# Imaged Capillary Isoelectric Focusing During Process Development and as a Characterization Tool

Liping Wang, Henrik Andersen
Bioprocess Development
ZymoGenetics

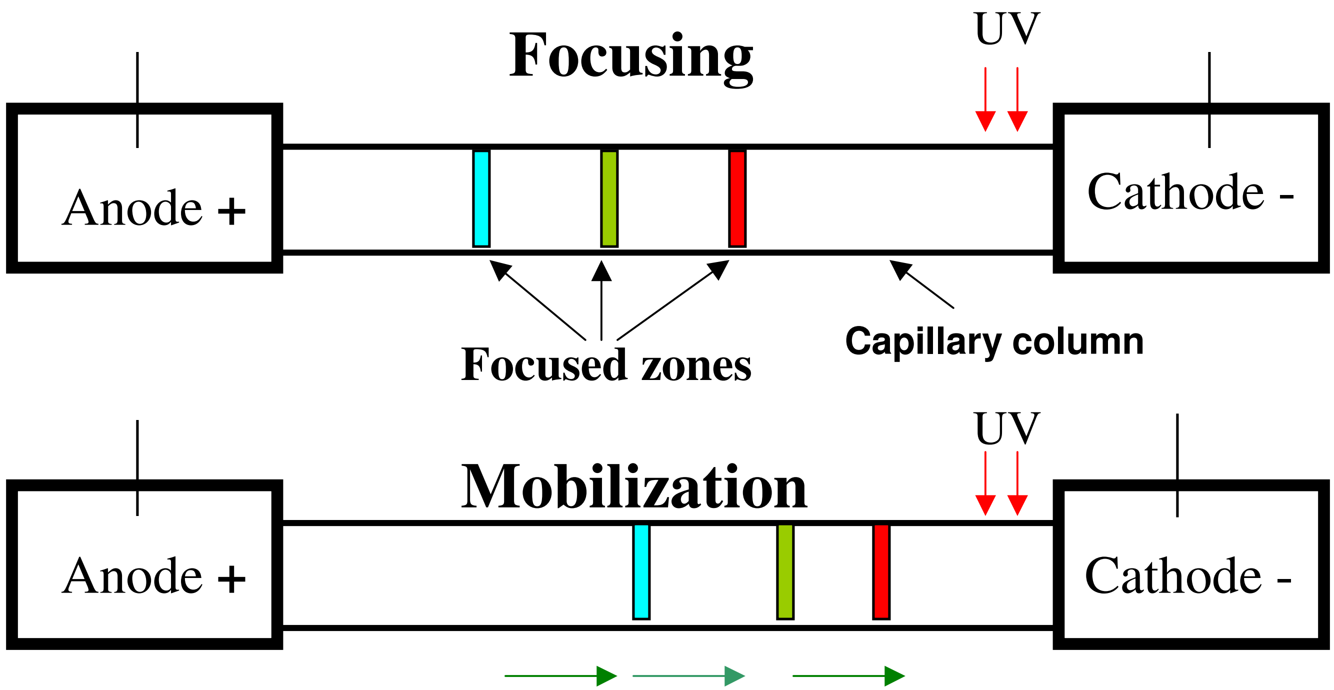
CE Pharm 2008, San Francisco
Oct 12-16, 2008

ZYMOGENETICS

# Imaged cIEF at ZymoGenetics

- Imaged cIEF was evaluated as a characterization tool at ZymoGenetics for charge isoform analysis for recombinant proteins, including recombinant fusion proteins, monoclonal antibodies, etc.

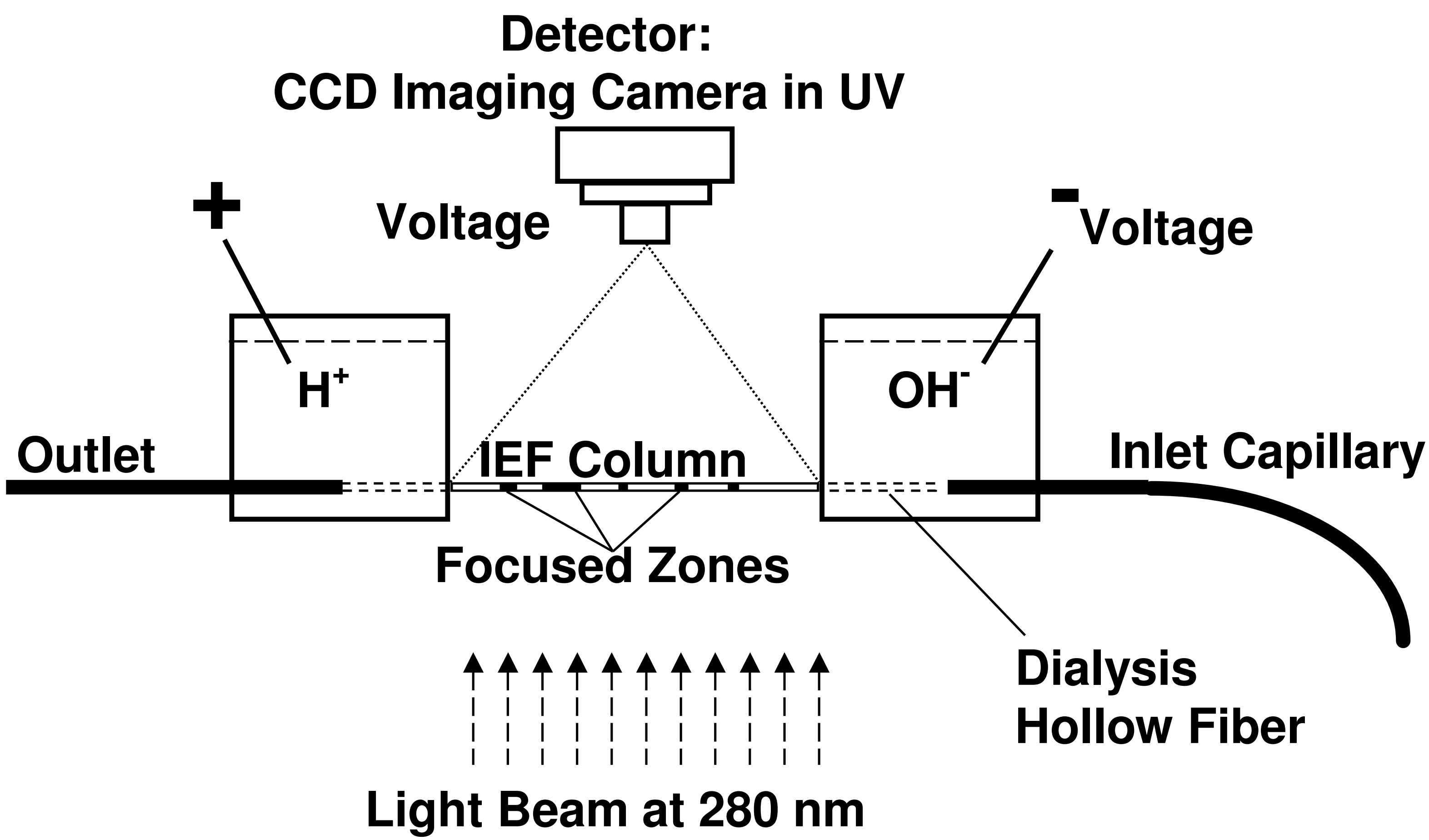
# Conventional cIEF
# - 2 Step Process




Problems:

- Less reproducibility due to uneven mobilization step.
- Long time for method development.
- Longer analysis time (1-2 runs/hour).

