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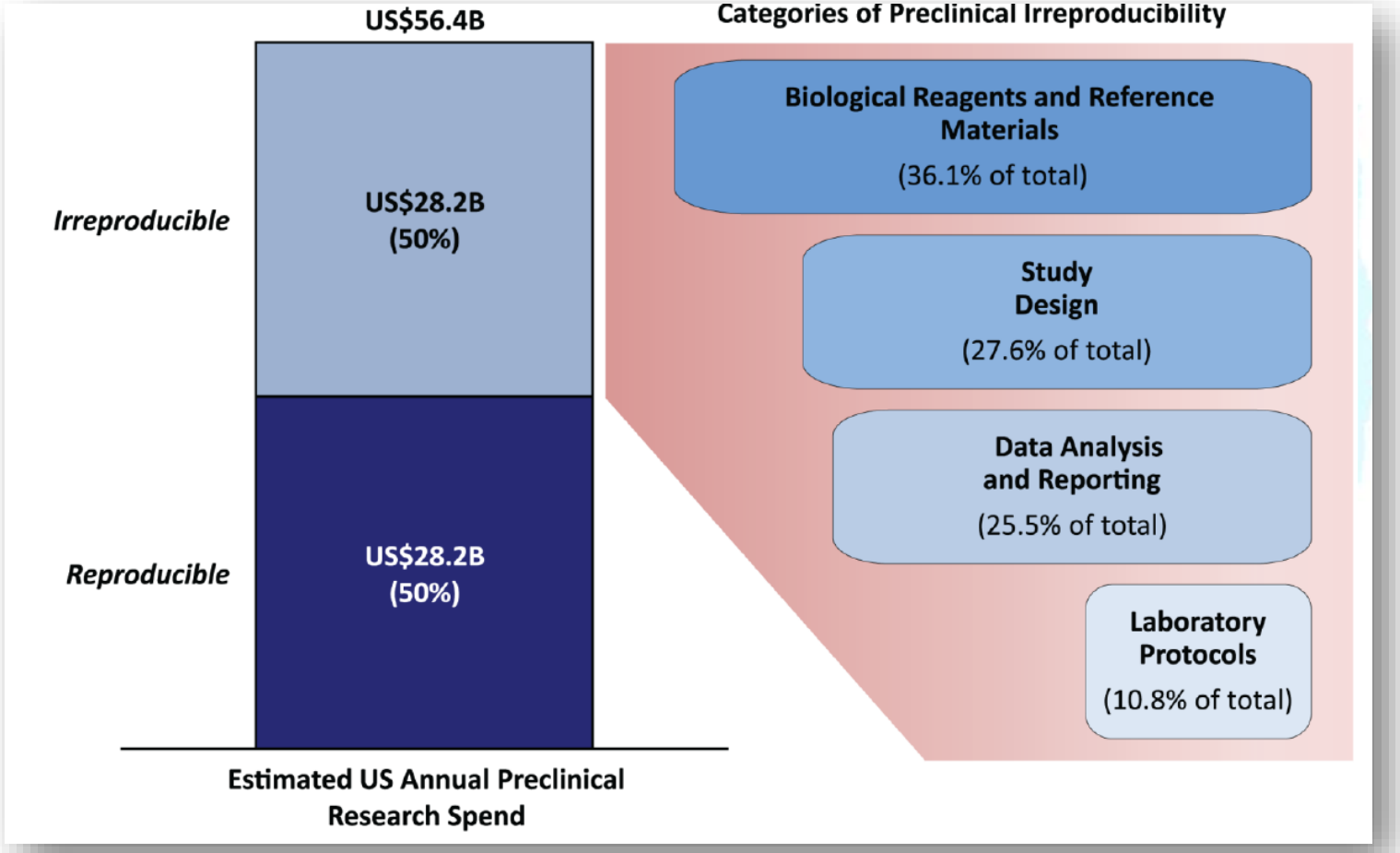
Data Quality and its Impact on Decision-Making

Natalia Markova, PhD

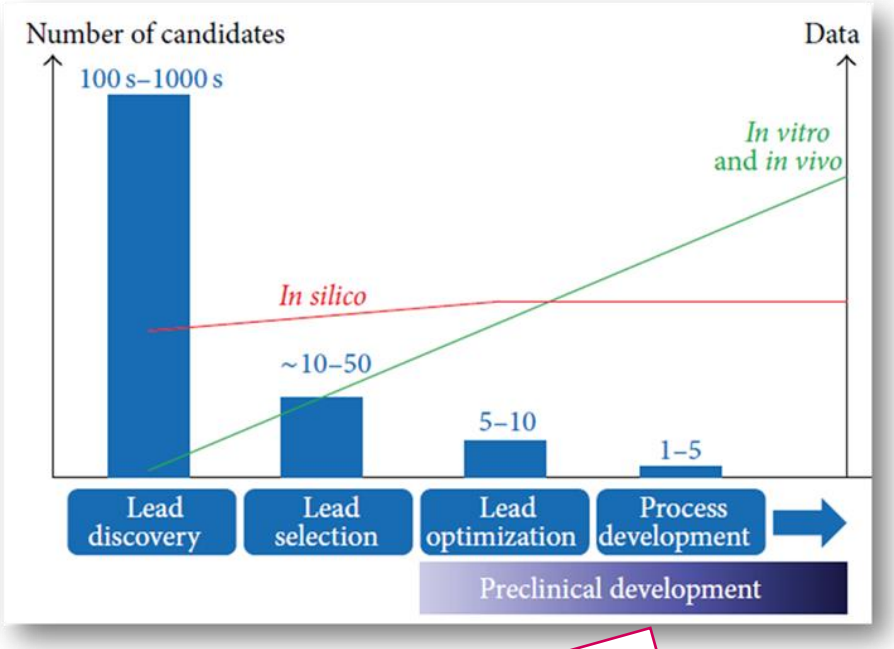
ANALYTICAL STRATEGIES FOR NOVEL THERAPEUTIC MODALITIES
Virtual Workshop - 22nd November 2021

Data quality – economy of scale

Room for improvments



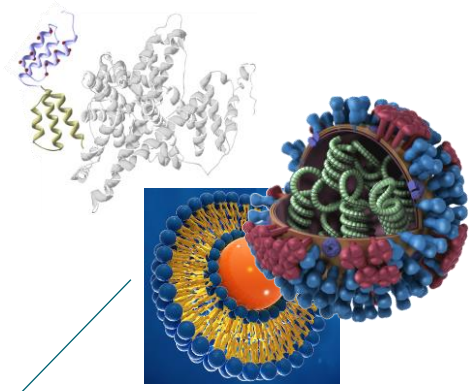
Friedman, Cockburn & Simcoe (2015) The Economics of Reproducibility in Preclinical Research. *PLOS Biology* 16(4)

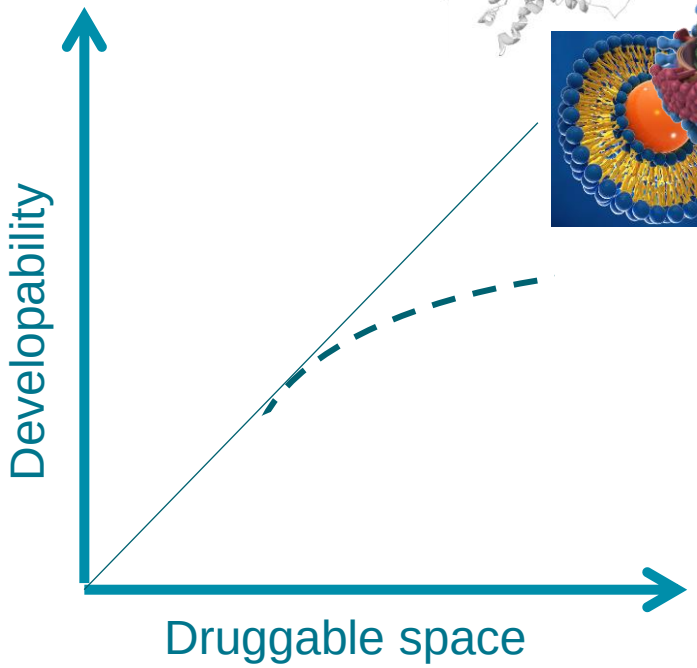


- Fit-for-purpose analytics.
- Product and method understanding.
- Orthogonal approach.

Challenges and drivers

Development of new modalities





Fit-for-purpose analytics.
Product and method understanding.
Orthogonal approach.

