

The Perfinity Workstation: Achieving Quality Through Automation

Scott A. Kuzdzal, Ph.D.

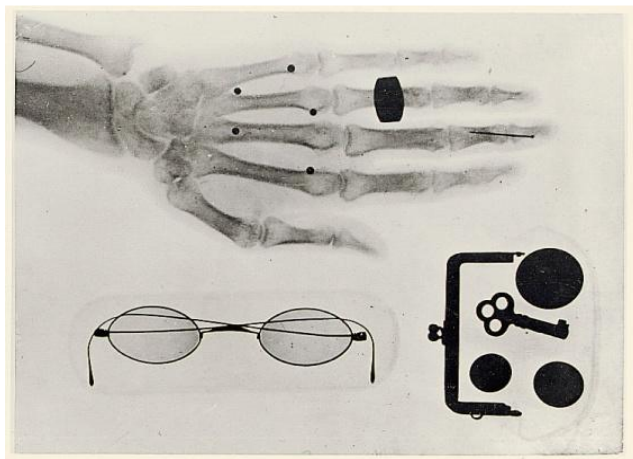
**Bioprocessing and Process Development
Symposium (BPD)**

**"Analytical Technologies"
13 October, 2011**

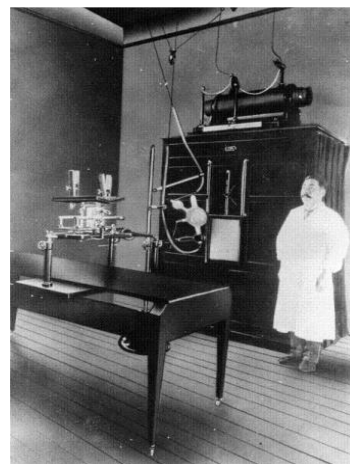
- 1875** Established in Nijo area of Kyoto's Kiyamachi district
Started manufacture and sales of physical and chemical instruments
- 1877** Succeeded in Japan's first manned balloon flight
- 1895** Started production of storage batteries
- 1896** Succeeded in taking radiographs
- 1909** Built Japan's first medical X-ray apparatus



Successful balloon flight (1877)



Early X-ray radiographs (1896)



**Delivered X-ray apparatus to
Japan Red Cross' Ohtsu
hospital (1911)**



**Founder
Genzo Shimadzu**



Genzo Shimadzu Jr.



LCMS-2020
Single Quad



LCMS-8030
Triple Quad



LCMS-IT-TOF
(Structural & Metabolite ID)



GCMS-QP2010 Ultra



PERFINITY WORKSTATION
(Automated Protein Sample Prep)



AXIMA MALDI

Shimadzu Platforms & Solutions

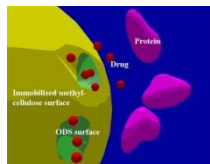
Shimadzu **PLATFORMS** provide the greatest versatility and performance...



Perfinity Workstation
Automated Protein
Sample Prep



MegaTOF
Pharmaceutical
Aggregates & Ultra
High Mass Samples



2D HPLC for Bioanalysis
Analyze LMW analytes
directly from complex fluids



AXIMA – iD^{Plus}
Microorganism ID

...And much, much more!

Protein/Peptide Analysis Challenges

Proteins and peptides have a growing impact on the areas of pharmaceutical development and disease diagnostics

Samples are extremely complex, containing thousands of proteins with target proteins occurring at trace levels

» Like looking for a needle in a haystack



Protein/Peptide Analysis Challenges

Sample purification – the time and quality bottleneck

- Traditional protein sample prep and analysis workflows often take upwards of 72 hours of multiple step processes
- Each step introduces variability in conversion and recovery
- Resulting in %CVs often in excess of 20-30+%

» **Hardly Quantitative!!!**

Protein/Peptide Analysis Challenges

Typical Processes in a Mass Spec Sample Preparation

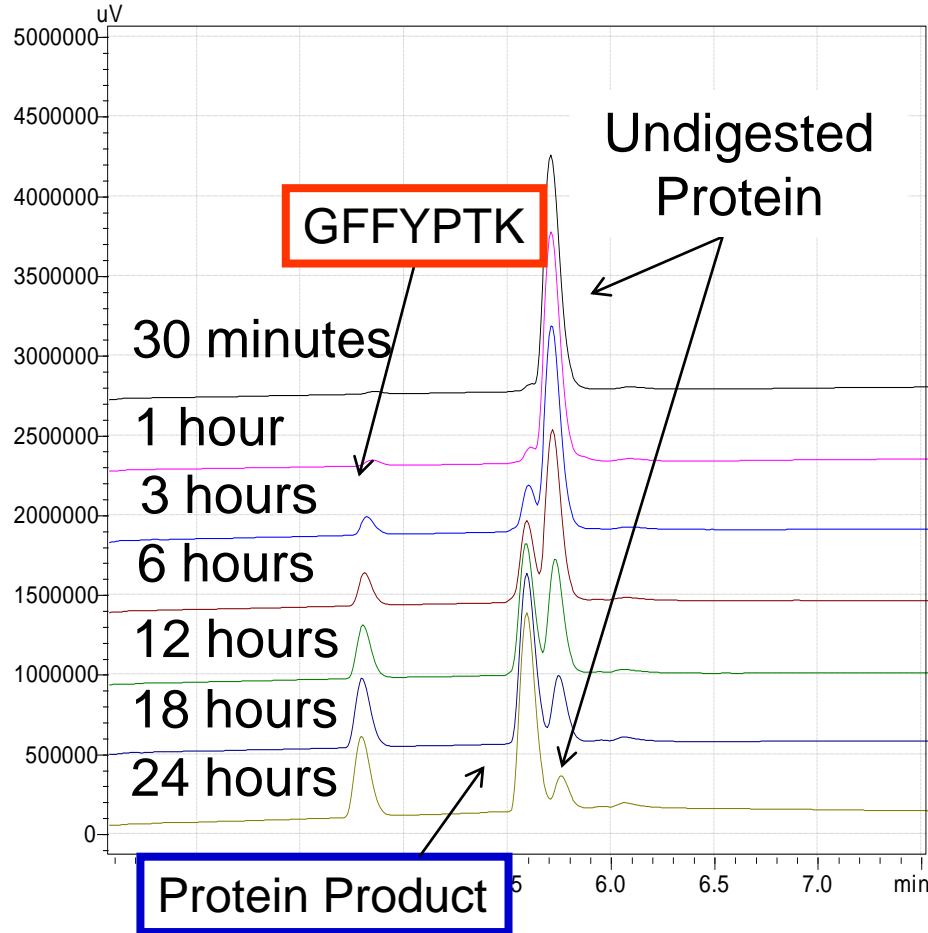
- Affinity Selection
- Buffer exchange
- Digestion
- Desalting
- Reverse phase separation

Protein/Peptide Analysis Challenges

Typical Processes in a Mass Spec Sample Preparation

- Affinity Selection
- Buffer exchange
- Digestion**
- Desalting
- Reverse phase separation

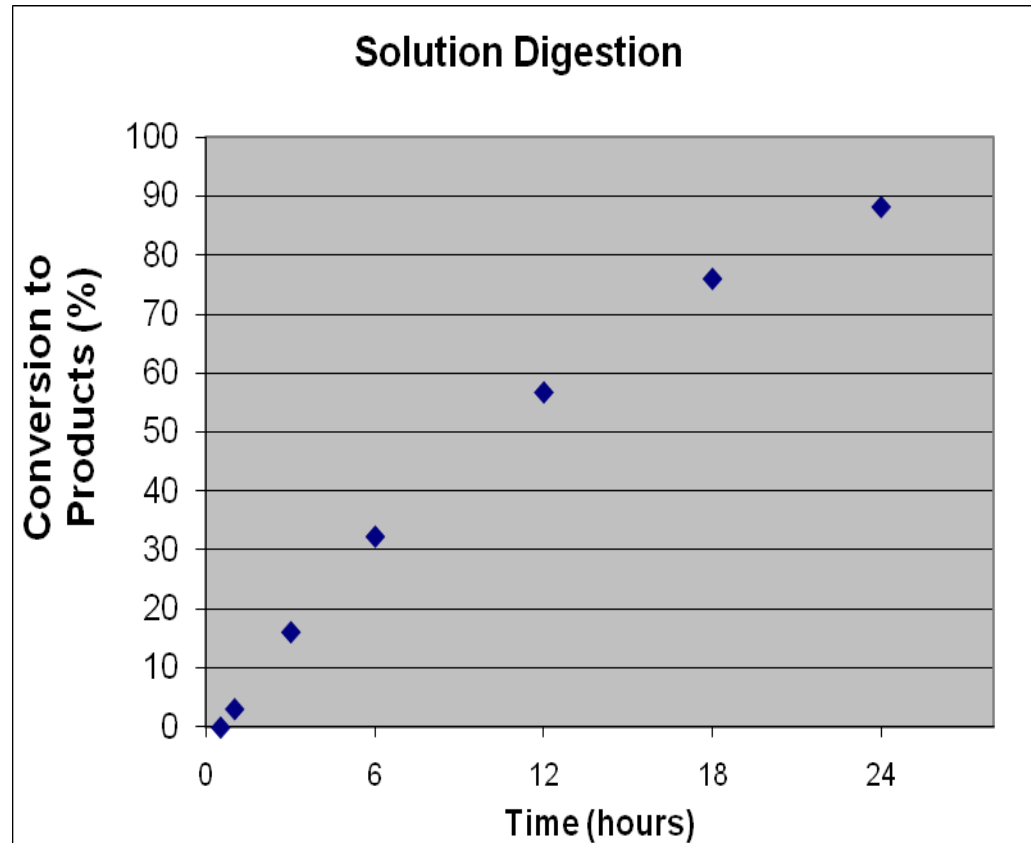
Protein Challenges: Digestion



Sample: 5ug insulin
 Column : HALO 2.1x100mm RPC
 Mobile Phase A: 2% ACN, 98% water, 0.1% Formic Acid
 Mobile Phase B: 90% ACN, 10% water, 0.1% Formic Acid
 Detection: UV/VIS at 214nm

