

# Fast Isoelectric Focusing Method Development with *imaged* CIEF (*i*CIEF) for Protein Analysis

Prepared by Tiemin Huang<sup>1</sup>, Martin Donker<sup>2</sup>, and Gerard Rozing<sup>3</sup>

<sup>1</sup>Advanced Electrophoresis Solutions Ltd., Cambridge, Ontario, Canada

<sup>2</sup>Isogen Bioscience, De Meern, Netherlands

<sup>3</sup>Gerard Rozing, ROZING.COM Consulting, Karlsruhe, Germany

## Outline

- Background & Fundamentals of Capillary Iso-Electric Focusing
- Whole Column Imaging Detection CIEF (*i*CIEF)
- Applications of *i*CIEF in Protein Analysis
- About AES (Advanced Electrophoresis Solutions Ltd.)

## Basics of Capillary IEF



At the beginning of a CIEF run, the whole capillary (coated FS) is filled with:

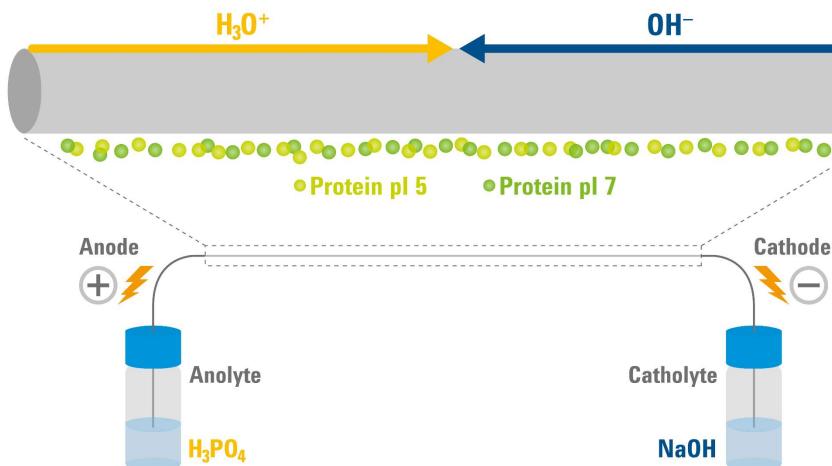
- Ampholytes (mixture of oligomers containing acidic and basic functional groups)
- Protein or peptide sample to be analyzed (ampholytes)
- pI standards (ampholytes)
- Optional additives to increase solubility of proteins
- Sacrificing ampholytes to suppress anodic & cathodic drift and keep the focused zones before the detection zone during focusing
- The inlet low pH (anolyte); the outlet high pH (catholyte)
- High positive voltage in the anolyte vial

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## Basic Principles of CIEF



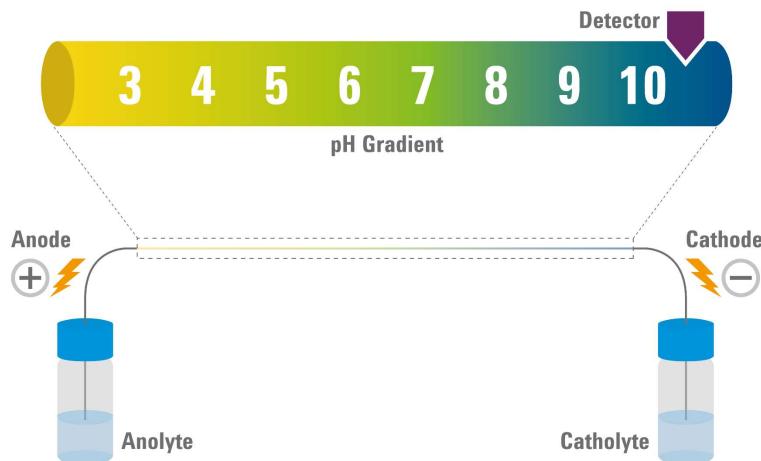
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## Basic Principles of CIEF



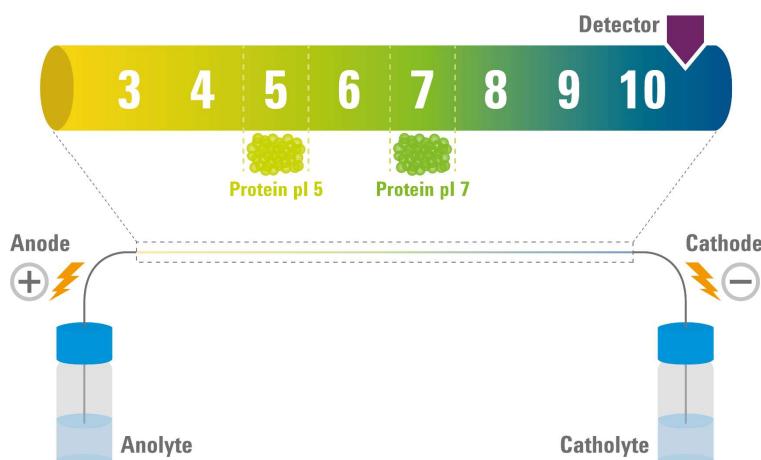
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## Basic Principles of CIEF



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## Basic Principles of CIEF

### Chemical Mobilization



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## Basic Principles of CIEF

### Chemical Mobilization



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## Conventional cIEF

### Pro's

- Uses existing commercial CE instruments
- Provides quantitative protein analysis in applicable concentration range (0.05 – 0.5 mg/mL)

### Con's

- Long cycle time (focusing & mobilization takes about 1 run/hour)
- Time-consuming method development
- Additives to suppress anodic & cathodic drift
- Non-reproducible peak pattern
- Resolution loss during mobilization step



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## Imaged CIEF

- First described by Pawliszyn and Wu, Anal. Chem. 1992, 64, 224-227
- Commercialized by Convergent Bioscience in 2000.
- Established market leader Protein Simple, ICE2 and ICE3
- Single vendor, undesirable situation for users

