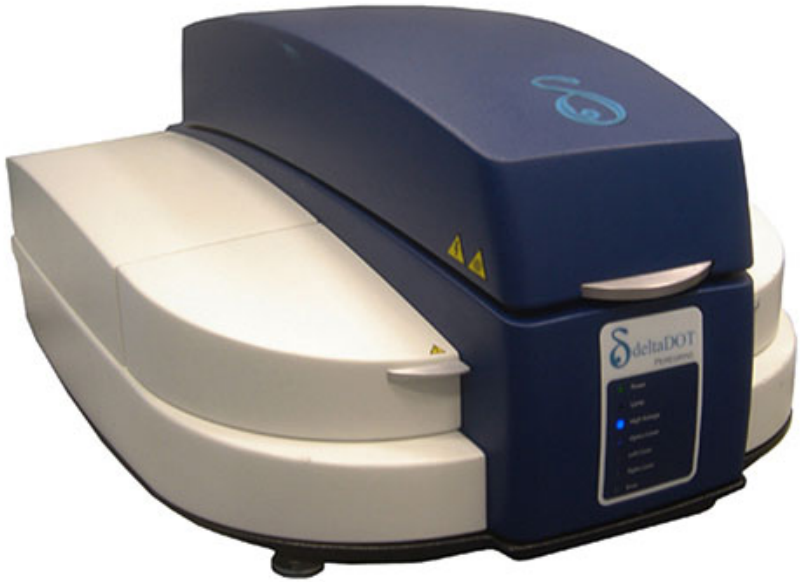
deltaDOT

See the molecule



Applications of deltaDOT's Label Free Intrinsic Imaging Technology

March 2012

Introduction to deltaDOT Ltd

deltaDOT is a biotechnology company selling into the biomedical and analytics markets.

At its core is the proprietary Label Free Intrinsic Imaging (LFII®) approach. LFII® has significant advantages over other approaches.

It has a wide spread of applications and customers in many sectors including biotech, academic, industrial and government sectors.

deltaDOT Ltd owns 19.9% of deltaDOT QSTP-LLC and receives about $900K per year from Qatar (plus cash from sales of any instruments).

The proposed route to market involves working with other established companies via collaborations and selling through distributors

Market Analysis

deltaDOT
See the molecule

Label Free Intrinsic Imaging brings advantages to:

- drug discovery and development
- QC/QA, biopharmaceutical production
- proteomics
- vaccine development
- general biotech
- threat identification
- forensics
- diagnostics
- analytical chemistry
- food and drink

Commercial offering

deltaDOT offers:

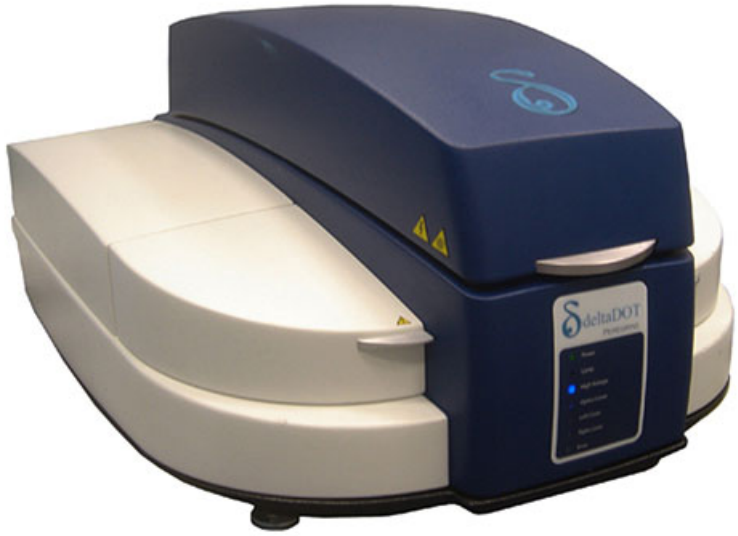
- Capital sales
- Collaboration deals
- Service Business
- Rental / leasing
- OEM deals
- Strategic Partnerships

The PEREGRINE I HPCE system

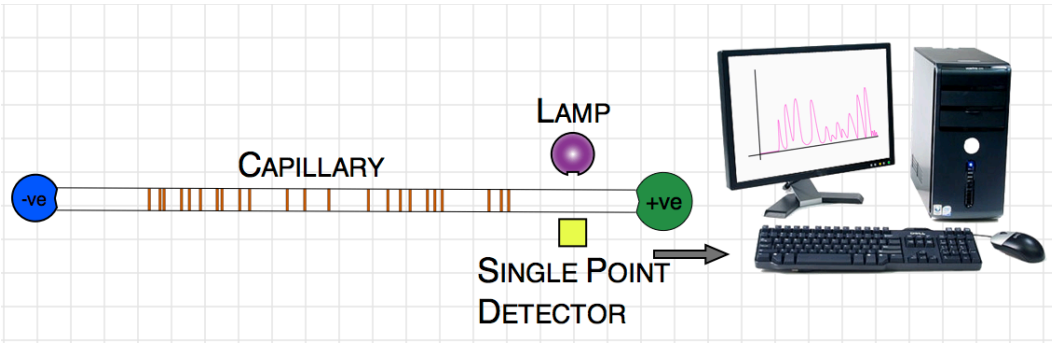
deltaDOT

See the molecule

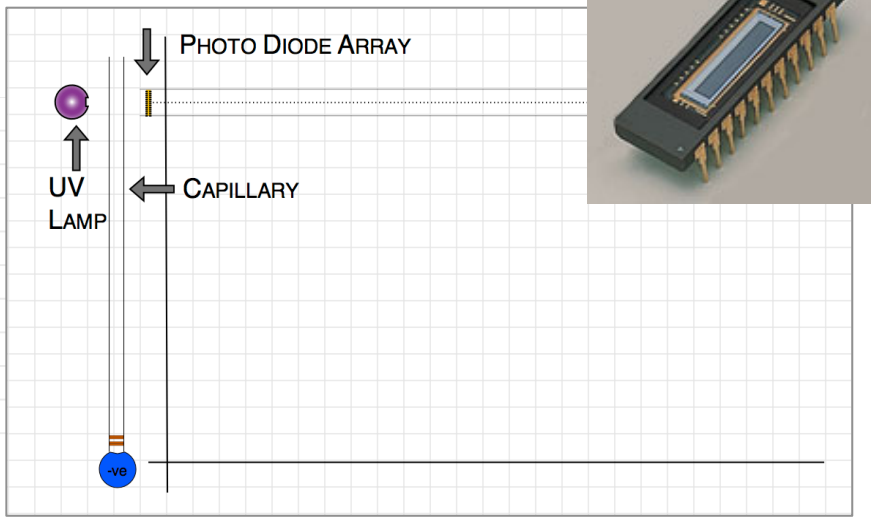
Label Free Intrinsic Imaging theory.



Capillary Electrophoresis systems



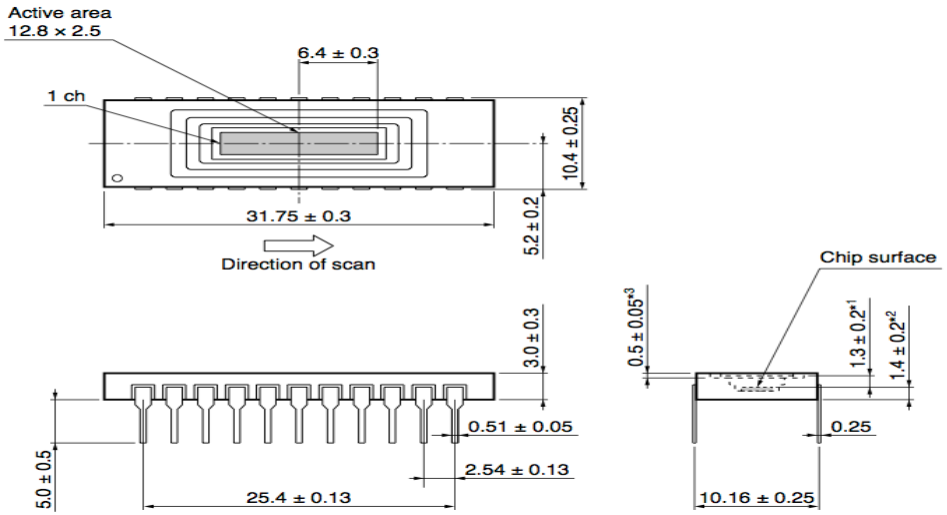
Conventional single point detection systems image each protein only once



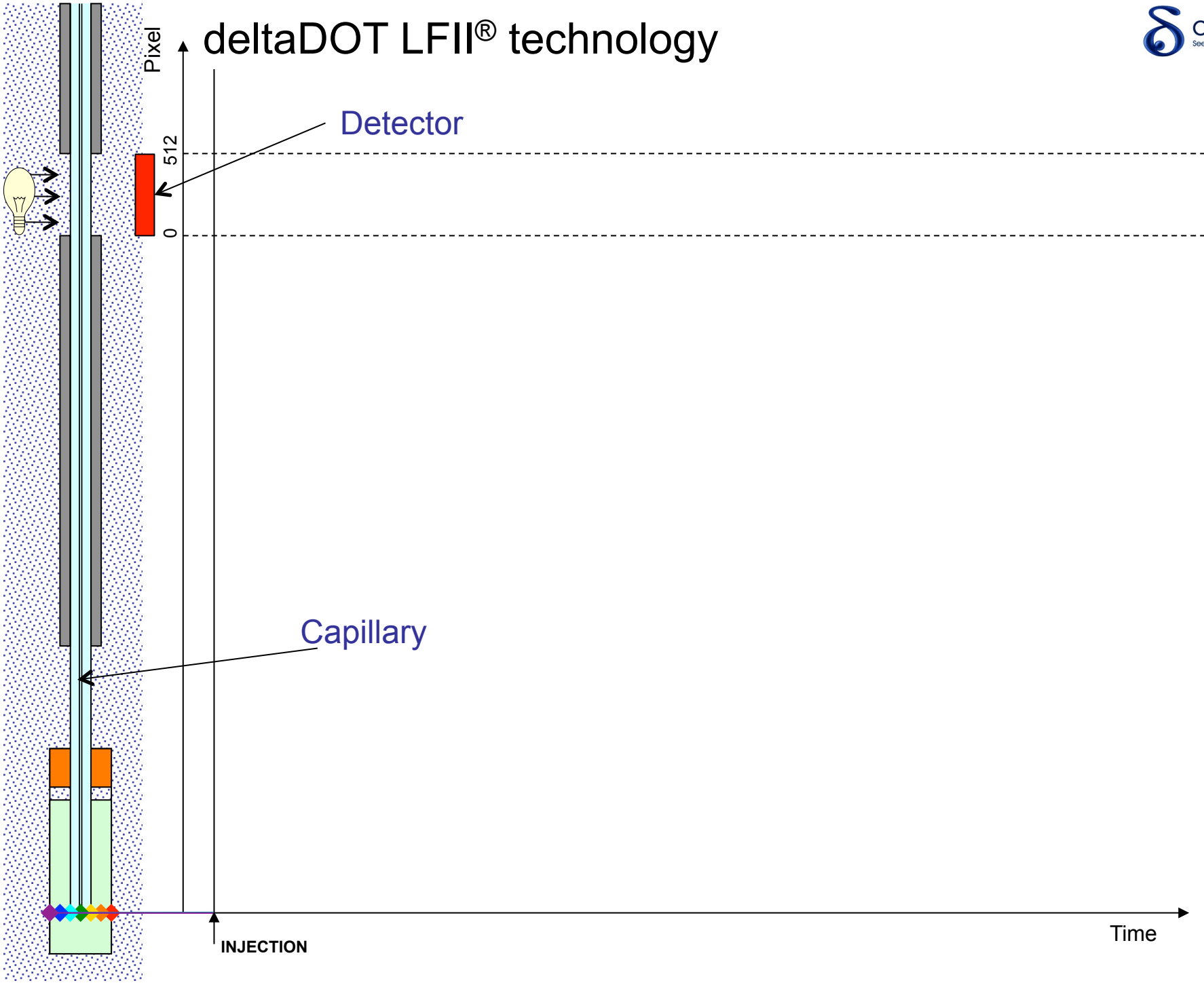
deltaDOT's LFII® technology images each protein band 512 times across a photo diode array at ~15 Hertz

It is our ability to track an entity as it passes through these multiple detectors and correlate the space/time data, seen as a sloped signal, that gives LFII® its power.

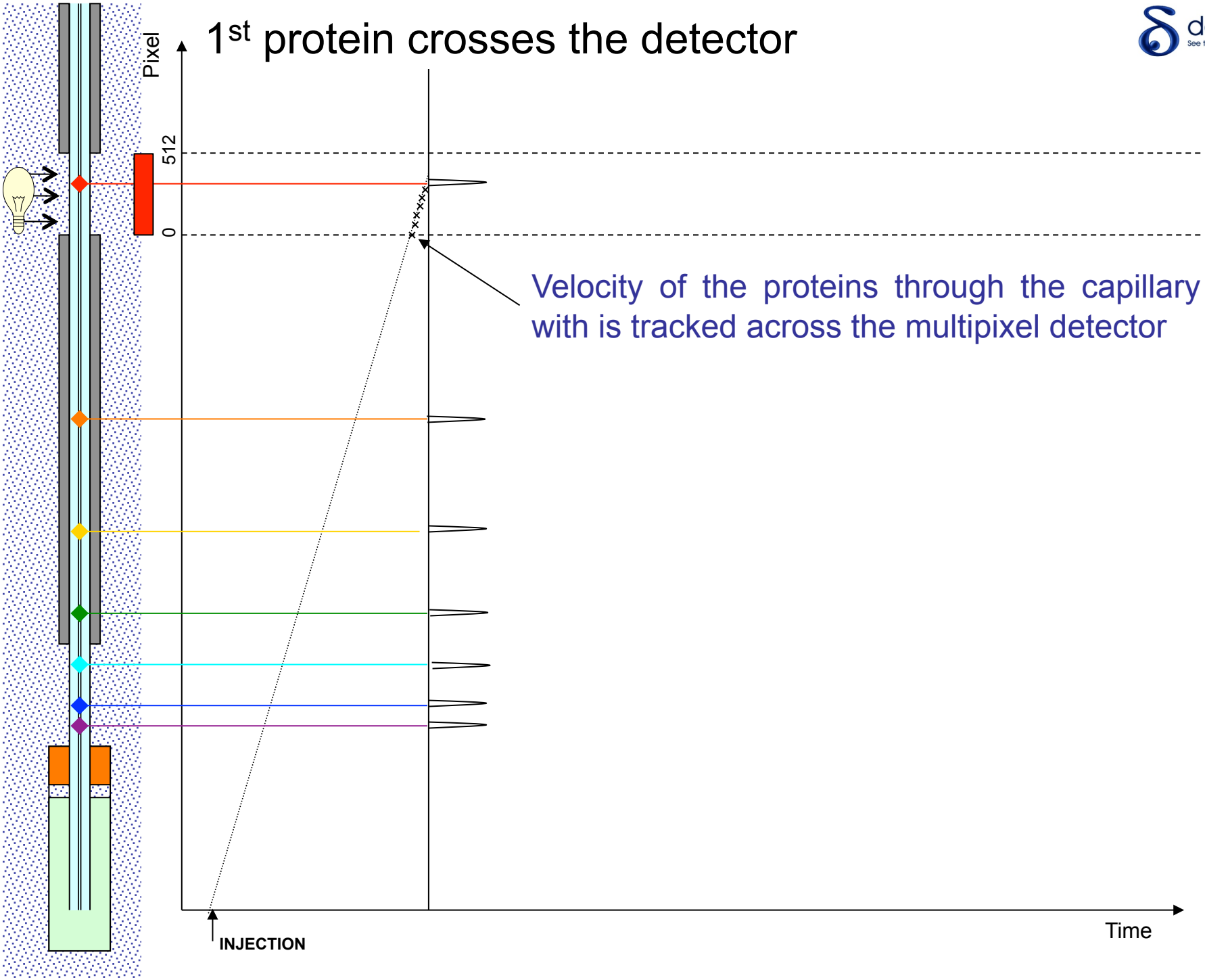
The junction of the signals is called the VERTEX. Any signals that do not hit the vertex are discarded.