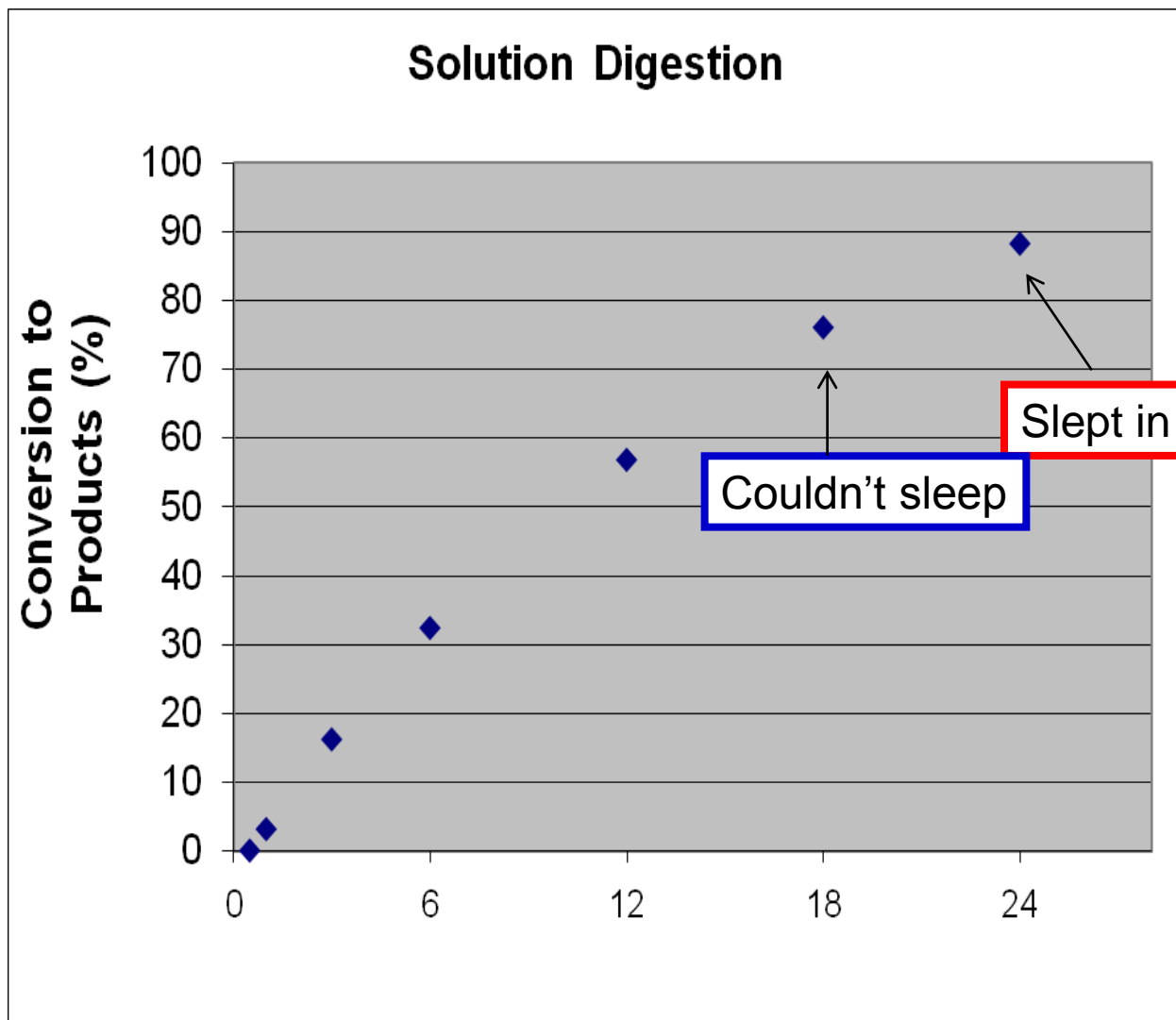
Digestion of Insulin Under Standard Conditions

The Traditional (solution) Way



Proteomics Challenges: Digestion

How does this affect your results?

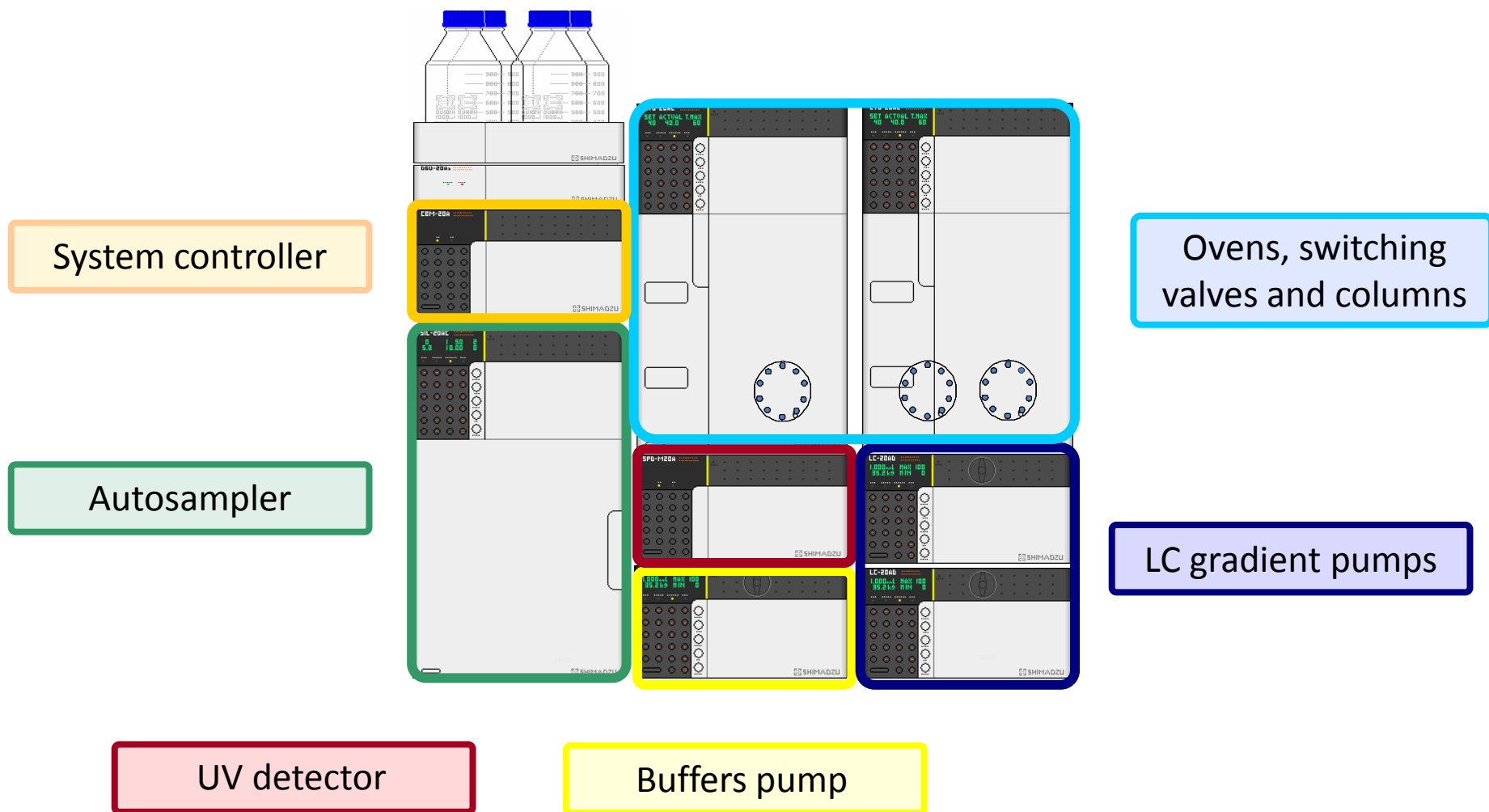


Perfinity Workstation – what is it?

