Doctoral Thesis

Proton transfer reactions in matrix-assisted laser desorption/ionization

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Proton Transfer Reactions in Matrix-assisted Laser Desorption/Ionization

Dissertation submitted to the Swiss Federal Institute of Technology Zürich for the degree of Doctor of Natural Sciences

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Abstract

In spite of the fact that matrix-assisted laser desorption/ionization (MALDI) has found widespread application in macromolecular analysis over the last decade, the mechanisms of ion formation have remained poorly understood. A number of potential ionization mechanisms have been suggested by several groups. However, only in a very few cases it was possible to quantify the required energies.

MALDI ions are often detected as protonated or deprotonated species, suggesting that proton transfer reactions between matrix and analyte play a dominant role in the MALDI process. The crucial parameter in quantifying energies in proton transfer reactions are the gas-phase basicities of the respective species involved. Especially for deprotonated matrix molecules, data have not been available so far. Therefore, the purpose of this work was to determine the energetics of potential proton transfer reactions in MALDI by measuring the gas-phase basicities of deprotonated matrix molecules and matrix clusters.

As part of this effort, two new bracketing-related methods have been developed. One is based on equilibrium and transition state theory and can be applied if quasi-equilibrium conditions are observed in the mass spectrometer. The other method is more generally applicable and based on structure-reactivity correlations.

It was found that the ground-state gas-phase basicities of the matrix anions under study cover a large range with differences of up to 1.7 eV. With the gas-phase basicity values obtained here and literature data on matrix neutrals, the energies required for proton transfer between neutral matrix species can be calculated to lie between 4 eV and 6 eV. Proton transfer between neutral matrix species and neutral analyte molecules can be estimated to cost between 2.8 eV and 5.2 eV. These energies are below the energy of two photons with 337 nm, the wavelength typically utilized in MALDI. These estimations show that ground-state proton transfer reactions in MALDI are energetically possible.
As an alternative to conventional MALDI, the "matrix-assisted laser desorption/chemical ionization" method was developed. Complex analytes with masses up to ca. 3000 Da can be studied with this method. By the choice of the reagent anion used for deprotonation of the analyte, controlled specific fragmentation is also obtained. Reagent anions with high proton affinity lead to strong fragmentation in a highly exothermic reaction, whereas reagent anions with low proton affinity yield little or no fragmentation. Molecular mass and structure information is obtained in a single mass spectrometric experiment.
Kurzfassung

Obwohl die Matrix-unterstützte Laser Desorption/Ionisation (MALDI) bereits seit Jahren breite Anwendung in der makromolekularen Analytik findet, sind die Mechanismen der Ionenbildung bis heute weitgehend ungeklärt. Eine Anzahl möglicher Ionsationsmechanismen wurde von verschiedenen Arbeitsgruppen vorgeschlagen. Es war bisher allerdings nur in wenigen Fällen möglich, die dazu benötigten Energien zu quantifizieren.


Es zeigte sich, dass die Gasphasenbasizitäten der untersuchten Matrixanionen im Grundzustand einen breiten Bereich aufspannen, mit Differenzen von bis zu 1.7 eV. Mit den hier erhaltenen Gasphasenbasizitäts-Werten und den Literaturdaten für neutrale Matrixteilchen können die für einen Protonentransfer zwischen neutralen Matrixspezies benötigten Energien zu 4 eV bis 6 eV berechnet werden. Die Kosten für Protonentransferreaktionen zwischen neutralen Matrixspezies und neutralen Analytmolekülen können auf 2.8 eV bis 5.2 eV abgeschätzt werden. Diese Energien liegen weit unter der Energie von zwei

1. Introduction

Understanding fundamental biochemical and chemical processes is a major challenge in modern research and development. Because the identification of large and complex molecules is a prerequisite for studying these processes, high-performance analytical methods are needed. Among these, matrix-assisted laser desorption/ionization (MALDI) mass spectrometry\textsuperscript{1-3} has been found to be a particularly suitable and straightforward technique applicable to a large variety of analytes such as peptides and proteins\textsuperscript{4,5}, nucleic acid oligo- and polymers\textsuperscript{6-7}, oligosaccharides\textsuperscript{8} and synthetic polymers\textsuperscript{9-11}.

With MALDI, non-volatile analyte molecules are transferred to the gas phase and ionized in a single step by means of pulsed laser desorption. The laser energy is primarily absorbed by matrix molecules, thus preventing decomposition of the thermally labile analyte molecules embedded therein. Upon matrix vaporization, intact analyte is co-desorbed. A quasi-thermal sublimation/desorption model was developed by Dreisewerd and co-workers\textsuperscript{12}, which was found to be in excellent agreement with experimental results. It is generally assumed that the matrix plays a key role not only in the desorption, but also in the ionization process. However, the mechanisms of ion formation in MALDI are only poorly understood and a matter of active research\textsuperscript{13}. As a consequence, the choice of suitable matrices is empirical rather than systematic\textsuperscript{14}. An enhanced knowledge of the ionization mechanisms in MALDI is desirable not only from a purely scientific standpoint. It is also indispensable for improving the method, e.g. maximizing ion yields, improving mass spectrometric performance, and gaining access to further analyte classes, for example by using more suitable matrices or sample preparation techniques.

Because most biological analyte molecules are predominantly detected in protonated or deprotonated form, proton transfer reactions between matrix and analyte are likely to play a key role in the MALDI ionization mechanisms. The aim of this work is to determine fundamental thermochemical data on matrix species in order to define possible proton transfer pathways of ion formation.
1.1. Matrix-assisted laser desorption/ionization

1.1.1. Mass spectrometric performance

MALDI mass spectrometry can be used for the analysis of low molecular weight compounds as well as for macromolecular mass determination. It is now routinely used in protein analysis, where peptides from chemical or enzymatic digests are separated by chromatographic means and analyzed by MALDI mass spectrometry ("peptide mapping"). Among the advantages of MALDI are its tolerance to buffer salts and other impurities, the accessible mass range of several hundred thousand Dalton, a sensitivity in the attomole-range, and the generation of intact molecular analyte ions. Additionally, analyte ions are predominantly detected in singly-charged monomeric form, simplifying mass spectral interpretation.

The key idea of MALDI is to dilute analyte molecules with an excess of a suitable matrix that absorbs the incident laser light. In typical MALDI preparations, the molar matrix-to-analyte ratio is about 10³ to 10⁶. Upon pulsed laser irradiation, the matrix-analyte mixture undergoes an instantaneous phase transition from the condensed into the gas phase. Besides neutral species, matrix and analyte ions are liberated in the MALDI process that can be detected with any appropriate mass spectrometer. The ratio of neutral to ionic products was found to be on the order of 10⁰⁰ or greater, and decreased with increasing laser irradiance. Thus, higher laser irradiances could in principle enhance sensitivity, but at the same time increase undesired analyte fragmentation and decrease mass resolution in time-of-flight instruments.

MALDI is usually considered a "soft" ionization method because prompt analyte fragmentation is rarely observed. In contrast, fast and metastable ion fragmentation are often substantial, but can be taken into advantage of for determining structural and sequence information. Whereas prompt fragmentation occurs on the time scale of the laser pulse duration of typically a few nanoseconds, fast fragmentation proceeds on a time scale of up to several hundreds of
nanoseconds. The efficiency of fast fragmentation, also termed in-source decay (ISD)\textsuperscript{32}, increases with increasing laser fluence\textsuperscript{30} and increasing residence time of the ions in the source region of the mass spectrometer\textsuperscript{32}. Moreover, it was found that both the matrix used\textsuperscript{30, 32} and the acidity and basicity of the analyte\textsuperscript{38} strongly influence the ISD fragment ion yield. The efficiency of metastable fragmentation, or post-source decay (PSD)\textsuperscript{33}, was also found to increase with increasing laser fluence\textsuperscript{31}. Additionally, the nature of the residual gas present in the mass spectrometer and its partial pressure were found to influence the PSD fragment ion yield; higher pressures and molecules with larger collisional cross sections increased the PSD efficiency\textsuperscript{34}. The MALDI matrix used also has an effect on the PSD yield\textsuperscript{34}. This suggests that the ion fragmentation observed in MALDI results from both internal energy deposition during the MALDI process and collision-induced reactions in the mass spectrometer\textsuperscript{34}.

1.1.2. MALDI matrices and sample preparation

Depending on their propensity to induce fragmentation, matrices can be classified as "hot" or "cold". For example, 3-hydroxypicolinic acid\textsuperscript{39} is considered a "cold" matrix, because even fragile analytes such as glycoproteins containing labile functional groups can be detected as intact ions\textsuperscript{40}. On the other hand, 4-hydroxy-\(\alpha\)-cyanocinnamic acid\textsuperscript{41} induces strong metastable fragmentation and is therefore considered a "hot" matrix\textsuperscript{40}. It may be that both thermochemical and physical matrix properties account for the observed differences in analyte fragmentation: An exoergic proton transfer reaction as well as a high matrix sublimation temperature should both increase analyte internal energy.

MALDI matrices must meet several requirements, including sufficient absorption at the laser wavelength used and vacuum stability. Many molecules fulfill these conditions, but only a few work well as MALDI matrices. Even isomers of a certain compound with comparable physical properties can yield MALDI spectra of substantially different quality. Horneffer and coworkers investigated structural isomers of dihydroxybenzoic acid with respect to their function as MALDI
matrices\textsuperscript{42}. Isomers containing an \textit{ortho}-hydroxy group were found to generally yield better quality MALDI spectra than isomers containing a \textit{meta}-hydroxy group\textsuperscript{42}. Schlunegger and coworkers found that \textit{meta}- and \textit{para}-isomers of hydroxybenzoic acids and hydroxycarbonyl compounds show either a significantly lower or no matrix activity when compared to the corresponding \textit{ortho}-isomers\textsuperscript{43}. This suggests that thermochemical matrix properties are responsible for the observed differences.

Because with common matrices the yield of desorbed neutrals in MALDI largely exceeds that of ionic species\textsuperscript{29}, the idea of separating desorption and ionization in order to independently optimize both events presents itself.

One approach is the use of comatrices as ionizing agents for enhancement of signal intensity and reproducibility. Among these are ammonium halides\textsuperscript{44, 45} and organic ammonium salts\textsuperscript{46}. These have been used as comatrices with conventional solid phase matrices for UV–MALDI mass spectrometry. For example, Cheng and Chan tested their use in MALDI mass spectrometry of oligonucleotides in negative ion mode\textsuperscript{44}. They found that all ammonium halides investigated display significant enhancement effects on the signal intensity of intact molecular DNA homopolymer anions. The fluoride salt exhibited the greatest enhancement. Currie and Yates investigated the use of a variety of ammonium salts as comatrices in MALDI mass spectrometry of oligodeoxynucleotides\textsuperscript{46}. They found that many of the alkylammonium salts prevented macroscopic crystallization, in which case no ions were observed. While the highly hygroscopic alkylammonium halides prevent crystallization, inorganic and other organic salts can be used as comatrices for solid MALDI matrices. Citrate, tartrate and oxalate ammonium salts were used in two-component matrix experiments performed by Zhu et al.\textsuperscript{47}. The addition of ammonium citrate to an UV-absorbing MALDI matrix (1:1 molar ratio) was found to greatly enhance both positive and negative ion signals from DNA molecules. Besides suppressing adducts of DNA–alkali ion, the ammonium salt is believed to have a protonation/deprotonation function for oligonucleotides, although the mechanism was not determined\textsuperscript{47}. Lebrilla and coworkers used sulfuric acid as a dopant for
MALDI analysis of neutral oligosaccharides$^{48}$. Depending on the $H_2SO_4$ concentration and the laser fluence applied, two types of quasimolecular anions were observed. At a low $H_2SO_4$ concentration and threshold laser fluence, a sulfate adduct ion, $[M + H_2SO_4]^{-}$, was formed. With higher $H_2SO_4$ concentration and elevated laser fluence, in situ derivatization of the oligosaccharides occurred, yielding $[M + HSO_4 - H_2O]^{-}$ type ions$^{48}$.

Another approach developed within the scope of this work is described in more detail in section 1.2. It is based on conventional chemical ionization mechanisms, with the key idea that preformed matrix ions liberated upon laser desorption act as chemical ionization agents for the co-desorbed analyte$^{49}$.

Most commonly used matrices are solids$^{14}$, but liquid matrices$^{16, 22, 50}$ or binary matrices$^{3, 49, 51-54}$, consisting of a liquid phase and solid particulates, can be valuable alternatives.

Depending on the sample preparation technique, the particular matrix used, and the analyte class, solid matrices can either homogeneously incorporate analyte molecules or form microcrystals with the analyte preferably distributed along the crystal surfaces$^{55, 56}$. Analyte incorporation has the advantage of a purification process in which salts and other impurities may stay outside the crystal volume, which is sometimes better for peptide analysis$^{57}$. For other analytes, e.g., for synthetic polymers, incorporation can be less desirable because here alkali salts usually act as ionizing agents, and better results can be obtained from amorphous preparations. Standard sample preparation techniques are the "dried-droplet"$^{58}$, the "crushed crystal"$^{19}$, and the "fast evaporation"$^{24}$ methods.

Requirements for liquid matrices are less stringent than those of solid matrices: solubility in common solvents and co-crystallization as criteria for matrix selection can be neglected. However, only a few compounds have yet been identified as effective matrices, among them 3-nitrobenzyl alcohol$^{50, 59}$ which absorbs in the ultraviolet and glycerol which absorbs in the infrared. By far the best results using a liquid matrix have been obtained with glycerol$^{16}$. Liquid mixtures consisting of
absorbing molecules dissolved in non-absorbing primary compounds as matrices for MALDI have also been reported\textsuperscript{60-63}.

Binary liquid/solid matrices were shown to be useful for the analysis of intermediate molecular weight substances\textsuperscript{3, 49, 51-53}. Since the liquid phase (for example glycerol) does not generally have an UV-chromophore, the laser energy is absorbed by the solid particulates and desorption occurs via rapid thermal evaporation of the liquid. Because a binary matrix consists of two different physical phases (liquid and solid), the method was termed two-phase MALDI\textsuperscript{64}.

The major advantage of binary matrices is that the laser energy is absorbed by particulates, and the liquid phase can be chosen without consideration of absorption criteria. The particles commonly used absorb laser wavelengths ranging from the near ultraviolet to the near infrared, and matrix performance was found to be largely independent of the laser wavelength used\textsuperscript{53}.

On the other hand, criteria for the choice of solid matrices include a sufficient absorption at the wavelength used. Optical absorption coefficients of sublimed UV-MALDI matrix films have been reported to be about 1·10\textsuperscript{5} cm\textsuperscript{-1} at the nitrogen laser wavelength (337 nm). A somewhat higher absorption coefficient of 2.2·10\textsuperscript{5} cm\textsuperscript{-1} was found for 4-hydroxy-\textalpha-cyanocinnamic acid\textsuperscript{65}. In general, the solid matrix films showed integrated cross-sections similar to those determined by measurements of the respective solutions\textsuperscript{65}.

1.1.3. Laser parameters

The first MALDI results were obtained by use of laser systems emitting ultraviolet irradiation at 266 nm and 355 nm (frequency-tripled and -quadrupled output of the Nd:YAG laser)\textsuperscript{1}. Because of their comparably low cost, nitrogen lasers emitting at 337 nm have since found widespread application in MALDI. Recently, UV-MALDI experiments at wavelengths in the range 360 – 450 nm emitted from a tunable titanium:sapphire laser were reported, and it was found that peptide analyte signals could be obtained with wavelengths of up to 435 nm\textsuperscript{66}.
Matrix ions were observed at even longer wavelengths of up to 450 nm\textsuperscript{66}. In these experiments, laser fluences of up to 272 mJ/cm\textsuperscript{2} were applied. A laser wavelength of 532 nm (frequency-doubled output of the Nd:YAG laser) was successfully used in MALDI experiments with rhodamine dyes as matrices\textsuperscript{67}. With a laser wavelength of 1064 nm (fundamental output of the Nd:YAG laser) and using IR dyes as matrices, no MALDI ions were observed, although the absorption of the IR dyes at 1064 nm was comparable to the absorption of the rhodamine dyes at 532 nm\textsuperscript{67}. In the latter studies, typical laser fluences were less than 200 mJ/cm\textsuperscript{2} to prevent production of a plasma or fragmentation\textsuperscript{67}.

In IR–MALDI experiments, Er:YAG (2.94 μm)\textsuperscript{68}, Er:YSGG (2.79 μm)\textsuperscript{22}, and CO\textsubscript{2} (10.6 μm)\textsuperscript{69} lasers are used. Moreover, tunable lasers such as optical parametric oscillators (OPO)\textsuperscript{70-73} and free–electron lasers\textsuperscript{74} have found application in fundamentally oriented studies.

In general, laser power density (or irradiance) thresholds for the observation of protein signals are about one order of magnitude higher in IR–MALDI when compared to UV–MALDI\textsuperscript{73}. UV–MALDI performance was found to be independent of the laser pulse width, at least for pulse durations of up to tens of nanoseconds, indicating that the desorption/ionization process is determined by the fluence, rather than irradiance\textsuperscript{13}. Typical fluences in UV–MALDI range from 50 to 300 J/m\textsuperscript{2}, and increase with decreasing laser spot size\textsuperscript{12}. In IR–MALDI, typical fluences are significantly higher and range from $10^3$ to $10^4$ J/m\textsuperscript{2}, depending on the matrix used and the laser spot size\textsuperscript{75}.

Yau and coworkers compared the threshold fluences required for ion production from liquid and solid matrices\textsuperscript{76}. In agreement with a lower sublimation temperature of the liquid matrix, they found that threshold fluences are lower in the case of the liquid matrix as compared to the solid matrix, but they did not report on analyte fragmentation. The threshold fluence required for production of both matrix ions and matrix neutrals from a solid matrix as a function of initial sample temperature was studied by Schürenberg and coworkers\textsuperscript{77}. A linear increase in threshold fluence with decreasing temperature was observed, and the results were found to be in agreement with the quasi–thermal desorption model developed in the same group\textsuperscript{12}. 
The influence of laser parameters such as fluence on the MALDI desorption process can be described by hydrodynamic\textsuperscript{78}, quasi-thermal\textsuperscript{12}, and other models\textsuperscript{79}, but their effect on the ion formation in MALDI is less clear. Possible routes for MALDI ion formation are discussed with respect to laser fluences and photon energies in sections 1.1.4. and 1.1.5.

1.1.4. Primary ion formation

In the following, the term "primary ion formation" stands for pathways in which neutral molecules (or aggregates) react to form charged products, whereas "secondary ion formation" refers to ion/molecule reactions involving charge transfer. Possible MALDI primary ion formation mechanisms are summarized in Table 1.1.

<table>
<thead>
<tr>
<th>MECHANISM</th>
<th>REACTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>single molecule multi-photon ionization</td>
<td>[ m \xrightarrow[n^{\text{hv}}]{} m^* + e^- ]</td>
</tr>
<tr>
<td>energy pooling</td>
<td>[ m \xrightarrow[^{hv}{}]{} m^* \rightarrow m^* + m^* \rightarrow m^* + m \rightarrow m^{++} + m + e^- ]</td>
</tr>
<tr>
<td>excited-state proton transfer</td>
<td>[ m \xrightarrow[^{hv}{}]{} m^* ], [ m^* + m \rightarrow [m-H]^+ + [m+H]^+ ]</td>
</tr>
<tr>
<td>proton transfer (excluding excited states)</td>
<td>[ m + A \xrightarrow[^{\Delta G}]{} [m-H]^+ + [m+H]^+ ]</td>
</tr>
<tr>
<td>thermal ionization</td>
<td>[ m + m \xrightarrow[^{\Delta G}]{} m^{++} + m^{+} ]</td>
</tr>
</tbody>
</table>

Table 1.1 Possible primary ion formation mechanisms in MALDI.

Electronic excitation of the matrix is believed by many to play a major role in the UV-MALDI ionization process; excited-state proton transfer\textsuperscript{80-}
and photoionization of matrix molecules\textsuperscript{14, 81} have been suggested as possible ionization mechanisms proceeding via excited states.

Ehring and coworkers studied the contribution of photochemical and thermal effects in MALDI ion formation by means of back side desorption\textsuperscript{83}. A quartz glass target with an Au layer of 2000 Å thickness was used in their experiments. With front side desorption, direct photochemical processes were possible, whereas back side desorption only allowed for thermal effects because the Au layer completely absorbed the 337 nm irradiation\textsuperscript{83}. Protonated peptide signals were observed with front side desorption, but only sodiated and potassiated ions were detected with back side desorption. It was concluded that photochemical reactions involving electronically excited matrix species play a significant role in the MALDI ionization process\textsuperscript{83}.

A single molecule multi-photon ionization (MPI) mechanism requires that the absorbed photon energy is higher than the ionization potential (IP) of the molecule. IP values for MALDI matrices are largely unknown, but some measurements have begun to appear. Karbach and Knochenmuss determined the IP of 2,5-dihydroxybenzoic acid (DHB) to be 8.05 eV, and the DHB dimer was found to have an IP of 7.93 eV\textsuperscript{13}. As pictured in Figure 1.1, this energy is not provided by one or two photons with typical laser wavelengths used in UV–MALDI (337 nm or 355 nm).

Nicotinic acid is used as a MALDI matrix in combination with a laser wavelength of 266 nm. Again, the energy of two photons (266 nm) is less than the nicotinic acid IP of 9.38 eV\textsuperscript{13}.

The above-mentioned near-UV–MALDI experiments at wavelengths in the range 360 – 450 nm were performed using β-indole acrylic acid, sinapic acid, and 4-hydroxy-α-cyanocinnamic acid as matrices. The IPs of these compounds are not known, but as can be seen in Figure 1.1, the energies provided by one or two 450 nm-photons are far below typical ionization potentials of small aromatic molecules of about 7 – 10 eV. Even more extreme is the situation at a wavelength of 532 nm: two photons have only 4.66 eV. From these energy considerations, it is obvious that multi-photon ionization as a possible mechanism in MALDI would require the absorption of at least three photons in one molecule, which is very unlikely with the typical fluences applied in MALDI.
Figure 1.1  Energy of a single (thin line) and two (thick line) photons as a function of wavelength. Dashed lines indicate the 2-photon energies at the laser wavelengths typically employed in UV–MALDI, i.e., 266 nm (frequency quadrupled Nd:YAG laser), 337 nm (nitrogen laser), and 355 nm (frequency tripled Nd:YAG laser). The other wavelengths (450 nm, 532 nm, and 1064 nm) are not typically used in MALDI. 450 nm is the longest laser wavelength reported for successful MALDI matrix ion generation from an UV–MALDI matrix66. A laser wavelength of 532 nm was successfully used in MALDI experiments with rhodamine dyes as matrices, but experiments using 1064 nm irradiation failed67.

Niu and coworkers compared UV- and IR-MALDI mass spectra of proteins that were obtained from the same sample73. In these experiments, sinapic acid and 4-hydroxy-α-cyanocinnamic acid were used as matrices at laser wavelengths of 355 nm and 2.94 μm. The resulting UV- and IR-MALDI spectra of a given protein were found to be strikingly similar73. Assuming matrix IPs of 8 eV, three 355 nm–photons or about twenty 2.94 μm–photons are needed to initiate MPI (a 3 μm–photon has only 0.4 eV), which does not seem to be a likely process. On the basis of these observations, the authors propose that ionization in MALDI takes place through proton–exchange reactions in the intermediate phase between solid and gas73. They further suggest that the protons derive from dissociation of the acidic matrix, and that the driving force for ionization is the relatively high proton affinity of the
protein molecules\textsuperscript{73}. However, they do not present any estimations on the energetics of such ground state proton transfer mechanisms.

Whereas typical irradiances used in MALDI are unfavorable for direct multi-photon absorption, an energy pooling mechanism is more likely\textsuperscript{13}. Multi-center models such as energy pooling are attractive because strong interactions between chromophores in solids are common, and because matrix clusters are known to be liberated from MALDI samples\textsuperscript{13}. If neighboring matrix molecules absorb one photon each, the energy can be pooled and become available for, e.g., matrix radical cation formation, which can be followed by further reactions\textsuperscript{14}. Energy pooling could also yield highly excited matrix molecules, which can react to form analyte ions by charge transfer reactions\textsuperscript{13}. Karbach and Knochenmuss showed for a 2,5-dihydroxybenzoic acid matrix using a photo/thermal model that even very modest energy pooling rates can lead to substantial ion production, much in excess of the ion yield without energy pooling\textsuperscript{84}.

Excited-state proton transfer (ESPT) reactions have also been proposed as MALDI ionization mechanisms\textsuperscript{80, 81}. Small organic molecules such as aromatic amines and phenols often exhibit an increased acidity upon electronic excitation when compared to their ground-state properties\textsuperscript{80}. Because many MALDI matrices are aromatic amines or can be viewed as substituted phenols, it was suggested that excited-state proton transfer occurs between acidic excited-state matrix and basic ground-state analyte molecules\textsuperscript{80}. Salicylates are known to be ESPT-active in solution, and salicylic acid was found to undergo intramolecular excited-state proton transfer in the gas phase\textsuperscript{13}. Despite its resemblance, the 5-hydroxy derivative of salicylic acid, 2,5-dihydroxybenzoic acid, does not undergo ESPT in solution, gas-phase, or cluster environments\textsuperscript{13}. Zenobi and Knochenmuss discuss the possible role of ESPT as a mechanism for MALDI ion formation in detail\textsuperscript{13}. They conclude that ESPT without intra- or intermolecular assistance is too costly for a single near-UV photon. However, too little is known to date about the local environment in a MALDI plume to evaluate an ESPT mechanism\textsuperscript{13}. A major argument against ESPT is that IR-MALDI performs as well as UV-MALDI\textsuperscript{73}; electronic excitation seems unlikely at
wavelengths as long as 10.6 \mu m, corresponding to a photon energy of only 120 meV\(^6\).

Proton transfer reactions not involving excited states are conceivable as well\(^{41, 85, 86}\). Gas-phase ground-state proton transfer reactions between neutral molecules are generally endoergic reactions at room temperature (300 K)\(^{87}\), but might proceed to a certain extent in the MALDI plume at elevated temperatures. Because the number of neutrals ejected in a MALDI plume is so much higher than the number of ions (by a factor of 10,000 or higher\(^2\)), it is imaginable that reactants in the high-energy tail of a Boltzmann energy distribution have sufficient energy to yield ionic products even of highly endoergic reactions. Figure 1.2 shows the fraction of reactants with Boltzmann energy distributions that have energies higher than a given energy \(E_0\) as a function of temperature. These curves were calculated from Eq. (1.1) for reaction endoergicities \(E_0 = 1\) eV, 2 eV, 3 eV, 4 eV, and 5 eV, respectively. Here, \(E\) is the energy, \(N(E > E_0)\) the number of reactants with energies \(E > E_0\), \(N_{\text{total}}\) the total number of reactants, \(T\) the temperature, and \(k\) the Boltzmann constant.

\[
\frac{N(E > E_0)}{N_{\text{total}}} = \frac{\int_{E_0}^{\infty} \exp(-E/k \cdot T)dE}{\int_{0}^{\infty} \exp(-E/k \cdot T)dE} = \exp\left(\frac{-E_0}{k \cdot T}\right)
\]  

From Figure 1.2, it can be seen that a reaction that is endoergic by 1 eV yields an ion/neutral ratio of \(10^{-4}\) at a temperature of 1260 K. The higher the temperature, the larger the fraction of reactants that yield products at a given endoergicity. With increasing endoergicity and at a given temperature, the reactive fraction becomes smaller. Put another way, the temperature required to yield a given fraction of reactive species increases with increasing endoergicity. For example, if the reaction is endoergic by 3 eV, 4 eV, or 5 eV, temperatures of 3780 K, 5040 K, or 6300 K are required for an ion/neutral ratio of \(10^{-4}\), respectively. Even lower temperatures are required for a smaller ion/neutral ratio of \(10^{-6}\): for reaction endoergicities of 3 eV, 4 eV, or 5 eV, temperatures of 2520 K, 3360 K, or 4200 K are required, respectively.
Figure 1.2 Fraction of reactants from Boltzmann energy distributions that have energies higher than 1 eV, 2 eV, 3 eV, 4 eV, and 5 eV, respectively, as a function of temperature. The dashed line indicates an ion/neutral ratio of $10^{-4}$, as is typical for UV-MALDI.