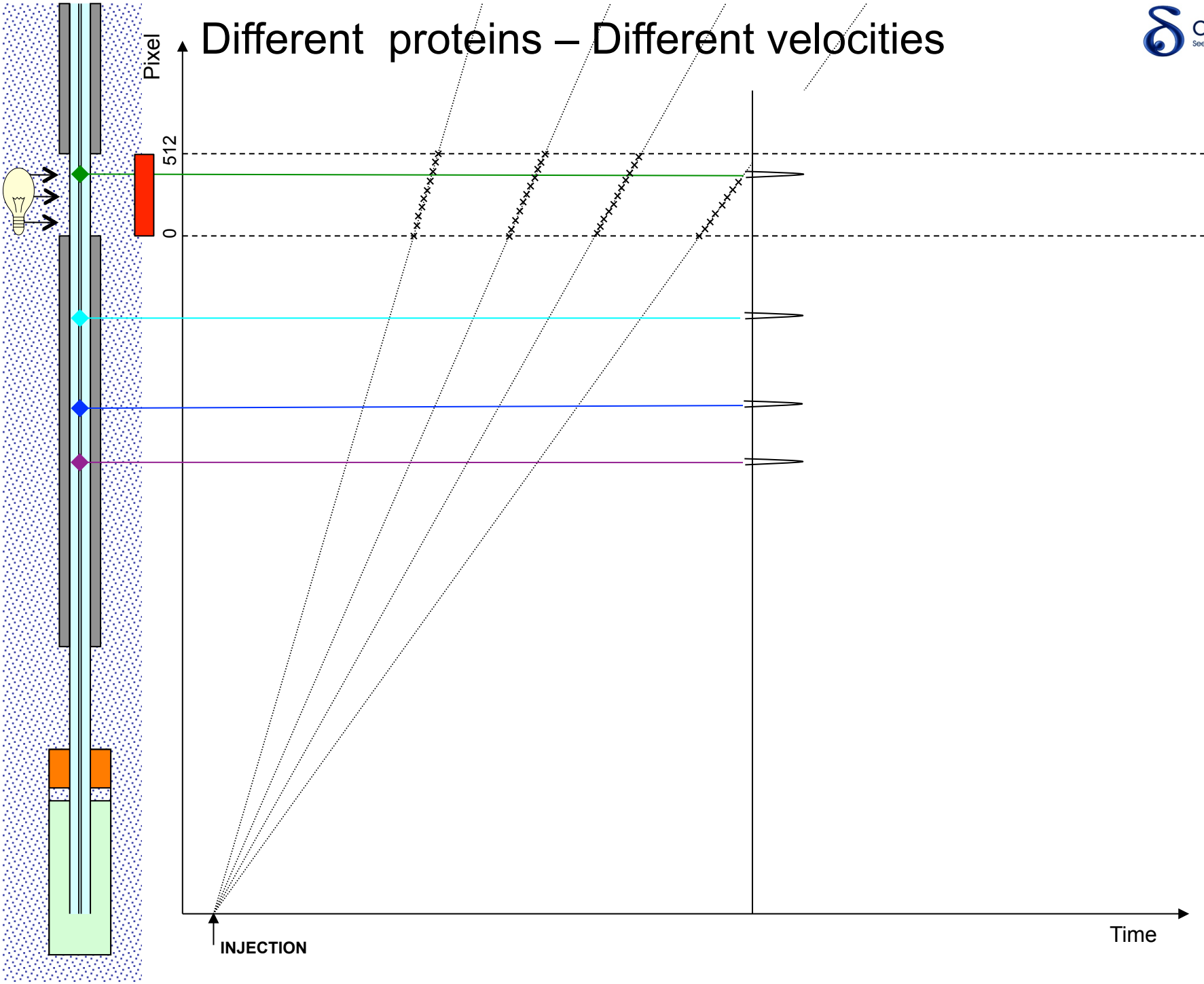
deltaDOT LFII® technology



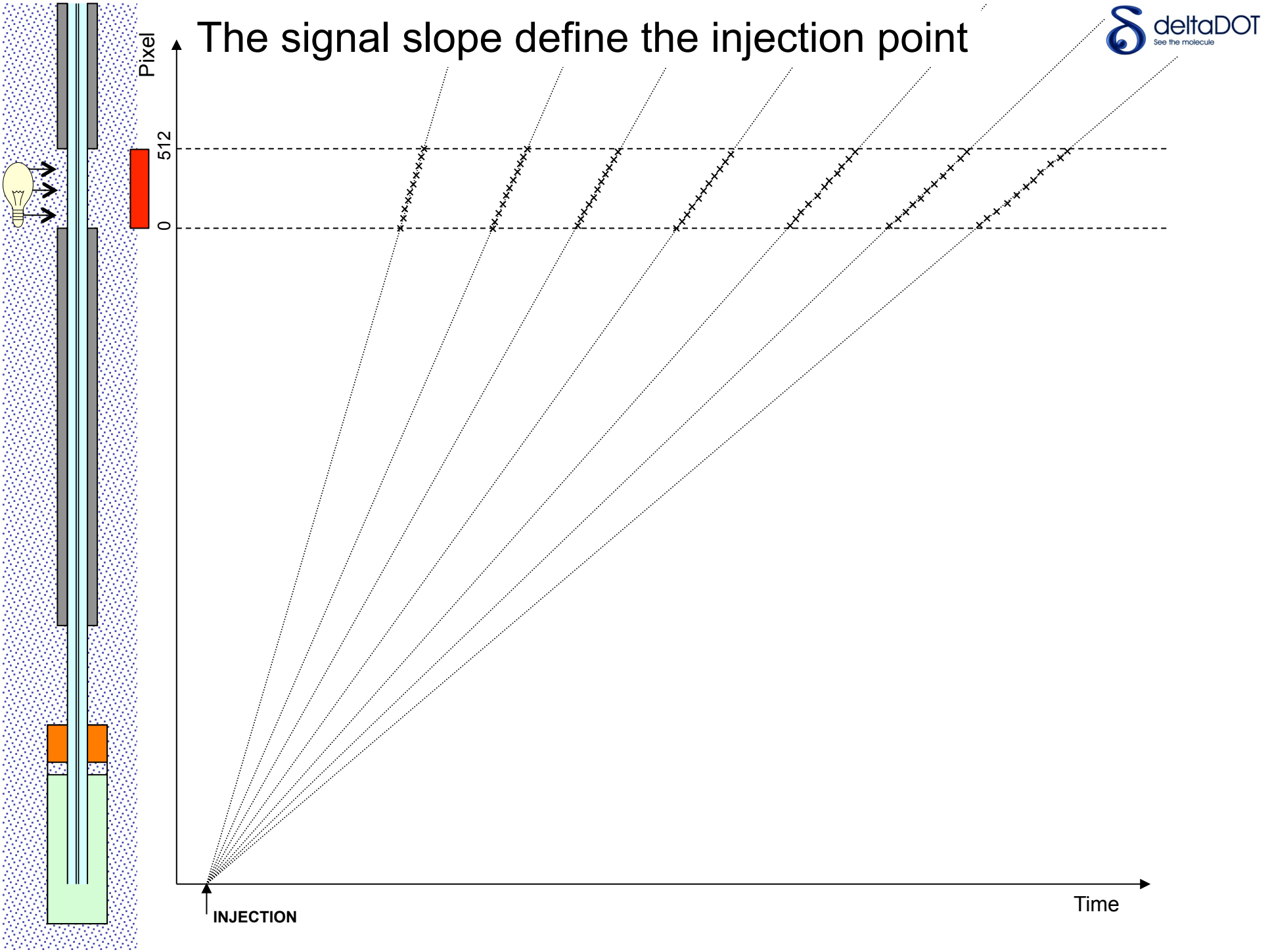
1st protein crosses the detector

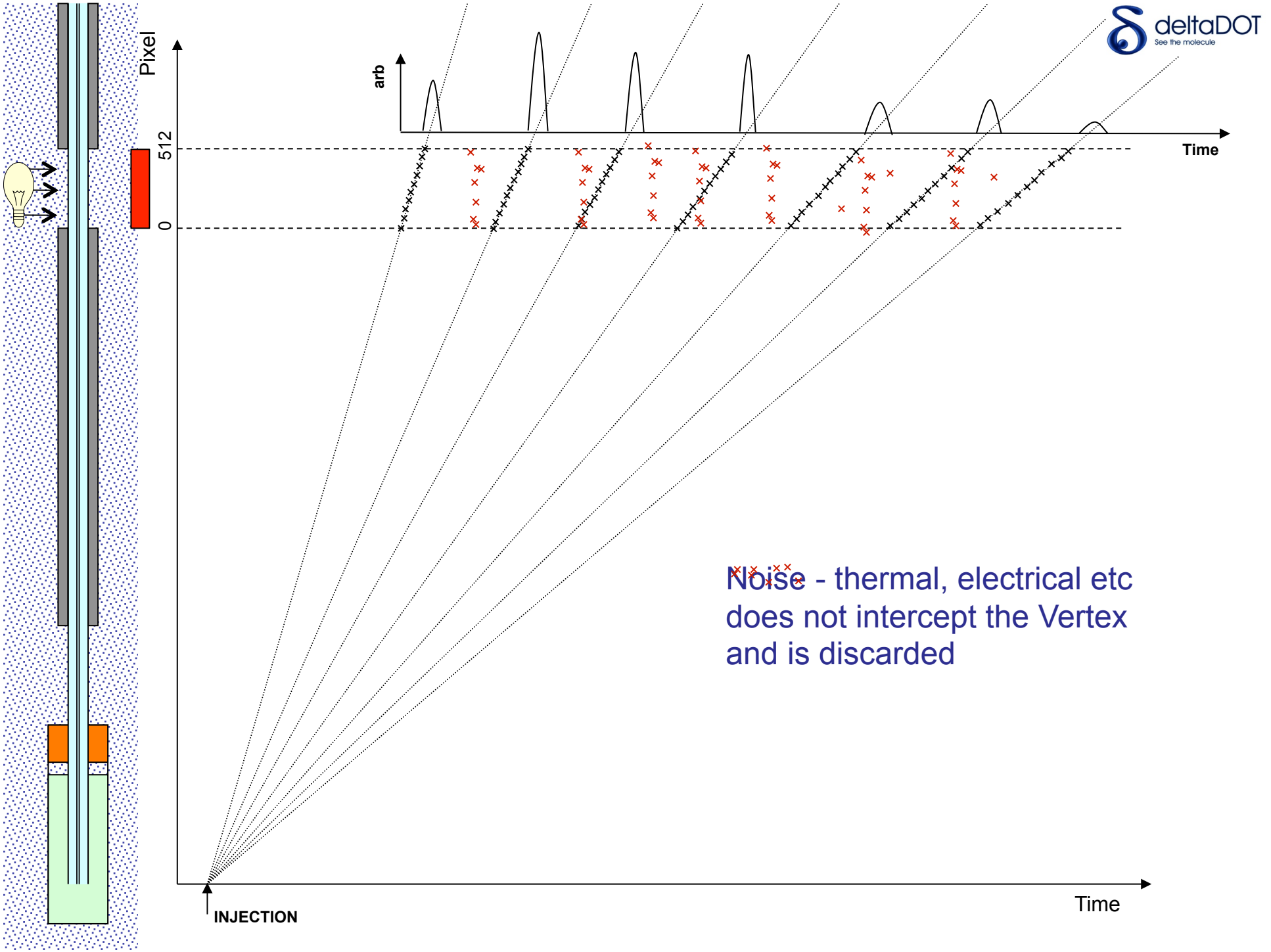


Different proteins – Different velocities



The signal slope define the injection point



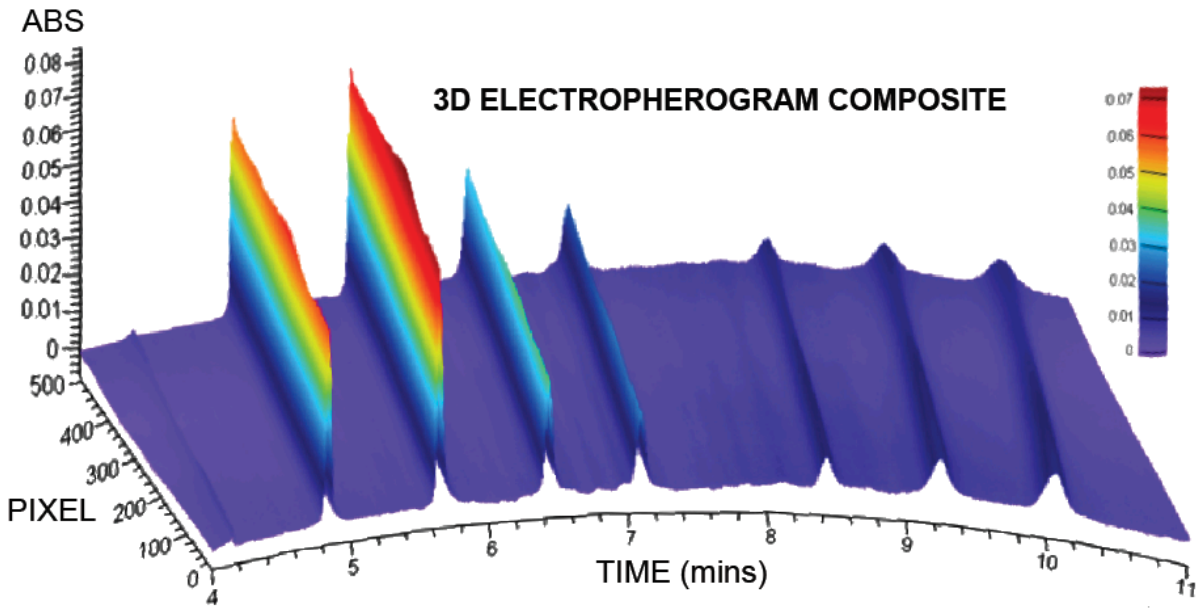

deltaDOT
See the molecule
Pixel
512
0
arb
Time
Noise - thermal, electrical etc
does not intercept the Vertex
and is discarded
INJECTION
Time

Label Free Intrinsic Imaging

The vertex created by the space/time data is used to filter noise and dramatically increase the data quality.

It also improves:-

- Quantification
- Resolution
- Reproducibility

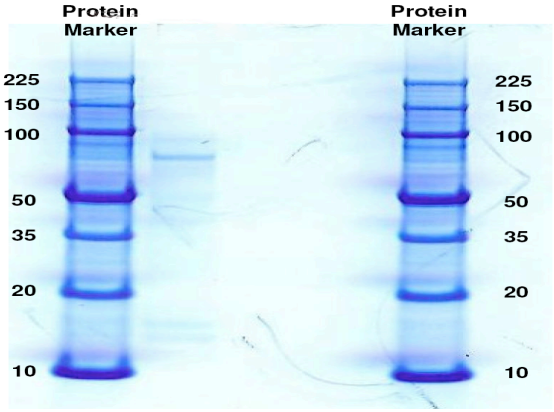


The amplitude of the peak directly correlates to the level of absorption from, for example, the peptide bond in proteins, and allows for highly accurate quantification.

Advantages of HPCE

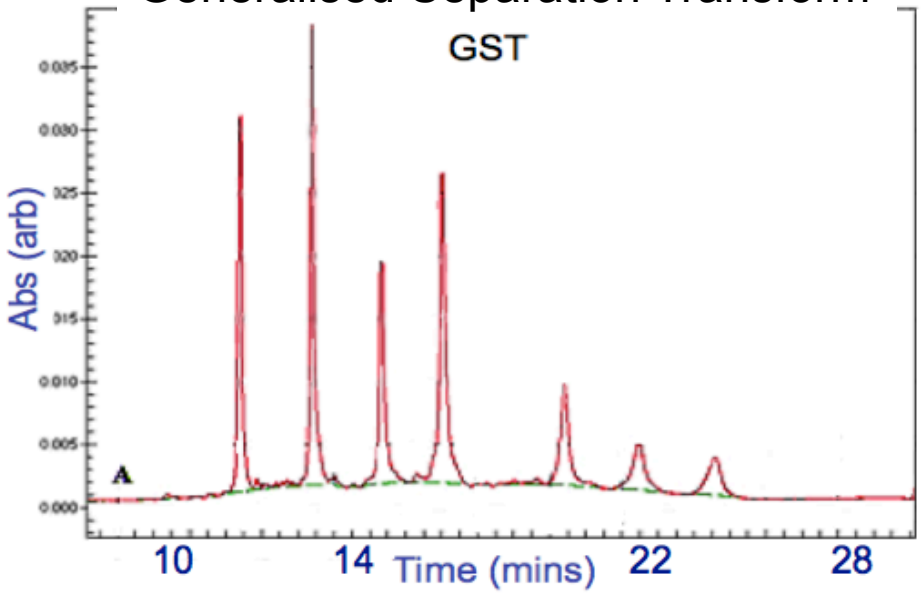
Traditional I-Dimensional Polyacrylamide gel electrophoresis

- 1D gels are 1950's Technology.
- They take hours to run
- They take hours to stain
- They take hours to destain
- They take hours to dry
- They take hours to analyse
- They take hours to document

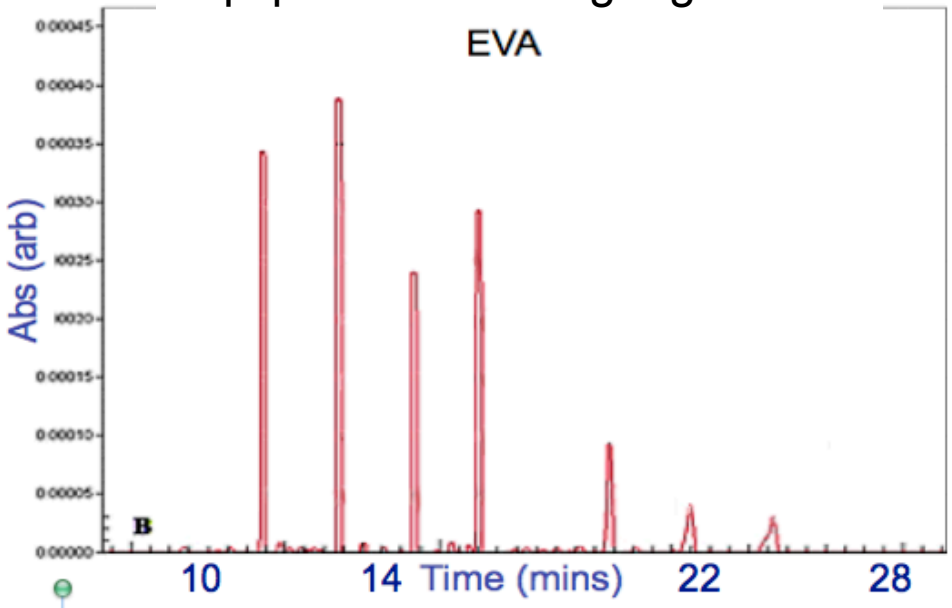


HPCE – Fast, accurate, inherently digital, quantitative, reproducible and high resolution.
LFII® has two major signal processing algorithms :-

Generalised Separation Transform



Equiphase Vertexing Algorithm



Beckman protein standard ladder

Features of LFII®

- Novel **Label Free** data acquisition:

  - No potential effects of fluorophores on molecules behaviour.
  - Reduce the costs of analysis

- Highest CE specs for resolution, sensitivity & reproducibility providing the customer with the best possible data:

  - Resolution (CGE – ~200Da, <10bp DNA)
  - Sensitivity (~1.0 μg/ml protein - LOQ)
  - Reproducibility (<4% RSD)
  - Quantitative (<2% RSD for simple mixtures, 5% for complex lysates etc.)
  - Linear Dynamic Range (5 orders concentration, 4 in MW)

- UV detection 214/254/280nm enabling the analysis of Protein, DNA, RNA virus particles and small molecules

- deltaDOT software is currently undergoing CFR21 11 compliance

deltaDOT and regulatory affairs

deltaDOT have been working with end-users for several years on regulatory issues for hardware, software and bioproduction issues.

deltaDOT has:-

•Helped a Cambridge company with Bioproduct regulatory steps for a vaccine

•satisfied our colleagues in Texas on IQ and OQ for the Caliber vaccine factory PEREGRINES

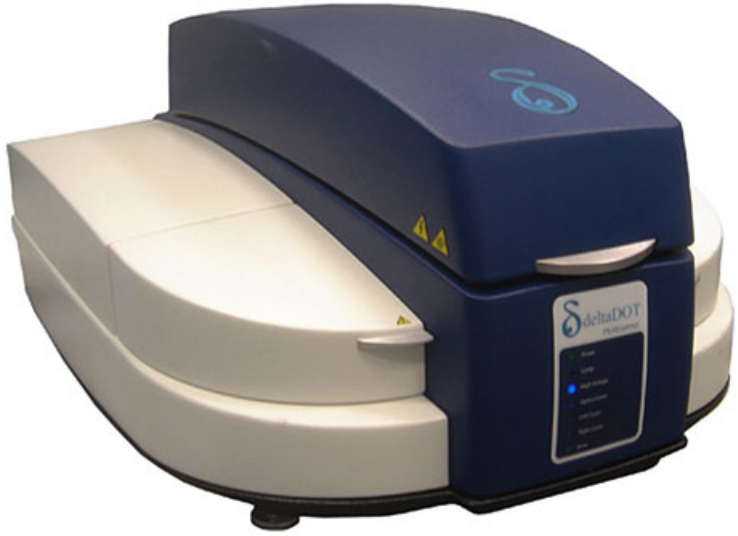
•Made significant progress on our CFR21/11 compliance

•Good experience of CE and other hardware conformity standards

deltaDOT

See the molecule

Measuring the Bioprocess using Label Free Intrinsic Imaging systems.



Areas of capability

deltaDOT
See the molecule

Capillary Gel Electrophoresis CGE

Molecular weight based separations

Capillary Zone Electrophoresis CZE

Charge based separation

direct UV absorption

Indirect UV absorption

- Capillary ElectroChromatography CEC

  Charge-Molecular weight and stationary phase based separations
- Micellar Electrokinetic Capillary Chromatography MECC

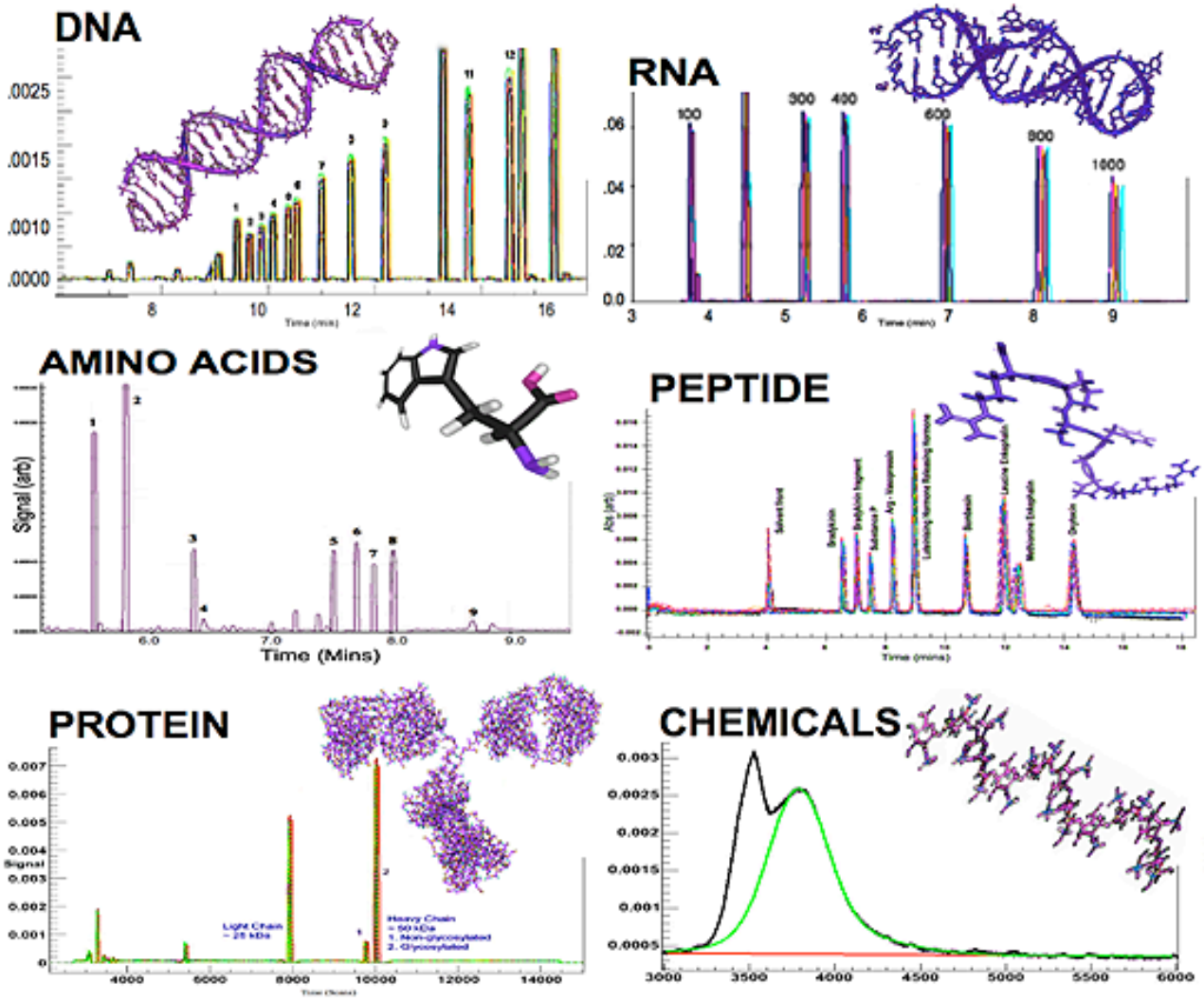
  Separation of neutral species
- Chiral Capillary Separation