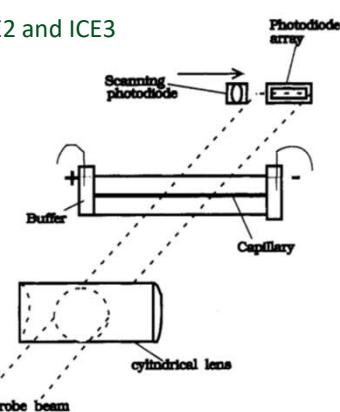


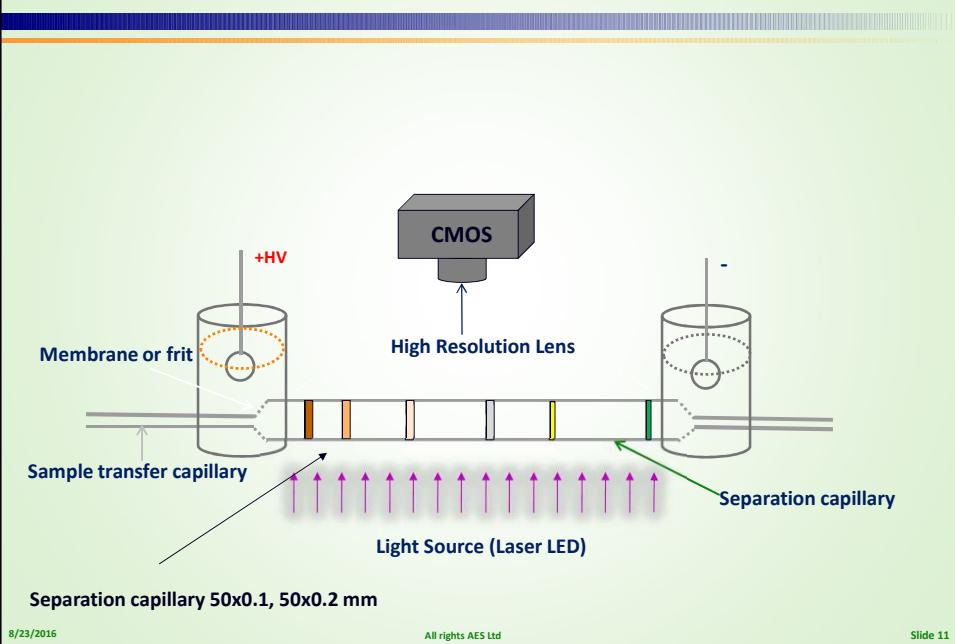
Figure 1. Diagram demonstrating the cartridge holding the capillary, and the probe beam alignment.

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## CEInfinite for *i*CIEF



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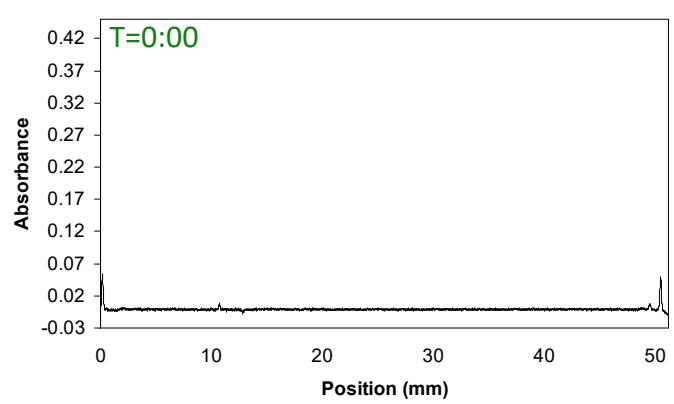
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## Development of Separation of five pI Markers

