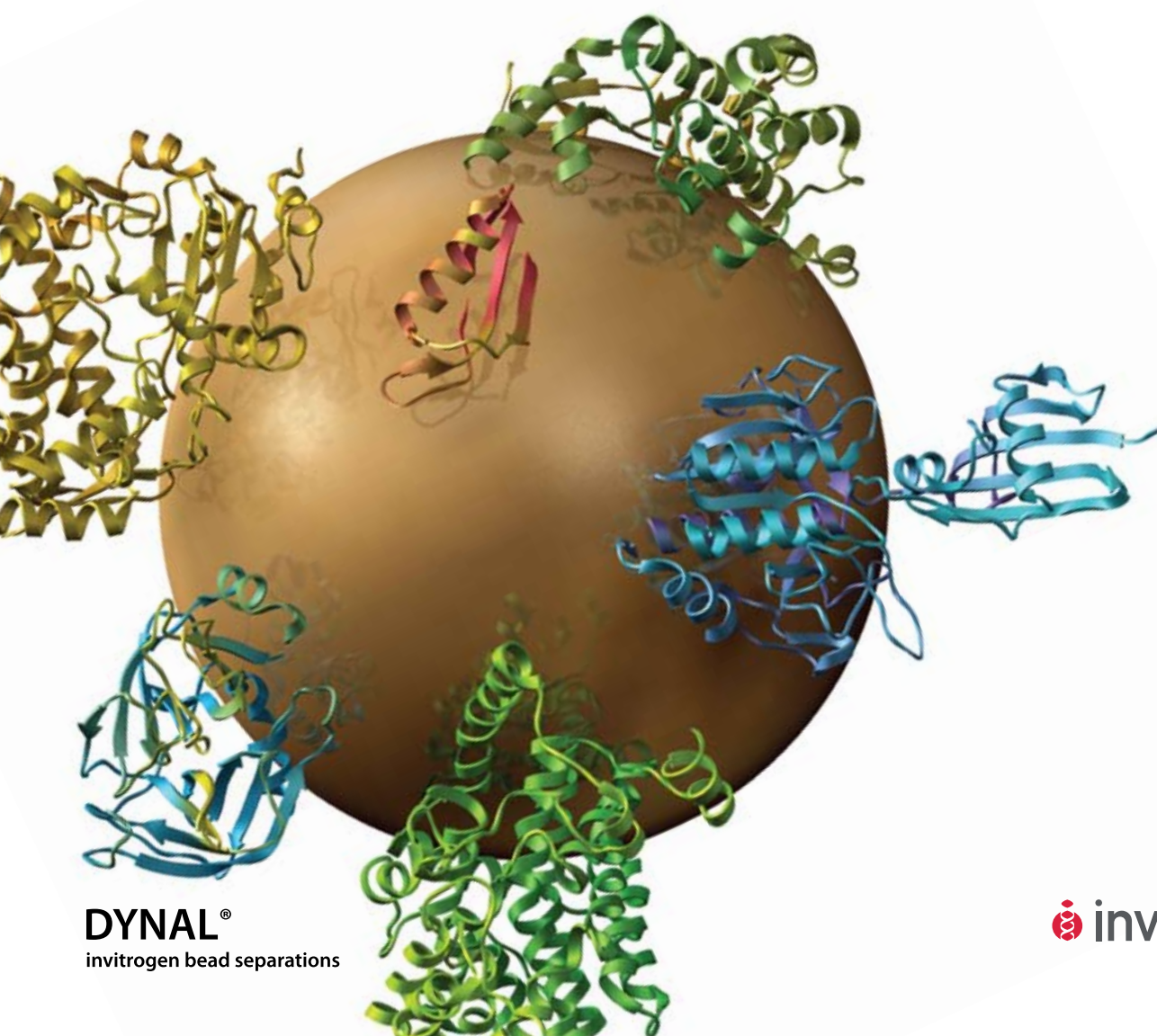




# A new approach to chromatography

Dynabeads® for proteomics





## Gentle and reproducible isolation of proteins and peptides from precious samples

- Reduce sample complexity—study smaller groups (fractions) of proteins or peptides in depth
- Perform serum profiling—a quick and reproducible way to qualify samples or discover and validate biomarkers, saving hours compared to conventional methods
- Remove contaminants such as salts or detergents from your sample to enable accurate and sensitive mass spectrometry analysis of peptides and proteins

Magnetic bead chromatography is a new technique that has been readily adopted in traditional and clinical proteomics research. It is being used to discover predictive biological markers as well as to capture groups of proteins for further studies (Figure 1).

