BIOMARIN

COMPARABILITY CONSIDERATIONS IN THE DEVELOPMENT OF GENE THERAPY PRODUCTS

CASSS Cell & Gene Therapy Products (CGTP):

Manufacturing, Quality and Regulatory Considerations

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VALOCTOCOGENE ROXAPARVOVEC (BMN 270) GENE THERAPY TREATMENT FOR HEMOPHILIA A

Clinical Results

- Safety
- Reduction in Annualized Bleeding Rate and Use of Recombinant Factors
- Increase in Quality of Life (QOL) Measures
- Sustained Factor VIII Levels 2 Years After a Single Infusion of BMN 270
- Summary and Next Steps

Process Comparability: Factors That Impact Potency

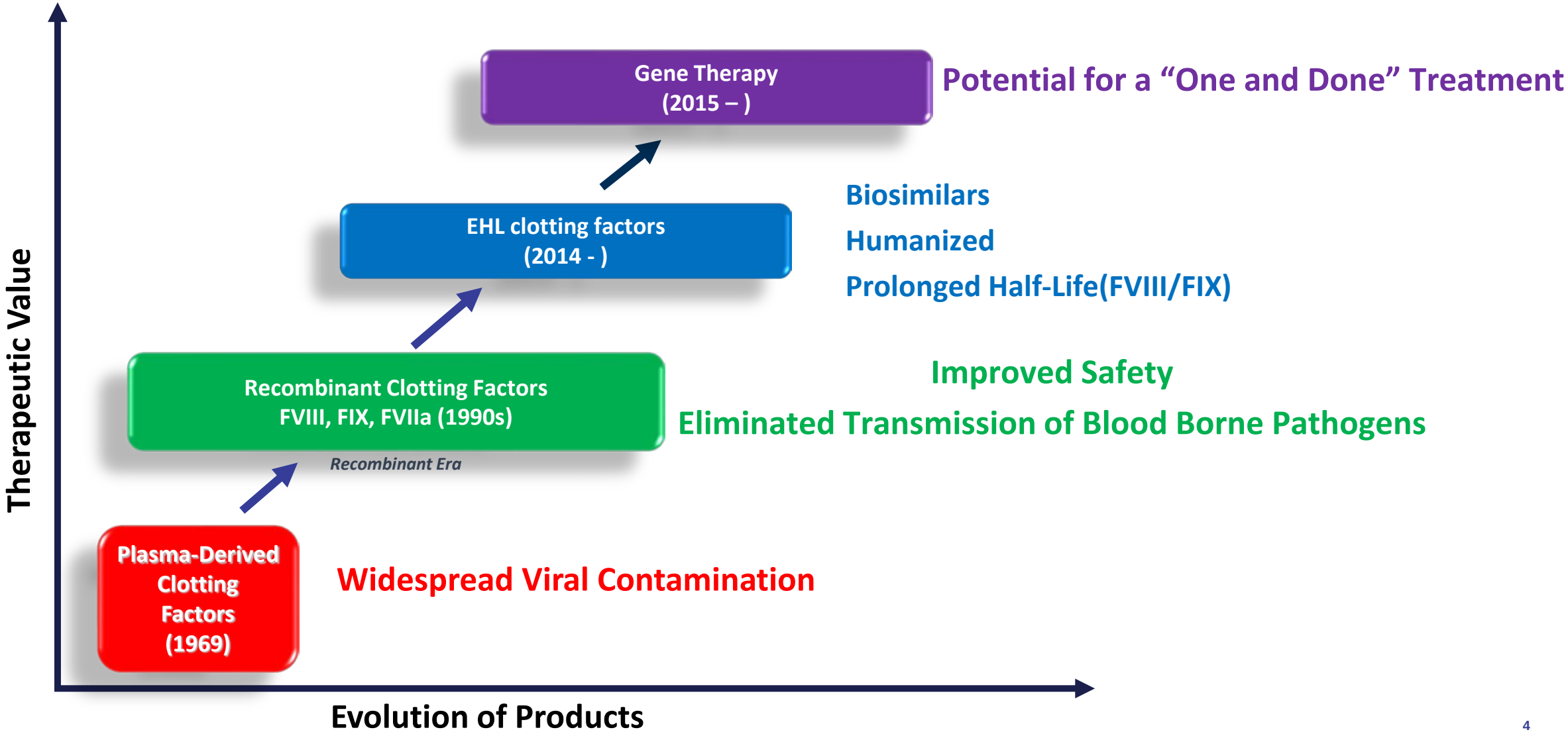
- Capsid Protein Content
- Deamidation of Capsid Proteins
- Presence of Empty Capsids

HEMOPHILIA IS THE OLDEST DESCRIBED GENETIC DISORDER

“. .if the first son of a woman is circumcised and he dies and the second son is circumcised and he dies, you must not circumcise the third son. .

additionally, the sons of the woman’s sister should not be circumcised but the sons of her brother can be circumcised. “ Talmud (Yebamot 64b)

EVOLUTION OF HEMOPHILIA TREATMENT



FAVORABLE SAFETY PROFILE FOR BMN 270

- Transient transaminitis
  - 73% of subjects; now all resolved
    - 93% Grade 1, 7 % Grade 2; Alanine Aminotransferase (ALT) elevation
  - Median onset at 7.6 weeks; duration <25.3 weeks post onset

- All patients are off corticosteroids

- No patients developed inhibitors to FVIII; no subject withdrew

- 2 SAEs, both self-limited
  - Pyrexia, resolved overnight
  - Total knee replacement for pre-existing arthropathy

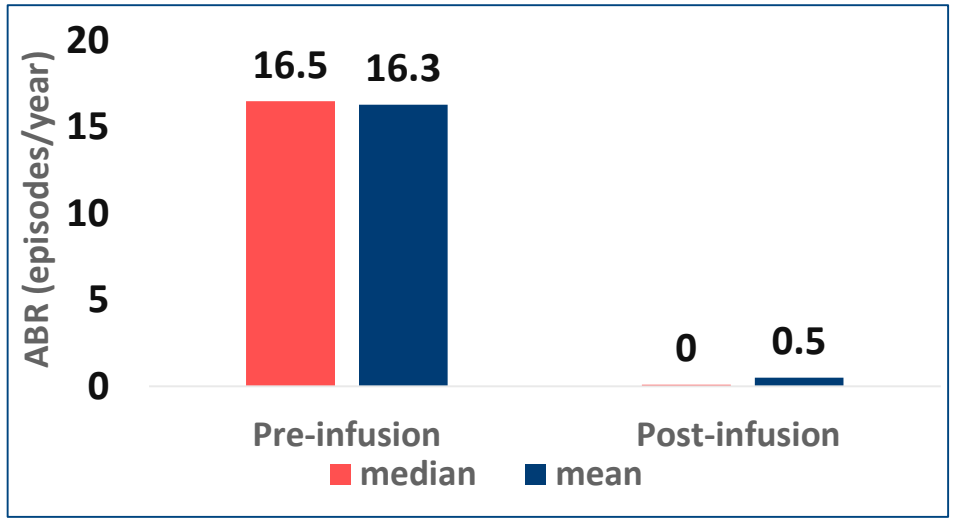
- Most common other AEs across all dose cohorts:
  - Arthralgia (60%); headache (47%), back pain (40%)
  - Viral upper respiratory tract infection (40%), fatigue, insomnia, pain in extremity (33%)

SUBSTANTIAL REDUCTION IN TREATED ANNUALIZED BLEED RATE (ABR)