# Whole-Column Detection cIEF - One Step Process




Convergent Bioscience, Toronto, Canada

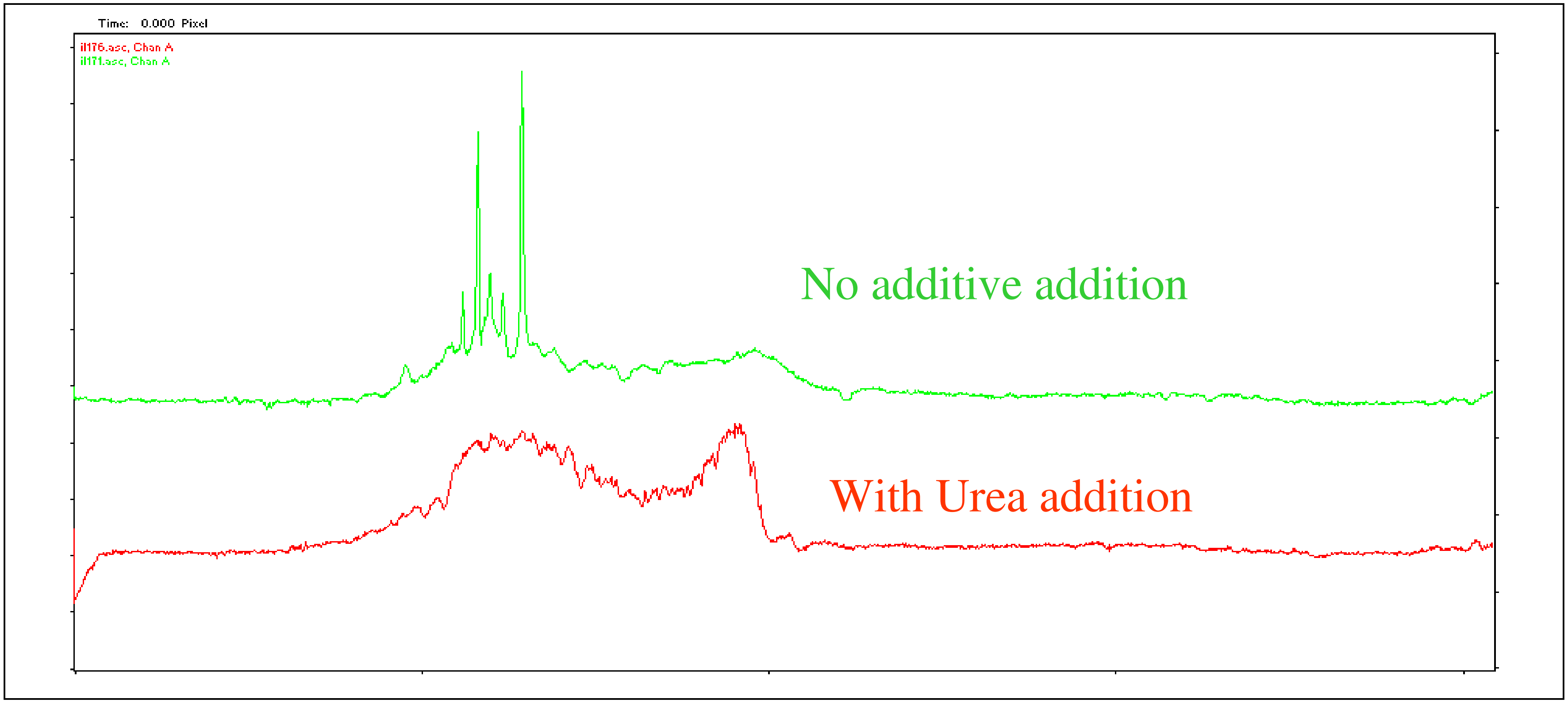
ZYMOGENETICS

# Imaged cIEF for a Recombinant Fusion Protein

- A recombinant fusion protein with 22 potential N-linked glycosylation sites
- Method Development:
  - Sample additive to increase protein solubility during focusing
  - Carrier ampholyte selection
  - pH range optimization for selected carrier ampholyte
  - Focusing time optimization
  - Sample concentration optimization
- A desialylation protocol was optimized for peak characterization
- Method Application
- Method Evaluation

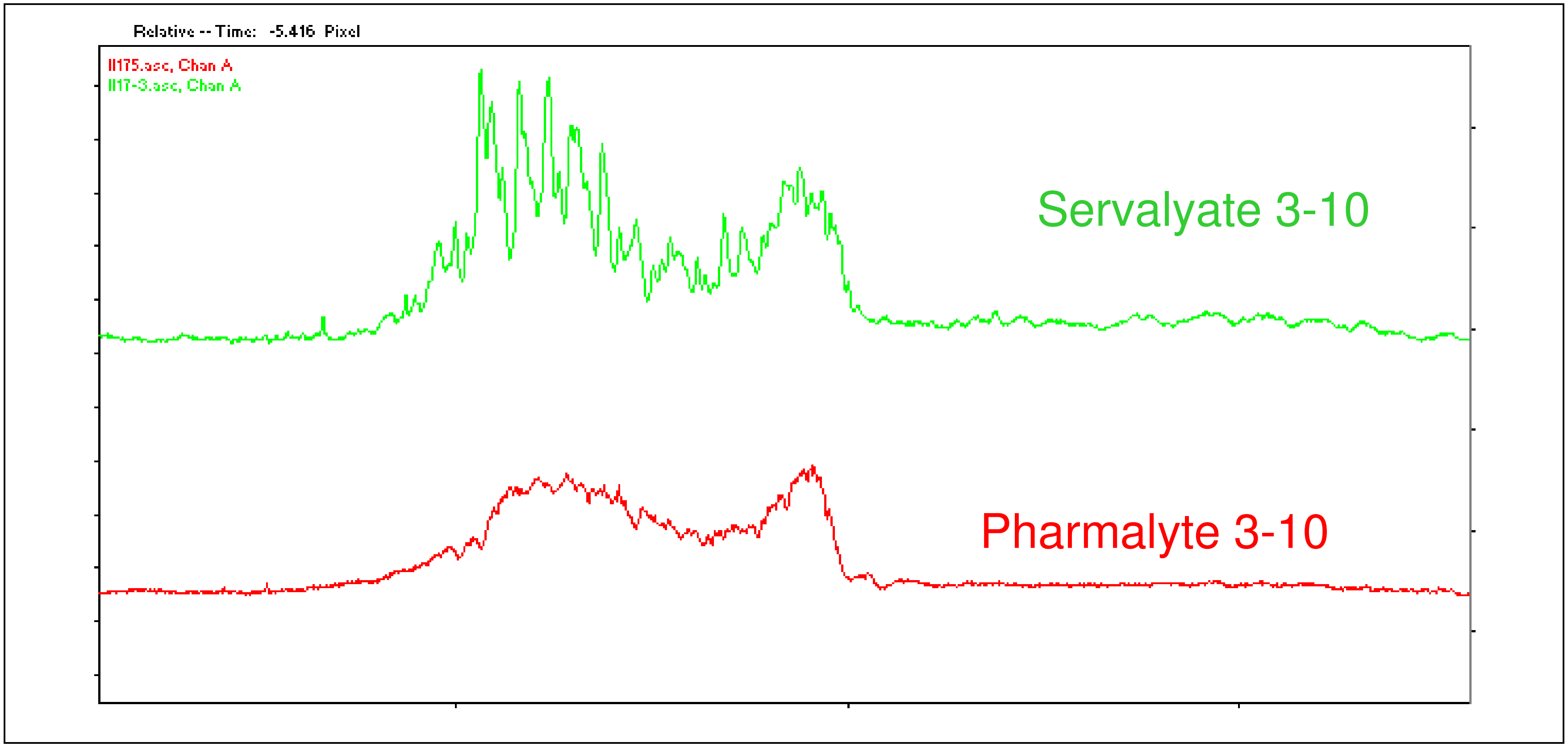
Method Development

# Urea Addition in the Sample Matrix




Without Urea addition to the sample matrix, spikes were observed**;** 8 M urea was added into sample matrix to increase protein solubility and reproducible profiles were thus obtained. Pharmalyte 3-10 was used in the method initially.