Design

Function

- Safety concerns
- Limited cell type specificity
- Low efficacy, high dose

Process development

Manufacturability

- Need for platform purification approach
- Lower yield, high cost
- Inter-batch variability

Formulation development

Stability

- Cold storage required
- Limited understanding of degradation mechanisms
- Needs for systematic formulation development

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Malvern Panalytical toolset overview

Analytical characterization of biologics

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DLS

Multi-detector SEC

NTA

MDRS

DSC

Concept Life Sciences

Viral titre

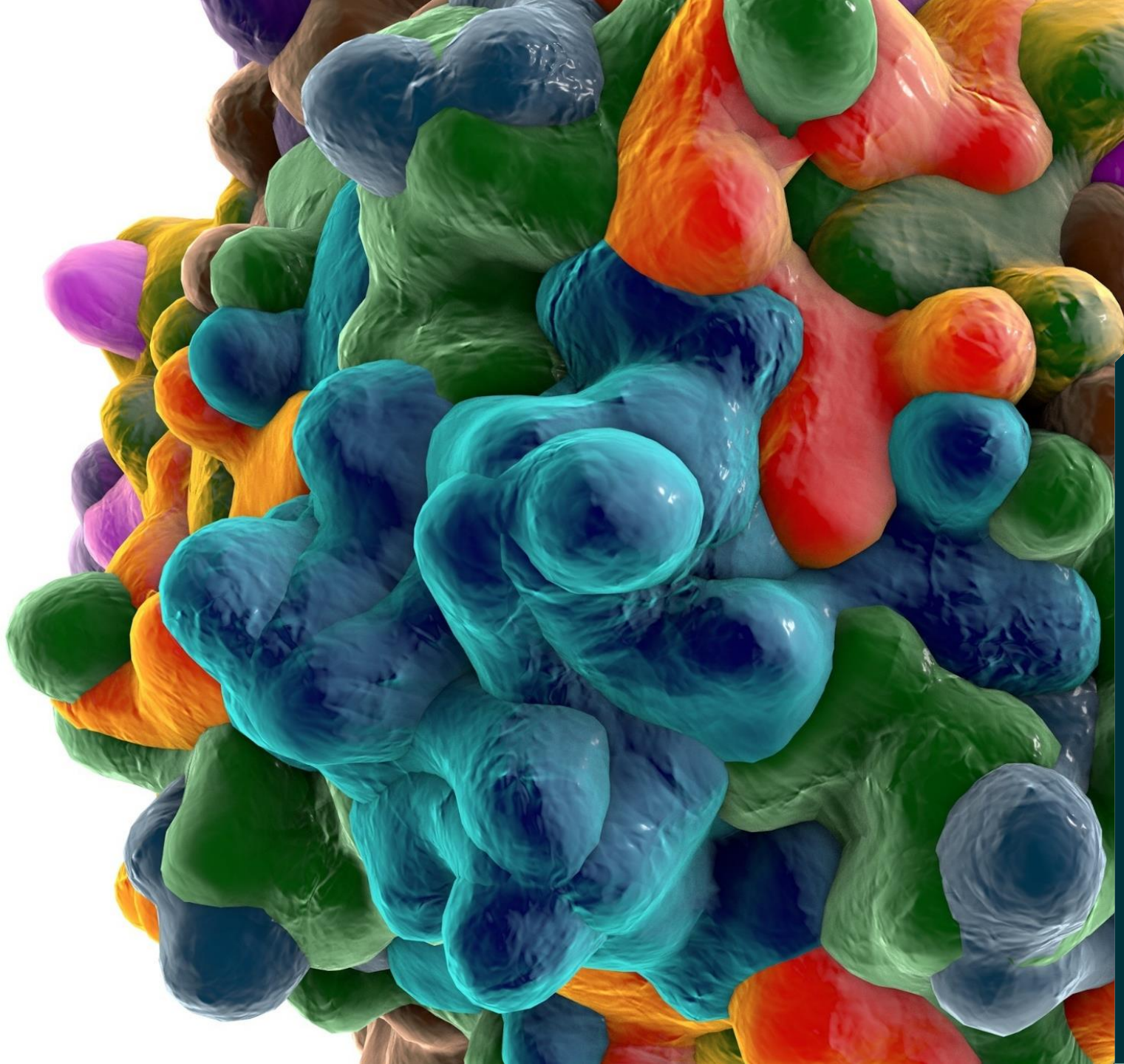
Composition
Glucosylation
Full/empty ratio

HOS, comparability of HOS, Serotype ID

Fragment content | Charge | Aggregate formation | Size | Thermal Stability

Stability

- **Biological activity, immunogenicity**
- **Biophysical characterization**
- **Amplify Analytics** (Method development, method validation, method transfer)



Characterization of viral vectors

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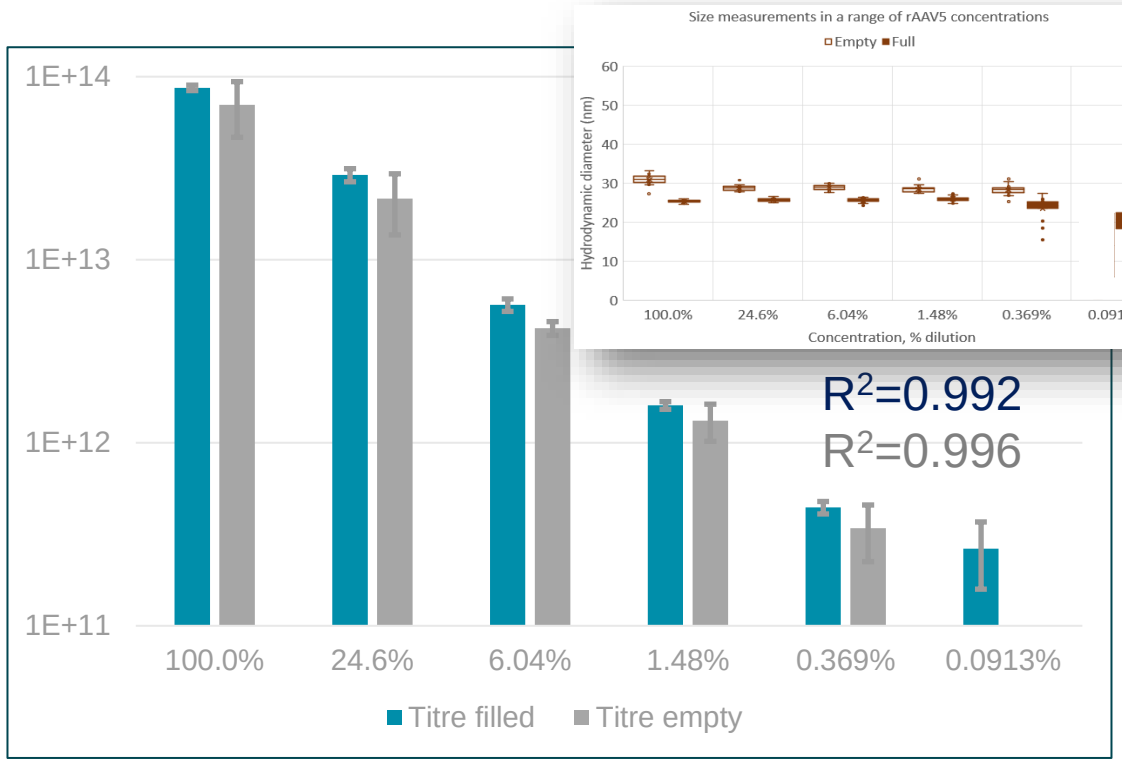
November 23, 2021

Capsid titer by MADLS for rAAV DS and DP characterization and formulation development

Linearity in 2-log concentration range. rAAV5



Sample	Capsid ELISA	MADLS titer	
		Virus peak (n=3)	%CV
ATCC AAV2 reference sample	0.92 x 10^{12}	1.14 x 10^{12}	45
Sample 1	6.14 x 10^{12}	4.82 x 10^{12}	15
Sample 2	4.29 x 10^{12} 2.83 x 10^{12} (assay ran twice)	4.92 x 10^{12}	13

	MADLS Titer	
	Range, cp/ml	CV%
Full	4.4e11÷8e13	3÷8
	@2.40E+11	40
Empty	3.4e11÷7e13	10÷37

- MADLS works as fast tracking tool for AAV particle concentration in DSP samples
- Lower resolution particle concentration for AAV samples of higher heterogeneity levels

Malvern Panalytical Application note: Measuring the concentration of Adeno-Associated Virus with multi-angle dynamic light scattering (MADLS).

