mRNA and CircRNA Integrity Analysis for Biopharmaceutical Development

Challenges and Opportunities

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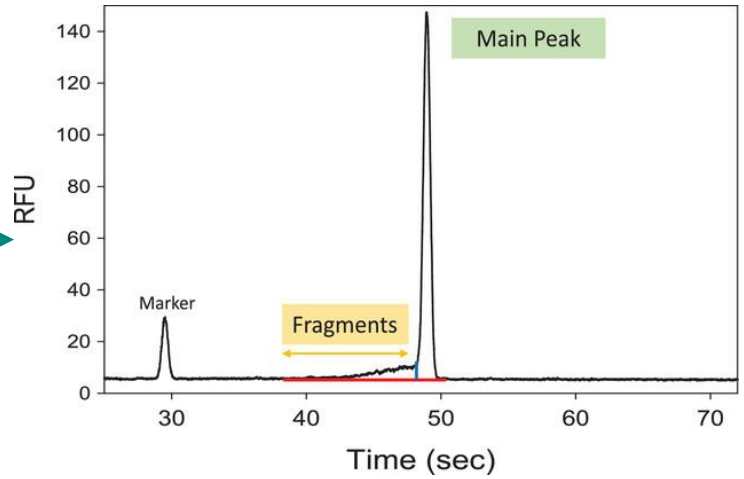
Outlines

- ❑ Integrity Analysis of mRNA drug substance and drug product
- ❑ Practical challenges for mono-valent mRNA vaccines
- ❑ Challenges for multi-valent mRNA vaccines
- ❑ Challenges for circular RNA samples

mRNA Integrity Measurement: CGE vs. IP-RPLC

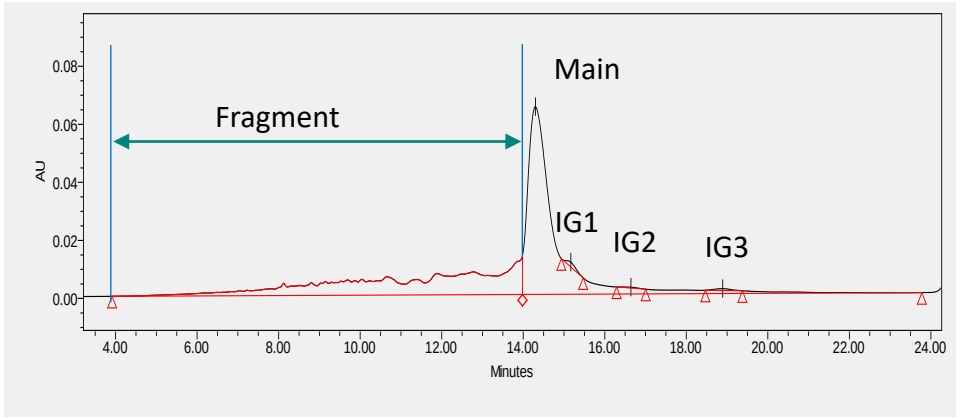
Pros:
Higher throughput
Higher sensitivity

Cons:
No separation of Lipidated mRNA (IG)



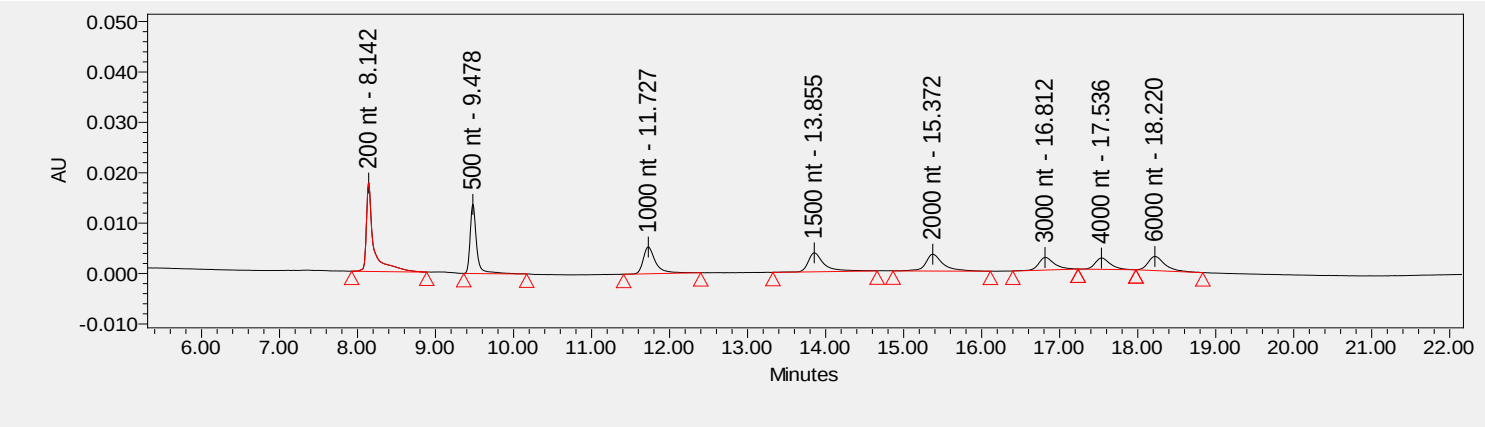
Pros:
Sound separation of IG
Higher resolution at lower MW