# Carrier Ampholyte Selection

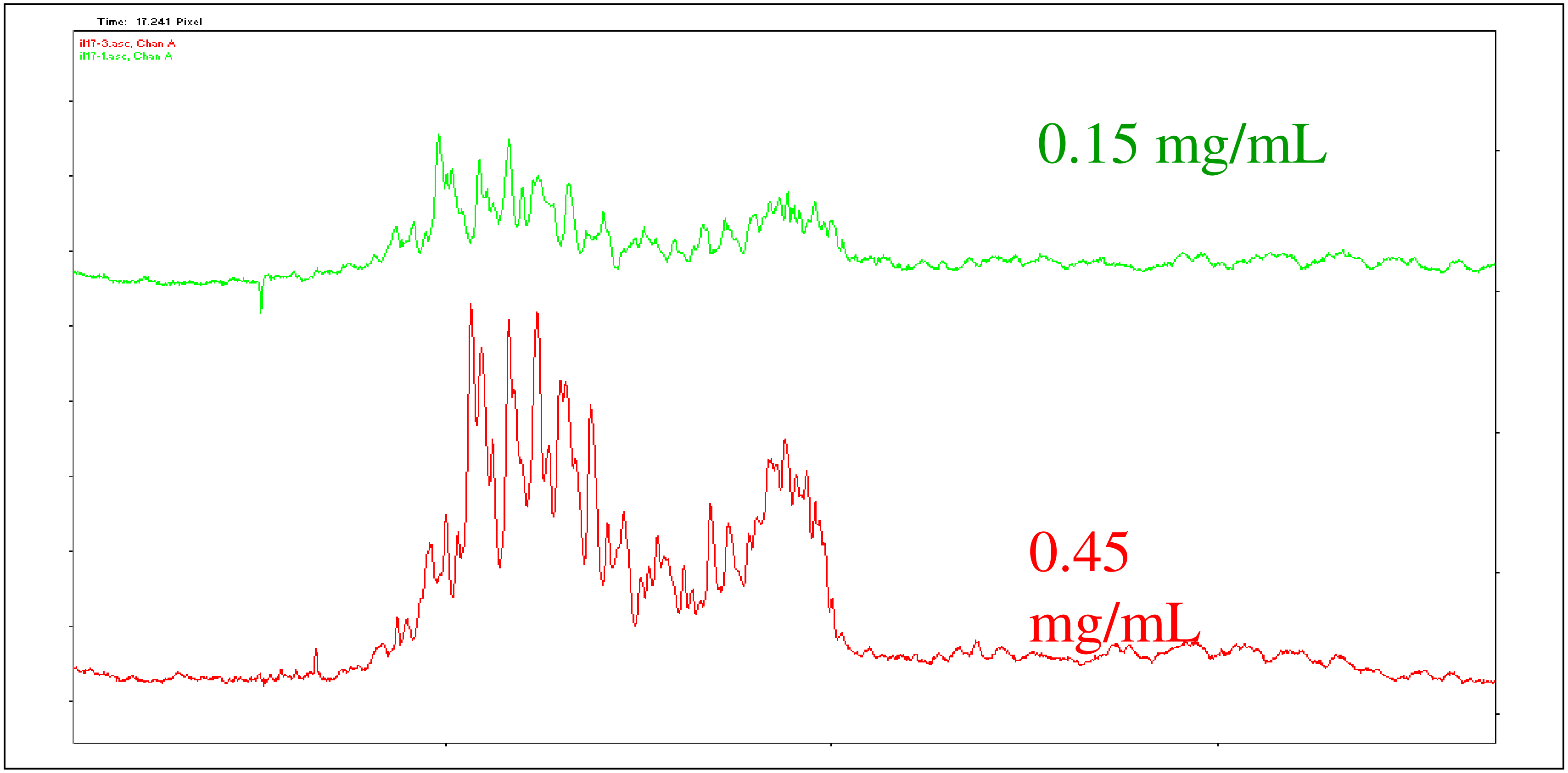



Servalyte gave rise to better resolution (other separation conditions are identical) and was selected for further method development.



ZYMOGENETICS

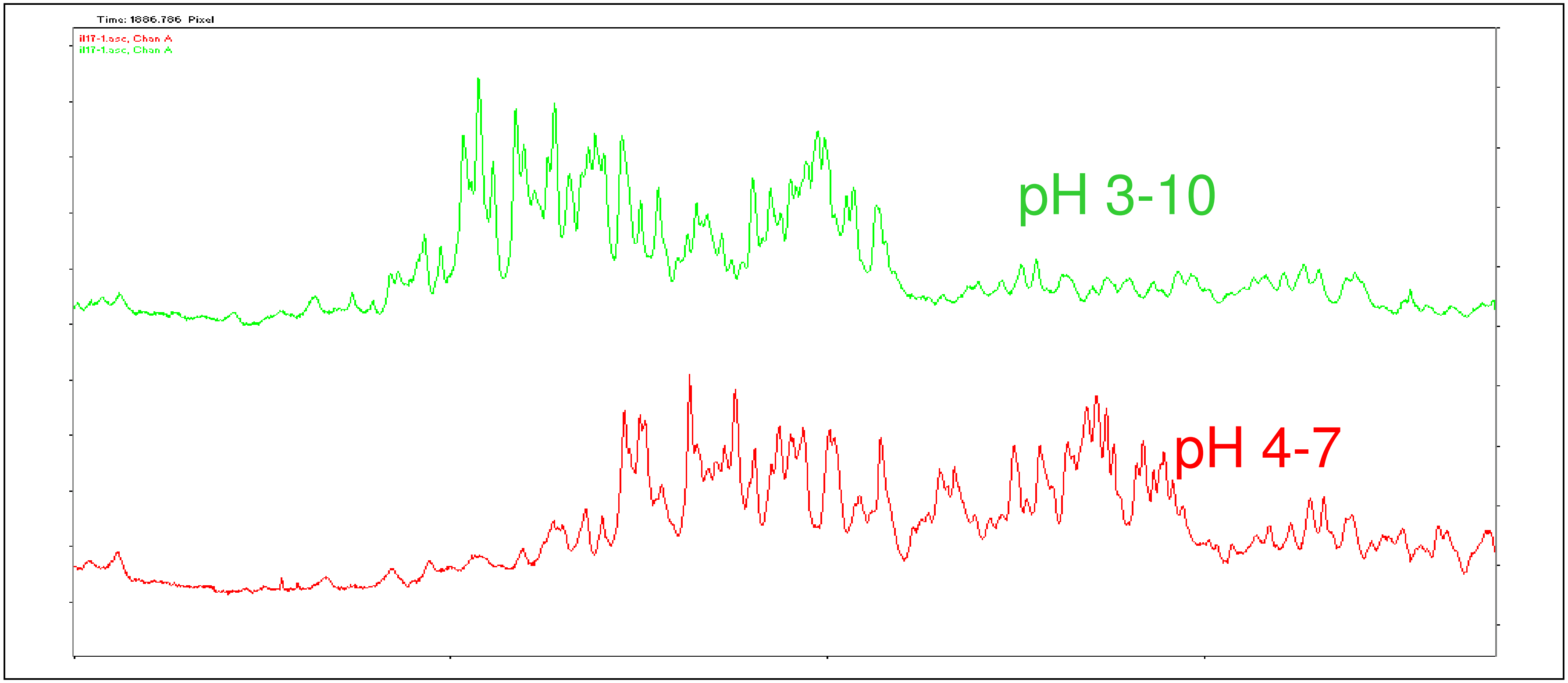
# Protein Concentration Optimization




Higher concentration 0.45 mg/mL gave better signal to noise ratio and was selected for further method development.

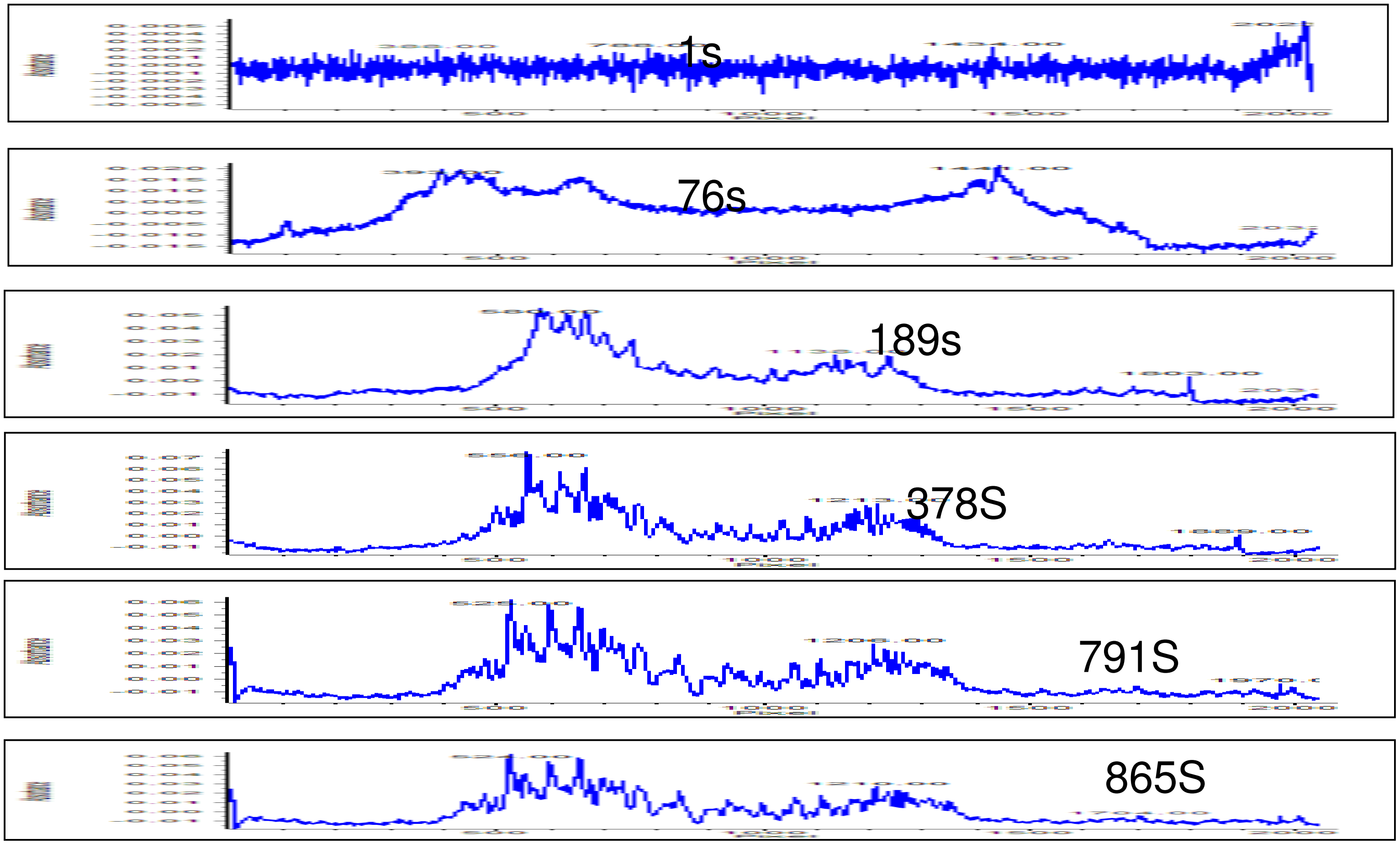
Confidential

# pH Range Selection




Better resolution was obtained with narrower pH range (pH 4-7), and thus selected in the method.

