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Analysis of Glycosylated Type II Interleukin-1 Receptor (IL-1R) by Imaged Capillary Isoelectric Focusing (i-clEF)

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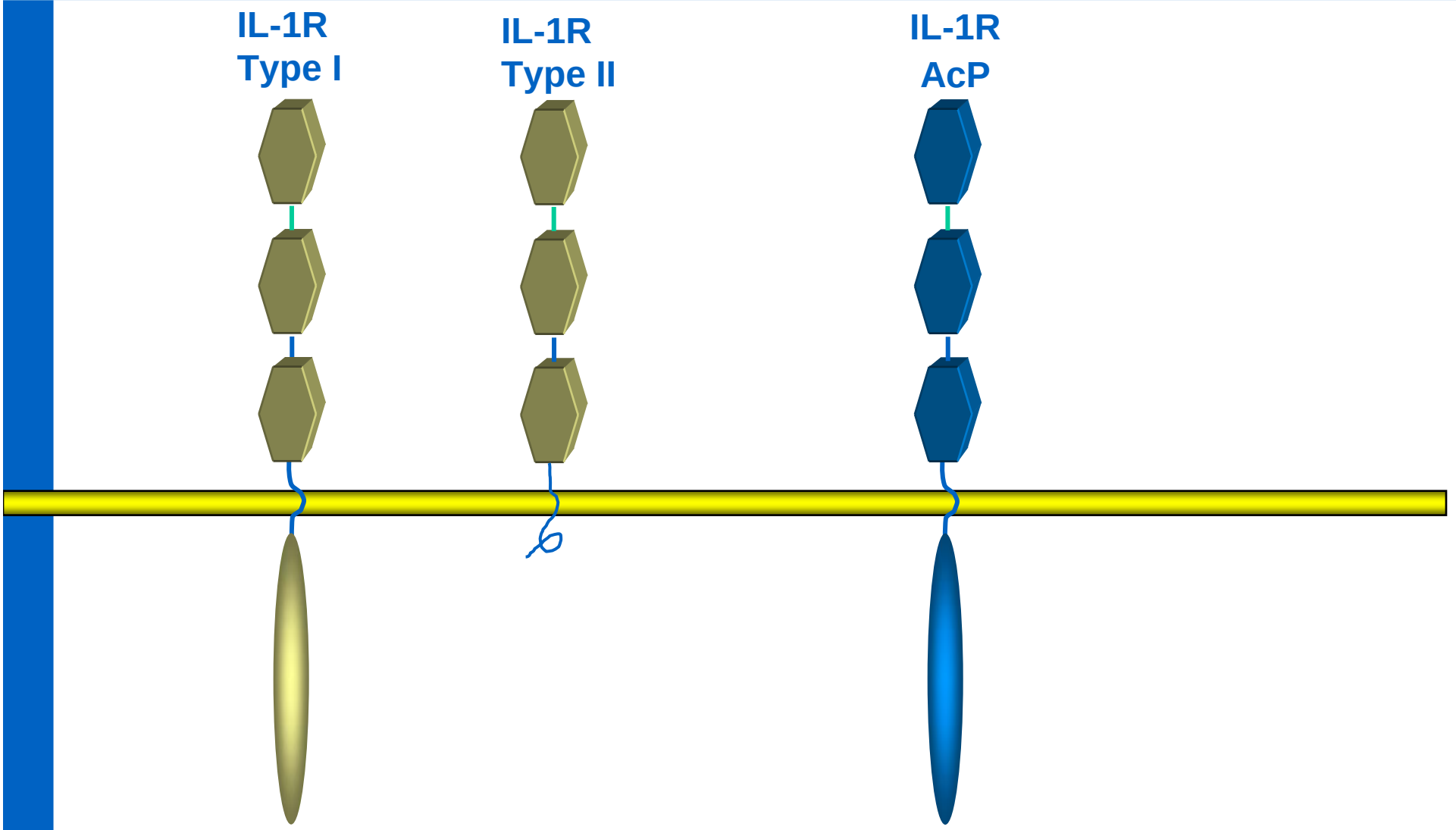
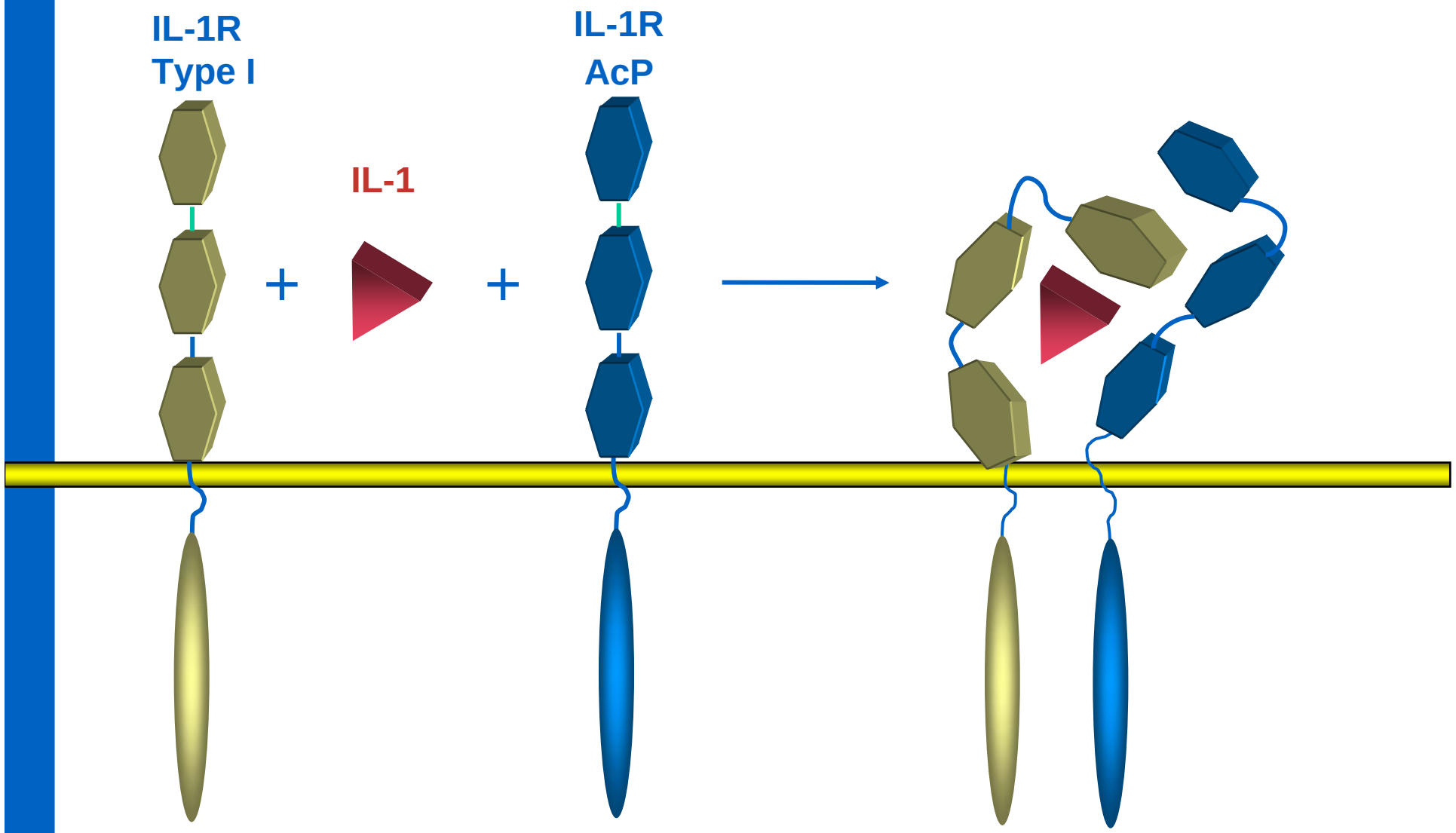
Overview