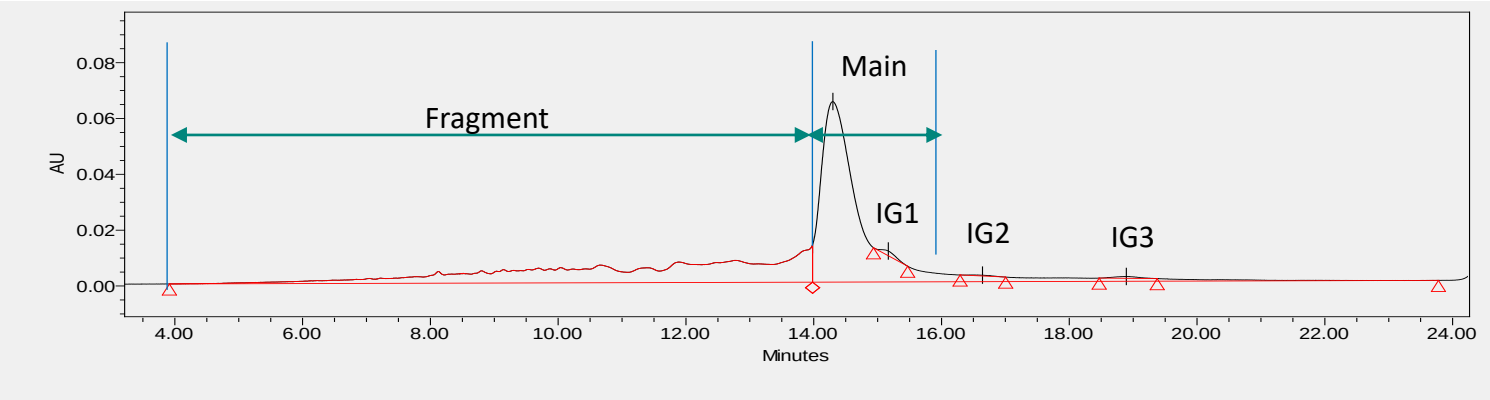
Cons:
Lower Throughput
Lower repeatability



INVENTING FOR LIFE

IP-RPLC for Purity Analysis: Quantitate Purity, Fragments and “IG” Peaks





- Elution order: nucleotide length of intact mRNA molecules, same as size-based separations (SEC, CGE)
- Separation mechanism: primarily based on hydrophobicity
- Lipidated mRNA has higher hydrophobicity than the intact mRNA counterparts

IP-RPLC: Shallow Gradient of Organic

- Column: DNAPac RP, Thermo Scientific
- System: Waters Acquity H Class Binary System
- MPA: 30/70 ACN/H_2O, 50 mM HAA

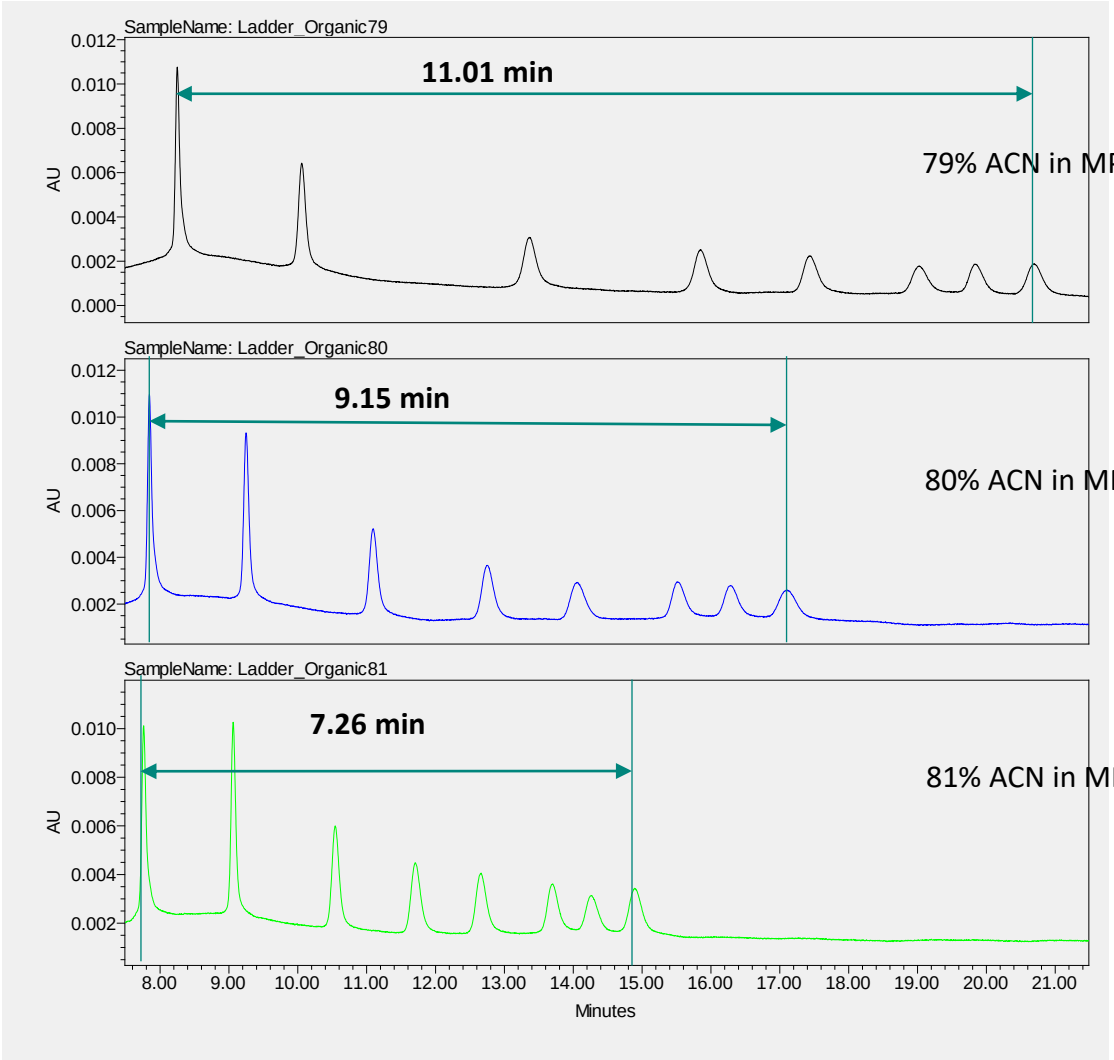
  MPB: 80/20 ACN/H_2O, 50 mM HAA
- Sample Temp: 5°C
- PDA: 260nm