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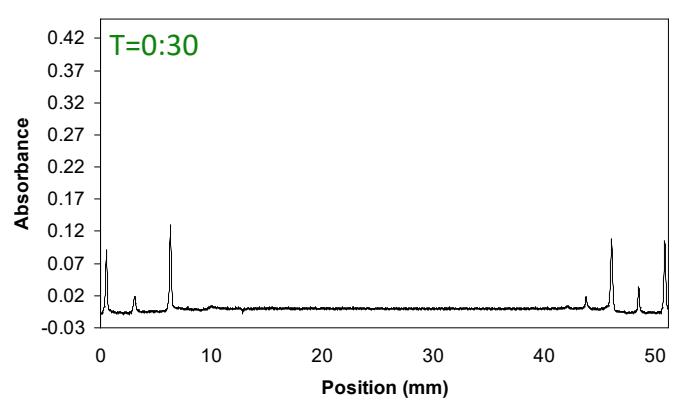
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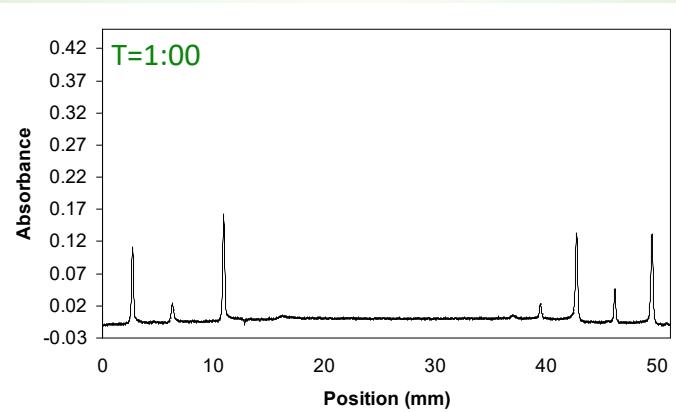
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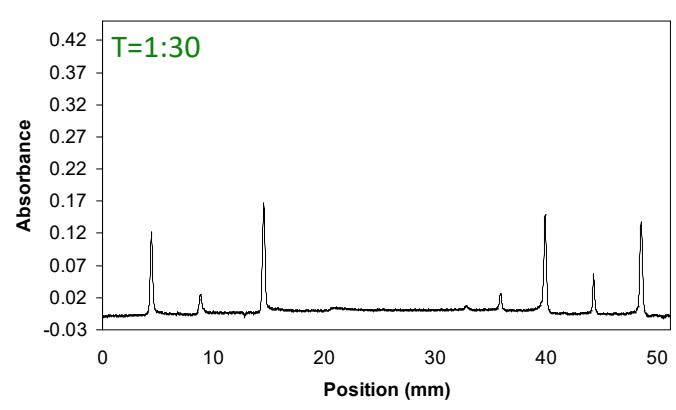
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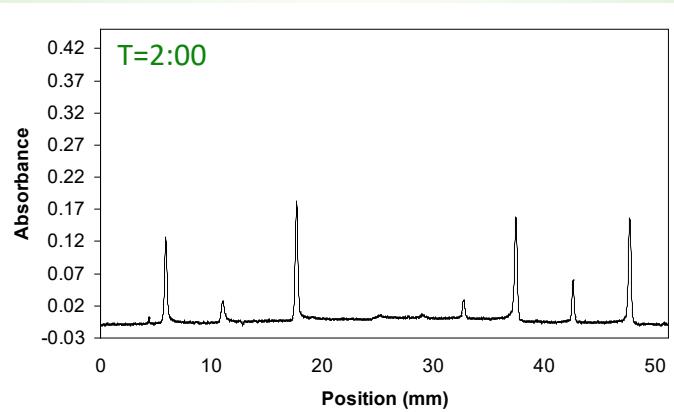
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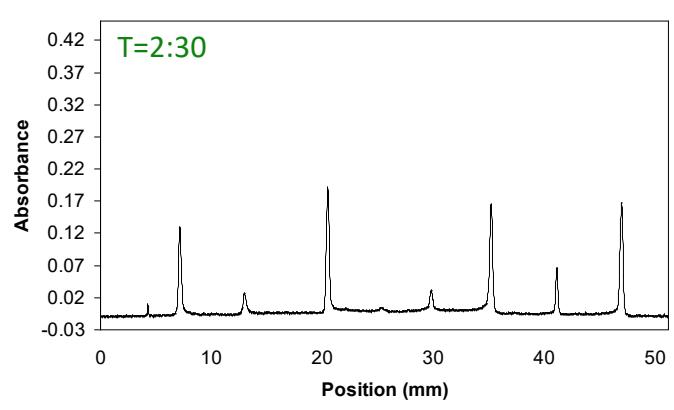
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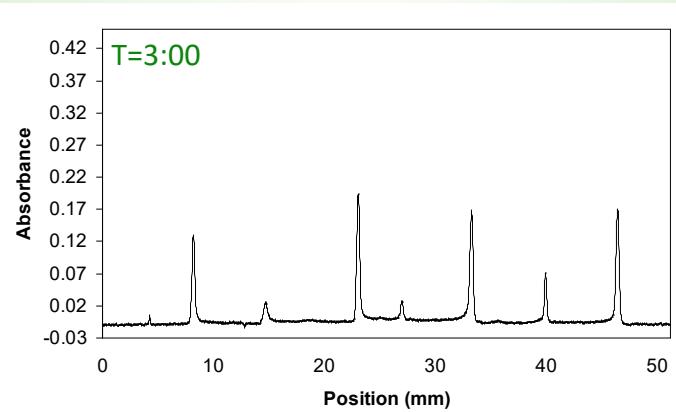
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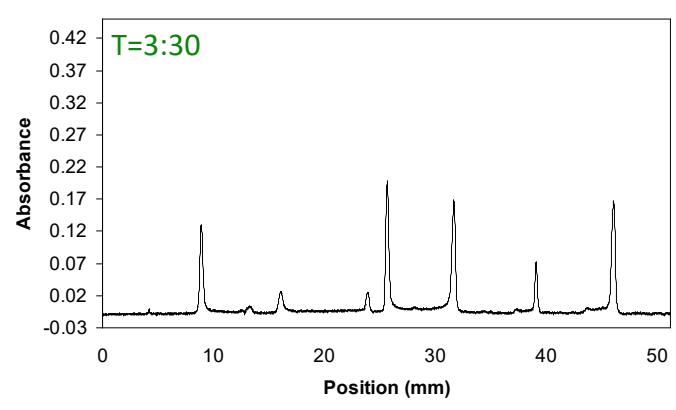
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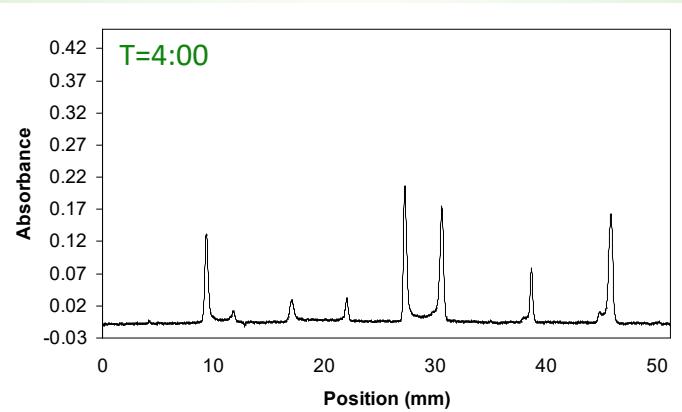