- Introduction
- i-cIEF Method Development
- i-cIEF Applications

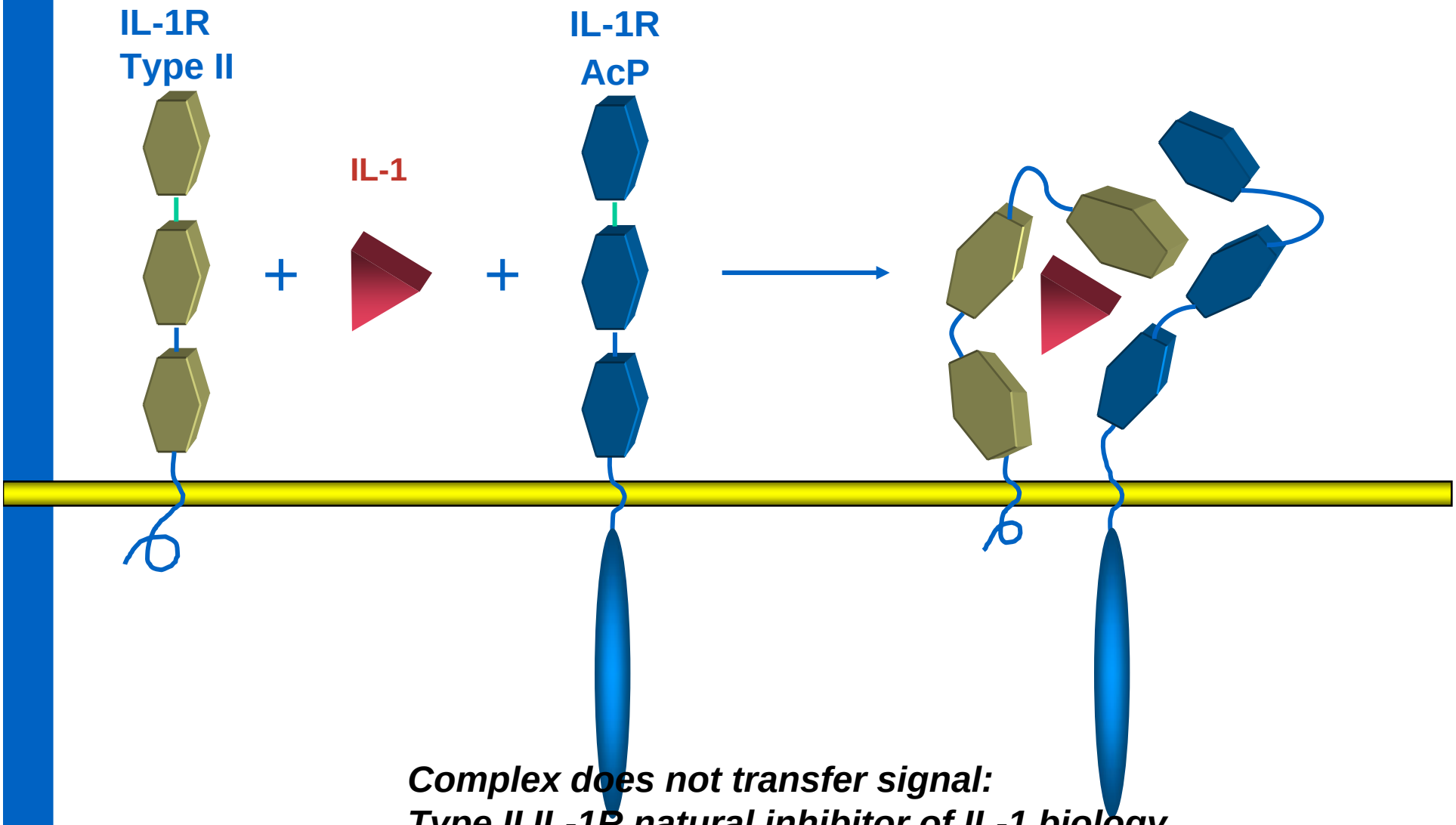
IL-1 Receptors



IL-1 Binding

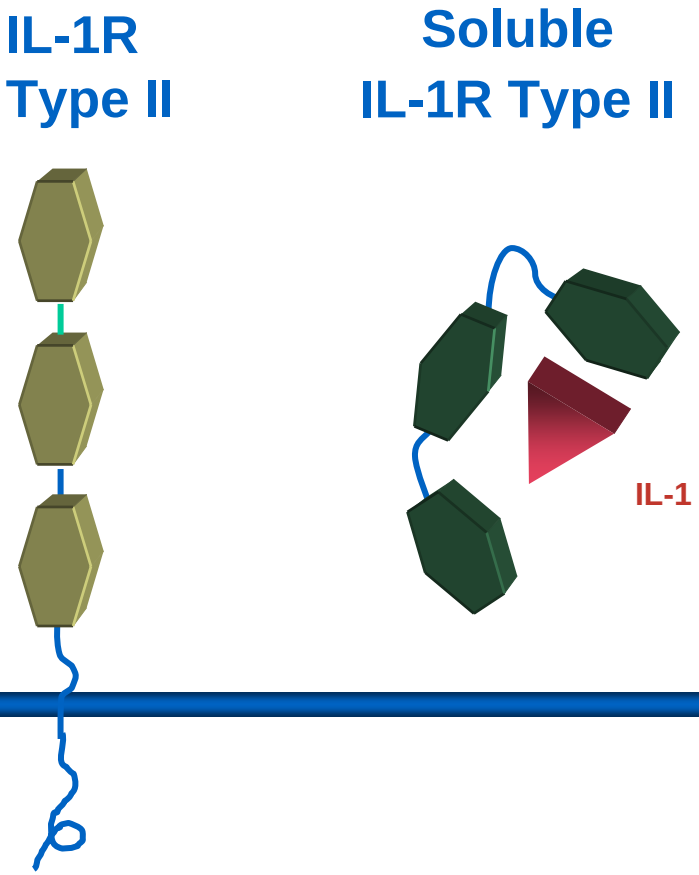


IL-1 Receptor Type II is a Decoy Receptor



Complex does not transfer signal:
Type II IL-1R natural inhibitor of IL-1 biology

Soluble IL-1 Receptor Type II



Main indication: rheumatoid arthritis (RA)

IL-1R Type II Molecule

- CHO expressed IL-1R II
- 333 Amino acids
- Peptide mass = 38,031 Da
- 4 disulfide bridges
- 5 Potential N-glycosylation sites
- Theoretical pI = 6.77
- Experimental pI range: 4.0-6.6
- Monomer in solution
- Glycoprotein mass = 46,520 Da

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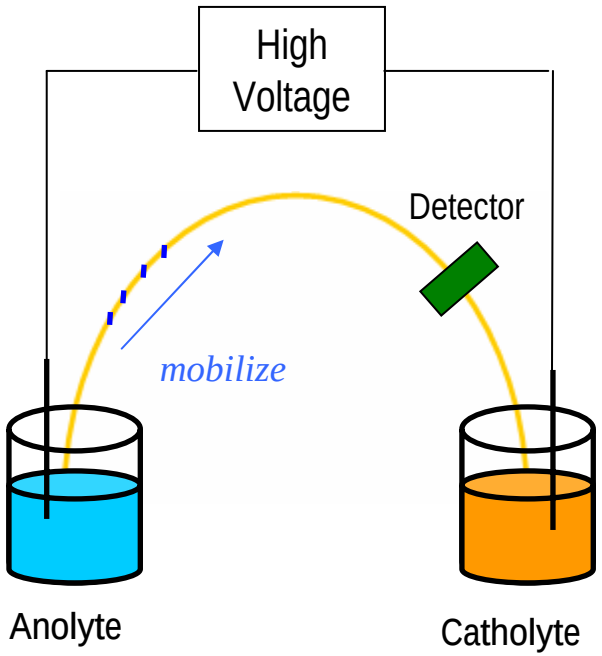
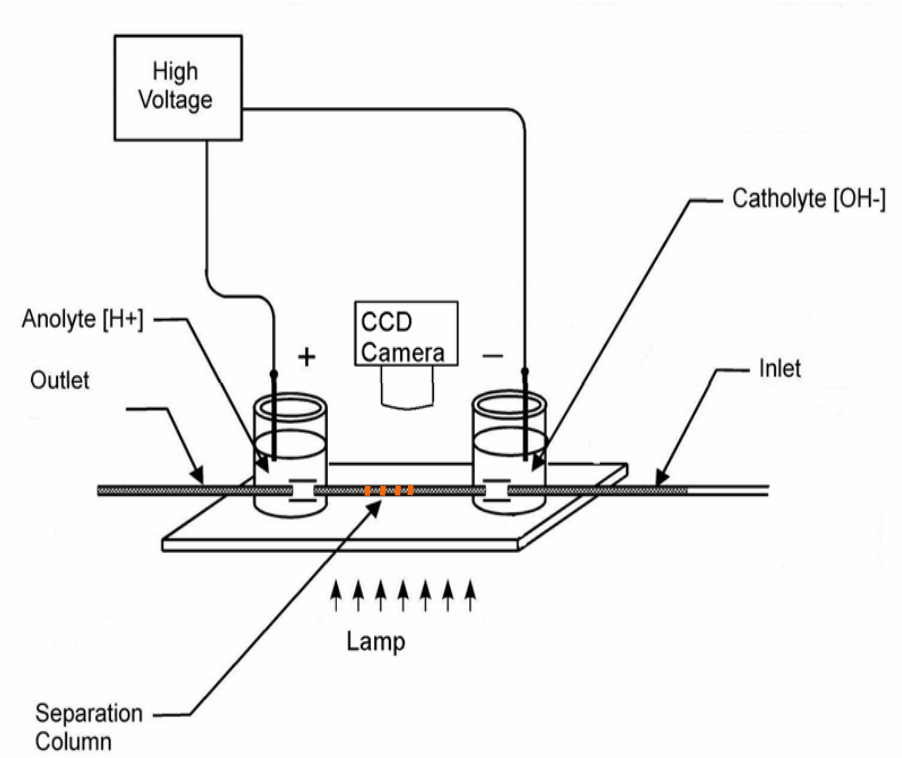
Imaged cIEF vs. Conventional cIEF

Conventional cIEF



Imaged cIEF (i-cIEF)



