

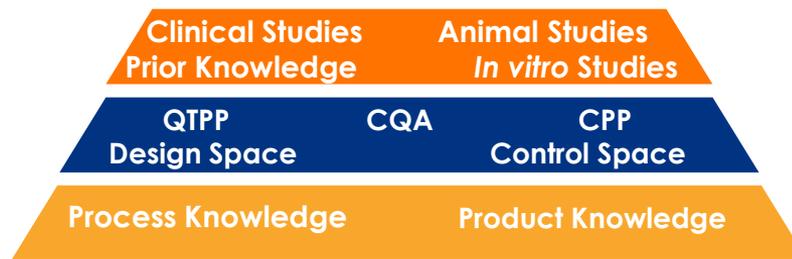


# Developability assessment of novel AAV capsids and payloads at early preclinical stage to enable development of AAV gene therapies

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## Critical Quality Attributes

- A physical, chemical, biological or microbiological characteristic/property within an appropriate range to ensure the desired product quality
- Defined by the QTPP, to establish a link between specific product attributes and expected clinical performance
- Determined through initial risk analysis followed by impact assessment



## Role of Developability

- Applied as a tool to filter out weaker candidates
- Earlier intervention of advanced analytics to de-risk *in vivo* selection studies
- Greater understanding of how product attributes influenced by the production process

	CQA/CDA	Stress/Degradation	Predictive Analytical Tool	Rationale
<b>Purity/ Heterogeneity</b>	Aggregation, fragmentation, hydrophobicity, charge	$\Delta$ Temp, $\Delta$ pH, F/T, high salt, ionic strength	SEC(MALS), DSF, CESDS, RP-HPLC(MS), HIC, CIEF, IEX, $\zeta$ -potential	Stability predictor, impact of viral inactivation/storage/handling, aggregation potential, process losses
<b>Conformational Stability</b>	Thermal unfolding, aggregation, particles	Temp ramp, $\Delta$ pH, formulation/excipients	DSF, DSC	Indicative of real-time/accelerated stability storage
<b>Colloidal Stability/ Self-association</b>	Viscosity, aggregation, particles	Temp ramp, $\Delta$ pH, $\Delta$ conc., formulation/excipients	AC-SINS, DLS, viscosity	Predictive of concentration dependent aggregation or viscosity/gel formation
<b>Solubility</b>	Solubility, concentration, aggregation, particles	0-40% PEG	PEG induced precipitation	Extrapolate solubility in formulation compositions or compare candidates.
<b>PTM/Chemical Stability</b>	Oxidation, Deamidation, Glycosylation, glycation, S-H	[Ox] (H <sub>2</sub> O <sub>2</sub> , TBHP, AAPH), pH, $\Delta$ temp, [red]	<i>In silico</i> analysis, peptide mapping	Impact on binding, function or aggregation
<b>Upstream Process</b>	Titer in CHO, cell viability	Representative/platform DOE	Octet or Protein A HPLC methods	Stable pool/ clone selection for high expression & desirable characteristics
<b>Downstream Process</b>	Purification unit process operations	Representative/platform DOE	In-process testing, yield and purity	Screen for breakthrough, retention, and performance. Prediction of control parameters and process sensitivity
<b>Formulation</b>	Formulation fit/all CQAs	Temp ramp, $\Delta$ pH, $\Delta$ conc. formulation/excipients	Stability in representative stress conditions	Reveal liabilities for storage and handling, estimate long-term storage stability
<b>Biological Attributes</b>	Affinity, specificity, $t_{1/2}$ , PK, functional activity	25°C and 37°C at pH 7.4	SPR, flow cytometry, ELISA, potency	Desirable affinity, half-life and off-target binding, Impact of pCQAs on function

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Narrowing of  
Development  
Candidates