STARTING FROM 4 WEEKS POST-INFUSION

6e13 Dose Through 104 Weeks; N=7

97% REDUCTION in MEAN ABR



% Patients Bleed Free

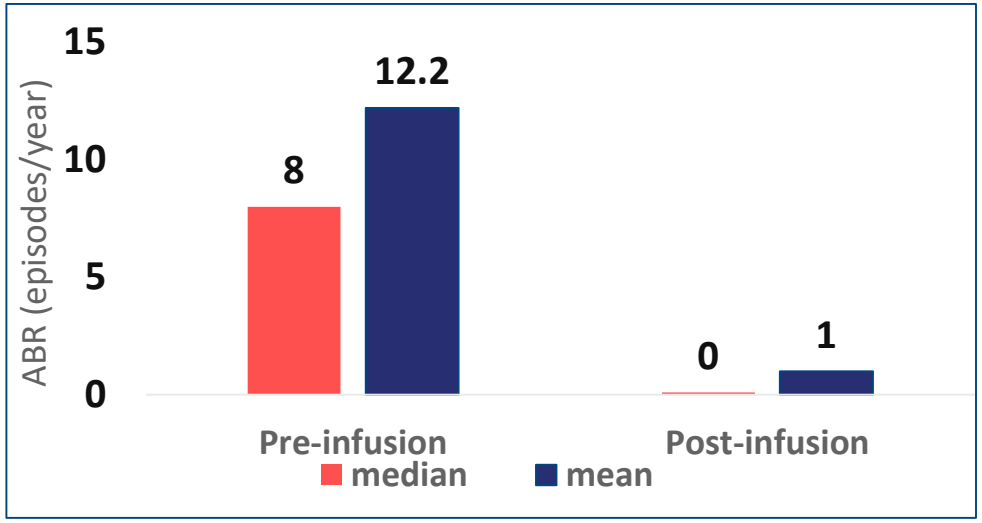
Baseline	Year 1	Year 2
15%	71%	86%

All patients off prophylaxis

100% resolution in target joints

4e13 Dose Through 52 Weeks; N=6

92% REDUCTION in MEAN ABR



% Patients Bleed Free

Baseline	Year 1
17%	83%

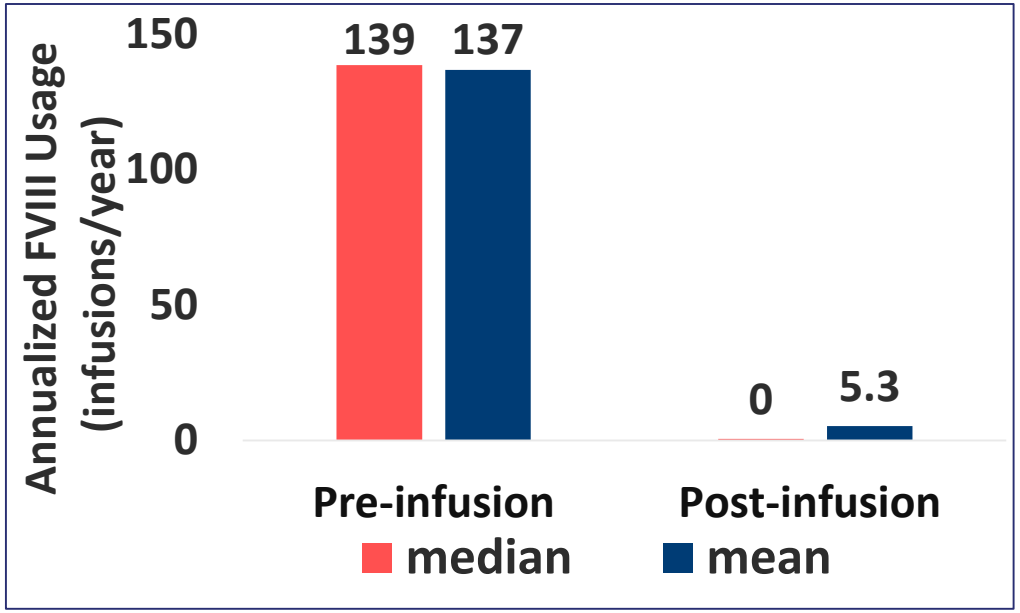
All patients off prophylaxis

SUBSTANTIAL REDUCTION IN MEAN ANNUALIZED FVIII USAGE

STARTING FROM 4 WEEKS POST- INFUSION

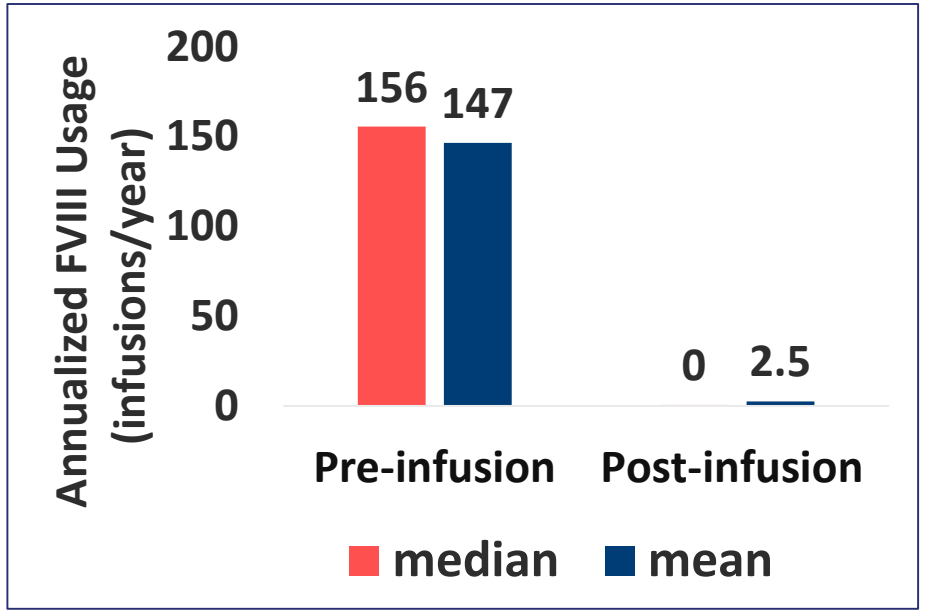
6e13 vg/kg Dose Through Week 104; N=7

96% REDUCTION in MEAN FVIII USAGE

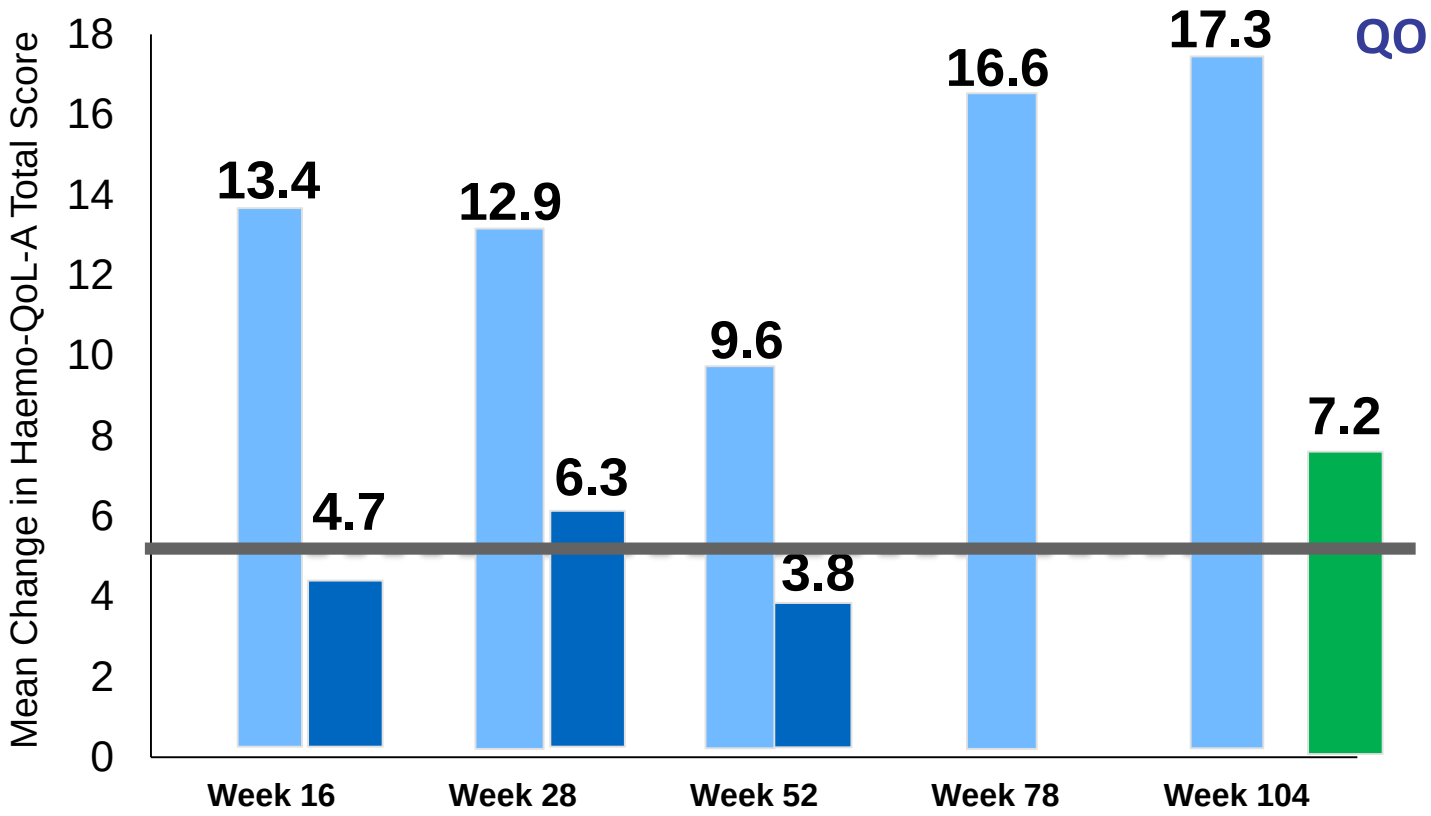


4e13 vg/kg Dose Through Week 52; N=6

98% REDUCTION in MEAN FVIII USAGE



BMN 270 SUBSTANTIALLY IMPROVED QUALITY OF LIFE



QOL improvement observed in all 6 domains;

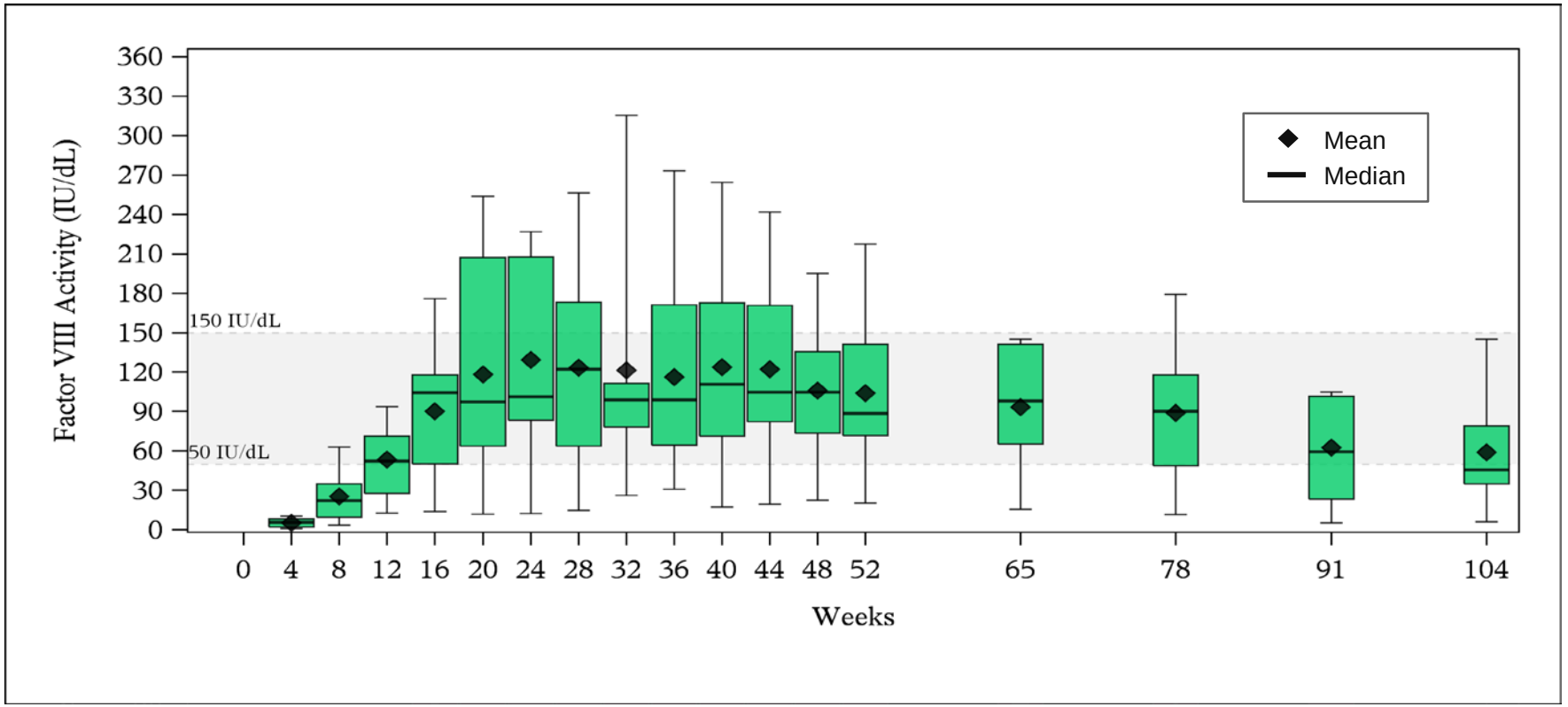
- Consequences of Bleeding
- Emotional Impact
- Physical Functioning
- Role Functioning
- Treatment Concern
- Worry

6e13 vg/kg cohort
4e13 vg/kg cohort
Spinart result at 3 years*

Grey line indicate distribution based minimally clinical important difference (MCID) at lower threshold* Pocoski J et al., 2014

Randomized, controlled, parallel-group trial of routine prophylaxis vs. on-demand treatment with sucrose-formulated recombinant factor VIII in adults with severe hemophilia A (SPINART). J Thromb Haemost. 2014 Jan;12(1):119-22.

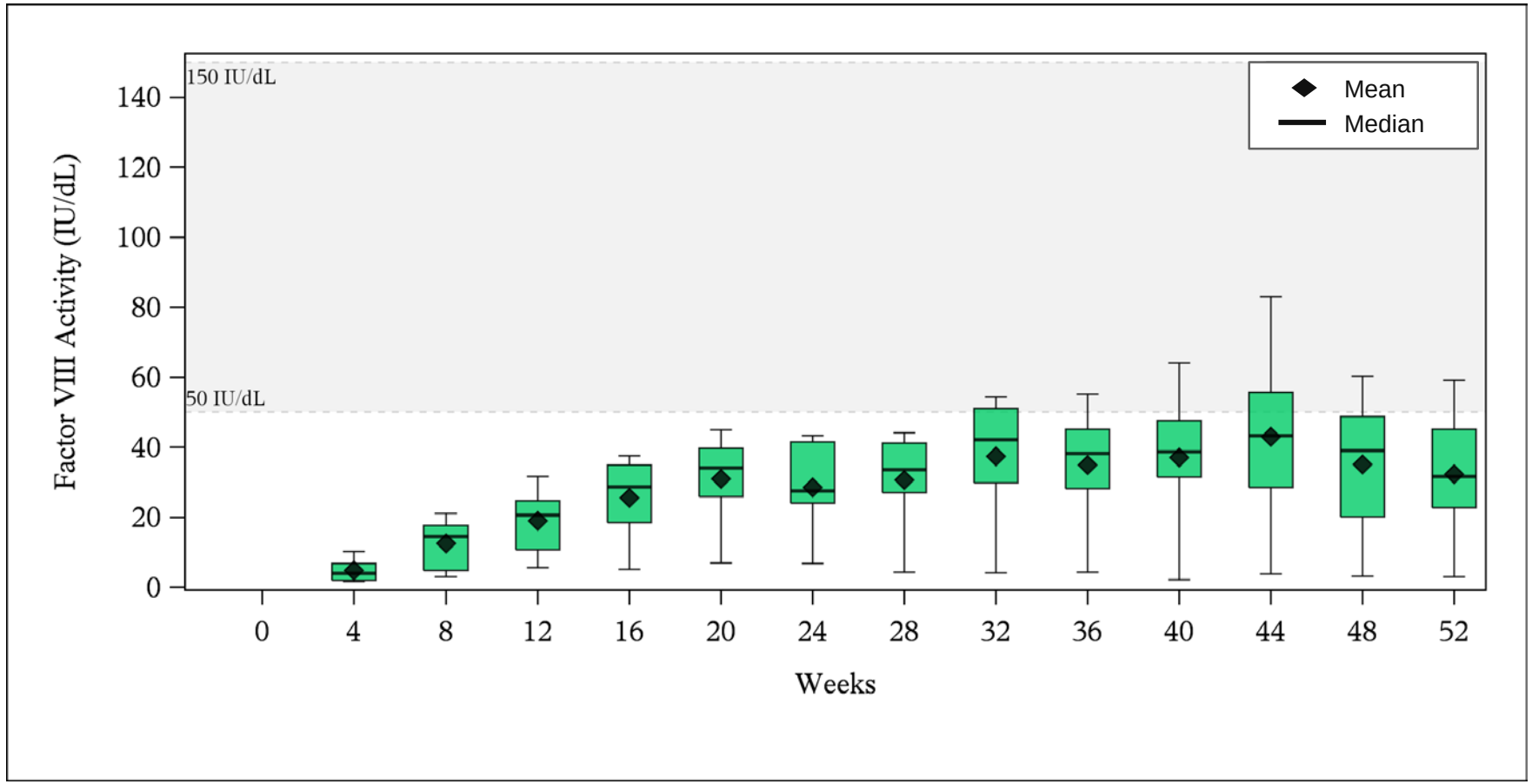
MEAN FVIII ACTIVITY LEVELS SETTLING IN NORMAL RANGE (6e13 VG/KG)