You can easily capture proteins and peptides from small amounts of complex samples such as serum, plasma, cerebrospinal fluid (CSF), and cell lysates. Protocols are scalable and analyses can be automated.

### The attraction is simply *magnetisk*

*Magnetisk* is the Norwegian word for magnetic, the property that makes Dynabeads® so irresistibly attractive for a wide range of research interests, including proteomics, nucleic acid isolation, cell separation and expansion, and IVD assay development. This rapid and flexible technology makes even complicated protocols simple.

Dynabeads® have become the first choice among researchers for magnetic separation technology. Pioneered in the 1980s by Dynal Biotech, now part of Invitrogen, Dynabeads® are based on microparticle technology developed by the late John Ugel-

stad, a professor of chemistry at the University of Trondheim, Norway. Ugelstad succeeded in making spherical polystyrene beads of exactly uniform size, a feat previously achieved only by NASA in the weightless conditions of space. When the uniform beads were made magnetizable, the outcome revolutionized separation methodologies and enabled researchers to realize results once considered unattainable.

Today, Dynabeads® are used in academic and industry laboratories worldwide. They're employed on more than 25,000 IVD instruments. Some 10,000 scientific articles have been published on studies that involve their use.

## How does it work?

Magnetic separation with Dynabeads® is quick, reproducible, and extremely gentle, with no need for columns or centrifugation.

Simply add your chosen beads to the sample and allow them to bind to the target protein or peptide—you can use a standard laboratory tube or a 96-well plate. Place the tube in a Dyna® magnet and watch the bead-bound target being pulled gently to the tube wall in minutes. Remove the supernatant to leave a pure, intact sample of your target. You can choose to elute the target from the beads by simply altering the solvent conditions.

Binding between the Dynabeads® and the target takes advantage of these bead properties:

- Ion exchange separation—exploits differences in net charge, where bead-bound charged groups adsorb oppositely charged molecules. By altering salt concentration or pH during binding or elution, you can separate proteins or peptides into different fractions, thus reducing sample complexity. Dynabeads® SCX are also ideal for removing detergents.
- Reversed-phase chromatography (RPC)—Dynabeads® bind to hydrophobic amino acids in proteins or peptides. This method is ideal for serum profiling, desalting, and reduction of sample complexity. A stepwise increase in acetonitrile concentration results in elution of fractions.

### Clinical proteomics research

#### **Serum profiling for biomarker discovery and validation or sample qualification**

Dynabeads® RPC-18  
Dynabeads® RPC Protein  
Dynabeads® SCX  
Dynabeads® SAX  
Dynabeads® WCX



### Traditional proteomics research

#### **Desalting**

Dynabeads® RPC-18 or  
Dynabeads® RPC Protein

#### **Detergent removal**

Dynabeads® SCX

#### **Reduction of sample complexity**

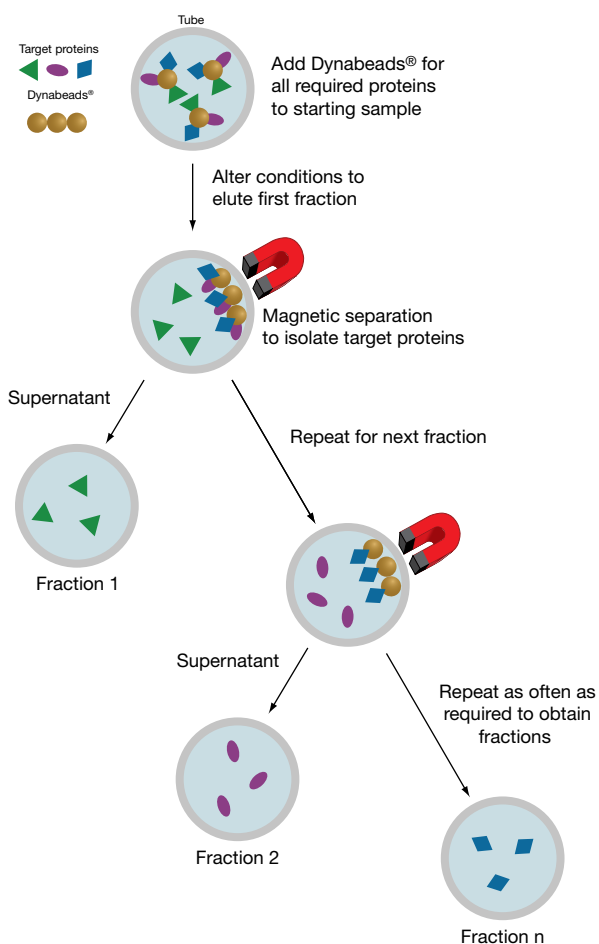
Dynabeads® RPC-18  
Dynabeads® RPC Protein  
Dynabeads® SCX  
Dynabeads® SAX  
Dynabeads® WCX