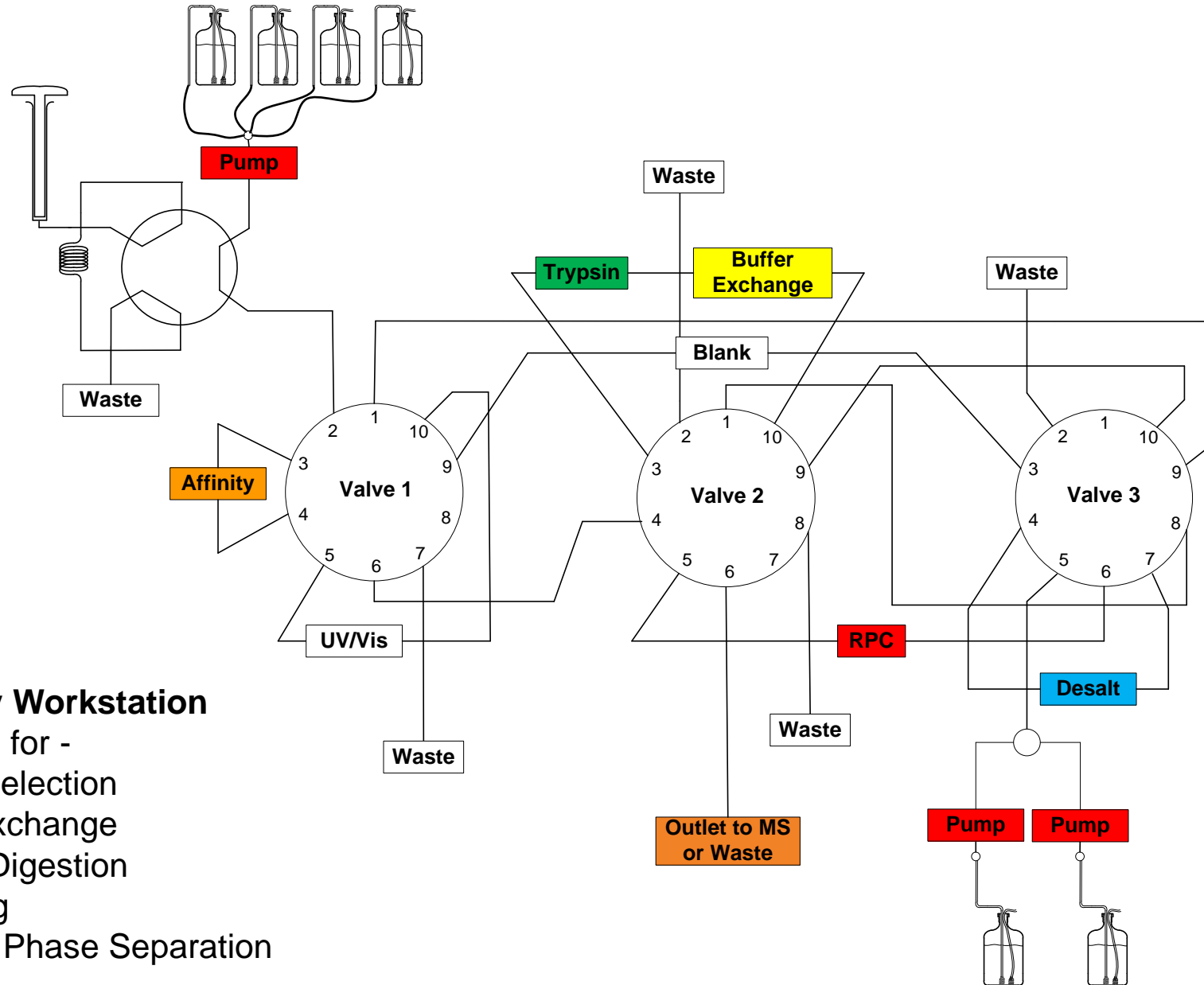
- Automated solution for targeted proteomics
- Affinity-based capture of target proteins and online digestion and reversed phase separation of peptides
- Can be also be used *without* affinity capture step i.e. automated, reproducible digestion & peptide separation only

Perfinity Workstation

Based on the Shimadzu modular HPLC system

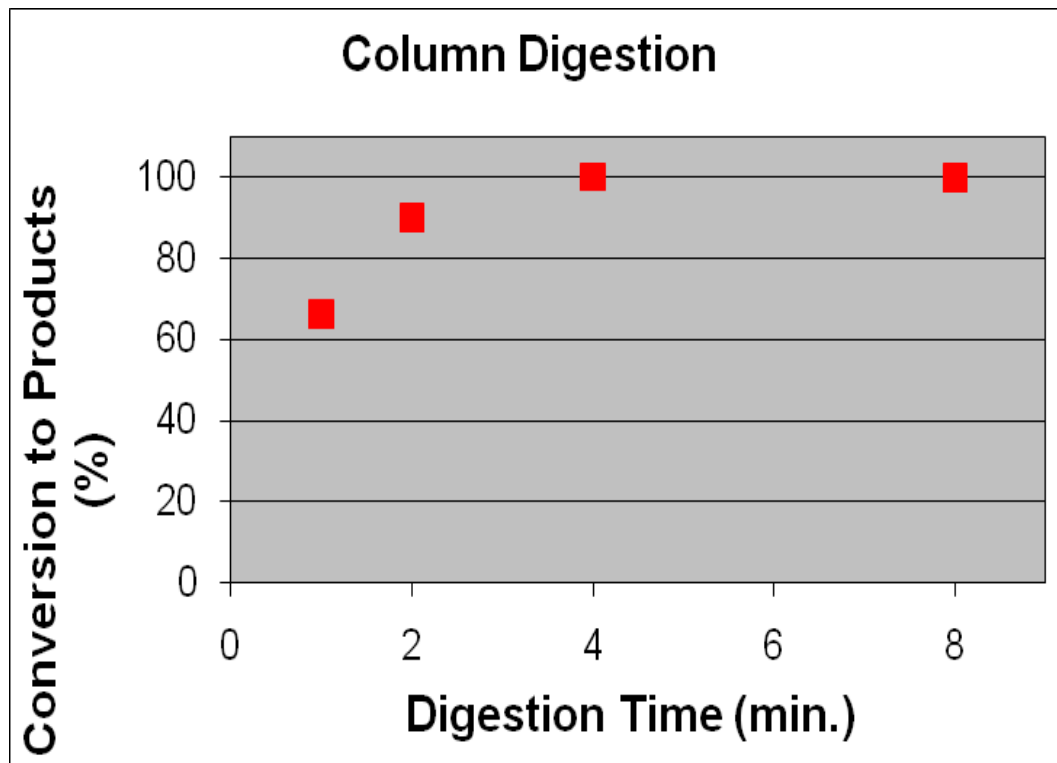
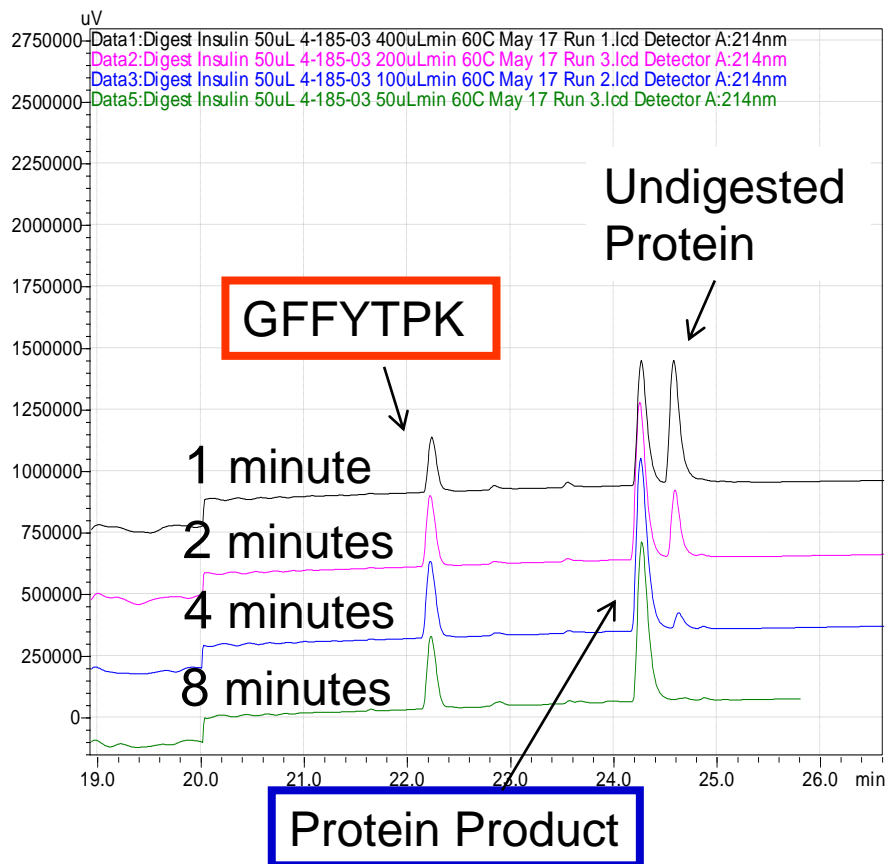


Perfinity Workstation



Perfinity Workstation
 Plumbed for -
 Affinity Selection
 Buffer Exchange
 Trypsin Digestion
 Desalting
 Reverse Phase Separation

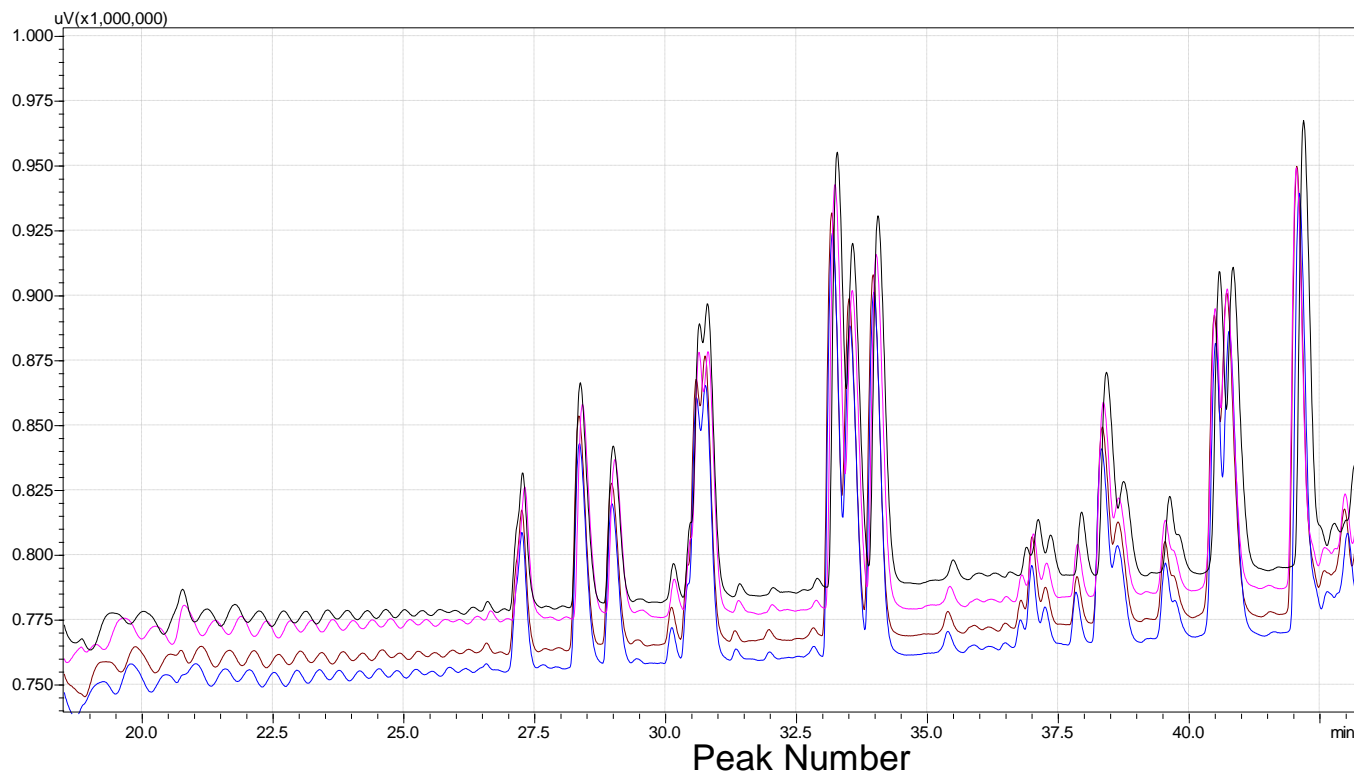
Perfinity Workstation: 4 min Digests!!!



