An evaluation of key analytical method parameters relevant to the reliable assessment of mRNA vaccine integrity by CGE-LIF and IP-RPLC

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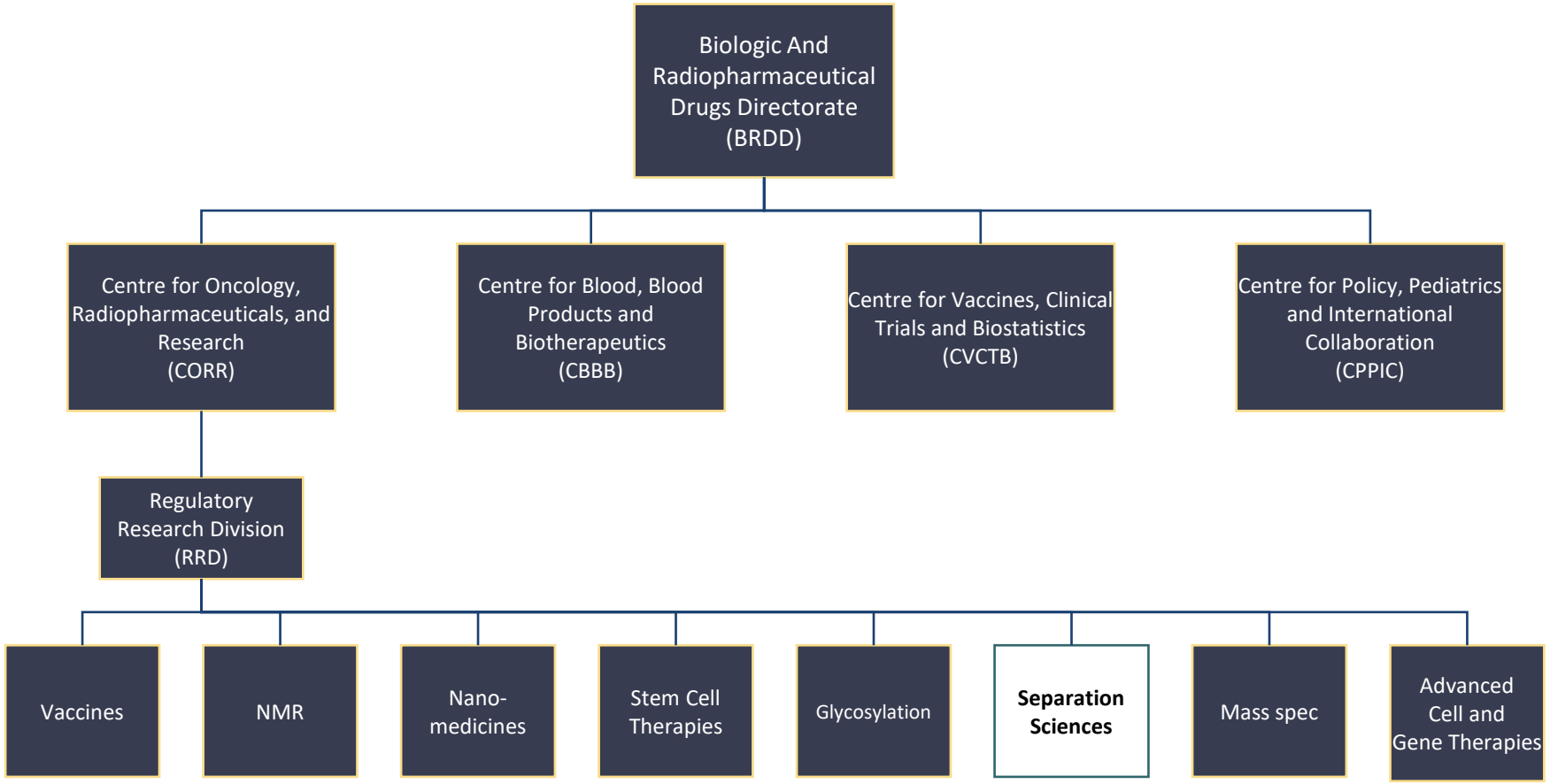
mRNA virtual summit

March 11, 2025

Disclaimers

- The views expressed in this presentation are those of the presenter and do not convey official Health Canada policy.
- The information in this presentation relates to novel research on the characterization of mRNA quality with physicochemical methods from a biologic drug perspective.

Health Canada: Regulatory Research Division



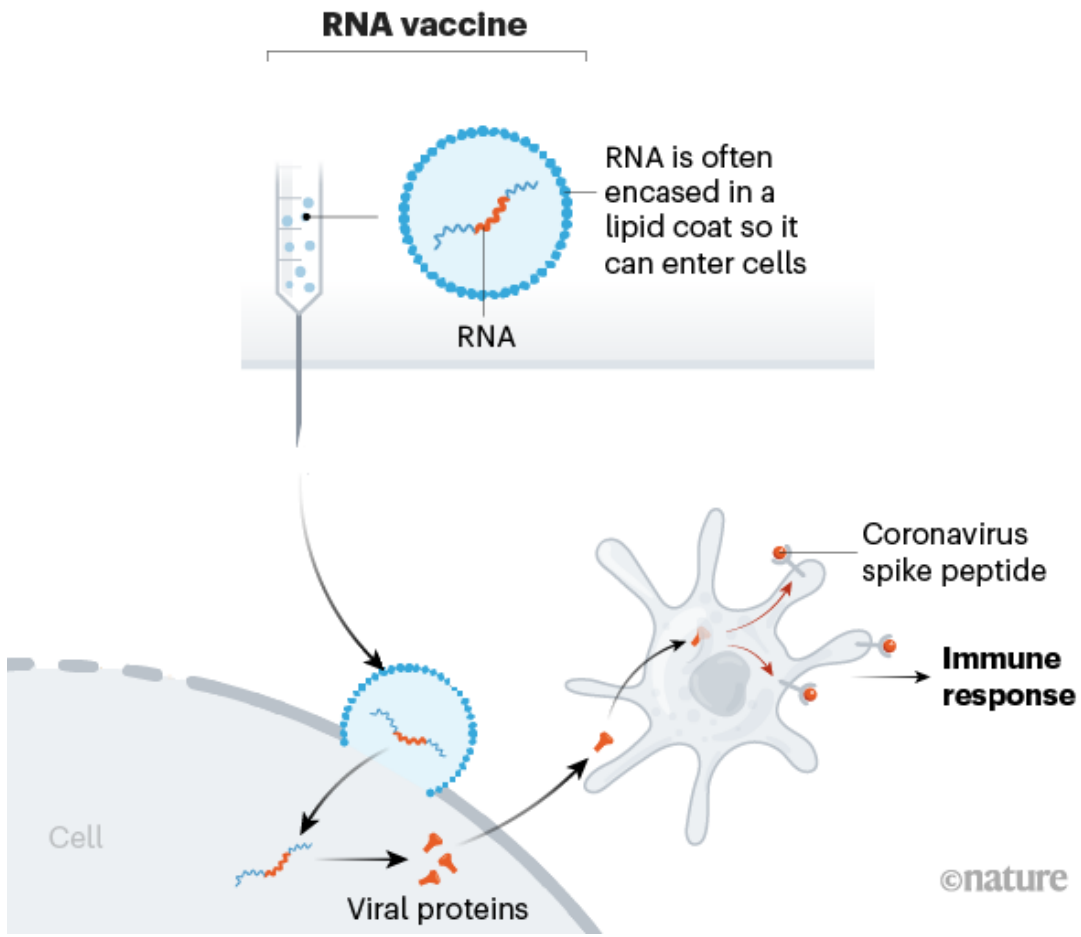
Separation Sciences Lab

- Analyzing peptides, proteins, viruses, nucleic acids with physicochemical approaches:
  - Chromatography
  - Capillary Electrophoresis
  - Mass Spectrometry
- Activities:
  - Pharmacopoeial monograph development.
  - Interlaboratory studies with academia and industry.
  - Regulatory research into assessing critical quality attributes of biotherapeutics.