Figure 1—Guide to choosing the right magnetic bead chromatography product.

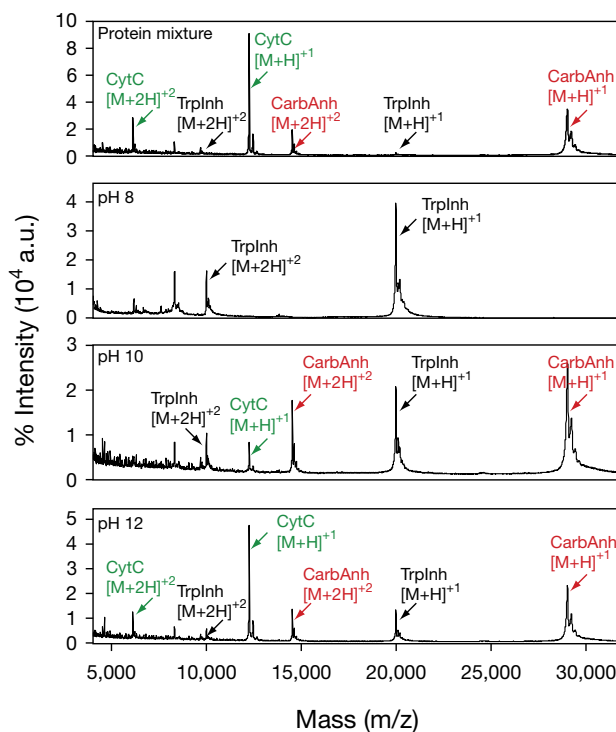


## Reduce sample complexity by fractionation

Fractionate smaller groups of proteins or peptides from a sample for in-depth study (Figures 2 and 3).



**Figure 2—Reducing sample complexity by fractionation.** Dynabeads® are added to the sample and the proteins/peptides allowed to adsorb. Changing the conditions (altering pH, salt concentration, or acetonitrile concentration) allows stepwise desorption of proteins into various fractions. Sample complexity is thereby reduced, allowing you to investigate specific groups of proteins. Different shapes represent different peptides and proteins.

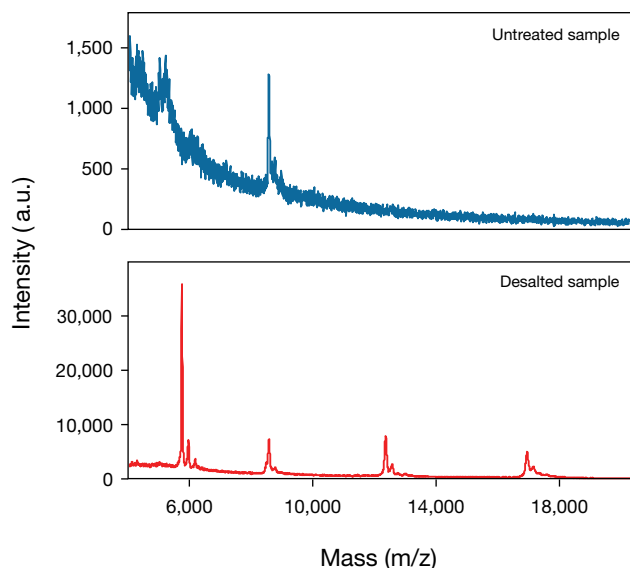


**Figure 3—Using adsorption pH to reduce sample complexity.** Different ion peaks become visible with changing pH. A mixture containing 100 pmol of cytochrome c, trypsin inhibitor, and carbonic anhydrase I was adsorbed by Dynabeads® SAX at pH 8, 10, or 12. After washing, proteins were eluted in 0.5% TFA. Aliquots of eluate were mixed with sinapinic acid matrix and analyzed using MALDI-TOF MS.

