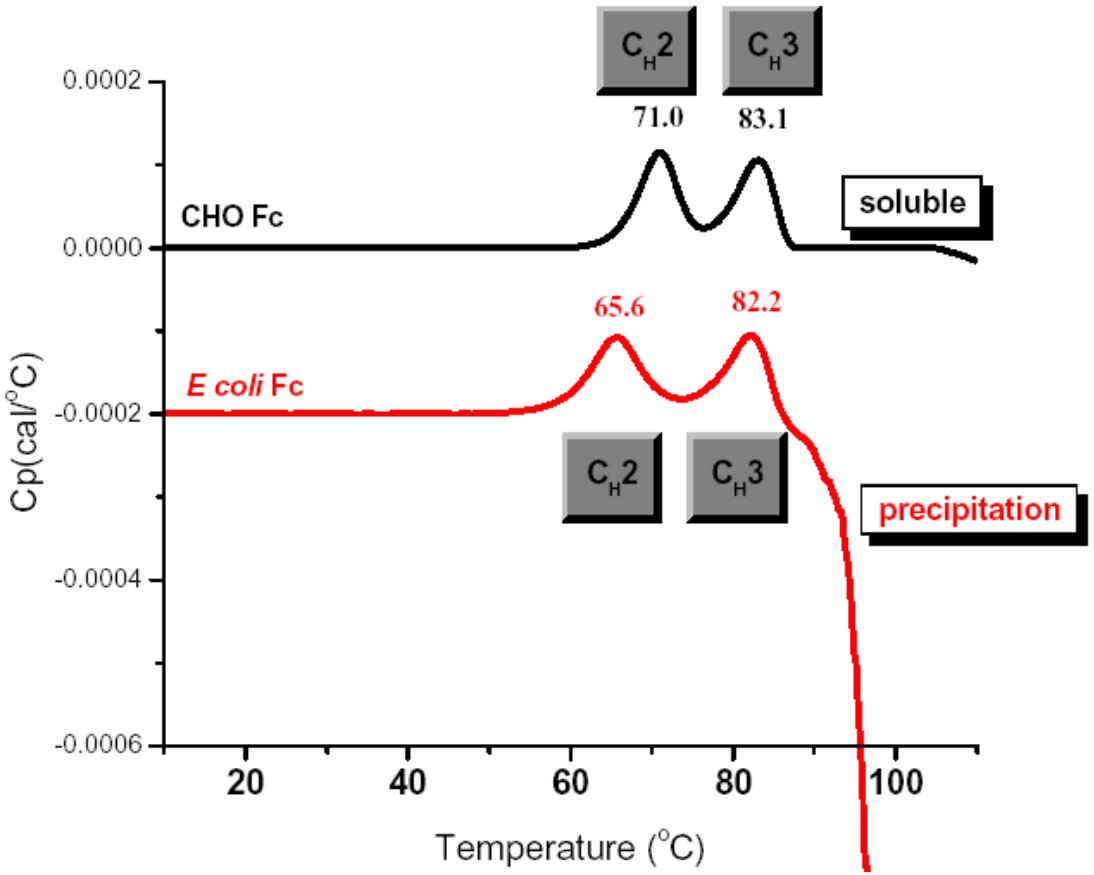
Sugar effect on stabilization of Fc domains

Two different protein expression systems



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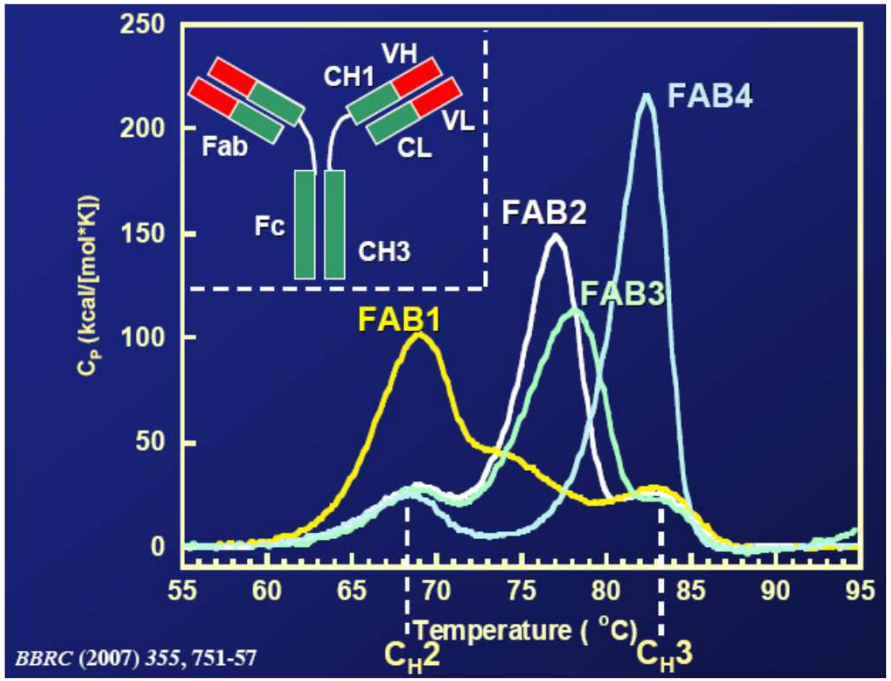
Stability contributions of individual domains identified

Individual domains are often masked

DSC uniquely identified individual stability contribution

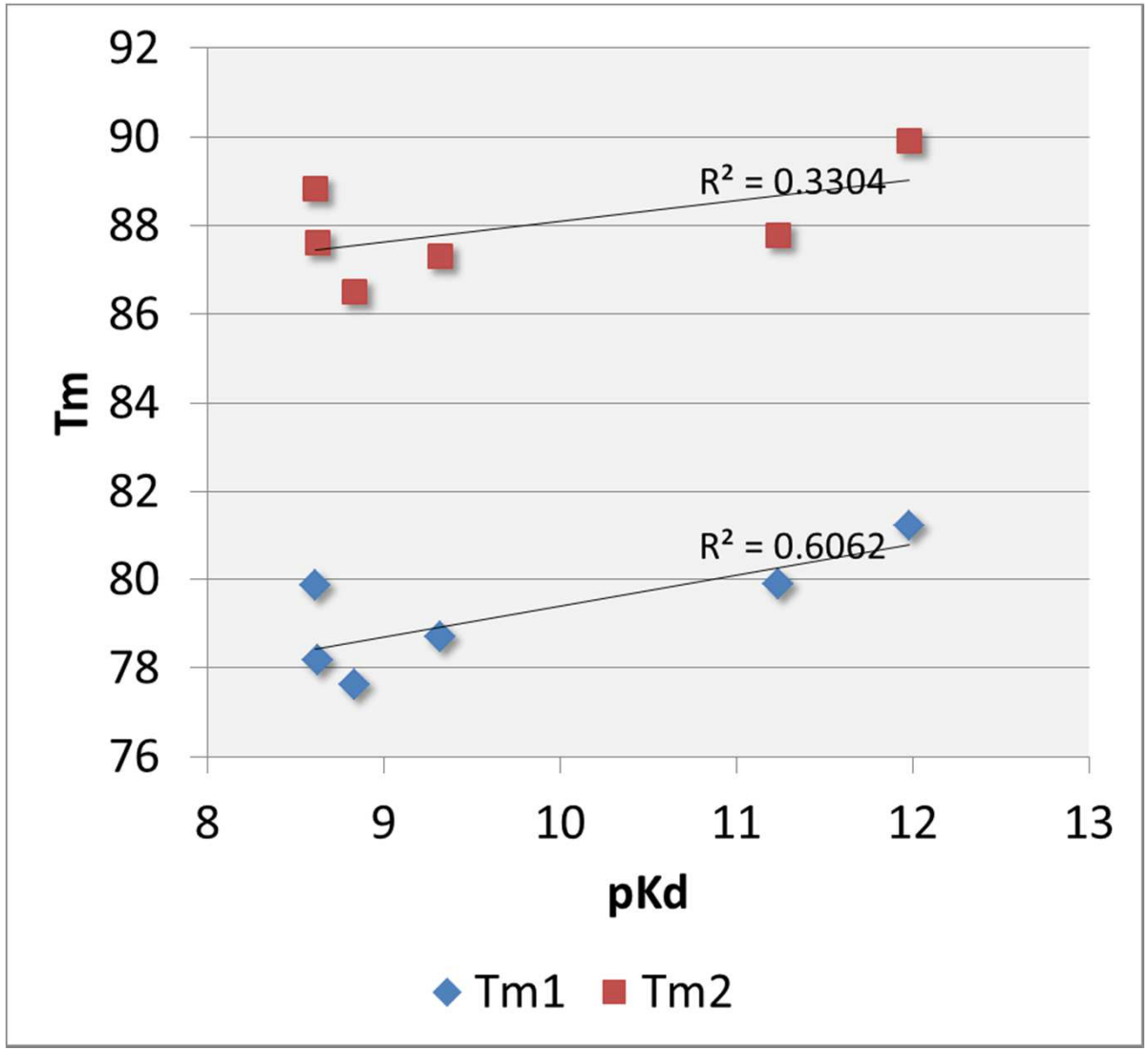
Increased stability correlates with increased functional protein fraction



Garber and Demarest, BBRC 355, 751-757 (2007); Demarest et al, MicroCal Application Note (2008)

mAb binding affinity to anti-Fc Ab correlates best with Tm1

Binding affinity determined with Biacore™ T200.