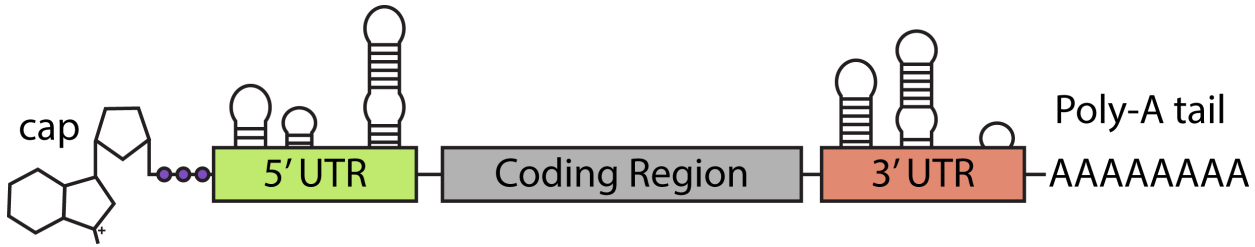
Outline

- Introduction
- Challenges to CGE-LIF
  - Disruption
  - Denaturants
  - Data analysis
- Discussion
- Conclusion

mRNA-LNP vaccines



Modified from Callaway, E. *Nature* 580, 576-577 (2020)

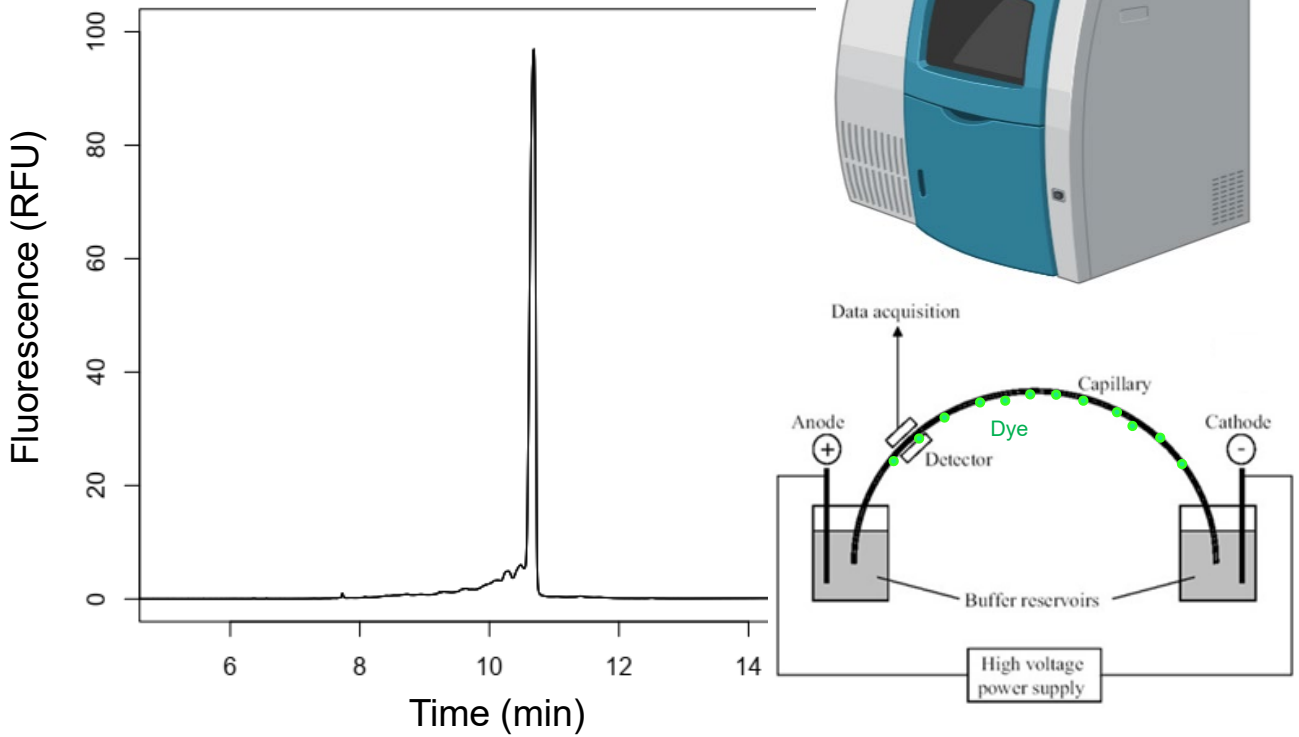


- Large sizes with complex structure.
- Sensitive to environmental degradation.
  - Lipid nanoparticles
- Potency

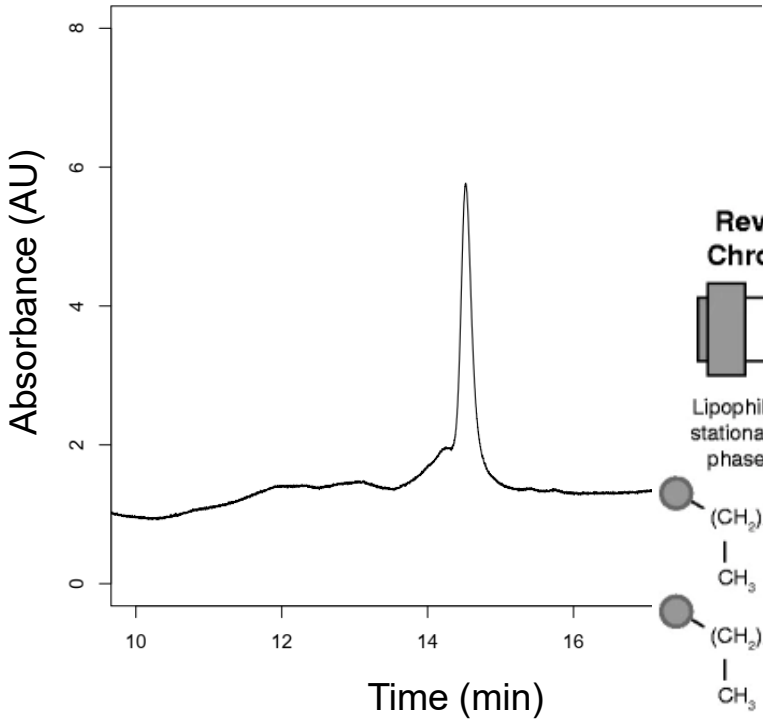
Physicochemical methods in mRNA integrity analysis

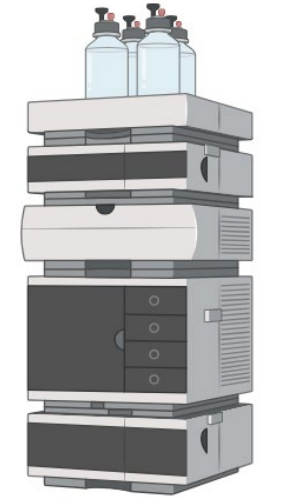
- Capillary gel electrophoresis with laser-induced fluorescence (CGE-LIF)
  - Detection requires dye-binding to mRNA
- Ion-pair reversed-phase liquid chromatography (IP-RPLC)
  - Detection of mRNA absorbance
- Separation by mRNA length.

