

## **CEInfinite** :

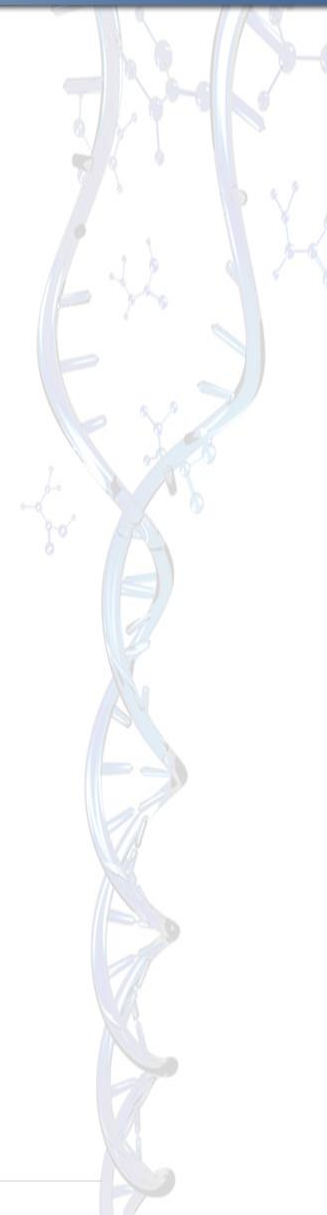
- Faster
- More sensitive

CIEF analyses

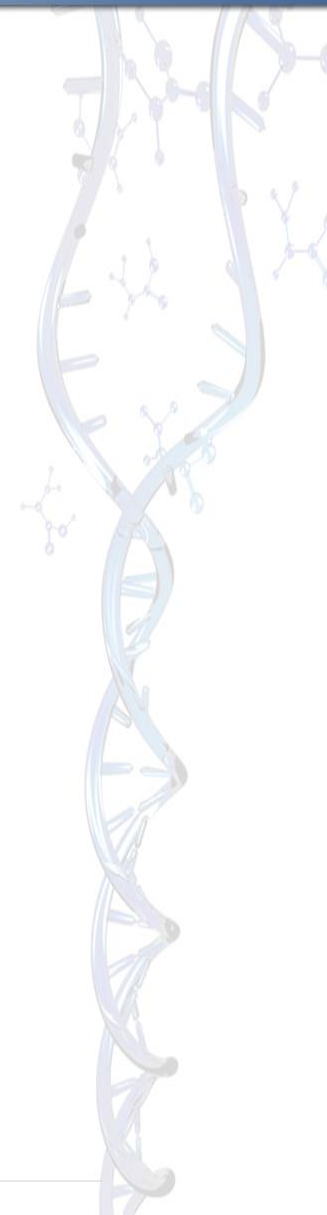
Now also available in  
***preparative*** configuration



- Conventional CIEF vs *i*CIEF
- CEInfinite – novelties:
  1. Improved sensitivity
  2. Higher sample throughput
  3. Preparative *i*CIEF

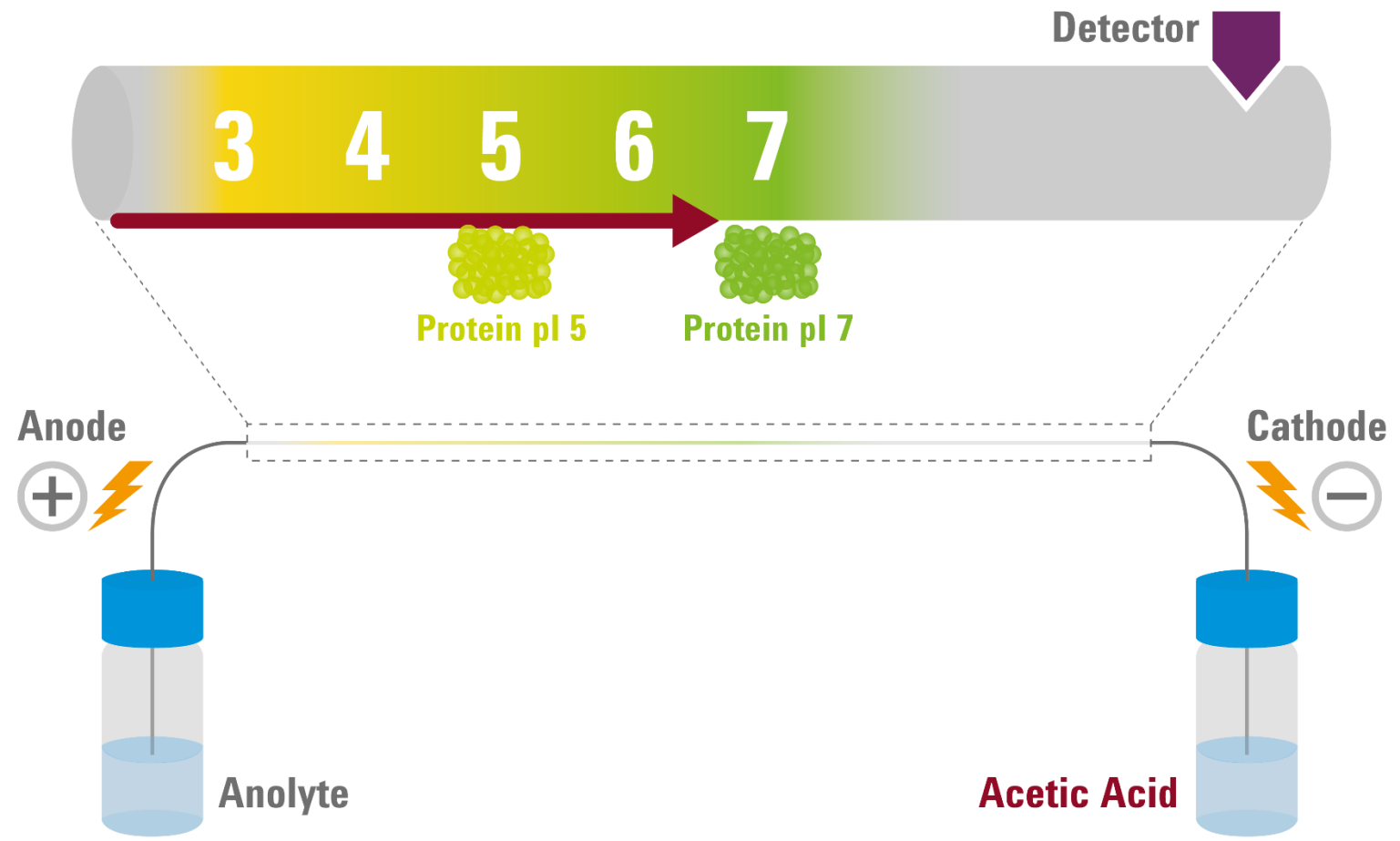


- What?
  - Charge-based characterisation of proteins, pI range 2.5-11
    - ✓ VLP, mAb, ADC, fusion proteins, peptides, ...
  - Lot release & stability analysis
  - Structural & degradation pathway characterisation
  - Kinetic & dynamic studies
- For whom?
  - QA/QC, analytical & process development



