When big is not beautiful;

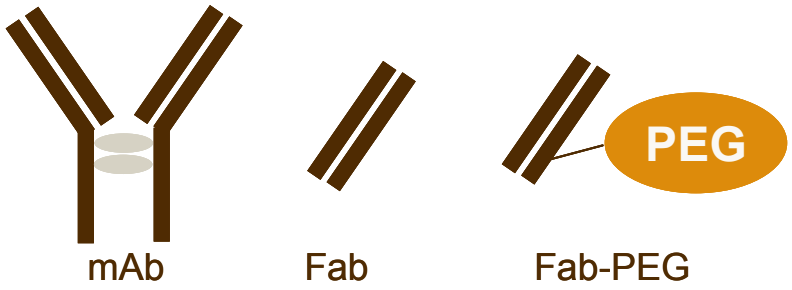
Aggregation minimisation and characterisation of biopharmaceuticals from discovery to commercial Phase

Clemens Stilling
Characterisation, Analytical Sciences for Biologicals, UCB Pharma S.A.

Alun, living with Parkinson's disease

Outline

- Short intro
- Aggregate characterisation during candidate selection and development
- Case Study: Candidate selection
  - Process flow
  - Feedback loop to ensure a inherently developable candidate is chosen for development
- Case Study: Aggregation understanding during development
  - Aggregation understanding by forced degradation studies
  - Increase in aggregates in a manufacturing batch and linking back to FDS
- Not included: formulation or process development

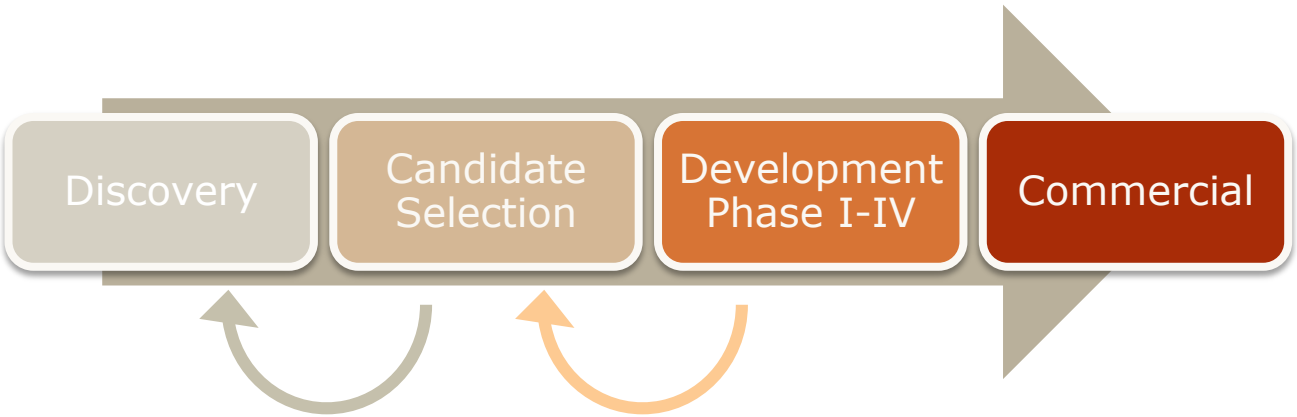


Introduction to aggregates and biopharms

- Aggregates are linked to immunogenicity

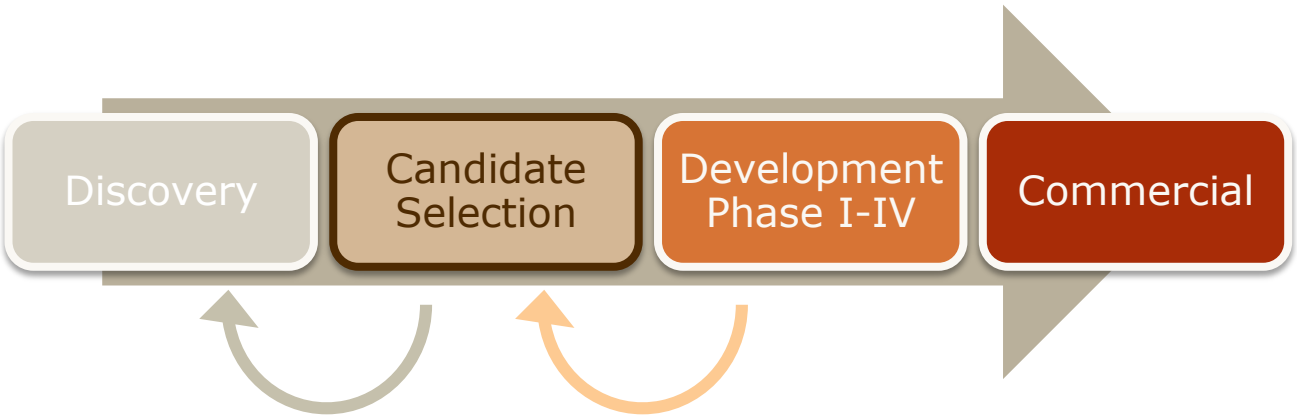
- Biopharma companies pro-actively aim to minimise the aggregate levels therefore minimising immunogenicity
  - Selecting candidates early with low inherent aggregation propensities
  - Developing a manufacturing process which reduces aggregates
  - Developing formulations which are unfavourable towards aggregation
  - Mapping out aggregation pathways and develop understanding of aggregates

Lifecycle management



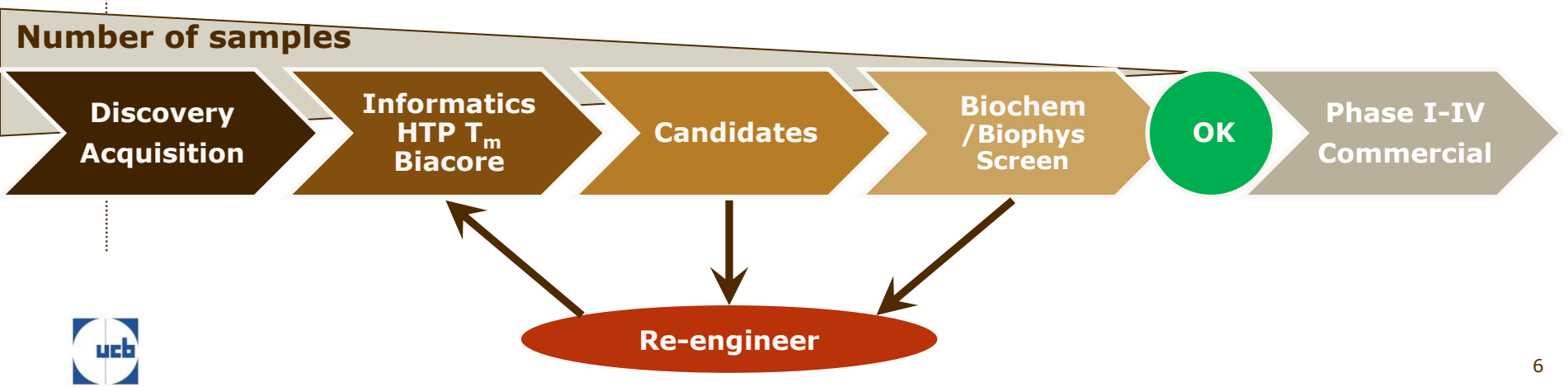
Aggregation propensity minimisation during candidate selection

- Case study: Aggregation screening informs candidate selection



Candidate selection

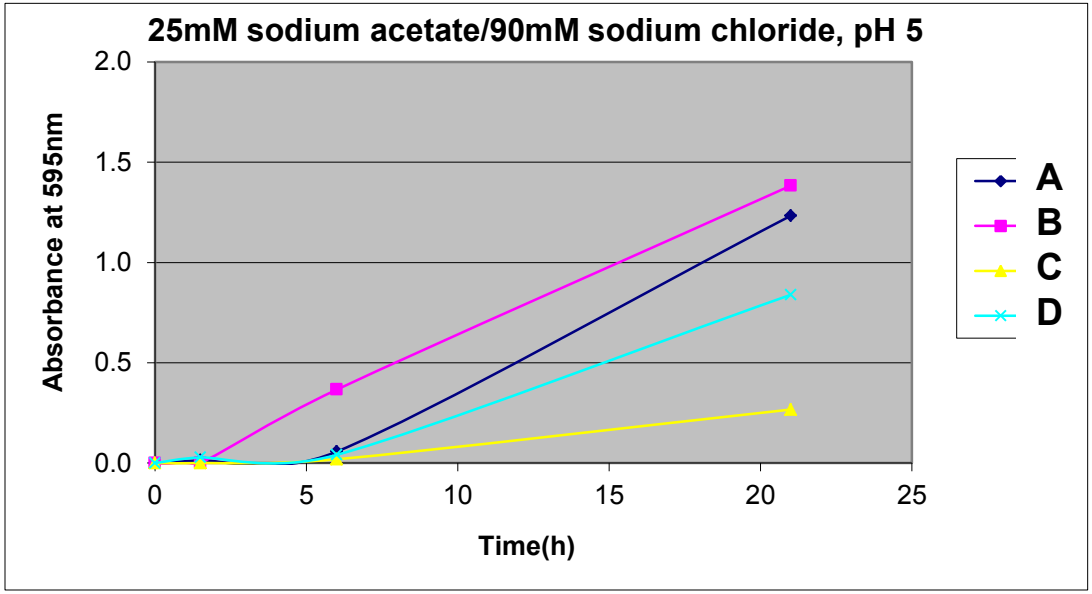
- Many candidates against a target are evaluated by various developability criteria
- Biophysical properties are evaluated early in the process to test for inherent aggregation propensity
- This occurs in tandem with biochemical screening
  - Aggregation, deamidation, oxidation, etc.
- This allows for re-engineering