Time (min)	Flow Rate (ml/min)	MPA	MPB
7	0.2	76	24
23	0.2	70	30

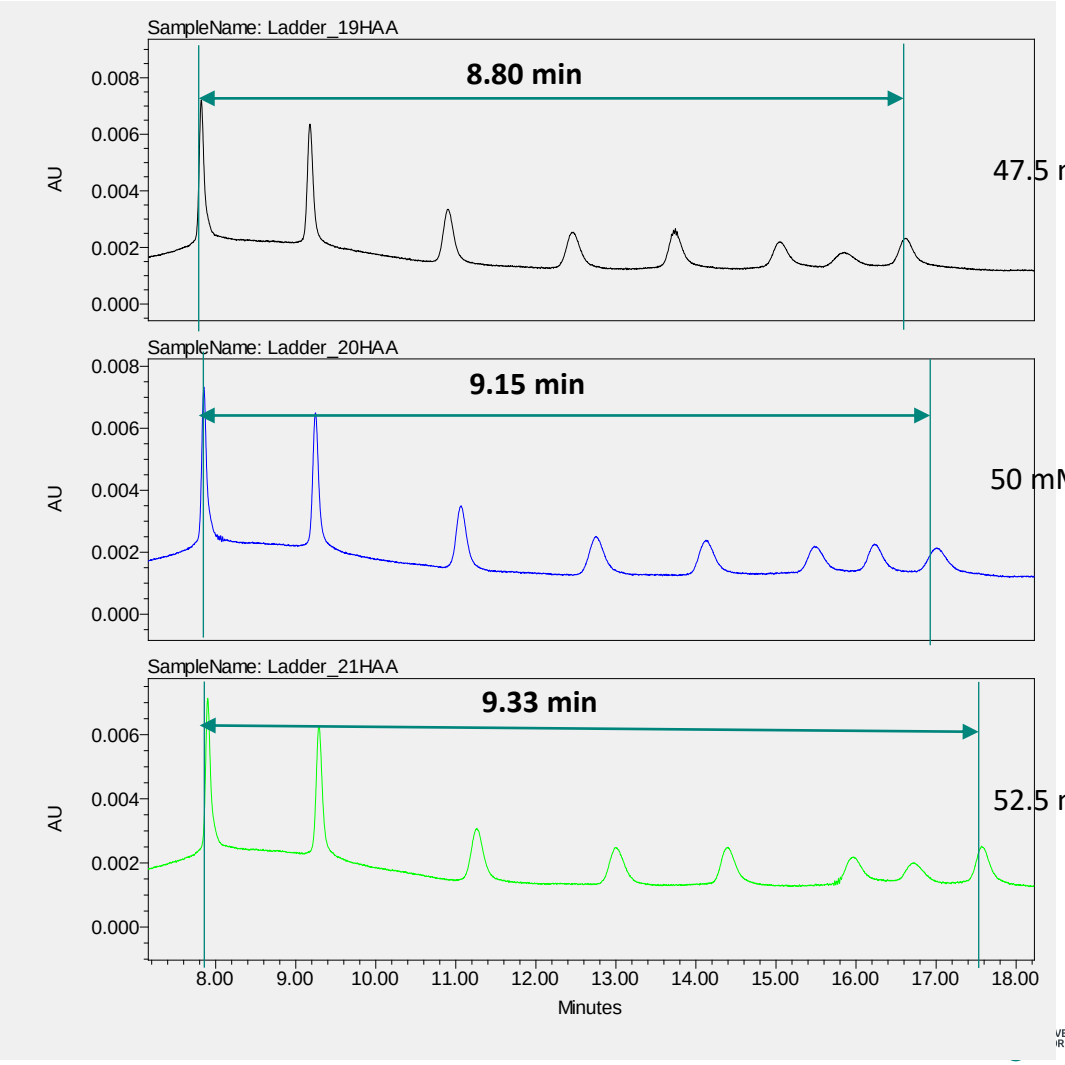
Shallow organic gradient: change of 3% ACN in 16min

Shifting Retention Times – Robustness of MP Composition

Change of Acetonitrile in MPB



Change of HAA

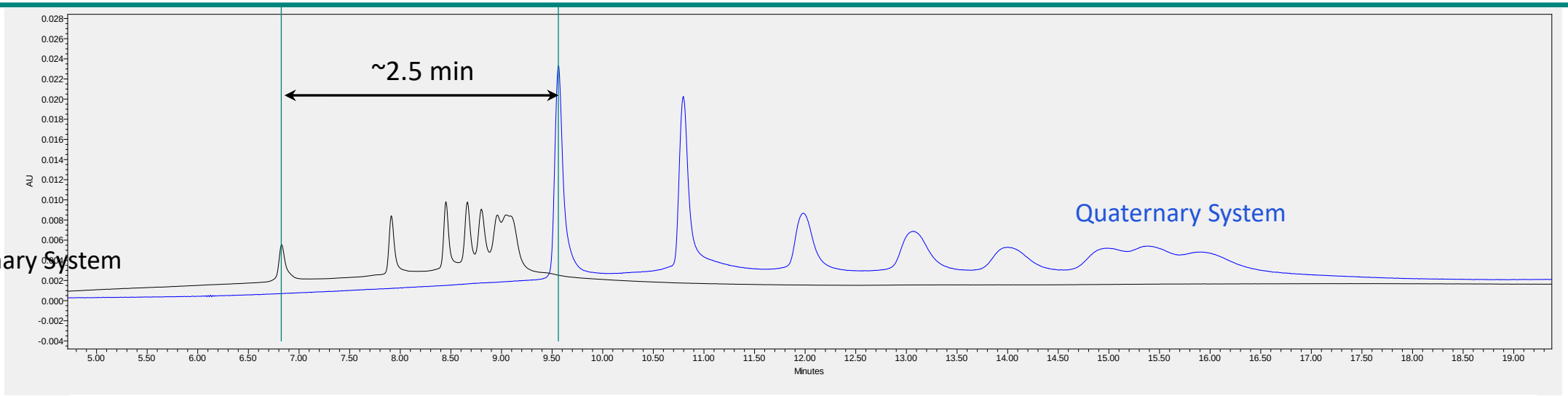


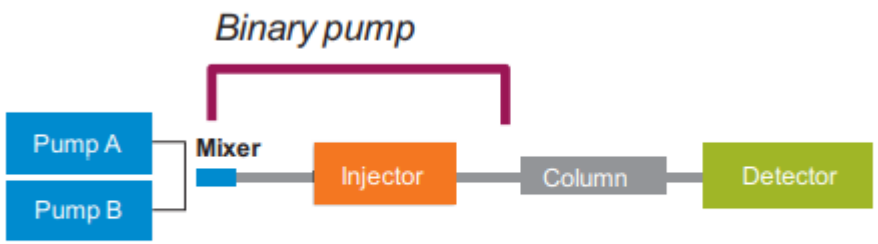
Resolution *vs.* Robustness of MP Preparation