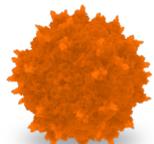
Increasing  
Toolkit  
Sophistication

## Initial Release Methods

ATTRIBUTE	METHOD
Vector titer	ddPCR
Genomic integrity	Agarose Gel
Capsid purity	SDS-PAGE Gel
Sub $\mu\text{m}$ aggregation	DLS
Safety	Endotoxin LAL
Compendial	pH, Osmo, Appearance



● DNA



● Capsid Protein

● Both

## Detailed Characterization

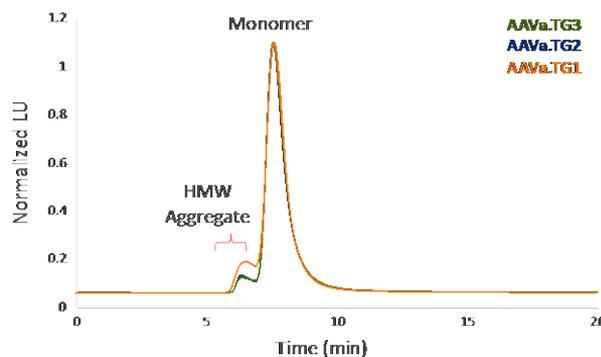
ATTRIBUTE	METHOD
Vector titer	ddPCR
Genome sequence	PacBio Sequencing/NGS
Capsid purity	CE-SDS (LIF)
Sub $\mu\text{m}$ aggregation	DLS, SEC-FLD
Sub-visible aggregation	HIAC, MFI
Primary sequence/PTM	LCMS peptide map
Safety	Endotoxin LAL
Product impurities	HCDNA, HCP, Plasmid etc.
Process impurities	Ligand, nuclease, surfactant
%Full	AUC, SEC-MALS
Relative Potency	<i>In vitro</i> function

Earlier application?

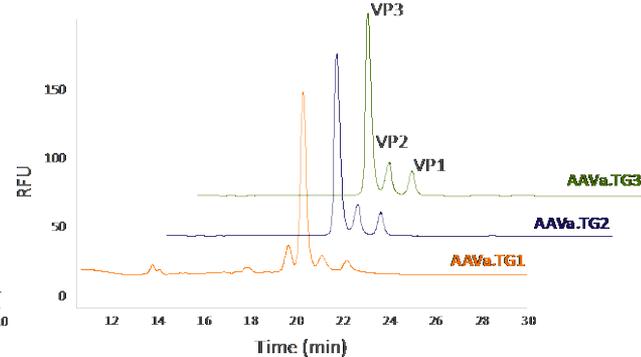
Capsid Identity	SEC-HPLC (%HMW)	AUC % (Full/Partial/Empty)	AUC (LOC/ HOC)	CESDS (VP3:VP2:VP1)	CESDS %Purity
AAVa.TG1	9.9	53/ 10/ 14	11/ 12	7:1:1	77%
AAVb.TG1	12.3	54 / 12 / 20	0 / 14	9:1:1	86%
AAVc.TG1	13.2	62 / 8 / 17	8 / 15	6:1:1	98%
AAVa.TG2	4.6	80 / 3 / 4	4 / 10	7:1:1	98%
AAVa.TG3	4.3	77 / 10 / 3	4 / 10	6:1:1	97%
AAVd.TG2	7.4	76 / 4 / 4	4 / 6	8:2:1	97%
AAVd.TG3	5.8	68 / 13 / 4	4 / 10	7:2:1	97%

- One set of constructs showed elevated levels of aggregation and fragmentation
- AUC indicates significant Loss of full peak with corresponding elevated High and Lower Order Capsids (HOC/LOC)
- Modification of incorporated transgene led to a more stable series of constructs