# Focusing Time Optimization

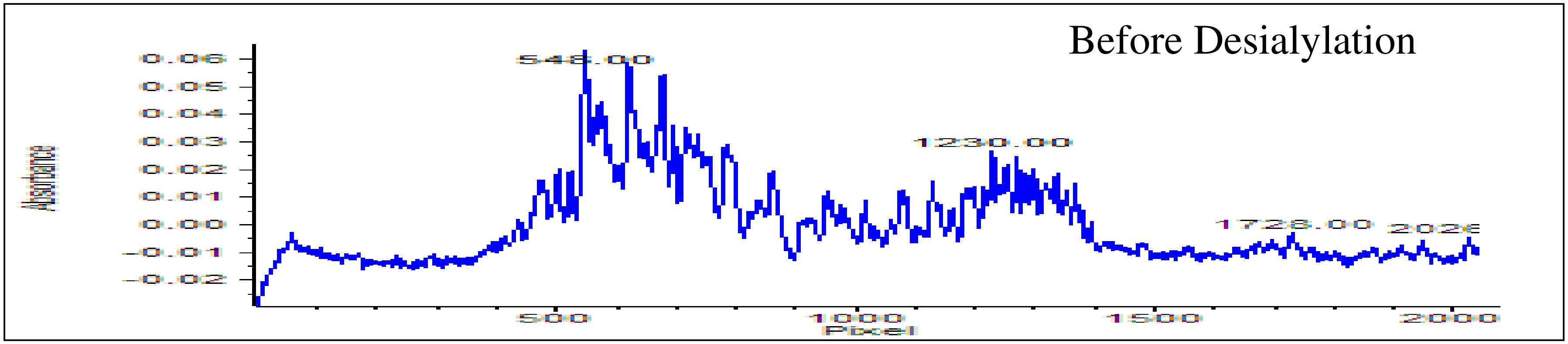


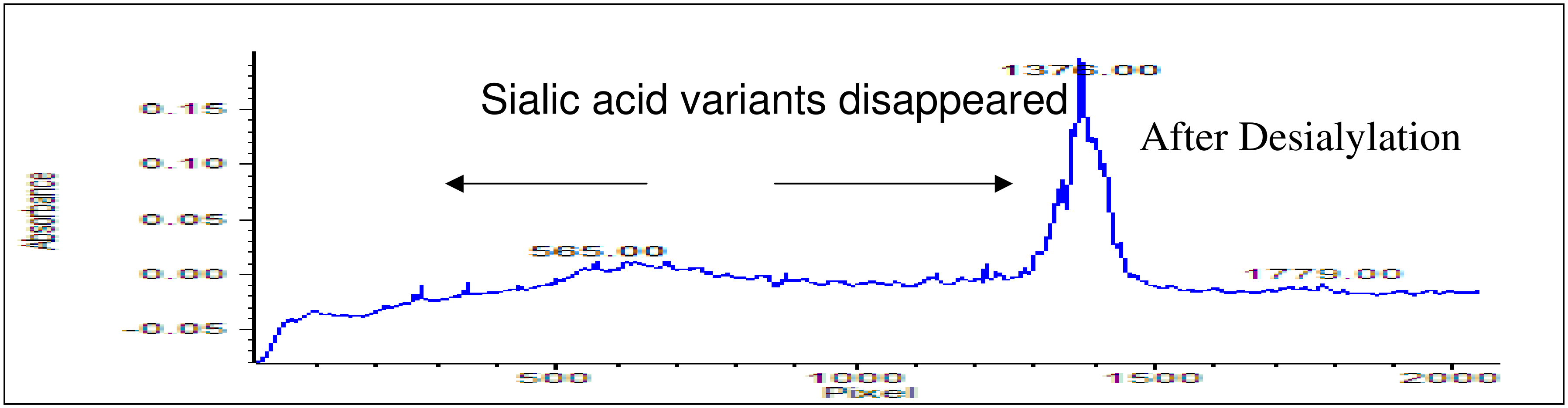

The image (electropherogram) was captured every 30 seconds during focusing procedure. Focusing time optimization can be finished within one run. 13 minutes was selected as final focusing time.

# Summary of Method Parameters

- Analyte: 80 mM phosphoric acid
- Catholyte: 100 mM NaOH
- Column: Fluorocarbon coated capillary, 100 µm x 50 mm
- Carrier ampholyte: 4% servalyte pH 4-7
- Sample additive: 8 M urea
- Focusing time: 13 minutes
- Focusing voltage: 3 kV
- Protein concentration: ~ 0.45 mg/mL

# Protein Characterization -Desialylation



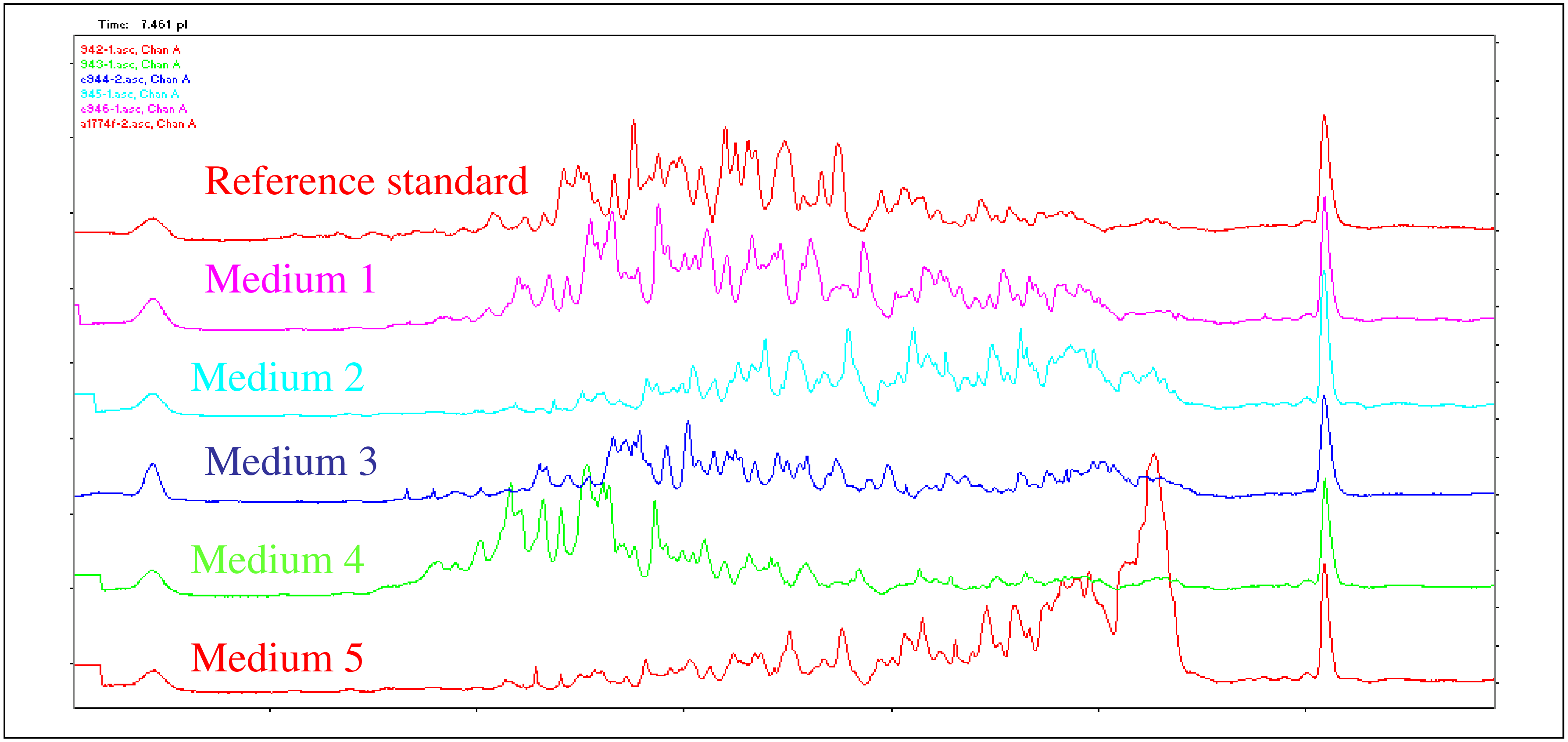




Desialyation with sialidase A of protein demonstrated that most of acidic variants are due to sialylation. The biological activity and pharmakinetics of a glycoprotein may be influenced by the sialic acid level. The developed assay was used to monitor the sialyation level of protein lots.