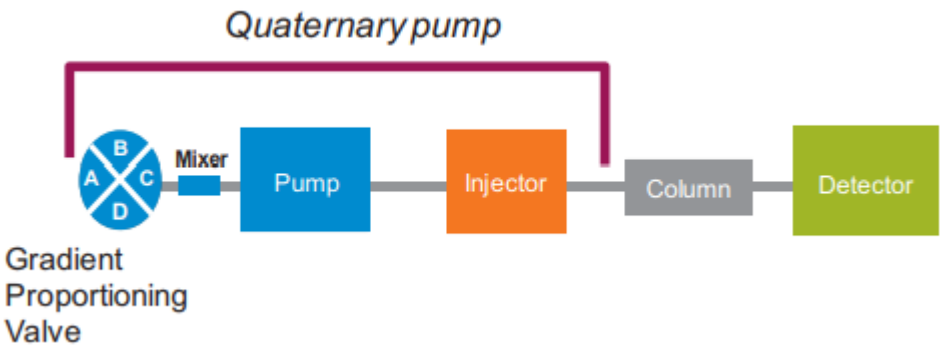
	Name	47.5mM HAA	50mM HAA	52.5mM HAA	79%ACN	80%ACN	81%ACN
1	200 nt	N/A	N/A	N/A	N/A	N/A	N/A
2	500 nt	11.1	11.0	11.0	11.2	11.0	10.8
3	1000 nt	9.1	9.9	9.5	12.0	9.9	9.3
4	1500 nt	5.6	6.0	5.6	6.9	6.0	5.1
5	2000 nt	4.2	3.6	4.1	4.1	3.6	3.5
6	3000 nt	4.1	3.8	4.4	4.0	3.8	3.6
7	4000 nt	2.1	2.1	1.9	2.1	2.1	1.8
8	6000 nt	2.0	1.9	2.2	2.2	1.9	1.8

- Resolution profile is comparable at low range of ladders (<2k nt)
- Resolution profile at higher range (>2k) differs
- Resolution is higher at lower range than higher range

