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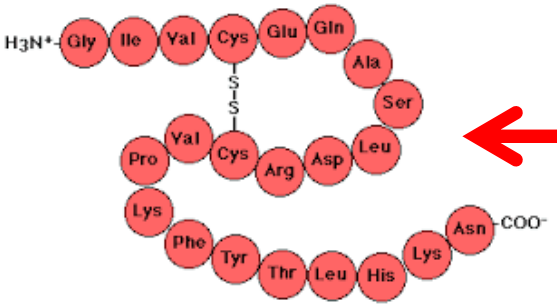
Stopping Aggregation

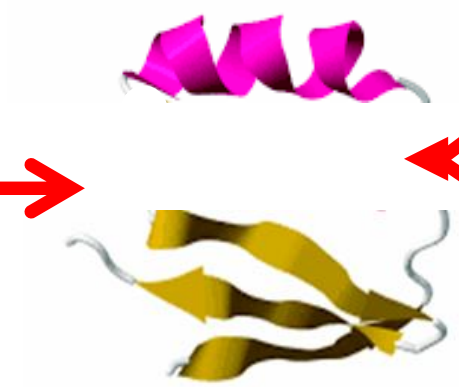
Measuring and Predicting Stability

Protein Folding

The aim of stabilisation is pushing the equilibrium towards the folded state



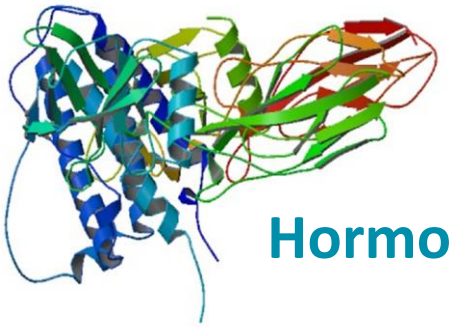
Primary Structure (1°)



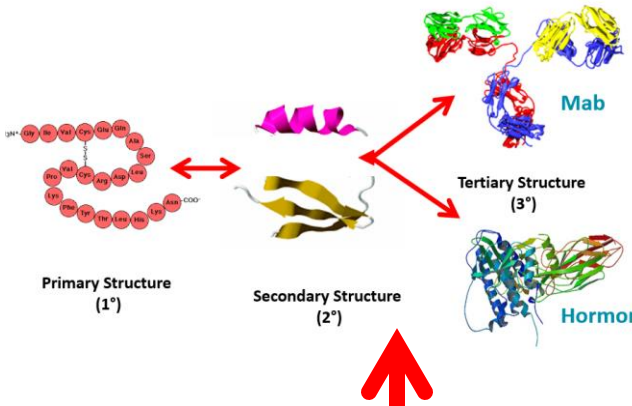
Secondary Structure (2°)

Mab

Tertiary Structure (3°)



Hormone



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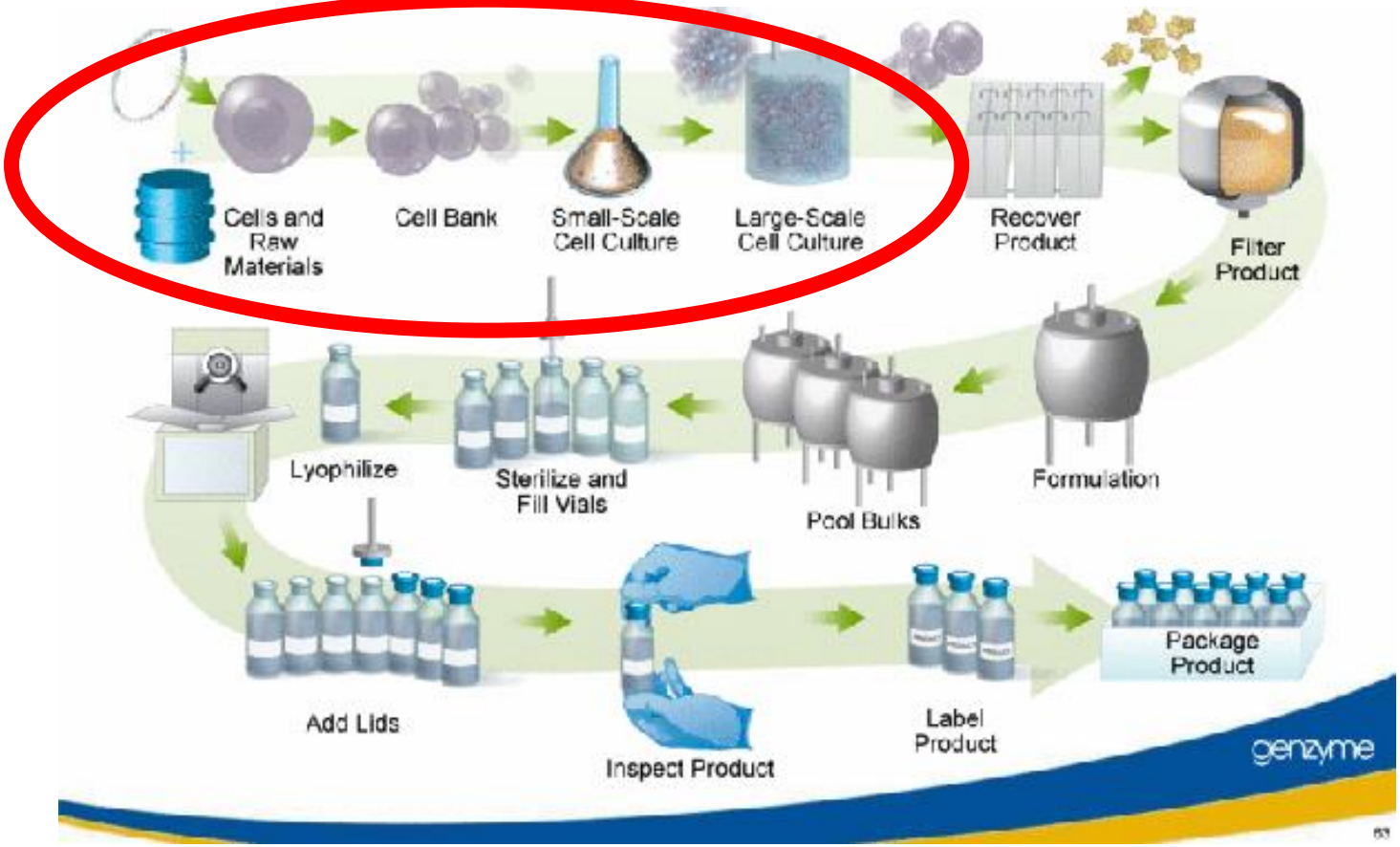


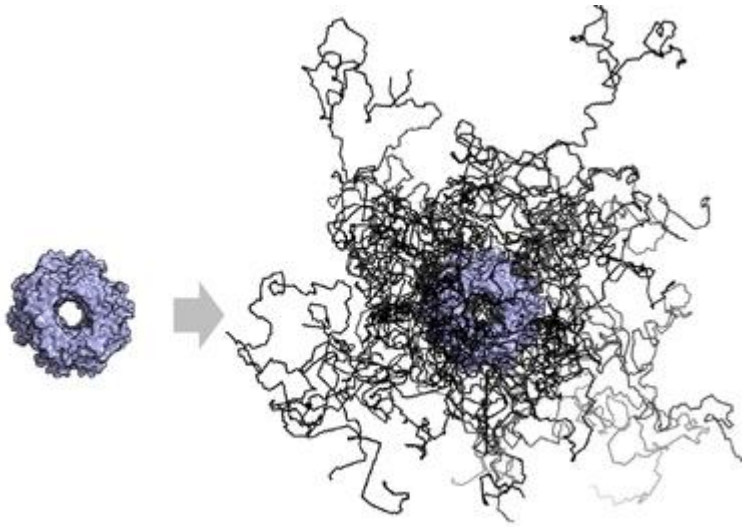
Protein Stabilisation

The mechanical option

- We can stabilise a protein by changing the primary structure
  - Genetic engineering of amino acid sequence in order to strengthen protein
  - Trade-off - Rigid protein is less functional

- We can also add polymer chains to the surface in order to sterically inhibit aggregation

- Pegylation, for instance, is often used to stabilise proteins
  - Pegloticase, for instance, is a stable pegylated Uricase used to treat gout.



PEGylated Proteins – SEC Compositional Analysis

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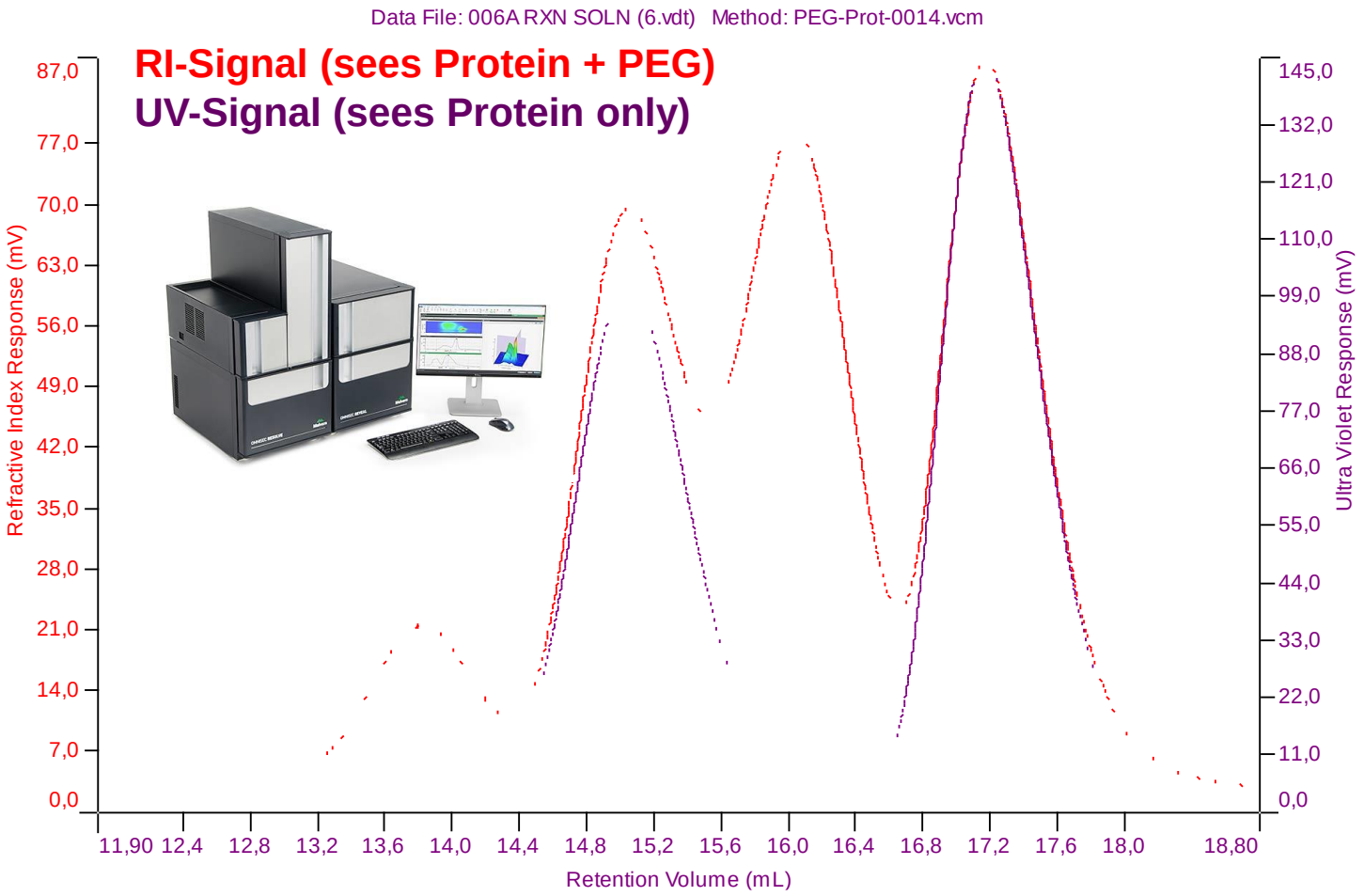
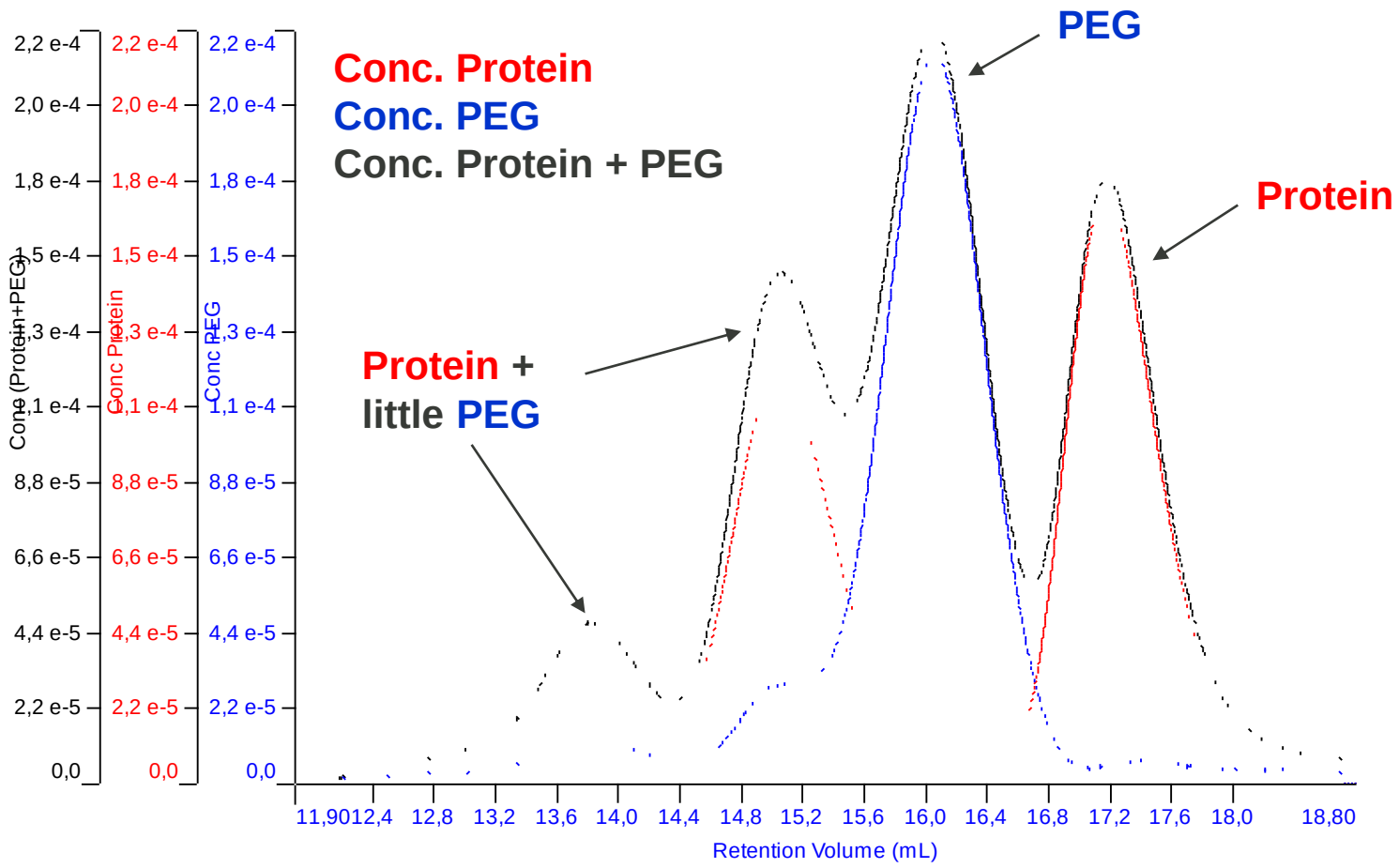
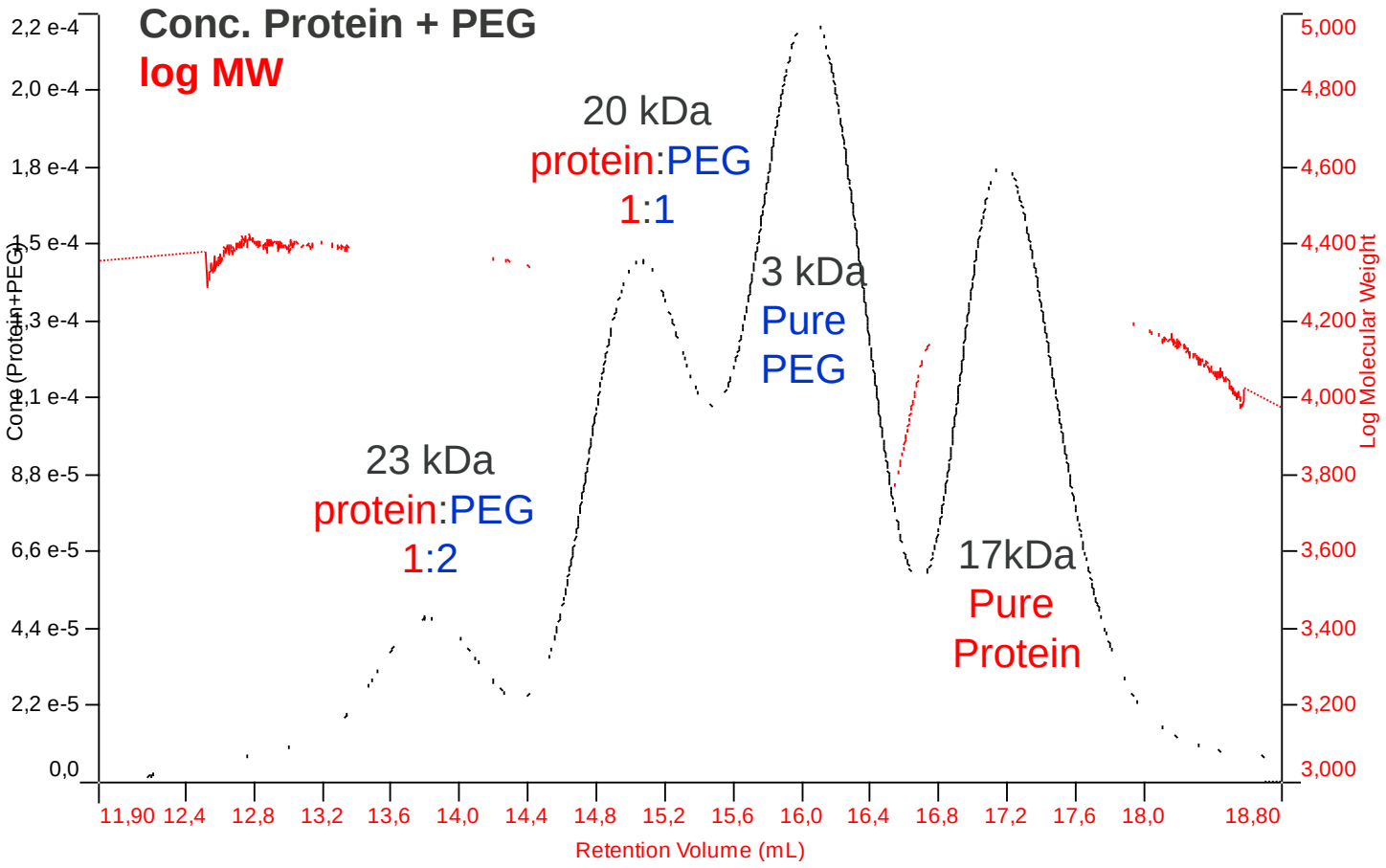


PEGylated Proteins – SEC Compositional Analysis

Malvern Panalytical

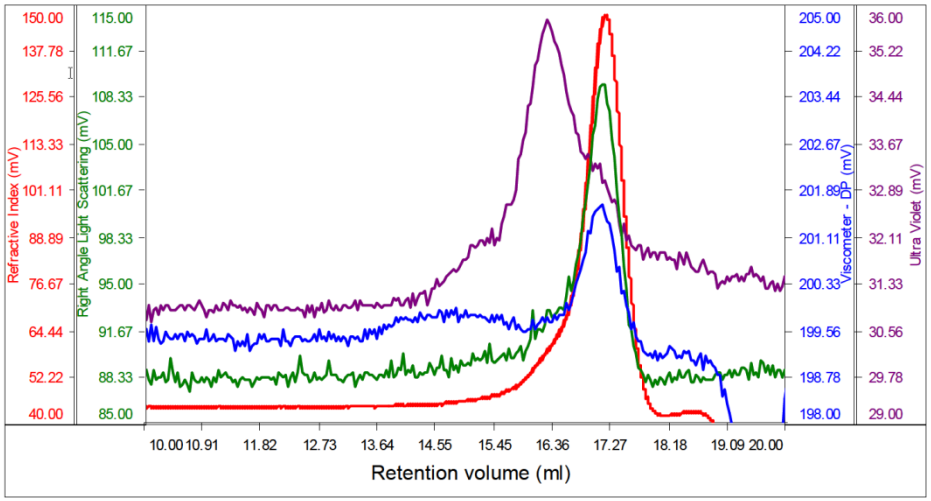


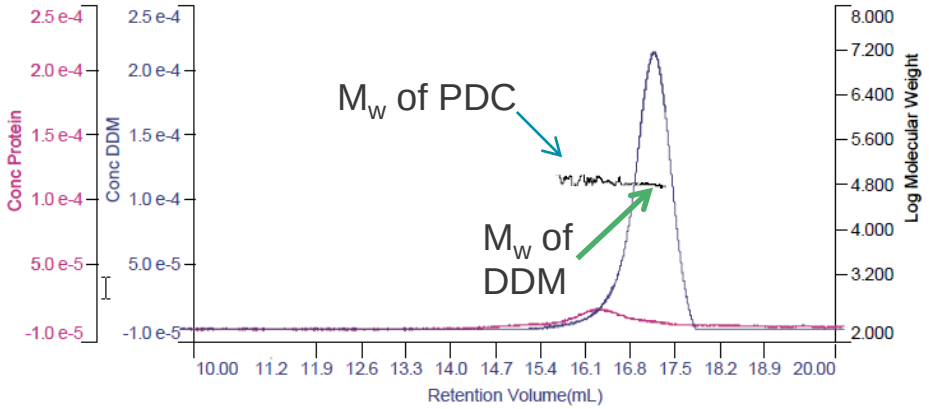
PEGylated Proteins – SEC Compositional Analysis



Membrane Proteins

- **Membrane protein** in detergent
- Micelles: M_W = 63 kDa
- Protein-Detergent-Complex (PDC): M_W = 75 kDa
- PDC contains 46 % protein and 54 % detergent