# Conventional CIEF

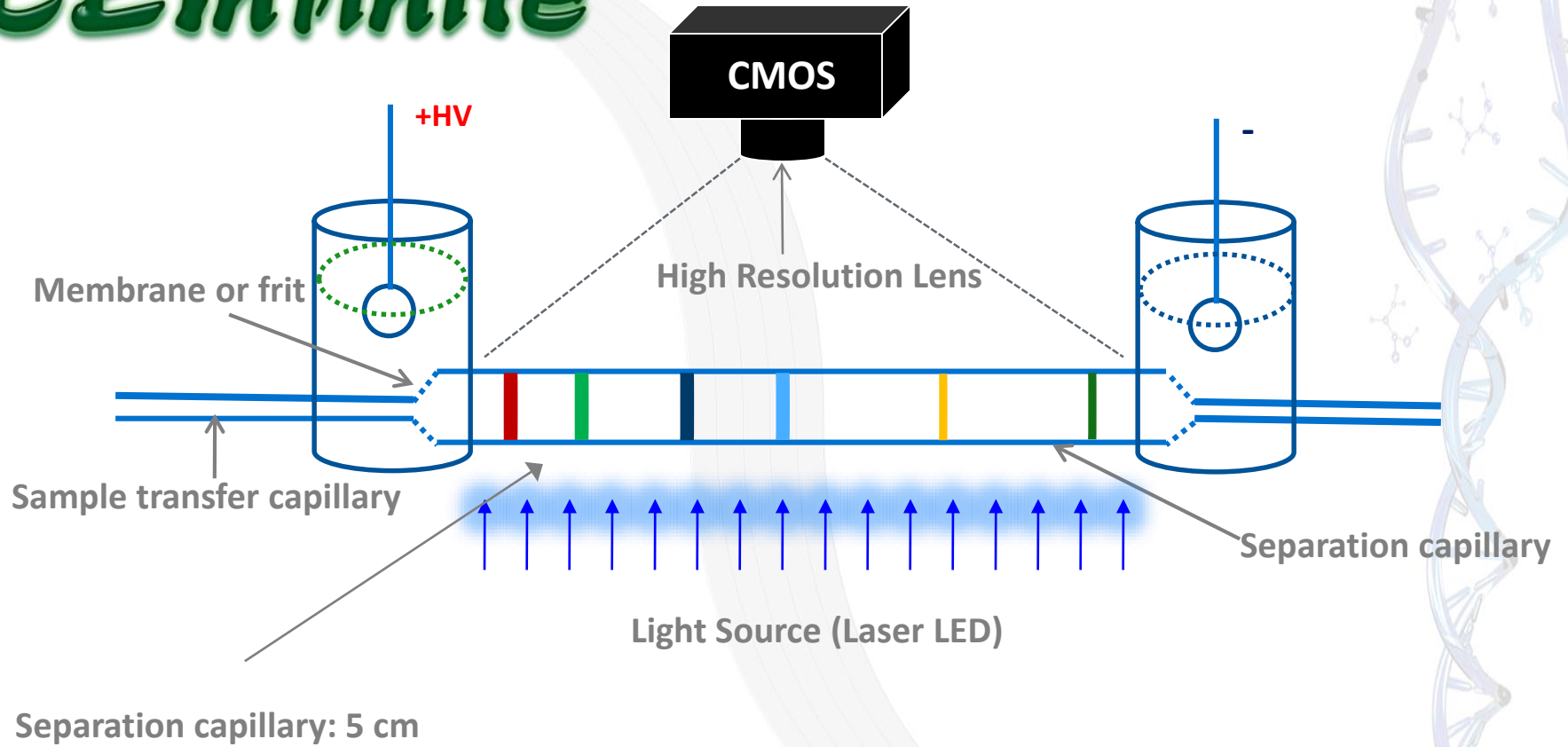
the innovator for science



Slide courtesy of Agilent Technologies

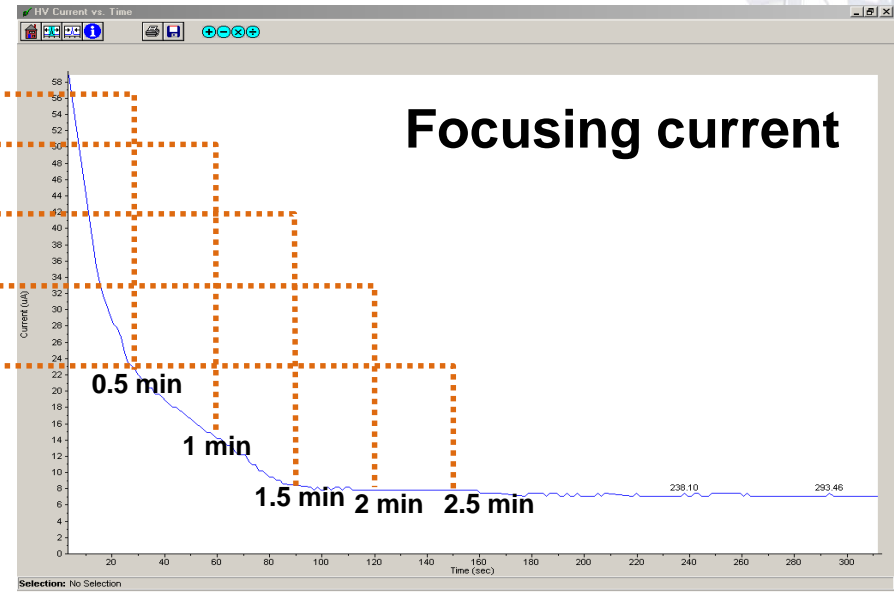
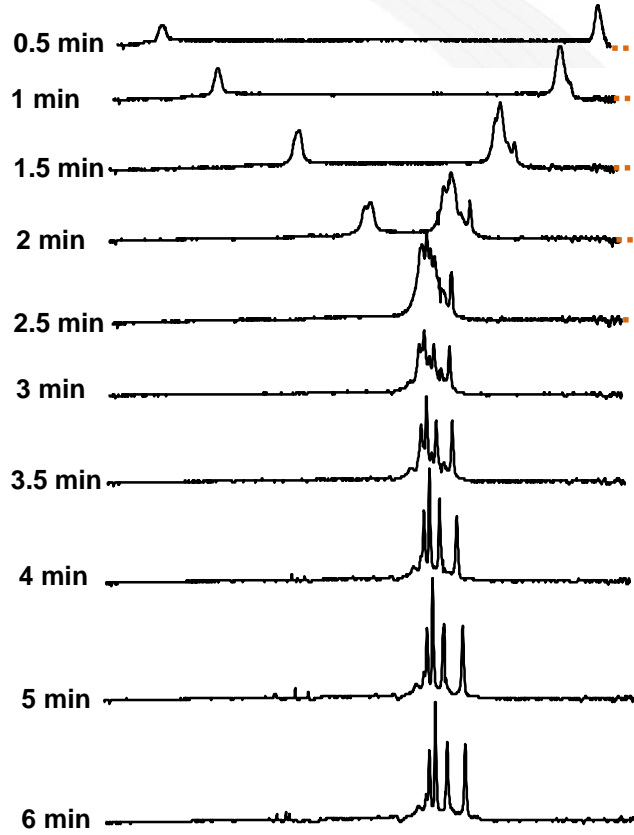