The temperature of the ejected material in MALDI is not known, and it is unclear if equilibrium conditions are established in the fast expanding plume. Mowry and Johnston probed the internal energy of neutral analyte molecules ejected in the MALDI process by photoionization with coherent vacuum-ultraviolet radiation\textsuperscript{88}. At irradiances near the threshold for desorption, the analyte neutrals contained between 0.6 eV and 0.9 eV internal energy, corresponding to temperatures between 440 K and 520 K. These temperatures were found to be comparable to the sublimation temperatures of the solid MALDI matrices used. Moreover, the analyte internal energy was found to increase with increasing desorption laser irradiance\textsuperscript{88}. However, these values reflect final rather than initial internal energies of neutral analyte molecules, and initial matrix temperatures might be much higher.

Allwood, Dyer, and Dreyfus modelled the excited-state thermoionic emission in a MALDI plume\textsuperscript{89}. Their calculations imply the equilibration of internal energy amongst the vibrational states and suggest matrix temperatures of up to 3000 K at typical fluences applied in UV–MALDI\textsuperscript{89}.
Vertes and coworkers used molecular dynamics to model the MALDI desorption process, and found that temperatures between 1500 K and 3000 K are required for analyte desorption\textsuperscript{90}.

Ehring and Sundqvist studied the UV–MALDI process by means of luminescence spectroscopy\textsuperscript{91, 92}. They found that for 2,5-dihydroxybenzoic acid and using 355 nm irradiation, the luminescence intensity depended less than linearly on the laser fluence in the range 20 to 1000 J/m\textsuperscript{2}, indicating quenching processes. With their experimental setup, the ion threshold was 160 J/m\textsuperscript{2} for 2,5-dihydroxybenzoic acid. Quenching was observed with all matrix molecules investigated, and the onset for quenching was below the ion threshold, between 10 to 40 J/m\textsuperscript{2}. The authors point out that the major relaxation pathway for all compounds tested is internal conversion of the electronic energy into vibrational states of the molecules and the lattice\textsuperscript{91}. Furthermore, several of their results such as large Stokes shifts and band broadening support the hypothesis that the observed luminescence originates from excimers, e.g. dimeric aggregates in their excited states\textsuperscript{91}.

These results support the hypothesis that the absorbed photon energy converts into internal (thermal) energy and becomes available for ground-state proton-transfer reactions. A potential primary ion formation mechanism proceeds via absorption of two photons in two neighboring matrix molecules forming a dimer species and energy relaxation via internal conversion, followed by a ground-state proton transfer reaction between the matrix dimer and another molecule. As will be shown in section 4, energetic considerations strongly support this mechanism.

The temperatures discussed above can also be used to estimate the contribution of thermal ionization in MALDI. A thermal ionization reaction is effectively an electron transfer reaction between two matrix molecules, and the required energy is given by the difference in ionization potential and electron affinity of the matrix. Zenobi and Knochenmuss estimated the electron affinities of matrix molecules to be quite low, about 1 eV\textsuperscript{13}. Together with matrix ionization potentials of about 8 eV, electron disproportionation reactions would then cost
roughly 7 eV, corresponding to temperatures of approximately 9000 K for ion/neutral yields of $10^{-4}$. These temperatures far exceed those of the model calculations, suggesting that thermal ionization is not a dominant ion formation mechanism in MALDI.

On the other hand, because electronic excitation does not generally take place in two-phase MALDI, ion formation here is assumed to proceed via thermal mechanisms\(^{13}\). Schürenberg estimated that peak temperatures of up to 10 000 K can be reached in two-phase MALDI\(^{53}\). As calculated above, these temperatures are sufficiently high to yield typical ion/neutral ratios of about $10^{-4}$, and thus thermal ionization can be considered a likely primary ion formation mechanism in two-phase MALDI.

1.1.5. Secondary ion formation

Secondary ion formation in MALDI is, at least in the case of proteins and oligonucleotides, believed to occur primarily via proton transfer reactions between matrix ions and neutral analyte molecules\(^{14, 57, 85, 86, 93-95}\). Moreover, Limbach and coworkers demonstrated that for analytes with low ionization potential such as ferrocene derivatives, electron transfer to matrix radical cations can also be a pathway of secondary ion formation in MALDI\(^{96}\).

<table>
<thead>
<tr>
<th>MECHANISM</th>
<th>REACTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>ground-state proton transfer</td>
<td>$[m + H]^+ + A \xrightleftharpoons{\Delta G} m + [A + H]^+$</td>
</tr>
<tr>
<td></td>
<td>$[m - H]^- + A \xrightleftharpoons{\Delta G} m + [A - H]^-$</td>
</tr>
<tr>
<td>ground-state electron transfer</td>
<td>$m^{++} + A \xrightleftharpoons{\Delta G} m + A^{++}$</td>
</tr>
</tbody>
</table>

Table 1.2 Possible MALDI secondary ion formation mechanisms.

All ionization mechanisms discussed above involve charge transfer in the gas phase. For compounds that are intrinsically ionic in character, the liberation of preformed ions upon laser irradiation might make a major contribution to the total ion yield\(^{13, 97}\).
1.1.6. Charge separation

No matter how primary ions are formed, the resulting complementary charge pairs ([m−H]+[m+H]+, m∗−m∗+m∗+e− etc.) finally need to be separated in the gas phase. Zenobi and Knochenmuss discussed the cost of overcoming Coulomb forces in MALDI, and conclude that solvation effects and energetic collisions in the fast expanding plume bring about charge separation. Depending on the initial distance between the charges, the Coulomb energy in vacuum can be very significant compared to the photon energies used in MALDI. For example, the energy required to separate a charge pair with an initial charge distance of 1 Å is 14.4 eV, as illustrated in Figure 1.3. With increasing initial distance between the charges to be separated, the required energy rapidly decreases: for an initial distance of 10 Å, the Coulomb energy is only 1.44 eV.

![Graph showing Coulomb energy vs initial distance between charges](image)

Figure 1.3 Coulomb energy (or energy required to separate an ion pair) in vacuum as a function of initial distance between the charges.

Thus, if the charge within the rather complex matrix or analyte ions formed by any of the above-mentioned mechanisms is delocalized, the Coulomb energy can be reduced substantially. For example, charge transfer between two matrix molecules can take place at certain sites, and resonance structures may exist in which charge is located at a different site, as pictured in Figure 1.4. Separation of the ions might then
cost only a relatively small amount of energy that is easily provided by collisions in the plume.

Figure 1.4 Schematics of a charge transfer reaction between two molecules. Separation of the resulting ions can be facilitated by a low Coulomb energy due to charge delocalization within the ion, as it is the case when resonance structures exist.
1.2. Chemical ionization and laser desorption/chemical ionization

Chemical ionization (CI) mass spectrometry, introduced by Munson and Field\textsuperscript{98} in 1966, was the first example of a soft gas-phase ionization method by which the formation of molecular ion species is enhanced relative to the formation of fragment ions. Analytes are ionized by reactions with reagent ions that are generated by electron bombardment of a neutral reagent gas. Typical CI ion sources operate at 0.5–2.0 torr, with the reagent gas present in at least a thousandfold molar excess. This requires pulsed reagent gas introduction or differential pumping in the mass spectrometer. Analyte molecules must be volatile at room temperature or be readily vaporized by heating\textsuperscript{99}. Because of this volatility requirement, the technique is not generally applicable. This limitation was partially lifted by introduction of laser desorption/chemical ionization (LD/CI), first reported by Cotter in 1980\textsuperscript{100}. The laser was used to desorb nonvolatile molecules which then undergo chemical ionization by collisions with conventional reagent ions prior to analysis in a magnetic sector instrument with differential pumping\textsuperscript{100}. In 1989, LD/CI experiments were reported employing a pulsed valve for reagent gas introduction in a Fourier transform mass spectrometer\textsuperscript{101}.

A further simplification of LD/CI that circumvents the external production of reagent ions, high pressure source conditions, and probe heating is co-desorption of reagent ions and analyte neutrals. Preformed reagent ions provided by salts and non-volatile neutral analyte molecules are simultaneously liberated in a single laser desorption event. Because the ion and neutral density is extremely high in the transition from the condensed into the gas phase, it constitutes an ideal situation for efficient charge-transfer reactions.

Most investigations concerning laser desorption/chemical ionization using salts were limited to studies on positive ions desorbed from the solid phase: Wood and Marshall used ammonium bromide for LD/CI of aromatic hydrocarbons which were thought to be protonated by the NH$_4^+$ ion\textsuperscript{102}. However, the efficiency of the proton transfer reaction was fairly poor. Silver nitrate LD/CI by Kahr and Wilkins was used for the
analysis of hydrocarbon polymers and silver-attached oligomer ion species were detected in high abundance. CI of perfluorinated polyethers (PFPE) was performed by Cromwell and coworkers using transition metal ions such as Cu$^+$ and Ni$^+$. In their studies, two pulsed lasers were employed: a low fluence laser for analyte desorption and a high fluence laser for metal ion formation. In general, LD/CI using salts works well if analyte ionization proceeds via transition, alkali, or metal cation attachment, but experiments involving proton transfer are critical.

A new approach for proton transfer LD/CI was developed within the scope of this work. The experimental results are presented in section 3.1. Here, deprotonated analyte molecules are generated in proton transfer reactions with reagent anions. These are provided by concentrated aqueous organic salt solutions which at the same time act as a liquid matrix assisting desorption. The laser energy is absorbed by small, dark particulates suspended therein. This binary matrix produces deprotonated analyte molecules and specific fragment ions, and the degree of fragmentation is controlled by the exothermicity of the deprotonation reaction. A relatively simple experimental setup is required and sample preparation is straightforward.
1.3. Proton transfer thermochemistry

The gas-phase basicity of a base $B$, $GB(B)$, is the negative of the free energy change $\Delta G$ of the protonation reaction (1.2),

$$B + H^+ \rightarrow BH^+$$

usually defined at 298 K. The proton affinity of $B$, $PA(B)$, is the negative of the enthalpy change $\Delta H$ of reaction (1.2). $\Delta G$ and $\Delta H$ are related through Eq. (1.3), where $T$ is the temperature and $\Delta S$ the change in entropy upon protonation.

$$\Delta G = \Delta H - T\Delta S$$

At 300 K and for molecules with masses higher than about 30 Da, the difference in $\Delta G$ and $\Delta H$ is typically about 30 kJ/mol. The major contribution to the entropy term in reaction (1.2), $T\Delta S$, is that of the proton ($\Delta S_{\text{proton}} = 108.95 \text{ J/mol-K}$, $T\Delta S_{\text{proton}}(300 \text{ K}) = 32.7 \text{ kJ/mol}$). More precise entropy values can be estimated from statistical mechanical methods. It is obvious from Eq. (1.3) that the free energy change of reaction (1.2) (and therefore the gas-phase basicity of $B$) is temperature dependent. Typical changes in gas-phase basicity upon temperature changes can be estimated from Eq. (1.3) by assuming typical values for $\Delta H$ and $\Delta S$. For example, if $\Delta H = -1000 \text{ kJ/mol}$ and $\Delta S = -100 \text{ J/mol-K}$, it follows that $T\Delta S = -30 \text{ kJ/mol}$ and $\Delta G(300 \text{ K}) = -970 \text{ kJ/mol}$. With the same enthalpy and entropy values but at 3000 K, the free energy change can be calculated to be $\Delta G(3000 \text{ K}) = -700 \text{ kJ/mol}$. Thus, the gas-phase basicity of $B$ decreases with increasing temperature.

The gas-phase basicities of bases $B_1$ and $B_2$ determine the overall free energy change $\Delta G$ of the proton transfer reaction (1.4):

$$B_1 + B_2H^+ \rightarrow B_1H^+ + B_2$$

for which

$$\Delta G = GB(B_2) - GB(B_1).$$

Reaction (1.4) proceeds towards products if $GB(B_1) > GB(B_2)$. Then, $\Delta G < 0$, and the reaction is exoergic; whereas $\Delta G > 0$ characterizes an
endoergic reaction. Reaction (1.4) can be viewed as a combination of two individual protonation reactions, namely protonation of $B_1$ and protonation of $B_2$ as in (1.2). Because the respective entropy contributions are typically comparable, the overall entropy contribution in reaction (1.4) is negligible, and $\Delta G = \Delta H$. As a consequence, the temperature has only a negligible effect on the proton transfer between $B_1$ and $B_2$.

The equilibrium constant $K_{eq}$ of reaction (1.4) is given by

$$
K_{eq} = \frac{[B_1H^+][B_2]}{[B_1][B_2H^+]} \quad (1.6)
$$

with $[B_1H^+]$, $[B_2H^+]$, $[B_1]$, and $[B_2]$ being the respective ion and neutral concentrations. $K_{eq}$ and $\Delta G$ are related through

$$
\Delta G = -RT \cdot \ln K_{eq} \quad (1.7)
$$

and therefore the equilibrium constant is a direct measure of the free energy change of a reaction.

As empirically observed$^{108}$ and generally accepted since the beginning of the 20th century$^{109}$, most reactions have rate constants that follow the Arrhenius equation$^{110}$:

$$
k = Z \cdot \exp \left( -\frac{G_a}{RT} \right) \quad (1.8)
$$

where $Z$ is a frequency factor, $G_a$ the activation energy, and $R$ the gas constant. For bimolecular gas-phase reactions, the frequency factor equals the collision rate constant.
1.4. Methods for determining thermochemical data

In most studies, relative rather than absolute values for gas-phase basicities and proton affinities are determined\textsuperscript{111}. Absolute values can conclusively be assigned if for at least one species in the relative scale reliable absolute data are available. Techniques that have been used to establish relative gas-phase basicities are the equilibrium\textsuperscript{112}, kinetic\textsuperscript{113}, and bracketing\textsuperscript{114} methods. These have been reviewed and discussed by Harrison\textsuperscript{115}. Witt and Grützmacher determined the gas-phase basicity and proton affinity of propionamide by the above methods and did not find significantly different values\textsuperscript{116}. In general, all methods discussed below can also be used to determine other thermochemical quantities such as electron or metal cation affinities as well as gas-phase basicities.

1.4.1. Equilibrium method

The equilibrium method is often considered the most reliable because equilibrium concentrations are measured that directly yield relative gas-phase basicities from Eqs. (1.6) and (1.7). By measuring the equilibrium constant as a function of temperature, proton affinities can be derived. If equilibrium concentrations cannot be measured directly, it may be possible to monitor the rate constants in the forward and reverse directions instead. Although the majority of gas-phase basicities were determined using the equilibrium method\textsuperscript{115}, it has substantial limitations. First, this method is only applicable to compounds that are sufficiently volatile, which is typically not the case for molecules used in MALDI. Second, large uncertainties in GB values can result because neutral concentrations and the temperature at which reactions occur need to be determined accurately, which is difficult in practice.

1.4.2. Kinetic method

Because proton-bound cluster ions can in many cases be easily prepared by desorption/ionization techniques such as fast atom
bombardment (FAB)\(^{117}\), MALDI or electrospray ionization (ESI)\(^{118}\), the kinetic method is ideal for the study of highly involatile species. With this method, the fragmentation of proton-bound heterodimer ions \([B_1\ldots H\ldots B_2]^+\), consisting of a reference and a test compound, is studied\(^{113}\).

\[
B_1H^+ + B_2 \rightleftharpoons ^{k_1} [B_1\ldots H\ldots B_2]^+ \rightleftharpoons ^{k_2} B_1 + B_2H^+ (1.9)
\]

In order to dissociate the weakly bound cluster species, techniques such as collisional\(^{119}\) or infrared multiple photon (IRMP)\(^{120, 121}\) activation need to be employed. Provided that the ratio of fragment ion signals is equal to the ratio of rate constants for the competing dissociation channels, gas-phase basicities and proton affinities can be derived. In Eq. (1.10), it is furthermore assumed that detection efficiencies for \(B_1H^+\) and \(B_2H^+\) are equal, that there are no reverse activation barriers, that the two channels studied are competitive, that entropy effects can be neglected, and that there are no other cluster decomposition channels involved.

\[
\ln \frac{[B_1H^+]}{[B_2H^+]} = \ln \frac{k_1}{k_2} - \frac{GB(B_1) - GB(B_2)}{RT_{\text{eff}}} = \frac{PA(B_1) - PA(B_2)}{RT_{\text{eff}}} (1.10)
\]

In Eq. (1.10), \(T_{\text{eff}}\) is the effective temperature and an empirical parameter, which was found to show a very good correlation with the mean internal ion energy\(^{122}\). One major advantage of the kinetic method is the large number of thermodynamic quantities that can easily be ascertained. For example, electron affinities, metal cation affinities, and ionization energies have been determined by use of the kinetic method\(^{123}\).

1.4.3. Bracketing method

In the bracketing method\(^{114}\), abrupt changes in reactivity with reference compounds are used to assign gas-phase basicities\(^{115}\). By reacting a test species with a variety of reference substances, its gas-phase basicity can be bracketed. Fast reactions are considered as exothermic and slow reactions are classified as endothermic\(^{115}\). In some studies using a FT–ICR mass spectrometer and pulsed reference gas
introduction, assignment of gas-phase basicities was done on the basis of occurrence or non-occurrence of proton transfer in the bracketing reactions instead of kinetics criteria. Reaction efficiencies (RE) can be used to distinguish fast and slow reactions. These are defined as the ratio of the experimental rate constant $k_{\text{exp}}$ and either the collision rate constant $k_{\text{coll}}$ or a theoretical collision rate constant $k_{\text{ADO}}$ obtained from average dipole orientation (ADO) theory. $RE = 1$ indicates an exoergic reaction ($AG < 0$), and $RE = 0$ an endoergic reaction ($AG > 0$). A RE of unity denotes the situation where every collision results in proton transfer, whereas a RE of zero means that independent of the number of collisions, proton transfer will not proceed. In near-thermoneutral reactions, reaction efficiencies between zero and unity are often found. There are two reasons that account for this: one is the non-zero temperature at which experiments are performed, and the other is activation energies.

Energy distributions of thermalized reactants at a given (non-zero) temperature can be described by Boltzmann statistics. Reactants in the high-energy tail of the distribution may have sufficient energy to react even if $AG > 0$, yielding relatively small but non-zero reaction efficiencies, as schematically illustrated in Figure 1.5.

![Figure 1.5](image)

Figure 1.5 Schematic energy diagram of a near-thermoneutral endoergic proton transfer reaction $X + YH^+ \rightarrow XH^+ + Y$. The major fraction of reactants (shaded light grey) does not yield products. Reactants in the high-energy tail of the Boltzmann distribution (shaded dark grey) have sufficient energy to react.

This implies that experiments should be performed at low temperature in order to narrow the Boltzmann distribution and minimize the fraction
of reactive species. However, at the typical experimental temperature of 300 K, the width of the distribution is already relatively small when compared with typical spacings between bracketing bases (~ 10 kJ/mol). From Eq. (1.1), it can be calculated that only 2.1% of the reactants have more than 10 kJ/mol internal energy at 300 K. This fraction can undergo proton transfer in a reaction that is endoergic by 10 kJ/mol, yielding a reaction efficiency of only 0.021. Thus, at an experiment temperature of 300 K, the contribution of thermal reactivity to the reaction efficiency is small.

Activation energies, on the other hand, can substantially decrease reaction efficiencies. Then, even if \( \Delta G < 0 \), reaction efficiencies much smaller than unity are obtained, meaning that a number of collisions are required for proton transfer to occur. One physical explanation for the latter effect is steric hindrance\(^{127}\); if protonation or deprotonation sites are not accessible from all directions, only a fractional number of collisions will yield reaction products. Thus, with increasing steric hindrance and activation energy, the number of collisions required for proton transfer to occur increases.

Values for \( k_{\text{exp}} \), \( k_{\text{coll}} \) and \( k_{\text{ADO}} \) can only be determined with relatively low precision. For example, experimental rate constants were reproducible to no better than 25% (relative standard deviation) in a study carried out by McKieman and coworkers\(^{128}\). Together with the fact that collision rate constants also can only be determined with limited accuracy, relatively large errors for the reaction efficiencies can result. This is reflected in the calculated reaction efficiencies, which then can have unphysical values significantly larger than unity (for example 1.78 was found by Wu and Lebrilla\(^{129}\)). The arbitrary criterion used to decide whether a reaction was considered exoergic or endoergic was if the reaction efficiency was larger or smaller than either 0.5\(^{129}\) or 0.1\(^{116}, 128\). Assigning a bracketing point on the basis of reaction efficiencies is therefore difficult when reaction efficiencies can only be determined with such low precision.

A fundamental problem with the bracketing method is that reaction rates are measured in order to determine a quantity, GB, that is defined under equilibrium conditions. Two new and different bracketing-related
approaches have been developed within the scope of this work that account for this difficulty. These are presented in the following sections 1.4.4. and 1.4.5.. The first approach ("equilibrium bracketing") is based on equilibrium theory, and the second ("bracketing by reactivity/ergodicity correlation", BREC) is based on structure-reactivity correlations.85, 86.

1.4.4. Equilibrium bracketing

With this approach, GB values can be deduced from relative ion abundances rather than from kinetics, provided that the abundances are probed after a sufficiently long reaction delay so that near-equilibrium conditions are established.85 Figure 1.6 illustrates such a case where near-equilibrium conditions were established in the FT-ICR cell after about 7 seconds reaction time.

![Kinetic plot of the reaction between cyanate (CNO⁻) and para-nitroaniline (PNA). Besides deprotonated PNA, [PNA+CNO]⁻ adduct ions are formed. Near-equilibrium conditions were established after about 7 seconds reaction time. The solid lines are exponential fit functions.](image)

The kinetic plot shows the relative intensities of the reference anion CNO⁻, deprotonated para-nitroaniline (PNA)⁻, and (PNA + CNO)⁻ complex ions, respectively. After relatively rapid changes during the first 7 seconds, the normalized ion abundances become almost constant.
The observed near-equilibrium conditions justify a theoretical treatment based on equilibrium and transition state theory as derived below. The equilibrium bracketing method avoids the assignment of a 'bracketing point' between two reference bases, which is difficult when near-thermoneutral reactions occur, but takes into account all bracketing results. Applying this method for a series of reference bases and fitting the complete data set yields reliable GB values, as shown in section 3.2.

In this work, gas-phase basicities of anions were determined using the equilibrium bracketing method by reacting reference anions with the neutral molecules under study. Therefore, the following theoretical treatment is formulated in terms of neutral molecules and reference anions. Reverse reactions and reactions involving proton transfer in positive polarity can be treated analogously.

Neutral molecules (M) were allowed to undergo reactions with reference bases (R⁻) of known gas-phase basicities. For the reaction

\[ R^- + M \leftrightarrow (M-H)^- + RH \]  (1.11)

the equilibrium constant \( K_{eq} \) is given by

\[ K_{eq} = \frac{[\text{Rh}] [R^-]}{[\text{M}] [R^-][\text{M}]} \]  (1.12)

By stoichiometry, the concentration of deprotonated molecules, (M - H)⁻, is equal to the concentration of protonated reagent ions RH at any stage of the reaction, including equilibrium. If the total ion intensities in each spectrum are normalized, the equilibrium constant for reaction (1.11) can be written as:

\[ K_{eq} = \frac{x^2}{(1-x) \cdot M_0} \]  (1.13)

where \( x \) is the normalized (M - H)⁻ concentration,

\[ x = \frac{[\text{M}^- - \text{H}^-]}{[\text{M}^- - \text{H}^-] + [R^-]} \]  (1.14)
and $M_0$ stands for the relative concentration of neutral molecules.

$$M_0 = \frac{[M]}{[M - H] + [R^-]} \quad (1.15)$$

Under equilibrium conditions, the equilibrium constant and the free energy change of reaction (1.11) are related through

$$\Delta G = -RT \cdot \ln K_{eq} \quad \text{or} \quad K_{eq} = \exp\left(\frac{-\Delta G}{RT}\right) \quad (1.16)$$

The free energy change of reaction (1.11) is given by the difference in gas–phase basicities of product and educt ions:

$$\Delta G = GB((M - H)^-) - GB(R^-) \quad (1.17)$$

Because ion intensities have been normalized, the value of $x$ will be between 0 and 1, $x \in [0,1]$, and it follows that $K_{eq} \in [0,\infty]$. This makes $K_{eq}$ an impractical parameter for data visualization, so the experimental data can instead be plotted as $R_k$ values defined as follows:

$$R_k = \frac{1}{1 + M_0 \cdot K_{eq}} = \left[1 + \frac{x^2}{1-x}\right]^{-1} \quad (1.18)$$

This has the advantageous properties that if $x$ approaches 0, the $R_k$ value will approach 1 and if $x$ approaches 1, $R_k$ will approach 0. The $R_k = 1$ value corresponds to no product ion yield, and the $R_k = 0$ value corresponds to 100% product ion yield. Using the expression for $K_{eq}$ in Eq. (1.16), and expanding $\Delta G$ as in Eq. (1.17), Eq. (1.18) gives the fit function for deriving $GB((M - H)^-)$ from $R_k$ for a series of test bases:

$$R_k[GB(R^-)] = \left[1 + M_0 \exp\left(-\frac{GB((M - H)^-) - GB(R^-)}{RT}\right)\right]^{-1} \quad (1.19)$$
1.4.5. Bracketing by reactivity/ergodicity correlation (BREC)

A more general approach for determining gas-phase basicities, "bracketing by reactivity/ergodicity correlation" (BREC), was also developed as part of this work. It is based on structure-reactivity correlations and can be applied even if near-equilibrium conditions are not established, as for example, in cases where reaction products are unstable. Here, gas-phase basicities are derived from reaction efficiencies, and the data treatment accounts for activation barriers in the bracketing reactions. For the purpose of determining reaction efficiencies, two sets of reactions are monitored: one set for probing the reactivity of the ion or molecule under study (B₁) with unknown gas-phase basicity, GB(B₁), and a second set for probing the reference compound partial pressure employing a base B₂ of known gas-phase basicity, GB(B₂):

\[
\begin{align*}
B₁ + R \rightarrow & \text{products} \\
B₂ + R \rightarrow & \text{products}
\end{align*}
\]

where \(k₁\) and \(k₂\) are the respective reaction rate constants. For a given reference compound partial pressure \([R]\), the integrated rate laws are:

\[
\begin{align*}
\ln \left( \frac{[B₁]}{[B₁]₀} \right) &= -k₁ \cdot t \cdot [R] \\
\ln \left( \frac{[B₂]}{[B₂]₀} \right) &= -k₂ \cdot t \cdot [R]
\end{align*}
\]

with \([B₁]₀\) and \([B₂]₀\) being the initial ion concentrations. Provided that no loss of ions occurs during the experiments, initial ion concentrations equal total ion concentrations at any stage of the respective reaction. Therefore, the measured \(B₁\) and \(B₂\) concentrations can be normalized to the initial ion concentrations:

\[
\begin{align*}
[B₁]ₙ &= \frac{[B₁]}{[B₁]₀} \\
[B₂]ₙ &= \frac{[B₂]}{[B₂]₀}
\end{align*}
\]

Combining (1.22) and (1.23), the rate laws can be written as:

\[
\begin{align*}
\ln[B₁]ₙ &= -k₁ \cdot t \cdot [R] \\
\ln[B₂]ₙ &= -k₂ \cdot t \cdot [R]
\end{align*}
\]

If both reactions are probed at \(t = tₓ\), then it follows that
If reaction (1.21) is exothermic and every collision results in proton transfer, then $k_2$ equals the collision rate constant $k_{\text{coll}}$, and the efficiency of reaction (1.20), $RE$, can be expressed in terms of normalized concentrations of $B_1$ and $B_2$:

$$RE = \frac{k_1}{k_{\text{coll}}} = \frac{\ln[B_1]_n}{\ln[B_2]_n}$$

(1.26)

This means that, instead of determining reaction rates, $RE$ can be simply determined by measuring the relative abundances of $B_1$ and $B_2$ at any arbitrary reaction time $t_x$. In practice, the reactions (1.20) and (1.21) were probed immediately one after the other in separate experiments. Probing relative abundances instead of determining reaction rates has the advantage that the reference compound partial pressure $[R]$ only needs to be constant on the relatively short timescale of the two experiments involving $B_1$ and $B_2$, but not on a long timescale as needed for rate determination.