Sample: 5ug insulin
 Column 1: Perfinity Optimized Trypsin Column
 Column 2: HALO 2.1x100mm RPC
 Mobile Phase A: 2% ACN, 98% water, 0.1% Formic Acid
 Mobile Phase B: 90% ACN, 10% water, 0.1% Formic Acid
 Detection: UV/VIS at 214nm

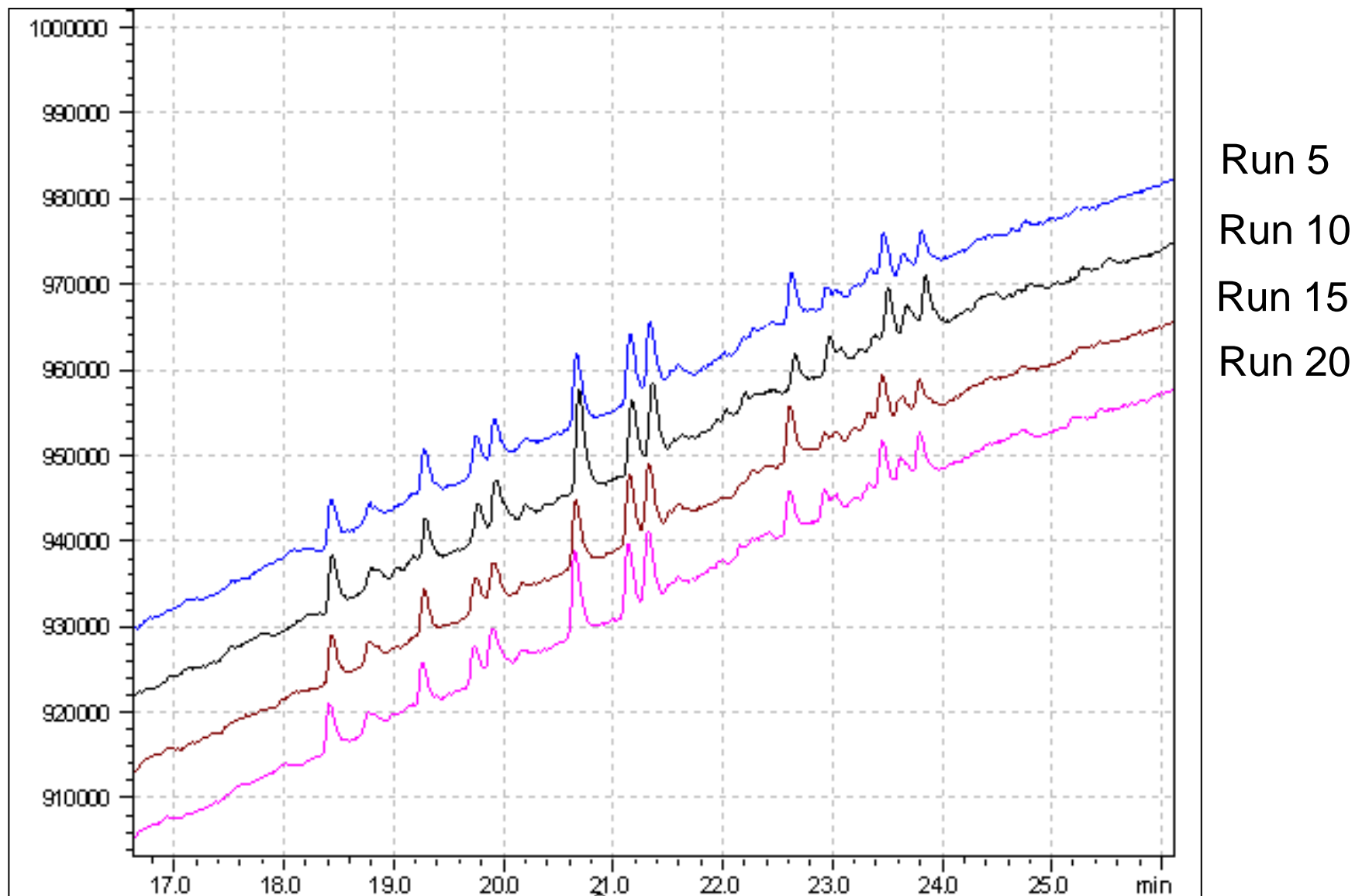
The Perfinity Way

Automated Digests w/ CV's < 10%

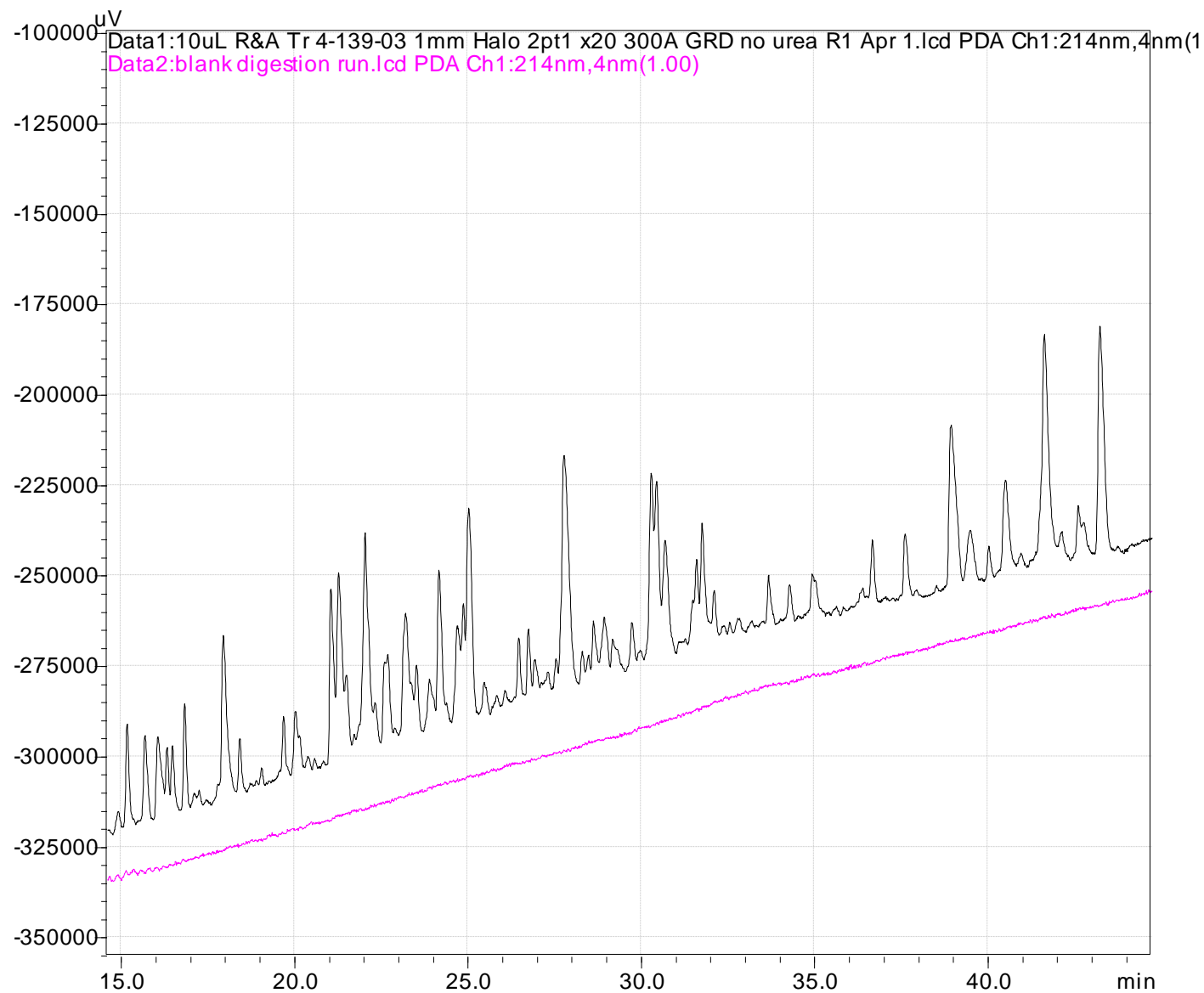


	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Run 1	814	1355	942	1305	1610	2469	2145	2353	1233	1535	1962	2805	714	439	1215
Run 2	793	1283	933	1261	1336	2287	1803	2052	1131	1376	2005	2516	649	378	1012
Run 3	745	1269	874	1218	1414	2310	1886	2084	1116	1485	1941	2648	664	393	1035
Run 4	865	1428	1022	1284	1601	2362	2064	2292	1223	1598	2245	2889	789	454	1156
Average	804	1334	943	1267	1490	2357	1975	2195	1176	1499	2038	2715	704	416	1105
StDev	50	73	61	37	137	81	157	150	61	94	140	166	63	36	97
CV(%)	6.2	5.5	6.5	2.9	9.2	3.4	8.0	6.8	5.2	6.3	6.9	6.1	9.0	8.7	8.8

Automated Protein Digests



Zero Carryover

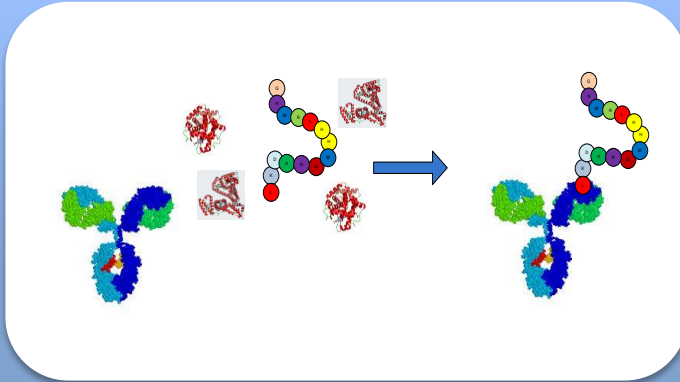


What About the Needle in the Haystack?