# CE Infinite



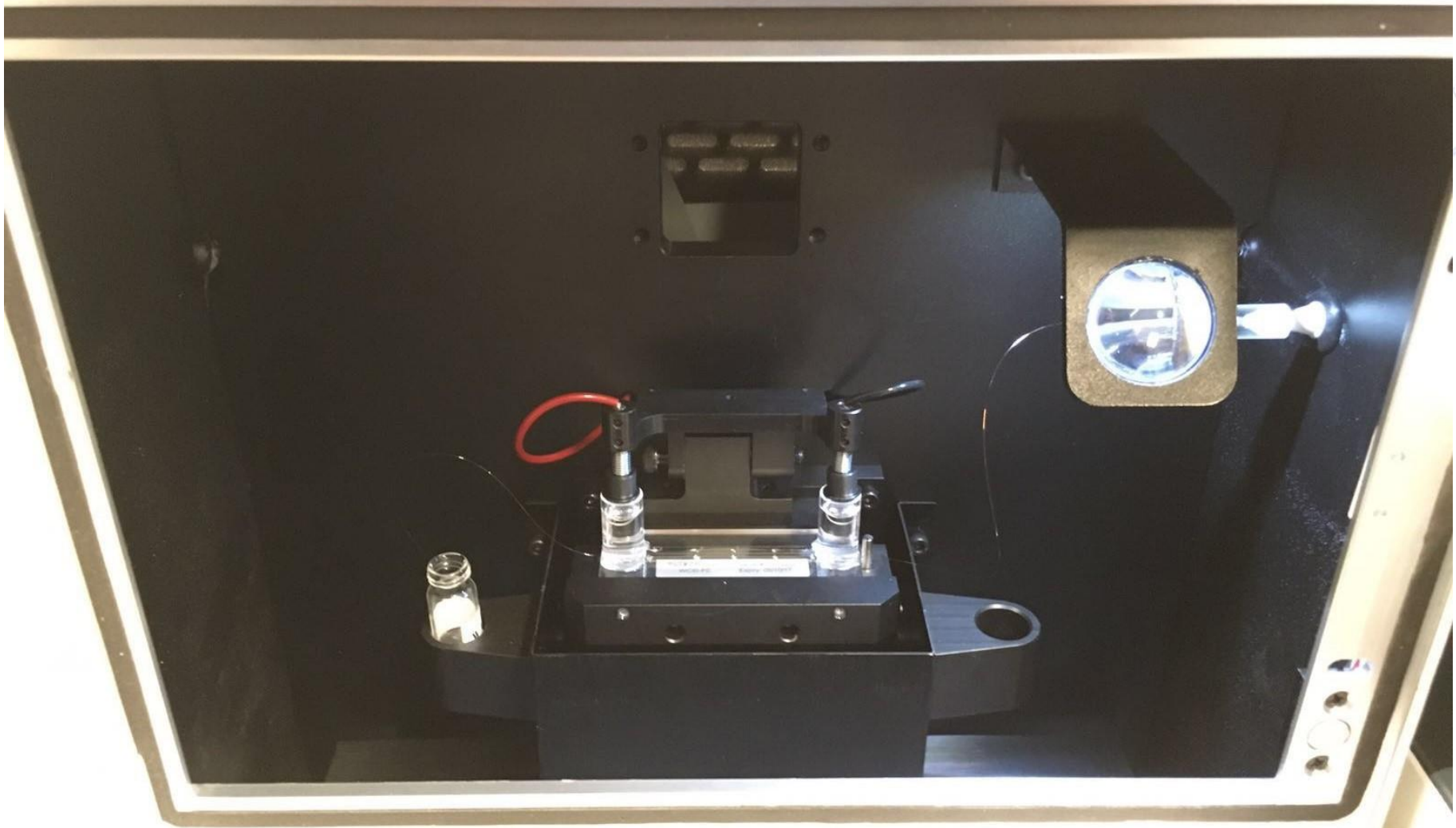
# iCIEF run of hemoglobin

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**Sample: Hemoglobin A, F, S, C**  
**8% Pharmalyte 3-10**  
**600 V/cm**





All consumables delivered with certificate



- Cartridges
- Carrier ampholytes
- Electrolytes
- Standards



Advanced Electrophoresis Solutions, Ltd  
www.aeslresolutions.com

CEInfinite

### CERTIFICATE OF PERFORMANCE

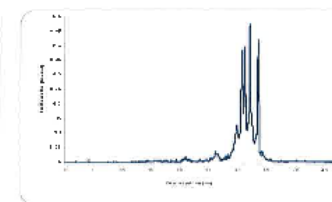
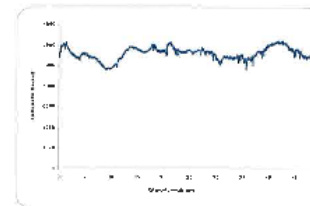
Product: WCID cartridge – FC coating	Part No: CP00202
Serial No: 1630022	Expiry Date: 05/29/2017
Quantity: 1	Date of manufacture: 05/29/2016


Each Cartridge was produced by Advanced Electrophoresis Solutions, Ltd.  
Each cartridge was tested individually by running a standard sample, and released by Advanced Electrophoresis Solutions' Quality Control Personnel

### Testing conditions

Sample	Carrier ampholyte	Additives	Anolyte	Catholyte	Focusing voltage
Hemoglobin A, F, S, C	4% Acelyc 3 - 10	0.35% methyl cellulose	80 mM H <sub>2</sub> PO <sub>4</sub> in 0.1% methyl cellulose	100 mM NaOH in 0.1% methyl cellulose	1 min:1kV 1 min:2kV 4 min:3kV

Test	Description	Results
E-grams	Four major peaks should be observed	pass
pH range	It should be about the pH range of the used carrier ampholyte (in this test, pH 3 – 10)	pass
Resolution	For the Hb standard, I-B4Lr shoulder should be observed	pass



Quality Control: 

Date: June 1, 2016



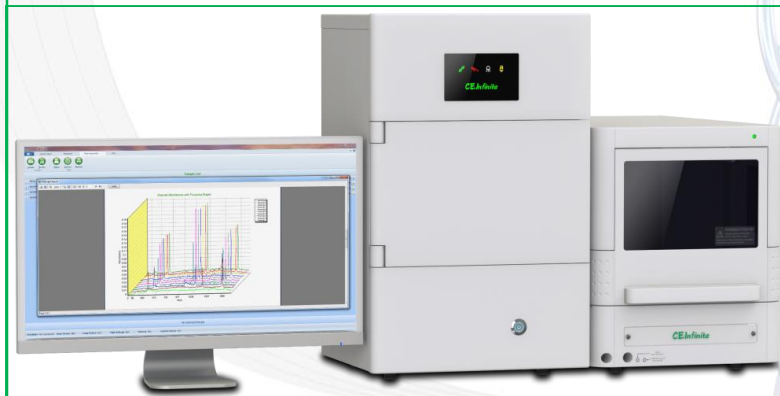
## Advantages of *i*CIEF

- Quantitative protein analysis
- Peak patterns: reproducible
- No hydrostatic flow
  
