Diving Deeper:

Using a SYBR Gold Capsid Ejection Assay as an Orthogonal Method of Measuring Potency and VP1 Deamidation in AAV Drug Product Samples

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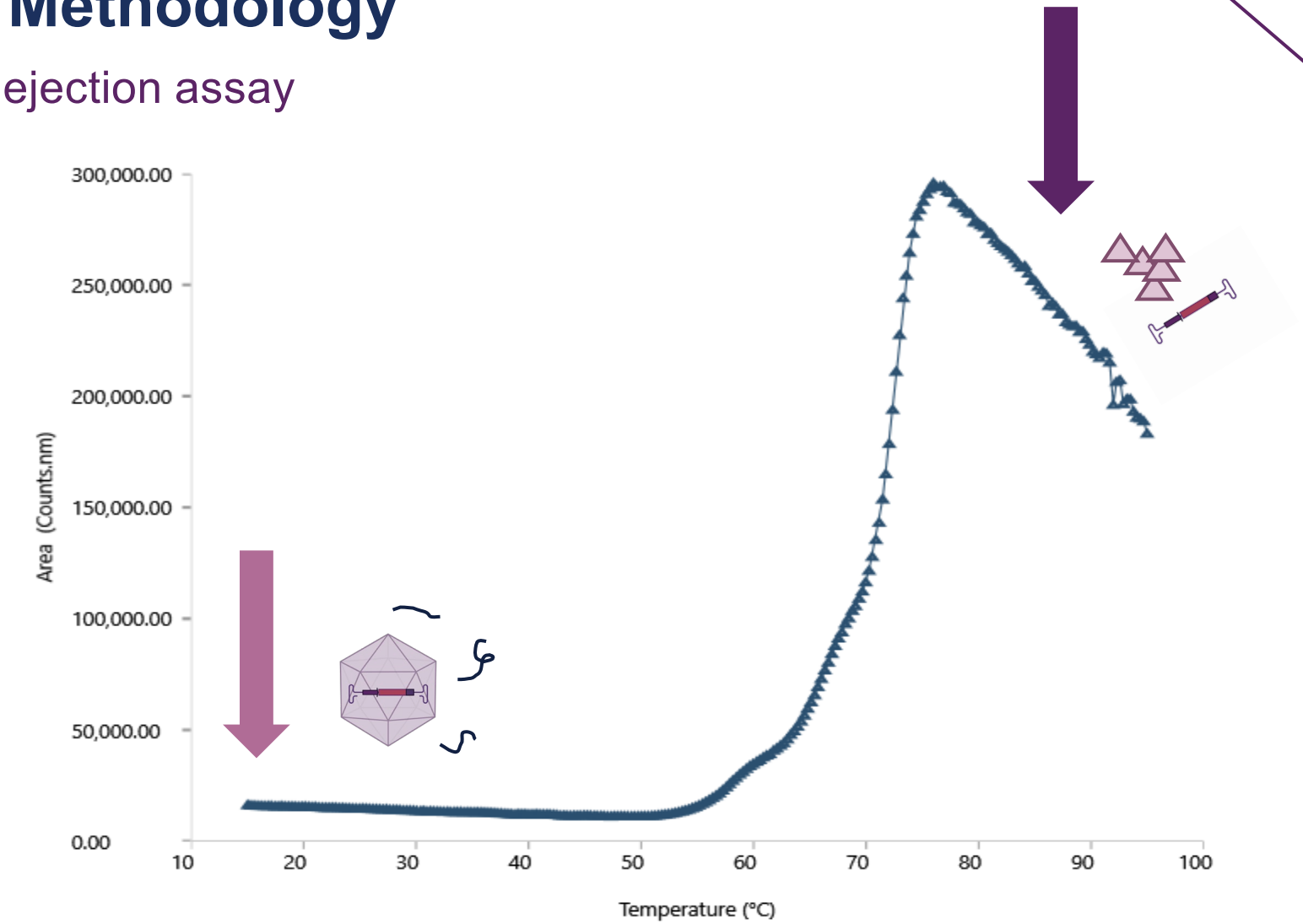
30	960+	45+	30+	65+
Years of manufacturing experience	Successful GMP batches since 2014	Client programmes	IND submissions	Successful audits

Agenda

1. Capsid ejection assay background information
2. Discrepancy between titer & fluorescence
3. Looking inside the capsid
4. Correlations to analytical assays
5. A step further with Next Generation Sequencing
6. Summary