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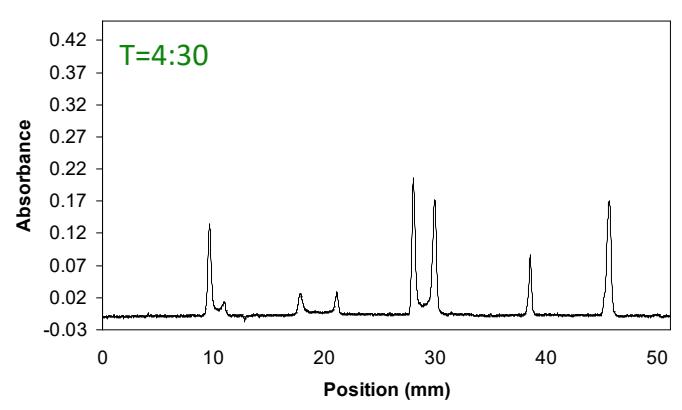
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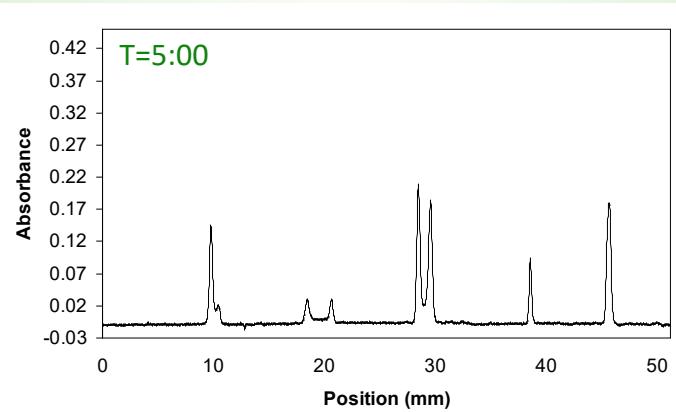
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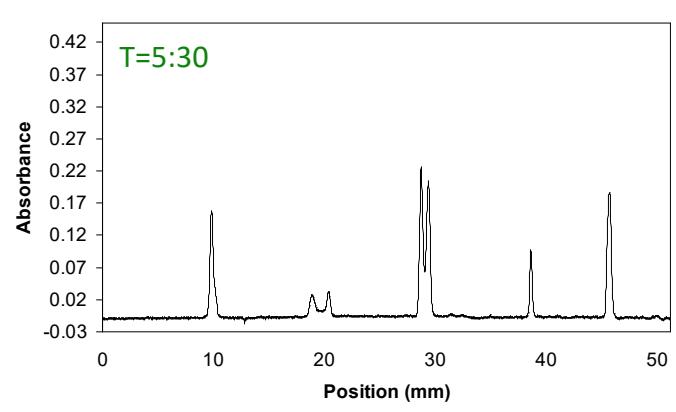
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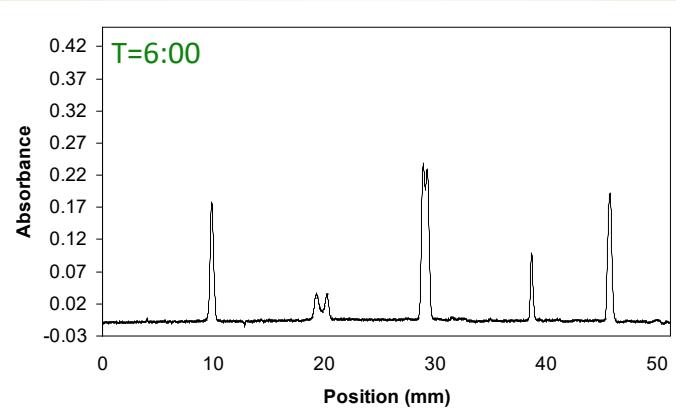
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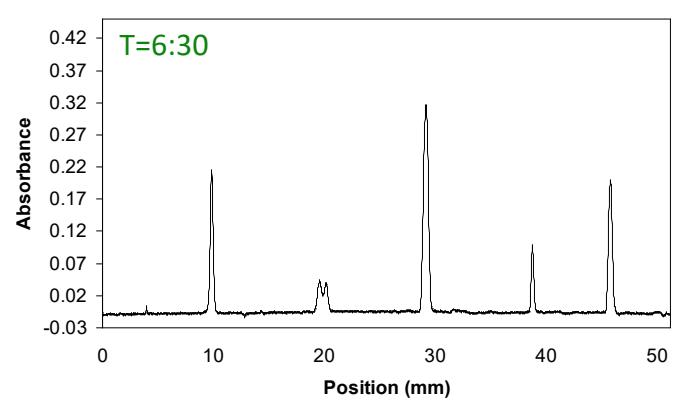
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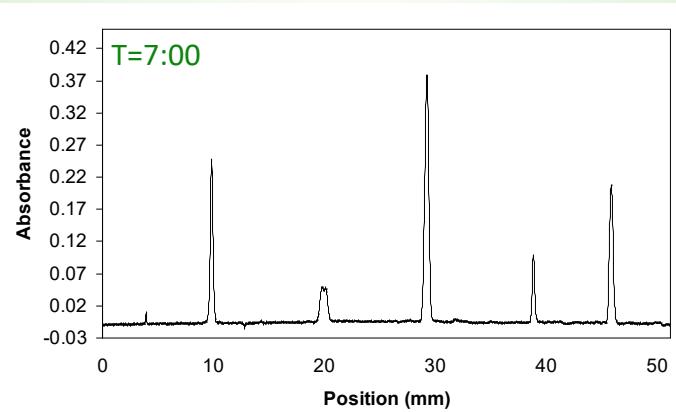
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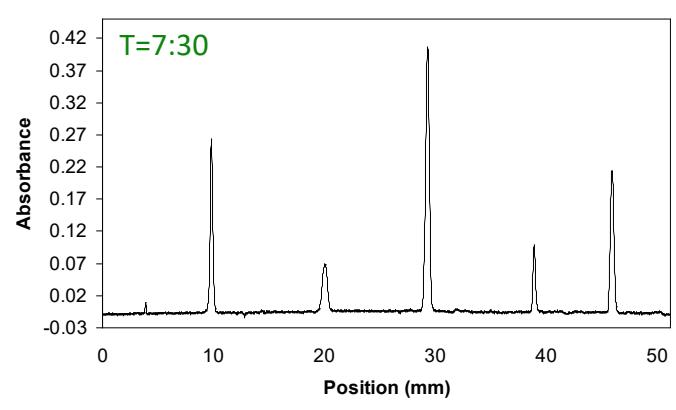
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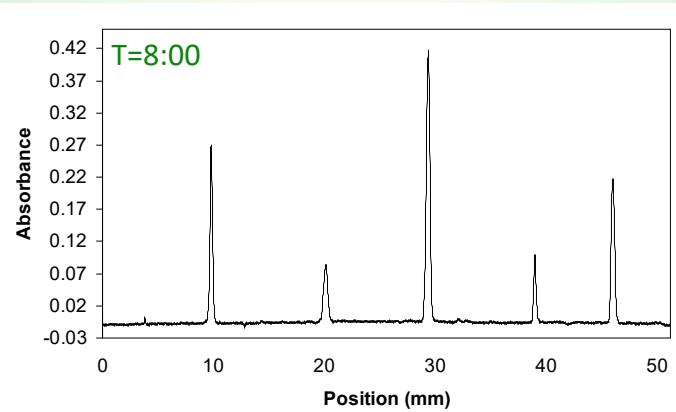
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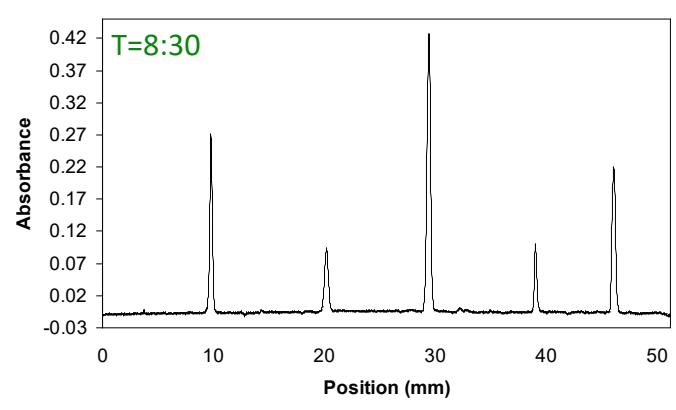