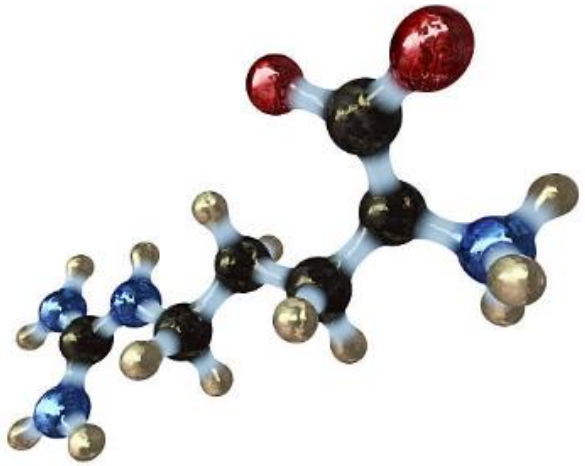
Protein Stabilisation

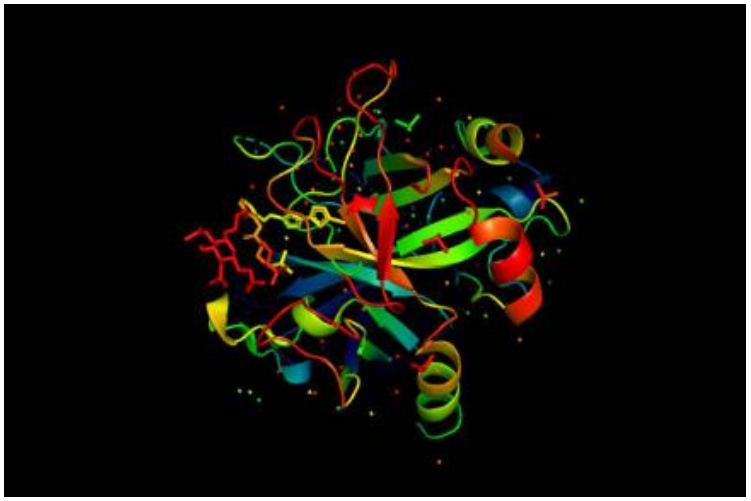
The formulation option

- Add formulation components that increase the favourability of the native, unaggregated state

- Arginine, for instance has been shown to inhibit the aggregation of numerous proteins

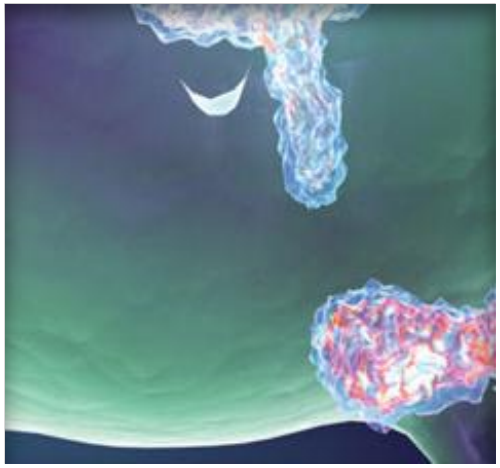
- The affect of any formulation component is heavily dependent on protein structure
  - Screening of formulations to measure their effect is essential





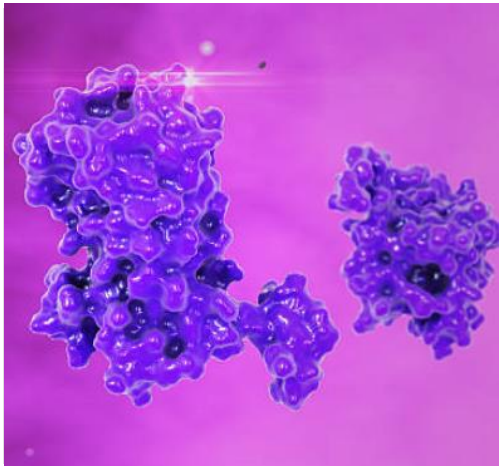
Stopping Aggregation

Formulation Optimisation



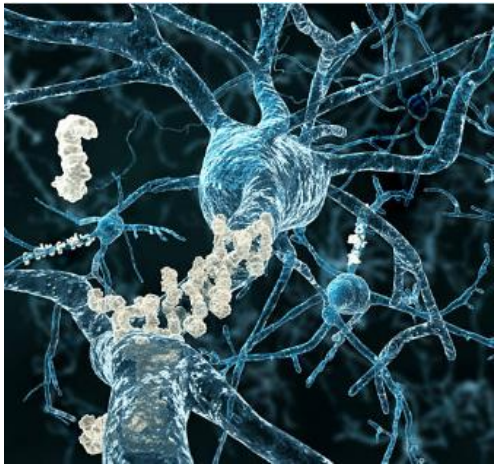
LATE STAGE SCREENING

Information is key



EARLY STAGE SCREENING

Sample Consumption is key



INTERACTION ANALYSIS

How are excipients interacting with protein

What is Differential Scanning Calorimetry (DSC)?

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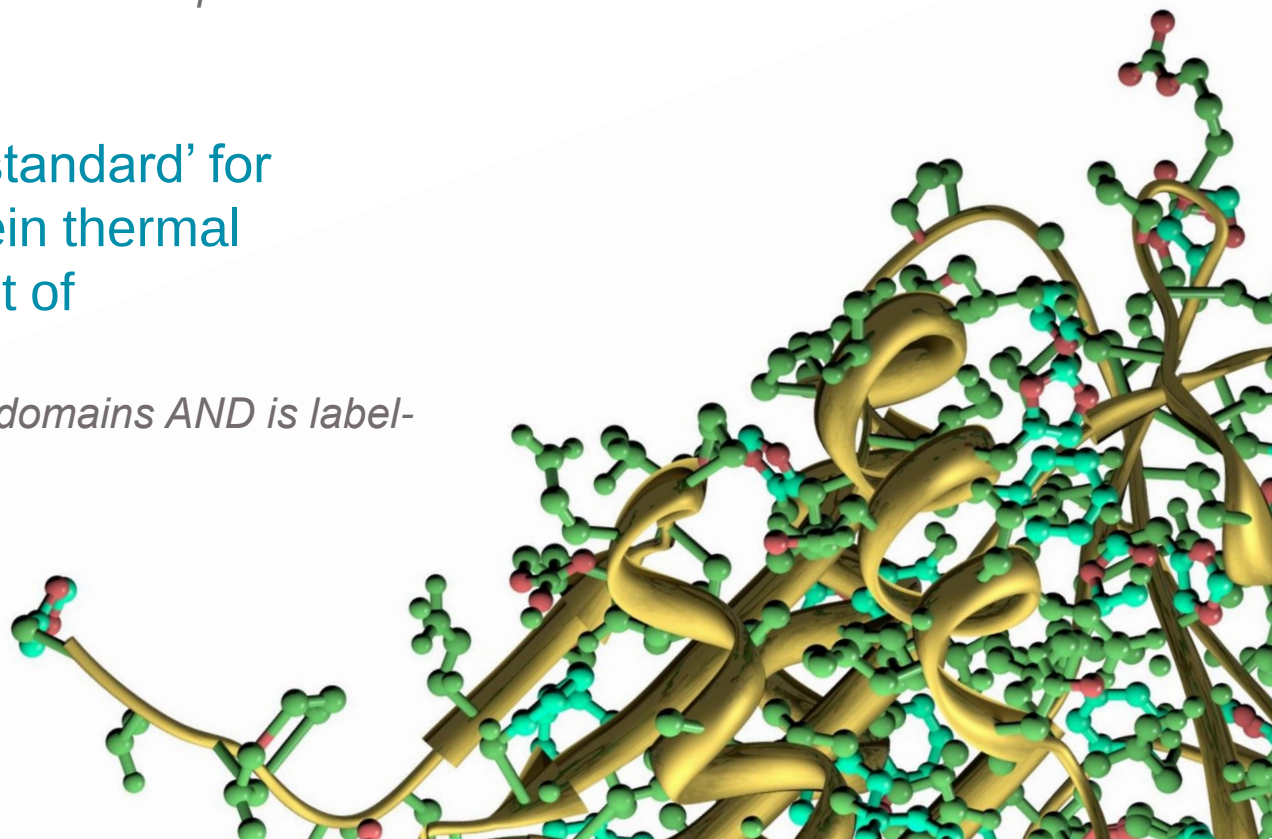
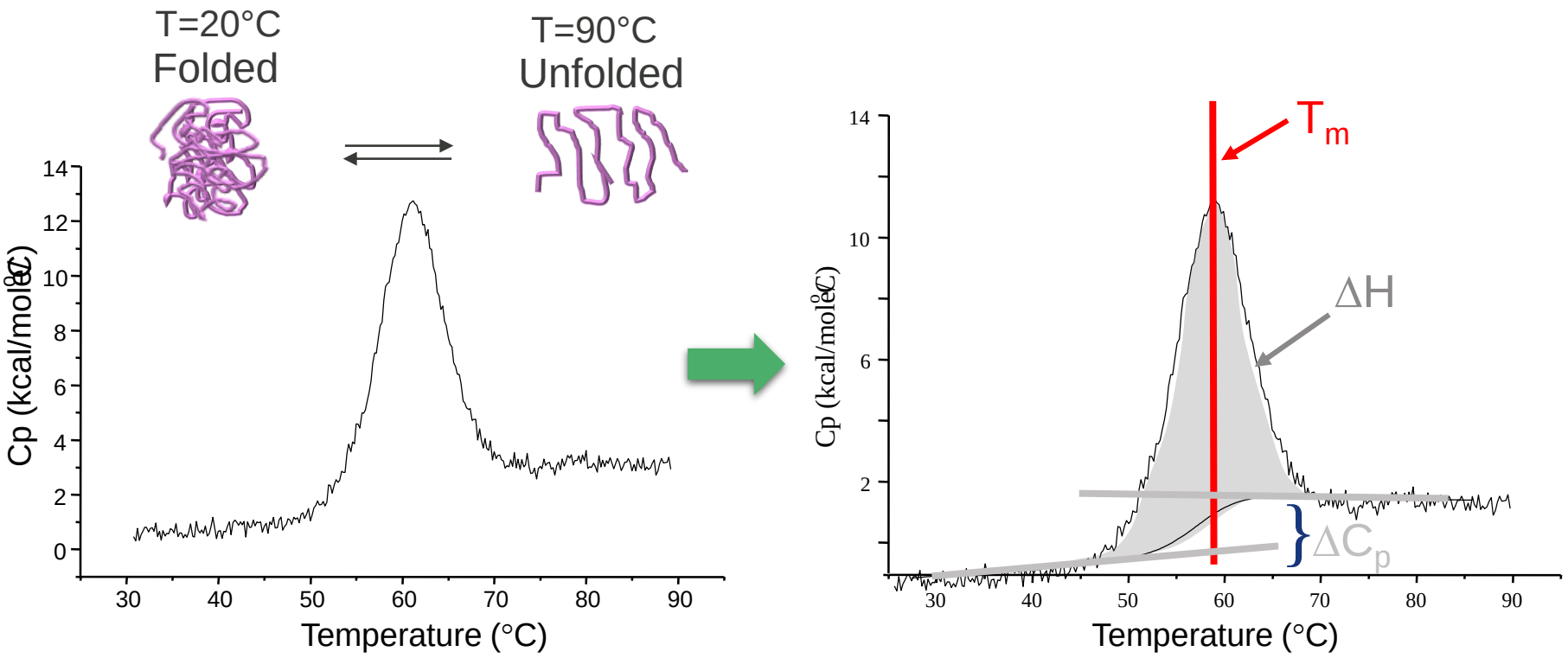
- Direct measurement of the heat absorbed during protein denaturation due to increasing temperature
  - *It detects the heat changes that occur when hydrogen bonds are broken and the protein unfolds*

- Considered the 'gold standard' for characterization of protein thermal stability and assessment of biocomparability
  - *'Sees' the unfolding of all domains AND is label-free – no dyes needed!*



How does DSC work?

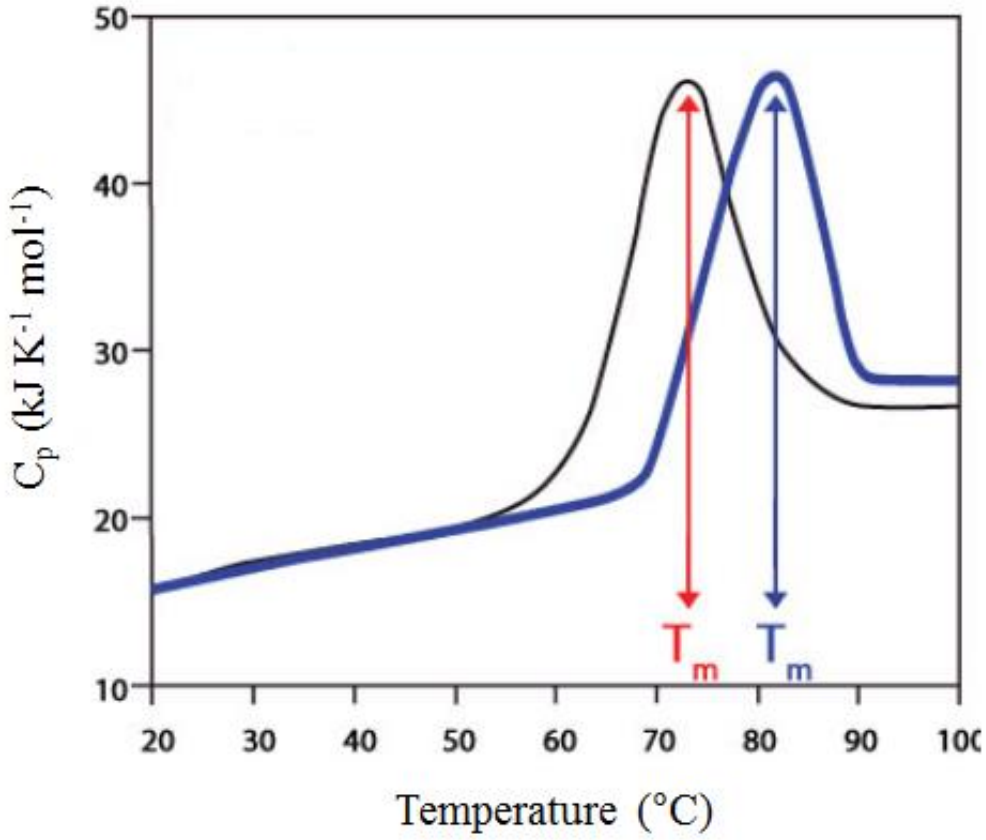
- Protein unfolding is studied **directly** by monitoring heat changes



T_m is an indicator of stability
ΔH is an indicator of stabilizing forces/energetics
ΔCp is in indicator of structural changes before and after

Why use DSC?

- The universal biotherapeutic stability platform



An increase in T_M correlates well with increased shelf life and the developability of a biotherapeutic candidate

MicroCal DSC:

The Gold Standard for HOS characterization

- In WHO Guidelines on the quality, safety, and efficacy of biotherapeutic protein products prepared by recombinant DNA technology (Replacement of Annex 3 of WHO Technical Report Series, No. 814) from 2013, intended to provide national regulatory authorities (NRAs) and manufacturers with guidance on the quality, safety and efficacy of rDNA-derived biotherapeutics for use in humans, it is stated:

> The higher-order structure of the product should be examined using appropriate procedures such as circular dichroism (CD), Fourier transform infrared spectroscopy (FT-IR), fluorescence, differential scanning calorimetry, proton nuclear magnetic resonance (1H-NMR) and/or other suitable techniques such as hydrogen-deuterium exchange MS. FT-IR and CD in the far ultraviolet range deliver information on the secondary structure, whereas CD in the near ultraviolet reflects to some extent the tertiary and quaternary structure. When using these methods, their capabilities and limitations need to be considered (e.g. impact of protein concentration).

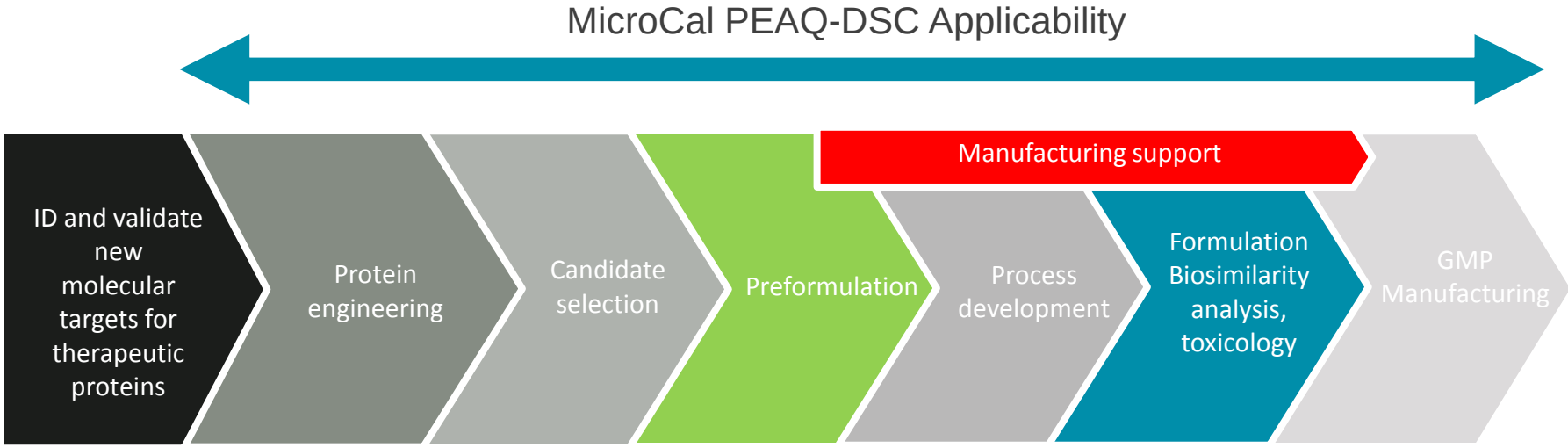
WHO recommends using DSC

MicroCal DSC: The Gold Standard for Biopharma

- **“DSC is likely the strongest, most informative and relevant of all biophysical methods currently available.”**
  - **Sorina Morar-Mitrica, Ph.D.** GlaxoSmithKline - **The BioProcessing Summit, 2012**

- **“Thermal stability is one of the key product attributes that determine the inherent stability of the molecule. DSC remains as an unparalleled technique to assess the thermodynamic stability of proteins in a given buffer condition.”**
  - **Gokarn , et al, ”Biophysical Techniques for Characterizing the Higher Order Structure and Interactions of Monoclonal Antibodies”** ***In:*** **State-of-the-Art and Emerging Technologies for Therapeutic Monoclonal Antibody Characterization , Biopharmaceutical Characterization: The NISTmAb Case Study**, Volume 1201, 2015

Where does DSC sit in the biotherapeutic development pipeline?



MicroCal PEAQ-DSC improves productivity, and quality and transferability of data

MicroCal PEAQ-DSC

MicroCal PEAQ-DSC

Automated

MicroCal PEAQ-DSC

Protein Engineering and Formulation Development

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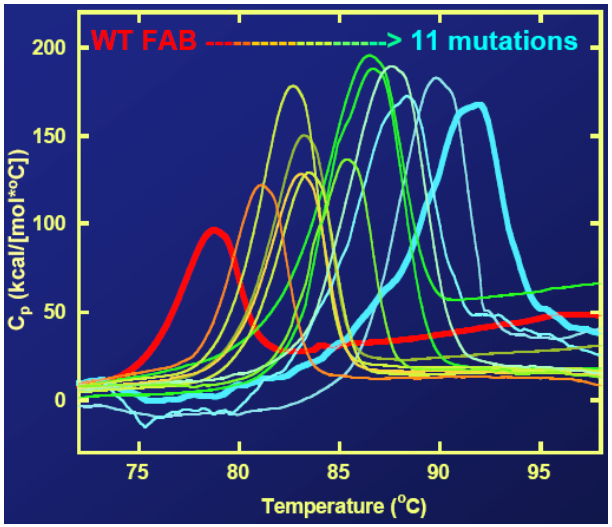
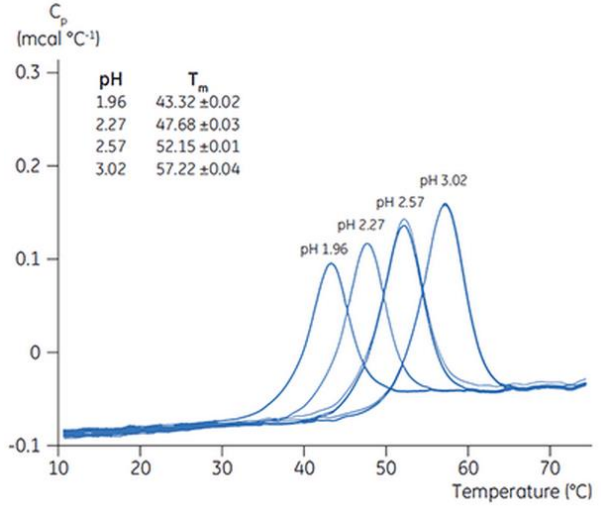
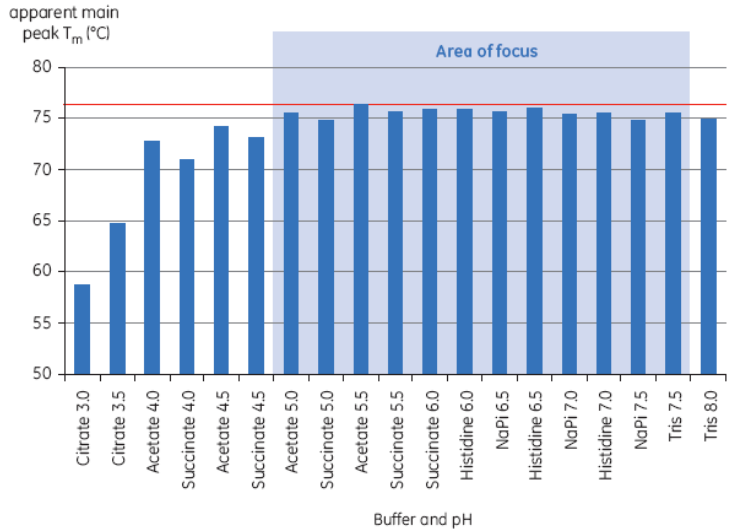
- Mutant selection
  - Simple comparison of the T_m indicates which mutants are most stable

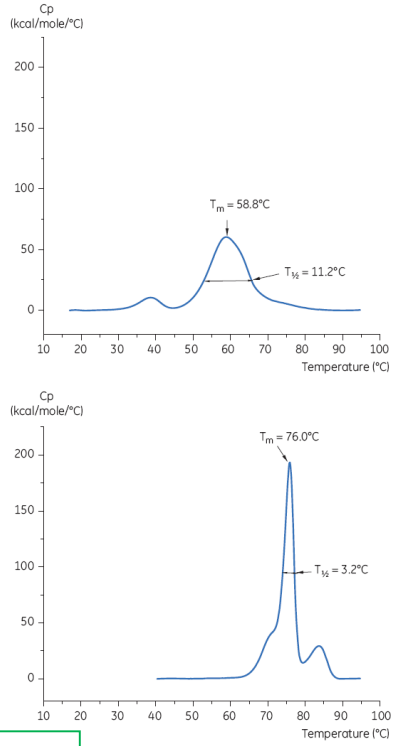


- Formulation selection
  - Simple comparison of the T_m indicates that the **pH 3.02 formulation is the most stable**



T_m screening: Initial pH/buffer screening during preformulation development with DSC





Antibody X at t = 0. T_m data for different pH and buffers
Top thermogram: AbX in citrate buffer pH 3.0
Lower thermogram: AbX in succinate buffer pH 6.0.