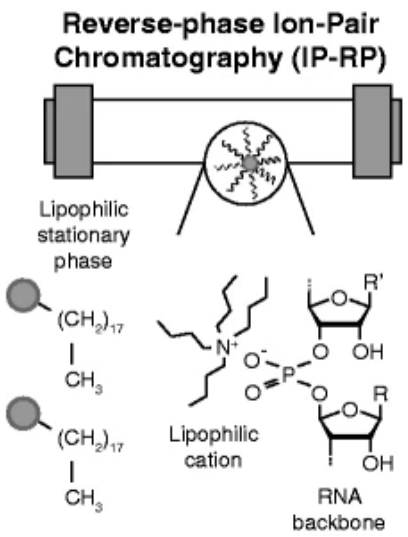
CGE-LIF of an mRNA-LNP



IP-RPLC of an mRNA-LNP







Questions about CGE-LIF during the pandemic

For Research Use Only. Not for Use In Diagnostic Procedures.

Technology

SCIEX

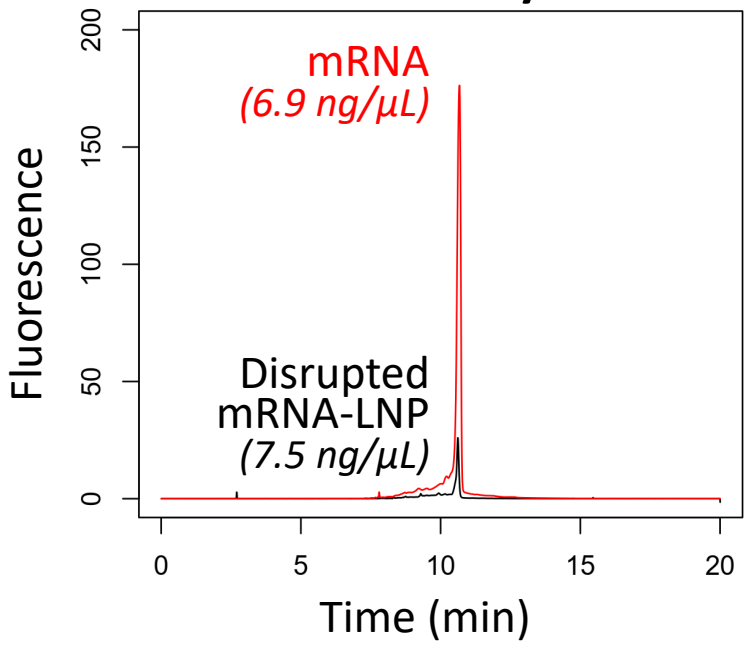
Method Evaluation for RNA Purity Analysis Using CE-LIF Technology

Tingting Li, Mukesh Malik, Handy Yowanto
SCIEX Separations, Brea, CA

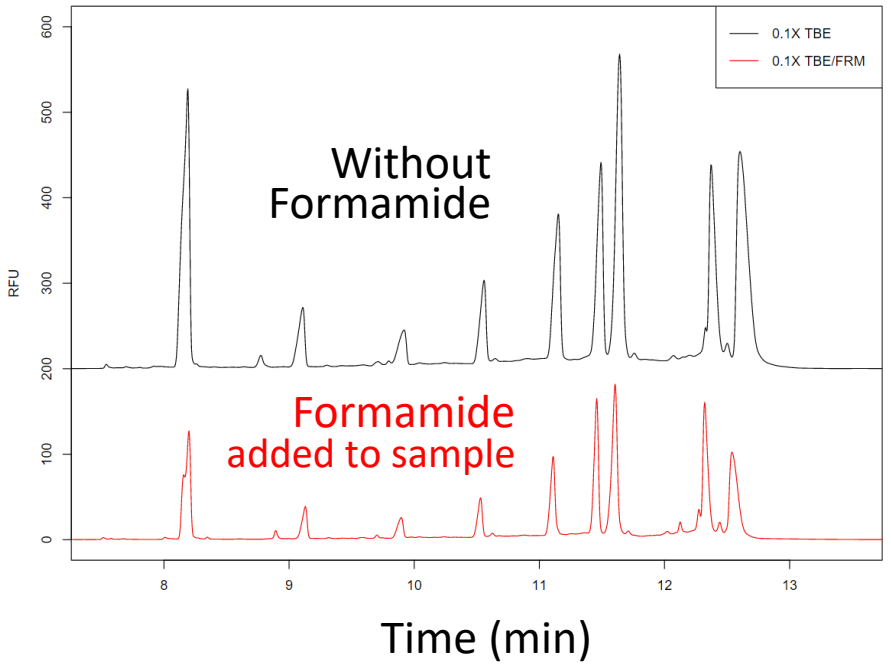
Parameter	Details
Gel buffer composition	1% PVP, 4M urea, 1X TBE
Dye	0.02% (v/v) SYBR Green II
Injection voltage	5.0 KV
Injection time	5 s
Separation voltage	6.0 KV

Sample treatment: formamide, detergent, and heat

Differences between mRNA and mRNA-LNP by CGE-LIF

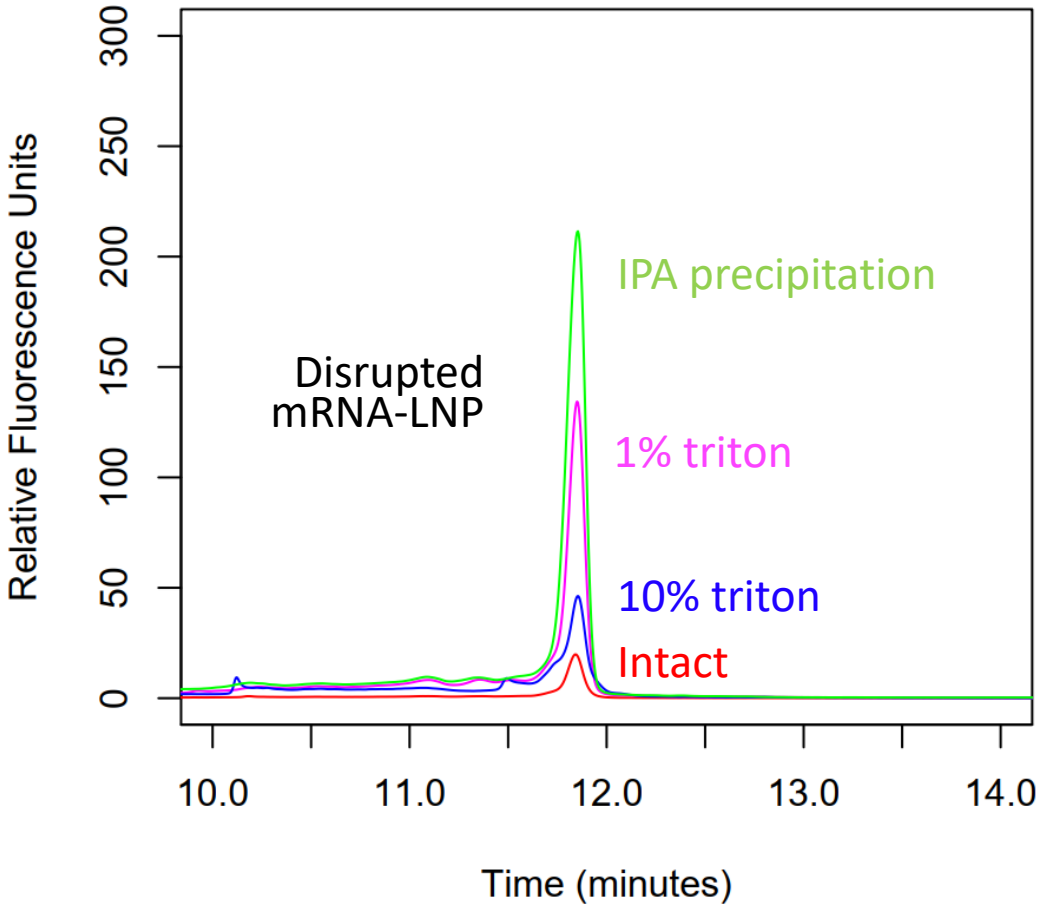


Utility of some reagents in CGE-LIF?