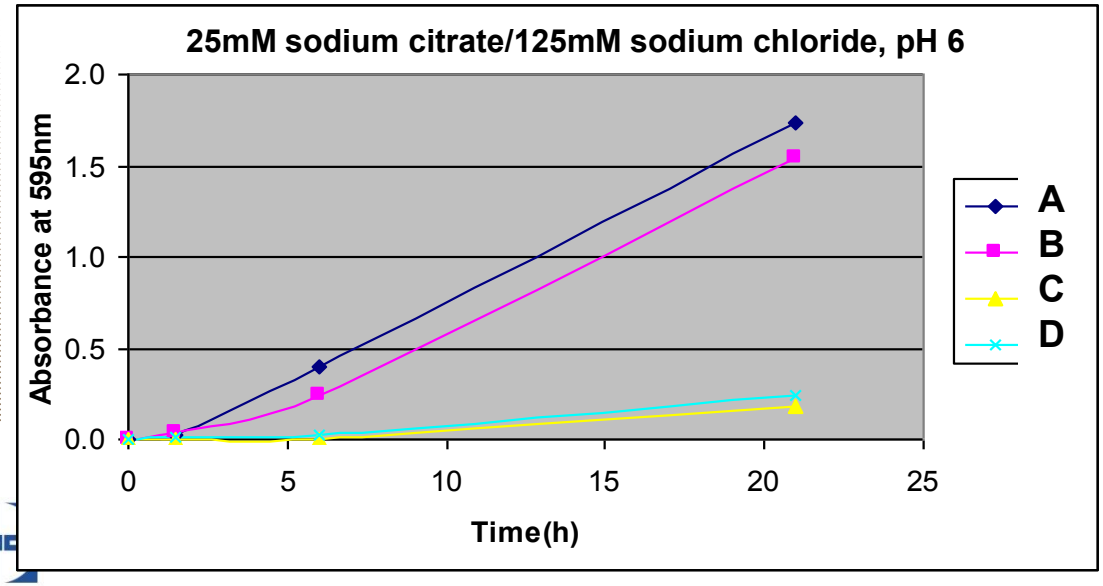


Bioinformatics during candidate selection

- Bioinformatics plays an increasingly important role in discovery and candidate selection.
  - Basic parameters
    - MW, chemical formulae, pI, exctinction coefficient
  - Homology modelling
  - Deamidation prediction
  - T_m prediction
  - Aggregation prediction
  - Secondary and tertiary structure prediction
  - Solvent accessibility
  - Disulfide connectivity

Aggregation by agitation: inherent properties *vs.* formulation





Vortexing 25°C 1400rpm, Nephelometry at 595 nm

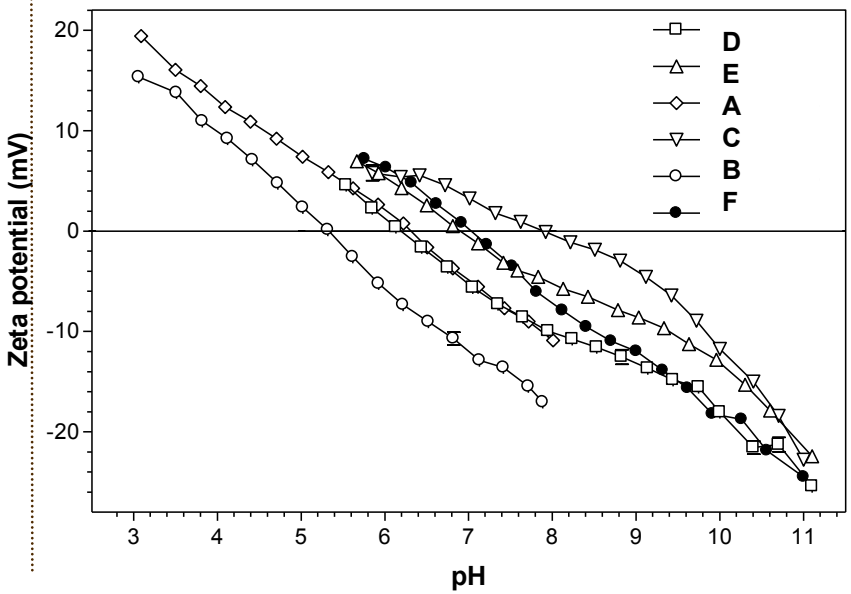
Stability at pH5: B<A<D<C

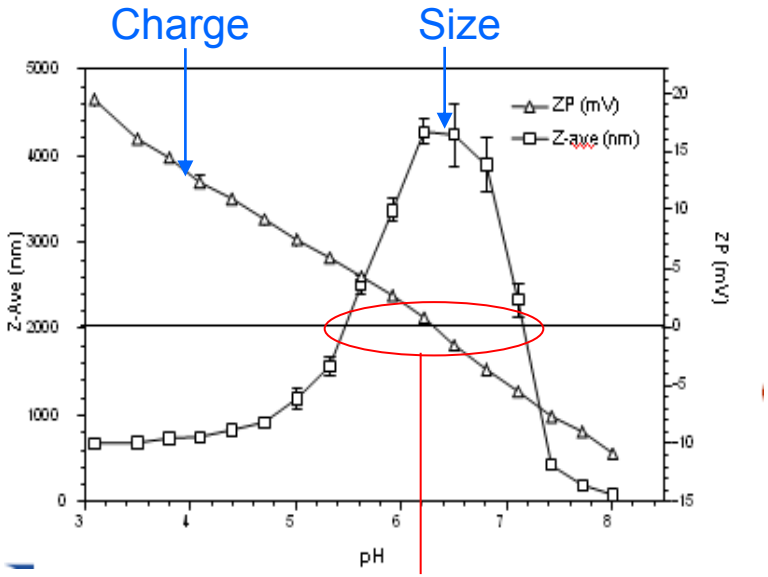
Stability at pH6: A<B<D<C

Distinct differences between molecules.

Molecular charge and aggregation: pI and zeta potential

- Zeta potential = molecular charge in standard buffer (10mM $NaPO_4$).
- Zero lower pH than pI
- Can increase tendency to aggregate if molecular charge approaches zero