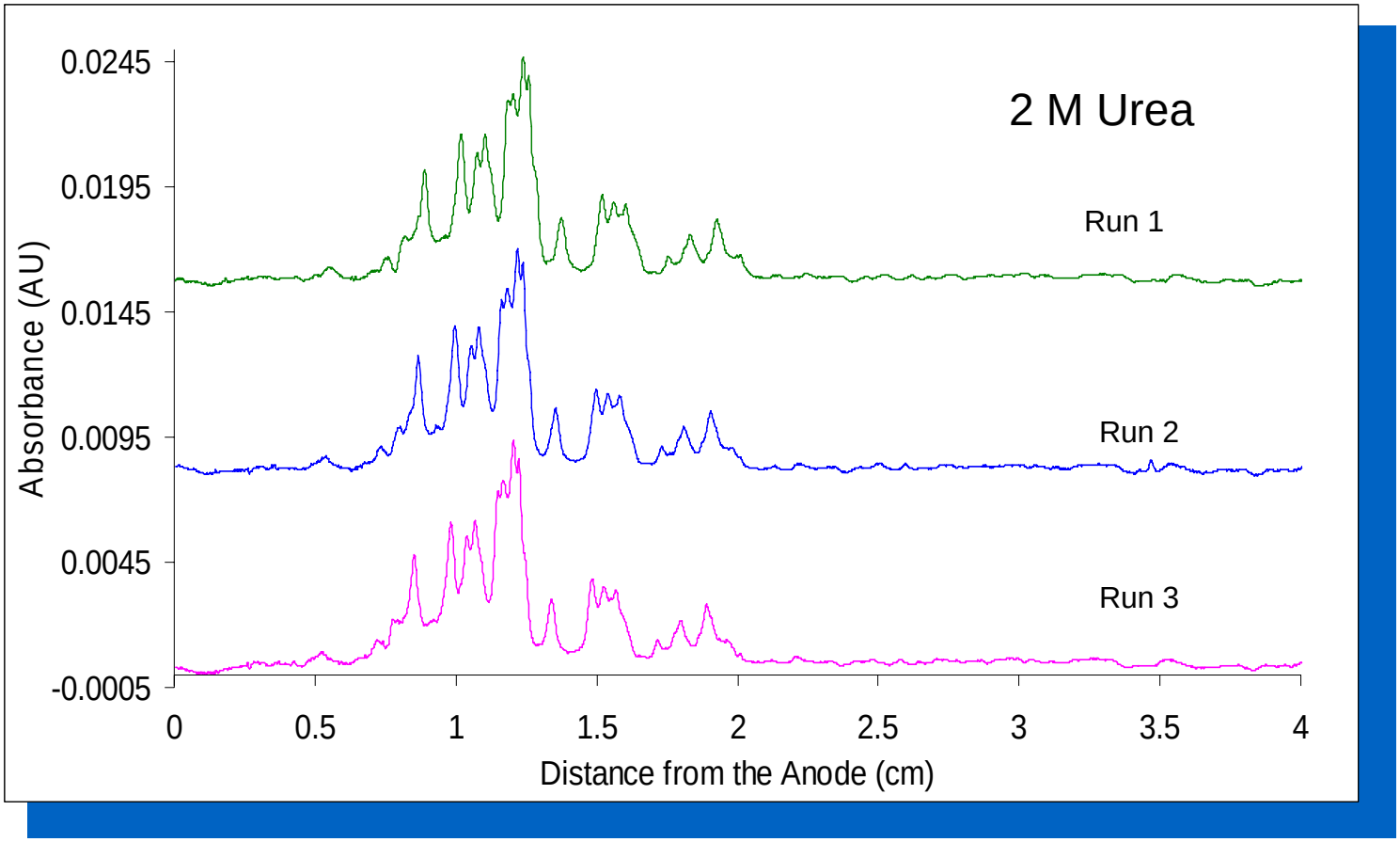
Experimental

- **iCE280 w/Alcott autosampler Model 714 (Convergent Biosciences).**
- **Capillary cartridge : 5 cm x 100 µm ID, FC coated (Convergent Biosciences).**
- **Detection: 280 nm.**
- **Software: iCE280 and EZChrom version 6.8**

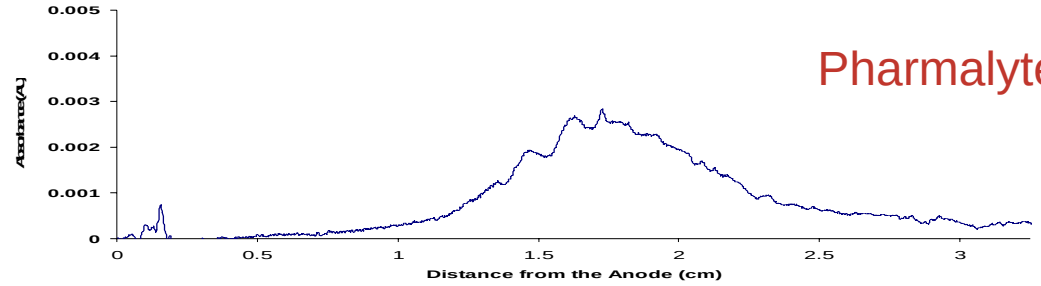
Method Development

- Cartridge pre-condition: different concentrations (0.35% vs. 0.50% methyl cellulose) at different times (2, 4,… and 24 hours)
- Different anolyte and catholyte concentrations
- Different ampholytes: servalyt pH 2-11, pharmalyte 3-10, Ampholines pH 3.5-9.5 and mixed with narrow pH ranges
- Different additives and different urea final concentrations: 0, 1, 2, 3, 4 and 8 M
- Different focusing time: 4, 5, 6 and 7 minutes
- Injection linearity
- Intermediate precision
- Identity