An Uncompromised Approach

Immunoassay



High selectivity (+)

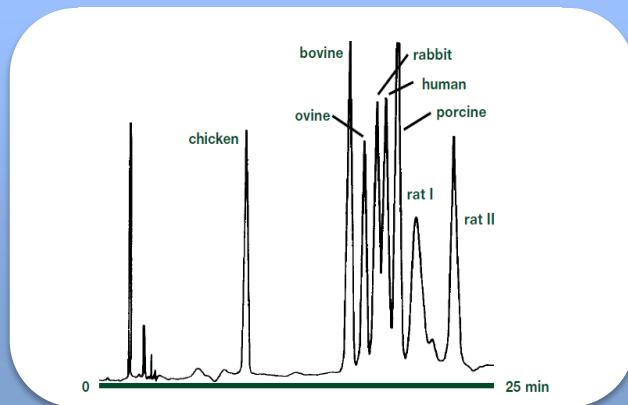
- Antibodies can be used to isolate proteins from biological extracts.

Poor Resolution (-)

- Immunological contact areas (epitopes) are very small

+

Chromatography



Reverse phase separation of insulin variants, some of which differ by a single amino acid.

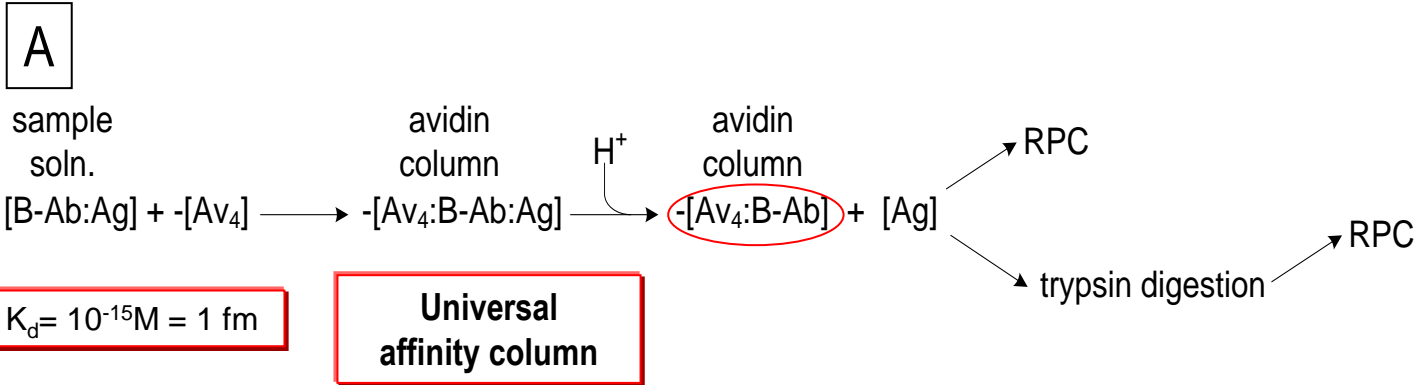
Poor selectivity (-)

- Proteins must be extracted or samples fractionated prior to analysis

High resolution (+)

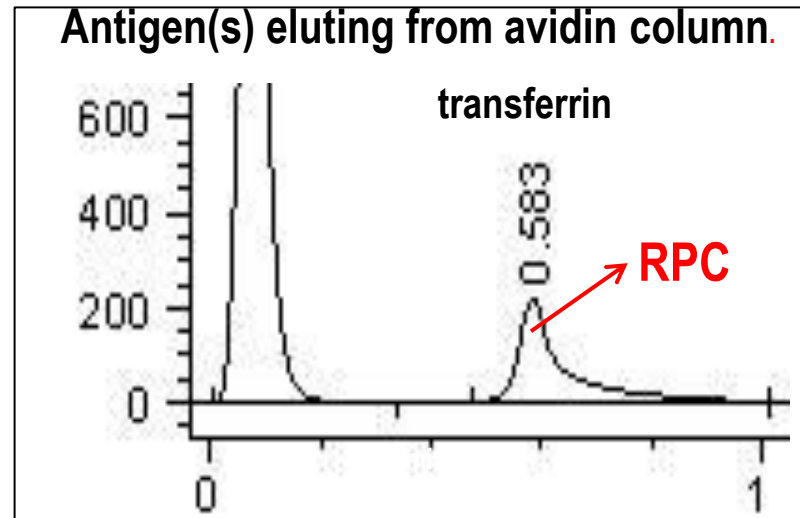
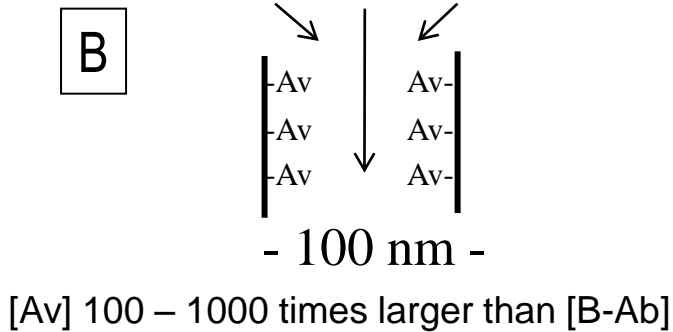
- Similar proteins differing by small changes in structure can easily be resolved.