The rather simple treatment leading to Eq. (1.26) requires that at a given partial pressure $[R]$, $B_1$ and $B_2$ undergo the same number of collisions in a given volume and time interval. However, if bases $B_1$ and $B_2$ have different masses, they will have different thermal velocities. As a consequence, the collision rate constants for reactions (1.20) and (1.21) will be different. In order to account for this, a correction factor derived from average dipole orientation (ADO) theory was introduced. In the ADO model, the collision rate constant in an ion/molecule reaction is given by

$$k_{\text{coll}} = \frac{2 \cdot \pi \cdot q}{\sqrt{\mu}} \cdot \left( \sqrt{\alpha + c \cdot \mu_D} \cdot \sqrt{\frac{2}{\pi \cdot k \cdot T}} \right)$$

(1.27)

where $q$ is the ionic charge, $\mu$ the reduced mass of ion and neutral, $k$ the Boltzmann constant and $T$ the temperature. All other parameters in Eq. (1.27) refer to the neutral molecule only, i.e. its polarizability $\alpha$, its dipole moment $\mu_D$, and the parameter $c$ which compensates for the effectiveness of “charge locking” in the dipole. If reaction (1.20)
occurs between collision partners with reduced mass \( \mu_1 \), and reaction (1.21) occurs between collision partners with reduced mass \( \mu_2 \), then the ratio of collision rate constants can be expressed in terms of their reduced masses. The expression in brackets in Eq. (1.27) cancels out in the rate constant ratio because here in both reactions the ions collide with the same neutral species.

\[
\frac{k_{\text{coll}}}{k_{\text{coll}2}} = \sqrt{\frac{\mu_2}{\mu_1}} \tag{1.28}
\]

Assuming that reaction (1.21) is highly exoergic, then \( k_2 \) equals \( k_{\text{coll}2} \), but what is needed to know is \( k_{\text{coll}1} \). From Eq. (1.28), \( k_{\text{coll}1} \) can be calculated, and the reaction efficiencies obtained from relative ion abundances can be expressed as in Eq. (1.29):

\[
RE = \frac{k_i}{k_{\text{coll}i}} = \sqrt{\frac{\mu_1}{\mu_2}} \frac{\ln[B_1]_n}{\ln[B_2]_n} \tag{1.29}
\]

An expression for reaction efficiencies that can be used to derive gas-phase basicities was suggested by Bouchoux and coworkers:\textsuperscript{130-132}

\[
RE = \left( 1 + \exp \left( \frac{\Delta G + \Delta G_a}{RT} \right) \right)^{-1} \tag{1.30}
\]

where \( \Delta G \) is the difference in GB of test and reference compounds, \( R \) the gas constant, \( T \) the temperature and \( \Delta G_a \) accounts for an "intrinsic barrier" separating reactants and products. In reference 130, \( \Delta G_a \) is assumed to be nearly constant and probably small for proton transfer reactions. In bracketing experiments, though, both the GB of the test compound and \( \Delta G_a \) are unknown, and cannot independently be determined from reaction efficiencies using Eq. (1.30). It was also argued by Bouchoux and coworkers that the temperature \( T \) is an "effective temperature" of internally excited ions rather than the temperature at which the experiment is performed. Proton transfer reactions with higher exoergicity should then result in higher effective ion temperatures. However, effective ion temperatures are not correlated with exoergicities in Eq. (1.30).
In reference 130, temperatures of up to 860 K were derived from analyses using Eq. (1.30). Witt and Grützmacher also applied Eq. (1.30) to two sets of bracketing data and found temperatures of 633 K and 859 K\(^1\)\(^1\)\(^6\). When applying Eq. (1.30) to the data reported in this work, temperatures of up to 1400 K were obtained. Besides the above-mentioned fact that an uniform internal ion temperature cannot be expected from a series of reactions with differing exoergicities, these temperatures seem to be too high to be reasonable.

To avoid the above problems, a different approach to analyzing RE data and derive GB values was used in this work. Instead of correlating reaction rates directly with \(\Delta G\), rates are expressed in terms of activation energies \(G_a\). \(\Delta G\) is a purely thermochemical parameter, whereas \(G_a\) is a purely kinetic parameter and a measure of reactivity. By use of structure-reactivity correlations, \(G_a\) can then be correlated with \(\Delta G\), e.g. for a given series of reactions, \(G_a\) can be expressed as a function of \(\Delta G\)\(^1\)\(^3\)\(^3\). A well-known case in structure-reactivity correlations is frequently observed for slow reactions over a relatively small \(\Delta G\)-range: the so-called linear free energy relations (LFER)\(^1\)\(^0\)\(^8\), in which \(G_a\) is assumed to be linearly related to \(\Delta G\). The LFER approach, applied in both solution and gas-phase chemistry, is based on the idea that as a reaction becomes thermodynamically more favorable, its activation energy linearly decreases. However, it was shown that the LFER model is not in agreement with experimental data when reactions are fast and/or the \(\Delta G\)-range is broad\(^1\)\(^3\)\(^3\). This makes the LFER approach unsuitable for a bracketing experiment, because fast reactions certainly occur in a bracketing series, and the range of reference basicities is chosen to be as large as possible.

A correlation of activation energies, \(G_a\), and free energy changes, \(\Delta G\), in concerted reactions that entirely describes experimental data over a broad \(\Delta G\)-range (covering fast and slow reactions) was suggested by Agmon and Levine\(^1\)\(^3\)\(^3\), \(^1\)\(^3\)\(^4\). This correlation was taken to be appropriate for the conditions in a bracketing experiment:

\[
G_a = \Delta G - \lambda \cdot \ln \frac{1}{1 + \exp(-\Delta G / \lambda)}
\]  

(1.31)
The independent parameter \( \lambda \) is positive and constant for a given reaction series. Lambda is related to the "intrinsic barrier" of a reaction series, that is the activation energy at thermoneutrality\(^{133}\):

\[
G^0_a = G_a(\Delta G = 0) = \lambda \cdot \ln 2 \tag{1.32}
\]

For gas–phase proton transfer reactions, values for \( \lambda \) between 14.5 kJ/mol and 19.3 kJ/mol have been reported\(^{133}\). The latter values were reported for the gas–phase reactions of the type \( D^- + HX \rightarrow DH + X^- \) with \( HX \) being \( CH_3OH, H_2O, HCl, C_2H_2, H_2S, CCl_3H, H_2, NH_3, \) and \( ND_3 \)\(^{135}\), and for proton transfer reactions from ketones\(^{136}\), respectively. From reactions of the type \( X^- + CH_3CN \rightarrow XH + CH_3CN^- \) with \( XH \) being \( H_2, DH, NH_3, H_2O, C_2H_2, \) and \( CH_3OH \)\(^{137}\), and for anionic proton transfer reactions involving amines\(^{138}\), \( \lambda \)-values of 15.7 kJ/mol and 15.1 kJ/mol were derived, respectively.

The intrinsic barrier accounts for the fact that thermoneutral reactions do not always proceed spontaneously and that even highly exoergic reactions can proceed with reduced rates. Characteristics that are known to decrease reaction rates are charge delocalization and steric hindrance\(^{127}\). For example, Dodd and coworkers found reaction efficiencies smaller than unity for gas–phase proton transfer reactions between alkoxide anions and neutral alcohols with 25 kJ/mol exothermicity\(^{127}\), and attributed this to the presence of substantial intrinsic barriers.

A relationship between reaction efficiencies and intrinsic barriers can be derived as follows. The rate constant \( k \) of a reaction is determined by its activation energy \( G_a \),

\[
G_a = -RT \cdot \ln \left( \frac{k}{Z} \right) \quad \text{or} \quad \frac{k}{Z} = \exp \left( -\frac{G_a}{RT} \right) \tag{1.33}
\]

where \( Z \) is the largest possible rate constant, corresponding to \( G_a = 0 \)\(^{133}\). In gas–phase reactions, \( Z \) equals the collision rate constant. For a base \( B_1 \) reacting with various reference compounds, \( R \), it follows that:
\[
\frac{k_{B_i}}{k_{\text{coll}_i}} = \exp\left( \frac{-G_{a,B_i}}{RT} \right) \tag{1.34}
\]

where \( G_{a,B_i} \) are the activation energies for each reaction:

\[
G_{a,B_i} = \Delta G_i - \lambda_i \cdot \ln \frac{1}{1 + \exp(-\Delta G_i / \lambda_i)} \tag{1.35}
\]

with \( \Delta G_i = GB([R-H]^-) - GB(B_i) \).

Here \( GB(B_i) \) is the GB of the base under study, and \( GB([R-H]^-) \) is the GB of the reference anion.

The reaction for probing the partial pressure of the reference compound is performed with base \( B_2 \):

\[
\frac{k_{B_2}}{k_{\text{coll}_2}} = \exp\left( \frac{-G_{a,B_2}}{RT} \right) \tag{1.37}
\]

The activation energies \( G_{a,B_2} \) are given by:

\[
G_{a,B_2} = \Delta G_2 - \lambda_2 \cdot \ln \frac{1}{1 + \exp(-\Delta G_2 / \lambda_2)} \tag{1.38}
\]

with \( \Delta G_2 = GB([R-H]^-) - GB(B_2) \).

\( GB(B_2) \) is the GB of the base used for probing the partial reference compound pressure. Combining Eqs. (1.28), (1.29), (1.34), and (1.37), a new expression for the reaction efficiencies is obtained:

\[
RE = \sqrt{\mu_1} \cdot \frac{k_{B_1}}{k_{\text{coll}_1}} = \sqrt{\mu_2} \cdot \frac{\ln[B_1]_n}{\ln[B_2]_n} = \exp\left( \frac{G_{a,B_2} - G_{a,B_1}}{RT} \right) \tag{1.40}
\]

Substituting (1.35) and (1.38) for the activation energies, reaction efficiencies can be written as:

\[
RE = \exp\left[ \frac{1}{RT} \left( \Delta G_2 - \Delta G_1 - \lambda_2 \cdot \ln \frac{1}{1 + \exp(-\Delta G_2 / \lambda_2)} + \lambda_1 \cdot \ln \frac{1}{1 + \exp(-\Delta G_1 / \lambda_1)} \right) \right] \tag{1.41}
\]
In Eq. (1.41), $\lambda_1$ and $GB(B_1)$ are independent parameters. Thus, by fitting experimental data with Eq. (1.41), both the gas-phase basicity of the base under study and the corresponding $\lambda$-value can be deduced, from which one can calculate activation energies. This, of course, requires that the reference reaction is well-characterized and that not only $GB(B_2)$, but also $\lambda_2$ is known.

The reaction efficiency in Eqs. (1.40) and (1.41) expresses the reactivity of $B_1$ with respect to the reactivity of $B_2$ and thus is a relative parameter. If the reaction of $B_2$ with $R$ is highly exoergic and proceeds at the collision rate, the RE in Eqs. (1.40) and (1.41) is the classical reaction efficiency. However, the reaction of $B_2$ with $R$ does not generally need to be highly exoergic. In such cases, the RE in Eqs. (1.40) and (1.41) will deviate from the classical reaction efficiency, but will still be a measure of relative reactivity of $B_1$, from which $\lambda_1$ and $GB(B_1)$ can be derived.
1.5. Principles of Fourier transform–ion cyclotron resonance mass spectrometry

A Fourier transform–ion cyclotron resonance (FT–ICR) mass spectrometer is a device for trapping ions and determination of ion mass-to-charge ratios by measuring their characteristic ion cyclotron frequencies.

It provides, in many aspects, superior instrumental performance when compared with other types of mass spectrometers. It can serve as an ion–molecule reactor with the capability of ion selection and isolation. In a purification step, undesired ions can be ejected out of the cell volume either prior to the actual experiment or continuously. For this purpose, stored waveform inverse Fourier transform (SWIFT) or chirp waveforms can be utilized\textsuperscript{139}. After isolation, ions can undergo reactions with selected molecules or unimolecular dissociation. The latter requires ion activation, which can be achieved by collisions with neutrals (collision–induced dissociation, CID)\textsuperscript{119}, surfaces (surface–induced dissociation, SID)\textsuperscript{140, 141}, by electron capture (electron capture dissociation, ECD)\textsuperscript{142, 143}, one–photon absorption (ultraviolet photodissociation, UVPD\textsuperscript{144}, infrared multi–photon dissociation, IRMPD\textsuperscript{120}, blackbody infrared dissociation, BIRD\textsuperscript{121, 145}) or combined techniques\textsuperscript{146}. Unimolecular ion dissociation provides sequence and structural information if analyzed qualitatively, or thermochemical information if analyzed quantitatively as in the kinetic method.

The high mass resolving power and mass accuracy realized in FT–ICR mass spectrometry enable a variety of experimental strategies. Mass resolving power (or mass resolution), MRP, is usually defined as the ratio of ionic mass and the full width of a spectral peak at half maximum (FWHM)\textsuperscript{147}:

\[
\text{MRP} = \frac{m}{\Delta m_{\text{FWHM}}} \quad (1.42)
\]

Because unit mass resolution is routinely achieved up to m/z ~ 10 000\textsuperscript{148}, gas–phase H/D exchange reactions involving small to moderate sized
biomolecules can be monitored, from which structural information is obtained\textsuperscript{149-153}. Moreover, sub-unit mass resolution allows unambiguous mass assignment. For example, ultrahigh resolution MALDI FT–ICR mass spectra with mass resolved $^{34}$S and $^{13}$C\textsubscript{2} species of disulfide–containing peptides were used to determine the number of disulfide bonds\textsuperscript{154}. A mass resolving power of $\approx 1\ 500\ 000$ was reported at m/z 1670 for $\alpha$–melanocyte stimulating hormone ions generated by MALDI\textsuperscript{155}, and 228 000 was reported for laser–desorbed melittin at m/z 2850\textsuperscript{156}.

Mass accuracy is generally high in FT–ICR mass spectrometry; mass measurement errors are between 10 and 50 ppm for external and about 2 ppm with internal calibration\textsuperscript{157}. Taking advantage of the high mass accuracy, endgroups of synthetic homopolymers can be identified by correlating the measured component mass with the degree of polymerization\textsuperscript{158}.

For a typical instrument and typical operating parameters, the detection limit in FT–ICR can be calculated to be $\sim 187$ charges for an observed signal–to–noise ratio of 3:1\textsuperscript{159}. Experimentally, the detection limit was found in one study to be $\sim 177$ charges\textsuperscript{160}. In this respect, the sensitivity of FT–ICR detection is smaller than conventional detection in mass spectrometry using electron multipliers, which are capable of detecting single charges\textsuperscript{161}. However, because ion detection in FT–ICR mass spectrometry is non–destructive, a given ion population can undergo multiple measurement sequences\textsuperscript{162-164}, thereby greatly enhancing sensitivity. For example, it was shown that peptide ions from a single laser shot can be subjected to multistage mass analysis by subsequent measurement cycles consisting of isolation, dissociation, and detection steps\textsuperscript{165}.

The maximum number of charges that can be detected in a FT–ICR mass spectrometer was reported to be $\sim 5.6 \times 10^{6}$\textsuperscript{160}. From that number and the reported detection limit, the dynamic range of a FT–ICR mass spectrometer is $\sim 3 \times 10^{4}$. This relatively small dynamic range is one of the few limitations of FT–ICR, but can partly be overcome by ejecting abundant ions before detection.
The principle of ion trapping in FT-ICR mass spectrometry is based on the ion motion in the presence of a spatially uniform static magnetic field and a non-uniform static electrical field. Figure 1.7 shows the schematics of a cylindrical FT-ICR cell with the magnetic field directed along the z-axis. Small electric potentials are applied to the trapping electrodes, and the other two electrode pairs are used for ion excitation and detection.

Ions with thermal or kinetic velocities experience the Lorentz force. Due to its component arising from the static magnetic field, ion motion is confined to directions perpendicular to the magnetic field lines. Along the magnetic field lines, ions are trapped in an electrostatic potential. Ion motion in a FT-ICR cell to a good approximation can be described by a superposition of three independent ones, namely the trapping oscillation, the cyclotron and the magnetron motions. The overall force acting on the ions is given by Eq. (1.43):

\[
\mathbf{F} = m \frac{d\mathbf{v}}{dt} = q\mathbf{E} + q\mathbf{v} \times \mathbf{B} 
\]

with \( \mathbf{F} \) the force, \( m \) the ionic mass, \( q \) its charge, \( \mathbf{E} \) the electric field, \( \mathbf{B} \) the magnetic field, and \( \mathbf{v} \) the ion velocity. The static voltage \( V_T \) applied to the trapping electrodes generates an approximately quadrupolar electrostatic potential, given by (1.44):
\[ \Phi(x, y, z) = \nabla_T \left( \gamma + \frac{2\alpha}{d^2} (2z^2 - x^2 - y^2) \right) \]  

(1.44)

where \( d \) is the trapping electrode separation, and \( \gamma \) and \( \alpha \) are constants that depend on the trap shape. For cylindrical cells with unit aspect ratio, i.e. for which the cell diameter equals the trapping electrode separation, \( \gamma = 0.2787 \) and \( \alpha = 2.8404^{159} \). Other trap geometries used in FT-ICR mass spectrometry and advances in trap design have been discussed by Vartanian, Anderson, and Laude\(^{165} \), and geometric trap parameters such as \( \gamma \)– and \( \alpha \)–values are listed in a recent review article\(^{159} \). The electric field along the \( z \)-axis can be calculated from Eq. (1.45):

\[ E(z) = -\frac{\partial \Phi(x, y, z)}{\partial z} = -\frac{8\alpha \nabla_T}{d^2} z \]  

(1.45)

The force exerted on an ion with mass \( m \) and charge \( q \) in the \( z \)-direction therefore is

\[ F(z) = m \frac{d^2 z}{dt^2} = q \cdot \frac{8\alpha \nabla_T}{d^2} z \]  

(1.46)

from which the frequency of the trapping oscillation \( \omega_T \) can be derived:

\[ \omega_T = \sqrt{\frac{8q\alpha \nabla_T}{md^2}} \]  

(1.47)

Trapping frequencies typically range from several hundred Hz to a few kHz and can be effectively damped by collisions with neutrals. As the axial oscillation amplitude decreases, the ions become localized in the center of the cell \( (z = 0) \).

Whereas the trapping oscillation arises from the axial component of the electric field, its radial component determines cyclotron and magnetron motion. With \( r^2 = x^2 + y^2 \), the radial electric field is given by Eq. (1.48):

\[ E(r) = -\frac{\partial \Phi(r, z)}{\partial r} = \frac{4\alpha \nabla_T}{d^2} r = E_0 \cdot r, \]  

(1.48)
and the radial forces acting on the ions can be written as:

\[ F(r) = m \frac{d^2 r}{dt^2} = q \cdot \frac{dr}{dt} \cdot \mathbf{B} - q \cdot E_0 \cdot r \]  \hspace{1cm} (1.49)

The radial electric field produces an outward-directed electric force that opposes the inward-directed magnetic force, which is reflected in the opposite signs in Eq. (1.49). Because \( B_0 = |\mathbf{B}| \) and \( E_0 \) are constant, and \( v = \omega \cdot r \), it follows that:

\[ m \cdot \omega^2 \cdot r = q \cdot \omega \cdot r \cdot B_0 - q \cdot E_0 \cdot r \]  \hspace{1cm} (1.50)

In Eq. (1.50), \( r \) cancels from each term, meaning that ICR orbital frequencies are independent of ICR orbital radii in a quadrupolar electrostatic field. Eq. (1.50) can equivalently be written in the form:

\[ \omega^2 - \frac{q \cdot B_0 \cdot \omega}{m} + \frac{q}{m} \cdot E_0 = 0 \]  \hspace{1cm} (1.51)

The two solutions of Eq. (1.51) yield the perturbed cyclotron frequency \( \omega_+ \) and the magnetron frequency \( \omega_- \), respectively:

\[ \omega_+ = \frac{q \cdot B_0 + \sqrt{q^2 \cdot B_0^2 - 4 \cdot q \cdot m \cdot E_0}}{2m} \]  \hspace{1cm} (1.52)

\[ \omega_- = \frac{q \cdot B_0 - \sqrt{q^2 \cdot B_0^2 - 4 \cdot q \cdot m \cdot E_0}}{2m} \]  \hspace{1cm} (1.53)

If the electric field is zero, it follows that \( \omega_- \) is zero, i.e. no magnetron motion occurs, and Eqs. (1.52) or (1.53) yield the unperturbed cyclotron frequency \( \omega_C \):

\[ \omega_C = \frac{q \cdot B_0}{m} \]  \hspace{1cm} (1.54)

Because the radial electric force is typically much weaker than the magnetic force, magnetron frequencies are much smaller than cyclotron frequencies. Typical magnetron frequencies are a few hundred Hz, whereas cyclotron frequencies lie in the kHz and MHz range.
The perturbed and unperturbed cyclotron frequencies usually differ by less than 0.1%.

Combining Eqs. (1.52) and (1.53) with (1.54) gives:

\[ \omega_+ = \frac{\omega_C}{2} \left( 1 + \sqrt{1 - \frac{m}{m_{\text{critical}}}} \right) \] (1.55)

and

\[ \omega_- = \frac{\omega_C}{2} \left( 1 - \sqrt{1 - \frac{m}{m_{\text{critical}}}} \right) \] (1.56)

with

\[ m_{\text{critical}} = \frac{q \cdot B_0^2 \cdot d^2}{16 \cdot \alpha \cdot V_t} \] (1.57)

The perturbed cyclotron and magnetron frequencies in Eqs. (1.55) and (1.56) are real quantities, and therefore \( m/q \) cannot be larger than \( m_{\text{critical}}/q \). For \( m/q \) exceeding this limit, ion motion in the trap is no longer stable, and the ions spiral outward until they are lost from the trap.

In order to monitor a large number of oscillations in a short time, the fast cyclotron motion is typically used for ion detection. The idea of detecting the frequencies of ion motion in a FT-ICR cell is to monitor an image charge induced by the rotating ions at the two opposed detection electrodes. However, the radii of ion cyclotron motion at thermal velocities and 3 Tesla magnetic field strength are generally smaller than one mm and decrease with increasing magnetic field strength\textsuperscript{159}. This is too small for typical cell dimensions and detection electrode spacings that are on the order of several centimeters.

Moreover, the phase of each ion's cyclotron motion is initially random, so that the net charge detected between the two electrodes is zero. Thus, ion packets are coherently excited to larger radii for detection of ion cyclotron motion by applying dipolar rf voltages between the two opposed excitation electrodes. Upon dipolar excitation, ions pick up energy and their cyclotron radii increase with excitation duration \( t_{\text{exc}} \) and magnitude of the oscillating electric field excitation \( E_{\text{exc}} \).
The laboratory frame translational energy $E_{\text{kin,lab}}$ imparted into the ions can be calculated from Eq. (1.59):

$$r = \frac{E_{\text{exc}} \cdot t_{\text{exc}}}{2B_0} \quad (1.58)$$

$$E_{\text{kin,lab}} = \frac{q^2 \cdot E_{\text{exc}}^2 \cdot t_{\text{exc}}^2}{8m} \quad (1.59)$$

$E_{\text{exc}}$ is given by:

$$E_{\text{exc}} = \frac{\beta \cdot U_{\text{pp}}}{d} \quad (1.60)$$

where $\beta$ is a scale factor depending on cell geometry, $U_{\text{pp}}$ is the applied peak-to-peak voltage, and $d$ the detection electrode separation, equal to the trapping electrode separation for cells with unity aspect ratio. For cylindrical cells with unit aspect ratio, the geometrical factor $\beta$ is $0.80818159$. Typical ion radii for detection are between 0.5 and 2.0 cm, and can be optimized for high resolution analysis\textsuperscript{167}. Coupling of cyclotron and trapping motions during dipolar excitation can lead to axial ion ejection\textsuperscript{168}, which can be overcome by special cell designs\textsuperscript{169,170}.

The oscillating image charge induced at the detection electrodes is converted into an AC signal by use of operational amplifiers, followed by further amplification. Corresponding to the harmonic ion cyclotron motion, the recorded time-domain signal is sinusoidal.

Under idealized conditions, this signal can in principle last infinitely long. Because frequencies can be determined better, the longer the transient is monitored, infinitely high frequency-domain resolution could then be obtained. However, cyclotron motion is always of finite duration due to several limiting factors. First, the ion cyclotron motion is damped by inelastic collisions with neutrals or ions\textsuperscript{171}. Second, the coherently oscillating ion packet can undergo dephasing due to elastic ion-neutral collisions\textsuperscript{171}. Both effects depend on the ion-neutral collision frequency and lead to an exponential decrease in signal amplitude with time. This is why mass resolving power in FT-ICR mass
spectrometry decreases with increasing background pressure. Third, loss of phase coherence can occur even in the absence of collisions when ions in a packet have slightly different ICR frequencies, as is the case for closely spaced isotope ions.\textsuperscript{171}

In addition to these damping and dephasing mechanisms, ion leakage out of the cell volume during detection can decrease signal intensity. Axial ion ejection usually occurs prior to detection and should not affect the transient shape. Radial ion ejection occurs if the magnetron radius increases above the cell dimensions, due to ion–neutral collisions. On the other hand, Peurrung and Kouzes argued that under most experimental conditions, magnetron motion does not initiate, but rather prevents ion loss.\textsuperscript{172}

Data reduction in FT–ICR mass spectrometry is performed with computers. Because computers are designed to deal with discrete rather than continuous data, measured data first need to be digitized by use of analog-to-digital converters (ADC). These time-domain digitized data sets need to be Fourier transformed to yield frequency-domain spectra. The frequency domain spectra can then be converted into m/z data sets using a calibration formula, e.g. Eq. (1.52). Computation times for Fourier transformation of typical data sets in FT–ICR mass spectrometry can become prohibitively long.\textsuperscript{171} Fortunately, computation times can be significantly reduced if a fast Fourier transform (FFT) algorithm is employed. This algorithm can only be applied to data sets in which the number of time–domain data points is a power of two.\textsuperscript{171} Thus, typical time–domain data sets in FT–ICR contain 64, 128, 256, 512, or 1024 etc. data points.

According to the Nyquist criterion, the sampling rate should be at least twice the signal frequency. In the case of under-sampling, that is sampling at frequencies smaller than twice the signal frequency, an apparent frequency smaller than the true frequency will result. This effect is known as "aliasing" or "foldover.\textsuperscript{171}

The amplified time–domain signal can be sampled directly or after passing through a mixer/low-pass-filter.\textsuperscript{171} Direct sampling (broadband detection) is suitable for wide-range mass spectral detection, but at
the cost of mass resolution. For example, if an ion with m/z = 50 is trapped in a 4.7 T magnetic field, its cyclotron frequency is 1443 kHz, and the sampling rate should be at least 2886 kHz to avoid foldover. Thus, if the random-access-memory (RAM) for time-domain data storage is 64 K, then the maximum time-domain acquisition period is only 22 ms. The maximum achievable mass resolution in the low-pressure limit (where the acquisition period is smaller than the time-domain exponential damping constant) can in this case be calculated from Eq. (1.61)\textsuperscript{171} to be only \( \sim 26500 \).

\[
\left( \frac{m}{\Delta m} \right)_{\text{max}} = \frac{0.132 \cdot q \cdot B \cdot t}{m} \quad (1.61)
\]

In Eq. (1.61), \( t \) is the data acquisition period. Moreover, it can be shown that in the low-pressure limit, mass resolving power in FT-ICR mass spectrometry varies inversely with m/z\textsuperscript{139}. This is due to the fact that with increasing m/z, the peak width remains relatively constant, but the peaks are closer together, because the ICR frequency varies inversely with m/z\textsuperscript{171}.