- Wide pI range (pH 2.5-11)
- High resolution -  $\Delta pI > 0.02$
- Short analysis:  $> 5$  runs/hr
- Method development: hr - days

***CE Infinite***

## Conventional CIEF

- Quantitative protein analysis
- Low resolution - mobilisation
- Long: +/- 1 run/hr
- Method development: months
- Lower reproducibility
- Requires additives



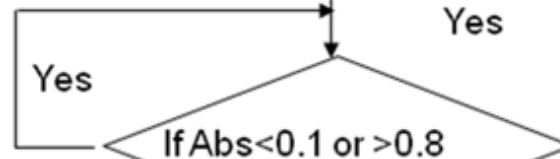
## Method development

1. Try 2, 4, 8 M urea
2. Try SH pH 3-10 with 0, 2, 4, 8 M urea
3. Try Servalyt pH 2-9 with 0, 2, 4, 8 M urea

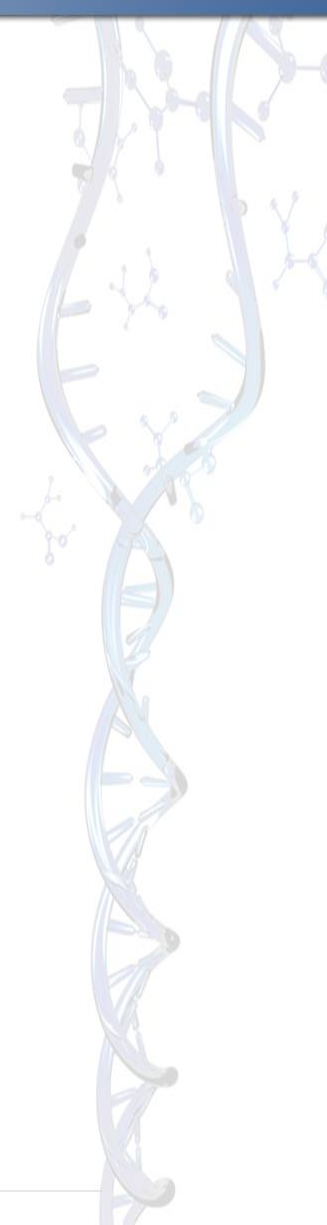
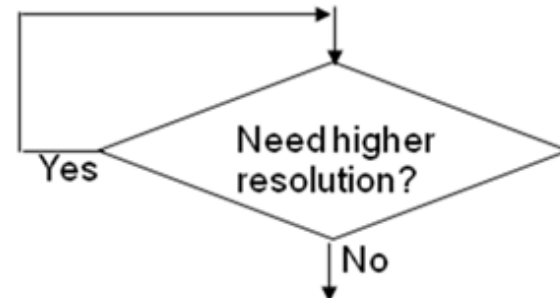
Adjust sample concentration

Narrow range ampholytes or mixture of carrier ampholytes

Initial condition: 0.1mg/ml  
4% HR pH3-10 Aeslyte, 10 min



Select two pI markers and determine sample pI



# 1. Improved sensitivity

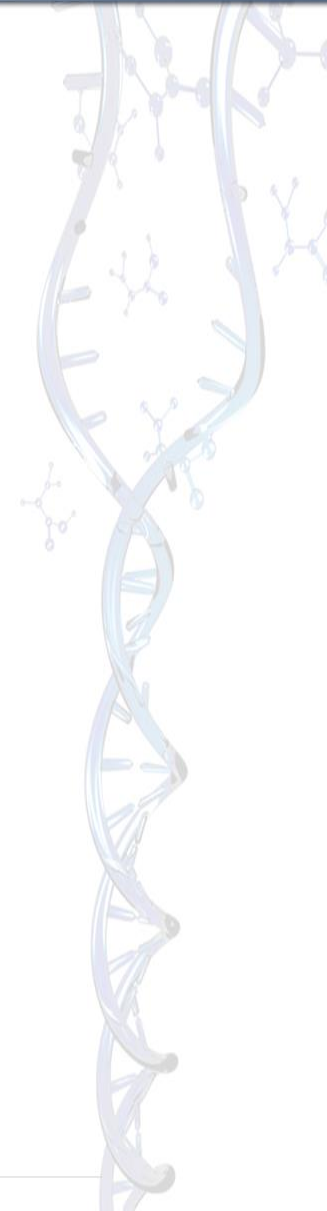
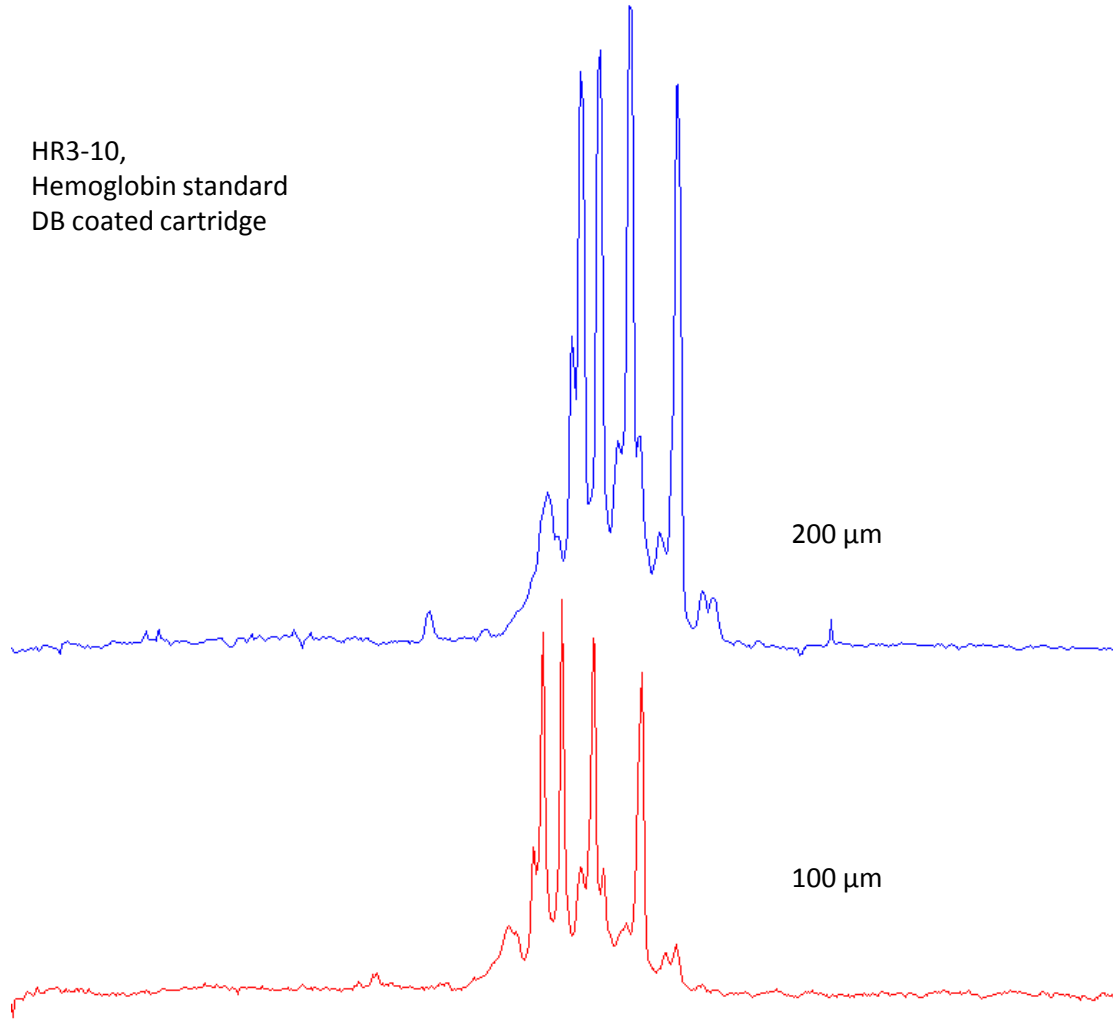
The cartridge *diameter*