Retention Time Shifting – System Variability

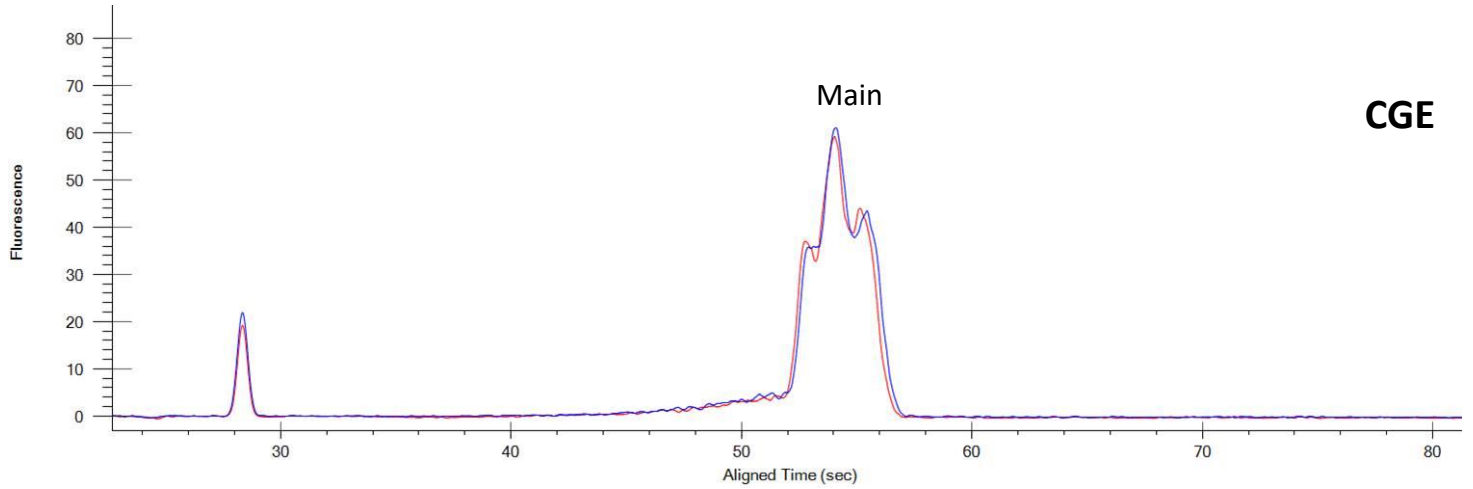


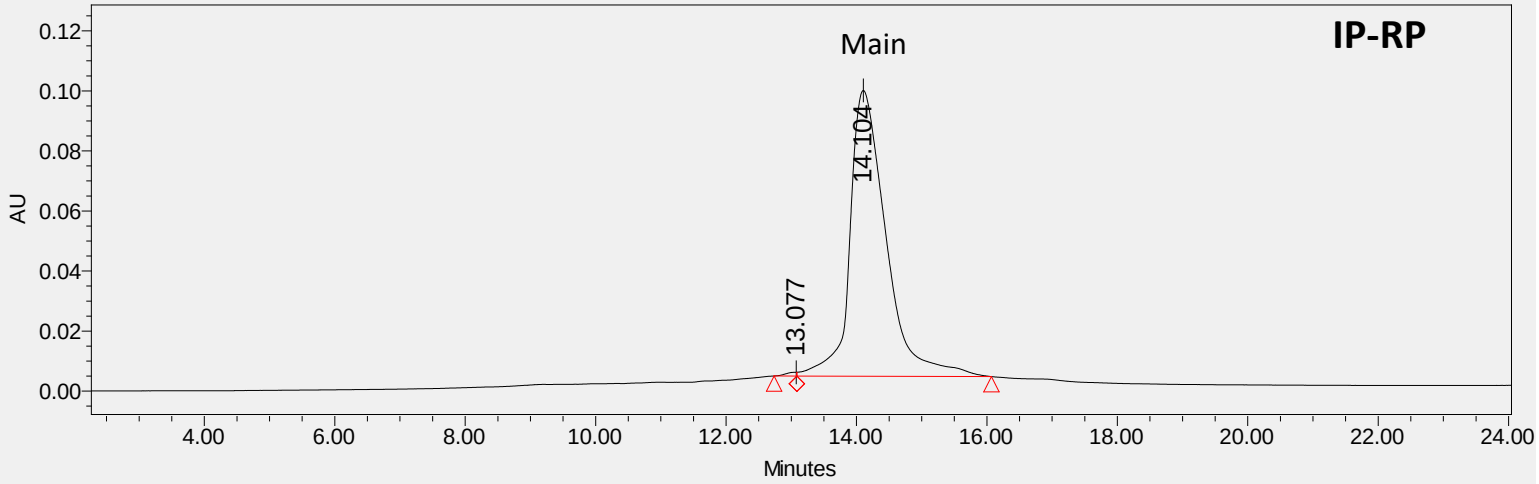




	System Dwell Volume (µL)	Extension Loop (µL)	Total Dwell Volume (µL)
Binary Acquity H Class	75	0	75
Quaternary Acquity H Class	375	100	475