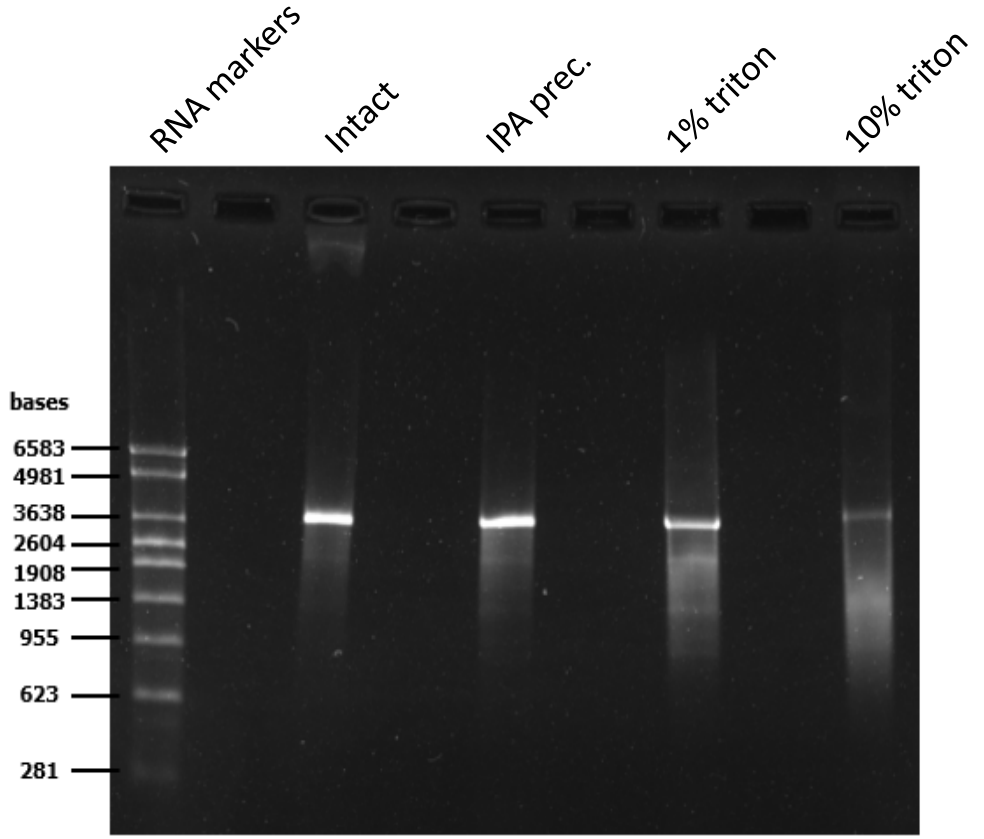


Impact of lipid disruption

CGE-LIF of mRNA-LNP samples treated with different disruption methods

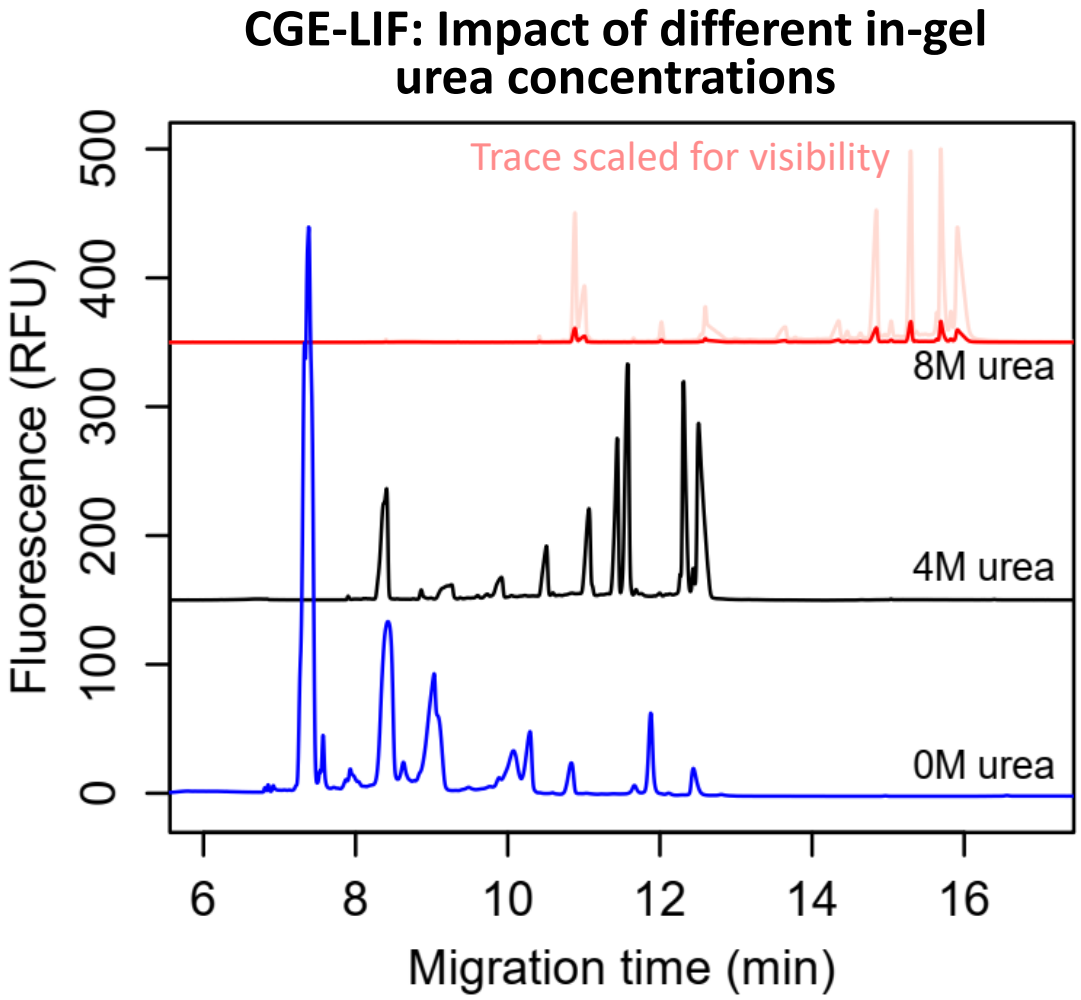