No FVIII Activity Above Upper Limit of Normal at 2 Years

The upper and lower box bounds represent 25th and 75th percentiles.
The whisker lines represent the minimum and maximum values.

MEAN FVIII ACTIVITY LEVELS AT HIGH END OF MILD RANGE (4e13 VG/KG)



No FVIII Activity Above Normal at 1 Year

The upper and lower box bounds represent 25th and 75th percentiles.
The whisker lines represent the minimum and maximum values.

SUMMARY OF CLINICAL RESULTS

- **BMN 270 was Well-Tolerated with Favorable Safety Profile; Currently:**
  - No subjects in either cohort have FVIII activity levels above the upper limit of normal
  - ALT levels within normal limits in all subjects
  - All subjects remain off corticosteroids
  - No inhibitors to FVIII
  - Only 2 serious adverse events as previously reported
- **Annualize Bleeding Rate: Profound Reduction with Both Cohorts**
  - 6e13 vg/kg sustained 2 years, 4e13 vg/kg sustained 1 year and counting
- **FVIII Usage: Profound Reduction with Both 6e13 and 4e13 vg/kg Cohorts**
- **Quality of Life Improvement in Six Domains Reflects:**
  - Cessation of Bleeding, Freedom From Worry, Independence From Treatment and Improved Functioning
- **FVIII Activity Levels**
  - 6e13 vg/kg cohort, settling within normal range
  - 4e13 vg/kg cohort, at upper range of mild hemophilia
- **Patterns of FVIII Activity Levels Consistent with Other Clinical and Pre-Clinical Reports**
- **Gene Therapy Has the Potential to Transform the Standard of Care in Hemophilia A**

NEXT STEPS

- New goal is to prove superiority of BMN 270 to prophylactic therapy
- GENEr8-1 (6e13 vg/kg) sample size now powered for superiority
  - 90% power to demonstrate reduction in bleeding events from ABR of 3.5 to 1.0
  - N = 130 (90 additional patients)
  - Expect to complete enrollment in Q1 2019
- GENEr8-2 (4e13 vg/kg) study design unchanged
  - N = 40
  - FVIII primary endpoint
  - Targeted to finish enrollment 1-2 quarters after GENEr8-1
- Comprehensive program underway
  - AAV5+ study initiated
  - Global seroprevalance study
  - Ongoing follow-up of 201 patients
  - Initiating use of full commercial scale material from BioMarin manufacturing facility

Process Comparability: Factors That Can Impact Potency

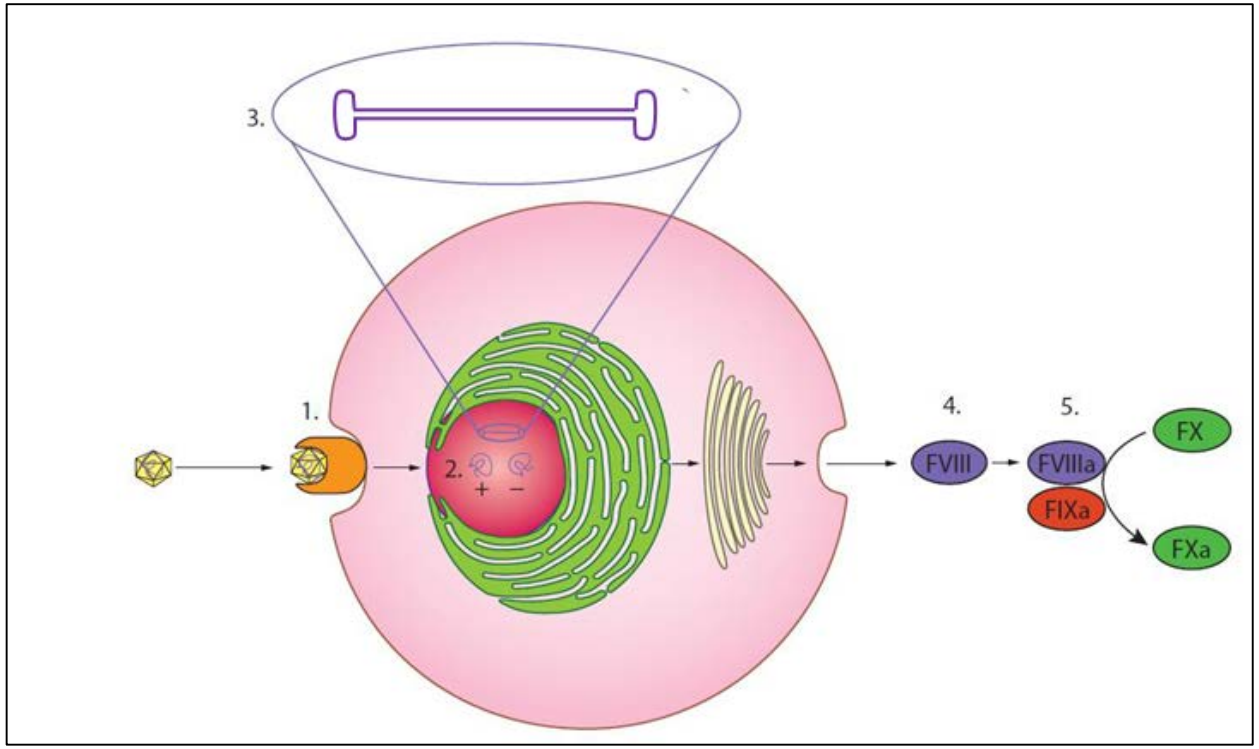
- Capsid Protein Content
- Deamidation of Capsid Proteins
- Presence of Empty Capsids

Potency Assay Overview

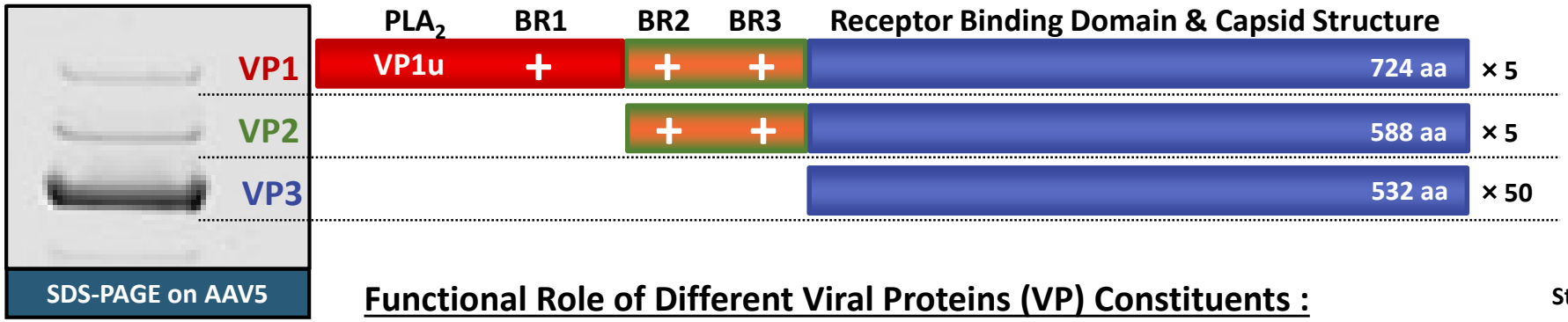
- **Method Details**
  - Cell-based assay confirms mechanism of action and enables monitoring of consistency and comparability between processes
  - Relies on vg titer to determine assay loading
  - Readout is a Potency Ratio (test sample read-out normalized to reference standard read-out).
  - Potency is assessed via measurement of FVIII activity and protein levels (separate potency ratios for both)

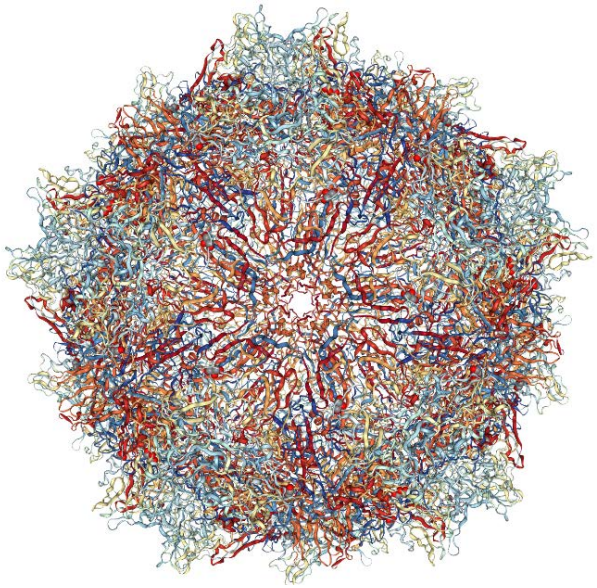
What is Biologically Required to Demonstrate Potency?

