

LC-MALDI target spotting and microfraction collection with nanoCOLLECT

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The nanoCOLLECT was used, as part of an LC-MALDI workflow, to spot eluate from a nanoscale LC peptide separation onto a MALDI target. Peptides were collected and spotted accurately and reproducibly, allowing them to be identified automatically by MALDI-MS/MS.

Introduction

With the increasing robustness of LC separations, such as those using Ettan™ MDLC and Ettan nanoLC, and the high performance of MS/MS systems, LC-MS/MS for proteomic applications has become routine in many laboratories, even overtaking MALDI-ToF MS as the routine workhorse for protein identification. Further, with the advent of MALDI MS/MS instruments enabling both peptide mass fingerprinting and sequence analysis with high throughput, sensitivity, and resolution, there has been an increasing requirement for coupling high-sensitivity nanoscale LC with MALDI MS/MS instruments for use in routine proteomics analysis (LC-MALDI).

Offline coupling of LC with MALDI as opposed to online electrospray ionization (ESI) has distinct advantages:

- > Fixation of samples to the MALDI target, allowing longer data collection and sample re-interrogation at any time, removing the constraints of the LC dimension
- > Conservation of samples
- > Decoupling of the LC and MS systems, enabling optimization of both
- > Easier separation of isobaric peptides (i.e. peptides with the same apparent molecular weight), which might have different hydrophobicities and would therefore be resolved by LC
- > Increased sequence coverage for higher confidence in protein identification

However, offline LC-MALDI requires a robust, automated spotting system that can deliver the low-nanoliter volumes produced by nanoscale chromatography, with high positional accuracy and reproducibility—the nanoCOLLECT.

This article describes the use of the nanoCOLLECT for spotting accurately and reproducibly onto MALDI targets and for the microfraction collection of samples from submillimeter i.d. columns, as part of the Ettan chromatography systems workflow (Fig 1). This LC workflow also includes the Ettan MDLC and Ettan nanoLC for peptide separation.

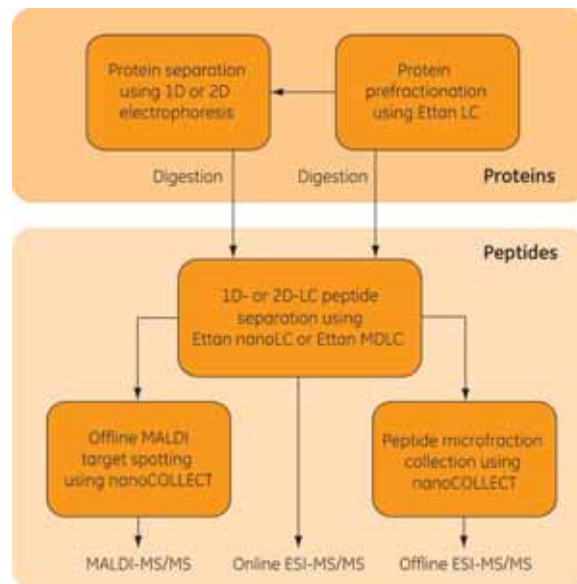


Fig 1. Ettan chromatography systems workflow.

Methods

<i>LC system:</i>	Ettan MDLC
<i>Mobile phase:</i>	A: 0.1% TFA, B: 84% acetonitrile/0.1% TFA
<i>Gradient:</i>	0–56% B in 48 min
<i>Flow rate:</i>	250 nL/min
<i>Columns:</i>	Ion exchange (IEX): BioBasic™ SCX, 2.1 × 250 mm (Thermo Electron) Reversed-phase chromatography (RPC), trap: Zorbax™ 300 SB C18, 0.3 × 5 mm (Agilent) RPC, analytical: Zorbax 300 SB C18, 0.075 × 150 mm (Agilent)
<i>Sample:</i>	Salivary gland extract of the leech <i>Hirudo medicinalis</i>
<i>Spotting device:</i>	nanoCOLLECT, fractions collected every 15 s for 45 min
<i>Matrix:</i>	αCHCA (LaserBio Labs), 3 mg/ml in 70% acetonitrile/0.1% TFA; syringe pump 1 μL/min (250-μL syringe)
<i>MS target:</i>	scoutMTP™ MALDI target (Bruker Daltonics)
<i>MS:</i>	Ultraflex™ I TOF/TOF (Bruker Daltonics)
<i>Protein ID:</i>	Mascot™ software (1) (Matrix Science)

Results

A polypeptide extract (1–3 kDa) from the leech *Hirudo medicinalis* was fractionated by IEX using the Ettan MDLC offline method. One fraction was taken, run on an analytical RPC column using the Ettan MDLC high-throughput method, and then spotted onto a MALDI target. Figure 2 shows good separation of the polypeptides, which were easily identified in the Ultraflex I TOF/TOF MALDI mass spectrometer (Fig 3).

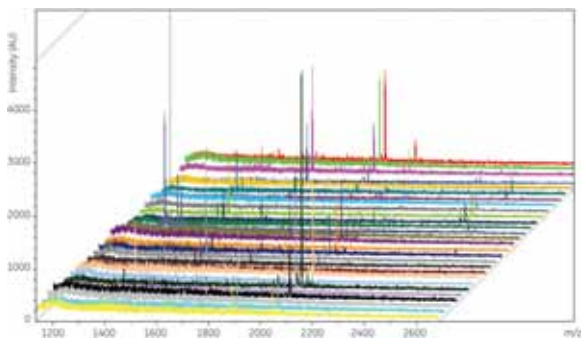


Fig 2. MALDI mass spectra derived from the Ettan MDLC fractionation of the salivary gland extract of the leech *Hirudo medicinalis*.