  Separation of enantiomer species

Multiple Analysis Capabilities

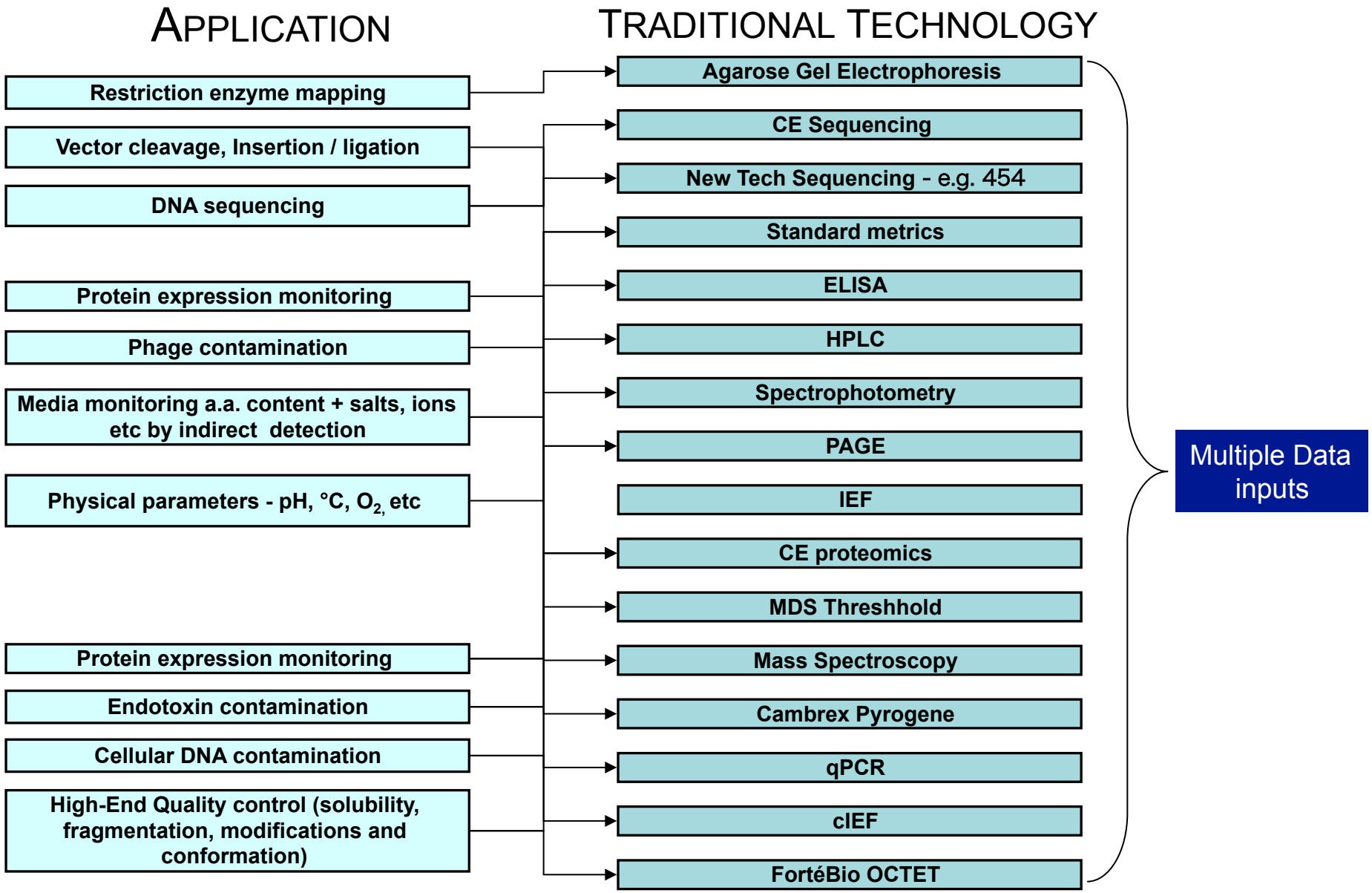
Low bias and label independent molecule analysis

- Proteomics
- Genomics
- Viral expression titre
- Small Molecules
- Carbohydrates
- Inorganic Acids



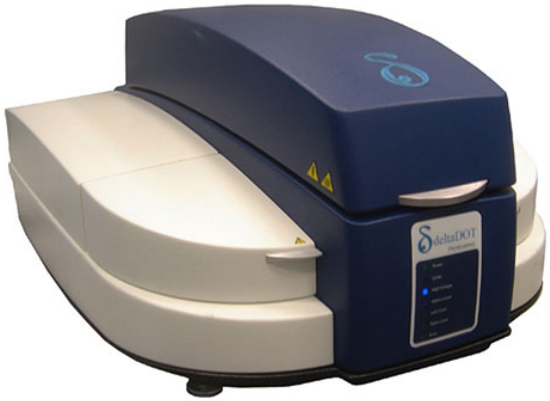
Bioprocessing

Manufacturing therapeutic proteins



Bioprocessing

Manufacturing therapeutic proteins



deltaDOT PEREGRINE I system

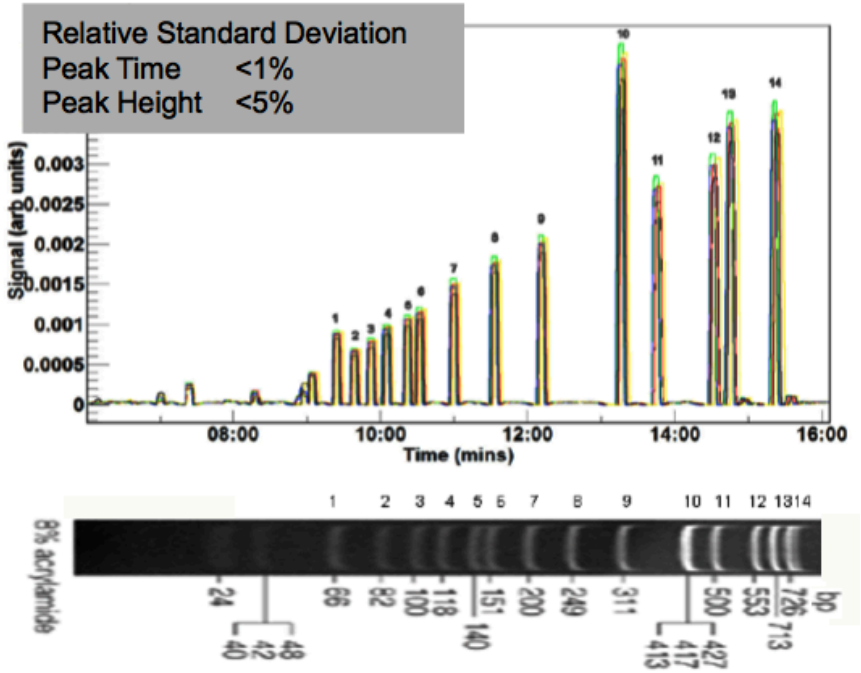
- provides *comparable data* to many of these multiple data input systems and excellent data compared to many.

TRADITIONAL TECHNOLOGY

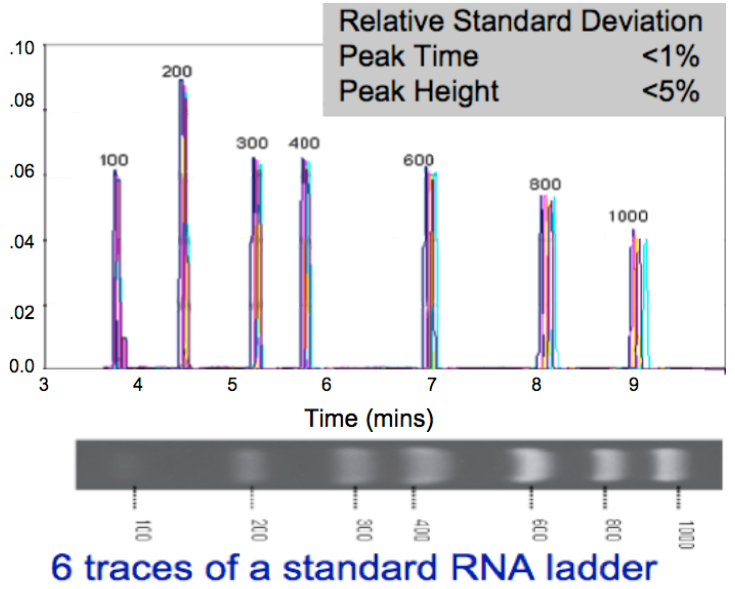
- Agarose Gel Electrophoresis $20K + Cons
- CE Sequencing $150K per system
- New Tech Sequencing - e.g. 454 $?+ Cons
- Standard metrics $100K per lab?
- ELISA $30K per seat + Cons
- HPLC $50K per system + Cons
- Spectrophotometry $12K per system
- PAGE $10K per system + Cons
- IEF $30K plus per system?
- CE proteomics $80K per system + cons
- MDS Threshhold $75K per system
- Mass Spectroscopy $150K per system?
- Cambrex Pyrogene $100K?
- qPCR $35K per RT system + Cons
- cIEF $?
- FortéBio OCTET $?

Single data inputs

Nucleic acid analysis



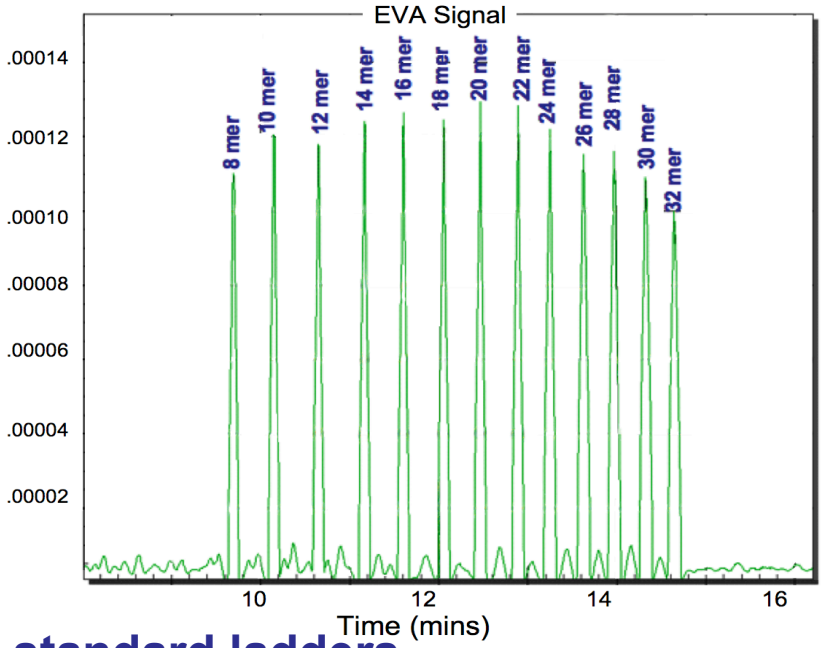
6 traces of a standard DNA ladder



6 traces of a standard RNA ladder

Oligonucleotide separation

Using a commercially available buffer oligonucleotides ranging from 8-32 mers were separated on a 22 cm long bare fused silica of 75μm ID. Sample was injected at 9kV for 5 seconds and run at a voltage of 12kV.



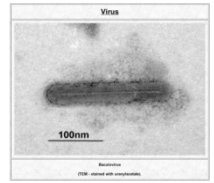
Promega and custom standard ladders

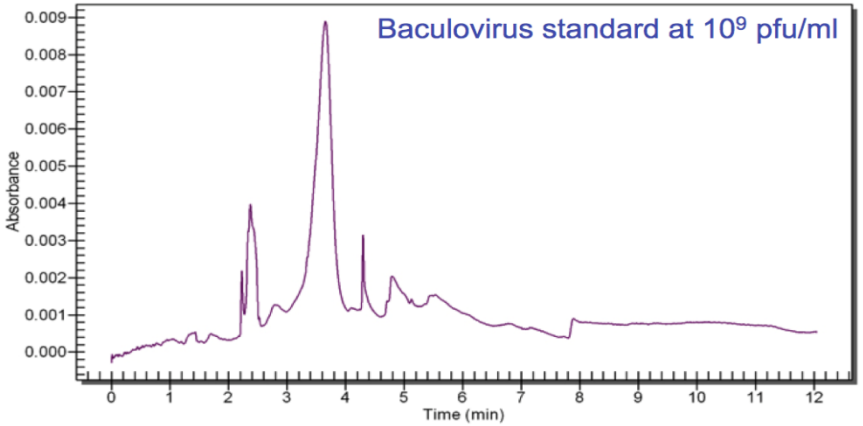
Baculovirus Analysis

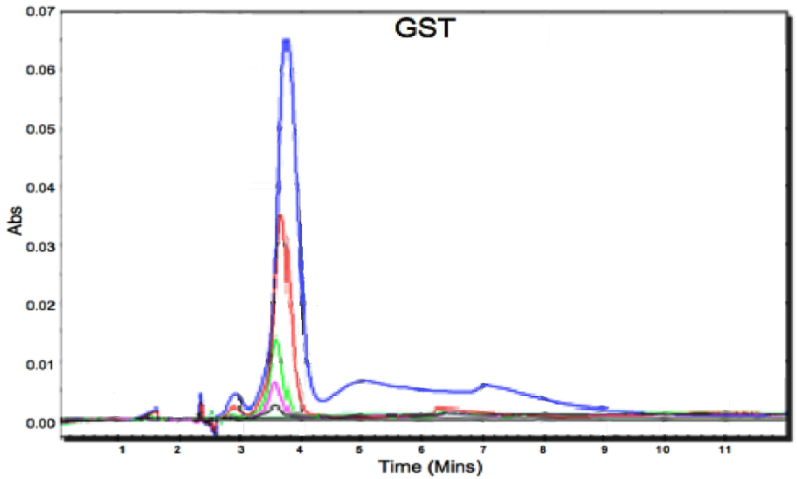
A collaboration with BRAD GlaxoSmithKline

deltaDOT
See the molecule

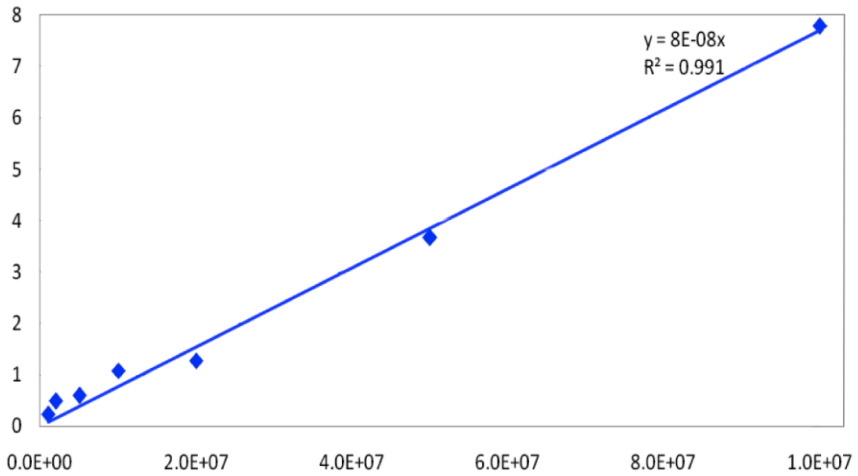
- LFII® systems enables samples to be analyzed in 1 hour
- Throughput - 10 samples per hour
- Real time monitoring of baculovirus titers







Excellent linearity is shown calibration curve in a concentration range between $1.2 \cdot 10^6$ and $1.2 \cdot 10^9$ pfu ml^{-1}



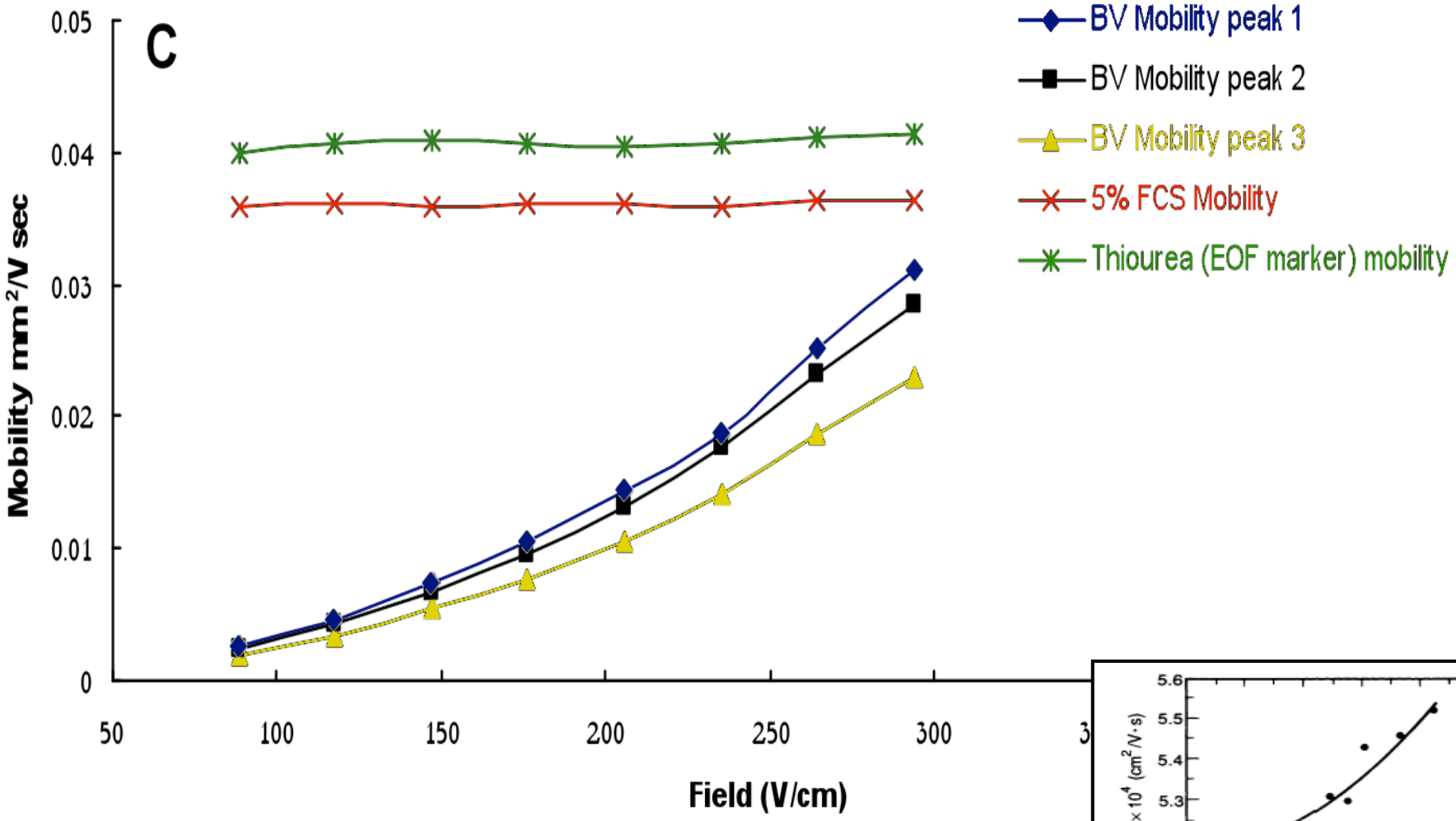
Dilution series on stock 6.7×10^8 pfu/ml.1:2, 1:5, 1:10, 1:100

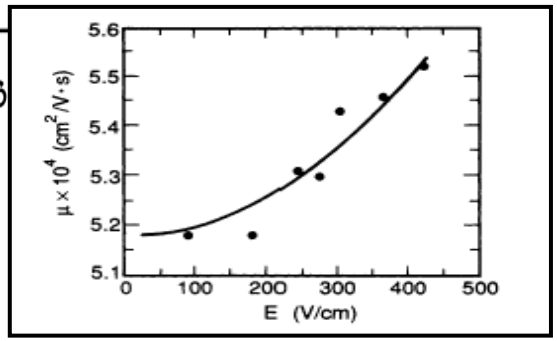
Baculoviruses are extensively used as eukaryotic expression vectors for proteins requiring post translational modifications such as glycosylation, proteolytic cleavage and fatty acylation.

GSK virus sample

Orientation Of Baculovirus

The main virus peak resolves into three separate peaks all of which show orientation with increasing field strength. The plateau seen at higher field strengths is when the virus is parallel to the field and no further rotation is observed leading to a consistent mobility value.