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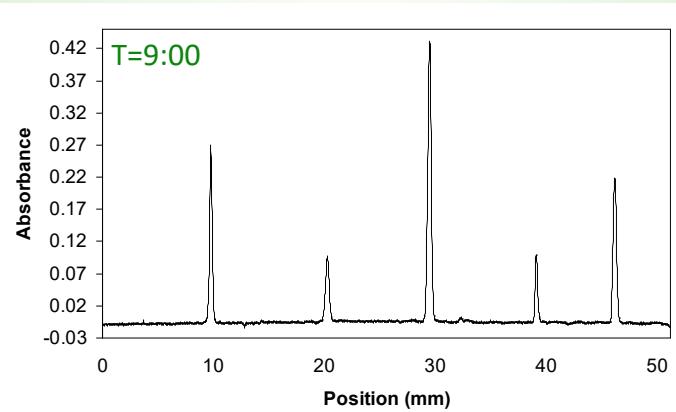
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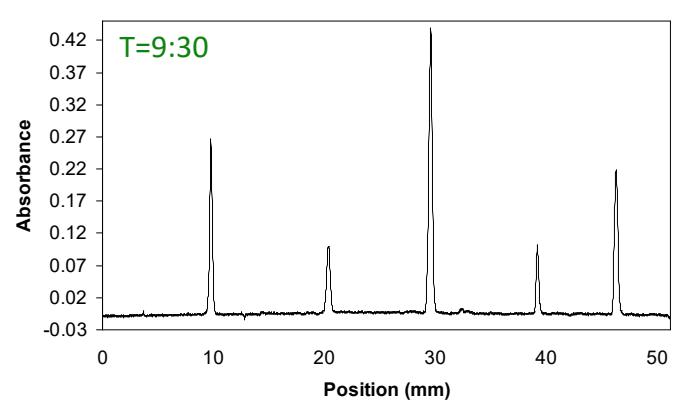
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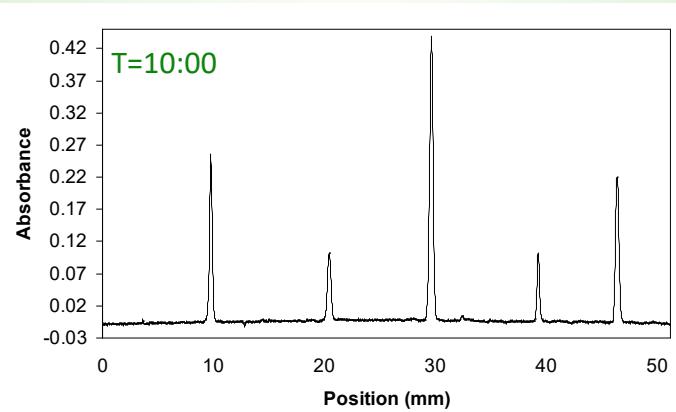
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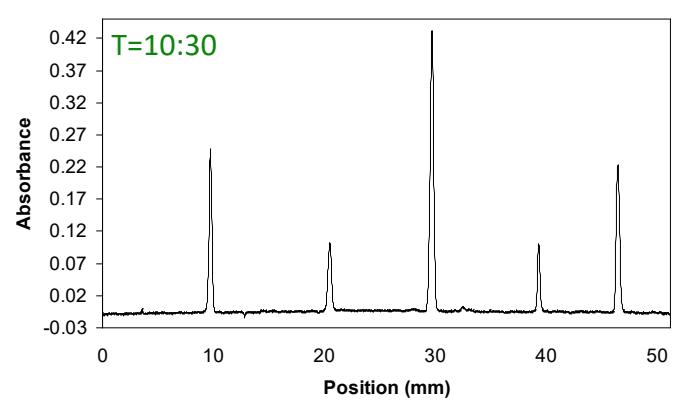
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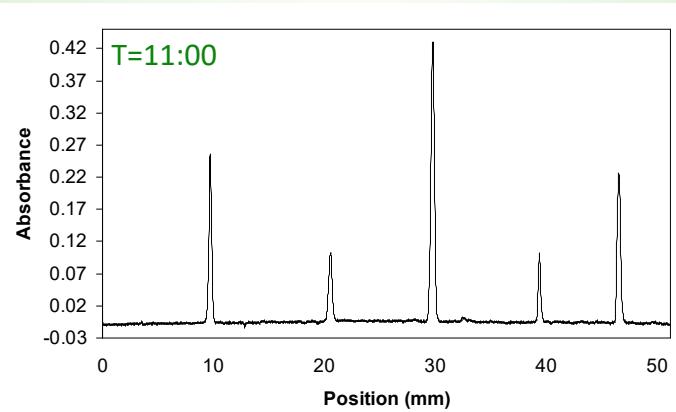
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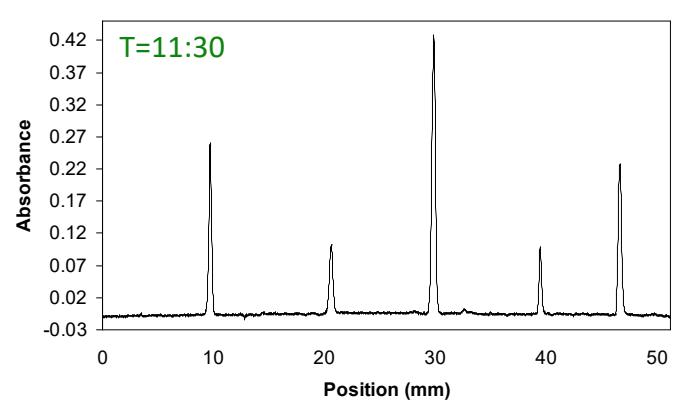
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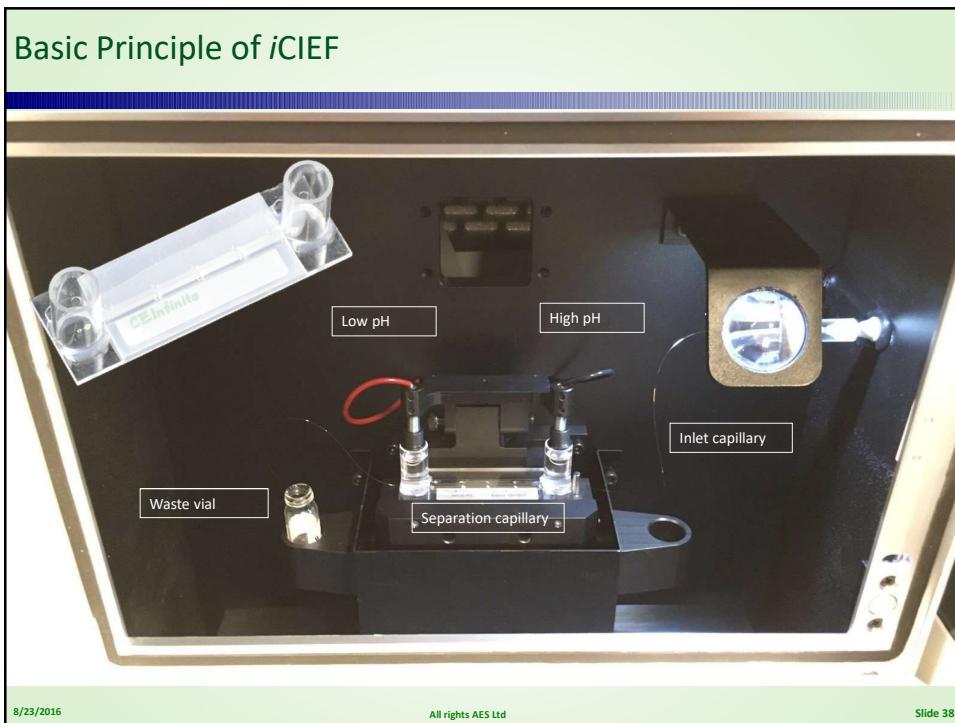
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## The Cartridge



