

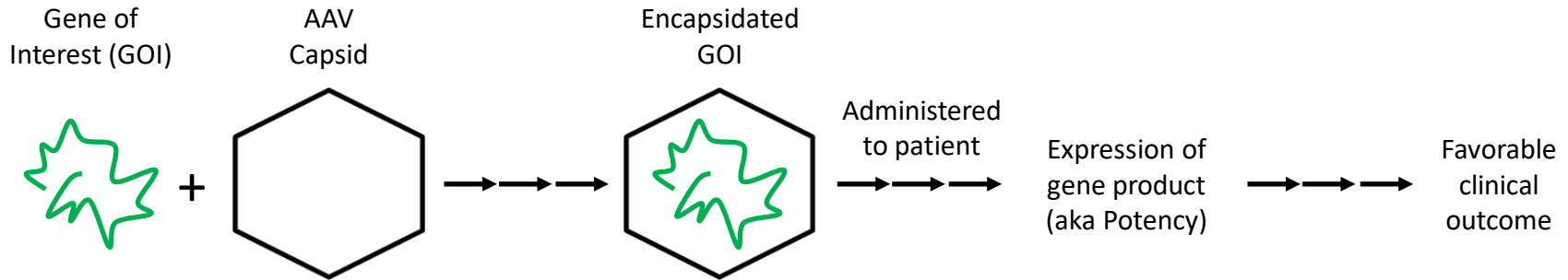


# Approaches to Capsid Characterization for AAV

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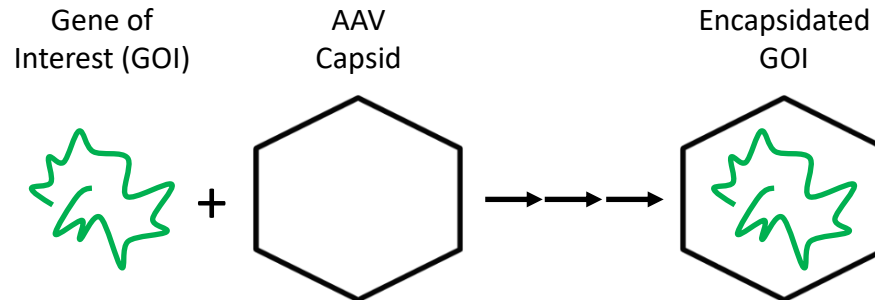
# Background

- Replace or supplement a missing or altered gene product by introducing a biologically functional copy of the gene of interest (GOI)
- Once inside the cell, the native cell machinery will take over and produce the administered GOI product
- Often need a carrier to deliver the GOI to the target cells (e.g. AAV)
- AAV encapsidates the GOI and provides protection, targeting, and stability
- Several different AAV serotypes



# Background

- Production of encapsidated GOI is a complex process
- Requires several steps which may introduce:
  - mechanical stress
  - thermal stress
  - Incubation at extreme pH
  - freeze-thaw cycling
  - non-GOI genetic material
  - excipients
- Not to mention biological inefficiencies
- All of these may alter or damage the capsid and/or GOI
  - Changes are often subtle and difficult to measure and/or remove
  - A single sample will likely contain some amount of altered capsids/GOI



# Capsid Basics

Icosahedral

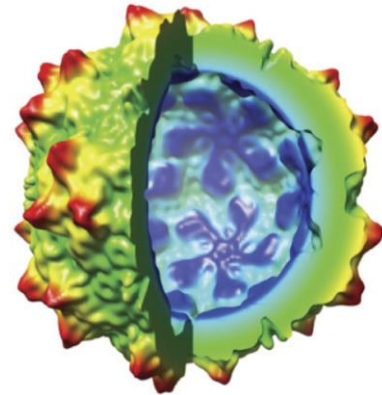
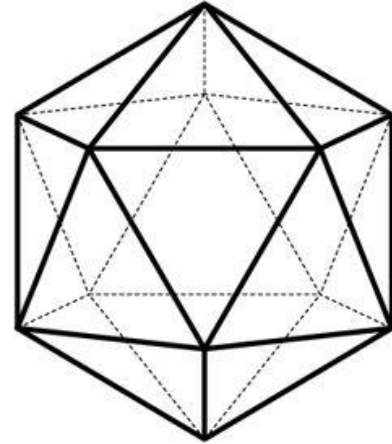
25 nm diameter