Challenge for Pentavalent Vaccine A on CGE: Split Peaks

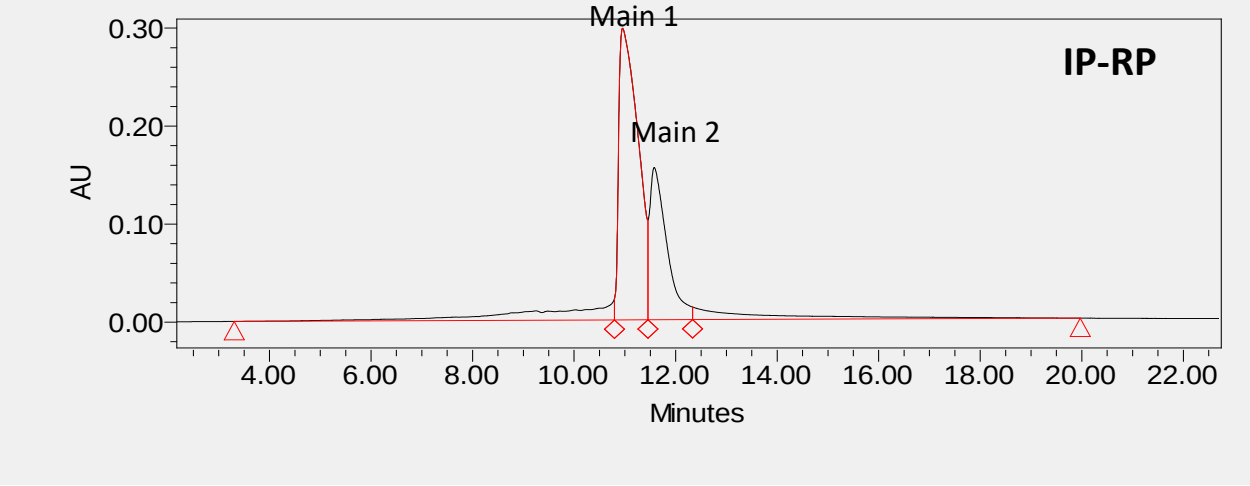


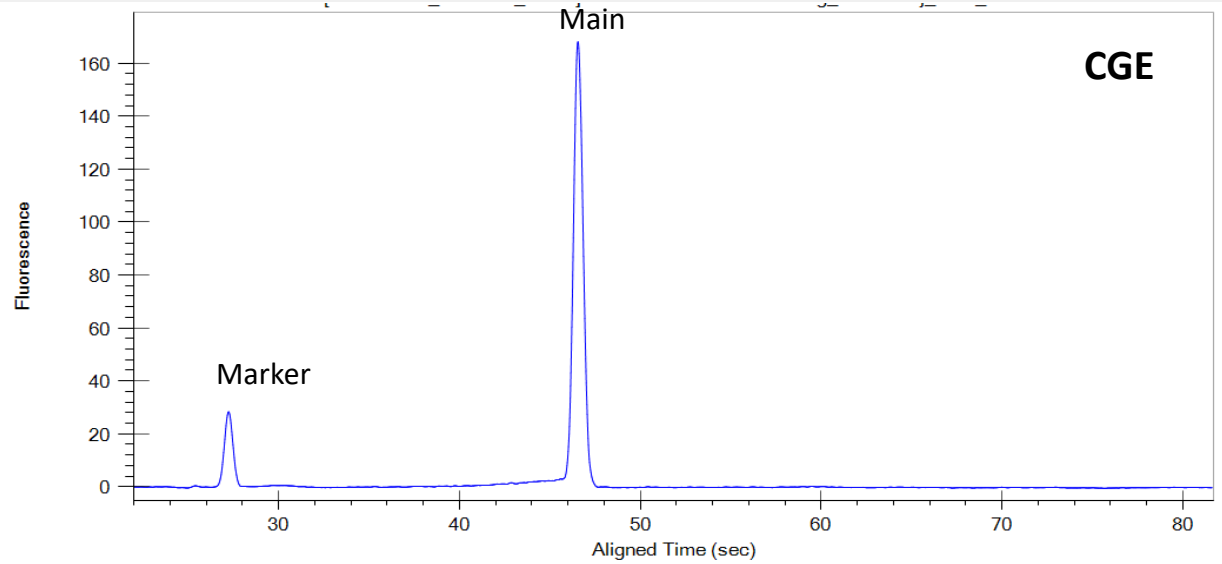


Pentavalent Vaccine A

V1	3.7k nt
V2	4.2k nt
V3	4.3knt
V4	4.4k nt
V5	4.1 nt
Δ (nt)	600

Split Peaks for Pentavalent Vaccine B on IP-RPLC



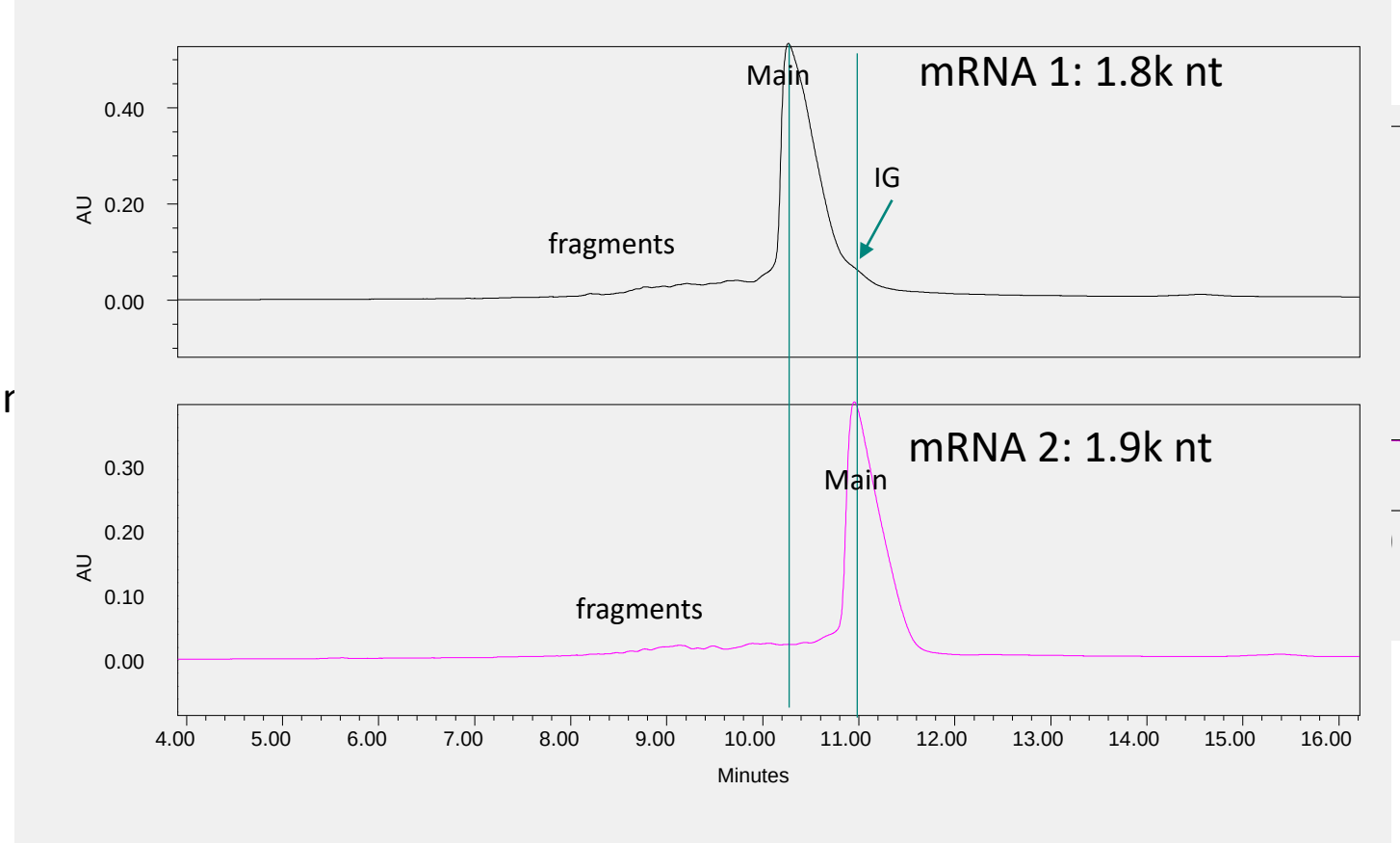


Pentavalent Vaccine B

V1	1.80k nt
V2	1.85k nt
V3	1.90k nt
V4	1.86k nt
V5	1.89k nt
Δ (nt)	100

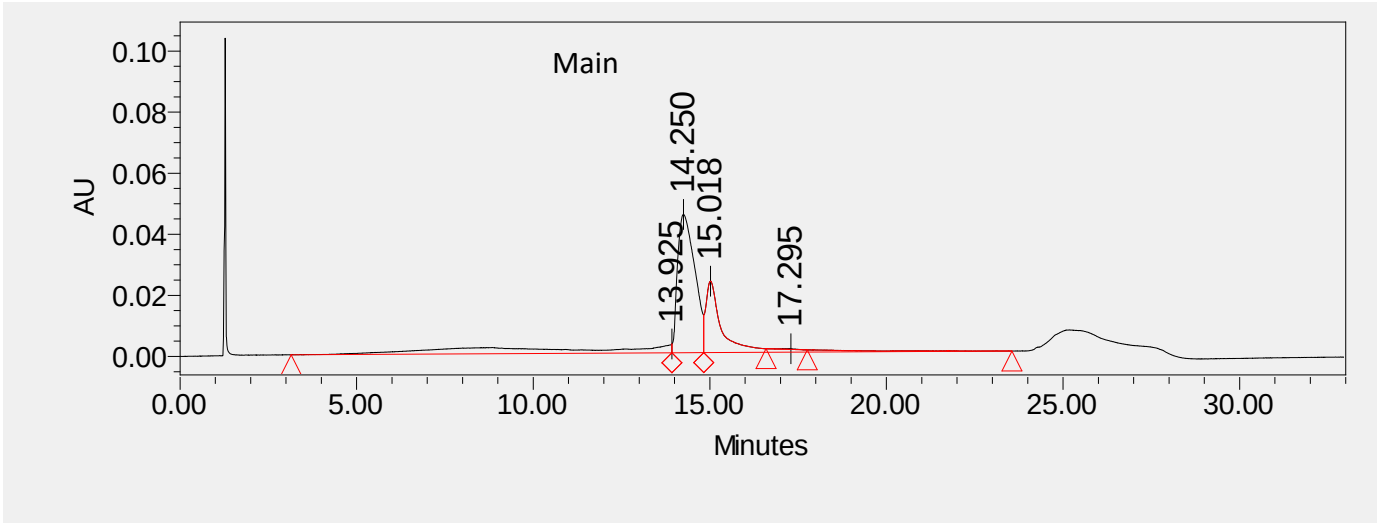
Potential Solution: Decease Resolution to Merge Main Peaks