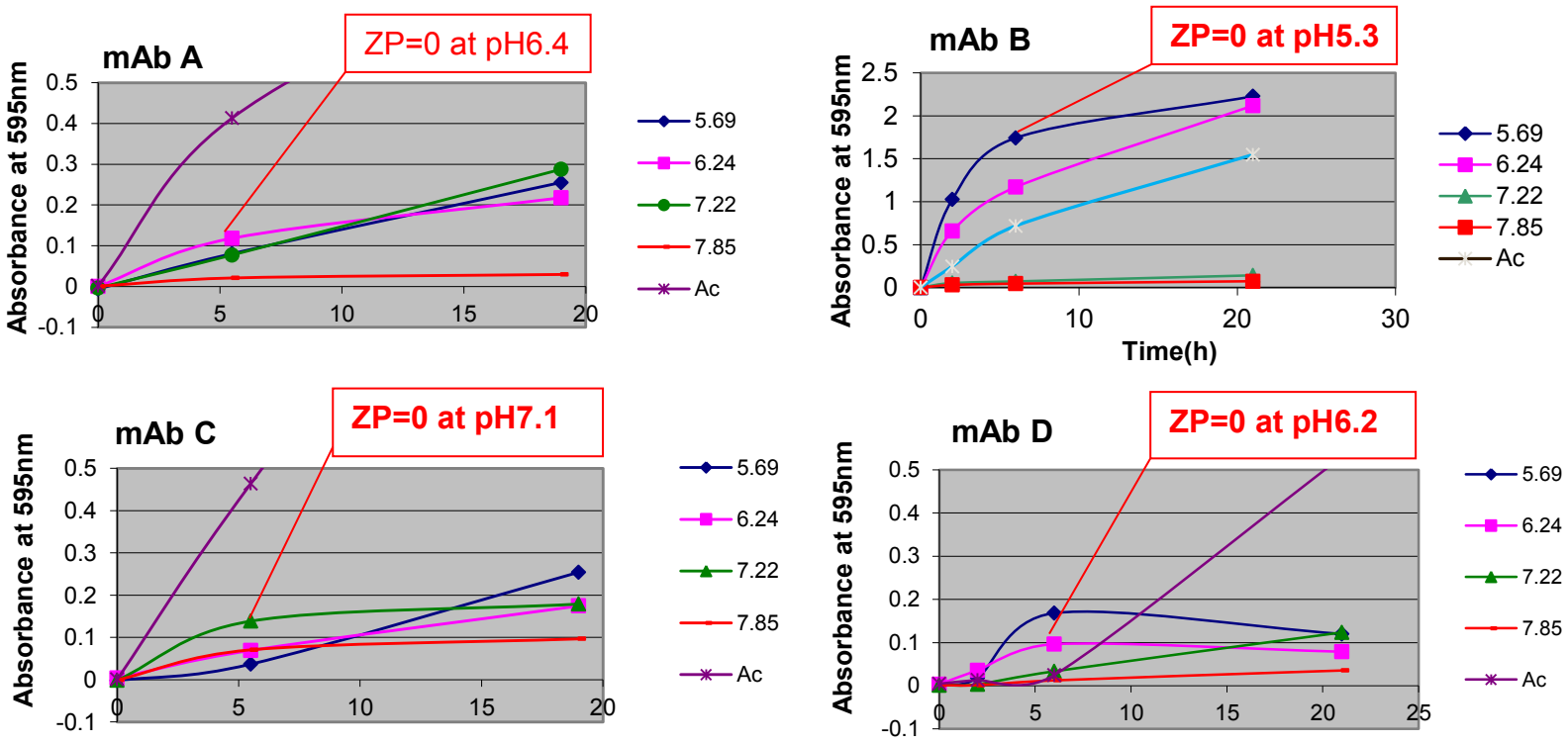




Most likely to aggregate

when Zeta = 0

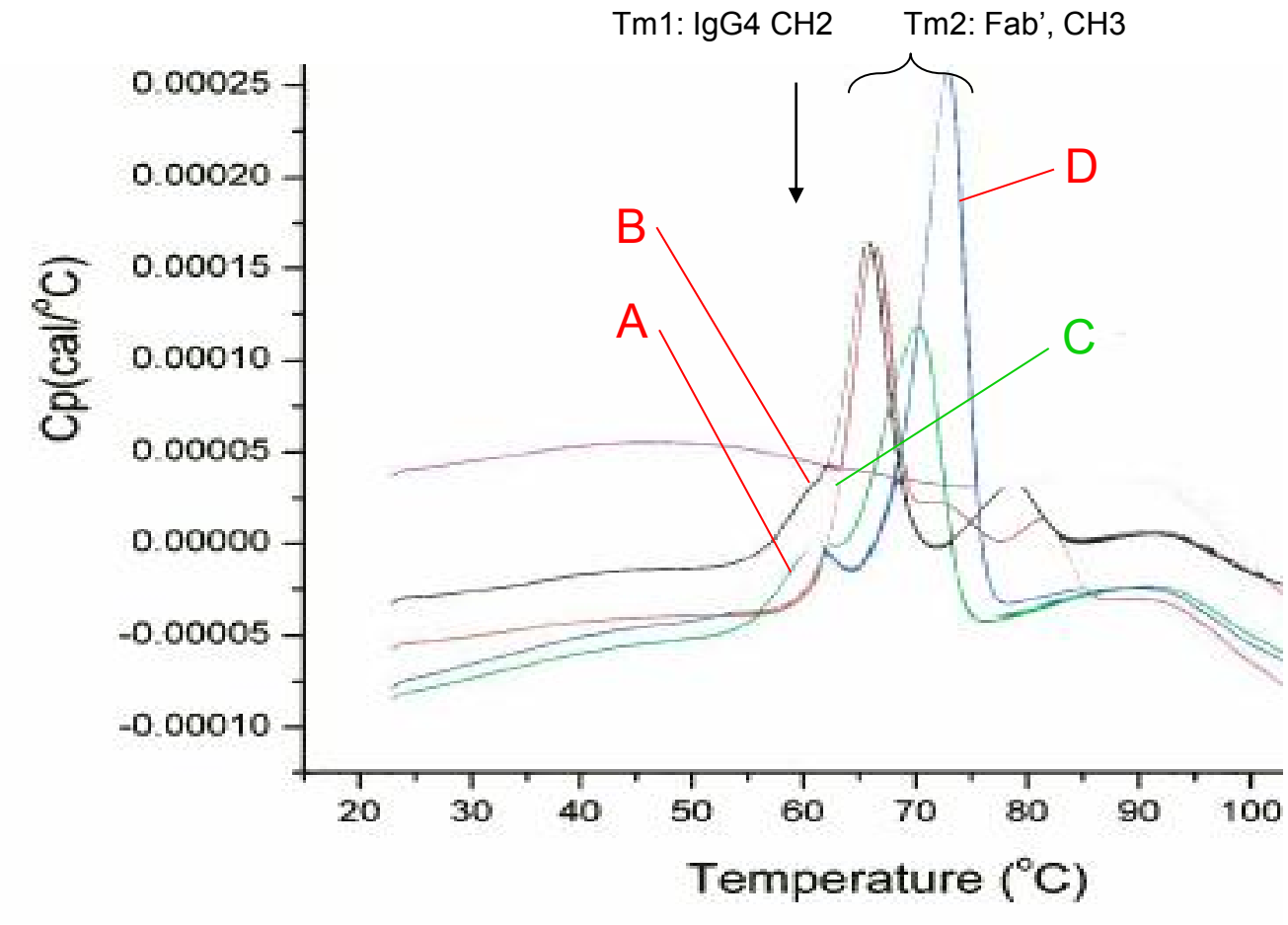
Effect of buffers on aggregation by vortexing



- 10mM phosphate (zeta conditions) or acetate pH5 (Ac)
- In 10mM Na phosphate, fastest initial aggregation rate at pH closest to Zeta 0
- Greater propensity to aggregate in 115mM (acetate) buffer, pH 5, than 10mM Na phosphate buffer pH 5.69.

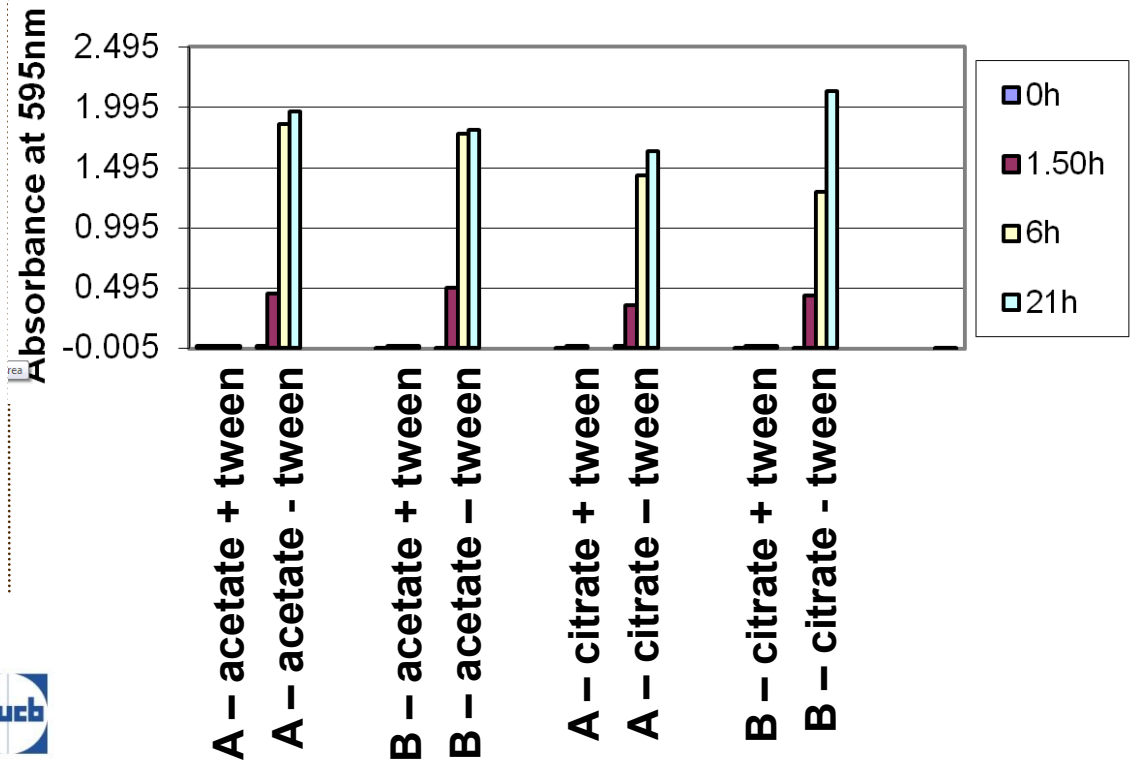
Melting point / start of melting as an indicator of stability