ZYMOGENETICS

# Imaged cIEF Support Cell Culture Development

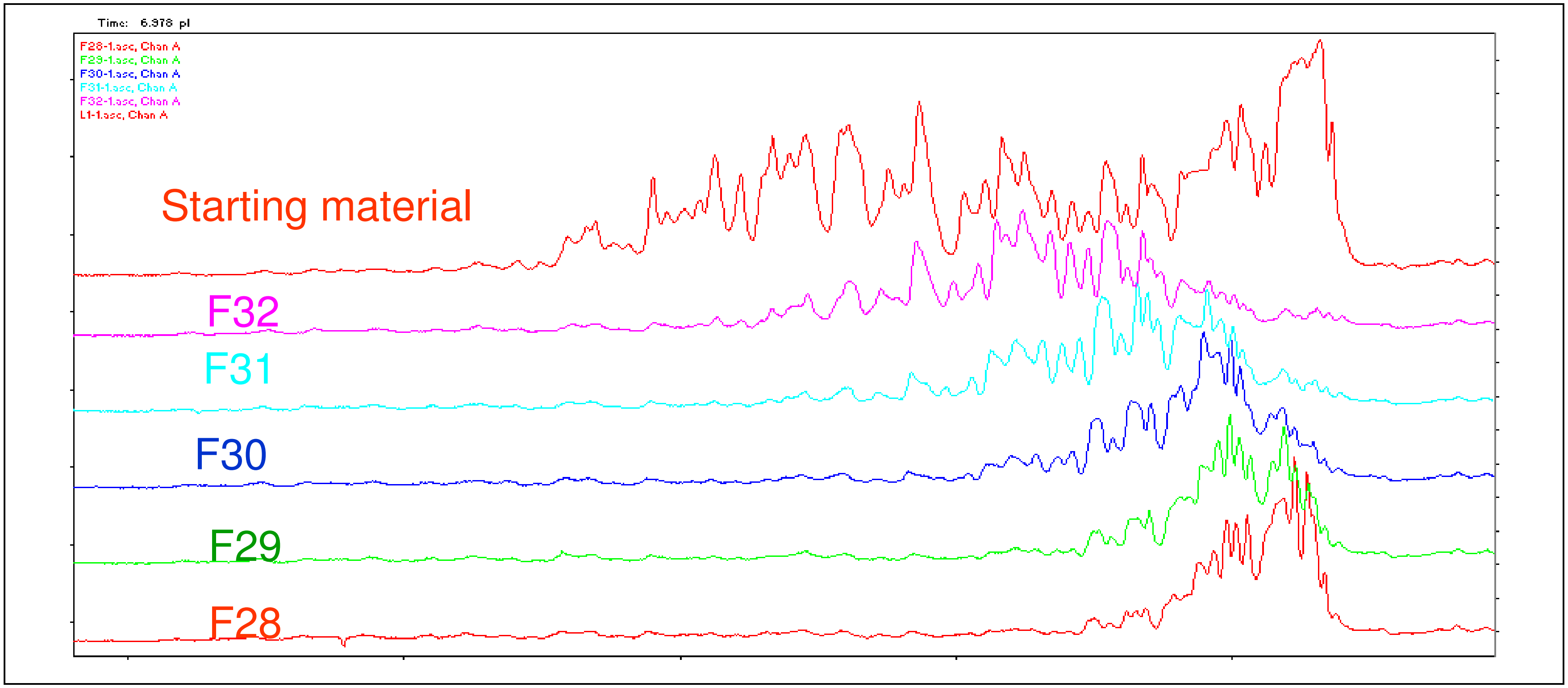



The analysis showed major quality (charge isoform) differences of protein as a function of the cell culture medium and growth conditions.



ZYMOGENETICS

# Imaged cIEF Support Downstream Purification Development




Isolation of specific charge isoform. The iCE280 was instrumental in aiding process development in enriching material to further test in biological assays. Charge profiles for fractions isolation of a Mono Q HR 5/5 anion exchange column are shown.



ZYMOGENETICS

# Imaged cIEF Support Downstream Process Development

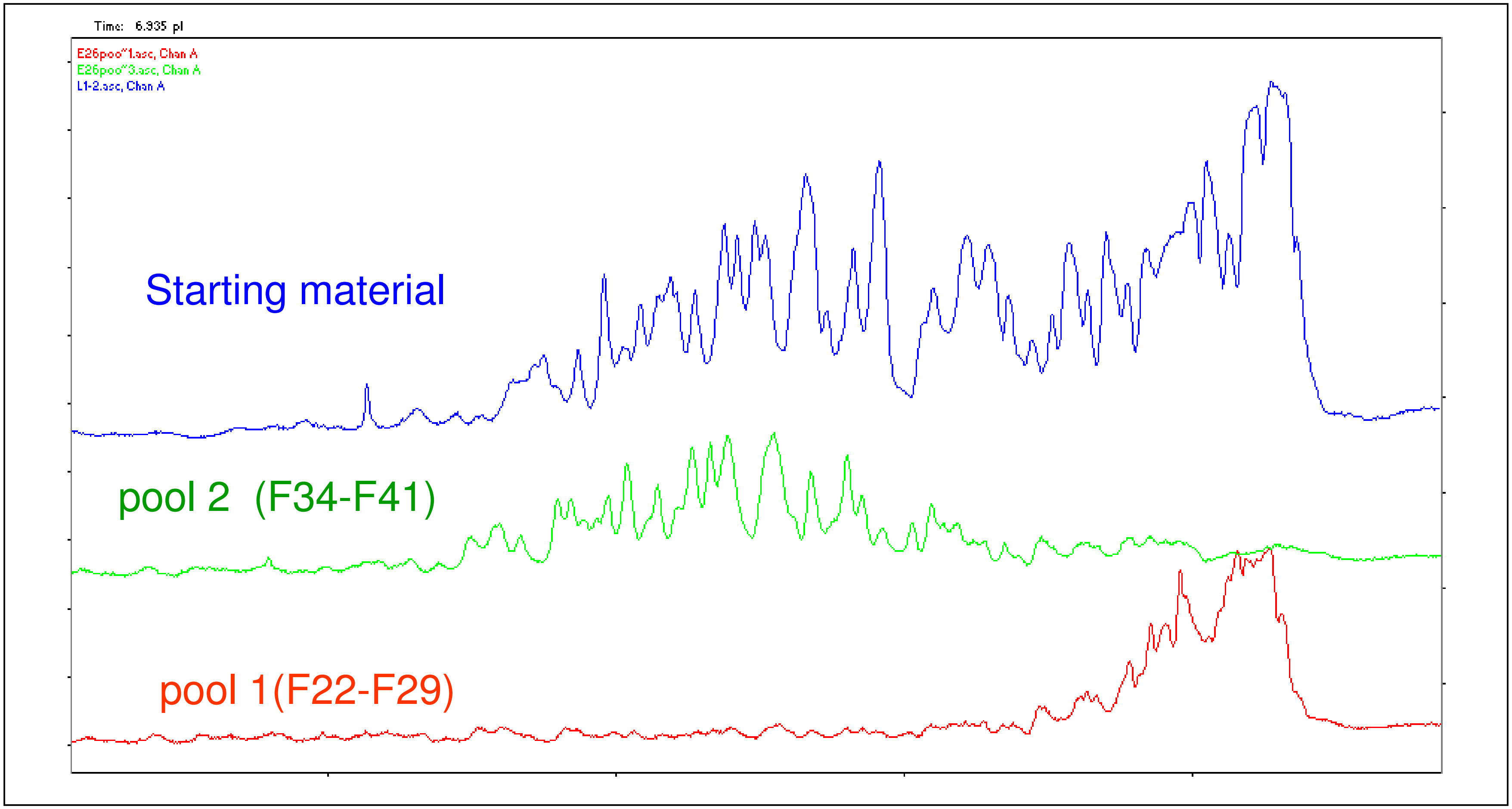



Figure 8B. Resulting iCE profiles generated from mono Q pools of the acidic and basic isoforms according to iCE profiles of fractions.

