Body fluid peptide profiling using Dynabeads®

Body fluid peptide and protein profiling with high-throughput sample preparation followed by time-of-flight (TOF) mass spectrometry (MS) analysis is an emerging approach for biomarker discovery and for disease diagnostics and monitoring. Key requirements are reproducibility and throughput. Automated peptide capture using Dynabeads® helps meet these requirements, and can be used to identify potential biomarkers of specific diseases and to qualify patient samples before clinical studies.

Body fluids such as blood and cerebrospinal fluid are a convenient source of potentially useful protein biomarkers. Body fluid peptide and protein profiling with high-throughput sample preparation followed by TOF-MS analysis is an emerging approach to discover biomarkers and to diagnose and monitor disease. Comparing peptide and protein profiles generated from at least two groups (for example, sick versus healthy or before versus after intervention) can help to uncover disease-specific patterns or identify biomarkers. However, analytical reproducibility is a substantial challenge for peptide and protein profiling coupled to MS, and clinical proteomics approaches have been hampered by a lack of standardized, reproducible methods.

Here we describe an approach to body fluid peptide and protein profiling using high-resolution matrix-assisted laser desorption/ionization (MALDI)-TOF MS instruments with magnetic Dynabeads for automated serum-peptide capture (Fig. 1).

For body fluid profiling to be useful for biomarker research in cross-site clinical studies in which changes in peptide or protein biomarker amounts are monitored, methods allowing reproducible detection of ion peaks and their intensities are needed. Clinical studies often take place over a period of months or even years, and involve several different laboratories, so it is essential that analytical variation is not introduced through lot-to-lot variation of reagents. Automation is also a prerequisite, permitting reproducible, parallel processing of many samples. We show that the use of Dynabeads combined with automated sample processing provides a reproducible method for generating body fluid peptide and protein profiles.

Body fluid profiling is reproducible using Dynabeads

To measure the reproducibility of this approach we generated peptide profiles by repeatedly processing and analyzing a single serum sample using three different lots of Dynabeads RPC 18 for serum peptide capture. The coefficient of variance (CV) for the number of ion peaks obtained per spectrum was 9% (n = 40), indicating a high level of reproducibility for this type of method1. The total peak count was significantly and consistently higher using all three lots of Dynabeads RPC 18 than with an equivalent product from another supplier (Fig. 2a). More peptides detected in the profiles gives a greater chance of detecting a biomarker of interest.

If body fluid profiling is to be a useful method for monitoring changes in peptide or protein biomarker levels, ion peak intensities must be reproducible. Reproducibility of peak intensities is a problem with MALDI-TOF MS because peak intensity is not only related to the concentration of the individual peptide or protein, but also to the primary structure of the peptide or protein and to the complexity of the sample. Figure 2b shows the variability in peak intensity for 10 ion peaks common to peptide profiles obtained from a single serum sample when processed with either Dynabeads RPC 18 or a product from another supplier. The data illustrate that the reproducibility of ion intensity is somewhat dependent upon the ion itself (CV ~4–28% using Dynabeads RPC 18). Reproducibility of

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**Figure 1** | Body fluid profiling workflow. The entire sample preparation procedure has been automated on Tecan® and KingFisher® (Thermo Scientific) platforms.
peak intensities was greater using Dynabeads RPC 18 as compared with the alternative product (Fig. 2b). Interexperimental variation does not greatly impact the total variation when using Dynabeads RPC 18 (Fig. 2b). Principal component analysis (PCA) of the serum profiling reproducibility experiments shows that analytical variability is lower with Dynabeads RPC 18 (Fig. 2c). Variability in peak intensities after magnetic bead sample preparation is small enough that significant changes in peptide or protein levels can be assigned with confidence.

**Profiling with many bead surfaces**

Use of multiple bead types, each with different peptide and protein adsorption characteristics, will generate distinct profiles for each sample. This approach will therefore increase the total number of peptide or protein peaks profiled.

To illustrate this, we profiled serum using Dynabeads RPC 18, Dynabeads® SAX and Dynabeads® SCX. PCA shows that the profiling data cluster tightly according to the bead type used (Fig. 3a), indicating reproducible sample preparation within each bead type with distinct peptide profiles for each bead type. We compared the total mass lists of ions detected for each bead type to quantify the differences between the peptide profiles (n = 40 for each bead type). In total, 2,494 different ions were detected. Of these 9, 18 and 26% were unique to Dynabeads RPC 18, Dynabeads SAX and Dynabeads SCX, respectively (Fig. 3b). Consequently, by combining three types of Dynabeads rather than just using Dynabeads RPC 18, the number of ions detected increased by a factor of 2.2.

**Conclusions**

The use of Dynabeads combined with automated sample processing provides a reproducible method for generating body fluid peptide and protein profiles that is suitable for cross-site clinical studies. This method has the potential to be used for identifying disease-specific biomarkers and as a screening method for patient sample qualification.


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