- DSC – calorimetry of unfolding induced by heating
- IgG4's melt at lower temp (more unstable) than IgG1, due to Fc (CH2)
- Overall stability includes Fab' – IgG A and B worse than D



Formulation strategy to minimise aggregation

- Commonly aggregation is partially controlled by the addition of excipients to the final formulation
- Low concentrations of surfactant (common excipient) inhibit denaturation and aggregation at air-liquid interface
- Aggregation still has to be controlled in the manufacturing process



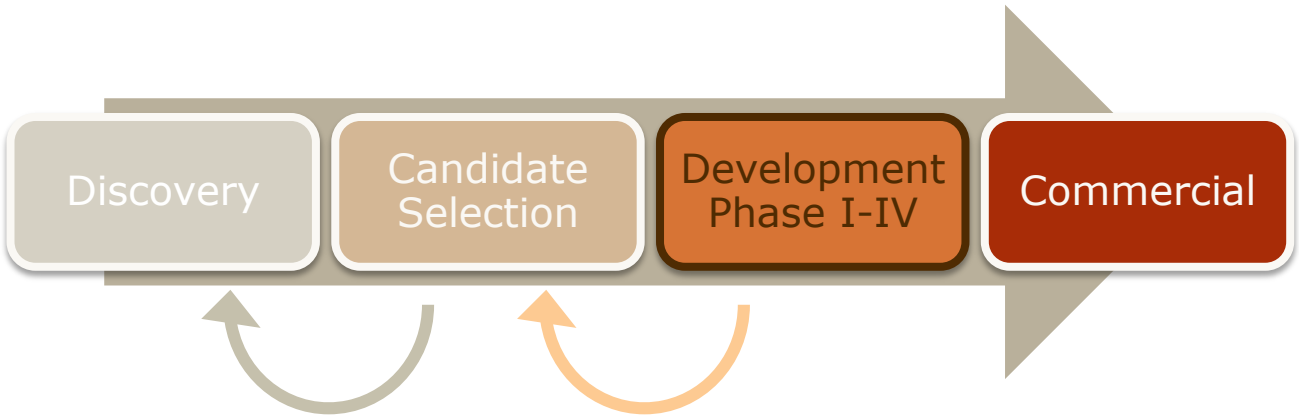
Effect of vortexing
25°C 1400rpm 1mg/ml
+/- 0.02% tween 80

Summary for candidate selection

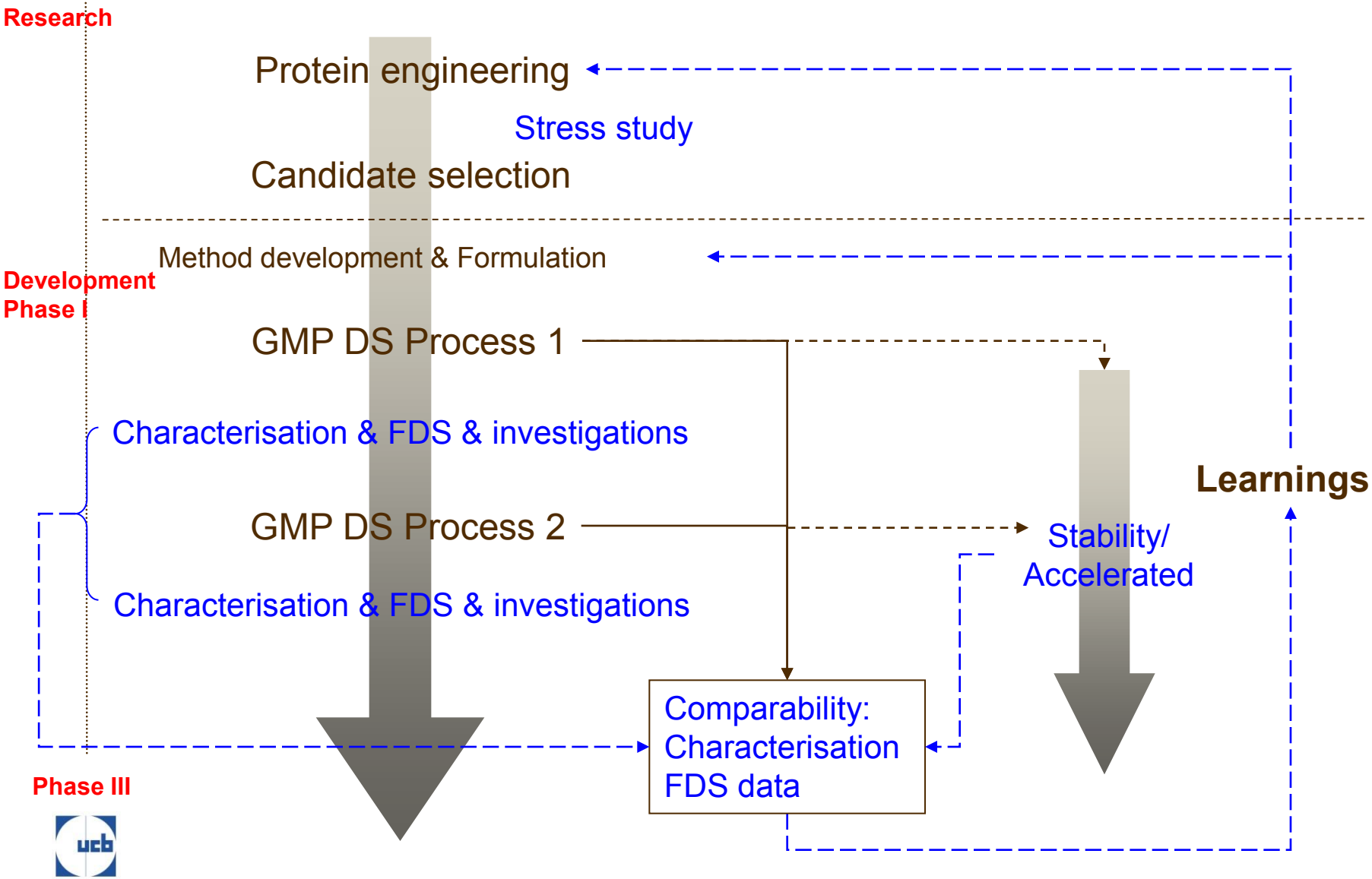
- Molecular charge and pH:
  - Choose buffer pH and type to avoid zero molecular charge
  - Select/engineer molecule pI that allows required formulation pH
- Molecular stability: in given conditions, higher Tm = less aggregation tendency
  - Select/engineer higher Tm
  - Adopt more stable format, e.g. IgG1 or Fab'-PEG
- Combinations of stresses may exacerbate aggregation
  - Avoid combinations e.g. zero molecular charge and agitation
- Protect final DS formulation with surfactant