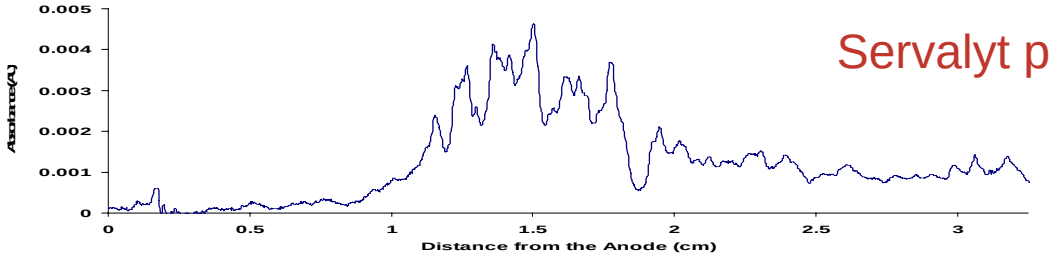
Obtaining Repeatable Profiles



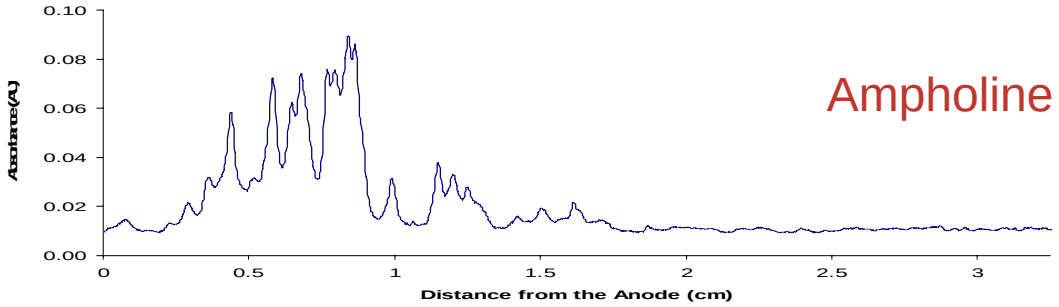
Optimizing Resolution



Pharmalyte pH 3-10

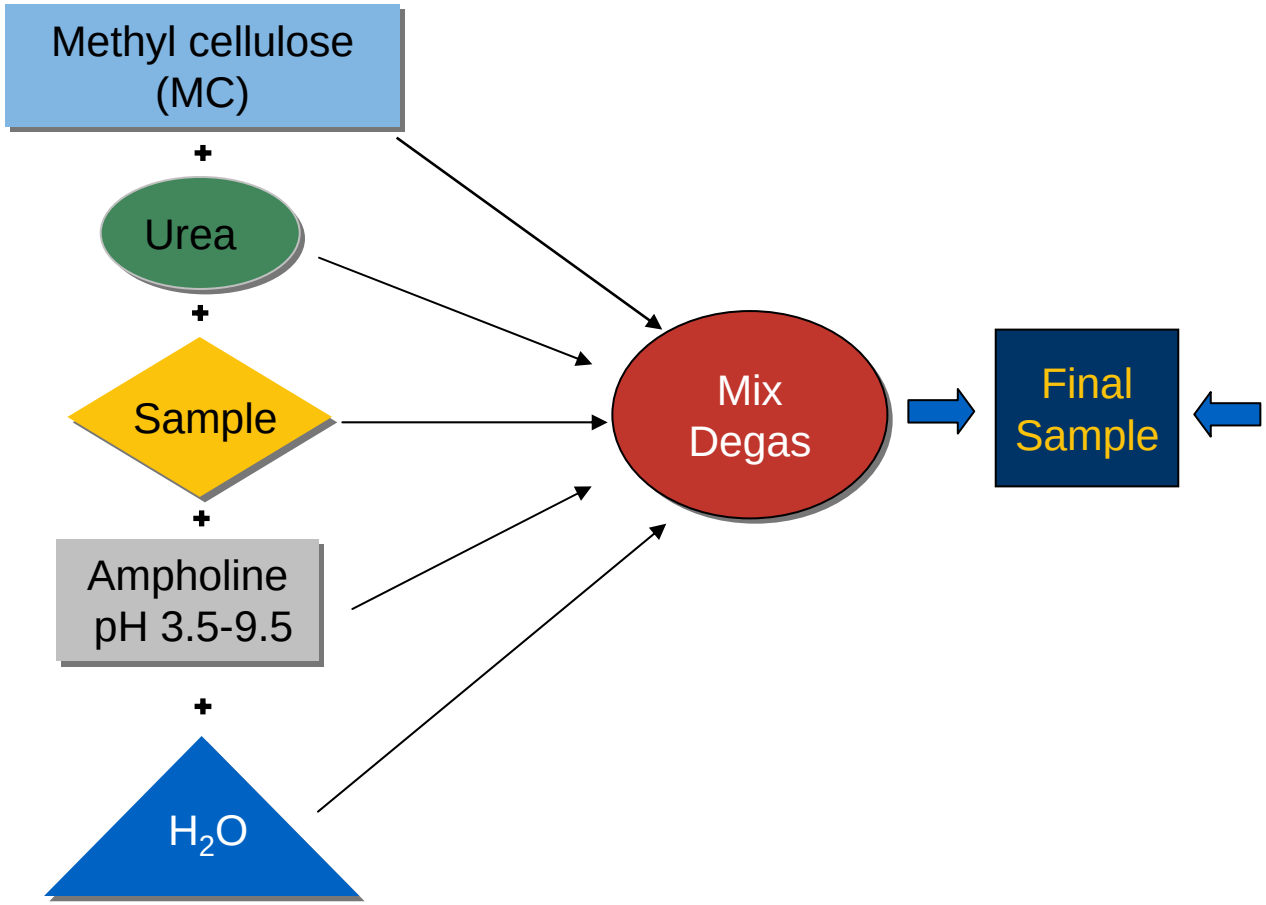


Servalyt pH 2-11



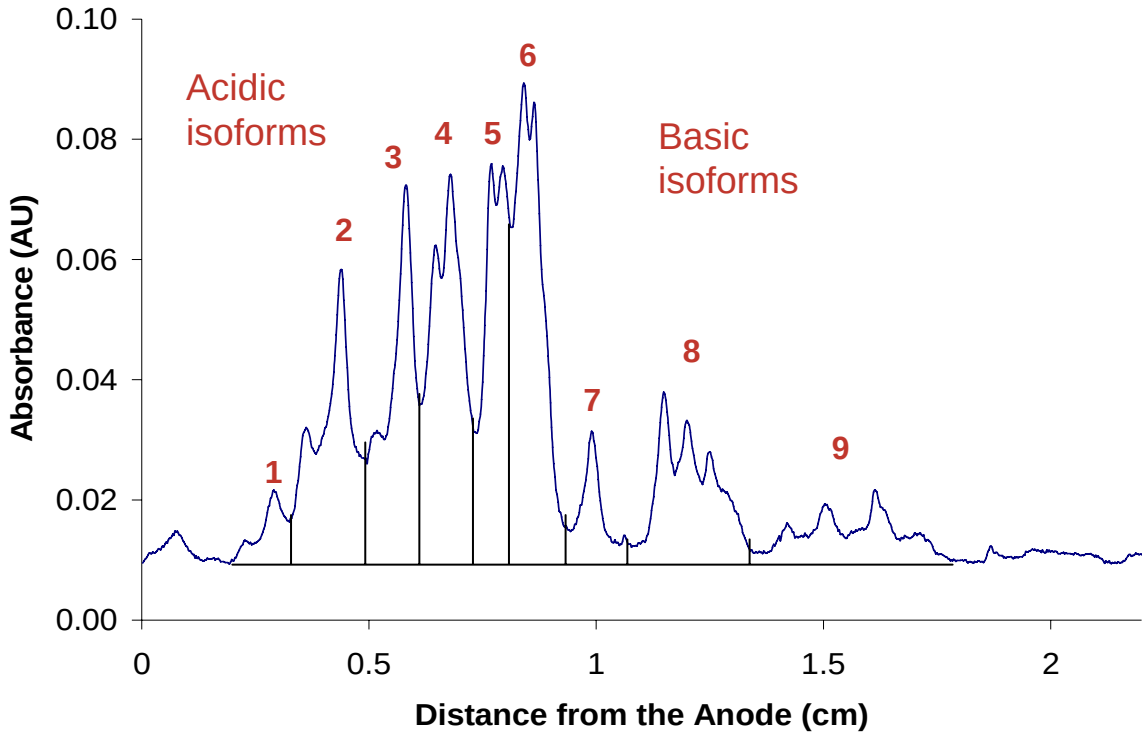
Ampholine pH 3.5-9.5

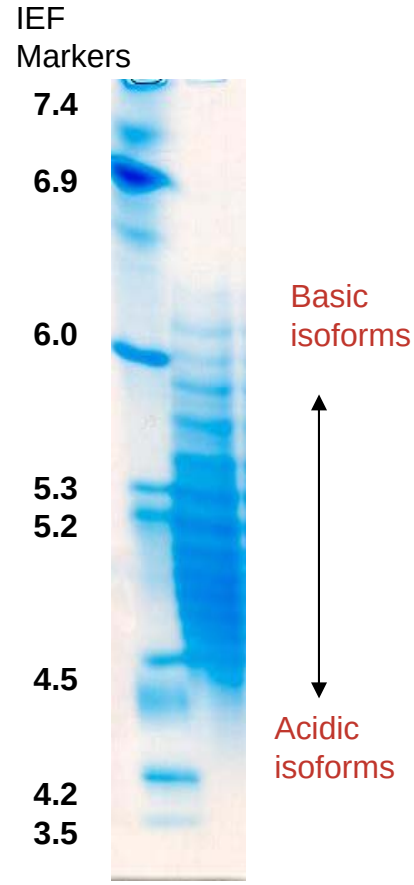
Final Sample Preparation Condition



- 0.35% MC
- 2 M Urea
- 0.3 mg/mL final protein concentration
- 4% ampholine
- 6 minutes focusing
- 3000 Volts
- Anolyte: 80 mM H_3PO4 in 0.1% MC
- Catholyte: 50 mM NaOH in 0.1% MC

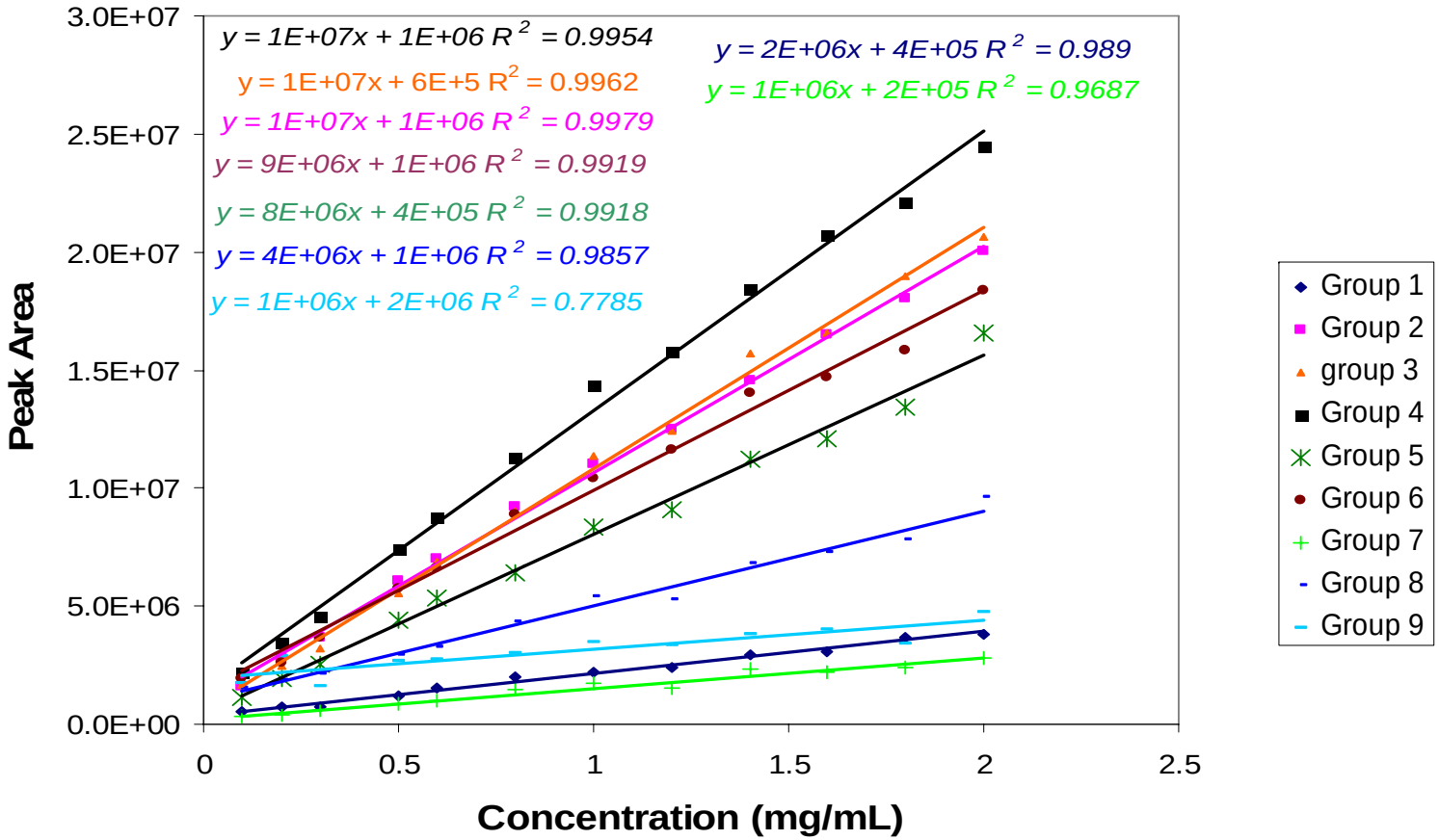
Typical i-cIEF Profile Compared to the Slab Gel