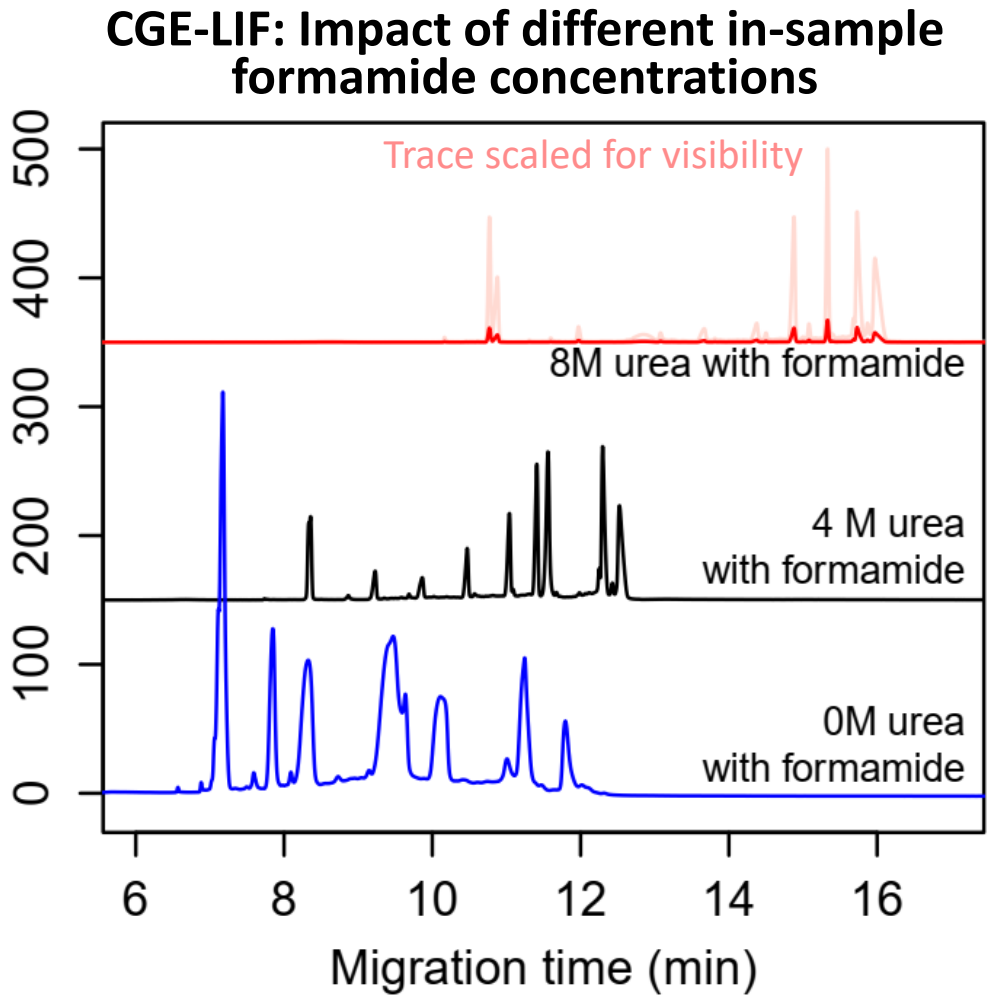
Agarose gel of mRNA-LNP samples treated with different disruption methods



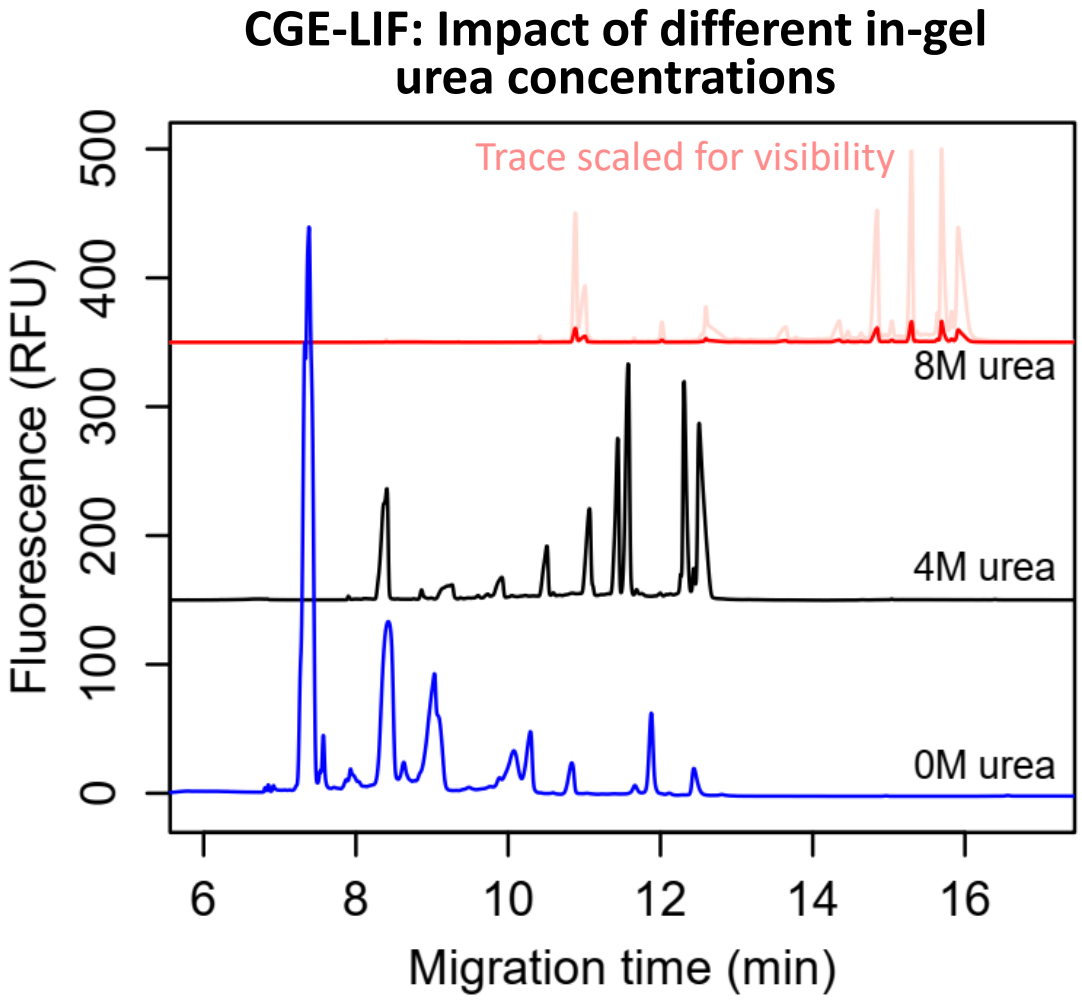
Tran et al., Electrophoresis. 2025.

