

# SELDI-TOF MS as Novel HTS Tool for Analysis of Protein Samples in Process Development

Ralf Bogumil<sup>1</sup>, Pierre Schulze Wierling<sup>2</sup>, Jürgen Hubbuch<sup>2</sup>, Lothar Britsch<sup>3</sup>, Andreas Wiesner<sup>1</sup>

<sup>1</sup>Ciphergen Biosystems GmbH, 16761 Hennigsdorf, <sup>2</sup>Institute of Biotechnology, Forschungszentrum Jülich GmbH, 52425 Jülich, <sup>3</sup>Atoll GmbH, 88250 Weingarten

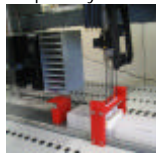
E-mail: rbogumil@ciphergen.com

## Summary

Optimization of chromatographic separation conditions is normally a time consuming task in the large scale production of proteins. In this study, we used customized 96 well-formatted MediaScout® mini columns together with robotic automation for the fast testing of different chromatographic process parameters. Analysis of the numerous fractions was performed with the Surface Enhanced Laser Desorption/Ionization - Time Of Flight - Mass Spectrometry (SELDI-TOF-MS)-based ProteinChip® System. This combined approach enables to speed up the process significantly with the capacity to run and analyze several hundreds samples in parallel per day.



MediaScout columns  
in 96 well format



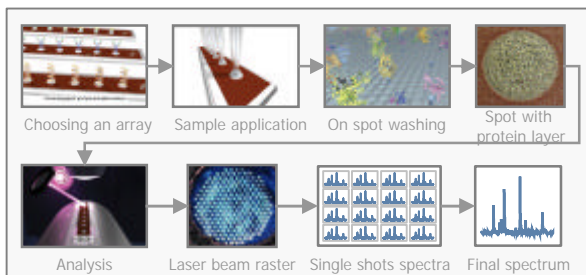
Robotic automation  
of minicolumns



ProteinChip® Reader

## The SELDI process

After choosing an array from a selection of chromatographic ProteinChip Arrays, samples are applied and incubated on the spots, subsequent on-spot washing ensures efficient sample cleanup before matrix application, resulting in a homogenous layer of co-crystallized proteins. In the ProteinChip Reader, a laser beam is directed on the spots causing desorption and ionization of the proteins. A defined laser beam raster is used to selectively cover the entire spot surface. Final spectra show the mass-to-charge ratios of the ionized proteins and the corresponding signal intensities are well correlated to analyte concentration allowing to work in a quantitative manner.



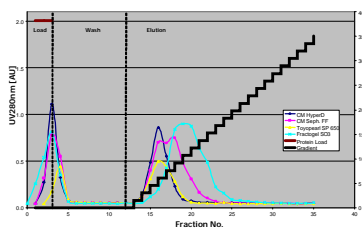
## Parallel purification of IgG with different resins

A monoclonal antibody preparation (IgG) was purified from a host cell protein (HCP) preparation. Four different cation exchange resins (CM Hyper D, CM Seph. FF, Toyopearl SP650, Fractogel SO<sub>3</sub>) were tested in parallel.

Elution profile of the four mini columns monitored by UV detection at 280 nm

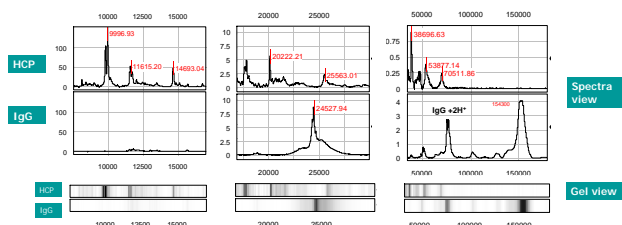
Buffer 20 mM Bicine pH 8,  
25 mM NaCl  
Gradient elution:  
25 mM NaCl - 500 mM NaCl

Fraction Volume = Column Volume = 200 µl



## SELDI analysis of IgG and Host Cell Proteins

SELDI-TOF MS spectra of IgG and HCP in three different mass regions



The HCP preparation is characterized by a high number of protein species. The highest signal intensities are observed in the mass range from 6 to 17 kDa. In contrast the purified IgG exhibits a typical spectrum with dominant peak of intact IgG at 154 kDa together with the double charge peak at 77 kDa.

