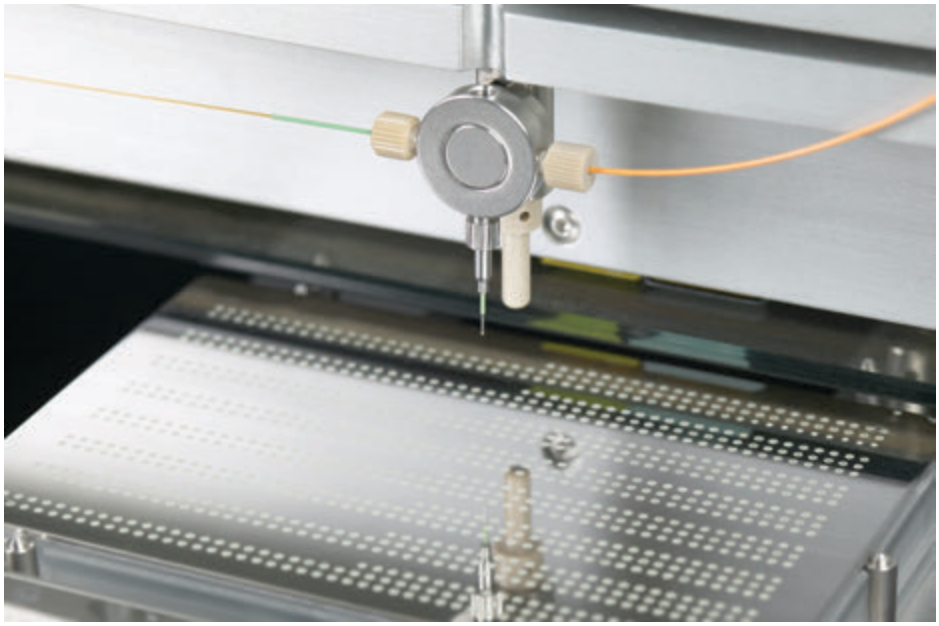
Interfacing Liquid Chromatography and Tandem MALDI Mass Spectrometry

Mass spectrometers with matrix-assisted laser desorption/ionization (MALDI) sources having collision cells suitable for high-energy collision-induced dissociation (CID) of peptides may be used to acquire fragmentation information suitable for peptide sequencing. MALDI tandem mass spectrometers with QqTOF, TOF-TOF,[1] ion trap-TOF, and ion trap-Orbitrap hybrid configurations were used extensively for this purpose until a preference for electrospray ionization (ESI) sources emerged over the course of the last decade. Never the less, MALDI tandem mass spectrometers remain a source of reasonably high quality peptide sequencing data. In the Proteomics Core Facility, the AB SCIEX 5800 TOF/TOF System is often employed for projects of moderate scope requiring peptide sequencing data that do not require the greater mass accuracy and resolution afforded by the Orbitrap mass spectrometer.

To reduce sample complexity and suppress matrix effects in a manner analogous to LC-MS/MS, MALDI tandem mass spectrometers have been interfaced with liquid chromatographs. This hyphenated approach is referred to as liquid chromatography–MALDI mass spectrometry (LC-MALDI-MS or just LC-MALDI).[2,3] Since laser desorption/ionization requires samples be deposited in MALDI matrix, a solution of MALDI matrix (α-cyano-4-hydroxcinnamic acid) injected from a syringe pump is mixed with the effluent from a C18 reverse phase capillary liquid chromatography column at a "T" just prior to collecting chromatographic factions as spots on the surface of a MALDI plate (*see* photo). In the Proteomics Core Facility, the AB SCIEX Tempo LC-MALDI system is used for this purpose. Spot set data including spot number, spot location (relative to fiducial markings), retention time, UV absorbance at 214 nm, and gradient composition (%Solvent A and %Solvent B) for each spot is recorded, and this data is transferred to the AB SCIEX 5800 TOF/TOF system to facilitate the acquisition of mass spectral data as well as provide combined chromatographic and mass spectral data comparable to LC-MS/MS (*see* Figure 1).



Spot deposition on AB SCIEX Tempo LC-MALDI. Capillary column (150 µm ID X 15 cm) effluent enters from the left and MALDI matrix from the right. Using an average flow rate of 1.0 µL/min, a spot is collected every 10 seconds corresponding to an approximate elution volume of 0.17 µL.

Unlike ESI-LC-MS/MS, MS1 data is first acquired from every spot and lists of precursor ions associated with individual spots (up to 25 per spot) are compiled before MS/MS analysis begins.