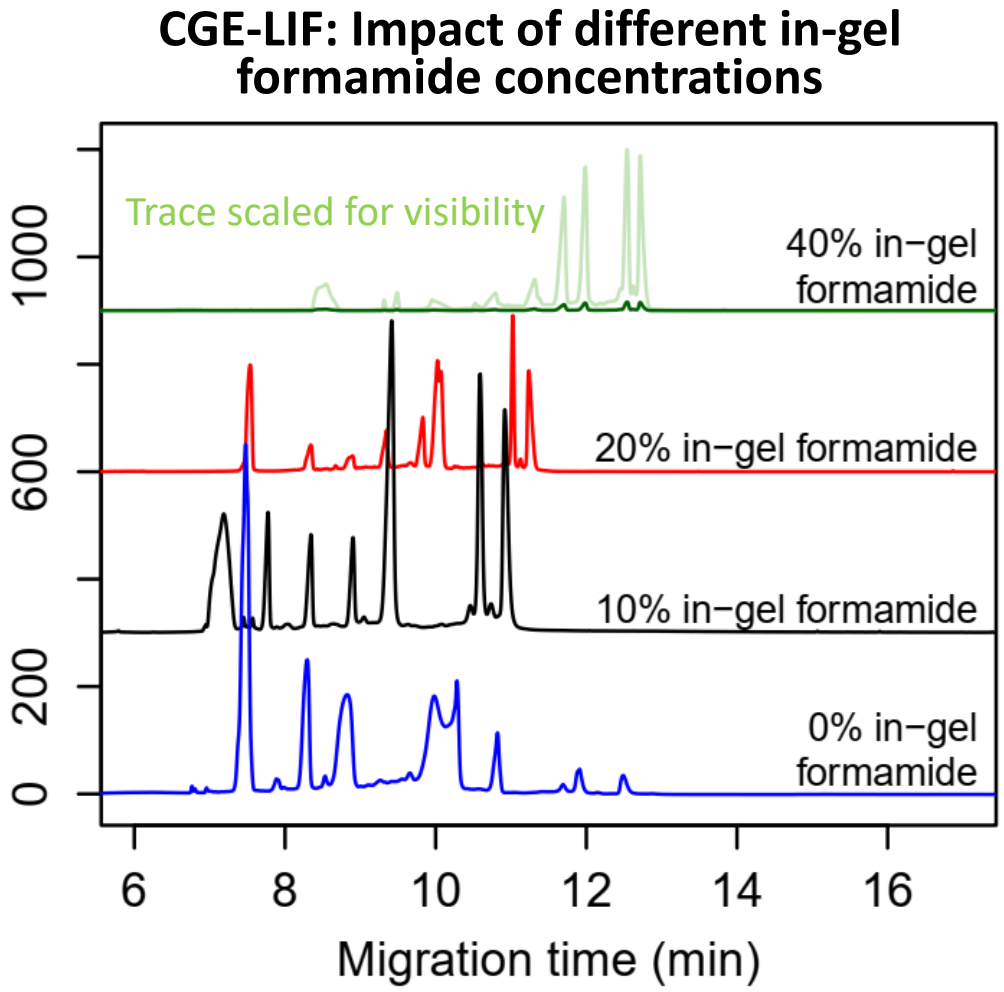
Urea and In-sample Formamide in CGE-LIF





Urea and In-gel Formamide in CGE-LIF



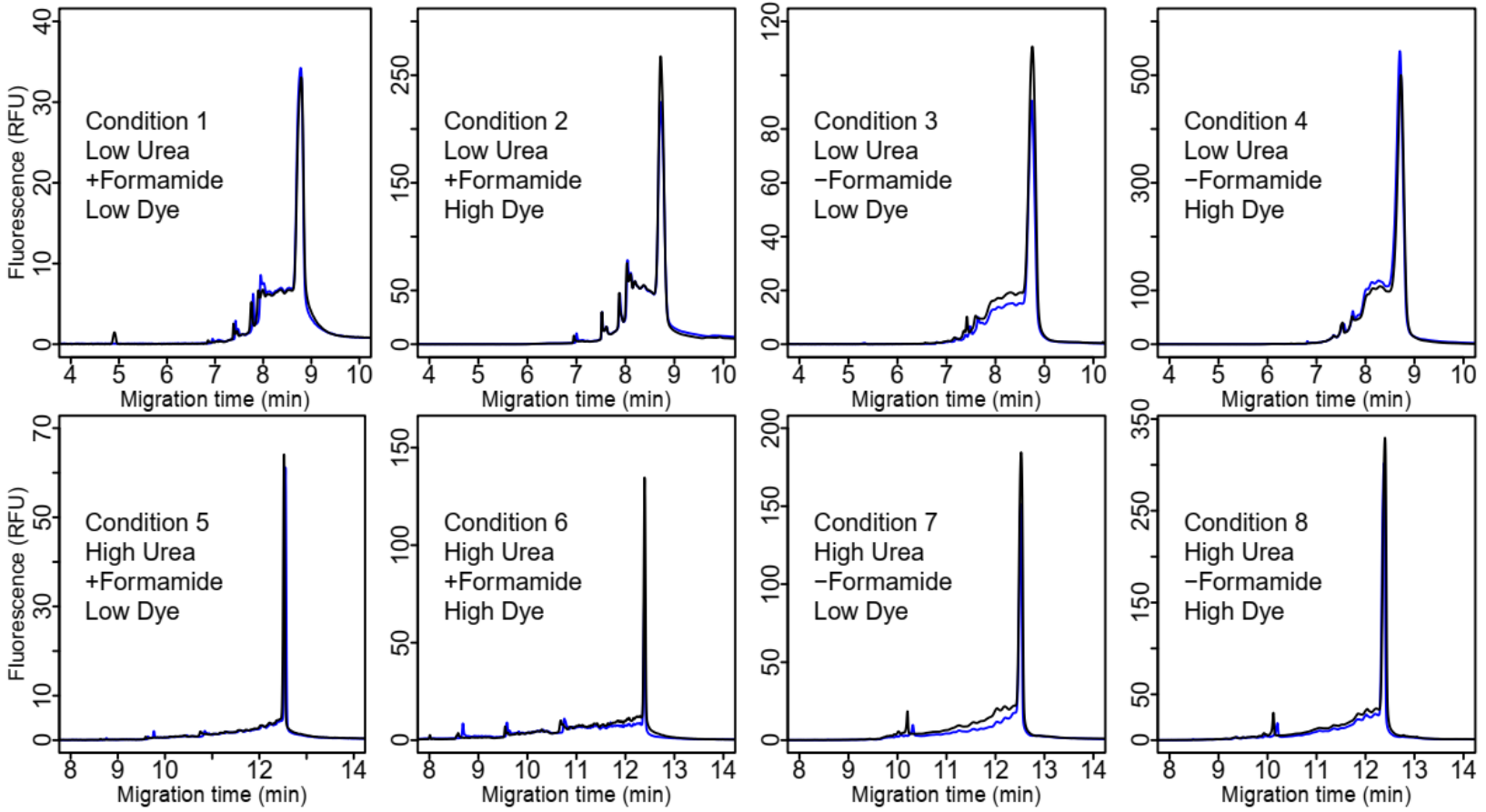


Dye, Formamide and Urea electropherograms

Full factorial design with 3 factors at 2 levels each

No.	[Urea] (M)	Formamide presence	[Dye] (%)
1	Low (2 M)	Yes	Low (0.005%)
2	Low (2 M)	Yes	High (0.04%)
3	Low (2 M)	No	Low (0.005%)
4	Low (2 M)	No	High (0.04%)
5	High (6 M)	Yes	Low (0.005%)
6	High (6 M)	Yes	High (0.04%)
7	High (6 M)	No	Low (0.005%)
8	High (6 M)	No	High (0.04%)

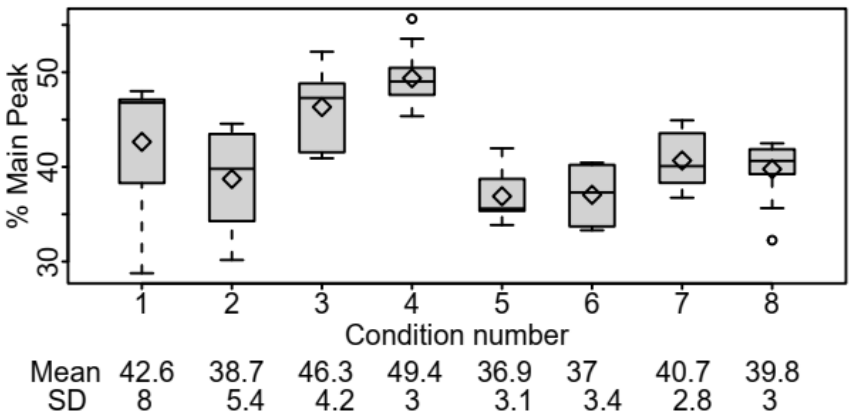
CGE-LIF design of experiments: urea, formamide, dye



Dye, Formamide and Urea integration analysis

Full factorial design with 3 factors at 2 levels each

No.	[Urea] (M)	Formamide presence	[Dye] (%)
1	Low (2 M)	Yes	Low (0.005%)
2	Low (2 M)	Yes	High (0.04%)
3	Low (2 M)	No	Low (0.005%)
4	Low (2 M)	No	High (0.04%)
5	High (6 M)	Yes	Low (0.005%)
6	High (6 M)	Yes	High (0.04%)
7	High (6 M)	No	Low (0.005%)
8	High (6 M)	No	High (0.04%)