### SEC-FLD: Aggregation



### CESDS: VP Ratio & %Purity



## Capsid Identity

## SEC-FLD (%HMW)

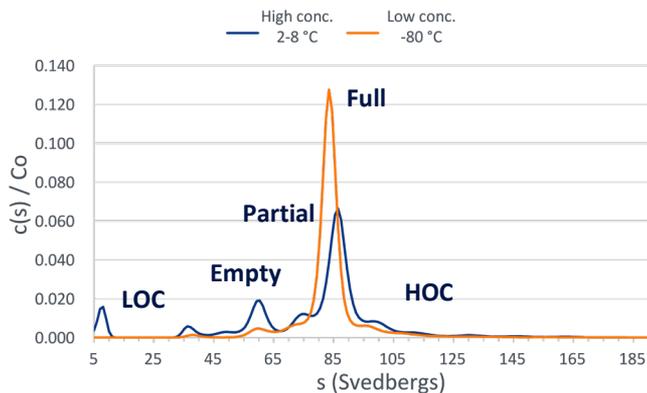
## SEC-MALS (%Full)

## AUC (% Full/ Partial/ Empty)

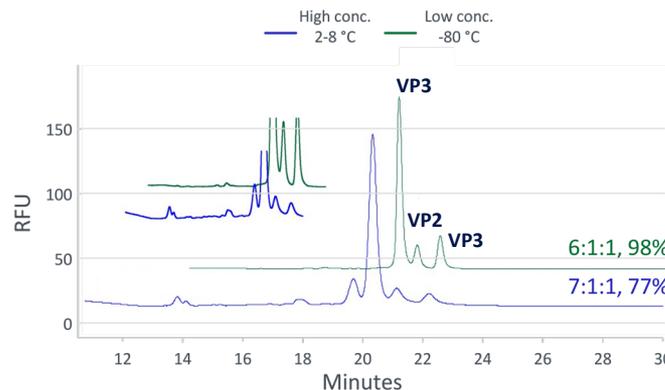
## AUC (LOC/ HOC)

Capsid Identity	SEC-FLD (%HMW)	SEC-MALS (%Full)	AUC (% Full/ Partial/ Empty)	AUC (LOC/ HOC)
AAVa.TG1, high conc., 2-8 °C	9.9	69	53/ 10 / 14	11 / 12
AAVa.TG1, lower conc., -80 °C	1.8	83	82 / 5 / 4	1 / 9

## AUC: Capsid Occupancy

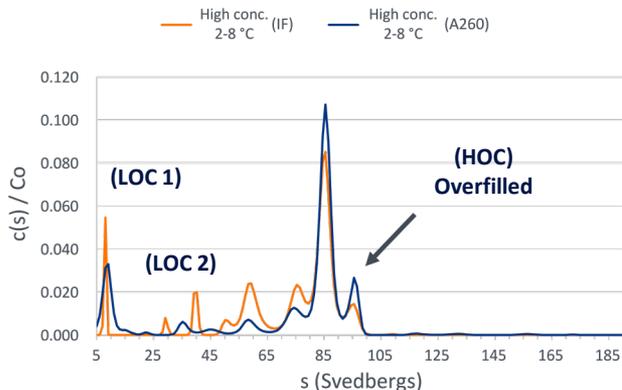


## CESDS: VP Ratio & %Purity

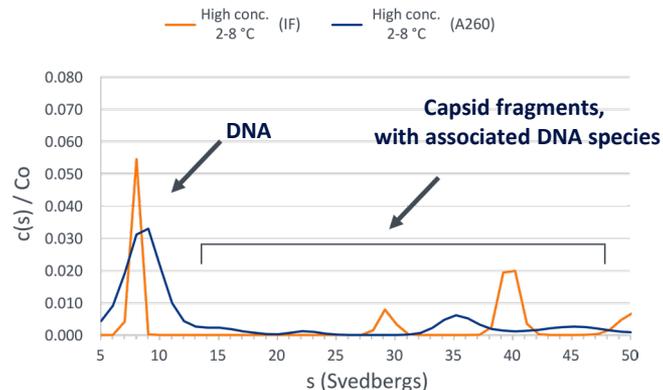


- Combined impact of higher conc. /storage temp. observed in LOC & HOC regions, with loss of capsid occupancy
- These changes confirmed by orthogonal SEC (%HMW), and CESDS (%purity) analysis