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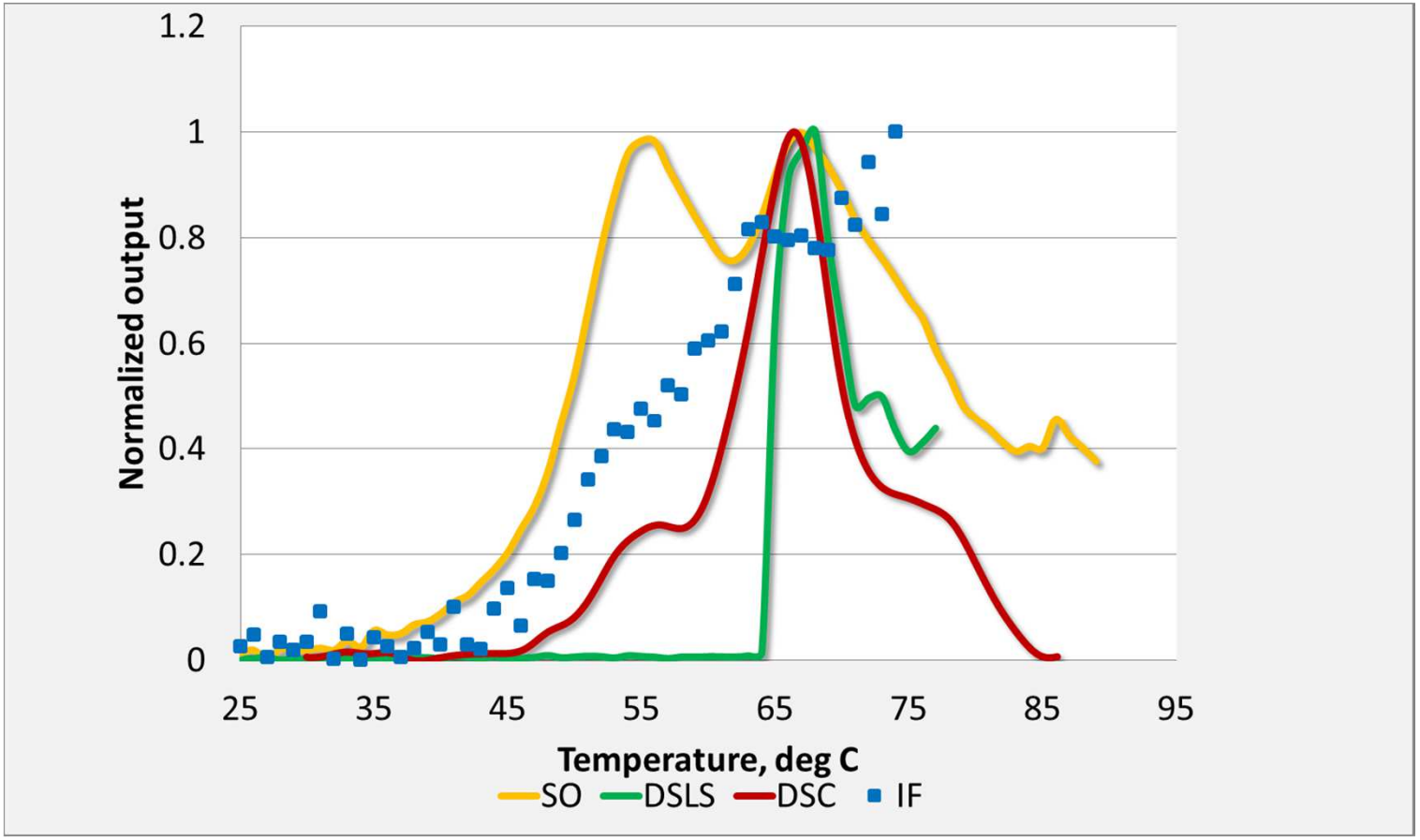
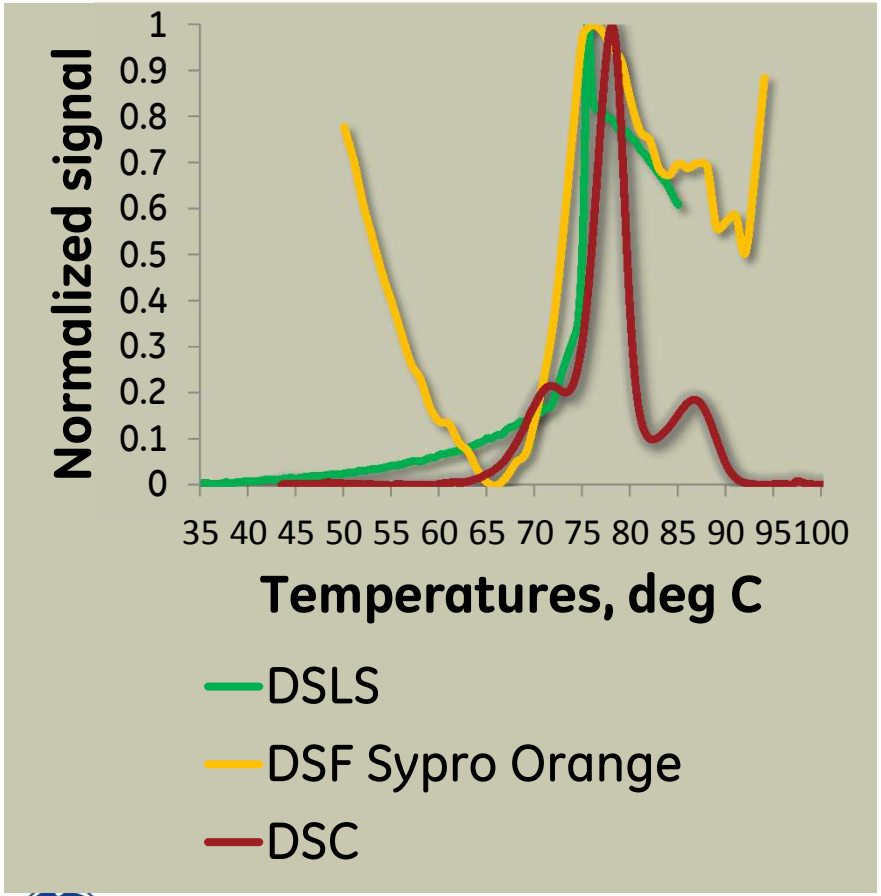
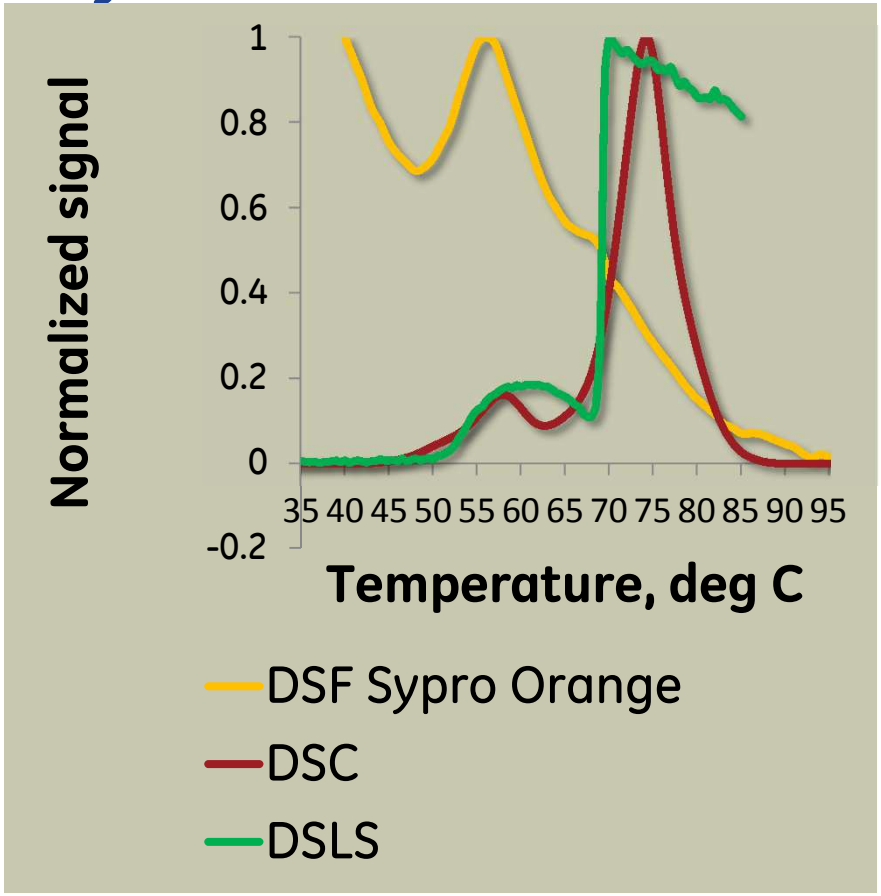
Domain resolution with different thermal stability techniques



Robustness of baseline definition and domain resolution differs between thermal stability assays





Addressing complexity with DSC

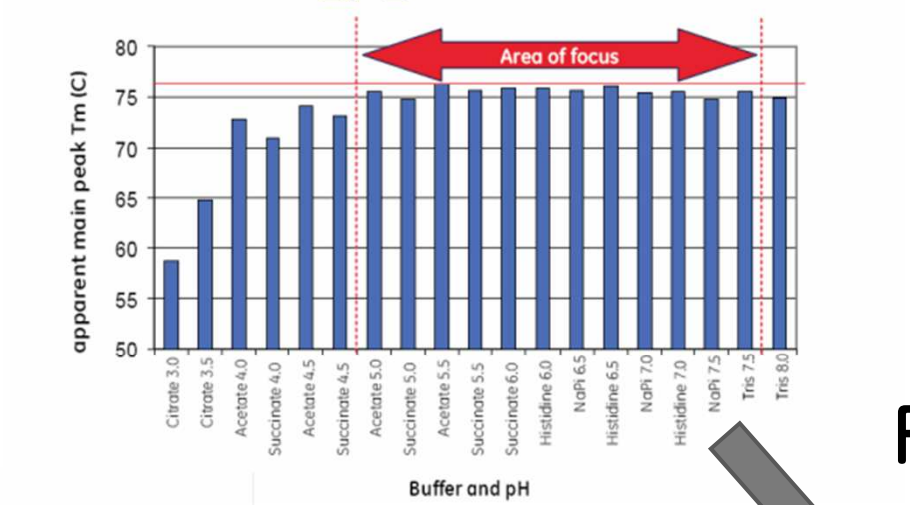
Individual domain stabilization

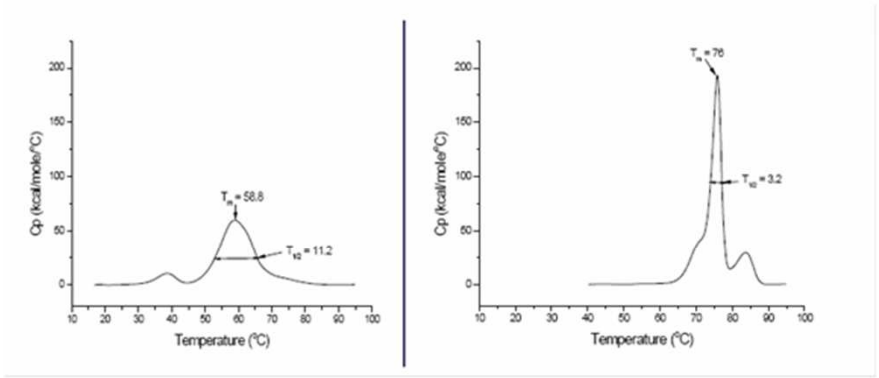
Using multiple descriptors

Using thermogram as a fingerprint

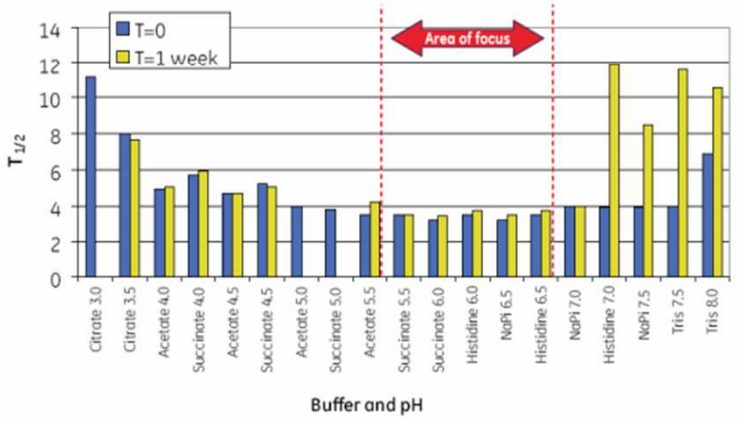
Pre-formulation decisions funnel utilizing Tm and $\Delta T_{1/2}$

T_m





Focus with $\Delta T_{1/2}$



These data were kindly provided by Dr Kathrine E. Bowers, Diosynth Biotechnology, a part of Schering Plough Corporation.

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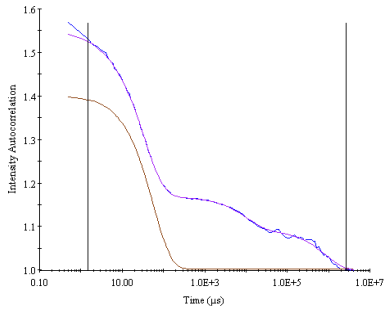
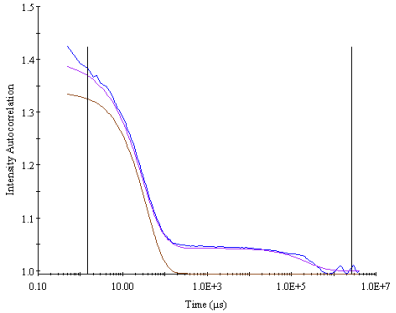
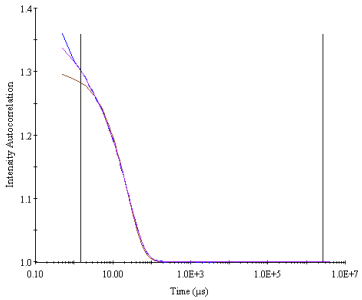
Thermogram shape: indication of heterogeneity of higher order structure