Perfinity Workstation: Affinity Selection

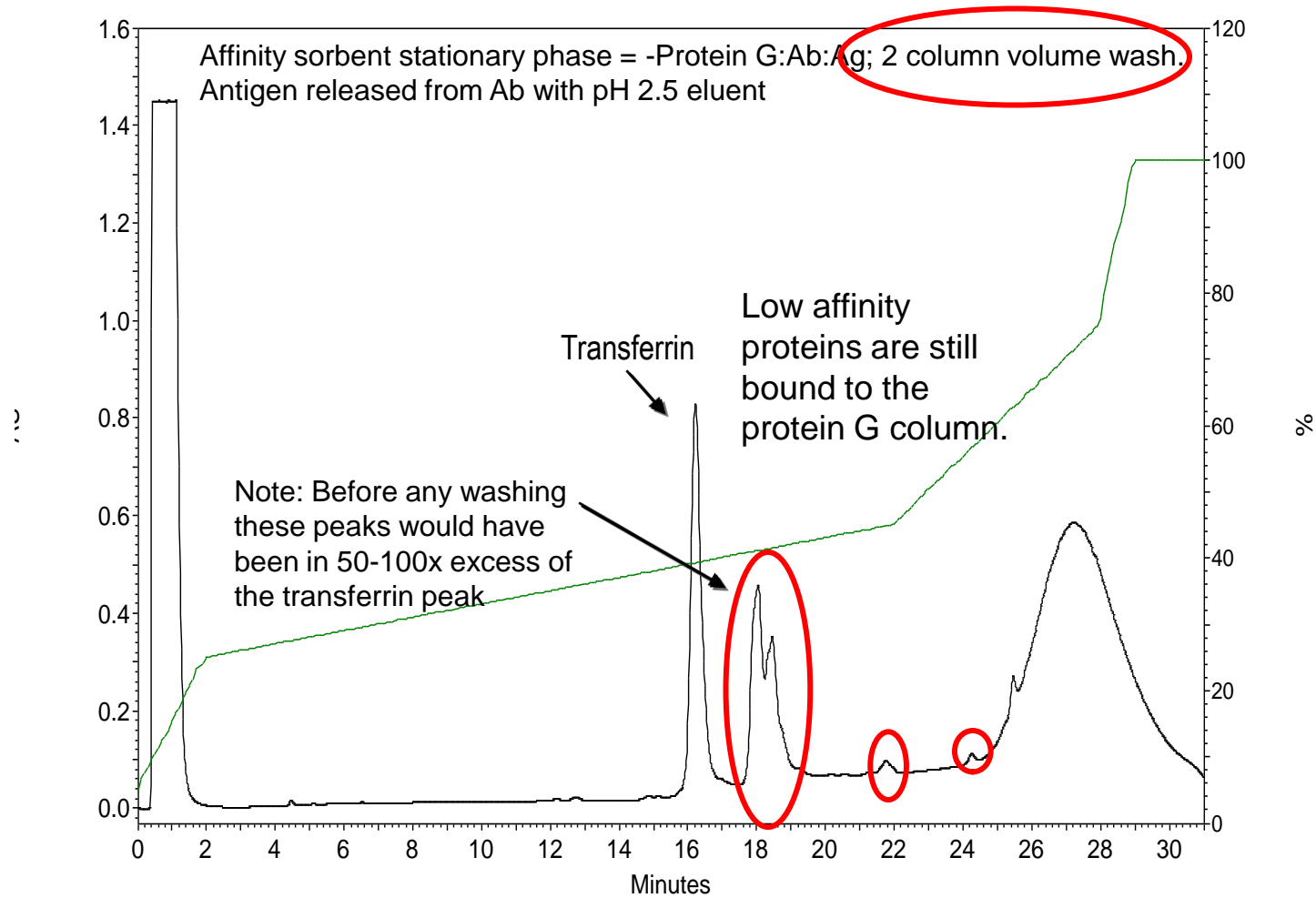


Enhancing Capture Kinetics

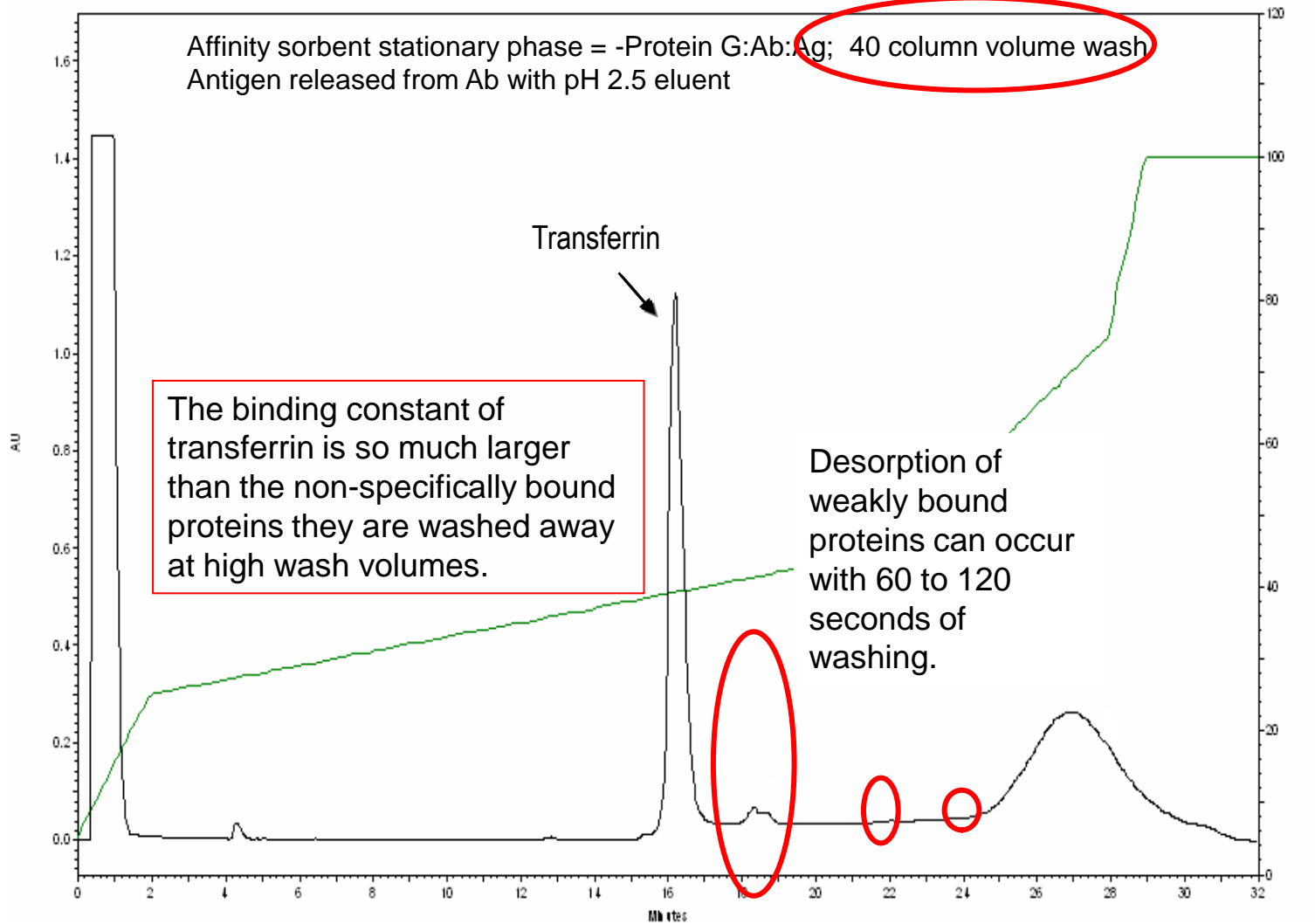
- Allow binding to occur in solution
- Force antigens through an -Av tunnel



