Comparison of CE-SDS Platforms to Quantitate Purity and Viral Protein Ratios of Adeno-associated Virus (AAV) Capsids

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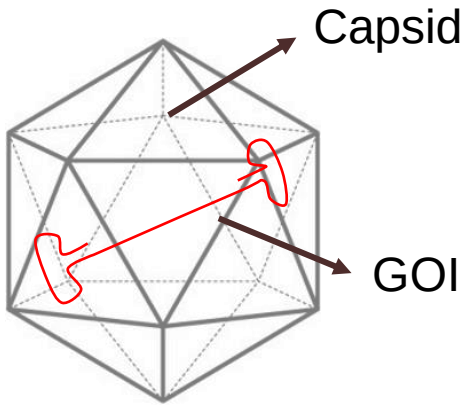
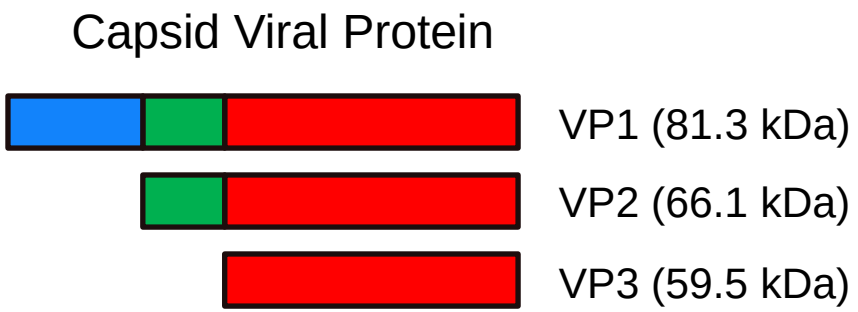
Adeno-associated Virus (AAV)

- Adeno Associated Virus (AAV) for Gene Therapy
  - Genome editing for curative therapies
  - Instead of binding to a receptor or protein, the AAV gene therapy helps create a missing or deficient protein
  - AAV is an icosahedral 60-mer made of 3 monomer variants (VP1, VP2, VP3) encasing a gene of interest
  - A consistent VP ratio and purity profile is important for infectivity and potency

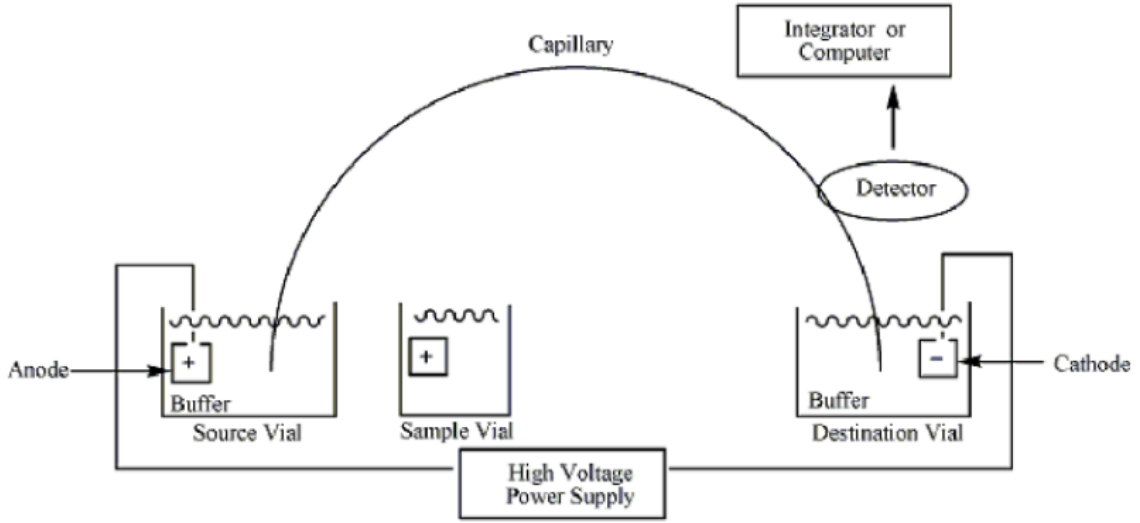


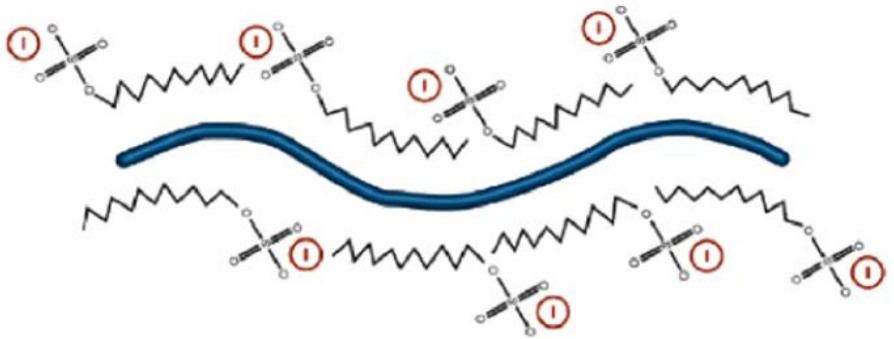


Theoretical VP ratio
1:1:10 (5:5:50)