Experimental Methodology

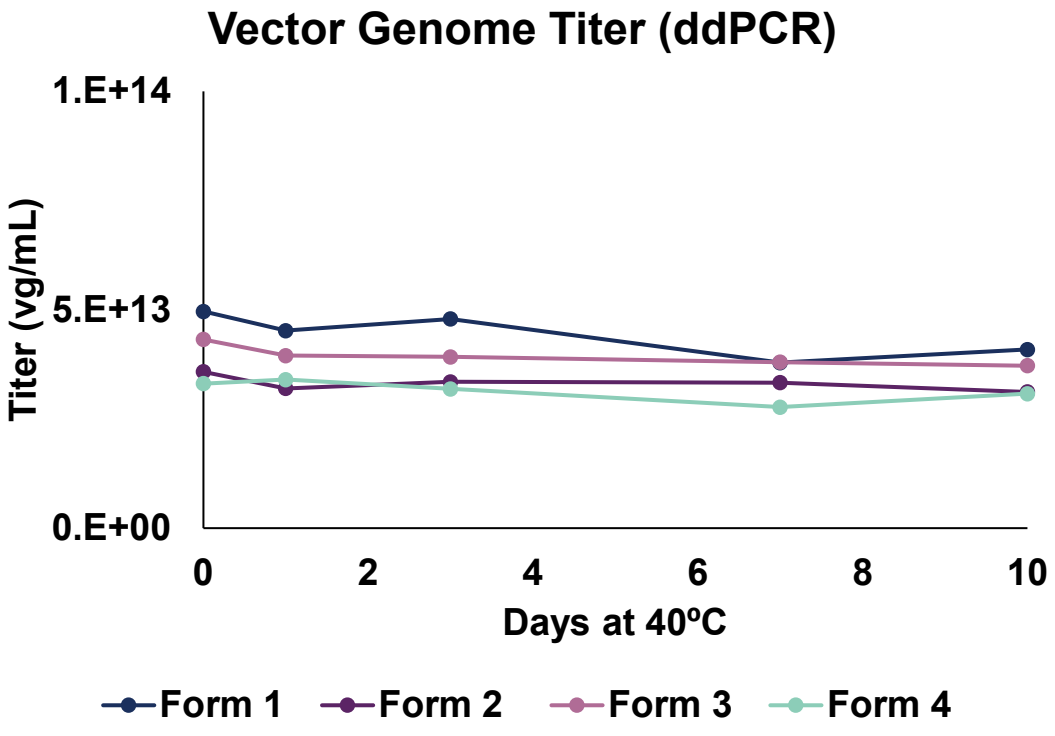
SYBR gold / capsid ejection assay

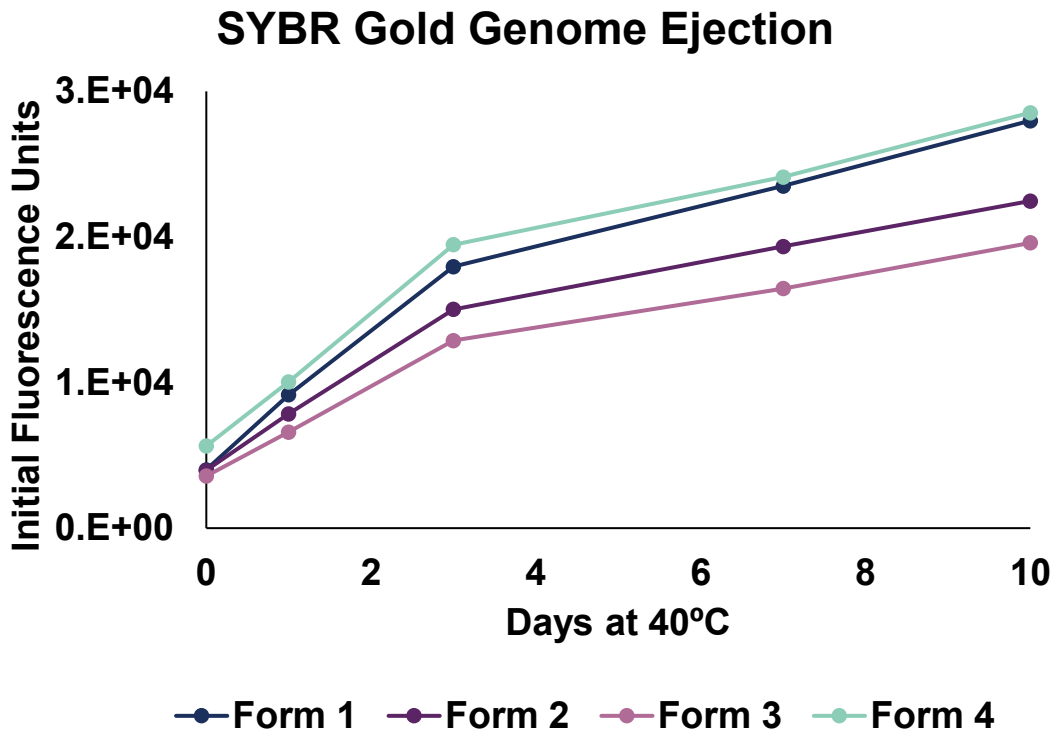


Avg initial fluorescence = free DNA (before thermal ramp)
Avg final fluorescence = final DNA (after thermal ramp and capsid degradation)