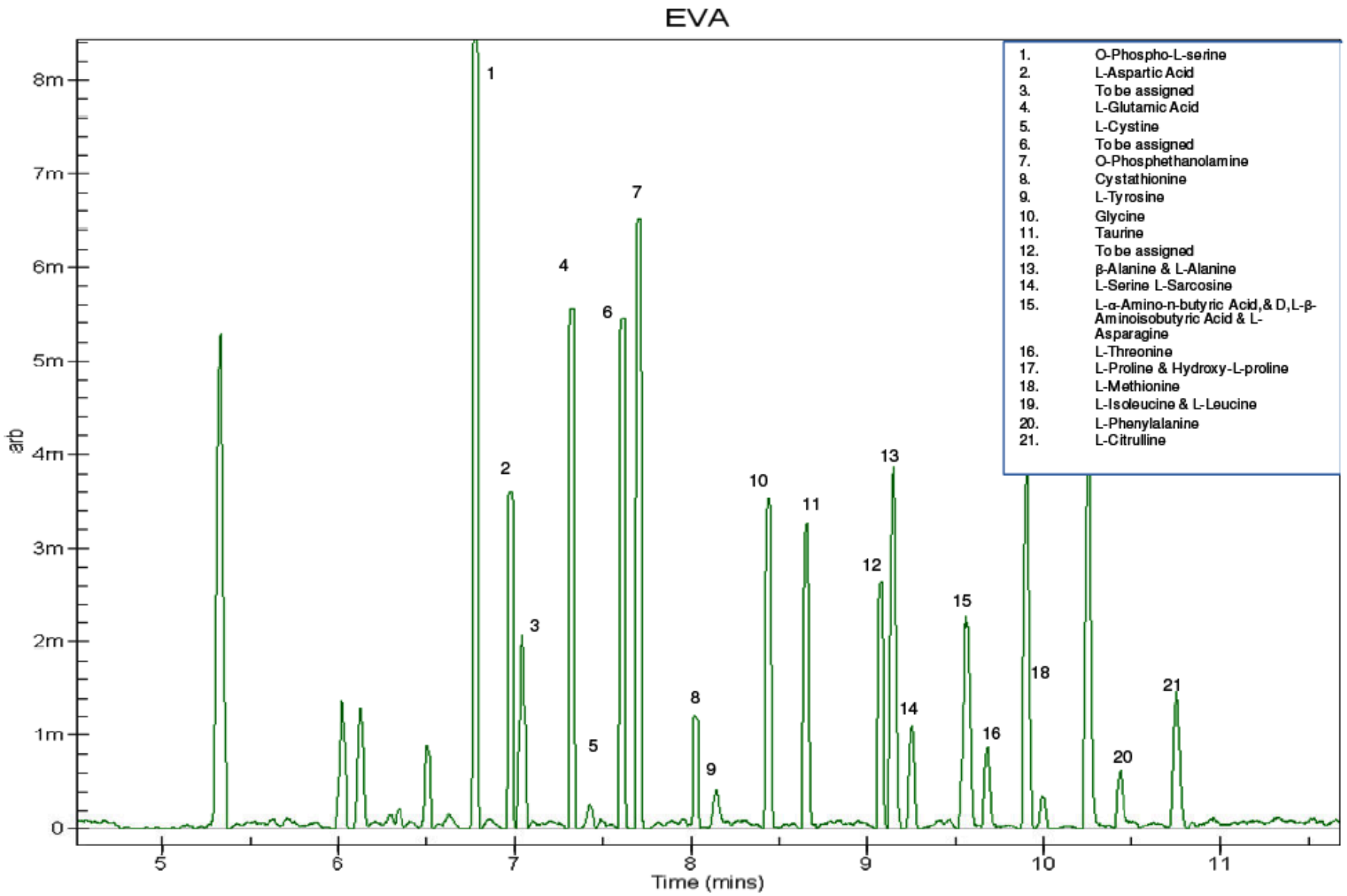




Grossman and Soane Result using Tobacco Mosaic Virus

Grossman, P. D., Soane, D. S., *Analytical Chemistry* 1990, *62*, 1592-1596.

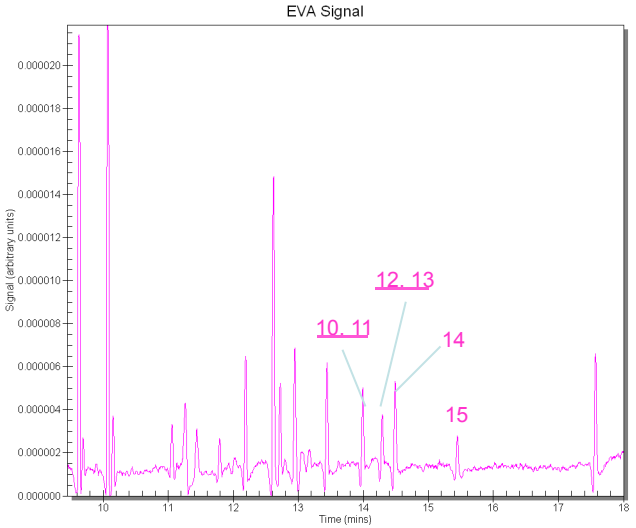
Indirect UV Amino Acid Analysis



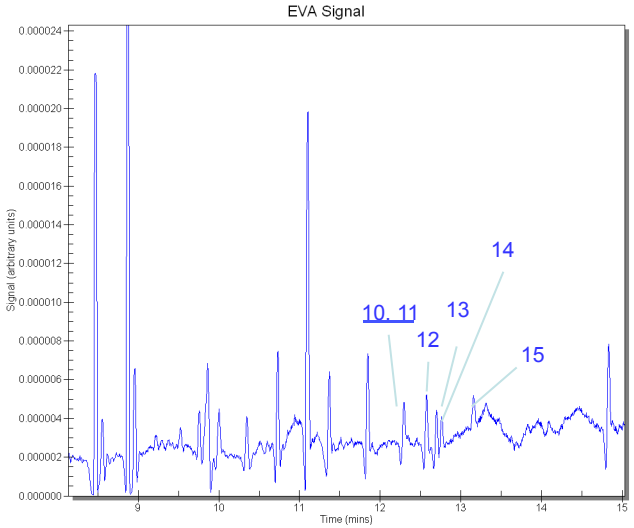
EVA processed data of amino acid standard solution (Sigma A6407)

Voltage Conditions on Amino Acid Profile

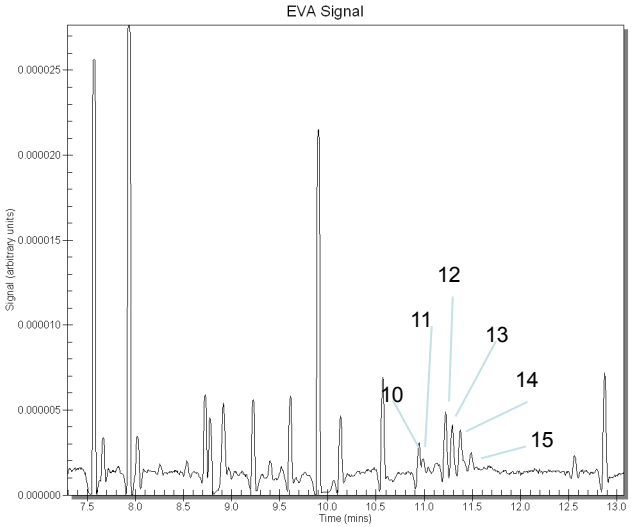
EVA Trace



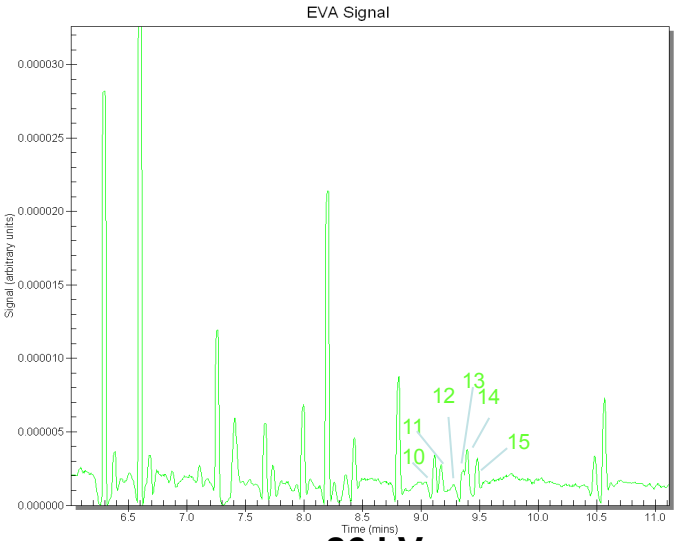
18 kV



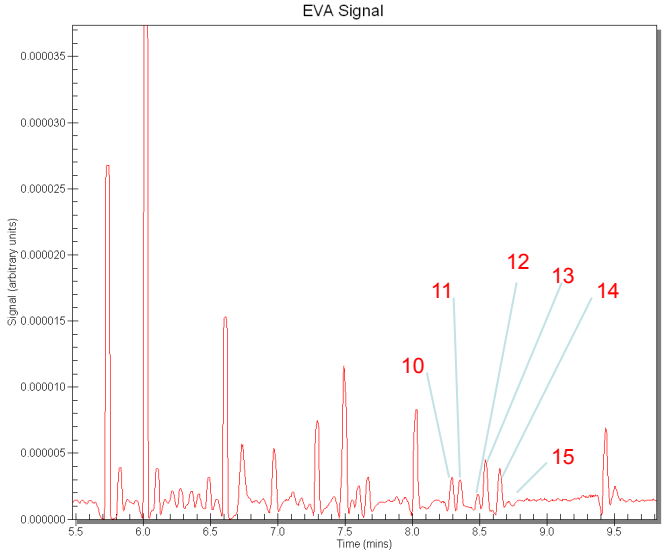
20 kV



22 kV

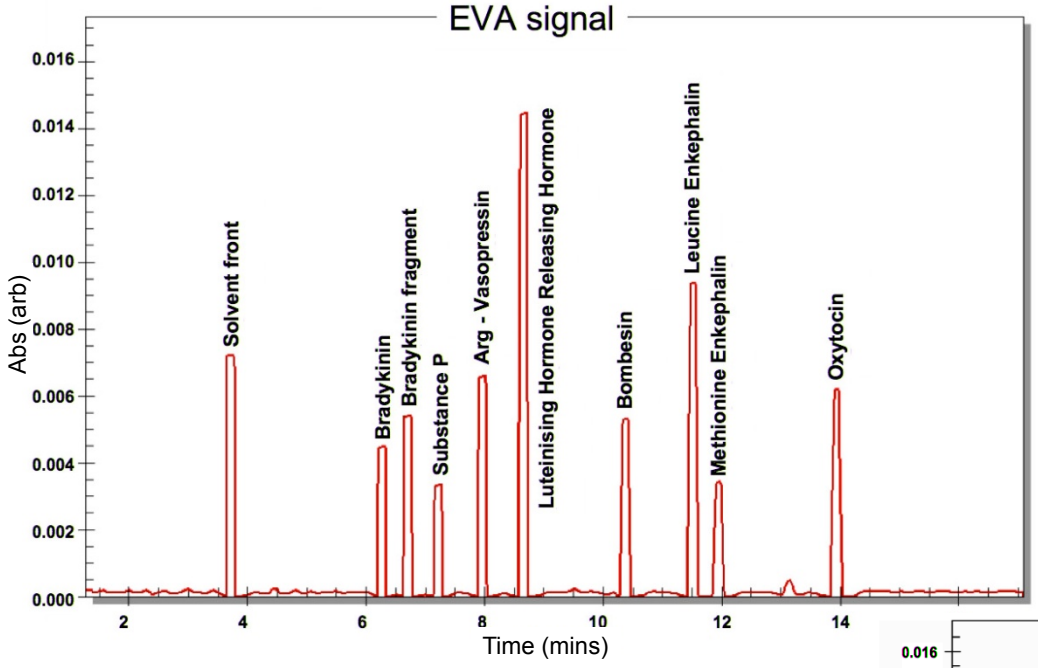


26 kV



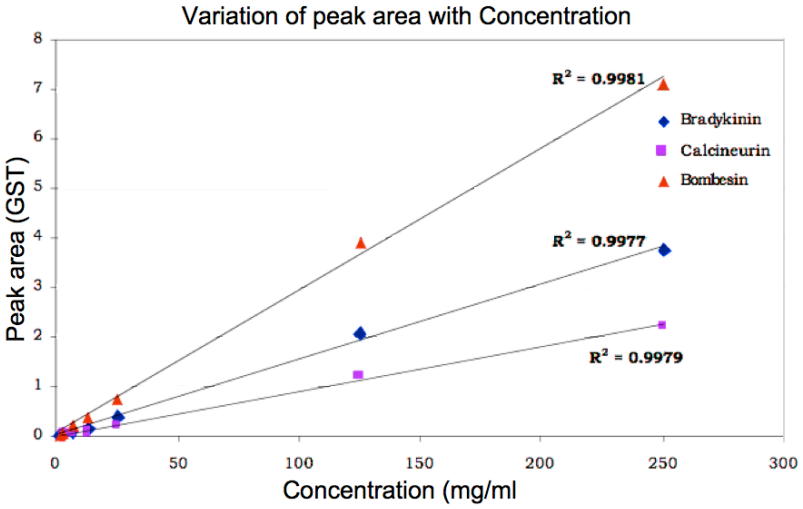
28 kV

Peptide Analysis

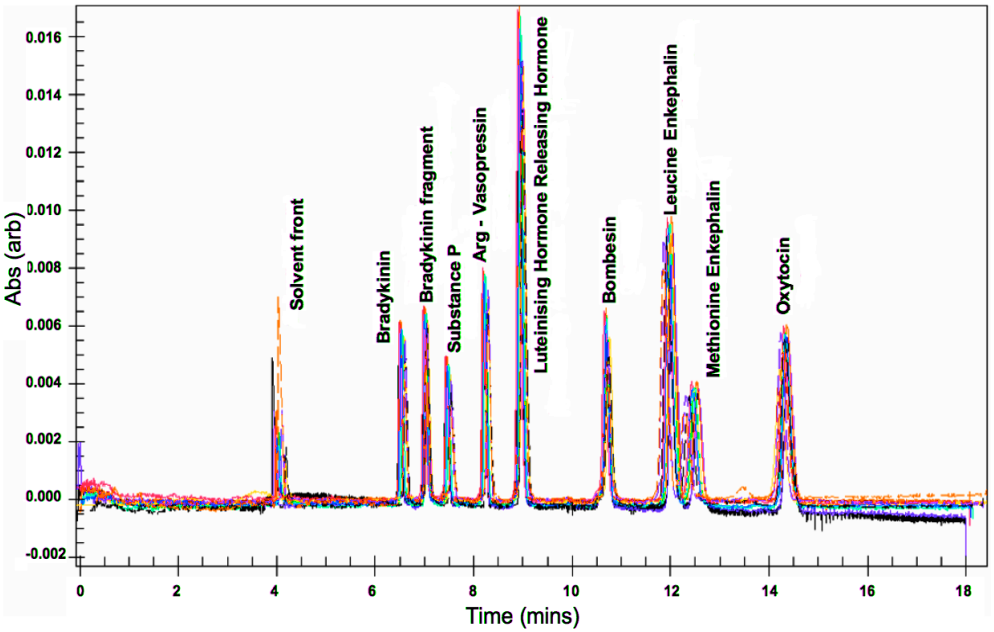


GST Data		
Peak	Peak Time % RSD (in minutes)	Peak Area % RSD
1	1.932	1.747
2	2.167	2.568
3	1.652	2.66
4	1.57	2.865
5	1.56	1.944
6	1.415	2.547
7	1.891	1.98
8	2.063	2.698
9	1.581	2.614

Percentage RSD values for peak time and peak area for all nine peaks of the peptide standard mixture (N=10).



Plot of sample concentration versus peak area.



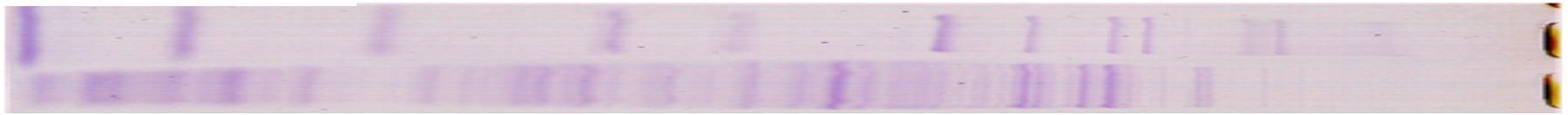
Reproducibility.