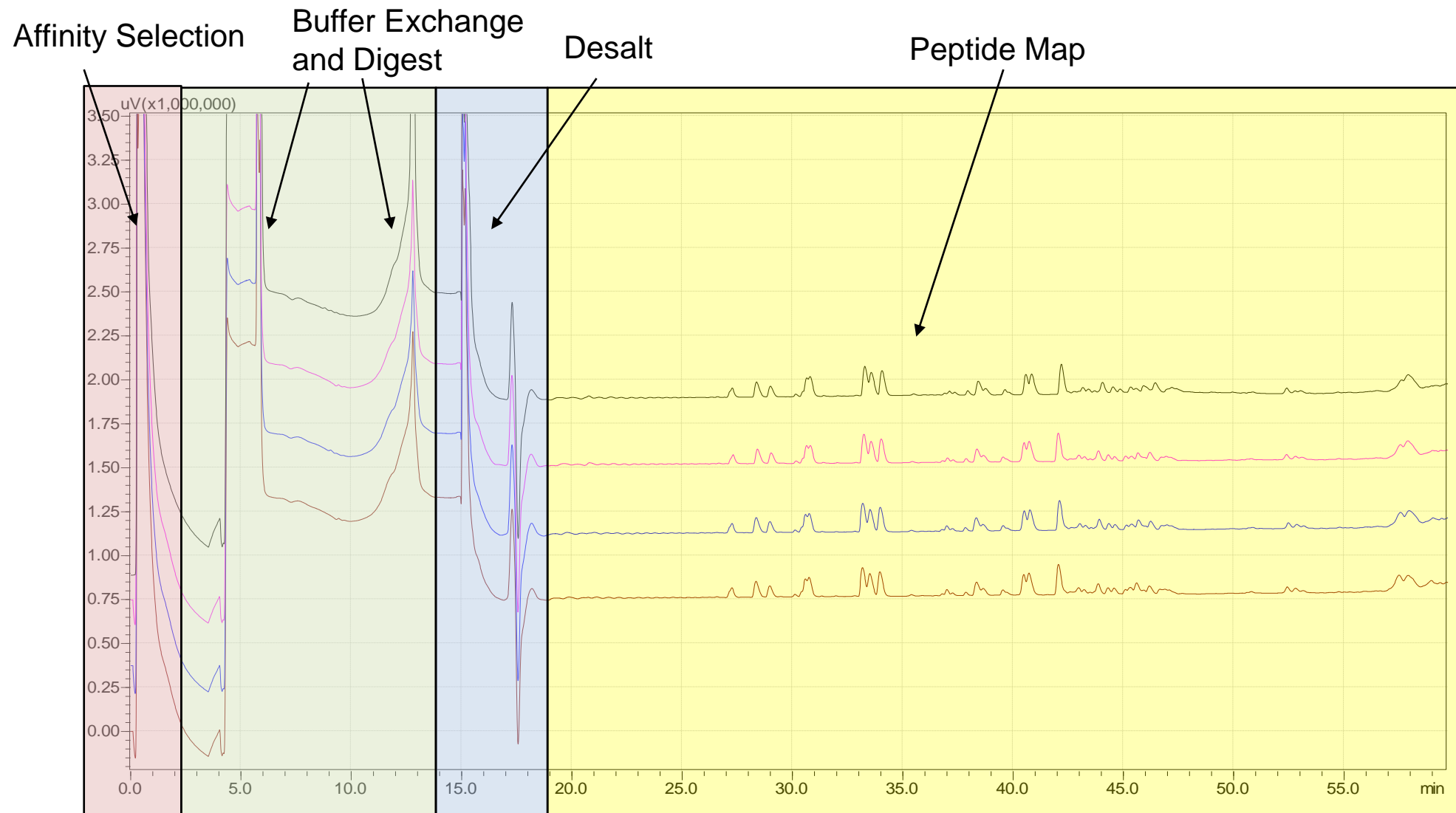
High Volume Column Washing Benefits



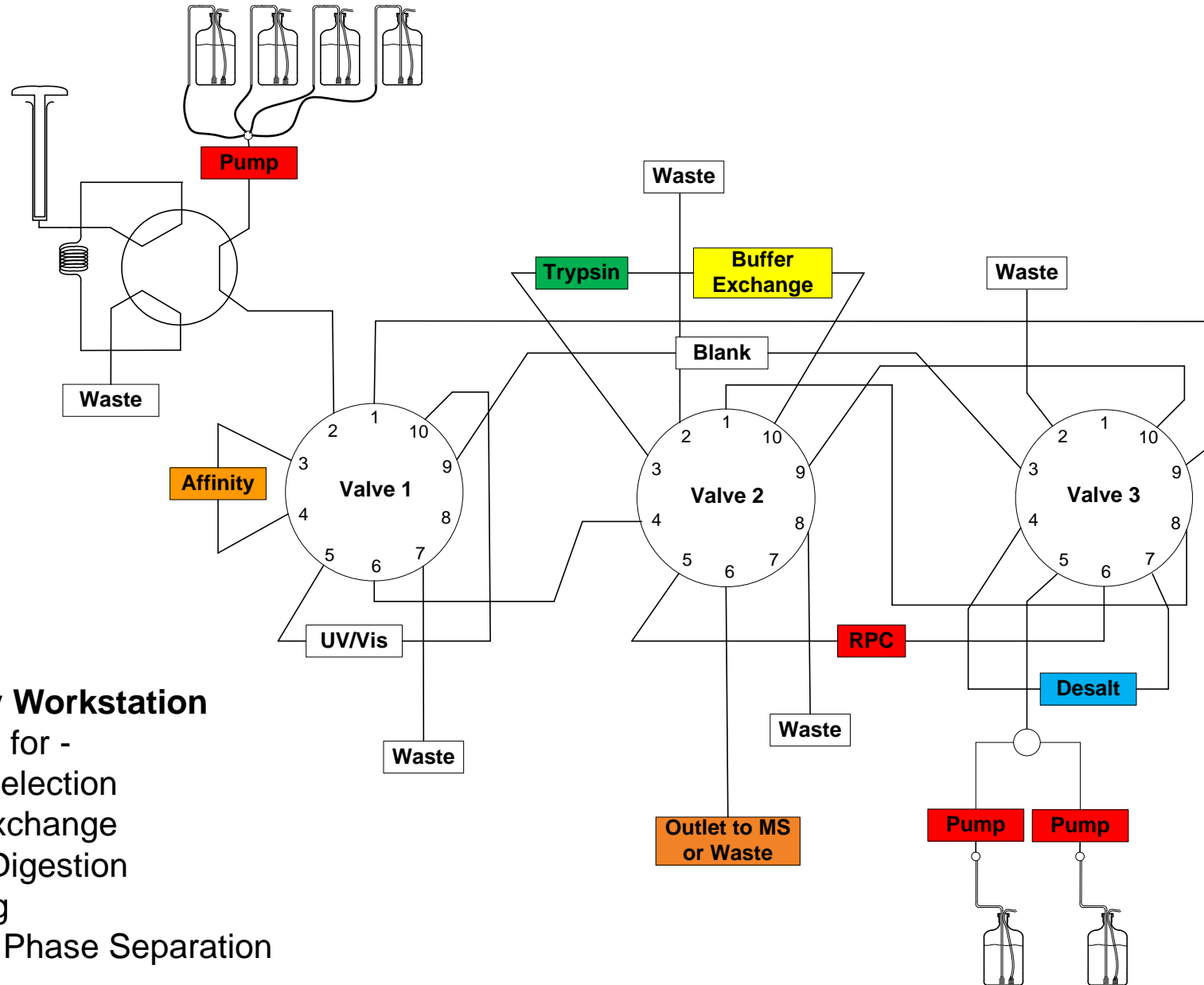
High Volume Column Washing Benefits



Integrated Proteomics Processes



Perfinity Workstation



Perfinity Workstation
 Plumbed for -
 Affinity Selection
 Buffer Exchange
 Trypsin Digestion
 Desalting
 Reverse Phase Separation

Perfinity Workstation

Simple software – you don't have to be an LC expert to use the system

The screenshot displays the Perfinity v1.0 software interface, which is divided into several sections for method optimization and sample management.

Methods Section: A sidebar on the left lists three methods: 1) Serum Assay (highlighted), 2) Digest and RPC, and 3) Affinity Selection and RPC.

Optimize Your Method Here Section: This section is titled "1) Serum Assay - Method Input" and contains several input fields for method parameters:

- Reverse Phase Diameter (mm): 2.1
- Reverse Phase Length (mm):
- Reverse Phase Flow Rate (mL/min):
- Reverse Phase Initial (%B):
- Reverse Phase Final (%B):
- Gradient Length (min):

Run Sample(s) Section: This section is titled "Run Sample(s)" and contains a table for defining the sample batch. The table has the following columns: Method, Sample Name, File Name, Vial #, and Injection Vol.

Method	Sample Name	File Name	Vial #	Injection Vol
Serum Assay	Diblurblesquirtase June 6 Run 1	Diblurblesquirtase June 6 Run 1	1	20
Serum Assay	Diblurblesquirtase June 6 Run 2	Diblurblesquirtase June 6 Run 2	2	20
Serum Assay	Diblurblesquirtase June 6 Run 3	Diblurblesquirtase June 6 Run 3	3	20
Serum Assay	Diblurblesquirtase June 6 Run 4	Diblurblesquirtase June 6 Run 4	4	20
Serum Assay	Diblurblesquirtase June 6 Run 5	Diblurblesquirtase June 6 Run 5	5	20
Serum Assay	Diblurblesquirtase June 6 Run 6	Diblurblesquirtase June 6 Run 6	6	20
Serum Assay	Diblurblesquirtase June 6 Run 7	Diblurblesquirtase June 6 Run 7	7	20
Serum Assay	Diblurblesquirtase June 6 Run 8	Diblurblesquirtase June 6 Run 8	8	20
Serum Assay	Diblurblesquirtase June 6 Run 9	Diblurblesquirtase June 6 Run 9	9	20
Serum Assay	Diblurblesquirtase June 6 Run 10	Diblurblesquirtase June 6 Run 10	10	20
Serum Assay	Diblurblesquirtase June 6 Run 11	Diblurblesquirtase June 6 Run 11	11	20
Serum Assay	Diblurblesquirtase June 6 Run 12	Diblurblesquirtase June 6 Run 12	12	20


Annotations: Three green boxes highlight key steps in the workflow:

- Step 1: define the** (points to the "Optimize Your Method Here" section)
- Step 2: create the sample table** (points to the "Run Sample(s)" table)
- Step 3: Start the experiment** (points to the "Run" button, which is also highlighted with a red circle)


Additional interface elements include "Modify Methods", "Run Sample(s)", and "Re-Order Supplies" buttons, as well as a "Method Description" field at the bottom left.

Perfinity Workstation



 Automates and integrates five key proteomics workflow steps:
Affinity Selection,
Buffer Exchange,
Trypsin Digestion,
Desalting &
Reverse Phase HPLC.

 Reduces sample preparation times from 72 hours to < 1 hour.

 Achieves exceptional reproducibility (CVs < 10%)

Mass Spec Detection (Immuno-MS)

- Can be coupled with mass spectrometric detection
- Two different ways of generating ions from the sample:
 1. Electrospray ionisation (ESI) – online detection
 2. MALDI – offline
- Can perform MS to detect all masses e.g. peptide masses
 - » Identify/quantitate proteins present in the sample
- With the Shimadzu LCMS-8030, can perform targeted acquisitions to detect and quantify known components (increased sensitivity, selectivity)

Application: Identification of Hb Variants



LCMS-IT-TOF

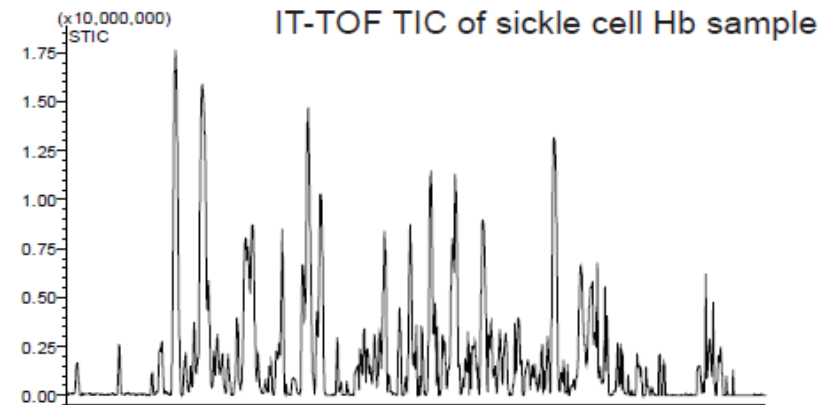
- Structural Identification of targets

MASCOT Results

Matched peptides shown in **Bold Red**

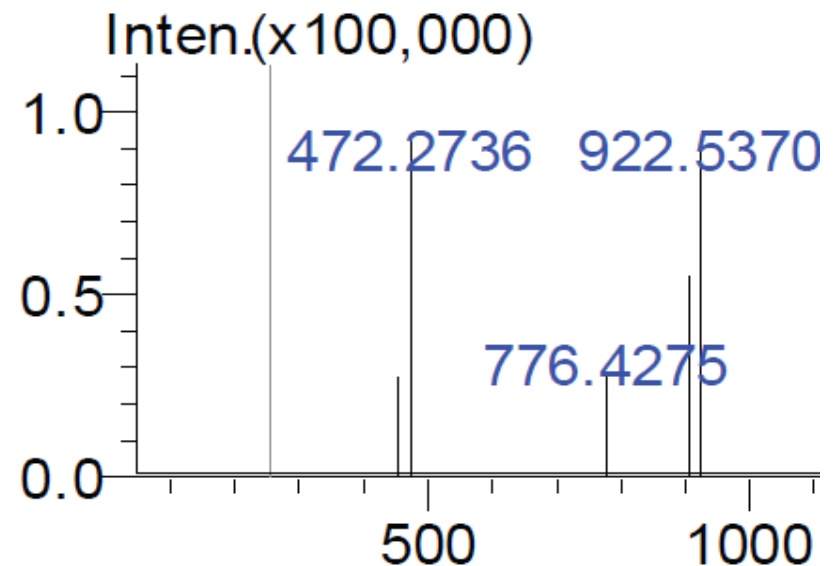
```

MVHLTPEEKS AVTALWGKVH VDEVGGEALG RLLVVYPWTQ RFESEFGDLS
TPDAVMGNPK VKAHGKKVLG AFSDGLAHLD NLKGTFATLS ELHCDKLHVD
PENFRLLGNV LVCVLAHHFG KEFTPPVQAA YQKVVAGVAN ALAHKYH
    
```



Rapid identification of VHLTP**V**EK sickle cell peptide isoform by IT-TOF

- 91% sequence coverage
- Rapid identification of sickle cell variants
- Fully automated extraction, digestion and analysis