Figure 1.8 illustrates the broadband detection mode. The data acquisition rate here was 1454.545 kHz, determining the time-domain acquisition period of 45 ms with the 64 K memory used. The directly sampled transient in Figure 1.8 a) shows two striking characteristics. First, the signal decays exponentially with a relatively short time constant of 31 ms. This can be explained by the relatively high instrument background pressure of 8 \( \cdot 10^{-8} \) mbar and a corresponding large number of ion-neutral collisions during data acquisition. Second, the transient is modulated with a relatively small frequency of about \( 1/(5.65 \text{ ms}) = 177 \) Hz. The frequency spectrum in Figure 1.8 b) aids in understanding the latter effect: two ions with closely spaced ICR frequencies contribute to the time-domain signal, and their ICR frequency difference of 177 Hz agrees well with the observed modulation period. Figure 1.8 c) shows the m/z spectrum obtained after mass calibration of the frequency spectrum. The detected thiophenolate and 2-amino-3-hydroxypyridine anion both have a nominal mass of 109 Da. Despite the relatively high pressure, the mass difference of 0.029 Da is well resolved. The possible mass resolution
Figure 1.8  Broadband detection of the thiophenolate and 2-amino-3-hydroxy-pyridine anions. The C_{12}-isotopes of these ions have masses of 109.0117 Da and 109.0407 Da, respectively, which are routinely resolved in a 4.7 Tesla instrument even at pressures as high as 8·10^{-8} mbar.

a) time-domain signal, b) frequency-domain, and c) m/z spectrum.
calculated from Eq. (1.61) was ~ 25 000, but the actual mass resolution obtained was only about 14 000. This is because Eq. (1.61) applies in the low-pressure limit, but it can be seen in Figure 1.8 a) that the time-domain exponential damping constant is 31 ms and thus smaller than the acquisition time of 45 ms and low-pressure conditions no longer hold.

In contrast to broadband detection where acquisition times are relatively small, much longer data acquisition periods can be realized in narrowband (or heterodyne) detection under otherwise equal conditions, i.e. using the same RAM. In narrowband detection, the time-domain signal is passed through a mixer and a low-pass-filter prior to sampling. The mixer is an operational amplifier that multiplies the analog time-domain ICR signal at frequency $\omega_C$ with a defined time-domain radio-frequency signal at reference frequency $\omega_{ref}$. The resulting signal consists of one component at frequency $\omega_C - \omega_{ref}$ and another one at frequency $\omega_C + \omega_{ref}$, as can be seen from the trigonometric relationship (1.62):

$$\cos(\omega_C \cdot t) \cdot \cos(\omega_{ref} \cdot t) = \frac{1}{2} \cdot \left( \cos(\omega_C + \omega_{ref}) \cdot t + \cos(\omega_C - \omega_{ref}) \cdot t \right) \quad (1.62)$$

After frequency mixing, the signal is filtered such that only the low frequency component is kept. Thus, the true frequency is effectively transformed into a much smaller frequency, allowing for sampling at considerably reduced rates.

Figure 1.9 shows a spectrum of protonated substance P detected in heterodyne mode. The mass resolution is about 400 000. The mass accuracy was better than 15 ppm with external calibration, as shown in Table 1.3.

The time-domain data set yielding the mass spectrum in Figure 1.9 was manipulated by zero-filling and apodization prior to Fourier transformation. The zero-filling procedure is simply adding $n \cdot N$ (n = integer) zeroes after the last data point of the original data set consisting of N data points. Fourier transformation of the new $(n+1) \cdot N$ time-domain data set gives a corresponding increased number of
frequency-domain data points by keeping the spectral frequency range, thus enhancing spectral resolution.\(^{171}\)

![Graph](image)

Figure 1.9 Narrowband detection FT–ICR mass spectrum of protonated substance P (H-Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH\(_2\)) generated by MALDI (355 nm). The time domain data were zero–filled four times, followed by Blackman-Harris apodization and Fourier transformation (FFT and magnitude calculation). The mass resolution is about 400 000.

<table>
<thead>
<tr>
<th>calculated mass [Da]</th>
<th>observed mass [Da]</th>
<th>deviation [Da]</th>
<th>m/Δm (FWHM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1347.735423</td>
<td>1347.716915</td>
<td>0.018508 (14 ppm)</td>
<td>386246</td>
</tr>
<tr>
<td>1348.738778</td>
<td>1348.721488</td>
<td>0.017290 (13 ppm)</td>
<td>386583</td>
</tr>
<tr>
<td>1349.742133</td>
<td>1349.732687</td>
<td>0.009446 (7 ppm)</td>
<td>445107</td>
</tr>
</tbody>
</table>

Table 1.3 Calculated and observed mass, mass deviation, and mass resolution for the isotope signals of protonated substance P in Figure 1.9.

It can be shown that the zero–filling procedure uses information that is otherwise lost. The frequency-domain data obtained by Fourier transformation of the real time-domain data set are complex numbers that can be represented as the sum of real and imaginary components. A presentation of the real amplitude versus frequency is the absorption spectrum, and the imaginary amplitude versus frequency is the...
dispersion spectrum. In mass spectrometry, the absorption spectrum is usually considered, whilst information contained in the dispersion spectrum is discarded. Zero-filling effectively recovers this information in the adsorption spectrum. Apodization (or windowing) is a procedure for weighting the time-domain data set in order to suppress artifactual wiggles (known as Gibbs oscillations) in the frequency-domain spectrum.

As already mentioned above, ion detection in FT-ICR mass spectrometry is non-destructive, and its sensitivity can be greatly improved by making multiple measurements of the same ions. This requires that ions be forced to move back to the center of the cell after dipolar excitation and detection in a process called axialization.

The most simple approach is to axialize the ions by collisions with background gas. Each collision will reduce the ion kinetic energy, causing a corresponding decrease in cyclotron radius so that the ions return back to the center of their original orbits where the measurement process can be repeated. The time required for this collisional deactivation process decreases with an increase in background gas pressure. As a matter of course, the deactivation time also decreases with a decrease in ion radius during detection and therefore with dipolar excitation power. Remeasurement efficiencies of 98% using collisional relaxation were reported for laser desorbed m/z 2000 ions.

Remeasurement efficiencies in experiments with simple collisional deactivation for axialization of laser desorbed ions are shown in Figure 1.10. Ion species of 2,5-dihydroxybenzoic acid ([DHB]+, [DHB+H]+, [DHB+Na]+, [DHB–H2O+H]+, [2DHB–2H2O+H]+) simultaneously generated in a single laser desorption/ionization event were trapped in the ICR cell. After a variable time delay during which the ions were allowed to collide with neutral background molecules, the induced ion current was detected. The background gas mainly consisted of neutral DHB molecules sublimed from the solid sample, at a pressure of 8.10^-9 mbar as indicated on the pressure meter. The cool/excite/detect sequence was repeated 19 times without application of any further laser shots. The term "normalized intensity" in connection with remeasurement
experiments refers to the normalization to initial ion concentrations, i.e. normalization to the signal intensity detected in the first measurement cycle. The remeasurement efficiency increased with increasing cool delay time, as pictured in Figure 1.10. A remeasurement efficiency of above 80% was achieved for delay times of at least 20 seconds.

![Normalized intensity of laser-desorbed DHB cations](image)

Figure 1.10 Normalized intensity of laser-desorbed DHB cations ([DHB]+, [DHB+H]+, [DHB+Na]+, [DHB−H2O+H]+, and [2DHB−2H2O+H]+) for different cool delays as a function of measurement cycle. The dashed lines are calculated remeasurement efficiencies, $V_{\text{trap}} = 4V$.

These data illustrate a major disadvantage of pure collisional damping, that is, the relatively long time required for efficient cooling when using static background pressures. This time can be significantly reduced by using pulsed cooling gas introduction, which allows for a high number of collisions in a comparatively short time and without degrading FT-ICR performance. However, pulsed gas introduction does not avoid the possibility of undesired charge transfer reactions with background molecules.
Collisions with a buffer gas will tend to equilibrate the kinetic energy of the ion with that of the gas, thus damping ion motion and leading to reduced axial amplitude and cyclotron radius\textsuperscript{173}. In contrast, the magnetron radius increases with decreasing energy, directing the ions out of the cell volume. Because every collision results in increased magnetron radii, ion loss here is not affected by the absolute cooling time, but rather by the total number of collisions. Thus, with simple collisional cooling, loss of ions due to increased magnetron radii cannot be prevented even when employing pulsed gas introduction.

Collision-induced increase in magnetron radius may be overcome by coupling the magnetron motion to the cyclotron motion\textsuperscript{174}. Coupling of radial ion motions in a Penning trap can be achieved by applying a quadrupolar electric field in the $xy$-plane. The radial motion of an ion in the Penning trap can be described as a linear combination of cyclotron motion $\omega_+$ of radius $p_+$ and magnetron motion $\omega_-$ of radius $p_-$\textsuperscript{173}:

\begin{align}
    x &= p_+ \sin(\omega_+ t) + p_- \sin(\omega_- t) \\
    y &= p_+ \cos(\omega_+ t) + p_- \cos(\omega_- t)
\end{align}

In the case of a quadrupolar electric field in the $xy$-plane, ions can pick up energy from the applied field at the three frequencies $2\omega_+$, $2\omega_-$ and $\omega_+ + \omega_-\textsuperscript{175}$. A quadrupolar field at the unperturbed cyclotron frequency $\omega_c = \omega_+ + \omega_-$ couples the magnetron and the cyclotron motion, resulting in a periodical interconversion of both modes\textsuperscript{174}. Upon collisions with neutrals, the ion cyclotron radii, $p_+$, decrease rapidly, whereas ion magnetron radii, $p_-$, increase only slowly with time\textsuperscript{176}. Thus, interconversion of the cyclotron and magnetron motions in the presence of collisions effectively cools and axializes initially off-axis ions. This effect called "quadrupolar excitation/collisional cooling", or simplified "quadrupolar axialization", requires only that the interconversion of magnetron and cyclotron motion be faster than the collision-induced increase of the magnetron radius\textsuperscript{173}.

For quadrupolar axialization experiments, unperturbed cyclotron frequencies $\omega_c$ must be determined by measuring the apparent cyclotron frequency as a function of the voltage applied to the trap.
electrodes ("magnetron shift") and linear interpolation to 0V trapping potential. From Eq. (1.51), it is obvious that the measured cyclotron frequency, $\omega_+\text{,}$ does not depend linearly on $E_0$. However, $\omega_+(E_0)$ (Eq. (1.52)) can be expanded in a Taylor series about $E_0 = 0$, corresponding to $V_T = 0$. Disregarding higher order terms, which is justified because $E_0 q/m$ is typically much smaller than $\omega_+^2/4$, the first two expansions lead to Eq. (1.65). Here, $\omega_+$ depends linearly on $E_0$, and the intercept is $\omega_C$. 

$$
\omega_+(E_0) = \omega_C - \frac{E_0}{B_0}
$$

(1.65)

Because $E_0$ is linearly related to $V_T$, $\omega_C$ can as well be determined by measuring $\omega_+$ as a function of trapping voltage and linear interpolation. In order to avoid frequency shifts and signal broadening due to coulomb interaction, the total number of ions should be kept small in such experiments.

**Figure 1.11** Perturbed cyclotron frequencies of the benzene molecular radical cation as a function of voltage applied to the trapping electrodes. Error bars represent the FWHM of the respective signal. A linear extrapolation to 0V trapping potential yields the unperturbed cyclotron frequency of 921 946 ± 24 Hz.

As an example, Figure 1.11 shows the observed cyclotron frequency of the benzene molecular radical cation as a function of voltage applied
to the trapping electrodes, from which its unperturbed cyclotron frequency in a 4.7 Tesla magnetic field can be extrapolated to be \(921.946 \pm 24\) Hz. The error bars in Figure 1.11 represent the full width at half maximum of each signal obtained in broadband detection mode.

The background pressure in the FT–ICR instrument is often sufficiently high so that the application of an additional cooling gas in quadrupolar excitation/collisional cooling experiments is needless. For instance, most MALDI matrix samples sublime in the vacuum system, yielding partial matrix pressures of about \(10^{-8}\) mbar\(^8\). This partial matrix pressure can then be used as cooling gas, as in the following example.

In Figure 1.12, the effect of the quadrupolar excitation amplitude on the remeasurement efficiency of laser–desorbed 2,5–dihydroxybenzoic acid (DHB) fragment ions, \([\text{DHB–H}_2\text{O}]^+\), is shown. Neutral DHB molecules evaporated from the solid sample (positioned approximately 1 cm from the ICR cell) were used as cooling gas. An optimal quadrupolar excitation amplitude is observed because a too low amplitude fails to axialize the ions and a too high amplitude gives initially off–axis ions so much kinetic energy that they may react or collisionally dissociate upon collisions with neutrals\(^8\).

![Figure 1.12](image)

**Figure 1.12** Signal intensity of the DHB fragment ion \([\text{DHB–H}_2\text{O}]^+\) for different quadrupolar excitation amplitudes as a function of measurement cycle.
Quadrupolar axialization can be used to remeasure a number of ions of differing mass by applying broadband quadrupolar excitation waveforms. It can also serve as a purification technique by selectively remeasuring a single m/z. For this purpose, a single frequency quadrupolar excitation waveform can be employed. The effect of selective remeasurement of a single m/z is illustrated in Figures 1.13 and 1.14.

![Laser desorption spectra of rhodamine 6G](image)

**Figure 1.13** Laser desorption spectra of rhodamine 6G. Initial ion population in the first measurement cycle (top), ion population in the 20th measurement cycle using quadrupolar excitation at 162370 Hz, corresponding to m/z 443 (middle), and ion population in the 20th measurement cycle without quadrupolar excitation (bottom). The three spectra have the same scaling. Peak-to-peak voltage, applied: 475 mV; duration of quadrupolar excitation: 2s; $V_{trap} = 4V$.

The upper trace in Figure 1.13 shows the initial ion population obtained by laser desorption of rhodamine 6G using a laser wavelength of 355 nm. The dominant signal is that of the molecular radical cation (m/z 443). The fragment ion corresponding to loss of ethylene (m/z 415) is also observed. Whereas without quadrupolar excitation both the molecular ion and its fragment ion completely disappear after twenty cycles due to increased magnetron radii and radial loss (see bottom trace), application of a single frequency quadrupolar excite waveform at
162370 Hz – corresponding to m/z 443 – results in efficient axialization and substantial molecular ion recovery (see middle trace). The fragment ion is completely lost after a few remeasurement cycles because the quadrupolar excitation frequency matches only the unperturbed cyclotron frequency of the molecular ion, but not that of the fragment ion, illustrating the mass selectivity of this method.

In Figure 1.14, the normalized signal intensities of the rhodamine 6G ions are depicted as a function of measurement cycle. The remeasurement efficiency for the rhodamine 6G radical cation produced by a single laser desorption event is about 96%. On the other hand, the fragment ion signal after ca. 15 cycles is indistinguishable from the noise. The fact that the remeasurement efficiency for the molecular ion is below 100% is most likely due to axial ion ejection arising from the increased space charge that results from compaction of the ion cloud at the center of the trap as suggested by Marshall and coworkers180.

![Normalized intensities of the rhodamine 6G molecular and fragment ions as a function of measurement cycle. V_trap = 4V, with quadrupolar excitation of the molecular ion at m/z 443. Peak-to-peak voltage, applied: 475 mV, frequency 162370 Hz; duration of the single-frequency quadrupolar excite waveform: 2s.](image)
1.6. Principles of time-of-flight mass spectrometry

Time-of-flight (TOF) mass spectrometry has a number of distinctive features, among them an essentially unlimited mass range, simultaneous detection of all ions, and potentially high sensitivity\textsuperscript{181}. Its major disadvantage lies in the limited mass resolution and accuracy. The principles of TOF mass spectrometry\textsuperscript{181-183} and recent developments\textsuperscript{184-187} have been the subject of numerous publications.

Ions formed in the ionization region of the ion source are accelerated towards the detector by one or a series of static or pulsed electric fields. In the case of a single static extraction field and a linear field-free drift region, ion flight times $t_D$ can be approximated by (1.66):

$$t_D = \frac{D}{\sqrt{2 \cdot q \cdot U}} 
$$

where $D$ is the length of the field-free drift region, $m$ the mass, and $q$ the charge of the ion. $U$ is the voltage applied between ion source and extraction electrode.
2. Experimental

The experiments reported in this work were carried out on both time-of-flight (TOF) and Fourier transform-ion cyclotron resonance (FT-ICR) mass spectrometers.

2.1. The Fourier transform-ion cyclotron resonance mass spectrometer

Bracketing, collision-induced dissociation, and ultrahigh mass resolution experiments were performed utilizing a Fourier transform-ion cyclotron resonance mass spectrometer with a 4.69 Tesla superconducting magnet (Bruker, Fällanden, Switzerland). The laboratory-built vacuum system comprised a closed cylindrical ion cell and a sample transfer device for insertion of solid material. The instrument base pressure was below $10^{-8}$ mbar. Figure 2.1 shows a schematic side view of the cylindrical FT-ICR cell and the metal target positioned approximately 15 mm from the cell.

![Schematic side view of the target supporting solid samples and the FT-ICR cell. The laser beam is guided through holes in the trapping plates and hits the target at a 90° angle with respect to the target surface. Ions generated upon laser desorption move into the cell volume due to their initial desorption velocities. After the ions reach the cell volume, the electric potential on the trap plates is raised and ions are trapped.](image)

The RF electronics and Odyssey data acquisition system were from Finnigan (Finnigan FT/MS, Madison, WI, USA). The ADC converter allowed a maximum data acquisition rate of 8 MHz, corresponding to a
smallest detectable m/z of 17.99 in a 4.69 T magnetic field. With the array processor used, the acquisition of up to 1 M points per scan was possible.

For laser desorption, a Nd:YAG laser (Continuum, Minilite ML-10, Santa Clara, CA, USA) operated at 355 nm was employed. The typical laser irradiance used was $4 \times 10^6$ W/cm² or less. The laser beam was directed onto the metal target through holes in the trapping plates.

The desorbed ions drifted, guided by the magnetic field, away from the target surface and reached the cell volume after several tens of microseconds up to a few hundreds of microseconds. During desorption and transfer, the trapping plate next to the target was kept at ground potential, whereas the other was at trapping potential. After the ions reached the cell volume, the potential on the trapping plate next to the target was raised.

2.2. The time-of-flight mass spectrometer

Time-of-flight experiments were carried out with a home-built linear mass spectrometer ($D = 2$ m). The instrument base pressure was $5 \times 10^{-7}$ mbar with a liquid nitrogen trap. Five minutes after sample introduction, this pressure was reached again. Ions were accelerated by a static potential ($U = 25$ kV) and detected with a micro-channel plate detector. Mass spectra acquired consisted of a sum of typically 50 consecutive single spectra in order to enhance the signal-to-noise ratio. The typical mass resolution (FWHM-definition) in laser desorption experiments was about $m/\Delta m = 220$ and $m/\Delta m = 110$ at mass 350 and 1350, respectively.

Desorption in TOF experiments was performed using a nitrogen laser (337 nm) with a pulse width of 3 ns (Laser Science Inc., VSL–337ND-T, Cambridge, MA, USA). Attenuation of the laser was achieved using glass plates and an adjustable iris. Laser pulse energies were in the range of 15 to 60 µJ.
2.3. Reference ion generation

Reference ions are usually generated by electron bombardment of a volatile gas, which is introduced through a pulsed valve. This has some substantial disadvantages. First, the pressure pulse degrades mass spectral resolution and sensitivity, and very pure gases are required in order to avoid undesired reactions. Second, the electron emitting filament can heat up the ion cell, resulting in temperature gradients and uncertainties, which increases errors in the determination of thermochemical data. Third, competing gas-phase reactions such as clustering can completely suppress the production of the desired ions. Reference anions are also especially difficult to generate under electron impact conditions.

For the generation of a variety of organic and inorganic reference anions, a new, simple and straightforward method has been developed. It avoids any gas load to the vacuum system and the use of a filament.

The reference anions were generated from organic tetrabutylammonium (TBA) salts by means of laser desorption. Hygroscopic TBA salts were chosen as the liquid component of a binary liquid/solid matrix because of their ability to form hydrates that melt around room temperature. Silicon particulates were chosen as the solid component because they do not give rise to any interfering negative ion peaks. Although graphite particulates were shown to be equally effective as energy transfer media, production of intense carbon-cluster anions makes them less useful for the generation of reference anions. Highly concentrated aqueous TBA salt solutions were mixed with silicon particulates which absorb the laser energy. Laser desorption from these vacuum-stable binary matrices yields only the desired reference anions when using low laser fluences between 10 to 50 mJ/cm².

TBA salt solutions were prepared by adding approximately 70% by volume of water to the salt crystals. Subsequently, an approximately equal volume of silicon particulates was added. These mixtures were then mechanically shaken for at least 15 minutes in order to obtain a
homogeneous suspension. A volume of ca. 0.5 µl (in the TOF experiments) or 5 µl (in the FT-ICR experiments) was applied to the sample holder and allowed to dry. As already reported by Dale and co-workers, best results from binary matrices are obtained if a 'flat' appearing surface is prepared. It is therefore necessary that the slurry spreads evenly over the probe prior to evaporation of excess water. The result is a particulate/liquid matrix mixture with dry appearance. If the preparation looks wet, too much of the salt was used and hardly any signals can be obtained. All salts used form hydrates with melting points slightly above room temperature. For example, TBA hydroxide·4H₂O has a melting point of 26°C. The concentrated solutions used were found to be vacuum stable.

To illustrate the performance of this method, Figure 2.2 shows FT-ICR mass spectra using TBA cyanide, TBA acetate, TBA methanesulfonate, TBA hydrogen sulfate, and TBA benzoate matrices, respectively. Intense reference ion signals were obtained in all cases, and no interfering ions were detected.

![Figure 2.2](image)

Figure 2.2 Negative ion FT-ICR mass spectra of TBA cyanide, TBA acetate, TBA methanesulfonate, TBA hydrogen sulfate, and TBA benzoate matrices, respectively (from top to bottom) laser-desorbed from silicon particulates.

Tetrabutylammonium salts (TBA hydroxide 30-hydrate, TBA fluoride trihydrate, TBA hydrogen sulfide, TBA cyanide, TBA acetate, TBA
cyanate, TBA thiophenolate, TBA benzoate, TBA chloride, TBA bromide, TBA nitrate, TBA rhodanide, TBA methanesulfonate, TBA iodide, TBA hydrogen sulfate, TBA trifluoromethanesulfonate, TBA perchlorate) were purchased from Fluka (Buchs, Switzerland). Silicon particulates (325 mesh, corresponding to 45 μm diam.) were purchased from Aldrich (Buchs, Switzerland).

The TBA salt/silicon binary matrices can also be used as a reagent anion source for analyte deprotonation49, as described in section 3.1.. For these experiments, analyte solutions were prepared as $10^{-3} - 10^{-2}$ M solutions in water, methanol, water/methanol, or water/acetonitrile mixtures, and 0.5 μl to 5 μl were applied on top of the 'dry' binary matrix. After evaporation of the solvent, the sample holder was introduced into the vacuum chamber of the mass spectrometer.

Substance P was purchased from Sigma (Buchs, Switzerland). γ-cyclodextrin, adenosine-5'-monophosphate monohydrate and bombesin were purchased from Fluka (Buchs, Switzerland). All chemicals were used without further purification.

2.4. Equilibrium bracketing experiments

In the equilibrium bracketing experiments, gas-phase basicities of deprotonated MALDI matrix molecules were determined by reacting reference anions with neutral matrix molecules in the FT-ICR mass spectrometer. The reference anions were generated as described above, and the matrix molecules were present in the vacuum system due to sublimation from solid samples.

Before starting a series of bracketing experiments, the vacuum system was cleaned with a bakeout procedure. After the system cooled to room temperature (299 K ± 1 K), a solid sample of the MALDI matrix under investigation was inserted into the main chamber. The sublimed material was then allowed to adsorb on the vacuum system and cell walls during a one hour period, resulting in a good wall coverage. For
each reference base, a target carrying both solid matrix material which was placed on the outer rim of the target and a two-phase sample for reference anion generation (placed in the center) was prepared, as schematically shown in Figure 2.3. This target was then positioned about 15 mm from one trapping plate of the cell. The laser was aligned such that only the two-phase sample was irradiated, but not the MALDI matrix under study.

![Figure 2.3 Schematic side view of the metal target used in the equilibrium bracketing experiments. The laser is focused onto the two-phase sample for generation of reference ions. Neutral MALDI matrix molecules sublime from solid crystals placed on the outer rim of the target.](image)

The gas-phase basicities of the reference anions used in the equilibrium bracketing experiments were from the NIST database\textsuperscript{105}, and in the case where more than one value was listed, average values were used, as summarized in Table 2.1. Literature values with standard deviations larger than 20 kJ/mol were disregarded.

Reference ions generated by laser desorption were trapped within a −1V static potential and allowed to axialize for 5 s prior to SWIFT isolation, a variable reaction delay, chirp excitation, and subsequent detection. In a few cases, the cooling delay was reduced to no less than 1 s. This was necessary for highly exothermic reactions (ΔGB > 40 kJ/mol) together with relatively high matrix vapor pressures. These reactions proceeded so quickly that the reference anions would otherwise be reacted away during the cooling period.
In order to exactly determine the relative concentration of neutrals, \( M_0 \), in Eq. (1.19), both the absolute ion and neutral concentrations needed to be measured accurately. The latter was constant for a given matrix, but vapor pressures are not known for these compounds. Employing the electron filament for neutral density determination would increase the temperature of the cell and the metal target supporting the solid matrix, resulting in increased sublimation and an overestimation of the partial matrix pressure compared to the GB measurements with no filament.

The relative matrix concentration \( M_0 \) in Eq. (1.19) was therefore estimated from ion densities and exoergic reaction rates using collision theory. Judging from signal intensities, estimated instrument sensitivity, and lack of space-charge effects, a reasonable value for the number
of ions trapped in the ICR cell was \(10^4\), equivalent to an ion density of \(1.22 \times 10^{11} \text{ L}^{-1}\) in the active cell volume of \(8.2 \times 10^{-8} \text{ L}\). The latter is given by the ICR orbital radius of a \(m/z = 100\) ion at thermal energies and \(4.7 \text{ T}\), the trapping potential of \(-1 \text{ V}\), and the cell dimensions. With the assumed ion density \(\rho = 1.22 \times 10^{11} \text{ L}^{-1}\) and an estimated collisional cross-section \(\sigma\) of \(10^{-18} \text{ m}^2\), \(M_0\) can be calculated from collision theory. For reactions that proceed with unit efficiency, the rate of change in \(R^-\) concentration, \([R^-]\), is given by the collision density \(Z\),

\[
Z = -\frac{d[R^-]}{dt} = \sigma \sqrt{\frac{8kT}{\pi \mu}} \rho \cdot [M] \tag{2.1}
\]

where \([M]\) is the matrix concentration, \(T\) the temperature, \(k\) the Boltzmann constant, and \(\mu\) the reduced mass of reference anion and matrix molecule. Because rates were obtained from normalized reference ion intensities \([R^-]_n\) and the normalization factor was the same as used for normalization of the matrix concentration, \(2.1\) can be written as:

\[
-\frac{d[R^-]_n}{dt} = \sigma \sqrt{\frac{8kT}{\pi \mu}} \rho \cdot M_0, \tag{2.2}
\]

from which \(M_0\)-values can be extracted. The rates were obtained from the kinetics of highly exoergic reactions, where every collision results in proton transfer. For all matrices except for \(para\)-nitroaniline and \(2,5\)-dihydroxybenzoic acid, \(M_0\) values between 1 and 10 were obtained. For \(para\)-nitroaniline, a higher value of 48 was determined, corresponding to the significantly higher vapor pressure of \(para\)-nitroaniline. On the other end of the scale, the \(M_0\) value for \(2,5\)-dihydroxybenzoic acid was found to be 0.65. Partial matrix pressures in the cell volume can then be back-calculated to range from \(3 \times 10^{-9} \text{ mbar}\) to \(2 \times 10^{-7} \text{ mbar}\).

The \(M_0\) values from the kinetic analysis were used in the fitting functions for deriving GB values. As part of the error analysis, two extreme cases were considered. In the first case, a small ion number of \(10^3\) was hypothesized together with a tenfold higher partial matrix pressure than found above, giving a relative matrix concentration of \(100 \cdot M_0\). In the second case, the ion number was assumed to be high \((10^5)\), and the
partial matrix pressure was taken to be an order of magnitude lower, resulting in a relative matrix concentration of 0.01·M₀. These conservative bounds for M₀ span four orders of magnitude and far exceed possible uncertainties resulting from ion–dipole interactions, which can enhance maximum reaction rate constants by at most a factor of 2 to 4. A covariance analysis showed that the matrix GB error derived from the reference GB uncertainty was much smaller than the matrix GB error due to these conservative bounds for M₀.