1. Effectively enter the cell
   a) Available Receptors
   b) Functional Capsid
2. Traffic DNA to the Nucleus
3. Forms complete Double Stranded DNA
4. FVIII Efficiently Expressed and Secreted (ELISA)
5. FVIII Activity (Chromogenic Assay)
   a) Requires effective expression, correct folding and postranslational modifications



STRUCTURAL ASSEMBLY OF AAV5 CAPSID





AAV5 Crystal Structure

Structural Insights into Adeno-Associated Virus Serotype 5
Govindaswamy et.al (2013) J.Virol. 87: 11187-11199

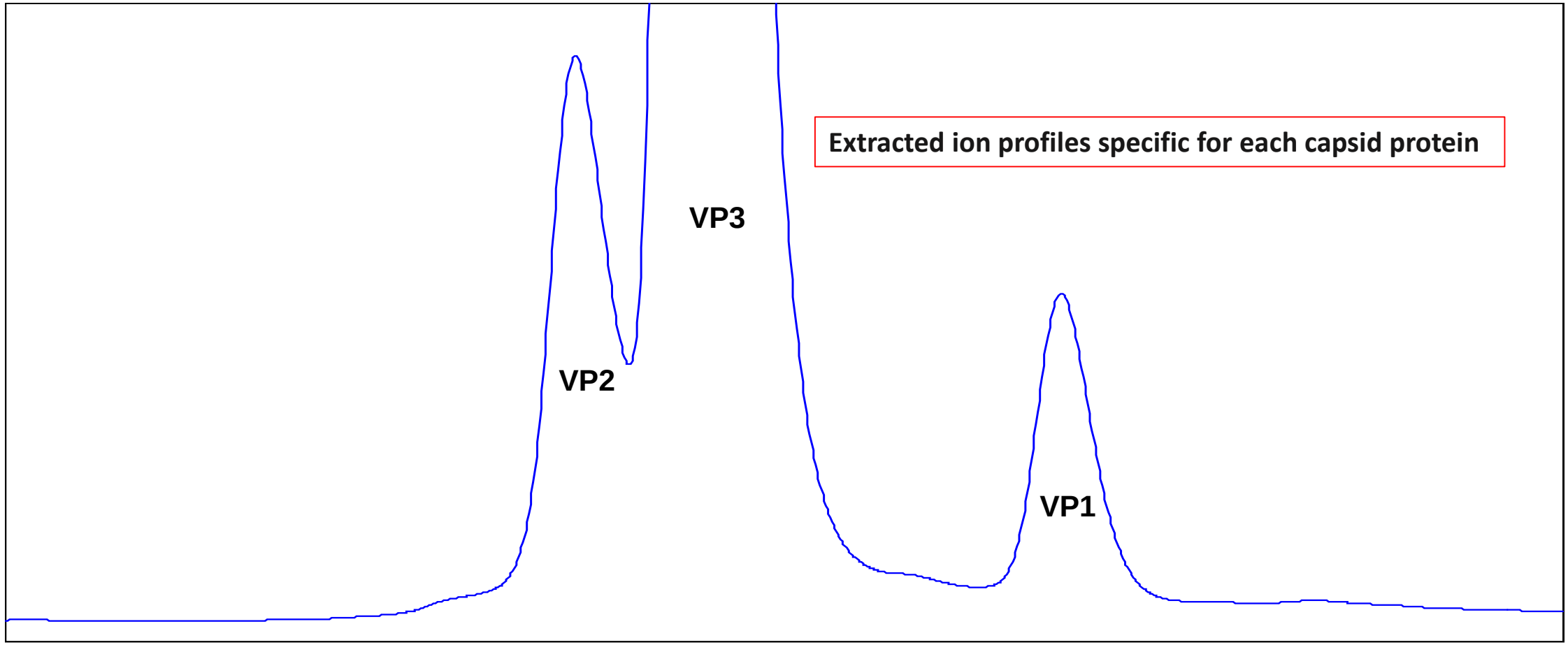
Functional Role of Different Viral Proteins (VP) Constituents :

- Phospholipase A_2 (PLA_2): Membrane Disruption of Endosome (VP1)
- Basic Region (BR): Putative Nuclear Localization Signals (VP1, VP2)
- Receptor Binding Domain: Capsid Interaction with Cell Surface Receptors (VP1, VP2 & VP3)
- Capsid Assembly: Icosahedral Structure (VP1, VP2 & VP3)

Mutagenesis of Adeno-Associated Virus Type 2 Capsid Protein VP1 Uncovers New Roles for Basic Amino Acids in Trafficking and Cell-Specific Transduction
Johnson et.al (2010) J.Virol. 84: 8888-8902

Impact of VP1-Specific Protein Sequence Motifs on Adeno-Associated Virus Type 2 Intracellular Trafficking and Nuclear Entry
Popa-Wagner (2012) J.Virol. 86: 9163-9174

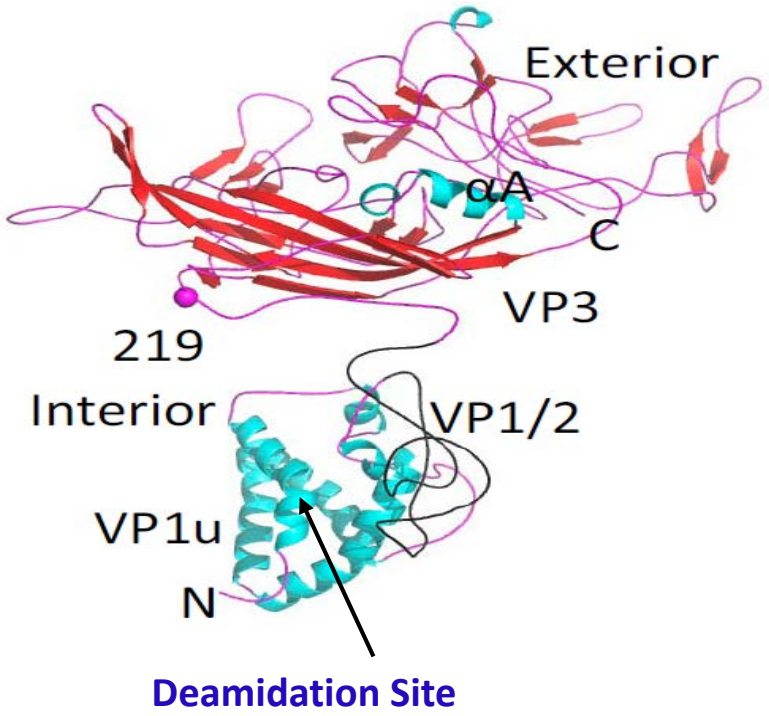
SEPARATING CAPSID PROTEINS BY REVERSED PHASE HPLC & MONITORING BY MASS SPECTROMETRY



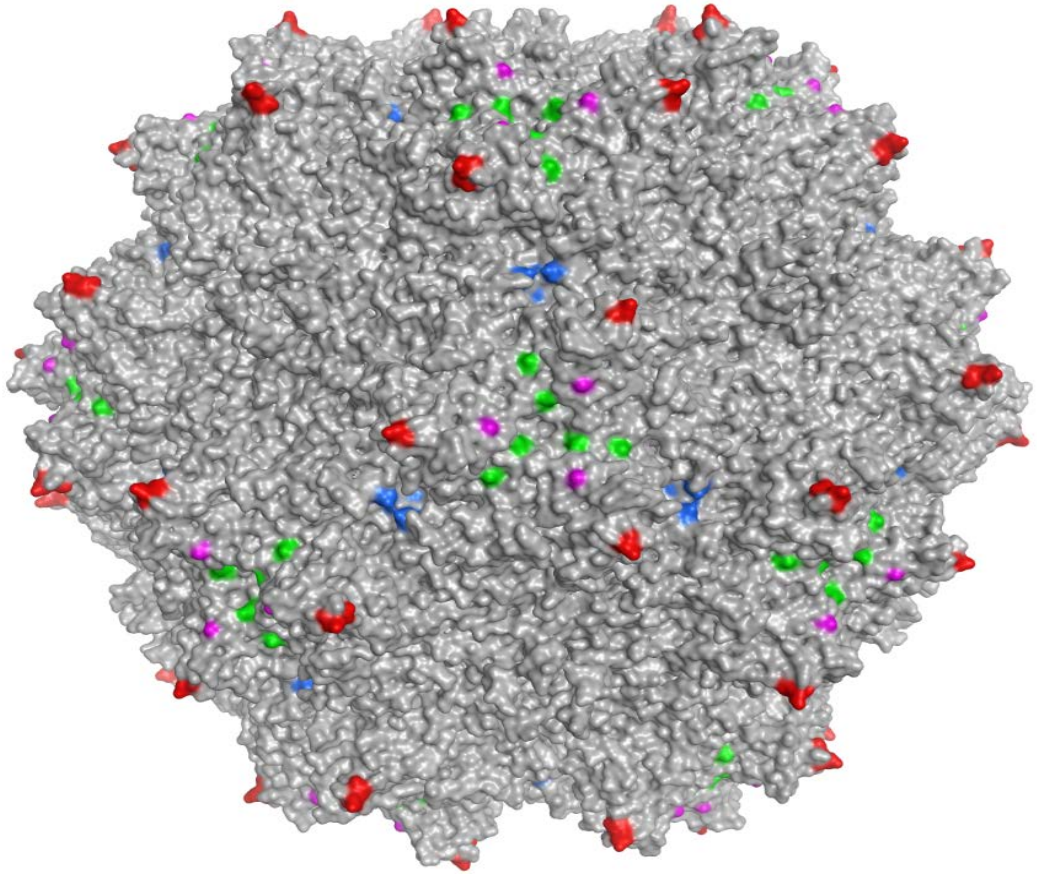
Viral capsid proteins are detected using UV wavelength 214 and identified using mass spec analysis.

CAPSID DEAMIDATION SITES

VP1 N-Terminal Site



Surface Domain



Deamidation Site A

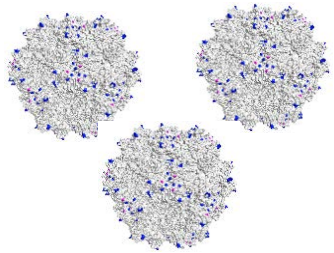
Deamidation Site B

Deamidation Site C

Sialic Acid Binding Site

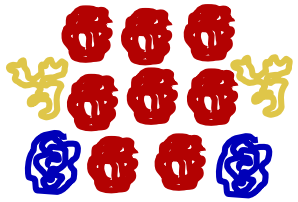
Structure and Dynamics of Adeno-Associated Virus Serotype 1 VP1-Unique N-Terminal Domain and Its Role in Capsid Trafficking
Venkatakrishnan et al J. Virology (2013) 87(24):13150-60.

