

Interfacing Capillary/Nano LC with MALDI/MS for High-Throughput Proteomics

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Introduction

Besides higher efficiency and sensitivity, another advantage of capillary/nano LC is the volume compatibility with the μ -liter fraction requirements of MALDI targets. One of the challenges is the collection of nanoliter volumes without remixing the separated compounds and losing chromatographic separation. In this work, a high-precision X/Y/Z robotic system, Probot[™], was used that allows for the collection of tiny nanoliter volumes with zero chromatographic dispersion.

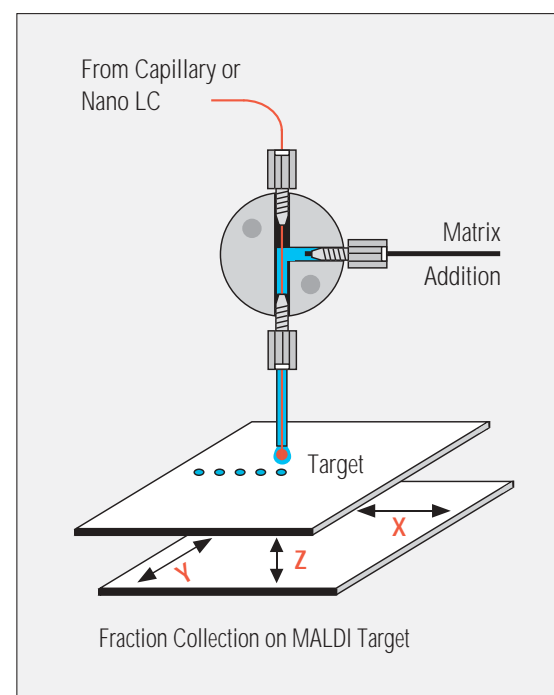


Figure 1. X, Y, Z table movement with static needle for microfraction collection and coaxial matrix addition.



Figure 2. UltiMate[™] capillary/nano LC system with Probot: from left to right a FAMOS[™] micro autosampler, Switchos[™] micro-column switching module, UltiMate micropump and detection module, and Probot.

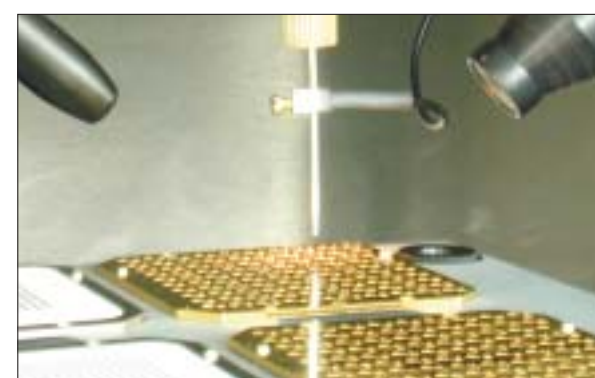


Figure 3. Close-up of fraction collection onto MALDI targets.

Experimental

Nano LC System: UltiMate, FAMOS, Switchos, Probot
Sample: 5 μ L of 200 fmol/ μ L transferrin digest
Flow Rates: 15 μ L/min for loading on μ -Guard 800
nL/min for nano LC
 μ -Guard Column: 300 μ m i.d. \times 1 mm
PepMap C18, 5 μ m
Analytical Column: 100 μ m i.d. \times 15 cm
PepMap C18, 3 μ m

Methods

Small nanoliter volumes are collected onto MALDI targets with zero chromatographic dispersion. By moving only the collection table of the robotic system and not the collection needle, a high precision of $\pm 2 \mu$ m is achieved routinely. The use of a static needle allows for the collection of small nanoliter volumes, which is impossible with a moving needle due to capillary forces. For optimal crystallization, the needle setup allows for coaxial and postcolumn addition of matrix solution, generating a perfect sweet spot. For high throughput using MALDI/TOF/TOF MS, up to six high-density targets can be placed on the table deck. Using nano LC with typical 200 nL/min flow rates, collection times as short as 2 s are possible that result in collection (spotting) volumes as low as 7 nL. Under these conditions, no chromatographic dispersion is observed (zero dead volume), and therefore highest MALDI/MS sensitivity is achieved. Another advantage of this approach includes the sample preservation, allowing for optimizing work flows and sample reanalysis.