20 µg load

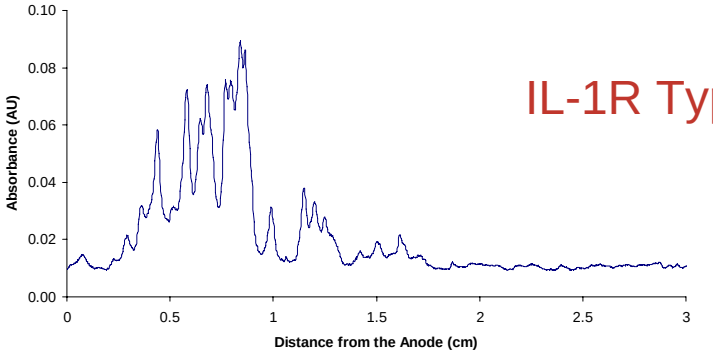
Linearity Study of IL-1R II Charge Isoforms



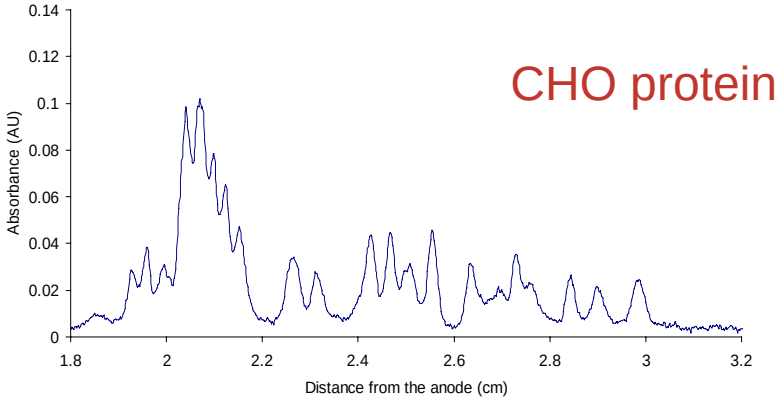
Intermediate Precision

Run #	% Peak Area								
	1	2	3	4	5	6	7	8	9
1	3.53	17.34	16.21	22.34	12.29	15.59	2.46	6.86	3.40
2	3.39	16.73	15.95	21.85	12.01	15.80	2.43	7.46	4.38
3	3.23	16.95	14.97	21.51	12.14	15.56	3.00	7.78	4.86
4	3.31	16.59	14.70	21.51	12.93	16.63	2.91	8.04	3.38
5	3.61	16.38	14.70	19.46	12.09	15.77	3.55	9.07	5.37
6	3.66	16.67	14.36	19.07	12.78	15.45	3.63	9.51	4.87
7	3.77	17.73	14.28	20.40	13.11	14.94	3.32	8.20	4.28
8	3.81	18.28	13.64	19.16	12.75	15.77	3.20	7.96	5.45
9	3.06	15.57	13.86	20.13	13.39	16.00	3.40	8.72	5.86
Average	3.49	16.92	14.74	20.60	12.61	15.72	3.10	8.18	4.65
STD	0.26	0.79	0.87	1.24	0.50	0.45	0.44	0.82	0.87
%RSD	7.32	4.68	5.89	6.00	3.93	2.88	14.14	9.99	18.78