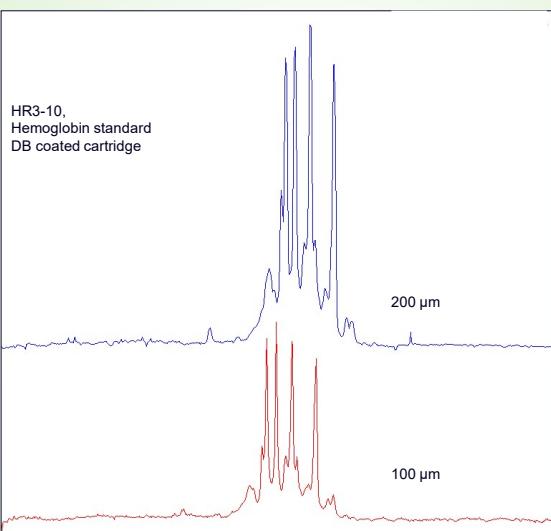
Available in  
100 and 200 µm i.d.

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## High sensitive cartridge



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## CEInfinite by Advance Electrophoresis Solutions Ltd.

### Definition

- CE analyzer specially designed for cIEF – CEInfinite

### Advantages

- Provides quantitative protein analysis
- High resolution –  $\Delta pI \geq 0.02$  units
- Short analysis time (up to 6 runs/hour)
- Fast method development
- Reproducible peak pattern
- Proprietary pI markers and ampholytes with wide pI range (pH 2-11)
- Method development support and services

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## Major Fields of Application

### iCIEF versatile, essential separation method

- pI measurement
- Assessment of charge heterogeneity, purity
- Determination of PTMs or degradation, de-amidation (Asn, Gln), oxidation(Met, Cys, Trp, Tyr) of recombinant proteins, mABs or other biopharmaceuticals
- Quality control in protein biologics production.
- Identify and characterize allergenic proteins in food products

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## iCIEF Method Development Guidelines

- Carrier ampholytes
  - 4% pH3-10 AESlyte
- Sample concentration
  - 0.1 mg/ml for single major peak sample, >0.1 mg for samples with multiple major peaks
- Additives
  - 0.35% methyl cellulose
- Focusing voltage and time
  - ~600 v/cm
  - 6-8 minutes for wide pH range carrier ampholytes
  - Always use pre focusing

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## Easy Focusing Time Optimization iCIEF

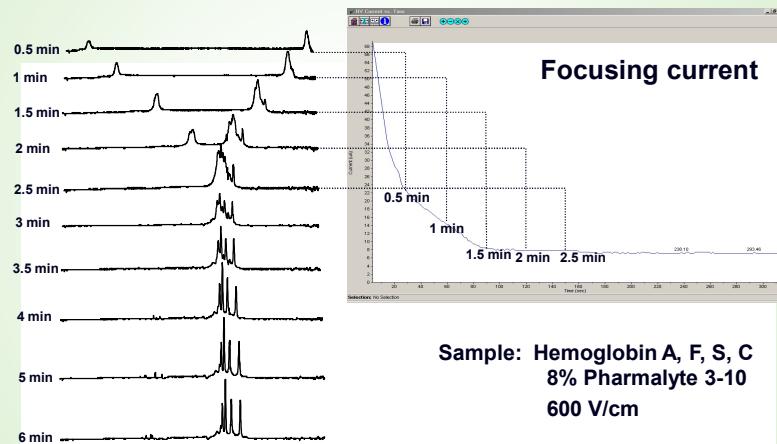
- Optimization of focusing time by a single run
  - The whole-column detector monitors the IEF process within the separation column
  - IEF process can be stopped at any time when the focusing is complete and the peak pattern is stable
  - Sample aggregation and precipitation during IEF can be identified
  - Additives are selected according to the identified problems

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## Focusing Time



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## Narrow pH Range Carrier Ampholytes

- Use narrow pH range carrier ampholytes to improve resolution
- The narrow pH range carrier ampholytes can be used alone or mixed with wide pH range carrier ampholytes

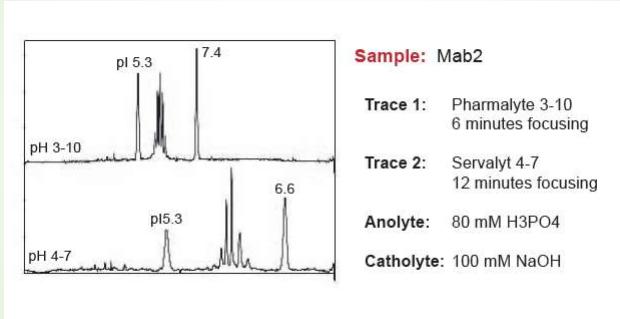
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## Narrow pH Range Carrier Ampholytes