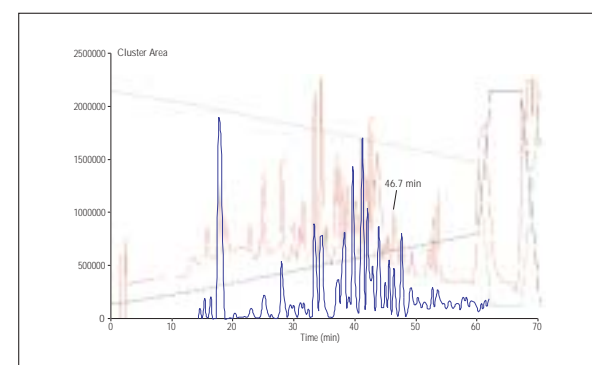


Figure 4. UV (214 nm) and MALDI (BPI) trace of 1 pmol transferrin digest, illustrating zero chromatographic dispersion (frequency: 28 s/spot). Peptide eluting at 46.7 min collected on well #100.

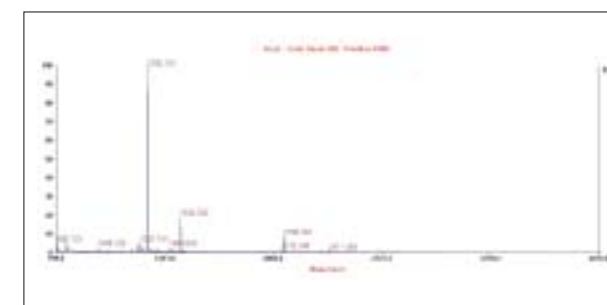


Figure 5. MALDI/MS spectrum of spot #100 eluting at 46.7 min (m/z 1336). MS data can be recorded from any spot.

Results

With the Probot microfraction collector, on-line collection of μ L- and nL-fractions eluting from capillary/nano LC systems is possible with the highest reproducibility. Fractions can be collected on many different target types.

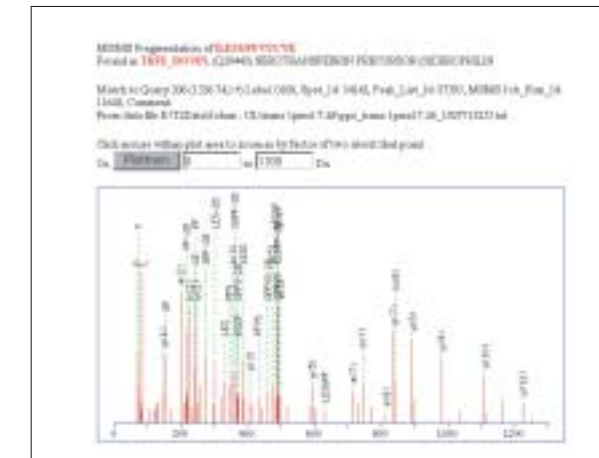


Figure 6. MS/MS spectrum from peak m/z 1336. Very strong and complete y-ion series with immonium ions and internal fragments for sequence verification are obtained, allowing for unambiguous identification by database search.

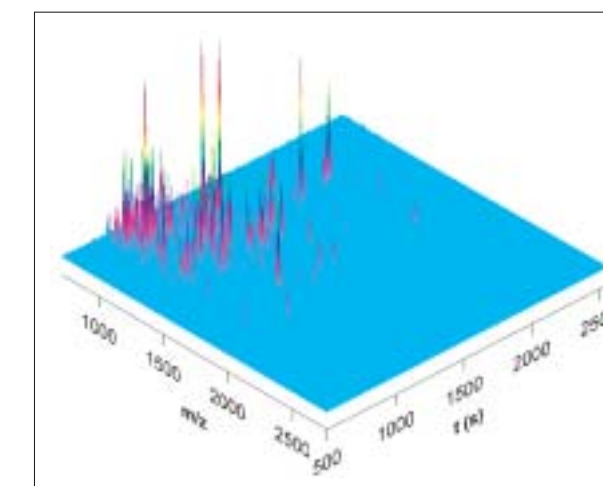


Figure 7. A 3-D mass map of Yeast digest fraction #8 after a 2-D separation on SAX and RP (5 s fractions).

Conclusions

A method has been developed to collect entire chromatograms onto MALDI targets. Transferrin has been successfully separated using a nano LC system, coupled to a Probot microfraction collector. The analytes eluting from the nano LC column are directly collected onto MALDI targets for subsequent MALDI/MS analysis. The use of Probot allows for a high degree of automation with zero chromatographic dispersion, and the transcription and storage of the entire chromatogram on MALDI targets.

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