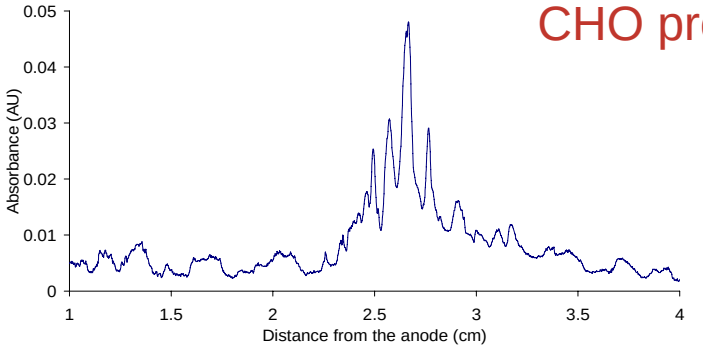
Identity



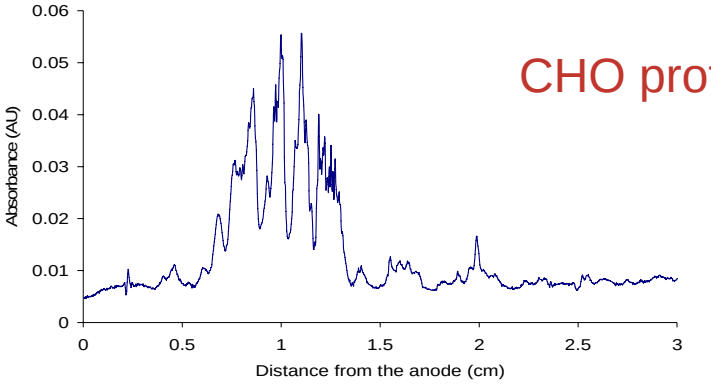
IL-1R Type II



CHO protein 1



CHO protein 2



CHO protein 3

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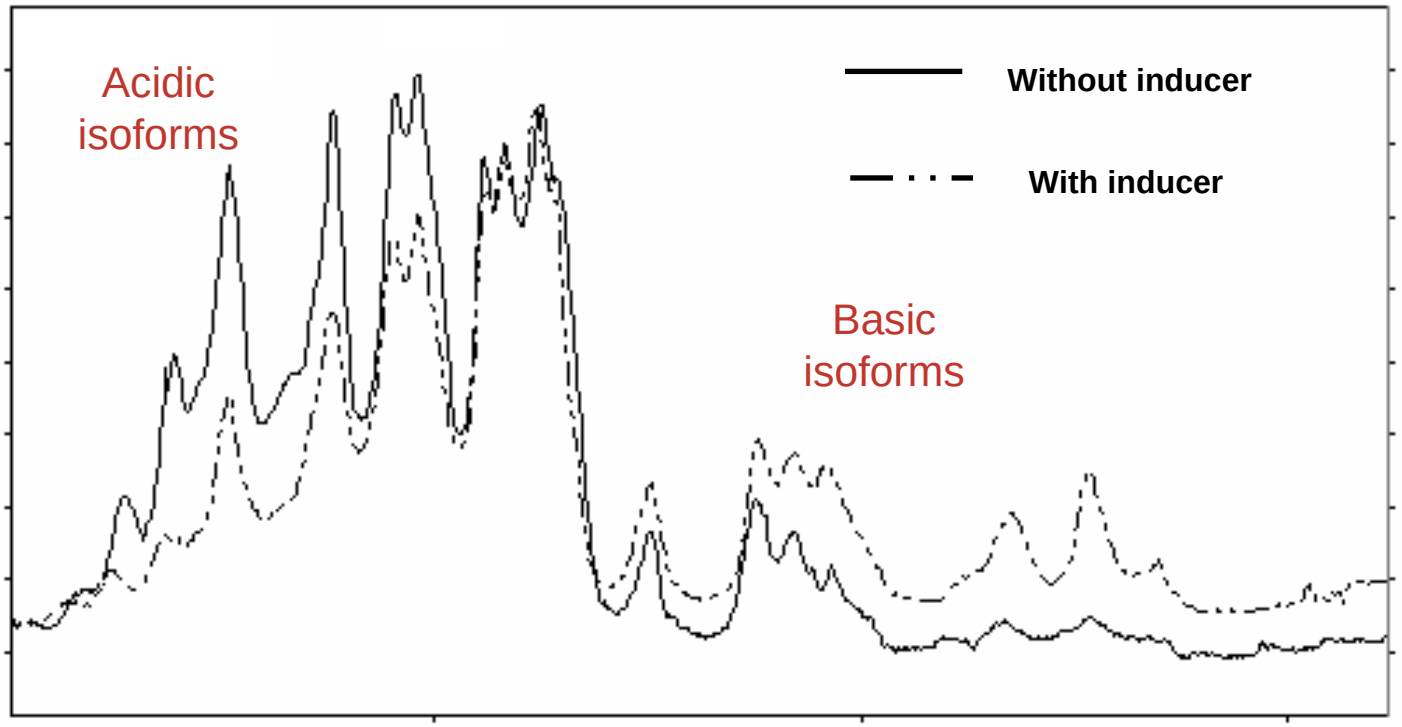
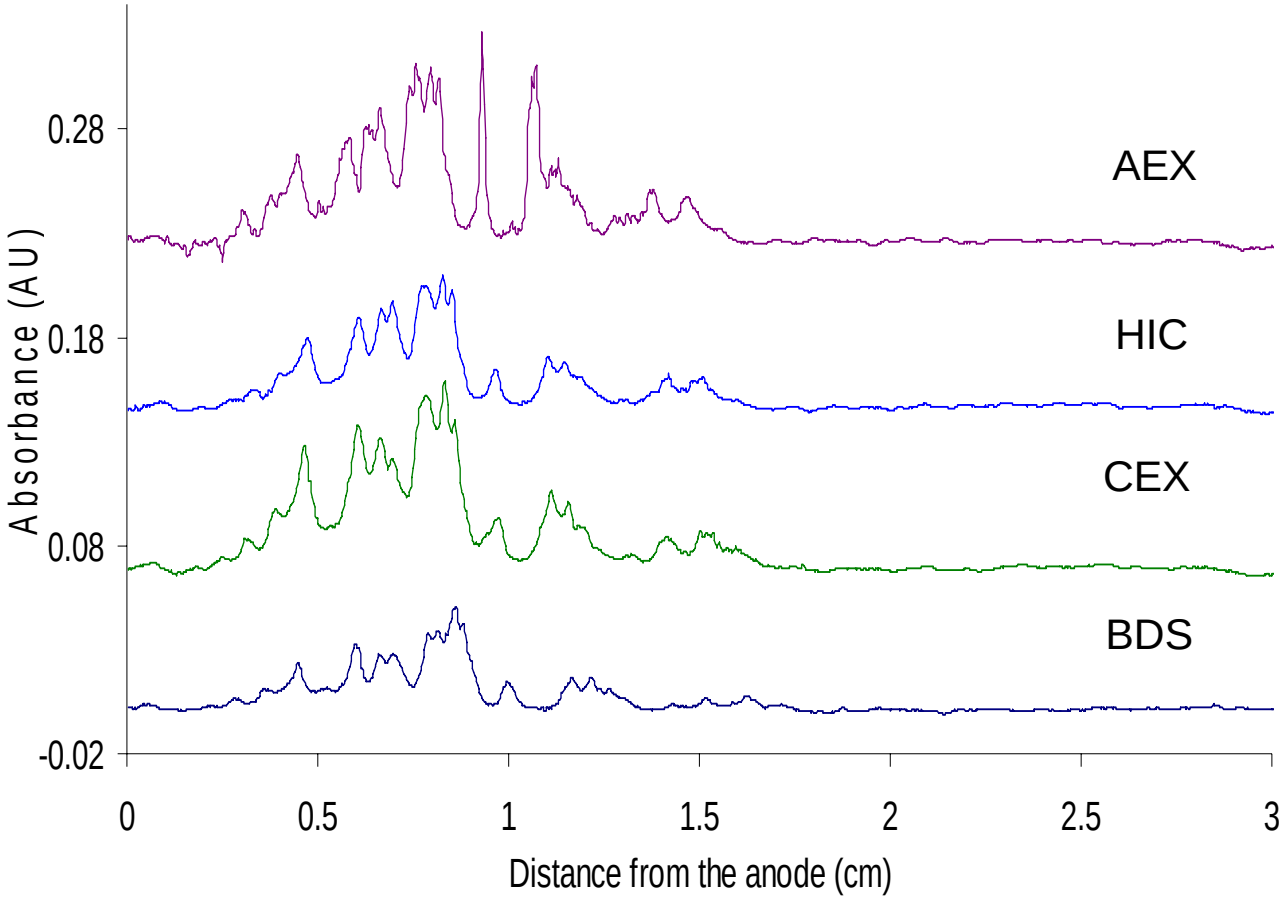
Effect of Inducer on i-cIEF Profile



	% Peak Area								
	1	2	3	4	5	6	7	8	9
w/o inducer	3.19	17.5	17.4	21.7	13.6	15.8	2.09	6.44	2.36
w/ inducer	1.59	9.40	11.9	19.3	15.0	16.6	3.79	12.9	9.44

Monitoring Downstream Purification Process



Harvest

AEX

↓

HIC

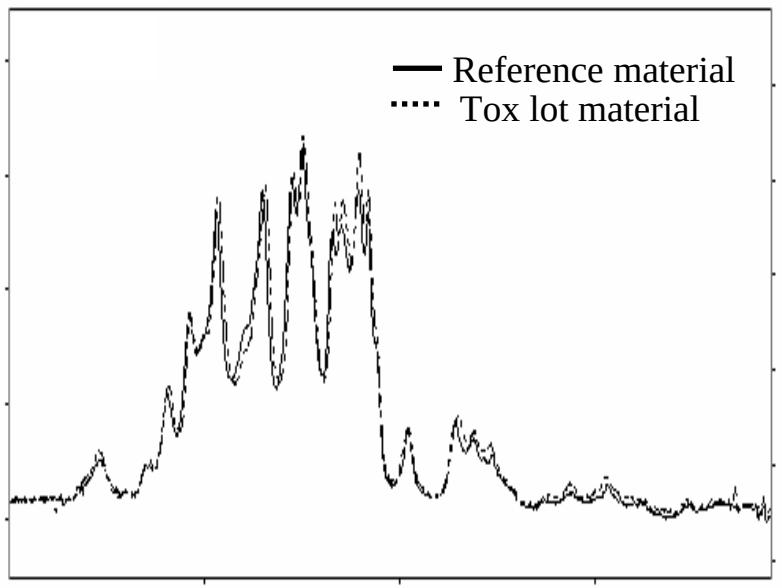
↓

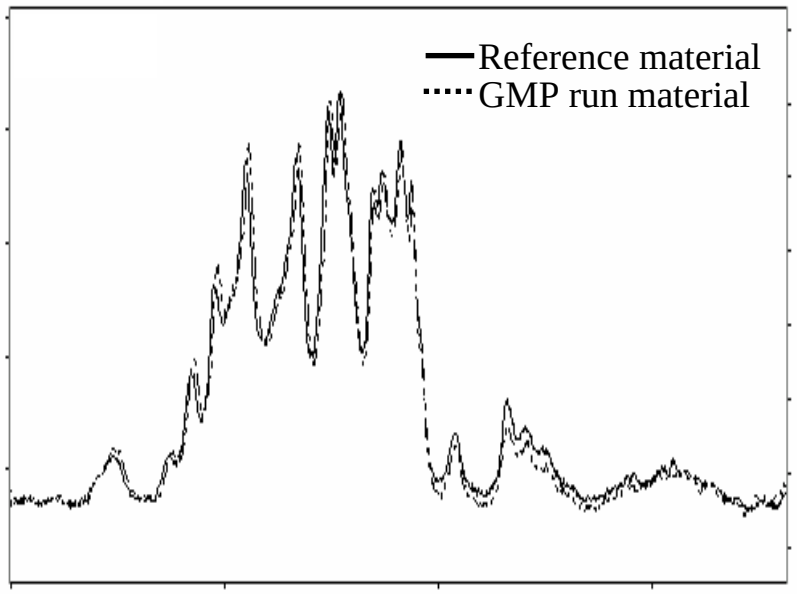
CEX

↓

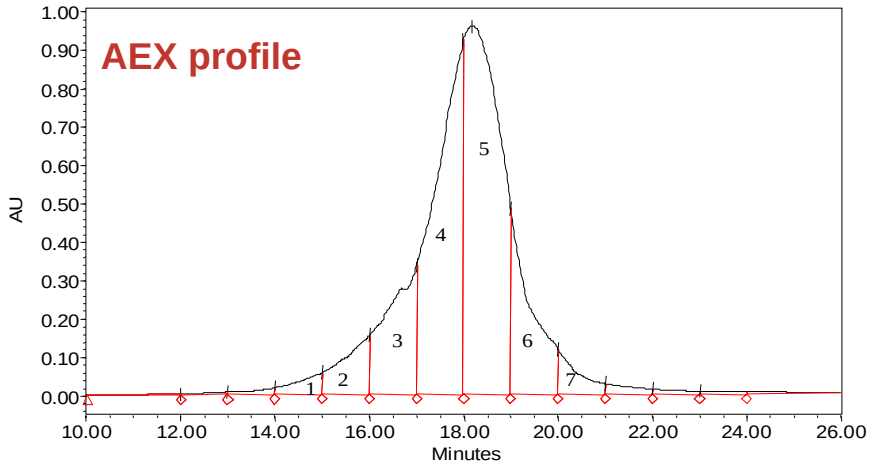
BDS

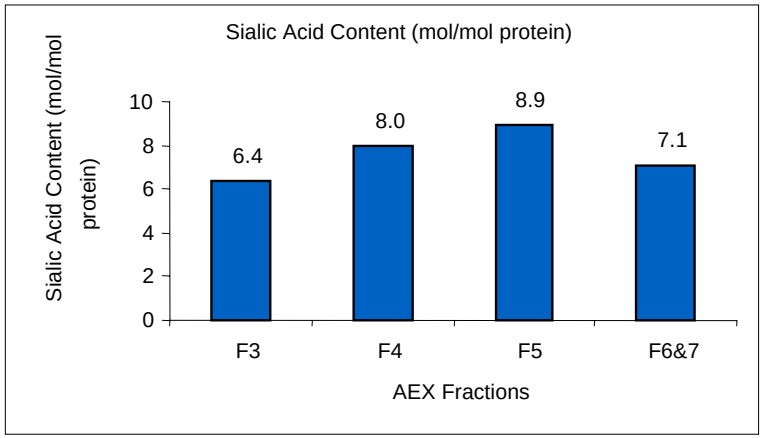
Assessment of Lot-to-Lot Reproducibility