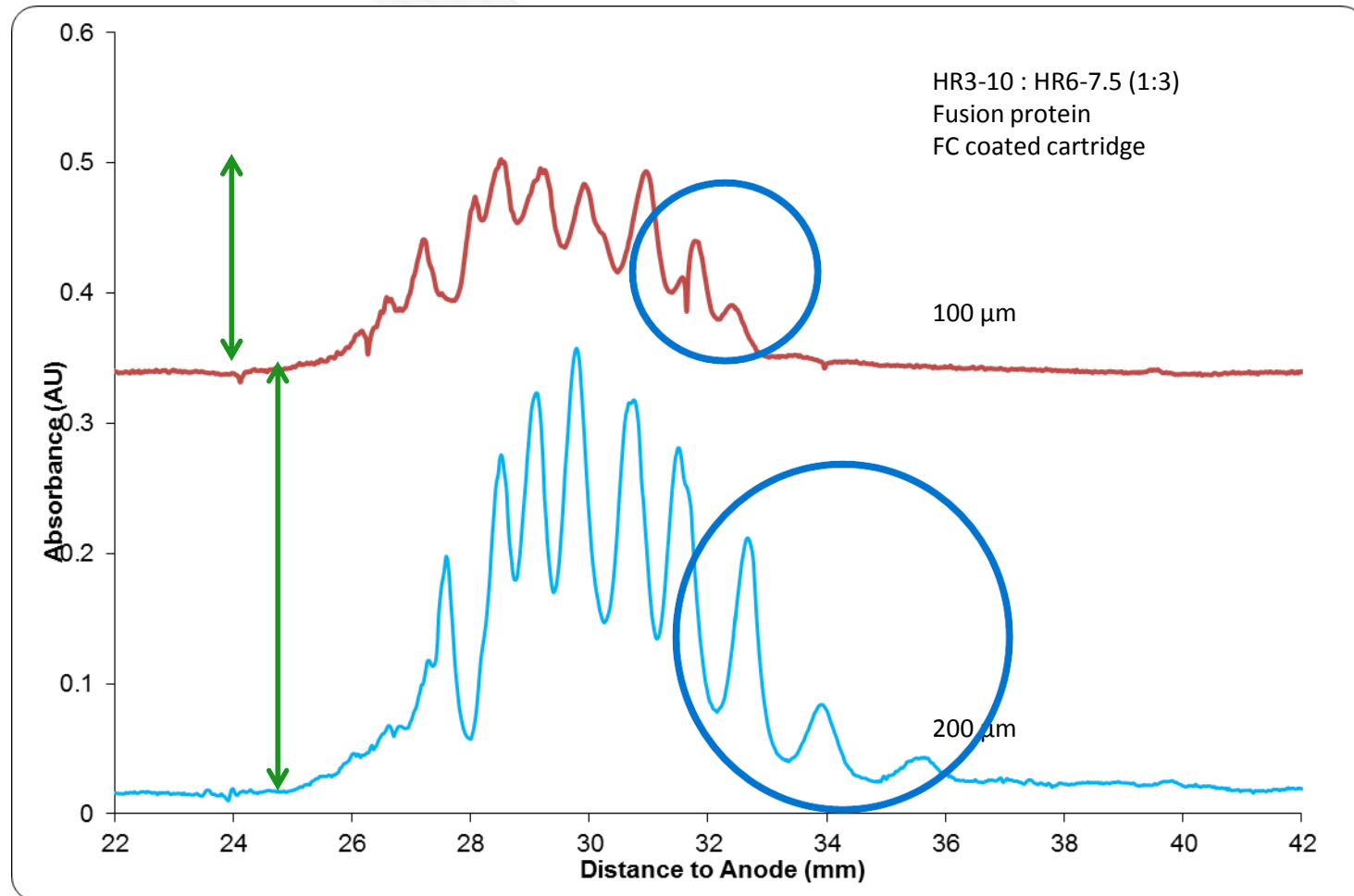


# High-sensitivity cartridge

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HR3-10,  
Hemoglobin standard  
DB coated cartridge