Aggregation characterisation in Development

- Case study: a change in aggregation profile during manufacturing in a mAb



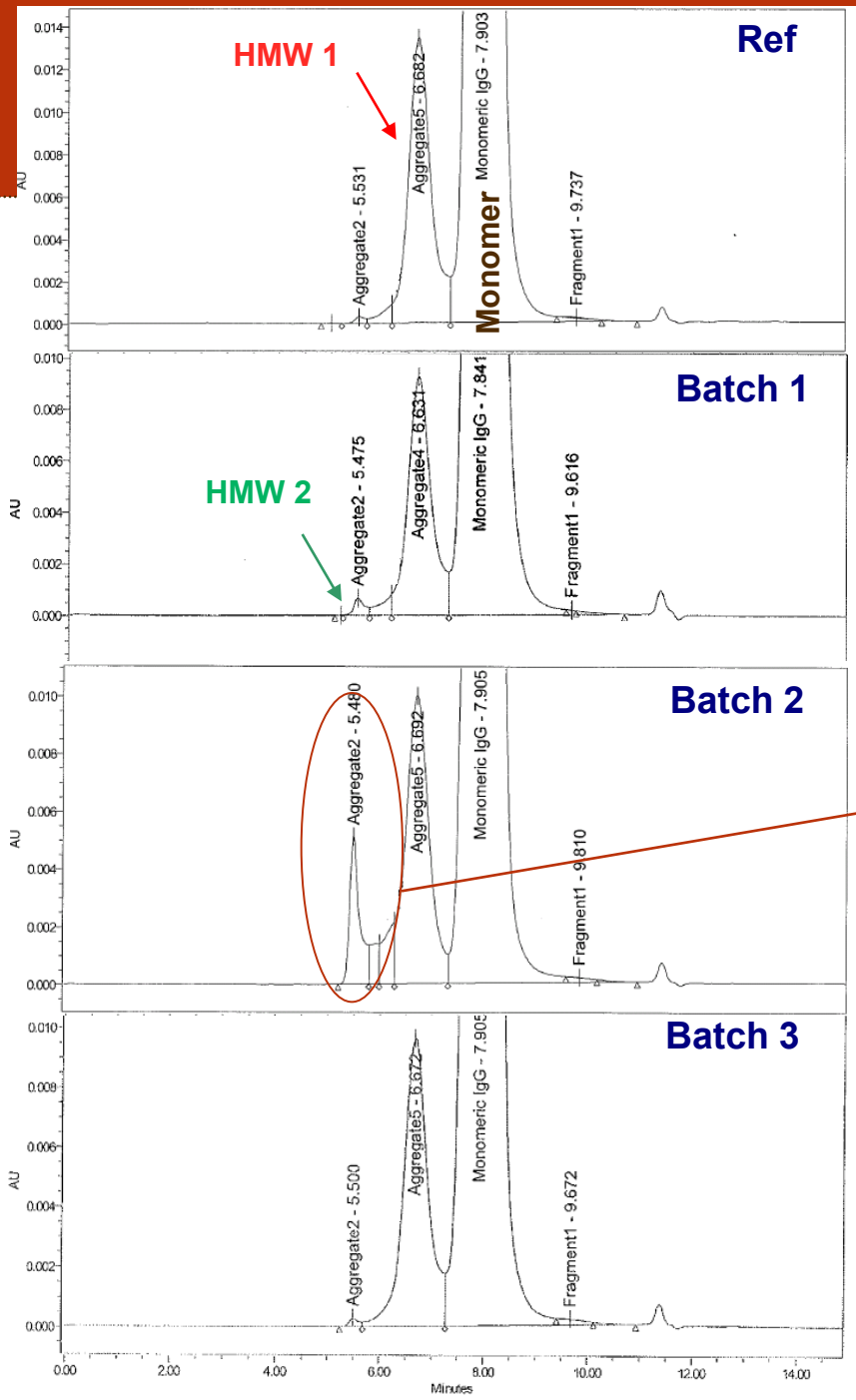
Incorporating Characterisation and FDS Studies into the Product Lifecycle



Observation of HMW species

- Initial observation from batch release data
  - Increased level of HMW species
  - Question 1: Are these new species?
  - Question 2: Do we know the pathway?
- Investigation undertaken (purification & characterisation)
  - Semi-prep SE-HPLC
  - SDS-PAGE, native PAGE, DLS, SEC-MALLS, MALDI-MS
- Results
- Compare with learnings from FDS
  - Question 3: could FDS data have prevented the investigation?
- Outcome

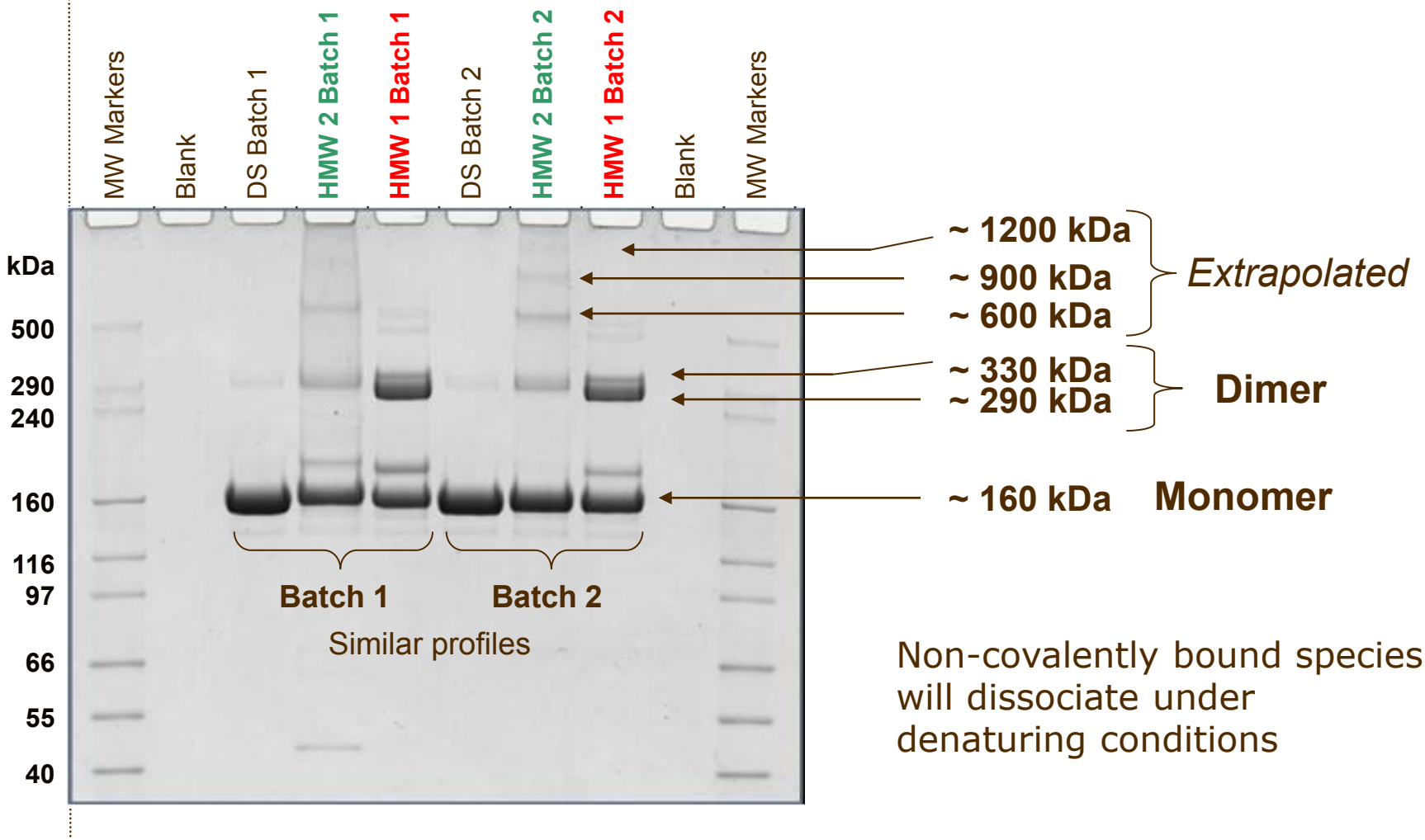
Comparison of Batch SE-HPLC Profiles (Drug Substance)



Batch	Percent Peak Area (%)				
	Aggregates				Monomer
	HMW 2	HMW1/2	HMW 1	Total	
Ref	0.05	0.09	2.0	2.2	97.8
Batch 1	0.05	0.08	1.5	1.6	98.4
Batch 2	0.33	0.24	1.5	2.1	97.9
Batch 3	0.02	-	1.5	1.6	98.4

→ Formation of aggregates can potentially affect activity and immunogenicity profiles of biopharmaceuticals