May contain up to 4.7 kb of ssDNA

Each capsid consists of 60 total proteins

- VP1 (87 kDa)
- VP2 (73 kDa)
- VP3 (62 kDa)
  - 1 : 1 : 8-10 ratio





Characterization: quantitating measurables of capsids and VPs and monitoring changes



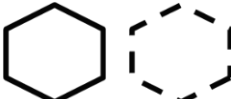



Cryo-EM cross-section<sup>1</sup>

# Altered Capsid/GOI Species

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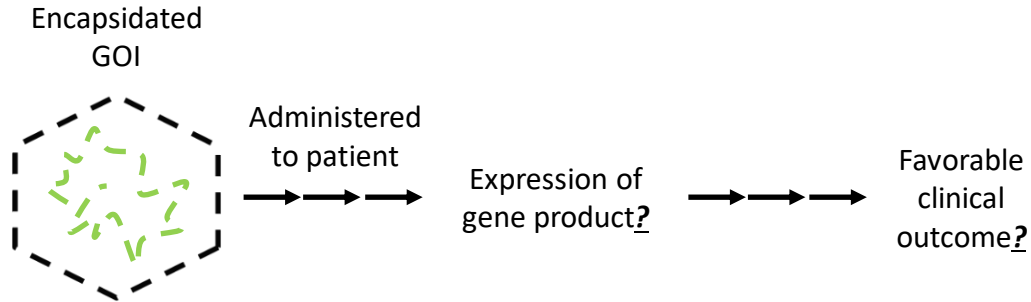
	Classification	Capsid	Packaged GOI	
1	Full	Intact	Intact	
2		Intact	Damaged	
3		Altered	Intact	
4		Altered	Damaged	

	Classification	Capsid	Packaged GOI	
5	Partial	Intact	Fragment	
6		Altered	Fragment	
7	Empty	Intact or Altered	No GOI	
8	Unpackaged	No Capsid	Intact, Damaged, Fragment	

# Why should we care?

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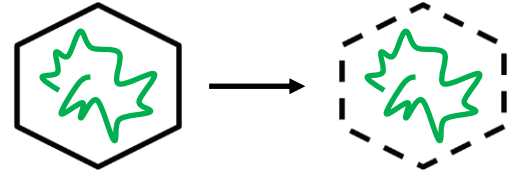
- Potential effect on potency
- Increased risk of adverse event
  
- Informs manufacturing decisions and thresholds
  
- Informs on short- and long-term storage



# Altered Capsid/GOI Phenomena

Formation of these species can be grouped into three processes

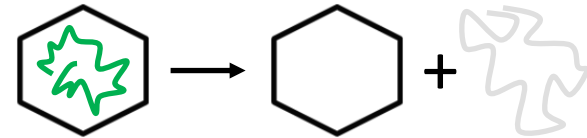
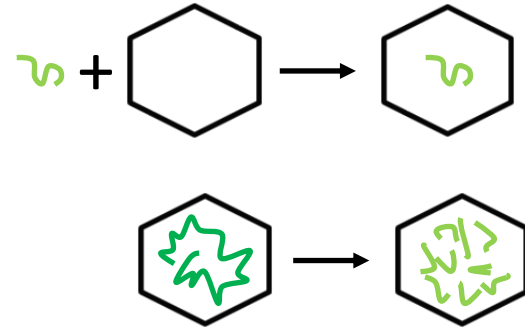
1. Capsid Alteration: capsid or VPs are altered or damaged
  - Could occur during cell packaging, production, and/or storage
  - Characterization/Measurables:
    - Capsid MW (empty:partial:full) – CDMS, AUC, AEX, cryoTEM
    - Capsid Concentration – MADLS, ELISA, NTA
    - Capsid particle size and distribution – MADLS, DLS, SEC, FlowCam
    - Capsid thermal stability – IF, DSC
    - VP ratio/content, MWs, purity – CE, RP-HPLC, Mass Spec
    - VP surface charge – cIEF, Mass Spec
    - VP higher order structure – CD
2. DNA Degradation: GOI breaks down
3. DNA Ejection: GOI is forced outside of the capsid