Routine Formulation Screening Uncovered a Trend

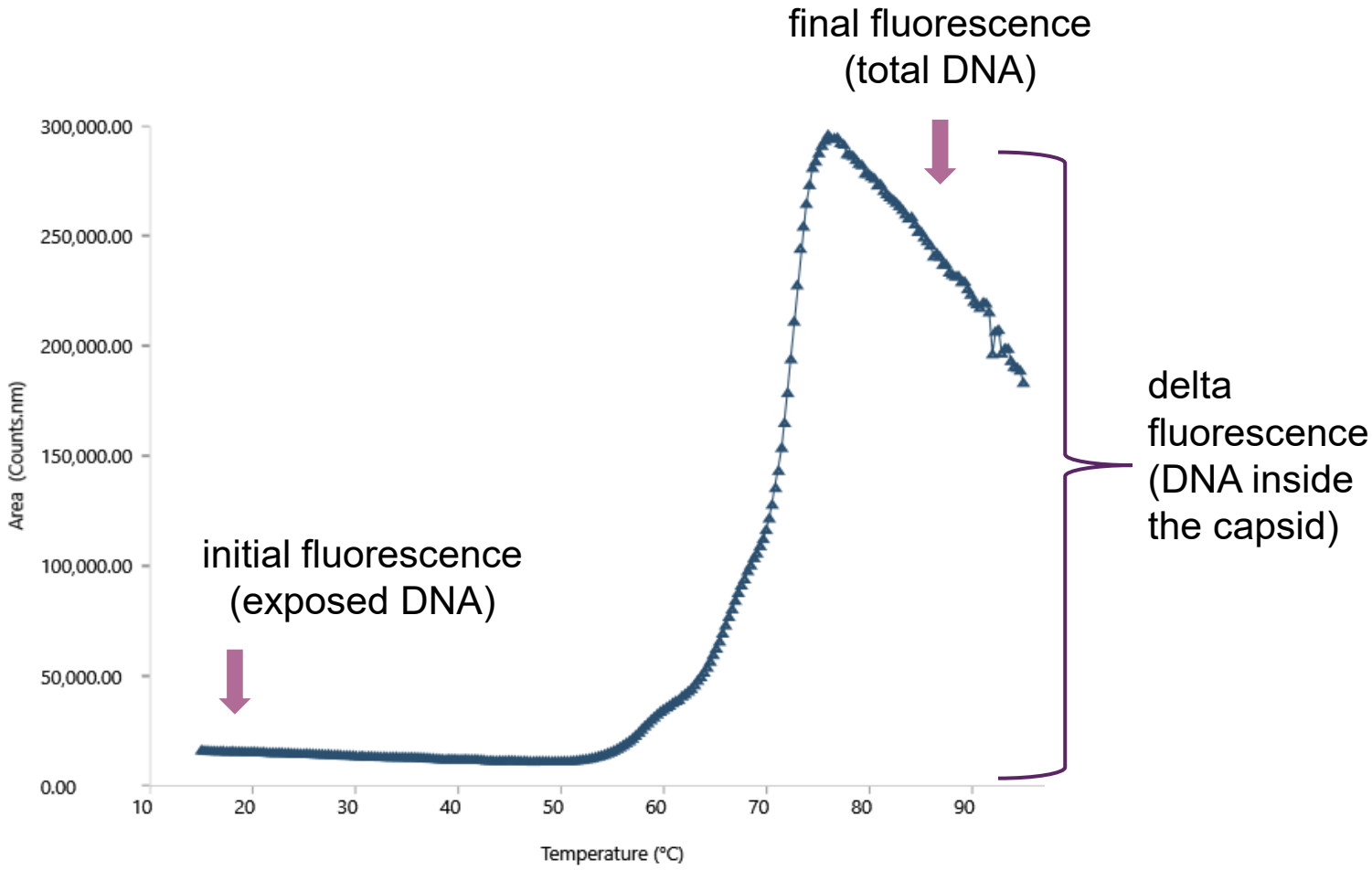
Discrepency between vector genome titer and SYBR gold fluorescence values...





If genome is leaking out of the capsid, why are we not detecting the loss with a vg titer assay?

Looking Inside the Capsid



vg/mL	fluor from DNA inside the capsid	vg/mL	fluor from DNA outside the capsid
3.88E13	619227	**2.9E12**	46916