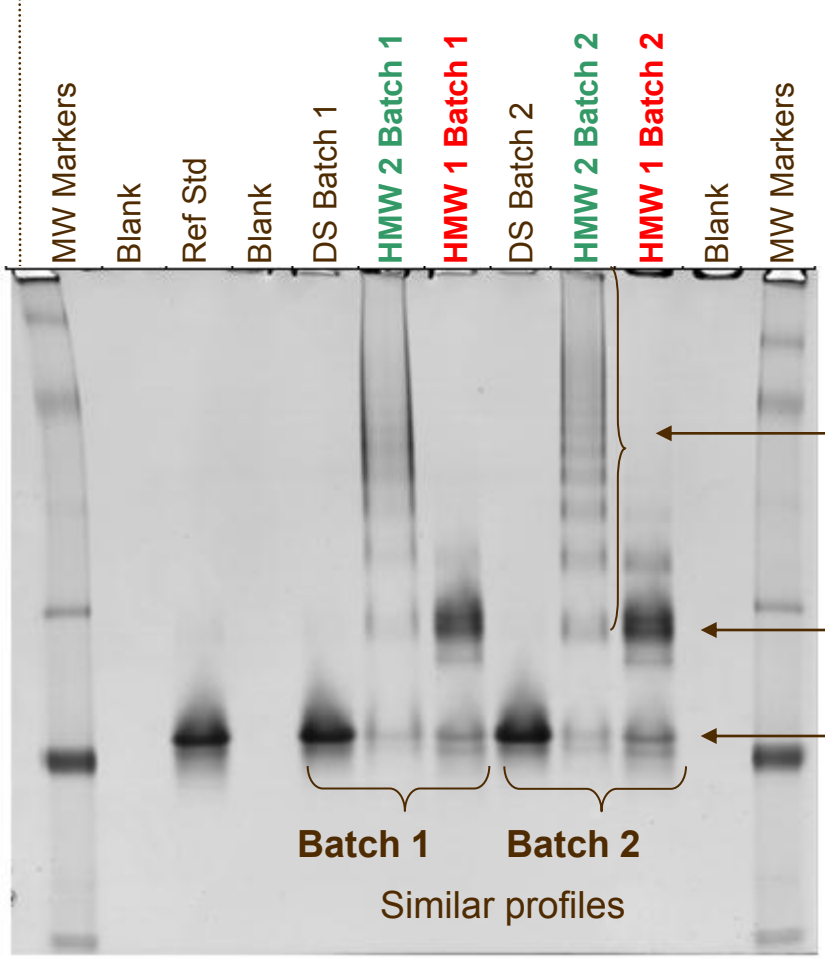
Non-Reduced SDS-PAGE (3-8% Tris-Acetate) – Denaturing conditions

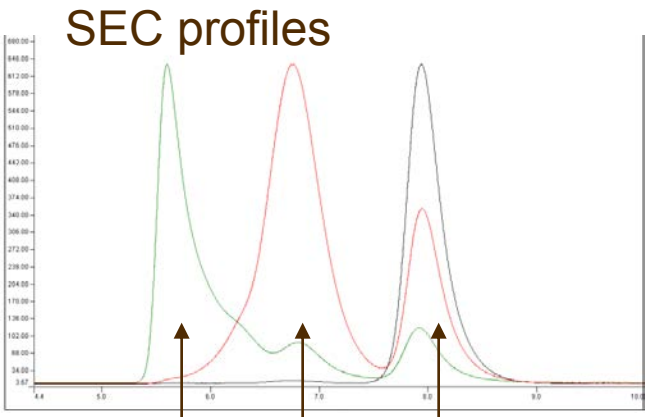


Non-covalently bound species will dissociate under denaturing conditions

- **Lane 5 & 8:** ~ half of **HMW 1** (dimer) are non-covalently bound
- **Lanes 4 & 7: HMW 2** species are predominately (~80%) non-covalently bound

Clear Native Gel (3-8% Tris-Acetate) – reserves integrity of non-covalent species





Mixture of oligomers

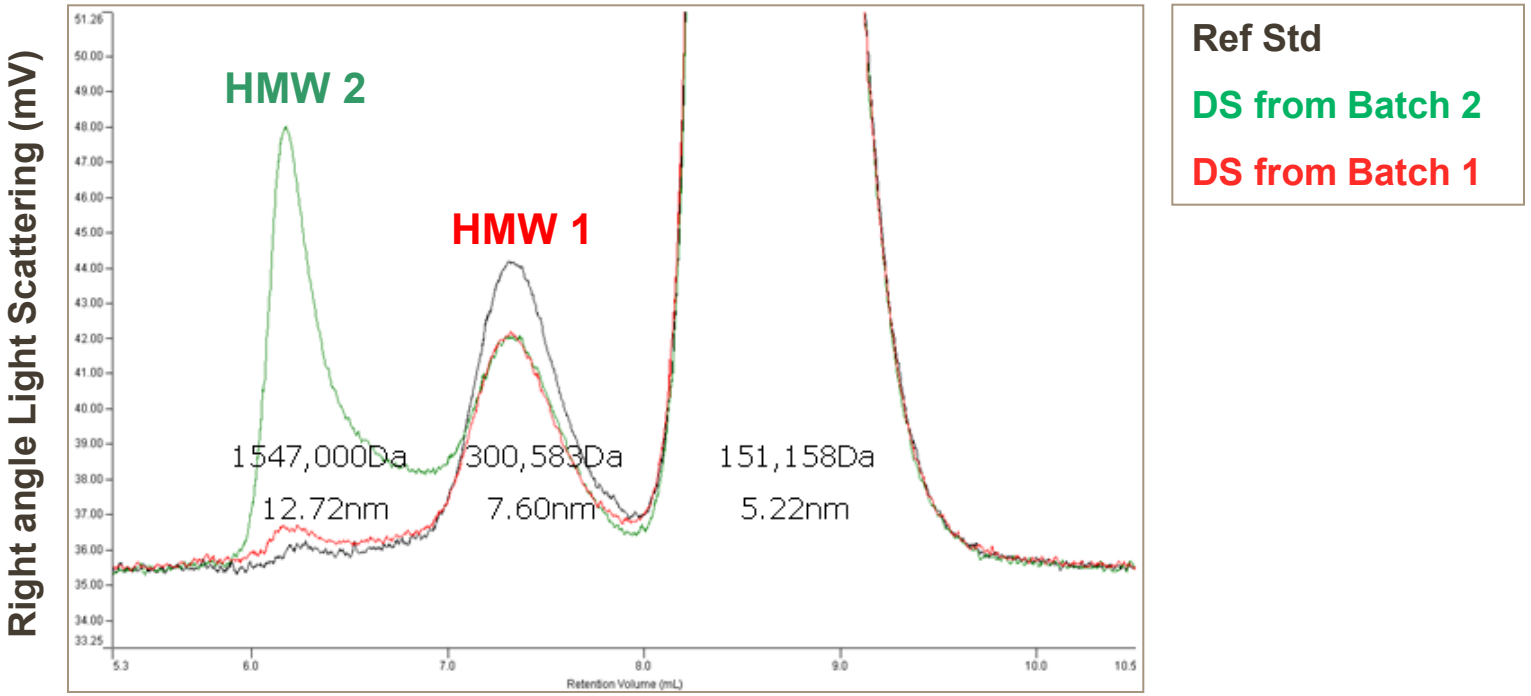
Dimer

Monomer

- **Lane 7 & 10:** Confirms **HMW 1** are mainly a dimeric species
- **Lanes 6 & 9:** confirms **HMW 2** species is a mixture of oligomers (di-,tetra-, hexamer…)