The MALDI matrices investigated with the equilibrium bracketing method were 3-aminoquinoline, para-aminophenol, 2-amino-3-hydroxypyridine, 4-hydroxybenzoic acid, nicotinic acid, 2,5-dihydroxybenzoic acid, purchased from Fluka (Buchs, Switzerland), and para-nitroaniline, 2-amino-5-nitropyridine, 3-hydroxypicolinic acid, 2,4,6-trihydroxyacetophenone, purchased from Aldrich (Buchs, Switzerland).

2.5. Bracketing by reactivity/ergodicity correlation (BREC) experiments

The BREC method was used to determine the gas-phase basicities of monomeric and dimeric deprotonated ferulic and sinapic acids. As described in section 1.4.5., two sets of reactions were monitored in the BREC experiments. One set was that of base B₁ (with GB(B₁) to be determined), and the other was that of base B₂ (with GB(B₂) known) reacting with the reference compounds.

The reference compounds used in the BREC experiments and the gas-phase basicities of their corresponding anions are listed in Table 2.2. Reference compounds on the low end of the anion basicity scale are sparse, so dimer bracketing was of necessity performed with fewer reference compounds than monomer bracketing.

The reference compounds for the bracketing reactions were sublimed in the vacuum system from a solid sample, which provided a constant partial pressure of the reference compound. However, in many cases
the partial pressure of the reference compounds was too high \( > 10^{-7} \text{ mbar} \) to allow experiments with the solid material. In these instances, the reference material was inserted into the main chamber and allowed to sublime and cover the instrument walls prior to the actual experiment. After this "contamination" period of 10 to 30 minutes, the remaining solid material was removed from the vacuum system. A suitable working pressure was then obtained after a subsequent pumpdown of approximately 1–5 hours. During the entire experiment, the partial pressure of the reference compound continued to drop slowly, by approximately 50% in 1.5 hours. However, the reference compound partial pressure was continuously probed by monitoring products from an exoergic reaction as described in section 1.4.5. The reference compound pressure drop between probing the bracketing reaction and probing the partial pressure was always less than 5.5 % (4.4 % on average). The reactions were monitored over a time period of up to 40 seconds.

<table>
<thead>
<tr>
<th>reference compound</th>
<th>gas-phase basicity of the corresponding anion [kJ/mol]</th>
</tr>
</thead>
<tbody>
<tr>
<td>para-nitroaniline</td>
<td>1407 ± 8.4 (^a)</td>
</tr>
<tr>
<td>2,4,6-trimethylbenzoic acid</td>
<td>1389 ± 8.4 (^a)</td>
</tr>
<tr>
<td>4-hydroxybenzoic acid</td>
<td>1376 ± 8.4 (^a)</td>
</tr>
<tr>
<td>3-hydroxypicolinic acid</td>
<td>1365 ± 11.5 (^b)</td>
</tr>
<tr>
<td>3-nitrobenzoic acid</td>
<td>1347.5 ± 8.4 (^a)</td>
</tr>
<tr>
<td>4-cyanobenzoic acid</td>
<td>1342 ± 8.4 (^a)</td>
</tr>
<tr>
<td>2-hydroxybenzoic acid</td>
<td>1330 ± 8.4 (^a)</td>
</tr>
<tr>
<td>2-chloro-4-nitrophenol</td>
<td>1322 ± 8.4 (^a)</td>
</tr>
<tr>
<td>2,3-dinitrophenol</td>
<td>1295 ± 8.4 (^a)</td>
</tr>
<tr>
<td>2,4-dinitrophenol</td>
<td>1291 ± 8.4 (^a)</td>
</tr>
<tr>
<td>picric acid</td>
<td>1267 ± 8.4 (^a)</td>
</tr>
</tbody>
</table>

Table 2.2  Gas-phase basicities of the reference anions used in the BREC experiments. \(^a\) from reference 111, \(^b\) from reference 85

The reactions of \( \text{B}_1 \) and \( \text{B}_2 \) with the reference compound \( \text{R} \) as in reactions (1.20) and (1.21) were probed immediately one after the other. This was possible because \( \text{B}_1 \) and \( \text{B}_2 \) could be generated by laser
desorption from one and the same sample, as illustrated in Figure 2.4. Here, B$_1$ was deprotonated ferulic acid, and B$_2$ was acetate.

![Figure 2.4](image)

**Figure 2.4** Negative ion FT-ICR mass spectrum of ferulic acid laser-desorbed from a tetrabufylammonium acetate/silicon particulate binary matrix. Intense signals of acetate and [FA-H]$^-$ were detected.

In the matrix monomer bracketing experiments, the reference compound partial pressure was probed by reacting the reference compound with acetate ($G_B = 1429 \pm 8.4 \text{kJ/mol}$). This is a highly exoergic reaction ($\Delta G_B > 40 \text{kJ/mol}$) in all cases, except with para-nitroaniline. Sinapic acid (SA) or ferulic acid (FA) was mixed with tetrabutylammonium acetate (molar ratio approximately 1:2) and dissolved in H$_2$O. Silicon particulates were suspended in the resulting solution to form a binary matrix as described above. From this mixture, intense signals of both acetate and matrix monomer anions were obtained by laser desorption (see Figure 2.4).

Ions were trapped within a −1V static potential. Thermalization of the ions was performed by applying a nitrogen or argon gas pulse. During the 10 second cooling delay, the pressure increased to $5 \times 10^{-5}$ mbar and then dropped to the base pressure. The ion of interest was isolated using SWIFT and/or chirp excitation and allowed to react with the reference compound, as illustrated in Figure 2.5. FA and SA dimer anions were not formed by laser desorption from this binary matrix. However, both monomeric and dimeric SA and FA anions were formed by laser desorption from pure solid SA or FA samples. Therefore, the exoergic monomer reactions were used to probe the reference compound partial pressure in the dimer bracketing experiments. This procedure
allows rapid probing of the reference compound partial pressure without changing the sample or employing other ionization methods.

![Mass spectra diagram](image)

Figure 2.5 Negative ion FT-ICR mass spectra of isolated \([FA-H]^-\) (left) and acetate (right) after 0 s (top) and 8 s (bottom) reaction with the reference compound R (3-nitrobenzoic acid).

The BREC method as applied here was restricted to matrices with vapor pressures significantly lower than that of the reference compounds. These matrices were ferulic acid, sinapic acid, 2-(4-hydroxyphenylazo)-benzoic acid (HABA), alpha-cyano cinnamic acid and 2,4,6-trihydroxyacetophenone. From this rather small selection, only ferulic acid and sinapic acid formed dimer anions which were sufficiently stable on the extended time scale of the FT-ICR experiments. In contrast, 2,4,6-trihydroxyacetophenone (THAP) dimer anions could not be detected with sufficient intensity. In a linear TOF instrument where ions only have to survive in the extraction region for some microseconds, \([M_2-H]^-\) and \([M-H]^-\)-type ions of THAP formed upon laser desorption can be detected with relative intensities of approximately 1:4. Using the FT-ICR instrument with a time scale of several seconds, the relative intensities of dimeric and monomeric ions were only about 1:100. This suggests that the THAP matrix dimer anions generated in the MALDI-process are relatively weakly bound and dissociate upon colliding with neutrals to form neutral matrix molecules and the observed monomer anions.

Sinapic acid, ferulic acid, 2,4,6-trimethylbenzoic acid, 4-hydroxybenzoic acid, 3-nitrobenzoic acid, 4-cyanobenzoic acid, 2-hydroxybenzoic acid, 2,3-dinitrophenol, 2,4-dinitrophenol, and picric
acid were purchased from Fluka (Buchs, Switzerland). 3-hydroxypicolinic acid, \textit{para}-nitroaniline, and 2-chloro-4-nitrophenol were from Aldrich (Buchs, Switzerland).

2.6. Collision–induced dissociation experiments

Collision–induced dissociation (CID)\textsuperscript{119} experiments were performed in order to investigate the nature of the bonding in the sinapic and ferulic acid homodimer anions. An argon pressure pulse provided the collision gas. During the high pressure period, a single frequency RF waveform was applied. Ion excitation was performed on resonance at 160.930 kHz and 185.880 kHz for the sinapic and ferulic acid dimer anions, respectively. The laboratory frame translational energy $E_{\text{kin,lab}}$ imparted into the ions was calculated from Eq. (1.59). The duration of the applied waveforms was 400 μs. In these experiments, the kinetic energy of the ions was changed by varying the applied voltage $U_{pp}$. The center-of-mass energy was calculated according to Eq. (2.3)\textsuperscript{191}:

$$E_{\text{CM}} = E_{\text{kin,lab}} \frac{m_{\text{gas}}}{m_{\text{gas}} + m_{\text{ion}}}$$

(2.3)

where $m_{\text{gas}}$ is the mass of the collision gas and $m_{\text{ion}}$ is the mass of the ion to be dissociated. The CID waveform was followed by a 10 s pumpdown delay, after which the product ions were excited to larger radii by applying a chirp waveform and subsequently detected.

In order to ensure that the translational energy imparted into the ions was completely converted into internal energy, an experiment was carried out in which CID dissociation products were monitored as a function of number of collisions. This was determined by changing the time delay between the pulsed valve trigger ($t = 0s$) and application of the CID waveform. The relative argon concentration in the ICR cell was determined by electron impact ionization and detection of the resulting argon ions. CID product ion ratios and the relative argon concentration as a function of the time delay between the pulsed
valve trigger and application of the CID waveform are shown in Figure 2.6.

In this CID experiment, the sinapic acid dimer anion was excited to an energy of 2.1 eV. Under our instrumental conditions, the product ion ratio did not change significantly for delay times between 0.13 s and 0.7 s, although the pressure in the cell kept rising. It can be concluded that for delay times longer than 0.13 s the argon pressure in the ICR cell was sufficiently high to promote complete conversion of translational into internal energy. Additional collisions, corresponding to longer time delays, did not further increase the fragment ion ratio.

The argon pressure reached its maximum value only after about 1.5 s. This is due to the fact that the pulsed valve is located 2 meters away from the ICR cell, with flow restrictions between. For delay times longer than 0.7 s, the efficiency of the dissociation process decreased, which
can be explained by continuous damping of the ions. As the pressure increases to very high values, excitation of ion cyclotron motion and thus ion activation is prevented by the large number of collisions\textsuperscript{192}. The CID experiments presented in section 3.3. were performed with a 0.3 s delay between the pulsed valve trigger and application of the CID waveform, well within the region of complete energy conversion.
Seite Leer / Blank leaf
3. Results

3.1. Controlled analyte ion fragmentation

In this section, the performance of a new method for performing chemical ionization following laser desorption is presented. It is a straightforward technique and was developed within the scope of this work. Highly concentrated aqueous tetrabutylammonium salt solutions served as a reagent anion source for matrix-assisted laser desorption/chemical ionization (MALD/CI). The method yields molecular weights and controlled, specific fragmentation in a single step, and was successfully applied to several classes of analytes. The degree of fragmentation was found to depend on the proton affinity (PA) of the reagent anion used. The experiments presented in this section were carried out on the TOF mass spectrometer, except for the high mass resolution spectra as in Figure 3.5.

Figure 3.1 shows spectra of y-cyclodextrin desorbed from TBA bromide, TBA chloride and TBA acetate matrices. The main types of ions observed with this method are: (a) quasimolecular ions formed by halide ion attachment, (b) deprotonated molecules, and (c) specific fragment ion species. The bromide salt can be used for molecular mass information, it provides the [M + Br]⁻ quasimolecular ion. Whereas the bromide ion forms a stable addition complex, the acetate salt yields deprotonated analyte ions. The chloride ion, with a PA in between that of bromide and acetate, forms both the addition complex and the deprotonated molecule plus fragment ions, but causes less intense fragmentation than acetate. By choice of the salt matrix, selective mass spectral information is obtained.

These results clearly show that the observed fragmentation is not a simple result of laser exposure. Since y-cyclodextrin is not UV-absorbing, photofragmentation can be excluded. Pyrolysis can also be excluded since no fragment ions were observed with the bromide matrix at the same laser pulse energy.
Figure 3.1  Negative ion TOF mass spectra of γ-cyclodextrin (M) desorbed from various TBA salt matrices. From top to bottom: TBA bromide, chloride and acetate salt. The bromide matrix provides molecular mass information, whereas the chloride and acetate matrices additionally provide structural information. The laser pulse energy was 55 μJ. In the middle trace, Gramicidin S (GS) was used as an internal calibrant. Bx- and Cx-type series ions primarily differ in the fate of the glycosidic oxygen at the point of cleavage. In the Bx-series, the oxygen remains on the neutral fragment, whereas in the Cx-series it stays on the ionic fragment\textsuperscript{193}.

For comparison, neither a diethanolamine/silicon nor a glycerol/graphite binary matrix yielded any negative γ-cyclodextrin ion species, only an intense [M + Na]\textsuperscript{+} signal. At elevated laser pulse energies, low abundance positive fragment ions were observed with glycerol/graphite\textsuperscript{52}, but spectral quality was decreased and the signal-to-noise ratio for the fragment ions was only about 5. The observed fragment ions corresponded to successive cleavage of the γ-
cyclodextrin at its glycosidic linkages and can be attributed to pyrolysis products.

In the case of the TBA acetate matrix, pyrolysis effects do not seem to play a major role. Not only are more abundant fragment ions found, but also a different fragmentation pattern. In addition to the deprotonated molecule, an initial loss of 118 Da and further losses of 162 Da were observed. Moreover, a series of fragments starting with \([M - 102 - 162]^-\) and subsequent losses of 162 Da was observed. This is similar to the pattern obtained under infrared laser desorption conditions in positive ion mode, where an initial loss of 102 Da from both sodiated and potassiated molecular ions is followed by further losses of 162 Da\(^{194}\).

Spengler and coworkers suggest that the most prominent fragmentation pathway involves a series of retro-aldol reactions which are assumed to take place prior to ionization, in their case by attachment of an alkali-metal ion\(^{194}\). Although the \([M - 102 - H]^-\) ion itself was not observed in the MALD/CI experiment, subsequent single sugar unit losses (\(\Delta m = 162\) Da) are detected (indicated with C\(_x\)\(^{193}\)). The series of \([M - 118 - x\cdot162 - H]^-\) ions (indicated with B\(_x\)\(^{193}\)) can be explained by an additional loss of oxygen. These fragment ions only show up when using the TBA salt matrices with anion PAs higher than that of \(\text{Br}^-\), but not when using the TBA bromide, diethanolamine, or glycerol matrices.

Matrix ions were mainly detected as reagent anions (R\(^-\)), proton bound dimers of R\(^-\), and \([(C_4H_9)_4NR + R]^-\) cluster ion species. The R\(^-\) ion was usually the most abundant. In the case of the fluoride and the hydroxide salts, the anticipated reagent anions could only be detected in minor or negligible amounts, whereas proton bound dimers (e.g. \([2F + H]^-\), \([20H + H]^-\)) were the dominant peaks. \([(C_4H_9)_4NR + R]^-\) type ions are most likely created in the gas-phase by reagent anion attachment to the matrix molecule. It is therefore fair to assume that besides the observed reagent anions, intact matrix molecules are also liberated during the desorption event. Cluster ions appear mostly at higher laser pulse energies (50–60 \(\mu\)J) and can interfere with desirable fragment ions. However, these energies were only needed for desorption of
relatively large molecules (MW > 1200 Da), and the mass range free of interference was still quite large (above 300–400 Da).

In positive ion mode, the most prominent ion detected, even at elevated laser irradiance, was the \((\text{C}_4\text{H}_9)_4\text{N}^+\) cation (m/z 242). This was occasionally accompanied by weak fragments at m/z 186 (\([\text{C}_4\text{H}_9]\text{N} - \text{C}_4\text{H}_8]^+\)) and m/z 142 (\([\text{C}_4\text{H}_9]\text{N} - \text{C}_4\text{H}_8 - \text{C}_3\text{H}_8]^+\)). These results suggest that salt ions are generated during laser exposure by thermally induced dissociation. In no experiments were positive analyte ions observed.

In some, but not all cases, attachment of \(\text{C}_4\text{H}_8\) to both the deprotonated molecule and deprotonated fragment ions was observed. It is known that larger alkyl homologs of tetramethylammonium fluoride decompose above 80 °C\(^\text{195}\). The same should apply to tetrabutylammonium salts in general, so it is not surprising that alkyl fragments are generated under laser desorption conditions. These can then attach to either neutral or ionic species.

The observed ion types are expected to result from ion–molecule reactions similar to those in chemical ionization. Reagent anions of relatively low proton affinity such as chloride and bromide usually cannot react by proton transfer, but may form stable addition complexes as observed for \(\gamma\)-cyclodextrin with the TBA bromide matrix\(^\text{196}\):

\[
X^- + M \rightarrow MX^-
\]

where \(X^-\) is the halide anion and \(M\) is the analyte molecule. With saccharose as analyte, the TBA chloride matrix yielded some \([M - H]^-\) ions, but formed mainly chloride adducts. Chloride addition complexes with saccharides from conventional CI are also known\(^\text{197}\). The reaction leading to deprotonation of analyte molecules as observed for the \(\gamma\)-cyclodextrin/TBA acetate system is expected to be a proton transfer reaction:

\[
R^- + M \rightarrow [M - H]^- + RH
\]

where \(R^-\) stands for reagent anions. This type of reaction was shown to proceed in the gas phase with unit efficiency under equilibrium
conditions, provided that the exoergicity of the reaction is at least 10 kcal/mol\textsuperscript{198}. Fragment ions, the third type of observed species, are thought to be secondary products of deprotonation reactions with high exothermicity.

One general advantage of CI as an ionization method is that the energy deposited into the analyte molecule has a well-defined upper limit given the enthalpy of the proton transfer reaction. Thus, unimolecular fragmentation can be controlled by choice of the reagent ion. Speir and Amster studied the fragmentation mechanisms of protonated peptide molecules generated by LD/CI and found that although the internal energy of laser desorbed molecules has not yet been established quantitatively, the major contribution leading to fragmentation results from the CI process rather than from desorption\textsuperscript{199, 200}. In order to investigate the fragmentation behaviour in deprotonation reactions with increasing exothermicity, adenosine-5'-monophosphate (AMP) was used as a probe. AMP is a relatively fragile molecule that yields specific fragment anions at m/z 79 (PO\textsubscript{3}⁻), m/z 97 (H₂PO₄⁻), m/z 134 (deprotonated adenine), and m/z 211 ([AMP – adenine – H]⁻) under direct laser desorption conditions (248 nm). Besides an intense molecular ion, these fragments were also observed with the TBA salt matrices.

As shown in Figure 3.2, the TBA hydroxide matrix yielded comparatively intense AMP fragment ion signals, whereas in the case of the TBA chloride matrix the overall fragment ion intensity was much smaller. Mass spectra of AMP were recorded from the various TBA salt matrices. The gas-phase proton affinities of the selected anions are listed in Table 3.1. Using a constant laser pulse energy of 20 μJ, the normalized fragment ion abundance was found to increase systematically as the proton affinity of the reagent anion increases. Concurrently, the normalized molecular ion abundance decreased with increasing reagent anion proton affinity, as illustrated in Figure 3.3. Although the bromide isotope at m/z 79 and the PO\textsubscript{3}⁻ ion could not be resolved with the TOF instrument, the intensity of the latter was determined by calculating the ⁷⁹Br contribution from the ⁸¹Br peak. The TBA perchlorate matrix did not yield any analyte ions under these conditions.
Figure 3.2 Negative ion TOF mass spectra of adenosine-5'-monophosphate (AMP) desorbed from tetrabutylammonium chloride matrix (upper trace) and tetrabutylammonium hydroxide matrix (lower trace). The higher PA of the hydroxide compared to the chloride matrix imparts elevated internal energy into the AMP molecule during ionization, resulting in enhanced specific fragmentation. Fragment ions can be assigned to phosphoric acid, adenine, and base loss. Peaks not annotated are matrix signals. The laser pulse energy was 20 μJ.

<table>
<thead>
<tr>
<th>reagent anion</th>
<th>proton affinity</th>
<th>mass</th>
</tr>
</thead>
<tbody>
<tr>
<td>OH⁻</td>
<td>1634.7 ± 0.4⁺</td>
<td>17</td>
</tr>
<tr>
<td>F⁻</td>
<td>1553.5 ± 8.8⁺</td>
<td>19</td>
</tr>
<tr>
<td>CN⁻</td>
<td>1457.0 ± 8.4⁺</td>
<td>26</td>
</tr>
<tr>
<td>CH₃COO⁻</td>
<td>1457.5 ± 10.6⁺</td>
<td>59</td>
</tr>
<tr>
<td>C₆H₅CO₂⁻</td>
<td>1423.0 ± 9.2⁺</td>
<td>121</td>
</tr>
<tr>
<td>CI⁻</td>
<td>1396.2 ± 8.8⁺</td>
<td>35, 37</td>
</tr>
<tr>
<td>Br⁻</td>
<td>1353.0 ± 8.8⁺</td>
<td>79, 81</td>
</tr>
<tr>
<td>ClO₄⁻</td>
<td>1300.0 ± 59.0⁺</td>
<td>99, 101</td>
</tr>
</tbody>
</table>

Table 3.1 Reagent anion proton affinities
⁺ from reference 111, + from reference 189
Figure 3.3  Normalized molecular ion intensity of adenosine-5'-monophosphate, as a function of the reagent anion proton affinity. The normalized molecular ion intensity was calculated from the absolute molecular ion and fragment ion intensities, respectively, where the fragment ion intensity was determined by summing the integrals of the signals at m/z 79 (PO₃⁻), m/z 97 (H₂PO₄⁻), m/z 134 (deprotonated adenine), and m/z 211 ([AMP adenine H]⁻). The relative molecular ion intensity correlates with the PA of the reagent anion used. The solid curve shows a fit function of the type (3.7). The laser pulse energy was 20 μJ. Data points surrounded by dashed lines are those obtained by use of the TBA cyanide matrix.

The [M – H]⁻ product initially formed in reaction (3.2) clearly retained a large portion of the reaction energy, which then enabled fragmentation:

$$[M - H]^- \rightarrow \text{fragments} \quad (3.3)$$

Fragmentation energies for AMP-related dinucleotides were measured to be in the range 80-100 kJ/mol, with activation energies of about 120 kJ/mol²⁰¹. Ho and Kebarle studied the dissociation mechanisms of deprotonated mononucleotides by energy resolved collision-induced dissociation²⁰². They determined the activation energy for the dissociation of [AMP – H]⁻ into [AMP – adenine – H]⁻ to be 155 kJ/mol²⁰².
However, they consider this value unreliable because of the very low ion yield in the experiment, which also explains the deviation from the theoretical value of 130 kJ/mol²⁰².

The proton affinity of [AMP – H]⁻ can be estimated to lie between that of the bromide (1353 kJ/mol) and the perchlorate (1200 kJ/mol) anion since the TBA perchlorate matrix did not yield an [AMP – H]⁻ signal whereas the bromide matrix did. The proton affinity of the PO₃⁻ anion is 1300 kJ/mol which further suggests a PA of [AMP – H]⁻ not greater than 1300 kJ/mol¹¹¹. Assuming a PA of 1250 kJ/mol for the AMP anion, the maximum energy from deprotonation, PA(R⁻) – PA([AMP – H]⁻), is greater than the activation energy for fragmentation for all reagents with a PA greater than that of bromide. For example, using acetate as the reagent, the PA difference is about 208 kJ/mol.

As can be seen in Figure 3.2, the [AMP – H]⁻ ion decomposes via multiple fragmentation channels. Because the activation energies for these different channels are small compared to the enthalpy deposited by proton transfer and the variation among them is even smaller, all activation energies can be treated as about equal (E₀). In this case, the total unimolecular dissociation rate constant, kuni, can be expressed as the sum of the microscopic rate constants kᵢ for each channel²⁰³:

\[
k_{uni} = \sum_{i} k_i = \sum_{i} A_i \cdot \exp \left( -\frac{E_i}{k_B T} \right) = \exp \left( -\frac{E_0}{k_B T} \right) \sum_{i} A_i \tag{3.4}
\]

Reaction (3.3) will be driven further toward fragments when greater excess enthalpy is deposited by the prior proton transfer step. Assuming that this energy is thermalized within the ions and neglecting the room temperature internal energy of k_BT(300 K) = 2.5 kJ/mol, the enthalpy deposited by proton transfer gives the effective temperature of [AMP – H]⁻ prior to fragmentation: T_eff = ΔHₚ / k_B. It follows that:

\[
k_{uni} \propto \exp \left( -\frac{E_0}{\Delta H_{pT}} \right) \tag{3.5}
\]

For first order unimolecular decay, the relative number of undissociated molecular ions at a given time t is:
Combining (3.4) through (3.6) gives:

\[
\frac{N(t)}{N_0} = \exp\left[-k_{uni} \cdot t\right]
\]

where \( \Delta H_{PT} \) is given by the difference in PA of reagent anion and deprotonated molecule, \( PA(R^-) - PA([AMP - H]^-) \).

Because only ions formed within a short characteristic time after the laser desorption event will be focused on the detector of the mass spectrometer, Eq. (3.7) gives the observed relative \([AMP - H]^-\) abundance as a function of \( \Delta H_{PT} \) at this time. Eq. (3.7) was fit to the data of Figure 3.3. Within the experimental scatter, the fit is quite good. An \([AMP - H]^-\) proton affinity of 1279 kJ/mol was derived from the fit, corresponding well with the estimated value of 1250 kJ/mol. The \( E_0 \) value from the fit is 157 kJ/mol, also in good agreement with the activation energies reported above.

The amount of internal energy deposited into the analyte ions by MALD/Cl can be varied in relatively small steps of about 50 kJ/mol. In principle, the step size for excess energy deposition can be decreased further by employment of additional bases. Increased internal energy shows an effect on the degree of fragmentation. However, the width of the internal energy distribution is not known. Since the reagent anions are liberated by a thermal desorption process, a relatively broad internal energy distribution cannot be excluded. An indication for this is given by the fact that the chloride anion forms both addition complexes and deprotonated sugar ion species.

A variety of peptides were investigated using TBA salts in MALD/Cl: Phe-Gly-Gly-Phe, Val-Ala-Ala-Phe, (Phe)\(_5\), (Ala)\(_6\), (Tyr)\(_6\), bombesin, substance P and gramicidin S. All yielded intense signals of the deprotonated molecule by use of the TBA acetate matrix. (Ala)\(_6\) and Val-Ala-Ala-Phe yielded spectra completely devoid of fragmentation. For the other peptides, fragment ions were also observed, as shown in
Figure 3.4 for substance P. These fragments can be assigned to deprotonated Y-, X-, C- and Z-type fragments, and do not appear in spectra obtained with a diethanolamine matrix\textsuperscript{64}. The TBA chloride matrix yielded a signal of deprotonated substance P, but fragmentation was observed to be less intense, as can be seen in the lower trace in Figure 3.4.

Figure 3.4 Substance P (Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH\textsubscript{2}) desorbed from a TBA acetate matrix (upper trace) and a TBA chloride matrix (lower trace), in negative ion mode. Useful structural information is obtained by controlled selective fragmentation of the peptide. Peptide fragment nomenclature from reference 204. The laser pulse energy was 40 \mu J.
A similar effect of matrix anion proton affinity on the degree of fragmentation was found for the peptide bombesin. Just as in the case of γ-cyclodextrin, AMP and substance P, fragmentation was found to increase as the proton affinity increases in the series F⁻ > CH₃COO⁻ > Cl⁻. The hydroxide salt matrix did not give increased fragmentation compared to the fluoride salt matrix. However, the cyanide salt gave the same fragmentation as the fluoride and the hydroxide salt matrices, in contrast to what can be expected from its PA. The same effect was already observed with AMP as analyte (see Figure 3.3), but no explanation could be found yet.

Because the mass resolution in the TOF instrument was not sufficient to unambiguously assign ion signals in the higher mass region, reference experiments were carried out using the FT–ICR mass spectrometer. Isotopic profiles obtained by use of the salt matrices confirmed mass assignments of the [M – H]⁻ peptide signals. In the FT–ICR mass spectrometer, the analyte signal was exhausted after about ten shots from the same sample position. This can be attributed to dehydration of the liquid phase during the 30 minute pumpdown in the FT–ICR mass spectrometer. In contrast, in the TOF mass spectrometer, where the pumpdown takes only a few minutes, ion signals were stable for up to several hundred shots.