Bowers, Malvern application note

DSC – Effect of Formulation Excipients on Stability

Sugars	Conc. (g/ml) in Buffer	T_m (°C)
Mannitol	0.0517	46.7
Lactose	0.0972	49.7
Sucrose	0.0972	49.7
Glucose	0.0512	49.6
Salts	**Conc. (g/ml) in Buffer**	**T_m (°C)**
NaCl	0.00584	53.1
$CaCl_2$	0.0111	41.1
Combination	**Conc. (g/ml) in Buffer**	**T_m (°C)**
Glucose/NaCl	0.0512/0.00584	52.2

Control T_m (no excipients) = 48.1 °C

DSC – Effect of Formulation Excipients on Stability

Polymers	Conc. (g/ml) in Buffer	T_m (°C)
PVP (10,000)	0.01	48.9
PEG (300)	0.0003	49.4
PEG (1000)	0.001	49.1
PEG (3350)	0.00335	48.7
Dextran 40	0.0392	48.0
Polyols	**Conc. (g/ml) in Buffer**	**T_m (°C)**
Glycerol	0.01	48.7
Ethanol	0.0051	48.6
Ethanol	0.05	43.8

Control T_m (no excipients) = 48.1 °C

DSC – Effect of Formulation Excipients on Stability

Amino Acids	Conc. (g/ml) in Buffer	T_m (°C)
Glycine	0.01	46.2
L-Lysine	0.01947	48.3
L-Cysteine	0.01614	51.3
L-Alanine	0.01187	46.2
L-Arginine	0.0232	49.1
Surfactants	**Conc. (g/ml) in Buffer**	**T_m (°C)**
Pluronic™ F68	0.0001	46.6
Tween™ 80	0.001	45.8

Control T_m (no excipients) = 48.1 °C

Microcal DSC cell is Extremely Inert

Allows wide array of excipients and proteins to be tested accurately and efficiently

Malvern Panalytical

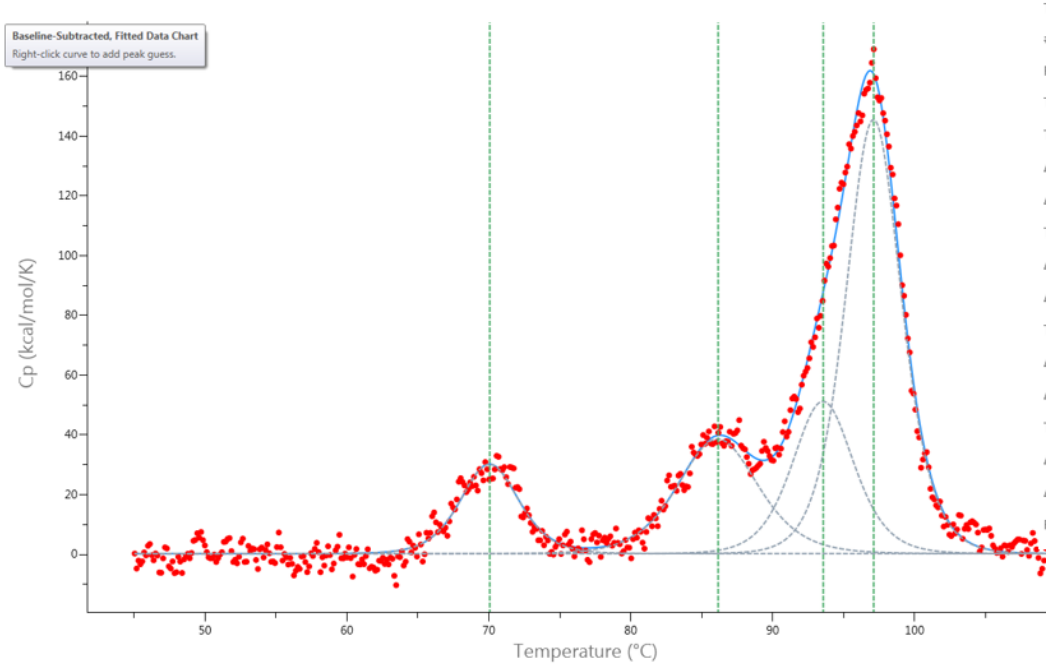
- Microcal DSC are made of Tantalum, a highly rare and corrosion resistant element
- **More inert**, in the context of DSC experiments, than platinum
- **Maximises the range of excipients** that can be assessed – compatible with all common bioformulation components

MicroCal PEAQ-DSC

candidate selection

- Improved cleaning routines increase productivity
- NISTMab was run at ~0.05 mg/mL. 56 T_Ms were obtained from 64 measurements in 2 days (18 μg loaded in each well/96 well plate)
- Detect the transitions that spectroscopic techniques miss!

Use the Gold Standard first – save time and sample

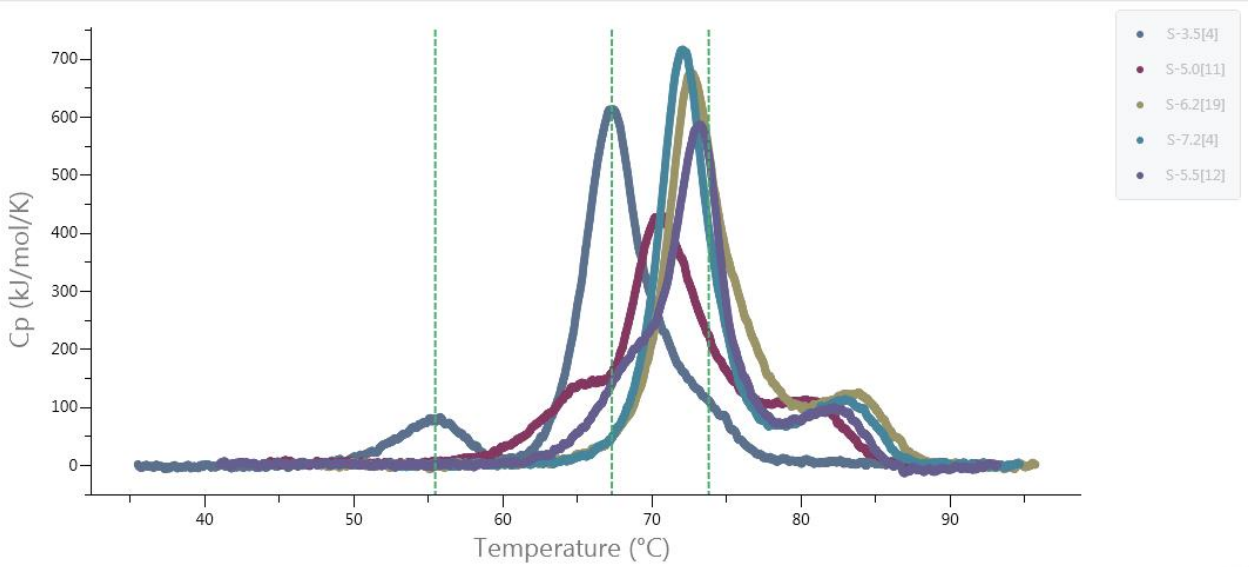


4x productivity of current DSC
Robust 'Gold Standard' data for confident decision making

MicroCal PEAQ-DSC

Formulation Screening

- Reference scans run intermittently through the series mean verifiable, optimal performance
- Efficient cleaning routines increase productivity
- ‘Rule of thumb’: samples of 0.5 mg/mL (325 μL) can often be analyzed automatically- no user input required



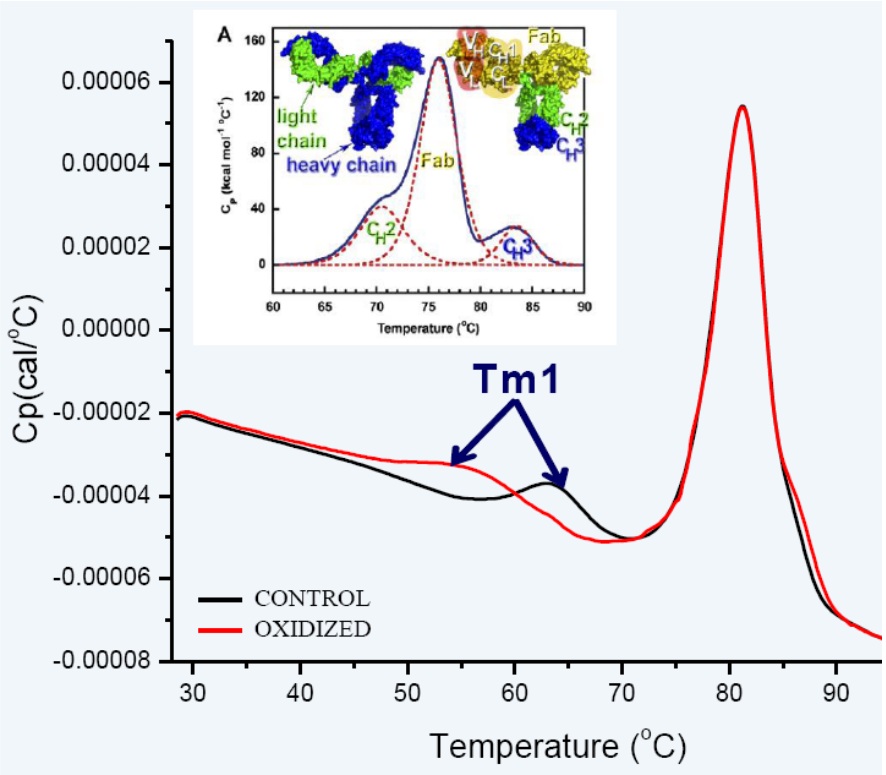
Avastin in buffer at pH 3.5, 5.0, 5.5, 6.2, and 7.2. Data supplied by John Carpenter, University of Colorado - MicroCal PEAQ-DSC Automated system

Automatic analysis = 2x productivity and more robust decision making, with less expertise needed!

MicroCal PEAQ-DSC

Manufacturing Support

- DSC has been found to be the best technique for monitoring even **primary structure** changes or deterioration in a biotherapeutic product



Now these methods can be transferred from support functions into manufacturing (GxP) departments (PEAQ-Smart and PEAQ-Compliance)

- Morar-Mitrica *et al*., BioPharma Asia, July/August 2013, 46-55
- Arthur *et al*. J Pharm Sci, vol 104, 1584-1554 (2014)

MicroCal PEAQ-DSC

Manufacturing Support

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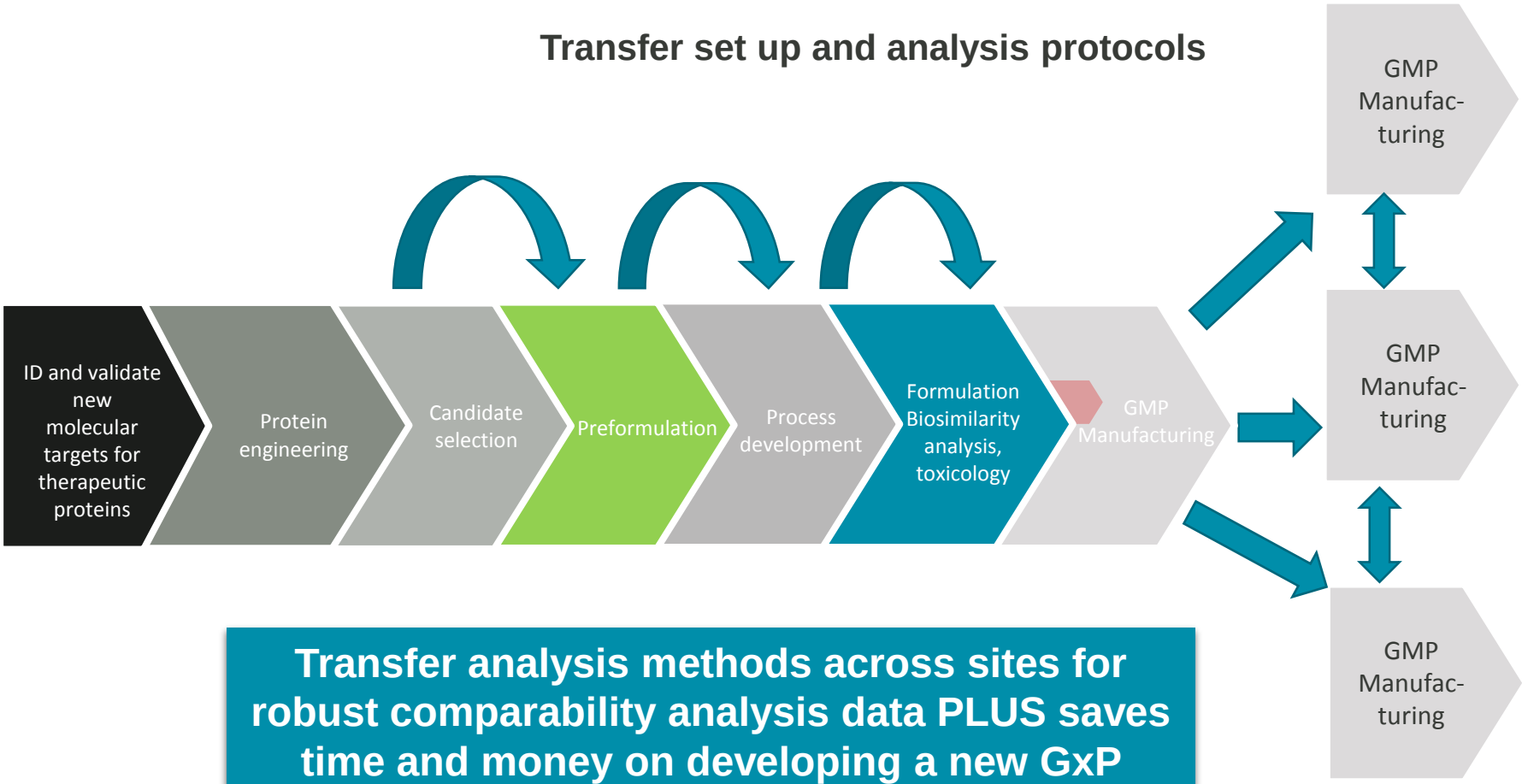
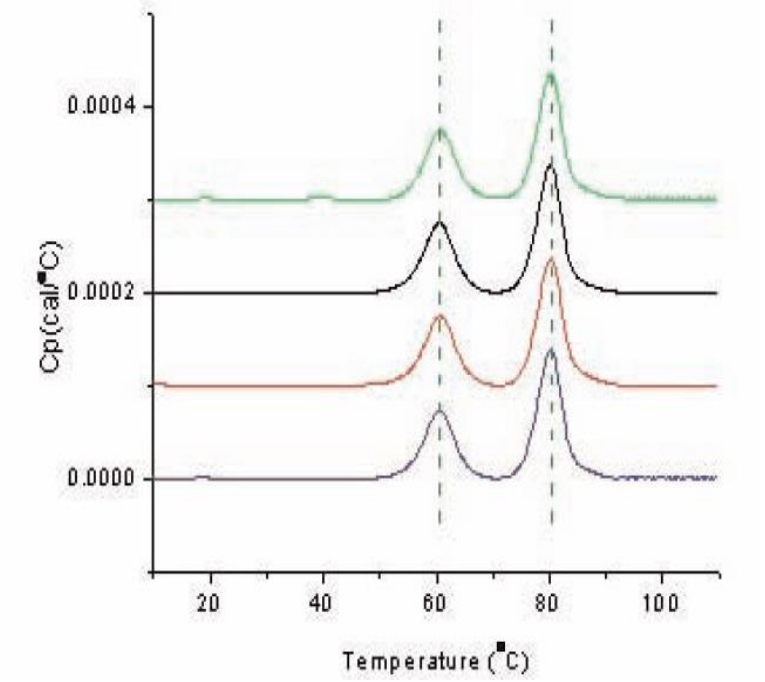


Transfer analysis methods across sites for robust comparability analysis data PLUS saves time and money on developing a new GxP assay

MicroCal PEAQ-DSC

Manufacturing Support

- DSC is sensitive and reproducible enough to assess batch to batch **biocomparability** in manufacturing



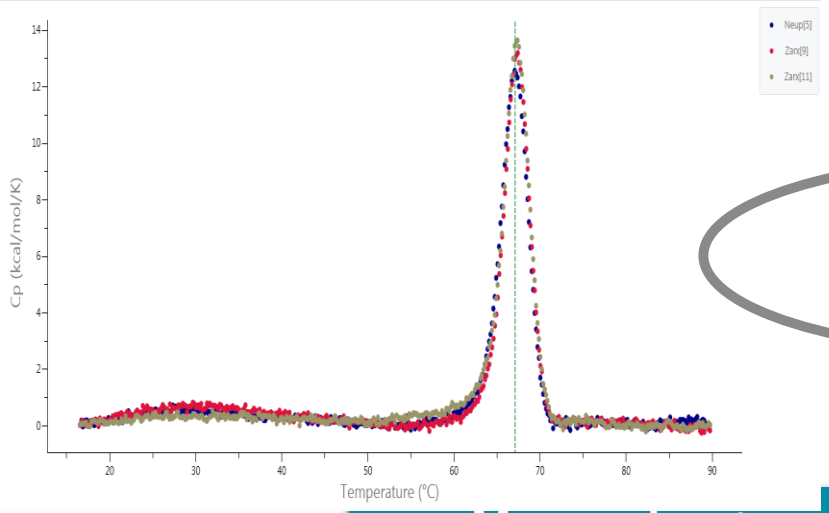
Jiang and Nahri, American Pharmaceutical Review, 2006 (online)

Change in process did not affect product quality….. and now can be used directly by manufacturing

MicroCal PEAQ-DSC

Biocomparability studies for biosimilar approval – Tm & ΔH

- A number of successful applications have used DSC data to support biosimilarity submissions- for example
  - Amgen biosimilar for Adalimumab
  - Benepali for Embrel
  - Remsima for Remicade
- Neopogen (innovator) vs Zarzio (biosimilar)



Parameters

Analysis Software Version 1.0.14200
Measurement Software Version N/A
Measurement Date Not Available
Instrument Serial Number 1

Scripted Table

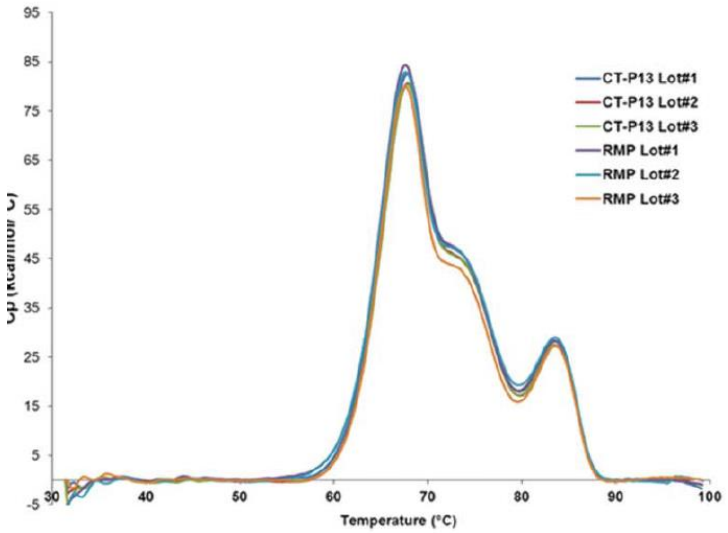
Name	Similarity Score	Total Area (kcal/mole)	Tm (°C)
Neup[5]	N/A	64.16	67.03
Zarx[9]	0.977	64.81	67.26
Zarx[11]	0.973	66.31	67.23

They're similar (both Tm and ΔH)!

More robust applications for biosimilar approval

MicroCal PEAQ-DSC

Biocomparability studies for biosimilar approval – Tm & ΔH



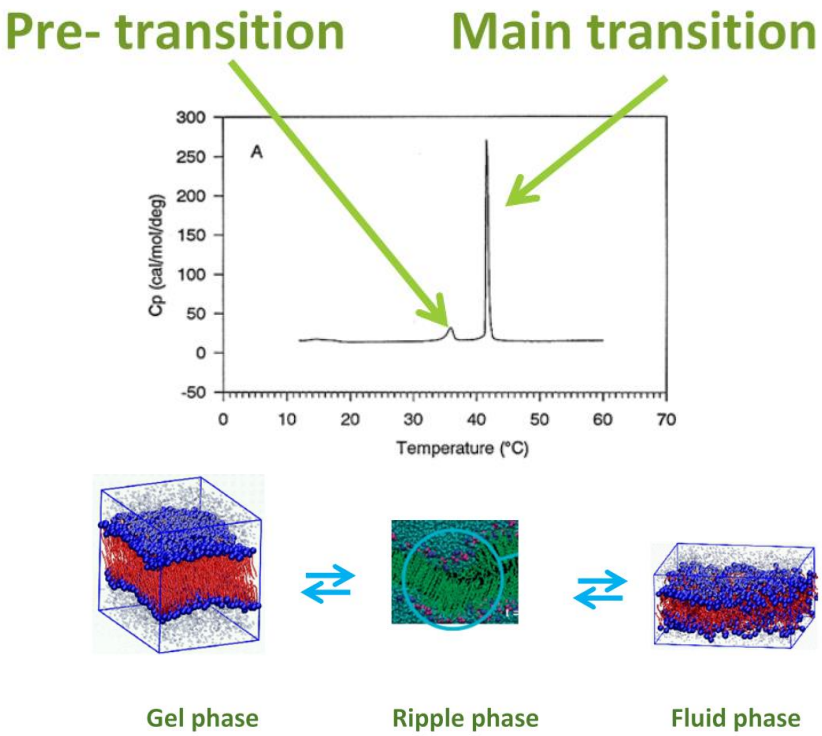
DSC thermograms of different lots of CT-P13 (Remsima) and the RMP (Remicade reference product).

The similar thermal unfolding profiles and thermal transition midpoint temperature suggest that the thermal stability and conformation of CTP13 batches are comparable to those of the RMP.

Jung, et al, mAbs, 6, 1163 (2014)

MicroCal PEAQ-DSC

Comparability Studies even for DDS – Liposome Phase Transition Temp.



Good practices for studying the stability of liposomes…; Margaride Bastos, Nanostructures & Self-Organisation R&D Group, Dept. Chem and Biochem, University of Porto.

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Liposome Drug Products

Chemistry, Manufacturing, and Controls; Human Pharmacokinetics and Bioavailability; and Labeling Documentation

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)

April 2018
Pharmaceutical Quality/CMC

2. *Physicochemical Properties*

a. Morphology of the liposomes including, if applicable, lamellarity determination.

b. Surface characteristics of the liposomes, as applicable, e.g., pegylation.

c. Net charge, typically measured as zeta potential of the liposomes.

d. Drug product viscosity.

e. Parameters of the contained drug.

f. Particle size (i.e., mean and distribution profile), preferably defined on the basis of volume or mass if particle density is known.

g. Liposome phase transition temperature.

