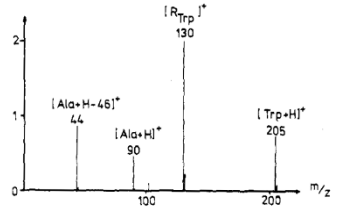
MILESTONE 18

Enter the matrix

By the early 1980s, mass spectrometry was a well-established laboratory technique for the characterization of small organic molecules (**Milestone 7**). But larger ones—particularly biological molecules, such as proteins, DNA and complex carbohydrates—were proving to be a challenge. Because mass analysis relies on the detection of ionized species in the gas phase, the key problem was imparting sufficient energy to these large molecules to send them into the gas phase without destroying them. Although some progress had been made using fast atom bombardment (FAB) and ^{252}Cf-plasma desorption (see **Milestone 2**), at the time the gold standard was the CO_2 laser desorption of organic molecules with masses up to 1,000 daltons—so the world of proteins was largely inaccessible.

Michael Karas and Franz Hillenkamp, in Germany, were two of the earliest adopters of lasers for mass spectrometry, starting efforts in this area when laser technology was relatively immature. In 1985, while irradiating an equimolar mixture of alanine and tryptophan, they observed signals in the mass spectrum for both amino acids. Although this does not sound particularly surprising, Karas and Hillenkamp knew that one needed to use a much higher laser energy at that wavelength to form gaseous ions of alanine—so they only expected to observe signals for tryptophan. They postulated that the alanine was 'riding piggyback' on the tryptophan and called this phenomenon "matrix-assisted laser desorption." Although the term matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) would not be coined for a few years, the premise—the use of a matrix material to adsorb laser energy and transfer it to an admixed analyte of interest, facilitating the vaporization and ionization of that analyte—was born.

Karas and Hillenkamp went on to use their matrix-assisted laser-desorption technique to compare the solids tryptophan and nicotinic acid (another small organic molecule) with liquid matrices that had been developed for FAB-MS. They obtained mass spectra for three 'medium-sized' antibiotics (stachyose, erythromycin and gramicidin S) and achieved significantly better results—less fragmentation and a more intense signal from the parent ion—with the solid than with the liquid matrices. More promising results also came when analyzing vitamin B_{12} in a nicotinic acid matrix: no desorption was observed without a matrix, and there were solubility problems when using the liquid matrices. Evidence was mounting that a solid, UV-adsorbing small-molecule matrix might be the tool needed to obtain mass spectra of large proteins.



The mass spectrum obtained by Karas and Hillenkamp of a mixture of alanine (Ala) and tryptophan (Trp). Reprinted with permission from Karas, M., Bachmann, D. & Hillenkamp, F., Influence of the wavelength in high-irradiance ultraviolet laser desorption mass spectrometry of organic molecule, *Anal. Chem.* **57**, 2935–2939 (1985).

On the other side of the world, Koichi Tanaka and colleagues from the Shimadzu Corporation in Japan were working on a similar problem, and in 1988, they published the mass spectra of several proteins and synthetic polymers obtained from a laser ionization mass spectrometer they had built. Signals for molecules up to ~25,000 daltons, and oligomeric molecules up to *m/z* 100,000, could be detected using their instrument. Tanaka and colleagues developed an unconventional sample preparation approach in which they dispersed an ultra-fine cobalt powder in glycerol. As the Shimadzu sample preparation method relied on inorganic nanoparticles, rather than small organic molecules, for photon adsorption, it was not technically MALDI, but it was nonetheless a landmark development. Tanaka was awarded a portion of the 2002 Nobel Prize for his work on laser-induced soft desorption/ionization of large molecules that led to modern-day MALDI techniques.

Karas and Hillenkamp further developed the use of solid small molecules as matrices, and later in the same year they published the mass spectra of four proteins, obtained using nicotinic acid as a matrix. Several other matrices have been developed in the past 25 years, as have alternative mechanisms to facilitate the mild ionization and desorption of macromolecules. Today, MALDI and its laser-desorption cousins are workhorses in most chemistry and biochemistry labs.

Claire Hansell, Associate Editor, Nature

ORIGINAL RESEARCH PAPERS Karas, M., Bachmann, D. & Hillenkamp, F. Influence of the wavelength in high-irradiance ultraviolet laser desorption mass spectrometry of organic molecules. *Anal. Chem.* **57**, 2935–2939 (1985) | Karas, M., Bachmann, D., Bahr, U. & Hillenkamp, F. Matrix-assisted ultraviolet laser desorption of non-volatile compounds. *Int. J. Mass Spectrom. Ion Processes* **78**, 53–68 (1987) | Tanaka, K. *et al.* Protein and polymer analyses up to *m/z* 100,000 by laser ionization time-of-flight mass spectrometry. *Rapid Commun. Mass Spectrom.* **2**, 151–153 (1988) | Karas, M. & Hillenkamp, F. Laser desorption ionization of proteins with molecular masses exceeding 10,000 daltons. *Anal. Chem.* **60**, 2299–2301 (1988)