CE-SDS principle

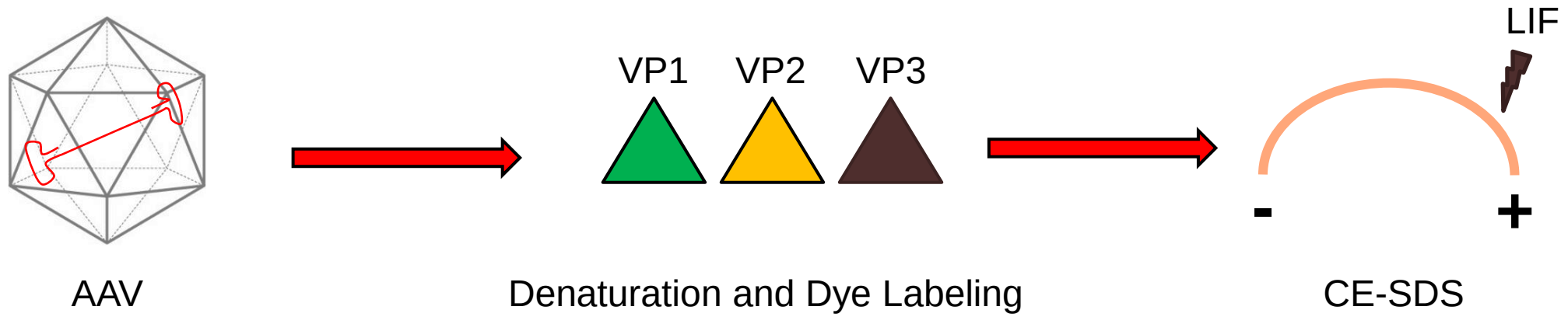
- Capillary Electrophoresis (CE) is a separation technique that uses an applied voltage to separate ions based on their electrophoretic mobility.
- In Capillary Gel Electrophoresis, molecules are separated via electric current through a polymeric gel matrix
- Movement through the gel is based on the molecules size, shape and charge
- Sodium Dodecyl Sulfate (SDS) denatures most proteins and binds the protein at an equal ratio based on their size resulting in a uniform charge to mass ratio.





CE-SDS for AAV

- Used as alternative to SDS-PAGE for determining AAV capsid purity and viral protein (VP) ratio
- The AAV capsids are denatured to their three VP components: VP1, VP2 and VP3.
- Due to low protein content in AAV samples, laser induced fluorescence (LIF) detector is preferred to increase the sensitivity of detection.



Reporting

What CE-SDS tells us:

- Time corrected area of each named peak (VP1, VP2, VP3, or other identifiable species)
- Total fragments (%)
- Total aggregates (%) in rare cases
- %Purity = %VP1 + %VP2 + %VP3
- VP ratio (VP1:VP2:VP3) = %VP1 : %VP2 : %VP3 (normalized to VP1)

CE-SDS Platforms

Instruments:

Sciex PA800+

Sciex Biophase 8800

ProteinSimple Maurice
(CE-SDS Plus)

Revvity LabChip
(ProteinExpress Kit)

Serotypes studied:

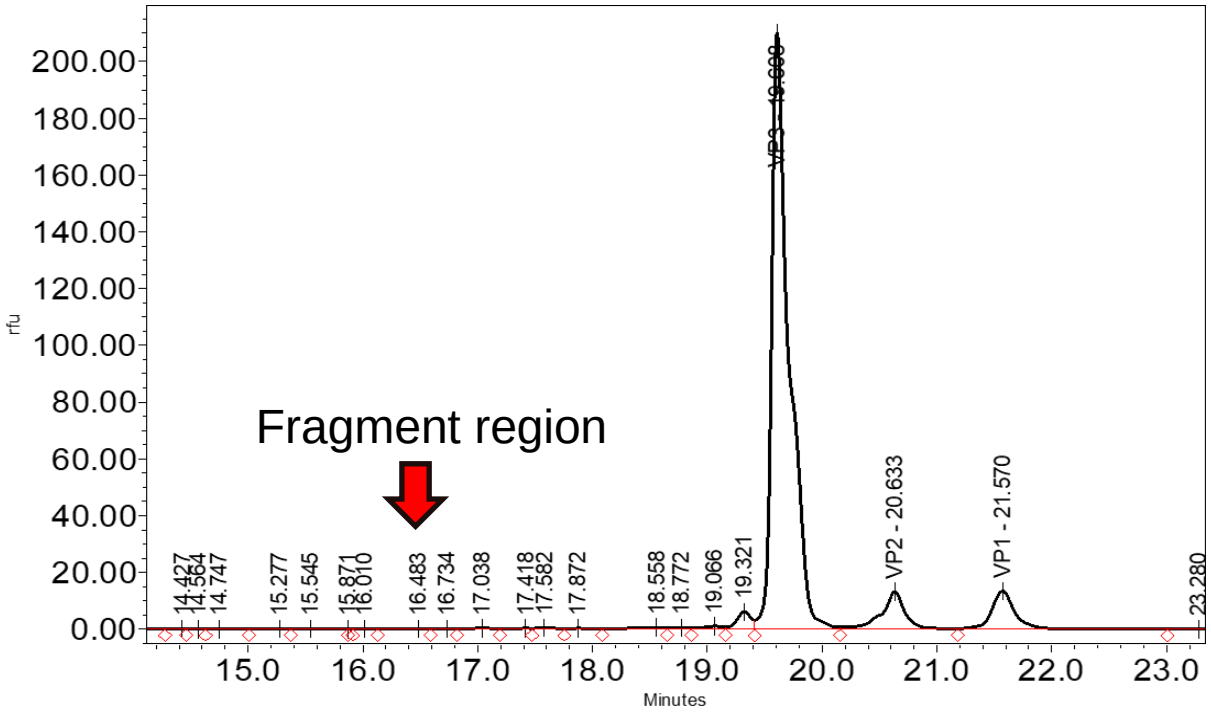
- AAV6 and AAV9

Permission obtained for image use

Sciex Workflow

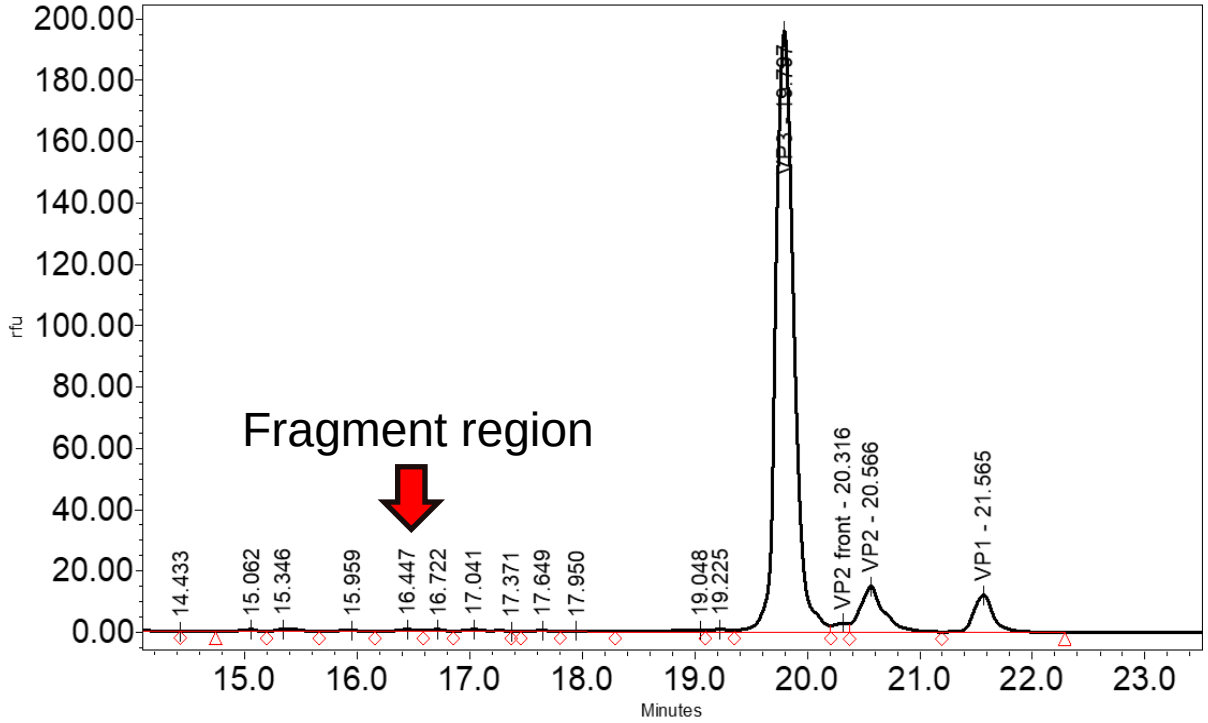
- Use buffer and gel provided in SDS-MW Analysis Kit
- Sample is denatured in SDS buffer (reducing agent can also be added if necessary) at 75^{o}C
- Fluorescent dye is used (Chromeo P503) in the denaturing step to label the protein contents so that the VPs can detected by LIF.
- The concentration ratio of dye to sample is important.
- LIF detector: 488 nm laser excitation, 600 nm emission filter
- Both EK and pressure injection mode work