Nanoparticle Tracking Analysis informs development of viral vectors

Higher resolution size and particle concentration

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Bioparticle characterization

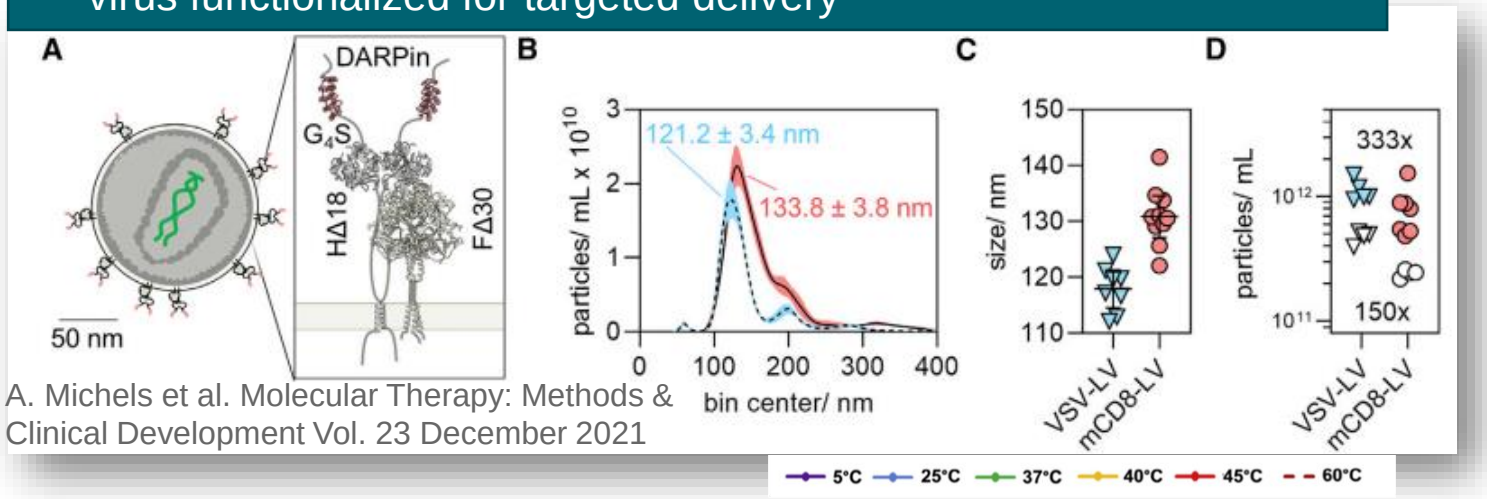
Exosomes

Virus and vaccines

Drug Delivery & Gene therapy

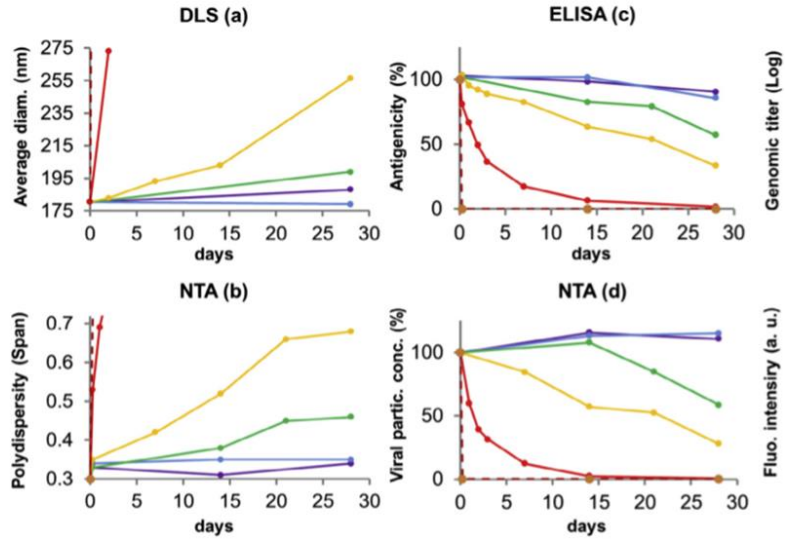
…simultaneously, ‘real time’, particle-by-particle

- Design. Informing potency assay with robust physical titer of lenti virus functionalized for targeted delivery



A. Michels et al. Molecular Therapy: Methods & Clinical Development Vol. 23 December 2021

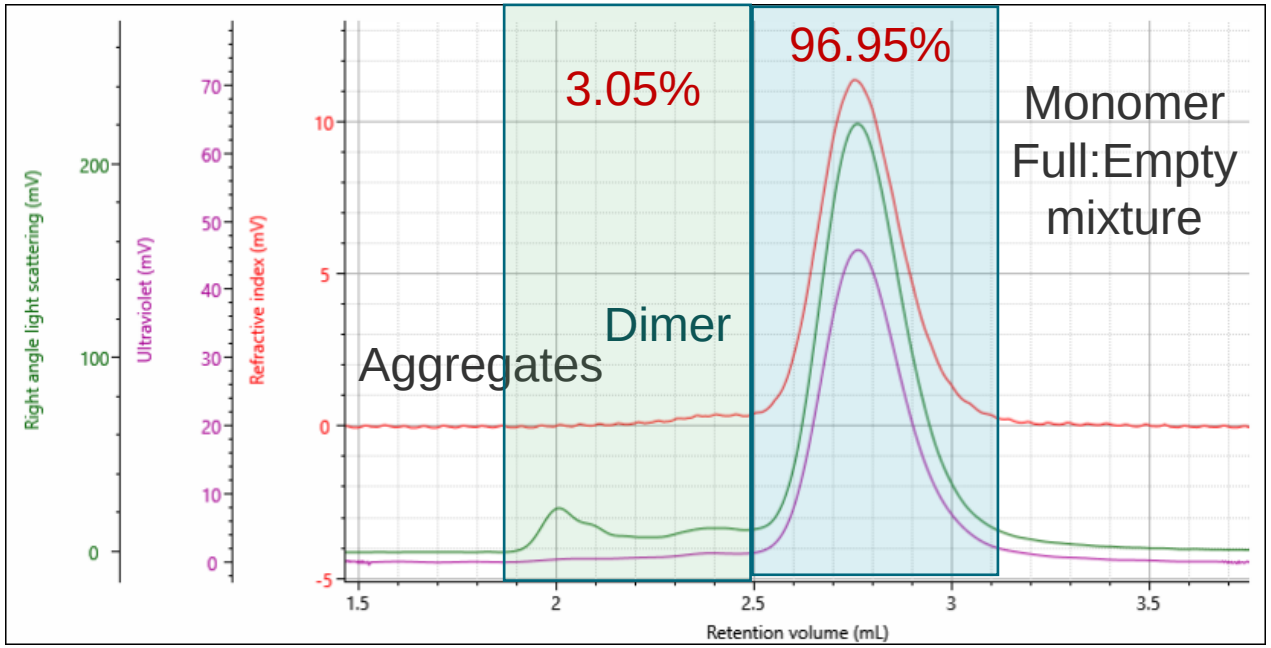
- Formulation development. Enabling comparison and ranking of formulations based on physical stability to stress.
- NTA and ELISA showed mirrored loss of RABV antigenicity during forced degradation studies



D. Clénet et al. European Journal of Pharmaceutics and Biopharmaceutics 132 (2018) 62–69\

Characterization of rAAVs with OMNISEC. DS process and formulation development

Multiple quality attributes at high precision in one run.



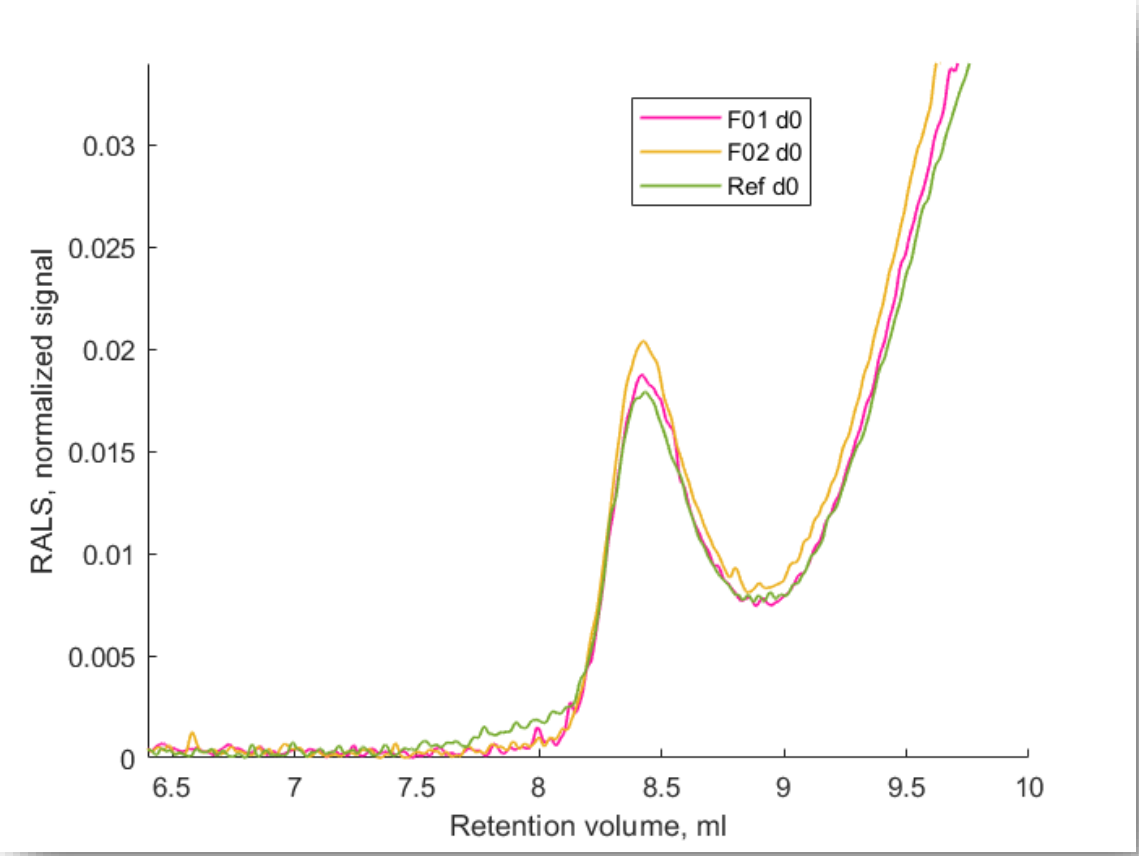
	Peak 1 Mean	% RSD
Mw (g/mol)	4,502,000	0.4752
Mw/Mn	1.008	0.2073
Weight Fraction (A - Capsid) (%)	83.9	0.1331
% Full AAV	77.49	0.4742
cp/vg ratio	1.291	0.4735
Total AAV Titer (cp/mL)	3.08×10^{13}	5.645