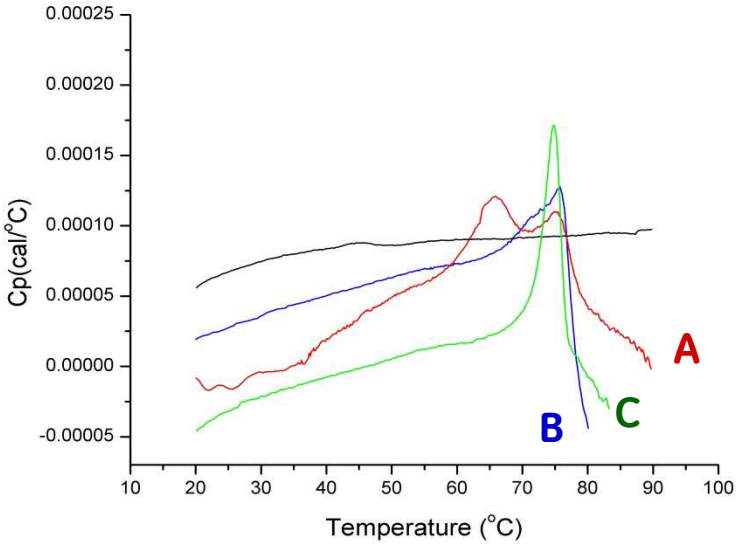
Batch A



Batch B



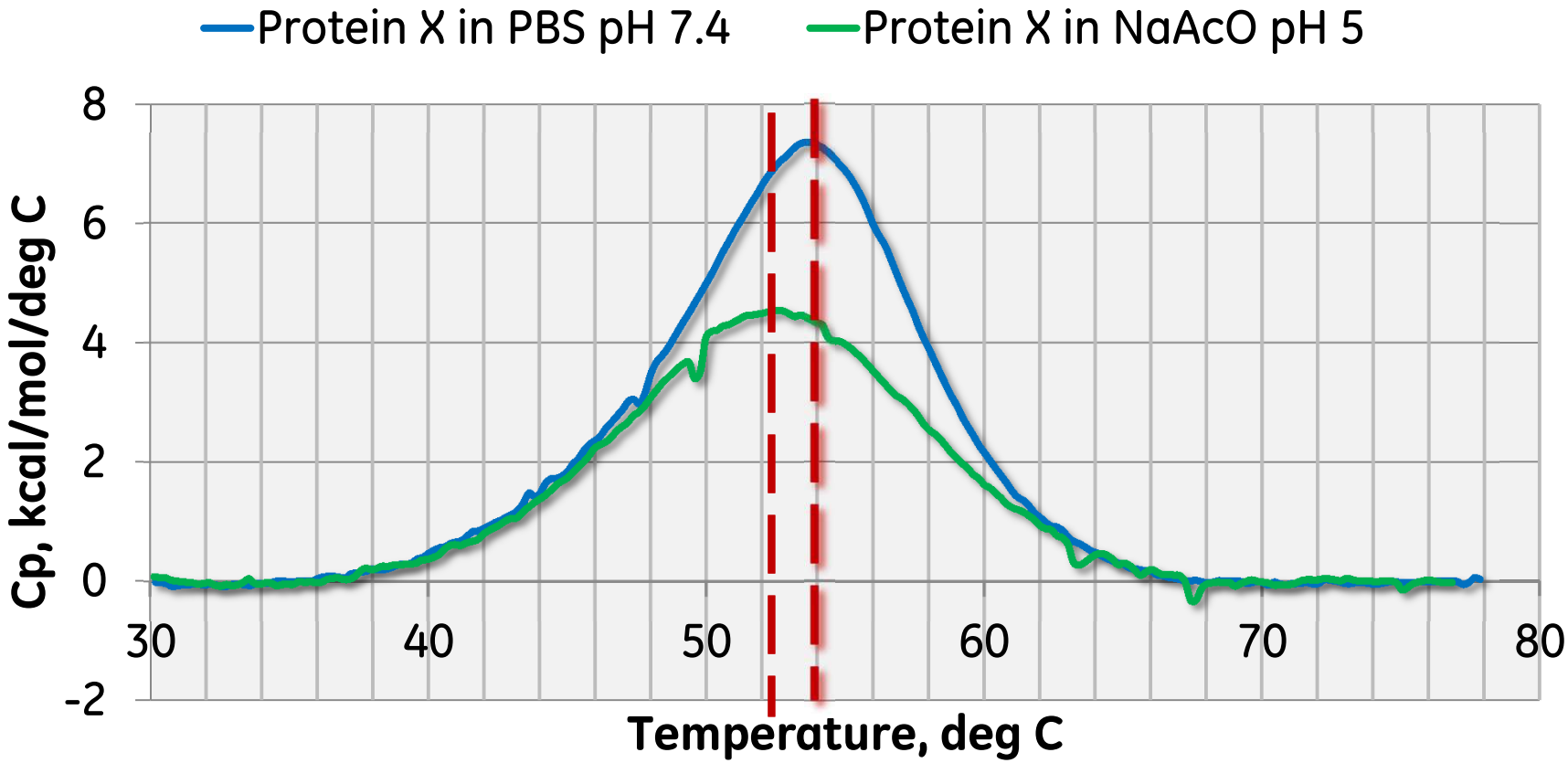
Batch C



DLS and DSC:

- Batch A: conformational heterogeniety, thermal lability.
- Batch B: decreased heterogeneity.
- Batch C: homogenous and most stable.

Thermogram shape: indication of heterogeneity. On-going dissiciation of protein oligomers?



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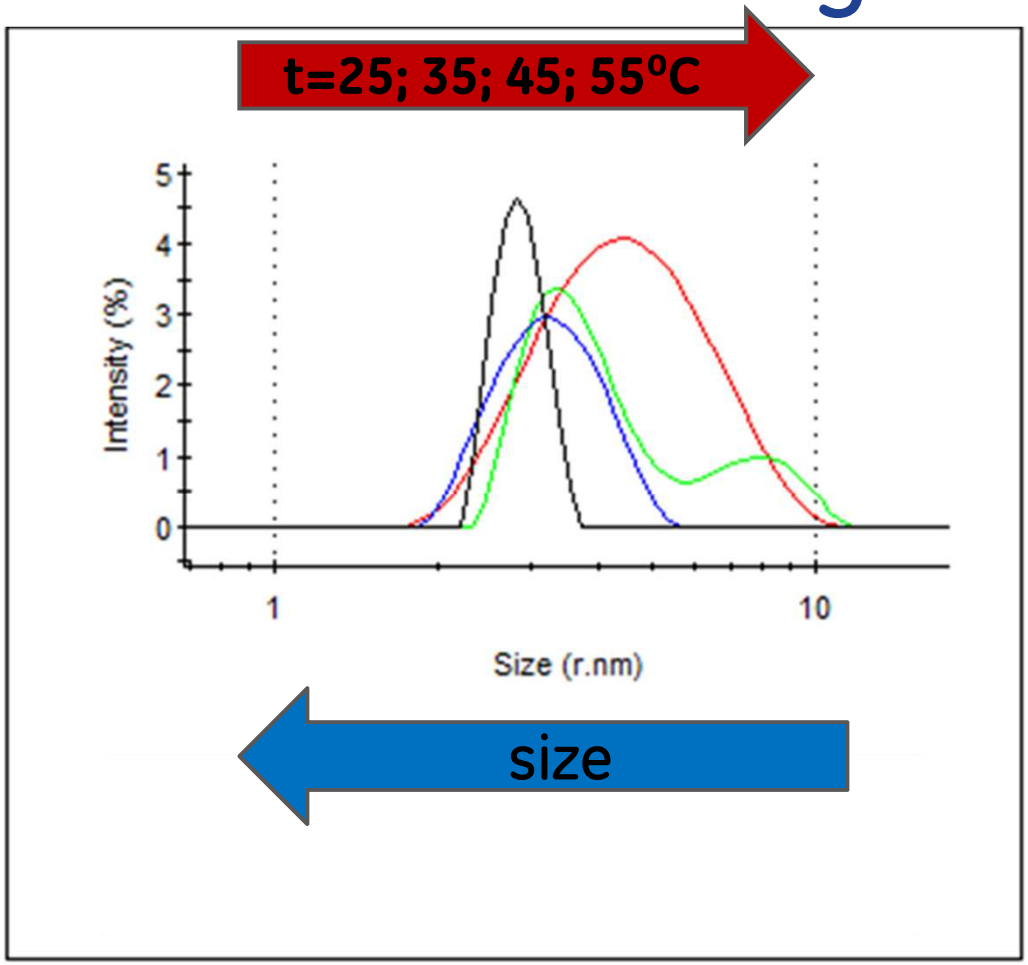
Convergence of DLS and DSC data: in PBS Protein X undergoes temperature induced dissociation of oligomers



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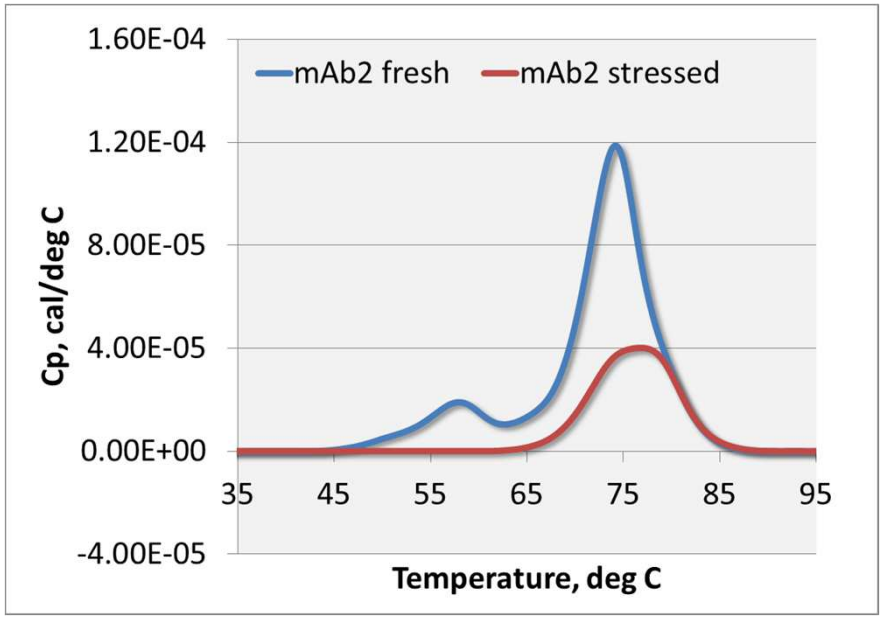
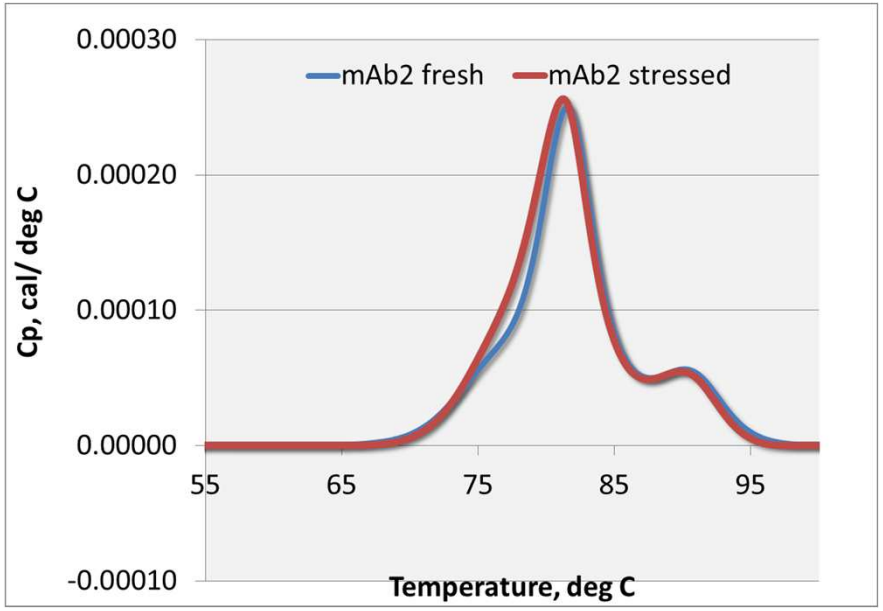
Thermogram shape and area as means to assess protein viability