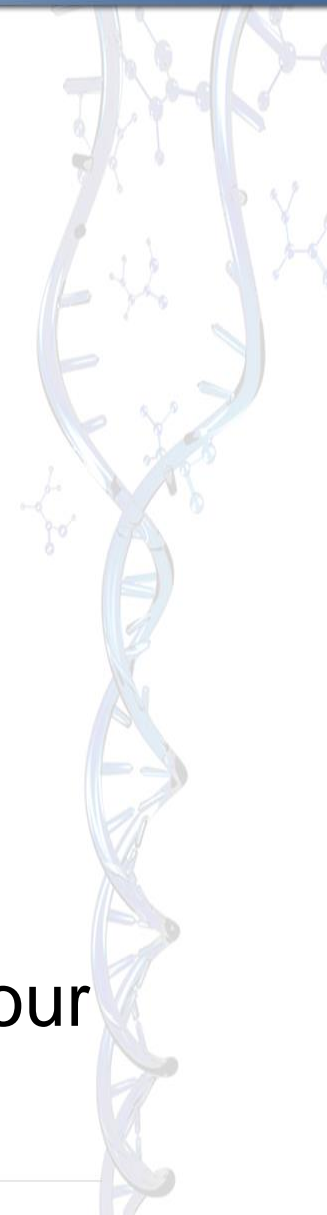


## 2. Increased throughput

The cartridge *coating*

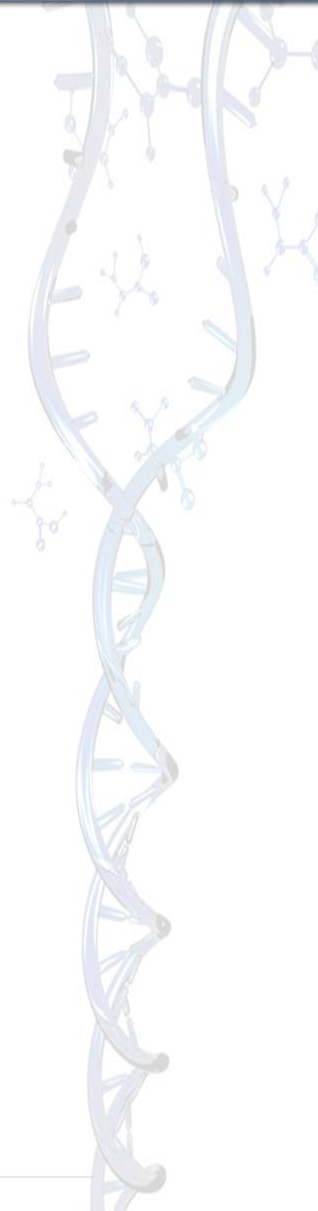


- **Limitations** of traditional cartridges:
    - FC-coating → need methylcellulose → viscosity ↑
  
  - **Solution:** other coatings
    - PA or DB: no methylcellulose needed! → viscosity ↓
      - Improved pipetting reproducibility
      - Increased injection speed
      - Reduction in separation time CIEF
- From 5 runs per hour to 10 runs per hour