## AAVa.TG1



## AAVa.TG1



- UV A260 and interference (IF) collected on the same sample, data analyzed using SEDFIT
- DNA containing species generates stronger A260 signal than IF
- Empty capsid A260/IF at 0.5, DNA species LOC and HOC identified as being DNA rich

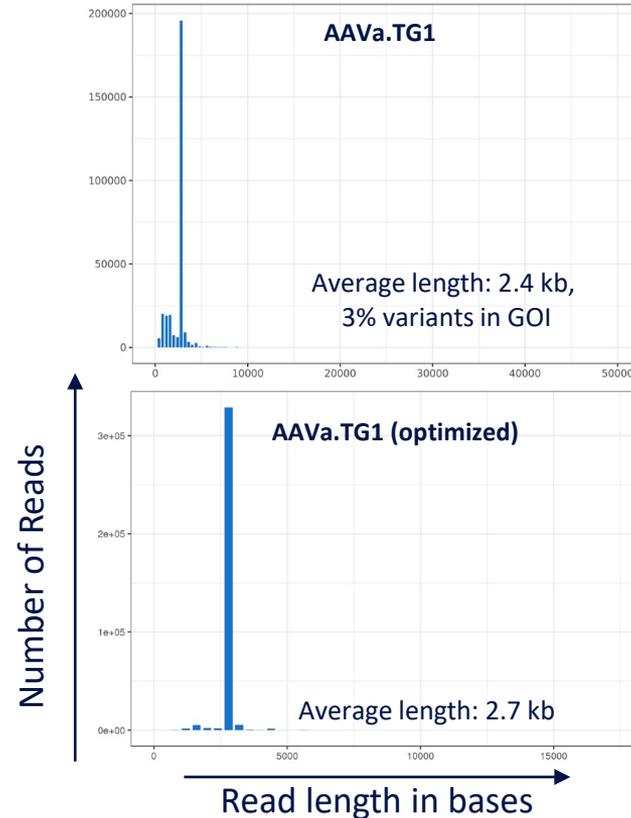
Signal	LOC 1 (Area)	LOC 2 (Area)	Empty (Area)	Partial (Area)	Full (Area)	HOC (Area)
A260	0.125	0.048	0.042	0.086	0.472	0.098
IF	0.030	0.044	0.081	0.078	0.236	0.038
A260/IF	4.2	1.1	0.5	1.1	2.0	2.6

## NGS: Short sequence and variants

TG1	Population mapped to reference	% Consensus similarity to Reference
AAVa	93.5%	100
AAVb	89.1%	100
AAVc	94.7%	99.93
AAVa (opt)	94.7%	100

- Short read sequencing revealed matching to reference sequence with one significant variant: single base (G) deletion at the 5' ITR (<1%)
- Optimized AAVa showed expected TG length with low levels of fragments and variants, during long read sequencing