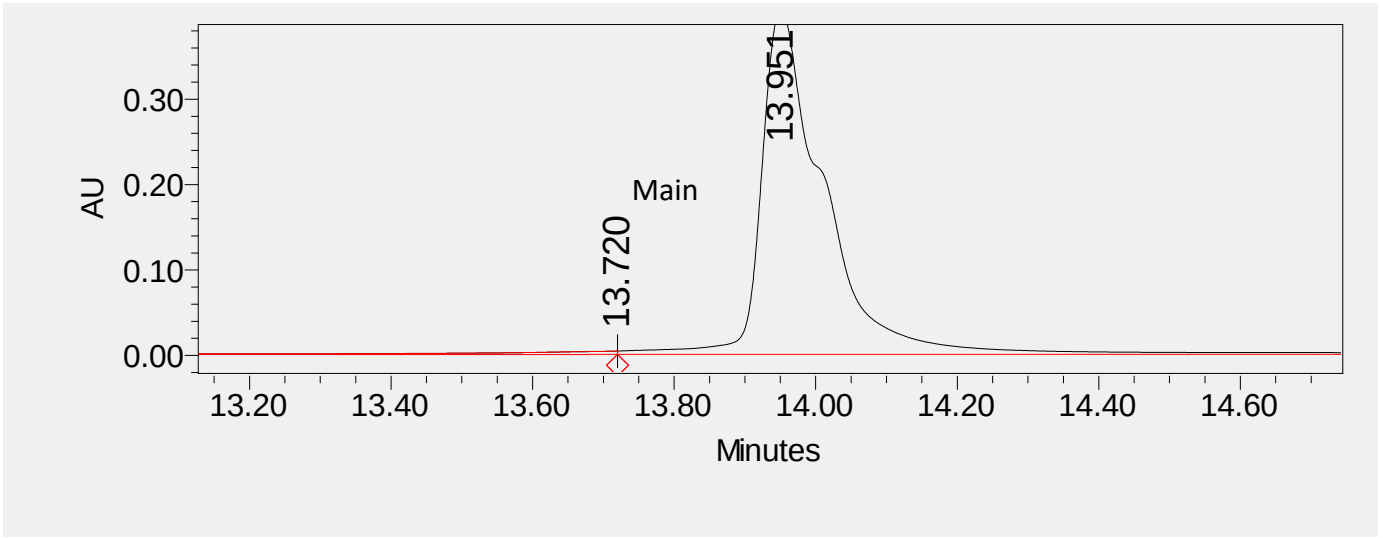
Fragments slide ur



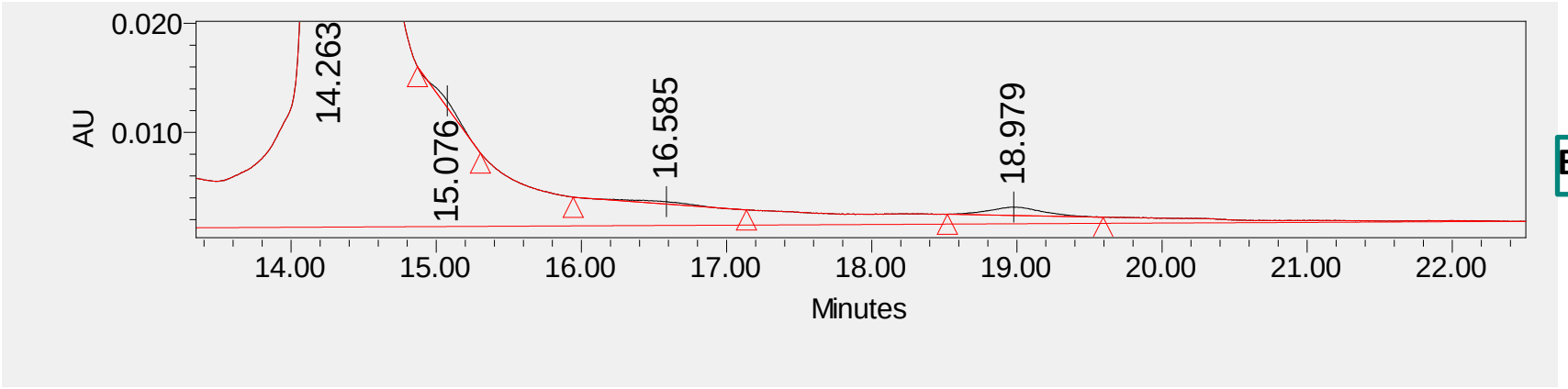
Main Peaks Merged with Shoulder

Rs

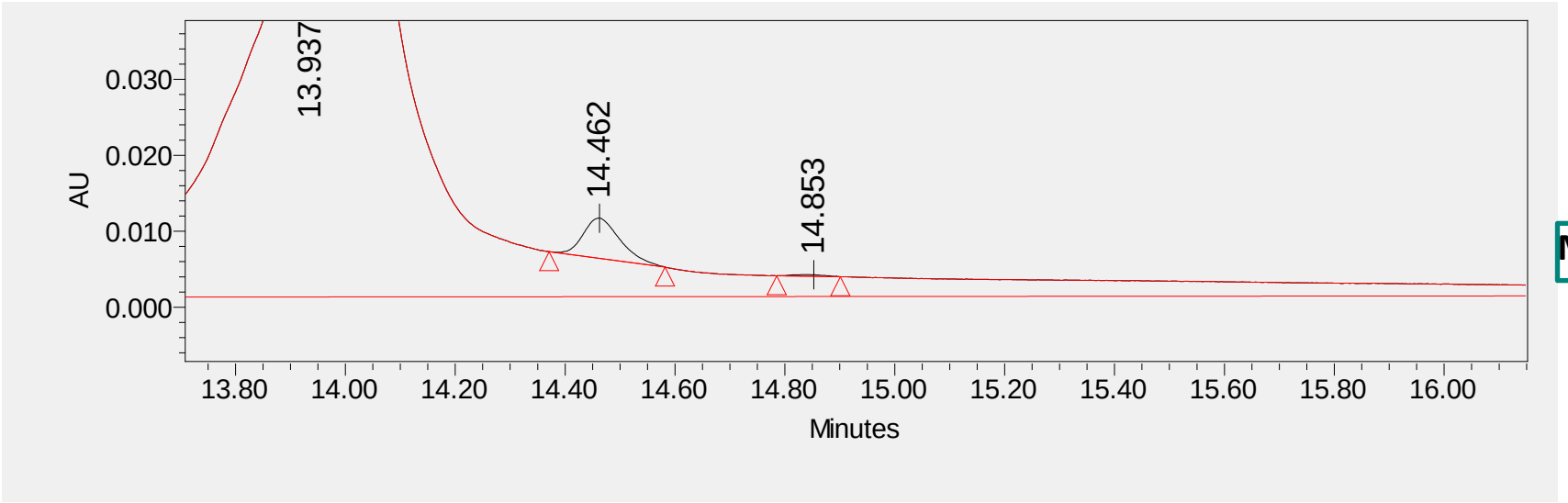




Comparison of IG Separation of the Two Methods



Base Method



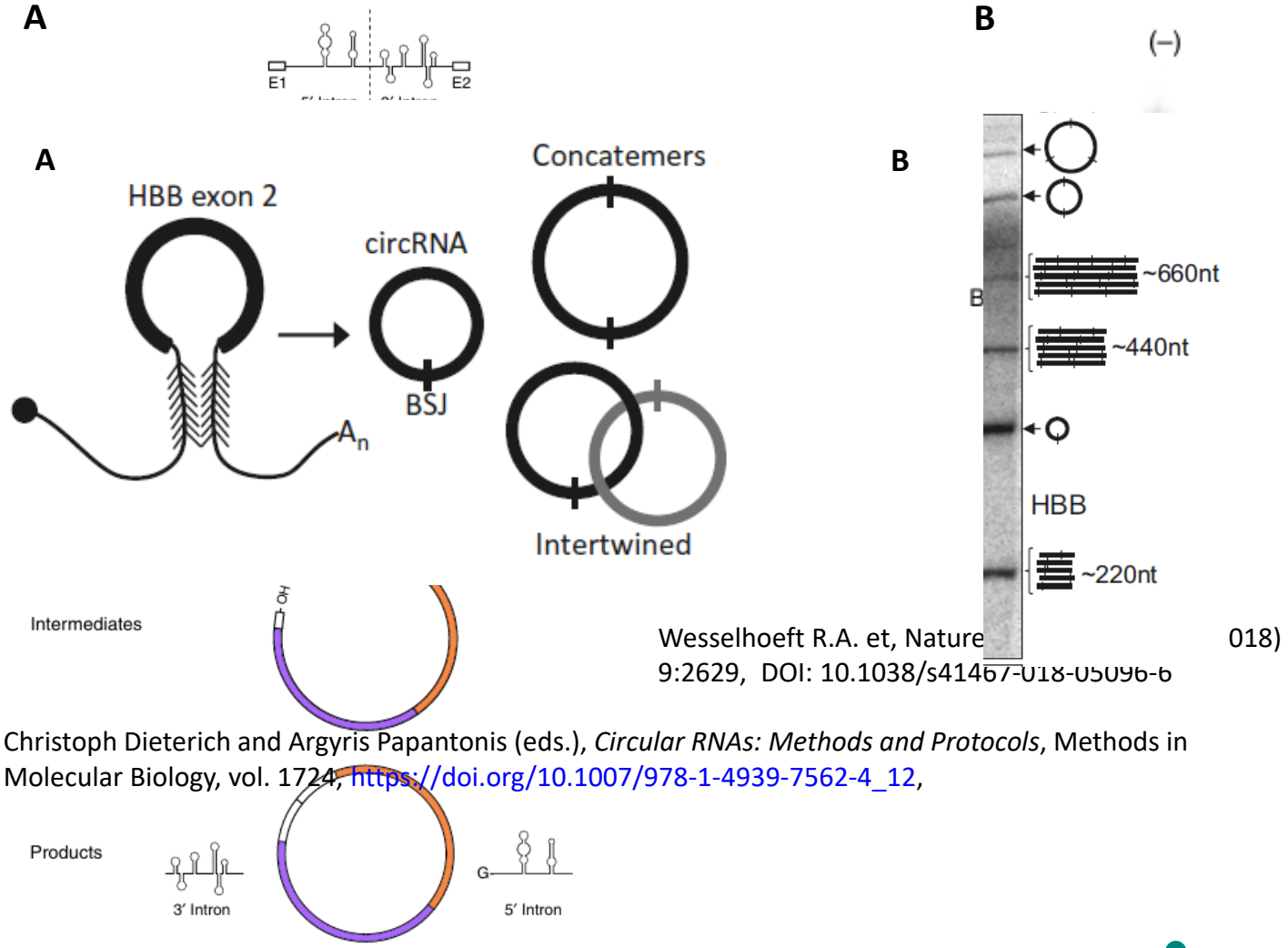
Modified Method

Comparability of Two Methods: DP Samples

	Base Method			Modified Method			Δ (Modified - Base)		
Sample	%Main	%Fragment	%IG	%Main	%Fragment	%IG	%Main	%Fragment	%IG
v1 25C 14D	86.9	12.6	0.5	86.3	12.8	0.9	-0.6	0.2	0.4
v2 25C 14D	85.4	14.3	0.4	85.4	13.9	0.7	0.0	-0.4	0.3
v3 25C 14D	84.1	15.4	0.5	89.0	10.2	0.9	4.8	-5.2	0.4
v4 25C 14D	90.2	9.6	0.2	93.1	6.6	0.3	2.9	-3.0	0.1
v5 25C 14D	83.9	15.2	0.9	88.5	10.8	0.8	4.6	-4.4	-0.1
Calculated Average	**86.1**	**13.4**	**0.5**	88.4	10.9	0.7	2.3	-2.6	0.2
Measured Penta DP	N/A			**88.5**	**10.8**	**0.7**	2.4	-2.7	0.2

Inherent Separation Challenges for Circular RNA Integrity Analysis

- Separation of linear and circular components
- Separation of lipidated RNA component from intact circular component
- Separation of circular peak and concatemer peaks
- Stability of circular component on column



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Christoph Dieterich and Argyris Papantonis (eds.), *Circular RNAs: Methods and Protocols*, Methods in Molecular Biology, vol. 1724, https://doi.org/10.1007/978-1-4939-7562-4_12,

Summary

- Analytical challenges exist to establish IR-RPLC as the platform method for RNA integrity analysis
- Further evaluation of feasibility of IR-PRLC for circRNA is needed
- IP-RPLC method is a feasible method to analyze the fragments, main components and high molecular/hydrophobic components

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