MicroCal PEAQ-DSC
Application Advantages

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Applicatures	Supporting features	Benefits
Candidate selection	PEAQ-Performance, PEAQ-Smart	• Increased productivity • Less expertise required • More gold standard DSC • No reliance on less robust stability assays • Better Decisions
Formulations	PEAQ-Performance, PEAQ-Smart	
Process Development	PEAQ-Performance, PEAQ-Smart	
Manufacturing support	PEAQ-Performance, PEAQ-Smart PEAQ-Compare, (PEAQ-Compliance)	• Quantitative tools for comparing data • Transferability of experimental protocols • Transferability of analysis methods between people, departments and sites • DSC is more sensitive for detecting changes in HOS
Biosimilarity	PEAQ, Performance, PEAQ-Smart PEAQ-Compare, (PEAQ-Compliance)	
Manufacturing	PEAQ Performance, PEAQ-Smart PEAQ-Compare, PEAQ- Compliance	• 21 CFR part 11 compliant data for regulatory submissions • Less method development • Improved methods for monitoring certain CTQs (critical to quality attributes)

Summary

- High data integrity
  - 21 CFR Part 11
- Increased productivity
  - More use of ‘gold standard’ thermal stability assay; minimized reliance on less robust spectroscopic methods
  - Facilitates demonstration that method is ‘fit for purpose’
- Increased transferability of knowledge
  - More robust decisions
  - Less time spent on method development and validation
- Strengthened biosimilarity and biocomparability submissions
  - Pass/fail biocomparability tool
  - DSC is now more sensitive to changes in Higher Order Structure (HOS)

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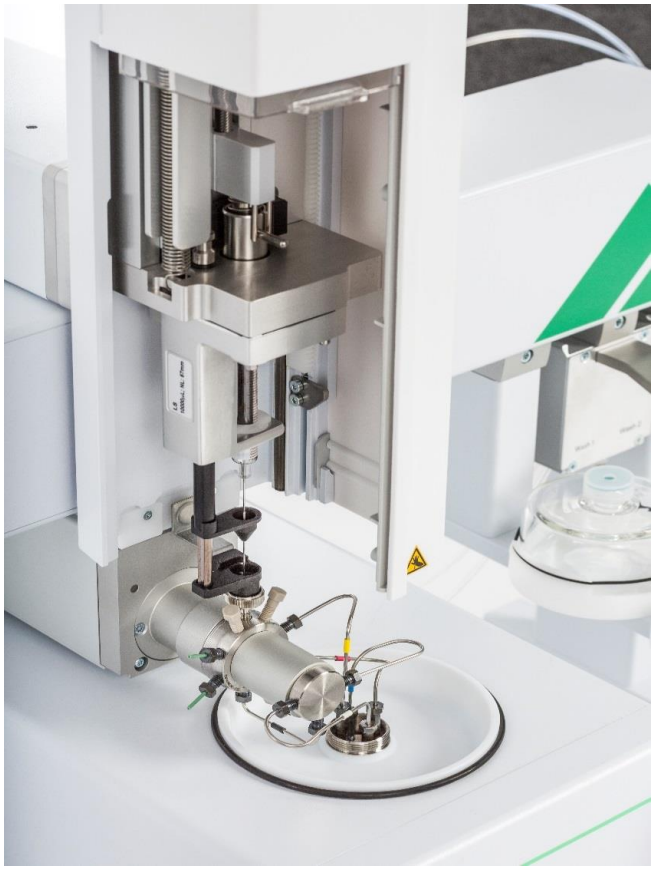


A DSC for pharmaceutical development

Pays for itself in 1 hr to 6 months

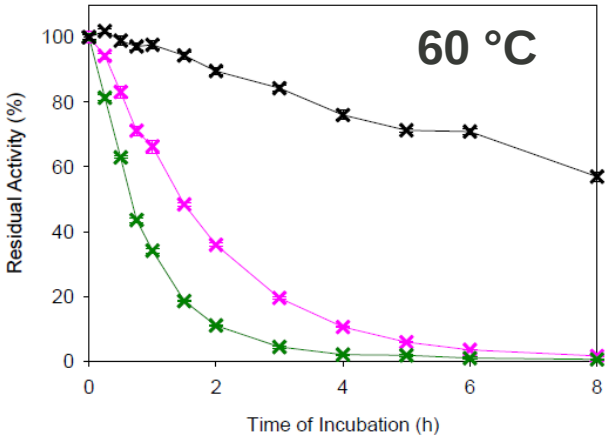
Applications	Assumptions-per project	Savings-per project	Annual savings for dept. with 20 projects per year
Candidate selection Formulations Process Development	• 20 % increase productivity of 2 people - 1 MW • False positives- 1 MW • Removing analytical step- 1 MW • False negatives-?	• $3 K • $3K • $3K • ?	• $180K
Manufacturing support Biosimilarity	• Method development costs- 4 MW • 2 fold increase productivity of an expert – 2MW • Decrease time of submissions- 4 MW	• $12K per project • $6K per project • $12K per project	• $600K
Manufacturing	• Method development costs 4 MW • Reduced submission time/ less resubmissions -4 MW	• $12K per project • $12K per project	• ~$480K
Alternatively……	• $1.5 Billion year sales • 200 ‘selling days’ per year	$7.5 M daily revenue will yield at least $1.5 M in profit	• Reach market 1 day earlier- ROI in about 1 hour
In terms of pure research	• 20 % increase in impact factor (citation points per paper)		

Different temp. different effect?

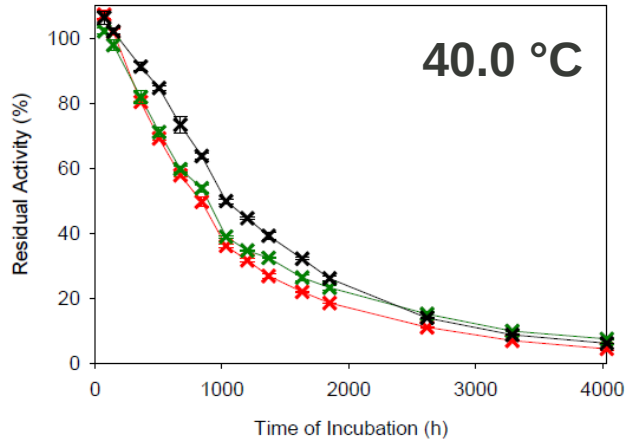
Is temperature-stress data always representative of what happens at lower temp?

- Though DSC allows us to assess stability **directly**, formulation behaviour is dependent on temperature
  - For example, NaCl destabilse Urc at 60 °C but not at 40 °C

- How can we screen stability at lower temperatures?



NaCl conc.
0 mM
150 mM
500 mM

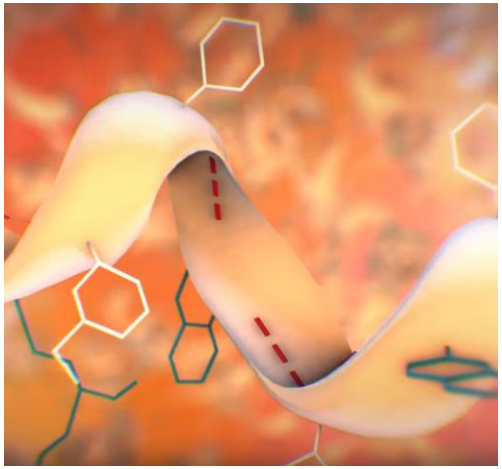


NaCl conc.
0 mM
150 mM
500 mM

Caves et al. (2013) **Biochemistry 52**: 497-507

Stopping Aggregation

Formulation Optimisation

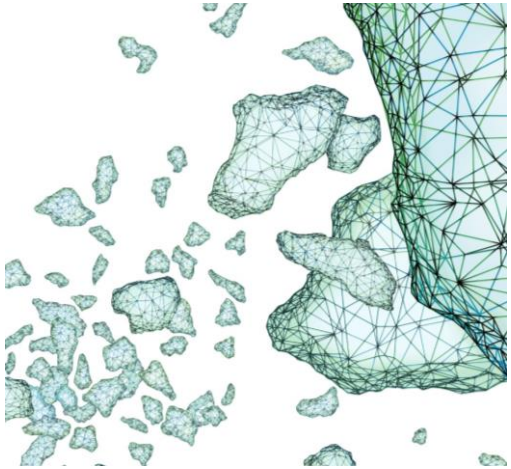


LATE STAGE SCREENING

Information is key

EARLY STAGE SCREENING

Sample Consumption is key



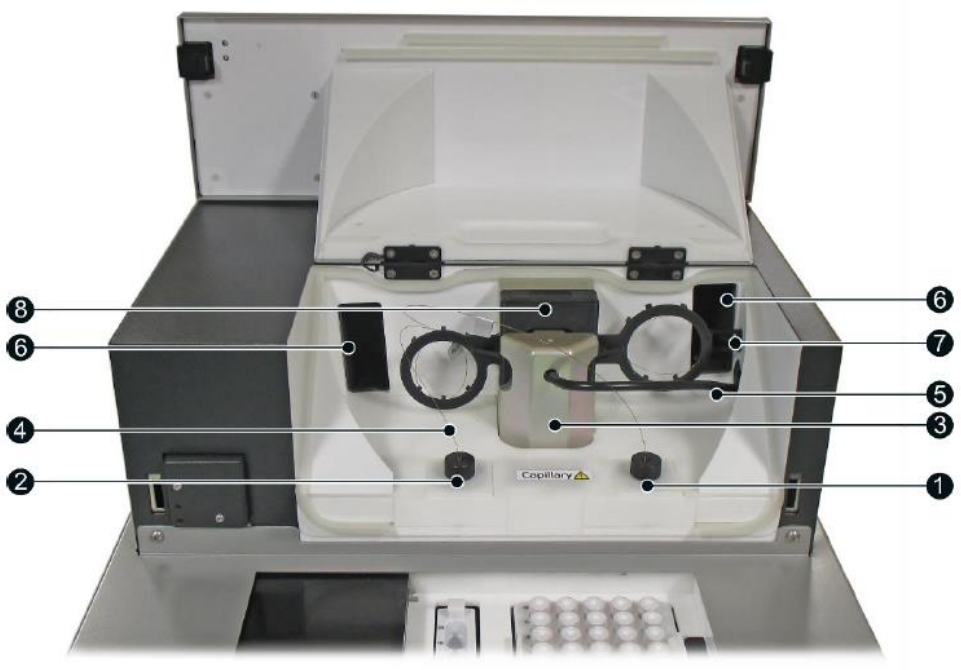
INTERACTION ANALYSIS

The fundamentals of formulation

Viscosizer TD - Overview

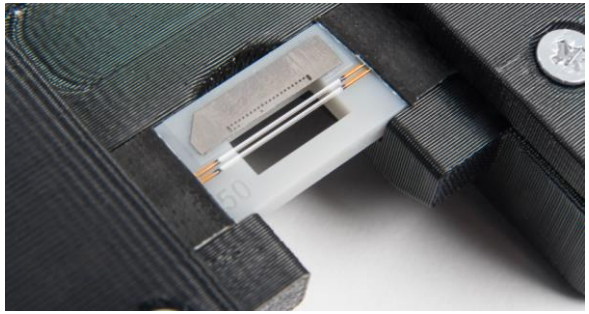
- Orthogonal biophysical technique for solution characterization
  - ***Molecular size, conformational stability and self-association, and relative viscosity***
- Low concentration measurements (µg quantities)
  - *Ultra-low sample volumes of 40nl (sizing) and 6 µl (viscosity)*
- Automated, multi-sample analysis with walk-away operation
  - *Precise environmental control for sample storage and measurement (4□C - 40□C)*
- Sample analysis without dilution or filtration
  - *Measurements not adversely affected by the presence of a small amount of aggregates, excipients or surfactants*
- Label-free characterization of target biomolecules in complex solutions
  - *Small molecules, peptides and proteins, and samples with mixtures of these species*

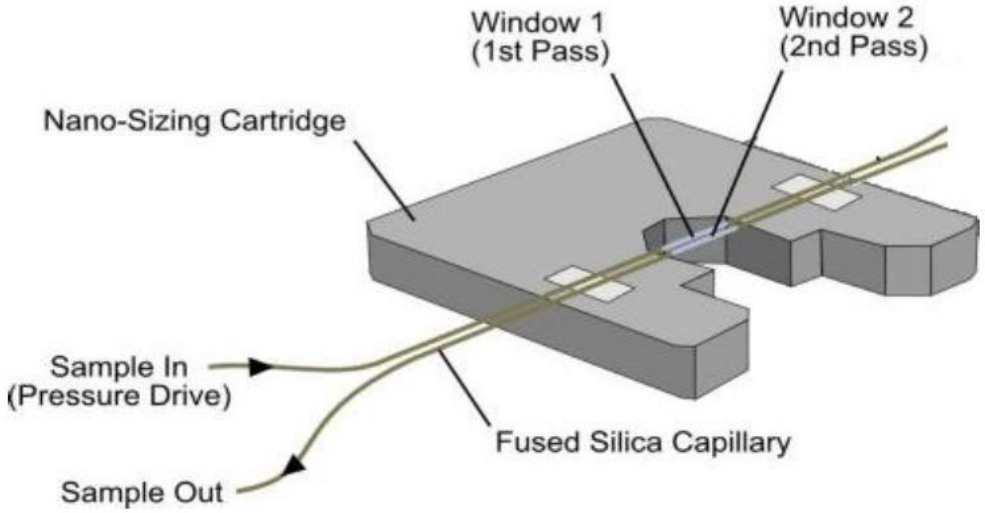
Viscosizer TD – Oven (detector head and capillary)



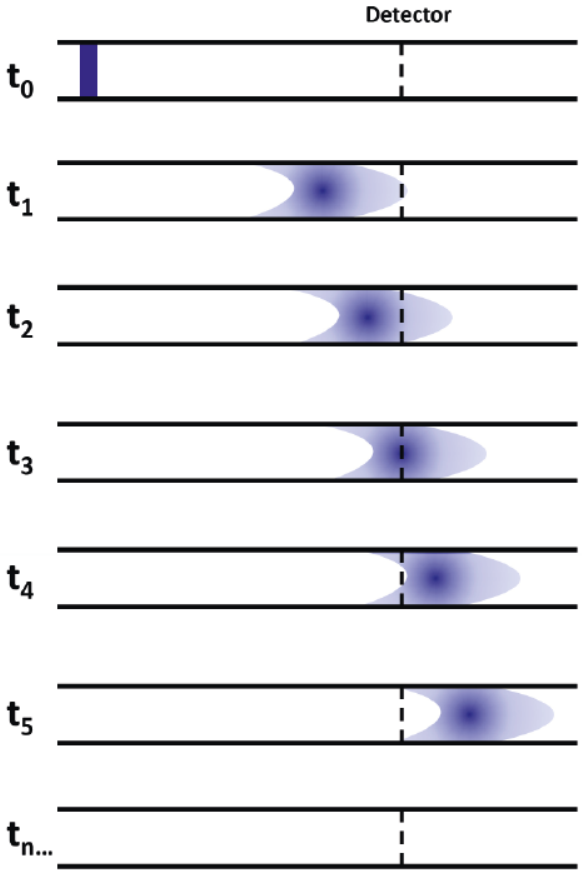
1	Capillary Inlet	5	Fiber Optic Cable
2	Capillary Outlet	6	Air Flow
3	Detector Head	7	Temperature Sensor
4	Capillary	8	Cartridge

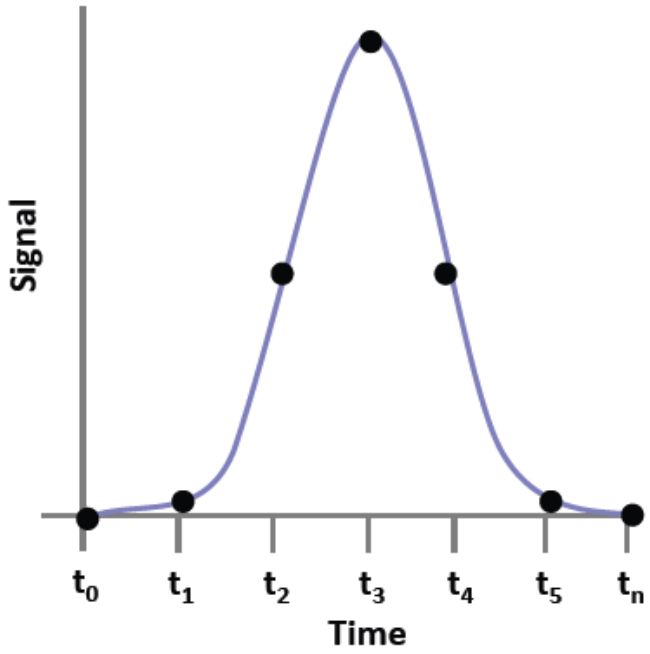
Viscosizer TD – Capillary cartridge





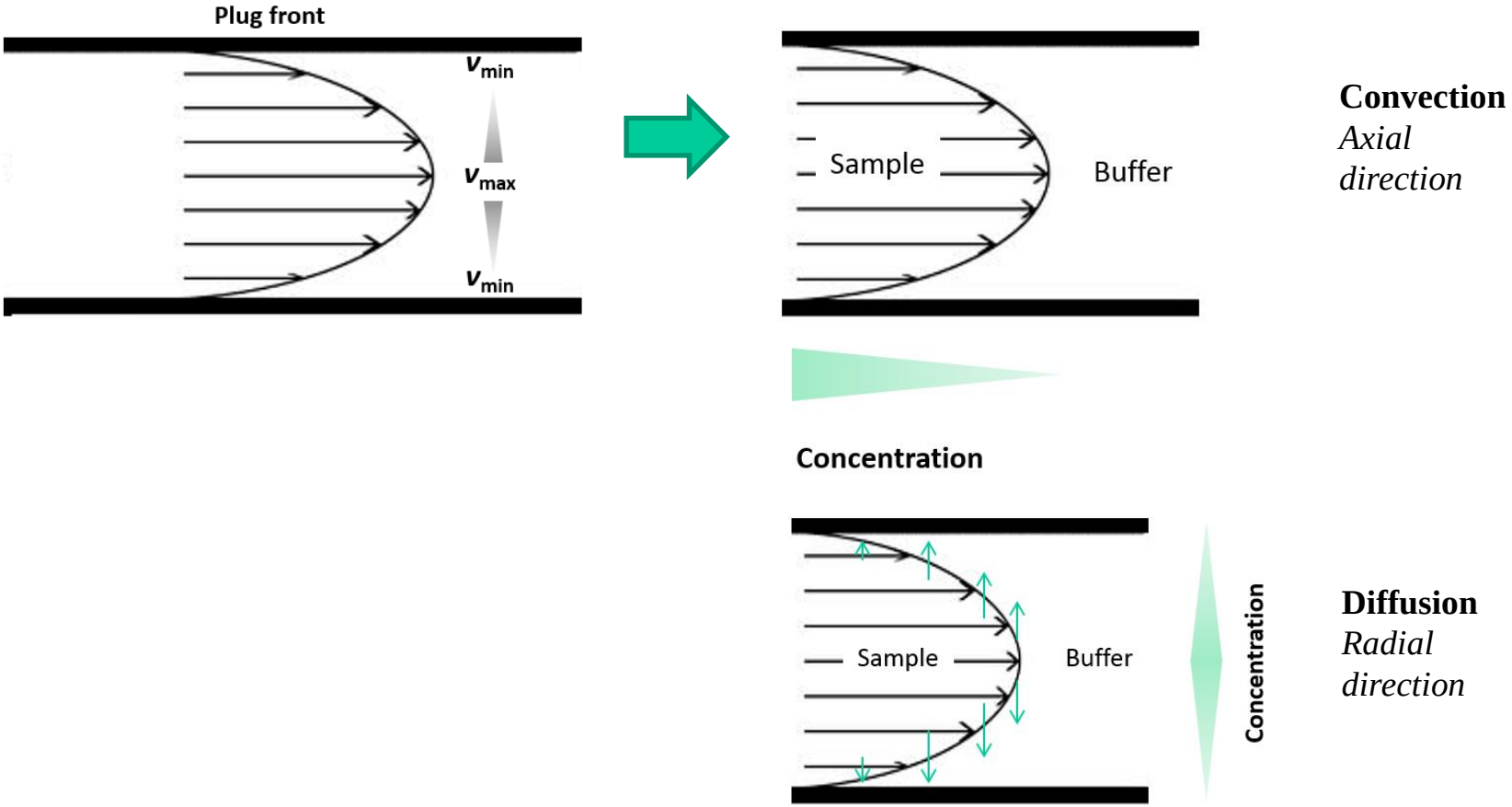
Taylor Dispersion Analysis





Understanding Taylor Dispersion Analysis, White paper; download from www.malvern.com

Taylor Dispersion Analysis

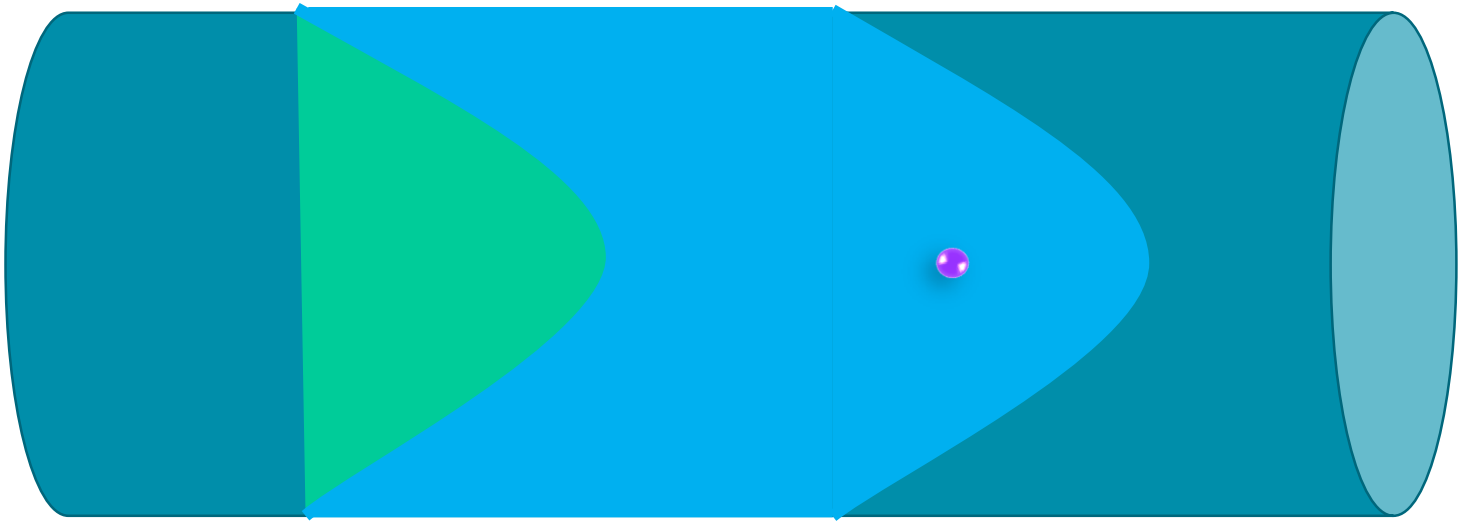


Convection
Axial direction

Diffusion
Radial direction

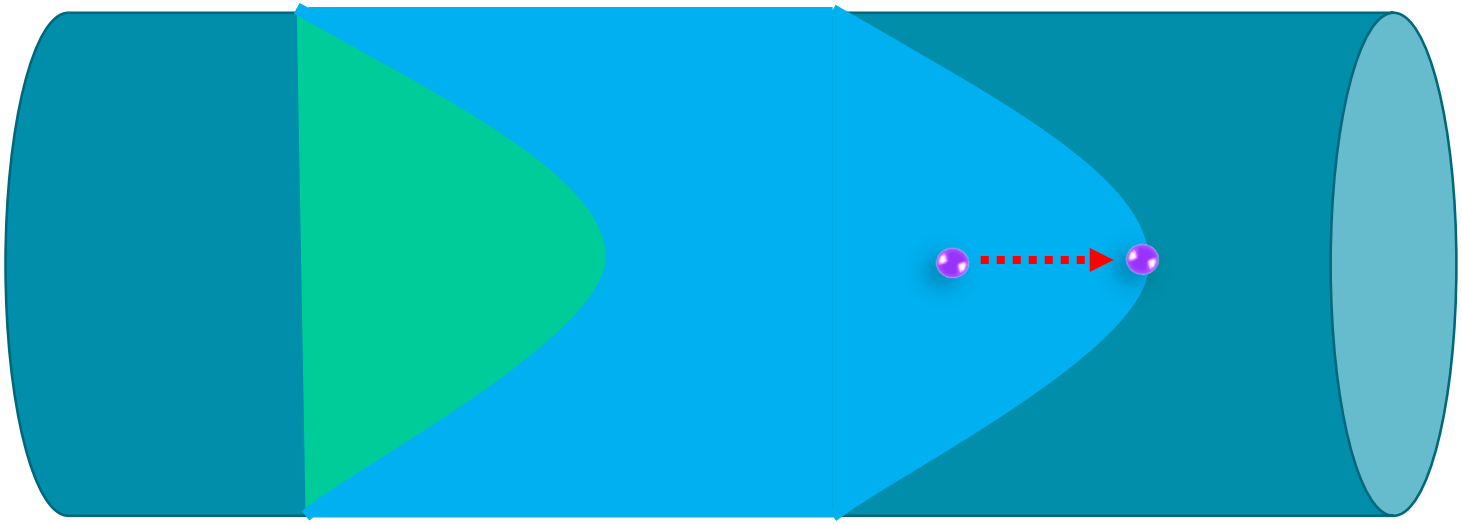
Understanding Taylor Dispersion Analysis, White paper; download from www.malvern.com