GLMSELECT-StepWise (% Main Peak)

Parameter	DF	Estimate	Standard Error	t Value	Pr > |t|
Intercept	1	39.516230	0.603920	65.43	<.0001
urea_conc*formamide 2 0	1	8.471641	1.122470	7.55	<.0001

GLMSELECT-StepWise (% Shoulder)

Parameter	DF	Estimate	Standard Error	t Value	Pr > |t|
Intercept	1	55.647895	0.698129	79.71	<.0001
urea_conc 2	1	-6.964474	0.987303	-7.05	<.0001

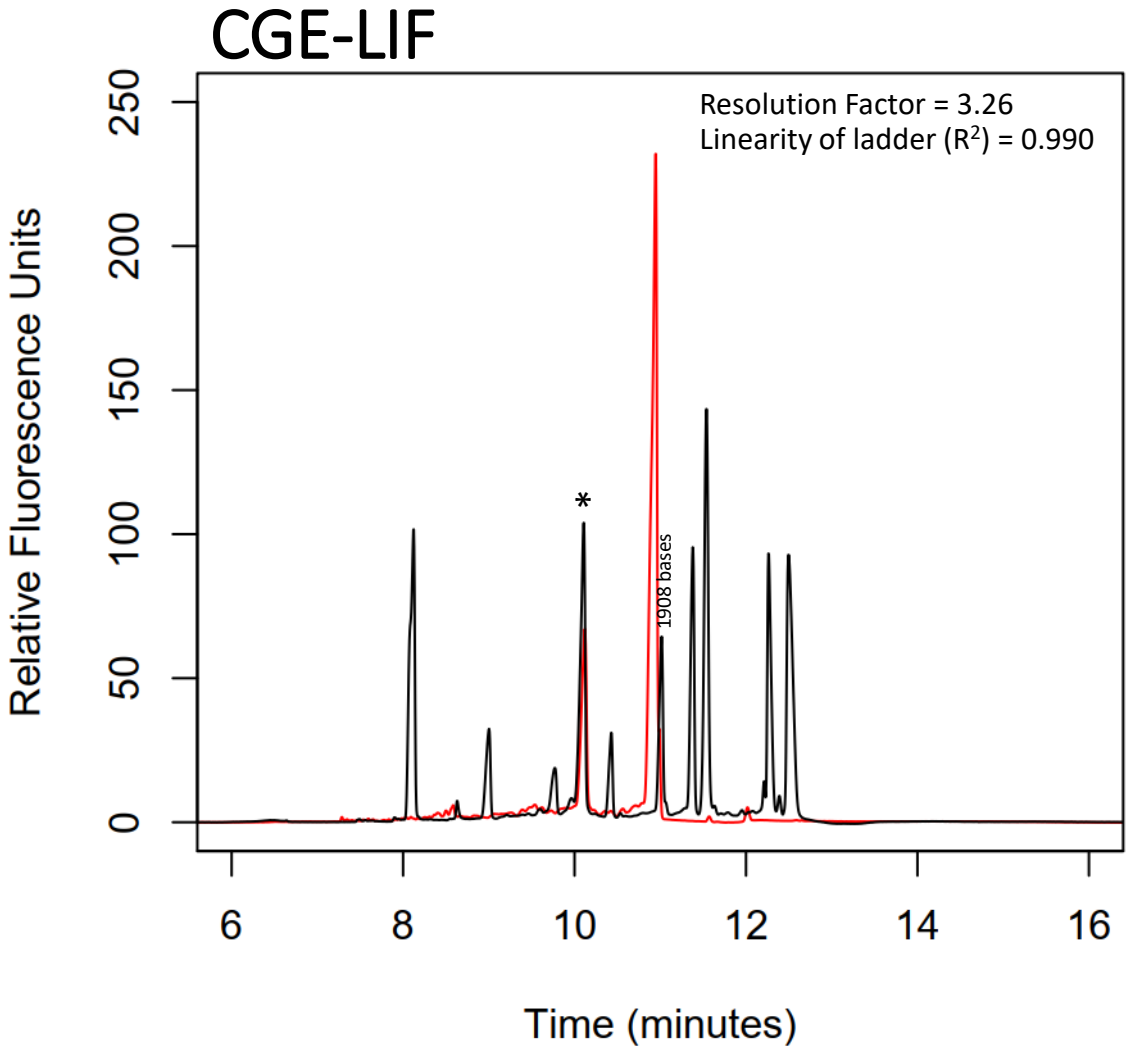
GLMSELECT-StepWise (% LMS)

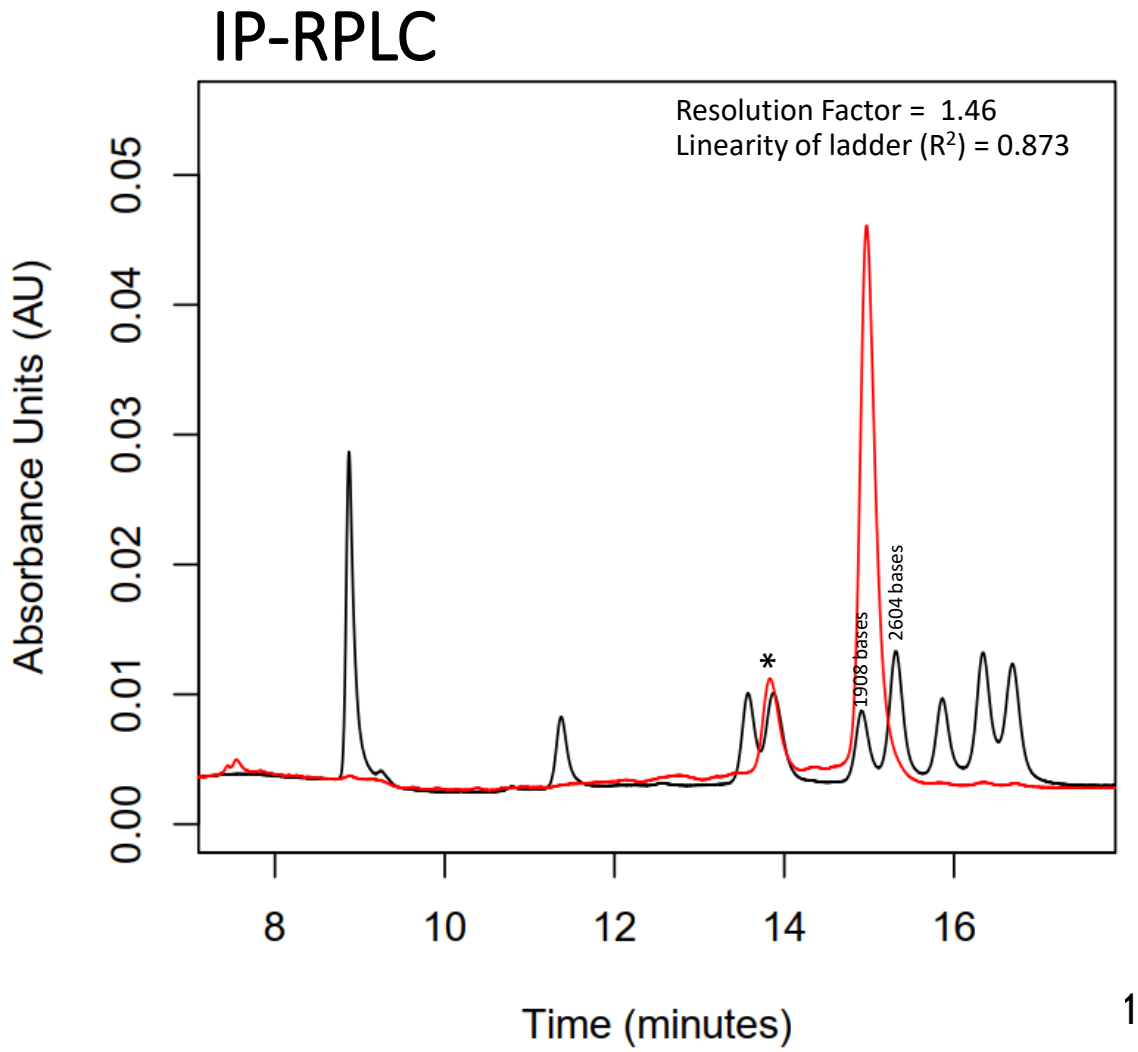
Parameter	DF	Estimate	Standard Error	t Value	Pr > |t|
Intercept	1	9.188667	0.668824	13.74	<.0001
formamide 0	1	-5.490841	0.859686	-6.39	<.0001