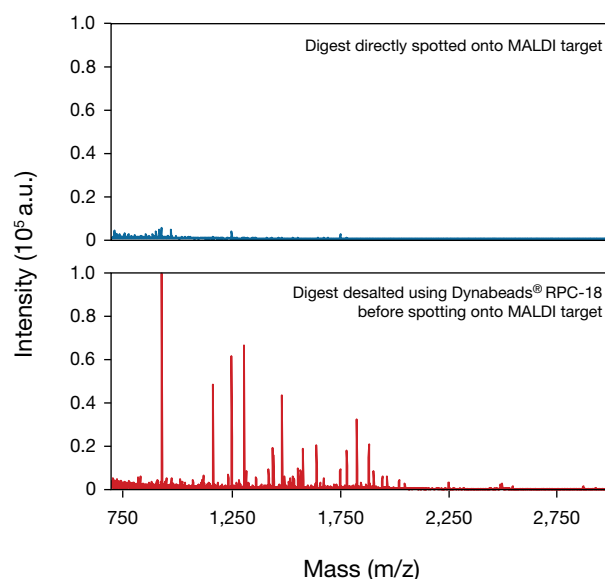
## Desalting and detergent removal

Remove contaminants such as salts or detergents from your sample to enable accurate and sensitive mass spectrometry analysis of peptides and proteins.

The target peptides or proteins are adsorbed to the Dynabeads®, then pulled to the tube wall by a magnet. Any contaminants, such as salts (Figures 4 and 5) or detergents, (Figure 6) can be washed away. The proteins or peptides are then desorbed and can be analysed via MALDI-MS.



**Figure 4—Desalting with Dynabeads® RPC Protein.** A mixture contained insulin, ubiquitin I, cytochrome c, myoglobin, and 2.2 M NaCl. The top panel shows how noise on the baseline obscures visibility of the ion peaks. Once salt is removed, ion peaks that were hidden become visible (bottom panel). An aliquot of a protein/peptide mixture or eluate (1 µl, in 50% acetonitrile) was mixed with an equal amount of MALDI matrix, and 0.5 µl of the sample/matrix mixture was spotted onto a MALDI target plate.



Contaminant	Measurement	Spotting after clean-up with Dynabeads® RPC-18	Direct spotting on MALDI target
6 M urea	Sequence coverage (%)	35 ± 1	No I.D.
	Mascot® score	199 ± 7	
	Peptides matched	19 ± 1	
6 M guanidine	Sequence coverage (%)	25 ± 2	No I.D.
	Mascot® score	142 ± 10	
	Peptides matched	14 ± 2	
2 M NaCl	Sequence coverage (%)	30 ± 8	9 ± 1
	Mascot® score	17 ± 52	37 ± 8
	Peptides matched	16 ± 4	5 ± 1
Digestion buffer	Sequence coverage (%)	40 ± 2	No I.D.
	Mascot® score	238 ± 19	
	Peptides matched	22 ± 2	

**Figure 5—Desalting digests with Dynabeads® RPC-18.** Tryptic BSA digests contained 6 M urea, 6 M guanidine, 2 M NaCl, or digestion buffer (50 mM NH<sub>4</sub>HCO<sub>3</sub>, 0.5 M urea, 1 mM DTT, 0.1 % TFA). The top panel shows signal quenched by salt. Desalting removes salt so that the ion peaks become visible (bottom panel). An aliquot of digest or eluate (1 µl, in 50 % acetonitrile) was mixed with an equal amount of MALDI matrix, and 0.5 µl of the sample/matrix mixture was spotted onto a MALDI target plate.



### Serum profiling

Magnetic bead chromatography is a quick and reproducible way to qualify samples or discover and validate biomarkers, saving hours compared to conventional methods.