SIGMA standard peptide ladder

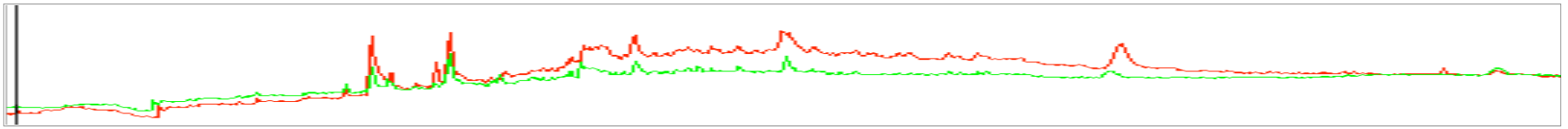
E.coli Analysis

deltaDOT
See the molecule

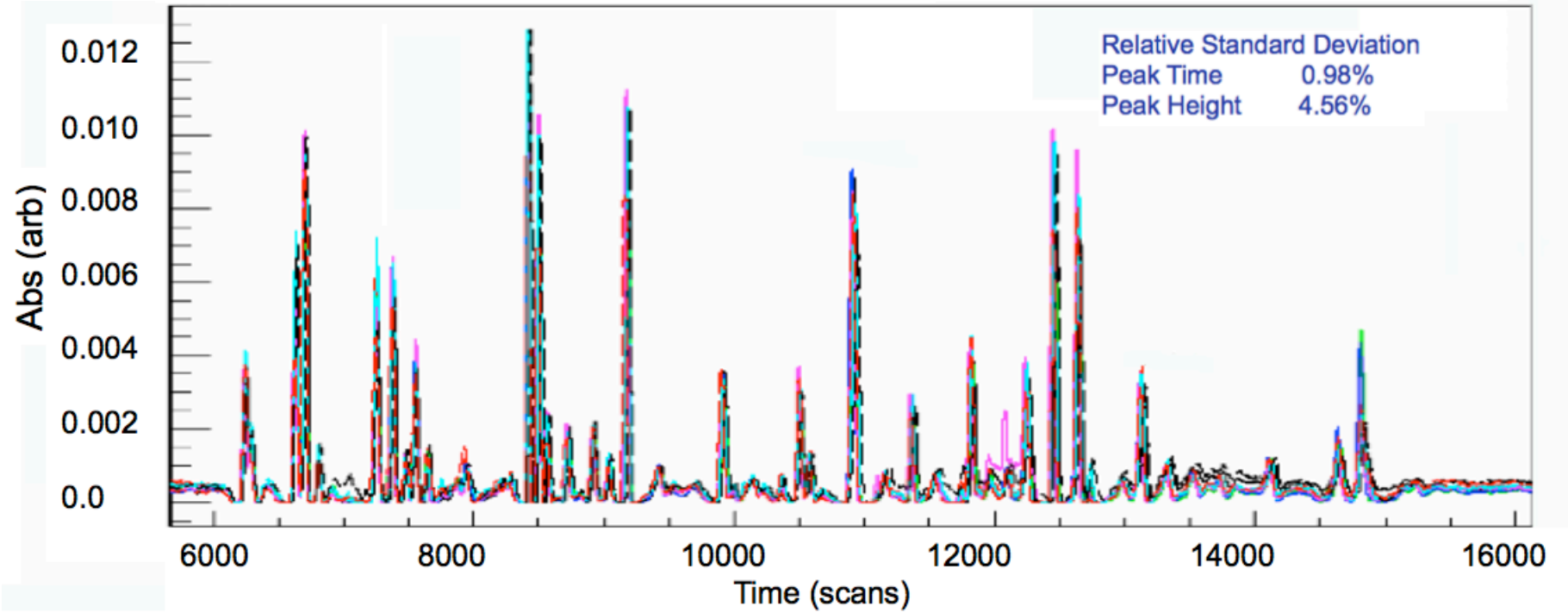
Conventional PAGE



Conventional CE



Label Free Intrinsic Imaging

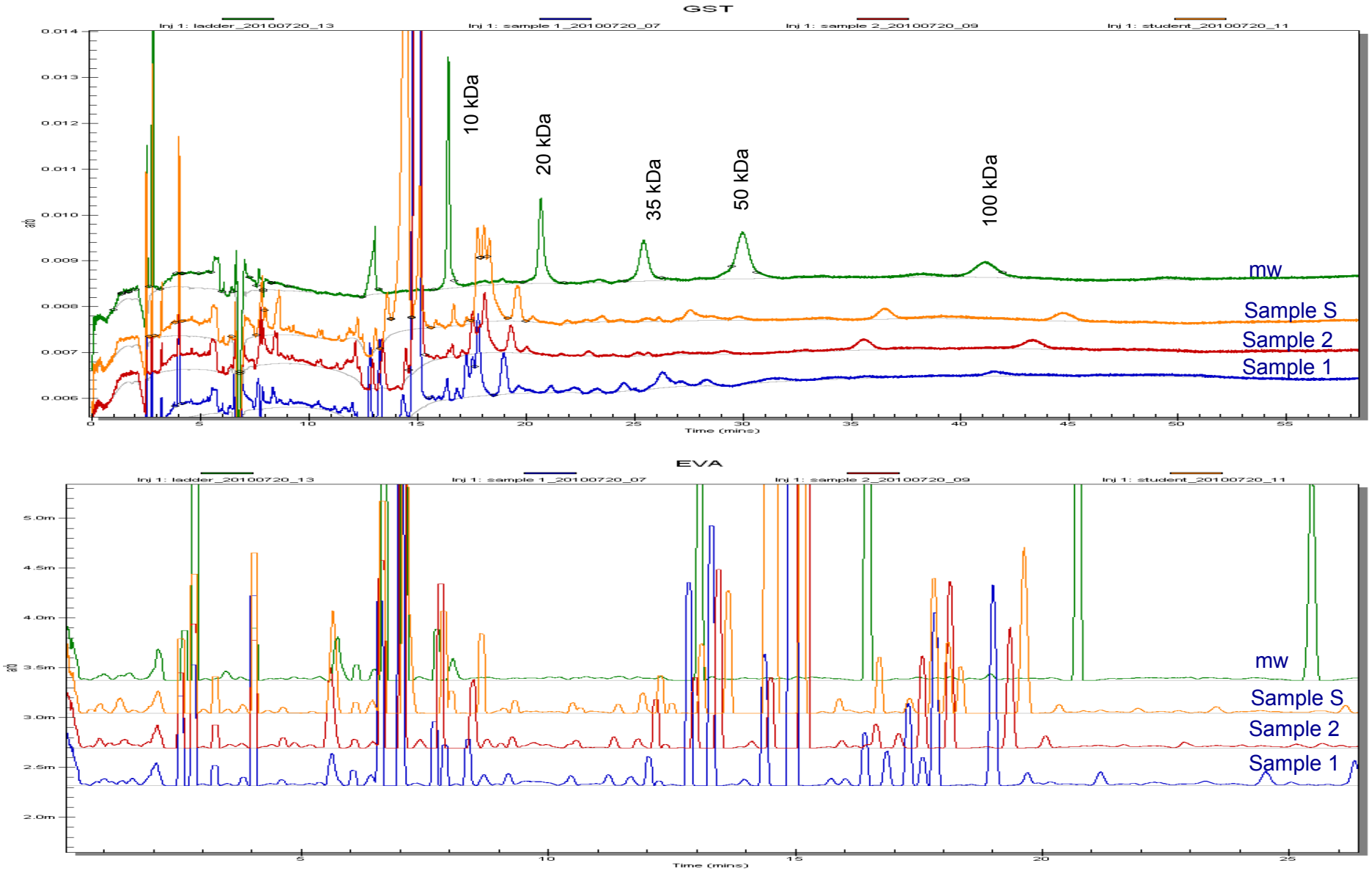


Overlay of the EVA processed data of nine consecutive *E. coli* lysate runs all separated under the same conditions.

BioRad *E.coli* Cell lysate standard

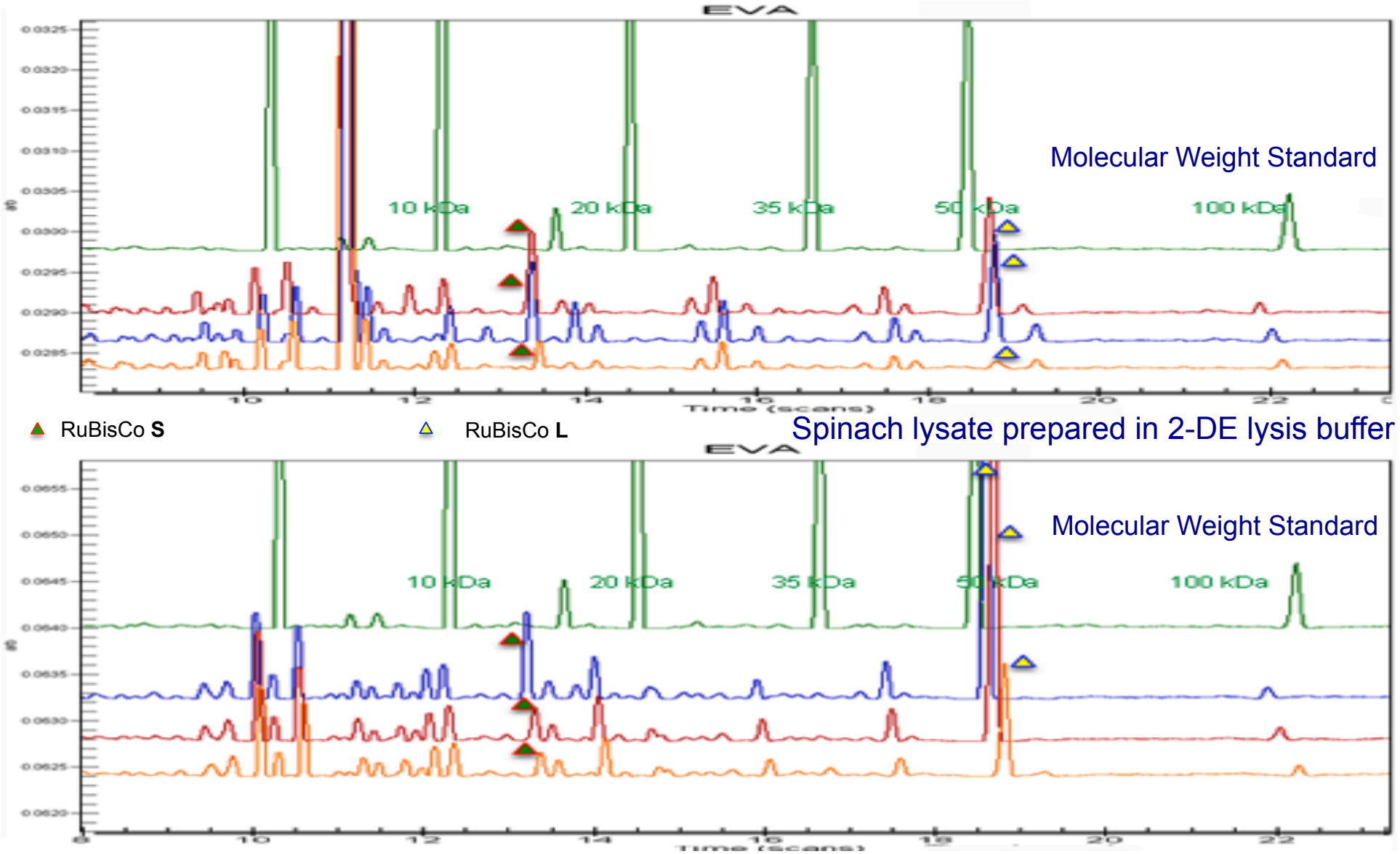
Heart Lysate Analysis

deltaDOT
See the molecule



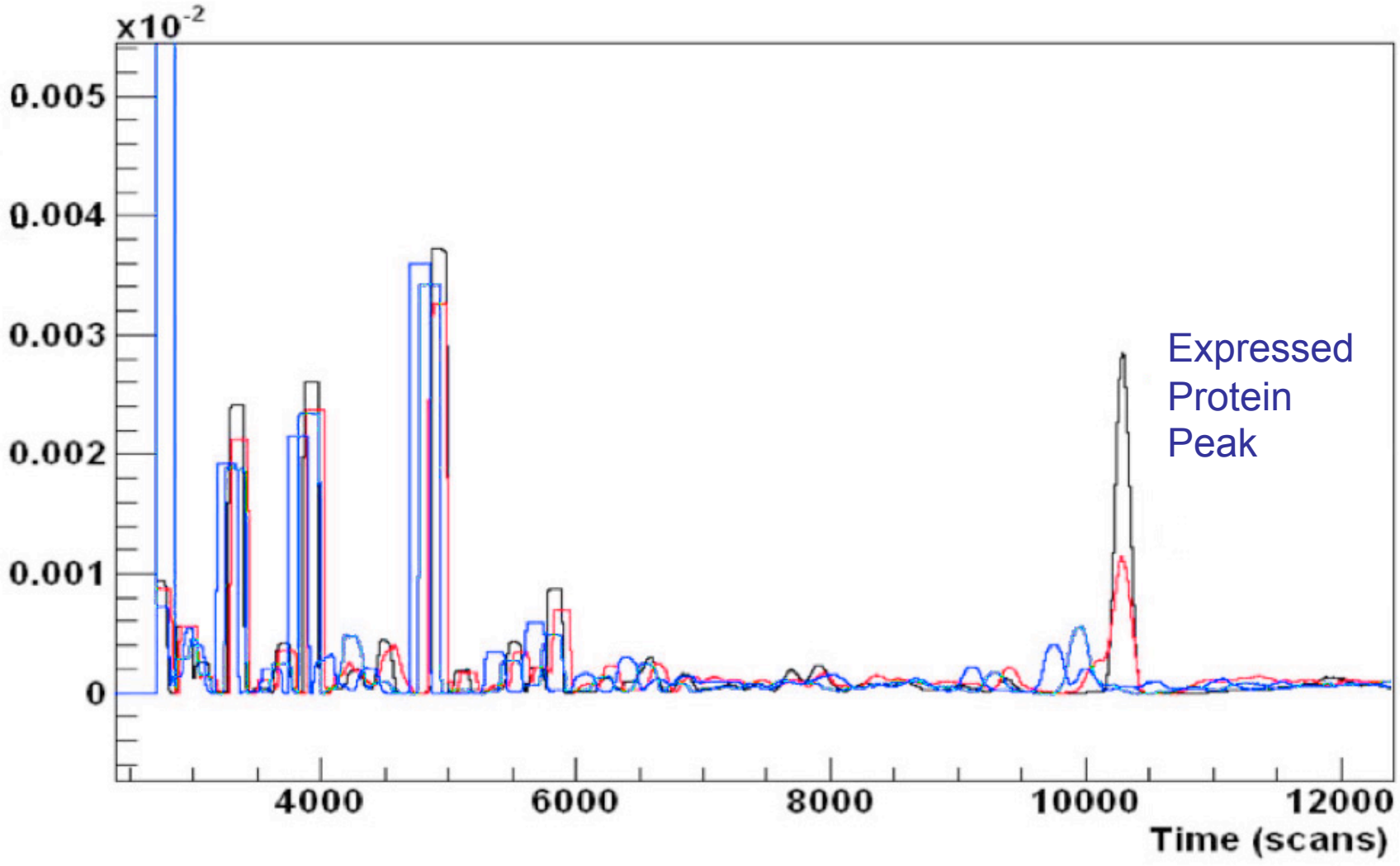
Protein Profile of Spinach

- Different buffer regimes



Spinach lysate prepared in Beckman sample buffer

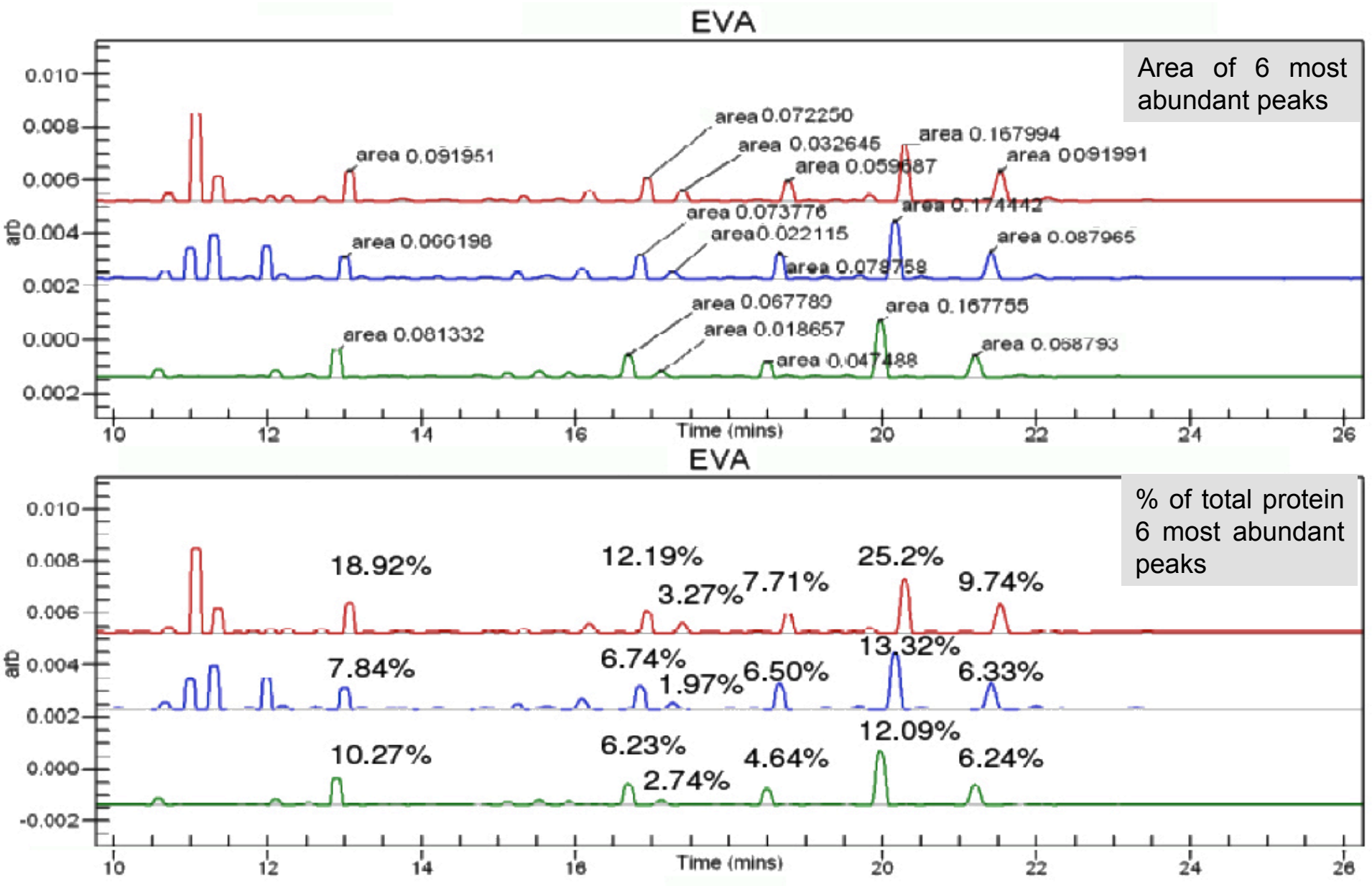
Amylase expressed in *E.coli*



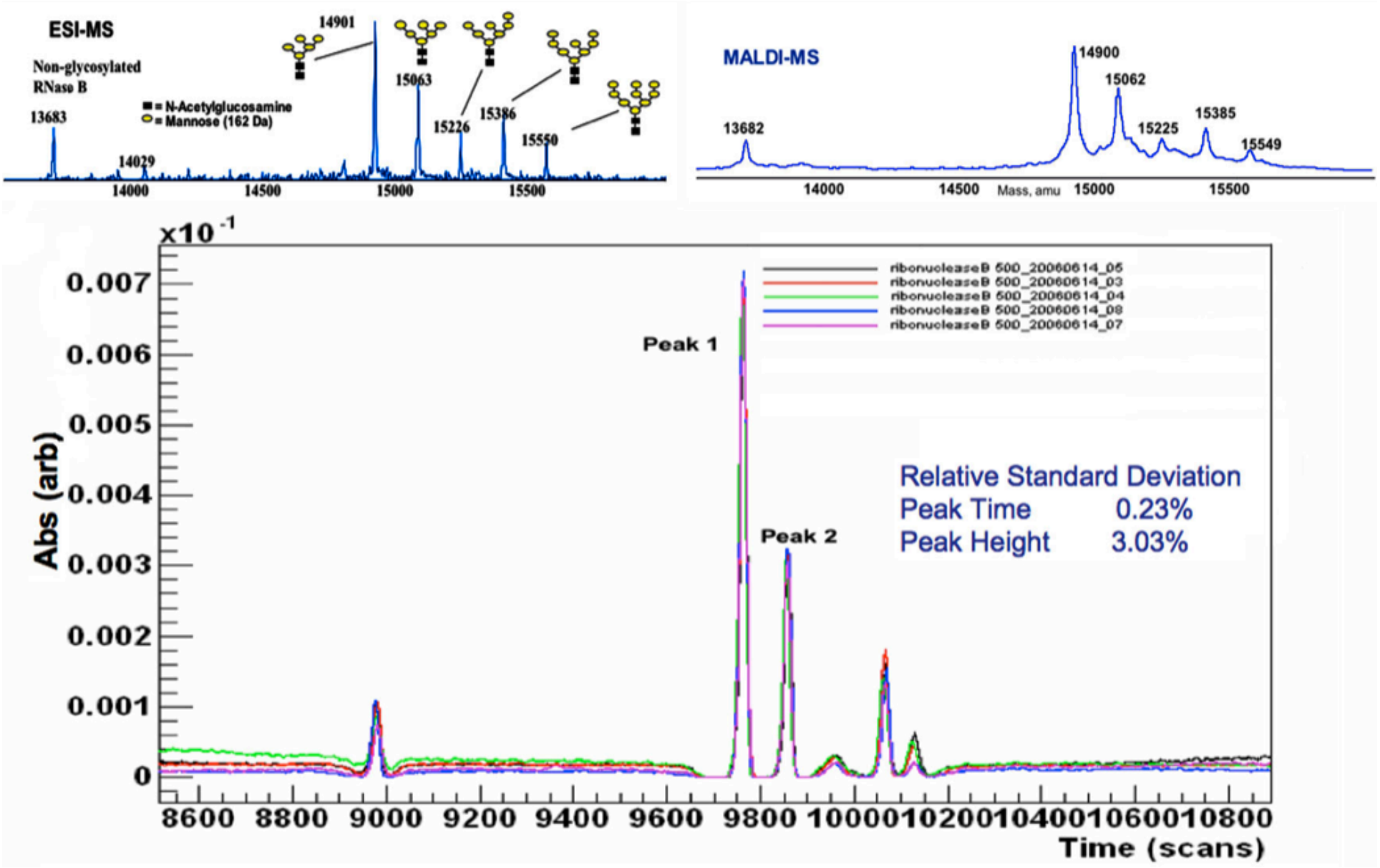
Expressed protein quantification in *E.coli* cell lysate