From Eqs. (3.6) and (3.7), it can be expected that the extent of analyte fragmentation increases with increasing time delay between the initial proton transfer step and ion detection. As can be seen in Figure 3.5, this prediction proves to be true: the degree of fragmentation was found to be higher in the FT–ICR mass spectrometer with a time scale of at least several tens of microseconds when compared to the linear TOF instrument, where ions only have to survive in the extraction region for some microseconds.

In general, mainly C-terminal peptide fragment ions are generated in MALD/Cl, whereas Kaufmann and coworkers observe mainly N-terminal fragment ions in PSD analysis of protonated peptides generated with MALDI. This may give a hint about the site of protonation and deprotonation, respectively. Protonation is likely to occur at the terminal NH₂-group, therefore A-, B- and C-type fragment ions can be
expected. On the other hand, if deprotonation occurs at the C-terminus, X-, Y- and Z-type fragment ions can be expected. However, substance P and bombesin are CONH$_2$- rather than COOH-terminated and the site of deprotonation is more likely to be an acidic amino acid side chain.

Figure 3.5  Negative ion mode FT-ICR (black) and TOF (grey) mass spectra of substance P desorbed from a TBA acetate/silicon particulate binary matrix. As a consequence of the longer time scale of the FT-ICR experiment, the relative fragment ion abundance in the FT-ICR mass spectrum is higher than in the TOF mass spectrum.

Kaufmann and coworkers found that the choice of a solid matrix in MALDI/post-source decay analysis can influence the parent ion stability, but they attribute this effect to crystallization behavior rather than thermochemical matrix properties$^{34}$. They further emphasize that the major part of the activation energy imparted to analyte ions stems from ion/neutral collisions occurring in the expanding plume of ions and neutrals. However, the choice of a suitable MALDI matrix for specific analyte fragmentation in PSD analysis can only be made empirically, because the MALDI ionization mechanism is not yet understood. In contrast, ionization in MALD/CI using TBA salts proceeds via well-defined pathways and fragmentation is easily controllable by choice of the reagent anion used.

It is interesting to note that PSD analysis of cationized species was reported to be less efficient than PSD of protonated peptides;
cationized ions were found to be much more stable. It was found here that analytes that usually yield sodium and potassium adducts in positive ion mode (γ-cyclodextrin, maltotriose, valinomycin) can readily be deprotonated and yield abundant specific fragmentation patterns by use of the appropriate salt matrix. Moreover, the fragment ion yield can be adjusted without changing instrumental parameters by choice of the salt matrix, contrary to PSD measurements where acceleration field strength, final ion kinetic energy and residual gases all influence the degree of fragmentation.

Calculation of the absolute energy deposited into the analyte molecules by means of chemical ionization requires thermochemical data on the analyte anions, which are not available. For peptides and proteins, the proton affinities of deprotonated α-amino acids can be used to make an estimate. O'Hair, Bowie and Gronert found values between 1385 kJ/mol (L-histidine) and 1431 kJ/mol (glycine). Proton affinities of the deprotonated amino acid side chains were estimated to lie in the range of 1439 kJ/mol to above 1632 kJ/mol. At least for deprotonated peptide molecules the PA can be assumed to lie in the range between 1385 kJ/mol and 1431 kJ/mol, and therefore it is consistent with the data that even a relatively weak base such as the chloride anion (PA 1396.2 kJ/mol) is able to abstract a proton from a peptide molecule. Assuming a peptide anion PA of 1400 kJ/mol, excess energies ranging from 25 kJ/mol up to 240 kJ/mol can be imparted into the analyte ion by use of TBA benzoate and hydroxide matrix, respectively.

As pointed out in section 1.1.5., it is probable that proton transfer reactions yielding deprotonated analyte in conventional MALDI proceed via an analogous path to that in MALD/CI. To address this point, gas-phase basicities or, equivalently, proton affinities of the MALDI matrix anions need to be known. Several MALDI matrix anion GBs have been determined in this work, as presented in the following section.
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3.2. Gas-phase basicities of MALDI matrix anions

3.2.1. Results from equilibrium bracketing experiments

The ion/molecule reaction products detected in the equilibrium bracketing experiments were either deprotonated matrix anions (M - H\(^-\)), MR\(^-\) heterodimer ions, or both species simultaneously. Figure 3.6 illustrates typical spectra obtained after a 30 second reaction time.

![Diagram of mass spectra]

**methanesulfonate**
\[ \text{GB} = 1319.5 \text{ kJ/mol} \]

\[ \text{CH}_3\text{SO}_3^- \]

\[ [\text{2A5NP}+\text{CH}_3\text{SO}_3^-]^- \]

**benzoate**
\[ \text{GB} = 1393.3 \text{ kJ/mol} \]

\[ \text{C}_6\text{H}_5\text{CO}_2^- \]

\[ [\text{2A5NP}+\text{C}_6\text{H}_5\text{CO}_2^-]^- \]

**thiophenolate**
\[ \text{GB} = 1397 \text{ kJ/mol} \]

\[ \text{C}_6\text{H}_5\text{S}^- \]

Figure 3.6 Typical FT-ICR mass spectra obtained in equilibrium bracketing experiments, illustrated for reactions of reference anions with the MALDI matrix 2-amino-5-nitropyridine (2A5NP). The reaction time was 30 seconds. Upper trace: Adduct ion formation with 2A5NP depletes the methanesulfonate anion, and no deprotonated matrix is observed. Middle trace: the benzoate anion forms both adduct ions and deprotonated 2A5NP. Lower trace: the thiophenolate anion reacts by deprotonating 2A5NP, whereas the adduct species is not observed. The relative yield of deprotonated matrix is correlated with the reference anion basicity, which increases from top to bottom.

Depending on their gas-phase basicities, the various reference anions yield different types of product ions when reacting with the matrix molecules. As shown in the upper trace of Figure 3.6, the
methanesulfonate anion is depleted solely by complex formation with the MALDI matrix 2-amino-5-nitropyridine (2A5NP), so it can be concluded that the proton transfer reaction is endoergic. The benzoate anion forms both complex ions and deprotonated matrix, indicating a near-thermoneutral proton transfer reaction (see middle trace). In the lower trace, a pure deprotonation reaction between the thiophenolate anion and 2A5NP is shown, which can be classified as exoergic. The relative yield of deprotonated matrix increases with increasing reference anion basicity.

Especially in near-thermoneutral proton transfer reactions, heterodimer ions are often detected\textsuperscript{128,129}. Since proton transfer and not adduct formation is of interest here, the intermediate RM\textsuperscript{−} is considered as unreacted material formally equivalent to R\textsuperscript{−}. Therefore, the term [R\textsuperscript{−}] in Eqs. (1.11) through (1.15) includes the measured RM\textsuperscript{−} concentration.

As illustrated in section 1.4.4, for CNO\textsuperscript{−} reacting with \textit{para}-nitroaniline (PNA), near-equilibrium conditions were established in the FT-ICR cell after reaction times of about ten seconds or less. The reactions of the various reference anions with PNA were monitored as a function of time, and from the relative ion abundances, R\textsubscript{K} values were calculated. Using Equation (1.19), GB values at 300 K were obtained. A non-linear, least-square fitting procedure was employed for data evaluation. Experimental data were weighted with the reference base GB errors in the fitting procedure. Experimental data and the corresponding R\textsubscript{K} fit functions are depicted in Figure 3.7 for reaction times of 1, 5, and 30 seconds.

After a relatively rapid change of R\textsubscript{K} values during the first seconds, corresponding to non-equilibrium conditions, no further significant change was observed. The R\textsubscript{K} values after 10, 20, and 30 seconds reaction times were almost undistinguishable. The same was true for the derived gas-phase basicities, as illustrated in Figure 3.8. The data in Figure 3.8 reflect the kinetics of the various reactions and were fitted with an exponential function. The gas-phase basicity of the \textit{para}-nitroaniline anion (PNA – H)\textsuperscript{−} determined with this method is 1410.6 ± 11.5 kJ/mol, which is in very good agreement with the literature value of 1407.0 ± 8.4 kJ/mol\textsuperscript{111}. 
Figure 3.7 Rk values as a function of reference anion basicity for reactions of para-nitroaniline (PNA) with several reference anions (hydrogen sulfide, cyanide, acetate, cyanate, thiophenolate, benzoate, chloride, bromide, nitrate, rhodanide, methanesulfonate, iodide, hydrogen sulfate). Reaction times were 1, 5, and 30 seconds, respectively. The solid lines are fit functions of the type (1.19) for 300 K and a relative matrix concentration $M_0 = 48$. The vertical reference line at GB = 1402 kJ/mol helps to see the shifting of fit functions towards lower GB with increasing reaction time. The shift during the first 5 seconds was much larger than the shift between 5 and 30 seconds.

Figure 3.8 Derived apparent gas-phase basicities of [PNA – H]– as a function of reaction time. After a relatively rapid initial decrease, the GB clearly approaches a limiting value. The solid line is an exponential fit function with an asymptotic value of 1410.6 kJ/mol. Error bars show the error in GB obtained from a covariance analysis.
In order to further validate the method, the gas-phase basicities of \textit{para}-aminophenol (pAP) and 4-hydroxybenzoic acid (4HBA) were determined. These molecules are not used as MALDI-matrices, but are chemically similar to some matrices and literature values are available. The (4HBA – H)^- anion basicity found, 1378.0 ± 11.5 kJ/mol, is in excellent agreement with the literature value of 1376.0 ± 8.4 kJ/mol\textsuperscript{111}. For \textit{para}-aminophenol, a GB value of 1438.3 ± 11.5 kJ/mol was determined. The literature value is larger, 1450 ± 8.4 kJ/mol\textsuperscript{111}, but within the errors still in agreement. Literature values and the data obtained here from the analysis of R\textsubscript{k}-values are summarized in Table 3.2.

In a few cases, the deprotonated matrix molecule was found to form a (2M – H)^-–type complex by attachment of an additional matrix molecule. This ion appeared only slowly and was always minor in abundance for reaction times up to 30 seconds. It was treated in the analysis as a (M – H)^-– type matrix anion.

2,5-dihydroxybenzoic acid (DHB) and 2,4,6-trihydroxyacetophenone (THAP) were the only matrices investigated here for which a 30 second reaction delay was not sufficiently long to determine the corresponding anion GBs. The exoergic reactions with DHB were only complete after a 90 second reaction time. On this extended time scale, the formation of (2M – H)^-–type homodimer ions became significant. The ratio of (DHB – H)^- and (2DHB – H)^- ions after a 90 second reaction time was about one. The homodimer ion was treated as deprotonated matrix. With this assumption, the derived gas-phase basicity of (DHB – H)^- is 1329.4 ± 11.5 kJ/mol.

The bromide and nitrate anions reacted with DHB both by deprotonation and complex formation, whereas the rhodanide anion reacted only by heterodimer complex formation. From a qualitative bracketing viewpoint, then, the derived gas-phase basicity of 1329.4 kJ/mol is therefore reasonable. However, on this longer time scale some departure from equilibrium cannot be ruled out since neutral product molecules will slowly be pumped out of the cell volume, driving the reaction further towards products.
<table>
<thead>
<tr>
<th>[M–H]⁻ derived from</th>
<th>GB([M–H]⁻) [kJ/mol]</th>
<th>literature value [kJ/mol]</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-aminoquinoline</td>
<td>1450.7 ± 11.5</td>
<td>–</td>
</tr>
<tr>
<td>para-aminophenol</td>
<td>1438.3 ± 11.5</td>
<td>1450 ± 8.4*</td>
</tr>
<tr>
<td>para-nitroaniline</td>
<td>1410.6 ± 11.5</td>
<td>1407 ± 8.4*</td>
</tr>
<tr>
<td>2-amino-5-nitropyridine</td>
<td>1398.8 ± 11.5</td>
<td>–</td>
</tr>
<tr>
<td>2-amino-3-hydroxypyridine</td>
<td>1398.5 ± 11.5</td>
<td>–</td>
</tr>
<tr>
<td>4-hydroxybenzoic acid</td>
<td>1378.0 ± 11.5</td>
<td>1376 ± 8.4*</td>
</tr>
<tr>
<td>nicotinic acid</td>
<td>1365.5 ± 11.5</td>
<td>–</td>
</tr>
<tr>
<td>3-hydroxypicolinic acid</td>
<td>1365.3 ± 11.5</td>
<td>–</td>
</tr>
<tr>
<td>2,5-dihydroxybenzoic acid</td>
<td>1329.4 ± 11.5</td>
<td>–</td>
</tr>
<tr>
<td>2,4,6-trihydroxyacetophenone</td>
<td>1324.3 ± 7.1**</td>
<td>–</td>
</tr>
</tbody>
</table>

Table 3.2 Gas-phase basicities of MALDI matrix anions and related compounds determined with the equilibrium method. * from reference 111, ** see text

For THAP, the observed reactions were even slower. After a 30 second reaction delay, the product ion yield was only about 20%. The bromide and rhodanide (CNS⁻) anion both reacted by formation of (THAP – H)⁻ and heterodimer complex anions in approximately equal abundance.
The nitrate anion yielded exclusively (THAP-H)~ ions, whereas the methanesulfonate exclusively formed heterodimer anions. THAP was the only matrix here which was deprotonated by the rhodanide anion. The GB of the (THAP-H)~ anion was therefore determined by bracketing between rhodanide (GB = 1329 kJ/mol) and methanesulfonate (GB = 1319.5 kJ/mol), giving rise to a (THAP-H)~ gas-phase basicity of 1324.3 ± 7.1 kJ/mol. The relative order of (DHB-H)~ and (THAP-H)~ anion basicities was confirmed in a proton transfer experiment. The (DHB-H)~ anion reacted with THAP in a pure deprotonation reaction.

The method described here could not be utilized for determination of anion GBs of sinapic acid, ferulic acid, and 2-(4-hydroxyphenylazo)benzoic acid (HABA), since these matrices were found to have insufficient vapor pressures. For example, the reaction between acetate and ferulic acid, which by analogy to the other carboxylic acids can reasonably be assumed to be exoergic, was far from completion even after 1000 second reaction time. The GBs of deprotonated ferulic and sinapic acids were instead determined using the BREC method.

3.2.2. Results from BREC experiments

The BREC method was used to determine the gas-phase basicities of monomeric and dimeric deprotonated ferulic and sinapic acids. The chemical structures of ferulic and sinapic acid are shown in Figure 3.9.

```
H3CO
\H--O
| \O
\H  \H
```

Figure 3.9 Chemical structures of ferulic acid (left) and sinapic acid (right).

In the BREC method, gas-phase basicities are derived from reaction efficiencies, as comprehensively described in section 1.4.5.0. Efficiencies of the various bracketing reactions calculated from Eq. (1.26) were monitored as a function of reaction time. In Figure 3.10 a), efficiencies
for the ferulic acid dimer anion reaction with 2,4-dinitrophenol are depicted. The average efficiency of this deprotonation reaction was small, 0.078 ± 0.005. In contrast, the efficiency for the ferulic acid monomer anion reaction with 3-hydroxypicolinic acid was 0.55 ± 0.02 (Figure 3.10 b)).

![Graph](image)

**Figure 3.10** Reaction efficiencies calculated from relative ion abundances as a function of reaction time for reactions with different efficiencies. 

- **a)** RE = 0.078 ± 0.005 for [FA₂ - H]⁻ reacting with 2,4-dinitrophenol.
- **b)** RE = 0.55 ± 0.02 for [FA - H]⁻ reacting with 3-hydroxypicolinic acid.
- **c)** RE = 0.9 ± 0.1 for [FA₂ - H]⁻ reacting with picric acid.

Solid lines in a) and b) are linear fit functions with zero slope. Apparent reaction efficiencies as a function of reaction time for the picric acid deprotonation reaction through the ferulic acid dimer anion are shown in c). Apparent REs much larger than unity were initially obtained due to back-reactions of product ions with laser-desorbed neutral matrix molecules. Neutral matrix molecules will slowly be pumped out of the cell volume, preventing the back-reaction at longer reaction times. The exponential fit function (solid line in c)) approaches an asymptotic value after sufficiently long reaction times.
As expected from the theory in section 1.4.5., the reaction efficiencies for a given reaction do not depend on the time $t_x$ after which the reaction is probed. With this method, the average standard deviation in determining reaction efficiencies was 6%. This is much less than typical errors in determining reaction efficiencies from rate constants, which were up to 25%\textsuperscript{128}.

While the reaction efficiencies did not generally depend on the time after which the reaction was probed, the FA and SA dimer reactions with picric acid were an exception. The efficiencies of the picric acid deprotonation reactions by FA and SA dimer anions were found to depend on the reaction time as depicted in Figure 3.10 c). The apparent RE values were initially much larger than unity and decreased with increasing reaction time.

This behavior appears problematic, but can be understood by taking a closer look at the chemistry which can occur inside the FT-ICR cell. The reaction efficiencies are defined as the ratio of the forward reaction rate constant and the collision rate constant. Back-reactions from products to educts have been neglected. For most of the experiments presented here, this is a reasonable assumption since the sublimation pressures of solid FA and SA are negligible and do not provide significant numbers of matrix molecules or neutral matrix dimers for the back-reaction. The two-phase sample preparations also did not evaporate detectable amounts of any neutral molecules involved in the reactions. However, as surveyed in section 1.1.1., it is known that a large number of neutrals is liberated upon laser desorption/ionization from solid MALDI matrices, typically a ten thousand-fold excess above the number of ions\textsuperscript{29}. With the experimental setup used, this neutral matrix material can reach the cell volume and is then available for the reverse of reaction (3.8).

\begin{align}
[M - H]^+ + R &\rightarrow M + [R - H]^+ \quad (3.8) \\
[M_2 - H]^+ + R &\rightarrow M_2 + [R - H]^+ \quad (3.9)
\end{align}

In reactions (3.8) and (3.9), $M$ stands for monomeric and $M_2$ for dimeric FA or SA acid, and $R$ for the reference compound. The reverse of
reaction (3.9) will not proceed because laser-desorbed neutral matrix dimers are, due to energetic collisions in the expanding plume, unlikely to survive on the longer time scale of the FT-ICR experiment. In contrast to the relatively strong ion-dipole bonding in the matrix dimer anions (see section 3.3.), only hydrogen bonding keeps the neutral matrix dimers together, which are thereby less stable. If the reverse of reaction (3.8) is possible, but not of reaction (3.9), then reaction (3.8) will appear to proceed slower than reaction (3.9). For the dimer bracketing, the reaction (3.8) was used as the reference reaction. This explains the RE values larger than unity for the picric acid deprotonation reaction by the FA and SA dimer anions. The laser-desorbed molecules will then slowly be pumped out of the cell volume, which is in agreement with the decreasing reaction efficiencies at longer reaction delays.

In accordance with typical exponential pressure drops, the reaction efficiencies for the picric acid deprotonation were fitted with exponential functions, and the long-time asymptotes were used as the 'true' reaction efficiencies. This 'decay' in reaction efficiency was only observed in the dimer deprotonation reactions with picric acid, but neither in the monomer bracketing nor in the dimer reactions with 2,3-dinitrophenol or 2,4-dinitrophenol. It must be assumed that laser-desorbed SA and FA were also available in the latter reactions, but here the back-reactions obviously did not affect the relatively small reaction efficiencies (see Figure 3.10 a).

In the BREC experiments reported here, the ADO correction factor was between 1.14 and 1.48. As a consequence, the corrected reaction efficiencies were always higher than the uncorrected reaction efficiencies. The uncorrected (from Eq. (1.26)) and corrected reaction efficiencies (from Eq. (1.29)) are given in Table 1. The error in reaction efficiency for RE = 0 was estimated from the smallest signal-to-noise ratio, which was about 250, resulting in an absolute error of ± 0.004.

The uncorrected reaction efficiencies did not exceed unity, but corrected efficiencies significantly larger than 1 were obtained in two cases. This is most likely the result of an overestimated ADO correction factor. ADO theory is derived from a point charge model, and
deviations due to the finite ion size may occur, especially if charge is
delocalized\textsuperscript{126}. Charge delocalization certainly occurs in deprotonated
carboxylic acid groups, and can be even more significant if the alkyl
group is conjugated.

<table>
<thead>
<tr>
<th>reference compound</th>
<th>[FA - H]\textsuperscript{−}</th>
<th>[SA - H]\textsuperscript{−}</th>
<th>[FA\textsubscript{2} - H]\textsuperscript{−}</th>
<th>[SA\textsubscript{2} - H]\textsuperscript{−}</th>
</tr>
</thead>
<tbody>
<tr>
<td>PNA</td>
<td>0 ± 0.004</td>
<td>0 ± 0.004</td>
<td>0 ± 0.004</td>
<td>0 ± 0.004</td>
</tr>
<tr>
<td>TMBA</td>
<td>0.46 ± 0.02</td>
<td>0.34 ± 0.03</td>
<td>0 ± 0.004</td>
<td>0 ± 0.004</td>
</tr>
<tr>
<td>(0.32 ± 0.01)</td>
<td>(0.23 ± 0.02)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4HBA</td>
<td>0.50 ± 0.02</td>
<td>0.51 ± 0.02</td>
<td>0 ± 0.004</td>
<td>0 ± 0.004</td>
</tr>
<tr>
<td>(0.36 ± 0.01)</td>
<td>(0.36 ± 0.02)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HPA</td>
<td>0.77 ± 0.02</td>
<td>0.90 ± 0.06</td>
<td>0 ± 0.004</td>
<td>0 ± 0.004</td>
</tr>
<tr>
<td>(0.55 ± 0.02)</td>
<td>(0.63 ± 0.04)</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>NBA</td>
<td>0.84 ± 0.03</td>
<td>0.83 ± 0.02</td>
<td>0 ± 0.004</td>
<td>0 ± 0.004</td>
</tr>
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<td>(0.59 ± 0.02)</td>
<td>(0.56 ± 0.01)</td>
<td></td>
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</tr>
<tr>
<td>CBA</td>
<td>1.29 ± 0.07</td>
<td>0.89 ± 0.03</td>
<td>0 ± 0.004</td>
<td>0 ± 0.004</td>
</tr>
<tr>
<td>(0.91 ± 0.05)</td>
<td>(0.61 ± 0.02)</td>
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</tr>
<tr>
<td>2HBA</td>
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<td>0.88 ± 0.03</td>
<td>0 ± 0.004</td>
<td>0 ± 0.004</td>
</tr>
<tr>
<td>(0.83 ± 0.04)</td>
<td>(0.62 ± 0.02)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CNP</td>
<td>0 ± 0.004</td>
<td>0 ± 0.004</td>
<td></td>
<td></td>
</tr>
<tr>
<td>23DNP</td>
<td>-</td>
<td>-</td>
<td>0.011 ± 0.005</td>
<td>0.016 ± 0.005</td>
</tr>
<tr>
<td>(0.009 ± 0.004)</td>
<td>(0.014 ± 0.004)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24DNP</td>
<td>-</td>
<td>-</td>
<td>0.090 ± 0.006</td>
<td>0.055 ± 0.006</td>
</tr>
<tr>
<td>(0.078 ± 0.005)</td>
<td>(0.048 ± 0.005)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PIC</td>
<td>-</td>
<td>-</td>
<td>1.05 ± 0.1\textsuperscript{*}</td>
<td>1.0 ± 0.1\textsuperscript{*}</td>
</tr>
<tr>
<td>(0.9 ± 0.1\textsuperscript{*})</td>
<td>(0.86 ± 0.1\textsuperscript{*})</td>
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</tr>
</tbody>
</table>

Table 3.3 Corrected reaction efficiencies calculated from Equation (1.29) and
uncorrected reaction efficiencies (in brackets) calculated from
Equation (1.26). PNA = \textit{para}-nitroaniline, TMBA = 2,4,6-trimethyl
benzoic acid, 4HBA = 4-hydroxybenzoic acid, HPA = 3-hydroxypicolinic
acid, NBA = 3-nitrobenzoic acid, CBA = 4-cyanobenzoic acid, 2HBA =
2-hydroxybenzoic acid, CNP = 2-chloro-4-nitrophenol, 23DNP =
2,3-dinitrophenol, 24DNP = 2,4-dinitrophenol, and PIC = picric acid.
\textsuperscript{*} see text

\textit{Para}-nitroaniline could not be deprotonated by either monomer or
dimer anions from ferulic or sinapic acid, even though the monomer
anions formed intense heterodimer complex anions. The deprotonation
reactions with para-nitroaniline were all endoergic. With 2,4,6-trimethylbenzoic acid, 4-hydroxybenzoic acid, 3-hydroxypicolinic acid, and 3-nitrobenzoic acid, the FA and SA monomer reactions were near-thermoneutral, whereas the dimer reactions were all endoergic. The FA and SA monomer reactions with all other reference compounds were classified as exoergic. Deprotonation by FA and SA dimer anions was observed with 2,3-dinitrophenol, 2,4-dinitrophenol and picric acid. Because of their small efficiencies, the dinitrophenol reactions were classified as near-thermoneutral, whereas the reactions with picric acid were clearly exoergic.

As discussed in section 1.4.3., reactions with efficiencies smaller than 0.1 are usually considered endoergic, and efficiencies greater than 0.1 indicate exoergic reactions in conventional bracketing experiments\textsuperscript{115, 116, 128}. From this standpoint, both the FA and SA monomer anion basicities are bracketed between para-nitroaniline (1407 kJ/mol) and 2,4,6-trimethylbenzoic acid (1389 kJ/mol), indicating a GB value for both $[\text{FA}^- - \text{H}]^-$ and $[\text{SA}^- - \text{H}]^-$ of 1398 ± 9 kJ/mol. This locates the deprotonated SA and FA matrix anions on the higher end of the matrix anion basicity scale determined with the equilibrium bracketing method\textsuperscript{85}. The FA and SA dimer anions, $[\text{FA}_2^- - \text{H}]^-$ and $[\text{SA}_2^- - \text{H}]^-$, were bracketed between 2,4-dinitrophenol (1291 kJ/mol) and picric acid (1267 kJ/mol), yielding GB values of 1279 ± 12 kJ/mol.

Equation (1.41) was applied to the RE data sets keeping T constant at the true experimental temperature of 300 K. A non-linear least square fitting procedure was utilized and the RE standard deviations were used for weighting the RE fit. The corrected reaction efficiencies as a function of reference anion GB and the obtained fit functions of the type (1.41) are shown in Figures 3.11 a) and b) for FA and SA, respectively.

From these fit functions, gas-phase basicities of SA and FA monomer and dimer anions were derived. In the monomer bracketing experiments, reactions with the acetate anion were employed as reference reactions. The GB of the acetate anion is known\textsuperscript{111}, but the $\lambda$-value or, equivalently, the intrinsic barrier for the series of gas-phase acetate reactions is not. To a first approximation, it was therefore
assumed that the λ-value for the acetate reactions is within the region of reported values, between 14.5 kJ/mol and 19.3 kJ/mol, e.g. λ₂ was estimated to be 16.9 kJ/mol. This choice did not strongly affect the derived GB(B₁) and λ₁⁻ values: with λ₂ = 14.5, 16.9 or 19.3 kJ/mol, the derived gas-phase basicities of SA monomer anion were 1400.8 ± 1.3, 1399.8 ± 1.2, or 1399.2 ± 1.1 kJ/mol, and λ₁⁻-values were 14.0 ± 0.5, 14.6 ± 0.5, or 15.6 ± 0.4 kJ/mol, respectively. The λ-values for the monomer reactions, which served as reference reactions in the dimer bracketing, were obtained from the monomer bracketing data analysis.

![Graph](image)

Figure 3.11 Corrected reaction efficiencies calculated from Equation (1.29) as a function of reference anion gas-phase basicity for the ferulic acid reactions (a)) and the sinapic acid reactions (b)). The dashed lines are fit functions of the type (1.41) for the monomeric FA and SA anions, and solid lines are fit functions of the type (1.41) for the dimeric FA and SA anions.

Table 3.4 summarizes the results of this analysis. Standard deviations for GB-values and intrinsic barriers were obtained from a covariance analysis. The GBs of the FA and SA monomer anions were within the error limits found to be similar and ca. 1400 kJ/mol. The GBs of the dimer
anions of SA and FA were also found to be similar and about 1285 kJ/mol. The GB values obtained from data analysis using Equation (1.41) at T = 300 K are in excellent agreement with the qualitative bracketing results discussed above.