Sciex PA800+



AAV6

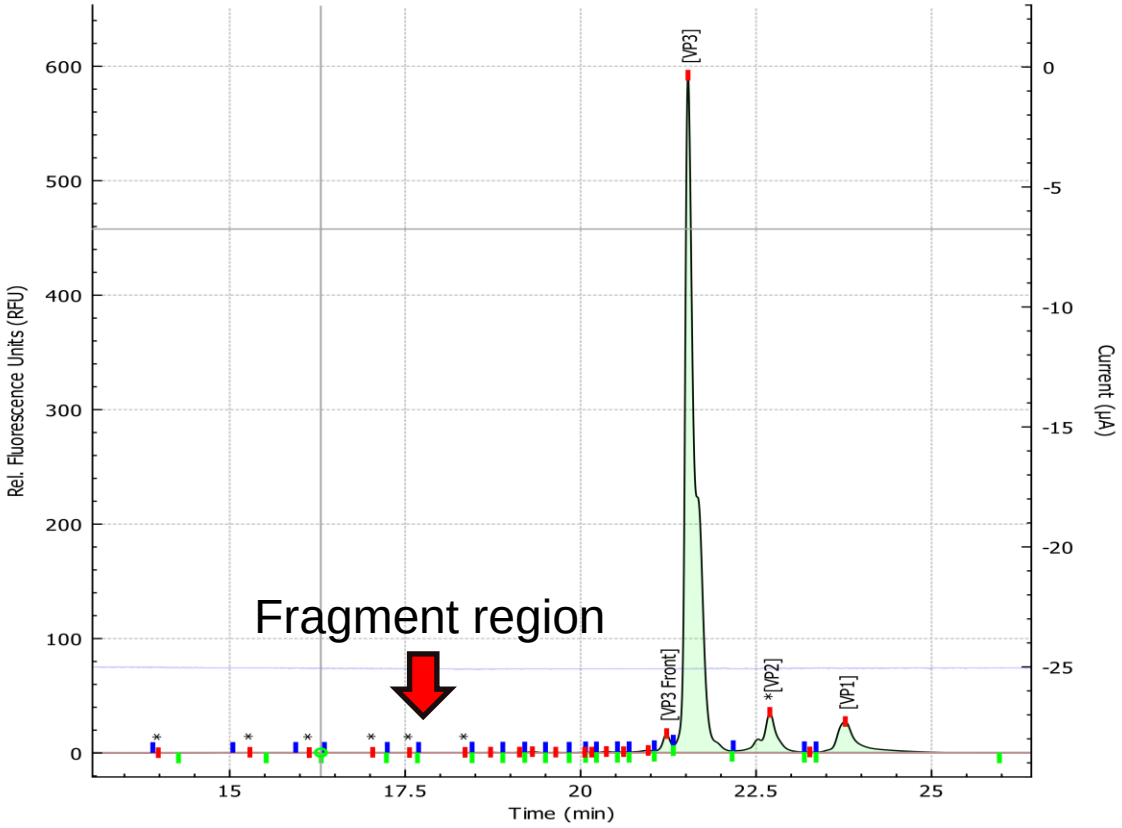
%VP1	%VP2	%VP3	%Purity	%Total Fragments
6.3	6.6	82.2	95.1	4.9



AAV9

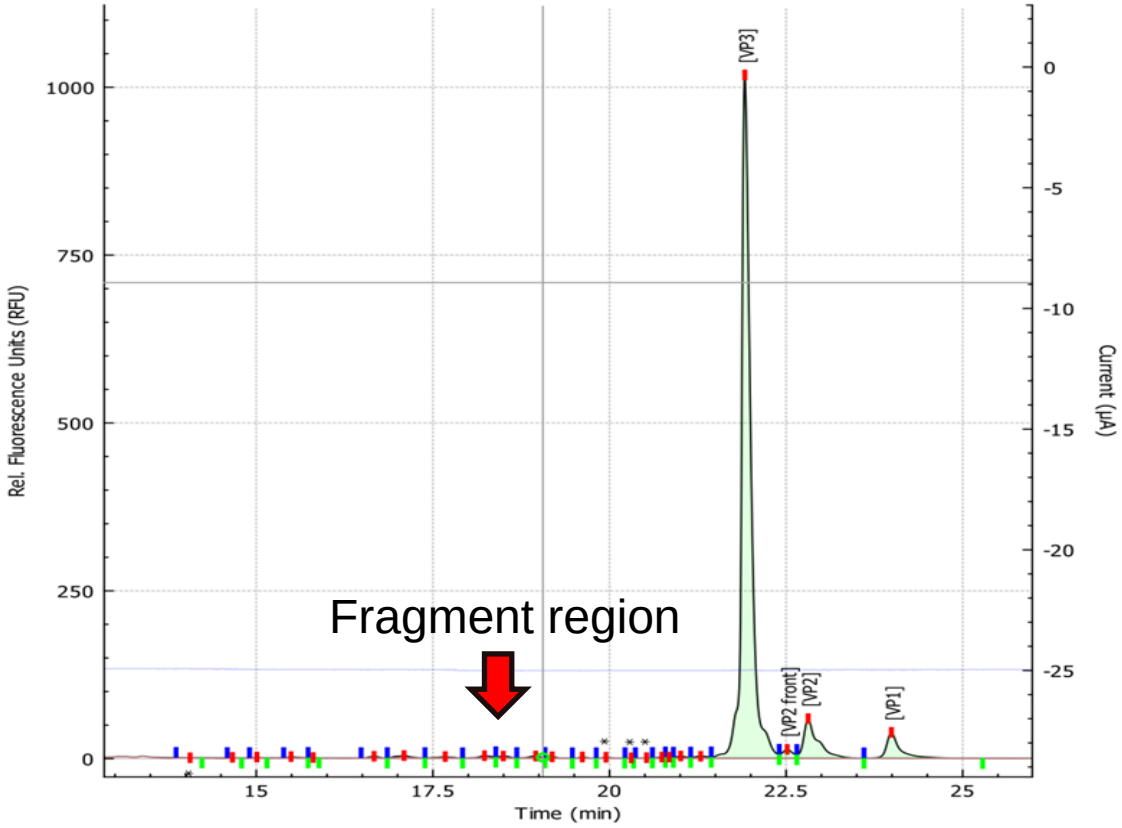
%VP1	%VP2	%VP3	%Purity	%Total Fragments
4.9	7.6	81.4	93.9	6.1