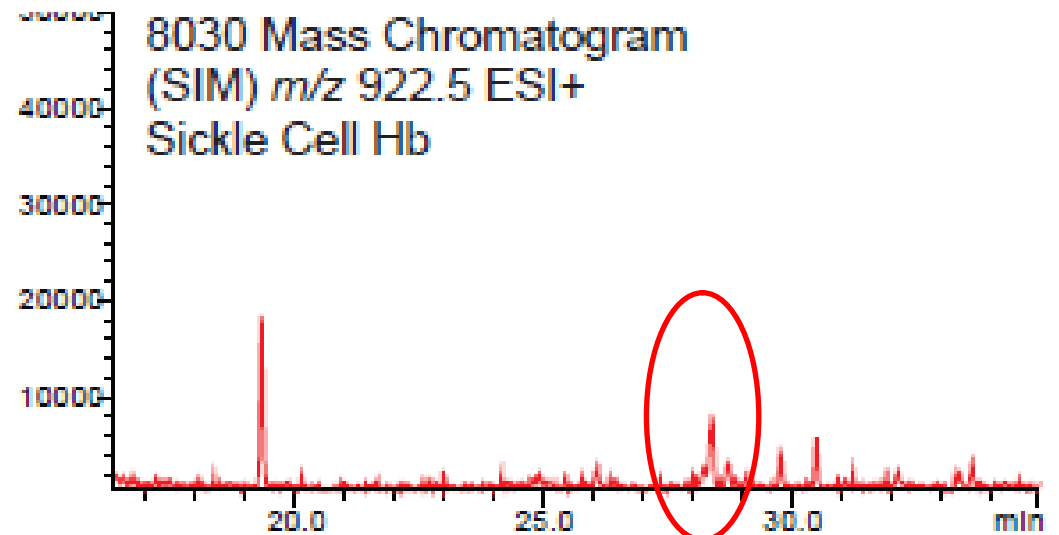
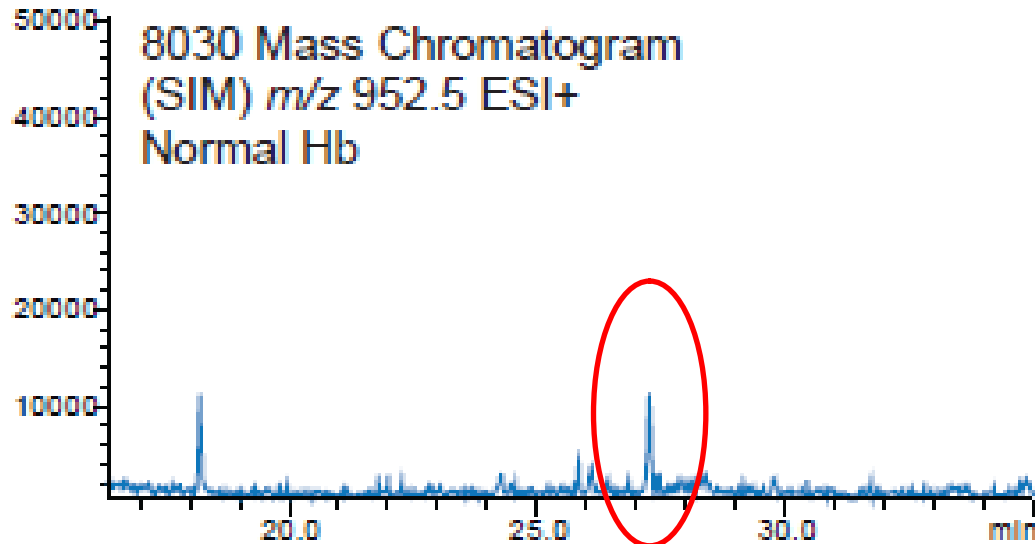
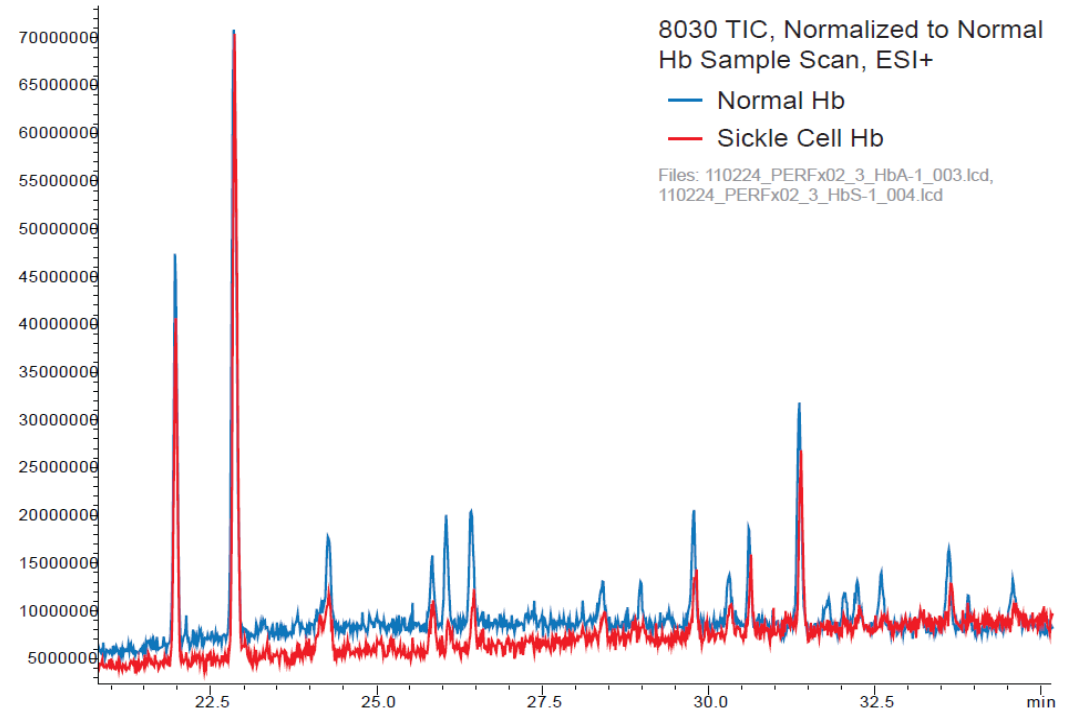


Quantitation: Sickle Cell Anemia Hb

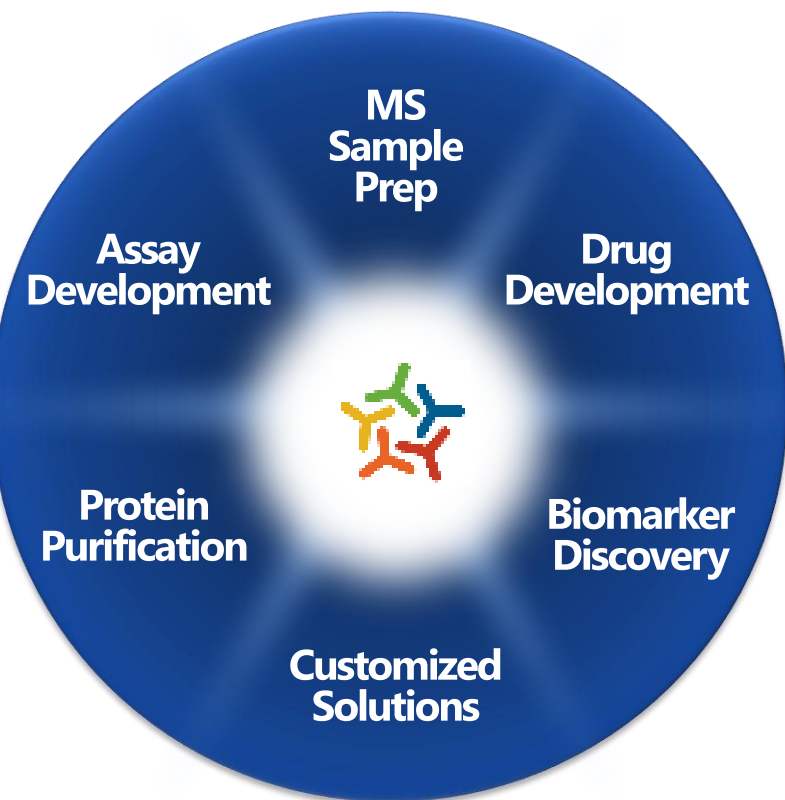


LCMS-8030

- Quantitation of purified targets
- Selected ion monitoring



Benefits of the Perfinity Workstation



Three day workflow complete in under 1 hour



Serum to purified peptides in < 10 minutes



High quality, reproducible (CVs < 10%)



Hand free operation – fully automated



Easily customizable

Acknowledgements

Perfinity Biosciences

Fred Regnier

Kevin Meyer

Nick Herold

Steve Plump

Shimadzu

Terry Adams

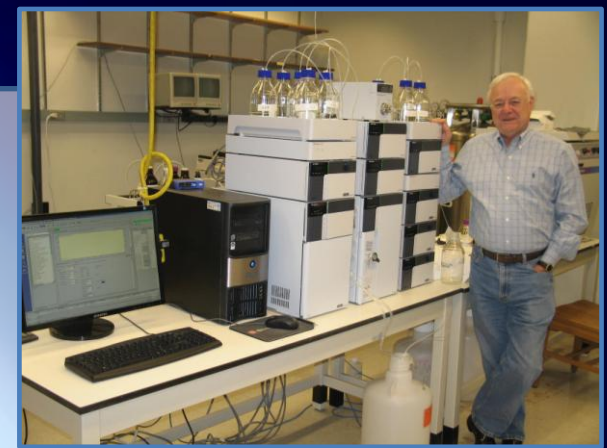
Curtis Campbell

Rachel Lieberman

Jason Harrington

Tom Hayes

PerfinityWorkstation.com



THANK YOU!

Summary