<table>
<thead>
<tr>
<th></th>
<th>gas-phase basicity [kJ/mol]</th>
<th>intrinsic barrier [kJ/mol]</th>
</tr>
</thead>
<tbody>
<tr>
<td>[FA – H]⁻</td>
<td>1399.4 ± 1.0</td>
<td>9.3 ± 0.3</td>
</tr>
<tr>
<td>[FA₂ – H]⁻</td>
<td>1285.6 ± 1.1</td>
<td>2.4 ± 1.4</td>
</tr>
<tr>
<td>[SA – H]⁻</td>
<td>1399.8 ± 1.2</td>
<td>10.1 ± 0.3</td>
</tr>
<tr>
<td>[SA₂ – H]⁻</td>
<td>1285.0 ± 1.6</td>
<td>3.4 ± 1.6</td>
</tr>
</tbody>
</table>

Table 3.4  Gas-phase basicities of ferulic and sinapic acid monomer and dimer anions and activation energies at thermoneutrality derived from the data analysis as described in the text.

The λ₁-values for the matrix monomer reactions were 13.4 kJ/mol and 14.6 kJ/mol, well within the typical region. The λ₁-values for the dimer bracketing were substantially lower, 3.5 kJ/mol and 4.8 kJ/mol. The fact that only a few reference bases were available in the dimer bracketing experiments results in relatively large errors for λ₁, but the difference in λ₁-values for monomer and dimer reactions is unambiguous. The activation energies at thermoneutrality, the "intrinsic barriers", were calculated from Eq. (1.32). These energies are 9.3 ± 0.3 kJ/mol, 2.4 ± 1.4 kJ/mol, 10.1 ± 0.3 kJ/mol, and 3.4 ± 1.6 kJ/mol for the FA monomer anion, the FA dimer anion, the SA monomer anion, and the SA dimer anion, respectively.

With the data derived from the fit functions, activation energies for the various reactions can be calculated from Equation (1.31). Activation energies for the sinapic acid monomer and dimer anion reactions as a function of exoergicity are shown in Figure 3.12. In both cases, the activation energy decreases non-linearly as the reaction becomes thermochemically more favorable. For the monomer reactions, the activation energies are higher than for the dimer reactions and also their decrease with increasing exoergicity is slower when compared to the dimer reactions.
Figure 3.12 Calculated activation energies $G_a$ for deprotonation of reference molecules via the SA monomer (dashed line) and SA dimer (solid line) anions as a function of reference anion gas-phase basicity. The curves were calculated from Equation (1.31) using the GB and $\lambda$-values from Table 3.4. The dashed and solid vertical lines indicate the activation energies for the respective thermoneutral reactions.
3.3. Collision-induced dissociation of matrix dimer anions

Collision-induced dissociation (CID) experiments were performed in order to investigate the nature of the bonding in the FA and SA homodimer anions. Figure 3.13 shows mass spectra obtained after isolation and excitation of the ferulic acid dimer anion, \([\text{FA}_2 - \text{H}]^-\). The center-of-mass energies were 0.02 eV, 1.74 eV, and 2.58 eV, respectively. Complete dissociation of the dimer anion was observed with an excitation energy of about 2.6 eV.

![FT-ICR CID spectra of the ferulic acid dimer anion \([\text{FA}_2 - \text{H}]^-\) with 0.02 eV (upper trace), 1.74 eV (middle trace), and 2.58 eV (lower trace) excitation energies. The main dissociation product is the monomer anion \([\text{FA} - \text{H}]^-\).](image)

The breakdown curves from the CID experiments for the ferulic and sinapic acid dimer anions are depicted in Figures 3.14 and 3.15, respectively. Both dimer anions show a similar dissociation behavior, with the main dissociation product being the monomer anions. The energies required for 50% dissociation of the dimer anion are approximately 1.8 eV for FA and 1.7 eV for SA, with an onset for dissociation of about 1 eV. Interestingly, CO$_2$ loss from the dimer anion is also observed at relatively low dissociation energies. Release of CO$_2$
might also occur under MALDI conditions, thereby facilitating the desorption process.

**Figure 3.14** Breakdown curves for collision-induced dissociation of the ferulic acid dimer anion [FA$_2$ - H]$^-$.  

**Figure 3.15** Breakdown curves for collision-induced dissociation of the sinapic acid dimer anion [SA$_2$ - H]$^-$. 
4. Discussion

4.1. Gas-phase basicities of MALDI matrix anions

The gas-phase basicities of the monomeric MALDI matrix anions studied exhibit substantial differences, ranging from very (\(\text{GB(3-aminoquinoline anion)} = 1451 \text{ kJ/mol}\)) to slightly basic (\(\text{GB(2,4,6-trihydroxyacetophenone anion)} = 1324 \text{ kJ/mol}\)). The gas-phase basicities of the FA and SA matrix dimer anions were found to be even smaller (\(\text{GB([FA}_2 \text{ - H}]^- \approx \text{GB([SA}_2 \text{ - H}]^-) = 1285 \text{ kJ/mol}\)). As a general trend, the anion gas-phase basicities (that can equivalently be viewed as gas-phase acidities of the neutral molecules) follow the aqueous solution phase acidities; amines are less acidic than carboxylic acids\(^{13}\). Only a few solution pH-values for the compounds investigated here are available from the literature, which are listed in Table 4.1.

<table>
<thead>
<tr>
<th>compound</th>
<th>(\text{pK}_a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>para-aminophenol</td>
<td>8.50</td>
</tr>
<tr>
<td>nicotinic acid</td>
<td>4.75</td>
</tr>
<tr>
<td>4-hydroxybenzoic acid</td>
<td>4.582</td>
</tr>
<tr>
<td>2,5-dihydroxybenzoic acid</td>
<td>2.97</td>
</tr>
</tbody>
</table>

Table 4.1 pH-values in aqueous solution at 25 °C (from reference 208).

For these four compounds, a linear correlation of anion gas-phase basicities with \(\text{pK}_a\)-values clearly exists, as can be seen in Figure 4.1. The \(\text{pK}_a\)-values correspond to \(\Delta G_{\text{acid}}\)-values that can be calculated from the relationship \(\Delta G_{\text{acid}} = 2.303 \cdot R \cdot T \cdot \text{pK}_a\), where \(R\) is the gas constant and \(T\) the temperature\(^{108}\). The slope of the linear fit in Figure 4.1 is 3.3, meaning that the increase in acidity due to molecular structure is much higher in the gas phase when compared to the solution phase. This relative attenuation observed in solution can be explained by the fact that stabilization of the anions is mainly due to the solvent, so that the intrinsic substituent effects are smaller\(^{209}\).
Figure 4.1  Gas-phase basicities of deprotonated MALDI matrix molecules, [M – H]–, versus the acidity constants pKₐ and the derived ΔGₘₚₐₐₐ values of the neutral molecules in water at 300 K. The solid line is a linear fit function, GB([M – H]–) = 1278.8 kJ/mol + 3.3133 * ΔGₐcid.

Among the matrices with carboxylic functional groups, deprotonated ferulic and sinapic acids exhibit the highest gas-phase basicities of about 1400 kJ/mol, whereas the gas-phase basicity of deprotonated 2,5-dihydroxybenzoic acid is 70 kJ/mol lower (GB([DHB – H]–) = 1329 kJ/mol). This may be rationalized by comparing with the acidities of substituted benzoic acids and phenols, as determined by McMahon and Kebarle. The data from their measurements for substituted benzoic acids and phenols are summarized in Tables 4.2 and 4.3, respectively. These data were calculated from equilibrium constants that were measured at 600 K, and anchored to the gas-phase basicity of the chloride anion. A direct comparison with the data determined in this work is complicated by the higher temperature at which the equilibrium measurements were performed, but general trends are nevertheless apparent.

As can be seen from Table 4.2, the ortho- and para-hydroxybenzoic acids are substantially more acidic than benzoic acid. McMahon and Kebarle propose that the acidic proton in the ortho- and para-
hydroxybenzoic acids is the hydroxy but not the carboxy proton, which they attribute to resonance stabilization of the anions. Moreover, the ortho-hydroxybenzoic acid anion has the lowest gas-phase basicity due to intramolecular hydrogen bonding between the COOH group and the O- anion. This is consistent with the data presented in this work; the carboxylic acid matrix anions with the lowest gas-phase basicities are those derived from 2,5-dihydroxybenzoic acid and 3-hydroxypicolinic acid, both exhibiting a hydroxy group next to the carboxy group.

<table>
<thead>
<tr>
<th>substituent</th>
<th>H</th>
<th>CH₃</th>
<th>OCH₃</th>
<th>OH</th>
<th>NH₂</th>
<th>NO₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>ortho</td>
<td>1411</td>
<td>1408</td>
<td>1409</td>
<td>1353</td>
<td>1400</td>
<td>1372</td>
</tr>
<tr>
<td>meta</td>
<td>1411</td>
<td>1414</td>
<td>1409</td>
<td>1406</td>
<td>1418</td>
<td>1371</td>
</tr>
<tr>
<td>para</td>
<td>1411</td>
<td>1416</td>
<td>1414</td>
<td>1394</td>
<td>1421</td>
<td>1362</td>
</tr>
</tbody>
</table>

Table 4.2 Gas-phase basicities (in kJ/mol) of deprotonated substituted benzoic acids. Data are from reference 210.

<table>
<thead>
<tr>
<th>substituent</th>
<th>H</th>
<th>CH₃</th>
<th>OCH₃</th>
<th>OH</th>
<th>NH₂</th>
<th>NO₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>ortho</td>
<td>1451</td>
<td>1449</td>
<td>1448</td>
<td>1407</td>
<td>1443</td>
<td>1393</td>
</tr>
<tr>
<td>meta</td>
<td>1451</td>
<td>1453</td>
<td>1445</td>
<td>1430</td>
<td>1455</td>
<td>1386</td>
</tr>
<tr>
<td>para</td>
<td>1451</td>
<td>1457</td>
<td>1455</td>
<td>-</td>
<td>1469</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 4.3 Gas-phase basicities (in kJ/mol) of deprotonated substituted phenols. Data are from reference 210.

Similar arguments can be used to explain the relatively low gas-phase basicity of deprotonated 2,4,6-trihydroxyacetophenone. Resonance structures of 2,4,6-trihydroxyacetophenone deprotonated at the ortho- and para-hydroxy groups are shown in Figure 4.2. Strong resonance stabilization lowers the anion basicity, with the charge either located at the hydroxy oxygen or at the acetaldehyde oxygen. As in the case of ortho-hydroxybenzoic acid, intramolecular hydrogen bonding is enhanced by electrostatic contributions from the negatively charged oxygen interacting with the ortho-hydroxy group.
The ferulic and sinapic acid anions, on the other hand, were found to have relatively high gas-phase basicities. Assuming that deprotonation occurs at the hydroxy and not the carboxy group, resonance structures are also readily drawn for these ions. However, stabilization of the anions will be significantly reduced when compared to the above examples because stabilization due to intramolecular hydrogen bonding cannot take place.

As shown in Figure 3.9, ferulic and sinapic acid differ only in the number of methoxy groups next to the para-hydroxy group. Ferulic acid has one, and sinapic acid has two methoxy groups, but molecular acidities were found to be the same within experimental error. This is in agreement with the change in acidity upon methoxy substitution found for both benzoic acids and phenols, which was generally small and at most 6 kJ/mol (see Tables 4.2 and 4.3).

The decrease in basicity of the ferulic and sinapic acid homodimer anions by about 115 kJ/mol versus the monomer anions can qualitatively be explained by resonance stabilization. Deprotonation of the FA and SA dimers at the phenolic rather than the carboxylic sites enables a much stronger stabilization of the dimer anion than of the corresponding monomer anion due to ion–dipole interactions, as pictured in Figure 4.3.
Figure 4.3 Resonance structures for the ferulic acid dimer anion deprotonated at the phenolic sites. The resonance structures involving intermolecular ion-dipole interaction probably account for the strong stabilization of the dimer anion.

The energies required for dissociation of the matrix homodimer anions (~1.7 eV) determined in the CID studies substantiate the proposed strong ion-dipole interactions. They exceed typical contributions from hydrogen bonds, which have strengths of about 20 kJ/mol or 0.21 eV each\textsuperscript{108}, but are lower than typical energies needed for the rupture of covalent bonds (~3 eV)\textsuperscript{211}. Besides hydrogen bonding between the carboxylic groups of the matrix species in the cluster ions, longer range ion-dipole interactions presumably account for the relatively high dissociation energies. The fact that loss of a neutral matrix molecule is the main dissociation product in the CID experiments also suggests that the bond nature is noncovalent.

The ferulic acid dimer and monomer anions were on average found to be slightly more reactive than the sinapic acid anions. This also suggests that deprotonation occurs at the phenolic rather than at the carboxylic hydroxy group. Then proton transfer to the phenolic oxygen is more sterically hindered in SA than FA, because SA has two neighboring methoxy groups and FA only has one.

It was shown in section 3.2.2, that the intrinsic barriers for proton transfer involving SA and FA dimer anions are about a factor of three lower than those for the monomer reactions. This means that for a given reaction exoergicity, reactions involving FA or SA dimer anions should proceed faster than the monomer reactions.
Seite Leer /
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4.2. Consequences for primary ion formation in MALDI

Possible proton transfer reactions for primary matrix ion generation include:

\[ M + M \rightarrow [M - H]^- + MH^+ \]  \hspace{1cm} (4.1)

\[ M + F \rightarrow [M - H]^- + FH^+ \]  \hspace{1cm} (4.2)

\[ M_2 + M \rightarrow [M_2 - H]^- + MH^+ \]  \hspace{1cm} (4.3)

\[ M_2 + F \rightarrow [M_2 - H]^- + FH^+ \]  \hspace{1cm} (4.4)

where M stands for a matrix molecule, \( M_2 \) for a matrix dimer, and F for a neutral matrix fragment. With the literature data and the results reported here, the energetics of reactions (4.1) through (4.4) can now be calculated for some matrices. The data for reactions of the type (4.1) are summarized in Table 4.4. The energies required for proton disproportionation reactions lie between 5 eV and 6 eV for the matrices investigated here.

<table>
<thead>
<tr>
<th>compound</th>
<th>GB([M - H]⁻) [kJ/mol]</th>
<th>GB(M) [kJ/mol]</th>
<th>( \Delta GB ) [kJ/mol]</th>
<th>[eV]</th>
</tr>
</thead>
<tbody>
<tr>
<td>para-nitroaniline</td>
<td>1410.6*</td>
<td>834.2t</td>
<td>576.4</td>
<td>5.97</td>
</tr>
<tr>
<td>sinapic acid</td>
<td>1399.8*</td>
<td>860.5‡</td>
<td>539.3</td>
<td>5.59</td>
</tr>
<tr>
<td>ferulic acid</td>
<td>1399.4*</td>
<td>849†t</td>
<td>550.4</td>
<td>5.71</td>
</tr>
<tr>
<td>nicotinic acid</td>
<td>1365.5*</td>
<td>877†t</td>
<td>488.5</td>
<td>5.06</td>
</tr>
<tr>
<td>3-hydroxybenzoic acid</td>
<td>1365.3*</td>
<td>866†t</td>
<td>499.3</td>
<td>5.18</td>
</tr>
<tr>
<td>2,5-dihydroxybenzoic acid</td>
<td>1329.4*</td>
<td>822.5‡</td>
<td>506.9</td>
<td>5.25</td>
</tr>
<tr>
<td>2,4,6-trihydroxyacetophenone</td>
<td>1324.3*</td>
<td>852x</td>
<td>472.3</td>
<td>4.90</td>
</tr>
</tbody>
</table>

Table 4.4 Gas-phase basicities of matrix species and calculated endoergicities \( \Delta G \) for reaction (4.1).

* this work, † reference 105, ‡ reference 95, †† estimated from proton affinity from reference 93, x estimated from proton affinity from reference 212, GB ~ PA ~ 30 kJ/mol.
<table>
<thead>
<tr>
<th>compound</th>
<th>GB([M - H]−) [kJ/mol]</th>
<th>GB(F) [kJ/mol]</th>
<th>ΔGB [kJ/mol] [eV]</th>
</tr>
</thead>
<tbody>
<tr>
<td>sinapic acid</td>
<td>1399.8*</td>
<td>900.5†</td>
<td>499.3</td>
</tr>
<tr>
<td>2,5-dihydroxybenzoic acid</td>
<td>1329.4*</td>
<td>882.0†</td>
<td>447.4</td>
</tr>
</tbody>
</table>

Table 4.5 Gas-phase basicities of matrix species and calculated endoergicities ΔG for reaction (4.2). F = M − H2O. * this work, † reference 95.

<table>
<thead>
<tr>
<th>compound</th>
<th>GB([M2 − H]−) [kJ/mol]</th>
<th>GB(M) [kJ/mol]</th>
<th>ΔGB [kJ/mol] [eV]</th>
</tr>
</thead>
<tbody>
<tr>
<td>sinapic acid</td>
<td>1285.0*</td>
<td>860.5†</td>
<td>424.5</td>
</tr>
<tr>
<td>ferulic acid</td>
<td>1285.6*</td>
<td>849†</td>
<td>436.6</td>
</tr>
</tbody>
</table>

Table 4.6 Gas-phase basicities of matrix species and calculated endoergicities ΔG for reaction (4.3). * this work, † reference 95, ‡ estimated from proton affinity from reference 93, GB ~ PA − 30 kJ/mol.

<table>
<thead>
<tr>
<th>compound</th>
<th>GB([M2 − H]−) [kJ/mol]</th>
<th>GB(F) [kJ/mol]</th>
<th>ΔGB [kJ/mol] [eV]</th>
</tr>
</thead>
<tbody>
<tr>
<td>sinapic acid</td>
<td>1285.0*</td>
<td>900.5†</td>
<td>384.5</td>
</tr>
</tbody>
</table>

Table 4.7 Gas-phase basicities of sinapic acid species and calculated endoergicities ΔG for reaction (4.4). F = M − H2O. * this work, † reference 95.

As can be seen from Table 4.5, this energy can be reduced by about 0.5 eV if a basic matrix fragment is the proton acceptor as in reaction (4.2). The energy reduction is even larger if matrix dimers instead of monomers function as proton donors. Then the required energies are only about 4 eV to 4.5 eV. Furthermore, charge separation of the ionic products can be facilitated by a low Coulomb energy due to charge delocalization in the case of matrix dimers, as discussed in section 1.1.6.

Sinapic acid is now the MALDI matrix best characterized with respect to thermochemical data on proton transfer, and the only matrix for which the entire set of proton transfer energetics for reactions (4.1) through (4.4) is known. The data for ferulic acid are similar, but the
ferulic acid fragment ion GB has not been determined yet. As can be seen from Tables 4.4 to 4.7, the energies required for proton transfer decrease in the order $M+M > M+F > M_2+M > M_2+F$. The matrix dimers were found to be the most acidic species, whereas the neutral matrix fragments were the most basic species. At least in the case of sinapic acid, the energetically most favorable reaction is reaction (4.4), which only costs about 4 eV.

This is consistent with experimental findings. The most abundant ion signals in a sinapic acid LDI mass spectrum arise from the products of reaction (4.4). In other words, the thermochemically most favorable reaction seems to dominate the observed final ion products in a MALDI plume, as illustrated in Figure 4.4. The mass spectra of ferulic and para-coumaric acid exhibit essentially the same characteristics as that of sinapic acid: the most abundant cation is the protonated fragment, and the most abundant anion is the deprotonated dimer. Obviously, the presence or absence of methoxy groups located next to the phenolic hydrogen does not strongly influence the mass spectral pattern, which is in agreement with the considerations in section 4.1. that methoxy substitution will not strongly change the acidity of the matrix dimers.

In addition to the deprotonated matrix dimer, matrix monomer anions are also observed. These can be assumed to stem from the dissociation of matrix dimer anions induced by collisions as described below. The relative kinetic energies of matrix ions and neutrals ejected in the MALDI process can be calculated from their respective average initial velocities.

For matrix ions, these were reported to be about $1140 \text{ m/s}^{213}$, $1000 \text{ m/s}^{214}$, and $1200 \text{ m/s}^{215}$ for sinapic acid, nicotinic acid and 2,5-dihydroxybenzoic acid, respectively. These velocities correspond to kinetic energies of 1.51 eV, 0.64 eV, and 1.15 eV, respectively. Juhasz and coworkers found somewhat lower average initial ion velocities between $350 \text{ m/s}$ and $670 \text{ m/s}$ for $\alpha$-cyano-4-hydroxycinnamic acid, sinapic acid, 2,5-dihydroxybenzoic acid mixed with 10% 2-hydroxy-5-methoxybenzoic acid, 2(-4-hydroxyphenylazo)-benzoic acid, and 3-hydroxypicolinic acid$^{216}$. On average, initial matrix ion velocities were found to be roughly 1000 m/s.
Matrix neutrals, on the other hand, were reported to have lower average initial velocities. For laser desorbed neutral 2,5-dihydroxybenzoic acid molecules, an average initial velocity of 540 m/s was found, corresponding to a kinetic energy of 0.23 eV. Huth-Fehre and Becker determined the average initial velocity of laser-desorbed
ferulic acid neutrals to be 300 m/s, corresponding to a kinetic energy of 0.09 eV\textsuperscript{218}. Spengler and coworkers found that laser desorbed tryptophan neutrals have average initial velocities of 200 m/s, corresponding to an average kinetic energy of only 0.04 eV\textsuperscript{219}. They obtained comparable results for a number of other substances and state that these values are in sharp contrast to the initial kinetic energies of the ions desorbed at the same irradiances, which are typically in the 1 eV to 20 eV range\textsuperscript{219}. On average, initial matrix neutral velocities were found to be roughly 350 m/s.

Velocity distributions of laser-desorbed neutrals and ions can be described by Maxwell-Boltzmann distributions on a stream velocity as in Eq. (4.5)\textsuperscript{218, 220, 221}:

\[
\frac{dN(v)}{dv} \sim v^3 \cdot \exp\left(-\frac{m}{2kT} \cdot (v - v_s)^2\right)
\]  

where \(v_s\) is the stream velocity. In the upper trace of Figure 4.5, velocity distributions calculated from Eq. (4.5) are shown. The corresponding kinetic energy distributions are shown in the middle trace, and the bottom trace shows the relative center-of-mass kinetic energies between neutral monomers and dimer ions. In accordance with the results obtained by Huth-Fehre and Becker, a plume temperature of 800 K was assumed in these simulations, and the stream velocity of the neutral monomer with a mass of 200 Da was assumed to be 100 m/s\textsuperscript{218}. The stream velocity of the dimer ion (400 Da) was assumed to be higher and 1000 m/s.

The velocity distributions in Figure 4.5 are in good agreement with measured data for matrix neutrals\textsuperscript{218} and matrix ions\textsuperscript{213}. The average kinetic energy of the monomer in this simulation is about 0.2 eV, and that of the dimer is about 2.5 eV. These simulations can be used as an estimate for the maximum translational energy that can be converted into internal energy upon collisions of matrix ions with neutrals in the expanding MALDI plume. From the CID experiments it is evident that the onset for dissociation of FA and SA matrix dimer anions is at about 1 eV. Thus, the relative center-of-mass energies of dimer anions and
monomeric neutrals shown in the bottom trace of Figure 4.5 would be sufficient to partially dissociate the matrix dimer anions.

![Image of velocity distributions](image)

Figure 4.5 Velocity distributions calculated from eq. (4.5) (upper trace) and the corresponding kinetic energy distributions (middle trace). The solid lines represent the neutral matrix monomers (200 Da) with a stream velocity of 100 m/s, and the dashed lines represent the matrix dimer anions (400 Da) with a stream velocity of 1000 m/s. In these calculations, a plume temperature of 800 K was assumed. In the bottom trace, the relative center-of-mass kinetic energy distribution of neutral matrix monomers and matrix dimers anion is shown.

Direct formation of SA monomer anions via reaction (4.2) costs 5.18 eV (see Table 4.5). On the other hand, dimer anion formation via reaction (4.4) costs only 3.99 eV (see Table 4.7), and the energy for dissociation into monomer anion and neutral can be provided by energetic collisions. One could argue that the total energy for each channel is essentially the same in both cases, which is about 5 eV for both direct monomer anion formation and dimer anion formation (~ 4 eV) followed
by dissociation (~1 eV). However, direct monomer anion formation is a concerted reaction in which the total energy of above 5 eV needs to be available all at once, whereas dimer anion formation followed by dissociation is a stepwise reaction that only requires about 4 eV in the initial proton transfer step. The energy needed for dissociation of the dimer can then be imparted in successive collisions. Thus, if the initial proton transfer step is a thermal process, dimer anion formation followed by dissociation is more likely than direct formation of monomer anions.

Because gas-phase basicities of matrix and analyte molecules and their corresponding anions usually do not differ so much, the proposed proton transfer reactions could also lead to direct ionization of analytes instead of matrix molecules. The energetically most favorable reactions involve the most acidic [M$_2$] and most basic (F) matrix species, as in reactions (4.6) and (4.7).

\[
M_2 + A \rightarrow [M_2 - H]^+ + AH^+ \quad (4.6)
\]

\[
F + A \rightarrow FH^+ + [A - H]^- \quad (4.7)
\]

The proton affinities of α-amino acids have been measured$^{124, 222}$, from which gas-phase basicities between 850 and 990 kJ/mol can be derived$^{115}$. Gorman and Amster determined the gas-phase basicities of dipeptides that contain valine to be at most 950 kJ/mol$^{125}$, and dipeptides of serine and glycine were found to have GBs not exceeding 885 kJ/mol$^{128}$. The GBs of selected di- and tripeptides of histidine and lysine were determined to be at most 965 kJ/mol$^{223}$. Oligomers of alanine, valine$^{129}$, and glycine$^{224}$ have been studied with respect to their gas-phase basicities as a function of oligomer length. In all cases, the gas-phase basicities were found to increase with increasing oligomer chain length, reaching a maximum value for the tetramer of at most 951 kJ/mol$^{129, 224}$. Wu and Fenselau determined the gas-phase basicities of glycine and alanine oligomers and also observed an increase in gas-phase basicity with increasing oligomer length of up to 992 kJ/mol for the glycine decamer$^{225}$. They further found that peptides containing very basic amino acid residues such as arginine, lysine, and histidine exhibit gas-phase basicities of up to 1012 kJ/mol$^{225}$. These
data suggest peptide and protein GBs between 850 kJ/mol and 1010 kJ/mol. Typical gas-phase basicities of oligonucleotides were estimated to be roughly 955 kJ/mol. Thus, reaction (4.6) involving analyte molecules and SA or FA dimers requires energies between 275 kJ/mol (2.85 eV) and 435 kJ/mol (4.51 eV) to proceed.

The gas-phase basicities of deprotonated α-amino acids were determined to lie between 1356 kJ/mol and 1402 kJ/mol, which can be used as an estimate for peptide anion gas-phase basicities. Deprotonated amino acid side chain proton affinities were estimated to be higher, ranging from 1439 kJ/mol to above 1632 kJ/mol, which corresponds to GBs of about 1409 kJ/mol and 1602 kJ/mol, respectively.

The gas-phase basicities of deprotonated oligonucleotides have not yet been determined and are the subject of current investigations. However, from the LD/CI experiments reported in section 3.1., an adenosine-5'-monophosphate (AMP) anion proton affinity of about 1280 kJ/mol was derived from unimolecular dissociation analysis. The GB of (AMP-H)− should be about 30 kJ/mol lower, due to the typical entropy contribution at 300 K. Because all nucleic acids are identical with respect to their acidic functionality, the phosphate group, oligonucleotide GBs should not deviate much from this value of 1250 kJ/mol. With these data, reaction (4.7) involving analyte molecules and the neutral SA fragment requires between 350 kJ/mol (3.62 eV) and 502 kJ/mol (5.20 eV).