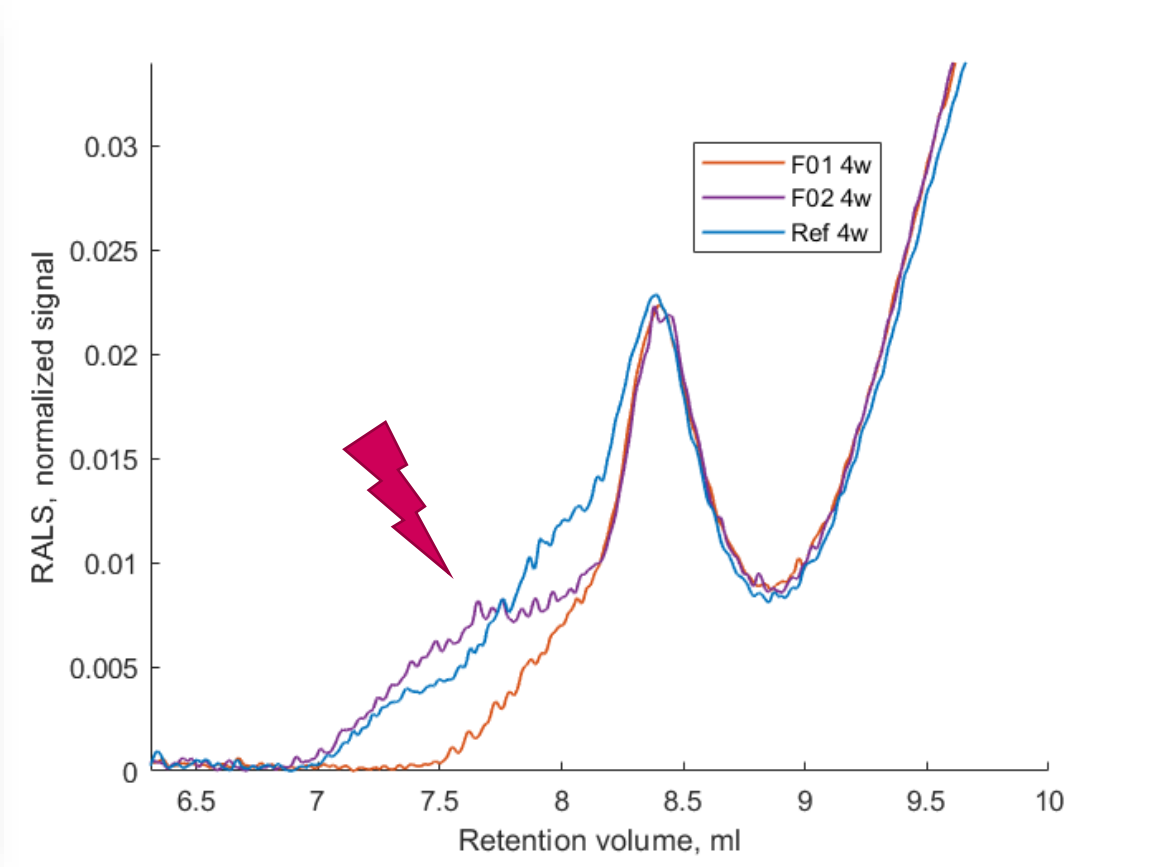
Analytical method	Required virus amount	Determined genome load [% full]		Expected genome load [% full]
		Mean	SD	
MADLS	++	2.3**	n.a.	3.3 (*qPCR+ELISA)
SEC-MALS	+	3.9	0.1	
AUC	+++	6.0	n.a.	

- Serotype independent
- Label-free and reference standards-free
- Multiple quality attributes in one run
- Rapid analysis
- Reliable results through orthogonality

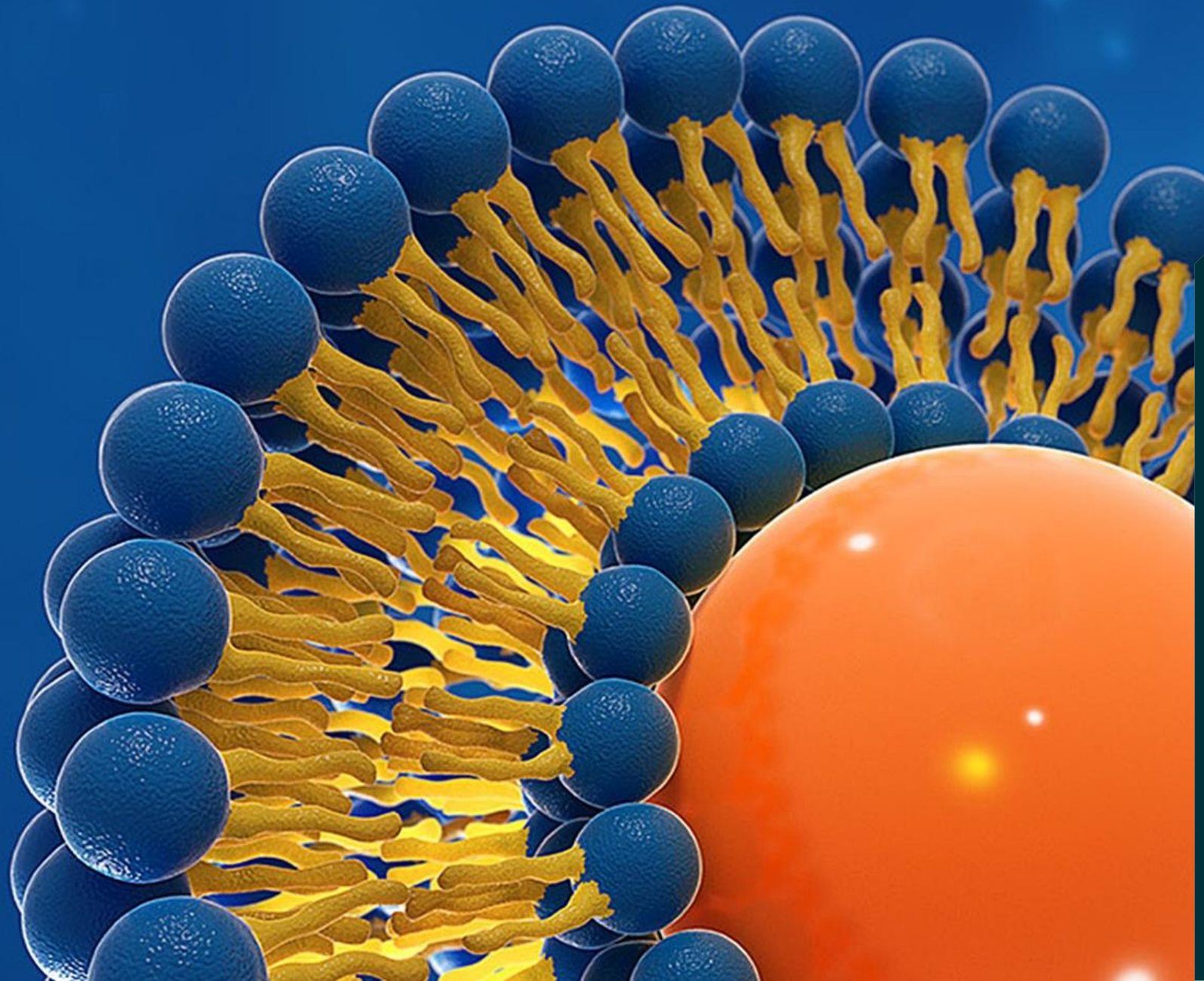
OMNISEC in formulation development: stress stability tests

Time 0 and 4 w samples in 3 conditions overlayed





- Aggregation profiles differ between the 3 formulations
- In all cases a small increase in high molecular weight aggregation is observed
- F01 appears to have lower level of HMWA



Lipid-based delivery vectors

In collaboration with SINTEF

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mRNA-LNP size and particle concentration

