# Laser Pulse Length Dependence of Ionization Processes and Internal Energy Transfer in UV-MALDI-MS

Akos Vertes, Yong Chen, Guanghong Luo, Ioan Marginean, Louise Ye
Department of Chemistry, George Washington University, Washington, DC 20052, USA

Recent internal energy (IE) measurements for various analytes in matrix-assisted laser desorption ionization (MALDI) have indicated that the amount of IE transferred to analytes not only depends on the matrix but also on the nature of the analyte. Common matrixes, such as α-cyano-4-hydroxycinnamic acid (CHCA), 3,5-dimethoxy-4-hydroxycinnamic acid (sinapinic acid, SA) and 2,5-dihydroxybenzoic acid (DHB), had been characterized as "cold" or "hot" according to the IEs of analyte ions produced in the corresponding MALDI plume. In this contribution, we present evidence that IE transfer in MALDI depends on matrix, analyte as well as laser pulse properties. We also explore primary matrix ion formation using ns and ps excitation.

Seven substituted benzylpyridinium salts as thermometer molecules (TM) and short peptides, such as leucine enkephalin (LE), were investigated in CHCA, SA and DHB matrixes. Nitrogen laser (3 ns pulse length) and frequency tripled Nd:YAG laser (22 ps pulse length) were used as excitation sources at various fluences. Survival yields (SY) of the analyte molecular ions were extracted from the spectra and the corresponding IEs were obtained from RRKM theory. Matrix ion yields and SY were measured for DHB and CHCA.

For all three matrixes, the log-log slopes of the total ion yield vs. laser fluence are very similar for both lasers. The threshold fluence for the ps laser is ~1, ~1.5 and ~2 times the value for ns laser for CHCA, SA and DHB, respectively. The survival yields of molecular matrix ion, i.e., the ratio of molecular ion yield to total ion yield, indicate that the ps laser systematically preserves higher molecular ion content whereas the ns laser induces more fragmentation of the matrix. Considering the similarity in fluence and in absorption coefficient at the laser wavelengths, the discrepancy of survival yields may result from the vast difference in pulse length. This manifests in different pumping rates and enables different channels for relaxation. The alternative explanation is that the longer pulse can sequentially desorb and ionize the matrix, thus more collisions are allowed during the longer plume-laser interaction time (especially between neutral molecules and accelerated molecular ions). These findings enable more accurate modeling of the plume dynamics and the ionization mechanism of MALDI.

All analyte spectra were collected and SYs were calculated for all TMs and LE in CHCA, SA and DHB matrixes with both nitrogen laser and mode locked 3×ω Nd:YAG laser. The SYs indicate the IEs of analyte ions in MALDI are analyte, matrix and laser source dependent. The ion generation threshold fluences follow the same order for both lasers: CHCA < SA < DHB, but for all the analytes the mode locked Nd:YAG laser source requires higher threshold fluence than the nitrogen laser. Despite the higher fluence, the SYs are generally higher (the corresponding IEs are lower) for the Nd:YAG laser than for nitrogen laser. The SYs of all the TM molecular ions decrease with the increase of fluence for both the ns laser and the ps laser. Compared to the TMs, the order of IE transfer from the different matrixes is reversed for LE.