SEC-MALLS Data

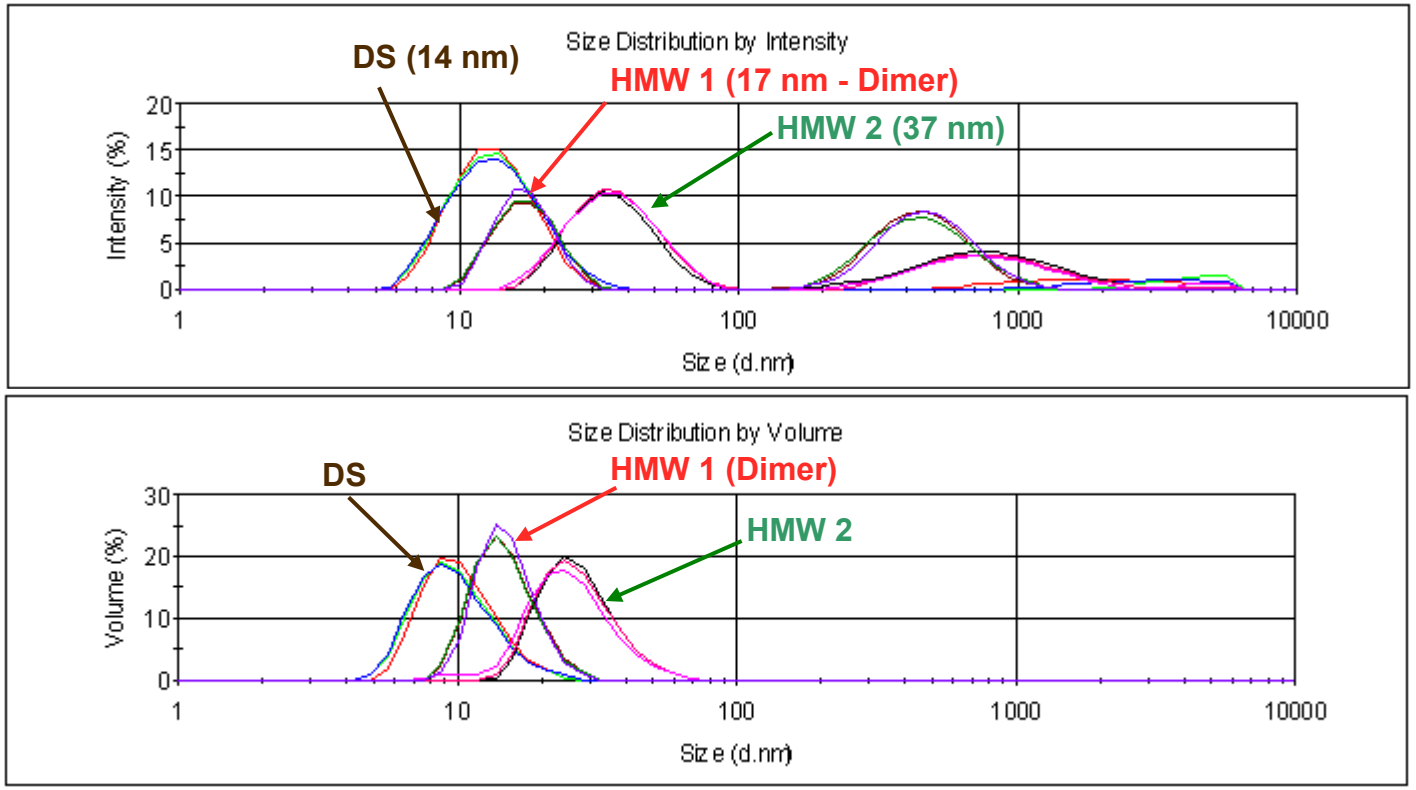
- SEC with multi-angle laser light-scattering (MALLS), viscometer and refractive index detectors
- Provides MW, hydrodynamic radii, intrinsic viscosity and % aggregates



- Estimated average MW of HMW 2 > 1,500,000 Da (limit of working range of SEC column)

Dynamic Light Scattering

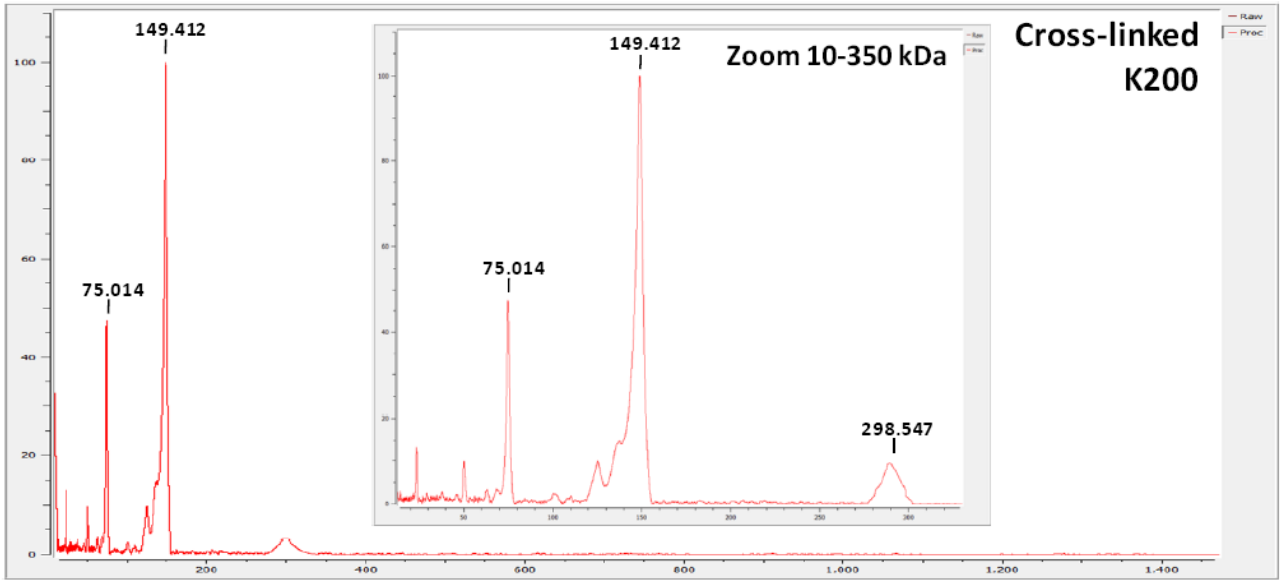
- Measures intensity of laser light that is scattered from particles
- Larger particles scatter light >> smaller particles
- Provides an estimation of diameter size of particles



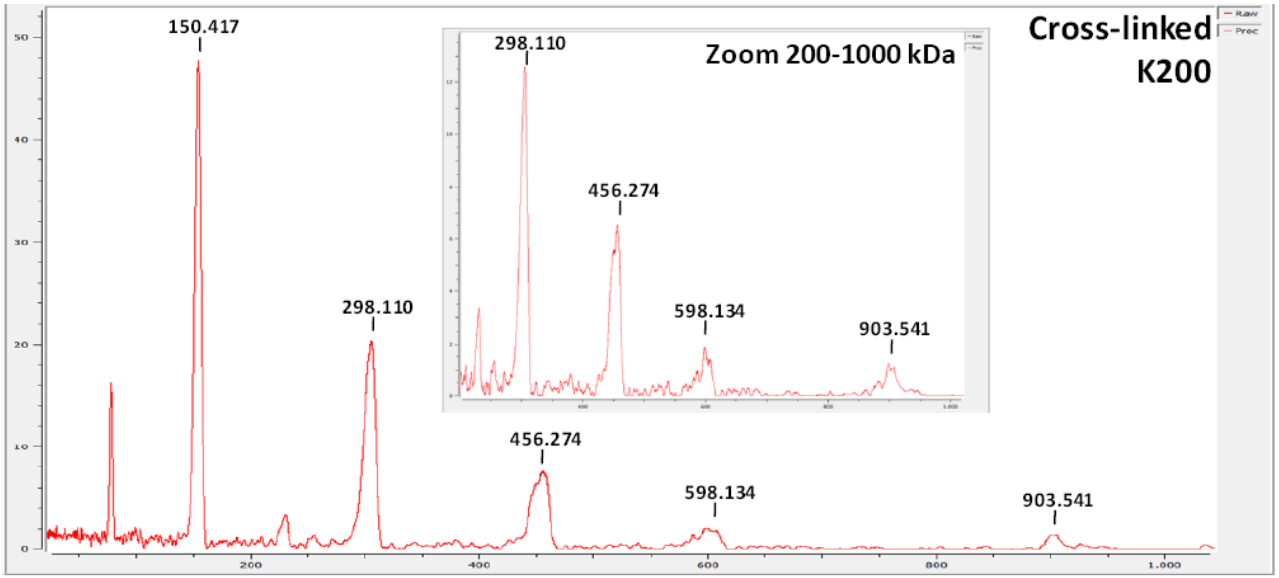
- Confirms that HMW 1 are a dimeric species; **HMW 2** data suggest that average MW > decamer