Buffer #1



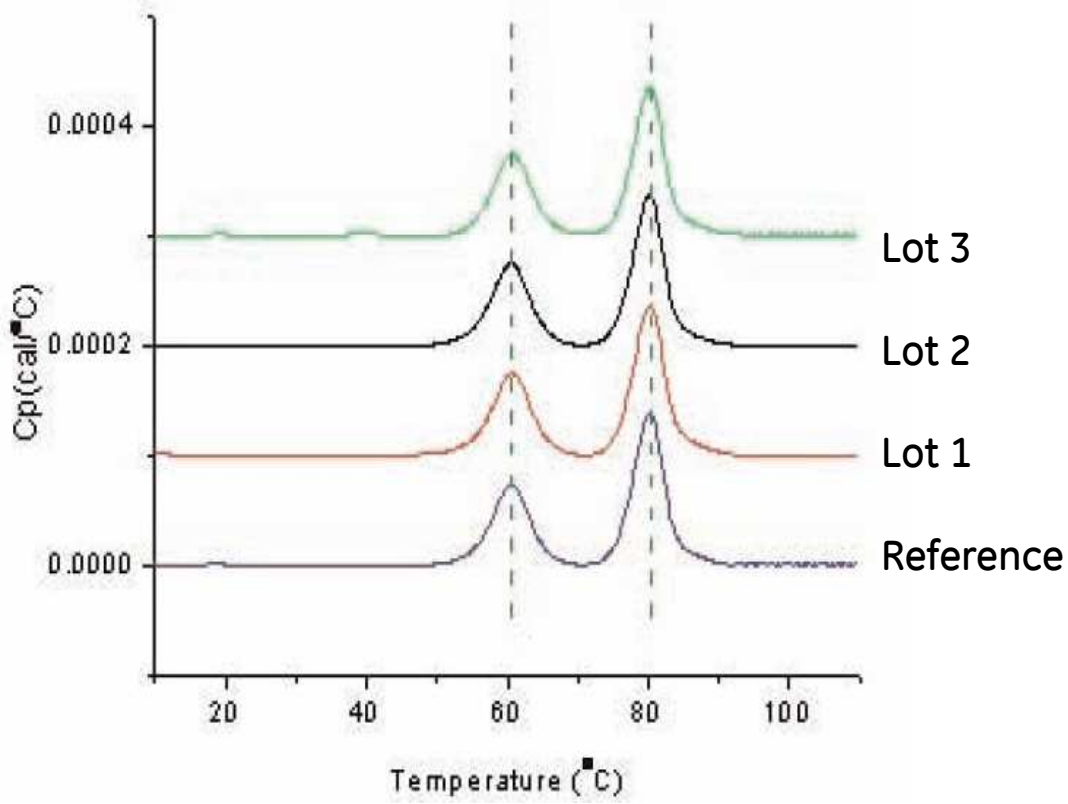
Buffer #2



Easily Assess Biocomparability with DSC

Three lots manufactured at different sites

DSC verifies no difference in stability and solubility betweer lots



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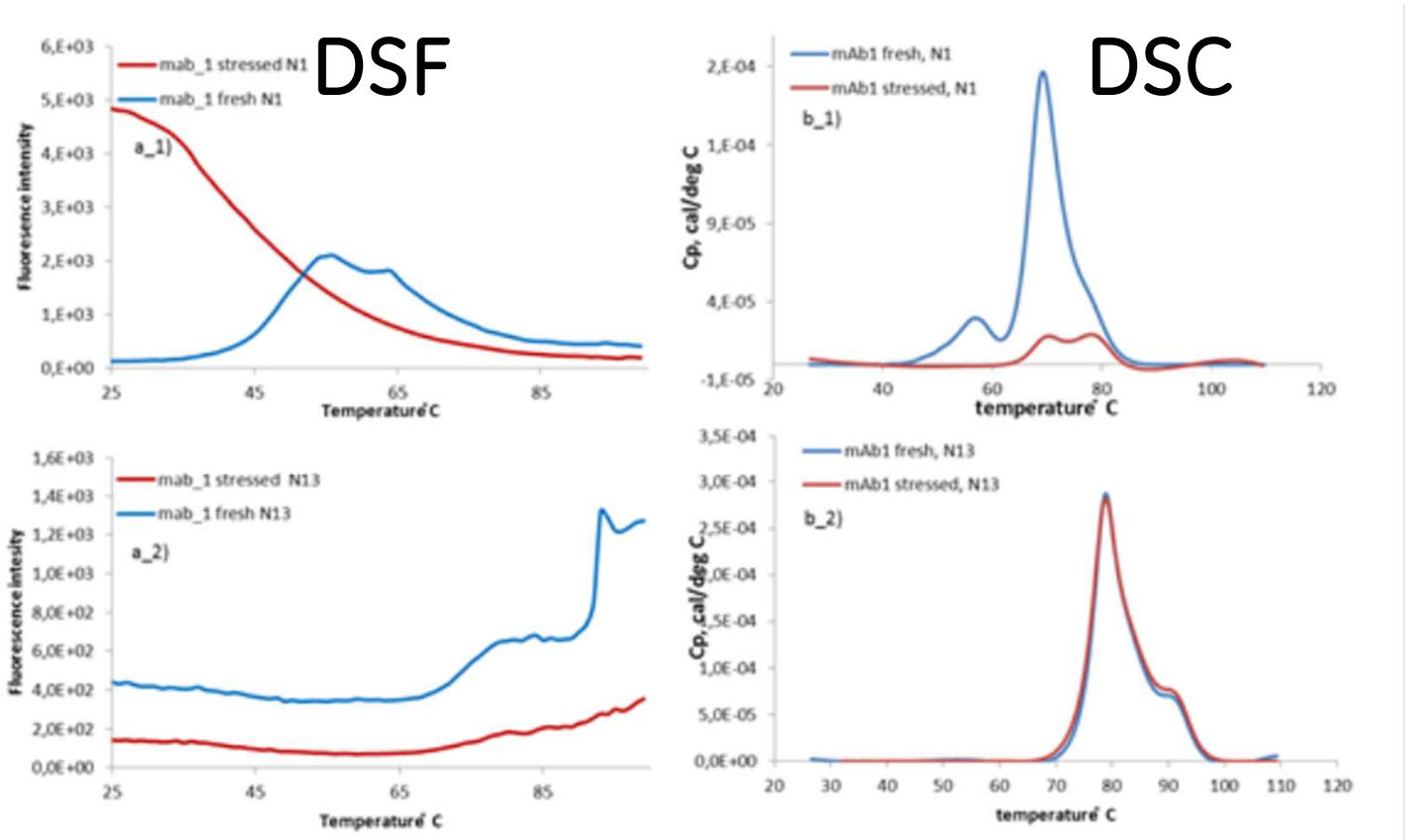
courtesy Amgen

Advantages

- Generic . Almost all transitions have an enthalpy change
- Broadest dynamic range of attainable temperatures, scan rates and possibility for re-scans
- Temperature resolution to 0.2 ºC
- No optical probes
- **No labelling, allows to study the sample as it is**
- **Possible to measure on turbid solutions**

Robustness of qualitative and quantitative comparison of fresh and stressed mAb1 samples



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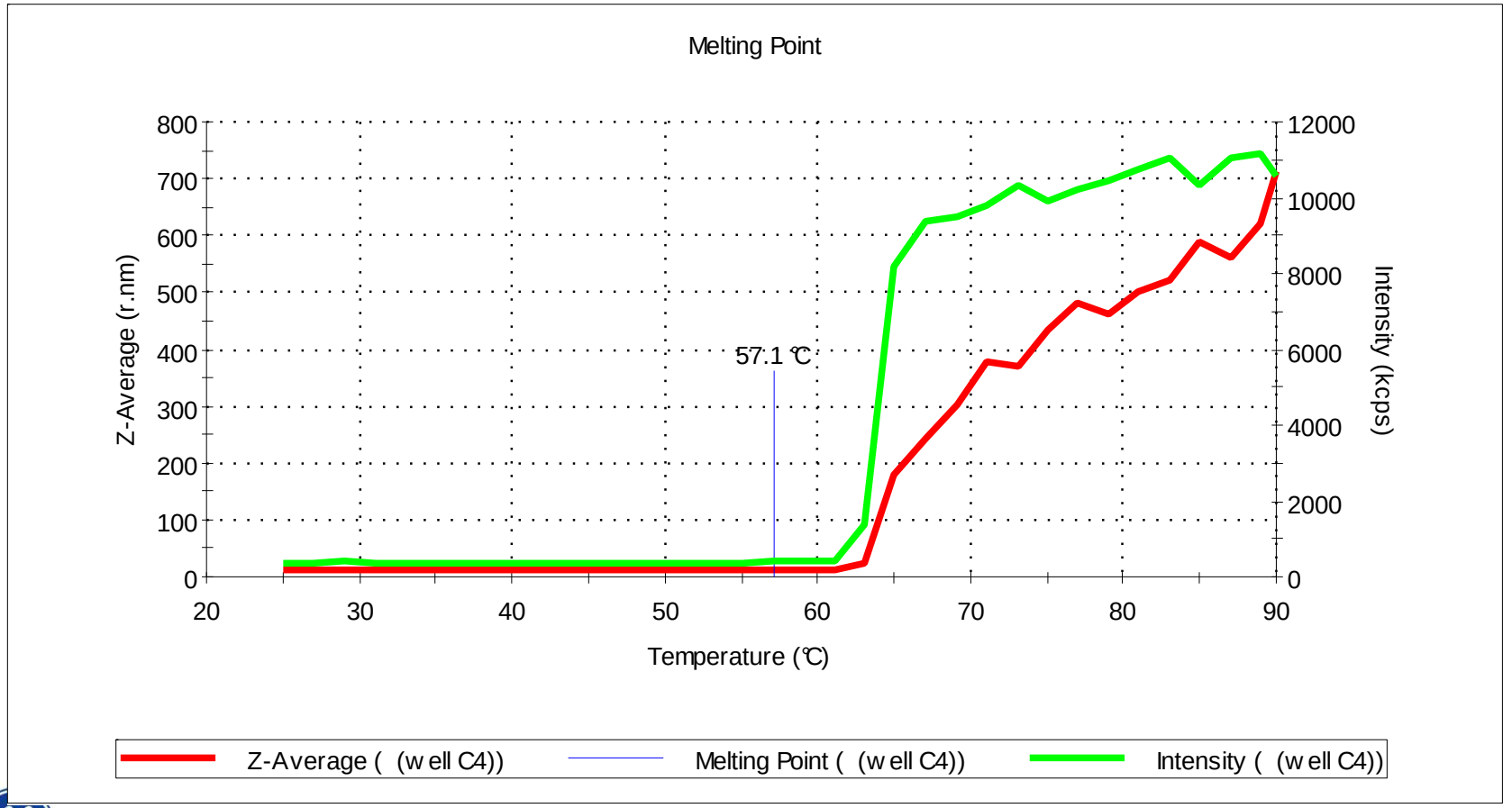
Screening with DSC – testing the effects of diverse excipients with no interference in instrumental readout