DEAMIDATION ANALYSIS BY LC/MS



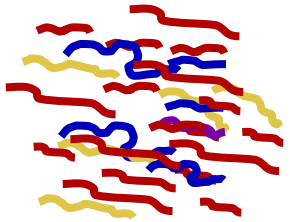
Capsid particles

Denaturation



Viral protein mixture

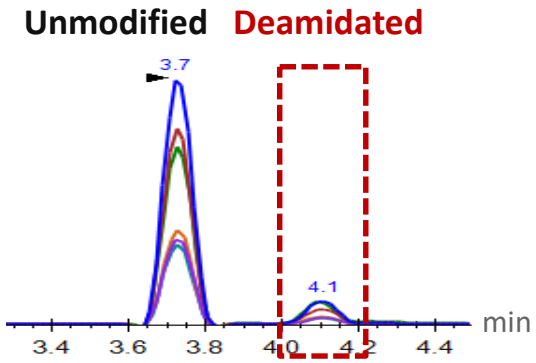
Digestion



Peptides

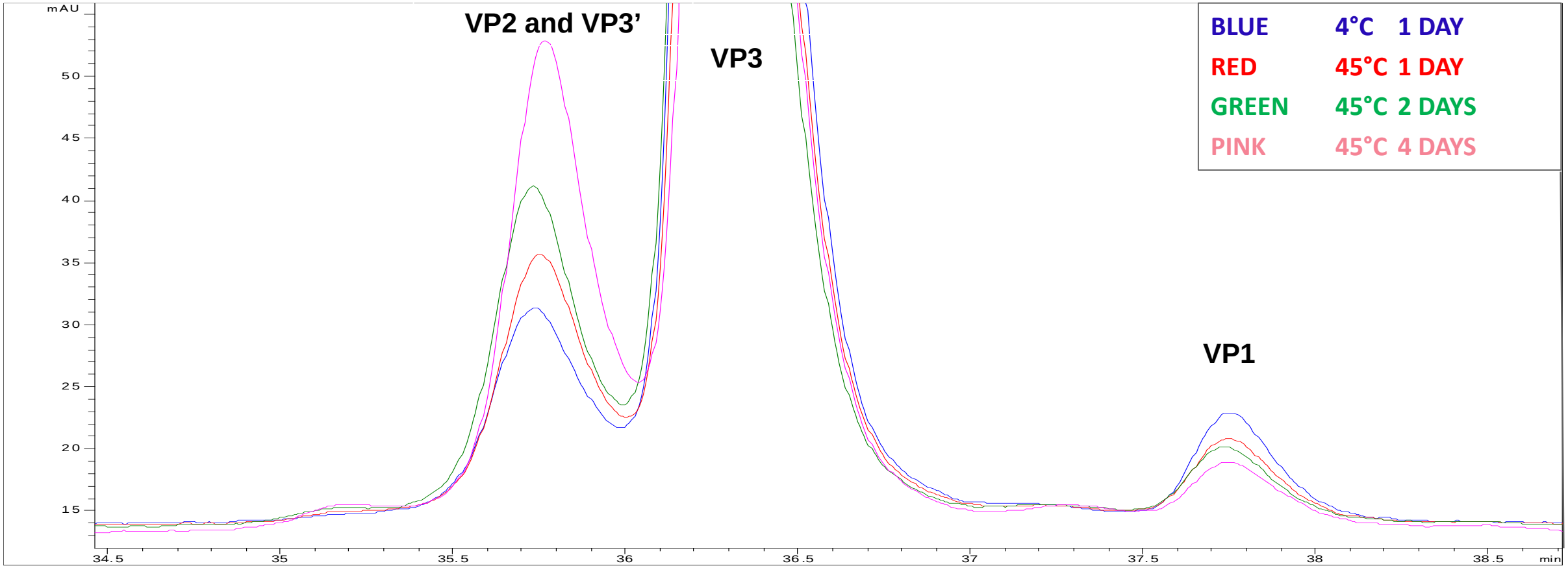
LC separation

Mass Spectrometry



Analysis

DETECTION OF DEAMIDATION REVERSED PHASE HPLC & MONITORING BY MASS SPECTROMETRY



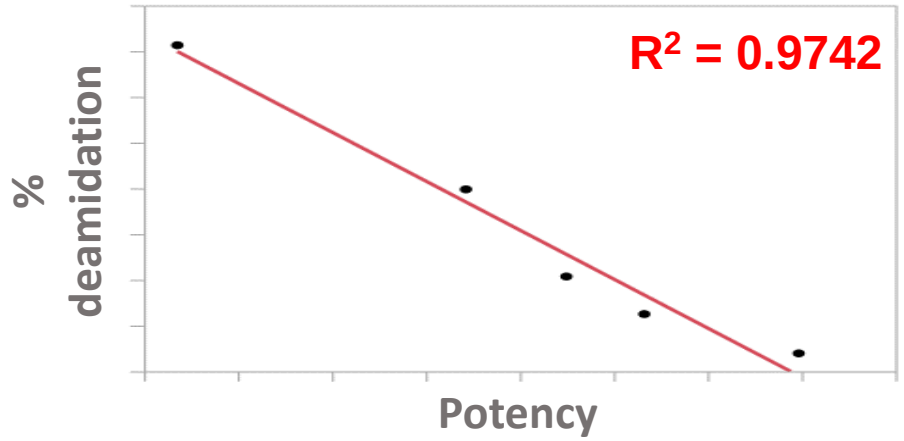
Thermal degradation results in deamidation of the capsid virus proteins

In the RP-HPLC, deamidation results in the decrease of VP1 and VP3 peak areas and an increase of VP2 apparent peak area

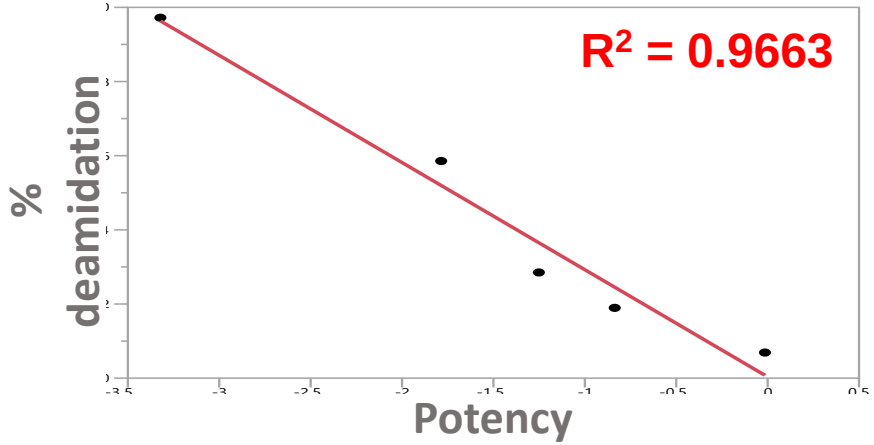
It has been determined by various MS methods that deamidated VP3 species (shown here as VP3') co-eludes with VP2