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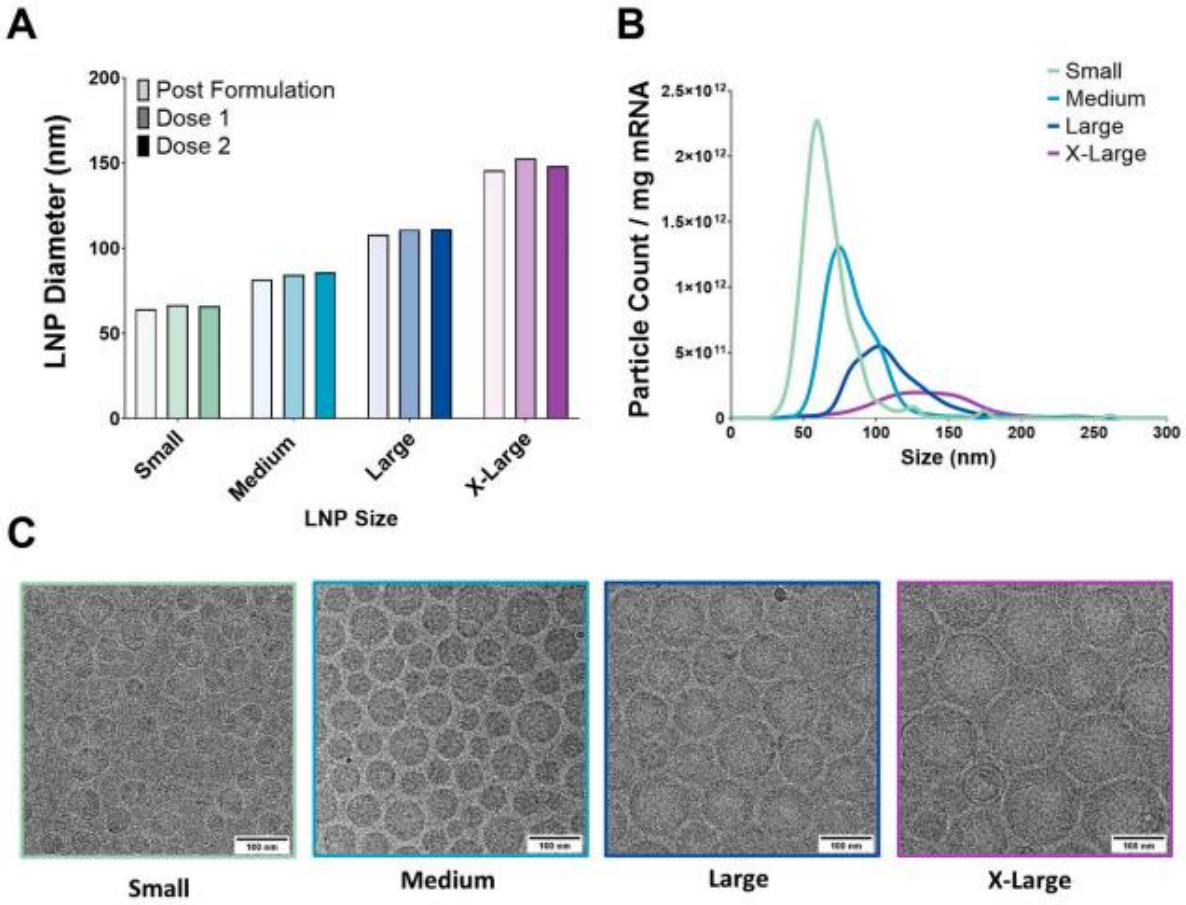
Manufacturability | Drug Delivery | Quality, Safety & Efficacy

Control mRNA LNP particle size at Moderna Inc.



Journal of Controlled Release 335 (2021) 237–246

- DLS and MADLS for a quick screen of **sample as is**
- Looking closer into the particle size distribution with NTA

LNP1 Median: $7.4*10^{12}$
Mean: $11.5*10^{12} \pm 9*10^{12}$
78% RSD

LNP1 main peak Median: $2.4*10^{12}$
Mean: $2.6*10^{12} \pm 0.5*10^{12}$
19% RSD

Follow LNP sample stability

Z-average size and polydispersity to track stability

Manufacturability Quality, Safety & Efficacy

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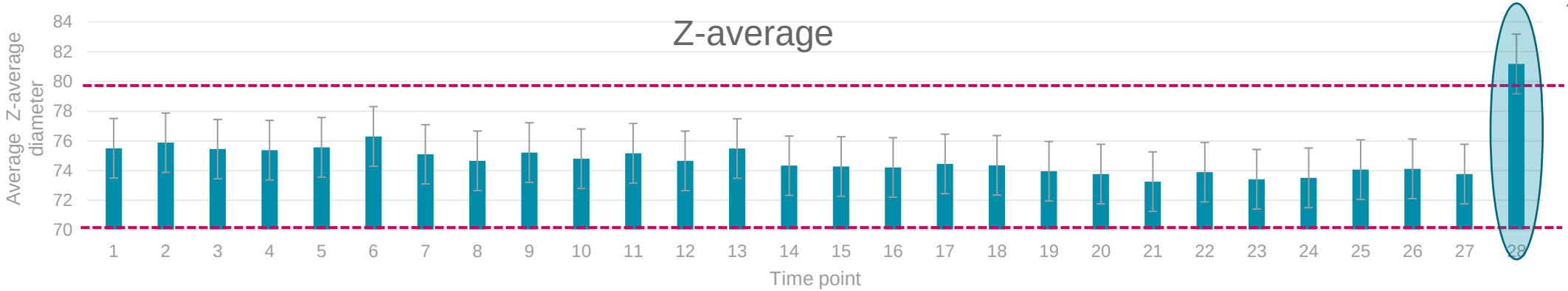

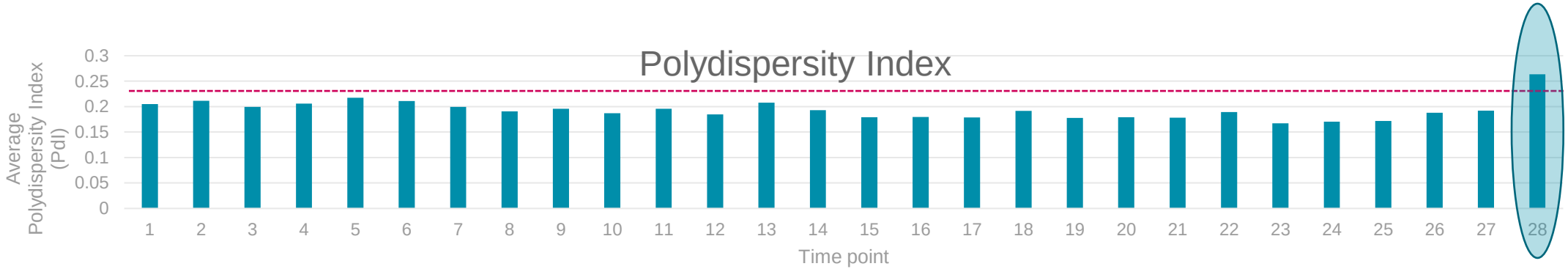

Track changes: batch-to-batch, over stress condition such as time, storage, etc

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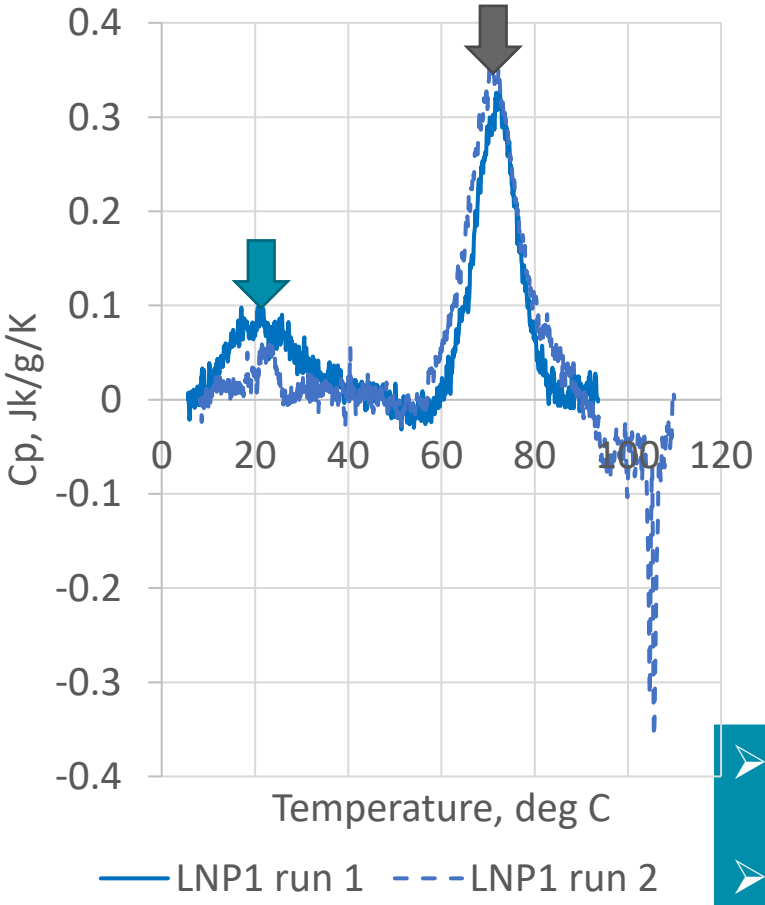
Formulation and batch comparability of LNPs