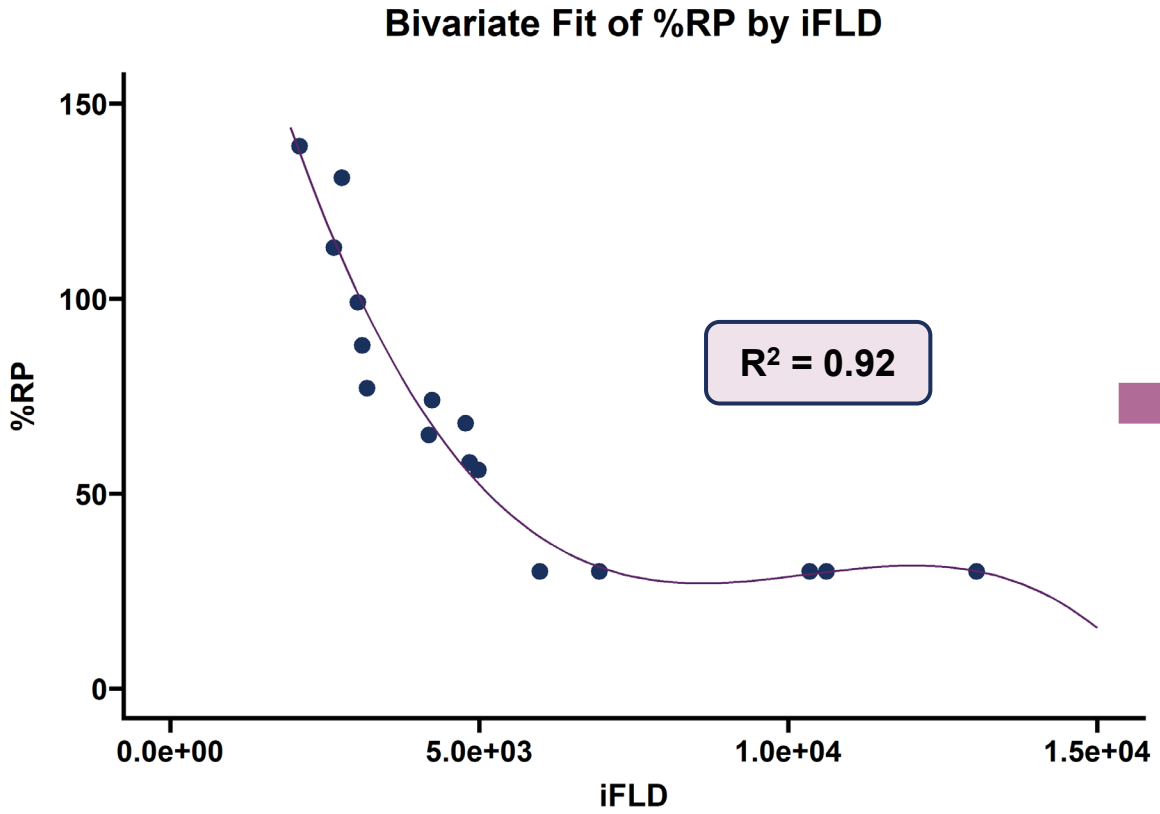
starting		*loss*		*final*
3.88E13	-	2.9E12	=	3.58E13

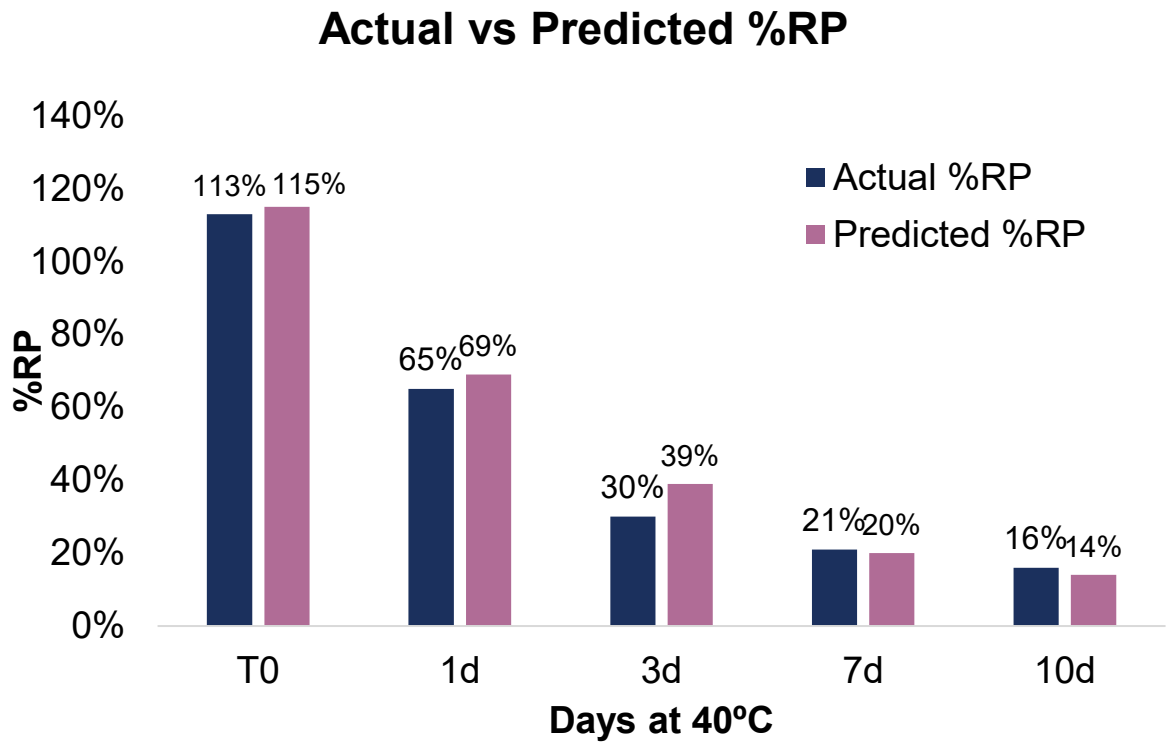
What are the impacts of losing potentially ~8% of the product's genome?