# Altered Capsid/GOI Phenomena

Formation of these species can be grouped into three processes

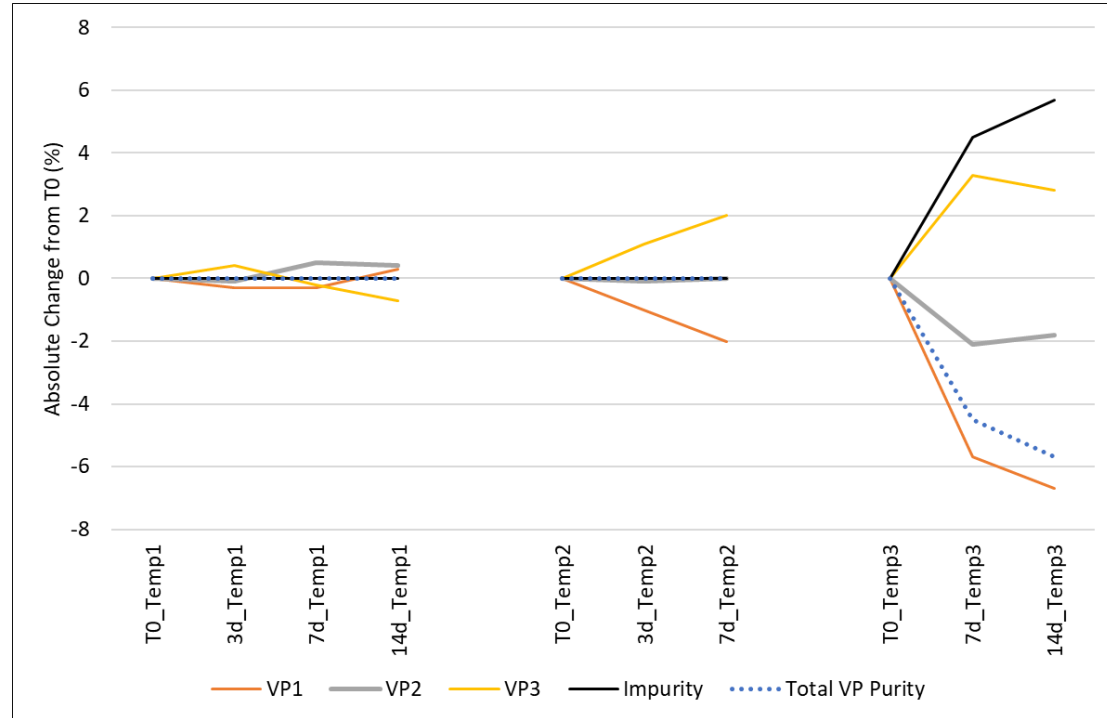
1. **Capsid Alteration:** capsid or VPs are altered or damaged
2. **DNA Degradation:** GOI breaks down
  - Could occur during cell packaging, production, and/or storage
  - Characterization/Measurables:
    - GOI titer – ddPCR, qPCR
    - GOI integrity – Multiplex ddPCR, Fragment analysis, Next Gen Sequencing
3. **DNA Ejection:** GOI is forced outside of the capsid
  - Could occur during production and/or storage
  - Characterization/Measurables:
    - Capsid MW (empty:partial:full) – CDMS, AUC, AEX, cryoTEM
    - Unpackaged GOI titer – ddPCR, qPCR
    - DNA Exposure – SYBR-Gold