# Summary of cIEF Method for Fc Fusion Protein

- Overall, a high throughput and reproducible cIEF assay was developed for the charge isoform analysis of a recombinant fusion protein with potential 22 N-glycosylation sites.
- It facilitated the process development of the protein, and was used as a characterization assay for lot release.

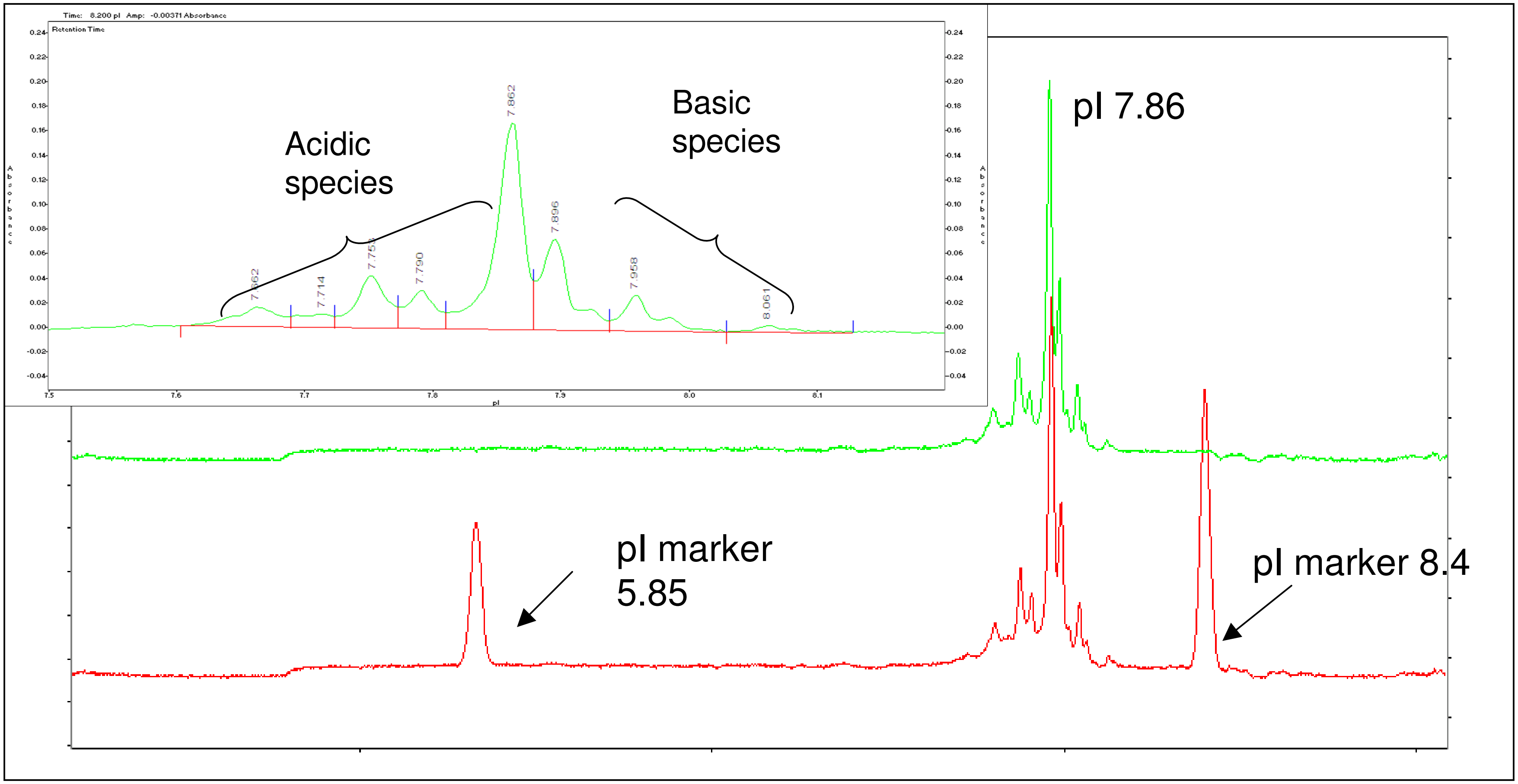
# Applications of Imaged cIEF for Charge Isoform Analysis of a Monoclonal Antibody

ZymoGenetics

# Summary of Method Parameters

- 4% pharmalyte pH 5-8: pH 3-10 (4:1)
- 6 M urea additive to keep protein solubility during focusing
- 0.35% methyl cellulose in sample matrix
- Focusing time 8 minutes
- Focusing voltage 3 Kv
- pI marker 5.85, 8.4
- Final protein concentration ~0.2 mg/mL

# pI Calibrated Imaged cIEF Profile for a mAb



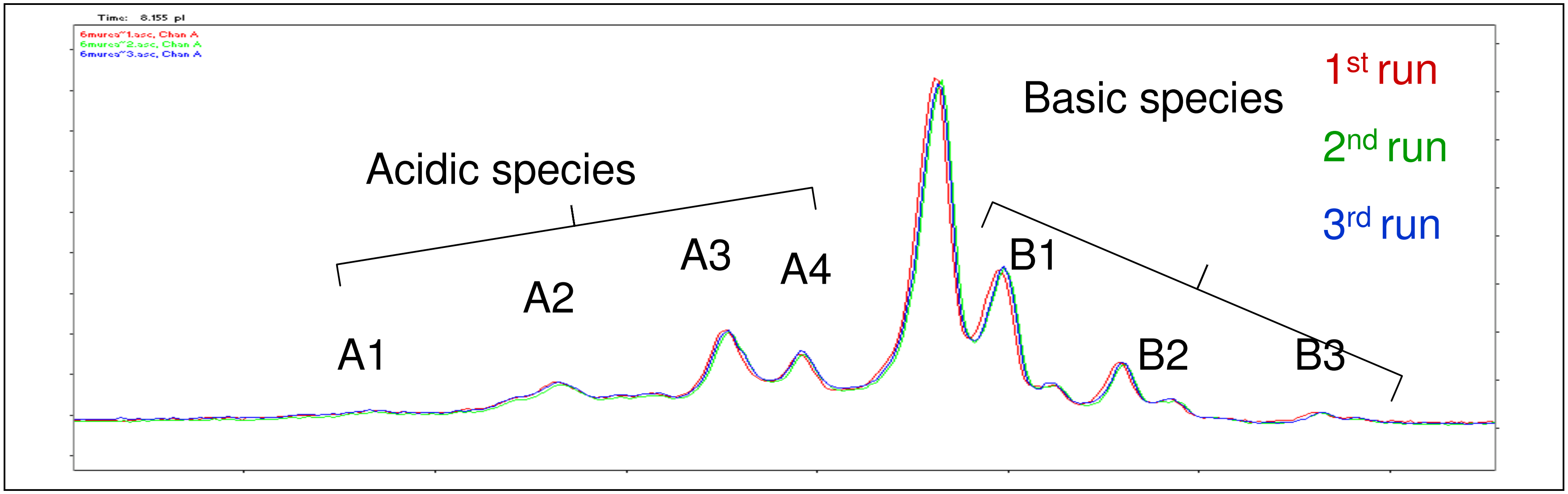

pI range from 7.6-8.06 for mAb components.