SDS-CGE stability analysis – quantification

Comparing batch-to-batch variations protein load normalized at sample prep stage

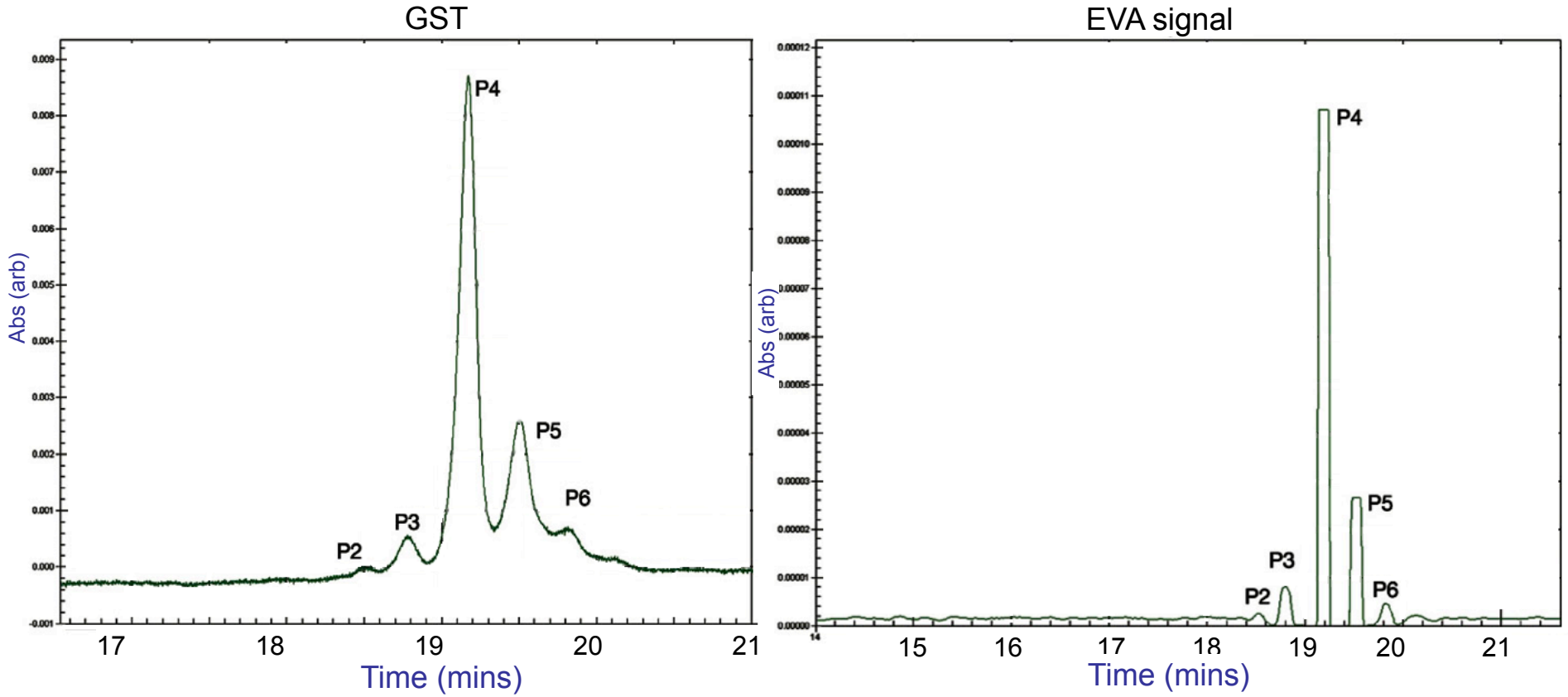


Glycoproteins



Ribonuclease B Glycoforms

Glycoproteins

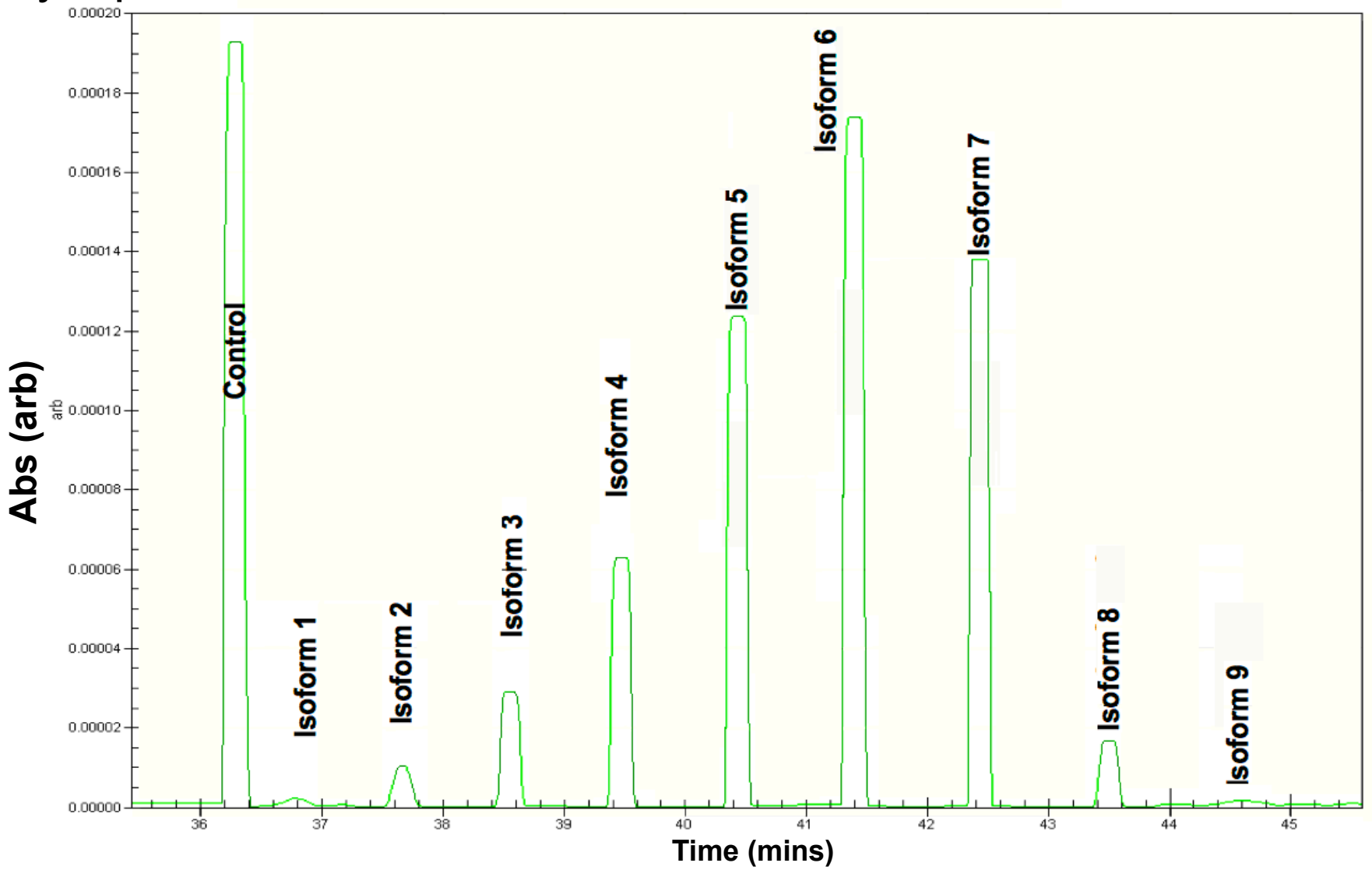


Using the Successive Multiple Ionic Layer (SMIL) coating developed by Katayama *et al* 1998 protein absorption was reduced and the Transferrin isoforms were separated based on differences in their net charge. The analysis of the distribution of sialoforms has been demonstrated.

Peak identification: P2 –disialo-Tf, P3 –trisialo–Tf, P4 –tetrasialo –Tf, P5 –pentasialo –Tf and P6 – hexasialo –Tf.

Human Holo-Transferrin

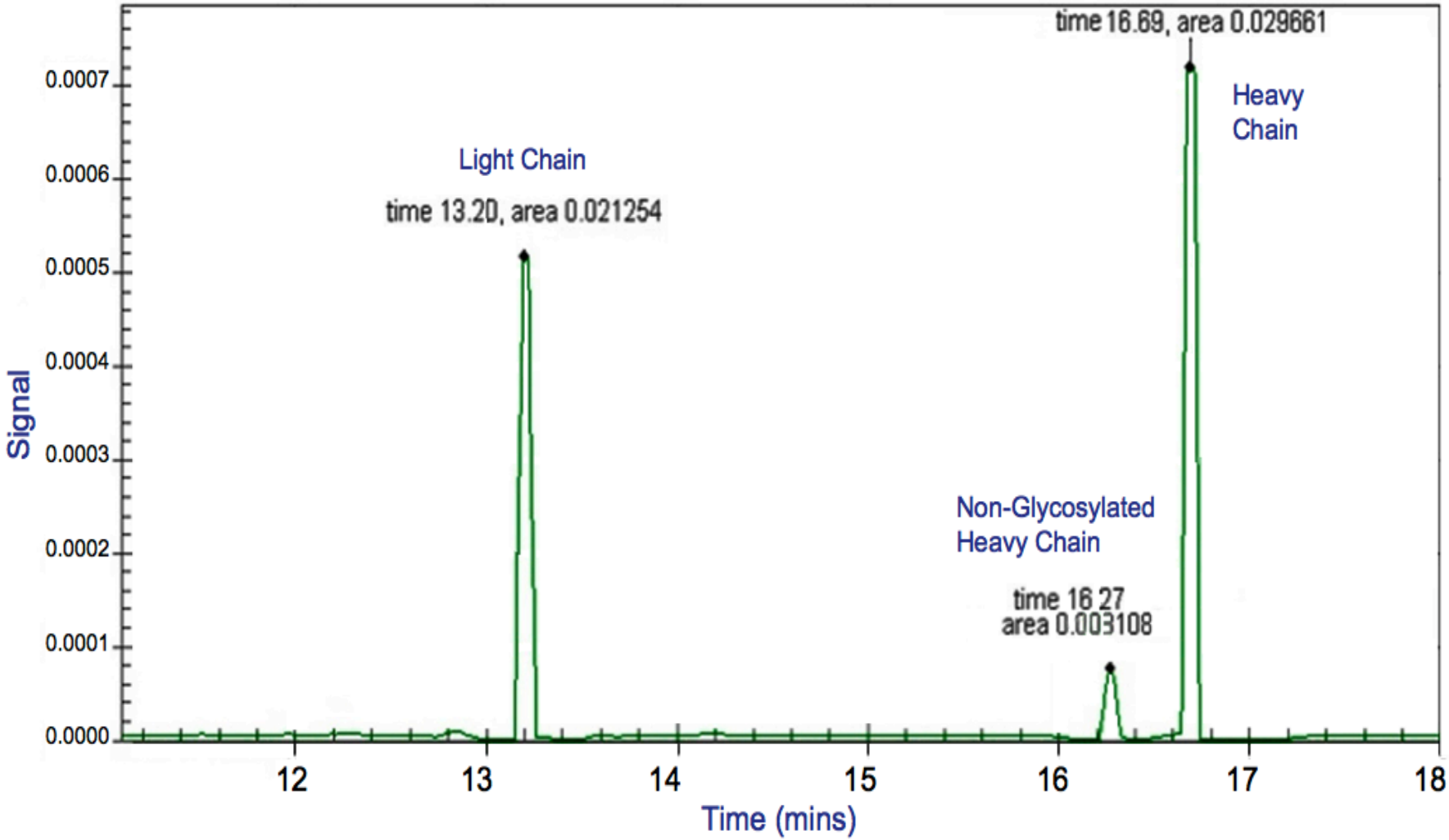
Glycoproteins



EVA processed data for recombinant human EPO. There are many variation in the number of isoforms, but the main set 1-8 are used in the European Pharmacopeia analysis parameters

Erythropoietin

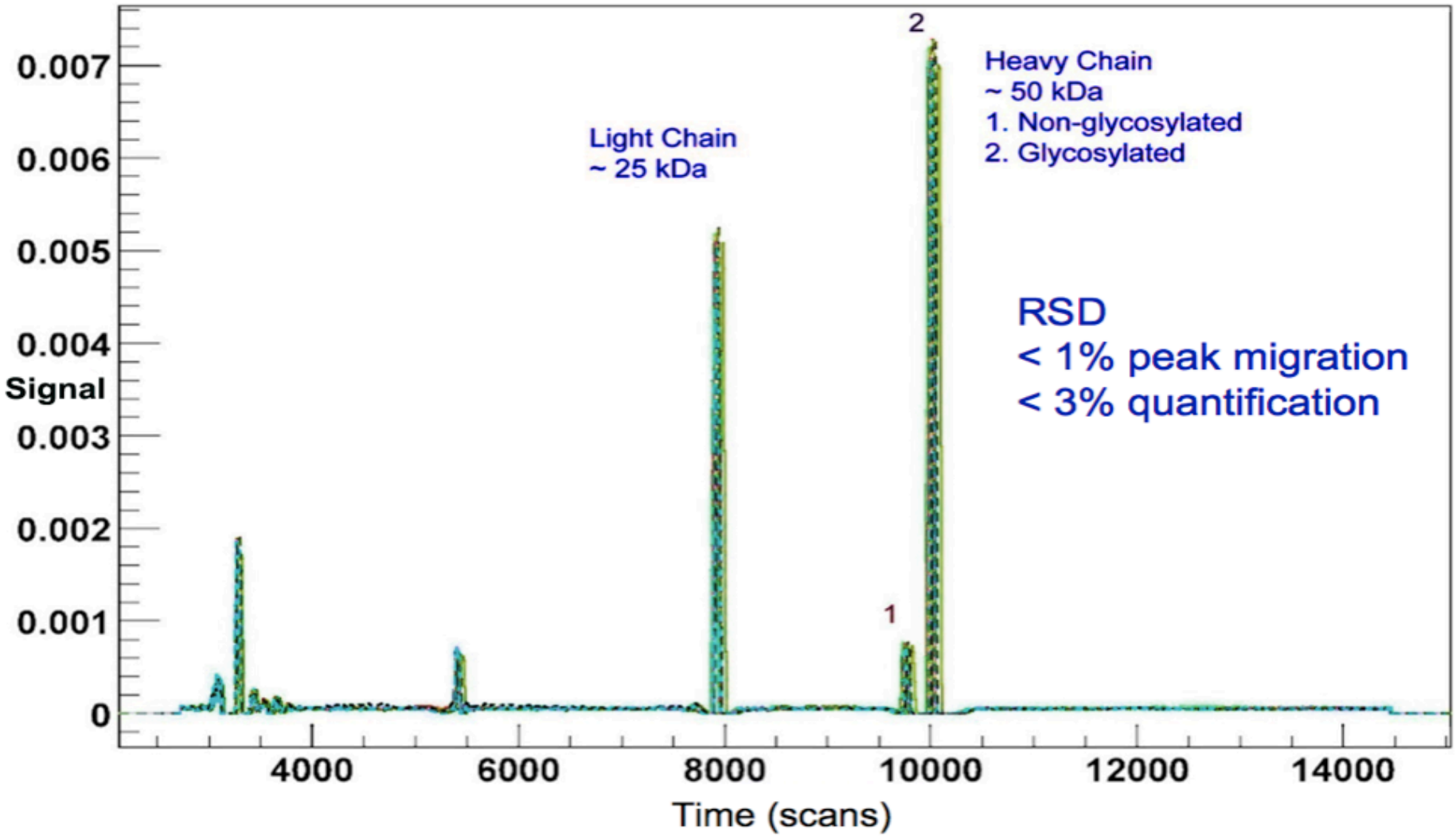
Antibodies



An antibody control standard analysed under denaturing conditions. The non-glycosylated heavy chain is clearly distinguishable from the glycosylated heavy chain. The reproducibility can be seen to be excellent over nine consecutive runs.

Antibody standard

Antibodies

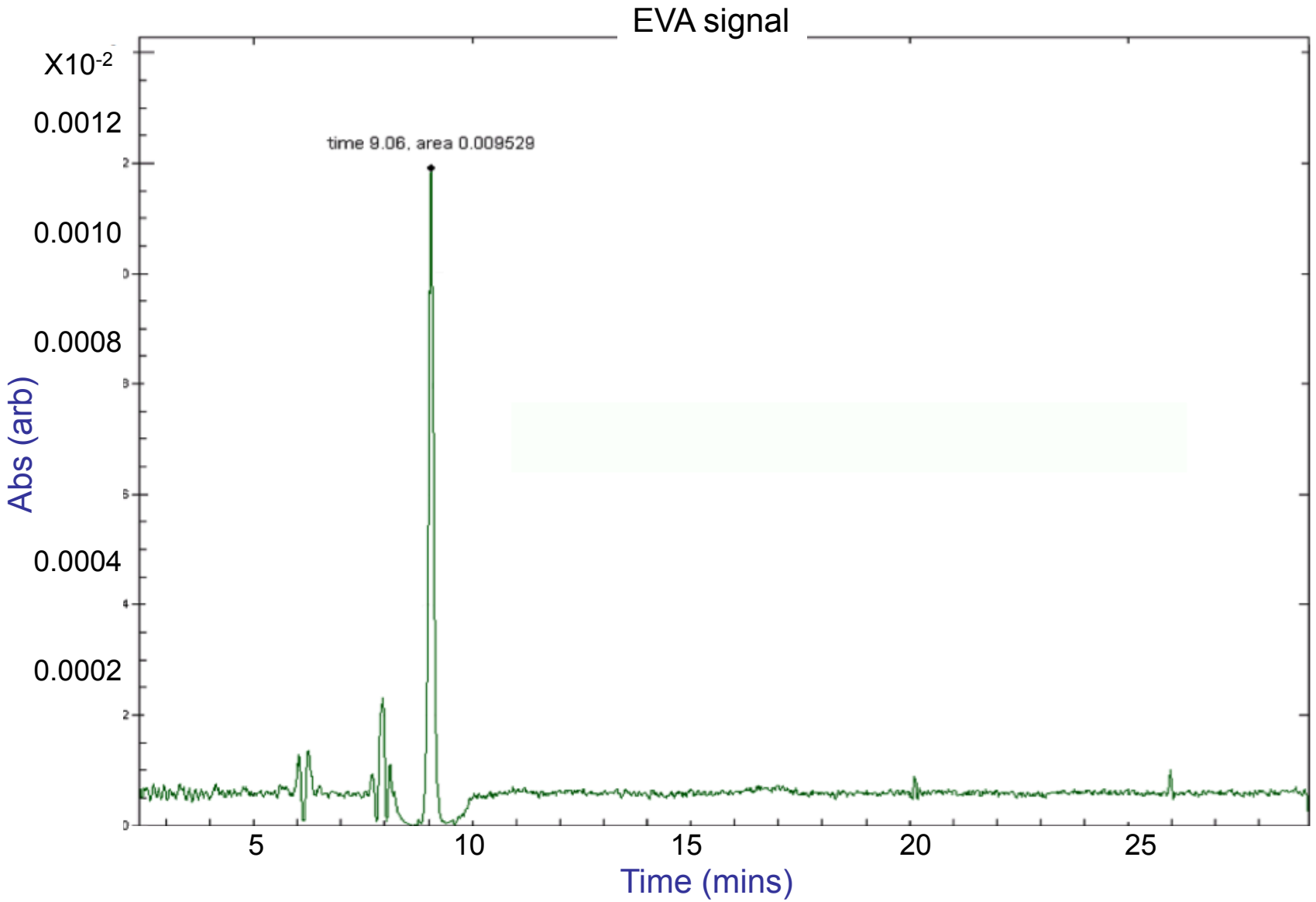


The reproducibility can be seen to be excellent over nine consecutive runs.