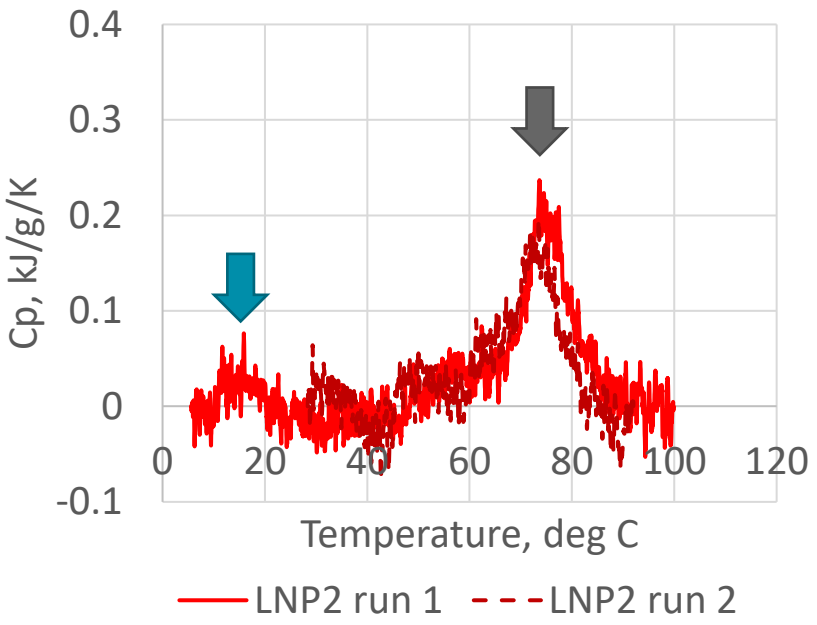
Manufacturability | Drug Delivery | Quality, Safety & Efficacy

Higher Order Structure & stability from DSC

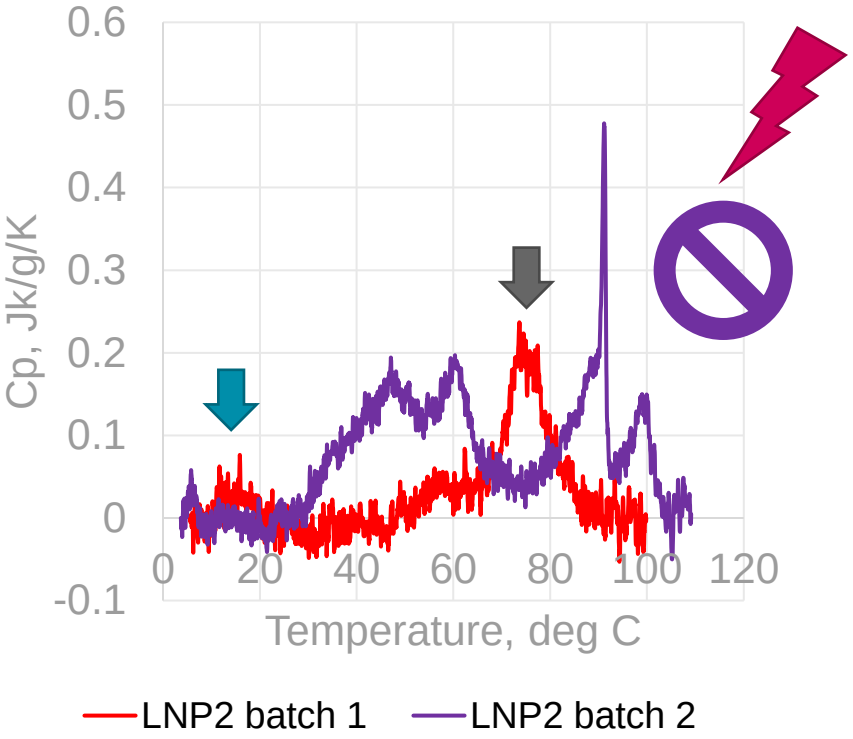
LNP1 run 1 and run 2



LNP2 run 1 and 2



LNP2 batch 1 and LNP2 batch 2



- Well-reproduced qualitatively similar DSC profiles with at least two transitions for LNP1 and LNP2 batch 1
- Qualitative and quantitative differences between LNP2 batch 1 and batch 2. Changed composition and increased structural heterogeneity in the sample?

Fusion proteins

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Affibody – The Best of Two Worlds

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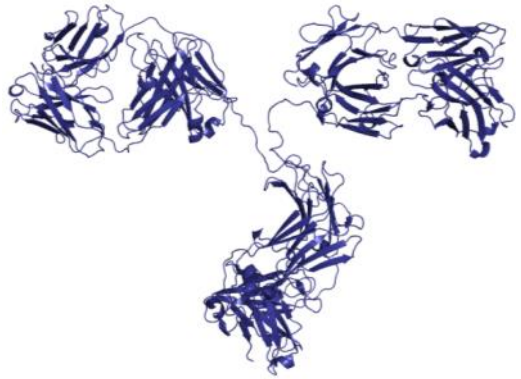
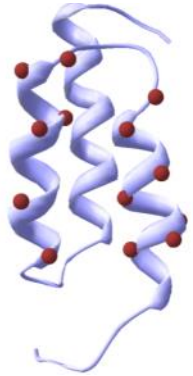
Biopharmaceutical	Next generation therapeutics	Traditional pharmaceuticals
		
Monoclonal Antibodies	**Affibody® Molecules**	**Small Molecules**
• High specificity • High COGS • Size: 150 kDa	• High specificity • Low COGS • Size: 6.5 kDa	• Low specificity • Low COGS • Size: <0.5 kDa

The Affibody® technology is uniquely positioned in the market

Affibody® and Albumod™ Platforms

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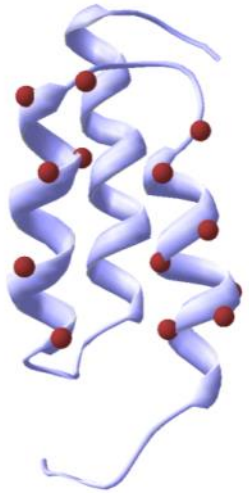
Affibody® Platform



- Antibody alternative with superior properties
- Highly functional 10^{10} library
- IP protection until 2034

Albumod™ Platform

- Extending the half-life of biotherapeutics
- Sub pM affinity to albumin
- IP protection until 2030

Proven technology and excellent IP position leads to business opportunities

Early-on stability profiling and construct selection.
Workflow attempted for characterization of Protein X stability