### Acidic Mab



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## Recombinant Proteins

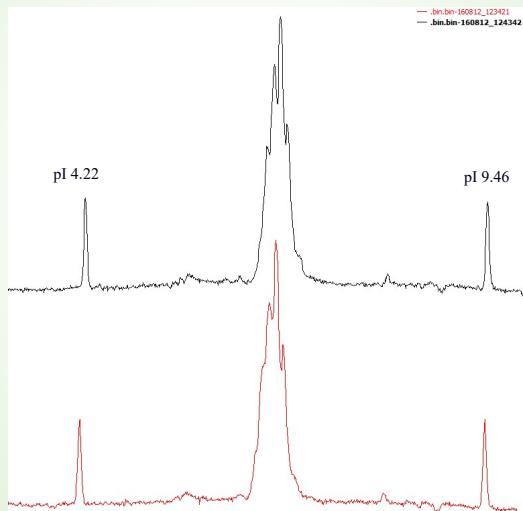
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## IgG, pI 6.76



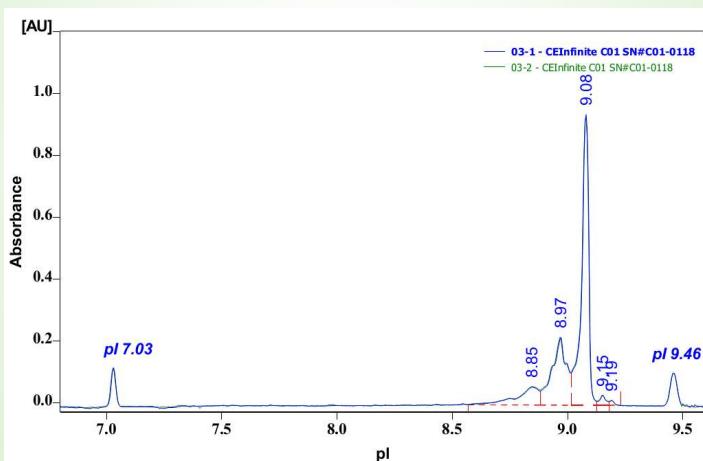
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## Trastuzumab (Herceptin)

Overlay of 2 analyses



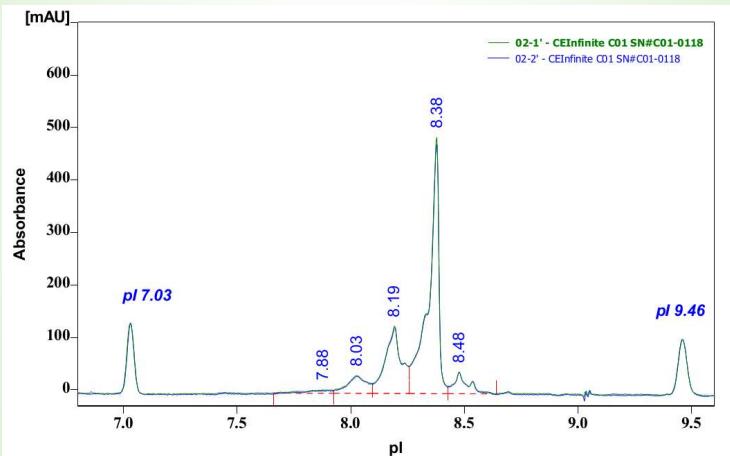
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## Bevacizumab (Avastin)

Overlay of 2 analyses



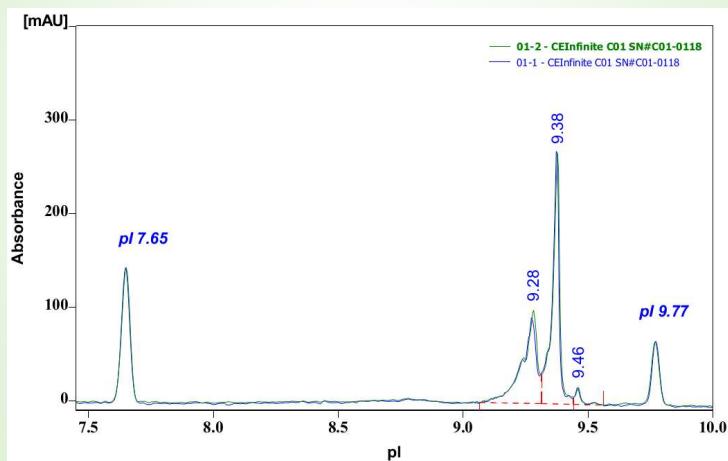
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## Rituximab (Rituxan)

Overlay of 2 analyses



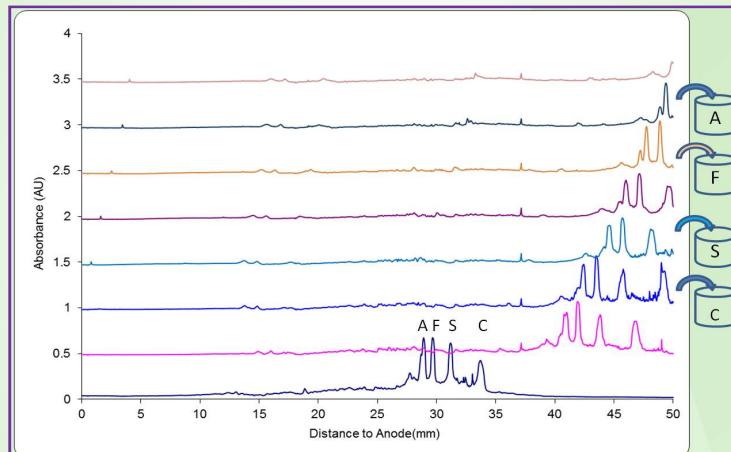
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## Other Applications

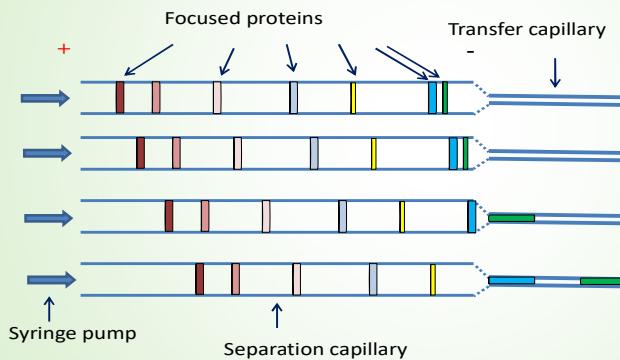
### Preparative iCIEF\*



\*patent pending

## Preparative iCIEF

Changing the diameter with a factor of 4 will increase the length of the peak by a factor of 16 in the outlet capillary