Discovery and validation of protein and peptide biomarkers in clinical proteomics requires large numbers of patient samples. Traditional methods are cumbersome and lack standardization. Magnetic bead chromatography offers quick, reproducible screening (Figure 7) and can be automated to run 96 samples in a day. Due to the consistent bead properties and results, the methods can be standardized, with opportunities for inter-laboratory collaborations.

**“Dynabeads® RPC-18 allow for efficient biofluid peptide capture and yield rich serum peptide mass profiles in MALDI-TOF MS, with good batch-to-batch reproducibility of ion intensities.”**

Connie Jimenez, PhD  
Head, OncoProteomics Laboratory  
VU University Medical Center, Amsterdam, The Netherlands

### Dynal® Peptide Profiler

Magnetic chromatography beads, such as Dynabeads® RPC-18, capture peptides from serum (or other biological fluids) for analysis by MALDI mass spectrometry. This can now be automated using the new Dynal® Peptide Profiler, a high-throughput serum profiling package on Tecan® robotic platforms.

- Quickly and accurately screen 96 samples in just 27 hours
- Capture peptides directly from serum or plasma for mass spectrometry
- Generate disease-specific peptide profiles for biomarker discovery and diagnostic research
- Analyze large numbers of samples simultaneously

The Dynal® Peptide Profiler contains two magnets for use with 96-well microtiter plates, plus a CD containing a protocol algorithm for high-throughput serum profiling using Tecan® robotic platforms.

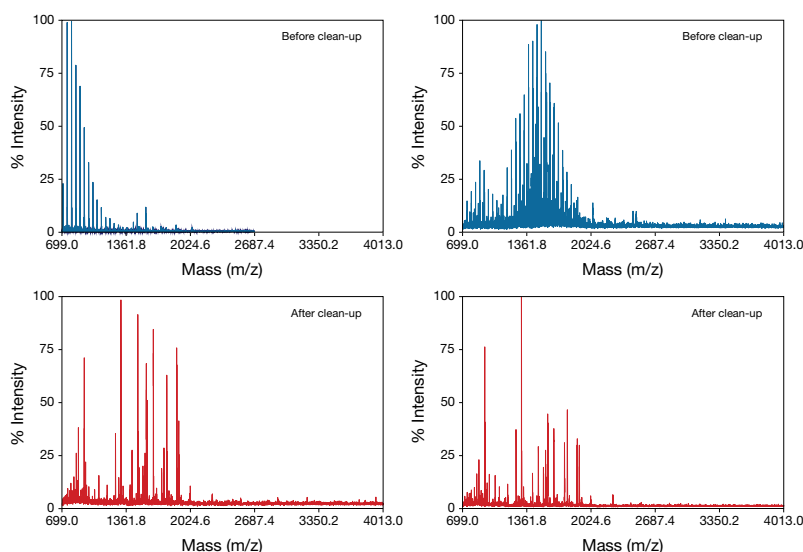


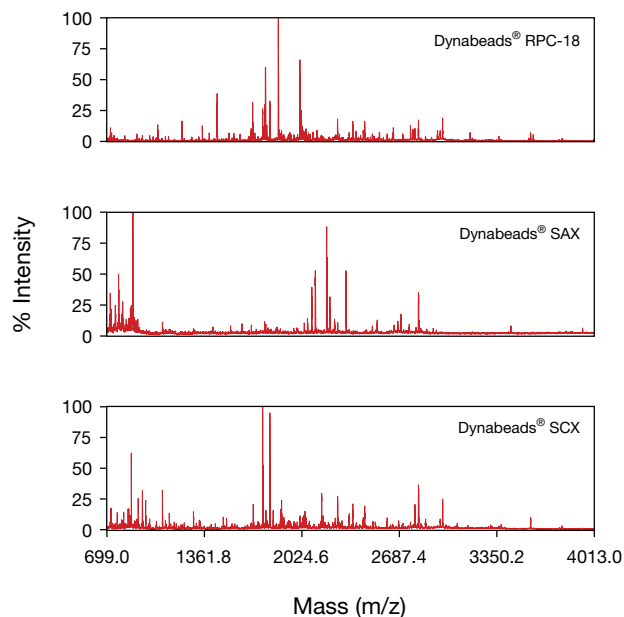
Figure 6—Removal of detergents using Dynabeads® SCX.