Taylor Dispersion Analysis



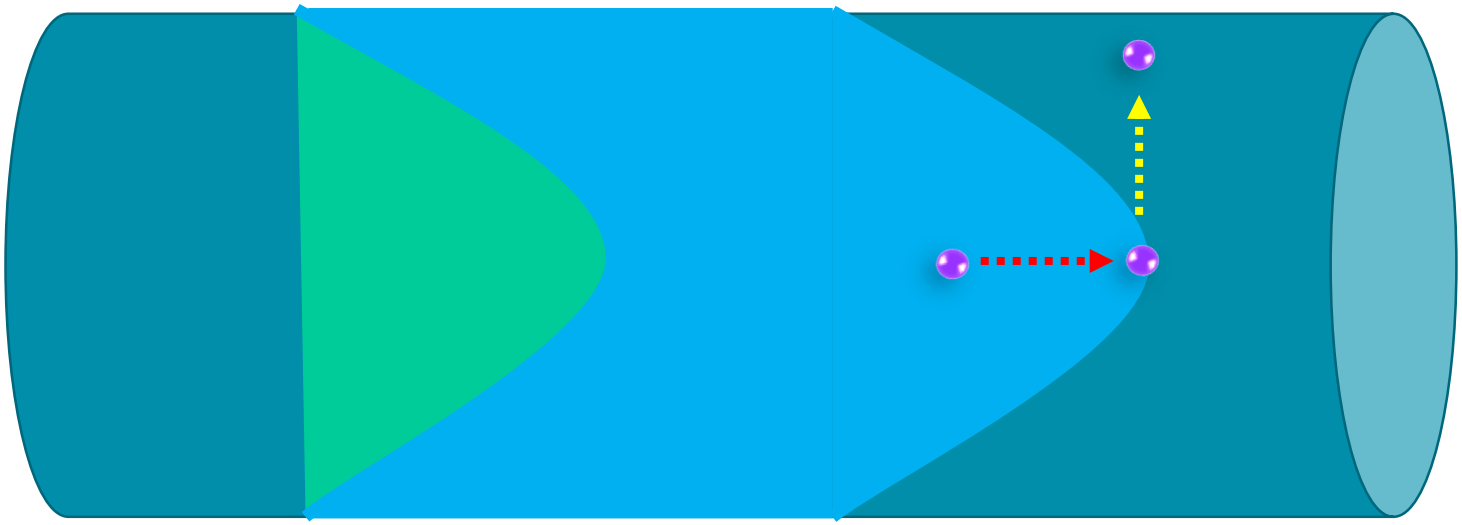
Convection ⇢ Diffusion ⇢

Taylor Dispersion Analysis



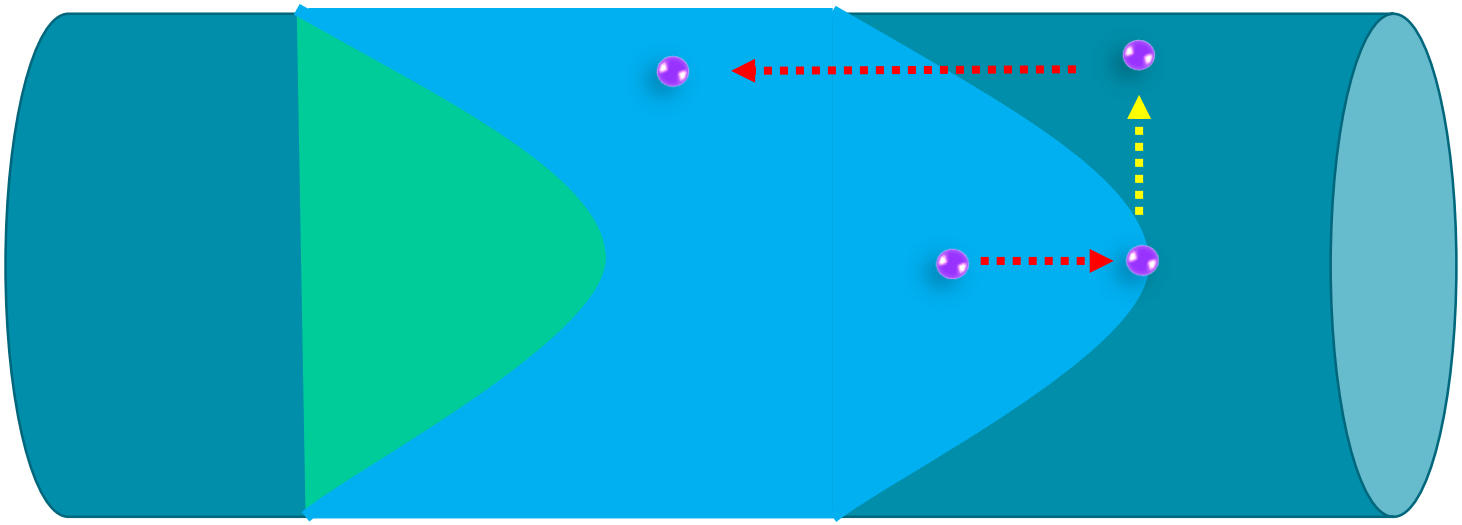
Convection ▸ Diffusion ▸

Taylor Dispersion Analysis



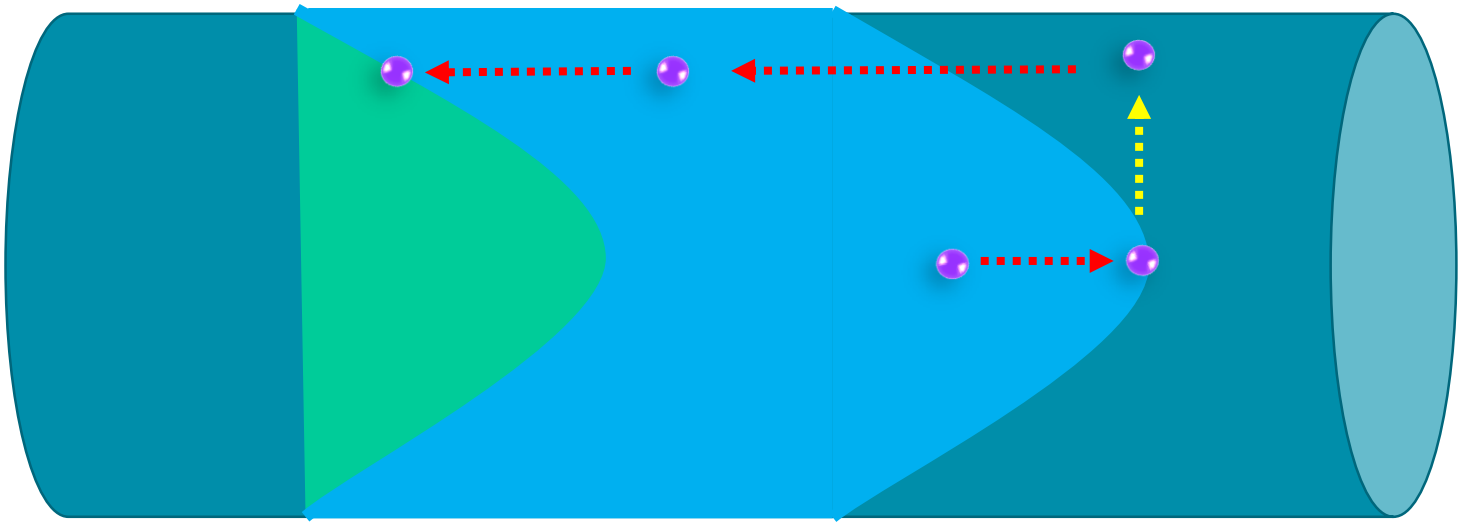
Convection ▸ Diffusion ▸

Taylor Dispersion Analysis



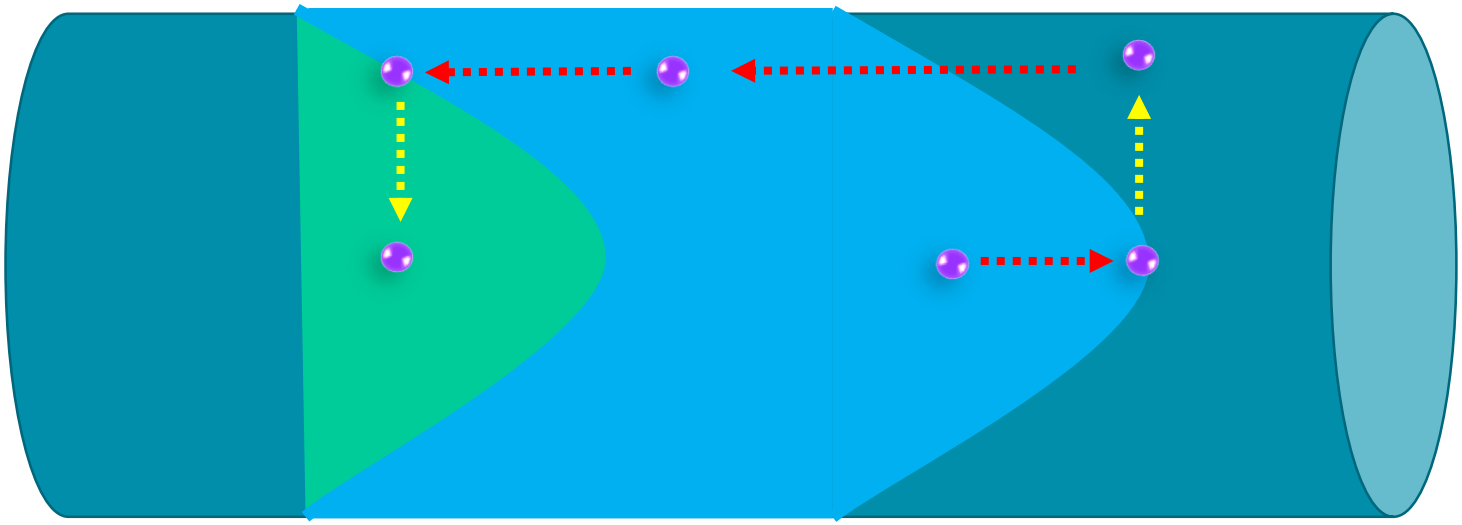
Convection ▸ Diffusion ▸

Taylor Dispersion Analysis



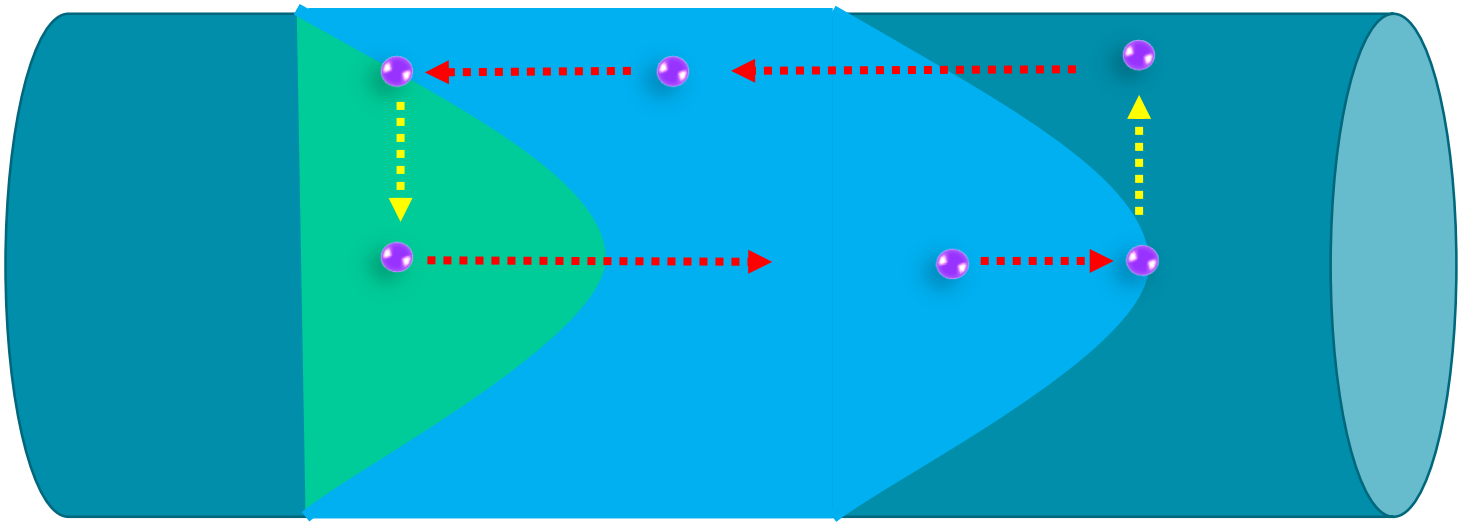
Convection ▸ Diffusion ▸

Taylor Dispersion Analysis



Convection ▸ Diffusion ▸

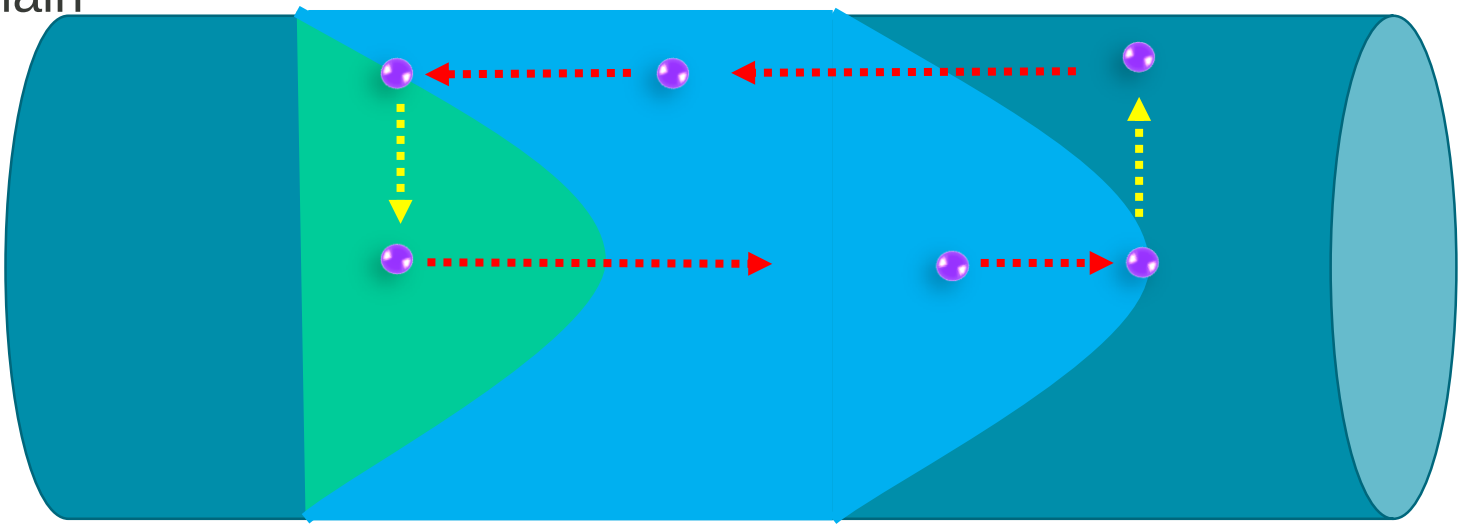
Taylor Dispersion Analysis



Convection ▸ Diffusion ▸

Taylor Dispersion Analysis

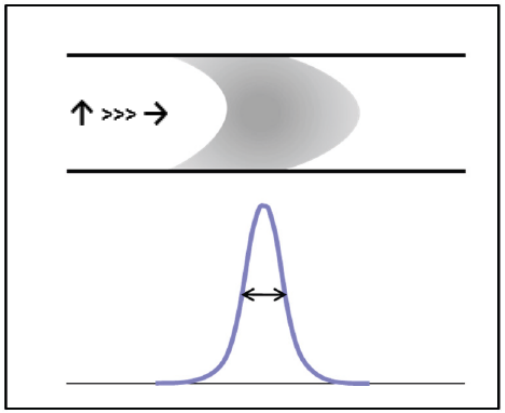
- The faster this diffusion process occurs, the narrower the plug will remain



Convection Diffusion

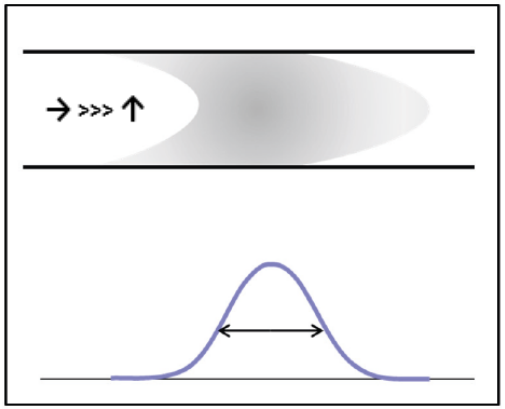
Taylor Dispersion Analysis

SMALL MOLECULES



- Small molecules diffuse quickly in the radial direction
- Diffusion works against dispersion
- Keeps the plug compact and peak narrow

LARGE MOLECULES



- Diffusion occurs slowly in the radial direction
- Plug dispersion is more dominant
- Peak broadens

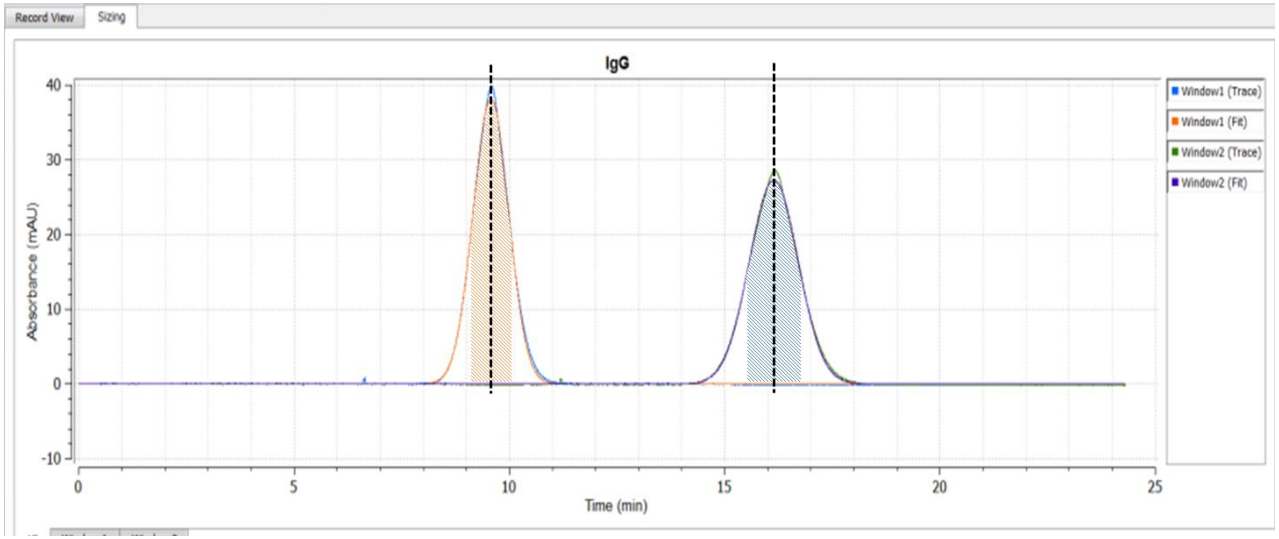
$$D = \frac{R_c^{\,2} t_o}{24 \sigma_t^{\,2}}$$

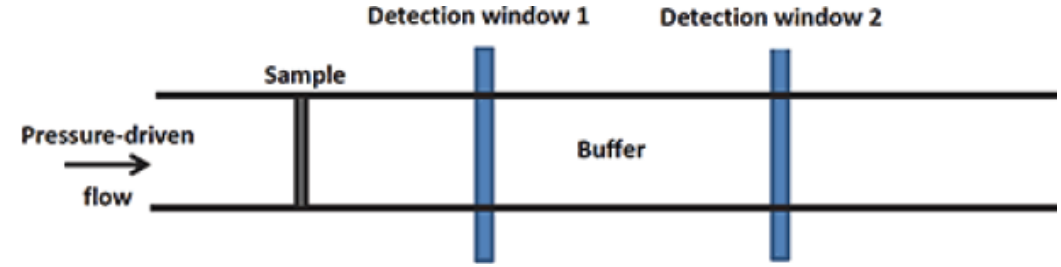
Molecular diffusion coefficient (D) is inversely proportional to peak width (σ_t) at the detection window

R_c – capillary radius
t_0 – residence time

Understanding Taylor Dispersion Analysis, White paper; download from www.malvern.com

Taylor Dispersion Analysis





$$R_h = \frac{4k_B T(\tau_{2^2} - \tau_{2^2})}{\pi\eta r^2(t_1 - t_2)}$$

R_h = Hydrodynamic radius
k_B = Boltzmann's constant
T = Temperature
τ = Peak width
η = Viscosity
r = Capillary radius
t = Peak elution time