DSC can screen a wide variety of excipients

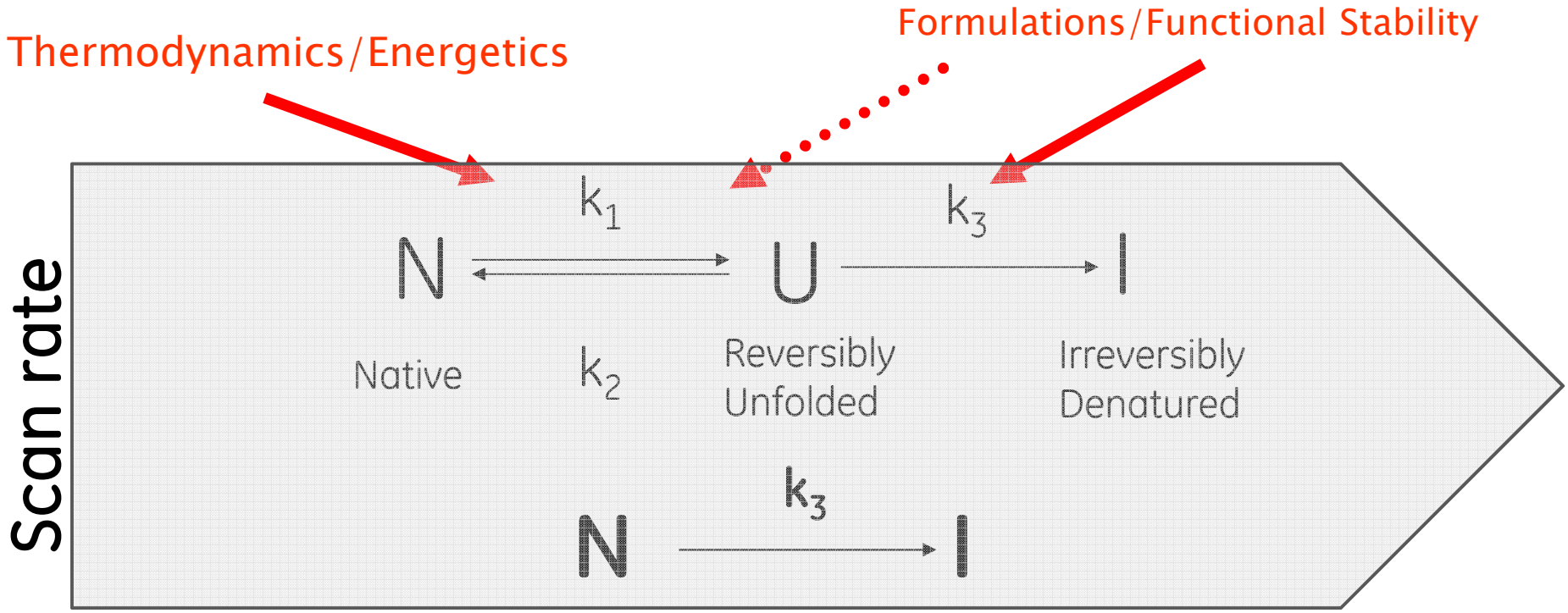
	Excipient	Tm (°C)
Control	-	48.1
Sugars	Manitol	46.7
	Glucose	49.6
Polymers / Polyols	PEG (300)	49.4
	Ethanol	48.7
	Ethanol	43.8
Salts	NaCl	53.1
	$CaCl_2$	41.1
Surfactants	Pluronic F68	46.6
	Tween 80	45.8
Effects of Excipients on Stability of IL-R		

Adopted from Remmele, et al, Pharm. Res. 15, 200-208 (1998)

Some excipients can corrupt thermal stability assays based on optical readout: Tween 20 in DLS

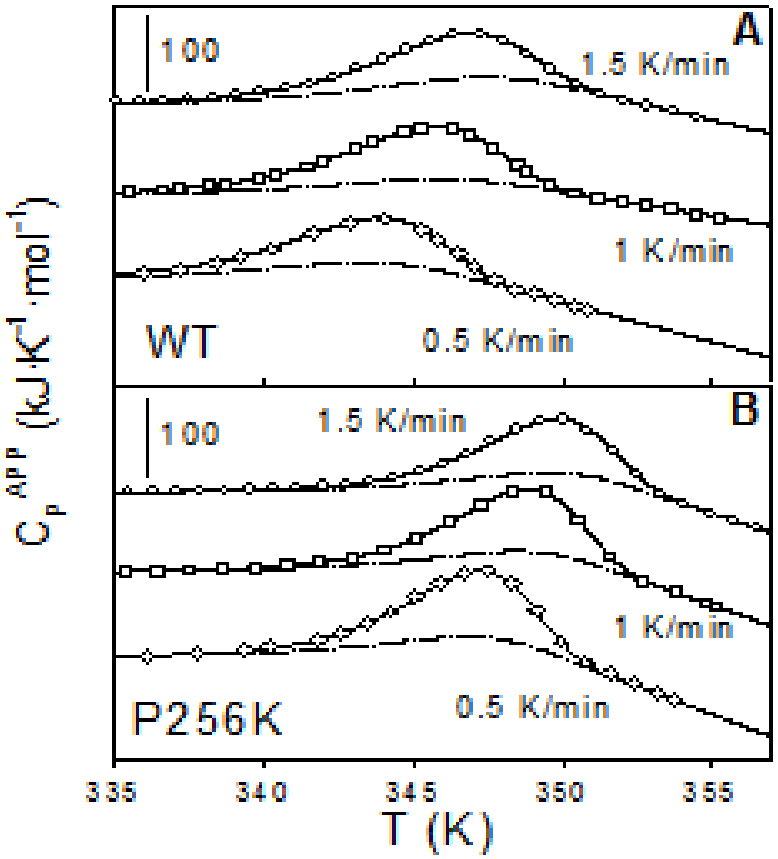


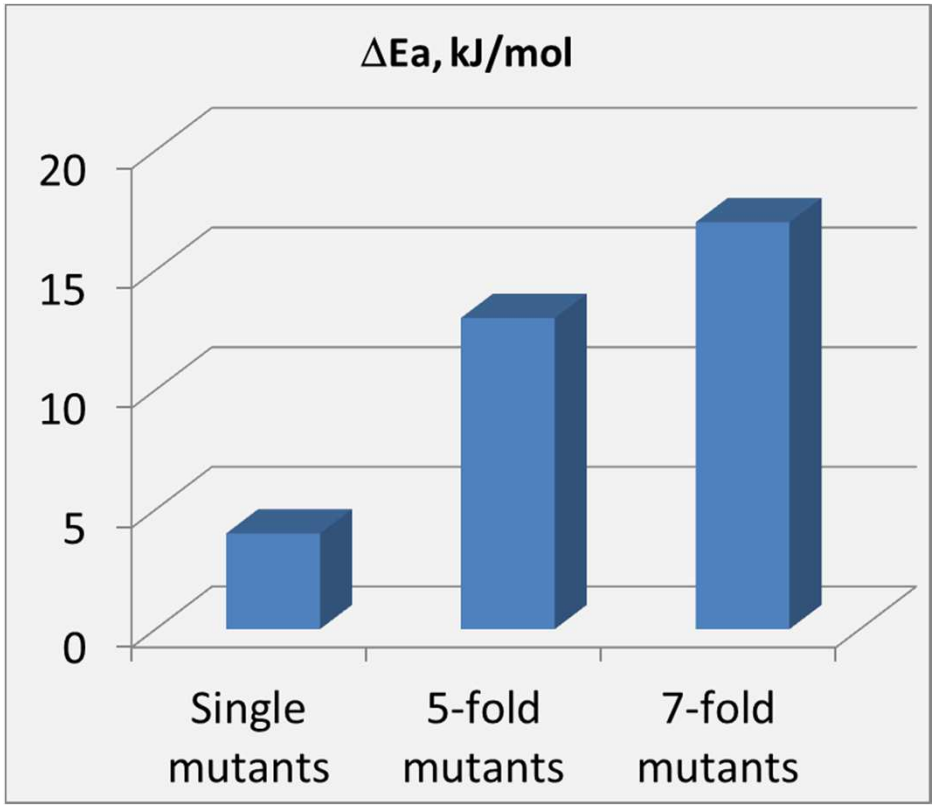
Protein denaturation in non-simplified form



Irreversible unfolding N->D, where k_3 is a first-order kinetic constant

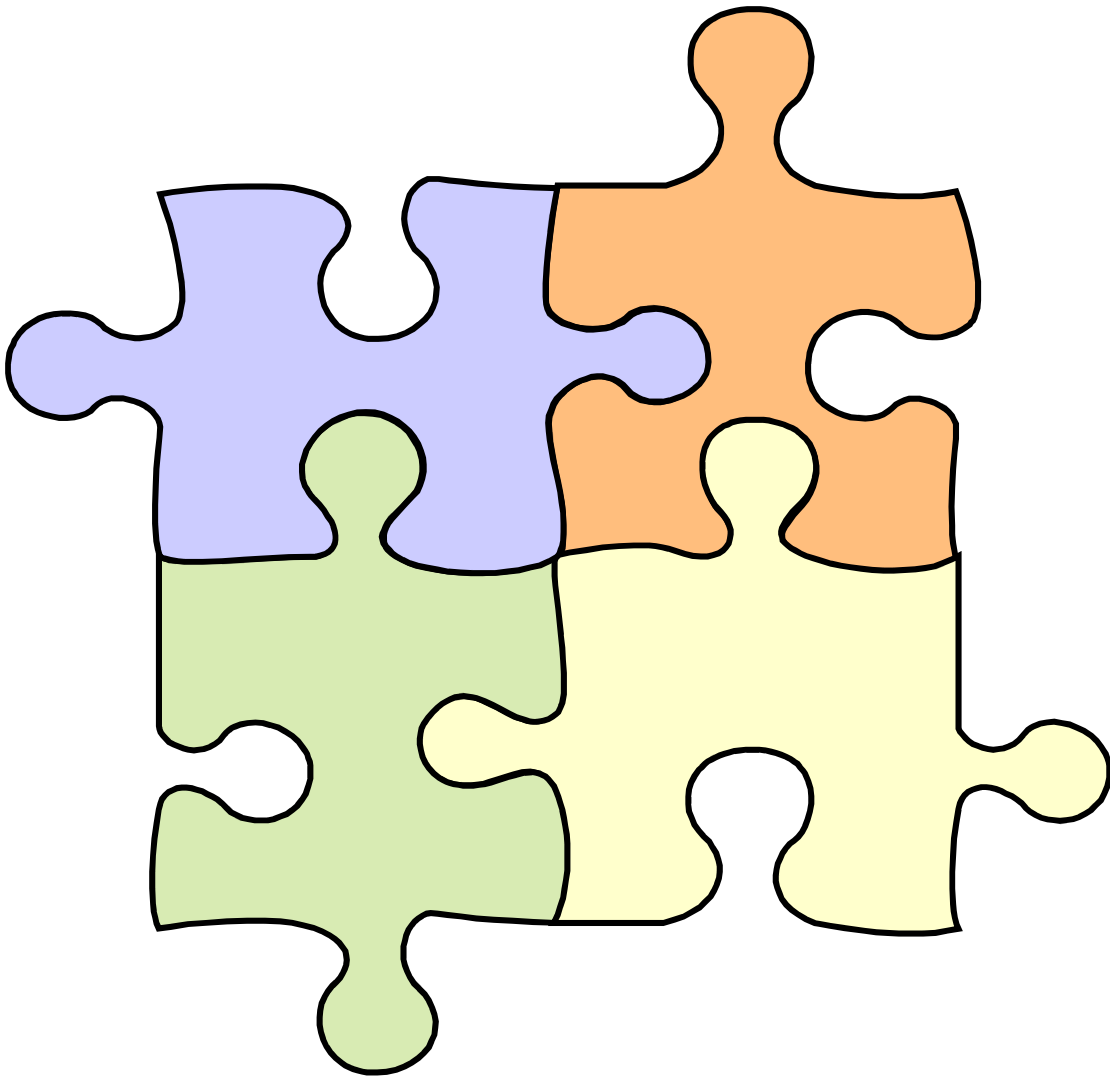
Kinetic stabilization confirmed through scan rate dependence of Tm in DSC





Rodriguez-Larrea et al. J. Mol. Biol. (2006) 360, 715-724

Isothermal Titration Calorimetry



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Isothermal titration calorimetry (ITC) provides

Assays for biological activity by monitoring in one simple assay:

- Binding affinity
- Stoichiometry of binding

Assays for formulation development by monitoring:

- Protein – excipient interactions

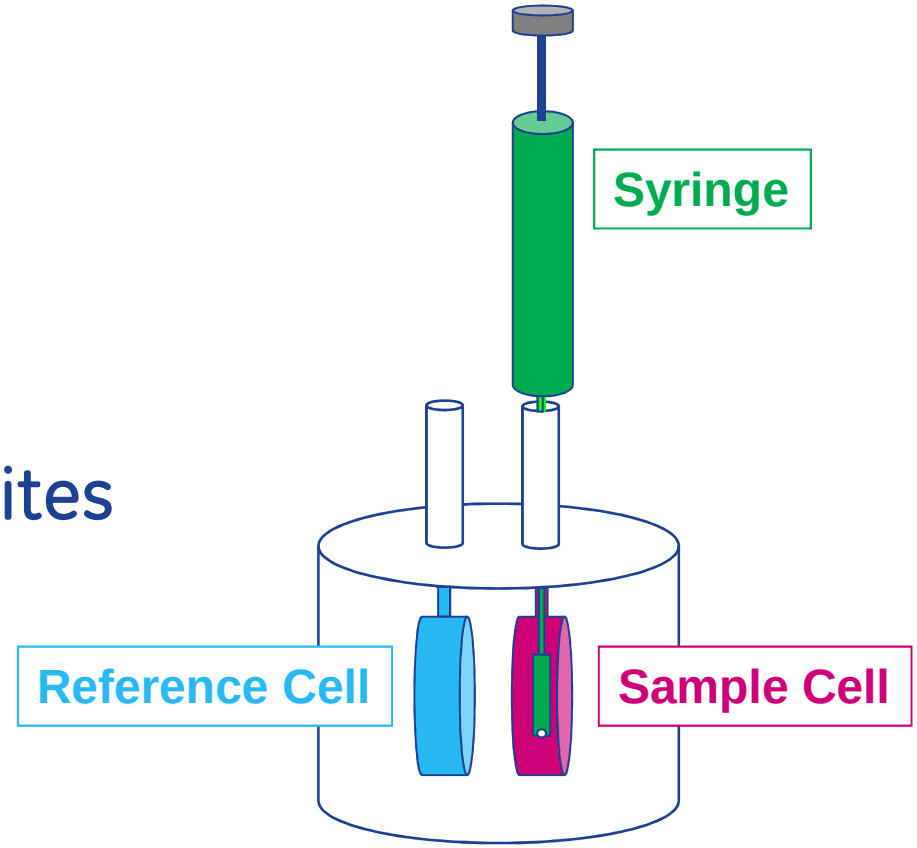
MicroCal™ iTC200

How does ITC work?