Difference = ~8%

Loss of Potency Directly Correlates with Increased Initial Fluorescence in AAV9 Drug Product Samples

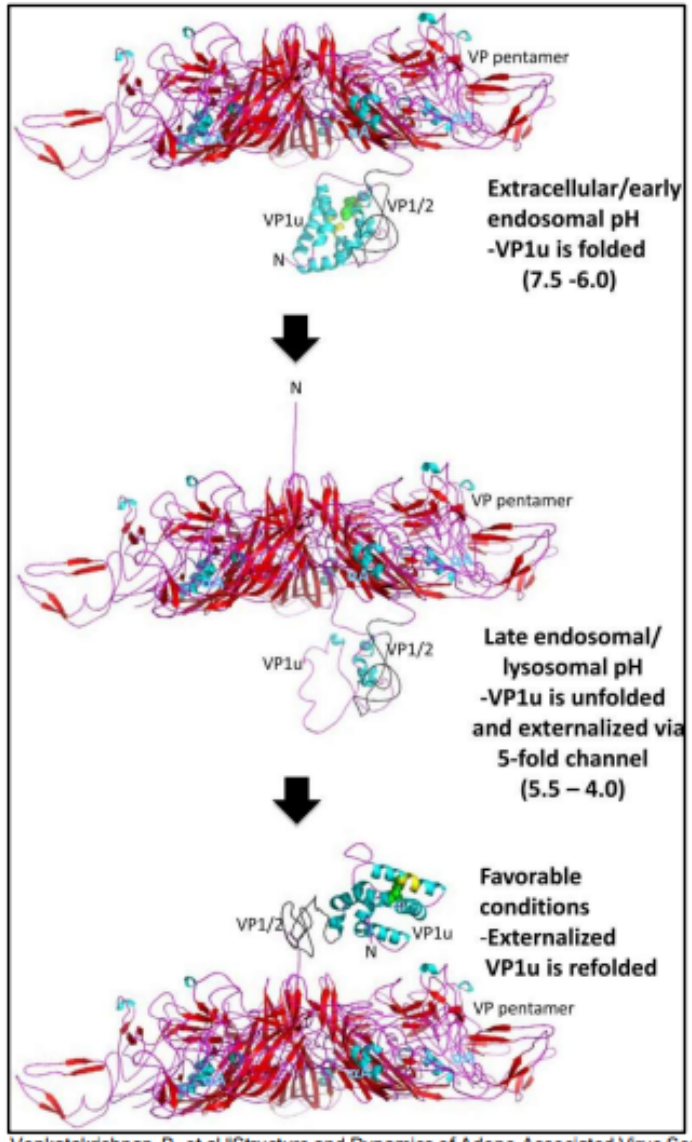
Four independent stability studies supplied ~20 datapoints to generate an equation linking the two assays with a statistically strong fit





The equation was applied to future studies, predicted values validated by actual potency values.