## PacBio long read: Transgene Sequence

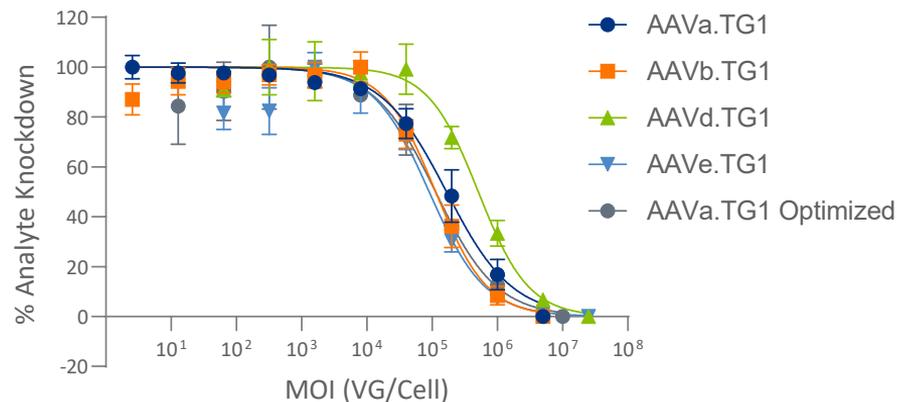


## Capsid.Transgene

## (LogIC<sub>50</sub>)

AAVa.TG1	5.22
AAVb.TG1	5.05
AAVd.TG1	5.71
AAVe.TG1	4.96
AAVa.TG1 Optimized	5.05

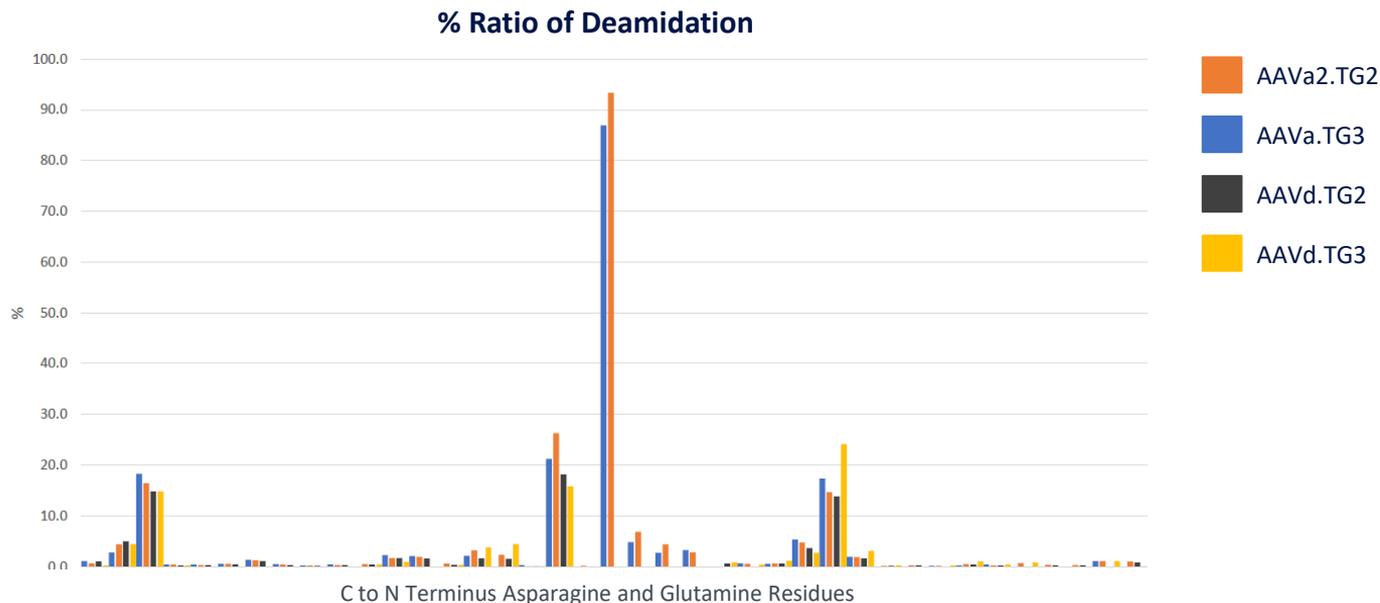
## Relative Potency: Functional Output



- Comparable *in vitro* activity observed across the constructs within the variability of the method
- Despite elevated levels of aggregation dose dependent response observed
- In this iteration of the method, with a majority of active capsid required to show a consistent knock down