Measures heat of interaction

Single ITC experiment

- Affinity
- Binding mechanism
- Number of binding sites



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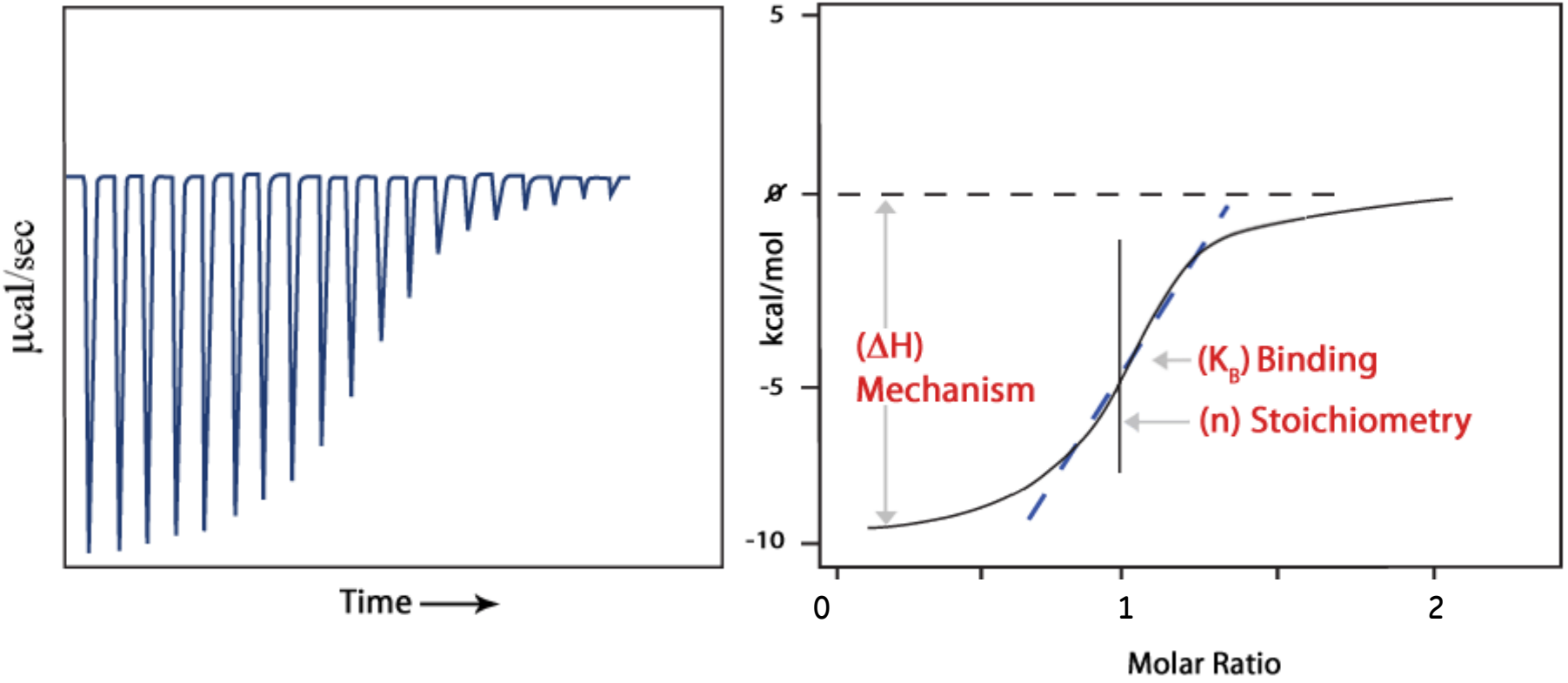
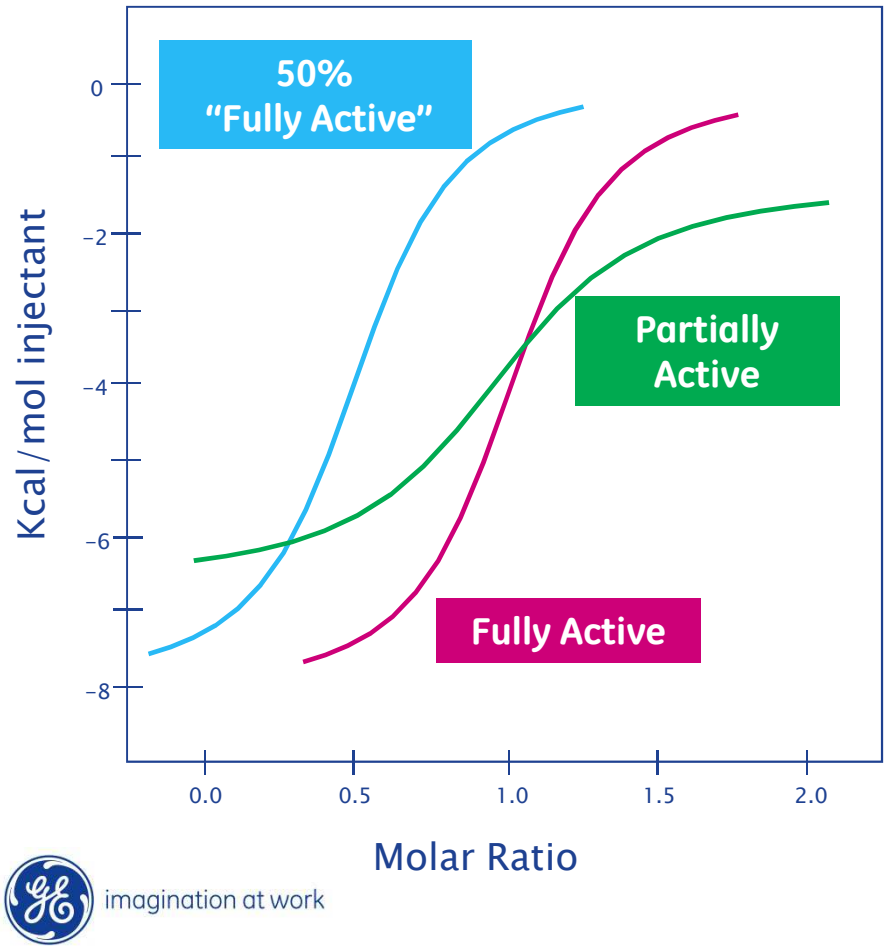
Fitting ITC data

For a binding isotherm, integrate the area for each peak



Affinity and stoichiometry



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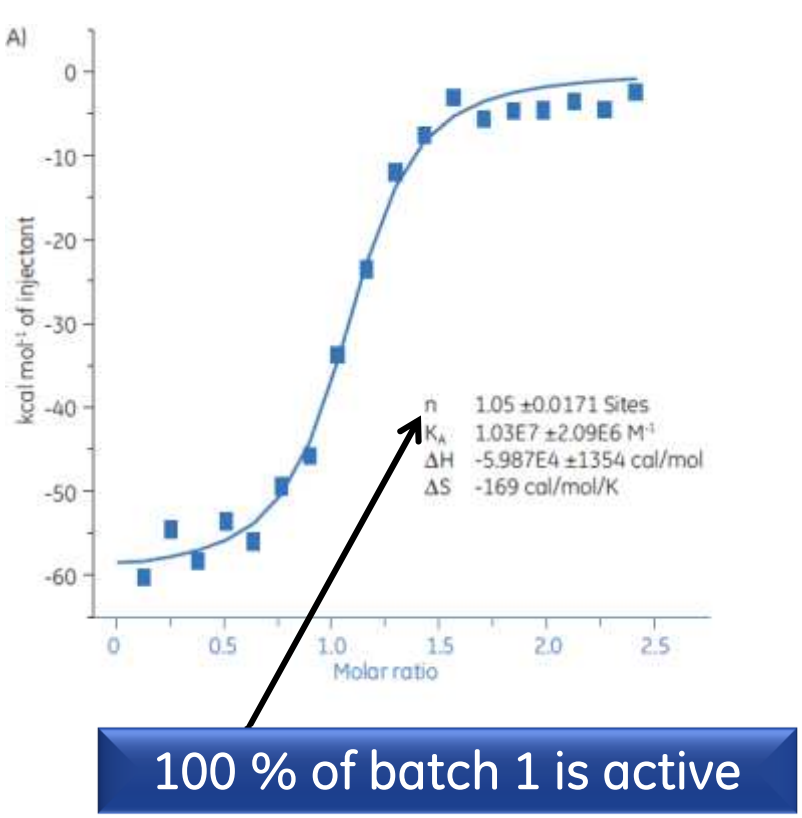
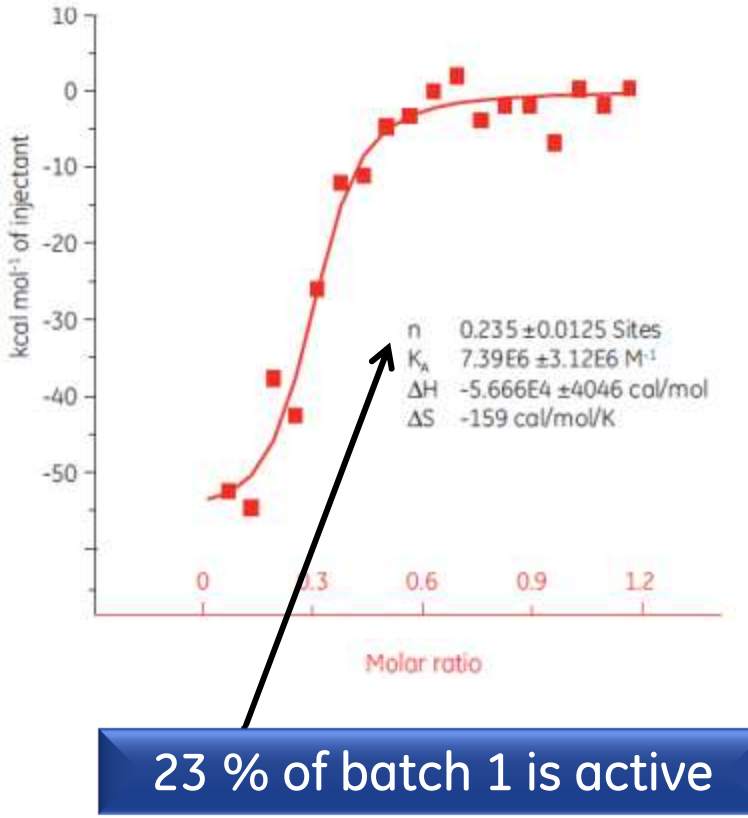
Protein Quality