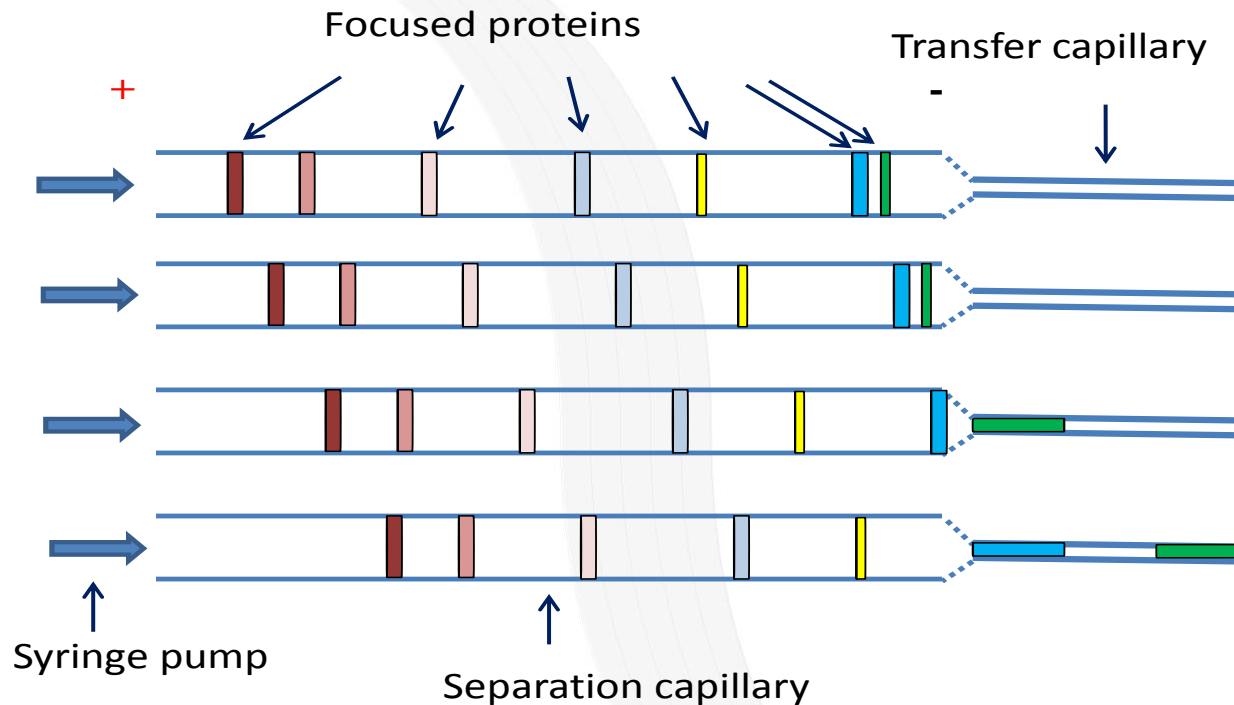
# 3. Preparative *i*CIEF

**The CEInfinite**

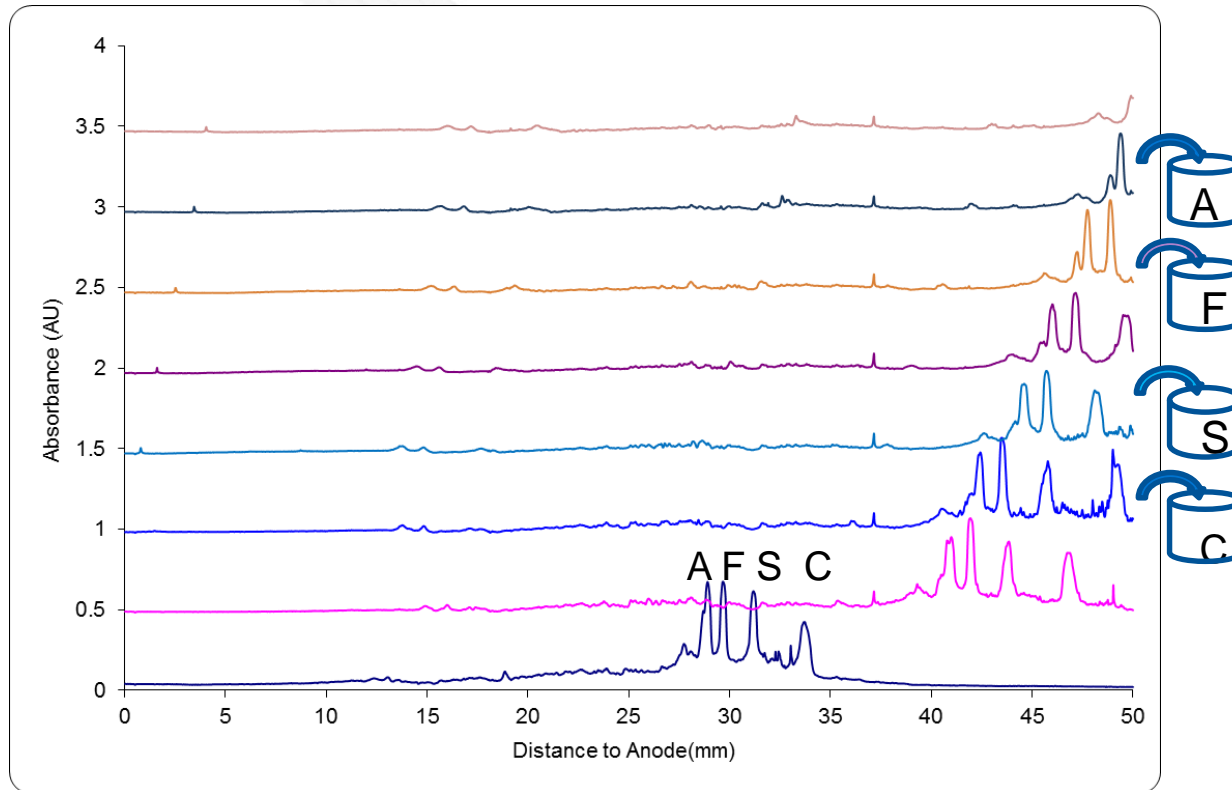




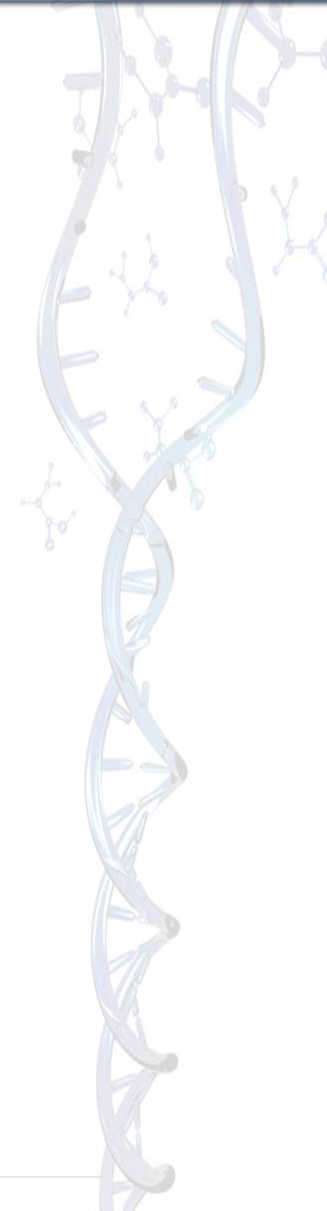
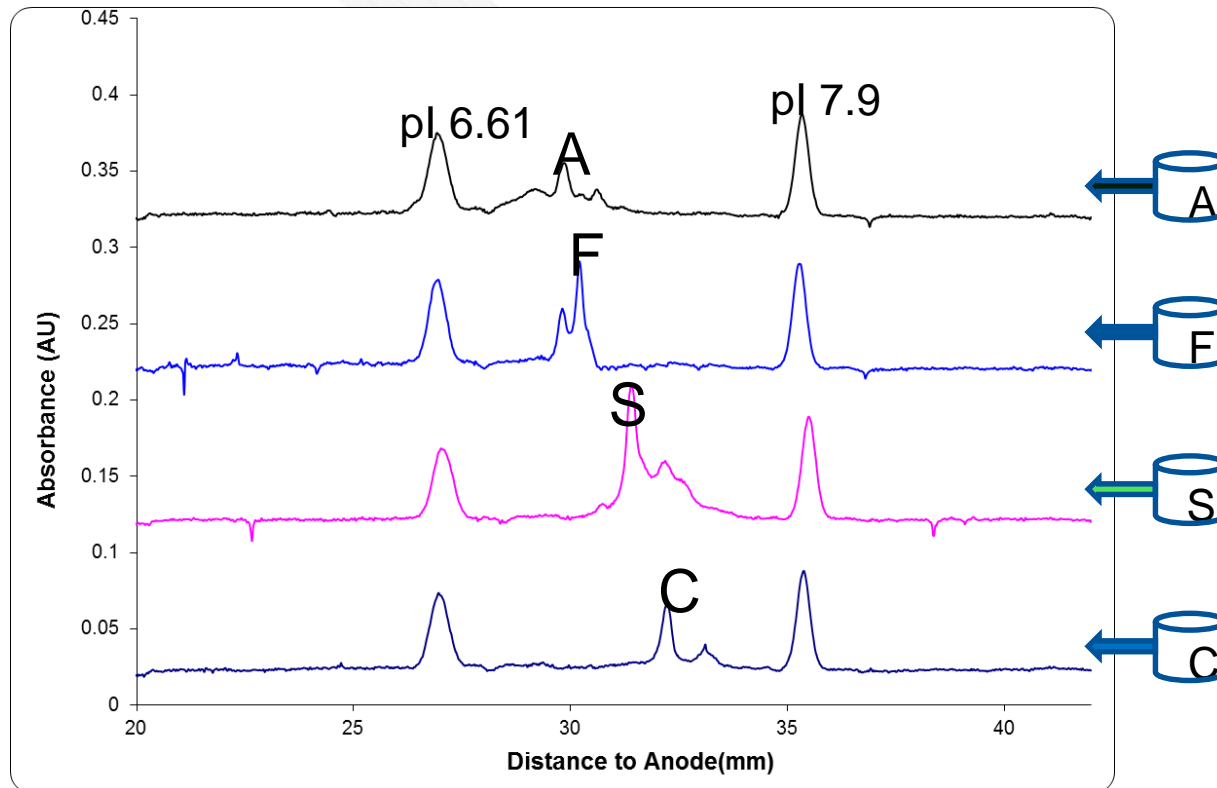
Recuperate fractions with the software-controlled pump and then inject in your MS or other device for more



Changing diameter with **factor 4** increases length peak **factor 16** in outlet capillary → better time control

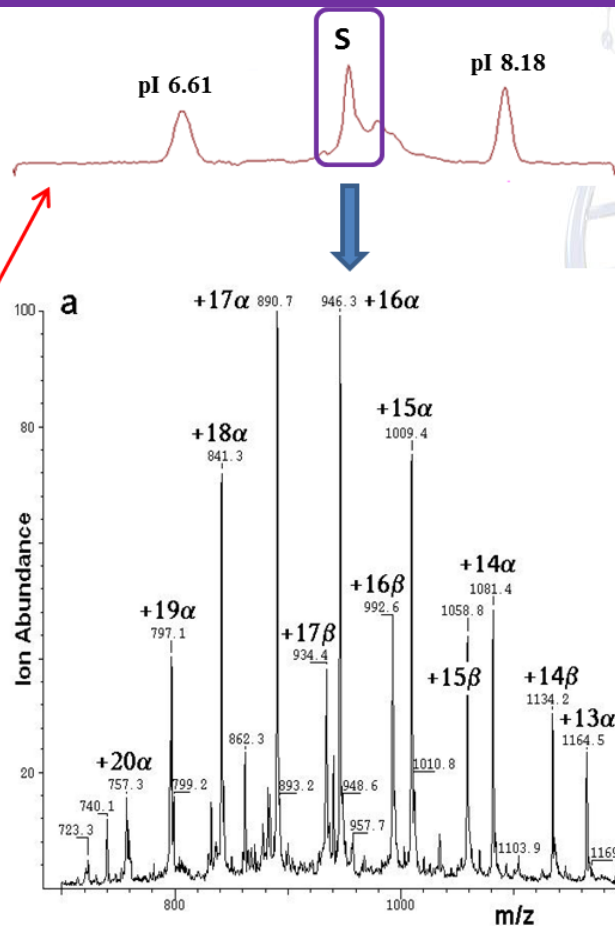
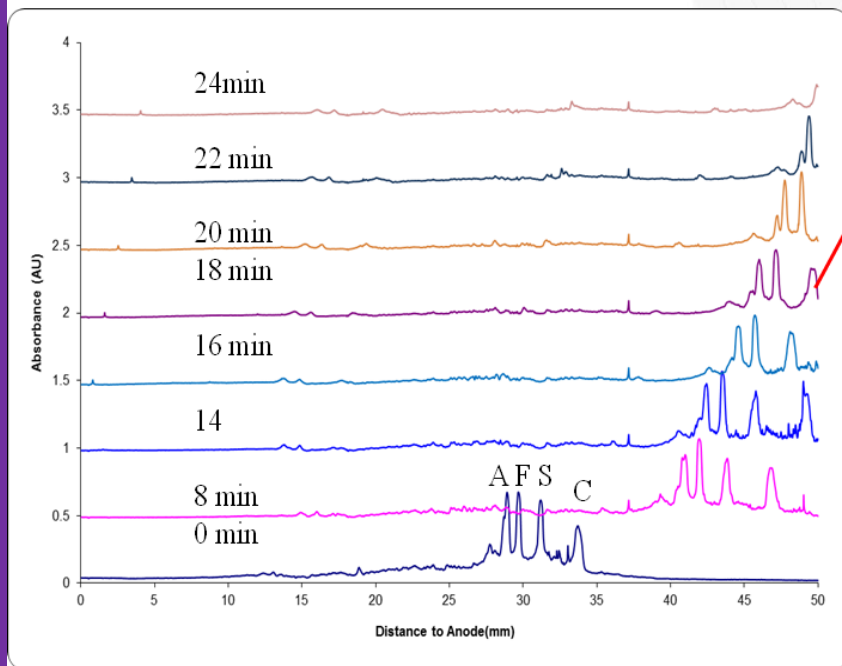