Antibody standard

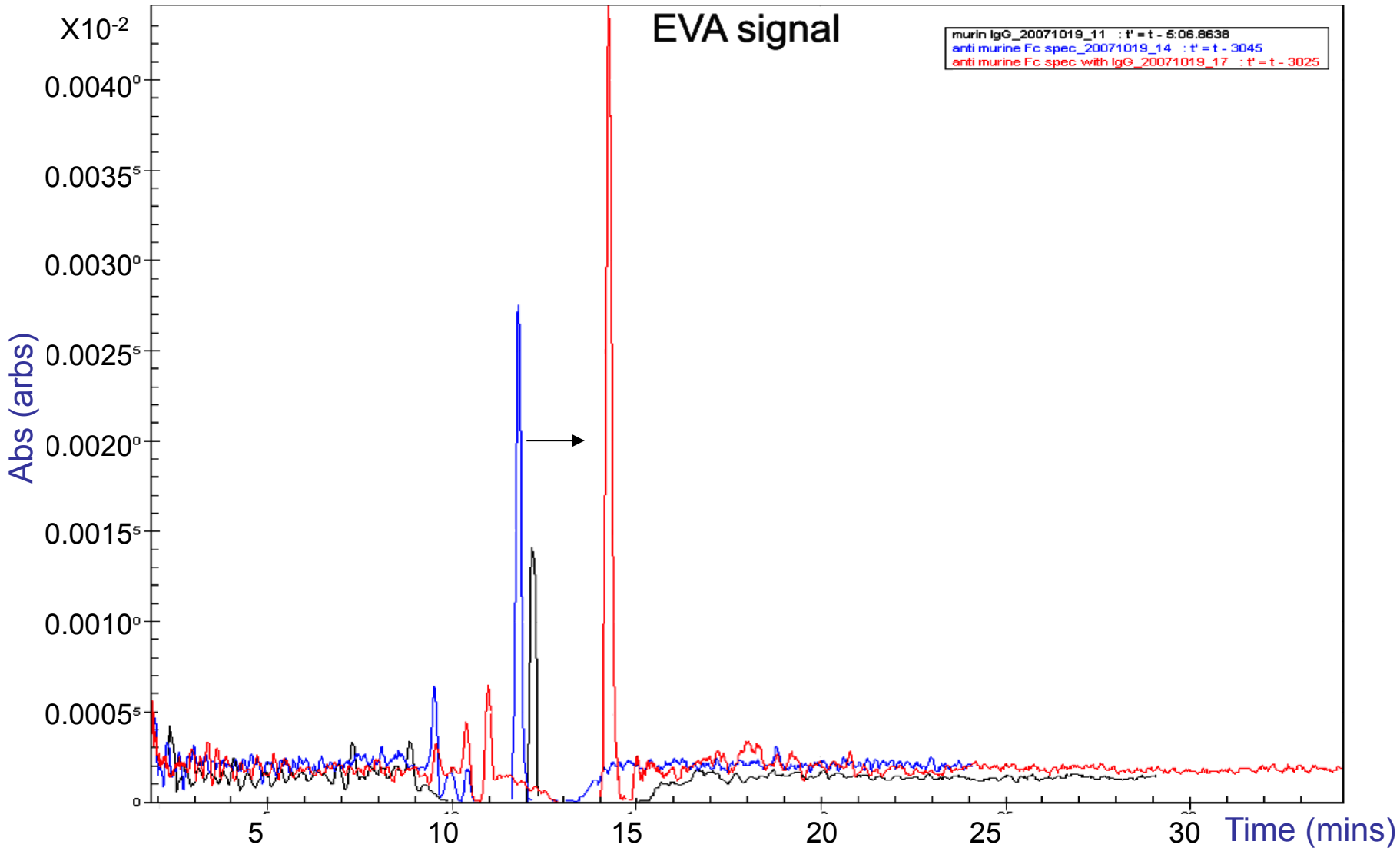
Antibodies

Capillary Zone Electrophoresis



Antibodies

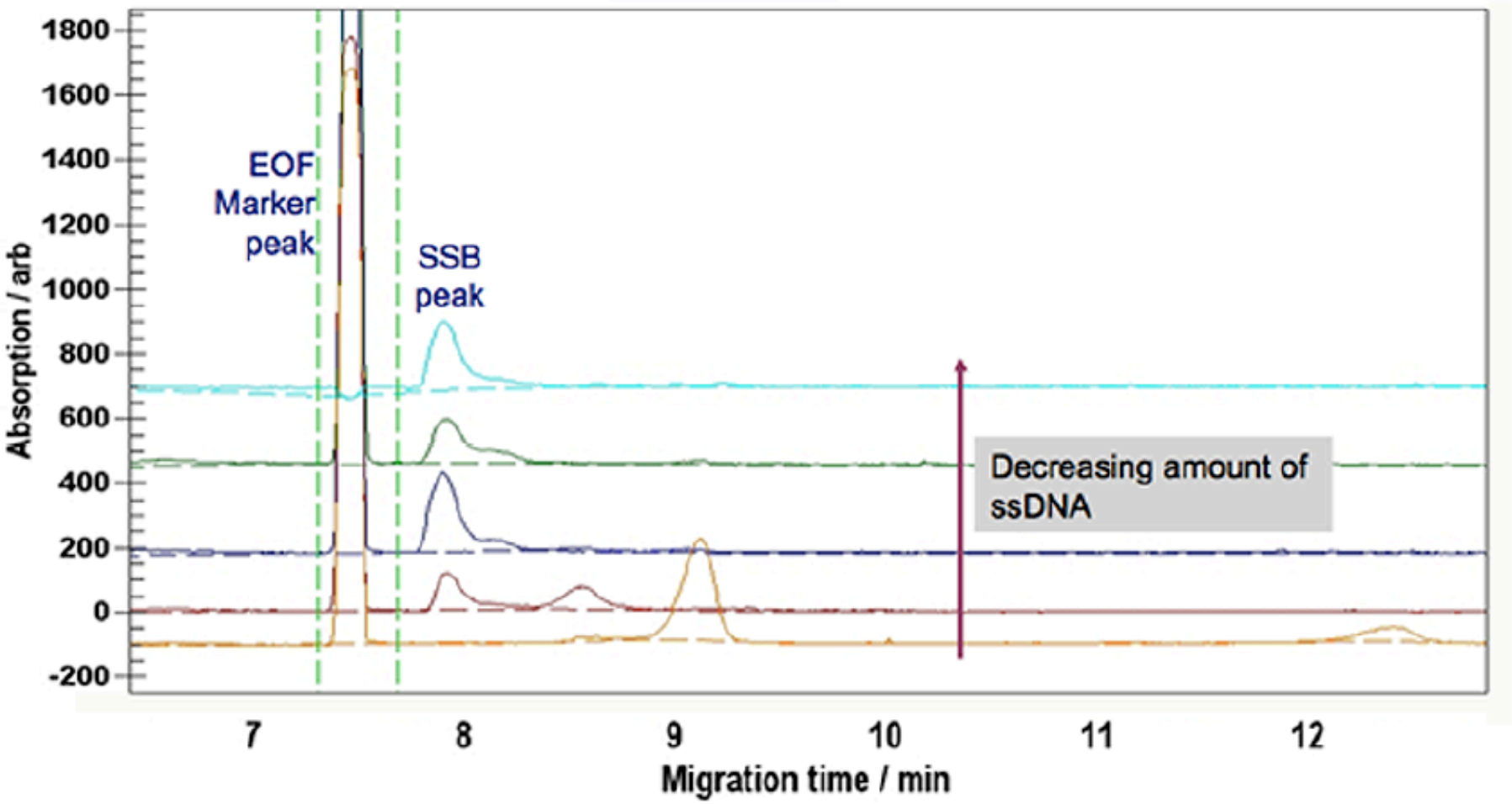
Capillary Zone Electrophoresis



This preliminary data shows a clear shift for the complexed proteins

IgG and anti-murine IgG

Protein/Nucleic acid interactions

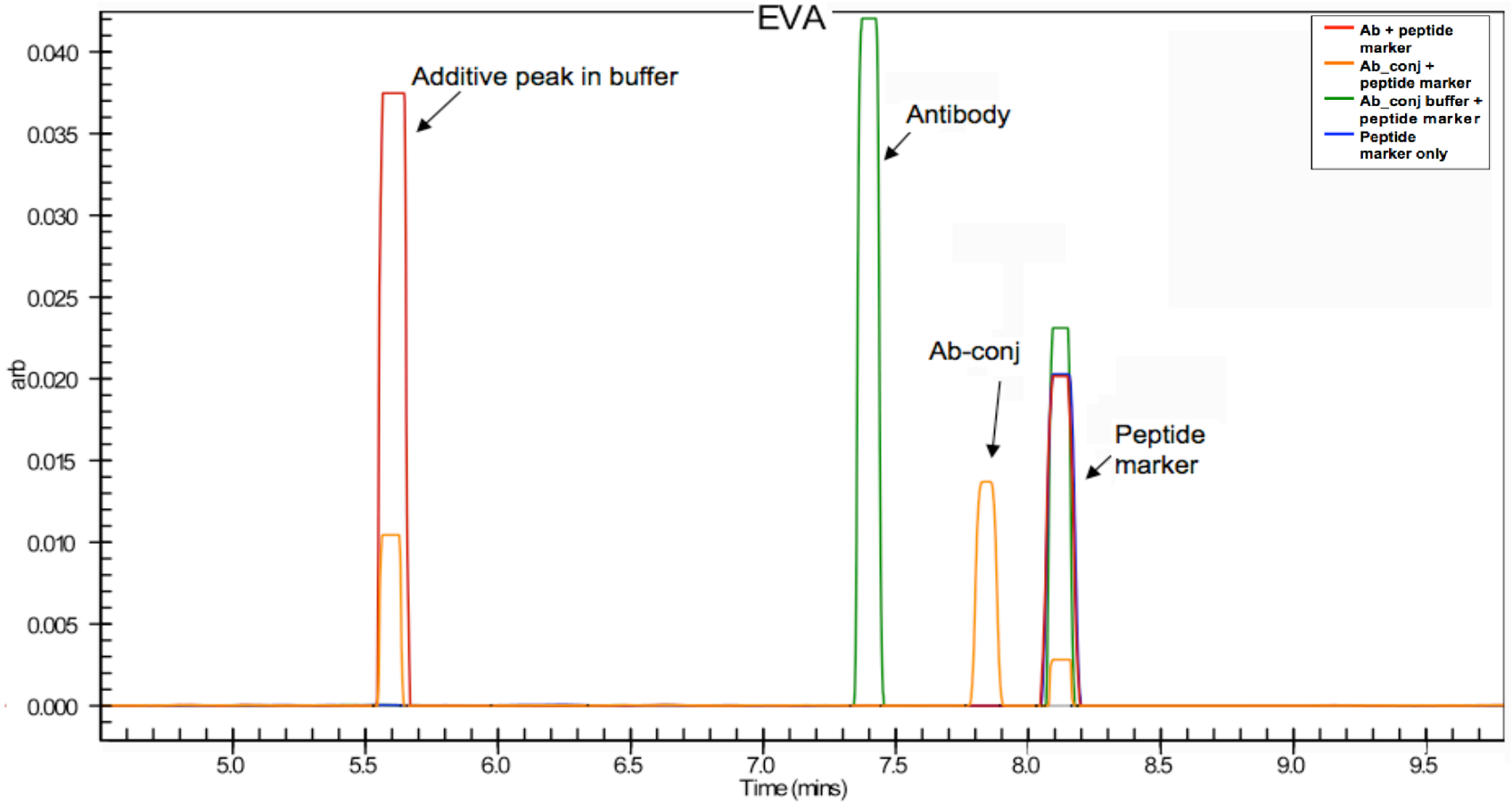


The change in single-stranded DNA binding protein (SSB) migration pattern in different concentrations of ssDNA.
Thiourea is added as an Electroosmotic Flow (EOF) marker.

Antibody/Conjugate interaction

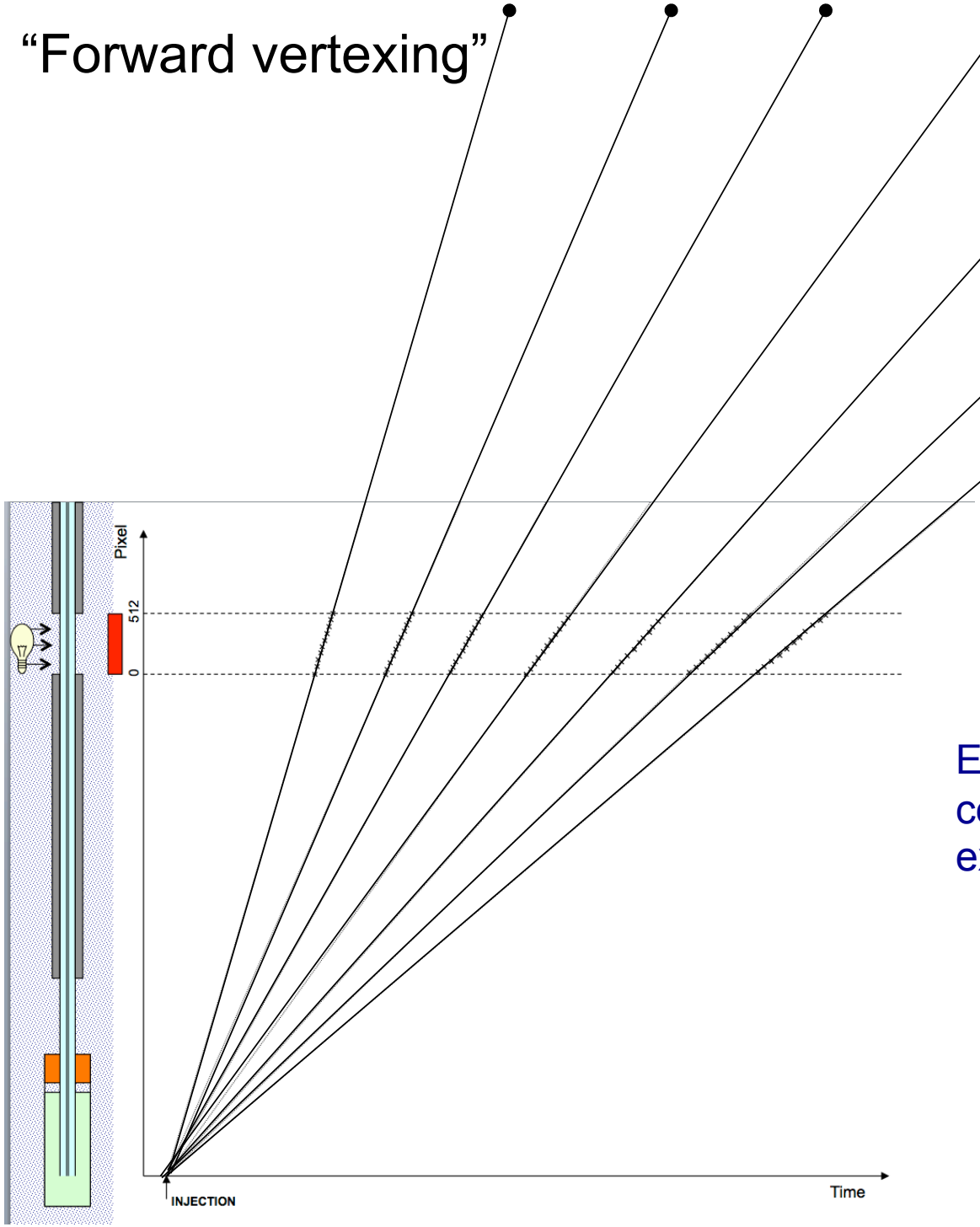
- Capillary Zone Electrophoresis

Distinct separation patterns were observed for the antibody, antibody-conjugate and peptide marker samples. One single major peak was observed in the CZE separation of the antibody sample while an additional small peak with faster migration time was observed in the CZE separation of the antibody-conjugate sample.



GST-processed CZE separation profile of the antibody (red), the antibody-conjugate (orange) and antibody-conjugate buffer samples (green) in the presence of the peptide internal marker overlaid with the separation profile of the peptide marker on its own (blue).

“Forward vertexing”



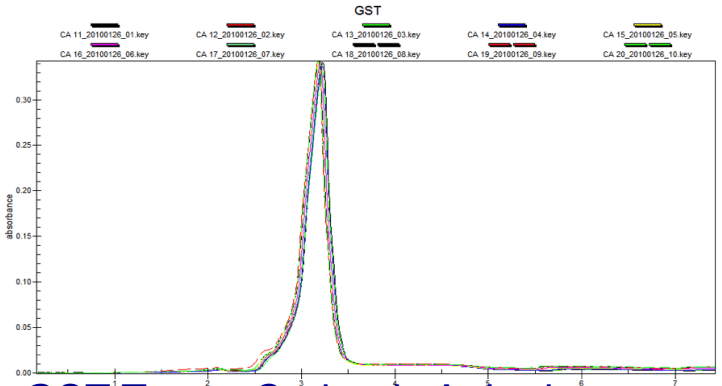
Extrapolate the space/time correlation forward to the peak exit moment

Recovery of sample from PEREGRINE I

- for further analysis in e.g. Mass Spec

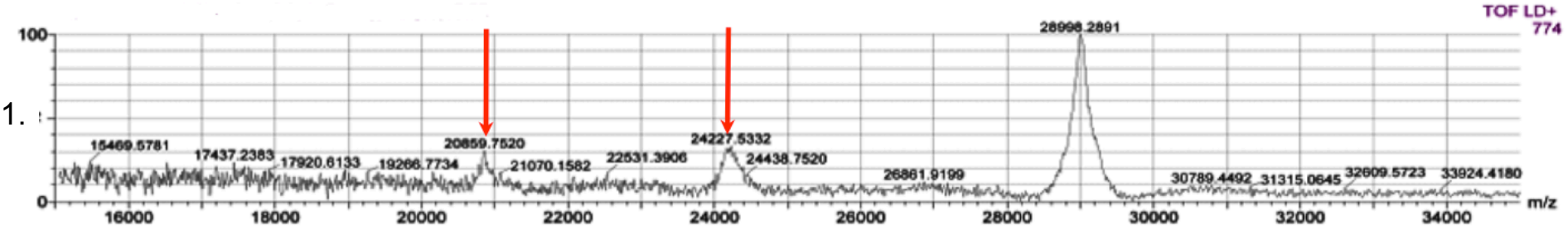
Samples separated in the PEREGRINE I can be further analysed by collecting the target peaks.

This is achieved by forward vertexing the peak position and collecting it in a “waste” vial.



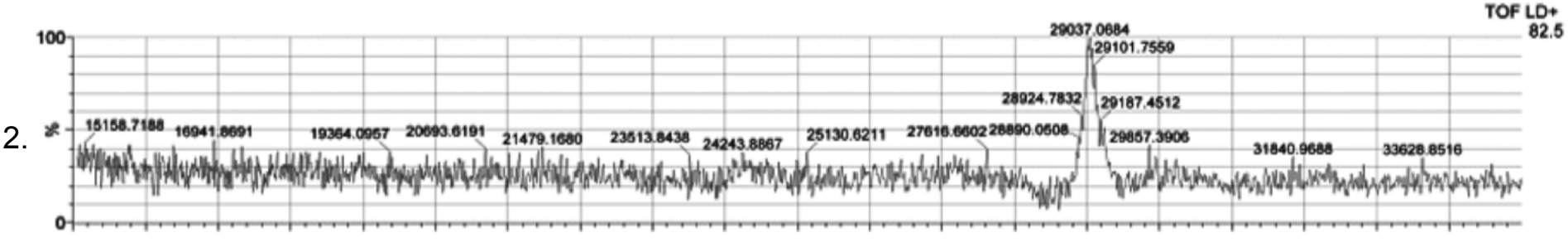
GST Trace. Carbonic Anhydrase analysed in the PEREGRINE I system

1.



MS Trace 1. MALDI spectrum of carbonic anhydrase stock control.

2.

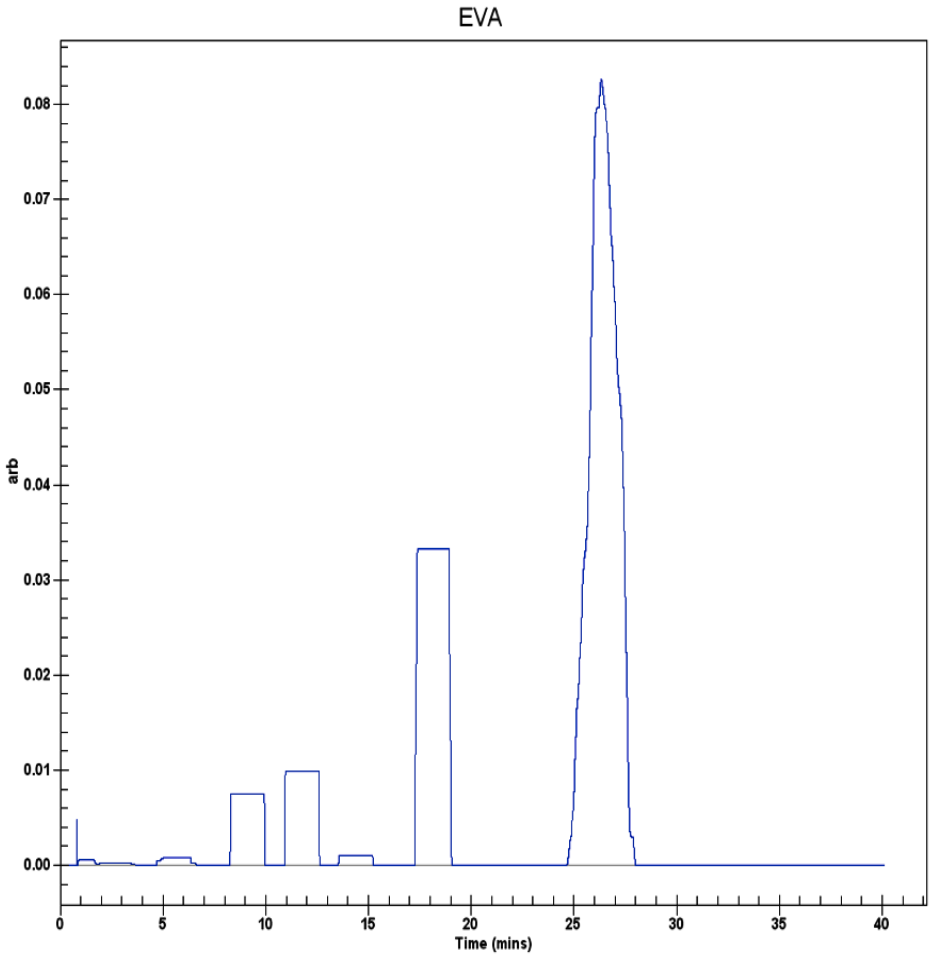


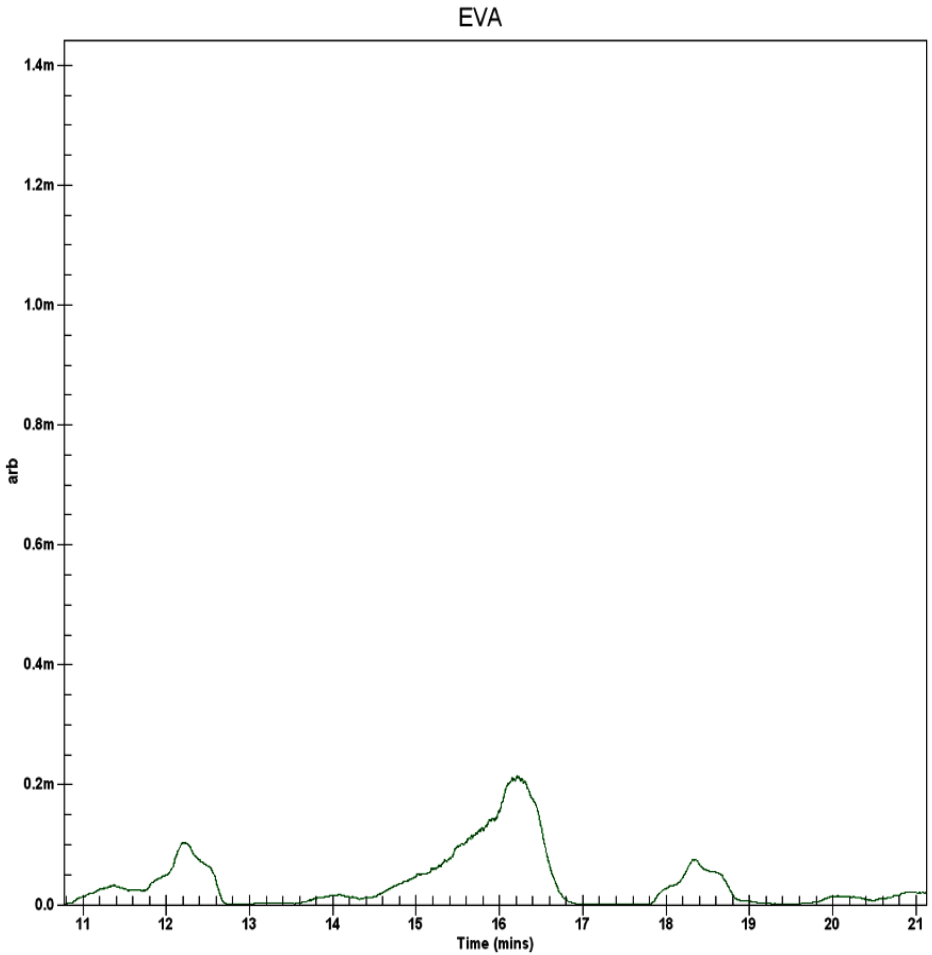
MS Trace 2. MALDI spectrum of carbonic anhydrase following collection of 20 peaks from the PEREGRINE I