- Measure active concentrations
- Compare protein batches
- Investigate MOA

Protein quality/assay development



100 % of batch 1 is active



23 % of batch 1 is active

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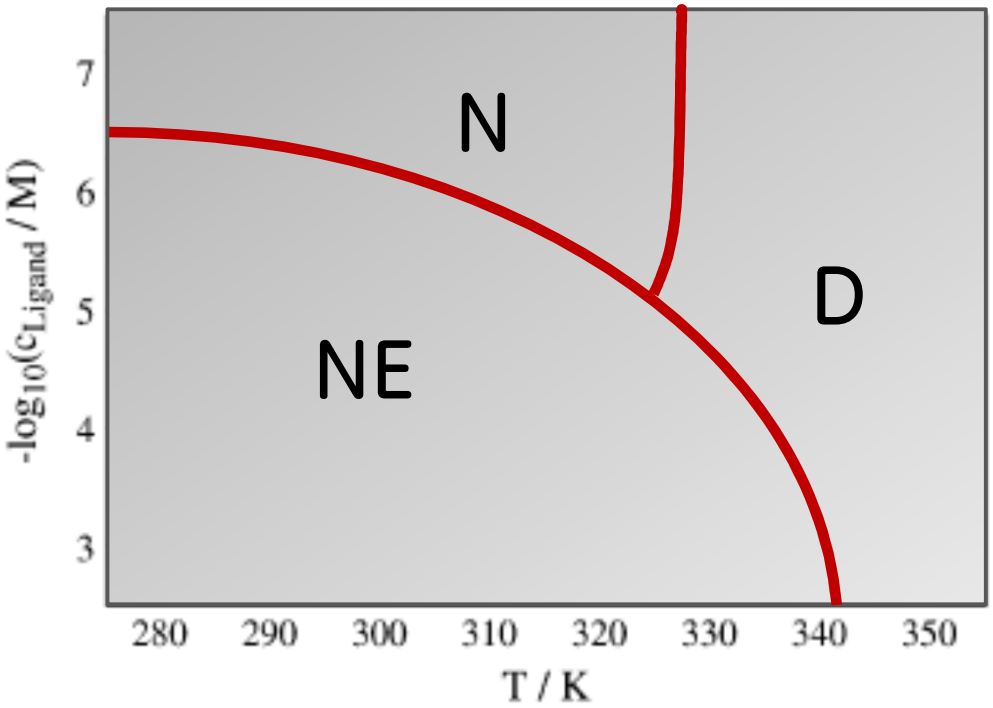
Getting a bigger picture on protein thermal stability and interaction with excipient: Phase diagram



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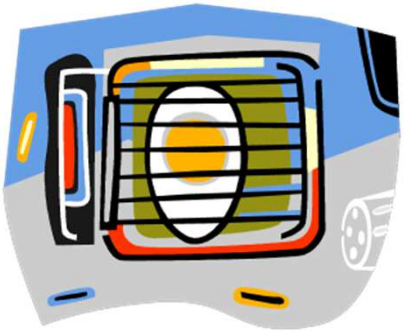
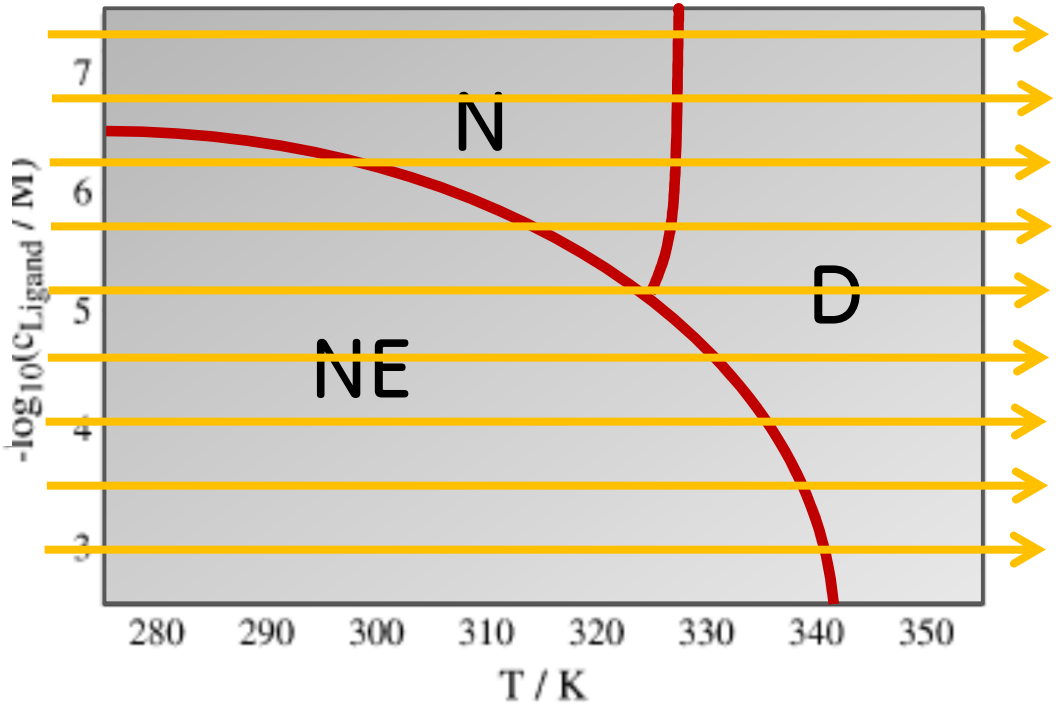
Mapping stability profile of a protein



MicroCal™
Auto Cap DSC



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Profiling protein-ligand interaction

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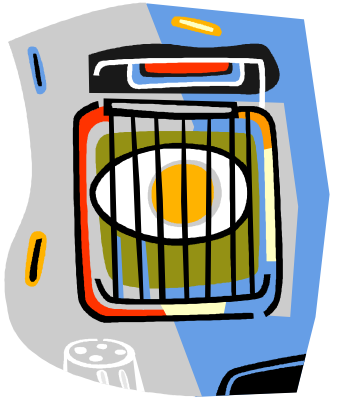
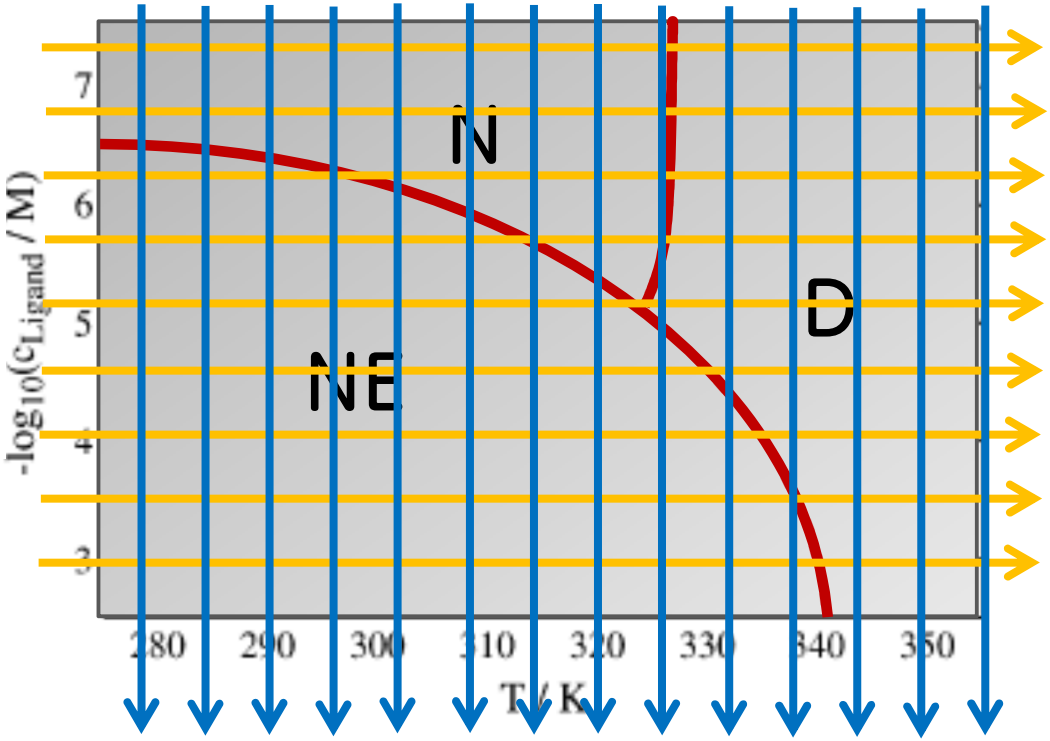
Auto iTC200



MicroCal™
Auto Cap DSC



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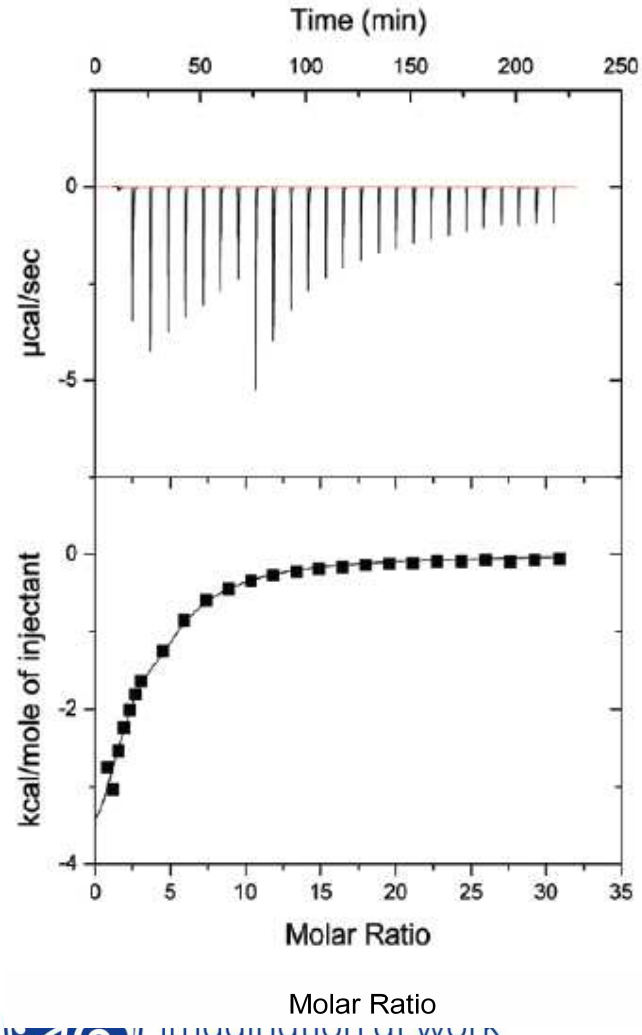
Explore protein - excipient binding



Molar Ratio

Binding of polysorbate-80 to Protein X

Binding saturation of ~10 moles of polysorbate-80 per mole of Protein X

Weak interaction: polysorbate-80/Protein X complex more likely to dissociate in vivo

ITC data suggests minimum excipient concentration needed to stabilize Protein X in formulation

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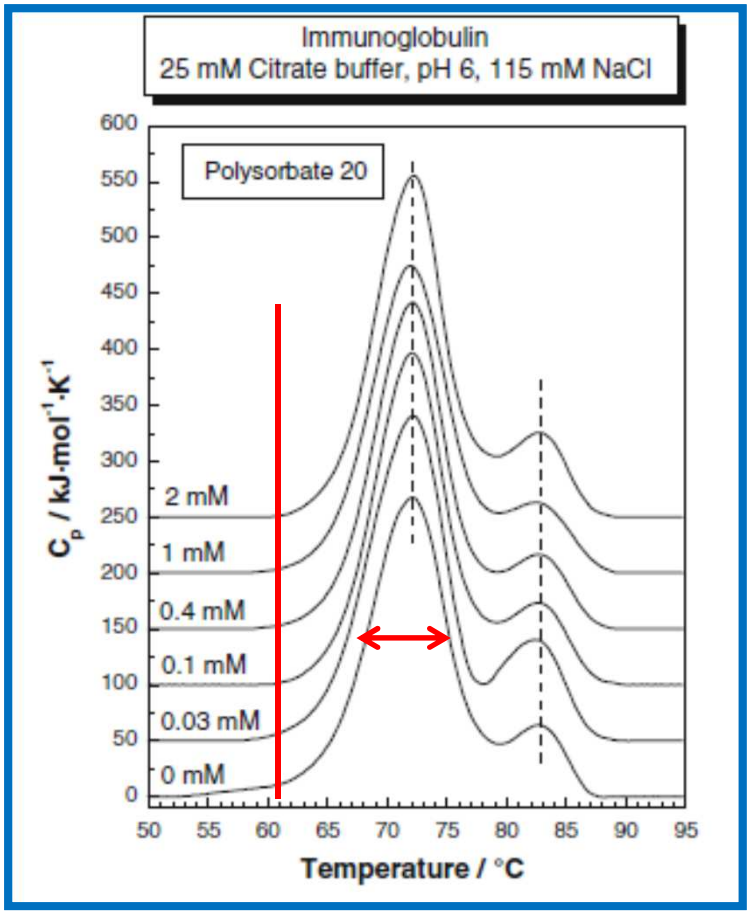
Quality and quantity: DSC thermograms offer multiple descriptors of Ab1 unfolding profile