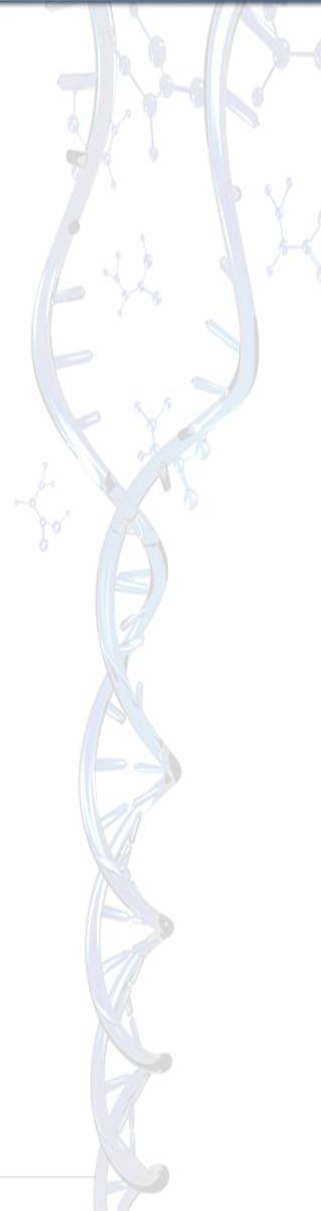
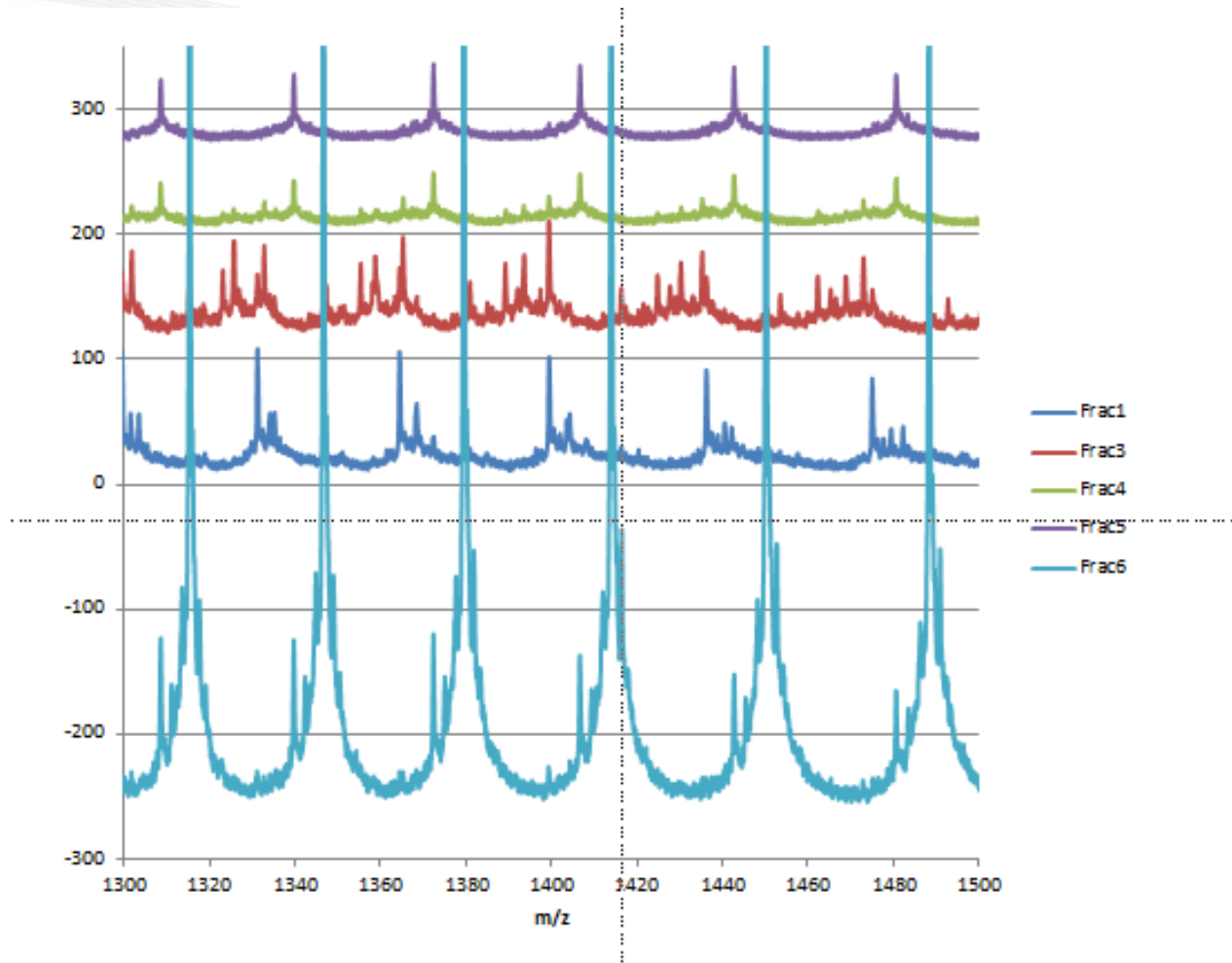
High voltage during mobilization. Preserve sharp peaks





**cIEF-WCID tandem MS (ESI & MALDI)**





## Questions?

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