Sciex Biophase 8800



AAV6

%VP1	%VP2	%VP3	%Purity	%Total Fragments
7.0	6.7	82.7	96.4	3.6



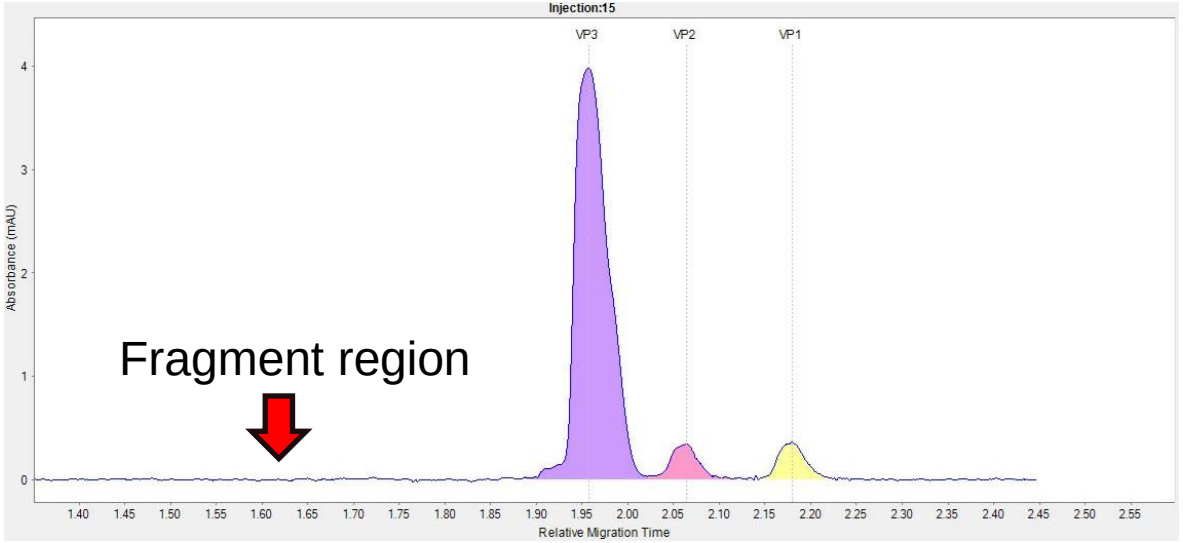
AAV9

%VP1	%VP2	%VP3	%Purity	%Total Fragments
4.2	7.1	82.8	94.2	5.8

ProteinSimple Maurice Workflow

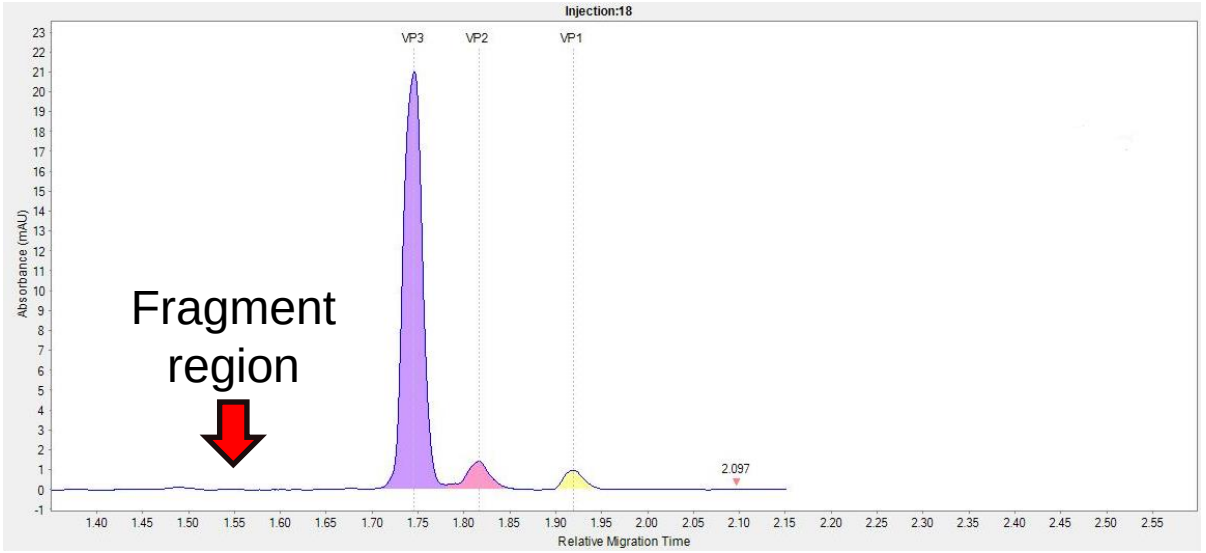
- Use Maurice CE-SDS plus cartridge
- Since Maurice CE-SDS cartridge does not support fluorescence detection, the sample needs to be concentrated to yield sufficient signal
- Desalting may be required for lowering the current