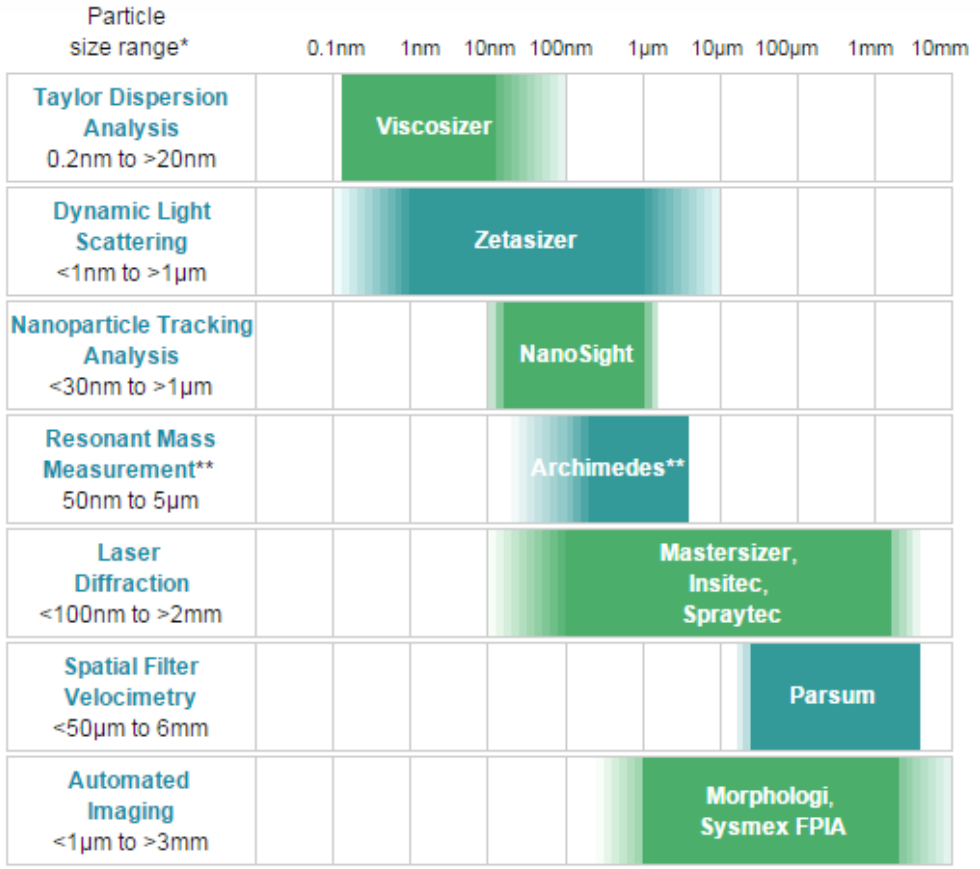
Understanding Taylor Dispersion Analysis, White paper; download from www.malvern.com

Taylor Dispersion Analysis

Malvern Panalytical

Compatible:

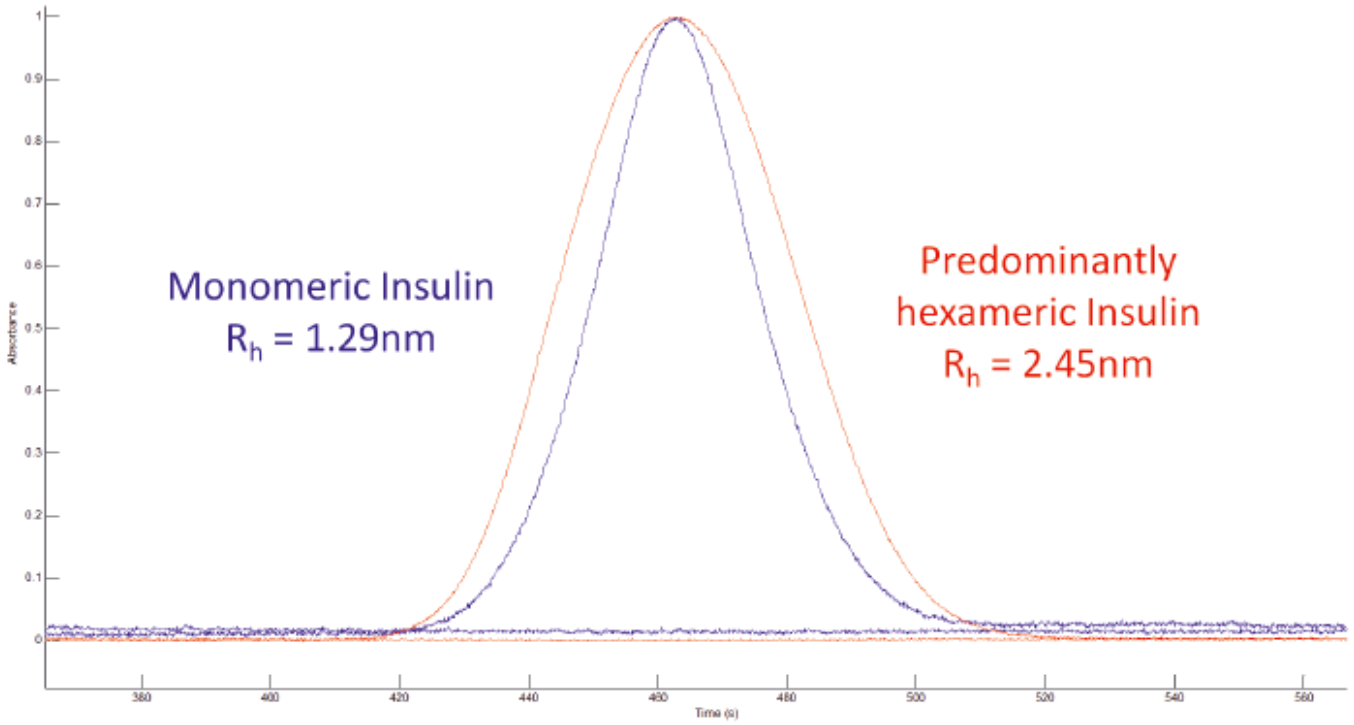
- ✓ Surfactants
- ✓ Turbid solutions
- ✓ Coloured solutions and protein samples
- ✓ High concentrations
- ✓ Non-aqueous solvents (e.g. 100% DMSO)
- ✓ Samples with aggregates
- ✓ High refractive index samples
- ✓ Small Molecules >75 Da
- ✓ Low concentrations



*all particle size ranges are sample dependent

**particle size ranges are sample and sensor dependent.

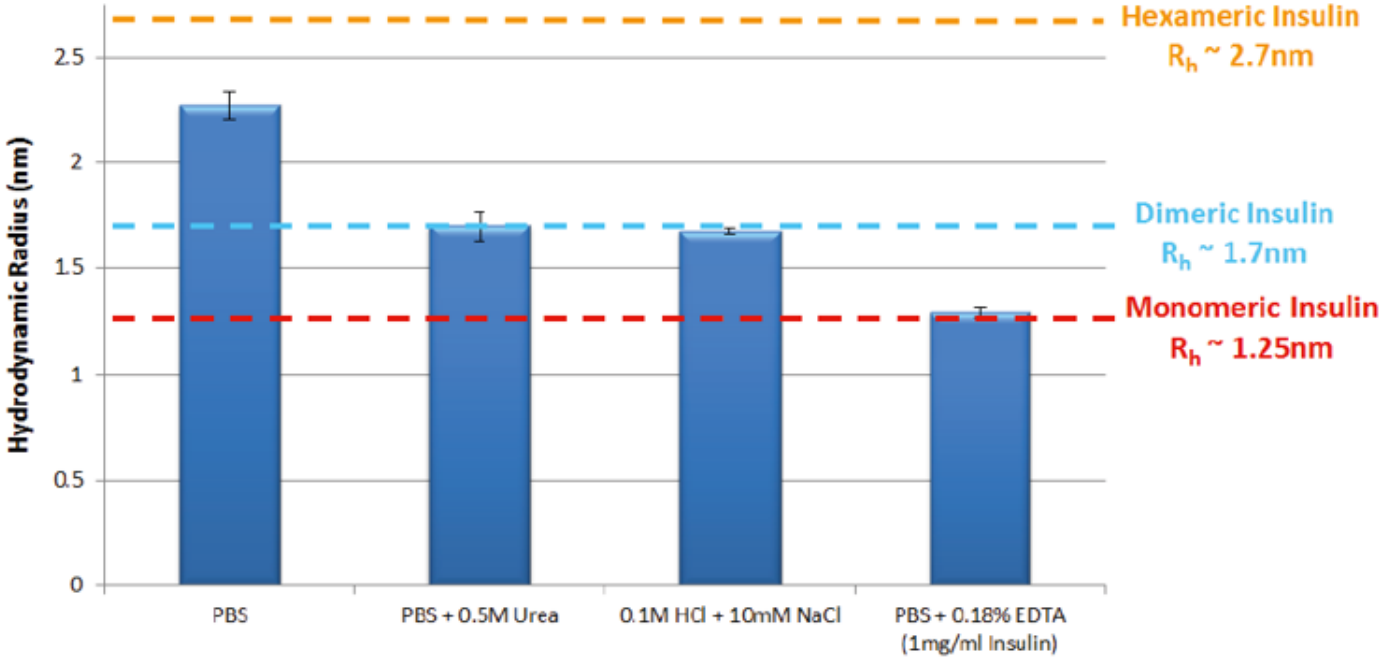
Taylor Dispersion Analysis - Proteins



Conformational change with cofactor molecules

Overlay of Taylorgrams showing change in hydrodynamic radius of Insulin after the addition of EDTA to remove Zinc ions (Absorbance normalised to 1)

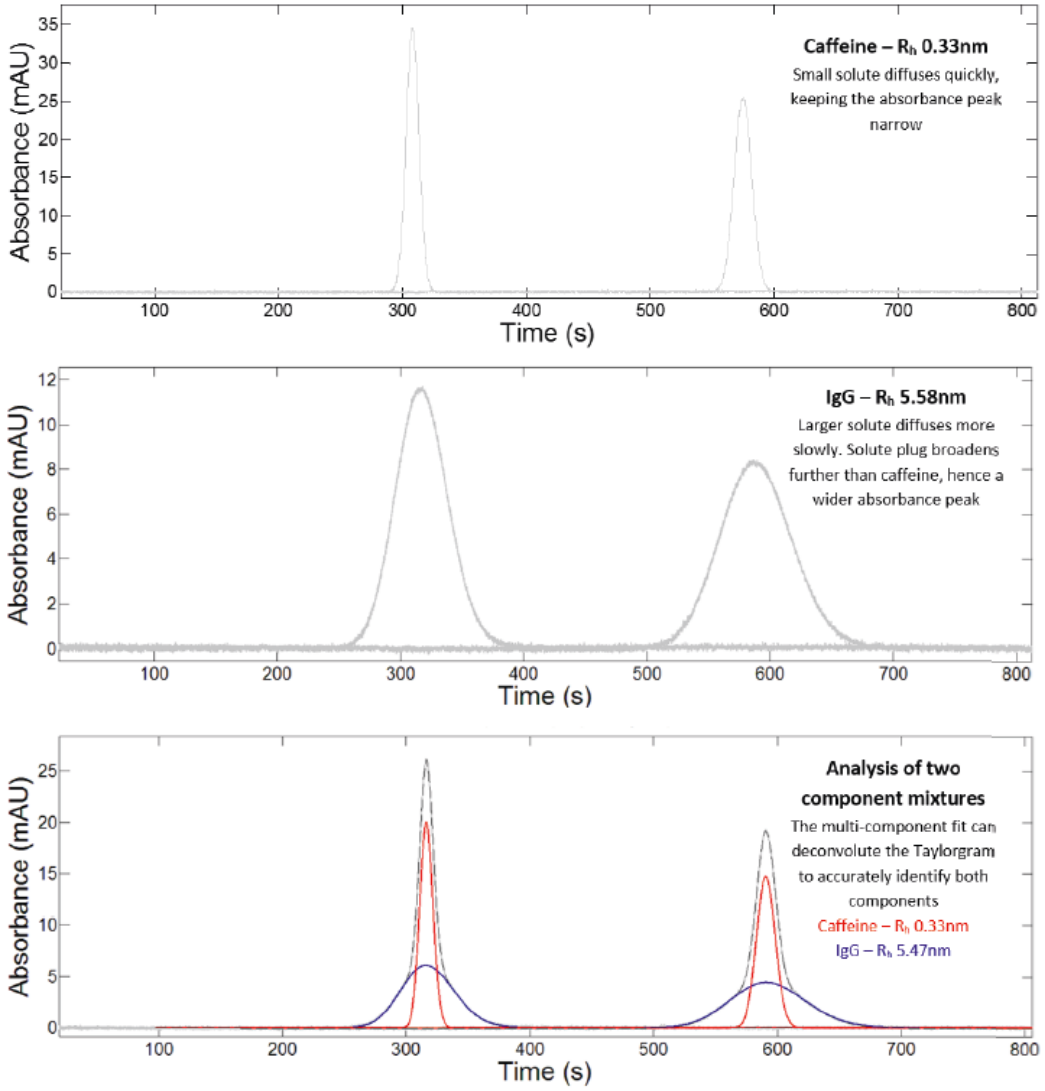
Taylor Dispersion Analysis - Proteins



Hydrodynamic radius of Insulin measured by TDA in a range of buffer conditions. Insulin concentration is 2mg/ml (unless stated).

Assessing the self-association and stability of Insulin under varying formulation conditions using Taylor Dispersion Analysis, Application Note; download from www.malvern.com

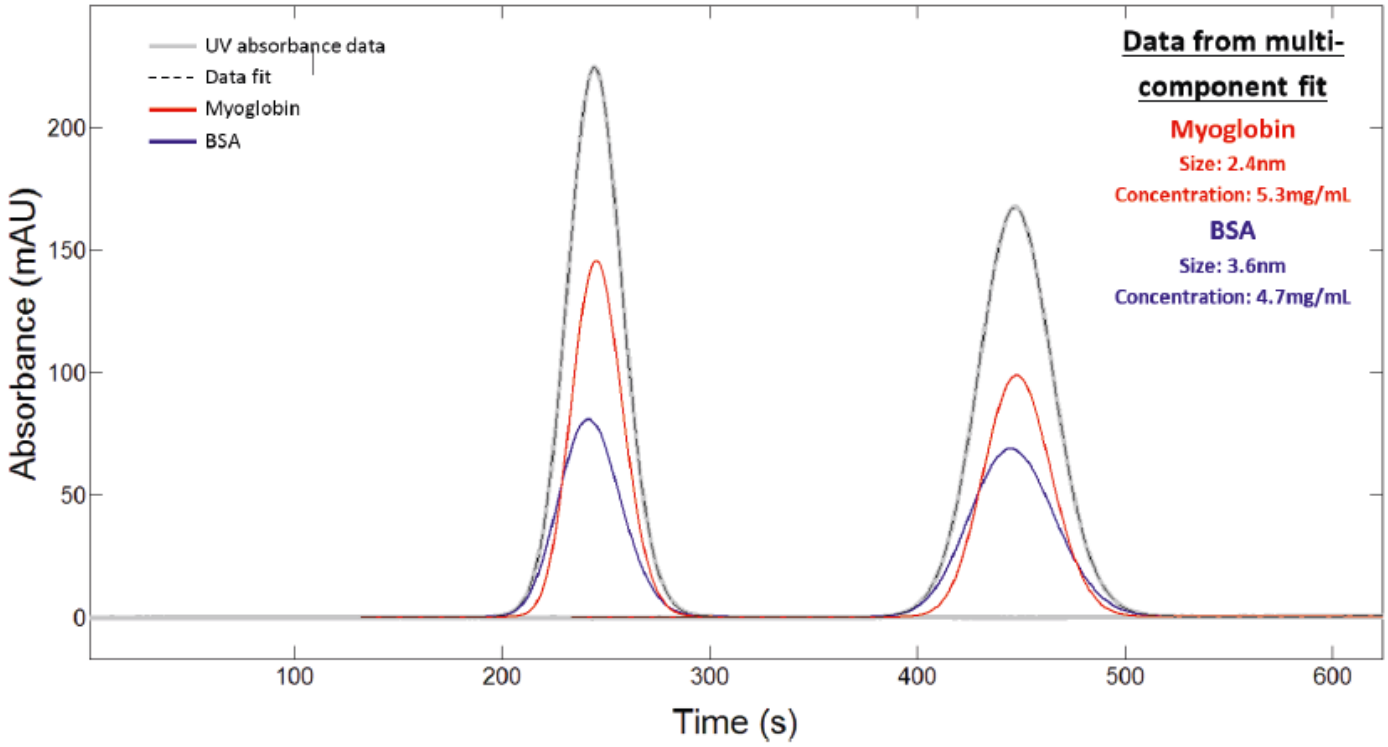
Taylor Dispersion Analysis - Mixtures



Taylor Dispersion Analysis - Mixtures

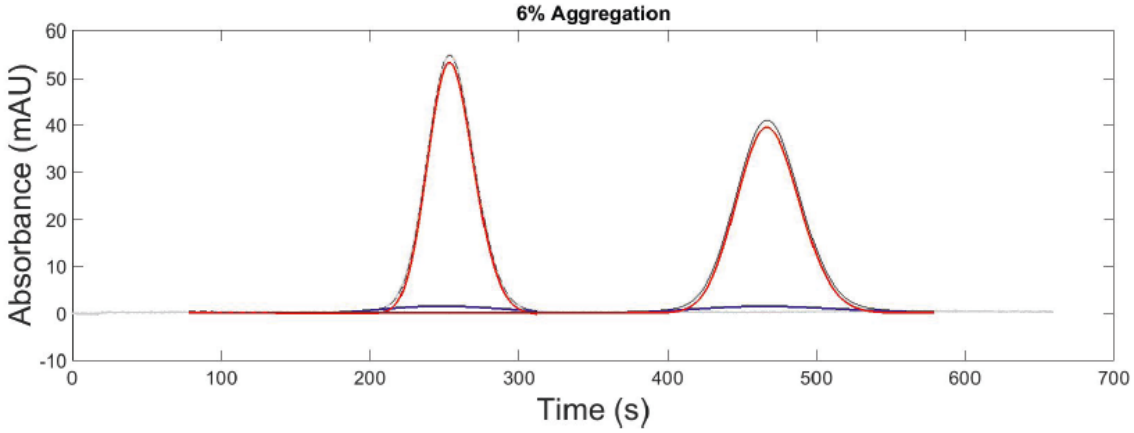
Malvern Panalytical

Taylorgram of fits to BSA (3.6nm, ~5mg/mL) and Myoglobin (2.1nm, ~5mg/mL)



Taylor Dispersion Analysis can resolve components close in size without separation

Taylor Dispersion Analysis - Mixtures



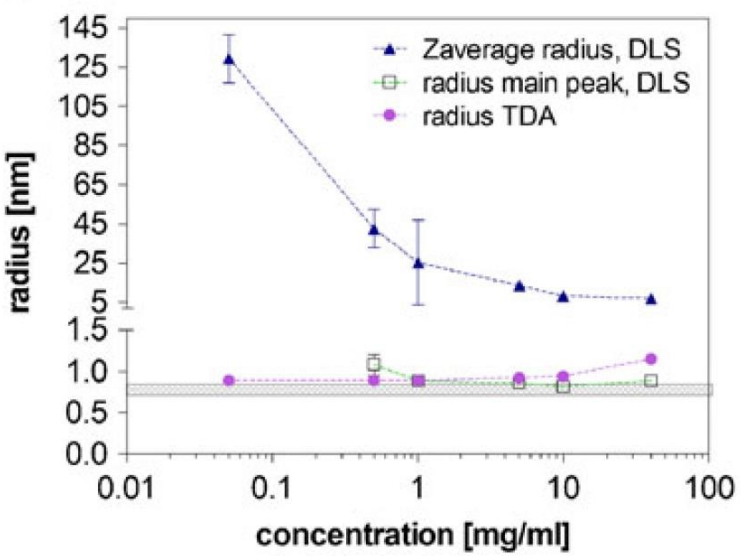
R_h of monomer/nm	Approximate sample aggregation (%)	Measured sample aggregation (%)
3.5 (PBS buffer)	3	3.5 ± 0.2
	4	3.9 ± 0.3
	6	6.8 ± 0.4
	9	7.7 ± 0.7
5 (Arginine buffer)	50	57.0 ± 0.3
	75	81.8 ± 0.2
	100	97.2 ± 5.7

Taylor Dispersion Analysis resolves monomers in the presence of aggregates, and determines % sample aggregation

TDA for Small Molecule Therapeutics

- **TDA** able to measure low concentrations of small peptides, such as **0.05 mg/ml oxytocin**

  (peptide hormone drug used to induce labour)



- Viscosizer TD can measure **sub nanometre molecules** at low concentrations with M_w < 0.1 kDa

Small Molecule	Measured D_h (nm)	Molecular Mass (Da)
Glycine	0.50	75
Nicotinic Acid	0.56	123
Caffeine	0.64	194
Warfarin	0.92	308

Hawe et al. (2011) **Pharm. Res. 28:** 2302-2310

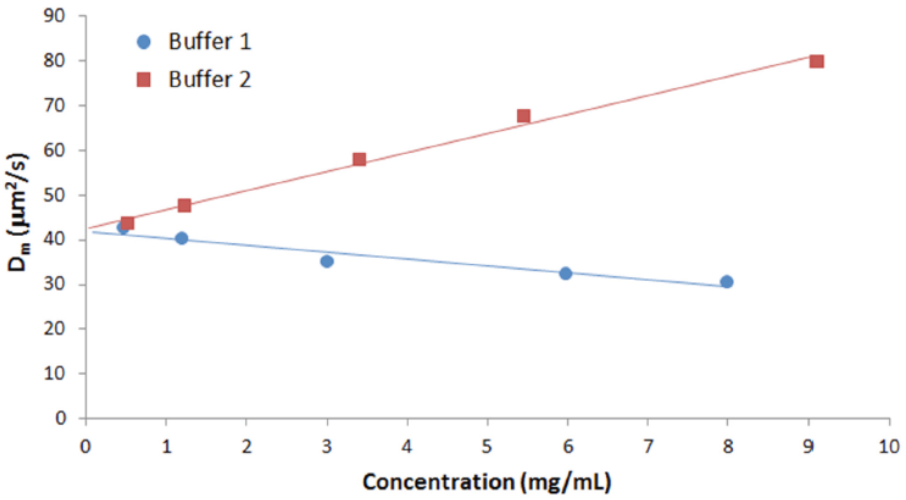
Measurement of KD

Dynamic Virial Coefficient

- Virial coefficients used to find wide application to, for instance, crystal screening
  - The more positive the coefficient, the higher the stability of the formulation

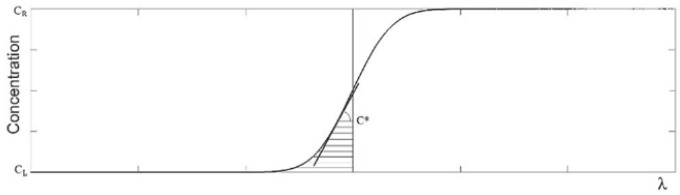
- Improvements in Crystallisation and formulation robotics, however, made the old way of measuring virial coefficients redundant