The matrix–matrix reactions (4.1) through (4.4) and the matrix–analyte reactions (4.6) and (4.7) require comparable amounts of energy to proceed. Matrix and analyte can thus compete for protons donated by acidic species such as matrix dimers in the case of positive ion formation, and they can compete for basic proton acceptors such as neutral matrix fragments in the case of negative ion formation. Such processes might be important for observation of the matrix-suppression effect. Another aspect of the reactions suggested above which is consistent with the proposed explanation for the matrix-suppression effect is the participation of at least two matrix molecules in the primary ionization events.
In order to determine if the thermochemistry of the proposed reactions controls analyte ion formation in a MALDI plume, the tripeptide Gly-Gly-His (T) was used as a probe. The chemical structure of Gly-Gly-His is shown in Figure 4.6. The gas–phase basicity of T is 979.5 kJ/mol\textsuperscript{105}. The GB of deprotonated T, GB([T – H]\textsuperscript{−}), is not known, but can be reasonably well estimated from the GB of deprotonated histidine to be about 1356 kJ/mol\textsuperscript{111}. With these data and the data for sinapic acid, the energetics of the proton transfer reactions (4.6) and (4.7) can be calculated. Reaction (4.6) between SA\textsubscript{2} and T costs 305.5 kJ/mol (3.17 eV), and reaction (4.7) between (SA – H\textsubscript{2}O) and T costs about 455.5 kJ/mol (4.72 eV).

![Figure 4.6 Chemical structure of the tripeptide Gly-Gly-His.](image)

The GBs of SA and the SA fragment are 860.5 kJ/mol and 900.5 kJ/mol, respectively. If these neutral SA species compete with the tripeptide T (GB = 979.5 kJ/mol) for protons donated by neutral SA dimers, then thermochemistry predicts that tripeptide protonation will be favored over matrix protonation by about 0.82 eV, and an intense [T + H]\textsuperscript{+} signal can be expected. Because the protons will be donated by the neutral matrix dimers, this should be accompanied by an intense [SA\textsubscript{2} – H]\textsuperscript{−} signal in negative polarity.

On the other hand, competition for proton acceptors such as (SA – H\textsubscript{2}O) will favor matrix instead of tripeptide deprotonation. Reaction (4.4) costs 3.99 eV in the case of sinapic acid, and reaction (4.7) yielding analyte anions costs 4.72 eV, which is 0.73 eV more.

Overall, reaction (4.6) yielding [T + H]\textsuperscript{+} and [SA\textsubscript{2} – H]\textsuperscript{−} costs only 3.17 eV and is by far the least expensive reaction. If thermochemical matrix and analyte properties control ion formation in MALDI, then this reaction should predominate.
As can be seen in Figure 4.7, these expectations are in agreement with what is observed experimentally. The SA matrix was mixed with the tripeptide at the highest analyte/matrix ratio allowing for crystallization, which was about 1:5. This analyte/matrix ratio should allow for matrix suppression for the small analyte molecule Gly-Gly-His\textsuperscript{227}. As expected, matrix suppression occurs in positive polarity. Only very small signals due to analyte fragmentation, sodium ion attachment, and heterodimer formation appear.

In negative polarity, the [SA\textsubscript{2} - H]	extsuperscript{-} ion is the dominant signal. Additionally, comparably small [SA - H]	extsuperscript{-} and [T - H]	extsuperscript{-} signals are detected. The latter ions most likely stem from dimer dissociation and secondary ionization, as discussed in more detail in section 4.3. As already observed for the pure matrix spectra, the experimentally observed ions result from the thermochemically most favorable reaction.
The fact that the energetically most favorable reactions studied here predict products that are actually observed in the MALDI spectra should not distract from the fact that all reactions proposed in this section are endoergic. These reactions might become exoergic if the matrix dimer anion GB is lowered as it can be the case in an electronically excited state. However, the change in GB upon electronic excitation would have to be on the order of 300 kJ/mol, which does not seem likely. Calculated differences in GBs of excited and ground states have been reported to be at most 267 kJ/mol and generally much lower\textsuperscript{229}.

Nevertheless, the photon energy absorbed by the matrix molecules is undoubtedly needed to promote primary ionization. Considering only total absolute energy and not how it is distributed, requirements for the proposed proton transfer reactions can now be calculated in terms of photon energy. Reaction (4.6) yielding \([T + H]^+\) and \([SA_2 - H]^-\) costs 3.17 eV, which is the energy of one photon with a wavelength of 391 nm. A 355 nm-photon has even more energy, 3.49 eV. If the entire photon energy becomes available for proton transfer, then the absorption of only one photon with a wavelength shorter than 391 nm should be sufficient to initiate reaction (4.6). This is in contrast to the successful MALDI experiments using 532 nm irradiation\textsuperscript{67}; photons with a wavelength of 532 nm have only 2.33 eV. Moreover, the absorbed photon energy is also partly needed in the desorption process, and therefore it is more likely that two photons are involved. Assuming that two photons initiate MALDI ionization, then even 532 nm photons (corresponding to 4.66 eV) provide more than enough energy for the proposed proton transfer reaction, and the excess energy could be consumed in the desorption process.

It is not clear yet how the absorbed photon energy becomes available for proton transfer reactions. A possible mechanism is internal conversion. As discussed in section 1.1.4., ions can be formed out of the high energy reactants in a Boltzmann distribution. Then, a (local) temperature of about 4000 K is needed to yield 0.01 % products in a reaction with 3.17 eV endoergicity. Potential mechanisms still have to be examined, for example by means of spectroscopic investigations.
The energetically most favorable reaction proposed here involves acidic matrix dimer molecules, but the corresponding product, \([M_2 - H]^-\), is not generally observed with all MALDI matrices. Matrix dimer anions were observed to different extents with 3-aminoquinoline, para-nitroaniline, 2,4,6-trihydroxyacetophenone, 2-amino-5-nitropyridine, 2-amino-3-hydroxypyridine, ferulic and sinapic acid, but not with 2,5-dihydroxybenzoic acid (DHB). This can either mean that DHB dimer anions rapidly dissociate to form monomer anions (as observed with 2,4,6-trihydroxyacetophenone), or it is possible that the neutral matrix monomer instead acts to protonate analyte or matrix. The latter hypothesis is supported by the relatively high gas-phase acidity of DHB \((\text{GB}((\text{DHB} - H)^-) = 1329 \text{ kJ/mol})\) when compared to sinapic or ferulic acid monomers \((\text{GB}((\text{FA} - H)^-) = \text{GB}((\text{SA} - H)^-) = 1400 \text{ kJ/mol})\).

Based on their comparatively high acidities, matrix dimers of FA and SA are proposed here to act as proton donors as in reactions (4.4) and (4.6). Two structural features of these acids were used to explain their high acidity in section 4.1.: first, the carboxylic acid group that enables dimer formation via hydrogen bonding, and second, the para-hydroxy group that was considered as the proton donor group. These structural features are also present in related cinnamic acid derivatives that function well as MALDI matrices.

Ehring, Karas, and Hillenkamp studied five cinnamic acid derivatives with respect to their ability to protonate peptides and proteins under UV-MALDI conditions. They found that the compounds exhibiting a para-hydroxy group (3,4-dihydroxycinnamic acid (caffeic acid), 3,5-dimethoxy-4-hydroxycinnamic acid (sinapic acid), 4-hydroxy-3-methoxycinnamic acid (ferulic acid)) yielded strong protein signals, whereas compounds not exhibiting a para-hydroxy group (2,4-dimethoxycinnamic acid, 3-hydroxy-4-methoxycinnamic acid) yielded only weak protein signals. Moreover, \(\alpha\)-cyano-4-hydroxycinnamic acid was found to be a highly effective MALDI matrix for the generation of protonated peptide and protein ions. These findings all support the hypothesis that at least in the case of cinnamic acid derivatives, matrix dimers act as protonating agents in MALDI.
In contrast, Amster and coworkers stated that neither the carboxy nor the para-hydroxy proton is required to form positively charged analyte molecules in a MALDI experiment. They synthesized the methyl ether, the methyl ester, and the doubly methylated derivative of sinapic acid and used these as MALDI matrices with gramicidin S and insulin as analyte. With all derivatives, analyte signals were obtained in positive polarity. However, the mass resolution of the linear TOF mass spectrometer used was always below 230 in the case of insulin (5734 Da), which is not sufficient to distinguish between protonated and sodiated insulin ions.

Schlunegger and coworkers tested small aromatic molecules as MALDI matrices that were selected using structural criteria. They chose several hydroxycarbonyl compounds and hydroxybenzoic acids and found that ortho-substituted molecules such as ortho-hydroxyacetophenones and ortho-hydroxybenzophenones were good MALDI matrices, whereas the corresponding meta- and para-isomers showed a significantly lower or no matrix activity, in agreement with the results for dihydroxybenzoic acid isomers reported by Hillenkamp and coworkers. The authors attribute this to the compounds' capability for intramolecular proton transfer along an intramolecular hydrogen bond upon UV irradiation. They suggest that the ortho-hydroxy proton is transferred to the carbonyl oxygen, forming a metastable excited state matrix zwitterion. This zwitterion is believed to donate the labile proton to analyte molecules, yielding deprotonated matrix and protonated analyte.

However, their findings can also be interpreted differently. With the exceptions of 4-hydroxyacetophenone and 4-hydroxybenzophenone, the compounds that were found to function well as MALDI matrices in Schluneggers' study have in common that the corresponding anions exhibit strong stabilization due to intramolecular hydrogen bonding as discussed in section 4.1..

For example, they found that 2,4-dihydroxyacetophenone is an excellent MALDI matrix, but 2-hydroxyacetophenone is not. Strong resonance stabilization involving intramolecular hydrogen bonding of the anion is possible in the case of 2,4-dihydroxyacetophenone, but not
with 2-hydroxyacetophenone, as illustrated in Figure 4.8. This suggests that 2,4-dihydroxyacetophenone is much more acidic when compared to 2-hydroxyacetophenone, and that 2,4-dihydroxyacetophenone is the better proton donor. On the other hand, if intramolecular proton transfer along the hydrogen bond is a crucial prerequisite in MALDI ionization, both molecules should function equally well.

![Figure 4.8: Resonance structures of deprotonated 2,4-dihydroxyacetophenone (top) and deprotonated 2-hydroxyacetophenone (bottom). Strong resonance stabilization is possible in the case of 2,4-dihydroxyacetophenone due to intramolecular hydrogen bonding in the anion, whereas for the 2-hydroxyacetophenone anion, only a relatively small resonance stabilization is possible.](image)

Among the 26 compounds tested by Schlunegger and coworkers, 20 molecules exhibit ortho-hydroxy groups, and out of these, only 13 were found to be good MALDI matrices. Thus, the presence of an ortho-hydroxy group alone does not seem to be a guarantee for good MALDI matrix performance. The 13 compounds that worked well as protonating agents for proteins under MALDI conditions differ from the 7 that did not work well by the presence of a second labile proton in addition to the proton of the ortho-hydroxy group, as in the above example. This supports the hypothesis of enhanced matrix acidity due to strong resonance stabilization of the corresponding anions involving intramolecular hydrogen bonding rather than Schlunegger's hypothesis of intramolecular proton transfer.

Unfortunately, only very few gas-phase acidity values of the molecules investigated by Schlunegger and coworkers are available as follows: 2,4,6-trihydroxyacetophenone (THAP, $G_B(\text{THAP} - \text{H}^-) = 1324$ kJ/mol, this
work), 2,5-dihydroxybenzoic acid (DHB, \( GB((DHB - H)^-) = 1329 \text{ kJ/mol} \)), 2-hydroxybenzoic acid (2HBA, \( GB((2HBA - H)^-) = 1330 \text{ kJ/mol}^{111} \)), 4-hydroxybenzophenone (4HBP, \( GB((4HBP - H)^-) = 1364 \text{ kJ/mol}^{111} \)), 4-hydroxyacetophenone (4HAP, \( GB((4HAP - H)^-) = 1375 \text{ kJ/mol}^{111} \)), 4-hydroxybenzoic acid (4HBA, \( GB((4HBA - H)^-) = 1376 \text{ kJ/mol}^{111} \)), and 3-hydroxybenzoic acid (3HBA, \( GB((3HBA - H)^-) = 1387 \text{ kJ/mol}^{111} \)). Among these, THAP, DHB, and 2HBA have relatively high acidities, which can be viewed as an argument for the proton transfer reactions proposed here. However, these molecules also have an ortho-hydroxy group, which can as well be taken as an argument for Schlunegger's hypothesis of intramolecular proton transfer. 4-HAP and 4-HBP have neither an ortho-hydroxy group, nor do they have a particularly high acidity, so both explanations fail for these compounds.

In general, the findings in the literature and the data presented in this section suggest that a suitable MALDI matrix should be capable of forming acidic neutral matrix species that can function as proton donors for analyte ion formation in positive polarity. Deprotonated analyte can analogously be formed if the matrix is susceptible of forming basic neutral matrix species that can function as proton acceptors.

The proton transfer reactions suggested here do not exclude the possibility of delayed analyte ionization, meaning that the products of reactions (4.1) – (4.4), (4.6), and (4.7) can further react with neutral analyte. This is discussed in more detail in the following section 4.3..
4.3. Consequences for secondary ion formation in MALDI

Possible proton transfer reactions for secondary analyte ion generation include:

\[ [M + H]^+ + A \rightarrow M + AH^+ \]  \hspace{1cm} (4.8)

\[ [M - H]^- + A \rightarrow M + [A - H]^- \]  \hspace{1cm} (4.9)

where A is the analyte and M is the matrix molecule. Reaction (4.8) proceeds towards products if \( GB(A) > GB(M) \), and reaction (4.9) is exoergic for \( GB([M - H]^-) > GB([A - H]^-) \). The secondary analyte ion formation mechanisms via reactions (4.8) and (4.9) may then make a major contribution to the MALDI ion signal in cases where primary analyte ion formation via reactions (4.6) and (4.7) is unfavorable.

It was recently shown that protonated matrix in many cases can protonate analyte molecules via ground-state exoergic proton transfer reactions as in (4.8)\(^9^3\)-\(^9^5\). With the data on matrix anions determined in this work, the corresponding analyte deprotonation reactions as in (4.9) can now be quantified.

An analyte class which is predominantly detected in negative polarity is that of nucleic acid oligomers\(^6\). In typical negative ion MALDI mass spectra, oligonucleotides appear as deprotonated molecules which can result from a deprotonation reaction with matrix anions via reaction (4.9). Matrix anion GBs were found to lie between 1324 kJ/mol and 1451 kJ/mol, significantly higher than the estimated oligonucleotide anion GB in all cases. Therefore, with all matrices investigated here, a ground-state oligonucleotide deprotonation via reaction (4.9) is energetically favorable.

In order to avoid or minimize analyte fragmentation, the excess energy released in an exoergic deprotonation reaction should be kept as small as possible, meaning that the differences in matrix and analyte anion GBs should be small. For oligonucleotides, a 'soft' deprotonation reaction occurs if the GB of the matrix anion is higher than, but close to
the GB of the oligonucleotide anion (GB $\approx 1250$ kJ/mol). The GB of the 2,4,6-trihydroxyacetophenone (THAP) anion is closest to the estimated value ($\Delta GB \approx -75$ kJ/mol), and from the point of view of chemical ionization and neglecting excited-state chemistry, it constitutes the softest reagent anion for oligonucleotide deprotonation found here. The next least basic anion is that of 2,5-dihydroxybenzoic acid (DHB) ($\Delta GB \approx -80$ kJ/mol). The 3-hydroxypicolinic acid (HPA) anion basicity is about 40 kJ/mol higher ($\Delta GB \approx -115$ kJ/mol). HPA should still be a good candidate for soft nucleotide deprotonation since activation energies for dinucleotide anion fragmentation can be estimated to be about 130 kJ/mol, as discussed in section 3.1.

This is consistent with experimental findings. THAP$^{231}$ and HPA$^{39}$ were found to be particularly suitable for nucleic acid analysis in negative ion mode. Both matrix anions are located on the lower end of the basicity scale, which is in agreement with the above considerations. Tang and coworkers found that DHB is a good matrix for short oligonucleotides up to 5–6 bases in negative ion mode, but for larger oligomers, strong fragmentation was observed$^{232,233}$. Moreover, for larger oligonucleotides, DHB induced more fragmentation than HPA in negative mode$^{234}$. The results for small oligonucleotides are consistent with the above considerations, but a thermodynamic explanation fails for larger oligonucleotides. Zhu and coworkers suggest that the observed fragmentation is initiated by protonation of the nucleic acid bases, followed by base loss and phosphodiester backbone cleavage$^{234}$. In this complex, multi-step process, the ability of a matrix to protonate the bases should therefore be a key factor for the observed fragmentation in negative ion mode. Since the proton affinity of DHB is 853 kJ/mol, 42 kJ/mol lower than that of HPA, their model can be used to explain the observed stronger fragmentation in the case of DHB when compared to HPA. However, it is not clear why this mechanism is only relevant when analyzing larger oligonucleotides.

Ammonium salts have become important as comatrices in oligonucleotide MALDI mass spectrometry, among them ammonium acetate$^{46}$ and ammonium fluoride$^{44}$. Zhu and coworkers suggest that besides suppressing alkali-ion adducts, ammonium salts have both a protonation and deprotonation function$^{47}$. In the LD/CI studies reported...
in section 3.1., it was shown that the acetate and fluoride anions cause substantial fragmentation of the relatively fragile nucleotides. It is therefore unlikely that ammonium acetate or fluoride should be potent deprotonation agents without inducing fragmentation at the same time. Li and coworkers did not observe fragmentation when using a HPA/NH₄F matrix. Interestingly, they did observe fragmentation under the same conditions when using a 2-amino-5-nitropyridine (2A5NP)/NH₄F matrix. This is consistent with the data reported here, since the 2A5NP anion GB is 1399 kJ/mol, 34 kJ/mol higher than that of HPA, making the deprotonation reaction exoergic with about 149 kJ/mol. Although ammonium salts are capable of improving the signal-to-noise ratio in MALDI mass spectra, it is more likely that analyte ionization is controlled by the thermochemical properties of the MALDI matrix used. A possible alternative explanation for the observed signal enhancement is an improved incorporation of analyte molecules into the matrix crystals caused by the salt addition.

MALDI analysis of peptides and proteins is usually performed in positive mode. However, with many matrices, MALDI ion generation is possible in negative polarity as well as in positive polarity. With peptide and protein anion GBs estimated from amino acid anions (between 1356 kJ/mol and 1402 kJ/mol), it can be calculated that depending on the peptide or protein composition, a ground-state deprotonation reaction of the type (4.9) is energetically favorable with a variety of matrix anions, especially with the more basic ones.

In a MALDI spectrum, ionic products from primary and secondary formation pathways are usually indistinguishable. However, the SA/Gly–Gly–His system introduced in section 4.2. can be used to study the respective contributions. In the experiment shown in Figure 4.6, not only an intense [SA₂ – H]⁻ signal, but also smaller [T – H]⁻ and [SA – H]⁻ signals were observed. As discussed in section 4.2., the [SA – H]⁻ ion is most likely formed upon dimer anion dissociation. Whereas the [SA₂ – H]⁻ ion with a basicity of 1285 kJ/mol will not be able to abstract a proton from the tripeptide T (GB([T – H]⁺) ~ 1356 kJ/mol), the GB of [SA – H]⁻ is 1400 kJ/mol and sufficiently high to promote tripeptide deprotonation. It can therefore be assumed that the [T – H]⁻ ion in Figure 4.7 is a result of secondary ion formation.
If this is true, higher dissociation rates of \([SA_2 - H]^-\) should yield more \([SA - H]^-\), which in turn should spontaneously react with neutral tripeptide to form \([T - H]^-\). Experimentally, increased dissociation of the dimer anions can be induced by higher laser fluences. Spengler and Bökelmann studied initial matrix ion velocities as a function of laser fluence and found that average initial velocities strongly increased with increasing fluence. When raising the fluence by a factor of six, initial matrix ion kinetic energies increased by about a factor of seven. This behavior was not observed for desorption of neutral matrix molecules. Thus, upon using higher laser fluences, matrix ion/neutral collisions in the MALDI plume should be more energetic, and it can be expected that the \([SA_2 - H]^-\) ion dissociates more efficiently due to an increased dissociation rate.

As can be seen in Figure 4.9, these expectations are again in full agreement with what is observed experimentally. The SA dimer anion signal is now very small, presumably due to dissociation into monomeric SA anions. These can then rapidly react with neutral tripeptide to form the observed abundant \([T - H]^-\) signal.

![Figure 4.9](image)

**Figure 4.9** MALDI FT-ICR mass spectra of the tripeptide Gly-Gly-His \([T]\) in positive (top) and negative (bottom) ion mode. Sinapic acid (SA) was used as the matrix, and the tripeptide/matrix ratio was approximately 1:5. The laser fluence was approximately 500 J/m².
In positive polarity, the dominant signal is that of the protonated tripeptide. When compared to the spectrum in Figure 4.7, more sodiated tripeptide and more fragment ions from dissociation of the protonated peptide are observed here. The latter are most likely a result of increased analyte internal energy due to the increased laser fluence, as also observed by Mowry and Johnston for alkylamine analytes\(^8\).

As stated above, ionic products from primary and secondary formation pathways in MALDI are usually indistinguishable. However, secondary ion formation can only follow primary ionization, which implies that these are separated in time. Evidence for contributions of both mechanisms in MALDI was recently found in MALDI time-of-flight studies\(^2, 15, 236\). The flight time profile analysis of bovine insulin cluster ions produced by MALDI suggested that a MALDI plume consists of two separate components\(^236\). Flight time analysis suggested that one component is best described as prompt ionization, and the second component is best described as delayed ionization\(^236\). A bimodal plume profile was also observed in an angular resolved time-of-flight study, with a significant quenching of matrix ions in areas of high peptide ion densities\(^2, 15\). These findings suggest that analyte ions in MALDI can be formed in a primary ion formation mechanism or a secondary ion formation mechanism or both.
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5. Summary

Among the possible ion formation mechanisms in MALDI, proton transfer reactions between matrix and analyte are widely believed to play a major role. Based on the thermochemical data on matrix species determined in this work, the energetics of such processes can now be calculated.

The gas-phase basicities of ten monomeric MALDI matrix anions were determined. The most basic matrix anion found was that of 3-aminoquinoline (GB = 1451 kJ/mol) and the least basic was the 2,4,6-trihydroxyacetophenone anion (GB = 1324 kJ/mol), spanning an energy range of 127 kJ/mol, or 1.3 eV. Additionally, the gas-phase basicities of dimeric deprotonated ferulic and sinapic acids were determined. The matrix dimer anions (GB = 1285 kJ/mol) were found to be significantly less basic than the corresponding monomer anions (GB = 1400 kJ/mol), by about 115 kJ/mol, or 1.2 eV.

With these data, the energies for ground-state proton transfer between neutral matrix species and analyte molecules were estimated to lie between 2.8 eV and 5.2 eV. Secondary ground-state proton transfer reactions between matrix ions and neutral analyte were found to be energetically favorable in most cases. In a case study it was shown that the thermochemically most favorable among the proposed proton transfer reactions yielded products that were observed in the MALDI spectra.

For the purpose of determining gas-phase basicities of matrix species, two new bracketing-related methods that yield reliable thermochemical data have been developed. The "equilibrium bracketing" method is based on equilibrium and transition state theory, and the "bracketing by reactivity/ergodicity correlation" method uses structure-reactivity correlations for deriving gas-phase basicities from reaction efficiencies. Both methods were applied in conjunction with a new technique for the generation of reference anions that was also developed within the scope of this work. The reference anions were generated from a two-phase liquid/solid mixed matrix by laser
desorption, allowing measurements at a well-defined temperature and under low pressure conditions.

The reference anions employed in the bracketing experiments can also serve as reagent anions in MALD/CI experiments. For this purpose, a simple, controlled, and efficient method for performing chemical ionization following laser desorption has also been developed as part of this work. It is a straightforward technique yielding molecular ions and controlled fragmentation simultaneously. The method was successfully applied to several classes of analytes. Fragmentation is controlled by taking advantage of the differing proton affinities of the reagent anions. Proton abstraction from the analyte molecules then makes differing amounts of energy available for unimolecular dissociation.

Future investigations of the ionization mechanisms in MALDI should include the determination of analyte thermochemical properties such as gas-phase basicities and acidities. Moreover, spectroscopic studies of matrix and matrix/analyte clusters should be carried out in order to determine the mechanisms of photon energy conversion.
Literature


### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>2A5NP</td>
<td>2-amino-5-nitropyridine</td>
</tr>
<tr>
<td>2HBA</td>
<td>2-hydroxybenzoic acid</td>
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<tr>
<td>3HBA</td>
<td>3-hydroxybenzoic acid</td>
</tr>
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<td>4HAP</td>
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<td>4HBA</td>
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<td>4HBP</td>
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<td>23DNP</td>
<td>2,3-dinitrophenol</td>
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<tr>
<td>24DNP</td>
<td>2,4-dinitrophenol</td>
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<tr>
<td>AC</td>
<td>alternating current</td>
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<tr>
<td>ADC</td>
<td>analog-to-digital converter</td>
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<td>ADO</td>
<td>average dipole orientation</td>
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<td>AMP</td>
<td>adenosine-5'-monophosphate</td>
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<tr>
<td>BIRD</td>
<td>blackbody infrared dissociation</td>
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<tr>
<td>BREC</td>
<td>bracketing by reactivity/ergodicity correlation</td>
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<td>CBA</td>
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<td>CI</td>
<td>chemical ionization</td>
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<td>CID</td>
<td>collision-induced dissociation</td>
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<td>CNP</td>
<td>2-chloro-4-nitrophenol</td>
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<td>DHB</td>
<td>2,5-dihydroxybenzoic acid</td>
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<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
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<tr>
<td>ECD</td>
<td>electron capture dissociation</td>
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<tr>
<td>Er:YSGG</td>
<td>Erbium Yttrium Scandium Gallium Garnet</td>
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<td>ESI</td>
<td>electrospray ionization</td>
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<tr>
<td>ESPT</td>
<td>excited-state proton transfer</td>
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<td>FA</td>
<td>ferulic acid</td>
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<td>FAB</td>
<td>fast atom bombardment</td>
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<td>FFT</td>
<td>fast Fourier transform</td>
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<tr>
<td>FT-ICR</td>
<td>Fourier transform–ion cyclotron resonance</td>
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<tr>
<td>FWHM</td>
<td>full width at half maximum</td>
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<tr>
<td>GB</td>
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<td>H/D</td>
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<td>ICR</td>
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<tr>
<td>IR</td>
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<td>Abbreviation</td>
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<tr>
<td>IRMP</td>
<td>infrared multiple photon</td>
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<td>IRMPD</td>
<td>infrared multi-photon dissociation</td>
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<td>LD/Cl</td>
<td>laser desorption/chemical ionization</td>
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<td>LFER</td>
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<td>MALD/Cl</td>
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<td>MALDI</td>
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<td>multi-photon ionization</td>
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<td>MRP</td>
<td>mass resolving power</td>
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<tr>
<td>NBA</td>
<td>3-nitrobenzoic acid</td>
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<tr>
<td>Nd:YAG</td>
<td>Neodymium Yttrium Aluminum Garnet</td>
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<td>NIST</td>
<td>National Institute of Standards and Technology</td>
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<tr>
<td>OPO</td>
<td>optical parametric oscillator</td>
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<tr>
<td>PA</td>
<td>proton affinity</td>
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<td>pAP</td>
<td>para-aminophenol</td>
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<td>PFPE</td>
<td>perfluorinated polyethers</td>
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<td>PSD</td>
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<td>RAM</td>
<td>random-access-memory</td>
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<tr>
<td>RE</td>
<td>reaction efficiency</td>
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<td>RF</td>
<td>radio frequency</td>
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<td>sinapic acid</td>
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<td>surface-induced dissociation</td>
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<td>SWIFT</td>
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<td>ultraviolet photodissociation</td>
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Publications


K. Breuker, R. Knochenmuss, R. Zenobi, 'Matrix-assisted laser desorption/chemical ionization with reagent ion generation directly from a liquid matrix', International Journal of Mass Spectrometry 176, 149–159 (1998). This publication was voted by the Scientific Selection committee as jointly the most significant contribution to mass spectrometry published in the International Journal of Mass Spectrometry in 1998 (Best Student Paper Award 1999 for the best paper on technique development).


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Curriculum Vitae

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1986 – 1994 physics studies, Münster, Germany

1987 – 1990 Research Assistant in several projects, Jowat Lober u. Frank GmbH & Co KG, Detmold, Germany
"Development of adhesives by computer simulations", July 1987 – October 1987
"Synthesis of polyamides and determination of physical properties", February 1989 – April 1989
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1993 – 1994 Student Assistant, Westfälische Wilhelms-Universität Münster, Germany

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