Mass Spectrometry: Cross-linking aggregates

- Reference standard sample



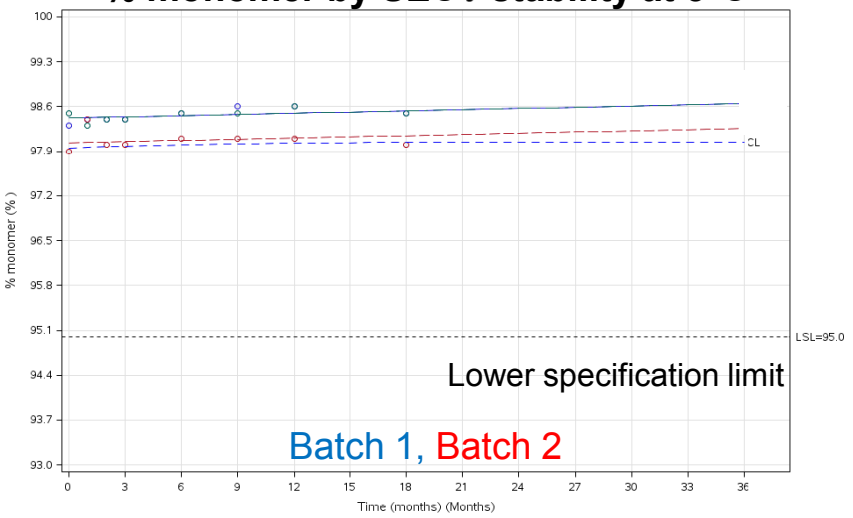
- HMW purified sample



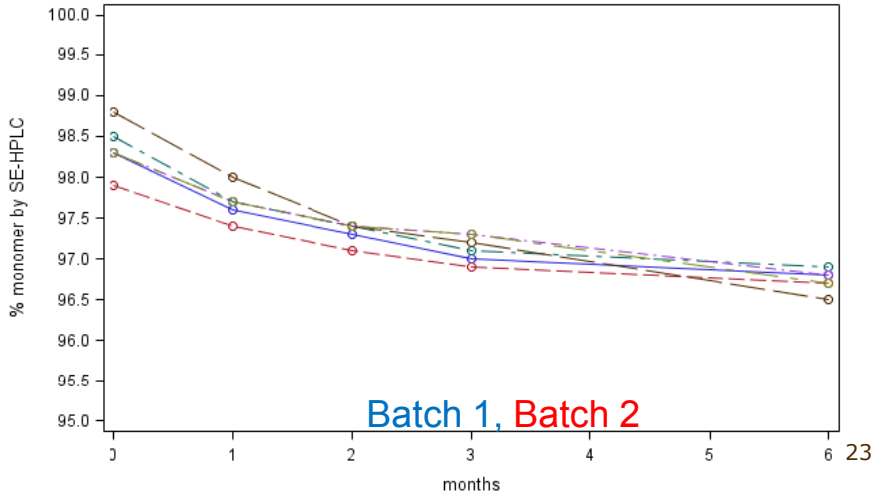
Stability and clinical data

- Batch 2 with increased HMW2
  - within specification for aggregates at manufacture
  - within specification for aggregates at end of shelf-life
- On stability and accelerated stability studies:
  - -70°C, 5°C and 25°C
  - batch 2 did not form aggregates at a faster rate than other batches.

% monomer by SEC - stability at 5°C



% monomer by SEC - accelerated stability at 25°C

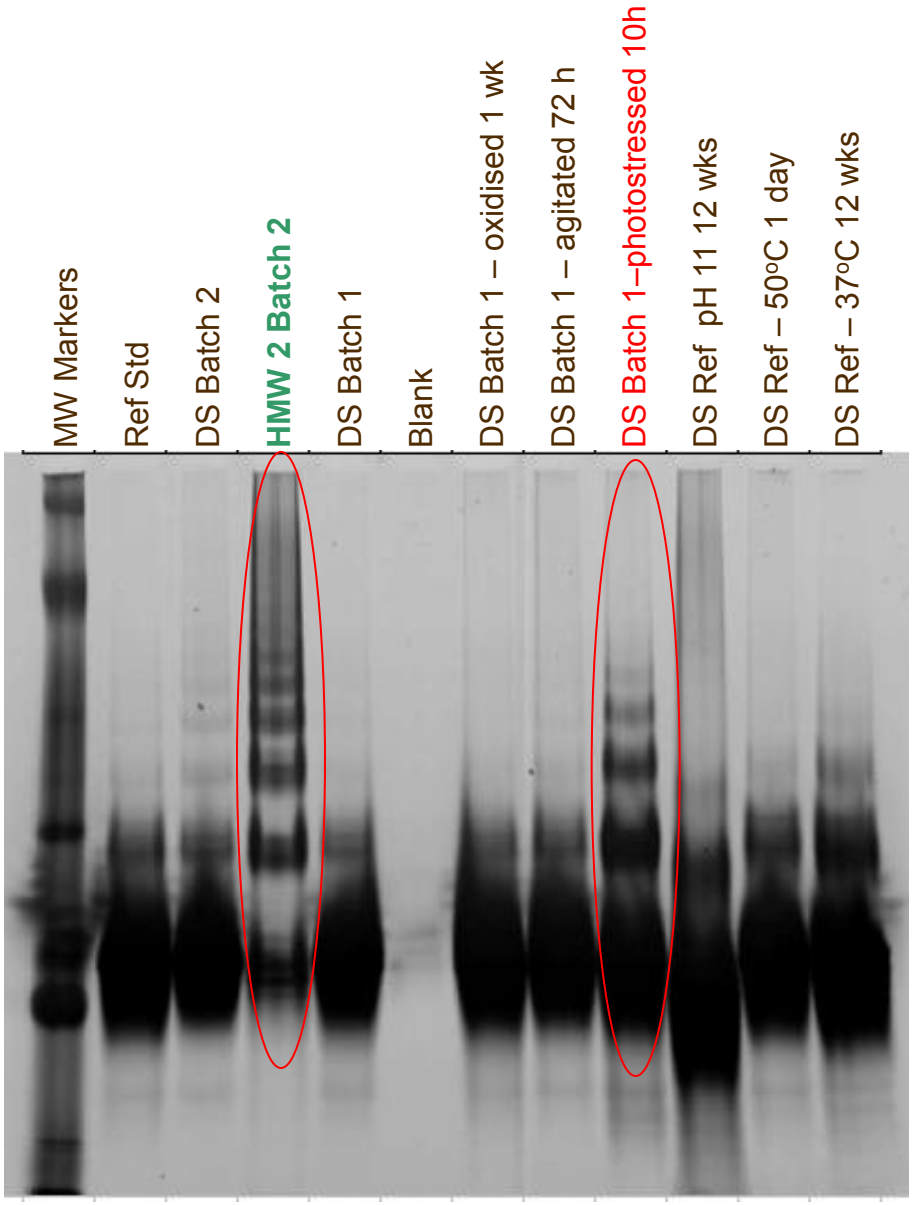


HMW 1 and 2: Characterisation Summary

- **HMW 1:**
  - Data consistent for a dimer (MS, AUC, SEC-MALLS ,SDS and native PAGE, and DLS)
  - About half of the dimer species is made from non-covalent bonds
  - 97% of species was reducible to Heavy and Light chain species
- **HMW 2:**
  - Analysed by SDS and native PAGE, SEC-MALLS, MS and DLS
  - Predominantly (80%) non-covalently linked species
  - Fully reducible to Heavy and Light chain species
  - Mixture of oligomers with MW up to decamers

- **Q1: Are they the same species?**
- **A1:** Same species present in all batches but levels vary (0.02% to 0.33% for HMW 2)

Native PAGE (Silver Stain) of FDS Samples



Mixture of oligomers

Dimer

Monomer

Development case study summary

- Increase of HMW2 at time of release
- HMW1 and HMW2 were purified and characterised by an array of thechniques.
  - HMW1 represents dimer
  - HMW2 represents oligomers up to decamer
- An initial FDS screen was performed to identify conditions which mimick HMW2
- Identification of simple and informative methods - clear native PAGE (Silver/Sypro Ruby)
- Confirmation of identified stress conditions using orthogonal techniques e.g. native gels and SE-HPLC

Development case study summary

- **Q2:** Can we determine the degradation pathway?
- **A2:** Answer: photostability appears to mimic closely the observed aggregation pattern. However, aggregation are difficult pathways and to truly understand pathways considerably work has to be performed.

- **Q3:** Could we have used the FDS samples to prevent an investigation?
- **A3:** This depends on confidence levels
  - The FDS gave 5 different options.
  - Analysing the sample using the same methods allowed better understanding. Thus in this instance the investigation was still necessary
  - Experience: number of studies, platform technology, sequence predictions etc
  - Aggregation is a complex pathway – often overlap between Photostability, agitation and oxidation….

Conclusions

- Candidate Selection
  - Biophysics/biochemical screen early in project (including other factors: pH stability, chemical stability, etc)
  - Select / re-engineer candidate to improve – easier process development, more stable product
  - Characterisation informs process development and formulation
  - **Don’t diagnose problem, avoid it!**
- Development
  - Understand the process
  - Have the tools to understand aggregation
  - Perform stress studies early
  - **Investigations into abnormal events can make or break projects**

Acknowledgements

- Characterisation
  - John O'Hara
  - Xavier Perraud, Jenni Halley, Martin Hampel, Smita Thobhani, Jun Gan, Kieran Dawkins
- Protein Biophysics
  - Alison Turner
  - Bryan Smith, Kaushik Sarkar
- Structural Biology
  - Alistair Henry
  - James Heads
- Analytical Sciences for Biologicals
  - Carine Dortu