CORRELATION BETWEEN SITE SPECIFIC DEAMIDATION AND IN VITRO POTENCY

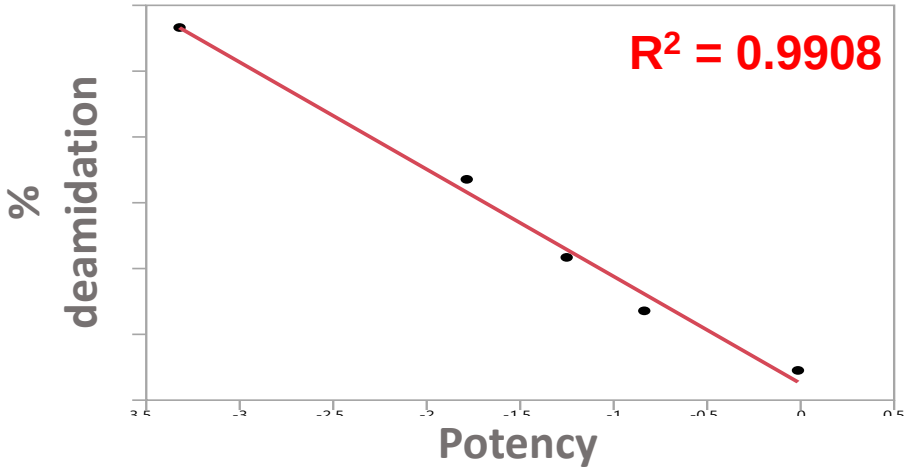
VP1 N-Terminal Site



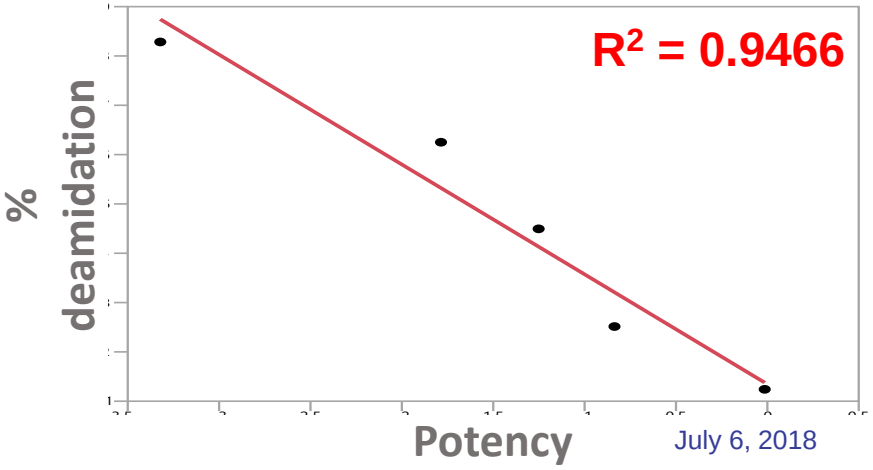
Surface Site A



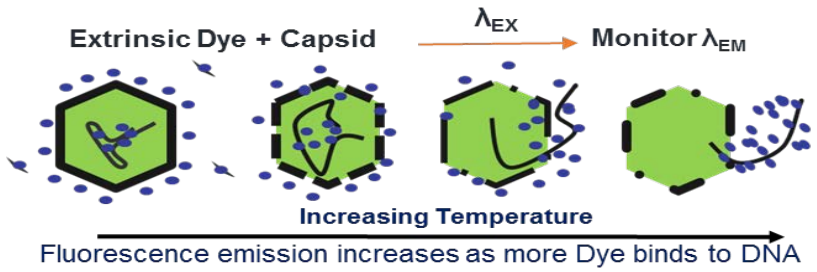
Surface Site B

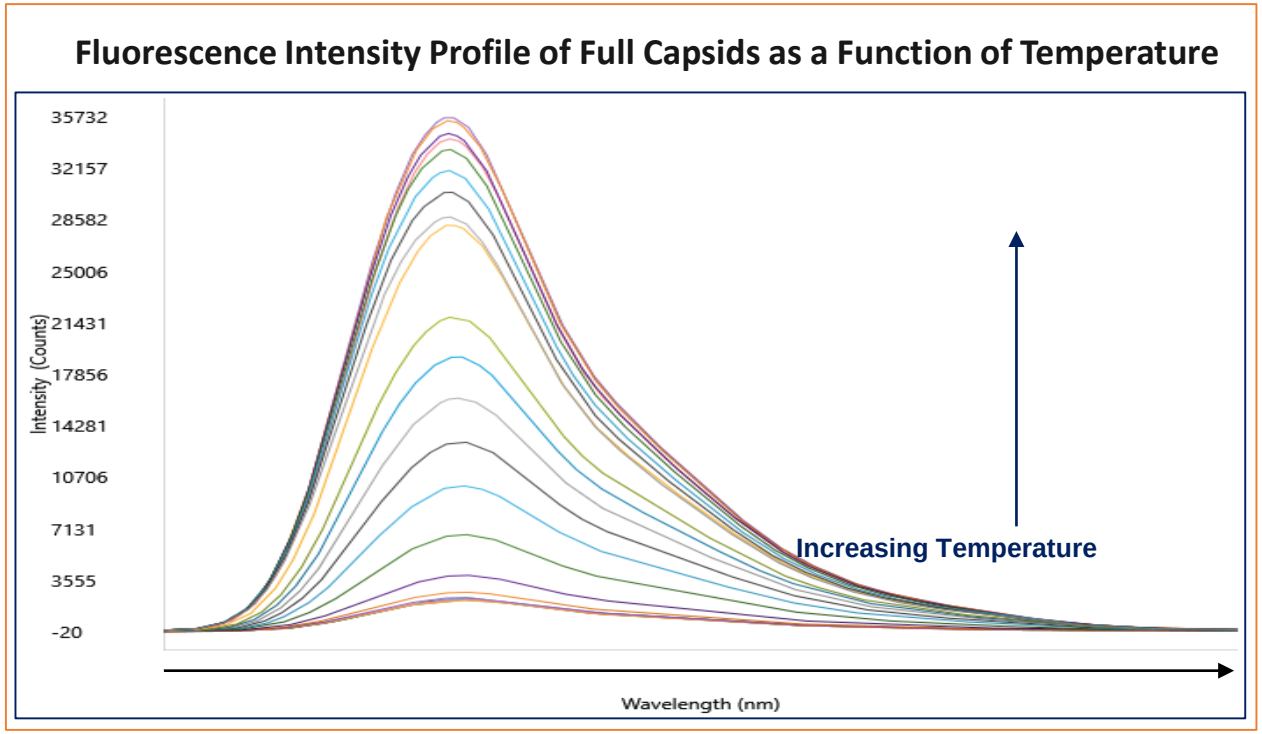


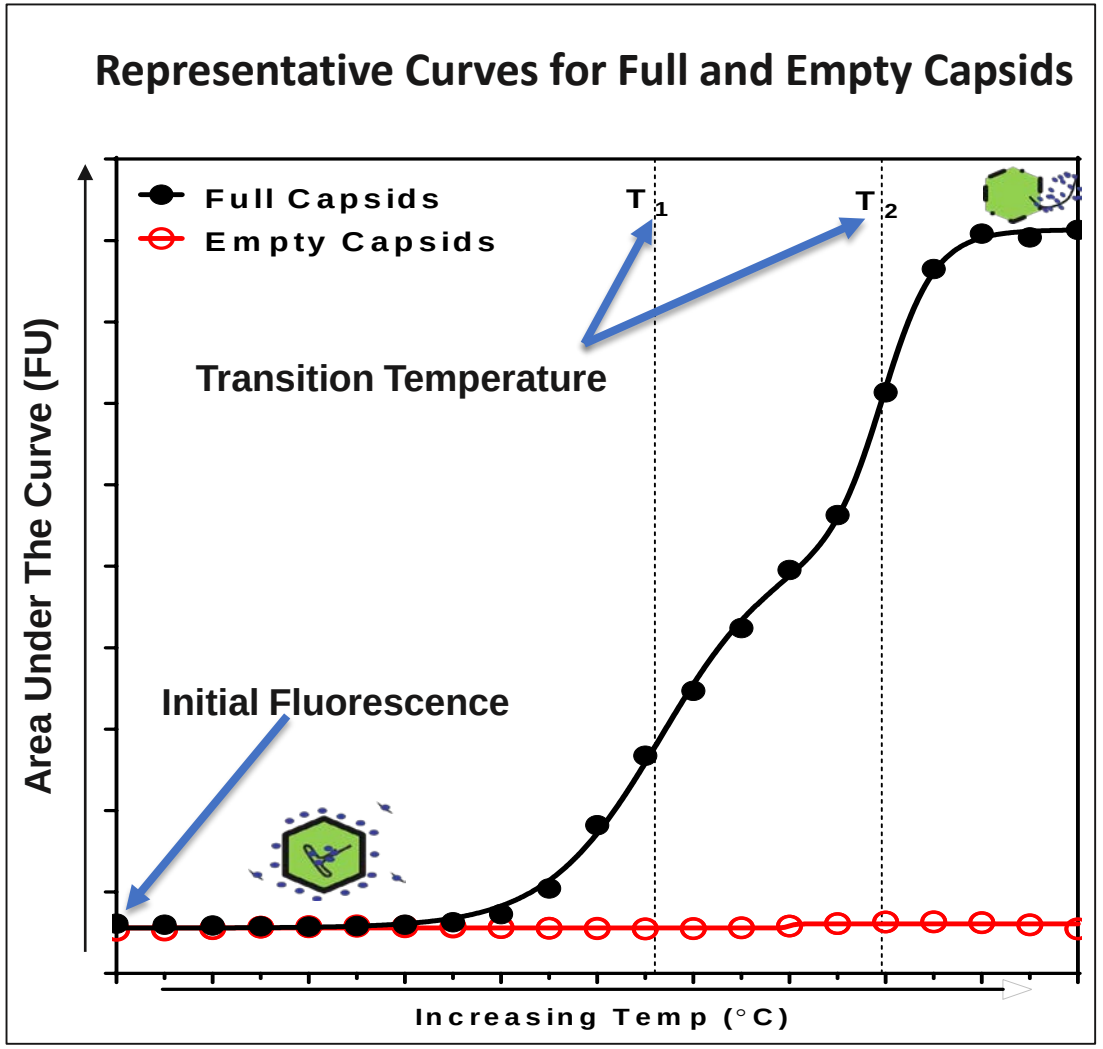
Surface Site C



CAPSID INTEGRITY ANALYSIS USING FLUORESCENCE SPECTROSCOPY

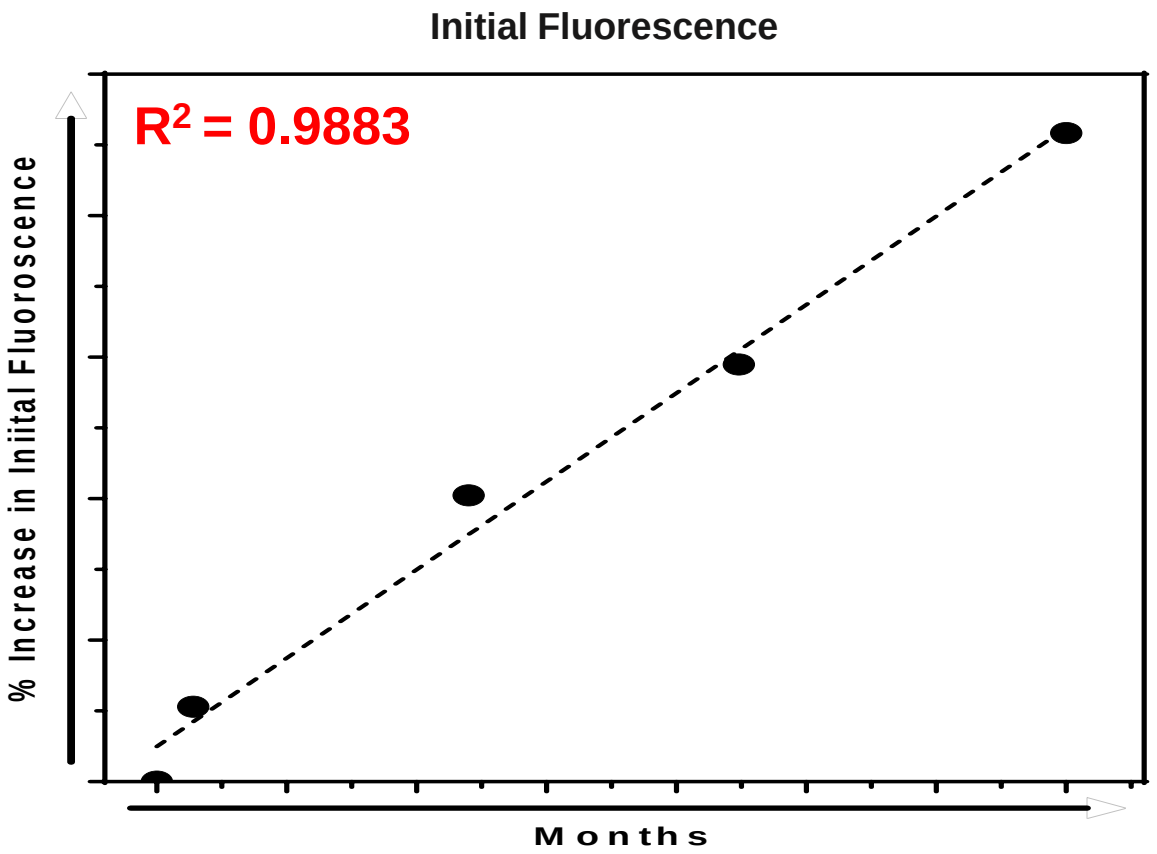


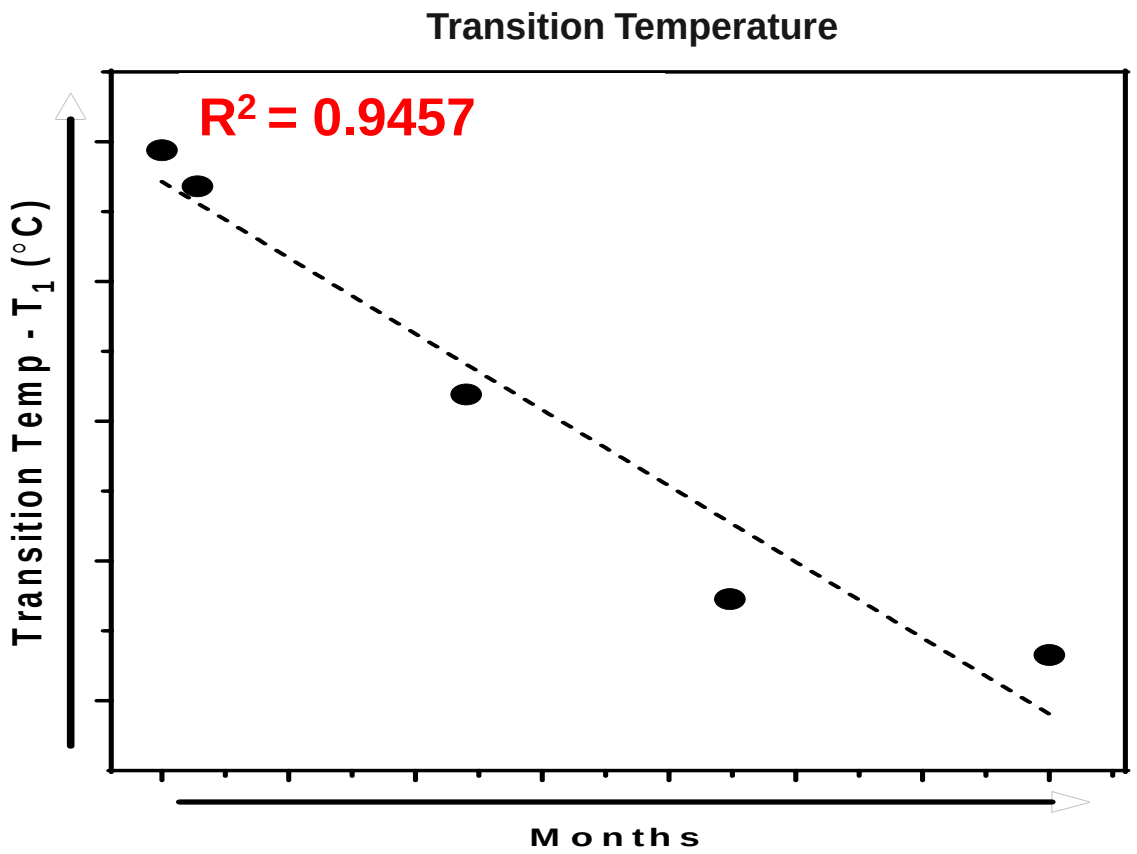




Transition Temperature Inflections Detected By Fluorescence Emission Increases Are Indicative of Capsid Instability