- Magnet 1—DynaL MPC™-96DPP-S contains bar magnets to collect bead pellets at the sides of the wells; working volumes range from 5 µl to 200 µl
- Magnet 2—DynaL MPC™-96DPP-B contains a flat magnet to collect bead pellets at the bottoms of the wells; working volumes range from 5 µl to 200 µl

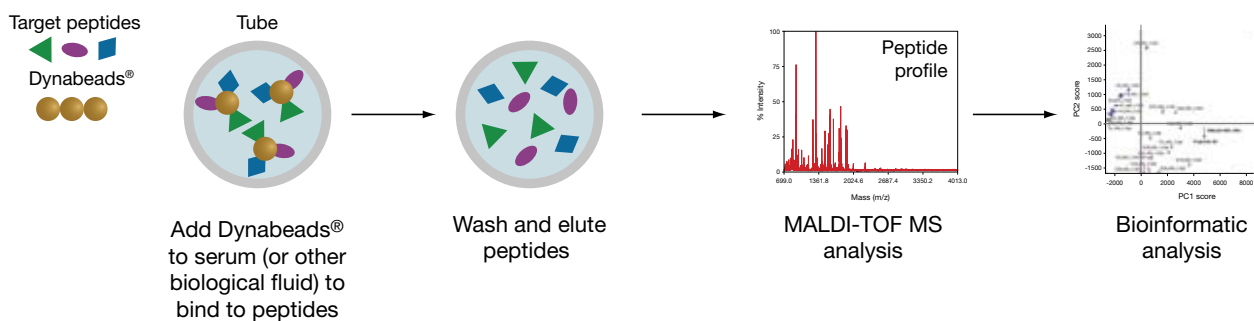
Several research groups have already developed protocols for commonly used automated platforms for high-throughput serum profiling.<sup>1,2</sup>

## Multiple surface screening

Use different bead surfaces to adsorb peptides from a single serum sample (Figure 8). This will increase the number of ion peaks observed and give you more information about your sample. This will also allow you to choose the surface that best displays the biomarkers you are looking for.



**Figure 8—Multiple surface screening.** Multiple surface screening of serum samples increases the number of ion peaks observed in a sample. Serum profiling was done using Dynabeads® RPC-18, Dynabeads® SAX, and Dynabeads® SCX. Analyses were performed for each bead type using a single serum sample. Aliquots of eluted peptides were analyzed by MALDI-TOF mass spectrometry, and typical spectra are shown. Distinct spectra were obtained from a single serum sample for each bead type.



**Figure 7—Typical workflow for serum profiling.**

## Ordering information

Product	Quantity	Concentration	Cat. no.
<b>RPC Dynabeads®</b>			
Dynabeads® RPC-18	2 ml	12.5 mg/ml	02.11D
	20 ml	12.5 mg/ml	102.12D
Dynabeads® RPC Protein	2 ml	12.5 mg/ml	102.16D
	20 ml	12.5 mg/ml	102.17D
<b>Ion exchange Dynabeads®</b>			
Dynabeads® SCX	2 ml	12.5 mg/ml	105.13D
	20 ml	12.5 mg/ml	105.14D
Dynabeads® SAX	2 ml	12.5 mg/ml	105.15D
	20 ml	12.5 mg/ml	105.16D
Dynabeads® WCX	2 ml	12.5 mg/ml	105.11D
	20 ml	12.5 mg/ml	105.12D
<b>Automation</b>			
Dynal® Peptide Profiler	1 unit*		120.40D
* 2 magnets and a CD with protocol algorithm for installation onto Tecan® robotic platforms.			

A wide range of Dynabeads® for protein research are available; please visit [www.invitrogen.com/dynabeads](http://www.invitrogen.com/dynabeads).

## References

1. Villanueva, J. et al. (2006) Automated serum peptide profiling. *Nat Protoc* 1(2): 880–891.
2. Jimenez, C.R. et al. (2007) Automated serum peptide profiling using novel magnetic C18 beads off-line coupled to MALDI-TOF-MS. *Proteomics – Clinical Applications* 1(6): 598–604.