# Capillary Electrophoresis (CE)

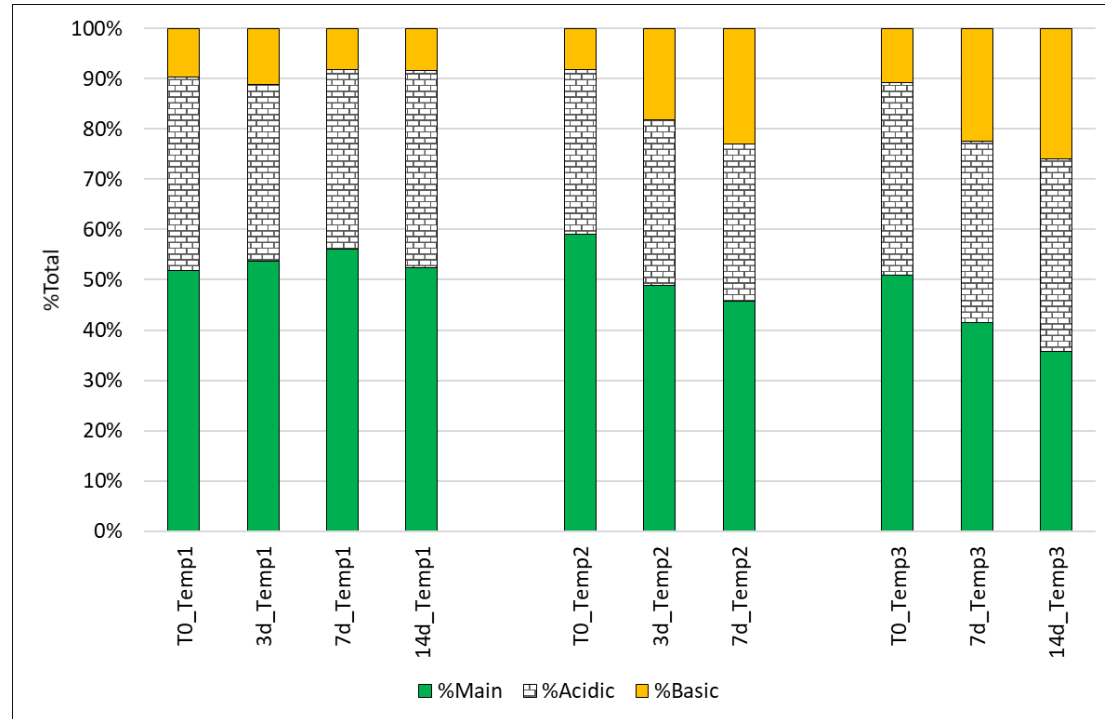
- Separates VPs based on electrophoretic mobility and MW
- Takeaways:
  - the relative amounts of VP1, VP2, and VP3
  - the relative purity (VP1+VP2+VP3)
    - relative amounts of impurities
- Advantages:
  - Low sample volume requirement
  - Sample prep kits available (capillary, MW markers, buffers, etc.)
- Disadvantages:
  - No fractionation
  - Need a clean sample (similar MW proteins may interfere)



Increasing Temp1 -> Temp3

# Capillary Isoelectric Focusing (cIEF)

- Separates VPs based on electrophoretic mobility and pI
- Takeaways:
  - the relative amounts of Acidic, Main, and Basic species (i.e. altered charge states of VPs)
- Advantages:
  - Short analysis time
  - Sample prep kits available (capillary, pI markers, buffers, etc.)
- Disadvantages:
  - No separation of VPs, only charge states
  - No fractionation
  - Need a clean sample (similar pI proteins may interfere)



Increasing Temp1 -> Temp3

# Particle Count (MADLS & Capsid ELISA)

- Multi-angle dynamic light scattering (MADLS): DLS + Brownian motion determines total particle concentration, particle size, and distribution
- ELISA: antibody-based capture of AAV + colorimetric detection determines capsid concentration

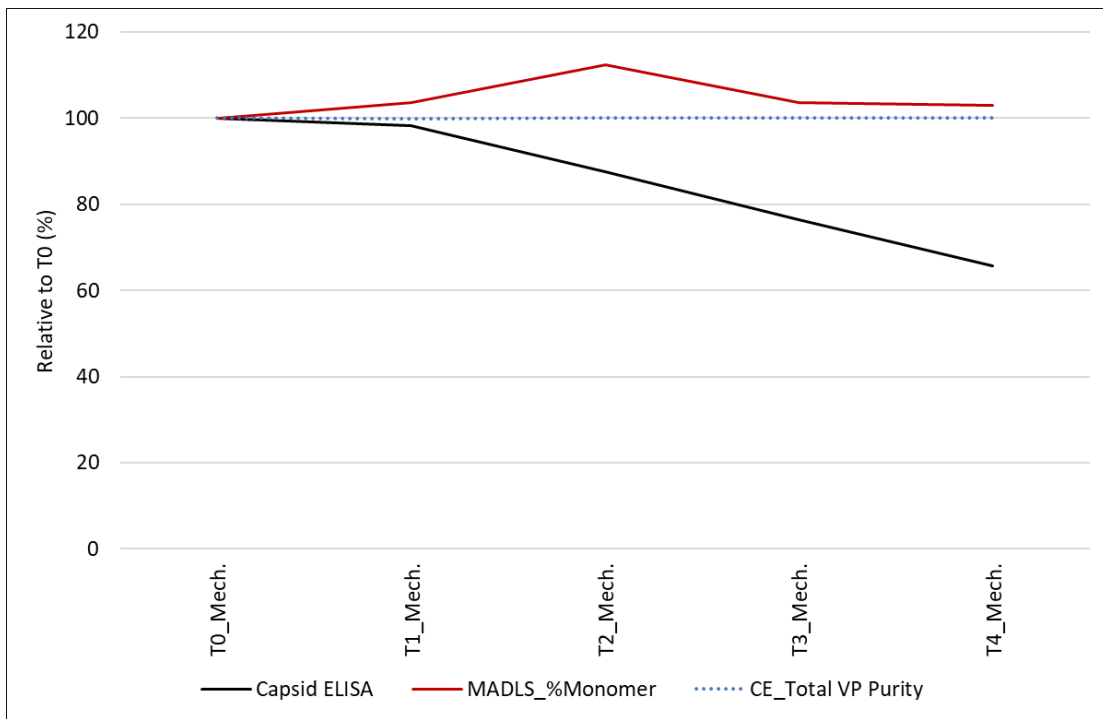
➤ Takeaways: Integrity of epitope ~ capsid structural alterations