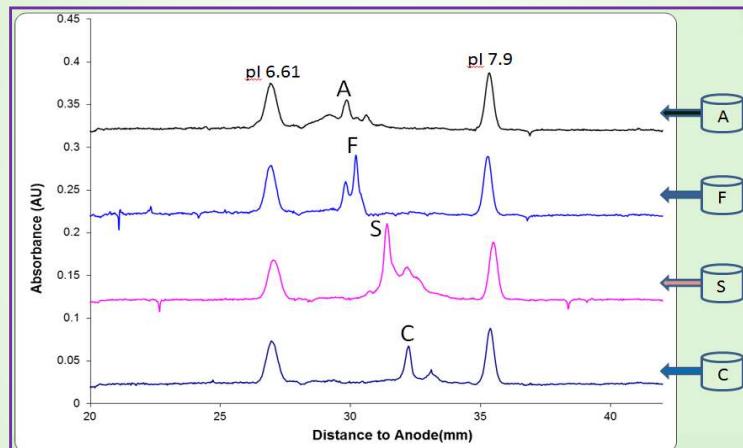


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## Preparative iCIEF - Reinjection



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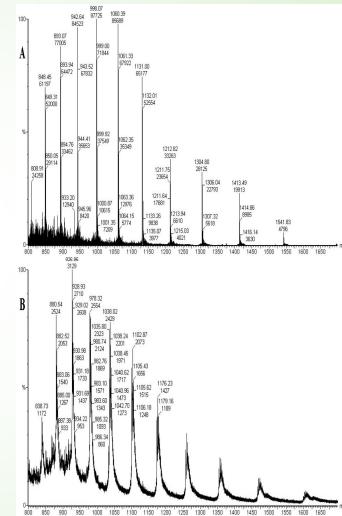
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## iCIEF Coupling with MS

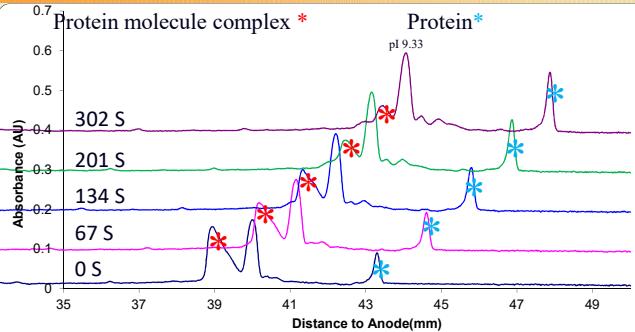


MALDI

ESI



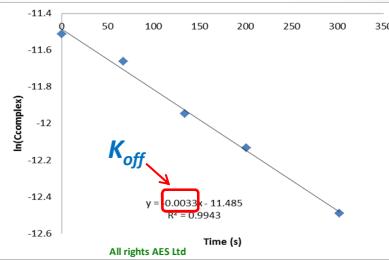
## Determination of Binding Rates and Constant



### Calculation method for KD

1. The initial protein concentration was 0.0001 M, and small molecule at 0.0001 M.
2. The equilibrium protein concentration can be calculated 0.00009M.
3. The equilibrium small molecule concentration was calculated 0.00009M.
4. The  $K_d$  can be calculated to be  $8.1 \times 10^{-4}$  M, and  $K_b 1.2 \times 10^3$  M<sup>-1</sup>

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Calculation of  $K_d$  and  $k_{on}$ ,  $k_{off}$  can be done with a novel method.

1. Make protein and small molecule solution in IEF solution with protein at 0.0001 M, and small molecule at 0.0001 M.
2. The increase of protein peak and decrease of protein complex were observed in the E-gram.

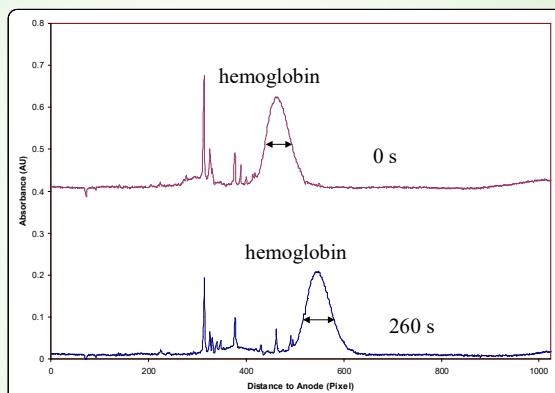
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## Determination of hydrodynamic radius

$$W_h = 2.355\sigma$$

$$D = \frac{R_c^2(t_2 - t_1)}{24(\sigma_z^2 - \sigma_y^2)}$$

$$R_h = \frac{4k_B T (\tau_2^2 - \tau_1^2)}{\pi \eta r^3 (t_2 - t_1)}$$



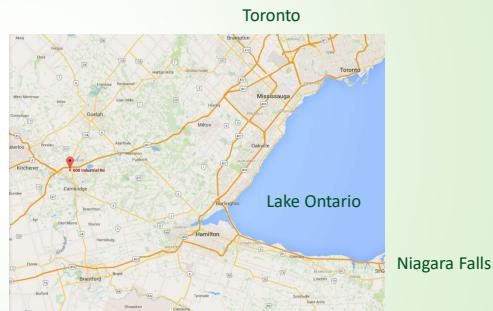
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## About AES

- Founded in 2010 by former Convergent Bioscience employees.
- As the CEO, Dr. Huang is the (co-)inventor of patents for whole column image detection CIEF.
- Currently based in Canada's Technology Triangle, Cambridge, Ontario
- Core technology WCID CE instrumentation, proprietary capillary cartridges, ampholytes, pI standards and buffers
- iCIEF based total solution provider for life science research and biopharmaceutical industry



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## More Information and Contact

Product Website  
[ceinfinite.com](http://ceinfinite.com)

USA and Canada

**Advanced Electrophoresis Solutions Ltd.**  
1600 Industrial Road, Cambridge, ON N3H 4W5, Canada  
Dr. Tiemin Huang  
✉ +1 (519) 653-6888      ✉ [info@aeslifesciences.com](mailto:info@aeslifesciences.com)

Europe

**IsoGen Life Science**  
Veldzigt 2a, NL 3454 PW De Meern, The Netherlands  
Mr. Martin Donker  
✉ +31 30 6880771      ✉ [info@isogen-lifescience.com](mailto:info@isogen-lifescience.com)

**ROZING.COM Consulting**

Gerberastrasse 2, DE 76228 Karlsruhe, Germany  
Dr. Gerard Rozing  
✉ +49 721 47639816      ✉ [gerard@rozing.com](mailto:gerard@rozing.com)

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