## Monitoring of IgG elution by SELDI-MS

The same protein fractions monitored using 280 nm absorption were analyzed by SELDI-MS. The elution of the IgG and the amount in the flow-through can be clearly monitored. Analysis time using SELDI-MS method is about 2 h for 96 samples.

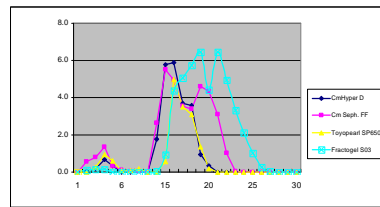


Table 1 : Relative quantification of IgG by SELDI

Resin	Sample volume loaded/µl	Summed IgG signal intensities in flow through and wash	Summed IgG signal intensities in eluate
CM Hyper D	248	1.2	22.0 (F14-F20)
CM Seph. FF	396	3.3	33.1 (F14-F22)
Toyopearl SP 650	168	1.9	13.7 (F15-F20)
Fractogel SO <sub>3</sub>	507	0.4	45.1 (F15-F25)

## Relative quantification of HCP by SELDI-MS

The sum of the signal intensities of four dominant peaks at 9.8, 10.0, 11.6 and 14.6 kDa was taken to reflect the total HCP content.

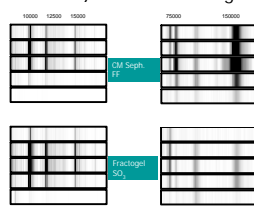
Most of the HCP were detected in the flow-through. The amount of HCP correlates with the amount of protein loaded to the column

Table 2 : Relative quantification of HCP by SELDI

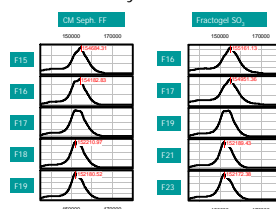
Resin	Sample volume [µl]	Summed HCP signal intensities in fraction 1 to 5
CM Hyper D	248	1190
CM Seph. FF	396	1330
Toyop. SP 650	168	502
Fractogel SO <sub>3</sub>	507	2090

## Comparison of two resins by SELDI-MS

Analysis of flow-through



Analysis of eluate



Higher amounts of IgG (band at 150 kDa) are found in the flow-through fraction of CM Seph. FF in comparison to Fractogel. In both cases, most HCP (bands in left panel) are found in the flow-through.

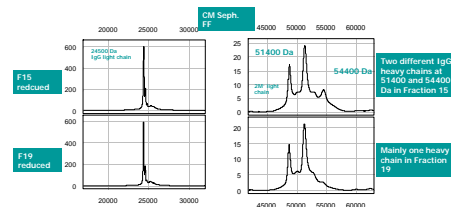
The product elutes earlier from the CM Seph. FF in comparison to the Fractogel resin.

SELDI analysis reveals a shift in the mass of the eluted IgG. The IgG with higher mass elutes earlier.

## Analysis of IgG glycosylation by SELDI-MS

To get more information about the IgG heterogeneity, selected fractions were reduced on-spot with 1.4 - Dithiothreitol (DTT).

This allows to detect the light- and heavy chains separately.



SELDI analysis shows that the mass variation of the monoclonal IgG can be explained by a heterogeneous glycosylation of the heavy chain.

## Conclusions

The new combination of robotic-supported MediaScout® mini-columns with ProteinChip® technology revealed to be a promising tool for process optimization at a high throughput scale.

ProteinChip® technology provides detailed information about

- Binding and elution of IgG and HCP
- Relative quantification of the IgG and HCP
- Amount of glycosylation of IgG
- Exact masses of detected protein

Acknowledgement: We thank Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach for providing the IgG and HCP preparation for this study.