- Advantages

- MADLS: non-destructive; serotype independent
- ELISA: kit-based; high specificity

- Disadvantages

- MADLS: manual cuvette reading
- ELISA: need specific anti-AAV antibodies

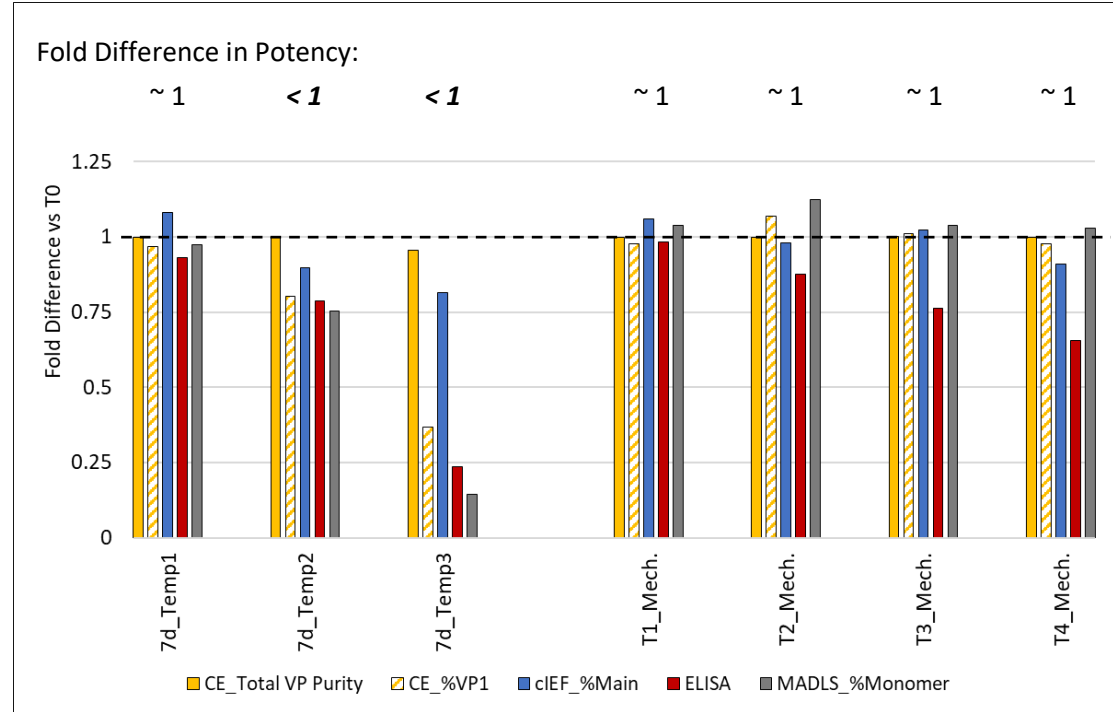


Increasing mechanical stress T0 -> T4

# Potency

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- Potency reports gene product expression
- Informs on infection of the target cell and translation of GOI into protein
- Effect on potency is the sum of capsid and DNA integrity



Increasing Temp1 -> Temp3; Increasing mechanical time T0 -> T4

Fold Difference = stressed / T0

1.0: no change (-----); > 1.0: increase; < 1.0: decrease

# Summary

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- Several techniques are available to characterize capsids as well as VPs
- Changes in capsid analytics does not necessarily correlate to an effect on potency
- GOI characterization is necessary to complete the picture

## Acknowledgements

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