VP1 Extrusion – a Link Between Potency and Deamidation

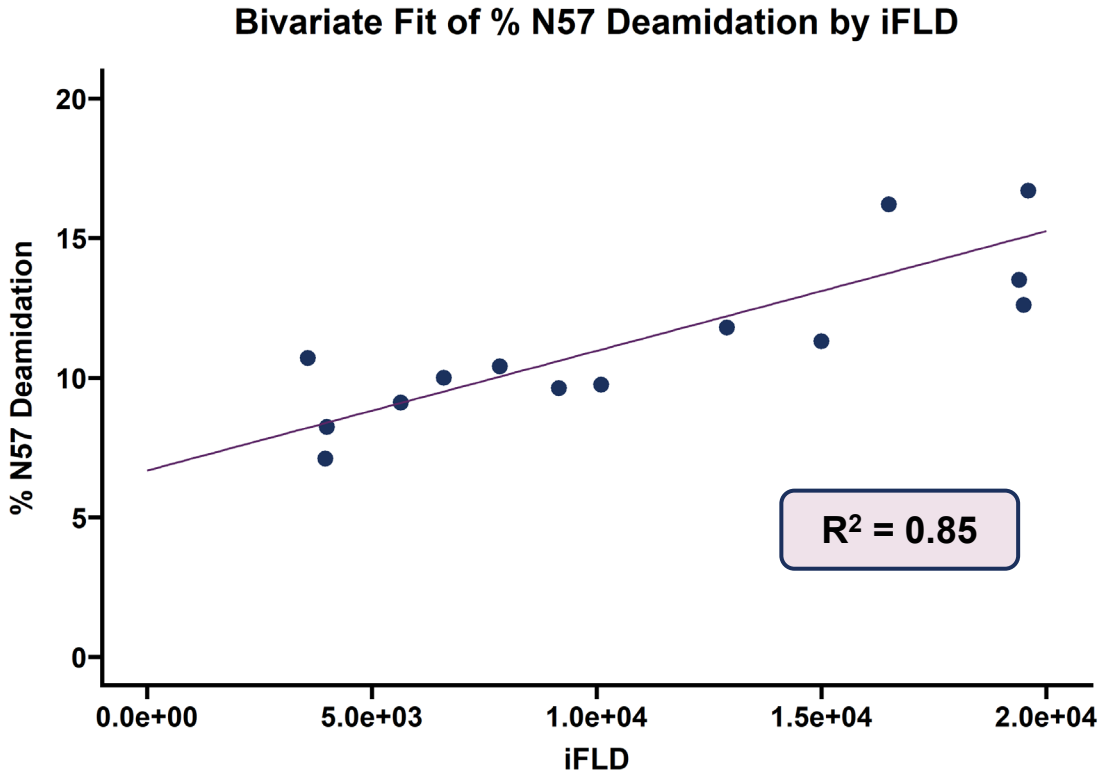


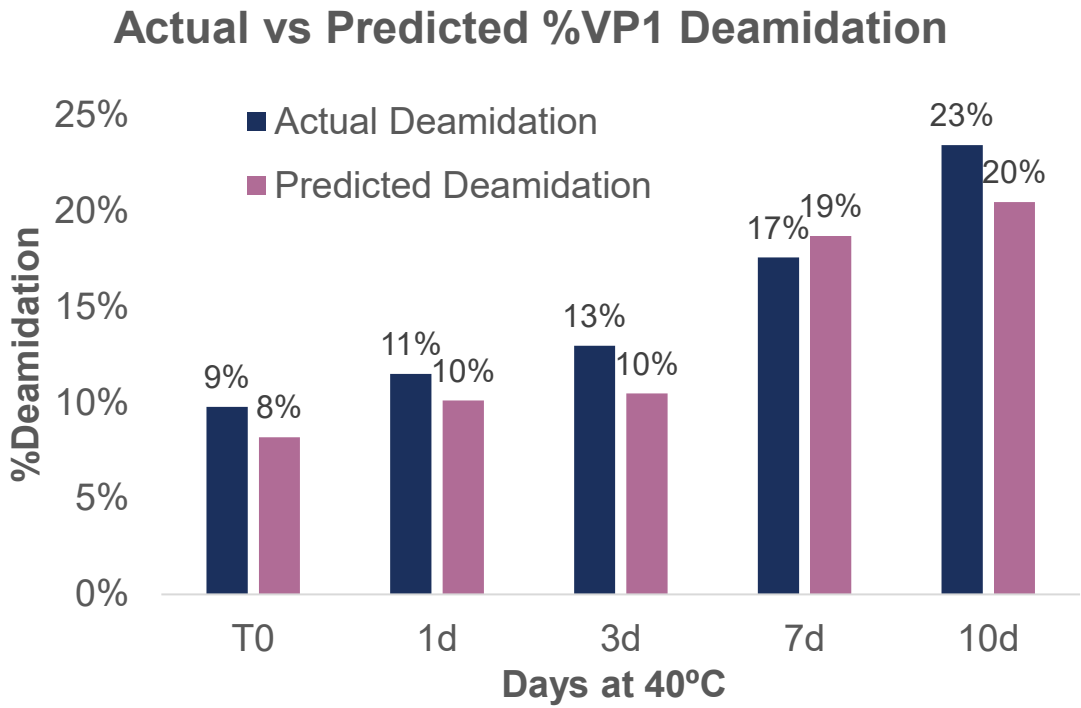
Venkatakrishnan, B. et al "Structure and Dynamics of Adeno-Associated Virus Serotype 1 VP1-Unique N-Terminal Domain and Its Role in Capsid Trafficking"

- VP1 is extruded when the capsid is exposed to higher temperature environments (37-40° C)
- The extrusion of VP1u exposes the PLA2 domain, and N57 is deamidated
- The deamidation of N57 and the hydrophobic nature of the PLA2 domain make the capsid more negatively charged and hydrophobic due to surface exposure
- N57 deamidation decreases PLA2 activity which results in diminished infectivity/gene expression

Increase in VP1 N57 Deamidation Directly Correlates with Increased Initial Fluorescence

Four independent stability studies supplied ~20 datapoints to generate an equation linking the two assays with a statistically strong fit