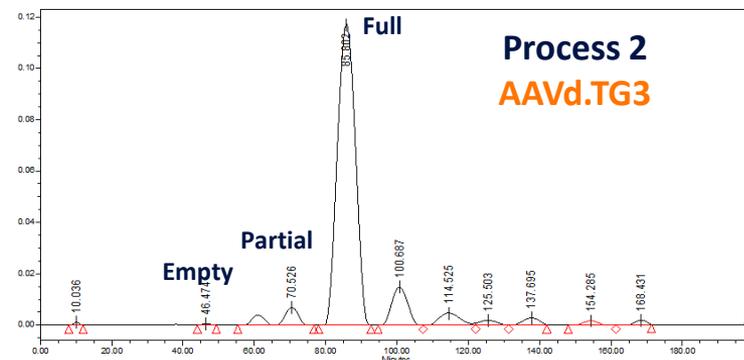
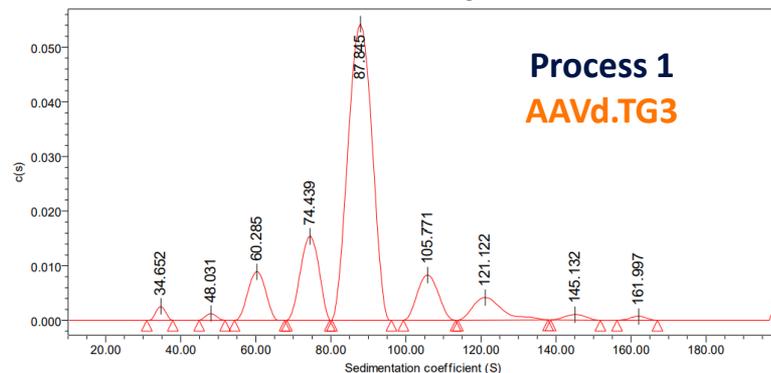
## Other PTMs (Oxidation, methylation, phosphorylation) were examined, focus on deamidation

- Majority high deamidations contain NG motif<sup>1</sup>
- Average for each construct has  $\leq 5\%$
- Changes at key deamidation sites have the potential to impact stability and function



Construct	Source	AUC %(Full/Partial/Empty)	SEC-FLD %HMW	CESDS %Purity
AAVa.TG2	Process 1	70 / 7 / 5	4.6%	98%
	Process 2	78 / 2 / 1	0.1%	98%
AAVa.TG3	Process 1	76 / 9 / 5	4.3%	97%
	Process 2	84 / 2 / 1	0.1%	98%
AAVd.TG2	Process 1	68 / 11 / 9	7.3%	97%
	Process 2	78 / 3 / 2	0.1%	98%
AAVd.TG3	Process 1	62 / 14 / 8	5.8%	97%
	Process 2	81 / 4 / 1	0.3%	98%

- The impact of two production processes can be readily assessed by employing three techniques (AUC combined with SEC and CESDS)
- Despite comparable yields process 2 superior in terms of occupancy, aggregation and purity



- To de-risk early selection activities recommend the inclusion of key analytics prior to *in vivo* studies
- Essential to correlate structural data and *in vivo* activity to continually assess and build analytical toolkit
- Need to bring in earlier manufacturability/developability assessments to accelerate future development activities

## Comparison of structural features from sets of constructs indicated significant differences:

- AUC analysis key, loss of %full and presence of fragments (present in CESDS data)
- Higher levels of aggregation observed in SEC, confirmed by AUC
- Packaged transgene matched reference sequence by NGS, Long read identified small population of fragmented species and low-level deletions/mutations in GOI

## Subsequent batches demonstrated a more stable, structurally sound construct:

- High levels of %full and purity and lower sub- $\mu$ m aggregation
- Improved chances of successful *in vivo* performance
- Functional differences between sets of constructs, further work needed on the analytics to parse this out

## Voyager

- The Research Team
  - Collaboration and guidance
- Process Analytics Team
  - SEC-MALS, AUC & CESDS data
- Vector Production & Pilot Plant Team
  - Steadfast providers of high-quality material

## BioAnalysis

- AUC and LCMS analysis

## Azenta

- NGS and PacBio analysis



voyager  
therapeutics