The precursor ions associated with specific peptides are often found in two or more adjacent spots. Data acquired on the 5800 TOF/TOF System is analyzed using AB SCIEX ProteinPilot software.[4]

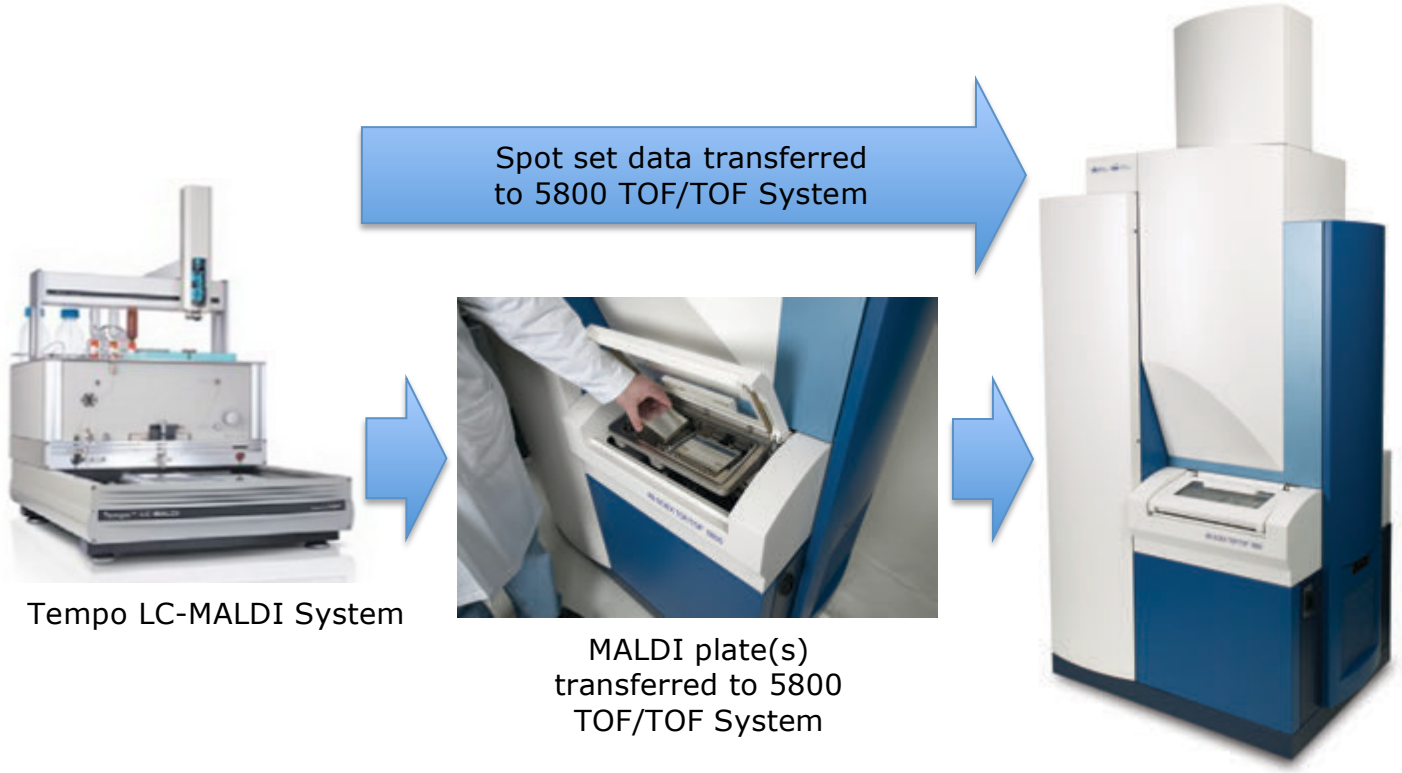


Figure 1. LC-MALDI workflow is depicted. Spot set data is transferred to 5800 TOF/TOF System in conjunction with MALDI plate(s). From 3 to 6 chromatographic fractionations can be stored on each plate.

It is interesting to note that unlike ESI-LC-MS/MS, wherein the sample is consumed, very little of the sample in each of the individual spots on the MALDI plate is consumed during acquisition of MS and MS/MS data. Consequently, the chromatographic fractionation can be "frozen" on the surface of the MALDI plate and if the plate is properly stored can be analyzed or re-analyzed days, months or even years later.

Complementarity of ESI and MALDI

ESI and MALDI are complementary with respect to protein identification in complex samples.[5] Whereas ESI is biased with respect to detection of lysine-terminated tryptic peptides; MALDI is biased with respect to detection of arginine-terminated tryptic peptides. Consequently, when complex samples are analyzed on both ESI and MALDI platforms, ESI affords a number of peptide IDs for lysine-terminated peptides not detected by MALDI; MALDI affords a number of peptide IDs for arginine-terminated peptides not detected by ESI; and taken together a greater depth of sequence coverage can be obtained. Generally speaking, a greater number of novel arginine-terminated peptides are detected as compared with novel lysine-terminated peptides. This is not because MALDI affords greater sensitivity, but rather because on average there are more arginine-terminated peptides present in tryptic digests. However, these are generalizations as a direct comparison of results would require access to a tandem mass spectrometer with interchangeable ESI and MALDI sources and few such systems are available a present. The sensitivity of lysine-terminated peptides with respect to MALDI detection can be improved by chemical reaction of tryptic digests with O-methylisourea that modifies the ε-amino group of lysine-terminated peptides to afford the corresponding homoarginine-terminated peptides that are detected by MALDI with sensitivities comparable to arginine-terminated peptides.[6]

References

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