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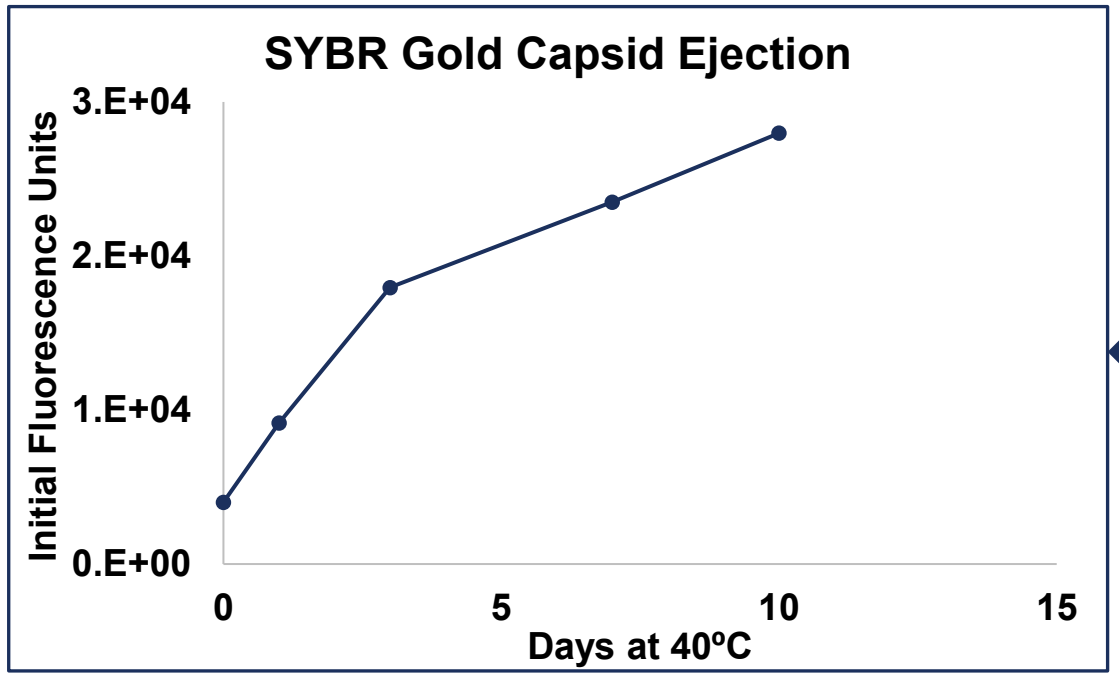
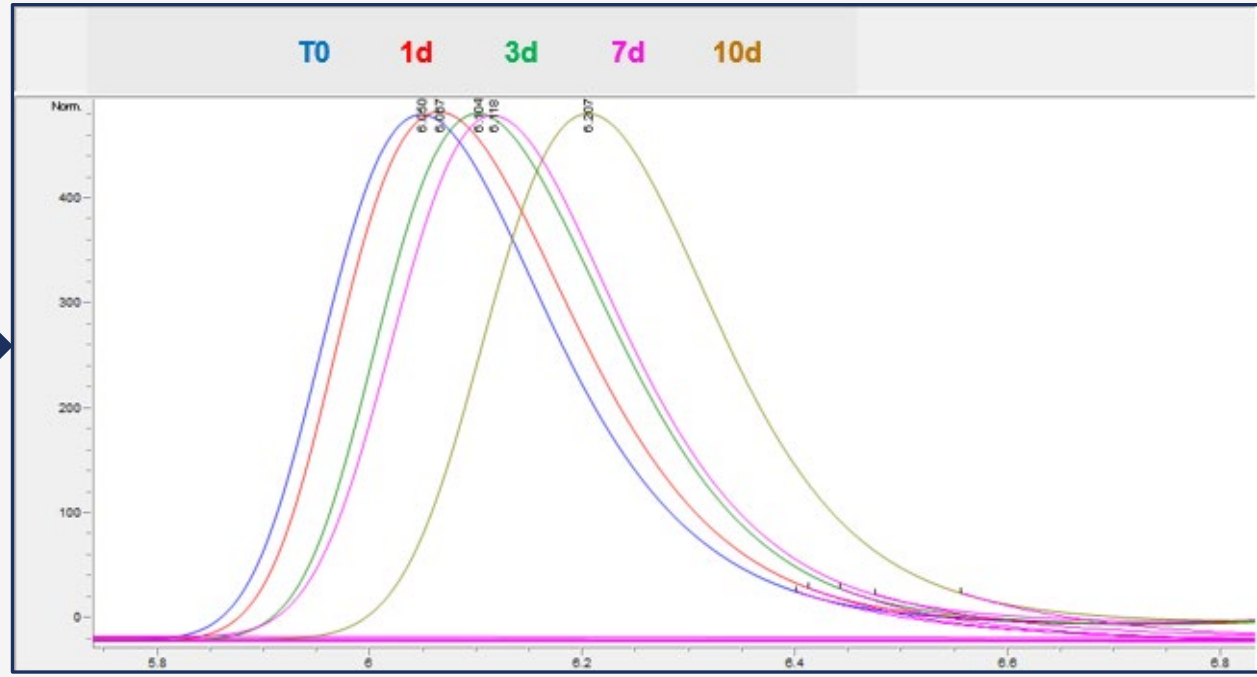
Initial Fluorescence is Correlated with Hydrophobicity

Samples exposed to 40ºC show a visible shift in retention time as the capsid undergoes conformational changes