ProteinSimple Maurice



AAV6

%VP1	%VP2	%VP3	%Purity	%Total Fragments
5.9	6.0	88.1	100	0



AAV9

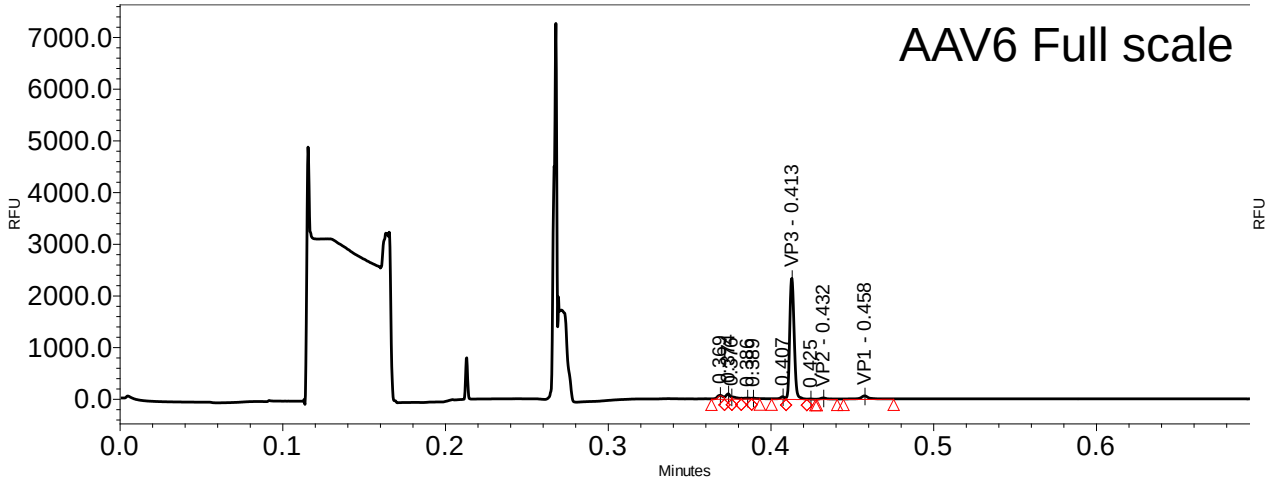
%VP1	%VP2	%VP3	%Purity	%Total Fragments
4.0	7.5	88.6	100	0