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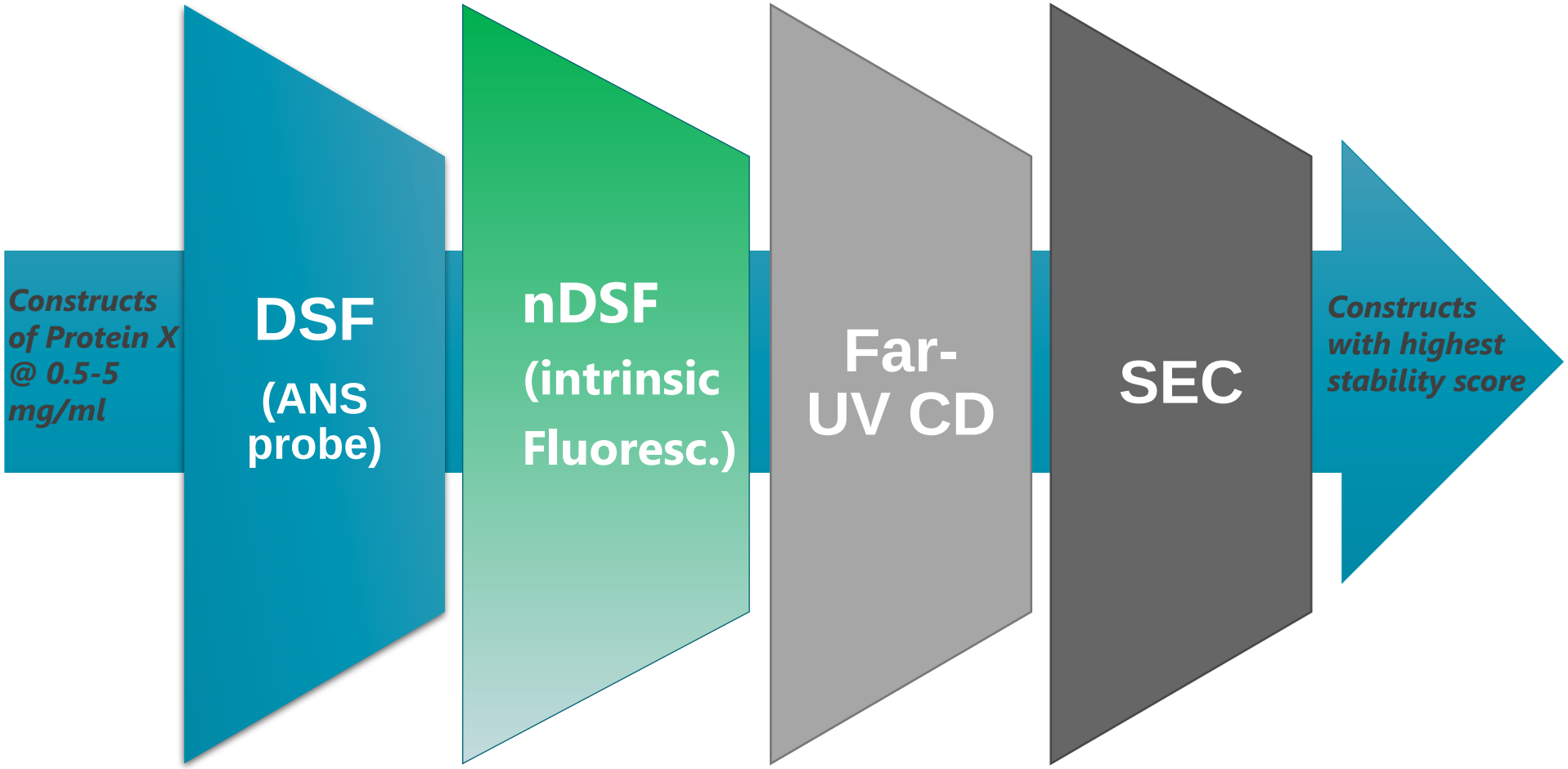
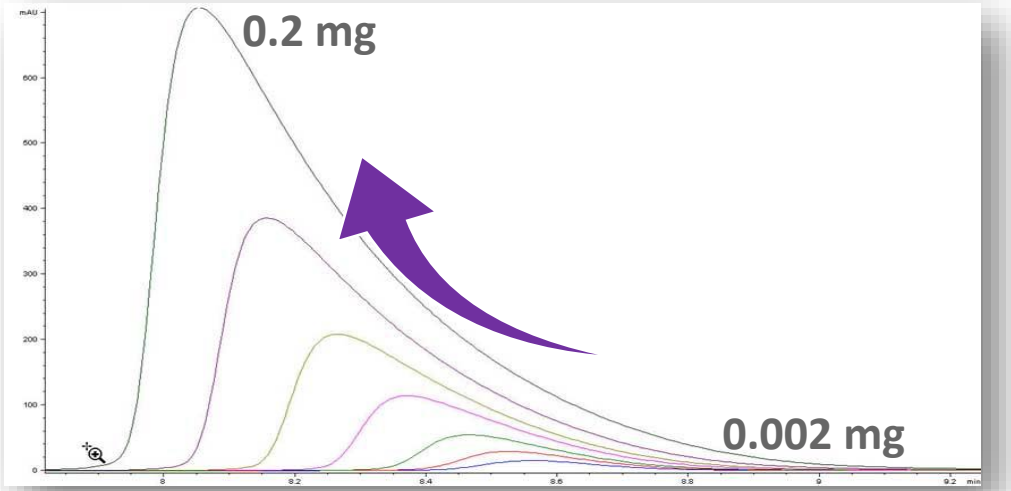
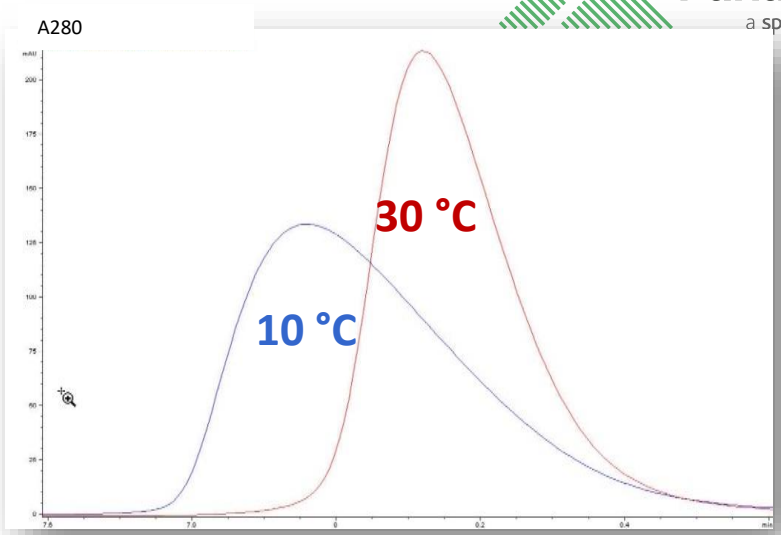
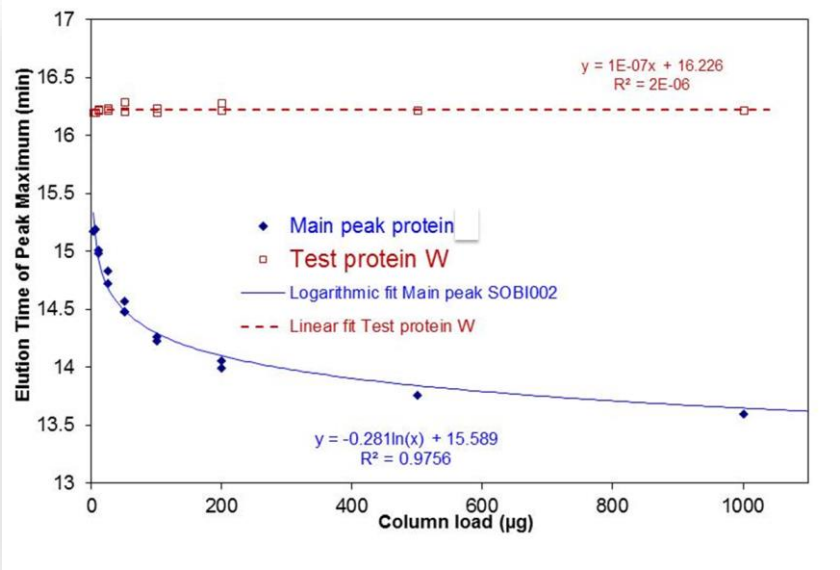


Project setbacks.

Early-on SEC data found insufficient for quantitative and qualitative comparison of size and aggregation levels of Protein X constructs



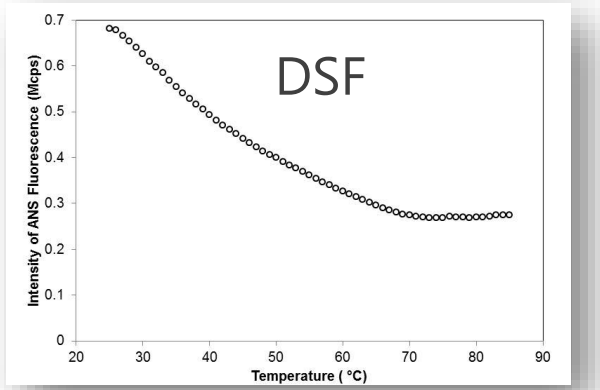


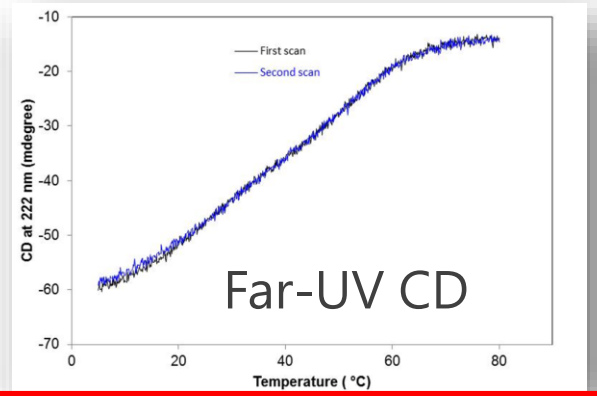


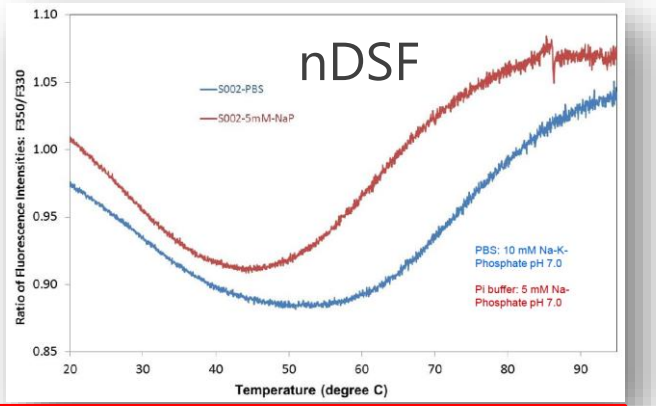
- ✓ SEC profile of Protein X was dependent on protein load and column temperature.
- ✓ SEC chromatograms gave limited means to assess monomeric purity and aggregation level of protein X constructs

Project setbacks.

Inconclusive data on thermal stability of Protein X constructs probed with Differential Scanning Fluorimetry (DSF), CD spectropolarimetry and nDSF.