ZYMOGENETICS

Method Evaluation

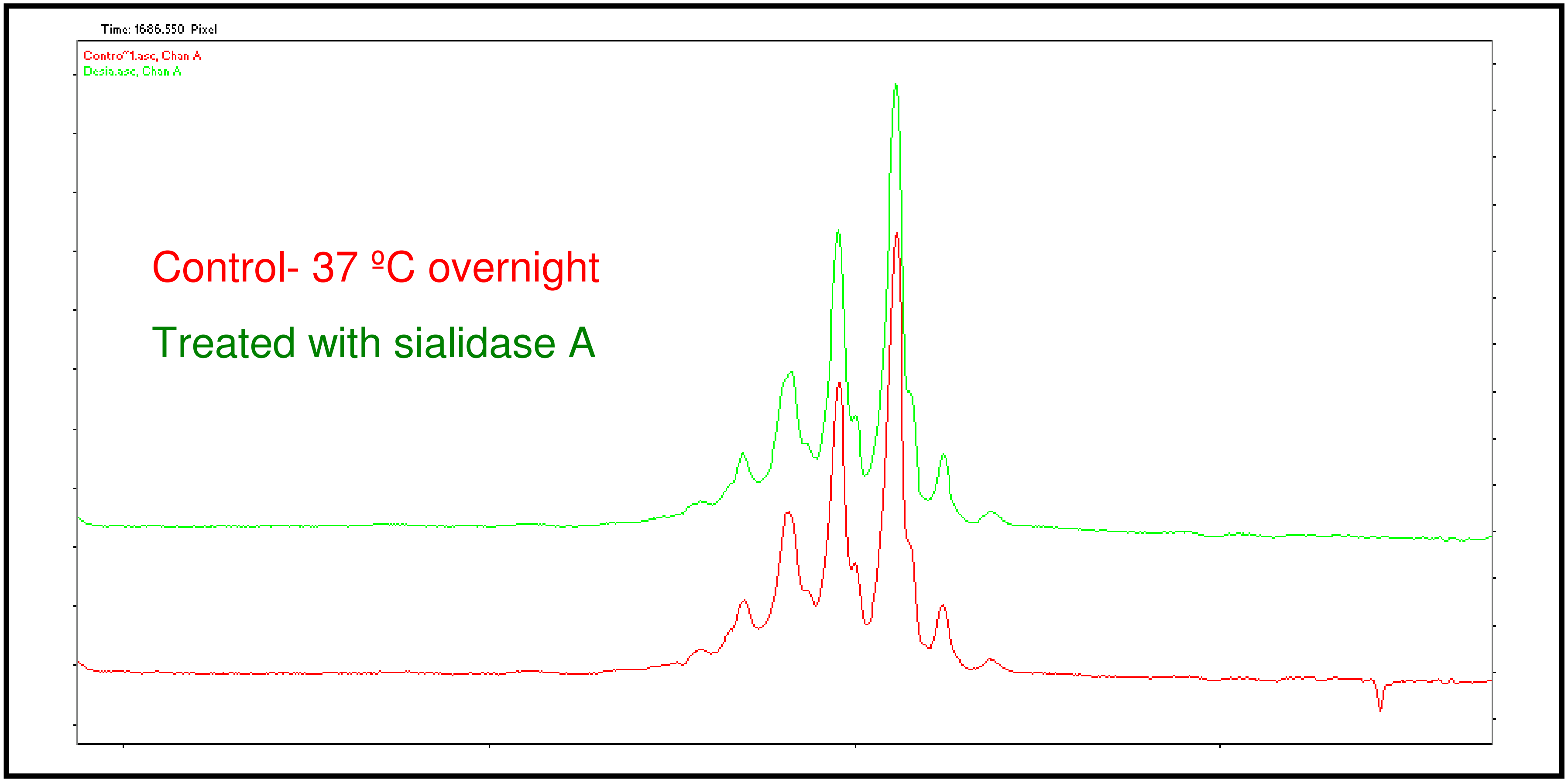
# Assay Reproducibility




| | % Peak Area | | | | pI | | | |
|---|---|---|---|---|---|---|---|---|
| Components | Run 1 | Run 2 | Run 3 | %RSD | Run 1 | Run 2 | Run 3 | STDEV |
| Major peak | 42.0 | 42.4 | 42.4 | 0.5 | 7.86 | 7.86 | 7.86 | 0.0 |
| Total acidic species | 28.6 | 29.0 | 29.4 | 1.4 | | | | |
| A1 | 6.0 | 5.8 | 6.0 | 1.9 | 7.66 | 7.67 | 7.66 | 0.0 |
| A2 | 3.7 | 3.8 | 4.0 | 4.0 | 7.71 | 7.71 | 7.71 | 0.0 |
| A3 | 11.5 | 11.8 | 11.3 | 2.2 | 7.75 | 7.75 | 7.75 | 0.0 |
| A4 | 7.7 | 7.9 | 8.1 | 2.5 | 7.79 | 7.79 | 7.79 | 0.0 |
| Total basic species | 29.4 | 28.6 | 28.0 | 2.5 | | | | |
| B1 | 19.3 | 18.8 | 18.0 | 3.5 | 7.90 | 7.90 | 7.90 | 0.0 |
| B2 | 8.4 | 8.3 | 8.5 | 1.3 | 7.96 | 7.96 | 7.96 | 0.0 |
| B3 | 1.6 | 1.5 | 1.5 | 4.9 | 8.06 | 8.06 | 8.06 | 0.0 |

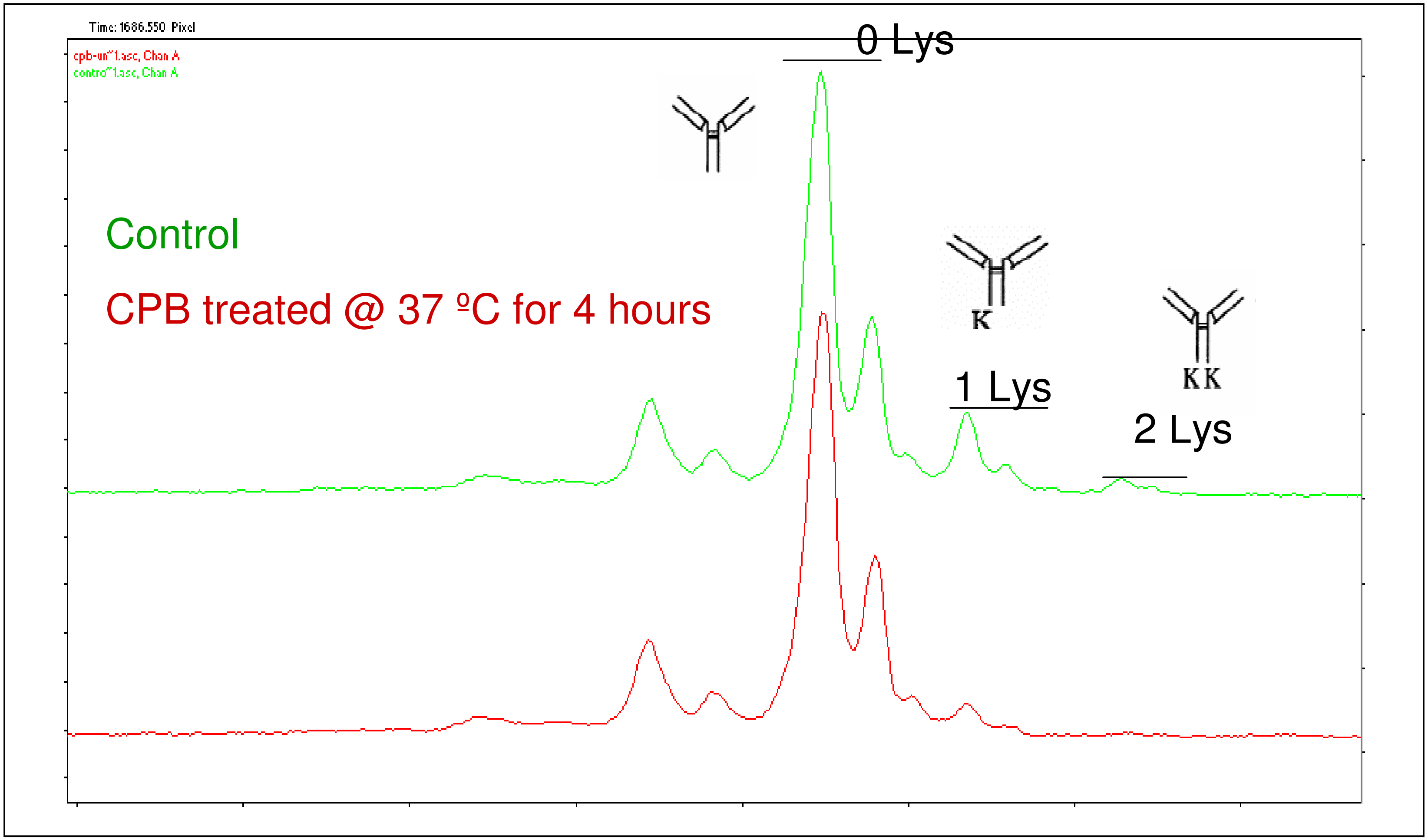
Overall, %RSD < 5.0% for percent peak area. pI of components are very consistent.

# Desialyation to Characterize Acidic Species




There is no apparent changes before and after desialylation, indicating there is no sialic acidic species in mAb. This was confirmed by LC-MS analysis.