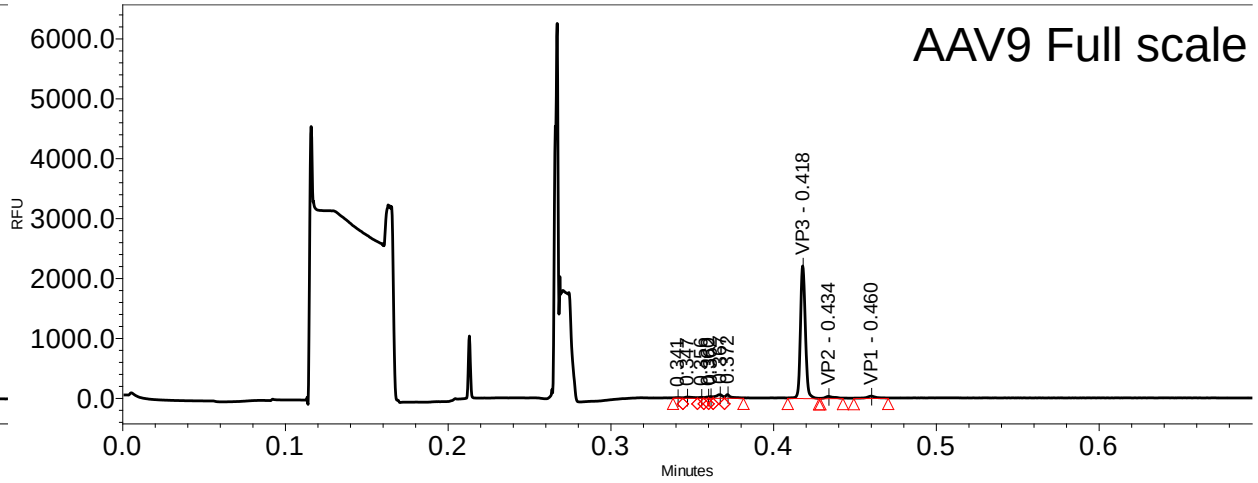
Data from Sarah Kudman

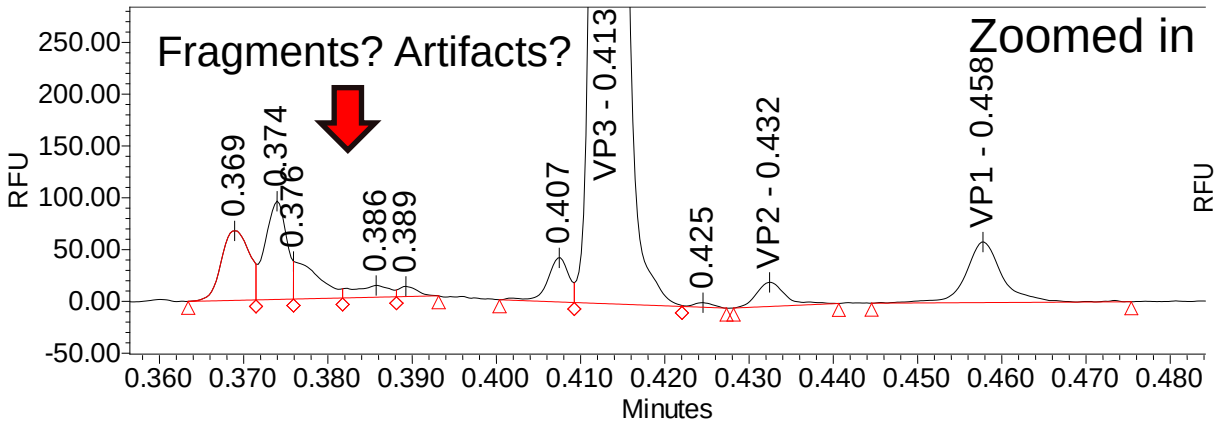
LabChip workflow

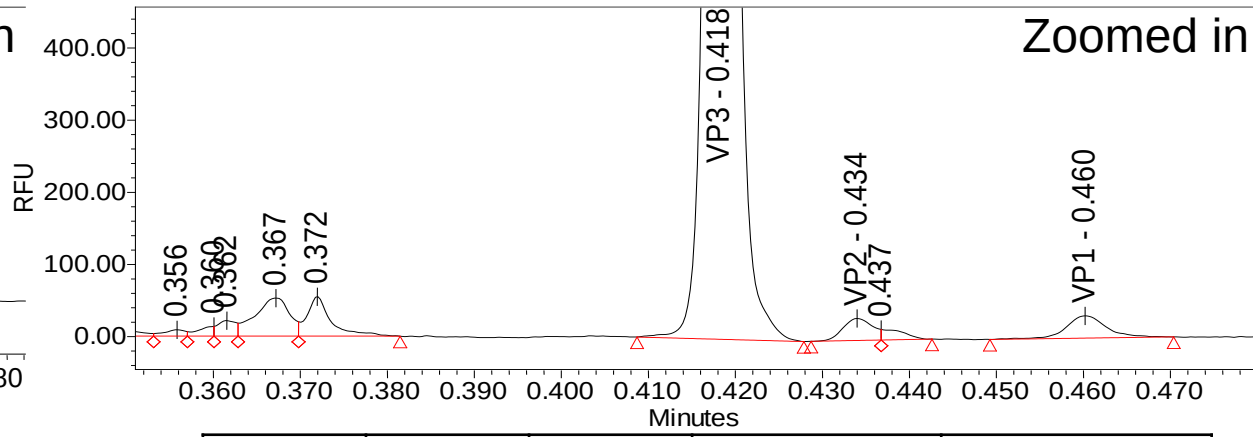
- Use ProteinExpress chip and reagent kit
- The sample is mixed with sample buffer (and reducing agent if needed) and heated at 70^{o}C for denaturation
- Follow the assay quick guide to set up the chip and execute the analysis
- The VPs are labeled with Protein Express dye in the gel matrix during the run and detected by a fluorescence detector.

LabChip









%VP1	%VP2	%VP3	%Purity	%Total Fragments
3.5	1.1	85.2	89.7	10.3

%VP1	%VP2	%VP3	%Purity	%Total Fragments
1.9	1.9	89.1	93.0	7.0

Data from Poulami Majumder

VP ratio calculation

%VP1	%VP2	%VP3	%Purity	%Total Fragments
6.3	6.6	82.2	95.1	4.9

- Peak area VP ratio (normalize to VP1)

$$VP1:VP2:VP3 = \frac{VP1}{VP1}:\frac{VP2}{VP1}:\frac{VP3}{VP1} = \frac{6.3}{6.3}:\frac{6.6}{6.3}:\frac{82.2}{6.3} = 1:1.0:13.0$$

- Molar VP ratio (normalize to VP1)

For AAV6, MW VP1 = 81.3 KDa, MW VP2 = 66.1 KDa, MW VP3 = 59.5 KDa.

$$VP1:VP2:VP3 = \frac{1}{\frac{MW(VP1)}{MW(VP1)}}:\frac{0.75}{\frac{MW(VP2)}{MW(VP1)}}:\frac{8.37}{\frac{MW(VP3)}{MW(VP1)}} = 1:\frac{1.0}{\frac{66.1\ KDa}{81.3\ KDa}}:\frac{13.0}{\frac{59.5\ KDa}{81.3\ KDa}} = 1:1.3:17.8$$

- Normalize to 60 VPs (ratio for entire capsid)

$$VP1:VP2:VP3 = \left(\frac{1}{1+1.3+17.8}:\frac{1.3}{1+1.3+17.8}:\frac{17.8}{1+1.3+17.8}\right) \times 60 = 3.0:3.8:53.2$$

Comparison of VP ratio

AAV9 Sample

Instrument	%VP1	%VP2	%VP3	VP1:VP2:VP3	Normalized to 60 VPs
PA800+	4.9	7.6	81.4	1 : 1.6 : 16.6	2.3 : 4.5 : 53.2
Biophase 8800	4.2	7.1	82.8	1 : 1.7 : 19.7	2.0 : 4.2 : 53.8
Maurice	4.0	7.5	88.6	1 : 1.9 : 22.2	1.8 : 4.1 : 54.1
Labchip	1.9	1.9	89.1	1 : 1.0 : 46.9	0.9 : 1.1 : 58.0
SDS-PAGE	6.3	12.0	81.7	1 : 1.9 : 13.0	2.8 : 6.7 : 50.5

Detection mode (LIF or UV) and dye selection will have significant impact on the VP ratio results

VP ratio considerations

Same AAV9 sample measured by PA800+ with different detections

Detector	VP1%	VP2%	VP3%	VP ratio
LIF	8.59	5.73	82.44	1 : 0.67 : 9.60
UV - 220 nm	6.60	5.14	87.60	1 : 0.78 : 13.27

- The addition of Chromeo P503 may overdetect VP1 and VP2. To ensure calculated molar VP ratio is accurate, Chromeo P503 should have same binding efficiency to VP1, VP2 and VP3, but some paper suggest it does not.