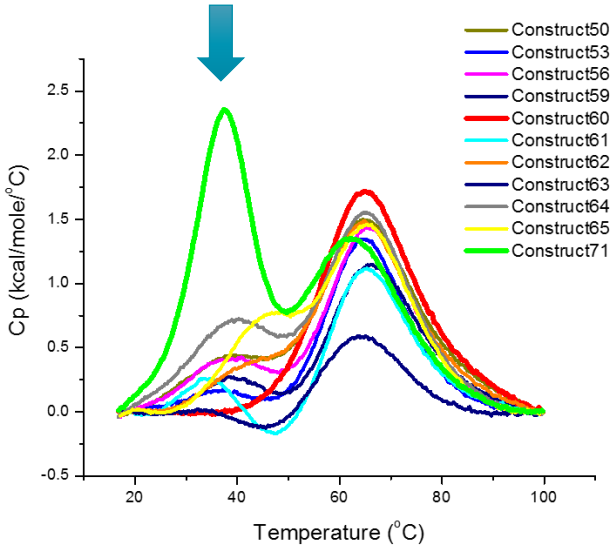
No means to rationalize the data and to rank conformational stability of constructs. Data convergence not achieved between techniques.

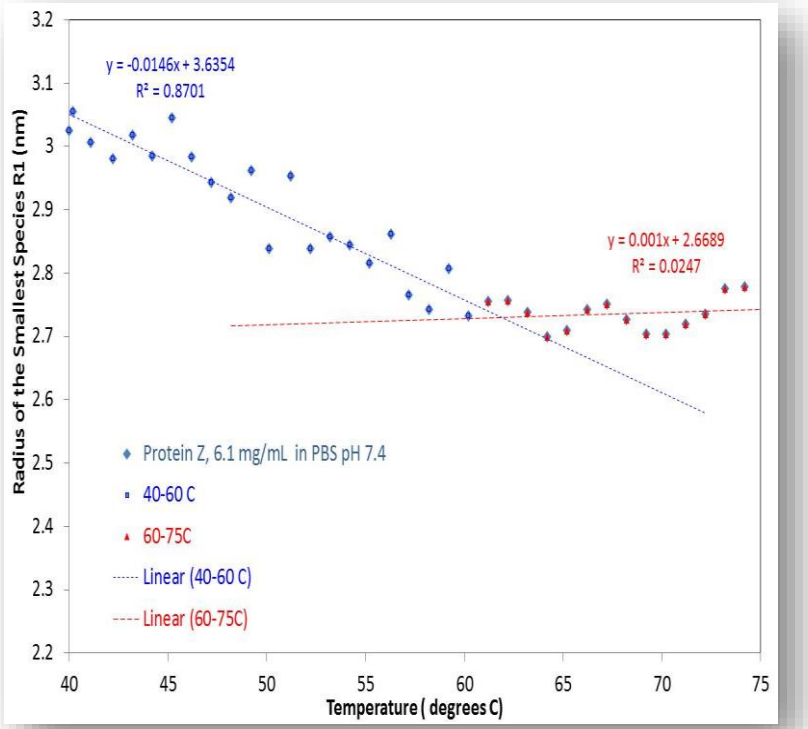
- ✓ No conclusions could be made on unfolding properties by monitoring fluorescence of hydrophobicity probe, ANS.
- ✓ Only decrease of fluorescence intensity was observed

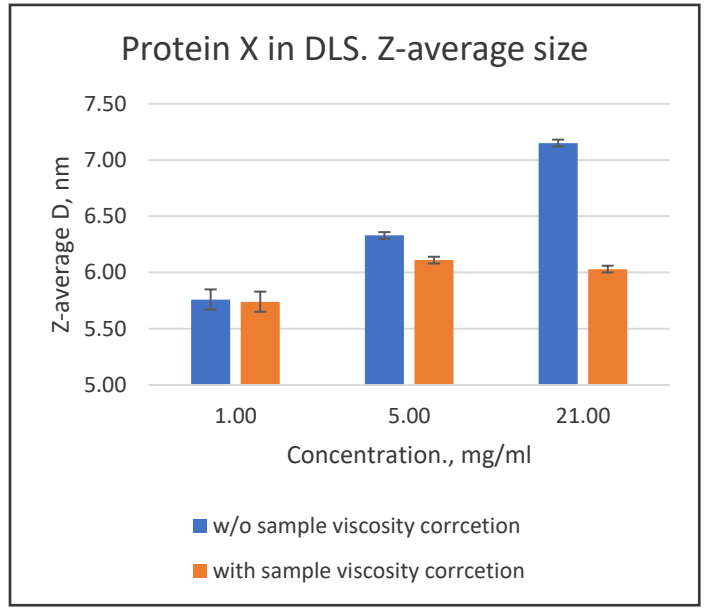
- ✓ Thermal unfolding monitored with far-UV CD was completely reversible.
- ✓ Temperature dependence curve was not sigmoidal, potentially indicating several simultaneous processes, lack of cooperativity or multiple domains.

- ✓ Tryptophan fluorescence showed complex temperature dependence.
- ✓ Fluorescence ratio displays blue shift at temperatures <45 °C and red shift at temperatures >45 °C.
- ✓ Structuring followed by gradual loss of structure at higher temperatures?

Markedly different conformational and colloidal stability of Protein X constructs revealed by DSC and DLS





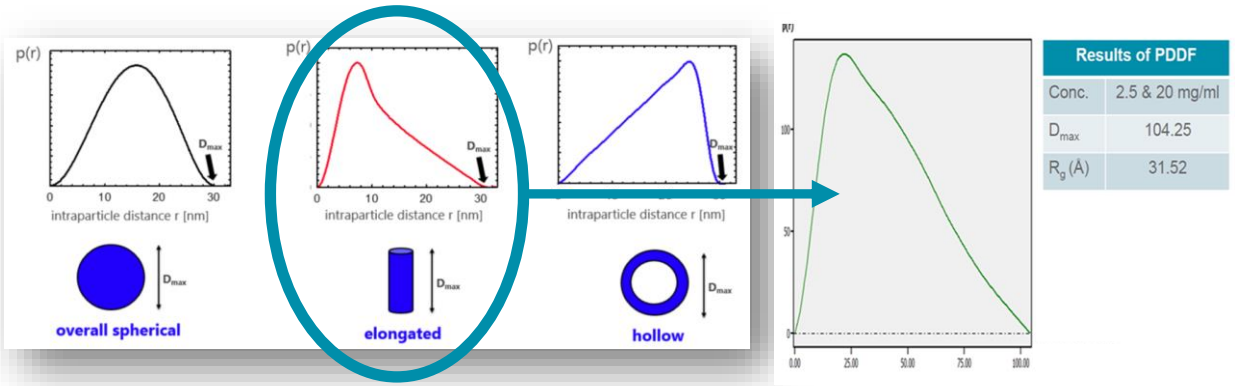


- ✓ Twelve constructs varying in one or a few amino acid positions yielded significantly different DSC thermograms.
- ✓ Two transitions were identified and most variability was observed for the first transition.

- ✓ Heating appears to induce dissociation of Protein X aggregates up to ~60 deg C.
- ✓ **Is protein X prone to specific or non-specific self-association?**
- ✓ **Can oligomerization be quantified?**

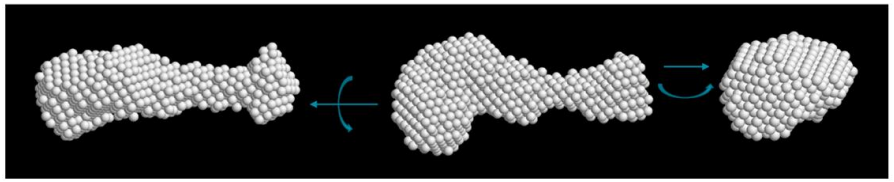
Size and Shape of Protein X in Solution Probed with SAXS.

- Could SAXS data help to address Protein X state in solution and converge contradicting results from:
  - SEC - short retention time – larger than monomer – oligomer?
  - SEC-MALS -monomer
  - DLS – size increase with concentration



Results of PDDF	
Conc.	2.5 & 20 mg/ml
D_{max}	104.25
R_g (Å)	31.52

Results of Guinier plot		
	Lysozyme	Protein X
I(0)	27702	85163
Mw (monomer)	14.3 kDa	19 kDa



- SAXS data indicated elongated shape
- The shape could explain the apparent larger size observed with SEC (suspected but not proved in the absence of SAXS data).
- **But is it a monomer or dimer in solution?**

- Apparent Mw of Protein X in solution established as 44 kDa.
- Protein X at 10 mg/ml exists as a multimer with a **mass close to that of a dimer**.
- The averaged bead model derived with EMBL-Hamburg ATSAS software suite highlights **asymmetric shape of Protein X species in solution.**

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…”The addition of multiple orthogonal techniques, such as ITC, DSC, DLS and SAXS, in combination with extensive support from Malvern Panalytical has been instrumental for us to better understand the behavior of our Affibody® molecules in solution and in standard analytical assays.”

David Bejker, CEO
Affibody AB


We are
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We're BIG on small™
Accelerating AAV Analysis