CAPSID STABILITY ANALYSIS AND CORRELATION WITH DEAMIDATION AND POTENCY



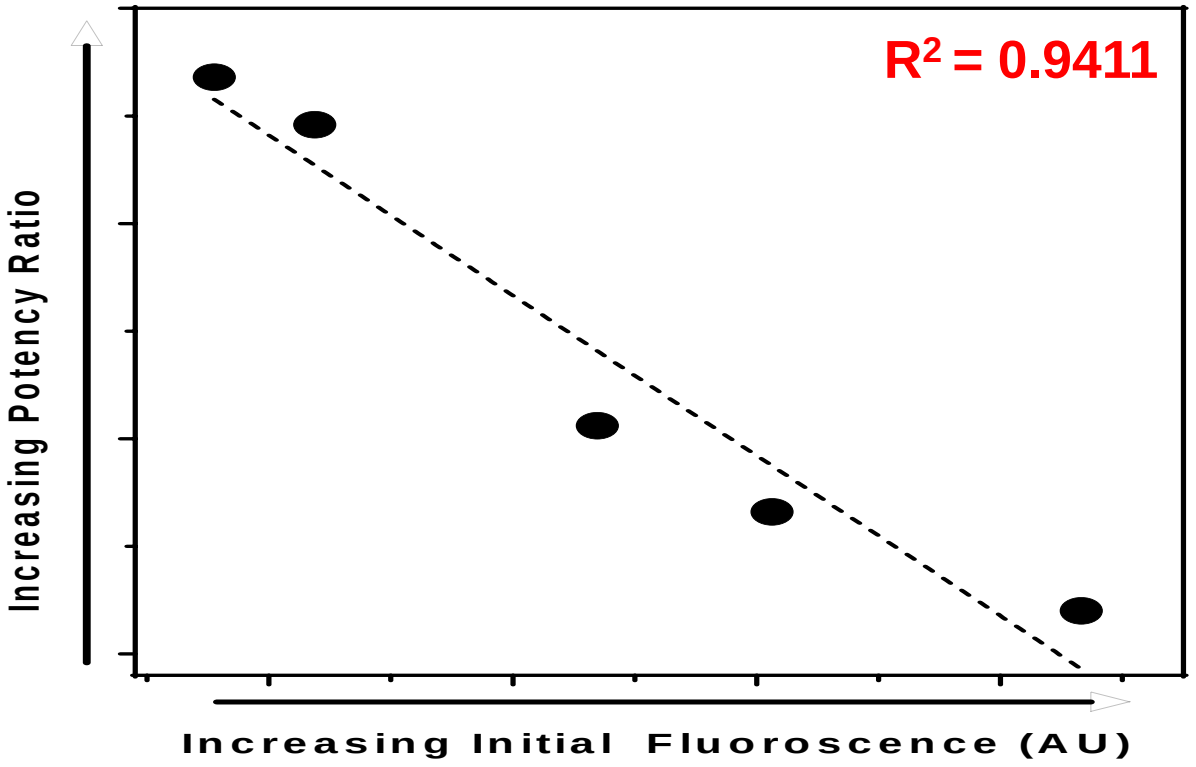


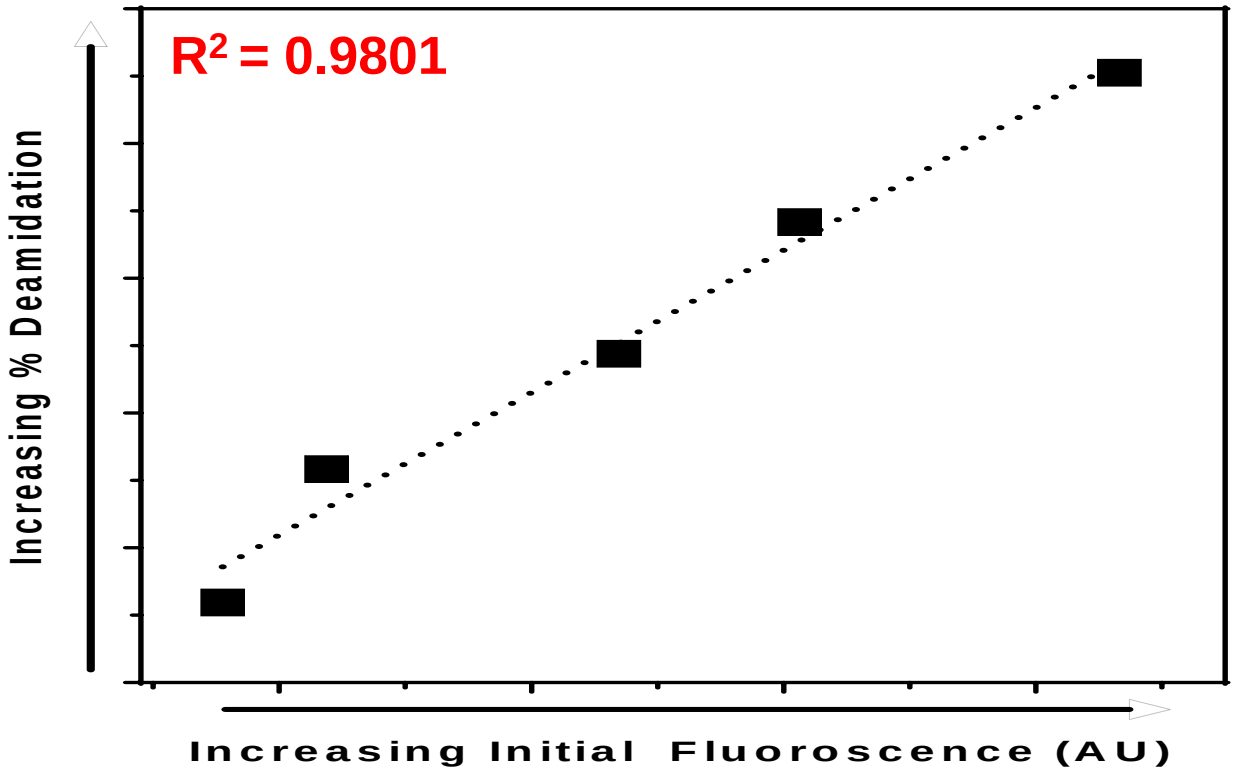
Initial Fluorescence and Transition Temperature

Change Over Time

Consistent With Capsid Instability

CAPSID STABILITY ANALYSIS AND CORRELATION WITH DEAMIDATION AND POTENCY

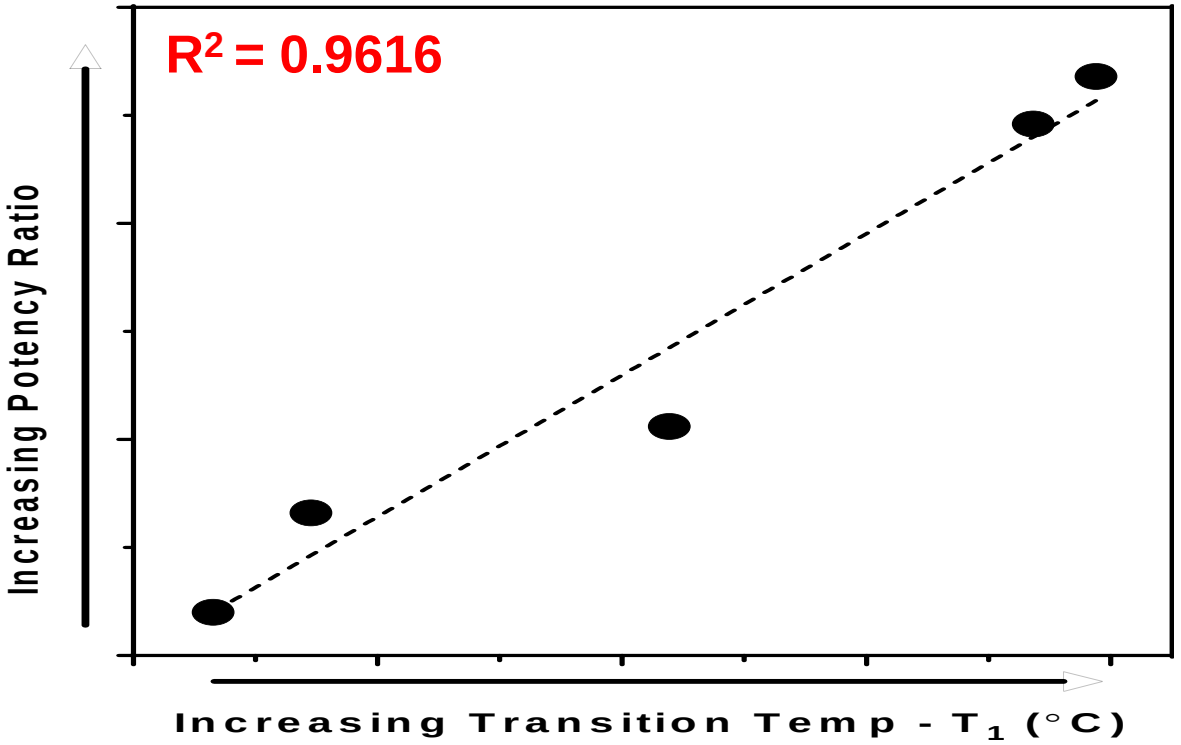


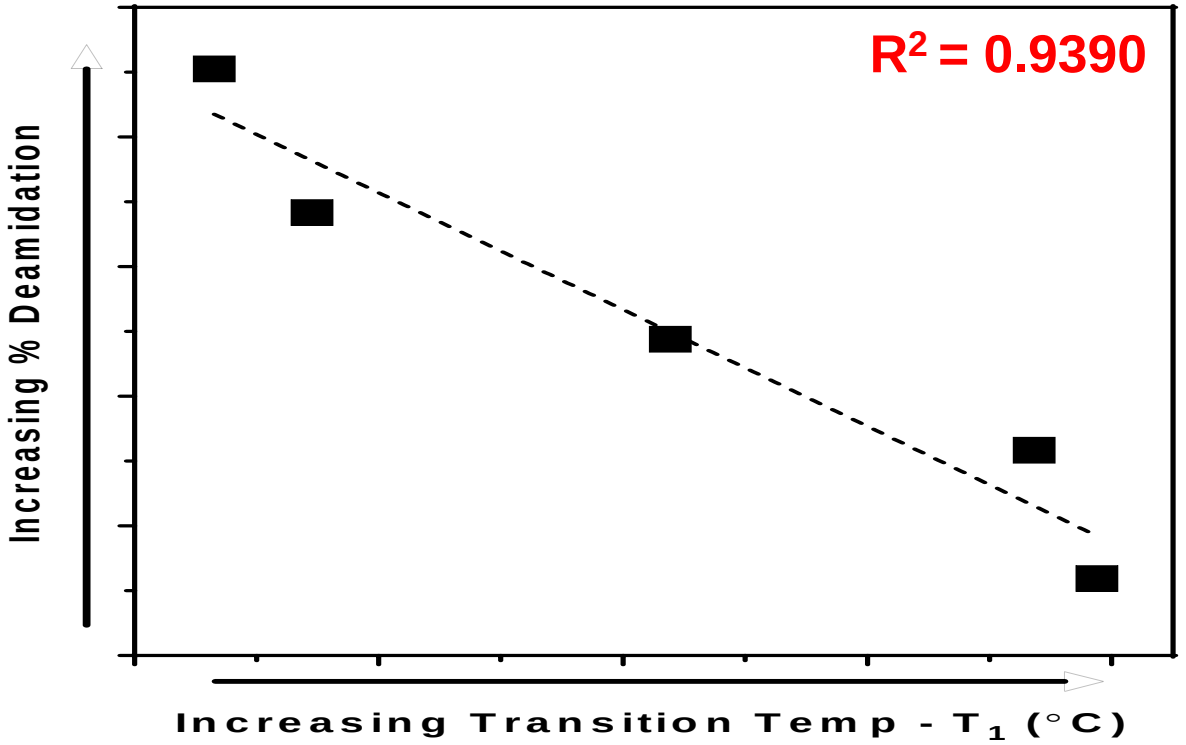


Initial Fluorescence Change is Correlated with

% Deamidation Increase and Potency Decrease of Capsid Vectors

CAPSID STABILITY ANALYSIS AND CORRELATION WITH DEAMIDATION AND POTENCY



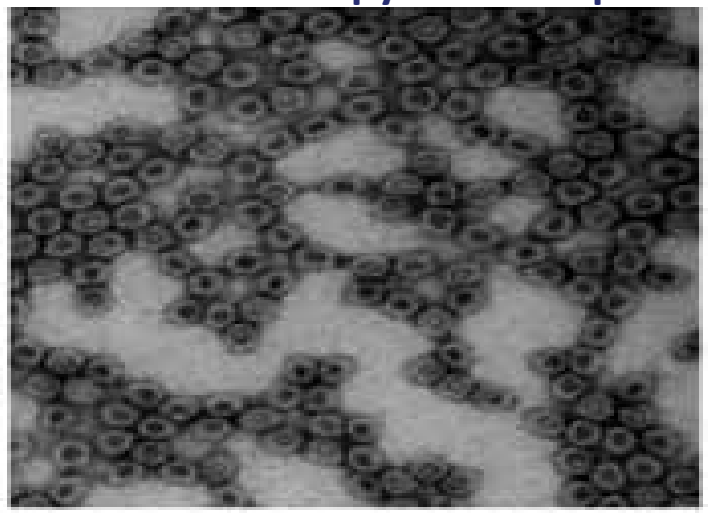


Transition Temperature Change is Correlated with

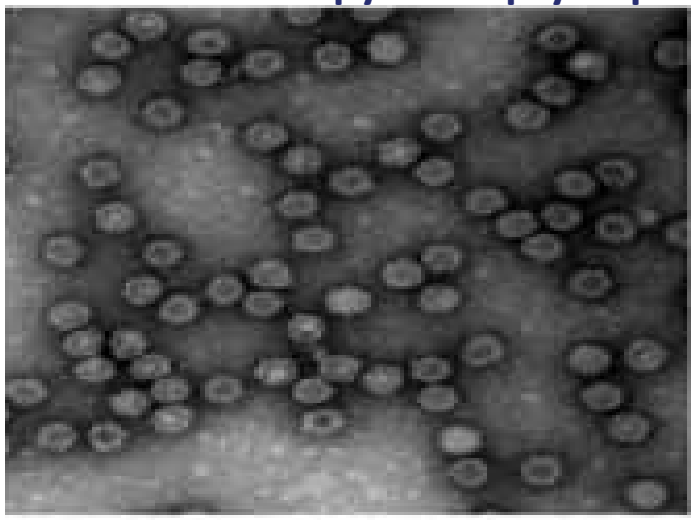
% Deamidation Increase and Potency Decrease of Capsid Vectors