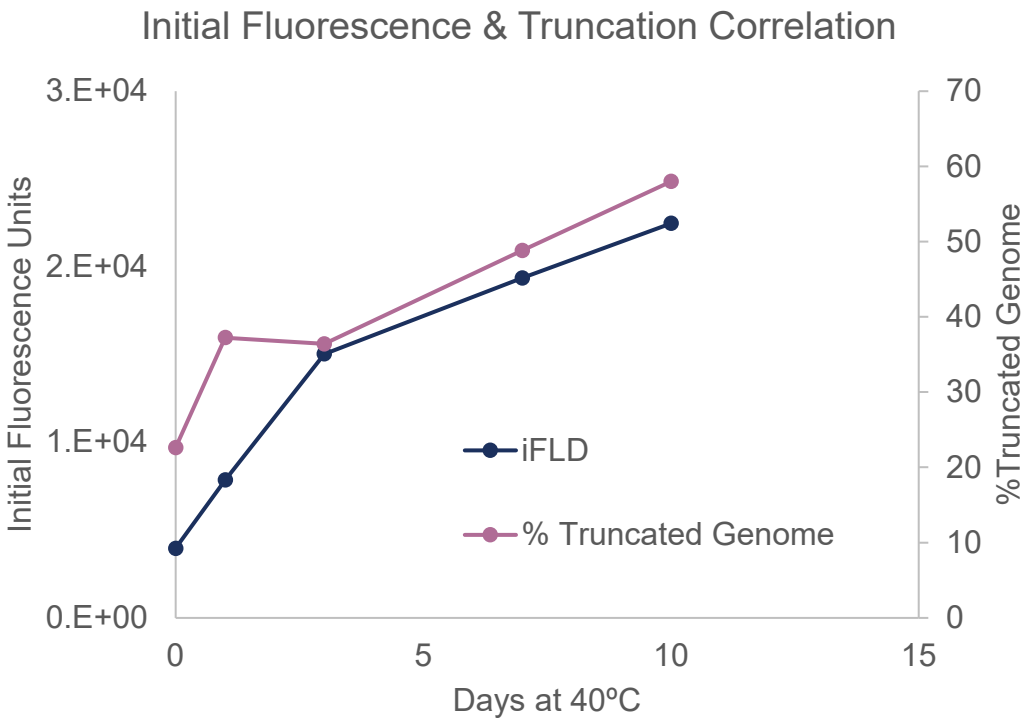




Higher initial fluorescence = VP1 deamidation = increase in RT = <u>decrease in potency</u>

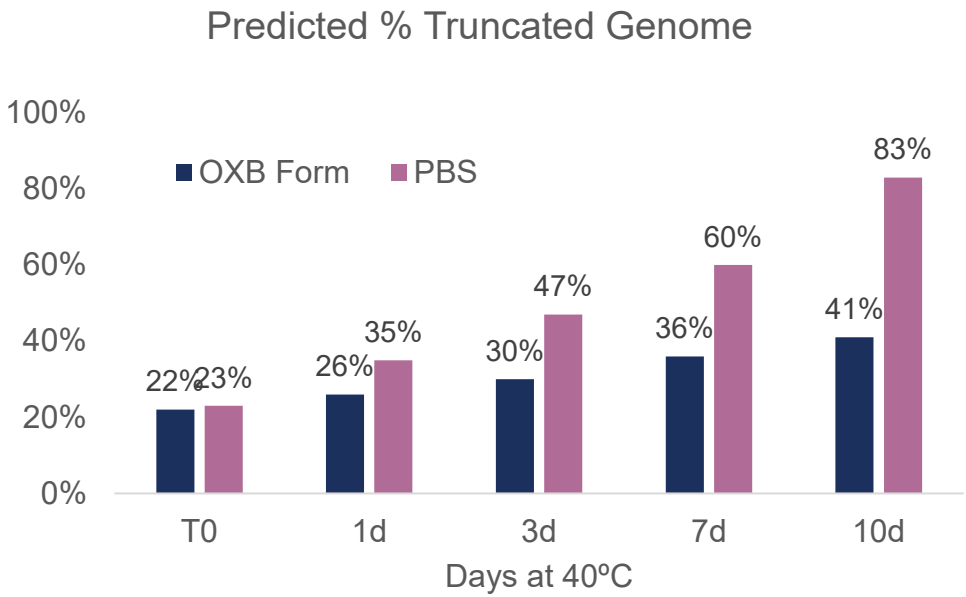
A Step Further with Next Generation Sequencing

Initial fluorescence and truncated genome increase in parallel in AAV9 drug product samples exposed to 40ºC



bivariate fit

$R^2 = 0.88$



Derived equation was applied to a formulation screen for an easy comparison between candidates

OXB's platform formulation demonstrates protective properties and product stability over a non-optimized formulation (PBS)

Summary

Using SYBR gold capsid ejection assay, we were able to derive equations linking initial fluorescence to relative potency, N57 deamidation, and genome truncation

- The establishment of these equations allows us to make faster, cheaper go/no-go decisions on process parameters and formulation candidates

Overall, we believe there is a strong correlation between initial fluorescence, N57 deamidation, hydrophobicity, relative potency, and genome truncation

- This observation allows us to better understand what is going on at the vector level as it undergoes thermal stress

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