Factors to consider when choosing detection mode:

- LIF detection with Chromeo P503: The lysine count of VP1, VP2 and VP3
- Intrinsic fluorescence (Ex/Em: 280/350): Tryptophan count of VP1, VP2 and VP3
- UV – 220 nm: Overall all amino acid (backbone) count of VP1, VP2 and VP3
- UV – 280 nm: Tyrosine and Tryptophan count of VP1, VP2 and VP3

Technique Comparison

	PA800+	Biophase 8800	Maurice	Labchip
Advantages	-Generic and QC Friendly -Superior peak separation and resolution	-Can run 8 samples simultaneously, so its throughput is technically 8x compared to PA800+	-Easy to use CE-SDS cartridges	-Very fast turnaround time and large sample capacity
Disadvantages	-Chromeo dye inconsistencies -Sometimes lab to lab reproducibility concerns	-The reliability of the instrument and cartridges needs further assessment	-Lack fluorescence detection	-Baseline Artifacts can be observed -Fixed Conditions
Recommended Application	Both purity and VP ratio in a QC lab setting.	High throughput screening and robustness studies.	Inexpensive characterization with little instrumental startup cost	High throughput screening for cell line selection or capsid engineering.

Conclusion and Future Perspectives

Summary:

- CE-SDS is a great alternative to SDS-PAGE for characterizing AAV purity profile and VP ratio.
- Different CE instruments have been evaluated on the AAV CE-SDS performance. Technical comparison has been summarized.
- VP ratio results are highly dependent on the sample labelling and detection.

Future improvement on the AAV CE-SDS platforms:

- Peak identification
- Improve the accuracy of VP ratio results
- Better sensitivity on small fragments
- Higher tolerance on the interference from in-process samples

Acknowledgement

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Sarah Kudman
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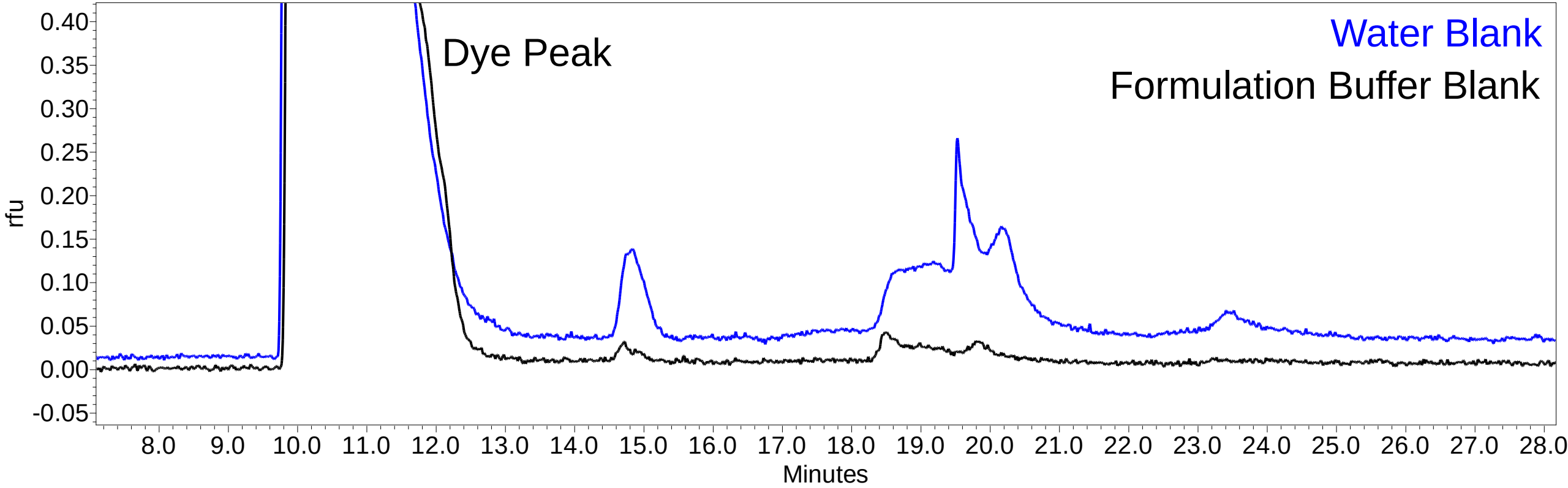
Questions?

Back up - Blank Interference



- Using water as blank could have interfering peaks in region of interest (sometimes larger than 1%, not sure whether it is carryover)
- Using formulation buffer as blank can significantly reduce this issue (<LOQ)
- Fresh prepared dye solution does not seem to have the interference.