BIOMARIN PROCESS IS ABLE TO REMOVE EMPTY CAPSIDS TO UNDETECTABLE LEVELS

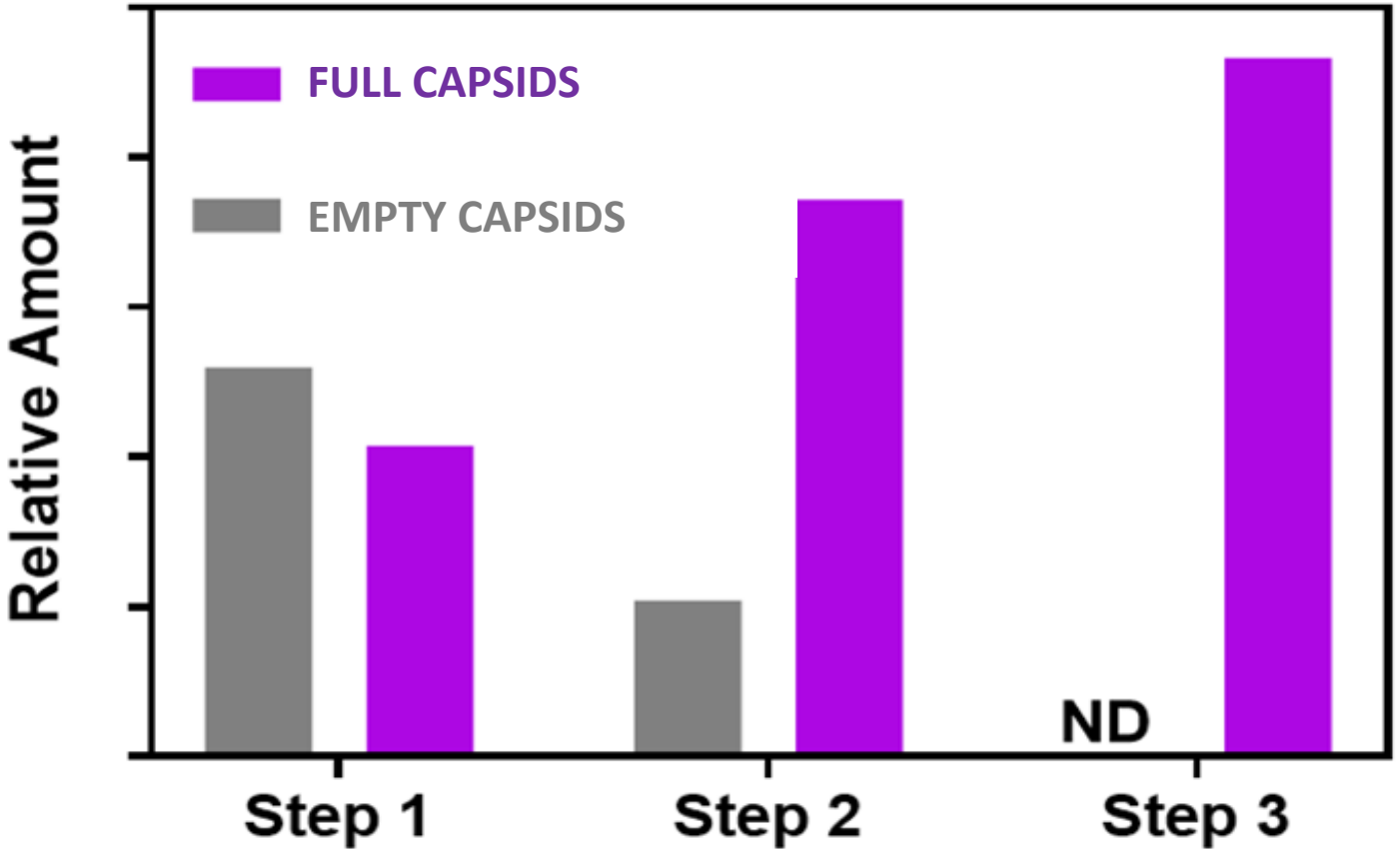
Electron Microscopy of Full Capsids



Electron Microscopy of Empty Capsids



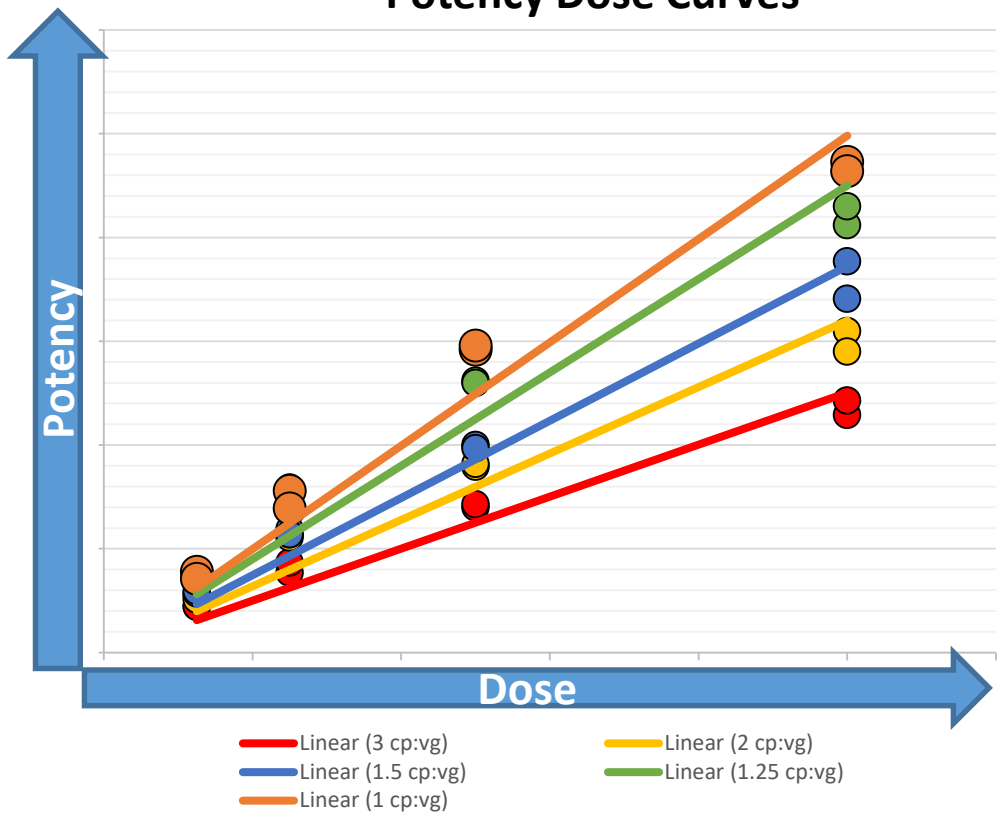
BioMarin Phase 3/Commercial Process



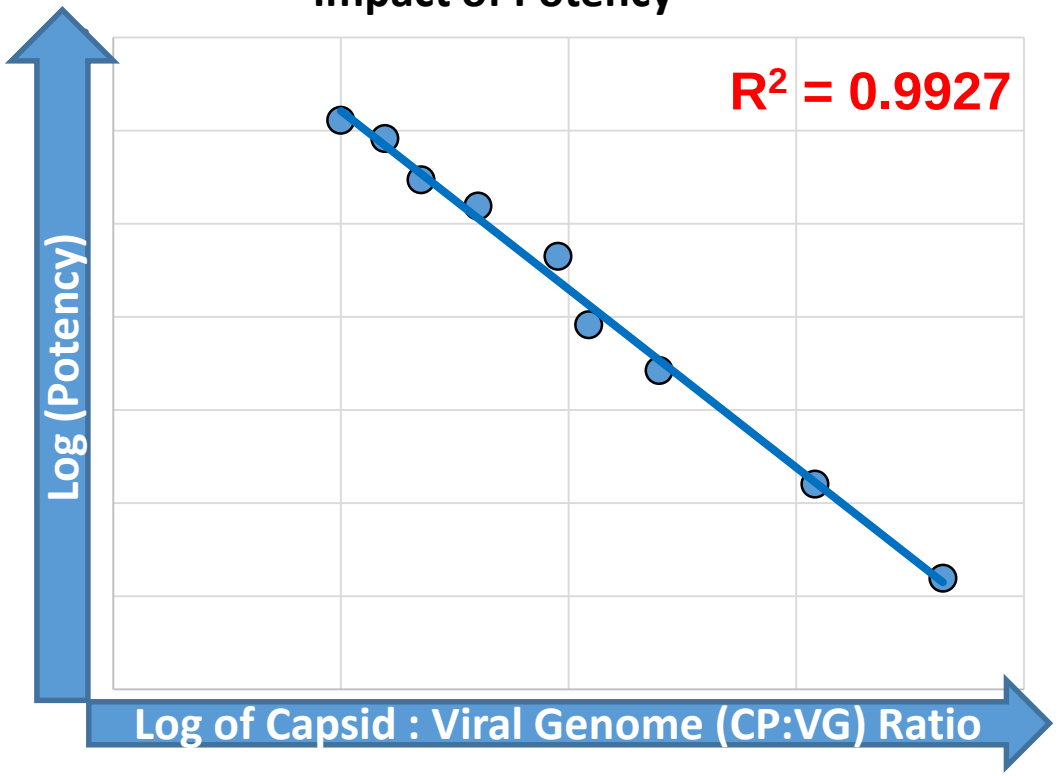
Removal of Empty Capsids from Type 1 Adeno-Associated Virus Vector Stocks by Anion-Exchange Chromatography Potentiates Transgene Expression, Masashi et al., Molecular Therapy, Volume 13, Issue 4, April 2006, Pages 823-828

CAPSID TO VIRAL GENOME CONCENTRATION NEGATIVELY IMPACTS POTENCY

Potency Dose Curves



Impact of Potency



Empty capsids mixed with Full capsids to generate material with increasing [CP:VG]

Cells were transduced with the same concentration of VG/cell regardless of CP:VG ratio

Empty Capsid Results in Reduce Potency Even at a Constant Viral Genome

Log transformed potency value exhibit a negative association with log transformed [CP:VG]

CP:VG ratios between 1 and 21 shown in graph above

SUMMARY OF COMPARABILITY APPROACH

- Biomimetic Cell Based Assays Most Appropriate for Measurement of Potency in Such a Complex Product
  - Cell Based Assays Monitors For:
    - Binding to the Cellular Receptor
    - Internalization of the Vector in to the Cell
    - Release and Translocation of the Transgene to the Nuclease (Nucleolus)
    - Expression of Functional Factor VIII as a Clinically Meaningful Readout
- Capsid Protein Content and Potency Assays Used to Monitor Comparability During
  - First In-Human Study
  - Process Robustness Initiatives
  - Facility & Scale Modifications
- Deamidation of Capsid Proteins
  - Strong Negative Correlation Between Deamidation and Potency
- Empty Capsid Content
  - Strong Negative Correlation Between Empty Capsid Content and Potency
  - Empty Capsid Results in Reduce Potency Even at a Constant Viral Genome Level

ELEMENTS AND BENEFITS OF A COMPREHENSIVE COMPARABILITY STRATEGY HAVE INCLUDED

PROCESS DEVELOPMENT

- We Have Developed a Robust Vector Manufacturing Process Consistent With ICH Guidance Facilitating World Wide Registration

ANALYTICAL CHARACTERIZATION

- We Have Developed a Battery of Biochemical, Cell Based and Animal Based Methods Consistent With ICH Guidance
- These Methods Provide Comprehensive Characterization of Purity, Potency, Consistency and Comparability

SCALE

- Fermentation, Purification & Filling Operations Performed at Commercial Scale Demonstrating Comparability

FACILITY

- Constructed, Commissioned and GMP Operational to Support Intended Commercial Production
- Recognized as ISPE 2018 Facility of the Year – Project Execution Category
- Material Generated Currently Supporting Clinical Development and Capable of Supporting Projected Commercial Demand
- Capable of Supporting at Least 3,000 Patients Per Year at the Highest Clinical Dose Tested to Date

THANK YOU