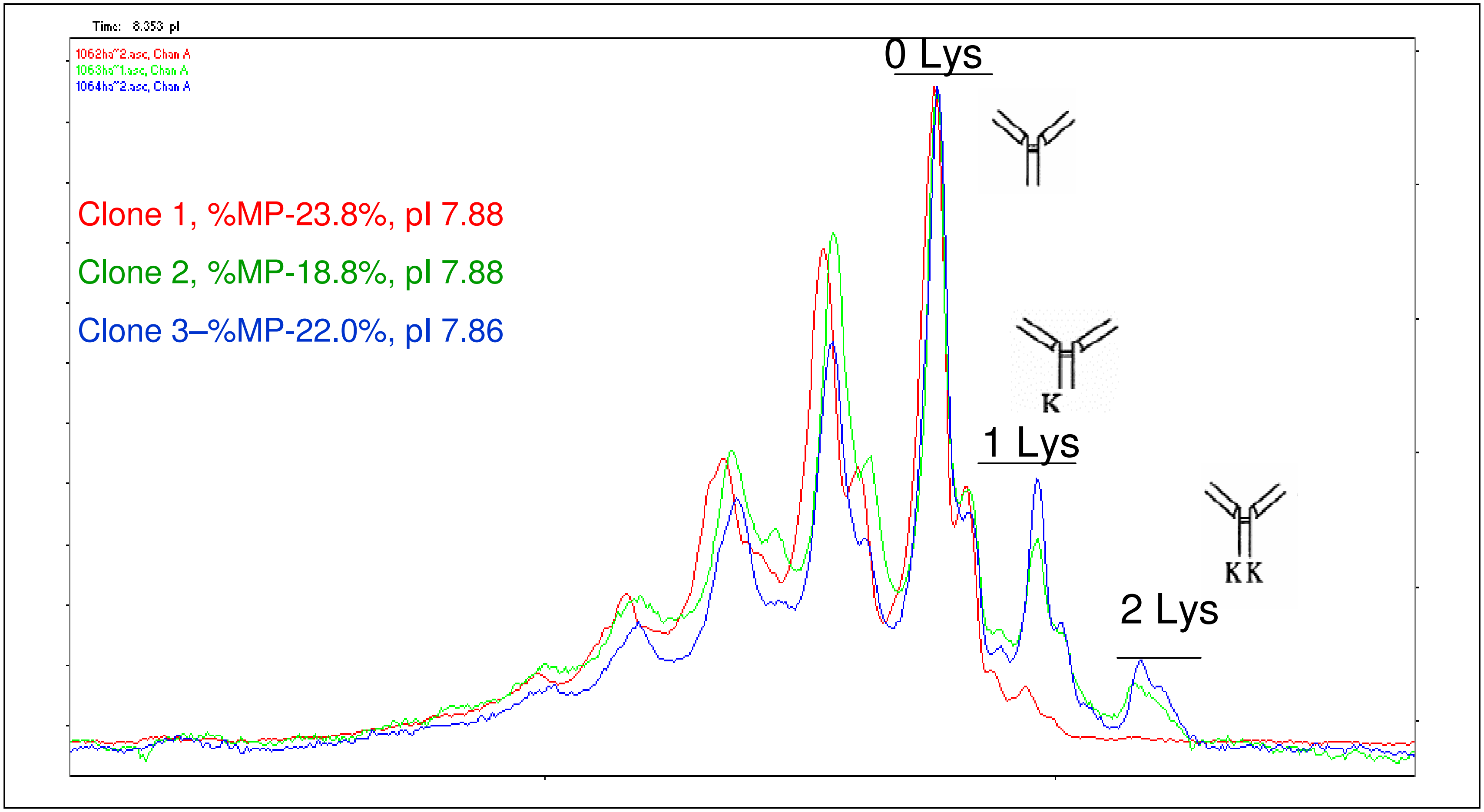
# Carboxypeptidase B (CPB) Enzyme Treatment for Peak Characterization




CPB treatment will cleave the C- terminal Lys of protein chains and the associated peaks will shift towards the acidic region. .

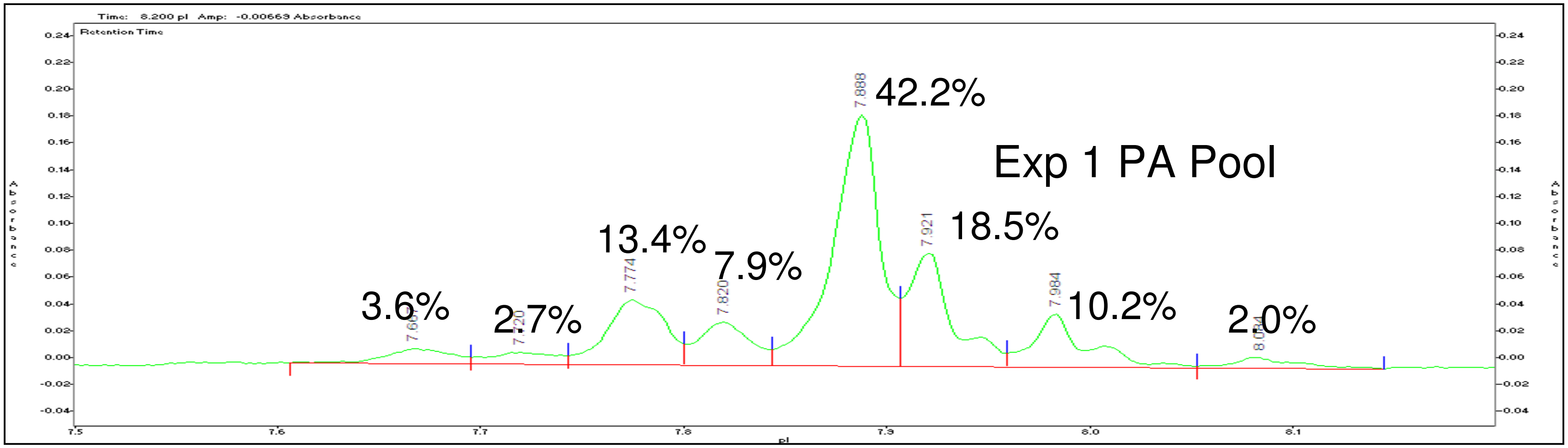
Method Application

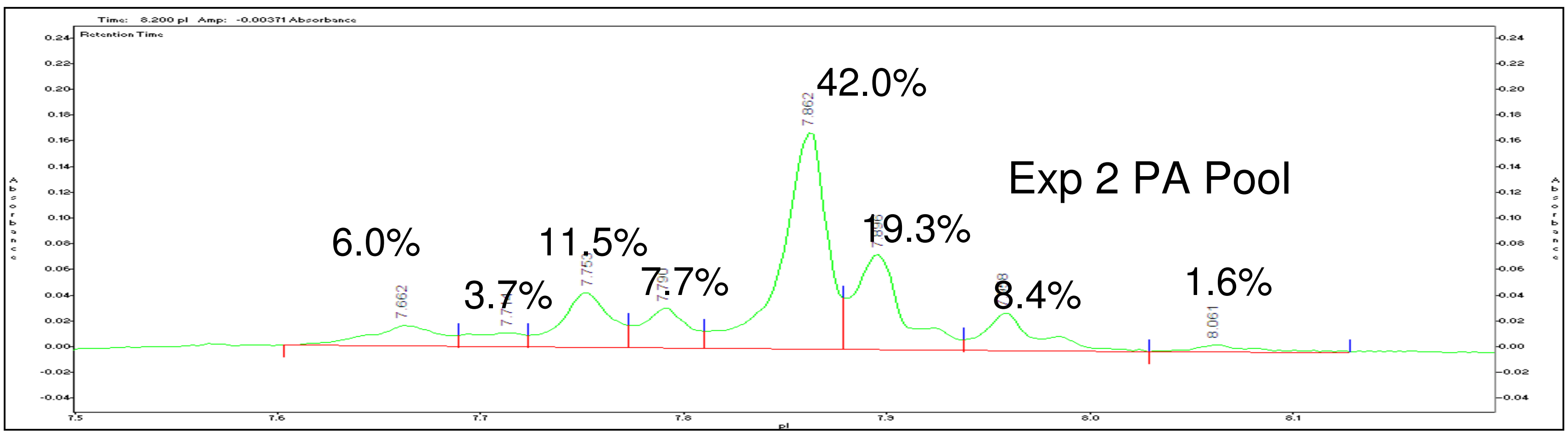
# Imaged cIEF to Support Cell Culture Development




- Overall, some quality differences (charge isoforms) were observed between different clones.
- Clone 1 resulted in mAb molecules with less heterogeneity. The major isoform has no C-terminal lysine variants.

# Imaged cIEF to Support Downstream Process Development




There was no apparent changes for charge isoform distribution.

# Conclusions

- High throughput and reproducible imaged cIEF assays were developed.
- Assays facilitated process development and are being used as characterization tools for lot characterization at ZymoGenetics

# Acknowledgements


- Steve Davin
- Geoff Lee
- Bob Mallet
- Gautam Nayar




ZYMOGENETICS