GLMSELECT-StepWise (width at half-height)

Parameter	DF	Estimate	Standard Error	t Value	Pr > |t|
Intercept	1	0.072266	0.009969	7.25	<.0001
urea_conc 2	1	0.230840	0.010416	22.16	<.0001
formamide 0	1	0.038103	0.010655	3.58	0.0006

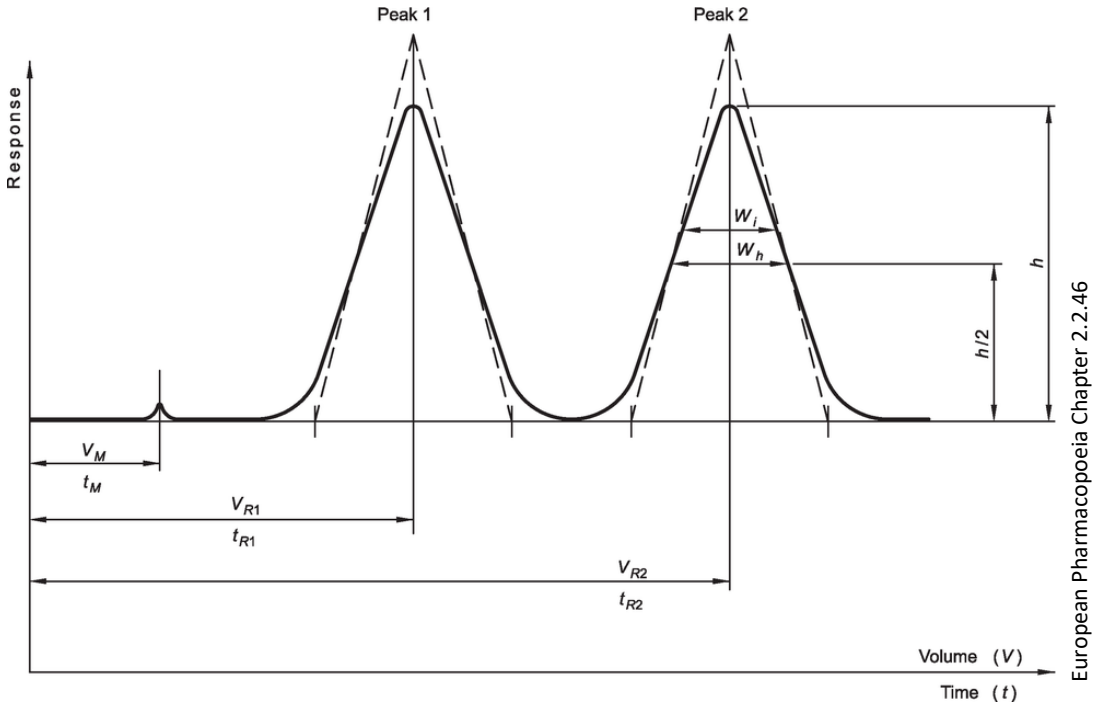
Resolution: CGE-LIF and IP-RPLC





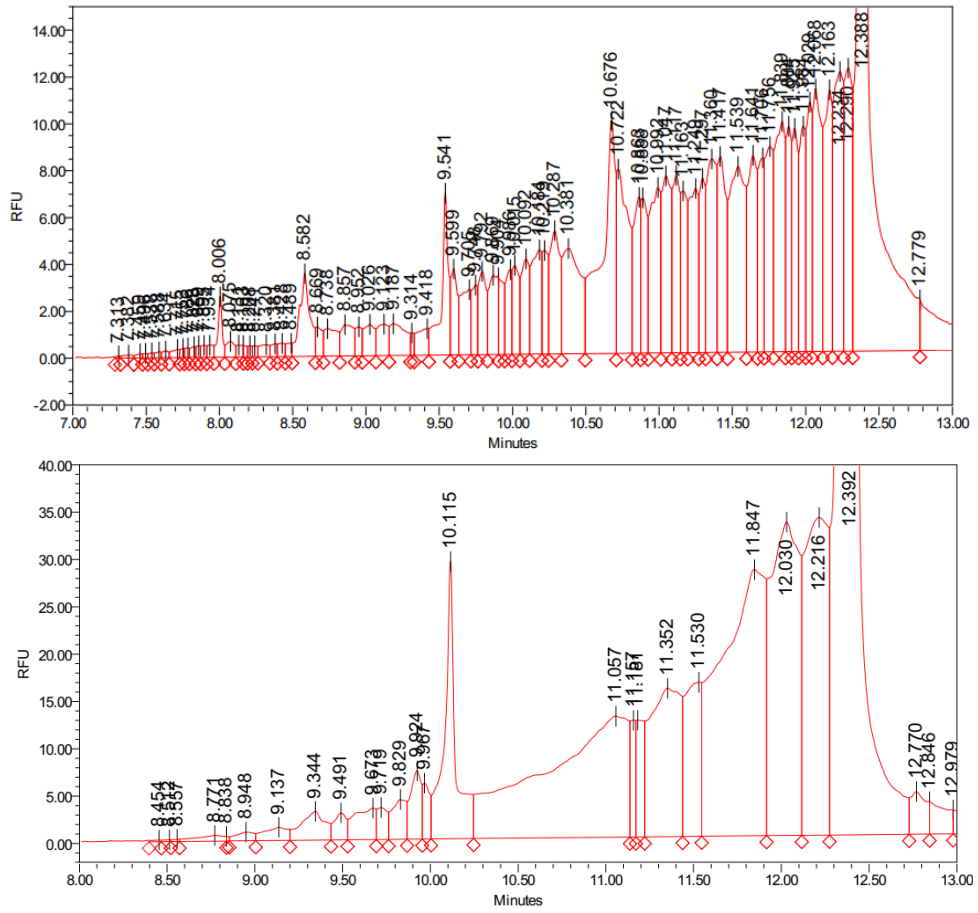
Discussion

Controlling for Resolution?



European Pharmacopoeia Chapter 2.2.46

Velocity-corrected averages?



Conclusions and lessons learned

- Important to consider sample matrix effects during CGE-LIF method development
  - Encapsulation and naked mRNA
  - In-process intermediates
- The type of denaturant and how it's used can affect separation
  - Urea concentration needs to be tightly controlled
  - Formamide needs to be in-gel to effectively act as a denaturant
- Standardizing data analysis
  - Incorporating resolution specifications into system suitability tests for IP-RPLC and CGE-LIF
  - Broader CE community discussion on integration standardization

Acknowledgements

Health Canada
- Jessica Tran
- Jun Gao
- Barry Lorbetskie
- Dr. Yun Wang
- Dr. Simon Sauve
- Dr. Michel Girard
- Casey Lansdell
- Dr. Michael Johnston
- Dr. Michael Wall