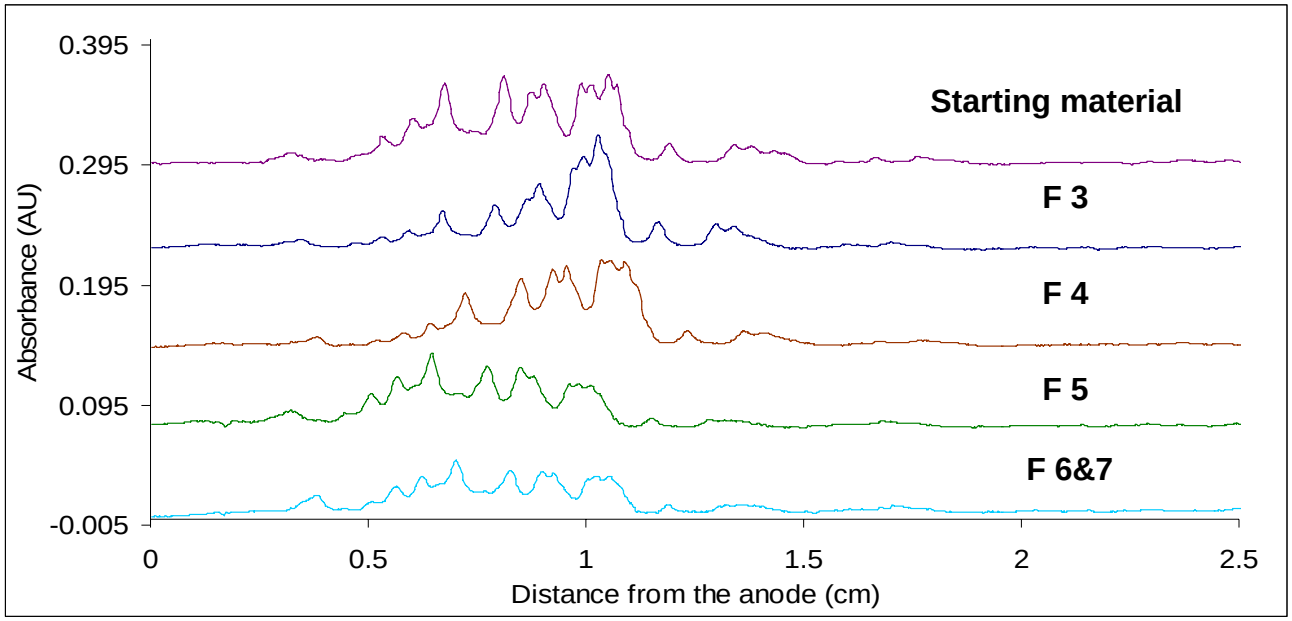




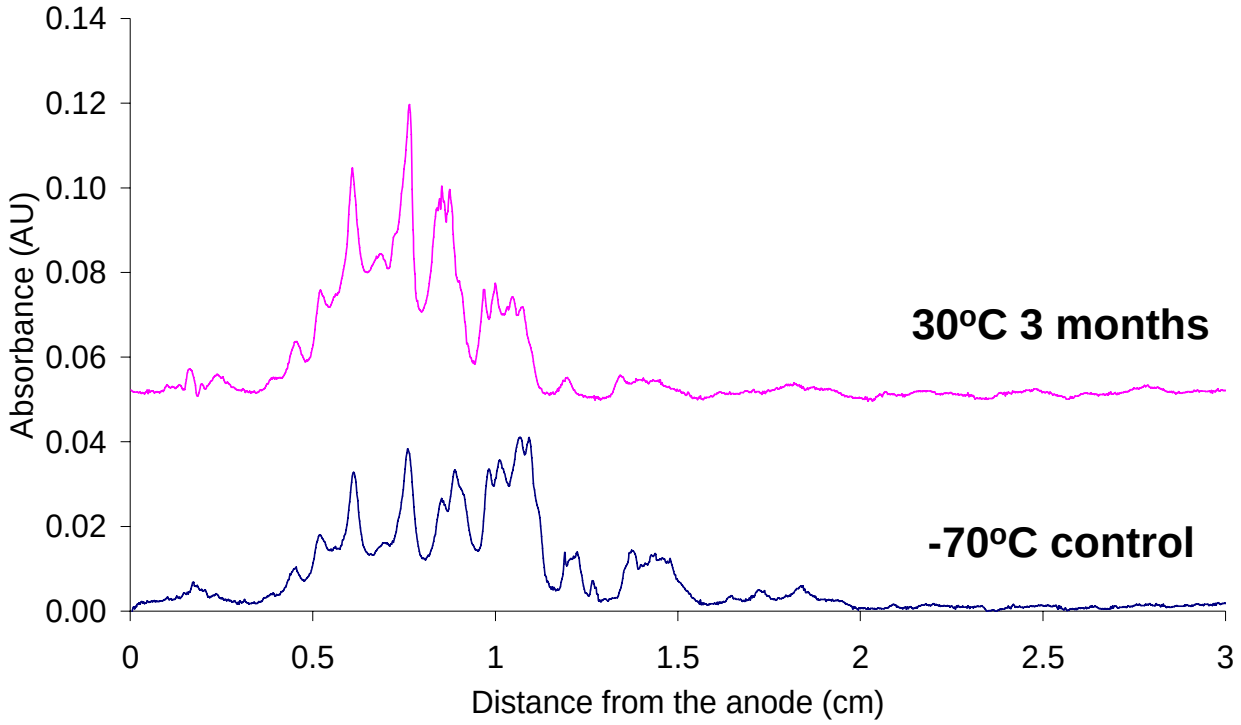
Monitoring Sialic Acid Distribution





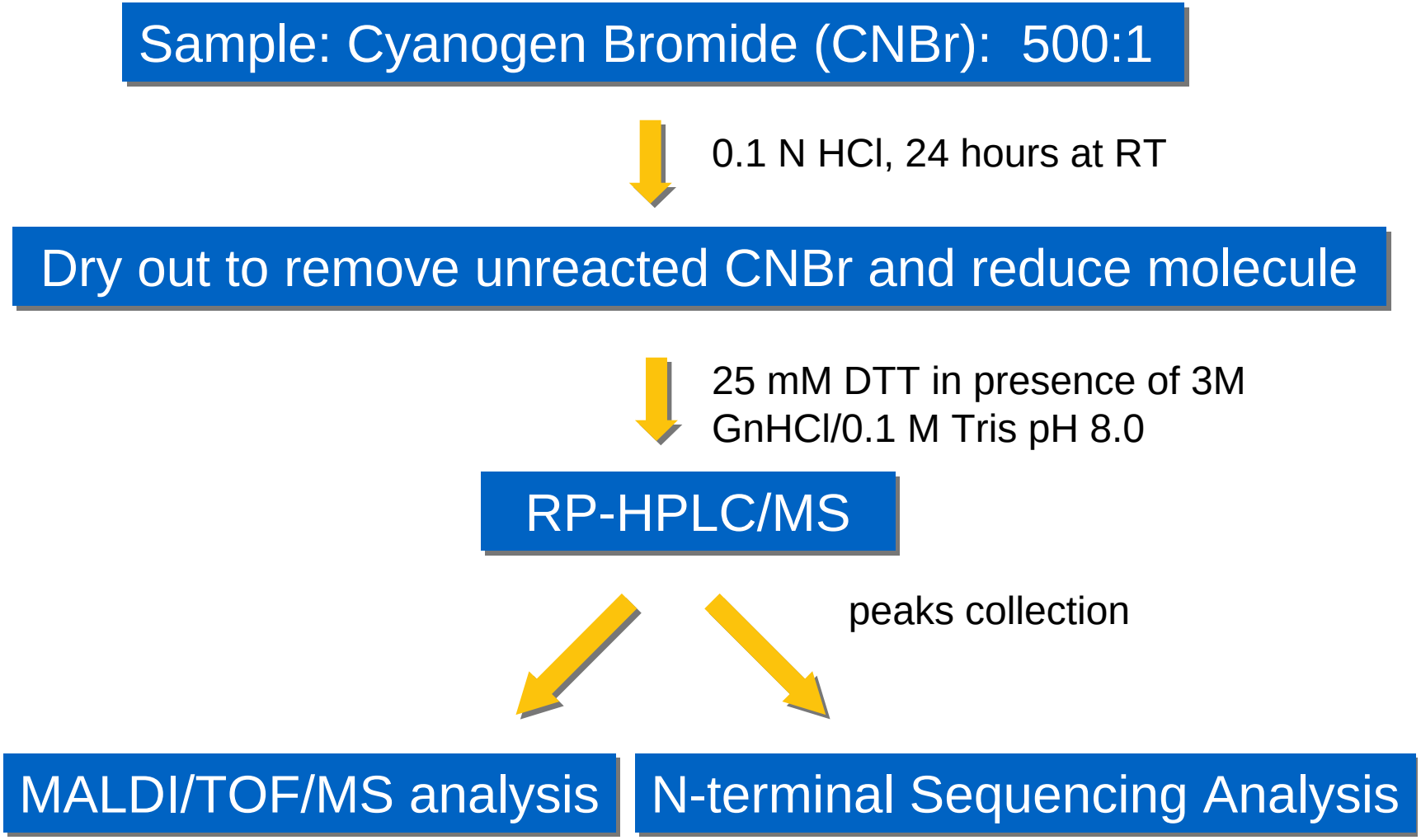


i-cIEF As a Stability Indicating Assay

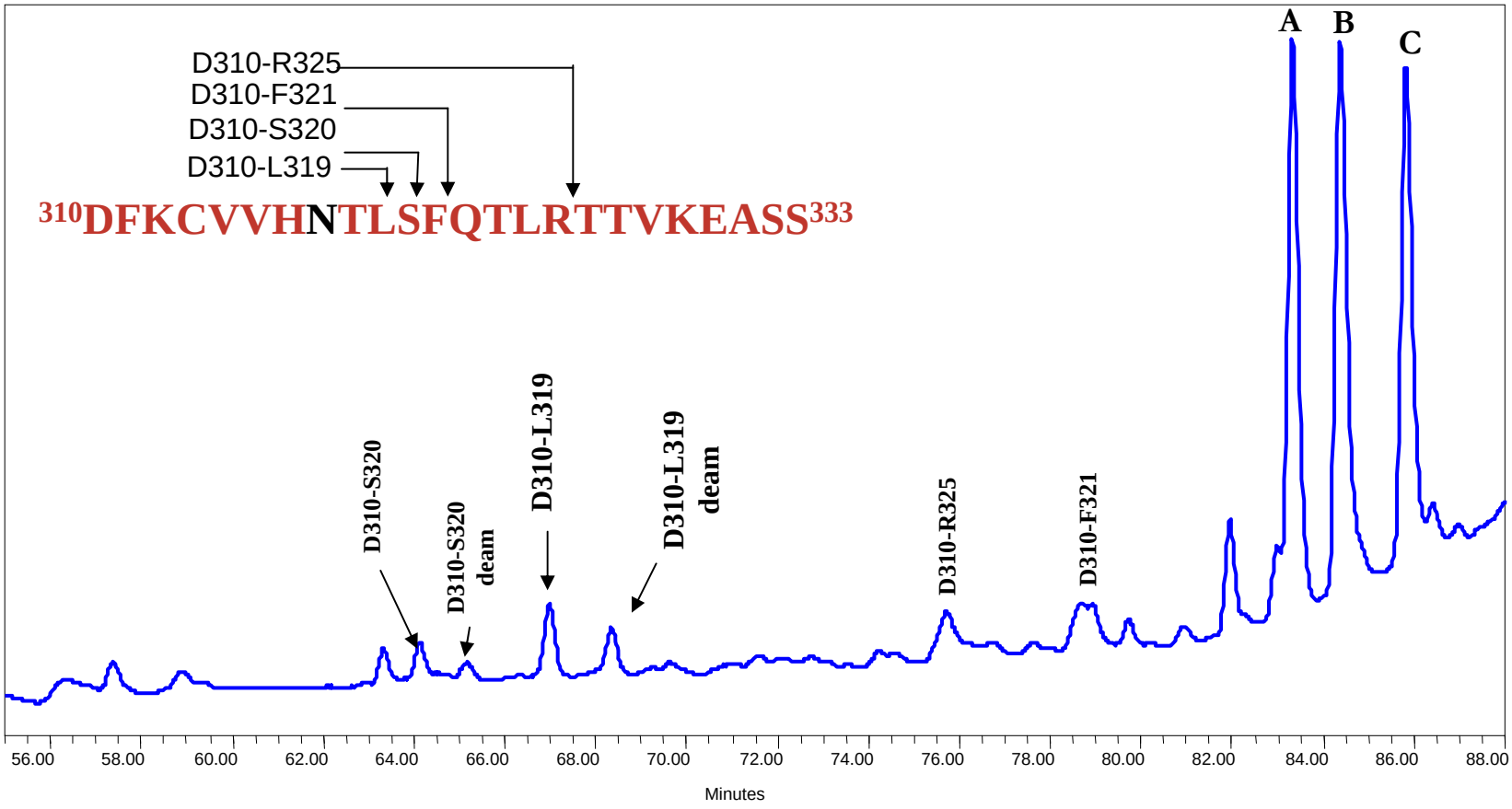


	% Peak Area								
	1	2	3	4	5	6	7	8	9
-70ºC control	2.43	15.27	14.80	16.45	12.52	17.53	4.62	10.80	5.58
30ºC 3 months	3.47	22.43	27.41	21.63	7.92	8.19	1.25	3.97	3.74

Characterization: Stability Sample

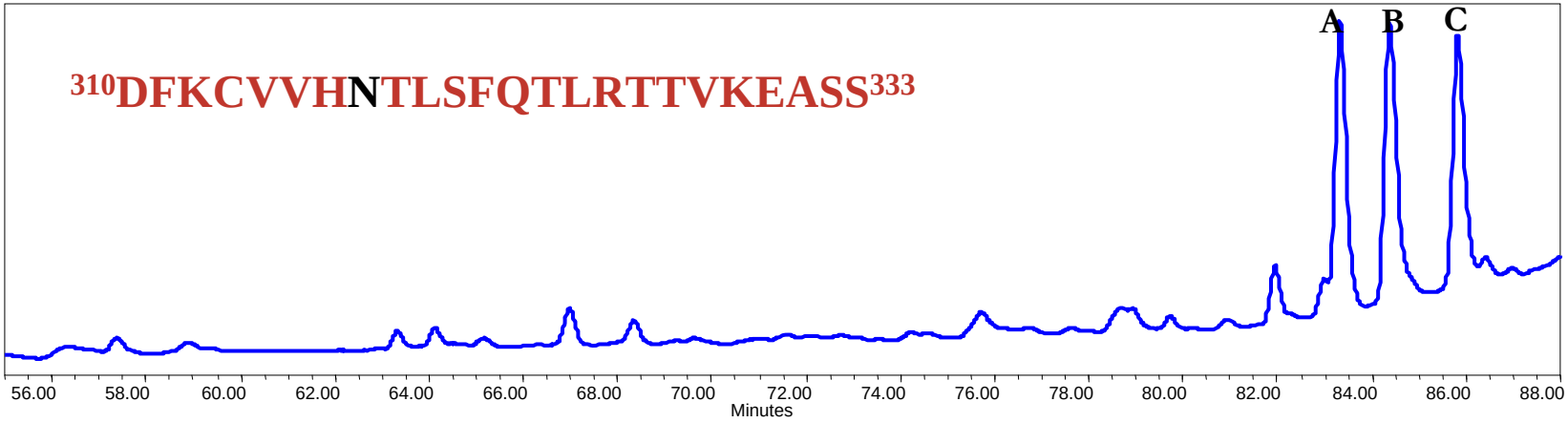


LC/MS Results of CNBr Digest of Stability Sample



30ºC, 3 months sample

MALDI/TOF/MS Results



CNBr Fractions	Theoretical Mass	Experimental Mass	Fragment (Modification)
A	2710.37	2711.28	D310-S333 (Isomerization)
B	2710.37	2710.28	D310-S333 (Unmodified)
C	2710.37	2711.27	D310-S333 (Deamidation)

N-terminal Sequencing Results

CNBr RP-HPLC fractions	NTS Results	Modification
Fraction A	X-V-V-H (sequence stopped)	isomerization
Fraction B	X-V-V-H-**N**-T-L-S-F-Q-T-	unmodified
Fraction C	X-V-V-H-**D**-T-L-S-F-Q-T-	deamidation

Conclusions

- **i-cIEF analysis is a fast and automated method to monitor IL-1R Type II charge profile.**
- **This assay has a large linear response range and the profiles are reproducible. Organizing the isoforms in series of groups helps comparative and quantitative analyses.**
- **The distinctive electropherogram can be used as an identity fingerprint.**
- **i-cIEF is an effective method for monitoring upstream and downstream process.**
- **Bulk Drug Substance (BDS) as well as in process samples can be analyzed by this technique.**
- **i-cIEF is an appropriate method for monitoring deamidation.**

Acknowledgements

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