Recovery of sample from PEREGRINE I

- for further analysis in e.g. SEM

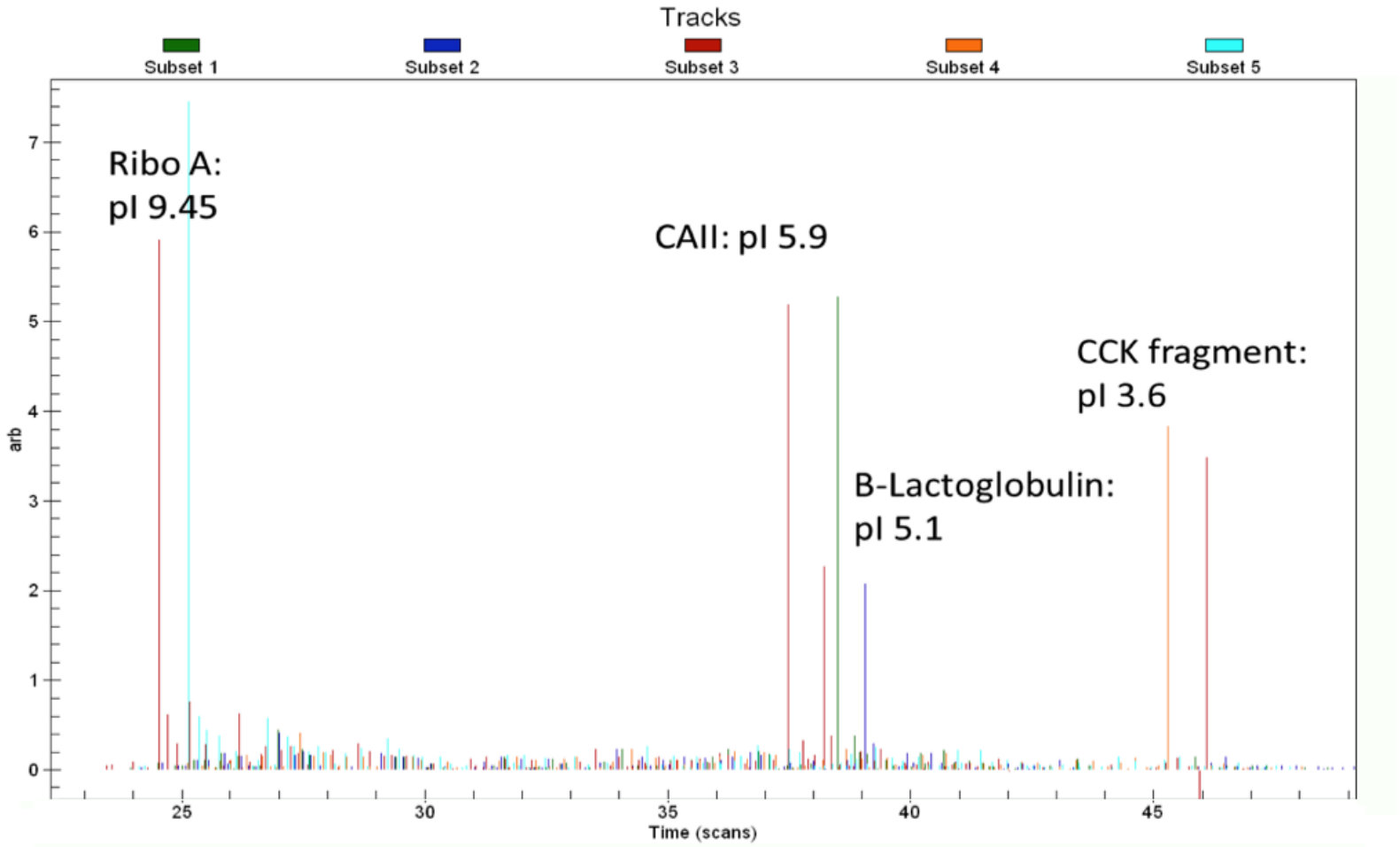
Samples separated in the PEREGRINE I can be further analysed by collecting the target peaks.
This is achieved by forward vertexing the peak position and collecting it in a “waste” vial.





IEF

-performed by capillary electrophoresis (cIEF) with chemical mobilization



cIEF separation of sample containing a mixture of Ribonuclease A, carbonic anhydrase II, β-lactoglobulin-A and CCK flanking peptide overlaid on a sample containing Ribonuclease A, carbonic anhydrase II, β-lactoglobulin-A and CCK flanking peptide.

Conclusions

In the Bioprocess, LFII® can be used to analyse

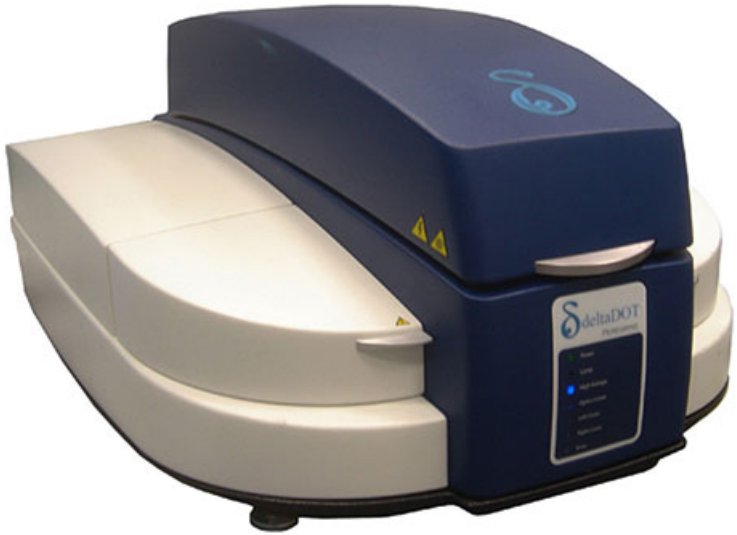
- cloning steps
- the titre of e.g. Baculovirus expression systems,
- the bioreactor media components
- the expression level of the target protein
- the final QA/QC steps.

This approach in the bioprocess workflow allows great economies of scale, reduces analytical bias and offers a step-change in power necessary for the huge increase in throughput required by the goal accelerating drug development.

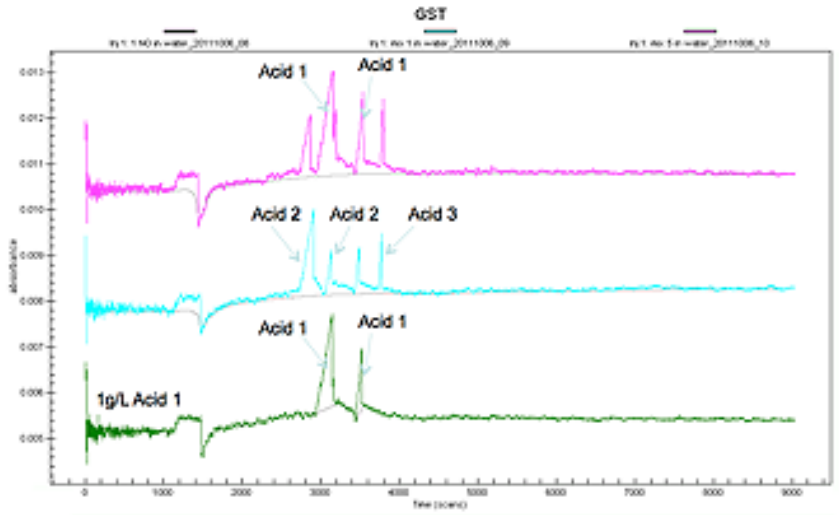
deltaDOT

See the molecule

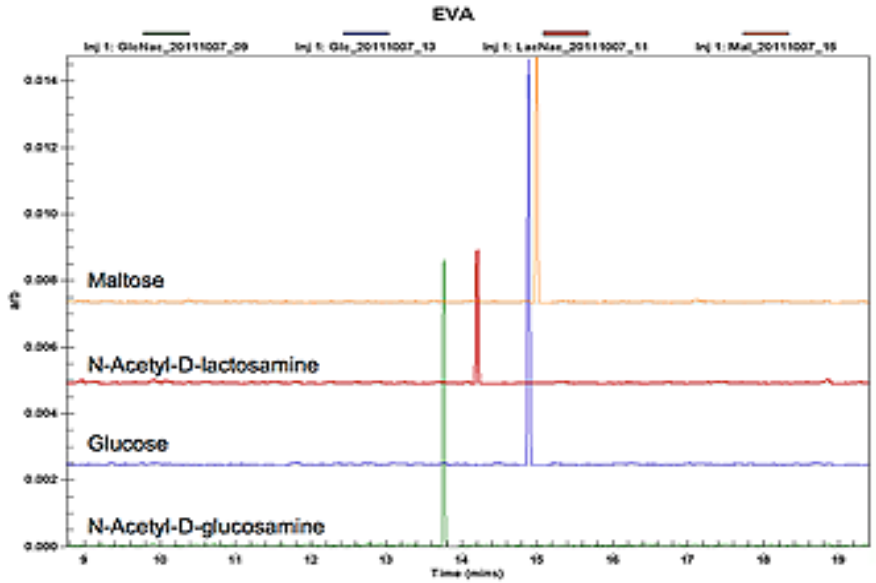
Other applications of LFII®



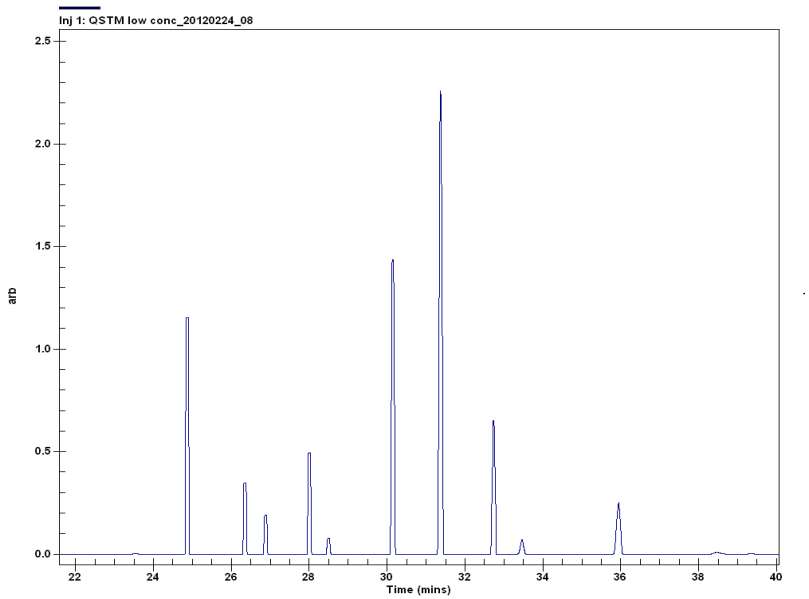
Other sample analyses



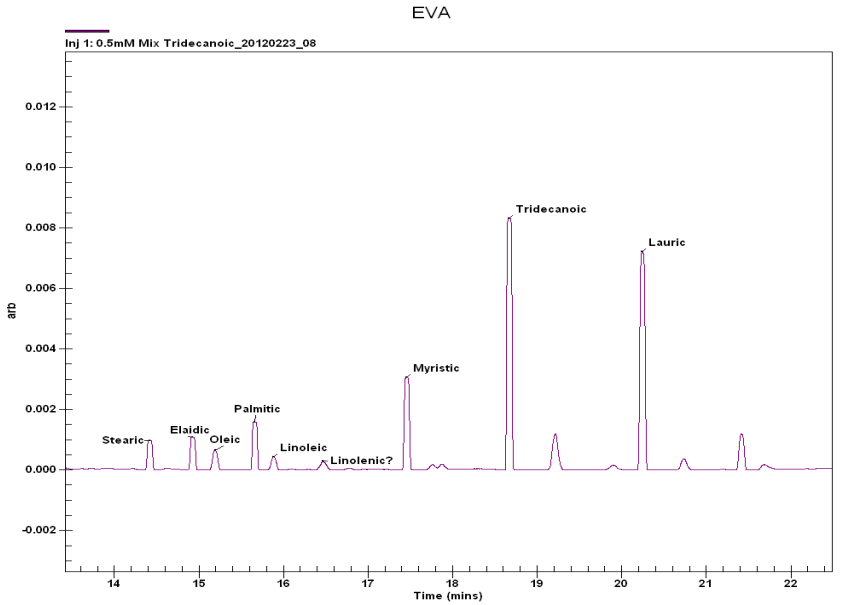
Various acids analysed by LFII®



Carbohydrate analysis

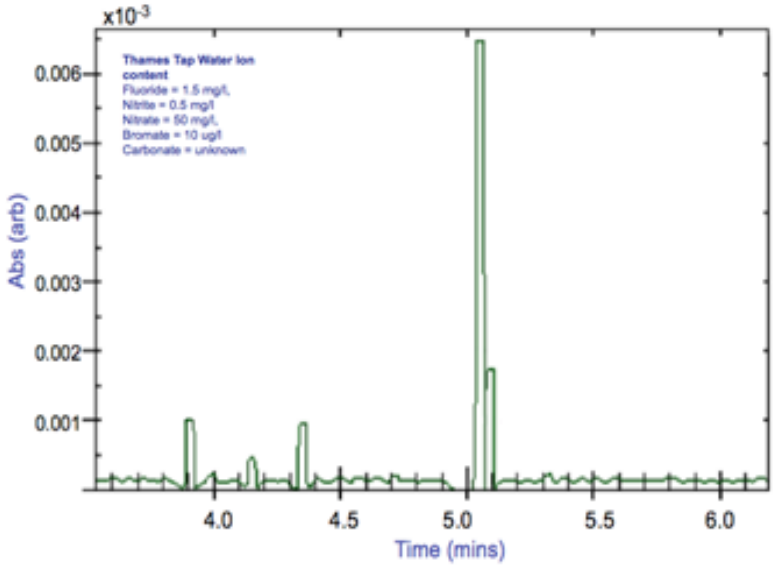


PolyCyclic Aromatic Hydrocarbon Mix

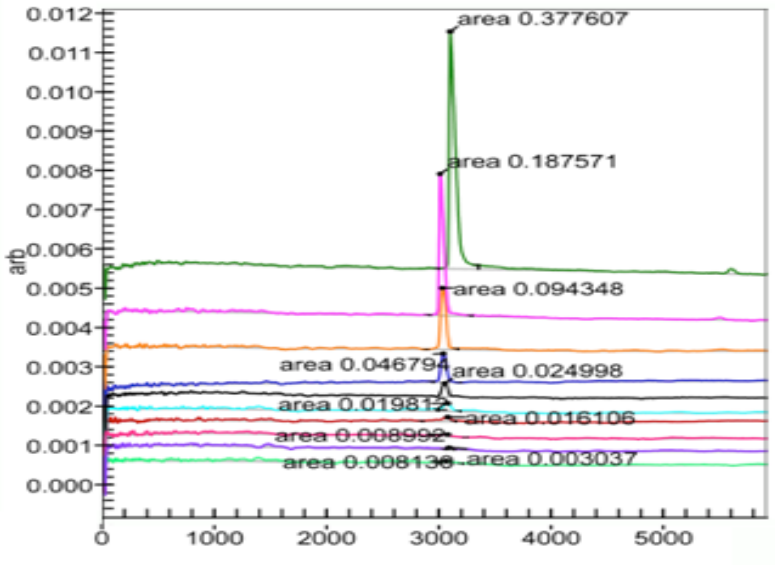


Fatty Acid standards at 0.5mM

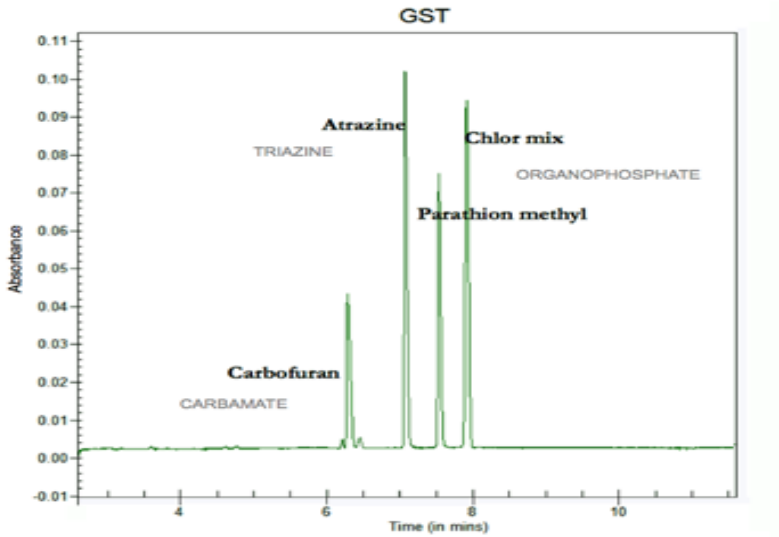
Other sample analyses



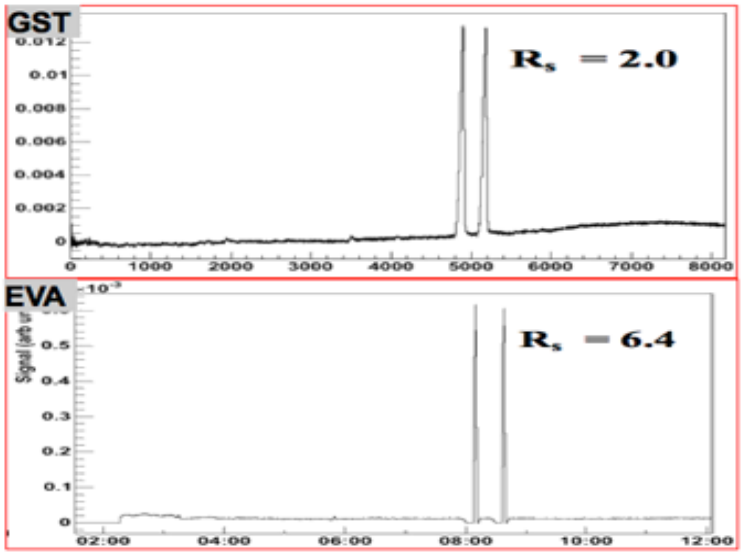
Indirect UV Analysis of Tap Water



Nicotine analysis



Pesticide sample mix



Chiral Analysis

Conclusions

LFII® can be used to analyse

- Small molecules
- Chiral drugs
- Inorganic acids
- Carbohydrates
- Anions in water

This universal LFII® approach to analyses that usually have to be performed on multiple platforms allows great economies of scale, reduces analytical bias and delivers enormous cost savings in capital equipment, service contracts, personnel costs and consumables