- Viscosizer gives us a newer, more efficient means of measurement



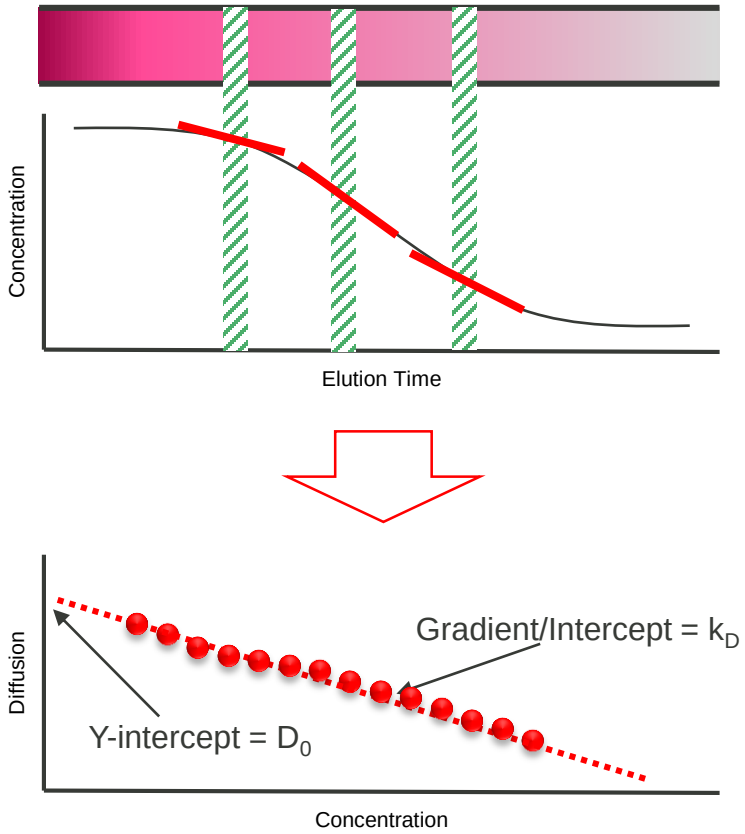
Exploiting the Gradient

- Boltzmann-Matano Analysis
  - This analysis model allows the calculation of Diffusion, from a gradient generated using Fick's Law of Diffusion
  - Calculation of diffusion distance as a function of concentration



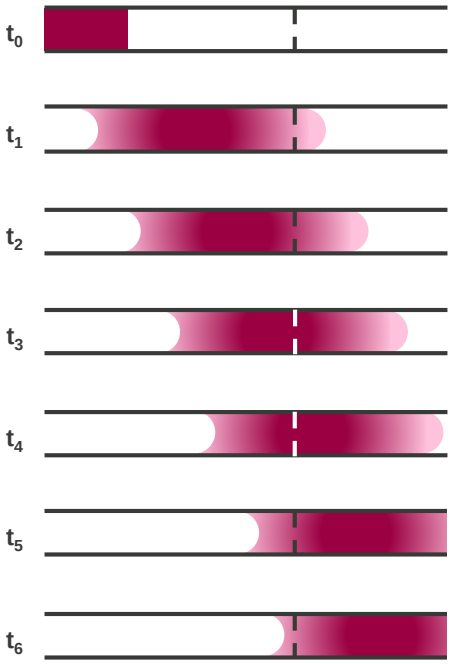
  - Value reported is Dispersion Coefficient (k) as sample is undergoing both convection and diffusion
  - Application of this value to Taylor's Diffusion equation provides Diffusion (D) as a function of concentration

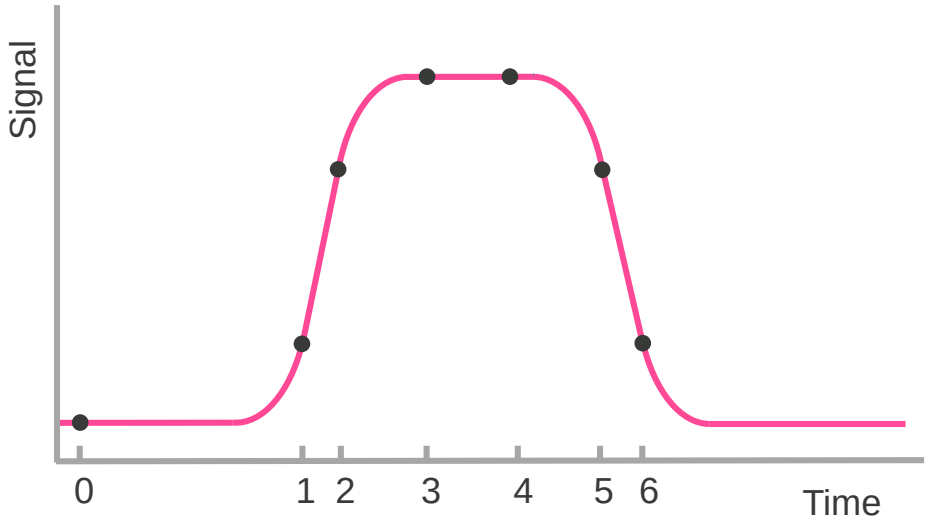
$$D = \frac{r^2 u^2}{48k}$$



Diffusion Interaction (k_D) Analysis

Calculated using the gradient





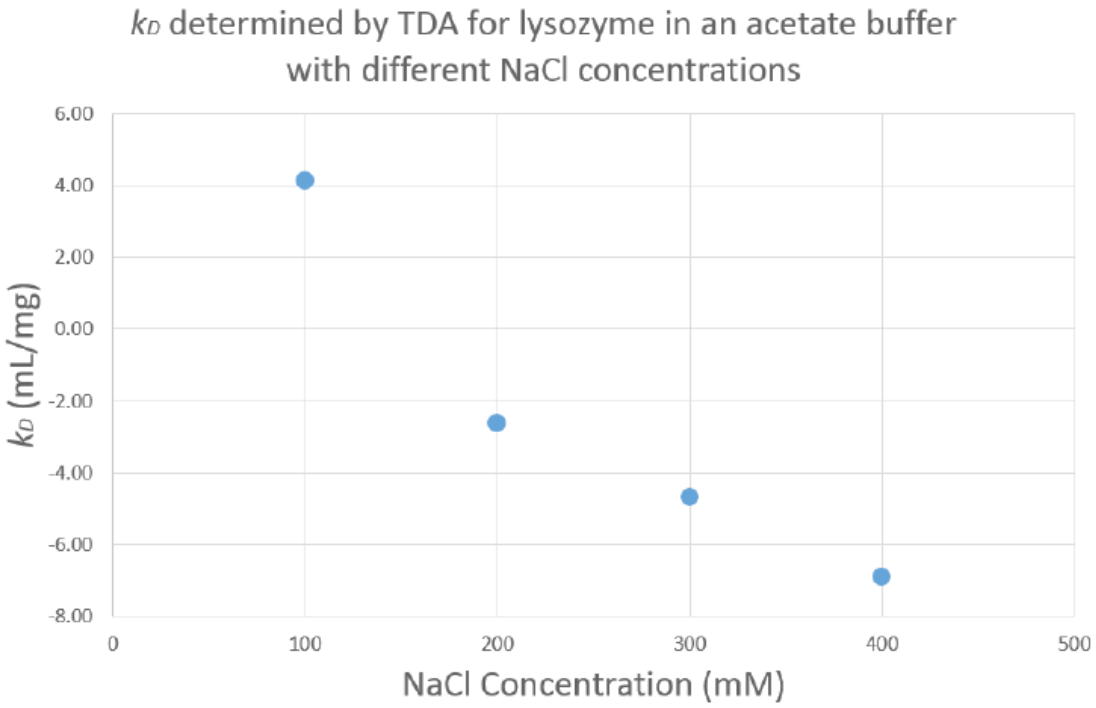
Diffusion Interaction (k_D) Analysis

- Comparison of k_D data from TDA and DLS

	k_D from TDA (10^{-2} mL mg^{-1})	k_D from DLS (10^{-2} mL mg^{-1})	D_0 from TDA (μm^2 s^{-1})	D_0 from DLS (μm^2 s^{-1})
BSA in Iodide	1.61 +/- 0.05	1.30 +/- 0.01	54.4 +/- 0.2	59.7 +/- 0.4
BSA in Sulfate	0.93 +/- 0.04	0.75 +/- 0.01	54.3 +/- 0.3	59.7 +/- 0.4

  - Results show consistent data between DLS and TDA assessment of k_D

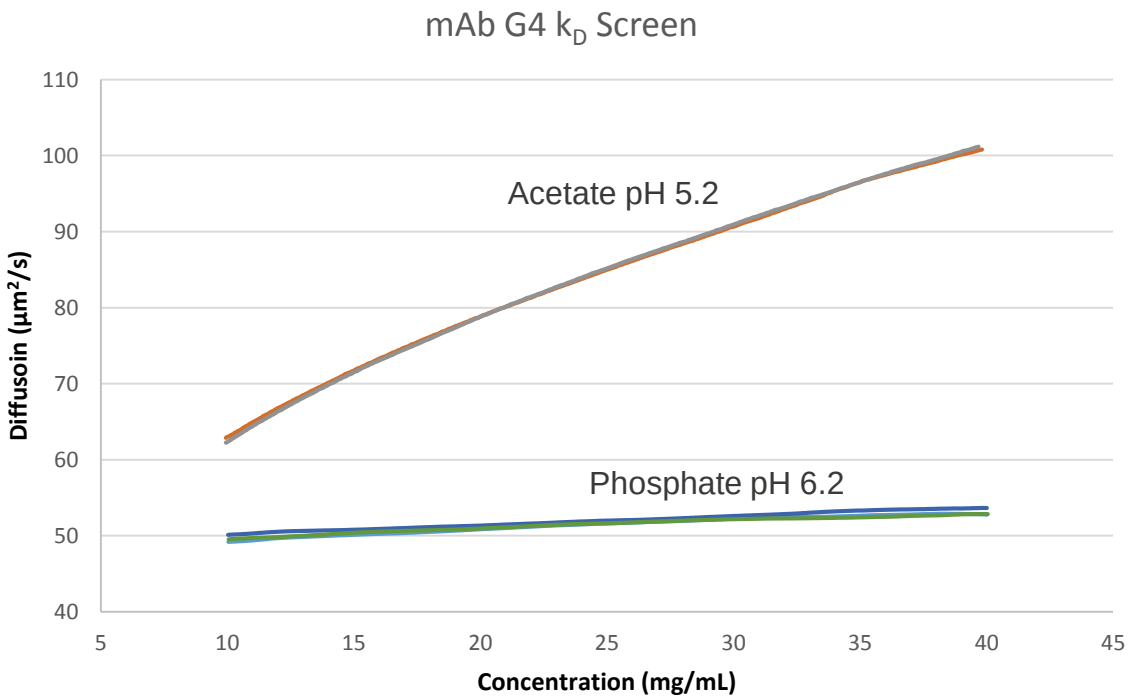
Diffusion Interaction (k_D) Analysis



- Increasing salt concentration results in a reduction in measured k_D value for lysozyme

Diffusion Interaction (k_D) Analysis

- Antibody (mAb – G4) in two buffer formulations
  - Buffer A – Acetate pH 5.2
  - Buffer B – Phosphate pH 6.2



Acetate pH 5.2	
Replicate 1	23.41
Replicate 2	23.69
Replicate 3	24.53
Average	**23.88**
StDev	**0.58**

Phosphate pH 6.2	
Replicate 1	2.59
Replicate 2	2.46
Replicate 3	2.24
Average	**2.43**
StDev	**0.18**

Visosizer TD – KD as a true stability screening method

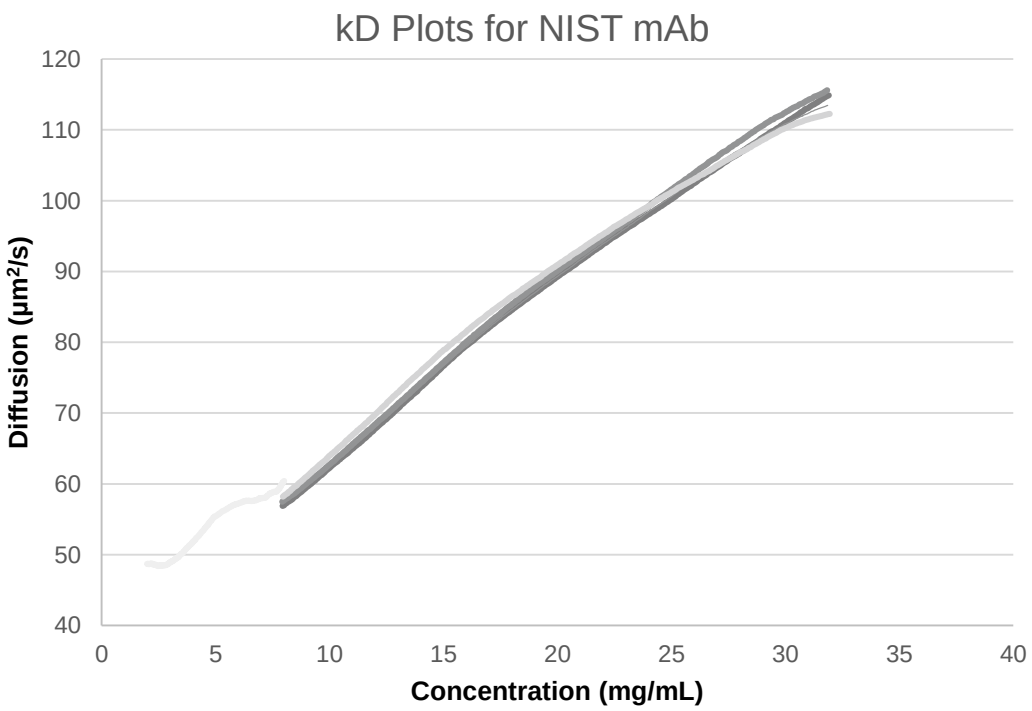
NIST mAb data

Data Summary

Sample	Gradient	Y-Intercept	kD	Average	SD
40mg/mL	2.445	38.896	62.868	60.7	4.3
	2.491	38.923	64.011		
	2.424	39.451	61.448		
	2.316	42.518	54.480		
10mg/mL	2.160	43.365	49.808	46.5	2.9
	2.052	44.084	46.557		
	2.076	44.056	47.116		
	1.945	45.591	42.658		

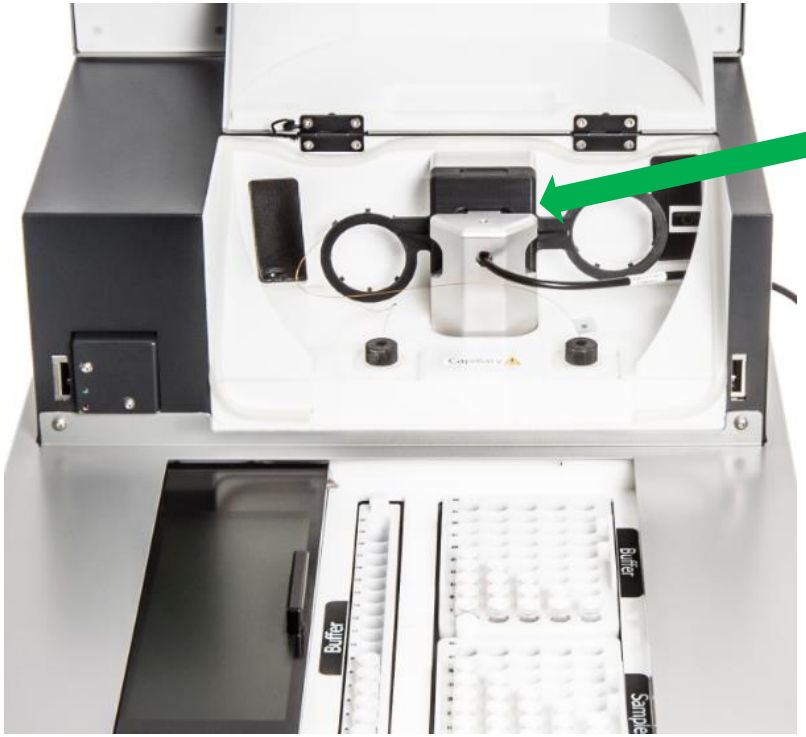
Sample Consumption

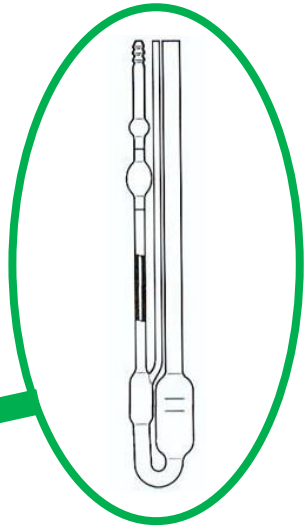
Test	Volume Used (µL)	Stock Conc (mg/mL)	Mass Used (µg)
DLS	50	40	2000
TDA High Conc	12	40	480
TDA Low Conc	12	10	120



Relative viscosity

Another Stability Indicator





Viscosizer TD - relative viscosity measurement concept

Microcapillary analogue of glass U-tube relative viscometer principle using Poiseuille's Law

Time sample flow between two windows under constant uP and reference with sample of known viscosity

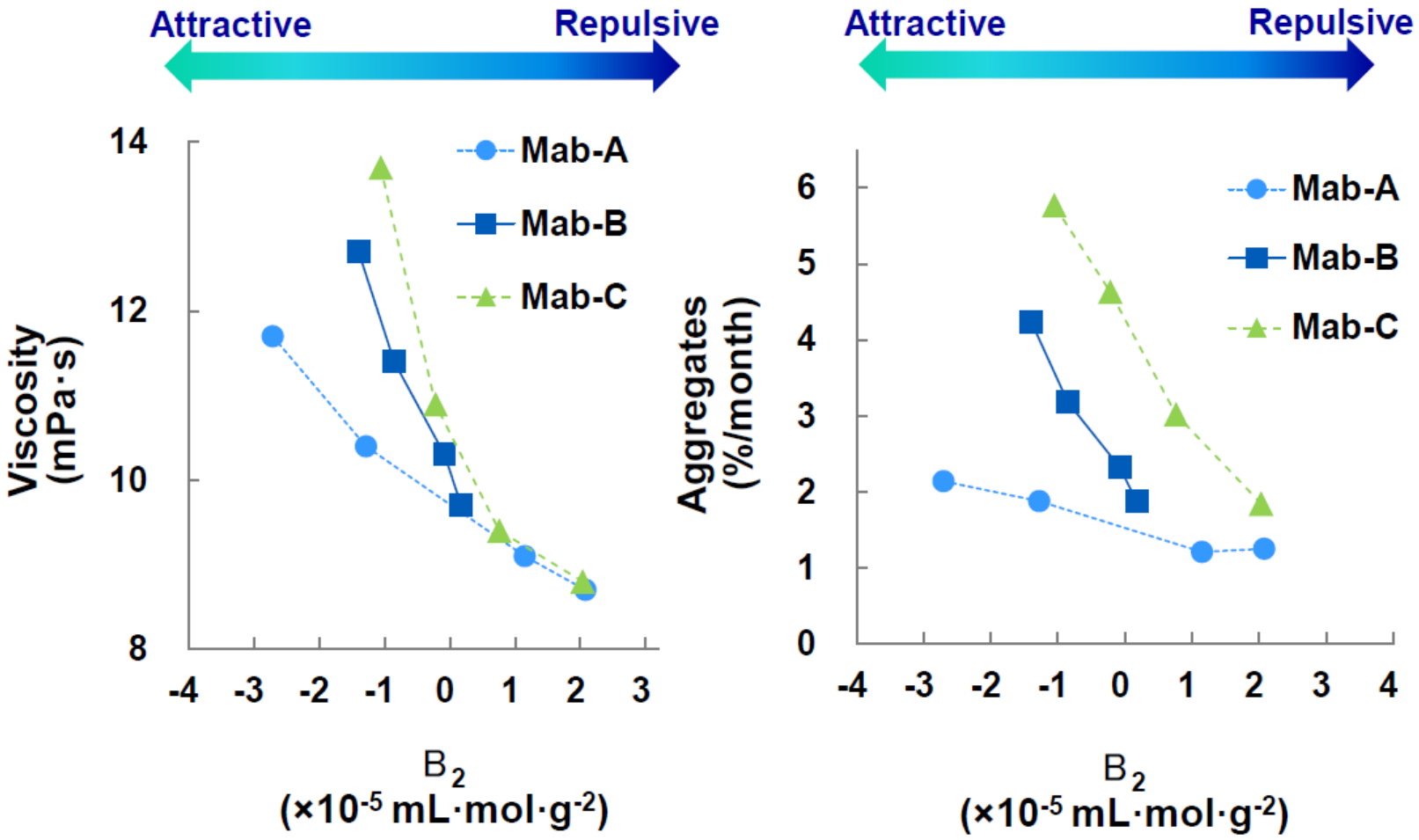
- ✓ **Low volume (~6ul per measurement)**
- ✓ **Automated**
- ✓ **Temperature controlled**
- ✓ **Newtonian liquids**
- ✓ **Suitable for low viscosity and low concentration protein solutions**
- ✓ **Fast measurements with high resolution and repeatability in this regime**

Principles of relative viscosity measurements using Viscosizer TD, Technical Note; download from www.malvern.com

Relative viscosity

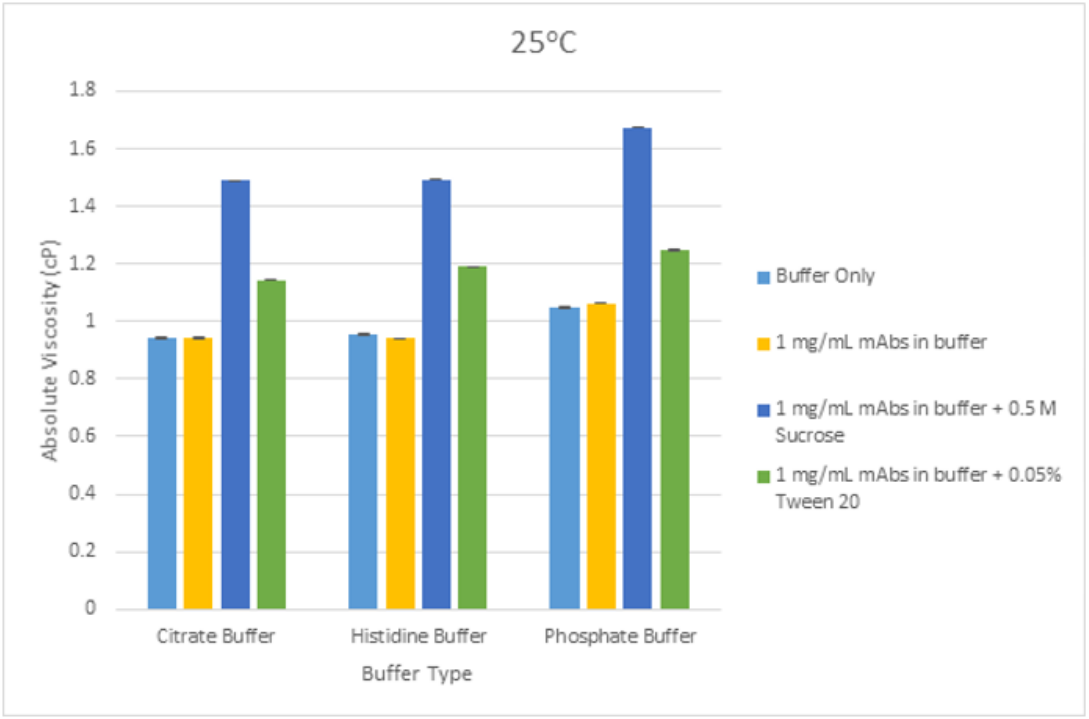
Another Stability Indicator

Malvern Panalytical



Saito, Uchiyama et al., 2012 Pharm. Res.