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Adopted from Hoffman et al. Eur. Biophys. J. (2009) 38: 557-568

T_ms unaffected **BUT** Tween 20 affects cooperativity of Ab1 unfolding

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Revisiting data from Hoffman et al. Eur. Biophys. J. (2009) 38: 557-568

Screening - evaluation of preservatives for IL-1R

DSC quickly and easily identifies most stable conditions for optimal liquid formulations

DSC stability indications correlate well with SEC results

	T_m	% Aggregation by SEC
Control	50.8	1.5
Phenol	50.3	3.0
m-Creosol	48.4	5.1
Benzyl alcohol	45.2	16.5

Remmele, et.al.,Pharmaceutical Res. 15, 200-208 (1998)

ITC and DSC used to study effect of preseravtives on an Ab

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Effect of phenol on Protein X formulations

- ✓ Phenol was found to have a decreased antimicrobial activity in the presence of Protein X

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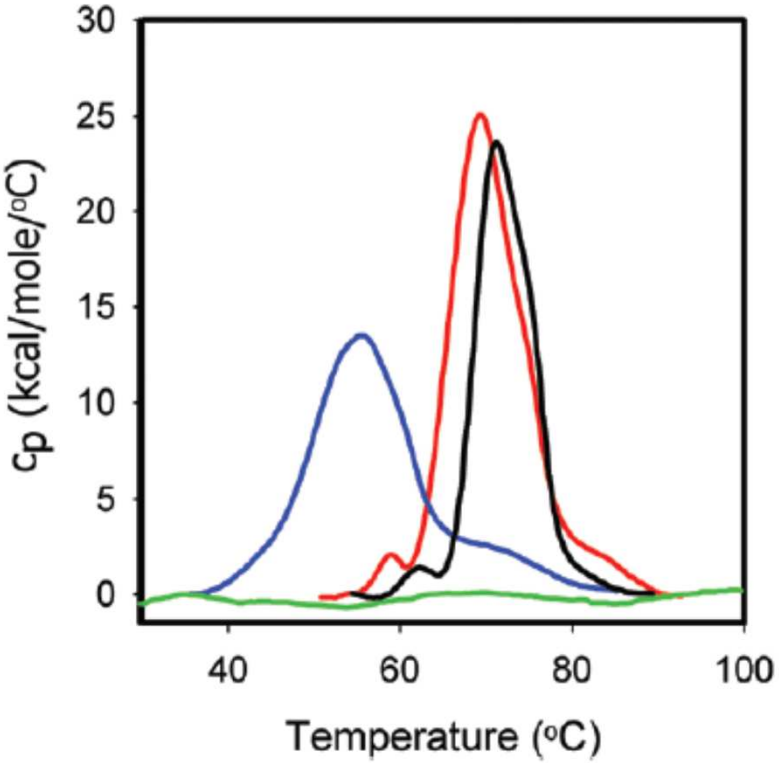
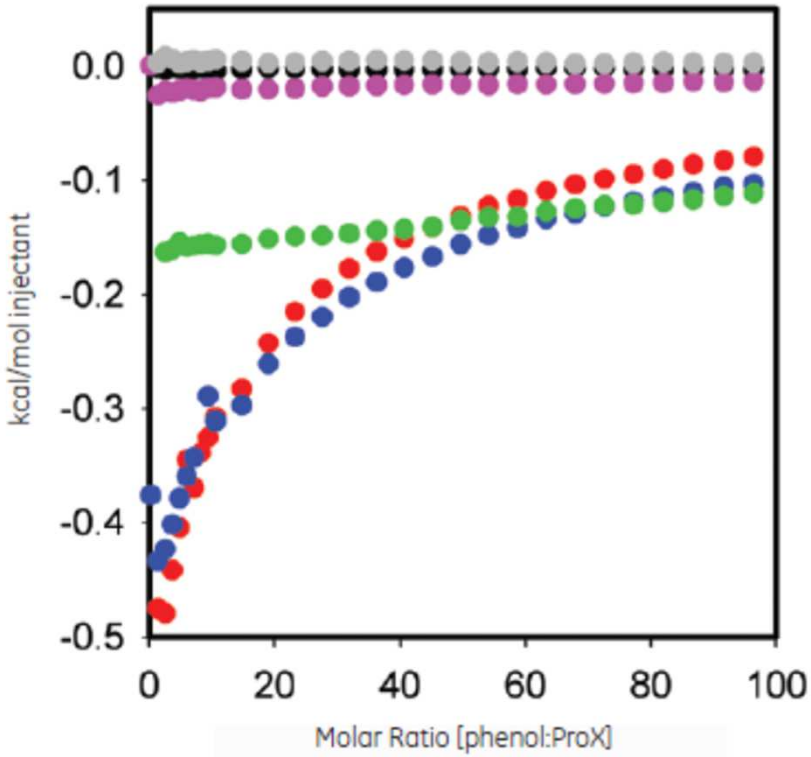
Phenol binds to Protein X and stabilizes it against thermal unfolding



DSC curves:
pH 5.7 (free protein - red line; protein+phenol black line), pH 4.5 (blue), pH 3.5 (green).



ITC data on protein X titration with phenol: pH 5.7 (red line), pH 4.5 (blue), pH 3.5 (green). Rest of the data represents reference titrations.

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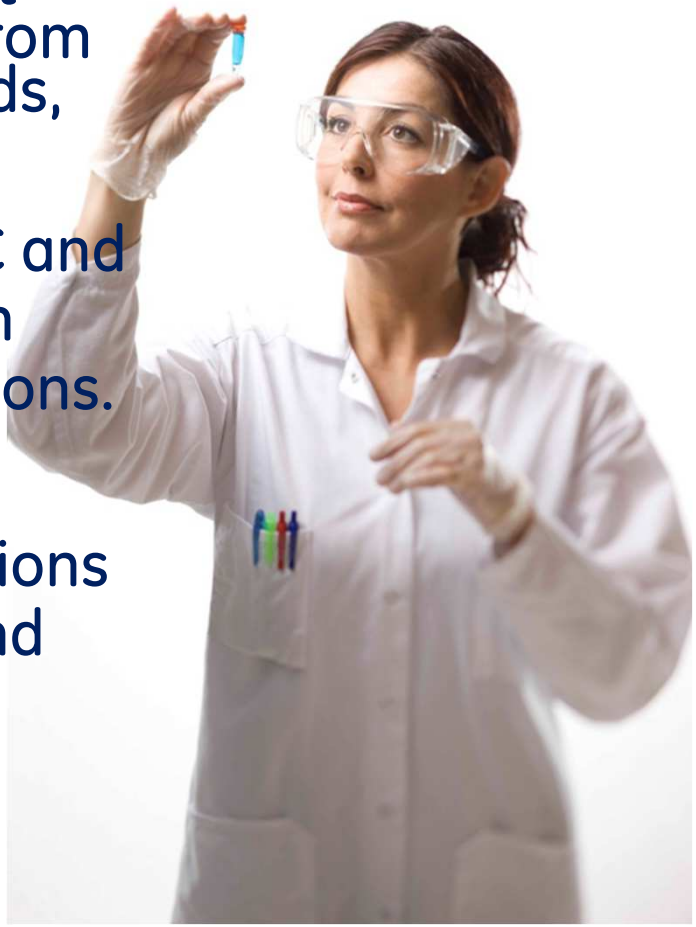
Conclusions

Profiling and optimization of protein stability is a complex task which needs to be approached by several orthogonal techniques.

Application of ITC and DSC can help in most steps of Biopharmaceutical development from initial selection and optimization of the leads, to formulation and QC.

As first principle biophysical techniques ITC and DSC can deliver insightful details on protein stability profile and protein-ligand interactions.

DSC is well suited for smaller scale buffer/excipient screens, DSF assay validations and in-depth characterization of protein and protein-ligand interactions.



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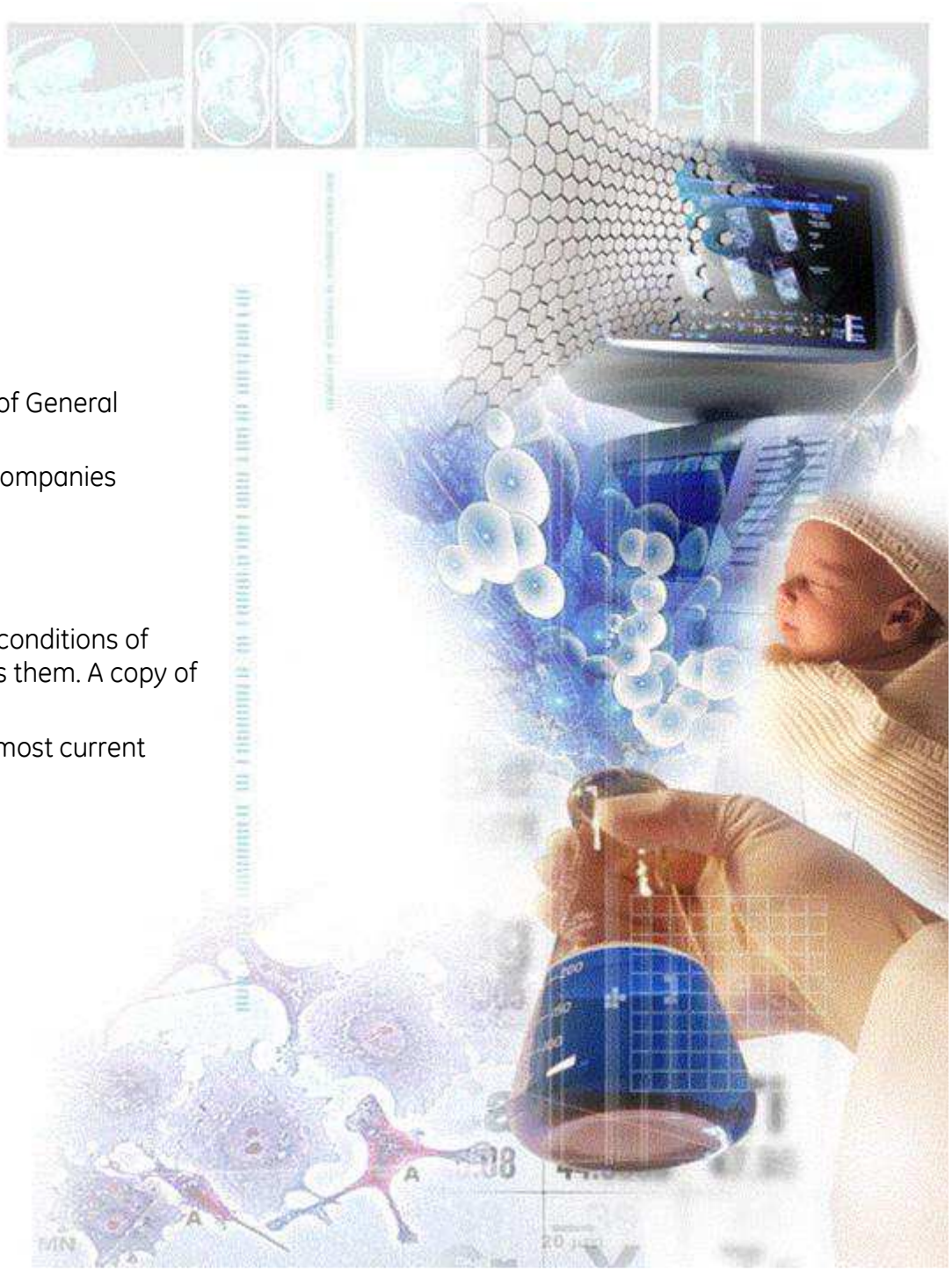
Thank you



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