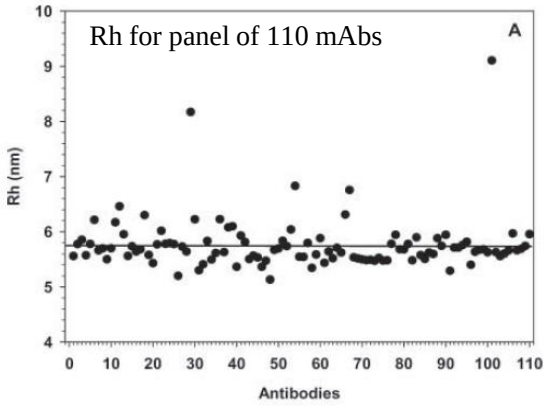
Relative viscosity – mAb formulations

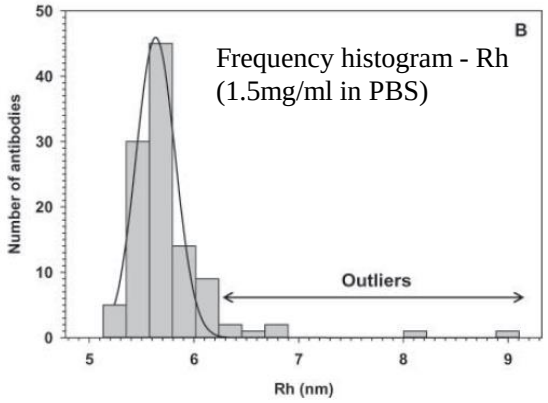


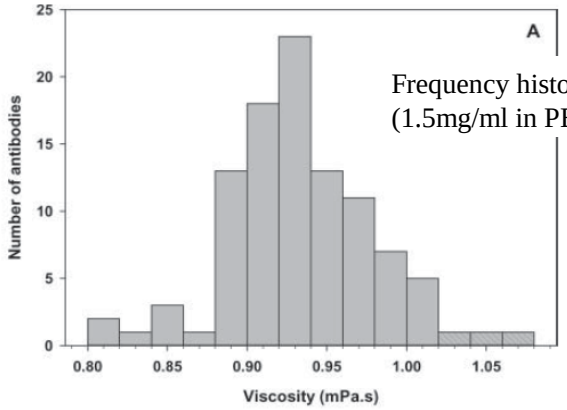
Bar chart showing the differences in mean viscosity for mAbs samples at 1mg/ml in different buffers with the addition of sucrose and Tween 20 as excipients

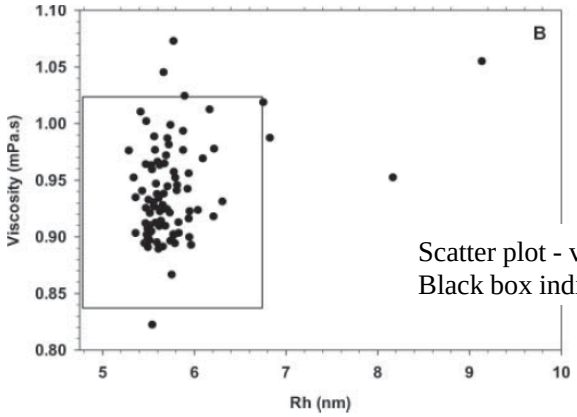
Relative viscosity screening of monoclonal antibody (mAb) formulations at low concentration and low viscosity for early stage developability, Application Note; download from www.malvern.com

Early developability screen of Ab candidates









Alexandra Lavoisier & Jean-Marc Schlaeppi (2015) Early developability screen of therapeutic antibody candidates using Taylor dispersion analysis and UV area imaging detection, mAbs, 7:1, 77-83, http://dx.doi.org/10.4161/19420862.2014.985544

Stopping Aggregation – Summary

- Viscosizer gives an excellent method (TDA) for early stage formulation screening
  - Extremely low sample requirements
  - 3 stability indicating parameters generated – orthogonal analysis
  - **Stability prediction** under storage conditions

- DSC the gold standard for later stage formulation, where sample availability is less of an issue
  - **Direct** ‘gold standard’ measure of stability
  - Critical for biocomparability studies also

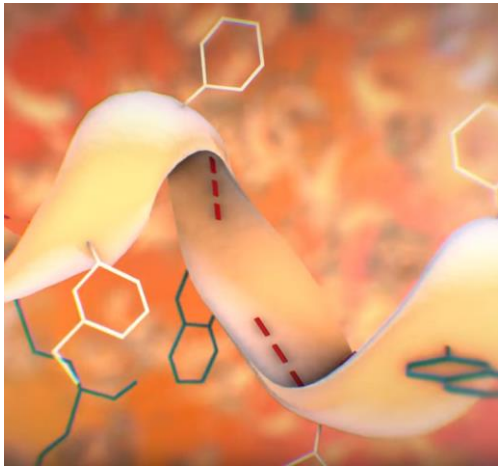
The fundamentals of Formulation
Excipient Binding

- We have discussed techniques that measure stability – but why do certain excipients stabilise certain protein
  - Do they bind in a specific way?
  - Is the excipient binding at all, or simply altering stability through, for instance, its affect on water structure

- Knowledge of excipient binding parameters gives a deeper understanding of the interactions that cause certain excipients to stabilize protein

Stopping Aggregation

Formulation Optimisation

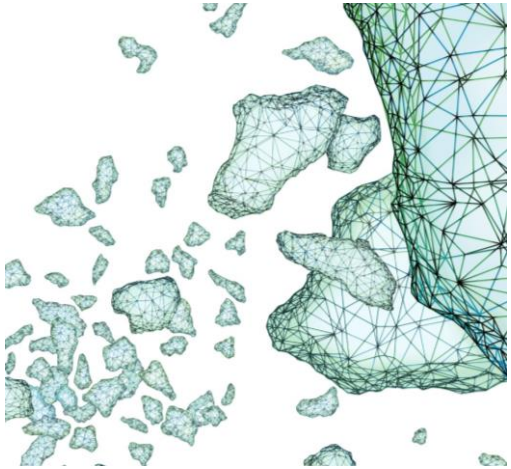


LATE STAGE SCREENING

Information is key

EARLY STAGE SCREENING

Sample Consumption is key



INTERACTION ANALYSIS

The fundamentals of formulation

The fundamentals of Formulation
Excipient Binding

- Allows determination of the lowest possible conc. of excipient needed to ‘saturate’ the protein and attain the stabilizing effect

- Minimizing conc. of excipient in a formulation **reduces costs**

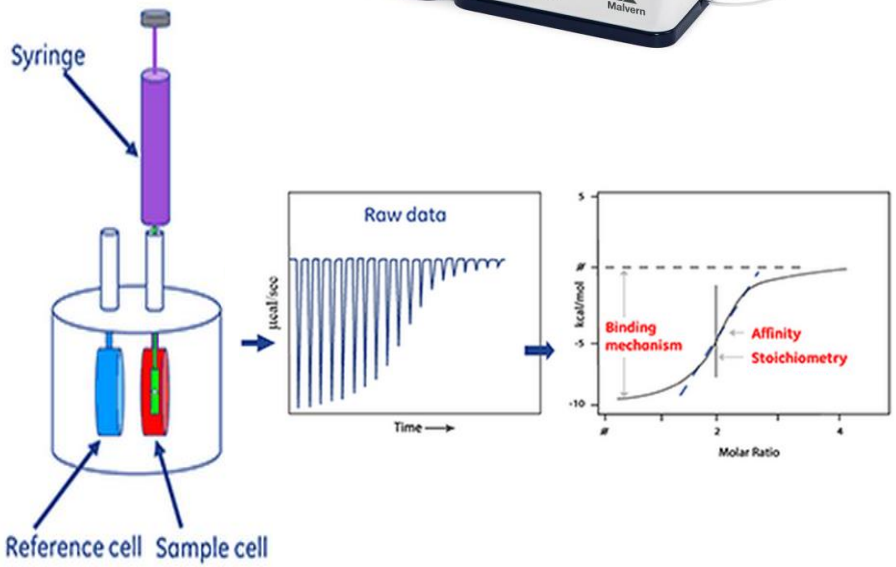
- Also reduces the level of additives to be administered to a patient

Microcal ITC

Isothermal Titration Calorimetry

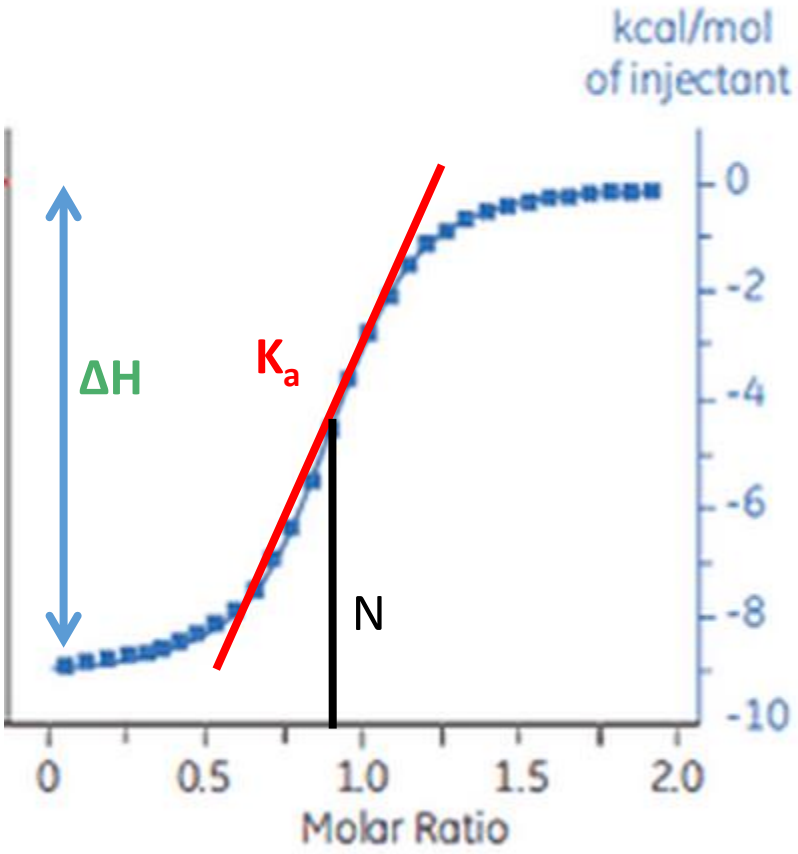
Malvern Panalytical

- Gives comprehensive label-free analysis of binding
- Information rich data allows intelligently designed **lead optimisation**
- Fully automatable – ideal for **screening** purposes



Microcal ITC Isotherm

- Stoichiometry (n)
  Molar Ratio (x-axis) at centre of isotherm
- Enthalpy change of binding (ΔH)
  Total energy change during experiment (Y-axis)
- Binding affinity (Ka)
  Slope
- Entropy change (ΔS) and Gibbs Free energy change (ΔG) calculated from these values



Formulations development: Protein-excipient binding

- Titration of 50 mM polysorbate-80 into 25 mg/ml ProX protein

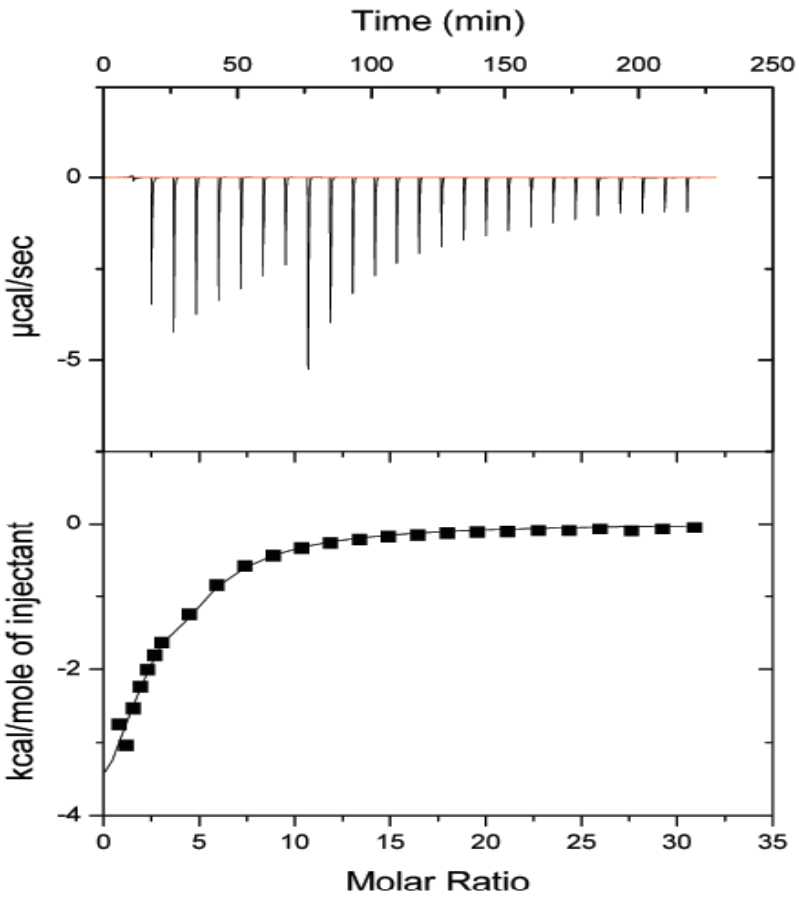
  Ka = 1430

  ΔH = -6.3 kcal/mol

  ΔS = -6.7 Kcal/mol

  n = 2.6

- 10-fold excess of polysorbate-80 needed to saturate ProX



Formulation Development – Excipient Binding Summary

- 10-fold molar excess of polysorbate-80 per molecule of protein was sufficient to saturate the protein – so only add a 10-fold excess to the formulation

- The binding parameters may also be used to predict the *in vivo* behavior of the protein-excipient complex:

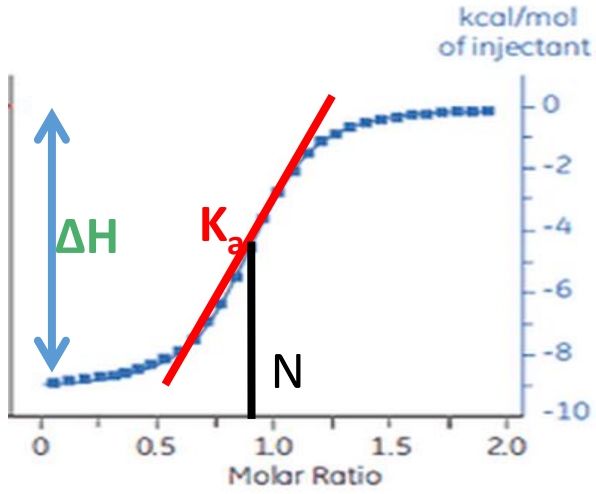
  Weak association constants measured by ITC suggest that the **ProX/polysorbate-80 complex will dissociate due to dilution** upon entering the bloodstream with no effect on the biological activity of the protein drug

Lead Optimisation

- Major application of PEAQ ITC is drug lead optimization

- Once a drug has been identified for a target, the developer may want to optimize its binding properties

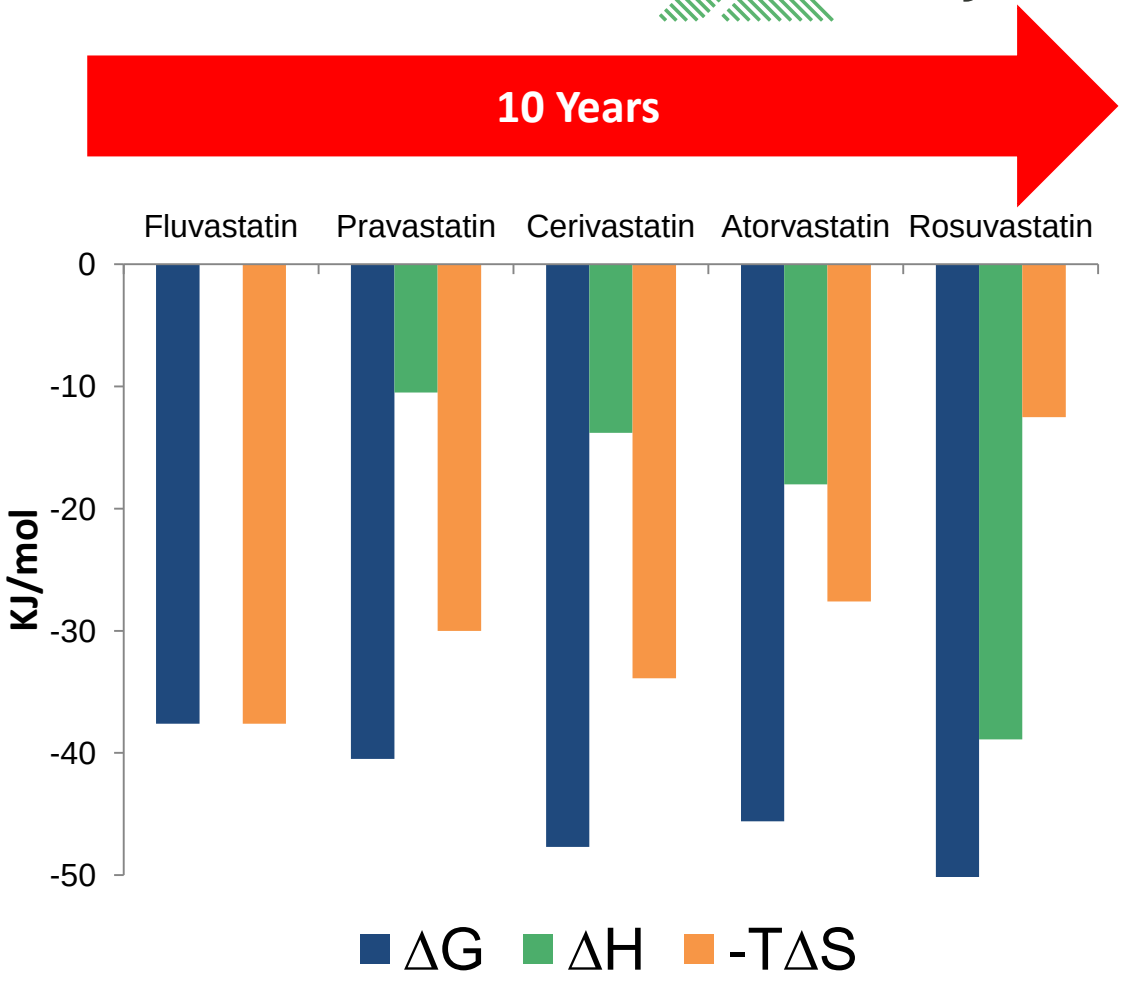
- The wealth of information given by ITC makes it ideally suited to the purpose.



The best drugs have more enthalpic binding

- **Enthalpic contribution increases** from the first statin to be approved to the most recent, **entropic contribution declines**

- Introducing **hydrogen bonds** may be more effective than introducing **hydrophobic bonds**

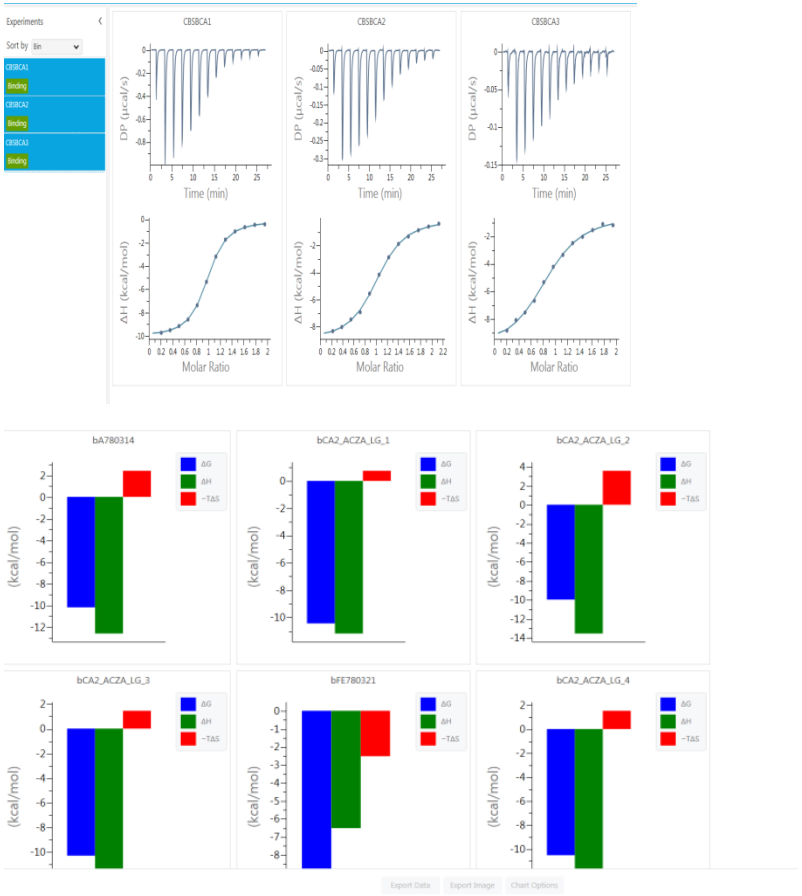


Ladbury et al. (2010) Nature Reviews Drug Discovery, 9: 23-27

Efficient assessment of Binding interactions

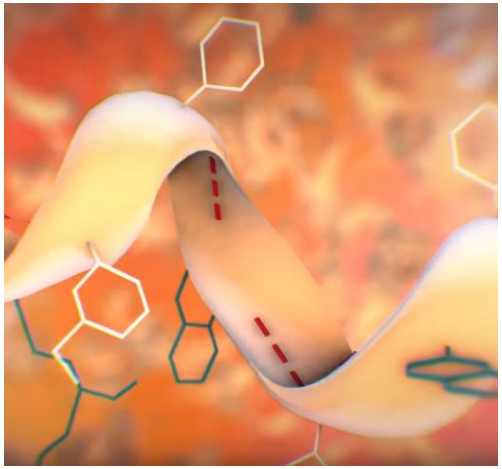
From excipients to drug-target interactions

- Label-free information rich analysis
- Automated data qualification
- Robust automated data analysis.
- Robust batch analysis of multiple data sets
- Multiple inbuilt tools to graphically visualize the data
- New features to support common applications such as Structure-activity relationships (SAR)



Stopping Aggregation

Formulation Optimisation



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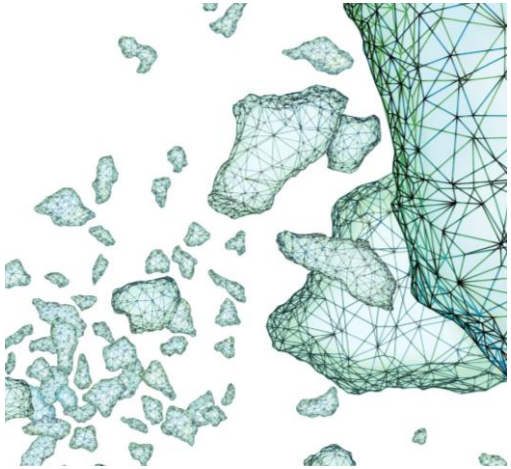

INTERACTION ANALYSIS

The fundamentals of formulation

Malvern Panalytical

Stopping Aggregation

Measuring and Predicting Stability